GLOBAL GENE EXPRESSION DURING MUSCLE HYPERTROPHY INDUCED BY MYOSTATIN SUPPRESSION AND/OR BETA-ADRENERGIC AGONIST

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ABSTRACT

Skeletal muscle growth is important for animal agriculture, particularly for meat-producing animals, and for human health as well. A better understanding of the mechanisms regulating skeletal muscle growth is expected to contribute to improving the efficiency of meat animal production and alleviating human suffereings caused by muscle atrorphic conditions. Currently, two molecules have been shown to have dramatic effects on skeletal muscle mass: myostatin and β-adrenergic agonists. Myostatin (Mstn), a member of the TGF-β superfamily proteins, acts as a potent negative regulator of skeletal muscle growth. Suppression of Mstn by varying means has shown to increase skeletal muscle mass of animals. Administration of β-adrenergic agonist (BAA) such as clenbuterol (CL) induces dramatic increase in skeletal muscle mass or inhibits muscle atrophy.

Although there have been numerous demonstrations of the muscle growth–promoting effect of BAA administration or Mstn suppression, the effect of a combination of BAA administration and Mstn inhibition on skeletal muscle growth has not been investigated. Similarly, very little is known about the molecular signaling pathways leading to muscle hypertrophy induced by the two stimuli and the genes that are commonly regulated by both Mstn and BAA administration. Therefore, the objectives of this study were 1) to investigate the combined effect of Mstn-suppression and BAA administration on skeletal muscle growth, 2) to examine the role of the Akt/mTOR pathway in the two muscle hypertrophic models, 3) to examine global changes in gene expression in skeletal muscle undergoing hypertrophy induced by chronic suppression of Mstn or BAA administration , and 4) to compare the changes in gene expression between these two muscle hypertrophic models.

We used a transgenic mouse strain that overexpresses the Mstn-prodomain

(Mstn-pro) and exhibits a significant increase in skeletal muscle mass regardless of age and sex. Clenbuterol (CL) was used as a BAA compound. Heterozygous Mstn-pro and wild-type littermates were produced and were given 0 or 20 ppm of CL in their drinking water. Phosphorylation of molecules involved in the Akt/mTOR pathway was examined by using the Western blot analysis. RNA samples of the gastrocnemius muscle in each group were subjected to microarray analysis using the Affymetrix GeneChip Mouse 430-2.0 platform.

CL increased body and muscle mass of male and female mice in both genotypes, indincating that the muscle-hypertrophic effect of CL is additive to the effect of Mstn suppression. Levels of phosphorylated muscle 4E-BP1 and p70S6k, two downstream effectors of the mTOR pathway, were higher in Mstn-pro mice than in wild type mice. Levels of phosphorylated muscle Akt, an upstream effector of the mTOR pathway, were also higher in Mstn-pro mice than in wild type mice, indicating that the Akt/mTOR anabolic pathway is involved in the regulation of muscle mass by Mstn. CL increased the phosphorylation of Akt, 4E-BP1 and p70S6k in both genotypes, resulting in the highest phosphorylation levels of Akt, 4E-BP1 and p70S6k in CL-fed Mstn-pro mice. This result suggests that like Mstn, BAA also regulates muscle hypertrophy through the Akt/mTOR pathway, and the pathways of Mstn and CL signaling converge to the Akt/mTOR anabolic pathway to regulate skeletal muscle hypertrophy.

Microarray analysis of global gene expression showed that Mstn suppression and CL administration induced significant changes in the mRNA abundance of various genes associated with muscle contraction, initiation of translation, transcription, and muscle hypertrophic signal pathway, suggesting that increased protein synthesis is partly responsible for the hypetrophy induced by Mstn and CL. Additionally, the alteration of Igf2 obsderved in Mstn suppressed mice, and the alterations of eIF4e, Acvr2b, FoxO and PTEN observed in mice treated with CL indicate that the pathways of Mstn and CL signaling converge to the Akt/mTOR anabolic pathway to regulate skeletal muscle hypertrophy.

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CHAPTER 1

LITERATURE REVIEW

1.1 SKELETAL MUSCLE GROWTH AND DEVELOPMENT

1.1.1 Embryonic development of skeletal muscle

During vertebrate embryogenesis, skeletal muscle forming cells arise from the somites, which are formed sequentially as epithelial spheres from the rostral part of the unsegmented paraxial mesoderm (reviewed by Stockdale et al., 2000). The somites mature according to a rostro-caudal gradient of differentiation and become subsequently subdivided into the ventrolateral sclerotome that gives rise to axial skeleton and ribs, and the dorsomedial dermomyotome that contributes to the formation of dermis and skeletal muscles (reviewed by Stockdale et al., 2000). Two distinctive compartments present in the dermomyotome give rise to separate lineage of skeletal muscles during embryogeneis: the dorsomedial epaxial domain gives rise to the deep back and intercostal muscles, and the lateral hypaxial domain gives rise to the rest of the musculature of the body and limbs. The hypaxial dermomyotome gives rise to muscles in two distinct ways: ventrolateral extension of the dermomyotome forms the body wall muscles and some migratory precursor cells leaving the dermomyotome form more distant muscle masses of the wing and limb (Ordahl and Ledouarin, 1992; Miller et al., 1999).

The myotomal compartments of somites eventually give rise to myoblasts, mono-nucleated muscle precursor cells. During embryonic muscle development, the mono-nucleated myoblasts fuse together to form multi-nucleated myotubes. Later, myotubes mature into highly specialized skeletal muscle fibers that show cross striation. The determination of myogenic cell lineage (myoblast) and control of terminal differentiation is regulated by the expression of myogenic regulatory transcription factors (reviewed by Perry and Rudnicki, 2000). In addition, the presence of some particular growth factors such as insulin, insulin like growth factor-1 (IGF-1), fibroblast growth factor (FGF) and transforming growth factor (TGF)-β, regulates sustained proliferation of myoblasts and subsequent withdrawal from the cell cycle as part of differentiation process (reviewed by Florini et al., 1996). The timing of entry of myoblasts into differentiation is likely to play a significant role in determining the number of muscle fibers, and subsequent postnatal muscle mass.

1.1.2 Myogenic regulatory factors (MRFs)

The myogenic regulatory factors (MRFs) form a family of basic helix-loop-helix (bHLH) transcription factors consisting of Myf5, MyoD, myogenin, and MRF4. The MRFs are expressed exclusively in the skeletal muscle lineage, and forcing their expression in a wide range of cultured cells induces the skeletal muscle differentiation program. They activate muscle specific gene expression by binding to the E-boxes (CANNTG) in the promoter region of target genes after dimmer formation with the E2A proteins, ubiquitously expressed bHLH proteins. Gene targeting studies that induced null mutations in Myf5, MyoD, myogenin and MRF4 into the germ line of mice revealed a hierarchical relationship among the MRFs and established that functional redundancy is a feature of the MRF regulatory network (reviewed by Rudnicki and Jaenisch, 1995). The MRF family can be divided two-tiered subclassification through sequence homology, expression patterns, and gene targeting expressions: Myf5 and MyoD are homologous genes, and are expressed in myoblasts before differentiation, and are required for the determination or survival of muscle progenitor cells. Myogenin and MRF4 are homologous genes, and are expressed upon differentiation. Myf5 and MyoD are classified as primary MRFs because they are required for the determination of skeletal myoblasts in proliferation and early differentiation stages, and myogenin and

MRF4 are classified as secondary MRFs because they are required in the later stages of myognic program for the terminal differentiation of myoblasts and myofibril synthesis (reviewed by Rudnicki and Jaenisch, 1995).

The MyoD null mice show no obvious abnormalities in skeletal muscle but express four-fold higher level of Myf5, suggesting the presence of functional redundancy between MyoD and Myf5 (Rudnicki et al., 1992). Newborn mice lacking Myf5 also have normal skeletal muscle but die prenatally because of severe rib abnormalities, indicating the requirement of Myf5 for normal rib development in addition to the role in myoblast determination and proliferation (Braun et al., 1992). Newborn mice lacking both MyoD and Myf5 have a complete absence of skeletal muscle (Rudnicki et al., 1993), indicating the requirement of either MyoD or Myf5 for myoblast proliferation. The myogenin gene null mice die at birth due to a lack of myofibers, suggesting that myogenin plays an important role in the differentiation of myoblast into myotube and myofibers (Hasty et al., 1993). The MRF4 gene null mice displayed normal Myf5 expression and a four-fold induction in myogenin, and is viable with seemingly normal skeletal muscle, suggesting that MRF4 may function late in the myogenic pathway, and its function may be substituted by the presence of myogenin (Zhang et al., 1995).

The expression of MRFs appears to be regulated by pax3, a paired-domain containing transcription factor, since forced expression of pax3 using retroviral expression systems in chick tissue explants revealed that pax3 activates the expression of MyoD, Myf5, and myogenin (Maroto et al., 1997). Another paired-domain containing transcription factor pax7 have shown to play an important role in the biology of satellites cells (reviewed by Hawke and Garry, 2001).

1.1.3 Postnatal development of skeletal muscle

The increase in skeletal muscle mass during postnatal growth is mainly due to an increase in muscle fiber size (hypertrophy). This process is accompanied by the proliferative activity of satellite cells, which are the source of new nuclei incorporated into the muscle fibers (reviewed by Rehfeldt et al., 2000). After birth, the number of skeletal muscle fiber in most mammals and avian species does not increase due to the completion of embryonic proliferation of skeletal muscle cells (Rowe and Goldspink, 1969). For example, no significant changes in postnatal fiber number have been found in mice (Rowe and Goldspink, 1969), rat (Rosenblatt and Woods, 1992), pig (Fiedler et al., 1983), cattle (Wegner et al., 2000) and chicken (Smith and Fletcher, 1988). However, some studies indicated that in some species, limited extent of muscle cell proliferation occurs after birth. For example, the increase in muscle fiber number was observed shortly after birth in rodents (Summers and Medrano, 1994) and pigs (Swatland, 1975). Others argued that the increase in fiber number during the first days of postnatal life was a result of maturation and elongation of the existing myotubes rather than due to a production of new fibers (Ontell and Kozeka, 1984). In this regard, the postnatal increase in skeletal muscle mass appears to be achieved mostly by an increase in fiber size and not much by an increase in fiber number. Since fiber size cannot be increased beyond a certain limit, the growth potential for skeletal muscle is virtually determined by the number of fibers established at around the time of birth. This relationship of muscle number and growth potential has been demonstrated in the enlarged muscles of double-muscled cattle (Swatland and Kieffer, 1974), in genetically different size of animals (Hanrahan et al., 1973), and in runts as compared with normal pigs (Powell and Aberle, 1981). Interestingly, some studies indicate that muscle hypertrophy is inversely correlated with muscle fiber number at the end of the intensive growth period. For example, the postnatal growth rate of the individual muscle fiber is lower when there are high numbers of fibers and higher when there are low numbers of fibers (reviewed by Rehfeldt et al., 2000).

1.1.4 Muscle hypertrophy and satellite cell

Muscle mass increases more than 10 fold from birth to mature body size with a slight decrease in DNA: protein ratio during this period, indicating that there is an increase in the number of myonuclei during the postnatal hypertrophy of skeletal muscle fibers. The increase in the number of myonuclei is solely contributed to satellite cells that are located between the basal lamina and sarcolemma of myofibers (Moss and Leblond, 1971). Satellite cells have one nucleus that occupies most of the cell volume and unlike the nuclei inside muscle fibers, the satellites cells retain the capacity to proliferate in response to stimuli, thus provide most of the myonuclei to adult muscles during growth to balance the cytoplasm/nuclei ratio. Satellites cells have long been considered monopotential only giving rise to cells of myogenic lineage (Bischoff and Heintz, 1994). However, recent evidence demonstrates that satellite cells are capable of forming osteocytes and adipocytes in response to different growth factors *in vitro*, indicating that satellites possess multipotential mesenchymal stem cell activity (Asakura et al., 2001).

The remarkable adaptability of skeletal muscle to physiological stimuli such as exercise training, stress and injury is attributable to the function of satellite cells. Usually, satellite cells are dormant, but they become activated when the muscle fiber receives any forms of trauma, damage or injury. The satellite cells then proliferate or multiply, and the daughter cells are drawn to the damaged muscle site to form new muscle fibers during the regeneration period (reviewed by Hawke and Garry, 2001).

While it is well established that satellite cells play a role in maintaining the relatively constant myonuclear domain size during growth and are source of new muscle fibers during regeneration, there has been a debate regarding whether satellite cell activation is prerequisite for muscle hypertrophy occurring in response to various hypertrophic stimuli or during growth. Recent studies suggest that muscle hypertrophy can occur without the activation of satellite cells. Blaauw et al., (2009) showed that the increase in skeletal muscle mass induced by overexpression of Akt occurred without satellite cell activcation. Postnatal blockade of myostatin, a negative regulator of muscle growth, induced massive skeletal muscle hypertrophy without satellite cell activation (Amthor et al., 2009). Some β_2-adrenergic agonists induced skeletal muscle hypertrophy by increased protein synthesis and decreased protein degradation without satellite cell activation in rats (Rehfeldt et al., 1994) and chickens (Rehfeldt et al., 1997). Furthermore, stretch-overload could induce hypertrophy in skeletal muscle whose satellite cells were inactivated by irradiation (Lowe and Always, 1999), supporting that satellite cell activation is not prerequisite for muscle hypertrophy. In contrast, Kawano et al. (2008) reported that satellite cell activation in response to mechanical load/or neural activity plays an essential role in muscle hypertrophy. While the debate on the role of satellite cells on muscle hypertrophy is not settled, it is generally agreed that experimental models (type of hypertrophic stimuli) and situations (magnitude of growth response and the age of animal, timing of measurement) influence the relationship between muscle hypertrophy and satellite cell activation (O'Connor et al., 2007).

1.2 SIGNALING PATHWAYS IN SKELETAL MUSCLE HYPERTROPHY AND ATROPHY

Changes in skeletal muscle mass are dictated through the process of myofibrillar protein turnover, which is the balance between protein synthesis and protein breakdown. Higher rate of protein synthesis relative to protein degradation must be maintained in order to achieve hypertrophy, whereas elevated protein breakdown will induce a loss in muscle mass (atrophy). Overall, these processes continuously operate and are controlled by the intimate relationship between the mitogen, growth factors, and hormone-responsive signaling pathways (reviewed by Richardson et al., 2004; Glass, 2005, Sandri, 2008). Figure 1.1 illustrates the major signaling pathways that control muscle fiber size.

1.2.1 Akt/mTOR signaling pathway

The mammalian target of rapamycin (mTOR) pathway has emerged as a key regulator of cell growth that integrates signals from growth factors, nutrients, and energy status to control protein synthesis and other cell functions (reviwed by Asnaghi et al., 2004). The serine/threonine kinase mTOR interacts with multiple protein partners, forming two distinctive multi-protein complexes, mTORC1 consisting of GβL, and raptor, and mTORC2 consisting of GβL, mSin, and rictor. The mTORC1 complex is selectively inhibited by rapamycin, a drug used as an immunosuppressant in organ transplantation: rapamycin binds to members of the FK binding protein (FKBP) family and the complex rapamycin/FKBP binds to mTORC1 and blocks its activity. The mTORC1 complex mediates functions that are sensitive to rapamycin such as protein translation, whereas mTORC2 mediates functions not sensitive to rapamycin. The major upstream regulator of mTOR is Akt, a serine/threonine kinase also known as protein kinase B. Akt activation of mTOR is indirect through the phosphorylation of tuberous sclerosis 2 (TSC2), a GTPase activating protein (GAP) that functions together with TSC1 to inactivate the small G protein Rheb. The phosphorylation of TSC2 activtes Rheb, leading to the activation of the mTOR signal transduction pathway.

The role of the mTOR pathway in muscle growth was demonstrated by *in vivo* studies, in which the hypertrophic responses induced by overload or regenerating muscle growth are blocked by rapamysin (Bodine et al., 2001; Pallafacchina et al., 2002). In addition, transgenic mice overexpressing TSC1 specifically in skeletal muscle showed a defect in muscle growth through the downregualtion of mTOR activation (Wan et al., 2006). It was also reported that muscle-specific ablation of mTORC1 (by ablating raptor) results in a dystrophic phenotype (Bentzinger et al., 2008).

One of the major upstream regulators of Akt is phosphatidylinositol-3-OH kinase (PI3K), a lipid kinase mediating the action of IGF-1. It is well established that IGF-1 is a potent stimulator of skeletal muscle hypertrophy as demonstrated by an increase in muscle mass and strength by forced expression of IGF-1 in mice (Coleman et al., 1995; Musaro et al., 2001). Studies have shown that the PI3K/Akt pathway plays an important role in mediating the hypertrophic action of IGF-1, as well as other anabolic stimuli, such as overloading, mechanical stimuli and insulin (reviewed by Nader, 2005; Glass, 2005). Expression of constructs encoding constitutively active forms of either PI3k or Akt induced muscle hypertrophy both *in vitro* (Rommel et al., 1999; Rommel et al., 2001) and *in vivo* (Bodine et al., 2001; Pallafacchina et al., 2002). Furthemore, activation of the Akt/mTOR pathway could oppose muscle atrophy induced by disuse (Bodine et al., 2001).

mTOR activation by Akt induces the phosphorylation of two downstream effectors, ribosomal protein p70S6k and 4E-BP1, a suppressor of the cap binding protein eIF4E, resulting in increased protein translation (reviewed by Sarbassov et al., 2005). Supporting the role of p70S6k as a downstream mediator for the mTOR anabolic pathway, p70S6k null mice has a smaller muscle fiber size and the hypertrophic response to IGF-1 and activated Akt is blocked in p70S6k null mice (Ohanna et al., 2005). Anabolic stimuli, such as overloading, resistance exercise, and essential amino acid ingestion, have been shown to increase the phosphorylation of p70S6k and 4E-BP1 along with a corresponding increase in protein synthesis (reviewed by Sarbassov et al., 2005). Conversely, atrophying muscles have decreased levels of phosphorylation of the downstream targets of mTOR (Bodine et al., 2001; Hornberger et al., 2001).

1.2.2 FoxO transcription factors

A major contributor for skeletal muscle atrophy (decrease in skeletal muscle mass) is the degradation of myofibrillar proteins via ubiquitin-proteasome system. Recent studies have shown that two muscle-specific ubiquitin ligases, atrogin-1/MAFbx and MurF1 are responsible for the increased protein breakdown via ubiquitin-proteasome system observed in various models of muscle atrophy including immobilization, denervation, hindlimb suspension, administration of IL-1, and administration of the glucocorticoid hormone (reviewed by Sandri, 2008). Studies have shown that muscle atrophy in various models could be blocked by IGF-1 treatment, and this was accompanied by a complete suppression of the induction of these two genes (Lee et al., 2004). Genetic activation of Akt, a downstream mediator of IGF-1 signaling, was shown to be sufficient to block the atrophy-associated increases in MAFbx and MuRF1 transcription (Stitt et al., 2004). These findings together demonstrated that Akt stimulation could dominantly inhibit the induction of atrophy signaling. The mechanism by which Akt inhibits MAFbx and MuRF1 upregulation involves the Forkhead box O (FoxO) family of transcription factors (Lee et al., 2004; Sandri et al., 2004; Stitt et al., 2004). FoxO transcription factors are excluded from the nucleus when phosphorylated by Akt, and translocate to the nucleus upon dephosphorylation. The nuclear translocation of FoxO transcription factors was required for upregulation of MuRF1 and MAFbx, and FoxO was found to be sufficient to induce atrophy when trasfected in skeletal muscles *in vivo* (Sandri et al., 2004). Moreover, the knockdown of FoxO expression by RNAi suppressed the upregulation of atrogin-1/MAFbx expression during atrophy and muscle loss (Liu et al., 2007). It should be noted that FoxO is not only regulated by Akt, but also many other Akt-independent regulatory pathways (Huang and Tindall, 2007), suggesting a presence of Akt/Foxo-independent atrophic pathways. According to a study by Southgate et al. (2007), FoxO can also induce atrophy by suppressing the anabolic mTOR pathway since activation of FoxO1 in skeletal muscle induced the expression of 4E-BP1 along with a reduction in the abundance of Raptor and mTOR protein.

1.2.3 NF-kB signaling pathway

The NF-κB transcription factors (p65/p50) belong to the Rel family of transcription factors, which play a major role as mediators of immunity and inflammation. The NF-κB transcription factors are also expressed in skeletal muscle and appear to play a role in regulating muscle differentiation and mediate the effect of inflammatory cytokines, in particular TNF-α, on muscle wasting and cachexia (reviewed by Glass, 2005). This NF-κB pathway is activated by phosphorylation, ubiquitination, and proteolysis of the inhibitory protein IκB, which in non-activated state binds to NF-κB in the cytosol to prevent nuclear translocation of NF-κB and suppress NF-κB-mediated gene trascriptions. The inactivation of IκB is through phosphorylation of the molecule on serine residue mediated by IK kinase complex (IKK). TNF-α-induced NF-κB activation leads to muscle protein loss and inhibition of NF-κB protects against TNF-α-induced protein degradation in cultured muscle cells (reviewed by Glass, 2005; Sandri, 2008). The activation of NF-κB through muscle-specific overexpression of IKKβ in transgenic mice led to severe muscle wasting (Cai et al., 2004). On the other hand, muscle atrophy induced by hindlimb unloading is reduced in mice with a knockout of the p105/p50 NF-κB1 gene (Hunter and Kandarian, 2004). Moreover, the electrotransfer of constitutively active IκB in soleus muscles inhibited unloading-induced fiber atrophy by 40% (Judge et al., 2007).

It appears that muscle atrophy mediated by NF-κB activation involves the activation of proteasome system. Inhibiting NF-κB activity attenuated the induction of proteasome expression in skeletal muscle (Wyke et al., 2004). In contrast, elevation in the expression of the ubiquitin-ligase MuRF1 was observed in wasting muscles of transgenic mice in which the NF-κB pathway was constitutively active through muscle-specific overexpression of IKKβ (Cai et al., 2004). Interestingly, the above study did not observe any changes in the expression of another muscle-speicif ubiquitin ligase MAFbx.

1.2.4 MAPK/ERK signaling pathway

The mitogen-activated protein kinase (MAPK) is a family of serine/threonine kinases that exists in all eukaryotes, and controls such fundamental cellular processes as proliferation, differentiation, survival and apoptosis (reviewed by Pearson et al., 2001). In mammals, three major MAPK cascades are known, the extracellular signal-regulated protein kinase (ERK) cascade, c-Jun amino-terminal protein kinase/stress-activated protein kinase (JNK/SAPK) cascade and p38MAPK cascade. Among those three MAPK cascades, the Ras/Raf/MEK (mitogen-activated protein kinase)/ERK pathway controls such fundamental cellular processes as cell proliferation, differentiation and survival. A wide variety of hormones, growth factors and differentiation factors activate Ras proteins by inducing the exchange GDP to GTP. The activated Ras functions as an adapter that binds to Raf kinases with high affinity and causes their translocation to the cell membrane, where Raf activation takes place. The activated Raf can activate MEK, and then MEK can activate the downstream ERK kinases. One effect of ERK activation is to alter the translation of mRNA to proteins, and phosphorylation of 40S ribosomal protein S6 kinase. Also, MAPK/ERK signaling pathway regulates the actvitiy of several transcription factors, such as C-myc, MNK, CREB, C-fos (reviewed by Moodie and

Wolfman, 1994; Kolch, 2000; Avruch et al., 2001). The MAPK/ERK signaling pathway appears to have a role in cardiac muscle growth and protein synthesis by stimulating initiation and elongation of protein translation. For example, Babu et al. (2000) demonstrated that *in vitro* phosphorylation of Elk-1 by the ERK kinase pathway is important for early gene activation during phenylephrine-induced cardiac myocyte hypertrophy. Huang et al. (2005) also demonstrated that the activation of ERK induced myocardial hypertrophy in rats (Huang et al., 2005). Moreover, Kim et al. (2006) demonstrated that the administration of isoproterenol induced cardiac hypertrophy by increased phosphorylation of ERK. In human skeletal muscles, phosphorylation of ERK1/2 and MEK1/2 increased in an exercise intensity-dependent manner, suggesting a potential role of the MAPK/ERK signaling pathway in skeletal muscle growth (Widegren et al., 2000).

1.3 REGULATION OF MUSCLE GROWTH BY MYOSTATIN

Myostatin (Mstn), also known as a growth differentiation factor-8 (GDF-8), was first identified by McPherron et al. (1997) in a study seeking novel mammalian members of the transforming growth factor-β (TGF-β) superfamily. The study reported that knock-out of the myostatin gene increased muscle mass up to three-fold in mice (McPherron et al., 1997). Subsequently, studies have demonstrated that nonfunctional mutations of Mstn are associated with dramatic muscularity in cattle (Grobet et al., 1997; Kambadur et al., 1997; McPherron and Lee, 1997), dogs (Mosher et al., 2007), sheep (Clop et al., 2006) and humans (Schuelke et al., 2004), collectively demonstrating that Mstn is a negative regulator of skeletal muscle growth. Studies have demonstrated that Mstn suppresses muscle growth in two ways: one way to limit muscle growth is by regulating the number of muscle fiber during development, and the other way is by regulating postnatal hypertrophy of skeletal muscle fibers. Because of its unique role on muscle growth and development, extensive investigations have been performed with related to myostatin, resulting in almost 1,000 publications in the last 13 years since its first identification in 1997. Many reviews on myostatin are available (Sharma et al., 2001; Kocamis and Killefer, 2002; Gonzalez-Cadavid and Bhasin, 2004; Lee, 2004; Patel and Amthor, 2005; Joulia-Ekaza and Cabello, 2007; McFarlane et al., 2008; Rodgers and Garikipati, 2008).

1.3.1 Role of myostatin in the control of myoblast proliferation and differentiation

Mstn is expressed in cells in the myotome during embryogenesis (McPherron et al., 1997). In pigs, Mstn mRNA was detectable in whole fetuses at 21 and 35 days of gestation and was markedly increased by 49 days (Ji et al., 1998). At birth, mRNA abundance in longissimus muscle had declined significantly from the level at day 105 of gestation and continued to decrease to its lowest level at 2 weeks postnatally (Ji et al., 1998). The reduction in Mstn mRNA abundance at birth and postnatal period coincides also with the period of reduction in myoblast mitogenic activity and differentiation. In skeletal muscle cell cultures, addition of recombinant Mstn or over-expression of Mstn inhibited the proliferation of mouse myoblasts (Thomas et al., 2000; Taylor et al., 2001), turkey embryonic myoblasts and satellite cells (McFarland et al., 2006), and chicken embryonic myoblasts (McFarland et al., 2007). Levels of Mstn mRNA expression during chicken embryonic development and in muscle cell culture support the inhibitory role of Mstn on myoblast proliferation because the lower level of Mstn mRNA coincides generally with the period of myoblast proliferation before myotube formation (Kocamis et al., 1999; Kocamis et al., 2001). The inhibitory role of Mstn on myoblast proliferation explains the hyperplasia of muscle fibers observed in mice, cattle, sheep and dogs carrying a non-functional myostatin gene.

The inhibition of the proliferation of myoblasts by Mstn appears to be through the prevention of the progression of myoblasts from the G1- to S- phase of the cell cycle regulated by retinoblastoma (Rb) protein hypophosphorylation (Thomas et al., 2000; Joulia et al., 2003). The maintenance of the proliferative capacity of myoblasts after removing Mstn from the cell culture indicates that the inhibitory effect of Mstn is reversible (Taylor et al., 2001). In addition, the Mstn-added muscle cell cultures showed the upregulation of p21, a cyclin-dependent kinase inhibitor, and down regulation of cyclin dependent kinase-2 (Cdk-2) with higher levels of hypophosphorylated Rb (Thomas et al., 2000). The upregulation of p21 was also observed in C2C12 muscle cell cultures over-expressing Mstn (Rios et al., 2001). Because Cdks are the key regulatory molecules for cell proliferation, these results indicate that Mstn inhibit myoblast proliferation through the Cdk system. Since Rb protein phosphorylation by Cdk physically releases E2F-DP1, a component that is needed for the transcription of S-phase-specific genes, by inhibiting the phosphorylation of Rb and its dissociation of E2F-DP1, Mstn ceases the cells in G0/G1 phase of cell cycle (Thomas et al., 2000; Joulia et al., 2003). These results suggest that myostatin probably arrests the myoblast at G0/G1 phase of the cell cycle in a phosphorylation of Rb-dependent pathway. Results from Langley et al. (2004) showed that myostatin inhibited myoblast proliferation by down regulating Cdk2 and Cyclin E, without altering phosphorylation status of Rb in rhabdosarcoma cell cultures, indicating a presence of Rb-independent pathway to inhibit myoblast proliferation. Yang et al. (2007) also demonstrated that Mstn treatment inhibited C2C12 cells proliferation by down regulating Cdk4 and Cyclin D1 through the Akt/GSK-3β pathway independent of Rb phosphorylation. In addition, a recent study indicated that inhibition of myoblast proliferation by Mstn is associated with upregulation of ubiquitin ligase RING finger protein 13 (RNF 13) at both the transcriptional and translational levels (Zhang et al., 2010).

Besides inhibiting myoblast proliferation, myostatin also appears to have a negative regulatory role in myoblast differentiation. Mstn inhibited myoblast differentiation in a dose-dependent manner in C2C12 myoblast culture, where down-regulation of MyoD, Myf5, and myogenin were observed in C2C12 cells by Mstn treatment (Langley et al., 2002; Rios et al., 2002; Joulia et al., 2003). Increased phosphorylation of Smad 2/3, a down-stream mediator of Mstn signaling, and Smad2/3-MyoD association were observed by Mstn treatment during myoblast differentiation (Bogdanovich et al., 2002; Langley et al., 2002). Conversely, when cultured C2C12 cells were treated with blocking antibodies generated against Mstn, cells treated with anti-myostatin antibody had decreased concentrations of phosphorylated Smad2 and 3 as compared to controls (Bogdanovich et al., 2002). Using siRNA to block Smad2/3, Trendelenburg et al., (2009) demonstrated that the Mstn's anti-differentiation effect requires both of the Smads. These studies together indicate that Mstn requires Smad 2/3 activation in order to inhibit myoblast differentiation. Recent evidences indicate that Mstn inhibition of myoblast differentiation requires the activation of the Akt/mammalian target of rapamycin (mTOR) pathway. For example, Trendelenburg et al., (2009) demonstrated that the Mstn's anti-differentiation effects can be blocked by blockade of the Akt/mTOR pathway by using small interfering RNA.

1.3.2 Role of myostatin in the control of post-natal muscle growth

In addition to the inhibitory role of Mstn in myoblast proliferation and differentiation during development, numerous studies indicate that Mstn has a role in skeletal muscle growth and maintenance in postnatal animals. Whittemore et al. (2003) produced monoclonal anti-Mstn antibody, and when they administered the antibody to mice, they observed that the antibody-treated mice gained approximately 10 % more weight than the control. The administration of monoclonal anti-Mstn antibodies to mdx mice (an animal model for Dunchenne muscular dystrophy) increased body weight, whole muscle cross sectional area and muscle fiber area after 3 months of treatment as compared to control animals (Bogdanovich et al., 2002). Mstn suppression by anti-Mstn antibody generated via DNA vaccine method in mice increased skeletal muscle mass up to 31%, as well as muscle function (Tang et al., 2007). Conversely, systemic administration of Mstn by injection of Mstn–producing CHO cells into the thigh muscle or ectopic expression of Mstn through the electrotransfer of Mstn-containing plasmid significantly decreased skeletal muscle mass in adult mice (Zimmers et al., 2002).

Studies in which genectic manipulation was used to modify postnatal Mstn concentration also provide evidence supporting the role of Mstn in postnatal skeletal muscles. Grobet et al. (2003) generated conditional Mstn knockout mice that demonstrated postnatal inactivation of Mstn. They reported that the conditional Mstn knockout mice had significantly increased muscle mass, as was demonstrated in the constitutive Mstn knockout mice generated by McPherron et al. (1997). However, unlike the constitutive myostatin knockout mice, they reported that the increase in skeletal muscle mass in the conditional myostatin knockout mice was primarily due to the hypertrophy of skeletal muscle fibers. Injection of plasmid expressing short-interfereing RNA (siRNA) targeting Mstn in adult mice increased tibialis muscle mass (10%) and fiber size (24%), along with a two-fold increase in satellite cell number (Magee et al., 2006). Direct injection of antisense RNA against Mstn also improved the muscle growth in adult normal and cachexia mice (Liu et al., 2008). Systemic overexpression of dominant negative form of Mstn in liver via aneno-associated virous method led to an increase in skeletal muscle mass in normal and dystrophic mice (Morine et al., 2010). Overexpression of dominant-negative latency-associated Mstn propeptide under the control of myosin light chain 1F promoter and 1/3 enhancer from the TSPY locus on the Y chromosome in transgenic mice induced a 5-20% increase in skeletal muscle mass (Pirottin et al., 2005).

In support of the negative regulation of Mstn on postnatal muscle hypertrophy, various studies reported a negative relationship between the level of Mstn and muscle mass under various physiological or pathological conditions that induced muscle loss or gain in many vertebrate species. Carlson et al. (1999) examined Mstn mRNA abundance in mice gatrocnemius and plantaris muscles undergoing hindlimb unloading at 1, 3 and 7 days. Significant muscle atrophy was observed at 3 day after unloading. Mstn mRNA increased significantly in mice gatrocnemius and plantaris muscles at 1 day after hindlimb unloading but not at 3 or 7 days of hindlimb unloading, suggesting that Mstn upregulation was associated with the atrophy of skeletal muscles. Wehling et al. (2000) also reported that 10 days of unloading caused a 16 % decrease in plantaris mass and a 110 % increase in Mstn mRNA. Consistent with the above results, the expression of Mstn mRNA level increased 30-fold in both chronic and acute disuse-induced muscle atrophy (Reardon et al., 2001). Mstn mRNA concentrations were significantly higher in skeletal muscles from the spaceflight rat than those in the ground-based controls, resulting in negative relationship between the myostatin mRNA expression and muscle mass (Lalani et al., 2000).

Some studies indicate that regulation of satellite cell proliferation by Mstn is associated with the negative role of Mstn on postnatal skeletal muscle growth. McCroskery et al. (2003) demonstrated that satellitce cell activation was inhibited by the addition of Mstn to myofiber explant cultures. The Mstn-deficient mice had higher number of satellite cells as compared with wild type mice. In contrast, addition of Mstn to myofiber explant cultures inhibited satellite cell activation. Cell cycle analysis showed that Mstn up-regulated p21 and decreased the levels and activity of Cdk2 protein in satellite cells. Hence, Mstn negatively regulates the G_1 to S progression of statellite cells and thus maintains the quiescent status of satellite cells. McFarland et al. (2006) also demonstrated that Mstn inhibts the proliferation and differentiation of myogenic satellite cells, indicating that Mstn is a potential negative regulator of the activation of satellite cells. Furthermore, McFarlane et al. (2008) demonstrated that exogenous addition of Mstn dramatically reduced Pax7 expression, which is required for satellite cell specification (self-renewal process) in C2C12 cells. In contrast, either functional blockade or complete genetic inactivation of Mstn resulted in an increase in Pax7 expression. These results suggest that Mstn signals via a Pax7-dependent mechanism to regulate satellite cell self-renewal.

Other studies indicate that Mstn regulates postnatal skeletal muscle hypertrophy through regulation of protein synthesis mediated by the Akt/mTOR signaling pathway. Amirouche et al. (2009) demonstrated that the overexpressed-Mstn in mice skeletal muscle by electrotransfer of Mstn expression vector attenuated the phosphorylation of Akt and 4E-BP1, indicating an mTOR-dependent downregulation of protein synthesis by myostatin. Welle et al. (2009) also demonstrated that the administration of anti-Mstn antibody suppressed Mstn activity through an increased phosphorylation of p70S6k, but not by an increased phosphorylation of Akt and 4E-BP1. Furthermore, genetic loss of Mstn in mice led to enhanced muscle expression of Akt and elevated activity of the components of the Akt/mTOR pathway (Lipina et al., 2010), and adenoviral-mediated overexpression of Mstn attenuated the IGF-1-induced myotube diameter through the down-regulation of Akt (Morissette et al., 2009).

1.3.3 Role of myostatin on muscle fiber type

In addition to the regulation of muscle mass, Mstn also appears to be involved in the determination of muscle fiber type composition and to regulate the hypertrophy of muscle fiber differentially dependent upon muscle fiber types. The expression of Mstn was higher in fast type muscle than in slow type muscle (Mendler et al., 2000). In agreement with this finding, the greatest amount of Mstn mRNA was also found in the white portion of mouse quadriceps muscle that was composed of 100 % type IIB (Carlson et al., 1999). Thus, Mstn appears to be more involved in the regulation of fast-twitch fibers rather than slow-twitch fibers. In support of the above hypothesis, mice overexpressing Mstn prodomain exhibited preferential hypertrophy of type II muscle fiber (Yang et al. 2001). Moreover, Mstn gene knockout mice and cattle with non-functional Mstn mutation showed an increased percentage of fast-twitch glycolytic (type IIB) muscle fibers (Carlson et al., 1999; Salerno et al., 2004; Hayashi et al., 2008).

While molecular mechanisms determining muscle fiber type differentiation is largely unknown, recent studies suggest that MyoD, calcineurin (a calcium dependent phosphatase), and MEF (a MADS box transcription factor) are involved in the regulation of muscle fiber type differentiation. It was demonstrated that MEF2 as a downstream effector of calcineurin-dependent signaling pathway is necessary for the formation of slow fibers since overexpression of MEF2 isoforms and calcineurin results in an increased number of type IIA and type I fibers (Wu et al., 2000). Similarly, in myotonic mice, in which an increased number of slow fibers are seen, there was an increased activity of MEF2 proteins (Wu et al., 2001). A study by Hughes et al. (1993) demonstrated that MyoD mRNA specifically accumulated in fast type IIB/IIX fiber of hindlimb muscles in mice, leading to a hypothesis that MyoD is required for the fast-fiber formation. Recently, Hennebry et al. (2009) examined the expression levels of MEF2, calcineurin, and MyoD in myostatin null mice to investigate whether Mstn regulates muscle fiber type differentiation via regulation of these molecules. They observed reduced MEF2 protein in nuclear extracts of Mstn null mice muscle and myoblast along with reduced MEF2-DNA complex in electrophoretic mobility-shift assay. Calcineurin mRNA expression depressed, but MyoD protein level increased in Mstn null mice. These results, thus, suggest that the fiber type differentiation regulated by Mstn is probably via the pathways involving MyoD, calcineurin, and MEF2 regulation.

1.3.4 Myostatin synthesis, processing, activation and inhibition

Mstn is mainly synthesized as a precursor protein composed of a signal sequence (24 amino acid (AA)), an N-terminal propeptide domain (prodomain, 240-243 AA) and a C-terminal active domain (mature myostatin, 109 AA) in skeletal muscles (Zimmers et al., 2002). Mstn undergoes two proteolytic processing events in order to generate the biologically active molecule. The first cleavage event removes the signal peptide, which plays a role in the transport of Mstn precursor protein from the cytoplasm into endoplasmic reticulum. The second cleavage event generating N-terminal propeptide and C-terminal active-form of myostatin occur at the tetrabasic (RSRR) site, which is mediated by a calcium-dependent serine protease called furin (Lee and McPherron, 2001; Thies et al., 2001). After the proteolytic processing of the precursor, Mstn is secreted as an inactive latent complex of a mature dimmer bound by two propeptides, thus the propeptide is functioning to suppress Mstn activity. In support of the role of propeptide to inhibit Mstn biological activity, the overexpression of Mstn propeptide in mice resulted in a dramatic increase in skeletal muscle mass (Lee and McPherron, 2001; Yang et al., 2001). In addition, the administration of recombinant Mstn propeptide (Li et al., 2010) or plasmid- or adeno-associated virus-mediated delivery of Mstn propeptide induced an increase in muscle mass (Matsakas et al., 2009; Hu et al., 2010).

Binding of mature Mstn to its receptor (activin type IIB receptor) for induction of intracellular signaling cascade, thus, requires dissociation of the propeptide from the latent complex. The propeptide binding to the mature region can be physically or biochemically destabilized after it is cleaved by protease, such as bone morphogenetic protein-1/tolloid (BMP-1/TLD) family of metalloproteinases (Wolfman et al., 2003; Lee, 2008). The importance of BMP-1/TLD metalloproteinase in regulating Mstn latency *in vivo* is supported by studies using a mutant form of the propeptide in which aspartate 76 was mutated to alanine (Wolfman et al., 2003). The mutant form of propeptide resistant to cleavage by BMP-1/TLD metalloproteinases caused a significant increase in muscle mass when injected into adult mice (Wolfman et al., 2003). Mice engineered to carry a germline point mutation of propeptide rendered to be resistant to the BMP-1/TLD metalloproteinases exhibited an increase in skeletal muscle mass (Lee, 2008), suggesting that the BMP-1/TLD metalloproteinases is involved in activating latent Mstn *in vivo* and that molecules capable of inhibiting these proteinases may be an effective therapeutic strategy for enhancing muscle growth in clinical settings of muscle loss and degeneration.

In addition to the propeptide, several other proteins have also been shown to be capable of binding and inhibitng the activity of mature Mstn. Several studies suggested that follistatin can function as a potent myostatin antagonist and plays an important role in modulating Mstn activity. Follistatin was capable of blocking Mstn activity in both receptor binding and reporter gene assays in CHO cells (Zimmers et al., 2002). Overexpression of follistatin in muscle induced dramatic increases in skeletal muscle growth in mice (Lee and McPherron, 2001). Amother et al. (2004) also demonstrated that follistatin and Mstn interact directly with high affinity during chick development, and Mstn inhibits terminal differential of muscle cells in high-density cell cultures of limb mesenchyme and that follistatin rescues muscle differentiation in a concentration-dependent manner. The results suggest that follistatin antagonizes Mstn by direct interaction, resulting in prevention of Mstn from executing its inhibitory effect on muscle development. Recently, gene-targeted approach of follistatin overexpression, such as adenoviral vector-mediated gene theraphy and cell-mediated theraphy using myogenic stem cells, showed that the over-expression of follistatin enhanced skeletal muscle growth in dystrophic mice (Nakatani et al., 2008; Tsuchida, 2008; Rodino-Klapac et al., 2009).

Follistatin related gene (FLRG) and growth and differentiation factor (GDF)-associated serum protein-1 (GASP-1) also appear to be involved in regulating the activity of mature Mstn (Hill et al., 2002; Hill et al., 2003; Takehara-Kasamatsu et al., 2007; Saremi et al., 2010). For example, Hill et al. (2002, 2003) demomstrated that both FLRG and GASP-1 is complexed to myostatin in the blood of mice and human, and studies with recombinant proteins have shown that both proteins can bind with high affinitiy to the Mstn and inhibit its acitivity as assessed by reporter gene assays. Moreover, Takehara-Kasamatsu et al. (2007) demonostrated that the overexpression of FLRG mRNA suppressed the Mstn activity as a negative regulator during fetal and adult mouse heart development. In addition, Saremi et al. (2010) demonstrated that resistance training caused a significant decrease in serum levels of Mstn and increase in GASP-1. The effect of resistance training on serum levels of Mstn and GASP-1, may explain the increased muscle mass.

In addition to FLRG and GASP-1, titin-cap (T-cap), decorin and perlecan also appear to be involved in regulating the activity of Mstn (Nicholas et al., 2002; Miura et al., 2006; Nishimura et al., 2007; Kishioka et al., 2008; Xu et al., 2010). For example, the overexpression of titin-cap, a sarcomeric protein, in C2C12 myoblasts induced an increase in the rate of cell proliferation through suppression of Mstn expression, indicating that titin-cap controls Mstn secretion in myogenic precursor cells without affecting the processing step of precursor Mstn (Nicholas et al., 2002). Decorin, which is a small leucine-rich proteoglycan, binds to myostatin and blocks the inhibitory effect of Mstn on muscle cell growth *in vitro* (Miura et al., 2006). Moreover, the overexpression of decorin enhances the proliferation and differentiation of C2C12 myoblasts through suppressing myostatin acitivty (Kishioka et al., 2008). In addtion, skeletal muscle-specific knock-out of perlecan, a component of the basement membrane that surrounds skeletal muscle, induced a decrease in Mstn expression and increased fiber cross-sectional area as compared with control mice, suggesting that perlecan is critical for maintaining skeletal muscle mass and fiber composition, and for regulating Mstn signaling (Xu et al., 2010).

1.3.5 Myostatin signal transduction

Most members of the TGF-β superfamily are believed to signal through Smad complex activated by heteromeric complexes of type I and type II serine/threonine kinase receptors (reviewed by Kawabata and Miyazono, 1999; Mehra and Wrana, 2002). Most ligands binding to type II receptor activates type I receptor, resulting in the initiation of signal transduction pathways by phosphorylation of intracellular mediators, such as Smad proteins. Eight different Smad proteins are divided into three functional groups: Smad 1, 2, 3, 5 and 8 belongs to the receptor regulated R-Smads, and Smad 4 belongs to the common partner Co-Smad, and Smad 6 and 7 belongs to the inhibitory I-Smads. R-Smads are phosphorylated by type I receptor serine kinase in response to the binding of TGF-β superfamily to type II receptors. The Co-Smad positively regulates the phosphorylated R-Smads by hetero-oligomer formation with the R-Smads. The entire complex of R-Smads and Co-Smad translocates into the nucleus. Inside nucleus, they interact with different cellular partners, bind to DNA, and regulate transcription of various downstream response genes. In contrast to R-Smads and Co-Smads, I-Smads inhibits the signaling of TGF-β superfamily proteins. The I-Smads interact with type I receptors, thus compete with R-Smads for activation by the type I receptors. In addition, I-Smads also recruit Smurf ubiquitin ligases to catalyze degradation of the receptor complex. The transcription of I-Smads is known to be initiated by TGF-β superfamily proteins, thus providing a negative feedback regulation.

Similar to many other TGF-β superfamily proteins, Mstn was shown to bind to the activin type IIB receptor (ActRIIB) in a specific and saturable manner (Lee and McPherron, 2001), suggesting that Mstn initiates its signaling cascade by binding to ActRIIB and subsequent activation of Smads pathway. Lee and McPherron (2001) have demonstrated that transgenic mice over-expressing a dominant negative form of ActRIIB had increased muscle growth similar to the Mstn null mice, suggesting that Mstn binds to ActRIIB receptor for signaling cascade. Recently, it was shown that lentiviral-mediated delivery of dominant negative mutated ActRIIB increased myobalst proliferation and fusion and changed the expression of myogenic regulatory factors (Fakhfakh et al., 2010). In support of the activation of Smad pathway by Mstn, Langly et al. (2002) demonstrated that the addition of Mstn in muscle cell culture up-regulated the level of phosphorylated Smad3 during the myoblast differentiation, and the addition of anti-Mstn antibodies down-regulated the level of phosphorylated Smad3 in C2C12 cells (Bogdanovich et al., 2002). Moreover, Zhu et al. (2004) investigated the Mstn signaling pathway using a myostain-responsive p(CAGA)12-MLP luciferase reporter assay system in which plasmids containing various Smads were co-transfected. Their results showed that the myostatin-induced transcription required the participation of R-Smads (Smad 2/3) and Co-Smad (Smad 4), and inhibitory Smad7 dramatically reduced the Mstn-induced transcription.

The activation of Smad signaling by mysotatin has been well characterized, but very little has been known about the molecular mechanisms connecting Smads activation to muscle protein metabolism. Recently, however, several studies indicate an existence of a cross-talk between the Smads signaling and the Akt/mammalian target of rapamycin (mTOR) pathway. For example, Sartori et al. (2009) observed an atrophy in muscle fibers that were transfected with constitutively active (c.a.) ALK4/5, a type I Mstn receptor, in mice, conforming the muscle atrophic effect of Mstn stimulation. To examine the involvement of Smad phosphorylation in the atrophic effect of ALK4/5 activation, they suppressed the Smad2 and 3 activities through siRNA method in the c.a. ALK transfected muscle fiber. They observed that inhibition of Smad 2 and 3 could completely block ALK4/5-mediated atrophy. Furthermore, induction of Akt could completely block the atrophic action of c.a. ALK4/5, indicating that Mstn signaling involves the Akt downstream of Smad regulation. It was also reported that Akt phosphorylation was inhibited by Mstn in human myotube, and the effect was dependent upon the presence of Smad2 and 3 (Trendelenburg et al., 2009). Blockade of the Akt/mTOR pathway, using small interfering RNA to regulatory-associated protein of mTOR (RAPTOR), a component of TOR signaling complex 1 (TORC1), increased Mstn-induced Smad phosphorylation, establishing a feed-forward mechanism: Mstn activates Smad2, which inhibits Akt, inhibiting TORC1, which in turn potentiates Mstn 's activation of Smad2 (Trendelenburg et al., 2009). Addition of IGF-1 dominantly blocked the effects of myostatin, when applied to either myoblasts or myotubes (Trendelenburg et al., 2009). The results combinded together indicate that Akt is a particular crossing point between the IGF-1 and Mstn signaling and that IGF-1 can rescue the activation of the PI3k/Akt pathway that is blunted by Mstn.

1.4 REGULATION OF MUSCLE GROWTH BY β-ADRENERGIC AGONISTS

1.4.1 Function of β-adrenergic agonists

The β-adrenergic agonists (β-gonists) regulate a variety of physiological functions in almost all organ systems, including cardiovascular, respiratory, gastrointestinal, urogenital, ocular, hormonal secretion, and central nervous systems through binding to β-adrenergic receptors (β-adrenoceptors) (reviewed by Hieble and Ruffolo Jr., 1991). Naturally occurring endogenous β-agonists in mammals are adrenaline (epinephrine) and noradrenaline (norepinephrine). Multiple metabolic responses are also controlled by β-adrenoceptor activation, including stimulation of lipolysis and glycogenolysis. Furthermore, anabolic effects of β-agonists have been widely studied for potential applications in the prevention of muscle atrophy, and improvement of the efficiency of muscle growth, feed efficiency, and carcass composition in production livestock (reviewed by Kim and Sainz, 1992). Although the degree of responses varies depending on the dose and type of β-agonists, animal breed, age, sex, nutritional support and experinmental period, muscle gain and feed efficiency were significantly increased by the treatment of β-agonists (Buttery, 1993). Moreover, β-agonists increase carcass protein content and decrease carcass fat deposition. The increase in protein content by β-agonists is 10-17% in ruminants (Allen et al., 1987), 7% in swine (Buttery, 1993), and 2-3% in poultry (Duquette et al., 1987). The decrease in carcass fat by β-agonists is up to 30% in ruminants (Hanrahan et al., 1987), 6-27% in swine (Bekaert et al., 1987), and 8-10% in poultry (Scholtyssek, 1987).

1.4.2 Skeletal muscle hypertrophic effect of β-adrenergic agonists

Several studies demonstrated that β-agonists have a specific anabolic effect on skeletal muscle in animals. In rats, for instance, fenoterol or clenbuterol increased skeletal muscle mass by 20-30% after 2-4 weeks (Ryall et al., 2002). In lambs, administration of cimaterol for 7-8 weeks increased muscle weight by 30-40% (Kim et al., 1987). In pigs, salbutamol significantly increased the weight of longissimus dorsi muscle by 17% after 44 days of administration (Oksbjerg et al., 1990). Lean muscle mass was increased up to 20% by clenbuterol administration in mice (Hayes and Williams, 1994). Moreover, the hypertrophic effect of β-agonists is selective to skeletal muscle fibers types. The cross-sectional area of fast muscle fiber type (type II) was increased by the hypertrophic effect of β-agonists in muscles of various animals. However, the effects of β-agonists on slow muscle fiber type (type I) are not consistent among studies (reviewed by Yang and McElligott, 1989; Kim and Sainz, 1992), indicating that the growth effects induced by β-agonists are more pronounced in predominantly fast-twitch muscle than slow-twitch muscle.

In addition to skeletal muscle hypertrophic effect in normally growing animals, β-agonists have been shown to prevent or inhibit weight loss or decrease in growth of animals induced by physiological or pharmacological disturbance. For example, clenbuterol increased the weight of the gastrocnemius and soleus muscles in dystrophic mice by 29% (Rothwell and Stock, 1985). Moreover, clenbuterol retarded atrophy in denervated muscles in rats (Zeman et al., 1987), and cimaterol delayed chicken muscle atrophy induced by stretch-release (Lee et al., 1994). In addition, administration of clenbuterol prevented glucocorticoid-induced muscle atrophy (Agbenyega and Wareham, 1992), and reduced the muscle loss caused by phytohaemaglutinin injection (Bardocz et al., 1992). Furthermore, clenbuterol enhanced the recovery of body weight gain from weight loss caused by endotoxemia in rats (Choo et al., 1989), and pretreatment of cimaterol significantly reduced the catabolic effect of corticosterone on carcass and muscle protein (Brown et al., 1992).

1.4.3 β-adrenergic receptors

β-adrenoceptors are G-protein-coupled receptors, which comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways, cellular responses, and specifically mediate physiological responses to epinephrine and norepinephrine. There are three different receptor subtypes: β_1-, β_2-, and β_3-adrenoceptors, each with a 65-70% homology in their amino acid composition. β_1-adrenoceptor is located at highest levels in the heart and brain (Frielle et al., 1987), β_2-adrenoceptor is widely expressed (Dixon et al., 1986), and β_3-adrenoceptor is located at highest levels in the adipose tissue (Emorine et al., 1989). These three receptors have two principal signal transduction pathways involving the adenyl cyclase pathway and the phophatidylinositol signal pathway (reviewed by Yang and McElligott, 1989; Hinkle et al., 2002; Hall, 2004; Lynch and Ryall, 2008).

1.4.4. Signaling pathways of β_2-adrenergic receptor leading to muscle hypertrophy

The activated β_2-adrenoceptor stimulates the adenylyl cyclase (AC) pathway via the activation of guanine nucleotide binding protein $G\alpha_s$, which stimulated G protein complex exchange GDP and GTP and is released from the complex, resulting in increased cyclic adenosine monophosphate (cAMP) levels. The production of cAMP results in the activation of numerous downstream signaling pathways, including the well-described protein kinase A (PKA) signaling pathways. The cAMP- PKA signaling pathway is one of the most common and versatile signaling pathway in eukaryotic cells and is involved in regulation of cellular functions in almost all tissues in mammals. Thus, the classic signaling pathway of β_2-adrenoceptor involves activation of adenyl cyclase (AC) and subsequent formation of cAMP (reviwed by Bos, 2003; Tasken and Aandahl, 2004; Hall, 2004). Nevertheless, the link between the classic β_2-adrenergic receptor/Gα/AC/cAMP signaling pathway and the change in protein metabolism induced by the administration of β-adrenergic agonists has remained elusive. Recent results, however, indicate that like MSTN, β-adrenergic agonists may induce muscle hypertrophy via the Akt/mTOR signaling pathway. For example, clenbuterol treatment induced protein accretion in mice via stimulation of protein translation through the phosphorylation of p70S6k and 4E-BP1, which are downstream effectors of the Akt/mTOR signaling pathway (Sneddon et al., 2001). Kline et al. (2007) also demonstrated that clenbuterol administration induced dramatic increase in skeletal muscle mass through the Akt phosphorylation and subsequent activation of the mTOR. In addition, Sable et al. (1997) demonstrated that *in vivo* the cAMP elevating agents led to a significant increase in the activity of Akt through a mechanism which is independent of PI3k, suggesting that cAMP can lead to the activation of Akt.

1.4.5 Adaptation of animals to growth promoting effects of β-adrenergic agonists

Some studies demonstrated that the growth-promoting effect of β-agonists is effective for a limited time period. The response is attenuated or disappears with prolonged treatment (reviewed by Yang and McElligott, 1989; Kim and Sainz, 1992). For example, the onset of the anabolic effect is rapidly observed within 2 days after administration of clenbuterol and reached a maximum within 8 days in rats. However, the response was attenuated after 14 days of treatment with the same daily gain as control (Mcelligott et al., 1989). Chronic treatment with clenbuterol for 18 days reduced β-adrenoceptor density by 50% in rat skeletal muscles (Rothwell et al., 1987). Chronic treatment with cimaterol also reduced β-adrenoceptor density in mice skeletal muscle after 3 days and remained lower thereafter (Kim et al., 1992). Ryall et al. (2002) also demonstrated that β-adrenoceptor density was decreased by fenoterol and clenbuterol treatment for 4 weeks in EDL muscles (51 and 34%, respectively) and in soleus muscles (42 and 44%, respectively). Taken together, the decrease in growth-promoting effect of β-agonists is probably due to the desensitization process operated by down-regulation of β-aerenoceptors (reviewed by Ferguson, 2001).

1.5 DNA MICROARRAY TECHNOLOGY

DNA microarray technology has provided researchers with the ability to measure the expression levels of thousands of genes simultaneously in a single experiment. The ability to probe a sample for hundred to million different genes simultaneously is very powerful in evaluating cellular functions and genetic variations in a systemic way. For this reason, DNA microarray assay has become an important tool in many genomics research laboratories, as well as other various fields of life science laboratories (reviewed by Barrett and Kawasaki, 2003).

1.5.1 Principles of DNA microarray

DNA microarray is a multiplex technology used in molecular biology. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, known as probes (or reporters). These can be a short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA sample (called target) under high-stringency conditions. The probes are attached via surface engineering to a solid surface by a covalent bond to a chemical matrix (via epoxy-silane, amino-silane, lysine, polyacrylamide or others). Surface engineering includes printing with fine-pointed pins onto glass slides, photolithography using pre-made masks, dynamic micromirror devices, ink-jet printing, or electrochemistry on microelectrode arrays (reviewed by Schena et al., 1998; Lausted et al., 2004).

The main advantage of DNA microarray is that it obviates the need for expensive sequencing of entire genomes since it allows the evaluation of gene expression in an organism for which little sequence data is available. The next microarray technology to emerge involved in situ-synthesized oligonucleotide arrays using photolithographic technology (Affymetrix). Each gene target is proved by a number of distinct probes collectively termed a probe set, while some probes within a set are known to overlap in sequence. Each probe with the same 25 bp segment of a target gene consists of millions of single strands of DNA of exact length and sequence, and is confined to a small square area. Some of the probes on the surface have sequences that are perfectly complementary to particular target sequences and are referred to as perfect match (PM) probes. Also, present on the chip are probes whose sequence is deliberately selected not to be perfectly complementary to a target sequence. Such probes are referred to as mismatched (MM) control probes. Thus, the biological sample such as an mRNA sample can be analyzed for gene expression for hybridization to above-described microarray chip (reviewed by Naef et al., 2002; Sasik et al., 2004). The presence of RNA sequences that bind to the oligonucleotide probes on the chips are then detected by methods such as tagging with a fluorescence material and then detecting the fluorescence (Pease et al., 1994). Since sequences that are different from the target sequences may also bind to the PM probes that correspond to such target sequences, the fluorescence signals from such sequences would appear as noise. Signal to noise ratio is improved by calculating the difference from signals from the sequences that bind to the PM probes and the signals form sequences that bind to the MM probes (Pease et al., 1994).

1.5.2 Two-channel and one-channel microarray detection

Two-channel microarray detection is typically hybridized with cDNA prepared from two samples to be compared and that are labeled with two different fluorophores (Shalon et al., 1996). Fluorescent dyes commonly used for cDNA labeling include Cy3, which has a fluorescence emission wavelength of 570 nm (corresponding to the green part of the light spectrum), and Cy5 with a fluorescence emission wavelength of 670 nm (corresponding to the red part of the light spectrum). The two Cy-labelled cDNA samples are mixed and hybridized to a single microarray that is then scanned in a microarray scanner to visualize fluorescence of the two fluorophores after excitation with a laser beam of a defined wavelength. Relative intensities of each fluorophore may then be used in ratio-based analysis to identify up-regulated and down-regulated genes (Tang et al., 2007).

The single-channel microarray detection provides intensity data for each probe or probe set indicating a relative level of hybridization with the labeled target. However, they do not truly indicate abundance levels of a gene but rather relative abundance when compared to other samples or conditions when processed in the same experiment (Patterson et al., 2006). The advantage of single-channel microarray system is that an aberrant sample cannot affect the raw data derived from other samples, because each array chip is exposed to only one sample. Another benefit is that data are more easily compared to arrays from different experiments, and the absolute values of gene expression may be compared between studies conducted months or years apart. However, disadvantage of the one-color system is that, when compared to the two-color system, twice as many microarrays are needed to compare samples within an experiment (reviewed by Barrett and Kawasaki, 2003).

1.5.3 Use of microarray analysis to examine global gene expression during skeletal muscle growth

Bey et al. (2003) analyzed the patterns of global gene expression in rat skeletal muscle during unloading and low-intensity ambulatory activaity using microarray. They identified 121 (≥1.5 fold) genes being differentially expressed, and analyis of these genes indicated that key signaling proteins and transcription factors involved in protein synthesis/degradation and energy metabolism were regulated in rat soleus muscle during unloading and low-intensity amululatory activaity. They also identified potential new targets that may initiate muscle alterations during inactivity. Carson et al. (2002) analyzed the differential gene expression in the rat soleus muscle during early work overload-induced hypertrophy. They identified 112 genes being differentially expressed, and these genes were classified into cell proliferation, autocrine/paracrine, extracellular matrix, immune response, intracellular signaling, metabolism, neural, protein synthesis/degradation, structural, and transcription. Jagoe et al. (2002) analyzed the patterns of gene expression in atrophying skeletal muscles response to food deprivation. The results indicated that the about 200 genes (≥1.8 fold) were differentially expressed. Functional analysis of those genes showed increased patterns of polyubiquitin, ubiquitin extension proteins and ligase, atrogin-1 and many proteasome subunits, and decreased patterns of myosin binding protein H, IGF binding protein 5, glycolytic enzymes and phosphorylase kinase subunits. Lecker et al. (2004) compared changes in gene expression between normal and atrophying muscle casued by fasting, cancer cachexia, streptozotocin-induced diabetes and uremia. They found that genes encoding myofibrillar proteins were not affected by the catabolic conditions, but they observed an upregulation of genes involved in protein degradation genes, including polyubiquitions, ubiquitin fusion proteins, atrogin-1/MAFbx, MuRF-1, proteasome subunits and cathepsin L in muscles of the catabolic states. Many genes required for ATP production and late steps in glycolysis, and extracellular matrix proteins were down-regulated. Campbell et al. (2001) analyzed the differential global gene expression in red and white skeletal muscle. They identified 49 genes that were differentially expressed between white and red skeletal muscles. The list of genes included genes reflecting the metabolic and contractile difference between the fast and slow muscle fiber type, as well as newly identified transcription factors that may be involved in the determination of the phenotypic differences between muscle fiber types.

Microarray anlaysis also have used to examine the changes in global gene expression induced by Mstn or β-adrenergic agonists. Steelman et al. (2006) analyzed the patterns of gene expression in pectoral muscles of wild type and Mstn-null mice, and identified 717 genes ($p < 0.05$, 1.5 fold change) being differentially expressed. They observed a downregulation of genes encoding slow isoforms of myofibrillar contractile proteins, and fucntional analysis of the differentially expressed genes indicated that Mstn may act upstream of Wnt pathway. Wnt4 is known to stimulate satellite cell proliferation, indicating a role of Mstn in the growth and maintenance of postnatal skeletal mucle through Wnt/calcium signaling. Welle et al. (2009) reported that 124 genes ($p < 0.01$, 1.5 fold change) were differentially expressed in gastrocnemius muscles between wild type and Mstn null mice. Unlike the results of Steelman et al. (2006), they did not observe downregulation of genes encoding slow isoforms of myofibrillar contractile proteins or genes encoding proteins involved in energy metabolism. Most of the other genes affected by Mstn depletion have not been previously linked to Mstn signaling, thus the results indicated that Smads are not the only transcription factors with reduced activity after Mstn depletion. Spurlock et al. (2006) reported that over 50 genes were differentially expressed (FDR < 10%) in mouse skeletal muscle 24 hours and 10 days after administration of CL. Functional characterization of differentially expressed genes revealed several categories that participate in biological processes important to skeletal muscle growth, including regulators of transcription and translation, mediators of cell-signaling pathways, and genes involved in polyamine metabolism. Pearson et al. (2009) analyzed the skeletal muscle gene expression following systemic administration of formoterol at both 1 and 4 hours. They identified 23 genes at one hour and 112 genes at four hours, and these genes were classified into skeletal muscle hypertrophy, myoblast differentiation, metabolism, circadian rhythm, transcription, histones, and oxidative stress, providing new insights into many of the underlying changes in gene expression that mediated β-agonists-induced skeletal muscle hypertrophy and altered metabolism.

1.6 POTENTIALS OF MYOSTATIN AND BETA-ADRENERGIC AGONISTS IN ANIMAL PRODUCTION AND HUMAN HEALTH

An increase in skeletal muscle growth and decrease in fat deposition are important aspects in increasing animal productivity efficiency for animal agriculture, particularly for meat-producing animals (reviewed by Beitz, 1985). Moreover, normal development of skeletal muscle growth is important for human health, because abnormal development of the skeletal muscle growth results in appearance defects and function of the body, causing serious health problems to humans, such as sarcopenia, cachexia, and muscular dystrophy (reviewed by Gelfi et al., 2011; Whittemore et al., 2003). Therefore, expanding our understanding of the mechanisms regulating skeletal muscle growth is expected to contribute to developing novel means to improve the efficiency of meat production and to alleviate human suffering caused by muscle atrophic conditions.

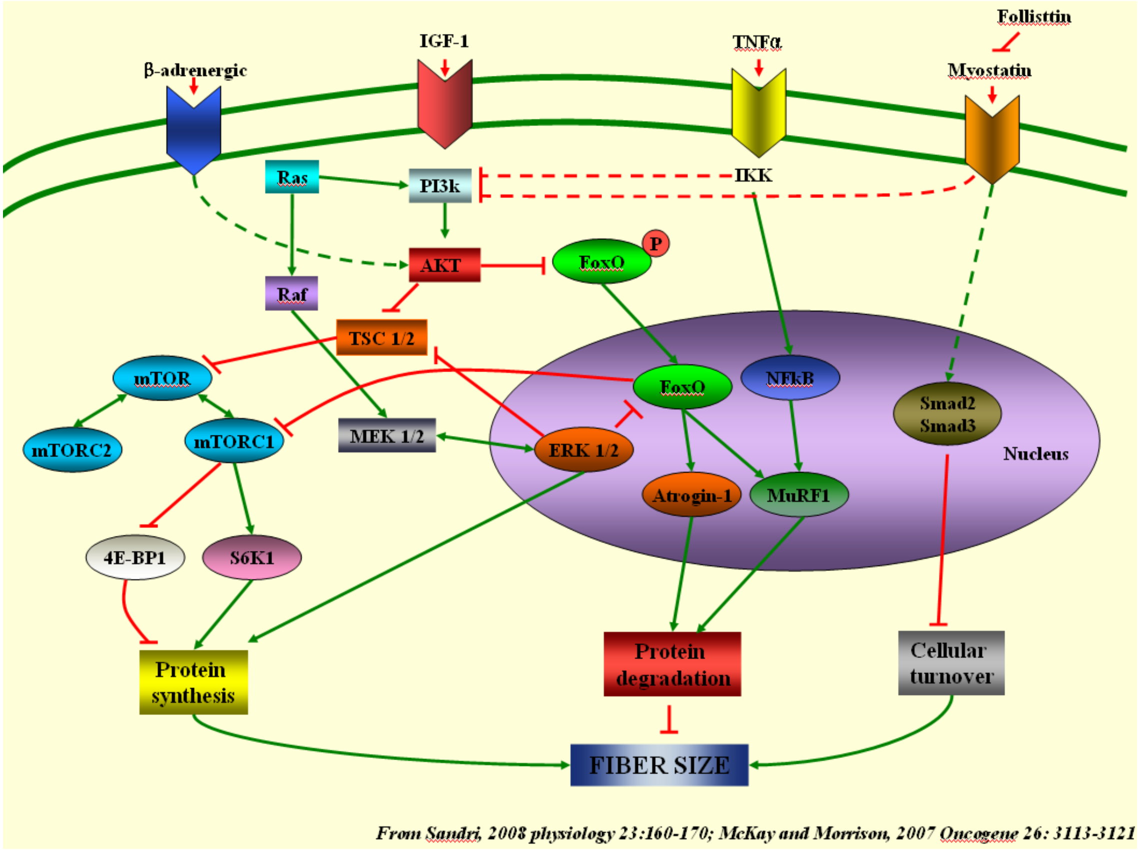
Because of the potent role of Mstn on skeletal muscle growth, there have been many interests in Mstn-based technologies to improve skeletal muscle growth of agriculturally-important species and to treat muscle atrophic conditions in humans. The strategies have mostly focused on utilizing Mstn-inhibiting molecules, such as anti-Mstn antibody, soluble activin typeIIB receptor, follistatin, and Mstn prodomain. Kim et al. (2006) have shown that broilers from eggs that had anti-Mstn antibody injected into the yolk had significantly heavier body (4.2%) and muscle (5.5%) mass than the controls in both male and female birds, demonstrating for the first time that skeletal muscle growth of meat-producing animals can be improved by blocking Mstn activity. Medeiros et al. (2009) produced transgenic rainbow trout overexpressing follistatin, to investigate the effect of this protein on muscle development and growth. The study demonstrated that transgenic overexpression of follistatin in trout muscle tissue increased epaxial and hypaxial muscling similar to that observed in double-muscled cattle and Mstn null mice. Carpio et al. (2008) examined the growth effect of administering a soluble form of goldfish activin typeIIB receptor to juvenile and larval goldfish, and found that the recombinant protein improved body weight and muscle mass in teleost fish in a dose-dependent manner. Lee et al. (2010) produced Mstn prodomain in soluble forms in E. coli, to examine its capacity to suppress Mstn activity in vitro, and to investigate the effect of treating fish with the Mstn prodomain on fish growth. This study indicates that Mstn prodomain suppresses Mstn activity and improve rainbow trout growth. These studies together indicate that inhibition of Mstn might help to promote skeletal muscle growth and improve the production efficiency of agriculturally important animal species.

Mstn blockade has been considered as a mean to treat muscular dystrophy. Bogdanovich et al. (2002) administered monoclonal anti-Mstn antibody to mdx mice, and found a significant alleviation of dystrophic pathophysiology by 3 months of treatment with an increased whole muscle cross sectional area and muscle fiber area. Wagner et al. (2002) also demonstrated that Mstn null mutation in the mdx mice showed the improving muscle regeneration. It has been shown that Mstn-null mice undergo reduced atrophy during aging along with improved muscle regenerative capacity (Siriett et al., 2006), illustrating the therapeutic potential of Mstn inhibition to treat sarcopenia. Indeed, inhibiting Mstn via the administration of an aniti-Mstn antibody in aged mice significantly attenuated the decline of skeletal muscle mass and function (LeBrasseur et al., 2009; Murphy et al., 2010). In human trials, Wagner et al. (2008) conducted a safety trial of a neutralizing antibody to Mstn, MYO-029, in adult muscular dystrophy patients.

This study showed that the MYO-029 had good safety and tolerability with the exception of cutaneous hypersensitivity at the 10 and 30 mg/kg doses without improving muscular dystrophic conditions, indicating that the systemic administration of Mstn inhibitors provides an adequate safety margin for clinical studies. As discussed before, many Mstn-suppressing molecules are known including anti-Mstn antibody, soluble activin typeIIB receptor, follistatin, and Mstn prodomain. Therefore, further studies needs to explore the potentials of these molecules in improving muscle growth in meat-producing animals and treating muscle-wasting conditions in humans.

The anabolic effects of β-adrenergic agonists have been widely studied for potential applications in the improvement of animal growth rate, feed efficiency, and carcass composition in production livestock animals (reviewed by Yang and McElligott, 1989; Kim and Sainz, 1992). Currently, ractopamine, a beta-adrenergic compound, is commercialized and approved by the FDA to be used in pig and cattle to improve growth rate and carcass composition. Moreover, β-adrenergic agonists have been shown to prevent or inhibit weight loss or retardation of animal growth induced by physiological or pharmacological disturbances. For example, clenbuterol increased the weight of the gastrocnemius and soleus muscles in dystrophic mice by 29% (Rothwell and Stock, 1985). Moreover, clenbuterol retarded atrophy in denervated muscles in rats (Zeman et al., 1987), and cimaterol delayed chicken muscle atrophy induced by stretch-release (Lee et al., 1994). Besides, administration of clenbuterol prevented glucocorticoid-induced muscle atrophy (Agbenyega and Wareham, 1992), and reduced the muscle loss caused by phytohaemaglutinin injection (Bardocz et al., 1992). In addition, clenbuterol enhanced the recovery of body weight gain from weight loss caused by endotoxemia in rats (Choo et al., 1989), and pretreatment of cimaterol significantly reduced the catabolic effect of corticosterone on carcass and muscle protein (Brown et al., 1992). Furthermore, Hayes and Williams (1998) examined the effects of clenbuterol on dystrophic skeletal muscle in mice. This study indicates that clenbuterol significantly reduced the natural death rate of dystrophic mice, and significantly increased the relative mass ($P<0.001$) and relative tetanic force production ($P<0.01$) of the soleus of dystrophic animals, suggesting that clenbuterol could be a valuable adjunct to treatments of muscle wasting diseases such as muscular dystrophy. In humans, trials with salbutamol, a β-adrenergic agonist approved for humans, have shown limited positive effect in muscular dystrophy patients (Kissel et al., 2001; Fowler et al., 2004; van der Kooi et al., 2004). New generation of adrenergic agonists, such as formoterol and salmeterol, have shown more powerful skeletal muscle anabolic effects with less side effects (Busquets et al., 2004; Ryall et al., 2006; Harcourt et al., 2007).

Given that both Mstn and β-adrenergic agonists have a strong effect of on skeletal muscle growth, future studies probably need to consider investigating the potentials of the combination of the two agents in improving skeletal muscle growth in meat-producing animals and in treatment of muscle atrophic condition in humans.



From Sandri, 2008 physiology 23:160-170; McKay and Morrison, 2007 Oncogene 26: 3113-3121

Figure 1.1 Diagram illustrating the major pathways that control fiber size.

CHAPTER 2

THE MUSCLE-HYPERTROPHIC EFFECT OF CLENBUTEROL IS ADDITIVE TO THE HYPERTROPHIC EFFECT OF MYOSTATIN SUPPRESSION

2.1 ABSTRACT

In this study, we investigated the combined effect of myostatin (Mstn) suppression and β-agonist (clenbuterol) administration on muscle hypertrophy and the phosphorylation of muscle 4E-BP1 and p70S6k, two downstream effectors of the Akt/mTOR anabolic pathway. Female heterozygous Mstn-prodomain transgenic mice (Mstn-pro) and wild-type littermates were given 0 or 20 ppm of clenbuterol (CL) in their drinking water, and muscle samples were collected at 1 and 2 weeks after treatment. CL increased body and muscle mass in both genotypes. Levels of phosphorylated muscle 4E-BP1 and p70S6k were higher in Mstn-pro mice than in wild type mice. CL increased the phosphorylation of 4E-BP1 and p70S6k in both genotypes. The muscle-hypertrophic effect of CL is additive to the effect of Mstn suppression. The combination of Mstn suppression and treatment with β-agonists may be an effective therapeutic approach to combat muscle-wasting conditions.

2.2 INTRODUCTION

Loss of muscle mass can adversely affect human health and viability, and molecules involved in the regulation of skeletal muscle growth are valuable targets in developing therapeutic strategies for muscle-wasting conditions. Myostatin (Mstn) and some β-adrenergic agonists, such as clenbuterol (CL), have emerged as powerful regulators of skeletal muscle growth and mass (Lee et al., 2004; Lynch and Ryall, 2008). Both CL administration and Mstn inhibition have individually demonstrated their therapeutic potential for preventing or reversing muscle loss in various conditions that lead to muscle atrophy or wasting (Carter et al., 1991; Kissel et al., 2001; Lynch et al., 2001; Wagner et al., 2002; Fowler et al., 2004; van der Kooi et al., 2004; Bogdanovich et al., 2005; Li et al., 2005; Wagner, 2005; Jespersen et al., 2006).

Mstn, a member of the transforming growth factor-beta (TGF- β) superfamily of growth and differentiation factors, is a potent negative regulator of myogenesis during development, and it also inhibits postnatal skeletal muscle growth (Lee et al., 2004). Mstn is synthesized mainly in skeletal muscle as a precursor form that is proteolytically cleaved into an N-terminal prodomain (Mstn-pro) and a C-terminal active form. The cleaved Mstn-pro remains noncovalently associated with the active Mstn and inhibits its biological activity by preventing binding to its receptor (Thies et al., 2001; Hill et al., 2002; Lee et al., 2004). The important role of Mstn-pro in regulating Mstn activity was demonstrated by a dramatic increase in skeletal muscle mass in transgenic mice that overexpress Mstn-pro (Lee and McPherron, 2001; Yang et al., 2001).

Mstn binds to activin receptor type IIB (ActRIIB) to exert its biological activity (Thies et al., 2001; Rebbapragada et al., 2003; Lee et al., 2005). The binding of Mstn to its receptor leads to phosphorylation of transcription factors Smad2 and Smad3 and complex formation with Smad4, resulting in nuclear translocation of the Smad complex and consequent regulation of transcription of downstream target genes (Bogdanovich et al., 2002; Langley et al., 2002; Zhu et al., 2004). Although the activation of Smad signaling by Mstn has been well characterized, very little is known about the molecular mechanisms that connect Smads activation to muscle protein metabolism. Recent studies, however, indicate there is cross-talk between Smads signaling and the Akt/mammalian target-of-rapamycin (mTOR) anabolic pathway. For example, muscle fiber atrophy induced by Smad2/3 activation was prevented by the presence of constitutively active Akt (Sartori et al., 2009). It was also reported that Akt phosphorylation was inhibited by Mstn in human myotubes, and the effect was dependent upon the presence of Smad2 and 3 (Trendelenburg et al., 2009). The mTOR, a downstream target of Akt, is an integral control point for various signals involved in muscle hypertrophy and atrophy (Bodine et al., 2001; Proud, 2007; Drummond et al., 2009). Activation of mTOR promotes the phosphorylation of two key downstream effectors that are closely associated with translational regulation of protein synthesis: p70S6 kinase (p70S6k) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1) (Wang and Proud, 2006). Recent results have indicated that myostatin inhibits muscle hypertrophy in part through inhibition of protein synthesis mediated by the Akt/mTOR pathway (Welle et al., 2006; Amirouche et al., 2009; Welle et al., 2009a).

It is well documented that administration of β-adrenergic agonists such as CL induces a dramatic increase in skeletal muscle growth in various mammalian species (Kim and Sainz, 1992; Mersmann, 1998; Kissel et al., 2001) . The growth- and muscle-promoting action of β-adrenergic agonists is short-lived, as the effect disappears during prolonged treatment (McElligott et al., 1989; Kim and Sainz, 1992; Maltin et al., 1992; Kissel et al., 2001). The temporal nature of the growth-promoting responsiveness to β-adrenergic agonists is caused by receptor desensitization operating at both the receptor level and downstream from receptors (Pierce et al., 2002). Studies with transgenic mice lacking the β1-, β2-, or both β1- and β2-adrenergic receptors have indicated that β-adrenergic agonists mediate the hypertrophic effect through binding to the β2-adrenergic receptor (Hinkle et al., 2002). The β2-adrenergic receptor is a member of the G-protein–coupled receptor superfamily, and ligand binding induces GDP-to-GTP exchange on the Gα subunit and subsequent activation of Gα. Thus, the classic signaling pathway of β2-adrenergic receptor involves activation of adenyl cyclase (AC) and subsequent formation of cAMP (Pierce et al., 2002). The link between the classic β2-adrenergic receptor/Gα/AC/cAMP signaling pathway and the change in protein metabolism induced by the administration of β-adrenergic agonists has remained elusive, but recent results indicate that, like Mstn, β-adrenergic agonists may induce muscle hypertrophy via the Akt/mTOR signaling pathway (Sneddon et al., 2001; Kline et al., 2007).

Although there have been numerous demonstrations of the muscle growth–promoting effect of CL administration or Mstn suppression, the effect of a combination of CL administration and Mstn inhibition on skeletal muscle growth has not been investigated. Therefore, we designed an experiment in which CL was fed to wild-type and Mstn suppressed mice to assess the combined effect of CL administration and Mstn suppression on body and muscle growth. At the same time, we examined the phosphorylation of 4E-BP1 and p70S6k, two key downstream effectors of the mTOR pathway, during treatment.

2.3 MATERIALS AND METHODS

2.3.1 Animals and sample collection

All procedures using experimental animals were approved by the Institutional Animal Care and Use Committee at the University of Hawaii. All mice were maintained in temperature- and humidity-controlled conditions with a 12 h light/dark cycle and had free access to food (10% kcal fat, ME3.85 kcal/g) and water. For Mstn suppression, we used a transgenic mouse strain that overexpress the Mstn-prodomain (Mstn-pro) and exhibits a significant increase in skeletal muscle mass, regardless of age and gender (Yang et al., 2001). To produce the heterozygous Mstn-pro transgenic and wild-type littermate genotypes used in this study, female B6SJL F1 mice were mated to heterozygous Mstn-pro transgenic male mice. Pups were weaned at 28 days of age, and tail tissue samples were collected for genotyping at the time of weaning (Appendix 2.1). At 35 days of age, female mice were separated by their genotypes, and randomly assigned to one of the two groups: 0 or 20 ppm CL. CL was administered in the drinking water for 14 days, and body weights were monitored periodically. Previous studies have demonstrated the effectiveness of the 20-ppm dose (about 5 mg/kg body weight/day) of CL via drinking water on promoting body and muscle growth (Lynch et al., 1996). At 7 and 14 days after the administration, animals were sacrificed (about half in each killing) by CO_2 asphyxiation, and gastrocnemius, plantaris, soleus, and extensor digitorum longus muscles of the hind leg were rapidly dissected out, weighed, snap-frozen in liquid nitrogen and stored at -80°C until analysis.

2.3.2 Genotyping

DNA was extracted from tail samples by phenol/chloroform extraction after solubilization of the tissue in a Tris buffer (50 mM, pH 8.0) containing 0.5% sodium dodecylsulfate (SDS), 0.1 M ethylene-diamine tetraacetic acid (EDTA), and proteinase K (0.7 mg/ml). The extracted DNA was subjected to polymerase chain reaction (PCR) amplification with a primer set unique to transgenic mice. The forward and reverse primers were 5’ GACAGCAGTGATGGCTCT 3’ and 5’ CTTGTCATCGTCGTCCTTGTAATCGGTAC 3’, respectively. PCR condition was the same as described previously (Yang et al., 2001). The PCR products were subjected to electrophoresis in a 1.2 % agarose gel and stained with ethidium bromide to examine for the presence of a transgenic PCR product (Appendix 2.1).

2.3.3 Measurement of skeletal muscle DNA and RNA concentration

Plantaris muscle samples were homogenized in 20 volumes of ice-cold distilled water, then duplicate aliquots of the homogenates were drawn and added to 0.5 volume of 0.6 N ice-cold perchloric acid. The aforementioned mixtures were used to separate DNA and RNA following the procedure of Munro and Fleck (1966). RNA concentration was measured by absorption at 260 nm, and DNA concentration was measured by fluorescence spectrophotometry method using Hoechst H33258 dye as described by previously (Brunk et al., 1979). Protein concentrations in muscle homogenates were determined using the modified Lowry protein assay kit (Pierce, Rockford, IL) to estimate muscle protein content.

2.3.4 Western blot analysis of the phosphorylation of 4E-BP1 and p70s6k

The procedure described by Dreyer et al. (2006) was used for Western blot analysis. Briefly, extensor digitorum longus muscle samples were homogenized at 4°C in 9 volumes of ice-cold lysis buffer [50 mM Tris-HCl (pH 7.4), 250 mM mannitol, 50 mM NaF, 5 mM Na pyrophosphate, 1 mM EDTA, 1 mM ethylene-glycol teraacetic acid (EGTA), 1% Triton X-100, 1 mM dithiothreitol (DTT), 1 mM benxamidine, 0.1 mM phenylmethylsulfonylfluoride (PMSF), and 5 µg/ml soybean trypsin inhibitor]. The homogenates were centrifuged at 6,000 g for 10 min at 4°C, and aliquots of the supernatants were saved for Western blot detection of p70S6k. For Western blot detection of 4E-BP1, 50 µl of the above supernatants were heated at 100^{o}C for 10 min, centrifuged for 30 min at 10,000 g at 4^{o}C, and the supernatants were saved. Protein concentrations in supernatants were determined using the modified Lowry protein assay kit (Pierce, Rockford, IL) to estimate protein content. The protein concentration of supernatants was not significantly ($P < 0.01$) affected by the genotype and CL administration, and thus equal volumes of supernatants were loaded per lane with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Twenty µl of the protein extract for p70S6k and 4E-BP1were subjected to 7.5% and 15% SDS-PAGE, respectively, and then proteins were transferred to polyvinylidene difluoride (PVDF) membranes. The blots were blocked for 3 hrs at room temperature in Tris-buttered saline (20 mM Tris-HCI, 150 mM NaCI at pH 7.5) containing 3 % bovine serum albumin (BSA), followed by incubation with primary antibody in Tris-buffered saline plus 0.01% Tween-20 (TBST, 20 mM Tris-HCL, 150 mM NaCl at pH 7.5) at 4°C overnight. The primary antibodies were rabbit anti-phospho-p70S6k (Thr^{389}; 1:1,000; Cell signaling, Beverly, MA), and rabbit anti-phospho-4E-BP1 (Thr$^{37/46}$; 1:1,000; Cell signaling). The blots were washed twice with TBST and incubated with horseradish peroxidase-conjugated anti-rabbit antibody (1:2,500, Invitrogen, Carlsbad, CA) in TBST for 2 hrs at room temperature. After washing, the membrane was developed with the enhanced chemiluminescent reagent (ECL plus Western Detection System; GE Healthcare Biosciences; Pittsburg, PA), followed by apposition of the membrane to auto-radiographic films (Hyperfilm ECL; Kodak, CA). After obtaining an appropriate image, band intensity was quantitatively analyzed using Quantity One analysis software (Bio-Rad, Hercules, CA).

2.3.5 Statistical analysis

All values are expressed as mean ± SEM. Two-way analysis of variance (ANOVA) was performed using Prism5 software (GraphPad, San Diego, CA) to examine the effects of genotype, CL, and interaction on body and muscle weights; muscle DNA and RNA concentrations; and the levels of phosphorylation of 4E-BP1 and p70S6k.

2.4 RESULTS

2.4.1 Effects of CL on body and muscle weights of wild type and Mstn-pro mice

Consistent with our previous report (Yang et al., 2001), Mstn-pro mice were heavier than wild-type mice at the time of CL administration ($P < 0.001$; Fig. 2.1), and they grew significantly faster than wild-type mice over the 14-day period ($P < 0.001$,

Fig. 2.1). The growth-promoting effect of CL was evident in both wild-type and Mstn-pro mice (Fig. 2.1), demonstrating that the growth-promoting effect of CL is additive to the growth-promoting effect of Mstn suppression. The temporal effect of CL on body weight gain was evident in both genotypes (Fig. 2.1): the increase in body weight gain occurred at up to 7–10 days of CL administration, and no further increase was observed thereafter.

Skeletal muscle weights of wild-type and Mstn-pro mice as affected by CL administration for 7 and 14 days are summarized in Table 2.1 and 2.2. As expected, gastrocnemius, plantaris, extensor digitorum longus (EDL), and soleus muscles of Mstn-pro mice were significantly ($P < 0.001$) heavier than those of wild-type mice during the experimental period. The protein concentration of plantaris muscle of Mstn-pro mice was greater than that of wild-type mice ($P < 0.001$). CL administration significantly increased the four muscle weights of both Mstn-pro and wild-type mice after both the 7- and 14-day administration periods. CL administration also increased the protein concentration of plantaris muscle in both Mstn mice and wild-type mice ($P < 0.001$), leading to the highest muscle protein concentration in CL-fed Mstn-pro mice. When concentrations of soluble proteins in lysis buffer were measured from EDL muscle during Western blot analysis, genotype and CL administration had no effect on soluble protein concentration. Thus, it is postulated that the increase in total muscle protein concentration in Mstn-pro mice or by CL administration is probably due to an increase in insoluble myofibrillar proteins. The increases in weight gain of the gastrocnemius (23%) and plantaris (27%) at 7 day CL administration in Mstn-pro mice were significantly ($P < 0.05$) greater than those in wild-type mice (8.5% for gastrocnemius and 6.4% for plantaris). However, at 14 days after CL administration, the increases in weight gain of the gastrocnemius (26.9%) and plantaris (34.9%) by CL in

Mstn-pro mice were not different from those in the wild-type mice (25.6% for gastrocnemius and 38.9% for plantaris), suggesting that a synergistic effect of Mstn suppression and CL administration on the growth of these muscles may occur only during the early period of CL administration.

2.4.2 Effects of CL on muscle DNA and RNA concentrations of wild type and Mstn-pro mice

DNA and RNA concentrations in plantaris muscle were measured to determine indirectly the myonuclei domain size and protein synthetic capacity, respectively (Table 2.3). No significant difference in muscle RNA concentration was observed between wild type and Mstn-pro mice during the experimental period. Muscle DNA concentration of Mstn-pro mice was significantly ($P < 0.001$) lower than that of wild type mice during the experimental period (5.9% at day 7 and 7.6% at day 14), As a result of the lower muscle DNA concentration, the muscle RNA/DNA ratio of Mstn-pro mice was significantly ($P < 0.05$) greater than that of wild-type mice.

CL administration significantly ($P < 0.05$) increased muscle RNA concentrations of both the wild-type and Mstn-pro mice at 7 days, but the effect disappeared at 14 days after administration. Likewise, CL administration significantly ($P < 0.001$) decreased muscle DNA concentrations of both wild-type and Mstn-pro mice, leading to a lowest DNA concentration in Mstn-pro mice treated with CL. The extent of decrease in muscle DNA concentration was greater at 14 days after CL administration (13.9% in wild-type and 9.5% in Mstn-pro) than at 7 days of CL administration (5.3% in wild-type and 5.9% in Mstn-pro). Because CL administration lowered DNA concentration, it significantly ($P < 0.05$) increased the muscle RNA/DNA ratio in both wild-type and Mstn-pro mice, resulting in the highest RNA/DNA ratio in the Mstn-pro mice treated with CL.

2.4.3 Effect of CL administration on the phosphorylation of muscle 4E-BP1 and

p70S6k in wild type and Mstn-pro mice.

The phosphorylations of 4E-BP1 and p70S6k have been shown to play an important role in regulating protein synthesis, and thus the levels of phosphorylations of these proteins in EDL muscles were examined using Western blot analysis (Fig. 2.2 and 2.3). In our Western blot analysis of the phosphorylation of 4E-BP1 and p70S6k, we did not perform immunoblot against the total amount of these proteins. Accordingly, the levels of phosphorylation expressed in this study represent the phosphorylated amount of those proteins per unit tissue volume, not the phosphorylated proportion of these proteins. Thus, these results cannot reveal whether the changes in phosphorylated 4E-BP1 or p70S6k observed in this study occurred in the absence of changes in the total amount of these proteins. However, a recent study showed that myostatin blockage did not significantly affect muscle concentrations of p70S6k or 4E-BP1 (Welle et al., 2006), indicating that modulation of Mstn activity does not affect the muscle concentration of these proteins.

The levels of both phosphorylated 4E-BP1 and p70S6k were significantly ($P < 0.001$) greater in Mstn-pro mice than in wild-type mice during the experimental period. CL administration significantly ($P < 0.001$) increased the levels of phosphorylated 4E-BP1 and p70S6k regardless of genotype, leading to the highest levels of phosphorylation of these proteins in Mstn-pro mice treated with CL. This result suggests that both Mstn suppression and CL administration stimulate the phosphorylation of 4E-BP1 and p70S6k, two downstream effectors of the mTOR pathway, and the phosphorylations of 4E-BP1 and p70S6k are summed when the Mstn suppression and CL administration are combined.

The increase in phosphorylated 4E-BP1 by CL administration was greater at 7 days of CL administration (38.2% for wild-type and 69.2% for Mstn-pro) than at 14 days of CL administration (24.0% for wild-type and 13.9% for Mstn-pro). Likewise, the increase in phosphorylated p70S6k by CL administration was greater at 7 days of CL administration (70.1% for wild-type and 41.7% for Mstn-pro) than at 14 days of CL administration (22.2% for wild-type and 18.5% for Mstn-pro). This result suggests that, like other cellular responses, the effect of CL on phosphorylation of 4E-BP1 and p70S6k is diminished during long-term administration of CL.

2.5 DISCUSSION

The results from this study show that the muscle hypertrophic effects of CL is additive to the muscle hypertrophic effect of Mstn suppression, an important implication for future therapeutic approaches aimed to reverse muscle-wasting conditions. Our study, however, did not examine the change in muscle function such as grip strength and endurance during the hypertrophy induced by the combination of CL administration and Mstn-suppression, and therefore future studies will need to investigate muscle functionality as affected by the combination of β-agonist treatment and Mstn suppression.

The temporal nature of the growth-promoting effect of CL was observed in both wild-type and Mstn-pro mice, suggesting that the desensitization process of muscle β-adrenoceptors appears not be influenced by chronic suppression of Mstn activity. It also appears that, during the early period of CL administration, CL may act synergistically with the hypertrophic effect of Mstn suppression, because the increase in body and muscle weight gains of Mstn-pro mice by CL was significantly greater than that of wild-type mice at 7 days after CL administration, whereas the increase at 14 days of administration was similar between the two genotypes.

Some studies have indicated that Mstn regulates postnatal muscle growth and repair via satellite cell activation because lack of Mstn increased satellite activation and resulted in improved muscle repair in mice (McCrosky et al., 2003 and 2005). In contrast, recent results by Sartori et al. (2009) and Amthor et al. (2009) suggest that muscle hypertrophy induced by Mstn suppression is not due to the activation of satellite cells and addition of new nuclei into the adult fibers. When new myonuclei formation was measured by BrdU incorporation method, no new myonuclei addition occurred in muscles expressing dominant negative ActRIIB (Sartori et al., 2009). Myonuclei number per muscle fiber was significantly lower in hypertrophic muscles induced by Mstn blockade than in non-hypertrophic muscles, along with no difference in the number of Pax7 expressing satellite cells in muscles or satellite cell proliferation and differentiation between those two muscles (Amthor et al., 2009; Matsakas et al., 2009). Our current results show that the muscle DNA concentration in Mstn-pro mice was lower than that of wild-type mice but without a difference in RNA concentration, resulting in higher RNA/DNA ratios in Mstn-pro mice. These results are consistent with a previous study that showed muscle DNA concentration and RNA/DNA ratio of Mstn-deficient transgenic mice are lower and higher, respectively, than those of wild-type mice, without differences in muscle RNA concentration between the two groups (Welle et al., 2006). The total fiber number in gastrocnemius muscle of Mstn-pro mice was not different from that of wild-type mice (Yang et al., 2001), indicating that the increase in muscle mass in the Mstn-pro group was mostly due to an increase in fiber size (hypertrophy) and not an increase in fiber number (hyperplasia). Taken together, these results indicate that the myonuclear domain size of Mstn-pro mice probably was not maintained during the enhanced muscle hypertrophy of the Mstn-pro mice, thus suggest that satellite cell activation is probably not a primary factor for muscle hypertrophy induced by Mstn-suppression. β-Adrenergic agonists have been shown to increase muscle RNA concentration during the early period of administration and decrease muscle DNA concentration (Kim and Sainz, 1992). This is consistent with our results that show CL administration for 7 days increased muscle RNA concentrations and decreased muscle DNA concentrations of both the wild-type and Mstn-pro mice, resulting in an increased RNA/DNA ratio by CL administration. This suggests that, like the Mstn suppression, satellite cell activation in not involved in the muscle hypertrophy induced by CL administration.

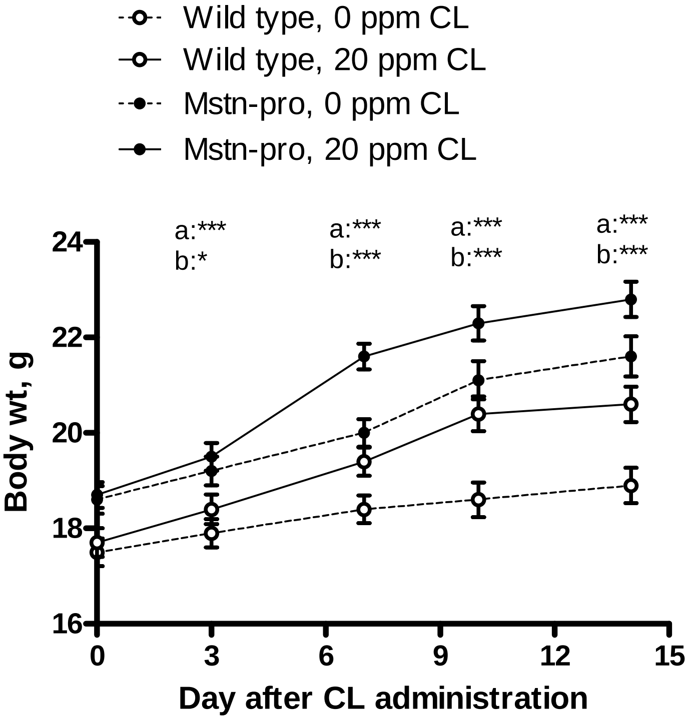
The mTOR pathway has emerged as a critical mediator of cell growth and proliferation by integrating signals from growth factors, nutrients, and the energy status of cells (Sarbassov et al., 2005). The serine/threonine kinase mTOR interacts with multiple protein partners, forming two distinctive multiprotein complexes, mTORC1 and mTORC2. The mTORC1 complex mediates functions that are sensitive to rapamycin, such as protein translation, whereas mTORC2 mediates functions not sensitive to rapamycin. mTORC1 activation by Akt induces the phosphorylation of two downstream effectors, ribosomal protein p70S6k and 4E-BP1, a suppressor of the cap binding protein eIF4E, resulting in increased protein translation (Sarbassov et al., 2005). Muscle growth also appears to be regulated by the mTOR pathway, as demonstrated by studies showing that the hypertrophic response induced by overload or regenerating muscle growth was blocked by rapamycin, an inhibitor of mTOR (Bodine et al., 2001; Pallafacchina et al., 2002). Anabolic stimuli, such as overloading, resistance exercise, and essential amino acid ingestion, have been shown to increase the phosphorylation of p70S6k and 4E-BP1 along with a corresponding increase in protein synthesis (Bodine et al., 2001; Dreyer et al., 2006; Fujita et al., 2007; Dreyer et al., 2008; Drummond et al., 2008). Conversely, atrophying muscles have decreased levels of phosphorylation of the downstream targets of mTOR (Bodine et al., 2001; Hornberger et al., 2001).

In our study, we observed that levels of phosphorylated 4E-BP1 (Thr$^{37/46}$) and p70S6k (Thr^{389}) were greater in Mstn-pro transgenic mice compared with wild-type mice, suggesting that Mstn also regulates muscle mass probably through the mTOR pathway. This result is in agreement with a recent report that showed Mstn overexpression in mice skeletal muscle by electrotransfer of Mstn-expression vector attenuated the phosphorylation of 4E-BP1 (Thr$^{37/46}$) (Wang and Proud, 2006). In addition to the decrease in 4E-BP1 phosphorylation, the study also observed a decrease in the phosphorylation of TSC2 (Thr^{1462}), ribosomal protein S6 (Ser$^{235/236}$), and Akt (Thr^{308}) in response to Mstn overexpression, supporting the role of the Akt/mTOR signaling pathway in Mstn regulation of skeletal muscle mass. Interestingly, in another study using a different model, where Mstn activity was suppressed by the administration of anti-Mstn antibody in mice (Welle et al., 2009a), Mstn suppression did not affect the phosphorylation of Akt (Thr^{308} and Ser^{473}) and 4E-BP1 (Thr$^{36/45}$, Ser^{64}, and Thr^{69}), but it increased the phosphorylation of p70S6k (Thr^{389}) as well as rpS6, a substrate for p70S6k. In that study it was also noted that rapamycin, a pharmacological blocker of mTOR, eliminated the phosphorylation of p70S6k and rpS6, but it did not block the increase in muscle protein synthetic rate induced by Mstn suppression (Welle et al., 2009a). These results suggest that Mstn regulation of muscle protein synthesis can be independent of Akt/mTOR signaling even though we cannot rule out the possibility that the discrepancy resulted from differences in Mstn modulation in conjunction with sampling factors. The mTOR pathway is known to take diverse signals and produce a myriad of responses (Sarbassov et al., 2005), and thus further studies are needed to clearly define the details of the involvement of the Akt/mTOR pathway in Mstn signaling.

Like Mstn, β-adrenergic agonists also induce muscle hypertrophy through the Akt/mTOR signaling pathway (Sneddon et al., 2001; Kline et al., 2007). For example, CL treatment in mice promoted phosphorylation of 4E-BP1 (Thr^{70}) and p70S6k (Thr^{412}) (Sneddon et al., 2001). In addition to 4E-BP1 and p70S6k phosphorylation, CL treatment also promoted the phosphorylation of Akt (Kline et al., 2007). Furthermore, the CL effect was suppressed by rapamycin (Kline et al., 2007). In agreement with the results of previous studies, increased levels of phosphorylated 4E-BP1 and p70S6k were observed by CL administration in our study, supporting the role of the Akt/mTOR pathway in β-adrenergic agonist–induced skeletal muscle hypertrophy. Notably, our result demonstrated that CL administration increased the phosphorylation of 4E-BP1 and p70S6k not only in wild-type mice but also in Mstn-pro mice, resulting in a summation of the levels of phosphorylation of the two molecules. The findings further show that skeletal muscle masses of treatment groups were closely related to the levels of phosphorylation of 4E-BP1 and p70S6k and the RNA/DNA ratio of treatment groups. It is thus suggested that the post-receptor signaling of both Mstn suppression and β-receptor activation by CL induces the phosphorylation of 4E-BP1 and p70S6k to enhance protein translation, and the combination of Mstn suppression and β-receptor activation induces the summation of 4E-BP1 and p70S6k phosphorylation and subsequent protein translation. In this study we did not extensively examine signaling intermediates of the Akt/mTOR pathway, and more studies are needed to understand the role of the Akt/mTOR pathway in the convergence of Mstn and β-adrenergic agonist signaling to 4E-BP1 and p70S6k phosphorylation.

In conclusion, the results of this study demonstrate that the skeletal muscle hypertrophic effect of CL is additive to the hypertrophic effect of Mstn suppression. Although CL is not approved for use in humans, a few trials with salbutamol, a β-adrenergic agonist approved for human use, have shown a limited positive effect in muscular dystrophy patients (Kissel et al., 2001; Fowler et al., 2004; van der Kooi et al., 2004). A new generation of adrenergic agonists, including formoterol and salmeterol, appear to have more powerful skeletal muscle anabolic effects, with fewer side effects (Busquets et al., 2004; Ryall et al., 2006; Harcourt et al., 2007). Our findings thus indicate the potential of the combination of Mstn suppression and treatment with β-agonists in the prevention or reversal of muscle-wasting conditions. The results on 4E-BP1 and p70S6k phosphorylation also show that the pathways of Mstn and CL signaling converge to the phosphorylation of 4E-BP1 and p70S6k, two downstream effectors of the Akt/mTOR anabolic pathway, to regulate skeletal muscle hypertrophy.

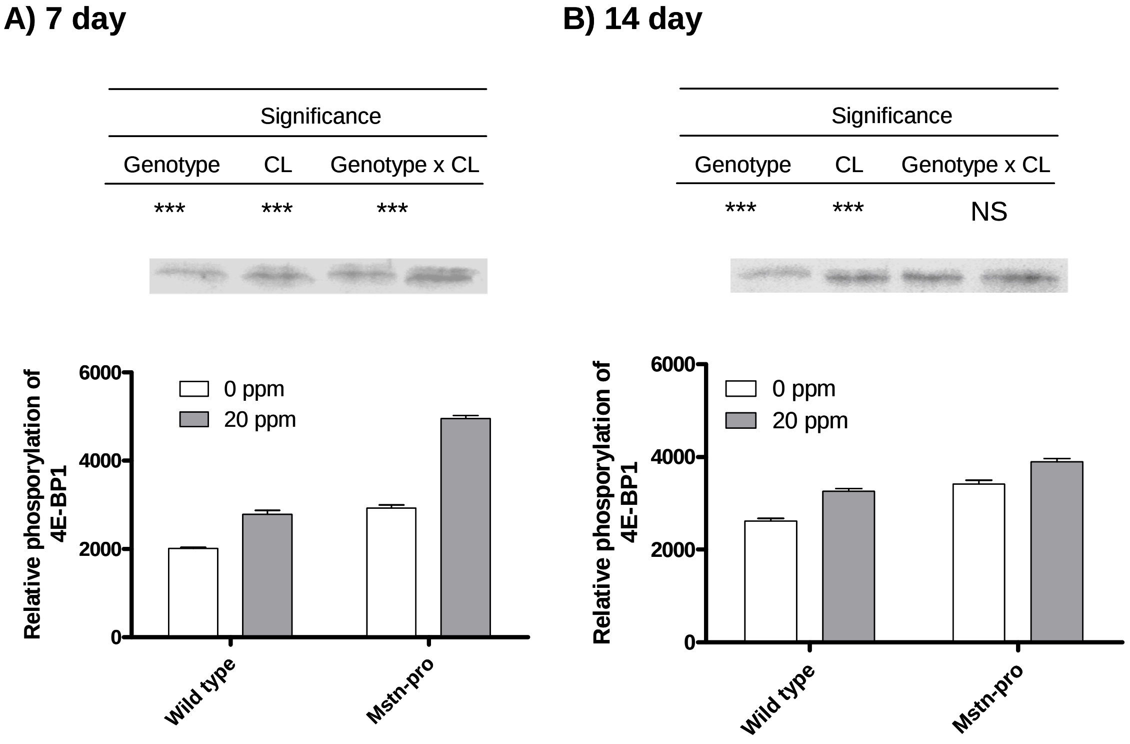
A)



B)

Weight gain							
	Wild type		Mstn-pro		Significance		
Days	0 ppm	20 ppm	0 ppm	20 ppm	GT	CL	GTxCL
0-3	0.4 (0.16)	0.7 (0.16)	0.6 (0.16)	0.9 (0.15)	NS	*	NS
3-7	0.4 (0.15)	1.1 (0.15)	0.8 (0.15)	2.1 (0.14)	***	***	*
7-10	0.3 (0.15)	0.8 (0.15)	0.7 (0.17)	1.0 (0.15)	+	**	NS
10-14	0.3 (0.14)	0.2 (0.14)	0.5 (0.15)	0.5 (0.14)	*	NS	NS

Figure 2.1 Effect of CL administration on body weights of wild type and Mstn-pro mice. Solid and dotted lines indicate 20 and 0 ppm CL administration, respectively, to wild type (open circle) and Mstn-pro mice (closed circle). Values are means (SEM). (A): Growth curve of female mice during 14 days (a, difference between wild type and Mstn-pro; b, difference between 0 ppm and 20 ppm CL administration (***, $P < 0.001$, **, $P < 0.01$)). (B): Body weight gain of female mice during 14 days (***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$, $^{+}$, $P < 0.1$, NS, not significance). CL, clenbuterol; GT, genotype.



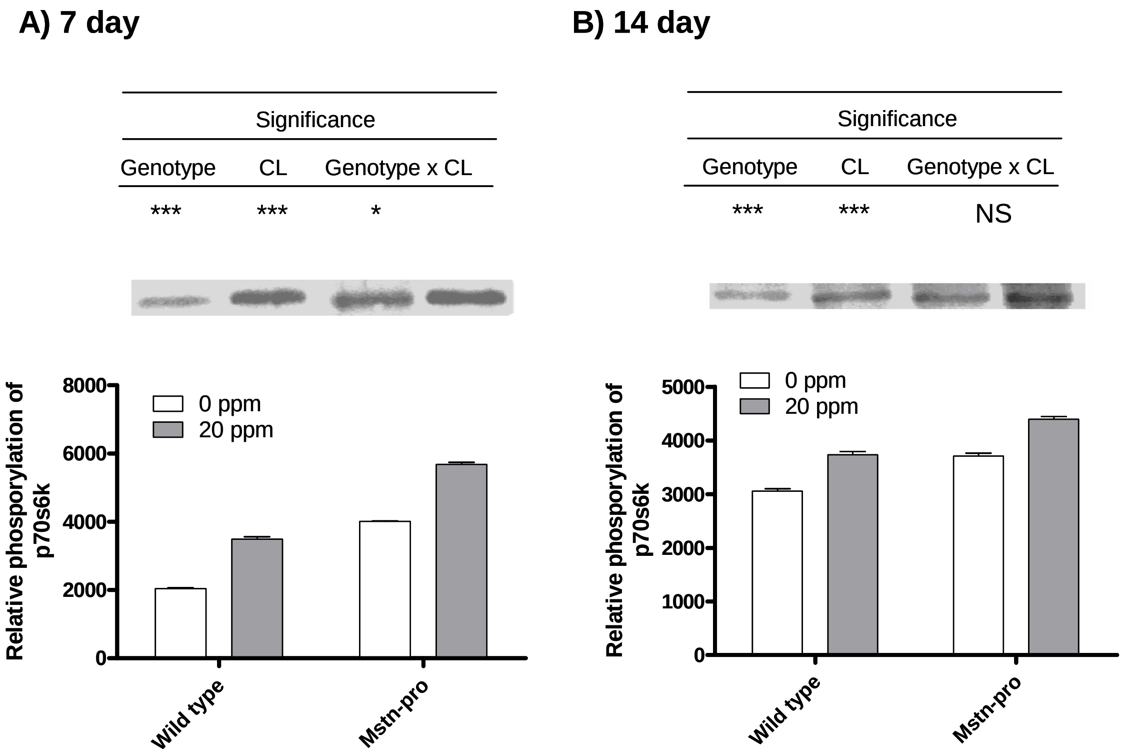
A) 7 day

Genotype	CL	Genotype x CL
***	***	***

B) 14 day

Genotype	CL	Genotype x CL
***	***	NS

Figure 2.2 Effect of CL administration on 4E-BP1 at Thr37/46 phosphoryalation in extensor digitorum longus muscles of wild type and Mstn-pro mice. Representative immunoblot images are shown above the bar graph. Each lane sequentially represents the group in the bar graph. Values are expressed as mean ±SEM. ***, P<0.001.



A) 7 day

Significance		
Genotype	CL	Genotype x CL
***	***	*

B) 14 day

Significance		
Genotype	CL	Genotype x CL
***	***	NS

Figure 2.3 Effect of CL administration on p70S6k phosphoryalation at Thr389 in extensor digitorum longus muscles of wild type and Mstn-pro mice. Representative immunoblot images are shown above the bar graph. Each lane sequentially represents the group in the bar graph. Values are expressed as mean ±SEM. *, P<0.05; ***, P<0.001.

Table 2.1 Muscle weights of wild type and Mstn-pro mice as affected by CL administration for 7 days.

	Wild type		Mstn-pro		Significance		
	0 ppm	20 ppm	0 ppm	20 ppm	GT	CL	GT x CL
7 days after administration							
Number of animals	12	10	15	15			
Initial body Wt, g	17.7 (0.47)	17.7 (0.37)	18.5 (0.49)	18.7 (0.37)	*	NS	NS
Final body Wt, g	18.5 (0.44)	19.3 (0.40)	19.7 (0.47)	21.8 (0.40)	***	**	NS
Gastrocnemius Wt, mg	87.6 (3.73)	95.0 (3.41)	110.8 (4.30)	136.6 (3.51)	***	***	*
Plantaris Wt, mg	9.8 (0.52)	10.4 (0.68)	12.6 (0.45)	15.9 (0.55)	***	***	*
EDL Wt, mg	6.9 (0.51)	7.5 (0.37)	9.4 (0.40)	11.2 (0.23)	***	**	NS
Soleus Wt, mg	4.2 (0.38)	5.9 (0.38)	4.85 (0.295)	6.1 (0.36)	NS	***	NS
Muscle protein, %[1]	21.1 (0.14)	25.9 (0.20)	24.7 (0.17)	28.5 (0.20)	***	***	NS

Values are means (SEM). CL, clenbuterol; GT, genotype; Wt, weight; EDL, extensor digitorum longus; NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. [1]Muscle protein concentration was measured in plantaris muscle using the modified Lowry method.

Table 2.2 Muscle weights of wild type and Mstn-pro mice as affected by CL administration for 14 days.

	Wild type		Mstn-pro		Significance		
	0 ppm	20 ppm	0 ppm	20 ppm	GT	CL	GT x CL
14 days after administration							
Number of animals	12	14	11	14			
Initial body Wt, g	17.7 (0.36)	17.6 (0.30)	18.8 (0.56)	18.6 (0.37)	**	NS	NS
Final body Wt, g	19.1 (0.34)	20.6 (0.24)	21.6 (0.60)	22.8 (0.37)	***	**	NS
Gastrocnemius Wt, mg	84.9 (2.75)	106.6 (2.49)	119.1 (4.10)	151.1 (3.99)	***	***	NS
Plantaris Wt, mg	9.5 (0.34)	13.2 (0.50)	13.9 (0.43)	18.7 (0.72)	***	***	NS
EDL Wt, mg	6.3 (0.34)	9.1 (0.31)	8.6 (0.61)	11.2 (0.34)	***	***	NS
Soleus Wt, mg	4.46 (0.287)	5.97 (0.229)	5.13 (0.192)	6.96 (0.311)	**	***	NS
Muscle protein, %[1]	20.1 (0.12)	25.2 (0.17)	24.1 (0.14)	27.4 (0.15)	***	***	NS

Values are means (SEM). CL, clenbuterol; GT, genotype; Wt, weight; EDL, extensor digitorum longus; NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. [1]Muscle protein concentration was measured in plantaris muscle using the modified Lowry method.

Table 2.3 Muscle DNA and RNA concentrations in wild-type and MSTN-pro mice as affected by CL administration.

	Wild type		Mstn-pro		Significance		
	0 ppm	20 ppm	0 ppm	20 ppm	GT	CL	GT x CL
7 day after administration							
Number of animals	12	10	15	15			
DNA (mg/g wet muscle)	1.22 (0.010)	1.15 (0.015)	1.14 (0.030)	1.08 (0.012)	***	***	NS
RNA (mg/g wet muscle)	1.13 (0.044)	1.27 (0.030)	1.24 (0.031)	1.36 (0.031)	NS	*	NS
RNA/DNA ratio	1.01 (0.035)	1.11 (0.032)	1.09 (0.037)	1.27 (0.036)	**	***	NS
14 day after administration							
Number of animals	12	14	11	14			
DNA (mg/g wet muscle)	0.95 (0.031)	0.87 (0.026)	0.88 (0.030)	0.79 (0.012)	**	*	NS
RNA (mg/g wet muscle)	1.13 (0.032)	1.15 (0.027)	1.15 (0.022)	1.15 (0.019)	NS	NS	NS
RNA/DNA ratio	1.22 (0.072)	1.35 (0.055)	1.32 (0.035)	1.45 (0.034)	*	*	NS

Values are means (SEM). CL, clenbuterol; GT, genotype; NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

CAPTER 3

INVESTIGATION OF DIFFERENTIAL GENE EXPRESSION IN HYPERTROPHIC MUSCLES INDUCED BY MYOSTATIN SUPPRESSION OR CLENBUTEROL, A BETA-ADRENERGIC AGONIST

3.1 ABSTRACT

In this study, we employed microarray analysis to acquire a comprehensive picture of gene expression occurring in hypertrophic muscles induced by Mstn suppression (Mstn-prodomain overexpression) and/or clenbuterol (CL). Involvement of the Akt/mTOR pathway was also investigated in the regulation of muscle hypertrophy occurring in the two models. Male heterozygous Mstn-pro and wild-type littermates were given 0 or 20 ppm of CL in their drinking water, and muscle samples were collected at 3 and 14 days after treatment. Phosphorylation levels of Akt, 4E-BP1 and p70S6k in EDL muscle were analyzed using the Western blot analysis. Four or five pooled RNA samples of the gastrocnemius muscle in each group were subjected to microarray analysis using the Affymetrix GeneChip Mouse 430-2.0 platform, and data were analyzed by t-test and fold changes. The skeletal muscle hypertrophic effect of CL was additive to the hypertrophic effect of Mstn suppression, and the results on Akt, 4E-BP1 and p70S6k phosphorylation indicate that muscle hypertrophy induced by Mstn suppression or CL administration is through the activation of the Akt/mTOR anabolic pathway, and Mstn and CL signaling converge to the Akt/mTOR pathway to regulate skeletal muscle hypertrophy. Microarray analysis showed that genes differentially expressed by Mstn-suppression (108 at 1.5 fold cutoff) is far less than those differentially expressed by CL administration (1241 at 1.5 fold cutoff), reflecting the

diverse physiological role of CL. Functional anlysis of those differentially expressed gene showed that Mstn suppression and CL administration induced significant changes in the mRNA abundance of various genes associated with muscle contraction, initiation of translation, transcription, and muscle hypertrophic signal pathway, suggesting that increased protein synthesis is partly responsible for the hypetrophy induced by Mstn and CL.. Additionally, the alteration of Igf2 obsderved in Mstn suppressed mice, and the alterations of eIF4e, Acvr2b, FoxO and PTEN observed in mice treated with CL indicate that the pathways of Mstn and CL signaling converge to the Akt/mTOR anabolic pathway to regulate skeletal muscle hypertrophy.

3.2 INTRODUCTION

The ability to increase skeletal muscle mass is highly desirable for both human health and agriculture applications. Of major interest in this study is the growth factor myostatin (Mstn) and β-adrenergic agonist (BAA) such as clenbuterol (CL). Mstn is a member of the TGF-β superfamily that acts as a potent negative regulator of skeletal muscle growth. Mice with constitutive knockout of the Mstn gene have a remarkable double-muscled phenotype caused by both muscle cell hypertrophy and hyperplasia (McPherron et al., 1997). Mstn also regulates muscle development in other mammals, including human (Mosher et al., 2007; Grobet et al., 1997; Kambadur et al., 1997; McPherron and Lee, 1997; Clop et al., 2006; Schuelke et al., 2004). Mstn binds to activin type IIb receptor to exert its biological activity (Lee and McPherron, 2001; Thies et al., 2001; Rebbapragada et al., 2003) and initiate a signaling cascade through the Smad pathway (Lee and McPherron, 2001; Zhu et al., 2004; Tsuchida et al., 2008). The activation of Smad signaling by Mstn has been well characterized. Recent studies, however, indicated that there is a cross-talk between Smads signaling and the

Akt/mammalian target-of-rapamycin (mTOR) anabolic pathway, which plays an important role in mediating the muscle hypertrophy (Sartori et al., 2009; Trendelenburg et al., 2009; Welle et al., 2006; Amirouche et al., 2009; Morissette et al., 2009; Welle et al., 2009a).

Anabolic effects of BAA have been widely studied for potential applications in the prevention of muscle atrophy in humans and improvement of the efficiency of muscle growth in livestock (Kim and Sainz, 1992; Mersmann, 1998; Kissel et al., 2001). The muscle hypertrophic effect of BAA is mediated through the binding of ligands to β_2-adrenergic receptor (β_2AR), and subsequent activation of the adenylyl cyclase (AC)/cyclic adenosine monophosphate (cAMP) signaling pathway (Hinkle et al., 2002). Although the β_2AR /AC/cAMP pathway is responsible for the β_2AR -mediated hypertrophy in skeletal muscle (Navegantes et al., 2000; Hinkle et al., 2002), the link between the classic β_2AR /AC/cAMP signaling pathway and the change in protein metabolism induced by the administration of BAAs has remained elusive. Recent results, however, indicate that like Mstn, BAAs may induce muscle hypertrophy via the Akt/mTOR anabolic pathway (Sneddon et al., 2001; Kline et al., 2007).

Microarray technology enables a broad overview of the impact of treatments on expression of all known genes, and this technology has already been used to examine the effects of constitutive Mstn deficiency or the effects of acute BAA stimulation in mice (Steelman et al., 2006; Welle et al., 2009; Spurlock et al., 2006; Pearson et al., 2009). For example, Steelman et al. (2006) reported that 717 genes ($p < 0.05$, 1.5 fold change) were differentially expressed in pectoral muscles of wild type and Mstn null mice, and Welle et al. (2009) reported that 124 genes ($p < 0.01$, 1.5 fold change) were differentially expressed in gastrocnemius muscles between wild type and Mstn null mice. Moreover, Spurlock et al. (2006) reported that over 50 genes were differentially expressed (FDR < 10%) in mouse skeletal muscle 24 hours and 10 days after administration of CL, and Pearson et al. (2009) reported that 23 and 112 genes were differentially expressed by systemic administration of the formoterol at both 1 and 4 hours in mice.

Even though Mstn and CL bind to different receptors, their receptor activations have a dramatic effect on skeletal muscle growth. Thus, it was hypothesized that comparing global gene expression between the two different models of muscle hypertrophy (Mstn-suppression and CL administration) would allows us to acquire a comprehensive picture of transcriptional changes in skeletal muscles undergoing hypertrophy, which may lead to the identification of key transcriptional adaptations and signaling pathways involved in muscle hypertrophy. Therefore, the objective of this study was 1) to examine global changes in gene expressions of skeletal muscle undergoing hypertrophy induced by genetic suppression of Mstn or administration of BAA, 2) to compare the gene expression changes between the two muscle hypertrophic models, and 3) to examine the role of the Akt/mTOR pathway in the two muscle hypertrophic models.

3.3 MATERIALS AND METHODS

3.3.1 Animals and sample collection

All procedures using experimental animals were approved by the Institutional Animal Care and Use Committee, University of Hawaii. All mice were maintained in temperature- and humidity-controlled conditions with a 12 h light/dark cycle and had free access to food (10% kcal fat, ME3.85 kcal/g) and water *ad libitum*. For Mstn suppression, we used transgenic mice overexpressing Mstn prodomain (Mstn-pro). The generation and phenotype of the Mstn-pro transgenic mice was previously reported (Yang et al., 2001). To produce heterozygous Mstn-pro transgenic mice and wild-type littermate, female B6SJL F1 mice were mated to heterozygous Mstn-pro transgenic male mice. Pups were weaned at 28 days, then males and females were housed separately. Tail tissue samples were collected at weaning for genotyping (Appendix 3.1). At 35 days of age, male mice were separated by their genotypes, and mice in each genotype were randomly assigned to one of the two groups: 0 or 20 ppm clenbuterol (CL). Mice were administered with CL in the drinking water (20 ppm) during the experimental period, and body weights were monitored periodically. Previous studies have demonstrated the effectiveness of the 20-ppm dose (about 5 mg/kg body weight/day) of CL via drinking water on promoting body and muscle growth (Lynch et al., 1996). At 3 and 14 days after the CL administration, animals were sacrificed (about half in each sacrifice) by CO_2 asphyxiation, and gastrocnemius, plantaris, soleus, and extensor digitorum longus (EDL) muscles of the lower hind leg were rapidly dissected out, weighed, snap-frozen in liquid nitrogen and stored at -80°C until analysis.

3.3.2 Genotyping

Genotyping was determined by the same PCR procedure described in the Chapter 2. The PCR products were subjected to electrophoresis in a 1.2 % agarose gel and stained with ethidium bromide to examine the presence of a transgenic PCR product (Appendix 3.1).

3.3.3 Measurement of skeletal muscle DNA and RNA concentration

DNA and RNA concentrations of plantaris muscle samples were estimated by the same procedure described in Chapter 2.

3.3.4 Western blot analysis of Akt, 4E-BP1 and p70S6k phosphoryalation

The procedure described by Dreyer et al. (2006) was used for Western blot analysis. Briefly, EDL muscle samples were homogenized at 4°C in 9 volumes of ice-cold lysis buffer [50 mM Tris-HCl (pH 7.4), 250 mM mannitol, 50 mM NaF, 5 mM Na pyrophosphate, 1 mM EDTA, 1 mM ethylene-glycol teraacetic acid (EGTA), 1% Triton X-100, 1 mM dithiothreitol (DTT), 1 mM benxamidine, 0.1 mM phenylmethylsulfonylfluoride (PMSF), and 5 μg/ml soybean trypsin inhibitor]. The homogenates were centrifuged at 6,000 g for 10 min at 4°C, and aliquots of the supernatants were saved for Western blot detection of Akt and p70S6k phosphorylation. For Western blot detection of 4E-BP1, 100 μl of the above supernatants were heated at 100 ^{o}C for 10 min, centrifuged for 30 min at 10,000 g at 4 ^{o}C, and the supernatants were saved. Protein concentrations in supernatants were determined using the modified Lowry protein assay kit (Pierce, Rockford, IL) to estimate protein content. The protein concentration of supernatants was not significantly ($P < 0.01$) affected by the genotype and CL administration, and thus equal volumes of supernatants were loaded per lane with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Twenty μl of the protein extract for Akt, p70S6k and 4E-BP1were subjected to 7.5%, 7.5% and 15% SDS-PAGE, respectively, and then proteins were transferred to polyvinylidene difluoride (PVDF) membranes. The blots were blocked for 3 hrs at room temperature in Tris-buttered saline (20 mM Tris-HCI, 150 mM NaCI at pH 7.5) containing 3 % bovine serum albumin (BSA), followed by incubation with primary antibody in Tris-buffered saline plus 0.01% Tween-20 (TBST, 20 mM Tris-HCL, 150 mM NaCl at pH 7.5) at 4°C overnight. The primary antibodies were rabbit anti-phospho-Akt (Thr^{308}; 1:1,000; Cell signaling, Beverly, MA), rabbit anti-phospho-p70S6k (Thr^{389}; 1:1,000; Cell signaling), rabbit anti-phospho-4E-BP1 (Thr$^{37/46}$; 1:1,000;

Cell signaling), rabbit anti-Akt (1:1,000; Santa Cruz Biotechnology, Delaware, CA), rabbit anti-p70S6k (1:1,000; Santa Cruz Biotechnology), and rabbit anti-4E-BP1 (1:1,000; Santa Cruz Biotechnology). The blots were washed twice with TBST and incubated with horseradish peroxidase-conjugated anti-rabbit antibody (1:2,500, Invitrogen, Carlsbad, CA) in TBST for 2 hrs at room temperature. After washing, the membrane was developed with the enhanced chemiluminescent reagent (ECL plus Western Detection System; GE Healthcare Biosciences; Pittsburg, PA), followed by apposition of the membrane to auto-radiographic films (Hyperfilm ECL; Kodak, CA). After obtaining an appropriate image, band intensity was quantitatively analyzed using Quantity One analysis software (Bio-Rad, Hercules, CA).

3.3.5 RNA Extraction, Gene Chip Hybridization, and Data Acquisition

Total RNA was extracted from the gastrocnemius muscle using Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacture's instructions. RNA integrity was confirmed using the Agilent Bionalyzer 2100 (Agilent Technologies, Palo Alto, CA). Total RNA samples from three or four individual mice were pooled together for microarray analysis to minimize the effect of individual variability in gene expression. Four or five pooled samples for each group were subjected to microarray analysis. The sample labeling, gene chip hybridization and data acquisition were preformed using the standard Affymetrix protocol at the University of Hawaii Biotech/Molecular Biology Core facility. Briefly, about 18 micrograms of pooled total RNA were converted into double-stranded cDNAs using the SuperScript Choice system (Invitrogen, Carlsbad, CA) with an oligo-dT$_{24}$ primer containing the T7 polymerase promoter attached to a poly-dT sequence. In vitro transcription was performed using a commercially available RNA transcript biotin labeling kit (Affymetrix, Santa Clara, CA). Biotin-labeled cRNA were purified using a commercially available kit (Affymetrix, Santa Clara, CA), then fragmented. Labeled cRNAs were hybridized first to Tester arrays (Affymetrix, Santa Clara, CA), then to Mouse 430-2 arrays after examination of the results of Tester array hybridization. Briefly, ten micrograms of fragmented cRNAs were hybridized for 16 hours on the Affymetrix Mouse 430-2 microarray chips. After incubation, Affymetrix Fluidics Station 450 was used for washing and staining according to the manufacturer's instructions with its reagents. Briefly, non-hybridized materials were removed, then the hybridized chips were incubated with streptavidin-phycoerythrin (SAPE) to detect hybridized cRNA. The signal intensity was amplified by a second staining with biotin-labeled anti-streptavidin antibody followed by SAPE staining. Fluorescent images were scanned before and after amplification using the Affymetrix Gene Chip Scanner 3000.

3.3.6 Microarray Data Processing and Analysis

The Affymetrix Mouse 430-2 microarray contains probe spots for over 45,000 genes. The microarray images were processed with the GenChip® Operating Software v. 1.4 (GCOS, Affymetrix, Santa Clara, CA). Gene expression signals were scaled to a target intensity of 500, and detection values were determined with the default settings of the GCOS. The probes sets having at least an absent call in microarrays of each group were eliminated from the GCOS-generated raw data. To examine differential gene expression during muscle hypertrophy induced by chornic myostatin suppression, signal intensities of wild type and Mstn-pro mice were subjected to t-test ($p<0.01$), then various fold change cutoffs were applied to select genes that were differentially expressed between the two groups. To examine differential gene expression during muscle hypertrophy induced by the administration of CL, signal intensities of wild type and wild type-CL mice were subjected to the same approach. To determine the biological significance of the differentially expressed genes, genes were categorized based on Gene Ontology (GO) biological process categories and over-represented categories were identified using the DAVID software (Dennis, Jr. *et al.* 2003).

3.3.7 Validation of Microarray Results with Real-time PCR

Ten genes were selected among the differentially expressed genes, and primers were synthesized (Table 3.4) for a validation of microarray expression results using the real-time PCR method. Total RNAs isolated for microarray hybridization were used for PCR. Five µg of total RNAs were reverse transcribed using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) with oligo-dT primers in a 50 µL total volume. Five µL of the resulting cDNA was used for real-time PCR in a 50 µL total volume at the University of Hawaii Biotech/Molecular Biology Core facility using the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA) following the manufacturer's instruction. Threshold cycle (Ct) of each sample was obtained and analyzed by Prism5 software for statistical significance (Grapad, San Diego, CA).

3.3.8 Statistical analysis

All values are expressed as mean ± SEM. Two-way analysis of variance (ANOVA) was performed using Prism5 software (GraphPad, San Diego, California) to examine the effects of genotype, CL, and interaction on body and muscle weights; muscle DNA and RNA concentrations; and the levels of phosphorylation of Akt, 4E-BP1 and p70S6k. Statistical significance was determined to be $P < 0.05$ unless otherwise indicated.

3.4 RESULTS

3.4.1 Effects of CL on body and muscle weights of wild type and Mstn-pro mice

The effect of CL administration on body weights of wild type and Mstn-pro mice is summarized in Fig. 3.1. As expected from a previous report (Yang et al., 2001), Mstn-pro mice were heavier than wild type mice at the time of CL administration ($P < 0.001$), and they grew significantly faster than wild type mice over the 14 day period ($P < 0.001$). The growth-promoting effect of CL was evident in both genotypes (Fig. 3.1), indicating that the growth-promoting effect of CL is additive to the growth-promoting effect of Mstn-pro. The temporal effect of CL on body weight gain was evident in both genotypes: the increase in body weight gain of Mstn-pro mice occurred at up to 7 days of CL administration in both genotypes and no further increase was observed afterward.

Skeletal muscle weights of wild type and Mstn-pro mice as affected by CL administration for 3 and 14 days are summarized in Table 3.1 and 3.2. As expected, the skeletal muscle weights of Mstn-pro mice were significantly ($P < 0.001$) heavier than those of wild type mice over the experimental period. The protein concentration of plantaris muscle of Mstn-pro mice was greater than that of wild type mice ($P < 0.001$). CL administration significantly ($P < 0.001$) increased the skeletal muscle weights of both Mstn-pro and wild type mice over the experimental period. CL administration also increased the protein concentrations of plantaris muscle in both Mstn-pro and wild type mice ($P < 0.001$), leading to the highest muscle protein concentration in CL-fed Mstn-pro mice. When concentrations of soluble proteins in lysis buffer were measured from the EDL muscle during Western blot analysis, genotype and CL administration had no effect on soluble protein concentration. Thus, it is postulated that the increase in total muscle protein concentration in Mstn-pro mice or by CL administration is probably due to an increase in insoluble myofibrilar proteins.

3.4.2 Effects of CL on muscle RNA and DNA concentrations of wild type and Mstn-pro mice

RNA and DNA concentrations in plantaris muscle were measured to examine indirectly the myonuclei domain size and protein synthetic capacity (Table 3.3). Muscle RNA concentration of Mstn-pro was not significantly different from those in the wild type mice at 3 and 14 days. However, muscle RNA concentrations of the wild type and Mstn-pro mice were significantly ($P < 0.05$) increased at 3 days after CL administration (6.4% for wild type and 15.9% for Mstn-pro), but the effect disappeared at 14 days after CL administration. Muscle DNA concentration of Mstn-pro mice was significantly ($P < 0.001$) lower than that of wild type mice during the experimental period (10.2% at 3 days and 9.3 % at 14 days), and CL administration also significantly ($P < 0.05$) decreased the muscle DNA concentrations of both wild type and Mstn-pro mice. As a result of lower DNA concentration of Mstn-pro mice, the muscle RNA/DNA ratio of Mstn-pro mice was significantly ($P < 0.001$) higher than that of wild type mice. Similarly, CL administration significantly ($P < 0.05$) increased the muscle RNA/DNA ratio of both wild type and Mstn-pro mice, leading to the highest RNA/DNA ratio in CL-fed Mstn-pro mice. The increased RNA/DNA ratio by both Mstn-pro and CL administration suggests that overall capacity of protein synthesis was enhanced by both Mstn-pro and/or CL administration.

3.4.3 Effect of CL administration on the phosphorylation of muscle Akt, 4E-BP1 and p70S6k in wild type and Mstn-pro mice

The phosphorylation of Akt, 4E-BP1 and p70S6k have been shown to play an important role in regulating protein synthesis, thus the phosphorylation levels of Akt, 4E-BP1 and p70S6k in EDL muscles were examined using Western blot analysis (Fig. 3.2-3.4). The levels of phosphorylated Akt, 4E-BP1 and p70S6k were significantly ($P < 0.001$) higher in Mstn-pro mice than in wild-type mice, but no difference was observed in the levels of total Akt, 4E-BP1 and p70S6k between the groups during the experimental period. Regardless of genotype, CL administration also significantly (P <0.001) increased the phosphorylation levels of Akt, 4E-BP1 and p70S6k without affecting the total levels of Akt, 4E-BP1 and p70S6k, leading to the highest levels of phosphorylation of these proteins in CL-fed Mstn-pro mice.

Notably, at 3day of CL administration, significant effects of genotype and CL interaction on levels of phosphorylated Akt, 4E-BP1 and p70S6k were observed. The increase in the levels of phosphorylated Akt, 4E-BP1 and p70S6k induced by CL administration was greater in Mstn-pro mice than in wild type mice. However, the interaction effects disappeared at 14 days of CL administration. These results suggest that the effect of CL on phosphorylation of Akt, 4E-BP1 and p70S6k diminished during the 14 day administration of CL.

3.4.4 Identification of differential gene expression

We analyzed gene expression profiles to gain a better understanding of the genetic basis of the muscle hypertrophic effects of either Mstn-pro or CL administration by using the Affymetrix 430-2.0 microarray containing ~45,000 probe sets. The probe sets not expressed in skeletal muscles were removed from analysis by eliminating probes sets having at least an absent call in microarrays of each group.

Differential gene expression profile as affected by the effect of genotype (Wild type vs Mstn-pro mice)

We identified that 108 genes (92 overexpressed genes and 16 underexpressed genes), represented by 134 probe sets, were differentially expressed between the Mstn-pro and wild type mice at 1.5 fold change cutoff. Among the 108 different genes, 11 genes were ESTs, resulting identification of 97 known genes (87 overexpressed genes and 10 underexpressed genes, Appendix 3.5). With a fold change cutoff of 3.0 being applied, the list of genes showing differential expression was reduced to 8 overexpressed genes (Table 3.5). To examine the validity of the microarray results, five genes that showed significantly differential expression in microarray analysis were selected for real-time PCR assay. Those genes included Mt3 (5.7 fold), Tnnt2 (3.5 fold), S100a4 (2.9 fold), Ctss (2.3 fold) and Cgref1 (1.6 fold). Equal amount of total RNA samples of individual animal used in the microarray analysis were subjected to the real-time PCR analysis. The real time PCR results confirmed the microarray results (Table 3.10).

Differential gene expression profile as affected by the effect of genotype under CL administration (CL treated wild type mice vs CL treated Mstn-pro mice)

We identified that 96 genes (43 overexpressed genes and 53 underexpressed genes), represented by 121 probe sets, were differentially expressed between the CL-treated wild type and CL-treated Mstn-pro mice. Among the 96 different genes, 3 genes were ESTs, resulting in the identification of 93 known genes (40 overexpressed genes and 53 underexpressed genes, Appendix 3.6). With a fold change cutoff of 3 being applied, the list of gene showing differential expression was reduced to 3 overexpressed genes (Table 3.6). To examine the validity of the microarray results, five genes that showed significantly differential expression in microarray analysis were selected for real-time PCR assay. Those genes included Tubb2a (4.4 fold), Myof (2.2 fold), Atf3 (2.1 fold), Palm2 (1.7 fold) and Cgref1 (1.5 fold). Equal amount of total RNA samples of individual animal used in the microarray analysis were subjected to the real-time PCR analysis. The real time PCR results confirmed the microarray results (Table 3.10).

To examine whether the differential gene expression induced by Mstn-pro overexpression is affected by CL administration, the 108 genes differentially expressed between the wild type and Mstn-pro groups were compared to the 96 differentially expressed genes between CL-treated wild type and CL-treated Mstn-pro groups (Fig. 3.5c). Only two genes were shown to be overlapping between the two lists of differentially expressed genes, indicating that differential gene expression caused by Mstn-pro is dramatically altered by CL administration.

Differential gene expression profile as affected by the effect of CL in wild type mice (Wild type mice vs CL treated wild type mice)

We identified that 1241 genes (650 overexpressed genes and 591 underexpressed genes), represented by 1652 probe sets, were differentially expressed between the wild type and CL-treated wild type mice. Among the 1241 different genes, 90 genes were ESTs, resulting in the identification of 1151 known genes (616 overexpressed genes and 535 underexpressed genes, Appendix 3.7). With a fold change cutoff of 3.0 being applied, the list of genes showing differential expression was reduced to 104 overexpressed genes (Table 3.7). To examine the validity of the microarray results, five genes that showed significantly differential expression in microarray analysis were selected for real-time PCR assay. Those genes included Krt8 (390.3 fold), Rrad (54.8 fold), Gdf5 (50.83 fold), Myog (8.6 fold) and Myo10 (3.3 fold). Equal amount of total RNA samples of individual animal used in the microarray analysis were subjected to the real-time PCR analysis. The real time PCR results confirmed the microarray results (Table 3.10).

Differential gene expression profile as affected by the effect of CL in Mstn-pro (Mstn-pro mice vs CL treated Mstn-pro mice)

We identified that 1522 genes (682 overexpressed genes and 840 underexpressed genes), represented by 1907 probe sets, were differentially expressed between Mstn-pro and CL-treated Mstn-pro mice. Among the 1522 different genes, 115 genes were ESTs, resulting in the identification of 1407 known genes (618 overexpressed genes and 789 underexpressed genes, Appendix 3.8). With a fold change cutoff of 3.0 being applied, the list of genes showing differential expression was reduced to 99 overexpressed genes (Table 3.8). To examine the validity of the microarray results, five genes that showed significantly differential expression in microarray analysis were selected for real-time PCR assay. Those genes included Krt8 (413.4 fold), Runx1 (41 fold), Orm2 (20.6 fold), Myog (8.4 fold) and Tubb2a (4.7 fold). Equal amount of total RNA samples of individual animal used in the microarray analysis were subjected to the real-time PCR analysis. The real time PCR results confirmed the microarray results (Table 3.10).

To examine whether the differential gene expression induced by CL administration is affected by chronic Mstn-pro overexpression, we compared 1241 genes differentially expressed between wild type and CL-treated wild type groups to the 1522 genes differentially expressed between Mstn-pro and CL-treated Mstn-pro mice groups. Six hundred sixty genes were shown to be overlapping between the two lists of differentially expressed genes (Fig. 3.5b). The result indicates about half of the genes differentially expressed by CL administration is altered by the chronic overexpression of Mstn-pro.

Differentially expressed gene profile as affected by both Mstn-pro overexpression and CL administration

To examine the genes that are differentially expressed during muscle hypertrophy induced by both chronic Mstn suppression and CL administration, the profile of differentially expressed genes induced by Mstn-pro overexpression was compared to the profile of differentially expressed genes induced by CL administration. Forty genes (36 overexpressed genes and 4 underexpressed genes) were identified to be overlapping between the two models of muscle hypertophy (Fig. 3.5a). The 40 genes represent 37% of the differentially expressed genes induced by Mstn-pro overexpression and 3.2% of the differentially expressed genes induced by CL administration (Table 3.9).

3.4.5 Functional clustering of differentially expressed genes

To gain insight into the physiological relevance of the differential gene expression caused by Mstn-pro overexpression and/or CL administration, we functionally classified the differentially expressed genes with more than 1.5 fold change using the DAVID software that categorizes genes based on Gene Ontology (GO) biological process and identifies over-represented clusters.

Functional clustering of genes differentially expressed by the effect of Mstn-pro overexpression (Wild type mice vs Mstn-pro mice)

The 108 genes were categorized into overexpression of 27 clusters, and underexpresssion of 2 clusters (Table 3.11). Among these clusters, cell differentiation (24 genes), organ development (20 genes), transport (19 genes), system development (18 genes), neuron development (14 genes), immune response (14 genes) and cell cycle (12 genes) contained large number of overexpression genes. Underexpression functional clusters include lipid metabolic process (3 genes) and cell redox homeostasis (2 genes).

Functional clustering of genes differentially expressed by the effect of genotype under CL (CL treated wild type mice vs CL treated Mstn-pro mice)

The 96 genes were categorized into overexpression of 8 clusters and underexpression of 8 clusters (Table 3.11). Among these clusters, regulation of cellular process (24 genes), nucleic acid metabolic process (21 genes), transcription, DNA-dependent (18 genes), regulation of transcription (18 genes), regulation of gene expression (18 genes), regulation of cellular metabolic process (18 genes) and regulation of metabolic process (18 genes) contain large number of overexpression genes. Transport (23 genes) and protein localization (12 genes) contain large number of underexpression genes. The result indicates that functional clusters of differentially expressed genes caused by Mstn-pro overexpression are dramatically altered under CL administration.

Functional clustering of genes differentially expressed by the effect of CL (Wild type mice vs CL treated wild type mice)

The 1241 genes were categorized into overexpression of 36 clusters and underexpression of 5 clusters (Table 3.12). Among these clusters, cellular metabolic process (403 genes), metabolic process (275 genes), transport (222 genes), protein metabolic process (182 genes), organ development (156 genes), cellular component organization and biogenesis (110 genes), developmental process (107 genes), cellular localization (106 genes) and protein localization (87 genes) contain large number of overexpression genes. Underexpression functional clusters include electron transport (19 genes), reproductive structure development (5 genes), regulation of heart contraction (5 genes), menstrual cycle phase (4 genes) and menstrual cycle process (4 genes).

Functional clustering of genes differentially expressed by the effect of CL in Mstn-pro mice (Mstn-pro mice vs CL treated Mstn-pro mice)

The 1522 genes were categorized into overexpression of 26 clusters and underexpression of 9 clusters (Table 3.12). Among these clusters, cellular metabolic process (427 genes), metabolic process (258 genes), transport (188 genes), protein metabolic process (181 genes), organ development (119 genes), developmental process (103 genes), biological process (99 genes), cellular localization (87 genes), cell differentiation (84 genes) and protein localization (75 genes) contain large number of overexpression genes. Underexpression functional clusters include negative regulation of biological process (53 genes), negative regulation of cellular process (51 genes), electron transport (32 genes), regulation of catalytic activity (22 genes), hemopoietic or lymphoid organ development (17 genes), lipid transport (13), negative regulation of growth (6 genes), regulation of binding (5 genes) and opsonization (2 genes). The result indicates that the functional clusters of differentially expressed genes induced by CL administration were much greater than those induced by Mstn-suppression, reflecting the divese physiological functions regulated by β-adrenergic agonists.

Functional clustering of genes differentially expressed by both Mstn-pro overexpression and CL administration

The 40 genes were categorized into overexpression of 17 clusters (Table 3.13). Among these clusters, system development (11 genes), organ development (9 genes) and cell differentiation (8 genes) contain large number of overexpression genes.

3.4.6 Functional clustering of differentially expressed genes based on skeletal muscle growth

To gain insight into the relevance of the differential gene expression occurred during muscle hypertrophy in the two models to skeletal muscle growth, we functionally classified the differentially expressed gene based on carboxylic acid and amino acid metabolism, protein metabolic process, transcription, translation, muscle contraction, cell proliferation and cell cycle, cell differentiation, skeletal muscle development and signal transduction pathway using DAVID software. We assumed those functional clusters are key regulators involved in skeletal muscle growth.

Functional clustering of genes differentially expressed by the effect of Mstn-overexpression (Wild type mice vs Mstn-pro mice)

The 108 genes were categorized into carboxylic acid and amino acid metabolism (6 genes), protein metabolic process (12 genes), transcription (3 genes), cell proliferation and cell cycle (7 genes), cell differentiation (16 genes), skeletal muscle development (3 genes) and signal transduction (10 genes) (Table 3.14).

Functional clustering of genes differentially expressed by the effect of Mstn-overexpression under CL (CL treated wild type mice vs CL treated Mstn-pro mice)

The 96 genes were categorized into carboxylic acid and amino acid metabolism (6 genes), protein metabolic process (16 genes), transcription (12 genes), cell proliferation and cell cycle (19 genes), cell differentiation (5 genes) and signal transduction (15 genes) (Table 3.15).

Functional clustering of genes differentially expressed by the effect of CL (Wild type mice vs CL treated wild type mice)

The 1241 genes were categorized into carboxylic acid and amino acid metabolism (6 genes), protein metabolic process (50 genes), translation (22 genes), transcription (49 genes), cell proliferation and cell cycle (32 genes), cell differentiation (37 genes), skeletal muscle development (11 genes) and signal transduction (59 genes) (Table 3.16).

Functional clustering of genes differentially expressed by the effect of CL (Mstn-pro mice vs CL treated Mstn-pro mice)

The 1522 genes were categorized into muscle contraction (6 genes), carboxylic acid and amino acid metabolism (12 genes), protein metabolic process (53 genes), translation (20 genes), transcription (22 genes), cell proliferation and cell cycle (43 genes), cell differentiation (32 genes), skeletal muscle development (8 genes) and signal transduction (41 genes) (Table 3.17).

Functional clustering of genes differentially expressed by both Mstn-pro overexpression and CL administration

The 40 genes were categorized into carboxylic acid and amino acid metabolism (5 genes), protein metabolic process (3 genes), cell differentiation (3 genes) and skeletal muscle development (3 genes) (Table 3.18).

3.5 DISCUSSION

3.5.1 Effect of CL administration on skeletal muscle growth of Mstn-pro overexpression mice

The results of this study show that the muscle hypertrophic effects of CL is additive to the muscle hypertrophic effect of Mstn suppression in male mice. In our previous experiment (chapter 2) with female mice, we also observed that muscle hypertrophic effects of CL is additive to the muscle hypertrophic effect of Mstn suppression, indicating that the additive hypertrophic effect of CL administration is not affected by gender. Furthermore, the results suggest that during the early period of CL administration, CL may act synergistically to the hypertrophic effect of Mstn suppression since the increase in body and muscle weight gains of Mstn-pro mice by CL was significantly greater than that of wild type mice at 3-day CL administration, whereas the increase of body and muscle weight gains at 14-day CL administration was similar between the two genotypes. This result is in agreement with our previous female experiment (chapter 2), indicating that there is no gender influence on this synergistic effect.

In agreement with our previous female study (chapter 2), the current results also show that muscle DNA concentration of Mstn-pro mice is lower than that of wild-type mice, without a difference in RNA concentration, indicating that the increase in muscle mass in the Mstn-pro group was probably due to an increase in fiber size (hypertrophy) not by an increase in fiber number (hyperplasia). Welle et al. (2006) also reported that muscle DNA concentration was reduced in Mstn-deficient mice, whereas RNA concentration was not affected, and as a result, the ratio of RNA to DNA of Mstn-deficient mice was 30% greater than that of wild-type mice.

CL administration increased muscle RNA concentration of both genotypes at 3day, but the muscle RNA concentration was not affected by the CL administration at 14 days. The muscle DNA concentration of both genotypes was decreased by the CL administration during experimental period, leading to the higher RNA/DNA ratio in CL-treated mice. This result is consistent with our previous female experiment (chapter 2) and others, which showed that BAAs increases muscle RNA concentration and decrease muscle DNA concentration during the early period of administration (Kim and Sainz, 1992). The decrease in muscle DNA concentration by CL administration indicated that the increase in muscle mass in the CL administration was probably due to an increase in fiber size (hypertrophy) not by an increase in fiber number (hyperplasia). Taken together, the current results indicate that the myonuclear domain size was not maintained during the enhanced muscle hypertrophy induced by Mstn suppression or CL administration, thus suggest that satellite cell activation is probably not a primary factor for muscle hypertrophy induced by Mstn-suppression and CL administration.

3.5.2 Changes in the mTOR signaling pathway as affected by Mstn-pro

overexpression and CL administration

The mammalian target of rapamycin (mTOR) pathway is considered as a central regulator of ribosome biogenesis, protein synthesis, cell growth and proliferation by integrating signals from growth factors, nutrients, and energy status of cells (Asnaghi et al., 2004; Sarbassov et al., 2005). The mTOR activation by Akt induces the phosphorylation of two downstream effectors, ribosomal protein p70S6k and 4E-BP1, a suppressor of cap binding protein eIF4E, resulting in increased protein translation (Sarbassov et al., 2005). Studies have shown that the mTOR pathway plays an important role in regulating skeletal muscle growth. Anabolic stimuli, such as overloading, resistance exercise, and essential amino acid ingestion, have been shown to increase the phosphorylation of p70S6k and 4E-BP1 along with a corresponding increase in protein synthesis (Bodine et al., 2001; Dreyer et al., 2006; Fujita et al., 2007; Dreyer et al., 2008; Drummond et al., 2008). Conversely, atrophying muscles have decreased levels of the phosphorylation of the downstream targets of mTOR (Bodine et al., 2001; Hornberger et al., 2001). Supporting the role of the mTOR pathway on skeletal muscle growth, p70S6k null mice have a smaller muscle fiber size and the hypertrophic response to IGF-1 was blocked in p70S6k null mice (Ohanna et al., 2005). Recent results have indicated that the mTOR pathway is also involved in the regulation of muscle mass induced by Mstn (Amirouche et al., 2009; Sartori et al., 2009) or BAAs (Sneddon et al., 2001; Kline et al., 2007).

As was observed in our previous experiment with female mice (chapter 2), we also observed that the levels of phosphorylated 4E-BP1 at Thr$^{37/46}$ and p70S6k at Thr^{389} were greater in Mstn-pro male mice compared with wild type male mice. In addition, we also observed that the levels of phosphorylated Akt at Thr^{308} were greater in Mstn-pro mice compared with wild type mice, suggesting that the increased phosphorylations of 4E-BP1 and p70S6k observed in Mstn-pro mice were probably through the Akt/mTOR pathway. This result is consistent with a previous study, which showed that a decrease in the phosphorylation of both 4E-BP1 at Thr$^{37/46}$ and p70S6k at Ser$^{235/236}$ along with a decrease in the phosphorylation of Akt at Thr^{308} in Mstn overexpression mice (Amirouche et al., 2009), and supports that Akt/mTOR pathway is involved in the regulation of muscle mass by Mstn. Interestingly, Welle et al. (2009) observed an increase in the phosphorylation of p70S6k at Thr^{389}, but not of 4E-BP1 at Thr$^{36/45}$, Ser^{64}, orThr^{69}, nor Akt at Thr^{308} or Ser^{473} in anti-Mstn antibody administration mice. At the same time, it was also noted that rapamycin did not suppress the effect of Mstn suppression on increasing myofiber size or the rate of muscle protein synthesis (Sartori et al., 2009; Welle et al., 2009), suggesting that Mstn regulation of muscle protein synthesis can be independent of Akt/mTOR pathway signaling or other yet unidentified components are involved in mTOR phosphorylation of 4E-BP1 and p70S6k.

Like in our previous female study (chapter 2), we also observed that the levels of phosphorylated 4E-BP1 at Thr$^{37/46}$ and p70S6k at Thr^{389} were also significantly increased by CL administration regardless of genotype. In addition, we also observed that the levels of phosphorylated Akt at Thr^{308} were significantly increased by CL administration regardless of genotype, suggesting that like Mstn, BAA is also regulate muscle hypertrophy probably through the Akt/mTOR pathway. In support of our results, Senddon et al. (2001) demonstrated that CL treatment induced protein accretion in mice via stimulation of protein translation through the phosphorylation of p70S6k at Thr^{412} and 4E-BP1 at Thr^{70}. It is also noted that CL administration induced dramatic increase in skeletal muscle mass through the Akt phosphorylation and subsequent activation of the mTOR, supporting the role of the Akt/mTOR pathway in BAA-induced skeletal muscle hypertrophy (Kline et al., 2007). Notably, the increase in levels of phosphorylated Akt, 4E-BP1 and p70S6k induced by CL administration was less at 14 days CL administration (Akt, 26.1% for wild type and 26% for Mstn-pro; 4E-BP1, 22.1% for wild type and 16.3% for Mstn-pro; p70S6k, 23.9% for wild type and 15.4% for Mstn-pro) than at 3 days CL administration (Akt, 29.7% for wild type and 31.1% for Mstn-pro; 4E-BP1, 24.2% for wild type and 39.8% for Mstn-pro; p70S6k, 33% for wild type and 37% for Mstn-pro) in both genotypes, reflecting the temporal responsiveness of muscle hypertrophy to BAAs.

3.5.3 Differential gene expression during muscle hypertrophy induced by Mstn suppression or clenbuterol administration

Differential gene expression induced by chronic Mstn-pro suppression

There have been studies using microarray to investigate the global gene expression in skeletal muscle hypertrophy induced by Mstn inhibition (Steelman et al., 2006; Sadkowski et al., 2008; Welle et al., 2009b). Steelman et al. (2006) reported that 717 genes were differentially expressed ($p < 0.05$, 1.5 fold change) in pectoral muscles between wild type and Mstn null mice. Welle et al. (2009) reported that 124 genes ($p < 0.01$, 1.5 fold change) were differentially expressed in gastrocnemius muscles between wild type and Mstn null mice. Our current results showed that 108 genes ($p < 0.01$, 1.5 fold change) were differentially expressed in gastrocnemius muscle by chronic suppression of Mstn.

Functional analysis revealed that the differentially expressed genes represent a wide variety of biological processes, including cell differentiation, organ development, transport, system development, neuron development, immune response, cell cycle, lipid metabolic process, and cell redox homeostasis, indicating that a wide variety of biological processes are altered during muscle hypertohphy induced by Mstn-pro overexpresseion. However, the limitation of microarray analysis is that it is not possible to tell whether the alteration of the biological process was due to a direct effect of the modification of Mstn signaling or due to indirect consequences of muscle hypertrophy induced by Mstn-pro overexpression. Therefore, we further focused on gene clusters which we assume to be involved in skeletal muscle development, and these include carboxylic acid and amino acid metabolism, protein metabolic process, transcription, cell proliferation and cell cycle, cell differentiation, skeletal muscle development and signal transduction.

We have also included a more detaile table (Table 3.19 and 3.20), which compares the microarray information in this study to previously published studies on chronic Mstn-suppression-induced hypertrophy.

Genes encoding muscle contractile proteins

Steelman et al. (2006) have reported a decreased expression of genes for slow isoforms of myosin (Myh7 [slowest myosin heavy chain isoform, type I], Myl2, Myl3) and troponin (Tnnc1, Tnni1, Tnnt1, Tpm3) in constitutive Mstn null mice as compared to wild type mice. In bovine late fetus, Cassar-Malek et al. (2007) reported that constitutive non-functional mutation of Mstn decreased the expression of slow forms of contractile proteins (Myh7, Myl2, Tpm3). Sadkowski et al. (2008) also observed that there was increased expression of genes for fast isoforms of myosin (Myh3) in skeletal muscle carrying a SNP in the 5'flanking region of the Mstn gene in bulls, while slow isoforms of myosin (Myh8 [myosin heavy chain neonatial isoform 2], Myo5a, Myl6) were decreased. However, in this study, we could not observe any alteration in gene expression ecoding myofibrillar in Mstn-pro mice except the differential expression of troponin T2 (Tnnt2, 3.5 fold), which is mostly expressed during embryonic development. Similar to our results, Welle et al. (2009) reported that the expression of myofibrillar genes was not significantly altered in mice with post-developmental Mstn knockout. The above inconsistency appears to be related to differences in the developmental stages of Mstn-suppression. While Steelman et al. (2006) and Cassar-Malek et al. (2007) used constitutive Mstn-null animal models, the current study and Welle et al. (2009) used postnatal Mstn-suppression models. The suppression of Mstn from embryonic development (constitutive Mstn knockout models) induces greater development of fast, glycolytic fiber types at the expense of slow, oxidative fibers (Girgenrath et al. 2005), explaining the decreased expression of slow isoforms of myofibrillar contractile proteins in constitutive Mstn-null animal modles. Unlike the constitutive Mstn-knockout nodels, the muscle hypertrophy induce by postnatal Mstn-suppression is much less slective on muscle fiber types, thus less effect on the expression of genes encoding isoforms of myofibrillar proteins.

Genes encoding proteins involved in transcription

In this study, we observed that the 3 transcription factor genes were differentially expressed by Mstn-pro overexpression, including runt related transcription factor 1 (Runx1, 2.2 fold), DNA methyltransferase (Dnmt1, 1.6 fold) and sterol regulatory element binding transcription factor 1 (Sreb1, -1.5 fold). However, studies by other groups did not observe that these genes were differentially regulated by Mstn modulation (Steelman et al., 2006; Welle et al., 2009). Zhu et al. (1994) reported that a 50-100 fold upregulation in runx1 expression was observed after 5 days of denervation *in vivo*. Similarly, Wang et al. (2005) also reported that a 73 fold upregulation in runx1 expression was observed after 7 days of denervation using micrarray analysis in mice muscle. It has been shown that this up-regulation is an attempt to prevent the autophagy and to minimize muscle wasting. The Sreb1 gene is a transcription factor involved in adipocyte differentiation (Briggs et al., 1993) as well as in the biosynthesis of cholesterol and fatty acids (Brown and Goldstein, 1997). Shimano et al. (1999) reported that the Sreb1 gene knockout mice showed the low levels of all lipogenic enzymes and severely impaired the marked induction of hepatic mRNAs of fatty acid synthetic genes, such as acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase, indicating that Sreb1 plays a crucial role in the induction of lipogenesis. Taken together, it is postulated that the muscle hypertrophy induced by Mstn-suppression is through the alteration of Runx1 and Sreb1.

Genes encoding proteins involved in immune response

In the present study, we observed that metallothionein 3 (Mt3) was differentially overexpressed, and many genes involved in immune response were overrepresented. The expression of Mt3 is enhanced by heavy metals, cytokines, glucocorticoids, oxidative stress, and other stresses, and under some conditions, Mt3 may protect cells against DNA damage from apoptosis (Davis and Cousins, 2000; Coyle et al., 2002). It is not clear how the overexpression of Mt3 and genes involved in immune response are related to muscle hypertrophy induced by Mstn-pro overexpression. Mendias et al. (2008) and Eliasson et al. (2009) recently demonstrated that Mstn-deficient rodents have abnormally large muscles, but also small, stiff and brittle tendons with a higher peak stress and a lower peak strain during stretch, indicating a potential for high-strain muscle damage during excessive muscle contraction. If Mstn suppression indeed cuases stiff and brittle tendons, it is speculated that Mstn-pro mice is highly susceptible to muscle damage due to a weakness in muscle tendons, leading to enhanced immune responses during repair of the damaged skeletal muscles.

Genes encoding proteins involved in muscle hypertrophic signal pathway

In this study, we observed that insulin-like growth factor 2 (Igf2, 2.1 fold) was differentially expressed in the skeletal muscle of Mstn-pro mice even though previous studies (Steelman et al., 2006; Cassar-Malek et al., 2007; Welle et al., 2009) have not observed that Igf2 is regulated by Mstn. Like Igf1, Igf2 is known to regulate muscle hypertrophy. For example, Miyake et al. (2010) demonstrated that IGF-2 mRNA expression was higher in the regenerating muscle of double-muscled cattle than those of normal cattle. An addition of recombinant mouse Mstn to myoblast cultures attenuated IGF-2 mRNA expression and decreased myotube formation, but not IGF-1 mRNA expression, indicating that Mstn may negatively regulate myoblast differentiation by suppressing IGF-2 expression. Recently, several studies indicated that Mstn inhibits muscle hypertrophy in part through inhibition of protein synthesis mediated by the Akt/mTOR pathway (Welle et al., 2006; Amirouche et al., 2009; Morissette et al., 2009; Welle et al., 2009a). Igf2 appears to activate the Akt/mTOR pathway since administration of Igf2 to human chondrocytes increased the phosphorylation levels of Akt and GSK3β (Hamamura et al., 2008). Taken together, it is postulated that Igf2 is a target for Mstn signaling, and the muscle hypertrophy induced by Mstn-suppression is through the Igf2-induced activation of the Akt/mTOR anabolic pathway.

Differential gene expression induced by CL administration

Several studies have previously used microarray technology to examine skeletal muscle gene expression following chronic BAAs administration (Spurlock et al., 2006;

Pearen et al., 2009). For example, Spurlock et al. (2006) reported that 52 genes were differentially expressed (FDR < 10%) in mouse skeletal muscle 24 hours and 10 days after administration of CL. Pearson et al. (2009) reported that 23 genes at one hour and 112 genes at four hours were significantly ($p < 0.05$, 1.85 fold change) altered in mice after systemic administration of formoterol. Our current results showed that 1241 genes ($p < 0.01$, 1.5 fold change) were differentially expressed in gastrocnemius muscle by chronic administration of CL.

Functional analysis revealed that the differentially expressed genes represent a wide variety of biological processes, including cellular metabolic process, metabolic process, transport, protein metabolic process, organ development, cellular component organization and biogenesis, developmental process, cellular localization, protein localization, electron transport, reproductive structure development, regulation of heart contraction, menstrual cycle phase, and menstrual cycle process, indicating that a wide variety of biological processes are altered during muscle hypertrophy induced by CL administration. However, the limitation of microarray analysis is that it is not possible to tell whether the alteration of the biological process was due to a direct effect of the modification of CL signaling or due to indirect consequences of muscle hypertrophy induced by CL administration. Therefore, we further focused on gene clusters which we assume to be involved in skeletal muscle development, and these include carboxylic acid and amino acid metabolism, protein metabolic process, transcription, translation, cell proliferation and cell cycle, cell differentiation, skeletal muscle development and signal transduction.

We have also included a more detaile table (Table 3.21 and 3.22), which compares the microarray information in this study to previously published studies on

BAA-induced hypertrophy.

Genes encoding muscle contractile proteins

Previous studies have demonstrated that BAAs increase the expression of several contractile proteins (Oishi et al., 2002; Spurlock et al., 2006). For example, Oishi et al. (2002) have reported that CL administration resulted in muscle fiber hypertrophy, stimulated a de novo expression of type IIx MHC and increased the percentage of fibers containing multiple MHC isoforms in the rat soleus muscle. Spurlock et al. (2006) have reported that muscle contractile proteins, including myosin isoforms (Myo1b, Myo18a) and actin isoform (Actn4), were altered in mouse skeletal muscle at 24 hours and 10 days after administration of CL. In contrast, Pearen et al. (2009) demonstrated that no significant changes were observed in muscle contractile proteins at 1 and 4 hours after administration formoterol in mice muscle. However, they found that alterations in Stat3, Smad 3, and Acvr2b three genes directly associated with the regulation of muscle hypertophy at molecular level. In this study, we showed that myosin heavy polypeptide 8 (Myh8, 5 fold), troponin T2 (Tnnt2, 1.9 fold), myosin light polypeptide 2 (Myl2, -1.8 fold) and myosin light polypeptide kinase 2 (Mylk2, -1.7 fold) were altered by CL administration, which are myofibrillar proteins mostly expressed during embryonic development. Thus, it is reasonable to hypothesize that in the current study the altered expression of Myh8, Tmnt2, Myl2, and Mylk2 lead to an increase in structural proteins which are associated with muscular hypertrophy. Similar to Pearen et al. (2009) study, we also found the differentially expressed activin receptor IIB (Acvr2b, -2.4 fold) gene, which is a well-characterized negative mediator of downstream of Mstn on muscle growth. The dominant-negative form of Acvr2b by using a skeletal muscle-specific promoter exhibited dramatic increase skeletal muscle mass in mice (Lee and McPherron, 2001). Given that this result could provide a partial mechanistic basis for muscle hypertrophy and the underexpression of Acvr2b would be another target for CL signaling.

Genes encoding proteins involved in translation and transcription

Previous studies have used microarray technology to examine skeletal muscle gene expression associated with translation and transcription following CL administration. For example, Spurlock et al. (2006) reported that the increase expressions of 13 genes including eukaryotic initiation factors (Eif2, Eif2b, Eif2b4, Eif2b2, Eif5, Eif4g3), and 18 genes including protein transcription factors (Hod, Yy1, Mef2c, and Myog) were observed mainly at 24 h after CL administration. Pearen et al. (2009) reported that the increase expressions of 11 genes including transcriptional regulators (Fos, Klf2/4, Crem, Cebpb, Nurr1, Fosl2, Maff, Aft3, Tbx3, and Litaf) were observed at 1 and 4 hours by CL administration in mice muscle, but no significant changes were observed in translation proteins. In this study, we observed a regulation of translational and transcriptional factors responsible for increased protein synthesis. The 22 translation genes, including 40S ribosomal protein S20 pseudogene (Gm6607, 2.5 fold), ribosomal protein L3 (Rpl3, 2.4 fold), ribosomal protein L13A (Rpl13a, 2.2 fold), ribosomal protein L23 (Rpl23, 1.8 fold), eukaryotic translation initiation factor 2b (Eif2b, 1.5 fold), eukaryotic translation initiation factor 1 (Eif1, 2.0 fold), eukaryotic translation initiation factor 4a1 (Eif4a1, 1.9 fold), eukaryotic translation initiation factor 4e (Eif4e, 1.9 fold), and 48 transcription genes, including three genes with known functions in muscle growth and development: myogenin (Myog, 8.6 fold), myogenic differentiation 1 (Myod1, 2.7 fold), myocyte enhancer factor 2A (Mef2a, 2.0 fold), were differentially expressed by CL administration. Although, the four ribosomal proteins

(Rpl3, Rpl13a, Rpl23, and Gm6607) are differentially expressed by CL administration, the functions of ribosomal proteins are largely unknown. Similar to Spurlock et al. (2006) study, we also observed the differentially expressed Eif2b gene by CL administration. The function of Eif2b is that a new cycle of initiation of translation requires Eif2b to catalyze the exchange of eukaryotic initiation factor 2 (Eif2)-bound GDP for GTP, which is an important step in the regulation of translation initiation (Hershey, 1991; Proud, 2004). Therefore, the increased abundance of Eif2b by CL administration may contiribute to increased activity of Eif2 and the global upregulation of protein synthesis in skeletal muscle. In addition, eukaryotic translation initiation factor Eif1, Eif4a1, and Eif4e also increased after CL administration. These genes are the most abundant in translation initiation factors, involved in the initation of eukaryotic translation (Fletcher et al., 1999; Rogers et al., 2002). Furthermore, we also observed differentially expressed transcription factors (Myog, Myod1, Mef2a) by CL administration. The Myogenin, Myod1, and Mef2a are well characterized transcription factors known to be essential for myoblast differentiation (Ridgeway et al., 2000; Ma et al., 2005). Although significant alteration of satellite cell differentiation and recruitment into muscle fibers in the effect of CL administration has not been described, the observed increase in mRNA of transcription factors that contribute to muscle cell differentiation suggest the recruitment and differentiation of pre-myogenic cells may be involved in the physiological response of skeletal muscle to CL administration. Taken together, these current results could provide a general upregulation of translational and transcriptional machinery occurring after the administration of CL.

Genes encoding proteins involved in muscle hypertrophic signal pathway

Spurlock et al. (2006) have reported that insulin-like growth factor1 (Igf1) and

TSC22 domain family member1 (Tss22d1) were altered in mouse skeletal muscle 24 hours after administration of CL. Pearen et al. (2009) have reported that forkhead box O1 (FoxO1) was altered in mice after systemic administration of formorterol. In this study, we observed that eukaryotic translation initiation factor 4E (eIF4E, 1.9 fold), forkhead box (FoxO, -2.0 fold) and PTEN (-1.5 fold) were differentially expressed in the muscle hypertrophy induced by CL administration even though previous studies (Spurlock et al., 2006; Pearen et al., 2009) have not observed that the alteration of eIF4E and PTEN are regulated by BAA administration. The eIF4E is the interacting protein of the eukaryotic initiation factor (eIF) 4E binding proteins (4E-BPs), indicating that the overexpressed gene induced the increase of protein translation. In other words, the eIF4E binds to eIF4G, a scaffolding protein required for formation of the eIF4F complex and the form of eIF4F complex involved in a critical protein synthesis (Lawrence and Abraham, 1997). The differentially underexpressed PTEN and FoxO genes also might be related to the skeletal muscle hypertrophy. For example, the PI3k dependent Akt activation can be regulated through the tumor suppressor PTEN. The PTEN acts as a tumor suppressor gene through the action of its phosphatase protein product (Chu and Tarnawski, 2004). The PTEN negatively regulates intracellular levels of PIP3 in cells and functions as a tumor suppressor by negatively regulating PI3k/Akt signaling pathway (Sun et al., 1999). Also, the activation of Akt inhibits FoxO expression, which acts on the atrogin-1 promoter to cause atrogin-1 transcription and dramatic atrophy of myotubes and muscle fibers. Thus, FoxO play a critical role in the development of muscle atrophy, and inhibition of FoxO factors is an attractive approach to prevent muscle atrophy (Sandri et al., 2004). Taken together, it is postulated that eIF4E, FoxO and PTEN are targets for CL signaling, and the muscle hypertrophy incuded by CL administration is through the alteration of eIF4E, FoxO and PTEN-induced activation of the Akt/mTOR anabolic pathway.

Differential expression overelapping genes induced by both chronic Mstn-pro suppression and CL administration

Between the differential genes expressed by Mstn-pro and CL administration, we found forty genes which overlapped. We believe these forty genes, including Ahnak2, Anxa4, Blnk, Cgref1, Chrna1, Col19a1, Cyb5r3, D11Wsu99e, Ddc, Gatm, Hmox1, Hn1, Ift122, Kif5c, Lgals3, Lgmn, Obfc2a, Pak1, Pdia3, Psat1, Rab27b, Rnh1, Rtn4, Runx1, Slc25a24, Sln, Snx5, Stbd1, Tnnt2, Tspo, Ttll1, Tubb2a, Tubb6, Vash2, Txnl1, Rps6ka5 and Acot6, might be common targets in the skeletal muscle hypertrophy induced by Mstn-pro and CL administration. However, the full mechanisms of forty genes are not previously reported to be elated to skeletal muscle hypertrophy, suggesting that further studies will be needed to examine these genes expression change for their biological relevance in skeletal muscle hypertrophy.

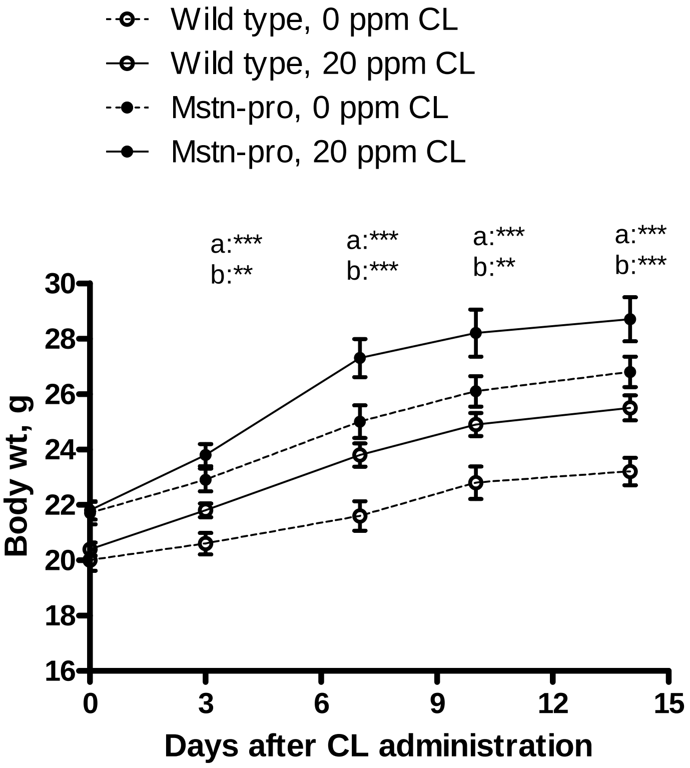
3.6 CONCLUSIONS

The results of this study demonstrate that the muscle hypertrophic effect of Mstn suppression and CL administration led to significant increase in body and muscle weight gain. The skeletal muscle hypertrophic effect of CL was additive to the hypertrophic effect of Mstn suppression. The pathways of Mstn and CL signaling appears to converge to the Akt/mTOR anabolic pathway to regulate skeletal muscle hypertrophy. Global evaluation of gene expression after CL administration and Mstn suppression by microarray analysis showed that genes involved in many biological processes are differentially expressed by the Mstn supperession and CL administration. Muscle contraction, initiation of translation, transcription, and muscle hypertrophic signal pathway were represnted among those biological processes, suggesting that increased porotein syntheis is partly responsible for the hypertophy induced by Mstn suppression and CL administration. Alterations of the mRNA level of Igf2 observed in Mstn suppressed mice and the mRNA levels of eIF4e, Acvr2b, FoxO and PTEN observed in mice treated with CL indicate that the pathways of Mstn and CL signaling converge to the Akt/mTOR anabolic pathway to regulate skeletal muscle hypertrophy.

Future study

Microarray analysis in this study revealed that numerous genes involved in various biological processes were differentially expressed by Mstn suppression and CL administration, providing a comprehensive picture of changes in gene expression during muscle hypertophy induced by the two stimuli. However, it is not possible to tell what genes are directly regulated by the stimuli or what genes are indirectly regulated as the consequence of the muscle hypertrophy. At the same time, muscle tissue contains various cell types from blood vessels, nerve fibers and cellular matrix in addition to muscle fiber, thus it is expected that some changes in gene expression observed in this study were contributed from other cell types. Therefore, further studies need to sort out among those differentially expressed genes, which genes are directly involved in the mechanisms regulating muscle hypertrophy.

A)



B)

Weight gain							
	Wild type		Mstn-pro		Significance		
Days	0 ppm	20 ppm	0 ppm	20 ppm	GT	CL	GTxCL
0-3	0.6 (0.13)	1.4 (0.13)	1.3 (0.19)	2.1 (0.16)	***	***	NS
3-7	0.7 (0.14)	1.8 (0.06)	1.2 (0.21)	2.5 (0.29)	***	***	NS
7-10	1.2 (0.17)	1.5 (0.13)	1.5 (0.11)	1.5 (0.26)	NS	NS	NS
10-14	0.4 (0.18)	0.7 (0.15)	0.7 (0.13)	0.3 (0.21)	NS	NS	NS

Figure 3.1 Effect of CL administration on body weights of wild type and Mstn-pro mice. Solid and dotted lines indicate 20 and 0 ppm CL administration, respectively, to wild type (open circle) and Mstn-pro mice (closed circle). Values are means (SEM). (A): Growth curve of male mice during 14 days (a, difference between wild type and Mstn-pro; b, difference between 0 ppm and 20 ppm CL administration (***, $P < 0.001$, **, $P < 0.01$)). (B): Body weight gain of male mice during 14 days (***, $P < 0.001$, NS, not significance). CL, clenbuterol; GT, genotype.

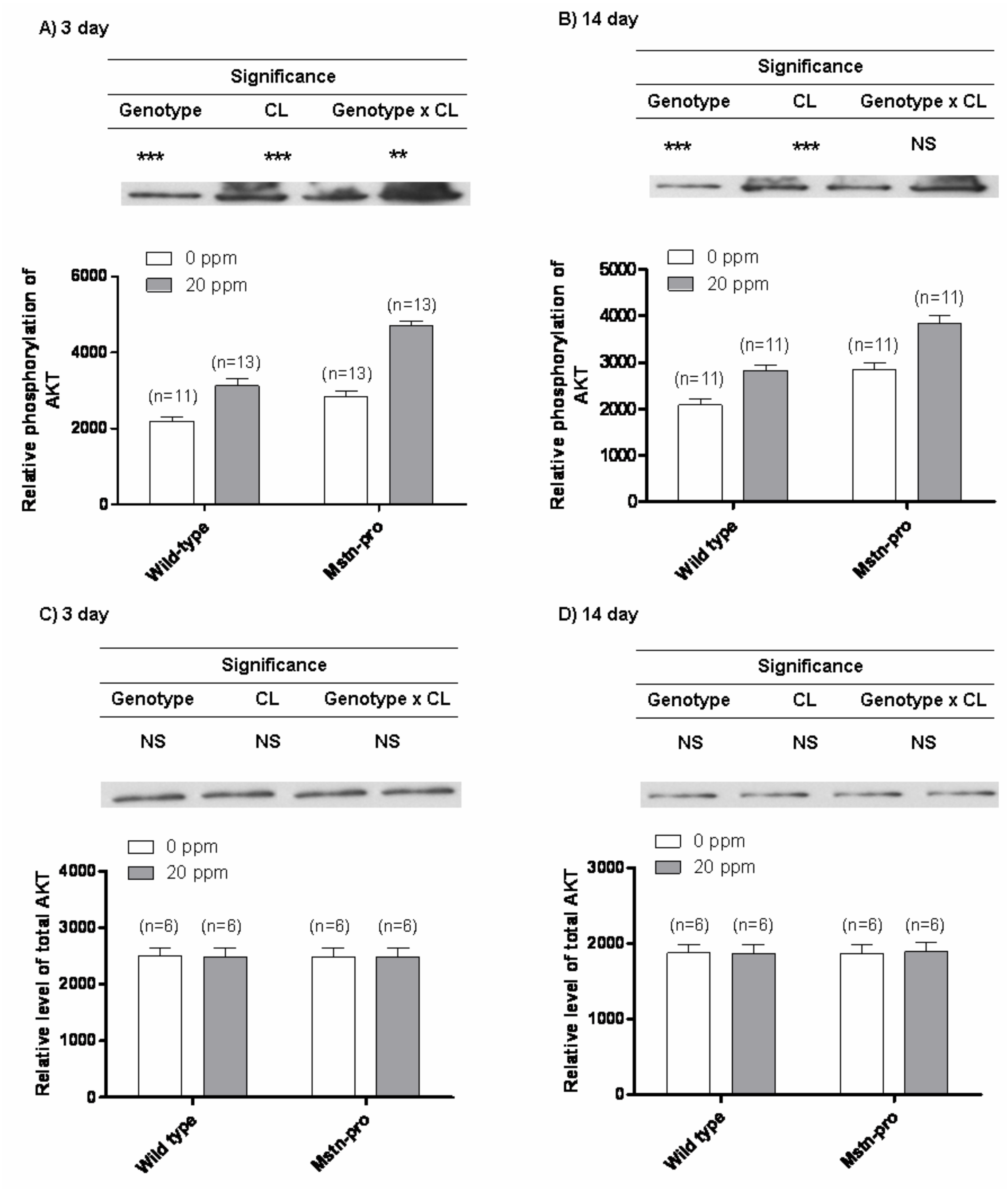


Figure 3.2 Effect of CL administration on AKT at Thr^{308} phosphoryalation in extensor digitorum longus muscles of wild type and Mstn-pro mice. Representative immunoblot images are shown above the bar graph. Each lane sequentially represents the group in the bar graph. A): 3day phosphorylation levels of Akt. B): 14day phosphorylation levels of Akt. C): 3day non-phosphorylation levels of Akt. D): 14day non-phosphorylation levels of Akt. Values are expressed as mean ±SEM. ***, P<0.001; **, P<0.01; NS, no significance.

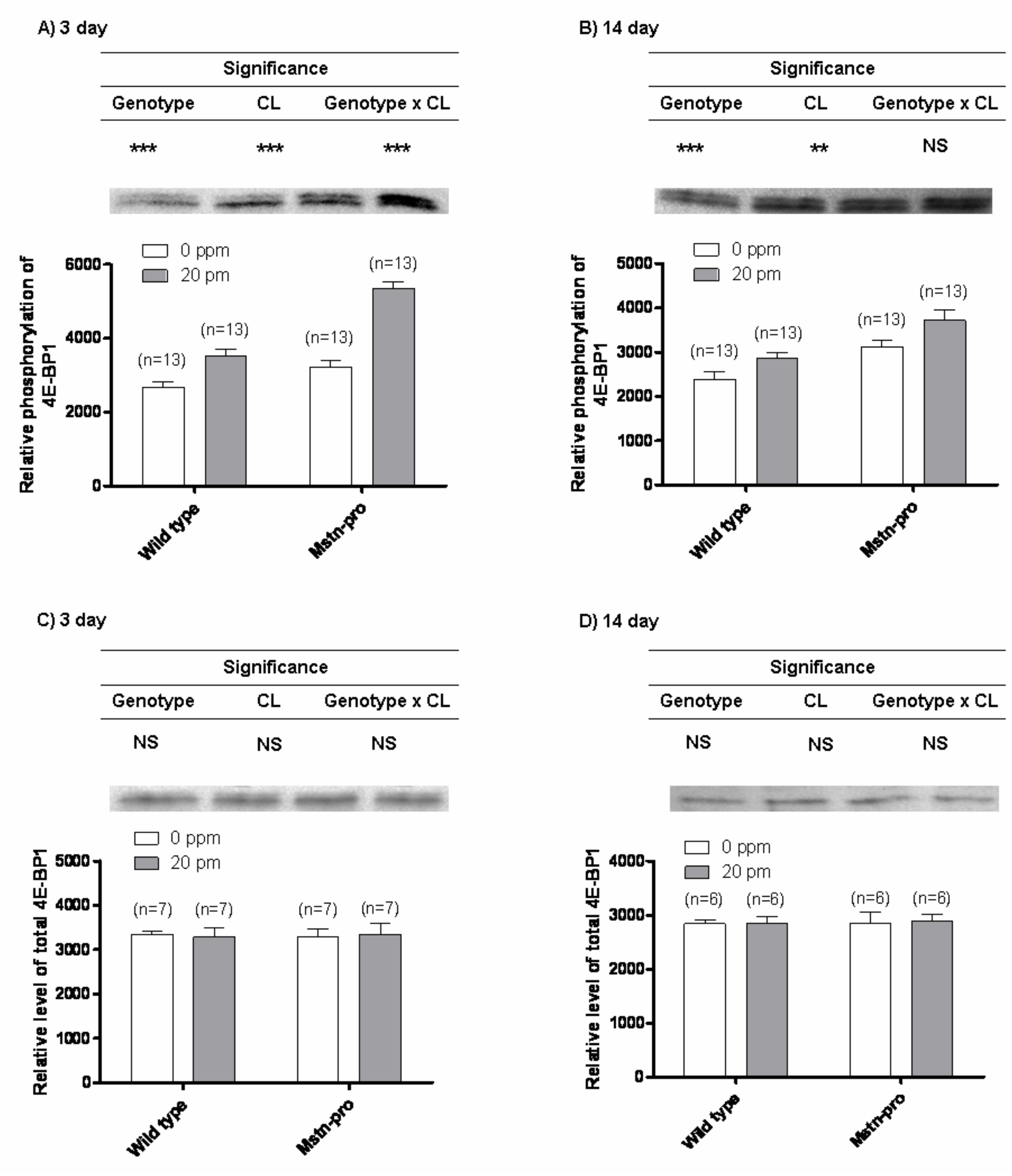


Figure 3.3 Effect of CL administration on 4E-BP1 at Thr$^{37/46}$ phosphoryalation in extensor digitorum longus muscles of wild type and Mstn-pro mice. Representative immunoblot images are shown above the bar graph. Each lane sequentially represents the group in the bar graph. A): 3day phosphorylation levels of 4E-BP1. B): 14day phosphorylation levels of 4E-BP1. C): 3day non-phosphorylation levels of 4E-BP1. D): 14day non-phosphorylation levels of 4E-BP1. Values are expressed as mean ±SEM. ***, P<0.001; **, P<0.01; NS, no significance.

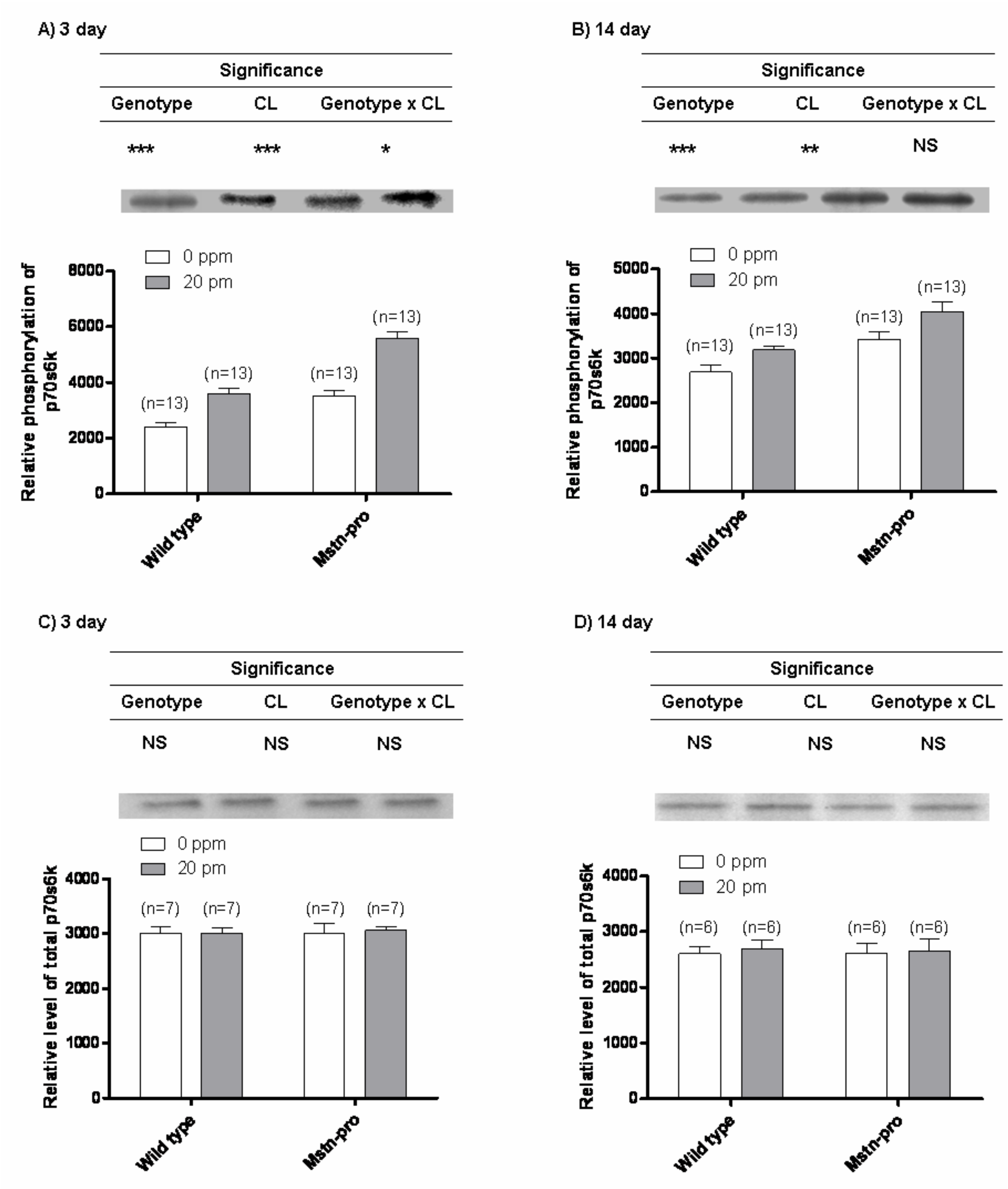


Figure 3.4 Effect of CL administration on p70S6k phosphoryalation at Thr^{389} in extensor digitorum longus muscles of wild type and Mstn-pro mice. Representative immunoblot images are shown above the bar graph. Each lane sequentially represents the group in the bar graph. A): 3day phosphorylation levels of p70S6k. B): 14day phosphorylation levels of p70S6k. C): 3day non-phosphorylation levels of p70S6k. D): 14day non-phosphorylation levels of p70S6k. Values are expressed as mean ±SEM. ***, P<0.001; **, P<0.01; *, P<0.05; NS, no significance.

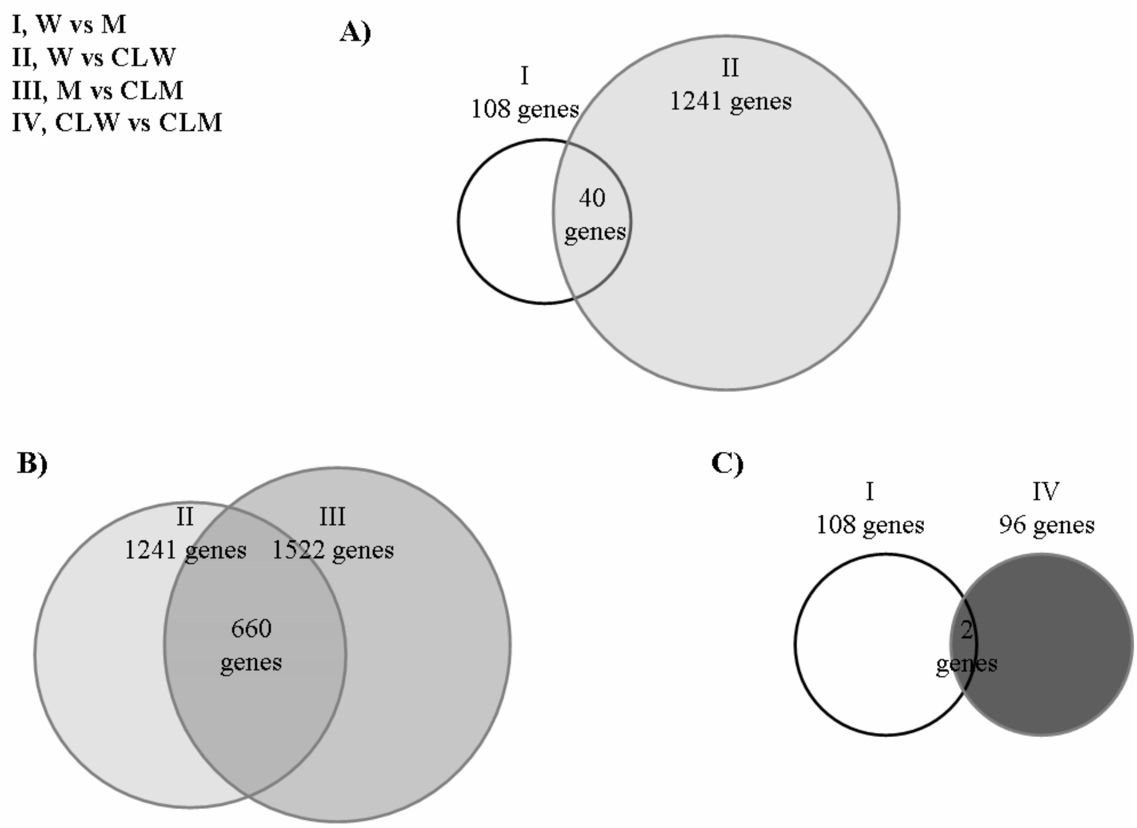


Figure 3.5 Comparison of genes that were differentially expressed by Mstn-pro overexpression and/or CL administration. A) Comparison of differentially expressed genes induced by Mstn-pro overexpression with those induced by CL administration B) comparison of differentially expressed genes induced by CL administration under the influence of Mstn-pro overexpression, C) comparison of differentially expressed genes induced by Mstn-pro overexpression under the influence of CL treatment. W, wild type mice; M, Mstn-pro mice; CLW, clenbuterol treated wild mice; CLM, clenbuterol treated Mstn-pro mice.

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Table 3.1 Muscle weights of wild type and Mstn-pro mice as affected by CL administration for 3 days.

	Wild type		Mstn-pro		Significance		
	0 ppm	20 ppm	0 ppm	20 ppm	GT	CL	GT x CL
Number of animals	11	25	15	19			
Initial body Wt, g	19.9 (0.63)	20.6 (0.31)	21.6 (0.56)	21.8 (0.37)	**	NS	NS
Final body Wt, g	20.2 (0.58)	22.0 (0.31)	22.5 (0.50)	23.5 (0.39)	***	**	NS
Gastrocnemius Wt, mg	99.0 (6.08)	115.55 (3.45)	131.4 (5.55)	158.8 (5.09)	***	***	NS
% Gastrocnemius[1]	0.49 (0.018)	0.52 (0.011)	0.58 (0.018)	0.67 (0.014)	***	***	NS
Plantaris Wt, mg	11.5 (0.76)	14.9 (0.55)	15.1 (0.65)	20.2 (0.96)	***	***	NS
% Plantaris[1]	0.056 (0.0026)	0.067 (0.0020)	0.067 (0.0024)	0.085 (0.0031)	***	***	NS
EDL[2] Wt, mg	7.4 (0.35)	9.7 (0.33)	10.3 (0.23)	12.9 (0.35)	***	***	NS
%EDL[1]	0.037 (0.0011)	0.044 (0.0011)	0.046 (0.0010)	0.055 (0.0011)	***	***	NS
Soleus Wt, mg	5.4 (0.37)	6.1 (0.24)	5.3 (0.36)	6.4 (0.37)	NS	*	NS
% Soleus[1]	0.026 (0.0013)	0.028 (0.0010)	0.023 (0.0014)	0.027 (0.0014)	NS	NS	NS
Muscle protein, %[#]	23.1 (0.77)	26.9 (0.28)	27.4 (0.99)	29.2 (0.43)	***	***	NS
Soluble muscle protein, %[+]	2.2 (0.02)	2.2 (0.02)	2.2 (0.01)	2.2 (0.01)	NS	NS	NS

Values are means (SEM). CL, clenbuterol; GT, genotype; EDL, extensor digitorum longus; NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. [1]% weight of muscle to body weight. [2]EDL: extensor digitorum longus muscle. [#]Total muscle protein concentration was measured in plantaris muscle using the modified Lowry method. [+] The EDL muscle was homogenized in 9 volumes of ice-cold lysis buffer, centrifuged at 6,000 g for 10 min at 4°C, and supernatant protein concentration was measured using the modified Lowry method.

Table 3.2 Muscle weights of wild type and Mstn-pro mice as affected by CL administration for 14 days.

	Wild type		**Mstn-pro**		**Significance**		
	0 ppm	**20 ppm**	**0 ppm**	**20 ppm**	**GT**	**CL**	**GT x CL**
Number of animals	13	18	13	14			
Initial body Wt, g	20.1 (0.47)	20.1 (0.34)	21.8 (0.60)	21.9 (0.62)	***	NS	NS
Final body Wt, g	23.2 (0.50)	25.5 (0.45)	26.8 (0.55)	28.7 (0.80)	***	***	NS
Gastrocnemius Wt, mg	121.4 (5.28)	138.1 (2.30)	175.7 (5.40)	199.8 (6.10)	***	***	NS
% Gastrocnemius[1]	0.52 (0.014)	0.54 (0.005)	0.66 (0.015)	0.70 (0.010)	***	**	NS
Plantaris Wt, mg	13.5 (0.68)	17.1 (0.64)	20.3 (0.91)	25.7 (1.22)	***	***	NS
% Plantaris[1]	0.058 (0.0020)	0.067 (0.0024)	0.076 (0.0026)	0.089 (0.0021)	***	***	NS
EDL[2] Wt, mg	8.7 (0.51)	10.8 (0.43)	12.8 (0.43)	15.1 (0.49)	***	***	NS
%EDL[1]	0.037 (0.0017)	0.042 (0.0013)	0.048 (0.0014)	0.053 (0.0010)	***	***	NS
Soleus Wt, mg	5.3 (0.32)	8.1 (0.28)	6.6 (0.21)	8.4 (0.46)	*	***	NS
% Soleus[1]	0.023 (0.0012)	0.031 (0.0009)	0.025 (0.0008)	0.029 (0.0015)	NS	***	*
Muscle protein, %[#]	21.5 (0.32)	25.4 (0.39)	25.5 (0.39)	28.0 (0.34)	***	***	NS
Soluble muscle protein, %[+]	2.0 (0.01)	2.0 (0.01)	2.0 (0.01)	2.0 (0.01)	NS	NS	NS

Values are means (SEM). CL, clenbuterol; GT, genotype; EDL, extensor digitorum longus; NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. [1]% weight of muscle to body weight. [2]EDL: extensor digitorum longus muscle. [#] Total muscle protein concentration was measured in plantaris muscle using the modified Lowry method. [+] The EDL muscle was homogenized in 9 volumes of ice-cold lysis buffer, centrifuged at 6,000 g for 10 min at 4°C, and supernatant protein concentration was measured using the modified Lowry method.

Table 3.3 Muscle DNA and RNA concentrations in wild-type and MSTN-pro mice as affected by CL administration.

	Wild type		Mstn-pro		Significance		
	0 ppm	20 ppm	0 ppm	20 ppm	GT	CL	GT x CL
3 day after administration							
Number of animals	8	25	11	17			
DNA (mg/g wet muscle)	1.25 (0.042)	1.07 (0.022)	1.12 (0.021)	0.95 (0.011)	***	***	NS
RNA (mg/g wet muscle)	1.08 (0.046)	1.15 (0.049)	1.13 (0.040)	1.31 (0.034)	NS	*	NS
RNA/DNA ratio	0.87 (0.041)	1.08 (0.043)	1.01 (0.047)	1.37 (0.035)	***	***	NS
14 day after administration							
Number of animals	13	18	11	10			
DNA (mg/g wet muscle)	0.99 (0.028)	0.95 (0.011)	0.90 (0.028)	0.83 (0.013)	***	*	NS
RNA (mg/g wet muscle)	1.21 (0.041)	1.24 (0.017)	1.22 (0.023)	1.22 (0.030)	NS	NS	NS
RNA/DNA ratio	1.24 (0.048)	1.31 (0.020)	1.38 (0.048)	1.47 (0.028)	***	*	NS

Values are means (SEM). CL, clenbuterol; GT, genotype; NS, not significant; *, $P<0.05$; ***, $P<0.001$.

Table 3.4 Sequence of PCR primers used for real-time PCR.

Probe set ID (Gene symbol)	Forward Primers (5’-3’)	Reverse Primers (5’-3’)
1420575_at (Mt3)	GGACTGGATATGGACCCTGAGACCTG	CACACACAGTCCTTGGCACACTT-
1418726_a_at (Tnnt2)	TCACAACCTGGAGGCTGAGAACTT	TCATCTATTTCCAACGCCCGGTGA
1424542_at (S100a4)	CATGGCAAGACCCTTGGAGGAGGCCCTGGA	AGCTTCATCTGTCCTTTTCCCCAGGAAGCT
1448591_at (Ctss)	TGGATGCTTCTGTGACAAGCTCCGATTTC	CAGAGATCCCAATGGTAGTCCAGGGTAGGG
1424529_s_at (Cgref1)	GCCAAAGAACTGCTGGTGGAAACA	TTCAACACCATGCTTCTGCTCTGC
1423691_x_at (Krt8)	TATGAGGAATTGCAGACCCTGGCT	TCTGGCCTTTGAGGGCTTCAATCT
1419139_at (Gdf5)	TGAATATTTGTTCAGCCAGCGGCG	ACTCAAGAGGTGCGATGATCCAGT
1422562_at (Rrad)	TATGCAGCACACACCCTTGTCTCA	AGAAAGGCAGAGTCCTGTCCACAA
1419391_at (Myog)	ACAATCTGCACTCCCTTACGTCCA	TCTCAGTTGGGCATGGTTTCGTCT
1450650_at (Myo10)	ATTGGCCATGGCACTTTATGCTCG	ATCCGAAGATGTCAAGGATGCCGA
1418625_s_at (Gapdh)	TCAACAGCAACTCCCACTCTTCCA	ACCCTGTTGCTGTAGCCGTATTCA

Table 3.5 Differential gene expression profiles as affected by the effect of genotype at 3-fold change (W vs M).

Probe Set ID	Fold change	Gene Title	Gene Symbol
1420575_at	5.75	metallothionein 3	Mt3
1420884_at	4.37	sarcolipin	Sln
1426808_at	4.36	lectin, galactose binding, soluble 3	Lgals3
1427076_at	3.94	macrophage expressed gene 1	Mpeg1
1418726_a_at	3.54	troponin T2, cardiac[a]	Tnnt2
1416431_at	3.49	tubulin, beta 6[a]	Tubb6
1459860_x_at	3.18	tripartite motif-containing 2	Trim2
1427201_at	3.13	musculoskeletal, embryonic nuclear protein 1	Mustn1

[a] This gene appears two times; W, wild type mice; M, Mstn-pro mice.

Table 3.6 Differential gene expression profiles as affected by the effect of genotype under CL administration at 3-fold change (CLW vs CLM).

Probe Set ID	Fold change	Gene Title	Gene Symbol
1427838_at	4.38	tubulin, beta 2A	Tubb2a
1451054_at	3.75	orosomucoid 1	Orm1
1449700_at	3.01	Immunoglobulin (CD79A) binding protein 1	Igbp1
1437915_at	3.35	target of myb1-like 2 (chicken)	Tom1l2
1426340_at	3.23	solute carrier family 1, member 3	Slc1a3

CLW, clenbuterol treated wild type mice; CLM, clenbuterol treated Mstn-pro mice.

Table 3.7 Differential gene expression profiles as affected by the effect of CL at 3-fold change (W vs CLW).

Probe Set ID	Fold change	Gene Title	Gene Symbol
1423691_x_at	390.33	keratin 8[b]	Krt8
1448169_at	187.70	keratin 18	Krt18
1440878_at	65.37	runt related transcription factor 1[b]	Runx1
1422562_at	54.82	Ras-related associated with diabetes	Rrad
1435053_s_at	52.42	Pleckstrin homology domain [b]	Plekh
1419139_at	50.83	growth differentiation factor 5	Gdf5
1434709_at	30.46	neuron-glia-CAM-related cell adhesion molecule[a]	Nrcam
1420991_at	23.76	ankyrin repeat domain 1 (cardiac muscle) [a]	Ankrd1
1424831_at	23.02	copine II	Cpne2
1448975_s_at	21.68	renin 1 structural	Ren1
1426808_at	16.80	lectin, galactose binding, soluble 3	Lgals3
1424245_at	16.56	carboxylesterase 2	Ces2
1458813_at	16.54	sodium channel, voltage-gated, type V, alpha	Scn5a
1418852_at	15.79	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	Chrna1
1428640_at	14.23	heat shock transcription factor 2 binding protein	Hsf2bp
1430086_at	13.92	cholinergic receptor, nicotinic, alpha polypeptide 9	Chrna9
1449519_at	13.70	growth arrest and DNA-damage-inducible 45 alpha	Gadd45a
1421698_a_at	9.43	collagen, type XIX, alpha 1[a]	Col19a1
1418460_at	9.23	SH3 domain protein [b]	Sh3
1440085_at	9.14	ectodysplasin A2 isoform receptor	Eda2r
1435176_a_at	8.97	inhibitor of DNA binding 2 [a]	Id2
1451287_s_at	8.93	allograft inflammatory factor 1-like [a]	Aif1l
1419391_at	8.63	myogenin	Myog
1420884_at	8.55	sarcolipin	Sln
1422580_at	8.43	myosin, light polypeptide 4	Myl4
1455649_at	7.72	tetratricopeptide repeat domain 9	Ttc9
1421852_at	7.71	potassium channel, subfamily K, member 5	Kcnk5
1419394_s_at	7.71	S100 calcium binding protein A8 (calgranulin A)	S100a8
1455342_at	7.59	prune homolog 2 (Drosophila)[a]	Prune2
1421269_at	7.41	UDP-glucose ceramide glucosyltransferase [b]	Ugcg
1453125_at	7.12	SRY-box containing gene 11[b]	Sox11
1423186_at	6.99	T-cell lymphoma invasion and metastasis 2[a]	Tiam2
1427910_at	6.94	cystatin E/M	Cst6

1418571_at	6.86	tumor necrosis factor receptor superfamily, member 12a[a]	Tnfrsf12a
1438988_x_at	6.59	hematological and neurological expressed sequence 1[b]	Hn1
1420444_at	6.56	solute carrier family 22 (organic cation transporter), member 3	Slc22a3
1416431_at	6.53	tubulin, beta 6	Tubb6
1422629_s_at	6.38	shroom family member 3	Shroom3
1423719_at	6.28	similar to long palate	LOC632073
1434513_at	5.92	ATPase type 13A3[a]	Atp13a3
1460290_at	5.82	lipin 2[b]	Lpin2
1420938_at	5.80	heparan sulfate 6-O-sulfotransferase 2[a]	Hs6st2
1455771_at	5.47	benzodiazapine receptor associated protein 1	Bzrap1
1448660_at	5.38	Rho GDP dissociation inhibitor (GDI) gamma	Arhgdig
1421679_a_at	5.19	Cyclin-dependent kinase inhibitor 1A(P21)[a]	Cdkn1a
1426650_at	5.02	myosin, heavy polypeptide 8, skeletal muscle, perinatal	Myh8
1451036_at	4.80	spastic paraplegia 21 homolog (human)	Spg21
1420438_at	4.72	orosomucoid 2	Orm2
1418158_at	4.50	transformation related protein 63[a]	Trp63
1442590_at	4.49	tumor necrosis factor receptor superfamily, member 22 [a]	Tnfrsf22
1437224_at	4.36	reticulon 4	Rtn4
1449533_at	4.36	transmembrane protein 100	Tmem100
1425543_s_at	4.34	pleckstrin homology domain containing, family A member 5	Plekha5
1422573_at	4.33	adenosine monophosphate deaminase 3	Ampd3
1434754_at	4.30	GTPase activating RANGAP domain-like 4	Garnl4
1452232_at	4.23	UDP-N-acetyl-alpha-D-galactosamine	Galnt7
1428834_at	4.15	dual specificity phosphatase 4	Dusp4
1421499_a_at	4.10	protein tyrosine phosphatase, non-receptor type 14	Ptpn14
1448605_at	4.06	ras homolog gene family, member C[a]	Rhoc
1426337_a_at	3.99	TEA domain family member 4	Tead4
1454674_at	3.96	fasciculation and elongation protein zeta 1 (zygin I)	Fez1
1422929_s_at	3.91	atonal homolog 7 (Drosophila)	Atoh7
1430062_at	3.89	hedgehog interacting protein-like 1	Hhipl1
1451160_s_at	3.88	poliovirus receptor[c]	Pvr
1449852_a_at	3.86	EH-domain containing 4	Ehd4
1424613_at	3.83	G protein-coupled receptor, family C, group 5, member B [a]	Gprc5b
1442827_at	3.83	Toll-like receptor 4 [b]	Tlr4
1421175_at	3.78	myelin transcription factor 1-like	Myt1l
1420682_at	3.70	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)	Chrnb1
1422286_a_at	3.60	TGFB-induced factor homeobox 1	Tgif1

1451105_at	3.59	vasohibin 2	Vash2
1427347_s_at	3.57	tubulin, beta 2A	Tubb2a
1452799_at	3.55	FGGY carbohydrate kinase domain containing	Fggy
1429786_a_at	3.52	ZW10 interactor[c]	Zwint
1460521_a_at	3.51	oligonucleotide/oligosaccharide-binding fold containing 2A[d]	Obfc2a
1416401_at	3.51	CD82 antigen	Cd82
1417014_at	3.49	heat shock protein 8 [a]	Hspb8
1421042_at	3.46	rho/rac guanine nucleotide exchange factor (GEF) 2[a]	Arhgef2
1436507_at	3.35	interleukin-1 receptor-associated kinase 2	Irak2
1450650_at	3.34	myosin X [b]	Myo10
1428803_at	3.32	acyl-CoA thioesterase 6	Acot6
1427005_at	3.29	polo-like kinase 2 (Drosophila)	Plk2
1424354_at	3.25	transmembrane protein 140	Tmem140
1451680_at	3.24	sulfiredoxin 1 homolog (S. cerevisiae) [b]	Srxn1
1435184_at	3.21	natriuretic peptide receptor 3[a]	Npr3
1426471_at	3.15	zinc finger protein 52 [a]	Zfp52
1450241_a_at	3.13	ecotropic viral integration site 2a	Evi2a
1432075_a_at	3.12	tektin 1	Tekt1
1424507_at	3.12	Ras and Rab interactor 1	Rin1
1418649_at	3.11	EGL nine homolog 3 (C. elegans)[a]	Egln3
1433571_at	3.10	serine incorporator 5	Serinc5
1427483_at	3.10	solute carrier family 25, member 24[a]	Slc25a24
1435948_at	3.09	transmembrane protein 181A	Tmem181a
1434526_at	3.09	epoxide hydrolase 4	Ephx4
1416231_at	3.09	Vac14 homolog (S. cerevisiae)	Vac14
1423596_at	3.06	NIMA (never in mitosis gene a)-related expressed kinase 6[a]	Nek6
1420895_at	3.02	transforming growth factor, beta receptor I	Tgfbr1
1417481_at	6.03	receptor (calcitonin) activity modifying protein 1	Ramp1
1451382_at	4.67	ChaC, cation transport regulator-like 1 (E. coli)	Chac1
1437250_at	4.23	melanoregulin	Mreg
1449876_at	3.92	protein kinase, cGMP-dependent, type I	Prkg1
1428025_s_at	3.68	phosphatidylinositol transfer protein, cytoplasmic 1	Pitpnc1
1434499_a_at	3.65	lactate dehydrogenase B	Ldhb
1449422_at	3.55	cadherin 4	Cdh4
1458635_at	3.54	RIKEN cDNA 4832428D23 gene	4832428D23Rik
1439143_at	3.42	RIKEN cDNA A930018M24 gene	A930018M24Rik
1422798_at	3.40	contactin associated protein-like 2	Cntnap2

1427329_a_at	3.35	immunoglobulin heavy chain 6 (heavy chain of IgM)	Igh-6
1450048_a_at	3.33	isocitrate dehydrogenase 2 (NADP+), mitochondrial	Idh2
1424393_s_at	3.28	alcohol dehydrogenase, iron containing, 1	Adhfe1
1435370_a_at	3.18	carboxylesterase 3	Ces3
1434645_at	3.17	RIKEN cDNA C530008M17 gene	C530008M17Rik
1416023_at	3.12	fatty acid binding protein 3, muscle and heart	Fabp3
1448300_at	3.12	microsomal glutathione S-transferase 3	Mgst3
1440335_at	3.11	hypothetical protein LOC100046468	LOC100046468
1423454_a_at	3.10	sema domain, transmembrane domain (TM)	Sema6c
1444504_at	3.02	dehydrogenase/reductase (SDR family) member 7C	Dhrs7c

[a] This gene appears two times; [b] This gene appears three times; [c] This gene appears four times; [d] This gene appears five times; W, wild type mice; CLW, clenbuterol treated wild type mice. Gray box is underexrpessed genes.

Table 3.8 Differential gene expression profiles as affected by the effect of CL under Mstn-pro at 3 fold change (M vs CLM).

Probe Set ID	Fold change	Gene Title	Gene Symbol
1423691_x_at	413.40	keratin 8[b]	Krt8
1448169_at	144.41	keratin 18	Krt18
1419139_at	77.47	growth differentiation factor 5	Gdf5
1440878_at	41.09	runt related transcription factor 1[c]	Runx1
1422562_at	38.79	Ras-related associated with diabetes	Rrad
1424245_at	32.27	carboxylesterase 2	Ces2
1458813_at	31.87	sodium channel, voltage-gated, type V, alpha	Scn5a
1420992_at	21.26	ankyrin repeat domain 1 (cardiac muscle)[a]	Ankrd1
1420438_at	20.69	orosomucoid 2	Orm2
1434709_at	18.91	neuron-glia-CAM-related cell adhesion molecule[a]	Nrcam
1435053_s_at	16.27	pleckstrin homology domain[a]	Plekh
1419394_s_at	12.54	S100 calcium binding protein A8 (calgranulin A)	S100a8
1435176_a_at	12.29	inhibitor of DNA binding 2	Id2
1421852_at	12.14	potassium channel, subfamily K, member 5	Kcnk5
1451054_at	11.27	orosomucoid 1	Orm1
1449519_at	10.97	growth arrest and DNA-damage-inducible 45 alpha	Gadd45a
1448975_s_at	10.72	renin 1 structural	Ren1
1418852_at	9.72	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	Chrna1
1420444_at	9.18	solute carrier family 22 (organic cation transporter), member 3	Slc22a3
1451287_s_at	9.15	allograft inflammatory factor 1-like[a]	Aif1l
1421269_at	9.04	UDP-glucose ceramide glucosyltransferase [b]	Ugcg
1419391_at	8.47	myogenin	Myog
1428640_at	7.96	heat shock transcription factor 2 binding protein	Hsf2bp
1456953_at	6.78	collagen, type XIX, alpha 1[a]	Col19a1
1430062_at	6.74	hedgehog interacting protein-like 1	Hhipl1
1424638_at	6.72	Cyclin-dependent kinase inhibitor 1A(P21)[a]	Cdkn1a
1442145_at	6.72	ATPase type 13A3[a]	Atp13a3
1449084_s_at	6.72	SH3 domain protein[b]	Sh3
1440085_at	6.65	ectodysplasin A2 isoform receptor	Eda2r
1426808_at	6.63	lectin, galactose binding, soluble 3	Lgals3
1427910_at	6.58	cystatin E/M	Cst6
1437669_x_at	6.52	Chemokine (C-C motif) receptor-like 1[a]	Ccrl1
1423186_at	6.50	T-cell lymphoma invasion and metastasis 2	Tiam2

1424831_at	6.29	copine II	Cpne2
1436790_a_at	6.17	SRY-box containing gene 11[c]	Sox11
1437460_x_at	5.99	Ras and Rab interactor 1[a]	Rin1
1434754_at	5.84	GTPase activating RANGAP domain-like 4	Garnl4
1455649_at	5.70	tetratricopeptide repeat domain 9	Ttc9
1455771_at	5.58	benzodiazapine receptor associated protein 1	Bzrap1
1422629_s_at	5.58	shroom family member 3	Shroom3
1421499_a_at	5.14	protein tyrosine phosphatase, non-receptor type 14[a]	Ptpn14
1423719_at	5.02	similar to long palate	LOC632073
1422573_at	4.95	adenosine monophosphate deaminase 3	Ampd3
1427838_at	4.77	tubulin, beta 2A[a]	Tubb2a
1418572_x_at	4.77	tumor necrosis factor receptor superfamily, member 12a	Tnfrsf12a
1455342_at	4.76	prune homolog 2 (Drosophila)[a]	Prune2
1454674_at	4.69	fasciculation and elongation protein zeta 1 (zygin I)	Fez1
1442827_at	4.54	Toll-like receptor 4 [b]	Tlr4
1424354_at	4.46	transmembrane protein 140	Tmem140
1428834_at	4.46	dual specificity phosphatase 4	Dusp4
1440999_at	4.34	zinc finger protein 697	Zfp697
1452836_at	4.33	lipin 2[a]	Lpin2
1426337_a_at	4.24	TEA domain family member 4	Tead4
1437062_s_at	4.15	phytanoyl-CoA hydroxylase interacting protein-like	Phyhipl
1430086_at	4.14	cholinergic receptor, nicotinic, alpha polypeptide 9	Chrna9
1416028_a_at	4.06	hematological and neurological expressed sequence 1[b]	Hn1
1424517_at	3.97	coiled-coil domain containing 12[a]	Ccdc12
1435184_at	3.95	natriuretic peptide receptor 3	Npr3
1451006_at	3.95	xanthine dehydrogenase	Xdh
1457157_at	3.94	phospholipase C, eta 1	Plch1
1419621_at	3.92	ankyrin repeat domain 2 (stretch responsive muscle)	Ankrd2
1428803_at	3.91	acyl-CoA thioesterase 6[a]	Acot6
1451411_at	3.89	G protein-coupled receptor, family C, group 5, member B[a]	Gprc5b
1441055_at	3.89	paralemmin 2	Palm2
1449852_a_at	3.89	EH-domain containing 4	Ehd4
1452799_at	3.80	FGGY carbohydrate kinase domain containing	Fggy
1419184_a_at	3.77	four and a half LIM domains	Fhl
1433731_at	3.70	insulin-like growth factor 2 mRNA binding protein 3	Igf2bp3
1450047_at	3.70	heparan sulfate 6-O-sulfotransferase 2	Hs6st2
1455149_at	3.64	SH3 domain containing ring finger 1[a]	Sh3rf1

1437224_at	3.55	reticulon 4[a]	Rtn4
1429086_at	3.45	grainyhead-like 2 (Drosophila)	Grhl2
1418840_at	3.43	programmed cell death 4	Pdcd4
1438795_x_at	3.38	FK506 binding protein 1	Fkbp1
1460392_a_at	3.38	enhancer of yellow 2 homolog (Drosophila)[a]	Eny2
1417103_at	3.37	D-dopachrome tautomerase	Ddt
1431281_at	3.37	dysferlin interacting protein 1	Dysfip1
1416065_a_at	3.36	ankyrin repeat domain 10 [b]	Ankrd10
1436362_x_at	3.34	CCR4 carbon catabolite repression 4-like (S. cerevisiae)	Ccrn4l
1418158_at	3.32	transformation related protein 63[a]	Trp63
1420884_at	3.32	sarcolipin	Sln
1452232_at	3.29	UDP-N-acetyl-alpha-D-galactosamine	Galnt7
1456291_x_at	3.29	scleraxis[a]	Scx
1451757_at	3.27	cDNA sequence BC003883	BC003883
1448660_at	3.25	Rho GDP dissociation inhibitor (GDI) gamma	Arhgdig
1418853_at	3.22	apolipoprotein N	Apon
1425964_x_at	3.21	heat shock protein 1[a]	Hspb1
1425543_s_at	3.14	pleckstrin homology domain containing, family A member 5	Plekha5
1420895_at	3.13	transforming growth factor, beta receptor I[a]	Tgfbr1
1448793_a_at	3.10	syndecan 4 [a]	Sdc4
1435394_s_at	3.10	ras homolog gene family, member C	Rhoc
1426329_s_at	3.09	brain and acute leukemia, cytoplasmic	Baalc
1418648_at	3.08	EGL nine homolog 3 (C. elegans)[a]	Egln3
1443667_at	3.07	expressed sequence C79407	C79407
1458067_at	3.06	Family with sequence similarity 179, member B	Fam179b
1433972_at	3.02	calmodulin binding transcription activator 1[a]	Camta1
1422286_a_at	3.02	TGFB-induced factor homeobox 1	Tgif1
1421479_at	3.02	zinc finger protein 318[c]	Zfp318
1416401_at	3.01	CD82 antigen	Cd82
1425546_a_at	5.88	transferrin	Trf
1458635_at	5.71	RIKEN cDNA 4832428D23 gene	4832428D23Rik
1444504_at	5.42	dehydrogenase/reductase (SDR family) member 7C	Dhrs7c
1425089_at	5.42	potassium voltage gated channel member 4	Kcnc4
1420575_at	5.36	metallothionein 3	Mt3
1436644_x_at	5.27	transmembrane protein 25	Tmem25
1421979_at	5.03	phosphate regulating gene with homologies to endopeptidases on the X chromosome	Phex
1437273_at	4.95	HtrA serine peptidase 4	Htra4

1436986_at	4.89	syntrophin, basic 2	Sntb2
1417481_at	4.68	receptor (calcitonin) activity modifying protein 1	Ramp1
1439821_at	4.57	Lrp2 binding protein	Lrp2bp
1453904_at	4.52	coiled-coil domain containing 146	Ccdc146
1439491_at	4.41	leucine rich repeat containing 38	Lrrc38
1427919_at	4.34	sushi-repeat-containing protein, X-linked 2	Srpx2
1439426_x_at	4.33	lysozyme 1	Lyz1
1442251_at	4.19	valosin containing protein (p97)	Vcpip1
1451715_at	4.18	v-maf musculoaponeurotic fibrosarcoma oncogene family	Mafb
1432107_at	4.18	RIKEN cDNA 2310010M20 gene	2310010M20Rik
1427329_a_at	4.12	immunoglobulin heavy chain 6 (heavy chain of IgM)	Igh-6
1435370_a_at	4.10	carboxylesterase 3	Ces3
1436359_at	4.10	ret proto-oncogene	Ret
1434911_s_at	4.07	Rho GTPase activating protein 19	Arhgap19
1416863_at	4.04	abhydrolase domain containing 8	Abhd8
1439311_at	3.97	RIKEN cDNA B830012L14 gene	B830012L14Rik
1427076_at	3.95	macrophage expressed gene 1	Mpeg1
1451305_at	3.92	chibby homolog 1 (Drosophila)	Cby1
1442739_at	3.92	cDNA sequence BC031441	BC031441
1440435_at	3.92	kyphoscoliosis peptidase	Ky
1434210_s_at	3.90	leucine-rich repeats and immunoglobulin-like domains 1	Lrig1
1450243_a_at	3.81	regulator of calcineurin 2	Rcan2
1419456_at	3.78	dicarbonyl L-xylulose reductase	Dcxr
1429012_at	3.77	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	Arhgef6
1450048_a_at	3.76	isocitrate dehydrogenase 2 (NADP+), mitochondrial	Idh2
1448300_at	3.75	microsomal glutathione S-transferase 3	Mgst3
1418589_a_at	3.74	myeloid leukemia factor 1	Mlf1
1455224_at	3.68	angiopoietin-like 1	Angptl1
1438431_at	3.63	ATP-binding cassette, sub-family D (ALD), member 2	Abcd2
1453851_a_at	3.63	growth arrest and DNA-damage-inducible 45 gamma	Gadd45g
1424393_s_at	3.49	alcohol dehydrogenase, iron containing, 1	Adhfe1
1455220_at	3.45	frequently rearranged in advanced T-cell lymphomas 2	Frat2
1420346_at	3.42	ankyrin repeat and SOCS box-containing 12	Asb12
1416318_at	3.42	serine (or cysteine) peptidase inhibitor, clade B, member 1a	Serpinb1a
1456397_at	3.38	cadherin 4	Cdh4
1437915_at	3.36	target of myb1-like 2 (chicken)	Tom1l2
1438396_at	3.35	oculocerebrorenal syndrome of Lowe	Ocrl

1451989_a_at	3.35	microtubule-associated protein, RP/EB family, member 2	Mapre2
1451322_at	3.35	carboxymethylenebutenolidase-like (Pseudomonas)	Cmbl
1451500_at	3.34	Usher syndrome 1C binding protein 1	Ushbp1
1426981_at	3.29	proprotein convertase subtilisin/kexin type 6	Pcsk6
1428025_s_at	3.26	phosphatidylinositol transfer protein, cytoplasmic 1	Pitpnc1
1429474_at	3.23	prostaglandin reductase 2	Ptgr2
1421878_at	3.21	mitogen-activated protein kinase 9	Mapk9
1452766_at	3.17	tubulin polymerization promoting protein	Tppp
1452474_a_at	3.16	ADP-ribosyltransferase 3	Art3
1436763_a_at	3.16	Kruppel-like factor 9	Klf9
1422605_at	3.14	protein phosphatase 1, regulatory (inhibitor) subunit 1A	Ppp1r1a
1439836_at	3.13	ankyrin repeat and SOCS box-containing 15	Asb15
1427053_at	3.11	ABI gene family, member 3 (NESH) binding protein	Abi3bp
1449547_at	3.09	ankyrin repeat and SOCS box-containing 14	Asb14
1449078_at	3.08	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	St3gal6
1422798_at	3.06	contactin associated protein-like 2	Cntnap2
1426208_x_at	3.06	pleiomorphic adenoma gene-like 1	Plagl1
1451991_at	3.00	Eph receptor A7	Epha7

[a] This gene appears two times; [b] This gene appears three times; [c] This gene appears four times; M, Mstn-pro mice; CLM, clenbuterol treated Mstn-pro mice.

Table 3.9 The expression of overlapping genes between the effect of genotype and CL (W vs M VS W vs CLW) at 1.5 fold change.

Probe Set ID	Gene Symbol	Gene Title
1436520_at	Ahnak2	AHNAK nucleoprotein 2
1424176_a_at	Anxa4	annexin A4
1451780_at	Blnk	B-cell linker
1424529_s_at	Cgref1	cell growth regulator with EF hand domain 1
1418852_at	Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)
1421698_a_at	Col19a1	collagen, type XIX, alpha 1
1422186_s_at	Cyb5r3	cytochrome b5 reductase 3
1449258_at	D11Wsu99e	DNA segment, Chr 11, Wayne State University 99, expressed
1426215_at	Ddc	dopa decarboxylase
1423569_at	Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
1448239_at	Hmox1	heme oxygenase (decycling) 1
1438988_x_at	Hn1	hematological and neurological expressed sequence 1
1441259_s_at	Ift122	intraflagellar transport 122 homolog (Chlamydomonas)
1455266_at	Kif5c	kinesin family member 5C
1426808_at	Lgals3	lectin, galactose binding, soluble 3
1448883_at	Lgmn	Legumain
1455679_at	Obfc2a	oligonucleotide/oligosaccharide-binding fold containing 2A
1450070_s_at	Pak1	p21 protein (Cdc42/Rac)-activated kinase 1
1423423_at	Pdia3	protein disulfide isomerase associated 3
1451064_a_at	Psat1	phosphoserine aminotransferase 1
1439610_at	Rab27b	RAB27b, member RAS oncogene family
1451201_s_at	Rnh1	ribonuclease/angiogenin inhibitor 1
1437224_at	Rtn4	reticulon 4
1422864_at	Runx1	runt related transcription factor 1
1452717_at	Slc25a24	solute carrier family 25, member 24
1420884_at	Sln	Sarcolipin
1417647_at	Snx5	sorting nexin 5
1434442_at	Stbd1	starch binding domain 1
1418726_a_at	Tnnt2	troponin T2, cardiac
1438948_x_at	Tspo	translocator protein
1436833_x_at	Ttll1	tubulin tyrosine ligase-like 1
1427347_s_at	Tubb2a	tubulin, beta 2A
1416431_at	Tubb6	tubulin, beta 6
1451105_at	Vash2	vasohibin 2

1437906_x_at	Txnl1	thioredoxin-like 1
1440343_at	Rps6ka5	ribosomal protein S6 kinase, polypeptide 5
1460388_at	Acot6	acyl-CoA thioesterase 6

A total 40 genes was identified between the effects of genotype and CL (36 overexpressed genes and 4 underexpressed genes). Among the 40 genes, 3 genes were ESTs resulting in 37 known gene identified (34 overexpressed genes and 3 underexpressed genes). W, wild type mice; M, Mstn-pro mice; CLM, clenbuterol treated Mstn-pro mice. Gray box is underexpressed genes.

Table 3.10 Comparison of gene expression changes measured by microarray analysis and RT-PCR.

Probe set ID (Gene symbol)	Ratio CT/CLT by array analysis	Threshold cycle of real-time PCR[1]		Group
		Control group	Comparison group	
1420575_at (Mt3)	5.75	28.3 ± 1.24	18.9 ± 0.17***	I
1418726_a_at (Tnnt2)	3.54	23.4 ± 0.61	16.9 ± 0.26***	I
1424542_at (S100a4)	2.9	23.5 ± 0.87	17.9 ± 0.36***	I
1448591_at (Ctss)	2.3	23.6 ± 0.80	15.6 ± 0.36***	I
1424529_s_at (Cgref1)	1.69	23.2 ± 0.63	18.9 ± 0.16***	I
1418625_s_at (Gapdh)	-	9.9 ± 0.28	9.7 ± 0.16	I
1423691_x_at (Krt8)	390.33	27.4 ± 0.46	14.9 ± 0.22***	II
1422562_at (Rrad)	54.82	21.4 ± 0.55	14.0 ± 0.34***	II
1419139_at (Gdf5)	50.83	25.0 ± 0.55	17.8 ± 0.21***	II
1419391_at (Myog)	8.63	24.1 ± 0.58	17.2 ± 0.39***	II
1450650_at (Myo10)	3.34	23.5 ± 0.54	19.7 ± 0.12***	II
1418625_s_at (Gapdh)	-	10.7 ± 0.37	10.4 ± 0.38	II
1423691_x_at (Krt8)	413.4	27.4 ± 0.43	14.0 ± 0.42***	III
1440878_at (Runx1)	41.29	24.0 ± 0.43	16.9 ± 0.40***	III
1420438_at (Orm2)	20.69	19.4 ± 0.26	12.4 ± 0.40***	III
1419391_at (Myog)	8.47	21.4 ± 0.15	15.4 ± 0.46***	III
1427838_at (Tubb2a)	4.77	22.1 ± 0.22	19.5 ± 0.24***	III
1418625_s_at (Gapdh)	-	9.1 ± 0.06	9.6 ± 0.11	III
1427838_at (Tubb2a)	4.38	22.4 ± 0.17	19.5 ± 0.24***	IV
1452455_at (Myof)	2.15	21.4 ± 0.15	16.7 ± 0.24***	IV
1449363_at (Atf3)	2.13	20.1 ± 0.18	17.1 ± 0.19***	IV
1441055_at (Palm2)	1.66	23.5 ± 0.87	18.0 ± 0.16***	IV
1424529_s_at (Cgref1)	1.5	25.5 ± 0.32	22.2 ± 0.21***	IV
1418625_s_at (Gapdh)	-	9.6 ± 0.06	9.5 ± 0.16	IV

[1] Threshold cycle (Ct) of each sample of the wild type mice (W, n=18), Mstn-pro mice (M, n=18), CL treated wild type mice (CLW, n=18) and CL treated Mstn-pro mice (CLM, n=12) was obtained and analyzed by Prism5 software (Grapad, San Diego, CA). ***, P<0.001. I, W vs M; II, W vs CLW; III, M vs CLM; IV, CLW vs CLM.

Table 3.11 Differentially expressed gene clusters by the effect of genotype based on Gene Ontology (GO) biological process terms.

W vs M		
GO Biological Process Terms	**Number of Genes**	**p-Value**
Overexpression		
Antigen processing and presentation of exogenous antigen or peptide	12	0.00037
Neuron development	14	0.00076
Cell differentiation	24	0.0011
Regulation of cell activation	5	0.0012
System development	18	0.0016
Lymphocyte activation	6	0.0029
Organ development	20	0.0039
Leukocyte activation	6	0.0041
Immune response	14	0.01
Regulation of multicellular organismal process	6	0.013
Extracellular structure organization and biogenesis	4	0.014
Dendrite development	3	0.014
Transport	19	0.04
Muscle fiber development	3	0.041
Cell death	8	0.045
Cell cycle	12	0.053
Mononuclear cell proliferation	3	0.056
Organic acid metabolic process	6	0.08
Other categories[2]	41	-
Underexpression		
Cell redox homeostasis	2	0.03
Lipid metabolic process	3	0.049

CLW vs CLM		
GO Biological Process Terms	**Number of Genes**	**p-Value**
Overexpression		
transcription, DNA-dependent	18	0.0026
regulation of transcription	18	0.004
regulation of gene expression	18	0.0078
regulation of cellular process	24	0.0081
regulation of cellular metabolic process	18	0.011
regulation of metabolic process	18	0.016
nucleic acid metabolic process	21	0.027

one-carbon compound metabolic process	3	0.05
Underexpression		
protein localization	12	0.0091
post-embryonic hemopoiesis	2	0.012
cellular component assembly	8	0.017
Transport	23	0.057
organic acid metabolic process	7	0.084
cell-substrate adhesion	3	0.084
cell maturation	3	0.094
lipid metabolic process	8	0.099

W vs M: A total 108 genes identified as differentially over and underexpressed genes by DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on Gene Ontology biological process terms. Count and EASE thresholds were set at 2 and 0.1, respectively. For overexpressed 48 genes were unclassified. For underexpressed 14 genes were unclassified. [2]The other categories include negative T cell selection, inflammatory response to antigenic stimulus, regulation of programmed cell death, thymic T cell selection, cell wall metabolic process, external encapsulating structure organization and biogenesis, anatomical structure morphogenesis, negative regulation of biological process and cellular structure morphogenesis. CLW vs CLM: A total 96 genes identified as differentially over and underexpressed genes by DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on Gene Ontology biological process terms. Count and EASE thresholds were set at 2 and 0.1, respectively. For overexpressed 65 genes were unclassified. For underexpressed 94 genes were unclassified. W, wild type mice; M, Mstn-pro mice; CLW, clenbuterol treated wild type mice; CLM, clenbuterol treated Mstn-pro mice.

Table 3.12 Differentially expressed gene clusters by the effect of CL based on Gene Ontology (GO) biological process terms.

W vs CLW		
GO Biological Process Terms	**Number of Genes**	**p-Value**
Overexpression		
Transport	222	0.0000036
Cellular localization	106	0.000024
Cellular metabolic process	403	0.000043
Protein metabolic process	182	0.000058
Regulation of biological process	104	0.00012
Cell proliferation	26	0.00048
Protein localization	87	0.00049
Developmental process	107	0.0017
Muscle fiber development	9	0.002
Metabolic process	275	0.0023
Cell cycle	88	0.005
Cell death	37	0.0064
Cell differentiation	91	0.0069
Cell redox homeostasis	7	0.0083
Cellular component organization and biogenesis	110	0.011
Signal transduction	25	0.011
Ribonucleoprotein complex biogenesis and assembly	15	0.012
Embryonic development	38	0.014
Cell growth	11	0.014
Organ development	156	0.017
Phosphorus metabolic process	42	0.019
Cell maturation	8	0.021
Anatomical structure development and formation	63	0.034
Neurogenesis	29	0.034
Homeostatic process	20	0.035
Translational initiation	6	0.041
Cell migration	6	0.082
Regulation of locomotion	6	0.098
Other categories[1]	103	-
Underexpression		
Regulation of heart contraction	5	0.027
Menstrual cycle phase	4	0.078

Menstrual cycle process	4	0.083
Reproductive structure development	5	0.098
Electron transport	19	0.096

M vs CLM

GO Biological Process Terms	Number of Genes	p-Value
Overexpressed		
Cellular metabolic process	427	0.0000096
Protein metabolic process	181	0.000033
Transport	188	0.00024
Cell differentiation	84	0.00086
Metabolic process	258	0.0011
Developmental process	103	0.0019
Signal transduction	27	0.0028
Biological process	99	0.0035
Cellular localization	87	0.0076
Protein localization	75	0.0078
Cell cycle	50	0.0096
Ribonucleoprotein complex biogenesis and assembly	15	0.011
Cell death	57	0.017
Muscle fiber development	7	0.026
Neurogenesis	40	0.027
Anatomical structure development and formation	63	0.031
Organ development	119	0.044
Angiogenesis	10	0.057
Cell maturation	7	0.058
Cellular component organization and biogenesis	62	0.082
Phosphorus metabolic process	38	0.085
Homeostatic process	18	0.099
Other categories[2]	185	-
Underexpressed		
lipid transport	13	0.00045
electron transport	32	0.007
negative regulation of growth	6	0.025
regulation of binding	5	0.035
negative regulation of cellular process	51	0.069
regulation of catalytic activity	22	0.08
Opsonization	2	0.082

negative regulation of biological process	53	0.09
hemopoietic or lymphoid organ development	17	0.09

W vs CLW: A total 1241 genes identified as differentially over and uncerexpressed genes by DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on Gene Ontology biological process terms. Count and EASE thresholds were set at 2 and 0.1, respectively. 280 overexpressed genes were unclassified. 309 underexpressed genes were unclassified. [1] other categories include regulation of catalytic activity, macromolecule catabolic process, response to tumor cell, lymphocyte homeostasis, macromolecule biosynthetic process, extracellular structure organization and biogenesis, formation of primary germ layer and cytokinesis. M vs CLM: A total 1522 genes identified as differentially over and underexpressed genes by DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on Gene Ontology biological process terms. Count and EASE thresholds were set at 2 and 0.1, respectively. 383 overexpressed genes were unclassified. 384 underexpressed genes were unclassified. [2] other categories include macromolecule biosynthetic process, tissue development, rRNA processing and nucleic acid metabolic process. W, wild type mice; M, Mstn-pro mice; CLW, clenbuterol treated wild type mice; CLM, clenbuterol treated Mstn-pro mice.

Table 3.13 Differentially expressed gene clusters by the effect of genotype and CL (W vs M VS W vs CLW) based on Gene Ontology (GO) biological process terms.

GO Biological Process Terms	Number of Genes	p-Value
Overexpression		
system development	11	0.0043
response to nutrient	3	0.01
organ development	9	0.014
microtubule-based movement	3	0.017
regulation of multicellular organismal process	6	0.018
regulation of anti-apoptosis	2	0.026
response to extracellular stimulus	3	0.029
cell differentiation	8	0.032
cell development	5	0.032
positive regulation of angiogenesis	2	0.034
tissue development	5	0.037
regulation of anatomical structure morphogenesis	3	0.054
cellular macromolecular complex assembly	3	0.07
protein complex assembly	3	0.076
cellular macromolecular complex subunit organization	3	0.086
regulation of angiogenesis	2	0.087
synapse organization	2	0.094

A total 40 genes identified as differentially over and underexpressed genes by DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on Gene Ontology biological process terms. Count and EASE thresholds were set at 2 and 0.1, respectively. 19 overexpressed genes were unclassified. W, wild type mice; M, Mstn-pro mice; CLW, clenbuterol treated wild type mice.

Table 3.14 Differentially expressed gene clusters by the effect of genotype (W vs M) based on skeletal muscle growth at 1.5 fold change.

Ontology/Gene symbol	Gene name	Fold change
Carboxylic acid and amino acid metabolism		
Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	2.58
Cd74	CD74 antigen	2.34
Arrb2	arrestin, beta 2	2.06
Alox5ap	arachidonate 5-lipoxygenase activating protein	1.92
Psat1	phosphoserine aminotransferase 1	1.66
Ddc	dopa decarboxylase	1.60
Protein metabolic process		
Tubb6	Tubulin, beta 6	3.54
Serpinb1a	serine (or cysteine) peptidase inhibitor, clade B, member 1a	2.94
Cd74	CD74 antigen	2.34
Ctss	cathepsin S	2.30
Lgmn	legumain	2.15
Arrb2	arrestin, beta 2	2.06
Tubb2a	tubulin, beta 2A	1.87
Ptprc	protein tyrosine phosphatase, receptor type, C	1.79
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.63
Rab40b	Rab40b, member RAS oncogene family	1.60
Ttll1	tubulin tyrosine ligase-like 1	1.54
Ctsz	cathepsin Z	1.51
Transcription		
Runx1	runt related transcription factor 1	2.17
Dnmt1	DNA methyltransferase (cytosine-5) 1	1.62
Srebf1	sterol regulatory element binding transcription factor 1	-1.51
Cell proliferation and cell cycle		
Tubb6	Tubulin, beta 6	3.54
S100a4	S100 calcium binding protein A4	2.90
Igf2	insulin-like growth factor 2	2.05
Fcgr2b	Fc receptor, IgG, low affinity IIb	1.93
Ptprc	protein tyrosine phosphatase, receptor type, C	1.79
Cgref1	cell growth regulator with EF hand domain 1	1.69
Smc4	structural maintenance of chromosomes 4	1.61
Grn	granulin	1.59
Cell differentiation		

Mt3	metallothionein 3	5.75
Tnnt2	troponin T2, cardiac	3.54
Lyz1	lysozyme 1	2.92
H2-Aa	histocompatibility 2, class II antigen A, alpha	2.83
Lyz2	lysozyme 2	2.47
Kif5c	kinesin family member 5C	2.19
Runx1	runt related transcription factor 1	2.17
Rtn4	reticulon 4	2.00
Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	1.90
Ptprc	protein tyrosine phosphatase, receptor type, C	1.79
Col19a1	collagen, type XIX, alpha 1	1.78
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.77
Naip5	NLR family, apoptosis inhibitory protein 5	1.76
Pdia3	protein disulfide isomerase associated 3	1.60
Ndrg4	N-myc downstream regulated gene 4	1.55
Mal	myelin and lymphocyte protein, T-cell differentiation protein	-1.54
Skeletal muscle development		
Tnnt2	troponin T2, cardiac	3.54
Col19a1	collagen, type XIX, alpha 1	1.78
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.77
Signal transduction		
Arrb2	arrestin, beta 2	2.06
Igf2	insulin-like growth factor 2	2.05
Dclk1	doublecortin-like kinase 1	1.91
Ptprc	protein tyrosine phosphatase, receptor type, C	1.79
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.77
Emr1	EGF-like module containing, hormone receptor-like sequence 1	1.73
Hmox1	heme oxygenase 1	1.73
Rab40b	Rab40b, member RAS oncogene family	1.60
Csf1r	colony stimulating factor 1 receptor	1.59
Rab27b	RAB27b, member RAS oncogene family	1.53

A total 108 genes identified as differentially expressed genes were uploaded to DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on skeletal muscle growth. W, wild type mice; M, Mstn-pro mice.

Table 3.15 Differentially expressed gene clusters by the effect of genotype in CL (CLW vs CLM) based on skeletal muscle growth.

Ontology/Gene symbol	Gene name	Fold change
Carboxylic acid metabolic process		
Atf3	activating transcription factor 3	2.13
Shmt2	serine hydroxymethyltransferase 2 (mitochondrial)	1.91
Ddt	D-dopachrome tautomerase	1.85
Acaa2	acetyl-Coenzyme A acyltransferase 2	-1.75
Plp1	proteolipid protein (myelin) 1	-1.64
Slc37a4	solute carrier family 37 (glucose-6-phosphate transporter), member 4	-1.60
Protein metabolic process		
Tubb2a	tubulin, beta 2A	4.38
Tubb2b	tubulin, beta 2B	2.21
Hspa4l	heat shock protein 4 like	2.01
Rpl41	ribosomal protein L41	1.73
Dyn3	dynamin 3	1.70
Itga2b	integrin alpha 2b	1.61
Ttll5	tubulin tyrosine ligase-like family, member 5	1.55
Prkg2	protein kinase, cGMP-dependent, type II	1.54
Erf	Ets2 repressor factor	1.50
Ret	ret proto-oncogene	-2.58
Gsn	gelsolin	-2.03
Lamc1	laminin, gamma 1	-1.70
Colec12	collectin sub-family member 12	-1.69
Prcp	prolylcarboxypeptidase (angiotensinase C)	-1.62
Mme	membrane metallo endopeptidase	-1.59
Phkg1	phosphorylase kinase gamma 1	-1.57
Transcription		
Atf3	activating transcription factor 3	2.13
Fubp1	far upstream element (FUSE) binding protein 1	2.07
Zfp697	zinc finger protein 697	1.90
Elk3	ELK3, member of ETS oncogene family	1.72
Meox1	mesenchyme homeobox 1	1.59
Sbcg11	sry-box containing gene 11	1.59
Erf	Ets2 repressor factor	1.50
Gtf2a1	general transcription factor II A, 1	-1.84
Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	-1.70

Txnip	thioredoxin interacting protein	-1.65
Ikzf2	IKAROS family zinc finger 2	-1.58
Lrrfip1	leucine rich repeat (in FLII) interacting protein 1	-1.55
Cell proliferation and cell cycle Cell cycle		
Igbp1	Immunoglobulin (CD79A) binding protein 1	3.01
Wnt5a	wingless-related MMTV integration site 5A	1.89
Gng3	guanine nucleotide binding protein (G protein), gamma 3	1.75
Snx4	sorting nexin 4	1.75
Rap2b	RAP2B, member of RAS oncogene family	1.74
Mras	muscle and microspikes RAS	1.64
Itga2b	integrin alpha 2b	1.61
Cgref1	cell growth regulator with EF hand domain 1	1.50
Ret	ret proto-oncogene	-2.58
Calm2	calmodulin 2	-2.06
Cxcr7	chemokine (C-X-C motif) receptor 7	-1.75
Grb14	growth factor receptor bound protein 14	-1.73
Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	-1.70
Colec12	collectin sub-family member 12	-1.69
Cd47	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	-1.65
Txnip	thioredoxin interacting protein	-1.65
Plp1	proteolipid protein (myelin) 1	-1.64
Atrnl1	attractin like 1	-1.56
Stac3	SH3 and cysteine rich domain 3	-1.56
Cell differentiation		
Cbf	core-binding factor, runt domain, alpha subunit 2	1.94
Ret	ret proto-oncogene	-2.58
Plp1	proteolipid protein (myelin) 1	-1.64
Txnip	thioredoxin interacting protein	-1.65
Slc37a4	solute carrier family 37 (glucose-6-phosphate transporter), member 4	-1.60
Signal transduction		
Igbp1	Immunoglobulin (CD79A) binding protein 1	3.01
Wnt5a	wingless-related MMTV integration site 5A	1.89
Gng3	guanine nucleotide binding protein (G protein), gamma 3	1.75
Snx4	sorting nexin 4	1.75
Rap2b	RAP2B, member of RAS oncogene family	1.74
Mras	muscle and microspikes RAS	1.64
Itga2b	integrin alpha 2b	1.61

Ret	ret proto-oncogene	-2.58
Calm2	calmodulin 2	-2.06
Cxcr7	chemokine (C-X-C motif) receptor 7	-1.75
Grb14	growth factor receptor bound protein 14	-1.73
Colec12	collectin sub-family member 12	-1.69
Cd47	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	-1.65
Atrnl1	attractin like 1	-1.56
Stac3	SH3 and cysteine rich domain 3	-1.56

A total 96 genes identified as differentially expressed genes were uploaded to DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on skeletal muscle growth. CLW, clenbuterol treated wild type mice; CLM, clenbuterol treated Mstn-pro mice.

Table 3.16 Differentially expressed gene clusters by the effect of CL (W vs CLW) based on skeletal muscle growth at 1.5 fold change.

Ontology/Gene symbol	Gene name	Fold change
Carboxylic acid and amino acid metabolism		
Acot10	acyl-CoA thioesterase 10	2.20
Egf	epidermal growth factor	1.69
Prkce	protein kinase C, epsilon	1.64
Gars	glycyl-tRNA synthetase	1.59
Vars	valyl-tRNA synthetase	1.57
Pdzd2	PDZ domain containing 2	-1.74
Protein metabolic process		
Krt8	keratin 8	390.33
Tubb	tubulin	6.53
Ptpn14	protein tyrosine phosphatase, non-receptor type 14	4.10
Tlr4	toll-like receptor 4	3.48
Tgfbr1	transforming growth factor, beta receptor I	3.02
Musk	muscle, skeletal, receptor tyrosine kinase	2.46
Map3k14	mitogen-activated protein kinase kinase kinase 14	2.41
Ppap2a	phosphatidic acid phosphatase type 2A	2.17
Rbm3	RNA binding motif protein 3	2.13
Map4k4	mitogen-activated protein kinase kinase kinase kinase 4	2.00
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.97
Prkar1a	protein kinase, cAMP dependent regulatory, type I, alpha	1.85
Mapk1	mitogen-activated protein kinase 1	1.84
Map1lc3a	microtubule-associated protein 1 light chain 3 alpha	1.79
Egf	epidermal growth factor	1.69
Cdk7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.67
Prkce	protein kinase C, epsilon	1.64
Mknk2	MAP kinase-interacting serine/threonine kinase 2	1.63
Mapk6	mitogen-activated protein kinase 6	1.62
Atf6	activating transcription factor 6	1.58
Gtpbp4	GTP binding protein 4	1.54
Arf6	ADP-ribosylation factor 6	1.53
Ptprd	protein tyrosine phosphatase, receptor type, D	1.50
Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	-2.68
Acvr2b	activin receptor IIB	-2.38
Pparr	peroxisome proliferative activated receptor-γ	-2.38

Cdc2l6	cell division cycle 2-like 6 (CDK8-like)	-2.19
Camk2a	calcium/calmodulin-dependent protein kinase II alpha	-2.18
Socs2	suppressor of cytokine signaling 2	-2.15
Cabc1	chaperone, ABC1 activity of bc1 complex like (S. pombe)	-2.15
Ppm1k	protein phosphatase 1K (PP2C domain containing)	-1.94
Cul3	cullin 3	-1.76
Capn3	calpain 3	-1.74
Inhbb	inhibin beta-B	-1.72
Ppm1l	protein phosphatase 1 (formerly 2C)-like	-1.72
Apba3	amyloid beta (A4) precursor protein-binding, family A, member 3	-1.70
Ppp1cb	protein phosphatase 1, catalytic subunit, beta isoform	-1.70
Mylk2	myosin, light polypeptide kinase 2, skeletal muscle	-1.68
Acvr1b	activin A receptor, type 1B	-1.66
Smad6	MAD homolog 6 (Drosophila)	-1.63
Fkbp	FK506 binding protein	-1.63
Crebbp	CREB binding protein	-1.61
Map3k5	mitogen-activated protein kinase kinase kinase 5	-1.61
Ctsc	cathepsin C	-1.59
Ube2n	ubiquitin-conjugating enzyme E2N	-1.59
Prss23	protease, serine, 23	-1.57
Kcmf1	potassium channel modulatory factor 1	-1.56
Smad1	MAD homolog 1 (Drosophila)	-1.55
Pten	PTEN	-1.54
Ubr2	ubiquitin protein ligase E3 component n-recognin 2	-1.53
Ribosome biogenesis and assembly (Translation)		
Runx1	runt related transcription factor 1	23.76
Trp63	transformation related protein 63	4.50
Myt1l	myelin transcription factor 1-like	3.78
Gm6607	40S ribosomal protein S20 pseudogene	2.47
Rb1	retinoblastoma 1	2.47
Rpl3	ribosomal protein L3	2.39
Rpl13a	ribosomal protein L13A	2.19
Scx	Scleraxis	2.07
E2f4	E2F transcription factor 4	1.97
Etf1	eukaryotic translation termination factor 1	1.95
Eif4e	eukaryotic translation initiation factor 4E	1.86
Eif4a1	eukaryotic translation initiation factor 4A1	1.86

Rpl23	ribosomal protein L23	1.81
Eif6	eukaryotic translation initiation factor 6	1.77
Cdk7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.67
Eif1a	eukaryotic translation initiation factor 1A	1.64
Eef1a1	eukaryotic translation elongation factor 1 alpha 1	1.63
Eif2b2	eukaryotic translation initiation factor 2B, subunit 2 beta	1.54
Trp53	transformation related protein 53	1.54
Esr1	estrogen receptor 1 (alpha)	-1.73
Crebbp	CREB binding protein	-1.61
Bcor	BCL6 interacting corepressor	-1.58
Transcription		
Myog	myogenin	8.63
Sox11	SRY-box containing gene 11	7.12
Trp63	transformation related protein 63	4.50
Tead4	TEA domain family member 4	3.99
Myt1l	myelin transcription factor 1-like	3.78
Myod1	myogenic differentiation 1	2.73
Rb1	retinoblastoma 1	2.47
Hdac4	histone deacetylase 4	2.44
Junb	Jun-B oncogene	2.26
Scx	scleraxis	2.08
Eaf1	ELL associated factor 1	1.97
E2f4	E2F transcription factor 4	1.97
Mef2a	myocyte enhancer factor 2A	1.95
Baz1a	bromodomain adjacent to zinc finger domain 1A	1.93
Bzw1	basic leucine zipper and W2 domains 1	1.92
Myc	myelocytomatosis oncogene	1.90
Crem	cAMP responsive element modulator	1.79
Lbh	limb-bud and heart	1.73
Xbp1	X-box binding protein 1	1.73
Ndn	necdin	1.72
Gtf2f2	general transcription factor IIF, polypeptide 2	1.71
Jun	Jun oncogene	1.69
Cdk7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.67
Pcgf6	polycomb group ring finger 6	1.64
Fubp1	far upstream element (FUSE) binding protein 1	1.64
Atf6	activating transcription factor 6	1.58

Runx2	runt related transcription factor 2	1.55
Trp53	transformation related protein 53	1.54
Trp53bp1	transformation related protein 53 binding protein 1	1.53
Tceal1	transcription elongation factor A (SII)-like 1	1.52
Foxo	forkhead box	-1.99
Lbx1	ladybird homeobox homolog 1 (Drosophila)	-1.82
Hdac5	histone deacetylase 5	-1.80
Esr1	estrogen receptor 1 (alpha)	-1.73
Trim24	tripartite motif-containing 24	-1.65
Dnmt3a	DNA methyltransferase 3A	-1.65
Nfia	nuclear factor I/A	-1.64
Mxi1	Max interacting protein 1	-1.64
Smad6	MAD homolog 6 (Drosophila)	-1.63
Jarid2	jumonji, AT rich interactive domain 2	-1.59
Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	-1.58
Bcor	BCL6 interacting corepressor	-1.58
Smad1	MAD homolog 1 (Drosophila)	-1.55
Rcor3	REST corepressor 3	-1.55
Rere	arginine glutamic acid dipeptide (RE) repeats	-1.54
Ncor1	nuclear receptor co-repressor 1	-1.54
Gatad2b	GATA zinc finger domain containing 2B	-1.53
Rxra	retinoid X receptor alpha	-1.51
Mitf	microphthalmia-associated transcription factor	-1.50
Cell proliferation and cell cycle		
Tubb6	tubulin, beta 6	6.53
Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	5.19
Trp63	transformation related protein 63	4.50
Plekh	pleckstrin homology domain	2.64
Rb1	retinoblastoma 1	2.47
Prc1	protein regulator of cytokinesis 1	2.33
Ppap2a	phosphatidic acid phosphatase type 2A	2.17
Ckap2	cytoskeleton associated protein 2	2.09
Cgref1	cell growth regulator with EF hand domain 1	2.06
E2f4	E2F transcription factor 4	1.97
Col8a1	collagen, type VIII, alpha 1	1.90
Prkar1a	protein kinase, cAMP dependent regulatory, type I, alpha	1.85
Mapk1	mitogen-activated protein kinase 1	1.84

Egf	epidermal growth factor	1.69
Cdk7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)	1.67
Mapk6	mitogen-activated protein kinase 6	1.62
Runx2	runt related transcription factor 2	1.55
Il6st	interleukin 6 signal transducer	1.55
Trp53	transformation related protein 53	1.54
Gtpbp4	GTP binding protein 4	1.54
Shb	src homology 2 domain-containing transforming protein B	1.51
Mlf1	myeloid leukemia factor 1	-2.76
Pttg1	pituitary tumor-transforming gene 1	-2.70
Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	-2.68
Camk2a	calcium/calmodulin-dependent protein kinase II alpha	-2.18
Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	-2.11
Foxo	forkhead box	-1.99
Cul3	cullin 3	-1.76
Acvr1b	activin A receptor, type 1B	-1.66
Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	-1.58
Smad1	MAD homolog 1 (Drosophila)	-1.55
Ubr2	ubiquitin protein ligase E3 component n-recognin 2	-1.53
Cell differentiation		
Krt8	keratin 8	390.33
Krt18	keratin 18	187.70
Gdf5	growth differentiation factor 5	50.83
Runx1	runt related transcription factor 1	23.76
Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	15.79
Col19a1	collagen, type XIX, alpha 1	9.43
Myog	myogenin	8.63
Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	5.19
Trp63	transformation related protein 63	4.50
Tgfbr1	transforming growth factor, beta receptor I	3.02
Myod1	myogenic differentiation 1	2.73
Casp3	Caspase 3	2.49
Rb1	retinoblastoma 1	2.47
Musk	muscle, skeletal, receptor tyrosine kinase	2.46
Myf6	myogenic factor 6	2.29
Ckap2	cytoskeleton associated protein 2	2.09
Mapt	microtubule-associated protein tau	2.04

Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.97
Mef2a	myocyte enhancer factor 2A	1.95
Tnnt2	troponin T2, cardiac	1.92
Mapk1	mitogen-activated protein kinase 1	1.84
Stk17b	serine/threonine kinase 17b (apoptosis-inducing)	1.74
Akt1	thymoma viral proto-oncogene 1	1.59
Runx2	runt related transcription factor 2	1.55
Trp53	transformation related protein 53	1.54
Eif2b2	eukaryotic translation initiation factor 2B, subunit 2 beta	1.54
Mlf1	myeloid leukemia factor 1	-2.76
Tiam1	T-cell lymphoma invasion and metastasis 1	-2.70
Gadd45g	growth arrest and DNA-damage-inducible 45	-2.68
Cdh4	cadherin 4	-2.67
Prkg1	protein kinase, cGMP-dependent, type I	-2.47
Pparr	peroxisome proliferative activated receptor-γ	-2.38
Socs2	suppressor of cytokine signaling 2	-2.15
Foxo	forkhead box	-1.99
Myl2	Myosin, light polypeptide 2, regulatory, cardiac, slow	-1.78
Capn3	Calpain 3	-1.74
Plp1	proteolipid protein (myelin) 1	-1.69
Skeletal muscle development		
Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	15.79
Col19a1	collagen, type XIX, alpha 1	9.43
Myog	myogenin	8.63
Myod1	myogenic differentiation 1	2.73
Musk	muscle, skeletal, receptor tyrosine kinase	2.46
Myf6	myogenic factor 6	2.29
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.97
Tnnt2	troponin T2, cardiac	1.92
Mubl1	muscleblind-like 1 (drosophila)	-1.83
Myl2	myosin, light polypeptide 2, regulatory, cardiac, slow	-1.78
Capn3	calpain 3	-1.74
Signal transduction		
Krt8	keratin 8	390.33
Krt18	keratin 18	187.70
Rrad	Ras-related associated with diabetes	54.82
Gdf5	growth differentiation factor 5	50.83

Cdkn1a	Cyclin-dependent kinase inhibitor 1A (P21)	5.19
Trp63	transformation related protein 63	4.50
Tlr4	toll-like receptor 4	3.48
Myo10	myosin X	3.34
Rin1	Ras and Rab interactor 1	3.12
Tgfbr1	transforming growth factor, beta receptor I	3.02
Pde10a	phosphodiesterase 10A	2.99
Casp3	caspase 3	2.49
Musk	muscle, skeletal, receptor tyrosine kinase	2.46
Ppap2a	phosphatidic acid phosphatase type 2A	2.17
Map4k4	mitogen-activated protein kinase kinase kinase kinase 4	2.00
Fgf13	fibroblast growth factor 13	1.99
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1	1.97
Tgfb3	transforming growth factor, beta 3	1.95
Eif4a1	eukaryotic translation initiation factor 4A1	1.86
Eif4e	eukaryotic translation initiation factor 4E	1.86
Prkar1a	protein kinase, cAMP dependent regulatory, type I, alpha	1.85
Mapk1	mitogen-activated protein kinase 1	1.84
Adcy7	adenylate cyclase	1.84
Stam	Signal transducing adaptor molecule (SH3 domain and ITAM motif)	1.83
Eif6	eukaryotic translation initiation factor 6	1.77
Gprc5b	G protein-coupled receptor, family C, group 5, member B	1.70
Egf	epidermal growth factor	1.69
Prkce	protein kinase C, epsilon	1.64
Mknk2	MAP kinase-interacting serine/threonine kinase 2	1.63
Akt1	thymoma viral proto-oncogene 1	1.59
Atf6	activating transcription factor 6	1.58
Grb2	growth factor receptor bound protein 2	1.57
Il6st	interleukin 6 signal transducer	1.55
Trp53	transformation related protein 53	1.54
Gtpbp4	GTP binding protein 4	1.54
Rgs12	regulator of G-protein signaling 12	1.52
Tiam1	T-cell lymphoma invasion and metastasis 1	-2.70
Gadd45	growth arrest and DNA-damage-inducible 45	-2.68
Acvr2b	activin receptor IIB	-2.38
Plcl2	phospholipase C-like 2	-2.38
Pparr	peroxisome proliferative activated receptor-γ	-2.38

Timp3	tissue inhibitor of metalloproteinase 3	-2.15
Rcan2	regulator of calcineurin 2	-2.13
Foxo	forkhead box	-1.99
Iigp1	interferon inducible GTPase 1	-1.96
Atp2a2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	-1.95
Tns1	Tensin 1	-1.88
Wif1	Wnt inhibitory factor 1	-1.85
Itga6	integrin alpha 6	-1.75
Ppm1l	protein phosphatase 1 (formerly 2C)-like	-1.72
Inhbb	inhibin beta-B	-1.72
Acvr1b	activin A receptor, type 1B	-1.66
Smad6	MAD homolog 6 (Drosophila)	-1.63
Pkia	protein kinase inhibitor, alpha	-1.62
Crebbp	CREB binding protein	-1.61
Ube2n	ubiquitin-conjugating enzyme E2N	-1.59
Cited2	Cbp/p300-interacting transactivator, Glu/Asp-rich carboxy-terminal domain, 2	-1.58
Smad1	MAD homolog 1 (Drosophila)	-1.55
Pten	PTEN	-1.54

A total 1242 genes identified as differentially expressed genes were uploaded to DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on skeletal muscle growth. W, wild type mice; CLW, clenbuterol treated wild type mice.

Table 3.17 Differentially expressed gene clusters in the effect of CL in Mstn-pro (M vs CLM) based on skeletal muscle growth.

Ontology/Gene symbol	Gene name	Fold change
Muscle contraction		
Cnn3	Calponin 3, acidic	2.49
Utrn	utrophin	1.90
Trim63	tripartite motif-containing 63	1.81
Gaa	glucosidase, alpha, acid	1.59
Tnnc1	troponin C, cardiac/slow skeletal	-2.19
Tpm3	tropomyosin 3, gamma	-2.10
Carboxylic acid and amino acid metabolism		
Atf3	activating transcription factor 3	2.45
Tyrp1	tyrosinase-related protein 1	2.27
Gars	glycyl-trna synthetase	1.59
Asns	asparagine synthetase	1.56
Igh-6	immunoglobulin heavy chain 6 (heavy chain of igm)	-4.12
Ces3	carboxylesterase 3	-4.10
Pparr	peroxisome proliferative activated receptor-γ	-1.99
Mlycd	malonyl-coa decarboxylase	-1.84
Pdzd2	pdz domain containing 2	-1.79
Mdh1	malate dehydrogenase 1, nad (soluble)	-1.55
Polr3h	polymerase (rna) iii (dna directed) polypeptide h	-1.53
Idh1	isocitrate dehydrogenase 1 (nadp+), soluble	-1.53
Protein metabolic process		
Krt8	keratin complex 2, basic, gene 8	34.06
Ren1	renin 1 structural	10.72
Ptpn14	protein tyrosine phosphatase, non-receptor type 14	5.14
Tubb	tubulin, beta	4.77
Fkbp1	fk506 binding protein 12-rapamycin associated protein 1	3.38
Gm6607	40s ribosomal protein s20 pseudogene	2.98
Tgfbr1	transforming growth factor, beta receptor i	2.91
Arhgef2	rho/rac guanine nucleotide exchange factor (gef) 2	2.72
Eif4a1	eukaryotic translation initiation factor 4a, pseudogene 4	2.64
Musk	muscle, skeletal, receptor tyrosine kinase	2.50
Tlr4	toll-like receptor 4	2.30
Tubb6	tubulin, beta 6	2.28
Ppme1	protein phosphatase methylesterase 1	2.12

Ptprd	protein tyrosine phosphatase, receptor type, d	2.04
Rpl3	ribosomal protein l3	1.99
Eif1a	eukaryotic translation initiation factor 1a	1.94
Arpc5l	actin related protein 2/3 complex, subunit 5-like	1.93
Mapt	microtubule-associated protein tau	1.90
Prkg2	protein kinase, cgmp-dependent, type ii	1.86
Gprk5	G protein-coupled receptor kinase 5	1.79
Atf6	activating transcription factor 6	1.74
Map2k2	mitogen activated protein kinase kinase 2	1.73
Rpl37a	ribosomal protein l37a	1.73
Eif4g2	eukaryotic translation initiation factor 4, gamma 2	1.70
Ttf2	transcription termination factor, rna polymerase ii	1.68
Pa2g4	proliferation-associated 2g4	1.68
Eif4e	eukaryotic translation initiation factor 4e	1.65
Eif2b3	eukaryotic translation initiation factor 2b, subunit 3	1.59
Cdk7	cyclin-dependent kinase 7 (homolog of xenopus mo15 cdk-activating kinase)	1.57
Vcpip1	valosin containing protein (p97)/p47 complex interacting protein 1	-4.19
Gadd45g	growth arrest and dna-damage-inducible 45 gamma	-3.63
Capn3	calpain 3	-2.78
Serpinb1a	serine (or cysteine) peptidase inhibitor, clade b, member 1a	-2.62
Acvr2b	activin receptor iib	-2.35
Pten	PTEN	-2.27
Ube2d1	ubiquitin-conjugating enzyme e2d 1, ubc4/5 homolog (yeast)	-2.23
Mylk2	myosin, light polypeptide kinase 2, skeletal muscle	-2.22
Ppp2	protein phosphatase 2	-2.19
Acvr1b	activin a receptor, type 1b	-2.12
Acvr1	activin a receptor, type 1	-2.12
Usp15	ubiquitin specific peptidase 15	-2.07
Ppargc1a	caspase 7	-1.93
Ctsc	cathepsin c	-1.92
Gpx3	glutathione peroxidase 3	-1.90
Pdk2	pyruvate dehydrogenase kinase, isoenzyme 2	-1.89
Ctsb	cathepsin b	-1.88
Pias2	protein inhibitor of activated stat 2	-1.85
Ppm1j	protein phosphatase 1j	-1.66
Cebpg	ccaat/enhancer binding protein (c/ebp), gamma	-1.60
Prkrir	protein-kinase, interferon-inducible double stranded rna dependent inhibitor	-1.59

Gm15361	ubiquitin-conjugating enzyme e2d 3 (ubc4/5 homolog, yeast)	-1.59
Dstn	destrin	-1.50
Ribosome biogenesis and assembly (translation)		
Gm6607	40s ribosomal protein s20 pseudogene	2.98
Eif4a1	eukaryotic translation initiation factor 4a, pseudogene 4	2.64
Tlr4	toll-like receptor 4	2.30
Rpl3	ribosomal protein l3	1.99
Nhp2	nucleolar protein family a, member 2	1.97
Eif1a	eukaryotic translation initiation factor 1a	1.94
Gart	phosphoribosylglycinamide formyltransferase	1.84
Rpl37a	ribosomal protein l37a	1.73
Gfm2	G elongation factor, mitochondrial 2	1.72
Eif4g2	eukaryotic translation initiation factor 4, gamma 2	1.70
Pa2g4	proliferation-associated 2g4	1.68
Eif4e	eukaryotic translation initiation factor 4e	1.65
Rpl13a	ribosomal protein l13a	1.61
Gm2614	ribosomal protein s17	1.61
Wdr46	wd repeat domain 46	1.60
Eif2b3	eukaryotic translation initiation factor 2b, subunit 3	1.59
Gars	glycyl-trna synthetase	1.59
Rpl3l	ribosomal protein l3-like	-2.28
Eif1ay	eukaryotic translation initiation factor 1a, y-linked	-2.07
Cebp	ccaat/enhancer binding protein (c/ebp)	-1.60
Transcription		
Runx1	runt related transcription factor 1	15.90
Sox11	sry-box containing gene 11	6.17
Trp63	transformation related protein 63	3.32
Scx	scleraxis	3.29
Atf3	activating transcription factor 3	2.45
Nfat5	nuclear factor of activated t-cells 5	2.29
Runx2	runt related transcription factor 2	2.17
Rb1	retinoblastoma 1	1.92
Mef2a	myocyte enhancer factor 2a	1.88
Med15	positive cofactor 2, multiprotein complex, glutamine/q-rich-associated protein	1.80
Edf1	endothelial differentiation-related factor 1	1.75
Ttf2	transcription termination factor, rna polymerase ii	1.68
Pa2g4	proliferation-associated 2g4	1.68

Cdk7	cyclin-dependent kinase 7 (homolog of xenopus mo15 cdk-activating kinase)	1.57
Tcea3	transcription elongation factor a (sii), 3	-2.46
Ppp2	protein phosphatase 2	-2.19
Tsc	TSC domain	-2.12
Pparr	peroxisome proliferative activated receptor-γ	-1.99
Pias2	protein inhibitor of activated stat 2	-1.85
Foxn3	checkpoint suppressor 1	-1.81
Ctcf	ccctc-binding factor	-1.62
Cebp	ccaat/enhancer binding protein (c/ebp)	-1.60
Cell proliferation and cell cycle		
Krt18	keratin complex 1, acidic, gene 18	144.41
Gdf5	growth differentiation factor 5	77.47
Krt8	keratin complex 2, basic, gene 8	34.06
Runx1	runt related transcription factor 1	15.90
Myog	myogenin	8.47
Cdkn1a	cyclin-dependent kinase inhibitor 1a (p21)	6.72
Fkbp1	fk506 binding protein 12-rapamycin associated protein 1	3.38
Trp63	transformation related protein 63	3.32
Tgfbr1	transforming growth factor, beta receptor i	2.91
Tgfa	transforming growth factor alpha	2.91
Notch2	notch gene homolog 2 (drosophila)	2.71
Musk	muscle, skeletal, receptor tyrosine kinase	2.50
Sgms1	transmembrane protein 23	2.40
Tubb6	tubulin, beta 6	2.28
Plekh	pleckstrin homology domain	2.26
Runx2	runt related transcription factor 2	2.17
Shb	src homology 2 domain-containing transforming protein b	2.14
Neb	nebulin	2.10
Rb1	retinoblastoma 1	1.92
Cgref1	cell growth regulator with ef hand domain 1	1.82
Rhou	ras homolog gene family, member u	1.77
Eif4g2	eukaryotic translation initiation factor 4, gamma 2	1.70
Eif2b3	eukaryotic translation initiation factor 2b, subunit 3	1.59
Cdk7	cyclin-dependent kinase 7 (homolog of xenopus mo15 cdk-activating kinase)	1.57
Mlf1	myeloid leukemia factor 1	-3.74
Gadd45g	growth arrest and dna-damage-inducible 45 gamma	-3.63
Cdh4	cadherin 4	-3.38

Mapre2	microtubule-associated protein, rp/eb family, member 2	-3.35
Capn3	calpain 3	-2.78
Gadd45b	growth arrest and dna-damage-inducible 45 beta	-2.69
Calm2	calmodulin 1	-2.28
Sesn1	sestrin 1	-2.21
Acvr1b	activin a receptor, type 1b	-2.12
Acvr1	activin a receptor, type 1	-2.12
Ppargc1a	caspase 7	-1.93
Socs2	suppressor of cytokine signaling 2	-1.82
Foxo	forkhead box	-1.81
Mtus1	mitochondrial tumor suppressor 1	-1.72
Bnip3l	bcl2/adenovirus e1b interacting protein 3-like	-1.67
Pds5b	androgen-induced proliferation inhibitor	-1.67
Cdkn2d	cyclin-dependent kinase inhibitor 2d (p19, inhibits cdk4)	-1.66
Ctcf	ccctc-binding factor	-1.62
Cebpg	ccaat/enhancer binding protein (c/ebp), gamma	-1.60
Cell differentiation		
Krt18	keratin complex 1, acidic, gene 18	144.41
Gdf5	growth differentiation factor 5	77.47
Krt8	keratin complex 2, basic, gene 8	34.06
Runx1	runt related transcription factor 1	15.90
Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	9.72
Myog	myogenin	8.47
Cdkn1a	cyclin-dependent kinase inhibitor 1a (p21)	6.72
Col19a1	procollagen, type xix, alpha 1	5.13
Fkbp1	fk506 binding protein 12-rapamycin associated protein 1	3.38
Trp63	transformation related protein 63	3.32
Tgfbr1	transforming growth factor, beta receptor i	2.91
Notch2	notch gene homolog 2 (drosophila)	2.71
Musk	muscle, skeletal, receptor tyrosine kinase	2.50
Sgms1	transmembrane protein 23	2.40
Runx2	runt related transcription factor 2	2.17
Shb	src homology 2 domain-containing transforming protein b	2.14
Neb	nebulin	2.10
Rb1	retinoblastoma 1	1.92
Mef2a	myocyte enhancer factor 2a	1.88
Myf6	myogenic factor 6	1.86

Acsl6	acyl-coa synthetase long-chain family member 6	1.83
Edf1	endothelial differentiation-related factor 1	1.75
Eif4g2	eukaryotic translation initiation factor 4, gamma 2	1.70
Eif2b3	eukaryotic translation initiation factor 2b, subunit 3	1.59
Rtn4	reticulon 4	1.57
Gadd45g	growth arrest and dna-damage-inducible 45 gamma	-3.63
Cdh4	cadherin 4	-3.38
Capn3	calpain 3	-2.78
Acvr1	activin a receptor, type 1	-2.12
Tirap	toll-interleukin 1 receptor (tir) domain-containing adaptor protein	-2.09
Pparr	peroxisome proliferative activated receptor-γ	-1.99
Bnip3l	bcl2/adenovirus e1b interacting protein 3-like	-1.67
Skeletal muscle development		
Myog	myogenin	8.47
Col19a1	procollagen, type xix, alpha 1	5.13
Musk	muscle, skeletal, receptor tyrosine kinase	2.50
Neb	nebulin	2.10
Fkbp12	fk506 binding protein 12-rapamycin associated protein 1	1.89
Myf6	myogenic factor 6	1.86
Capn3	calpain 3	-2.78
Dmd	dystrophin, muscular dystrophy	-2.08
Signal transduction		
Gdf5	growth differentiation factor 5	77.47
Rrad	ras-related associated with diabetes	38.79
Cdkn1a	cyclin-dependent kinase inhibitor 1a (p21)	6.72
Plch1	phospholipase c-like 3	3.94
Fkbp1	fk506 binding protein 12-rapamycin associated protein 1	3.38
Trp63	transformation related protein 63	3.32
Rhoc	ras homolog gene family, member c	3.10
Tgfbr1	transforming growth factor, beta receptor i	2.91
Tgfa	transforming growth factor alpha	2.91
Gprc5b	G protein-coupled receptor, family c, group 5, member b	2.89
Myo10	myosin x	2.82
Notch2	notch gene homolog 2 (drosophila)	2.71
Musk	muscle, skeletal, receptor tyrosine kinase	2.50
Sh3	sh3 domain	2.30
Tlr4	toll-like receptor 4	2.30

Runx2	runt related transcription factor 2	2.17
Shb	src homology 2 domain-containing transforming protein b	2.14
Itsn1	intersectin 1 (sh3 domain protein 1a)	2.13
Fgf13	fibroblast growth factor 13	2.08
Ptprd	protein tyrosine phosphatase, receptor type, d	2.04
Rgs12	regulator of g-protein signaling 12	1.88
Gprk5	G protein-coupled receptor kinase 5	1.79
Rhou	ras homolog gene family, member u	1.77
Stam2	signal transducing adaptor molecule (sh3 domain and itam motif) 2	1.77
Adcy2	adenylate cyclase 2	1.63
Ltbp3	latent transforming growth factor beta binding protein 3	1.55
Pik3r3	phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3 (p55)	1.54
Gadd45g	growth arrest and dna-damage-inducible 45 gamma	-3.63
Ppp1r1a	protein phosphatase 1, regulatory (inhibitor) subunit 1a	-3.14
Acvr2b	activin receptor iib	-2.35
Calm2	calmodulin 1	-2.28
Pten	PTEN	-2.27
Ppp2	protein phosphatase 2	-2.19
Acvr1b	activin a receptor, type 1b	-2.12
Acvr1	activin a receptor, type 1	-2.12
Timp3	tissue inhibitor of metalloproteinase 3	-2.02
Pkia	protein kinase inhibitor, alpha	-2.01
Socs2	suppressor of cytokine signaling 2	-1.82
Hipk3	homeodomain interacting protein kinase 3	-1.76
Rsu1	ras suppressor protein 1	-1.59
Raf1	v-raf-leukemia viral oncogene 1	-1.56

A total 1521 genes identified as differentially expressed genes were uploaded to DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on skeletal muscle growth. M, Mstn-pro mice; CLM, clenbuterol treated Mstn-pro mice.

Table 3.18 Differentially expressed gene clusters by the effect of genotype and CL (W vs M VS W vs CLW) based on skeletal muscle growth.

Ontology/Gene symbol	Gene name
Carboxylic acid and amino acid metabolism	
Ddc	dopa decarboxylase
Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1
Tubb2a	tubulin, beta 2A
Tubb6	tubulin, beta 6
Protein metabolic process	
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1
Tubb2a	tubulin, beta 2A
Tubb6	tubulin, beta 6
Cell differentiation	
Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1
Tnnt2	troponin T2, cardiac
Skeletal muscle development	
Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)
Col19a1	collagen, type XIX, alpha 1
Pak1	p21 protein (Cdc42/Rac)-activated kinase 1

A total 40 genes identified as differentially expressed genes were uploaded to DAVID bioinformatics software (Dennis et al., 2003) to functionally classify the genes based on skeletal muscle growth. W, wild type mice; M, Mstn-pro mice; CLW, clenbuterol treated wild type mice.

Table 3.19 Summary of previous reports describing the known molecular effect of Mstn-suppresion-induced hypertrophy in skeletal muscle.

Title	References	Comparison to our study
Genes encoding muscle contractile proteins	**Steelman et al. (2006):** There was a decreased expression of genes for slow isoforms of myosin (Myh7, Myl2, and Myl3) and troponin (Tnnc1, Tnni1, Tnnt1, and Tpm3) In muscle from myostatin null mice. **Cassar-Malek et al. (2007):** Constitutive non-functional mutation of Mstn decreased the expression of slow forms of contractile proteins (Myh7, Myl2, and Tpm3). **Sadkowski et al. (2008):** There was increased expression of genes for fast isoforms of myosin (Myh3) In skeletal muscle carrying a SNP in the 5'flanking region of the Mstn gene in bulls, while slow isoforms of myosin (Myh8, Myo5a, Myl6) were decreased. **Welle et al. (2009):** Gene encoding slow isoforms of several contractile proteins (Myh7, Myl2, Myl3, Tnnc1, Tnni1, Tnnt1 and Tpm3) are downregulated 4- to 10-fold in mice muscle with constitutive Mstn deficiency, none of the effects was statistically significant.	In this study, we could not observe any alteration in gene expression encoding myofibrillar proteins in Mstn-pro mice similar to previous studies, except the differential expression of Tnnt2, which is mostly expressed during embryonic development.
Genes encoding proteins involved in transcription	**Steelman et al. (2006):** In muscle from myostatin null mice, there was an increased expression of Wnt4 gene, which is known to play a role in embryonic myogensis. **Welle et al. (2009):** They did not observe any genes encoding transcription.	In this study, we observed that the 3 transcription factor genes were differentially expressed by Mstn-pro overexpression, including Runx1, Dnmt1, and Sreb1. However, studies by other groups did not observe that these genes were differentially regulated by Mstn modulation
Genes encoding proteins involved in immune response	**Welle et al. (2009) and Steelman et al. (2006):** They did not observe any genes encoding immune response.	In this study study, we observed that immune response genes were differentially expressed by Msnt-pro overexpression, including Mt3. However, studies by other groups did not observe that this gene was differentially regulated by Mstn modulation
Genes encoding proteins involved in muscle hypertrophic signal pathway	**Steelman et al. (2006) and Welle et al. (2009):** They did not observe any genes encoding muscle hypertriphic signal pathway.	In this study, we observed that Igf2 was differentially expressed in the skeletal muscle of Mstn-pro mice even though previous studies have not observed that Igf2 is regulated by Mstn.

Table 3.20 Comparison of the genes describing the effect of Mstn-suppression-induced hypertrophy in skeletal muscle between previous studies and current study.

Gene	Steelman et al. (2006)	Welle et al. (2009)	In current study
Myosin heavy polypeptide 7	Down	NS	-
Myosin light polypeptide 2	Down	NS	-
Myosin light polypeptide 3	Down	NS	-
Troponin C, cardiac/slow skeletal	Down	NS	-
Troponin I, skeletal, slow 1	Down	NS	-
Troponin T1, skeletal, slow	Down	NS	-
Tropomyosin 3, gamma	Down	NS	-
Troponin T2, cardiac	-	-	Down
Wnt4	-	Up	-
Runt related trasncription factor 1	-	-	Up
sterol regulatory element binding transcription factor 1	-	-	Down
metallothionein 3	-	-	Up
insulin-like growth factor 2	-	-	Up
nitric oxide synthase 1	-	Down	-
dimethylarginine dimethylaminohydrolase 1	-	Down	-
dynein light chain LC8-type 1	-	Down	-
prosaposin	-	Down	-
HtrA serine peptidase 2	-	Down	-
3-hydroxyisobutyryl-coenzyme A hydrolase	-	Up	-
glutamate-ammonia ligase (glutamine synthetase)	-	Up	-
cytochrome b5 type B	-	Up	-
acyl-coenzyme A dehydrogenase, short/branched chain	-	Up	-
cimilar to UDP glycosyltransferase family	-	Up	-
peroxisome proliferator-activated receptor gamma, coactivator 1 α	-	Down	-
protein phosphatase 1, regulatory (inhibitor) subunit 3C	-	Up	-
acyl-CoA synthetase long-chain family member 1	-	Up	-
elastin	-	Down	-
procollagen C-endopeptidase enhancer protein	-	Down	-
zinc finger, MYND-type containing 17	-	Down	-
family with sequence similarity 19, member A5	-	Down	-
cerebral endothelial cell adhesion molecule	-	Down	-
BTB (POZ) domain containing 1	-	Down	-
Ras-related associated with diabetes	-	Down	-

myosin, light chain 4, alkali	-	Down	-
prostate transmembrane protein, androgen induced 1	-	Down	-
sulfotransferase family 5A, member 1	-	Down	-
keratin 18	-	Down	-
deoxynucleotidyltransferase, terminal	-	Up	-
zinc finger protein 318	-	Up	-
chemokine (C-C motif) ligand 25	-	Up	-
receptor accessory protein 6	-	Up	-
rhophilin, Rho GTPase binding protein 2	-	Up	-
nephronectin	-	Up	-
cadherin 4	-	Up	-
Collagen I,α1	Down	-	-
Collagen I,α2	Down	-	-
Collagen III,α1	Down	-	-
Collagen IV,α1	Down	-	-
Collagen IV,α4	Down	-	-
Collagen V,α1	Down	-	-
Collagen V,α2	Down	-	-
Collagen VI,α1	Down	-	-
Collagen VI,α2	Down	-	-
Collagen VI,α3	Down	-	-
Collagen XVI,α1	Down	-	-

Up, upregulated gene; Down, downregulated gene, NS, not significant.

Table 3.21 Summary of previous reports describing the known molecular effect of CL administration-induced hypertrophy in skeletal muscle.

Title	References	Comparison to our study
Genes encoding muscle contractile proteins	**Oishi et al. (2002):** CL administration resulted in muscle fiber hypertrophy, stimulated a de novo expression of type IIx MHC and increased the percentage of fibers containing multiple MHC isoforms in the rat soleus muscle. **Spurlock et al. (2006):** The differentially expressed genes by CL administration increase the expression of several contractile proteins, including fast isoforms of myosin (Myo1b, Myo18a), actin (Actn4) in male mice **Pearen et al. (2009):** No significant changes were observed in contractile proteins at 1 and 4 hours by beta-agonists in mice muscle. However, they found that alterations in Stat3, Smad 3, and Acvr2b three genes directly associated with the regulation of muscle hypertophy.	In this study, we observed that Myh8, Tnnt2, Myl2, and Mylk2 were altered by CL administration even though previous studies have not observed that the genes are regulated by CL administration. At molecular levels, we found the differentially expressed Acvr2b gene by CL administration similar to Pearen et al. (2009) study.
Genes encoding proteins involved in translation and transcription	**Spurlock et al. (2006):** The increase expressions of 13 genes encoding initiator of protein translation factors (Eif2, Eif2b, Eif2b4, Eif2b2, Eif5, and Eif4g3) and 18 genes encoding protein transcription factors (Hod, Yy1, Mef2c, and Myog) were observed mainly at 24h after CL administration. **Pearen et al. (2009):** The increase expressions of 11 genes encoding transcriptional regulators (Fos, Klf2/4, Crem, Cebpb, Nurr1, Fosl2, Maff, Aft3, Tbx3, and Litaf), but no significant changes were observed in translation proteins at 1 and 4 hours by CL administration in mice muscle.	In this study, we observed a regulation of translational and transcriptional factors responsible for increasing protein synthesis. The 22 translation genes, including Gm6607, Rpl3, Rpl13a, Rpl23, Eif1, Eif4a1, Eif4e, and the 48 transcription genes, including Myod1, Mef2a, were differentially expressed by CL administration even though previous studies have not observed that the genes are regulated by CL administration. However, we found the differentially expressed Eif2b and Myog gene similar to Spurlock et al. (2006) study.
Genes encoding proteins involved in muscle hypertrophic signal pathway	**Spurlock et al. (2006):** They reported that Igf1 and Tss22d1 genes were altered in mouse skeletal muscle 24 hours after administration of CL. **Pearen et al. (2009):** They are reported that FoxO1 gene was altered in mice after systemic administration of formorterol.	In this study, we observed that eIF4E and PTEN were differentially expressed in the skeletal muscle of CL administration even though previous studies have not observed that eIF4E and PTEN are regulated by CL administration. However, we found the differentially expressed FoxO gene by CL administration similar to Pearen et al. (2009) study.

Table 3.22 Comparison of the genes describing the effect of BAA administration-induced hypertrophy in skeletal muscle between previous studies and current study.

Gene Name	Pearen et al. (2009)	Spurlock et al. (2006)		In current sutdy
		Change 24h	Change10D	
40S ribosomal protein S20 pseudogene	-	-	-	Up
activin receptor IIB	-	-	-	Down
ADP-ribosyltransferase 1	-	Down	NS	Down
Alkaline phosphatase 2	Up	-	-	-
Alpha Tubulin 6	Up	-	-	-
Alpha-kinase 2	Up	-	-	-
Angiotensinogen	-	Up	Up	-
ankyrin repeat and BTB (POZ) domain containing 2	-	-	-	Down
ankyrin repeat and SOCS box-containing 10	-	Down	NS	Down
ankyrin repeat and SOCS box-containing 14	-	Down	NS	Down
Ankyrin repeat and SOCS box-containing protein 15	-	Down	NS	-
Aryl hydrocarbon receptor nuclear translocator-like	-	Up	NS	-
ATPase, H+ transporting, V0 subunit E	-	Up	Up	-
ATPase, H+ transporting, V1 subunit B, isoform 2	Up	-	-	-
Avian musculoaponeurotic fibrosarcoma (v-maf)	Up	-	-	Down
Bromodomain containing 4	-	Down	Down	-
cAMP responsive element modulator	Up	-	-	Up
CCAAT/enhancer binding protein beta	Up	-	-	-
CCR4 carbon catabolite repression 4-like	Up	-	-	-
cell growth regulator with EF hand domain 1	-	-	-	Up
Chemokine (C-C motif) ligand 9	Up	-	-	-
Chemokine ligand 12	-	Up	NS	-
Contactin associated protein-like 2	-	Down	Down	Down
Cryptochrome 2	Up	-	-	-
Cyclin-dependent kinase inhibitor 1A	Up	Up	Up	Up
Cyclin-dependent kinase inhibitor 1C	Down	-	-	-
Cytokine induced apoptosis inhibitor 1	Up	-	-	-
D site albumin promoter binding protein	Down	-	-	-
Dehydrogenase/reductase (SDR family) member 7	-	Down	Down	Down
DNA segment, Chr 19, Wayne State University 162	Up	-	-	-
DNA-damage-inducible transcript 4-like	Down	Up	Up	-
Down syndrome critical region gene 1-like 1	-	Down	Down	-
Downstream of Stk11	Up	-	-	-

EGL nine homolog 3	Up	-	-	Up
Emerin	Up	Up	NS	-
Endothelin 1	-	Up	Up	-
Enhancer of yellow 2 homolog	-	Up	NS	Up
eukaryotic translation elongation factor 1	-	-	-	Up
eukaryotic translation initiation factor 1A	-	-	-	Up
Eukaryotic translation initiation factor 2B, subunit 2 beta	-	Up	NS	Up
Eukaryotic translation initiation factor 2B, subunit 4 delta	-	Up	NS	-
Eukaryotic translation initiation factor 4 gamma, 3	-	Down	NS	-
Eukaryotic translation initiation factor 4A1	-	Up	Up	Up
eukaryotic translation initiation factor 4E	-	-	-	Up
eukaryotic translation initiation factor 4E member 2	-	-	-	Up
Eukaryotic translation initiation factor 5	-	Up	NS	-
Excision repair cross-complementing rodent repair deficiency	Up	-	-	-
F-box only protein 34	Up	-	-	-
Fibroblast growth factor 1	Up	-	-	-
forkhead box	-	-	-	Down
Forkhead box O1	Up	-	-	-
Fos-like antigen 2	Up	-	-	-
Fructose bisphosphatase 2	-	Up	Up	-
Glutamic pyruvate transaminase 2	Up	-	-	-
Growth arrest and DNA-damage-inducible 45 alpha	Up	-	-	Up
Growth arrest and DNA-damage-inducible 45 gamma	-	Down	NS	Down
Growth hormone receptor	-	Down	Down	-
heat shock protein 1	-	-	-	Up
heat shock protein 4	-	-	-	Up
heat shock protein 8	-	-	-	Up
heat shock protein 90, alpha (cytosolic), class A member 1	-	-	-	Up
heat shock protein family, member 7	-	-	-	Up
heat shock transcription factor 2 binding protein	-	-	-	Up
Hexokinase 2 (Hk2)	Up	-	-	-
Histidyl-tRNA synthetase	-	Up	NS	-
Histone 1, H2ai	Down	-	-	-
Histone 1, H2ao	Down	-	-	-
Homebox only domain	-	Up	NS	-
Imprinted and ancient	Up	-	-	Up
Inhibitor of DNA binding 1	Up	-	-	-

Insulin-like growth factor 1	-	Up	NS	-
Insulin-like growth factor 2 receptor	-	Down	Down	-
Insulin-like growth factor binding protein 5	-	Down	Down	-
Integrin beta 1 binding protein 3	Up	-	-	-
Interferon gamma inducible protein 30	Up	-	-	-
Interleukin 6 signal transducer	-	Up	Up	Up
Kelch repeat and BTB (POZ) domain containing 5	-	Up	NS	-
Kinesin family member 1B (Kif1b), transcript variant 1	Down	-	-	Down
Kruppel-like factor 4 (gut)	Up	-	-	-
Lady bird-like homeobox 1 homolog	-	Down	Down	Down
Lectin, galactose binding, soluble 3	Up	-	-	Up
Lipin 1 (Lpin1)	Up	-	-	Down
LPS-induced TN factor (Litaf)	Up	-	-	-
Lymphocyte antigen 6 complex, locus A	Up	-	-	-
MAP kinase-interacting serine/threonine kinase 2	Up	-	-	-
Metallothionein (Mt1)	Up	Up	Up	-
Metallothionein 2 (Mt2)	Up	-	-	-
Microfibrilar-associated protein 3-like	-	NS	Up	-
Microtubule-associated protein 1 light chain 3 alpha	Up	-	-	Up
Midnolin (Midn)	Up	-	-	-
Mitogen activated protein kinase kinase 4	-	Up	NS	-
Mitogen activated protein kinase kinase 6	-	Down	NS	Down
Mitogen-activated protein kinase kinase kinase 6	Up	-	-	-
Myeloid/lymphoid or mixed-lineage leukaemia	-	Down	NS	-
myocyte enhancer factor 2A	-	-	-	Up
Myocyte enhancer factor 2C	-	Down	NS	-
myogenic differentiation 1	-	-	-	Up
Myogenin	-	Up	NS	Up
Myosin IB	-	Down	NS	-
myosin IC	-	-	-	Up
myosin X	-	-	-	Up
Myosin XVIIIa	-	Up	Up	-
myosin, heavy polypeptide 8, skeletal muscle, perinatal	-	-	-	Up
myosin, light polypeptide 2, regulatory, cardiac, slow	-	-	-	Down
myosin, light polypeptide 4	-	-	-	Up
Myosin, light polypeptide kinase 2, skeletal muscle	-	Down	NS	-
myosin, light polypeptide kinase 2, skeletal muscle	-	-	-	Down

Neuraminidase 1	-	Up	NS	-
Nuclear factor, interleukin 3, regulated	Up	-	-	-
Nuclear receptor subfamily 4, group A, member 1	-	Down	Down	Down
Nuclear receptor subfamily 4, group A, member 2	Up	-	-	-
Optineurin	Up	-	-	-
Ornithine decarboxylase, structural	-	Up	Up	Up
pleckstrin homolog domains 1	-	Up	Up	-
Pannexin 1	-	Up	Up	Up
Peroxisome proliferator activator receptor delta (Pparδ)	Up	-	-	-
Phosphodiesterase 4D (Pde4d)	Up	-	-	-
Phosphomevalonate kinase (Pmvk)	Up	-	-	-
Plasma membrane associated protein	Up	-	-	-
Platelet derived growth factor alpha	Down	-	-	-
Polo-like kinase 3 (Plk3)	Up	-	-	-
PPARγ coactivator 1 alpha (Pgc-1α)	Up	-	-	-
Protein kinase, cGMP-dependent, type 1	-	Down	NS	Down
Protein phosphatase 1 (formerly 2C)-like	-	Down	NS	Down
Protein phosphatase 1 regulatory subunit 3C	Up	-	-	-
PTEN	-	-	-	Down
Pyruvate dehydrogenase kinase 4	Up	-	-	-
retinoblastoma 1	-	-	-	Up
ribosomal protein L13A	-	-	-	Up
ribosomal protein L23	-	-	-	Up
ribosomal protein L3	-	-	-	Up
ribosomal protein L3-like	-	-	-	Down
ribosomal protein S25	-	-	-	Up
ribosomal protein S6 kinase, polypeptide 2	-	-	-	Down
ribosomal protein S6 kinase, polypeptide 5	-	-	-	Down
Ring finger protein 128	-	Up	Up	Up
S100 calcium binding protein A8	Up	-	-	Up
S-adenosylmethionine decarboxylase 1	-	Down	Down	Down
Scavenger receptor class B member 1	Down	-	-	-
Serine (or cysteine) preptidase inhibitor, clade A, member 1b	-	Up	Up	-
Seven in absentia 2	-	Up	Up	-
SH3 domain protein	-	-	-	Up
Signal transducer and activator of transcription 3	Up	-	-	-
Small chemokine (C-C motif) ligand 11	Up	-	-	-

Small mothers against decapentaplegic homolog 1	Down	-	-	-
Small mothers against decapentaplegic homolog 3	Up	-	-	-
Sodium-dependent vitamin C transporter 2 (Slc23a2)	Up	-	-	-
solute carrier family 10, member 3	Up	-	-	Up
Solute carrier family 7, member 2	-	Down	NS	Down
Solute carrier family 7, member 5	-	Up	Up	-
Sorbin and SH3-domains containing 1	Up	-	-	-
Spermine oxidase	-	Down	NS	-
Ssemaphorin 3F	Up	-	-	-
Sulfiredoxin 1 homolog	Up	-	-	-
Synaptopodin 2-like	Down	-	-	Down
Syndecan 4	Up	-	-	Up
T-box 3 (Tbx3), transcript variant 2	Up	-	-	-
TG interacting factor	Up	-	-	-
Thimet oligopeptidase 1	-	Up	NS	-
TSC22 domain family, member 1	-	Down	NS	-
TSR 1, 20S rRNA accumulation, homolog	-	Up	NS	Up
Tubulin, alpha 1A	-	-	-	Up
Tubulin, alpha 1B	--	-	-	Up
Tubulin, alpha 4A	-	-	-	Up
Tubulin, beta 2A	-	-	-	Up
Tubulin, beta 2B	-	-	-	Up
Tubulin, beta 2C	-	-	-	Up
Tubulin, beta 5	-	Up	Up	-
Tubulin, beta 6	-	Up	Up	Up
Tubulointerstitial nephritis antigen-like	-	Up	Up	-
Tumor necrosis factor receptor superfamily, member 12a	Up	Up	NS	Up
Tweety homolog 1 (Drosophila)	Up	-	-	-
Ubiquitin C	Up	-	-	-
Ubiquitin G	Up	-	-	-
Ubiquitin protein ligase E3 component n-recognin 1	-	n/s	Up	-
Ubiquitin specific protease 2 (Usp2), transcript variant 2	Up	-	-	-
Uncoupling protein 3	Up	-	-	-
Vasodilator-stimulated phosphoprotein	Down	-	-	-
Vinculin	-	Up	Up	-
v-maf musculoaponeurotic fibrosarcoma oncogene, protein B	Up	-	-	Down
Y box protein 3 (Ybx3)	Up	-	-	-

YY1 transcription factor	-	Up	Up	-
Zinc finger protein 46 (Zfp46)	Down	-	-	-

Up, upregulated gene; Down, downregulated gene, NS, not significant

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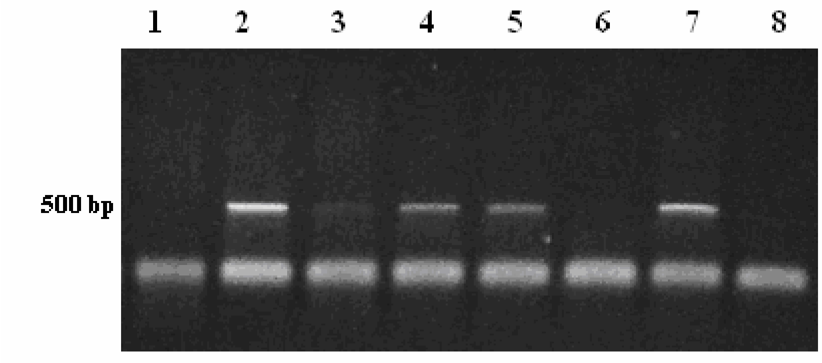
4.2 APPENDICES

APPENDIX 2.1 Agarose gel electrophoresis of genotype



1.2% agarose gel electrophoresis gel stained with ethidium bromide. Lane 1, Wild type mice; Lane 2, Transgnice mice; Lane 3, Transgnice mice; Lane 4, Transgnice mice; Lane 5, Transgnice mice; Lane 6, Wild type mice; Lane 7, Transgnice mice; Lane 8, Wild type mice.

APPENDIX 2.2 The female raw data at 7day.

		wt	wt	C0d	C3d	C7d					mg/g of muscle							Adj. Vol CNT*mm2			confirm
No	wean	3d	5d	7d	10d	14d	GAS	Sol	Plan	E D L	RNA	DNA	Ratio	%Gas	%pl	%sol	%EDL	4EBP1	p70S6k	CL	Geno
689	12.1	14.1	14.7	16.2	16.2	16.2	0.0717	0.0036	0.0079	0.0056	1.2311	1.1674	1.0546	0.4426	0.0488	0.0222	0.0346	1976.3	2074.6	con	Wild
690	12.1	14.6	15.6	17.2	17.2	17.2	0.0730	0.0035	0.0080	0.0061	1.3281	1.2306	1.0792	0.4244	0.0465	0.0203	0.0352	1901.5	1929.5	con	Wild
691	13.2	15.4	16.4	19.4	19.2	19.6	0.0968	0.0030	0.0093	0.0065	1.3657	1.2125	1.1263	0.4939	0.0474	0.0153	0.0329	2016.5	2031.7	con	Wild
708	14.9	17.4	18.5	19.4	19.3	19.4	0.1067	0.0054	0.0127	0.0085	1.3283	1.1923	1.114	0.5500	0.0655	0.0278	0.0436	2032.3	2087.1	con	Wild
716	18.7	19.4	20	21.3	21.3	21.6	0.1108	0.0068	0.0132	0.0115	1.162	1.1658	0.9967	0.5130	0.0611	0.0315	0.0532	2047.7	1924.8	con	Wild
728	13.8	13.9	14.3	16.3	16.4	17.1	0.0694	0.0036	0.0077	0.0046	1.3512	1.1998	1.1262	0.4058	0.0450	0.0211	0.0269	2006.9	-	con	Wild
756	12.6	15.2	15.8	16.3	16.8	17.6	0.0846	0.0051	0.0095	0.0060	1.3354	1.2632	1.0571	0.4807	0.0540	0.0290	0.0338	2019.4	2058.4	con	Wild
787	13.4	15.4	16	16.6	17	17.7	0.0826	0.0038	0.0089	0.0063	1.4308	1.2703	1.1263	0.4667	0.0503	0.0215	0.0356	2095.4	1909.2	con	Wild
782	15.9	17.3	17.6	18.5	17.6	19.3	0.0858	0.0026	0.0100	0.0075	1.1294	1.2095	0.9338	0.4446	0.0518	0.0135	0.0386	2041.9	2021.2	con	Wild
797	15.7	16.4	16.6	17	18.4	18.1	0.0901	0.0024	0.0088	0.0061	1.0342	1.2569	0.8228	0.4978	0.0486	0.0133	0.0337	-	2339.9	con	Wild
798	15.8	16.4	17	17.2	18.6	19.9	0.091	0.0054	0.011	0.0062	1.0117	1.2335	0.8202	0.4573	0.0553	0.0271	0.0309	-	2144.1	con	Wild
801	15.5	16.5	16.6	16.6	17	17.7	0.0885	0.0046	0.0104	0.0075	1.0053	1.1918	0.8435	0.5000	0.0588	0.0260	0.0424	-	2012.2	con	Wild
698	18.1	19.6	20.5	21.4	21.5	22.2	0.1339	0.0056	0.0142	0.0097	1.3418	1.1753	1.1417	0.6032	0.0640	0.0252	0.0435	2877.4	3986.3	con	Trans
699	18.2	19	19.5	20.8	20.8	20.8	0.1076	0.0050	0.0117	0.0087	1.302	1.1601	1.1223	0.5173	0.0563	0.0240	0.0418	2855.1	3887.0	con	Trans
715	18.7	19.2	19.6	20	20.3	21.3	0.1278	0.0047	0.0129	0.0107	1.2766	1.1452	1.1148	0.6000	0.0606	0.0221	0.0502	2362.9	4002.9	con	Trans
709	14.9	17.1	18.1	19.2	19.8	20.3	0.1072	0.0052	0.0131	0.0102	1.2162	1.1021	1.1034	0.5281	0.0645	0.0256	0.0502	2870.5	4232.3	con	Trans
726	14.5	14.9	15.1	17.1	17.4	18.3	0.0998	0.0040	0.0111	0.0082	1.4473	1.2079	1.1982	0.5454	0.0607	0.0219	0.0448	2869.0	3906.6	con	Trans
727	16	15.9	16.3	18.6	19.2	19.4	0.1065	0.0050	0.0160	0.0094	1.4124	1.0492	1.3462	0.5490	0.0825	0.0258	0.0485	2888.5	4024.2	con	Trans
770	15.8	18.1	19.6	19.8	18.6	19.4	0.0860	0.0062	0.0104	0.0065	1.2456	1.1600	1.0738	0.4433	0.0536	0.0320	0.0332	2954.4	3937.8	con	Trans
786	14.5	16	17.1	17.8	17.8	19.5	0.1046	0.0043	0.0123	0.0093	1.2239	1.0875	1.1255	0.5364	0.0631	0.0221	0.0477	3742.8	4025.6	con	Trans
800	17.2	18.8	18.8	19.8	22	22.9	0.1359	0.0058	0.0145	0.0124	1.2161	1.1290	1.0772	0.5934	0.0633	0.0253	0.0541	3130.9	3990.8	con	Trans
818	13.3	15	15.5	16.1	17.3	18.2	0.1004	0.0034	0.0102	0.0086	1.3918	1.2095	1.1508	0.5516	0.0560	0.0187	0.0470	2968.8	3958.9	con	Trans
819	13.9	16.5	17.1	17.5	18	19.3	0.1197	0.0048	0.0125	0.0100	1.3462	1.0461	1.2869	0.6202	0.0648	0.0249	0.0516	-	4037.8	con	Trans
831	17	18	19.3	19.4	18.9	20.5	0.1202	0.006	0.0129	0.0096	1.2756	1.2022	1.0611	0.5863	0.0629	0.0293	0.0466	-	4087.4	con	Trans
832	12.4	13.4	14.8	14.7	14.6	16	0.0862	0.002	0.0118	0.0070	1.2633	1.2294	1.0276	0.5388	0.0738	0.0125	0.0438	-	4044.0	con	Trans
834	13.5	14.9	15.9	16.2	16.2	17.2	0.0941	0.0045	0.0103	0.0091	0.9843	1.1978	0.8218	0.5471	0.0599	0.0262	0.0526	-	4024.3	con	Trans
807	16.2	17.9	18.2	18.8	20.5	20.7	0.1323	0.0063	0.0148	0.0115	1.1366	1.1612	0.9788	0.6391	0.0715	0.0304	0.0553	-	-	con	Trans
692	12.8	16	17.1	18.9	19.5	19.8	0.1029	0.0081	0.0114	0.0101	1.2625	0.9252	1.3645	0.5197	0.0576	0.0409	0.0510	2744.5	3890.3	Clen	Wild
707	15.4	17	17.1	18.3	19.6	19.6	0.0945	0.0057	0.0114	0.0074	1.2085	1.1479	1.0528	0.4821	0.0582	0.0291	0.0378	2953.6	3814.6	Clen	Wild
761	13.2	14.8	15.2	16	16.2	18.6	0.0947	0.0051	0.0112	0.0064	1.3122	1.2158	1.0792	0.5091	0.0602	0.0274	0.0341	2216.3	3204.9	Clen	Wild
783	15.6	17.5	17.9	18.9	18.9	20.5	0.1085	0.0072	0.0118	0.0081	1.2246	1.1186	1.0947	0.5293	0.0576	0.0351	0.0395	2773.7	3439.6	Clen	Wild
788	15.2	16.4	17.5	18.3	17.7	19.6	0.1062	0.0067	0.0128	0.0082	1.2512	1.1315	1.1058	0.5418	0.0653	0.0342	0.0416	2555.5	3837.4	Clen	Wild
817	14.9	16.8	17.5	18.2	17.8	19.9	0.1042	0.0055	0.0121	0.0081	1.3173	1.2703	1.037	0.5236	0.0608	0.0276	0.0405	2870.3	3499.2	Clen	Wild
820	12.6	15	15.2	16.1	15.7	17.2	0.0801	0.0047	0.0095	0.0064	1.3354	1.1468	1.1645	0.4657	0.0552	0.0273	0.0369	3251.1	3246.3	Clen	Wild
833	15.8	17.1	17.5	16.4	16.4	18.4	0.0845	0.0065	0.0072	0.0067	1.2311	1.1674	1.0546	0.4592	0.0391	0.0353	0.0364	2588.8	3291.6	Clen	Wild
835	16.6	18	18.9	18.7	18.1	21.2	0.095	0.0056	0.0104	0.0072	0.9843	1.0839	0.9081	0.4481	0.0491	0.0264	0.0337	3097.4	3091.0	Clen	Wild
839	15.4	16.5	16.5	17.3	16.3	17.7	0.0789	0.0042	0.0063	0.0065	1.2582	1.2361	1.0179	0.4458	0.0356	0.0237	0.0364	2879.3	-	Clen	Wild
717	17.2	18.5	19.3	20.3	21.1	22	0.1527	0.0069	0.0166	0.0108	1.3237	1.0927	1.2114	0.6941	0.0755	0.0314	0.0491	4821.2	5590.8	Clen	Trans

724	13.9	14.2	14.7	15.9	16.9	18.1	0.1122	0.0057	0.0159	0.0099	1.5591	1.0014	1.557	0.6199	0.0878	0.0315	0.0544	4992.7	5778.0	Clen	Trans
725	15.4	14.7	15.5	17.8	19.7	20.3	0.1311	0.0062	0.0161	0.0112	1.4064	1.1340	1.2402	0.6458	0.0793	0.0305	0.0552	4846.3	5575.7	Clen	Trans
758	16.7	17.8	19	19.1	20.7	22.6	0.1455	0.0062	0.0154	0.0097	1.6253	1.0723	1.5157	0.6438	0.0681	0.0274	0.0429	4861.6	5780.5	Clen	Trans
759	14.2	14.2	16.8	17.6	18.1	21	0.1335	0.0037	0.0166	0.0121	1.4124	1.0492	1.3462	0.6357	0.0790	0.0176	0.0576	5464.7	5792.0	Clen	Trans
769	15.3	16.9	18.6	19.1	20.4	23.2	0.1315	0.0091	0.0114	0.0109	1.3515	1.0596	1.2755	0.5668	0.0491	0.0392	0.0468	5594.6	5857.2	Clen	Trans
784	15.1	16.6	17.3	18.2	19.5	21.8	0.1463	0.0070	0.0187	0.0120	1.3244	1.0614	1.2478	0.6711	0.0858	0.0321	0.0548	4938.5	5444.1	Clen	Trans
785	16.2	17.1	18.6	19.7	19.6	22.5	0.1623	0.0070	0.0191	0.0127	1.3049	1.0791	1.2092	0.7213	0.0849	0.0311	0.0564	4597.6	5761.4	Clen	Trans
789	15.4	17.2	18.4	18.9	19.4	21.8	0.1264	0.0040	0.0154	0.0109	1.4718	1.0515	1.3997	0.5798	0.0706	0.0183	0.0500	4905.1	5324.0	Clen	Trans
790	17.3	18.9	19.5	20.1	21.3	23.2	0.1539	0.0054	0.0174	0.0119	1.2142	1.0406	1.1668	0.6634	0.0750	0.0233	0.0513	4870.7	5223.4	Clen	Trans
837	16.1	18.5	19.1	20.1	20	23.9	0.1382	0.0066	0.0186	0.0121	1.3221	1.1620	1.1378	0.5782	0.0778	0.0276	0.0504	4816.9	5954.1	Clen	Trans
838	16.9	18.4	19.7	20.9	20.1	23.9	0.1402	0.0076	0.0133	0.0117	1.297	1.1475	1.1303	0.5866	0.0556	0.0318	0.0490	4970.7	5881.3	Clen	Trans
840	14.9	16.6	17.2	18.1	17.7	21	0.126	0.0049	0.0134	0.0103	1.2162	1.0728	1.1336	0.6000	0.0638	0.0233	0.0488	0.0119	5757.4	Clen	Trans
757	13.2	14.4	15.7	16.3	18	20.2	0.1288	0.0066	0.0160	0.0105	1.3847	1.0152	1.364	0.6376	0.0792	0.0327	0.0517	0.0155	5854.4	Clen	Trans
836	15.6	17.8	18.7	18.7	18	21.4	0.1209	0.005	0.0149	0.0116	1.2356	1.1051	1.118	0.5650	0.0696	0.0234	0.0540	0.0125	-	Clen	Trans

Animals were weaned at 4 week. C0day, start of CL administration; C3day, 3day of CL administration; C7day, 7day of CL administrationl; Geno, Genotype; CL, Clenbuterol; Gas, Gastrocnemius; Pln, Plantaris; EDL, extensor digitorum longus; Sol, Soleus Con, control; Clen, Clenbuterol administration; Wild, wild type mice; Trans, Transgenic mice.

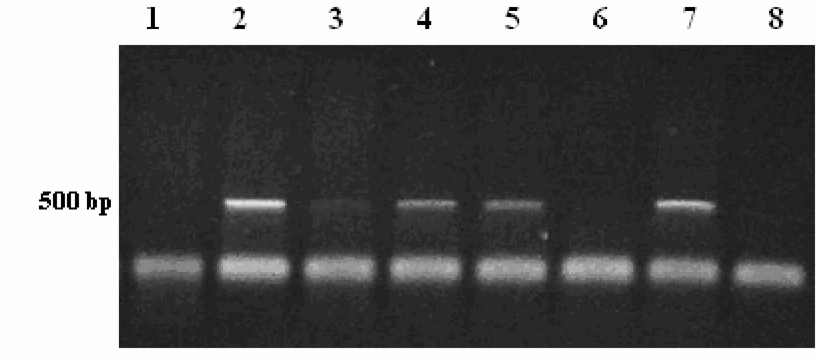
APPENDIX 2.3 The female raw data at 14 day.

	wt	wt	wt	wt	0day	3day	7day	10day	14day					mg/g of muscle							Adj. Vol CNT*mm2			
No	wean	3day	5day	7day	10day	14day	18day	21day	25day	GAS	Sol	Plan	E D L	RNA	DNA	Ratio	%Gas	%pl	%sol	%EDL	4EBP1	p70S6k	CL	Treat
852	17.1	17.7	17.7	19	19.5	21	21.3	21.3	21.3	0.0906	0.0059	0.011	0.0070	1.096	1.119	0.980	0.4254	0.0516	0.0277	0.0329	2859.9	2840.8	con	Wild
853	15.8	17.6	17.9	18.5	19.4	19.8	20.1	20.3	20.8	0.0992	0.0063	0.0101	0.0071	1.244	0.934	1.333	0.4769	0.0486	0.0303	0.0339	2815.8	3162.1	con	Wild
854	13.8	15.1	15.2	16.3	17	18.4	19	19.3	19.3	0.0803	0.0031	0.0089	0.0062	1.029	0.935	1.101	0.4161	0.0461	0.0161	0.0321	2865.7	3162.1	con	Wild
949	12.7	14.6	16.1	16.6	17.1	17.3	17.9	18	18	0.0791	0.0042	0.0092	0.0060	1.033	1.081	0.956	0.4394	0.0511	0.0233	0.0333	2909.9	2910.9	con	Wild
950	13	13.9	15.5	15.6	16.2	16.7	16.9	17.5	18	0.0763	0.0038	0.0095	0.0059	1.009	1.046	0.965	0.4239	0.0528	0.0211	0.0328	2863.7	3087.8	con	Wild
951	11.4	12.7	14.1	14.4	15.9	16.4	17.1	17.1	17.3	0.0812	0.0037	0.0091	0.0061	1.108	0.928	1.194	0.4694	0.0526	0.0214	0.0353	2653.6	2937.6	con	Wild
961	14.7	16.0	16.5	17.6	18.1	18.2	18.6	18.6	19.4	0.0976	0.0049	0.0092	0.0068	1.150	0.915	1.257	0.5031	0.0474	0.0253	0.0348	2131.7	3842.8	con	Wild
962	12.8	14.2	15.0	15.7	16.6	17.2	18	18	18.1	0.0784	0.004	0.0077	0.0045	1.312	0.762	1.723	0.4331	0.0425	0.0221	0.0249	2682.7	2971.5	con	Wild
25	12.1	14.1	15.3	16.1	17.8	18.7	18.8	19.2	19.6	0.1016	0.004	0.012	0.0091	1.037	1.014	1.023	0.5184	0.0612	0.0204	0.0464	2078.8	2545.6	con	Wild
26	14.2	16.4	17.6	18.4	19.4	19.5	19.7	19.7	19.5	0.0797	0.0047	0.009	0.0051	1.294	0.941	1.375	0.4087	0.0462	0.0241	0.0259	2304.4	3354.2	con	Wild
30	11.3	14.2	15.8	16.4	18	18.3	18.3	18.4	18.9	0.0773	0.0035	0.0083	0.0067	1.232	0.770	1.600	0.4090	0.0439	0.0185	0.0354	-	3228.5	con	Wild
32	12.3	14.3	15.7	16.3	17.6	17.7	18.2	18.4	19	0.0775	0.0054	0.0104	0.0054	1.061	0.935	1.135	0.4079	0.0547	0.0284	0.0282	-	3045.4	con	Wild
865	12.4	13.9	15.3	17.2	18.9	20	20.5	21.2	22	0.1193	0.0043	0.0118	0.0091	1.329	0.914	1.454	0.5423	0.0536	0.0195	0.0414	2957.1	3463.5	con	Trans
960	15	16.5	17.2	17.4	18.2	18.4	19.1	20.7	20.9	0.1152	0.0055	0.0133	0.0094	1.085	0.911	1.191	0.5512	0.0636	0.0263	0.0447	3682.6	3356.5	con	Trans
948	12.3	13.2	14.6	14.8	17.2	19.4	20.1	20.1	21.4	0.1128	0.0053	0.0133	0.0095	1.191	0.937	1.271	0.5271	0.0621	0.0248	0.0444	3244.3	3743.2	con	Trans
17	11.4	13.1	14.2	15.2	17.2	17.8	19.1	19.6	20.1	0.1236	0.0054	0.0148	0.0098	1.121	0.906	1.238	0.6149	0.0736	0.0269	0.0488	3566.6	3743.2	con	Trans
20	14.1	16.9	18.7	18.9	20.1	20.8	21.6	21.5	22.1	0.12	0.0044	0.0145	0.0100	1.209	0.895	1.350	0.5430	0.0656	0.0199	0.0452	3578.1	3905.5	con	Trans
23	12.3	14.7	16.7	16.7	19.5	20.6	21.1	21.6	21.6	0.1252	0.0057	0.0134	0.0104	1.150	0.882	1.304	0.5796	0.0620	0.0264	0.0479	3594.7	3748.5	con	Trans
28	12.7	13.0	14.2	14.4	15.6	15.6	16.6	17.2	17.6	0.0868	0.0043	0.0125	0.0080	1.031	0.875	1.179	0.4932	0.0710	0.0244	0.0452	3929.5	3791.2	con	Trans
31	15.9	17.3	17.3	17.1	18.6	18.7	20.1	21	21	0.1162	0.0047	0.0135	0.0100	1.010	0.867	1.164	0.5533	0.0643	0.0224	0.0476	3924.3	3199.4	con	Trans
72	12.9	14.6	16.3	17.1	21.9	22.9	22.7	23.8	24.1	0.1328	0.006	0.0146	0.0095	1.186	0.898	1.321	0.5510	0.0606	0.0249	0.0392	3208.7	3658.4	con	Trans
85	17.9	19.9	20.4	20.9	21.4	22.4	23.2	24.1	25.3	0.1411	0.0049	0.0172	0.0108	1.198	0.729	1.644	0.5577	0.0680	0.0194	0.0427	3476.6	3926.9	con	Trans
29	12	14.0	15.2	16.6	18.7	19.4	19.6	20.8	21.1	0.1162	0.0059	0.0134	0.0095	1.120	0.655	1.711	0.5507	0.0635	0.0280	0.0450	-	3574.2	con	Trans
846	13.9	15.5	15.5	15.5	15.6	18.3	18.7	19.5	20.1	0.1008	0.0054	0.0097	0.0077	1.294	1.159	1.116	0.5015	0.0483	0.0269	0.0381	2894.9	3852.7	Clen	Wild
847	16.1	16.7	16.7	17.1	17.2	19.3	20	20.9	21.1	0.1071	0.006	0.0107	0.0071	1.282	1.056	1.213	0.5076	0.0507	0.0284	0.0334	2744.1	3872.5	Clen	Wild
848	15.1	16.8	16.6	17.4	17.6	20.2	20.7	21.8	22.3	0.1357	0.0076	0.017	0.0114	1.138	0.886	1.286	0.6085	0.0762	0.0341	0.0509	3200.0	3482.5	Clen	Wild
864	12	14	15	16.2	17.4	18.3	19.3	21.1	21.4	0.1076	0.0053	0.0132	0.0087	1.169	0.911	1.284	0.5028	0.0617	0.0248	0.0404	3678.9	3482.5	Clen	Wild
872	14	15.2	15.8	16.7	17.2	18.5	19.8	20.3	20.1	0.1046	0.007	0.0131	0.0074	1.173	0.797	1.472	0.5204	0.0652	0.0348	0.0368	3217.3	3763.4	Clen	Wild
873	12.2	14.2	15.0	15.6	17.3	17.9	19.2	20.3	19.1	0.106	0.0066	0.0136	0.0089	1.083	0.758	1.428	0.5550	0.0712	0.0346	0.0463	3575.0	3992.0	Clen	Wild
880	13.9	14.6	15.3	16.4	17.6	18.7	19.3	20.1	20.5	0.1047	0.0064	0.016	0.0087	1.037	0.863	1.203	0.5107	0.0780	0.0312	0.0424	3289.9	2626.9	Clen	Wild
882	13.4	13.9	14.8	15.8	16.8	18.4	18.7	19.2	19.7	0.0984	0.0058	0.0137	0.0078	1.120	0.896	1.250	0.4995	0.0695	0.0294	0.0396	2970.5	3706.3	Clen	Wild
886	13.6	15.5	16.3	16.8	18.7	19.9	20.7	21.4	21.1	0.1043	0.0058	0.0126	0.0084	1.096	0.853	1.285	0.4943	0.0597	0.0275	0.0398	2940.3	3775.4	Clen	Wild
893	15.1	14.9	15.9	17.1	18.3	19.8	20.1	21.2	20.7	0.104	0.0042	0.0141	0.0074	1.244	0.834	1.492	0.5024	0.0681	0.0203	0.0357	3047.2	3773.4	Clen	Wild
18	10.6	11.4	13.7	13.9	16.8	17.8	18.7	19.3	19.4	0.0962	0.0052	0.0116	0.0066	1.146	0.790	1.451	0.4959	0.0598	0.0268	0.0338	-	3969.5	Clen	Wild
19	13	14.8	16.2	16.3	18.7	19.3	18.7	19.2	20.1	0.1022	0.0067	0.0131	0.0073	1.030	0.797	1.293	0.5085	0.0652	0.0333	0.0363	-	3699.6	Clen	Wild
21	12	13.6	15.8	15.8	18.1	18.4	19.3	20	20.5	0.1111	0.0056	0.0132	0.0081	1.164	0.758	1.535	0.5420	0.0644	0.0273	0.0395	-	4491.7	Clen	Wild
24	14.4	15.8	17.1	17.5	19.6	19.8	20.5	21	21.6	0.1092	0.006	0.0137	0.0084	1.065	0.903	1.179	0.5056	0.0634	0.0278	0.0387	-	-	Clen	Wild
874	14.3	15.5	16.6	17	18.2	18.9	20.6	21	22.6	0.1472	0.0069	0.0183	0.0115	1.092	0.799	1.366	0.6513	0.0810	0.0305	0.0507	3481.9	3644.6	Clen	Trans

883	14.4	16.0	16.6	17	17.8	18.7	19.6	22	23.3	0.1558	0.0082	0.0195	0.0131	1.115	0.822	1.356	0.6687	0.0837	0.0352	0.0560	3981.6	3634.7	Clen	Trans
884	14.7	16.9	18.0	18.4	20.5	21.5	22.9	24.2	24.4	0.1604	0.0072	0.0186	0.0130	1.133	0.807	1.405	0.6574	0.0762	0.0295	0.0531	3920.7	4273.4	Clen	Trans
885	10.8	12.0	13.3	14.9	15.6	17.4	19.3	19.8	19.8	0.1345	0.0049	0.0154	0.0114	1.120	0.787	1.423	0.6793	0.0778	0.0247	0.0573	4084.2	4273.4	Clen	Trans
891	15.8	17.9	18.9	20.4	20.2	21.5	22.4	22.9	22.9	0.1617	0.0074	0.0218	0.0124	1.075	0.823	1.306	0.7061	0.0952	0.0323	0.0541	3810.0	4222.3	Clen	Trans
892	16.3	16.3	17.3	18.7	18.5	19.7	20.9	23.3	23.3	0.1574	0.0075	0.018	0.0130	1.114	0.852	1.308	0.6755	0.0773	0.0322	0.0558	3847.0	4215.2	Clen	Trans
894	14.5	16.4	17.4	19.2	20.1	20.6	22.3	23.7	23.7	0.1653	0.0081	0.021	0.0118	1.055	0.807	1.307	0.6975	0.0886	0.0342	0.0496	4926.2	4414.0	Clen	Trans
881	15.3	16.5	17.5	18.9	18.9	20.1	21	22.9	22.5	0.1644	0.0078	0.0207	0.0116	1.142	0.785	1.455	0.7307	0.0920	0.0347	0.0516	3171.4	4486.5	Clen	Trans
896	13.9	15.2	16.2	18.4	19.8	21.5	22	22.4	23.4	0.1647	0.0071	0.019	0.0123	1.238	0.809	1.531	0.7038	0.0812	0.0303	0.0524	3840.1	5340.1	Clen	Trans
862	11.9	13.8	15.1	16.6	17.6	19.4	20.9	21.9	22.9	0.1492	0.0049	0.0171	0.0118	1.198	0.841	1.425	0.6515	0.0747	0.0214	0.0515	3854.5	4566.9	Clen	Trans
895	12.9	14.2	15.2	16.7	16.7	18.8	19.7	19.9	19.9	0.1109	0.0051	0.0114	0.0090	1.060	0.677	1.565	0.5573	0.0573	0.0256	0.0450	-	4507.1	Clen	Trans
40	10.1	12.0	13.6	15.8	18.3	19.4	22.5	22.7	24.3	0.1426	0.0082	0.0199	0.0112	1.219	0.805	1.515	0.5868	0.0819	0.0337	0.0459	-	4175.4	Clen	Trans
44	11.1	13.7	15.0	17.3	18.8	19	22.4	22.7	23.2	0.1442	0.0072	0.0198	0.0097	1.223	0.755	1.621	0.6216	0.0853	0.0310	0.0418	-	4375.2	Clen	Trans
22	14	16.1	16.9	16.9	19.4	19.4	22.4	22.9	23.2	0.1574	0.0069	0.021	0.0122	1.263	0.729	1.733	0.6784	0.0905	0.0297	0.0526	-	-	Clen	Trans

Animals were weaned at 4 week. C0day, start of CL administration; C3day, 3day of CL administration; C7day, 7day of CL administration; C10day, 10day of CL administration; C14day, 14day of CL administration; Geno, Genotype; CL, Clenbuterol; Gas, Gastrocnemius; Pln, Plantaris; EDL, extensor digitorum longus; Sol, Soleus Con, control; Clen, Clenbuterol administration; Wild, wild type mice; Trans, Transgenic mice.

APPENDIX 3.1 Agarose gel electrophoresis of genotype



1.2% agarose gel electrophoresis gel stained with ethidium bromide. Lane 1, Wild type mice; Lane 2, Transgnice mice; Lane 3, Transgnice mice; Lane 4, Transgnice mice; Lane 5, Transgnice mice; Lane 6, Wild type mice; Lane 7, Transgnice mice; Lane 8, Wild type mice.

Appendix 3.2 The male raw data at 3day.

		wt	wt	C0d	C3d					mg/g of muscle							Adj. Vol CNT*mm2					
No	wean	3d	5d	7d	10d	GAS	Sol	Plan	E D L	RNA	DNA	Ratio	%Gas	%pl	%sol	%EDL	Akt	4EBP1	p70S6k	CL	Geno	
721	15.4	16.5	15.6	17.9	18.7	0.0800	0.0054	0.0094	0.0066	0.985	1.379	0.714	0.4278	0.0503	0.0289	0.0350	2483.9	2116.2	2978.5	con	Wild	*
667	14.8	15.9	15.6	16.0	16.9	0.0629	0.0038	0.0088	0.0054				0.3722	0.0521	0.0225	0.0320	2703.8	3015.6	2537.9	con	Wild	*
702	17.2	18.3	18.9	19.6	20.0	0.1100	0.0067	0.0126	0.0083				0.5500	0.0630	0.0335	0.0413	2297.1	2581.6	2189.2	con	Wild	
773	19.7	20.8	22.3	22.3	23.0	0.1193	0.0067	0.0153	0.0079	0.935	1.178	0.794	0.5187	0.0665	0.0291	0.0343	2432.3	2027.3	2038.8	con	Wild	
774	15.7	16.8	17.7	18.6	19.1	0.0845	0.0041	0.0109	0.0069	1.282	1.196	1.072	0.4424	0.0571	0.0215	0.0361	2418.5	3569.0	2066.9	con	Wild	*
776	17.4	18.5	20.5	21.4	21.8	0.1229	0.0056	0.0152	0.0091	1.011	1.108	0.912	0.5638	0.0697	0.0257	0.0417	1841.4	3290.9	3285.4	con	Wild	*
779	17.5	18.6	19.9	20.1	20.2	0.1044	0.0048	0.0126	0.0084	0.992	1.152	0.861	0.5168	0.0624	0.0238	0.0416	1827.9	3047.1	2714.4	con	Wild	
781	16.3	17.4	18.5	19.5	20.6	0.0976	0.0059	0.0115	0.0070				0.4738	0.0558	0.0286	0.0340	2269.7	2371.3	2367.7	con	Wild	
821	16.1	17.2	18.8	19.7	19.3	0.0892	0.0045	0.0092	0.0067	1.102	1.262	0.873	0.4622	0.0477	0.0233	0.0347	1429.7	2492.9	2232.0	con	Wild	*
822	18.2	19.3	23	23.7	23.4	0.1285	0.0076	0.0130	0.0089	1.137	1.464	0.777	0.5491	0.0556	0.0325	0.0378	2354.7	2646.7	1853.2	con	Wild	*
825	15	16.1	18.9	19.7	19.4	0.0899	0.0042	0.0079	0.0065	1.262	1.275	0.990	0.4634	0.0407	0.0216	0.0335	2061.9	2158.8	2217.2	con	Wild	
673	23.7	24.8	24.3	24.8	24.8	0.1822	0.0044	0.0213	0.0127				0.7347	0.0859	0.0177	0.0510	-	-	-	con	Trans	*
703	19.4	20.5	20.6	22.3	22.6	0.1491	0.0061	0.0168	0.0112	0.841	1.157	0.727	0.6597	0.0743	0.0270	0.0496	3781.8	2474.4	3531.5	con	Trans	
720	20.0	21.1	20.1	22.7	24.0	0.1446	0.0065	0.0154	0.0100	1.079	1.164	0.927	0.6025	0.0642	0.0271	0.0417	2718.6	2047.0	2821.7	con	Trans	*
704	18.2	19.3	20.8	19.0	20.9	0.1248	0.0062	0.0156	0.0103	1.262	1.029	1.227	0.5971	0.0746	0.0297	0.0493	2443.7	3866.3	2386.9	con	Trans	*
823	17	18.1	21	22.9	22.3	0.1262	0.0036	0.0144	0.0096				0.5659	0.0646	0.0161	0.0428	-	-	-	con	Trans	*
829	14.2	15.3	18.8	20.4	20	0.1163	0.0050	0.0151	0.0107	1.176	1.144	1.028	0.5815	0.0755	0.0250	0.0535	3221.3	3806.0	2819.1	con	Trans	*
830	16.8	17.9	21.2	23.3	22.9	0.1394	0.0054	0.0154	0.0102	1.358	1.055	1.287	0.6087	0.0672	0.0236	0.0443	2976.7	2615.6	3070.5	con	Trans	*
772	14.5	15.6	17.0	18.9	20.9	0.1291	0.0050	0.0153	0.0105	1.163	1.155	1.007	0.6177	0.0732	0.0239	0.0502	3432.7	3117.4	4887.5	con	Trans	
824	20	21.1	23.7	25.1	24.6	0.1575	0.0079	0.0172	0.0116	1.110	1.158	0.958	0.6402	0.0699	0.0321	0.0472	3286.7	4098.9	4792.6	con	Trans	
826	15.9	17.0	17.2	18.6	18.7	0.1031	0.0024	0.0127	0.0088	1.161	1.042	1.114	0.5513	0.0679	0.0128	0.0471	2560.7	3803.9	3745.3	con	Trans	
2	13.9	15.0	18.9	20	21.7	0.1104	0.0046	0.0118	0.0094				0.5088	0.0544	0.0212	0.0433	2834.4	3246.6	3526.0	con	Trans	
11	16.4	17.5	21.8	23.5	25.2	0.1190	0.0052	0.0153	0.0105	1.074	1.236	0.870	0.4722	0.0607	0.0206	0.0415	2268.3	3064.4	3118.5	con	Trans	

13	14	15.1	20.8	22.4	24.9	0.1455	0.0073	0.0169	0.0109	1.033	1.045	0.988	0.5843	0.0679	0.0293	0.0438	2806.0	3265.6	3650.2	con	Trans	
15	14	15.1	17.9	19.4	21.3	0.1092	0.0056	0.0127	0.0094	1.192	1.180	1.010	0.5127	0.0596	0.0263	0.0439	2640.0	4101.4	3979.9	con	Trans	
16	14.4	15.5	18.9	20.4	22.7	0.1150	0.0045	0.0110	0.0094				0.5066	0.0485	0.0198	0.0412	2006.6	2336.0	3299.4	con	Trans	
710	16.8	17.9	18.5	18.8	20.4	0.1193	0.0061	0.0139	0.0081	1.537	1.173	1.311	0.5848	0.0681	0.0299	0.0397	4040.4	3172.3	3247.2	Clen	Wild	
711	20.9	22.0	19.1	20.7	21.4	0.1246	0.0095	0.0141	0.0097	1.367	1.151	1.188	0.5822	0.0659	0.0444	0.0451	3111.6	2650.8	3706.4	Clen	Wild	
792	20.7	21.8	23.0	23.8	25.4	0.1416	0.0069	0.0182	0.0112	1.410	1.137	1.239	0.5575	0.0717	0.0272	0.0441	2665.7	3952.9	2681.9	Clen	Wild	
794	20.0	21.1	21.6	22.3	23.9	0.1337	-	0.0147	0.0120	1.212	1.042	1.163	0.5594	0.0615	-	0.0500	-	-	-	Clen	Wild	
795	16.9	18.0	18.9	19.6	21.4	0.1172	0.0059	0.0146	0.0113	1.474	1.161	1.269	0.5477	0.0682	0.0276	0.0528	3506.1	4134.8	3112.6	Clen	Wild	
804	17	18.1	19.3	20	21.6	0.1151	0.0057	0.0121	0.0087	1.271	1.091	1.166	0.5329	0.0560	0.0264	0.0400	3323.8	3133.0	2942.1	Clen	Wild	*
805	17.3	18.4	20.3	21.4	24	0.1460	0.0060	0.0178	0.0119	1.065	1.165	0.914	0.6083	0.0742	0.0250	0.0496	3436.8	3782.2	4738.7	Clen	Wild	*
806	17.1	18.2	17.6	18	19.1	0.1005	0.0043	0.0124	0.0078	1.224	0.808	1.516	0.5262	0.0649	0.0225	0.0408	2819.3	4027.7	5303.2	Clen	Wild	*
812	15.3	16.4	21.3	22.7	22.6	0.1402	0.0052	0.0183	0.0126	1.630	1.117	1.460	0.6204	0.0810	0.0230	0.0558	3702.7	4220.2	3517.0	Clen	Wild	*
813	16.8	17.9	20.8	21.5	21.8	0.1212	0.0042	0.0141	0.0089	1.669	1.160	1.439	0.5560	0.0647	0.0193	0.0406	4189.3	3582.7	2942.3	Clen	Wild	*
816	14.6	15.7	19.9	20.5	21.3	0.1029	0.0062	0.0126	0.0096	1.294	1.189	1.088	0.4831	0.0592	0.0291	0.0448	1817.2	3118.3	3481.8	Clen	Wild	
869	14.4	15.5	16.7	17.6	19.6	0.0816	0.0043	0.0106	0.0073	1.064	1.040	1.023	0.4163	0.0541	0.0219	0.0372	2566.2	3252.7	3234.2	Clen	Wild	
887	12.5	13.6	15.8	18.4	20	0.0950	0.0050	0.0119	0.0085	1.129	1.150	0.982	0.4750	0.0595	0.0250	0.0423	2713.3	4299.7	4343.5	Clen	Wild	*
889	15.5	16.6	18.2	19.8	21.2	0.1048	0.0056	0.0128	0.0087	0.963	1.091	0.883	0.4943	0.0604	0.0264	0.0410	2700.6	2506.0	3395.2	Clen	Wild	*
890	17.3	18.4	20.8	21.5	23	0.1262	0.0076	0.0174	0.0102	0.864	1.023	0.844	0.5487	0.0757	0.0330	0.0443	-	-	-	Clen	Wild	*
986	15.3	16.4	19.5	21.5	24.4	0.1372	0.0060	0.0198	0.0126	1.016	1.067	0.952	0.5623	0.0811	0.0246	0.0514	-	-	-	Clen	Wild	*
994	17.2	18.3	21.2	22	22.9	0.1105	0.0068	0.0125	0.0084	1.018	1.049	0.970	0.4825	0.0546	0.0297	0.0365	-	-	-	Clen	Wild	*
402	17	18.1	20.7	20.9	23	0.1025	0.0063	0.0132	0.0092	0.999	1.052	0.950	0.4457	0.0574	0.0274	0.0400	-	-	-	Clen	Wild	*
403	16.7	17.8	20	20.7	21.5	0.1018	0.0072	0.0186	0.0082	0.922	0.710	1.299	0.4735	0.0865	0.0335	0.0379	-	-	-	Clen	Wild	*
404	14.6	15.7	20.4	20.7	22.9	0.1064	0.0063	0.0171	0.0085	1.033	1.030	1.003	0.4646	0.0747	0.0275	0.0371	-	-	-	Clen	Wild	*
405	15.6	16.7	19.2	19.7	21.2	0.1037	0.0065	0.0164	0.0098	1.052	1.040	1.011	0.4892	0.0774	0.0307	0.0460	-	-	-	Clen	Wild	*
406	17.3	18.4	19.8	19.3	20.7	0.1019	0.0061	0.0124	0.0085	1.003	1.099	0.913	0.4923	0.0599	0.0295	0.0411	-	-	-	Clen	Wild	*
413	18.2	19.3	21.4	21.7	22.4	0.1047	0.0059	0.0130	0.0087	0.865	1.023	0.846	0.4674	0.0580	0.0263	0.0386	-	-	-	Clen	Wild	*

414	15	16.1	17.4	19.6	20.6	0.1053	0.0055	0.0136	0.0090	0.789	1.100	0.718	0.5112	0.0660	0.0267	0.0437	-	-	-	Clen	Wild	*
415	15.8	16.9	19.2	22.9	23.6	0.1448	0.0071	0.0202	0.0127	0.940	1.149	0.818	0.6136	0.0856	0.0301	0.0538	-	-	-	Clen	Wild	
672	21.8	22.9	23.6	24.1	25.9	0.1963	0.0090	0.0273	0.0163				0.7579	0.1054	0.0347	0.0647				Clen	Trans	*
705	18.6	19.7	20.4	22.4	24.2	0.1817	0.0085	0.0254	0.0118				0.7508	0.1050	0.0351	0.0508				Clen	Trans	
706	19.9	21.0	21.1	23.3	24.8	0.1836	0.0064	0.0281	0.0121	1.228	1.018	1.206	0.7403	0.1133	0.0258	0.0508	5181.0	4741.2	5233.9	Clen	Trans	
791	16.5	17.6	19.7	21.0	22.6	0.1417	0.0065	0.0177	0.0133	1.307	0.980	1.334	0.6270	0.0783	0.0288	0.0586	4762.2	4078.9	5401.4	Clen	Trans	*
793	17.0	18.1	19.8	20.5	22.9	0.1549	0.0061	0.0181	0.0141	1.256	0.934	1.345	0.6764	0.0790	0.0266	0.0616	4391.3	6488.0	4163.8	Clen	Trans	*
802	17	18.1	21.4	22.3	23.6	0.1408	0.0068	0.0174	0.0122	1.310	0.912	1.437	0.5966	0.0737	0.0288	0.0536	4223.7	5307.4	6051.3	Clen	Trans	*
811	18.3	19.4	22	23.2	25.6	0.1881	0.0087	0.0247	0.0138	1.637	1.052	1.557	0.7348	0.0965	0.0340	0.0559	4942.5	4889.9	4352.8	Clen	Trans	*
844	19.2	20.3	21.8	22.5	24.1	0.1426	0.0036	0.0174	0.0125	1.224	0.950	1.288	0.5917	0.0722	0.0149	0.0560	4262.4	5777.9	6707.4	Clen	Trans	
803	14.2	15.3	18.5	19.9	21.7	0.1454	0.007	0.017	0.0112	1.461	0.925	1.579	0.6700	0.0783	0.0323	0.0537	5100.5	6017.8	6839.1	Clen	Trans	*
814	13.9	15.0	18.7	20.7	20.6	0.1379	0.0062	0.0172	0.0102	1.384	0.980	1.411	0.6694	0.0835	0.0301	0.0568	4275.4	5689.7	5624.3	Clen	Trans	*
842	19.1	20.2	20.5	20.8	22.4	0.1548	0.0073	0.0178	0.0117	1.364	0.971	1.406	0.6911	0.0795	0.0326	0.0520	4898.7	4995.6	5190.9	Clen	Trans	*
843	20.5	21.6	24.7	25.2	27.7	0.1989	0.0078	0.0279	0.0144	1.272	0.870	1.462	0.7181	0.1007	0.0282	0.0518	5429.3	5289.6	5337.4	Clen	Trans	
888	17.3	18.4	20.4	22.4	23.3	0.1782	0.0065	0.021	0.0127	1.115	0.951	1.172	0.7648	0.0901	0.0279	0.0567	4173.5	5528.3	5357.8	Clen	Trans	*
868	16.8	17.9	21.2	22.4	23.7	0.1621	0.0056	0.0193	0.0133	1.359	0.960	1.415	0.6840	0.0814	0.0236	0.0603	4809.0	6123.2	5686.6	Clen	Trans	*
871	14.8	15.9	18.4	19.8	21.9	0.1429	0.0055	0.0182	0.0110	1.377	0.947	1.454	0.6525	0.0831	0.0251	0.0525	4827.0	4565.2	6535.3	Clen	Trans	*
878	16	17.1	19.4	19.3	21.7	0.1244	0.0033	0.0172	0.0100	1.261	0.966	1.305	0.5733	0.0793	0.0152	0.0461				Clen	Trans	*
47	15.8	16.9	22.7	23.3	24.5	0.1571	0.0064	0.0175	0.0118	1.285	0.996	1.291	0.6412	0.0714	0.0261	0.0480				Clen	Trans	*
55	13.7	14.8	19.4	20.4	22.7	0.1410	0.0039	0.0169	0.0132	1.403	0.870	1.613	0.6211	0.0744	0.0172	0.0601				Clen	Trans	*
64	13.1	14.2	18.2	21	22.7	0.1444	0.0057	0.017	0.0110	1.007	0.926	1.087	0.6361	0.0749	0.0251	0.0507				Clen	Trans	*

Animals were weaned at 4 week. C0day, start of CL administration; C3day, 3day of CL administration; Geno, Genotype; CL, Clenbuterol; Gas, Gastrocnemius; Pln, Plantaris; EDL, extensor digitorum longus; Sol, Soleus Con, control; Clen, Clenbuterol administration; Wild, wild type mice; Trans, Transgenic mice; * indicates animals that were not used for microarray.

Appendix 3.3 The male raw data at 14 day.

		wt	wt	C0d	C3d	C7d	C10d	C14d					mg/g of muscle							Adj. Vol CNT*mm2				
No	**wean**	**3d**	**5d**	**7d**	**10d**	**14d**	**17d**	**21d**	**GAS**	**Sol**	**Plan**	**E D L**	**RNA**	**DNA**	**Ratio**	**%Gas**	**%pl**	**%sol**	**%EDL**	**Akt**	**4EBP1**	**p70S6k**	**CL**	**Geno**
855	13.6	15.1	16.5	17.6	17.8	18.9	20.8	21.2	0.1132	0.0035	0.0110	0.0095	1.128	0.862	1.308	0.5340	0.0519	0.0165	0.0446	2639.8	1346.3	3099.5	con	Wild
857	12.6	14.8	17.2	19.4	20.3	21.4	23.2	24.2	0.1157	0.0056	0.0123	0.0080	1.183	0.792	1.493	0.4781	0.0508	0.0231	0.0329	1823.9	2441.3	2787.9	con	Wild
858	13.7	16.0	17.4	19.4	20.0	21.3	21.8	22.2	0.1183	0.0057	0.0128	0.0092	1.052	0.940	1.118	0.5329	0.0577	0.0257	0.0414	1189.3	2265.7	4036.1	con	Wild
860	12.4	14.0	16.4	18.0	19.3	20.2	20.9	22.8	0.1078	0.0049	0.0101	0.0066	1.139	1.281	0.889	0.4728	0.0443	0.0215	0.0287	1887.0	2690.8	2655.6	con	Wild
870	15.9	17.9	18.7	18.9	19.8	19.8	20.4	21.3	0.1022	0.0046	0.0111	0.0068	1.620	1.013	1.600	0.4798	0.0521	0.0216	0.0317	2147.0	1714.9	2613.9	con	Wild
875	15.4	17.4	18.8	19.5	20.4	21.4	22.4	22.9	0.1228	0.0048	0.0136	0.0091	1.313	1.081	1.214	0.5362	0.0594	0.0210	0.0397	1945.9	2146.5	2702.5	con	Wild
876	17.2	19.2	20.0	20.3	20.9	21.3	22.1	22.4	0.1039	0.0052	0.0151	0.0078	1.245	1.061	1.173	0.4638	0.0674	0.0232	0.0348	1804.1	2747.3	3366.4	con	Wild
879	15.5	17.5	19.1	20.6	21.6	22.4	23.3	23.3	0.1253	0.0057	0.0135	0.0099	1.303	1.029	1.265	0.5378	0.0579	0.0245	0.0423	2414.4	2713.0	1741.0	con	Wild
145	17.4	20.1	22.6	22.6	24.1	23.8	24.5	24.9	0.1317	0.0081	0.0157	0.0088	1.204	1.084	1.110	0.5289	0.0631	0.0325	0.0351	2405.5	4012.6	2753.3	con	Wild
146	17.0	19.6	21.3	20.7	21.9	22.7	23.4	24.1	0.1181	0.0064	0.0138	0.0079	1.170	1.057	1.107	0.4900	0.0573	0.0266	0.0328	2378.3	2557.1	2344.8	con	Wild
438	16.7	19.3	22.1	22.8	22.3	23.7	25.2	24.6	0.1280	0.0051	0.0155	0.0082	1.192	1.064	1.119	0.5203	0.0630	0.0207	0.0333	2250.4	2156.6	1770.1	con	Wild
441	19.4	21.0	22.5	22.3	24.5	25.3	27.8	27.2	0.1774	0.0053	0.0193	0.0139	1.223	0.848	1.441	0.6522	0.0710	0.0195	0.0509	-	2037.9	2557.4	con	Wild
444	19.4	19.0	18.7	19.0	18.9	19.2	20.6	20.7	0.1134	0.0037	0.0121	0.0081	-	-	-	0.5478	0.0585	0.0179	0.0389	-	2239.7	2440.2	con	Wild
856	15.7	16.9	18.5	19.5	20.7	24.1	25.3	26.3	0.1782	0.0055	0.0199	0.0142	1.263	0.791	1.596	0.6776	0.0757	0.0209	0.0540	3007.4	2092.0	4181.4	con	Trans
859	14.7	16.0	17.9	19.5	20.5	22.7	24.1	25.1	0.1865	0.0070	0.0202	0.0135	1.395	0.847	1.646	0.7430	0.0805	0.0279	0.0538	2941.6	2623.4	3297.4	con	Trans
861	13.5	15.5	18.3	19.5	21.7	22.5	23.8	24.3	0.1672	0.0064	0.0186	0.0119	1.153	0.838	1.376	0.6881	0.0765	0.0263	0.0488	3173.5	3206.4	3540.1	con	Trans
953	14.3	16.9	17.7	18.9	20.3	21.1	22.4	22.6	0.1436	0.0057	0.0159	0.0103	1.219	0.764	1.595	0.6354	0.0704	0.0252	0.0456	2720.9	3341.8	3695.2	con	Trans
75	17.7	18.9	22.0	22.5	24.2	26.2	27.2	27.5	0.1702	0.0072	0.0214	0.0132	1.276	0.739	1.725	0.6189	0.0778	0.0262	0.0480	3599.5	2500.3	2809.0	con	Trans
77	15.3	17.4	18.8	19.6	21.3	23.3	24.4	26.1	0.1581	0.0072	0.0176	0.0130	1.178	0.737	1.597	0.6057	0.0674	0.0276	0.0498	3059.4	3317.9	2974.2	con	Trans
84	17.5	19.0	22.6	23.5	23.9	25.6	26.8	27.2	0.1733	0.0059	0.0186	0.0125	1.144	0.687	1.663	0.6371	0.0684	0.0217	0.0458	1985.2	3349.0	3534.1	con	Trans
144	18.8	20.9	23.2	23.4	24.8	27.1	28.3	29.6	0.1958	0.0075	0.0217	0.0141	1.209	0.906	1.334	0.6615	0.0733	0.0253	0.0476	2787.6	4169.3	4775.2	con	Trans
155	19.4	21.3	22.8	22.9	23.8	24.5	25.8	26.5	0.1395	0.0074	0.0152	0.0092	1.338	0.873	1.533	0.5264	0.0574	0.0279	0.0347	2817.9	2715.7	3462.9	con	Trans
171	19.6	-	-	21.1	24.3	25.9	27.1	28.0	0.1940	0.0054	0.0274	0.0128	1.332	0.981	1.357	0.6929	0.0979	0.0193	0.0457	2951.4	2965.7	2816.2	con	Trans

424	18.9	21.4	23.5	23.8	25.8	27.2	28.7	29.2	0.1912	0.0068	0.0225	0.0129	1.263	0.976	1.293	0.6548	0.0771	0.0233	0.0440	2262.4	4182.0	2299.9	con	Trans
440	21.5	23.4	25.2	25.1	27.5	28.1	28.1	28.1	0.1993	0.0065	0.0236	0.0142	1.055	0.951	1.109	0.7093	0.0840	0.0231	0.0504	-	2958.3	3158.8	con	Trans
442	19.5	21.4	23.6	24.0	25.9	26.7	27.6	28.2	0.1871	0.0075	0.0217	0.0142	1.202	0.964	1.245	0.6635	0.0770	0.0266	0.0502	-	3009.4	3915.6	con	Trans
944	15.1	17.2	19.0	19.8	20.7	22.9	24.5	25.7	0.1438	0.0073	0.0185	0.0118	1.148	0.952	1.206	0.5595	0.0720	0.0284	0.0457	2267.7	2005.1	3031.1	Clen	Wild
947	12.4	15.0	16.1	17.5	19.4	21.3	22.7	22.8	0.1191	0.0058	0.0175	0.0097	1.213	0.914	1.326	0.5224	0.0768	0.0254	0.0423	2659.2	2461.1	3227.2	Clen	Wild
956	14.2	16.7	17.9	18.9	19.3	21.2	22.4	22.7	0.1230	0.0080	0.0157	0.0108	1.142	0.937	1.218	0.5419	0.0692	0.0352	0.0474	3204.7	2708.8	3044.7	Clen	Wild
958	15.6	18.2	18.5	19.3	20.1	22.3	23.8	24.4	0.1430	0.0077	0.0168	0.0109	1.239	0.948	1.306	0.5861	0.0689	0.0316	0.0447	3059.8	3098.4	2909.3	Clen	Wild
3	12.5	14.6	16.9	18.1	19.1	21.4	22.1	21.9	0.1210	0.0065	0.0136	0.0084	1.276	0.981	1.300	0.5525	0.0621	0.0297	0.0381	3014.8	2260.8	3069.4	Clen	Wild
7	12.0	15.1	18.2	19.2	22.6	24.3	24.9	25.3	0.1341	0.0071	0.0176	0.0105	1.289	0.925	1.392	0.5300	0.0696	0.0281	0.0415	2664.8	2893.3	2916.4	Clen	Wild
8	17.2	19.6	21.4	22.0	22.8	25.1	25.0	24.8	0.1304	0.0070	0.0192	0.0084	1.033	0.896	1.153	0.5258	0.0774	0.0282	0.0339	3155.9	2986.6	3250.9	Clen	Wild
10	17.2	20.2	21.9	22.7	22.9	24.8	25.3	25.5	0.1481	0.0088	0.0184	0.0088	1.219	0.915	1.333	0.5808	0.0722	0.0345	0.0343	2740.5	3353.3	3871.3	Clen	Wild
12	13.2	15.9	18.8	20.3	22.5	24.4	25.4	26.6	0.1452	0.0075	0.0185	0.0116	1.287	0.941	1.367	0.5459	0.0695	0.0282	0.0434	3616.3	3928.0	3932.6	Clen	Wild
153	16.8	17.4	19.1	19.4	20.3	22.5	23.3	25.3	0.1309	0.0081	0.0158	0.0090	1.256	0.887	1.415	0.5174	0.0625	0.0320	0.0354	2553.9	2841.8	2976.2	Clen	Wild
154	19.0	18.4	19.1	18.6	20.3	22.7	24.1	25.5	0.1335	0.0091	0.0177	0.0092	1.108	0.955	1.160	0.5235	0.0694	0.0357	0.0359	2181.3	2831.1	2917.2	Clen	Wild
164	20.9	22.3	-	21.7	22.2	24.7	26.3	27.4	0.1477	0.0091	0.0126	0.0129	1.319	0.930	1.416	0.5391	0.0460	0.0332	0.0469	-	2859.5	3095.1	Clen	Wild
165	19.3	18.0	-	18.8	21.5	24.5	25.3	25.9	0.1422	0.0077	0.0116	0.0124	1.175	0.942	1.247	0.5490	0.0448	0.0297	0.0479	-	2878.8	3104.1	Clen	Wild
172	17.8	-	-	20.4	21.6	24.0	25.7	26.3	0.1434	0.0109	0.0186	0.0129	1.353	0.953	1.420	0.5452	0.0707	0.0414	0.0490	-	-	-	Clen	Wild
173	18.2	-	-	20.3	20.4	23.6	24.5	26.2	0.1401	0.0085	0.0197	0.0128	1.246	0.848	1.469	0.5347	0.0752	0.0324	0.0487	-	-	-	Clen	Wild
425	15.2	17.9	20.0	22.7	24.6	26.8	28.1	28.3	0.1465	0.0095	0.0140	0.0126	1.273	1.062	1.199	0.5177	0.0495	0.0336	0.0445	-	-	-	Clen	Wild
427	18.3	19.4	21.6	21.4	22.1	24.6	25.0	25.4	0.1455	0.0081	0.0195	0.0090	1.237	0.905	1.367	0.5728	0.0768	0.0319	0.0352	-	-	-	Clen	Wild
429	16.9	19.0	21.5	21.8	25.7	27.6	29.0	29.8	0.1489	0.0082	0.0221	0.0139	1.304	1.025	1.272	0.4997	0.0742	0.0275	0.0465	-	-	-	Clen	Wild
943	15.7	18.5	20.4	21.2	22.7	25.1	25.8	27.8	0.1938	0.0079	0.0237	0.0156	1.358	0.744	1.823	0.6971	0.0853	0.0284	0.0559	3479.1	2835.8	4676.9	Clen	Trans
946	15.1	17.9	19.3	20.6	22.1	25.2	25.5	26.4	0.1866	0.0096	0.0258	0.0143	1.307	0.891	1.467	0.7068	0.0977	0.0364	0.0542	3579.0	3631.9	3360.8	Clen	Trans
952	14.4	17.3	19.0	20.2	21.8	25.7	25.6	26.0	0.1855	0.0082	0.0202	0.0146	1.332	0.664	2.005	0.7135	0.0777	0.0315	0.0562	3980.8	3430.5	2853.1	Clen	Trans
957	17.2	20.0	20.3	20.9	23.2	25.0	25.2	26.3	0.1858	0.0053	0.0227	0.0149	1.349	0.832	1.622	0.7065	0.0863	0.0202	0.0565	3778.0	3812.9	4054.1	Clen	Trans
959	14.2	16.2	17.0	18.2	20.2	25.2	25.6	26.1	0.1740	0.0074	0.0213	0.0134	1.147	0.803	1.428	0.6667	0.0816	0.0284	0.0513	3751.4	2785.9	3729.6	Clen	Trans

4	15.9	18.8	21.3	21.3	23.9	27.3	28.3	27.9	0.1966	0.0100	0.0208	0.0136	1.083	0.750	1.443	0.7047	0.0746	0.0358	0.0487	4728.0	3791.6	3321.9	Clen	Trans
5	16.2	18.8	21.1	22.1	24.9	27.7	28.5	27.4	0.1939	0.0097	0.0249	0.0145	1.151	0.820	1.403	0.7077	0.0909	0.0354	0.0527	4245.2	2828.2	3195.0	Clen	Trans
6	15.0	16.8	19.2	20.4	22.3	25.8	26.0	27.9	0.1942	0.0058	0.0262	0.0153	1.334	0.874	1.526	0.6961	0.0939	0.0208	0.0548	4208.3	4156.7	4221.1	Clen	Trans
14	12.4	15.5	18.5	20.9	23.0	25.9	26.9	26.8	0.1946	0.0091	0.0239	0.0130	1.275	0.797	1.597	0.7261	0.0892	0.0340	0.0485	4370.0	4146.1	4267.7	Clen	Trans
174	18.2	-	-	20.4	22.3	26.7	27.6	28.0	0.1882	0.0064	0.0243	0.0146	1.197	0.837	1.430	0.6721	0.0868	0.0229	0.0520	3059.4	3016.0	4380.5	Clen	Trans
984	16.5	18.9	23.0	23.9	26.9	28.4	31.5	31.1	0.2009	0.0078	0.0273	0.0158	1.313	0.827	1.587	0.6460	0.0878	0.0251	0.0506	3147.4	5066.7	4402.2	Clen	Trans
995	22.9	26.8	27.4	26.6	30.8	33.2	35.9	35.8	0.2566	0.0105	0.0364	0.0194	1.154	0.869	1.327	0.7168	0.1017	0.0293	0.0541	-	5411.3	4445.4	Clen	Trans
996	20.2	24.5	25.3	24.8	27.0	31.4	31.4	31.9	0.2448	0.0087	0.0298	0.0138	1.201	0.814	1.474	0.7674	0.0934	0.0273	0.0433	-	3410.6	5649.5	Clen	Trans
401	19.6	22.4	24.7	24.9	28.2	30.2	31.1	32.9	0.2022	0.0107	0.0322	0.0185	1.214	0.751	1.614	0.6146	0.0979	0.0325	0.0562	-	-	-	Clen	Trans

Animals were weaned at 4 week. C0day, start of CL administration; C3day, 3day of CL administration; C7day, 7day of CL administration; C10day, 10day of CL administration; C14day, 14day of CL administration; Geno, Genotype; CL, Clenbuterol; Gas, Gastrocnemius; Pln, Plantaris; EDL, extensor digitorum longus; Sol, Soleus Con, control; Clen, Clenbuterol administration; Wild, wild type mice; Trans, Transgenic mice

Appendix 3.4 The raw data of RT-PCR.

W VS M				W VS CLW				M VS CLM				CLW VS CLM			
Gene	Ct	Gene	Ct	Gene	Ct	Gene	Ct	Gene	Ct	Gene	Ct	Gene	Ct	Gene	Ct
A1	23	D1	24.8	G1	24.7	J1	26.6	M1	N/A	P1	20.9	S1	22	V1	20.3
A2	27.2	D2	21.3	G2	26.8	J2	22	M2	26.2	P2	21.9	S2	22.1	V2	21.2
A3	28.5	D3	22.9	G3	26.8	J3	23	M3	26.4	P3	21.6	S3	21.7	V3	21
A4	26	D4	22.8	G4	26.6	J4	24.2	M4	26.9	P4	21.9	S4	22.3	V4	21.3
A5	28.7	D5	21.2	G5	26.1	J5	22.8	M5	26.6	P5	21.1	S5	21.9	V5	21.1
A6	28.6	D6	21	G6	26.3	J6	23.9	M6	26.7	P6	21.4	S6	22.8	V6	22
A7	29.7	D7	22.9	G7	30.2	J7	24.6	M7	27.4	P7	21.5	S7	23.3	V7	25
A8	N/A	D8	24.6	G8	27.5	J8	22.6	M8	N/A	P8	21.2	S8	23.3	V8	32.1
A9	24.1	D9	23.7	G9	26.4	J9	22.5	M9	31.6	P9	21	S9	23.8	V9	20.3
A10	23.4	D10	22	G10	26.9	J10	22.5	M10	29.1	P10	21.6	S10	22.3	V10	20.2
A11	24	D11	21.7	G11	26.9	J11	21.1	M11	26.1	P11	21.2	S11	22.8	V11	22.4
A12	24.9	D12	24	G12	28.6	J12	23.3	M12	25.6	P12	21	S12	21.4	V12	21.1
A13	31.1	D13	23.1	G13	28.1	J13	22.2	M13	26.2	P13	21	S13	22	V13	24.8
A14	28.4	D14	35	G14	29.5	J14	26.5	M14	25.6	P14	21.2	S14	22.3	V14	23.7
A15	44.8	D15	23.9	G15	28.9	J15	25.2	M15	26.5	P15	20.7	S15	21.1	V15	32.2
A16	29.4	D16	26.3	G16	29.8	J16	29.6	M16	28.9	P16	20.4	S16	23.2	V16	25.1
A17	33.1	D17	20.7	G17	22.6	J17	21.8	M17	29.8	P17	22.7	S17	21.5	V17	26.8
A18	26.7	-	-	G18	29.8	J18	29.1	M18	28.8	P18	23	S18	22.5	V18	22.6
A19	N/A	D19	13.2	G19	15.9	J19	15.6	M19	17.3	P19	17.6	S19	19.2	V19	18.3
A20	N/A	D20	15.8	G20	14.1	J20	18.8	M20	13	P20	14	S20	18.5	V20	18
A21	19.5	D21	16.2	G21	15.2	J21	18.6	M21	15	P21	15	S21	20.4	V21	18.8
A22	18.8	D22	17.5	G22	16.8	J22	16.1	M22	13.6	P22	13.7	S22	19.7	V22	18.6
A23	18.5	D23	17.2	G23	15.2	J23	15.2	M23	12.3	P23	12.6	S23	20.6	V23	18.6
A24	18.4	D24	15.4	G24	14.1	J24	14.6	M24	13.6	P24	14.6	S24	20.5	V24	18.1
A25	17.8	D25	13.7	G25	14.7	J25	15	M25	13.2	P25	17.2	S25	20.2	V25	17.7
A26	19.2	D26	13.6	G26	13.7	J26	15.2	M26	16.1	P26	17.3	S26	19.4	V26	17.2
A27	19.9	D27	15.1	G27	16.4	J27	17.8	M27	13.6	P27	15.9	S27	19.7	V27	18.1
A28	N/A	D28	14.7	G28	14.3	J28	18.7	M28	14.6	P28	14.8	S28	19.5	V28	17
A29	19.5	D29	18.9	G29	16	J29	18.7	M29	13.2	P29	16.5	S29	18.6	V29	17.5
A30	18.4	D30	16.2	G30	15	J30	18.9	M30	13	P30	16	S30	18.1	V30	18.2
A31	19.8	D31	15.3	G31	14.9	J31	15.2	-	-	-	-	-	-	-	-
A32	18.9	D32	16	G32	14	J32	17.6	-	-	-	-	-	-	-	-
A33	18.7	D33	13.6	G33	14.8	J33	18.2	-	-	-	-	-	-	-	-

A34	18.2	D34	14.8	G34	14.3	J34	18.1	-	-	-	-	-	-	-	-
A35	19	D35	16.7	G35	14.9	J35	18.7	-	-	-	-	-	-	-	-
A36	N/A	D36	16.3	G36	13.2	J36	18.9	-	-	-	-	-	-	-	-
B1	22.6	E1	21.9	H1	20.7	K1	N/A	N1	18	Q1	22	T1	20.9	W1	23.7
B2	22.6	E2	21.6	H2	20.6	K2	22.5	N2	20.2	Q2	21.1	T2	21.9	W2	25
B3	22.8	E3	21.2	H3	19.7	K3	22	N3	19.2	Q3	21.7	T3	21.6	W3	23.2
B4	23.7	E4	21.4	H4	19.1	K4	21	N4	20.3	Q4	21.3	T4	21.9	W4	25.4
B5	23.1	E5	21.6	H5	20.2	K5	21.7	N5	20.8	Q1	21.9	T5	21.1	W5	24.1
B6	21.4	E6	23.9	H6	19.6	K6	23.5	N6	19	Q2	21.8	T6	21.4	W6	23.2
B7	21	E7	23.7	H7	22.4	K7	25.8	N7	18.7	Q3	23.3	T7	21.5	W7	24.9
B8	22.8	E8	21.1	H8	19.3	K8	N/A	N8	17.5	Q4	23.3	T8	21.2	W8	26
B9	23.3	E9	21.4	H9	21.5	K9	N/A	N9	20.1	Q1	23.8	T9	21	W9	25
B10	23.7	E10	21.1	H10	21.2	K10	21.6	N10	20.6	Q2	21.3	T10	21.6	W10	26.2
B11	23.2	E11	20.5	H11	19.4	K11	21.3	N11	19.7	Q3	22.8	T11	21.2	W11	26.6
B12	22.3	E12	23	H12	21.3	K12	22.7	N12	19	Q4	20.4	T12	21	W12	25.4
B13	23.2	E13	26.6	H13	20.8	K13	22	N13	17.5	Q1	22	T13	21	W13	25.9
B14	22.4	E14	25	H14	23.5	K14	26.3	N14	18.9	Q2	22.3	T14	21.2	W14	25.9
B15	33	E15	29.8	H15	23.8	K15	24.8	N15	19.6	Q3	21.1	T15	20.7	W15	25.8
B16	22.9	E16	21.1	H16	28.4	K16	26	N16	18.8	Q4	23.2	T16	20.4	W16	27.3
B17	25.3	E17	28.3	H17	21.6	K17	24	N17	19.9	Q1	21.5	T17	22.7	W17	27.1
B18	22.1	E18	23.8	H18	N/A	K18	27.6	N18	21.6	Q2	22.5	T18	23	W18	27.9
B19	16.6	E19	18.9	H19	12.3	K19	19.1	N1	14.8	Q19	19.2	T19	17.6	W19	20.6
B20	17.7	E20	N/A	H20	15.2	K20	19.8	N2	11.4	Q20	18.5	T20	17	W20	21.7
B21	17.8	E21	19.6	H21	14.9	K21	19.7	N3	12.7	Q21	20.4	T21	17	W21	21.3
B22	17.4	E22	18.5	H22	12.8	K22	19.8	N4	11.5	Q22	19.7	T22	17.7	W22	22.7
B23	18.4	E23	N/A	H23	12.2	K23	19.4	N5	11.4	Q23	20.6	T23	16.6	W23	22.9
B24	16.6	E24	18.8	H24	11.9	K24	20.3	N6	11.6	Q24	20.5	T24	16.6	W24	22.1
B25	15.1	E25	18.7	H25	12.4	K25	18.9	N7	11	Q25	20.2	T25	17.2	W25	22.2
B26	17.5	E26	18.3	H26	11.7	K26	19.4	N8	13	Q26	19.4	T26	17.3	W26	22.9
B27	16.9	E27	19.2	H27	15.2	K27	19	N9	11.3	Q27	19.7	T27	15.9	W27	22.4
B28	17.6	E28	19.9	H28	13.6	K28	19.7	N10	11.7	Q28	19.5	T28	14.8	W28	22.7
B29	16.8	E29	18.6	H29	13.7	K29	19.2	N11	14.6	Q29	18.6	T29	16.5	W29	22.8
B30	15.7	E30	18.4	H30	15	K30	20.1	N12	14.1	Q30	18.1	T30	16	W30	21.9
B31	15.8	E31	18.6	H31	15.8	K31	20.2	-	-	-	-	-	-	-	-
B32	16	E32	N/A	H32	16	K32	N/A	-	-	-	-	-	-	-	-
B33	17.6	E33	18	H33	15.2	K33	20.4	-	-	-	-	-	-	-	-

B34	14.6	E34	19.7	H34	14.1	K34	20.1	-	-	-	-	-	-	-	-
B35	18.3	E35	19.8	H35	14.9	K35	19.9	-	-	-	-	-	-	-	-
B36	17.7	E36	N/A	H36	15.2	K36	20.4	-	-	-	-	-	-	-	-
C1	20.3	F1	9.2	I1	22.9	L1	8.9	O1	22.7	R1	9.7	U1	20	X1	10.2
C2	21.2	F2	10	I2	24	L2	9.7	O2	23	R2	8.8	U2	20.2	X2	9.3
C3	21	F3	9.4	I3	23.1	L3	9.3	O3	23.2	R3	8.8	U3	19.2	X3	9.3
C4	21.3	F4	10.1	I4	23.6	L4	9.9	O4	24.4	R4	9.3	U4	20.3	X4	9.8
C5	21.1	F5	9.2	I5	22.1	L5	9.5	O5	24.1	R5	8.9	U5	20.8	X5	9.4
C6	22	F6	10.4	I6	23	L6	10.6	O6	23.2	R6	8.9	U6	19	X6	9.4
C7	25	F7	8.4	I7	28.4	L7	11.7	O7	21.9	R7	8.9	U7	18.7	X7	9.4
C8	32.1	F8	10.1	I8	23.8	L8	10.1	O8	22	R8	9	U8	20.5	X8	9.5
C9	20.3	F9	9.2	I9	23.8	L9	9	O9	25	R9	8.8	U9	20.1	X9	9.3
C10	20.2	F10	9.5	I10	23.7	L10	9.9	O10	26.2	R10	8.9	U10	20.6	X10	9.4
C11	22.4	F11	9.2	I11	24.2	L11	8.9	O11	26.6	R11	9.2	U11	19.7	X11	9.7
C12	21.1	F12	10.6	I12	27.7	L12	10.6	O12	23.4	R12	9	U12	19	X12	9.5
C13	24.8	F13	N/A	I13	27.5	L13	10.7	O13	21.9	R13	9	U13	20.5	X13	9.5
C14	23.7	F14	13	I14	27.7	L14	13.6	O14	23.9	R14	9.1	U14	20.9	X14	9.6
C15	32.2	F15	N/A	I15	26.9	L15	12.7	O15	23.8	R15	9.3	U15	19.6	X15	9.8
C16	25.1	F16	N/A	I16	28.1	L16	12.9	O16	22.3	R16	9.4	U16	20.8	X16	9.9
C17	26.8	F17	9.3	I17	21.3	L17	13.1	O17	27.1	R17	9	U17	19.9	X17	9.5
C18	22.6	F18	10.6	I18	27.5	L18	12.1	O18	27.9	R18	8.9	U18	21.6	X18	9.4
C19	17.6	F19	9.3	I19	18.3	L19	8.8	O19	19.6	R19	9.7	U19	15.8	X19	9.2
C20	21.9	F20	9.1	I20	18	L20	9.2	O20	17.7	R20	9.3	U20	17.4	X20	9.8
C21	18.5	F21	9.2	I21	18.8	L21	9.9	O21	17.3	R21	9.5	U21	15.7	X21	9
C22	14.1	F22	9.6	I22	18.6	L22	9.8	O22	16.7	R22	9.3	U22	17.5	X22	9.8
C23	18.1	F23	9.7	I23	18.6	L23	9.5	O23	15.9	R23	9.5	U23	17.4	X23	9
C24	17.6	F24	9.6	I24	18.1	L24	10	O24	16.1	R24	9.4	U24	17.6	X24	9.9
C25	17.7	F25	9.5	I25	17.7	L25	8.6	O25	16.2	R25	9.7	U25	17	X25	9.2
C26	17.5	F26	9.6	I26	17.2	L26	9.6	O26	17.9	R26	9.9	U26	17	X26	9.4
C27	17.6	F27	9.6	I27	18.1	L27	9	O27	15.4	R27	10.5	U27	17.3	X27	11
C28	18.8	F28	9.7	I28	17	L28	9.6	O28	16.7	R28	9	U28	17.7	X28	9.5
C29	18.6	F29	9.8	I29	17.5	L29	10.1	O29	18.8	R29	9.8	U29	17.6	X29	9.3
C30	18.9	F30	9.4	I30	18.2	L30	10.4	O30	14.9	R30	9.6	U30	17.1	X30	9.1
C31	17.1	F31	9	I31	17.4	L31	13	-	-	-	-	-	-	-	-
C32	17.6	F32	9.7	I32	15.1	L32	N/A	-	-	-	-	-	-	-	-
C33	17.6	F33	9.2	I33	18.9	L33	11.4	-	-	-	-	-	-	-	-

C34	15.9	F34	11	I34	18.2	L34	11.2	-	-	-	-	-	-	-	-
C35	18.1	F35	10.2	I35	17.7	L35	12	-	-	-	-	-	-	-	-
C36	18.7	F36	11.7	I36	17.5	L36	14.4	-	-	-	-	-	-	-	-

A, MT3; B, S100a4; C, Tnnt2; D, Ctss; E, Cgref1; F, Gapdh; G, Krt8; H, Rrad; I, Gdf5; J. Myog; K, Myo10; L, Gaphd; M, Krt8; N, Runx1; O, Orm2; P, Myog; Q, Tubb2; R, Gapdh; S, Tubb2a; T, Myof; U, Atf3; V, Palm2; W, Cgref1; X, Gapdh.. Ct, Threshold cycle; W, wild type mice M, Mstn-pro mice; CLW, clenbuterol treated wild type mice; CLM, clenbuterol treated Mstn-pro mice.

Appendix 3.5 Differential gene expression profiles as affected by the effect of genotype at 1.5 fold change (W vs M).

Probe Set ID	Fold change	Gene Symbol	Gene Title
1420575_at	5.75	Mt3	metallothionein 3
1420884_at	4.37	Sln	sarcolipin
1426808_at	4.36	Lgals3	lectin, galactose binding, soluble 3
1427076_at	3.94	Mpeg1	macrophage expressed gene 1
1418726_a_at	3.54	Tnnt2	troponin T2, cardiac[a]
1416431_at	3.49	Tubb6	tubulin, beta 6[a]
1459860_x_at	3.18	Trim2	tripartite motif-containing 2
1427201_at	3.13	Mustn1	musculoskeletal, embryonic nuclear protein 1
1416318_at	2.94	Serpinb1a	serine (or cysteine) peptidase inhibitor, clade B, member 1a[a]
1439426_x_at	2.92	Lyz1	lysozyme 1[a]
1424542_at	2.90	S100a4	S100 calcium binding protein A4
1443783_x_at	2.83	H2-Aa	histocompatibility 2, class II antigen A, alpha[b]
1436905_x_at	2.68	Laptm5	lysosomal-associated protein transmembrane 5
1419378_a_at	2.64	Fxyd2	FXYD domain-containing ion transport regulator 2
1423569_at	2.58	Gatm	glycine amidinotransferase
1423547_at	2.47	Lyz2	lysozyme 2
1425519_a_at	2.34	Cd74	CD74 antigen
1448591_at	2.30	Ctss	cathepsin S
1448303_at	2.29	Gpnmb	glycoprotein (transmembrane) nmb
1436778_at	2.29	Cybb	cytochrome b-245, beta polypeptide
1448617_at	2.27	Cd53	CD53 antigen
1451105_at	2.27	Vash2	vasohibin 2
1449164_at	2.24	Cd68	CD68 antigen
1432059_x_at	2.21	5031425E22Rik	RIKEN cDNA 5031425E22 gene
1455266_at	2.19	Kif5c	kinesin family member 5C
1422864_at	2.17	Runx1	runt related transcription factor 1[a]
1448883_at	2.15	Lgmn	legumain
1425477_x_at	2.13	H2-Ab1	histocompatibility 2, class II antigen A, beta 1[a]
1438988_x_at	2.12	Hn1	hematological and neurological expressed sequence 1[a]
1419599_s_at	2.09	Ms4a6d	membrane-spanning 4-domains, subfamily A, member 6D
1451987_at	2.06	Arrb2	arrestin, beta 2
1448152_at	2.05	Igf2	insulin-like growth factor 2
1448226_at	2.04	Rrm2	ribonucleotide reductase M2[a]

1419280_at	2.03	Pip4k2a	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha
1437224_at	2.00	Rtn4	reticulon 4
1437467_at	1.97	Alcam	activated leukocyte cell adhesion molecule
1436520_at	1.95	Ahnak2	AHNAK nucleoprotein 2
1418196_at	1.95	Tep1	telomerase associated protein 1
1450792_at	1.94	Tyrobp	TYRO protein tyrosine kinase binding protein
1444432_at	1.94	D330040H18Rik	RIKEN cDNA D330040H18 gene
1435477_s_at	1.93	Fcgr2b	Fc receptor, IgG, low affinity Iib[a]
1452016_at	1.92	Alox5ap	arachidonate 5-lipoxygenase activating protein
1451289_at	1.91	Dclk1	doublecortin-like kinase 1
1452717_at	1.91	Slc25a24	solute carrier family 25, member 24
1433893_s_at	1.90	Spag5	sperm associated antigen 5
1418852_at	1.90	Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1
1451064_a_at	1.87	Psat1	phosphoserine aminotransferase 1[a]
1427347_s_at	1.87	Tubb2a	tubulin, beta 2A
1429954_at	1.85	Clec4a3	C-type lectin domain family 4, member a3
1451201_s_at	1.84	Rnh1	ribonuclease/angiogenin inhibitor 1
1428420_a_at	1.83	1200009I06Rik	RIKEN cDNA 1200009I06 gene
1422124_a_at	1.79	Ptprc	protein tyrosine phosphatase, receptor type, C
1421698_a_at	1.78	Col19a1	collagen, type XIX, alpha 1
1450070_s_at	1.77	Pak1	p21 protein (Cdc42/Rac)-activated kinase 1[a]
1424987_at	1.76	5430435G22Rik	RIKEN cDNA 5430435G22 gene
1421525_a_at	1.76	Naip5	NLR family, apoptosis inhibitory protein 5
1417936_at	1.76	Ccl9	chemokine (C-C motif) ligand 9
1438948_x_at	1.73	Tspo	translocator protein[a]
1451161_a_at	1.73	Emr1	EGF-like module containing, hormone receptor-like sequence 1
1456014_s_at	1.73	Fermt3	fermitin family homolog 3 (Drosophila)
1448239_at	1.73	Hmox1	heme oxygenase (decycling) 1
1450234_at	1.72	Ms4a6c	membrane-spanning 4-domains, subfamily A, member 6C
1424529_s_at	1.69	Cgref1	cell growth regulator with EF hand domain 1
1426755_at	1.68	Ckap4	cytoskeleton-associated protein 4
1438596_at	1.68	1500017E21Rik	RIKEN cDNA 1500017E21 gene
1460555_at	1.64	Fam65b	family with sequence similarity 65, member B
1436188_a_at	1.64	Ndrg4	N-myc downstream regulated gene 4
1416698_a_at	1.63	Cks1b	CDC28 protein kinase 1b
1437771_at	1.63	Lrrn4cl	LRRN4 C-terminal like
1424176_a_at	1.63	Anxa4	annexin A4

1435122_x_at	1.62	Dnmt1	DNA methyltransferase (cytosine-5) 1
1455679_at	1.62	Obfc2a	oligonucleotide/oligosaccharide-binding fold containing 2A
1416844_at	1.61	Prmt2	protein arginine N-methyltransferase 2
1427275_at	1.61	Smc4	structural maintenance of chromosomes 4
1451780_at	1.60	Blnk	B-cell linker
1422186_s_at	1.60	Cyb5r3	cytochrome b5 reductase 3[a]
1423423_at	1.60	Pdia3	protein disulfide isomerase associated 3
1436566_at	1.60	Rab40b	Rab40b, member RAS oncogene family
1426215_at	1.60	Ddc	dopa decarboxylase
1417597_at	1.60	Cd28	CD28 antigen
1438629_x_at	1.59	Grn	granulin
1419873_s_at	1.59	Csf1r	colony stimulating factor 1 receptor[a]
1456377_x_at	1.59	Limd2	LIM domain containing 2
1441259_s_at	1.57	Ift122	intraflagellar transport 122 homolog (Chlamydomonas)
1434442_at	1.56	Stbd1	starch binding domain 1
1426615_s_at	1.55	Ndrg4	N-myc downstream regulated gene 4
1456783_at	1.55	Zdbf2	zinc finger, DBF-type containing 2
1417647_at	1.54	Snx5	sorting nexin 5
1436833_x_at	1.54	Ttll1	tubulin tyrosine ligase-like 1
1439610_at	1.53	Rab27b	RAB27b, member RAS oncogene family
1449258_at	1.52	D11Wsu99e	DNA segment, Chr 11, Wayne State University 99, expressed
1417868_a_at	1.51	Ctsz	cathepsin Z
1452554_at	2.29	Gm8479	thiopurine S-methyltransferase pseudogene
1437906_x_at	2.28	Txnl1	thioredoxin-like 1
1431491_at	2.11	9430087N24Rik	RIKEN cDNA 9430087N24 gene
1460388_at	1.83	Acot6	acyl-CoA thioesterase 6
1447567_at	1.83	D130007H15Rik	RIKEN cDNA D130007H15 gene
1438544_at	1.79	1700001G11Rik	RIKEN cDNA 1700001G11 gene
1440820_x_at	1.78	Tmco2	transmembrane and coiled-coil domains 2
1453026_at	1.67	Fam166a	family with sequence similarity 166, member A
1417275_at	1.54	Mal	myelin and lymphocyte protein, T-cell differentiation protein
1440335_at	1.53	LOC100046468	hypothetical protein LOC100046468
1440343_at	1.51	Rps6ka5	ribosomal protein S6 kinase, polypeptide 5
1447868_x_at	1.51	Glrx3	glutaredoxin 3
1426690_a_at	1.51	Srebf1	sterol regulatory element binding transcription factor 1

A total 108 gene identified from 134 Affymetrix probe sets after a 1.5 fold change cut off. 92 genes were overexpressed, and 16 genes were underexpressed (grey box). [a] This gene appears two times; [b] This gene appears three times; W, wild type mice; M, Mstn-pro mice.

Appendix 3.6 Differential gene expression profiles as affected by the effect of genotype under CL administration at 1.5 fold change (CLW vs CLM).

Probe Set ID	Fold change	Gene Symbol	Gene Title
1427838_at	4.38	Tubb2a	tubulin, beta 2A
1451054_at	3.75	Orm1	orosomucoid 1
1449700_at	3.01	Igbp1	Immunoglobulin (CD79A) binding protein 1
1430932_at	2.48	Slc9a8	solute carrier family 9, member 8
1444565_at	2.46	BB166591	expressed sequence BB166591
1455599_at	2.46	Gfod1	glucose-fructose oxidoreductase domain containing 1
1423887_a_at	2.37	Telo2	TEL2, telomere maintenance 2, homolog (S. cerevisiae)
1453673_at	2.29	LOC100046982	hypothetical protein LOC100046982
1452679_at	2.21	Tubb2b	tubulin, beta 2B
1452455_at	2.15	Myof	myoferlin
1449363_at	2.13	Atf3	activating transcription factor 3
1427205_x_at	2.08	Ccdc46	coiled-coil domain containing 46
1437544_at	2.07	Fubp1	far upstream element (FUSE) binding protein 1
1458385_at	2.01	Hspa4l	heat shock protein 4 like
1434204_x_at	1.91	Shmt2	serine hydroxymethyltransferase 2 (mitochondrial)
1440999_at	1.90	Zfp697	zinc finger protein 697
1456976_at	1.89	Wnt5a	wingless-related MMTV integration site 5A
1425362_at	1.87	Agfg2	ArfGAP with FG repeats 2
1417103_at	1.85	Ddt	D-dopachrome tautomerase
1457823_at	1.82	Cyr61	cysteine rich protein 61
1416396_at	1.75	Snx4	sorting nexin 4
1417428_at	1.75	Gng3	guanine nucleotide binding protein (G protein), gamma 3
1448885_at	1.74	Rap2b	RAP2B, member of RAS oncogene family
1454639_x_at	1.73	Rpl41	ribosomal protein L41
1445886_at	1.72	Elk3	ELK3, member of ETS oncogene family
1418908_at	1.70	Pam	peptidylglycine alpha-amidating monooxygenase
1444712_at	1.70	Hsf5	heat shock transcription factor family member 5
1431838_at	1.70	1700128E19Rik	RIKEN cDNA 1700128E19 gene
1441055_at	1.66	Palm2	paralemmin 2
1449590_a_at	1.64	Mras	muscle and microspikes RAS
1417758_at	1.61	Itga2b	integrin alpha 2b
1429509_at	1.60	Lsm12	LSM12 homolog (S. cerevisiae)
1417595_at	1.59	Meox1	mesenchyme homeobox 1

1454674_at	1.59	Fez1	fasciculation and elongation protein zeta 1 (zygin I)
1426275_a_at	1.57	Uxs1	UDP-glucuronate decarboxylase 1
1430469_at	1.55	Ttll5	tubulin tyrosine ligase-like family, member 5
1421354_at	1.54	Prkg2	protein kinase, cGMP-dependent, type II
1453854_at	1.53	Ptar1	protein prenyltransferase alpha subunit repeat containing 1
1434402_at	1.52	Samd8	sterile alpha motif domain containing 8
1447924_at	1.51	Nucks1	nuclear casein kinase and cyclin-dependent kinase substrate 1
1451505_at	1.51	Chchd5	coiled-coil-helix-coiled-coil-helix domain containing 5
1435561_at	1.50	Erf	Ets2 repressor factor
1424529_s_at	1.50	Cgref1	cell growth regulator with EF hand domain 1
1437915_at	3.35	Tom1l2	target of myb1-like 2 (chicken)
1426340_at	3.23	Slc1a3	solute carrier family 1, member 3
1436359_at	2.58	Ret	ret proto-oncogene
1427053_at	2.50	Abi3bp	ABI gene family, member 3 (NESH) binding protein
1436644_x_at	2.21	Tmem25	transmembrane protein 25
1430700_a_at	2.11	Pla2g7	phospholipase A2, group
1432001_at	2.10	Zmynd17	zinc finger, MYND domain containing 17
1422414_a_at	2.06	Calm2	calmodulin 2
1456312_x_at	2.03	Gsn	gelsolin
1434424_at	1.97	Mfsd7b	major facilitator superfamily domain containing 7B
1451047_at	1.94	Itm2a	integral membrane protein 2A
1426950_at	1.90	Parp16	poly (ADP-ribose) polymerase family, member 16
1448735_at	1.89	Cp	ceruloplasmin
1433511_at	1.84	Gtf2a1	general transcription factor II A, 1
1426109_a_at	1.81	Slc14a2	solute carrier family 14 (urea transporter), member 2
1429728_at	1.80	Zh2c2	zinc finger, H2C2 domain containing
1434093_at	1.77	Lysmd4	LysM, putative peptidoglycan-binding, domain containing 4
1428145_at	1.75	Acaa2	acetyl-Coenzyme A acyltransferase 2
1417625_s_at	1.75	Cxcr7	chemokine (C-X-C motif) receptor 7
1417673_at	1.73	Grb14	growth factor receptor bound protein 14
1451683_x_at	1.73	H2-D1	histocompatibility 2, D region locus 1
1435828_at	1.70	Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog
1423885_at	1.70	Lamc1	laminin, gamma 1
1419693_at	1.69	Colec12	collectin sub-family member 12
1434592_at	1.69	Slc16a10	solute carrier family 16, member 10
1455468_at	1.69	Dpy19l3	dpy-19-like 3 (C. elegans)
1448734_at	1.69	Cp	ceruloplasmin

1444504_at	1.68	Dhrs7c	dehydrogenase/reductase (SDR family) member 7C
1454806_at	1.66	Fam49a	family with sequence similarity 49, member A
1428107_at	1.65	Sh3bgrl	SH3-binding domain glutamic acid-rich protein like
1428187_at	1.65	Cd47	CD47 antigen
1415996_at	1.65	Txnip	thioredoxin interacting protein
1451718_at	1.64	Plp1	proteolipid protein (myelin) 1
1416072_at	1.62	Cd34	CD34 antigen
1452190_at	1.62	Prcp	prolylcarboxypeptidase (angiotensinase C)
1437313_x_at	1.62	Hmgb2	high mobility group box 2
1417042_at	1.60	Slc37a4	solute carrier family 37, member 4
1419442_at	1.60	Matn2	matrilin 2
1416968_a_at	1.59	Hsd3b7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7
1437868_at	1.58	Fam46a	family with sequence similarity 46, member A
1416225_at	1.58	Adh1	alcohol dehydrogenase 1 (class I)
1456956_at	1.58	Ikzf2	IKAROS family zinc finger 2
1433907_at	1.57	Pknox2	Pbx/knotted 1 homeobox 2
1425164_a_at	1.57	Phkg1	phosphorylase kinase gamma 1
1458087_at	1.56	Stac3	SH3 and cysteine rich domain 3
1451221_at	1.56	BC018507	cDNA sequence BC018507
1419922_s_at	1.56	Atrnl1	attractin like 1
1433842_at	1.55	Lrrfip1	leucine rich repeat (in FLII) interacting protein 1
1455739_at	1.54	Gm4980	predicted gene 4980
1455179_at	1.54	Mpp7	membrane protein, palmitoylated
1437041_at	1.53	Sfrs18	splicing factor, arginine/serine-rich 18
1416108_a_at	1.52	Tmed3	transmembrane emp24 domain containing 3
1417408_at	1.51	F3	coagulation factor III
1448421_s_at	1.51	Aspn	asporin

A total 97 gene identified from 302 Affymetrix probe sets after a 1.5 fold change cut off. 43 genes were overexpressed, and 54 genes were underexpressed (grey box). CLW, clenbuterol treated wild type mice; CLM, clenbuterol treated Mstn-pro mice.

Appendix 3.7 Differential gene expression profiles as affected by the effect of CL at 1.5 fold change (W vs CLW).

Probe Set ID	Fold change	Gene Symbol	Gene Title
1423691_x_at	390.33	Krt8	keratin 8 [b]
1448169_at	187.70	Krt18	keratin 18
1440878_at	65.37	Runx1	runt related transcription factor 1 [b]
1422562_at	54.82	Rrad	Ras-related associated with diabetes
1435053_s_at	52.42	Plekh	pleckstrin homology domain H [b]
1419139_at	50.83	Gdf5	growth differentiation factor 5
1434709_at	30.46	Nrcam	neuron-glia-CAM-related cell adhesion molecule [a]
1420991_at	23.76	Ankrd1	ankyrin repeat domain 1 (cardiac muscle) [a]
1455271_at	23.06	Gm13889	predicted gene 13889
1424831_at	23.02	Cpne2	copine II
1448975_s_at	21.68	Ren1	renin 1 structural
1454632_at	21.58	6330442E10Rik	RIKEN cDNA 6330442E10 gene [a]
1426808_at	16.80	Lgals3	lectin, galactose binding, soluble 3
1424245_at	16.56	Ces2	carboxylesterase 2
1458813_at	16.54	Scn5a	sodium channel, voltage-gated, type V, alpha
1418852_at	15.79	Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)
1428640_at	14.23	Hsf2bp	heat shock transcription factor 2 binding protein
1430086_at	13.92	Chrna9	cholinergic receptor, nicotinic, alpha polypeptide 9
1449519_at	13.70	Gadd45a	growth arrest and DNA-damage-inducible 45 alpha
1427655_a_at	10.35	A630038E17Rik	RIKEN cDNA A630038E17 gene
1421698_a_at	9.43	Col19a1	collagen, type XIX, alpha 1 [a]
1418460_at	9.23	Sh3	SH3 domain protein[b]
1440085_at	9.14	Eda2r	ectodysplasin A2 isoform receptor
1435176_a_at	8.97	Id2	inhibitor of DNA binding 2 [a]
1451287_s_at	8.93	Aif1l	allograft inflammatory factor 1-like [a]
1419391_at	8.63	Myog	Myogenin
1420884_at	8.55	Sln	Sarcolipin
1422580_at	8.43	Myl4	myosin, light polypeptide 4
1455649_at	7.72	Ttc9	tetratricopeptide repeat domain 9
1421852_at	7.71	Kcnk5	potassium channel, subfamily K, member 5
1419394_s_at	7.71	S100a8	S100 calcium binding protein A8 (calgranulin A)
1455342_at	7.59	Prune2	prune homolog 2 (Drosophila) [a]
1421269_at	7.41	Ugcg	UDP-glucose ceramide glucosyltransferase [b]

1453125_at	7.12	Sox11	SRY-box containing gene 11 [b]
1423186_at	6.99	Tiam2	T-cell lymphoma invasion and metastasis 2 [a]
1427910_at	6.94	Cst6	cystatin E/M
1418571_at	6.86	Tnfrsf12a	tumor necrosis factor receptor superfamily, member 12a [a]
1438988_x_at	6.59	Hn1	hematological and neurological expressed sequence 1 [b]
1420444_at	6.56	Slc22a3	solute carrier family 22 (organic cation transporter), member 3
1416431_at	6.53	Tubb6	tubulin, beta 6
1422629_s_at	6.38	Shroom3	shroom family member 3
1423719_at	6.28	LOC632073	similar to long palate, lung and nasal epithelium carcinoma associated 1 isoform 2
1434513_at	5.92	Atp13a3	ATPase type 13A3 [a]
1460290_at	5.82	Lpin2	lipin 2 [b]
1420938_at	5.80	Hs6st2	Heparan sulfate 6-O-sulfotransferase 2 [a]
1455771_at	5.47	Bzrap1	benzodiazapine receptor associated protein 1
1448660_at	5.38	Arhgdig	Rho GDP dissociation inhibitor (GDI) gamma
1421679_a_at	5.19	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21) [a]
1450013_at	5.16	2900073G15Rik	RIKEN cDNA 2900073G15 gene [a]
1426650_at	5.02	Myh8	myosin, heavy polypeptide 8, skeletal muscle, perinatal
1451036_at	4.80	Spg21	spastic paraplegia 21 homolog (human)
1420438_at	4.72	Orm2	orosomucoid 2
1418158_at	4.50	Trp63	transformation related protein 63 [a]
1442590_at	4.49	Tnfrsf22	tumor necrosis factor receptor superfamily, member 22 [a]
1437224_at	4.36	Rtn4	reticulon 4
1449533_at	4.36	Tmem100	transmembrane protein 100
1425543_s_at	4.34	Plekha5	pleckstrin homology domain containing, family A member 5
1422573_at	4.33	Ampd3	adenosine monophosphate deaminase 3
1434754_at	4.30	Garnl4	GTPase activating RANGAP domain-like 4
1452232_at	4.23	Galnt7	UDP-N-acetyl-alpha-D-galactosamine
1458245_at	4.17	Gm12528	predicted gene 12528
1428834_at	4.15	Dusp4	dual specificity phosphatase 4
1421499_a_at	4.10	Ptpn14	protein tyrosine phosphatase, non-receptor type 14
1448605_at	4.06	Rhoc	ras homolog gene family, member C [a]
1426337_a_at	3.99	Tead4	TEA domain family member 4
1454674_at	3.96	Fez1	fasciculation and elongation protein zeta 1 (zygin I)
1422929_s_at	3.91	Atoh7	atonal homolog 7 (Drosophila)
1430062_at	3.89	Hhipl1	hedgehog interacting protein-like 1
1451160_s_at	3.88	Pvr	poliovirus receptor [c]
1449852_a_at	3.86	Ehd4	EH-domain containing 4

1424613_at	3.83	Gprc5b	G protein-coupled receptor, family C, group 5, member B [a]
1442827_at	3.83	Tlr4	Toll-like receptor 4 [b]
1457779_at	3.81	1110046J04Rik	RIKEN cDNA 1110046J04 gene
1421175_at	3.78	Myt1l	myelin transcription factor 1-like
1420682_at	3.70	Chrnb1	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
1422286_a_at	3.60	Tgif1	TGFB-induced factor homeobox 1
1451105_at	3.59	Vash2	vasohibin 2
1427347_s_at	3.57	Tubb2a	tubulin, beta 2A
1452799_at	3.55	Fggy	FGGY carbohydrate kinase domain containing
1429786_a_at	3.52	Zwint	ZW10 interactor [c]
1460521_a_at	3.51	Obfc2a	oligonucleotide/oligosaccharide-binding fold containing 2A [d]
1416401_at	3.51	Cd82	CD82 antigen
1417014_at	3.49	Hspb8	heat shock protein 8 [a]
1421042_at	3.46	Arhgef2	rho/rac guanine nucleotide exchange factor (GEF) 2 [a]
1436507_at	3.35	Irak2	interleukin-1 receptor-associated kinase 2
1450650_at	3.34	Myo10	myosin X [b]
1428803_at	3.32	Acot6	acyl-CoA thioesterase 6
1427005_at	3.29	Plk2	polo-like kinase 2 (Drosophila)
1424354_at	3.25	Tmem140	transmembrane protein 140
1451680_at	3.24	Srxn1	sulfiredoxin 1 homolog (S. cerevisiae) [b]
1435184_at	3.21	Npr3	natriuretic peptide receptor 3 [a]
1426471_at	3.15	Zfp52	zinc finger protein 52 [a]
1450241_a_at	3.13	Evi2a	ecotropic viral integration site 2a
1432075_a_at	3.12	Tekt1	tektin 1
1424507_at	3.12	Rin1	Ras and Rab interactor 1
1418649_at	3.11	Egln3	EGL nine homolog 3 (C. elegans) [a]
1433571_at	3.10	Serinc5	serine incorporator 5
1427483_at	3.10	Slc25a24	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 24 [a]
1435948_at	3.09	Tmem181a	transmembrane protein 181A
1434526_at	3.09	Ephx4	epoxide hydrolase 4
1416231_at	3.09	Vac14	Vac14 homolog (S. cerevisiae)
1438596_at	3.07	1500017E21Rik	RIKEN cDNA 1500017E21 gene
1423596_at	3.06	Nek6	NIMA (never in mitosis gene a)-related expressed kinase 6 [a]
1420895_at	3.02	Tgfbr1	transforming growth factor, beta receptor I
1439618_at	2.99	Pde10a	phosphodiesterase 10A
1457296_at	2.98	Cilp	cartilage intermediate layer protein, nucleotide pyrophosphohydrolase
1416065_a_at	2.97	Ankrd10	ankyrin repeat domain 10 [b]

1440008_at	2.95	2310043L19Rik	RIKEN cDNA 2310043L19 gene
1451814_a_at	2.93	Htatip2	HIV-1 tat interactive protein 2, homolog (human)
1460062_at	2.93	Plekhh2	pleckstrin homology domain containing, family H member 2
1434241_at	2.92	Wdr67	WD repeat domain 67
1449164_at	2.89	Cd68	CD68 antigen
1422101_at	2.87	Tnfrsf23	tumor necrosis factor receptor superfamily, member 23
1416379_at	2.84	Panx1	pannexin 1
1450437_a_at	2.83	Ncam1	neural cell adhesion molecule 1 [a]
1425329_a_at	2.83	Cyb5r3	cytochrome b5 reductase 3 [b]
1417856_at	2.81	Relb	avian reticuloendotheliosis viral (v-rel) oncogene related B
1453323_at	2.79	2900079G21Rik	RIKEN cDNA 2900079G21 gene
1460718_s_at	2.78	Mtch1	mitochondrial carrier homolog 1 (C. elegans)
1434382_at	2.76	Serinc2	serine incorporator 2
1424176_a_at	2.74	Anxa4	annexin A4 [b]
1418420_at	2.73	Myod1	myogenic differentiation 1
1423082_at	2.73	Derl1	Der1-like domain family, member 1 [b]
1420832_at	2.69	Qsox1	quiescin Q6 sulfhydryl oxidase 1
1417356_at	2.69	Peg3	paternally expressed 3
1425347_a_at	2.68	Zfp318	zinc finger protein 318 [c]
1428739_at	2.68	Enho	energy homeostasis associated [a]
1426576_at	2.64	Sgms1	sphingomyelin synthase 1 [b]
1424517_at	2.64	Ccdc12	coiled-coil domain containing 12 [a]
1417128_at	2.64	Plekho1	pleckstrin homology domain containing, family O member 1
1460392_a_at	2.63	Eny2	enhancer of yellow 2 homolog (Drosophila) [a]
1451064_a_at	2.63	Psat1	phosphoserine aminotransferase 1 [a]
1446342_at	2.61	2310001H17Rik	RIKEN cDNA 2310001H17 gene [a]
1449839_at	2.60	Casp3	caspase 3 [a]
1434442_at	2.57	Stbd1	starch binding domain 1
1434927_at	2.56	Hspb7	heat shock protein family, member 7 (cardiovascular) [a]
1438948_x_at	2.55	Tspo	translocator protein [b]
1449036_at	2.55	Rnf128	ring finger protein 128 [a]
1452242_at	2.55	Cep55	centrosomal protein 55
1436520_at	2.55	Ahnak2	AHNAK nucleoprotein 2
1436237_at	2.54	Ttc9	tetratricopeptide repeat domain 9
1436042_at	2.51	Tln1	talin 1
1421052_a_at	2.51	Sms	spermine synthase [b]
1455203_at	2.50	A930003A15Rik	RIKEN cDNA A930003A15 gene

1421654_a_at	2.49	Lmna	lamin A
1435981_at	2.47	Nav2	neuron navigator 2
1417850_at	2.47	Rb1	retinoblastoma 1
1425238_at	2.47	Gm6607	40S ribosomal protein S20 pseudogene
1451227_a_at	2.46	Slc10a3	solute carrier family 10 (sodium/bile acid cotransporter family), member 3
1450511_at	2.46	Musk	muscle, skeletal, receptor tyrosine kinase
1444786_at	2.46	Nol3	nucleolar protein 3 (apoptosis repressor with CARD domain)
1438133_a_at	2.45	Cyr61	cysteine rich protein 61 [a]
1436758_at	2.44	Hdac4	histone deacetylase 4 [a]
1431281_at	2.44	Dysfip1	dysferlin interacting protein 1
1417398_at	2.43	Rras2	related RAS viral (r-ras) oncogene homolog 2 [a]
1416251_at	2.43	Mcm6	minichromosome maintenance deficient 6 (S. cerevisiae)
1419028_at	2.42	Arpp21	cyclic AMP-regulated phosphoprotein, 21 [a]
1422776_at	2.42	Serpinb8	serine (or cysteine) peptidase inhibitor, clade B, member 8
1417005_at	2.41	Klc1	kinesin light chain 1
1434364_at	2.41	Map3k14	mitogen-activated protein kinase kinase kinase 14
1438429_at	2.40	2610319H10Rik	RIKEN cDNA 2610319H10 gene
1437669_x_at	2.40	Ccrl1	Chemokine (C-C motif) receptor-like 1 [a]
1436204_at	2.40	1110059G02Rik	RIKEN cDNA 1110059G02 gene [a]
1449323_a_at	2.39	Rpl3	ribosomal protein L3
1455350_at	2.39	Tmem62	transmembrane protein 62
1435039_a_at	2.37	Pip5k1a	phosphatidylinositol-4-phosphate 5-kinase, type 1 alpha [a]
1425964_x_at	2.35	Hspb1	heat shock protein 1 [a]
1429618_at	2.35	Cyld	cylindromatosis (turban tumor syndrome) [a]
1450959_at	2.35	D930014E17Rik	RIKEN cDNA D930014E17 gene
1423606_at	2.35	Postn	periostin, osteoblast specific factor
1424489_a_at	2.34	Trit1	tRNA isopentenyltransferase 1
1440999_at	2.34	Zfp697	zinc finger protein 697
1423774_a_at	2.33	Prc1	protein regulator of cytokinesis 1
1460302_at	2.32	Thbs1	thrombospondin 1 [b]
1419150_at	2.29	Myf6	myogenic factor 6
1448744_at	2.27	Galns	galactosamine (N-acetyl)-6-sulfate sulfatase
1421346_a_at	2.26	Slc6a6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6 [a]
1454962_at	2.26	Spire1	spire homolog 1 (Drosophila) [a]
1450062_a_at	2.26	Maged1	melanoma antigen, family D, 1
1415899_at	2.26	Junb	Jun-B oncogene
1441055_at	2.24	Palm2	paralemmin 2

1453238_s_at	2.23	1200016E24Rik	RIKEN cDNA 1200016E24 gene
1417375_at	2.23	Tuba4a	tubulin, alpha 4A [a]
1424292_at	2.21	Depdc1a	DEP domain containing 1a
1424875_at	2.21	Spg20	spastic paraplegia 20, spartin (Troyer syndrome) homolog (human)
1450686_at	2.21	Pon2	paraoxonase 2 [a]
1449968_s_at	2.20	Acot10	acyl-CoA thioesterase 10
1435137_s_at	2.19	1200015M12Rik	RIKEN cDNA 1200015M12 gene
1433928_a_at	2.19	Rpl13a	ribosomal protein L13A
1434369_a_at	2.19	Cryab	crystallin, alpha B [a]
1452478_at	2.18	Alpk2	alpha-kinase 2
1422620_s_at	2.17	Ppap2a	phosphatidic acid phosphatase type 2A [a]
1416606_s_at	2.17	Nhp2	NHP2 ribonucleoprotein homolog (yeast) [a]
1418498_at	2.17	Fgf13	fibroblast growth factor 13 [a]
1452719_at	2.16	Zdhhc24	zinc finger, DHHC domain containing 24 [a]
1423662_at	2.16	Atp6ap2	ATPase, H+ transporting, lysosomal accessory protein 2 [a]
1416192_at	2.16	Napa	N-ethylmaleimide sensitive fusion protein attachment protein alpha [a]
1441071_at	2.16	Kcnq5	potassium voltage-gated channel, subfamily Q, member 5
1419184_a_at	2.15	Fhl2	four and a half LIM domains 2
1448883_at	2.15	Lgmn	legumain
1423264_at	2.15	Bop1	block of proliferation 1
1450070_s_at	2.14	Pak1	p21 protein (Cdc42/Rac)-activated kinase 1 [b]
1423745_at	2.13	Snap47	synaptosomal-associated protein, 47 [a]
1416749_at	2.13	Htra1	HtrA serine peptidase 1
1451809_s_at	2.13	Rwdd3	RWD domain containing 3
1422660_at	2.13	Rbm3	RNA binding motif protein 3
1416034_at	2.12	Cd24a	CD24a antigen [b]
1418448_at	2.12	Rras	Harvey rat sarcoma oncogene, subgroup R
1448124_at	2.12	Gusb	glucuronidase, beta
1436305_at	2.11	Rnf217	ring finger protein 217 [a]
1424378_at	2.10	Ldlrap1	low density lipoprotein receptor adaptor protein 1
1434891_at	2.10	Ptgfrn	prostaglandin F2 receptor negative regulator
1419398_a_at	2.10	Reep5	receptor accessory protein 5 [a]
1434748_at	2.09	Ckap2	cytoskeleton associated protein 2
1426465_at	2.09	Dlgap4	discs, large homolog-associated protein 4 (Drosophila) [a]
1456291_x_at	2.08	Scx	scleraxis [a]
1436833_x_at	2.08	Ttll1	tubulin tyrosine ligase-like 1 [a]
1460235_at	2.08	Scarb2	scavenger receptor class B, member 2

1423122_at	2.07	Avpi1	arginine vasopressin-induced 1
1417654_at	2.07	Sdc4	syndecan 4 [a]
1432393_a_at	2.07	Thg1l	tRNA-histidine guanylyltransferase 1-like (S. cerevisiae)
1424529_s_at	2.06	Cgref1	cell growth regulator with EF hand domain 1
1418428_at	2.06	Kif5b	kinesin family member 5B [c]
1427932_s_at	2.05	1200003I10Rik	RIKEN cDNA 1200003I10 gene
1436684_a_at	2.05	Riok2	RIO kinase 2 (yeast) [a]
1451201_s_at	2.05	Rnh1	ribonuclease/angiogenin inhibitor 1
1417032_at	2.05	Ube2g2	ubiquitin-conjugating enzyme E2G 2 [a]
1424719_a_at	2.04	Mapt	microtubule-associated protein tau
1452761_a_at	2.04	8430436O14Rik	RIKEN cDNA 8430436O14 gene
1424538_at	2.04	Ubl4	ubiquitin-like 4
1416119_at	2.03	Txn1	thioredoxin 1
1435432_at	2.03	Agap1	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
1437828_s_at	2.03	Wdr46	WD repeat domain 46
1418073_at	2.02	Acot9	acyl-CoA thioesterase 9
1416138_at	2.01	Anxa7	annexin A7
1448968_at	2.01	Ubfd1	ubiquitin family domain containing 1
1452679_at	2.01	Tubb2b	tubulin, beta 2B
1434080_at	2.00	Aebp2	AE binding protein 2 [b]
1423607_at	2.00	Lum	lumican
1422615_at	2.00	Map4k4	mitogen-activated protein kinase kinase kinase kinase 4
1422650_a_at	1.99	Riok3	RIO kinase 3 (yeast) [a]
1416911_a_at	1.99	Akirin1	akirin 1 [a]
1452787_a_at	1.99	Prmt1	protein arginine N-methyltransferase 1
1423642_at	1.99	Tubb2c	tubulin, beta 2C
1426534_a_at	1.98	Arfgap3	ADP-ribosylation factor GTPase activating protein 3
1427364_a_at	1.98	Odc1	ornithine decarboxylase, structural 1 [b]
1426329_s_at	1.98	Baalc	brain and acute leukemia, cytoplasmic
1436710_at	1.97	Zswim4	zinc finger, SWIM domain containing 4
1433555_at	1.97	Eaf1	ELL associated factor 1
1449645_s_at	1.97	Cct3	chaperonin containing Tcp1, subunit 3 (gamma) [a]
1422155_at	1.97	Hist2h3c2	histone cluster 2, H3c2
1451480_at	1.97	E2f4	E2F transcription factor 4
1448271_a_at	1.96	Ddx21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
1449258_at	1.96	D11Wsu99e	DNA segment, Chr 11, Wayne State University 99, expressed
1450198_at	1.96	Dusp13	dual specificity phosphatase 13

1427186_a_at	1.95	Mef2a	myocyte enhancer factor 2A [b]
1451208_at	1.95	Etf1	eukaryotic translation termination factor 1
1423239_at	1.95	Impdh1	inosine 5'-phosphate dehydrogenase 1
1417455_at	1.95	Tgfb3	transforming growth factor, beta 3
1418186_at	1.95	Gstt1	glutathione S-transferase, theta 1
1423551_at	1.95	Cdh13	cadherin 13 [a]
1449073_at	1.94	Flnc	filamin C, gamma [a]
1451325_at	1.93	Fyttd1	forty-two-three domain containing 1
1426215_at	1.93	Ddc	dopa decarboxylase
1433599_at	1.93	Baz1a	bromodomain adjacent to zinc finger domain 1A
1419287_at	1.92	Tmem208	transmembrane protein 208
1451269_at	1.92	Pdzd11	PDZ domain containing 11
1426722_at	1.92	Slc38a2	solute carrier family 38, member 2
1418726_a_at	1.92	Tnnt2	troponin T2, cardiac [a]
1449275_at	1.92	Fam119a	family with sequence similarity 119, member A
1439708_at	1.92	Myom3	myomesin family, member 3
1450846_at	1.92	Bzw1	basic leucine zipper and W2 domains 1 [b]
1453137_at	1.92	Fbxo30	F-box protein 30
1428420_a_at	1.91	1200009I06Rik	RIKEN cDNA 1200009I06 gene
1437109_s_at	1.91	Lsm6	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae)
1424289_at	1.91	Osgin2	oxidative stress induced growth inhibitor family member 2
1434923_at	1.91	Cox19	COX19 cytochrome c oxidase assembly homolog (S. cerevisiae)
1448239_at	1.91	Hmox1	heme oxygenase (decycling) 1
1419058_at	1.90	Polr1e	polymerase (RNA) I polypeptide E
1427771_x_at	1.90	Itgb1	integrin beta 1 (fibronectin receptor beta) [b]
1455627_at	1.90	Col8a1	collagen, type VIII, alpha 1
1426485_at	1.90	Ubxn4	UBX domain protein 4 [b]
1423296_at	1.90	Psmd8	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8
1424942_a_at	1.90	Myc	myelocytomatosis oncogene
1427239_at	1.90	Ift122	intraflagellar transport 122 homolog (Chlamydomonas) [a]
1423158_at	1.89	Gnpnat1	glucosamine-phosphate N-acetyltransferase 1
1416213_x_at	1.89	Surf4	surfeit gene 4 [a]
1426403_at	1.89	Actr1b	ARP1 actin-related protein 1 homolog B, centractin beta (yeast)
1426600_at	1.89	Slc2a1	solute carrier family 2 (facilitated glucose transporter), member 1 [a]
1428847_a_at	1.89	Macf1	microtubule-actin crosslinking factor 1
1422789_at	1.89	Aldh1a2	aldehyde dehydrogenase family 1, subfamily A2
1416708_a_at	1.89	Gramd1a	GRAM domain containing 1A

1419524_at	1.89	Tph1	tryptophan hydroxylase 1
1423388_at	1.88	Ap1g1	adaptor protein complex AP-1, gamma 1 subunit
1420984_at	1.88	Pctp	phosphatidylcholine transfer protein
1419254_at	1.88	Mthfd2	methylenetetrahydrofolate dehydrogenase (NAD+ dependent)
1433972_at	1.88	Camta1	calmodulin binding transcription activator 1 [a]
1455405_at	1.88	Pstpip2	proline-serine-threonine phosphatase-interacting protein 2 [b]
1429307_s_at	1.87	Lzic	leucine zipper and CTNNBIP1 domain containing
1423810_at	1.87	Ppme1	protein phosphatase methylesterase 1
1418822_a_at	1.87	Arf6	ADP-ribosylation factor 6 [a]
1452011_a_at	1.87	Uxs1	UDP-glucuronate decarboxylase 1
1421824_at	1.87	Bace1	beta-site APP cleaving enzyme 1 [a]
1451257_at	1.86	Acsl6	acyl-CoA synthetase long-chain family member 6
1418454_at	1.86	Mfap5	microfibrillar associated protein 5 [a]
1450909_at	1.86	Eif4e	eukaryotic translation initiation factor 4E
1434985_a_at	1.86	Eif4a1	eukaryotic translation initiation factor 4A1
1430724_at	1.86	Ephx3	epoxide hydrolase 3
1417806_at	1.85	Popdc2	popeye domain containing 2
1416608_a_at	1.85	BC004004	cDNA sequence BC004004
1451891_a_at	1.85	Dysf	dysferlin
1431645_a_at	1.85	Gdi2	guanosine diphosphate (GDP) dissociation inhibitor 2 [a]
1436234_at	1.85	4732471D19Rik	RIKEN cDNA 4732471D19 gene
1460345_at	1.85	Aida	axin interactor, dorsalization associated
1425550_a_at	1.85	Prkar1a	protein kinase, cAMP dependent regulatory, type I, alpha
1423223_a_at	1.84	Prdx6	peroxiredoxin 6
1419568_at	1.84	Mapk1	mitogen-activated protein kinase 1
1423358_at	1.84	Ece2	endothelin converting enzyme 2
1452954_at	1.84	Ube2c	ubiquitin-conjugating enzyme E2C
1437524_x_at	1.84	Coro7	coronin 7
1423923_a_at	1.84	Wdr8	WD repeat domain 8
1425534_at	1.84	Stau2	staufen (RNA binding protein) homolog 2 (Drosophila)
1450065_at	1.84	Adcy7	adenylate cyclase 7
1417647_at	1.83	Snx5	sorting nexin 5
1416974_at	1.83	Stam2	signal transducing adaptor molecule (SH3 domain and ITAM motif) 2
1426645_at	1.83	Hsp90aa1	heat shock protein 90, alpha (cytosolic), class A member 1 [b]
1426931_s_at	1.83	D19Bwg1357e	DNA segment, Chr 19, Brigham & Women's Genetics 1357 expressed
1423865_at	1.83	Slc44a1	solute carrier family 44, member 1
1450138_a_at	1.82	Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a

1455210_at	1.82	Zhx2	zinc fingers and homeoboxes 2
1428754_at	1.82	Trmt6	tRNA methyltransferase 6 homolog (S. cerevisiae)
1448557_at	1.82	Fam13c	family with sequence similarity 13, member C [a]
1450423_s_at	1.81	Bxdc1	brix domain containing 1 [a]
1448824_at	1.81	Ube2j1	ubiquitin-conjugating enzyme E2, J1
1435034_at	1.81	Rpap2	RNA polymerase II associated protein 2
1418364_a_at	1.81	Ftl1	ferritin light chain 1
1421947_at	1.81	Gng12	guanine nucleotide binding protein (G protein), gamma 12
1424926_at	1.81	Sec63	SEC63-like (S. cerevisiae) [a]
1422859_a_at	1.81	Rpl23	ribosomal protein L23
1422508_at	1.80	Atp6v1a	ATPase, H+ transporting, lysosomal V1 subunit A [a]
1428386_at	1.80	Acsl3	acyl-CoA synthetase long-chain family member 3
1440822_x_at	1.80	Reps1	RalBP1 associated Eps domain containing protein
1417103_at	1.80	Ddt	D-dopachrome tautomerase
1422506_a_at	1.80	Cstb	cystatin B [a]
1426266_s_at	1.80	Zbtb8os	zinc finger and BTB domain containing 8 opposite strand
1455798_at	1.79	Galk2	galactokinase 2
1436828_a_at	1.79	Tpd52l2	tumor protein D52-like 2 [a]
1449037_at	1.79	Crem	cAMP responsive element modulator
1420745_a_at	1.79	Ccndbp1	cyclin D-type binding-protein 1
1451290_at	1.79	Map1lc3a	microtubule-associated protein 1 light chain 3 alpha [a]
1416038_at	1.79	Snd1	staphylococcal nuclease and tudor domain containing 1
1424620_at	1.79	Nop16	NOP16 nucleolar protein homolog (yeast)
1419446_at	1.79	Tbc1d1	TBC1 domain family, member 1
1423465_at	1.79	Frrs1	ferric-chelate reductase 1
1417143_at	1.78	Lpar1	lysophosphatidic acid receptor 1
1422906_at	1.78	Abcg2	ATP-binding cassette, sub-family G (WHITE), member 2
1451026_at	1.78	Ftsj3	FtsJ homolog 3 (E. coli)
1423735_a_at	1.78	Wdr36	WD repeat domain 36
1423706_a_at	1.78	Pgd	phosphogluconate dehydrogenase
1416748_a_at	1.78	Mre11a	meiotic recombination 11 homolog A (S. cerevisiae)
1423053_at	1.78	Arf4	ADP-ribosylation factor 4
1421529_a_at	1.78	Txnrd1	thioredoxin reductase 1
1425551_at	1.77	Hip1r	huntingtin interacting protein 1 related
1425027_s_at	1.77	Sft2d2	SFT2 domain containing 2
1422844_a_at	1.77	Wdr77	WD repeat domain 77
1419838_s_at	1.77	Plk4	polo-like kinase 4 (Drosophila)

1427578_a_at	1.77	Eif6	eukaryotic translation initiation factor 6
1433548_at	1.76	Mare	alpha globin regulatory element containing gene
1439040_at	1.76	Cenpe	centromere protein E
1419573_a_at	1.76	Lgals1	lectin, galactose binding, soluble 1 [a]
1455726_at	1.76	Gm71	predicted gene 71
1446750_at	1.76	Impact	imprinted and ancient [a]
1429588_at	1.76	2810474O19Rik	RIKEN cDNA 2810474O19 gene
1424819_a_at	1.76	Ric8	resistance to inhibitors of cholinesterase 8 homolog (C. elegans)
1427375_at	1.75	Rg9mtd2	RNA (guanine-9-) methyltransferase domain containing 2
1416178_a_at	1.75	Plekhb1	pleckstrin homology domain containing, family B (evectins) member 1
1419619_at	1.75	Krt80	keratin 80
1423621_a_at	1.74	Slc33a1	solute carrier family 33 (acetyl-CoA transporter), member 1
1417453_at	1.74	Cul4b	cullin 4B
1422813_at	1.74	Cacng1	calcium channel, voltage-dependent, gamma subunit 1
1417088_at	1.74	Zfp346	zinc finger protein 346
1423452_at	1.74	Stk17b	serine/threonine kinase 17b (apoptosis-inducing)
1416013_at	1.74	Pld3	phospholipase D family, member 3
1429370_a_at	1.74	Psmd11	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11
1448618_at	1.74	Mvp	major vault protein [a]
1415803_at	1.73	Cx3cl1	chemokine (C-X3-C motif) ligand 1
1453228_at	1.73	Stx11	syntaxin 11
1429088_at	1.73	Lbh	limb-bud and heart
1420886_a_at	1.73	Xbp1	X-box binding protein 1
1452066_a_at	1.73	Ndfip2	Nedd4 family interacting protein 2
1455390_at	1.73	Alkbh6	alkB, alkylation repair homolog 6 (E. coli)
1460198_a_at	1.73	Psmb3	proteasome (prosome, macropain) subunit, beta type 3 [a]
1421746_a_at	1.73	Fbxo17	F-box protein 17
1423846_x_at	1.73	Tuba1b	tubulin, alpha 1B
1416309_at	1.72	Nusap1	nucleolar and spindle associated protein 1
1431339_a_at	1.72	Efhd2	EF hand domain containing 2
1422389_at	1.72	Tas2r105	taste receptor, type 2, member 105
1451453_at	1.72	Dapk2	death-associated protein kinase 2
1455792_x_at	1.72	Ndn	necdin [c]
1459890_s_at	1.72	1110008P14Rik	RIKEN cDNA 1110008P14 gene
1455043_at	1.72	Tnpo1	transportin 1
1435537_at	1.72	Ptprd	protein tyrosine phosphatase, receptor type, D [b]
1434402_at	1.72	Samd8	sterile alpha motif domain containing 8

1416129_at	1.71	Errfi1	ERBB receptor feedback inhibitor 1
1426626_at	1.71	Gtf2f2	general transcription factor IIF, polypeptide 2
1435402_at	1.71	Gramd1b	GRAM domain containing 1B
1423605_a_at	1.71	Mdm2	transformed mouse 3T3 cell double minute 2
1435091_at	1.71	Zfp568	zinc finger protein 568
1417684_at	1.71	Thumpd3	THUMP domain containing 3
1434173_s_at	1.71	D19Bwg1357e	DNA segment, Chr 19, Brigham & Women's Genetics 1357 expressed
1436047_at	1.71	Gm672	predicted gene 672
1448279_at	1.71	Arpc3	actin related protein 2/3 complex, subunit 3
1435803_a_at	1.71	Eif4e2	eukaryotic translation initiation factor 4E member 2 [a]
1437993_x_at	1.71	Qdpr	quinoid dihydropteridine reductase [a]
1423423_at	1.70	Pdia3	protein disulfide isomerase associated 3
1415941_s_at	1.70	Zfand2a	zinc finger, AN1-type domain 2A [a]
1435472_at	1.70	Kremen1	kringle containing transmembrane protein 1
1426577_a_at	1.70	Lin37	lin-37 homolog (C. elegans)
1447662_x_at	1.70	D18Ertd653e	DNA segment, Chr 18, ERATO Doi 653, expressed
1455266_at	1.70	Kif5c	kinesin family member 5C
1448694_at	1.69	Jun	Jun oncogene
1428512_at	1.69	Bhlhb9	basic helix-loop-helix domain containing, class B9
1423234_at	1.69	Psmd5	proteasome (prosome, macropain) 26S subunit, non-ATPase, 5
1448595_a_at	1.69	Bex1	brain expressed gene 1
1429384_at	1.69	Csnk1g3	casein kinase 1, gamma 3
1448268_at	1.69	Tmed9	transmembrane emp24 protein transport domain containing 9
1454221_a_at	1.69	Kif2c	kinesin family member 2C
1418093_a_at	1.69	Egf	epidermal growth factor
1426333_a_at	1.68	Ikbkb	inhibitor of kappaB kinase beta
1451780_at	1.68	Blnk	B-cell linker
1448571_a_at	1.68	Gmfb	glia maturation factor, beta
1423552_at	1.68	Leprotl1	leptin receptor overlapping transcript-like 1
1424861_at	1.68	D930016D06Rik	RIKEN cDNA D930016D06 gene
1438480_a_at	1.68	Thyn1	thymocyte nuclear protein 1 [a]
1453833_a_at	1.68	Rnaseh1	ribonuclease H1
1423800_at	1.68	Dars	aspartyl-tRNA synthetase
1448730_at	1.68	Cpa3	carboxypeptidase A3, mast cell
1429712_at	1.68	Gm14288	predicted gene 14288
1416352_s_at	1.67	Tecr	trans-2,3-enoyl-CoA reductase
1428261_at	1.67	Ccdc134	coiled-coil domain containing 134

1451741_a_at	1.67	Cdk7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)
1435306_a_at	1.67	Kif11	kinesin family member 11
1452170_at	1.67	Chpf2	chondroitin polymerizing factor 2
1416655_at	1.67	C1galt1c1	C1GALT1-specific chaperone 1
1448438_at	1.67	Derl2	Der1-like domain family, member 2
1416426_at	1.66	Rab5a	RAB5A, member RAS oncogene family
1420654_a_at	1.66	Gbe1	glucan (1,4-alpha-), branching enzyme 1
1456747_x_at	1.66	Cd99l2	CD99 antigen-like 2
1444451_at	1.66	Pappa2	pappalysin 2
1439610_at	1.66	Rab27b	RAB27b, member RAS oncogene family
1458716_at	1.66	Dusp27	dual specificity phosphatase 27 (putative)
1418621_at	1.66	Rab2a	RAB2A, member RAS oncogene family
1448736_a_at	1.66	Hprt1	hypoxanthine guanine phosphoribosyl transferase 1
1424110_a_at	1.65	LOC100046344	similar to Nucleoside diphosphate kinase A (NDK A)
1425348_a_at	1.65	Srprb	signal recognition particle receptor, B subunit
1433682_at	1.65	Arhgef17	Rho guanine nucleotide exchange factor (GEF) 17
1425933_a_at	1.65	Nt5c2	5'-nucleotidase, cytosolic II
1415735_at	1.65	Ddb1	damage specific DNA binding protein 1
1417473_a_at	1.65	Ppcs	phosphopantothenoylcysteine synthetase
1450731_s_at	1.65	Tnfrsf21	tumor necrosis factor receptor superfamily, member 21
1434585_at	1.64	Tulp4	Tubby like protein 4
1454120_a_at	1.64	Pcgf6	polycomb group ring finger 6
1430700_a_at	1.64	Pla2g7	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
1433482_a_at	1.64	Fubp1	far upstream element (FUSE) binding protein 1
1460583_at	1.64	Golt1b	golgi transport 1 homolog B (S. cerevisiae)
1438094_x_at	1.64	Ola1	Obg-like ATPase 1 [a]
1424216_a_at	1.64	Papola	poly (A) polymerase alpha
1448947_at	1.64	Setd6	SET domain containing 6
1455725_a_at	1.64	H3f3b	H3 histone, family 3B
1433656_a_at	1.64	Gnl3	guanine nucleotide binding protein-like 3 (nucleolar)
1437861_s_at	1.64	Prkce	protein kinase C, epsilon
1424343_a_at	1.64	Eif1a	eukaryotic translation initiation factor 1A
1429776_a_at	1.63	Dnajb6	DnaJ (Hsp40) homolog, subfamily B, member 6 [a]
1428315_at	1.63	Ebna1bp2	EBNA1 binding protein 2
1418300_a_at	1.63	Mknk2	MAP kinase-interacting serine/threonine kinase 2
1424635_at	1.63	Eef1a1	eukaryotic translation elongation factor 1 alpha 1
1424210_at	1.63	Erlin1	ER lipid raft associated 1

1422516_a_at	1.63	Fibp	fibroblast growth factor (acidic) intracellular binding protein
1423648_at	1.63	Pdia6	protein disulfide isomerase associated 6
1428910_at	1.63	2310022B05Rik	RIKEN cDNA 2310022B05 gene
1415676_a_at	1.63	Psmb5	proteasome (prosome, macropain) subunit, beta type 5
1419648_at	1.63	Myo1c	myosin IC
1451120_at	1.63	Polr1d	polymerase (RNA) I polypeptide D [b]
1449002_at	1.63	Phlda3	pleckstrin homology-like domain, family A, member 3
1422567_at	1.63	Fam129a	family with sequence similarity 129, member A
1452707_at	1.63	Klhl30	kelch-like 30 (Drosophila)
1423920_at	1.62	Ncaph	non-SMC condensin I complex, subunit H
1434059_at	1.62	B230312A22Rik	RIKEN cDNA B230312A22 gene
1423299_at	1.62	Txnl1	thioredoxin-like 1 [a]
1423569_at	1.62	Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
1450720_at	1.62	Acp1	acid phosphatase 1, soluble
1416925_at	1.62	Kpnb1	karyopherin (importin) beta 1
1419169_at	1.62	Mapk6	mitogen-activated protein kinase 6
1449821_a_at	1.62	Memo1	mediator of cell motility 1
1449930_a_at	1.62	Ssr2	signal sequence receptor, beta
1450087_a_at	1.62	Nolc1	nucleolar and coiled-body phosphoprotein 1
1428011_a_at	1.62	Erbb2ip	Erbb2 interacting protein
1416291_at	1.61	Psmc4	proteasome (prosome, macropain) 26S subunit, ATPase, 4
1428335_a_at	1.61	Scfd1	Sec1 family domain containing 1
1435705_at	1.61	Zscan18	zinc finger and SCAN domain containing 18
1416537_at	1.61	Creld1	cysteine-rich with EGF-like domains 1
1421751_a_at	1.61	Psmd14	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14
1416146_at	1.61	Hspa4	heat shock protein 4
1451233_at	1.61	Traf2	TNF receptor-associated factor 2
1419548_at	1.60	Kpna1	karyopherin (importin) alpha 1 [a]
1416473_a_at	1.60	Igdcc4	immunoglobulin superfamily, DCC subclass, member 4 [a]
1436202_at	1.60	Malat1	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)
1425780_a_at	1.60	Tmem167	transmembrane protein 167
1452251_at	1.60	Nbea	neurobeachin
1451968_at	1.60	Xrcc5	X-ray repair complementing defective repair in Chinese hamster cells 5
1427885_at	1.60	Pold4	polymerase (DNA-directed), delta 4
1417817_a_at	1.60	Wwtr1	WW domain containing transcription regulator 1
1416657_at	1.59	Akt1	thymoma viral proto-oncogene 1
1415828_a_at	1.59	Serp1	stress-associated endoplasmic reticulum protein 1

1423784_at	1.59	Gars	glycyl-tRNA synthetase
1423453_at	1.59	Nol12	nucleolar protein 12
1452182_at	1.59	Galnt2	UDP-N-acetyl-alpha-D-galactosamine
1437495_at	1.59	Mbtps2	membrane-bound transcription factor peptidase, site 2
1423850_at	1.59	Nsun2	NOL1/NOP2/Sun domain family member 2
1419108_at	1.59	Ophn1	oligophrenin 1
1417408_at	1.59	F3	coagulation factor III
1423247_at	1.59	Erp44	endoplasmic reticulum protein 44
1428587_at	1.59	Tmem41b	transmembrane protein 41B
1418602_at	1.58	Cdh15	cadherin 15
1419029_at	1.58	Ero1l	ERO1-like (S. cerevisiae)
1418371_at	1.58	Dynll2	dynein light chain LC8-type 2
1426441_at	1.58	Slc11a2	solute carrier family 11, member 2
1424047_at	1.58	Dera	2-deoxyribose-5-phosphate aldolase homolog (C. elegans)
1435444_at	1.58	Atf6	activating transcription factor 6
1428635_at	1.58	Comtd1	catechol-O-methyltransferase domain containing 1
1416130_at	1.58	Prnp	prion protein
1436026_at	1.58	Zfp703	zinc finger protein 703
1449304_at	1.58	2310061J03Rik	RIKEN cDNA 2310061J03 gene
1420340_at	1.58	Cspp1	centrosome and spindle pole associated protein 1
1434303_at	1.58	Raph1	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1
1454004_at	1.58	5033414K04Rik	RIKEN cDNA 5033414K04 gene
1422609_at	1.58	Arpp19	cAMP-regulated phosphoprotein 19 [a]
1428534_at	1.57	Nr2c2ap	nuclear receptor 2C2-associated protein
1433766_at	1.57	C330023M02Rik	RIKEN cDNA C330023M02 gene
1416638_at	1.57	Sall2	sal-like 2 (Drosophila)
1455643_s_at	1.57	Tsr1	TSR1, 20S rRNA accumulation, homolog (yeast) [a]
1448472_at	1.57	Vars	valyl-tRNA synthetase
1424214_at	1.57	9130213B05Rik	RIKEN cDNA 9130213B05 gene [a]
1449111_a_at	1.57	Grb2	growth factor receptor bound protein 2
1416979_at	1.57	Pomp	proteasome maturation protein
1426873_s_at	1.57	Jup	junction plakoglobin
1417657_s_at	1.57	Dnajc2	DnaJ (Hsp40) homolog, subfamily C, member 2
1417057_a_at	1.56	Lamp3	lysosomal-associated membrane protein 3
1427108_at	1.56	9530068E07Rik	RIKEN cDNA 9530068E07 gene
1423142_a_at	1.56	Gtpbp4	GTP binding protein 4 [b]
1427035_at	1.56	Slc39a14	solute carrier family 39 (zinc transporter), member 14

1416529_at	1.56	Emp1	epithelial membrane protein 1
1451908_a_at	1.56	Sec14l1	SEC14-like 1 (S. cerevisiae)
1418102_at	1.56	Hes1	hairy and enhancer of split 1 (Drosophila)
1450722_at	1.56	Nup50	nucleoporin 50
1434543_a_at	1.56	Bola2	bolA-like 2 (E. coli)
1456139_at	1.56	Airn	antisense Igf2r RNA
1455605_at	1.56	Rufy3	RUN and FYVE domain containing 3
1425134_a_at	1.56	Pigx	phosphatidylinositol glycan anchor biosynthesis, class X
1434436_at	1.56	Morc4	microrchidia 4
1451207_at	1.55	Cbara1	calcium binding atopy-related autoantigen 1
1415892_at	1.55	Sgpl1	sphingosine phosphate lyase 1
1448715_x_at	1.55	Sgip1	SH3-domain GRB2-like (endophilin) interacting protein 1
1424894_at	1.55	Rab13	RAB13, member RAS oncogene family
1425389_a_at	1.55	Runx2	runt related transcription factor 2
1427934_at	1.55	Lyrm2	LYR motif containing 2
1420540_a_at	1.55	Rit1	Ras-like without CAAX 1
1433908_a_at	1.55	Cttn	cortactin
1432367_a_at	1.55	Ufd1l	ubiquitin fusion degradation 1 like
1423167_at	1.55	Mobkl3	MOB1, Mps One Binder kinase activator-like 3 (yeast)
1417252_at	1.55	Nt5c	5',3'-nucleotidase, cytosolic
1451223_a_at	1.55	Btf3l4	basic transcription factor 3-like 4
1452843_at	1.55	Il6st	interleukin 6 signal transducer
1445568_at	1.55	Inca1	Inhibitor of CDK, cyclin A1 interacting protein 1
1416563_at	1.55	Ctps	cytidine 5'-triphosphate synthase
1418937_at	1.55	Dio2	deiodinase, iodothyronine, type II
1437500_at	1.54	Noc3l	nucleolar complex associated 3 homolog (S. cerevisiae)
1451068_s_at	1.54	Rps25	ribosomal protein S25
1429328_at	1.54	Nsfl1c	NSFL1 (p97) cofactor (p47)
1455870_at	1.54	Akap2	A kinase (PRKA) anchor protein 2
1460344_at	1.54	Pbxip1	pre-B-cell leukemia transcription factor interacting protein 1
1427032_at	1.54	Herc4	hect domain and RLD 4
1453075_at	1.54	1600012P17Rik	RIKEN cDNA 1600012P17 gene
1427739_a_at	1.54	Trp53	transformation related protein 53
1422576_at	1.54	Atxn10	ataxin 10 [a]
1415780_a_at	1.54	Armcx2	armadillo repeat containing, X-linked 2
1451136_a_at	1.54	Eif2b2	eukaryotic translation initiation factor 2B, subunit 2 beta
1449674_s_at	1.54	Pdcd6ip	programmed cell death 6 interacting protein

1416861_at	1.54	Stam	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1
1418825_at	1.53	Irgm1	immunity-related GTPase family M member 1
1460556_at	1.53	Micall1	microtubule associated monoxygenase
1418387_at	1.53	Mphosph8	M-phase phosphoprotein 8
1425299_s_at	1.53	Trmt112	tRNA methyltransferase 11-2 homolog (S. cerevisiae)
1418794_at	1.53	Cds2	CDP-diacylglycerol synthase 2 [a]
1454901_at	1.53	Ypel2	yippee-like 2 (Drosophila)
1426956_a_at	1.53	Trp53bp1	transformation related protein 53 binding protein 1
1429400_at	1.53	Clcn5	chloride channel 5
1448480_at	1.53	Nip7	nuclear import 7 homolog (S. cerevisiae)
1416985_at	1.53	Sirpa	signal-regulatory protein alpha
1434809_at	1.53	Arhgap28	Rho GTPase activating protein 28
1455462_at	1.53	Adcy2	adenylate cyclase 2
1433982_at	1.53	Usp28	ubiquitin specific peptidase 28
1415683_at	1.53	Nmt1	N-myristoyltransferase 1
1416208_at	1.52	Usp14	ubiquitin specific peptidase 14
1425215_at	1.52	Ffar2	free fatty acid receptor 2
1422768_at	1.52	Syncrip	synaptotagmin binding, cytoplasmic RNA interacting protein
1453129_a_at	1.52	Rgs12	regulator of G-protein signaling 12
1438928_x_at	1.52	Ninj1	ninjurin 1
1456085_x_at	1.52	Cd151	CD151 antigen [a]
1450890_a_at	1.52	Abi1	abl-interactor 1
1455493_at	1.52	Syne1	synaptic nuclear envelope 1
1417235_at	1.52	Ehd3	EH-domain containing 3
1437242_at	1.52	Ttll12	tubulin tyrosine ligase-like family, member 12
1451113_a_at	1.52	Ik	IK cytokine
1454897_at	1.52	6330509M05Rik	RIKEN cDNA 6330509M05 gene
1419003_at	1.52	Bves	blood vessel epicardial substance
1455994_x_at	1.52	Elovl1	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1
1424634_at	1.52	Tceal1	transcription elongation factor A (SII)-like 1
1424222_s_at	1.52	Rad23b	RAD23b homolog (S. cerevisiae)
1434558_at	1.52	Wdr47	WD repeat domain 47
1417125_at	1.52	Ahcy	S-adenosylhomocysteine hydrolase
1460317_s_at	1.52	Gna13	guanine nucleotide binding protein, alpha 13 [a]
1423126_at	1.51	Atp1b3	ATPase, Na+/K+ transporting, beta 3 polypeptide
1460479_at	1.51	A330094K24Rik	RIKEN cDNA A330094K24 gene
1437033_a_at	1.51	Skp2	S-phase kinase-associated protein 2 (p45)

1434153_at	1.51	Shb	src homology 2 domain-containing transforming protein B
1422980_a_at	1.51	Bet1l	blocked early in transport 1 homolog (S. cerevisiae)-like
1454963_at	1.51	E430028B21Rik	RIKEN cDNA E430028B21 gene
1452196_a_at	1.51	Nckap1	NCK-associated protein 1
1449799_s_at	1.51	Pkp2	plakophilin 2
1449841_at	1.51	Kif3a	kinesin family member 3A
1435775_at	1.51	Clock	circadian locomoter output cycles kaput
1453796_a_at	1.51	Ergic2	ERGIC and golgi 2
1435822_at	1.51	D830012I24Rik	RIKEN cDNA D830012I24 gene
1436138_at	1.50	Ttc19	tetratricopeptide repeat domain 19
1455316_x_at	1.50	BC094435	cDNA sequence BC094435
1419428_a_at	1.50	Gaa	glucosidase, alpha, acid
1415670_at	1.50	Copg	coatomer protein complex, subunit gamma
1449507_a_at	1.50	Cd47	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)
1430999_a_at	1.50	Scoc	short coiled-coil protein
1424749_at	1.50	Wdfy1	WD repeat and FYVE domain containing 1
1435417_at	1.50	AI464131	expressed sequence AI464131
1450388_s_at	1.50	Twsg1	twisted gastrulation homolog 1 (Drosophila)
1450841_at	1.50	Stt3a	STT3, subunit of the oligosaccharyltransferase complex, homolog A
1417825_at	1.50	Esd	esterase D/formylglutathione hydrolase
1423519_at	1.50	Fam108c	family with sequence similarity 108, member C
1426187_a_at	1.50	Hax1	HCLS1 associated X-1
1433611_s_at	1.50	Bud31	BUD31 homolog (yeast)
1448533_at	1.50	Tbcb	tubulin folding cofactor B
1448232_x_at	1.50	Tuba1a	tubulin, alpha 1A
1417481_at	6.03	Ramp1	receptor (calcitonin) activity modifying protein 1
1451382_at	4.67	Chac1	ChaC, cation transport regulator-like 1 (E. coli)
1437250_at	4.23	Mreg	melanoregulin
1449876_at	3.92	Prkg1	protein kinase, cGMP-dependent, type I [a]
1428025_s_at	3.68	Pitpnc1	phosphatidylinositol transfer protein, cytoplasmic 1 [e]
1434499_a_at	3.65	Ldhb	lactate dehydrogenase B [c]
1449422_at	3.55	Cdh4	cadherin 4 [a]
1458635_at	3.54	4832428D23Rik	RIKEN cDNA 4832428D23 gene
1439143_at	3.42	A930018M24Rik	RIKEN cDNA A930018M24 gene
1422798_at	3.40	Cntnap2	contactin associated protein-like 2
1427329_a_at	3.35	Igh-6	immunoglobulin heavy chain 6 (heavy chain of IgM)
1450048_a_at	3.33	Idh2	isocitrate dehydrogenase 2 (NADP+), mitochondrial

1424393_s_at	3.28	Adhfe1	alcohol dehydrogenase, iron containing, 1 [a]
1435370_a_at	3.18	Ces3	carboxylesterase 3 [a]
1434645_at	3.17	C530008M17Rik	RIKEN cDNA C530008M17 gene
1416023_at	3.12	Fabp3	fatty acid binding protein 3, muscle and heart
1448300_at	3.12	Mgst3	microsomal glutathione S-transferase 3
1440335_at	3.11	LOC100046468	hypothetical protein LOC100046468
1423454_a_at	3.10	Sema6c	sema domain, transmembrane domain (TM), (semaphorin) 6C
1444504_at	3.02	Dhrs7c	dehydrogenase/reductase (SDR family) member 7C
1436736_x_at	2.96	D0H4S114	DNA segment, human D4S114 [a]
1438263_at	2.91	9430020K01Rik	RIKEN cDNA 9430020K01 gene [a]
1418421_at	2.88	Bcl6b	B-cell CLL/lymphoma 6, member B
1422153_a_at	2.84	Asb11	ankyrin repeat and SOCS box-containing 11
1451553_at	2.81	Art5	ADP-ribosyltransferase 5
1435605_at	2.81	Actr3b	ARP3 actin-related protein 3 homolog B (yeast)
1416835_s_at	2.80	Amd1	S-adenosylmethionine decarboxylase 1 [a]
1454867_at	2.78	Mn1	meningioma 1
1416468_at	2.78	Aldh1a1	aldehyde dehydrogenase family 1, subfamily A1
1441581_at	2.77	Asb10	ankyrin repeat and SOCS box-containing 10 [a]
1423405_at	2.76	Timp4	tissue inhibitor of metalloproteinase 4
1417877_at	2.76	Eepd1	endonuclease/exonuclease/phosphatase family domain containing 1
1418589_a_at	2.76	Mlf1	myeloid leukemia factor 1
1439491_at	2.73	Lrrc38	leucine rich repeat containing 38
1419145_at	2.73	Smtnl1	smoothelin-like 1
1439821_at	2.73	Lrp2bp	Lrp2 binding protein
1417061_at	2.72	Slc40a1	solute carrier family 40 (iron-regulated transporter), member 1 [b]
1424105_a_at	2.70	Pttg1	pituitary tumor-transforming gene 1 [a]
1418057_at	2.70	Tiam1	T-cell lymphoma invasion and metastasis 1
1416617_at	2.69	Acss1	acyl-CoA synthetase short-chain family member 1
1437273_at	2.69	Htra4	HtrA serine peptidase 4
1453904_at	2.69	Ccdc146	coiled-coil domain containing 146
1453851_a_at	2.68	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma
1439087_a_at	2.67	Pik3ip1	phosphoinositide-3-kinase interacting protein 1 [a]
1455622_at	2.60	Podxl2	podocalyxin-like 2
1423062_at	2.59	Igfbp3	insulin-like growth factor binding protein 3 [a]
1452766_at	2.59	Tppp	tubulin polymerization promoting protein
1424937_at	2.57	Plin5	perilipin 5 [a]
1457999_at	2.54	Fam82b	family with sequence similarity 82, member B [b]

1429144_at	2.51	Prei4	preimplantation protein 4 [a]
1455506_at	2.50	Slc25a34	solute carrier family 25, member 34
1422678_at	2.50	Dgat2	diacylglycerol O-acyltransferase 2 [a]
1453084_s_at	2.49	Col22a1	collagen, type XXII, alpha 1 [a]
1451716_at	2.48	Mafb	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)
1418181_at	2.46	Ptp4a3	protein tyrosine phosphatase 4a3
1452474_a_at	2.45	Art3	ADP-ribosyltransferase 3
1449834_at	2.43	Magix	MAGI family member, X-linked
1422811_at	2.40	Slc27a1	solute carrier family 27 (fatty acid transporter), member 1 [a]
1417163_at	2.40	Dusp10	dual specificity phosphatase 10
1417283_at	2.39	Lynx1	Ly6/neurotoxin 1 [a]
1430522_a_at	2.39	Vamp5	vesicle-associated membrane protein 5
1419140_at	2.38	Acvr2b	activin receptor IIB
1420346_at	2.38	Asb12	ankyrin repeat and SOCS box-containing 12
1427482_a_at	2.38	Car8	carbonic anhydrase 8
1430969_at	2.38	Mtch2	mitochondrial carrier homolog 2 (C. elegans)
1457721_at	2.38	PPAR-γ	peroxisome proliferative activated receptor-γ
1426450_at	2.38	Plcl2	phospholipase C-like 2
1418507_s_at	2.38	Socs2	suppressor of cytokine signaling 2 [a]
1423422_at	2.37	Asb4	ankyrin repeat and SOCS box-containing 4
1451313_a_at	2.36	1110067D22Rik	RIKEN cDNA 1110067D22 gene [a]
1450971_at	2.35	Gadd45b	growth arrest and DNA-damage-inducible 45 beta [a]
1435292_at	2.34	Tbc1d4	TBC1 domain family, member 4
1449290_at	2.34	Dpysl5	dihydropyrimidinase-like 5
1449547_at	2.33	Asb14	ankyrin repeat and SOCS box-containing 14
1425089_at	2.33	Kcnc4	potassium voltage gated channel, Shaw-related subfamily, member 4
1416203_at	2.32	Aqp1	aquaporin 1
1417645_at	2.31	Sspn	sarcospan [a]
1451322_at	2.31	Cmbl	carboxymethylenebutenolidase-like (Pseudomonas)
1422605_at	2.31	Ppp1r1a	protein phosphatase 1, regulatory (inhibitor) subunit 1A
1452962_at	2.28	Tmem25	transmembrane protein 25 [a]
1433532_a_at	2.25	Mbp	myelin basic protein [a]
1426729_at	2.24	Fam131a	family with sequence similarity 131, member A
1435745_at	2.24	5031439G07Rik	RIKEN cDNA 5031439G07 gene
1427213_at	2.24	Pfkfb1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1
1427100_at	2.24	Metrn	meteorin, glial cell differentiation regulator
1436361_at	2.24	Vgll2	vestigial like 2 homolog (Drosophila)

1453552_at	2.23	2310014F07Rik	RIKEN cDNA 2310014F07 gene
1454749_at	2.23	Pcnt	pericentrin (kendrin) [a]
1450490_at	2.23	Kcna7	potassium voltage-gated channel, shaker-related subfamily, member 7
1429071_at	2.22	Me3	malic enzyme 3, NADP(+)-dependent, mitochondrial
1455220_at	2.22	Frat2	frequently rearranged in advanced T-cell lymphomas 2
1426719_at	2.21	Apbb2	amyloid beta (A4) precursor protein-binding, family B, member 2 [a]
1434386_at	2.20	Atp2c1	ATPase, Ca++-sequestering
1428140_at	2.20	Oxct1	3-oxoacid CoA transferase 1 [b]
1434210_s_at	2.20	Lrig1	leucine-rich repeats and immunoglobulin-like domains 1 [a]
1432062_at	2.20	Mdga1	MAM domain containing glycosylphosphatidylinositol anchor 1
1420712_a_at	2.19	Hpn	hepsin
1455031_at	2.19	Cdc2l6	cell division cycle 2-like 6 (CDK8-like)
1454822_x_at	2.18	Apcdd1	adenomatosis polyposis coli down-regulated 1
1440343_at	2.18	Rps6ka5	ribosomal protein S6 kinase, polypeptide 5 [a]
1440435_at	2.18	Ky	kyphoscoliosis peptidase
1430176_at	2.18	5430433E21Rik	RIKEN cDNA 5430433E21 gene
1457311_at	2.18	Camk2a	calcium/calmodulin-dependent protein kinase II alpha [a]
1436763_a_at	2.18	Klf9	Kruppel-like factor 9 [c]
1440443_at	2.17	E030016H06Rik	RIKEN cDNA E030016H06 gene
1428864_at	2.17	5530400B01Rik	RIKEN cDNA 5530400B01 gene
1417590_at	2.17	Cyp27a1	cytochrome P450, family 27, subfamily a, polypeptide 1
1450251_a_at	2.16	Lnx1	ligand of numb-protein X 1
1428991_at	2.16	Hrasls	HRAS-like suppressor [a]
1449334_at	2.15	Timp3	tissue inhibitor of metalloproteinase 3 [b]
1430462_at	2.15	2310002L09Rik	RIKEN cDNA 2310002L09 gene
1417067_s_at	2.15	Cabc1	chaperone, ABC1 activity of bc1 complex like (S. pombe)
1429598_at	2.15	2310042D19Rik	RIKEN cDNA 2310042D19 gene
1432107_at	2.15	2310010M20Rik	RIKEN cDNA 2310010M20 gene
1438031_at	2.15	Rasgrp3	RAS, guanyl releasing protein 3 [a]
1444178_at	2.14	Gm9895	predicted gene 9895
1417542_at	2.14	Rps6ka2	ribosomal protein S6 kinase, polypeptide 2
1457881_at	2.13	Osbpl6	oxysterol binding protein-like 6 [a]
1429264_at	2.13	C030044B11Rik	RIKEN cDNA C030044B11 gene
1421425_a_at	2.13	Rcan2	regulator of calcineurin 2
1456812_at	2.13	Abcd2	ATP-binding cassette, sub-family D (ALD), member 2 [a]
1435733_x_at	2.12	Rnaseh2c	ribonuclease H2, subunit C
1419301_at	2.12	Fzd4	frizzled homolog 4 (Drosophila)

1437197_at	2.12	Sorbs2	sorbin and SH3 domain containing 2
1439096_at	2.12	Ddo	D-aspartate oxidase [a]
1435917_at	2.12	Ociad2	OCIA domain containing 2
1437473_at	2.11	Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog [c]
1416564_at	2.11	Sox7	SRY-box containing gene 7
1434354_at	2.09	Maob	monoamine oxidase B
1417680_at	2.09	Kcna5	potassium voltage-gated channel, shaker-related subfamily, member 5
1438201_at	2.09	Pdp1	pyruvate dehyrogenase phosphatase catalytic subunit 1 [a]
1449581_at	2.08	Emid1	EMI domain containing 1
1436440_at	2.08	Slc25a12	solute carrier family 25 (mitochondrial carrier, Aralar), member 12
1429796_at	2.07	Kalrn	kalirin, RhoGEF kinase
1428536_at	2.07	Kcng4	potassium voltage-gated channel, subfamily G, member 4
1456755_at	2.07	Trak1	trafficking protein, kinesin binding 1
1447934_at	2.07	9630033F20Rik	RIKEN cDNA 9630033F20 gene [b]
1419137_at	2.07	Shank3	SH3/ankyrin domain gene 3
1421961_a_at	2.06	Dnajb5	DnaJ (Hsp40) homolog, subfamily B, member 5 [a]
1416775_at	2.06	Atp5sl	ATP5S-like
1438012_at	2.05	Ppm1l	protein phosphatase 1 (formerly 2C)-like
1434454_at	2.05	Adcy9	adenylate cyclase 9 [c]
1446619_at	2.04	A130038J17Rik	RIKEN cDNA A130038J17 gene
1423104_at	2.04	Irs1	insulin receptor substrate 1
1429267_at	2.04	Acot11	acyl-CoA thioesterase 11
1424737_at	2.03	Thrsp	thyroid hormone responsive SPOT14 homolog (Rattus) [a]
1441727_s_at	2.03	Zfp467	zinc finger protein 467 [a]
1440007_at	2.03	D930003E18Rik	RIKEN cDNA D930003E18 gene
1455712_at	2.03	Hist3h2a	histone cluster 3, H2a
1452942_at	2.02	Tmem65	transmembrane protein 65
1448900_at	2.02	D16H22S680E	DNA segment, Chr 16, human D22S680E, expressed
1429918_at	2.02	Arhgap20	Rho GTPase activating protein 20 [a]
1453418_at	2.01	Col24a1	collagen, type XXIV, alpha 1
1418370_at	2.01	Tnnc1	troponin C, cardiac/slow skeletal
1456574_at	2.01	Zfp800	zinc finger protein 800
1416105_at	2.01	Nnt	nicotinamide nucleotide transhydrogenase [a]
1421498_a_at	2.01	2010204K13Rik	RIKEN cDNA 2010204K13 gene
1438294_at	1.99	Atxn1	ataxin 1
1424182_at	1.99	Acat1	acetyl-Coenzyme A acetyltransferase 1
1453094_at	1.99	Foxo	forkhead box [b]

1416666_at	1.99	Serpine2	serine (or cysteine) peptidase inhibitor, clade E, member 2
1436756_x_at	1.98	Hadh	hydroxyacyl-Coenzyme A dehydrogenase[b]
1430653_at	1.98	A930026B05Rik	RIKEN cDNA A930026B05 gene
1425344_at	1.98	Narf	nuclear prelamin A recognition factor[a]
1418328_at	1.98	Chkb-cpt1b	choline kinase beta, carnitine palmitoyltransferase 1b, muscle transcription unit
1456640_at	1.98	Sh3rf2	SH3 domain containing ring finger 2
1460652_at	1.98	Esrra	estrogen related receptor, alpha
1429012_at	1.98	Arhgef6	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6
1422852_at	1.98	Cib2	calcium and integrin binding family member 2
1431714_at	1.97	2310015D24Rik	RIKEN cDNA 2310015D24 gene
1424563_at	1.97	Slc25a4	solute carrier family 25, member 4
1448636_at	1.97	Myoz1	myozenin 1
1426043_a_at	1.96	Capn3	calpain 3[a]
1419042_at	1.96	Iigp1	interferon inducible GTPase 1
1426850_a_at	1.96	Map2k6	mitogen-activated protein kinase kinase 6
1450637_a_at	1.96	Aebp1	AE binding protein 1[a]
1448667_x_at	1.96	Tob2	transducer of ERBB2, 2[b]
1450044_at	1.96	Fzd7	frizzled homolog 7 (Drosophila)
1441636_at	1.96	Sec14l5	SEC14-like 5 (S. cerevisiae)
1427416_x_at	1.95	Dusp7	dual specificity phosphatase 7
1452985_at	1.95	Uaca	uveal autoantigen with coiled-coil domains and ankyrin repeats
1435464_at	1.95	1110003E01Rik	RIKEN cDNA 1110003E01 gene
1426027_a_at	1.95	Arhgap10	Rho GTPase activating protein 10
1418951_at	1.95	Txlnb	taxilin beta[a]
1440346_at	1.95	Kdm6b	KDM1 lysine (K)-specific demethylase 6B
1437797_at	1.95	Atp2a2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
1424367_a_at	1.94	Homer2	homer homolog 2 (Drosophila)
1452973_at	1.94	Ppm1k	protein phosphatase 1K (PP2C domain containing)
1441531_at	1.94	Plcb4	phospholipase C, beta 4[a]
1436387_at	1.94	C330006P03Rik	RIKEN cDNA C330006P03 gene
1415904_at	1.94	Lpl	lipoprotein lipase[a]
1418091_at	1.93	Tcfcp2l1	transcription factor CP2-like 1
1428444_at	1.93	Asb2	ankyrin repeat and SOCS box-containing 2
1456909_at	1.93	LOC676974	similar to Glucose-6-phosphate isomerase
1443923_at	1.93	Akap13	A kinase (PRKA) anchor protein 13
1457102_at	1.93	A030001D16Rik	RIKEN cDNA A030001D16 gene
1456069_at	1.93	Dtna	dystrobrevin alpha

1433453_a_at	1.92	Abtb2	ankyrin repeat and BTB (POZ) domain containing 2
1454397_at	1.92	4632418H02Rik	RIKEN cDNA 4632418H02 gene
1423785_at	1.92	Egln1	EGL nine homolog 1 (C. elegans) [a]
1452299_at	1.92	Wwp1	WW domain containing E3 ubiquitin protein ligase 1 [a]
1438292_x_at	1.92	Adk	adenosine kinase [a]
1442243_at	1.91	Per3	period homolog 3 (Drosophila)
1428301_at	1.91	100041874	predicted gene, 100041874 [a]
1445843_at	1.91	Chd2	chromodomain helicase DNA binding protein 2 [a]
1440635_at	1.91	Palld	palladin, cytoskeletal associated protein [a]
1416008_at	1.90	Satb1	special AT-rich sequence binding protein 1 [b]
1444612_at	1.90	Ppp2r3a	protein phosphatase 2, regulatory subunit B", alpha
1449964_a_at	1.90	Mlycd	malonyl-CoA decarboxylase
1433628_at	1.90	Coq10a	coenzyme Q10 homolog A (yeast)
1454873_at	1.90	Zfp775	zinc finger protein 775
1448927_at	1.89	Kcnn2	potassium intermediate/small conductance calcium-activated channel 2
1455475_at	1.89	3110057O12Rik	RIKEN cDNA 3110057O12 gene [a]
1449078_at	1.89	St3gal6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6
1449405_at	1.88	Tns1	tensin 1 [b]
1435357_at	1.88	D4Wsu53e	DNA segment, Chr 4, Wayne State University 53, expressed [a]
1448607_at	1.88	Nampt	nicotinamide phosphoribosyltransferase [a]
1448949_at	1.88	Car4	carbonic anhydrase 4
1428146_s_at	1.87	Acaa2	acetyl-Coenzyme A acyltransferase 2 [b]
1455698_at	1.87	Sec62	SEC62 homolog (S. cerevisiae)
1449818_at	1.87	Abcb4	ATP-binding cassette, sub-family B (MDR/TAP), member 4
1424504_at	1.86	Rab22a	RAB22A, member RAS oncogene family
1424531_a_at	1.86	Tcea3	transcription elongation factor A (SII), 3
1457304_at	1.85	D13Ertd787e	DNA segment, Chr 13, ERATO Doi 787, expressed
1426959_at	1.85	Bdh1	3-hydroxybutyrate dehydrogenase, type 1 [a]
1449398_at	1.85	Rpl3l	ribosomal protein L3-like
1448876_at	1.85	Evc	Ellis van Creveld gene homolog (human)
1423835_at	1.85	Zfp503	zinc finger protein 503
1417673_at	1.85	Grb14	growth factor receptor bound protein 14
1425425_a_at	1.85	Wif1	Wnt inhibitory factor 1
1450884_at	1.84	Cd36	CD36 antigen [a]
1417841_at	1.84	Pxmp2	peroxisomal membrane protein 2
1450700_at	1.83	Cdc42ep3	CDC42 effector protein (Rho GTPase binding) 3 [a]
1417702_a_at	1.83	Hnmt	histamine N-methyltransferase

1431199_at	1.83	Ggnbp1	gametogenetin binding protein 1
1427045_at	1.83	Synpo	synaptopodin
1422323_a_at	1.82	Lbx1	ladybird homeobox homolog 1 (Drosophila)
1453657_at	1.82	2310065F04Rik	RIKEN cDNA 2310065F04 gene
1434008_at	1.82	Scn4b	sodium channel, type IV, beta
1451740_at	1.82	Paip1	polyadenylate binding protein-interacting protein 1
1420098_s_at	1.82	D13Ertd787e	DNA segment, Chr 13, ERATO Doi 787, expressed
1445632_at	1.82	Ogdh	oxoglutarate dehydrogenase (lipoamide)
1456629_at	1.81	Kank3	KN motif and ankyrin repeat domains 3
1428432_at	1.81	Zcchc24	zinc finger, CCHC domain containing 24
1444982_at	1.81	C230007H23Rik	RIKEN cDNA C230007H23 gene
1434480_at	1.81	4930402E16Rik	RIKEN cDNA 4930402E16 gene
1435591_at	1.81	AI426330	expressed sequence AI426330
1435471_at	1.81	Zfp708	zinc finger protein 708
1417050_at	1.80	C1qtnf4	C1q and tumor necrosis factor related protein 4
1433762_at	1.80	C630043F03Rik	RIKEN cDNA C630043F03 gene
1415743_at	1.80	Hdac5	histone deacetylase 5
1454976_at	1.80	Sod2	superoxide dismutase 2, mitochondrial
1424553_at	1.80	Hhatl	hedgehog acyltransferase-like
1432562_at	1.79	1110006G14Rik	RIKEN cDNA 1110006G14 gene
1436538_at	1.79	Ankrd37	ankyrin repeat domain 37
1428016_a_at	1.79	Rasip1	Ras interacting protein 1
1416424_at	1.79	Plin3	perilipin 3
1424362_at	1.78	Ppapdc3	phosphatidic acid phosphatase type 2 domain containing 3
1415984_at	1.78	Acadm	acyl-Coenzyme A dehydrogenase, medium chain
1448394_at	1.78	Myl2	myosin, light polypeptide 2, regulatory, cardiac, slow
1429097_at	1.78	C030044C12Rik	RIKEN cDNA C030044C12 gene
1451932_a_at	1.78	Adamtsl4	ADAMTS-like 4
1448104_at	1.78	Aldh6a1	aldehyde dehydrogenase family 6, subfamily A1
1434647_at	1.78	Egflam	EGF-like, fibronectin type III and laminin G domains
1427436_at	1.77	Six2	sine oculis-related homeobox 2 homolog (Drosophila)
1454840_at	1.77	Mccc2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)
1425678_a_at	1.77	Snrk	SNF related kinase [a]
1452841_at	1.77	Pgm2l1	phosphoglucomutase 2-like 1 [a]
1425920_at	1.77	Cuedc1	CUE domain containing 1
1449062_at	1.76	Khk	ketohexokinase
1434671_at	1.76	B230337E12Rik	RIKEN cDNA B230337E12 gene

1449576_at	1.76	Eif1ay	eukaryotic translation initiation factor 1A, Y-linked
1448530_at	1.76	Gmpr	guanosine monophosphate reductase
1425891_a_at	1.76	Grtp1	GH regulated TBC protein 1
1422927_at	1.76	Yipf7	Yip1 domain family, member 7
1434717_at	1.76	Cul3	cullin 3 [c]
1424777_at	1.76	Dcaf4	DDB1 and CUL4 associated factor 4
1437869_at	1.76	Ppp2r3a	protein phosphatase 2, regulatory subunit B", alpha
1455087_at	1.76	D7Ertd715e	DNA segment, Chr 7, ERATO Doi 715, expressed
1453135_at	1.76	Fndc5	fibronectin type III domain containing 5 [a]
1451803_a_at	1.76	Vegfb	vascular endothelial growth factor B
1453141_at	1.75	0610009L18Rik	RIKEN cDNA 0610009L18 gene
1456903_at	1.75	Ptx3	pentraxin related gene
1422445_at	1.75	Itga6	integrin alpha 6 [a]
1454984_at	1.75	Lifr	leukemia inhibitory factor receptor
1449544_a_at	1.75	Kcnh2	potassium voltage-gated channel, subfamily H (eag-related), member 2
1417580_s_at	1.75	Selenbp1	selenium binding protein 1
1426951_at	1.75	Crim1	cysteine rich transmembrane BMP regulator 1 (chordin like)
1427982_s_at	1.74	Syne2	synaptic nuclear envelope 2
1435171_at	1.74	2810416G20Rik	RIKEN cDNA 2810416G20 gene
1452005_at	1.74	Dlat	dihydrolipoamide S-acetyltransferase
1435344_at	1.74	Tfdp2	transcription factor Dp 2
1437689_x_at	1.74	Clu	clusterin [b]
1422589_at	1.74	Rab3a	RAB3A, member RAS oncogene family
1435553_at	1.74	Pdzd2	PDZ domain containing 2
1439565_at	1.74	Fam123b	family with sequence similarity 123, member B
1435549_at	1.74	Trpm4	transient receptor potential cation channel, subfamily M, member 4
1429178_at	1.73	Odz3	odd Oz/ten-m homolog 3 (Drosophila) [a]
1456981_at	1.73	Tmc7	transmembrane channel-like gene family 7
1454850_at	1.73	Tbc1d10c	TBC1 domain family, member 10c
1460591_at	1.73	Esr1	estrogen receptor 1 (alpha) [a]
1435881_at	1.73	Pcbp2	poly(rC) binding protein 2
1422910_s_at	1.73	Smc6	structural maintenance of chromosomes 6
1433907_at	1.73	Pknox2	Pbx/knotted 1 homeobox 2
1448825_at	1.73	Pdk2	pyruvate dehydrogenase kinase, isoenzyme 2
1429108_at	1.72	Msl2	male-specific lethal 2 homolog (Drosophila)
1434537_at	1.72	Slco3a1	solute carrier organic anion transporter family, member 3a1
1422622_at	1.72	Nos3	nitric oxide synthase 3, endothelial cell

1426858_at	1.72	Inhbb	inhibin beta-B
1425753_a_at	1.72	Ung	uracil DNA glycosylase
1435787_at	1.72	Ppm1l	protein phosphatase 1 (formerly 2C)-like
1415698_at	1.72	Golm1	golgi membrane protein 1
1417846_at	1.71	Ulk2	Unc-51 like kinase 2 (C. elegans) [a]
1459903_at	1.71	Sema7a	sema domain, immunoglobulin domain (Ig), and GPI membrane anchor 7A
1418592_at	1.71	Dnaja4	DnaJ (Hsp40) homolog, subfamily A, member 4 [c]
1428081_at	1.71	Klhl21	kelch-like 21 (Drosophila)
1429768_at	1.71	Dtna	dystrobrevin alpha
1452227_at	1.70	2310045A20Rik	RIKEN cDNA 2310045A20 gene
1454720_at	1.70	Apba3	amyloid beta (A4) precursor protein-binding, family A, member 3
1434380_at	1.70	Gbp6	guanylate binding protein 6
1415959_at	1.70	Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4 [a]
1428704_at	1.70	Zfp661	zinc finger protein 661
1416846_a_at	1.70	Pdzrn3	PDZ domain containing RING finger 3
1424790_at	1.70	Slc25a42	solute carrier family 25, member 42
1453103_at	1.70	Ablim1	actin-binding LIM protein 1
1417056_at	1.70	Psme1	proteasome (prosome, macropain) 28 subunit, alpha
1431328_at	1.70	Ppp1cb	protein phosphatase 1, catalytic subunit, beta isoform
1457671_at	1.70	9330120H11Rik	RIKEN cDNA 9330120H11 gene
1425362_at	1.70	Agfg2	ArfGAP with FG repeats 2
1434815_a_at	1.70	Mapkapk3	mitogen-activated protein kinase-activated protein kinase 3
1423072_at	1.69	6720475J19Rik	RIKEN cDNA 6720475J19 gene
1429552_at	1.69	Wdr16	WD repeat domain 16
1448297_a_at	1.69	Tnk2	tyrosine kinase, non-receptor, 2
1422478_a_at	1.69	Acss2	acyl-CoA synthetase short-chain family member 2
1434099_at	1.69	Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha [a]
1426894_s_at	1.69	Fam102a	family with sequence similarity 102, member A
1451304_at	1.69	Tmem143	transmembrane protein 143
1451718_at	1.69	Plp1	proteolipid protein (myelin) 1
1434204_x_at	1.69	Shmt2	serine hydroxymethyltransferase 2 (mitochondrial)
1443078_at	1.69	6030439D06Rik	RIKEN cDNA 6030439D06 gene
1435207_at	1.69	Dixdc1	DIX domain containing 1
1449536_at	1.68	Kcnn1	potassium intermediate/small conductance calcium-activated channel, member 1
1439196_at	1.68	Hook3	hook homolog 3 (Drosophila)
1455091_at	1.68	Ppp2r3a	protein phosphatase 2, regulatory subunit B", alpha
1427556_at	1.68	Mylk2	myosin, light polypeptide kinase 2, skeletal muscle

1453384_at	1.68	Dennd1b	DENN/MADD domain containing 1B
1428930_at	1.67	Tmem29	transmembrane protein 29
1417661_at	1.67	Rdm1	RAD52 motif 1
1449553_at	1.67	Nkain1	Na+/K+ transporting ATPase interacting 1 [a]
1455155_at	1.67	Lsm14b	LSM14 homolog B (SCD6, S. cerevisiae)
1427151_at	1.67	Qser1	glutamine and serine rich 1
1438483_at	1.67	Nos1	nitric oxide synthase 1, neuronal [a]
1418888_a_at	1.67	Sepx1	selenoprotein X 1
1452332_at	1.67	Ccdc85a	coiled-coil domain containing 85A
1418170_a_at	1.67	Zcchc14	zinc finger, CCHC domain containing 14
1428467_at	1.67	Tardbp	TAR DNA binding protein
1428926_at	1.67	1110003O08Rik	RIKEN cDNA 1110003O08 gene
1423994_at	1.67	Kif1b	kinesin family member 1B [c]
1436219_at	1.67	4933403F05Rik	RIKEN cDNA 4933403F05 gene
1451372_a_at	1.67	Art1	ADP-ribosyltransferase 1 [a]
1426345_at	1.67	Prepl	prolyl endopeptidase-like
1420930_s_at	1.66	Ctnnal1	catenin (cadherin associated protein), alpha-like 1
1439283_at	1.66	Osbpl9	Oxysterol binding protein-like 9
1451523_a_at	1.66	Mif4gd	MIF4G domain containing
1418715_at	1.66	Pank1	pantothenate kinase 1
1420950_at	1.66	Znrf1	zinc and ring finger 1
1433725_at	1.66	Acvr1b	activin A receptor, type 1B
1423076_at	1.66	Snx9	sorting nexin 9
1451357_at	1.66	Mpnd	MPN domain containing
1425512_at	1.66	Map2k7	mitogen-activated protein kinase kinase 7
1424632_a_at	1.66	Rev3l	REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like
1458364_s_at	1.65	Tor1aip1	torsin A interacting protein 1
1430253_at	1.65	2900006B11Rik	RIKEN cDNA 2900006B11 gene
1418714_at	1.65	Dusp8	dual specificity phosphatase 8
1443835_x_at	1.65	Fam100b	family with sequence similarity 100, member B [a]
1418595_at	1.65	Plin4	perilipin 4
1427258_at	1.65	Trim24	tripartite motif-containing 24
1440187_at	1.65	Taf3	TAF3 RNA polymerase II, TATA box binding protein (TBP)-associated factor
1438317_a_at	1.65	Endog	endonuclease G
1434441_at	1.65	1110018J18Rik	RIKEN cDNA 1110018J18 gene
1438241_at	1.65	Rgma	RGM domain family, member A
1439036_a_at	1.65	Atp1b1	ATPase, Na+/K+ transporting, beta 1 polypeptide

1435117_a_at	1.65	Dnajc27	DnaJ (Hsp40) homolog, subfamily C, member 27
1450449_a_at	1.65	Rilpl1	Rab interacting lysosomal protein-like 1 [a]
1428614_at	1.65	Ldhd	lactate dehydrogenase D
1460056_at	1.65	1700109F18Rik	RIKEN cDNA 1700109F18 gene
1449530_at	1.65	Trps1	trichorhinophalangeal syndrome I (human)
1460324_at	1.65	Dnmt3a	DNA methyltransferase 3A
1433512_at	1.64	Fli1	Friend leukemia integration 1
1417793_at	1.64	Irgm2	immunity-related GTPase family M member 2
1426516_a_at	1.64	Lpin1	lipin 1 [a]
1456087_at	1.64	Nfia	nuclear factor I/A
1450376_at	1.64	Mxi1	Max interacting protein 1
1435203_at	1.64	Man2a2	mannosidase 2, alpha 2
1429084_at	1.64	Vezf1	vascular endothelial zinc finger 1
1443904_at	1.64	Fads6	fatty acid desaturase domain family, member 6
1417985_at	1.64	Nrarp	Notch-regulated ankyrin repeat protein
1424045_at	1.64	5730437N04Rik	RIKEN cDNA 5730437N04 gene
1429083_at	1.63	Agl	amylo-1,6-glucosidase, 4-alpha-glucanotransferase
1448130_at	1.63	Fdft1	farnesyl diphosphate farnesyl transferase 1
1422771_at	1.63	Smad6	MAD homolog 6 (Drosophila)
1416858_a_at	1.63	Fkbp3	FK506 binding protein 3
1439500_at	1.63	Scrn1	secernin 1
1418746_at	1.63	Pnkd	paroxysmal nonkinesiogenic dyskinesia
1427345_a_at	1.63	Sult1a1	sulfotransferase family 1A, phenol-preferring, member 1
1428160_at	1.63	Ndufab1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1
1449019_at	1.63	Akap1	A kinase (PRKA) anchor protein 1 [a]
1449121_at	1.63	Fusip1	FUS interacting protein (serine-arginine rich) 1
1425537_at	1.63	Ppm1a	protein phosphatase 1A, magnesium dependent, alpha isoform
1422493_at	1.63	Cpox	coproporphyrinogen oxidase [a]
1454801_at	1.62	Ankrd28	ankyrin repeat domain 28
1454838_s_at	1.62	Pkdcc	protein kinase domain containing, cytoplasmic [a]
1439505_at	1.62	Clic5	chloride intracellular channel 5 [b]
1417264_at	1.62	Coq5	coenzyme Q5 homolog, methyltransferase (yeast)
1420859_at	1.62	Pkia	protein kinase inhibitor, alpha
1434128_a_at	1.62	Zfp574	zinc finger protein 574
1453421_at	1.62	Srr	serine racemase
1448499_a_at	1.62	Ephx2	epoxide hydrolase 2, cytoplasmic
1457568_at	1.62	Hnrnpd	heterogeneous nuclear ribonucleoprotein D

1454637_at	1.62	Klhl8	kelch-like 8 (Drosophila)
1452294_at	1.62	Pcdh1	protocadherin 1
1423101_at	1.62	Paqr4	progestin and adipoQ receptor family member IV
1455680_at	1.62	9630025H16Rik	RIKEN cDNA 9630025H16 gene
1439066_at	1.62	Angpt1	angiopoietin 1
1415964_at	1.62	Scd1	stearoyl-Coenzyme A desaturase 1
1418716_at	1.61	Mrps25	mitochondrial ribosomal protein S25
1427992_a_at	1.61	Rab12	RAB12, member RAS oncogene family
1451364_at	1.61	Polr3gl	polymerase (RNA) III (DNA directed) polypeptide G like
1434633_at	1.61	Crebbp	CREB binding protein
1418780_at	1.61	Cyp39a1	cytochrome P450, family 39, subfamily a, polypeptide 1
1446288_at	1.61	C78692	expressed sequence C78692
1424671_at	1.61	Plekhf1	pleckstrin homology domain containing, family F member 1
1428394_at	1.61	Lrrc8a	leucine rich repeat containing 8A
1446244_at	1.61	Zyg11b	zyg-ll homolog B (C. elegans)
1421340_at	1.61	Map3k5	mitogen-activated protein kinase kinase kinase 5
1434977_at	1.61	4933403F05Rik	RIKEN cDNA 4933403F05 gene
1435836_at	1.60	Pdk1	pyruvate dehydrogenase kinase, isoenzyme 1
1433832_at	1.60	Unc84b	unc-84 homolog B (C. elegans)
1435004_at	1.60	Pank4	pantothenate kinase 4
1448943_at	1.60	Nrp1	neuropilin 1 [a]
1454747_a_at	1.60	Klhdc3	kelch domain containing 3 [a]
1433827_at	1.60	Atp8a1	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1 [a]
1452789_at	1.60	Snn	stannin
1435672_at	1.60	3830612M24	hypothetical protein 3830612M24
1416505_at	1.60	Nr4a1	nuclear receptor subfamily 4, group A, member 1
1416362_a_at	1.60	Fkbp4	FK506 binding protein 4
1444550_at	1.60	1110021J02Rik	RIKEN cDNA 1110021J02 gene
1417892_a_at	1.60	Sirt3	sirtuin 3 (silent mating type information regulation 2, homolog) 3 (S. cerevisiae)
1422501_s_at	1.60	Idh3a	isocitrate dehydrogenase 3 (NAD+) alpha [a]
1433657_at	1.60	Fam78a	family with sequence similarity 78, member A
1449108_at	1.60	Fdx1	ferredoxin 1
1428637_at	1.60	Dyrk2	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2
1426857_a_at	1.60	Hsdl2	hydroxysteroid dehydrogenase like 2 [a]
1435396_at	1.60	Stxbp6	syntaxin binding protein 6 (amisyn)
1437309_a_at	1.60	Rpa1	replication protein A1
1417116_at	1.60	Slc6a8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8

1418117_at	1.60	Ndufs4	NADH dehydrogenase (ubiquinone) Fe-S protein 4
1440926_at	1.60	Flt1	FMS-like tyrosine kinase 1
1437939_s_at	1.59	Ctsc	cathepsin C
1422698_s_at	1.59	Jarid2	jumonji, AT rich interactive domain 2 [a]
1453739_at	1.59	Tmem126b	transmembrane protein 126B
1418332_a_at	1.59	Agtpbp1	ATP/GTP binding protein 1
1420484_a_at	1.59	Vtn	vitronectin
1435384_at	1.59	Ube2n	ubiquitin-conjugating enzyme E2N
1425848_a_at	1.59	Dusp26	dual specificity phosphatase 26 (putative)
1417393_a_at	1.59	Fam132a	family with sequence similarity 132, member A
1417042_at	1.59	Slc37a4	solute carrier family 37 (glucose-6-phosphate transporter), member 4
1438562_a_at	1.59	Ptpn2	protein tyrosine phosphatase, non-receptor type 2
1424716_at	1.59	Retsat	retinol saturase (all trans retinol 13,14 reductase)
1452922_at	1.59	Ppp1r3d	protein phosphatase 1, regulatory subunit 3D
1434756_at	1.58	5430421B17	hypothetical protein 5430421B17
1456046_at	1.58	Cd93	CD93 antigen
1429146_at	1.58	Svip	small VCP/p97-interacting protein
1422692_at	1.58	Sub1	SUB1 homolog (S. cerevisiae)
1442033_at	1.58	C1qtnf9	C1q and tumor necrosis factor related protein 9
1455071_at	1.58	Zbtb7b	zinc finger and BTB domain containing 7B
1441444_at	1.58	Nbeal1	Neurobeachin like 1
1428014_at	1.58	Hist1h4h	histone cluster 1, H4h
1452207_at	1.58	Cited2	Cbp/p300-interacting transactivator domain 2
1433931_at	1.58	C030046I01Rik	RIKEN cDNA C030046I01 gene
1424359_at	1.58	Oplah	5-oxoprolinase (ATP-hydrolysing)
1418886_s_at	1.58	Idh3b	isocitrate dehydrogenase 3 (NAD+) beta
1429438_at	1.58	Bcor	BCL6 interacting corepressor
1452358_at	1.57	Rai2	retinoic acid induced 2
1434133_s_at	1.57	Dcaf8	DDB1 and CUL4 associated factor 8
1441987_at	1.57	Mbd5	methyl-CpG binding domain protein 5
1441047_at	1.57	Slc45a4	solute carrier family 45, member 4
1420909_at	1.57	Vegfa	vascular endothelial growth factor A
1420374_at	1.57	Foxj2	forkhead box J2
1455975_x_at	1.57	Rnf114	ring finger protein 114
1446560_at	1.57	Prss23	protease, serine, 23
1429433_at	1.57	Bat2d	BAT2 domain containing 1
1416737_at	1.57	Gys1	glycogen synthase 1, muscle

1452868_at	1.56	Usp24	ubiquitin specific peptidase 24
1437067_at	1.56	Phtf2	putative homeodomain transcription factor 2 [a]
1453022_at	1.56	Gpihbp1	GPI-anchored HDL-binding protein 1
1449180_at	1.56	Kcmf1	potassium channel modulatory factor 1
1428651_at	1.56	Klhl24	kelch-like 24 (Drosophila)
1435716_x_at	1.56	Snrpn	small nuclear ribonucleoprotein N
1435551_at	1.56	Fhod3	formin homology 2 domain containing 3
1452198_at	1.56	Kdm2b	lysine (K)-specific demethylase 2B
1430392_at	1.56	9530086O07Rik	RIKEN cDNA 9530086O07 gene
1418068_at	1.56	Ndufa10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10
1438641_x_at	1.56	Fam57b	family with sequence similarity 57, member B [a]
1460241_a_at	1.56	St3gal5	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
1456611_at	1.56	Fam13a	family with sequence similarity 13, member A
1431083_a_at	1.55	1810014B01Rik	RIKEN cDNA 1810014B01 gene
1436913_at	1.55	Cdc14a	CDC14 cell division cycle 14 homolog A (S. cerevisiae)
1455323_at	1.55	Rbak	RB-associated KRAB repressor
1459843_s_at	1.55	Smad1	MAD homolog 1 (Drosophila)
1427922_at	1.55	2310061C15Rik	RIKEN cDNA 2310061C15 gene
1451577_at	1.55	Zbtb20	zinc finger and BTB domain containing 20
1416275_at	1.55	Slc26a6	solute carrier family 26, member 6
1417175_at	1.55	Csnk1e	casein kinase 1, epsilon
1428342_at	1.55	Rcor3	REST corepressor 3
1438477_a_at	1.55	Mcee	methylmalonyl CoA epimerase
1420019_at	1.55	Tspan8	Tetraspanin 8
1433486_at	1.54	Clcn3	chloride channel 3
1418321_at	1.54	Dci	dodecenoyl-Coenzyme A delta isomerase
1455059_at	1.54	Helz	helicase with zinc finger domain
1451148_at	1.54	Pten	PTEN
1443905_at	1.54	Hpvc-ps	Human papillomavirus 18 E5 central sequence motif, pseudogene
1424223_at	1.54	1700020C11Rik	RIKEN cDNA 1700020C11 gene
1454670_at	1.54	Rere	arginine glutamic acid dipeptide (RE) repeats [a]
1420483_at	1.54	Cnnm3	cyclin M3
1450643_s_at	1.54	Acsl1	acyl-CoA synthetase long-chain family member 1
1423202_a_at	1.54	Ncor1	nuclear receptor co-repressor 1 [a]
1424746_at	1.54	Kif1c	kinesin family member 1C [b]
1421066_at	1.53	Jak2	Janus kinase 2
1426689_s_at	1.53	Sdha	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)

1436189_at	1.53	Nqo2	NAD(P)H dehydrogenase, quinone 2
1437474_at	1.53	Gatad2b	GATA zinc finger domain containing 2B
1429515_at	1.53	Ubr2	ubiquitin protein ligase E3 component n-recognin 2
1426822_at	1.53	Rhot2	ras homolog gene family, member T2
1448743_at	1.53	Ssx2ip	synovial sarcoma, X breakpoint 2 interacting protein
1453065_at	1.53	Aldh5a1	aldhehyde dehydrogenase family 5, subfamily A1
1445841_at	1.53	Lrrc39	leucine rich repeat containing 39
1436555_at	1.53	Slc7a2	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2
1447923_at	1.53	1810026B05Rik	RIKEN cDNA 1810026B05 gene
1416513_at	1.53	Lamb2	laminin, beta 2
1424166_at	1.53	Msh3	mutS homolog 3 (E. coli)
1437552_at	1.52	2410127L17Rik	RIKEN cDNA 2410127L17 gene
1428295_at	1.52	Synpo2l	synaptopodin 2-like
1423831_at	1.52	Prkag2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit
1417371_at	1.52	Peli1	pellino 1
1417480_at	1.52	Fbxo9	f-box protein 9
1428929_s_at	1.52	Slc25a26	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 26
1440884_s_at	1.52	A530047J11Rik	RIKEN cDNA A530047J11 gene
1431335_a_at	1.52	Wfdc1	WAP four-disulfide core domain 1
1416647_at	1.52	Bckdha	branched chain ketoacid dehydrogenase E1, alpha polypeptide
1420059_at	1.52	Fam54b	Family with sequence similarity 54, member B
1436919_at	1.52	Trp53i11	transformation related protein 53 inducible protein 11
1426094_at	1.52	Rhbdl1	rhomboid, veinlet-like 1 (Drosophila)
1421878_at	1.52	Mapk9	mitogen-activated protein kinase 9
1438658_a_at	1.52	S1pr3	sphingosine-1-phosphate receptor 3
1415998_at	1.52	Vdac1	voltage-dependent anion channel 1
1419369_at	1.52	Rnf138	ring finger protein 138
1435645_at	1.52	Mmd	monocyte to macrophage differentiation-associated
1425164_a_at	1.52	Phkg1	phosphorylase kinase gamma 1 [a]
1443932_at	1.52	Klhdc1	kelch domain containing 1
1419171_at	1.51	Fam174a	family with sequence similarity 174, member A
1455777_x_at	1.51	Hsd17b4	hydroxysteroid (17-beta) dehydrogenase 4
1454858_x_at	1.51	Mettl7a1	methyltransferase like 7A1
1448288_at	1.51	Nfib	nuclear factor I/B
1451669_at	1.51	Ppm1b	protein phosphatase 1B, magnesium dependent, beta isoform
1456048_at	1.51	Cpeb3	cytoplasmic polyadenylation element binding protein 3
1428519_at	1.51	2610528E23Rik	RIKEN cDNA 2610528E23 gene

1452821_at	1.51	Tial1	Tia1 cytotoxic granule-associated RNA binding protein-like 1
1448717_at	1.51	Gcdh	glutaryl-Coenzyme A dehydrogenase
1434551_at	1.51	Hnrnpul2	heterogeneous nuclear ribonucleoprotein U-like 2
1437287_at	1.51	1110020G09Rik	RIKEN cDNA 1110020G09 gene
1433503_at	1.51	Ptgr2	prostaglandin reductase 2
1451575_a_at	1.51	Nudt3	nudix (nucleotide diphosphate linked moiety X)-type motif 3
1444085_at	1.51	Pdss2	prenyl (solanesyl) diphosphate synthase, subunit 2
1427591_at	1.51	Clcn1	chloride channel 1
1416367_at	1.51	Fmc1	formation of mitochondrial complexes 1 homolog (S. cerevisiae)
1454773_at	1.51	Rxra	retinoid X receptor alpha
1415897_a_at	1.50	Mgst1	microsomal glutathione S-transferase 1
1437992_x_at	1.50	Gja1	gap junction protein, alpha 1
1417203_at	1.50	Ethe1	ethylmalonic encephalopathy 1
1416778_at	1.50	Sdpr	serum deprivation response
1424797_a_at	1.50	Pitx2	paired-like homeodomain transcription factor 2
1417585_at	1.50	Nup210	nucleoporin 210
1422025_at	1.50	Mitf	microphthalmia-associated transcription factor
1436358_at	1.50	Atpaf1	ATP synthase mitochondrial F1 complex assembly factor 1
1415949_at	1.50	Cpe	carboxypeptidase E
1421046_a_at	1.50	Pabpc4	poly(A) binding protein, cytoplasmic 4
1435391_at	1.50	Prr8	proline rich 8
1425403_at	1.50	Dnm3	dynamin 3
1427072_at	1.50	Stard8	START domain containing 8
1427191_at	1.50	Npr2	natriuretic peptide receptor 2

A total 1242 gene identified from 1652 Affymetrix probe sets after a 1.5 fold change cut off. 649 genes were overexpressed, and 593 genes were underexpressed (grey box). [a] This gene appears two times; [b] This gene appears three times; [c] This gene appears four times; [d] This gene appears five times; [e] This gene appears six times. W, wild type mice; CLW, clenbuterol treated wild type mice.

Appendix 3.8 Differential gene expression profiles as affected by the effect of CL under Mstn-pro at 1.5 fold change (M vs CLM).

Probe Set ID	Fold change	Gene Symbol	Gene Title
1423691_x_at	413.40	Krt8	keratin 8 [b]
1448169_at	144.41	Krt18	keratin 18
1419139_at	77.47	Gdf5	growth differentiation factor 5
1440878_at	41.09	Runx1	runt related transcription factor 1 [c]
1422562_at	38.79	Rrad	Ras-related associated with diabetes
1424245_at	32.27	Ces2	carboxylesterase 2
1458813_at	31.87	Scn5a	sodium channel, voltage-gated, type V, alpha
1420992_at	21.26	Ankrd1	ankyrin repeat domain 1 (cardiac muscle) [a]
1420438_at	20.69	Orm2	orosomucoid 2
1423933_a_at	19.24	1600029D21Rik	RIKEN cDNA 1600029D21 gene
1434709_at	18.91	Nrcam	neuron-glia-CAM-related cell adhesion molecule [a]
1435053_s_at	16.27	Plekhh1	pleckstrin homology domain containing, family H [a]
1455271_at	14.23	Gm13889	predicted gene 13889
1454632_at	12.56	6330442E10Rik	RIKEN cDNA 6330442E10 gene
1419394_s_at	12.54	S100a8	S100 calcium binding protein A8 (calgranulin A)
1435176_a_at	12.29	Id2	inhibitor of DNA binding 2
1421852_at	12.14	Kcnk5	potassium channel, subfamily K, member 5
1451054_at	11.27	Orm1	orosomucoid 1
1449519_at	10.97	Gadd45a	growth arrest and DNA-damage-inducible 45 alpha
1448975_s_at	10.72	Ren1	renin 1 structural
1418852_at	9.72	Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)
1458245_at	9.60	Gm12528	predicted gene 12528
1420444_at	9.18	Slc22a3	solute carrier family 22 (organic cation transporter), member 3
1451287_s_at	9.15	Aif1l	allograft inflammatory factor 1-like [a]
1421269_at	9.04	Ugcg	UDP-glucose ceramide glucosyltransferase [b]
1419391_at	8.47	Myog	myogenin
1428640_at	7.96	Hsf2bp	heat shock transcription factor 2 binding protein
1456953_at	6.78	Col19a1	collagen, type XIX, alpha 1 [a]
1430062_at	6.74	Hhipl1	hedgehog interacting protein-like 1
1424638_at	6.72	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21) [a]
1442145_at	6.72	Atp13a3	ATPase type 13A3 [a]
1449084_s_at	6.72	Sh3d19	SH3 domain protein D19 [c]
1450013_at	6.67	2900073G15Rik	RIKEN cDNA 2900073G15 gene [a]

1440085_at	6.65	Eda2r	ectodysplasin A2 isoform receptor
1426808_at	6.63	Lgals3	lectin, galactose binding, soluble 3
1427910_at	6.58	Cst6	cystatin E/M
1437669_x_at	6.52	Ccrl1	Chemokine (C-C motif) receptor-like 1 [a]
1423186_at	6.50	Tiam2	T-cell lymphoma invasion and metastasis 2
1424831_at	6.29	Cpne2	copine II
1436790_a_at	6.17	Sox11	SRY-box containing gene 11[c]
1456393_at	6.16	2310002J21Rik	RIKEN cDNA 2310002J21 gene
1437460_x_at	5.99	Rin1	Ras and Rab interactor 1 [a]
1434754_at	5.84	Garnl4	GTPase activating RANGAP domain-like 4
1455649_at	5.70	Ttc9	tetratricopeptide repeat domain 9
1455771_at	5.58	Bzrap1	benzodiazapine receptor associated protein 1
1422629_s_at	5.58	Shroom3	shroom family member 3
1421499_a_at	5.14	Ptpn14	protein tyrosine phosphatase, non-receptor type 14 [a]
1423719_at	5.02	LOC632073	similar to long palate, lung and nasal epithelium carcinoma associated 1 isoform 2
1422573_at	4.95	Ampd3	adenosine monophosphate deaminase 3
1427838_at	4.77	Tubb2a	tubulin, beta 2A [a]
1418572_x_at	4.77	Tnfrsf12a	tumor necrosis factor receptor superfamily, member 12a
1455342_at	4.76	Prune2	prune homolog 2 (Drosophila) [a]
1454674_at	4.69	Fez1	fasciculation and elongation protein zeta 1 (zygin I)
1457254_x_at	4.55	6330442E10Rik	RIKEN cDNA 6330442E10 gene
1442827_at	4.54	Tlr4	Toll-like receptor 4 [b]
1424354_at	4.46	Tmem140	transmembrane protein 140
1428834_at	4.46	Dusp4	dual specificity phosphatase 4
1440999_at	4.34	Zfp697	zinc finger protein 697
1452836_at	4.33	Lpin2	lipin 2 [a]
1435062_at	4.29	1500001A10Rik	RIKEN cDNA 1500001A10 gene
1426337_a_at	4.24	Tead4	TEA domain family member 4
1437062_s_at	4.15	Phyhipl	phytanoyl-CoA hydroxylase interacting protein-like
1430086_at	4.14	Chrna9	cholinergic receptor, nicotinic, alpha polypeptide 9
1416028_a_at	4.06	Hn1	hematological and neurological expressed sequence 1 [b]
1424517_at	3.97	Ccdc12	coiled-coil domain containing 12 [a]
1435184_at	3.95	Npr3	natriuretic peptide receptor 3
1451006_at	3.95	Xdh	xanthine dehydrogenase
1457157_at	3.94	Plch1	phospholipase C, eta 1
1419621_at	3.92	Ankrd2	ankyrin repeat domain 2 (stretch responsive muscle)
1428803_at	3.91	Acot6	acyl-CoA thioesterase 6 [a]

1451411_at	3.89	Gprc5b	G protein-coupled receptor, family C, group 5, member B [a]
1441055_at	3.89	Palm2	paralemmin 2
1449852_a_at	3.89	Ehd4	EH-domain containing 4
1452799_at	3.80	Fggy	FGGY carbohydrate kinase domain containing
1419184_a_at	3.77	Fhl	four and a half LIM domains
1433731_at	3.70	Igf2bp3	insulin-like growth factor 2 mRNA binding protein 3
1450047_at	3.70	Hs6st2	heparan sulfate 6-O-sulfotransferase 2
1455149_at	3.64	Sh3rf1	SH3 domain containing ring finger 1 [a]
1437224_at	3.55	Rtn4	reticulon 4 [a]
1429086_at	3.45	Grhl2	grainyhead-like 2 (Drosophila)
1418840_at	3.43	Pdcd4	programmed cell death 4
1438795_x_at	3.38	Fkbp1	fk506 binding protein 12-rapamycin associated protein 1
1460392_a_at	3.38	Eny2	enhancer of yellow 2 homolog (Drosophila) [a]
1417103_at	3.37	Ddt	D-dopachrome tautomerase
1431281_at	3.37	Dysfip1	dysferlin interacting protein 1
1416065_a_at	3.36	Ankrd10	ankyrin repeat domain 10 [b]
1436362_x_at	3.34	Ccrn4l	CCR4 carbon catabolite repression 4-like (S. cerevisiae)
1418158_at	3.32	Trp63	transformation related protein 63 [a]
1420884_at	3.32	Sln	sarcolipin
1452232_at	3.29	Galnt7	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7
1456291_x_at	3.29	Scx	scleraxis [a]
1451757_at	3.27	BC003883	cDNA sequence BC003883
1448660_at	3.25	Arhgdig	Rho GDP dissociation inhibitor (GDI) gamma
1418853_at	3.22	Apon	apolipoprotein N
1425964_x_at	3.21	Hspb1	heat shock protein 1 [a]
1425543_s_at	3.14	Plekha5	pleckstrin homology domain containing, family A member 5
1420895_at	3.13	Tgfbr1	transforming growth factor, beta receptor I [a]
1448793_a_at	3.10	Sdc4	syndecan 4 [a]
1435394_s_at	3.10	Rhoc	ras homolog gene family, member C
1426329_s_at	3.09	Baalc	brain and acute leukemia, cytoplasmic
1418648_at	3.08	Egln3	EGL nine homolog 3 (C. elegans) [a]
1443667_at	3.07	C79407	expressed sequence C79407
1458067_at	3.06	Fam179b	Family with sequence similarity 179, member B
1433972_at	3.02	Camta1	calmodulin binding transcription activator 1 [a]
1422286_a_at	3.02	Tgif1	TGFB-induced factor homeobox 1
1421479_at	3.02	Zfp318	zinc finger protein 318 [c]
1416401_at	3.01	Cd82	CD82 antigen

1421407_at	2.99	F2rl2	coagulation factor II (thrombin) receptor-like 2
1438621_x_at	2.98	Axl	AXL receptor tyrosine kinase
1425238_at	2.98	Gm6607	40S ribosomal protein S20 pseudogene
1428427_at	2.97	Fbxl2	F-box and leucine-rich repeat protein 2
1428294_at	2.97	Zfp259	zinc finger protein 259
1416379_at	2.95	Panx1	pannexin 1
1442340_x_at	2.94	Cyr61	cysteine rich protein 61 [b]
1444786_at	2.93	Nol3	nucleolar protein 3 (apoptosis repressor with CARD domain)
1420832_at	2.92	Qsox1	quiescin Q6 sulfhydryl oxidase 1
1436042_at	2.91	Tln1	talin 1
1421943_at	2.91	Tgfa	transforming growth factor alpha
1447662_x_at	2.89	D18Ertd653e	DNA segment, Chr 18, ERATO Doi 653, expressed
1444451_at	2.88	Pappa2	pappalysin 2
1426471_at	2.88	Zfp52	zinc finger protein 52 [a]
1426095_a_at	2.87	Tnfrsf22	tumor necrosis factor receptor superfamily, member 22 [a]
1449533_at	2.86	Tmem100	transmembrane protein 100
1434927_at	2.85	Hspb7	heat shock protein family, member 7 (cardiovascular)
1426288_at	2.83	Lrp4	low density lipoprotein receptor-related protein 4
1437109_s_at	2.83	Lsm6	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae)
1454731_at	2.82	Myo10	myosin X [a]
1451160_s_at	2.82	Pvr	poliovirus receptor [a]
1423172_at	2.81	Napb	N-ethylmaleimide sensitive fusion protein attachment protein beta [a]
1438701_at	2.81	Bicd1	bicaudal D homolog 1 (Drosophila)
1430929_at	2.80	9430082L08Rik	RIKEN cDNA 9430082L08 gene
1455573_at	2.79	Krt14	keratin 14
1417013_at	2.79	Hspb8	heat shock protein 8
1439708_at	2.75	Myom3	myomesin family, member 3
1422101_at	2.75	Tnfrsf23	tumor necrosis factor receptor superfamily, member 23
1439049_at	2.74	Dph5	DPH5 homolog (S. cerevisiae) [a]
1453323_at	2.74	2900079G21Rik	RIKEN cDNA 2900079G21 gene
1455605_at	2.74	Rufy3	RUN and FYVE domain containing 3
1450320_at	2.72	4931432E15Rik	RIKEN cDNA 4931432E15 gene
1421043_s_at	2.72	Arhgef2	rho/rac guanine nucleotide exchange factor (GEF) 2 [a]
1451889_at	2.71	Notch2	Notch gene homolog 2 (Drosophila)
1433360_at	2.71	1700023B13Rik	RIKEN cDNA 1700023B13 gene
1445439_at	2.68	Epb4.9	erythrocyte protein band 4.9
1421947_at	2.68	Gng12	guanine nucleotide binding protein (G protein), gamma 12

1441101_at	2.66	Hecw1	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1
1429618_at	2.65	Cyld	cylindromatosis (turban tumor syndrome)[a]
1435402_at	2.65	Gramd1b	GRAM domain containing 1B
1450541_at	2.64	Pvt1	plasmacytoma variant translocation 1
1434985_a_at	2.64	Eif4a1	eukaryotic translation initiation factor 4A1
1420769_at	2.63	Dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58
1424489_a_at	2.62	Trit1	tRNA isopentenyltransferase 1
1445709_at	2.61	Mdm1	transformed mouse 3T3 cell double minute 1
1438001_x_at	2.61	Reep5	receptor accessory protein 5
1445553_at	2.60	Ric3	resistance to inhibitors of cholinesterase 3 homolog (C. elegans)
1432570_at	2.60	6030458E02Rik	RIKEN cDNA 6030458E02 gene
1440008_at	2.59	2310043L19Rik	RIKEN cDNA 2310043L19 gene
1438824_at	2.58	Slc20a1	solute carrier family 20, member 1[a]
1445568_at	2.58	Inca1	Inhibitor of CDK, cyclin A1 interacting protein 1[a]
1436938_at	2.57	Rbms3	RNA binding motif, single stranded interacting protein
1433571_at	2.57	Serinc5	serine incorporator 5
1444089_at	2.57	Spnb2	spectrin beta 2
1431869_at	2.56	5730419F03Rik	RIKEN cDNA 5730419F03 gene
1427539_a_at	2.54	Zwint	ZW10 interactor[b]
1451453_at	2.54	Dapk2	death-associated protein kinase 2
1456387_at	2.54	Nol4	nucleolar protein 4
1452719_at	2.54	Zdhhc24	zinc finger, DHHC domain containing 24[a]
1418186_at	2.53	Gstt1	glutathione S-transferase, theta 1
1436235_x_at	2.53	4732471D19Rik	RIKEN cDNA 4732471D19 gene[a]
1439166_at	2.52	Nol11	nucleolar protein 11
1446470_at	2.51	Gm9799	predicted gene 9799
1450511_at	2.50	Musk	muscle, skeletal, receptor tyrosine kinase
1420377_at	2.50	St8sia2	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2
1422316_at	2.50	Gp1ba	glycoprotein 1b, alpha polypeptide
1454693_at	2.50	Hdac4	histone deacetylase 4[a]
1457587_at	2.49	Kcnq5	potassium voltage-gated channel, subfamily Q, member 5
1438354_x_at	2.49	Cnn3	Calponin 3, acidic
1436305_at	2.49	Rnf217	ring finger protein 217[a]
1441144_at	2.48	Arr3	arrestin 3, retinal
1448694_at	2.47	Jun	Jun oncogene
1454133_s_at	2.46	4930523O13Rik	RIKEN cDNA 4930523O13 gene
1449363_at	2.45	Atf3	activating transcription factor 3

1439483_at	2.44	AI506816	expressed sequence AI506816
1442957_at	2.44	G730013B05Rik	RIKEN cDNA G730013B05 gene
1449907_at	2.44	Bcmo1	beta-carotene 15,15'-monooxygenase
1449691_at	2.41	Zfp644	Zinc finger protein 644
1419916_at	2.41	Rnf20	Ring finger protein 20
1436499_at	2.40	Sgms1	sphingomyelin synthase 1 [a]
1428739_at	2.39	Enho	energy homeostasis associated
1434080_at	2.38	Aebp2	AE binding protein 2 [a]
1455577_at	2.38	Ccl28	chemokine (C-C motif) ligand 28
1420539_a_at	2.38	Chrdl2	chordin-like 2
1423596_at	2.38	Nek6	NIMA (never in mitosis gene a)-related expressed kinase 6 [a]
1422608_at	2.38	Arpp19	cAMP-regulated phosphoprotein 19
1418938_at	2.36	Dio2	deiodinase, iodothyronine, type II
1455493_at	2.35	Syne1	synaptic nuclear envelope 1
1433990_at	2.34	Lhfpl3	lipoma HMGIC fusion partner-like 3
1435987_x_at	2.34	1110059G02Rik	RIKEN cDNA 1110059G02 gene
1438307_at	2.34	Hmgb2	high mobility group box 2
1434402_at	2.33	Samd8	sterile alpha motif domain containing 8
1416119_at	2.33	Txn1	thioredoxin 1
1458504_at	2.32	Zc3h12d	zinc finger CCCH type containing 12D
1415803_at	2.32	Cx3cl1	chemokine (C-X3-C motif) ligand 1
1448744_at	2.32	Galns	galactosamine (N-acetyl)-6-sulfate sulfatase
1419305_a_at	2.31	Fbxo36	F-box protein 36
1426875_s_at	2.31	Srxn1	sulfiredoxin 1 homolog (S. cerevisiae) [b]
1438429_at	2.30	2610319H10Rik	RIKEN cDNA 2610319H10 gene
1437722_x_at	2.30	Pcbp3	Poly(rC) binding protein 3
1430724_at	2.30	Ephx3	epoxide hydrolase 3
1452909_at	2.29	Klhdc10	kelch domain containing 10
1439805_at	2.29	Nfat5	Nuclear factor of activated T-cells 5
1452478_at	2.29	Alpk2	alpha-kinase 2
1440688_at	2.28	Arhgap26	Rho GTPase activating protein 26
1426486_at	2.28	Ubxn4	UBX domain protein 4
1416431_at	2.28	Tubb6	tubulin, beta 6
1415862_at	2.27	Tyrp1	tyrosinase-related protein 1
1420682_at	2.27	Chrnb1	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
1447507_at	2.27	C80406	expressed sequence C80406
1417128_at	2.26	Plekho1	pleckstrin homology domain containing, family O member 1

1449839_at	2.26	Casp3	caspase 3
1451814_a_at	2.25	Htatip2	HIV-1 tat interactive protein 2, homolog (human)
1421928_at	2.25	Epha4	Eph receptor A4 [a]
1432171_at	2.25	4933409F18Rik	RIKEN cDNA 4933409F18 gene
1419344_at	2.25	Tcte1	t-complex-associated testis expressed 1
1455350_at	2.25	Tmem62	transmembrane protein 62
1432129_a_at	2.25	Prrx1	paired related homeobox 1
1419446_at	2.24	Tbc1d1	TBC1 domain family, member 1
1456251_x_at	2.24	Tspo	translocator protein [a]
1454520_at	2.23	5830415B17Rik	RIKEN cDNA 5830415B17 gene
1457296_at	2.23	Cilp	cartilage intermediate layer protein, nucleotide pyrophosphohydrolase
1421746_a_at	2.23	Fbxo17	F-box protein 17
1416606_s_at	2.22	Nhp2	NHP2 ribonucleoprotein homolog (yeast) [a]
1439396_x_at	2.22	Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)
1435936_at	2.22	Slc13a5	solute carrier family 13 (sodium-dependent citrate transporter), member 5
1432949_at	2.20	5330421F21Rik	RIKEN cDNA 5330421F21 gene
1448715_x_at	2.20	Sgip1	SH3-domain GRB2-like (endophilin) interacting protein 1
1453228_at	2.20	Stx11	syntaxin 11
1416911_a_at	2.20	Akirin1	akirin 1 [a]
1435303_at	2.20	Taf4b	TAF4B RNA polymerase II, TATA box binding protein (TBP)-associated factor
1427938_at	2.19	Mycbp	c-myc binding protein
1422301_at	2.19	Ftl1	ferritin light chain 1
1416708_a_at	2.19	Gramd1a	GRAM domain containing 1A
1438288_x_at	2.18	1110059G02Rik	RIKEN cDNA 1110059G02 gene
1454489_at	2.18	4930513L20Rik	RIKEN cDNA 4930513L20 gene
1438059_at	2.18	Ctxn3	cortexin 3
1425389_a_at	2.17	Runx2	runt related transcription factor 2
1434241_at	2.17	Wdr67	WD repeat domain 67
1418470_at	2.17	Yes1	Yamaguchi sarcoma viral (v-yes) oncogene homolog 1
1417236_at	2.17	Ehd3	EH-domain containing 3
1422363_at	2.17	Olfr65	olfactory receptor 65
1416916_at	2.17	Elf3	E74-like factor 3
1416231_at	2.16	Vac14	Vac14 homolog (S. cerevisiae)
1448571_a_at	2.16	Gmfb	glia maturation factor, beta
1445691_at	2.15	Chn1	chimerin (chimaerin) 1
1426159_x_at	2.15	Tcrb-J	T-cell receptor beta, joining region
1455284_x_at	2.14	Pigx	phosphatidylinositol glycan anchor biosynthesis, class X

1420096_at	2.14	Zscan21	Zinc finger and SCAN domain containing 21
1449304_at	2.14	2310061J03Rik	RIKEN cDNA 2310061J03 gene
1434153_at	2.14	Shb	src homology 2 domain-containing transforming protein B
1435981_at	2.14	Nav2	neuron navigator 2 [a]
1442637_at	2.13	Itsn1	Intersectin 1 (SH3 domain protein 1A)
1419557_a_at	2.13	Tmem9	transmembrane protein 9
1454354_at	2.13	8030476L19Rik	RIKEN cDNA 8030476L19 gene
1418908_at	2.13	Pam	peptidylglycine alpha-amidating monooxygenase
1457208_at	2.13	Nfxl1	nuclear transcription factor, X-box binding-like 1
1459003_at	2.13	Fhl1	four and a half LIM domains 1
1418318_at	2.12	Rnf128	ring finger protein 128 [a]
1450260_at	2.12	Grpr	gastrin releasing peptide receptor
1438021_at	2.12	Rbm47	RNA binding motif protein 47
1435049_s_at	2.12	AI854703	expressed sequence AI854703
1457775_at	2.12	Sacs	sacsin [a]
1423810_at	2.12	Ppme1	protein phosphatase methylesterase 1
1423923_a_at	2.11	Wdr8	WD repeat domain 8
1429990_at	2.11	Hyal4	hyaluronoglucosaminidase 4
1440522_at	2.11	Gm10454	predicted gene 10454
1424171_a_at	2.11	Hagh	hydroxyacyl glutathione hydrolase [a]
1426054_at	2.11	Npy1r	neuropeptide Y receptor Y1
1450959_at	2.11	D930014E17Rik	RIKEN cDNA D930014E17 gene
1446183_at	2.11	Als2	Amyotrophic lateral sclerosis 2 (juvenile) homolog (human)
1430294_at	2.11	Ssbp1	single-stranded DNA binding protein 1
1436761_s_at	2.11	Fam13c	family with sequence similarity 13, member C
1453414_at	2.10	Ypel2	yippee-like 2 (Drosophila)
1447819_x_at	2.10	Col8a1	collagen, type VIII, alpha 1
1428833_at	2.10	4930406D14Rik	RIKEN cDNA 4930406D14 gene
1451227_a_at	2.08	Slc10a3	solute carrier family 10 (sodium/bile acid cotransporter family), member 3
1436026_at	2.08	Zfp703	zinc finger protein 703
1436887_x_at	2.08	Grwd1	glutamate-rich WD repeat containing 1 [a]
1418497_at	2.08	Fgf13	fibroblast growth factor 13
1433211_at	2.08	Rptn	repetin
1448689_at	2.08	Rras2	related RAS viral (r-ras) oncogene homolog 2
1442858_at	2.08	Mycbp2	MYC binding protein 2
1455316_x_at	2.07	BC094435	cDNA sequence BC094435
1420626_at	2.07	Gtf3c6	general transcription factor IIIC, polypeptide 6, alpha

1433110_at	2.06	5830474E16Rik	RIKEN cDNA 5830474E16 gene
1424378_at	2.06	Ldlrap1	low density lipoprotein receptor adaptor protein 1
1434382_at	2.05	Serinc2	serine incorporator 2
1426873_s_at	2.05	Jup	junction plakoglobin
1444712_at	2.05	Hsf5	heat shock transcription factor family member 5
1432494_a_at	2.04	1700019E19Rik	RIKEN cDNA 1700019E19 gene
1441881_x_at	2.04	Fam101a	family with sequence similarity 101, member A
1427434_at	2.04	Birc1f	baculoviral IAP repeat-containing 1f
1424886_at	2.04	Ptprd	protein tyrosine phosphatase, receptor type, D [a]
1457681_at	2.04	2610301F02Rik	RIKEN cDNA 2610301F02 gene
1437058_at	2.04	Megf6	multiple EGF-like-domains 6
1424530_at	2.03	Sec14l2	SEC14-like 2 (S. cerevisiae)
1455661_at	2.03	Gm9958	predicted gene 9958
1433182_at	2.03	4930431N21Rik	RIKEN cDNA 4930431N21 gene
1437502_x_at	2.03	Cd24a	CD24a antigen [a]
1446344_at	2.03	Nyx	nyctalopin
1422218_at	2.02	P2rx7	purinergic receptor P2X, ligand-gated ion channel, 7
1455064_at	2.02	Rab36	RAB36, member RAS oncogene family
1423239_at	2.02	Impdh1	inosine 5'-phosphate dehydrogenase 1
1456586_x_at	2.02	Mvp	major vault protein [a]
1415993_at	2.02	Sqle	squalene epoxidase
1416350_at	2.01	Klf16	Kruppel-like factor 16
1445901_at	2.01	Lrp2	low density lipoprotein receptor-related protein 2
1425858_at	2.01	Ube2m	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)
1426403_at	2.01	Actr1b	ARP1 actin-related protein 1 homolog B, centractin beta (yeast)
1426722_at	2.01	Slc38a2	solute carrier family 38, member 2
1438176_x_at	2.00	Snap47	synaptosomal-associated protein, 47
1458385_at	2.00	Hspa4l	heat shock protein 4 like
1436710_at	2.00	Zswim4	zinc finger, SWIM domain containing 4
1447865_x_at	2.00	Pdzd11	PDZ domain containing 11
1453585_at	2.00	1600010M07Rik	RIKEN cDNA 1600010M07 gene
1453358_s_at	2.00	Amn1	antagonist of mitotic exit network 1 homolog (S. cerevisiae)
1436978_at	2.00	Wnt9a	wingless-type MMTV integration site 9A
1439556_at	1.99	Ncam1	neural cell adhesion molecule 1
1433367_at	1.99	8430401P03Rik	RIKEN cDNA 8430401P03 gene
1449323_a_at	1.99	Rpl3	ribosomal protein L3
1450686_at	1.99	Pon2	paraoxonase 2 [a]

1455203_at	1.99	A930003A15Rik	RIKEN cDNA A930003A15 gene
1446871_at	1.99	D7Ertd481e	DNA segment, Chr 7, ERATO Doi 481, expressed
1418448_at	1.99	Rras	Harvey rat sarcoma oncogene, subgroup R
1434724_at	1.99	Usp31	ubiquitin specific peptidase 31
1453854_at	1.98	Ptar1	protein prenyltransferase alpha subunit repeat containing 1
1442838_at	1.98	Gm11691	predicted gene 11691
1423850_at	1.98	Nsun2	NOL1/NOP2/Sun domain family member 2
1458370_at	1.97	Bmp2k	BMP2 inducible kinase
1423082_at	1.97	Derl1	Der1-like domain family, member 1
1450198_at	1.97	Dusp13	dual specificity phosphatase 13
1459914_at	1.97	Mcart6	mitochondrial carrier triple repeat 6
1437614_x_at	1.97	Zdhhc14	zinc finger, DHHC domain containing 14 [a]
1447868_x_at	1.96	Glrx3	glutaredoxin 3
1415676_a_at	1.96	Psmb5	proteasome (prosome, macropain) subunit, beta type 5
1422646_at	1.96	Mga	MAX gene associated
1419116_at	1.96	Alg14	asparagine-linked glycosylation 14 homolog (yeast)
1423846_x_at	1.95	Tuba1b	tubulin, alpha 1B
1449902_at	1.95	Lce1a2	late cornified envelope 1A2
1424860_at	1.95	D930016D06Rik	RIKEN cDNA D930016D06 gene [a]
1430429_at	1.94	Pgs1	phosphatidylglycerophosphate synthase 1
1446731_at	1.94	Fancf	Fanconi anemia, complementation group F
1424344_s_at	1.94	Eif1a	eukaryotic translation initiation factor 1A
1440941_at	1.93	LOC552912	hypothetical LOC552912
1441190_at	1.93	Arpc5l	actin related protein 2/3 complex, subunit 5-like
1416192_at	1.93	Napa	N-ethylmaleimide sensitive fusion protein attachment protein alpha
1455689_at	1.93	Fzd10	frizzled homolog 10 (Drosophila)
1440916_at	1.93	2510049J12Rik	RIKEN cDNA 2510049J12 gene
1418227_at	1.93	Orc2l	origin recognition complex, subunit 2-like (S. cerevisiae)
1441503_at	1.93	Dcdc2a	doublecortin domain containing 2a
1434585_at	1.93	Tulp4	Tubby like protein 4
1439060_s_at	1.93	Wipi1	WD repeat domain, phosphoinositide interacting 1
1430171_at	1.93	Fibp	fibroblast growth factor (acidic) intracellular binding protein
1417850_at	1.92	Rb1	retinoblastoma 1
1432418_a_at	1.92	Ckmt1	creatine kinase, mitochondrial 1, ubiquitous
1434891_at	1.92	Ptgfrn	prostaglandin F2 receptor negative regulator
1417453_at	1.92	Cul4b	cullin 4B
1455994_x_at	1.92	Elovl1	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1 [a]

1426465_at	1.91	Dlgap4	discs, large homolog-associated protein 4 (Drosophila)
1448345_at	1.91	Tomm34	translocase of outer mitochondrial membrane 34
1459230_at	1.91	B430319G15Rik	RIKEN cDNA B430319G15 gene
1439091_at	1.91	Fancd2	Fanconi anemia, complementation group D2
1455738_at	1.91	Ccdc55	coiled-coil domain containing 55
1420150_at	1.91	Spsb1	splA/ryanodine receptor domain and SOCS box containing 1
1455643_s_at	1.91	Tsr1	TSR1, 20S rRNA accumulation, homolog (yeast) [a]
1432569_at	1.90	Cby3	chibby homolog 3 (Drosophila)
1451862_a_at	1.90	Prf1	perforin 1 (pore forming protein)
1417428_at	1.90	Gng3	guanine nucleotide binding protein (G protein), gamma 3
1440990_at	1.90	Kif26b	kinesin family member 26B
1426577_a_at	1.90	Lin37	lin-37 homolog (C. elegans)
1442454_at	1.90	Top2a	topoisomerase (DNA) II alpha
1424718_at	1.90	Mapt	microtubule-associated protein tau
1452222_at	1.90	Utrn	utrophin
1434660_at	1.89	Alkbh1	alkB, alkylation repair homolog 1 (E. coli)
1447577_x_at	1.89	Pcca	propionyl-Coenzyme A carboxylase, alpha polypeptide
1432156_a_at	1.89	Rnf32	ring finger protein 32
1426426_at	1.89	Mak16	MAK16 homolog (S. cerevisiae)
1426275_a_at	1.88	Uxs1	UDP-glucuronate decarboxylase 1 [a]
1448271_a_at	1.88	Ddx21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
1452759_s_at	1.88	Ppfibp1	PTPRF interacting protein, binding protein 1 (liprin beta 1)
1453129_a_at	1.88	Rgs12	regulator of G-protein signaling 12
1427186_a_at	1.88	Mef2a	myocyte enhancer factor 2A [b]
1427364_a_at	1.88	Odc1	ornithine decarboxylase, structural 1 [a]
1433656_a_at	1.88	Gnl3	guanine nucleotide binding protein-like 3 (nucleolar)
1439399_a_at	1.87	Snhg1	small nucleolar RNA host gene (non-protein coding) 1
1433719_at	1.87	Slc9a9	solute carrier family 9 (sodium/hydrogen exchanger), member 9
1434963_at	1.87	Supt3h	suppressor of Ty 3 homolog (S. cerevisiae)
1427028_at	1.87	Lgr6	leucine-rich repeat-containing G protein-coupled receptor 6
1417005_at	1.87	Klc1	kinesin light chain 1 [a]
1421354_at	1.86	Prkg2	protein kinase, cGMP-dependent, type II
1431838_at	1.86	1700128E19Rik	RIKEN cDNA 1700128E19 gene
1450852_s_at	1.86	F2r	coagulation factor II (thrombin) receptor [a]
1422929_s_at	1.86	Atoh7	atonal homolog 7 (Drosophila)
1419150_at	1.86	Myf6	myogenic factor 6
1415941_s_at	1.86	Zfand2a	zinc finger, AN1-type domain 2A

1433849_at	1.85	Cdc27	cell division cycle 27 homolog (S. cerevisiae) [a]
1428847_a_at	1.85	Macf1	microtubule-actin crosslinking factor 1
1424469_a_at	1.85	Cpsf4	cleavage and polyadenylation specific factor 4
1437688_x_at	1.85	Atp6ap2	ATPase, H+ transporting, lysosomal accessory protein 2
1425253_a_at	1.85	Madcam1	mucosal vascular addressin cell adhesion molecule 1
1429049_at	1.84	Nuak2	NUAK family, SNF1-like kinase, 2
1427980_at	1.84	4933407C03Rik	RIKEN cDNA 4933407C03 gene [a]
1418429_at	1.84	Kif5b	kinesin family member 5B
1443008_at	1.84	Msi2	Musashi homolog 2 (Drosophila) [a]
1451233_at	1.84	Traf2	TNF receptor-associated factor 2
1455442_at	1.84	Slc6a19	solute carrier family 6 (neurotransmitter transporter), member 19
1441911_x_at	1.84	Gart	phosphoribosylglycinamide formyltransferase
1430978_at	1.84	Rps25	ribosomal protein S25
1448968_at	1.83	Ubfd1	ubiquitin family domain containing 1
1423605_a_at	1.83	Mdm2	transformed mouse 3T3 cell double minute 2
1436060_at	1.83	Ralyl	RALY RNA binding protein-like
1420048_at	1.83	C78859	expressed sequence C78859
1417817_a_at	1.83	Wwtr1	WW domain containing transcription regulator 1
1451257_at	1.83	Acsl6	acyl-CoA synthetase long-chain family member 6
1438699_at	1.83	Srd5a1	steroid 5 alpha-reductase 1
1441464_at	1.82	A630035D09Rik	RIKEN cDNA A630035D09 gene
1453233_s_at	1.82	Calr3	calreticulin 3
1424529_s_at	1.82	Cgref1	cell growth regulator with EF hand domain 1
1432665_at	1.82	2210416J07Rik	RIKEN cDNA 2210416J07 gene
1459764_x_at	1.82	Snx10	Sorting nexin 10
1433379_at	1.82	9430019H13Rik	RIKEN cDNA 9430019H13 gene
1437166_at	1.82	Tinf2	Terf1 (TRF1)-interacting nuclear factor 2
1440620_at	1.82	Rab8a	RAB8A, member RAS oncogene family
1453571_at	1.82	Depdc6	DEP domain containing 6
1444242_at	1.81	Slco2a1	Solute carrier organic anion transporter family, member 2a1
1438704_at	1.81	Trim63	tripartite motif-containing 63
1443787_x_at	1.81	Casp14	caspase 14
1460421_at	1.80	Zfp133	zinc finger protein 133
1425969_a_at	1.80	Htt	huntingtin [a]
1435775_at	1.80	Clock	circadian locomoter output cycles kaput
1457638_x_at	1.80	Rfc2	replication factor C (activator 1) 2
1438544_at	1.80	1700001G11Rik	RIKEN cDNA 1700001G11 gene

1448435_at	1.80	Med15	mediator complex subunit 15
1422567_at	1.80	Fam129a	family with sequence similarity 129, member A
1424985_a_at	1.79	Sox10	SRY-box containing gene 10
1419819_s_at	1.79	Sec63	SEC63-like (S. cerevisiae)
1454391_at	1.79	6030442K20Rik	RIKEN cDNA 6030442K20 gene
1416030_a_at	1.79	Mcm7	minichromosome maintenance deficient 7 (S. cerevisiae)
1416029_at	1.79	Klf10	Kruppel-like factor 10
1427880_at	1.79	Uqcrq	ubiquinol-cytochrome c reductase, complex III subunit VII
1420570_x_at	1.79	Tcl1b3	T-cell leukemia/lymphoma 1B, 3
1443746_x_at	1.79	Dmp1	dentin matrix protein 1
1452964_at	1.79	Ttll11	tubulin tyrosine ligase-like family, member 11
1429976_at	1.78	Clasp2	CLIP associating protein 2
1459696_at	1.78	Fry	Furry homolog (Drosophila)
1443159_at	1.78	9130221J17Rik	RIKEN cDNA 9130221J17 gene
1424087_at	1.77	Psmg3	proteasome (prosome, macropain) assembly chaperone 3
1431566_at	1.77	9030622O22Rik	RIKEN cDNA 9030622O22 gene
1457628_at	1.77	Ccdc53	coiled-coil domain containing 53
1417843_s_at	1.77	Eps8l2	EPS8-like 2
1436180_at	1.77	Dnajc5	DnaJ (Hsp40) homolog, subfamily C, member 5
1449027_at	1.77	Rhou	ras homolog gene family, member U
1430680_a_at	1.77	Gon4l	gon-4-like (C.elegans)
1448450_at	1.77	Ak2	adenylate kinase 2
1416974_at	1.77	Stam2	signal transducing adaptor molecule (SH3 domain and ITAM motif) 2
1417499_at	1.76	Timm10	translocase of inner mitochondrial membrane 10 homolog (yeast)
1428635_at	1.76	Comtd1	catechol-O-methyltransferase domain containing 1
1453324_at	1.76	6330509M23Rik	RIKEN cDNA 6330509M23 gene
1453451_at	1.75	6820402I19Rik	RIKEN cDNA 6820402I19 gene
1423567_a_at	1.75	Psma7	proteasome (prosome, macropain) subunit, alpha type 7
1435035_at	1.75	Rg9mtd2	RNA (guanine-9-) methyltransferase domain containing 2
1451809_s_at	1.75	Rwdd3	RWD domain containing 3
1440152_x_at	1.75	Edf1	endothelial differentiation-related factor 1
1425780_a_at	1.75	Tmem167	transmembrane protein 167
1439552_at	1.75	Trio	triple functional domain (PTPRF interacting)
1433555_at	1.75	Eaf1	ELL associated factor 1
1434532_at	1.75	Csrnp2	cysteine-serine-rich nuclear protein 2
1416419_s_at	1.75	Gabarapl1	gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1
1450377_at	1.74	Thbs1	thrombospondin 1

1456470_x_at	1.74	Tubb2c	tubulin, beta 2C
1435444_at	1.74	Atf6	activating transcription factor 6
1422906_at	1.74	Abcg2	ATP-binding cassette, sub-family G (WHITE), member 2
1435431_at	1.74	Psmg4	proteasome (prosome, macropain) assembly chaperone 4
1446145_at	1.74	Eif4g3	eukaryotic translation initiation factor 4 gamma, 3
1426362_at	1.74	Tmem144	transmembrane protein 144 [a]
1443919_at	1.74	Prrt3	proline-rich transmembrane protein 3
1421052_a_at	1.73	Sms	spermine synthase
1422508_at	1.73	Atp6v1a	ATPase, H+ transporting, lysosomal V1 subunit A
1416748_a_at	1.73	Mre11a	meiotic recombination 11 homolog A (S. cerevisiae)
1460260_s_at	1.73	Kpna1	karyopherin (importin) alpha 1 [a]
1438769_a_at	1.73	Thyn1	thymocyte nuclear protein 1 [a]
1428874_at	1.73	Fam96b	family with sequence similarity 96, member B
1451290_at	1.73	Map1lc3a	microtubule-associated protein 1 light chain 3 alpha
1439447_x_at	1.73	Rpl37a	ribosomal protein L37a
1422650_a_at	1.73	Riok3	RIO kinase 3 (yeast)
1426600_at	1.72	Slc2a1	solute carrier family 2 (facilitated glucose transporter), member 1
1426591_at	1.72	Gfm2	G elongation factor, mitochondrial 2
1424538_at	1.72	Ubl4	ubiquitin-like 4
1449841_at	1.72	Kif3a	kinesin family member 3A
1416146_at	1.72	Hspa4	heat shock protein 4
1435926_at	1.72	Chml	choroideremia-like
1455210_at	1.71	Zhx2	zinc fingers and homeoboxes 2
1436573_at	1.71	Scrn3	secernin 3 [a]
1419029_at	1.71	Ero1l	ERO1-like (S. cerevisiae)
1456738_s_at	1.71	Brp16	brain protein 16
1450319_at	1.71	Gabrb2	gamma-aminobutyric acid (GABA) A receptor, subunit beta 2
1416014_at	1.71	Abce1	ATP-binding cassette, sub-family E (OABP), member 1
1434258_s_at	1.71	Phactr4	phosphatase and actin regulator 4
1438497_at	1.70	Mfsd8	major facilitator superfamily domain containing 8 [a]
1423453_at	1.70	Nol12	nucleolar protein 12
1458431_at	1.70	Eif4g2	eukaryotic translation initiation factor 4, gamma 2 [b]
1426806_at	1.70	Obfc2a	oligonucleotide/oligosaccharide-binding fold containing 2A [b]
1416171_at	1.70	2310037I24Rik	RIKEN cDNA 2310037I24 gene
1434059_at	1.70	B230312A22Rik	RIKEN cDNA B230312A22 gene
1422706_at	1.70	Pmepa1	prostate transmembrane protein, androgen induced 1
1417375_at	1.69	Tuba4a	tubulin, alpha 4A

1419999_at	1.69	Igbp1	immunoglobulin (CD79A) binding protein 1
1436797_a_at	1.69	Surf4	surfeit gene 4
1436202_at	1.69	Malat1	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA) [a]
1448480_at	1.69	Nip7	nuclear import 7 homolog (S. cerevisiae)
1456761_at	1.68	D630030B22Rik	RIKEN cDNA D630030B22 gene
1428522_at	1.68	Ttf2	transcription termination factor, RNA polymerase II
1439270_x_at	1.68	Ran	RAN, member RAS oncogene family
1432984_at	1.68	1700026H06Rik	RIKEN cDNA 1700026H06 gene
1446096_at	1.68	2310001H17Rik	RIKEN cDNA 2310001H17 gene
1451505_at	1.68	Chchd5	coiled-coil-helix-coiled-coil-helix domain containing 5
1440715_s_at	1.68	Cdkn2aipnl	CDKN2A interacting protein N-terminal like [a]
1448736_a_at	1.68	Hprt1	hypoxanthine guanine phosphoribosyl transferase 1
1456422_at	1.68	Dennd5b	DENN/MADD domain containing 5B
1449037_at	1.68	Crem	cAMP responsive element modulator
1437080_s_at	1.68	Psmd11	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11
1450745_at	1.68	C1galt1	core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1
1435372_a_at	1.68	Pa2g4	proliferation-associated 2G4
1419562_at	1.67	Birc6	baculoviral IAP repeat-containing 6
1418999_at	1.67	2310033P09Rik	RIKEN cDNA 2310033P09 gene
1424047_at	1.67	Dera	2-deoxyribose-5-phosphate aldolase homolog (C. elegans)
1429307_s_at	1.67	Lzic	leucine zipper and CTNNBIP1 domain containing
1416429_a_at	1.67	Cat	catalase
1419495_at	1.67	Immp2l	IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae)
1423358_at	1.67	Ece2	endothelin converting enzyme 2
1426266_s_at	1.67	Zbtb8os	zinc finger and BTB domain containing 8 opposite strand
1426931_s_at	1.67	D19Bwg1357e	DNA segment, Chr 19, Brigham & Women's Genetics 1357 expressed
1427190_at	1.66	Appl1	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1
1441018_at	1.66	Usp24	ubiquitin specific peptidase 24
1416396_at	1.66	Snx4	sorting nexin 4
1460583_at	1.66	Golt1b	golgi transport 1 homolog B (S. cerevisiae)
1420630_at	1.66	8430419L09Rik	RIKEN cDNA 8430419L09 gene
1425649_at	1.66	Slc39a14	solute carrier family 39 (zinc transporter), member 14
1422216_at	1.65	Mid2	midline 2
1454346_at	1.65	4930540E01Rik	RIKEN cDNA 4930540E01 gene
1429109_at	1.65	Msl2	male-specific lethal 2 homolog (Drosophila)
1436947_a_at	1.65	Txnl1	thioredoxin-like 1
1434016_at	1.65	Znrf2	zinc and ring finger 2

1418794_at	1.65	Cds2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2
1435803_a_at	1.65	Eif4e	eukaryotic translation initiation factor 4E
1417684_at	1.65	Thumpd3	THUMP domain containing 3
1438514_at	1.65	Npc1l1	NPC1-like 1
1427678_at	1.65	Zim3	zinc finger, imprinted 3
1437238_x_at	1.65	Nmd3	NMD3 homolog (S. cerevisiae)
1435057_x_at	1.65	Polr1e	polymerase (RNA) I polypeptide E
1438726_at	1.65	Mical2	microtubule associated monoxygenase, calponin and LIM domain containing 2
1438566_at	1.64	St8sia6	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 6
1452838_at	1.64	Ddx10	DEAD (Asp-Glu-Ala-Asp) box polypeptide 10
1430042_at	1.64	A930012M21Rik	RIKEN cDNA A930012M21 gene
1430725_at	1.64	4933432I09Rik	RIKEN cDNA 4933432I09 gene
1425534_at	1.64	Stau2	staufen (RNA binding protein) homolog 2 (Drosophila)
1456339_at	1.64	2810410D24Rik	RIKEN cDNA 2810410D24 gene
1422813_at	1.64	Cacng1	calcium channel, voltage-dependent, gamma subunit 1
1423552_at	1.64	Leprotl1	leptin receptor overlapping transcript-like 1
1460053_at	1.64	Smyd4	SET and MYND domain containing 4
1434115_at	1.63	Cdh13	cadherin 13
1416920_at	1.63	Rbm4	RNA binding motif protein 4
1415911_at	1.63	Impact	imprinted and ancient
1455462_at	1.63	Adcy2	adenylate cyclase 2
1459756_at	1.63	Cnot10	CCR4-NOT transcription complex, subunit 10
1441976_at	1.63	5430434G16Rik	RIKEN cDNA 5430434G16 gene
1448836_s_at	1.62	Gpn2	GPN-loop GTPase 2
1452012_a_at	1.62	Exosc1	exosome component 1
1418305_s_at	1.62	Gar1	GAR1 ribonucleoprotein homolog (yeast)
1428197_at	1.62	Tspan9	tetraspanin 9
1432321_at	1.62	Zcchc13	zinc finger, CCHC domain containing 13
1418616_at	1.62	Mafk	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein K (avian)
1419287_at	1.62	Tmem208	transmembrane protein 208
1454784_at	1.61	Hs3st2	heparan sulfate (glucosamine) 3-O-sulfotransferase 2
1433928_a_at	1.61	Rpl13a	ribosomal protein L13A
1438501_at	1.61	Gm2614	predicted gene 2614
1452594_at	1.61	Dusp11	dual specificity phosphatase 11 (RNA/RNP complex 1-interacting)
1427885_at	1.61	Pold4	polymerase (DNA-directed), delta 4
1434442_at	1.61	Stbd1	starch binding domain 1
1450729_at	1.61	Hs2st1	heparan sulfate 2-O-sulfotransferase 1

1443681_at	1.61	Fbll1	Fibrillarin-like 1
1424174_at	1.61	Shkbp1	Sh3kbp1 binding protein 1
1419253_at	1.61	Mthfd2	methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase
1452118_at	1.61	Rrp1b	ribosomal RNA processing 1 homolog B (S. cerevisiae)
1450375_at	1.60	Pspn	persephin
1458164_at	1.60	LOC552913	hypothetical LOC552913
1416925_at	1.60	Kpnb1	karyopherin (importin) beta 1
1423865_at	1.60	Slc44a1	solute carrier family 44, member 1
1437828_s_at	1.60	Wdr46	WD repeat domain 46
1433599_at	1.59	Baz1a	bromodomain adjacent to zinc finger domain 1A
1434524_at	1.59	Eif2b3	eukaryotic translation initiation factor 2B, subunit 3
1433766_at	1.59	C330023M02Rik	RIKEN cDNA C330023M02 gene
1423784_at	1.59	Gars	glycyl-tRNA synthetase
1434035_at	1.59	Dnajb6	DnaJ (Hsp40) homolog, subfamily B, member 6
1429087_at	1.59	1110054O05Rik	RIKEN cDNA 1110054O05 gene
1436849_x_at	1.59	Gaa	glucosidase, alpha, acid
1430158_at	1.58	3110021A11Rik	RIKEN cDNA 3110021A11 gene
1418388_s_at	1.58	Mphosph8	M-phase phosphoprotein 8 [a]
1416540_at	1.58	Hgs	HGF-regulated tyrosine kinase substrate
1458466_at	1.58	Ctsa	cathepsin A [a]
1456072_at	1.58	Ppp1r9a	protein phosphatase 1, regulatory (inhibitor) subunit 9A
1437225_x_at	1.58	Gnai3	guanine nucleotide binding protein (G protein), alpha inhibiting 3
1438596_at	1.57	1500017E21Rik	RIKEN cDNA 1500017E21 gene
1435055_a_at	1.57	Tom1	target of myb1 homolog (chicken)
1428390_at	1.57	Wdr43	WD repeat domain 43
1415903_at	1.57	Slc38a1	solute carrier family 38, member 1
1424257_at	1.57	Cdk7	cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase)
1435705_at	1.57	Zscan18	zinc finger and SCAN domain containing 18
1444436_at	1.56	Hsf2	heat shock factor 2
1423563_at	1.56	Prrt1	proline-rich transmembrane protein 1
1424753_at	1.56	Nudt14	nudix (nucleoside diphosphate linked moiety X)-type motif 14
1415735_at	1.56	Ddb1	damage specific DNA binding protein 1
1419035_s_at	1.56	Csnk2a1	casein kinase 2, alpha 1 polypeptide
1428835_at	1.56	Myh14	myosin, heavy polypeptide 14
1455887_at	1.56	Alg8	asparagine-linked glycosylation 8 homolog (yeast, alpha-1,3-glucosyltransferase)
1437303_at	1.56	Il6st	interleukin 6 signal transducer
1451095_at	1.56	Asns	asparagine synthetase

1435528_at	1.56	Dnlz	DNL-type zinc finger
1416328_a_at	1.56	Atp6v0e	ATPase, H+ transporting, lysosomal V0 subunit E
1441814_s_at	1.56	Rpain	RPA interacting protein
1415683_at	1.55	Nmt1	N-myristoyltransferase 1
1438312_s_at	1.55	Ltbp3	latent transforming growth factor beta binding protein 3
1434753_at	1.55	Nfrkb	nuclear factor related to kappa B binding protein
1428374_at	1.55	Glce	glucuronyl C5-epimerase
1416216_at	1.55	Reps1	RalBP1 associated Eps domain containing protein [a]
1427199_at	1.55	Fryl	furry homolog-like (Drosophila)
1434619_at	1.55	Rptor	regulatory associated protein of MTOR, complex 1
1428011_a_at	1.55	Erbb2ip	Erbb2 interacting protein
1426480_at	1.55	Sbds	Shwachman-Bodian-Diamond syndrome homolog (human)
1435160_at	1.55	Ahsa2	AHA1, activator of heat shock protein ATPase homolog 2 (yeast)
1416483_at	1.54	Ttc3	tetratricopeptide repeat domain 3
1456854_at	1.54	Neurl1a	neuralized homolog 1A (Drosophila)
1426749_at	1.54	Prmt3	protein arginine N-methyltransferase 3
1459857_at	1.54	Usp32	ubiquitin specific peptidase 32
1424788_at	1.54	Kri1	KRI1 homolog (S. cerevisiae)
1438226_at	1.54	AU022252	expressed sequence AU022252
1423465_at	1.54	Frrs1	ferric-chelate reductase 1
1419217_at	1.54	Sergef	secretion regulating guanine nucleotide exchange factor
1440356_at	1.54	Ciapin1	cytokine induced apoptosis inhibitor 1
1456482_at	1.54	Pik3r3	phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3 (p55)
1442252_at	1.54	Kif13a	kinesin family member 13A
1455904_at	1.54	Gas5	growth arrest specific 5
1456466_x_at	1.54	Atxn10	ataxin 10
1423274_at	1.53	Ints6	integrator complex subunit 6
1460357_at	1.53	Ythdf2	YTH domain family 2
1427077_a_at	1.53	Ap2b1	adaptor-related protein complex 2, beta 1 subunit
1459992_x_at	1.53	Cln8	ceroid-lipofuscinosis, neuronal 8
1458930_at	1.53	A4gnt	alpha-1,4-N-acetylglucosaminyltransferase
1424193_at	1.52	Pwp2	PWP2 periodic tryptophan protein homolog (yeast)
1455787_x_at	1.52	Minpp1	multiple inositol polyphosphate histidine phosphatase 1
1436828_a_at	1.52	Tpd52l2	tumor protein D52-like 2
1452209_at	1.52	Pkp4	plakophilin 4
1423808_at	1.52	Wrap53	WD repeat containing, antisense to TP53
1416426_at	1.52	Rab5a	RAB5A, member RAS oncogene family

1449072_a_at	1.52	N6amt2	N-6 adenine-specific DNA methyltransferase 2 (putative)
1438180_x_at	1.52	Hax1	HCLS1 associated X-1
1416861_at	1.52	Stam	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1
1437735_at	1.52	Ppp1r12a	protein phosphatase 1, regulatory (inhibitor) subunit 12A
1436897_at	1.52	Mfhas1	malignant fibrous histiocytoma amplified sequence 1
1448564_at	1.51	Cib1	calcium and integrin binding 1 (calmyrin)
1433548_at	1.51	Mare	alpha globin regulatory element containing gene
1423457_at	1.51	Slc35a5	solute carrier family 35, member A5
1448824_at	1.51	Ube2j1	ubiquitin-conjugating enzyme E2, J1 [a]
1438853_x_at	1.51	Ddx54	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54
1436360_at	1.51	Zscan22	zinc finger and SCAN domain containing 22
1429508_at	1.51	2310057M21Rik	RIKEN cDNA 2310057M21 gene
1452384_at	1.51	Enpp3	ectonucleotide pyrophosphatase/phosphodiesterase 3
1437394_at	1.51	Agap1	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
1453166_at	1.50	Ccdc109a	coiled-coil domain containing 109A
1415742_at	1.50	Aup1	ancient ubiquitous protein 1
1437465_a_at	1.50	P4hb	prolyl 4-hydroxylase, beta polypeptide
1424216_a_at	1.50	Papola	poly (A) polymerase alpha
1440799_s_at	1.50	Farp2	FERM, RhoGEF and pleckstrin domain protein 2
1448657_a_at	1.50	Dnajb2	DnaJ (Hsp40) homolog, subfamily B, member 2
1425546_a_at	5.88	Trf	transferrin
1458635_at	5.71	4832428D23Rik	RIKEN cDNA 4832428D23 gene
1444504_at	5.42	Dhrs7c	dehydrogenase/reductase (SDR family) member 7C
1425089_at	5.42	Kcnc4	potassium voltage gated channel, Shaw-related subfamily, member 4 [a]
1436644_x_at	5.27	Tmem25	transmembrane protein 25 [a]
1421979_at	5.03	Phex	phosphate regulating gene with homologies to endopeptidases on the X chromosome
1437273_at	4.95	Htra4	HtrA serine peptidase 4
1436986_at	4.89	Sntb2	syntrophin, basic 2
1417481_at	4.68	Ramp1	receptor (calcitonin) activity modifying protein 1
1439821_at	4.57	Lrp2bp	Lrp2 binding protein
1453904_at	4.52	Ccdc146	coiled-coil domain containing 146
1439491_at	4.41	Lrrc38	leucine rich repeat containing 38
1427919_at	4.34	Srpx2	sushi-repeat-containing protein, X-linked 2
1439426_x_at	4.33	Lyz1	lysozyme 1 [a]
1442251_at	4.19	Vcpip1	valosin containing protein (p97)/p47 complex interacting protein 1
1451715_at	4.18	Mafb	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian) [a]
1432107_at	4.18	2310010M20Rik	RIKEN cDNA 2310010M20 gene

1427329_a_at	4.12	Igh-6	immunoglobulin heavy chain 6 (heavy chain of IgM)
1435370_a_at	4.10	Ces3	carboxylesterase 3
1436359_at	4.10	Ret	ret proto-oncogene
1434911_s_at	4.07	Arhgap19	Rho GTPase activating protein 19
1416863_at	4.04	Abhd8	abhydrolase domain containing 8
1439311_at	3.97	B830012L14Rik	RIKEN cDNA B830012L14 gene
1427076_at	3.95	Mpeg1	macrophage expressed gene 1
1451305_at	3.92	Cby1	chibby homolog 1 (Drosophila)
1442739_at	3.92	BC031441	cDNA sequence BC031441
1440435_at	3.92	Ky	kyphoscoliosis peptidase
1434210_s_at	3.90	Lrig1	leucine-rich repeats and immunoglobulin-like domains 1[a]
1450243_a_at	3.81	Rcan2	regulator of calcineurin 2[a]
1419456_at	3.78	Dcxr	dicarbonyl L-xylulose reductase
1429012_at	3.77	Arhgef6	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6
1450048_a_at	3.76	Idh2	isocitrate dehydrogenase 2 (NADP+), mitochondrial
1448300_at	3.75	Mgst3	microsomal glutathione S-transferase 3
1418589_a_at	3.74	Mlf1	myeloid leukemia factor 1
1455224_at	3.68	Angptl1	angiopoietin-like 1
1438431_at	3.63	Abcd2	ATP-binding cassette, sub-family D (ALD), member 2
1453851_a_at	3.63	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma
1424393_s_at	3.49	Adhfe1	alcohol dehydrogenase, iron containing, 1[a]
1455220_at	3.45	Frat2	frequently rearranged in advanced T-cell lymphomas 2
1420346_at	3.42	Asb12	ankyrin repeat and SOCS box-containing 12
1416318_at	3.42	Serpinb1a	serine (or cysteine) peptidase inhibitor, clade B, member 1a[a]
1456397_at	3.38	Cdh4	cadherin 4[a]
1437915_at	3.36	Tom1l2	target of myb1-like 2 (chicken)
1438396_at	3.35	Ocrl	oculocerebrorenal syndrome of Lowe
1451989_a_at	3.35	Mapre2	microtubule-associated protein, RP/EB family, member 2
1451322_at	3.35	Cmbl	carboxymethylenebutenolidase-like (Pseudomonas)
1451500_at	3.34	Ushbp1	Usher syndrome 1C binding protein 1
1426981_at	3.29	Pcsk6	proprotein convertase subtilisin/kexin type 6
1428025_s_at	3.26	Pitpnc1	phosphatidylinositol transfer protein, cytoplasmic 1[b]
1429474_at	3.23	Ptgr2	prostaglandin reductase 2[a]
1421878_at	3.21	Mapk9	mitogen-activated protein kinase 9
1452766_at	3.17	Tppp	tubulin polymerization promoting protein
1452474_a_at	3.16	Art3	ADP-ribosyltransferase 3
1436763_a_at	3.16	Klf9	Kruppel-like factor 9[a]

1422605_at	3.14	Ppp1r1a	protein phosphatase 1, regulatory (inhibitor) subunit 1A
1439836_at	3.13	Asb15	ankyrin repeat and SOCS box-containing 15
1427053_at	3.11	Abi3bp	ABI gene family, member 3 (NESH) binding protein
1449547_at	3.09	Asb14	ankyrin repeat and SOCS box-containing 14
1449078_at	3.08	St3gal6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6
1422798_at	3.06	Cntnap2	contactin associated protein-like 2
1426208_x_at	3.06	Plagl1	pleiomorphic adenoma gene-like 1
1451991_at	3.00	Epha7	Eph receptor A7
1450839_at	2.97	D0H4S114	DNA segment, human D4S114 [a]
1425621_at	2.95	Trim35	tripartite motif-containing 35 [a]
1435917_at	2.93	Ociad2	OCIA domain containing 2 [a]
1451063_at	2.93	Stxbp4	syntaxin binding protein 4
1437874_s_at	2.90	Hexb	hexosaminidase B
1450699_at	2.87	Selenbp1	selenium binding protein 1 [a]
1424362_at	2.86	Ppapdc3	phosphatidic acid phosphatase type 2 domain containing 3
1441636_at	2.85	Sec14l5	SEC14-like 5 (S. cerevisiae)
1439087_a_at	2.85	Pik3ip1	phosphoinositide-3-kinase interacting protein 1 [a]
1454867_at	2.85	Mn1	meningioma 1
1457881_at	2.84	Osbpl6	oxysterol binding protein-like 6 [a]
1429144_at	2.84	Prei4	preimplantation protein 4 [a]
1422153_a_at	2.82	Asb11	ankyrin repeat and SOCS box-containing 11
1454840_at	2.82	Mccc2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta) [a]
1416023_at	2.82	Fabp3	fatty acid binding protein 3, muscle and heart
1428750_at	2.80	Cdc42ep2	CDC42 effector protein (Rho GTPase binding) 2
1434424_at	2.78	Mfsd7b	major facilitator superfamily domain containing 7B
1424737_at	2.78	Thrsp	thyroid hormone responsive SPOT14 homolog (Rattus) [a]
1426043_a_at	2.78	Capn3	calpain 3 [a]
1438030_at	2.77	Rasgrp3	RAS, guanyl releasing protein 3
1437250_at	2.77	Mreg	melanoregulin
1417644_at	2.77	Sspn	sarcospan
1431057_a_at	2.77	Prss23	protease, serine, 23 [b]
1456069_at	2.76	Dtna	dystrobrevin alpha [a]
1437939_s_at	2.75	Ctsc	cathepsin C [a]
1433827_at	2.75	Atp8a1	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1 [a]
1434572_at	2.75	Hdac9	histone deacetylase 9
1426340_at	2.74	Slc1a3	solute carrier family 1 (glial high affinity glutamate transporter), member 3
1439422_a_at	2.74	Fam132a	family with sequence similarity 132, member A [a]

1440884_s_at	2.74	A530047J11Rik	RIKEN cDNA A530047J11 gene
1418666_at	2.71	Ptx3	pentraxin related gene [a]
1450675_at	2.71	Smap2	stromal membrane-associated GTPase-activating protein 2
1450971_at	2.69	Gadd45b	growth arrest and DNA-damage-inducible 45 beta
1427982_s_at	2.69	Syne2	synaptic nuclear envelope 2
1432062_at	2.68	Mdga1	MAM domain containing glycosylphosphatidylinositol anchor 1
1417625_s_at	2.67	Cxcr7	chemokine (C-X-C motif) receptor 7
1454699_at	2.67	Sesn1	sestrin 1 [b]
1455235_x_at	2.67	Ldhb	lactate dehydrogenase B [c]
1418328_at	2.65	Chkb-cpt1b	choline kinase beta, carnitine palmitoyltransferase 1b, muscle transcription unit
1448561_at	2.64	Ncf2	neutrophil cytosolic factor 2
1435292_at	2.64	Tbc1d4	TBC1 domain family, member 4
1433907_at	2.64	Pknox2	Pbx/knotted 1 homeobox 2
1415864_at	2.64	Bpgm	2,3-bisphosphoglycerate mutase [b]
1416666_at	2.64	Serpine2	serine (or cysteine) peptidase inhibitor, clade E, member 2
1422324_a_at	2.63	Pthlh	parathyroid hormone-like peptide
1449834_at	2.62	Magix	MAGI family member, X-linked
1426319_at	2.62	Pdgfd	platelet-derived growth factor, D polypeptide
1435828_at	2.62	Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog [c]
1415871_at	2.62	Tgfbi	transforming growth factor, beta induced
1419652_s_at	2.61	Nkain1	Na+/K+ transporting ATPase interacting 1 [a]
1423547_at	2.60	Lyz2	lysozyme 2
1439096_at	2.58	Ddo	D-aspartate oxidase
1421466_at	2.58	Asb10	ankyrin repeat and SOCS box-containing 10
1427157_at	2.56	Ccdc85a	coiled-coil domain containing 85A
1416225_at	2.55	Adh1	alcohol dehydrogenase 1 (class I)
1436918_at	2.55	LOC100044376	similar to Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2
1429084_at	2.55	Vezf1	vascular endothelial zinc finger 1
1424553_at	2.55	Hhatl	hedgehog acyltransferase-like
1434079_s_at	2.54	Mcm2	minichromosome maintenance deficient 2 mitotin (S. cerevisiae) [a]
1436566_at	2.53	Rab40b	Rab40b, member RAS oncogene family
1424933_at	2.53	Myo5c	myosin VC
1417963_at	2.52	Pltp	phospholipid transfer protein
1427410_at	2.52	Dleu2	deleted in lymphocytic leukemia, 2
1438858_x_at	2.52	H2-Aa	histocompatibility 2, class II antigen A, alpha
1437030_at	2.51	Plcd4	phospholipase C, delta 4
1416203_at	2.51	Aqp1	aquaporin 1

1428145_at	2.51	Acaa2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase) [a]
1418181_at	2.50	Ptp4a3	protein tyrosine phosphatase 4a3
1417542_at	2.50	Rps6ka2	ribosomal protein S6 kinase, polypeptide 2
1423104_at	2.49	Irs1	insulin receptor substrate 1
1427098_at	2.48	Wwp1	WW domain containing E3 ubiquitin protein ligase 1 [a]
1416617_at	2.47	Acss1	acyl-CoA synthetase short-chain family member 1
1453578_at	2.47	Pter	phosphotriesterase related
1435745_at	2.47	5031439G07Rik	RIKEN cDNA 5031439G07 gene
1439143_at	2.46	A930018M24Rik	RIKEN cDNA A930018M24 gene
1434647_at	2.46	Egflam	EGF-like, fibronectin type III and laminin G domains
1444550_at	2.46	1110021J02Rik	RIKEN cDNA 1110021J02 gene
1424531_a_at	2.46	Tcea3	transcription elongation factor A (SII), 3
1453084_s_at	2.45	Col22a1	collagen, type XXII, alpha 1 [a]
1417042_at	2.45	Slc37a4	solute carrier family 37 (glucose-6-phosphate transporter), member 4
1438292_x_at	2.45	Adk	adenosine kinase [a]
1425394_at	2.43	BC023105	cDNA sequence BC023105
1434866_x_at	2.42	Cpt1a	carnitine palmitoyltransferase 1a, liver
1426850_a_at	2.42	Map2k6	mitogen-activated protein kinase kinase 6
1426450_at	2.42	Plcl2	phospholipase C-like 2
1448721_at	2.42	D1Ertd622e	DNA segment, Chr 1, ERATO Doi 622, expressed [a]
1448104_at	2.42	Aldh6a1	aldehyde dehydrogenase family 6, subfamily A1
1419279_at	2.40	Pip4k2a	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha
1417056_at	2.40	Psme1	proteasome (prosome, macropain) 28 subunit, alpha
1451313_a_at	2.39	1110067D22Rik	RIKEN cDNA 1110067D22 gene [a]
1424716_at	2.39	Retsat	retinol saturase (all trans retinol 13,14 reductase)
1423835_at	2.39	Zfp503	zinc finger protein 503
1416846_a_at	2.39	Pdzrn3	PDZ domain containing RING finger 3
1426950_at	2.39	Parp16	poly (ADP-ribose) polymerase family, member 16
1450883_a_at	2.38	Cd36	CD36 antigen [a]
1418509_at	2.38	Cbr2	carbonyl reductase 2
1417673_at	2.38	Grb14	growth factor receptor bound protein 14
1455031_at	2.37	Cdc2l6	cell division cycle 2-like 6 (CDK8-like)
1419140_at	2.35	Acvr2b	activin receptor IIB
1425164_a_at	2.35	Phkg1	phosphorylase kinase gamma 1 [a]
1438012_at	2.35	Ppm1l	protein phosphatase 1 (formerly 2C)-like [a]
1429071_at	2.34	Me3	malic enzyme 3, NADP(+)-dependent, mitochondrial
1422558_at	2.34	Gamt	guanidinoacetate methyltransferase

1426272_at	2.34	Lmbr1	limb region 1 [a]
1434354_at	2.34	Maob	monoamine oxidase B
1416452_at	2.34	Oat	ornithine aminotransferase
1419145_at	2.34	Smtnl1	smoothelin-like 1
1434454_at	2.33	Adcy9	adenylate cyclase 9 [c]
1419382_a_at	2.32	Dhrs4	dehydrogenase/reductase (SDR family) member 4 [a]
1419565_a_at	2.32	Zfx	zinc finger protein X-linked
1417680_at	2.32	Kcna5	potassium voltage-gated channel, shaker-related subfamily, member 5
1417590_at	2.32	Cyp27a1	cytochrome P450, family 27, subfamily a, polypeptide 1
1417061_at	2.32	Slc40a1	solute carrier family 40 (iron-regulated transporter), member 1
1438201_at	2.31	Pdp1	pyruvate dehyrogenase phosphatase catalytic subunit 1 [a]
1457424_at	2.31	Eya1	eyes absent 1 homolog (Drosophila)
1426345_at	2.30	Prepl	prolyl endopeptidase-like
1431936_a_at	2.30	Neu2	neuraminidase 2
1419292_at	2.30	Htra3	HtrA serine peptidase 3
1424807_at	2.30	Lama4	laminin, alpha 4
1427258_at	2.29	Trim24	tripartite motif-containing 24
1448530_at	2.29	Gmpr	guanosine monophosphate reductase
1416592_at	2.28	Glrx	glutaredoxin
1422414_a_at	2.28	Calm2	calmodulin 2 [a]
1425719_a_at	2.28	Nmi	N-myc (and STAT) interactor
1425519_a_at	2.28	Cd74	CD74 antigen
1449398_at	2.28	Rpl3l	ribosomal protein L3-like
1457999_at	2.28	Fam82b	family with sequence similarity 82, member B [a]
1418595_at	2.27	Plin4	perilipin 4
1433453_a_at	2.27	Abtb2	ankyrin repeat and BTB (POZ) domain containing 2
1437724_x_at	2.27	Pitpnm1	phosphatidylinositol transfer protein, membrane-associated 1
1423885_at	2.27	Lamc1	laminin, gamma 1
1437868_at	2.27	Fam46a	family with sequence similarity 46, member A
1441937_s_at	2.27	PTEN	PTEN [a]
1455007_s_at	2.26	Gpt2	glutamic pyruvate transaminase (alanine aminotransferase) 2 [a]
1426719_at	2.26	Apbb2	amyloid beta (A4) precursor protein-binding, family B, member 2
1425407_s_at	2.26	Clec4a2	C-type lectin domain family 4, member a2
1422852_at	2.26	Cib2	calcium and integrin binding family member 2
1451932_a_at	2.25	Adamtsl4	ADAMTS-like 4
1436440_at	2.25	Slc25a12	solute carrier family 25 (mitochondrial carrier, Aralar), member 12 [a]
1417283_at	2.25	Lynx1	Ly6/neurotoxin 1

1429598_at	2.25	2310042D19Rik	RIKEN cDNA 2310042D19 gene
1425179_at	2.25	Shmt1	serine hydroxymethyltransferase 1 (soluble)
1418888_a_at	2.24	Sepx1	selenoprotein X 1
1451707_s_at	2.24	Slc41a3	solute carrier family 41, member 3[b]
1417847_at	2.24	Ulk2	Unc-51 like kinase 2 (C. elegans)[a]
1437197_at	2.24	Sorbs2	sorbin and SH3 domain containing 2
1430738_at	2.24	Myoz3	myozenin 3
1454873_at	2.23	Zfp775	zinc finger protein 775
1437689_x_at	2.23	Clu	clusterin[b]
1424062_at	2.23	Ube2d1	ubiquitin-conjugating enzyme E2D 1, UBC4/5 homolog (yeast)
1435591_at	2.23	AI426330	expressed sequence AI426330
1416799_at	2.23	Trpm7	transient receptor potential cation channel, subfamily M, member 7[a]
1427556_at	2.22	Mylk2	myosin, light polypeptide kinase 2, skeletal muscle
1450414_at	2.21	Pdgfb	platelet derived growth factor, B polypeptide
1433508_at	2.21	Klf6	Kruppel-like factor 6[a]
1422927_at	2.21	Yipf7	Yip1 domain family, member 7
1417050_at	2.21	C1qtnf4	C1q and tumor necrosis factor related protein 4
1420664_s_at	2.20	Procr	protein C receptor, endothelial
1426852_x_at	2.20	Nov	nephroblastoma overexpressed gene
1456312_x_at	2.20	Gsn	gelsolin[b]
1455091_at	2.19	Ppp2r3a	protein phosphatase 2, regulatory subunit B'', alpha[a]
1453836_a_at	2.19	Mgll	monoglyceride lipase
1418370_at	2.19	Tnnc1	troponin C, cardiac/slow skeletal
1450044_at	2.19	Fzd7	frizzled homolog 7 (Drosophila)
1448382_at	2.19	Ehhadh	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase
1449335_at	2.19	Timp3	tissue inhibitor of metalloproteinase 3[a]
1416968_a_at	2.19	Hsd3b7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7
1436778_at	2.19	Cybb	cytochrome b-245, beta polypeptide
1448666_s_at	2.18	Tob2	transducer of ERBB2, 2[b]
1448839_at	2.18	Kank3	KN motif and ankyrin repeat domains 3[a]
1416321_s_at	2.18	Prelp	proline arginine-rich end leucine-rich repeat
1435272_at	2.18	Itpkb	inositol 1,4,5-trisphosphate 3-kinase B
1431335_a_at	2.18	Wfdc1	WAP four-disulfide core domain 1
1452469_a_at	2.18	Smtn	smoothelin
1457227_at	2.18	AI843755	expressed sequence AI843755
1448484_at	2.18	Amd1	S-adenosylmethionine decarboxylase 1
1456944_at	2.17	Kcnc1	potassium voltage gated channel, Shaw-related subfamily, member 1

1416412_at	2.17	Nsmaf	neutral sphingomyelinase (N-SMase) activation associated factor
1417701_at	2.17	Ppp1r14c	protein phosphatase 1, regulatory (inhibitor) subunit 14c
1416007_at	2.17	Satb1	special AT-rich sequence binding protein 1 [a]
1428140_at	2.17	Oxct1	3-oxoacid CoA transferase 1 [b]
1415994_at	2.17	Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1
1422919_at	2.16	Hrasls	HRAS-like suppressor [a]
1423422_at	2.15	Asb4	ankyrin repeat and SOCS box-containing 4
1455087_at	2.15	D7Ertd715e	DNA segment, Chr 7, ERATO Doi 715, expressed
1418525_at	2.15	Pcm1	pericentriolar material 1
1433873_s_at	2.15	Pcnt	pericentrin (kendrin)
1450429_at	2.14	Capn6	calpain 6
1416737_at	2.14	Gys1	glycogen synthase 1, muscle
1423447_at	2.14	Clpx	caseinolytic peptidase X (E.coli)
1434900_at	2.14	Mkl1	MKL (megakaryoblastic leukemia)/myocardin-like 1
1425156_at	2.14	Gbp6	guanylate binding protein 6 [a]
1436405_at	2.14	Dock4	dedicator of cytokinesis 4
1433842_at	2.14	Lrrfip1	leucine rich repeat (in FLII) interacting protein 1 [a]
1428444_at	2.14	Asb2	ankyrin repeat and SOCS box-containing 2
1455698_at	2.13	Sec62	SEC62 homolog (S. cerevisiae)
1417500_a_at	2.13	Tgm2	transglutaminase 2, C polypeptide [b]
1457568_at	2.13	Hnrnpd	heterogeneous nuclear ribonucleoprotein D
1425678_a_at	2.12	Snrk	SNF related kinase [a]
1437287_at	2.12	1110020G09Rik	RIKEN cDNA 1110020G09 gene
1454637_at	2.12	Klhl8	kelch-like 8 (Drosophila)
1433628_at	2.12	Coq10a	coenzyme Q10 homolog A (yeast)
1426857_a_at	2.12	Hsdl2	hydroxysteroid dehydrogenase like 2 [a]
1454758_a_at	2.12	Tsc	TSC domain family [a]
1433725_at	2.12	Acvr1b	activin A receptor, type 1B
1427043_s_at	2.12	Enox2	ecto-NOX disulfide-thiol exchanger 2
1448460_at	2.12	Acvr1	activin A receptor, type 1
1435929_at	2.11	9630033F20Rik	RIKEN cDNA 9630033F20 gene
1448636_at	2.11	Myoz1	myozenin 1
1434671_at	2.11	B230337E12Rik	RIKEN cDNA B230337E12 gene
1444232_at	2.11	Prkg1	protein kinase, cGMP-dependent, type I
1434592_at	2.11	Slc16a10	solute carrier family 16 (monocarboxylic acid transporters), member 10
1448569_at	2.10	Mlec	malectin
1437637_at	2.10	Phtf2	putative homeodomain transcription factor 2 [b]

1416424_at	2.10	Plin3	perilipin 3
1428535_at	2.10	9430020K01Rik	RIKEN cDNA 9430020K01 gene
1427567_a_at	2.10	Tpm3	tropomyosin 3, gamma
1430462_at	2.10	2310002L09Rik	RIKEN cDNA 2310002L09 gene
1423489_at	2.10	Mmd	monocyte to macrophage differentiation-associated
1429918_at	2.10	Arhgap20	Rho GTPase activating protein 20[a]
1431056_a_at	2.10	Lpl	lipoprotein lipase
1420619_a_at	2.10	Aes	amino-terminal enhancer of split
1456573_x_at	2.10	Nnt	nicotinamide nucleotide transhydrogenase[a]
1449405_at	2.10	Tns1	tensin 1[a]
1427213_at	2.10	Pfkfb1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1
1436501_at	2.10	Mtus1	mitochondrial tumor suppressor 1[a]
1439505_at	2.09	Clic5	chloride intracellular channel 5[b]
1441384_at	2.09	Gadl1	glutamate decarboxylase-like 1
1448609_at	2.09	Tst	thiosulfate sulfurtransferase, mitochondrial
1457676_at	2.09	Tirap	toll-interleukin 1 receptor (TIR) domain-containing adaptor protein
1415984_at	2.08	Acadm	acyl-Coenzyme A dehydrogenase, medium chain
1443315_at	2.08	Dmd	dystrophin, muscular dystrophy
1435343_at	2.08	Dock10	dedicator of cytokinesis 10
1417190_at	2.08	Nampt	nicotinamide phosphoribosyltransferase
1425419_a_at	2.08	Raf1	v-raf-leukemia viral oncogene 1[a]
1437041_at	2.08	Sfrs18	splicing factor, arginine/serine-rich 18
1436555_at	2.08	Slc7a2	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2[a]
1428467_at	2.07	Tardbp	TAR DNA binding protein
1455842_x_at	2.07	Usp15	ubiquitin specific peptidase 15
1422012_at	2.07	Crhr2	corticotropin releasing hormone receptor 2
1449576_at	2.07	Eif1ay	eukaryotic translation initiation factor 1A, Y-linked
1436189_at	2.07	Nqo2	NAD(P)H dehydrogenase, quinone 2
1416775_at	2.07	Atp5sl	ATP5S-like
1452889_at	2.07	2310007H09Rik	RIKEN cDNA 2310007H09 gene
1436185_at	2.07	AI314180	expressed sequence AI314180
1452942_at	2.06	Tmem65	transmembrane protein 65
1440635_at	2.06	Palld	palladin, cytoskeletal associated protein[b]
1422445_at	2.05	Itga6	integrin alpha 6
1460411_s_at	2.05	Pkdcc	protein kinase domain containing, cytoplasmic[a]
1451221_at	2.05	BC018507	cDNA sequence BC018507[a]
1443078_at	2.05	6030439D06Rik	RIKEN cDNA 6030439D06 gene

1451047_at	2.05	Itm2a	integral membrane protein 2A [a]
1430308_at	2.05	5730409N16Rik	RIKEN cDNA 5730409N16 gene
1417877_at	2.04	Eepd1	endonuclease/exonuclease/phosphatase family domain containing 1
1452789_at	2.04	Snn	stannin
1458268_s_at	2.04	Igfbp3	insulin-like growth factor binding protein 3
1434815_a_at	2.04	Mapkapk3	mitogen-activated protein kinase-activated protein kinase 3
1455544_at	2.04	Zranb3	zinc finger, RAN-binding domain containing 3
1420484_a_at	2.04	Vtn	vitronectin
1443932_at	2.04	Klhdc1	kelch domain containing 1
1439675_at	2.03	Ppara	peroxisome proliferator activated receptor alpha
1439256_x_at	2.03	Gpr137b-ps	G protein-coupled receptor 137B, pseudogene
1418561_at	2.03	Sf3b1	splicing factor 3b, subunit 1
1451683_x_at	2.03	H2-D1	histocompatibility 2, D region locus 1
1416513_at	2.03	Lamb2	laminin, beta 2
1453343_s_at	2.03	Vrk2	vaccinia related kinase 2
1422323_a_at	2.03	Lbx1	ladybird homeobox homolog 1 (Drosophila) [a]
1424194_at	2.02	Rcsd1	RCSD domain containing 1
1426482_at	2.02	Prkrir	protein-kinase, interferon-inducible double stranded RNA dependent inhibitor
1423063_at	2.02	Dnmt3a	DNA methyltransferase 3A [a]
1456483_at	2.02	Zfp9	zinc finger protein 9
1436689_a_at	2.02	Aldh9a1	aldehyde dehydrogenase 9, subfamily A1
1451212_at	2.02	Ccdc21	coiled-coil domain containing 21
1418951_at	2.01	Txlnb	taxilin beta [a]
1420859_at	2.01	Pkia	protein kinase inhibitor, alpha
1427100_at	2.01	Metrn	meteorin, glial cell differentiation regulator
1436913_at	2.00	Cdc14a	CDC14 cell division cycle 14 homolog A (S. cerevisiae)
1422624_at	2.00	Rev1	REV1 homolog (S. cerevisiae)
1451140_s_at	2.00	Prkag2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit
1418990_at	1.99	Ms4a4d	membrane-spanning 4-domains, subfamily A, member 4D
1449944_a_at	1.99	Sec61a2	Sec61, alpha subunit 2 (S. cerevisiae)
1428323_at	1.99	Gpd2	glycerol phosphate dehydrogenase 2, mitochondrial
1452331_s_at	1.99	Qser1	glutamine and serine rich 1
1421065_at	1.99	Jak2	Janus kinase 2
1420387_at	1.98	Mpv17	Mpv17 transgene, kidney disease mutant
1423994_at	1.98	Kif1b	kinesin family member 1B [b]
1422642_at	1.98	Cdc42ep3	CDC42 effector protein (Rho GTPase binding) 3 [a]
1429300_at	1.98	Ankrd9	ankyrin repeat domain 9

1444178_at	1.98	Gm9895	predicted gene 9895
1423600_a_at	1.98	Tcof1	Treacher Collins Franceschetti syndrome 1, homolog
1423282_at	1.96	Pitpna	phosphatidylinositol transfer protein, alpha
1422603_at	1.96	Rnase4	ribonuclease, RNase A family 4
1454834_at	1.96	Nfib	nuclear factor I/B [b]
1417841_at	1.96	Pxmp2	peroxisomal membrane protein 2
1451718_at	1.95	Plp1	proteolipid protein (myelin) 1
1436387_at	1.95	C330006P03Rik	RIKEN cDNA C330006P03 gene
1422011_s_at	1.95	Xlr	X-linked lymphocyte-regulated complex
1417960_at	1.94	Cpeb1	cytoplasmic polyadenylation element binding protein 1
1433695_at	1.94	Cnrip1	cannabinoid receptor interacting protein 1
1422807_at	1.94	Arf5	ADP-ribosylation factor 5
1451523_a_at	1.94	Mif4gd	MIF4G domain containing
1422501_s_at	1.94	Idh3a	isocitrate dehydrogenase 3 (NAD+) alpha [a]
1433868_at	1.94	Btbd3	BTB (POZ) domain containing 3
1429234_s_at	1.94	11-Sep	septin 11
1435464_at	1.93	1110003E01Rik	RIKEN cDNA 1110003E01 gene
1434100_x_at	1.93	PPAR-γ	peroxisome proliferative activated receptor-γ [b]
1429111_at	1.93	Tln2	talin 2 [a]
1457035_at	1.93	AI607873	expressed sequence AI607873
1428016_a_at	1.93	Rasip1	Ras interacting protein 1
1434133_s_at	1.93	Dcaf8	DDB1 and CUL4 associated factor 8
1448316_at	1.93	Cmtm3	CKLF-like MARVEL transmembrane domain containing 3
1435836_at	1.92	Pdk1	pyruvate dehydrogenase kinase, isoenzyme 1
1452767_at	1.92	Rrbp1	ribosome binding protein 1
1454951_at	1.92	Zfp606	zinc finger protein 606
1433639_at	1.92	Fam117a	family with sequence similarity 117, memberA
1457031_at	1.92	Fsd2	fibronectin type III and SPRY domain containing 2
1440007_at	1.92	D930003E18Rik	RIKEN cDNA D930003E18 gene
1451357_at	1.92	Mpnd	MPN domain containing
1422678_at	1.91	Dgat2	diacylglycerol O-acyltransferase 2
1456388_at	1.91	Atp11a	ATPase, class VI, type 11A
1435339_at	1.91	Kctd15	potassium channel tetramerisation domain containing 15
1419270_a_at	1.91	Dut	deoxyuridine triphosphatase
1458456_x_at	1.91	6430571L13Rik	RIKEN cDNA 6430571L13 gene [a]
1449106_at	1.90	Gpx3	glutathione peroxidase 3
1460591_at	1.90	Esr1	estrogen receptor 1 (alpha) [a]

1429083_at	1.90	Agl	amylo-1,6-glucosidase, 4-alpha-glucanotransferase
1417290_at	1.90	Lrg1	leucine-rich alpha-2-glycoprotein 1
1427014_at	1.90	Dennd4b	DENN/MADD domain containing 4B
1423785_at	1.90	Egln1	EGL nine homolog 1 (C. elegans)
1423305_at	1.90	Extl1	exostoses (multiple)-like 1
1434956_at	1.90	Rnf170	ring finger protein 170
1436714_at	1.90	Lpp	LIM domain containing preferred translocation partner in lipoma
1433623_at	1.90	Zfp367	zinc finger protein 367
1451229_at	1.89	Hdac11	histone deacetylase 11
1454801_at	1.89	Ankrd28	ankyrin repeat domain 28
1448825_at	1.89	Pdk2	pyruvate dehydrogenase kinase, isoenzyme 2
1427149_at	1.89	Plekha6	pleckstrin homology domain containing, family A member 6
1452841_at	1.89	Pgm2l1	phosphoglucomutase 2-like 1
1424130_a_at	1.89	Ptrf	polymerase I and transcript release factor
1435551_at	1.89	Fhod3	formin homology 2 domain containing 3
1425646_at	1.89	BC016495	cDNA sequence BC016495
1428691_at	1.88	Chd2	chromodomain helicase DNA binding protein 2
1433512_at	1.88	Fli1	Friend leukemia integration 1
1447977_x_at	1.88	3100002L24Rik	RIKEN cDNA 3100002L24 gene
1456909_at	1.88	LOC676974	similar to Glucose-6-phosphate isomerase (GPI)
1424367_a_at	1.88	Homer2	homer homolog 2 (Drosophila)
1452005_at	1.88	Dlat	dihydrolipoamide S-acetyltransferase
1417492_at	1.88	Ctsb	cathepsin B [a]
1426094_at	1.88	Rhbdl1	rhomboid, veinlet-like 1 (Drosophila)
1428097_at	1.88	2510009E07Rik	RIKEN cDNA 2510009E07 gene
1438951_x_at	1.88	Nup54	nucleoporin 54
1415949_at	1.88	Cpe	carboxypeptidase E
1422429_at	1.87	Rnf14	ring finger protein 14
1428224_at	1.87	Hnrpdl	heterogeneous nuclear ribonucleoprotein D-like
1456755_at	1.87	Trak1	trafficking protein, kinesin binding 1
1436944_x_at	1.87	Pisd	phosphatidylserine decarboxylase
1430522_a_at	1.87	Vamp5	vesicle-associated membrane protein 5
1453734_at	1.87	Atrx	alpha thalassemia/mental retardation syndrome X-linked homolog (human)
1455009_at	1.87	Cpd	carboxypeptidase D
1448376_at	1.87	Wrnip1	Werner helicase interacting protein 1
1456640_at	1.86	Sh3rf2	SH3 domain containing ring finger 2
1454984_at	1.86	Lifr	leukemia inhibitory factor receptor

1417567_at	1.86	Ctnnbip1	catenin beta interacting protein 1
1453657_at	1.86	2310065F04Rik	RIKEN cDNA 2310065F04 gene
1419554_at	1.86	Cd47	CD47 antigen (Rh-related antigen, integrin-associated signal transducer) [a]
1424649_a_at	1.86	Tspan8	tetraspanin 8
1428725_at	1.85	Pias2	protein inhibitor of activated STAT 2
1455372_at	1.85	Cpeb3	cytoplasmic polyadenylation element binding protein 3 [a]
1429042_at	1.85	Tmem167b	transmembrane protein 167B
1451801_at	1.85	Trdn	triadin
1448297_a_at	1.85	Tnk2	tyrosine kinase, non-receptor, 2
1427345_a_at	1.85	Sult1a1	sulfotransferase family 1A, phenol-preferring, member 1
1434449_at	1.85	Aqp4	aquaporin 4
1434611_at	1.85	Rnf123	ring finger protein 123
1451811_at	1.85	Cacng6	calcium channel, voltage-dependent, gamma subunit 6
1454795_at	1.85	Cobll1	Cobl-like 1
1427467_a_at	1.84	Rpgr	retinitis pigmentosa GTPase regulator
1415996_at	1.84	Txnip	thioredoxin interacting protein
1458087_at	1.84	Stac3	SH3 and cysteine rich domain 3
1425458_a_at	1.84	Grb10	growth factor receptor bound protein 10
1434766_at	1.84	Prkaa2	protein kinase, AMP-activated, alpha 2 catalytic subunit
1452341_at	1.84	Echs1	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial
1460004_x_at	1.84	Stx6	syntaxin 6
1428679_s_at	1.84	0610010K14Rik	RIKEN cDNA 0610010K14 gene
1452016_at	1.84	Alox5ap	arachidonate 5-lipoxygenase activating protein
1449964_a_at	1.84	Mlycd	malonyl-CoA decarboxylase
1416536_at	1.83	Mum1	melanoma associated antigen (mutated) 1
1428111_at	1.83	Slc38a4	solute carrier family 38, member 4
1460565_at	1.83	Slc41a1	solute carrier family 41, member 1
1427226_at	1.83	Epn2	epsin 2
1416727_a_at	1.83	Cyb5	cytochrome b-5
1426895_at	1.83	Zfp191	zinc finger protein 191
1425075_at	1.83	Gatad2b	GATA zinc finger domain containing 2B
1453406_a_at	1.83	Rab28	RAB28, member RAS oncogene family [a]
1419518_at	1.83	Tuba8	tubulin, alpha 8
1423456_at	1.83	Bzw2	basic leucine zipper and W2 domains 2
1419442_at	1.83	Matn2	matrilin 2
1417892_a_at	1.83	Sirt3	sirtuin 3 (silent mating type information regulation 2, homolog) 3 (S. cerevisiae)
1438022_at	1.83	Rab11fip3	RAB11 family interacting protein 3 (class II)

1460652_at	1.83	Esrra	estrogen related receptor, alpha
1415939_at	1.83	Fmod	fibromodulin
1451084_at	1.82	Etfdh	electron transferring flavoprotein, dehydrogenase
1439658_at	1.82	Lmod3	leiomodin 3 (fetal)
1452985_at	1.82	Uaca	uveal autoantigen with coiled-coil domains and ankyrin repeats
1449109_at	1.82	Socs2	suppressor of cytokine signaling 2 [a]
1454670_at	1.82	Rere	arginine glutamic acid dipeptide (RE) repeats
1426774_at	1.82	Parp12	poly (ADP-ribose) polymerase family, member 12
1429223_a_at	1.82	Hfe2	hemochromatosis type 2 (juvenile) (human homolog)
1416713_at	1.82	Tppp3	tubulin polymerization-promoting protein family member 3
1450376_at	1.82	Mxi1	Max interacting protein 1
1452346_at	1.81	B3gnt1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1
1426247_at	1.81	Stk24	serine/threonine kinase 24 (STE20 homolog, yeast)
1417044_at	1.81	Lcmt1	leucine carboxyl methyltransferase 1
1434002_at	1.81	Foxo	forkhead box
1417582_s_at	1.81	Dhodh	dihydroorotate dehydrogenase [a]
1422375_a_at	1.81	Art1	ADP-ribosyltransferase 1 [a]
1435445_at	1.80	Ccnt2	cyclin T2 [a]
1421899_a_at	1.80	Mr1	major histocompatibility complex, class I-related
1436012_s_at	1.80	Scrn2	secernin 2
1420909_at	1.80	Vegfa	vascular endothelial growth factor A
1457218_at	1.80	Strbp	spermatid perinuclear RNA binding protein
1449875_s_at	1.80	H2-T10	histocompatibility 2, T region locus 10
1434537_at	1.80	Slco3a1	solute carrier organic anion transporter family, member 3a1
1451782_a_at	1.80	Slc29a1	solute carrier family 29 (nucleoside transporters), member 1
1417962_s_at	1.80	Ghr	growth hormone receptor [a]
1434717_at	1.80	Cul3	cullin 3 [a]
1418187_at	1.80	Ramp2	receptor (calcitonin) activity modifying protein 2
1422752_at	1.79	Polr3k	polymerase (RNA) III (DNA directed) polypeptide K
1435177_a_at	1.79	Anapc5	anaphase-promoting complex subunit 5
1452615_s_at	1.79	Trpt1	tRNA phosphotransferase 1
1424669_at	1.79	Zfyve21	zinc finger, FYVE domain containing 21
1426822_at	1.79	Rhot2	ras homolog gene family, member T2
1435553_at	1.79	Pdzd2	PDZ domain containing 2
1428829_at	1.79	6820401H01Rik	RIKEN cDNA 6820401H01 gene
1437363_at	1.79	Homer1	homer homolog 1 (Drosophila) [a]
1418057_at	1.79	Tiam1	T-cell lymphoma invasion and metastasis 1

1427041_at	1.79	BC013712	cDNA sequence BC013712
1418341_at	1.78	Rab4a	RAB4A, member RAS oncogene family
1449406_at	1.78	Cyhr1	cysteine and histidine rich 1 [a]
1428519_at	1.78	2610528E23Rik	RIKEN cDNA 2610528E23 gene
1453592_at	1.78	Lrrc39	leucine rich repeat containing 39
1416454_s_at	1.78	Acta2	actin, alpha 2, smooth muscle, aorta
1452670_at	1.78	Myl9	myosin, light polypeptide 9, regulatory
1434758_at	1.78	Crispld2	cysteine-rich secretory protein LCCL domain containing 2
1457672_at	1.78	Chd9	chromodomain helicase DNA binding protein 9
1443992_at	1.78	Nipbl	Nipped-B homolog (Drosophila)
1460303_at	1.78	Nr3c1	nuclear receptor subfamily 3, group C, member 1
1416072_at	1.78	Cd34	CD34 antigen
1455071_at	1.77	Zbtb7b	zinc finger and BTB domain containing 7B
1433657_at	1.77	Fam78a	family with sequence similarity 78, member A
1438629_x_at	1.77	Grn	granulin
1452823_at	1.77	Gstk1	glutathione S-transferase kappa 1
1423645_a_at	1.77	Ddx5	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5
1454730_at	1.77	Tapt1	transmembrane anterior posterior transformation 1
1450409_a_at	1.77	Slc48a1	solute carrier family 48 (heme transporter), member 1
1428479_at	1.77	Nfatc1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
1426819_at	1.76	Hipk3	homeodomain interacting protein kinase 3
1448835_at	1.76	E2f6	E2F transcription factor 6
1417327_at	1.76	Cav2	caveolin 2
1452241_at	1.76	Topbp1	topoisomerase (DNA) II binding protein 1
1434977_at	1.76	4933403F05Rik	RIKEN cDNA 4933403F05 gene [a]
1426440_at	1.76	Dhrs7	dehydrogenase/reductase (SDR family) member 7
1436954_at	1.76	Wipf1	WAS/WASL interacting protein family, member 1
1415743_at	1.76	Hdac5	histone deacetylase 5
1448900_at	1.76	D16H22S680E	DNA segment, Chr 16, human D22S680E, expressed
1435081_at	1.76	Sypl	synaptophysin-like protein
1423789_at	1.76	BC005624	cDNA sequence BC005624
1433655_at	1.76	Rnf141	ring finger protein 141
1423364_a_at	1.75	Aktip	thymoma viral proto-oncogene 1 interacting protein
1435588_at	1.75	Wdfy1	WD repeat and FYVE domain containing 1
1426459_s_at	1.75	AW549877	expressed sequence AW549877
1420821_at	1.75	Sgpp1	sphingosine-1-phosphate phosphatase 1
1436729_at	1.75	Afap1	actin filament associated protein 1

1437302_at	1.75	Adrb2	adrenergic receptor, beta 2
1448897_at	1.75	Mkrn2	makorin, ring finger protein, 2
1425580_a_at	1.75	Pik3c3	phosphoinositide-3-kinase, class 3
1420950_at	1.74	Znrf1	zinc and ring finger 1
1415897_a_at	1.74	Mgst1	microsomal glutathione S-transferase 1
1438169_a_at	1.74	Frmd4b	FERM domain containing 4B
1453094_at	1.74	Foxn3	forkhead box N3
1448623_at	1.74	Tmem123	transmembrane protein 123
1424426_at	1.74	Mtap	methylthioadenosine phosphorylase
1425328_at	1.74	Fam76a	family with sequence similarity 76, member A
1420098_s_at	1.74	D13Ertd787e	DNA segment, Chr 13, ERATO Doi 787, expressed
1425537_at	1.74	Ppm1a	protein phosphatase 1A, magnesium dependent, alpha isoform
1458364_s_at	1.74	Tor1aip1	torsin A interacting protein 1
1421046_a_at	1.74	Pabpc4	poly(A) binding protein, cytoplasmic 4
1433591_at	1.74	Ppp3r1	protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)
1428630_x_at	1.74	Haghl	hydroxyacylglutathione hydrolase-like
1455700_at	1.74	Mterfd3	MTERF domain containing 3
1456838_at	1.74	Lingo3	leucine rich repeat and Ig domain containing 3
1449510_at	1.74	Zfp467	zinc finger protein 467
1438647_x_at	1.74	Cetn2	centrin 2
1441792_at	1.74	A630033E08Rik	RIKEN cDNA A630033E08 gene
1426124_a_at	1.74	Clk1	CDC-like kinase 1
1418763_at	1.74	Nit2	nitrilase family, member 2
1456413_at	1.73	Pde4dip	phosphodiesterase 4D interacting protein (myomegalin)
1438241_at	1.73	Rgma	RGM domain family, member A
1417001_a_at	1.73	D4Wsu53e	DNA segment, Chr 4, Wayne State University 53, expressed
1453552_at	1.73	2310014F07Rik	RIKEN cDNA 2310014F07 gene
1451931_x_at	1.73	H2-L	histocompatibility 2, D region
1456981_at	1.73	Tmc7	transmembrane channel-like gene family 7
1423852_at	1.73	Shisa2	shisa homolog 2 (Xenopus laevis)
1441352_at	1.73	9430034N14Rik	RIKEN cDNA 9430034N14 gene
1428295_at	1.73	Synpo2l	synaptopodin 2-like
1433545_s_at	1.73	Acad11	acyl-Coenzyme A dehydrogenase family, member 11
1451219_at	1.72	Ormdl1	ORM1-like 1 (S. cerevisiae)
1428326_s_at	1.72	Hrsp12	heat-responsive protein 12
1451050_at	1.72	Nt5c3	5'-nucleotidase, cytosolic III
1450394_at	1.71	Golph3	golgi phosphoprotein 3

1422982_at	1.71	Ar	androgen receptor
1427416_x_at	1.71	Dusp7	dual specificity phosphatase 7
1426773_at	1.71	Mfn1	mitofusin 1 [a]
1416108_a_at	1.71	Tmed3	transmembrane emp24 domain containing 3
1437150_at	1.71	Fam110b	family with sequence similarity 110, member B
1417265_s_at	1.71	Coq5	coenzyme Q5 homolog, methyltransferase (yeast)
1418483_a_at	1.71	Ggta1	glycoprotein galactosyltransferase alpha 1, 3
1417067_s_at	1.71	Cabc1	chaperone, ABC1 activity of bc1 complex like (S. pombe)
1449383_at	1.71	Adssl1	adenylosuccinate synthetase like 1
1450380_at	1.71	Epdr1	ependymin related protein 1 (zebrafish)
1418505_at	1.71	Nudt4	nudix (nucleoside diphosphate linked moiety X)-type motif 4
1435026_at	1.71	Spock2	sparc/osteonectin, cwcv and kazal-like domains proteoglycan 2
1448211_at	1.71	Atp6v0e2	ATPase, H+ transporting, lysosomal V0 subunit E2
1423293_at	1.71	Rpa1	replication protein A1
1419137_at	1.71	Shank3	SH3/ankyrin domain gene 3
1428151_x_at	1.70	Ccbl1	cysteine conjugate-beta lyase 1
1437498_at	1.70	Gm9971	predicted gene 9971
1423732_at	1.70	Tram1	translocating chain-associating membrane protein 1
1430526_a_at	1.70	Smarca2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, member 2
1429382_at	1.70	Tomm40l	translocase of outer mitochondrial membrane 40 homolog-like (yeast)
1429122_a_at	1.70	1700040I03Rik	RIKEN cDNA 1700040I03 gene
1434325_x_at	1.70	Prkar1b	protein kinase, cAMP dependent regulatory, type I beta
1434401_at	1.70	Zcchc2	zinc finger, CCHC domain containing 2
1435261_at	1.69	Tmtc1	transmembrane and tetratricopeptide repeat containing 1
1455680_at	1.69	9630025H16Rik	RIKEN cDNA 9630025H16 gene
1439189_at	1.69	Fnip2	folliculin interacting protein 2
1435754_at	1.69	0	zyg-ll homolog B (C. elegans)
1425753_a_at	1.69	Ung	uracil DNA glycosylase
1428385_at	1.69	08-Mar	membrane-associated ring finger (C3HC4) 8
1460210_at	1.69	Pkd1	polycystic kidney disease 1 homolog
1449145_a_at	1.69	Cav1	caveolin 1, caveolae protein
1448890_at	1.68	Klf2	Kruppel-like factor 2 (lung)
1435115_at	1.68	Fndc5	fibronectin type III domain containing 5
1424183_at	1.68	Acat1	acetyl-Coenzyme A acetyltransferase 1 [a]
1455777_x_at	1.68	Hsd17b4	hydroxysteroid (17-beta) dehydrogenase 4
1448131_at	1.68	Mfn2	mitofusin 2
1436389_at	1.68	Atl3	Atlastin GTPase 3

1416923_a_at	1.67	Bnip3l	BCL2/adenovirus E1B interacting protein 3-like
1436161_at	1.67	Pds5b	PDS5, regulator of cohesion maintenance, homolog B (S. cerevisiae) [a]
1426382_at	1.67	Ppm1b	protein phosphatase 1B, magnesium dependent, beta isoform
1450490_at	1.67	Kcna7	potassium voltage-gated channel, shaker-related subfamily, member 7
1454893_at	1.67	Fam189b	family with sequence similarity 189, member B
1422514_at	1.66	Aebp1	AE binding protein 1
1435097_at	1.66	Mmab	methylmalonic aciduria (cobalamin deficiency) type B homolog (human)
1453127_at	1.66	Ppm1j	protein phosphatase 1J
1453200_at	1.66	Rai1	retinoic acid induced 1
1454236_a_at	1.66	Fam118b	family with sequence similarity 118, member B
1434829_at	1.66	Cbl	Casitas B-lineage lymphoma
1416253_at	1.66	Cdkn2d	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)
1449818_at	1.66	Abcb4	ATP-binding cassette, sub-family B (MDR/TAP), member 4 [a]
1457696_at	1.66	Rilp	Rab interacting lysosomal protein
1456039_at	1.66	Snx14	sorting nexin 14
1449066_a_at	1.66	Arhgef7	Rho guanine nucleotide exchange factor (GEF7)
1436495_s_at	1.66	Zfp260	zinc finger protein 260
1418288_at	1.66	Lpin1	lipin 1
1423266_at	1.66	2810405K02Rik	RIKEN cDNA 2810405K02 gene
1423707_at	1.66	Tmem50b	transmembrane protein 50B
1419023_x_at	1.66	Eno1	enolase 1, alpha non-neuron
1448735_at	1.65	Cp	ceruloplasmin
1421923_at	1.65	Sh3bp5	SH3-domain binding protein 5 (BTK-associated)
1428508_at	1.65	Tbc1d2b	TBC1 domain family, member 2B
1454959_s_at	1.65	Gnai1	guanine nucleotide binding protein (G protein), alpha inhibiting 1
1427138_at	1.65	Ccdc88c	coiled-coil domain containing 88C
1415708_at	1.65	Tug1	taurine upregulated gene 1
1436569_at	1.65	Prex2	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2
1456735_x_at	1.65	Acpl2	acid phosphatase-like 2
1455404_at	1.65	Jph2	junctophilin 2
1416653_at	1.65	Stxbp3a	syntaxin binding protein 3A
1449108_at	1.65	Fdx1	ferredoxin 1
1430038_at	1.64	Gphn	gephyrin
1427050_at	1.64	Txndc16	thioredoxin domain containing 16
1418232_s_at	1.64	Lims1	LIM and senescent cell antigen-like domains 1
1431928_at	1.64	Ttn	titin
1429097_at	1.64	C030044C12Rik	RIKEN cDNA C030044C12 gene

1453113_at	1.64	Wdsub1	WD repeat, SAM and U-box domain containing 1
1416766_at	1.64	Mosc2	MOCO sulphurase C-terminal domain containing 2
1435436_at	1.64	Epas1	endothelial PAS domain protein 1
1453022_at	1.64	Gpihbp1	GPI-anchored HDL-binding protein 1
1433816_at	1.64	Mcart1	mitochondrial carrier triple repeat 1
1432136_s_at	1.64	Zdhhc4	zinc finger, DHHC domain containing 4
1450327_at	1.64	P2rx6	purinergic receptor P2X, ligand-gated ion channel, 6
1433581_at	1.64	1190002N15Rik	RIKEN cDNA 1190002N15 gene
1450706_a_at	1.64	Arl3	ADP-ribosylation factor-like 3
1437193_s_at	1.64	Snrpb	small nuclear ribonucleoprotein B [a]
1419922_s_at	1.64	Atrnl1	attractin like 1
1439234_a_at	1.64	Tm2d2	TM2 domain containing 2 [a]
1454976_at	1.64	Sod2	superoxide dismutase 2, mitochondrial
1451975_at	1.64	2810453I06Rik	RIKEN cDNA 2810453I06 gene
1422811_at	1.63	Slc27a1	solute carrier family 27 (fatty acid transporter), member 1
1416273_at	1.63	Tnfaip2	tumor necrosis factor, alpha-induced protein 2 [a]
1456888_at	1.63	Pfkfb4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
1454753_at	1.63	Rnpepl1	arginyl aminopeptidase (aminopeptidase B)-like 1
1416978_at	1.63	Fcgrt	Fc receptor, IgG, alpha chain transporter
1451474_a_at	1.63	Parp8	poly (ADP-ribose) polymerase family, member 8
1453003_at	1.63	Sorl1	sortilin-related receptor, LDLR class A repeats-containing [a]
1422566_at	1.63	Tcfeb	transcription factor EB
1437829_s_at	1.63	Eef2k	eukaryotic elongation factor-2 kinase
1438428_at	1.63	Jph1	junctophilin 1
1434720_at	1.63	Zfp652	zinc finger protein 652
1416858_a_at	1.63	Fkbp3	FK506 binding protein 3
1415958_at	1.63	Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4
1427981_a_at	1.62	Csad	cysteine sulfinic acid decarboxylase
1437992_x_at	1.62	Gja1	gap junction protein, alpha 1
1415814_at	1.62	Atp6v1b2	ATPase, H+ transporting, lysosomal V1 subunit B2
1460464_at	1.62	2700089E24Rik	RIKEN cDNA 2700089E24 gene
1424124_at	1.62	Mospd2	motile sperm domain containing 2
1435096_at	1.62	Ric8b	resistance to inhibitors of cholinesterase 8 homolog B (C. elegans)
1434205_at	1.62	Ppp2r5c	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
1454397_at	1.62	4632418H02Rik	RIKEN cDNA 4632418H02 gene
1451413_at	1.62	Cast	calpastatin
1438199_at	1.62	AI316807	expressed sequence AI316807

1418330_at	1.62	Ctcf	CCCTC-binding factor
1422492_at	1.62	Cpox	coproporphyrinogen oxidase
1417208_at	1.62	Amacr	alpha-methylacyl-CoA racemase
1417474_at	1.62	1500035H01Rik	RIKEN cDNA 1500035H01 gene
1435171_at	1.62	2810416G20Rik	RIKEN cDNA 2810416G20 gene
1450938_at	1.61	Pnn	pinin
1436181_at	1.61	Ddef2	development and differentiation enhancing factor 2
1448221_at	1.61	Bat1a	HLA-B-associated transcript 1A
1417480_at	1.61	Fbxo9	f-box protein 9
1426734_at	1.61	Fam43a	family with sequence similarity 43, member A
1432158_a_at	1.61	Trappc2	trafficking protein particle complex 2
1419499_at	1.61	Gpam	glycerol-3-phosphate acyltransferase, mitochondrial
1440353_at	1.61	Ntf5	neurotrophin 5
1439713_at	1.60	Itga1	integrin alpha 1
1427677_a_at	1.60	Sox6	SRY-box containing gene 6
1429173_at	1.60	Dnase1l1	deoxyribonuclease 1-like 1
1434738_at	1.60	Tarsl2	threonyl-tRNA synthetase-like 2
1449297_at	1.60	Casp12	caspase 12
1424632_a_at	1.60	Rev3l	REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like (S. cerevisiae)
1425262_at	1.60	Cebpg	CCAAT/enhancer binding protein (C/EBP), gamma
1424223_at	1.60	1700020C11Rik	RIKEN cDNA 1700020C11 gene
1428293_at	1.60	Bod1	biorientation of chromosomes in cell division 1
1434510_at	1.60	Papss2	3'-phosphoadenosine 5'-phosphosulfate synthase 2
1433834_at	1.60	06-Mar	membrane-associated ring finger (C3HC4) 6
1428474_at	1.60	Ppp3cb	protein phosphatase 3, catalytic subunit, beta isoform
1429690_at	1.60	1300003B13Rik	RIKEN cDNA 1300003B13 gene
1456611_at	1.60	Fam13a	family with sequence similarity 13, member A
1433649_at	1.59	Aof1	amine oxidase, flavin containing 1
1440397_at	1.59	Cacna2d1	calcium channel, voltage-dependent, alpha2/delta subunit 1 [a]
1417724_at	1.59	Thoc4	THO complex 4
1417827_at	1.59	Ngly1	N-glycanase 1
1450859_s_at	1.59	Gm15361	ubiquitin-conjugating enzyme E2D 3 pseudogene
1451190_a_at	1.59	Sbk1	SH3-binding kinase 1 [a]
1426668_at	1.59	Slc30a9	solute carrier family 30 (zinc transporter), member 9
1419259_at	1.59	Rsu1	Ras suppressor protein 1
1423097_s_at	1.59	Capn7	calpain 7
1456087_at	1.59	Nfia	nuclear factor I/A

1419703_at	1.59	Col5a3	collagen, type V, alpha 3
1419421_at	1.59	Ank1	ankyrin 1, erythroid
1460121_at	1.58	9630010G10Rik	RIKEN cDNA 9630010G10 gene
1435554_at	1.58	Tmcc3	transmembrane and coiled coil domains 3
1428432_at	1.58	Zcchc24	zinc finger, CCHC domain containing 24
1429213_at	1.58	2310030N02Rik	RIKEN cDNA 2310030N02 gene
1434487_at	1.58	Mef2d	myocyte enhancer factor 2D
1429146_at	1.58	Svip	small VCP/p97-interacting protein
1425920_at	1.58	Cuedc1	CUE domain containing 1
1460694_s_at	1.58	Svil	supervillin
1452202_at	1.58	Pde2a	phosphodiesterase 2A, cGMP-stimulated
1452281_at	1.58	Sos2	son of sevenless homolog 2 (Drosophila)
1448130_at	1.58	Fdft1	farnesyl diphosphate farnesyl transferase 1
1448753_at	1.58	Srp9	signal recognition particle 9
1418413_at	1.58	Cav3	caveolin 3
1426979_at	1.58	Mlxip	MLX interacting protein
1456041_at	1.58	Snx16	sorting nexin 16
1434515_at	1.58	Ncoa1	nuclear receptor coactivator 1
1449738_s_at	1.57	Fam48a	family with sequence similarity 48, member A
1448224_at	1.57	Tfam	transcription factor A, mitochondrial
1436841_at	1.57	B230380D07Rik	RIKEN cDNA B230380D07 gene [a]
1427070_at	1.57	Snx21	sorting nexin family member 21
1426449_a_at	1.57	Pja1	praja1, RING-H2 motif containing [a]
1425323_a_at	1.57	Fam173a	family with sequence similarity 173, member A
1450688_at	1.57	Rgl2	ral guanine nucleotide dissociation stimulator-like 2
1417789_at	1.57	Ccl11	chemokine (C-C motif) ligand 11
1447693_s_at	1.57	Neo1	neogenin
1434551_at	1.57	Hnrnpul2	heterogeneous nuclear ribonucleoprotein U-like 2
1429141_at	1.57	Neurl2	neuralized-like 2 (Drosophila)
1416703_at	1.57	Mapk14	mitogen-activated protein kinase 14
1427191_at	1.57	Npr2	natriuretic peptide receptor 2
1416530_a_at	1.57	Pnp1	purine-nucleoside phosphorylase 1
1450870_at	1.56	Rala	v-ral simian leukemia viral oncogene homolog A (ras related)
1437730_at	1.56	Ppp2r2a	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform
1433782_at	1.56	Cldn12	claudin 12
1460192_at	1.56	Osbpl1a	oxysterol binding protein-like 1A
1458126_at	1.56	D030041H20Rik	RIKEN cDNA D030041H20 gene

1452660_s_at	1.56	Klhl7	kelch-like 7 (Drosophila)
1452169_a_at	1.56	Dgkz	diacylglycerol kinase zeta
1450782_at	1.56	Wnt4	wingless-related MMTV integration site 4
1415779_s_at	1.56	Actg1	actin, gamma, cytoplasmic 1
1448513_a_at	1.56	Npc2	Niemann Pick type C2 [a]
1425129_a_at	1.56	Taldo1	transaldolase 1
1425149_a_at	1.56	Pdcl	phosducin-like
1436187_at	1.56	1110054M08Rik	RIKEN cDNA 1110054M08 gene
1425508_s_at	1.55	Arfrp1	ADP-ribosylation factor related protein 1
1460167_at	1.55	Aldh7a1	aldehyde dehydrogenase family 7, member A1
1422064_a_at	1.55	Zbtb20	zinc finger and BTB domain containing 20
1422478_a_at	1.55	Acss2	acyl-CoA synthetase short-chain family member 2
1454925_x_at	1.55	Mdh1	malate dehydrogenase 1, NAD (soluble)
1417158_at	1.55	Zxdc	ZXD family zinc finger C
1433486_at	1.55	Clcn3	chloride channel 3
1424790_at	1.55	Slc25a42	solute carrier family 25, member 42
1416275_at	1.55	Slc26a6	solute carrier family 26, member 6
1418372_at	1.55	Adsl	adenylosuccinate lyase
1427407_s_at	1.55	Trip11	thyroid hormone receptor interactor 11
1416690_at	1.55	Gtpbp2	GTP binding protein 2
1452769_at	1.55	Rnf145	ring finger protein 145
1428651_at	1.55	Klhl24	kelch-like 24 (Drosophila)
1426581_at	1.55	Ptpmt1	protein tyrosine phosphatase, mitochondrial 1
1424033_at	1.54	Sfrs7	splicing factor, arginine/serine-rich 7
1449686_s_at	1.54	Scp2	sterol carrier protein 2, liver
1454919_at	1.54	Nmt2	N-myristoyltransferase 2
1448429_at	1.54	Gyg	glycogenin
1448664_a_at	1.54	Speg	SPEG complex locus
1455014_at	1.54	Hint3	histidine triad nucleotide binding protein 3
1426453_at	1.54	Pitrm1	pitrilysin metallepetidase 1
1438035_at	1.54	Fam82a1	family with sequence similarity 82, member A1
1434093_at	1.53	Lysmd4	LysM, putative peptidoglycan-binding, domain containing 4
1434107_at	1.53	Spata2	spermatogenesis associated 2
1451264_at	1.53	Frmd6	FERM domain containing 6
1435086_s_at	1.53	Klhdc2	kelch domain containing 2
1419401_at	1.53	Asb13	ankyrin repeat and SOCS box-containing 13
1460194_at	1.53	Phyh	phytanoyl-CoA hydroxylase

1426759_at	1.53	Map4k3	mitogen-activated protein kinase kinase kinase kinase 3
1435547_at	1.53	Mkl2	MKL/myocardin-like 2
1419821_s_at	1.53	Idh1	isocitrate dehydrogenase 1 (NADP+), soluble
1456625_at	1.53	Aasdhppt	aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase
1437552_at	1.53	2410127L17Rik	RIKEN cDNA 2410127L17 gene
1448373_at	1.53	Mrpl18	mitochondrial ribosomal protein L18
1421863_at	1.52	Vamp1	vesicle-associated membrane protein 1
1423908_at	1.52	Ndufs8	NADH dehydrogenase (ubiquinone) Fe-S protein 8
1449038_at	1.52	Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1
1439549_at	1.52	Prrg3	proline rich Gla (G-carboxyglutamic acid) 3 (transmembrane)
1423246_at	1.52	Erp44	endoplasmic reticulum protein 44
1460434_at	1.52	Fundc2	FUN14 domain containing 2
1428516_a_at	1.52	Alkbh7	alkB, alkylation repair homolog 7 (E. coli)
1436531_at	1.52	Metap2	methionine aminopeptidase 2
1424748_at	1.52	Galnt11	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11
1460548_a_at	1.52	Eral1	Era (G-protein)-like 1 (E. coli)
1420967_at	1.52	Slc25a15	solute carrier family 25 (mitochondrial carrier ornithine transporter), member 15
1450034_at	1.52	Stat1	signal transducer and activator of transcription 1
1451176_at	1.52	Mavs	mitochondrial antiviral signaling protein
1418886_s_at	1.52	Idh3b	isocitrate dehydrogenase 3 (NAD+) beta
1417565_at	1.51	Abhd5	abhydrolase domain containing 5
1427177_at	1.51	Fyco1	FYVE and coiled-coil domain containing 1
1452353_at	1.51	Gpr155	G protein-coupled receptor 155
1418210_at	1.51	Pfn2	profilin 2
1439389_s_at	1.51	Myadm	myeloid-associated differentiation marker
1423883_at	1.51	Acsl1	acyl-CoA synthetase long-chain family member 1
1424433_at	1.51	Msrb2	methionine sulfoxide reductase B2
1453766_a_at	1.51	Efcab6	EF-hand calcium binding domain 6
1424166_at	1.51	Msh3	mutS homolog 3 (E. coli)
1428755_at	1.51	Creb1	cAMP responsive element binding protein 1
1438164_x_at	1.51	Flot2	flotillin 2
1415961_at	1.51	Itm2c	integral membrane protein 2C
1418234_s_at	1.51	Bcas2	breast carcinoma amplified sequence 2
1434348_at	1.51	Fez2	fasciculation and elongation protein zeta 2 (zygin II)
1430786_at	1.50	1110002E22Rik	RIKEN cDNA 1110002E22 gene
1455610_at	1.50	Synm	synemin, intermediate filament protein
1455101_at	1.50	Phactr2	phosphatase and actin regulator 2

1452661_at	1.50	Tfrc	transferrin receptor
1421147_at	1.50	Terf2	telomeric repeat binding factor 2
1423200_at	1.50	Ncor1	nuclear receptor co-repressor 1
1417124_at	1.50	Dstn	destrin
1431885_a_at	1.50	Mus81	MUS81 endonuclease homolog (yeast)
1433830_at	1.50	Hnrnpa2b1	heterogeneous nuclear ribonucleoprotein A2/B1
1434390_at	1.50	LOC100270747	hypothetical LOC100270747

A total 1521 gene identified from 1907 Affymetrix probe sets after a 1.5 fold change cut off. 683 genes were overexpressed, and 838 genes were underexpressed (grey box).[a] This gene appears two times; [b] This gene appears three times; [c] This gene appears four times; [d] This gene appears five times. M, Mstn-pro mice; CLM, clenbuterol treated Mstn-pro mice.

Appendix 3.9 The expression of overlapping genes between the effects of CL (W vs CLW VS M vs CLM).

Probe Set ID	Gene Symbol	Gene Title
1422906_at	Abcg2	ATP-binding cassette, sub-family G (WHITE), member 2
1428803_at	Acot6	acyl-CoA thioesterase 6
1451257_at	Acsl6	acyl-CoA synthetase long-chain family member 6
1426403_at	Actr1b	ARP1 actin-related protein 1 homolog B, centractin beta (yeast)
1455462_at	Adcy2	adenylate cyclase 2
1434080_at	Aebp2	AE binding protein 2
1435432_at	Agap1	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
1451287_s_at	Aif1l	allograft inflammatory factor 1-like
1416911_a_at	Akirin1	akirin 1
1452478_at	Alpk2	alpha-kinase 2
1422573_at	Ampd3	adenosine monophosphate deaminase 3
1420992_at	Ankrd1	ankyrin repeat domain 1 (cardiac muscle)
1416065_a_at	Ankrd10	ankyrin repeat domain 10
1448660_at	Arhgdig	Rho GDP dissociation inhibitor (GDI) gamma
1427646_a_at	Arhgef2	rho/rac guanine nucleotide exchange factor (GEF) 2
1422608_at	Arpp19	cAMP-regulated phosphoprotein 19
1435444_at	Atf6	activating transcription factor 6
1422929_s_at	Atoh7	atonal homolog 7 (Drosophila)
1442145_at	Atp13a3	ATPase type 13A3
1437688_x_at	Atp6ap2	ATPase, H+ transporting, lysosomal accessory protein 2
1422508_at	Atp6v1a	ATPase, H+ transporting, lysosomal V1 subunit A
1456466_x_at	Atxn10	ataxin 10
1426329_s_at	Baalc	brain and acute leukemia, cytoplasmic
1433599_at	Baz1a	bromodomain adjacent to zinc finger domain 1A
1455316_x_at	BC094435	cDNA sequence BC094435
1455771_at	Bzrap1	benzodiazapine receptor associated protein 1
1422813_at	Cacng1	calcium channel, voltage-dependent, gamma subunit 1
1433972_at	Camta1	calmodulin binding transcription activator 1
1449839_at	Casp3	caspase 3
1424517_at	Ccdc12	coiled-coil domain containing 12
1437669_x_at	Ccrl1	Chemokine (C-C motif) receptor-like 1
1437502_x_at	Cd24a	CD24a antigen
1416401_at	Cd82	CD82 antigen
1434115_at	Cdh13	cadherin 13

1451741_a_at	Cdk7	cyclin-dependent kinase 7
1424638_at	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)
1418794_at	Cds2	CDP-diacylglycerol synthase 2
1424245_at	Ces2	carboxylesterase 2
1424529_s_at	Cgref1	cell growth regulator with EF hand domain 1
1418852_at	Chrna1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)
1430086_at	Chrna9	cholinergic receptor, nicotinic, alpha polypeptide 9
1420682_at	Chrnb1	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
1457296_at	Cilp	cartilage intermediate layer protein
1435775_at	Clock	circadian locomoter output cycles kaput
1456953_at	Col19a1	collagen, type XIX, alpha 1
1455627_at	Col8a1	collagen, type VIII, alpha 1
1428635_at	Comtd1	catechol-O-methyltransferase domain containing 1
1424831_at	Cpne2	copine II
1449037_at	Crem	cAMP responsive element modulator
1427910_at	Cst6	cystatin E/M
1417453_at	Cul4b	cullin 4B
1415803_at	Cx3cl1	chemokine (C-X3-C motif) ligand 1
1429618_at	Cyld	cylindromatosis (turban tumor syndrome)
1416039_x_at	Cyr61	cysteine rich protein 61
1451453_at	Dapk2	death-associated protein kinase 2
1415735_at	Ddb1	damage specific DNA binding protein 1
1417103_at	Ddt	D-dopachrome tautomerase
1448271_a_at	Ddx21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21
1424047_at	Dera	2-deoxyribose-5-phosphate aldolase homolog (C. elegans)
1423082_at	Derl1	Der1-like domain family, member 1
1418937_at	Dio2	deiodinase, iodothyronine, type II
1426465_at	Dlgap4	discs, large homolog-associated protein 4 (Drosophila)
1434035_at	Dnajb6	DnaJ (Hsp40) homolog, subfamily B, member 6
1450198_at	Dusp13	dual specificity phosphatase 13
1428834_at	Dusp4	dual specificity phosphatase 4
1431281_at	Dysfip1	dysferlin interacting protein 1
1433555_at	Eaf1	ELL associated factor 1
1423358_at	Ece2	endothelin converting enzyme 2
1440085_at	Eda2r	ectodysplasin A2 isoform receptor
1418648_at	Egln3	EGL nine homolog 3 (C. elegans)
1417235_at	Ehd3	EH-domain containing 3

1449852_a_at	Ehd4	EH-domain containing 4
1424343_a_at	Eif1a	eukaryotic translation initiation factor 1A
1434985_a_at	Eif4a1	eukaryotic translation initiation factor 4A1
1435803_a_at	Eif4e2	eukaryotic translation initiation factor 4E member 2
1455994_x_at	Elovl1	elongation of very long chain fatty acids-like 1
1428739_at	Enho	energy homeostasis associated
1460392_a_at	Eny2	enhancer of yellow 2 homolog (Drosophila)
1430724_at	Ephx3	epoxide hydrolase 3
1428011_a_at	Erbb2ip	Erbb2 interacting protein
1419029_at	Ero1l	ERO1-like (S. cerevisiae)
1422567_at	Fam129a	family with sequence similarity 129, member A
1436761_s_at	Fam13c	family with sequence similarity 13, member C
1421746_a_at	Fbxo17	F-box protein 17
1454674_at	Fez1	fasciculation and elongation protein zeta 1 (zygin I)
1418497_at	Fgf13	fibroblast growth factor 13
1452799_at	Fggy	FGGY carbohydrate kinase domain containing
1419184_a_at	Fhl2	four and a half LIM domains 2
1422516_a_at	Fibp	fibroblast growth factor (acidic) intracellular binding protein
1423465_at	Frrs1	ferric-chelate reductase 1
1418364_a_at	Ftl1	ferritin light chain 1
1419428_a_at	Gaa	glucosidase, alpha, acid
1449519_at	Gadd45a	growth arrest and DNA-damage-inducible 45 alpha
1448744_at	Galns	galactosamine (N-acetyl)-6-sulfate sulfatase
1452232_at	Galnt7	UDP-N-acetyl-alpha-D-galactosamine
1434754_at	Garnl4	GTPase activating RANGAP domain-like 4
1423784_at	Gars	glycyl-tRNA synthetase
1419139_at	Gdf5	growth differentiation factor 5
1458245_at	Gm12528	predicted gene 12528
1455271_at	Gm13889	predicted gene 13889
1425238_at	Gm6607	40S ribosomal protein S20 pseudogene
1448571_a_at	Gmfb	glia maturation factor, beta
1421947_at	Gng12	guanine nucleotide binding protein (G protein), gamma 12
1433656_a_at	Gnl3	guanine nucleotide binding protein-like 3 (nucleolar)
1460583_at	Golt1b	golgi transport 1 homolog B (S. cerevisiae)
1451411_at	Gprc5b	G protein-coupled receptor, family C, group 5, member B
1416708_a_at	Gramd1a	GRAM domain containing 1A
1435402_at	Gramd1b	GRAM domain containing 1B

1418186_at	Gstt1	glutathione S-transferase, theta 1
1426187_a_at	Hax1	HCLS1 associated X-1
1454693_at	Hdac4	histone deacetylase 4
1430062_at	Hhipl1	hedgehog interacting protein-like 1
1438988_x_at	Hn1	hematological and neurological expressed sequence 1
1448736_a_at	Hprt1	hypoxanthine guanine phosphoribosyl transferase 1
1450047_at	Hs6st2	heparan sulfate 6-O-sulfotransferase 2
1428640_at	Hsf2bp	heat shock transcription factor 2 binding protein
1416146_at	Hspa4	heat shock protein 4
1425964_x_at	Hspb1	heat shock protein 1
1434927_at	Hspb7	heat shock protein family, member 7 (cardiovascular)
1417013_at	Hspb8	heat shock protein 8
1451814_a_at	Htatip2	HIV-1 tat interactive protein 2, homolog (human)
1435176_a_at	Id2	inhibitor of DNA binding 2
1415911_at	Impact	imprinted and ancient
1423239_at	Impdh1	inosine 5'-phosphate dehydrogenase 1
1445568_at	Inca1	Inhibitor of CDK, cyclin A1 interacting protein 1
1448694_at	Jun	Jun oncogene
1426873_s_at	Jup	junction plakoglobin
1421852_at	Kcnk5	potassium channel, subfamily K, member 5
1441071_at	Kcnq5	potassium voltage-gated channel, subfamily Q, member 5
1449841_at	Kif3a	kinesin family member 3A
1418429_at	Kif5b	kinesin family member 5B
1417005_at	Klc1	kinesin light chain 1
1460260_s_at	Kpna1	karyopherin (importin) alpha 1
1416925_at	Kpnb1	karyopherin (importin) beta 1
1448169_at	Krt18	keratin 18
1423691_x_at	Krt8	keratin 8
1424378_at	Ldlrap1	low density lipoprotein receptor adaptor protein 1
1423552_at	Leprotl1	leptin receptor overlapping transcript-like 1
1426808_at	Lgals3	lectin, galactose binding, soluble 3
1426577_a_at	Lin37	lin-37 homolog (C. elegans)
1423719_at	LOC632073	lung and nasal epithelium carcinoma associated 1 isoform 2
1452836_at	Lpin2	lipin 2
1437109_s_at	Lsm6	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae)
1429307_s_at	Lzic	leucine zipper and CTNNBIP1 domain containing
1428847_a_at	Macf1	microtubule-actin crosslinking factor 1

1436202_at	Malat1	metastasis associated lung adenocarcinoma transcript
1451290_at	Map1lc3a	microtubule-associated protein 1 light chain 3 alpha
1424719_a_at	Mapt	microtubule-associated protein tau
1433548_at	Mare	alpha globin regulatory element containing gene
1423605_a_at	Mdm2	transformed mouse 3T3 cell double minute 2
1427186_a_at	Mef2a	myocyte enhancer factor 2A
1418387_at	Mphosph8	M-phase phosphoprotein 8
1416748_a_at	Mre11a	meiotic recombination 11 homolog A (S. cerevisiae)
1419254_at	Mthfd2	methylenetetrahydrofolate dehydrogenase (NAD+ dependent)
1450511_at	Musk	muscle, skeletal, receptor tyrosine kinase
1456586_x_at	Mvp	major vault protein
1419150_at	Myf6	myogenic factor 6
1454731_at	Myo10	myosin X
1419391_at	Myog	myogenin
1439708_at	Myom3	myomesin family, member 3
1416192_at	Napa	N-ethylmaleimide sensitive fusion protein attachment protein alpha
1435981_at	Nav2	neuron navigator 2
1439556_at	Ncam1	neural cell adhesion molecule 1
1423596_at	Nek6	NIMA (never in mitosis gene a)-related expressed kinase 6
1416606_s_at	Nhp2	NHP2 ribonucleoprotein homolog (yeast)
1448480_at	Nip7	nuclear import 7 homolog (S. cerevisiae)
1415683_at	Nmt1	N-myristoyltransferase 1
1423453_at	Nol12	nucleolar protein 12
1444786_at	Nol3	nucleolar protein 3 (apoptosis repressor with CARD domain)
1435184_at	Npr3	natriuretic peptide receptor 3
1434709_at	Nrcam	neuron-glia-CAM-related cell adhesion molecule
1423850_at	Nsun2	NOL1/NOP2/Sun domain family member 2
1426806_at	Obfc2a	oligonucleotide/oligosaccharide-binding fold containing 2A
1427364_a_at	Odc1	ornithine decarboxylase, structural 1
1420438_at	Orm2	orosomucoid 2
1441055_at	Palm2	paralemmin 2
1416379_at	Panx1	pannexin 1
1424216_a_at	Papola	poly (A) polymerase alpha
1444451_at	Pappa2	pappalysin 2
1451269_at	Pdzd11	PDZ domain containing 11
1425134_a_at	Pigx	phosphatidylinositol glycan anchor biosynthesis, class X
1425543_s_at	Plekha5	pleckstrin homology domain containing, family A member 5

1435053_s_at	Plekhh1	pleckstrin homology domain containing, family H member 1
1417128_at	Plekho1	pleckstrin homology domain containing, family O member 1
1427885_at	Pold4	polymerase (DNA-directed), delta 4
1419058_at	Polr1e	polymerase (RNA) I polypeptide E
1450686_at	Pon2	paraoxonase 2
1423810_at	Ppme1	protein phosphatase methylesterase 1
1455342_at	Prune2	prune homolog 2 (Drosophila)
1415676_a_at	Psmb5	proteasome (prosome, macropain) subunit, beta type 5
1429370_a_at	Psmd11	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11
1434891_at	Ptgfrn	prostaglandin F2 receptor negative regulator
1421499_a_at	Ptpn14	protein tyrosine phosphatase, non-receptor type 14
1424886_at	Ptprd	protein tyrosine phosphatase, receptor type, D
1451160_s_at	Pvr	poliovirus receptor
1420832_at	Qsox1	quiescin Q6 sulfhydryl oxidase 1
1416426_at	Rab5a	RAB5A, member RAS oncogene family
1417850_at	Rb1	retinoblastoma 1
1438001_x_at	Reep5	receptor accessory protein 5
1448975_s_at	Ren1	renin 1 structural
1440822_x_at	Reps1	RalBP1 associated Eps domain containing protein
1427375_at	Rg9mtd2	RNA (guanine-9-) methyltransferase domain containing 2
1453129_a_at	Rgs12	regulator of G-protein signaling 12
1435394_s_at	Rhoc	ras homolog gene family, member C
1424507_at	Rin1	Ras and Rab interactor 1
1422650_a_at	Riok3	RIO kinase 3 (yeast)
1418318_at	Rnf128	ring finger protein 128
1436305_at	Rnf217	ring finger protein 217
1433928_a_at	Rpl13a	ribosomal protein L13A
1449323_a_at	Rpl3	ribosomal protein L3
1451068_s_at	Rps25	ribosomal protein S25
1422562_at	Rrad	Ras-related associated with diabetes
1418448_at	Rras	Harvey rat sarcoma oncogene, subgroup R
1448689_at	Rras2	related RAS viral (r-ras) oncogene homolog 2
1437224_at	Rtn4	reticulon 4
1455605_at	Rufy3	RUN and FYVE domain containing 3
1440878_at	Runx1	runt related transcription factor 1
1425389_a_at	Runx2	runt related transcription factor 2
1451809_s_at	Rwdd3	RWD domain containing 3

1419394_s_at	S100a8	S100 calcium binding protein A8 (calgranulin A)
1434402_at	Samd8	sterile alpha motif domain containing 8
1458813_at	Scn5a	sodium channel, voltage-gated, type V, alpha
1456291_x_at	Scx	scleraxis
1448793_a_at	Sdc4	syndecan 4
1419819_s_at	Sec63	SEC63-like (S. cerevisiae)
1434382_at	Serinc2	serine incorporator 2
1433571_at	Serinc5	serine incorporator 5
1448715_x_at	Sgip1	SH3-domain GRB2-like (endophilin) interacting protein 1
1436499_at	Sgms1	sphingomyelin synthase 1
1449084_s_at	Sh3d19	SH3 domain protein D19
1434153_at	Shb	src homology 2 domain-containing transforming protein B
1422629_s_at	Shroom3	shroom family member 3
1451227_a_at	Slc10a3	solute carrier family 10, member 3
1420444_at	Slc22a3	solute carrier family 22 (organic cation transporter), member 3
1426600_at	Slc2a1	solute carrier family 2 (facilitated glucose transporter), member 1
1426722_at	Slc38a2	solute carrier family 38, member 2
1427035_at	Slc39a14	solute carrier family 39 (zinc transporter), member 14
1423865_at	Slc44a1	solute carrier family 44, member 1
1420884_at	Sln	sarcolipin
1421052_a_at	Sms	spermine synthase
1438176_x_at	Snap47	synaptosomal-associated protein, 47
1453125_at	Sox11	SRY-box containing gene 11
1420150_at	Spsb1	splA/ryanodine receptor domain and SOCS box containing 1
1451680_at	Srxn1	sulfiredoxin 1 homolog (S. cerevisiae)
1416861_at	Stam	signal transducing adaptor molecule 1
1416974_at	Stam2	signal transducing adaptor molecule 2
1425534_at	Stau2	staufen (RNA binding protein) homolog 2 (Drosophila)
1434442_at	Stbd1	starch binding domain 1
1453228_at	Stx11	syntaxin 11
1436797_a_at	Surf4	surfeit gene 4
1455493_at	Syne1	synaptic nuclear envelope 1
1419446_at	Tbc1d1	TBC1 domain family, member 1
1426337_a_at	Tead4	TEA domain family member 4
1420895_at	Tgfbr1	transforming growth factor, beta receptor I
1422286_a_at	Tgif1	TGFB-induced factor homeobox 1
1450377_at	Thbs1	thrombospondin 1

1417684_at	Thumpd3	THUMP domain containing 3
1438769_a_at	Thyn1	thymocyte nuclear protein 1
1423186_at	Tiam2	T-cell lymphoma invasion and metastasis 2
1436042_at	Tln1	talin 1
1442827_at	Tlr4	Toll-like receptor 4
1449533_at	Tmem100	transmembrane protein 100
1424354_at	Tmem140	transmembrane protein 140
1425780_a_at	Tmem167	transmembrane protein 167
1419287_at	Tmem208	transmembrane protein 208
1455350_at	Tmem62	transmembrane protein 62
1418572_x_at	Tnfrsf12a	tumor necrosis factor receptor superfamily, member 12a
1426095_a_at	Tnfrsf22	tumor necrosis factor receptor superfamily, member 22
1422101_at	Tnfrsf23	tumor necrosis factor receptor superfamily, member 23
1436828_a_at	Tpd52l2	tumor protein D52-like 2
1451233_at	Traf2	TNF receptor-associated factor 2
1424489_a_at	Trit1	tRNA isopentenyltransferase 1
1418158_at	Trp63	transformation related protein 63
1456251_x_at	Tspo	translocator protein
1455643_s_at	Tsr1	TSR1, 20S rRNA accumulation, homolog (yeast)
1455649_at	Ttc9	tetratricopeptide repeat domain 9
1423846_x_at	Tuba1b	tubulin, alpha 1B
1417375_at	Tuba4a	tubulin, alpha 4A
1427347_s_at	Tubb2a	tubulin, beta 2A
1423642_at	Tubb2c	tubulin, beta 2C
1416431_at	Tubb6	tubulin, beta 6
1434585_at	Tulp4	Tubby like protein 4
1416119_at	Txn1	thioredoxin 1
1423299_at	Txnl1	thioredoxin-like 1
1448824_at	Ube2j1	ubiquitin-conjugating enzyme E2, J1
1448968_at	Ubfd1	ubiquitin family domain containing 1
1424538_at	Ubl4	ubiquitin-like 4
1426486_at	Ubxn4	UBX domain protein 4
1421269_at	Ugcg	UDP-glucose ceramide glucosyltransferase
1452011_a_at	Uxs1	UDP-glucuronate decarboxylase 1
1416231_at	Vac14	Vac14 homolog (S. cerevisiae)
1437828_s_at	Wdr46	WD repeat domain 46
1434241_at	Wdr67	WD repeat domain 67

1423923_a_at	Wdr8	WD repeat domain 8
1417817_a_at	Wwtr1	WW domain containing transcription regulator 1
1454901_at	Ypel2	yippee-like 2 (Drosophila)
1426266_s_at	Zbtb8os	zinc finger and BTB domain containing 8 opposite strand
1452719_at	Zdhhc24	zinc finger, DHHC domain containing 24
1415940_at	Zfand2a	zinc finger, AN1-type domain 2A
1421479_at	Zfp318	zinc finger protein 318
1426471_at	Zfp52	zinc finger protein 52
1440999_at	Zfp697	zinc finger protein 697
1436026_at	Zfp703	zinc finger protein 703
1455210_at	Zhx2	zinc fingers and homeoboxes 2
1435705_at	Zscan18	zinc finger and SCAN domain containing 18
1436710_at	Zswim4	zinc finger, SWIM domain containing 4
1423724_at	Zwint	ZW10 interactor
1449507_a_at	Cd47	CD47 antigen
1423247_at	Erp44	endoplasmic reticulum protein 44
1424749_at	Wdfy1	WD repeat and FYVE domain containing 1
1429108_at	Msl2	male-specific lethal 2 homolog (Drosophila)
1452868_at	Usp24	ubiquitin specific peptidase 24
1449818_at	Abcb4	ATP-binding cassette, sub-family B (MDR/TAP), member 4
1438431_at	Abcd2	ATP-binding cassette, sub-family D (ALD), member 2
1428145_at	Acaa2	acetyl-Coenzyme A acyltransferase 2
1415984_at	Acadm	acyl-Coenzyme A dehydrogenase, medium chain
1424182_at	Acat1	acetyl-Coenzyme A acetyltransferase 1
1450643_s_at	Acsl1	acyl-CoA synthetase long-chain family member 1
1416617_at	Acss1	acyl-CoA synthetase short-chain family member 1
1422478_a_at	Acss2	acyl-CoA synthetase short-chain family member 2
1433725_at	Acvr1b	activin A receptor, type 1B
1419140_at	Acvr2b	activin receptor IIB
1451932_a_at	Adamtsl4	ADAMTS-like 4
1434454_at	Adcy9	adenylate cyclase 9
1424393_s_at	Adhfe1	alcohol dehydrogenase, iron containing, 1
1438292_x_at	Adk	adenosine kinase
1422514_at	Aebp1	AE binding protein 1
1429083_at	Agl	amylo-1,6-glucosidase, 4-alpha-glucanotransferase
1435591_at	AI426330	expressed sequence AI426330
1448104_at	Aldh6a1	aldehyde dehydrogenase family 6, subfamily A1

1448484_at	Amd1	S-adenosylmethionine decarboxylase 1
1454801_at	Ankrd28	ankyrin repeat domain 28
1426719_at	Apbb2	amyloid beta (A4) precursor protein-binding, family B, member 2
1416203_at	Aqp1	aquaporin 1
1429918_at	Arhgap20	Rho GTPase activating protein 20
1429012_at	Arhgef6	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6
1422375_a_at	Art1	ADP-ribosyltransferase 1
1452474_a_at	Art3	ADP-ribosyltransferase 3
1421466_at	Asb10	ankyrin repeat and SOCS box-containing 10
1422153_a_at	Asb11	ankyrin repeat and SOCS box-containing 11
1420346_at	Asb12	ankyrin repeat and SOCS box-containing 12
1449547_at	Asb14	ankyrin repeat and SOCS box-containing 14
1428444_at	Asb2	ankyrin repeat and SOCS box-containing 2
1423422_at	Asb4	ankyrin repeat and SOCS box-containing 4
1416775_at	Atp5sl	ATP5S-like
1433827_at	Atp8a1	ATPase, aminophospholipid transporter, class I, type 8A, member 1
1417067_s_at	Cabc1	chaperone, ABC1 activity of bc1 complex like (S. pombe)
1426043_a_at	Capn3	calpain 3
1453904_at	Ccdc146	coiled-coil domain containing 146
1452332_at	Ccdc85a	coiled-coil domain containing 85A
1450883_a_at	Cd36	CD36 antigen
1436913_at	Cdc14a	CDC14 cell division cycle 14 homolog A (S. cerevisiae)
1455031_at	Cdc2l6	cell division cycle 2-like 6 (CDK8-like)
1422642_at	Cdc42ep3	CDC42 effector protein (Rho GTPase binding) 3
1449422_at	Cdh4	cadherin 4
1435370_a_at	Ces3	carboxylesterase 3
1452991_at	Chd2	chromodomain helicase DNA binding protein 2
1418328_at	Chkb-cpt1b	choline kinase beta, carnitine palmitoyltransferase 1b
1422852_at	Cib2	calcium and integrin binding family member 2
1433486_at	Clcn3	chloride channel 3
1439505_at	Clic5	chloride intracellular channel 5
1437689_x_at	Clu	clusterin
1451322_at	Cmbl	carboxymethylenebutenolidase-like (Pseudomonas)
1422798_at	Cntnap2	contactin associated protein-like 2
1453084_s_at	Col22a1	collagen, type XXII, alpha 1
1433628_at	Coq10a	coenzyme Q10 homolog A (yeast)
1417264_at	Coq5	coenzyme Q5 homolog, methyltransferase (yeast)

1415949_at	Cpe	carboxypeptidase E
1456048_at	Cpeb3	cytoplasmic polyadenylation element binding protein 3
1422492_at	Cpox	coproporphyrinogen oxidase
1437939_s_at	Ctsc	cathepsin C
1425920_at	Cuedc1	CUE domain containing 1
1434717_at	Cul3	cullin 3
1417590_at	Cyp27a1	cytochrome P450, family 27, subfamily a, polypeptide 1
1434133_s_at	Dcaf8	DDB1 and CUL4 associated factor 8
1439096_at	Ddo	D-aspartate oxidase
1422677_at	Dgat2	diacylglycerol O-acyltransferase 2
1444504_at	Dhrs7c	dehydrogenase/reductase (SDR family) member 7C
1452005_at	Dlat	dihydrolipoamide S-acetyltransferase
1460324_at	Dnmt3a	DNA methyltransferase 3A
1456069_at	Dtna	dystrobrevin alpha
1427416_x_at	Dusp7	dual specificity phosphatase 7
1417877_at	Eepd1	endonuclease/exonuclease/phosphatase family domain containing 1
1434647_at	Egflam	EGF-like, fibronectin type III and laminin G domains
1423785_at	Egln1	EGL nine homolog 1 (C. elegans)
1449576_at	Eif1ay	eukaryotic translation initiation factor 1A, Y-linked
1460591_at	Esr1	estrogen receptor 1 (alpha)
1460652_at	Esrra	estrogen related receptor, alpha
1416023_at	Fabp3	fatty acid binding protein 3, muscle and heart
1417393_a_at	Fam132a	family with sequence similarity 132, member A
1456611_at	Fam13a	family with sequence similarity 13, member A
1433657_at	Fam78a	family with sequence similarity 78, member A
1457999_at	Fam82b	family with sequence similarity 82, member B
1417480_at	Fbxo9	f-box protein 9
1448130_at	Fdft1	farnesyl diphosphate farnesyl transferase 1
1449108_at	Fdx1	ferredoxin 1
1435551_at	Fhod3	formin homology 2 domain containing 3
1416858_a_at	Fkbp3	FK506 binding protein 3
1433512_at	Fli1	Friend leukemia integration 1
1453135_at	Fndc5	fibronectin type III domain containing 5
1434002_at	Foxn3	forkhead box N3
1455220_at	Frat2	frequently rearranged in advanced T-cell lymphomas 2
1450044_at	Fzd7	frizzled homolog 7 (Drosophila)
1450971_at	Gadd45b	growth arrest and DNA-damage-inducible 45 beta

1453851_a_at	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma
1437474_at	Gatad2b	GATA zinc finger domain containing 2B
1434380_at	Gbp6	guanylate binding protein 6
1437992_x_at	Gja1	gap junction protein, alpha 1
1444178_at	Gm9895	predicted gene 9895
1448530_at	Gmpr	guanosine monophosphate reductase
1453022_at	Gpihbp1	GPI-anchored HDL-binding protein 1
1417673_at	Grb14	growth factor receptor bound protein 14
1416737_at	Gys1	glycogen synthase 1, muscle
1436756_x_at	Hadh	hydroxyacyl-Coenzyme A dehydrogenase
1415743_at	Hdac5	histone deacetylase 5
1424553_at	Hhatl	hedgehog acyltransferase-like
1457568_at	Hnrnpd	heterogeneous nuclear ribonucleoprotein D
1434551_at	Hnrnpul2	heterogeneous nuclear ribonucleoprotein U-like 2
1424367_a_at	Homer2	homer homolog 2 (Drosophila)
1422919_at	Hrasls	HRAS-like suppressor
1455777_x_at	Hsd17b4	hydroxysteroid (17-beta) dehydrogenase 4
1426857_a_at	Hsdl2	hydroxysteroid dehydrogenase like 2
1437273_at	Htra4	HtrA serine peptidase 4
1450048_a_at	Idh2	isocitrate dehydrogenase 2 (NADP+), mitochondrial
1422501_s_at	Idh3a	isocitrate dehydrogenase 3 (NAD+) alpha
1418886_s_at	Idh3b	isocitrate dehydrogenase 3 (NAD+) beta
1458268_s_at	Igfbp3	insulin-like growth factor binding protein 3
1427329_a_at	Igh-6	immunoglobulin heavy chain 6 (heavy chain of IgM)
1423104_at	Irs1	insulin receptor substrate 1
1422445_at	Itga6	integrin alpha 6
1421066_at	Jak2	Janus kinase 2
1456629_at	Kank3	KN motif and ankyrin repeat domains 3
1417680_at	Kcna5	potassium voltage-gated channel, shaker-related subfamily, member 5
1450490_at	Kcna7	potassium voltage-gated channel, shaker-related subfamily, member 7
1425089_at	Kcnc4	potassium voltage gated channel, Shaw-related subfamily, member 4
1423994_at	Kif1b	kinesin family member 1B
1436763_a_at	Klf9	Kruppel-like factor 9
1443932_at	Klhdc1	kelch domain containing 1
1428651_at	Klhl24	kelch-like 24 (Drosophila)
1454637_at	Klhl8	kelch-like 8 (Drosophila)
1440435_at	Ky	kyphoscoliosis peptidase

1416513_at	Lamb2	laminin, beta 2
1422323_a_at	Lbx1	ladybird homeobox homolog 1 (Drosophila)
1455235_x_at	Ldhb	lactate dehydrogenase B
1454984_at	Lifr	leukemia inhibitory factor receptor
1456909_at	LOC676974	similar to Glucose-6-phosphate isomerase (GPI)
1418288_at	Lpin1	lipin 1
1431056_a_at	Lpl	lipoprotein lipase
1434210_s_at	Lrig1	leucine-rich repeats and immunoglobulin-like domains 1
1439821_at	Lrp2bp	Lrp2 binding protein
1439491_at	Lrrc38	leucine rich repeat containing 38
1445841_at	Lrrc39	leucine rich repeat containing 39
1417283_at	Lynx1	Ly6/neurotoxin 1
1435828_at	Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42
1451716_at	Mafb	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B
1449834_at	Magix	MAGI family member, X-linked
1434354_at	Maob	monoamine oxidase B
1426850_a_at	Map2k6	mitogen-activated protein kinase kinase 6
1421878_at	Mapk9	mitogen-activated protein kinase 9
1434815_a_at	Mapkapk3	mitogen-activated protein kinase-activated protein kinase 3
1454840_at	Mccc2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)
1432062_at	Mdga1	MAM domain containing glycosylphosphatidylinositol anchor 1
1429071_at	Me3	malic enzyme 3, NADP(+)-dependent, mitochondrial
1427100_at	Metrn	meteorin, glial cell differentiation regulator
1415897_a_at	Mgst1	microsomal glutathione S-transferase 1
1448300_at	Mgst3	microsomal glutathione S-transferase 3
1451523_a_at	Mif4gd	MIF4G domain containing
1418589_a_at	Mlf1	myeloid leukemia factor 1
1449964_a_at	Mlycd	malonyl-CoA decarboxylase
1435645_at	Mmd	monocyte to macrophage differentiation-associated
1454867_at	Mn1	meningioma 1
1451357_at	Mpnd	MPN domain containing
1437250_at	Mreg	melanoregulin
1424166_at	Msh3	mutS homolog 3 (E. coli)
1450376_at	Mxi1	Max interacting protein 1
1427556_at	Mylk2	myosin, light polypeptide kinase 2, skeletal muscle
1448636_at	Myoz1	myozenin 1
1417190_at	Nampt	nicotinamide phosphoribosyltransferase

1423202_a_at	Ncor1	nuclear receptor co-repressor 1
1456087_at	Nfia	nuclear factor I/A
1448288_at	Nfib	nuclear factor I/B
1419652_s_at	Nkain1	Na+/K+ transporting ATPase interacting 1
1456573_x_at	Nnt	nicotinamide nucleotide transhydrogenase
1427191_at	Npr2	natriuretic peptide receptor 2
1436189_at	Nqo2	NAD(P)H dehydrogenase, quinone 2
1435917_at	Ociad2	OCIA domain containing 2
1457881_at	Osbpl6	oxysterol binding protein-like 6
1428140_at	Oxct1	3-oxoacid CoA transferase 1
1421046_a_at	Pabpc4	poly(A) binding protein, cytoplasmic 4
1440635_at	Palld	palladin, cytoskeletal associated protein
1433873_s_at	Pcnt	pericentrin (kendrin)
1435836_at	Pdk1	pyruvate dehydrogenase kinase, isoenzyme 1
1448825_at	Pdk2	pyruvate dehydrogenase kinase, isoenzyme 2
1438201_at	Pdp1	pyruvate dehyrogenase phosphatase catalytic subunit 1
1435553_at	Pdzd2	PDZ domain containing 2
1416846_a_at	Pdzrn3	PDZ domain containing RING finger 3
1427213_at	Pfkfb1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1
1452841_at	Pgm2l1	phosphoglucomutase 2-like 1
1425164_a_at	Phkg1	phosphorylase kinase gamma 1
1437637_at	Phtf2	putative homeodomain transcription factor 2
1439087_a_at	Pik3ip1	phosphoinositide-3-kinase interacting protein 1
1451148_at	Pink1	PTEN induced putative kinase 1
1428025_s_at	Pitpnc1	phosphatidylinositol transfer protein, cytoplasmic 1
1460411_s_at	Pkdcc	protein kinase domain containing, cytoplasmic
1420859_at	Pkia	protein kinase inhibitor, alpha
1433907_at	Pknox2	Pbx/knotted 1 homeobox 2
1437030_at	Plcd4	phospholipase C, delta 4
1426450_at	Plcl2	phospholipase C-like 2
1416424_at	Plin3	perilipin 3
1418595_at	Plin4	perilipin 4
1451718_at	Plp1	proteolipid protein (myelin) 1
1424362_at	Ppapdc3	phosphatidic acid phosphatase type 2 domain containing 3
1457721_at	Ppara	peroxisome proliferator activated receptor alpha
1434100_x_at	Ppargc1a	peroxisome proliferative activated receptor, gamma
1425537_at	Ppm1a	protein phosphatase 1A, magnesium dependent, alpha isoform

1451669_at	Ppm1b	protein phosphatase 1B, magnesium dependent, beta isoform
1438012_at	Ppm1l	protein phosphatase 1 (formerly 2C)-like
1422605_at	Ppp1r1a	protein phosphatase 1, regulatory (inhibitor) subunit 1A
1455091_at	Ppp2r3a	protein phosphatase 2, regulatory subunit B", alpha
1429144_at	Prei4	preimplantation protein 4
1426345_at	Prepl	prolyl endopeptidase-like
1423831_at	Prkag2	protein kinase, AMP-activated, gamma 2 non-catalytic subunit
1444232_at	Prkg1	protein kinase, cGMP-dependent, type I
1446560_at	Prss23	protease, serine, 23
1417056_at	Psme1	proteasome (prosome, macropain) 28 subunit, alpha
1433503_at	Ptgr2	prostaglandin reductase 2
1418181_at	Ptp4a3	protein tyrosine phosphatase 4a3
1456903_at	Ptx3	pentraxin related gene
1417841_at	Pxmp2	peroxisomal membrane protein 2
1427151_at	Qser1	glutamine and serine rich 1
1417481_at	Ramp1	receptor (calcitonin) activity modifying protein 1
1438030_at	Rasgrp3	RAS, guanyl releasing protein 3
1428016_a_at	Rasip1	Ras interacting protein 1
1421425_a_at	Rcan2	regulator of calcineurin 2
1454670_at	Rere	arginine glutamic acid dipeptide (RE) repeats
1424716_at	Retsat	retinol saturase (all trans retinol 13,14 reductase)
1424632_a_at	Rev3l	REV3-like, catalytic subunit of DNA polymerase zeta RAD54 like
1438241_at	Rgma	RGM domain family, member A
1426094_at	Rhbdl1	rhomboid, veinlet-like 1 (Drosophila)
1426822_at	Rhot2	ras homolog gene family, member T2
1437309_a_at	Rpa1	replication protein A1
1449398_at	Rpl3l	ribosomal protein L3-like
1417542_at	Rps6ka2	ribosomal protein S6 kinase, polypeptide 2
1416007_at	Satb1	special AT-rich sequence binding protein 1
1441636_at	Sec14l5	SEC14-like 5 (S. cerevisiae)
1455698_at	Sec62	SEC62 homolog (S. cerevisiae)
1417580_s_at	Selenbp1	selenium binding protein 1
1418888_a_at	Sepx1	selenoprotein X 1
1416666_at	Serpine2	serine (or cysteine) peptidase inhibitor, clade E, member 2
1456640_at	Sh3rf2	SH3 domain containing ring finger 2
1419137_at	Shank3	SH3/ankyrin domain gene 3
1417892_a_at	Sirt3	sirtuin 3 (silent mating type information regulation 2, homolog) 3

1436440_at	Slc25a12	solute carrier family 25 (mitochondrial carrier, Aralar), member 12
1424790_at	Slc25a42	solute carrier family 25, member 42
1416275_at	Slc26a6	solute carrier family 26, member 6
1422811_at	Slc27a1	solute carrier family 27 (fatty acid transporter), member 1
1415958_at	Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4
1417042_at	Slc37a4	solute carrier family 37 (glucose-6-phosphate transporter), member 4
1417061_at	Slc40a1	solute carrier family 40 (iron-regulated transporter), member 1
1460565_at	Slc41a1	solute carrier family 41, member 1
1436555_at	Slc7a2	solute carrier family 7, member 2
1434537_at	Slco3a1	solute carrier organic anion transporter family, member 3a1
1419145_at	Smtnl1	smoothelin-like 1
1452789_at	Snn	stannin
1425678_a_at	Snrk	SNF related kinase
1449109_at	Socs2	suppressor of cytokine signaling 2
1454976_at	Sod2	superoxide dismutase 2, mitochondrial
1437197_at	Sorbs2	sorbin and SH3 domain containing 2
1417644_at	Sspn	sarcospan
1449078_at	St3gal6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6
1427345_a_at	Sult1a1	sulfotransferase family 1A, phenol-preferring, member 1
1429146_at	Svip	small VCP/p97-interacting protein
1427982_s_at	Syne2	synaptic nuclear envelope 2
1428295_at	Synpo2l	synaptopodin 2-like
1428467_at	Tardbp	TAR DNA binding protein
1435292_at	Tbc1d4	TBC1 domain family, member 4
1424531_a_at	Tcea3	transcription elongation factor A (SII), 3
1424737_at	Thrsp	thyroid hormone responsive SPOT14 homolog (Rattus)
1418057_at	Tiam1	T-cell lymphoma invasion and metastasis 1
1449335_at	Timp3	tissue inhibitor of metalloproteinase 3
1456981_at	Tmc7	transmembrane channel-like gene family 7
1452962_at	Tmem25	transmembrane protein 25
1452942_at	Tmem65	transmembrane protein 65
1448297_a_at	Tnk2	tyrosine kinase, non-receptor, 2
1418370_at	Tnnc1	troponin C, cardiac/slow skeletal
1449405_at	Tns1	tensin 1
1448667_x_at	Tob2	transducer of ERBB2, 2
1458364_s_at	Tor1aip1	torsin A interacting protein 1
1452766_at	Tppp	tubulin polymerization promoting protein

1456755_at	Trak1	trafficking protein, kinesin binding 1
1427258_at	Trim24	tripartite motif-containing 24
1420019_at	Tspan8	Tetraspanin 8
1418951_at	Txlnb	taxilin beta
1452985_at	Uaca	uveal autoantigen with coiled-coil domains and ankyrin repeats
1417847_at	Ulk2	Unc-51 like kinase 2 (C. elegans)
1425753_a_at	Ung	uracil DNA glycosylase
1430522_a_at	Vamp5	vesicle-associated membrane protein 5
1420909_at	Vegfa	vascular endothelial growth factor A
1429084_at	Vezf1	vascular endothelial zinc finger 1
1420484_a_at	Vtn	vitronectin
1431335_a_at	Wfdc1	WAP four-disulfide core domain 1
1427098_at	Wwp1	WW domain containing E3 ubiquitin protein ligase 1
1422927_at	Yipf7	Yip1 domain family, member 7
1451577_at	Zbtb20	zinc finger and BTB domain containing 20
1455071_at	Zbtb7b	zinc finger and BTB domain containing 7B
1428432_at	Zcchc24	zinc finger, CCHC domain containing 24
1449510_at	Zfp467	zinc finger protein 467
1423835_at	Zfp503	zinc finger protein 503
1454873_at	Zfp775	zinc finger protein 775
1420950_at	Znrf1	zinc and ring finger 1
1446244_at	Zyg11b	zyg-ll homolog B (C. elegans)

A total 660 genes was identified between the effects of genotype and CL (327 overexpressed genes and 333 underexpressed genes). Among the 660 genes, 50 genes were ESTs resulting in 610 known gene identified (310 overexpressed genes and 300 underexpressed genes). W, wild type mice; M, Mstn-pro mice; CLW, clenbuterol treated wild type mice. Grey box is underexpressed genes.

Appendix 3.10 The expression of overlapping genes between the effects of genotype and genotype in CL (W vs M VS CLW vs CLM).

Probe Set ID	Gene Symbol	Gene Title
1424529_s_at	Cgref1	cell growth regulator with EF hand domain 1
1427347_s_at	Tubb2a	tubulin, beta 2A

A total 2 genes was identified between the effects of genotype and CL (2 overexpressed genes). W, wild type mice; M, Mstn-pro mice; CLW, clenbuterol treated wild mice; CLM, clenbuterol treated Mstn-pro.