PHOSPHATIDYLINOSITOL-3 KINASE/AKT AND FOS LIKE PROTEIN 1 REGULATION OF TROPHOBLAST DIFFERENTIATION

BY

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Submitted to the graduate degree program in Pathology and Laboratory Medicine and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Hemochorial placentation is an effective strategy facilitating nutrient delivery and development of the fetus within the female reproductive tract. Remodeling of the uterine vasculature by trophoblast cells is one of the key processes necessary for a successful pregnancy. Several pathways have been implicated in the regulation of trophoblast invasion including the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway. In this research, we identified genes characteristic of trophoblast stem cell and differentiated states, including those involved in trophoblast invasion and vasculature remodeling. We also evaluated the role of the PI3K/AKT signaling pathway in the regulation of trophoblast cell differentiation. This investigation utilized the Rcho-1 trophoblast stem cells as a model system for trophoblast differentiation. These cells can be maintained in a trophoblast stem cell state or induced to differentiate into trophoblast giant cells, which have the capability to invade. mRNA expression profiling using microarrays provided insights about trophoblast stem cell renewal and differentiation. Genes linked to trophoblast invasion and/or vasculature remodeling were identified. These putative ‘invasion related genes’ were upregulated during differentiation, corresponding to an increase in PI3K/AKT signaling during differentiation. Using pharmacological inhibition of PI3K or AKT we determined that expression of several ‘invasion related genes’ was dependent on the PI3K/AKT signaling pathway. We also showed that the invasive ability of trophoblast cells was decreased when the PI3K/AKT signaling pathway was inhibited. Further analysis using lentiviral delivered shRNAs to

knockdown Akt1, Akt2 or Akt3 further validated the involvement of the PI3K/AKT pathway in the regulation of these events. Fos like antigen 1 (FOSL1 or FRA1) was upregulated during trophoblast cell differentiation and shown to be a candidate downstream mediator of the PI3K/AKT signaling pathway. Modulation of the PI3K/AKT signaling pathway resulted in a decrease in FRA1 protein level. Knockdown of FRA1 using lentiviral delivered shRNA resulted in a decrease in a subset of the PI3K/AKT sensitive ‘invasion related genes’ and decreased trophoblast invasion. In conclusion, the PI3K/AKT signaling pathway regulates trophoblast differentiation by regulating FRA1 and the expression of key genes involved in the invasion process. In this research, we have identified key factors contributing to the regulation of rodent hemochorial placentation. The conservation of these regulatory factors in the process of human placentation and their potential involvement in pregnancy-related diseases remain to be determined.

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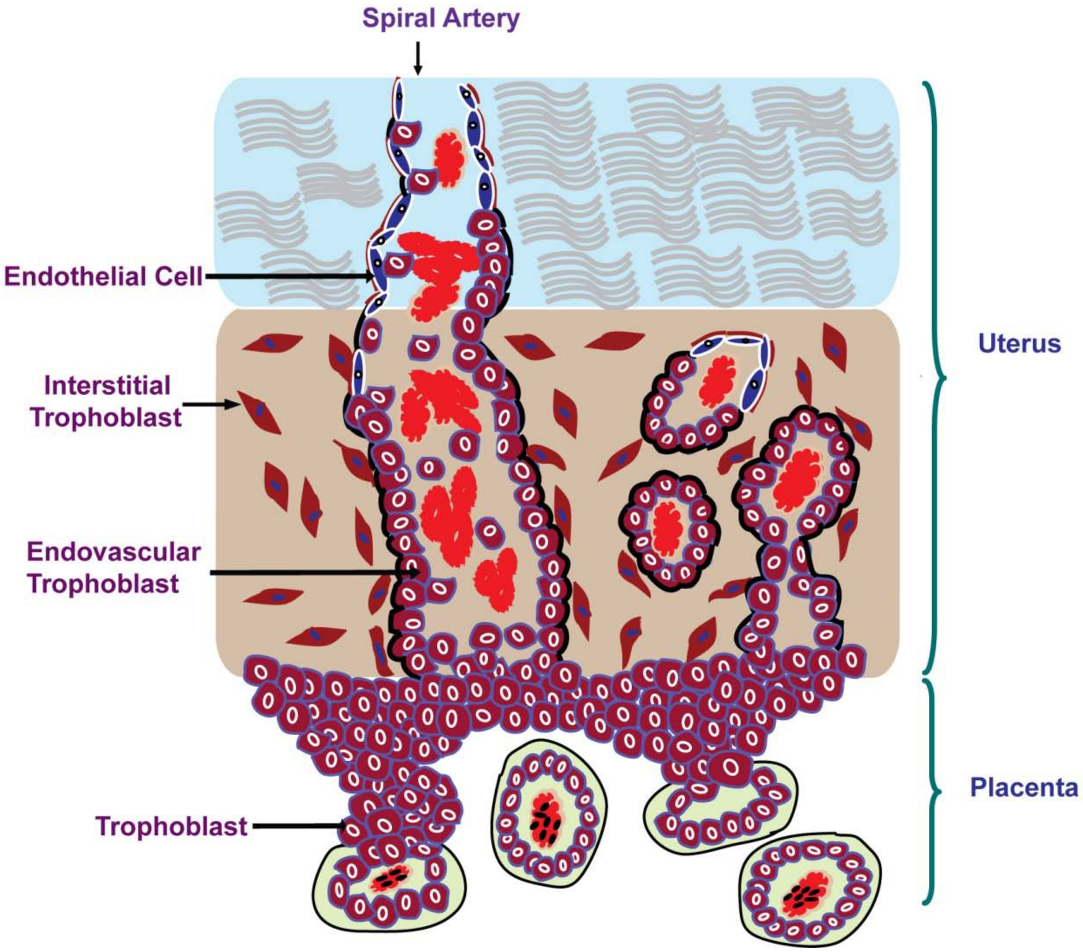
CHAPTER 1:

GENERAL INTRODUCTION

Mammalian pregnancy is made possible by the evolution of a unique organ called the placenta. The placenta is of extraembryonic origin and mediates interactions between the mother and fetus. The maternal-fetal interface is a dynamic site where uterine and placental structures cooperate to promote the development of the fetus. These specialized tissues facilitate efficient nutrient delivery. The rat, mouse, and human each possess a hemochorial placenta (Davies and Glasser 1968; Enders and Welsh 1993; Georgiades et al. 2002; **Fig. 1.1**). This type of placentation is characterized by erosion of the maternal uterine epithelium and vasculature permitting the direct flow of maternal nutrients to trophoblast. Trophoblast cells are the parenchymal cells of the placenta.

Trophoblast cells form a vital connection between mother and fetus. Some of the functions of trophoblast cells include: i) regulation of nutrient and waste transport between maternal and fetal compartments, ii) assisting in the creation of an immune privileged state, iii) production of hormones and cytokines which modify maternal metabolism, and iv) remodeling uterine spiral arteries via invasion. When trophoblast cells develop abnormally their ability to function as a link between mother and fetus is altered and can lead to pregnancy failure or diseases such as fetal growth restriction and preeclampsia (Pijnenborg et al., 1981; Kaufmann et al., 2003) or longer term adverse effects on postnatal development and health (Bateson et al., 2004; Gluckman and Hanson, 2004). Rodent models are useful tools for studying mammalian development and pregnancy.

Fig. 1.1 Schematic representation of the general organization of the hemochorial placenta. One characteristic of the hemochorial placenta trophoblast invasion into the uterine compartment. In the hemochorial placenta trophoblast cells invade into the uterus both interstitially and along spiral arteries. Spiral arteries are widened, the smooth muscle lining the arteries is lost and trophoblast cells replace the maternal endothelium.


Spiral Artery
Endothelial Cell
Interstitial Trophoblast
Endovascular Trophoblast
Trophoblast
Uterus
Placenta

Rodent Trophoblast Models

The use of animal model systems represents a valuable tool for dissecting molecular mechanisms controlling cellular development. The maternal-fetal interface is no exception. The premise of employing any animal model system is that if the process being studied is fundamental then some elements of the process should demonstrate conservation across species. Although, there are some differences in placentation in rodents versus primates, there are overriding similarities in the functions and lineages of cells comprising the maternal-fetal interface (Cross, 2005).

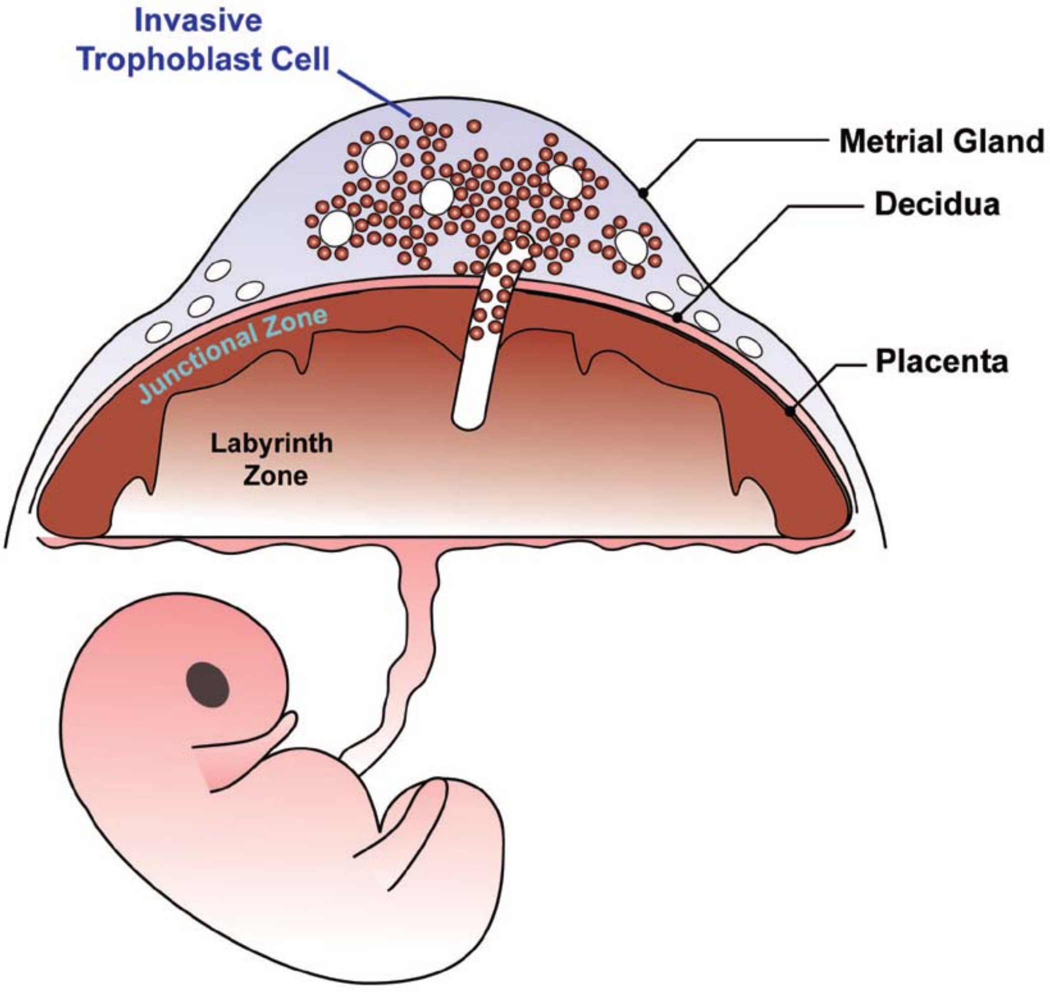
Early lineage decisions in the mammalian embryo

The first differentiation event to occur during development is the differentiation of the trophectoderm and the inner cell mass from a totipotent cell. The trophectoderm gives rise to the extraembryonic trophoblast cells while the inner cell mass is the origin of all fetal tissue (Yamanaka et al., 2006). Each of these cell layers can be cultured in vitro as stem cell populations. Trophoblast stem cells can be derived from the trophectoderm (Tanaka et al., 1998) and embryonic stem cells from the inner cell mass (Evans and Kaufman, 1981; Martin, 1981).

Placentation in the Rodent

The mature rodent placenta has two major components: the junctional zone and the labyrinth zone (**Fig. 1.2**). Trophoblast cells comprising the rat and

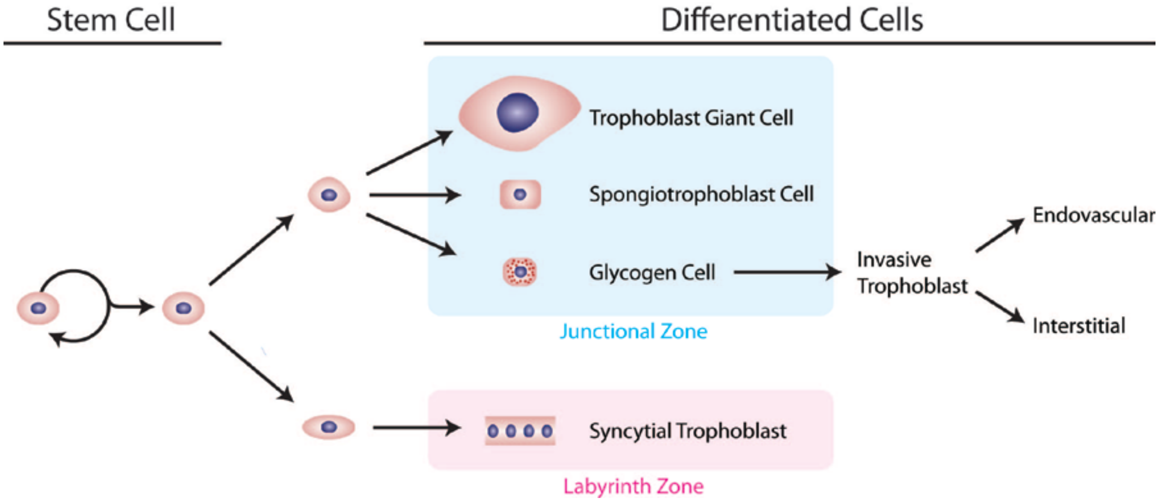
Fig. 1.2. Organization of the mature rodent placenta. The two zones of the late gestational rodent placenta (junctional and labyrinth) are shown, as are invasive trophoblast cells.


Invasive Trophoblast Cell
Metrial Gland
Decidua
Junctional Zone
Placenta
Labyrinth Zone

mouse placenta have the capacity to differentiate along a multi-lineage pathway. These lineages include trophoblast giant cells, spongiotrophoblast cells, glycogen cells, invasive trophoblast cells, and syncytial trophoblast (Soares et al., 1996; Rossant and Cross, 2001; Ain et al., 2003; **Fig. 1.3**). Each lineage has specialized functions that are necessary for a normal pregnancy.

The junctional zone is positioned at the maternal fetal-interface and has an outer layer of trophoblast giant cells with spongiotrophoblast and glycogen cells making up the largest portion of this zone. The invasive trophoblast lineage arises from precursors within the junctional zone and invades into the uterine mesometrial compartment. Trophoblast giant cells are the first trophoblast lineage to differentiate and have invasive and endocrine functions (Riley et al., 1998). Spongiotrophoblast also have endocrine functions. Glycogen cells accumulate glycogen, which is thought to be an important source of energy. Invasive trophoblast cells penetrate into the maternal uterus where they are hypothesized to alter the spiral arterioles increasing blood flow to the placenta and fetus. The labyrinth zone is derived from the fusion of the chorion with the allantois and contains both trophoblast cells and fetal blood vessels. Trophoblast cells found in the labyrinth zone are primarily syncytial trophoblast, which arise from fusion of labyrinth trophoblast precursors, and scattered populations of trophoblast giant cells. The syncytial trophoblast of the labyrinth zone are responsible for transport of nutrients and wastes between maternal and fetal compartments.

Fig. 1.3. Multi-lineage trophoblast pathway. In the mouse and rat trophoblast cells differentiate into several lineages. Junctional zone precursor cells can give rise to trophoblast giant cells, spongiotrophoblast and glycogen cells. Glycogen cells become invasive and are then termed invasive trophoblast cells. Labyrinth zone precursor cells differentiate into syncytial trophoblast.


Stem Cell
Differentiated Cells
Trophoblast Giant Cell
Spongiotrophoblast Cell
Glycogen Cell
Invasive Trophoblast
Endovascular
Interstitial
Junctional Zone
Syncytial Trophoblast
Labyrinth Zone

Trophoblast Invasion and Vascular Remodeling

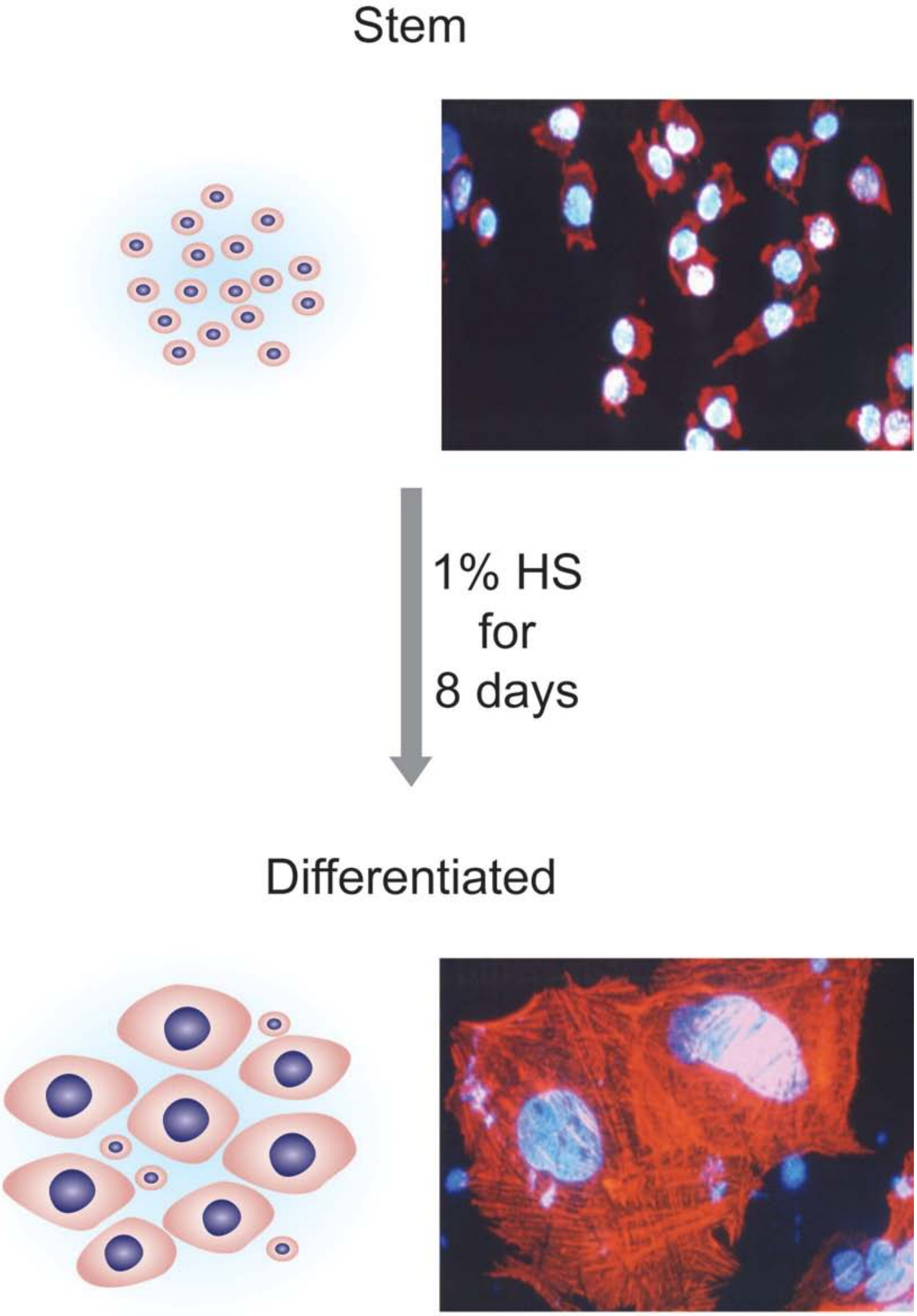
Trophoblast invasion and uterine vascular remodeling occur in the human as well as the mouse and rat. One of the key functions of invasive trophoblast cells is the remodeling of uterine spiral arterioles. This remodeling transforms the tightly coiled spiral arterioles into dilated vessels that are no longer under maternal control (Pijnenborg et al., 1981; Adamson et al. 2002; Kaufmann et al., 2003). In the human, poor trophoblast invasion has been linked to miscarriage, preeclampsia, pre-term birth, and fetal growth restriction (Harris, 2010).

Trophoblast invasion in the mouse and the rat occurs in two waves. The first wave starts during midgestation and consists of endovascular invasion into the spiral arterioles located in the central area of the placenta. Invasive trophoblast cells line these blood vessels, replacing the maternal endothelium (Pijnenborg et al., 1981). This early invasive population has been characterized in the mouse. These cells have a trophoblast giant cell morphology and express the trophoblast giant cell marker and angiogenic factor, proliferin (*Prl2c2;* Adamson et al., 2002; Hemberger et al. 2003; Simmons et al., 2007). Other angiogenic and vasodilatory factors are also expressed in invasive trophoblast giant cells including: adrenomedullin (ADM), proliferin-related protein (PRL7D1), and nitric oxide synthases (NOS2 and NOS3; Hemberger et al. 2003). The later population of invasive trophoblast cells begins invading around gestation d13.5 to d14.5 and has an interstitial pattern of invasion. This second wave of invasive trophoblast cells also expresses members of the prolactin gene family and exhibits features suggesting a glycogen cell origin (Ain et al., 2003; Bouillot et al., 2006; Coan et al. 2006).

Rcho-1 Trophoblast Stem Cells

Rcho-1 trophoblast stem (Rcho-1 TS) cells represent an excellent model for investigating regulatory pathways controlling trophoblast giant cell differentiation (Faria et al. 1991; Sahgal et al., 2006). Rcho-1 TS cells were derived from a serially transplanted rat choriocarcinoma (Teshima et al., 1983; Faria and Soares, 1991). The choriocarcinoma was induced by surgically removing the embryo and exposing the placenta to the peritoneal cavity (Teshima et al., 1983). Similar to blastocyst derived mouse trophoblast stem cells the Rcho-1 TS cells can be manipulated to proliferate or to differentiate along the trophoblast giant cell lineage (Faria and Soares, 1991; Hamlin et al., 1994; **Fig. 1.4**). Unlike blastocyst derived mouse trophoblast stem cells the Rcho-1 TS cells do not need addition of growth factors to maintain their stem cell state (Tanaka et al. 1998), possibly due to their polyploidy nature (Teshima et al., 1983; Faria and Soares, 1991). When proliferating Rcho-1 TS cells express TS cell markers. These markers are downregulated as the cells differentiate. Differentiated Rcho-1 TS cells possess both trophoblast giant cell morphology and function (Faria and Soares, 1991; Hamlin et al., 1994).

Fig. 1.4. Overview of the Rcho-1 trophoblast stem cell model. The Rcho-1 trophoblast stem cells (Rcho-1 TS) can be maintained in a stem cell state or induced to differentiate by switching from 20% fetal bovine serum to 1% Horse serum. Differentiated Rcho-1 TS cells have morphology, phenotype similar to trophoblast giant cells. The increase in volume and nuclear size during differentiation are illustrated.

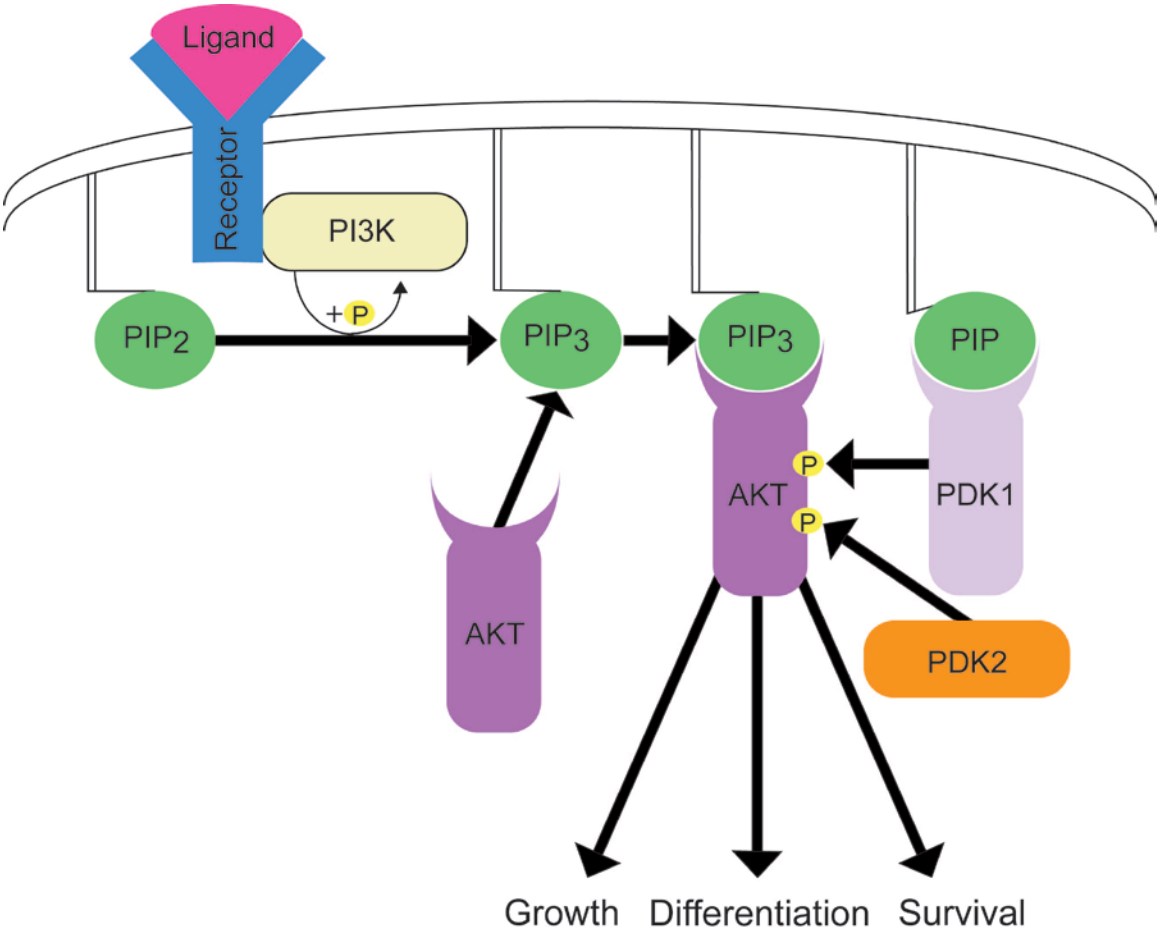

Stem
1% HS for 8 days
Differentiated

*Overview of the Phosphatidylinositol-3 Kinase (PI3K)/AKT Signaling Pathway (**Fig. 1.5**)*

PI3K is a lipid kinase that phosphorylates phosphatidylinositol and phosphoinositides (Engelman et al., 2006). The actions of several growth factors are mediated through activation of PI3K (Cantley, 2002). PI3K is a heterodimer consisting of p85 regulatory and p110 catalytic subunits. There are three p85 subunit genes. The first is *Pik3r1* which codes for three variants: p85α, p55α, and p50α. The other two genes are *Pik3r2* and *Pik3r3,* which code for p85β and p85γ, respectively. There are three genes coding for the p110 subunit. They are *Pik3ca*, *Pik3cb* and *Pik3cd,* which code for p110α, p110β and p110γ.

Analysis of PI3K mutant mice reveals that mice with mutations in the p85α or p85β subunits have altered insulin signaling (Fruman et al., 2000; Ueki et al., 2002). Mice engineered with mutations, which disrupt both p85α and p85β subunits die in utero at embryonic d12.5 (Brachmann et al., 2005). Mice that lack the p110α subunit of PI3K are also embryonic lethal and die between gestation d9.5 and d10.5 (Bi et al., 1999). Mutant mice deficient in the p110β subunit die on embryonic d3.5 (Bi et al., 2002). Trophoblast cell phenotypes have not been evaluated in these mice, but the embryonic phenotype has been described as general defects in proliferation which can be caused by problems with placentation. The phenotypes of these mutant models imply that the PI3K signaling pathway is essential for early stages of embryonic and extraembryonic development.

Fig. 1.5. Schematic of the phosphatidylinositol-3 Kinase (PI3K)/AKT signaling pathway. Activated receptors recruit PI3K to the membrane where it is activated. PI3K then phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 recruits protein dependent kinase 1 (PDK1) and AKT to the plasma membrane where AKT is phosphorylated at Thr308 by PDK1 and at Ser473 by multiple kinases termed here as PDK2. Once activated AKT can phosphorylate a multitude of substrates involved in many cellular processes including growth, differentiation, and cell survival.


Ligand
Receptor
PI3K
+P
PIP2
PIP3
PIP3
PIP
AKT
P
P
AKT
PDK1
PDK2
Growth
Differentiation
Survival

PI3K initiates a signaling cascade leading to AKT (also known as protein kinase B, PKB) activation (Cantley, 2002; Brazil et al., 2004). AKT is a serine threonine kinase with numerous substrates. These substrates are involved in many processes including: metabolism, cell cycle, cell survival, and protein synthesis (Engelman et al., 2006). There are three AKT isoforms in mammals, AKT1/PKBα, AKT2/PKBβ and AKT3/PKBγ (Brazil and Hemmings, 2001). These enzymes are conserved at the amino acid level and are thought to share substrate specificity (Coffer et al., 1998; Brazil and Hemmings, 2001). Mutant mouse models of these three AKT isoforms show different phenotypes. *Akt1* null mice exhibit disrupted placental development and intrauterine growth restriction (IUGR; Cho et al., 2001a; Cho et al., 2001b; Yang et al., 2003). *Akt2* null mice are insulin intolerant (Cho et al., 2001a), and *Akt3* null mice have decreased brain mass (Easton et al., 2005). Mice that are null for both *Akt1* and *Akt3* have altered vascular and placental development, are embryonic lethal, and die by gestation d12.5 (Yang et al., 2005).

PI3K/AKT Pathway in Placental and Trophoblast Cell Development and Invasion

The PI3K/AKT pathway has been implicated as a potential regulator of trophoblast cell development. Upon differentiation of trophoblast cells, PI3K is activated leading to the phosphorylation and constitutive activation of AKT (Kamei et al., 2002). LY294002 is a potent inhibitor of PI3K (Vanhaesebroeck and Waterfield, 1999). Inhibition of PI3K with LY294002 disrupts AKT activation and interferes with trophoblast cell differentiation (Kamei et al., 2002). The predominant isoform of AKT expressed in developing trophoblast giant cells is AKT1 (Kamei et al., 2002; Yang et al., 2003). In rodent trophoblast, PI3K/AKT signaling is involved in the regulation of peptide hormone biosynthesis [placental lactogen-I (PL-I or PRL3D1), PL-II (PRL3B1), and prolactin-like-protein-A (PLP-A or PRL4A1)] (Kamei et al., 2002; Kent et al., 2010; Chapter 2).

The PI3K/AKT signaling pathway has also been shown to be involved in regulating human trophoblast cell survival (Straszewski-Chavez et al., 2010), expression of proteins involved in immunological protection (Holets et al, 2009), and the expression of pro-invasion genes and the invasive process (Qiu et al., 2004a,b; Sonderegger et al. 2010). For example in two human trophoblast cell lines (HTR-8/SVneo and SGHPL-5), the PI3K/AKT pathway has been linked to matrix metalloproteinase 9 (MMP9) expression and invasion (Qiu et al., 2004a,b; Sonderegger et al. 2010).

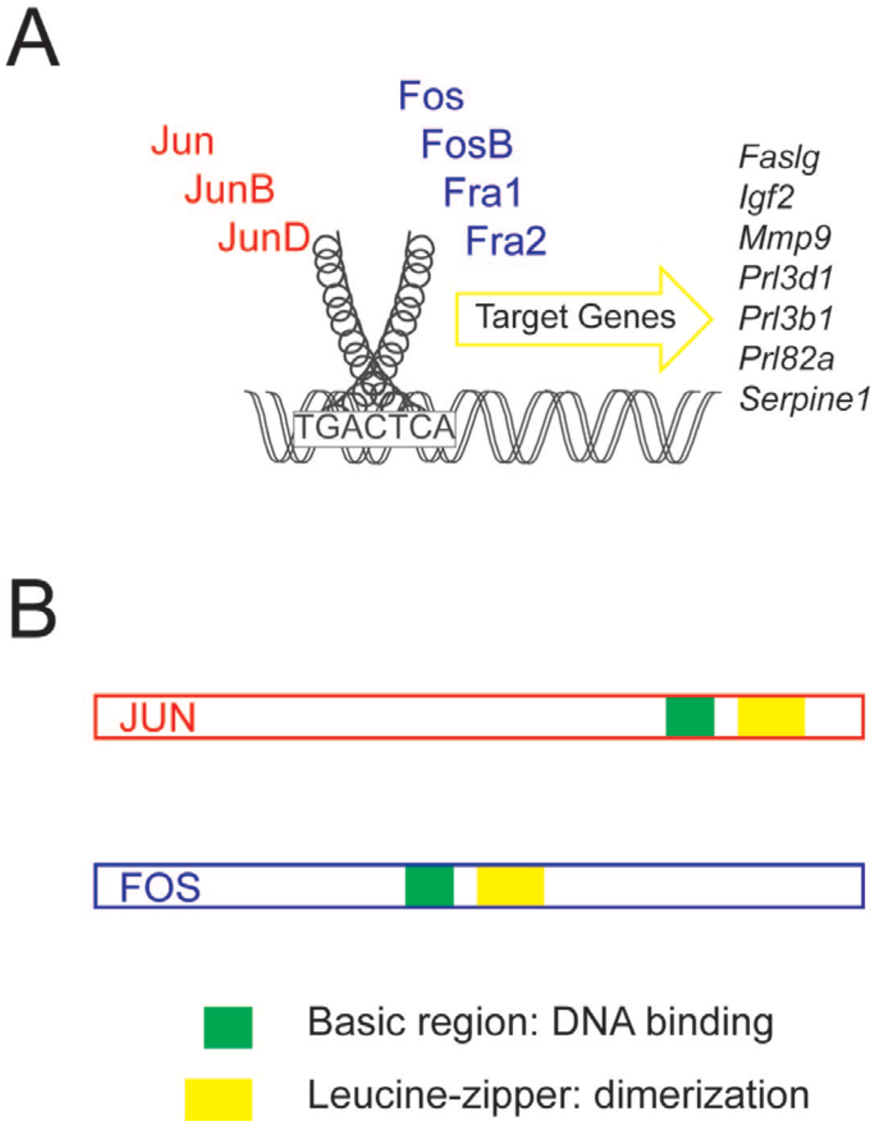
In summary, there is evidence suggesting that the PI3K/AKT pathway contributes to the regulation of placental and trophoblast cell development including the endocrine and invasive phenotypes of trophoblast cells. The PI3K/AKT signaling pathway potentially regulates trophoblast differentiation via actions on the availability and/or the activity of key proteins. Proteins regulated by PI3K/AKT could impact transcriptional or posttranscriptional events and regulate gene expression during trophoblast cell differentiation.

FOS Like Protein 1 (FOSL1 or FRA1)

Several transcription factors participate in the regulation of trophoblast cell differentiation (Simmons and Cross, 2005). Our efforts focus on the role of FRA1 in the regulation of trophoblast differentiation.

FRA1 is a component of activator protein-1 (AP-1). AP-1 is a dimeric transcriptional regulator comprised of proteins possessing a basic region-leucine zipper (bZIP) and includes members of the FOS and JUN families proteins (Eferl and Wagner, 2003). The FOS family consists of c-FOS, FOSB, FRA1, and FRA2, while JUN proteins include c-JUN, JUNB, and JUND (**Fig. 1.6**). AP-1 can consist of FOS-JUN heterodimers or JUN-JUN homo- or heterodimers. In addition JUN can also dimerize with other bZIP proteins (Eferl and Wagner, 2003). The activity and specificity of AP-1 varies, and is dependent on the composition of the dimer. AP-1 binds to the AP-1 response element, also known as the 12-O-tetradecanoylphorbol-13-acetate (TPA)-response element (TRE, TGA(C/G)TCA), and the cyclic adenosine monophosphate (cAMP)-response element (CRE, TGACGTCA) (Nakabeppu et al., 1988; Rauscher et al., 1988; Eferl and Wagner, 2003). FRA1 has been implicated in bone development (Wagner, 2010), tumorigenesis, cancer progression and cell invasion (Verde et al., 2007).

Fig. 1.6. Overview of the activator protein 1 (AP-1) family. The dimeric (AP-1) transcription factor complex is composed of JUN and FOS proteins. JUN proteins form homodimers or heterodimers with FOS proteins through their leucine-zipper domains. The different dimer combinations recognize different sequence elements in the promoters and enhancers of target genes. Only the classic TPA-responsive element with the consensus sequence TGACTCA is shown. AP-1 dimers recognize specific response elements via their basic domain that is adjacent to the leucine-zipper domain and exhibits an α-helical structure. **A)** Illustration of JUN and FOS dimerization and a list targets. **B)** Overview of the structure of JUN and FOS family proteins. Modified from Zenz et al. 2008.


A
Jun
JunB
JunD
Fos
FosB
Fra1
Fra2
TGACTCA
Target Genes
Faslg
Igf2
Mmp9
Prl3d1
Prl3b1
Prl82a
Serpine1
B
JUN
FOS
🟩 Basic region: DNA binding
🟨 Leucine-zipper: dimerization

PI3K/AKT Signaling Pathway and FRA1

Although the role of the PI3K/AKT signaling pathway in the regulation of FRA1 has not been reported for trophoblast, it has been shown to regulate *Fra1* expression in other cell types (Tiwari et al. 2003). FRA1 has also been shown to be a downstream mediator of the PI3K/AKT signaling pathway in regulating the expression of pro-invasion genes, including *Mmp9*, and cellular invasion (Cao et al. 2006; Ramachandran et al. 2010). The exact mechanism of the regulation of FRA1 by the PI3K/AKT signaling pathway is unclear. In some reports PI3K/AKT has been shown to regulate FRA1 mRNA (Tiwari et al. 2003), while other studies report a change in protein and or activity (Cao et al. 2006; Ramachandran et al. 2010). It is likely that the mechanism of regulation of FRA1 by the PI3K/AKT signaling pathway may be cell type dependent.

FRA1 in Placental and Trophoblast Cell Development

Fra1 null mice die at approximately gestation d10.5 due to placental defects (Schreiber et al., 2000). However, little is known about the specific biological actions of FRA1 in trophoblast cells. In cancer cells, FRA1 has been proposed as a potential regulator of the invasive phenotype, possibly via regulation of *Mmp9* (Kustikova et al., 1998; Bischof et al., 2002; Zhang et al., 2004; Belguise et al., 2005), which may provide a clue for its role in trophoblast cells.

Examination of the promoters for several genes expressed in differentiating trophoblast cells demonstrates the existence of AP-1 response elements. Promoter activation of *Prl3d1*, *Prl3b1,* and *Prl8a2* genes in differentiated Rcho-1 TS cells is dependent upon functional AP-1 response elements (Shida et al., 1993; Orwig and Soares, 1999; Sun and Duckworth, 1999). At this juncture, it is not known whether FRA1 contributes to the AP-1 complexes that are engaged in these important regulatory regions.

In conclusion, additional studies need to be conducted to determine if the PI3K/AKT signaling pathway regulates FRA1 in trophoblast cells. The role of FRA1 in trophoblast cell differentiation needs to be better defined, and gene targets for FRA1 identified.

Objectives of our Research

Trophoblast cells of the placenta are essential for the establishment and maintenance of pregnancy. An important function of trophoblast cells is to alter the maternal environment and create optimal conditions for fetal development. Remodeling of the uterine vasculature is one of the key changes that occur during pregnancy. Trophoblast cells, particularly invasive trophoblast, are involved in the remodeling of the uterine vasculature. Poor trophoblast invasion and/or vascular remodeling has been linked to disorders in the human such as fetal growth restriction and preeclampsia. In this study we examine changes in gene expression in trophoblast cells in order to identify genes that are involved in trophoblast invasion and vascular remodeling. We also analyze the role of the PI3K/AKT signaling pathway in the expression of invasive and remodeling genes and the invasive phenotype of trophoblast. The AP-1 transcription factor FRA1 is also evaluated as a putative mediator of PI3K/AKT signaling in trophoblast and as a potential regulator of the invasive potential of trophoblast cells.

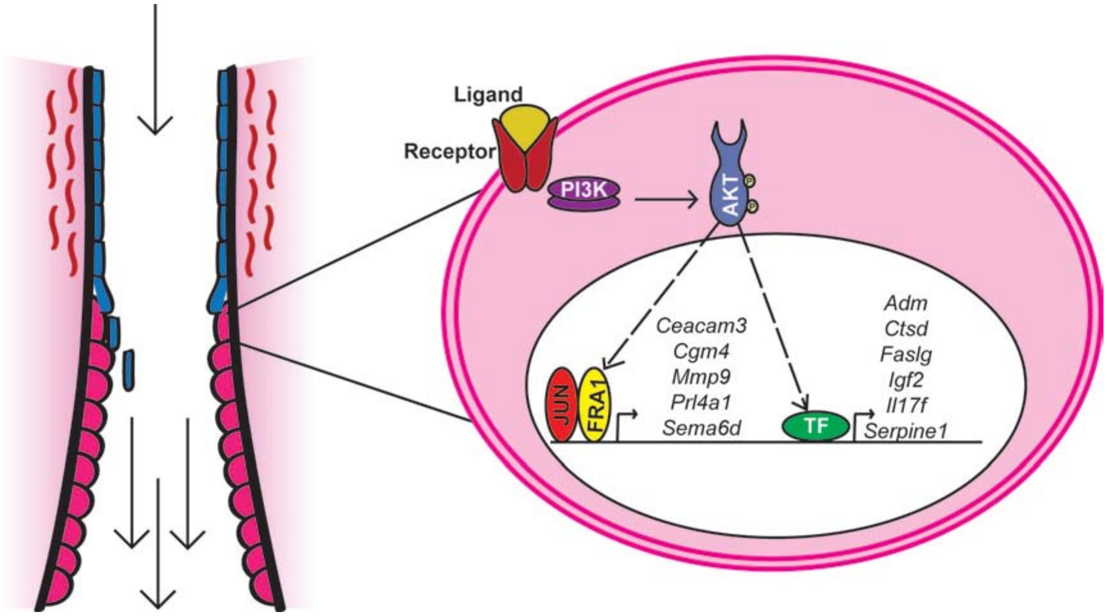
By studying signaling pathways and transcriptional regulators of trophoblast invasion we gain insight into mechanisms controlling these fundamental processes, which could serve as sites for disruption in diseases of pregnancy.

There are four specific aims in this research project (**Fig. 1.7**), they are:

1) To examine changes in gene expression between stem and differentiated trophoblast cells.
2) To evaluate the role of the PI3K/AKT signaling pathway in trophoblast differentiation and gene expression.
3) To determine if the PI3K/AKT signaling pathway regulates trophoblast invasion.
4) To assess the involvement of FRA1 as a mediator of the PI3K/AKT regulation of trophoblast invasion.

Experimental findings related to Specific Aims 1 and 2 are presented in Chapter 2 and those associated with Specific Aims 3 and 4 are presented in Chapter 3.

Fig. 1.7. Schematic diagram describing the objectives of the research. Illustration of trophoblast invasion into a maternal blood vessel (left): The invasive trophoblast cells (pink) move along maternal blood vessel and replace the endothelium (blue). During this process the blood vessel is restructured into a dilated, high flow, low pressure vessel lacking smooth muscle. Overview of PI3K/AKT/FRA1 signaling pathway in an invasive trophoblast cell inducing the expression of invasion and vascular remodeling related genes (right).


Ligand
Receptor
PI3K
AKT
JUN
FRA1
Ceacam3
Cgm4
Mmp9
Prl4a1
Sema6d
TF
Adm
Ctsd
Faslg
Igf2
Il17f
Serpine1

CHAPTER 2: PHOSPHATIDYLINOSITOL 3-KINASE MODULATION OF TROPHOBLAST CELL DIFFERENTIATION

ABSTRACT

The trophoblast lineage arises as the first differentiation event during embryogenesis. Trophoblast giant cells are one of several end-stage products of trophoblast cell differentiation in rodents. These cells are located at the maternal-fetal interface and are capable of invasive and endocrine functions, which are necessary for successful pregnancy. Rcho-1 trophoblast stem (Rcho-1 TS) cells can be effectively used as a model for investigating trophoblast cell differentiation. In this report, we evaluated the role of the phosphatidylinositol 3-kinase (PI3K) signaling pathway in the regulation of trophoblast cell differentiation. Transcript profiles from trophoblast stem cells, differentiated trophoblast cells, and differentiated trophoblast cells following disruption of PI3K signaling were generated and characterized. Prominent changes in gene expression accompanied the differentiation of trophoblast stem cells. PI3K modulated the expression of a subset of trophoblast cell differentiation-dependent genes. Among the PI3K-responsive genes were those encoding proteins contributing to the invasive and endocrine phenotypes of trophoblast giant cells. Genes have been identified with differential expression patterns associated with trophoblast stem cells and trophoblast cell differentiation; a subset of these genes are regulated by PI3K signaling, including those impacting the differentiated trophoblast giant cell phenotype.

INTRODUCTION

Hemochorial placental development is a complex process involving multiple signaling pathways. Effectively two placental compartments are established. One compartment contains trophoblast cells specialized for interactions with the maternal environment, while the other contains trophoblast cells directed toward the bidirectional transport of nutrients and wastes between the mother and the fetus. Trophoblast cells of the rat and mouse have the capacity to differentiate along a multi-lineage pathway. Cell lineages directed toward the maternal environment, include trophoblast giant cells, spongiotrophoblast, glycogen cells, and invasive trophoblast cells; whereas syncytial trophoblast regulate maternal-fetal nutrient and waste delivery (Soares et al., 1996; Rossant and Cross, 2001; Ain et al.,2003). Each lineage possesses specialized functions necessary for a normal pregnancy.

Trophoblast giant cells are the first trophoblast lineage to differentiate (Riley et al. 1998). Trophoblast giant cells are located at the maternal-fetal interface and have several functions. They produce steroid and peptide hormones (Soares et al., 2001) and have the ability to invade into the uterine vasculature (Adamson et al, 2002; Hemberger et al, 2003).

The phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), pathway is involved in trophoblast cell development (Kamei et al, 2002). Upon differentiation of trophoblast cells, PI3K is activated leading to the phosphorylation and constitutive activation of AKT (Kamei et al, 2002). Inhibition of PI3K disrupts AKT activation and interferes with trophoblast cell differentiation (Kamei et al, 2002; Nadra et al., 2006). The predominant isoform of AKT in developing trophoblast giant cells is AKT1 (Kamei et al, 2002; Yang et al. 2003). Mice possessing a null mutation at the *Akt1* locus exhibit defects in placental development (Yang et al. 2003). Their placentas are smaller and accumulate less glycogen than wild-type mice.

In this report, we utilize Rcho-1 trophoblast stem (TS) cells as an in vitro model to gain a better understanding of trophoblast cell differentiation. Rcho-1 TS cells are remarkable in that they can be maintained in a stem cell state or induced to differentiate along the trophoblast giant cell lineage (Faria and Soares, 1991; Hamlin et al., 1994; Yamamoto et al., 1994; Sahgal et al., 2006). This in vitro system represents an excellent model for investigating regulatory pathways controlling trophoblast giant cell differentiation. In order to gain new insights about trophoblast cell differentiation we performed genome wide screens for transcripts expressed in trophoblast stem cells, differentiating trophoblast cells, and differentiating trophoblast cells with disrupted PI3K signaling. Genes selected for further analyses exhibited high levels of expression, prominent differences among the experimental groups, and/or encoded proteins with actions potentially relevant to trophoblast biology. Expression patterns of a subset of genes identified from the array were verified by northern analysis and/or quantitative reverse transcription-polymerase chain reaction (qRT-PCR). In vivo placental expression patterns of the selected genes identified from the gene profiles were also determined. ‘Trophoblast stem cell-associated’, ‘differentiation-associated’, and ‘PI3K-regulated’ genes were identified. A subset of the ‘differentiation-associated’ genes is regulated by the PI3K signaling pathway and may contribute to the trophoblast cell phenotype.

MATERIALS AND METHODS

Reagents and cDNA generation

All reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. cDNAs to selected transcripts were obtained from Invitrogen (Carlsbad, CA), American Type Culture Collection (ATCC, Rockville, MD), or cloned using TOPO TA cloning kit (Invitrogen). Other cDNAs were gifts from the following investigators: *Atp1a1*, Dr. Gustavo Blanco, University of Kansas Medical Center (Kansas City, KS); *Cyp11a1*, Dr. JoAnne Richards, Baylor College of Medicine (Houston, TX); *Mmp9*, Dr. Ruth Muschel, University of Pennsylvania (Philadelphia, PA), and *Prl4a1*, Dr. Mary Lynn Duckworth, University of Manitoba (Winnipeg, Manitboa, Canada). **Tables 2.1-2.3** include information on the source of cDNAs and primer sequences used for the generation of cDNAs and for qRT-PCR.

Animals and tissue collection

Holtzman Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN). Animals were housed in an environmentally controlled facility with lights on from 0600-2000 h and were allowed free access to food and water. Timed pregnancies were generated by cohabitation of female and male animals. The presence of a copulatory plug or sperm in the vaginal smear was designated d0.5 of pregnancy. Rat placental tissues were collected on gestation d11.5 and d18.5. At d11.5 of gestation, the placenta contains a mixture of proliferating and

Table 2.1. Plasmid sources

Symbol	GenBank Accession No.	Est ID	Vendor/Source
Ccnd3	NM_012766	BI291261	Invitrogen
Ccne1	NM_001100821	BE112508	Invitrogen
Cd47	NM_019195	AI044792	Invitrogen
Cd9	NM_053018	BF555259	Invitrogen
Ceacam10	NM_173339	BF556893	Invitrogen
Cgm4	NM_012525	BI275410	Invitrogen
Cited2	NM_053698	AA900476	Invitrogen
Cyp11a1	NM_017286	---	Dr. JoAnne Richards, Baylor College of Medicine, Oonk RB et al 1989. J Biol Chem 264: 21934-21942
Ddit3	NM_001109986	BF395052	Invitrogen
Fn1	NM_019143	BI292076	Invitrogen
Grn	NM_017113	BF5555139	Invitrogen
H19	NR_027324	BE117886	Invitrogen
Hbp1	NM_013221	AA891261	ATCC
Hsd3b1	NM_001007719	BF559905	Invitrogen
Id1	NM_012797	BI292304	Invitrogen
Krt19	NM_199498	BI279605	Invitrogen
Mif	NM_031051	BG380752	Invitrogen
Mmp9	NM_031055	---	Dr. Ruth Muschel (University of Pennsylvania, Philadelphia, PA) Peters et al 1999
Nfe2l2	NM_031789	AI177161	Invitrogen
Pgam1	NM_053290	BI278242	Invitrogen
Prl4a1	NM_017036	---	ML Duckworth, Unv Manitoba, Duckworth et al J Bio Chem 261: 10879-10889, 1986
Slc16a3	NM_030834	BE112961	Invitrogen
Slc28a2	NM_031664	AI059393	Invitrogen
Dif EST #1	AI012949	AI012949	ATCC
Dif EST #2	AA964255	AA964255	Invitrogen
Tfpi	NM_017200	BF551171	Invitrogen

Table 2.2 Primers used for cDNA cloning

Symbol	GenBank Accession No.	Forward primer	Reverse primer
Ccna2	NM_053702	5'tgttgaatcaccccatgcta3'	5'agccaagtcaaaagcaagga3'
Ctsd	NM_134334	5'gccaagtttgatggcatctt3'	5'atgaagccactcaggcagat3'
Cyp17a1	NM_012753	5'atgtgggaacttgtgggtctc3'	5'tgtggcctctaggtgctaac3'
Ecm1	NM_053882	5'ccagaagacatccctgtgta3'	5'ttccatgtgcaagtgtggtt3'
Fra1	NM_012953	5'gtgcagaaaccgaagaaagg3'	5'cctcacaaagccaggagtgt3'
Hsd17b2	NM_024391	5'ttctctgcaaagcctggagt3'	5'aacaccttggtgacctcgac3'
Il17f	NM_001015011	5'tggtcaagtctctgctgctg3'	5'ttcggtatgtggctttgtca3'
Junb	NM_021836	5'atgtgcacgaaaatggaaca3'	5'gcaggcacataggagggata3'
Lgmn	NM_022226	5'attaccgacaccaggcagac3'	5'cccatactgcatgacgtgac3'
Maged1	NM_053409	5'cagctaggcagacaccatca3'	5'atgggcaccttcgtgtagtc3'
Msn	NM_030863	5'cccaaagagtcttggagcag3'	5'agctcttccttctcccgttc3'
Mt1a	NM_138826	5'caccagatctcggaatggac3'	5'tcggtagaaaacggggttta3'
Phlda2	NM_001100521	5'gcgacagcctgttccaggtatg3'	5'tgggttggaagcaggtaaccta3'
Plac1	NM_001024894	5'accctgctgcaagaatgaaa3'	5'cgcccatgttactgctaggt3'
Prl3b1	NM_012535	5'gcaccaaattaccgaatgtccact3'	5'tcagcagttgttgttataaatatcgcg3'
Rhox9	NM_001024874	5'gctgccaaagtttccaaaag3'	5'gatcttctcatccggaacca3'
Rsp1	NM_138537	5'ctgggcctctcatttgtgat3'	5'agacaactctgaaggattgatga3'
S1pr1	NM_017301	5'gaaactacacaacggcagca3'	5'atgatggggttggtacctga3'
Star	NM_031558	5'tctcaactggaagcaacactcta3'	5'taccagtcagtcctagtgtct3'
Gapdh	NM_017008	5'accacagtccatgccatcac3'	5'tccaccaccctgttgctgta3'

Table 2.3 Primers used for qRT-PCR

Symbol	GenBank Accession No.	Forward primer	Reverse primer
Adm	NM_012715	5'acgtctcggactttctgctt3'	5'gctgctggacgcttgtagtt3'
Aurbk	NM_053749	5'catccctgaggaggaagacca3'	5'actgtggctagggctctcaa3'
Ceacam3	NM_012702	5'tggtacaaagggctgacaaa3'	5'tccacaggtcaaagtggagaa3'
Cgm4	NM_012525	5'tagcccgatacagaacagcaa3'	5'agggtcacagcatgaggaaa3'
Ctsd	NM_134334	5'tacctgaacgtcacccgaaa3'	5'caggctggacaccttctcac3'
Ect2	NM_001108547	5'acgtcagaggagcttccaaa3'	5'ccctctgagctatgggatga3'
Fabp3	NM_024162	5'tttgacgaggtcacagcaga3'	5'acattgccatgggtgagagt3'
Fabp5	NM_145878	5'tggccaaaccagactgcatcat3'	5'tcgtcttcaccgtgctctcagt3'
Id2	NM_013060	5'gacatcagcatcctgtcctt3'	5'tctcctggtgaaatggttgat3'
Igf2	NM_031511	5'ggaagtcgatgttggtgctt3'	5'cttgcccacggggtatct3'
Klf2	NM_001007684	5'cctgtgaccgaggagaacaa3'	5'ccggctccgggtagtaga3'
Klf5	NM_053394	5'cacctcagcttcctccagtt3'	5'tacgcatggtctctgggatt3'
Mmp9	NM_031055	5'aacttcgacgctgacaagaa3'	5'tttagagccacgaccatacaga3'
Pik3cb	NM_053481	5'aaatttggcaggttccgccagt3'	5'tcaacatcagcgcaaacagggt3'
Prl4a1	NM_017036	5'gaccaccagatgccacactt3'	5'caggagctttatgtttgattcctt3'
Prl3b1	NM_012535	5'accatgcttctctgggacact3'	5'aggcttccagtggacattcggtaa3'
Rhob	NM_022542	5'aaacctccctccctctccc3'	5'tagaagtaccactgggctgggaa3'
Rsp1	NM_138537	5'gaggctaaagaggaccaggaa3'	5'tgcagcagaattgagcaagaa3'
Satb1	NM_001012129	5'tgagagggaaaggagcttga3'	5'tgttctctggcttcccattc3'
Seam6d	NM_001107768	5'ggccagtgatgctgtcattt3'	5'tatgttccacggcgatttct3'
Serpine1	NM_012620	5'agtctttccgaccaagagca3'	5'gtgccgaaccacaaagagaa3'

differentiating trophoblast cells, while at gestation d18.5, the placenta is fully mature and comprised of differentiated trophoblast cells. D11.5 tissue samples contained all trophoblast present within the placentation site, whereas d18.5 tissue samples were restricted to the junctional zone. Placentation site dissections were performed as previously described (Ain et al., 2006). Tissues for histological analysis were frozen in dry-ice cooled heptane and stored at -80°C. Tissue samples for RNA extraction were frozen in liquid nitrogen and stored at -80°C. The University of Kansas Animal Care and Use Committee approved protocols for the care and use of animals.

Maintenance of Rcho-1 TS cells

Rcho-1 TS cells were maintained at subconfluent conditions in Stem Medium [RPMI-1640 culture medium (Cellgro, Herndon, VA) supplemented with 20% fetal bovine serum (FBS; Atlanta Biologicals, Norcross, GA) 50 µM 2-mercaptoethanol, 1 mM sodium pyruvate (Cellgro), 100 µM penicillin, and 100 U/ml streptomycin (Cellgro)] as previously reported (Peters et al., 2000; Sahgal et al., 2006). Differentiation was induced by growing cells to near confluence in FBS-supplemented culture medium and then replacing the medium with Differentiation Medium [NCTC-135 medium (Sigma-Aldrich) supplemented with 1% horse serum (HS; Atlanta Biologicals), 50 µM 2-mercaptoethanol, 1 mM sodium pyruvate, 10 mM HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Fisher, Pittsburgh, PA), 38 mM sodium bicarbonate (Fisher), 100 µM penicillin and 100 U/ml streptomycin (Cellgro)]. High cell density and the absence of sufficient growth stimulatory factors (removal of FBS) facilitate trophoblast giant cell formation (Yamamoto et al., 1994; Sahgal et al., 2006). Trypsin (0.25%)-ethylenediamine tetraacetic acid (EDTA, 0.1% in Hank's Balanced Salt Solution, Cellgro) was used to passage the cells. Cells in the stem cell condition were grown in Stem Medium and collected 24 h after subculture to restrict the accumulation of spontaneously differentiating cells. Cells in the differentiation condition were grown for eight days in Differentiation Medium prior to harvesting unless otherwise noted. RNA samples were extracted using TRIzol (Invitrogen) according to the manufacturer's instructions.

Inhibition of PI3K

LY294002 (Calbiochem, La Jolla, CA) was used to inhibit PI3K (Vlahos et al., 1994). For chronic treatment experiments, Rcho-1 TS cells were grown to near confluence and then shifted to Differentiation Medium containing vehicle (0.1% final concentration of dimethyl sulfoxide, DMSO) or Differentiation Medium supplemented with LY294002 (10 μM). This LY294002 treatment regimen was based on our earlier report, which effectively disrupts PI3K signaling in Rcho-1 TS cells (Kamei et al, 2002). Cells were harvested after eight days of treatment. For acute inhibition of PI3K, cells were cultured for 6-12 days in Differentiation Medium and then shifted to Differentiation Medium containing vehicle (0.1% DMSO) or LY294002 (10 μM) for 48 h. Culture medium was replaced daily.

DNA microarray

Affymetrix 230 2.0 DNA microarray chips (Affymetrix, Santa Clara, CA) were probed with cDNAs generated from Rcho-1 TS cells grown under stem or differentiation conditions with chronic exposure to LY294002 or vehicle. Each treatment group was repeated in triplicate. RNA samples were hybridized to the Affymetrix 230 2.0 DNA microarray chip using the GeneChip® Hybridization Oven 640 (Affymetrix). Washing and staining of hybridized chips were conducted using the GeneChip® Fluidics Station 450 (Affymetrix). Chips were scanned using the Affymetrix GeneChip® Scanner 3000 (Affymetrix) with autoloader by the KUMC Biotechnology Support Facility. Hybridization signals were normalized with internal controls using the Mas5 algorithm in the Expression Console (Affymetrix) and fold change computed. Significant differences were determined by paired two-tailed Student *t*-tests. Microarray data was processed for functional analysis using Ingenuity Pathway Analysis (Redwood City, CA). Expression of genes in Rcho-1 TS cells and mouse trophoblast stem cells was compared using the "*Compare Lists of Genes*" program (http://elegans.uky.edu/MA/progs/Compare.html; Dr. James Lund, University of Kentucky, personal communication). Only genes annotated identically by Affymterix in both rat and mouse chips were included. Mouse trophoblast stem cell array data were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). TS 3.5 d0 (GSM325436) was compared to TS 3.5 d6 (GSM325442) (Ralston et al., 2010). Probe sets included in the analysis were restricted to those changing at least 1.5 fold between group comparisons with signal strengths of ≥800 for the maximal value.

Northern blotting

Northern blotting analysis was performed as previously described (Faria et al., 1990). Total RNA (20 µg) was separated in 1% formaldehyde-agarose gels and transferred to nitrocellulose membranes (Schleicher & Schuell Bioscience, Keene, NH). cDNA inserts were obtained by enzymatic digestion and labeled with [^{32}P] (NEN Life Science Products, Boston, MA) using Prime-it II random primer labeling kits (Stratagene, La Jolla, CA). See **Tables 2.1 and 2.2** for information on cDNAs. Probes were incubated with the blots at 42°C overnight and washed with 2XSSPE/0.1XSDS at 42°C twice for 25 min and 1XSSPE/0.1XSDS at 50°C for 35 min. Blots were then exposed to x-ray film at -80°C. Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used to assess RNA integrity and as a loading control.

qRT-PCR

cDNAs were reverse transcribed (RT) from RNA using reagents from Promega (Madison, WI) according to the manufacturer's instructions. SYBR GREEN PCR Master Mix (Applied Biosystems, Foster City, CA) was used in the PCR reaction. Reactions were run using a 7500 Real-Time PCR System (Applied Biosystems). Conditions included an initial holding stage (50°C for 2 min and 95°C for 10 min) and 40 cycles (95°C for 15 s and 60°C for 1 min) followed by a dissociation stage (95°C for 15 s, 60°C for 1 min, and then 95°C for 15 s). Primers are listed in **Table 2.3**. Expression of 18S ribosomal RNA was used as an internal control. At least four replicates were run for each condition. Samples were normalized to the control sample for each gene. Statistical comparisons of two means were evaluated with Student's *t*-test.

In situ hybridization

mRNAs were localized in placental tissues using nonradioactive in situ hybridization as previously described (Ain et al., 2003; Wiemers et al., 2003). Ten μm cryosections were prepared and stored at -80°C until used. Plasmids containing cDNAs were used as templates to synthesize sense and antisense digoxigenin-labeled riboprobes according to the manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, IN). Information on the cDNAs for probe generation is presented in **Tables 2.1 and 2.2**. Tissue sections were air dried and fixed in ice cold 4% paraformaldehyde in PBS. Prehybridization, hybridization, and detection of alkaline phosphatase-conjugated anti-digoxigenin were preformed as previously reported (Ain et al., 2003; Wiemers et al., 2003). Images were captured using a Leica MZFLIII stereomicroscope equipped with a Leica CCD camera (Leica Microsystems GmbH, Welzlar, Germany).

Immunocytochemistry

Rcho-1 TS cells were cultured on chamber slides under stem, differentiation, or differentiation conditions with chronic exposure to LY294002. Cells were fixed in ice-cold 4% paraformaldehyde**.** Actin filaments were visualized using rhodamine-conjugated phalloidin (Molecular Probes, Eugene, OR). Nuclei were stained with 4,6'-diamidino-2-phenylindole (DAPI, Molecular Probes). Bright field and fluorescence images were captured using either Leica MZFLIII stereomicroscope or DMI 4000 microscopes equipped with CCD cameras (Leica).

Analysis of DNA content

DNA content was estimated by flow cytometry (Quinn et al., 2006). Cells were trypsinized and fixed in 70% ethanol and then stained with propidium iodine and analyzed using a BDLSRIII flow cytometer (BD Biosciences, San Jose, CA**).**

Steroid hormone measurements

Steroid radioimmunoassays (RIAs) were performed as previously reported (Yamamoto et al., 1996). Androstenedione and progesterone concentrations were measured in Rcho-1 trophoblast cell conditioned medium with ^{125}I-labelled RIA kits (Diagnostic Products, Los Angeles, CA) and normalized to cellular DNA content. DNA samples were obtained by lysis of cells with digestion buffer containing proteinase K. Samples were then incubated at 37°C overnight and diluted 10X with water. DNA content was then measured with the PicoGreen®dsDNA Quantitation Kit (Molecular Probes) according to the manufacturer’s instructions. Statistical comparisons of two means were evaluated with Student’s *t*-test.

RESULTS

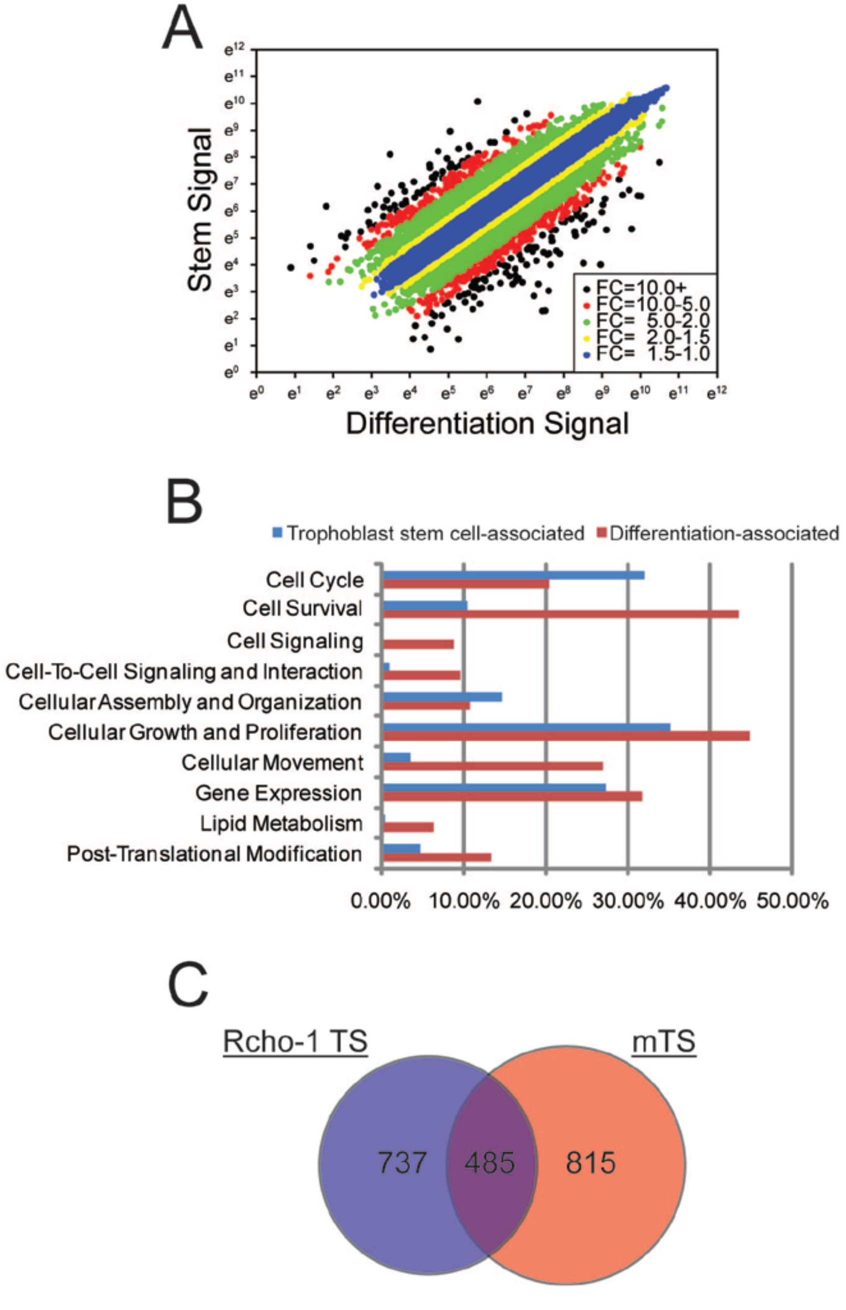
Identification of genes associated with trophoblast differentiation

Phenotypes of trophoblast cells connected to distinct developmental states were assessed by DNA microarray analysis. In summary, RNA was collected from Rcho-1 TS cells in the stem and differentiated states. Gene expression was compared between the two states using an Affymetrix DNA microarray. Samples were run in triplicate. Gene-restricted expression patterns associated with stem cell and differentiated states were identified (**Fig. 2.1A**). All DNA microarray data presented in this chapter have been deposited in the Gene Expression Omnibus (GEO) repository under the GSE21938 accession number (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE21938).

Trophoblast stem-associated genes

Approximately half of the genes differentially expressed between the stem cell- and differentiated cell-states were specific to the stem cell state, termed ‘trophoblast stem cell-associated’ genes. An abbreviated list of ‘trophoblast stem cell-associated’ genes is shown in **Table 2.4**. Genes listed in this table are those with arbitrary expression signal strengths ≥800 in the stem cell condition and displaying a significantly higher level of expression in the stem cell state versus the differentiated state (≥1.5 fold; P≤0.05). We used Ingenuity Pathway Analysis software to investigate ‘trophoblast stem cell-associated’ genes. Of the 1720 probe sets (complete list provided in **Table 6.1**), 584 genes were

Fig.2.1. Analysis of microarray data. A) Scatter graph showing the signal intensity of probe sets. **B)** Functional analysis of ‘trophoblast stem cell-associated’ genes and ‘differentiation-associated’ genes using Ingenuity Pathway Analysis software. **C)** Comparison of ‘trophoblast stem cell-associated’ genes in Rcho-1 TS cells and mouse trophoblast stem cells. **D)** Examples of ‘trophoblast stem cell-associated’ genes unique to or shared between Rcho-1 TS cells and mouse trophoblast stem cells.



D

	Trophoblast stem cell-associated genes
Unique to Rcho-1 TS	Atp1a1, Bex1, **Elf3**, **Hes1**, **Id3**, **Mif**, **Msx1**, **Pgam1**, **S1pr1**, **Spint1**, **Stat1**
mTS and Rcho-1 TS	Aurkb, Bmp4, Ccna2, Ccnd3, Cdx2, Chek1, Ect2, Eomes, Fabp3, Fgfr2, Id1, Id2, Klf5, Phlda2, Satb1, Slc16a3
Unique to mTS	Cyp26a1, Cyr61, Elf5, Fgfr1, Gata1, Grb10, Igf1r, Klf10, Msx2, Notch3, Parp1, Prom1, Smad1/3/4/6/7, Sox2, Vefga

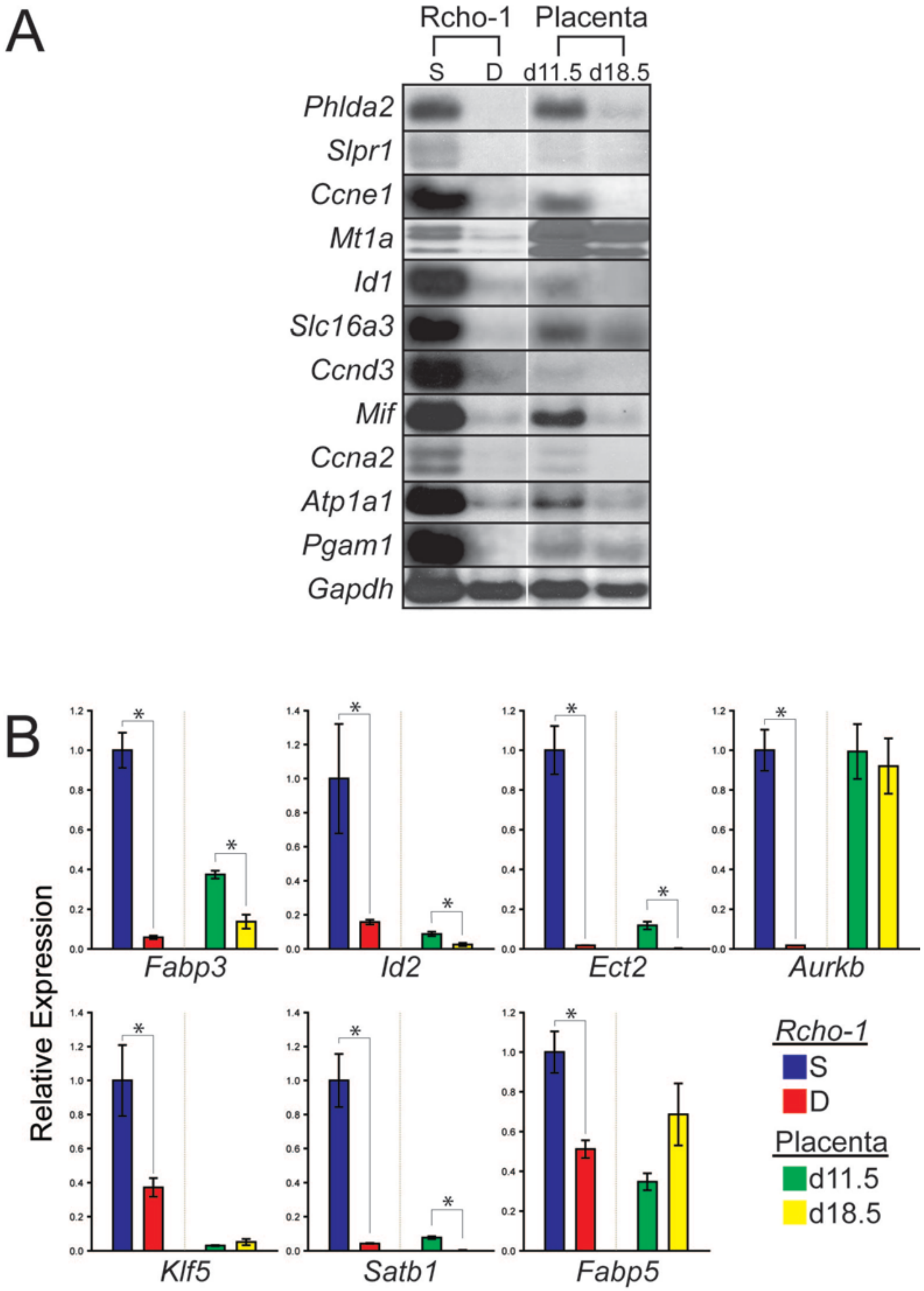
Table 2.4. Trophoblast stem cell associated genes

Gene name	Abbreviation	Synonyms	Functional Group	GenBank Accession No.	Fold Change S/D
Fatty acid binding protein 3	*Fabp3*		Fatty acid binding	NM_024162	71.41
Pleckstrin homology-like domain, family A, member 2	*Phlda2*	*Ipl, Tssc3*	Placental growth	NM_001100521	48.67
Inhibitor of DNA binding 2	*Id2*		Transcription regulator	NM_013060	21.03
Sphingosine-1-phosphate receptor 1	*S1pr1*	*Edg1, IpB1*	Lipid receptor	NM_017301	16.08
Cyclin E	*Ccne1*	*Ccne*	Cell cycle regulator	NM_001100821	11.52
Metallothionein 1a	*Mt1a*	*Mt*	Protection from oxidative stress	NM_138826	10.75
Ect2 oncogene	*Ect2*		Ras signaling	NM_001108547	10.06
Aurora kinase B	*Aurkb*	*Aim1, Stk12*	Kinase	NM_053749	9.67
Kruppel-like factor 5	*Klf5*	*IKLF, bteb2*	Transcription regulator	NM_053394	8.85
Inhibitor of DNA binding 1	*Id1*		Transcription regulator	NM_012797	8.64
Solute carrier family 16 (monocarboxylic acid transporters), member 3	*Slc16a3*	*Mct3, Mct4*	Transporter	NM_030834	6.57
Special AT-rich sequence binding protein 1	*Satb1*		DNA binding	NM_001012129	6.17
Cyclin D3	*Ccnd3*		Cell cycle regulator	NM_012766	6.40
Macrophage migration inhibitory factor	*Mif*	*GIF, Glif*	Ligand, chemokine	NM_031051	5.32
Cyclin A2	*Ccna2*	*Ccn1, Ccna, Cyca*	Cell cycle regulator	NM_053702	4.49
ATPase, Na+/K+ transporting, alpha 1 polypeptide	*Atp1a1*	*Nkaa1b, Atpa-1*	NA+/K+ pump	NM_012504	3.95
Phosphoglycerate mutase 1	*Pgam1*	*Pgmut*	Metabolism	NM_053290	3.03
Fatty acid binding protein 5, epidermal	*Fabp5*	*C-Fabp, E-Fabp*	Fatty acid binding	NM_145878	1.43

annotated by Ingenuity Pathway Analysis software. Functions associated with the annotated ‘trophoblast stem cell-associated’ genes included cellular growth and proliferation (35%), cell cycle (32%), and cellular assembly and organization (15%), (**Fig. 2.1B; Table 6.2**). Not surprisingly, the analysis indicates that a large percentage of ‘trophoblast stem cell-associated’ genes have functions that correlate with the proliferative phenotype of these cells.

A subset of ‘trophoblast stem cell-associated’ genes identified from the microarray analysis was further evaluated (**Table 2.4**). Transcript levels were estimated by northern analysis or qRT-PCR in Rcho-1 TS cells from stem and differentiated states. Each of the genes was expressed at higher levels in the trophoblast stem cell state (**Fig. 2.2**). Approximately half of the ‘trophoblast stem cell-associated’ genes showed elevated expression in midgestation versus late gestation trophoblast tissues (**Fig. 2.2**). The validated ‘trophoblast stem cell-associated’ genes encode proteins involved in cell cycle regulation (*Ccne1*, *Ccna2*, *Ccnd3, Klf5*, *Ect2*), inhibition of differentiation (*S1pr1*, *Id1, Id2*), inhibition of placental growth (*Phlda2*), and protection from cytotoxic agents (*Mt1a1*). Other ‘trophoblast stem cell-associated’ genes were previously detected in proliferative populations of trophoblast (*Slc16a3, Mif, Atp1a1*). Many of the ‘trophoblast stem cell-associated’ genes identified in Rcho-1 cells are also found in mouse trophoblast stem cells (**Fig. 2.1C, 2.1D**). Conspicuous among the genes unique to mouse trophoblast stem cells is *Elf5*, while *Atp1a1*, *Id3*, *Mif*, *Pgam1*, and *S1pr1* are unique to the Rcho-1 trophoblast stem cell population (**Fig. 2.1D**).

Fig. 2.2. Expression of a subset of ‘trophoblast stem cell-associated’ genes. A) Representative northern blot analysis of ‘trophoblast stem cell-associated’ genes identified by DNA microarray analysis. **B)** qRT-PCR analysis of ‘trophoblast stem cell-associated’ genes identified by DNA microarray analysis. See **Table 2.4** for a list and description of the mRNAs investigated. Rcho-1 TS cells were cultured under stem (S) or differentiating (D) conditions. Rat placental samples were also included in the analysis and are from gestation d11.5 trophoblast and d18.5 junctional zone. Student *t*-tests (*$P<0.05$).


A
Rcho-1
Placenta
S
D
d11.5
d18.5
Phlda2
Slpr1
Ccne1
Mt1a
Id1
Slc16a3
Ccnd3
Mif
Ccna2
Atp1a1
Pgam1
Gapdh
B
Relative Expression
Fabp3
Id2
Ect2
Aurkb
Klf5
Satb1
Fabp5
*
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Rcho-1
🟦 S
🟥 D
Placenta
🟩 d11.5
🟨 d18.5

Trophoblast differentiation-associated genes

The second collection of genes exhibiting changes in mRNA expression is upregulated in association with differentiation and referred to as ‘differentiation-associated’ genes. An abbreviated list of ‘differentiation-associated’ genes is shown in **Table 2.5** and the complete list is presented in **Table 6.3**. Genes listed in this category are those with arbitrary expression signal strengths ≥800 in the differentiated cell condition and displaying a significantly higher level of expression in the differentiated cell state versus the stem cell state (≥1.5 fold; P≤0.05). Of the 1585 probe sets listed in **Table 6.3**, 537 genes were annotated by Ingenuity Pathway Analysis software. Functions associated with the annotated ‘differentiation-associated’ genes included cellular growth and proliferation (45%), cell survival (43%), gene expression (32%), cellular movement (27%), and lipid metabolism (6%) (**Fig. 2.1B; Table 6.2**). Many of the genes associated with the cellular growth and proliferation classification encode growth factors, cytokines, and peptide hormones (e.g. *Igf2, Grn*, members of the prolactin, PRL, family, etc); and represent features of the endocrine phenotype of trophoblast giant cells. Genes linked to cell movement and lipid metabolism, include those encoding proteins contributing to the invasive and steroid hormone producing phenotypes of trophoblast giant cells.

A sampling of ‘differentiation-associated’ genes identified from the microarray analysis was further examined (**Table 2.5**). Transcript levels were estimated by northern analysis or qRT-PCR in Rcho-1 TS cells from stem and

Table 2.5. Trophoblast differentiation associated genes

Gene name	Abbreviation	Synonyms	Functional Group	GenBank Accession No.	Fold Change D/S
Keratin complex 1, acidic, gene 19	*Krt19*	*EndoC, K19*	Cytoskeletal protein	NM_199498	149.07[t]
Carcinoembryonic antigen gene family 4	*Cgm4*	*Psg16*	Secretory protein, unknown function	NM_012525	89.73
Carcinoembryonic antigen-related cell adhesion molecule 3	*Ceacam3*	*Cgm1*	Cell adhesion molecule	NM_012702	88.71
Cytochrome P450, family 11, subfamily a, polypeptide 1	*Cyp11a1*	*P450scc*	Steroidogenic enzyme	NM_017286	47.11
Prolactin family 4, subfamily a, member 1	*Prl4a1*	*PLP-A*	Ligand/cytokine	NM_017036	33.05
Spleen protein 1 precursor	*Rsp1*	*Sslp1, LOC171573*	Unknown	NM_138537	30.29
Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	*Slc28a2*	*Cnt2*	Transporter, nucleotide	NM_031664	23.94
Interleukin 17F	*Il17f*	*ML1*	Ligand/cytokine	NM_001015011	22.49
Fibronectin 1	*Fn1*	*Fn*	Extracellular matrix protein	NM_019143	19.68
H19 fetal liver mRNA	*H19*	*ASM1*	Unknown	NR_027324	13.10
Cytochrome P450, family 17, subfamily a, polypeptide 1	*Cyp17a1*	*Cyp17, p450c17*	Steroidogenic enzyme	NM_012753	10.76
Hydroxysteroid (17-beta) dehydrogenase 2	*Hsd17b2*		Steroidogenic enzyme	NM_024391	10.56
Placenta-specific 1	*Plac1*	*Epcs26*	Cell-cell comunication	NM_001024894	9.62
CEA-related cell adhesion molecule 10	*Ceacam10*	*C-CAM4*	Cell adhesion molecule	NM_173339	8.66
Differentaly expressed X chromosome EST 1	*Dif EST 1*			AI012949	8.61
Prolactin family 3, subfamily b, member 1	*Prl3b1*	*PL-II, Csh2*	Ligand/cytokine	NM_012535	8.38
CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	*Cd47*	*IAP, Itgp*	Receptor, thrombospondin	NM_019195	7.90
Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	*Sema6d*		Receptor	NM_001107768	8.38
Differentaly expressed X chromosom EST 2	*Dif EST 2*	LOC681066		AA964255	7.40
Serine (or cysteine) peptidase inhibitor, clade E, member 1	*Serpine1*	*Pai1, Planh*	blood coagulation, angiogenesis	NM_012620	6.41
Extracellular matrix protein 1	*Ecm1*		Extracellular protein	NM_053882	6.11
Adrenomedullin	*Adm*		Hypotensive peptide	NM_012715	5.81

Moesin	*Msn*		Cell-cell comunication	NM_030863	5.66
DNA-damage inducible transcript 3	*Ddit3*	*Chop10, Gadd153*	Cell stress/death	NM_001109986	5.65
Melanoma antigen, family D, 1	*Maged1*	*Nrage, Dlxin1*	Apoptosis, cell cycle, transcription	NM_053409	5.61
Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	*Cited2*	*Mrg1, Msg2, p35srj*	Transcription regulator	NM_053698	5.49
Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	*Hsd3b1*		Steroidogenic enzyme	NM_001007719	5.15
Fos-like antigen 1	*Fra1*	*Fosl1*	Transcription regulator	NM_012953	5.04
Legumain	*Lgmn*	*Prsc1*	Putative cysteine protease	NM_022226	4.73
Insulin-like growth factor 2	*Igf2*	*Igf-II*	Ligand, growth factor	NM_031511	4.38
Tribbles homolog 3 (Drosophila)	*Trib3*	*Trb3, Ifld2, Nipk*	Metabolism	NM_144755	4.37
High mobility group box transcription factor 1	*Hbp1*	*HMGB1*	Transcription regulator	NM_013221	4.36
Cathepsin D	*Ctsd*	*CD, CatD*	Lysosomal aspartic endopeptidase	NM_134334	4.13
Matrix metallopeptidase 9	*Mmp9*	*Gelatinase B*	Extracellular matrix remodeling	NM_031055	4.00
Fas ligand (TNF superfamily, member 6)	*Faslg*	*Faslg, Tnfsf6*	Ligand/membrane anchored	NM_012908	3.80
Reproductive homeobox on X chromosome, 9	*Rhox9*	*Gpbox, Psx2*	Transcription regulator	NM_001024874	3.43
Granulin	*Grn*	*Gep, Pcdgf, Pgrn*	Ligand, growth factor	NM_017113	3.39
CD9 antigen	*Cd9*	*Tspan29*	Cell surface glycoprotein	NM_053018	3.14
RhoB gene	*Rhob*	*Arhb*	Ras family	NM_022542	3.07
Phosphatidylinositol 3-kinase, catalytic, beta polypeptide	*Pik3cb*		Inositol lipid kinase	NM_053481	2.75
Kruppel-like factor 2 (lung)	*Klf2*		Transcription regulator	NM_001007684	2.73
Jun-B oncogene	*Junb*		Transcription regulator	NM_021836	2.35
Tissue factor pathway inhibitor	*Tfpi*	*EPI, LACI*	Kunitz family serine protease inhibitor	NM_017200	2.20
Nuclear factor, erythroid derived 2, like 2	*Nfe2l2*	*Nrf2*	Transcription regulator	NM_031789	2.09

[t] Not significant by Student t-test

differentiated states. Each of the genes was expressed at higher levels in the differentiated cell state (**Fig. 2.3**). All of the ‘differentiation-associated’ genes were detected in placental tissues and approximately half showed elevated expression in late gestation versus midgestation trophoblast tissues (**Fig. 2.3**). Several of the validated ‘differentiation-associated’ genes (**Table 2.5; Fig. 2.3**) have been previously reported as upregulated during trophoblast giant cell development, while others have not been associated with trophoblast lineages (e.g. *Rsp1, Sema6d, Ceacam10, Cd47, Maged1, Trib3, Hbp1* and *Pik3cb*). Functions of the ‘differentiation-associated’ genes have been connected to the regulation of cell movement and invasion (*Serpine1*, *Adm, Msn*, *Maged1*, *Cited2*, *Fra1*, *Ifg2*, *Hbp1*, *Mmp9*, *Grn*, *Cd9*), interactions with maternal immune and vascular systems, (*Cgm4*, *Prl4a1*, *Cd47, Ecm1*, *Ctsd, Faslg*, *Grn*, *Cd9*, *Tfpi*), and the endocrine phenotype of trophoblast giant cells (PRL family and steroid biosynthesis).

A subset of ‘differentiation-associated’ mRNAs highly expressed in rat placental samples (**Fig. 2.3**) were localized to the placentation site via in situ hybridization (**Fig 2.4**). ‘Differentiation-associated’ transcripts were all found in trophoblast giant cells and in most instances other trophoblast lineages. *Ecm1* mRNA is expressed in trophoblast giant cells and some progenitor trophoblast cells on gestation d11.5. *Tfpi, Cited2,* and *Rsp1* transcripts were localized to trophoblast giant cells on gestation d11.5, including those penetrating into the uterine spiral arterioles. On gestation d18.5, *Tfpi, Cited2,* and *Rsp1* were also identified in spongiotrophoblast. *Cgm4* and *Grn* transcripts were expressed in

Fig. 2.3. Expression of a subset of trophoblast ‘differentiation-associated’ genes. A) Representative northern blot analysis of trophoblast ‘differentiation-associated’ genes identified by DNA microarray analysis. **B)** qRT-PCR analysis of trophoblast ‘differentiation-associated’ genes identified by DNA microarray analysis. See **Table 2.5** for a list and description of the mRNAs investigated. Rcho-1 TS cells were cultured under stem (S) or differentiating (D) conditions. Rat placental samples were also included in the analysis and are from gestation d11.5 trophoblast and d18.5 junctional zone. Student *t*-tests (*P<0.05).

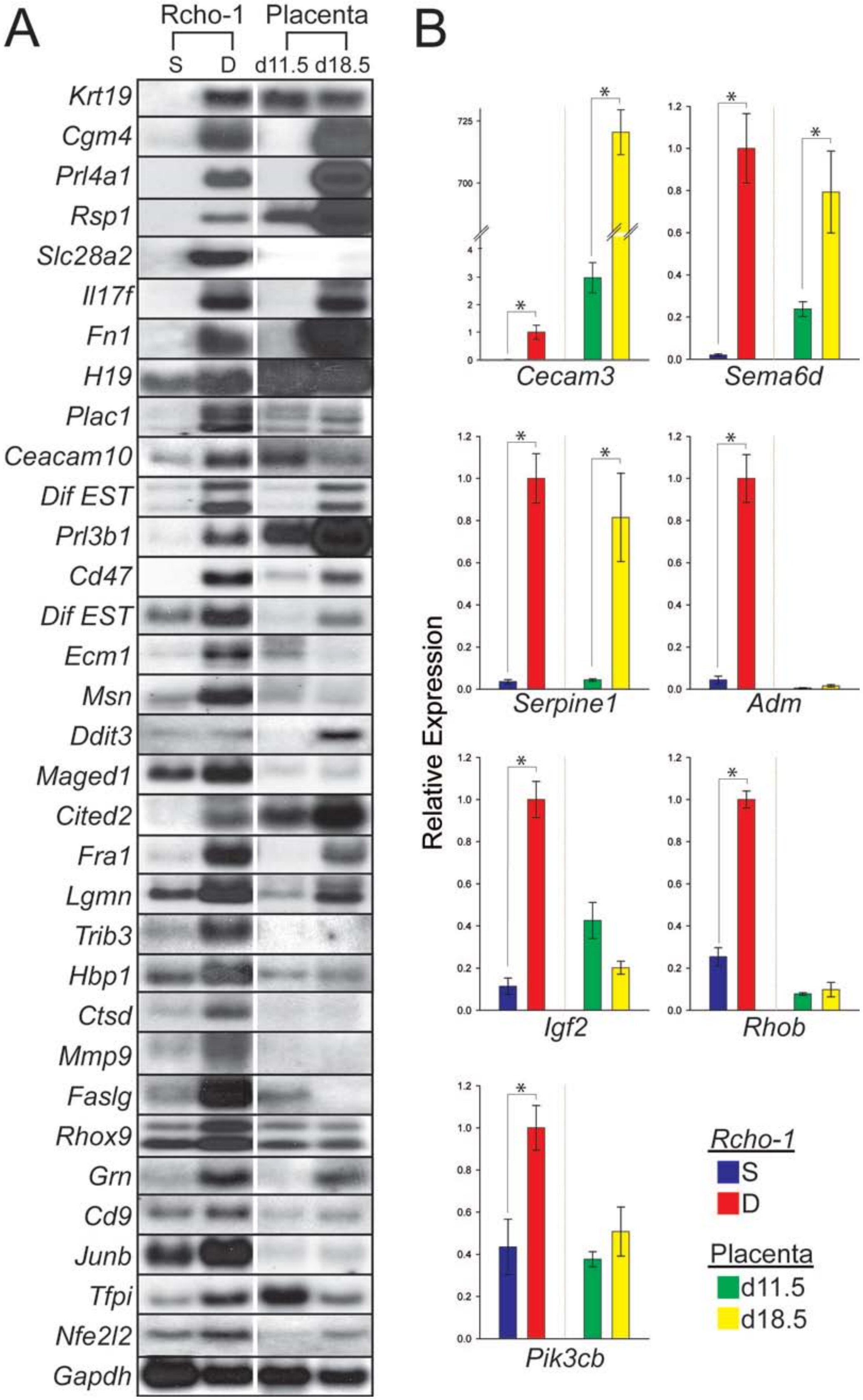
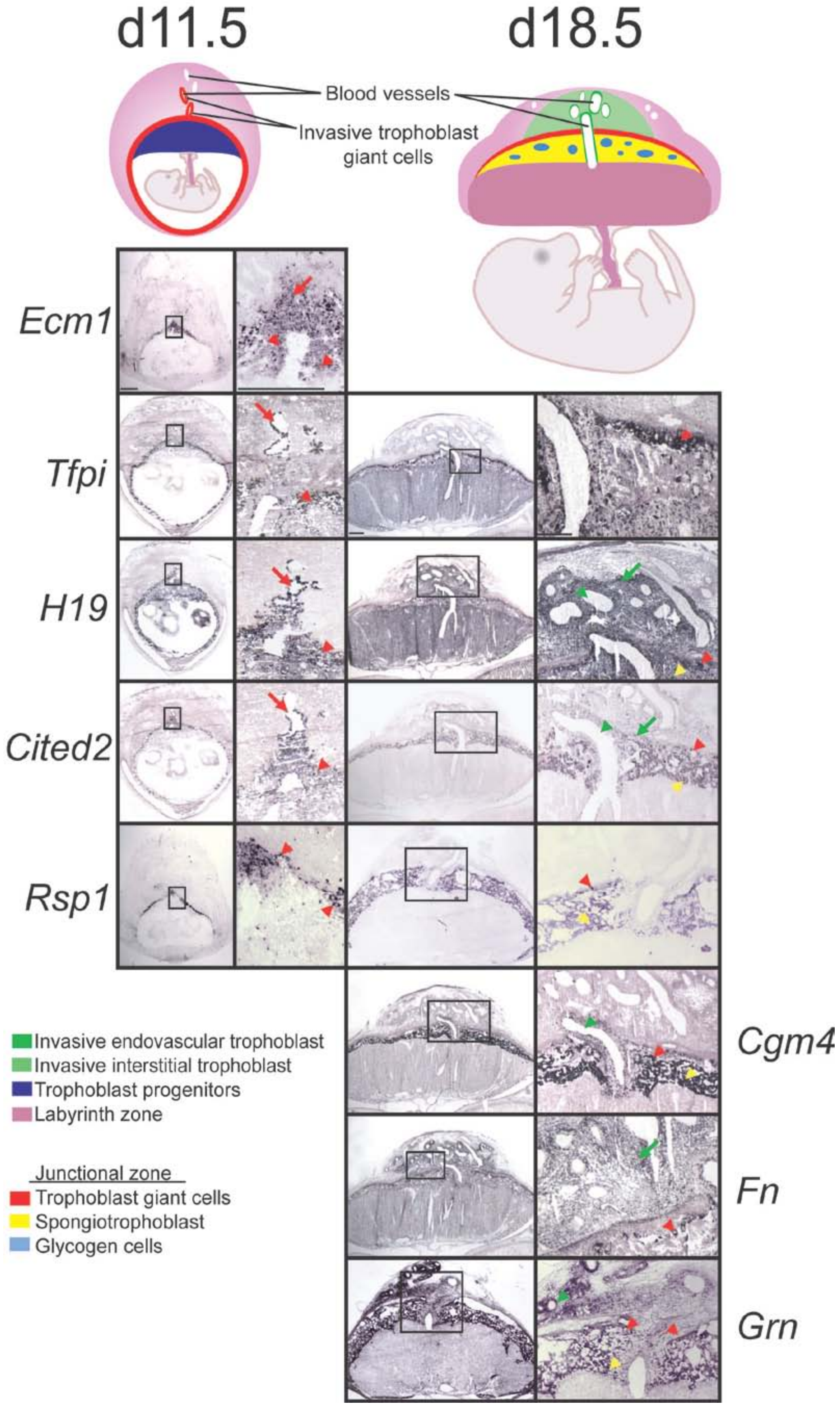

A
Rcho-1
Placenta
S
D
d11.5
d18.5
Krt19
Cgm4
Prl4a1
Rsp1
Slc28a2
Il17f
Fn1
H19
Plac1
Ceacam10
Dif EST
Prl3b1
Cd47
Dif EST
Ecm1
Msn
Ddit3
Maged1
Cited2
Fra1
Lgmn
Trib3
Hbp1
Ctsd
Mmp9
Faslg
Rhox9
Grn
Cd9
Junb
Tfpi
Nfe2l2
Gapdh
B
Relative Expression
725
700
4
3
2
1
0
Cecam3
Sema6d
Serpine1
Adm
Igf2
Rhob
Pik3cb
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Rcho-1
S
D
Placenta
d11.5
d18.5

Fig. 2.4. 'Differentiation-associated' genes are expressed by trophoblast cells developing within the chorioallantoic placenta. In situ detection of mRNA expression of 'differentiation-associated' genes in gestational d11.5 and d18.5 rat placentation sites is presented. Bars = 1 mm. Left panels, d11.5 placentation sites, far left: low magnification; middle left: high magnification of boxed area. Right panels, d18.5 placentation sites, middle right: low magnification; far right: high magnification of boxed area. Red arrowheads, trophoblast giant cells; red arrows, invasive trophoblast giant cells; yellow arrows, spongiotrophoblast; green arrowheads, endovascular invasive trophoblast; green arrows, interstitial invasive trophoblast.


d11.5
d18.5
Blood vessels
Invasive trophoblast giant cells
Ecm1
Tfpi
H19
Cited2
Rsp1
Cgm4
Fn
Grn
🟩 Invasive endovascular trophoblast
🟩 Invasive interstitial trophoblast
🟦 Trophoblast progenitors
🟪 Labyrinth zone
Junctional zone
🟥 Trophoblast giant cells
🟨 Spongiotrophoblast
🟦 Glycogen cells

trophoblast giant cells, spongiotrophoblast, and invasive trophoblast cells on gestation d18.5. *H19* mRNA was expressed in all trophoblast lineages on gestation d11.5 and d18.5. *Fn* mRNA was expressed in all trophoblast lineages on d18.5.

PI3K signaling and trophoblast differentiation

The PI3K signaling pathway has been implicated in the regulation of trophoblast differentiation (Kamei et al., 2002; Nadra et al., 2006) and was further investigated in this report. Initially we examined the effect of disruption of PI3K during trophoblast differentiation on the distribution of actin filaments and DNA content (**Fig. 2.5**). Actin filaments were not significantly affected by the PI3K inhibitor treatment regimen used (LY294002, 10 µM; **Fig. 2.5A**). However, inhibition of PI3K did affect ploidy. Disruption of PI3K resulted in a significant fraction of cells with increased DNA content, and thus the generation of giant cells with elevated ploidy levels (**Fig. 2.5B, 2.5C**). The findings suggest that PI3K restricts the formation of trophoblast giant cells with high ploidy levels (>32N). Higher concentrations of PI3K inhibitors interfere with actin filament distributions and cell survival (data not shown). Phenotypes of differentiating trophoblast cells treated with the PI3K inhibitor (LY294002, 10 µM) or vehicle were also assessed by DNA microarray analysis. Some genes identified were negatively regulated and others positively regulated by PI3K signaling (**Fig. 2.6A**).

Fig. 2.5. PI3K impact on trophoblast cell differentiation: morphology and DNA content. Morphology and DNA content were assessed in Rcho-1 TS cells cultured in the following conditions: stem (Stem), differentiating (Dif), differentiating with vehicle exposure (0.1% DMSO; Dif+V), or LY294002 (10 μM; Dif+LY). **A)** Morphology was determined by bright field microscopy (top panels). Actin filaments were stained with rhodamine-conjugated phalloidin; nuclei were visualized with DAPI (bottom). Bar= 50 μm. **B)** DNA content was estimated by propidium iodine staining followed by flow cytometry. Due to the tetraploid nature of the Rcho-1 TS cells, 4N and 8N cell populations correspond to dividing cells, whereas cell populations with more than 8N DNA content have undergone endoreduplication**. C)** Graphic representation of three independent flow cytometry experiments. Student t-tests (*P<0.05).

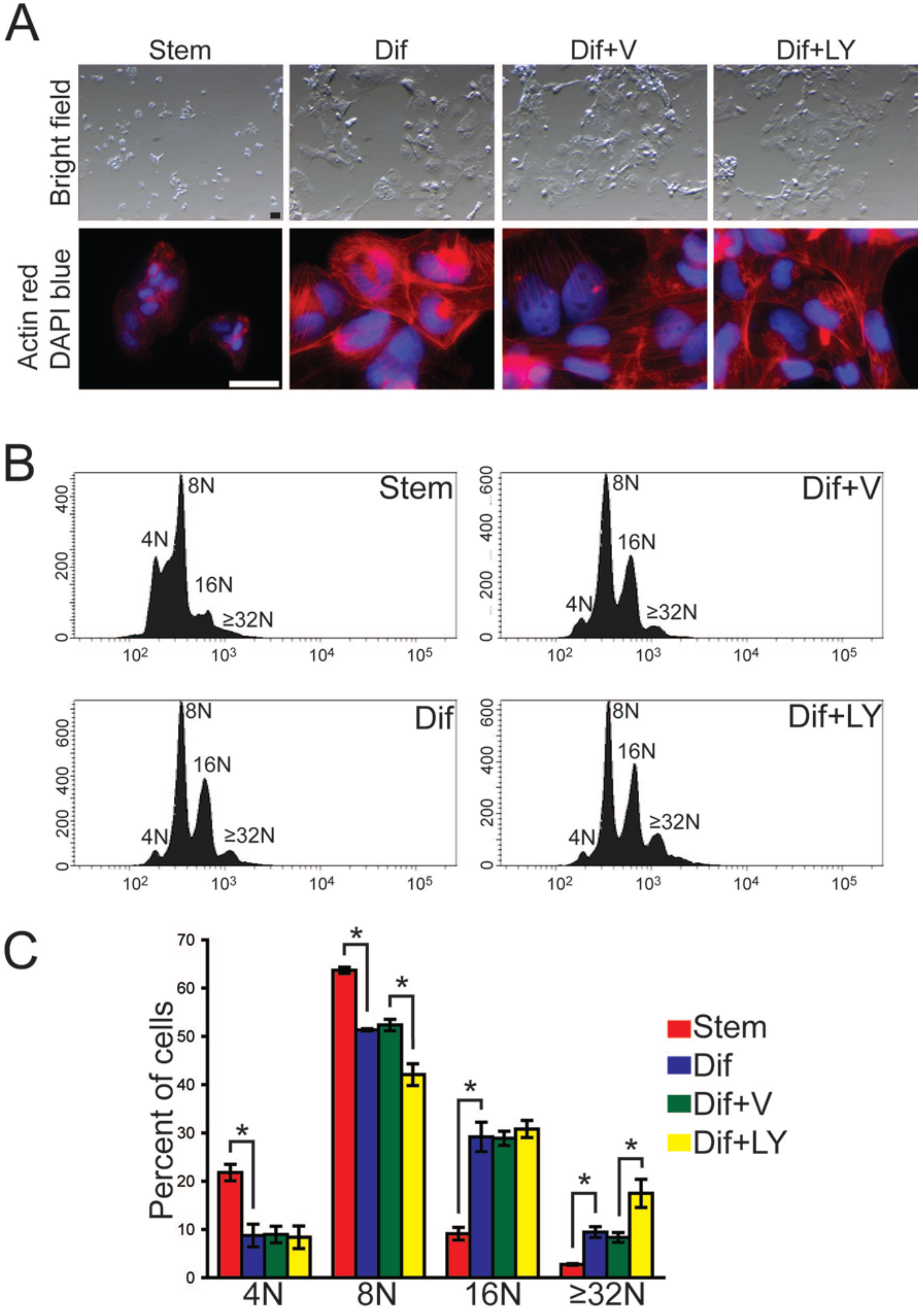
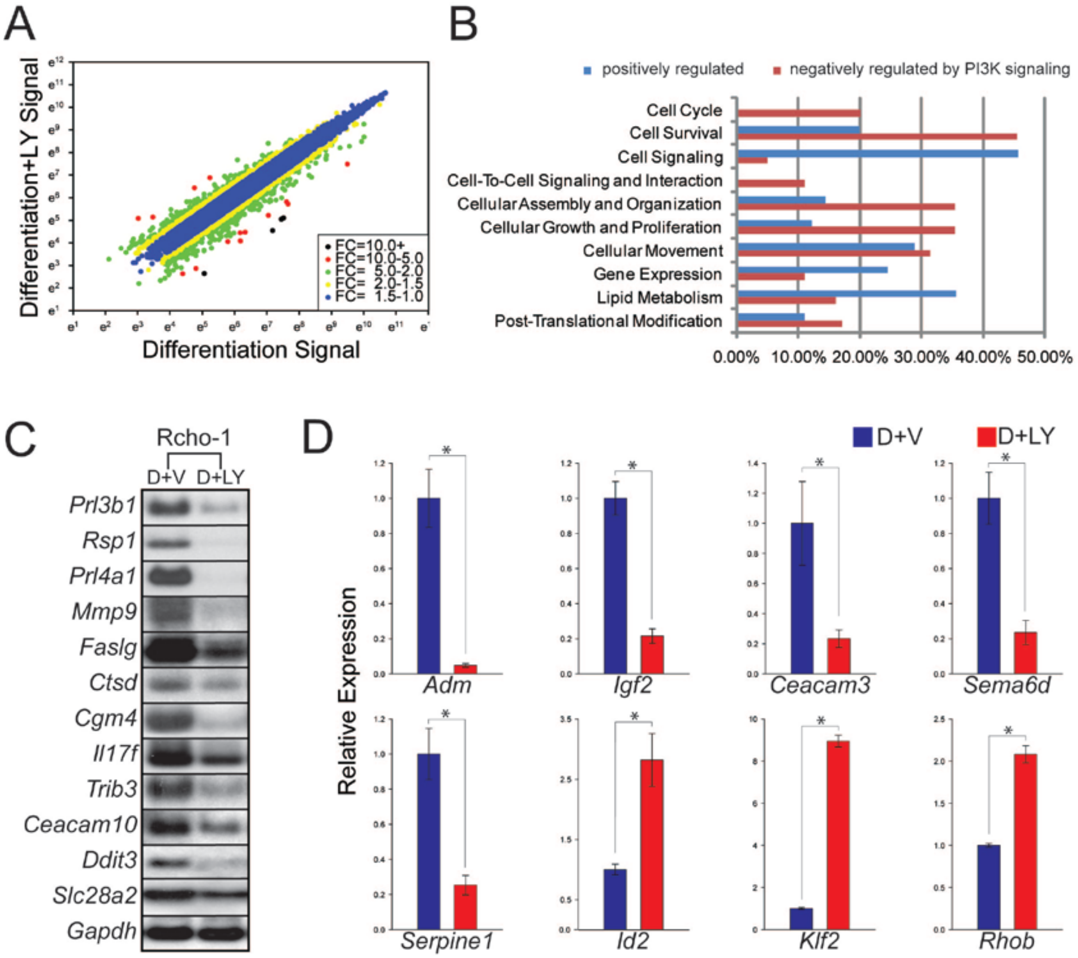

A
Stem
Dif
Dif+V
Dif+LY
Bright field
Actin red
DAPI blue
B
Stem
Dif+V
Dif
Dif+LY
4N
8N
16N
≥32N
0
200
400
600
10^2
10^3
10^4
10^5
C
Percent of cells
0
10
20
30
40
50
60
70
4N
8N
16N
≥32N
*
Stem
Dif
Dif+V
Dif+LY

Fig. 2.6. PI3K regulation: Analysis of microarray data and expression of a subset of PI3K regulated genes. A) Scatter graph showing the signal intensity of probe sets. **B)** Functional analysis of negatively and positively regulated genes by PI3K using Ingenuity Pathway Analysis software. **C)** Representative northern blot analyses of genes regulated by PI3K originally identified by DNA microarray analysis. **D)** qRT-PCR analysis of genes regulated by PI3K originally identified by DNA microarray analysis. See **Tables 2.6** for a list and description of the mRNAs investigated. Rcho-1 TS cells were cultured under differentiating conditions with exposure to vehicle (0.1% DMSO; D+V) or LY294002 (10 µM; D+LY). Student *t*-tests (*P<0.05).


A
Differentiation+LY Signal
Differentiation Signal
FC=10.0+
FC=10.0-5.0
FC= 5.0-2.0
FC= 2.0-1.5
FC= 1.5-1.0
B
positively regulated
negatively regulated by PI3K signaling
Cell Cycle
Cell Survival
Cell Signaling
Cell-To-Cell Signaling and Interaction
Cellular Assembly and Organization
Cellular Growth and Proliferation
Cellular Movement
Gene Expression
Lipid Metabolism
Post-Translational Modification
0.00%
10.00%
20.00%
30.00%
40.00%
50.00%
C
Rcho-1
D+V
D+LY
Prl3b1
Rsp1
Prl4a1
Mmp9
Faslg
Ctsd
Cgm4
Il17f
Trib3
Ceacam10
Ddit3
Slc28a2
Gapdh
D
D+V
D+LY
Relative Expression
Adm
Igf2
Ceacam3
Sema6d
Serpine1
Id2
Klf2
Rhob
*

PI3K signaling: negatively regulated genes

The ‘negatively regulated’ PI3K dependent genes are diverse in their expression patterns (**Table 6.4**). An abbreviated list of ‘negatively regulated’ PI3K dependent genes are shown in **Table 2.6.** Some are ‘trophoblast stem cell-associated’ genes; others are ‘differentiation-associated’ genes, while still others were not affected by differentiation state. Genes listed in **Table 6.4** are those with arbitrary expression signal strengths ≥800 in the differentiated cell condition and displaying a significantly lower level of expression in the differentiated cell state versus the differentiated cell state treated with the PI3K inhibitor (≥1.5 fold; P≤0.05). Of the 257 probe sets listed in **Table 6.4**, 99 genes were annotated by Ingenuity Pathway Analysis software. Functions associated with the annotated ‘negatively regulated’ genes included cell survival (45%), cellular assembly and organization (35%), cellular growth and proliferation (35%), cellular movement (31%), and lipid metabolism (16%) (**Fig. 2.6B; Table 6.2**). These functions overlap with those observed for both the ‘trophoblast stem cell associated’ and ‘differentiation-associated’ gene profiles (**Fig. 2.1**). Of the sixteen validated ‘trophoblast stem cell-associated’ genes only *Id2* was regulated by PI3K signaling (**Fig 2.6D**). *Klf2* and *Rhob* expression was not affected by differentiation state but was negatively regulated by PI3K (**Fig. 2.6D**).

Table 2.6. PI3K regulated genes

Genes negatively regulated by PI3K

Gene name	Abbreviation	Functional Group	GenBank Accession No.	Fold Change D/D+LY
Kruppel-like factor 2 (lung)	*Klf2*	Transcription regulator	NM_001007684	4.70
Inhibitor of DNA binding 2	*Id2*	Transcription regulator	NM_013060	3.53
RhoB gene	*Rhob*	Ras family	NM_022542	2.08*

Genes positively regulated by PI3K

Gene name	Abbreviation	Functional Group	GenBank Accession No.	Fold Change D/D+LY
Adrenomedullin	*Adm*	Hypotensive peptide	NM_012715	-14.40
Prolactin family 3, subfamily b, member 1	*Prl3b1*	Ligand/cytokine	NM_012535	-7.73
Cytochrome P450, family 17, subfamily a, polypeptide 1	*Cyp17a1*	Steroidogenic enzyme	NM_012753	-4.57
Spleen protein 1 precursor	*Rsp1*	Unknown	NM_138537	-4.43
Serine (or cysteine) peptidase inhibitor, clade E, member 1	*Serpine1*	blood coagulation, angiogenesis	NM_012620	-3.95*
Prolactin family 4, subfamily a, member 1	*Prl4a1*	Ligand/cytokine	NM_017036	-3.91
Insulin-like growth factor 2	*Igf2*	Ligand, growth factor	NM_031511	-3.74
Fatty acid binding protein 5, epidermal	*Fabp5*	Fatty acid binding	NM_145878	-3.23
Matrix metallopeptidase 9	*Mmp9*	Extracellular matrix remodeling	NM_031055	-3.11
Fas ligand (TNF superfamily, member 6)	*Faslg*	Ligand/membrane anchored	NM_012908	-3.06
Cathepsin D	*Ctsd*	Lysosomal aspartic endopeptidase	NM_134334	-3.00
Carcinoembryonic antigen gene family 4	*Cgm4*	Secretory protein, unknown function	NM_012525	-2.79
Interleukin 17F	*Il17f*	Ligand/cytokine	NM_001015011	-2.61
Tribbles homolog 3 (Drosophila)	*Trib3*	Metabolism	NM_144755	-2.09
CEA-related cell adhesion molecule 10	*Ceacam10*	Cell adhesion molecule	NM_173339	-1.86
DNA-damage inducible transcript 3	*Ddit3*	Cell stress/death	NM_001109986	-1.86
Carcinoembryonic antigen-related cell adhesion molecule 3	*Ceacam3*	Cell adhesion molecule	NM_012702	-1.75
Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	*Slc28a2*	Transporter, nucleotide	NM_031664	-1.71
Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	*Sema6d*	Receptor	NM_001107768	-1.56

* Fold change values based on qRT-PCR analysis

PI3K signaling: positively regulated genes

The majority of ‘positively regulated’ PI3K dependent genes are included in the ‘differentiation-associated’ gene set (**Table 6.5**). An abbreviated list of ‘positively regulated’ PI3K dependent genes is shown in **Table 2.6.** Genes listed in **Table 6.5** are those with arbitrary expression signal strengths ≥800 in the differentiated cell condition and displaying a significantly higher level of expression in the differentiated cell state versus the differentiated cell state treated with the PI3K inhibitor (≥1.5 fold; P≤0.05). Of the 226 probe sets listed in **Table 6.5**, 90 genes were annotated by Ingenuity Pathway Analysis software. Functions associated with the annotated ‘positively regulated’ genes included cell survival (46%), gene expression (36%), cellular growth and proliferation (29%), small molecule biochemistry (27%), cellular development (26%), cellular movement (24%), and lipid metabolism (11%) (**Fig. 2.6B; Table 6.2**). These results are similar to that observed for the ‘differentiation-associated’ data set (**Fig. 2.1; Table 6.2**). Not all ‘differentiation-associated’ genes are regulated by PI3K suggesting that other signaling pathways contribute to the regulation of trophoblast differentiation. A subset of the ‘positively regulated’ PI3K-dependent genes identified from the microarray analysis was further evaluated by northern analysis or qRT-PCR in Rcho-1 TS cells treated with the PI3K inhibitor or vehicle (**Fig. 2.6C, 2.6D**). The ‘differentiation-associated’ genes sensitive to PI3K regulation have potential roles in cell invasion (e.g. *Igf2, Mmp9, Serpine1*), immune and vascular cell regulation (e.g. *Adm*, *Cgm4, Ceacam 3, Ceacam10, Prl4a1, Il17f, Ctsd, Faslg, Sema6d*), and the endocrine phenotype of trophoblast giant cells (e.g. *Prl3b1*, *Prl4a1*, *Cyp17a1*). In an additional experiment, we sought to determine whether the effects of the PI3K inhibitor on trophoblast gene expression required exposure throughout the differentiation process or whether the inhibitor could act acutely to affect differentiated trophoblast cell function. Several of the ‘differentiation-associated’ genes were also sensitive to acute disruption of the PI3K signaling pathway (**Fig. 2.7**).

PI3K regulation of trophoblast steroidogenesis

Trophoblast giant cells are known sites for the biosynthesis of progesterone and androstenedione (Yamamoto et al., 1994; Yamamoto et al., 1996). Several genes encoding proteins involved in the biosynthesis of steroid hormones are upregulated during trophoblast differentiation (**Table 2.5; Fig. 2.8**). These include *Star*, which encodes a protein involved in transporting cholesterol to the mitochondria, and a series of genes encoding enzymes responsible for the production of progesterone and androstenedione (*Cyp11a1, Hsd3b1, Cyp17a1, Hsd17b2*; **Fig. 2.8A**). *Hsd3b1* and *Cyp17a1* expression were positively regulated by PI3K signaling (**Table 2.6; Fig. 2.8B**). Consistent with this observation, the production of androstenedione by differentiating trophoblast cells is also dependent upon PI3K (**Fig. 2.8C**).

Fig. 2.7. Acute inhibition of PI3K is sufficient to regulate expression of PI3K sensitive genes. qRT-PCR analysis of trophoblast 'differentiation-associated' genes regulated by PI3K under acute PI3K inhibition. Rcho-1 TS cells were cultured under differentiating conditions for six days and for an additional 48 h of treatment with vehicle (0.1% DMSO; D+V) or LY294002 (10 μM; D+LY). Student *t*-tests (*P<0.05).

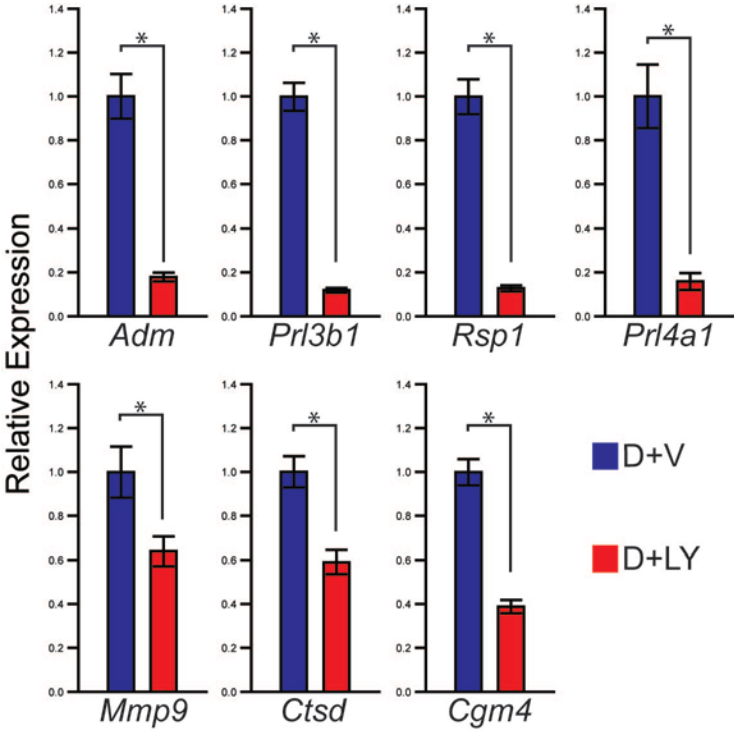
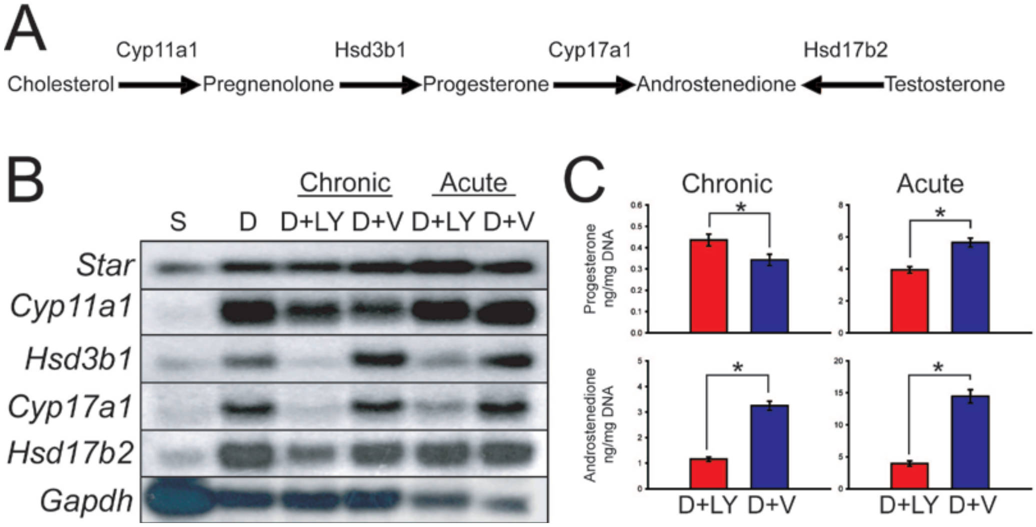

Relative Expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Adm
Prl3b1
Rsp1
Prl4a1
Mmp9
Ctsd
Cgm4
D+V
D+LY

Fig. 2.8. PI3K regulates steroidogenic potential of trophoblast cells. A) Overview of the steroidogenic pathway in trophoblast giant cells. **B)** Representative northern blot analysis of genes encoding components of the steroidogenic pathway in Rcho-1 TS cells. Rcho-1 TS cells were cultured under stem (S), differentiating (D) and differentiating with chronic or acute exposure to LY294002 (10 µM; D+LY) or vehicle (0.1% DMSO; D+V) conditions. **C)** Progesterone and androstenedione concentrations were measured by RIA in conditioned medium from Rcho-1 TS cells cultured in differentiating conditions with chronic or acute exposure to LY294002 (10 µM D+LY) or vehicle (0.1% DMSO; D+V). Acute conditions consisted of 12 days of differentiation and an additional 48 h of treatment with vehicle or LY294002 treatment. Conditioned media was collected from the last 24 hrs of each treatment period. Steroid measurements were normalized to DNA content. Student *t*-tests (*P<0.05).


A
Cyp11a1
Hsd3b1
Cyp17a1
Hsd17b2
Cholesterol
Pregnenolone
Progesterone
Androstenedione
Testosterone
B
Chronic
Acute
S
D
D+LY
D+V
D+LY
D+V
Star
Cyp11a1
Hsd3b1
Cyp17a1
Hsd17b2
Gapdh
C
Chronic
Acute
Progesterone ng/mg DNA
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
2
4
6
8
*
*
Androstenedione ng/mg DNA
0
1
2
3
4
5
0
5
10
15
20
*
*
D+LY
D+V
D+LY
D+V

DISCUSSION

Organization of the hemochorial placenta is the result of signaling pathways directing the expansion and differentiation of trophoblast stem cell and progenitor cell populations. This decision-making culminates in the systematic activation and inactivation of gene networks within trophoblast cell populations and elaboration of specific functions that facilitate redirection of resources from the mother to the fetus. In this report, we utilized the Rcho-1 TS cell model and induced differentiation through increased cell density and removal of growth stimuli. The growth factor deprivation may also lead to activation of stress pathways, which have been shown to influence trophoblast differentiation (Rappolee et al., 2007). Using this strategy, we have identified genes associated with trophoblast stem cell expansion, differentiation, and those impacted by the PI3K signaling pathway.

Trophoblast stem cell-associated genes

Stem cells possess the potential to proliferate and to differentiate. Several genes implicated in maintenance of the trophoblast stem cell state were identified in the Rcho-1 TS cells and are similarly present in mouse trophoblast stem cells. These include an assortment of genes implicated as cell cycle regulators in numerous cell types and also genes that have been more specifically shown to have a role in the specification and maintenance of trophoblast stem cells (e.g.

Cdx2, *Eomes, Id1*, *Id2*; Sasaki, 2010; Ralston and Rossant, 2005; Simmons and Cross, 2005).

Phlda2 displayed one of the most striking differences in its expression profile in stem versus differentiated cells. It was high in stem cells and virtually undetectable following differentiation, which is also found in mouse trophoblast stem cells. *Phlda2* is intriguing for a number of reasons. *Phlda2* is an imprinted gene exhibiting maternal allele-specific expression in extraembryonic and embryonic structures and in postnatal tissues, including the kidney (Qian et al., 1997; Frank et al., 1999). In the mouse, disruption of the *Phlda2* gene leads to placental overgrowth, while overexpression of *Phlda2* results in placental growth restriction (Frank et al., 2002; Salas et al. 2004; Tunster et al., 2010). Given that PHLDA2 restrains placental growth it seems counter-intuitive that it would be abundantly expressed in stem cell populations. Insights will likely be forthcoming when more is learned about the cellular actions of PHLDA2. The activities of PHLDA2 may be linked to its pleckstrin homology domain and its ability to bind phosphoinositides and could include an intracellular signal transduction function (Saxena et al., 2002).

Some differences in the behavior of mouse trophoblast stem cells and Rcho-1 TS cells are noteworthy. *Elf5*, a member of the ETS transcription factor family and a player in the derivation and maintenance of mouse trophoblast stem cells (Donnison et al., 2005; Ng et al., 2008; Kuckenberg et al., 2010) is not among the ‘trophoblast stem cell-associated’ genes of the Rcho-1 TS cell model. This may relate to differences in the requirements for exogenous factors to maintain trophoblast stem cell populations. Mouse trophoblast stem cells are dependent upon fibroblast growth factor-4 (FGF4)/FGF receptor 2 signaling (Tanaka et al., 1998), whereas maintenance of Rcho-1 TS cells does not require FGF4 (Faria and Soares, 1991). Evidence indicates that ELF5 may be a downstream effector of FGF4 signaling needed to sustain activation of *Cdx2* and *Eomes* genes and the trophoblast stem cell state (Ng et al., 2008). The requirement for *Elf5* must in some way be circumvented in Rcho-1 TS cell maintenance. In addition to Rcho-1 TS cells other recently derived trophoblast cell lines from the rat and common vole also grow in the absence of exogenous FGF4 (Grigor'eva et al., 2009; Chuykin et al., 2010). These observations do not reflect a fundamental species difference in the regulation of trophoblast stem cells. FGF4-dependent trophoblast stem cell lines can be established from the rat blastocyst (K. Asanoma and M.J. Soares, unpublished data). Instead, the FGF4 independence of the trophoblast stem cell populations is probably the consequence of genetic and/or epigenetic modifications and in vitro selection.

Several ‘trophoblast stem cell-associated genes’ were not shared with mouse trophoblast stem cells. Among these genes were *Mif* and *S1pr1*. *Mif* encodes a pro-inflammatory cytokine implicated in the regulation of angiogenesis (Amin et al., 2003), the migration and adhesion of monocytes (Amin et al., 2006), and modulation of uterine natural killer cell cytolytic activity (Arcuri et al., 2006). *S1pr1* encodes a Gi protein-coupled receptor for sphingosine 1-phosphate (S1P). S1P has been implicated in a range of functions, including controlling cell proliferation and differentiation (Skoura and Hla, 2009). In human trophoblast, S1P inhibits differentiation (Johnstone et al., 2005). Activation of some of the 'trophoblast stem cell-associated' genes may represent a developmental progression beyond the trophoblast stem cell state exhibited by mouse trophoblast stem cells or alternatively they may provide Rcho-1 cells with their tumorigenic features (Faria et al., 1990; Schreiber et al., 2000).

Trophoblast differentiation-associated genes

'Differentiation-associated' genes possess a broader range of functions than noted for the 'trophoblast stem cell-associated' gene cluster. Many of these genes are characteristic of the trophoblast giant cell phenotype. The trophoblast giant cell is conspicuous in its location at the maternal-fetal interface and its functions are in large part directed toward uterine structures and in facilitating maternal adaptations to pregnancy. These functions include endocrine activities (PRL family and steroidogenesis) and intrauterine invasion and modulation of the maternal vasculature and immune cells (*Il17f, Tfpi, Cgm4, Ecm1, Cd47, Fn, Lgmn, Mmp9, Grn, Igf2*).

Among the 'differentiation-associated' genes was a subgroup of genes encoding transcriptional regulators (*Hbp1, Ddit3, Rhox9, Nrf*2*, Fra1, Junb, Cited2*). Mouse mutagenesis experimentation has implicated a few of these genes (*Fra1, Junb*, *Cited2)* as regulators of placental development (Schorpp-Kistner et al., 1999; Schreiber et al., 2000; Withington et al., 2006). However, the specific roles of FRA1, CITED2, and the other transcriptional regulators in the regulation of trophoblast differentiation are yet to be determined. Some may participate in the regulation or maintenance of the differentiated trophoblast cell phenotype.

There is a connection between the ‘differentiation-associated’ genes and the PI3K/AKT signaling pathway. As trophoblast stem cells differentiate, the PI3K/AKT signaling pathway becomes constitutively activated (Kamei et al., 2002). IGF2 and GRN are candidate autocrine activators of the PI3K/AKT signaling pathway (Vincent and Feldman, 2002; Ong and Bateman 2003). *Trb3* and *Msn* were also classified as ‘differentiation-associated’ genes. They encode proteins with potential roles downstream of PI3K/AKT signaling pathway (Wu et al., 2004; Qi et al., 2006).

PI3K signaling-sensitive genes

PI3K regulates the phenotype of differentiating trophoblast cells (Kamei et al., 2002). Endoreduplication and/or survival of trophoblast giant cells are influenced by PI3K signaling. An active PI3K pathway favors trophoblast giant cells with lower ploidy levels. Trophoblast giant cells with lower ploidy levels may be more motile and phenotypically resemble midgestation trophoblast lining uterine spiral arteries (Adamson et al., 2002; Hemberger et al., 2003). PI3K signaling also possesses dramatic effects on gene expression patterns.

Overall, the functions of the PI3K-sensitive genes are biologically less diverse. Most interestingly, they include genes encoding proteins potentially impacting trophoblast invasion (*Mmp9*, [Peters et al., 1999]; *Igf2*, [Irwin et al., 1999; Aplin et al., 2000; Hiden et al., 2009]; *Serpine1*, [Graham, 1997]), directed to the maternal uterine environment influencing immune and vascular cells (*Cgm4, Faslg, Prl4a1, Adm, Il17f*), and also regulating androgen biosynthesis (*Hsd3b1, Cyp17a1*).

Cgm4 is one of the most abundant genes expressed by differentiating trophoblast cells. It encodes a member of the expanded pregnancy specific glycoprotein (PSG) family called PSG36. PSGs act on immune cells, potentially through CD9, to influence cytokine production (Wessells et al., 2000; Waterhouse et al., 2002; Ha et al., 2005; Wynne et al., 2006); they also target the vasculature and modulate endothelial cell function (Park et al., 2000). The presence of *Cd9* in differentiating trophoblast cells implies that PSGs may also possess autocrine/paracrine actions on trophoblast development, which may include regulating the trophoblast invasive phenotype (Park et al., 2000).

FAS ligand (FASLG), PRL-like protein A (PLP-A; *Prl4a1*), adrenomedullin (ADM), and interleukin 17f (IL17F) are cytokines produced by differentiating trophoblast that are exquisitely sensitive to PI3K regulation. FASLG binds to the FAS receptor and can initiate cell death. Trophoblast derived FASLG has been implicated as a modulator of intraplacental immune cell trafficking (Hunt et al., 1997; Abrahams et al., 2004) and is hypothesized to be a key participant in uterine spiral arteriole remodeling (Ashton et al., 2005; Harris et al., 2006). PLP-A targets natural killer cells and contributes to placentation site-specific adaptations to physiological stressors (Müller et al., 1999; Ain et al., 2003; Ain et al., 2004). ADM may possess an autocrine role regulating trophoblast invasion (Zhang et al., 2005) but also probably affects the uterine vasculature by regulating vessel diameter, permeability, and angiogenesis (Wilson et al., 2004; Kato et al., 2005; Temmesfeld-Wollbrück et al., 2007). Insights about IL17F and its potential role at the placentation site are limited. IL17F is proinflammatory with prominent effects on immune and vascular cells (Kolls and Lindén, 2004; Gaffen, 2008; Spolski and Leonard, 2009). Whether IL17F contributes to the organization of the hemochorial placentation site remains to be determined.

Key components of the enzymatic machinery required for trophoblast cell androgen biosynthesis are positively regulated by PI3K, including 17α hydroxylase (encoded by *Cyp17a1*). Trophoblast giant cells are the sites of androstenedione biosynthesis (Soares and Talamantes, 1983; Gibori et al., 1988). Androstenedione can serve as a prohormone for the biosynthesis of estrogens and more potent androgens, such as testosterone. Estrogens possess a vital luteotropic role essential for the maintenance of pregnancy (Gibori et al., 1988). Differentiating rodent trophoblast cells also express 17β hydroxysteroid dehydrogenase type 2 (encoded by *Hsd17b2*), which is responsible for converting testosterone to less biologically potent androgens, thereby protecting the fetus from excessive androgen exposure (Labrie et al., 1994; Labrie et al., 2000). Thus, PI3K signaling has a vital role in determining the steroid hormone milieu at the maternal-fetal interface.

In summary, the PI3K signaling pathway regulates the differentiated trophoblast cell phenotype. Under the direction of the PI3K signaling pathway, trophoblast cells produce a battery of cytokines and hormones. These extracellular signals modulate intrauterine immune and inflammatory cells, regulate vascular remodeling, and collectively ensure a successful pregnancy.

CHAPTER 3: PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALING PATHWAY AND FOS LIKE PROTEIN 1 IN THE REGULATION OF TROPHOBLAST INVASION AND VASCULAR REMODELING

ABSTRACT

Remodeling of the uterine vasculature by trophoblast cells is one of the key processes necessary for establishment of the hemochorial placenta and a successful pregnancy. This remodeling includes: invasion of trophoblast into the uterine vasculature, trophoblast cell replacement of maternal endothelium, trophoblast cell acquisition of an endothelial cell phenotype, and restructuring of the vessels to increase blood flow to the placenta and ultimately the fetus. Several signal transduction cascades have been implicated in the regulation of trophoblast invasion, including the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway. In this study we investigate the role of PI3K/AKT signaling in the regulation of expression of invasion related genes, transcription factor activation, and trophoblast cell invasion. We utilize Rcho-1 trophoblast stem (Rcho-1 TS) cells as a model system for trophoblast cell differentiation. These cells can be maintained in a stem cell state or induced to differentiate into trophoblast giant cells, which have the ability to invade. mRNA expression profiling using microarrays revealed numerous genes that have been implicated in trophoblast cell invasion and/or vasculature remodeling. These putative ‘invasion-vascular remodeling related’ genes are upregulated during differentiation, corresponding to an increase in PI3K/AKT signaling during differentiation. Using small molecules to inhibit the activity of PI3K or AKT we determined that expression of several ‘invasion-vascular remodeling related’ genes were dependent on the PI3K/AKT signaling pathway. We also showed

that the invasive ability of trophoblast cells was decreased when PI3K/AKT signaling was inhibited. These observations were further validated using shRNAs to knockdown AKT1, AKT2 or AKT3. The next step was to identify how the PI3K/AKT signaling pathway regulates gene expression. Fos like protein 1 (FOSL1 or FRA1) is upregulated during trophoblast cell differentiation and is a candidate downstream mediator of PI3K/AKT signaling. Down regulation of PI3K/AKT resulted in a decrease in the nuclear accumulation of FRA1 protein. Knockdown of FRA1 using shRNA led to a decrease in a subset of PI3K/AKT sensitive ‘invasion-vascular remodeling related’ genes and decreased trophoblast invasion when assessed using either in vitro or in vivo assays. In summary, the PI3K/AKT signaling pathway participates in the regulation of trophoblast cell differentiation through modulating FRA1 and the expression of key genes involved the invasion process.

INTRODUCTION

Hemochorial placental development is characterized by close contact between maternal and fetal tissues and occurs in primates and rodents such as the rat and mouse. Trophoblast cells are the functional units of the placenta. One of the key functions of trophoblast cells is the remodeling of uterine spiral arterioles. Specific lineages of trophoblast cells exit the placenta and enter into the uterine parenchyma where they interact with the vasculature. Vascular remodeling transforms tightly coiled uterine spiral arterioles into dilated vessels that are no longer under maternal control (Pijnenborg et al., 1981; Kaufmann et al., 2003). The restructuring of the maternal vasculature is essential for optimal delivery of nutrients and oxygen to the fetus. In the human, poor trophoblast invasion has been linked to miscarriage, preeclampsia, pre-term birth, and fetal growth restriction (Harris, 2010). Hemochorial placentation and especially trophoblast-directed vascular remodeling in the human and the rat are remarkably similar (Pijnenborg et al. 1981, 2006; Ain et al., 2003; Caluwaerts et al., 2005; Vercruysse et al., 2006; Konno et al., 2007; Geusens et al., 2008, 2010; Rosario et al., 2008, 2009). In both species, invasive trophoblast cells penetrate the uterus through endovascular and interstitial routes (Pijnenborg et al. 2006).

In the rat, trophoblast cell invasion occurs in two waves. The first wave is initiated during midgestation and consists of trophoblast cell invasion into the spiral arterioles situated within the central area of the uterine mesometrial implantation site. These invasive trophoblast cells are thought to be a type of trophoblast giant cell and line the blood vessels, effectively replacing the maternal endothelium (Hemberger et al., 2003; Simmons et al., 2007; Rosario et al. 2008). The second wave of trophoblast cell invasion arises from trophoblast glycogen cells that originate in the junctional zone of the placenta and begin invading around gestation d14.5. These cells primarily invade interstitially and are not restricted to the proximity of blood vessels (Ain et al., 2003; Wiemers et al. 2003; Bouillot et al., 2006). Signaling pathways controlling trophoblast invasion and vascular remodeling are not well understood.

The phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway is constitutively activated upon differentiation of in vitro trophoblast model systems such as mouse trophoblast stem cells and Rcho-1 trophoblast stem (Rcho-1 TS) cells (Kamei et al., 2002). This pathway has also been reported to regulate the expression of pro-invasion genes and to promote an invasive phenotype of immortalized human trophoblast cell lines and primary trophoblast culture systems (Qiu et al., 2004a,b; Sonderegger et al., 2010). PI3K is a lipid kinase that phosphorylates phosphatidylinositol and phosphoinositides (Engelman et al., 2006). The cellular actions of several growth factors are mediated through activation of PI3K (Cantley, 2002). PI3K initiates a signaling cascade leading to AKT (also known as protein kinase B, PKB) activation (Cantley, 2002; Brazil et al., 2004). AKT is a serine threonine kinase with a wide range of substrates. These substrates are involved in many cellular processes including: metabolism, cell cycle, survival, protein synthesis, and differentiation (Engelman et al., 2006). There are three AKT isoforms in mammals, AKT1/PKBα, AKT2/PKBβ, and

AKT3/PKBγ (Brazil and Hemmings, 2001). These enzymes are conserved at the amino acid level and share substrate specificity (Coffer et al., 1998; Brazil and Hemmings, 2001). Null mouse models for the three AKT isoforms exhibit a range of phenotypes, including impacts on placentation, fetal growth, postnatal metabolism, and growth (Cho et al., 2001a,b; Yang et al., 2003, 2005).

An earlier report from our laboratory identified a set genes regulated by PI3K that may contribute to the invasive phenotype of trophoblast cells (Chapter 2; Kent et al., 2010). One of these genes is matrix metalloproteinase 9 (*Mmp9*). Substrates for MMP9 include components of the extracellular matrix. MMP9 is pro-invasive in trophoblast cells of the rat as well as the human (Peters et al., 1999, Qiu et al., 2004a) and has been reported to be involved in the regulation of cancer cell invasion and metastasis (Kustikova et al., 1998; Bischof et al., 2002; Zhang et al., 2004; Belguise et al., 2005). In cancer cells, MMP9 can be regulated by the transcription factor Fos like protein 1 (FOSL1 or FRA1). FRA1 has been implicated as a downstream mediator of PI3K/AKT regulation of invasion related genes (e.g. *Mmp9*) and invasive abilities (Cao et al. 2006; Chandrasekar et al., 2006; Ramachandran et al. 2010). *Fra1* null mice are embryonic lethal due to placental defects (Schreiber et al., 2000). Thus, FRA1 represents an intriguing candidate mediator of the PI3K/AKT signaling pathway in trophoblast cells.

In this study, we utilize Rcho-1 trophoblast stem cells as a model system to investigate trophoblast cell differentiation (Faria and Soares, 1991). Rcho-1 TS cells are experimentally valuable in that they can be maintained in a stem cell state or induced to differentiate along the trophoblast giant cell lineage (Faria and Soares, 1991; Hamlin et al., 1994; Yamamoto et al., 1994; Sahgal et al., 2006). This in vitro system was used to evaluate the role of a pathway involving PI3K/AKT and FRA1 in the regulation of trophoblast cell differentiation, including acquisition of the invasive phenotype. Disruption of the PI3K/AKT signaling pathway using small molecule inhibitors and short hairpin RNAs (shRNAs) show that this pathway regulates a set of ‘invasion-vascular remodeling related’ genes, the invasive ability of trophoblast cells, and the nuclear accumulation of FRA1. Knockdown of FRA1 expression with shRNAs leads to decreases in expression of a subset of the PI3K/AKT regulated ‘invasion related’ genes and the invasive characteristics of trophoblast cells in vitro. Trophoblast specific knockdown of FRA1 in the rat embryo also leads to an inhibition of trophoblast invasion as assessed in vivo. Collectively, the experimental findings demonstrate that PI3K/AKT and FRA1 participate in a signal transduction cascade controlling a pro-invasive and vascular remodeling trophoblast phenotype.

METHODS AND MATERIALS

Animals and tissue collection

Holtzman Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN). Animals were housed in an environmentally controlled facility with lights on from 0600-2000 h and were allowed free access to food and water. Timed pregnancies were generated by cohabitation of female and male animals. The presence of a copulatory plug or sperm in the vaginal smear was designated d0.5 of pregnancy. Rat placental tissues were collected on gestation d7.5, d11.5 and d18.5. Tissues for histological analysis were frozen in dry-ice cooled heptane and stored at -80°C. Tissue samples for RNA or protein extraction were frozen in liquid nitrogen and stored at -80°C. Female rats were made pseudopregnant by mating with vasectomized males. Presence of seminal plugs was designated d0.5 of pseudopregnancy. The University of Kansas Animal Care and Use Committee approved protocols for the care and use of animals.

Maintenance of Rcho-1 TS cells

Rcho-1 TS cells were maintained at subconfluent conditions in Stem Medium [RPMI-1640 culture medium (Cellgro, Herndon, VA) supplemented with 20% fetal bovine serum (FBS; Atlanta Biologicals, Norcross, GA) 50 µM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), 1 mM sodium pyruvate (Cellgro), 100 µM penicillin and 100 U/ml streptomycin (Cellgro)] as previously reported (Peters et al., 2000; Sahgal et al., 2006). Differentiation was induced by growing cells to near confluence in FBS-supplemented culture medium and then replacing the medium with Differentiation Medium [NCTC-135 medium supplemented with 1% horse serum (HS; Atlanta Biologicals) 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 10 mM HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Fisher, Pittsburgh, PA), 38 mM sodium bicarbonate (Fisher), 100 μM penicillin and 100 U/ml streptomycin (Cellgro)]. High cell density and the absence of sufficient growth stimulatory factors (removal of FBS) facilitate trophoblast giant cell formation (Yamamoto et al., 1994, Sahgal et al., 2006). Trypsin (0.25%)-ethylenediamine tetraacetic acid (EDTA, 0.1% in Hank's Balanced Salt Solution, Cellgro) was used to passage the cells. Cells in the stem cell condition were grown in Stem Medium and collected 24 h after subculture to restrict the accumulation of spontaneously differentiating cells. Cells in the differentiation condition were grown for eight days in Differentiation Medium prior to harvesting unless otherwise noted.

Inhibition of PI3K and AKT

LY294002 (Calbiochem, La Jolla, CA) was used to inhibit PI3K (Vlahos et. al., 1994). AKT inhibitor IX (API-59CJ-OMe, abbreviated: AI9) was used to inhibit AKT activities (Calbiochem). Rcho-1 TS cells were grown to near confluence and then shifted to Differentiation Medium containing vehicle (0.1% final concentration of dimethyl sulfoxide, DMSO), LY294002 (10 μM), or AI9 (10 μM). Cells were harvested after eight days of treatment. Culture medium and small molecule inhibitors were replaced daily.

qRT-PCR

RNA samples were extracted using TRIzol (Invitrogen) according to the manufacturer's instructions. cDNAs were reverse transcribed (RT) from RNA using reagents from Promega (Madison, WI) according to the manufacturer's instructions. SYBR GREEN PCR Master Mix (Applied Biosystems, Foster City, CA) was used in the PCR reaction. Reactions were processed using a 7500 Real-Time PCR System (Applied Biosystems). Conditions included an initial holding stage (50°C for 2 min and 95°C for 10 min) and 40 cycles (95°C for 15 s and 60°C for 1 min) followed by a dissociation stage (95°C for 15 s, 60°C for 1 min, and then 95°C for 15 s). Primers are listed in **Table 3.1**. Expression of 18S ribosomal RNA was used as an internal control. At least four replicates were processed for each condition. Samples were normalized to the control sample for each gene.

Immunohistochemistry

Immunohistochemical analyses were used to localize proteins in placental tissues. Ten µm cryosections of placentation sites were prepared and stored at -80°C until used. Sections were fixed in ice-cold 4% paraformaldehyde and blocked in 10% normal goat serum for 30 min at room temperature. Primary antibody incubation was for 1 h at room temperature with antibodies specific for FRA1 (dilution of 1:50; Santa Cruz Biotechnology, Santa Cruz, CA), placental lactogen-I (PL-I or PRL3D1, 1:50; Hamlin et al. 1994), PL-II (PRL3B1,1:50; Deb

Table 3.1. qRT-PCR primers

Symbol	GenBank Accession No.	Forward Primer	Reverse Primer
18s		5'gcaattattccccatgaacg3'	5'ggcctcactaaaccatccaa3'
Akt1	NM_033230	5'ggcacctttattggctacaag3'	5'agcacctgagttgtcactg3'
Akt2	NM_017093	5'tggcaggatgtggtacagaa3'	5'aggagaactgggggaagtgt3'
Akt3	NM_031575	5'tggttcgagagaaggcaagt3'	5'acagctctcccccattaaca3'
Adm	NM_012715	5'acgtctcggactttctgctt3'	5'gctgctggacgcttgtagtt3'
Ceacam3	NM_012702	5'tggtacaaagggctgacaaa3'	5'tccacaggtcaaagtggagaa3'
Cgm4	NM_012525	5'tagcccgatacagaacagcaa3'	5'agggtcacagcatgaggaaa3'
Ctsd	NM_134334	5'tacctgaacgtcacccgaaa3'	5'caggctggacaccttctcac3'
Faslg	NM_012908	5'tctggttggaatggggttag3'	5'cttggctttttggtttcagag3'
Fra1	NM_012953	5'atccccgacctctgacctat3'	5'caaggcgttccttctgctt3'
Igf2	NM_031511	5'ggaagtcgatgttggtgctt3'	5'cttgcccacggggtatct3'
Il17f	NM_001015011	5'caaaaccagggcatttctgt3'	5'gaccaggatttcttgctgga3'
Mmp9	NM_031055	5'aacttcgacgctgacaagaa3'	5'tttagagccacgaccatacaga3'
Prl4a1	NM_017036	5'gaccaccagatgccacactt3'	5'caggagctttatgtttgattcctt3'
Sema6d	NM_001107768	5'ggccagtgatgctgtcattt3'	5'tatgttccacggcgatttct3'
Serpine1	NM_012620	5'agtctttccgaccaagagca3'	5'gtgccgaaccacaaagagaa3'

et al. 1989), or pan-cytokeratin (1:300; Sigma-Aldrich). Sections were incubated with secondary antibodies for 30 min at room temperature. For bright field immunohistochemical analysis, a secondary goat anti-rabbit antibody conjugated with biotin (Sigma-Aldrich) was used. Avidin-peroxidase was added for 30 min at room temperature followed by color-development with an AEC kit (Zymed Laboratories, San Francisco, CA). Tissues were counterstained with Mayer's hematoxylin. For fluorescence detection, a secondary goat anti-rabbit antibody conjugated with Cy3 bis-NHS ester (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) or a secondary goat anti-mouse antibody conjugated with fluorescein isothiocyanate (FITC, Sigma-Aldrich) were used. Nuclei were visualized with 4',6-diamidino-2-phenylindole (DAPI, Molecular Probes, Carlsbad, CA). Bright field and fluorescence images were captured using Leica MZFLIII stereomicroscope and DMI 4000 microscopes equipped with CCD cameras (Leica Microsystems GmbH, Welzlar, Germany).

Western blotting

Whole cell lysates were prepared in radioimmunoprecipitation buffer (10 mM Tris-HCl, pH 7.2, 1% Triton X-100 or 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 5 mM EDTA, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin). Nuclear and cytoplasmic lysates were collected using the NE-PER kit (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer’s instructions. Whole cell lysates (50 µg), cytoplasmic lysates (50 µg) or nuclear lysates (20 µg) were fractionated by SDS-PAGE. Separated proteins were electrophoretically transferred to nitrocellulose. Filters were probed with the designated antibodies overnight at 4°C. Antibodies specific for pan-AKT, phospho-Ser 473 AKT, AKT1 and AKT2 were obtained from Cell Signaling Technology (Danvers, MA). Antibodies for FRA1 and TATA box binding protein (TBP or TFIID) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The antibody for β-ACTIN (ACTB) clone AC-15 was obtained from Sigma. Antibodies were used at the following dilutions: pan-AKT (1:2000), phospho-Ser 473 AKT (1:2000), AKT1 (1:1000), AKT2 (1:1000), FRA1 (1:500), TBP (1:2000), and β–ACTIN (1:8000). Blots were incubated with horseradish peroxidase-conjugated antibodies to rabbit (Cell signaling) or mouse (Sigma) IgG for 1 h at room temperature. Reaction products were visualized by incubation with enhanced chemiluminescence according to the manufacturer's instructions (ECL, Amersham Pharmacia, Piscataway, NJ). Signal intensity for selected western blots was measured by calculating integrated density using Photoshop CS5 extended edition (Adobe Systems Incorporated, San Jose, CA)

AKT kinase assay

The activity of AKT was measured in Rcho-1 TS cell lysates using an AKT Activity Immunoassay Kit (Calbiochem). AKT activity was measured in lysates from Rcho-1 TS cells treated with LY294002, AI9, or vehicle control. The assay was preformed as per the manufacturer's instructions.

Matrigel invasion assay

The invasive ability of Rcho-1 TS cells were measured as previously described (Peters et al., 1999). Rcho-1 TS cells were differentiated for six days, trypsinized and placed in Matrigel invasion chambers (BD Biosciences, San Jose, CA) at a density of 2.5 x 10^4 cells per chamber. Cells were initially plated in Stem Cell Medium and switched to Differentiation Medium and allowed to invade for 72 h. The experimental design for analysis of the effects of LY294002, AI9, or the vehicle control (0.1% DMSO) consisted of exposure during the 72 h invasion period. Control, AKT1, AKT2, AKT3, and FRA1 knockdown Rcho-1 TS cells were also evaluated during a 72 h invasion period. After 72 h, membranes were collected and stained with Diff-Quick (Dade Behring, Newark, DE). Invading cells were visualized by light microscopy and counted for each sample. Sample sizes of eight to nine Matrigel invasion chambers were analyzed per treatment group.

Production of lentivirus and shRNA constructs

Akt shRNA constructs in the pLKO.1 vector were obtained from Open Biosystems (Huntsville, AL). Several shRNAs were tested for each gene. The sequence of the shRNAs included in these analyses are as follows: *Akt1*, 5'CCGGGCACATCAAGATAACGGACTTCTCGAGAAGTCCGTTATCTTGATGTGCTTTTT3', *Akt2,* 5'CCGGGATGGATCTTTCATTGGGTATCTCGAG ATACCCAATGAAAGATCCATCTTTTT3' and *Akt3,* 5'CCGGGCTCTTGATAAAGGATCCAAACTCGAGTTTGGATCCTTTATCAAGAG

CTTTTT3’. A *Fra1* shRNA was generated by ligating the following oligonucleotides: 5’CCGGTGACAAGTTGGAGGATGAGAAATACTCGAGAATTTCTCATCCTCCAACTTGTCTTTTTTG3’ and 5’AATTCAAAAAAGACAAGTTGGAGGATGAGAAATTCTCGAGTATTTCTCATCCTCCAACTTGTCA3’ and subcloning the ligated segment into the pLKO.1 vector using *Age1* and *EcoR1*. Control shRNA, which targets no known mammalian gene, pLKO.1-shSCR (Plasmid 1864), was obtained from Addgene (Cambridge, MA) and has the following sequence 5’CCTAAGGTTAAGTCGCCCTCGCTCTAGCGAGGGCGACTTAACCTTAGG3’ (Sarbassov et al., 2005). Third generation lentiviral packaging vectors were purchased from Addgene and included: pMDLg/pRRE (Plasmid 12251), pRSV-Rev (Plasmid 12253) and pMD2.G (Plasmid 12259). Lentiviral particles were produced as previously reported in (Lee et al., 2009). To summarize, 293FT cells (Invitrogen, Carlsbad, CA) were transiently transfected using Lipofectamine 2000 (Invitrogen) with the following plasmids: shRNA containing transducing vector, third-generation packaging system plasmids (pMDLg/pRRE and pRSV-Rev; Dull et al., 1998), and a VSVG envelope plasmid (pMD2.G). After transfection, cells were maintained in 50% DMEM with high glucose (Cellgro), 45% Opti-MEM I (Invitrogen, Carlsbad, CA) and 5% FBS. Culture supernatants containing lentiviral particles were harvested every 24 h for two to three days. Supernatants were centrifuged to remove cell debris, filter sterilized, concentrated by ultracentrifugation, and stored at -80°C until used. Lentiviral vector titers were determined by measurement of p24 gag antigen by ELISA (Advanced Bioscience Laboratories, Kensington, MD).

In vitro and in vivo lentiviral transduction

Rcho-1 TS cells were exposed to lentiviral particles and selected with puromycin dihydrochloride (Sigma, 2 µg/µl) for 2 to 4 days and then maintained in puromycin dihydrochloride (1 µg/µl). The puromycin selective pressure was removed during Rcho-1 TS cell differentiation.

Rat embryos were transduced with lentiviral particles as previously described (Lee et al., 2009). Briefly, rat embryos were collected on gestation d4.5. Recovered blastocysts were washed with KSOM medium (Millipore). Zonae pellucidae were removed with Pronase (10 mg/ml for 10 min) and incubated with concentrated lentiviral particles (750 ng of p24/ml) for 4.5 h. Transduced blastocysts were transferred to uteri of d3.5 pseudopregnant rats for subsequent evaluation of gene knockdown and placentation site phenotypes on gestation d11.5.

Measurement of in vivo FRA1 expression

Placentation sites derived from embryos treated with control or *Fra1* shRNAs were processed for detection of *Fra1* mRNA by qRT-PCR and FRA1 protein by western blotting and immunofluorescence analsysis. Trophoblast dissections were performed as previously described (Ain et al. 2006) and qRT-PCR and western blotting conducted as presented above. FRA1 expression levels detected on tissue sections with immunofluorescence were measured by calculating integrated density using Photoshop CS5 extended edition (Adobe Systems Incorporated). All values were normalized to control samples. Placentation sites exhibiting a decrease in FRA1 protein expression were included for additional analyses. A decrease in FRA1 protein expression was defined as samples in which FRA1 protein expression was one standard deviation below the mean of control samples.

Measurement of in vivo invasion

Invasive trophoblast in placentation sites derived from embryos treated with control or *Fra1* shRNAs were monitored by immunostaining with antibodies to pan-cytokeratin. An invasion index was calculated by measuring the ratio between the depth of trophoblast invasion into the decidua and the total depth of the decidua using National Institutes of Health ImageJ software (Bethesda, MD). All values were normalized to control samples.

Statistical analysis

Statistical comparisons of two means were performed with Student's *t*-test or Welch's *t*-test. Comparisons of multiple groups were evaluated with analysis of variance. The source of variation from significant F-ratios was determined with Tukey's HSD Multiple Comparison Test. Statistical analyses were performed using the R Statistical Package (http://www.r-project.org/).

RESULTS

The PI3K/AKT pathway and trophoblast cell invasion

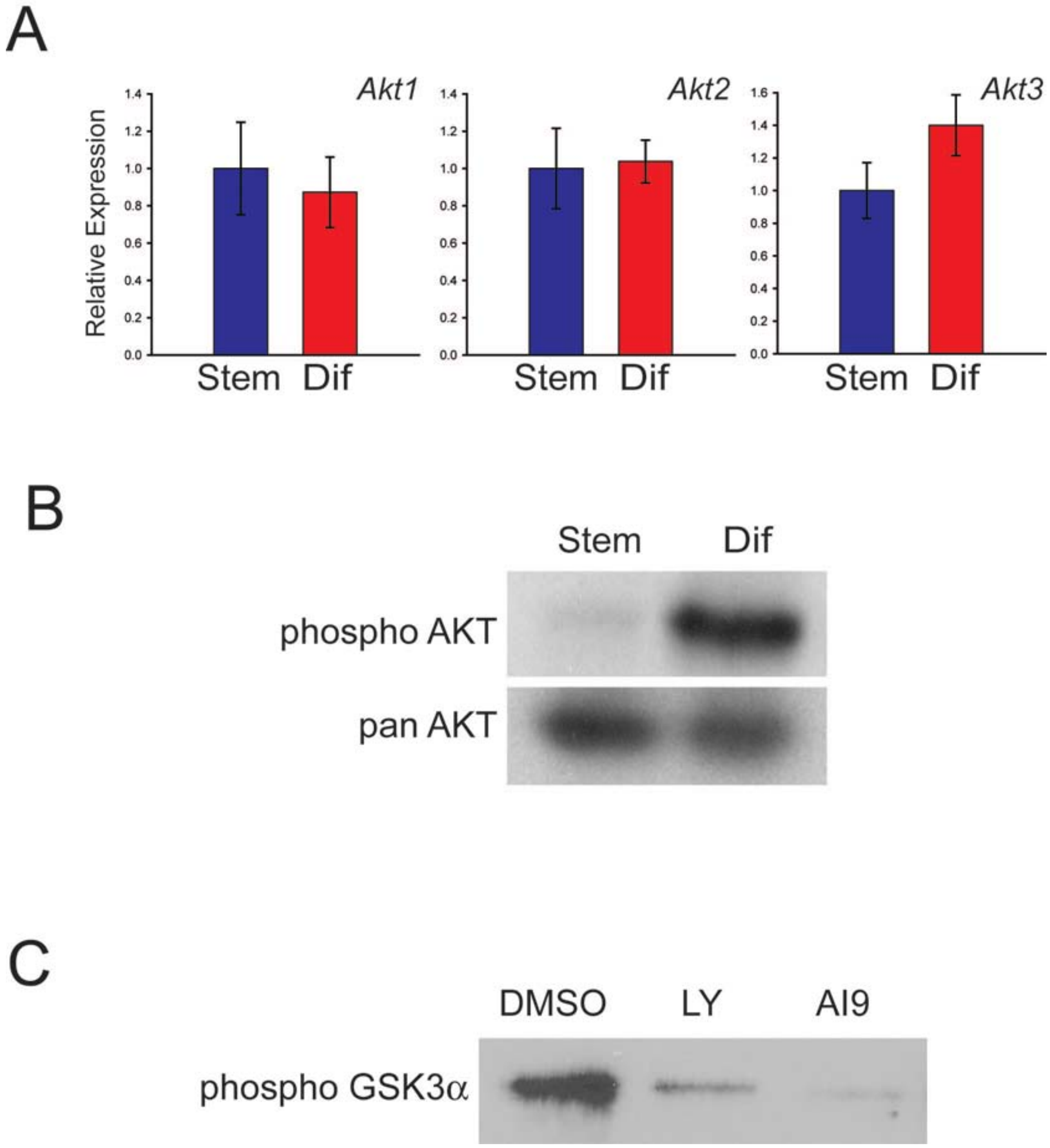
The PI3K/AKT pathway is activated during trophoblast differentiation (Kamei et al., 2002). In the following experiments we examine roles for the PI3K/AKT pathway in the regulation of trophoblast cell differentiation and the trophoblast invasive phenotype.

Trophoblast AKT is expressed, active, and sensitive to small molecule inhibition

Examination of *Akt* expression in Rcho-1 TS cells showed that each of the three isoforms of *Akt* (*Akt1*, *Akt2*, and *Akt3*) was expressed (**Fig. 3.1A**) with *Akt1* being the predominate isoform (Kamei et al, 2002; Yang et al. 2003; data not shown). AKT is activated upon phosphorylation of serine 473. In Rcho-1 TS cells phosphorylation of AKT on serine 473 AKT was increased after differentiation (**Fig. 3.1B**; Kamei et al., 2002) indicating that AKT activity is increased in differentiated Rcho-1 TS cells.

AKT activity was blocked using a small molecule inhibitor of PI3K (LY293002; abbreviated LY) or using an inhibitor that blocks the activity of all AKT isofoms (Jin et al., 2004; AKT inhibitor IX; API-59CJ-OMe; abbreviated AI9). Treatment of Rcho-1 TS cells with LY decreased both AKT phosphorylation (not shown) and kinase activity (**Fig. 3.1C**), while cells treated with AI9 retain AKT phosphorylation but their AKT is no longer able to phosphorylate substrates in a kinase assay (**Fig. 3.1C**). This strategy for using small molecule inhibitors to disrupt PI3K and/or AKT was used in experiments described below to investigate

Fig. 3.1. AKT expression and activity in trophoblast cells. A) qRT-PCR analysis of *Akt1, Akt2* and *Akt3* in stem and differentiated Rcho-1 TS cells. **B)** Western analysis of total AKT (pan-AKT) and phosphorylated AKT (phospho-AKT) in lysates from stem and differentiated Rcho-1 TS cells. **C)** Measurement of AKT activity in lysates from differentiated Rcho-1 TS cell treated with LY (10 μM), AI9 (10 μM), or vehicle control (DMSO, 0.01%). AKT activity was measured by the ability of AKT in cell lysate to phosphorylate GSK3α (phospho- GSK3α).


A
Relative Expression
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Akt1
Stem
Dif
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Akt2
Stem
Dif
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Akt3
Stem
Dif
B
Stem
Dif
phospho AKT
pan AKT
C
DMSO
LY
AI9
phospho GSK3α

the role of the PI3K/AKT signaling pathway in the regulation of the trophoblast invasive cell phenotype.

Small molecule inhibition of PI3K and AKT in trophoblast cells decreases expression of "invasion-vascular remodeling related" genes

Previous gene profiling in trophoblast cells led to the identification of a subset of genes that are upregulated during differentiation and sensitive to PI3K inhibition (Chapter 2; Kent et al., 2010). In this study we focused on a group of eleven PI3K-sensitive genes that are potential regulators of trophoblast invasion and/or vascular remodeling (*Adm, Ceacam3, Cgm4, Ctsd, Faslg, Igf2, Il17f, Mmp9, Prl4a1, Sema6d, and Serpine1;* **see Table 3.2**). In **Fig. 3.2**, we demonstrate that these eleven genes are also sensitive to disruption of AKT activation. These observations are consistent with a role of the PI3K/AKT signaling pathway in the regulation of the invasive and vascular remodeling phenotypes of differentiating trophoblast cells.

Small molecule inhibition of PI3K or AKT decreases MMP9 activity and invasive ability of trophoblast cells

Of the eleven 'invasion-vascular remodeling related' genes, one gene in particular *Mmp9*, which encodes matrix metalloproteinase 9, has been associated with invasion in both trophoblast (Librach et al., 1991; Peters et al., 1999; Qiu et al., 2004b) and cancer cells (Coussens et al., 2002). *Mmp9* mRNA expression increases during differentiation (**Fig. 3.3A**). Disruption of PI3K and/or

Table 3.2. Potential regulators of trophoblast invasion and/or vascular remodeling

Gene name	Abbreviation	Synonyms	Functional Group	GenBank Accession No.
Adrenomedullin	*Adm*		Hypotensive peptide	NM_012715
Carcinoembryonic antigen-related cell adhesion molecule 3	*Ceacam3*	*Cgm1*	Cell adhesion molecule	NM_012702
Carcinoembryonic antigen gene family 4	*Cgm4*	*Psg16*	Secretory protein, unknown function	NM_012525
Cathepsin D	*Ctsd*	*CD, CatD*	Lysosomal aspartic endopeptidase	NM_134334
Fas ligand (TNF superfamily, member 6)	*Faslg*	*Faslg, Tnfsf6*	Ligand/membrane anchored	NM_012908
Insulin-like growth factor 2	*Igf2*	*Igf-II*	Ligand, growth factor	NM_031511
Interleukin 17F	*Il17f*	*ML1*	Ligand/cytokine	NM_001015011
Matrix metallopeptidase 9	*Mmp9*	*Gelatinase B*	Extracellular matrix remodeling	NM_031055
Prolactin family 4, subfamily a, member 1	*Prl4a1*	*PLP-A*	Ligand/cytokine	NM_017036
Sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D	*Sema6d*		Receptor	NM_001107768
Serine (or cysteine) peptidase inhibitor, clade E, member 1	*Serpine1*	*Pai1, Planh*	Blood coagulation, angiogenesis	NM_012620

Fig. 3.2. Expression of 'invasion-vascular remodeling related' genes in Rcho-1 TS cells treated with PI3K and AKT inhibitors. qRT-PCR analysis of 'invasion-vascular remodeling related' genes in Rcho-1 TS cells after eight days of differentiation treated with LY294002 (LY, 10 μM), AKT inhibitor IX (AI9, 10 μM), or vehicle control (DMSO, 0.01%). All samples were normalized to control samples. Bars represent means ± the standard error of the mean. Expression values significantly different from the control are indicated with an asterisk (*P<0.05).

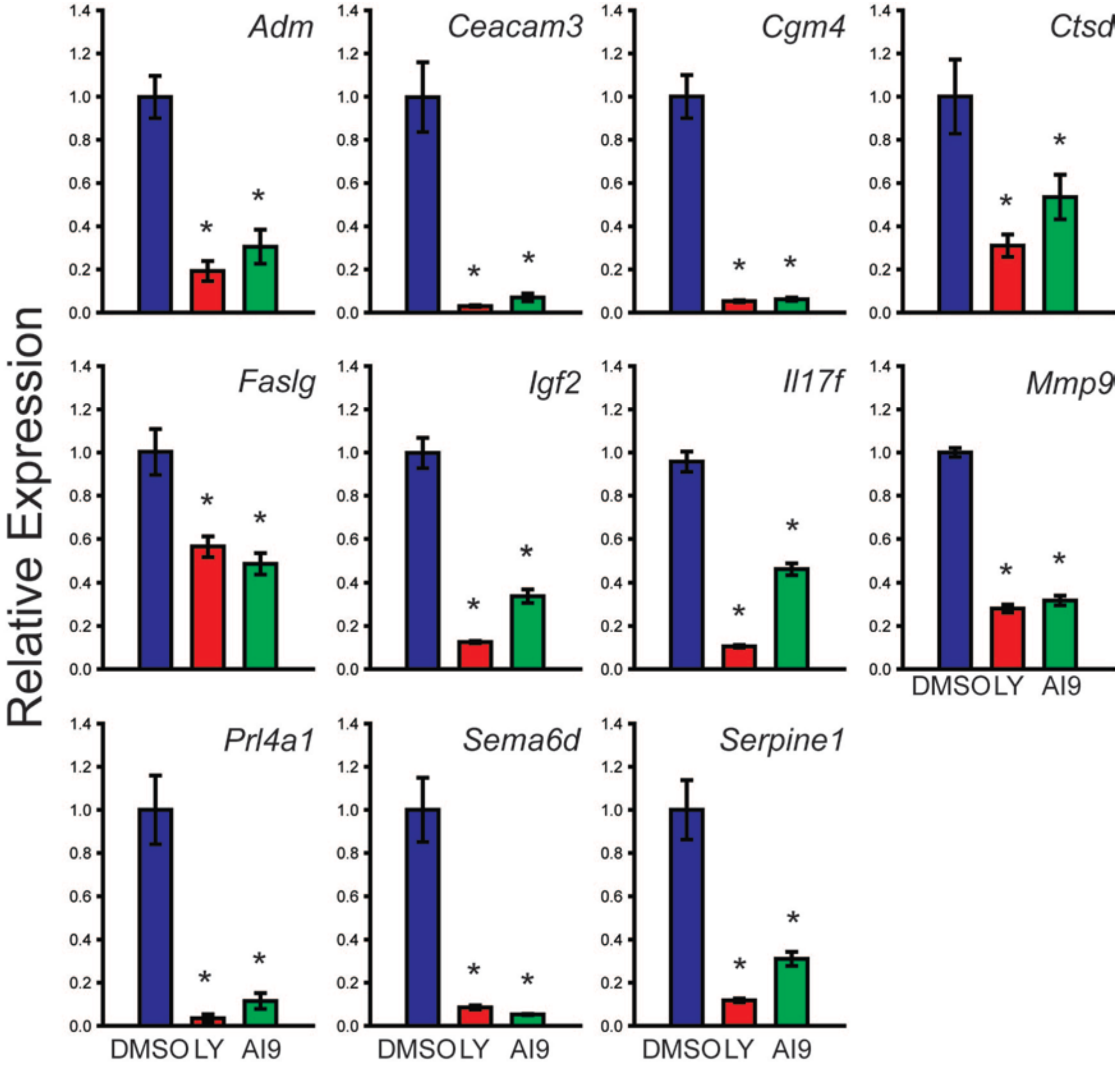
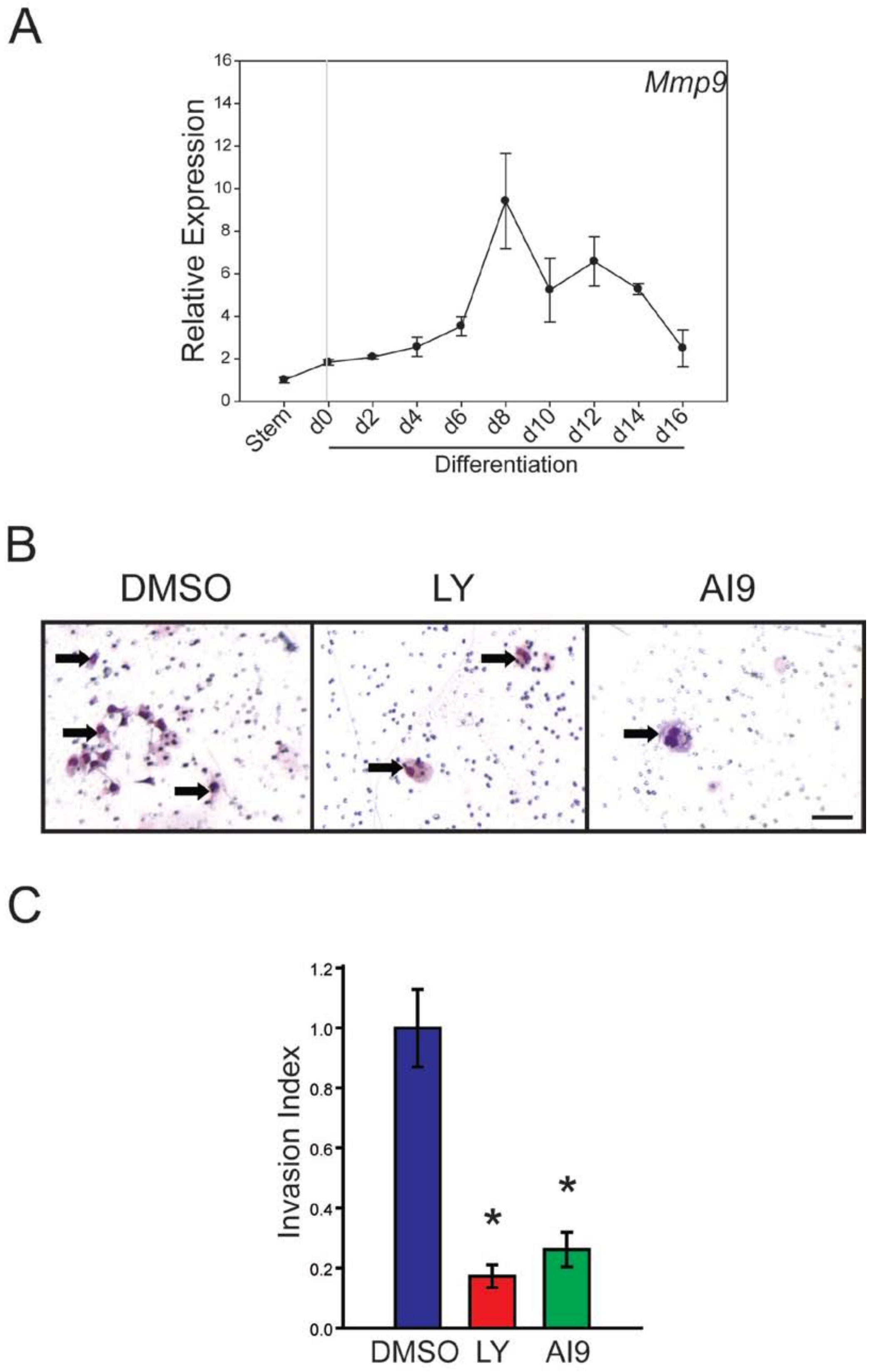

Relative Expression
Adm
Ceacam3
Cgm4
Ctsd
Faslg
Igf2
Il17f
Mmp9
Prl4a1
Sema6d
Serpine1
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
*
DMSO
LY
AI9

Fig. 3.3. Inhibition of PI3K or AKT decreases the invasive abilities of trophoblast cells. A) qRT-PCR analysis of *Mmp9* expression during Rcho-1 TS differentiation. **B)** The invasive ability of differentiated Rcho-1 TS treated with LY (10 µM), AI9 (10 µM), or vehicle control (DMSO, 0.01%) was measured by Matrigel invasion chamber assay. Representative filters illustrating effects of the treatments are shown, cells are marked with arrows. Bar= 100 µm. **C)** Graphical presentation of results from the Matrigel invasion chamber assays. Cells were counted from nine replicates and normalized to control samples. Invasion index represents the number of invading cells in each group relative to the number of cells invading in the control group. Bars represent means ± the standard error of the mean. Expression values significantly different from controls are indicated with an asterisk (*P<0.05).


A
Mmp9
Relative Expression
0
2
4
6
8
10
12
14
16
Stem
d0
d2
d4
d6
d8
d10
d12
d14
d16
Differentiation
B
DMSO
LY
AI9
C
Invasion Index
0.0
0.2
0.4
0.6
0.8
1.0
1.2
*
*
DMSO
LY
AI9

AKT activities impaired trophoblast cell invasion as assessed by Matrigel invasion chamber assays (**Fig. 3.3B and C**). These experimental results indicate that PI3K/AKT signaling promotes the trophoblast cell invasive phenotype, potentially through modulation of expression of *Mmp9* and other pro-invasive genes.

AKT isoform regulation of the expression of 'invasion-vascular remodeling related' genes and the invasive ability of trophoblast

Cellular AKT activity is the net result of the activities of three AKT isoenzymes (AKT1, AKT2, AKT3; Brazil and Hemmings, 2001). To identify which AKT isoform is responsible for regulating the expression of the 'invasion-vascular remodeling related' genes, the expression of each AKT isoform was specifically inhibited using lentiviral delivery of isoform-specific shRNAs. Validation of the isoform-specific knockdown is shown in **Fig. 3.4**. Disruption of each individual AKT isoform yielded an isoform-specific effect on expression of the 'invasion-vascular remodeling related' genes. Knockdown of AKT1 resulted in a significant decrease in *Cgm4, Faslg, Prl4a1, Sema6d,* and *Serpine1*; while AKT2 disruption inhibited *Cgm4, Igf2, Mmp9, Prl4a1,* and *Sema6d*; and AKT3 contributed to the regulation of *Cgm4, Igf2, Mmp9,* and *Sema6d* (**Fig. 3.5**). Expression of some 'invasion-vascular remodeling related' genes (*Adm, Ceacam3*, *Ctsd* and *Il17f*) was not sensitive to knockdown of any single AKT isoform. Furthermore knockdown of any individual AKT isoform was not sufficient to disrupt the invasive abilities of trophoblast cells as assessed in the Matrigel invasion

Fig. 3.4. Analysis of shRNA knockdown of AKT isoforms. A) qRT-PCR analysis for *Akt1, Akt2* and *Akt3* in Rcho-1 TS cells expressing control shRNA or shRNAs specific for *Akt1*, *Akt2* or *Akt3* after eight days of differentiation. All samples were normalized to control samples. Bars represent means ± the standard error of the mean. Expression values significantly different from controls are indicated with an asterisk (*P<0.05). **B)** Western blot analysis of Phosphorylated AKT (phospho- AKT), total AKT (pan-AKT), AKT1 and AKT2 in Rcho-1 TS cells expressing control shRNA or shRNA specific for *Akt1*, *Akt2* or *Akt3*. β-ACTIN was included as a loading control.

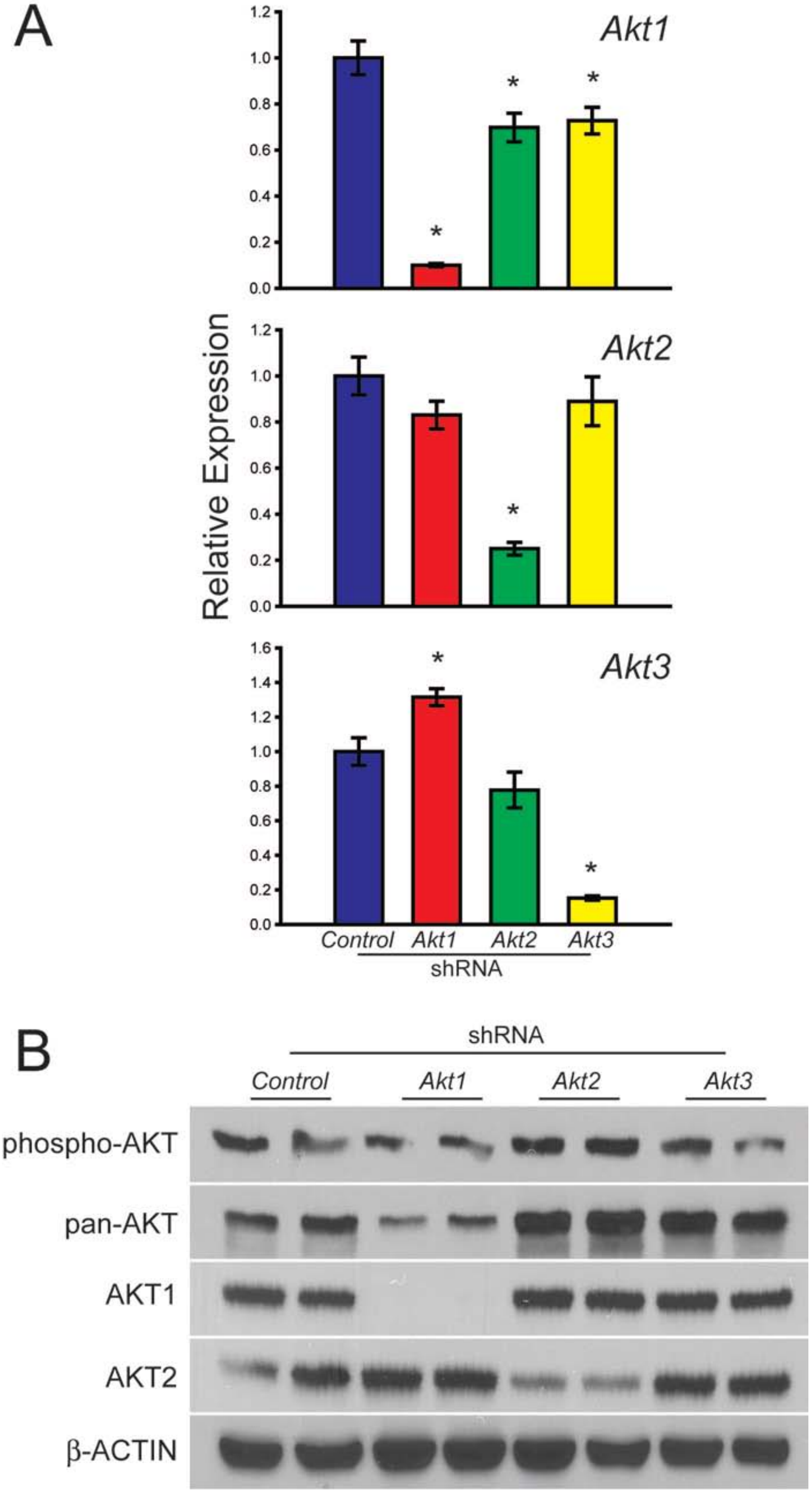
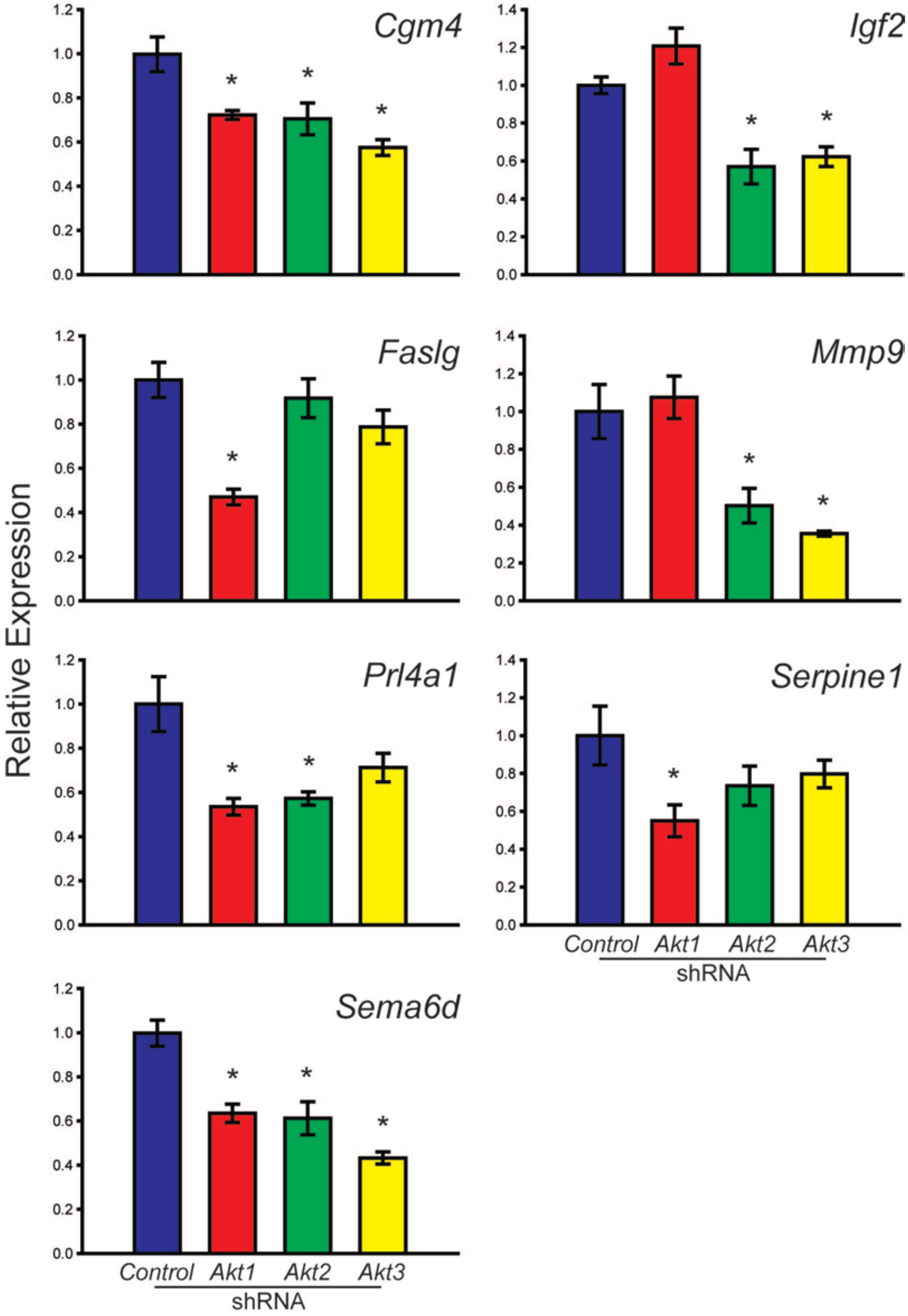

A
Akt1
Akt2
Akt3
Relative Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.6
1.4
*
Control
Akt1
Akt2
Akt3
shRNA
B
shRNA
Control
Akt1
Akt2
Akt3
phospho-AKT
pan-AKT
AKT1
AKT2
β-ACTIN

Fig. 3.5. Expression of ‘invasion-vascular remodeling related’ genes in Rcho-1 TS cells expressing *Akt1*, *Akt2,* or *Akt3* specific shRNAs. qRT-PCR analyses of ‘invasion-vascular remodeling related’ genes in Rcho-1 TS cells expressing control shRNA or shRNAs specific for *Akt1*, *Akt2*, or *Akt3* after eight days of differentiation. All samples were normalized to control samples. Bars represent means ± the standard error of the mean. Expression values significantly different from controls are indicated with an asterisk (*P<0.05).


Relative Expression
Cgm4
Igf2
Faslg
Mmp9
Prl4a1
Serpine1
Sema6d
*
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Control
Akt1
Akt2
Akt3
shRNA

chamber assay (**Fig. 3.6**). Trophoblast cell phenotypes resulting from the knockdown of individual AKT isoforms provide further support for the involvement of the PI3K/AKT signaling pathway in the regulation of trophoblast invasion and vascular remodeling. However, the results also demonstrate that there is a potentially complex interplay of each isoform in modulating the trophoblast cell phenotype.

FRA1 and PI3K/AKT signaling

We next sought to identify potential downstream mediators of the PI3K/AKT signaling pathway responsible for regulating the trophoblast cell invasive and vascular remodeling phenotype. In cancer cells, FRA1 is regulated by PI3K/AKT signaling and this integrated pathway is linked to cell invasion (Cao et al. 2006; Chandrasekar et al., 2006; Ramachandran et al. 2010). The exact mechanism of PI3K/AKT regulation has not yet been worked out. FRA1 has also been linked to placentation (Schreiber et al., 2000) and is expressed in a differentiation-dependent pattern in rat trophoblast cells (Chapter 1; Kent et al., 2010).

FRA1 is expressed in trophoblast giant cells and invasive trophoblast cells

Expression of FRA1 was examined in trophoblast cell populations maintained in vitro and developing in vivo. *Fra1* mRNA increases in Rcho-1 TS cells during differentiation (**Fig. 3.7A**) and FRA1 protein can be detected in

Fig. 3.6. Invasive abilities of Rcho-1 TS cells expressing *Akt1*, *Akt2,* or *Akt3* specific shRNAs. Graphical presentation of results from the Matrigel invasion chamber assays. Cells were counted from eight replicates and normalized to control samples. Bars represent means ± the standard error of the mean.

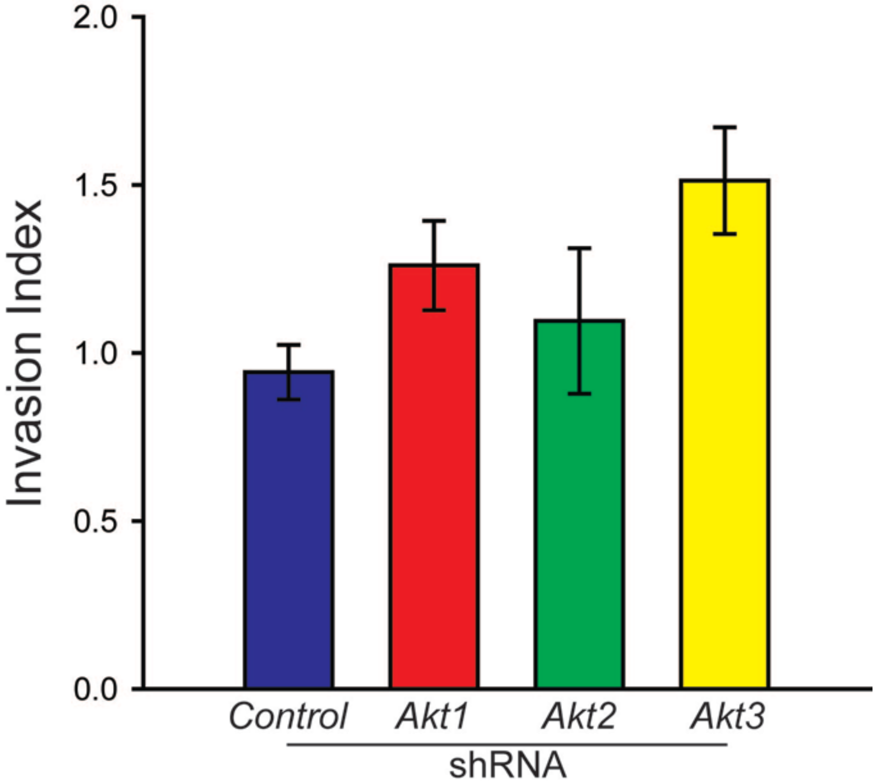
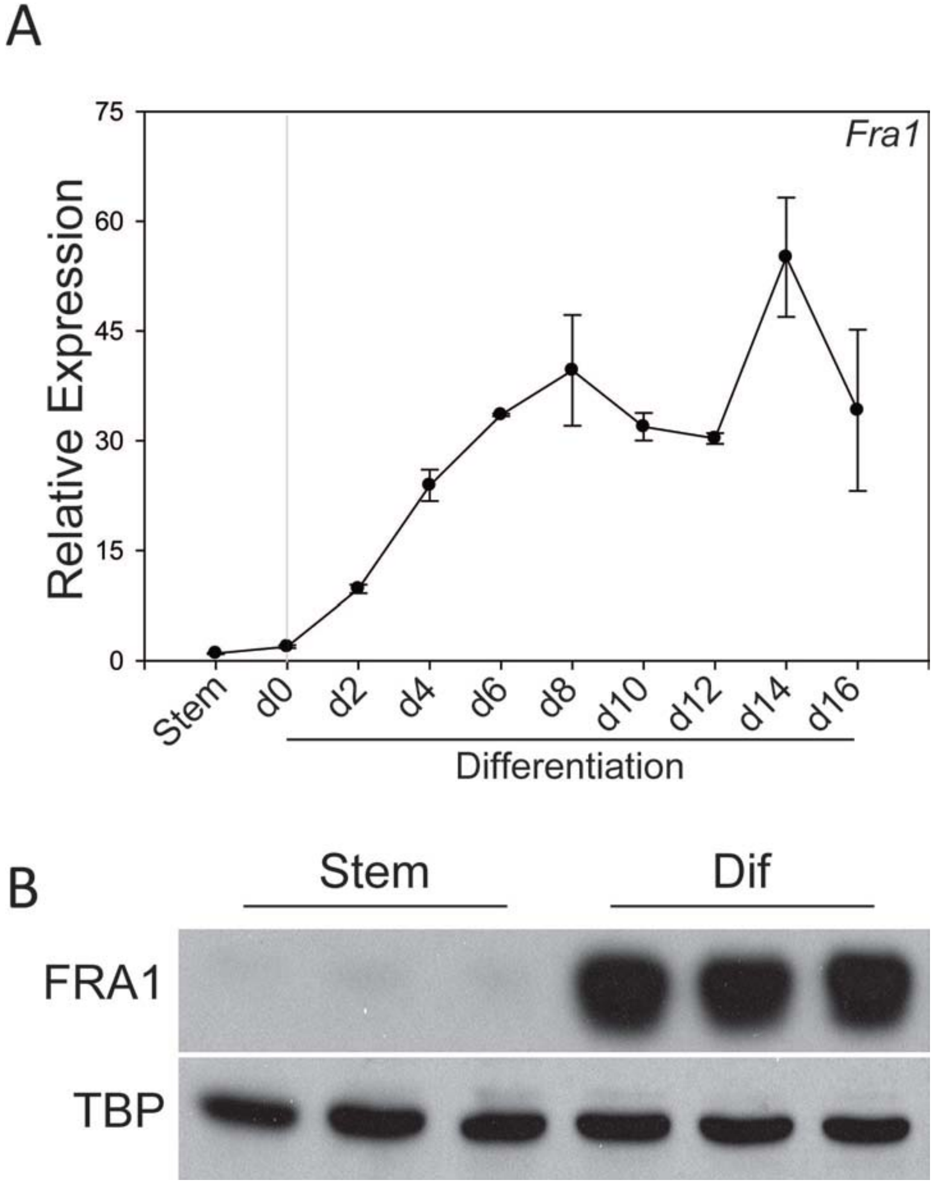

2.0
1.5
1.0
0.5
0.0
Invasion Index
Control
Akt1
Akt2
Akt3
shRNA

Fig. 3.7. Expression of FRA1 in Rcho-1 TS cells. A) qRT-PCR analysis of *Fra1* expression during Rcho-1 TS cell differentiation. **B)** Western blot analysis of FRA1 protein in nuclear lysates from Rcho-1 TS cells in stem (Stem) and differentiated (Dif) cell states. TATA box binding protein (TBP) was included as a loading control.


A
75
60
45
30
15
0
Relative Expression
Fra1
Stem
d0
d2
d4
d6
d8
d10
d12
d14
d16
Differentiation
B
Stem
Dif
FRA1
TBP

differentiated Rcho-1 TS cells (**Fig. 3.7B**). FRA1 is expressed in the rat placenta on gestation d11.5 and d18.5 as assessed by western blotting. On d18.5 the FRA1 protein was restricted to the junctional zone of the chorioallantoic placenta (**Fig. 3.8A**). FRA1 was localized to the nuclei of trophoblast giant cells by immunohistochemistry on gestation d7.5, d11.5, and d18.5. Trophoblast giant cells were identified morphologically and by their expression of PRL3D1and PRL3B1 (**Fig. 3.8B**). Co-staining with anti-FRA1 and anti-CYTOKERATIN (an epithelial cell marker routinely used to identify trophoblast cells) demonstrated that FRA1 is also expressed in the nuclei of invasive trophoblast cells on gestation d11.5 (**Fig. 3.8C**). These findings suggest that FRA1 could be a candidate regulator of trophoblast cell invasion and/or vascular remodeling.

PI3K/AKT signaling regulates FRA1

To determine if the PI3K/AKT signaling pathway can regulate FRA1 we examined the presence of FRA1 protein in nuclear and cytoplasmic lysates from control and PI3K/AKT disrupted Rcho-1 TS cells. FRA1 expression was greatly diminished in cells treated with PI3K or AKT inhibitors in nuclear (**Fig. 3.9A**) but not cytoplasimic (**Fig. 3.9C**) lysates. These findings indicate that the PI3K/AKT signaling pathway regulates nuclear accumulation of FRA1; the site of FRA1 action.

Fig. 3.8. Localization of FRA1 protein in the rat placenta. A) Analysis of protein levels of FRA1 in placental tissues by western blotting. Nuclear extracts were collected from gestation d11.5 and d18.5 rat placentas. d18.5 placental tissues were dissected into junctional and labyrinth zones. TBP was included as a loading control. **B)** Immunohistochemical characterization of FRA1 in rat placentation sites. Top panels: FRA1 expression on gestational d7.5, 11.5 and 18.5. Boxes indicate area of middle panels. Bars =1 mm. Middle panels: FRA1 is localized to the nucleus of trophoblast giant cell (arrows). Lower panels: PRL3D1 (d7.5) and PRL3B1 (d11.5 and d18.5) localization was used to illustrate the position of trophoblast giant cells (arrows). Bars =100 μm. **C)** Left: Immunohistochemical analysis of CYTOKERATIN localization on gestational d11.5. The boxed area is an example of trophoblast lined blood vessels similar to the region shown in the right panels. Right: Dual immunofluorescence localization of FRA1 (red) and CYTOKERATIN (green) in invasive trophoblast cells within gestation d11.5 placentation sites. Nuclei were visualized with DAPI (blue). Bar= 100 μm.

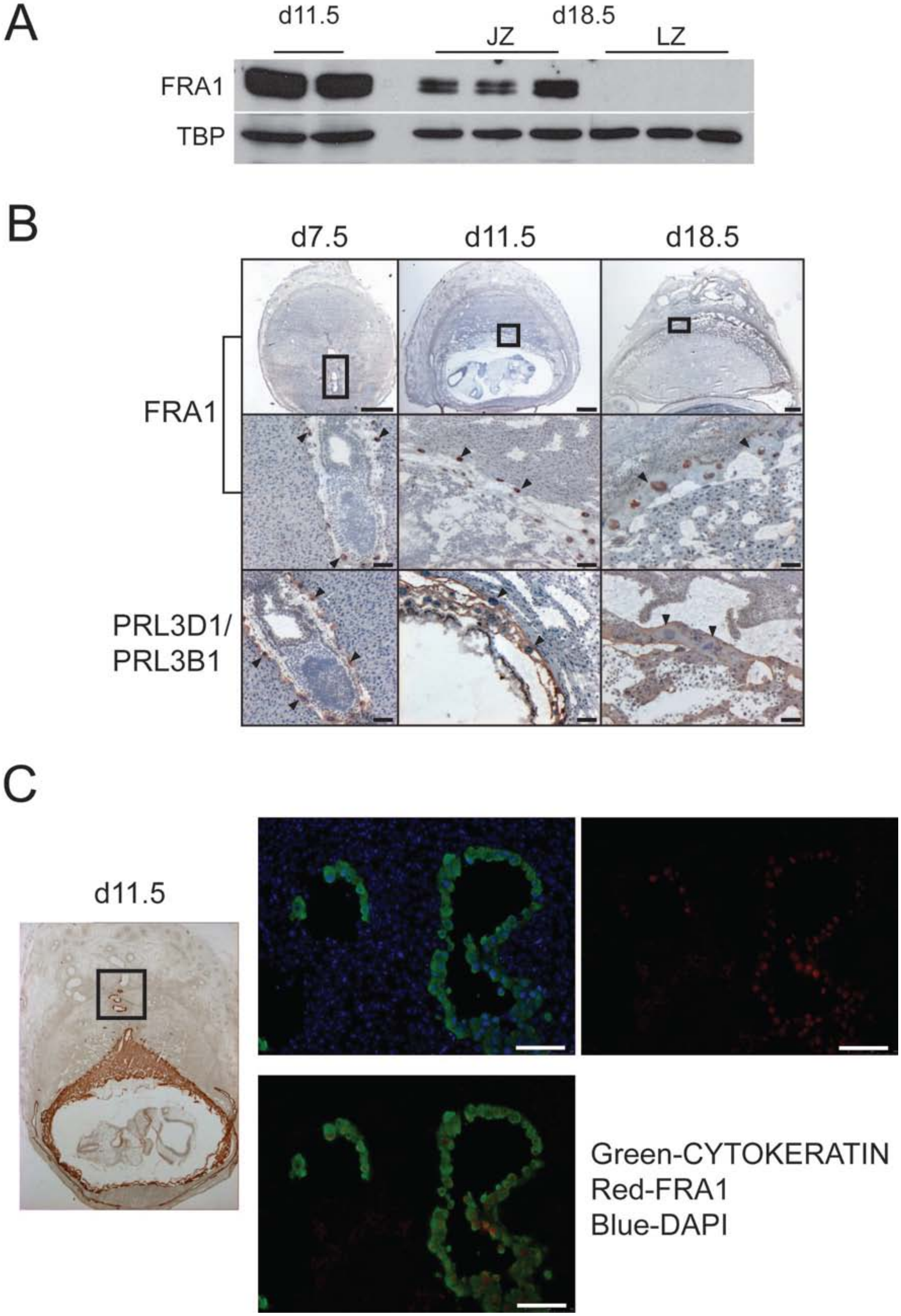
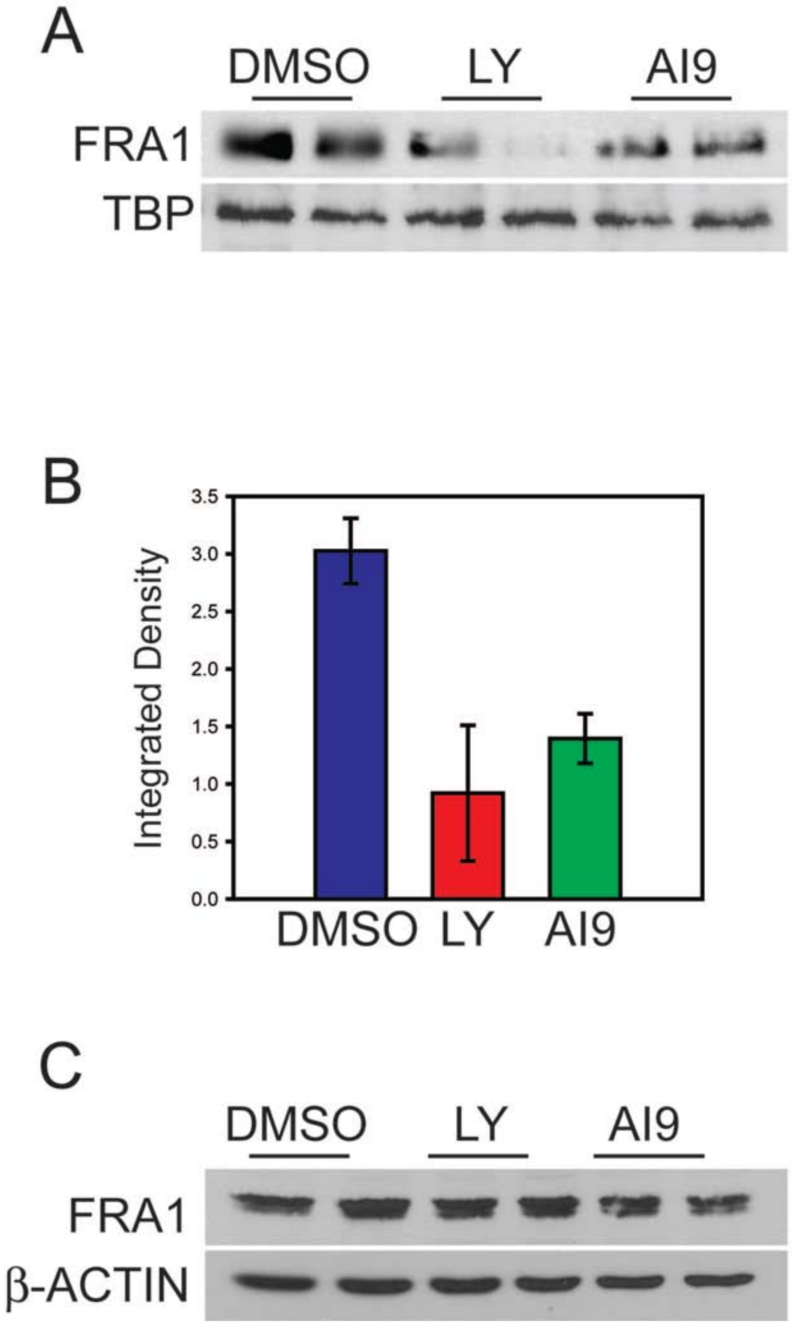

A
d11.5
d18.5
JZ
LZ
FRA1
TBP
B
d7.5
d11.5
d18.5
FRA1
PRL3D1/
PRL3B1
C
d11.5
Green-CYTOKERATIN
Red-FRA1
Blue-DAPI

Fig. 3.9. Modulation of the PI3K/AKT signaling pathway inhibits nuclear accumulation of FRA1. A) Analysis of FRA1 protein in nuclear lysates of differentiating Rcho-1 TS cells treated with LY294002 (LY, 10 μM), AKT inhibitor IX (AI9, 10 μM), or vehicle control (DMSO, 0.01%) by western blotting. **B)** Quantificaion of western blot analysis shown in **A**. TBP was included as a loading control. **C)** Analysis of FRA1 protein in cytoplasmic lysates of differentiating Rcho-1 TS cells treated with LY294002 (LY, 10 μM), AKT inhibitor IX (AI9, 10 μM), or vehicle control (DMSO, 0.01%) by western blotting.


A
DMSO
LY
AI9
FRA1
TBP
B
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Integrated Density
DMSO
LY
AI9
C
DMSO
LY
AI9
FRA1
β-ACTIN

FRA1 and trophoblast invasion

In the next series of experiments, we investigated the role of FRA1 in regulating the trophoblast cell invasive phenotype.

FRA1 regulates expression of PI3K/AKT-sensitive 'invasion-vascular remodeling related' genes

The biological actions of FRA1 were investigated following knockdown of FRA1 in Rcho-1 TS cells. *Fra1* mRNA and FRA1 protein were effectively decreased in Rcho-1 TS cells using lentiviral-delivered shRNAs (**Fig. 3.10**). Knockdown of FRA1 resulted in a decrease in expression of several 'invasion-vascular remodeling related' genes including: *Ceacam3, Cgm4, Mmp9, Prl4a1,* and *Sema6d*. Other 'invasion-vascular remodeling related' genes were unaffected by FRA1 knockdown (*Ctsd, Faslg, Igf2, Il17f* and *Serpine1*; data not shown), while expression *Adm* was significantly upregulated (**Fig. 3.11**).

FRA1 regulates in vitro trophoblast cell invasion

The effects of FRA1 on the regulation of a subset of 'invasion-vascular remodeling related' genes prompted an analysis of the role of FRA1 in the regulation of in vitro trophoblast cell invasion. Similar to modulation of the PI3K/AKT signaling pathway, loss of FRA1 decreased the in vitro invasive ability of Rcho-1 TS cells as assessed using the Matrigel chamber invasion assay (**Fig. 3.12**). These results indicate that FRA1 mediates some actions of the PI3K/AKT signaling pathway in vitro, including indices of invasive cell behavior.

Fig. 3.10. Analysis of FRA1 knockdown. A) qRT-PCR analysis of *Fra1* in Rcho-1 TS cells expressing control shRNA or shRNA specific for *Fra1* after eight days of differentiation. All samples were normalized to control samples. Bars represent means ± the standard error of the mean. Expression values significantly different from controls are indicated with an asterisk (*P<0.05). **B)** Western blot analysis of FRA1 in Rcho-1 TS cells expressing control shRNA or an shRNA specific for Fra1 after eight days of differentiation. TBP was included as a loading control.

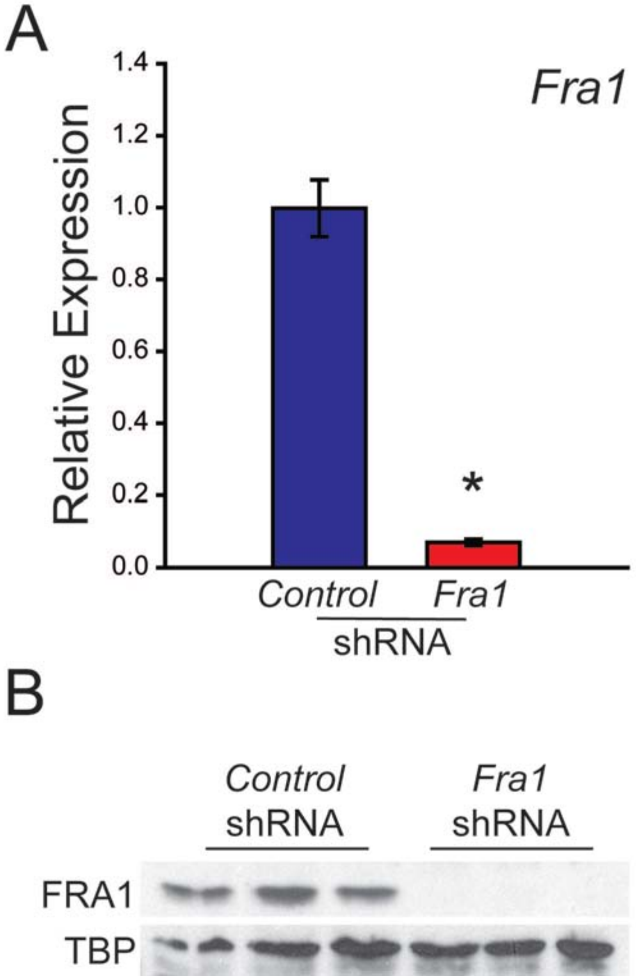

A
Fra1
Relative Expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Control
Fra1
shRNA
B
Control
shRNA
Fra1
shRNA
FRA1
TBP

Fig. 3.11. Expression of ‘invasion-vascular remodeling related’ genes in Rcho-1 TS cells expressing a *Fra1* specific shRNA. qRT-PCR analysis of ‘invasion-vascular remodeling related’ genes in Rcho-1 TS cells expressing control shRNA or an shRNA specific for *Fra1* after eight days of differentiation. All samples were normalized to control samples. Bars represent means ± the standard error of the mean. Expression values significantly different from controls are indicated with an asterisk (*P<0.05).

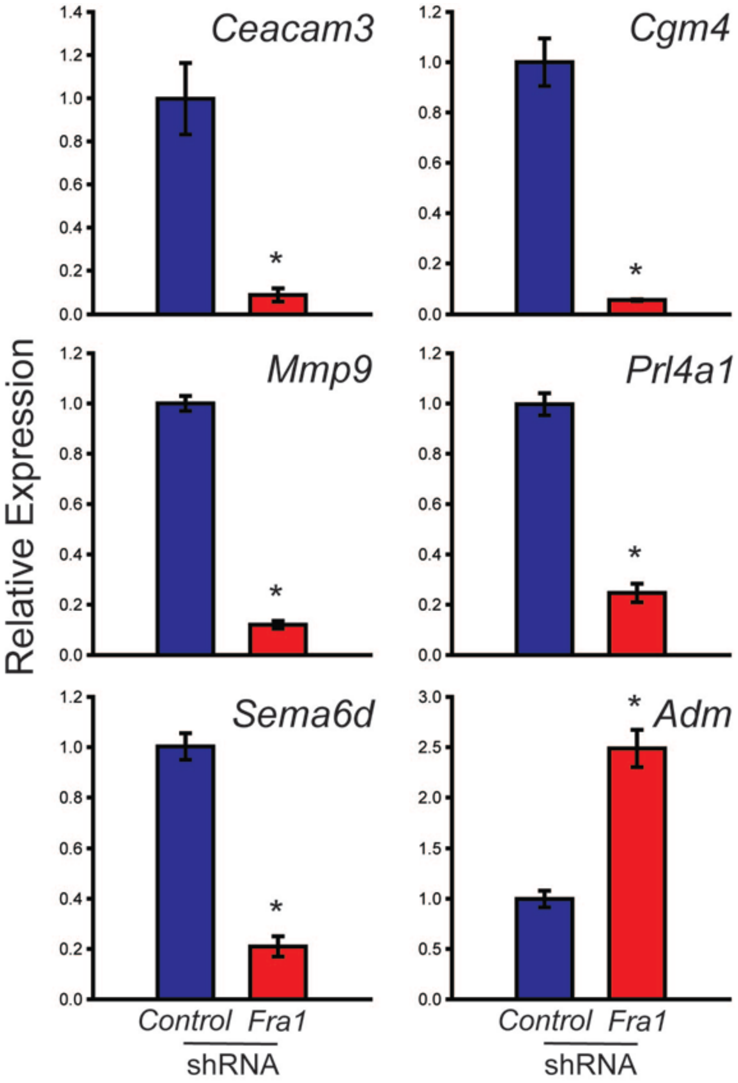
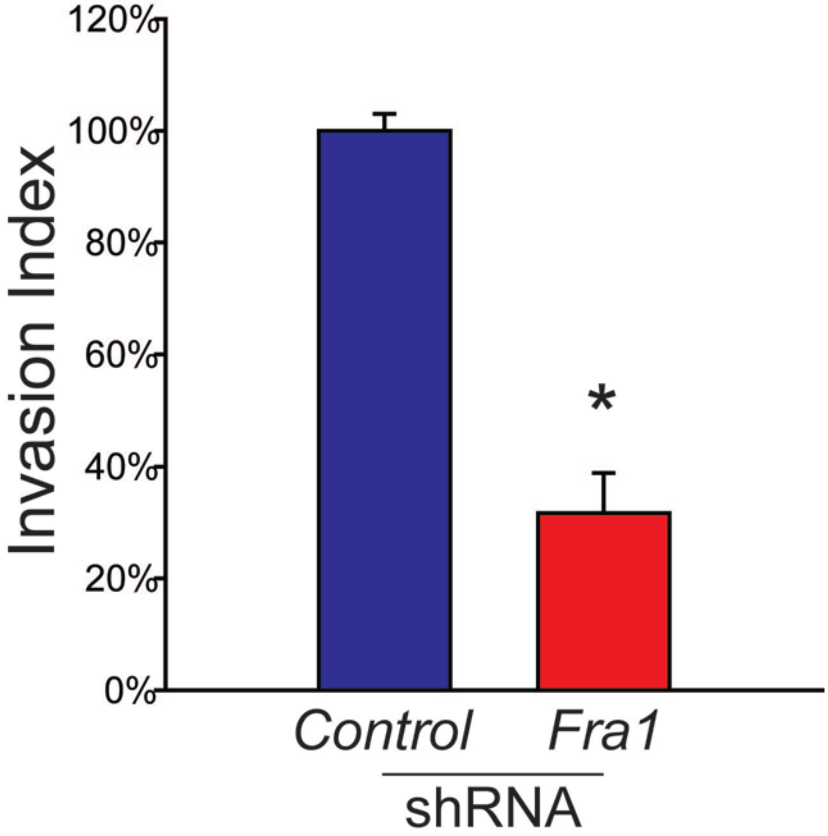

Relative Expression
Ceacam3
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Cgm4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Mmp9
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Prl4a1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Sema6d
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Adm
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
Fra1
shRNA
Control
Fra1
shRNA

Fig. 3.12. Effects of FRA1 on in vitro trophoblast cell invasion. Graphical presentation of results from the Matrigel invasion chamber assays. Cells were counted from nine replicates and normalized to control samples. Bars represent means ± the standard error of the mean. Expression values significantly different from controls are indicated with an asterisk (*P<0.05).


Invasion Index
120%
100%
80%
60%
40%
20%
0%
*
Control
Fra1
shRNA

FRA1 regulates in vivo trophoblast cell invasion

We next evaluated a role for FRA1 in regulating in vivo trophoblast cell invasion. A trophoblast cell-specific lentiviral system was used to deliver a *Fra1*-specific shRNA (Lee et al. 2009). Gestation d4.5 rat embryos were exposed to lentiviral particles expressing control shRNA or shRNA specific for *Fra1* and then transferred to d3.5 pseudopregnant female rats and allowed to develop until d11.5. The in vivo lentiviral system effectively knocked down *Fra1* mRNA and FRA1 protein expression in gestation d11.5 trophoblast tissue (**Fig. 3.13**). Histological analysis demonstrated that the trophoblast invasion index (depth of invasion/height of decidua) was significantly less at placentation sites with FRA1 knockdown (**Fig. 3.14**), implicating FRA1 as a component of the in vivo regulatory pathway controlling trophoblast cell invasion.

Collectively our findings indicate that a regulatory pathway involving PI3K/AKT and FRA1 controls the trophoblast invasive phenotype.

Fig. 3.13. Analysis of FRA1 knockdown in vivo.

FRA1 expression in placentas exposed to *control* or *Fra1* specific shRNA was examined on gestational day 11.5. **A)** *Fra1* mRNA level was measured by qRT-PCR in dissected placental samples. Dashed lines mark the mean. Mean of the *Fra1* shRNA expressing placentas were significantly different from control (*P<0.05). Solid lines indicate ± the standard error of the mean. **B)** FRA1 protein level was measured by western blot analysis in dissected placental samples. TBP is included as a loading control.

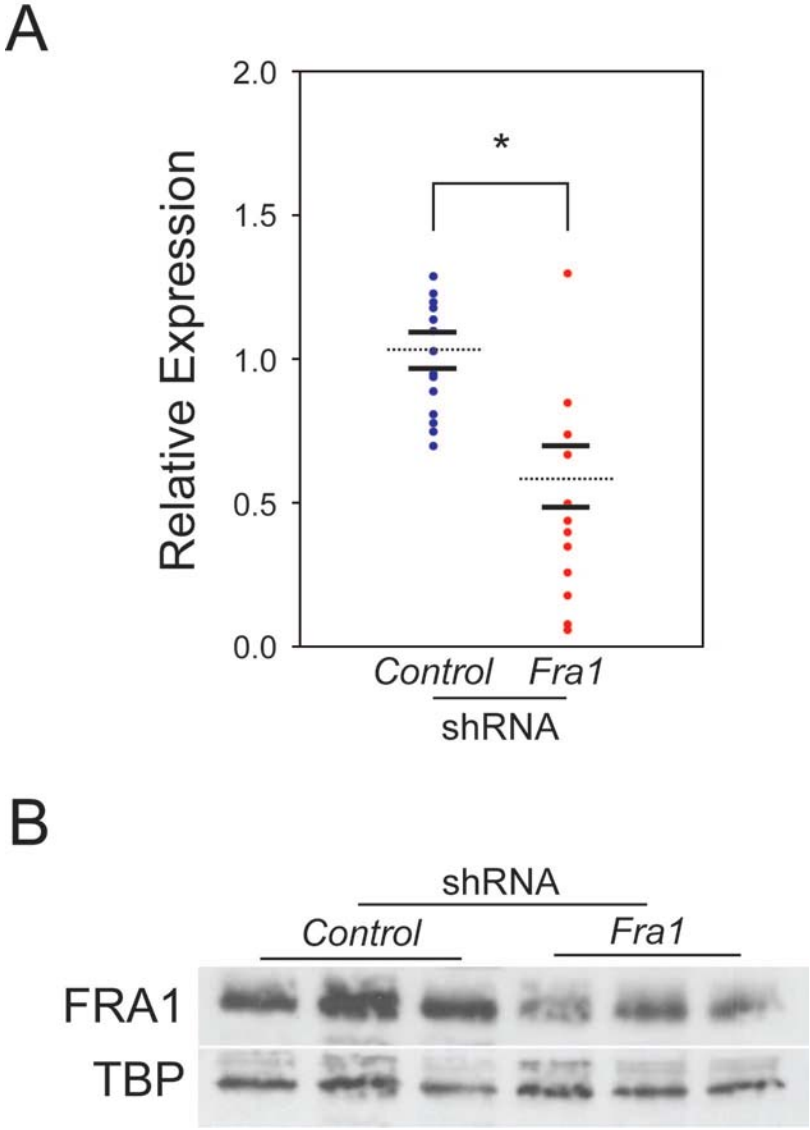
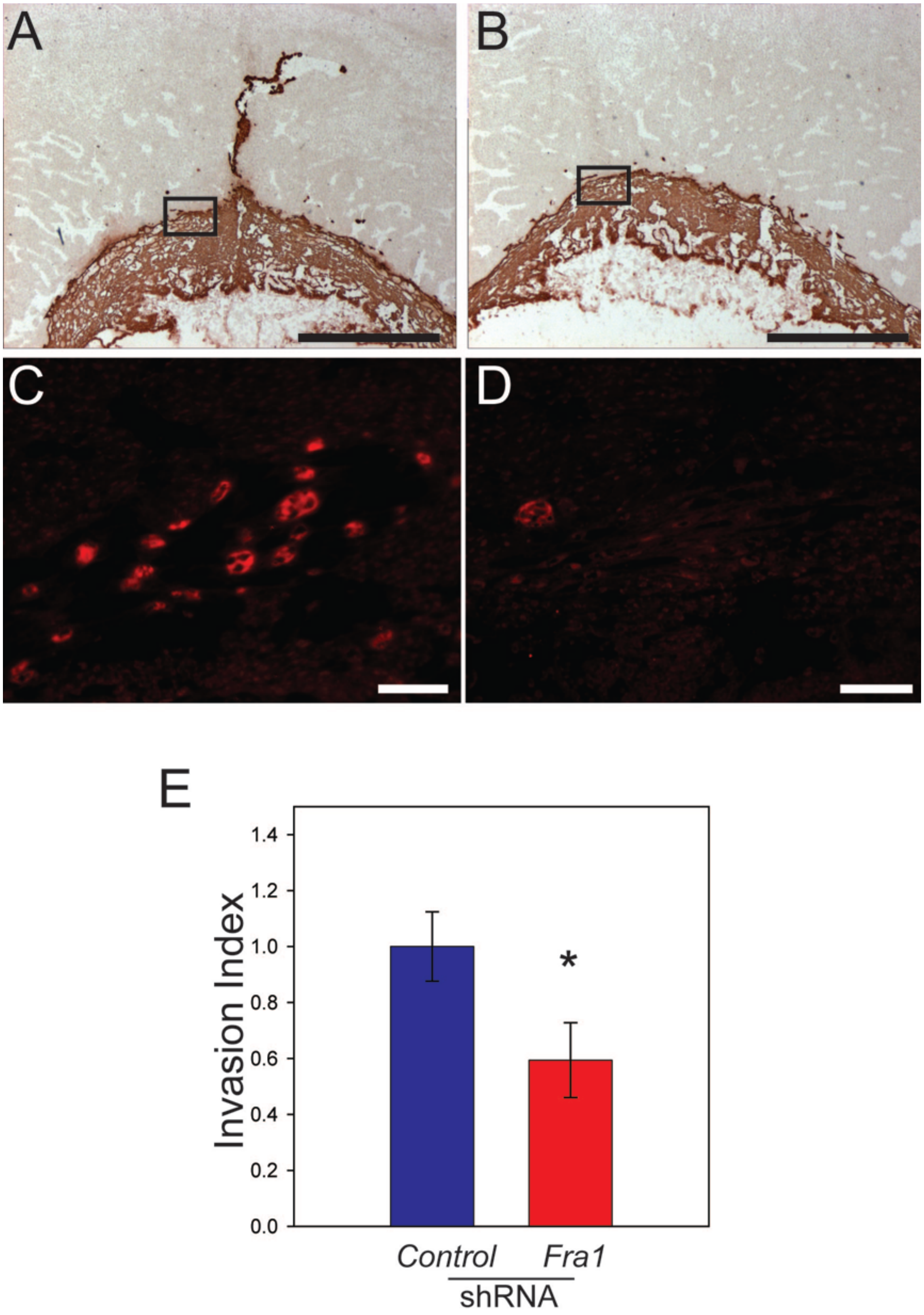

A
2.0
1.5
1.0
0.5
0.0
Relative Expression
*
Control
Fra1
shRNA
B
shRNA
Control
Fra1
FRA1
TBP

Fig. 3.14. In vivo role of FRA1 in regulating trophoblast cell invasion. Immunohistochemistry of rat placentation sites was performed using anti-CYTOKERATIN **(A,B)** to identify trophoblast or anti-FRA1 **(C,D)** to illustrate FRA1 knockdown. Representative sections of gestational day 11.5 placentation sites for embryos treated with *control* shRNA **(A,C)** or shRNA specific for *Fra1* **(B,D)** are shown. Boxes in **(A,B)** show approximate position of **(C,D)**. **(A,B)** Bar= 1 mM, **(C,D)** Bar= 100 μM. **E)** Quantification of the invasion index of trophoblast treated with *control* shRNA or shRNA specific for *Fra1*. Expression values significantly different from control are indicated with an asterisk (*P<0.05).


A
B
C
D
E
Invasion Index
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
*
Control
Fra1
shRNA

DISCUSSION

Intrauterine trophoblast invasion and remodeling of the uterine vasculature are important features of hemochorial placentation. Blood vessels are restructured permitting increased blood flow and nutrient delivery to the placenta and ultimately the fetus. In this study we have identified a cellular signaling pathway involving PI3K/AKT and FRA1 that regulates trophoblast cell invasive and vascular remodeling phenotypes. Small molecule inhibitors were effective in interfering with PI3K/AKT pathway. This strategy of nullifying the actions of PI3K or AKT in trophoblast cells led to the inhibition of pro-invasion and vascular remodeling genes. Blockade of the PI3K/AKT pathway also decreased nuclear accumulation of the FRA1 protein. Disruptions of PI3K, AKT, or FRA1 interfered with trophoblast cell invasion.

PI3K/AKT regulation of 'invasion-vascular remodeling related' genes

PI3K/AKT signaling activates the expression of a set of eleven genes encoding proteins implicated in cell invasion and/or vascular remodeling. The 'invasion-vascular remodeling related' set of genes can be placed into two groups, genes encoding proteins that affect trophoblast movement and genes encoding proteins that alter the vasculature. In the first group, the genes encode proteins that can induce cell movement (IGF2; Irwin et al., 1999; Aplin et al., 2000; Hiden et al., 2009), guide cells (SEMA6D, Toyofuku et al. 2004ab), and remodel the extracellular matrix (MMP9, Peters et al., 1999; SERPINE1, Graham, 1997). The second group consists of genes encoding proteins that directly affect endothelial cells and blood vessels (ADM, CGM4, CTSD, FASLG, IL17F: Park et al., 2000; Wilson et al., 2004; Ashton et al., 2005; Kato et al., 2005; Harris et al., 2006; Temmesfeld-Wollbrück et al., 2007; Gaffen, 2008) or indirectly modulate blood vessel remodeling by altering immune cell cytokine production (CGM4, IL17F, PRL4A1: Müller et al., 1999; Wessells et al., 2000; Waterhouse et al., 2002; Ain et al., 2003; Ain et al., 2004; Kolls and Lindén, 2004; Ha et al., 2005; Wynne et al., 2006; Spolski and Leonard, 2009). Although, each of the eleven proteins encoded by these genes has the potential to influence trophoblast invasion and/or vascular remodeling, the relative roles of each regulator and the orchestration of these genes and their respective encoded proteins are unknown.

AKT isoforms and trophoblast cell biology

Three AKT isoforms are expressed in trophoblast cells and regulate trophoblast cell biology in a unique and complicated overlapping manner. Knockdown of individual AKT isoforms affected overlapping subsets of PI3K/AKT-sensitive ‘invasion-vascular remodeling related’ genes and led to a unique expression profile. Expression of some ‘invasion-vascular remodeling related’ genes (*Adm, Ceacam3*, *Ctsd,* and *Il17f*), nuclear accumulation of FRA1 protein, and trophoblast cell invasion were not affected by interfering with the expression of a single AKT isoform. The retention of these functions in single

AKT knockdown cells was associated with the presence of active cellular AKT, suggesting a potentially complex interplay of each AKT isoform.

Even though knockdown of individual AKT isoforms was not sufficient to decrease trophoblast cell invasion and FRA1 nuclear protein accumulation, we gained some insight into the role of individual AKT isoforms in regulating expression of the ‘invasion-vascular remodeling related’ genes. Two of the eleven ‘invasion-vascular remodeling related’ genes (*Cgm4; Sema6d*) are sensitive to disruptions in any of the three AKT isoforms indicating that a high level of AKT activity is needed for regulating their expression. Other ‘invasion-vascular remodeling related’ genes were sensitive to disruption of a single isoform. *Faslg* and *Serpine1* were only sensitive to interference of AKT1, implying that there must be some AKT1 substrate specificity associated with their regulation. *Prl4a1* was sensitive to either AKT1 or AKT2 knockdown and *Mmp9* and *Igf2* were sensitive to either AKT2 or AKT3 knockdown. These findings indicate a mixture of substrate specificity and promiscuity. *Adm*, *Ceacam3*, *Ctsd*, and *Il17f* were exquisitely sensitive to small molecule inhibition of PI3K or AKT but were not affected by knockdown of any single AKT isoform. Following the above logic, we would propose that activation of this subset of genes requires lower levels of AKT activity and/or exhibits complete AKT substrate promiscuity. The same conclusion could be made for the regulation of FRA1 protein nuclear accumulation and trophoblast invasion. The balance of each individual AKT isoform may also be critical for the regulation of the trophoblast cell phenotype, as has been shown in breast cancer cells (Iliopoulos et al., 2009). Disruption of multiple AKT isoforms in the trophoblast cells may provide additional insights; however, based on preliminary experiments this approach may be challenging due to effects on cell survival. Finally, subcellular location of each AKT isoform could be a key to understanding roles for each isoform in trophoblast cells. Santi and Lee (2010) have described specific intracellular sites for each AKT isoform in HEK293 and HEK293T cells. Intracellular AKT isoform location impacts access to substrates and activation of specific regulatory pathways.

PI3K/AKT regulation of FRA1

PI3K/AKT signaling regulates FRA1 in trophoblast cells. Similar findings have been reported for other cell types (Tiwari et al. 2003; Belguise et al., 2005; Ramos-Nino et al. 2008). FRA1 has also been shown to be a downstream mediator of the PI3K/AKT signaling pathway in regulating the expression of pro-invasion genes, including *Mmp9*, and cellular invasion (Cao et al. 2006; Chandrasekar et al., 2006; Ramachandran et al. 2010). The precise mechanism of PI3K/AKT regulation of FRA1 has not been clarified but here we show that loss of PI3K/AKT signaling results in a decrease of the nuclear accumulation of FRA1 protein. FRA1 may be a substrate for AKT and its phosphorylation may influence its nuclear accumulation and/or activity. Alternatively, the role of the PI3K/AKT signaling pathway in regulating FRA1 may be indirect. Cross-talk between PI3K/AKT and other cellular signaling pathways in trophoblast has been described (Qui et al., 2004a,b; Sonderegge et al., 2010) and may contribute to the stabilization of FRA1 protein in the nucleus. FRA1 undergoes ubiquitin-independent degradation by the proteasome, which can be prevented by ERK1/2 phosphorylation within the C-terminal stabilizing region of the FRA1 protein (Basbous et al. 2008). Whether AKT can act similarly or whether it activates another kinase (such as ERK1/2) to stabilize FRA1 remains to be determined.

FRA1 and placentation

FRA1 has been implicated as a key regulator of placentation. A null mutation in the *Fra1* gene disrupts morphogenesis of the mouse placenta and results in prenatal lethality (Schreiber et al., 2000). Defective vascularization of the labyrinth zone of the mouse chorioallantoic placenta was viewed as the insult responsible for the prenatal demise of the fetus. In our report, invasive trophoblast cell populations were shown to express FRA1, including those possessing an endovascular location, and FRA1 was demonstrated to regulate the expression of a subset of trophoblast 'invasion-vascular remodeling related' genes (*Ceacam3, Cgm4, Mmp9, Prl4a1, Sema6d*). FRA1 responsive genes include: genes sensitive to knockdown of any AKT isoform (*Cgm4, Sema6d*), a gene regulated by AKT1 and AKT2 (*Prl4a1*), and a gene regulated by AKT2 and AKT3 (*Mmp9*) but not the genes that are only responsive to AKT1 knockdown (*Faslg, Serpine1*). This implies that FRA1 is a potential mediator of AKT2 and/or AKT3 signaling. Both in vitro and in vivo experiments, also implicated FRA1 as a regulator of the invasive trophoblast phenotype. In vivo trophoblast-specific knockdown of FRA1 led to significantly decreased intrauterine endovascular trophoblast invasion. Disruptions in labyrinthine vascularization may have also been evident in the FRA1 hypomorphs but were not examined in our report. Trophoblast invasion is less prominent in the mouse versus the rat (Adamson et al. 2002; Ain et al. 2003; Coan et al. 2006). In the *Fra1* null mouse, trophoblast invasion was probably of secondary significance to interruptions in nutrient and waste exchange (Schreiber et al., 2000).

In conclusion, we have generated experimental evidence indicating that the PI3K/AKT and FRA1 signaling pathway is involved in regulating trophoblast invasive and vascular remodeling phenotypes.

CHAPTER 4: GENERAL DISCUSSION

I. Model

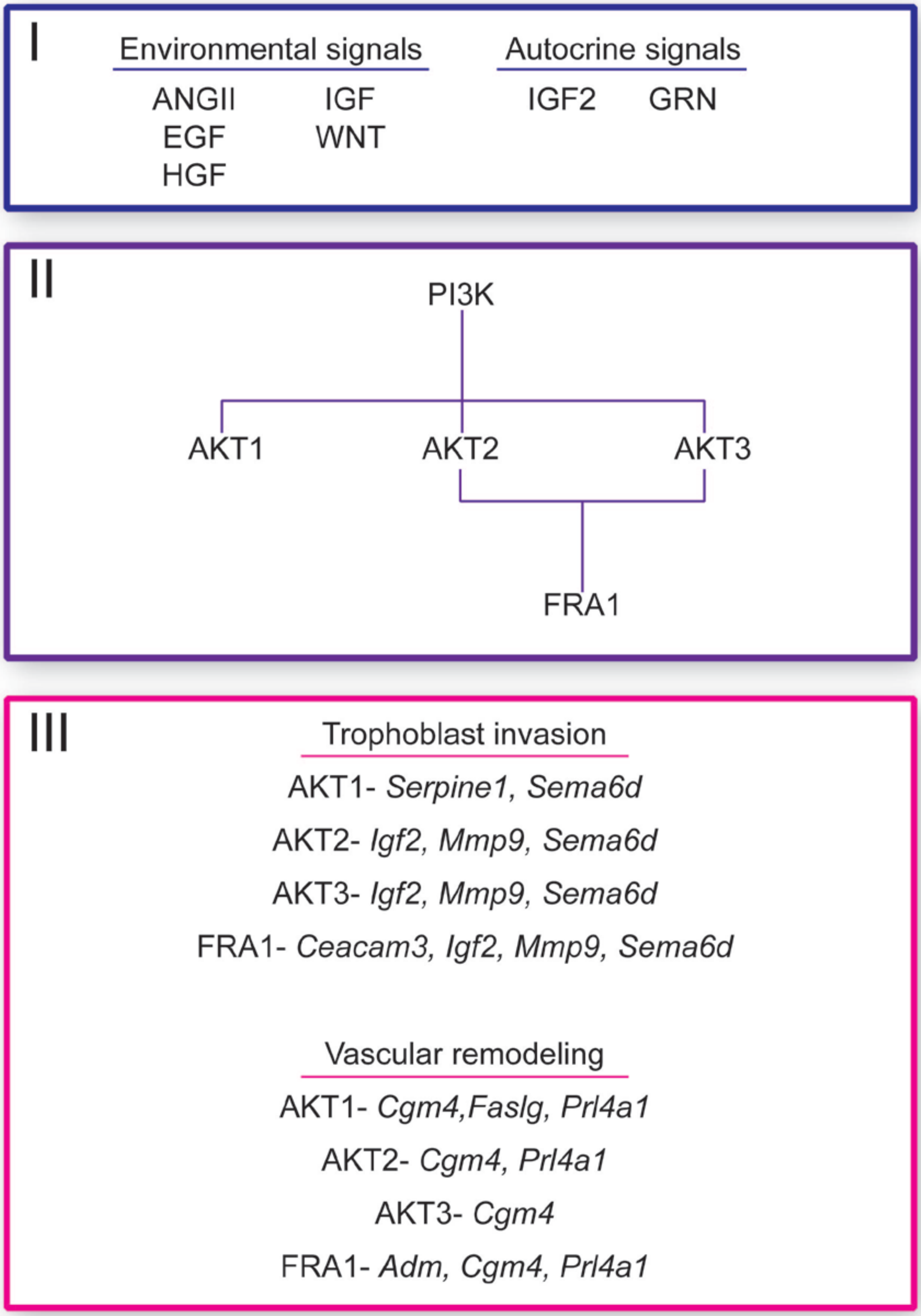
Figure 4.1 summarizes our findings. Part I illustrates known factors that can activate phosphatidylinositol 3-kinase (PI3K) in trophoblast cells. These factors can be found in the maternal environment or produced by the trophoblast. Part II is an overview of the AKT (also known as PKB) isoforms and their potential regulation of transcription. Part III is an overview of putative invasion and vascular remodeling genes that are controlled by the PI3K/AKT signaling pathway. The AKT isoform predicted to regulate each target gene is shown.

II. Regulation of trophoblast differentiation and invasion by the PI3K/AKT signaling pathway

Activation of the PI3K/AKT signaling pathway in trophoblast cells

There are many ligand-receptor complexes that activate the PI3K/AKT signaling pathway. They include growth factors and tyrosine kinase receptors and G proteins and G protein coupled receptors, among others (Cantley, 2002). Sources of ligands that activate PI3K can originate within the maternal environment or alternatively within trophoblast cells through an autocrine signaling mechanism. Several ligands have been implicated in promoting trophoblast invasion by activating the PI3K/AKT signaling pathway such as: angiotensin II (Ang II; Ishimatsu et al., 2006), epidermal growth factor (EGF; Qiu et al., 2004a,b), hepatocyte growth factor (HGF; Cartwright et al. 2002) and wingless 3A (WNT3A; Sonderegger et al., 2010).

Fig.4.1. Overview of the regulation of invasion and vascular remodeling related genes by the PI3K/AKT/FRA1 signaling pathway in trophoblast cells. Abbreviations: Part I - angiotensin II (ANGII), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), wingless (WNT) granulin (GRN) and insulin-like growth factor 2 (IGF2); Part II - phosphatidylinositol 3-kinase (PI3K), AKT (also known as protein kinase B, PKB) and fos like 1 (FOSL1 or FRA1); Part III - adrenomedullin (*Adm*), carcinoembryonic antigen-related cell adhesion molecule 3 (*Ceacam3*), carcinoembryonic antigen gene family 4 (*Cgm4),* cathepsin D (*Ctsd*), fas ligand (TNF superfamily, member 6; *Faslg)*, interleukin 17F (*Il17f*), matrix metalloproteinase-9 (*Mmp9*), prolactin family 4, subfamily a, member 1 (*Prl4a1)* sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D*, (Sema6d*), serine (or cysteine) peptidase inhibitor, clade E, member 1(*Serpine1*).


I
Environmental signals
ANGII
IGF
EGF
WNT
HGF
Autocrine signals
IGF2
GRN
II
PI3K
AKT1
AKT2
AKT3
FRA1
III
Trophoblast invasion
AKT1- *Serpine1, Sema6d*
AKT2- *Igf2, Mmp9, Sema6d*
AKT3- *Igf2, Mmp9, Sema6d*
FRA1- *Ceacam3, Igf2, Mmp9, Sema6d*
Vascular remodeling
AKT1- *Cgm4,Faslg, Prl4a1*
AKT2- *Cgm4, Prl4a1*
AKT3- *Cgm4*
FRA1- *Adm, Cgm4, Prl4a1*

Our work has revealed several additional ligands produced by trophoblast giant cells that can activate the PI3K/AKT signaling pathway (granulin, GRN and insulin-like growth factor 2, IGF2; **Chapter 2**). These ligands are produced by trophoblast giant cells may serve as an autocrine mechanism for constitutive activation of PI3K/AKT in trophoblast giant cells.

Constitutive activation of the PI3K/AKT in trophoblast giant cells could also be due to upregulation of one of the catalytic subunits of PI3K (phosphatidylinositol 3-kinase, catalytic, beta polypeptide, PIK3CB) during differentiation (**Chapter 2**). PIK3CB mutant blastocysts fail to show trophoblast giant cell outgrowth when cultured, indicating that this PI3K subunit may be essential for trophoblast giant cell differentiation (Bi et al., 2002).

Isoform specific roles for AKT in trophoblast cells

Akt isoforms in placental and embryonic development

There are three AKT isoforms (AKT1 /PKBα, AKT2/PKBβ and AKT3/PKBγ) downstream of PI3K (Brazil and Hemmings, 2001). These enzymes are conserved at the amino acid level and are thought to share substrate specificity (Coffer et al., 1998; Brazil and Hemmings, 2001). Analysis of mutant mouse models (summarized in **Table 4.1**) makes it clear that the three AKT isoforms have both overlapping and specific functions. Overall AKT1 is the most vital isoform for fetal survival with some contribution of AKT3 and to a lesser extent, AKT2.

Table 4.1. An overview of the phenotypes of AKT mutant mice

Mutant mouse model	Phenotype	Source
AKT1-/-	Viable with some fetal loss, disrupted placental development and intrauterine growth restriction.	Cho et al., 2001a; Yang et al., 2003
AKT2-/-	Viable with intolerance to glucose and insulin.	Cho et al., 2001b
AKT3-/-	Viable with decreased brain mass.	Easton et al., 2005
AKT1-/-, AKT2-/-	Lethal postnatally with multiple defects and intrauterine growth restriction.	Peng et al., 2003
AKT1-/-, AKT3-/-	Embryonic lethal (gestational day 12.5). Vascular and placental developmental defects.	Yang et al., 2005
AKT2-/-, AKT3-/-	Viable with intrauterine growth restriction and intolerance to glucose and insulin.	Dummler et al., 2006
AKT1-/-, AKT2-/-, AKT3-/-	Embryonic lethal.	Dummler et al., 2006

AKT isoforms in invasion

There is an apparent role for the AKT pathway in inducing cellular invasion, but the roles of the individual isoforms remains unclear. In the cancer field most research has focused on AKT1 and AKT2. In most breast cancer models AKT1 inhibits invasion while AKT2 induces invasion (Dillon and Muller, 2010). In other model systems (fibroblast, fibrosarcoma and squamous carcinoma) AKT1 is clearly pro-invasive (Chin and Toker, 2010). A role for AKT3 in cancer cell invasion is still unclear but it is known to play a role in melanoma development (Madhunapantula and Robertson, 2009).

Modest efforts have been directed to dissecting individual AKT isoforms as regulators of trophoblast invasion. One study showed that overexpression of constitutively active Akt2 was not sufficient to stimulate trophoblast invasion (Qui et al., 2004b). Likewise overexpression of constitutively active Akt1, Akt2 or Akt3 was not sufficient to upregulate expression of invasion related genes: matrix metalloproteinase 9 (MMP9) or tissue inhibitor of metalloproteinase-1 (TIMP1) (Qui et al., 2004a). However both studies showed that chemical inhibition of PI3K was sufficient to decrease invasion and expression of MMP9 and TIMP1.

Similar to Qui et al., (2004a,b) we demonstrated that inhibition of PI3K/AKT signaling using small molecule inhibitors specific for PI3K or AKT is sufficient to decrease expression of genes involved invasion and vascular remodeling and trophoblast cell invasion. Knockdown of single AKT isoforms using isoform specific short hairpin RNAs (shRNAs) did not have an effect on trophoblast invasion, suggesting that there are overlapping functions between the isoforms (**Chapter 3**). If possible, knockdown of multiple isoforms at the same time might give more insight into the role of each isoform in regulating invasive ability. Thus far, we have not succeeded in generating stable trophoblast cells with significant knockdown of more than one AKT isoform. Conditional/inducible shRNA strategies for AKT isoform knockdown may represent a preferred option.

Individual AKT isoform disruption yielded distinct patterns of gene expression (**Chaper 3, Fig. 4.1**). Two genes were regulated solely by AKT1: Fas ligand (TNF superfamily, member 6, *Faslg*) and Serine (or cysteine) peptidase inhibitor, clade E, member 1, (*Serpine1*). Identification of specific targets downstream of AKT1 was not unexpected. Of the AKT mutant mouse models, deletion of AKT1 causes the most severe effects on placental development, fetal growth and viability and is therefore the most likely isoform to have specific targets. The functions of the proteins encoded by the AKT1-specific target genes give insight into possible roles for AKT1 in trophoblast cells. FASLG is involved in trophoblast-replacement of the endothelium from uterine spiral arteriole genes, while SERPINE1 modulates the activities of matrix remodeling enzymes. Thus similar to breast cancer cells AKT1 may not be pro-invasive in trophoblast, but instead may have a role in vascular remodeling.

PI3K/AKT mediators of trophoblast invasion

Regulation of Fos like protein 1 (FOSL1 or FRA1) by the PI3K/Akt signaling pathway

In this work, we have identified FRA1 as a mediator of PI3K/AKT action. We show that FRA1 expression is regulated by the PI3K/AKT pathway and that FRA1 regulates the expression of several PI3K/AKT regulated genes involved in invasion and vascular remodeling.

Although the role of the PI3K/AKT signaling pathway in the regulation of FRA1 has not been reported for trophoblast it has been shown to regulate *Fra1* expression in other cell types. In smooth muscle cells PI3K inhibition was shown to decrease FRA1 protein (Cao et al. 2006) and inhibition of PI3K or AKT decreases FRA1 activity (Ramachandran et al. 2010).

In a prostate cancer cell line, AKT3 was shown to increase *Fra1* mRNA while high levels of AKT1 actually decreased *Fra1* mRNA levels (Tiwari et al. 2003). In Rcho-1 TS cells we observed no change in FRA1 expression after knockdown of any of the AKT isoforms indicating that they may each contribute to the regulation of FRA1 and that one isoform may compensate for the loss of other isoforms (data not shown).

The link between AKT regulation and FRA1 is still unknown. In Rcho-1 TS cells this regulation is likely at the protein level, because changes in *Fra1* mRNA are modest in comparison to changes at the protein level (data not show). This leads us to hypothesize that FRA1 may be phosphorylated by AKT. Although FRA1 does not contain a classic AKT consensus phosphorylation site (RXRXXT/S), it does contain a site that can be phosphorylated by ERK (Basbous et al. 2008). This site regulates FRA1 protein stability. This site (SSAHRKSSSSSGDPS) shares some similarity with atypical AKT2 and AKT3 phosphorylation sites. Thus, there is merit in examining kinase-substrate relationships for each AKT isoform and FRA1

Crosstalk between PI3K/AKT/FRA1 and other signaling pathways and trophoblast invasion

Other pathways may also be involved in regulating trophoblast invasion. Two of the most likely signaling cascades are the mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway and the WNT pathway. The MAPK pathway is known to interact with the PI3K/AKT pathway. Two major points of cross talk between these pathways are: i) the same ligand/receptor complexes can activate each pathway; ii) PI3K can activate MAPK. MAPK signaling has also been implicated in trophoblast invasion (Qui et al., 2004a,b) and regulation of FRA1 protein stability (Basbous et al., 2007). Likewise the WNT signaling pathway can also regulate trophoblast invasion (Sonderegger et al., 2010). AKT can phosphorylate several components of the WNT pathway [glycogen synthase kinase 3 beta, GSK3B (Katoh and Katoh, 2006) and βCATENIN] and βCATENIN is known to regulate FRA1 expression (Mann et al., 1999).

Evaluation of the MAPK/ERK and WNT signaling pathways in Rcho-1 TS cells would be an interesting avenue to pursue, as would evaluation of the role of these pathways in the regulation of Rcho-1 TS cell invasion.

The role of FRA1 in trophoblast invasion

Identifying targets of FRA1

In this study we have identified several genes that are regulated by FRA1 knockdown (*Adm, Ceacam3, Cgm4, Mmp9, Prl4a1* and *Sema6d)* the next step is to determine if FRA1 directly regulates these genes. To achieve this, chromatin immunoprecipitation assays (ChIPs) could be used to localize FRA1 on regulatory sequences of impacted genes. A good starting point would be *Mmp9.* FRA1 has already been shown to bind the human *Mmp9* promoter (Chandrasekar et al., 2006) and may engage the *Mmp9* promoter in trophoblast cells, as well.

The AP-1 transcription factor complex has also been implicated in regulating the expression of prolactin family genes in trophoblast cells (Shida et al., 1993; Orwig and Soares, 1999; Sun and Duckworth, 1999). Thus, it would be informative to take a broader look at the role of FRA1 in trophoblast cells. Profiling gene expression changes in cells treated with *Fra1* shRNA using DNA microarray analysis could be a useful tool to better define the role of FRA1 in trophoblast cells. Additionally, identifying targets through a genome wide examination of FRA1-DNA interactions via techniques such as ChIP-sequencing would provide important complementary insights on potential FRA1 targets.

Potential binding partners of FRA1

FRA1 must form a dimer with a member of the JUN family to regulate gene expression. Of the JUN family members JUN, JUNB and JUND are expressed in Rcho-1 trophoblast cells. JUN and JUNB are upregulated during differentiation and the JUNB mutant mouse is the only JUN protein mutant mouse that has placental defects. This makes a potential FRA1-JUNB dimer an interesting prospect for further study. The role of JUNB in trophoblast cells could be studied using shRNA mediated knockdown of JUNB. ChIP assays could also be useful in determining if FRA1 and JUNB bind the same target sites.

The function of FRA1 target genes in trophoblast invasion

In this study we show that five genes involved in invasion and vascular remodeling *(Ceacam3, Cgm4, Mmp9, Prl4a1* and *Sema6d)* are positively regulated by FRA1 (**Chapter 3**). Of these FRA1 regulated genes MMP9 and SEMA6D are the most likely to influence cell movement. MMP9 is an important factor in trophoblast invasion and can remodel the extracellular matrix facilitating cell invasion (Peters et al., 1999). SEMA6D has not been previously associated with trophoblast but it can guide the movement of neurons (Qu et al., 2002) and is involved in movement of cells during cardiac development (Toyofuku et al., 2004a,b).

CGM4 and PRL4A1 are likely to be involved in vascular remodeling by directly affecting endothelial cells and blood vessels or indirectly affecting vessel remodeling by altering cytokine production by immune cells (Müller et al., 1999; Park et al., 2000, Wessells et al., 2000; Waterhouse et al., 2002; Ain et al., 2003; Ain et al., 2004; Ha et al., 2005; Wynne et al., 2006).

Significance of the invasion and vascular remodeling related genes

The eleven PI3K/AKT regulated genes can be linked to cellular invasion and vascular remodeling in various cell models but their involvement in the regulation of trophoblast cell invasion and uterine vascular remodeling is unknown. Research directed toward understanding the roles for each of these genes at the placentation site has considerable merit. Gene targeting via homologous recombination in embryonic stem cells is a common strategy that has been effectively used in the mouse to create null mutations within genes of interest to investigate biology. The mouse exhibits modest trophoblast invasion and vascular remodeling and is not a suitable model for investigating trophoblast-uterine interactions. As demonstrated in our research, the rat has significant advantages for experimentation on trophoblast invasion and uterine vascular remodeling. However, gene targeting of embryonic stem cells has not yet become a viable technique for creating rat models. Recent advances with other genetic modification techniques in the rat have considerable promise. These strategies include trophoblast-specific knockdown of genes using lentiviral delivered shRNAs as described in **Chapter 3** and gene targeting via zinc-finger nucleases (Geurts et al., 2009) or Sleeping Beauty transposons (Lu et al., 2007). Gene expression or polymorphisms could also be evaluated in the Brown Norway rat strain. The Brown Norway rat has limited trophoblast invasion and high fetal loss and is a useful model to study the regulation of placentation (Konno et al., 2007). Our lab is currently evaluating the impact of the loss of

FASLG in vivo using rats with disrupted FASLG that were generated using the Sleeping Beauty transposon system.

III. Human pregnancy related disorders

Preeclampsia and intrauterine growth restriction

There are several pregnancy related disorders in humans that are associated with dysfunctional trophoblast development or poor trophoblast invasion. Two of the most common are preeclampsia and intrauterine growth restriction (IUGR). Preeclampsia occurs in 3 to 5% of pregnant women. The disorder is characterized by maternal hypertension and proteinuria starting during mid to late gestation and represents a leading cause of maternal and neonatal morbidity and mortality. The only known cure is delivery of the placenta. Preeclampsia is characterized by poor trophoblast invasion and upregulation of placental anti-angiogenic factors that result in disruptions in the maternal endothelium (reviewed in Young et al., 2010). IUGR is defined by small for gestation babies and has many causes, including inadequate placental development and/or trophoblast invasion. IUGR can result in fetal death or disability and a lifelong increased risk for several disorders including, hypertension, cardiovascular disease and renal disease (Scifres and Nelson, 2009).

The PI3K/AKT/FRA1 pathway in preeclampsia and IUGR

There is evidence that the PI3K/AKT pathway is involved in preeclampsia and IUGR. In placentas from preeclamptic pregnancies PI3K is upregulated and AKT activity is elevated. Preeclampsia is thought to induce hypoxia in the placenta. AKT activity is increased in models of placental hypoxia and is thought to have a role in elevating the expression of soluble fms-like tyrosine kinase 1 (sFlt1), which plays a major role in the pathogenesis of preeclampsia (Park et al., 2010). This indicates that placental hypoxia may activate the PI3K/AKT signaling pathway in an attempt to induce trophoblast invasion or vascular remodeling to improve blood flow to the embryo.

Recently storkhead box 1 (STOX1) has been identified as a preeclampsia susceptibility gene that negatively regulates trophoblast invasion. A recent study has shown that AKT phosphorylates and induces ubiquitin mediated degradation of STOX1 indicating that AKT may be able to negate the effect of STOX1 on trophoblast invasion (van Dijk et al., 2010).

Mutant mouse models of AKT (**Table 4.1**) can result in IUGR. AKT has also been examined in human cases of IUGR. In such a study, components of the AKT signaling pathway were shown to be downregulated. Expression of AKT1 and AKT2 was decreased by approximately 40%, AKT3 expression and overall kinase activity was reduced by over 90% due to translational inhibition (Yung et al., 2008). These findings further support a role for AKT in the etiology of IUGR.

Invasion and vascular remodeling related genes in preeclampsia and IUGR

Some of the eleven PI3K/AKT regulated invasion and vascular remodeling related genes have also been studied in preeclampsia, including CTSD, FASLG and MMP9.

CTSD activity is elevated in placentas from preeclamptic pregnancies (González et al., 2007). An important function of CTSD is to cleave prolactin to produce vasoinhibins (Clapp et al., 2006). Vasoinhibins are elevated in preeclampsia and can act on endothelial cells to inhibit vasodilatation and angiogenesis (González et al., 2007). Expression of the FAS/FASLG system has also been reported to be altered during preeclampsia and is thought to increase trophoblast cell death (Neale and Mor, 2005). MMP9 shows elevated expression levels in placentas from preeclamptic pregnancies (Wang et al. 2009). The significance of MMP9 in the etiology of preeclampsia is unknown.

In summary, several elements of the PI3K/AKT signaling pathway and PI3K/AKT regulated invasion and vascular remodeling genes show increased expression or activity in placentas from preeclamptic pregnancies. It is likely that this pathway is activated by: i) changes in the maternal environment such as expression of activating ligands; ii) increased placental hypoxia due to poor trophoblast invasion and spiral artery remodeling; or iii) defects in trophoblast cells. Activation of this pathway is most likely an attempt to increase trophoblast invasion and vascular remodeling but may actually contribute to the pathogenesis of preeclampsia via AKT-mediated sFLT1, CTSD creation of vasoinhibins, and FASLG promotion of trophoblast cell death. However, the negative regulation of preeclampsia susceptibility gene STOX1 by AKT shows that AKT may also have a role in preventing the development of preeclampsia.

FRA1 in the human placenta

Knowledge of the role of FRA1 in human trophoblast is limited. FRA1 has been localized to human trophoblast by immunostaining but FRA1 expression was low compared to other AP-1 transcription factors which were highest in the extravillous (invasive) trophoblast (Bamberger et al., 2004). FRA1 expression has also been reported in human choriocarcinomas (Briese et al., 2005). A more thorough and quantitative approach is need to evaluate a role for FRA1 in human trophoblast cells. There are several human trophoblast cell lines that are phenotypically similar to extravillous trophoblast cells. The expression and function of FRA1 could be assessed in one or more of the cell lines similar to the analysis we conducted in the Rcho-1 TS cells.

Although a biological role for FRA1 in placentation cannot be tested directly in humans, studies could be done looking at the expression, activity, and mutations associated with FRA1 in human placental disorders.

IV. Further lessons to be learned from gene profiling experiments in trophoblast cells

Evaluation of gene profiling experiments from **Chapter 2** show that many of the genes that are differentially expressed in trophoblast cells are not sensitive to inhibition of PI3K. Additional studies examining roles for MAPK, WNT, and transforming growth factor, beta (TGFβ) signaling pathways in trophoblast differentiation and gene expression would greatly increase our understanding of the regulation of hemochorial placentation.

Analysis of transcriptional regulators downstream of the aforementioned signaling pathways could also be useful. In this study, we show that FRA1 is a key transcription factor in regulating the expression of a subset of differently expressed genes. Analysis of the expression of known transcriptional regulators from our gene profiling experiments (**Chapter 2**) reveals a number of transcription factors upregulated during differentiation (Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2, *Cited2*; Heart and neural crest derivatives expressed 1, *Hand1*; High mobility group box transcription factor 1, *Hbp1*; GATA binding protein 2, *Gata2*; Jun-B oncogene, *Junb*; Kruppel-like factor 2, *Klf2*; Nuclear factor, erythroid derived 2, like 2, *Nfe2l2;* Reproductive homeobox on X chromosome, 9, *Rhox9*) or that may have altered activity in differentiated trophoblast cells (v-ets erythroblastosis virus E26 oncogene homolog 2, *Ets2*; GATA binding protein 3, *Gata3*).

Additionally, the DNA microarray experiments have led to the identification of several genes that were not previously known to be functionally involved in trophoblast cells (*Rsp1*, *Cd47*, among others). These genes may have potentially interesting roles in trophoblast biology and should be studied in more detail.

V. Overview of the importance of this work

Starting with gene profiling experiments in rodent trophoblast cells we have identified a signaling pathway (PI3K/AKT), a transcriptional mediator of the PI3K/AKT pathway (FRA1), and target genes involved in trophoblast invasion and vascular remodeling in the rat in vitro and in vivo. We are now poised to apply this new knowledge to elucidate specific mechanisms underlying the regulation of hemochorial placentation in normal and diseased pregnancies.

CHAPTER 5: REFERENCES

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CHAPTER 6: APPENDIX

Appendix A

Curriculum Vitae

Lindsey N. Kent (Canham)

Present position

Postdoctoral fellow
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University of Kansas Medical Center

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Education

Truman State University
Kirksville, Missouri
August 1996-December 2000
Bachelor of Science Degree in Biology

University of Kansas Medical Center
Department of Pathology & Laboratory Medicine
Kansas City, Kansas
August 2004-August 2010
Ph.D. in Pathology/Reproductive Biology

Work Experience

August 1999-December 2000
Lab/Herbarium Assistant
Truman State University
Supervisor: Dr. Elisabeth Hooper

January 2001- August 2004
Research Assistant
University of Kansas Medical Center
Principal Investigator: Dr. Michael J. Soares

Awards

University of Kansas Medical Center student travel award 2005, 2006, 2007

International Federation of Placental Associations, Trainee Poster Award, 2007

NIH Travel Award to the International Federation of Placental Associations Annual Meeting, Kingston, Ontario, 2007

University of Kansas Medical Center Biomedical Graduate Student Training Award, 2007 – 2009

KUMC student research forum, first place presentation in the reproduction session, 2008.

Abstracts Presented

1. Sahgal, N., **Kent, L.N**., Dai, G., and Soares, M.J. (2002) Experimental modulation of trophoblast stem cell and trophoblast giant cell phenotypes. 84th Annual Meeting of the Endocrine Society, San Francisco, California.

2. Sahgal, N., **Canham, L.N**., and Soares, M.J. (2003) Fetal regulation of trophoblast cell invasion into the maternal uterine mesometrial vascular compartment. Annual Meeting of the Society for Pediatric Research, Seattle, Washington

3. **Canham, L.N**., Sahgal, N., Wolfe, M.W., and Soares, M.J. (2003) Selective involvement of the phosphatidylinositol 3-kinase/Akt signaling pathway in the regulation of trophoblast cell differentiation. 36th Annual Meeting of the Society for the Study of Reproduction, Cincinnati, Ohio

4. Ain, R., **Canham, L.N**., and Soares, M.J. (2003) Migratory trophoblast cell fate: modulation by glucocorticoids. 9th International Federation of Placental Associations and 10th European Placental Group Meeting, Mainz, Germany

5. Alam, S.M.K., Konno, T., Sahgal, N., Li, C., Lu, L., **Canham, L.N**., and Soares, M.J. (2004) Decidual cells produce a heparin-binding cytokine, prolactin-like protein-J: expression and biological targets. 37th Annual Meeting of the Society for the Study of Reproduction, Vancouver, British Columbia, Canada

6. **Canham, L.N**., Sahgal, N., and Soares, M.J. (2006) Regulation of steroidogenesis in differentiating trophoblast giant cells: a role for the phosphatidylinositol 3-kinase/akt signaling pathway. 39th Annual Meeting of the Society for the Study of Reproduction, Omaha, Nebraska

7. **Canham, L.N**., Sahgal, N., and Soares, M.J. (2007) Discovery of genes differentially regulated during trophoblast cell differentiation. 13th International Federation of Placenta Associations, Kingston, Ontario, Canada.

8. Rumi, M.A.K., **Canham, L.N.**, Asanoma, K., Gowda, S., and Soares, M.J. (2008) A differentiation state-specific promoter for tracking trophoblast cell decisions. 6th International Society for Stem Cell Research Annual Meeting, Philadelphia, PA

9. **Canham, L.N.**, Rumi, M.A.K., Asanoma, K., and Soares, M.J. (2008) Cell lineage decisions in the differentiation of rat trophoblast stem cells: involvement of the phosphatidylinositol 3-kinase signaling pathway and fos-like antigen 1. 6th International Society for Stem Cell Research Annual Meeting, Philadelphia, PA

10. Rumi M.A., **Kent L.N.**, Lee D.S., Chakraborty D., Konno T. and Soares M.J. (2010) Rat placental lactogen II promoter directs trophoblast giant cell specific expression. Gordon Research Conference on Reproductive Tract Biology, Andover, NA.

11. **Kent, L.N.,** Rumi, M.A.K., Lee, D.S. and Soares, M.J. (2010) PI3K/Akt signaling pathway and Fral1 in the regulation of trophoblast invasion and vascular remodeling. Gordon Research Conference on Reproductive Tract Biology, Andover, NA.

12. **Kent, L.N.,** Rumi, M.A.K., Lee, D.S. and Soares, M.J. (2010) PI3K/Akt Signaling pathway and Fra1 regulate trophoblast invasion and vascular remodeling. Fifth Annual NIH National Graduate Student Research Festival, Bethesda, MD.

Publications

1. Ain, R., **Canham, L.N.**, and Soares, M.J. (2003) Gestational stage-dependent intrauterine trophoblast cell invasion in the rat and mouse: novel endocrine phenotype and regulation. *Developmental Biology* **260**, 176-190.

2. Ain, R., **Canham, L.N.**, and Soares, M.J. (2005) Dexamethasone-induced intrauterine growth restriction impacts the placental prolactin family and the insulin-like growth factor-II/Akt signaling pathway. *Journal of Endocrinology* **185**, 253-263.

3. Sahgal, N., **Canham, L.N**., Konno, T., Wolfe, M.W., and Soares, M.J. (2005) Modulation of trophoblast stem cell and trophoblast giant cell phenotypes. *Differentiation* **9-10**, 452-62.

4. Sahgal, N., **Canham, L.N.**, and Soares, M.J. (2006) Rcho-1 trophoblast cells: a model for studying trophoblast differentiation. In: *Placenta and Trophoblast: Methods and Protocols, Vol I*. M.J. Soares and J.S. Hunt (eds), Humana Press, New Jersey.159-178.

5. Ain, R., Konno, T., **Canham, L.N.**, and Soares, M.J. (2006) Phenotypic analysis of the placenta in the rat. In: *Placenta and Trophoblast: Methods and Protocols, Vol I*. M.J. Soares and J.S. Hunt (eds), Humana Press, New Jersey. 295-313.

6. Ray,S., Dutta, D., Rumi, M.A., **Kent, L.N.**, Soares, M.J., and Paul S. (2008) Context-dependent function of regulatory elements and a switch in chromatin occupancy between GATA3 and GATA2 regulate Gata2 transcription during trophoblast differentiation. *Journal of Biological Chemistry* **284**, 4978-88.

7. **Kent, L.N.**, Konno, T., and Soares, M.J. (2010) Phosphatidylinositol 3-Kinase Modulation of Trophoblast cell differentiation. *BMC Developmental Biology. *In Press**

Appendix B

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1374863_at	similar to retinoid binding protein 7 (predicted)	RGD1562168_predicted	3217	33	**97.63**
1367660_at	fatty acid binding protein 3	Fabp3	23051	323	**71.41**
1378106_at	pleckstrin homology-like domain, family A, member 2 (predicted)	Phlda2_predicted	7644	157	**48.67**
1374273_at	leucine zipper-EF-hand containing transmembrane protein 1	Cxadr	980	24	**41.22**
1370884_at	sepiapterin reductase	Spr	3630	115	**31.45**
1391509_at	tumor-associated calcium signal transducer 2	Tacstd2	2215	93	**23.92**
1367919_at	nuclear pore membrane glycoprotein 210	Pom210	1013	45	**22.72**
1368870_at	inhibitor of DNA binding 2	Id2	1958	93	**21.03**
1369063_at	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	Anp32a	3956	197	**20.05**
1389378_at	CDC42 effector protein (Rho GTPase binding) 5 (predicted)	Cdc42ep5_predicted	2771	145	**19.08**
1389253_at	vanin 1	Vnn1	1163	61	**18.94**
1372326_at	Solute carrier family 2 (facilitated glucose transporter), member 3	Slc2a3	4491	258	**17.44**
1372629_at	similar to KIAA0925 protein	LOC300768	819	48	**16.95**
1371840_at	endothelial differentiation sphingolipid G-protein-coupled receptor 1	Edg1	4220	263	**16.08**
1390566_a_at	creatine kinase, mitochondrial 1, ubiquitous	Ckmt1	1538	108	**14.21**
1378262_at	Transcribed locus	---	2266	160	**14.13**
1387135_at	a disintegrin and metallopeptidase domain 15 (metargidin)	Adam15	1370	99	**13.87**
1390839_at	PQ loop repeat containing 3	Pqlc3	11449	843	**13.58**
1367654_at	fat tumor suppressor homolog (Drosophila)	Fath	1695	130	**13.06**
1389609_at	transmembrane 7 superfamily member 3	Tm7sf3	1347	104	**13.00**
1387707_at	solute carrier family 2 (facilitated glucose transporter), member 3	Slc2a3	1986	156	**12.72**
1375532_at	inhibitor of DNA binding 2	Id2	14665	1162	**12.62**
1371986_at	adaptor-related protein complex AP-4, sigma 1 (predicted)	Anp32a	2066	167	**12.37**
1373530_at	cyclin E	Ccne1	2506	218	**11.52**
1383496_at	similar to hypothetical protein FLJ20171 (predicted)	RGD1560481_predicted	5046	451	**11.19**
1388781_at	vitamin D receptor interacting protein	Ssbp3	2807	252	**11.13**
1368165_at	phosphoribosyl pyrophosphate synthetase 1	Prps1	1978	181	**10.94**
1383935_at	Transcribed locus	---	859	79	**10.89**
1371237_a_at	metallothionein 1a	Mt1a	7363	685	**10.75**
1383290_at	serine peptidase inhibitor, Kunitz type 1	Spint1	1350	129	**10.45**
1386120_at	hypothetical protein LOC689147	LOC689147	2139	205	**10.43**
1387232_at	bone morphogenetic protein 4	Bmp4	1035	100	**10.36**
1367735_at	acetyl-Coenzyme A dehydrogenase, long-chain	Acadl	3366	327	**10.29**
1368812_at	lamin B1	Lmnb1	936	91	**10.27**
1383747_at	ect2 oncogene (predicted)	Ect2_predicted	5018	499	**10.06**
1391063_at	kinesin family member 23 (predicted)	Kif23_predicted	2262	228	**9.93**
1389085_at	Transcribed locus	---	1576	162	**9.75**
1368260_at	aurora kinase B	Aurkb	2482	257	**9.67**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1382830_at	suppressor of variegation 3-9 homolog 2 (Drosophila) (predicted)	Suv39h2_predicted	2720	284	**9.56**
1377940_at	hypothetical LOC287534	RGD1359691	1303	138	**9.43**
1368689_at	gap junction membrane channel protein beta 5	Gjb5	1488	159	**9.35**
1383926_at	budding uninhibited by benzimidazoles 1 homolog, beta (S. cerevisiae)	Bub1b	2547	275	**9.26**
1387812_at	proprotein convertase subtilisin/kexin type 6	Pcsk6	2968	327	**9.08**
1370432_at	POU domain, class 3, transcription factor 1	Pou3f1	2034	228	**8.93**
1368363_at	Kruppel-like factor 5	Klf5	5887	665	**8.85**
1368517_at	single stranded DNA binding protein 3	Ssbp3	1536	175	**8.79**
1387028_a_at	inhibitor of DNA binding 1	Id1	8485	982	**8.64**
1377725_at	Transcribed locus	---	861	100	**8.58**
1393904_at	similar to lin-28 homolog (predicted)	RGD1566408_predicted	2594	304	**8.53**
1368437_at	carbonic anhydrase 4	Ca4	3337	396	**8.42**
1395652_at	glycosyltransferase-like 1B	Gyltl1b	1437	172	**8.37**
1393816_at	hypothetical protein LOC689399	LOC689399	2099	252	**8.32**
1368180_s_at	glutathione-S-transferase, alpha type2	Gsta2	3070	374	**8.22**
1389440_at	Transcribed locus	---	3105	383	**8.10**
1380611_at	FK506 binding protein 5	Fkbp5	5674	701	**8.09**
1379448_at	Ttk protein kinase (predicted)	Ttk_predicted	2359	296	**7.97**
1377743_at		Spred1	1578	203	**7.78**
1375937_a_at	similar to RIKEN cDNA G630055P03 gene	RGD1309383	1095	142	**7.71**
1390454_at	4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (C. elegans)	Nipsnap1	1186	157	**7.57**
1376084_a_at	extra spindle poles like 1 (S. cerevisiae) (predicted)	Espl1_predicted	2037	271	**7.52**
1374540_at	cell division cycle associated 7	Cdca7	2333	313	**7.46**
1389054_at	similar to RIKEN cDNA 0610040J01	LOC498368	860	116	**7.41**
1375277_at	similar to Notch-regulated ankyrin repeat protein	LOC499745 /// LOC68824	1058	146	**7.23**
1394039_at	Kruppel-like factor 5	Klf5	8621	1233	**6.99**
1370894_at	claudin 7	Cldn7	1889	273	**6.92**
1385668_at	---	---	827	120	**6.90**
1391317_at	similar to RIKEN cDNA 2810433K01 (predicted)	RGD1310784_predicted	2011	292	**6.89**
1378006_at	Transcribed locus, moderately similar to XP_893119.1 PREDICTED: similar to melanoma antigen [Mus musculus]	---	1239	181	**6.83**
1368811_at	lamin B1	Lmnb1	2374	351	**6.77**
1379380_at	sprouty homolog 1 (Drosophila) (predicted)	Spry1_predicted	1279	190	**6.72**
1391051_at	gene rich cluster, C2f gene (predicted)	Gjb3	9250	1378	**6.71**
1371976_at	hypothetical protein LOC682248 /// hypothetical protein LOC685545	LOC682248 /// LOC68554	1491	223	**6.68**
1387747_at	gap junction membrane channel protein beta 3	Gjb3	5356	810	**6.61**
1368965_at	solute carrier family 16 (monocarboxylic acid transporters), member 3	Slc16a3	3437	523	**6.57**
1382138_at	similar to Notch-regulated ankyrin repeat protein	LOC499745 /// LOC68824	918	141	**6.51**
1379386_at	similar to B99 protein (predicted)	RGD1563164_predicted	1960	301	**6.51**
1385733_at	exonuclease 1 (predicted)	Exo1_predicted	1839	284	**6.47**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1369935_at	cyclin D3	Ccnd3	13904	2172	**6.40**
1397341_at	PDZ binding kinase (predicted)	Pbk_predicted	1083	171	**6.32**
1385781_at	similar to SNF2/RAD54 family protein (predicted)	RGD1565734_predicted	911	144	**6.32**
1393581_at	asp (abnormal spindle)-like, microcephaly associated (Drosophila) (predicted)	Aspm_predicted	1827	290	**6.30**
1389668_at	spindle pole body component 25 homolog (S. cerevisiae)	Spbc25	5121	817	**6.27**
1391626_at	similar to RIKEN cDNA 2610034E18 gene (predicted)	RGD1309007_predicted	1235	198	**6.24**
1373026_at	spindle pole body component 24 homolog (S. cerevisiae) (predicted)	Spbc24_predicted	2274	366	**6.22**
1378629_at	special AT-rich sequence binding protein 1	Satb1	9335	1514	**6.17**
1389735_at	ribosomal protein S6 kinase polypeptide 6 (predicted)	Rps6ka6_predicted	1004	163	**6.14**
1369629_at	adenosine kinase	Adk	1184	193	**6.13**
1385522_at	origin recognition complex, subunit 1-like (S.cereviaiae)	Orc1l	1646	270	**6.10**
1368799_at	baculoviral IAP repeat-containing 5	Birc5	2192	360	**6.10**
1371455_at	phosphomannomutase 1	Pmm1	3113	517	**6.02**
1383696_at	similar to protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a isoform 1	LOC680115 /// LOC68391	858	143	**6.01**
1379057_at	hypothetical protein LOC683460	LOC683460	801	134	**5.98**
1374449_at	cell division cycle associated 3 /// similar to cell division cycle associated 3 (predicted)	Cdca3 /// RGD1562067_p	2984	502	**5.95**
1389800_at	PWWP domain containing 2 (predicted)	Pwwp2_predicted	1820	307	**5.93**
1387895_s_at	cell division cycle 20 homolog (S. cerevisiae)	Cdc20	1853	315	**5.89**
1373754_at	Transcribed locus	---	3011	524	**5.75**
1392065_at	TAR DNA binding protein (predicted)	RGD1561740_predicted	929	162	**5.72**
1373897_at	Lamin B1	Lmnb1	10711	1877	**5.71**
1374388_at	EF hand domain containing 2	Efhd2	9193	1615	**5.69**
1390838_at	---	---	2424	427	**5.68**
1388650_at	topoisomerase (DNA) 2 alpha	Top2a	4847	854	**5.68**
1385961_at	Kruppel-like factor 5	Klf5	2942	520	**5.66**
1387769_a_at	inhibitor of DNA binding 3	Id3	1364	243	**5.62**
1374794_at	kinesin family member 15	Kif15	1758	316	**5.57**
1374119_at	E74-like factor 3	Elf3	1523	275	**5.53**
1389457_at	myeloblastosis oncogene-like 2 (predicted)	Mybl2_predicted	2695	487	**5.53**
1371928_at	cell division cycle associated 8	Cdca8	2081	377	**5.52**
1383578_at	similar to DNA repair protein RAD51 homolog 1 (predicted)	RGD1563603_predicted	5067	922	**5.49**
1384231_at	similar to Shc SH2-domain binding protein 1	LOC687121	2895	529	**5.47**
1374775_at	antigen identified by monoclonal antibody Ki-67 (predicted)	Mki67_predicted	4566	841	**5.43**
1385086_at	budding uninhibited by benzimidazoles 1 homolog (S. cerevisiae) (predicted)	Bub1_predicted	4644	858	**5.41**
1374912_at	kinesin family member 2C	Kif2c	1428	264	**5.41**
1375224_at	pleckstrin homology-like domain, family A, member 3	Phlda3	1113	207	**5.38**
1382627_at		Satb1	2659	498	**5.34**
1367609_at	macrophage migration inhibitory factor	Mif	9661	1816	**5.32**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1373625_at	serine hydroxymethyltransferase 1 (soluble)	Shmt1	1695	319	**5.31**
1373722_at	kinesin family member 20A (predicted)	Kif20a_predicted	2655	504	**5.27**
1387264_at	potassium channel, subfamily K, member 6 /// similar to potassium channel, subfamily K, member 6	Kcnk6 /// LOC501662	1065	202	**5.27**
1391518_at	---	---	921	177	**5.20**
1389033_at	similar to RIKEN cDNA 2900010M23 (predicted)	RGD1306917_predicted	2774	536	**5.18**
1373761_at	similar to Protein FAM60A (Tera protein)	LOC686611	3128	607	**5.15**
1393202_a_at	Insulin-like growth factor 2, binding protein 3	Igf2bp3	1286	250	**5.15**
1390050_at	similar to Golgi phosphoprotein 2 (Golgi membrane protein GP73)	LOC680692 /// LOC68286	1366	266	**5.14**
1390918_at	GH regulated TBC protein 1	Grtp1	2363	460	**5.14**
1394419_at	similar to RIKEN cDNA 6530401L14 gene	RGD1309107	7080	1392	**5.09**
1388882_at	FK506 binding protein 3 (predicted)	Fkbp3_predicted	4544	894	**5.08**
1389756_at	maternal embryonic leucine zipper kinase (predicted)	Melk_predicted	2288	457	**5.01**
1389858_at	thymidine kinase 1	Tk1	1664	332	**5.01**
1368226_at	Nucleoside 2-deoxyribosyltransferase domain containing protein RGD620382	RGD620382	2129	429	**4.96**
1388901_at	FK506 binding protein 5	Fkbp5	2531	510	**4.96**
1393947_at	Solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15	Slc25a15	1941	393	**4.93**
1388547_at	claudin 4	Cldn4	2905	591	**4.92**
1388853_at	mitochondrial ribosomal protein L54 (predicted)	Mrpl54_predicted	2833	581	**4.88**
1373283_at	Transcribed locus, strongly similar to NP_035735.1 thymopoietin [Mus musculus]	---	1637	340	**4.82**
1367721_at	syndecan 4	Sdc4	4809	1004	**4.79**
1378674_at	similar to engrailed protein (predicted)	RGD1561842_predicted	1125	235	**4.78**
1369380_at	kinesin family member 2C	Kif2c	945	199	**4.75**
1369213_at	L1 cell adhesion molecule	L1cam	912	192	**4.74**
1373451_at	similar to hypothetical protein FLJ11712	MGC94780	1255	265	**4.73**
1375849_at	RGM domain family, member A (predicted)	Rgma_predicted	1092	231	**4.73**
1367585_a_at	ATPase, Na+/K+ transporting, alpha 1 polypeptide	Atp1a1	5807	1228	**4.73**
1370823_at	BMP and activin membrane-bound inhibitor, homolog (Xenopus laevis)	Bambi	1536	325	**4.72**
1398326_at	similar to Nur77 downstream protein 2	MGC105647	2918	622	**4.69**
1369630_at	adenosine kinase	Adk	2952	630	**4.69**
1383940_at	cell division cycle associated 1	Cdca1	3879	828	**4.68**
1385222_at	eomesodermin	LOC316052	3490	748	**4.66**
1392899_at	protein regulator of cytokinesis 1 (predicted)	Prc1_predicted	2944	635	**4.63**
1375559_at	similar to thyroid receptor-interacting protein 6	LOC686323	2741	593	**4.62**
1370308_at	RS21-C6 protein	Rs21c6	2713	588	**4.61**
1387024_at	dual specificity phosphatase 6	Dusp6	1485	323	**4.60**
1371539_at	nucleolar protein family A, member 2 (predicted)	Nola2_predicted	9562	2104	**4.54**
1388436_at	small nuclear ribonucleoprotein polypeptide A	Snrpa	2298	507	**4.54**
1375205_at	p300/CBP-associated factor	Pcaf	970	214	**4.53**
1379582_a_at	cyclin A2	Ccna2	11416	2544	**4.49**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1367987_at	arginyl aminopeptidase (aminopeptidase B)	Rnpep	7055	1576	**4.48**
1370158_at	myosin, heavy polypeptide 10, non-muscle	Myh10	4095	916	**4.47**
1372692_at	tyrosine kinase, non-receptor, 2	Tnk2	1000	225	**4.45**
1371862_at	ribonucleotide reductase M1	Rrm1	3974	894	**4.44**
1383316_at	HD domain containing 2 (predicted)	Hddc2_predicted	998	226	**4.42**
1378640_at	ubiquitin-like, containing PHD and RING finger domains, 1	Uhrf1	928	210	**4.41**
1389748_at	Transcribed locus	---	832	190	**4.39**
1392061_at	minichromosome maintenance deficient 10 (S. cerevisiae) (predicted)	Mcm10_predicted	1238	283	**4.38**
1373829_at	fibroblast growth factor receptor 2	Fgfr2	3813	877	**4.35**
1373250_at	similar to Anillin (predicted)	RGD1566097_predicted	3502	806	**4.34**
1387361_s_at	phosphoglycerate kinase 1	Pgk1	12161	2814	**4.32**
1368400_at	translocase of inner mitochondrial membrane 8 homolog a (yeast)	Timm8a	2175	504	**4.31**
1372186_a_at	topoisomerase (DNA) 2 alpha	Top2a	2955	685	**4.31**
1372685_at		Cdkn3_predicted	1218	283	**4.30**
1380775_at	M-phase phosphoprotein 1 (predicted)	Mphosph1_predicted	3193	743	**4.30**
1378296_at	similar to Condensin complex subunit 2 (Barren homolog protein 1) (Chromosome-associated protein H) (mCAP-H) (XCAP-H homolog)	LOC680089	2386	558	**4.27**
1376690_at	---	---	2013	477	**4.22**
1372250_at	SCF apoptosis response protein 1	LOC499941	2446	580	**4.21**
1368649_at	dyskeratosis congenita 1, dyskerin	Dkc1	1351	321	**4.21**
1374195_at	ladinin (predicted)	Lad1_predicted	1318	313	**4.21**
1370999_at	sperm associated antigen 5	Spag5	1897	454	**4.18**
1392981_at	Iroquois related homeobox 4 (Drosophila) (predicted)	Irx4_predicted	3397	816	**4.16**
1372997_at		Csnk2b	2011	488	**4.12**
1368756_at	thioesterase domain containing 1	Thedc1	1222	297	**4.11**
1382778_at	Dual specificity phosphatase 6	Dusp6	982	240	**4.09**
1368002_at	mutS homolog 2 (E. coli)	Msh2	2306	570	**4.05**
1372516_at	kinesin family member 22	Kif22	2796	692	**4.04**
1368470_at	gamma-glutamyl hydrolase	Ggh	1992	495	**4.03**
1382493_at	Transcribed locus, strongly similar to XP_223060.3 PREDICTED: similar to LEK1 [Rattus norvegicus]	---	2105	524	**4.02**
1371277_at	caudal type homeo box 1	Cdx1	1231	309	**3.98**
1367834_at	spermidine synthase	Srm	1512	381	**3.97**
1393342_at	integrin alpha 9	Slc25a15	1629	418	**3.90**
1368356_a_at	type 1 tumor necrosis factor receptor shedding aminopeptidase regulator	Arts1	2203	567	**3.89**
1392953_at	similar to protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a isoform 1	LOC680115 /// LOC68391	1081	278	**3.89**
1372903_at	similar to 3000004C01Rik protein	RGD1310360	1894	488	**3.88**
1367780_at	pituitary tumor-transforming 1	Pttg1	3013	778	**3.87**
1377832_at	polo-like kinase 4 (Drosophila) (predicted)	Plk4_predicted	2442	630	**3.87**
1387206_at	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6	B4galt6	2592	669	**3.87**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1367593_at	selenoprotein W, muscle 1	Sepw1	1002	259	**3.87**
1388488_at	LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae) (predicted)	Lsm3_predicted	5704	1474	**3.87**
1382096_at	chemokine-like factor super family 5 (predicted)	Cklfsf5_predicted	2126	550	**3.87**
1378592_at	tripartite motif-containing 59 (predicted)	Trim59_predicted	1759	455	**3.86**
1385437_at	carbamyl phosphatate synthetase 2	Cad	860	223	**3.86**
1387889_at	folate receptor 1 (adult)	Folr1	5347	1392	**3.84**
1367576_at	glutathione peroxidase 1	Gpx1	7955	2075	**3.83**
1392580_at	Transcribed locus	---	900	235	**3.83**
1389471_at	translocase of outer mitochondrial membrane 34 (predicted)	Tomm34_predicted	2336	613	**3.81**
1370345_at	cyclin B1	Ccnb1	4214	1105	**3.81**
1376951_at	MAD2 (mitotic arrest deficient, homolog)-like 1 (yeast) (predicted)	Mad2l1_predicted	1166	307	**3.80**
1392754_at	similar to cysteine-rich glycoprotein (predicted)	RGD1566394_predicted	845	222	**3.80**
1389809_at	transmembrane, prostate androgen induced RNA (predicted)	Tmepai_predicted	1269	335	**3.79**
1389419_at	Transcribed locus, weakly similar to XP_001005337.1 PREDICTED: similar to zinc finger protein 97 [Mus musculus]	---	1795	474	**3.78**
1373482_at	Tnf receptor-associated factor 3 (predicted)	Traf3_predicted	822	218	**3.78**
1382459_at	---	---	1591	423	**3.76**
1383675_at	Transcribed locus, strongly similar to NP_955518.1 hypothetical protein LOC107094 [Mus musculus]	---	1781	476	**3.74**
1398868_at	translocase of inner mitochondrial membrane 13 homolog (yeast)	Timm13	8115	2170	**3.74**
1392818_at	growth arrest specific 5	Gas5	2842	760	**3.74**
1383008_at	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	Smc4l1	1088	291	**3.74**
1382604_at	similar to DNA-directed RNA polymerase III 32 kDa polypeptide (RNA polymerase III C32 subunit)	LOC685465	1351	362	**3.73**
1379530_at	vascular endothelial zinc finger 1 (predicted)	Itga9	4644	1244	**3.73**
1387196_at	KH domain containing, RNA binding, signal transduction associated 3	Khdrbs3	1223	329	**3.72**
1373884_at	kelch-like 2, Mayven (Drosophila) (predicted)	Klhl2_predicted	1320	355	**3.71**
1390891_at	kinesin family member 11	Kif11	1592	431	**3.70**
1375060_at	Transcribed locus, strongly similar to XP_345605.2 PREDICTED: similar to RIKEN cDNA 2610040C18 [Rattus norvegicus]	---	844	230	**3.66**
1370376_a_at	cold shock domain protein A	Csda	2931	804	**3.64**
1384042_at	Transcribed locus	---	2039	561	**3.64**
1376939_at	Transcribed locus	---	1236	340	**3.63**
1368115_at	claudin 3	Cldn3	2665	735	**3.63**
1371873_at	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	Anp32e	1966	543	**3.62**
1387036_at	hairy and enhancer of split 1 (Drosophila)	Hes1	1950	538	**3.62**
1371392_at	glucose phosphate isomerase	Gpi	6328	1749	**3.62**
1388484_at	ubiquitin-conjugating enzyme E2C (predicted)	Ube2c_predicted	1652	457	**3.61**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1389969_at	translocase of outer mitochondrial membrane 40 homolog (yeast)	Tomm40	2972	825	**3.60**
1389566_at	cyclin B2	Ccnb2	2794	776	**3.60**
1388932_at	laminin, alpha 5	Lama5	3854	1071	**3.60**
1390137_at	TRAF4 associated factor 1	Traf4af1	817	227	**3.60**
1372297_at	glutathione S-transferase, alpha 4	Gsta4	2262	630	**3.59**
1393848_at	ribonucleotide reductase M2	Rrm2	8398	2344	**3.58**
1374800_at	karyopherin (importin) beta 1	Slc25a15	2079	581	**3.58**
1390415_at	retinoic acid induced 3	Trip13	2689	754	**3.57**
1376185_at	kinesin family member C1	Kifc1	2575	724	**3.56**
1374840_at	similar to peptidyl prolyl isomerase H (predicted)	RGD1564921_predicted	2624	741	**3.54**
1368181_at	methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthase	Mthfd1	7208	2043	**3.53**
1370294_a_at	cell division cycle 20 homolog (S. cerevisiae)	Cdc20	1480	420	**3.53**
1384230_at	similar to keratinocytes associated protein 3	LOC683980	819	233	**3.52**
1374805_at	similar to hypothetical protein MGC5528 (predicted)	RGD1561749_predicted	1438	409	**3.52**
1377802_at	similar to MYST histone acetyltransferase monocytic leukemia 4 (predicted)	RGD1566399_predicted	1595	454	**3.52**
1392597_at	similar to ionized calcium binding adapter molecule 2 (Iba2) (predicted)	RGD1305081_predicted	3501	997	**3.51**
1390602_a_at	similar to RIKEN cDNA C430004E15	LOC499749	1839	524	**3.51**
1392514_at	brix domain containing 1 (predicted)	Bxdc1_predicted	2439	696	**3.51**
1383938_at	similar to nuclear receptor coactivator 7 (predicted)	RGD1566426_predicted	1360	388	**3.50**
1372886_at	transforming, acidic coiled-coil containing protein 3	Tacc3	2348	671	**3.50**
1370346_at	cyclin B1	Ccnb1	5262	1510	**3.48**
1393293_at	checkpoint with forkhead and ring finger domains	Chfr	1149	331	**3.47**
1370297_at	polo-like kinase 1 (Drosophila)	Plk1	1730	498	**3.47**
1386996_at	myosin light chain, regulatory B	Mrlcb	4629	1334	**3.47**
1388353_at	proliferation-associated 2G4	Pa2g4	4020	1162	**3.46**
1388753_at	sulfatase 2	Sulf2	3640	1055	**3.45**
1388318_at	phosphoglycerate kinase 1	Pgk1	18066	5238	**3.45**
1389446_at	FtsJ homolog 2 (E. coli) (predicted)	Snrpa1_predicted	4751	1379	**3.44**
1386867_at	brain protein 44-like	Brp44l	6161	1793	**3.44**
1383315_at	hepatic protein EIIH	Eiih	957	280	**3.42**
1385220_at	similar to Zwilch	LOC691493	2546	747	**3.41**
1372620_at	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	Anp32e	3886	1140	**3.41**
1373902_at	similar to mKIAA1238 protein (predicted)	RGD1560851_predicted	1499	440	**3.41**
1370330_at	signal-induced proliferation-associated 1 like 1	Sipa1l1	1195	351	**3.40**
1373074_at	similar to RIKEN cDNA 2700002I20	RGD1307279	2597	766	**3.39**
1384407_at	similar to RIKEN cDNA 1110025F24 (predicted)	RGD1311451_predicted	1290	381	**3.38**
1373068_at	interferon-related developmental regulator 2 (predicted)	Ifrd2_predicted	1705	505	**3.38**
1377350_at	Transcribed locus	---	1440	428	**3.36**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	**Gene Title**	**Gene Symbol**	**Stem Ave**	**Dif Ave**	**Stem/ Dif**
1387793_at	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 1	Slc9a3r1	2455	731	**3.36**
1381124_at	BRCA2 and CDKN1A interacting protein (predicted)	Slc25a15	1133	338	**3.35**
1398903_at	esterase D/formylglutathione hydrolase	Esd	5348	1601	**3.34**
1374121_at	Transcribed locus	---	961	288	**3.34**
1376231_at	casein kinase 2, beta subunit	RGD1562246_predicted	977	294	**3.32**
1372431_at	ribosomal protein, mitochondrial, L12	Mrpl12	4942	1487	**3.32**
1388341_at	RAN GTPase activating protein 1	Rangap1	2055	619	**3.32**
1373001_at	coiled-coil-helix-coiled-coil-helix domain containing 4	Chchd4	1485	447	**3.32**
1388443_at	CDK2 (cyclin-dependent kinase 2)-associated protein 1 (predicted) /// similar to CDK2 (cyclin-dependent kinase 2)-associated protein 1	Cdk2ap1_predicted /// LO	3694	1114	**3.32**
1374799_at	similar to mKIAA0159 protein (predicted)	RGD1562596_predicted	1248	377	**3.31**
1367662_at	hydroxyacyl-Coenzyme A dehydrogenase type II	Hadh2	877	266	**3.30**
1393041_at	SMC2 structural maintenance of chromosomes 2-like 1 (yeast) (predicted)	Smc2l1_predicted	1760	534	**3.30**
1390579_at	similar to RIKEN cDNA 1810029B16 (predicted)	RGD1305222_predicted	4290	1302	**3.29**
1370630_a_at	fibroblast growth factor receptor 2	Fgfr2	988	300	**3.29**
1368036_at	protein tyrosine phosphatase, receptor type, F	Ptprf	993	302	**3.29**
1388320_at	serine protease inhibitor, Kunitz type 2	Spint2	4211	1280	**3.29**
1379360_at	similar to Putative protein 15E1.2 (predicted)	RGD1309698_predicted	1354	413	**3.28**
1377172_at	similar to Pins (predicted)	RGD1560967_predicted	917	280	**3.27**
1371035_at	general transcription factor III A	Gtf3a	1284	392	**3.27**
1376052_at	similar to hypothetical protein FLJ20512 (predicted)	RGD1564549_predicted	3570	1092	**3.27**
1398602_at	MAD2 (mitotic arrest deficient, homolog)-like 1 (yeast) (predicted)	Mad2l1_predicted	2481	759	**3.27**
1395376_at	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, S. cerevisiae) (predicted)	Ddx11_predicted	1292	395	**3.27**
1371113_a_at	transferrin receptor	Tfrc	7349	2255	**3.26**
1390481_a_at	ubiquitin-conjugating enzyme E2T (putative) (predicted)	Ube2t_predicted	2134	657	**3.25**
1386897_at	heterogeneous nuclear ribonucleoproteins methyltransferase-like 2 (S. cerevisiae)	Hrmt1l2	6975	2147	**3.25**
1382674_a_at	similar to KIAA1582 protein (predicted)	RGD1310027_predicted	1131	348	**3.25**
1372071_at	CD320 antigen	Cd320	1686	520	**3.24**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/Dif
1389600_at	hypothetical protein LOC363306 /// similar to Discs large homolog 5 (Placenta and prostate DLG) (Discs large protein P-dlg) /// hypothetical LOC363433 /// hypothetical LOC501092 /// hypothetical LOC501226 /// hypothetical LOC501250 /// hypothetical protein LOC680443 /// hypothetical protein LOC684587 /// hypothetical protein LOC685573 /// hypothetical protein LOC685668 /// similar to similar to RIKEN cDNA 1700001E04 /// hypothetical protein LOC685863 /// hypothetical protein LOC686842 /// region containing hypothetical protein LOC501396; similar to RIKEN cDNA 1700001E04 /// hypothetical protein LOC688586 /// similar to hypothetical protein LOC363306	LOC363306	829	257	**3.23**
1381403_at	malic enzyme 2, NAD(+)-dependent, mitochondrial (predicted)	Me2_predicted	1167	362	**3.22**
1382802_x_at	---	---	1612	501	**3.22**
1383301_at	hypothetical protein LOC498154	LOC498154	837	260	**3.22**
1371484_at	hypothetical protein LOC686518 /// hypothetical protein LOC690349	LOC686518 /// LOC69034	4227	1314	**3.22**
1388802_at	brain expressed X-linked 1	Bex1	12559	3905	**3.22**
1367908_at	glycine cleavage system protein H (aminomethyl carrier)	Gcsh	2549	793	**3.21**
1381252_at	Transcribed locus, weakly similar to XP_001158686.1 PREDICTED: SA hypertension-associated homolog isoform 3 [Pan troglodytes]	---	922	287	**3.21**
1378507_at	Transcribed locus, moderately similar to NP_007230.1 ATP synthase F0 subunit 6 [Rattus norvegicus]	---	3348	1044	**3.21**
1372343_at	exosome component 8 (predicted)	Exosc8_predicted	2842	888	**3.20**
1383313_at	checkpoint with forkhead and ring finger domains	Chfr	2849	891	**3.20**
1389302_at	similar to RIKEN cDNA D030070L09	RGD1310199	1062	332	**3.20**
1373439_at		Lbr	7573	2381	**3.18**
1371108_a_at	ATPase, Na+/K+ transporting, alpha 1 polypeptide	Atp1a1	4255	1342	**3.17**
1384280_at	nucleolar and spindle associated protein 1 (predicted)	Nusap1_predicted	1571	499	**3.15**
1390378_at	---	---	1900	604	**3.15**
1393494_at	c-myc binding protein (predicted)	Pdgfa	957	305	**3.14**
1371797_at	carbamyl phosphatate synthetase 2	Cad	3468	1104	**3.14**
1374465_at	ubiquitously expressed transcript	Uxt	1154	367	**3.14**
1367901_at	glucuronidase, beta	Gusb	1484	473	**3.14**
1373935_at	polymerase (DNA directed), delta 2, regulatory subunit	Pold2	3114	993	**3.14**
1397508_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18	Ddx18	1802	575	**3.13**
1374424_at	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	Skb1_predicted	2089	668	**3.13**
1372811_at	kinectin 1 (predicted)	Ktn1_predicted	2610	835	**3.13**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1371683_at	LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae) (predicted)	Lsm4_predicted	3638	1166	**3.12**
1395645_at	---	---	1141	366	**3.12**
1376065_at	RRS1 ribosome biogenesis regulator homolog (S. cerevisiae) (predicted)	Rrs1_predicted	2372	763	**3.11**
1376868_at	Cobl-like 1 (predicted)	Cobll1_predicted	2972	957	**3.11**
1385788_at	Eph receptor B3 (predicted)	Ephb3_predicted	1036	334	**3.10**
1384565_at	---	---	2919	940	**3.10**
1368669_at	uncoupling protein 2 (mitochondrial, proton carrier)	Ucp2	1103	356	**3.10**
1393684_at	TBC1D12: TBC1 domain family, member 12 (predicted)	Tbc1d12_predicted	2099	677	**3.10**
1389009_at	arginine/serine-rich coiled-coil 1	Rsrc1	1438	464	**3.10**
1368387_at	3-hydroxybutyrate dehydrogenase, type 1	Bdh1	978	317	**3.09**
1398732_at	hypothetical protein LOC688273	LOC688273	1774	574	**3.09**
1377833_at	similar to Rap1-interacting factor 1 (predicted)	RGD1562474_predicted	2739	888	**3.09**
1367983_at	flap structure-specific endonuclease 1	Fen1	2248	731	**3.08**
1376483_at	LSM5 homolog, U6 small nuclear RNA associated (S. cerevisiae) (predicted)	Lsm5_predicted	4520	1472	**3.07**
1371839_at	similar to splicing factor, arginine/serine-rich 2	Sfrs2	4840	1581	**3.06**
1387268_at	RNA polymerase 1-2	Rpo1-2	1838	605	**3.04**
1393203_at	similar to RIKEN cDNA 2010309E21 (predicted)	RGD1304825_predicted	4595	1514	**3.04**
1391555_at	nuclear receptor coactivator 3	Ncoa3	2094	691	**3.03**
1386864_at	phosphoglycerate mutase 1	Pgam1	19832	6545	**3.03**
1380143_at	Transcribed locus	---	804	265	**3.03**
1388154_at	E2F transcription factor 5	E2f5	1911	636	**3.00**
1384068_at	cytoskeleton associated protein 2 (predicted)	Ckap2_predicted	3029	1009	**3.00**
1379375_at	Platelet derived growth factor, alpha	Pdgfa	972	324	**3.00**
1374531_at	Transcribed locus	---	1419	474	**2.99**
1397644_at	Methylthioadenosine phosphorylase (predicted)	Mtap_predicted	2360	789	**2.99**
1398401_at	tudor domain containing 3	Tdrd3	2357	789	**2.99**
1392534_at	transmembrane, prostate androgen induced RNA (predicted)	Tmepai_predicted	1092	366	**2.98**
1371480_at	RGD1561797 (predicted)	RGD1561797_predicted	5993	2014	**2.98**
1371982_at	AIP1	Aip1	7702	2589	**2.98**
1381298_at	minichromosome maintenance deficient 10 (S. cerevisiae) (predicted)	Mcm10_predicted	1155	392	**2.95**
1373054_at	CDW92 antigen	Cdw92	961	326	**2.95**
1377616_at	similar to RIKEN cDNA 6720467C03 (predicted)	RGD1310681_predicted	2120	720	**2.95**
1371879_at	leucine rich repeat containing 42	Lrrc42	1528	519	**2.94**
1370314_at	solute carrier family 20 (phosphate transporter), member 1	Slc20a1	8015	2725	**2.94**
1370910_at	replication factor C (activator 1) 2	Rfc2	2252	767	**2.94**
1387062_a_at	checkpoint kinase 1 homolog (S. pombe)	Chek1	2151	734	**2.93**
1372156_at	transmembrane protein 97	Tmem97	3766	1285	**2.93**
1392589_at	replication factor C (activator 1) 5 (predicted)	Rfc5_predicted	2251	772	**2.92**
1393982_at	polymerase (DNA directed), epsilon 2 (p59 subunit) (predicted)	Pole2_predicted	1193	409	**2.92**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1382216_at	gem (nuclear organelle) associated protein 6	Gemin6	1978	678	**2.92**
1371449_at	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1 (predicted)	Pin1_predicted	5297	1824	**2.90**
1387878_at	glutamate dehydrogenase 1	Glud1	4675	1614	**2.90**
1374577_at	transforming growth factor beta regulated gene 4	Tbrg4	3111	1076	**2.89**
1387726_at	caudal type homeo box 2	Cdx2	2687	930	**2.89**
1374777_at	hypothetical protein LOC502894	LOC502894	1326	459	**2.89**
1375259_at	eukaryotic translation initiation factor 4E binding protein 2	Eif4ebp2	2903	1006	**2.88**
1390831_at	similar to KIAA1582 protein (predicted)	RGD1310027_predicted	1422	493	**2.88**
1371615_at	diacylglycerol O-acyltransferase homolog 2 (mouse)	Dgat2	1647	571	**2.88**
1381933_at	replication factor C (activator 1) 5 (predicted)	Rfc5_predicted	3286	1140	**2.88**
1380668_at	Hypothetical LOC316976	MGC72974	899	312	**2.88**
1377797_at	EMG1 nucleolar protein homolog (S. cerevisiae) (predicted)	Emg1_predicted	1384	481	**2.88**
1393119_at	Transcribed locus	---	6344	2204	**2.88**
1368632_at	forkhead box G1	Foxg1	1226	426	**2.88**
1377872_at	similar to chromosome 7 open reading frame 30 (predicted)	RGD1306936_predicted	4545	1586	**2.87**
1367683_at	karyopherin (importin) alpha 2	Kpna2	9211	3217	**2.86**
1372556_at	hypothetical protein LOC502374	LOC502374	2346	820	**2.86**
1375686_at	peptidylprolyl isomerase (cyclophilin)-like 3	Ppil3	3221	1126	**2.86**
1376240_at	similar to MYST histone acetyltransferase monocytic leukemia 4 (predicted)	RGD1566399_predicted	910	319	**2.86**
1389425_at	similar to 5(3)-deoxyribonucleotidase, cytosolic type (Cytosolic 5,3-pyrimidine nucleotidase) (Deoxy-5-nucleotidase 1) (dNT-1)	LOC688261	923	323	**2.86**
1393987_s_at	G protein-coupled receptor kinase 6	Gprk6	5740	2010	**2.85**
1379835_at	coxsackie virus and adenovirus receptor	Soat1	972	340	**2.85**
1370323_at	thimet oligopeptidase 1	Thop1	1767	619	**2.85**
1387865_at	deoxyuridine triphosphatase	Dut	7201	2524	**2.85**
1373004_at	DnaJ (Hsp40) homolog, subfamily C, member 2	Dnajc2	1910	670	**2.85**
1374953_at	similar to CG12279-PA	LOC500420	1305	458	**2.85**
1398448_at	FUS interacting protein (serine-arginine rich) 1	Tscot_predicted	806	283	**2.85**
1368674_at	liver glycogen phosphorylase	Pygl	3267	1147	**2.85**
1396205_at	Transcribed locus	---	840	295	**2.85**
1384465_at	paraspeckle protein 1	Pspc1	4450	1565	**2.84**
1378117_at	Transcribed locus	---	2221	782	**2.84**
1370127_at	polymerase (DNA directed), delta 1, catalytic subunit	Pold1	1143	405	**2.82**
1389385_at	endothelial precursor protein B9 (predicted)	Eppb9_predicted	1683	596	**2.82**
1393627_at	similar to ovary-specific acidic protein	LOC689931	1198	425	**2.82**
1389537_at	Treacher Collins Franceschetti syndrome 1, homolog (predicted)	Tcof1_predicted	1510	536	**2.82**
1386669_at	similar to Protein KIAA0690	LOC679127	812	288	**2.82**
1372611_at	similar to RIKEN cDNA 2010305A19 (predicted)	RGD1305274_predicted	853	303	**2.81**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1392670_at	similar to BH3-only member B protein (predicted)	RGD1559427_predicted	850	302	**2.81**
1390823_at	similar to S-phase kinase-associated protein 2 (F-box protein Skp2) (F-box/WD-40 protein 1) (predicted)	RGD1562456_predicted	1889	674	**2.80**
1378753_at	occludin	Ocln	1282	458	**2.80**
1392385_at	nuclear receptor coactivator 3	Ncoa3	2611	934	**2.80**
1374857_at	similar to nucleolar protein family A, member 1	LOC499709	4078	1458	**2.80**
1368436_at	nuclear distribution gene C homolog (Aspergillus)	Nudc	7465	2676	**2.79**
1376828_at	G protein-coupled receptor, family C, group 5, member A	Gprc5a	4364	1565	**2.79**
1377760_at	nucleolar complex associated 4 homolog (S. cerevisiae)	Noc4l	2101	753	**2.79**
1388321_at	IMP3, U3 small nucleolar ribonucleoprotein, homolog (yeast) (predicted)	Imp3_predicted	2528	907	**2.79**
1372108_at	similar to RIKEN cDNA 2810422B04	LOC500199	3933	1411	**2.79**
1377708_at	hypothetical protein LOC499339	LOC499339	2771	997	**2.78**
1367817_at	hepatoma-derived growth factor	Hdgf	4880	1761	**2.77**
1374748_at	serine hydroxymethyltransferase 1 (soluble)	Shmt1	1054	381	**2.77**
1367926_at	prohibitin	Phb	913	332	**2.75**
1386870_at	glutamate-ammonia ligase (glutamine synthase)	Glul	961	350	**2.74**
1383601_at	THO complex 4 (predicted)	Dufd1_predicted	1247	454	**2.74**
1367604_at	cysteine-rich protein 2	Crip2	8761	3194	**2.74**
1371608_at	mitochondrial ribosomal protein S34 (predicted)	Mrps34_predicted	1730	631	**2.74**
1384224_at	---	---	890	325	**2.74**
1373280_at	RuvB-like 2 (E. coli)	Ruvbl2	6971	2545	**2.74**
1390218_at	similar to hypothetical protein (predicted)	RGD1310440_predicted	1433	523	**2.74**
1372043_at	similar to ribosomal protein P0-like protein; 60S acidic ribosomal protein PO; ribosomal protein, large, P0-like (predicted)	RGD1311709_predicted	3555	1299	**2.74**
1398358_a_at	integrin, beta 5	Itgb5	4150	1516	**2.74**
1379422_at	similar to RIKEN cDNA 4833435D08	LOC300284	1462	535	**2.73**
1369962_at	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	Atic	6392	2340	**2.73**
1390163_at	---	---	905	332	**2.73**
1393104_at	Similar to hypothetical protein FLJ33977 (predicted)	RGD1306423_predicted	1533	562	**2.73**
1375430_at	hypothetical LOC303211	RGD1311260	954	350	**2.73**
1377042_at	similar to polycomb group ring finger 5	LOC681178 /// LOC68773	2173	797	**2.72**
1388338_at	protein phosphatase 2A, regulatory subunit B (PR 53) (predicted)	Ppp2r4_predicted	1087	399	**2.72**
1383845_at	---	---	1010	371	**2.72**
1383685_at	HEAT repeat containing 1 (predicted)	Heatr1_predicted	2760	1015	**2.72**
1398994_at	tyrosylprotein sulfotransferase 2	Tpst2	2705	995	**2.72**
1378056_at	geminin (predicted)	Gmnn_predicted	2924	1077	**2.72**
1383106_at	cullin 2 (predicted)	Cul2_predicted	1651	609	**2.71**
1389228_at	similar to RIKEN cDNA 2010309E21 (predicted)	RGD1304825_predicted	11325	4186	**2.71**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1392906_at	ubiquitin-like 4a (predicted)	Ubl4a_predicted	1130	418	**2.70**
1377846_a_at	protein kinase, cAMP-dependent, regulatory, type 2, alpha	Msh6_predicted	3408	1261	**2.70**
1393719_at	Transcribed locus	---	1386	513	**2.70**
1370136_at	lamin B receptor	Lbr	1913	710	**2.70**
1368318_at	homer homolog 1 (Drosophila)	Homer1	870	323	**2.69**
1370213_at	Y box protein 1 /// similar to Nuclease sensitive element-binding protein 1 (Y-box-binding protein 1) (Y-box transcription factor) (YB-1) (CCAAT-binding transcription factor I subunit A) (CBF-A) (Enhancer factor I subunit A) (EFI-A) (DNA-binding protein B) (DBPB)...	LOC686530 /// Ybx1	14927	5541	**2.69**
1383305_at	---	---	1895	703	**2.69**
1367960_at	ADP-ribosylation factor-like 4A	Arl4a	2286	851	**2.69**
1368404_at	drebrin 1	Dbn1	3542	1318	**2.69**
1382117_at	similar to hypothetical protein FLJ21148 (predicted)	RGD1560538_predicted	1069	398	**2.69**
1377727_at	bromodomain adjacent to zinc finger domain, 1A (predicted)	Baz1a_predicted	2109	785	**2.69**
1372331_at	similar to eukaryotic translation initiation factor 1A, Y-linked (predicted)	RGD1560198_predicted	2641	985	**2.68**
1372076_at	hepatitis B virus x interacting protein (predicted)	Hbxip_predicted	8606	3210	**2.68**
1376206_at	Transcribed locus	---	2575	961	**2.68**
1378127_at	cullin 2 (predicted)	Cul2_predicted	1539	574	**2.68**
1398349_at	adenylate kinase 2	Ak2	5669	2120	**2.67**
1392732_at	nucleolar and spindle associated protein 1 (predicted)	Nusap1_predicted	1546	578	**2.67**
1372697_at	mitochondrial ribosomal protein S15	Mrps15	1705	638	**2.67**
1371246_at	nuclear transport factor 2	NTF2	4854	1818	**2.67**
1370964_at	argininosuccinate synthetase	Ass	19682	7375	**2.67**
1371365_at	ubiquitin-conjugating enzyme E2S (predicted)	Ube2s_predicted	11552	4336	**2.66**
1391050_at	metal response element binding transcription factor 2	Mtf2	2229	838	**2.66**
1383238_at	queuine tRNA-ribosyltransferase 1	Qtrt1	1119	421	**2.66**
1383202_at	similar to U6 snRNA-associated Sm-like protein LSm6 (Sm protein F) (predicted)	RGD1561937_predicted	4984	1877	**2.66**
1373980_at	similar to XPA binding protein 1	LOC688393	864	326	**2.65**
1391045_at	diacylglycerol O-acyltransferase homolog 2 (mouse)	Dgat2	1401	530	**2.65**
1395394_at	golgi associated, gamma adaptin ear containing, ARF binding protein 2	Gga2	2895	1095	**2.64**
1388750_at	transferrin receptor	Tfrc	15757	5961	**2.64**
1399047_at	mitochondrial ribosomal protein L27 (predicted)	Mrpl27_predicted	4746	1796	**2.64**
1367818_at	coenzyme Q3 homolog, methyltransferase (yeast)	Coq3	1621	614	**2.64**
1391573_at	tumor necrosis factor receptor superfamily, member 21 (predicted)	Tnfrsf21_predicted	1681	639	**2.63**
1370006_at	NADH dehydrogenase (ubiquinone) Fe-S protein 6	LOC679739 /// LOC69205	4613	1754	**2.63**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/Dif
1369931_at	pyruvate kinase, muscle /// similar to Pyruvate kinase isozyme M2 /// similar to pyruvate kinase (EC 2.7.1.40) isozyme M2 - rat (predicted) /// similar to Pyruvate kinase isozymes M1/M2 (Pyruvate kinase muscle isozyme)	LOC499261 /// LOC68113	15366	5847	**2.63**
1384185_at	similar to RIKEN cDNA 2410016O06 (predicted)	RGD1307704_predicted	1995	760	**2.62**
1367997_at	ClpB caseinolytic peptidase B homolog (E. coli)	Clpb	1013	386	**2.62**
1378104_at	similar to RIKEN cDNA 8430406I07	RGD1307465	1289	492	**2.62**
1372408_at	golgi associated, gamma adaptin ear containing, ARF binding protein 2	Gga2	6454	2463	**2.62**
1388959_at	---	---	2138	817	**2.62**
1373400_at		Prkar2a	2801	1071	**2.61**
1387151_at	nucleoporin 107	Nup107	1680	644	**2.61**
1388960_at	pyrophosphatase	Pyp	11909	4567	**2.61**
1379348_at	BCL2-associated transcription factor 1	Exosc2_predicted	1218	467	**2.61**
1377676_at	nuclear ubiquitous casein kinase and cyclin-dependent kinase substrate	Nucks	9138	3508	**2.61**
1374802_at	similar to chromosome 14 open reading frame 94	RGD1305288	1558	598	**2.60**
1371935_at	transmembrane protein 9 (predicted)	Tmem9_predicted	931	357	**2.60**
1398997_at	phosphohistidine phosphatase 1 (predicted)	Phpt1_predicted	2189	841	**2.60**
1390335_at	Transcribed locus	---	2257	868	**2.60**
1372398_at	similar to potassium channel tetramerisation domain containing 15	LOC499129	2168	834	**2.60**
1374072_at	homolog of zebrafish ES1	Vezf1_predicted	4488	1728	**2.60**
1370200_at	glutamate dehydrogenase 1	Glud1	2412	929	**2.60**
1389758_at	transcriptional adaptor 2 (ADA2 homolog, yeast)-like	Tada2l	1023	394	**2.60**
1398303_s_at	tropomyosin 3, gamma	Tpm3	7427	2864	**2.59**
1370353_at	translocase of inner mitochondrial membrane 22 homolog (yeast)	Timm22	1416	547	**2.59**
1388528_at	fibrillarin	Fbl	13455	5200	**2.59**
1378264_at	Nuclear autoantigenic sperm protein (histone-binding)	Nasp	2371	917	**2.58**
1374713_at	transforming growth factor, beta receptor II	Hrmt1l6_predicted	1972	764	**2.58**
1398753_at	aldo-keto reductase family 1, member A1	Akr1a1	4402	1707	**2.58**
1371777_at	poly A binding protein, cytoplasmic 4	Pabpc4	7615	2964	**2.57**
1371853_at	mitochondrial ribosomal protein L42 (predicted)	Mrpl42_predicted	2290	892	**2.57**
1379541_at	similar to hypothetical protein FLJ14624 (predicted)	RGD1560183_predicted	1146	446	**2.57**
1367548_at	similar to FLJ00052 protein (predicted)	RGD1305986_predicted	1804	703	**2.56**
1367575_at	enolase 1, alpha	Eno1	19853	7744	**2.56**
1373138_at	nudix (nucleoside diphosphate linked moiety X)-type motif 5	Nudt5	3049	1189	**2.56**
1376055_at	minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae) (predicted)	Mcm5_predicted	2234	873	**2.56**
1389869_at	similar to C184L-22	LOC684594 /// LOC68939	1581	618	**2.56**
1373823_at	similar to Cyclin-dependent kinases regulatory subunit 2 (CKS-2) (predicted)	RGD1562047_predicted	17206	6725	**2.56**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1397647_at	solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15	Slc25a15	1056	413	**2.55**
1389983_at	similar to cDNA sequence AF155546	RGD1309387	1789	700	**2.55**
1389625_at	coiled-coil-helix-coiled-coil-helix domain containing 4	Chchd4	1224	479	**2.55**
1389337_at	Transcribed locus	---	2159	846	**2.55**
1367798_at	S-adenosylhomocysteine hydrolase	Ahcy	4817	1889	**2.55**
1371754_at	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	Slc25a25	1174	461	**2.55**
1370303_at	solute carrier family 35, member A4	Slc35a4	1428	561	**2.55**
1372730_at	similar to Mitochondrial 39S ribosomal protein L39 (L39mt) (MRP-L39)	LOC684304	3121	1226	**2.55**
1369775_at	nuclear ubiquitous casein kinase and cyclin-dependent kinase substrate	Nucks	4326	1703	**2.54**
1372893_at	Yip1 domain family, member 1	Yipf1	1877	740	**2.54**
1370057_at	cysteine and glycine-rich protein 1	Csrp1	2701	1064	**2.54**
1371850_at	3'-phosphoadenosine 5'-phosphosulfate synthase 1 (predicted)	Papss1_predicted	1574	621	**2.54**
1399143_at	ubiquitin-conjugating enzyme E2N	Ube2n	6512	2568	**2.54**
1367479_at	similar to nucleolar protein family A, member 3	LOC691534	9673	3816	**2.53**
1377627_at	similar to down-regulated in metastasis (predicted)	RGD1560606_predicted	1096	434	**2.53**
1373654_at	annexin A8	Anxa8	3488	1380	**2.53**
1390902_at	Transcribed locus	---	1670	661	**2.53**
1383116_at	similar to RIKEN cDNA 4833435D08	LOC300284	1643	651	**2.52**
1374393_at	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b (predicted) /// similar to protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	LOC690040 /// Ptplb_pred	2081	824	**2.52**
1376579_at	leucine aminopeptidase 3	Lap3	1343	533	**2.52**
1389765_at	notchless homolog 1 (Drosophila) (predicted)	Nle1_predicted	1364	542	**2.52**
1389279_at	similar to Hypothetical UPF0080 protein KIAA0186 (predicted)	Ran	3050	1212	**2.52**
1388641_at	phosphoribosylglycinamide formyltransferase	Gart	1913	761	**2.51**
1395967_at	similar to LDL receptor adaptor protein (predicted)	RGD1563417_predicted	863	344	**2.51**
1389265_at	glucan (1,4-alpha-), branching enzyme 1	Gbe1	1717	685	**2.51**
1374415_at	polymerase (RNA) III (DNA directed) polypeptide E (predicted)	Polr3e_predicted	1077	431	**2.50**
1367663_at	proteasome (prosome, macropain) 28 subunit, alpha	Psme1	1410	565	**2.50**
1379626_at	Special AT-rich sequence binding protein 1	Satb1	16935	6786	**2.50**
1395560_at	Transcribed locus	---	2234	895	**2.50**
1389544_at	similar to translocase of outer mitochondrial membrane 7 homolog	LOC685620	6826	2736	**2.50**
1390272_at	DPH5 homolog (S. cerevisiae)	Dph5	1204	483	**2.49**
1380062_at	membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6) (predicted)	Mpp6_predicted	1727	693	**2.49**
1393241_at	protease, serine, 32 (predicted)	Prss32_predicted	3991	1605	**2.49**
1368054_at	lamin A	Lmna	808	325	**2.49**
1381672_at	similar to LDL receptor adaptor protein (predicted)	RGD1563417_predicted	1734	699	**2.48**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1379592_at	similar to citrin (predicted)	RGD1565889_predicted	974	393	**2.48**
1368302_at	homeo box, msh-like 1	Msx1	1797	725	**2.48**
1375268_at	Transcribed locus, strongly similar to XP_996369.1 PREDICTED: similar to 40S ribosomal protein S27-like protein [Mus musculus]	---	1694	683	**2.48**
1382471_at	similar to hypothetical protein MGC10993	RGD1310320	846	342	**2.48**
1382975_at	CEA-related cell adhesion molecule 1	Ceacam1	1176	475	**2.48**
1396013_at	leucine zipper-EF-hand containing transmembrane protein 1	Letm1	921	372	**2.48**
1370030_at	glutamate cysteine ligase, modifier subunit	Gclm	3435	1389	**2.47**
1376407_a_at	LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae) (predicted)	Lsm7_predicted	2025	820	**2.47**
1370699_a_at	epidermal growth factor receptor /// peptidase D	Egfr /// Pepd	1524	618	**2.47**
1389408_at	ribonucleotide reductase M2	Rrm2	20710	8415	**2.46**
1382443_at	similar to Pabpc4_predicted protein (predicted) /// poly A binding protein, cytoplasmic 4	Pabpc4 /// RGD1562451_	3223	1313	**2.46**
1371390_at	tubulin, beta 2c	Tubb2c	16301	6640	**2.45**
1388560_at	WD repeat domain 77	Wdr77	6672	2723	**2.45**
1370008_at	proteasome (prosome, macropain) 26S subunit, ATPase 3, interacting protein	Psmc3ip	1061	433	**2.45**
1391466_at	mutS homolog 6 (E. coli) (predicted)	Nucks	1978	808	**2.45**
1374844_at	RNA binding motif protein 14	Rbm14	2344	958	**2.45**
1378638_a_at	similar to 2700029M09Rik protein (predicted)	RGD1311747_predicted	2523	1031	**2.45**
1389622_at	similar to citrin (predicted)	RGD1565889_predicted	2305	945	**2.44**
1370309_a_at	heterogeneous nuclear ribonucleoprotein A/B	Hnrpab	13579	5566	**2.44**
1373452_at	RNA terminal phosphate cyclase-like 1	Rcl1	2048	840	**2.44**
1399005_at	protein phosphatase 2, regulatory subunit B (B56), alpha isoform (predicted)	Ppp2r5a_predicted	1193	490	**2.44**
1390237_at	translocase of inner mitochondrial membrane 8 homolog a (yeast)	Timm8a	2135	877	**2.44**
1388482_at	similar to RIKEN cDNA 9130404D14	RGD1307018	2983	1225	**2.44**
1371962_at	Tu translation elongation factor, mitochondrial (predicted)	Tufm_predicted	3502	1438	**2.44**
1382679_at	similar to WD-repeat protein 43	LOC362703	2751	1130	**2.43**
1371632_at	similar to Coronin, actin binding protein 1C (predicted)	RGD1564490_predicted	7727	3176	**2.43**
1371752_at	similar to Ran-interacting protein MOG1 (predicted)	RGD1563195_predicted	1576	648	**2.43**
1371548_at	mitochondrial ribosomal protein S25	Mrps25	1959	806	**2.43**
1388178_at	nuclear receptor coactivator 3	Ncoa3	1741	717	**2.43**
1399161_a_at	type 1 tumor necrosis factor receptor shedding aminopeptidase regulator	Arts1	1631	672	**2.43**
1382592_at	similar to RIKEN cDNA 2600001J17	RGD1306908	1394	574	**2.43**
1376606_a_at	similar to e(y)2 protein	LOC682575 /// LOC68525	3324	1370	**2.43**
1388744_at	minichromosome maintenance deficient 7 (S. cerevisiae)	Mcm7	5481	2262	**2.42**
1385020_at	similar to DNA segment, Chr 19, Brigham & Womens Genetics 1357 expressed (predicted) /// hypothetical protein LOC499339 /// similar to D19Bwg1357e protein	LOC499339 /// LOC68367	1625	671	**2.42**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1379296_at	Transcribed locus	---	926	382	**2.42**
1396086_at	transmembrane protein 97	Tmem97	2427	1004	**2.42**
1375246_at	hypothetical protein LOC679747 /// hypothetical protein LOC685117	LOC679747 /// LOC68511	10244	4243	**2.41**
1393972_at	metal response element binding transcription factor 2	Mtf2	860	356	**2.41**
1372554_at	similar to RW1 protein (predicted)	RGD1309266_predicted	1256	521	**2.41**
1392717_at	exosome component 2 (predicted)	Kif1b	831	345	**2.41**
1371350_at	similar to S-adenosylmethionine synthetase isoform type-2 (Methionine adenosyltransferase 2) (AdoMet synthetase 2) (Methionine adenosyltransferase II) (MAT-II)	LOC683283	4877	2027	**2.41**
1376202_at	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6 (predicted)	B3gnt6_predicted	804	334	**2.41**
1373705_at	similar to 39S ribosomal protein L28, mitochondrial precursor (L28mt) (MRP-L28)	LOC497876	2378	992	**2.40**
1371693_at	similar to AHA1, activator of heat shock 90kDa protein ATPase homolog 1	LOC681996	4975	2075	**2.40**
1389288_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2 (predicted)	Ndufa2_predicted	3394	1418	**2.39**
1367586_at	lactate dehydrogenase A	Ldha	18791	7858	**2.39**
1371967_at	mitochondrial ribosomal protein L16	Mrpl16	2451	1026	**2.39**
1375630_at	similar to NHP2-like protein 1 (High mobility group-like nuclear protein 2 homolog 1) ([U4/U6.U5] tri-snRNP 15.5 kDa protein) (OTK27)	MGC72932	9084	3811	**2.38**
1390587_x_at	sprouty protein with EVH-1 domain 1, related sequence	Musk	3632	1525	**2.38**
1385853_at	bromodomain adjacent to zinc finger domain, 1A (predicted)	Baz1a_predicted	1049	441	**2.38**
1367883_at	survival of motor neuron 1, telomeric	Smn1	2995	1260	**2.38**
1373870_at	similar to RIKEN cDNA 2810405J04	RGD1305486	2779	1169	**2.38**
1388129_at	structure specific recognition protein 1	Ssrp1	4805	2022	**2.38**
1371662_at	lysyl-tRNA synthetase	Kars	7801	3283	**2.38**
1377745_at	similar to hypothetical protein FLJ20331	LOC310946	1652	696	**2.37**
1397255_at	similar to hypothetical protein MGC2574	MGC94720	939	396	**2.37**
1391205_at	similar to hypothetical protein FLJ20397	MGC125214	1805	761	**2.37**
1372185_at	similar to RIKEN cDNA 2610205E22	RGD1306582	3503	1479	**2.37**
1372644_at	similar to CG14286-PA	LOC686765	1850	781	**2.37**
1371743_at	guanylate kinase 1	Guk1	3132	1324	**2.37**
1388709_at	similar to WD-repeat protein 43	LOC362703	2806	1186	**2.37**
1373538_at	ubiquitin specific peptdiase 1	Usp1	1864	789	**2.36**
1383477_at	ubiquitin carboxyl-terminal hydrolase L5	Uchl5	5344	2264	**2.36**
1389964_at	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1 (predicted) /// similar to Acyl carrier protein, mitochondrial precursor (ACP) (NADH-ubiquinone oxidoreductase 9.6 kDa subunit) (CI-SDAP)	LOC683884 /// Ndufab1_p	6280	2663	**2.36**
1377706_x_at	---	---	1508	640	**2.36**
1374836_at	RNA, U3 small nucleolar interacting protein 2 (predicted)	Rnu3ip2_predicted	1952	828	**2.36**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1395404_at	Similar to Hypothetical protein 9030012M21 (predicted)	RGD1304570_predicted	800	340	**2.35**
1396250_at	similar to Coronin, actin binding protein 1C (predicted)	RGD1564490_predicted	868	369	**2.35**
1389738_at	uracil-DNA glycosylase	Ung	1840	783	**2.35**
1372631_at	thymidine kinase 1	Tk1	898	382	**2.35**
1388532_at	similar to hypothetical protein	RGD1310571	1167	497	**2.35**
1385428_at	exportin 4 (predicted)	Xpo4_predicted	1714	730	**2.35**
1372430_at	similar to RIKEN cDNA 1700037H04	RGD1311739	1161	495	**2.35**
1376098_a_at	myosin IG	Myo1g	3387	1443	**2.35**
1379330_s_at	similar to Pterin-4-alpha-carbinolamine dehydratase 2 (PHS 2) (4-alpha-hydroxy-tetrahydropterin dehydratase 2) (DcoH-like protein DCoHm) (Dimerization cofactor of hepatocyte nuclear factor 1 from muscle) (HNF1-alpha dimerization cofactor)...	LOC683319	1929	822	**2.35**
1368032_at	nucleolar and coiled-body phosphoprotein 1	Nolc1	6136	2615	**2.35**
1374537_at	carbohydrate (chondroitin) synthase 1 (predicted)	Chsy1_predicted	5370	2291	**2.34**
1389393_at	similar to RIKEN cDNA 2210412D01	RGD1308210	2689	1148	**2.34**
1368042_a_at	high mobility group box 1 /// similar to High mobility group protein 1 (HMG-1) (predicted) /// similar to Hmgb1 protein (predicted) /// hypothetical protein LOC678705 /// similar to High mobility group protein 1 (HMG-1) (High mobility group protein B1) (Amphoterin) (Heparin-binding protein p30)	Hmgb1 /// LOC678705 ///	13965	5971	**2.34**
1375639_at	Transcribed locus, strongly similar to XP_947674.1 PREDICTED: similar to E2F transcription factor 6 isoform 1 [Homo sapiens]	---	1590	680	**2.34**
1393584_at	tumor necrosis factor receptor superfamily, member 21 (predicted)	Tnfrsf21_predicted	2821	1207	**2.34**
1389815_at	protein phosphatase 1, regulatory (inhibitor) subunit 14B	Ppp1r14b	5331	2281	**2.34**
1376395_at	Transcribed locus	---	2623	1123	**2.34**
1373585_at		RGD1310781_predicted	1621	695	**2.33**
1373017_at	succinate-Coenzyme A ligase, GDP-forming, beta subunit	Suclg2	2706	1160	**2.33**
1370462_at	hyaluronan mediated motility receptor (RHAMM)	Hmmr	972	417	**2.33**
1384281_at	Transcribed locus	---	3159	1358	**2.33**
1383251_at	poly (ADP-ribose) polymerase family, member 2 (predicted)	Parp2_predicted	1571	675	**2.33**
1388988_at	abhydrolase domain containing 14b	Abhd14b	1025	441	**2.33**
1390805_at	hypothetical protein LOC687491 /// hypothetical protein LOC689755	LOC687491 /// LOC68975	985	424	**2.32**
1391415_at	putatative 28 kDa protein	LOC289809	5165	2225	**2.32**
1367751_at	dolichol-phosphate (beta-D) mannosyltransferase 2	Dpm2	1277	550	**2.32**
1395719_at	Tu translation elongation factor, mitochondrial (predicted)	Tufm_predicted	2030	875	**2.32**
1372188_at	similar to hypothetical protein D15Ertd785e	MGC114417	1071	462	**2.32**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1367870_at	thioredoxin-like 2	Txnl2	12009	5194	**2.31**
1373516_at	similar to RIKEN cDNA 2600005C20 (predicted)	RGD1305633_predicted	1555	673	**2.31**
1373954_at	similar to FLJ00052 protein (predicted)	RGD1305986_predicted	4349	1886	**2.31**
1371887_at	similar to high mobility group protein homolog HMG4 (predicted)	RGD1564407_predicted	1266	549	**2.30**
1383353_at	ephrin B2 (predicted)	Efnb2_predicted	1354	588	**2.30**
1368647_at	G protein-coupled receptor kinase 6	Gprk6	1147	498	**2.30**
1373965_at	similar to hypothetical protein BC013949 (predicted)	RGD1310931_predicted	1353	588	**2.30**
1371847_at	---	---	1494	649	**2.30**
1374877_at	similar to translocase of inner mitochondrial membrane 50 homolog	LOC687295	3425	1488	**2.30**
1390384_at	similar to Histone H2A.x (H2a/x) (predicted)	RGD1566119_predicted	2443	1062	**2.30**
1392668_at	recombining binding protein suppressor of hairless (Drosophila) (predicted) /// similar to Recombining binding protein suppressor of hairless (J kappa-recombination signal binding protein) (RBP-J kappa)	LOC679028 /// Rbpsuh_pr	1303	566	**2.30**
1388410_at	UDP-glucose pyrophosphorylase 2	Ugp2	8462	3681	**2.30**
1368055_a_at	lamin A	Lmna	4609	2005	**2.30**
1386973_a_at	mitogen activated protein kinase 8 interacting protein	Mapk8ip	913	398	**2.30**
1392747_at	caspase 8 associated protein 2 (predicted)	Fbxo30	891	388	**2.29**
1394397_at	sterol O-acyltransferase 1	Gspt1	5170	2256	**2.29**
1386954_at	adenylate kinase 2	Ak2	2615	1141	**2.29**
1392454_at	similar to hypothetical protein FLJ20729 (predicted)	RGD1308723_predicted	1467	640	**2.29**
1389658_at	NOL1/NOP2/Sun domain family, member 2 (predicted)	Nsun2_predicted	6305	2753	**2.29**
1372001_at	similar to Protein FAM96B	LOC680987	1343	587	**2.29**
1370461_at	hyaluronan mediated motility receptor (RHAMM)	Hmmr	968	423	**2.29**
1386586_at	similar to PAK/PLC-interacting protein 1	MGC125015	5299	2317	**2.29**
1375059_at	similar to zinc finger protein 652 (predicted)	RGD1566329_predicted	1305	571	**2.28**
1376684_at	discs, large homolog 7 (Drosophila) (predicted)	Dlg7_predicted	1914	839	**2.28**
1368977_a_at	fractured callus expressed transcript 1	Fxc1	847	372	**2.28**
1372406_at	minichromosome maintenance deficient 3 (S. cerevisiae) (predicted) /// similar to DNA replication licensing factor MCM3 (DNA polymerase alpha holoenzyme-associated protein P1) (P1-MCM3)	LOC367976 /// Mcm3_pre	2268	996	**2.28**
1371838_at	similar to splicing factor, arginine/serine-rich 2	Sfrs2	10034	4406	**2.28**
1373668_at	polymerase (RNA) II (DNA directed) polypeptide I (predicted)	Polr2i_predicted	3281	1442	**2.28**
1381885_at	Transcribed locus	---	1604	705	**2.28**
1382144_at	mitochondrial ribosomal protein L47	Mrpl47	2123	933	**2.28**
1393431_at	similar to Splicing factor U2AF 35 kDa subunit (U2 auxiliary factor 35 kDa subunit) (U2 snRNP auxiliary factor small subunit)	LOC687575	8603	3783	**2.27**
1371448_at	CDNA clone IMAGE:7303896	---	2178	958	**2.27**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1398608_at	similar to hypothetical gene supported by BC007071	RGD1311868	1666	733	**2.27**
1387085_at	phosphoribosyl pyrophosphate synthetase 1	Prps1	2275	1003	**2.27**
1371503_at	nucleotide binding protein 1	Nubp1	1993	880	**2.26**
1389587_at	uridine monophosphate synthetase	Umps	4132	1826	**2.26**
1371908_at	NTF2-related export protein 1 (predicted)	Nxt1_predicted	3025	1338	**2.26**
1388567_at	THUMP domain containing 1	Thumpd1	2013	890	**2.26**
1374199_at	similar to Disco-interacting protein 2 homolog	LOC687682 /// LOC69021	922	408	**2.26**
1375915_at	interleukin-1 receptor-associated kinase 1 binding protein 1 (predicted)	Irak1bp1_predicted	1217	539	**2.26**
1391432_at	WD repeat domain 75	Wdr75	2055	911	**2.26**
1395246_at	aquarius (predicted)	Prkar2a	872	386	**2.26**
1373744_at	anaphase promoting complex subunit 1 (predicted)	Anapc1_predicted	1938	860	**2.25**
1394884_s_at	single-stranded DNA binding protein 1	Ssbp1	3475	1543	**2.25**
1377299_at	nuclear autoantigenic sperm protein (histone-binding)	Nasp	3622	1610	**2.25**
1374288_at	FtsJ homolog 3 (E. coli)	Ftsj3	3341	1488	**2.25**
1392494_at	similar to hypothetical protein D030056L22	LOC499331	1857	827	**2.24**
1398894_at	COMM domain containing 3	Commd3	7264	3238	**2.24**
1388473_at	Transcribed locus	---	956	427	**2.24**
1389450_at	similar to Williams-Beuren syndrome critical region protein 22 /// similar to Putative methyltransferase WBSCR22 (Williams-Beuren syndrome chromosome region 22 protein homolog)	LOC360830 /// LOC36808	4106	1834	**2.24**
1367617_at	aldolase A	Aldoa	15478	6926	**2.23**
1382435_at	fizzy/cell division cycle 20 related 1 (Drosophila) (predicted)	Fzr1_predicted	959	429	**2.23**
1375895_at	growth factor receptor bound protein 2-associated protein 1 (predicted)	Srr	6256	2805	**2.23**
1388783_at	high mobility group box 1	Hmgb1	5329	2391	**2.23**
1398877_at	stress-induced phosphoprotein 1	Stip1	5241	2351	**2.23**
1388163_at	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	Slc25a5	16999	7627	**2.23**
1367661_at	S100 calcium binding protein A6 (calcyclin)	S100a6	6024	2709	**2.22**
1376703_at	nucleoporin 37 (predicted)	Nup37_predicted	3291	1481	**2.22**
1367927_at	prohibitin	Phb	8571	3857	**2.22**
1371907_at	ADP-ribosylation factor-like 6 interacting protein 4	Arl6ip4	2423	1090	**2.22**
1386096_at	metal response element binding transcription factor 2	Mtf2	2364	1065	**2.22**
1388954_at	similar to hypothetical protein MGC25461 (predicted)	RGD1306717_predicted	1233	556	**2.22**
1373682_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51 (predicted)	Ddx51_predicted	1566	706	**2.22**
1375551_at	similar to hypothetical protein MGC2574	MGC94720	1013	457	**2.22**
1389312_at	similar to RIKEN cDNA 0610016J10 gene	RGD1309929	3453	1557	**2.22**
1399067_at	Guanine nucleotide binding protein-like 3 (nucleolar)-like	Gnl3l	1942	876	**2.22**
1372709_at	B-cell receptor-associated protein 29	Bcap29	3365	1521	**2.21**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1371074_a_at	minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)	Mcm6	5760	2603	**2.21**
1383144_at	tRNA nucleotidyl transferase, CCA-adding, 1	Trnt1	3070	1388	**2.21**
1371094_at	LIM homeobox protein 2 (predicted)	Lhx2_predicted	1112	504	**2.21**
1390356_at	Transcribed locus	---	5307	2407	**2.20**
1382275_at	similar to PAK/PLC-interacting protein 1	MGC125015	5297	2404	**2.20**
1393043_at	WD repeat domain 50 (predicted)	Wdr50_predicted	1449	658	**2.20**
1374161_at	importin 11 (predicted)	Ipo11_predicted	1627	739	**2.20**
1383323_at	similar to RIKEN cDNA 1700022L09	LOC499933	835	379	**2.20**
1368201_at	natriuretic peptide receptor 1	Npr1	1807	821	**2.20**
1388304_at	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5 (predicted)	Ndufb5_predicted	4935	2247	**2.20**
1394003_at	polymerase (DNA directed), epsilon 3 (p17 subunit)	Pole3	1607	732	**2.19**
1371735_at	Transcribed locus, strongly similar to XP_579758.1 PREDICTED: hypothetical protein XP_579758 [Rattus norvegicus]	---	9213	4205	**2.19**
1372688_at	exosome component 7	Exosc7	4198	1922	**2.18**
1377945_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18	Ddx18	3534	1621	**2.18**
1388126_at	multiple inositol polyphosphate histidine phosphatase 1	Minpp1	2529	1160	**2.18**
1370386_at	RuvB-like protein 1	Ruvbl1	2183	1001	**2.18**
1392983_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	Psmd12	4779	2194	**2.18**
1371443_at	similar to RIKEN cDNA A430005L14	RGD1304567	1308	601	**2.18**
1373454_at	similar to RIKEN cDNA 0610037P05	RGD1305823	2528	1162	**2.18**
1372715_at	sideroflexin 1	Sfxn1	3005	1383	**2.17**
1398308_at	replication protein A3 (predicted)	Rpa3_predicted	4149	1910	**2.17**
1388397_at	EBNA1 binding protein 2	Ebna1bp2	6415	2956	**2.17**
1389117_at	O-sialoglycoprotein endopeptidase	Osgep	1132	522	**2.17**
1371296_at	succinate dehydrogenase complex, subunit D, integral membrane protein	Sdhd	2391	1103	**2.17**
1371813_at	HIRA interacting protein 3	Hirip3	1205	556	**2.17**
1391461_at	similar to hypothetical protein (predicted)	RGD1306576_predicted	1259	581	**2.17**
1398867_at	PRP19/PSO4 pre-mRNA processing factor 19 homolog (S. cerevisiae)	Prpf19	2383	1100	**2.17**
1388683_at	similar to hypothetical protein MGC14151 (predicted)	RGD1559617_predicted	3194	1474	**2.17**
1379542_at	similar to Small nuclear ribonucleoprotein F (snRNP-F) (Sm protein F) (Sm-F) (SmF)	LOC680737 /// LOC68784	7198	3323	**2.17**
1371421_at	similar to 3-oxoacid CoA transferase 1	LOC678860 /// LOC69010	1248	576	**2.17**
1367687_a_at	peptidylglycine alpha-amidating monooxygenase	Pam	3620	1672	**2.17**
1367766_at	expressed in non-metastatic cells 2	Nme2	11911	5504	**2.16**
1398944_at	apoptotic chromatin condensation inducer 1	Acin1	1621	749	**2.16**
1378259_at	lamin B receptor	Tardbp_predicted	1817	841	**2.16**
1372645_at	presenilin associated, rhomboid-like	Psarl	2487	1152	**2.16**
1372693_at	heterogeneous nuclear ribonucleoprotein A1	Hnrpa1	4392	2034	**2.16**
1374390_at	similar to Protein UNQ655/PRO1286 homolog precursor	LOC686324 /// LOC69034	826	383	**2.16**
1389021_at	similar to HCV NS3-transactivated protein 1 (predicted)	RGD1306332_predicted	3044	1411	**2.16**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1371782_at	nipsnap homolog 3A (C. elegans)	Nipsnap3a	1766	820	**2.15**
1389200_at	bystin-like	Bysl	2188	1016	**2.15**
1378020_at	Transcribed locus	---	4701	2184	**2.15**
1372142_at	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	Asna1	2962	1377	**2.15**
1395642_at	nucleolar protein 9	Nol9	1952	907	**2.15**
1370421_a_at	cullin-associated and neddylation-dissociated 2 (putative)	Cand2	812	378	**2.15**
1393583_at	similar to RIKEN cDNA 5730466H23 (predicted)	RGD1560957_predicted	1020	474	**2.15**
1376316_at	Origin recognition complex, subunit 6-like (S. cerevisiae)	Orc6l	902	420	**2.15**
1392702_at	GH regulated TBC protein 1	Musk	917	427	**2.15**
1372488_at	similar to RIKEN cDNA 2310057D15	RGD1309016	1576	734	**2.15**
1386951_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 5	Ndufa5	1459	680	**2.15**
1367497_at	phosphatidylserine synthase 1	Ptdss1	3148	1469	**2.14**
1390692_at	cytidine 5'-triphosphate synthase (predicted)	Ctps_predicted	1918	896	**2.14**
1371391_at	thioredoxin domain containing 5 (predicted)	Txndc5_predicted	2320	1084	**2.14**
1371567_at	aldehyde dehydrogenase family 7, member A1	Aldh7a1	3636	1699	**2.14**
1371663_at	similar to expressed sequence AW556797 (predicted)	RGD1305138_predicted	2199	1028	**2.14**
1374051_at	similar to hypothetical protein D15Ertd785e	MGC114417	1290	604	**2.14**
1367999_at	aldehyde dehydrogenase 2	Aldh2	4884	2285	**2.14**
1374532_at	prostaglandin E synthase 2 (predicted)	Ptges2_predicted	1384	648	**2.14**
1372793_at	single-stranded DNA binding protein 1	Ssbp1	1561	731	**2.14**
1389582_at	similar to Nucleoporin Nup43	LOC683983	3165	1483	**2.13**
1398809_at	nuclear distribution gene E homolog 1 (A nidulans)	Nde1	1485	696	**2.13**
1375197_at	similar to ubiquinol-cytochrome c reductase subunit	LOC686951	3562	1670	**2.13**
1370976_at	Ras-GTPase-activating protein SH3-domain binding protein	G3bp	7665	3596	**2.13**
1368204_at	ligase I, DNA, ATP-dependent	Lig1	1660	779	**2.13**
1371387_at	cytochrome c oxidase subunit VIIb	Cox7b	12196	5731	**2.13**
1371591_at	mitochondrial ribosomal protein S18A	Mrps18a	2363	1111	**2.13**
1367772_at	chloride channel, nucleotide-sensitive, 1A	Clns1a	5443	2558	**2.13**
1389344_at	ubiquitin specific protease 39 (predicted)	Usp39_predicted	1940	913	**2.12**
1391481_at	---	---	6780	3199	**2.12**
1388564_at	similar to hypothetical protein FLJ20436	RGD1303127	3638	1717	**2.12**
1372401_at	N-acetylneuraminic acid synthase (sialic acid synthase) (predicted)	Nans_predicted	2331	1100	**2.12**
1375579_at	hypothetical protein LOC688300 /// hypothetical protein LOC690871	LOC688300 /// LOC69087	1500	708	**2.12**
1392446_at	kelch-like 2, Mayven (Drosophila) (predicted)	Klhl2_predicted	2191	1034	**2.12**
1385133_at	similar to spermatogenesis associated 5-like 1	LOC691729	887	419	**2.12**
1375411_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7 (B14.5a) (predicted)	Ndufa7_predicted	3057	1445	**2.12**
1389050_at	similar to Dual specificity protein phosphatase 3 (T-DSP11) (predicted)	RGD1560049_predicted	1439	681	**2.11**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1372271_at	RNA-binding region (RNP1, RRM) containing 1 (predicted)	Rnpc1_predicted	1525	722	**2.11**
1370393_at	coiled-coil domain containing 5	Ccdc5	1347	638	**2.11**
1388911_at	DNA primase, p58 subunit	Prim2	1245	590	**2.11**
1398458_at	similar to protein kinase, lysine deficient 1; kinase deficient protein (predicted)	RGD1307284_predicted	1661	788	**2.11**
1372173_at	similar to U5 snRNP-specific protein (Prp8-binding) (predicted)	RGD1309198_predicted	1978	939	**2.11**
1374063_at	splicing factor, arginine/serine-rich 3 (SRp20) (predicted)	Sfrs3_predicted	11543	5478	**2.11**
1389274_at	dephospho-CoA kinase domain containing	Dcakd	1433	680	**2.11**
1373263_at	similar to WD repeat domain 74	LOC686449	3076	1460	**2.11**
1382135_at	similar to CG9346-PA (predicted)	RGD1307882_predicted	6733	3201	**2.10**
1373824_at	craniofacial development protein 1	Cfdp1	5223	2485	**2.10**
1385534_at	nerve growth factor receptor (TNFRSF16) associated protein 1	Ngfrap1	998	475	**2.10**
1398910_at	STIP1 homology and U-Box containing protein 1	Stub1	2687	1279	**2.10**
1389055_at	peptidylprolyl isomerase E (cyclophilin E)	Ppie	1852	882	**2.10**
1370235_at	diazepam binding inhibitor	Dbi	5833	2778	**2.10**
1369785_at	phosphoribosyl pyrophosphate amidotransferase	Ppat	2979	1419	**2.10**
1385592_at	Bcl6 interacting corepressor (predicted)	Bcor_predicted	5144	2451	**2.10**
1398943_at	similar to Eso3 protein (predicted)	RGD1562476_predicted	1543	735	**2.10**
1393035_at	---	---	1650	786	**2.10**
1373869_at	sterol O-acyltransferase 1	Soat1	2982	1422	**2.10**
1379715_at	similar to CG9346-PA (predicted)	RGD1307882_predicted	1809	863	**2.10**
1389367_at	schwannomin interacting protein 1	Schip1	1430	682	**2.10**
1372324_at	similar to thyroid hormone receptor interactor 3	LOC497975	1780	850	**2.09**
1371896_at	growth arrest and DNA-damage-inducible, gamma interacting protein 1	Gadd45gip1	1900	908	**2.09**
1382316_at	similar to histone cell cycle regulation defective homolog A isoform 1	LOC363849	1605	767	**2.09**
1392144_at	5'-nucleotidase domain containing 1 (predicted)	Nt5dc1_predicted	920	440	**2.09**
1381130_at	minichromosome maintenance deficient 8 (S. cerevisiae) (predicted)	Mcm8_predicted	817	391	**2.09**
1372999_at	DCN1, defective in cullin neddylation 1, domain containing 5 (S. cerevisiae)	Dcun1d5	3559	1704	**2.09**
1376100_at	tubulin, beta 6	Tubb6	4216	2019	**2.09**
1386910_a_at	apurinic/apyrimidinic endonuclease 1	Apex1	3501	1677	**2.09**
1374446_at	TCDD-inducible poly(ADP-ribose) polymerase (predicted)	Tiparp_predicted	903	432	**2.09**
1372116_at	mitochondrial ribosomal protein S2 (predicted)	Mrps2_predicted	4390	2103	**2.09**
1398891_at	Rho GTPase activating protein 5	Mrpl15_predicted	2658	1274	**2.09**
1371342_at	cytochrome c-1 (predicted)	Cyc1_predicted	7425	3561	**2.08**
1387872_at	heterogeneous nuclear ribonucleoprotein A1	Hnrpa1	10677	5123	**2.08**
1378180_at	similar to 6030466N05Rik protein	LOC290741	1239	594	**2.08**
1371467_at	similar to RIKEN cDNA 0610007P06	LOC293103	3843	1845	**2.08**
1388870_at	similar to RNA-binding protein Musashi2-S (predicted)	RGD1560397_predicted	3118	1499	**2.08**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1388405_at	cytoskeleton associated protein 5	Ckap5	3140	1509	**2.08**
1371553_at	mitochondrial ribosomal protein L36 (predicted)	Mrpl36_predicted	2116	1018	**2.08**
1374196_at	lanC (bacterial lantibiotic synthetase component C)-like 1	Lancl1	3457	1663	**2.08**
1389010_at	leukotriene A4 hydrolase	Lta4h	2536	1221	**2.08**
1374323_at	angiopoietin 1	Bccip_predicted	5407	2605	**2.08**
1376401_at	Transcribed locus	---	902	434	**2.08**
1388930_at	transmembrane protein 123	Tmem123	1220	588	**2.07**
1376212_at	similar to Zinc finger protein RP-8 CG3260-PA	LOC687670 /// LOC68963	1114	537	**2.07**
1377735_at	pleckstrin homology domain-containing, family A (phosphoinositide binding specific) member 3	Plekha3	4755	2294	**2.07**
1372286_at	tetraspanin 6	Tspan6	1650	797	**2.07**
1383429_at	similar to T-Brain-1	LOC311078	9352	4516	**2.07**
1372259_at	DEK oncogene (DNA binding)	Dek	4960	2396	**2.07**
1379234_a_at	similar to cell division cycle 45 homolog (S. cerevisiae)-like	LOC287961	1048	506	**2.07**
1371915_at	poly(rC) binding protein 4 (predicted)	Pcbp4_predicted	1480	715	**2.07**
1394854_at	telomeric repeat binding factor 1	Terf1	918	444	**2.07**
1368033_at	nucleolar and coiled-body phosphoprotein 1	Nolc1	2227	1077	**2.07**
1383261_at	Transcribed locus	---	894	432	**2.07**
1375417_at	protein kinase C binding protein 1	Prkcbp1	3309	1600	**2.07**
1373174_at	similar to 1110001K21Rik protein	LOC497867	1534	743	**2.07**
1392499_at	phosphoribosylformylglycinamidine synthase (FGAR amidotransferase) (predicted)	Pfas_predicted	2230	1080	**2.07**
1367603_at	triosephosphate isomerase 1	Tpi1	10098	4891	**2.06**
1388328_at	similar to Eukaryotic translation initiation factor 3 subunit 2 (eIF-3 beta) (eIF3 p36) (eIF3i) (TGF-beta receptor-interacting protein 1) (TRIP-1)	LOC682390	6695	3247	**2.06**
1369950_at	cyclin-dependent kinase 4	Cdk4	3197	1552	**2.06**
1371649_at	mitochondrial ribosomal protein S24 (predicted)	Mrps24_predicted	2732	1326	**2.06**
1368184_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	Psmd9	1778	864	**2.06**
1371399_at	hypothetical protein LOC681123	LOC681123	7748	3766	**2.06**
1383618_at	mitochondrial ribosomal protein L38	Mrpl38	1362	662	**2.06**
1385783_at	similar to BH3-only member B protein (predicted)	RGD1559427_predicted	810	394	**2.06**
1373003_at	squamous cell carcinoma antigen recognized by T-cells 3 (predicted)	Sart3_predicted	1992	969	**2.05**
1373135_at	similar to hypothetical protein MGC2744	LOC619440	891	434	**2.05**
1388634_at	phosphoglucomutase 1	Pgm1	1042	507	**2.05**
1383324_at	membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6) (predicted)	Mpp6_predicted	1045	508	**2.05**
1379302_at	recombining binding protein suppressor of hairless (Drosophila) (predicted) /// similar to Recombining binding protein suppressor of hairless (J kappa-recombination signal binding protein) (RBP-J kappa)	LOC679028 /// Rbpsuh_pr	1469	715	**2.05**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1391427_at	similar to RIKEN cDNA 1500031M22 (predicted)	RGD1311752_predicted	2585	1259	**2.05**
1383182_at	serine/arginine-rich protein specific kinase 1	Srpk1	1727	842	**2.05**
1373640_at	pyrroline-5-carboxylate reductase family, member 2	Pycr2	1103	537	**2.05**
1396185_at	RNA binding motif protein 17	Rbm17	1889	921	**2.05**
1371737_at	tripartite motif protein 27 (predicted)	Trim27_predicted	8213	4005	**2.05**
1398960_at	chaperonin subunit 6a (zeta) /// similar to chaperonin subunit 6a (zeta)	Cct6a /// LOC303526 /// L	10896	5316	**2.05**
1374287_at	paladin	Pald	829	404	**2.05**
1376985_at	---	---	1087	531	**2.05**
1372711_at	similar to cDNA sequence BC005537	LOC498750	2398	1171	**2.05**
1391062_at	similar to elongation protein 4 homolog	LOC687694	1033	506	**2.04**
1397708_at	hypothetical LOC288978	LOC288978	817	400	**2.04**
1374113_at	bromodomain adjacent to zinc finger domain, 1A (predicted)	Baz1a_predicted	4784	2343	**2.04**
1372939_at	NudC domain containing 2	Nudcd2	1709	837	**2.04**
1389497_at	SET and MYND domain containing 5 (predicted)	Smyd5_predicted	1038	509	**2.04**
1389389_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 56	Ddx56	1638	803	**2.04**
1378220_at	similar to RIKEN cDNA 4930517K11	LOC497860	3669	1800	**2.04**
1372815_at	mago-nashi homolog, proliferation-associated (Drosophila) (predicted)	Magoh_predicted	8582	4213	**2.04**
1373222_at	hexosaminidase A	Hexa	1794	881	**2.04**
1372150_at	ubiquitin specific protease 10	Usp10	3201	1572	**2.04**
1379853_at	similar to Placental protein 25 homolog (PP25)	LOC685284	959	471	**2.04**
1372182_at	phosphofructokinase, platelet	Pfkp	13681	6729	**2.03**
1388728_at	lysosomal-associated protein transmembrane 4B	Laptm4b	1395	686	**2.03**
1390141_at	breast cancer 1	Fthfsdc1_predicted	916	451	**2.03**
1369617_at	ubiquitin-conjugating enzyme E2N	Ube2n	844	416	**2.03**
1382419_at	SoxLZ/Sox6 leucine zipper binding protein in testis (predicted)	Solt_predicted	2353	1160	**2.03**
1392530_at	similar to NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1	LOC689938	2850	1405	**2.03**
1388141_at	centrin 3	Cetn3	2157	1064	**2.03**
1379406_at	similar to chromosome 14 open reading frame 21 (predicted)	RGD1308396_predicted	995	491	**2.03**
1398921_at	mitochondrial ribosomal protein L37	Mrpl37	2238	1105	**2.03**
1372362_at	galactokinase 2	Galk2	938	463	**2.03**
1369984_at	cytochrome c oxidase, subunit XVII assembly protein homolog (yeast)	Cox17	3377	1668	**2.03**
1371323_at	similar to NADH-ubiquinone oxidoreductase MLRQ subunit (Complex I-MLRQ) (CI-MLRQ)	LOC681024	12638	6241	**2.02**
1375767_at	similar to common-site lymphoma leukemia guanine nucleotide exchange factor like (5N754) (predicted)	RGD1308089_predicted	916	452	**2.02**
1374620_at	CEA-related cell adhesion molecule 1	Ceacam1	2120	1048	**2.02**
1383068_at	deoxythymidylate kinase (predicted)	Dtymk_predicted	3221	1595	**2.02**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1381969_at	similar to Recombining binding protein suppressor of hairless (J kappa-recombination signal binding protein) (RBP-J kappa)	LOC679028	3246	1609	**2.02**
1398974_at	chromosome segregation 1-like (S. cerevisiae) (predicted)	Cse1l_predicted	4472	2219	**2.02**
1372876_at	selenophosphate synthetase 2	Sephs2	2072	1028	**2.02**
1371620_at	similar to px19-like protein	RGD1308082	4354	2162	**2.01**
1379235_x_at	similar to cell division cycle 45 homolog (S. cerevisiae)-like	LOC287961	1874	931	**2.01**
1393114_at	alpha-2,6-sialyltransferase ST6GalNAc IV	siat7D	922	458	**2.01**
1384878_at	N-myristoyltransferase 2	Nmt2	3199	1591	**2.01**
1395058_at	WD repeat domain 75	Wdr75	2027	1008	**2.01**
1367495_at	similar to prefoldin 4 (predicted)	RGD1560211_predicted	7938	3949	**2.01**
1371597_at	ring finger protein 187 (predicted)	Rnf187_predicted	2094	1042	**2.01**
1379312_at	peroxisome proliferative activated receptor, gamma, coactivator-related 1 (predicted)	Pprc1_predicted	2958	1472	**2.01**
1372677_at	similar to RIKEN cDNA 1110033C18	RGD1304783	1810	901	**2.01**
1376572_a_at	supervillin (predicted)	Svil_predicted	1050	523	**2.01**
1385711_at	similar to RIKEN cDNA 1500031M22 (predicted)	RGD1311752_predicted	1277	636	**2.01**
1371899_at	protein kinase, interferon inducible double stranded RNA dependent activator	Prkra	1255	626	**2.00**
1372290_at	RD RNA-binding protein	Rdbp	1504	751	**2.00**
1375230_at	Endogenous retrovirus mRNA, partial sequence	---	5919	2957	**2.00**
1369940_at	transaldolase 1	Taldo1	4314	2156	**2.00**
1393009_at	similar to RIKEN cDNA 2410002F23	RGD1309326	1223	611	**2.00**
1387737_at	methionine adenosyltransferase II, alpha	Mat2a	1956	978	**2.00**
1383867_at	fizzy/cell division cycle 20 related 1 (Drosophila) (predicted)	Eif5a2_predicted	2042	1022	**2.00**
1393154_at	Transcribed locus	---	2450	1228	**2.00**
1371470_at	Alcohol dehydrogenase 4 (class II), pi polypeptide	Adh4	3046	1527	**1.99**
1383208_at	polymerase (DNA directed), epsilon 3 (p17 subunit)	Pole3	1438	721	**1.99**
1371581_at	similar to splicing factor, arginine/serine-rich 1 (ASF/SF2)	LOC688114 /// LOC68989	8848	4440	**1.99**
1372143_at	ubiquitin-conjugating enzyme E2 variant 2	Ube2v2	5153	2588	**1.99**
1373557_at	minichromosome maintenance deficient 4 homolog (S. cerevisiae)	Mcm4	2654	1333	**1.99**
1373458_at	brain expressed X-linked 4	Bex4	7577	3810	**1.99**
1374733_at	symplekin	Sympk	914	460	**1.99**
1371345_at	methyl-CpG binding domain protein 3 (predicted)	Mbd3_predicted	2430	1223	**1.99**
1370159_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	Smarcd2	1406	707	**1.99**
1395338_at	leucine-rich PPR-motif containing	Lrpprc	1138	573	**1.99**
1386946_at	carnitine palmitoyltransferase 1a, liver	Cpt1a	1279	644	**1.99**
1372501_at	splicing factor 3b, subunit 3 (predicted)	Sf3b3_predicted	5281	2660	**1.99**
1398902_at	similar to mKIAA0664 protein (predicted)	RGD1307222_predicted	1717	865	**1.99**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1397740_at	sideroflexin 1	Sfxn1	2193	1105	**1.98**
1373489_at	similar to Chromatin assembly factor 1 subunit A (CAF-1 subunit A) (Chromatin assembly factor I p150 subunit) (CAF-I 150 kDa subunit) (CAF-Ip150)	LOC363333	1440	726	**1.98**
1379476_at	nucleoporin 35	Nup35	2747	1385	**1.98**
1388326_at	NADH dehydrogenase (ubiquinone) Fe-S protein 8 (predicted)	Ndufs8_predicted	2074	1047	**1.98**
1390787_at	phosphoribosylglycinamide formyltransferase	Gart	2650	1339	**1.98**
1372498_at	cytokine induced apoptosis inhibitor 1	Ciapin1	3892	1968	**1.98**
1379862_at	similar to CG33331-PA	LOC362419	1961	992	**1.98**
1372902_at	asparagine-linked glycosylation 12 homolog (yeast, alpha-1,6-mannosyltransferase) (predicted)	Alg12_predicted	1061	537	**1.98**
1378112_at	Transcribed locus	---	2282	1155	**1.98**
1387771_a_at	mitogen activated protein kinase 3	Mapk3	936	474	**1.98**
1371869_at	proteasome (prosome, macropain) subunit, alpha type 7	Psma7	10336	5236	**1.97**
1372336_at	Unknown sequence	---	6543	3315	**1.97**
1376687_at	ubiquitin specific peptdiase 1	Usp1	3735	1893	**1.97**
1389126_at	coiled-coil-helix-coiled-coil-helix domain containing 1 (predicted)	Chchd1_predicted	4468	2264	**1.97**
1367833_at	peptidase (prosome, macropain) 26S subunit, ATPase 5	Psmc5	4737	2402	**1.97**
1372339_at	cell division cycle 26	Cdc26	1362	691	**1.97**
1372128_at	mitochondrial ribosomal protein S12 (predicted)	Mrps12_predicted	2401	1220	**1.97**
1389518_at	hypothetical protein LOC680080	LOC680080	6874	3492	**1.97**
1368330_at	apoptosis antagonizing transcription factor	Aatf	1983	1008	**1.97**
1388294_at	succinate dehydrogenase complex, subunit D, integral membrane protein	Sdhd	4718	2399	**1.97**
1388370_at	cyclin I (predicted)	Ccni_predicted	5316	2704	**1.97**
1390305_at	protein kinase C binding protein 1	Prkcbp1	1173	597	**1.97**
1383067_a_at	deoxythymidylate kinase (predicted)	Dtymk_predicted	1636	832	**1.97**
1393058_at	similar to CREBBP/EP300 inhibitor 2	LOC685691 /// LOC68735	2065	1051	**1.97**
1388402_at	similar to 2410001H17Rik protein (predicted)	RGD1305824_predicted	1276	649	**1.96**
1371698_at	elongation factor Tu GTP binding domain containing 2	Eftud2	3777	1923	**1.96**
1371622_at	similar to Dph2l1 protein (predicted) /// similar to candidate tumor suppressor OVCA2 (predicted)	RGD1562694_predicted //	2161	1100	**1.96**
1375186_at	similar to zinc finger, CSL domain containing 2	LOC680594 /// LOC68211	3316	1689	**1.96**
1370443_at	deoxyribonuclease II	Dnase2	1421	725	**1.96**
1375859_a_at	Transcribed locus, strongly similar to XP_218473.3 PREDICTED: similar to zinc finger protein 565 [Rattus norvegicus]	---	4976	2539	**1.96**
1377689_at	kinetochore associated 1 (predicted)	Kntc1_predicted	886	452	**1.96**
1389363_at	acireductone dioxygenase 1	Adi1	1577	807	**1.96**
1390168_a_at	zinc finger, CSL-type containing 3 (predicted)	Zcsl3_predicted	1114	570	**1.95**
1372087_at	hypertrophic agonist responsive protein B64	Harpb64	2890	1479	**1.95**
1373658_at	Rac GTPase-activating protein 1 (predicted)	Racgap1_predicted	5590	2860	**1.95**
1384762_at	Transcribed locus	---	1577	807	**1.95**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1392972_at	triple functional domain (PTPRF interacting)	Trio	2139	1096	**1.95**
1368309_at	thioredoxin reductase 2	Txnrd2	802	411	**1.95**
1368101_at	calmodulin 3	Calm3	1789	917	**1.95**
1369013_a_at	mitochondrial ribosomal protein L17	Mrpl17	6636	3402	**1.95**
1390709_at	triple functional domain (PTPRF interacting)	Trio	2026	1041	**1.95**
1393238_at	similar to DKFZP434B168 protein (predicted)	RGD1308014_predicted	1039	534	**1.95**
1371525_at	solute carrier family 12 (potassium/chloride transporters), member 7	Slc12a7	3091	1588	**1.95**
1375701_at	similar to Hypothetical UPF0049 protein ZK1128.2 in chromosome III	LOC360568	2897	1488	**1.95**
1398837_at	transcription elongation factor B (SIII), polypeptide 2	Tceb2	11683	6003	**1.95**
1372052_at	budding uninhibited by benzimidazoles 3 homolog (S. cerevisiae)	Bub3	7691	3952	**1.95**
1389551_at	lactamase, beta 2	Lactb2	849	436	**1.95**
1371658_at	COX4 neighbor	Cox4nb	2269	1167	**1.94**
1372021_at	nucleoporin 188	Nup188	1028	529	**1.94**
1373273_at	PRP38 pre-mRNA processing factor 38 (yeast) domain containing A (predicted)	Prpf38a_predicted	2171	1117	**1.94**
1367831_at	tumor protein p53	Tp53	1371	706	**1.94**
1391905_at	similar to PWP2 periodic tryptophan protein homolog	LOC687601 /// LOC69029	1258	648	**1.94**
1372705_at	calcium homeostasis endoplasmic reticulum protein (predicted)	Cherp_predicted	1894	975	**1.94**
1373121_at	similar to Mitochondrial 28S ribosomal protein S28 (S28mt) (MRP-S28)	LOC679330	939	484	**1.94**
1373351_at	ankyrin 2, neuronal	Ank2	1313	676	**1.94**
1373500_at	leucine-rich PPR-motif containing	Lrpprc	3504	1805	**1.94**
1398411_at	---	---	12921	6659	**1.94**
1367820_at	barrier to autointegration factor 1	Banf1	5522	2850	**1.94**
1376250_at	nuclear fragile X mental retardation protein interacting protein 1	Nufip1	3478	1795	**1.94**
1384256_at	similar to multi sex combs CG12058-PA	LOC686883	4574	2362	**1.94**
1386983_at	hydroxymethylbilane synthase	Hmbs	1422	735	**1.93**
1395297_at	similar to hypothetical protein FLJ10652	RGD1309621	1904	985	**1.93**
1389832_at	glutathione S-transferase omega 1	Gsto1	6141	3176	**1.93**
1374622_at	Transcribed locus	---	3720	1925	**1.93**
1388309_at	high mobility group AT-hook 1	Hmga1	2421	1253	**1.93**
1379521_at	asparagine-linked glycosylation 3 homolog (yeast, alpha-1,3-mannosyltransferase)	Alg3	1203	623	**1.93**
1368234_at	prolyl endopeptidase	Prep	1366	708	**1.93**
1376201_at	similar to Hypothetical UPF0049 protein ZK1128.2 in chromosome III	LOC360568	1639	850	**1.93**
1389326_at	replication factor C (activator 1) 3	Rfc3	1459	756	**1.93**
1370313_at	acyl-CoA thioesterase 7	Acot7	4483	2326	**1.93**
1376196_a_at	mediator of RNA polymerase II transcription, subunit 4 homolog (yeast)	Med4	1321	685	**1.93**
1390650_at	pericentrin 1	Pcnt1	2009	1043	**1.93**
1368588_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 52	Ddx52	2152	1117	**1.93**
1372181_at	Replication protein A1	Rpa1	3069	1594	**1.92**
1372059_at	similar to RIKEN cDNA 2610528E23	RGD1309437	1389	722	**1.92**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1370191_at	antizyme inhibitor 1	Azin1	2575	1338	**1.92**
1367613_at	peroxiredoxin 1	Prdx1	20194	10503	**1.92**
1371634_at	similar to RIKEN cDNA 1810020E01	RGD1305677	3422	1785	**1.92**
1377461_at	hypothetical protein LOC680485	LOC680485	2048	1068	**1.92**
1398385_at	similar to RIKEN cDNA 1500006O09 (predicted)	RGD1305475_predicted	4435	2314	**1.92**
1390285_at	similar to BC026645 protein (predicted)	RGD1560911_predicted	896	468	**1.92**
1388514_at	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	Ppm1g	5843	3050	**1.92**
1390124_at	similar to hypothetical protein FLJ38426 (predicted)	RGD1564603_predicted	1605	838	**1.91**
1371463_at	PHD finger protein 5A	Phf5a	5158	2695	**1.91**
1389184_at	similar to Ribonuclease P protein subunit p30 (RNaseP protein p30) (RNase P subunit 2)	LOC685332 /// LOC68777	1275	666	**1.91**
1380794_at		Aqr_predicted	1567	819	**1.91**
1386073_at	Similar to T-Brain-1	LOC311078	1420	743	**1.91**
1375645_at	similar to DNA segment, Chr 4, ERATO Doi 22, expressed (predicted)	RGD1560286_predicted	1464	766	**1.91**
1371612_at	tRNA splicing endonuclease 34 homolog	TSEN34	7330	3836	**1.91**
1372519_at	nucleoporin 93	Nup93	2823	1478	**1.91**
1374897_at	similar to THO complex subunit 4 (Tho4) (RNA and export factor binding protein 1) (REF1-I) (Ally of AML-1 and LEF-1) (Aly/REF)	LOC688305	7160	3748	**1.91**
1382923_at	synaptotagmin binding, cytoplasmic RNA interacting protein	Syncrip	3068	1606	**1.91**
1392522_at	similar to hypothetical protein D10Ertd438e (predicted)	RGD1307879_predicted	1765	924	**1.91**
1398730_at	similar to arsenate resistance protein 2	LOC686980	907	475	**1.91**
1367670_at	fumarate hydratase 1	Fh1	8998	4716	**1.91**
1388344_at	similar to RIKEN cDNA 1110005A23	RGD1305692	4173	2187	**1.91**
1367941_at	transcription factor A, mitochondrial	Tfam	3239	1699	**1.91**
1371739_at		Letm1	1251	656	**1.91**
1369948_at	nerve growth factor receptor (TNFRSF16) associated protein 1	Ngfrap1	6189	3246	**1.91**
1390681_at	Transcribed locus	---	1432	751	**1.91**
1372543_at	similar to RIKEN cDNA 2610029G23 (predicted)	RGD1562502_predicted	2944	1545	**1.91**
1398439_a_at	origin recognition complex, subunit 6-like (S. cerevisiae)	Orc6l	935	491	**1.90**
1373383_at	MTERF domain containing 1	Mterfd1	3989	2096	**1.90**
1390390_at	transcription factor 20	Tcf20	2022	1062	**1.90**
1389535_at	death associated protein 3	Dap3	1200	630	**1.90**
1377060_at	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	Mccc2	1148	604	**1.90**
1390048_at	serine/arginine repetitive matrix 2 (predicted)	Srrm2_predicted	4978	2618	**1.90**
1398980_at	suppressor of Ty 16 homolog (S. cerevisiae) (predicted)	Supt16h_predicted	5037	2651	**1.90**
1382521_at	glutaminase	Gls	2936	1545	**1.90**
1373260_at		Lims2_predicted	3377	1777	**1.90**
1379849_at	THO complex 3 (predicted)	Thoc3_predicted	1398	736	**1.90**
1398847_at	nudix (nucleoside diphosphate linked moiety X)-type motif 4	Nudt4	1372	723	**1.90**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1371848_at	similar to small nuclear ribonucleoprotein D3	LOC687711	4557	2404	**1.90**
1390489_at	Synaptotagmin binding, cytoplasmic RNA interacting protein	Syncrip	1532	808	**1.90**
1395152_at	RNA binding motif protein 13	Rbm13	1155	610	**1.89**
1376681_at	similar to RIKEN cDNA 1500003O22	RGD1308302	1181	624	**1.89**
1372732_at	inosine triphosphatase (nucleoside triphosphate pyrophosphatase)	Itpa	3047	1613	**1.89**
1370298_at	similar to hypothetical protein D11Ertd99e (predicted)	RGD1564337_predicted	3764	1993	**1.89**
1385066_a_at	Transcribed locus	---	844	447	**1.89**
1372932_at	novel nuclear protein 1	Nnp1	2591	1372	**1.89**
1372402_at	N-acetylneuraminic acid synthase (sialic acid synthase) (predicted)	Nans_predicted	1726	914	**1.89**
1387850_at	transmembrane protein with EGF-like and two follistatin-like domains 1	Tmeff1	1017	538	**1.89**
1367837_at	proteasome (prosome, macropain) subunit, alpha type 4	Psma4	3600	1907	**1.89**
1371955_at	mitochondrial ribosomal protein L35 (predicted)	Mrpl35_predicted	2042	1082	**1.89**
1370295_at	expressed in non-metastatic cells 1	Nme1	4089	2168	**1.89**
1371830_at	ubiquitin-like 1 (sentrin) activating enzyme E1A	Uble1a	2287	1212	**1.89**
1383577_at	stratifin (predicted)	Rabl3_predicted	2878	1527	**1.88**
1383945_at	uridine-cytidine kinase 2	Uck2	3077	1633	**1.88**
1377952_at	ADP-dependent glucokinase	Adpgk	923	490	**1.88**
1393388_at	Zinc finger, DHHC domain containing 3	Zdhhc3	978	519	**1.88**
1370230_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6	Atp5j	7327	3889	**1.88**
1368079_at	pyruvate dehydrogenase kinase, isoenzyme 1	Pdk1	836	444	**1.88**
1374289_at	RNA polymerase 1-1	Rpo1-1	4883	2592	**1.88**
1373741_at	pseudouridine synthase 1	Pus1	1143	607	**1.88**
1383380_at	ribonuclease P/MRP 38 subunit (human)	Rpp38	1873	995	**1.88**
1371914_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	Smarcb1	1320	701	**1.88**
1388158_at	HLA-B-associated transcript 1A	Bat1a	7855	4175	**1.88**
1372272_at	hypothetical protein LOC692032	LOC692032	828	440	**1.88**
1398884_at	prefoldin 5 (predicted)	Pfdn5_predicted	3151	1676	**1.88**
1393753_at	NudC domain containing 1 (predicted)	Nudcd1_predicted	874	465	**1.88**
1389844_at	FK506 binding protein 4	Fkbp4	3353	1784	**1.88**
1370931_at	X-ray repair complementing defective repair in Chinese hamster cells 5	Xrcc5	1072	570	**1.88**
1367676_at	high mobility group box 2 /// similar to High mobility group protein 2 (HMG-2) (predicted) /// similar to High mobility group protein 2 (HMG-2)	Hmgb2 /// LOC680704 ///	14981	7975	**1.88**
1387779_at	MYB binding protein (P160) 1a	Mybbp1a	4385	2334	**1.88**
1386963_at	thyroid hormone receptor interactor 10	Trip10	1863	993	**1.88**
1370316_at	hsp70-interacting protein	Hspbp1	1520	810	**1.88**
1383986_at	tropomyosin 3, gamma	Tpm3	8620	4602	**1.87**
1388576_at	eukaryotic translation initiation factor 3, subunit 9 (eta)	Eif3s9	5576	2977	**1.87**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1370915_s_at	deoxynucleotidyltransferase, terminal, interacting protein 1	Dnttip1	1588	848	**1.87**
1388884_at	similar to RIKEN cDNA 1810022C23	RGD1310224	907	485	**1.87**
1383962_at	Cd27 binding protein (Hindu God of destruction) (predicted)	Siva_predicted	1805	965	**1.87**
1385109_at	Josephin domain containing 3	Josd3	1873	1003	**1.87**
1369635_at	sorbitol dehydrogenase	Sord	1345	720	**1.87**
1389065_at	RNA binding motif protein 34	Rbm34	966	517	**1.87**
1371712_at	similar to snRNP core protein SMX5	LOC684148	3448	1850	**1.86**
1367463_at	prohibitin 2	Phb2	9292	4984	**1.86**
1398316_at	similar to LEYDIG CELL TUMOR 10 KD PROTEIN	LOC288913	1187	638	**1.86**
1376317_at	origin recognition complex, subunit 6-like (S. cerevisiae)	Orc6l	1877	1008	**1.86**
1367756_at	G elongation factor	Gfm	1091	586	**1.86**
1380185_at	similar to hypothetical protein FLJ22965 (predicted)	RGD1564541_predicted	1101	591	**1.86**
1377772_at	transmembrane protein with EGF-like and two follistatin-like domains 1	Tmeff1	2545	1368	**1.86**
1367493_at	similar to DNA segment, Chr 18, Wayne State University 98, expressed (predicted)	RGD1560212_predicted	4050	2178	**1.86**
1384588_at	similar to 4931433E08Rik protein (predicted)	RGD1565838_predicted	1183	637	**1.86**
1389063_at	exportin 6	Xpo6	3153	1697	**1.86**
1389338_at	hypothetical LOC293114 (predicted)	RGD1308371_predicted	2033	1094	**1.86**
1376488_at	similar to KIAA1582 protein (predicted)	RGD1310027_predicted	1115	601	**1.86**
1374632_at	jumonji domain containing 6	Jmjd6	1292	697	**1.85**
1376091_at	adenylosuccinate lyase (predicted)	Adsl_predicted	1756	947	**1.85**
1376005_at	similar to RIKEN cDNA 1190017O12 (predicted)	Kif1b	1112	600	**1.85**
1397551_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	Ddx27	2243	1210	**1.85**
1376986_at	methyltransferase-like 3	Mettl3	934	504	**1.85**
1388414_at	NADH dehydrogenase (ubiquinone) Fe-S protein 5b, 15kDa (NADH-coenzyme Q reductase)	Ndufs5b	5005	2702	**1.85**
1369002_at	sterol O-acyltransferase 1	Soat1	3883	2096	**1.85**
1398763_at	translocase of inner mitochondrial membrane 23 homolog (yeast)	Timm23	9569	5168	**1.85**
1390149_at	transforming, acidic coiled-coil containing protein 2	Tacc2	2573	1390	**1.85**
1379829_at	baculoviral IAP repeat-containing 6 (predicted)	Mycbp_predicted	1557	842	**1.85**
1383434_at	pyrroline-5-carboxylate reductase 1 (predicted)	Pycr1_predicted	1213	656	**1.85**
1379665_at	peptidylprolyl isomerase domain and WD repeat containing 1 (predicted)	Ppwd1_predicted	1624	879	**1.85**
1370258_at	basic leucine zipper and W2 domains 2	Bzw2	1357	734	**1.85**
1399098_at	glyoxylase 1	Glo1	5348	2894	**1.85**
1372028_at	similar to Protein CGI-117 (Protein HSPC111) (predicted)	RGD1305727_predicted	3823	2070	**1.85**
1388458_at	replication factor C (activator 1) 4 (predicted)	Rfc4_predicted	3500	1896	**1.85**
1398875_at	polymerase (RNA) III (DNA directed) polypeptide K	Polr3k	3064	1661	**1.84**
1373046_at	DAZ associated protein 1	Dazap1	2164	1174	**1.84**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1371352_at	high mobility group nucleosomal binding domain 2	Hmgn2	9747	5287	**1.84**
1379424_at	similar to DKFZP434B168 protein (predicted)	RGD1308014_predicted	844	458	**1.84**
1399077_at	similar to Metaxin 1, isoform 2	LOC295241	2439	1323	**1.84**
1377998_at	coproporphyrinogen oxidase	Cpox	1820	989	**1.84**
1371980_at	ATPase family, AAA domain containing 3A	Atad3a	817	444	**1.84**
1374527_at	enoyl Coenzyme A hydratase domain containing 2 (predicted)	Echdc2_predicted	3618	1970	**1.84**
1389286_at	glutaredoxin 5 homolog (S. cerevisiae) (predicted)	Glrx5_predicted	2649	1444	**1.84**
1398259_at	nucleoporin 155	Nup155	3676	2004	**1.83**
1372100_at	transmembrane protein 50A (predicted)	Tmem50a_predicted	1259	687	**1.83**
1372073_at	GATA zinc finger domain containing 2A	Gatad2a	2545	1388	**1.83**
1388797_at	U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) 2	U2af2	4844	2645	**1.83**
1391014_at	zinc finger, MYND domain containing 19	Zmynd19	956	522	**1.83**
1388701_at	myeloid/lymphoid or mixed lineage-leukemia translocation to 6 homolog (Drosophila) (predicted)	Mllt6_predicted	1441	788	**1.83**
1379417_at	midasin homolog (yeast)	Mdn1	1773	969	**1.83**
1393242_at	similar to chromosome 16 open reading frame 33; minus -99 protein (predicted)	RGD1310922_predicted	992	542	**1.83**
1393365_at	chromodomain helicase DNA binding protein 1 (predicted)	Chd1_predicted	990	541	**1.83**
1372218_at	WD repeat domain 12	Wdr12	3267	1786	**1.83**
1398860_at	neural precursor cell expressed, developmentally down-regulated gene 8	Nedd8	12592	6887	**1.83**
1379269_at	CD2-associated protein	Cd2ap	3030	1658	**1.83**
1372347_at	Transcribed locus	---	2149	1176	**1.83**
1376321_at	family with sequence similarity 38, member A (predicted)	Fam38a_predicted	1762	964	**1.83**
1369978_at	phosphoribosyl pyrophosphate synthetase-associated protein 2	Prpsap2	1468	804	**1.83**
1390035_at	similar to hypothetical protein MGC29875; novel putative protein similar to YIL091C yeast hypothetical 84 kD protein from SGA1-KTR7	LOC305076	1576	864	**1.82**
1387031_at	endoplasmic reticulum protein 29	Erp29	1045	573	**1.82**
1371437_at	SEC13-like 1 (S. cerevisiae)	Sec13l1	6260	3436	**1.82**
1384259_at	Rac GTPase-activating protein 1 (predicted)	Racgap1_predicted	1233	677	**1.82**
1394082_at	similar to T-Brain-1	LOC311078	6175	3392	**1.82**
1370919_at	heterogeneous nuclear ribonucleoprotein M	Hnrpm	3856	2120	**1.82**
1371486_at	U1 small nuclear ribonucleoprotein 1C (predicted) /// similar to U1 small nuclear ribonucleoprotein C (U1 snRNP protein C) (U1C protein) (U1-C)	LOC682020 /// LOC68527	6284	3458	**1.82**
1381217_at	similar to CGI-09 protein (predicted)	RGD1308877_predicted	1081	595	**1.82**
1377705_at	---	---	1423	784	**1.82**
1384804_at	DNA methyltransferase 3A	Dnmt3a	1234	680	**1.81**
1384087_at	---	---	1196	659	**1.81**
1374036_at	minichromosome maintenance deficient 2 mitotin (S. cerevisiae) (predicted)	Mcm2_predicted	2558	1410	**1.81**
1372698_at	similar to CG2662-PA (predicted)	RGD1305094_predicted	814	449	**1.81**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1388478_at	transducin (beta)-like 1 X-linked (predicted)	Tbl1x_predicted	4133	2280	**1.81**
1388517_at	mitochondrial ribosomal protein L40	Mrpl40	1047	578	**1.81**
1387019_at	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e	Atp5i	1777	982	**1.81**
1372260_at	leucine zipper domain protein	MGC116147	806	445	**1.81**
1373387_at	similar to CG33714-PB, isoform B	LOC688717	4822	2665	**1.81**
1390419_a_at	tumor suppressor candidate 3	Tusc3	2021	1117	**1.81**
1384330_at	similar to hypothetical protein FLJ20546	LOC303067	1674	925	**1.81**
1384136_at	similar to mKIAA0704 protein (predicted)	RGD1564287_predicted	1561	863	**1.81**
1374631_at	oligonucleotide/oligosaccharide-binding fold containing 2B	Obfc2b	1186	656	**1.81**
1388327_at	similar to DNA segment, Chr 10, ERATO Doi 214, expressed (predicted)	RGD1309529_predicted	6720	3725	**1.80**
1367911_at	isocitrate dehydrogenase 3 (NAD+) alpha	Idh3a	1760	976	**1.80**
1371551_at	Tnf receptor associated factor 4 (predicted)	Traf4_predicted	808	448	**1.80**
1376849_at	ubiquitin specific protease 48	Usp48	951	528	**1.80**
1370378_at	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	Atp5a1	10505	5835	**1.80**
1383250_at	UTP14, U3 small nucleolar ribonucleoprotein, homolog A (yeast)	Utp14a	1254	697	**1.80**
1399034_at	pecanex homolog (Drosophila)	Pcnx	1138	633	**1.80**
1389101_at	ubiquitin-like 1 (sentrin) activating enzyme E1B	Ccnc	1042	580	**1.80**
1368136_at	thymopoietin	Tmpo	1389	773	**1.80**
1393158_at	similar to 2610528M18Rik protein	LOC363198	932	519	**1.80**
1387113_at	C-terminal binding protein 2	Ctbp2	3896	2170	**1.80**
1377480_at	similar to RIKEN cDNA D030060M11	RGD1308076	1359	757	**1.80**
1371367_at	TAR DNA binding protein	Tardbp	6252	3483	**1.80**
1368049_at	t-complex protein 1	Tcp1	16713	9320	**1.79**
1377619_at	WD repeat domain 36 (predicted)	Wdr36_predicted	1216	678	**1.79**
1376298_at	UPF3 regulator of nonsense transcripts homolog B (yeast) (predicted)	Upf3b_predicted	902	503	**1.79**
1398963_at	N-myristoyltransferase 2	Taf10_predicted	2465	1377	**1.79**
1374467_at	TNF receptor-associated protein 1	Trap1	2751	1537	**1.79**
1376723_a_at	Transcribed locus	---	2268	1268	**1.79**
1378501_at	similar to gem (nuclear organelle) associated protein 5	LOC686616	838	469	**1.79**
1389519_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	Psmd8	5155	2884	**1.79**
1398301_at	ribosomal protein L36 /// similar to ribosomal protein L36 (predicted) /// hypothetical LOC364105 /// hypothetical gene supported by NM_022504 (predicted) /// similar to ribosomal protein L36	LOC364105 /// LOC68902	1524	853	**1.79**
1378346_at	Transcribed locus	---	1446	809	**1.79**
1383491_at	interferon stimulated exonuclease gene 20-like 1 (predicted)	Isg20l1_predicted	973	545	**1.79**
1385620_at	heat shock 105kDa/110kDa protein 1	Hsph1	1892	1060	**1.79**
1382166_at	signal recognition particle receptor, B subunit	Srprb	1182	662	**1.79**
1375516_at	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2	Ndufc2	4647	2604	**1.78**
1373319_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 1	Ddx1	10461	5865	**1.78**
1369929_at	prosaposin	Psap	12821	7191	**1.78**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1377594_at	Src homology 2 domain-containing transforming protein C1	Shc1	2924	1641	**1.78**
1367480_at	similar to DEAD (Asp-Glu-Ala-Asp) box polypeptide 48	rCG_33565	7844	4406	**1.78**
1383961_a_at	DnaJ (Hsp40) homolog, subfamily C, member 11 (predicted)	Dnajc11_predicted	1073	603	**1.78**
1372207_at	bromodomain containing 8	Brd8	1191	669	**1.78**
1377137_at	tyrosyl-DNA phosphodiesterase 1	Tdp1	800	450	**1.78**
1375641_at	actin related protein 2/3 complex, subunit 5-like /// similar to actin related protein 2/3 complex, subunit 5-like (predicted)	Arpc5l /// RGD1560362_p	3800	2139	**1.78**
1387790_at	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	Paics	10439	5876	**1.78**
1368083_at	cyclin H	Ccnh	3505	1973	**1.78**
1388199_at	tumor-associated calcium signal transducer 1	Tacstd1	3655	2058	**1.78**
1387077_at	cAMP-regulated phosphoprotein 19	Arpp19	3272	1843	**1.78**
1388364_at	NADH dehydrogenase (ubiquinone) Fe-S protein 3 (predicted)	Ndufs3_predicted	3928	2213	**1.78**
1370062_at	HIG1 domain family, member 1A	Higd1a	2949	1662	**1.77**
1398784_at	complement component 1, q subcomponent binding protein	C1qbp	13939	7856	**1.77**
1388837_at	Solute carrier family 44, member 2 (predicted)	Slc44a2_predicted	1030	581	**1.77**
1371971_at	---	---	2768	1560	**1.77**
1392937_at	cyclin I (predicted)	Ccni_predicted	1182	666	**1.77**
1368233_at	general transcription factor IIF, polypeptide 2	Gtf2f2	1462	825	**1.77**
1377577_at	guanine monphosphate synthetase	Gmps	3867	2184	**1.77**
1378357_at	Transcribed locus	---	1521	859	**1.77**
1373568_at	Polycomb group ring finger 4 (predicted)	Pcgf4_predicted	1878	1061	**1.77**
1367557_s_at	glyceraldehyde-3-phosphate dehydrogenase /// similar to glyceraldehyde-3-phosphate dehydrogenase /// similar to glyceraldehyde-3-phosphate dehydrogenase (predicted)	Gapdh /// LOC366623 /// L	29358	16591	**1.77**
1392979_at	calcyclin binding protein	Cacybp	4801	2716	**1.77**
1367808_at	translocase of inner mitochondrial membrane 8 homolog b (yeast)	Timm8b	5729	3242	**1.77**
1374712_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11 (predicted)	Psmd11_predicted	7964	4506	**1.77**
1373609_at	similar to Mitochondrial 28S ribosomal protein S21 (S21mt) (MRP-S21)	LOC689432	2737	1549	**1.77**
1377827_at	serum response factor binding protein 1	Srfbp1	2158	1222	**1.77**
1392425_x_at	LUC7-like (S. cerevisiae)	Luc7l	1020	577	**1.77**
1388898_at	heat shock 105kDa/110kDa protein 1	Hsph1	4392	2491	**1.76**
1371785_at	tumor necrosis factor receptor superfamily, member 12a	Tnfrsf12a	2101	1192	**1.76**
1388132_at	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	Sfpq	3709	2104	**1.76**
1382093_at	Similar to DKFZP434B168 protein (predicted)	RGD1308014_predicted	803	456	**1.76**
1388742_at	---	---	984	559	**1.76**
1390178_at	signal recognition particle receptor, B subunit	Srprb	2254	1280	**1.76**
1379043_at	transmembrane protein 8 (five membrane-spanning domains) (predicted)	Grtp1	1335	758	**1.76**

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Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1373649_at	similar to COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase	LOC687381 /// LOC69185	933	530	**1.76**
1388695_at	serine hydroxymethyltransferase 2 (mitochondrial)	Shmt2	5493	3122	**1.76**
1367467_at	similar to nitrogen fixation cluster-like (predicted)	RGD1309562_predicted	2767	1573	**1.76**
1374299_at	DEAH (Asp-Glu-Ala-His) box polypeptide 9 (predicted)	Dhx9_predicted	2862	1627	**1.76**
1371371_at	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa	Ndufb4	4663	2652	**1.76**
1368835_at	signal transducer and activator of transcription 1	Stat1	908	516	**1.76**
1371348_at	proteasome (prosome, macropain) subunit, beta type 5	Psmb5	9441	5370	**1.76**
1367695_at	quinoid dihydropteridine reductase	Qdpr	2291	1304	**1.76**
1371498_at	JTV1	MGC125271	4333	2466	**1.76**
1385458_a_at	similar to C11orf17 protein (predicted)	RGD1306959_predicted	1191	678	**1.76**
1371395_at	chromobox homolog 3 (HP1 gamma homolog, Drosophila)	Cbx3	9180	5227	**1.76**
1383466_at	Transcribed locus	---	1042	593	**1.76**
1372596_at	similar to RIKEN cDNA 1110007L15 (predicted)	RGD1311660_predicted	1545	880	**1.76**
1388355_at	RNA binding motif protein 17	Rbm17	7694	4384	**1.75**
1376816_at	mitochondrial ribosomal protein S28	Brca1	865	493	**1.75**
1369971_a_at	heterogeneous nuclear ribonucleoprotein D	Hnrpd	5627	3208	**1.75**
1371478_at	similar to RIKEN cDNA 1110008F13	RGD1307752	8671	4943	**1.75**
1373850_at	sphingomyelin phosphodiesterase, acid-like 3B	Smpdl3b	2601	1483	**1.75**
1375546_at	cyclin-dependent kinase inhibitor 3 (predicted)	Fzr1_predicted	1162	663	**1.75**
1388495_at	Transcribed locus	---	1075	613	**1.75**
1373445_at	nucleolar protein 8 (predicted)	Nol8_predicted	1389	793	**1.75**
1372261_at	Transcribed locus	---	1149	657	**1.75**
1388640_at	similar to Ubiquitin-like protein SMT3A precursor (Ubiquitin-related protein SUMO-2)	LOC499417	7946	4543	**1.75**
1398756_at	nucleophosmin 1 /// similar to Nucleophosmin (NPM) (Nucleolar phosphoprotein B23) (Numatrin) (Nucleolar protein NO38)	LOC300303 /// Npm1	11610	6637	**1.75**
1372550_at		Kpnb1	5979	3422	**1.75**
1389252_at	Transcribed locus	---	1427	817	**1.75**
1371037_at	protein S (alpha)	Pros1	5533	3168	**1.75**
1374976_a_at	Sterol O-acyltransferase 1	Soat1	7519	4306	**1.75**
1387860_at	calpain 2	Capn2	5477	3137	**1.75**
1389387_at	similar to Proteasome inhibitor PI31 subunit	LOC682071 /// LOC68985	2072	1186	**1.75**
1390558_at	staufen, RNA binding protein, homolog 2 (Drosophila)	Stau2	1409	807	**1.75**
1371638_at	ring finger protein 7 (predicted)	Rnf7_predicted	4222	2421	**1.74**
1370340_x_at	tropomyosin 3, gamma	Tpm3	7023	4027	**1.74**
1371845_at	processing of precursor 4, ribonuclease P/MRP family, (S. cerevisiae)	Pop4	1590	912	**1.74**
1367933_at	S-adenosylmethionine decarboxylase 1	Amd1	2331	1341	**1.74**

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Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1368507_at	proteasome (prosome, macropain) subunit, alpha type 3 /// proteasome subunit alpha type 3-like	Psma3 /// Psma3l	13252	7626	**1.74**
1383107_at	small nuclear ribonucleoprotein D1 (predicted)	Snrpd1_predicted	3122	1797	**1.74**
1371398_at	similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2	LOC684567 /// LOC69044	9891	5694	**1.74**
1374945_at	GCD14/PCMT domain containing protein RGD1359191	RGD1359191	1273	734	**1.73**
1384913_at	Transcribed locus	---	1293	746	**1.73**
1371923_at	acyltransferase like 2 (predicted)	Aytl2_predicted	1515	874	**1.73**
1388794_at	RNA binding motif protein, X chromosome retrogene (predicted)	Rbmxrt_predicted	4676	2700	**1.73**
1375368_at	similar to RIKEN cDNA 1700012G19 gene (predicted)	RGD1307773_predicted	1809	1045	**1.73**
1376648_at	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	Mycn	4165	2406	**1.73**
1368195_at	Hspb associated protein 1	Hspbap1	2313	1336	**1.73**
1370365_at	glutathione synthetase	Gss	1296	750	**1.73**
1388913_at	phosphatidic acid phosphatase type 2c	Ppap2c	2396	1387	**1.73**
1371546_at	similar to TR4 orphan receptor associated protein TRA16	LOC361128	1575	912	**1.73**
1373169_at	1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon) (predicted)	Agpat5_predicted	1498	867	**1.73**
1373620_at	similar to hypothetical protein D10Ertd438e (predicted)	RGD1307879_predicted	3214	1862	**1.73**
1376598_at	PAX interacting (with transcription-activation domain) protein 1 (predicted)	Paxip1_predicted	919	533	**1.73**
1373758_at	flavin adenine dinucleotide synthetase	Flad1	929	538	**1.73**
1383643_at	similar to UPF0197 protein C11orf10 homolog (predicted)	RGD1560328_predicted	3436	1993	**1.72**
1373768_at	BMS1-like, ribosome assembly protein (yeast)	Bms1l	1950	1132	**1.72**
1371576_at	mitochondrial ribosomal protein S36 (predicted)	Mrps36_predicted	2690	1563	**1.72**
1373583_at	Transcribed locus	---	1989	1155	**1.72**
1388136_at	translocase of inner mitochondrial membrane 9 homolog (yeast)	Timm9	2418	1406	**1.72**
1388489_at	similar to NADH-ubiquinone oxidoreductase B9 subunit (Complex I-B9) (CI-B9)	LOC683547 /// LOC69100	2561	1490	**1.72**
1399002_at	mitochondrial ribosomal protein S17 (predicted)	Mrps17_predicted	4972	2894	**1.72**
1375967_a_at	dual specificity phosphatase 22 (predicted)	Dusp22_predicted	1275	742	**1.72**
1367709_at	CD63 antigen	Cd63	12715	7409	**1.72**
1371321_at	similar to Cytochrome c oxidase polypeptide VIb (Cytochrome c oxidase subunit AED) (predicted)	RGD1565270_predicted	8197	4777	**1.72**
1389343_at	similar to Hypothetical UPF0327 protein (predicted)	RGD1560187_predicted	7498	4372	**1.72**
1380373_at	guanine monphosphate synthetase	Gmps	2048	1194	**1.71**
1388555_at	thioredoxin-like 5 (predicted)	Txnl5_predicted	4886	2851	**1.71**
1398432_at	similar to Ankyrin repeat domain protein 28 (predicted)	RGD1559931_predicted	1704	995	**1.71**
1377967_at	retroviral integration site 2 (predicted)	Ris2_predicted	3711	2167	**1.71**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1388362_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13 (predicted)	Ndufa13_predicted	4555	2662	**1.71**
1383502_at	similar to mKIAA0259 protein (predicted)	RGD1562949_predicted	1585	927	**1.71**
1392131_at	---	---	834	488	**1.71**
1387805_at	BCL2/adenovirus E1B 19 kDa-interacting protein 3	Bnip3	1500	877	**1.71**
1374886_at	BCS1-like (yeast)	Bcs1l	1203	704	**1.71**
1371641_at	chaperonin subunit 7 (eta) (predicted)	Cct7_predicted	10540	6170	**1.71**
1388601_at	activator of basal transcription 1	Abt1	2196	1286	**1.71**
1392938_s_at	similar to C11orf17 protein (predicted)	RGD1306959_predicted	1661	972	**1.71**
1372364_a_at	similar to N-terminal asparagine amidohydrolase	RGD1305160	2524	1478	**1.71**
1375554_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 47	Ddx47	2677	1568	**1.71**
1388716_at	euchromatic histone lysine N-methyltransferase 2	Ehmt2	1915	1123	**1.71**
1388931_at	mitochondrial ribosomal protein L13	Mrpl13	2622	1537	**1.71**
1371617_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13 (predicted)	Psmd13_predicted	2183	1280	**1.71**
1367506_at	mitochondrial ribosomal protein L11	mrpl11	2118	1242	**1.71**
1373941_at	similar to YEATS domain containing 2 (predicted)	RGD1566176_predicted	1286	755	**1.70**
1371239_s_at	tropomyosin 3, gamma	Tpm3	7750	4549	**1.70**
1373182_at	claudin 12	Cldn12	1086	637	**1.70**
1372466_at		Tgfbr2	966	568	**1.70**
1367671_at	proliferating cell nuclear antigen	Pcna	16664	9793	**1.70**
1387055_at	amyloid beta precursor protein binding protein 1	Appbp1	2045	1202	**1.70**
1374793_at	WD repeat domain 3 (predicted)	Wdr3_predicted	1943	1144	**1.70**
1369946_at	PDRP protein	Pdrp	948	558	**1.70**
1367471_at	similar to DNA-directed RNA polymerase II 23 kDa polypeptide (RPB25) (RPB5) (RPABC1)	LOC687055 /// LOC69096	2346	1382	**1.70**
1382287_at	ribonucleic acid binding protein S1	Rnps1	3864	2276	**1.70**
1398890_at	similar to 2310044H10Rik protein	MGC93975	943	555	**1.70**
1395595_at	Mki67 (FHA domain) interacting nucleolar phosphoprotein	Mki67ip	3784	2230	**1.70**
1371451_at	ribonuclease H2, large subunit	Rnaseh2a	1320	778	**1.70**
1390788_a_at	WD repeat domain 36 (predicted)	Wdr36_predicted	2722	1605	**1.70**
1373731_at	similar to phosphoseryl-tRNA kinase (predicted)	RGD1564300_predicted	806	476	**1.69**
1370304_at	translocator of inner mitochondrial membrane 17a	Timm17a	8435	4981	**1.69**
1390717_at	similar to chromosome 20 open reading frame 155	RGD1311037	1790	1058	**1.69**
1372214_at	mitochondrial ribosomal protein S33 (predicted)	Mrps33_predicted	2525	1493	**1.69**
1388645_at	similar to RIKEN cDNA 2810409H07	RGD1307982	13361	7904	**1.69**
1391577_at	phosphoglycerate mutase family member 5	Pgam5	2597	1537	**1.69**
1375460_at	similar to hypothetical protein FLJ30656 (predicted)	RGD1309441_predicted	909	538	**1.69**
1372312_at	LTV1 homolog (S. cerevisiae)	Ltv1	1745	1034	**1.69**
1367819_at	glutamate oxaloacetate transaminase 2, mitochondrial	Got2	10108	5986	**1.69**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1372721_at	---	---	4660	2763	**1.69**
1373103_at	metastasis-associated gene family, member 2	Mta2	1292	766	**1.69**
1398772_at	NSFL1 (p97) cofactor (p47)	Nsfl1c	5656	3355	**1.69**
1380030_at	zinc finger protein 593 (predicted)	Znf593_predicted	914	543	**1.68**
1371428_at	similar to Protein transport protein SEC61 gamma subunit	LOC689134	12115	7194	**1.68**
1377021_at	similar to CGI-09 protein (predicted)	RGD1308877_predicted	1106	657	**1.68**
1372506_at	proteaseome (prosome, macropain) 28 subunit, 3	Psme3	6151	3658	**1.68**
1392629_a_at	zinc finger CCHC-type and RNA binding motif 1	Zcrb1	1161	690	**1.68**
1374443_at	immature colon carcinoma transcript 1 (predicted)	Ict1_predicted	1898	1129	**1.68**
1388736_at	coiled-coil domain containing 43	Ccdc43	1017	605	**1.68**
1382782_at	mitochondrial ribosomal protein S2 (predicted)	Mrps2_predicted	870	517	**1.68**
1383175_a_at	similar to C11orf17 protein (predicted)	RGD1306959_predicted	1025	610	**1.68**
1367795_at	interferon-related developmental regulator 1	Ifrd1	4735	2820	**1.68**
1384295_at	heat shock 70kDa protein 14	Hspa14	4370	2602	**1.68**
1372642_at	Transcribed locus	---	3545	2111	**1.68**
1377506_at	longevity assurance homolog 1 (S. cerevisiae) (predicted) /// growth differentiation factor 1 (predicted)	Gdf1_predicted /// Lass1_	2750	1638	**1.68**
1367578_at	peroxiredoxin 2	Prdx2	13440	8008	**1.68**
1372085_at	ADP-ribosylation factor-like 6 interacting protein 2	Arl6ip2	3330	1985	**1.68**
1372460_at	SET translocation (predicted) /// similar to SET protein (Phosphatase 2A inhibitor I2PP2A) (I-2PP2A) (Template-activating factor I) (TAF-I) (Liver regeneration-related protein LRRGR00002)	LOC502130 /// LOC68189	10867	6481	**1.68**
1382114_at	tousled-like kinase 1 (predicted)	Tlk1_predicted	1673	998	**1.68**
1377889_at	similar to Hypothetical protein KIAA0539 (predicted)	RGD1304621_predicted	1129	674	**1.68**
1372827_at	peptidylprolyl isomerase D (cyclophilin D)	Ppid	9716	5802	**1.67**
1394136_at	PAP associated domain containing 1 (predicted)	Papd1_predicted	1567	936	**1.67**
1388529_at	RNA terminal phosphate cyclase domain 1	Rtcd1	5030	3005	**1.67**
1393014_at	similar to hypothetical protein FLJ30656 (predicted)	RGD1309441_predicted	838	501	**1.67**
1378568_a_at	similar to chromosome 14 open reading frame 138 (predicted)	RGD1306228_predicted	2412	1441	**1.67**
1389608_at	ATP-binding cassette, sub-family F (GCN20), member 2 (predicted)	Abcf2_predicted	1521	909	**1.67**
1388134_at	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	Eef1d	10171	6084	**1.67**
1370861_at	cytochrome c oxidase, subunit VIa, polypeptide 1	Cox6a1	9588	5737	**1.67**
1385806_at	Retinitis pigmentosa 9 homolog (human) (predicted)	Rp9h_predicted	1087	650	**1.67**
1382059_at	F-box protein 30	Fbxo30	1624	972	**1.67**
1395044_at	hypothetical protein LOC679298 /// hypothetical protein LOC688832	LOC679298 /// LOC68883	1649	988	**1.67**
1382567_at	phosphatidylserine receptor	Nmt2	2432	1456	**1.67**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1386711_a_at	DnaJ (Hsp40) homolog, subfamily C, member 11 (predicted)	Dnajc11_predicted	931	558	**1.67**
1389336_at	processing of precursor 5, ribonuclease P/MRP family (S. cerevisiae) (predicted)	Pop5_predicted	911	546	**1.67**
1367907_a_at	clathrin, light polypeptide (Lcb)	Cltb	2336	1401	**1.67**
1388180_at	phosphorylated adaptor for RNA export	Phax	2587	1551	**1.67**
1398899_at	polymerase (RNA) II (DNA directed) polypeptide C	Polr2c	3298	1979	**1.67**
1373143_at	similar to hypothetical protein FLJ10652	RGD1309621	4037	2422	**1.67**
1390257_at	vesicle-associated membrane protein, associated protein B and C	Vapb	2236	1342	**1.67**
1372311_at	---	---	2729	1639	**1.67**
1371714_at	Serine/arginine-rich protein specific kinase 1	Srpk1	800	480	**1.67**
1399029_at	ubiquitin specific protease 48	Usp48	1480	889	**1.66**
1377202_at	SMC6 structural maintenance of chromosomes 6-like 1 (yeast) (predicted)	Smc6l1_predicted	1076	647	**1.66**
1384005_at	down-regulator of transcription 1	Dr1	805	484	**1.66**
1389510_at	Ly1 antibody reactive clone	Lyar	5073	3048	**1.66**
1373155_at	mitochondrial ribosomal protein L46	Mrpl46	1303	783	**1.66**
1374711_at	cleavage and polyadenylation specificity factor 3	Cpsf3	3684	2215	**1.66**
1376599_at	ATPase family, AAA domain containing 2 (predicted)	Atad2_predicted	877	527	**1.66**
1398786_at	proteasome (prosome, macropain) subunit, beta type 2	Psmb2	7293	4388	**1.66**
1390019_at	H3 histone, family 3B	H3f3b	10958	6595	**1.66**
1371403_at	chaperonin subunit 3 (gamma)	Cct3	11933	7186	**1.66**
1379262_at	similar to acyl-CoA thioesterase	LOC302640	1381	832	**1.66**
1392473_at	family with sequence similarity 29, member A (predicted)	Nmt2	4842	2917	**1.66**
1390039_at	zinc finger, DHHC domain containing 3	Zdhhc3	921	555	**1.66**
1372634_at	ADP-ribosylhydrolase like 2 (predicted)	Adprhl2_predicted	1174	708	**1.66**
1383703_at	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	Fusip1	1437	867	**1.66**
1387773_at	cytochrome c, somatic	Cycs	8307	5012	**1.66**
1373552_at	LAS1-like (S. cerevisiae) (predicted)	Las1l_predicted	1074	648	**1.66**
1372628_at		Ap4s1_predicted	3740	2257	**1.66**
1370356_at	RNA binding motif protein 10	Rbm10	1356	819	**1.66**
1386894_at	heat shock protein 1 (chaperonin)	Hspd1	23644	14287	**1.65**
1368031_at	nucleolar and coiled-body phosphoprotein 1	Nolc1	1634	987	**1.65**
1368230_a_at	nuclear protein E3-3	RGD708545	1262	763	**1.65**
1383073_at	ubiquitin specific protease 14	Usp14	8774	5303	**1.65**
1382052_at		Fusip1	1385	837	**1.65**
1373389_at	acyl-Coenzyme A dehydrogenase family, member 9	Acad9	1791	1083	**1.65**
1389646_at	CDC23 (cell division cycle 23, yeast, homolog)	Cdc23	1322	799	**1.65**
1382131_at	SKB1 homolog (S. pombe) (predicted)	Arhgap5	1025	620	**1.65**
1369636_at	sorbitol dehydrogenase	Sord	1079	653	**1.65**
1372517_at	peptidylprolyl isomerase (cyclophilin)-like 1	Ppil1	1646	996	**1.65**
1392464_at	RAE1 RNA export 1 homolog (S. pombe)	Rae1	3367	2038	**1.65**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1394371_at	nuclear fragile X mental retardation protein interacting protein 1	Nufip1	853	517	**1.65**
1383752_at	nucleolar protein 1 (predicted)	Nol1_predicted	1947	1179	**1.65**
1382043_at	unc-93 homolog B1 (C. elegans)	Unc93b1	1441	872	**1.65**
1389577_at	cirrhosis, autosomal recessive 1A (human)	Cirh1a	5281	3199	**1.65**
1379810_at	small inducible cytokine subfamily E, member 1	Scye1	3145	1905	**1.65**
1373294_at	COMM domain containing 8 (predicted)	Commd8_predicted	3705	2245	**1.65**
1374804_at	guanine nucleotide binding protein-like 2 (nucleolar)	Gnl2	1655	1002	**1.65**
1389760_at	polycomb group ring finger 6	Pcgf6	3357	2034	**1.65**
1372867_at	RNA (guanine-7-) methyltransferase	Rnmt	1020	618	**1.65**
1371958_at	poly(A) binding protein, nuclear 1	Pabpn1	1169	708	**1.65**
1373633_at	Transcribed locus	---	857	520	**1.65**
1392829_at	similar to cleavage stimulation factor, 3 pre-RNA subunit 2	LOC683927	882	535	**1.65**
1373653_at	PAP associated domain containing 1 (predicted)	Papd1_predicted	1928	1170	**1.65**
1388642_at	etoposide induced 2.4 mRNA	Ei24	1967	1193	**1.65**
1371790_at	mitochondrial ribosomal protein L45 (predicted)	Mrpl45_predicted	2476	1503	**1.65**
1389222_at	similar to Serum amyloid A-3 protein precursor	LOC687992 /// LOC69114	1594	968	**1.65**
1393042_at	Ubiquitin specific protease 7 (herpes virus-associated)	Usp7	2685	1631	**1.65**
1384390_at	similar to jumonji protein	LOC681740	1660	1009	**1.65**
1388793_at	phosphatidylinositol glycan, class Q	Pigq	1431	869	**1.65**
1382009_at	chromodomain helicase DNA binding protein 1 (predicted)	Chd1_predicted	2226	1352	**1.65**
1372942_at	exosome component 5 (predicted)	Exosc5_predicted	820	499	**1.64**
1398762_at	syndecan binding protein	Sdcbp	12589	7658	**1.64**
1374227_at	similar to hypothetical protein FLJ20272 (predicted)	RGD1305264_predicted	1775	1080	**1.64**
1367931_a_at	polypyrimidine tract binding protein 1	Ptbp1	7160	4359	**1.64**
1389291_at	coiled-coil-helix-coiled-coil-helix domain containing 3 (predicted)	Chchd3_predicted	2962	1804	**1.64**
1375439_at	WD repeat domain 18	Wdr18	1094	667	**1.64**
1388366_at	mitochondrial ribosomal protein L4 (predicted)	Mrpl4_predicted	2008	1224	**1.64**
1371888_at	mitochondrial ribosomal protein L24	mrpl24	2255	1375	**1.64**
1379028_at	sperm associated antigen 7 (predicted)	Spag7_predicted	1209	737	**1.64**
1385471_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	Ddx27	916	558	**1.64**
1370844_at	heterogeneous nuclear ribonucleoprotein F	Hnrpf	8182	4992	**1.64**
1374326_at	peter pan homolog (Drosophila)	Ppan	1835	1120	**1.64**
1389215_at	selenophosphate synthetase 1	Sephs1	1411	861	**1.64**
1399020_at	similar to family with sequence similarity 40, member A	LOC362012	812	496	**1.64**
1370939_at	acyl-CoA synthetase long-chain family member 1	Acsl1	849	518	**1.64**
1373868_at		Bclaf1	1800	1100	**1.64**
1383271_at	coiled-coil domain containing 59 (predicted)	Ccdc59_predicted	859	525	**1.64**
1379320_at	Small nuclear ribonucleoprotein D1 (predicted)	Snrpd1_predicted	1754	1072	**1.64**
1372575_at	WW domain binding protein 11	Wbp11	2360	1442	**1.64**
1372017_at	diablo homolog (Drosophila)	Diablo	1706	1044	**1.63**

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Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1389237_at	zinc finger, C3HC-type 1 (predicted)	Zc3hc1_predicted	1083	662	**1.63**
1390333_at	protein phosphatase 4, catalytic subunit	Ppp4c	2055	1257	**1.63**
1374279_at	small inducible cytokine subfamily E, member 1	Scye1	3123	1912	**1.63**
1382661_at	nucleoporin 160 (predicted)	Nup160_predicted	3595	2204	**1.63**
1395328_at	similar to hypothetical protein FLJ20003 (predicted)	RGD1309025_predicted	966	592	**1.63**
1375906_at	hypothetical protein LOC683034	LOC683034	830	509	**1.63**
1396187_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21a	Ddx21a	4770	2926	**1.63**
1393033_at	tyrosyl-tRNA synthetase 2 (mitochondrial)	Yars2	1579	969	**1.63**
1383160_at	cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1 (predicted)	Chordc1_predicted	4313	2648	**1.63**
1385765_at	similar to lin-9 homolog (C. elegans)	LOC360888	1705	1047	**1.63**
1388976_at	similar to BolA domain-containing protein like (11.4 kD) (1P25) (predicted)	RGD1305975_predicted	1095	673	**1.63**
1374082_at	similar to PRO1853 homolog	RGD1311578	1293	795	**1.63**
1373913_at	polyribonucleotide nucleotidyltransferase 1	Pnpt1	2591	1593	**1.63**
1388582_at	proteaseome (prosome, macropain) 28 subunit, 3	Psme3	4396	2703	**1.63**
1367678_at	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	Sdha	5088	3132	**1.62**
1371699_at	Transcribed locus, moderately similar to NP_005132.2 fibrinogen, beta chain preproprotein [Homo sapiens]	---	3415	2103	**1.62**
1399130_at	similar to RIKEN cDNA 2610022G08	LOC502782	821	506	**1.62**
1372918_at		RGD1566291_predicted	877	541	**1.62**
1382921_at	SDA1 domain containing 1	Sdad1	1256	775	**1.62**
1374927_at	Transcribed locus	---	1017	627	**1.62**
1372496_at	similar to hypothetical protein MGC19604 (predicted)	RGD1561264_predicted	1660	1024	**1.62**
1371505_at	heterogeneous nuclear ribonucleoprotein C	Hnrpc	10716	6614	**1.62**
1370243_a_at	prothymosin alpha	Ptma	23845	14726	**1.62**
1385073_at	---	---	5174	3195	**1.62**
1368573_at	karyopherin (importin) beta 1	Kpnb1	2756	1703	**1.62**
1371789_at	similar to Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial precursor (Endopeptidase Clp)	LOC301117	1922	1188	**1.62**
1383028_at	similar to CDV-3B	LOC315970	1038	641	**1.62**
1387899_at	collapsin response mediator protein 1	Crmp1	800	494	**1.62**
1395455_at	heterogeneous nuclear ribonucleoprotein H3 (2H9) (predicted)	Hnrph3_predicted	1531	947	**1.62**
1388368_at	mediator of RNA polymerase II transcription, subunit 28 homolog (yeast) (predicted)	Med28_predicted	3200	1979	**1.62**
1370253_at	ribosomal protein L22	Rpl22	22344	13818	**1.62**
1390040_at	brain and reproductive organ-expressed protein	Bre	1461	904	**1.62**
1372151_at	transcription elongation regulator 1 (CA150) (predicted)	Tcerg1_predicted	3745	2318	**1.62**
1372077_at	serine/threonine kinase receptor associated protein	Strap	6724	4161	**1.62**
1382051_at	similar to J-type co-chaperone HSC20 (predicted)	RGD1311005_predicted	1132	700	**1.62**
1383625_a_at	similar to zinc finger protein (predicted)	RGD1562173_predicted	3346	2071	**1.62**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1389108_at	similar to tubulin-specific chaperone d	LOC363309	1106	686	**1.61**
1383051_at	Synaptotagmin binding, cytoplasmic RNA interacting protein	Syncrip	3142	1948	**1.61**
1371311_at	succinate dehydrogenase complex, subunit C, integral membrane protein	Sdhc	5857	3632	**1.61**
1374186_at	Transcribed locus, weakly similar to XP_001475219.1 PREDICTED: similar to Lrrc47 protein [Mus musculus]	---	1519	942	**1.61**
1371586_at	mitochondrial ribosomal protein L48 (predicted)	Mrpl48_predicted	1160	719	**1.61**
1388182_at	DNA primase, p49 subunit	Prim1	10518	6524	**1.61**
1391201_at	WD repeat and HMG-box DNA binding protein 1 (predicted)	Wdhd1_predicted	1144	710	**1.61**
1372461_at	SET translocation (predicted)	Set_predicted	16307	10118	**1.61**
1388833_at	polymerase (DNA directed), epsilon 3 (p17 subunit)	Pole3	1175	729	**1.61**
1376811_a_at	cleavage and polyadenylation specific factor 6, 68kDa (predicted)	Cpsf6_predicted	2279	1415	**1.61**
1379858_at	methyltransferase like 2 (predicted)	Mettl2_predicted	1479	918	**1.61**
1389247_at	similar to polymerase (RNA) III (DNA directed) (155kD)	RGD1305574	1199	744	**1.61**
1372529_at	Transcribed locus	---	1677	1042	**1.61**
1372962_at	TAR (HIV) RNA binding protein 2	Tarbp2	987	613	**1.61**
1389442_at	Transcribed locus	---	1852	1151	**1.61**
1371544_at	similar to Enhancer of rudimentary homolog	LOC681415	12287	7637	**1.61**
1394120_a_at	Transcribed locus	---	987	614	**1.61**
1388398_at	similar to 40S ribosomal protein S4, X isoform	LOC690845	8263	5137	**1.61**
1389182_at	similar to hypothetical protein FLJ37953 (predicted)	RGD1311269_predicted	833	518	**1.61**
1387299_at	Msx-interacting-zinc finger	Miz1	3273	2036	**1.61**
1367543_at	similar to Protein SYS1 homolog	LOC685079	1429	889	**1.61**
1375901_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21a	Ddx21a	9023	5614	**1.61**
1392830_at	chromodomain helicase DNA binding protein 1 (predicted)	Chd1_predicted	1188	739	**1.61**
1367509_at	similar to general transcription factor IIH, polypeptide 5 (predicted)	RGD1560991_predicted	2380	1481	**1.61**
1373437_at	protein phosphatase 1, regulatory (inhibitor) subunit 8 (predicted)	Ppp1r8_predicted	2072	1290	**1.61**
1388417_at	anaphase-promoting complex subunit 5 (predicted)	Anapc5_predicted	4522	2817	**1.61**
1376941_at	Williams-Beuren syndrome chromosome region 16 homolog (human) (predicted)	Wbscr16_predicted	993	619	**1.60**
1371657_at	ubiquitin-like 1 (sentrin) activating enzyme E1B	Uble1b	8499	5299	**1.60**
1382101_at	heparan sulfate 2-O-sulfotransferase 1	Hs2st1	1691	1055	**1.60**
1374316_at	similar to arsenate resistance protein 2	LOC686980	2901	1810	**1.60**
1389633_at	polycystic kidney and hepatic disease 1-like 1	E2f4_predicted	2211	1379	**1.60**
1372882_at	similar to CG12379-PA (predicted)	RGD1308635_predicted	866	540	**1.60**
1374714_at	---	---	2209	1379	**1.60**
1394095_at	similar to RIKEN cDNA 4930429M06Rik	RGD1307449	1923	1200	**1.60**
1382161_at	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein) (predicted)	Mphosph10_predicted	1576	984	**1.60**
1382755_at	Transcribed locus	---	3011	1882	**1.60**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1376357_at	similar to Ankyrin repeat and IBR domain-containing protein 1	LOC368062	1758	1099	**1.60**
1369996_at	polymerase (RNA) II (DNA directed) polypeptide F	Polr2f	2762	1728	**1.60**
1373384_at	Similar to Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit gamma isoform (PP2A, B subunit, B gamma isoform) (PP2A, B subunit, B56 gamma isoform) (PP2A, B subunit, PR61 gamma isoform) (PP2A, B subunit, R5 gamma isoform) (PP2A, B su...	LOC691318	842	528	**1.60**
1398946_at	similar to mitochondrial ribosomal protein S16	LOC688912	1981	1241	**1.60**
1373075_at	similar to Cell division protein kinase 8 (Protein kinase K35) (predicted)	RGD1560888_predicted	3861	2420	**1.60**
1368890_at	glyceronephosphate O-acyltransferase	Gnpat	910	571	**1.59**
1370238_at	upregulated during skeletal muscle growth 5	Usmg5	11802	7400	**1.59**
1371341_at	similar to small nuclear ribonucleoprotein D2	LOC680309	8855	5553	**1.59**
1373077_at	similar to hypothetical protein D11Ertd497e	RGD1308696	2514	1576	**1.59**
1370284_at	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	Atp5e	8511	5339	**1.59**
1371720_at	similar to mitochondrial ribosomal protein L20	LOC680747	2254	1414	**1.59**
1372141_at	similar to Prefoldin subunit 2	LOC678975 /// LOC68560	5961	3740	**1.59**
1398343_at	similar to DnaJ (Hsp40) homolog, subfamily A, member 4	LOC498996	1215	762	**1.59**
1398804_at	MAK10 homolog, amino-acid N-acetyltransferase subunit, (S. cerevisiae)	Mak10	1948	1222	**1.59**
1392999_at	neuropilin (NRP) and tolloid (TLL)-like 2 (predicted)	Neto2_predicted	1199	753	**1.59**
1374716_at	similar to RIKEN cDNA 2810430M08	RGD1306106	1271	798	**1.59**
1395758_at	solute carrier family 25 (mitochondrial carrier, ornithine transporter) member 15	Syncrip	929	583	**1.59**
1367720_at	aminolevulinate, delta-, dehydratase	Alad	3543	2224	**1.59**
1376037_at	achalasia, adrenocortical insufficiency, alacrimia (Allgrove, triple-A) (predicted)	Aaas_predicted	816	513	**1.59**
1388622_at	nucleolar protein 5A	Nol5a	7764	4877	**1.59**
1389154_at	lipoic acid synthetase	Lias	1467	922	**1.59**
1391786_at	Similar to Murine homolog of human ftp-3	LOC308650	1294	813	**1.59**
1388306_at	similar to RIKEN cDNA 1810042K04 (predicted)	RGD1305593_predicted	1157	727	**1.59**
1388953_at	guanine nucleotide binding protein-like 3 (nucleolar)	Gnl3	8635	5430	**1.59**
1371655_at	mannose-P-dolichol utilization defect 1	Mpdu1	1266	796	**1.59**
1383965_at	similar to SMP3 mannosyltransferase	LOC684506	6105	3840	**1.59**
1398883_at	hypothetical protein LOC685548	LOC685548	8176	5144	**1.59**
1390408_at	holocytochrome c synthetase (predicted)	Hccs_predicted	984	619	**1.59**
1371255_at	Harvey rat sarcoma viral (v-Ha-ras) oncogene homolog	Hras	1371	864	**1.59**
1367754_s_at	argininosuccinate lyase	Asl	883	557	**1.59**
1392936_at	RNA binding motif protein 25 (predicted) /// similar to RNA binding motif protein 25 (predicted)	Rbm25_predicted /// RGD	1301	820	**1.59**
1373707_at	Transcribed locus	---	1737	1095	**1.59**
1368063_a_at	splicing factor YT521-B	Yt521	3505	2210	**1.59**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1370911_at	A kinase (PRKA) anchor protein 8	Akap8	3820	2409	**1.59**
1383520_at	solute carrier family 25 (mitochondrial carrier, ornithine transporter) member 15	Sfrs12	1675	1057	**1.58**
1370888_at	cytochrome c oxidase, subunit Va	Cox5a	13237	8352	**1.58**
1373875_at	similar to RIKEN cDNA 1190005P17 (predicted) /// hypothetical protein LOC690089	LOC690089 /// RGD13082	1020	644	**1.58**
1395354_at	lanC (bacterial lantibiotic synthetase component C)-like 1	Lancl1	1195	755	**1.58**
1372167_at	similar to small nuclear ribonucleoprotein polypeptide G	LOC681031 /// LOC68767	7422	4689	**1.58**
1398583_at	similar to small nuclear RNA activating complex, polypeptide 5	LOC691501	1173	742	**1.58**
1386918_a_at	opioid receptor, sigma 1	Oprs1	1103	697	**1.58**
1392460_at	---	---	821	519	**1.58**
1367769_at	polymerase (RNA) II (DNA directed) polypeptide G	Polr2g	5415	3424	**1.58**
1374182_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 1 (predicted)	Smarcc1_predicted	1619	1024	**1.58**
1389569_at	brix domain containing 2	Bxdc2	5623	3556	**1.58**
1371745_at	hypothetical LOC300361	RGD1310669	2973	1880	**1.58**
1373475_at	coiled-coil domain containing 58 (predicted)	Ccdc58_predicted	5177	3275	**1.58**
1372764_at	similar to density-regulated protein	LOC687565 /// LOC68960	4753	3006	**1.58**
1371302_at	similar to Small EDRK-rich factor 2 (4F5rel)	LOC502663	8849	5597	**1.58**
1375029_at	hypothetical LOC288447 (predicted)	RGD1310835_predicted	1981	1253	**1.58**
1398834_at	mitogen activated protein kinase kinase 2	Map2k2	1238	783	**1.58**
1371582_at	interleukin enhancer binding factor 2	Ilf2	3712	2352	**1.58**
1383011_at	eukaryotic translation initiation factor 2A	Eif2a	3988	2527	**1.58**
1379353_at	aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase (predicted)	Aasdhppt_predicted	2550	1617	**1.58**
1388354_at	activating signal cointegrator 1 complex subunit 3-like 1	Ascc3l1	3134	1987	**1.58**
1368004_at	mitochondrial ribosomal protein L23	Mrpl23	1592	1010	**1.58**
1371324_at	similar to Splicing factor 3B subunit 5 (SF3b5) (Pre-mRNA splicing factor SF3b 10 kDa subunit)	LOC680891 /// LOC68449	3959	2514	**1.58**
1367740_at	creatine kinase, brain	Ckb	3362	2135	**1.57**
1371822_at	polymerase (RNA) III (DNA directed) polypeptide D	Polr3d	2515	1598	**1.57**
1373037_at	ubiquitin-conjugating enzyme E2L 6	Ube2l6	1071	681	**1.57**
1380447_a_at	mitochondrial ribosomal protein S18C (predicted)	Mrps18c_predicted	955	607	**1.57**
1391604_at	similar to mKIAA0056 protein	LOC315508	1084	689	**1.57**
1370842_at	branched chain ketoacid dehydrogenase kinase	Bckdk	1267	806	**1.57**
1388516_at	LRRGT00141	LOC499235	7180	4568	**1.57**
1383341_at	calcium binding protein 39-like	Cab39l	856	544	**1.57**
1388803_at	deoxyhypusine synthase	Dhps	1588	1011	**1.57**
1372174_at	PEF protein with a long N-terminal hydrophobic domain	Peflin	1339	852	**1.57**
1382139_at	similar to hypothetical protein FLJ12994 (predicted)	RGD1308795_predicted	1025	653	**1.57**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1382783_at	Bloom syndrome homolog (human) (predicted)	Blm_predicted	1608	1024	**1.57**
1388562_at	serine racemase	Stard7_predicted	4584	2919	**1.57**
1372723_at	Importin 9 (predicted)	Ipo9_predicted	4707	2999	**1.57**
1389031_at	similar to Coatomer gamma-2 subunit (Gamma-2 coat protein) (Gamma-2 COP) (predicted)	RGD1566215_predicted	1083	691	**1.57**
1378181_at	ribonuclease P 40 subunit (human)	Rpp40	1302	830	**1.57**
1395640_at	CDC23 (cell division cycle 23, yeast, homolog)	Cdc23	1069	682	**1.57**
1372931_at	PRA1 domain family 2 (predicted)	Praf2_predicted	801	511	**1.57**
1382598_at	heat shock factor 2	Hsf2	1782	1137	**1.57**
1373502_at	dymeclin (predicted)	Dym_predicted	1228	784	**1.57**
1397697_at	Eukaryotic translation initiation factor 4A2	Eif4a2	1430	913	**1.57**
1367938_at	UDP-glucose dehydrogenase	Ugdh	4034	2577	**1.57**
1370075_at	dihydrofolate reductase	Dhfr	1063	679	**1.57**
1373119_at	potassium channel tetramerisation domain containing 3	Kctd3	1082	691	**1.57**
1384442_at	claudin 6 (predicted)	Cldn6_predicted	1803	1152	**1.57**
1387039_at	glypican 1	Gpc1	990	632	**1.57**
1391410_at	Transcribed locus	---	1098	702	**1.56**
1386890_at	S100 calcium binding protein A10 (calpactin)	S100a10	5076	3249	**1.56**
1373408_at	tubulin cofactor a	Tbca	10349	6626	**1.56**
1393881_at	NMDA receptor-regulated gene 1 (predicted)	Narg1_predicted	2951	1889	**1.56**
1369939_at	cytochrome c, somatic	Cycs	11102	7111	**1.56**
1399062_at	CDNA clone IMAGE:7317308	---	2160	1384	**1.56**
1367642_at	succinate-CoA ligase, GDP-forming, alpha subunit	Suclg1	2441	1564	**1.56**
1386856_a_at	sterile alpha motif domain containing 4B	Samd4b	2175	1394	**1.56**
1371396_at	tropomyosin 3, gamma	Tpm3	8584	5504	**1.56**
1393088_at	RNA (guanine-9-) methyltransferase domain containing 1	Rg9mtd1	2449	1570	**1.56**
1392544_at	rcd1 (required for cell differentiation) homolog 1 (S. pombe)	Rqcd1	1607	1031	**1.56**
1397767_at	Transcribed locus	---	830	532	**1.56**
1372879_at	AKT1 substrate 1 (proline-rich) (predicted)	Akt1s1_predicted	1629	1045	**1.56**
1387918_at	tropomyosin 3, gamma	Tpm3	6470	4153	**1.56**
1388956_at	exportin 5 (predicted)	Xpo5_predicted	1327	852	**1.56**
1381224_at	similar to Zinc finger protein 551 (Zinc finger protein KOX23)	LOC684878 /// LOC69139	1195	767	**1.56**
1370339_at	tropomyosin 3, gamma	Tpm3	13014	8358	**1.56**
1390382_at	similar to RIKEN cDNA 2310003F16 (predicted)	RGD1311457_predicted	6367	4090	**1.56**
1372522_at	similar to hypothetical protein FLJ10154	RGD1310061	4640	2982	**1.56**
1372155_at	tripartite motif protein 28	Trim28	7109	4571	**1.56**
1371892_at	ATP synthase mitochondrial F1 complex assembly factor 2 (predicted)	Atpaf2_predicted	860	553	**1.56**
1389547_at	periphilin 1 (predicted)	Pphln1_predicted	1591	1023	**1.55**
1370171_at	heterogeneous nuclear ribonucleoprotein U	Hnrpu	6578	4231	**1.55**
1389980_at	similar to Protein HSPC163 (predicted)	RGD1559740_predicted	4186	2693	**1.55**
1367591_at	peroxiredoxin 3	Prdx3	4309	2773	**1.55**
1389327_at	mitochondrial ribosomal protein L32 (predicted)	Mrpl32_predicted	3689	2374	**1.55**
1387950_at	nuclear import 7 homolog (S. cerevisiae)	Nip7	4006	2583	**1.55**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1392912_at	calcyclin binding protein	Cacybp	7518	4848	**1.55**
1393451_at	similar to RIKEN cDNA 2610510J17	RGD1310953	2544	1641	**1.55**
1385988_at	H6 homeo box 1 (predicted)	Hmx1_predicted	827	534	**1.55**
1388638_at	sirtuin 6 (silent mating type information regulation 2, homolog) 6 (S. cerevisiae)	Sirt6	843	544	**1.55**
1384149_at	similar to hypothetical protein FLJ38348 (predicted)	RGD1311424_predicted	1506	972	**1.55**
1393118_at	armadillo repeat containing 1 (predicted)	Armc1_predicted	1056	681	**1.55**
1392736_at	---	---	6163	3979	**1.55**
1371430_at	dystroglycan 1	Dag1	1484	959	**1.55**
1385553_at	similar to SMP3 mannosyltransferase /// similar to nuclear cap binding protein subunit 2	LOC684506 /// LOC68911	4171	2694	**1.55**
1370899_at	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	Sfpq	1366	883	**1.55**
1371881_at	zinc finger, HIT domain containing 2 (predicted)	Znhit2_predicted	864	558	**1.55**
1389289_at	Ewing sarcoma breakpoint region 1	Ewsr1	3676	2375	**1.55**
1372453_at	down-regulator of transcription 1	Dr1	3092	1998	**1.55**
1380009_at	vaccinia related kinase 1	Vrk1	2727	1763	**1.55**
1373185_at	signal sequence receptor, beta (predicted)	Ssr2_predicted	3107	2011	**1.55**
1389197_at	similar to RIKEN cDNA 9630046K23	RGD1306248	1674	1084	**1.54**
1372109_at	---	---	1010	654	**1.54**
1371904_at	SET and MYND domain containing 2	Smyd2	1342	869	**1.54**
1398810_at	PDGFA associated protein 1	Pdap1	9271	6005	**1.54**
1371418_at	chaperonin containing TCP1, subunit 2 (beta)	Cct2	12509	8103	**1.54**
1374517_at	RecQ protein-like	Recql	1763	1142	**1.54**
1372563_at	similar to D330021B20 protein	RGD1308143	1898	1229	**1.54**
1378493_at	similar to RIKEN cDNA 1810020G14 (predicted)	Fam29a_predicted	1246	807	**1.54**
1370007_at	protein disulfide isomerase associated 4	Pdia4	1772	1148	**1.54**
1371633_at	beta catenin-like 1	Ctnnbl1	2486	1613	**1.54**
1385521_at	Transcribed locus	---	1306	848	**1.54**
1369976_at	dynein light chain LC8-type 1	Dynll1	12259	7960	**1.54**
1371381_at	similar to ubiquinol-cytochrome c reductase complex 7.2kDa protein isoform b	LOC685322	9460	6145	**1.54**
1389866_at	similar to 6.8 kDa mitochondrial proteolipid	LOC691427	4630	3008	**1.54**
1376704_a_at	necdin-like 2	Ndnl2	1060	689	**1.54**
1374301_at	component of oligomeric golgi complex 1 (predicted)	Cog1_predicted	950	618	**1.54**
1394027_at	similar to Nucleoporin 62 (predicted)	RGD1564353_predicted	5421	3524	**1.54**
1370785_s_at	translocase of outer mitochondrial membrane 20 homolog (yeast)	Tomm20	8061	5242	**1.54**
1376069_at	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11 (predicted)	Psmd11_predicted	4079	2652	**1.54**
1379438_at	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1` (predicted)	Smarcad1_predicted	1078	701	**1.54**
1387080_at	chondroitin sulfate proteoglycan 6	Cspg6	5598	3641	**1.54**
1372215_at	similar to mitochondrial ribosomal protein S11 (predicted)	RGD1559901_predicted	884	575	**1.54**

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Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1367714_at	eukaryotic translation initiation factor 2B, subunit 2 beta	Eif2b2	1818	1183	**1.54**
1392627_x_at	thiopurine methyltransferase	Zfp84_predicted	16155	10517	**1.54**
1370873_at	calmodulin 3	Calm3	1918	1249	**1.54**
1371585_at	G1 to S phase transition 1	Gspt1	7789	5073	**1.54**
1371876_at	tumor necrosis factor superfamily, member 5-induced protein 1 (predicted)	Tnfsf5ip1_predicted	3347	2180	**1.54**
1388423_at	hypothetical protein MGC:15854	RGD1302996	1323	863	**1.53**
1398778_at	proteasome (prosome, macropain) subunit, alpha type 1	Psma1	6683	4356	**1.53**
1373371_a_at	similar to RIKEN cDNA 1110001J03	MGC112899	1263	824	**1.53**
1371806_at	DiGeorge syndrome critical region gene 8 (predicted)	Dgcr8_predicted	1079	704	**1.53**
1388316_at	RGD1566320 (predicted)	RGD1566320_predicted	1240	810	**1.53**
1392908_at	hypothetical protein LOC595134	LOC595134	1198	782	**1.53**
1389041_at	Vac14 homolog (S. cerevisiae)	Vac14	831	543	**1.53**
1373063_at	huntingtin interacting protein 2 (predicted)	Hip2_predicted	7730	5051	**1.53**
1371609_at	homolog of zebrafish ES1	RGD1303003	1119	732	**1.53**
1371073_at	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1	B4galt1_predicted	4104	2683	**1.53**
1388752_at	kinesin family member 1B	Bclaf1	3291	2152	**1.53**
1389061_at	NOL1/NOP2/Sun domain family, member 5 (predicted)	Nsun5_predicted	1477	966	**1.53**
1395479_at	brix domain containing 2	Bxdc2	1019	666	**1.53**
1388990_at	Mki67 (FHA domain) interacting nucleolar phosphoprotein	Mki67ip	4281	2801	**1.53**
1387366_at	interleukin enhancer binding factor 3	Ilf3	1475	965	**1.53**
1391440_at	testis expressed gene 10 (predicted)	Tex10_predicted	1799	1178	**1.53**
1388112_at	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	Slc25a4	13635	8935	**1.53**
1390340_a_at	eukaryotic translation initiation factor 4 gamma, 1	Eif4g1	907	594	**1.53**
1371651_at	similar to Vacuolar protein sorting protein 25 (ELL-associated protein of 20 kDa)	LOC681059 /// LOC68819	2259	1481	**1.53**
1393193_at	Transcribed locus	---	1183	776	**1.53**
1399065_at	Similar to RNA binding protein with multiple splicing 2 (predicted)	RGD1561222_predicted	974	639	**1.52**
1373704_at	aquarius (predicted)	Aqr_predicted	2171	1424	**1.52**
1367877_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	Slc11a2	839	551	**1.52**
1398932_at	histidine triad nucleotide binding protein 1 /// histidine triad nucleotide binding protein 1 (predicted) /// similar to Histidine triad nucleotide-binding protein 1 (Adenosine 5-monophosphoramidase) (Protein kinase C inhibitor 1) (Protein kinase C-interacting protein 1) (PKCI-1)	Hint1 /// Hint1_predicted //	10550	6923	**1.52**
1367943_at	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, beta	Nfkbib	1283	842	**1.52**
1393045_at	Rho-associated coiled-coil forming kinase 2	Rock2	1012	664	**1.52**
1388160_a_at	isocitrate dehydrogenase 3 (NAD+) beta	Idh3B	3791	2490	**1.52**
1374135_at	importin 4 (predicted)	Ipo4_predicted	1903	1250	**1.52**
1386891_at	phosphatidylethanolamine binding protein 1	Pebp1	4786	3144	**1.52**

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Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1375019_at	heterogeneous nuclear ribonucleoprotein H3 (2H9) (predicted)	Hnrph3_predicted	1253	824	**1.52**
1390848_at	RNA binding motif protein 19 (predicted) /// similar to RNA binding motif protein 19	LOC687727 /// Rbm19_pr	845	556	**1.52**
1367551_a_at	similar to CG14980-PB	RGD1306410	3175	2088	**1.52**
1371424_at	similar to RIKEN cDNA 3110005O21	RGD1304823	882	580	**1.52**
1389979_at	transportin 3	Tnpo3	991	652	**1.52**
1381364_at	transportin 1	Tnpo1	1945	1280	**1.52**
1374009_at	similar to RIKEN cDNA 5830415L20	RGD1306302	1696	1116	**1.52**
1393807_at	Transcribed locus	---	1496	985	**1.52**
1388614_at	splicing factor 3b, subunit 2 (predicted)	Sf3b2_predicted	3385	2228	**1.52**
1371704_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 23 (predicted)	Ddx23_predicted	1824	1201	**1.52**
1398977_at	special AT-rich sequence binding protein 1	Ide	894	589	**1.52**
1388476_at	Tial1 cytotoxic granule-associated RNA binding protein-like 1	Tial1	2650	1746	**1.52**
1372255_at	arginyl-tRNA synthetase (predicted)	Rars_predicted	7509	4948	**1.52**
1376567_at	similar to Mitotic spindle assembly checkpoint protein MAD1 (Mitotic arrest deficient-like protein 1) (MAD1-like 1)	LOC680006 /// LOC68641	1075	708	**1.52**
1377551_at	Transcribed locus	---	1069	704	**1.52**
1374876_at	leptin receptor overlapping transcript-like 1	Leprotl1	1259	830	**1.52**
1398572_at	RNA (guanine-7-) methyltransferase	Rnmt	4805	3168	**1.52**
1389319_at	similar to Endoplasmic reticulum-Golgi intermediate compartment protein 1 (ER-Golgi intermediate compartment 32 kDa protein) (ERGIC-32)	LOC287177 /// LOC68283	1158	764	**1.52**
1389168_at	McKusick-Kaufman syndrome protein	Mkks	1436	947	**1.52**
1371423_at	mitochondrial ribosomal protein L41	Mrpl41	3737	2465	**1.52**
1367653_a_at	malate dehydrogenase 1, NAD (soluble)	Mdh1	4263	2813	**1.52**
1371488_at	similar to SPBPJ4664.02 (predicted) /// RGD1562079 (predicted)	RGD1559845_predicted //	2696	1780	**1.51**
1375520_at	coatomer protein complex, subunit zeta 1 (predicted)	Copz1_predicted	2435	1608	**1.51**
1379671_at	major facilitator superfamily domain containing 1 (predicted)	Mfsd1_predicted	1519	1004	**1.51**
1373556_at	similar to CG14903-PA (predicted)	RGD1561792_predicted	1923	1271	**1.51**
1389655_at	similar to A230072I16Rik protein	RGD1306819	3611	2389	**1.51**
1374597_at	Transcribed locus, strongly similar to XP_574009.1 PREDICTED: similar to CDNA sequence BC034664 [Rattus norvegicus]	---	812	537	**1.51**
1367500_at	similar to 26 proteasome complex subunit DSS1 (Deleted in split hand/split foot protein 1) (Split hand/foot deleted protein 1 homolog)	LOC680532	5470	3621	**1.51**
1393458_s_at	similar to PHD finger protein 14 isoform 1 (predicted)	RGD1563764_predicted	1938	1283	**1.51**
1379322_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 7 (predicted)	Psmd7_predicted	2351	1557	**1.51**
1368199_at	nucleoporin 88	Nup88	3556	2354	**1.51**
1367502_at	mitochondrial ribosomal protein L21 (predicted)	Mrpl21_predicted	2159	1430	**1.51**

Table 6.1. Trophoblast stem-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	Stem/ Dif
1373200_at	eukaryotic translation elongation factor 1 epsilon 1 (predicted) /// similar to eukaryotic translation elongation factor 1 epsilon 1 (predicted)	Eef1e1_predicted /// RGD	5548	3676	**1.51**
1392678_a_at	DNA methyltransferase 3A	Dnmt3a	1322	876	**1.51**
1390572_at	THO complex 3 (predicted)	Thoc3_predicted	811	537	**1.51**
1371726_at	Transcribed locus	---	1629	1080	**1.51**
1378080_at	similar to SCO cytochrome oxidase deficient homolog 1 (yeast) (predicted)	RGD1559538_predicted	1796	1191	**1.51**
1399131_at	similar to ribosomal protein, mitochondrial, S22	LOC683519	1309	868	**1.51**
1370908_at	histone deacetylase 2	Hdac2	4025	2669	**1.51**
1367484_at	ubiquitin-conjugating enzyme E2E 2 (UBC4/5 homolog, yeast)	Ube2e2	3804	2522	**1.51**
1374848_at	Similar to RIKEN cDNA 2410016F19 (predicted)	RGD1307434_predicted	1238	821	**1.51**
1371999_at	Transcribed locus	---	1126	747	**1.51**
1398858_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	Psmd2	6379	4235	**1.51**
1371987_at	polymerase (DNA directed) sigma (predicted)	Pols_predicted	1107	735	**1.51**
1371557_at	THAP domain containing 4	Thap4	2077	1379	**1.51**
1388441_at	hypothetical protein LOC689574	LOC689574	1381	918	**1.51**
1372581_at	SNF8, ESCRT-II complex subunit, homolog (S. cerevisiae)	Snf8	1608	1068	**1.50**
1372665_at	phosphoserine aminotransferase 1	Psat1	10633	7071	**1.50**
1388428_at	histidyl tRNA synthetase 2 (predicted)	Hars2_predicted	1933	1285	**1.50**
1399142_at	Transcribed locus	---	1780	1184	**1.50**
1388570_at	similar to RIKEN cDNA 2310005O14	LOC498909	1881	1252	**1.50**
1384131_at	ADP-ribosylation factor-like 6 interacting protein 2	Arl6ip2	2180	1450	**1.50**
1388668_at	Transcribed locus	---	3237	2155	**1.50**
1398916_at	aurora kinase A interacting protein 1	Aurkaip1	2502	1666	**1.50**
1373394_at	similar to RIKEN cDNA 4122402O22	RGD1310674	1218	812	**1.50**
1374806_at	similar to 14-3-3 protein sigma /// stratifin (predicted)	LOC298795 /// Sfn_predic	6985	4656	**1.50**

Table 6.2. Gene functions

Function	Group	Genes
Cell Cycle	Trophoblast stem cell-associated	BMP4, PIAS2, MAPK3, NOLC1, CCNB2, DSN1, BAZ1A, HRAS, HES1, GPSM2, RECQL, CHAF1A, CD2AP, BMI1, CSE1L, SMARCB1, CDKN3, POLR3D, TP53, CALM3, CDCA8, ESPL1, PCGF6, CKS2 (includes EG:1164), ZC3HC1, HIRA, HMGA1, FGFR2, CKAP2 (includes EG:26586), FOXG1, RAE1, SKP2, CCDC5, PCNA, CCND3, HELLS, TARDBP, H2AFX, EHMT2, ANAPC5, TFRC, EBNA1BP2, SGOL1, BRE, XRCC5, UBE2C, KIF20A, NCAPD3, CD320, AATF, AKAP8, CDT1, SUV39H2, KPNA2, DKC1, GAPDH (includes EG:2597), DTD1, NUF2, CDX1, CHEK1, PTMA, ID1, PSMG2, PROS1, DAP3, TOP2A, E2F5, MCM10, KIF2C, EGFR, CDC45L, MYCN, CCNB1, DBI, NAE1, CDK2AP1, PSMD13, SPC25, TIAL1, PLK1, NCAPD2, POLD1, ID3, FZR1, CEACAM1, KPNB1, KIF22, BUB1 (includes EG:699), MAD2L1, GPI, CCNE1, MCM2, TLK1, MSH2, CUL2, NDE1, NOL1, MPHOSPH1, CHFR, CCT7, EDG1, PRMT5, NHP2L1, PSAP, PIN1, SFN, LIG1, KIF23, DLG7, SFRS2, SRPK1, CCNI, MLLT6, PTTG1, PARP2, SMC4, UBE2V2, NUDC, TAF10, KNTC1, PCBP4, AURKB, SCYE1, BIRC5, GSPT1, MAP2K2, MYBL2, CAST, ECT2, NUCKS1, KIFC1, MCM5, SMC3, MIF, PSMB5, EWSR1, HDAC2, NUBP1, MTA2, ORC6L, LMNA, PAXIP1, ZRF1, BUB1B, SSRP1, NCOA3, ERCC6L, PCAF, NASP, SMC2, CDK4, HIRIP3, CAPN2, GNL3, DIABLO, TMPO, POLE3, BUB3, ID2, PA2G4, CDC20, DTYMK, PTPRF, SHC1, CCNA2, ASPM, GMNN, DR1, BLM, STAT1, TERF1, TACC3, NUSAP1, UHRF1,
	Differentiation-associated	MAGED1, CAMK1, CREM, HBP1, SOD2, TCEB3, TSC2, FOSL1, VCPIP1, IRS3, SNAI1, JUNB, STAT3, IER3, CSH1, ATF2, DAXX, HELLS, JDP2, FOXN3, BTG2, CDC2L6, MORC3, ADM, FN1, DDIT3, ILK, ZNF655, CRK, DMTF1, NOTCH2, JUN, HK2, GADD45A, PCTK2, PDGFRA, RB1CC1, CITED2, ITGB1, GRB2, EGR1, XBP1, PPP1R15A (includes EG:23645), CEACAM1, GRN, FIS1, GNAI3, LYN, APBB2, PLAU, NCOR2, CSH2, PSEN1, HSPA1A, CUL4A, KLF6, C13ORF15, PIP5K3, GATA2, PPM1D, MYC, USP8, TOP1, CAMK2D, PLAC1, RHOB, EZH2, ITGAV, CDKN1C, ZMYM2, FGFR1, PAWR, BCAR3, KLF4, SEL1L, BCL2L1, IGF2, CCNG2, MDM4, MNT, DUSP1, CEBPD, CASP2, MDK, SOCS5, VHL, JARID1B, ATF5, RBBP8, HIF1A, ING4, NFKBIA, CLK1, SP1, FLNA (includes EG:2316), PLK2, ANXA1, SPHK1, ARID3A, PBEF1, PPARD, PLAUR, CEBPB, IVNS1ABP, GNAI2, PIK3CB, CDKN1B, ING1, MMP9, LGALS1
	Positively regulated by PI3K	AKAP12, CUL5, CREM, DDIT3, BAZ1A, CDC123, SCIN, AURKA, CSH1, MYC, BCL2L1, DAXX, HMOX1, IGF2, SREBF1, GATA3, CSH2, MMP9
	Negatively regulated by PI3K	AMACR, TPM1, ATF3, ING5, HSPA1A, EGR1, TAF10, LATS2, CYP26B1, CD2AP, H2AFX, SPRY2, PRMT5, SPHK1, UBE2D3, PRKCH, CAST, TCF7L2, ID4, PPP3CA
Cell Survival	Trophoblast stem cell-associated	LIG1, NPM1 (includes EG:18148), PRPF19, PIAS2, BMP4, DNMT3A, MSX1, TRAP1, HRAS, HES1, BIRC5, BMI1, TP53, TNFRSF21, PRKRA, HDAC2, RRM2, HMGA1, PAXIP1, LMNA, BUB1B, PCNA, CCND3, DIABLO, XRCC5, PRDX2, S100A6, ID2, NME1, SMN1, EMILIN2, CDC20, NUF2, PTMA, CHEK1, ID1, SHC1, MT1E, HMGB1 (includes EG:25459), MCM10, PDK1, BLM, EGFR, DHX9, MYCN, RBM17, TACC3, CCNB1, UCP2, UHRF1, SPC25, PLK1, MAD2L1, CCNE1, TPM3, MSH2, CHFR, COX5A, EDG1, SFN, TXNDC17

Table 6.2. Gene functions

Function	Group	Genes
Cell Survival (cont)	Differentiation-associated	MAGED1, TGFBR1, CREM, MAP3K7IP2, GCLC, TEGT, ODC1, C16ORF5, MAML1, POR, PKN2, NSF, SOD2, FHL2, TSC2, VAMP3, ADA, ATF4, FOSL1, SERPINE1, DNM2, DDIT4, CSH1, STAT3, ATF2, DAXX, CDH5, JDP2, HELLS, BTG2, CTSB, ZYX, SQSTM1, HTATIP2, ARMC10, HSPA1B, DDIT3, ILK, HSPB8, GLIPR1, TGM2, CASP6, APPL1, NOTCH2, CD47, GADD45A, BBC3, ZFAND5, RNF19A, NFE2L2, B4GALT5, TMSB4X, OPTN, GRB2, TNFRSF1A, ABCG2, TRIB3, SLC25A24, GRN, LDLR, BFAR, LYN, HAP1, PSEN1, PINK1, DAD1, JAK1, KLF6, BAG3, GATA2, CEBPG, PDCD7, PPM1D, MYC, FDFT1, CTSD, UBE2B, RHOB, SNN, PRR13, CDKN1C, KLF11, STK17B, ATG12, STXBP1, IL2RG, ZMYM2, FEM1B, RDX, BTG1, KLF4, BCL2L1, IGF2, MDM4, SERINC3, GCNT1, DUSP1, SGPP1, MDK, IRF8, HFE, VHL, HSPB1, B2M, KLF10, MAP2K1IP1, NFAT5, PBEF1, CD24, PXN, SLC12A2, G6PD, NSD1, HERPUD1, PLAUR, ALS2, NFE2L1, IVNS1ABP, PRKCI, NRTN, CDKN1B, ING1, CTSL2, A4GALT, BCL2L2, SMG1, IRS3, FASLG, PEG3 (includes EG:5178), TNKS, JUNB, IER3, LDOC1, CD9, RTN4, CDC2L6, ADM, CA2, TCF4, FN1, ZFP36, CRK, PSENEN, NFIL3, JUN, HK2, TFAP2A, RB1CC1, PDGFRA, ACSL4, MAP1LC3B, CITED2, ITGB1, EGR1, ELL, XBP1, ANXA4, PPP1R15A (includes EG:23645), F3, RABGEF1, CEACAM1, ZNF148, ITGB2, FIS1, KRT8, DHCR24, APBB2, PLAU, NCOR2, SLC31A1, NCOA6, CLN8, HSPA1A, TICAM2, CUL4A,
	Positively regulated by PI3K	ADM, CA2, HTATIP2, TCF4, CUL5, CREM, DDIT3, ATF5, GADD45G, HSPB8, GCLC, GLIPR1, MYC, CTSD, HMOX1, HSP90B1, GCLM, FASLG, PLSCR1, DDIT4, SLC2A1, HSPA9, HERPUD1, NSD1, TRIB3, SCIN, AURKA, CSH1, HCLS1, LDOC1, CEACAM1, BCL2L1, DAXX, IGF2, YARS, RIPK3, CYB5A, DNAJB6, SQSTM1, MMP9, HSPB1
	Negatively regulated by PI3K	TPM1, S100A6, HSPA1B, ING5, SGK1, HSPA1A, DUSP22, TAF10, LATS2, TNFRSF12A, NCK2, FDFT1, CYP26B1, CD2AP, PPP3CB, CLDN4, STUB1, A4GALT, SPHK1, CYB5R3, PRR13, DAG1, CAST, DNAJB1, DUSP14, SERPINE1, PPM2C, KLF2, PPP3CA, S100A10, PRNP, STXBP1, ATF3, EGR1, PERP (includes EG:64065), ITGA6, PSIP1, AKAP13, MBTPS1, BNIP3L, PRKCH, ZFP36L2, HFE, CLDN3, ID4
Cell Signaling	Trophoblast stem cell-associated	
	Differentiation-associated	LITAF, JAK1, ZFP36, TICAM2, CRK, AKAP11, TGM2, GNB4, MAP2K1IP1, NLK, JUN, GADD45A, FLNA (includes EG:2316), TNIP2, PLK2, ITGAV, RB1CC1, MAPKAPK2, CD24, TMEM9B, HGS, GNG12, STK17B, ITGB1, GRB2, FGFR1, DAPK3, MKNK2, STAT3, STAMBP, ATF2, GNAI2, DAXX, STK38, NRTN, NDFIP1, WNK1, PIK3CB, STAT2, DOK1, MAP4K5, SQSTM1, SOCS5, ECM1, PINK1, IRAK2, LGALS1
	Positively regulated by PI3K	
	Negatively regulated by PI3K	CD2AP, SLC9A3R1, DAG1, PARD3, HFE
Cell-To-Cell Signaling and Interaction	Trophoblast stem cell-associated	LAMA5, PHB2, TFRC, L1CAM, HES1, S100A10
	Differentiation-associated	NCOA6, MYC, PKN2, CTSL2, ESAM, FHL2, SOD2, RHOB, SCARB1, TSC2, ITGAV, JUP, MGAT1, SERPINE1, LCP1, IL2RG, FERMT2, STAT3, IGF2, CD9, CDH5, ITGA1, IRF8, TFPI, C1GALT1, VHL, SPN, FN1, PVRL3, PTPN14, ILK, UGCG, CRK, PTPN12, TGM2, CD47, ANXA1, STX4, CD24, ITGB1, CD59, EGR1, PLAUR, ITGA3, F3, ITGB2, ADAM10, LYN, CALD1, PIK3CB, PLAU, ITGAX
Cell-To-Cell Signaling and Interaction	Positively regulated by PI3K	ADM, CEACAM1, MYC, HMOX1, BCL2L1, IGF2, HSP90B1, PBX3, ENTPD2, PRL4A1, GATA3, MMP9, FASLG

Table 6.2. Gene functions

Function	Group	Genes
(cont)	Negatively regulated by PI3K	NCK2, F11R, STXBP1, HSPA1A, PIP5K1C, SPRY2, EGR1, ITGA6, TACSTD1, CAST, SERPINE1
Cellular Assembly and Organization	Trophoblast stem cell-associated	KIF23, DLG7, SRPK1, PTTG1, PARP2, SMC4, TXNL4A, CCNB2, DSN1, BAZ1A, GPSM2, AURKB, BIRC5, CHAF1A, CKB, SNRPD1, ECT2, KIFC1, TP53, PPID, ESPL1, NUBP1, MTA2, CKAP2 (includes EG:26586), BUB1B, ERCC6L, SKP2, RAE1, PRDX3, TFAM, SMC2, HELLS, H2AFX, EHMT2, HMGN2, EBNA1BP2, CAPN2, HIRIP3, SGOL1, XRCC5, BUB3, POLE3, SMN1, NCAPD3, CDT1, UBE2N, SUV39H2, CKMT1B, NUF2, SLC25A5, USP39, ID1, CCNA2, GMNN, DR1, TOP2A, KIF2C, BLM, TERF1, MYCN, NUSAP1, TACC3, CCNB1, SLC25A4, UHRF1, MRPL15, PRC1, SPC25, CKAP5, PLK1, MRPL17, NCAPD2, NCAPH2, KIF22, KPNB1, BUB1 (includes EG:699), MAD2L1, CCNE1, B4GALT1, TLK1, NDE1, NHP2L1, PIN1, SFN, KIF18A, DDX11
	Differentiation-associated	EPS15, TOM1, RABEP1, MAP1B, PIP5K3, LIMK2, MYC, NSF, SOD2, RHOB, BAIAP2, ITGAV, FASLG, DSP, PTP4A3, RDX, CD9, CDH5, SQSTM1, ELMO1, CTTN, VHL, ENAH, FN1, PICALM, PTPN14, UGCG, CRK, REPS1, PTPN12, FLNA (includes EG:2316), TINAGL1, ARHGEF2, GOSR1, CD24, HGS, ITGB1, STX12, PXN, LOC290704, RAB5A, GRB2, DDEF1, STX5, PLAUR, ALS2, RABGEF1, ITGB2, EPB41, WIPF1, PRKCI, SYNJ1, STX7, HAP1, CALD1, PLAU, CDKN1B, MSN
	Positively regulated by PI3K	ADM, MYC, BCL2L1, IGF2, GCLC, BAZ1A, AURKA, GCLM, GATA3, FASLG, PLSCR1
	Negatively regulated by PI3K	FLNB, TPM1, MYH10, HSPA1B, HSPA1A, CSRP1, TNFRSF12A, NCK2, DHCR7, CD2AP, A4GALT, STMN4, BAIAP2, CYB5R3, SPHK1, PLDN, CAST, DAG1, SERPINE1, KLF2, PPP3CA, LASP1, STXBP1, ATF3, ARHGEF12, DDEF1, ITGA6, AKAP13, CORO1C, CHRAC1, PIP5K1C, H2AFX, SPRY2, HMGN2, PARD3
Cellular Growth and Proliferation	Trophoblast stem cell-associated	NPM1 (includes EG:18148), DBN1, PIAS2, HMMR, PLK4, ELF3, SMARCB1, TRAF4, RUVBL2, MYBBP1A, AHCY, SART3, TNFRSF21, TP53, ESPL1, CDCA8, HMGA1, GFM1, RRM2, FGFR2, ANP32A, SKP2, NDNL2, ANAPC5, TFRC, DHFR, HNRPAB, XRCC5, UBE2C, KIF20A, NME1, AATF, CD320, PHLDA2, GADD45GIP1, TMEFF1, CLDN4, BCCIP, HNRNPU, THG1L, UCP2, CDK2AP1, LASS1, WNK2, CDCA7, HNRPD, ID3, MCM3, MAD2L1, CCNE1, ENO1, CCT7, PSAP, SHMT1, PIN1, SFN, CXADR, DLG7, SFRS2, ILF3, MLLT6, PTTG1, SF3B2, GNPAT, CCT2, NME2, SCYE1, GGA2, SAE1, MAP2K2, MYBL2, CAST, EIF5A2, PGK1, SMC3, HNRPF, LAPTM4B, EWSR1, HDAC2, CDX2, BAMBI, PFDN5, ZRF1, NCOA3, TACSTD1, DIABLO, PPAT, TMEPAI, ID2, HDGF, SHC1, CCNA2, STUB1, STAT1, TERF1, LAMA5, SLC9A3R1, ADAM15, HSPD1 (includes EG:3329), MTHFD1, PPM1G, ATIC, JOSD3, PSMD2, CKS1B, EIF3I, BMP4, MAPK3, TRAP1, HRAS, HES1, GSS, SET, EEF1D, OCLN, ACIN1, BNIP3 (includes EG:664), BMI1, NFKBIB, NOL8, ITGB5, CALM3, METTL3, TNK2, FOXG1, CCND3, PSMB2, CCT3, SPINT2, LDHA, EMILIN2, CDT1, DUSP6, GPX1, RUVBL1, SYMPK, EIF4G1, CDX1, SF3B3, EIF2B2, CHEK1, BYSL, PTMA, ID1, MT1E, TCOF1 (includes EG:6949), DAP3, EGFR, MYCN, CCNB1, TRIM28, DDX56, TIAL1, RRM1, FOLR1, CEACAM1, MCM2, NDE1, MPHOSPH1, CHFR, RSL1D1, EDG1, FKBP4, C6ORF108, PRPF19, CCNI, PCBP4, PBK, CD63, PSMC5, BIRC5,

Table 6.2. Gene functions

Function	Group	Genes
Cellular Growth and Proliferation (cont)	Differentiation-associated	MAGED1, TGFBR1, CREM, RAB2A, MAP3K7IP2, ODC1, MAML1, LAMC1, POR, SOD2, FHL2, TSC2, ADA, ATF4, FOSL1, SERPINE1, MGAT1, DNM2, LCP1, FLOT1, STAT3, CSH1, ATF2, CDC73, DAXX, CDH5, BTG2, CTSB, ZYX, ITGA1, DOK1, ECM1, ARMC10, PIM3, DDIT3, PTPN14, SEMA6A, ILK, HSPB8, TGM2, APPL1, DMTF1, HADHB, CD47, NOTCH2, GADD45A, BBC3, TMSB4X, OPTN, ENTPD1, TNFRSF1A, GRB2, GRN, WIPF1, TMEM49, LYN, NRIP1, RNF14, CSH2, PSEN1, JAK1, ARL6IP5, KLF6, GATA2, PPM1D, GPNMB, HDAC6, MYC, CTNNBIP1, CTSD, FDFT1, USP8, UBE2B, ANXA11, RHOB, CDKN1C, STK17B, KLF11, TOP3B, IL2RG, ZMYM2, BTG1, KLF4, PRL7A2, SLC3A2, BCL2L1, MDM4, IGF2, SERINC3, DUSP1, GCNT1, IRF8, MDK, TFPI, S100A11 (includes EG:6282), VHL, LITAF, YPEL3, KLF10, DHCR7, NFAT5, ARID3A, PBEF1, CD24, G6PD, ERO1L, PLAUR, IVNS1ABP, SHB, PRKCI, ING1, CDKN1B, ITGAX, TCIRG1, HBP1, CTSL2, TCEB3, BCL2L2, FASLG, TNKS, PTP4A3, USP9X, JUNB, IER3, BMYC, LDOC1, CD9, CDC2L6, RAP1B, ADM, TCF4, RALA, FN1, B3GNT2, CRK, DNAJA1, HK2, JUN, TFAP2A, PDGFRA, RB1CC1, HGS, CITED2, BMP1, GLMN, ITGB1, RGS2, EGR1, ELL, XBP1, PFN2, PPP1R15A (includes EG:23645), NAP1L1, ITGA3, RABGEF1, ZNF148, CEACAM1, CUX1, FIS1, ITGB2, NUB1, KRT8, DHCR24, SREBF1, ADAM10, APBB2, NCOR2, PLAU, EPS15, NCOA6, INSIG1, CUL4A, CDA, TCF7, TOP1, PLAC1, SCARB1, EZH2, ITGAV,
	Positively regulated by PI3K	AKAP12, ADM, CREM, DDIT3, GADD45G, HSPB8, DNAJC3, MYC, CTSD, HMOX1, FASLG, PLSCR1, LCP1, TSNAX, CSH1, AURKA, ATIC, SLC3A2, CEACAM1, BCL2L1, IGF2, FABP5, SREBF1, DNAJB6, CSH2, MMP9
	Negatively regulated by PI3K	GBX2, FLNB, MYH10, S100A6, ING5, HSPA1A, DUSP22, IGBP1, TNFRSF12A, NCK2, FDFT1, DHCR7, CLDN4, STUB1, SPHK1, UBE2D3, SERPINE1, IRX3 (includes EG:79191), KLF2, PPP3CA, S100A10, PRNP, AMACR, ATF3, EGR1, ITGA6, F11R, AKAP13, SPRY2, PRMT5, BNIP3L, TACSTD1, ZFP36L2, HMGCR, ID4
Cellular Movement	Trophoblast stem cell-associated	KIF23, TP53, KIF20A, NUSAP1, CCNB1, CDC20, RACGAP1, PRC1, HRAS, PLK1, AURKB, ID3, ID1, CD2AP, ELF3, MAP2K2, MPHOSPH1, CHFR, TOP2A, ECT2, EGFR
	Differentiation-associated	TGFBR1, TCIRG1, HBP1, ODC1, LAMC1, CTSL2, LGMN, SOD2, FHL2, A4GALT, TSC2, CBLL1, FOSL1, SERPINE1, GNG12, DNM2, FASLG, LCP1, PTP4A3, CREB3, FERMT2, SNAI1, JUNB, STAT3, CSH1, ARHGDIB, CDH5, CD9, RTN4, CTSB, ZYX, ITGA1, DOK1, ADM, ENAH, FLNB, RALA, FN1, PTPN14, ILK, TPM4, CRK, DNAJA1, PTPN12, TGM2, PRKX, CD47, ACTR3, JUN, GADD45A, PPAP2B, PDGFRA, SARS, NFE2L2, B4GALT5, TMSB4X, ITGB1, TNFRSF1A, EGR1, ITGA3, F3, GRN, CEACAM1, GNAI3, ITGB2, PEX5, LDLR, SREBF1, ADAM10, LYN, APBB2, PLAU, PSEN1, JAK1, EPS15, MAP1B, HEBP1, HDAC6, MYC, CTSD, ESAM, RHOB, PLAC1, EZH2, ITGAV, JUP, FXYD5, FGFR1, BCAR3, TMOD3, RALBP1, KLF4, SLC3A2, SEL1L, IGF2, ARRB2, PREX1, GCNT1, CXCR7, DUSP1, SEMA6D, SGPP1, MDK, TFPI, CTTN, ELMO1, VHL, HSPB1, PAFAH1B2, SPN, PICALM, ING4, HIF1A, DNMBP, PDCD4, NFKBIA, NFAT5, SP1, FLNA (includes EG:2316), ANXA1, SPHK1, CCRL1, MAPKAPK2, CD24, PRNP, CD59, PXN, DDEF1, PPARD, DAPK3, G6PD, PLAUR, GNAI2, PRKCI, NRTN, SYNJ1, MYLIP, CALD1, PIK3CB, CDKN1B, HMGCR, MMP9, MSN, LGALS1, ITGAX
	Positively regulated by PI3K	ADM, LCP1, CUL5, AURKA, CSH1, SLC3A2, CEACAM1, MYC, CTSD, ESAM, NARS, WARS, IGF2, FABP5, YARS, SEMA6D, PPAP2B, GATA3, SARS, ELMO1, FASLG, MMP9
	Negatively regulated by PI3K	GBX2, MYH10, FLNB, TPM1, S100A6, IGBP1, LATS2, TNFRSF12A, NCK2, CD2AP, CLDN4, A4GALT, SPHK1, CAST, SERPINE1, KLF2, LASP1, S100A10, PRNP, ATF3, SLC9A3R1, DDEF1, EGR1, APLP2, ITGA6, F11R, PIP5K1C, MRLC2, SPRY2, HMGCR, CLDN3

Table 6.2. Gene functions

Function	Group	Genes
Gene Expression	Trophoblast stem cell-associated	POLR2F, PRIM1, PIAS2, BMP4, MAPK3, MSX1, SMARCD2, MED21, BAZ1A, HRAS, SFPQ, HES1, PPP4C, SET, EEF1D, CHAF1A, ELF3, BMI1, GATAD2A, SMARCB1, TCERG1, MYBBP1A, NFKBIB, APEX1, TARBP2, HMX1, TP53, BCOR, PCGF6, NUFIP1, HIRA, HMGA1, MAPK8IP1, ANP32A, NDNL2, ZNF238, CCND3, SUPT16H, TARDBP, EHMT2, HMGN2, SLC44A2, POLR2I, HNRPAB, XRCC5, HMGB2, DEK, NME1, PRDX1, KPNA2, PSMD9, RUVBL1, MED28, CDX1, PTMA, VRK1, NARG1, ID1, RNPS1, TRIM27, TCOF1 (includes EG:6949), CTBP2, E2F5, TOP2A, POLR3G, PSMD14, HMGB1 (includes EG:25459), ABT1, EGFR, ORC1L, MYCN, CCNB1, NAE1, PPP1R8, PRIM2, TRIM28, MRPL12, TIAL1, PLK1, ID3, CCNE1, ZNF593, ENO1, EDG1, PIN1, MED4, GTF3A, TRAF3, ILF3, GTF3C6, MLLT6, DNMT3A, PTTG1, HSF2, NME2, CBX3, PSMC5, RDBP, TCEB2, PTGES2, PPIE, ECT2, MIF, EWSR1, HDAC2, PHF5A, CDX2, MTA2, PHB2, PFDN5, LMNA, BRD8, TBL1X, PUS1, SSRP1, NCOA3, IFRD1, PCAF, TFAM, TADA2L, NSEP1, PABPN1, TMPO, PEBP1, POU3F1, SNF8, ID2, PA2G4, GTF2F2, PDGFA, BCLAF1, CCNA2, POLR1C, SHC1, POLR2C, DNTTIP1, STUB1, NEDD8, DR1, SUDS3, STAT1, TCF20, SLC20A1, TRIP13, CCNH, VEZF1, UHRF1, CSDA, PSMC3IP, STRAP, CENPK, POLR2G, NOC2L, ZNHIT3, KLF5, SATB1, PNPT1, SMARCC1, MCM7
	Differentiation-associated	MED13, MAGED1, CREM, TGFBR1, MAP3K7IP2, GCLC, GTF2E2, HBP1, ATP8B1, PKN2, MAML1, MYCBP2, FHL2, SOD2, TSC2, TCEB3, ATF4, FOSL1, SERPINE1, FASLG, JUNB, CSH1, STAT3, ZNF496, ATF2, DAXX, JDP2, CCNDBP1, FOXN3, BTG2, SQSTM1, ECM1, ADM, TCF4, HTATIP2, SCAP, FN1, DDIT3, ZFP36, ZNF143, ILK, CRK, AFF4, SMURF1, ARID4B, APPL1, DMTF1, NFIL3, NOTCH2, JUN, ACTR3, GADD45A, TFAP2A, HGS, NFE2L2, CITED2, GLMN, ITGB1, GRB2, TNFRSF1A, EGR1, ELL, XBP1, TRIB3, NAP1L1, NOSTRIN, GRN, ZNF148, CUX1, WIPF1, GPBP1, SREBF2, SREBF1, ATF7IP, LYN, UBN1, AEBP2, APBB2, NCOR2, NRIP1, RNF14, STAG2, PSEN1, JAK1, NCOA6, TICAM2, JMJD1C, CUL4A, KLF6, HAND1, UIMC1, LRRFIP2, GATA2, TCF7, CEBPG, PPM1D, MYC, CTNNBIP1, TOP1, TCF25, CAMK2D, RHOB, EZH2, ITGAV, JUP, CDKN1C, KLF11, EID1, FGFR1, BTG1, PAWR, RALBP1, STAMBP, DDX17, ELF1, EPN2, BCL2L1, MDM4, IGF2, MNT, DUSP1, CEBPD, GMCL1, TLE3, PDLIM1, STAT2, LCOR, IRF8, VHL, JARID1B, LITAF, DYNLT3, ATF5, KLF10, RBBP8, MEIS2, HIF1A, ING4, HIC1, PDCD4, NFX1, MAP2K1IP1, NFKBIA, NFAT5, SP1, MED15, FLNA (includes EG:2316), PLK2, ARID3A, MAPKAPK2, BLZF1, ARID5B, PXN, TAF6, MSL3L1, PPARD, NSD1, CIR (includes EG:9541), CEBPB, BIRC4, NFE2L1, IVNS1ABP, HTATSF1, RCOR1, ERBB2IP, PRKCI, WNK1, CDKN1B, ING1, LGALS1
	Positively regulated by PI3K	ADM, AKAP12, HTATIP2, TCF4, CREM, DDIT3, GADD45G, ATF5, BAZ1A, GCLC, ATP8B1, MYC, HSP90B1, GRINL1A, ATF4, FASLG, MED20, PCGF6, NSD1, TRIB3, CSH1, HCLS1, DAXX, BCL2L1, IGF2, FABP5, SREBF1, CYB5A, DNAJB6, SQSTM1, GATA3, ELF5
	Negatively regulated by PI3K	AKAP13, ARHGEF12, ATF3, EGR1, ITGA6, PRKCH, DNAJB1, PARD3, KLF2, TCF7L2, PRNP
Lipid Metabolism	Trophoblast stem cell-associated	PRDX3, GPX1, APEX1
	Differentiation-associated	HSD3B1, SCAP, CLN8, IDI1, INSIG1, CYP17A1, FDFT1, DHCR7, CD47, SCARB1, SMG1, PBEF1, CD24, CYP51A1, PLSCR1, CYP11A1, ITGB1, APOB48R, ABHD5, RAB5A, PPARD, STARD3, CEBPB, FDPS, LDLR, SYNJ1, DHCR24, SREBF2, SREBF1, PIK3CB, SLC27A1, DGAT1, HMGCR, LGALS1
	Positively regulated by PI3K	ADM, CYP17A1, BCL2L1, FABP5, CYB5A, CSH1, CSH2, HSD17B2, FASLG, PLSCR1
	Negatively regulated by PI3K	AMACR, SQLE, ACAT2, DGAT2, FDFT1, CYP26B1, DHCR7, A4GALT, MBTPS1, PCYT2, SPHK1, SERPINE1, HMGCR, MOGAT2, CYP51A1, PRNP

Table 6.2. Gene functions

Function	Group	Genes
Post-Translational Modification	Trophoblast stem cell-associated	HSPBP1, DNAJA4, SCO1, SF3B3, OCLN, CHAF1A, CD2AP, TCEB2, STUB1, TCP1, RUVBL2, DAG1, VAPB, TBCD, FKBP5, PPID, TP53, BCS1L, MKKS, TBCA, SLC9A3R1, HMGA1, CCT6A, ERP29, FKBP4, CCT7, CCT3, HFE
	Differentiation-associated	RNF139, TGFBR1, JAK1, DPM1, PIP5K3, PPM1D, MYC, HDAC6, SOD2, CAMK2D, SCARB1, EZH2, TSC2, CBLL1, ITGAV, SMG1, ATG12, PTPRE, TNKS, FGFR1, ELF1, SLC3A2, BCL2L1, ARRB2, WHSC1L1, CD9, DUSP1, RNF25, UBE2G1, BACE2, TLK2 (includes EG:11011), VHL, ADM, PIM3, SPN, FN1, PTPN14, ILK, PSENEN, CRK, ING4, DNAJA1, BAG2, USP2, PTPN12, UBE4A, SMURF1, HK1, CD47, NLK, CLK1, PPP3CB, RB1CC1, CD24, PLOD3, MTMR3, ITGB1, PPARD, ERO1L, NSD1, CLPX, USP33, BIRC4, SHB, ITGB2, STK38, CMAS, MYLIP, LYN, PSEN1, IRAK2, LGALS1
	Positively regulated by PI3K	
	Negatively regulated by PI3K	SLC9A3R1, APLP2, ITGA6, DUSP22, NCK2, CD2AP, PPP3CB, STUB1, PCMT1, SPHK1, UBE2D3, DAG1, DNAJB1, PPM2C, PARD3, PPP3CA, HFE

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1390511_at	LOC308394	Cgm4	10	1863	**193.51**
1378534_at	similar to brain carcinoembryonic antigen	LOC308394	10	1746	**183.10**
1388433_at	keratin complex 1, acidic, gene 19	Krt1-19	53	7958	**149.07**
1369029_at	phospholipid scramblase 1	Plscr1	20	2050	**101.12**
1389856_at	carcinoembryonic antigen gene family 4	Cgm4	57	5073	**89.73**
1368996_at	carcinoembryonic antigen-related cell adhesion molecule 3	Ceacam3	202	17879	**88.71**
1392832_at	similar to angiopoietin-like 1	LOC684489	17	1398	**83.66**
1377666_at	choline dehydrogenase	Chdh	26	1571	**60.59**
1368468_at	cytochrome P450, family 11, subfamily a, polypeptide 1	Cyp11a1	196	9216	**47.11**
1376934_x_at	similar to brain carcinoembryonic antigen	Cgm4	53	2146	**40.22**
1390525_a_at	stimulated by retinoic acid gene 6 homolog (mouse)	Stra6	28	1037	**37.44**
1382690_at	carcinoembryonic antigen gene family 4	Cgm4	81	2729	**33.86**
1367809_at	prolactin family 4, subfamily a, member 1	Prl4a1	683	22573	**33.05**
1383458_at	calcium channel, voltage-dependent, L type, alpha 1D subunit	Cacna1d	26	802	**30.89**
1370852_at	spleen protein 1 precursor	LOC171573	692	20968	**30.29**
1376036_at	transporter	LOC314323	29	875	**29.86**
1370227_at	prolactin family 8, subfamily a, member 7	Prl8a7	33	937	**28.78**
1374488_at	GRAM domain containing 1B (predicted)	Gramd1b_predicted	89	2514	**28.11**
1380077_at	Transcribed locus	---	191	5186	**27.18**
1376845_at	putative ISG12(b) protein	isg12(b)	158	4110	**26.02**
1387180_at	interleukin 1 receptor, type II	Il1r2	81	2075	**25.57**
1385925_at	Transcribed locus	---	262	6573	**25.12**
1387144_at	integrin alpha 1	Itga1	79	1976	**24.98**
1389092_at	interleukin 2 receptor, gamma (severe combined immunodeficiency)	Il2rg	62	1490	**24.09**
1368227_at	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	Slc28a2	240	5743	**23.94**
1383765_at	similar to RIKEN cDNA 4921520P21	MGC114388	36	863	**23.86**
1377264_at	interleukin 17F	Il17f	179	4036	**22.49**
1378483_at	prolactin family 7, subfamily b, member 1	Prl7b1	374	8240	**22.01**
1379598_at	adenylate cyclase 7	Adcy7	53	1153	**21.75**
1380768_at	Transcribed locus	---	85	1798	**21.23**
1370234_at	fibronectin 1	Fn1	712	14010	**19.68**
1391756_at	Transcribed locus, strongly similar to XP_344867.2 PREDICTED: similar to carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) [Rattus norvegicus]	---	856	16683	**19.50**
1369182_at	coagulation factor III	F3	46	857	**18.69**
1389394_at	Transcribed locus	---	129	2255	**17.50**
1373841_at	rac/cdc42 guanine nucleotide exchange factor 6	arhgef6	129	2219	**17.23**
1389780_at	tissue factor pathway inhibitor	Tfpi	497	8452	**17.01**
1376958_at	Similar to serine (or cysteine) proteinase inhibitor, clade B, member 9 (predicted)	RGD1562844_predict	294	4825	**16.40**
1389474_at	myosin regulatory light chain interacting protein (predicted)	Mylip_predicted	64	1016	**15.81**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1373900_at	keratin complex 2, basic, gene 7	Krt2-7	334	5219	**15.62**
1384934_at	Solute carrier family 41, member 2 (predicted)	Slc41a2_predicted	87	1354	**15.56**
1388471_at	t-complex 11 (mouse) like 2	Tcp11l2	145	2202	**15.20**
1368579_at	Prolactin family 2, subfamily a, member 1	Prl2a1	2414	36672	**15.19**
1377912_at	Transcribed locus	---	149	2207	**14.80**
1370471_at	prolactin family 6, subfamily a, member 1	Prl6a1	102	1481	**14.55**
1368623_at	CEA-related cell adhesion molecule 9	Ceacam9	618	8892	**14.38**
1376457_at	cysteine-rich secretory protein LCCL domain containing 2	Crispld2	101	1375	**13.59**
1371298_at	H19 fetal liver mRNA	H19	329	4313	**13.10**
1378400_at	similar to RIKEN cDNA 4632404H22 (predicted)	RGD1560129_predict	292	3703	**12.69**
1391693_at	ATPase, Class I, type 8B, member 1 (predicted)	Atp8b1_predicted	195	2456	**12.60**
1378134_at	ATPase, Class I, type 8B, member 1 (predicted)	Atp8b1_predicted	338	4260	**12.59**
1395267_at	CDNA clone IMAGE:7366335	---	95	1174	**12.41**
1369874_at	G protein-coupled receptor 1	Gpr1	77	956	**12.37**
1383474_at	interleukin-1 receptor-associated kinase 2	Irak2	103	1241	**12.01**
1375934_at	similar to RIKEN cDNA D330045A20 (predicted)	RGD1566282_predict	262	3127	**11.93**
1379390_at	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	St6galnac2	194	2191	**11.28**
1388703_at	endothelial cell adhesion molecule	Esam	160	1748	**10.92**
1379636_at	similar to hypothetical protein FLJ32954	LOC313840	266	2880	**10.81**
1387123_at	cytochrome P450, family 17, subfamily a, polypeptide 1	Cyp17a1	413	4444	**10.76**
1387156_at	hydroxysteroid (17-beta) dehydrogenase 2	Hsd17b2	507	5354	**10.56**
1382868_at	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A (predicted)	Sema6a_predicted	582	6041	**10.38**
1367841_a_at	prolactin family 8, subfamily a, member 9	Prl8a9	402	4135	**10.29**
1383554_at	similar to RIKEN cDNA D330045A20 (predicted)	RGD1566282_predict	309	3166	**10.26**
1387282_at	heat shock 22kDa protein 8	Hspb8	91	935	**10.24**
1390190_at	placenta-specific 1	Plac1	1268	12838	**10.13**
1376937_at	similar to 4631422O05Rik protein (predicted)	RGD1565927_predict	93	933	**10.08**
1388721_at	heat shock 22kDa protein 8	Hspb8	210	2061	**9.80**
1367577_at	heat shock 27kDa protein 1	Hspb1	97	940	**9.73**
1382949_at	retinol dehydrogenase 12 (predicted)	Rdh12_predicted	89	857	**9.64**
1370371_a_at	CEA-related cell adhesion molecule 1 /// CEA-related cell adhesion molecule 10	Ceacam1 /// Ceacam1	514	4941	**9.62**
1395408_at	nitric oxide synthase trafficker	Nostrin	299	2864	**9.59**
1381428_a_at	hemochromatosis	Hfe	344	3275	**9.51**
1385361_at	ATPase, class V, type 10A	Atp10a	585	5563	**9.51**
1381473_at	---	---	236	2237	**9.47**
1369957_at	regulator of G-protein signaling 5	Rgs5	136	1287	**9.44**
1390406_at	Rho GTPase activating protein 18 (predicted)	Arhgap18_predicted	434	4093	**9.42**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1379356_at	similar to RIKEN cDNA C230093N12 (predicted)	RGD1310037_predict	376	3386	**9.00**
1370874_at	Prolactin family 3, subfamily b, member 1	Prl3b1	1556	13469	**8.66**
1382017_at	Transcribed locus, moderately similar to XP_985665.1 PREDICTED: hypothetical protein LOC72575 [Mus musculus]	---	1611	13864	**8.60**
1375635_at	Transcribed locus	---	134	1144	**8.54**
1384277_at	pleckstrin homology domain containing, family H (with MyTH4 domain) member 1 (predicted)	Plekhh1_predicted	525	4478	**8.54**
1389353_at	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D (predicted)	Sema6d_predicted	496	4154	**8.38**
1379982_at	nuclear receptor interacting protein 1 (predicted)	Nrip1_predicted	131	1095	**8.35**
1384406_at	similar to C-C chemokine receptor type 11 (C-C CKR-11) (CC-CKR-11) (CCR-11) (Chemokine receptor-like 1) (CCRL1) (CCX CKR)	LOC685243	355	2960	**8.35**
1385926_at	Transcribed locus	---	119	993	**8.32**
1391871_at	similar to Serologically defined colon cancer antigen 13 (predicted)	RGD1564816_predict	109	895	**8.25**
1385251_at	hypothetical protein LOC500638	LOC500638	104	839	**8.08**
1391424_at	Transcribed locus	---	237	1875	**7.92**
1369559_a_at	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	Cd47	653	5153	**7.90**
1373433_at	nucleosome binding protein 1 (predicted) /// similar to Nucleosome binding protein 1 (Nucleosome binding protein 45) (NBP-45) (GARP45 protein)	LOC680182 /// LOC68	1093	8590	**7.86**
1370695_s_at	tribbles homolog 3 (Drosophila)	Trib3	293	2284	**7.81**
1395020_at	pleckstrin homology domain containing, family H (with MyTH4 domain) member 1 (predicted)	Plekhh1_predicted	235	1824	**7.75**
1371959_at	histone cluster 2, H2aa (predicted) /// similar to H2A histone family, member O	Hist2h2aa_predicted /	180	1393	**7.74**
1382225_at	Transcribed locus	---	290	2220	**7.65**
1373504_at	GLI pathogenesis-related 1 (glioma)	Glipr1	131	971	**7.43**
1376671_at	similar to Ferritin heavy chain (Ferritin H subunit) (Proliferation-inducing gene 15 protein)	LOC681066	1965	14549	**7.40**
1370941_at	platelet derived growth factor receptor, alpha polypeptide	Pdgfra	314	2316	**7.38**
1372084_at	protein tyrosine phosphatase 4a3 (predicted)	Ptp4a3_predicted	186	1371	**7.36**
1395557_at	core promoter element binding protein	Copeb	433	3134	**7.23**
1368321_at	early growth response 1	Egr1	170	1230	**7.23**
1390655_at	antisense paternally expressed gene 3	Apeg3	162	1149	**7.08**
1368735_a_at	transient receptor potential cation channel, subfamily V, member 2	Trpv2	231	1625	**7.03**
1379396_at	engulfment and cell motility 1, ced-12 homolog (C. elegans) (predicted)	Elmo1_predicted	116	804	**6.94**
1375707_at	---	---	1389	9639	**6.94**
1390021_at	histone cluster 1, H2bh	Hist1h2bh	482	3341	**6.93**
1390529_at	CD83 antigen (predicted)	Cd83_predicted	425	2926	**6.88**
1395010_at	Transcribed locus	---	194	1326	**6.83**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1382345_at	PCTAIRE-motif protein kinase 2	Pctk2	864	5892	**6.82**
1392547_at	hypothetical LOC302884	MGC105649	352	2391	**6.80**
1376498_at	similar to 2900002H16Rik protein (predicted)	RGD1307973_predict	558	3788	**6.79**
1386059_at	Transcribed locus	---	519	3497	**6.74**
1373881_at	Rho, GDP dissociation inhibitor (GDI) beta	Arhgdib	614	4099	**6.67**
1383531_at	similar to adult retina protein (predicted)	RGD1310862_predict	209	1367	**6.55**
1368080_at	response gene to complement 32	Rgc32	206	1333	**6.48**
1378126_at	similar to RIKEN cDNA B930096L08 (predicted)	RGD1306142_predict	189	1215	**6.42**
1368519_at	serine (or cysteine) peptidase inhibitor, clade E, member 1	Serpine1	349	2238	**6.41**
1388432_at	optineurin	Optn	161	1027	**6.39**
1376117_at	solute carrier family 44, member 4	Slc44a4	521	3326	**6.38**
1394599_at	similar to cDNA sequence BC022133 (predicted)	RGD1561296_predict	188	1177	**6.25**
1377880_at	Transcribed locus	---	292	1820	**6.23**
1395381_at	---	---	1060	6602	**6.23**
1373826_at	yippee-like 5 (Drosophila)	Ypel5	332	2049	**6.17**
1372308_at	CDNA clone IMAGE:7366335	---	285	1746	**6.12**
1367970_at	profilin 2	Pfn2	411	2514	**6.12**
1388698_at	extracellular matrix protein 1	Ecm1	1255	7660	**6.11**
1379724_at	similar to pleckstrin homology-like domain, family B, member 2	LOC685611	260	1572	**6.06**
1394931_at	muscleblind-like 2 (predicted)	Mbnl2_predicted	170	1027	**6.03**
1389538_at	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	Nfkbia	580	3402	**5.86**
1387219_at	adrenomedullin	Adm	229	1327	**5.81**
1378094_at	Transcribed locus	---	364	2110	**5.80**
1380622_at	Sil1 protein	RGD:735103	1153	6689	**5.80**
1367733_at	carbonic anhydrase 2	Ca2	340	1935	**5.69**
1372328_at	kinesin light chain 4	Klc4	192	1090	**5.67**
1371933_at	---	---	239	1355	**5.66**
1368948_at	moesin	Msn	438	2476	**5.66**
1369590_a_at	DNA-damage inducible transcript 3	Ddit3	582	3287	**5.65**
1386895_at	melanoma antigen, family D, 1	Maged1	2198	12337	**5.61**
1370950_at	phosphatidic acid phosphatase type 2B	Ppap2b	164	922	**5.61**
1380338_at	Transcribed locus	---	174	972	**5.60**
1386922_at	carbonic anhydrase 2	Ca2	244	1363	**5.59**
1387630_at	ELOVL family member 5, elongation of long chain fatty acids (yeast)	Elovl5	941	5256	**5.58**
1382118_at	Transcribed locus, strongly similar to NP_001029332.1 hypothetical protein LOC619476 [Rattus norvegicus]	---	748	4177	**5.58**
1389502_at	---	---	322	1796	**5.57**
1368488_at	nuclear factor, interleukin 3 regulated	Nfil3	180	995	**5.53**
1398319_at	Nclone10 mRNA	---	498	2744	**5.52**
1376304_at	similar to AI661453 protein (predicted)	RGD1561662_predict	194	1069	**5.50**
1367601_at	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	Cited2	1390	7635	**5.49**
1383715_at	similar to hypothetical protein D4Ertd89e	RGD1305703	352	1931	**5.49**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1394948_at	arrestin domain containing 3	Arrdc3	181	987	**5.46**
1386075_at	Myc induced nuclear antigen	Mina	153	830	**5.42**
1389430_at	Transcribed locus	---	230	1241	**5.39**
1397680_at	Transcribed locus	---	198	1053	**5.31**
1377405_at	Ral GEF with PH domain and SH3 binding motif 2	Ralgps2	299	1578	**5.28**
1384653_at	similar to melanoma ubiquitous mutated protein	LOC684135 /// LOC69	265	1397	**5.28**
1392264_s_at	serine (or cysteine) peptidase inhibitor, clade E, member 1	Serpine1	233	1226	**5.26**
1384769_a_at	similar to Zinc finger X-linked protein ZXDB (predicted) /// similar to Zinc finger X-linked protein ZXDB	LOC683508 /// LOC68	274	1434	**5.23**
1368275_at	sterol-C4-methyl oxidase-like	Sc4mol	1030	5389	**5.23**
1371575_at	moesin	Msn	1509	7862	**5.21**
1367696_at	interferon induced transmembrane protein 2 (1-8D)	Ifitm2	292	1514	**5.18**
1387498_a_at	Fibroblast growth factor receptor 1	Fgfr1	166	859	**5.17**
1367602_at	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	Cited2	4265	21976	**5.15**
1384037_at	signal-induced proliferation-associated 1 like 2	Sipa1l2	249	1284	**5.15**
1368578_at	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	Hsd3b1	1636	8421	**5.15**
1368679_a_at	Yamaguchi sarcoma viral (v-yes-1) oncogene homolog	Lyn	712	3645	**5.12**
1384216_at	---	---	184	938	**5.11**
1381942_at	hypermethylated in cancer 1 (predicted)	Hic1_predicted	638	3251	**5.09**
1374137_at	E74-like factor 1	Elf1	258	1313	**5.09**
1382891_at	similar to ubiquitin specific protease 27, X chromosome (predicted)	RGD1564628_predict	274	1392	**5.09**
1372760_at	CDNA clone IMAGE:7321089	---	241	1228	**5.09**
1387015_at	profilin 2	Pfn2	1014	5151	**5.08**
1388986_at	core promoter element binding protein	Copeb	626	3179	**5.08**
1371951_at	four and a half LIM domains 2	Fhl2	290	1464	**5.05**
1368489_at	fos-like antigen 1	Fosl1	997	5028	**5.04**
1383137_at	SRY-box containing gene 4 (predicted)	Sox4_predicted	266	1342	**5.04**
1389594_at	similar to Protein C20orf22 homolog	LOC499913	311	1557	**5.01**
1373270_at	WD repeat domain, phosphoinositide interacting 1 (predicted)	Wipi1_predicted	612	3044	**4.98**
1388921_at	Transcribed locus	---	223	1109	**4.97**
1369166_at	matrix metallopeptidase 9	Mmp9	245	1209	**4.93**
1390459_at	Transcribed locus	---	222	1095	**4.93**
1374480_at	dishevelled associated activator of morphogenesis 1 (predicted)	Daam1_predicted	1329	6544	**4.92**
1367894_at	insulin induced gene 1	Insig1	780	3836	**4.92**
1375910_at	CDC42 effector protein (Rho GTPase binding) 3 (predicted)	Cdc42ep3_predicted	492	2406	**4.90**
1371332_at	similar to Histone H1.2 (H1 VAR.1) (H1c)	LOC684681	1284	6284	**4.89**
1370688_at	glutamate-cysteine ligase, catalytic subunit	Gclc	1317	6423	**4.88**
1389554_at	nuclear receptor subfamily 2, group F, member 2	Nr2f2	532	2593	**4.88**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1372101_at	phosphatidic acid phosphatase type 2B	Ppap2b	462	2245	**4.86**
1368262_at	PH domain and leucine rich repeat protein phosphatase	Phlpp	642	3108	**4.84**
1368189_at	7-dehydrocholesterol reductase	Dhcr7	216	1037	**4.81**
1378128_at	Transcribed locus	---	314	1509	**4.81**
1390412_at	solute carrier family 39 (iron-regulated transporter), member 1	Slc40a1	177	850	**4.80**
1372097_at	Interferon regulatory factor 8	Irf8	465	2231	**4.80**
1387060_at	Kruppel-like factor 6	Klf6	819	3915	**4.78**
1375962_at	Transcribed locus	---	775	3694	**4.77**
1382955_at	G protein-coupled receptor 126 (predicted)	Gpr126_predicted	630	2983	**4.73**
1368430_at	legumain	Lgmn	3873	18305	**4.73**
1395745_at	aspartate-beta-hydroxylase (predicted)	Asph_predicted	574	2693	**4.69**
1383229_at	ATP-binding cassette, sub-family A (ABC1), member 7	Abca7	202	944	**4.68**
1371729_at	yippee-like 5 (Drosophila)	Ypel5	302	1404	**4.66**
1372069_at	ankyrin repeat domain 15	Ankrd15	490	2279	**4.66**
1372447_at	Fibroblast growth factor receptor 1 /// similar to fibroblast growth factor receptor 1 isoform 9 precursor	Fgfr1 /// LOC500109	306	1426	**4.65**
1369313_at	four and a half LIM domains 2	Fhl2	283	1313	**4.64**
1393050_at	hypothetical LOC302495	LOC302495	660	3062	**4.64**
1376057_at	Phosphodiesterase 8A	Pde8a	340	1572	**4.63**
1372523_at	glutamate-cysteine ligase, catalytic subunit	Gclc	1617	7445	**4.60**
1369248_a_at	baculoviral IAP repeat-containing 4	Birc4	726	3337	**4.60**
1398450_at	Transcribed locus	---	177	813	**4.60**
1390803_at	HIV TAT specific factor 1 (predicted)	Htatsf1_predicted	4311	19468	**4.52**
1389453_at	RAD52 motif 1 (predicted)	Rdm1_predicted	201	905	**4.50**
1389918_at	Transcribed locus, strongly similar to XP_979526.1 PREDICTED: similar to palladin isoform 1 [Mus musculus]	---	608	2733	**4.50**
1383216_at	similar to mKIAA0431 protein	RGD1305781	563	2518	**4.48**
1370351_at	tudor domain containing 7	Tdrd7	342	1530	**4.47**
1388109_at	G protein-coupled receptor 116	Gpr116	1465	6540	**4.46**
1370379_at	protease, serine, 8 (prostasin)	Prss8	264	1173	**4.43**
1372836_at	Transcribed locus	---	371	1643	**4.43**
1368885_at	ectonucleoside triphosphate diphosphohydrolase 1	Entpd1	491	2177	**4.43**
1370694_at	tribbles homolog 3 (Drosophila)	Trib3	266	1176	**4.42**
1382535_at	potassium channel tetramerisation domain containing 12 (predicted)	Kctd12_predicted	513	2266	**4.42**
1381353_at	similar to melanoma antigen family A, 5	MGC114427	875	3863	**4.41**
1375140_at	muscleblind-like 2 (predicted)	Mbnl2_predicted	209	920	**4.41**
1367571_a_at	insulin-like growth factor 2	Igf2	3273	14329	**4.38**
1386321_s_at	tribbles homolog 3 (Drosophila)	Trib3	695	3032	**4.37**
1368549_at	high mobility group box transcription factor 1	Hbp1	722	3146	**4.36**
1379371_at	Transcribed locus	---	352	1532	**4.35**
1372175_at	similar to DNA segment, Chr 10, University of California at Los Angeles 1 (predicted)	RGD1560401_predict	677	2942	**4.35**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1367741_at	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	Herpud1	642	2781	**4.33**
1393346_at	similar to RIKEN cDNA 5830436D01 (predicted)	RGD1561673_predict	384	1663	**4.33**
1369358_a_at	huntingtin-associated protein 1	Hap1	433	1872	**4.32**
1371883_at	monocyte to macrophage differentiation-associated	Mmd	847	3656	**4.31**
1368009_at	glucosamine	Gne	514	2214	**4.31**
1388991_at	similar to SEC14 and spectrin domains 1 (predicted)	RGD1562244_predict	417	1795	**4.30**
1389318_at	dishevelled associated activator of morphogenesis 1 (predicted)	Daam1_predicted	2514	10742	**4.27**
1398312_s_at	solute carrier family 14 (urea transporter), member 2	Slc14a2	473	2020	**4.27**
1377925_at	Transcribed locus	---	245	1045	**4.27**
1372390_at	Transcribed locus	---	2908	12366	**4.25**
1388348_at	ELOVL family member 5, elongation of long chain fatty acids (yeast)	Elovl5	5188	22056	**4.25**
1367614_at	annexin A1	Anxa1	3856	16381	**4.25**
1389500_at	similar to ras homolog gene family, member U	LOC678766	430	1823	**4.24**
1377213_at	similar to protein kinase/endoribonuclease(IRE1) alpha (predicted)	RGD1559716_predict	543	2299	**4.23**
1372475_at	PTEN induced putative kinase 1 (predicted)	Pink1_predicted	267	1128	**4.22**
1389255_at	Cadherin 5 (predicted)	Cdh5_predicted	1488	6278	**4.22**
1389301_at	muscleblind-like 2 (predicted)	Mbnl2_predicted	2885	12146	**4.21**
1389986_at	CDNA clone IMAGE:7321089	---	1401	5882	**4.20**
1383131_at	integrin beta 2	Itgb2	719	3016	**4.19**
1374379_at	dual specificity phosphatase 23 (predicted)	Dusp23_predicted	221	923	**4.18**
1367650_at	lipocalin 7	Lcn7	736	3075	**4.18**
1367628_at	lectin, galactose binding, soluble 1	Lgals1	1807	7510	**4.16**
1373286_at	filamin binding LIM protein 1	Fblim1	1129	4688	**4.15**
1388464_at	similar to cullin 7	LOC680835	1167	4841	**4.15**
1398397_at	similar to Parg1-pending protein	RGD1306185	1049	4348	**4.15**
1371537_at	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5 (predicted)	B4galt5_predicted	3881	16060	**4.14**
1367651_at	cathepsin D	Ctsd	2606	10770	**4.13**
1383528_at	similar to mKIAA0215 protein (predicted)	RGD1563945_predict	391	1614	**4.12**
1398333_at	endothelial PAS domain protein 1	Epas1	209	861	**4.12**
1382210_at	hypothetical LOC302495	LOC302495	304	1251	**4.11**
1399069_at	Transcribed locus	---	241	982	**4.07**
1372160_at	bladder cancer associated protein homolog (human)	Blcap	1090	4427	**4.06**
1390486_at	ubiquitin specific protease 32 (predicted)	Usp32_predicted	688	2788	**4.05**
1368254_a_at	sphingosine kinase 1	Sphk1	218	883	**4.05**
1374489_at	GTP binding protein 2	Gtpbp2	204	825	**4.05**
1369788_s_at	Jun oncogene	Jun	459	1852	**4.04**
1395353_at	similar to KIAA0368 (predicted)	RGD1306148_predict	237	956	**4.04**
1374429_at	Transcribed locus	---	547	2194	**4.01**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1398275_at	matrix metallopeptidase 9	Mmp9	530	2120	**4.00**
1373818_at	potassium channel tetramerisation domain containing 12 (predicted)	Kctd12_predicted	1257	5024	**4.00**
1391672_at	Transcribed locus	---	241	961	**3.99**
1384652_at	HIV TAT specific factor 1 (predicted)	Htatsf1_predicted	272	1082	**3.98**
1368884_at	ectonucleoside triphosphate diphosphohydrolase 1	Entpd1	355	1410	**3.98**
1381705_at	pantothenate kinase 3 (predicted)	Pank3_predicted	707	2792	**3.95**
1384508_at	Transcribed locus, moderately similar to NP_033341.1 TAF6 RNA polymerase II, TATA box binding protein (TBP)-associated factor [Mus musculus]	---	568	2237	**3.94**
1368106_at	polo-like kinase 2 (Drosophila)	Plk2	309	1211	**3.92**
1394086_at	SUMO1/sentrin specific protease 7 (predicted)	Senp7_predicted	354	1386	**3.92**
1372977_at	similar to mitogen-activated protein kinase kinase kinase kinase 5 isoform 2 (predicted)	RGD1562028_predict	712	2789	**3.92**
1389107_at	similar to KIAA1749 protein (predicted)	RGD1304623_predict	914	3575	**3.91**
1371115_at	protein tyrosine phosphatase, receptor type, E	Ptpre	253	991	**3.91**
1369032_at	bladder cancer associated protein homolog (human)	Blcap	417	1626	**3.90**
1373019_at	epidermal growth factor receptor pathway substrate 15	Eps15	563	2190	**3.89**
1372299_at	cyclin-dependent kinase inhibitor 1C (P57)	Cdkn1c	9367	36398	**3.89**
1371440_at	Beta-2 microglobulin	B2m	312	1207	**3.87**
1396036_at	Ral GEF with PH domain and SH3 binding motif 2	Ralgps2	276	1068	**3.87**
1376600_at	Transcribed locus	---	1810	7008	**3.87**
1380229_at	Transcribed locus	---	312	1201	**3.85**
1371692_at	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 11	Mllt11	208	802	**3.85**
1367932_at	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	Hmgcs1	511	1966	**3.85**
1373363_at	microtubule-associated protein 1b	Map1b	976	3759	**3.85**
1373231_at	OTU domain containing 5	Otud5	1026	3947	**3.85**
1391495_at	Similar to RIKEN cDNA 2010012O05 (predicted)	RGD1311783_predict	359	1378	**3.84**
1394451_at	annexin A1	Anxa1	227	871	**3.84**
1367519_at	oxysterol binding protein-like 2	Osbpl2	536	2051	**3.83**
1383744_at	Transcribed locus	---	567	2163	**3.82**
1390832_at	transmembrane and coiled-coil domain family 3 (predicted)	Tmcc3_predicted	239	913	**3.82**
1388403_at	similar to NADP+-specific isocitrate dehydrogenase	LOC361596	260	993	**3.81**
1392267_at	similar to RIKEN cDNA B930096L08 (predicted)	RGD1306142_predict	253	965	**3.81**
1393037_at	SH3 domain binding glutamic acid-rich protein like 2 (predicted)	Sh3bgrl2_predicted	281	1071	**3.81**
1387587_at	Fas ligand (TNF superfamily, member 6)	Faslg	1816	6909	**3.80**
1382086_at	Transcribed locus	---	413	1568	**3.79**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1377810_at	Ral GEF with PH domain and SH3 binding motif 2	Ralgps2	262	989	**3.78**
1375548_at	similar to RIKEN cDNA 4732418C07 (predicted)	RGD1310351_predict	368	1387	**3.77**
1370898_at	stannin	Snn	298	1121	**3.76**
1370156_at	prion protein	Prnp	1723	6476	**3.76**
1387502_at	serine/threonine kinase 17b (apoptosis-inducing)	Stk17b	577	2162	**3.75**
1367839_at	farnesyl diphosphate farnesyl transferase 1	Fdft1	402	1503	**3.74**
1371765_at	histone 2a	H2a	592	2215	**3.74**
1376040_at	signal-induced proliferation-associated 1 like 2	Sipa1l2	2200	8228	**3.74**
1375170_at	S100 calcium binding protein A11 (calizzarin)	S100a11	4687	17470	**3.73**
1388725_at	leptin receptor overlapping transcript	Leprot	339	1261	**3.72**
1395274_at	dystonin (predicted)	Dst_predicted	641	2380	**3.71**
1368241_a_at	flotillin 1	Flot1	853	3152	**3.69**
1369305_at	RAB3A interacting protein (rabin3)-like 1	Rab3il1	310	1143	**3.69**
1380334_at	hypothetical RNA binding protein RGD1359713	RGD1359713	1317	4830	**3.67**
1374943_at	similar to RIKEN cDNA 2010011I20 (predicted)	RGD1311378_predict	420	1535	**3.66**
1373962_at	ubiquitin specific protease 32 (predicted)	Usp32_predicted	1241	4519	**3.64**
1383478_at	Janus kinase 1	Jak1	780	2841	**3.64**
1392785_at	potassium channel tetramerisation domain containing 12 (predicted)	Kctd12_predicted	1914	6965	**3.64**
1388169_at	jumonji domain containing 1C	Jmjd1c	1200	4369	**3.64**
1395887_at	similar to RIKEN cDNA 1600029D21	LOC363060	3928	14289	**3.64**
1394639_at	similar to CDC42 small effector 2 (predicted) /// hypothetical protein LOC686503 /// hypothetical protein LOC691031	LOC686503 /// LOC69	651	2365	**3.64**
1382060_at	Transcribed locus	---	366	1325	**3.62**
1375672_at	Transcribed locus	---	449	1622	**3.61**
1387550_a_at	solute carrier family 14 (urea transporter), member 2	Slc14a2	1770	6365	**3.60**
1377923_at	START domain containing 8 (predicted)	Stard8_predicted	324	1165	**3.59**
1382001_at	similar to Myeloid/lymphoid or mixed-lineage leukemia protein 3 homolog (Histone-lysine N-methyltransferase, H3 lysine-4 specific MLL3)	LOC502710	778	2795	**3.59**
1391012_at	Transcribed locus	---	266	955	**3.59**
1368025_at	DNA-damage-inducible transcript 4	Ddit4	814	2917	**3.59**
1389507_at	neural precursor cell expressed, developmentally down-regulated 4-like	Nedd4l	228	816	**3.58**
1371689_at	eukaryotic translation elongation factor 1 alpha 1	Eef1a1	302	1079	**3.58**
1372115_at	similar to ubiquitin protein ligase E3 component n-recognin 2	LOC363188	817	2920	**3.58**
1398302_at	prolactin family 7, subfamily a, member 3	Prl7a3	10970	39208	**3.57**
1389791_at	ceroid-lipofuscinosis, neuronal 8	Cln8	949	3393	**3.57**
1393345_at	aspartate-beta-hydroxylase (predicted)	Asph_predicted	713	2546	**3.57**
1379934_at	lysosomal trafficking regulator	Lyst	227	811	**3.57**
1397509_x_at	reproductive homeobox on X chromosome, 9	Rhox9	3486	12417	**3.56**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1388251_at	protein kinase C, iota	Prkci	250	888	**3.56**
1374642_at	zinc finger protein 64	Zfp64	1510	5359	**3.55**
1395770_at	Transcribed locus	---	364	1289	**3.55**
1399152_at	epidermal growth factor receptor pathway substrate 15	Eps15	702	2486	**3.54**
1374404_at	Jun oncogene	Jun	259	912	**3.53**
1389315_at	G protein-coupled receptor kinase-interactor 2	Git2	705	2489	**3.53**
1393845_a_at	transmembrane channel-like gene family 4	Tmc4	278	982	**3.53**
1374675_at	similar to D7H11orf15 protein (predicted)	RGD1310775_predict	295	1039	**3.53**
1386998_at	aldolase C	Aldoc	283	997	**3.53**
1371431_at	peroxisome biogenesis factor 5 (predicted)	Pex5_predicted	726	2550	**3.51**
1387260_at	Kruppel-like factor 4 (gut)	Klf4	395	1387	**3.51**
1375899_at	similar to HTPAP protein (predicted)	RGD1559978_predict	250	878	**3.51**
1391083_at	Rho GTPase activating protein 22 (predicted)	Arhgap22_predicted	586	2052	**3.50**
1391279_at	Scinderin	Scin	1137	3965	**3.49**
1393334_at	Transcribed locus	---	302	1049	**3.47**
1380909_at	Transcribed locus	---	939	3251	**3.46**
1373184_at	similar to chondroitin sulfate GalNAcT-2 (predicted)	RGD1563660_predict	400	1384	**3.46**
1375658_at	Transcribed locus	---	1215	4200	**3.46**
1376861_at	similar to RIKEN cDNA 1810018L05 /// similar to motile sperm domain containing 1	LOC317312 /// LOC68	308	1062	**3.44**
1395579_at	DEAH (Asp-Glu-Ala-His) box polypeptide 32 (predicted)	Dhx32_predicted	417	1433	**3.44**
1389528_s_at	Jun oncogene	Jun	1206	4145	**3.44**
1394346_at	mannosidase, alpha, class 1A, member 2 (predicted)	Man1a2_predicted	478	1641	**3.43**
1368147_at	dual specificity phosphatase 1	Dusp1	420	1441	**3.43**
1386662_at	similar to Sestrin 2 (Hi95) (predicted)	RGD1566319_predict	318	1090	**3.43**
1385089_at	grainyhead-like 1 (Drosophila) (predicted)	Grhl1_predicted	659	2258	**3.43**
1388945_at	similar to 1300014I06Rik protein	RGD1311307	496	1691	**3.41**
1392542_at	similar to CDC42 small effector 2 (predicted) /// hypothetical protein LOC686503 /// hypothetical protein LOC691031	LOC686503 /// LOC69	1785	6058	**3.39**
1383880_at	integrin alpha V (predicted)	Itgav_predicted	427	1449	**3.39**
1386893_at	granulin	Grn	3709	12577	**3.39**
1387703_a_at	ubiquitin specific peptidase 2	Usp2	374	1270	**3.39**
1373373_at	glutamine fructose-6-phosphate transaminase 1	Gfpt1	914	3097	**3.39**
1387870_at	zinc finger protein 36	Zfp36	2194	7419	**3.38**
1379275_at	sorting nexin 10	Snx10	373	1259	**3.37**
1384060_at	Janus kinase 1	Jak1	318	1071	**3.37**
1378420_at	Transcribed locus	---	338	1137	**3.37**
1373546_at	Putative UA20 protein	Ua20	723	2429	**3.36**
1389401_at	---	---	309	1036	**3.35**
1387227_at	Wiskott-Aldrich syndrome protein interacting protein	Waspip	374	1252	**3.35**
1392802_at	similar to RIKEN cDNA 4930431E10 (predicted) /// similar to Smad ubiquitination regulatory factor 1 isoform 2	LOC690516 /// RGD1	289	966	**3.35**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1367538_at	---	---	246	821	**3.34**
1377968_at	Transcribed locus	---	1748	5831	**3.34**
1398287_at	plasminogen activator, urokinase	Plau	488	1623	**3.33**
1390842_at	transcription factor AP-2, alpha (predicted)	Tcfap2a_predicted	727	2419	**3.33**
1376728_at	protein phosphatase 1, regulatory (inhibitor) subunit 8 (predicted)	Ppp1r8_predicted	1426	4728	**3.31**
1393234_at	Transcribed locus, moderately similar to XP_994560.1 PREDICTED: similar to Rho guanine exchange factor 16 [Mus musculus]	---	514	1704	**3.31**
1397729_x_at	similar to RIKEN cDNA 1600029D21	LOC363060	5827	19300	**3.31**
1395320_s_at	reproductive homeobox on X chromosome, 9	Rhox9	3386	11184	**3.30**
1387065_at	phospholipase C, delta 4	Plcd4	906	2990	**3.30**
1388714_at	elongation factor RNA polymerase II (predicted)	Ell_predicted	532	1754	**3.30**
1373066_at	Transcribed locus	---	359	1185	**3.30**
1370930_at	---	---	402	1326	**3.30**
1393294_at	cytoplasmic polyadenylation element binding protein 4 (predicted)	Cpeb4_predicted	569	1874	**3.29**
1377141_at		LOC498685	257	845	**3.29**
1392929_at	RGD1565616 (predicted)	RGD1565616_predict	323	1054	**3.27**
1393683_at	similar to KIAA0368 (predicted)	RGD1306148_predict	487	1588	**3.26**
1377911_at	Transcribed locus	---	826	2684	**3.25**
1389230_at	arrestin domain containing 3	Arrdc3	987	3196	**3.24**
1387101_at	acyl-CoA synthetase long-chain family member 4	Acsl4	1107	3582	**3.24**
1377636_at	Transcribed locus, strongly similar to XP_579796.1 PREDICTED: hypothetical protein XP_579796 [Rattus norvegicus]	---	277	894	**3.23**
1398598_at	dystonin (predicted)	Dst_predicted	2248	7260	**3.23**
1383590_at	similar to chondroitin beta1,4 N-acetylgalactosaminyltransferase (predicted)	RGD1307618_predict	480	1551	**3.23**
1386581_at	integrin alpha V (predicted)	Itgav_predicted	492	1586	**3.22**
1371684_at	pelota homolog	Pelo	938	3023	**3.22**
1369954_at	isocitrate dehydrogenase 1 (NADP+), soluble	Idh1	387	1248	**3.22**
1388130_at	zyxin	Zyx	2053	6605	**3.22**
1371071_at	guanine nucleotide binding protein beta 4 subunit	Gnb4	745	2396	**3.22**
1377307_at	similar to mammary tumor virus receptor 2 isoform 2	RGD1309879	376	1210	**3.22**
1393236_at	RIO kinase 3 (yeast) (predicted)	Riok3_predicted	2953	9487	**3.21**
1387675_at	plasminogen activator, urokinase	Plau	762	2444	**3.21**
1393159_at	glutaredoxin 2 (thioltransferase)	Glrx2	715	2293	**3.21**
1398327_at	pleckstrin homology domain containing, family C (with FERM domain) member 1	Plekhc1	2131	6832	**3.21**
1370128_at	heart and neural crest derivatives expressed transcript 1	Hand1	1169	3745	**3.20**
1371781_at	signal transducer and activator of transcription 3	Stat3	360	1152	**3.20**
1373313_at	Sel1 (suppressor of lin-12) 1 homolog (C. elegans)	Sel1h	736	2354	**3.20**
1373088_at	hypothetical protein LOC682888	LOC682888	2270	7243	**3.19**
1382807_at	similar to hypothetical protein	RGD1310450	415	1322	**3.18**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1394047_at	pregnancy specific beta-1-glycoprotein 4	Psg4	772	2444	**3.17**
1384865_at	Transcribed locus, strongly similar to XP_346369.1 PREDICTED: similar to leucine zipper, down-regulated in cancer 1 [Rattus norvegicus]	---	1759	5568	**3.16**
1384413_at	similar to solute carrier family 35, member A5 (predicted)	RGD1564361_predict	290	917	**3.16**
1374903_at	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	Gcnt2	495	1560	**3.15**
1373872_at	Transcribed locus, strongly similar to XP_574462.1 PREDICTED: similar to hypothetical protein C230069C04 [Rattus norvegicus]	---	340	1068	**3.14**
1384791_at	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1 (predicted)	B3gnt1_predicted	558	1752	**3.14**
1371499_at	CD9 antigen	Cd9	2729	8573	**3.14**
1383686_at	synaptogyrin 1	Syngr1	778	2435	**3.13**
1386967_at	ras homolog gene family, member Q	Rhoq	3994	12485	**3.13**
1398719_at	chromodomain protein, Y chromosome-like 2 (predicted)	Cdyl2_predicted	266	830	**3.12**
1390987_at	Transcribed locus	---	316	984	**3.11**
1392995_at	similar to zinc finger protein 322a	LOC680201 /// LOC68	362	1127	**3.11**
1385052_at	RAB3A interacting protein (rabin3)-like 1	Rab3il1	275	856	**3.11**
1384182_at	pleckstrin homology domain containing, family C (with FERM domain) member 1	Plekhc1	562	1746	**3.11**
1368213_at	P450 (cytochrome) oxidoreductase	Por	701	2170	**3.10**
1389971_at	sarcoglycan, epsilon	Sgce	1394	4316	**3.10**
1387109_at	P450 (cytochrome) oxidoreductase	Por	677	2095	**3.10**
1390117_at	Transcribed locus	---	294	909	**3.09**
1398362_at	Notch gene homolog 2 (Drosophila)	Notch2	1932	5957	**3.08**
1372854_at	tetratricopeptide repeat domain 17	Ttc17	308	947	**3.08**
1369958_at	ras homolog gene family, member B	Rhob	2343	7202	**3.07**
1381111_at	Transcribed locus	---	263	808	**3.07**
1372771_at	Transcribed locus	---	329	1010	**3.07**
1375421_a_at	praja 2, RING-H2 motif containing	Pja2	711	2181	**3.07**
1394706_at	pantothenate kinase 3 (predicted)	Pank3_predicted	716	2193	**3.06**
1387130_at	solute carrier family 39 (iron-regulated transporter), member 1	Slc40a1	545	1669	**3.06**
1383628_at	Transcribed locus	---	324	990	**3.06**
1387883_a_at	thymosin, beta 4	Tmsb4x	1203	3671	**3.05**
1373626_at	Transcribed locus	---	2412	7341	**3.04**
1394511_at	myb-like, SWIRM and MPN domains 1 (predicted)	Mysm1_predicted	266	808	**3.04**
1369943_at	transglutaminase 2, C polypeptide	Tgm2	319	968	**3.04**
1389483_at	Dpy-19-like 1 (C. elegans) (predicted)	Dpy19l1_predicted	1622	4925	**3.04**
1372888_at	ubiquitination factor E4A, UFD2 homolog (S. cerevisiae)	Ube4a	380	1151	**3.03**
1387867_at	aldehyde dehydrogenase family 9, subfamily A1	Aldh9a1	2060	6241	**3.03**
1388700_at	CDNA clone IMAGE:7317367	---	781	2359	**3.02**
1384205_at	N-glycanase 1	Ngly1	342	1032	**3.02**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1382818_at	similar to hypothetical protein D630010C10	RGD1309752	570	1721	**3.02**
1374066_at	cell division cycle 2-like 6 (CDK8-like) (predicted)	Cdc2l6_predicted	298	898	**3.02**
1389639_at	similar to Shb protein (predicted)	RGD1565350_predict	315	949	**3.01**
1370245_at	cathepsin L	Ctsl	3411	10278	**3.01**
1389418_at	---	---	306	920	**3.01**
1375637_at	similar to RIKEN cDNA 1110003E01	RGD1311122	348	1044	**3.00**
1371755_at	similar to RIKEN cDNA B230219D22 (predicted)	RGD1566359_predict	1323	3969	**3.00**
1382044_at	hypothetical protein LOC498796	LOC498796	397	1187	**2.99**
1367857_at	fatty acid desaturase 1	Fads1	281	837	**2.98**
1372223_at	cytoplasmic polyadenylation element binding protein 4 (predicted)	Cpeb4_predicted	890	2640	**2.97**
1389511_s_at	---	---	558	1650	**2.96**
1372106_at	EH-domain containing 4	Ehd4	428	1265	**2.96**
1387848_at	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Hmgcr	407	1203	**2.96**
1371249_at	X-box binding protein 1	Xbp1	3233	9555	**2.96**
1375944_at	acyl-CoA synthetase short-chain family member 2 (predicted)	Acss2_predicted	1423	4202	**2.95**
1376489_at	son of sevenless homolog 2 (Drosophila)	Sos2	1224	3608	**2.95**
1389617_at	ELK3, member of ETS oncogene family (predicted)	Elk3_predicted	938	2761	**2.94**
1396191_at	Eukaryotic translation initiation factor 5B	Eif5b	859	2527	**2.94**
1369953_a_at	CD24 antigen	Cd24	6918	20201	**2.92**
1378154_at	Transcribed locus	---	325	948	**2.92**
1394815_at	Transcribed locus	---	304	888	**2.92**
1377820_a_at	arginine-tRNA-protein transferase 1 (predicted)	Ate1_predicted	2862	8342	**2.91**
1368126_at	acetoacetyl-CoA synthetase	Aacs	304	887	**2.91**
1388448_at	Hypothetical protein LOC691031	LOC691031	2314	6710	**2.90**
1368146_at	dual specificity phosphatase 1	Dusp1	1321	3831	**2.90**
1367646_at	cathepsin B	Ctsb	6967	20177	**2.90**
1388690_at	breast cancer metastasis-suppressor 1-like (predicted)	Brms1l_predicted	1492	4316	**2.89**
1393824_at	Transcribed locus	---	309	893	**2.89**
1370129_at	meningioma expressed antigen 5 (hyaluronidase)	Mgea5	750	2164	**2.89**
1398923_at	similar to D11Bwg0434e protein (predicted)	RGD1305687_predict	2244	6474	**2.88**
1397417_at	Transcribed locus	---	536	1545	**2.88**
1374433_at	similar to Ferritin light chain (Ferritin L subunit) (predicted)	RGD1563902_predict	908	2617	**2.88**
1367759_at	H1 histone family, member 0	H1f0	2742	7894	**2.88**
1375349_at	similar to sorbin and SH3 domain containing 1 isoform 3 /// sorbin and SH3 domain containing 1 (predicted)	LOC678826 /// Sorbs1	437	1259	**2.88**
1379555_at	Transcribed locus	---	782	2248	**2.88**
1374808_at	similar to hypothetical protein FLJ21156 (predicted)	RGD1309823_predict	313	900	**2.87**
1373164_at	serine/threonine kinase 17b (apoptosis-inducing)	Stk17b	1294	3710	**2.87**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1381480_at	sterile alpha and TIR motif containing 1 (predicted)	Sarm1_predicted	309	885	**2.87**
1375027_at	Cell cycle progression 1 (predicted)	Ccpg1_predicted	403	1150	**2.86**
1373127_at	tripartite motif-containing 44	Trim44	683	1951	**2.86**
1382500_at	similar to Sestrin 2 (Hi95) (predicted)	RGD1566319_predict	772	2198	**2.85**
1389488_at	---	---	5790	16463	**2.84**
1371953_at	cyclin G2 (predicted)	Ccng2_predicted	300	851	**2.84**
1392955_at	Sel1 (suppressor of lin-12) 1 homolog (C. elegans)	Sel1h	429	1216	**2.83**
1393643_at	reticulocalbin 1 (predicted)	Rcn1_predicted	633	1791	**2.83**
1375700_at	kelch-like 22 (Drosophila) (predicted)	Klhl22_predicted	1141	3226	**2.83**
1372779_at	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1 (predicted)	B3gnt1_predicted	1543	4355	**2.82**
1368011_at	ferredoxin reductase	Fdxr	407	1147	**2.82**
1368207_at	FXYD domain-containing ion transport regulator 5	Fxyd5	663	1866	**2.81**
1399030_at	WD repeat domain 45	Wdr45	306	859	**2.81**
1370010_at	lysosomal membrane glycoprotein 2	Lamp2	3193	8940	**2.80**
1374525_at	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1 (predicted)	Raph1_predicted	531	1483	**2.79**
1368947_at	growth arrest and DNA-damage-inducible 45 alpha	Gadd45a	301	840	**2.79**
1385888_at	Transcribed locus	---	319	888	**2.78**
1375174_at	dpy-19-like 1 (C. elegans) (predicted)	Dpy19l1_predicted	979	2719	**2.78**
1390731_at	Transcribed locus	---	825	2292	**2.78**
1376990_at	similar to HCDI protein (predicted)	RGD1309307_predict	471	1306	**2.77**
1375723_at	Transcribed locus	---	535	1485	**2.77**
1390103_at	PHD finger protein 2 (predicted)	Phf2_predicted	459	1271	**2.77**
1388872_at	isopentenyl-diphosphate delta isomerase	Idi1	569	1575	**2.77**
1369027_at	alpha 1,4-galactosyltransferase	A4galt	958	2643	**2.76**
1380665_at	CGI-58-like protein	LOC316122	637	1756	**2.76**
1387847_at	phosphatidylinositol 3-kinase, catalytic, beta polypeptide	Pik3cb	916	2517	**2.75**
1367936_at	serine/threonine kinase 10	Stk10	1232	3385	**2.75**
1372129_at	similar to TBC1 domain family, member 8; BUB2-like protein 1; vascular Rab-GAP/TBC-containing (predicted)	RGD1310147_predict	668	1836	**2.75**
1390128_at	similar to chromatin modifying protein 1B	LOC682926 /// LOC68	755	2073	**2.74**
1384089_at	RAB guanine nucleotide exchange factor (GEF) 1 (predicted)	Rabgef1_predicted	399	1094	**2.74**
1393915_at	membrane bound O-acyltransferase domain containing 5	Mboat5	1067	2927	**2.74**
1370071_at	adenosine deaminase	Ada	1983	5441	**2.74**
1367667_at	farnesyl diphosphate synthase	Fdps	1784	4893	**2.74**
1389282_at	Integrin alpha 3 (predicted)	Itga3_predicted	414	1134	**2.74**
1367874_at	ras homolog gene family, member Q	Rhoq	2717	7443	**2.74**
1387116_at	DnaJ (Hsp40) homolog, subfamily B, member 9	Dnajb9	1095	2995	**2.74**
1385691_at	similar to RIKEN cDNA 5830435K17 (predicted)	RGD1560454_predict	306	835	**2.73**
1368878_at	isopentenyl-diphosphate delta isomerase	Idi1	1910	5214	**2.73**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1382901_at	growth factor independent 1B (predicted)	Gfi1b_predicted	338	924	**2.73**
1372781_at	WD repeat domain 47	Wdr47	484	1317	**2.72**
1369689_at	N-ethylmaleimide sensitive fusion protein	Nsf	659	1792	**2.72**
1389150_at	Transcribed locus	---	547	1488	**2.72**
1373742_at	splA/ryanodine receptor domain and SOCS box containing 2	Spsb2	385	1046	**2.72**
1376315_at	similar to putative alpha-mannosidase	RGD1303074	1313	3551	**2.70**
1372638_at	Rho guanine nucleotide exchange factor 7	Arhgef7	696	1881	**2.70**
1367715_at	tumor necrosis factor receptor superfamily, member 1a	Tnfrsf1a	1317	3548	**2.70**
1391607_at	similar to Sestrin 2 (Hi95) (predicted)	RGD1566319_predict	1017	2739	**2.69**
1373775_at	NEDD8 ultimate buster-1	Nub1	321	862	**2.69**
1373650_at	cytidine monophospho-N-acetylneuraminic acid synthetase	Cmas	1946	5231	**2.69**
1371783_at	heat shock protein	LOC290549	2201	5915	**2.69**
1392653_at	Transcribed locus	---	493	1321	**2.68**
1383799_at	Transcribed locus, strongly similar to NP_001026482.1 phosphoinositide-3-kinase, catalytic, beta polypeptide [Gallus gallus]	---	734	1967	**2.68**
1367869_at	oxidation resistance 1	Oxr1	319	854	**2.68**
1377644_at	similar to RIKEN cDNA 4921524J17 (predicted)	RGD1308706_predict	1886	5051	**2.68**
1375889_at	spermine synthase	Sms	504	1348	**2.68**
1369279_at	dehydrogenase/reductase (SDR family) member 9	Dhrs9	698	1865	**2.67**
1371975_at	RNA binding motif, single stranded interacting protein 2	Rbms2	563	1504	**2.67**
1383655_at	TNFAIP3 interacting protein 2	Tnip2	361	963	**2.66**
1397386_at	AF4/FMR2 family, member 4 (predicted)	Aff4_predicted	528	1399	**2.65**
1393718_at	similar to DKFZP564P1916 protein (predicted)	RGD1311704_predict	401	1061	**2.65**
1390114_at	myelin protein zero-like 1	Mpzl1	3057	8085	**2.64**
1374964_at	dipeptidylpeptidase 8 (predicted)	Dpp8_predicted	447	1182	**2.64**
1373996_at	Transcribed locus	---	568	1501	**2.64**
1384399_at	ankyrin repeat domain 50 (predicted)	Ankrd50_predicted	342	905	**2.64**
1373484_at	kelch-like 13 (Drosophila)	Klhl13	445	1173	**2.64**
1370812_at	Bcl2-like 1	Bcl2l1	339	892	**2.63**
1370476_at	Stam binding protein	Stambp	305	802	**2.63**
1388426_at	sterol regulatory element binding factor 1	Srebf1	870	2288	**2.63**
1374987_at	pantothenate kinase 3 (predicted)	Pank3_predicted	2320	6104	**2.63**
1390422_at	PX domain containing serine/threonine kinase	Pxk	365	959	**2.63**
1372812_at	similar to mKIAA0256 protein (predicted)	RGD1559930_predict	384	1008	**2.63**
1367528_at	F-box and WD-40 domain protein 8 (predicted)	Fbxw8_predicted	982	2578	**2.63**
1389355_at	immediate early response 5	Ier5	916	2397	**2.62**
1377713_at	checkpoint suppressor 1 (predicted)	Ches1_predicted	340	889	**2.62**
1387269_s_at	plasminogen activator, urokinase receptor	Plaur	432	1131	**2.61**
1368297_at	GATA binding protein 2	Gata2	556	1451	**2.61**
1382254_at	phosphodiesterase 4D interacting protein (myomegalin)	Pde4dip	616	1607	**2.61**
1369006_at	hexokinase 2	Hk2	798	2080	**2.60**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1389640_at	SH3 domain binding glutamic acid-rich protein like 2 (predicted)	Sh3bgrl2_predicted	2464	6412	**2.60**
1372772_at	nuclear factor of activated T-cells 5 (predicted)	Nfat5_predicted	430	1119	**2.60**
1373208_at	Transcribed locus	---	372	968	**2.60**
1388712_at	pleckstrin homology, Sec7 and coiled-coil domains 3	Pscd3	809	2103	**2.60**
1383770_at	Transcribed locus	---	756	1964	**2.60**
1390000_at	jumonji domain containing 3 (predicted)	Jmjd3_predicted	327	849	**2.60**
1379811_at	phosphodiesterase 4D interacting protein (myomegalin)	Pde4dip	312	810	**2.60**
1376840_at	Transcribed locus	---	1276	3305	**2.59**
1370940_at	tight junction protein 2	Tjp2	1018	2635	**2.59**
1375343_at	Transcribed locus	---	1066	2755	**2.59**
1372601_at	activating transcription factor 5	Atf5	521	1345	**2.58**
1389392_at	Chloride channel 6 (predicted)	Clcn6_predicted	409	1056	**2.58**
1368177_at	acyl-CoA synthetase long-chain family member 3	Acsl3	770	1987	**2.58**
1383362_at	Transcribed locus	---	324	836	**2.58**
1373385_at	similar to mahogunin, ring finger 1, mahoganoid	RGD1311862	703	1813	**2.58**
1389541_at	Malignant fibrous histiocytoma amplified sequence 1 (predicted)	Mfhas1_predicted	395	1017	**2.57**
1372164_at	similar to hypothetical protein FLJ20154	RGD1305793	1159	2981	**2.57**
1387074_at	regulator of G-protein signaling 2	Rgs2	902	2320	**2.57**
1384559_at	placenta specific homeobox 1 (predicted)	Psx1_predicted	1826	4694	**2.57**
1371104_at	sterol regulatory element binding factor 1	Srebf1	336	863	**2.57**
1389277_at	similar to KIAA0368 (predicted)	RGD1306148_predict	1722	4419	**2.57**
1373657_at	solute carrier family 31, member 2	Slc31a2	317	811	**2.56**
1374696_at	---	---	810	2073	**2.56**
1370987_at	sialophorin	Spn	3734	9556	**2.56**
1391613_at	ELK4, member of ETS oncogene family (predicted)	Elk4_predicted	754	1929	**2.56**
1385108_at		RGD1309752_predict	335	857	**2.56**
1390825_at	solute carrier family 35, member B3 (predicted)	Slc35b3_predicted	621	1587	**2.56**
1372462_at	acetyl-Coenzyme A acetyltransferase 2	Acat2	736	1876	**2.55**
1389187_at	Transcribed locus, strongly similar to NP_001007709.1 hypothetical protein LOC315126 [Rattus norvegicus]	---	884	2251	**2.55**
1369583_at	Jun dimerization protein 2	Jundp2	741	1887	**2.55**
1375921_at	Reticulocalbin 1 (predicted)	Rcn1_predicted	341	867	**2.54**
1387183_at	carnitine O-octanoyltransferase	Crot	488	1238	**2.54**
1377709_at	heart and neural crest derivatives expressed transcript 1	Hand1	9351	23698	**2.53**
1369643_a_at	latrophilin 2	Lphn2	1109	2809	**2.53**
1388824_at	similar to RIKEN cDNA 4930431E10 (predicted) /// similar to Smad ubiquitination regulatory factor 1 isoform 2	LOC690516 /// RGD1	471	1192	**2.53**
1373592_at	similar to SPI6	MGC94010	5086	12866	**2.53**
1369319_at	ADP-ribosylation factor-like 6 interacting protein 5	Arl6ip5	561	1418	**2.53**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1373219_at	snail homolog 1 (Drosophila)	Snai1	1095	2765	**2.53**
1388887_at	glycoprotein galactosyltransferase alpha 1, 3	Ggta1	1458	3679	**2.52**
1392704_at	sorting nexin 4 (predicted)	Snx4_predicted	406	1026	**2.52**
1367844_at	guanine nucleotide binding protein, alpha inhibiting 2	Gnai2	660	1663	**2.52**
1384292_at	docking protein 1	Dok1	347	874	**2.52**
1388732_at	solute carrier family 35, member F5 (predicted)	Slc35f5_predicted	889	2237	**2.52**
1382174_at	Transcribed locus, strongly similar to NP_001026482.1 phosphoinositide-3-kinase, catalytic, beta polypeptide [Gallus gallus]	---	1503	3784	**2.52**
1370347_at	PDZ and LIM domain 7	Pdlim7	1953	4916	**2.52**
1398340_at	cyclin D-type binding-protein 1	Ccndbp1	640	1611	**2.52**
1379914_at	transcription factor CP2-like 2	Tcfcp2l2	476	1197	**2.52**
1375739_at	EH-domain containing 4	Ehd4	1625	4072	**2.51**
1372030_at	zinc finger, FYVE domain containing 21 (predicted)	Zfyve21_predicted	641	1604	**2.50**
1397866_at	serine (or cysteine) proteinase inhibitor, clade B, member 6b	Serpinb6b	2755	6896	**2.50**
1379785_at	Transcribed locus	---	421	1052	**2.50**
1375969_at	torsin A interacting protein 2	Tor1aip2	344	860	**2.50**
1374594_at	similar to RIKEN cDNA 1600029D21	LOC363060	8755	21865	**2.50**
1398771_at	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	Slc3a2	3741	9339	**2.50**
1387446_at	core 1 UDP-galactose:N-acetylgalactosamine-alpha-R beta 1,3-galactosyltransferase	C1galt1	437	1091	**2.49**
1389439_at	---	---	457	1139	**2.49**
1373393_at	similar to Ext1	LOC299907	510	1272	**2.49**
1368144_at	regulator of G-protein signaling 2	Rgs2	702	1746	**2.49**
1368336_at	ferredoxin 1	Fdx1	1142	2839	**2.49**
1384186_at	similar to mKIAA0212 protein (predicted)	RGD1563633_predict	982	2440	**2.48**
1397349_at	UBX domain containing 6 (predicted)	Ubxd6_predicted	403	1000	**2.48**
1383585_s_at	Transcribed locus	---	658	1630	**2.48**
1396059_at	UBX domain containing 2	Ubxd2	769	1904	**2.48**
1396195_at	ankyrin repeat domain 13	Ankrd13	1266	3135	**2.48**
1372237_at	similar to testes development-related NYD-SP22 isoform 1 (predicted)	RGD1561916_predict	572	1413	**2.47**
1388539_at	plakophilin 2	Pkp2	1752	4328	**2.47**
1371645_at	stromal cell derived factor 2 (predicted)	Sdf2_predicted	1916	4725	**2.47**
1389546_at	angiomotin like 2	Amotl2	1460	3599	**2.46**
1372733_at	Similar to intracellular membrane-associated calcium-independent phospholipase A2 gamma (predicted)	RGD1311444_predict	686	1690	**2.46**
1370224_at	signal transducer and activator of transcription 3	Stat3	496	1221	**2.46**
1387903_at	praja 2, RING-H2 motif containing	Pja2	884	2178	**2.46**
1389482_at	similar to EST AA792894	RGD1306495	518	1275	**2.46**
1373395_at	tripartite motif protein 41 (predicted)	Trim41_predicted	933	2298	**2.46**
1383223_at	ubiquitin-like 3	Ubl3	404	995	**2.46**
1371766_at	Transcribed locus	---	454	1116	**2.46**
1367682_at	midkine	Mdk	2222	5464	**2.46**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1371587_at	mitogen-activated protein kinase kinase 1 interacting protein 1	Map2k1ip1	2729	6703	**2.46**
1388653_at	similar to Paired amphipathic helix protein Sin3b (Transcriptional corepressor Sin3b) (Histone deacetylase complex subunit Sin3b)	LOC683381	762	1871	**2.45**
1395741_at	Meningioma expressed antigen 5 (hyaluronidase)	Mgea5	1447	3552	**2.45**
1382112_at	similar to chromatin modifying protein 1B	LOC682926 /// LOC68	453	1112	**2.45**
1391304_at	similar to Ubr2 protein (predicted)	RGD1561637_predict	371	910	**2.45**
1381335_at	---	---	3553	8710	**2.45**
1381162_at	Similar to septin 10 isoform 1	LOC309891	434	1064	**2.45**
1386962_at	phospholipase C, beta 4	Plcb4	414	1014	**2.45**
1373178_at	Transcribed locus	---	2062	5052	**2.45**
1390423_at	pam, highwire, rpm 1 (predicted)	Phr1_predicted	1219	2987	**2.45**
1385887_at	Transcribed locus	---	383	936	**2.44**
1384899_at	Transcribed locus	---	5406	13211	**2.44**
1382568_at	Transcribed locus	---	640	1565	**2.44**
1369472_a_at	activating transcription factor 2	Atf2	475	1159	**2.44**
1390430_at	nuclear receptor subfamily 1, group D, member 2	Nr1d2	360	877	**2.44**
1383025_at	meningioma expressed antigen 5 (hyaluronidase)	Mgea5	613	1491	**2.43**
1370357_at	solute carrier family 30 (zinc transporter), member 4	Slc30a4	651	1579	**2.43**
1373624_at	Transcribed locus	---	663	1608	**2.43**
1383306_at	similar to RIKEN cDNA 5830435K17 (predicted)	RGD1560454_predict	1100	2665	**2.42**
1373225_at	myeloid cell leukemia sequence 1	Mcl1	1803	4368	**2.42**
1367915_at	diacylglycerol O-acyltransferase 1	Dgat1	824	1994	**2.42**
1394363_at	RIO kinase 3 (yeast) (predicted)	Riok3_predicted	825	1997	**2.42**
1374399_at	EH-domain containing 4	Ehd4	997	2412	**2.42**
1373047_at	protein kinase C, iota	Prkci	1838	4439	**2.42**
1380028_at	similar to ADP-ribosylation factor-like protein 4C (ADP-ribosylation factor-like 7)	LOC367311	339	818	**2.42**
1376758_at	inhibitor of growth family, member 1	Ing1	1435	3466	**2.41**
1389316_at	ubiquitin specific peptidase 9, X chromosome (predicted)	Usp9x_predicted	866	2090	**2.41**
1392579_at	similar to 5830411E10Rik protein	RGD1306658	3490	8418	**2.41**
1368308_at	myelocytomatosis viral oncogene homolog (avian)	Myc	1806	4355	**2.41**
1367853_at	solute carrier family 12, member 2	Slc12a2	2294	5528	**2.41**
1398909_at	hypothetical LOC301124	LOC301124	1215	2925	**2.41**
1372840_at	zinc finger, BED domain containing 4 (predicted) /// similar to zinc finger, BED domain containing 4	LOC678931 /// LOC68	621	1494	**2.40**
1376969_at	Transcribed locus	---	627	1505	**2.40**
1393738_s_at	malignant fibrous histiocytoma amplified sequence 1 (predicted)	Mfhas1_predicted	576	1382	**2.40**
1375852_at	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Hmgcr	1695	4067	**2.40**
1367929_at	CD59 antigen	Cd59	9949	23857	**2.40**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1374774_at	Protein tyrosine phosphatase, non-receptor type 14 (predicted)	Ptpn14_predicted	337	806	**2.39**
1386569_at	Transcribed locus	---	462	1105	**2.39**
1372225_at	centrosome-associated protein 350	Cap350	479	1146	**2.39**
1373501_at	NIMA (never in mitosis gene a)-related expressed kinase 7 (predicted)	Nek7_predicted	566	1353	**2.39**
1398969_at	Transcribed locus	---	2560	6118	**2.39**
1388751_at	CAP, adenylate cyclase-associated protein, 2 (yeast)	Cap2	806	1925	**2.39**
1387975_at	UDP-glucose ceramide glucosyltransferase	Ugcg	808	1926	**2.38**
1379283_at	Transcribed locus	---	1487	3542	**2.38**
1373149_at	similar to yippee-like 3 (predicted)	RGD1564579_predict	394	937	**2.38**
1383169_at	Transcribed locus	---	1623	3853	**2.37**
1373120_at	Transcribed locus, strongly similar to NP_446127.3 spermatogenesis associated 2 [Rattus norvegicus]	---	364	864	**2.37**
1373459_at	serine/threonine kinase 11 interacting protein (predicted)	Stk11ip_predicted	522	1239	**2.37**
1369690_at	N-ethylmaleimide sensitive fusion protein	Nsf	983	2332	**2.37**
1382884_at	Transcribed locus, strongly similar to XP_001006562.1 PREDICTED: similar to phosphatase subunit gene g4-1 [Mus musculus]	---	346	820	**2.37**
1372040_at	calmodulin binding transcription activator 2 (predicted)	Camta2_predicted	356	845	**2.37**
1387035_a_at	Rho GTPase activating protein 17	Arhgap17	1371	3248	**2.37**
1383742_at	sorting nexin 7	Snx7	417	988	**2.37**
1398332_at	Transcribed locus, strongly similar to XP_579788.1 PREDICTED: hypothetical protein XP_579788 [Rattus norvegicus]	---	780	1844	**2.37**
1373375_at	Rab6 interacting protein 1 (predicted)	Rab6ip1_predicted	808	1910	**2.36**
1370286_at	solute carrier family 38, member 2	Slc38a2	4742	11201	**2.36**
1377112_at	cytidine deaminase (predicted)	Cda_predicted	513	1211	**2.36**
1367789_at	solute carrier family 27 (fatty acid transporter), member 1	Slc27a1	489	1154	**2.36**
1383679_at	ring finger protein 31 (predicted)	Rnf31_predicted	357	842	**2.36**
1367657_at	B-cell translocation gene 1, anti-proliferative	Btg1	4285	10088	**2.35**
1372592_at	histone deacetylase 6	Hdac6	503	1183	**2.35**
1382245_at	Clone UI-R-FJ0-cpv-n-11-0-UI unknown mRNA	---	1374	3232	**2.35**
1377664_at	KARP-1 binding protein 1	Kab	769	1806	**2.35**
1387788_at	Jun-B oncogene	Junb	1974	4638	**2.35**
1374719_at	Transcribed locus, strongly similar to XP_228025.3 PREDICTED: similar to ICBP90 binding protein 1 [Rattus norvegicus]	---	556	1306	**2.35**
1389728_at	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (predicted)	Meis2_predicted	483	1133	**2.34**
1371644_at	protein tyrosine kinase 9	Ptk9	3225	7537	**2.34**
1377410_at	E2F transcription factor 8	E2f8	684	1597	**2.34**
1373672_at	Similar to SPI6	MGC94010	6343	14802	**2.33**
1389001_at	karyopherin (importin) alpha 4	Kpna4	3800	8855	**2.33**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1393956_at	serine/threonine kinase 17b (apoptosis-inducing)	Stk17b	830	1933	**2.33**
1392368_at	---	---	506	1178	**2.33**
1374490_at	thyroid hormone receptor interactor 12	Trip12	485	1130	**2.33**
1395725_at	Similar to intracellular membrane-associated calcium-independent phospholipase A2 gamma (predicted)	RGD1311444_predict	856	1993	**2.33**
1388330_at	vitamin K epoxide reductase complex, subunit 1	Vkorc1	787	1832	**2.33**
1377750_at	Rho guanine nucleotide exchange factor (GEF) 3 (predicted)	Arhgef3_predicted	499	1160	**2.33**
1370192_at	syntaxin 12	Stx12	1487	3458	**2.32**
1393186_at	similar to RIKEN cDNA 2010200O16 (predicted)	RGD1564454_predict	684	1586	**2.32**
1373457_at	inhibitor of growth family, member 1	Ing1	767	1776	**2.32**
1380408_at	Transcribed locus	---	1751	4057	**2.32**
1373059_at	ankyrin repeat domain 13	Ankrd13	2975	6890	**2.32**
1383062_at	glutaredoxin 2 (thioltransferase)	Glrx2	849	1966	**2.32**
1373520_at	CDNA clone IMAGE:7367270	---	420	972	**2.31**
1371805_at	ankyrin repeat domain 46	Ankrd46	400	925	**2.31**
1373072_at	Transcribed locus	---	1821	4211	**2.31**
1373305_at	sorting nexin 4 (predicted)	Snx4_predicted	879	2033	**2.31**
1383093_at	---	---	540	1246	**2.31**
1376650_at	golgi autoantigen, golgin subfamily a, 5	Golga5	514	1186	**2.31**
1369737_at	cAMP responsive element modulator	Crem	412	951	**2.31**
1377390_at	beta-site APP-cleaving enzyme 2	Bace2	1678	3869	**2.31**
1389521_at	influenza virus NS1A binding protein (predicted)	Ivns1abp_predicted	2569	5922	**2.31**
1385719_at	Transcribed locus	---	349	805	**2.31**
1390177_at	Transcribed locus	---	554	1278	**2.30**
1367979_s_at	cytochrome P450, subfamily 51	Cyp51	1385	3190	**2.30**
1374652_at	solute carrier family 25, member 26 (predicted)	Slc25a26_predicted	689	1587	**2.30**
1371547_at	similar to C10 (predicted)	RGD1309695_predict	1291	2971	**2.30**
1391348_at	pantothenate kinase 3 (predicted)	Pank3_predicted	373	859	**2.30**
1367595_s_at	beta-2 microglobulin	B2m	6965	16016	**2.30**
1388217_a_at	calumenin	Calu	2667	6129	**2.30**
1384290_at	Retinoblastoma binding protein 6	Rbbp6	501	1151	**2.30**
1371038_at	CCAAT/enhancer binding protein (C/EBP), gamma	Cebpg	481	1105	**2.30**
1383519_at	Hexokinase 2	Hk2	1722	3949	**2.29**
1374387_at	ADP-ribosylation factor-like 6 interacting protein 5	Arl6ip5	1018	2333	**2.29**
1368076_at	von Hippel-Lindau syndrome homolog	Vhl	1350	3093	**2.29**
1373533_at	---	---	759	1740	**2.29**
1384560_x_at	placenta specific homeobox 1 (predicted)	Psx1_predicted	1973	4512	**2.29**
1372244_at	calcium binding protein 39 (predicted)	Cab39_predicted	1164	2662	**2.29**
1373289_at	ankyrin repeat and SOCS box-containing protein 8 (predicted)	Asb8_predicted	576	1316	**2.29**
1379377_at	ganglioside-induced differentiation-associated-protein 2	Gdap2	596	1362	**2.29**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1376073_at	Sel1 (suppressor of lin-12) 1 homolog (C. elegans)	Sel1h	534	1219	**2.28**
1371382_at	filamin, alpha (predicted)	Flna_predicted	958	2185	**2.28**
1389571_at	signal transducer and activator of transcription 2	Stat2	358	816	**2.28**
1397174_at		Kiaa0415	551	1256	**2.28**
1372487_at	hypothetical protein LOC619573	LOC619573	389	886	**2.28**
1398421_at	nuclear factor of activated T-cells 5 (predicted)	Nfat5_predicted	1037	2361	**2.28**
1368908_at	annexin A4	Anxa4	1620	3682	**2.27**
1390628_at	cytoplasmic polyadenylation element binding protein 2 (predicted)	Cpeb2_predicted	1195	2714	**2.27**
1375858_at	tumor suppressor candidate 4	Tusc4	612	1388	**2.27**
1382420_at	similar to hypothetical protein FLJ14681 (predicted)	RGD1307907_predict	373	845	**2.26**
1372890_at	sphingosine phosphate lyase 1	Sgpl1	596	1348	**2.26**
1373488_at	Transcribed locus	---	1585	3583	**2.26**
1397618_at	sperm associated antigen 9 (predicted)	Spag9_predicted	553	1249	**2.26**
1388659_at	calcium regulated heat stable protein 1	Carhsp1	380	856	**2.26**
1398780_at	Rab acceptor 1 (prenylated)	Rabac1	989	2230	**2.25**
1389146_at	hypothetical protein LOC498796	LOC498796	2467	5559	**2.25**
1389047_at	Similar to Bcl2-associated athanogene 2	LOC690038	1721	3879	**2.25**
1376620_at	Transcribed locus	---	669	1505	**2.25**
1387072_at	protein kinase, lysine deficient 1	Prkwnk1	1204	2705	**2.25**
1368842_at	transcription factor 4	Tcf4	592	1329	**2.24**
1398374_at	similar to RIKEN cDNA 2810002D13 gene	RGD1307128	466	1047	**2.24**
1382070_at	activating transcription factor 7 interacting protein (predicted)	Atf7ip_predicted	467	1046	**2.24**
1380383_at	ADP-ribosylation factor 4-like (predicted)	Arf4l_predicted	576	1291	**2.24**
1375130_at	hypothetical protein LOC679612 /// hypothetical protein LOC687408	LOC679612 /// LOC68	396	888	**2.24**
1388868_at	zinc finger protein 216 (predicted)	Zfp216_predicted	3844	8611	**2.24**
1384584_at	Transcribed locus, moderately similar to NP_007227.1 cytochrome c oxidase subunit I [Rattus norvegicus]	---	390	873	**2.24**
1390648_at	hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 2 (predicted)	Herc2_predicted	522	1168	**2.24**
1387432_at	ROD1 regulator of differentiation 1 (S. pombe)	Rod1	929	2076	**2.24**
1392536_at	Transcribed locus	---	447	999	**2.23**
1388947_at	eukaryotic translation initiation factor 5B /// similar to Eukaryotic translation initiation factor 5B (eIF-5B) (Translation initiation factor IF-2)	Eif5b /// LOC689581	3312	7388	**2.23**
1372863_at	pam, highwire, rpm 1 (predicted)	Phr1_predicted	2187	4877	**2.23**
1392973_at	Transcribed locus, strongly similar to XP_579919.1 PREDICTED: hypothetical protein XP_579919 [Rattus norvegicus]	---	480	1070	**2.23**
1368486_at	insulin receptor substrate 3	Irs3	1104	2459	**2.23**
1374884_at	protein phosphatase 1D magnesium-dependent, delta isoform (predicted)	Ppm1d_predicted	441	982	**2.23**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1388995_at	ring finger protein 14	Rnf14	1779	3960	**2.23**
1388383_at	Transcribed locus	---	1135	2522	**2.22**
1398360_at	similar to Elongation of very long chain fatty acids protein 1	LOC679532	1089	2418	**2.22**
1371978_at	oxysterol binding protein-like 9 (predicted)	Osbpl9_predicted	1034	2296	**2.22**
1376440_at	ring finger protein 139 (predicted)	Rnf139_predicted	682	1513	**2.22**
1371664_at	paxillin	Pxn	3008	6674	**2.22**
1384366_at	COP9 (constitutive photomorphogenic) homolog, subunit 2 (Arabidopsis thaliana)	Cops2	1173	2601	**2.22**
1381542_at	UBX domain containing 2	Ubxd2	806	1785	**2.21**
1373874_at	sphingosine-1-phosphate phosphatase 1	Sgpp1	2206	4880	**2.21**
1389345_at	Transcribed locus	---	872	1929	**2.21**
1387087_at	CCAAT/enhancer binding protein (C/EBP), beta	Cebpb	2235	4935	**2.21**
1379439_at	BTB (POZ) domain containing 15	Btbd15	375	828	**2.21**
1398927_at	similar to 0610010K06Rik protein	RGD1307161	2366	5216	**2.20**
1390432_at	transmembrane and coiled-coil domains 3 (predicted)	Tmco3_predicted	737	1625	**2.20**
1389481_at	similar to GI:13385412-like protein splice form I	RGD735065	487	1074	**2.20**
1374682_at	SRY (sex determining region Y)-box 13 (predicted)	Sox13_predicted	1450	3193	**2.20**
1378896_at	solute carrier family 30 (zinc transporter), member 2	Slc30a2	634	1394	**2.20**
1368323_at	tissue factor pathway inhibitor	Tfpi	17979	39474	**2.20**
1376347_at	similar to PLU1 (predicted)	RGD1565602_predict	864	1896	**2.20**
1370172_at	superoxide dismutase 2, mitochondrial	Sod2	1639	3598	**2.19**
1375882_at	protein phosphatase 3, catalytic subunit, beta isoform	Ppp3cb	645	1416	**2.19**
1388308_at	hypothetical protein LOC687090	LOC687090	1008	2204	**2.19**
1392475_at	Transcribed locus	---	638	1396	**2.19**
1372871_at	hypothetical protein MGC:72616	RGD735175	493	1077	**2.19**
1399102_at	similar to hypothetical protein MGC15523	RGD1306356	823	1797	**2.18**
1385314_at	RAB14, member RAS oncogene family	Rab14	877	1914	**2.18**
1392531_at	similar to 3110080A02Rik protein (predicted)	RGD1305754_predict	667	1456	**2.18**
1392806_at	Wolf-Hirschhorn syndrome candidate 1-like 1 (predicted)	Whsc1l1_predicted	587	1279	**2.18**
1382027_at	integrin beta 3	Itgb3	1478	3211	**2.17**
1373812_at	Cyclin-dependent kinase inhibitor 1B	Cdkn1b	559	1214	**2.17**
1374413_at	ubiquitin-like 3	Ubl3	1337	2897	**2.17**
1383197_at	junction adhesion molecule 2	Jam2	812	1759	**2.17**
1385228_x_at	development and differentiation enhancing (predicted)	Ddef1_predicted	941	2038	**2.17**
1374544_at	hypothetical protein LOC679150 /// hypothetical protein LOC690899	LOC679150 /// MGC1	1398	3024	**2.16**
1391572_at	cysteinyl-tRNA synthetase (predicted)	Cars_predicted	1091	2361	**2.16**
1395454_at	similar to osteopetrosis associated transmembrane protein 1	LOC499474	550	1190	**2.16**
1387753_s_at	membrane associated guanylate kinase, WW and PDZ domain containing 3	Magi3	620	1337	**2.16**
1372082_at	NECAP endocytosis associated 2	Necap2	1227	2647	**2.16**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1372528_at	N-ethylmaleimide sensitive fusion protein	Nsf	985	2124	**2.16**
1373509_at	NOL1/NOP2/Sun domain family, member 4 (predicted)	Nsun4_predicted	425	915	**2.16**
1375979_at	Transcribed locus	---	841	1813	**2.15**
1372990_at	cAMP responsive element binding protein 3	Creb3	1115	2402	**2.15**
1369141_at	Prolactin family 3, subfamily d, member 1 /// Prolactin family 3, subfamily d, member 2	Prl3d1 /// Prl3d2	10452	22492	**2.15**
1389333_at	similar to F-box only protein 3 isoform 1	LOC690634	2031	4370	**2.15**
1382199_at	microtubule-associated proteins 1A/1B light chain 3	Map1lc3b	908	1953	**2.15**
1374599_at	hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 1 (predicted)	Herc1_predicted	688	1478	**2.15**
1375714_at	similar to Erbb2 interacting protein isoform 2 (predicted)	RGD1562952_predict	536	1147	**2.14**
1367974_at	annexin A3	Anxa3	2177	4660	**2.14**
1370174_at	myeloid differentiation primary response gene 116	Myd116	1244	2662	**2.14**
1387169_at	transducin-like enhancer of split 3, E(spl) homolog (Drosophila)	Tle3	908	1942	**2.14**
1376994_at	Transcribed locus, strongly similar to XP_988139.1 PREDICTED: similar to F-box and leucine-rich repeat protein 20 isoform 5 [Mus musculus]	---	442	946	**2.14**
1398481_at	Transcribed locus	---	728	1555	**2.14**
1368946_at	ADP-ribosylation factor 2	Arf2	686	1464	**2.13**
1372877_at	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	Plod3	1490	3178	**2.13**
1388336_at	similar to hypothetical protein D2Ertd391e (predicted)	RGD1310685_predict	515	1096	**2.13**
1371872_at	Nucleosome assembly protein 1-like 1	Nap1l1	5723	12181	**2.13**
1390845_at	Transcribed locus	---	386	821	**2.13**
1389304_at	RE1-silencing transcription factor	Rest	2866	6097	**2.13**
1399037_at	similar to Tetratricopeptide repeat protein 19 (TPR repeat protein 19)	LOC687312 /// LOC69	546	1161	**2.13**
1397670_at	autophagy-related 12 (yeast)	Atg12	842	1790	**2.13**
1392471_at	guanine nucleotide binding protein (G protein), gamma 12	Gng12	1190	2528	**2.12**
1374058_at	TMEM9 domain family, member B (predicted)	Tmem9b_predicted	632	1344	**2.12**
1375221_at	thioredoxin domain containing 13	Txndc13	1423	3021	**2.12**
1393341_at	Transcribed locus	---	1248	2649	**2.12**
1384331_at	sulfiredoxin 1 homolog (S. cerevisiae)	Srxn1	615	1306	**2.12**
1389297_at	ERO1-like (S. cerevisiae)	Ero1l	2562	5437	**2.12**
1389478_at	kelch-like 22 (Drosophila) (predicted)	Klhl22_predicted	1923	4075	**2.12**
1382332_at	stromal antigen 2 (predicted)	Stag2_predicted	1824	3864	**2.12**
1393024_at	Transcribed locus	---	459	973	**2.12**
1393226_at	Transcribed locus	---	883	1869	**2.12**
1371127_at	bone morphogenetic protein 1	Bmp1	432	914	**2.12**
1399153_at	RAB5B, member RAS oncogene family (predicted)	Rab5b_predicted	447	946	**2.12**
1387964_a_at	ERO1-like (S. cerevisiae)	Ero1l	437	924	**2.12**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1385540_at	protein inhibitor of activated STAT 1 (predicted)	Pias1_predicted	553	1169	**2.11**
1388874_at	metastasis suppressor 1 (predicted)	Mtss1_predicted	665	1405	**2.11**
1387653_at	translin-associated factor X	Tsnax	606	1280	**2.11**
1389444_at	Similar to RIKEN cDNA 5033406L14	RGD1305755	757	1597	**2.11**
1389194_at	Transcribed locus, strongly similar to NP_001020450.1 hypothetical protein LOC306014 [Rattus norvegicus]	---	554	1168	**2.11**
1379560_at	nuclear receptor binding SET domain protein 1 (predicted) /// similar to Histone-lysine N-methyltransferase, H3 lysine-36 and H4 lysine-20 specific (H3-K36-HMTase) (H4-K20-HMTase) (Nuclear receptor binding SET domain containing protein 1) (NR-binding SET domain containing protein)	LOC686060 /// Nsd1_	594	1251	**2.11**
1377964_at	similar to RIKEN cDNA 2010200O16 (predicted)	RGD1564454_predict	789	1662	**2.11**
1384929_at	similar to apolipoprotein B48 receptor (predicted)	RGD1565169_predict	830	1749	**2.11**
1398935_at	similar to Sorting nexin-9	LOC683687	1075	2263	**2.10**
1393268_at	Transcribed locus	---	688	1447	**2.10**
1369496_at	protein tyrosine phosphatase, non-receptor type 12	Ptpn12	786	1651	**2.10**
1378987_at	Transcribed locus	---	414	870	**2.10**
1375315_at	similar to hypothetical protein BC013035 (predicted)	RGD1560996_predict	481	1008	**2.10**
1367826_at	nuclear factor, erythroid derived 2, like 2	Nfe2l2	2960	6192	**2.09**
1383698_at	pyruvate dehydrogenase E1 alpha 1	Pdha1	518	1082	**2.09**
1374422_at	similar to Erbb2 interacting protein isoform 2 (predicted)	RGD1562952_predict	498	1042	**2.09**
1372410_at	C1q and tumor necrosis factor related protein 6	C1qtnf6	2364	4935	**2.09**
1381018_a_at	development and differentiation enhancing (predicted)	Ddef1_predicted	458	956	**2.09**
1392472_at	similar to myocyte enhancer factor 2C	LOC309957	446	931	**2.09**
1387346_at	integrin beta 1 (fibronectin receptor beta)	Itgb1	2875	5994	**2.09**
1376754_at	cysteinyl-tRNA synthetase (predicted)	Cars_predicted	920	1916	**2.08**
1387436_at	septin 7	Sept7	3327	6930	**2.08**
1397179_at	similar to DIP13 alpha (predicted)	RGD1309388_predict	404	841	**2.08**
1371857_at	potassium channel tetramerization domain containing 10	Kctd10	1008	2098	**2.08**
1371338_at	presenilin enhancer 2 homolog (C. elegans)	Psenen	849	1767	**2.08**
1377999_at	tropomodulin 3 (predicted)	Tmod3_predicted	1175	2442	**2.08**
1398512_at	nuclear transcription factor, X-box binding 1	Nfx1	774	1608	**2.08**
1389028_at	nuclear receptor coactivator 6	Ncoa6	1491	3091	**2.07**
1368365_at	aldehyde dehydrogenase family 3, subfamily A2	Aldh3a2	906	1878	**2.07**
1368702_at	PRKC, apoptosis, WT1, regulator	Pawr	771	1599	**2.07**
1390385_at	similar to D-glucuronyl C5-epimerase (predicted)	RGD1565253_predict	847	1755	**2.07**
1389534_at	ubiquitin-conjugating enzyme E2E 3, UBC4/5 homolog (yeast) (predicted)	Ube2e3_predicted	5666	11740	**2.07**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1388161_at	a disintegrin and metalloprotease domain 10	Adam10	569	1179	**2.07**
1369644_at	latrophilin 2	Lphn2	2974	6158	**2.07**
1399049_at	---	---	1961	4057	**2.07**
1374405_at	heterogeneous nuclear ribonucleoprotein A3	Hnrpa3	1498	3100	**2.07**
1371979_at	sterol regulatory element binding factor 2 (predicted)	Srebf2	661	1367	**2.07**
1388733_at	bifunctional apoptosis regulator	Bfar	807	1668	**2.07**
1376640_at	Hypothetical protein LOC680259	LOC680259	2157	4457	**2.07**
1380019_at	T-cell, immune regulator 1	Tcirg1	524	1081	**2.06**
1368770_at	glucosaminyl (N-acetyl) transferase 1, core 2	Gcnt1	1044	2155	**2.06**
1367946_at	PDZ and LIM domain 1 (elfin)	Pdlim1	4580	9449	**2.06**
1385658_at	zinc finger protein 313	Zfp313	1262	2602	**2.06**
1376658_at	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1 (predicted)	Raph1_predicted	416	858	**2.06**
1372510_at	sulfiredoxin 1 homolog (S. cerevisiae)	Srxn1	1104	2274	**2.06**
1388542_at	similar to RIKEN cDNA 2010315L10 (predicted) /// similar to USE1-like protein (Hematopoietic stem/progenitor cells protein MDS032) (Putative MAPK-activating protein PM26) (Protein p31)	LOC292282 /// RGD13	548	1128	**2.06**
1373115_at	similar to 2310061I09Rik protein (predicted)	RGD1304653_predict	652	1341	**2.06**
1375936_at	desmocollin 2	Dsc2	1659	3414	**2.06**
1372020_at	target of myb1 homolog (chicken)	Tom1	500	1029	**2.06**
1374075_at	N-ethylmaleimide-sensitive factor attachment protein, gamma	Napg	511	1051	**2.06**
1384163_at	Transcribed locus	---	1059	2175	**2.05**
1374034_at	cysteinyl-tRNA synthetase (predicted)	Cars_predicted	1339	2750	**2.05**
1381019_x_at	development and differentiation enhancing (predicted)	Ddef1_predicted	583	1198	**2.05**
1397634_at	ankyrin repeat and SAM domain containing 1 (predicted)	Anks1_predicted	1804	3701	**2.05**
1388635_at	similar to hypothetical protein FLJ20507 (predicted)	RGD1309744_predict	531	1090	**2.05**
1370141_at	myeloid cell leukemia sequence 1	Mcl1	2334	4788	**2.05**
1372909_at	similar to hypothetical protein FLJ36090 (predicted)	RGD1565619_predict	612	1256	**2.05**
1398866_at	membrane associated guanylate kinase, WW and PDZ domain containing 3	Magi3	1989	4080	**2.05**
1398375_at	metastasis associated 3 (predicted)	Mta3_predicted	1701	3486	**2.05**
1384376_at	Transcribed locus, strongly similar to XP_980162.1 PREDICTED: similar to DnaJ (Hsp40) homolog, subfamily B, member 14 isoform 1 [Mus musculus]	---	421	862	**2.05**
1374641_at	Transcribed locus	---	1394	2851	**2.05**
1388407_at	similar to BC003940 protein (predicted)	RGD1311925_predict	714	1458	**2.04**
1369912_at	v-crk sarcoma virus CT10 oncogene homolog (avian)	Crk	659	1346	**2.04**
1379500_at	similar to cDNA sequence BC018601	LOC498404	834	1702	**2.04**
1378925_at	CAMP responsive element modulator	Crem	1912	3897	**2.04**
1377697_at	similar to amyloid beta (A4) precursor protein-binding, family B, member 2 (predicted)	RGD1562438_predict	399	813	**2.04**
1371401_at	ing finger protein 5 pseudogene	rCG_38334	961	1958	**2.04**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1397317_at	Transcribed locus	---	1451	2955	**2.04**
1371322_at	laminin, gamma 1	Lamc1	1275	2596	**2.04**
1396353_at	Transcribed locus, strongly similar to XP_001059486.1 PREDICTED: similar to Protein phosphatase 2A, 59 kDa regulatory subunit B (PP2A PR59) (PP2A B-PR59) [Rattus norvegicus]	---	454	925	**2.04**
1380577_at	ATP-binding cassette, sub-family G (WHITE), member 2	Abcg2	732	1491	**2.04**
1374554_at	similar to HT014 (predicted)	RGD1308048_predict	3557	7234	**2.03**
1389305_at	annexin A4	Anxa4	2108	4285	**2.03**
1378037_at	similar to RIKEN cDNA 0610009J22 (predicted)	RGD1310738_predict	647	1314	**2.03**
1388873_at	thyroid hormone receptor interactor 12	Trip12	4960	10078	**2.03**
1371798_at	guanine nucleotide binding protein, alpha 12	Gna12	604	1228	**2.03**
1369661_at	dynamin 2	Dnm2	406	825	**2.03**
1390250_x_at	similar to Probable phospholipid-transporting ATPase ID (ATPase class I type 8B member 2)	LOC685152	448	908	**2.03**
1383368_at	Unknown (protein for MGC:72610)	MGC72610	4077	8267	**2.03**
1388813_at	ADP-ribosylation factor 2	Arf2	2117	4293	**2.03**
1374117_at	brain-specific angiogenesis inhibitor 1-associated protein 2	Baiap2	599	1215	**2.03**
1372568_at	EPM2A (laforin) interacting protein 1 (predicted)	Epm2aip1_predicted	677	1371	**2.03**
1389539_at	similar to DIP13 alpha (predicted)	RGD1309388_predict	527	1067	**2.03**
1379567_at	thyroid hormone receptor associated protein 1 (predicted)	Thrap1_predicted	678	1372	**2.02**
1367878_at	syntaxin 5a	Stx5a	787	1593	**2.02**
1374798_at	Transcribed locus	---	3236	6549	**2.02**
1388218_at	low density lipoprotein receptor	Ldlr	1523	3081	**2.02**
1376727_at	Yip1 domain family, member 4	Yipf4	3098	6267	**2.02**
1383874_at	RGD1560812 (predicted)	RGD1560812_predict	525	1062	**2.02**
1374536_at	Transcribed locus, strongly similar to XP_579981.1 PREDICTED: hypothetical protein XP_579981 [Rattus norvegicus]	---	835	1687	**2.02**
1377949_s_at	ankyrin repeat and SAM domain containing 1 (predicted)	Anks1_predicted	558	1127	**2.02**
1382196_at	similar to hypothetical protein ET (predicted)	RGD1307394_predict	483	976	**2.02**
1388401_at	filamin, beta (predicted)	Flnb_predicted	2636	5318	**2.02**
1372023_at	Transcribed locus, strongly similar to XP_579847.1 PREDICTED: hypothetical protein XP_579847 [Rattus norvegicus]	---	604	1217	**2.02**
1370905_at	dedicator of cytokinesis 9	Dock9	1695	3418	**2.02**
1368426_at	carnitine O-octanoyltransferase	Crot	1268	2554	**2.01**
1395165_at	PC4 and SFRS1 interacting protein 1	Psip1	735	1479	**2.01**
1392573_at	ubiquitin-conjugating enzyme E2A, RAD6 homolog (S. cerevisiae)	Ube2a	1102	2219	**2.01**
1391558_at	zinc finger protein 608 (predicted)	Zfp608_predicted	1063	2139	**2.01**
1389540_at	similar to IQ motif and Sec7 domain 1	LOC686590	490	987	**2.01**
1373071_at	similar to RIKEN cDNA 1810054G18 (predicted)	RGD1308901_predict	512	1030	**2.01**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1398549_at	Cyclin L2	Ccnl2	484	973	**2.01**
1391671_at	Hypothetical protein LOC689165	LOC689165	451	905	**2.01**
1392951_at	platelet-activating factor acetylhydrolase, isoform 1b, alpha2 subunit	Pafah1b2	899	1800	**2.00**
1395147_at	excision repair cross-complementing rodent repair deficiency, complementation group 3	Ercc3	527	1052	**2.00**
1393449_at	junction adhesion molecule 2	Jam2	585	1167	**2.00**
1367890_at	caspase 2	Casp2	693	1383	**1.99**
1398779_at	actin related protein 2/3 complex, subunit 1A	Arpc1a	2375	4737	**1.99**
1388929_at	Clone UI-R-FJ0-cpv-n-11-0-UI unknown mRNA	---	2097	4180	**1.99**
1372533_at	similar to mKIAA0212 protein (predicted)	RGD1563633_predict	2023	4032	**1.99**
1371562_at	epsin 2	Epn2	571	1138	**1.99**
1393162_at	solute carrier family 39 (metal ion transporter), member 6	Slc39a6	929	1849	**1.99**
1373864_at	mitogen-activated protein kinase kinase kinase kinase 4 (predicted)	Map4k4_predicted	1137	2263	**1.99**
1373278_at	nuclear factor, erythroid derived 2,-like 1 (predicted)	Nfe2l1_predicted	650	1294	**1.99**
1376465_at	Transcribed locus	---	421	838	**1.99**
1382689_at	similar to D7Ertd743e protein	RGD1310870	1859	3696	**1.99**
1371325_at	protective protein for beta-galactosidase	Ppgb	3857	7667	**1.99**
1368826_at	catechol-O-methyltransferase	Comt	1421	2824	**1.99**
1389567_at	SREBP cleavage activating protein (predicted)	Scap_predicted	493	978	**1.99**
1398536_at	zinc finger protein 655	Zfp655	940	1866	**1.99**
1374418_at	similar to CG8009-PA (predicted)	RGD1308816_predict	1302	2586	**1.99**
1368217_at	ralA binding protein 1	Ralbp1	1626	3224	**1.98**
1398335_at	tweety homolog 3 (Drosophila) (predicted)	Ttyh3_predicted	443	878	**1.98**
1391719_at	---	---	609	1206	**1.98**
1389965_at	trans-golgi network protein 2	Tgoln2	2293	4540	**1.98**
1398948_at	Tax1 (human T-cell leukemia virus type I) binding protein 1	Tax1bp1	3667	7259	**1.98**
1384380_at	zinc finger, CCHC domain containing 7 (predicted)	Zcchc7_predicted	486	962	**1.98**
1372776_at	F-box and leucine-rich repeat protein 5 (predicted)	Fbxl5_predicted	1471	2910	**1.98**
1388587_at	immediate early response 3	Ier3	3290	6496	**1.97**
1383837_at	Yip1 domain family, member 3	Yipf3	473	934	**1.97**
1398998_at	similar to CG4768-PA (predicted)	RGD1309748_predict	1484	2925	**1.97**
1386994_at	B-cell translocation gene 2, anti-proliferative	Btg2	4782	9422	**1.97**
1388957_at	CDNA clone IMAGE:7368410	---	857	1688	**1.97**
1374447_at	ubiquitin specific peptidase 9, X chromosome (predicted)	Usp9x_predicted	1597	3144	**1.97**
1371823_at	stromal antigen 2 (predicted)	Stag2_predicted	2978	5860	**1.97**
1372885_at	CCAAT/enhancer binding protein (C/EBP), gamma	Cebpg	1138	2238	**1.97**
1389686_at	protein kinase, X-linked	Prkx	517	1018	**1.97**
1389573_at	ChaC, cation transport regulator-like 1 (E. coli) (predicted)	Chac1_predicted	422	830	**1.97**
1375960_at	germ cell-less homolog 1 (Drosophila)	Gmcl1	1441	2833	**1.97**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1383942_at	similar to Sorting nexin-9	LOC683687	3997	7848	**1.96**
1393363_at	similar to zinc finger protein 198	LOC305913	609	1195	**1.96**
1370928_at	LPS-induced TN factor	Litaf	10984	21526	**1.96**
1375870_a_at	RNA binding motif, single stranded interacting protein 1	Rbms1	1752	3431	**1.96**
1392517_at	similar to Golgin 45 (Basic leucine zipper nuclear factor 1)	LOC498266	568	1110	**1.96**
1389189_at	actinin, alpha 1	Actn1	3016	5899	**1.96**
1393707_at	Bcl2-like 2	Bcl2l2	790	1544	**1.95**
1390171_at	similar to hypothetical protein BC008163 (predicted)	RGD1305162_predict	730	1426	**1.95**
1371353_at	sequestosome 1	Sqstm1	7025	13722	**1.95**
1388274_at	brain expressed myelocytomatosis oncogene	Bmyc	654	1278	**1.95**
1389778_a_at	transcription elongation factor B (SIII), polypeptide 3	Tceb3	974	1899	**1.95**
1371601_at	similar to Endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase (ER alpha-1,2-mannosidase) (predicted)	RGD1563595_predict	1051	2049	**1.95**
1387090_a_at	LIM motif-containing protein kinase 2	Limk2	1784	3477	**1.95**
1382556_a_at	similar to DIP13 alpha (predicted)	RGD1309388_predict	1015	1977	**1.95**
1367680_at	acyl-Coenzyme A oxidase 1, palmitoyl	Acox1	1840	3584	**1.95**
1371522_at	similar to mKIAA0945 protein (predicted)	RGD1563127_predict	590	1150	**1.95**
1387076_at	hypoxia inducible factor 1, alpha subunit	Hif1a	10340	20134	**1.95**
1379346_at	cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)	Cdc73	3839	7472	**1.95**
1383819_at	COP9 (constitutive photomorphogenic) homolog, subunit 2 (Arabidopsis thaliana)	Cops2	1267	2466	**1.95**
1396262_at	pre-B-cell colony enhancing factor 1	Pbef1	721	1403	**1.94**
1372808_at	similar to Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase, mitochondrial precursor	LOC680308	1993	3873	**1.94**
1377849_at	valosin containing protein (p97)/p47 complex interacting protein 1	Vcpip1	478	928	**1.94**
1378524_at	ring finger protein (C3HC4 type) 19 (predicted)	Rnf19_predicted	678	1317	**1.94**
1388468_at	CDC42 small effector 1	Cdc42se1	794	1542	**1.94**
1389590_at	Transcribed locus	---	995	1932	**1.94**
1385168_at	similar to receptor-interacting factor 1	RGD1306520	714	1385	**1.94**
1371996_at	AE binding protein 2 (predicted)	Aebp2_predicted	1066	2068	**1.94**
1377347_at	forkhead box J3 (predicted)	Foxj3_predicted	1978	3835	**1.94**
1391770_at	Ubiquitin specific peptidase 33	Usp33	583	1130	**1.94**
1374809_at	Transcribed locus	---	1099	2130	**1.94**
1382993_at	Bcl-2 binding component 3	Bbc3	743	1439	**1.94**
1378027_at	Pvrl3_predicted	poliovirus receptor-rel	2075	4019	**1.94**
1372497_at	neighbor of Brca1 gene 1	Nbr1	2370	4591	**1.94**
1374729_at	Similar to UV radiation resistance associated	LOC308846	755	1461	**1.94**
1373589_at	myotubularin related protein 3	Mtmr3	713	1380	**1.94**
1384958_at	Transcribed locus	---	566	1096	**1.94**
1374494_at	Transcribed locus	---	1312	2537	**1.93**
1399071_at	---	---	472	912	**1.93**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1373473_a_at	nucleosome assembly protein 1-like 1	Nap1l1	5166	9974	**1.93**
1389139_at	similar to Ttc15 protein (predicted)	RGD1566054_predict	648	1249	**1.93**
1377613_at	similar to RIKEN cDNA 5031400M07	RGD1307343	1524	2937	**1.93**
1395699_at	RIO kinase 3 (yeast) (predicted)	Riok3_predicted	1134	2186	**1.93**
1385158_at	similar to chromatin modifying protein 1B	LOC682926 /// LOC68	851	1638	**1.93**
1389210_at	lymphocyte cytosolic protein 1	Lcp1	1546	2976	**1.92**
1381349_a_at	similar to ubiquitin protein ligase E3 component n-recognin 2	LOC363188	1141	2194	**1.92**
1381100_at	Rho guanine nucleotide exchange factor (GEF) 12	Arhgef12	589	1132	**1.92**
1381980_at	Transcribed locus	---	773	1485	**1.92**
1382735_at	autophagy-related 12 (yeast)	Atg12	511	981	**1.92**
1377192_a_at	caseinolytic peptidase X (E.coli)	Clpx	990	1902	**1.92**
1368427_at	A kinase (PRKA) anchor protein 11	Akap11	544	1045	**1.92**
1373175_at	similar to RIKEN cDNA 1100001H23	RGD1308734	4260	8177	**1.92**
1393267_at	PC4 and SFRS1 interacting protein 1	Psip1	2077	3984	**1.92**
1385070_at	similar to Opa-interacting protein 5 (predicted)	RGD1564263_predict	922	1768	**1.92**
1371439_at	similar to erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked) isoform 1 (predicted)	RGD1564762_predict	669	1281	**1.92**
1388896_at	ubiquitin specific peptidase 33	Usp33	2048	3924	**1.92**
1372509_at	Transcribed locus	---	418	800	**1.91**
1374911_at	oxidative stress responsive gene	RGD1303142	2069	3954	**1.91**
1388847_at	positive cofactor 2, multiprotein complex, glutamine/Q-rich-associated protein (predicted)	Pcqap_predicted	524	1000	**1.91**
1373825_at	SCY1-like 2 (S. cerevisiae) (predicted)	Scyl2_predicted	934	1783	**1.91**
1372004_at	heme binding protein 1 (predicted)	Hebp1_predicted	581	1108	**1.91**
1369414_at	syntaxin binding protein 3	Stxbp3	428	816	**1.91**
1390102_at	disrupted in renal carcinoma 2 homolog (human)	Dirc2	677	1288	**1.90**
1373600_at	Similar to CG9996-PA	LOC300173	1089	2072	**1.90**
1399052_at	toll interacting protein (predicted)	Tollip_predicted	958	1821	**1.90**
1373160_at	similar to phosphatidic acid phosphatase type 2 domain containing 1A	LOC680466 /// LOC68	1034	1967	**1.90**
1388506_at	desmoplakin	Dsp	4061	7717	**1.90**
1387777_at	integrin linked kinase	Ilk	1573	2987	**1.90**
1368650_at	Kruppel-like factor 10	Klf10	1542	2921	**1.89**
1371446_at	MAP kinase-activated protein kinase 2	Mapkapk2	740	1402	**1.89**
1376314_at	ubiquitin-conjugating enzyme E2Q (putative) 2 (predicted)	Ube2q2_predicted	3789	7167	**1.89**
1392922_at	RAP2B, member of RAS oncogene family	Rap2b	777	1469	**1.89**
1375929_at	max binding protein (predicted)	Mnt_predicted	599	1132	**1.89**
1373781_a_at	similar to suprabasal-specific protein suprabasin (predicted)	RGD1562305_predict	12194	23036	**1.89**
1370305_at	Yip1 interacting factor homolog (S. cerevisiae)	Yif1	683	1289	**1.89**
1390786_at	rho/rac guanine nucleotide exchange factor (GEF) 2	Arhgef2	1730	3267	**1.89**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1392051_at	similar to hypothetical protein FLJ14681 (predicted)	RGD1307907_predict	487	919	**1.89**
1379866_at	septin 6 (predicted)	Sept6_predicted	514	969	**1.89**
1368433_at	SAC1 (suppressor of actin mutations 1, homolog)-like (S. cerevisiae)	Sacm1l	1134	2134	**1.88**
1374522_at	similar to melanoma inhibitory activity 3	LOC683007	2527	4756	**1.88**
1372569_at	four and a half LIM domains 3 (predicted)	Fhl3_predicted	1000	1881	**1.88**
1390208_at	HIV-1 tat interactive protein 2, homolog (human) (predicted)	Htatip2_predicted	753	1416	**1.88**
1376593_at	Transcribed locus	---	879	1653	**1.88**
1379574_at	similar to Inner nuclear membrane protein Man1 (LEM domain containing protein 3)	LOC680066	761	1431	**1.88**
1372221_at	Transcribed locus, strongly similar to XP_994234.1 PREDICTED: similar to Ubiquitin-conjugating enzyme E2 H (Ubiquitin-protein ligase H) (Ubiquitin carrier protein H) (UBCH2) (E2-20K) [Mus musculus]	---	1292	2429	**1.88**
1389111_at	Transcribed locus	---	533	1002	**1.88**
1399088_at	tousled-like kinase 2 (Arabidopsis) (predicted)	Tlk2_predicted	1467	2757	**1.88**
1387926_at	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (S. cerevisae)	Sc5d	1138	2138	**1.88**
1380513_at	similar to Expressed sequence AW060207	RGD1309034	1057	1984	**1.88**
1378001_at	Transcribed locus	---	2109	3953	**1.87**
1376796_at	RAB14, member RAS oncogene family	Rab14	1621	3038	**1.87**
1383077_at	similar to RIKEN cDNA 1110063F24 (predicted)	RGD1311622_predict	481	901	**1.87**
1372724_at	glutamate receptor, ionotropic, N-methyl D-asparate-associated protein 1 (glutamate binding)	Grina	1215	2276	**1.87**
1389322_at	programmed cell death protein 7 (predicted)	Pdcd7_predicted	676	1266	**1.87**
1373738_at	similar to M phase phosphoprotein 6	LOC686999	2951	5522	**1.87**
1374629_at	mediator of RNA polymerase II transcription, subunit 8 homolog (yeast) (predicted)	Med8_predicted	616	1151	**1.87**
1372754_at	similar to DIP13 beta (predicted)	RGD1563028_predict	488	911	**1.87**
1375006_at	CDNA clone IMAGE:7318427	---	2538	4741	**1.87**
1367889_at	calcium/calmodulin-dependent protein kinase I	Camk1	448	836	**1.87**
1383884_at	Transcribed locus	---	668	1248	**1.87**
1388999_at	transcription factor 12	Tcf12	936	1748	**1.87**
1368247_at	heat shock 70kD protein 1A /// heat shock 70kD protein 1B (mapped)	Hspa1a /// Hspa1b	1095	2044	**1.87**
1370507_at	discs, large homolog-associated protein 4 (Drosophila)	Dlgap4	520	970	**1.87**
1374824_at	vacuolar protein sorting 53 (yeast) (predicted)	Vps53_predicted	630	1176	**1.87**
1395555_at	golgi SNAP receptor complex member 1	Gosr1	504	939	**1.86**
1367783_at	GABA(A) receptor-associated protein like 2	Gabarapl2	2156	4015	**1.86**
1370912_at	heat shock 70kD protein 1B (mapped)	Hspa1b	839	1562	**1.86**
1392772_at	cysteine-rich protein 3 (predicted)	Crip3_predicted	876	1628	**1.86**
1373507_at	similar to acyl-Coenzyme A binding domain containing 5	LOC679565	1742	3238	**1.86**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1394414_at	Yip1 domain family, member 4 /// hypothetical gene supported by BC088468; NM_001009712 (predicted)	RGD1565766_predict	2959	5493	**1.86**
1377254_a_at	Cohen syndrome homolog 1 (predicted)	Cohh1_predicted	450	835	**1.86**
1391994_at	Transcribed locus	---	467	866	**1.86**
1397824_at	similar to WAC (predicted)	RGD1562407_predict	2222	4119	**1.85**
1372612_at	dynein light chain LC8-type 2	Dynll2	3741	6932	**1.85**
1397363_at	Poliovirus receptor-related 3 (predicted)	Pvrl3_predicted	1659	3072	**1.85**
1370807_at	transmembrane protein 49	Tmem49	1459	2701	**1.85**
1372500_at	tropomodulin 3	Tmod3	2781	5141	**1.85**
1395574_at	ROD1 regulator of differentiation 1 (S. pombe)	Rod1	2534	4685	**1.85**
1398294_at	actinin, alpha 1	Actn1	804	1486	**1.85**
1374296_at	leucine-rich repeat-containing 8	Lrrc8	1171	2161	**1.85**
1370123_a_at	cortactin	Cttn	1563	2879	**1.84**
1382040_at	glutamyl-prolyl-tRNA synthetase	Eprs	2218	4085	**1.84**
1367855_at	scavenger receptor class B, member 1	Scarb1	444	817	**1.84**
1385059_at	zinc finger protein 655	Zfp655	1201	2210	**1.84**
1371386_at	similar to Protein C9orf10 (predicted) /// hypothetical protein LOC683420 /// similar to Protein CXorf17 homolog	LOC683420 /// LOC69	9838	18098	**1.84**
1383023_at	ubiquitin-conjugating enzyme E2H (predicted)	Ube2h_predicted	614	1128	**1.84**
1381118_at	similar to RIKEN cDNA 2700007P21	RGD1311463	461	847	**1.84**
1372000_at	neuroepithelial cell transforming gene 1	Net1	2734	5017	**1.84**
1388487_at	adducin 1 (alpha)	Add1	1363	2500	**1.83**
1389048_at	bone morphogenetic protein 1	Bmp1	821	1505	**1.83**
1372650_at	similar to Dynamin-binding protein (Scaffold protein Tuba)	LOC309362	521	954	**1.83**
1373096_at	ARP8 actin-related protein 8 homolog (S. cerevisiae) (predicted)	Actr8_predicted	1633	2992	**1.83**
1383076_at	similar to RIKEN cDNA 1200016B10 (predicted)	RGD1308695_predict	1324	2427	**1.83**
1371028_at	trans-golgi network protein 2	Tgoln2	586	1073	**1.83**
1388983_at	similar to hypothetical protein (predicted)	RGD1305045_predict	1703	3114	**1.83**
1371380_at	pyruvate dehydrogenase E1 alpha 1 /// pyruvate dehydrogenase E1 alpha 1 pseudogene	LOC685778 /// Pdha1	1065	1947	**1.83**
1373244_at	similar to RIKEN cDNA 2010008E23 gene	RGD1304758	868	1585	**1.83**
1398879_at	transmembrane protein 66	Tmem66	1747	3192	**1.83**
1367694_at	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	Hadhb	3697	6751	**1.83**
1367534_at	G protein-coupled receptor 21 (predicted)	Gpr21_predicted	447	816	**1.83**
1371627_at	Angiomotin-like 1 (predicted)	Amotl1_predicted	3352	6120	**1.83**
1374920_at	Transcribed locus	---	1792	3269	**1.82**
1388830_at	protein kinase N2	Pkn2	1775	3236	**1.82**
1375843_at	iduronate 2-sulfatase	Ids	2095	3816	**1.82**
1372088_at	PTPRF interacting protein, binding protein 1 (liprin beta 1) (predicted)	Ppfibp1_predicted	1355	2464	**1.82**
1387521_at	programmed cell death 4	Pdcd4	942	1714	**1.82**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1387641_at	RAB5A, member RAS oncogene family	Rab5a	1720	3126	**1.82**
1374947_at	breast cancer anti-estrogen resistance 3 (predicted)	Bcar3_predicted	503	914	**1.82**
1382065_at	Transcribed locus	---	1032	1872	**1.81**
1382013_at	CAP, adenylate cyclase-associated protein, 2 (yeast)	Cap2	634	1148	**1.81**
1376984_at	gene trap locus 6	Gtl6	539	976	**1.81**
1372934_at	similar to 1700019E19Rik protein (predicted)	RGD1307392_predict	849	1537	**1.81**
1381564_at	glomulin, FKBP associated protein	Glmn	471	853	**1.81**
1399031_at	similar to CG31855-PA	LOC684106 /// LOC68	714	1293	**1.81**
1373894_at	RAB31, member RAS oncogene family	Rab31	867	1568	**1.81**
1370163_at	ornithine decarboxylase 1	Odc1	10035	18146	**1.81**
1388444_at	UBX domain containing 2	Ubxd2	3259	5888	**1.81**
1374047_at	similar to RIKEN cDNA 2400010D15	RGD1311805	484	874	**1.81**
1368838_at	tropomyosin 4	Tpm4	5901	10655	**1.81**
1393110_at	Mpv17 transgene, kidney disease mutant-like (predicted)	Mpv17l_predicted	1150	2075	**1.81**
1388140_at	RAB13, member RAS oncogene family	Rab13	579	1044	**1.80**
1373346_at	similar to hypothetical protein CL25084 (predicted)	RGD1306508_predict	1057	1905	**1.80**
1368819_at	integrin beta 1 (fibronectin receptor beta)	Itgb1	6048	10898	**1.80**
1368305_at	caspase 6	Casp6	2111	3804	**1.80**
1376197_at	transcription factor 7, T-cell specific (predicted)	Tcf7_predicted	543	978	**1.80**
1391980_at	similar to ubiquitin specific protease 34	LOC360990	863	1554	**1.80**
1387454_at	niban protein	Niban	609	1096	**1.80**
1376274_at	BTB (POZ) domain containing 10	Btbd10	901	1621	**1.80**
1382423_at	Transcribed locus	---	595	1069	**1.80**
1372320_at	male-specific lethal-3 homolog 1 (Drosophila)	Msl31	600	1077	**1.80**
1372206_at	similar to chromosome 14 open reading frame 9	RGD1307475	646	1159	**1.79**
1373561_at	chondroitin polymerizing factor	D1bwg1363e	984	1766	**1.79**
1388503_at	similar to CREBBP/EP300 inhibitory protein 1 (predicted)	RGD1562702_predict	2434	4366	**1.79**
1376832_at	similar to FLJ20689 (predicted)	RGD1308907_predict	834	1496	**1.79**
1390063_at	microfibrillar-associated protein 3	Mfap3	674	1207	**1.79**
1387782_at	dynein light chain LC8-type 2	Dynll2	1619	2899	**1.79**
1385741_at	similar to RIKEN cDNA 1700052N19	RGD1305235	530	948	**1.79**
1389014_at	pre-B-cell colony enhancing factor 1	Pbef1	2638	4717	**1.79**
1374215_at	pleckstrin homology domain containing, family J member 1	Plekhj1	691	1235	**1.79**
1398934_at	mitogen-activated protein kinase kinase kinase 7 interacting protein 2	Map3k7ip2	2156	3853	**1.79**
1398959_at	inositol polyphosphate-5-phosphatase E	Inpp5e	881	1574	**1.79**
1398493_at	CDNA clone IMAGE:7318263	---	735	1313	**1.79**
1367966_at	dipeptidylpeptidase 3 /// similar to Dipeptidyl-peptidase 3 (Dipeptidyl-peptidase III) (DPP III) (Dipeptidyl aminopeptidase III) (Dipeptidyl arylamidase III)	Dpp3 /// LOC678760	936	1671	**1.79**
1387086_at	calcium modulating ligand	Camlg	819	1462	**1.79**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1388823_at	RAB5B, member RAS oncogene family (predicted)	Rab5b_predicted	1312	2343	**1.79**
1381065_at	hypothetical protein LOC499120	LOC499120	1186	2116	**1.78**
1375955_at	zinc finger protein 313	Zfp313	1385	2469	**1.78**
1393072_at	ubiquitin-conjugating enzyme E2Q (putative) 2 (predicted)	Ube2q2_predicted	3324	5923	**1.78**
1387061_at	junction plakoglobin	Jup	2754	4905	**1.78**
1368385_a_at	growth factor receptor bound protein 2	Grb2	3053	5436	**1.78**
1378098_at	RGD1309748_predicted	similar to CG4768-PA	1717	3057	**1.78**
1390952_at	REST corepressor 1 (predicted)	Rcor1_predicted	2159	3844	**1.78**
1373529_at	mitochondrial carrier triple repeat 1	Mcart1	616	1097	**1.78**
1375642_at	ubiquitin-conjugating enzyme E2B, RAD6 homolog (S. cerevisiae)	Ube2b	1216	2165	**1.78**
1389412_at	---	---	674	1200	**1.78**
1371552_at	Transcribed locus	---	592	1054	**1.78**
1372346_at	zinc finger protein 513	Zfp513	1038	1846	**1.78**
1384148_at	similar to Ras-related protein Rab-20	LOC686866 /// LOC68	635	1130	**1.78**
1371614_at	autophagy-related 12 (yeast)	Atg12	1615	2870	**1.78**
1370000_at	nucleobindin 2	Nucb2	675	1200	**1.78**
1369738_s_at	cAMP responsive element modulator	Crem	577	1026	**1.78**
1376089_at	low density lipoprotein receptor	Ldlr	8931	15861	**1.78**
1374169_at	similar to chromosome 16 open reading frame 5	RGD1310686	622	1105	**1.78**
1387857_at	syntaxin 7	Stx7	595	1056	**1.78**
1378257_at	three prime repair exonuclease 1	Trex1	653	1160	**1.78**
1369452_a_at	phosphatidylinositol binding clathrin assembly protein	Picalm	920	1633	**1.77**
1376230_at	similar to Hypothetical protein MGC37938 (predicted)	RGD1566169_predict	492	873	**1.77**
1381972_at	cereblon	Crbn	1299	2304	**1.77**
1388796_at	Golgi SNAP receptor complex member 1	Gosr1	534	945	**1.77**
1395802_at	similar to SEC24 related gene family, member C	LOC685144	689	1220	**1.77**
1372012_at	24-dehydrocholesterol reductase	Dhcr24	503	890	**1.77**
1373418_at	glutamyl-prolyl-tRNA synthetase	Eprs	3871	6850	**1.77**
1386929_at	hexokinase 1	Hk1	464	821	**1.77**
1368712_at	zinc finger protein 386 (Kruppel-like)	Znf386	776	1373	**1.77**
1375561_at	Transcribed locus	---	499	882	**1.77**
1373476_at	hypothetical protein LOC500251	LOC500251	503	889	**1.77**
1390524_at	ring finger protein 12	Rnf12	3383	5970	**1.76**
1390989_at	similar to Mospd2 protein (predicted)	RGD1563952_predict	993	1753	**1.76**
1390157_at	Transcribed locus, strongly similar to XP_994234.1 PREDICTED: similar to Ubiquitin-conjugating enzyme E2 H (Ubiquitin-protein ligase H) (Ubiquitin carrier protein H) (UBCH2) (E2-20K) [Mus musculus]	---	895	1579	**1.76**
1388474_at	Ubiquitin-conjugating enzyme E2I	Ube2i	1104	1946	**1.76**
1376813_at	membrane bound O-acyltransferase domain containing 5	Mboat5	898	1582	**1.76**
1374687_at	extra spindle poles like 1 (S. cerevisiae) (predicted)	Espl1_predicted	3420	6024	**1.76**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1374514_at	Transcribed locus	---	1525	2683	**1.76**
1371810_at	Coatomer protein complex, subunit gamma	Copg	534	939	**1.76**
1383059_a_at	G kinase anchoring protein 1	Gkap1	908	1598	**1.76**
1377092_at	Transcribed locus	---	992	1743	**1.76**
1372717_at	Transcribed locus	---	639	1123	**1.76**
1374007_at	similar to step II splicing factor SLU7; DNA segment, Chr 11, ERATO Doi 730, expressed; DNA segment, Chr 3, Brigham & Womens Genetics 0878 expressed	LOC303057	1324	2326	**1.76**
1367856_at	glucose-6-phosphate dehydrogenase X-linked	G6pdx	2116	3717	**1.76**
1377166_at	Amyotrophic lateral sclerosis 2 (juvenile) homolog (human)	Als2	515	905	**1.76**
1373282_at	similar to mitochondrial carrier protein MGC4399	LOC691431	703	1234	**1.76**
1391827_at	filamin, beta (predicted)	Flnb_predicted	2213	3885	**1.76**
1389205_at	Transcribed locus	---	640	1123	**1.75**
1372262_at	RGD1563087 (predicted)	RGD1563087_predicte	1075	1886	**1.75**
1374584_at	similar to Serine/threonine protein kinase 24 (predicted)	RGD1561742_predicte	1073	1881	**1.75**
1367512_at	chromatin modifying protein 5	Chmp5	5834	10230	**1.75**
1373918_at	retinol dehydrogenase 11	Rdh11	617	1081	**1.75**
1386880_at	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	Acaa2	1360	2383	**1.75**
1372825_at	Formin binding protein 1	Fnbp1	844	1478	**1.75**
1372400_at	similar to cullin 4A (predicted)	RGD1563853_predicte	634	1110	**1.75**
1370121_at	adducin 1 (alpha)	Add1	489	855	**1.75**
1375412_at	arylsulfatase B	Arsb	963	1684	**1.75**
1370826_at	nucleosome assembly protein 1-like 1	Nap1l1	7107	12426	**1.75**
1390152_at	transmembrane emp24 protein transport domain containing 7 /// similar to transmembrane emp24 protein transport domain containing 7	LOC679060 /// Tmed7	820	1431	**1.75**
1377739_at	guanine nucleotide binding protein (G protein), gamma 12	Gng12	991	1731	**1.75**
1370876_at	similar to proline arginine rich coiled coil 1	LOC681287	942	1644	**1.75**
1374028_at	similar to CDNA sequence BC024479	LOC500974	751	1312	**1.75**
1388365_at	ATPase, H+ transporting, V0 subunit D isoform 1	Atp6v0d1	2527	4410	**1.75**
1389685_at	zinc finger protein 655	Zfp655	3582	6248	**1.74**
1374428_at	kinesin family member 3B (predicted)	Kif3b_predicted	548	957	**1.74**
1399122_at	Transcribed locus	---	544	947	**1.74**
1374914_at	peroxisome proliferator activated receptor delta	Ppard	704	1226	**1.74**
1388520_at	sperm associated antigen 9 (predicted)	Spag9_predicted	2743	4775	**1.74**
1394871_at	solute carrier family 31 (copper transporters), member 1	Slc31a1	553	960	**1.74**
1368824_at	caldesmon 1	Cald1	1558	2705	**1.74**
1385595_at	similar to antigenic determinant of rec-A protein	LOC683353 /// LOC68	582	1008	**1.73**
1386995_at	B-cell translocation gene 2, anti-proliferative	Btg2	9996	17313	**1.73**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1367848_at	dynactin 1	Dctn1	859	1488	**1.73**
1373142_at	Growth hormone inducible transmembrane protein	Ghitm	2283	3952	**1.73**
1387855_at	guanosine diphosphate dissociation inhibitor 1	Gdi1	1343	2325	**1.73**
1370244_at	cathepsin L	Ctsl	14237	24644	**1.73**
1367545_at	G protein-coupled receptor 21 (predicted)	Gpr21_predicted	596	1030	**1.73**
1373279_at	similar to beta-catenin-interacting protein ICAT	LOC503000	1102	1905	**1.73**
1375964_at	phosphoserine phosphatase	Psph	1055	1824	**1.73**
1372862_at	RAB22A, member RAS oncogene family (predicted)	Rab22a_predicted	1112	1923	**1.73**
1393055_at	protein kinase N2	Pkn2	789	1362	**1.73**
1382846_at	Transcribed locus	---	1220	2106	**1.73**
1395237_at	eukaryotic translation initiation factor 5B /// similar to Eukaryotic translation initiation factor 5B (eIF-5B) (Translation initiation factor IF-2)	Eif5b /// LOC689581	1120	1933	**1.73**
1379689_at	Transcribed locus	---	1015	1750	**1.72**
1372201_at	zinc finger protein 403	Zfp403	1572	2709	**1.72**
1377214_a_at	hypothetical protein LOC292764	RGD1303117	907	1560	**1.72**
1367532_at	DAZ associated protein 2	Dazap2	2567	4414	**1.72**
1387654_at	myosin IC	Myo1c	799	1373	**1.72**
1371555_at	sorting nexin 12 (predicted)	Snx12_predicted	609	1047	**1.72**
1384319_at	Tousled-like kinase 2 (Arabidopsis) (predicted)	Tlk2_predicted	1426	2449	**1.72**
1372624_at	transmembrane protein 16F (predicted)	Tmem16f_predicted	737	1265	**1.72**
1383894_at	Transcribed locus	---	1290	2215	**1.72**
1371594_at	fission 1 (mitochondrial outer membrane) homolog (yeast)	Fis1	1274	2185	**1.71**
1375185_at	importin 7 (predicted)	Ipo7_predicted	4469	7659	**1.71**
1372038_at	MAP kinase-interacting serine/threonine kinase 2	Mknk2	524	897	**1.71**
1388937_at	ring finger protein (C3HC4 type) 19 (predicted)	Rnf19_predicted	702	1202	**1.71**
1376660_at	Transcribed locus	---	526	900	**1.71**
1372758_at	Transcribed locus	---	951	1627	**1.71**
1367624_at	activating transcription factor 4	Atf4	9715	16615	**1.71**
1388755_at	SEC23A (S. cerevisiae) (predicted)	Sec23a_predicted	966	1652	**1.71**
1373491_at	similar to Glucosylceramidase precursor (Beta-glucocerebrosidase) (Acid beta-glucosidase) (D-glucosyl-N-acylsphingosine glucohydrolase)	LOC684536	675	1153	**1.71**
1392462_at	v-crk sarcoma virus CT10 oncogene homolog (avian)	Crk	3605	6159	**1.71**
1377886_at	Transcribed locus, strongly similar to XP_926592.1 PREDICTED: similar to Tripartite motif protein 6 isoform 3 [Mus musculus]	---	1684	2876	**1.71**
1370265_at	arrestin, beta 2	Arrb2	564	964	**1.71**
1398999_at	similar to es 64	LOC363675	924	1574	**1.70**
1374548_at	AF4/FMR2 family, member 4 (predicted)	Aff4_predicted	2322	3956	**1.70**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1393843_at	feminization 1 homolog b (C. elegans) (predicted)	Fem1b_predicted	1723	2934	**1.70**
1388445_at	annexin A11	Anxa11	1796	3057	**1.70**
1369994_at	calcitonin gene-related peptide-receptor component protein	Crcp	695	1182	**1.70**
1398905_at	ATPase, H transporting, lysosomal V1 subunit G1 (predicted)	Atp6v1g1_predicted	877	1490	**1.70**
1374212_at	Transcribed locus	---	1525	2592	**1.70**
1388771_at	CGG triplet repeat binding protein 1 (predicted)	Cggbp1_predicted	984	1671	**1.70**
1383206_at	component of oligomeric golgi complex 3	Cog3	511	868	**1.70**
1382721_at	zinc finger protein 403	Zfp403	2933	4978	**1.70**
1372120_at	ubiquitin-activating enzyme E1-domain containing 1	Ube1dc1	1039	1762	**1.70**
1399114_at	general transcription factor II E, polypeptide 2 (beta subunit) (predicted)	Gtf2e2_predicted	1602	2718	**1.70**
1373862_at	transmembrane emp24 protein transport domain containing 7	Tmed7	6674	11320	**1.70**
1390868_at	similar to RIKEN cDNA 1200016B10 (predicted)	RGD1308695_predict	842	1428	**1.70**
1371337_at	cytochrome c oxidase subunit VIIa polypeptide 2 like (predicted)	Cox7a2l_predicted	6498	11020	**1.70**
1368386_at	growth factor receptor bound protein 2	Grb2	1995	3381	**1.69**
1383060_at	G kinase anchoring protein 1	Gkap1	643	1089	**1.69**
1391040_at	similar to Retinoblastoma-binding protein 8 (RBBP-8) (CtBP interacting protein) (CtIP) (Retinoblastoma-interacting protein and myosin-like) (RIM) (predicted)	RGD1308872_predict	650	1100	**1.69**
1372177_at	molybdenum cofactor synthesis 2	Mocs2	882	1492	**1.69**
1388512_at	phosphodiesterase 6D, cGMP-specific, rod, delta (predicted)	Pde6d_predicted	640	1082	**1.69**
1379909_at	G kinase anchoring protein 1	Gkap1	2918	4931	**1.69**
1376153_at	Transcribed locus	---	1355	2288	**1.69**
1396128_at	similar to RIKEN cDNA 1700034P14 (predicted)	RGD1305492_predict	1862	3141	**1.69**
1377103_at	midnolin (predicted)	Midn_predicted	1681	2836	**1.69**
1371653_at	Tropomyosin 4	Tpm4	12657	21341	**1.69**
1393569_at	RNA binding motif protein 18 (predicted)	Rbm18_predicted	1231	2076	**1.69**
1368030_at	guanine nucleotide binding protein, alpha inhibiting 3 /// guanine nucleotide binding protein, alpha inhibiting 1	Gnai1 /// Gnai3	2223	3744	**1.68**
1389730_at	Transcribed locus	---	1141	1921	**1.68**
1367854_at	ATP citrate lyase	Acly	5221	8787	**1.68**
1386229_at	similar to CBF1 interacting corepressor	RGD1309199	755	1270	**1.68**
1372414_at	microtubule-associated proteins 1A/1B light chain 3	Map1lc3b	1006	1692	**1.68**
1373301_at	protein phosphatase 1, regulatory (inhibitor) subunit 13B (predicted)	Ppp1r13b_predicted	2118	3563	**1.68**
1373206_at	fibronectin type III domain containing 3B (predicted)	Fndc3b_predicted	2706	4551	**1.68**
1385889_at	Similar to hypothetical protein MGC52110 (predicted)	RGD1565095_predict	5680	9547	**1.68**
1372196_at	nuclear receptor co-repressor 2 (predicted)	Ncor2_predicted	647	1088	**1.68**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1371983_at	Josephin domain containing 1	Josd1	571	959	**1.68**
1374048_at	neurturin	Nrtn	596	1000	**1.68**
1368834_at	calcium/calmodulin-dependent protein kinase II, delta	Camk2d	629	1055	**1.68**
1372791_at	Protein kinase, lysine deficient 1	Prkwnk1	2505	4201	**1.68**
1387152_at	nuclear receptor binding factor 2	Nrbf2	607	1017	**1.68**
1372283_at	trafficking protein particle complex 6B (predicted)	Trappc6b_predicted	1783	2986	**1.67**
1367884_at	RAB14, member RAS oncogene family	Rab14	4853	8125	**1.67**
1378500_at	AT rich interactive domain 3A (Bright like) (predicted)	Arid3a_predicted	922	1544	**1.67**
1387016_a_at	stromal cell derived factor receptor 1	Sdfr1	4995	8360	**1.67**
1399151_at	similar to hypothetical protein FLJ11526 (predicted)	RGD1309585_predicte	2295	3840	**1.67**
1394993_at	Transcribed locus	---	1916	3203	**1.67**
1369879_a_at	testis enhanced gene transcript	Tegt	4890	8177	**1.67**
1383167_at	pregnancy-specific beta 1-glycoprotein	RGD:727932	545	912	**1.67**
1376627_at	Transcribed locus	---	1188	1984	**1.67**
1387800_at	Fas death domain-associated protein	Daxx	1011	1688	**1.67**
1393310_at	itchy homolog E3 ubiquitin protein ligase	Itch	552	921	**1.67**
1398986_at	serine incorporator 3	Serinc3	4558	7603	**1.67**
1372548_at	Transcribed locus, strongly similar to XP_999783.1 PREDICTED: similar to cryptochrome 2 (photolyase-like) isoform 5 [Mus musculus]	---	557	930	**1.67**
1391497_at	TBC1D12: TBC1 domain family, member 12 (predicted)	Tbc1d12_predicted	651	1086	**1.67**
1383265_at	TBC1 domain family, member 23 (predicted)	Tbc1d23_predicted	938	1563	**1.67**
1368984_at	septin 2	Sept2	2334	3887	**1.67**
1373867_at	similar to Retinoblastoma-binding protein 2 (RBBP-2)	LOC312678	2155	3588	**1.66**
1377807_a_at	Similar to IQ motif and WD repeats 1 (predicted)	RGD1561961_predicte	983	1636	**1.66**
1367547_at	similar to zinc finger protein 198	LOC305913	1856	3085	**1.66**
1374462_at	kinesin-associated protein 3 (predicted)	Kifap3_predicted	583	970	**1.66**
1372789_at	zinc finger protein 637	Zfp637	694	1153	**1.66**
1389531_at	zinc finger protein 330 (predicted)	Zfp330_predicted	3956	6568	**1.66**
1383431_at	similar to KIAA1841 protein (predicted)	RGD1305110_predicte	668	1108	**1.66**
1389383_at	SEC24 related gene family, member A (S. cerevisiae) (predicted)	Sec24a_predicted	898	1488	**1.66**
1383697_at	Transcribed locus	---	661	1095	**1.66**
1389233_at	RalBP1 associated Eps domain containing protein (predicted)	Reps1_predicted	1566	2595	**1.66**
1376262_at	UDP-glucuronate decarboxylase 1	Uxs1	1653	2738	**1.66**
1371465_at	cortactin	Cttn	2967	4914	**1.66**
1371723_at	Ras-related GTP binding C (predicted)	Rragc_predicted	1890	3127	**1.65**
1383455_at	glutamyl-prolyl-tRNA synthetase	Eprs	4207	6957	**1.65**
1372395_at	similar to KIAA0597 protein (predicted)	RGD1565757_predicte	1326	2193	**1.65**
1380808_at	toll interacting protein (predicted)	Tollip_predicted	753	1244	**1.65**
1391632_at	inositol polyphosphate-5-phosphatase E	Inpp5e	777	1283	**1.65**
1394654_at	zinc finger protein 451	Zfp451	572	944	**1.65**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1390852_x_at	Similar to IQ motif and WD repeats 1 (predicted)	RGD1561961_predict	1007	1661	**1.65**
1383327_at	programmed cell death 4	Pdcd4	820	1352	**1.65**
1382365_at	Similar to nemo like kinase (predicted)	RGD1561602_predict	695	1146	**1.65**
1370025_at	phosphatidylinositol-4-phosphate 5-kinase, type II, gamma	Pip5k2c	1671	2751	**1.65**
1374608_at	similar to TatD DNase domain containing 2	LOC500295	1105	1819	**1.65**
1376043_at	similar to PHD zinc finger containing protein JUNE1	MGC94192	2104	3464	**1.65**
1379412_at	Transcribed locus	---	567	933	**1.65**
1385596_at	TBC1 domain family, member 15	Tbc1d15	1653	2716	**1.64**
1390199_at	Transcribed locus	---	874	1434	**1.64**
1399085_at	zinc metalloproteinase, STE24 homolog (S. cerevisiae) (predicted)	Zmpste24_predicted	628	1028	**1.64**
1388408_at	similar to RIKEN cDNA 1110020C13	RGD1307129	3435	5624	**1.64**
1384387_at	Transcribed locus	---	900	1473	**1.64**
1373791_at	similar to RIKEN cDNA 2310011J03	RGD1359127	1221	1999	**1.64**
1390115_at	SEC63-like (S. cerevisiae) (predicted)	Sec63_predicted	849	1390	**1.64**
1376080_at	REST corepressor 1 (predicted)	Rcor1_predicted	1431	2340	**1.64**
1391837_at	similar to chr2 synaptotagmin (predicted)	RGD1565705_predict	954	1559	**1.64**
1382045_at	TBC1 domain family, member 15	Tbc1d15	2441	3988	**1.63**
1382756_at	karyopherin (importin) alpha 1	Kpna1	617	1007	**1.63**
1375666_at	cyclin D binding myb-like transcription factor 1	Dmtf1	1108	1809	**1.63**
1370014_at	syntaxin 4A (placental)	Stx4a	1369	2235	**1.63**
1383328_x_at	programmed cell death 4	Pdcd4	1122	1831	**1.63**
1380110_at	Janus kinase 2	Jak2	760	1240	**1.63**
1373284_at	salvador homolog 1 (Drosophila) (predicted)	Sav1_predicted	1096	1788	**1.63**
1367796_at	mannoside acetylglucosaminyltransferase 1	Mgat1	523	853	**1.63**
1373817_at	inhibitor of growth family, member 4	Ing4	720	1173	**1.63**
1378637_at	nuclear transcription factor, X-box binding 1	Nfx1	3568	5813	**1.63**
1399123_at	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26	Ddx26	1498	2440	**1.63**
1379699_at	similar to FLJ20689 (predicted)	RGD1308907_predict	758	1231	**1.62**
1390352_at	polycomb group ring finger 1	Pcgf1	605	983	**1.62**
1379513_at	transmembrane protein 30B (predicted)	Tmem30b_predicted	781	1268	**1.62**
1379282_at	leucine rich repeat (in FLII) interacting protein 2	Lrrfip2	524	850	**1.62**
1379259_at	Transcribed locus	---	1022	1659	**1.62**
1369631_at	myosin IC /// similar to unconventional myosin Myr2 I heavy chain	LOC686250 /// Myo1c	1880	3051	**1.62**
1373416_at	fibronectin type III domain containing 3B (predicted)	Fndc3b_predicted	2831	4590	**1.62**
1371595_at	Transcribed locus, weakly similar to XP_984565.1 PREDICTED: hypothetical protein LOC66961 [Mus musculus]	---	919	1489	**1.62**
1379450_at	---	---	909	1472	**1.62**
1374412_at	F-box and WD-40 domain protein 11 (predicted)	Fbxw11_predicted	1811	2931	**1.62**
1382537_at	Ras-related GTP binding C (predicted)	Rragc_predicted	1486	2406	**1.62**
1372341_at	similar to solute carrier family 25, member 36	LOC501039	559	905	**1.62**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1382417_at	transformed mouse 3T3 cell double minute 4	Mdm4	836	1350	**1.61**
1384901_at	zinc finger protein 451	Zfp451	586	946	**1.61**
1372904_at	MOB1, Mps One Binder kinase activator-like 2B (yeast) (predicted)	Mobkl2b_predicted	674	1088	**1.61**
1390448_at	similar to 1110065L07Rik protein (predicted)	RGD1308317_predict	592	955	**1.61**
1390131_at	serine racemase	Srr	781	1259	**1.61**
1373553_at	topoisomerase (DNA) III beta (predicted)	Top3b_predicted	649	1045	**1.61**
1369421_at	topoisomerase (DNA) I	Top1	1456	2347	**1.61**
1399022_at	CDC-like kinase 1	Clk1	3024	4872	**1.61**
1371893_at	procollagen, type IV, alpha 3 (Goodpasture antigen) binding protein (predicted)	Col4a3bp_predicted	1302	2096	**1.61**
1370501_at	ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, C. elegans)	Ube2g1	3293	5303	**1.61**
1390142_at	microrchidia 3 (predicted)	Morc3_predicted	1194	1922	**1.61**
1381043_at	spermatogenesis associated, serine-rich 1	Spats1	630	1014	**1.61**
1377262_at	similar to KIAA2010 protein (predicted)	RGD1309450_predict	1814	2916	**1.61**
1378405_at	zinc finger protein 143	Zfp143	640	1029	**1.61**
1375423_at	hypothetical protein LOC689959	LOC689959	729	1172	**1.61**
1380148_at	Wolf-Hirschhorn syndrome candidate 1 (predicted)	Whsc1_predicted	1996	3207	**1.61**
1367865_at	limkain b1	Lkap	527	847	**1.61**
1367940_at	chemokine orphan receptor 1	Cmkor1	1704	2736	**1.61**
1370070_at	synaptojanin 1	Synj1	740	1189	**1.61**
1389729_at	similar to cDNA sequence BC003324	MGC94142	583	936	**1.60**
1367840_at	HGF-regulated tyrosine kinase substrate	Hgs	1361	2183	**1.60**
1389717_at	similar to KIAA0157 gene product is novel. (predicted)	RGD1308918_predict	635	1019	**1.60**
1397617_at	similar to Retinoblastoma-binding protein 2 (RBBP-2)	LOC312678	734	1177	**1.60**
1384439_at	peptidylprolyl isomerase D	RGD:1303174	1476	2367	**1.60**
1372824_at	pleckstrin homology domain containing, family F (with FYVE domain) member 2 (predicted)	Plekhf2_predicted	1132	1814	**1.60**
1399090_at	dynein, cytoplasmic, light intermediate chain 1	Dncli1	4588	7351	**1.60**
1389402_at	AXIN1 up-regulated 1 (predicted)	Axud1_predicted	1323	2119	**1.60**
1386525_at	THO complex 2 (predicted)	Thoc2_predicted	547	876	**1.60**
1375699_at	centaurin, beta 2	Centb2	540	865	**1.60**
1382308_at	Transcribed locus	---	1110	1777	**1.60**
1380560_at	Transcribed locus	---	541	866	**1.60**
1388027_a_at	reticulon 4	Rtn4	3982	6370	**1.60**
1392038_at	similar to PI-3-kinase-related kinase SMG-1 isoform 2 (predicted) /// similar to PI-3-kinase-related kinase SMG-1	LOC691397 /// RGD15	595	952	**1.60**
1367621_at	death-associated protein kinase 3	Dapk3	796	1273	**1.60**
1383538_at	Transcribed locus, strongly similar to XP_996029.1 PREDICTED: zinc finger protein 650 [Mus musculus]	---	1011	1617	**1.60**
1386042_at	Transcribed locus	---	624	997	**1.60**
1390475_at	RNA guanylyltransferase and 5'-phosphatase (predicted)	Rngtt_predicted	563	900	**1.60**
1393587_a_at	similar to RIKEN cDNA 2310047O13 (predicted)	RGD1309605_predict	870	1391	**1.60**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1392681_at	RAB2, member RAS oncogene family	Rab2	2316	3701	**1.60**
1390777_at	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (S. cerevisae)	Sc5d	1147	1832	**1.60**
1367980_at	rabaptin, RAB GTPase binding effector protein 1	Rabep1	829	1323	**1.60**
1369986_at	hydroxyacyl glutathione hydrolase	Hagh	511	815	**1.59**
1367725_at	serine/threonine-protein kinase pim-3	Pim3	1261	2011	**1.59**
1372719_at	---	---	1052	1677	**1.59**
1398865_at	unc-50 homolog (C. elegans)	Unc50	2724	4342	**1.59**
1371530_at	keratin complex 2, basic, gene 8	Krt2-8	14974	23855	**1.59**
1397405_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	Ddx17	514	818	**1.59**
1371878_at	mastermind like 1 (Drosophila) (predicted)	Maml1_predicted	581	925	**1.59**
1373535_at	enabled homolog (Drosophila)	Enah	2060	3280	**1.59**
1382242_at	zinc finger protein 451	Zfp451	574	913	**1.59**
1387222_at	postsynaptic protein Cript	Cript	972	1545	**1.59**
1369642_at	platelet-activating factor acetylhydrolase, isoform 1b, alpha2 subunit	Pafah1b2	1427	2268	**1.59**
1388002_at	TAO kinase 1	Taok1	759	1205	**1.59**
1385210_at	dedicator of cytokinesis 5 (predicted)	Dock5_predicted	862	1369	**1.59**
1373950_at	zinc finger protein 496 (predicted)	Zfp496_predicted	1169	1856	**1.59**
1372964_at	AT rich interactive domain 5B (Mrf1 like) (predicted)	Arid5b_predicted	3086	4896	**1.59**
1368837_at	AT rich interactive domain 4B (Rbp1 like)	Arid4b	849	1345	**1.59**
1394731_at	casein kinase 1, gamma 3	Csnk1g3	652	1034	**1.59**
1379449_at	RB1-inducible coiled-coil 1 (predicted)	Rb1cc1_predicted	1356	2149	**1.58**
1378096_at	kinesin family member 5B	Kif5b	889	1407	**1.58**
1386952_a_at	dynein, cytoplasmic, intermediate chain 2	Dncic2	2752	4355	**1.58**
1389138_at	SEC22 vesicle trafficking protein-like 1 (S. cerevisiae)	Sec22l1	710	1124	**1.58**
1390526_at	kelch-like 9 (Drosophila) (predicted)	Klhl9_predicted	1168	1847	**1.58**
1369070_at	peroxisomal biogenesis factor 12	Pex12	677	1069	**1.58**
1373548_at	WD repeat domain 68 (predicted)	Wdr68_predicted	1648	2601	**1.58**
1368056_at	tuberous sclerosis 2	Tsc2	697	1099	**1.58**
1371956_at	similar to praja1, RING-H2 motif containing	LOC683077	1124	1772	**1.58**
1370949_at	myristoylated alanine rich protein kinase C substrate /// similar to Myristoylated alanine-rich C-kinase substrate (MARCKS) (ACAMP-81) /// similar to Myristoylated alanine-rich C-kinase substrate (MARCKS) (Protein kinase C substrate 80 kDa protein)	LOC294446 /// LOC68	2617	4123	**1.58**
1392917_at	ADP-ribosylation factor interacting protein 1	Arfip1	1728	2722	**1.58**
1398970_at	translocation protein 1	Tloc1	2212	3483	**1.57**
1396692_at	similar to RIKEN cDNA 4933435A13	RGD1306402	1074	1692	**1.57**
1399126_at	kinesin light chain 1	Klc1	1136	1788	**1.57**
1392486_at	transmembrane protein 106B	Tmem106b	1101	1732	**1.57**
1374485_at	Transcribed locus	---	2800	4404	**1.57**
1389162_at	histone cell cycle regulation defective interacting protein 5 (predicted)	Hirip5_predicted	826	1298	**1.57**
1393027_at	similar to RIKEN cDNA 1700052N19	RGD1305235	1758	2761	**1.57**
1379271_at	similar to Suppressor of cytokine signaling 5 (predicted)	RGD1564914_predict	1043	1638	**1.57**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1385526_at	similar to autophagy 5-like	LOC365601	656	1029	**1.57**
1383617_at	similar to RIKEN cDNA 4921511I16	RGD1305302	662	1038	**1.57**
1371952_at	RNA binding motif protein 18 (predicted)	Rbm18_predicted	2207	3463	**1.57**
1377808_at	Similar to IQ motif and WD repeats 1 (predicted)	RGD1561961_predict	542	850	**1.57**
1368272_at	glutamate oxaloacetate transaminase 1	Got1	2781	4359	**1.57**
1373908_at	---	---	4860	7617	**1.57**
1377593_at	dolichol-phosphate (beta-D) mannosyltransferase 1 (predicted)	Dpm1_predicted	4184	6555	**1.57**
1369571_at	golgi phosphoprotein 3	Golph3	1414	2215	**1.57**
1381878_at	ubinuclein 1 (predicted)	Ubn1_predicted	857	1342	**1.56**
1375297_at	similar to RIKEN cDNA 0610008C08 (predicted)	RGD1565289_predict	723	1132	**1.56**
1379739_at	Transcribed locus	---	1509	2360	**1.56**
1390027_at	ubiquitin specific protease 8 (predicted)	Usp8_predicted	1125	1760	**1.56**
1374692_at	Sorting nexin 14 (predicted)	Snx14_predicted	543	849	**1.56**
1375687_at	RAB14, member RAS oncogene family	Rab14	8937	13964	**1.56**
1395886_at	ARP3 actin-related protein 3 homolog (yeast)	Actr3	3318	5184	**1.56**
1368029_at	guanine nucleotide binding protein, alpha inhibiting 3 /// guanine nucleotide binding protein, alpha inhibiting 1	Gnai1 /// Gnai3	5317	8306	**1.56**
1371707_at	transportin 2 (importin 3, karyopherin beta 2b) (predicted)	Tnpo2_predicted	2055	3210	**1.56**
1388307_at	serine incorporator 1	Serinc1	4998	7805	**1.56**
1396386_at	Beta-site APP-cleaving enzyme 2	Bace2	1537	2399	**1.56**
1376885_at	chemokine-like factor super family 4 (predicted)	Cklfsf4_predicted	1025	1599	**1.56**
1389317_at	similar to hypothetical protein FLJ20507 (predicted)	RGD1309744_predict	1064	1659	**1.56**
1377723_at	Transcribed locus	---	1200	1870	**1.56**
1373087_at	Membrane-associated ring finger (C3HC4) 7	Mar7	4802	7484	**1.56**
1375441_at	seryl-aminoacyl-tRNA synthetase 1	Sars1	4173	6504	**1.56**
1389064_at	fem-1 homolog c (C.elegans) (predicted)	Fem1c_predicted	1055	1643	**1.56**
1372055_at	similar to P-Rex1 (predicted)	RGD1306534_predict	835	1300	**1.56**
1370993_at	laminin, gamma 1	Lamc1	590	918	**1.56**
1389742_at	Transcribed locus	---	957	1489	**1.56**
1398330_at	syntaxin binding protein 1	Stxbp1	602	936	**1.56**
1372986_at	Jun dimerization protein 2	Jundp2	2616	4069	**1.56**
1399113_at	similar to chr2 synaptotagmin (predicted)	RGD1565705_predict	1223	1900	**1.55**
1383825_at	radixin	Rdx	993	1543	**1.55**
1398814_at	RAB11a, member RAS oncogene family	Rab11a	3105	4822	**1.55**
1376665_at	Transcribed locus	---	1318	2047	**1.55**
1373598_at	ubinuclein 1 (predicted)	Ubn1_predicted	730	1133	**1.55**
1372243_at	calcium binding protein 39 (predicted)	Cab39_predicted	1261	1958	**1.55**
1371610_at	tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase (predicted)	Tnks_predicted	2371	3678	**1.55**
1377147_at	TBC1 domain family, member 7 (predicted)	Tbc1d7_predicted	524	812	**1.55**
1392509_at	Casitas B-lineage lymphoma-like 1 (predicted)	Cbll1_predicted	778	1206	**1.55**
1371969_at	caldesmon 1	Cald1	4294	6656	**1.55**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1382154_at	Transcribed locus, moderately similar to NP_035333.2 protein tyrosine phosphatase, non-receptor type 12 [Mus musculus]	---	6473	10033	**1.55**
1390026_at	Bcl2-associated athanogene 3	Bag3	765	1185	**1.55**
1389556_at	kinesin-associated protein 3 (predicted)	Kifap3_predicted	761	1179	**1.55**
1376636_at	transforming growth factor, beta receptor 1	Tgfbr1	1419	2199	**1.55**
1387806_at	RAS related protein 1b	Rap1b	6218	9627	**1.55**
1392621_at	similar to FKSG26 protein (predicted)	RGD1309054_predict	644	997	**1.55**
1368067_at	zinc finger protein 148	Zfp148	1146	1774	**1.55**
1391431_at	transducer of ERBB2, 2	Tob2	568	878	**1.54**
1373998_at	Transcribed locus	---	1107	1710	**1.54**
1388800_at	RAB5A, member RAS oncogene family	Rab5a	2623	4051	**1.54**
1374285_at	Transcribed locus	---	810	1250	**1.54**
1368187_at	glycoprotein (transmembrane) nmb	Gpnmb	3142	4848	**1.54**
1390488_a_at	serine/threonine kinase 38	Stk38	682	1051	**1.54**
1383091_at	amyloid beta precursor protein (cytoplasmic tail) binding protein 2	Appbp2	1694	2609	**1.54**
1373534_at	similar to SR rich protein	RGD1307395	1228	1890	**1.54**
1377595_at	sperm specific antigen 2 (predicted)	Ssfa2_predicted	4668	7180	**1.54**
1391262_at	similar to SUMO/sentrin specific protease 5 (predicted) /// similar to SUMO/sentrin specific protease 5	LOC686268 /// LOC69	1011	1555	**1.54**
1394388_at	hypothetical LOC100125371	LOC100125371	1927	2958	**1.54**
1394741_at	heterogeneous nuclear ribonucleoprotein A3	Hnrpa3	552	848	**1.54**
1374224_at	eukaryotic translation initiation factor 2 alpha kinase 4 (predicted)	Eif2ak4_predicted	1176	1805	**1.53**
1373441_at	dynactin 5	Dctn5	2538	3895	**1.53**
1374408_at	similar to CBF1 interacting corepressor	RGD1309199	716	1097	**1.53**
1398445_at	autism susceptibility candidate 2 (predicted)	Auts2_predicted	3100	4749	**1.53**
1384122_at	---	---	715	1095	**1.53**
1386287_at	similar to RIKEN cDNA 5033405K12 (predicted)	RGD1311593_predict	690	1056	**1.53**
1373946_at	similar to RIKEN cDNA 2810037C14	RGD1305915	565	866	**1.53**
1372895_at	similar to RIKEN cDNA 5730469M10	RGD1309676	700	1072	**1.53**
1388677_at	ubiquitin-associated protein 1	Ubap1	820	1255	**1.53**
1371722_at	similar to CGI-96 protein; gastric cancer antigen Zg14	RGD1311547	963	1473	**1.53**
1379251_at	Transcribed locus	---	582	891	**1.53**
1388801_at	Similar to RIKEN cDNA 9030221M09 gene (predicted)	RGD1305469_predict	889	1359	**1.53**
1373290_at	similar to Enhancer of zeste homolog 2 (ENX-1)	LOC312299	1051	1605	**1.53**
1390684_at	Transcribed locus	---	1046	1598	**1.53**
1369562_at	hippocalcin-like 1	Hpcal1	4157	6347	**1.53**
1368852_at	DnaJ (Hsp40) homolog, subfamily A, member 1	Dnaja1	2044	3119	**1.53**
1370348_at	ninjurin 1	Ninj1	2024	3086	**1.52**
1370199_at	nucleobindin 1	Nucb1	3689	5622	**1.52**
1388345_at	p21 (CDKN1A)-activated kinase 2	Pak2	3610	5500	**1.52**
1368929_at	nuclear protein localization 4 homolog (S. cerevisiae)	Nploc4	655	998	**1.52**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1376129_at	similar to vacuolar protein sorting 13C protein (predicted)	RGD1560364_predict	929	1414	**1.52**
1374456_at	similar to Protein disulfide-isomerase TXNDC10 precursor (Thioredoxin domain-containing protein 10)	LOC682967	2652	4035	**1.52**
1399159_a_at	vesicle-associated membrane protein 3	Vamp3	3519	5352	**1.52**
1373569_at	Transcribed locus	---	863	1312	**1.52**
1371667_at	similar to RIKEN cDNA C530043G21 gene	RGD1311162_predict	1306	1985	**1.52**
1367465_at	defender against cell death 1	Dad1	2452	3725	**1.52**
1389053_at	similar to hypothetical protein FLJ20627 (predicted)	RGD1309546_predict	1242	1885	**1.52**
1383070_at	UBX domain containing 8	Ubxd8	1276	1936	**1.52**
1382076_at	solute carrier family 37 (glycerol-3-phosphate transporter), member 1	Slc37a1	919	1394	**1.52**
1372136_at	similar to tetraspanin similar to TM4SF9 (predicted)	RGD1305714_predict	6519	9883	**1.52**
1369641_at	platelet-activating factor acetylhydrolase, isoform 1b, alpha2 subunit	Pafah1b2	1165	1767	**1.52**
1373150_at	catechol-O-methyltransferase domain containing 1 (predicted)	Comtd1_predicted	604	915	**1.52**
1372484_at	Transcribed locus, strongly similar to XP_234508.3 PREDICTED: similar to polyA polymerase [Rattus norvegicus]	---	2505	3792	**1.51**
1374513_at	septin 7	Sept7	9641	14589	**1.51**
1390228_at	amine oxidase, flavin containing 1 (predicted)	Aof1_predicted	828	1253	**1.51**
1373391_at	transmembrane and coiled-coil domains 1	Tmco1	3354	5075	**1.51**
1375853_at	similar to CG13957-PA (predicted)	RGD1309995_predict	1646	2490	**1.51**
1398995_at	similar to retinoid x receptor interacting protein	RGD1307009	926	1399	**1.51**
1387798_a_at	complement receptor related protein	Crry	2780	4200	**1.51**
1379384_at	Sp1 transcription factor	Sp1	1025	1549	**1.51**
1374086_at	Rho GTPase activating protein 21 (predicted)	Arhgap21_predicted	3814	5760	**1.51**
1376075_at	Presenilin 1	Psen1	1281	1935	**1.51**
1372966_at	hypothetical LOC298504 (predicted)	RGD1310174_predict	1312	1980	**1.51**
1371880_at	Sp1 transcription factor	Sp1	2213	3340	**1.51**
1384938_at	Rho GTPase activating protein 1 (predicted)	Arhgap1_predicted	637	962	**1.51**
1386846_at	similar to hypothetical protein MGC36325 (predicted)	RGD1307374_predict	674	1017	**1.51**
1390029_at	transmembrane protein 110	Tmem110	683	1029	**1.51**
1368046_at	solute carrier family 31 (copper transporters), member 1	Slc31a1	543	819	**1.51**
1388563_at	similar to RIKEN cDNA 1700034P14 (predicted)	RGD1305492_predict	3780	5699	**1.51**
1383232_at	RAB33B, member of RAS oncogene family (predicted)	Rab33b_predicted	617	929	**1.51**
1398893_at	Nedd4 family interacting protein 1	Ndfip1	4417	6651	**1.51**
1388893_at	glycosyltransferase 8 domain containing 1	Glt8d1	550	827	**1.50**
1371637_at	heterochromatin protein 1, binding protein 3	Hp1bp3	764	1149	**1.50**
1387440_at	iron responsive element binding protein 2	Ireb2	943	1417	**1.50**
1375916_at	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2 (predicted)	Pcmtd2_predicted	583	876	**1.50**

Table 6.3. Trophoblast differentiation-associated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	dif/ stem
1388903_at	T-complex associated-testis-expressed 1-like (Protein 91/23)	Tcte1l	612	919	**1.50**
1383099_at	similar to RIKEN cDNA 1700034P14 (predicted)	RGD1305492_predict	1315	1975	**1.50**
1398925_at	similar to RIKEN cDNA 1300018I05	RGD1307801	830	1247	**1.50**
1372901_at	similar to RIKEN cDNA 8030451K01 (predicted)	RGD1565414_predict	623	934	**1.50**
1373974_at	oxysterol binding protein (predicted)	Osbp_predicted	797	1195	**1.50**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1394022_at	inhibitor of DNA binding 4	Id4	584	191	955	**5.00**
1376569_at	Kruppel-like factor 2 (lung) (predicted)	Klf2_predicted	163	447	2098	**4.70**
1375532_at	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	Id2	14665	1162	4365	**3.76**
1390205_at	---	---	124	301	1011	**3.36**
1377273_at	Transcribed locus	---	319	319	945	**2.97**
1390151_at	hypothetical protein LOC683034	LOC683034	431	365	1055	**2.89**
1373680_at	Transcribed locus	---	216	335	928	**2.77**
1388547_at	claudin 4	Cldn4	2905	591	1564	**2.65**
1389616_at	Transcribed locus	---	710	970	2567	**2.65**
1383480_at	similar to Gene model 784 (predicted)	RGD1560060_predicted /	53	423	1061	**2.51**
1389683_at	---	---	338	340	805	**2.37**
1367802_at	serum/glucocorticoid regulated kinase	Sgk	445	484	1128	**2.33**
1368189_at	7-dehydrocholesterol reductase	Dhcr7	216	1037	2392	**2.31**
1374531_at	Transcribed locus	---	1419	474	1093	**2.30**
1385350_at	Synaptogenesis-related mRNA sequence 6	---	365	597	1344	**2.25**
1371694_at	dihydropyrimidinase-like 2	Dpysl2	443	773	1727	**2.23**
1373754_at	Transcribed locus	---	3011	524	1168	**2.23**
1382873_at	CTTNBP2 N-terminal like (predicted)	Cttnbp2nl_predicted	421	759	1678	**2.21**
1372510_at	sulfiredoxin 1 homolog (S. cerevisiae)	Srxn1	1104	2274	5010	**2.20**
1373066_at	Transcribed locus	---	359	1185	2606	**2.20**
1377975_at	Transcribed locus	---	128	408	893	**2.19**
1370315_a_at	stathmin-like 4	Stmn4	183	441	957	**2.17**
1389033_at	similar to RIKEN cDNA 2900010M23 (predicted)	RGD1306917_predicted	2774	536	1154	**2.15**
1387017_at	squalene epoxidase	Sqle	197	626	1347	**2.15**
1368247_at	heat shock 70kD protein 1A /// heat shock 70kD protein 1B (mapped)	Hspa1a /// Hspa1b	1095	2044	4372	**2.14**
1370158_at	myosin, heavy polypeptide 10, non-muscle	Myh10	4095	916	1948	**2.13**
1384331_at	sulfiredoxin 1 homolog (S. cerevisiae)	Srxn1	615	1306	2775	**2.12**
1375906_at	hypothetical protein LOC683034	LOC683034	830	509	1080	**2.12**
1374864_at	sprouty homolog 2 (Drosophila)	Spry2	2766	2366	4974	**2.10**
1395957_at	Transcribed locus	---	198	580	1218	**2.10**
1369830_at	protein kinase C, eta	Prkch	626	453	950	**2.10**
1372603_at	protein kinase C and casein kinase substrate in neurons 3	Pacsin3	313	396	830	**2.10**
1383173_at	killer cell lectin-like receptor subfamily C, member 1	Klrc1	164	425	884	**2.08**
1387772_at	calmodulin 1	Calm1	2012	2310	4774	**2.07**
1388836_at	protein kinase C, eta	Prkch	1200	875	1789	**2.05**
1367932_at	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	Hmgcs1	511	1966	3999	**2.03**
1395044_at	hypothetical protein LOC679298 /// hypothetical protein LOC688832	LOC679298 /// LOC68883	1649	988	2000	**2.03**
1378734_at	gastrulation brain homeobox 2	Gbx2	738	858	1718	**2.00**
1394470_at	similar to hypothetical protein DKFZp566A1524 (predicted)	RGD1305961_predicted	214	458	907	**1.98**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1368910_at	protein phosphatase 2C, magnesium dependent, catalytic subunit	Ppm2c	475	645	1276	**1.98**
1370313_at	acyl-CoA thioesterase 7	Acot7	4483	2326	4501	**1.94**
1389367_at	schwannomin interacting protein 1	Schip1	1430	682	1315	**1.93**
1377156_at	similar to transcription factor 7-like 2, T-cell specific, HMG-box /// similar to Transcription factor 7-like 2 (HMG box transcription factor 4) (T-cell-specific transcription factor 4) (TCF-4) (hTCF-4)	LOC679869 /// LOC68373	1768	1525	2928	**1.92**
1368321_at	early growth response 1	Egr1	170	1230	2355	**1.92**
1393037_at	Transcribed locus	---	281	1071	2049	**1.91**
1392264_s_at	serine (or cysteine) peptidase inhibitor, clade E, member 1	Serpine1	233	1226	2347	**1.91**
1383979_at	similar to monoacylglycerol O-acyltransferase 2	LOC683355	1092	638	1218	**1.91**
1372044_at	similar to Ser/Thr-rich protein T10 in DGCR region (predicted)	RGD1310348_predicted	209	447	850	**1.90**
1393558_at	integrin, alpha 6	Itga6	836	919	1747	**1.90**
1391167_at	Transcribed locus	---	1333	1409	2674	**1.90**
1376419_at	Transcribed locus	---	775	684	1293	**1.89**
1390441_at	Development and differentiation enhancing (predicted)	Ddef1_predicted	332	676	1278	**1.89**
1396803_at	THO complex 2 (predicted)	Thoc2_predicted	525	518	975	**1.88**
1393238_at	similar to DKFZP434B168 protein (predicted)	RGD1308014_predicted	1039	534	1003	**1.88**
1376606_a_at	similar to e(y)2 protein	LOC682575 /// LOC68525	3324	1370	2568	**1.87**
1390416_at	Solute carrier family 25, member 30	Slc25a30	1284	1351	2527	**1.87**
1372554_at	similar to RW1 protein (predicted)	RGD1309266_predicted	1256	521	974	**1.87**
1375358_at	Transcribed locus	---	3220	4009	7455	**1.86**
1389172_at	enigma homolog	Enh	418	666	1239	**1.86**
1390929_at	Transcribed locus	---	310	627	1161	**1.85**
1395762_at	Transcribed locus	---	535	428	791	**1.85**
1380148_at	Wolf-Hirschhorn syndrome candidate 1 (predicted)	Whsc1_predicted	1996	3207	5905	**1.84**
1398447_at	Transcribed locus, strongly similar to XP_913560.1 PREDICTED: hypothetical protein LOC109169 [Mus musculus]	---	264	576	1058	**1.84**
1388742_at	---	---	984	559	1025	**1.83**
1390207_at	large tumor suppressor 2 (predicted)	Lats2_predicted	1414	1996	3653	**1.83**
1379450_at	similar to cDNA sequence BC003236 (predicted)	RGD1310822_predicted	909	1472	2690	**1.83**
1386978_at	BCL2/adenovirus E1B 19 kDa-interacting protein 3-like	Bnip3l	1980	2369	4321	**1.82**
1388888_at	---	---	562	553	1007	**1.82**
1386890_at	S100 calcium binding protein A10 (calpactin)	S100a10	5076	3249	5918	**1.82**
1371785_at	tumor necrosis factor receptor superfamily, member 12a	Tnfrsf12a	2101	1192	2167	**1.82**
1381428_a_at	Transcribed locus	---	344	3275	5923	**1.81**
1383353_at	ephrin B2 (predicted)	Efnb2_predicted	1354	588	1061	**1.81**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1371186_at	integrin, alpha 6	Itga6	665	707	1276	**1.80**
1398333_at	Transcribed locus	---	209	861	1554	**1.80**
1388882_at	FK506 binding protein 3 (predicted)	Fkbp3_predicted	4544	894	1610	**1.80**
1387279_at	F11 receptor	F11r	1232	963	1732	**1.80**
1378697_at	RNA binding motif, single stranded interacting protein 2	Rbms2	298	600	1079	**1.80**
1379719_at	Transcribed locus	---	2366	2314	4157	**1.80**
1382680_at	adipose differentiation-related protein	ADRP	258	625	1122	**1.80**
1389164_at	hect domain and RLD 3 (predicted)	Herc3_predicted	458	520	931	**1.79**
1380644_at	Transcribed locus	---	444	555	992	**1.79**
1368115_at	claudin 3	Cldn3	2665	735	1311	**1.78**
1370808_at	cytochrome b5 reductase 3	Cyb5r3	2514	2170	3868	**1.78**
1398389_at	Transcribed locus	---	1229	1443	2570	**1.78**
1373829_at	fibroblast growth factor receptor 2	Fgfr2	3813	877	1557	**1.78**
1372507_at	T-cell leukemia translocation altered gene	Tcta	169	485	860	**1.77**
1371424_at	similar to RIKEN cDNA 3110005O21	RGD1304823	882	580	1028	**1.77**
1375523_at	similar to Myristoylated alanine-rich C-kinase substrate (MARCKS) (ACAMP-81) /// similar to Myristoylated alanine-rich C-kinase substrate (MARCKS) (Protein kinase C substrate 80 kDa protein)	LOC294446 /// LOC68125	2074	2782	4906	**1.76**
1398910_at	STIP1 homology and U-Box containing protein 1	Stub1	2687	1279	2252	**1.76**
1373807_at	Vascular endothelial growth factor A	Vegfa	391	490	862	**1.76**
1398427_at	Myocyte enhancer factor 2D	Mef2d	828	479	841	**1.76**
1379506_at	Transcribed locus	---	349	492	865	**1.76**
1379424_at	similar to DKFZP434B168 protein (predicted)	RGD1308014_predicted	844	458	802	**1.75**
1391935_at	similar to eukaryotic translation initiation factor 4E member 3	LOC297481	206	486	852	**1.75**
1388918_at	high density lipoprotein binding protein	Hdlbp	1637	1483	2595	**1.75**
1388157_at	myristoylated alanine rich protein kinase C substrate	Marcks	836	1313	2296	**1.75**
1371776_at	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1	Pik3r1	1033	1168	2042	**1.75**
1376768_at	Transcribed locus	---	250	570	996	**1.75**
1372586_at	Transcribed locus	---	500	542	947	**1.75**
1388837_at	Solute carrier family 44, member 2 (predicted)	Slc44a2_predicted	1030	581	1014	**1.75**
1397164_at	Transcribed locus, weakly similar to XP_984565.1 PREDICTED: hypothetical protein LOC66961 [Mus musculus]	---	430	632	1101	**1.74**
1369304_at	6-pyruvoyl-tetrahydropterin synthase	Pts	276	517	900	**1.74**
1388853_at	mitochondrial ribosomal protein L54 (predicted)	Mrpl54_predicted	2833	581	1010	**1.74**
1373181_at	similar to actin filament associated protein; actin filament-associated protein, 110 kDa (predicted)	RGD1311580_predicted	1667	1302	2260	**1.74**
1371358_at	glycoprotein, synaptic 2	Gpsn2	1676	1965	3408	**1.73**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1393407_at	F-box and WD-40 domain protein 2 (predicted)	Fbxw2_predicted	743	579	1004	**1.73**
1372718_at	similar to anaphase promoting complex subunit 13	LOC685029	968	688	1192	**1.73**
1384059_at	PC4 and SFRS1 interacting protein 1	Psip1	286	546	945	**1.73**
1375707_at	---	---	1389	9639	16684	**1.73**
1371536_at	calcium regulated heat stable protein 1	Carhsp1	886	1184	2046	**1.73**
1373106_at	zinc finger protein 36, C3H type-like 2	Zfp36l2	1507	1036	1789	**1.73**
1383240_at	integrin, alpha 6	Itga6	2332	2567	4428	**1.73**
1368519_at	serine (or cysteine) peptidase inhibitor, clade E, member 1	Serpine1	349	2238	3858	**1.72**
1367839_at	farnesyl diphosphate farnesyl transferase 1	Fdft1	402	1503	2587	**1.72**
1368277_at	protein phosphatase 3, catalytic subunit, alpha isoform	Ppp3ca	555	669	1150	**1.72**
1373676_at	Transcribed locus	---	319	769	1323	**1.72**
1390902_at	Transcribed locus	---	1670	661	1137	**1.72**
1388943_at	chromatin accessibility complex 1 (predicted)	Chrac1_predicted	681	484	831	**1.72**
1379471_at	similar to RIKEN cDNA 5730469D23 (predicted)	RGD1308324_predicted	2251	2111	3626	**1.72**
1372771_at	Transcribed locus	---	329	1010	1736	**1.72**
1381969_at	similar to Recombining binding protein suppressor of hairless (J kappa-recombination signal binding protein) (RBP-J kappa)	LOC679028	3246	1609	2762	**1.72**
1372096_at	oxidative-stress responsive 1 (predicted)	Oxsr1_predicted	874	965	1656	**1.72**
1372556_at	hypothetical protein LOC502374	LOC502374	2346	820	1405	**1.71**
1368438_at	phosphodiesterase 10A	Pde10a	144	530	908	**1.71**
1372106_at	EH-domain containing 4	Ehd4	428	1265	2163	**1.71**
1368275_at	sterol-C4-methyl oxidase-like	Sc4mol	1030	5389	9202	**1.71**
1373571_at	reticulon 3	Rtn3	581	658	1122	**1.70**
1399150_at	similar to RIKEN cDNA A630054L15; hypothetical protein MGC38041 (predicted)	RGD1306064_predicted	414	624	1062	**1.70**
1372228_at	acetylserotonin O-methyltransferase-like (predicted)	Asmtl_predicted	829	1002	1703	**1.70**
1383096_at	amyloid beta (A4) precursor-like protein 2	Aplp2	550	551	937	**1.70**
1384392_at	cytochrome P450, family 26, subfamily b, polypeptide 1	Cyp26b1	719	912	1550	**1.70**
1370912_at	heat shock 70kD protein 1B (mapped)	Hspa1b	839	1562	2655	**1.70**
1382175_at	Similar to Wilms tumor 1-associating protein (WT1-associated protein) homolog) (predicted)	RGD1563824_predicted	1487	1779	3022	**1.70**
1373858_at	karyopherin (importin) beta 1	Kpnb1	1345	1180	2002	**1.70**
1387636_a_at	P11 protein	Cdtw1	921	785	1331	**1.69**
1372861_at	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	Pip5k1c	934	1115	1888	**1.69**
1398330_at	syntaxin binding protein 1	Stxbp1	602	936	1584	**1.69**
1369268_at	activating transcription factor 3	Atf3	545	517	873	**1.69**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1395502_at	protein phosphatase 2, regulatory subunit B (B56), delta isoform	Ppp2r5d	727	821	1386	**1.69**
1399166_a_at	similar to B-cell CLL/lymphoma 7B	LOC368001	346	560	944	**1.69**
1372419_at	vaccinia related kinase 3	Vrk3	302	531	895	**1.68**
1367663_at	proteasome (prosome, macropain) 28 subunit, alpha	Psme1	1410	565	949	**1.68**
1376026_at	downstream neighbor of SON	Donson	2162	1990	3323	**1.67**
1376655_at	Transcribed locus	---	896	1012	1690	**1.67**
1388199_at	tumor-associated calcium signal transducer 1	Tacstd1	3655	2058	3435	**1.67**
1371615_at	diacylglycerol O-acyltransferase homolog 2 (mouse)	Dgat2	1647	571	953	**1.67**
1398833_at	membrane-bound transcription factor peptidase, site 1	Mbtps1	1054	802	1338	**1.67**
1371352_at	high mobility group nucleosomal binding domain 2	Hmgn2	9747	5287	8794	**1.66**
1376812_at	Transcribed locus	---	747	740	1228	**1.66**
1390218_at	similar to hypothetical protein (predicted)	RGD1310440_predicted	1433	523	867	**1.66**
1372931_at	PRA1 domain family 2 (predicted)	Praf2_predicted	801	511	844	**1.65**
1376882_at	---	---	476	703	1162	**1.65**
1383253_at	solute carrier family 30 (zinc transporter), member 1	Slc30a1	2375	2130	3519	**1.65**
1384188_at	---	---	90	486	802	**1.65**
1367661_at	S100 calcium binding protein A6 (calcyclin)	S100a6	6024	2709	4467	**1.65**
1377617_at	purine rich element binding protein A (predicted)	Pura_predicted	2274	2792	4598	**1.65**
1375970_at	Transcribed locus	---	910	847	1391	**1.64**
1390048_at	serine/arginine repetitive matrix 2 (predicted)	Srrm2_predicted	4978	2618	4299	**1.64**
1388945_at	similar to 1300014I06Rik protein	RGD1311307	496	1691	2775	**1.64**
1370287_a_at	tropomyosin 1, alpha	Tpm1	2221	1727	2832	**1.64**
1373479_at	Protein phosphatase 3, catalytic subunit, alpha isoform	Ppp3ca	997	1487	2438	**1.64**
1387130_at	solute carrier family 39 (iron-regulated transporter), member 1	Slc40a1	545	1669	2731	**1.64**
1373324_at	dual specificity phosphatase 14 (predicted) /// similar to Dual specificity protein phosphatase 14 (Mitogen-activated protein kinase phosphatase 6) (MAP kinase phosphatase 6) (MKP-6)	Dusp14_predicted /// LOC	234	550	898	**1.63**
1370358_at	two pore channel 1	Tpcn1	1722	1189	1940	**1.63**
1386996_at	myosin light chain, regulatory B	Mrlcb	4629	1334	2173	**1.63**
1388722_at	DnaJ (Hsp40) homolog, subfamily B, member 1 (predicted)	Dnajb1_predicted	1560	1621	2638	**1.63**
1376784_at	Formin binding protein 1	Fnbp1	279	541	878	**1.62**
1370156_at	prion protein	Prnp	1723	6476	10511	**1.62**
1375967_a_at	dual specificity phosphatase 22 (predicted)	Dusp22_predicted	1275	742	1204	**1.62**
1372462_at	acetyl-Coenzyme A acetyltransferase 2	Acat2	736	1876	3037	**1.62**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1378668_at	Transcribed locus	---	485	526	849	**1.62**
1387793_at	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulator 1	Slc9a3r1	2455	731	1180	**1.61**
1392469_at	F-box and WD-40 domain protein 2 (predicted)	Fbxw2_predicted	1077	925	1489	**1.61**
1372743_at	sorting nexin 5 (predicted)	Snx5_predicted	4394	4324	6964	**1.61**
1392668_at	recombining binding protein suppressor of hairless (Drosophila) (predicted) /// similar to Recombining binding protein suppressor of hairless (J kappa-recombination signal binding protein) (RBP-J kappa)	LOC679028 /// Rbpsuh_p	1303	566	912	**1.61**
1382268_at	A kinase (PRKA) anchor protein 13	Akap13	492	572	918	**1.60**
1384442_at	claudin 6 (predicted)	Cldn6_predicted	1803	1152	1844	**1.60**
1387844_at	LIM and SH3 protein 1	Lasp1	1894	1447	2316	**1.60**
1375723_at	Transcribed locus	---	535	1485	2372	**1.60**
1371966_at	protein-L-isoaspartate (D-aspartate) O-methyltransferase 1	Pcmt1	614	572	913	**1.60**
1387261_at	protein phosphatase 3, catalytic subunit, beta isoform	Ppp3cb	324	551	877	**1.59**
1381976_at	kinesin family member 21A (predicted)	Kif21a_predicted	490	674	1072	**1.59**
1388872_at	isopentenyl-diphosphate delta isomerase	Idi1	569	1575	2504	**1.59**
1392818_at	growth arrest specific 5	Gas5	2842	760	1206	**1.59**
1376894_at	pallidin	Pldn	444	611	968	**1.59**
1375396_at	pumilio 1 (Drosophila) (predicted)	Pum1_predicted	1870	1886	2990	**1.58**
1383635_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 59	Ddx59	743	595	941	**1.58**
1395613_at	Transcribed locus	---	591	657	1039	**1.58**
1398441_at	non-catalytic region of tyrosine kinase adaptor protein 2 (predicted)	Nck2_predicted	421	639	1011	**1.58**
1367538_at	---	---	246	821	1295	**1.58**
1389430_at	Transcribed locus	---	230	1241	1953	**1.57**
1371918_at	CD99 antigen	Cd99	508	659	1035	**1.57**
1395455_at	heterogeneous nuclear ribonucleoprotein H3 (2H9) (predicted)	Hnrph3_predicted	1531	947	1487	**1.57**
1398963_at	---	---	2465	1377	2162	**1.57**
1395165_at	PC4 and SFRS1 interacting protein 1	Psip1	735	1479	2322	**1.57**
1374117_at	brain-specific angiogenesis inhibitor 1-associated protein 2	Baiap2	599	1215	1904	**1.57**
1388492_at	TNFAIP3 interacting protein 1 (predicted)	Tnip1_predicted	840	829	1299	**1.57**
1371975_at	Baz2a_predicted	Rbms2_predicted	563	1504	2355	**1.57**
1367775_at	alpha-methylacyl-CoA racemase	Amacr	364	558	873	**1.57**
1372484_at	ddx5 gene	Ddx5	2505	3792	5920	**1.56**
1390489_at	Synaptotagmin binding, cytoplasmic RNA interacting protein	Syncrip	1532	808	1262	**1.56**
1373466_at	calpastatin	Cast	390	570	888	**1.56**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1372609_at	protein phosphatase 2, regulatory subunit B (B56), delta isoform	Ppp2r5d	1451	1702	2652	**1.56**
1371304_a_at	myosin, light polypeptide 6, alkali, smooth muscle and non-muscle (predicted) /// similar to myosin, light polypeptide 6, alkali, smooth muscle and non-muscle /// similar to myosin light chain 1 slow a	LOC684520 /// LOC68586	6120	4219	6565	**1.56**
1390776_at	Iroquois related homeobox 3 (Drosophila) (predicted)	Irx3_predicted	2143	1451	2256	**1.55**
1375879_at	G protein-coupled receptor 48	Gpr48	512	507	787	**1.55**
1370057_at	cysteine and glycine-rich protein 1	Csrp1	2701	1064	1653	**1.55**
1393068_at	Transcribed locus	---	824	834	1294	**1.55**
1393131_at	similar to Flt3 interacting zinc finger protein 1 (predicted)	RGD1306359_predicted	885	626	971	**1.55**
1375692_at	mitogen activated protein kinase 1	Mapk1	3071	2421	3754	**1.55**
1384213_at	programmed cell death 6 interacting protein	Pdcd6ip	819	1131	1753	**1.55**
1395352_at	COP9 (constitutive photomorphogenic) homolog, subunit 3 (Arabidopsis thaliana)	Cops3	1436	1052	1628	**1.55**
1372100_at	transmembrane protein 50A (predicted)	Tmem50a_predicted	1259	687	1063	**1.55**
1376593_at	Transcribed locus	---	879	1653	2554	**1.54**
1368122_at	ring finger protein 103	Rnf103	369	602	930	**1.54**
1369978_at	phosphoribosyl pyrophosphate synthetase-associated protein 2	Prpsap2	1468	804	1240	**1.54**
1374424_at	SKB1 homolog (S. pombe) (predicted)	Skb1_predicted	2089	668	1028	**1.54**
1376868_at	Cobl-like 1 (predicted)	Cobll1_predicted	2972	957	1472	**1.54**
1398907_at	ORM1-like 2 (S. cerevisiae) (predicted)	Ormdl2_predicted	971	1113	1711	**1.54**
1382144_at	mitochondrial ribosomal protein L47	Mrpl47	2123	933	1434	**1.54**
1389984_at	similar to jumonji protein	LOC681740	1800	1230	1890	**1.54**
1389337_at	Transcribed locus	---	2159	846	1299	**1.54**
1375739_at	EH-domain containing 4	Ehd4	1625	4072	6252	**1.54**
1373178_at	Transcribed locus	---	2062	5052	7755	**1.54**
1369981_at	immunoglobulin (CD79A) binding protein 1	Igbp1	820	974	1492	**1.53**
1379269_at	CD2-associated protein	Cd2ap	3030	1658	2536	**1.53**
1367898_at	BCL2/adenovirus E1B 19 kDa-interacting protein 3-like	Bnip3l	1915	2079	3174	**1.53**
1377818_at	Transcribed locus	---	185	548	836	**1.53**
1383062_at	glutaredoxin 2 (thioltransferase)	Glrx2	849	1966	2998	**1.52**
1381100_at	Rho guanine nucleotide exchange factor (GEF) 12	Arhgef12	589	1132	1725	**1.52**
1371430_at	dystroglycan 1	Dag1	1484	959	1461	**1.52**
1371632_at	similar to Coronin, actin binding protein 1C (predicted)	RGD1564490_predicted	7727	3176	4830	**1.52**
1387848_at	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Hmgcr	407	1203	1828	**1.52**
1374846_at	cardiac lineage protein 1	Clp1	4288	4790	7265	**1.52**
1388677_at	ubiquitin-associated protein 1	Ubap1	820	1255	1903	**1.52**

Table 6.4. PI3K signaling: negatively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1388408_at	similar to RIKEN cDNA 1110020C13	RGD1307129	3435	5624	8529	**1.52**
1372801_at	COMM domain containing 10	Commd10	1689	1463	2218	**1.52**
1384413_at	similar to solute carrier family 35, member A5 (predicted)	RGD1564361_predicted	290	917	1390	**1.52**
1372544_at	Transcribed locus	---	554	670	1014	**1.51**
1367545_at	G protein-coupled receptor 21 (predicted)	Gpr21_predicted	596	1030	1559	**1.51**
1398933_at	HIG1 domain family, member 2A (predicted)	Higd2a_predicted	2565	1949	2947	**1.51**
1369027_at	alpha 1,4-galactosyltransferase	A4galt	958	2643	3995	**1.51**
1393915_at	membrane bound O-acyltransferase domain containing 5	Mboat5	1067	2927	4418	**1.51**
1368254_a_at	sphingosine kinase 1	Sphk1	218	883	1333	**1.51**
1390384_at	similar to Histone H2A.x (H2a/x) (predicted)	RGD1566119_predicted	2443	1062	1602	**1.51**
1379028_at	sperm associated antigen 7 (predicted)	Spag7_predicted	1209	737	1112	**1.51**
1372131_at	ubiquilin 2 (predicted)	Ubqln2_predicted	781	1120	1687	**1.51**
1387020_at	cytochrome P450, subfamily 51	Cyp51	338	648	976	**1.51**
1370288_a_at	tropomyosin 1, alpha	Tpm1	1074	966	1453	**1.50**
1370282_at	cysteine and glycine-rich protein 2	Csrp2	737	568	854	**1.50**
1390526_at	kelch-like 9 (Drosophila) (predicted)	Klhl9_predicted	1168	1847	2775	**1.50**
1372423_at	PERP, TP53 apoptosis effector (predicted)	Perp_predicted	1991	1815	2724	**1.50**
1368100_at	phosphate cytidylyltransferase 2, ethanolamine	Pcyt2	695	938	1408	**1.50**
1393267_at	PC4 and SFRS1 interacting protein 1	Psip1	2077	3984	5977	**1.50**
1376004_at	Transcribed locus	---	1305	1345	2018	**1.50**
1388575_at	similar to Opa-interacting protein 5 (predicted)	RGD1564263_predicted	1667	2001	3002	**1.50**
1390032_at	RNA binding motif, single stranded interacting protein 2	Rbms2	2009	2100	3149	**1.50**

Table 6.5. PI3K signaling: positively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1387219_at	adrenomedullin	Adm	229	1327	92	**14.40**
1390511_at	---	---	10	1863	161	**11.59**
1378534_at	similar to brain carcinoembryonic antigen	LOC308394	10	1746	153	**11.44**
1370874_at	Prolactin family 3, subfamily b, member 1	Prl3b1	1556	13469	1742	**7.73**
1376934_x_at	---	---	53	2146	298	**7.19**
1393449_at	junction adhesion molecule 2	Jam2	585	1167	180	**6.48**
1369029_at	phospholipid scramblase 1	Plscr1	20	2050	328	**6.25**
1387123_at	cytochrome P450, family 17, subfamily a, polypeptide 1	Cyp17a1	413	4444	974	**4.57**
1383197_at	junction adhesion molecule 2	Jam2	812	1759	391	**4.49**
1370852_at	spleen protein 1 precursor	LOC171573	692	20968	4736	**4.43**
1370471_at	prolactin family 6, subfamily a, member 1	Prl6a1	102	1481	357	**4.15**
1367809_at	prolactin family 4, subfamily a, member 1	Prl4a1	683	22573	5771	**3.91**
1376845_at	putative ISG12(b) protein	isg12(b)	158	4110	1061	**3.87**
1383765_at	similar to RIKEN cDNA 4921520P21	MGC114388	36	863	229	**3.76**
1367571_a_at	insulin-like growth factor 2	Igf2	3273	14329	3836	**3.74**
1369166_at	matrix metallopeptidase 9	Mmp9	245	1209	331	**3.66**
1374488_at	GRAM domain containing 1B (predicted)	Gramd1b_predicted	89	2514	697	**3.61**
1367733_at	carbonic anhydrase 2	Ca2	340	1935	551	**3.51**
1382690_at	carcinoembryonic antigen gene family 4	Cgm4	81	2729	800	**3.41**
1373282_at	similar to mitochondrial carrier protein MGC4399	LOC691431	703	1234	363	**3.40**
1370950_at	phosphatidic acid phosphatase type 2B	Ppap2b	164	922	272	**3.39**
1370281_at	fatty acid binding protein 5, epidermal	Fabp5	9936	6954	2153	**3.23**
1398275_at	matrix metallopeptidase 9	Mmp9	530	2120	683	**3.11**
1372101_at	phosphatidic acid phosphatase type 2B	Ppap2b	462	2245	733	**3.06**
1387587_at	Fas ligand (TNF superfamily, member 6)	Faslg	1816	6909	2259	**3.06**
1370869_at	branched chain aminotransferase 1, cytosolic	Bcat1	1480	1819	595	**3.06**
1390525_a_at	stimulated by retinoic acid gene 6 homolog (mouse)	Stra6	28	1037	342	**3.03**
1369520_a_at	branched chain aminotransferase 1, cytosolic	Bcat1	645	891	294	**3.03**
1367651_at	cathepsin D	Ctsd	2606	10770	3585	**3.00**
1386922_at	carbonic anhydrase 2	Ca2	244	1363	479	**2.85**
1389856_at	carcinoembryonic antigen gene family 4	Cgm4	57	5073	1819	**2.79**
1369957_at	regulator of G-protein signaling 5	Rgs5	136	1287	462	**2.78**
1372665_at	phosphoserine aminotransferase 1	Psat1	10633	7071	2576	**2.75**
1392981_at	Iroquois related homeobox 4 (Drosophila) (predicted)	Irx4_predicted	3397	816	298	**2.74**
1372601_at	activating transcription factor 5	Atf5	521	1345	494	**2.72**
1379275_at	sorting nexin 10	Snx10	373	1259	481	**2.62**
1377264_at	interleukin 17F	Il17f	179	4036	1546	**2.61**
1391051_at	Transcribed locus	---	9250	1378	541	**2.55**
1370695_s_at	tribbles homolog 3 (Drosophila)	Trib3	293	2284	899	**2.54**
1383987_at	Transcribed locus	---	1153	970	389	**2.49**
1389210_at	lymphocyte cytosolic protein 1	Lcp1	1546	2976	1209	**2.46**
1387747_at	gap junction membrane channel protein beta 3	Gjb3	5356	810	331	**2.45**
1373504_at	GLI pathogenesis-related 1 (glioma)	Glipr1	131	971	403	**2.41**

Table 6.5. PI3K signaling: positively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1377880_at	Transcribed locus	---	292	1820	762	**2.39**
1370817_at	Sec11-like 3 (S. cerevisiae)	Sec11l3	1299	1080	463	**2.33**
1394363_at	RIO kinase 3 (yeast) (predicted)	Riok3_predicted	825	1997	898	**2.22**
1376036_at	transporter	LOC314323	29	875	397	**2.20**
1388703_at	endothelial cell adhesion molecule	Esam	160	1748	793	**2.20**
1369141_at	Prolactin family 3, subfamily d, member 1 /// Prolactin family 3, subfamily d, member 2	Prl3d1 /// Prl3d2	10452	22492	10213	**2.20**
1387156_at	hydroxysteroid (17-beta) dehydrogenase 2	Hsd17b2	507	5354	2444	**2.19**
1375071_at	nucleoporin 133 (predicted)	Nup133_predicted	1476	830	379	**2.19**
1384769_a_at	similar to Zinc finger X-linked protein ZXDB (predicted) /// similar to Zinc finger X-linked protein ZXDB	LOC683508 /// LOC68	274	1434	657	**2.18**
1384865_at	Transcribed locus, strongly similar to XP_346369.1 PREDICTED: similar to leucine zipper, down-regulated in cancer 1 [Rattus norvegicus]	---	1759	5568	2605	**2.14**
1373418_at	glutamyl-prolyl-tRNA synthetase	Eprs	3871	6850	3216	**2.13**
1386662_at	similar to Sestrin 2 (Hi95) (predicted)	RGD1566319_predict	318	1090	515	**2.12**
1386321_s_at	tribbles homolog 3 (Drosophila)	Trib3	695	3032	1449	**2.09**
1393983_at	exportin, tRNA (nuclear export receptor for tRNAs) (predicted)	Xpot_predicted	2069	2580	1235	**2.09**
1370080_at	heme oxygenase (decycling) 1	Hmox1	3614	4953	2373	**2.09**
1371818_at	exportin, tRNA (nuclear export receptor for tRNAs) (predicted)	Xpot_predicted	2596	2913	1414	**2.06**
1376620_at	Transcribed locus	---	669	1505	731	**2.06**
1375962_at	Transcribed locus	---	775	3694	1824	**2.03**
1370422_at	receptor-interacting serine-threonine kinase 3	Ripk3	2417	1710	847	**2.02**
1370694_at	tribbles homolog 3 (Drosophila)	Trib3	266	1176	584	**2.01**
1379396_at	engulfment and cell motility 1, ced-12 homolog (C. elegans) (predicted)	Elmo1_predicted	116	804	402	**2.00**
1398247_at	protease, serine, 15	Prss15	3109	4523	2279	**1.98**
1380743_at	similar to Cat eye syndrome critical region protein 2 (predicted)	RGD1564182_predict	742	1170	591	**1.98**
1395699_at	RIO kinase 3 (yeast) (predicted)	Riok3_predicted	1134	2186	1106	**1.98**
1389659_at	similar to ctla-2-beta protein (141 AA) (predicted)	RGD1565540_predict	5855	3066	1552	**1.98**
1383585_s_at	sorting nexin 10	Snx10	658	1630	828	**1.97**
1372808_at	similar to Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase, mitochondrial precursor	LOC680308	1993	3873	1981	**1.96**
1376660_at	Transcribed locus	---	526	900	461	**1.95**
1384963_at	similar to Cat eye syndrome critical region protein 2 (predicted)	RGD1564182_predict	1634	2383	1220	**1.95**
1377923_at	START domain containing 8 (predicted)	Stard8_predicted	324	1165	604	**1.93**
1391012_at	Transcribed locus	---	266	955	495	**1.93**
1383455_at	glutamyl-prolyl-tRNA synthetase	Eprs	4207	6957	3618	**1.92**
1370848_at	solute carrier family 2 (facilitated glucose transporter), member 1	Slc2a1	4906	5678	2957	**1.92**

Table 6.5. PI3K signaling: positively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1368025_at	DNA-damage-inducible transcript 4	Ddit4	814	2917	1520	**1.92**
1382500_at	similar to Sestrin 2 (Hi95) (predicted)	RGD1566319_predict	772	2198	1150	**1.91**
1374113_at	bromodomain adjacent to zinc finger domain, 1A (predicted)	Baz1a_predicted	4784	2343	1228	**1.91**
1372145_at	threonyl-tRNA synthetase	Tars	2951	3492	1832	**1.91**
1370845_at	ectonucleoside triphosphate diphosphohydrolase 2	Entpd2	602	842	442	**1.90**
1372812_at	similar to mKIAA0256 protein (predicted)	RGD1559930_predict	384	1008	534	**1.89**
1391607_at	similar to Sestrin 2 (Hi95) (predicted)	RGD1566319_predict	1017	2739	1453	**1.89**
1389450_at	similar to Williams-Beuren syndrome critical region protein 22 /// similar to Putative methyltransferase WBSCR22 (Williams-Beuren syndrome chromosome region 22 protein homolog)	LOC360830 /// LOC36	4106	1834	973	**1.88**
1388574_at	tryptophanyl-tRNA synthetase	Wars	1687	2051	1091	**1.88**
1384449_at	Transcribed locus	---	942	868	464	**1.87**
1370379_at	protease, serine, 8 (prostasin)	Prss8	264	1173	628	**1.87**
1393796_at	centrosomal protein 152 (predicted)	Cep152_predicted	676	928	497	**1.87**
1369590_a_at	DNA-damage inducible transcript 3	Ddit3	582	3287	1764	**1.86**
1372642_at	Transcribed locus	---	3545	2111	1135	**1.86**
1370371_a_at	CEA-related cell adhesion molecule 1 /// CEA-related cell adhesion molecule 10	Ceacam1 /// Ceacam1	514	4941	2662	**1.86**
1371040_at	solute carrier family 1 (neutral amino acid transporter), member 5	Slc1a5	1286	1488	802	**1.86**
1386904_a_at	cytochrome b-5	Cyb5	3520	3893	2105	**1.85**
1368623_at	CEA-related cell adhesion molecule 9	Ceacam9	618	8892	4815	**1.85**
1387653_at	translin-associated factor X	Tsnax	606	1280	697	**1.84**
1395124_at	tryptophanyl-tRNA synthetase	Wars	789	1009	550	**1.84**
1389554_at	nuclear receptor subfamily 2, group F, member 2	Nr2f2	532	2593	1413	**1.83**
1367841_a_at	prolactin family 8, subfamily a, member 9	Prl8a9	402	4135	2260	**1.83**
1382040_at	glutamyl-prolyl-tRNA synthetase	Eprs	2218	4085	2252	**1.81**
1372009_at	tyrosyl-tRNA synthetase	Yars	4596	5405	2981	**1.81**
1388519_at	Sec61 beta subunit (predicted)	Sec61b_predicted	2830	3571	1973	**1.81**
1388695_at	serine hydroxymethyltransferase 2 (mitochondrial)	Shmt2	5493	3122	1724	**1.81**
1368308_at	myelocytomatosis viral oncogene homolog (avian)	Myc	1806	4355	2410	**1.81**
1369525_at	GATA binding protein 3	Gata3	2657	2930	1623	**1.81**
1395730_at	glycyl-tRNA synthetase /// similar to Glycyl-tRNA synthetase (predicted)	Gars /// RGD1559871	1032	1325	736	**1.80**
1398771_at	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	Slc3a2	3741	9339	5189	**1.80**
1392547_at	hypothetical LOC302884	MGC105649	352	2391	1334	**1.79**
1375964_at	phosphoserine phosphatase	Psph	1055	1824	1022	**1.78**
1371731_at	Similar to Coatomer gamma-2 subunit (Gamma-2 coat protein) (Gamma-2 COP) (predicted)	RGD1566215_predict	4385	3128	1756	**1.78**
1377998_at	coproporphyrinogen oxidase	Cpox	1820	989	555	**1.78**
1383974_at	E74-like factor 5 (predicted)	Elf5_predicted	8631	6083	3416	**1.78**

Table 6.5. PI3K signaling: positively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1382901_at	growth factor independent 1B (predicted)	Gfi1b_predicted	338	924	520	**1.78**
1368842_at	transcription factor 4	Tcf4	592	1329	748	**1.78**
1372661_at	transducin (beta)-like 3	Tbl3	1417	1134	642	**1.77**
1386900_at	ribosome associated membrane protein 4	RAMP4	3143	4778	2704	**1.77**
1388715_at	glycyl-tRNA synthetase	Gars	4334	5182	2933	**1.77**
1391279_at	Scinderin	Scin	1137	3965	2250	**1.76**
1370000_at	nucleobindin 2	Nucb2	675	1200	685	**1.75**
1368996_at	carcinoembryonic antigen-related cell adhesion molecule 3	Ceacam3	202	17879	10229	**1.75**
1390447_at	Transcribed locus	---	898	1114	640	**1.74**
1371709_at	mitochondrial ribosomal protein L3 (predicted)	Mrpl3_predicted	3662	2838	1632	**1.74**
1389573_at	ChaC, cation transport regulator-like 1 (E. coli) (predicted)	Chac1_predicted	422	830	478	**1.74**
1377016_at	cysteine-rich with EGF-like domains 2	Creld2	1618	1846	1063	**1.74**
1377644_at	similar to RIKEN cDNA 4921524J17 (predicted)	RGD1308706_predict	1886	5051	2910	**1.74**
1374118_at	leucyl-tRNA synthetase	Lars	3031	4253	2452	**1.73**
1374329_at	mcf.2 transforming sequence-like	Mcf2l	987	1064	615	**1.73**
1388426_at	sterol regulatory element binding factor 1	Srebf1	870	2288	1322	**1.73**
1387282_at	heat shock 22kDa protein 8	Hspb8	91	935	543	**1.72**
1389782_at	similar to RIKEN cDNA 2010107G23 (predicted) /// hypothetical protein LOC679430	LOC679430 /// RGD1	1761	1531	895	**1.71**
1373448_at	acylphosphatase 1, erythrocyte (common) type (predicted)	Acyp1_predicted	1172	858	502	**1.71**
1368227_at	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	Slc28a2	240	5743	3359	**1.71**
1373035_at	---	---	695	860	504	**1.71**
1379560_at	nuclear receptor binding SET domain protein 1 (predicted) /// similar to Histone-lysine N-methyltransferase, H3 lysine-36 and H4 lysine-20 specific (H3-K36-HMTase) (H4-K20-HMTase) (Nuclear receptor binding SET domain containing protein 1) (NR-binding SET domain containing protein)	LOC686060 /// Nsd1_	594	1251	732	**1.71**
1371883_at	monocyte to macrophage differentiation-associated	Mmd	847	3656	2142	**1.71**
1391572_at	cysteinyl-tRNA synthetase (predicted)	Cars_predicted	1091	2361	1383	**1.71**
1375441_at	seryl-aminoacyl-tRNA synthetase 1	Sars1	4173	6504	3829	**1.70**
1390208_at	HIV-1 tat interactive protein 2, homolog (human) (predicted)	Htatip2_predicted	753	1416	840	**1.69**
1367815_at	solute carrier family 5 (sodium-dependent vitamin transporter), member 6	Slc5a6	1571	1738	1032	**1.69**
1389381_at	sequestosome 1	Sqstm1	7322	9069	5388	**1.68**
1394705_at	Transcribed locus	---	1590	1858	1104	**1.68**
1381118_at	similar to RIKEN cDNA 2700007P21	RGD1311463	461	847	505	**1.68**
1390406_at	Rho GTPase activating protein 18 (predicted)	Arhgap18_predicted	434	4093	2444	**1.67**
1380201_at	nucleolar protein 10	Nol10	1808	1647	986	**1.67**

Table 6.5. PI3K signaling: positively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1371574_at	growth hormone inducible transmembrane protein	Ghitm	6548	9016	5401	**1.67**
1372230_at	transmembrane protein 147	Tmem147	4009	3597	2164	**1.66**
1389594_at	similar to Protein C20orf22 homolog	LOC499913	311	1557	941	**1.65**
1370073_at	DnaJ (Hsp40) homolog, subfamily C, member 3	Dnajc3	740	1017	615	**1.65**
1397584_at	Transcribed locus	---	1413	988	599	**1.65**
1374437_at	asparaginyl-tRNA synthetase	Nars	9640	13264	8080	**1.64**
1389733_at	---	Mars_predicted	3160	3829	2335	**1.64**
1385073_at	similar to SERTA domain containing 4 (predicted)	RGD1564561_predict	5174	3195	1952	**1.64**
1373142_at	Growth hormone inducible transmembrane protein	Ghitm	2283	3952	2416	**1.64**
1373549_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 10 (predicted)	Ddx10_predicted	1567	1196	732	**1.63**
1379785_at	Transcribed locus	---	421	1052	644	**1.63**
1384368_at	Transcribed locus	---	581	867	531	**1.63**
1395363_at	methionine-tRNA synthetase (predicted)	Mars_predicted	1126	1601	982	**1.63**
1367741_at	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	Herpud1	642	2781	1710	**1.63**
1383831_at	glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1	Qrsl1	1361	1421	876	**1.62**
1370252_at	arginine vasopressin-induced 1	Avpi1	2688	2451	1514	**1.62**
1369068_at	cullin 5	Cul5	722	882	546	**1.62**
1389760_at	polycomb group ring finger 6	Pcgf6	3357	2034	1259	**1.62**
1367577_at	heat shock 27kDa protein 1	Hspb1	97	940	583	**1.61**
1372451_at	Trf (TATA binding protein-related factor)-proximal protein homolog (Drosophila) /// similar to ubiquitin specific protease 49 (predicted)	LOC501098 /// Trfp	1261	1776	1103	**1.61**
1375343_at	Transcribed locus	---	1066	2755	1715	**1.61**
1372876_at	selenophosphate synthetase 2	Sephs2	2072	1028	641	**1.60**
1367484_at	ubiquitin-conjugating enzyme E2E 2 (UBC4/5 homolog, yeast)	Ube2e2	3804	2522	1573	**1.60**
1391422_at	DnaJ (Hsp40) homolog, subfamily B, member 6	Dnajb6	833	931	581	**1.60**
1392475_at	Transcribed locus	---	638	1396	872	**1.60**
1371828_at	similar to RIKEN cDNA 1500011H22	RGD1310861	1265	1727	1078	**1.60**
1383715_at	similar to hypothetical protein D4Ertd89e	RGD1305703	352	1931	1210	**1.60**
1379337_at	similar to RIKEN cDNA 2810421I24	RGD1307883	2301	1622	1017	**1.59**
1374034_at	cysteinyl-tRNA synthetase (predicted)	Cars_predicted	1339	2750	1725	**1.59**
1389151_at	Transcribed locus	---	2146	1540	969	**1.59**
1370688_at	glutamate-cysteine ligase, catalytic subunit	Gclc	1317	6423	4040	**1.59**
1388105_at	D123 gene product	D123	3265	2617	1649	**1.59**
1382044_at	hypothetical protein LOC498796	LOC498796	397	1187	748	**1.59**
1370134_at	solute carrier family 33 (acetyl-CoA transporter), member 1	Slc33a1	882	1294	816	**1.59**
1384115_at	Mitochondrial acyl-CoA thioesterase 1	Mte1	1406	1686	1065	**1.58**
1398312_s_at	solute carrier family 14 (urea transporter), member 2	Slc14a2	473	2020	1278	**1.58**

Table 6.5. PI3K signaling: positively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1368037_at	carbonyl reductase 1	Cbr1	1858	1880	1191	**1.58**
1376039_at	aurora kinase A	Aurka	3166	2300	1462	**1.57**
1398384_at	exosome component 9	Exosc9	1838	1224	779	**1.57**
1373556_at	similar to CG14903-PA (predicted)	RGD1561792_predict	1923	1271	810	**1.57**
1373053_at	---	---	6035	4042	2580	**1.57**
1373841_at	rac/cdc42 guanine nucleotide exchange factor 6	arhgef6	129	2219	1418	**1.57**
1376418_a_at	isoleucine-tRNA synthetase (predicted)	Iars_predicted	3654	3950	2526	**1.56**
1390448_at	similar to 1110065L07Rik protein (predicted)	RGD1308317_predict	592	955	612	**1.56**
1369962_at	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	Atic	6392	2340	1502	**1.56**
1378134_at	ATPase, Class I, type 8B, member 1 (predicted)	Atp8b1_predicted	338	4260	2735	**1.56**
1371380_at	pyruvate dehydrogenase E1 alpha 1 /// pyruvate dehydrogenase E1 alpha 1 pseudogene	LOC685778 /// Pdha1	1065	1947	1250	**1.56**
1389353_at	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D (predicted)	Sema6d_predicted	496	4154	2670	**1.56**
1393845_a_at	transmembrane channel-like gene family 4	Tmc4	278	982	632	**1.55**
1383698_at	pyruvate dehydrogenase E1 alpha 1	Pdha1	518	1082	697	**1.55**
1370030_at	glutamate cysteine ligase, modifier subunit	Gclm	3435	1389	897	**1.55**
1389146_at	hypothetical protein LOC498796	LOC498796	2467	5559	3597	**1.55**
1375230_at	Endogenous retrovirus mRNA, partial sequence	---	5919	2957	1913	**1.55**
1372523_at	glutamate-cysteine ligase, catalytic subunit	Gclc	1617	7445	4819	**1.54**
1371955_at	mitochondrial ribosomal protein L35 (predicted)	Mrpl35_predicted	2042	1082	701	**1.54**
1382309_at	Transcribed locus, moderately similar to XP_238213.3 PREDICTED: similar to delangin [Rattus norvegicus]	---	897	1180	765	**1.54**
1380521_at	hematopoietic cell specific Lyn substrate 1	Hcls1	581	813	527	**1.54**
1368868_at	A kinase (PRKA) anchor protein (gravin) 12	Akap12	1013	809	525	**1.54**
1369737_at	cAMP responsive element modulator	Crem	412	951	619	**1.54**
1371051_at	glutamate receptor, ionotropic, N-methyl D-aspartate-like 1A	Grinl1a	2927	2065	1347	**1.53**
1388394_at	alanyl-tRNA synthetase	Aars	4267	5166	3377	**1.53**
1392446_at	kelch-like 2, Mayven (Drosophila) (predicted)	Klhl2_predicted	2191	1034	676	**1.53**
1388721_at	heat shock 22kDa protein 8	Hspb8	210	2061	1349	**1.53**
1387800_at	Fas death domain-associated protein	Daxx	1011	1688	1105	**1.53**
1367624_at	activating transcription factor 4	Atf4	9715	16615	10882	**1.53**
1390508_at	Transcribed locus	---	1323	1674	1098	**1.52**
1388331_at	tumor rejection antigen gp96 (predicted)	Tra1_predicted	6438	5444	3575	**1.52**
1376754_at	cysteinyl-tRNA synthetase (predicted)	Cars_predicted	920	1916	1259	**1.52**
1387065_at	phospholipase C, delta 4	Plcd4	906	2990	1969	**1.52**
1370690_at	heat shock 70kDa protein 9A (predicted)	Hspa9a_predicted	6543	6718	4426	**1.52**

Table 6.5. PI3K signaling: positively regulated genes

Affymetrix ID	Gene Title	Gene Symbol	Stem Ave	Dif Ave	LY Ave	dif/ly
1374503_at	pre B-cell leukemia transcription factor 3 (predicted)	Pbx3_predicted	1329	1378	909	**1.52**
1368848_at	lectin, mannose-binding, 1	Lman1	1274	1449	958	**1.51**
1372502_at	neurofibromatosis 2	Nf2	1017	943	623	**1.51**
1388366_at	mitochondrial ribosomal protein L4 (predicted)	Mrpl4_predicted	2008	1224	809	**1.51**
1388792_at	growth arrest and DNA-damage-inducible 45 gamma	Gadd45g	1426	1806	1194	**1.51**
1370812_at	Bcl2-like 1	Bcl2l1	339	892	590	**1.51**
1373115_at	similar to 2310061I09Rik protein (predicted)	RGD1304653_predict	652	1341	888	**1.51**
1372170_at	aminoacylase 1	Acy1	1923	1322	878	**1.51**
1393645_at	hypothetical protein LOC317274	LOC317274	549	864	574	**1.51**
1378400_at	similar to RIKEN cDNA 4632404H22 (predicted)	RGD1560129_predict	292	3703	2460	**1.51**
1380257_at	SMC5 structural maintenance of chromosomes 5-like 1 (yeast) (predicted)	Smc5l1_predicted	1009	1199	798	**1.50**
1393214_at	similar to hypothetical protein A230042K10 (predicted)	RGD1566144_predict	1259	1116	743	**1.50**
1398961_at	similar to ring finger protein 13	LOC681578	1271	1608	1072	**1.50**