UNIVERSIDAD AUTÓNOMA DE MADRID

DEPARTAMENTO DE BIOQUÍMICA

TESIS DOCTORAL

THYROID HORMONE REGULATION
OF BRAIN GENE EXPRESSION:
ROLE OF THYROID HORMONE RECEPTORS

PILAR GIL IBÁÑEZ

Madrid 2014

DEPARTAMENTO DE BIOQUÍMICA

FACULTAD DE MEDICINA

UNIVERSIDAD AUTÓNOMA DE MADRID

THYROID HORMONE REGULATION OF BRAIN GENE EXPRESSION:

ROLE OF THYROID HORMONE RECEPTORS

Memoria que presenta la licenciada en Bioquímica Pilar Gil Ibáñez para optar al grado de Doctor Internacional por la Universidad Autónoma de Madrid

Directores de Tesis: Juan Bernal Carrasco y Beatriz Morte Molina

Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC-UAM

Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER)

ACKNOWLEDGMENT

Hace 6 años empecé esta aventura en la que he crecido como persona y como científica. Al pararme a pensar me doy cuenta de que las experiencias más duras, con el paso del tiempo se han convertido en mis momentos más valiosos, y es que echando la vista atrás, solo me quedan buenos recuerdos. Ser científico es duro, pero no lo es tanto si estas rodeado de gente que te ayuda y acompaña cuando los necesitas. Yo he tenido la suerte de compartir este camino con los que estáis leyendo este apartado, y a todos vosotros os doy las gracias.

Juan, me siento muy afortunada de haber trabajado contigo, y es que eres un gran científico y una gran persona. Aprender de ti ha sido muy enriquecedor. Científicos como tu hacen que mi visión de la investigación sea más positiva y optimista. Valoro mucho tu esfuerzo, dedicación y cercanía. Muchas gracias por todo lo que me has enseñado.

Bea, he aprendido muchas cosas trabajando contigo. Muchas gracias por tus consejos, ayuda y disposición para enseñarme. Me alegro mucho de que hayas codirigido mi tesis.

En el laboratorio 2.10 me siento como en casa, con el paso del tiempo hemos tenido grandes perdidas pero también buenos fichajes, a todas os doy las gracias. En especial a Barbarita, he tenido mucha suerte por recorrer este camino contigo sin soltarnos de la mano, has llenado mi rutina de risas, eres única y espero que no cambies. Mari Carmen, gracias por siempre estar ahi y ser, al principio un referente, luego una compañera y ahora una gran amiga. Ainhoa, gracias por tu ayuda los primeros años de mi tesis y tu amistad transatlántica los últimos. Raquel, muchas gracias por tu ayuda y apoyo en los últimos momentos de la tesis. Ana G, muchas gracias por tus consejos y amistad durante tanto tiempo. Anita, muchas gracias por ayudarme en estos momentos de estrés, ya te sabes la tesis mejor que yo!. Dani, gracias por ser siempre tan alegre y buena, es un placer trabajar a tu lado, y por ultimo Sol, después de tanto tiempo… me alegro muchísimo de que la vida nos haya vuelto a juntar, gracias por tu ayuda y disfruta que la tesis se pasa volando!

I would also like to thank everyone from the lab in Chicago. Specially Dr. Refetoff, I am grateful to have had the chance to work with an exceptional scientist and person. It is difficult not to feel passionate about science by your side. Thanks also to Heather, Dr. Weiss, Xhiao Hui, Massimiliano, Alexandra and Yolanda, you all made me feel at home.

No hubiese podido abarcar tanto ratón, confocal, cuantitativa o diseño de tesis sin la ayuda de Irene, Diego, Lucia, Diana, Javi y Antonio, muchas gracias a todos por vuestra ayuda, con gente tan maja es un placer trabajar. Gracias también a Paco Garcia y Joaquin Dopazo por ayudarnos a analizar el RNAseq.

El día a día no hubieran sido lo mismo sin amigos con los que comer, salir y bailar! Lara gracias por tu amistad y tus buenos consejos, León es imposible aburrirse contigo, gracias por ser un buen amigo; Ana y Sandra, muchas gracias por vuestra compañia y alegria. En especial, muchas gracias Petri, por estar siempre a mi lado, tu amistad es la sorpresa más valiosa de estos años. No todo el mundo tiene la suerte de ir al trabajo, reir, llorar y bailar todos los dias con su mejor amiga, gracias Mary por aguantarme y quererme. Gracias también a las chicas del 2.5.2 por vuestros consejos y acogerme todos los dias!

Fuera del IIB, hay mucha gente que desde hace muchos años han estado a mi lado apoyándome. En primer lugar gracias a Esther y Fátima, vuestro ejemplo me ha guiado muchos años en el camino científico. Tanto en Chicago como en Madrid muchas gracias a todos los Petaos, a Laura y Esther. Gracias a Esther y Carlos, juntos en la universidad empezamos este camino, espero seguir teniéndoos cerca muchos años más. Y como no...gracias a los de siempre!: Cris, Marta, Bel, Chuso, Pedro, Valerio, Martis y Andrés.

Por último, quiero dar las gracias a mi familia. Estos agradecimientos serian interminables si tengo que escribir en todo lo que habéis hecho por mi estos años. Muchas gracias a todos: a mis tios, primos, cuñados y sobri, a Blanca por ir abriendo camino y ser un ejemplo para mi, a Inesita por estar a mi lado toda la vida y

apoyarme siempre que lo he necesitado, a Carlitos, tu has hecho que tenga una vida llena de retos, amor y alegría gracias a ti habrá 3 doctoras en la familia. A mis padres, María José y Juan Carlos, gracias a los dos por quererme tanto. A Carlos gracias por acompañarme en este viaje, en los que ya hemos vivido y en los que nos quedan por vivir.

Pilar

SUMMARY

Thyroid hormones are important during development of the mammalian brain. They are involved in neuronal and glial cell differentiation and migration, axonal myelination, and synaptogenesis. The effects of thyroid hormones on brain development and function are largely mediated by the control of gene expression. This is achieved by the binding of the genomically active T3 to transcriptionally active nuclear thyroid hormone receptors. There are three functional receptor isoforms in mice (TRα1, TRβ1 and TRβ2). T3 can either enhance ("positive" genes) or reduce ("negative" genes) gene expression.

To analyze the contribution of the receptor subtypes TRα1 and TRβ (i.e., TRβ1 and TRβ2) in the regulation of gene expression during brain development, we have measured by qPCR the expression of a set of positive and negative genes in the postnatal cerebral neocortex and striatum as well as in primary cerebrocortical cells, from thyroid hormone receptor knock out mice. Our results show that on most genes TRα1 exerts a predominant but not exclusive role in the regulation of thyroid hormone dependent genes. *Dio3* and *Aldh1a1* were induced by T3 only in cells expressing TRα1. In our *in vivo* experiment, a fraction of the genes analyzed are not or only mildly affected by the total absence of thyroid hormone receptors, indicating that the effect of hypothyroidism on gene expression is mainly due to the activity of unliganded receptors.

To get further insight on the genes and pathways regulated by T3 in the developing brain at the cellular level, we used mouse cerebrocortical cells in primary culture. This culture maintains, to some extent, the original phenotypic cell diversity and therefore, reflects the T3 action on the cortex *in vivo*. For example, in 10% of the neurons, calbindin can be detected, which is a marker of a subpopulation of cortical GABAergic neurons. Using RNA-seq assays we have identified 1,145 genes whose expression depend on the presence of T3 (FRD<0.05). Gene Ontology analysis revealed that T3 specifically upregulates genes involved in transmission of the nerve impulse, ion transport, ephrin receptor activity, cell adhesion, chemotaxis, myelin assembly, protein localization at the paranodal region, and astrocyte differentiation. On the contrary, T3 specifically downregulates genes involved in cell division, M Phase of cell cycle, chromosome segregation and organization. In general, T3 favors the adult versus fetal pattern of cortex gene expression.

RESUMEN

Las hormonas tiroideas son importantes para el desarrollo del cerebro en mamíferos y están implicadas en diferenciación de neuronas y células gliales, mielinización axonal y synaptogenesis. La T3 modula el desarrollo y funcionamiento del cerebro mediante su unión a sus receptores nucleares y la regulación de la expresión génica. En ratón existen 3 isoformas de receptor de hormonas tiroideas funcionalmente activas (TRα1, TRβ1 y TRβ2). La T3 estimula o atenúa la expresión génica, y se denomina a sus genes diana "positivos" o "negativos" respectivamente.

Con la finalidad de analizar la contribución de las distintas isoformas de los receptores en la regulación de la expresión génica durante el desarrollo del cerebro hemos medido por PCR cuantitativa la expresión de genes positivos y genes negativos en el estriado y corteza cerebral posnatal (neocorteza) así como, en cultivos primarios de células cerebrocorticales de ratones Knock out para los receptores de hormonas tiroideas. En la regulación de la expresión de la mayoría de los genes estudiados, TRα1 tiene un papel predominante aunque no exclusivo. Los genes *Dio3* y *Aldh1a1* son inducidos por T3 solo en células que expresan TRα1. La expresión de algunos de los genes estudiados no varia en ausencia total de receptores, indicando que el efecto del hipotiroidismo se debe a la actividad de los receptores en ausencia de ligando.

Para profundizar en los genes y procesos regulados por la T3 en el desarrollo del cerebro a nivel celular, hemos empleado cultivos primarios de células cerebrocorticales de ratón. El cultivo primario mantiene gran parte de la diversidad fenotípica original; en el 10% de las neuronas del cultivo se detecta calbindina, el cual es un marcador de una subpoblación de neuronas GABAérgicas de la corteza. La acción de la T3 en el cultivo reflejaría su papel en la corteza *in vivo*. Mediante RNAseq hemos identificado 1.145 genes regulados por T3 (FRD<0.05) en el cultivo. El posterior análisis funcional de Gene Ontology mostró que los genes inducidos por T3 están enriquecidos en procesos de transmisión del impulso nervioso, transporte iónico, adhesión celular, quimiotaxis, receptores de efrinas, mielinización, diferenciación de los astrocitos y proteínas de la región paranodal. Por otro lado, los genes reprimidos por T3 están enriquecidos en procesos de proliferación celular y segregación y organización de los cromosomas. En general la T3 contribuye, en cierta medida, a la consecución de un perfil de expresión génica propio de la corteza adulta frente al de la corteza fetal.

INDEX

ABBREVIATIONS

ApoTR	Unliganded thyroid hormone receptor
BBB	Blood-brain barrier
CCK	Cholecystokinin
CHX	Cycloheximide
CSFB	Cerebrospinal fluid barrier
D1	Type 1 deiodinase
D2	Type 2 deiodinase
D3	Type 3 deiodinase
DAPI	4',6-Diamidino-2-phenylindole
DE	Differentially expressed
E	Embryonic day
EG	Expressed genes
FC	Fold Change
FDR	False discovery rate
GABA	Gamma amino butyric acid
Gfap	Glial fibrillary acidic protein
GO	Gene Ontology
KO	Knock out
MCM	Mini chromosome maintenance
MCT	Monocarboxylate anion transporters
MGE	Median Ganglionic Eminence
MMI	1-methyl-2-mercapto-imidazol
NeuN	neuronal specific nuclear protein
OATP	Na+ independent organic anion transporting polypeptides
P	Postnatal day
PNOC	Prepronociceptin
qPCR	Real-Time PCR
RA	Retinoic acid
RNA-Seq	RNA Sequencing
rT3	3,3´,5´-triiodothyronine
RTH	Resistance to thyroid hormone
RXR	9-cis retinoic acid receptor
T2	3,3´-Diiodothyronine
T3	3,5,3'-triiodo-L-thyronine
T4	Thyroxine
TH	Thyroid hormones
TRE	Thyroid hormone response element
TRH	Thyrotropin releasing hormone
TRs	Thyroid hormone receptors
TSH	Thyroid stimulating hormone
Wt	Wild type

INTRODUCTION

1. Thyroid Hormones

Thyroid hormones (TH) are essential for the proper development and function of many tissues of vertebrates, and in particular the brain.

In the human being adult onset hypothyroidism leads to an ample array of clinical manifestations that are mostly reversible with a proper treatment. On the other hand, TH deficiency during development may lead to irreversible brain damage, only preventable with a timely hormonal replacement treatment as in the case of congenital hypothyroidism [1].

1.1 Synthesis, secretion and tissue distribution

TH, thyroxine (3,5,3',5'-tetraiodo-L-thyronine, or T4) and triiodothyronine (3,5,3' triiodo-L-thyronine, or T3), are iodinated amino acids synthesized in the thyroid gland. The thyroid gland secretion consists of around 93% of T4 and 7% of T3. Most of the TH are transported through the bloodstream to the target tissues bound to serum proteins: albumin, thyroxine binding globulin, and transthyretin, with a small fraction as free TH, in equilibrium with the free hormone of the extracellular fluid. The free T4 concentration in blood is around 30 pM, and the free T3 is 8 pM.

TH synthesis and secretion are regulated by the hypothalamus-pituitary-thyroid axis. The hypothalamus synthesizes and secretes thyrotropin releasing hormone (TRH), which in the pituitary promotes the synthesis and secretion of the thyroid stimulating hormone (TSH). In the thyroid, TSH induces the synthesis and release of TH. Both, TRH and TSH, are negatively regulated by TH.

1.2 Metabolism

The main metabolic route of TH is the sequential deiodination. Deiodinases are selenoproteins that catalyze the removal of iodine atoms from the tyrosil (or "inner") ring, the 5 deiodinase (D3), and the phenolic (or "outer") ring, the 5' deiodinases (D1 and D2). These 3 deiodinases differ in their substrate specificity, specific inhibitors, Km and tissular distribution [2, 3].

D1 and D2 catalyse the conversion of T4 to the active hormone T3. They also catalyze further metabolization of reverse T3 (3,3′,5′-triiodothyronine, or rT3) to T2 (3,3′-diiodothyronine, or T2). D3 catalyzes the conversion of T4 and T3 to rT3 and T2 respectively. D1 also displays significant tyrosil ring activity (Figure 1).

D1 is expressed in tissues with a rapid exchange with blood such as liver, kidney and thyroid. D2 is expressed in tissues where the exchange with the bloodstream is slow and the intracellular concentration of T3 is critical: brain, anterior pituitary, and brown adipose tissue, and in human thyroid, heart and skeletal muscle. At least half of the T3 present in D2-expressing tissues is produced locally from deiodination of T4. D3 is expressed in brain and skin, and is crucial during development, with abundant expression in uterus, placenta, and fetal tissues. Its activity decreases after birth [4].
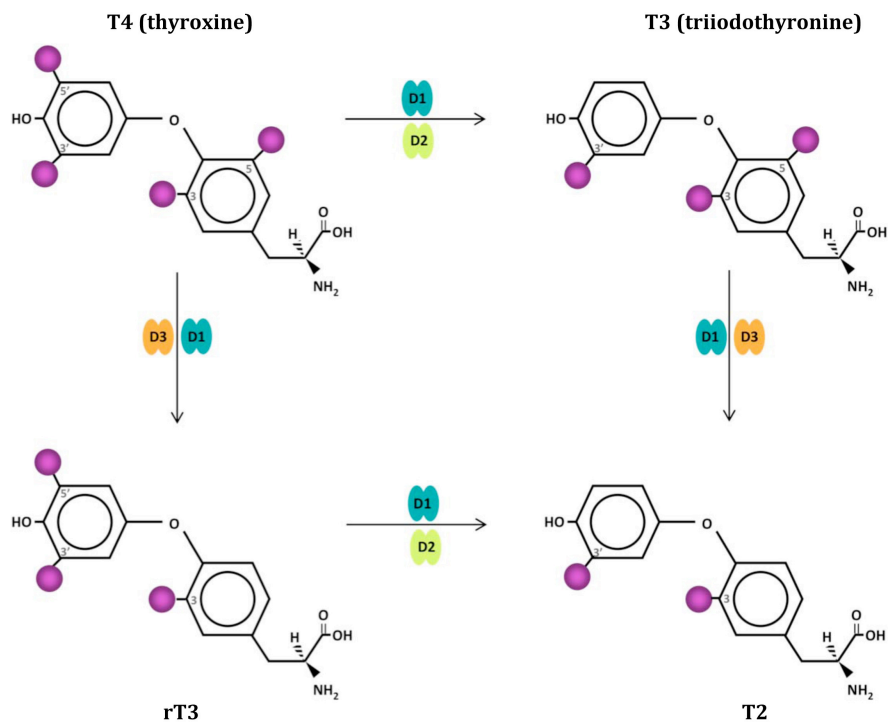
Figure 1. Thyroid hormone structure and metabolism. TH are molecules made of two phenolic rings bound by a oxygen atom; they have between 2 and 4 iodine atoms (purple). The figure shows the TH deiodination by the deiodinases (D1, D2 and D3).

In brain the genes encoding D2 (*Dio2*) and D3 (*Dio3*) are expressed in different cell types. *Dio2* is expressed in two glial cell types: astrocytes and tanycytes [5], and is one of the top 50 specific genes of astrocytes [6]. The protein D2 is localized in the endoplasmic

reticulum [7]. *Dio3* is expressed in neurons [8, 9] and the protein, D3, is localized in the plasma membrane allowing a fast degradation of TH excess [3, 10].

The brain local T3 concentration is critical and therefore, to maintain correct levels of TH, both deiodinases are strongly regulated by the thyroid status [11, 12]. T4 regulates D2 activity at the post-translational level, increasing protein degradation in proteasomes [13]. In addition, T3 has a small inhibitory effect on *Dio2* expression [14]. T3 also increases *Dio3* expression [15]. Consequently in situations of hypothyroidism D2 activity and *Dio2* expression are increased [16], whereas *Dio3* expression and D3 activity are decreased. The opposite situation occurs in hyperthyroidism.

1.3 Transport to the brain

Transporters are important for T4 and T3 delivery to brain cells. The passage of substances from the blood to the brain is restricted by the blood-brain barrier (BBB), the cerebrospinal fluid barrier (CSFB) and the plasma membrane of the target cells. At present different families of TH transporters are known differing in their substrate specificity and tissue distribution: the Na^+ dependent organic anion transporter, the Na^+ independent organic anion transporting polypeptides (OATP), the heterodimeric amino acid transporters, and the monocarboxylate anion transporters (MCT) [17]. Among the later, MCT8 has a specific role in T4 and T3 transport and has a high pathophysiological importance. The finding of patients with mutations in the MCT8 transporter suffering a neurological and endocrine syndrome highlights the relevance of this transporter in human brain development [18, 19].

In the mouse brain Mct8 is present in tanycytes, neurons, and strongly in the BBB and choroid plexus [20, 21]. The Oatp1c1 transporter is also expressed in the BBB and the choroid plexus. OATP1C1 is much less abundant in the human BBB than Oatp1c1 in rodents [21], and it is absent in primates [22]. Finally, Lat2, a member of the heterodimeric amino acid transporters, is expressed in neurons and choroid plexus [23]. OATP1C1 has high T4 affinity, whereas MCT8 and LAT2 have preference for T3 [24-26].

1.4 Mechanism of thyroid hormone availability in the brain

According to existing knowledge so far, our laboratory has proposed the following model of T3 availability in the rodent brain (Figure2).

Circulating T4 and T3 cross the BBB through Mct8, and are delivered to the interstitial fluid from where they can reach the neural cells. T4 is also transported specifically by Oatp1c1, a transporter which is present in the astrocytic end feet. In this way, T4 enters directly to the astrocytes where it is converted to T3 by D2. T3 generated in the astrocyte is subsequently transported to the neurons by Mct8, Lat2, or other transporters. In the neuron, T3 exert its action and can be degraded by D3. During fetal development, circulating T3 access to the brain is impaired even in the presence of Mct8 [20, 27], this could be due to the degradation by D3, which is expressed in the plasma membrane of neurons [28].
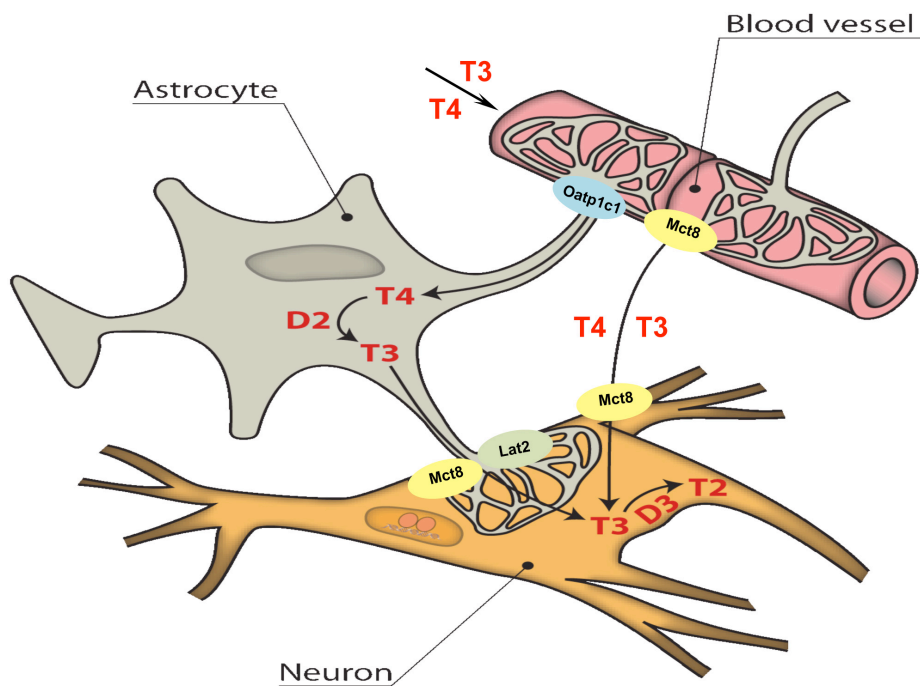
Figure 2. Route of entry of TH to the brain cells. TH cross the membrane of the blood vessels (BBB) through specific TH transporters as Oatp1c1and Mct8. T4 can enter into the astrocyte through the Oatp1c1 transporter where it is deiodinated to T3 by the D2, T3 passes from the astrocyte to the neuron by the Mct8 or Lat2 transporters. Mct8 can also transport TH directly to the interstitial fluid where they can be inactivated by D3, or enter into the neuron and exert their action.

1.5 Action in the target cell

TH may act in the nucleus, at the plasma membrane, in the cytoplasm or the mitochondria of the target cell [29].

The main action of the TH is exerted by T3 in the nucleus of the target cell modulating the expression of TH responsive genes. TH responsive genes can be directly regulated by T3 at the transcriptional level or indirectly regulated as a result of the direct changes. These genomic actions are mediated by the binding of T3 to thyroid hormone receptors (TRs). TRs are transcription factors that bind to the regulatory region of the TH target genes modulating their transcription.

2. Thyroid Hormone Receptors: TRs

2.1 Structure and isoforms

TRs are transcription factors modulated by ligand belonging to the nuclear receptor superfamily. TRs are encoded in two different genes: the *THRA/Thra* gene, located on chromosome 17 in human and chromosome 11 in mice, encodes the TRα subtype. The *THRB/Thrb* gene, located on chromosome 3 in human and 14 in mice, encodes the TRβ subtype. Each subtype has different receptor isoforms produced by different promoter usage and alternative splicing.

They are all modular proteins with three distinct domains [30-33]:

- Amino terminal domain: It is the part of the receptor most variable in size and sequence. Within the same gene, this region may vary due to different promoters or alternative splicing. This domain has promoter and cell-specific activity, which contributes to the specificity of each isoform and its interaction with other cell type-specific factors.

- DNA binding domain: It is the most conserved domain capable of recognizing TH response elements (TRE). These are sequences in the regulatory region of target genes, to which the receptor binds and modulates gene expression. This region contains two zinc fingers involved not only in recognition of the TRE sequence, but also in dimerization.

- Ligand binding domain: It is a multifunctional domain. Besides binding the ligand, it is involved in dimerization and binding coactivator and corepressor proteins which modulate the transcription of the TH target genes.

There are four TRα isoforms: TRα1, TRα2, ΔTRα1 and ΔTRα2. TRα1 is the only isoform with ligand binding capacity. The TRα1 and TRα2 isoforms are generated by alternative splicing, and differ in the carboxyl terminal region. TRα2 has DNA binding domain, but lacks a functional ligand binding domain. ΔTRα1 and ΔTRα2 are truncated TRα1 and TRα2 isoforms generated from an internal promoter located in an intron, both isoforms without receptor function [34].

TRβ consists of two isoforms, TRβ1 and TRβ2, and an additional TRβ3 and truncated ΔTRβ3 in the rat [35, 36]. They are transcribed from different promoters and undergo alternative splicing, forming part of each isoform specific exons. TRβ1 and TRβ2 (and rat TRβ3) are functional receptors with ability to bind T3 and DNA (Figure 3).
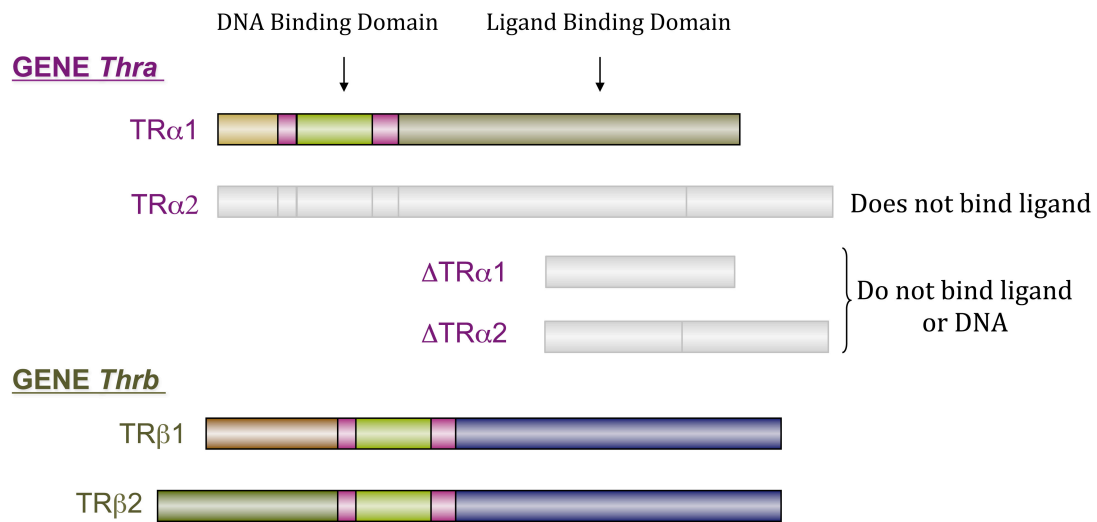
Figure 3. Mouse thyroid hormone receptor isoforms. The isoforms of the TRs are encoded in two different genes: *Thra* and *Thrb*. *Thra* encodes TRα1, TRα2, ΔTRα1 and ΔTRα2; TRα1 is the only one with functional DNA and ligand binding domain. *Thrb* encodes TRβ1 and TRβ2, both able to bind DNA and ligand.

TRs are present in the nucleus regulating the transcription of TH target genes, but they are also present in the cytoplasm mediating extragenomic actions of TH [29].

16

2.2 Mechanism of regulation of gene expression

The genes induced by T3 and repressed in hypothyroidism are called "positive genes". Conversely, the genes which are repressed in the presence of hormone and induced in hypothyroidism are called "negative genes".

In the nucleus TRs bind to the TRE sequence in the regulatory region of target genes [37, 38]. TREs are sequences of a pair of the consensus hexanucleotide AGGTCA; these repeats may be arranged as direct repeats separated by 4 nucleotides, as everted repeats separated by 6 nucleotides or as inverted repeats without intervening nucleotides, i.e, a palindromic sequence [39]. The receptor can bind to the DNA as monomer, as homodimer or most often as heterodimer with the 9-cis retinoic acid receptor (RXR).

TR activity is modulated by T3. In a simplified view, the mechanism of regulation of the positive target genes is represented in Figure 4.

 TH dependent genes have a basal transcription in the absence of TRs. TRs can be bound to the TRE with T3 or in the absence of hormone as unliganded TR (apoTR). Because of the intrinsic activity of the TRs, in the absence of TH when the heterodimer RXR-apoTR is bound to the TRE *in vitro* there is usually repression of transcription. The RXR-apoTR heterodimer interacts with different corepressor proteins with histone deacetylase activity. This complex of proteins maintains compacted the chromatin inhibiting transcription of the TH target gene [40, 41].

In the presence of T3, ligand binding to the TR causes a conformational change in the receptor that leads to the release of the corepressor proteins and the recruitment of the coactivators proteins. The coactivator complex has histone acetylase activity, which allows chromatin remodeling and transcriptional activation of the target gene [41, 42].

The corepressors and coactivators are protein complexes containing several enzyme activities besides acetyltransferases and deacetylases, such methylases, kinases and phosphatases. Relevant for T3 action are SRC-1 as coactivator and NCoR and SMRT as corepressors [42].
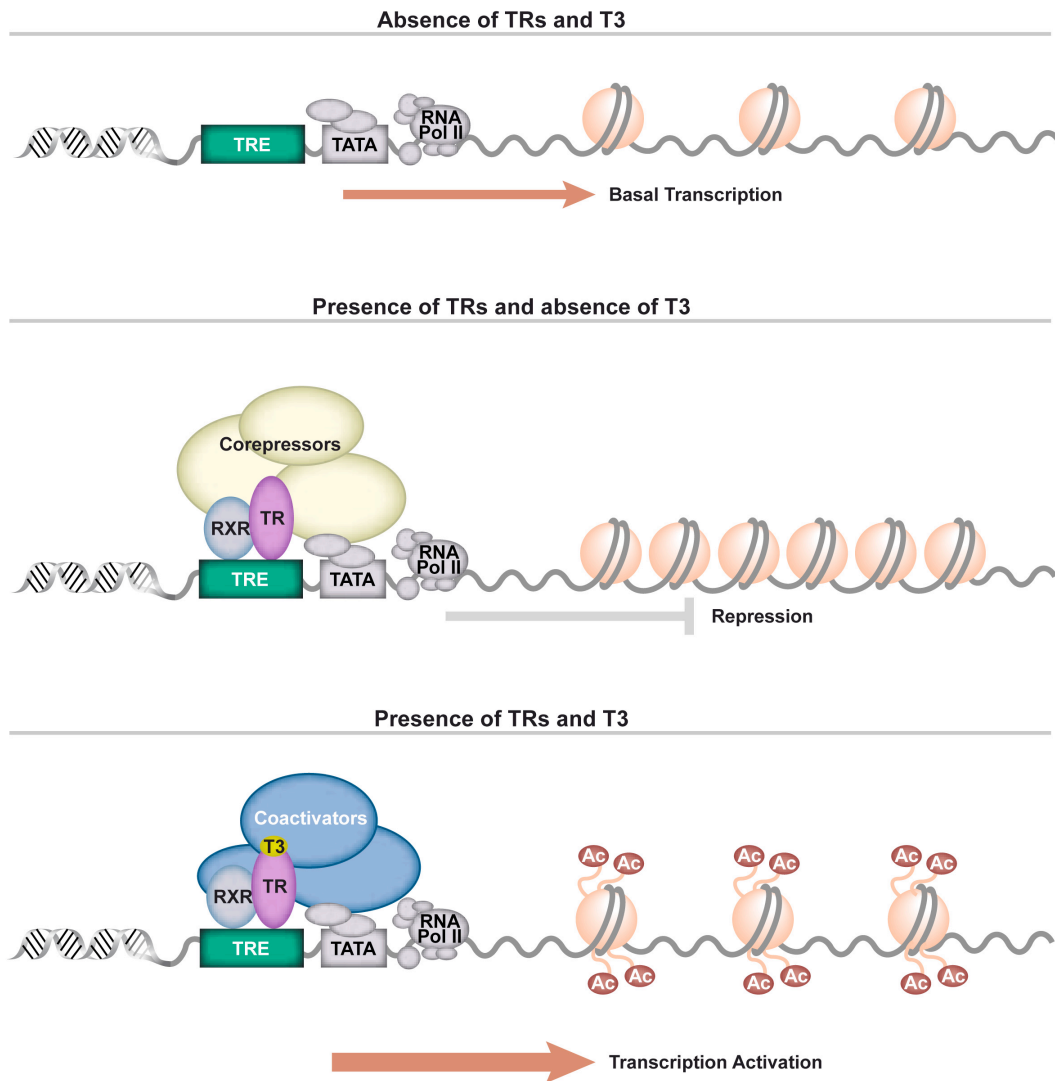
Figure 4. Mechanism of TRs and T3 genomic action. In the absence of TRs and T3, TH target genes have a basal transcription. In the presence of TRs, and absence of T3, the receptor binds to TRE sequences as a heterodimer with the RXR, they bind to a corepresor complex with enzymatic activities that lead to the repression of the transcription. The binding of T3 leads to a conformational change of the receptor releasing the corepresors and allowing the interaction with the coactivators. These proteins acetylate the chromatin activating the transcription.

The mechanism of regulation of negative genes by T3 is not well known [43]. Several studies have shown that in negative genes, such as the TSHβ, the TRs are bound to the TRE sequence, so it is possible that this negative regulation by T3 is mediated by a different set of co-regulatory proteins [44]. In a study in TRβ Knock in mice, that express the R429Q mutation affecting the ligand-binding domain but able to maintain TH binding, there was selective resistance of the negative genes in all tissues studied. Although the molecular mechanism for this selective impairment of gene expression is unknown, the authors concluded that the interaction of TRβ with co-regulators, and the receptor dimerization play an important role in the negative regulation of gene expression by TH [45].

2.3 Tissue distribution

Comparing the amount of TRs in different tissues at the protein level is particularly complex due to the absence of validated specific antibodies for the different isoforms, so most of the studies are done using the mRNA expression level. Nevertheless quantification assays have been done by kinetic assays or direct immunoprecipitation of receptor-bound to 125I-T3 [46-52].

In general, all isoforms are expressed in all tissues but in different proportions. The isoform most abundantly expressed in each tissue is usually considered responsible for mediating the action of TH in those cells.

TRα1 expression is ubiquitous from development, with a predominant role in brain, heart, intestine, muscle and bone [53, 54]. TRα2 is expressed in most tissues with expression levels many fold higher than those of TRα1 [55, 56]. TRβ1 is expressed slightly later during the development in brain, pituitary, lung, intestine, thyroid, cochlea, liver, kidney and heart [51]. TRβ2 expression is mainly restricted to the pituitary [35], hypothalamus, inner ear and retina [57-61].

There are a few situations where in a cell type just one TR isoform is expressed, this is the case of TRα1 in reticulocytes [53] or TRβ2 in the cone photoreceptors [59].

2.3.1 Distribution of TRs in the brain

In the brain all functional isoforms of TRs are expressed, although TRα1 has a more predominant role because it is expressed in higher proportion. At the protein level TRα1 isoform accounts for 70-80% of total T3 binding capacity in the rat brain.

TRα1 has been detected in the human brain at 10th week of gestation [62] and in the rat brain by embryonic day 14 (E14) [63]. TRα1 is expressed throughout the central nervous system of the mouse from E13.5 to adulthood. A recent study in mice described TRα1 localization in all neurons except cerebellar granular cells from the external germinal layer and mature Purkinje cells. There was no expression of TRα1 in the glial cells with the exception of the tanycytes [52]. On the contrary, another study showed that cerebellar astrocytes express TRα1 and TRβ1 in the postnatal rat [64].

TRβ1 expression in the brain is very low during the fetal period increasing progressively in the postnatal period until adulthood. The expression pattern of TRα1 and TRβ1 overlaps often, but there are cases where either expression is restricted to one cell type. For example, in the cerebellum, the differentiated granular cells, of the inner granular layer, only express TRα1 while Purkinje cells express TRβ1 in the postnatal rat[57].

In rodents (rat) TRβ2 amounts to 10% of receptors in the brain [65]. At the protein level, TRβ2 has been found in brain regions where its mRNA has not been detected as the layers II-VI of the cerebral cortex [49] and Purkinje cells of the cerebellum (unpublished results from our laboratory).

3. Specificity of the TR isoforms

The molecular diversity of TRs raises the question of whether different isoforms have redundant functions or otherwise they have specific intrinsic properties. To date there are different studies: clinical, genetically modified mice and *in vitro* studies, which have attempted to clarify this issue. The main conclusion is that the TR isoforms are mostly equivalent, but with different physiological roles depending on the relative expression patterns [66-68].

3.1 Clinical studies

In 1989 the first patient with a mutation in the *THRB* gene was found, suffering resistance to thyroid hormone (RTH). This condition is characterized by high levels of T3 and T4 in the serum as well as an unsuppressed TSH, goiter and no symptoms of thyrotoxicosis [69]. These patients may have learning disabilities, reduced IQ, and increased incidence of Attention Deficit and Hyperactivity Disorder. The mode of inheritance of this disease is autosomal dominant. The mutant receptor has a dominant negative effect. As most of the mutations affect the ligand binding domain, the mutated receptor can still be bound to the DNA disabling the correct function of the unaffected copy of the receptor. There are currently over 3000 patients identified with mutations in *THRB* worldwide.

It was not until 2012 when the first patient with a mutation in the *THRA* gene was diagnosed, presenting minimal alterations of circulating TH: slightly reduced T4, slightly increased T3 and normal TSH, severe constipation, mild cognitive deficits, and delayed growth and development [70]. The clinical manifestations of this patient suggested deficiency of TH in tissues expressing TRα as bone, digestive tract, heart, muscle and central nervous system. Like patients with mutations in *THRB*, inheritance of this disease is autosomal dominant and the TRα1 protein displays strong dominant negative activity.

The different clinical features of patients with mutations in *THRB* and *THRA*, affecting different organs, reflect the fact that both receptor isoforms have different functions.

3.2 Studies on genetically modified mice

The generation of genetically modified mice has been a very important tool in the study of TR specificity. Currently there are 22 lines of transgenic mice with point mutations or with one or more TR isoforms deleted [71].

In 1996 the first TRβ knock out (KO) mouse was generated with a deletion of part of exon 3, common to TRβ1 and TRβ2. These mice had hearing impairment and their thyroid status was similar to that of patients with mutations in THRB, characterized by high levels of T3, T4 and TSH in serum by a dysregulation of the hypothalamic pituitary thyroid axis [72]. These mice were a model of the RTH due to absence of receptor, the first case of RTH described, and the only one to date [73]. The model for the common

forms of RTH had to await the development of knock-in mice expressing mutated forms of the receptor.

Later, in 1998 the first TRα1 KO mouse was generated, with a deletion of a specific part of exon 9 common to the TRα1 and TRαΔ1 isoforms, without altering the expression of TRα2 and TRαΔ2 isoforms. These mice presented reduced heart rate and body temperature, and mild hypothyroidism (normal T3 and T4 levels, and slightly low TSH) [74]. It has to be pointed out that these mice lacking TRα1 expression are different from the patients, which express a mutated TRα1 with dominant negative properties.

As in clinical studies, the different phenotypes of the TRα1 and TRβ KO mice reflect the different tissue localizations and the different function of both receptor isoforms.

In 1999, combining the TRα1 and TRβ KO mice, the double TRα1/TRβ KO mice were generated. These mice presented a more severe phenotype than the single KO characterized by reduced fertility in females, delayed growth and bone maturation, goiter and a extreme hyperactivity of the hypothalamic pituitary thyroid axis resulting in extremely high levels of T3, T4 and TSH [75].

In contrast to the specificity of the different TRs isoforms, there are several studies that support redundancy in tissues that normally express TRα1 and TRβ isoforms. The double TRα1/TRβ KO mice present a more severe phenotype in the absence of both receptors compared with the phenotype seen in mice expressing only one receptor, indicating that the expressed receptor compensates for the absence of the other. This aspect has been studied in different tissues such as the regulation of the hypothalamic pituitary thyroid axis, the muscle, the bone growth and the maturation in the proliferation of the skin cells [75-77].

A large-scale study using microarrays to examine hepatic gene expression profiles showed that, interestingly, single TRα or TRβ KO mice present similar gene expression patterns to wild type (Wt) mice, suggesting that these isoforms co-regulate most hepatic target genes although the TRβ is the predominant isoform expressed in liver [66].

3.3 *In vitro* studies

The specific actions of each isoform raise the question of whether each receptor specifically regulates different sets of genes. The technological advances in large-scale gene expression analysis have made possible to go deeper into this topic. Recently, it has

been shown that expressing TRα1 or TRβ isoform separately in the same cell type, both receptors regulate the same sets of genes with differences only in the regulation kinetics [67, 78]. Conversely, there are some exceptions where the regulation of the expression by T3 of a concrete gene is restricted to a single TR isoform. This is the case of *Dio3* which is only regulated by TRα1 [15].

These results indicate that in some cases TRα1 and TRβ can be exchanged to mediate some of the T3 actions, but there are also actions mediated by a specific TR isoform [29].

One of the possible explanations of the distinct transcriptional regulatory properties of the TRs is based on the divergent DNA sequences of the different isoforms. A recent work has successfully found novel isoform-selective coregulators that could mediate the transcriptional and biological properties of each isoform. The relative expression of TRs and coregulators in different tissues could be the clue for the distinct transcriptional properties of TRs isoforms in each tissue [79].

4. TR activity in the absence of ligand

TRs bind to the TRE sequences not only in the presence of T3 but also as apoTRs in the absence of ligand. On some genes, TRs have transcriptional activity in the absence of T3 in the opposite direction to that exerted when bound to ligand [29]. In physiological situations, 50-80% of TRs are ligand-bound so that the rest of receptors may be acting as aporeceptors [80, 81]. There are two main lines of evidence supporting that TRs have physiological functions in the absence of ligand:

4.1 Absence of TRs is not equivalent to hypothyroidism

TRs mediate the response of T3 by regulating gene expression, so one would expect that the absence of receptors would be equivalent to the absence of hormone. Surprisingly, it has been shown in several studies that this is not the case. Hypothyroidism has a much more severe effect than the absence of receptors due to the intrinsic activity of the apoTRs [75].

23

The morphological changes caused by hypothyroidism in the development of the cerebellum of Wt mice, do not occur in hypothyroid TRα1 KO mice [82]. Furthermore, mice with congenital hypothyroidism due to a deletion of the *Pax8* gene do not survive unless they are treated with TH. In contrast, if the TRα1 gene is additionally deleted, the double KO mice increase their survival rate, indicating that the deleterious effect of congenital hypothyroidism is mediated by the apoTRα1 [83].

There are also gene expression studies supporting this evidence. A large-scale study was performed using microarray analysis to examine hepatic gene expression profiles of Wt, TRα and TRβ KO mice under different TH conditions. The analysis showed that a subpopulation of target genes repressed their basal transcription in the absence of ligand. Gene expression patterns of TRα/TRβ double-KO mice and TH-deprived Wt mice showed that absence of receptor and absence of hormone have different outcomes [66].

4.2 TR expression before onset of thyroid secretion

TRs are expressed before complete maturation of the thyroid gland and the synthesis of fetal TH during development in several animal species. This may indicate that in the period since the TRs are expressed till the onset of thyroid secretion, the apoTRs can perform early developmental actions. However we have to keep in mind that even if there is no fetal TH secretion in this period, maternal TH cross the placenta and reach the fetus, so that the proportion of aporeceptors may not be as high as suspected. But on the other hand, during development D3 activity in fetal tissues and placenta is very high to protect the fetus from excessive TH [84]. This mechanism would favour low receptor occupancy. In addition, D2 activity increases after birth, allowing higher concentrations of T3 in the brain [11, 85]. Total brain receptor occupancy by the hormone increases in parallel with plasma and cytosol total and free T3 with a maximum of 50-60% on postnatal day 15 (P15) [86] (Figure 5).
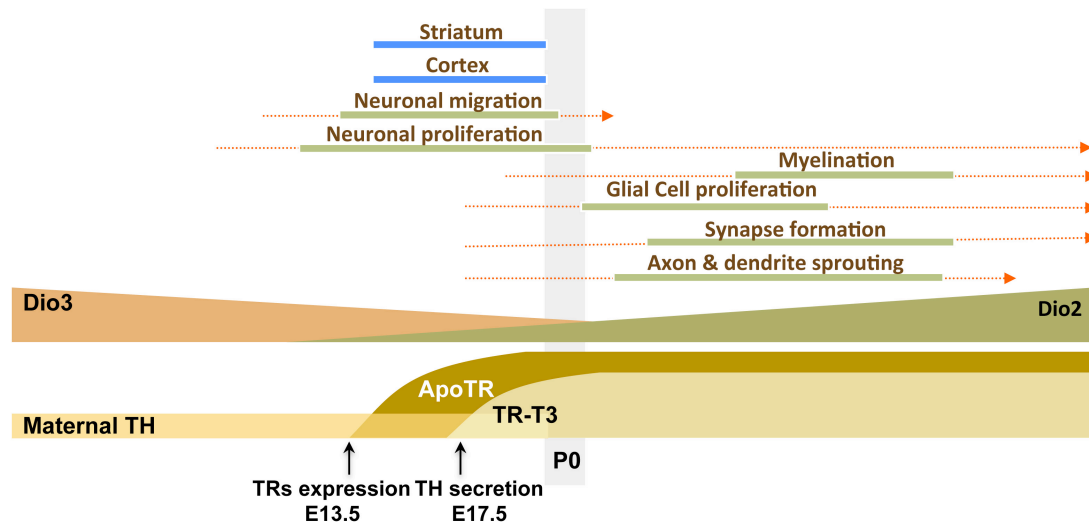
Figure 5. Scheme of mouse brain development. In the upper part: summary of timing of neurological process during mouse ontogeny. A broken line means that the process is active, a bold line indicated that the process is very active. In the lower part: summary of the TH related events around the perinatal period. D3 expression is very high and decreases during development while D2 expression increases. Maternal and individual TH levels are represented in yellow together with the proportion of receptor as ApoTR or TR bound to T3. TRs expression begins at E13.5 and fetal TH secretion at E17.5.

5. Thyroid Hormone action in the brain

The hypothyroid brain presents structural defects. TH have an effect on neurogenesis, but are mostly involved in late events of brain development, such as migration and terminal differentiation of neurons and glia (Figure 5). Hypothyroidism results in a delay in the radial glia maturation, less defined cortical layers as well as delayed migration of granular cells to the internal granular layer in the cerebellum.

TH also participate in the myelination process. In hypothyroidism, the differentiation of oligodendrocytes and myelin production is delayed and reduced, while hyperthyroidism accelerates these processes.

The morphological and functional alterations observed in hypothyroid brains are at least partially to a great extent, the result of an altered expression of the T3 target genes. Several TH-dependent brain genes have been studied. In the rat, most genes regulated by TH were first identified during the postnatal period. These studies revealed that each

of the regulated genes has a defined window of TH sensitivity and are regulated in a spatial and temporal fashion. Some of these genes are involve in myelination (*PLP, MBP*), cell migration (*Reelin*), cell signalling (*RC3/Neurogranin*), and transcription factors or splicing regulators (*Klf9, Hr, Musashi-1*).

5.1 Regulation of gene expression by TH in the developing brain

Despite the extensive work on the actions of TH in brain development there are still many uncertainties as how TH influence maturation of the developing brain. One of the difficulties in approaching these problems was the almost complete lack of knowledge on the molecular targets of TH in the developing fetal brain. The patterns of TR and deiodinase expression and T3 accumulation during brain development suggest that there must be many unknown TH target genes (Figure 5). During the second trimester of human pregnancy there is a selective accumulation of T3 in the cerebral cortex [62]. There are also many clinical and epidemiological data indicating that TH exert an important role in brain maturation before mid gestation in humans (corresponding to the end of the fetal period in the rodents) as evidenced by neurological cretinism and related syndromes [28, 87].

Nowadays, the availability of genomic sequence information and high-throughput advances in experimental techniques has allowed the genome-wide transcriptome analysis and the identification of TH target genes in the cerebral cortex during fetal [88, 89] and postnatal period [90] or in adult animals [91].

5.2 Brain gene expression of genetically modified mice

Levels of expression of certain TH target genes have been used as indicators of thyroid status in the brain to assess the effect of the deletion of specific proteins involved in TH metabolism using genetically modified mice.

Transgenic mice were generated to clarify the role of specific components of TH distribution and metabolism in the brain. For example, *Mct8*, *Oatp1c1*, *Dio2,* and *Dio3* have been inactivated, either alone or in combination.

Inactivation of the *Mct8* gene leads to a reduction in the passage of T3, but not T4, through the BBB. The deficient entry of T3 to the brain is compensated by increased D2

activity, producing more local T3 from T4. The final effect is that gene expression in the cerebral cortex is minimally affected [90]. *Oatp1c1* inactivation leads to altered expression of some TH positive target genes in the brain, highlighting the importance of this transporter in T4 transport through the BBB [92].

Dio2 inactivation leads to brain TH levels similar to those in hypothyroid mice although changes in gene expression are not as severe as in hypothyroidism [93]. Moreover, inactivation of *Dio2* preferentially affects genes regulated negatively by T3 [90]. The D3 KO mice have increased T3 levels in the brain. This brain hyperthyroidism has a stronger effect on the negative than on the positive genes. However in hyperthyroid Wt mice most affected genes are the positively regulated ones. Observations in the D2 and D3 KO mice highlight the importance of the route of TH entry to the brain in the regulation of gene expression [94].

5.3 Brain direct thyroid hormone target genes

Most of the brain TH target genes have been identified comparing euthyroid to hypo or hyperthyroid animals. The expression changes of these genes may be the result of TH action at the cellular level or a secondary and indirect effect of hypo/hyperthyroidism.

In fact, there is limited knowledge of brain TH target genes at the cellular level. To date, just two recent studies have used chromatin occupancy analysis performed at a genome-wide scale with transfected TRs, one in neural cell lines and the other in mouse hepatocytes, identifying a set of very likely directly TRs target genes [68, 95]. Both studies conclude that the number of genes occupied by TRs exceeds the number of T3 responsive genes. We should keep in mind that in both studies the TRs concentration excess the normal levels of the tissue. Studies focused on the primary brain cells at the cellular level have only been done in cerebellar granular cells identifying limited number of TH target genes [96].

OBJECTIVES

- To analyze the expression of thyroid hormone dependent genes in the cerebral cortex and striatum of TR knock out mice: role of TR subtype and comparisons with hypothyroidism.

- To analyze the specific role of TRα1 and TRβ in the regulation of selected thyroid hormone-dependent genes in mouse cerebrocortical primary cell culture.

- To get a further insight into the role of thyroid hormone on brain development through global transcriptome analysis in cerebrocortical primary cells.

MATERIALS AND METHODS

1. Handling of animals

Protocols for animal handling were approved by the local institutional Animal Care Committee, following the rules of the European Union. Animals were housed in temperature (22 ± 2 ºC) and light (12:12 light-dark cycle; lights on at 7 a.m.) controlled conditions and had free access to food and water. Mice of a hybrid genetic background of 129/OLa[+]129/Sv[+] BALB/c[+]C57BL/6 [72, 74-76] were used. We started by crossing *TRα1[+/-]TRβ^{+/-}* male mice with females of the same genotype (F0) to generate all possible genotypic combinations (F1). The mice used in the experiments were obtained by appropriate crossings of F1, to generate Wt, *TRα1^{-/-}TRβ^{+/+}, TRα1^{+/+}TRβ^{-/-}*, and *TRα1^{-/-}TRβ^{-/-}* mice (F2), and were indistinctly male or female (Figure 6). The *TRα1* deletion was detected by using the following combination of primers: forward 5´caagatcgagaagagtcagga3', reverse 5'gtatgggagctgcatctatccaag3' and the *TRα1^{-/-}* specific reverse primer 5'cactgcattctagttgtggt3'. The *TRβ* deletion was detected by using the following combination of primers: forward 5'gcacaggcaggaagtaggctgttct3', reverse 5'ccctggaggccaaaggtcatcaatg3' and the *TRβ^{-/-}* specific reverse primer 5'gtgccagcggggctgctaaag3'. In the *in vivo* experiments, hypothyroidism was induced in pregnant and lactating dams by administering a drinking solution containing 0.02% 1-methyl-2-mercapto-imidazol (MMI, Sigma Chemical Co, St Louis, MO) plus 1% $KClO_4$ *ad libitum*. These antithyroid compounds were given from gestational day 9 and throughout the lactating period, until the end of the experiment on P21. These compounds cross the placenta and are present in the milk, so that the pups derived from the treated dams were also hypothyroid. The pups were killed by decapitation on P21. The cerebral cortex and the striatum were rapidly dissected out, frozen on dry ice, and kept at -80 °C until RNA isolation.
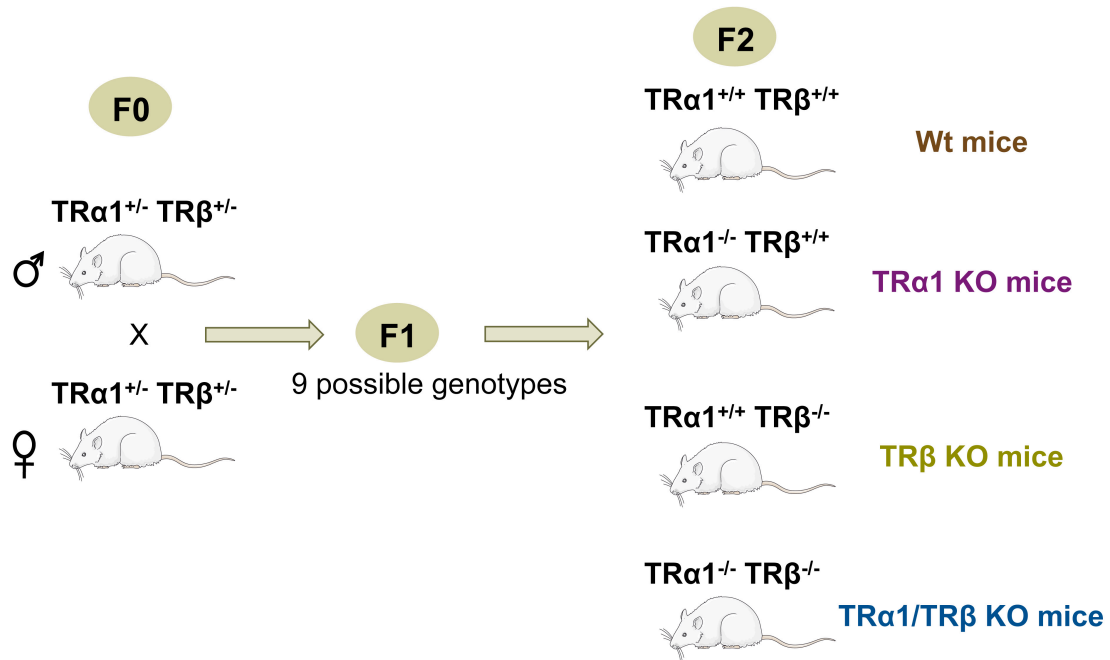
Figure 6. Generation of the Wt, *TRα1⁻/⁻TRβ⁺/⁺*, *TRα1⁺/⁺TRβ⁻/⁻*, and *TRα1⁻/⁻TRβ⁻/⁻* mice. We started by crossing TRα1⁺/⁻TRβ⁺/⁻ male mice with female of the same genotype (F0) to generate all possible genotypic combinations (F1). The mice used in the experiments were obtained by appropriate crossings of F1 to generate Wt, *TRα1⁻/⁻TRβ⁺/⁺*, *TRα1⁺/⁺TRβ⁻/⁻*, and *TRα1⁻/⁻TRβ⁻/⁻* mice (F2), and were indistinctly male or female.

2. Primary cerebrocortical cell culture

Pregnant dams were euthanized with CO_2 on gestational day 17.5, and the fetuses were extracted and euthanized by decapitation. The cerebral cortices were dissected in PBS containing 1% BSA and 0.1% Glucose. The tissue was disaggregated by enzymatic digestion with 0.4 mg/ml Papain (Roche) and passed through a 0.9 mm syringe in the presence of DNAse I (Roche). The homogenate was centrifuged and the cells were resuspended in serum free culture medium; Neurobasal Medium (Gibco) supplemented with 2% B27 (Gibco), Glutamax (Gibco), 10 U/ml Penicillin, and 10 U/ml Streptomycin. The cells were seeded on poly-L-ornithine-coated 12-well plates (Sigma). The wells were previously preincubated with a mixture of Horse Serum and culture medium (1:1), and Laminin (1μg/ml) (Sigma). Cells were added to this media in culture medium (6×10^5 cells per well). After 9 days, the cells were incubated 24h in the same medium without B27 supplement before adding the treatment: 1 or 10 nM T3, depending on the experiment. Incubation time was 24h in the presence of fetal calf serum without TH (1:1000 dilution). To examine the response to T3 in the presence of inhibition of protein

synthesis, cycloheximide (CHX) (Sigma) was added to the cultures at a concentration of 8 µg/ml 30 minutes before T3 (10 nM) and the cells were harvested 6 hours after T3 addition. Control cultures without treatment were incubated in parallel.

For the study of the role of thyroid hormone receptor subtypes, cerebrocortical cell cultures were obtain from a pool of mice cortices. For the RNAseq assay, cortices from each individual fetus originate two samples of the cerebrocortical cell culture, one with T3 treatment and one without treatment.

3. Immunofluorescence

The composition of the primary cerebrocortical cell cultures was analyzed by immunofluorescence as follows: Cells plated on glass coverslips were fixed with absolute acetone for 7 min at -20°C. After permeabilization for 5 min with 0.5% Triton X-100 in PBS, the cells were incubated for 1 h in 1% BSA 2% cold fish skin gelatin (Sigma), 0.05% Triton X-100, in PBS to block non-specific antibody binding sites. For immunofluorescence labeling, the cells were stained by overnight incubation at 4°C in the blocking buffer with the following combination of primary antibodies diluted 1:500 mouse monoclonal anti glial fibrillary acidic protein (GFAP) (Clone G-A-5, Sigma) for astrocytes, rabbit polyclonal anti neuronal specific nuclear protein (NeuN) [97] (Millipore) for neurons and mouse monoclonal anti calbindin D28K (Sigma) for calbindin positive neurons. The secondary antibodies were donkey anti mouse Alexa 488 (green) and donkey anti rabbit Alexa 555 (red) and were used at 1:500 dilution. Cells were then washed in PBS and incubated for 10 min with 4',6-diamidino-2-phenylindole (DAPI) (Gibco® Life Technologies) 0.1 µg/ml in PBS to label the nucleus. Omitting the first antibodies in the incubation reaction gave no signal. Confocal images were acquired using an inverted Zeiss LSM 710 laser scanning microscope with a plan-apochromatic objective 63x/N.A 1.3. Sequential scanning mode was used to avoid crosstalk between channels. All images shown correspond to the maximum intensity projection of a z-stack. Images were processed with Zen 2009 software and Adobe Photoshop. To calculate the relative abundance of neurons, astrocytes and calbindin positive neurons in the different cultures, antibody-labeled cells and DAPI-stained nuclei were counted in photographs taken using a 40x objective. A total number of 100-200 cells were counted in sextuplicate for each culture. The relative number of neurons, astrocytes and calbindin positive neurons was calculated as a percentage of DAPI-stained nuclei.

4. Primary astrocyte cell culture

Mice were euthanized by decapitation on P3. The cerebral cortices were dissected at 4°C in PBS. The tissue was disaggregated in culture medium (DMEM supplemented with Glutamax, 10 U/ml Penicillin, and 10 U/ml Streptomycin, fungizona and fetal calf serum without TH (1:10 dilution). The homogenate was centrifuged and the cells were resuspended in culture medium. The cells were seeded on poly-L-ornithine-coated 12-well plates (Sigma). After 7 days, the cells were incubated 24h in the same medium adding 1 nM T3. Control cultures without treatment were incubated in parallel. After treatment RNA extraction was performed to measure gene expression.

5. RNA preparation and quantification

From the primary cerebrocortical cell culture, primary astrocyte culture, striatum and cerebral cortex, total RNA was isolated using the Trizol procedure (Invitrogen, Carlsbad, CA) with an additional step of chloroform extraction. For the RNA sequencing (RNA-Seq) analysis, the RNA was isolated using RNeasy Plus Micro Kit (Quiagen). The quality of RNA was analyzed using a BioAnalyzer (Agilent, Santa Clara, CA). Complementary DNA was prepared from 250 ng of RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). qPCR assays were performed on microfluidic cards or single tube PCR. For the microfluidic cards we used TaqMan® low-density arrays (Applied Biosystems), format 48a (P/N 4342253). cDNA aliquots corresponding to 10 ng of starting RNA from individual mice were used, with TaqMan Universal PCR Master Mix, No Amp Erase UNG (Applied Biosystems) on a 7900HT Fast Real-Time PCR System (Applied Biosystems). The PCR program consisted in a hot start of 95°C for 10 minutes, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. For analysis we used the 2-Ct method. As internal control we included 18S RNA and *Ppia* (Peptidylprolyl cis/trans isomerase A or Cyclophilin A). The use of either reference RNA gave similar results, so that the data were all normalized to the 18S RNA. For single tube PCR a cDNA aliquot corresponding to 5 ng of the starting RNA was used, with Taqman Assay-on-Demand primers and the Taqman Universal PCR Master Mix, No Amp Erase UNG (Applied Biosystems) on a 7900HT Fast Real-Time PCR System (Applied Biosystems). Expression of *Thra1* and *Thrb* was measured using SYBR Green qPCR with the following primers: Thra forward: 5´AGCTGCTGATGAAGGTGACTGA3´, Thra reverse: 5´TGAGGCTTTAGACTTCCTGATCCT3´,

Thrb forward: 5´AAGCCACAGGGTACCACTATCG3´ and, Thrb reverse: 5´GCGGGTGACTTTGTCTATGATG3´.

The PCR program consisted in a hot start of 95°C for 10 minutes, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. PCRs were performed in triplicates, using the 18S gene as internal standard and the 2-Ct method for analysis. Data were expressed relative to the values obtained for the control Wt, which was given a value of 1.0 after correction for 18S RNA.

The Taqman probes used in this Thesis (Applied Biosystems) are listed below:

Gene Symbol	Complete Gene Name	Probe ID
18S	18S ribosomal RNA	Hs99999901_s1
Abcd2	ATP-binding cassette, sub-family D (ALD), member 2	Mm00496455_m1
Agbl3	ATP/GTP binding protein-like 3	Mm00618199_m1
Agxt2l1	Alanine-glyoxylate aminotransferase 2-like 1	Mm00510840_m1
Aldh1a1	Aldehyde dehydrogenase family 1, subfamily A1	Mm00657317_m1
Aldh1a3	Aldehyde dehydrogenase family 1, subfamily A3	Mm00474049_m1
Angptl4	Angiopoietin-like 4	Mm00480431_m1
Bcar3	Breast cancer anti-estrogen resistance 3	Mm00600213_m1
Bcl2l11	BCL2-like 11 (apoptosis facilitator)	Mm00437796_m1
Bcl6	B-cell leukemia/lymphoma 6	Mm00477633_m1
Cadm2	Cell adhesion molecule 2	Mm00618780_m1
Calb1	Calbindin 1	Mm00486645_m1
Camk4	Calcium/calmodulin-dependent protein kinase IV	Mm01135329_m1
Cbr2	Carbonyl reductase 2	Mm00483074_g1
Cd72	CD72 antigen	Mm00514264_g1
Cirbp	Cold inducible RNA binding protein	Mm00483331_m1
Cnn1	Calponin 1	Mm00487032_m1
Cntn2	Contactin 2	Mm00516138_m1
Col6a1	Collagen, type VI, alpha 1	Mm00487160_m1
Col6a2	Collagen, type VI, alpha 2	Mm00521578_m1
Cxadr	Coxsackie virus and adenovirus receptor	Mm00438361_m1
Cxcl14	Chemokine (C-X-C motif) ligand 14	Mm00444699_m1
Cyp26b1	Cytochrome P450, family 26, subfamily b, polypeptide 1	Mm00558507_m1
Dbc1	Deleted in bladder cancer 1 (human)	Mm00517359_m1
Dbp	D site albumin promoter binding protein	Mm00498056_m1
Dio3	Deiodinase, iodothyronine type III	Mm00548953_s1
Epha3	Eph receptor A3	Mm00580743_m1
Flywch2	FLYWCH family member 2	Mm00513052_m1
Fxyd6	FXYD domain-containing ion transport regulator 6	Mm00445583_m1
Gabrd	Ggamma-aminobutyric acid (GABA-A) receptor, subunit delta	Mm00433476_m1
Gbp3	Guanylate binding protein 3	Mm00497606_m1
Gls2	Glutaminase 2 (liver, mitochondrial)	Mm01164862_m1
Gpc3	Glypican 3	Mm00516722_m1
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Mm00550050_m1
Hr	Hairless	Mm00498963_m1
Htr7	5-hydroxytryptamine (serotonin) receptor 7	Mm00434133_m1
Ier5	Immediate early response 5	Mm01295615_s1
Itih3	Inter-alpha trypsin inhibitor, heavy chain 3	Mm00434548_m1
Kcnj10	Potassium inwardly-rectifying channel, subfamily J, member 10	Mm00445028_m1
Klf9	Kruppel-like factor 9	Mm00495172_m1
Ly75	Lymphocyte antigen 75	Mm00522144_m1
Mafb	V-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)	Mm00627481_s1
Mamdc2	MAM domain containing 2	Mm00805078_m1
Nefh	Neurofilament, heavy polypeptide	Mm01191456_m1
Nefl	Neurofilament, light polypeptide	Mm01315666_m1
Nefm	Neurofilament, medium polypeptide	Mm00456201_m1
Nr3c1	Nuclear receptor subfamily 3, group C, member 1	Mm00433832_m1
Nrgn	Neurogranin	Mm00480741_m1
Nrtn	Neurturin	Mm03024002_m1
Nt5e	5' nucleotidase, ecto	Mm00501910_m1
Paqr6	Progestin and adipoQ receptor family member VI	Mm01223417_m1
Pdp1	Pyruvate dehydrogenase phosphatase catalytic subunit 1	Mm01217532_m1
Pla2g5	Phospholipase A2, group V	Mm00448162_m1
Ppia	Peptidylprolyl isomerase A (cyclophilin A)	Mm02342429_g1
Pvalb	Parvalbumin	Mm00443100_m1
Rassf9	Ras association (RalGDS/AF-6) domain family (N-terminal) member 9	Mm00455442_m1
Rgs4	Regulator of G-protein signaling 4	Mm00501389_m1
Samd14	Sterile alpha motif domain containing 14	Mm00461337_m1
Sema3c	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	Mm00443121_m1
Sema7a	Sema domain, immunoglobulin domain (Ig), and GPI membrane anchor, (semaphorin) 7A	Mm00441361_m1
Shh	Sonic hedgehog	Mm00436528_m1
Vdr	Vitamin D receptor	Mm00437297_m1

40

6. Statistical analyses

Differences between means were obtained by one way ANOVA, two way ANOVA or two-tailed, unpaired Student's *t*-test depending on the experiment, and the Tukey or Bonferroni's post hoc tests, respectively. The statistical analysis used in each experiment is indicated in the figure legend. Calculations were done using the Graph-Pad Prism software (http://www.graphpad.com/prism/). The experimental groups were formed with about the same number of male and female pups, and sex of the animals was not considered a factor in statistical analyses.

7. RNA-Seq Illumina sequencing

RNA-Seq was performed at the Genomics Unit in the Centro Nacional de Investigaciones Cardiovasculares.

Total RNA was quantified and purity checked using a NanoDrop ND-1000 (Thermo Scientific, Waltham, MA, USA). RNA integrity was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). 500 ng of total RNA were used with the TruSeq RNA Sample Preparation v2 Kit (Illumina, San Diego, CA) to construct index-tagged cDNA libraries. Libraries were quantified using a Quant-iT™ dsDNA HS assay with the Q-bit fluorometer (Life Technologies, Carlsbad, California). Average library size and the size distribution were determined using a DNA 1000 assay in an Agilent 2100 Bioanalyzer. Libraries were normalized to 10nM using Tris-Cl 10mM, pH 8.5 with 0.1% Tween 20. Libraries were applied to an Illumina flow cell for cluster generation (True Seq SR Cluster Kit V2 cBot) and sequence-by-synthesis single reads of 75 base length using the TruSeq SBS Kit v5 (Illumina) were generated on the Genome Analyzer IIx following the standard RNA sequencing protocol. Reads were further processed using the CASAVA package (Illumina) to split reads according to adapter indexes and produce fastq files.

8. Sequence bioinformatics and differential expression analysis

Bioinformatic and differential expression analysis (Figure 7) was performed by Francisco Garcia-Garcia from Dr. Joaquín Dopazo group (Centro de Investigación Principe Felipe in Valencia).

Read quality was determined by analyzing reads with the application FastQC [98]. We used the mouse-sequenced genome: GRCm38.

The fasta file containing sequences of this genome was downloaded from Ensembl (http://www.ensembl.org/Mus_musculus/Info/Index). This genome was indexed from Bowtie [99] and sequence reads were aligned using TopHat [100]. Mapping data quality was evaluated from Qualimap [101]. We quantified reads to specific genes and transcripts using the Python module HT-SEQ [102]. We explored gene expression data by Principal Component Analysis and Clustering methods. Also we performed exploratory plots to evaluate saturation, count distribution, and type of detected features using the Bioconductor package NOISeq [103]. RNA-Seq data were normalized using Trimmed Mean of M values [104]. Length of genes and transcripts was estimated from only coding regions. For this, length of each exon was determined from Ensembl and length of each transcript was calculated by adding length of its exons. Finally, gene length was obtained as the median value of its transcripts. The expression level was estimated considering the gene length.

The paired design was analyzed from the Bioconductor package edgeR [105], fitting a Negative Binomial Generalized Linear Model where design matrix included two factors: group (treated and non treated) and pair. This test detects genes that are differentially expressed in response to T3 treatment compared to the control, adjusting for baseline differences between animals. Conventional multiple testing p-value correction procedure proposed by Benjamini-Hockberg was used to derive adjusted p-values [106].

Enrichment analysis was carried out for the Gene Ontology (GO) terms using the Bioconductor package GOSeq [107]. We corrected for multiple testing by Benjamini-Hochberg procedure. Significant GO terms were represented from CellMaps (http://cellmaps.babelomics.org/).
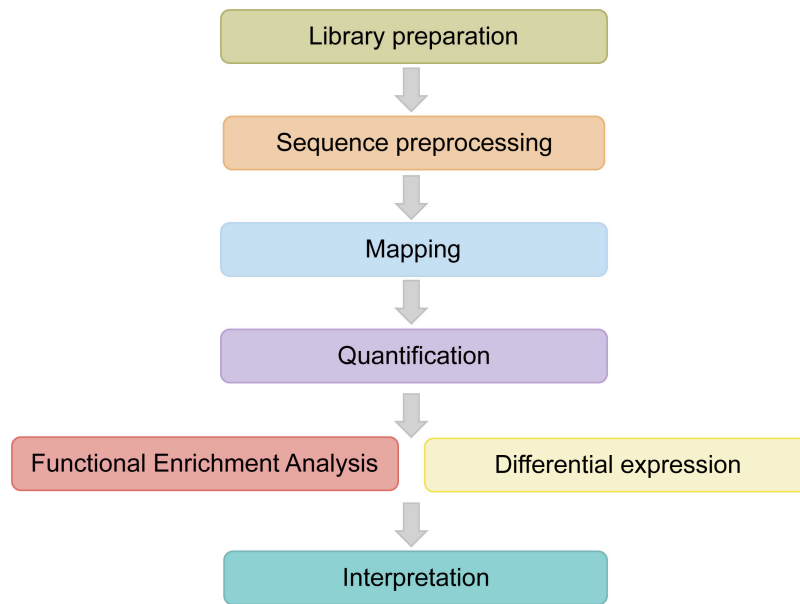
Figure 7. RNA-Seq data analysis pipeline.

RESULTS

1. Role of thyroid hormone receptor subtypes α1 and β on gene expression in the cerebral cortex and striatum of postnatal mice [108]

The goal of this work was to analyze the relative roles of TRα1 and TRβ on the expression of TH dependent genes in the cerebral cortex and the striatum of P21 mice. We analyzed 27 genes regulated positively and 14 genes regulated negatively by TH. These genes were identified in previous microarray analysis of the hypothyroid cerebral cortex [90]. The criterion to define these genes as positively or negatively regulated by TH was that hypothyroidism induced a decreased or increased expression respectively. The expression of each gene was measured by qPCR in RNA samples of individual mice from four genotypes: Wt, *TRα1-/-, TRβ-/-*, and *TRα1-/-TRβ-/-*. As a reference for the TH dependence of each gene we also included a group of Wt mice rendered hypothyroid from prenatal stages. The effects of hypothyroidism on the cortex expression of most genes were as previously described [94].

1.1 Effects of hypothyroidism or TR inactivation

Figures 8-11 show the relative changes of gene expression in reference to the expression in the Wt, given a value of 1.0 and represented as a dotted line in the figures. The genes were ordered in the figures in relation to the value obtained for the hypothyroid Wt mice. For the positive genes (figure 8 for the cortex, and figure9 for the striatum) the strongest effect of hypothyroidism was on *Agxt2l1* in the cortex and *Cd72* in the striatum with more than 90% reduction. There was no quantitative correlation between the effects of hypothyroidism in the cortex and striatum (r = 0.13, P = 0.52). As extreme examples, *Cd72* and *Vdr* were among the strongest affected genes in the striatum whereas in the cortex they were little or not affected. Neither the lack of TRα1 nor the lack of TRβ induced consistent changes, although the mean expression was below the Wt value for the *TRα1-/-* and above the Wt for the *TRβ-/-*. The absence of αβ had in general a strongest effect than the single inactivation and in the same direction as hypothyroidism. The effect of αβ deficiency on some genes approached that of hypothyroidism. This was the case for *Hr*, *Pvalb*, *Kcnj10*, *Nefm*, *Nefh*, or *Sema7a* in the cortex and for *Cd72*, *Vdr*, *Pvalb*, *Aldh1a1*, *Nefm*, *Hr*, or *Nefh* in the striatum. Other genes remained at or near Wt levels (*Flywch2, Ier5, Itih3, Nrtn* and *Paqr6* in the cortex, and *Agxt2l1*, *Cbr2*, *Flywch2*, *Ier5*, *Itih3*, *Klf9*, and *Pdp1* in the striatum). The results partially

agree with a predominant role of TRα1 in TH-mediated brain gene expression. The absence of TRβ was associated with normal or increased gene expression, probably due to the increased TH levels in these mice [72]. In the absence of TRα1 however, TRβ was able to sustain gene expression to near Wt euthyroid levels.
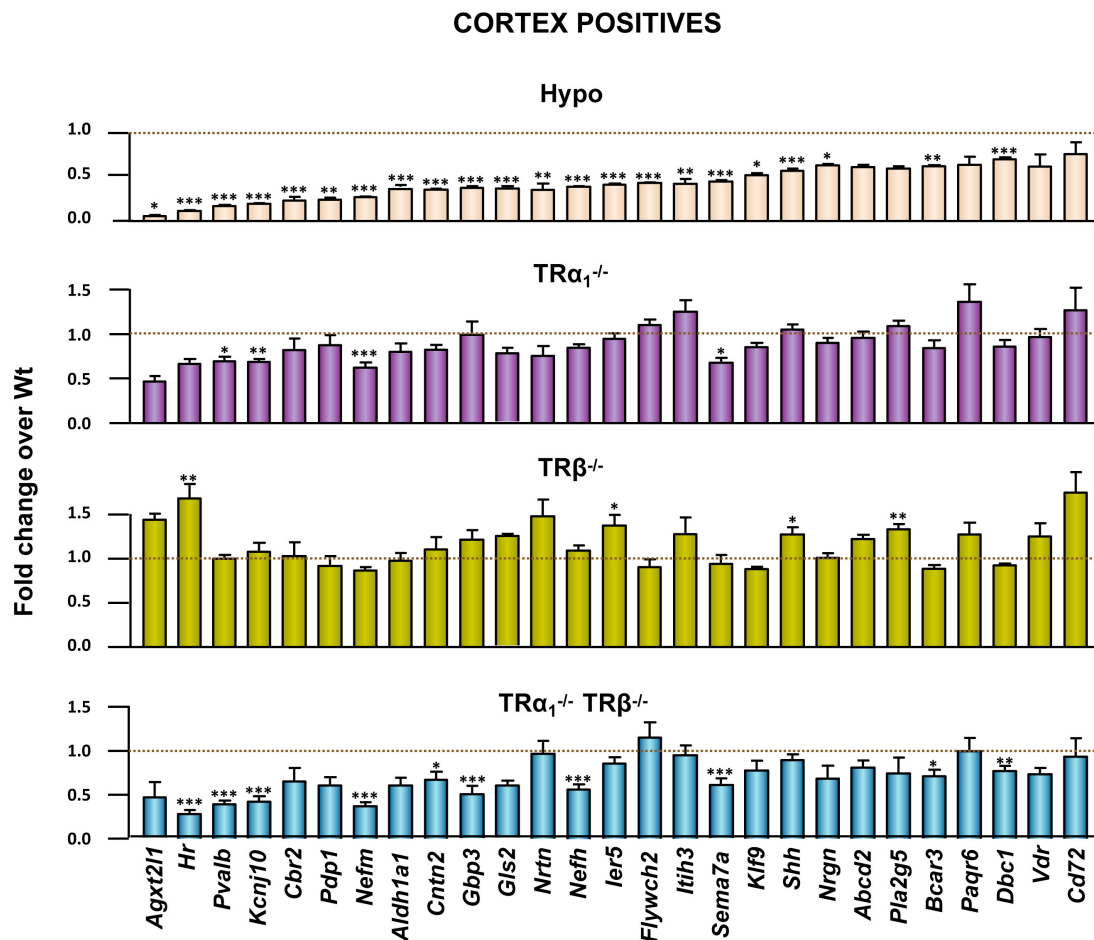
Figure 8. Expression of positive genes in the cerebral cortex of hypothyroid, *TRα1⁻/⁻, TRβ⁻/⁻* and *TRα1⁻/⁻TRβ⁻/⁻* mice using microfluidic cards (TaqMan arrays). The figure shows the comparisons of the Wt mice (n=7) which were given a value of 1.0, represented by the dotted line with the Wt hypothyroid mice (n = 6), *TRα1⁻/⁻* mice (n = 6), *TRβ⁻/⁻* mice (n = 6) and *TRα1⁻/⁻TRβ⁻/⁻* mice (n = 5). Statistical comparisons were made by one-way ANOVA followed by the Tukey test, between Wt, Hypo, and αβ and between Wt, α, and β; * = P<0.05; **=P<0.01; ***=P<0.001.

Figure 9. Expression of positive genes in the striatum of hypothyroid, *TRα1⁻/⁻*, *TRβ⁻/⁻* and *TRα1⁻/⁻TRβ⁻/⁻* mice using microfluidic cards (TaqMan arrays). The figure shows the comparisons of the Wt mice (n=7) which were given a value of 1.0, represented by the dotted line with the Wt hypothyroid mice (n = 6), *TRα1⁻/⁻* mice (n = 6), *TRβ⁻/⁻* mice (n = 6) and *TRα1⁻/⁻TRβ⁻/⁻* mice (n = 5). Statistical comparisons were made by one-way ANOVA followed by the Tukey test, between Wt, Hypo, and αβ and between Wt, α, and β; * = P<0.05; **=P<0.01; ***=P<0.001.

For the negative genes (figure 10 for the cortex, and figure 11 for the striatum) the effects of hypothyroidism showed a positive correlation between the cortex and the striatum (*r* = 0.76, *P* = 0.0014). Compared to the positive genes, a strongest effect of the absence of TRα1 was observed as a whole. Hypothyroidism increased the expression of 13 genes in the cortex from 1.5 to 4-fold, and 14 genes in the striatum from 1.3 to 5.5-fold. The absence of TRα1 increased the expression of several genes in the cortex and in the striatum at least 1.5-fold. In contrast, the absence of TRβ induced minimal changes. The absence of αβ was similar to the absence of TRα1, with 9 genes increasing at least 2-fold in the cortex and 6 genes increasing at least 2-fold in the striatum. We

may conclude that also for the negative genes TRα1 was more relevant for gene expression than TRβ. As for the positive genes, the effects of hypothyroidism were in general stronger than the absence of TRs.
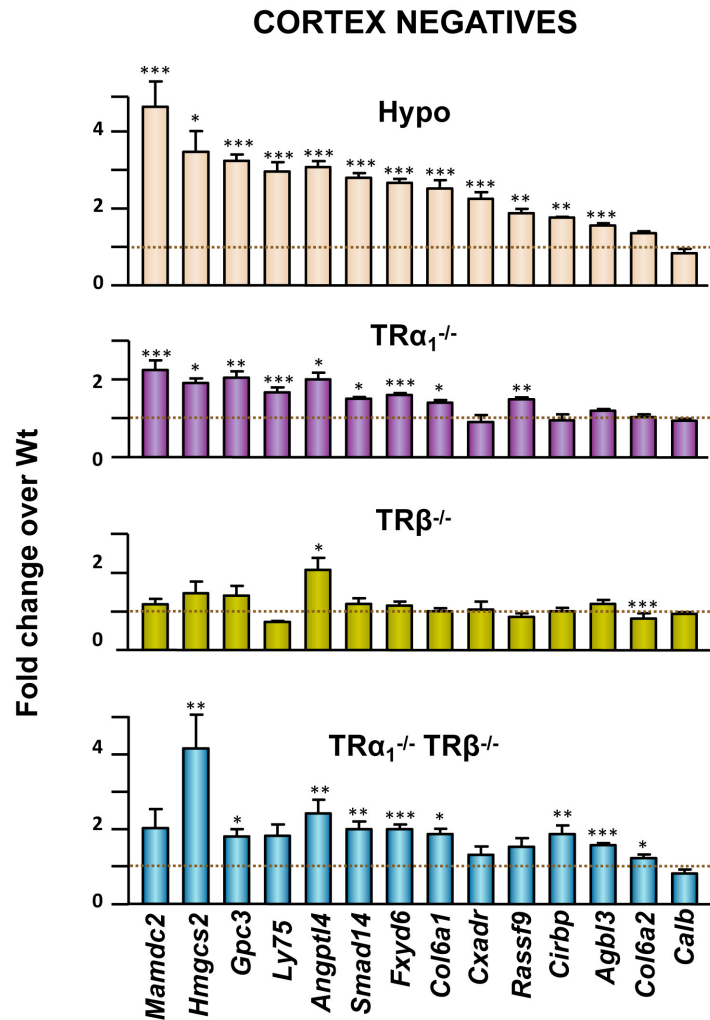
Figure 10. Expression of negative genes in the cerebral cortex of hypothyroid, *TRα1-/-*, *TRβ-/-* and *TRα1-/-TRβ-/-* mice using microfluidic cards (TaqMan arrays). The figure shows the comparisons of the Wt mice (n=7) which were given a value of 1.0, represented by the dotted line with the Wt hypothyroid mice (n = 6), *TRα1-/-* mice (n = 6), *TRβ-/-* mice (n = 6) and *TRα1-/- TRβ-/-* mice (n = 5). Statistical comparisons were made by one-way ANOVA followed by the Tukey test, between Wt, Hypo, and αβ and between Wt, α, and β; * = P<0.05; **=P<0.01; ***=P<0.001.

STRIATUM NEGATIVES

Figure 11. **Expression of negative genes in the striatum of hypothyroid, *TRα1⁻ᐟ⁻*, *TRβ⁻ᐟ⁻* and *TRα1⁻ᐟ⁻TRβ⁻ᐟ⁻* mice using microfluidic cards (TaqMan arrays).** The figure shows the comparisons of the Wt mice (n=7) which were given a value of 1.0, represented by the dotted line with the Wt hypothyroid mice (n = 6), *TRα1⁻ᐟ⁻* mice (n = 6), *TRβ⁻ᐟ⁻* mice (n = 6) and *TRα1⁻ᐟ⁻TRβ⁻ᐟ⁻* mice (n = 5). Statistical comparisons were made by one-way ANOVA followed by the Tukey test, between Wt, Hypo, and αβ and between Wt, α, and β; * = P<0.05; **=P<0.01; ***=P<0.001.

By comparing the expression in *TRα1⁻ᐟ⁻* and in *TRβ⁻ᐟ⁻* mice relative to the Wt, we conclude that both receptor subtypes are involved in the regulation of gene expression in brain. TRα1 appears to have a primary role, but the lack of this receptor affects only a subset of the genes. Absence of both receptor types increases the number of genes affected, and in many cases the effect approaches quantitatively the effect attained by hypothyroidism, indicating that in the absence of TRα1, TRβ maintains gene expression near normal levels. On the other hand, the absence of TRβ results in little changes, with increased expression of a few positive genes, and decreased expression of a few

negative genes. Figure 12 shows the expression patterns in cortex and striatum of four selected genes, *Kcnj10*, *Cbr2*, *Cirbp*, and *Angptl4*. The four genes selected are examples illustrating the observed patterns of regulation (see also figure 14). When hypothyroidism was performed in the Wt mice expression of *Kcnj10* and *Cbr2* decreased, whereas expression of *Cirbp* and *Angptl4* increased, classifying these genes among the positive and negative genes respectively. The effect of hypothyroidism may be compared to the effect of the combined absence of TRα1 and TRβ (abbreviated as αβ). In the cortex and the striatum, the absence of αβ increases the expression of the negatively regulated gene *Cirbp* to the same level as in hypothyroidism. For *Kcnj10* the effect of αβ deficiency was in the same direction but not as strong as the effect of hypothyroidism. In contrast to the strong effects of hypothyroidism, αβ deficiency was without effect on *Cbr2* in the cortex and the striatum, and on *Angptl4* in the striatum. The effect of αβ deficiency on cortex *Angptl4* was less clear. Single inactivation of *TRα1* or *TRβ* had variable effects, with a decreased expression of *Kcnj10* and *Cirbp* in the striatum, and increased expression of *Angptl4* in the cortex of TRα1 or TRβ deficient mice.
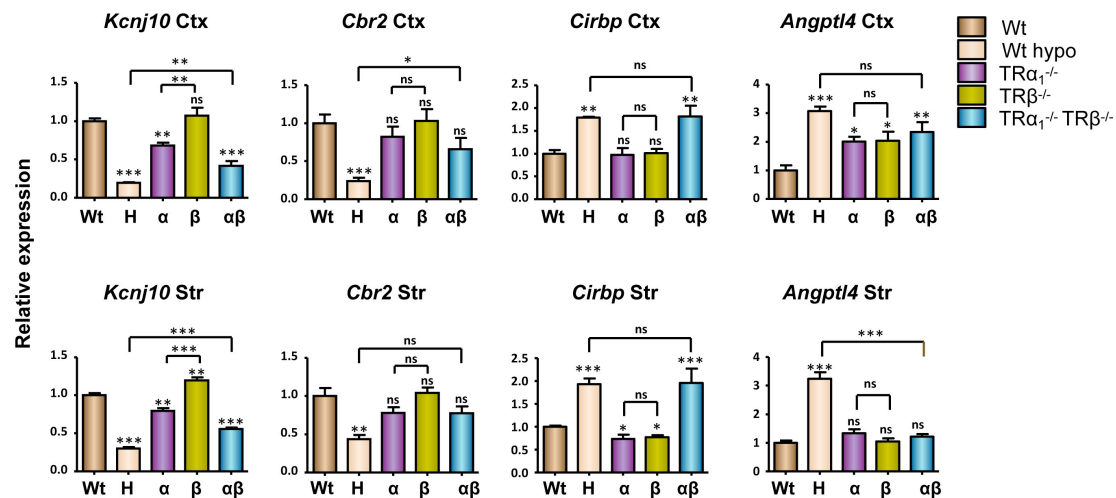
Figure 12. Expression of selected genes in the P21 mouse cerebral cortex and striatum. Effects of hypothyroidism and TR deficiency. Wt: Wild type (n = 7); H: Wt hypothyroid mice (n = 6); α: *TRα1⁻ᐟ⁻* mice (n = 6); β: *TRβ⁻ᐟ⁻* mice (n = 6). αβ: *TRα1⁻ᐟ⁻ TRβ⁻ᐟ⁻* mice (n = 5). Statistical comparisons were made by one-way ANOVA followed by the Tukey test, between Wt, H, and αβ and between Wt, α, and β; ns = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Ctx: Cortex. Str: Striatum.

The effects of hypothyroidism on gene expression could be due to two factors. Firstly, the reduction of T3 signaling directly related to the reduction of TR occupancy and transactivation. In this case, the absence of receptors should be similar to the effects of TH deprivation. Secondly, the unliganded TRs might have intrinsic activity and directly inhibit or stimulate the expression of positive or negative genes respectively.
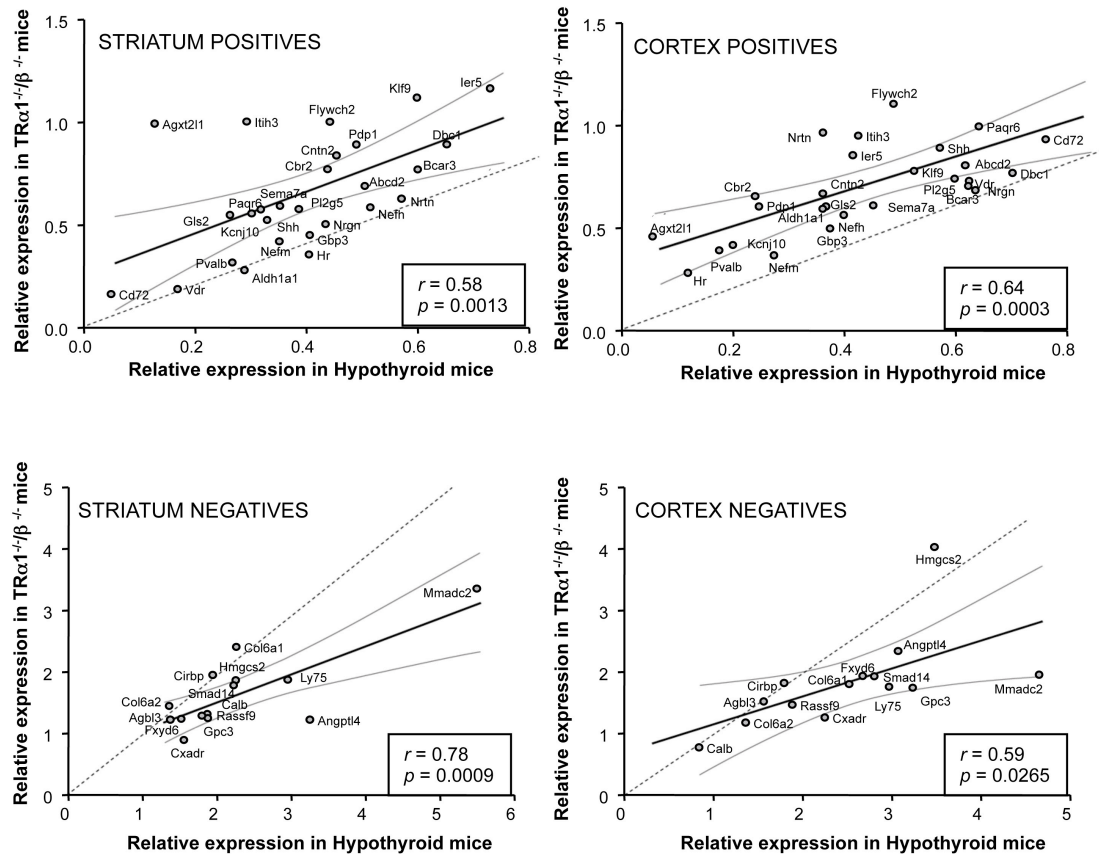
Figure 13. Correlations of gene expression between hypothyroid Wt mice and TRα1^{-/-}TRβ^{-/-} mice. The data correspond to expression of positive and negative genes in the cerebral cortex and the striatum. Shown are the best fit for linear regression and the 95% confidence limits. The dotted line represents the equation y = *x*.

The correlations between the effects of the lack of TRs and hypothyroidism for the positive and negative genes are shown in figure 13. In all cases the correlations were significant, but the slopes of the regression lines were lower than 1, indicating that the effect of hypothyroidism was stronger than the effect of TR deficiency. From the values of the *y* intercepts it may be calculated that the effect of TR deprivation, i.e., the loss of T3 signaling accounted for about 70-80% of the effect of hypothyroidism on the positive genes, and 60% for the negative genes, on average.

1.2 Influence of TRα1 inactivation on the effect of hypothyroidism

To confirm that the effects of hypothyroidism on some genes were due at least in part by the activity of unliganded receptors, especially TRα1, we analyzed the effect of hypothyroidism on gene expression in TRα1-deficient mice. Figure 14 shows the response of the genes described in figure 12 (*Kcnj10*, *Cbr2*, *Cirbp* and *Angptl4*). In Wt hypothyroid mice the expression of *Kcnj10* and *Cbr2* decreased, and the expression of *Cirbp* and *Angptl4* increased. On the other hand, whereas hypothyroidism had a similar effect on the expression of *Kcnj10* and *Cirbp* in the presence or absence of TRα1, it was without effect on *Cbr2* and *Angptl4* in the absence of TRα1 indicating that the effects of hypothyroidism on these two genes was due to the repressing (*Cbr2*) or inducing (*Angptl4*) activity of the apoTRα1.
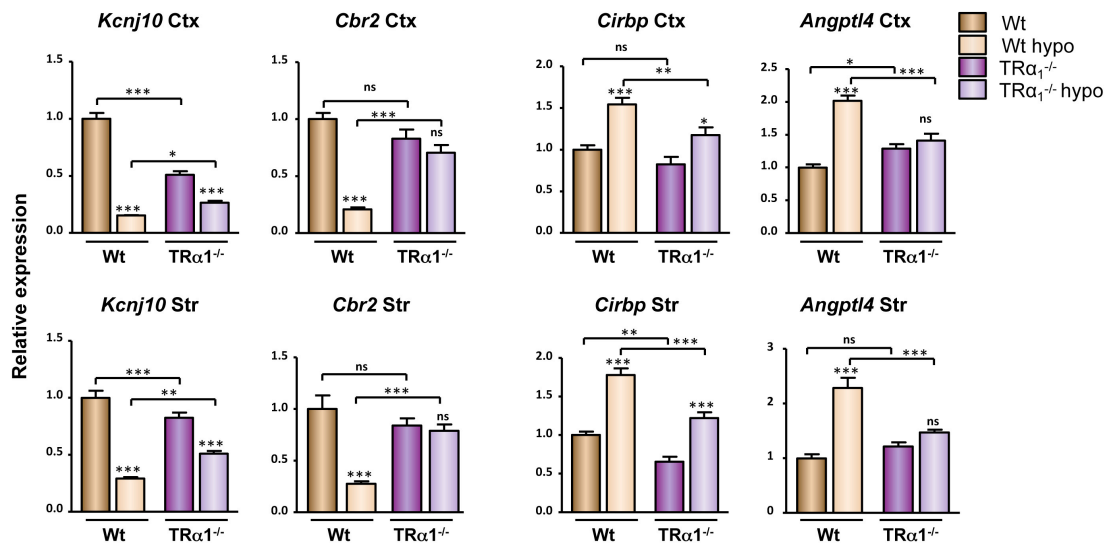
Figure 14. Effects of hypothyroidism in the presence and absence of TRα1. This figure shows the data from four different genes, two of them positively regulated (*Kcnj10* and *Cbr2*), and two of them negatively regulated (*Cirbp* and *Angptl4*) in the cerebral cortex and the striatum of Wt and TRα1$^{-/-}$ mice in the basal state and after induction of hypothyroidism (n = 8 for each experimental condition). Statistical comparisons were by two way ANOVA; ns = P>0.05; * = P<0.05; **=P<0.01; ***=P<0.001. Significance symbols above the bars represent the comparison between hypothyroidism (lighter bars) with the respective untreated Wt or *TRα1$^{-/-}$* mice (darker bars). Ctx: Cortex. Str: Striatum.

The fact that on some genes hypothyroidism had no effect on the *TRα1⁻/⁻* mice agrees with the hypothesis that the effects of hypothyroidism on the expression of some genes is due to the activity of the apoTR, consisting of downregulation of positive genes, and upregulation of negative genes.

2. Thyroid hormone regulation of gene expression in primary cerebrocortical cells: role of thyroid hormone receptor subtypes [109]

It is likely that TH action in the brain is modulated by many interacting physiological factors acting on micro domains. The bulk effect observed *in vivo* in individual brain regions would be an aggregate result of the interaction of T3 with additional factors that may be modulated differently in each micro domain, depending on the cellular composition, expression of TR, transporters, deiodinases, and the crosstalk with other signaling pathways. For that reason studies *in vivo* may not represent a direct cellular action of TH. To better understand specificity of TRs in the action of TH at the cellular level on neural gene expression we have analyzed the expression of six TH dependent genes in primary cultures of mouse cerebrocortical cells. Specifically we studied the effects of TRs knock down *in vivo* on the effects of T3 to better define the relative roles of TRα1 and TRβ.

2.1 Characterization of the primary cerebrocortical cell culture

Primary cerebrocortical cells were isolated from the E17.5 mouse cerebral cortex using standard procedures. The cellular composition of the cultures was analyzed by immunofluorescence. The results are presented in Table 1. These cultures are enriched in neurons (NeuN) and there were no differences in the percentage of cells stained as neurons in the three cultures. The percentage of astrocytes (Gfap) was slightly higher in the *TRα1⁻/⁻* and the *TRβ⁻/⁻* than in the Wt, although only statistically significant when comparing Wt and *TRα1⁻/⁻*. As in other studies [110] less than 10 percent of the cells was not stained as astrocytes or neurons (figure 15).
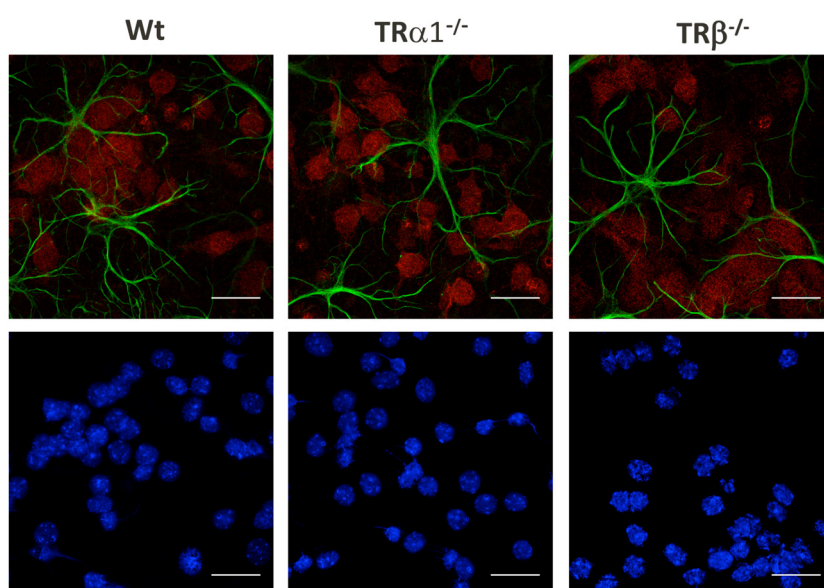
Figure 15. **Confocal images of the cerebrocortical cultures from Wt, *TRα1*-/-, and *TRβ*-/- mice.** Upper panels: Cells stained with antibodies against GFAP (green) for astrocytes and NeuN (red) for neurons. Lower panels: Nuclei stained with DAPI. Scale bar = 25 μm.

	% Neurons	95% CI	**% Astrocytes**	95% CI
Wt	74.3 ± 4.9	69.2 - 79.4	15.6 ± 2.2	14.5 – 33.2
***TRα1*-/-**	76.2 ± 3.6	72.4 – 80.0	19.3 ± 2.6 (*)	16.5 - 22.0
***TRβ*-/-**	78.3 ± 4.3	73.8 – 82.9	18.8 ± 2.2	16.4 - 21.1

Table 1. **Cellular composition of the primary cultures from Wt, *TRα1*-/-, and *TRβ*-/- mice.** Shown are the percentage of neurons and astrocytes relative to the total number of DAPI-stained nuclei. Data are mean ± SD (n = 6), and 95% Confidence Interval (CI). (*): $P<0.05$ compared to Wt. Other comparisons were not significant.

In the Wt cell culture, the relative proportion of *TRα1* and *TRβ* expression was similar as measured by SYBR Green qPCR. The quantitative assessment by RNA-Seq (see later) gave similar results.

2.2 TRα1 and TRβ in the regulation of gene expression at the cellular level

The goal of these experiments was to analyze the role of TRα1 and TRβ in the effect of T3 on gene expression in cultured primary cells. As T3 targets we selected some genes studied in the previous *in vivo* study, and that likely play a prominent role in mediating the effects of TH on neural development, as follows: 1) the transcription factor and TR co-repressor *Hr* (Hairless), which was originally shown to be under transcriptional regulation by T3 in the cerebellum [111]. *Hr* has since then been a widely studied T3 target to analyze the effects of TH on the brain. 2) The developmental morphogen *Shh* (Sonic hedgehog), which is regulated by TH in the rat and mouse embryonic and adult brain, presumably at the transcriptional level [112]. 3) The transcription factor *Klf9* (Krüpfel-like transcription factor 9, also known as Basic Transcription Element Binding Protein or BTEB), which is regulated by T3 at the transcriptional level during tadpole metamorphosis, oligodendrocyte differentiation, and in the rodent brain [113]. 4) *Dio3*, which is regulated specifically by TRα1 [15]. 5) *Aldh1a1* (Aldehyde dehydrogenase 1a1), a gene sensitive to hypothyroidism produced by blocking TH formation [88]. 6) *Aldh1a3*, a gene negatively regulated by T3 [114], which participates in the same retinoic acid (RA) synthesis pathway as *Aldh1a1* [115]. To search for clues that may explain response to T3 at the cellular level we analyzed the TR subtypes specificity.

The relative role of TR subtypes in the gene responses to T3 is illustrated in figure 16. For this experiment we isolated E17.5 cerebrocortical cells from Wt, *TRα1$^{-/-}$*, and *TRβ$^{-/-}$* mice. Gene expression was measured in the absence of T3, and 24 h after addition of 1 nM T3 to the cultures. In previous experiments we found that increasing the T3 concentration up to 100 nM did not increase the response above that attained with 1 nM T3. As an experimental control of the effect of receptor inactivation we measured the expression of *Dio3*, which is regulated by T3 in a TRα1 isoform-specific way [15]. *Dio3* expression in cells lacking TRα1 or TRβ was similar to Wt cells in the absence of T3. Addition of T3 increased *Dio3* expression by 4-fold in the Wt cells, and had no effect on the TRα1-deficient cells. TRβ deficiency potentiated the effect of T3, with an 8-fold induction, to twice the level obtained in the Wt cells. From the rest of the genes studied, only *Aldh1a1* was unresponsive in cells deficient of TRα1. T3 increased *Aldh1a1* expression in the Wt cells, but had no effect on the TRα1-deficient cells. In the TRβ-deficient cells the basal expression of *Aldh1a1* in the absence of T3 was increased with respect to the untreated Wt cells, and was further increased by T3.

Hr, *Klf9*, and *Shh* showed no TR isoform specificity, and were induced by T3 in the three culture types. *Hr* and *Shh* were induced by 5-fold and 6-fold, respectively in Wt cells and

about 2-fold in the TRα1-deficient cells. Similarly *Klf9* increased 2-fold after T3 addition to the Wt cells and 1.3-fold in the TRα1-deficient cells. In the TRβ-deficient cells T3 induced a similar effect as in the Wt cells on *Shh*, but the effects on *Hr* and Klf9 were increased.
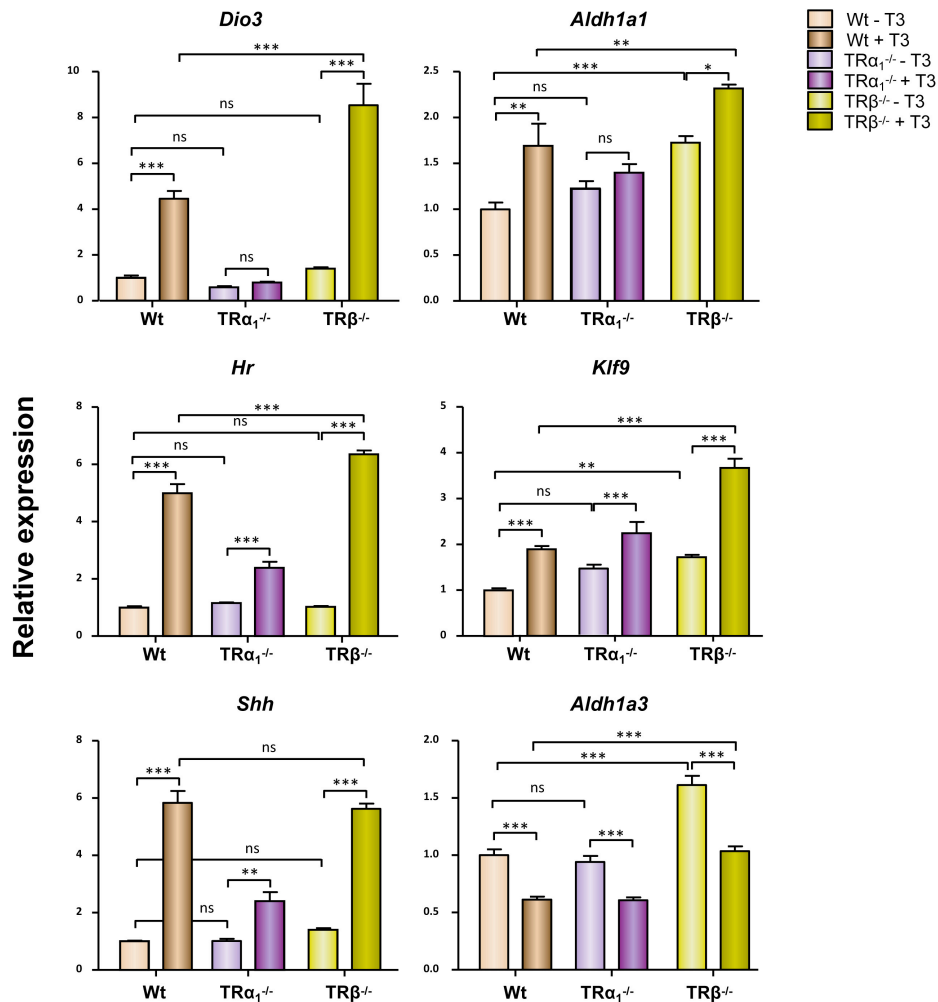
Figure 16. Effect of T3 on gene expression in primary mouse cerebrocortical cell cultures in the presence or absence of TRα1 or TRβ. Cells (n=6) from Wt, *TRα1-/-*, or *TRβ-/-* mice were incubated for 24 hours in the absence (lighter bars) or in the presence of 1 nM T3 (darker bars). Statistical analysis was by two-way ANOVA; ns = P>0.05; * = P<0.05; **=P<0.01; ***=P<0.001.

The Aldh1a1 enzyme is involved in RA synthesis, catalyzing conversion of all-trans-retinal to all-trans-retinoic acid. Another aldehyde dehydrogenase Aldh1a3 is also involved in this pathway. In contrast to *Aldh1a1*, *Aldh1a3* is negatively regulated by T3 (figure 16), with a 40% reduction after T3 addition to the Wt cells. TRα1-deficient cells behave exactly as the Wt cells. In the absence of TRβ the basal expression increased by about 60% and T3 induced a similar effect as in the Wt cells.

58

2.3 Effect of T3 in the presence or absence of cycloheximide

In the previous experiment we validated the regulation of the selected genes by TH as well as the contribution of the TR subtypes at the cellular level. To check whether the effects of T3 were due to a direct action on gene transcription, or mediated through increased expression of T3-dependent auxiliary proteins or transcription factors, we analyzed the effect of T3 in a shorter time, in the presence of the protein synthesis inhibitor CHX (Figure 17). After 6 hours in the presence of T3 an increased expression of *Hr*, *Klf9*, and *Shh* was already observed. The effect on any of these genes was not blocked by CHX pretreatment, indicating a direct effect of T3 on these genes. In contrast T3 had no significant effects on *Dio3*, *Aldh1a1* and *Aldh1a3* at 6 hours. Therefore no conclusions about the mechanism of induction could be made for *Dio3* and *Aldh1a1*. CHX had a strong stabilizing effect on *Aldh1a3* mRNA, and in the cells pretreated with CHX T3 was able to reduce *Aldh1a3* expression, suggesting that also for this gene the effect was direct.

It is also relevant to point out that, together with these experiments, we also studied interactions of T3 with RA and glucocorticoids in the regulation of gene expression. T3 had opposing influences on RA synthesizing enzymes, increasing the expression of *Aldh1a1*, and decreasing *Aldh1a3*, while increasing the RA degrading enzyme *Cyp26b1*. Dexamethasone increased *Klf9* and *Aldh1a1* expression. The effects of T3 and dexamethasone on *Aldh1a1* were highly synergistic, with mRNA increments of up to 20 fold. The results provide new data on the importance of TH interactions with RA and glucocorticoids during neural development. These results are not part of the Thesis because of space requirements but they are included in the accompanying paper [114] (Annex V).
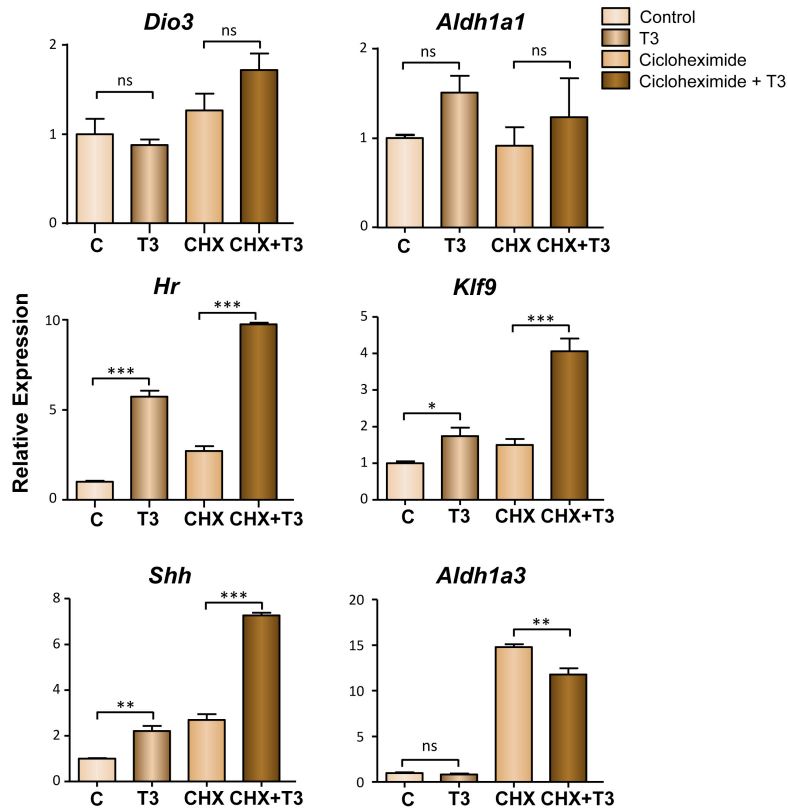
Figure 17. **Effect of T3 in the presence or absence of cycloheximide (CHX) on gene expression in primary mouse cerebrocortical cell cultures from wild type mice.** Cells (n=4) were incubated for 30 min with or without CHX (8 μg/ml) before adding T3 (10 nM), and then incubated for 6 hours before the RNA extraction. Statistical analysis was by one-way ANOVA; ns = P>0.05; * = P<0.05; **=P<0.01; ***=P<0.001.

3. Insight into the role of thyroid hormone on brain development through global transcriptome analysis in cerebrocortical cells *[116]*

The purpose of this study was to obtain a global view of TH action on the mammalian brain and to get further insight on the genes and pathways regulated by T3 using a simple system of cultured primary cerebrocortical cells. To identify TH-dependent genes we performed transcriptomic assays using RNA-Seq technology.

3.1 Characterization of the primary cerebrocortical cell culture

As explained in the results section 2.1, cells were isolated from the E17.5 mouse cerebral cortex. The culture system contained around 80% neurons and 20% astrocytes (Figure 18A). Transcriptome analysis by RNA-Seq was performed using cells incubated in the absence or presence of T3. The cultures contained a high degree of cellular phenotype complexity, and it was possible to identify minority cell populations expressing some of the markers used to define cellular phenotypes *in vivo*. As an example, figure 18B and 18C show the result of staining with an antibody against calbindin. Calbindin is expressed *in vivo* by a subpopulation of gamma amino butyric acid (GABAergic) interneurons. In the primary cultures calbindin was present occasionally in individual cells representing approximately 10% of the neurons present in the culture. These data indicate that individual cellular phenotypes were maintained in the cultures. Further support was obtained from RNA-Seq data described below showing expression of markers of different cerebral cortex layers and neuron populations.
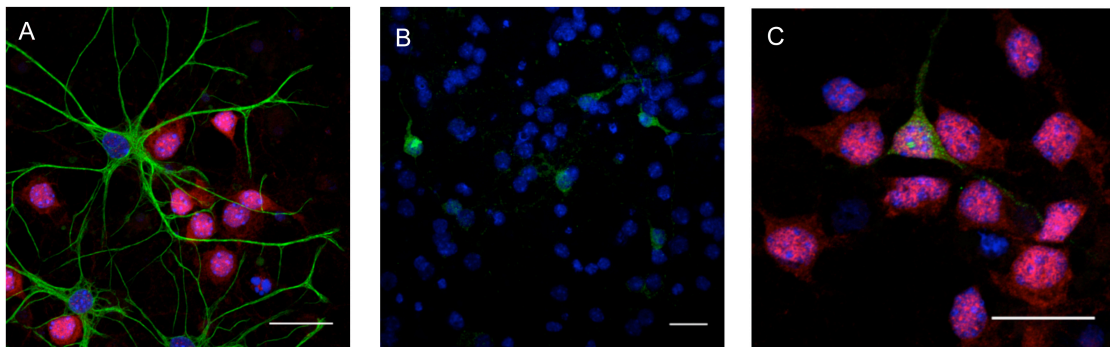
Figure 18. Primary cerebrocortical cells maintain individual cellular phenotypes and T3 responsiveness. Confocal images of the primary cerebrocortical cell culture. Nuclei stained with DAPI. Scale bar = 25 μm. **A:** Cells stained with antibodies against GFAP (green) for astrocytes and NeuN (red) for neurons. **B:** Cells stained with antibody against calbindin (green). **C:** Cells stained with antibodies against calbindin (green) and NeuN (red).

3.2 Transcriptome analysis of the effect of T3

Transcriptome analysis leaded us to a total of 14,801 expressed genes in the primary cerebrocortical cell culture. From this set we identified the genes encoding all the proteins involved in TH action in the brain. The transporters *Mct8*, *Lat1*, *Oatp1c1* and *Lat2* are expressed in the cells, *Mct8* accounting for 81% of total transporters. As for the receptors, *TRα1* and *TRβ1* were present in similar amounts. The non-T3 binding splicing product of the *Thra* gene, *TRα2* was present with an expression level 25 fold over that of *TRα1* or *TRβ1*. Finally *Dio2* and *Dio3* were also expressed as well as TRs corregulators *Ncor* and *SRC1*.

Differential gene expression between cells incubated in the absence or presence of T3 was obtained by RNA-Seq and analyzed using a negative binomial test. A total of 1,145 differentially expressed genes with p<0.05 after correction for false discovery rate (FDR) were obtained (Figure 19A). From these 619 were more highly expressed in the T3-treated cells, and 526 more in the untreated cells. We refer to these genes as positive and negative genes, respectively (Annex I). Table 2 represents the set of differentially expressed genes with a log fold change over 0.7 (positive genes) or below -0.7 (negative genes).

Validation of gene expression changes induced by T3 treatment was performed by qPCR in biological replicates using RNA from independent cultures. We focused on the set of genes, with available Taqman probes, with the highest fold changes and relative abundance, as well as some of the previously identified genes sensitive to TH *in vivo*. As reference RNAs for the qPCR we used *18S rRNA* and *Ppia* with essentially the same results [109]. Figure 19C shows the relative expression of 24 positive genes and 6 negative genes in the T3-treated cells compared to the untreated cells. There was a good correlation between the results obtained by RNA-Seq and qPCR (*Pearson r* = 0.908, *P<0.0001*) (Figure 19B). This results show that the RNA-Seq data have a predictive validity and a high level of confidence.
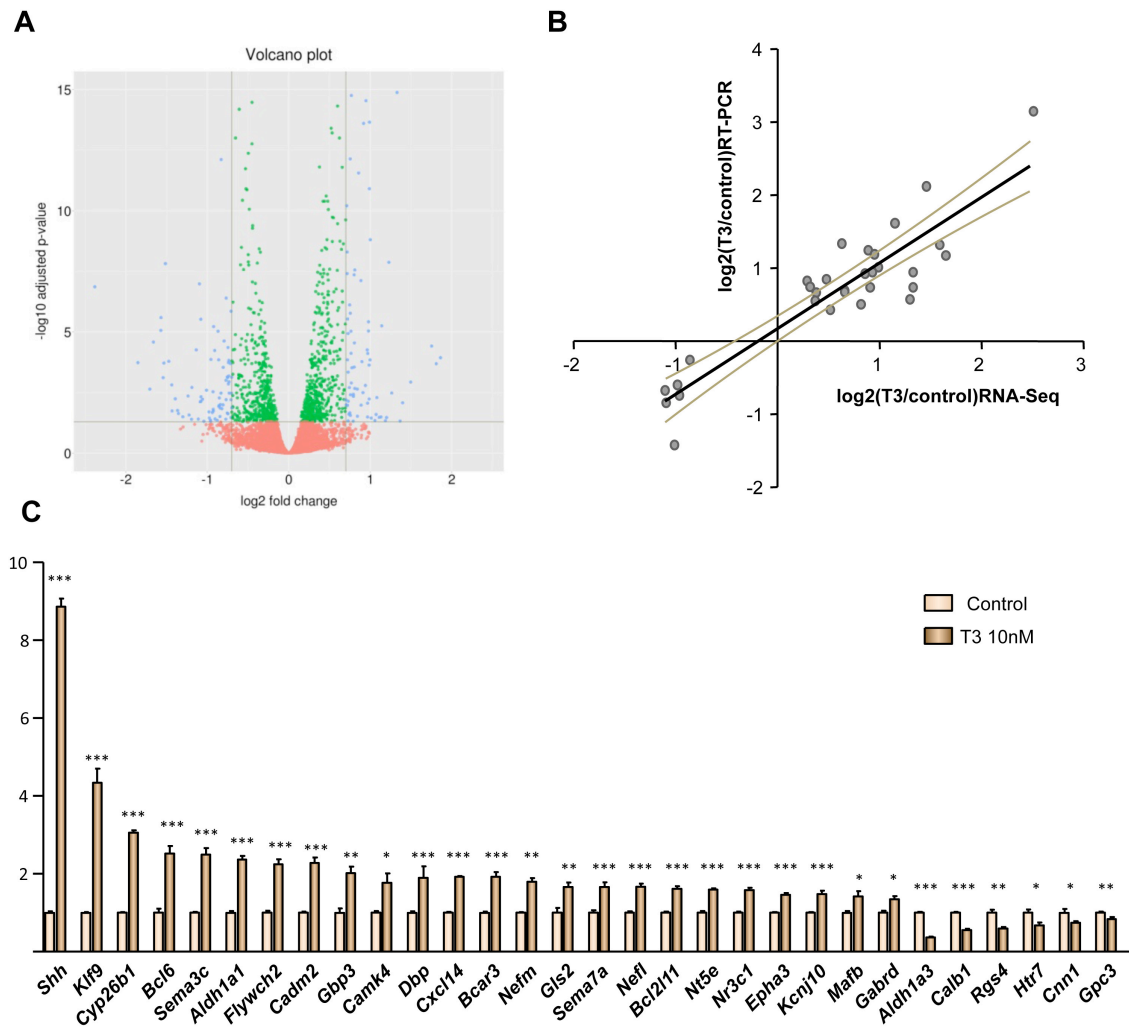
Figure 19. A: Volcano plot. Cells treated with T3 *versus* control. Scattered points represent genes. The x-axis is the log2 fold change for the ratio +T3/-T3. The y-axis is -log10 adjusted p-value and shows significant differential gene expression. Orange dots represent not significant genes, green dots represent significant genes and blue dots represent significant genes with a log2 fold change ± 0.7. **B: Biological and technical correlation of the data.** Log2 of fold expression (T3/control) using the RNA-Seq data compared to qPCR data in an independent experiment for 30 genes. In this representation *18S* was used for normalization. Shown are the best fit for linear regression and the 95% confidence limits. Pearson r = 0.908, P<0.0001. **C: qPCR validation of RNA-Seq results in a biological replicate.** Expression of selected genes by qPCR of primary cerebrocortical cells with and witout T3 (10 nM). Results are expressed as mean ± SEM relative to the control (lighter bars) value set as 1.0 (n = 5). Significance of differences was calculated by the Student's *t* –test; * = P<0.05; **=P<0.01; ***=P<0.001.

Positive genes

Gene Name	logFC	E. level	Gene Name	logFC	E. level
Cyp11a1	3.33	0.08	Sema7a	0.91	18.84
Hr	3.09	0.72	Prss35	0.91	2.21
Shh	2.51	0.47	Reep4	0.91	0.53
Dio3	2.16	0.47	Chrm3	0.91	5.61
AC165246.1	2.06	0.72	Glycam1	0.90	2.40
Sptssb	1.86	0.12	Aldh1a1	0.89	0.37
Gjc3	1.81	0.06	Adra1b	0.89	0.95
Stat5a	1.76	0.08	Cntn3	0.88	1.63
Flywch2	1.65	4.23	Nrarp	0.87	3.04
Sema3c	1.59	14.44	Dbp	0.86	5.99
Hcrtr1	1.56	0.77	Gm16421	0.84	0.43
Gpr30	1.50	0.20	Syt10	0.84	0.61
Klf9	1.46	5.10	Mafb	0.82	2.92
Il1r2	1.40	0.19	Ucp2	0.81	9.43
Gli1	1.37	0.05	Olfm4	0.81	0.76
Ret	1.35	0.57	Col19a1	0.81	0.52
Bcar3	1.33	1.12	Frzb	0.80	0.90
Gls2	1.33	2.52	Acot11	0.80	7.07
Rasd2	1.30	2.23	Npnt	0.80	5.85
Kcnj10	1.30	8.11	Tmem100	0.79	0.67
Gpr17	1.28	0.92	5033430I15Rik	0.79	1.66
Sept4	1.28	3.59	Cldn12	0.79	8.69
Gpr133	1.27	0.10	Slc5a5	0.79	0.23
Lgi3	1.23	0.41	Sema6c	0.79	25.63
Mc5r	1.20	0.23	Igf1	0.79	14.36
Car7	1.19	0.16	Trpv6	0.77	0.63
Cyp26b1	1.15	1.07	Has3	0.77	3.02
Dock5	1.14	0.10	Rasl10a	0.77	1.76
4930522L14Rik	1.13	0.24	Gpr37l1	0.77	1.07
Gm23935	1.11	759.65	Pllp	0.76	1.54
Stac2	1.09	4.17	Pcolce2	0.76	0.53
Acox2	1.08	0.74	AL672276.1	0.76	1.49
Col9a2	1.07	0.51	Dmrtb1	0.76	2.74
Frat1	1.06	0.58	Suv420h2	0.76	13.59
Ccl17	1.01	0.78	Ifih1	0.75	0.34
Asap3	1.00	0.64	Slc30a3	0.75	1.87
Gpr83	1.00	0.31	Slc13a5	0.74	2.58
Daam2	0.99	1.37	Itih3	0.74	0.47
Gbp3	0.99	1.94	Entpd2	0.73	0.77
Mr1	0.99	0.27	Gm6395	0.73	0.55
Slc22a3	0.99	0.30	Sned1	0.73	1.71
Rdh5	0.98	2.47	Rab3il1	0.73	2.30
Fam20a	0.98	0.89	Plekho2	0.72	1.27
Cntnap1	0.97	0.40	Slc24a6	0.72	0.82
Aldh1a7	0.97	0.31	Mpp7	0.72	0.51
Tmem238	0.97	0.35	Cdc42ep1	0.72	2.21
Them6	0.96	0.87	Itga7	0.72	1.58
Gdf10	0.95	3.06	Tmem132e	0.71	2.31
Cadm2	0.95	84.82	Bdh2	0.71	1.30
Lims2	0.94	0.53	Tesc	0.70	1.70
Bmp3	0.94	4.36	Mmp17	0.70	18.68
Cxcl14	0.93	12.19	Pnoc	0.70	4.89
Mfsd2a	0.92	4.74			

Negative genes

Gene Name	logFC	E. level	Gene Name	logFC	E. level
Popdc2	-3.51	2.65	Bub1	-0.92	5.53
Clca1	-2.38	0.24	Slfn9	-0.91	0.71
Obscn	-1.85	0.21	Spink10	-0.90	1.63
4930444P10Rik	-1.71	0.58	1110002E22Rik	-0.88	0.26
Ppp1r3b	-1.66	0.29	Nuf2	-0.87	1.60
Dsg2	-1.57	0.38	Kera	-0.86	1.43
Col14a1	-1.57	0.21	Gpc3	-0.86	4.88
Nrk	-1.54	0.28	Kcnh4	-0.86	0.37
Dscc1	-1.53	0.62	Arhgef15	-0.85	0.95
Rbpms	-1.52	1.43	Dnase1l2	-0.85	0.97
Pgm5	-1.47	1.13	Il33	-0.85	1.91
Adamts19	-1.44	0.17	Pdlim1	-0.84	1.04
1700010I14Rik	-1.43	0.30	Pgam2	-0.83	5.32
Arhgap6	-1.39	0.17	Pcdh18	-0.83	2.08
Slc38a4	-1.37	0.89	Rgs5	-0.82	0.60
Gzmk	-1.35	0.52	Klhl14	-0.81	0.41
Gm13131	-1.30	1.66	Mki67	-0.81	2.17
Cmya5	-1.24	0.08	2810417H13Rik	-0.79	2.12
Hck	-1.22	0.29	Crhr2	-0.77	0.87
Edn1	-1.19	0.24	Htr2a	-0.77	0.89
Tpm2	-1.19	1.17	Gm11837	-0.77	2.63
Myo18b	-1.14	0.50	Ndst3	-0.77	3.28
2410018L13Rik	-1.11	0.68	Mapk15	-0.76	1.02
Htr7	-1.10	1.02	Casc5	-0.75	0.53
Calb1	-1.09	41.12	Mc4r	-0.75	1.06
Dll4	-1.09	0.45	Rbl1	-0.75	1.88
Ncapg	-1.08	0.95	Anxa11	-0.75	0.63
Gja4	-1.08	0.65	Cdk1	-0.74	9.23
Gna14	-1.08	0.31	Drd2	-0.74	1.75
Tfpi	-1.06	0.55	Espl1	-0.74	0.50
Pygm	-1.04	1.72	Wdr76	-0.74	1.36
Aldh1a3	-1.01	28.36	Kif20a	-0.73	1.07
Spa17	-1.01	2.01	Msrb3	-0.73	5.04
Rgs4	-0.98	53.48	Cntnap5c	-0.72	0.97
Mfap4	-0.98	0.84	Esco2	-0.71	2.90
Tmc6	-0.98	0.97	C630043F03Rik	-0.71	2.47
Hes7	-0.96	0.45	Tec	-0.71	0.61
Cmbl	-0.96	1.16	Stbd1	-0.70	1.31
Cnn1	-0.96	1.41	Cep55	-0.70	0.88
A730046J19Rik	-0.94	0.23	Pygl	-0.70	3.75
Hif3a	-0.93	2.31	Sgol2	-0.70	0.38
Cdh18	-0.93	0.61	Ccna2	-0.70	6.35
A730049H05Rik	-0.93	0.52			

Table2. Positive and negative TH target genes. List of differentially expressed genes with p<0.05 after correction for false discovery rate (FDR). Genes are ordered by log fold change, over 0.7 (positive genes) or below -0.7 (negative genes). Expression levels (E.Level) of the genes are also represented.

3.3 Neuron *versus* astrocyte TH target genes

Cahoy and coworkers provided a detailed global characterization and comparison of the genes expressed by acutely isolated neurons and astrocytes of postnatal mice [6]. Given the cellular composition of our culture, containing up to 20% astrocytes, we compared our set of T3 differentially expressed gene with the transcriptome database of genes enriched in astrocytes and neurons population. Taking a value of 5-fold enrichment as the lower limit to consider a gene as being astrocyte or neuron-enriched, 137 T3 induced genes were enriched in neurons and 77 in astrocytes. On the other hand 84 T3 repressed genes were enriched in neurons and 10 in astrocytes. Even astrocyte or neuron-specific genes, i.e., those having more than 40-fold enrichment in either cell type were also differentially expressed by T3 (Table 3).

To assess whether astrocytes are also cellular targets of T3, we established pure astrocyte cultures and measured the response of *Aldh1a1* to T3. *Aldh1a1* is enriched 11-fold in astrocytes, and responded to T3 with a 3-fold increase in expression (Figure 20).
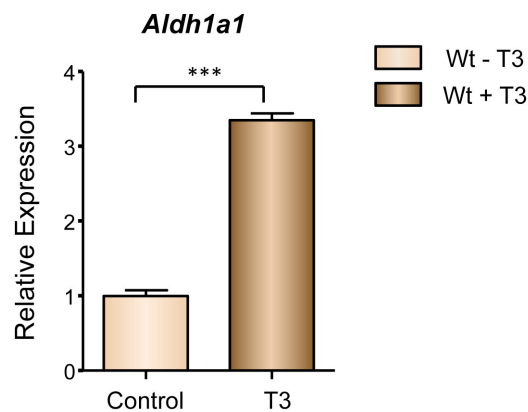
Figure 20. Effect of T3 in the regulation of *Aldh1a1* expression in primary astrocyte cell culture. qPCR of the effect of T3 treatment (1 nM) on *Aldh1a1* expression in the primary cultures of P3 cortex astrocytes (n=3). Significance of differences was calculated by the Student's *t* –test; ***=P<0.001.

A. TH Positive genes

Neuron enriched genes			
Gene Name	Enrichment	logFC	E.Level
Sla	62.7	0.49	25.26
Sstr2	32	0.48	7.83
Scg2	30.3	0.48	44.16
Camk4	27.6	0.29	20.98
Slc6a7	27	0.39	3.5
Nefm	26.5	0.48	9.67
Nefl	26.5	0.32	18.5
Cdh8	26.2	0.26	19.19
Trhde	25.5	0.37	2.08
Lpl	24.4	0.36	39.9
Clstn2	23.4	0.23	21.08
Sema3c	17.7	1.59	14.44
Gfra2	15.7	0.31	3.28
Cx3cl1	15.6	0.34	221.55
Epha3	15.1	0.37	12.23
Rasl10a	14.5	0.77	1.76
Spock1	13.9	0.33	27.63
Lin7b	13.4	0.38	18.19
Reln	12.7	0.23	65.83
Hs3st5	10.9	0.66	0.6
Ache	10.9	0.21	78.98
Kcnip4	10.8	0.54	3.28
Col19a1	10.5	0.81	0.52
Slco5a1	10.5	0.36	5.02
Calb2	10.4	0.44	30.47
Ccbe1	10.4	0.25	10.03
Tmsb10	10	0.21	69.1
Arg2	9.9	0.52	5.17
Cntn4	9.4	0.39	4.28
Chga	9.3	0.25	42.67
Cadps	8.9	0.38	35.72
Gpr83	8.7	1	0.31
Cyp11a1	8.6	3.33	0.08
Plcxd2	8	0.47	12.27
Cacna1a	7.8	0.37	54.75
Camk2a	7.6	0.19	197.64
Tm6sf1	7.4	0.68	0.77
Nxph3	7.4	0.58	1.7
Prss12	7.2	0.43	12.61
Atp2b2	7.1	0.29	28.26
Npnt	6.6	0.8	5.85
Gabrd	6.6	0.52	1.54
Nefh	6.5	0.42	1.11
Cabp1	6.2	0.38	8.02
Mmp17	6.1	0.7	18.68
Tesc	6	0.7	1.7
Slc30a3	5.9	0.75	1.87
Cyp26b1	5.8	1.15	1.07
Npy	5.8	0.33	29.45
Igf1	5.7	0.79	14.36
Epha4	5.6	0.56	25.89
Dbc1	5.6	0.38	22.61
Rph3a	5.5	0.19	21.33
Arhgap20	5.3	0.2	35.67

Astrocite enriched genes			
Gene Name	Enrichment	logFC	E.Level
Gfap	84.9	0.29	72.12
Aqp4	79.8	0.27	10.78
Pla2g7	65.1	0.38	114.92
Aldh1l1	54	0.3	81.88
Acsbg1	50	0.66	77.6
Slc4a4	46.9	0.43	22.16
Slc1a2	46.7	0.43	496.6
F3	40.6	0.43	6.97
Hapln1	32.7	0.38	4.44
Rfx4	32.7	0.28	6.86
Acot11	30.9	0.8	7.07
Atp1a2	29.8	0.48	49.31
Tmem47	29.3	0.29	38.32
Ntsr2	28.1	0.45	2.12
Entpd2	25.7	0.73	0.77
Tlcd1	24	0.37	8.48
Pbxip1	23.4	0.3	69.98
Htra1	19.3	0.42	8.77
Hsd11b1	18.4	0.43	3.27
Lrig1	15.7	0.28	47.74
Gstm1	14.7	0.24	108.97
Abcd2	14.5	0.67	1.44
Sdc4	14.3	0.26	12.92
Nrarp	13	0.87	3.04
E130114P18Rik	12	0.44	3.18
Slc13a5	11.9	0.74	2.58
P4ha3	11.8	0.45	2.07
Aldh1a1	11.7	0.89	0.37
Acsl6	11.6	0.22	22.81
Fbxo2	10.2	0.35	13.22
Cxcl14	9.9	0.93	12.19
Slc38a3	9.8	0.58	17.9
Atp1b2	9.2	0.35	233.95
Sfxn5	9.1	0.27	74.16
Gpc6	8.9	0.33	3.71
Itga7	8.7	0.72	1.58
Kcnk1	8.7	0.29	8.16
Mfap3l	8.6	0.41	10.59
Tst	8.3	0.55	5.5
Frmpd1	8.3	0.52	0.72
Rdh5	7.9	0.98	2.47
Prdx6	7.8	0.23	162.83
Eya4	7.6	0.39	1.4
Ednrb	7.6	0.25	17.61
Celsr1	7.4	0.39	0.91
Angptl4	7.2	0.54	5.33
Gas1	7.2	0.41	5.53
Sat1	7.1	0.33	12.12
Mt3	6.8	0.26	100.65
Paqr7	6.7	0.34	17.95
Rftn2	6.4	0.31	5.9
Megf10	6.3	0.36	2.38
Glycam1	6.1	0.9	2.4
Ppap2b	6.1	0.48	22.55
Slc41a1	6.1	0.23	116.85
Dkk3	6	0.32	23.76
Dtna	5.9	0.42	18.91
Daam2	5.8	0.99	1.37
Gpam	5.8	0.3	13.77
Myo10	5.8	0.25	40.29
Bcar3	5.6	1.33	1.12
Gdf10	5.2	0.95	3.06
Tor3a	5.2	0.43	5.46
Ppargc1a	5.2	0.24	25.3
Peli2	5.1	0.22	4
Cst3	5	0.22	359.74

B . Negative genes

Neuron enriched genes								Astrocite enriched genes			
Gene Name	Enrichment	logFC	E. Level	Gene Name	Enrichment	logFC	E.Level	Gene Name	Enrichment	logFC	E.Level
Glra2	79.4	-0.36	14.54	Nxph2	10.2	-0.36	13.73	Dio2	56	-0.5	2.2
Nov	65.5	-0.36	22.53	Reps2	9.8	-0.21	14.94	Thrsp	19.1	-0.4	9.34
9130024F11Rik	54.9	-0.52	8.08	Pde1a	9.5	-0.34	114.82	Il33	13.4	-0.9	1.91
Gabra5	47.4	-0.32	50.89	Rab9b	9.1	-0.2	15.75	Grm3	11.2	-0.3	6.12
Satb2	41.5	-0.25	38.27	Zcchc12	9	-0.6	56.55	Elovl2	11.1	-0.3	17.98
Gda	37.4	-0.41	84.65	Ehbp1l1	9	-0.34	4.76	Gabrg1	9.4	-0.2	6.66
Slc17a6	36.7	-0.29	10.64	Ssbp2	8.6	-0.33	110.95	Igfbp5	7.3	-0.3	37.65
Calb1	35.2	-1.09	41.12	Rit2	8.5	-0.23	19.91	Pygm	7.2	-1	1.72
Pcsk2	30.3	-0.29	27.2	Nol4	8.4	-0.22	45.79	Nuf2	7	-0.9	1.6
Vsnl1	29.8	-0.14	109.61	Adcyap1	8.3	-0.41	3.74	Pygl	5.2	-0.7	3.75
Syt4	29.2	-0.29	99.81	Socs2	8.1	-0.45	36.56				
Cck	28	-0.29	88.78	Lrfn5	8	-0.26	27.41				
Myo5b	26.8	-0.25	63.55	Car10	7.8	-0.66	2.03				
Nell1	26.6	-0.22	24.64	A030009H04Rik	7.7	-0.25	47.92				
Sv2b	26.1	-0.34	39.54	Cacna1e	7.7	-0.25	8.57				
Rgs4	25.2	-0.98	53.48	Dcn	7.5	-0.5	9.38				
Kcnc2	24.1	-0.33	3.67	Efnb2	7.5	-0.39	58.03				
Cxadr	23	-0.17	124.91	Arhgap6	7.4	-1.39	0.17				
Grp	21.6	-0.41	30.57	Slitrk4	7.4	-0.36	12.88				
Hs6st2	21.6	-0.25	23.43	B3galt2	7.4	-0.33	7.85				
Sst	20.2	-0.32	224.43	Gria1	7.4	-0.19	73.68				
3110047P20Rik	19.9	-0.43	50.19	Myo1b	7.1	-0.34	42.81				
Trhr	18.8	-0.63	1.11	Npy2r	7	-0.61	0.8				
Ablim3	16.7	-0.21	17.87	AF529169	7	-0.43	1.89				
Tac2	16.6	-0.54	4.31	Sms	6.9	-0.23	50.78				
Cntnap4	16.3	-0.3	11.05	Smarca1	6.5	-0.26	30.84				
Sema3a	15.9	-0.42	6.56	Gpr85	6.4	-0.37	53.96				
Rspo2	15.7	-0.53	13.38	Slc35f4	6.3	-0.38	3.24				
AW551984	15.6	-0.61	61.07	Hap1	6.2	-0.39	46.47				
Hpcal4	15.4	-0.19	329.97	Kitl	5.8	-0.46	48.89				
Ntf3	14.5	-0.54	10.83	Dpysl3	5.7	-0.15	1956.1				
Olfm3	14.4	-0.45	1.54	Cobl	5.6	-0.23	13.19				
Unc5d	14.1	-0.48	9.51	Gucy1b3	5.6	-0.23	48.18				
Rasgrf1	13.9	-0.33	14.39	Cnr1	5.6	-0.23	46.25				
Diras2	13.7	-0.45	31.99	Fat4	5.5	-0.29	9.81				
St8sia4	13	-0.65	11.91	Ntsr1	5.5	-0.21	18.81				
Cxcl12	12.1	-0.66	3.11	Tmem35	5.4	-0.3	81.6				
D130043K22Rik	12	-0.25	9.96	AI504432	5.4	-0.25	11.63				
Nsg2	11.3	-0.17	493.73	Dnm3	5.4	-0.18	156.44				
Scn3a	11	-0.22	16.68	Fosl2	5.3	-0.51	19.73				
Neto1	10.4	-0.27	18.21	March1	5.3	-0.35	28.95				
Npr3	10.2	-0.47	3.59	Tmem74	5.2	-0.29	13				

Table 3. **T3 effect on astrocyte and neuron. A:** T3 positive genes enriched above 5 fold in astrocytes or neurons. **B:** T3 negative genes enriched above 5 fold in astrocytes or neurons. Expression levels (E. Level), and log fold change by T3 of the genes are also represented. Genes are ordered by enrichment in neurons or astrocytes.

3.4 Relative cellular type composition of the primary culture

Doyle and coworkers identified the transcriptome of cerebral cortex cell types isolated using the translating ribosome affinity purification approach, where they reported thousands of cell-specific mRNAs [117]. This study permits the molecular phenotyping of genetically defined cell population. We compared our data set of expressed genes and differentially expressed genes by T3 in the primary cerebrocortical cells with this database of genes expressed in different neural cell populations.

Figure 21A shows genes expressed at least 5-fold in the cortical layers 6, 5b, or 5a. It also shows specific neuronal populations expressing cholecystokinin (CCK), cortistatin, or prepronociceptin (PNOC). Roughly about 50% of genes of enriched expression in these specific sites were expressed in the culture. Furthermore, a fraction of these genes, between 7% in the CCK neurons and 24% in the PNOC neurons were sensitive to T3. As an example, from 114 genes enriched in PNOC neurons 58 were expressed in the primary cultures and 14 were differentially expressed by T3 treatment such as *Cxcl14*. Or from 594 genes expressed in corticothalamic layer 6, 333 were expressed in the primary cultures, and 43 were differentially expressed, such as *Cyp26b1.* Images of Cxcl14 and Cyp26b1 expression from the Allen Brain Atlas are in Figure 21B.

A

5X enrichment cell type	n° genes	E genes 14801	DE genes 1145	% DEgenes /Egenes
Corticothalamic.layer 6	594	333	43	12,91
Corticospinal.layer 5b	1177	662	59	8,91
Corticostriatal.layer 5a	3695	1823	168	9,22
CCK neurons	571	240	17	7,08
CORTISTATIN neurons	482	232	30	12,93
PNOC neurons	114	58	14	24,14

B

Cxcl14
PNOC neurons

Cyp26b1
Corticothalamic layer6
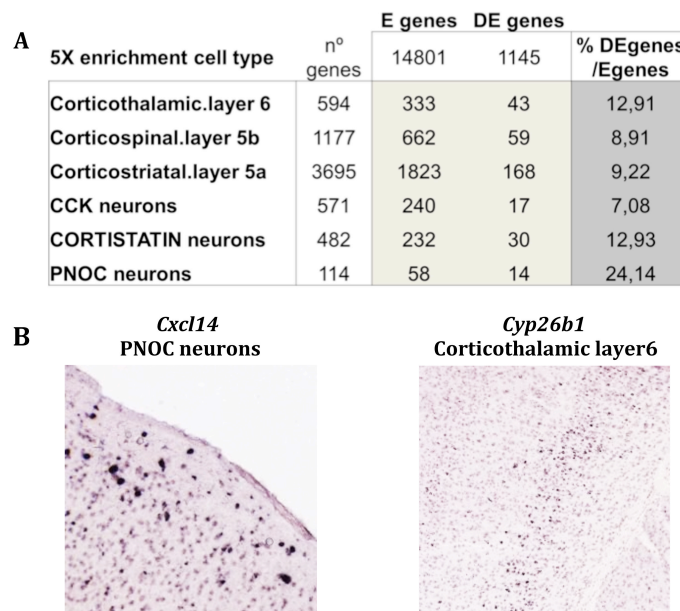
Figure 21. Relative cellular type composition of the primary culture. A: Overlap between our expressed genes (EG) and differentially expressed genes (DE genes) data set with a transcriptome database of acutely isolated cerebral cortex cell types [117]. **B:** Expression in the Allen Brain Atlas (http://mouse.brain-map.org/) of *Cxcl14* and *Cyp26b1* validated T3 DE genes that are enriched in PNOC neurons and corticothalamic layer 6 respectively.

3.5 Gene Ontology analysis

GO enrichment analysis was performed using in one set all differentially expressed genes. We also found convenient to perform GO analysis using the up-regulated and the down-regulated genes in two separate sets. All the significant categories (*P-adjust*<0.05) included in the analysis are specified in Annex II and Annex III. From the set combining the positive and negative genes the functions represented included response to stimulus and signal transduction, especially processes related to G-protein coupled receptor activity, regulation of nervous system development, cell communication and axon guidance. In addition Ca^{2+} signaling pathways are also highly represented.

Some of the enriched GO categories were specifically represented in one of the sets of positively or negatively regulated genes. For better visualization of the data, the most representative categories for Molecular Function, Biological Processes and Cellular Component are summarized in figure 22 for the up-regulated and in figure 23 for the down-regulated genes. The color gradient, from yellow to red, represents the degree of significance of the categories and the circle size represents the level of enrichment of the categories.

T3 specifically upregulates genes involved in transmission of the nerve impulse, processes involving ion transmembrane transport, ephrin receptor activity, cell adhesion and chemotaxis. Among the genes up-regulated by T3 are also present genes involved in myelin assembly and in protein localization at the paranodal region.

The negatively regulated genes by T3 are specifically enriched in cell division, M Phase of cell cycle, chromosome segregation and organization. Regulation of chemokine-mediated signaling pathway is also highly represented.

Genes involved in neurogenesis and neuron differentiation are represented in both set of genes up and down regulated by T3, but astrocyte differentiation is a GO category specifically induced by T3.

Considering the cellular component, T3 induces mainly transmembrane and axonal proteins, neurofilaments, and extracellular proteins, and specifically down regulates nuclear proteins related to the condensed chromosome and the mini chromosome maintenance (MCM) complex, and genes encoding proteins of the motile cilium.

Gene Ontology categories overrepresented in the positive genes
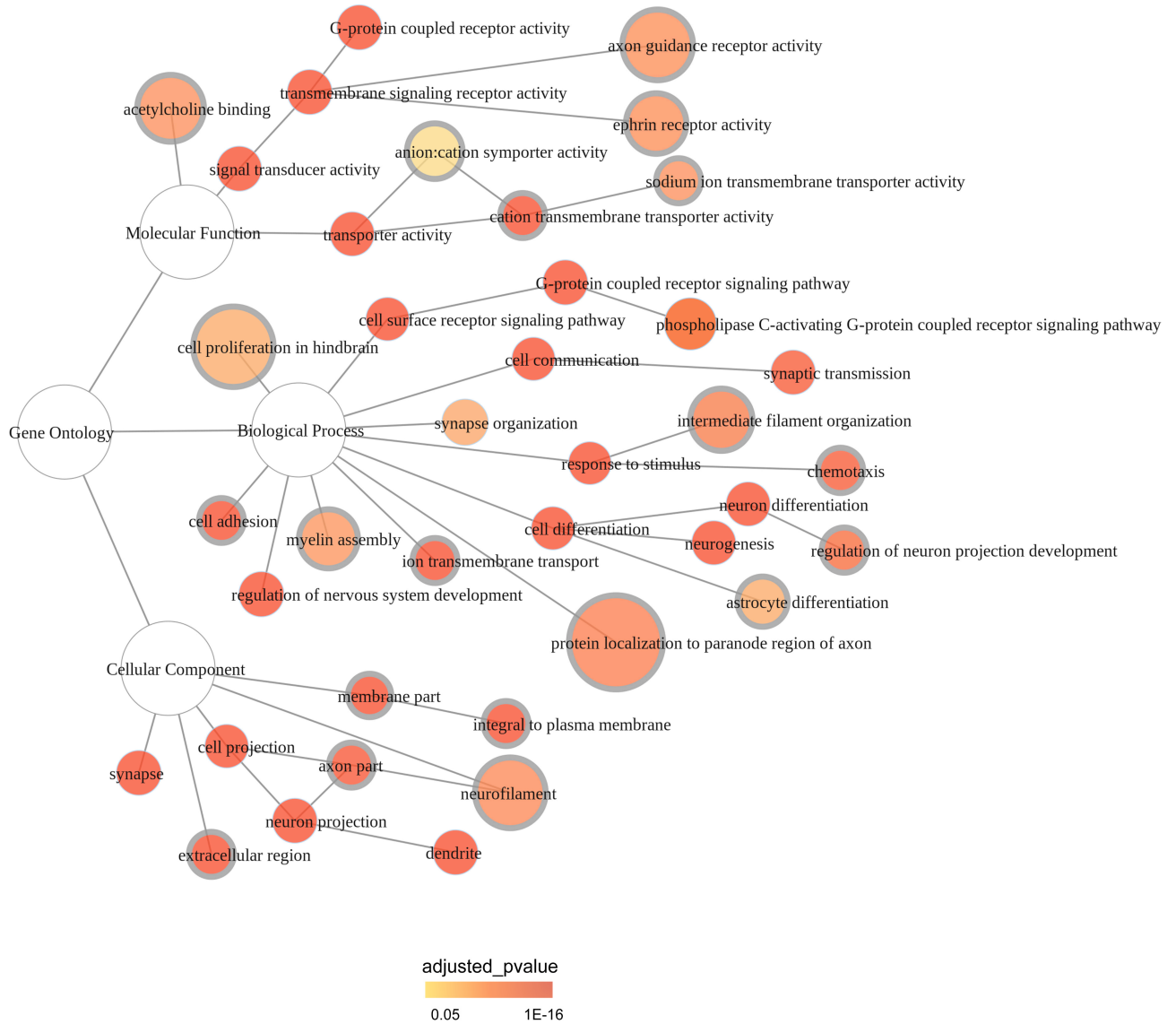
Figure 22. Selected Gene Ontology categories significantly overrepresented in the positive genes (adjusted Pvalue < 0.05) Color bar: significance level for categories by hypergeometric test with Benjamini Hochberg FDR correction. Circle size: enrichment of the category. Thicker border: specific GO categories only present in the positive genes.

Gene Ontology categories overrepresented in the negative genes
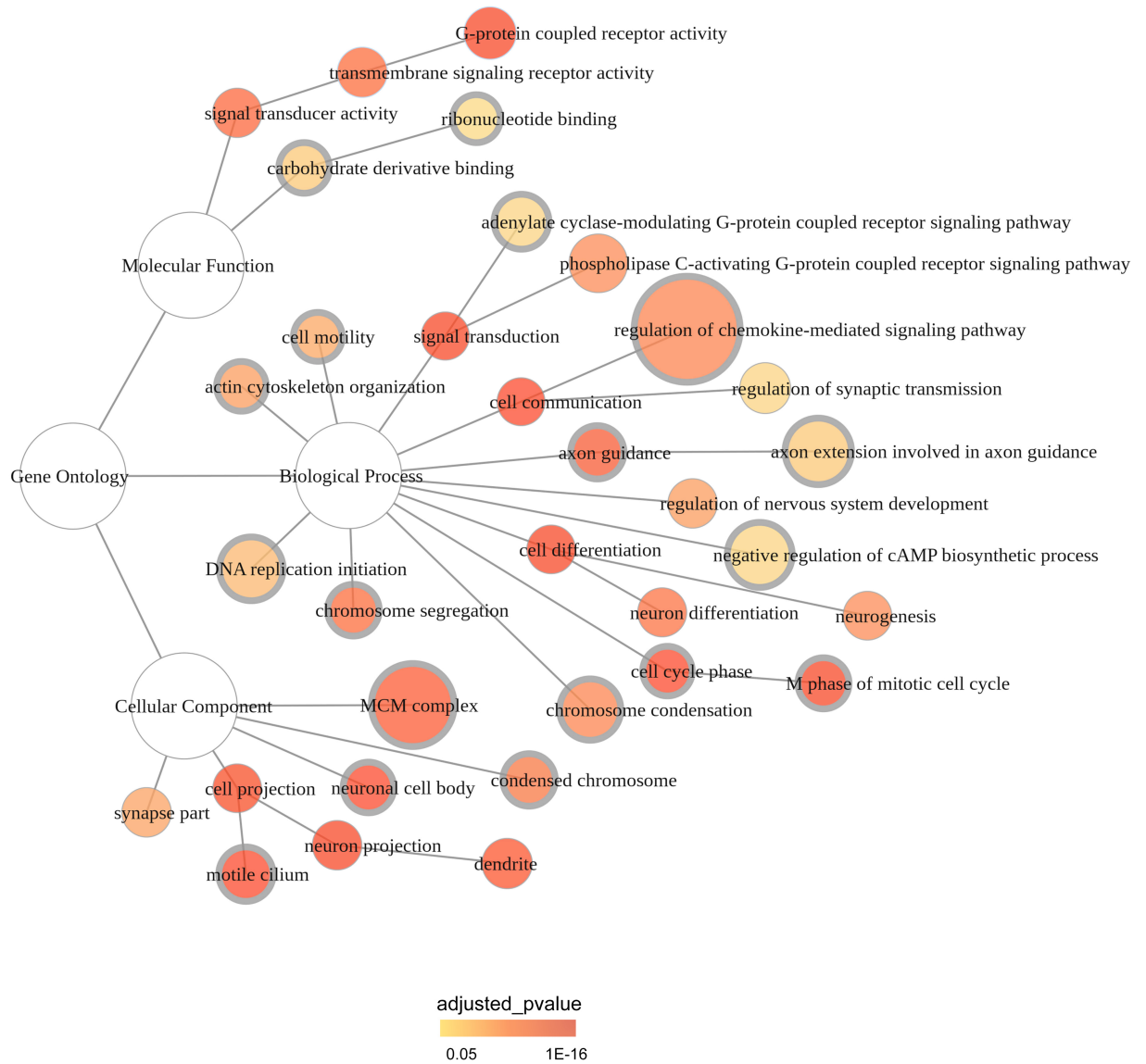
Figure 23. **Selected Gene Ontology categories significantly overrepresented in the negative genes** (adjusted Pvalue < 0.05) Color bar: significance level for categories by hypergeometric test with Benjamini Hochberg FDR correction. Circle size: enrichment of the category. Thicker border: specific GO categories only present in the negative genes.

Results

List of genes involved in some of the GO categories previously highlighted:

Regulation of nervous system development *Cdh4, Shh, D130043K22Rik, Hap1, Cav1, Met, Celsr1, Rarb, Pmp22, Sema6a, Sgk1, Socs2, Igf1, Cobl, Acsl6, Ncoa1, Gfap, Akap5, Rgs6, Fezf2, Il6st, Dpysl2, Nefm, Nefl, Ednrb, Robo1, Ache, Sox8, Adcyap1, Nr3c1, Dpysl3, Htr7, Gli1, Nrp1, Epha4, Dll4, Ephb2, Hdac1, Sema3a, Ret, Sfrp1, Slit2, Mt3, Drd2, Ephb1, Aspm, Cacna1a, Mycn, Sema7a, Atf5,L par1, Camk1d, Prex1, Olig2, Hey1, Reln, Nrep, Cnr1, Vwc2l, Tenm4, Ndnf, Ntf3, Oxtr, Grm5, Lrrc4c, Epha3, Robo2, Arhgef15, Zcchc24, Spock1, Chn1, Ntm, Cend1, Cxcl12, Sox21, Nlgn1, Cntn4, Lgals1, Zhx2*

G-protein coupled receptor signaling pathway *Gabra2, Gabrg1, Crhr2, Grm3, Prkacb, Mc5r, Entpd2, Celsr1, Glra2, Crhr1, Nsg2, Ntsr2, Chga, Rgs6, Edn1, Adcy2, Ednrb, Npr3, Fzd6, Grm8, Adcyap1, Grp, Gna14, Htr7, Tac2, Htr4, Gpr37l1, Rgs7, Rgs5, Ntsr1, Npy2r, Lphn2, Hcrtr1, Cort, Gpr125, Rgs12, Npy, Rgs10, Gpr83, Drd2, Hcrtr2, Cck, Mgll, Rpgrip1l, Adra2a, Npbwr1, Cacna1a, Bai1, Htr2a, Sstr1, Rgs4, Lpar1, Trhr, Gabbr2, Gpr161, Adrbk2, Gpr133, Gpr146, Cnr1, Cxcr7, Pnoc, Adra1a, Gpr158, Chrm3, Mc4r, Sstr2, Gpr85, Prex2, Oxtr, Grm5, Adra1b, Gpr17, Gpr21, Gpr30, Atrnl1, Gabrg3, Gabra5, Rxfp3*

Axon guidance *Cdh4, Shh, Etv1, Sema6a, Unc5b, Evl, Fezf2, Sema5a, Alcam, Robo1, Nrp1, Epha4, Nfasc, Nr4a3, Ephb2, Sema3c, Sema3a, Ptpro, Slit2, Ephb1, Sema6c, Reln, Efna5, Ntf3, Tenm2, Robo2, Gas1, Slit3, Chn1, Unc5c, Cxcl12*

Calcium ion binding *Cdh4, Shh, Calb2, Man1a, Stat5a, Letm1, Sulf2, Celsr1, Rhbdl3, Vcan, Cdhr1, Anxa11, Micu2, Bmp1, Cdh10, Enpp2, Syt4, Dtna, Fbn2, Pam, Dll4, Calb1, Padi2, Kcnip4, Tesc, Mmp17, Cabp1, Rph3a, Ret, Atp2b2, Ttyh1, Gas6, Slit2, Clstn2, Melk, Fstl4, Cdh8, Pcdh18, Slc24a2, Cdh12, Reps2, Npnt, Dsg2, Hpcal4*

Ion transmembrane transport *Slc5a5, Slc1a2, Atp1a2, Slc38a3, Cacna2d2, Cacng2, Acsl6, Slc13a5, Mink1, Abcc1, Slc22a3, Itgav, Chrna4, Trpc3, Tmem38b, Kcnq4, Kcnip4, Ppargc1a, Tesc, Trpv6, Atp2b2, Gas6, Slc24a6, Asic4, Sfxn5, Kcnk1, Cacna1a, Kcnk9, Slc24a2, Slc43a2, Cacng5, Slc7a1, Reln, Kcnj6, Kcnj10, Mmgt2, Atp5k, Slc6a7, Kcnip1, Gabrg3, Slc4a4*

Ephrin receptor activity *Epha4, Ephb2, Ephb6, Ephb1, Epha3*

Cell adhesion *Cdh4, Stat5a, Prlr, Celsr1, Sdc4, Cntnap1, Vmp1, Mink1, Hapln1, Rgcc, Olfm4, Myo10, Glycam1, Bcl6, Igsf11, Ache, Megf10, Ptprj, Itga7, Col19a1, Epha4, Nfasc, Prkcq, Itgav, Bcl2l11, Col11a1, Ppap2b, Pcdh7, Tesc, Cntn3, Ret, Cd9, Ttyh1, Gas6, Cx3cl1, Kifc3, Clstn2, Ephb1, Cdh8, Ssx2ip, Mycn, Spon1, Rapgef1, Npnt, Atp1b2, Sdk2, Reln, Cxcr7, Sned1, Epha3, Robo2, Cdh22, Rnd1, Spock1, Ntm, Cadm2, Cntn4, Cntnap5a, Fat3, Nrarp*

Chemotaxis *Cdh4, Shh, Evl, Ednrb, Sema5a, Enpp2, Abcc1, Pla2g7, Ptprj, Epha4, Nfasc, Ephb2, Sema3c, Gas6, Cx3cl1, Ccl17, Slc37a4, Ephb1, Lpar1, Sema6c, Camk1d, Reln, Scg2, Robo2, Gas1, Chn1*

Myelin assembly and protein localization to paranode region of axon *Pmp22, Cd9, Cntnap1, Nfasc, Ugt8a*

Cell cycle *Uhrf1, Mcm2, Fam5b, Prkacb, Mcm5, Pole, Met, Kif11, Ncapg, Brca1, Mybl2, Esr1, Tnfaip3, Cdk1, E2f7, Prmt2, Top2a, Edn1, Trip13, Cep72, Anxa11, Esco2, Dscc1, Mcm4, Racgap1, Adcyap1, Mapre2, Cep55, Hells, Plk4, Sgol2, Nabp1, Mcm6, Cenpf, Nuf2, Casc5, Knstrn, Bub1, Tpx2, Rbl1, Ccna2, Iqgap3, Smc2, Nr4a3, Cdc7, Ccng2, Mki67, Sfrp1, Casp3, Chek1, Usp37, Ccp110, Aspm, Fancd2, Ncaph, Fignl1, Melk, Cited2, Rhou, Timeless, Bub1b, 2810417H13Rik, Mcm3, Ncapg2, Clspn, Cenpe, E2f8, Mlf1, Cdca2, Lig1, Espl1*

Regulation of chemokine-mediated signaling pathway *Robo1, Slit2, Slit3*

Astrocyte differentiation *Shh, Gfap, Il6st, Sox8, Epha4, Mt3, Mycn, Atf5*

Neurofilament *Nefh, Nefm, Nefl, Nrp1, Shank2*

Motile cilium *Spa17, Bbs1, Met, Ahi1, Sept4, Cdhr1, Rsph9, Atp2b4, Spef1, Ccdc39, Prom1, Drd2, Rpgrip1l, Nme5, Shank2, Adrbk2, Ttc26, Alms1, Wdr35*

3.6 Correlation between the action of T3 *in vivo* and in primary cells

Several studies have focused on the action of T3 in the brain *in vivo* by analyzing patterns of gene expression between euthyroid and hypothyroid rats or mice. From these studies different sets of TH-dependent genes were identified. We compared the current study with the previous data sets of differentially expressed genes in the rat and mouse cerebral cortex during the fetal [88, 89] and postnatal periods [90] (Figure 24). From the *in vivo* studies on postnatal cerebral cortex gene expression, 1,275 genes were sensitive to hypothyroidism. We made an assessment on how many of these genes could be direct cellular responses to T3 and not a secondary effect of hypothyroidism. We found that from the *in vivo* data set 932 genes were also expressed in the primary cultures, and from these 201 were sensitive to T3 (Annex IV). Therefore we may conclude that about 21% of the responses *in vivo* to hypothyroidism were genes directly regulated by T3 at the cellular level. Furthermore, taking the whole set of 932 genes sensitive to TH *in vivo* and expressed in our cultures there was a good positive correlation (r =0.4535; P <0.0001) between the direction and magnitude of the gene expression changes in both conditions.

From similar comparisons using the effects of hypothyroidism on gene expression in the fetal cortex, 652 genes from a total set of 1,080 in the in vivo studies were expressed in the cultures. From these, 128 genes were regulated by T3. Therefore, in the fetal cortex also about 20 % of the genes sensitive in vivo to hypothyroidism were sensitive to T3 in primary culture (Annex IV). Similar correlations as above were also found (r= 0.1380; P<0.006).

3.7 T3 favors an adult *versus* fetal profile of gene expression

The transition between the embryonic and adult brain involves substantial changes in the expression of genes related to developmental processes. Dillman A. and coworkers [118] performed comparisons of transcriptome profiles between these two stages and defined a set of 1,185 genes highly expressed (5-fold or greater) in the embryonic *versus* the adult cerebral cortex and another set of 2,943 genes enriched in the adult compared to the embryonic cortex. Given the importance of TH in brain maturation, it was of interest to analyze whether T3 was involved in the relative expression of these two gene sets in the primary cultures. For this reason we analyzed the overlap between our gene expression data set and the over-represented embryonic and adult cortex transcriptomes. From a total of 14,801 expressed genes (EG) in the primary

cerebrocortical cells (Figure 24A) approximately half of the 5-fold enriched genes in the embryonic (649 genes) and adult (1,563 genes) cortex were expressed in our cultures. As indicated above, T3 treatment induced positive or negative changes in gene expression. 16% (107 of 649) of the genes enriched in the embryonic cortex were negatively regulated by T3, in contrast to only 4% (67 of 1,563) of the adult cortex enriched genes. Conversely, T3 positively regulated 12% (196 of 1,563) of the adult cortex genes *versus* 3% (22 of 649) of the embryonic enriched genes (Figure 24B).
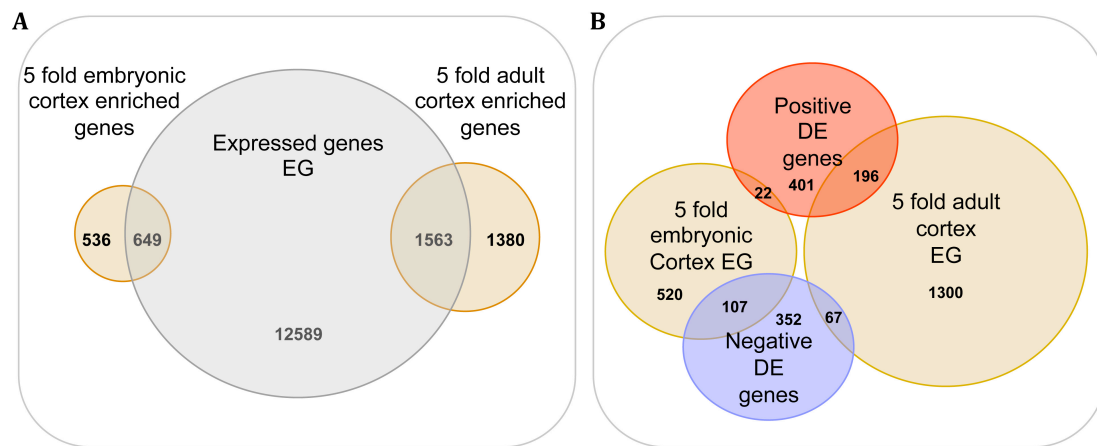
Figure 24. A: Overlap between the primary cerebrocortical cells expressed genes (EG) and previous data sets of 5-fold embryonic or adult cortex enriched genes [118]. **B:** Comparison of the overlap expressed genes from panel A and the positive or negative differentially express (DE) genes in the primary cerebrocortical cells after T3 treatment.

DISCUSSION

1. TRα1 and TR β specificity in the regulation of brain gene expression

In the present Thesis we have analyzed the role of TRα1 and TRβ in the control of brain gene expression. We have used two different approaches; the *in vivo* model, comparing the effect of hypothyroidism with that of receptor inactivation, and the *in vitro* model, comparing the response to T3 in primary cerebrocortical cells from Wt, *TRα1$^{-/-}$* and *TRβ $^{-/-}$* mice. *In vivo* we have measured the expression of genes that were previously identified in our laboratory as TH-dependent [90]. The genes analyzed cover a wide range of physiological and biochemical processes, reflecting the complexity of TH action in the brain and the pleiotropic effects of hypothyroidism.

1.1 The *in vivo* studies

In the *in vivo* approach we can conclude that both receptor subtypes are involved in the regulation of brain gene expression. TRα1 appears to play a primary role, but the lack of this receptor affects only a subset of the genes. Absence of both receptor types increases the number of genes affected, indicating that in the absence of TRα1, TRβ maintains gene expression near normal levels. On the other hand, the absence of TRβ results in little changes, with increased expression of a few positive genes, and decreased expression of a few negative genes.

In these effects of receptor deficiency in mice, we have to take into account possible changes of TH concentrations that might have contributed to the observed changes. The effects of TRα1 deficiency are not probably due to lower T3 concentration, since cerebral cortex concentrations of T4 and T3 are not modified in the *TRα1$^{-/-}$* mice [82]. However, the increased or decreased expression of some genes observed in the absence of TRβ is most likely due to the known enhancement of TH production in the *TR$\beta$$^{-/-}$* mice [72]. This would result in increased T3 action through the remaining TRα1, and increase or decrease expression of positive and negative genes respectively.

Another important concern is how the cellular heterogeneity of the brain regions might have influenced the gene expression changes. Indeed genetic studies have revealed a cellular complexity that goes well beyond the classical cell type subdivisions of the brain based on morphology and neurotransmitter production [119, 120]. Different cell groups might respond differently to TH in the regulation of expression of individual genes. Also, the responsive cells might be a minor component of the total cellular

repertoire of the region under study. It is well known that some individual genes may be sensitive to TH in some cell populations and not in others despite expressing TRs in adequate amounts [5]. As an example *Nrgn*, a gene regulated directly by T3 at the transcriptional level [121] is very sensitive to TH in the striatum, dentate gyrus and layer 6 of cerebral cortex, whereas other layers of the cortex and hippocampus are not sensitive [122]. This is the reason for the lower effect of hypothyroidism on *Nrgn* expression, and on other genes such as *Vdr* and *Cd72* in the whole cortex compared to the striatum. Only quantitative *in situ* hybridization techniques, with a detailed account of the gene expression responses by individual cell groups should be able to provide a complete picture.

With the above limitations in mind, we found little evidence for receptor subtype specificity among the positive genes. Some of the genes analyzed in this Thesis have also been examined in other cellular contexts. In HepG2 cells *Kcnj10* was positively regulated by T3 through TRα1 and *Gpc3* was negatively regulated in general agreement with our findings [67]. In contrast, *Angptl4*, which behaves as a negative gene in cortex and striatum, in HepG2 cells is a TRβ-specific positive gene [123]. Concerning the negative genes, the absence of TRα1 was in general more effective than for the positive genes, indicating that TRα1 was more involved in negative regulation of brain genes than TRβ. These results contrast with the predominant effect of TRβ on negative regulation of the *Tshb* gene [124].

1.2 T3 action in primary cerebrocortical cells

In order to get a deeper insight in the contribution of TR subtypes in the regulation of gene expression we also used primary cerebrocortical cells. This experimental set up allowed us to study the specificity of TRs at the cellular level avoiding distal effects that may occur *in vivo*. We measured T3 response in primary cells derived from the cerebral cortex of E17.5 TRα1$^{-/-}$ and TRβ$^{-/-}$ mice. The results confirm that at the cellular level both TRα1 and TRβ mediate the effects of T3, with two exceptions, *Dio3* and *Aldh1a1*. *Dio3* was already shown to be regulated specifically by TRα1 [15], and we confirm this fact in the cultured cells. Also *Aldh1a1* appears to be regulated specifically by TRα1, since no induction by T3 was observed in cells derived from TRα1$^{-/-}$ mice. To the best of our knowledge *Aldh1a1* was not known to be regulated specifically by TRα1. On the other hand, in the cortex we did not observe significant expression changes in this gene comparing *TRα1$^{-/-}$*, *TRβ $^{-/-}$* and *TRα1$^{-/-}$TRβ $^{-/-}$* with the Wt mice. The Aldh1a1 enzyme is

involved in RA synthesis, catalyzing conversion of all-trans-retinal to all-trans-retinoic acid. It is also relevant to point out that in studies included in the accompanying paper [114] we found *Aldh1a1* to be under glucocorticoid regulation having an effect comparable to that of T3. Surprisingly when added together there was a synergistic effect and *Aldh1a1* expression increased 20-fold, the largest induction produced by T3 that we have knowledge on any gene in neural cells. The glucocorticoid effect may also be the reason why double inactivation of *Mct8* and *Dio2,* which produces selective brain hypothyroidism*,* does not affect *Aldh1a1* expression in contrast to the downregulation observed in this gene when hypothyroidism is induced by thyroid gland blockade. Interactions of TH with glucocorticoids, retinoids and many other pathways are likely to be operating in different cell population micro domains at different stages of brain development. In the case of *Aldh1a1* this crosstalk may be the reason that blur out the specific regulation by TRα1 *in vivo*.

The regulation of *Klf9* and *Aldh1a3* showed no preference for each of the TR subtypes in the cerebrocortical cells. This agrees with the regulation of Klf9 observed in the cortex where we did not find differences in the expression of this gene between $TR\alpha1^{-/-}$, $TR\beta^{-/-}$, $TR\alpha1^{-/-}TR\beta^{-/-}$ and the Wt mice.

In the primary cells both receptor subtypes can regulate *Hr* and *Shh* although the magnitude of response to T3 is more affected in the absence of TRα1. They were both also analyzed in the P21 cortex. *Shh* displayed no TRs specificity in the comparison between all the different mice. In the case of Hr, although its expression was maintained in the single KO mice, there was an important reduction between the expression in the $TR\beta^{-/-}$ and the $TR\alpha1^{-/-}TR\beta^{-/-}$ mice, indicating the importance of TRα1 in the regulation of this gene.

A recent global analysis of TR specificity in HeLa cells expressing exogenous TRα1 or TRβ1 [78] concluded that there are no complete TR subtype specificity, although TRα1 or TRβ1 showed some gene preferences, depending on the time of exposure and the dose of T3. In established neural cell lines TRα1 or TRβ1 expression leads to substantial differences in the gene network regulated by T3, without correlation with differential chromatin occupancy [68].

1.3 Direct *versus* indirect regulation

As pointed out above, we found differences between the *in vivo* and the *in vitro* model. We think that the reason of some of these discrepancies may indicate that regulation by T3 is indirect, as a consequence of the effect on other signaling pathways, such as retinoids and glucocorticoids.

However, several lines of evidence indicate that many of the genes studied in this work are most probably direct targets of TH. Some of them have been specifically studied in this regard, with confirmation of direct regulation at the cellular level, and identification of TRE sequences. These include *Nrgn* [121, 125], *Hr* [111], *Klf9* [113, 126], *Dio3* [15], *Gbp3* [96], *Shh* [112] and *Angptl4* [123]. Others respond to the administration of a single T3 dose to adult rats (*Aldh1a1*, *Bcar3*, *Hr*, *Itih3,* and *Klf9*) [91]. Chromatin occupancy by the TR has been shown for *Hr, Klf9, Ier5, Cbr2, Cirbp, Cxadr, Bcar3, Col6a2, Smad14* and *Vdr* in established neural cell lines [68]. Here we confirm that also in cerebrocortical primary cells *Hr*, *Klf9*, and *Shh* are direct transcriptional targets of T3, given that the effect of the hormone was not blocked by previous treatment with CHX to inhibit protein synthesis.

Dio3 is especially interesting in this regard. We could not determine whether *Dio3* was also transcriptionally regulated by T3 because it was not stimulated by T3 at 6 hours of incubation, preventing to test the effect of CHX. However, a specific TRα1 binding site has been demonstrated in the upstream region of the gene [15]. The fact that we could not determine whether the effect of T3 was direct, suggests the possibility that the full effect of T3 requires interaction with other intermediate proteins. *Dio3* is a transcriptional target of Shh [127], which as mentioned above is a direct T3 target. This raises the possibility that full induction of *Dio3* by T3 is the result of a direct transcriptional effect of TRα1 potentiated by the T3-dependent accumulation of Shh protein.

A final conclusion concerning TRα1 and TRβ specificity is that TRα1 exerts a predominant, but not exclusive role in the regulation of gene expression in the cerebral cortex and the striatum. This may be a direct consequence of the higher abundance of TRα1 relative to TRβ in brain. At the cellular level we can conclude that from the genes analyzed we found two specifically regulated by TRα1 (*Dio3 and Aldh1a1*) but the rest can be regulated by both receptors although in some cases TRα1 exerts a predominant regulation; this may be a consequence of the differences observed in the action kinetics

between the two receptor subtypes, as pointed out recently for non neural cell lines in culture [67, 78].

1.4 Hypothyroidism *versus* lack of TRs

Another factor to keep in mind in the interpretation of the *in vivo* results is the role of the unliganded TRs in the regulation of TH dependent genes. Compared to the effects of hypothyroidism, the absence of TRα1 and TRβ, and therefore complete lack of T3 signaling through the TRs, led to either no changes in gene expression or to changes that were in the same direction as in hypothyroidism, but generally of much less severity. The double TRα1$^{-/-}$ and TRβ$^{-/-}$ mice are known to have highly increased TH levels [75]. Although unlikely, we cannot discard that overactivation of non genomic pathways [29] might play a role in these mice.

Considering the responses of all genes as a group, the effects of hypothyroidism and of *TRα1* and *TRβ* inactivation were correlated, but the regression line indicated a stronger effect of hypothyroidism. The results are compatible with the idea that the effects of hypothyroidism on the expression of some genes is due to the activity of the apoTR, consisting of repression of positive genes, and activation of negative genes. It is likely that the effects of apoTR in the hypothyroid brain are primarily due to TRα1. This was demonstrated by showing that on some genes hypothyroidism had no effect on the *TRα1$^{-/-}$* mice. A clear example was *Cbr2* and *Angptl4* in cortex and striatum. On others there was a significant effect of hypothyroidism on the *TRα1$^{-/-}$* mice but milder than the effect on the Wt, for example *Kcnj10* in both regions. This may indicate that the apoTRβ might also play a role in hypothyroidism in agreement with the effects of a mutant TRβ1 [128, 129].

In mice, as explained in the introduction, morphological changes in the development of the cerebellum or survival rate alterations during hypothyroidism have been attributed to the apoTRα1 [82, 83]. Also, in agreement with our results, a microarray analysis to examine hepatic gene expression profiles using *TRα1$^{-/-}$*, *TRβ $^{-/-}$* and *TRα1$^{-/-}$TRβ $^{-/-}$* mice has shown that absence of receptor and absence of hormone have different outcomes [66].

The regulation of gene expression by the apoTRs has also been widely studied during amphibian pre-metamorphosis. The pattern of receptor expression in relation to thyroid gland activity suggests that the apoTRs maintain TH responsive genes in the repressed

state during pre-metamorphosis. The later increase thyroidal secretion switches the TRs to the liganded state with recruitment of coactivators and induction of the T3 transcriptional program leading to metamorphosis [81].

The results of this Thesis confirm a role apoTRα1 in the regulation brain gene expression. The developmental implications of the regulation of gene expression in the mice by the apoTR during brain development remains to be explored.

2. The transcriptomics of T3 action during neural development

There have been many studies aimed at defining the gene network regulated by TH during brain development. To have a real physiological picture, the studies have been performed *in vivo.* However the results are greatly dependent on the specific timing of development studied as well as the specific region of the brain. In addition, as we have seen, secondary responses due to organismic hypothyroidism may lead to confusing results. We think that established lines of transformed cells are also not appropriate for this task. Therefore we evaluate the potential of primary cerebrocortical cells to obtain a global picture of the trascriptomics of T3 action during brain development.

2.1 Primary cerebrocortical cells maintain individual cellular phenotypes and T3 responsiveness

Our study on gene expression using primary cerebrocortical cells led us to recognize the potential value of this system to answer more general questions on the role of TH in brain development. Cerebrocortical cell culture might be a useful tool to reveal the potential and global T3 action at the cellular level, allowing us to extrapolate the results to particular cell populations in discrete brain regions and developmental stages.

One example is the influence of TH on RA metabolism. In cultured cerebrocortical cells T3 regulates the expression of several enzymes of the RA metabolism. These enzymes are: *Aldh1a1 and Aldh1a3* that metabolize all-trans-retinal to all-trans-retinoic acid (the mayor active form of RA) and *Cyp26b1* that metabolize excess RA. Thereby T3 is influencing the retinoid pathway at different steps [109]. *In vivo*, the net effect would depend on the differential, regional and timely, expression of each of these enzymes in the brain.

82

The primary cell culture contains necessary elements for T3 responsiveness. We analyzed several families of RNAs encoding proteins important in TH action: transporters, TRs, and coregulators. Among the transporters *Mct8* is expressed in the highest proportion followed by *Lat1*, *Oatp1c1* and *Lat2*. These four transporters are expressed in the cortex in vivo [20, 23, 130]. As for the TRs, TRα1 and TRβ are expressed in the primary cells in similar proportions. Within the coregulator family, the well known *Src* and *Ncor* are also expressed in the primary culture.

Our primary cerebrocortical cell culture is composed of 80% neurons and 20% astrocytes and we have shown that in both cell types TH regulates gene expression. So the TH target genes differentially expressed in the culture can be regulated in neurons, astrocytes or both.

One possible disadvantage of the culture would be the likely homogenization of cellular phenotypes in response to the artificial conditions of the culture system. Interestingly this is apparently not the case, at least to some extent. As an example, we have detected a cell subpopulation of calbindin positive neurons, which accounts for about 10% of total neurons in the culture. *In vivo* calbindin is a marker of a subpopulation of cortical GABAergic neurons. To extend this observation, we compared our data set with a transcriptome database of cerebral cortex cell types isolated using the translating ribosome affinity purification approach [117]. This study permits the molecular phenotyping of genetically defined cell populations. The primary cerebrocortical cell culture expresses 50% of the genes enriched in specific neuronal populations of CCK neurons, cortistatin neurons, or PNOC neurons as well as genes enriched in cortical layers 5b, 5a or 6.

Although the gene expression regulation by TH in astrocytes has not been studied it is known that TH deficiency induces a delay in astrocyte differentiation and maturation [64, 131]. As explained in the results, we compared our set of primary cells TH target genes with the Cahoy and coworkers transcriptome database of acutely purified astrocytes and neurons of postnatal mice [6]. We found that T3 up regulates both, genes enriched in astrocytes as well as enriched in neurons. Surprisingly we found big differences between the amount of T3 down regulated genes in both cell types. T3 down regulates 84 neuronal enriched genes *versus* 10 astrocyte enriched genes.

2.2 Transcriptome analysis

From the transcriptome analysis in the primary cerebrocortical cells we obtained a large data set of genes whose expression depends on the presence of T3 (1,145 differentially expressed genes). Among these, 27% (223 positives and 84 negatives) have been reported to contain a TRE in established neural cell lines expressing TRα1 or TRβ1 [68], suggesting a direct T3 regulation. Some of the direct responses to T3 encode transcription factors which would be responsible for indirect T3 actions on gene expression, for example: *Klf9, Bcl6, Mafb, Shh, Nr3c1*, and *Hr*.

To get a deeper analysis and extrapolate our cellular T3 target genes to *in vivo* regulation we compared the differentially express genes in the primary cerebrocortical cells with the previous data sets of differentially expressed genes in the rat and mouse cerebral cortex during the fetal [88, 89] and postnatal periods [90]. From these comparisons we can conclude that around 20% of the genes sensitive to hypothyroidism *in vivo*, both in fetal and adult brain, are cellular targets of T3 and not regulated as a secondary effect of hypothyroidism. The overlapping list of 329 TH target genes, both at the cellular level and *in vivo*, represents a valuable data set to break through the genomic targets of TH involved in brain development.

Looking for the biological significance of the regulation of gene expression by T3 in the primary cells we performed a GO analysis using all the differentially expressed genes. We found genes enriched in neurogenesis, neuron differentiation, cell comunication, response to stimuli and signal transduction. Interestingly, in the GO analysis using just the T3 positive genes we found genes that encode for proteins mainly localized at the plasma membrane. This includes genes involved in transmission of the nerve impulse, ion transport, chemotaxis, myelin assembly and the paranodal region. On the other hand, in the GO analysis of the T3 negative genes we found genes that encode proteins mainly localized in the nucleus, including genes involved in cell division, with the category of the M Phase of cell cycle specially enriched, and chromosome segregation and organization.

This is a very valuable analysis in order to understand the physiological actions of TH in cortex development. For example, there are many GO categories related to neuronal migration processes as, chemotaxis, cell communication, cell adhesion, axón guidance and, Roundabout signaling pathway. It is well known that hypothyroidism during cortical development alters neuronal migration [132, 133]. Specifically, *in vitro* studies have shown that transient maternal hypothyroxinemia at onset of corticogenesis alters

tangential migration of medial ganglionic eminence-derived neurons [134]. The tangential migration of GABAergic interneurons from the median ganglionic eminence (MGE) to the final destination in the cortex is modulated by expression of particular combinations of transcription factors in the progenitors cells, motogenic factors, guidance molecules present in the extracellular environment and the complements receptors expressed in the migrating interneurons. Interestingly we have found many differentially expressed genes encoding proteins that are involved in this process. For example, *Shh* morphogen which appears to play a critical role on the establishment of the interneurons progenitors transcription factor Nkx2.1 in the MGE, transcription factors implicated in interneuron development (*bMaf, Etv1, Npas1*), receptors that regulate migration (*ErbB4, Cxcr7*), the chemoattractant for MGE derived interneurons *Cxcl12*, chemorepulsive molecules (*Slit2, Slit3, Robo1, Robo2, EphrinA5* and *EphA4*), as well as *Sema3a* and *Nrp1* receptor which are important to maintain the interneuron migrating route. The altered expression of these genes could be the molecular basis responsible for the altered interneuronal migration observed in hypothyroidism.

2.3 Embryonic *versus* adult cerebral cortex gene expression profile

Many of the differentially expressed genes after T3 treatment are involved in nervous system development. A high resolution transcriptome analysis has been done between the embryonic and adult brain [118]. As explained in the results, we compared the 5 fold embryonic or adult cortex enriched genes with our set of TH target genes in our culture. From the overlapping genes we can conclude that T3 mainly up-regulates genes enriched in the adult cortex and down-regulates genes enriched in the embryonic cortex. The embryonic brain is enriched in genes involved in cell division, M Phase of cell cycle, chromosome segregation and organization whereas the mature nervous system is enriched in genes involved in neurotransmission and ion transport. The GO categories more highly represented in the set of genes down-regulated by T3 in our primary cerebrocortical cell culture highly overlap with the enriched functions in the embryonic brain. Conversely, the categories more represented in the set of up-regulated genes overlap with the enriched functions of the mature brain.

Cell type diversity in the nervous system is defined by expression of cell-surface proteins, such as channels and receptors, and also specific transcription factors and calcium-binding proteins [117]. In this Thesis we have shown that genes encoding these proteins are significatively regulated by T3. These data indicate that changes from the

embryonic brain to the mature brain with a stable neurotransmission system and specific cellular identity are controlled at least in part by TH.

In conclusion, T3 appears to be needed at an appropriate time for the gene expression transition from the embryonic to the adult brain. This transition is associated with a T3-dependent down-regulation of genes enriched in early developmental stages and the up-regulation of genes more represented in the mature brain (Figure 25).

EMBRYONIC → Gene expression pattern → **ADULT**

Cell division
M Phase of cell cycle
Microtubule cytoskeleton organization
Chromosome organization and segregation

- T3 +

Neurotransmission
Synaptic transmission
Chemical homeostasis
Ion transport
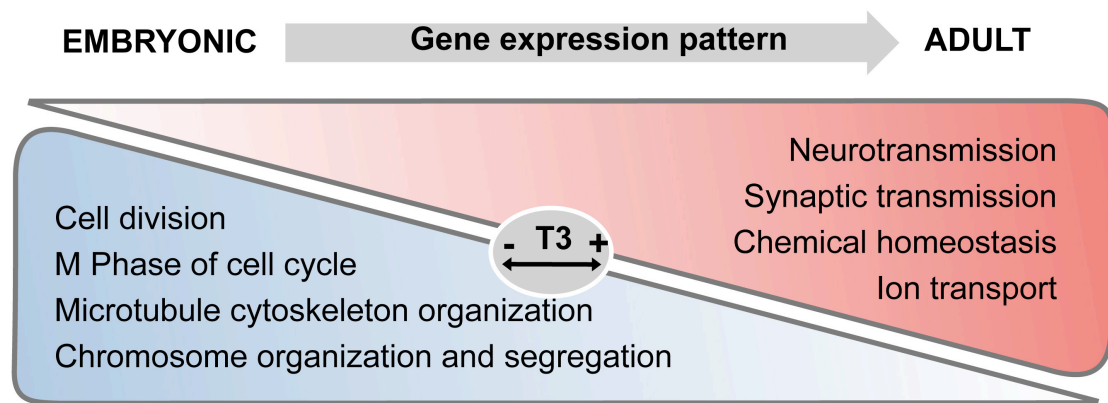
Figure 25. Scheme of the processes down-regulated (blue) and up-regulated (red) after T3 treatment in our study involved in the transition from embryonic to adult pattern of gene expression.

CONCLUSIONS

Conclusions

1. TH deficiency *in vivo* (hypothyroidism) affects negatively or positively gene expression in the cerebral cortex and striatum of developing mice. The quantitative effect depends on the specific gene and the region analyzed.

2. The effects of hypothyroidism are much stronger than T3 receptor inactivation, either single or combined.

3. Single inactivation of TR induces little changes in gene expression, but generally the effects of *TRα1* inactivation are more predominant than the effects of *TRβ* inactivation.

4. At the cellular level we found *Dio3 and Aldh1a1* specifically regulated by TRα1, the rest of the genes analyzed can be regulated by both receptors although in some cases TRα1 exerts a predominant role.

5. The stronger effects induced by hypothyroidism are due in some cases to the activity of unliganded receptor or apoTR, since hypothyroidism has lesser effects in TRα1 knock-out mice.

6. Cerebrocortical cells in primary culture maintain complex cellular phenotypes and T3 responsiveness. T3 induces gene expression directly and indirectly.

7. The transcriptomic response to T3 in primary cerebrocortical cells involves a set of 1,145 genes, from which 619 are positively regulated and 526 are negatively regulated. Twentyseven percent of these genes contain a TRE, suggesting direct regulation.

8. T3 specifically upregulates genes involved in transmission of the nerve impulse, processes involving ion transport, ephrin receptor activity, cell adhesion, chemotaxis, myelin assembly, in protein localization at the paranodal region and astrocyte differentiation.

9. T3 specifically downregulates genes enriched in cell division, M Phase of cell cycle, chromosome segregation and organization.

10. In general, T3 favors the adult *versus* fetal pattern of cortex gene expression.

BIBLIOGRAPHY

1.   Morreale de Escobar, G., M.J. Obregon, and F. Escobar del Rey, *Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia?* J Clin Endocrinol Metab, 2000. **85**(11): p. 3975-87.

2.   Kohrle, J., *Iodothyronine deiodinases.* Methods Enzymol, 2002. **347**: p. 125-67.

3.   Gereben, B., et al., *Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling.* Endocr Rev, 2008. **29**(7): p. 898-938.

4.   Mortimer, R.H., et al., *Maternal to fetal thyroxine transmission in the human term placenta is limited by inner ring deiodination.* J Clin Endocrinol Metab, 1996. **81**(6): p. 2247-9.

5.   Guadano-Ferraz, A., et al., *Transcriptional induction of RC3/neurogranin by thyroid hormone: differential neuronal sensitivity is not correlated with thyroid hormone receptor distribution in the brain.* Brain Res Mol Brain Res, 1997. **49**(1-2): p. 37-44.

6.   Cahoy, J.D., et al., *A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function.* J Neurosci, 2008. **28**(1): p. 264-78.

7.   Baqui, M.M., et al., *Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy.* Endocrinology, 2000. **141**(11): p. 4309-12.

8.   Tu, H.M., et al., *Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone.* Endocrinology, 1999. **140**(2): p. 784-90.

9.   Escamez, M.J., et al., *Type 3 iodothyronine deiodinase is selectively expressed in areas related to sexual differentiation in the newborn rat brain.* Endocrinology, 1999. **140**(11): p. 5443-6.

10.   Baqui, M., et al., *Human type 3 iodothyronine selenodeiodinase is located in the plasma membrane and undergoes rapid internalization to endosomes.* J Biol Chem, 2003. **278**(2): p. 1206-11.

11.   Obregon, M.J., et al., *Outer ring iodothyronine deiodinases and thyroid hormone economy: responses to iodine deficiency in the rat fetus and neonate.* Endocrinology, 1991. **129**(5): p. 2663-73.

12.   Escobar-Morreale, H.F., et al., *Regulation of iodothyronine deiodinase activity as studied in thyroidectomized rats infused with thyroxine or triiodothyronine.* Endocrinology, 1997. **138**(6): p. 2559-68.

13.   Steinsapir, J., J. Harney, and P.R. Larsen, *Type 2 iodothyronine deiodinase in rat pituitary tumor cells is inactivated in proteasomes.* J Clin Invest, 1998. **102**(11): p. 1895-9.

14.   Croteau, W., et al., *Cloning of the mammalian type II iodothyronine deiodinase. A selenoprotein differentially expressed and regulated in human and rat brain and other tissues.* J Clin Invest, 1996. **98**(2): p. 405-17.

15.   Barca-Mayo, O., et al., *Thyroid hormone receptor alpha and regulation of type 3 deiodinase.* Mol Endocrinol, 2011. **25**(4): p. 575-83.

16.   Guadano-Ferraz, A., et al., *Expression of type 2 iodothyronine deiodinase in hypothyroid rat brain indicates an important role of thyroid hormone in the development of specific primary sensory systems.* J Neurosci, 1999. **19**(9): p. 3430-9.

17. Friesema, E.C., et al., *Thyroid hormone transporters.* Vitam Horm, 2005. **70**: p. 137-67.

18. Dumitrescu, A.M., et al., *A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene.* Am J Hum Genet, 2004. **74**(1): p. 168-75.

19. Friesema, E.C., et al., *Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation.* Lancet, 2004. **364**(9443): p. 1435-7.

20. Grijota-Martinez, C., et al., *Lack of action of exogenously administered T3 on the fetal rat brain despite expression of the monocarboxylate transporter 8.* Endocrinology, 2011. **152**(4): p. 1713-21.

21. Roberts, L.M., et al., *Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier.* Endocrinology, 2008. **149**(12): p. 6251-61.

22. Ito, K., et al., *Quantitative membrane protein expression at the blood-brain barrier of adult and younger cynomolgus monkeys.* J Pharm Sci, 2011. **100**(9): p. 3939-50.

23. Núñez B, M.d.M.R., Obregon MJ, Font-Llitjós M, Nunes V, Palacín M, Dumitrescu AM, Morte B, Bernal J, *Cerebral cortex hyperthyroidism of newborn Mct8-deficient mice transiently suppressed by Lat2 inactivation.* PLoS One, 2014.

24. Pizzagalli, F., et al., *Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter.* Mol Endocrinol, 2002. **16**(10): p. 2283-96.

25. Friesema, E.C., et al., *Thyroid hormone transport by the heterodimeric human system L amino acid transporter.* Endocrinology, 2001. **142**(10): p. 4339-48.

26. Friesema, E.C., et al., *Thyroid hormone transport by the human monocarboxylate transporter 8 and its rate-limiting role in intracellular metabolism.* Mol Endocrinol, 2006. **20**(11): p. 2761-72.

27. Calvo, R., et al., *Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain.* J Clin Invest, 1990. **86**(3): p. 889-99.

28. Bernal, J., *Thyroid hormones in brain development and function* Thyroid Disease manager, 2012.

29. Cheng, S.Y., J.L. Leonard, and P.J. Davis, *Molecular aspects of thyroid hormone actions.* Endocr Rev, 2010. **31**(2): p. 139-70.

30. Aranda, A. and A. Pascual, *Nuclear hormone receptors and gene expression.* Physiol Rev, 2001. **81**(3): p. 1269-304.

31. Yen, P.M., *Physiological and molecular basis of thyroid hormone action.* Physiol Rev, 2001. **81**(3): p. 1097-142.

32. Flamant, F., et al., *International Union of Pharmacology. LIX. The pharmacology and classification of the nuclear receptor superfamily: thyroid hormone receptors.* Pharmacol Rev, 2006. **58**(4): p. 705-11.

33. Oetting, A. and P.M. Yen, *New insights into thyroid hormone action.* Best Pract Res Clin Endocrinol Metab, 2007. **21**(2): p. 193-208.

34. Chassande, O., et al., *Identification of transcripts initiated from an internal promoter in the c-erbA alpha locus that encode inhibitors of retinoic acid receptor-alpha and triiodothyronine receptor activities.* Mol Endocrinol, 1997. **11**(9): p. 1278-90.

35. Hodin, R.A., et al., *Identification of a thyroid hormone receptor that is pituitary-specific.* Science, 1989. **244**(4900): p. 76-9.

36. Williams, G.R., *Cloning and characterization of two novel thyroid hormone receptor beta isoforms.* Mol Cell Biol, 2000. **20**(22): p. 8329-42.

37. Umesono, K., et al., *Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors.* Cell, 1991. **65**(7): p. 1255-66.

38. Williams, G.R., et al., *Thyroid hormone receptor binds with unique properties to response elements that contain hexamer domains in an inverted palindrome arrangement.* Endocrinology, 1994. **134**(4): p. 1888-96.

39. Desvergne, B., *How do thyroid hormone receptors bind to structurally diverse response elements?* Mol Cell Endocrinol, 1994. **100**(1-2): p. 125-31.

40. Grunstein, M., *Histone acetylation in chromatin structure and transcription.* Nature, 1997. **389**(6649): p. 349-52.

41. Li, Q., et al., *Modification of Chromatin Structure by the Thyroid Hormone Receptor.* Trends Endocrinol Metab, 1999. **10**(4): p. 157-164.

42. Glass, C.K. and M.G. Rosenfeld, *The coregulator exchange in transcriptional functions of nuclear receptors.* Genes Dev, 2000. **14**(2): p. 121-41.

43. Santos, G.M., L. Fairall, and J.W. Schwabe, *Negative regulation by nuclear receptors: a plethora of mechanisms.* Trends Endocrinol Metab, 2011. **22**(3): p. 87-93.

44. Wang, D., et al., *Negative regulation of TSHalpha target gene by thyroid hormone involves histone acetylation and corepressor complex dissociation.* Mol Endocrinol, 2009. **23**(5): p. 600-9.

45. Machado, D.S., et al., *A thyroid hormone receptor mutation that dissociates thyroid hormone regulation of gene expression in vivo.* Proc Natl Acad Sci U S A, 2009. **106**(23): p. 9441-6.

46. Strait, K.A., et al., *Immunofluorescence localization of thyroid hormone receptor protein beta 1 and variant alpha 2 in selected tissues: cerebellar Purkinje cells as a model for beta 1 receptor-mediated developmental effects of thyroid hormone in brain.* Proc Natl Acad Sci U S A, 1991. **88**(9): p. 3887-91.

47. Schwartz, H.L., et al., *Quantitation of rat tissue thyroid hormone binding receptor isoforms by immunoprecipitation of nuclear triiodothyronine binding capacity.* J Biol Chem, 1992. **267**(17): p. 11794-9.

48. Schwartz, H.L., M.A. Lazar, and J.H. Oppenheimer, *Widespread distribution of immunoreactive thyroid hormone beta 2 receptor (TR beta 2) in the nuclei of extrapituitary rat tissues.* J Biol Chem, 1994. **269**(40): p. 24777-82.

49. Ercan-Fang, S., H.L. Schwartz, and J.H. Oppenheimer, *Isoform-specific 3,5,3'-triiodothyronine receptor binding capacity and messenger ribonucleic acid content in rat adenohypophysis: effect of thyroidal state and comparison with extrapituitary tissues.* Endocrinology, 1996. **137**(8): p. 3228-33.

50. Zhang, X.Y., et al., *Differential expression of thyroid hormone receptor isoforms dictates the dominant negative activity of mutant Beta receptor.* Mol Endocrinol, 2002. **16**(9): p. 2077-92.

51. Keijzer, R., et al., *Expression of thyroid hormone receptors A and B in developing rat tissues; evidence for extensive posttranscriptional regulation.* J Mol Endocrinol, 2007. **38**(5): p. 523-35.

52. Wallis, K., et al., *The thyroid hormone receptor alpha1 protein is expressed in embryonic postmitotic neurons and persists in most adult neurons.* Mol Endocrinol, 2010. **24**(10): p. 1904-16.

53. Forrest, D., M. Sjoberg, and B. Vennstrom, *Contrasting developmental and tissue-specific expression of alpha and beta thyroid hormone receptor genes.* EMBO J, 1990. **9**(5): p. 1519-28.

54. Strait, K.A., et al., *Relationship of c-erbA mRNA content to tissue triiodothyronine nuclear binding capacity and function in developing and adult rats.* J Biol Chem, 1990. **265**(18): p. 10514-21.

55. Hodin, R.A., M.A. Lazar, and W.W. Chin, *Differential and tissue-specific regulation of the multiple rat c-erbA messenger RNA species by thyroid hormone.* J Clin Invest, 1990. **85**(1): p. 101-5.

56. Mitsuhashi, T. and V.M. Nikodem, *Regulation of expression of the alternative mRNAs of the rat alpha-thyroid hormone receptor gene.* J Biol Chem, 1989. **264**(15): p. 8900-4.

57. Bradley, D.J., H.C. Towle, and W.S. Young, 3rd, *Spatial and temporal expression of alpha- and beta-thyroid hormone receptor mRNAs, including the beta 2-subtype, in the developing mammalian nervous system.* J Neurosci, 1992. **12**(6): p. 2288-302.

58. Cook, C.B., et al., *Expression of thyroid hormone receptor beta 2 in rat hypothalamus.* Endocrinology, 1992. **130**(2): p. 1077-9.

59. Sjoberg, M., B. Vennstrom, and D. Forrest, *Thyroid hormone receptors in chick retinal development: differential expression of mRNAs for alpha and N-terminal variant beta receptors.* Development, 1992. **114**(1): p. 39-47.

60. Bradley, D.J., H.C. Towle, and W.S. Young, 3rd, *Alpha and beta thyroid hormone receptor (TR) gene expression during auditory neurogenesis: evidence for TR isoform-specific transcriptional regulation in vivo.* Proc Natl Acad Sci U S A, 1994. **91**(2): p. 439-43.

61. Jones, I., et al., *An intron control region differentially regulates expression of thyroid hormone receptor beta2 in the cochlea, pituitary, and cone photoreceptors.* Mol Endocrinol, 2007. **21**(5): p. 1108-19.

62. Bernal, J. and F. Pekonen, *Ontogenesis of the nuclear 3,5,3'-triiodothyronine receptor in the human fetal brain.* Endocrinology, 1984. **114**(2): p. 677-9.

63. Perez-Castillo, A., et al., *The early ontogenesis of thyroid hormone receptor in the rat fetus.* Endocrinology, 1985. **117**(6): p. 2457-61.

64. Manzano, J., J. Bernal, and B. Morte, *Influence of thyroid hormones on maturation of rat cerebellar astrocytes.* Int J Dev Neurosci, 2007. **25**(3): p. 171-9.

65. Lechan, R.M., et al., *Immunocytochemical delineation of thyroid hormone receptor beta 2-like immunoreactivity in the rat central nervous system.* Endocrinology, 1993. **132**(6): p. 2461-9.

66.   Yen, P.M., et al., *Effects of ligand and thyroid hormone receptor isoforms on hepatic gene expression profiles of thyroid hormone receptor knockout mice.* EMBO Rep, 2003. **4**(6): p. 581-7.

67.   Chan, I.H. and M.L. Privalsky, *Isoform-specific transcriptional activity of overlapping target genes that respond to thyroid hormone receptors alpha1 and beta1.* Mol Endocrinol, 2009. **23**(11): p. 1758-75.

68.   Chatonnet, F., et al., *Genome-wide analysis of thyroid hormone receptors shared and specific functions in neural cells.* Proc Natl Acad Sci U S A, 2013. **110**(8): p. E766-75.

69.   Dumitrescu, A.M. and S. Refetoff, *The syndromes of reduced sensitivity to thyroid hormone.* Biochim Biophys Acta, 2013. **1830**(7): p. 3987-4003.

70.   Bochukova, E., et al., *A mutation in the thyroid hormone receptor alpha gene.* N Engl J Med, 2012. **366**(3): p. 243-9.

71.   Flamant, F. and J. Samarut, *Thyroid hormone receptors: lessons from knockout and knock-in mutant mice.* Trends Endocrinol Metab, 2003. **14**(2): p. 85-90.

72.   Forrest, D., et al., *Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function.* Embo J, 1996. **15**(12): p. 3006-15.

73.   Refetoff, S., L.T. DeWind, and L.J. DeGroot, *Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone.* J Clin Endocrinol Metab, 1967. **27**(2): p. 279-94.

74.   Wikstrom, L., et al., *Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1.* EMBO J, 1998. **17**(2): p. 455-61.

75.   Gothe, S., et al., *Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation.* Genes Dev, 1999. **13**(10): p. 1329-41.

76.   Contreras-Jurado, C., et al., *The thyroid hormone receptors as modulators of skin proliferation and inflammation.* J Biol Chem, 2011. **286**(27): p. 24079-88.

77.   Yu, F., et al., *Effects of thyroid hormone receptor gene disruption on myosin isoform expression in mouse skeletal muscles.* Am J Physiol Regul Integr Comp Physiol, 2000. **278**(6): p. R1545-54.

78.   Lin, J.Z., et al., *Gene specific actions of thyroid hormone receptor subtypes.* PLoS One, 2013. **8**(1): p. e52407.

79.   Hahm, J.B., A.C. Schroeder, and M.L. Privalsky, *The two major isoforms of thyroid hormone receptor, TRalpha1 and TRbeta1, preferentially partner with distinct panels of auxiliary proteins.* Mol Cell Endocrinol, 2014. **383**(1-2): p. 80-95.

80.   Chassande, O., *Do unliganded thyroid hormone receptors have physiological functions?* J Mol Endocrinol, 2003. **31**(1): p. 9-20.

81.   Bernal, J. and B. Morte, *Thyroid hormone receptor activity in the absence of ligand: physiological and developmental implications.* Biochim Biophys Acta, 2013. **1830**(7): p. 3893-9.

82.   Morte, B., et al., *Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism.* Proc Natl Acad Sci U S A, 2002. **99**(6): p. 3985-9.

83.   Flamant, F., et al., *Congenital hypothyroid Pax8(-/-) mutant mice can be rescued by inactivating the TRalpha gene.* Mol Endocrinol, 2002. **16**(1): p. 24-32.

84.   Bates, J.M., D.L. St Germain, and V.A. Galton, *Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat.* Endocrinology, 1999. **140**(2): p. 844-51.

85.   Ruiz de Ona, C., et al., *Developmental changes in rat brain 5'-deiodinase and thyroid hormones during the fetal period: the effects of fetal hypothyroidism and maternal thyroid hormones.* Pediatr Res, 1988. **24**(5): p. 588-94.

86.   Ferreiro, B., R. Pastor, and J. Bernal, *T3 receptor occupancy and T3 levels in plasma and cytosol during rat brain development.* Acta Endocrinol (Copenh), 1990. **123**(1): p. 95-9.

87.   Berbel, P., et al., *Iodine supplementation during pregnancy: a public health challenge.* Trends Endocrinol Metab, 2007. **18**(9): p. 338-43.

88.   Morte, B., et al., *Thyroid hormone regulation of gene expression in the developing rat fetal cerebral cortex: prominent role of the Ca2+/calmodulin-dependent protein kinase IV pathway.* Endocrinology, 2010. **151**(2): p. 810-20.

89.   Dong, H., et al., *Transient Maternal Hypothyroxinemia Potentiates the Transcriptional Response to Exogenous Thyroid Hormone in the Fetal Cerebral Cortex Before the Onset of Fetal Thyroid Function: A Messenger and MicroRNA Profiling Study.* Cereb Cortex, 2014.

90.   Morte, B., et al., *Thyroid hormone-regulated mouse cerebral cortex genes are differentially dependent on the source of the hormone: a study in monocarboxylate transporter-8- and deiodinase-2-deficient mice.* Endocrinology, 2010. **151**(5): p. 2381-7.

91.   Diez, D., et al., *Thyroid hormone action in the adult brain: gene expression profiling of the effects of single and multiple doses of triiodo-L-thyronine in the rat striatum.* Endocrinology, 2008. **149**(8): p. 3989-4000.

92.   Mayerl, S., et al., *Impact of Oatp1c1 deficiency on thyroid hormone metabolism and action in the mouse brain.* Endocrinology, 2012. **153**(3): p. 1528-37.

93.   Galton, V.A., et al., *Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development.* Endocrinology, 2007. **148**(7): p. 3080-8.

94.   Hernandez, A., et al., *Critical role of types 2 and 3 deiodinases in the negative regulation of gene expression by T(3)in the mouse cerebral cortex.* Endocrinology, 2012. **153**(6): p. 2919-28.

95.   Ramadoss, P., et al., *Novel mechanism of positive versus negative regulation by thyroid hormone receptor beta1 (TRbeta1) identified by genome-wide profiling of binding sites in mouse liver.* J Biol Chem, 2014. **289**(3): p. 1313-28.

96.   Chatonnet, F., et al., *Genome-wide search reveals the existence of a limited number of thyroid hormone receptor alpha target genes in cerebellar neurons.* PLoS One, 2012. **7**(5): p. e30703.

97.   Mullen, R.J., C.R. Buck, and A.M. Smith, *NeuN, a neuronal specific nuclear protein in vertebrates.* Development, 1992. **116**(1): p. 201-11.

98.   S, A., *FastQC A Quality Control tool for High Throughput Sequence Data* http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

98

99. Langmead, B., et al., *Ultrafast and memory-efficient alignment of short DNA sequences to the human genome.* Genome Biol, 2009. **10**(3): p. R25.
100. Trapnell, C., L. Pachter, and S.L. Salzberg, *TopHat: discovering splice junctions with RNA-Seq.* Bioinformatics, 2009. **25**(9): p. 1105-11.
101. Garcia-Alcalde, F., et al., *Qualimap: evaluating next-generation sequencing alignment data.* Bioinformatics, 2012. **28**(20): p. 2678-9.
102. Anders. S, T.P.P., Hube. W, *HTSeq – A Python framework to work with high-throughput sequencing data.* BioRxiv preprint 2014.
103. Tarazona, S., et al., *Differential expression in RNA-seq: a matter of depth.* Genome Res, 2011. **21**(12): p. 2213-23.
104. Robinson, M.D. and A. Oshlack, *A scaling normalization method for differential expression analysis of RNA-seq data.* Genome Biol, 2010. **11**(3): p. R25.
105. Robinson, M.D., D.J. McCarthy, and G.K. Smyth, *edgeR: a Bioconductor package for differential expression analysis of digital gene expression data.* Bioinformatics, 2010. **26**(1): p. 139-40.
106. Young, M.D., et al., *Gene ontology analysis for RNA-seq: accounting for selection bias.* Genome Biol, 2010. **11**(2): p. R14.
107. Benjamini, Y., & Hochberg, Y, *Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing.* Journal of the Royal Statistical Society, 1995. **Series B (Methodological), 57(1), 289-300**.
108. Gil-Ibanez, P., B. Morte, and J. Bernal, *Role of thyroid hormone receptor subtypes alpha and beta on gene expression in the cerebral cortex and striatum of postnatal mice.* Endocrinology, 2013. **154**(5): p. 1940-7.
109. Gil-Ibanez, P., J. Bernal, and B. Morte, *Thyroid hormone regulation of gene expression in primary cerebrocortical cells: role of thyroid hormone receptor subtypes and interactions with retinoic Acid and glucocorticoids.* PLoS One, 2014. **9**(3): p. e91692.
110. Ruiz, F., et al., *Cyclosporin A targets involved in protection against glutamate excitotoxicity.* Eur J Pharmacol, 2000. **404**(1-2): p. 29-39.
111. Thompson, C.C., *Thyroid hormone-responsive genes in developing cerebellum include a novel synaptotagmin and a hairless homolog.* J Neurosci, 1996. **16**(24): p. 7832-40.
112. Desouza, L.A., et al., *Thyroid hormone regulates the expression of the sonic hedgehog signaling pathway in the embryonic and adult Mammalian brain.* Endocrinology, 2011. **152**(5): p. 1989-2000.
113. Denver, R.J. and K.E. Williamson, *Identification of a thyroid hormone response element in the mouse Kruppel-like factor 9 gene to explain its postnatal expression in the brain.* Endocrinology, 2009. **150**(8): p. 3935-43.
114. Gil-Ibañez, P., J. Bernal, and B. Morte, *Thyroid hormone action on cerebrocortical neurons in primary culture.* 94th Annual Meeting of the Endocrine Society. Houston, TX 2012
115. Kumar, S., et al., *Alcohol and aldehyde dehydrogenases: retinoid metabolic effects in mouse knockout models.* Biochim Biophys Acta, 2012. **1821**(1): p. 198-205.
116. Gil-Ibañez, P.G.-G., F.; Dopazo, J.; Bernal, J.; Morte, B., *Insight into the role of thyroid hormone on brain development through global transcriptome analysis in cerebrocortical cells.* 2014.

117. Doyle, J.P., et al., *Application of a translational profiling approach for the comparative analysis of CNS cell types.* Cell, 2008. **135**(4): p. 749-62.
118. Dillman, A.A., et al., *mRNA expression, splicing and editing in the embryonic and adult mouse cerebral cortex.* Nat Neurosci, 2013. **16**(4): p. 499-506.
119. Sugino, K., et al., *Molecular taxonomy of major neuronal classes in the adult mouse forebrain.* Nat Neurosci, 2006. **9**(1): p. 99-107.
120. Diaz, E., *A functional genomics guide to the galaxy of neuronal cell types.* Nat Neurosci, 2006. **9**(1): p. 10-2.
121. Morte, B., et al., *Thyroid hormone-regulated expression of RC3/neurogranin in the immortalized hypothalamic cell line GT1-7.* J Neurochem, 1997. **69**(3): p. 902-9.
122. Iniguez, M.A., et al., *Cell-specific effects of thyroid hormone on RC3/neurogranin expression in rat brain.* Endocrinology, 1996. **137**(3): p. 1032-41.
123. Yuan, C., et al., *Identical gene regulation patterns of T3 and selective thyroid hormone receptor modulator GC-1.* Endocrinology, 2012. **153**(1): p. 501-11.
124. Chiamolera, M.I., et al., *Fundamentally distinct roles of thyroid hormone receptor isoforms in a thyrotroph cell line are due to differential DNA binding.* Mol Endocrinol, 2012. **26**(6): p. 926-39.
125. Martinez de Arrieta, C., et al., *The human RC3 gene homolog, NRGN contains a thyroid hormone-responsive element located in the first intron.* Endocrinology, 1999. **140**(1): p. 335-43.
126. Denver, R.J., et al., *Basic transcription element-binding protein (BTEB) is a thyroid hormone-regulated gene in the developing central nervous system. Evidence for a role in neurite outgrowth.* J Biol Chem, 1999. **274**(33): p. 23128-34.
127. Dentice, M., et al., *Sonic hedgehog-induced type 3 deiodinase blocks thyroid hormone action enhancing proliferation of normal and malignant keratinocytes.* Proc Natl Acad Sci U S A, 2007. **104**(36): p. 14466-71.
128. Hashimoto, K., et al., *An unliganded thyroid hormone receptor causes severe neurological dysfunction.* Proc Natl Acad Sci U S A, 2001. **98**(7): p. 3998-4003.
129. Portella, A.C., et al., *Thyroid hormone receptor beta mutation causes severe impairment of cerebellar development.* Mol Cell Neurosci, 2010. **44**(1): p. 68-77.
130. Braun, D., et al., *Developmental and cell type-specific expression of thyroid hormone transporters in the mouse brain and in primary brain cells.* Glia, 2011. **59**(3): p. 463-71.
131. Morte, B., et al., *Aberrant maturation of astrocytes in thyroid hormone receptor alpha 1 knockout mice reveals an interplay between thyroid hormone receptor isoforms.* Endocrinology, 2004. **145**(3): p. 1386-91.
132. Berbel, P., et al., *Role of thyroid hormones in the maturation and organisation of rat barrel cortex.* Neuroscience, 2001. **107**(3): p. 383-94.
133. Auso, E., et al., *A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration.* Endocrinology, 2004. **145**(9): p. 4037-47.

134.   Cuevas, E., et al., *Transient maternal hypothyroxinemia at onset of corticogenesis alters tangential migration of medial ganglionic eminence-derived neurons.* Eur J Neurosci, 2005. **22**(3): p. 541-51.

ANNEX I

Differentially expressed genes after T3 induction in primary cerebrocortical cell culture

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Hr	3.09	7.10E-192	1.05E-187	5278	0.72
Sema3c	1.59	4.44E-114	3.28E-110	1549	14.44
Kcnj10	1.30	2.44E-83	1.20E-79	5407	8.11
Klf9	1.46	2.78E-74	1.03E-70	3263	5.10
Rgs4	-0.98	6.93E-67	2.05E-63	2575	53.48
Shh	2.51	2.06E-54	5.09E-51	2692	0.47
Cadm2	0.95	1.55E-45	3.28E-42	2038	84.82
AC165246.1	2.06	3.35E-37	6.20E-34	2169	0.72
Flywch2	1.65	5.18E-36	8.51E-33	694	4.23
Gpr17	1.28	4.22E-31	6.24E-28	5195	0.92
Calb1	-1.09	6.21E-31	8.35E-28	736	41.12
Sorl1	0.67	2.95E-30	3.64E-27	712	116.70
Ret	1.35	2.60E-29	2.96E-26	6703	0.57
Aldh1a3	-1.01	2.97E-29	3.14E-26	1066	28.36
Cxcl14	0.93	3.34E-29	3.30E-26	1823	12.19
Sept4	1.28	1.27E-28	1.18E-25	1278	3.59
Zcchc12	-0.60	2.37E-28	2.06E-25	2135	56.55
Npnt	0.80	7.65E-28	6.29E-25	3383	5.85
Dio3	2.16	1.45E-26	1.13E-23	1740	0.47
Popdc2	-3.51	1.80E-26	1.34E-23	835	2.65
Igf1	0.79	7.21E-26	5.08E-23	1368	14.36
Rasd2	1.30	8.96E-26	6.03E-23	2055	2.23
Bmp3	0.94	1.26E-25	8.10E-23	3112	4.36
Sfrp1	-0.59	1.86E-25	1.15E-22	4372	47.23
Bcar3	1.33	1.22E-24	7.23E-22	3298	1.12
Stac2	1.09	2.81E-24	1.60E-21	1800	4.17
Sema7a	0.91	5.96E-24	3.27E-21	3296	18.84
Sema6c	0.79	2.30E-23	1.22E-20	1867	25.63
Mmp17	0.70	3.00E-23	1.53E-20	2422	18.68
Cyp26b1	1.15	1.33E-22	6.55E-20	4726	1.07
Cldn12	0.79	2.69E-22	1.29E-19	3477	8.69
Chrm3	0.91	7.58E-22	3.51E-19	1770	5.61
Hcrtr1	1.56	1.79E-21	8.04E-19	2174	0.77
Epha4	0.56	5.58E-21	2.43E-18	6328	25.89
Ust	0.60	7.79E-20	3.30E-17	4284	8.28
Mafb	0.82	1.00E-19	4.12E-17	3389	2.92
Cyp11a1	3.33	1.36E-19	5.45E-17	1775	0.08
Cntn3	0.88	1.41E-19	5.50E-17	5183	1.63
Ephb1	0.66	7.89E-19	3.00E-16	4101	5.41
Nrarp	0.87	1.40E-18	5.19E-16	2573	3.04
Prss35	0.91	1.49E-18	5.37E-16	2868	2.21
Gls2	1.33	3.75E-18	1.32E-15	815	2.52
Has3	0.77	5.08E-18	1.75E-15	4150	3.02
Gdf10	0.95	8.60E-18	2.89E-15	2507	3.06
Cadm1	-0.45	1.03E-17	3.38E-15	1784	293.24
Hccs	0.60	1.50E-17	4.82E-15	2473	9.47
AW551984	-0.61	2.07E-17	6.50E-15	906	61.07
Daam2	0.99	7.19E-17	2.22E-14	6045	1.37
Mfsd2a	0.92	8.23E-17	2.48E-14	1197	4.74
Adarb1	0.52	1.34E-16	3.97E-14	3289	24.24
Spon1	0.53	2.12E-16	6.15E-14	4579	11.28
St8sia4	-0.65	3.53E-16	9.91E-14	5411	11.91

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Deptor	0.62	3.55E-16	9.91E-14	3300	5.19
Diras2	-0.45	6.34E-16	1.74E-13	4348	31.99
L3mbtl3	-0.50	1.58E-15	4.26E-13	4500	18.10
Suv420h2	0.76	2.75E-15	7.28E-13	765	13.59
Pcdh18	-0.83	2.98E-15	7.73E-13	5168	2.08
Cadps	0.38	6.12E-15	1.56E-12	5460	35.72
Bcl2l11	0.66	6.42E-15	1.61E-12	1956	10.04
Rspo2	-0.53	7.48E-15	1.84E-12	3324	13.38
Dbp	0.86	1.14E-14	2.76E-12	912	5.99
Gbp3	0.99	5.21E-14	1.24E-11	1786	1.94
Plxnc1	-0.53	5.26E-14	1.24E-11	701	236.38
Fosl2	-0.51	5.86E-14	1.36E-11	5855	19.73
Shisa6	0.46	1.08E-13	2.47E-11	1578	26.58
Thsd7a	-0.57	1.65E-13	3.71E-11	3331	20.75
Dtna	0.42	1.85E-13	4.03E-11	4006	18.91
Scg2	0.48	1.85E-13	4.03E-11	2485	44.16
Camk2n1	0.44	1.88E-13	4.03E-11	4444	35.89
Tmem132e	0.71	2.95E-13	6.23E-11	4105	2.31
Unc5d	-0.48	4.14E-13	8.64E-11	8922	9.51
Rbfox1	0.49	4.32E-13	8.87E-11	2638	55.48
Cd9	0.54	9.12E-13	1.85E-10	904	34.49
Pmp22	0.55	9.67E-13	1.93E-10	733	29.33
Pnoc	0.70	1.20E-12	2.37E-10	2157	4.89
Sox8	0.60	1.75E-12	3.42E-10	928	13.99
Atp2b4	-0.44	2.12E-12	4.07E-10	4579	51.46
Gabra2	-0.45	2.80E-12	5.31E-10	2392	69.29
Asap3	1.00	8.35E-12	1.56E-09	4225	0.64
Plcxd2	0.47	8.44E-12	1.56E-09	7140	12.27
Hlf	0.63	9.88E-12	1.81E-09	1686	5.93
Plxnd1	0.54	1.03E-11	1.85E-09	700	113.64
Abcd2	0.67	1.30E-11	2.31E-09	5527	1.44
Arid5b	-0.58	1.85E-11	3.25E-09	4494	4.14
Tmtc1	0.45	1.91E-11	3.33E-09	8269	4.71
Nrp1	-0.36	2.15E-11	3.70E-09	5907	23.66
Kazn	0.47	2.23E-11	3.80E-09	1858	21.01
Prkcq	0.65	2.32E-11	3.90E-09	3313	3.00
Vmp1	0.41	2.58E-11	4.30E-09	603	115.62
Itga7	0.72	3.06E-11	5.03E-09	4013	1.58
6330403A02Rik	-0.35	3.14E-11	5.11E-09	3554	71.30
Cntnap3	-0.50	4.05E-11	6.52E-09	4868	5.21
Rfx3	-0.50	8.11E-11	1.29E-08	2682	51.63
Lgi3	1.23	8.55E-11	1.35E-08	3180	0.41
Efnb2	-0.39	9.27E-11	1.44E-08	2488	58.03
Rbpms	-1.52	9.78E-11	1.51E-08	2443	1.43
Lancl3	-0.59	1.12E-10	1.70E-08	3991	3.90
Grin3a	-0.49	1.15E-10	1.74E-08	5678	11.86
Sybu	0.38	1.18E-10	1.77E-08	3124	64.85
Abcc1	0.50	1.74E-10	2.57E-08	2642	6.57
Slc1a2	0.43	1.82E-10	2.66E-08	539	496.60
Col23a1	-0.55	1.83E-10	2.66E-08	3090	6.62
Ucp2	0.81	1.91E-10	2.75E-08	670	9.43
Adra2a	0.60	2.01E-10	2.86E-08	3801	2.95

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Lpl	0.36	2.44E-10	3.44E-08	2162	39.90
Slc24a2	0.43	2.75E-10	3.84E-08	2171	21.70
Cdc42ep1	0.72	2.82E-10	3.89E-08	2542	2.21
Frmpd4	0.39	2.91E-10	3.99E-08	8305	6.10
Actr3b	0.48	3.06E-10	4.16E-08	1696	28.52
Acot11	0.80	3.26E-10	4.39E-08	596	7.07
Enpp2	0.41	4.01E-10	5.34E-08	2991	10.36
Rnf112	0.49	5.45E-10	7.20E-08	1001	22.04
Adra1b	0.89	5.79E-10	7.58E-08	3047	0.95
Fam19a2	-0.45	6.64E-10	8.62E-08	4364	17.76
Cacna1a	0.37	6.79E-10	8.74E-08	1152	54.75
Ephb2	0.42	7.76E-10	9.91E-08	3086	25.08
Gda	-0.41	7.84E-10	9.92E-08	2450	84.65
Htr7	-1.10	8.25E-10	1.03E-07	3068	1.02
Trp53inp2	0.35	9.55E-10	1.19E-07	825	160.42
Clca1	-2.38	1.11E-09	1.37E-07	3661	0.24
Slc38a3	0.58	1.37E-09	1.68E-07	2604	17.90
Acsbg1	0.66	1.69E-09	2.05E-07	407	77.60
Slc16a6	0.49	1.77E-09	2.13E-07	548	33.76
Pitpnc1	0.33	1.80E-09	2.15E-07	3064	29.92
F730043M19Rik	0.56	1.87E-09	2.21E-07	1090	12.79
Sertm1	-0.40	2.15E-09	2.53E-07	2991	33.04
Chn1	0.37	2.31E-09	2.69E-07	952	77.54
Fam163b	0.41	2.35E-09	2.72E-07	2959	23.15
Ppap2b	0.48	2.51E-09	2.88E-07	3159	22.55
Tgfa	0.49	2.64E-09	3.01E-07	4229	3.67
Prss12	0.43	3.39E-09	3.83E-07	2607	12.61
Ndst3	-0.77	3.58E-09	4.01E-07	1980	3.28
Gucy1a3	-0.36	4.77E-09	5.31E-07	4653	55.00
Hspb6	0.54	5.11E-09	5.65E-07	1331	8.69
Dgkk	-0.69	5.38E-09	5.87E-07	6991	1.08
Fam211a	0.66	5.40E-09	5.87E-07	649	8.40
Nefm	0.48	5.47E-09	5.91E-07	3507	9.67
Slc4a4	0.43	5.74E-09	6.16E-07	2894	22.16
Nr4a3	-0.33	5.82E-09	6.20E-07	3179	42.84
Galnt14	0.48	6.74E-09	7.12E-07	2713	5.69
Slc30a3	0.75	7.33E-09	7.69E-07	2089	1.87
Atp1b2	0.35	8.91E-09	9.29E-07	993	233.95
Plod1	0.61	9.62E-09	9.96E-07	932	10.03
Rgma	0.47	1.00E-08	1.03E-06	3421	22.31
Dbc1	0.38	1.01E-08	1.03E-06	3212	22.61
Cdh12	-0.49	1.03E-08	1.04E-06	5639	5.13
Npr3	-0.47	1.14E-08	1.14E-06	6927	3.59
Sv2b	-0.34	1.22E-08	1.22E-06	5418	39.54
Nr3c1	0.38	1.31E-08	1.30E-06	2137	16.03
Inf2	0.60	1.32E-08	1.30E-06	4600	1.66
C630043F03Rik	-0.71	1.42E-08	1.40E-06	2581	2.47
Gabbr2	0.33	1.58E-08	1.54E-06	3079	69.63
3110047P20Rik	-0.43	1.65E-08	1.60E-06	2821	50.19
Sla	0.49	1.71E-08	1.63E-06	2626	25.26
Gpr125	0.37	1.72E-08	1.63E-06	4475	7.35
Ankrd44	-0.42	1.72E-08	1.63E-06	1731	21.50

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Pak1	0.34	1.76E-08	1.66E-06	2887	63.36
Sgk1	0.41	1.77E-08	1.66E-06	749	33.87
Dmrtb1	0.76	1.81E-08	1.68E-06	1327	2.74
Ptch1	0.47	1.98E-08	1.83E-06	4305	3.45
Gpr85	-0.37	2.03E-08	1.87E-06	2174	53.96
Kcnip1	0.40	2.08E-08	1.90E-06	2322	19.21
Col14a1	-1.57	2.81E-08	2.55E-06	6452	0.21
Rasl11b	0.49	3.24E-08	2.92E-06	1799	7.55
Plekho2	0.72	3.25E-08	2.92E-06	3271	1.27
Ncapg	-1.08	3.34E-08	2.98E-06	3154	0.95
Ssbp2	-0.33	4.13E-08	3.64E-06	880	110.95
Sstr2	0.48	4.13E-08	3.64E-06	1537	7.83
Rdh5	0.98	4.36E-08	3.82E-06	668	2.47
Tspan5	0.31	4.75E-08	4.13E-06	885	121.90
Tmem35	-0.30	4.95E-08	4.29E-06	1918	81.60
Emx1	0.69	5.44E-08	4.68E-06	1410	6.98
Sphkap	0.31	5.79E-08	4.95E-06	5757	22.97
Fam107a	0.60	5.81E-08	4.95E-06	2622	2.92
Kitl	-0.46	5.92E-08	5.01E-06	1260	48.89
Lrrc55	0.50	6.01E-08	5.05E-06	2559	6.27
Dock5	1.14	6.70E-08	5.60E-06	10335	0.10
Arl4d	0.52	7.06E-08	5.87E-06	1382	6.32
Bub1	-0.92	7.21E-08	5.97E-06	609	5.53
Met	-0.35	7.38E-08	6.07E-06	3541	51.87
Mfap3l	0.41	7.94E-08	6.49E-06	3179	10.59
Dhrs1	0.39	8.33E-08	6.78E-06	993	42.49
Dot1l	0.42	8.49E-08	6.86E-06	2820	10.03
Elmo1	-0.35	9.33E-08	7.51E-06	3639	21.64
Nek6	0.31	1.03E-07	8.25E-06	1513	43.19
Dsg2	-1.57	1.08E-07	8.61E-06	3590	0.38
Fam20a	0.98	1.16E-07	9.12E-06	1685	0.89
Calb2	0.44	1.16E-07	9.12E-06	1430	30.47
Pllp	0.76	1.20E-07	9.36E-06	1889	1.54
Nuf2	-0.87	1.21E-07	9.41E-06	2236	1.60
Atp1a2	0.48	1.26E-07	9.73E-06	3385	49.31
Nkain4	0.67	1.36E-07	1.05E-05	450	10.52
Vat1l	-0.29	1.37E-07	1.05E-05	2066	49.22
Olig1	0.41	1.47E-07	1.12E-05	2171	30.12
Rab3il1	0.73	1.52E-07	1.15E-05	1427	2.30
Pcdh7	0.37	1.64E-07	1.24E-05	5231	5.61
Vcan	-0.51	1.69E-07	1.27E-05	2273	25.65
Cxcl12	-0.66	1.72E-07	1.29E-05	3013	3.11
Socs2	-0.45	1.78E-07	1.32E-05	884	36.56
Scrt2	0.47	1.90E-07	1.40E-05	3395	3.39
Rgs6	0.45	2.10E-07	1.55E-05	1754	10.95
Pgap1	-0.34	2.15E-07	1.58E-05	10579	14.05
Pcdh17	-0.32	2.28E-07	1.66E-05	9509	18.16
Pcx	0.56	2.37E-07	1.72E-05	4137	4.19
Glra2	-0.36	2.51E-07	1.81E-05	3150	14.54
Syt17	-0.58	2.64E-07	1.90E-05	1617	5.80
Unc5c	-0.45	2.83E-07	2.02E-05	3498	15.23
Kcnj6	0.66	3.09E-07	2.20E-05	3086	1.27

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Arhgap28	-0.46	3.20E-07	2.27E-05	2037	7.55
Sh3rf1	-0.29	3.35E-07	2.35E-05	5181	17.69
Pde8b	0.53	3.37E-07	2.35E-05	2365	2.96
Chst11	0.28	3.39E-07	2.35E-05	5527	15.16
Kcnj2	-0.53	3.40E-07	2.35E-05	5444	1.79
2810417H13Rik	-0.79	3.40E-07	2.35E-05	2188	2.12
Gabra5	-0.32	3.45E-07	2.37E-05	2653	50.89
Hunk	0.40	3.70E-07	2.54E-05	5009	4.36
Lhfp	0.43	3.72E-07	2.54E-05	1932	8.00
Syt9	-0.41	3.76E-07	2.56E-05	2536	9.10
Ppp1r3b	-1.66	3.86E-07	2.61E-05	4206	0.29
Arg2	0.52	4.06E-07	2.73E-05	1428	5.17
Gpr158	0.42	4.13E-07	2.77E-05	5376	2.99
Col19a1	0.81	4.25E-07	2.83E-05	4266	0.52
Gpr21	0.53	4.31E-07	2.86E-05	1923	4.36
Drc1	-0.41	4.38E-07	2.89E-05	2484	7.64
Slc4a8	0.27	4.66E-07	3.07E-05	641	176.31
Lynx1	0.46	4.73E-07	3.10E-05	3952	4.96
6330403K07Rik	-0.31	4.76E-07	3.11E-05	1494	58.19
Tenm2	-0.31	4.80E-07	3.12E-05	2408	110.94
Epha3	0.37	4.96E-07	3.21E-05	5659	12.23
3110035E14Rik	-0.37	5.11E-07	3.29E-05	3256	44.07
Vwc2l	-0.59	5.39E-07	3.45E-05	4257	2.01
Tmeff2	-0.39	5.81E-07	3.70E-05	2718	13.15
Stat5a	1.76	5.99E-07	3.81E-05	3605	0.08
Fam65b	-0.29	6.07E-07	3.84E-05	2342	33.41
Cacng2	0.40	6.13E-07	3.86E-05	5510	8.50
Dio2	-0.52	6.87E-07	4.31E-05	5813	2.20
Rbl1	-0.75	7.01E-07	4.38E-05	2549	1.88
Mcm6	-0.43	7.50E-07	4.67E-05	2901	7.93
Mro	0.63	8.22E-07	5.09E-05	1825	2.34
Car10	-0.66	8.41E-07	5.18E-05	2613	2.03
Myo18b	-1.14	8.90E-07	5.46E-05	8280	0.50
Them6	0.96	9.01E-07	5.51E-05	1488	0.87
Ugt8a	0.63	9.05E-07	5.51E-05	3583	1.11
Uhrf1	-0.68	9.08E-07	5.51E-05	3391	1.83
F3	0.43	9.29E-07	5.61E-05	1876	6.97
9130024F11Rik	-0.52	9.42E-07	5.67E-05	1221	8.08
Rrp12	0.43	9.46E-07	5.67E-05	4319	7.10
Slfn9	-0.91	9.72E-07	5.80E-05	3862	0.71
Diap3	0.50	9.95E-07	5.91E-05	4049	1.89
Abcc4	0.45	1.04E-06	6.13E-05	5617	1.83
Asic4	0.59	1.05E-06	6.21E-05	2566	2.30
Sema5a	0.35	1.07E-06	6.27E-05	10809	3.03
Crhr1	0.56	1.11E-06	6.47E-05	2460	2.14
Sidt1	0.50	1.19E-06	6.94E-05	1824	3.99
Crim1	-0.28	1.30E-06	7.52E-05	5995	15.89
Schip1	-0.28	1.31E-06	7.58E-05	1625	108.99
Klf6	-0.31	1.32E-06	7.61E-05	4217	26.51
Chchd10	0.51	1.33E-06	7.65E-05	939	9.09
Gpr37l1	0.77	1.34E-06	7.65E-05	2269	1.07
Cntnap1	0.97	1.35E-06	7.67E-05	3052	0.40

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Slit2	-0.39	1.35E-06	7.67E-05	1930	25.44
Grp	-0.41	1.37E-06	7.76E-05	862	30.57
St3gal5	0.37	1.40E-06	7.85E-05	2342	15.93
Desi1	0.29	1.50E-06	8.38E-05	1368	46.71
Rapgef5	-0.32	1.51E-06	8.43E-05	3987	11.07
Dcn	-0.50	1.53E-06	8.51E-05	1840	9.38
Rin2	0.46	1.78E-06	9.85E-05	473	22.29
Trpc3	0.60	1.78E-06	9.85E-05	361	13.53
Htra1	0.42	1.80E-06	9.92E-05	1572	8.77
Casp3	-0.29	1.81E-06	9.94E-05	1455	105.78
Cdc42se1	0.32	1.94E-06	0.00010583	653	64.32
Sptssb	1.86	2.11E-06	0.00011499	1771	0.12
Tor3a	0.43	2.14E-06	0.00011588	2518	5.46
Dkk3	0.32	2.18E-06	0.00011779	3357	23.76
Pcdh10	-0.25	2.30E-06	0.0001237	6051	19.66
Gas1	0.41	2.37E-06	0.00012706	2961	5.53
Wdr66	-0.55	2.40E-06	0.00012842	3679	2.09
Atp2b2	0.29	2.47E-06	0.00013109	4586	28.26
Bcl6	0.63	2.47E-06	0.00013109	3729	1.04
Robo2	0.28	2.49E-06	0.00013133	4046	24.77
Pcsk2	-0.29	2.50E-06	0.00013133	4205	27.20
Dgat2	0.37	2.50E-06	0.00013133	2251	14.86
Lingo3	0.46	2.52E-06	0.0001318	3433	5.54
Cnn1	-0.96	2.62E-06	0.00013644	2368	1.41
Camk1d	0.26	2.69E-06	0.00013987	974	139.78
Kera	-0.86	2.73E-06	0.00014117	1950	1.43
Oxtr	0.60	2.74E-06	0.00014123	4568	0.88
Tril	0.37	2.76E-06	0.00014123	5355	3.99
Adcy8	0.44	2.77E-06	0.00014123	5032	3.49
Mpp7	0.72	2.78E-06	0.00014123	4975	0.51
1700001L19Rik	-0.50	2.78E-06	0.00014123	1808	6.41
Pfkfb3	0.28	2.81E-06	0.00014191	2008	27.12
Rab31	0.26	2.81E-06	0.00014191	3476	31.23
Gpr83	1.00	2.82E-06	0.00014191	3586	0.31
Tst	0.55	2.86E-06	0.00014334	1096	5.50
1700030J22Rik	-0.59	2.94E-06	0.00014687	2971	2.13
Wdr76	-0.74	2.98E-06	0.00014851	2789	1.36
Ccna2	-0.70	3.17E-06	0.00015749	1336	6.35
Pla2g7	0.38	3.24E-06	0.00016053	926	114.92
Pgm5	-1.47	3.31E-06	0.00016345	926	1.13
Iglon5	0.39	3.39E-06	0.00016673	2619	12.71
Glycam1	0.90	3.41E-06	0.00016673	625	2.40
Stk32b	-0.31	3.41E-06	0.00016673	3437	11.59
Ctsl	0.28	3.56E-06	0.00017347	1971	62.73
Slco5a1	0.36	3.60E-06	0.00017446	3653	5.02
C2cd4c	-0.41	3.72E-06	0.00018008	6681	3.22
Cdk1	-0.74	3.80E-06	0.00018299	695	9.23
Pbk	-0.68	3.81E-06	0.00018299	451	9.83
Obscn	-1.85	3.84E-06	0.00018398	14205	0.21
Mycn	0.28	3.87E-06	0.00018466	2522	20.55
Camk4	0.29	3.89E-06	0.00018499	12331	20.98
Maf	0.33	3.91E-06	0.00018499	4792	8.98

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Spata13	0.39	3.91E-06	0.00018499	869	22.46
Nxph2	-0.36	3.96E-06	0.00018662	2579	13.73
Negr1	-0.32	3.99E-06	0.00018755	2024	89.76
Opcml	0.30	4.06E-06	0.00019023	1554	232.37
Dscc1	-1.53	4.10E-06	0.00019117	1499	0.62
Slitrk4	-0.36	4.11E-06	0.00019117	3513	12.88
Vstm2a	-0.30	4.13E-06	0.00019136	2505	32.97
Nov	-0.36	4.14E-06	0.00019136	2869	22.53
Trp53i11	-0.36	4.20E-06	0.00019352	1371	20.73
Zcchc24	0.29	4.24E-06	0.00019491	4375	11.69
Adamts2	0.51	4.32E-06	0.00019786	1154	5.37
Pcp4l1	0.56	4.37E-06	0.00019961	1577	3.05
Cntnap5c	-0.72	4.44E-06	0.00020218	3918	0.97
Zfp423	-0.45	4.47E-06	0.00020308	746	21.02
Gjc3	1.81	4.52E-06	0.00020441	3515	0.06
Syt4	-0.29	4.69E-06	0.00021157	3901	99.81
Ntf3	-0.54	4.77E-06	0.00021477	1372	10.83
Slit3	-0.36	4.92E-06	0.0002205	865	28.32
Adcy2	0.29	4.98E-06	0.00022257	4211	11.33
Cdk19	-0.31	5.09E-06	0.00022688	5741	12.42
Slc22a3	0.99	5.22E-06	0.00023189	3501	0.30
Tenm1	-0.30	5.25E-06	0.00023261	6457	14.18
Plbd2	0.32	5.31E-06	0.00023476	4208	7.09
Hdac9	-0.32	5.39E-06	0.00023737	4422	15.48
Spock1	0.33	5.48E-06	0.00024065	1320	27.63
Large	-0.24	5.66E-06	0.00024801	3669	52.46
Adrbk2	-0.29	5.83E-06	0.00025464	6537	7.70
Gpam	0.30	5.98E-06	0.00026032	3832	13.77
Bmp1	0.36	6.13E-06	0.00026613	3757	4.45
Dnalc1	-0.28	6.34E-06	0.00027458	818	84.84
Frzb	0.80	6.54E-06	0.00028222	1990	0.90
Dpysl2	-0.21	6.58E-06	0.00028305	4520	75.93
Rcan1	0.29	6.79E-06	0.00029089	2258	22.40
Mki67	-0.81	6.80E-06	0.00029089	10075	2.17
Ssx2ip	0.28	6.95E-06	0.00029649	2244	31.52
Trpv6	0.77	7.04E-06	0.00029933	2926	0.63
C230081A13Rik	-0.33	7.07E-06	0.00029967	10977	3.27
Pde1a	-0.34	7.23E-06	0.00030589	1667	114.82
Myo1b	-0.34	7.55E-06	0.00031841	969	42.81
Cdk5r2	0.45	7.83E-06	0.00032914	2704	33.84
Kdm6b	0.36	8.40E-06	0.00035241	3699	11.82
Pter	-0.37	9.24E-06	0.00038643	916	27.62
B3galt2	-0.33	1.00E-05	0.00041767	5080	7.85
Wdr35	-0.30	1.04E-05	0.00043218	2921	14.80
Robo1	-0.29	1.06E-05	0.00044083	7563	13.22
Hap1	-0.39	1.09E-05	0.0004499	976	46.47
Sned1	0.73	1.10E-05	0.00045413	1236	1.71
Sema3a	-0.42	1.12E-05	0.00046152	2285	6.56
Ezr	0.39	1.25E-05	0.00051391	1904	32.50
Fbn2	-0.69	1.36E-05	0.00055447	10480	1.48
Pde1c	-0.62	1.42E-05	0.00057762	6485	0.72
Ctsb	0.23	1.44E-05	0.00058354	4739	70.14

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Ier5	0.34	1.45E-05	0.00058802	3270	5.39
Ypel2	-0.60	1.47E-05	0.00059327	4847	1.18
Rasgrf1	-0.33	1.47E-05	0.00059327	2613	14.39
D17H6S56E-5	-0.61	1.52E-05	0.00060956	1221	4.46
Zcchc18	-0.22	1.55E-05	0.00062322	2259	96.10
Nefl	0.32	1.58E-05	0.0006314	3380	18.50
Smarca2	-0.26	1.60E-05	0.00063736	929	281.18
Cx3cl1	0.34	1.60E-05	0.00063736	719	221.55
A030009H04Rik	-0.25	1.62E-05	0.00064314	1235	47.92
Adamts18	-0.42	1.63E-05	0.0006433	5642	2.11
Cdkl1	-0.58	1.65E-05	0.00064994	1689	2.97
Megf6	0.60	1.66E-05	0.00065334	2187	1.81
Espl1	-0.74	1.67E-05	0.000657	6630	0.50
Slc16a2	-0.22	1.70E-05	0.00066702	659	387.23
Elovl5	0.27	1.73E-05	0.00067511	662	122.68
Ckap2l	-0.58	1.73E-05	0.00067511	3137	2.04
Rgcc	0.53	1.83E-05	0.00071168	918	5.56
Casc5	-0.75	1.86E-05	0.00072148	5527	0.53
Pygl	-0.70	1.91E-05	0.00073848	925	3.75
Cntnap5a	0.54	1.92E-05	0.00074043	4783	1.17
Mc4r	-0.75	1.96E-05	0.00075462	2758	1.06
March1	-0.35	1.99E-05	0.00076279	3181	28.95
Klf8	-0.37	2.00E-05	0.00076389	4407	6.30
Nrk	-1.54	2.00E-05	0.00076389	6604	0.28
Nnmt	-0.61	2.07E-05	0.00078624	737	5.95
Cacna2d2	0.38	2.15E-05	0.00081708	5183	2.64
Grm8	-0.62	2.16E-05	0.00081815	3065	1.46
Epas1	0.41	2.19E-05	0.00082788	5309	1.97
Map3k5	0.36	2.28E-05	0.00085848	720	19.28
Zfp771	0.49	2.32E-05	0.00087015	703	8.97
Magi3	-0.26	2.37E-05	0.00088908	5912	8.62
Nxph3	0.58	2.48E-05	0.00092755	2099	1.70
Cdh22	0.45	2.52E-05	0.00093917	3112	2.22
Prnp	-0.23	2.53E-05	0.00094045	1252	432.17
Ptprj	0.27	2.64E-05	0.00098042	5661	6.94
Kcnip4	0.54	2.68E-05	0.00098986	1611	3.28
Htr2a	-0.77	2.68E-05	0.00098986	2971	0.89
Mical2	0.30	2.69E-05	0.00099173	3744	17.51
Gm13716	0.55	2.73E-05	0.00100004	808	5.18
Cntnap4	-0.30	2.73E-05	0.00100004	3257	11.05
Nr1d1	0.35	2.80E-05	0.00102433	1751	11.53
Pygo1	-0.25	2.89E-05	0.00105053	7916	13.51
Sulf2	0.23	2.89E-05	0.00105053	897	127.83
Esr1	-0.55	2.90E-05	0.00105053	2673	2.05
Cck	-0.29	2.90E-05	0.00105109	685	88.78
Cand2	-0.47	2.92E-05	0.00105513	4810	1.82
Flrt3	-0.34	2.96E-05	0.00106479	3334	17.60
Nt5c	0.42	2.97E-05	0.00106635	836	9.77
Kcnk9	0.43	3.01E-05	0.00107997	1209	6.47
Kcnc2	-0.33	3.10E-05	0.00110767	6196	3.67
Rnd1	0.33	3.14E-05	0.00111822	1034	23.13
Kif20a	-0.73	3.17E-05	0.00112867	3566	1.07

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Rell1	0.41	3.25E-05	0.00115241	530	18.96
Gpr30	1.50	3.30E-05	0.00116684	1515	0.20
Ube2e3	-0.28	3.35E-05	0.00118472	632	235.86
Ccng2	-0.27	3.43E-05	0.00120769	1747	35.02
Zmym3	-0.23	3.46E-05	0.00121706	1548	75.95
4833422C13Rik	-0.38	3.62E-05	0.00126884	2083	7.67
Slc43a2	0.29	3.63E-05	0.00126884	841	78.78
Zfp57	-0.26	3.63E-05	0.00126884	762	53.70
Rasl10a	0.77	3.66E-05	0.00127221	902	1.76
Fam163a	0.51	3.66E-05	0.00127221	3882	1.18
Hapln1	0.38	3.94E-05	0.00136574	5055	4.44
Extl2	0.25	3.97E-05	0.0013743	1741	27.90
Drd2	-0.74	4.03E-05	0.00139071	1549	1.75
Rsbn1l	-0.31	4.05E-05	0.0013947	5550	4.37
Nfic	0.32	4.25E-05	0.00146115	2409	9.94
Nt5e	0.66	4.32E-05	0.00148176	3580	0.63
Ttk	-0.65	4.46E-05	0.00151996	2904	1.24
Fndc9	-0.49	4.46E-05	0.00151996	2290	2.96
Dpp7	0.58	4.50E-05	0.00152302	930	3.48
Tenm4	-0.26	4.50E-05	0.00152302	1337	78.83
Adamts19	-1.44	4.50E-05	0.00152302	4666	0.17
Agap1	0.26	4.56E-05	0.0015401	9502	16.80
Ptchd2	0.40	4.59E-05	0.00154845	595	16.81
Gbe1	0.32	4.66E-05	0.00156703	2846	11.57
Cacng5	0.39	4.73E-05	0.00158651	3692	2.97
Cntn4	0.39	4.78E-05	0.00160118	2377	4.28
Ptar1	0.56	4.79E-05	0.00160118	2064	1.65
Qrsl1	0.48	4.82E-05	0.00160747	1981	2.80
Lims2	0.94	4.85E-05	0.00161359	1688	0.53
Filip1	-0.62	4.89E-05	0.00162125	3775	1.20
Lonrf1	-0.28	4.94E-05	0.00163565	3930	10.38
Lrfn5	-0.26	5.05E-05	0.0016691	3812	27.41
Rps6ka6	-0.33	5.07E-05	0.00167137	750	46.39
Man1a	-0.35	5.51E-05	0.0018119	2842	11.25
Slc25a5	0.23	5.62E-05	0.00184155	989	82.34
Hey1	0.33	5.62E-05	0.00184155	2203	8.22
Csrnp3	-0.24	5.69E-05	0.00185836	2231	100.64
Rgs10	0.40	5.74E-05	0.00186909	464	18.91
Mpp6	0.28	5.75E-05	0.00186909	1281	33.22
Ank1	0.35	5.78E-05	0.00187605	6257	2.33
Nme5	-0.53	5.84E-05	0.0018891	786	7.19
Tvp23a	0.32	5.85E-05	0.0018891	838	33.37
Tmem200a	-0.31	5.86E-05	0.0018891	4115	6.25
Asrgl1	0.26	6.01E-05	0.00192926	2258	26.45
Srm	0.27	6.02E-05	0.00192926	841	41.78
Flrt2	-0.28	6.04E-05	0.00192926	5161	11.58
Fam111a	-0.56	6.05E-05	0.00192926	3477	1.56
Arhgap44	0.28	6.05E-05	0.00192926	3687	12.28
Hells	-0.48	6.07E-05	0.00192926	781	9.27
Per3	0.52	6.07E-05	0.00192926	4253	1.00
Tpm2	-1.19	6.12E-05	0.00193582	1355	1.17
Angptl4	0.54	6.12E-05	0.00193582	1217	5.33

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Slc13a5	0.74	6.18E-05	0.00194974	640	2.58
Rnf152	0.29	6.23E-05	0.00196255	8468	4.30
Ndufa5	0.29	6.30E-05	0.0019799	443	64.75
Gpcpd1	0.27	6.47E-05	0.00202803	3431	24.17
Neto1	-0.27	6.76E-05	0.00211468	3531	18.21
Btbd17	0.62	6.86E-05	0.00214301	677	3.74
Olfm4	0.81	6.89E-05	0.00214408	1640	0.76
Grm5	-0.24	6.90E-05	0.00214408	3612	82.77
Cacna1e	-0.25	7.01E-05	0.00217431	12697	8.57
Kcnq4	0.49	7.15E-05	0.00221311	2328	1.90
Npc2	0.27	7.21E-05	0.0022278	3261	30.90
Syt10	0.84	7.35E-05	0.00226689	1845	0.61
Fat4	-0.29	7.42E-05	0.00228477	9552	9.81
Igfbp5	-0.28	7.47E-05	0.00229425	5144	37.65
4930444P10Rik	-1.71	7.49E-05	0.00229425	1001	0.58
5430435G22Rik	0.68	7.80E-05	0.00238565	607	3.38
Acta2	-0.59	7.90E-05	0.00241088	1781	29.06
Myo5b	-0.25	7.95E-05	0.00242139	764	63.55
Pnkp	0.44	8.00E-05	0.0024326	948	6.86
Slc17a6	-0.29	8.08E-05	0.00245013	4319	10.64
Grm3	-0.33	8.25E-05	0.00249799	3536	6.12
Mapk10	-0.22	8.31E-05	0.00250954	2357	72.91
Spa17	-1.01	8.34E-05	0.00251312	689	2.01
Hivep3	0.31	8.39E-05	0.00252428	1237	46.01
Gbp7	0.50	8.42E-05	0.00252832	5541	0.90
Trib2	0.22	8.46E-05	0.00253552	2504	33.71
Traf3	0.24	8.54E-05	0.00255303	6896	10.18
Cgrrf1	0.68	8.58E-05	0.00256006	1094	1.75
Usp49	-0.34	8.65E-05	0.00257686	994	17.20
Jmy	0.23	9.00E-05	0.0026755	8780	8.89
Morc4	0.46	9.09E-05	0.00269695	1475	3.56
Mgll	0.33	9.21E-05	0.00272654	1237	29.36
Frmd4b	0.33	9.38E-05	0.00277228	1419	11.15
Lpar1	0.53	9.40E-05	0.00277274	3333	1.15
Sh3kbp1	-0.25	9.67E-05	0.00284544	2598	31.27
Paqr7	0.34	9.82E-05	0.00288319	780	17.95
Fam101b	-0.35	0.00010001	0.00292741	3512	4.63
Hif3a	-0.93	0.00010024	0.00292741	1334	2.31
Ttc9c	-0.27	0.00010028	0.00292741	1389	30.52
Abtb2	0.37	0.00010521	0.00306547	2875	3.41
Gm5454	-0.41	0.00010723	0.003118	1455	6.29
Ube2l6	-0.48	0.00010778	0.00312805	848	7.41
Reep4	0.91	0.00011001	0.00318634	1668	0.53
Tle1	-0.30	0.00011365	0.00328438	2885	18.43
Sfxn5	0.27	0.00011392	0.00328438	618	74.16
Zim1	-0.38	0.00011417	0.00328438	3157	3.45
Gins1	-0.64	0.00011428	0.00328438	625	5.12
Akirin1	0.27	0.00011514	0.00330135	763	34.05
Gpr133	1.27	0.00011534	0.00330135	3665	0.10
Chst15	-0.29	0.00011554	0.00330135	4813	5.69
Msrb3	-0.73	0.00011704	0.00333748	896	5.04
Pgam2	-0.83	0.00011726	0.00333748	840	5.32

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Mknk1	0.33	0.00011791	0.00334959	680	20.15
Stbd1	-0.70	0.0001188	0.0033686	1985	1.31
Mcm2	-0.36	0.00012138	0.00343503	3370	3.85
Ccdc148	-0.40	0.00012263	0.00346368	3977	3.10
Slc6a7	0.39	0.00012346	0.0034805	3342	3.50
Rxfp3	-0.47	0.00012417	0.0034941	4252	1.60
Gm11189	0.67	0.00012508	0.00351058	375	4.98
Pnmal1	-0.27	0.00012523	0.00351058	1686	22.32
Zbtb20	0.29	0.00012557	0.00351346	736	184.74
Epb4.1l2	0.23	0.00012723	0.003553	4335	12.00
Mcm5	-0.40	0.0001279	0.00356512	3422	3.95
AF529169	-0.43	0.00013133	0.00365384	4241	1.89
Bub1b	-0.57	0.00013238	0.00367599	618	7.10
Timeless	-0.50	0.00013268	0.00367753	807	6.48
Cox18	0.41	0.00013437	0.00371743	754	10.15
5033430I15Rik	0.79	0.00013534	0.00373503	721	1.66
Stac	-0.58	0.00013551	0.00373503	2241	1.75
Sat1	0.33	0.0001398	0.00384615	1162	12.12
Pcolce2	0.76	0.0001416	0.00388302	2531	0.53
Ephb6	0.32	0.00014167	0.00388302	690	53.57
Afap1l2	0.36	0.00014269	0.00390372	1724	6.46
Ncoa1	0.22	0.00014302	0.00390551	7328	16.45
Frmpd1	0.52	0.00014379	0.00391939	4812	0.72
Cnr1	-0.23	0.0001443	0.00392596	5761	46.25
Gria1	-0.19	0.00014618	0.00396984	5361	73.68
Heca	-0.28	0.00015031	0.00407453	3667	12.35
Rin1	-0.51	0.00015432	0.00416901	4176	1.21
Npbwr1	-0.69	0.00015436	0.00416901	3692	0.71
N4bp2	-0.31	0.00015672	0.00421773	632	44.91
Megf10	0.36	0.00015673	0.00421773	5460	2.38
Got1	0.24	0.00015911	0.00427363	1047	50.68
C1ql3	-0.32	0.00015938	0.00427363	2476	7.40
Slc12a4	0.37	0.00016038	0.00429243	715	17.20
Trim59	-0.34	0.00016406	0.00438097	411	39.66
Galnt18	0.38	0.00016428	0.00438097	2553	5.24
Cmya5	-1.24	0.00016537	0.00440215	11850	0.08
Tmem100	0.79	0.00017051	0.00453099	1767	0.67
Spsb1	0.40	0.00017106	0.00453221	527	13.12
Elovl2	-0.29	0.00017117	0.00453221	3762	17.98
Arhgef26	0.26	0.00017181	0.00454093	901	55.55
Chadl	0.60	0.0001725	0.00455104	2548	0.94
Scn3a	-0.22	0.0001754	0.00461946	6026	16.68
Gja4	-1.08	0.00017876	0.00469954	1685	0.65
Stil	-0.57	0.00018593	0.00487922	1434	2.62
Smarca1	-0.26	0.00018816	0.00492594	2304	30.84
Fam57b	0.32	0.00018837	0.00492594	2113	26.07
AC119212.1	-0.38	0.00019104	0.00498682	1231	8.04
Acox2	1.08	0.00019477	0.00507538	747	0.74
Akap5	-0.30	0.0001956	0.00508795	6685	4.69
Acsl6	0.22	0.00019603	0.00509026	2432	22.81
Gpr146	0.49	0.00019827	0.0051395	3054	1.40
Myo6	0.27	0.00020106	0.00520271	875	58.97

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Trhr	-0.63	0.00020176	0.00521166	2861	1.11
Arhgef15	-0.85	0.00020484	0.00528193	1732	0.95
4930452B06Rik	-0.40	0.00020524	0.00528316	2681	3.27
Rbfox3	0.27	0.00020725	0.00532562	1548	46.07
Insm1	0.41	0.00020921	0.00536646	3096	1.96
Fezf2	-0.31	0.00021017	0.00538198	2304	7.69
Steap2	0.35	0.00021429	0.00547789	3470	2.95
Sst	-0.32	0.00021502	0.00548716	599	224.43
Tmem2	-0.31	0.00021661	0.00551814	6626	4.42
Fas	0.63	0.00022247	0.00565779	1021	2.01
Lphn2	-0.23	0.00022316	0.00566558	2585	57.83
Shank2	0.26	0.00022456	0.00569117	7666	14.84
Arhgap6	-1.39	0.00022624	0.00572401	4053	0.17
Eif1b	-0.23	0.00022811	0.00576145	917	149.36
Vav2	0.23	0.00022884	0.00577013	848	59.10
Mr1	0.99	0.00023217	0.00584408	2509	0.27
Hcrtr2	-0.55	0.00023349	0.00586734	3705	1.35
Nipsnap3b	-0.29	0.00023428	0.00587732	848	42.83
Sowaha	-0.32	0.00023993	0.00600539	3617	4.35
Blvrb	0.41	0.00024035	0.00600539	782	14.77
Tfpi	-1.06	0.00024061	0.00600539	2495	0.55
Dpf2	0.27	0.0002411	0.00600767	1115	23.32
Scand1	0.63	0.00024471	0.00608741	759	2.96
Tm6sf1	0.68	0.00024577	0.00610347	2094	0.77
Gm11837	-0.77	0.00024944	0.00618426	761	2.63
Tesc	0.70	0.0002509	0.00621	883	1.70
Esco2	-0.71	0.00025189	0.00622416	805	2.90
Mme	0.49	0.00025255	0.00622993	4240	0.96
Acss2	0.35	0.00025334	0.00623898	1896	6.88
Arhgap20	0.20	0.00025486	0.00626619	2670	35.67
Prex2	0.44	0.00026166	0.00642249	11054	0.66
Ppargc1a	0.24	0.00026222	0.00642578	1773	25.30
Greb1l	-0.39	0.0002642	0.00645631	2850	3.12
Ednrb	0.25	0.00026434	0.00645631	3934	17.61
Tspan4	0.31	0.00026736	0.00651914	654	25.87
Cdh4	0.25	0.00026813	0.00652739	747	85.94
Astn2	0.28	0.00027183	0.0066064	4817	4.17
Zfp184	-0.50	0.00027246	0.00661096	2459	2.15
Chn2	-0.25	0.0002746	0.00665105	1227	32.44
Mtss1	-0.18	0.00027501	0.00665105	4965	49.37
Wwc1	0.25	0.00027721	0.00669325	728	192.31
Igf1r	0.24	0.00028089	0.006771	4489	7.27
Npy	0.33	0.0002902	0.00698403	566	29.45
Carhsp1	-0.21	0.00029395	0.007063	2849	30.67
Entpd2	0.73	0.00029791	0.00714639	1784	0.77
Micu2	-0.25	0.00030003	0.00718561	2310	24.00
Adcyap1	-0.41	0.00030223	0.00722656	2088	3.74
Rhbdl3	0.48	0.00030365	0.00724884	827	4.72
Gna14	-1.08	0.00030482	0.00726502	3383	0.31
Scrt1	0.36	0.00030584	0.00726875	3744	8.86
Fam195a	0.48	0.00030595	0.00726875	816	4.66
Rbm48	-0.44	0.00031069	0.00736952	641	10.69

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Mcm3	-0.38	0.00031306	0.00740567	2991	3.63
Rfx1	-0.42	0.00031322	0.00740567	4172	1.93
Dll4	-1.09	0.0003192	0.00753502	2220	0.45
Lgals1	-0.40	0.00032178	0.00758378	800	16.56
Brca1	-0.53	0.00032491	0.00764545	2027	2.25
Naaa	0.36	0.00032616	0.00766261	1629	7.03
Hs3st4	0.34	0.00032876	0.00771161	3189	3.14
Rarb	-0.44	0.00033041	0.00773798	3034	2.20
Hnrnpl	0.19	0.00033293	0.00778473	1850	72.25
Top2a	-0.52	0.00033358	0.00778762	803	39.23
Il6st	0.23	0.00034337	0.00800349	5207	11.83
Tspan12	0.30	0.00034505	0.00803	727	28.15
Clstn2	0.23	0.00034839	0.00809495	4270	21.08
Nudt18	0.35	0.00034999	0.00811952	3716	3.39
Hdac4	0.29	0.00035242	0.00816304	3937	5.99
Gpc6	0.33	0.00035495	0.00820868	3817	3.71
Fstl4	0.40	0.00036022	0.00831767	4380	1.61
Gm10169	0.34	0.00036108	0.00832458	445	27.11
Tnfaip3	-0.53	0.00036589	0.00841406	2549	1.59
Il1r2	1.40	0.0003661	0.00841406	1337	0.19
Syndig1l	0.38	0.00036768	0.00843721	2499	3.07
Tmem74	-0.29	0.0003746	0.00858284	1472	13.00
Aldh1a7	0.97	0.00037986	0.00868983	2066	0.31
Tshz3	0.31	0.00038094	0.0087011	2761	8.02
Olfm3	-0.45	0.0003834	0.00874374	3928	1.54
Nsun5	0.47	0.00038755	0.00882481	729	5.42
Aldh1l1	0.30	0.00039033	0.00887443	653	81.88
Syt3	0.32	0.000392	0.00889868	1318	16.04
Syt12	0.60	0.00039551	0.00895216	1288	1.58
Fam78b	-0.21	0.00039652	0.00895216	2426	27.71
Gm5540	0.41	0.00039669	0.00895216	1890	3.27
2310061I04Rik	0.29	0.00039682	0.00895216	658	23.76
Me1	0.21	0.0003975	0.00895216	3257	18.46
Cdh10	-0.26	0.00039798	0.00895216	3292	14.19
Gas6	0.26	0.00040416	0.00907723	2593	22.79
Lipg	-0.36	0.00040901	0.00917238	3787	3.05
Ccdc39	-0.37	0.00040975	0.00917508	2630	3.64
Mmgt2	0.38	0.00041751	0.0093286	388	17.53
Gucy1b3	-0.23	0.00041787	0.0093286	3251	48.18
Kcnk1	0.29	0.0004198	0.00933555	2301	8.16
Klhdc7a	0.47	0.00041999	0.00933555	5854	0.73
Tns3	0.27	0.00042007	0.00933555	2174	16.35
Nfasc	0.23	0.00043478	0.00964794	9630	10.41
Cdo1	-0.25	0.00043585	0.00965726	1530	24.74
Mfsd6	0.25	0.00044115	0.00976002	3493	19.41
Hsdl2	0.28	0.00044593	0.00985111	2611	8.43
Pdlim1	-0.84	0.00044717	0.00986383	1503	1.04
Gfap	0.29	0.00046282	0.0101666	2600	72.12
Etv1	-0.33	0.00046298	0.0101666	1578	16.40
Slc37a4	0.27	0.00046366	0.0101666	2083	9.38
Igsf9	0.30	0.00046465	0.0101666	796	21.82
Klhl14	-0.81	0.00046488	0.0101666	4221	0.41

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Kifc3	0.31	0.00046514	0.0101666	3266	8.23
Slc7a1	0.19	0.00046571	0.0101666	1792	56.83
Bdh2	0.71	0.00046824	0.01020672	1100	1.30
Ttc26	-0.42	0.00048009	0.0104497	706	10.43
Aldh1a1	0.89	0.00048205	0.01047696	2053	0.37
Fam5b	-0.21	0.00048276	0.01047699	4099	19.73
Lpin3	0.59	0.00048806	0.01056504	3365	0.61
Alcam	-0.24	0.00048824	0.01056504	1621	77.20
Ehbp1l1	-0.34	0.00049098	0.01060865	4988	4.76
Slc24a6	0.72	0.00049456	0.01065819	1624	0.82
Dclk3	-0.33	0.00049471	0.01065819	3495	3.77
Rps6ka5	-0.25	0.00049701	0.0106863	4406	9.95
Nsg2	-0.17	0.00049746	0.0106863	1974	493.73
Ndufa1	0.27	0.0005099	0.01093777	419	61.97
Zbtb6	-0.25	0.00051315	0.01098721	4806	9.62
Hs6st1	0.24	0.00051392	0.01098721	3719	15.83
Lurap1	-0.35	0.00051462	0.01098721	3101	3.88
Kcnh4	-0.86	0.00051558	0.01098721	3833	0.37
Pgrmc1	-0.20	0.00051592	0.01098721	1857	208.19
Nfil3	-0.31	0.00051857	0.01102775	2026	8.36
Cabp1	0.38	0.00052426	0.01113276	927	8.02
Gabrg3	0.26	0.00052658	0.01116279	1724	16.63
P4ha3	0.45	0.00052718	0.01116279	2123	2.07
Gpr161	-0.24	0.00052993	0.01120508	2974	10.73
Dnahc5	-0.48	0.00054079	0.01141824	15630	0.30
Fank1	-0.55	0.00054388	0.01146711	824	4.11
Phlda3	0.32	0.00054564	0.01148787	1002	16.68
Pak3	-0.25	0.00054777	0.01150042	8149	31.21
Tox2	-0.31	0.00054779	0.01150042	1675	9.59
Rabgap1l	-0.25	0.00055101	0.0115517	4856	54.54
Gpc3	-0.86	0.00055736	0.01166835	2048	4.88
Cobl	-0.23	0.00056248	0.01175877	3976	13.19
Itpkb	0.35	0.00056932	0.01188506	6235	1.67
Fignl1	-0.47	0.00057549	0.01199689	2807	2.01
Fam64a	-0.69	0.00057982	0.01207017	681	3.16
Zfp395	-0.44	0.00058658	0.01219367	4229	1.65
Thrsp	-0.37	0.0005921	0.01229119	1277	9.34
Mmd	0.20	0.00059339	0.01230077	1654	98.13
Tmc7	0.40	0.00060489	0.01252163	1434	3.89
Ntm	0.20	0.00061373	0.0126868	879	242.85
Pam	-0.23	0.00061706	0.01272284	684	250.00
Gabrd	0.52	0.00061749	0.01272284	1921	1.54
Klhl29	0.24	0.00061805	0.01272284	7041	3.49
Satb2	-0.25	0.00062022	0.01273655	4694	38.27
Fabp3-ps1	-0.30	0.00062165	0.01273655	656	35.65
Mob3b	0.52	0.00062168	0.01273655	3527	0.88
1700016K19Rik	-0.69	0.00062216	0.01273655	921	2.33
Sms	-0.23	0.00062939	0.01286675	1540	50.78
Sorbs2	-0.32	0.0006381	0.01302694	1079	81.11
Col11a1	0.29	0.00064022	0.01305222	2341	6.88
Cep55	-0.70	0.00065696	0.01337494	2385	0.88
Prkacb	-0.16	0.00065874	0.01339291	4001	156.12

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Pdzrn3	0.27	0.00065995	0.01339915	1818	14.77
Camk2a	0.19	0.00066897	0.01356359	1734	197.64
Macrod2	-0.22	0.00067009	0.01356774	1192	62.50
Iqgap3	-0.63	0.00067203	0.0135883	5676	0.51
Il33	-0.85	0.00067501	0.01363005	725	1.91
Cst3	0.22	0.00067594	0.01363028	758	359.74
Cox5a	0.20	0.0006802	0.01369752	690	67.90
Lpo	0.69	0.0006861	0.01379342	570	2.28
Zhx3	0.21	0.00068683	0.01379342	3532	16.60
Ahi1	-0.20	0.00068781	0.01379442	3459	32.67
Creg2	-0.23	0.00069442	0.01390817	5799	9.29
Tuba4a	0.20	0.00069939	0.01398882	2053	36.56
Ncapg2	-0.32	0.000711	0.01419989	6604	2.28
Rftn2	0.31	0.00071187	0.01419989	1976	5.90
Prom1	0.30	0.00071932	0.01432926	3557	5.37
Chek1	-0.54	0.00072455	0.01441414	1139	3.06
Ubfd1	-0.20	0.00072677	0.01443884	3001	37.60
Adam12	-0.32	0.00072984	0.01446284	675	19.46
Hs3st5	0.66	0.00072993	0.01446284	2408	0.60
Neat1	0.62	0.00073241	0.01449245	2017	0.88
Nav2	0.25	0.00073477	0.01451979	7271	9.05
Farp2	0.43	0.00073935	0.0145909	3908	1.10
Rgs5	-0.82	0.00074147	0.01461316	2411	0.60
Kcnh3	-0.28	0.00074672	0.01469711	3583	9.20
Lrig1	0.28	0.00075739	0.01488723	700	47.74
Angel1	0.39	0.00076223	0.0149625	585	10.78
Atad2	-0.40	0.00076473	0.0149918	5683	2.14
Cxxc5	0.34	0.0007708	0.01509069	2271	6.23
Mpv17l2	0.38	0.00077499	0.0151527	955	8.48
Mink1	0.25	0.00077721	0.01516578	777	56.18
Eda2r	0.29	0.00077771	0.01516578	1793	13.42
Pgk1	0.27	0.00078203	0.01523001	1764	9.78
Nol4	-0.22	0.0007883	0.01533204	2175	45.79
Tac2	-0.54	0.00079851	0.01549112	766	4.31
Asap1	0.20	0.0007989	0.01549112	3435	21.11
Ablim3	-0.21	0.00080044	0.01549112	4373	17.87
Cdh18	-0.93	0.00080067	0.01549112	1865	0.61
Zfp287	-0.27	0.00080198	0.01549624	2403	8.09
Dcaf12l1	-0.27	0.00080534	0.01554093	3502	6.99
Faah	0.24	0.0008215	0.01583214	2342	14.82
Nup62	0.25	0.0008357	0.01608293	2708	11.90
Prdx6	0.23	0.00083669	0.01608293	913	162.83
Snx33	0.34	0.00084314	0.01618594	4564	1.86
Nkain2	-0.35	0.00087057	0.01669089	273	32.48
Hpcal4	-0.19	0.00087324	0.01670609	600	329.97
Lig1	-0.32	0.00087441	0.01670609	1005	14.46
Tmsb4x	-0.16	0.00087512	0.01670609	768	923.57
Trhde	0.37	0.00087588	0.01670609	3557	2.08
Cort	0.39	0.00089939	0.01711748	677	9.53
Bop1	0.29	0.00089976	0.01711748	2476	11.61
1110065P20Rik	0.51	0.00091113	0.01729679	762	3.87
Trip13	-0.42	0.00091153	0.01729679	2267	2.50

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Slc38a4	-1.37	0.00091444	0.0173299	2920	0.89
Rfx4	0.28	0.00092115	0.01743473	2382	6.86
Racgap1	-0.27	0.00092265	0.01744075	768	27.37
Mboat2	0.24	0.00093128	0.01758152	2754	9.48
Ggt7	0.27	0.00093417	0.01761348	887	57.84
Bhlhe22	-0.19	0.00094095	0.01771885	3185	48.13
1700010I14Rik	-1.43	0.00096269	0.01810512	1769	0.30
Cenpf	-0.51	0.00096689	0.01816115	1805	4.92
A730049H05Rik	-0.93	0.00097045	0.0182049	2159	0.52
Lin7b	0.38	0.00097934	0.01834844	405	18.19
D130043K22Rik	-0.25	0.00098386	0.01840983	2675	9.96
Sash1	0.23	0.00098856	0.0184744	7183	5.63
Trnp1	0.24	0.00099088	0.01849435	1171	30.97
Fbxo2	0.35	0.00099337	0.01851747	1288	13.22
Gm1673	0.51	0.00099842	0.0185788	451	29.38
Smc2	-0.36	0.00099917	0.0185788	1660	11.56
Spag6	-0.32	0.00100337	0.01863036	887	12.27
Npy2r	-0.61	0.00100446	0.01863036	3194	0.80
Zhx2	0.28	0.00101236	0.01873167	2294	6.87
Pbxip1	0.30	0.00101428	0.01873167	699	69.98
Gm9763	0.47	0.0010143	0.01873167	270	11.98
Grik1	-0.29	0.00101499	0.01873167	2688	7.19
Hsd17b4	0.21	0.00101995	0.01879986	585	84.07
Prlr	0.69	0.00103138	0.01898679	1244	1.06
Hsph1	0.19	0.00104293	0.01917572	3097	61.51
Adamts17	0.24	0.00104688	0.01922445	1885	15.61
Sox21	0.35	0.00106111	0.01946158	3447	2.16
Clspn	-0.51	0.00106362	0.01948348	785	4.48
Zc4h2	-0.21	0.0010687	0.01955239	2156	20.36
Kif11	-0.50	0.00108177	0.01974834	4819	1.86
Maged2	-0.19	0.00108298	0.01974834	1427	64.47
Reps2	-0.21	0.00108353	0.01974834	7146	14.94
Mfap4	-0.98	0.00108549	0.01974834	1331	0.84
Rrm1	-0.26	0.00108609	0.01974834	3997	10.23
Cenpe	-0.40	0.00109071	0.01980806	7813	0.89
Pstpip2	0.65	0.00109461	0.01985449	1395	1.02
Chga	0.25	0.00110284	0.01997926	1882	42.67
Nrep	0.16	0.00110941	0.02007389	2185	367.09
Afap1	0.17	0.00111086	0.02007557	1994	60.38
Abhd4	0.24	0.00111487	0.02012333	2534	50.90
Hspa1a	0.27	0.00112194	0.02021244	2554	9.53
Sgol2	-0.70	0.00112253	0.02021244	4939	0.38
Aldoart2	0.35	0.00114219	0.02054145	1658	5.50
Mcm4	-0.30	0.00114625	0.02058931	3589	5.07
Usmg5	0.38	0.00115691	0.02075558	341	20.14
Zbtb41	-0.21	0.00117001	0.02094231	8360	7.65
Epb4.1l3	-0.20	0.00117014	0.02094231	4058	31.32
A730046J19Rik	-0.94	0.00117317	0.02097111	4707	0.23
Bdh1	0.21	0.00117709	0.02101575	1152	72.46
Mms22l	-0.55	0.00119138	0.0212454	2287	1.30
Zfp354b	-0.53	0.00119314	0.02125107	1806	1.77
Tmpo	-0.32	0.00119676	0.02128997	3413	11.41

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Cdh8	0.26	0.0011997	0.02131657	3216	19.19
Slc39a10	-0.22	0.00120847	0.02143993	3935	25.73
Igsf11	0.35	0.00121077	0.02143993	2511	3.43
Rpgrip1l	-0.26	0.00121099	0.02143993	4279	5.66
Ifih1	0.75	0.0012243	0.02164983	2904	0.34
Tpst1	0.25	0.00123848	0.0218744	1457	15.18
Gfra2	0.31	0.00124208	0.02191191	3462	3.28
Adamts1	-0.25	0.00124745	0.02198032	2338	13.27
Mapk15	-0.76	0.00125587	0.02210234	1517	1.02
Eya4	0.39	0.00125938	0.02213778	3790	1.40
Ncaph	-0.46	0.00126187	0.02215539	1282	3.43
Aqp4	0.27	0.00126864	0.02224779	5082	10.78
Rgs7	0.24	0.00127923	0.02240698	1418	29.02
Crlf3	0.36	0.00128447	0.02247212	1169	5.21
Sdc4	0.26	0.0012871	0.02249162	1628	12.92
Csmd1	-0.22	0.00129915	0.02267538	2911	11.74
Neb	-0.60	0.00130163	0.02269188	2303	1.06
AI429214	-0.49	0.00131168	0.02280742	1957	1.93
Myo10	0.25	0.00131202	0.02280742	748	40.29
Gm12892	0.26	0.00131288	0.02280742	1146	69.91
Cdhr1	-0.32	0.0013271	0.02302736	4303	2.55
Gzmk	-1.35	0.00133901	0.0232069	1028	0.52
Ift74	-0.27	0.00134429	0.02327113	1713	10.98
Erbb4	-0.21	0.00135193	0.02337605	2196	85.02
2410018M08Rik	-0.37	0.00135462	0.02339524	1611	4.49
Mapre2	-0.14	0.00136143	0.02348541	2169	286.48
Anxa11	-0.75	0.00137774	0.02373914	2505	0.63
C030006K11Rik	0.41	0.00138335	0.02380807	1758	2.38
Shq1	0.39	0.0013875	0.0238517	1814	2.72
Nkain3	-0.30	0.00139333	0.02392426	3833	4.31
Alms1	-0.30	0.00139735	0.0239655	9986	1.23
Styk1	-0.42	0.00140671	0.02408737	1089	4.76
Mybl2	-0.66	0.00140771	0.02408737	789	2.51
Gm10269	0.43	0.00141512	0.02418615	372	9.99
Npas1	0.46	0.00142576	0.02433981	2091	1.47
Kif1c	0.27	0.00143841	0.02452755	5168	5.41
Itih3	0.74	0.00144703	0.02464608	2100	0.47
Slc24a4	-0.41	0.00145263	0.02471305	2337	2.37
Atrnl1	0.17	0.0014641	0.02485192	6581	16.52
Gstm1	0.24	0.00146415	0.02485192	883	108.97
Nefh	0.42	0.00146791	0.02488726	3994	1.11
3110039M20Rik	-0.36	0.00147396	0.02496115	854	8.96
Tspan2	-0.25	0.00147576	0.0249631	2723	8.93
Col9a2	1.07	0.00149837	0.02531657	752	0.51
Ndnf	-0.35	0.00151183	0.02551491	2616	3.48
Gmpr	0.28	0.00151412	0.02552446	871	15.04
Eva1a	0.46	0.00152219	0.02563132	788	4.12
Morn4	-0.19	0.00152679	0.02567949	1816	51.36
Tmem47	0.29	0.00153974	0.02586789	1801	38.32
Cited2	-0.21	0.00154608	0.02594512	1968	20.90
Unc5b	-0.44	0.00155418	0.02605135	5852	2.29
Rhou	-0.23	0.00156024	0.02612349	2077	26.05

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
2810468N07Rik	0.48	0.00156665	0.02618919	1235	2.15
Fancd2	-0.47	0.00156771	0.02618919	738	5.33
Sdk2	0.26	0.00157029	0.0262027	2933	9.30
Fkbp2	0.33	0.00158847	0.02645298	635	14.84
Pnrc1	-0.27	0.00158886	0.02645298	782	20.08
Hck	-1.22	0.00159334	0.02649776	2092	0.29
Srp72	0.16	0.00159518	0.02649866	764	178.77
Mex3a	-0.18	0.00159768	0.02651038	5778	12.02
Pcdh11x	-0.24	0.00161424	0.02670795	4422	19.29
Rfc3	-0.31	0.0016143	0.02670795	906	11.96
Cat	0.19	0.001615	0.02670795	1696	68.49
Ntsr1	-0.21	0.0016338	0.02698866	3256	18.81
Klhl32	-0.28	0.00164037	0.02705571	2506	6.15
Ppargc1b	0.53	0.00164151	0.02705571	3330	0.63
Hs6st2	-0.25	0.00164621	0.02710299	3800	23.43
Cdkl4	-0.45	0.00165035	0.02714092	2931	1.47
Oxr1	-0.20	0.00166005	0.02727006	4251	16.95
Sbsn	0.57	0.00167106	0.02742055	2285	0.77
Inpp5f	-0.16	0.00167649	0.02744087	2774	144.13
Vangl1	-0.40	0.00167775	0.02744087	718	8.34
Spock3	0.23	0.00167786	0.02744087	2942	15.79
AL672276.1	0.76	0.0016857	0.02753861	592	1.49
Nomo1	0.17	0.00169406	0.0276447	4259	23.63
Ttc39c	0.28	0.00170737	0.0278312	2261	5.65
G2e3	-0.27	0.0017097	0.02783858	2724	9.58
Ntsr2	0.45	0.00172032	0.0279807	1547	2.12
Cxadr	-0.17	0.00173437	0.02817823	3701	124.91
Scml2	-0.64	0.00173794	0.02819514	3440	0.59
Egflam	-0.68	0.00173922	0.02819514	3327	0.52
Lcorl	-0.27	0.00174408	0.02824295	3653	17.43
Gm12470	-0.32	0.00175065	0.0283185	1049	10.10
Bbs9	-0.25	0.00175324	0.02832933	1326	16.25
Nhsl2	-0.25	0.00176129	0.02838769	809	26.39
Slc35f4	-0.38	0.00176146	0.02838769	1931	3.24
Nlgn1	-0.22	0.0017626	0.02838769	4316	21.98
Zdhhc2	-0.20	0.00177066	0.02848642	1653	56.88
Rab9b	-0.20	0.00178444	0.0286425	3980	15.75
Gabrg1	-0.23	0.00178474	0.0286425	4771	6.66
2610001J05Rik	-0.20	0.00178617	0.0286425	2127	18.25
Olig2	0.23	0.00179103	0.02868941	2437	14.33
Mlf1	-0.63	0.00179962	0.02879586	877	3.91
Rrm2	-0.29	0.00181249	0.02894518	811	18.39
E2f7	-0.63	0.00181468	0.02894518	827	2.46
Rtn4ip1	0.35	0.00181482	0.02894518	2520	2.55
9530077C05Rik	-0.35	0.00181864	0.02897489	2155	3.43
Tsc22d4	0.31	0.00182578	0.02903971	1043	18.23
Adck4	0.33	0.00182703	0.02903971	1228	5.93
Nell1	-0.22	0.00182859	0.02903971	2010	24.64
Dlc1	-0.26	0.00183507	0.02911137	3423	8.47
Ttyh2	0.37	0.00185644	0.02941887	2091	2.88
Prune2	-0.28	0.00188007	0.02976136	12512	1.79
Pcsk5	0.28	0.00188532	0.0298127	6229	2.92

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Whrn	0.37	0.00189354	0.02991062	2632	2.17
Gm16421	0.84	0.00190178	0.03000885	1557	0.43
Tspan6	-0.23	0.00190735	0.03006458	562	138.50
Ppp1r18	-0.25	0.00190961	0.03006817	2937	8.47
Cntn5	-0.26	0.0019359	0.03042693	3297	6.08
Rimklb	-0.20	0.0019365	0.03042693	919	175.35
Dnmt3a	0.15	0.00194502	0.03051704	821	243.33
Slc6a8	0.18	0.00194636	0.03051704	1613	35.44
E130114P18Rik	0.44	0.00194849	0.03051807	1058	3.18
Armcx6	-0.34	0.00195501	0.03051807	1384	7.02
Rtkn2	-0.52	0.00195679	0.03051807	3521	0.86
Rgs12	-0.22	0.00195774	0.03051807	2628	13.34
Apaf1	-0.19	0.00196225	0.03051807	2153	26.74
Ccp110	-0.22	0.00196251	0.03051807	4716	8.52
Atp5k	0.26	0.00196284	0.03051807	371	61.10
Ache	0.21	0.00196292	0.03051807	424	78.98
Gm3788	0.20	0.00196932	0.03058543	136	382.35
Zbtb46	-0.29	0.00197271	0.03060593	2577	9.00
Emid1	0.43	0.00198069	0.03069753	855	4.21
Cachd1	0.21	0.0019915	0.03083286	2387	11.74
Dnase1l2	-0.85	0.00200181	0.03096008	1183	0.97
Plin2	0.40	0.00202358	0.03126405	707	9.20
Cdc7	-0.39	0.00202748	0.03129171	2864	1.98
Pnpla2	0.29	0.0020356	0.03138425	1799	6.25
Gm13131	-1.30	0.00204414	0.03148315	321	1.66
Ctsd	0.21	0.00208139	0.03202352	1360	222.84
Tmem38b	0.36	0.00208991	0.03212128	649	7.97
Zkscan16	-0.36	0.00209302	0.03213566	2794	2.40
4930522L14Rik	1.13	0.00210295	0.03225469	1300	0.24
Mdh1	0.17	0.00210728	0.03228766	622	472.16
Tmem238	0.97	0.00211084	0.03230871	1337	0.35
Tcn2	0.40	0.0021205	0.03239321	1945	2.31
Gm9790	0.33	0.00212074	0.03239321	442	16.10
Usp37	-0.24	0.00212424	0.03241324	7196	3.55
Itgav	0.21	0.00214555	0.03269592	1105	41.72
Wbscr27	0.32	0.00214718	0.03269592	1536	4.88
Aspm	-0.43	0.00215623	0.03279997	5023	1.14
Acaa1a	0.30	0.00218102	0.03314301	879	12.79
Grik4	0.23	0.00218938	0.03323593	4650	4.63
Gm23935	1.11	0.00220508	0.03343989	57	759.65
Ptpro	-0.18	0.00220828	0.03345413	6533	18.62
Ccbe1	0.25	0.00222123	0.03358263	2549	10.03
Slc35g1	0.65	0.0022213	0.03358263	3471	0.35
Hdac1	-0.52	0.00224051	0.0337424	803	3.78
Celsr1	0.39	0.00224089	0.0337424	11050	0.91
Phactr2	-0.45	0.00224134	0.0337424	2783	3.72
Mc5r	1.20	0.00224149	0.0337424	1162	0.23
Tec	-0.71	0.00224326	0.0337424	2556	0.61
Tk1	-0.69	0.00225581	0.03389669	786	2.14
Ncor2	0.29	0.00225886	0.03390802	5672	9.63
Hsd11b1	0.43	0.00226682	0.03399305	1010	3.27
Hs3st1	0.31	0.00229609	0.03439718	1224	6.73

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Mllt4	-0.16	0.00230168	0.03444613	1189	102.10
Dach2	-0.26	0.00230774	0.03446824	2707	17.20
Dcxr	0.39	0.00230782	0.03446824	901	5.29
Sox2ot	0.24	0.00231438	0.0344952	754	32.85
Cdca2	-0.63	0.00231484	0.0344952	2034	0.98
Pltp	0.37	0.00231662	0.0344952	1130	4.53
Fam20b	-0.17	0.00233117	0.03467706	4448	20.12
Paqr9	0.25	0.00235018	0.03492465	2305	7.52
Ccdc151	-0.60	0.00235939	0.03502634	2199	0.99
Pold3	-0.24	0.00236414	0.03506177	639	33.46
Shf	-0.30	0.00237721	0.0352203	1152	16.28
Piezo2	-0.69	0.00238262	0.03525727	3469	0.46
Pura	0.22	0.00238447	0.03525727	14943	11.56
Tmc6	-0.98	0.0023899	0.03530227	856	0.97
Ikzf4	-0.25	0.00239569	0.03535254	1352	13.13
Tlcd1	0.37	0.00241164	0.03554309	639	8.48
Ccl17	1.01	0.00241341	0.03554309	513	0.78
Prmt2	-0.20	0.00241594	0.03554503	2016	55.40
Rhoj	-0.47	0.00242849	0.03569424	2350	1.57
Fam76b	-0.25	0.00244152	0.03585014	2213	8.31
Cep72	-0.48	0.0024611	0.03610181	3632	0.94
Zbtb10	-0.23	0.00251247	0.03679196	7414	5.99
Nabp1	-0.31	0.00251312	0.03679196	2828	4.20
Rab39b	-0.21	0.00251885	0.03683947	3401	12.32
Bbs1	-0.21	0.0025607	0.03741447	5581	5.11
Fat3	0.24	0.00257115	0.03753016	18456	1.82
Rhobtb1	-0.32	0.00259796	0.03788418	719	13.58
Slc41a1	0.23	0.00261632	0.03811428	553	116.85
Dexi	0.34	0.00263334	0.03832455	1389	4.06
Trmt61a	0.31	0.00263753	0.03833215	2282	4.42
Mt3	0.26	0.00263904	0.03833215	538	100.65
Padi2	0.47	0.00265309	0.03848876	681	4.11
Krt10	0.37	0.00265503	0.03848876	2094	2.39
Alas1	0.20	0.00267614	0.03875688	1392	23.54
Spink10	-0.90	0.00268196	0.03880315	582	1.63
Lrrc4c	-0.23	0.00269752	0.03899029	3312	20.59
Letm1	0.20	0.00270945	0.0390883	2767	18.45
Tmsb10	0.21	0.00270959	0.0390883	597	69.10
2410018L13Rik	-1.11	0.00271564	0.03913752	1033	0.68
Dusp10	0.38	0.00273407	0.03934146	1758	2.45
Zc2hc1a	-0.21	0.00273723	0.03934146	3361	37.63
Rsph9	-0.29	0.00273777	0.03934146	670	22.61
Zfp882	-0.32	0.00274676	0.03938432	631	13.89
Crhr2	-0.77	0.00275043	0.03938432	1436	0.87
Gadd45b	0.32	0.00275083	0.03938432	1266	8.04
Naa38	-0.24	0.00275139	0.03938432	890	20.90
Abracl	-0.39	0.00276304	0.03950424	807	6.96
Rybp	-0.22	0.00276511	0.03950424	4458	5.85
Chrna4	0.27	0.00277133	0.03952238	1448	10.62
Rit2	-0.23	0.00277172	0.03952238	1740	19.91
Shisa2	-0.46	0.00277558	0.03953927	3144	1.26
AI504432	-0.25	0.00278309	0.03960816	2374	11.63

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Cend1	0.22	0.00278739	0.03963121	1685	41.29
Olfml2b	-0.50	0.00279197	0.03965826	3095	1.24
Kif26b	-0.30	0.00281251	0.0398829	6748	3.69
Cln5	0.29	0.00281317	0.0398829	2396	4.36
Vsnl1	-0.14	0.00283125	0.04009632	1934	109.61
Lrp3	0.25	0.00283517	0.04009632	2414	17.90
Edn1	-1.19	0.00283635	0.04009632	2344	0.24
Uchl4	0.28	0.00284226	0.04014148	1162	12.61
Fgl2	-0.49	0.00284766	0.04017936	3788	0.84
Sstr1	-0.31	0.00285725	0.04027636	1883	4.80
Taldo1	0.19	0.00286674	0.04033429	829	71.31
Prex1	0.21	0.00286681	0.04033429	1658	48.34
Dpy19l1	-0.21	0.00287575	0.04042166	3518	14.38
Cald1	-0.26	0.00289176	0.04060809	1010	31.65
Adra1a	0.47	0.00291716	0.04092592	4118	0.61
Mkl2	-0.19	0.00292053	0.04093449	3241	19.07
Tbcel	0.22	0.00292374	0.04094067	2327	17.20
Egfem1	-0.55	0.00292856	0.04096943	733	3.25
Fzd6	0.39	0.00297898	0.04163538	4079	1.01
Rnase4	0.39	0.00303475	0.04237482	1671	2.45
Bcas1	0.40	0.00305835	0.04262053	1014	4.06
Zdbf2	-0.27	0.0030585	0.04262053	12112	3.89
Rph3a	0.19	0.00306098	0.04262053	4127	21.33
Spin4	-0.51	0.00306758	0.04267222	4176	0.67
Mroh1	0.27	0.00307179	0.04269063	1244	20.67
Dpysl3	-0.15	0.00308605	0.04282273	1382	1956.07
E2f8	-0.66	0.00308708	0.04282273	2706	0.62
Eef2	0.21	0.0030965	0.04288478	3098	331.42
Tcf19	-0.48	0.00309964	0.04288478	1324	2.33
Cyp2j6	0.30	0.00310024	0.04288478	3605	2.39
Cxcr7	0.30	0.00314562	0.04347185	1735	5.60
Prrc1	-0.20	0.00316452	0.04363745	4801	8.18
Gm8327	0.40	0.00316479	0.04363745	702	5.51
Fam188b	-0.35	0.00316705	0.04363745	4270	1.65
Ckb	0.27	0.0031694	0.04363745	1478	148.73
Pask	-0.40	0.00317275	0.04364298	1701	2.73
Mpzl1	0.17	0.00319708	0.04393685	2303	27.21
Bai1	0.23	0.00321049	0.04400642	4455	19.41
Chrna5	0.38	0.00321056	0.04400642	2738	1.62
Tox3	-0.25	0.00321106	0.04400642	1109	19.34
Cav1	-0.26	0.00321856	0.04406841	472	46.79
Zfp719	0.23	0.00322275	0.04408501	2532	9.55
Mdm1	-0.42	0.00323381	0.04418377	3089	1.35
Gm5506	0.24	0.00323657	0.04418377	1757	11.90
1110002E22Rik	-0.88	0.00323893	0.04418377	9647	0.26
Rap1gap2	0.18	0.0032513	0.0442948	5765	13.35
Hes7	-0.96	0.00325305	0.0442948	1809	0.45
B230219D22Rik	-0.18	0.00326668	0.04443947	4651	38.31
Yjefn3	0.56	0.00328032	0.04458401	629	2.76
Htr4	-0.63	0.00328367	0.04458864	4657	0.38
Knstrn	-0.43	0.0033012	0.04478558	799	5.03
Tpx2	-0.27	0.003307	0.04482317	3483	3.77

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Ndufb10	0.20	0.00331863	0.04493967	540	76.12
Porcn	0.23	0.00333816	0.04512888	1854	10.35
Frat1	1.06	0.0033387	0.04512888	579	0.58
Gtse1	0.32	0.00335467	0.04530337	2721	2.55
Gypc	-0.61	0.00336111	0.04534899	2072	0.95
Sema6a	-0.16	0.00336517	0.04536238	3310	23.37
Car7	1.19	0.00340315	0.04583262	1522	0.16
Spef1	-0.32	0.00343393	0.04619594	2328	3.48
Zfyve16	0.26	0.00343637	0.04619594	2038	9.74
Snx18	0.21	0.00347271	0.04660293	4446	5.25
Gm6395	0.73	0.00347294	0.04660293	1491	0.55
Melk	-0.55	0.00347619	0.04660423	672	3.52
Pwwp2b	0.52	0.00349438	0.04680575	2488	0.74
Lfng	0.39	0.00350376	0.04688889	2282	1.88
Rapgef1	0.18	0.00353255	0.04723144	4223	32.70
Pole	-0.39	0.00353712	0.04724989	1745	2.77
Gli1	1.37	0.00354273	0.04728216	3665	0.05
Ttyh1	0.18	0.00356828	0.04758025	711	156.84
Evl	0.20	0.00357888	0.04764779	2037	48.29
Coq10b	0.23	0.00357978	0.04764779	1657	12.42
Arsg	0.32	0.00358813	0.04771604	1508	4.41
Slc25a33	0.28	0.0036093	0.04795287	1887	5.36
Mmd2	0.17	0.00361242	0.04795287	3310	35.29
Slitrk3	-0.18	0.00362297	0.0480498	3731	12.95
Reln	0.23	0.00362898	0.04808644	2556	65.83
Nop2	0.19	0.00363989	0.04812208	2623	12.49
Atf5	0.27	0.00364066	0.04812208	1775	31.24
Slc5a5	0.79	0.00364143	0.04812208	2928	0.23
Efr3b	0.17	0.00364763	0.04816104	6547	27.13
Arnt2	-0.16	0.00365845	0.04826091	5988	64.46
Cmbl	-0.96	0.00367184	0.0483696	690	1.16
Psmb3	0.23	0.00367434	0.0483696	766	26.61
Peli2	0.22	0.0036765	0.0483696	5849	4.00
Pygm	-1.04	0.00368486	0.04843656	1611	1.72
Ssbp4	0.28	0.00371114	0.0487087	1474	15.97
Tmem28	0.29	0.00371214	0.0487087	3909	2.43
Grb14	-0.25	0.00372078	0.04877881	755	29.09
Efna5	-0.51	0.00372779	0.04882746	2338	1.18
Plk4	-0.34	0.00373883	0.048892	2184	3.47
Gfpt2	0.32	0.00373933	0.048892	2906	2.58
Srxn1	0.18	0.00375197	0.04901404	1515	87.43
Pgam1	0.27	0.00377305	0.0492459	1831	6.53
Tufm	0.41	0.00379441	0.04948115	1732	1.89
Rrp9	0.28	0.00380007	0.0495113	655	18.87
Inpp5a	0.23	0.00381794	0.04970032	1525	14.46
Mblac2	-0.23	0.00382182	0.04970722	3881	17.61
Tubb4b	0.19	0.00384285	0.04991325	1215	68.78
Dnm3	-0.18	0.00384441	0.04991325	1389	156.44
Spata7	-0.27	0.00385344	0.04992649	1611	9.28
AC158956.1	-0.57	0.00385719	0.04992649	922	2.30
Asb13	0.24	0.00385927	0.04992649	707	26.92
Blmh	0.15	0.00386035	0.04992649	700	114.29

T3 differentially expressed genes

Gene Name	logFC	pvalue	FDR	Gene Length	Expression level
Ppapdc3	0.32	0.0038623	0.04992649	1439	4.64

Annex I. Differentially expressed genes by T3 induction with p<0.05 after a false discovery rate (FDR) correction. Expression levels, gene length and log fold change of the genes are also represented. Genes are ordered by FDR.

ANNEX II:

List of significant Gene Ontology categories

Molecular Function

Positive genes/Up regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0042731	PH domain binding	0.000374	0.032045	3	4	0.75
GO:0008046	axon guidance receptor activity	0.00018	0.018687	4	7	0.57
GO:0042166	acetylcholine binding	0.00019	0.019464	4	8	0.50
GO:0005003	ephrin receptor activity	0.000176	0.018466	5	13	0.38
GO:0015296	anion:cation symporter activity	0.000574	0.044894	5	17	0.29
GO:0005496	steroid binding	1.53E-06	0.000289	11	45	0.24
GO:0043178	alcohol binding	0.000309	0.027974	8	42	0.19
GO:0042562	hormone binding	0.000208	0.020879	9	49	0.18
GO:0015081	sodium ion transmembrane transporter activity	0.000197	0.019969	11	68	0.16
GO:0004888	transmembrane signaling receptor activity	1.76E-11	2.10E-08	49	380	0.13
GO:0004930	G-protein coupled receptor activity	1.71E-06	0.000319	26	203	0.13
GO:0033218	amide binding	7.07E-05	0.008562	17	138	0.12
GO:0042277	peptide binding	0.000135	0.014701	16	132	0.12
GO:0038023	signaling receptor activity	3.79E-11	3.26E-08	53	444	0.12
GO:0008509	anion transmembrane transporter activity	0.00016	0.017048	17	144	0.12
GO:0046873	metal ion transmembrane transporter activity	2.81E-06	0.000507	29	249	0.12
GO:0004872	receptor activity	4.40E-11	3.49E-08	59	532	0.11
GO:0005261	cation channel activity	0.000306	0.027974	20	184	0.11
GO:0008324	cation transmembrane transporter activity	1.23E-07	3.19E-05	40	372	0.11
GO:0015267	channel activity	1.55E-05	0.002381	29	271	0.11
GO:0022803	passive transmembrane transporter activity	1.55E-05	0.002381	29	271	0.11
GO:0015077	monovalent inorganic cation transmembrane transporter activity	0.000116	0.013412	21	197	0.11
GO:0022890	inorganic cation transmembrane transporter activity	5.11E-06	0.000899	31	293	0.11
GO:0022838	substrate-specific channel activity	2.94E-05	0.004067	28	266	0.11
GO:0004871	signal transducer activity	4.73E-11	3.49E-08	66	638	0.10
GO:0060089	molecular transducer activity	4.73E-11	3.49E-08	66	638	0.10
GO:0005216	ion channel activity	6.42E-05	0.007913	27	263	0.10
GO:0015075	ion transmembrane transporter activity	3.01E-08	1.04E-05	50	505	0.10
GO:0022891	substrate-specific transmembrane transporter activity	4.43E-08	1.41E-05	52	544	0.10
GO:0022857	transmembrane transporter activity	1.92E-08	7.61E-06	56	592	0.09
GO:0008289	lipid binding	1.48E-06	0.000282	46	507	0.09
GO:0005509	calcium ion binding	5.52E-05	0.007009	35	396	0.09
GO:0005215	transporter activity	2.46E-08	9.31E-06	65	746	0.09
GO:0022892	substrate-specific transporter activity	3.72E-07	8.87E-05	55	635	0.09
GO:0003674	molecular_function	0.000413	0.034473	568	12813	0.04

Molecular Function
Negative genes/Down regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0005179	hormone activity	0.000252	0.036526	6	32	0.19
GO:0005539	glycosaminoglycan binding	0.000389	0.047275	13	105	0.12
GO:0097367	carbohydrate derivative binding	0.000273	0.038155	14	118	0.12
GO:0004930	G-protein coupled receptor activity	0.000245	0.03577	21	203	0.10
GO:0004888	transmembrane signaling receptor activity	3.01E-05	0.009704	35	380	0.09
GO:0038023	signaling receptor activity	2.03E-05	0.007499	39	444	0.09
GO:0004872	receptor activity	8.32E-05	0.018217	42	532	0.08
GO:0004871	signal transducer activity	2.65E-05	0.008934	49	638	0.08
GO:0060089	molecular transducer activity	2.65E-05	0.008934	49	638	0.08
GO:0042802	identical protein binding	0.000222	0.03372	46	751	0.06
GO:0032553	ribonucleotide binding	0.000357	0.044977	82	1497	0.05
GO:0043168	anion binding	0.00027	0.038155	98	1868	0.05

Molecular Function
All DE genes/regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0004888	transmembrane signaling receptor activity	1.16E-15	1.13E-12	84	380	0.22
GO:0038023	signaling receptor activity	1.57E-15	1.35E-12	92	444	0.21
GO:0004871	signal transducer activity	3.20E-15	2.36E-12	115	638	0.18
GO:0060089	molecular transducer activity	3.20E-15	2.36E-12	115	638	0.18
GO:0004872	receptor activity	1.21E-14	7.80E-12	101	532	0.19
GO:0004930	G-protein coupled receptor activity	5.01E-10	1.32E-07	47	203	0.23
GO:0005496	steroid binding	5.21E-07	8.15E-05	15	45	0.33
GO:0042562	hormone binding	4.20E-06	0.00056	15	49	0.31
GO:0008528	G-protein coupled peptide receptor activity	4.30E-06	0.00057	16	51	0.31
GO:0001653	peptide receptor activity	6.34E-06	0.00081	16	53	0.30
GO:0015075	ion transmembrane transporter activity	8.64E-06	0.00108	71	505	0.14
GO:0005102	receptor binding	8.91E-06	0.0011	98	794	0.12
GO:0030594	neurotransmitter receptor activity	9.56E-06	0.00117	13	38	0.34
GO:0005509	calcium ion binding	1.47E-05	0.00174	58	396	0.15
GO:0001965	G-protein alpha-subunit binding	1.66E-05	0.00192	8	16	0.50
GO:0003674	molecular_function	2.50E-05	0.00283	1052	12813	0.08
GO:0022891	substrate-specific transmembrane transporter activity	2.56E-05	0.00287	73	544	0.13
GO:0022857	transmembrane transporter activity	2.65E-05	0.00293	78	592	0.13
GO:0017046	peptide hormone binding	5.91E-05	0.00548	10	28	0.36
GO:0015267	channel activity	7.03E-05	0.00637	44	271	0.16
GO:0022803	passive transmembrane transporter activity	7.03E-05	0.00637	44	271	0.16
GO:0022838	substrate-specific channel activity	9.42E-05	0.00818	43	266	0.16
GO:0005215	transporter activity	0.000122	0.01017	90	746	0.12

GO:0005216 ion channel activity	0.000155	0.01232	42	263	0.16
GO:0005179 hormone activity	0.0002	0.0146	9	32	0.28
GO:0008046 axon guidance receptor activity	0.000231	0.01634	5	7	0.71
GO:0008509 anion transmembrane transporter activity	0.000261	0.01812	25	144	0.17
GO:0008324 cation transmembrane transporter activity	0.000314	0.02021	51	372	0.14
GO:0005488 binding	0.000403	0.02506	713	8275	0.09
GO:0005230 extracellular ligand-gated ion channel activity	0.000516	0.03004	12	46	0.26
GO:0005539 glycosaminoglycan binding	0.000523	0.03036	20	105	0.19
GO:0022892 substrate-specific transporter activity	0.000572	0.03292	76	635	0.12
GO:0005515 protein binding	0.000689	0.03776	445	4940	0.09
GO:0046873 metal ion transmembrane transporter activity	0.00069	0.03776	38	249	0.15
GO:0008289 lipid binding	0.000702	0.03791	63	507	0.12
GO:0097367 carbohydrate derivative binding	0.00076	0.03991	21	118	0.18

Cellular Component

Positive genes/Up regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0005883	neurofilament	0.000158	0.016957	4	7	0.57
GO:0005902	microvillus	0.000146	0.015794	9	46	0.20
GO:0031225	anchored to membrane	4.83E-05	0.006232	12	71	0.17
GO:0033267	axon part	1.15E-05	0.0019	20	149	0.13
GO:0043235	receptor complex	0.000189	0.019464	15	117	0.13
GO:0030424	axon	5.09E-07	0.000116	33	287	0.11
GO:0005887	integral to plasma membrane	7.51E-09	3.23E-06	43	376	0.11
GO:0031226	intrinsic to plasma membrane	6.36E-09	2.99E-06	45	403	0.11
GO:0031012	extracellular matrix	4.67E-05	0.006081	27	265	0.10
GO:0030425	dendrite	1.06E-06	0.000219	39	385	0.10
GO:0005576	extracellular region	1.03E-14	2.28E-11	90	899	0.10
GO:0045202	synapse	3.09E-08	1.04E-05	51	514	0.10
GO:0005615	extracellular space	1.34E-06	0.000263	38	389	0.10
GO:0044421	extracellular region part	4.16E-09	2.08E-06	57	590	0.10
GO:0043005	neuron projection	1.89E-10	1.27E-07	70	727	0.10
GO:0005578	proteinaceous extracellular matrix	0.000627	0.047893	22	232	0.09
GO:0044456	synapse part	2.72E-05	0.003894	34	361	0.09
GO:0044459	plasma membrane part	3.16E-11	2.88E-08	83	900	0.09
GO:0097458	neuron part	2.31E-09	1.23E-06	72	805	0.09
GO:0009986	cell surface	0.000291	0.027563	29	333	0.09
GO:0044463	cell projection part	1.37E-05	0.002217	46	542	0.08
GO:0005886	plasma membrane	5.09E-19	1.97E-15	179	2170	0.08
GO:0030054	cell junction	5.97E-05	0.007459	45	550	0.08
GO:0071944	cell periphery	2.73E-18	8.47E-15	181	2241	0.08
GO:0042995	cell projection	9.54E-08	2.59E-05	89	1189	0.07
GO:0031224	intrinsic to membrane	2.40E-20	1.86E-16	232	3117	0.07
GO:0016021	integral to membrane	8.27E-18	2.14E-14	221	3047	0.07
GO:0044425	membrane part	4.67E-20	2.41E-16	256	3622	0.07
GO:0016020	membrane	7.78E-21	1.20E-16	317	4858	0.07
GO:0005623	cell	0.000631	0.047893	454	9893	0.05
GO:0044464	cell part	0.000631	0.047893	454	9893	0.05
GO:0005575	cellular_component	6.88E-08	2.01E-05	587	13014	0.05

Cellular Component

Negative genes/Down regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0042555	MCM complex	1.19E-05	0.005138	5	8	0.63
GO:0036126	sperm flagellum	0.00014	0.026085	4	9	0.44
GO:0031514	motile cilium	1.00E-06	0.00065	16	100	0.16
GO:0000793	condensed chromosome	4.86E-05	0.01348	13	101	0.13
GO:0043025	neuronal cell body	1.01E-06	0.00065	35	351	0.10
GO:0005929	cilium	6.70E-05	0.017176	21	215	0.10
GO:0044297	cell body	1.00E-06	0.00065	37	387	0.10
GO:0030425	dendrite	5.06E-06	0.002612	36	385	0.09

GO		pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0044456	synapse part	0.000143	0.026407	31	361	0.09
GO:0043005	neuron projection	3.33E-07	0.000344	58	727	0.08
GO:0097458	neuron part	7.60E-08	0.000161	64	805	0.08
GO:0044463	cell projection part	6.52E-05	0.017176	42	542	0.08
GO:0044427	chromosomal part	0.000123	0.024458	31	432	0.07
GO:0005694	chromosome	0.00015	0.026969	35	514	0.07
GO:0042995	cell projection	1.24E-06	0.000768	80	1189	0.07
GO:0005856	cytoskeleton	0.000341	0.043978	73	1244	0.06
GO:0043228	non-membrane-bounded organelle	7.71E-05	0.017563	111	2143	0.05
GO:0043232	intracellular non-membrane-bounded organelle	7.71E-05	0.017563	111	2143	0.05

Cellular Component

All DE genes/regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0005886	plasma membrane	8.54E-19	2.21E-15	292	2170	0.13
GO:0071944	cell periphery	6.93E-18	1.19E-14	296	2241	0.13
GO:0031224	intrinsic to membrane	7.67E-18	1.19E-14	374	3117	0.12
GO:0043005	neuron projection	1.35E-16	1.91E-13	128	727	0.18
GO:0005576	extracellular region	1.77E-16	2.10E-13	143	899	0.16
GO:0097458	neuron part	3.43E-16	3.80E-13	136	805	0.17
GO:0016021	integral to membrane	1.02E-15	1.05E-12	359	3047	0.12
GO:0044425	membrane part	2.50E-15	2.04E-12	407	3622	0.11
GO:0016020	membrane	1.38E-13	8.22E-11	504	4858	0.10
GO:0042995	cell projection	2.68E-13	1.54E-10	169	1189	0.14
GO:0030425	dendrite	4.66E-12	1.95E-09	75	385	0.19
GO:0044421	extracellular region part	4.97E-11	1.57E-08	94	590	0.16
GO:0045202	synapse	2.70E-10	7.48E-08	87	514	0.17
GO:0044459	plasma membrane part	3.75E-10	1.02E-07	129	900	0.14
GO:0005887	integral to plasma membrane	9.19E-10	2.33E-07	69	376	0.18
GO:0044463	cell projection part	9.72E-10	2.41E-07	88	542	0.16
GO:0031226	intrinsic to plasma membrane	9.81E-10	2.41E-07	72	403	0.18
GO:0044297	cell body	1.87E-09	4.38E-07	68	387	0.18
GO:0005615	extracellular space	2.39E-09	5.52E-07	65	389	0.17
GO:0030424	axon	2.99E-09	6.53E-07	56	287	0.20
GO:0005575	cellular_component	3.57E-09	7.67E-07	1080	13014	0.08
GO:0043025	neuronal cell body	4.20E-09	8.82E-07	63	351	0.18
GO:0044456	synapse part	4.47E-09	9.23E-07	65	361	0.18
GO:0033267	axon part	6.28E-07	9.73E-05	33	149	0.22
GO:0031012	extracellular matrix	7.08E-06	0.00089	45	265	0.17
GO:0030054	cell junction	9.65E-06	0.00117	78	550	0.14
GO:0031225	anchored to membrane	3.68E-05	0.00365	17	71	0.24
GO:0043204	perikaryon	0.000111	0.00932	13	49	0.27
GO:0043235	receptor complex	0.000131	0.01083	23	117	0.20
GO:0043197	dendritic spine	0.000168	0.01302	29	163	0.18
GO:0044309	neuron spine	0.000168	0.01302	29	163	0.18
GO:0005883	neurofilament	0.000177	0.01363	5	7	0.71
GO:0005578	proteinaceous extracellular matrix	0.000188	0.01431	37	232	0.16

GO:0008328	ionotropic glutamate receptor complex	0.000221	0.01587	12	42	0.29
GO:0009986	cell surface	0.000244	0.01713	47	333	0.14
GO:0044306	neuron projection terminus	0.000269	0.01831	17	78	0.22
GO:0043679	axon terminus	0.000279	0.01842	16	71	0.23
GO:0042555	MCM complex	0.000306	0.01994	5	8	0.63
GO:0031514	motile cilium	0.000342	0.02157	19	100	0.19
GO:0005623	cell	0.000486	0.02871	828	9893	0.08
GO:0044464	cell part	0.000486	0.02871	828	9893	0.08
GO:0008021	synaptic vesicle	0.000527	0.03046	19	102	0.19
GO:0097060	synaptic membrane	0.000689	0.03776	32	198	0.16
GO:0097386	glial cell projection	0.00078	0.04048	3	3	1.00
GO:0097449	astrocyte projection	0.00078	0.04048	3	3	1.00
GO:0045211	postsynaptic membrane	0.00089	0.04447	28	166	0.17

Biological Process

Positive genes/Up regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0002175	protein localization to paranode region of axon	0.000122	0.013751	3	3	1.00
GO:0030913	paranodal junction assembly	1.78E-05	0.002648	4	5	0.80
GO:0043383	negative T cell selection	0.000269	0.026007	3	4	0.75
GO:0045060	negative thymic T cell selection	0.000269	0.026007	3	4	0.75
GO:0021534	cell proliferation in hindbrain	0.000328	0.028878	3	4	0.75
GO:0021924	cell proliferation in external granule layer	0.000328	0.028878	3	4	0.75
GO:0021930	cerebellar granule cell precursor proliferation	0.000328	0.028878	3	4	0.75
GO:0045110	intermediate filament bundle assembly	0.000415	0.034473	3	4	0.75
GO:0021936	regulation of cerebellar granule cell precursor proliferation	0.000116	0.013412	4	7	0.57
GO:0072189	ureter development	0.000129	0.014213	4	7	0.57
GO:0045061	thymic T cell selection	0.000355	0.030894	4	9	0.44
GO:0045109	intermediate filament organization	0.000118	0.013472	5	12	0.42
GO:0006107	oxaloacetate metabolic process	0.00055	0.043454	4	10	0.40
GO:0032288	myelin assembly	0.000222	0.022011	5	14	0.36
GO:0046885	regulation of hormone biosynthetic process	0.000398	0.033694	5	16	0.31
GO:0042446	hormone biosynthetic process	0.000117	0.013412	7	28	0.25
GO:0007200	phospholipase C-activating G-protein coupled receptor signaling pathway	0.000128	0.014144	8	35	0.23
GO:0048708	astrocyte differentiation	0.0003	0.027967	8	41	0.20
GO:0007218	neuropeptide signaling pathway	4.15E-05	0.005537	11	59	0.19
GO:0006639	acylglycerol metabolic process	0.00042	0.034473	9	53	0.17
GO:0006638	neutral lipid metabolic process	0.000477	0.038491	9	54	0.17
GO:0006939	smooth muscle contraction	0.000304	0.027974	10	61	0.16
GO:0042445	hormone metabolic process	2.14E-05	0.003163	14	88	0.16
GO:0042471	ear morphogenesis	0.000374	0.032045	11	75	0.15
GO:0044242	cellular lipid catabolic process	0.000124	0.01377	13	92	0.14
GO:0043583	ear development	7.04E-05	0.008562	17	133	0.13
GO:0016042	lipid catabolic process	2.79E-05	0.003963	18	141	0.13
GO:0016358	dendrite development	0.000168	0.017838	16	128	0.13
GO:0050808	synapse organization	0.000281	0.027072	15	120	0.13
GO:0048839	inner ear development	0.000448	0.036359	14	114	0.12
GO:0042493	response to drug	0.000116	0.013412	17	141	0.12
GO:0048562	embryonic organ morphogenesis	0.000357	0.030911	16	139	0.12
GO:0015849	organic acid transport	0.000586	0.045298	16	148	0.11
GO:0046942	carboxylic acid transport	0.000586	0.045298	16	148	0.11
GO:0045664	regulation of neuron differentiation	8.35E-07	0.00018	35	327	0.11
GO:0006935	chemotaxis	2.69E-05	0.003887	26	246	0.11
GO:0042330	taxis	2.90E-05	0.004043	26	247	0.11
GO:0010975	regulation of neuron projection development	6.44E-05	0.007913	24	230	0.10
GO:0051960	regulation of nervous system development	3.00E-08	1.04E-05	45	432	0.10

GO:0007186	G-protein coupled receptor signaling pathway	6.00E-07	0.000133	40	389	0.10
GO:0050767	regulation of neurogenesis	8.30E-07	0.00018	39	387	0.10
GO:0034220	ion transmembrane transport	5.08E-07	0.000116	41	407	0.10
GO:0031344	regulation of cell projection organization	4.33E-05	0.005728	27	269	0.10
GO:0007600	sensory perception	0.000308	0.027974	22	221	0.10
GO:0022610	biological adhesion	1.59E-09	8.80E-07	62	626	0.10
GO:0016337	cell-cell adhesion	0.000416	0.034473	23	235	0.10
GO:0010817	regulation of hormone levels	0.000285	0.027231	22	225	0.10
GO:0007155	cell adhesion	6.78E-09	3.09E-06	60	620	0.10
GO:0006820	anion transport	0.000327	0.028878	23	243	0.09
GO:0060284	regulation of cell development	1.18E-06	0.000235	44	468	0.09
GO:0048878	chemical homeostasis	1.89E-08	7.61E-06	57	610	0.09
GO:0030001	metal ion transport	4.90E-06	0.000872	41	440	0.09
GO:0007268	synaptic transmission	1.54E-05	0.002381	37	400	0.09
GO:0019226	transmission of nerve impulse	1.78E-06	0.000328	45	487	0.09
GO:0015672	monovalent inorganic cation transport	0.000436	0.035534	24	262	0.09
GO:0055080	cation homeostasis	0.000334	0.029223	26	290	0.09
GO:0006812	cation transport	9.47E-07	0.000198	50	561	0.09
GO:0035637	multicellular organismal signaling	5.36E-06	0.000923	45	506	0.09
GO:0050877	neurological system process	4.68E-08	1.45E-05	63	717	0.09
GO:0007610	behavior	4.44E-05	0.00583	37	425	0.09
GO:0009725	response to hormone stimulus	0.000538	0.042748	26	300	0.09
GO:0050801	ion homeostasis	1.31E-05	0.002137	43	498	0.09
GO:0048812	neuron projection morphogenesis	0.000302	0.027974	31	360	0.09
GO:0048699	generation of neurons	3.34E-08	1.10E-05	68	794	0.09
GO:0045595	regulation of cell differentiation	2.12E-08	8.22E-06	69	807	0.09
GO:0030182	neuron differentiation	2.15E-07	5.29E-05	61	717	0.09
GO:0006811	ion transport	4.45E-08	1.41E-05	67	788	0.09
GO:0044057	regulation of system process	0.000292	0.027563	32	379	0.08
GO:0022008	neurogenesis	3.07E-08	1.04E-05	71	843	0.08
GO:0031175	neuron projection development	3.03E-05	0.004158	43	511	0.08
GO:0055085	transmembrane transport	6.15E-06	0.001048	49	584	0.08
GO:0051240	positive regulation of multicellular organismal process	0.000487	0.039064	30	358	0.08
GO:0045597	positive regulation of cell differentiation	0.000299	0.027967	32	384	0.08
GO:0009605	response to external stimulus	6.98E-06	0.001162	49	591	0.08
GO:0010740	positive regulation of intracellular protein kinase cascade	0.000641	0.048191	28	338	0.08
GO:0048666	neuron development	1.57E-05	0.002381	48	580	0.08
GO:0009611	response to wounding	0.000396	0.033668	31	377	0.08
GO:0055082	cellular chemical homeostasis	7.70E-05	0.009176	40	487	0.08
GO:0007267	cell-cell signaling	3.30E-05	0.004489	47	579	0.08
GO:0051239	regulation of multicellular organismal process	3.08E-11	2.88E-08	109	1343	0.08
GO:0043436	oxoacid metabolic process	1.55E-05	0.002381	45	563	0.08
GO:0050793	regulation of developmental process	7.48E-09	3.23E-06	88	1111	0.08
GO:0006082	organic acid metabolic process	2.19E-05	0.0032	45	571	0.08
GO:2000026	regulation of multicellular organismal development	4.61E-07	0.000108	69	877	0.08
GO:0006873	cellular ion homeostasis	0.000637	0.048111	35	451	0.08

GO:0051094	positive regulation of developmental process	0.000328	0.028878	40	524	0.08
GO:0040011	locomotion	2.89E-05	0.004043	57	751	0.08
GO:0003008	system process	2.63E-06	0.00048	72	955	0.08
GO:0042592	homeostatic process	5.26E-06	0.000915	66	876	0.08
GO:0007399	nervous system development	1.40E-07	3.54E-05	88	1174	0.07
GO:0023056	positive regulation of signaling	0.000174	0.018379	47	631	0.07
GO:0010647	positive regulation of cell communication	0.000195	0.019859	47	633	0.07
GO:0019752	carboxylic acid metabolic process	0.000253	0.024938	39	527	0.07
GO:0048468	cell development	8.84E-07	0.000188	82	1111	0.07
GO:0009967	positive regulation of signal transduction	0.000421	0.034473	43	585	0.07
GO:0048584	positive regulation of response to stimulus	5.30E-05	0.006784	56	763	0.07
GO:0030030	cell projection organization	0.000123	0.01377	54	739	0.07
GO:0006629	lipid metabolic process	7.68E-05	0.009176	51	698	0.07
GO:0065008	regulation of biological quality	2.38E-09	1.23E-06	120	1663	0.07
GO:0023051	regulation of signaling	2.72E-08	1.00E-05	109	1522	0.07
GO:0007166	cell surface receptor signaling pathway	2.35E-07	5.70E-05	99	1389	0.07
GO:0010646	regulation of cell communication	6.11E-08	1.86E-05	108	1527	0.07
GO:0042221	response to chemical stimulus	8.95E-08	2.48E-05	103	1464	0.07
GO:0044281	small molecule metabolic process	7.20E-08	2.04E-05	102	1470	0.07
GO:0048646	anatomical structure formation involved in morphogenesis	3.56E-05	0.004794	79	1153	0.07
GO:0007154	cell communication	2.10E-13	4.08E-10	203	2964	0.07
GO:0009966	regulation of signal transduction	6.33E-06	0.001066	90	1318	0.07
GO:0023052	signaling	1.33E-12	1.88E-09	196	2882	0.07
GO:0044700	single organism signaling	1.33E-12	1.88E-09	196	2882	0.07
GO:0048731	system development	1.53E-09	8.75E-07	153	2257	0.07
GO:0055114	oxidation-reduction process	0.000588	0.045298	46	686	0.07
GO:0048583	regulation of response to stimulus	1.16E-06	0.000233	107	1598	0.07
GO:0030154	cell differentiation	1.17E-07	3.06E-05	126	1890	0.07
GO:0007165	signal transduction	3.38E-10	2.18E-07	171	2569	0.07
GO:0044710	single-organism metabolic process	1.43E-09	8.53E-07	144	2168	0.07
GO:0070887	cellular response to chemical stimulus	0.000514	0.041075	59	892	0.07
GO:0048869	cellular developmental process	1.07E-07	2.86E-05	134	2043	0.07
GO:0044707	single-multicellular organism process	1.31E-11	1.69E-08	211	3251	0.06
GO:0009653	anatomical structure morphogenesis	0.00011	0.012965	91	1425	0.06
GO:0032879	regulation of localization	0.000566	0.044496	72	1133	0.06
GO:0032501	multicellular organismal process	9.64E-11	6.79E-08	211	3323	0.06
GO:0035556	intracellular signal transduction	0.000264	0.025927	83	1322	0.06
GO:0007275	multicellular organismal development	6.48E-08	1.93E-05	164	2615	0.06
GO:0051716	cellular response to stimulus	1.31E-09	8.09E-07	201	3237	0.06
GO:0044765	single-organism transport	1.64E-05	0.002468	118	1909	0.06
GO:0048856	anatomical structure development	1.42E-07	3.56E-05	164	2654	0.06
GO:1901564	organonitrogen compound metabolic process	0.000656	0.049077	71	1151	0.06
GO:0048513	organ development	0.000221	0.022011	97	1578	0.06
GO:0044767	single-organism developmental process	1.14E-06	0.000232	151	2461	0.06
GO:0050896	response to stimulus	2.29E-11	2.46E-08	243	3966	0.06
GO:0032502	developmental process	7.24E-08	2.04E-05	181	2975	0.06

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0048518	positive regulation of biological process	5.69E-05	0.007163	147	2557	0.06
GO:0044763	single-organism cellular process	2.38E-11	2.46E-08	365	6686	0.05
GO:0044699	single-organism process	8.87E-13	1.53E-09	393	7216	0.05
GO:0065007	biological regulation	5.00E-09	2.42E-06	324	5991	0.05
GO:0051179	localization	0.000417	0.034473	170	3168	0.05
GO:0050794	regulation of cellular process	1.40E-06	0.000272	289	5465	0.05
GO:0050789	regulation of biological process	5.76E-07	0.000129	302	5747	0.05
GO:0009987	cellular process	8.15E-09	3.41E-06	452	9162	0.05
GO:0008150	biological_process	1.51E-05	0.002381	578	12949	0.04

Biological Process

Negative genes/Down regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0035385	Roundabout signaling pathway	6.96E-05	0.017176	3	3	1.00
GO:0070099	regulation of chemokine-mediated signaling pathway	6.96E-05	0.017176	3	3	1.00
GO:0070100	negative regulation of chemokine-mediated signaling pathway	6.96E-05	0.017176	3	3	1.00
GO:0048846	axon extension involved in axon guidance	0.000278	0.038155	5	14	0.36
GO:0048521	negative regulation of behavior	2.36E-06	0.001395	8	24	0.33
GO:0050919	negative chemotaxis	0.000321	0.042846	5	15	0.33
GO:0030803	negative regulation of cyclic nucleotide biosynthetic process	0.00033	0.04334	5	15	0.33
GO:0030818	negative regulation of cAMP biosynthetic process	0.00033	0.04334	5	15	0.33
GO:0006270	DNA replication initiation	0.000227	0.034187	5	16	0.31
GO:0030809	negative regulation of nucleotide biosynthetic process	0.000408	0.048596	5	16	0.31
GO:1900372	negative regulation of purine nucleotide biosynthetic process	0.000408	0.048596	5	16	0.31
GO:0030800	negative regulation of cyclic nucleotide metabolic process	0.000425	0.049327	5	16	0.31
GO:0030815	negative regulation of cAMP metabolic process	0.000425	0.049327	5	16	0.31
GO:0008608	attachment of spindle microtubules to kinetochore	0.00037	0.046227	5	18	0.28
GO:0030261	chromosome condensation	8.46E-05	0.018217	6	22	0.27
GO:0007200	phospholipase C-activating G-protein coupled receptor signaling pathway	9.17E-05	0.019452	8	35	0.23
GO:0043627	response to estrogen stimulus	0.000427	0.049327	8	47	0.17
GO:0007188	adenylate cyclase-modulating G-protein coupled receptor signaling pathway	0.000315	0.042814	10	59	0.17
GO:0006261	DNA-dependent DNA replication	1.79E-05	0.006776	11	67	0.16
GO:0019233	sensory perception of pain	0.000179	0.029527	11	74	0.15
GO:0007411	axon guidance	1.30E-05	0.005447	17	119	0.14
GO:0050795	regulation of behavior	4.87E-05	0.01348	14	103	0.14
GO:0097305	response to alcohol	0.000274	0.038155	11	83	0.13
GO:0030072	peptide hormone secretion	0.000146	0.026566	14	114	0.12

GO:0002790	peptide secretion	0.000194	0.031624	14	116	0.12
GO:0007059	chromosome segregation	3.00E-05	0.009704	15	128	0.12
GO:0006260	DNA replication	1.82E-07	0.000202	25	216	0.12
GO:0015833	peptide transport	0.000415	0.049107	14	126	0.11
GO:0000087	M phase of mitotic cell cycle	4.61E-08	0.000143	31	290	0.11
GO:0000280	nuclear division	1.17E-07	0.000161	30	284	0.11
GO:0007067	mitosis	1.17E-07	0.000161	30	284	0.11
GO:0048589	developmental growth	4.66E-05	0.013368	23	222	0.10
GO:0050804	regulation of synaptic transmission	0.000321	0.042846	19	187	0.10
GO:0051969	regulation of transmission of nerve impulse	0.000132	0.025216	21	207	0.10
GO:0031644	regulation of neurological system process	0.000103	0.020985	22	219	0.10
GO:0048285	organelle fission	5.88E-07	0.000464	30	307	0.10
GO:0007186	G-protein coupled receptor signaling pathway	8.28E-07	0.00061	38	389	0.10
GO:0007600	sensory perception	0.000373	0.046227	21	221	0.10
GO:0003001	generation of a signal involved in cell-cell signaling	0.000215	0.033503	22	233	0.09
GO:0023061	signal release	0.000215	0.033503	22	233	0.09
GO:0000279	M phase	1.06E-07	0.000161	37	392	0.09
GO:0010817	regulation of hormone levels	0.000391	0.047275	20	225	0.09
GO:0044057	regulation of system process	8.31E-05	0.018217	32	379	0.08
GO:0030036	actin cytoskeleton organization	0.00013	0.025127	28	333	0.08
GO:0048667	cell morphogenesis involved in neuron differentiation	0.000344	0.043978	29	345	0.08
GO:0050767	regulation of neurogenesis	7.10E-05	0.017176	32	387	0.08
GO:0022403	cell cycle phase	4.91E-07	0.000451	44	537	0.08
GO:0051301	cell division	1.77E-05	0.006776	33	412	0.08
GO:0060284	regulation of cell development	6.19E-05	0.016814	37	468	0.08
GO:0051960	regulation of nervous system development	0.000124	0.024458	34	432	0.08
GO:0000278	mitotic cell cycle	6.91E-06	0.003148	39	501	0.08
GO:0007610	behavior	0.000238	0.035176	33	425	0.08
GO:0007010	cytoskeleton organization	2.43E-06	0.001395	51	657	0.08
GO:0031175	neuron projection development	0.000163	0.027854	39	511	0.08
GO:0048666	neuron development	7.05E-05	0.017176	44	580	0.08
GO:0007267	cell-cell signaling	0.000216	0.033503	42	579	0.07
GO:0030182	neuron differentiation	3.70E-05	0.011233	52	717	0.07
GO:0040011	locomotion	2.33E-05	0.008213	54	751	0.07
GO:0022402	cell cycle process	6.89E-06	0.003148	51	716	0.07
GO:0048870	cell motility	0.000164	0.027854	45	634	0.07
GO:0051674	localization of cell	0.000164	0.027854	45	634	0.07
GO:0048699	generation of neurons	3.61E-05	0.011189	56	794	0.07
GO:0006928	cellular component movement	7.26E-05	0.017312	54	778	0.07
GO:0007049	cell cycle	1.44E-07	0.000172	71	1026	0.07
GO:0030030	cell projection organization	0.000235	0.034972	50	739	0.07
GO:0022008	neurogenesis	9.50E-05	0.019629	57	843	0.07
GO:0048468	cell development	4.84E-06	0.002587	75	1111	0.07
GO:0006259	DNA metabolic process	4.21E-05	0.012496	43	638	0.07
GO:0032989	cellular component morphogenesis	0.000391	0.047275	49	728	0.07

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:2000026	regulation of multicellular organismal development	7.70E-05	0.017563	58	877	0.07
GO:0007399	nervous system development	1.57E-05	0.006385	77	1174	0.07
GO:0003008	system process	0.000136	0.025667	62	955	0.06
GO:0007166	cell surface receptor signaling pathway	5.51E-06	0.002756	89	1389	0.06
GO:0051239	regulation of multicellular organismal process	1.65E-05	0.006554	84	1343	0.06
GO:0050793	regulation of developmental process	0.000153	0.027303	68	1111	0.06
GO:0008283	cell proliferation	0.000275	0.038155	59	979	0.06
GO:0009653	anatomical structure morphogenesis	0.000218	0.033503	84	1425	0.06
GO:0007165	signal transduction	1.25E-07	0.000161	148	2569	0.06
GO:0023052	signaling	9.57E-08	0.000161	163	2882	0.06
GO:0044700	single organism signaling	9.57E-08	0.000161	163	2882	0.06
GO:0065008	regulation of biological quality	9.42E-05	0.019629	94	1663	0.06
GO:0048513	organ development	0.000357	0.044977	89	1578	0.06
GO:0044707	single-multicellular organism process	2.72E-08	0.000105	182	3251	0.06
GO:0032501	multicellular organismal process	2.52E-08	0.000105	185	3323	0.06
GO:0044767	single-organism developmental process	8.07E-06	0.003571	136	2461	0.06
GO:0007154	cell communication	5.40E-07	0.000464	163	2964	0.05
GO:0048869	cellular developmental process	0.000125	0.024458	112	2043	0.05
GO:0030154	cell differentiation	0.000335	0.043658	103	1890	0.05
GO:0007275	multicellular organismal development	2.10E-05	0.007565	142	2615	0.05
GO:0048731	system development	0.000179	0.029527	122	2257	0.05
GO:0048856	anatomical structure development	3.18E-05	0.01006	142	2654	0.05
GO:0051716	cellular response to stimulus	5.99E-07	0.000464	172	3237	0.05
GO:0032502	developmental process	4.28E-05	0.012496	155	2975	0.05
GO:0048523	negative regulation of cellular process	0.000212	0.033503	113	2186	0.05
GO:0048519	negative regulation of biological process	0.000218	0.033503	121	2367	0.05
GO:0016043	cellular component organization	0.000157	0.027669	152	3083	0.05
GO:0050896	response to stimulus	8.47E-05	0.018217	192	3966	0.05
GO:0050794	regulation of cellular process	4.95E-07	0.000451	262	5465	0.05
GO:0044763	single-organism cellular process	6.85E-09	5.30E-05	318	6686	0.05
GO:0044699	single-organism process	1.34E-09	2.08E-05	340	7216	0.05
GO:0050789	regulation of biological process	6.16E-06	0.002983	268	5747	0.05
GO:0065007	biological regulation	3.06E-06	0.001695	279	5991	0.05
GO:0009987	cellular process	0.000178	0.029527	378	9162	0.04
GO:0008150	biological_process	0.000277	0.038155	497	12949	0.04

Biological Process

All DE genes/regulated by T3

GO	description	pvalue	adjusted pvalue	ngenesDE in GO	ngenes in GO	enrichement
GO:0044699	single-organism process	3.29E-21	5.09E-17	733	7216	0.10
GO:0023052	signaling	5.19E-19	2.08E-15	359	2882	0.12
GO:0044700	single organism signaling	5.19E-19	2.08E-15	359	2882	0.12
GO:0044763	single-organism cellular process	5.38E-19	2.08E-15	683	6686	0.10
GO:0007154	cell communication	8.05E-19	2.21E-15	366	2964	0.12
GO:0044707	single-multicellular organism process	1.13E-18	2.50E-15	393	3251	0.12
GO:0032501	multicellular organismal process	7.65E-18	1.19E-14	396	3323	0.12

GO:0007165	signal transduction	1.51E-16	1.95E-13	319	2569	0.12
GO:0051239	regulation of multicellular organismal process	1.53E-15	1.35E-12	193	1343	0.14
GO:0051716	cellular response to stimulus	3.79E-15	2.67E-12	373	3237	0.12
GO:0050896	response to stimulus	1.10E-14	7.41E-12	435	3966	0.11
GO:0065007	biological regulation	4.41E-14	2.73E-11	603	5991	0.10
GO:0007186	G-protein coupled receptor signaling pathway	3.96E-13	2.19E-10	78	389	0.20
GO:0065008	regulation of biological quality	7.53E-13	4.02E-10	214	1663	0.13
GO:0048731	system development	1.09E-12	5.63E-10	275	2257	0.12
GO:0048699	generation of neurons	2.47E-12	1.23E-09	124	794	0.16
GO:0007166	cell surface receptor signaling pathway	2.64E-12	1.28E-09	188	1389	0.14
GO:0050794	regulation of cellular process	2.81E-12	1.32E-09	551	5465	0.10
GO:0007275	multicellular organismal development	3.07E-12	1.40E-09	306	2615	0.12
GO:0050793	regulation of developmental process	3.66E-12	1.62E-09	156	1111	0.14
GO:0007399	nervous system development	4.21E-12	1.81E-09	165	1174	0.14
GO:0009987	cellular process	5.25E-12	2.14E-09	830	9162	0.09
GO:0048468	cell development	5.51E-12	2.19E-09	157	1111	0.14
GO:0022008	neurogenesis	7.05E-12	2.73E-09	128	843	0.15
GO:0032502	developmental process	7.54E-12	2.85E-09	336	2975	0.11
GO:0051960	regulation of nervous system development	8.37E-12	3.09E-09	79	432	0.18
GO:0050789	regulation of biological process	1.01E-11	3.62E-09	570	5747	0.10
GO:0048856	anatomical structure development	1.05E-11	3.71E-09	306	2654	0.12
GO:0044767	single-organism developmental process	1.32E-11	4.55E-09	287	2461	0.12
GO:0030182	neuron differentiation	1.52E-11	5.13E-09	113	717	0.16
GO:0022610	biological adhesion	2.13E-11	7.02E-09	105	626	0.17
GO:0048869	cellular developmental process	3.60E-11	1.16E-08	246	2043	0.12
GO:0007155	cell adhesion	5.68E-11	1.76E-08	103	620	0.17
GO:0050767	regulation of neurogenesis	7.08E-11	2.15E-08	71	387	0.18
GO:2000026	regulation of multicellular organismal development	7.95E-11	2.37E-08	127	877	0.14
GO:0060284	regulation of cell development	8.59E-11	2.51E-08	81	468	0.17
GO:0030154	cell differentiation	1.23E-10	3.51E-08	229	1890	0.12
GO:0050877	neurological system process	1.54E-10	4.35E-08	110	717	0.15
GO:0003008	system process	4.58E-10	1.22E-07	134	955	0.14
GO:0040011	locomotion	7.39E-10	1.91E-07	111	751	0.15
GO:0048666	neuron development	1.19E-09	2.89E-07	92	580	0.16
GO:0045595	regulation of cell differentiation	1.73E-09	4.11E-07	115	807	0.14
GO:0045664	regulation of neuron differentiation	2.59E-09	5.91E-07	60	327	0.18
GO:0019226	transmission of nerve impulse	2.72E-09	6.10E-07	80	487	0.16
GO:0023051	regulation of signaling	2.76E-09	6.12E-07	187	1522	0.12
GO:0035637	multicellular organismal signaling	4.22E-09	8.82E-07	82	506	0.16
GO:0031175	neuron projection development	5.69E-09	1.16E-06	82	511	0.16
GO:0010646	regulation of cell communication	6.39E-09	1.28E-06	186	1527	0.12
GO:0008150	biological_process	6.57E-09	1.31E-06	1075	12949	0.08
GO:0007267	cell-cell signaling	8.47E-09	1.66E-06	89	579	0.15
GO:0007200	phospholipase C-activating G-protein coupled receptor signaling pathway	8.91E-09	1.73E-06	16	35	0.46

GO:0007268	synaptic transmission	1.09E-08	2.09E-06	68	400	0.17
GO:0007610	behavior	1.31E-08	2.47E-06	70	425	0.16
GO:0048878	chemical homeostasis	1.79E-08	3.34E-06	90	610	0.15
GO:0007411	axon guidance	1.90E-08	3.50E-06	31	119	0.26
GO:0009653	anatomical structure morphogenesis	2.42E-08	4.42E-06	175	1425	0.12
GO:0007218	neuropeptide signaling pathway	2.70E-08	4.86E-06	20	59	0.34
GO:0042221	response to chemical stimulus	3.07E-08	5.46E-06	173	1464	0.12
GO:0044057	regulation of system process	3.14E-08	5.50E-06	64	379	0.17
GO:0048646	anatomical structure formation involved in morphogenesis	3.16E-08	5.50E-06	148	1153	0.13
GO:0030030	cell projection organization	3.33E-08	5.72E-06	104	739	0.14
GO:0006928	cellular component movement	7.76E-08	1.32E-05	107	778	0.14
GO:0048513	organ development	8.03E-08	1.35E-05	186	1578	0.12
GO:0006935	chemotaxis	1.03E-07	1.72E-05	46	246	0.19
GO:0042330	taxis	1.18E-07	1.95E-05	46	247	0.19
GO:0048583	regulation of response to stimulus	1.96E-07	3.20E-05	184	1598	0.12
GO:0007600	sensory perception	2.07E-07	3.34E-05	43	221	0.19
GO:0010817	regulation of hormone levels	2.41E-07	3.85E-05	42	225	0.19
GO:0048518	positive regulation of biological process	3.90E-07	6.17E-05	268	2557	0.10
GO:0048667	cell morphogenesis involved in neuron differentiation	9.25E-07	0.00014	57	345	0.17
GO:0010975	regulation of neuron projection development	9.81E-07	0.00015	42	230	0.18
GO:0048870	cell motility	1.02E-06	0.00015	88	634	0.14
GO:0051674	localization of cell	1.02E-06	0.00015	88	634	0.14
GO:0048812	neuron projection morphogenesis	2.25E-06	0.00033	58	360	0.16
GO:0050795	regulation of behavior	2.25E-06	0.00033	24	103	0.23
GO:0006811	ion transport	2.42E-06	0.00035	102	788	0.13
GO:0048519	negative regulation of biological process	2.62E-06	0.00038	247	2367	0.10
GO:0031644	regulation of neurological system process	2.70E-06	0.00038	40	219	0.18
GO:0009605	response to external stimulus	2.72E-06	0.00038	80	591	0.14
GO:0050804	regulation of synaptic transmission	3.11E-06	0.00043	36	187	0.19
GO:0048522	positive regulation of cellular process	3.51E-06	0.00049	245	2337	0.10
GO:0007409	axonogenesis	3.62E-06	0.0005	49	288	0.17
GO:0031344	regulation of cell projection organization	3.88E-06	0.00053	45	269	0.17
GO:0016358	dendrite development	4.00E-06	0.00054	28	128	0.22
GO:0051969	regulation of transmission of nerve impulse	4.37E-06	0.00057	38	207	0.18
GO:0042592	homeostatic process	4.42E-06	0.00058	109	876	0.12
GO:0048523	negative regulation of cellular process	5.04E-06	0.00065	229	2186	0.10
GO:0055082	cellular chemical homeostasis	5.81E-06	0.00074	69	487	0.14
GO:0006939	smooth muscle contraction	9.61E-06	0.00117	17	61	0.28
GO:0048858	cell projection morphogenesis	1.17E-05	0.00141	67	459	0.15
GO:0009966	regulation of signal transduction	1.19E-05	0.00142	151	1318	0.11
GO:0051094	positive regulation of developmental process	1.49E-05	0.00175	71	524	0.14

GO ID	Description	P-value	Adj P-value	Count	Total	Ratio
GO:0044708	single-organism behavior	1.59E-05	0.00185	48	305	0.16
GO:0042445	hormone metabolic process	1.69E-05	0.00194	20	88	0.23
GO:0044710	single-organism metabolic process	2.16E-05	0.00246	215	2168	0.10
GO:0016337	cell-cell adhesion	2.63E-05	0.00293	41	235	0.17
GO:0007156	homophilic cell adhesion	2.67E-05	0.00293	17	58	0.29
GO:0032989	cellular component morphogenesis	2.72E-05	0.00297	93	728	0.13
GO:0050801	ion homeostasis	2.78E-05	0.00301	68	498	0.14
GO:0032990	cell part morphogenesis	2.90E-05	0.00312	67	474	0.14
GO:0000902	cell morphogenesis	2.96E-05	0.00316	88	676	0.13
GO:0019233	sensory perception of pain	3.02E-05	0.0032	18	74	0.24
GO:0009888	tissue development	3.20E-05	0.00338	108	882	0.12
GO:0007417	central nervous system development	3.36E-05	0.00352	63	443	0.14
GO:0001655	urogenital system development	3.40E-05	0.00353	33	182	0.18
GO:0051952	regulation of amine transport	3.45E-05	0.00353	14	50	0.28
GO:0023057	negative regulation of signaling	3.45E-05	0.00353	69	514	0.13
GO:0051128	regulation of cellular component organization	3.46E-05	0.00353	122	1062	0.11
GO:0045597	positive regulation of cell differentiation	3.59E-05	0.00364	55	384	0.14
GO:0035295	tube development	3.62E-05	0.00364	49	323	0.15
GO:0048584	positive regulation of response to stimulus	3.66E-05	0.00365	93	763	0.12
GO:0032879	regulation of localization	3.74E-05	0.00369	131	1133	0.12
GO:0048585	negative regulation of response to stimulus	3.79E-05	0.00371	76	588	0.13
GO:0008219	cell death	4.14E-05	0.00403	130	1187	0.11
GO:0051046	regulation of secretion	4.40E-05	0.00426	45	298	0.15
GO:0044281	small molecule metabolic process	4.82E-05	0.00464	153	1470	0.10
GO:0016265	death	4.88E-05	0.00467	130	1191	0.11
GO:0010648	negative regulation of cell communication	5.02E-05	0.00477	69	520	0.13
GO:0000904	cell morphogenesis involved in differentiation	5.13E-05	0.00484	63	448	0.14
GO:0035556	intracellular signal transduction	5.20E-05	0.00488	147	1322	0.11
GO:0016477	cell migration	5.45E-05	0.00509	77	590	0.13
GO:0048545	response to steroid hormone stimulus	6.07E-05	0.0056	24	125	0.19
GO:0048521	negative regulation of behavior	7.02E-05	0.00637	9	24	0.38
GO:0006812	cation transport	7.12E-05	0.00641	73	561	0.13
GO:0032101	regulation of response to external stimulus	8.32E-05	0.00745	37	240	0.15
GO:0007010	cytoskeleton organization	8.58E-05	0.00764	82	657	0.12
GO:0023056	positive regulation of signaling	8.74E-05	0.00773	79	631	0.13
GO:0060548	negative regulation of cell death	8.83E-05	0.00777	64	505	0.13
GO:0030036	actin cytoskeleton organization	8.87E-05	0.00777	48	333	0.14
GO:0009968	negative regulation of signal transduction	9.45E-05	0.00818	64	484	0.13
GO:0007215	glutamate receptor signaling pathway	9.90E-05	0.00852	15	56	0.27
GO:0035239	tube morphogenesis	0.0001	0.00856	37	228	0.16
GO:0050878	regulation of body fluid levels	0.000101	0.00856	25	135	0.19

| GO ID	Description				
GO:0010647	positive regulation of cell communication	0.000109	0.00926	79	633	0.12
GO:0040012	regulation of locomotion	0.000113	0.00949	51	360	0.14
GO:0048265	response to pain	0.000132	0.01085	8	19	0.42
GO:0016043	cellular component organization	0.000132	0.01086	298	3083	0.10
GO:0015850	organic hydroxy compound transport	0.000137	0.01114	17	78	0.22
GO:0030879	mammary gland development	0.00014	0.01133	19	90	0.21
GO:0019725	cellular homeostasis	0.000141	0.01138	70	554	0.13
GO:0034220	ion transmembrane transport	0.000145	0.01167	56	407	0.14
GO:0009887	organ morphogenesis	0.000155	0.01232	66	497	0.13
GO:0050769	positive regulation of neurogenesis	0.000165	0.01302	23	127	0.18
GO:0009725	response to hormone stimulus	0.000166	0.01302	43	300	0.14
GO:0006873	cellular ion homeostasis	0.000168	0.01302	60	451	0.13
GO:0031346	positive regulation of cell projection organization	0.000186	0.01423	26	149	0.17
GO:0051130	positive regulation of cellular component organization	0.000191	0.01445	59	452	0.13
GO:0006940	regulation of smooth muscle contraction	0.000191	0.01445	10	32	0.31
GO:0030001	metal ion transport	0.000193	0.0145	60	440	0.14
GO:0060429	epithelium development	0.000195	0.01453	57	414	0.14
GO:0007188	adenylate cyclase-modulating G-protein coupled receptor signaling pathway	0.000196	0.01453	15	59	0.25
GO:0072358	cardiovascular system development	0.000197	0.01453	73	570	0.13
GO:0072359	circulatory system development	0.000197	0.01453	73	570	0.13
GO:0002009	morphogenesis of an epithelium	0.0002	0.0146	43	285	0.15
GO:0033500	carbohydrate homeostasis	0.000206	0.01493	17	82	0.21
GO:0042593	glucose homeostasis	0.000206	0.01493	17	82	0.21
GO:0050920	regulation of chemotaxis	0.000221	0.01587	16	73	0.22
GO:0060562	epithelial tube morphogenesis	0.000224	0.01601	35	220	0.16
GO:0051240	positive regulation of multicellular organismal process	0.000228	0.01618	50	358	0.14
GO:0048167	regulation of synaptic plasticity	0.000239	0.01685	20	101	0.20
GO:0007214	gamma-aminobutyric acid signaling pathway	0.000255	0.0178	7	17	0.41
GO:0006260	DNA replication	0.000262	0.01812	32	216	0.15
GO:0060666	dichotomous subdivision of terminal units involved in salivary gland branching	0.000267	0.01831	4	5	0.80
GO:0003001	generation of a signal involved in cell-cell signaling	0.00027	0.01831	36	233	0.15
GO:0023061	signal release	0.00027	0.01831	36	233	0.15
GO:0030913	paranodal junction assembly	0.000271	0.01835	4	5	0.80
GO:0015837	amine transport	0.000274	0.01842	14	59	0.24
GO:0010627	regulation of intracellular protein kinase cascade	0.000277	0.01842	65	519	0.13
GO:0007187	G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger	0.000278	0.01842	16	67	0.24

GO:0001960	negative regulation of cytokine-mediated signaling pathway	0.000278	0.01842	6	13	0.46
GO:0048732	gland development	0.000279	0.01842	30	181	0.17
GO:0003012	muscle system process	0.000281	0.01844	26	152	0.17
GO:0050808	synapse organization	0.000287	0.01877	23	120	0.19
GO:0046058	cAMP metabolic process	0.000311	0.02017	16	69	0.23
GO:0030029	actin filament-based process	0.000313	0.02018	49	359	0.14
GO:0010740	positive regulation of intracellular protein kinase cascade	0.00032	0.02047	46	338	0.14
GO:0007420	brain development	0.000328	0.02089	49	348	0.14
GO:0022612	gland morphogenesis	0.000336	0.0213	18	87	0.21
GO:0050432	catecholamine secretion	0.000343	0.02157	9	30	0.30
GO:0001657	ureteric bud development	0.000389	0.02437	13	53	0.25
GO:0006639	acylglycerol metabolic process	0.000399	0.0249	13	53	0.25
GO:0001763	morphogenesis of a branching structure	0.000405	0.02512	25	143	0.17
GO:0006171	cAMP biosynthetic process	0.000414	0.02556	13	51	0.25
GO:0061138	morphogenesis of a branching epithelium	0.000425	0.02604	24	135	0.18
GO:0090257	regulation of muscle system process	0.000425	0.02604	18	89	0.20
GO:0043069	negative regulation of programmed cell death	0.000436	0.02659	58	474	0.12
GO:0012501	programmed cell death	0.000441	0.02678	118	1122	0.11
GO:0009967	positive regulation of signal transduction	0.00045	0.02723	71	585	0.12
GO:0033555	multicellular organismal response to stress	0.000455	0.02741	12	46	0.26
GO:0006638	neutral lipid metabolic process	0.000463	0.02782	13	54	0.24
GO:0071840	cellular component organization or biogenesis	0.000469	0.02807	302	3201	0.09
GO:0051937	catecholamine transport	0.000472	0.02813	10	37	0.27
GO:0043066	negative regulation of apoptotic process	0.000492	0.029	57	467	0.12
GO:0010977	negative regulation of neuron projection development	0.000501	0.02942	9	31	0.29
GO:0051048	negative regulation of secretion	0.000509	0.02978	17	88	0.19
GO:0009187	cyclic nucleotide metabolic process	0.000574	0.03292	19	96	0.20
GO:0050954	sensory perception of mechanical stimulus	0.000582	0.0332	17	81	0.21
GO:0006820	anion transport	0.000583	0.0332	35	243	0.14
GO:0001822	kidney development	0.000585	0.0332	23	126	0.18
GO:0035385	Roundabout signaling pathway	0.000593	0.03328	3	3	1.00
GO:0070099	regulation of chemokine-mediated signaling pathway	0.000593	0.03328	3	3	1.00
GO:0070100	negative regulation of chemokine-mediated signaling pathway	0.000593	0.03328	3	3	1.00
GO:0052652	cyclic purine nucleotide metabolic process	0.000601	0.03358	15	67	0.22
GO:0008283	cell proliferation	0.000635	0.03538	107	979	0.11
GO:0001504	neurotransmitter uptake	0.000673	0.03735	6	14	0.43
GO:0061061	muscle structure development	0.000688	0.03776	48	361	0.13
GO:0007243	intracellular protein kinase cascade	0.000696	0.03782	76	646	0.12

GO:0071705	nitrogen compound transport	0.000698	0.03782	48	370	0.13
GO:0032288	myelin assembly	0.000698	0.03782	6	14	0.43
GO:0031646	positive regulation of neurological system process	0.000707	0.03801	14	63	0.22
GO:0009190	cyclic nucleotide biosynthetic process	0.00072	0.03859	15	68	0.22
GO:0014013	regulation of gliogenesis	0.000724	0.03866	13	60	0.22
GO:0050433	regulation of catecholamine secretion	0.000733	0.03899	8	27	0.30
GO:0048568	embryonic organ development	0.000739	0.0392	34	234	0.15
GO:0046903	secretion	0.000748	0.03954	60	482	0.12
GO:0010720	positive regulation of cell development	0.000751	0.03958	25	157	0.16
GO:0060600	dichotomous subdivision of an epithelial terminal unit	0.000772	0.04041	5	10	0.50
GO:0014033	neural crest cell differentiation	0.000782	0.04048	10	37	0.27
GO:0006915	apoptotic process	0.000784	0.04048	115	1106	0.10
GO:0070098	chemokine-mediated signaling pathway	0.000791	0.0407	5	10	0.50
GO:0007413	axonal fasciculation	0.000795	0.04076	6	13	0.46
GO:0003014	renal system process	0.000803	0.04104	11	42	0.26
GO:0042127	regulation of cell proliferation	0.000809	0.04125	88	784	0.11
GO:0048754	branching morphogenesis of an epithelial tube	0.000812	0.04125	21	117	0.18
GO:0097305	response to alcohol	0.000838	0.0424	16	83	0.19
GO:0000165	MAPK cascade	0.000841	0.04243	50	383	0.13
GO:0050806	positive regulation of synaptic transmission	0.000854	0.0428	13	57	0.23
GO:0015844	monoamine transport	0.000854	0.0428	11	46	0.24
GO:0042446	hormone biosynthetic process	0.000894	0.04453	8	28	0.29
GO:0030817	regulation of cAMP biosynthetic process	0.000922	0.04579	12	49	0.24
GO:0051971	positive regulation of transmission of nerve impulse	0.000964	0.04772	13	58	0.22
GO:0060326	cell chemotaxis	0.000984	0.04856	17	91	0.19
GO:0051241	negative regulation of multicellular organismal process	0.001002	0.04928	32	221	0.14
GO:0030814	regulation of cAMP metabolic process	0.00101	0.04949	13	56	0.23

Annex II. Significantly enriched Gene Ontology categories (Molecular function, Cellular Component and Biological Process) in the set of positive, negative and all differentially expressed genes by T3 treatment.

ANNEX III:
Scheme of significant Gene Ontology categories

MF: T3 regulated genes
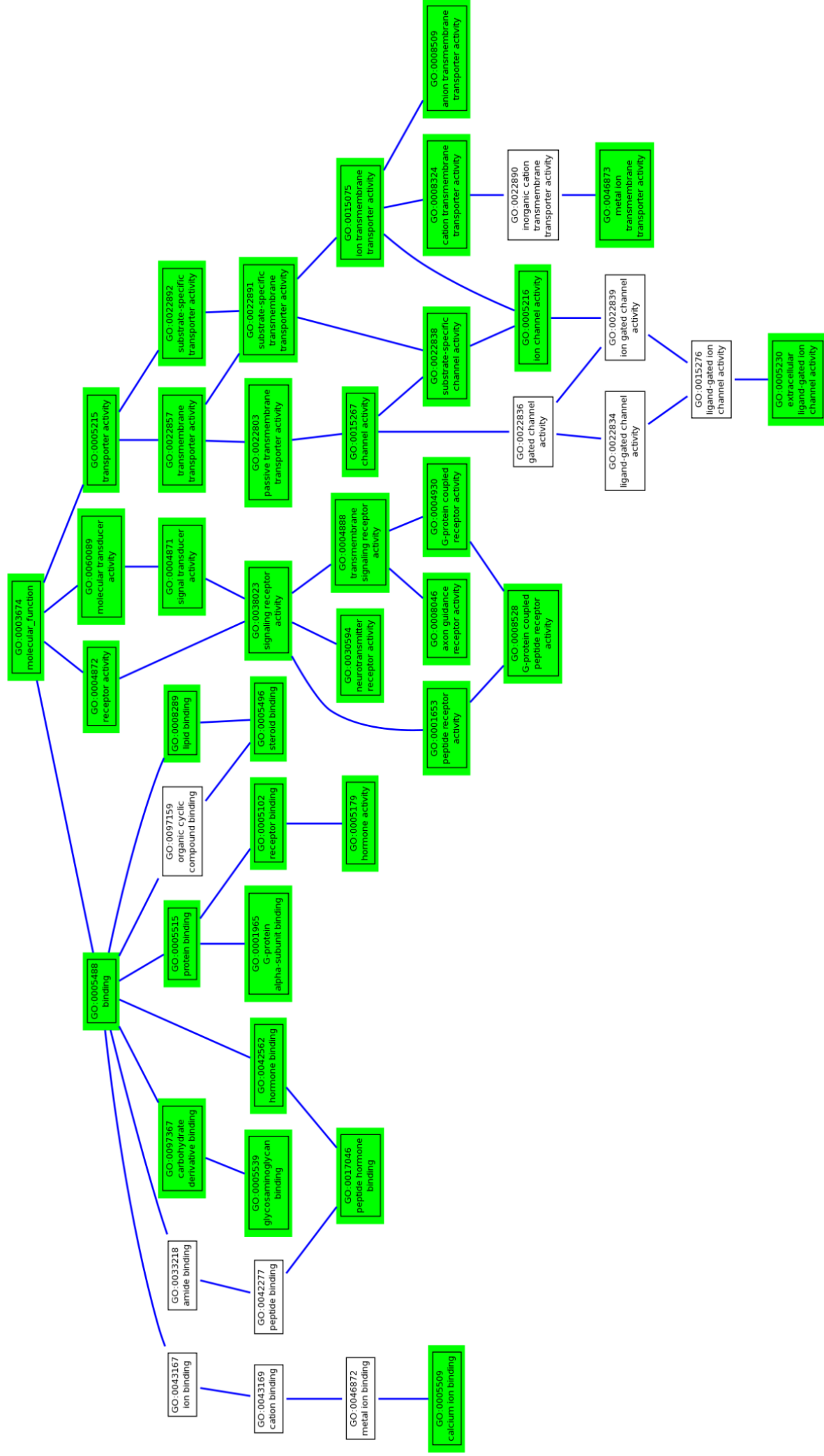
MF: T3 Up-regulated genes
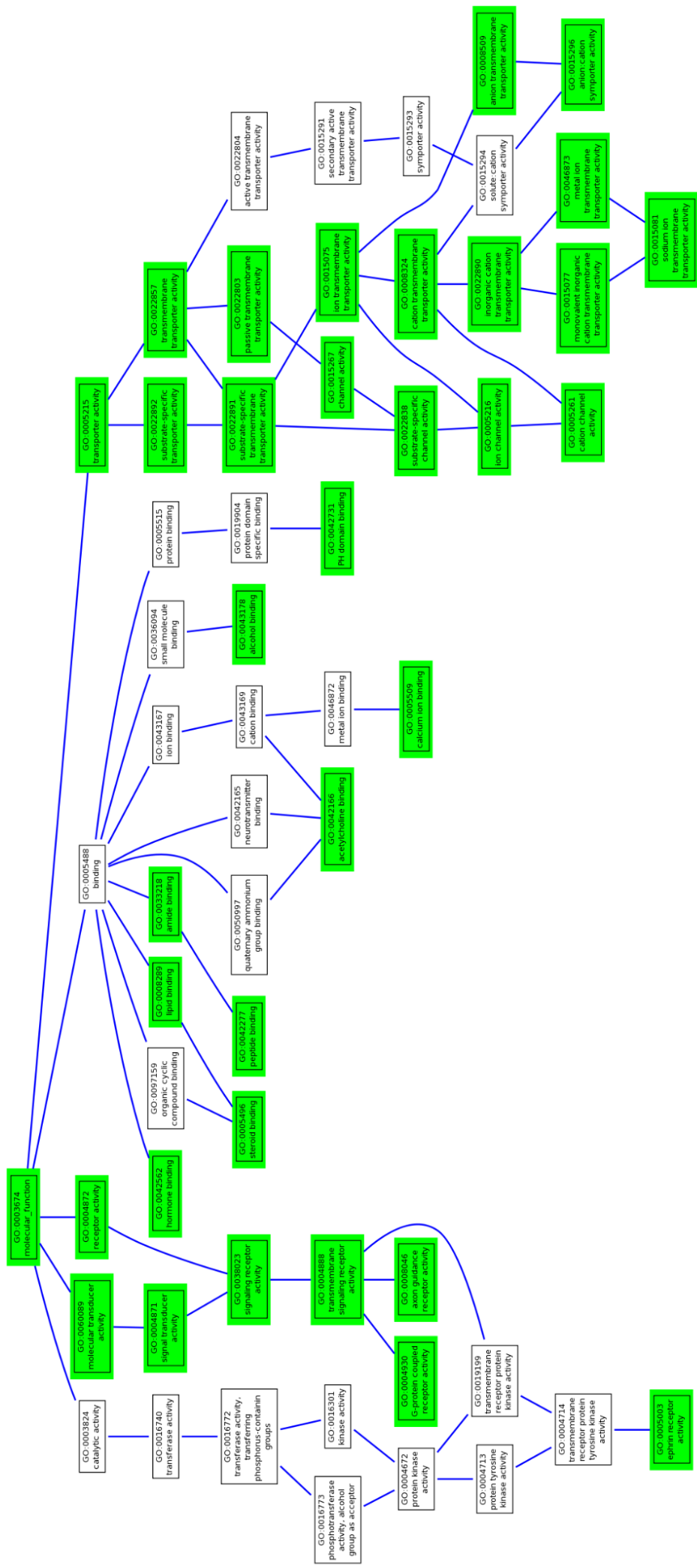
MF: T3 Down-regulated genes

CC: T3 regulated genes
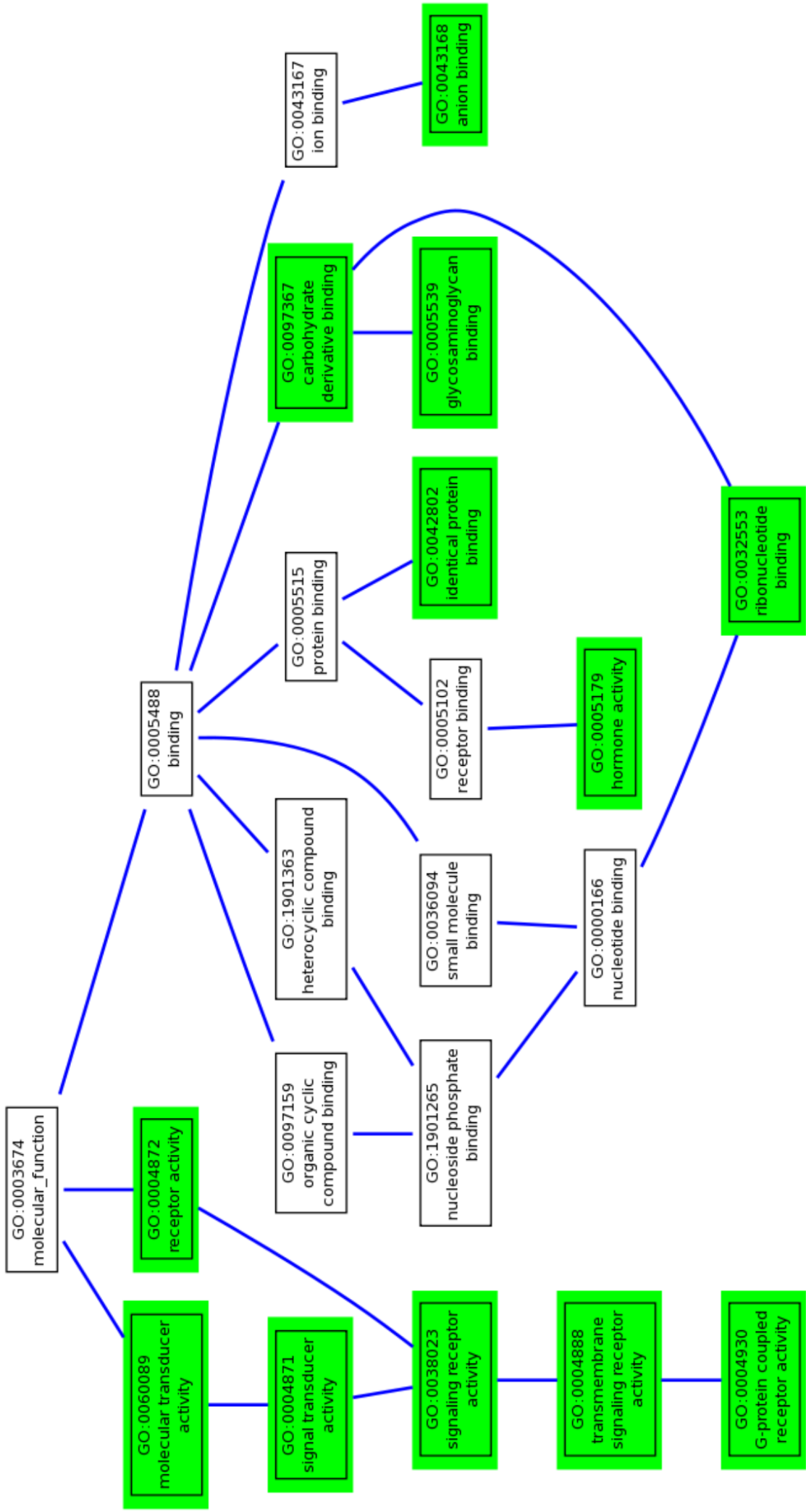
CC: T3 Up-regulated genes
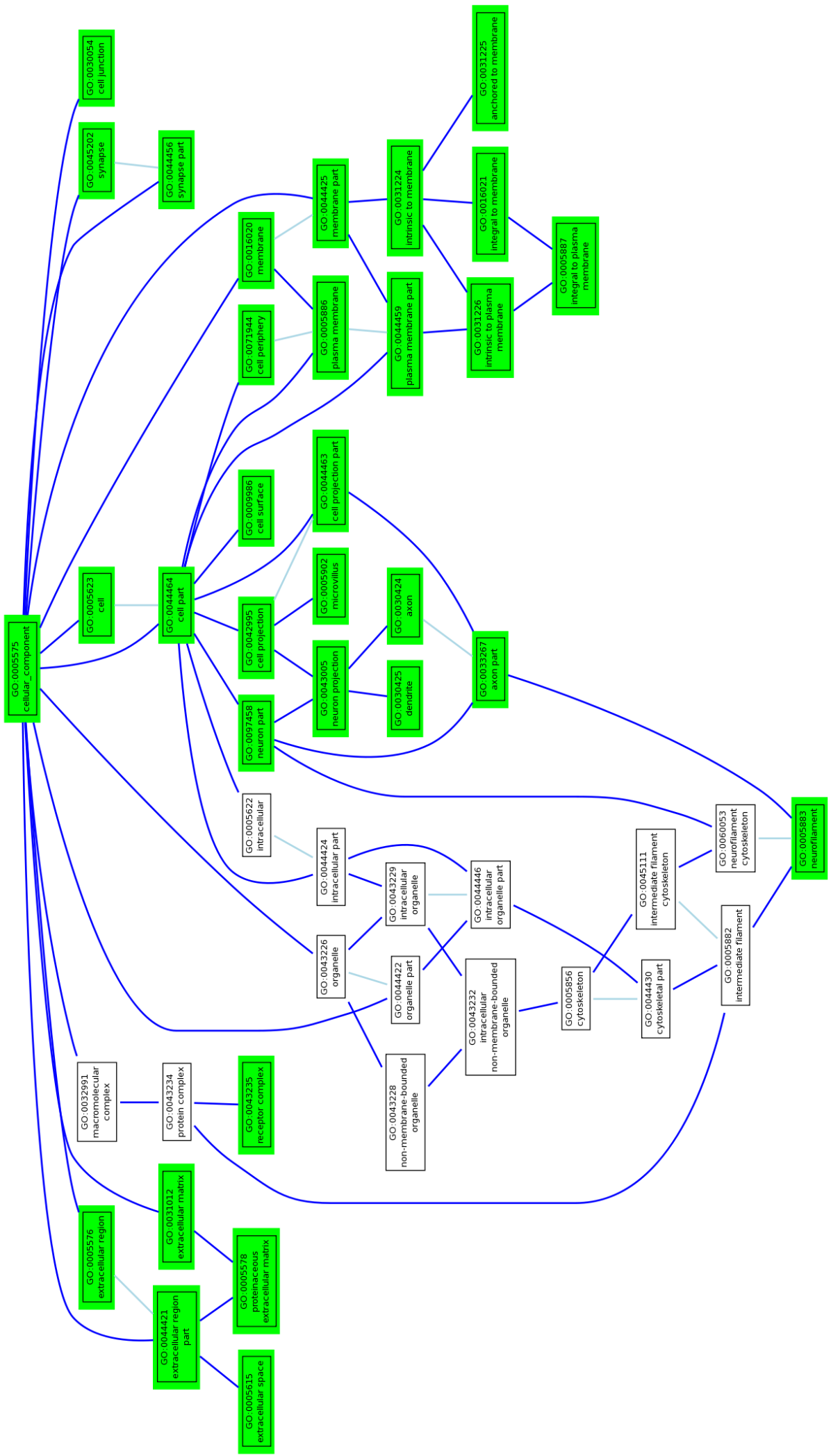
CC: T3 Down-regulated genes
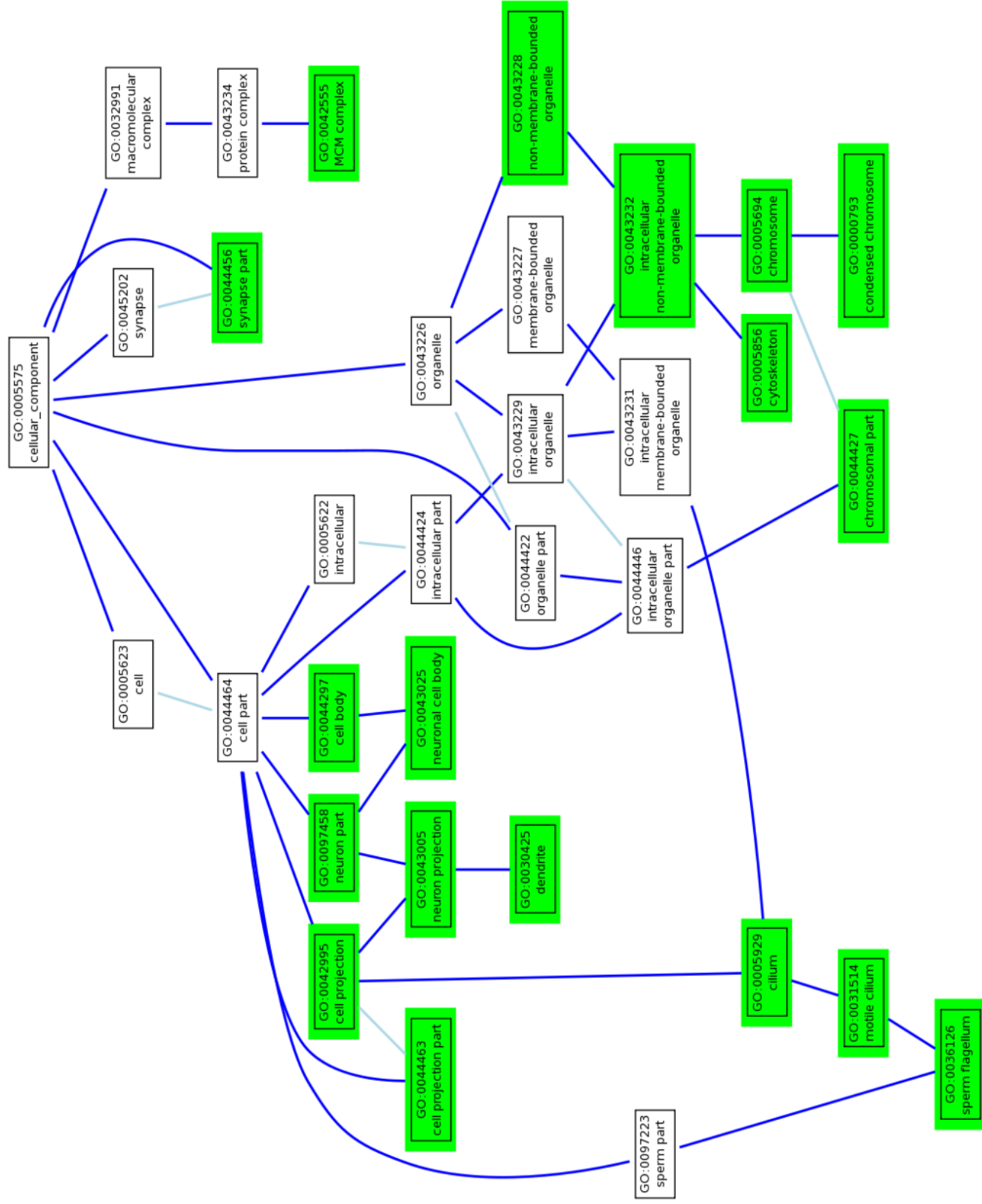
Annex III. Enriched Gene Ontology terms represented by GOgraphs. Graphs of Molecular Function and Cellular Component categories of all T3 regulated genes, T3 up-regulated and T3 down-regulated genes, are represented. Green nodes are significant functional terms associated to genes differentially expressed. The rest of the nodes without color are not significant but they give us an idea about the relationship between all categories. Biological Process is not represented due to the large amount of data.

ANNEX IV:

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid fetal or postnatal cortex

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid fetal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo fetal Ctx [88] logFC	Hypo+TH fetal Ctx [89] logFC
Hr	3.09	-0.53	
Sema3c	1.59	-1.33	
Klf9	1.46	-0.67	0.66
Bcar3	1.33	-0.28	
Cntnap1	0.97	-0.49	
Sema7a	0.91	-0.40	
Slc5a5	0.79	-0.96	
Sorl1	0.67	-0.36	
Atp1a2	0.48	0.63	
Nefm	0.48	-0.74	
Rgs6	0.45		1.20
Slc24a2	0.43		0.75
Ephb2	0.42		0.63
Nefh	0.42	-0.72	
Fstl4	0.40		0.80
Gpr125	0.37	-0.31	
Trp53inp2	0.35	-0.47	
Pitpnc1	0.33		0.73
Nfic	0.32		-0.68
Atp2b2	0.29	-0.97	
Tmem47	0.29	-0.49	
Col11a1	0.29	-0.79	
Mycn	0.28	-0.35	
Lrig1	0.28		0.82
Elovl5	0.27	-0.29	
Arhgap20	0.20	0.44	
Hsph1	0.19	-0.58	
Cxadr	-0.17		-0.59
Rgs12	-0.22	0.36	
Crim1	-0.28		0.67
Sh3rf1	-0.29		0.98
Grik1	-0.29	0.47	
Negr1	-0.32	0.43	
Fam101b	-0.35		0.63
Nov	-0.36	-0.92	
Sptssb	1.86	-1.27	
Col9a2	1.07	-0.61	
Cadm2	0.95	-0.79	
Prss35	0.91	1.18	
Dbp	0.86	-0.88	
Bcl6	0.63	-0.67	
Btbd17	0.62	-0.66	
Mme	0.49	-0.70	
Ppap2b	0.48		1.50
Actr3b	0.48	-0.82	
Plcxd2	0.47	-0.43	
Tmtc1	0.45	-0.64	
Adcy8	0.44	0.35	
Nt5c	0.42	0.40	
Rell1	0.41	-0.41	

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid fetal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo fetal Ctx [88] logFC	Hypo+TH fetal Ctx [89] logFC
Bcas1	0.40		0.61
Spata13	0.39		1.11
Cntn4	0.39	-0.74	
Frmpd4	0.39	-0.69	
Lin7b	0.38	-0.64	
Chrna5	0.38	0.45	
Cadps	0.38		0.64
Dbc1	0.38	-0.62	
Chn1	0.37	-0.46	
Kdm6b	0.36		0.75
Spock1	0.33		0.59
Fkbp2	0.33	-0.36	
Frmd4b	0.33		-0.61
Arsg	0.32	0.98	
Nefl	0.32	-0.70	
Tspan5	0.31		0.93
Hs3st1	0.31	-0.71	
Opcml	0.30	-0.32	
Prom1	0.30	0.47	
Camk4	0.29	-1.37	0.83
Zbtb20	0.29		0.85
Rnf152	0.29	-0.61	
Fat3	0.24	-0.38	
Igf1r	0.24		0.79
Faah	0.24		0.81
Got1	0.24	-0.33	
Epb4.1l2	0.23		0.82
Itgav	0.21		-0.90
Evl	0.20		1.02
Hnrnpl	0.19		0.60
Slc6a8	0.18	-0.53	
Afap1	0.17		0.99
Dnmt3a	0.15	0.43	
Prkacb	-0.16		-0.70
Dnm3	-0.18		0.61
Bhlhe22	-0.19	-0.51	
Dpysl2	-0.21		1.18
Cnr1	-0.23		-0.95
Large	-0.24		0.83
Rps6ka5	-0.25		-1.03
Ikzf4	-0.25		0.58
Rabgap1l	-0.25		-0.90
Rrm1	-0.26		0.59
Smarca2	-0.26		-0.71
Lrfn5	-0.26		1.45
G2e3	-0.27		-0.70
Robo1	-0.29		0.69
Alms1	-0.30		0.70
Mcm4	-0.30	-0.40	
Cdk19	-0.31		-0.74

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid fetal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo fetal Ctx [88] logFC	Hypo+TH fetal Ctx [89] logFC
Fezf2	-0.31		-0.66
C1ql3	-0.32	0.68	
Hdac9	-0.32		-0.78
Nr4a3	-0.33		-0.73
Flrt3	-0.34		-0.76
Gucy1a3	-0.36		-0.71
Thrsp	-0.37	-0.57	
Gpr85	-0.37	-1.26	
Slit2	-0.39	0.66	
Hap1	-0.39	0.49	
Pask	-0.40	0.50	
Sema3a	-0.42		-0.67
Unc5c	-0.45		1.38
Unc5d	-0.48	0.51	
Kif11	-0.50		-0.61
Olfml2b	-0.50	-0.31	
Vcan	-0.51		1.28
Efna5	-0.51		1.16
Tnfaip3	-0.53		-0.77
AW551984	-0.61	0.55	
Pde1c	-0.62	0.31	
St8sia4	-0.65		-1.09
Ndst3	-0.77		-0.71
Rgs5	-0.82		-0.61
Pcdh18	-0.83		-0.80
Cnn1	-0.96	0.45	
Calb1	-1.09	0.53	
Htr7	-1.10	0.59	

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid postnatal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo P21 Cxt [90] logFC
Hr	3.09	-2.14
Sema3c	1.59	-0.54
Klf9	1.46	-0.64
Bcar3	1.33	-0.94
Cntnap1	0.97	-0.50
Sema7a	0.91	-1.20
Slc5a5	0.79	0.65
Sorl1	0.67	-0.67
Atp1a2	0.48	-0.62
Nefm	0.48	-1.41
Rgs6	0.45	-0.53
Slc24a2	0.43	-0.55
Ephb2	0.42	-0.55
Nefh	0.42	-1.18
Fstl4	0.40	-1.15
Gpr125	0.37	-0.55
Trp53inp2	0.35	-0.57
Pitpnc1	0.33	-0.65
Nfic	0.32	-0.70
Atp2b2	0.29	-1.15
Tmem47	0.29	-0.68
Col11a1	0.29	-1.28
Mycn	0.28	-0.53
Lrig1	0.28	-0.51
Elovl5	0.27	-0.38
Arhgap20	0.20	-0.47
Hsph1	0.19	-0.57
Cxadr	-0.17	0.59
Rgs12	-0.22	0.44
Crim1	-0.28	0.44
Sh3rf1	-0.29	0.28
Grik1	-0.29	0.50
Negr1	-0.32	0.35
Fam101b	-0.35	-0.49
Nov	-0.36	0.61
Stat5a	1.76	-1.05
Flywch2	1.65	-0.79
Hcrtr1	1.56	-0.74
Gls2	1.33	-1.03
Rasd2	1.30	-1.19
Kcnj10	1.30	-2.22
Lgi3	1.23	-0.60
Cyp26b1	1.15	-1.41
Stac2	1.09	-1.34
Daam2	0.99	-0.55
Rdh5	0.98	-0.42
Aldh1a1	0.89	-2.05
Acot11	0.80	-0.71
Npnt	0.80	-1.31
Tmem100	0.79	0.59

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid postnatal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo P21 Cxt [90] logFC
Gpr37l1	0.77	-0.51
Suv420h2	0.76	-0.39
Itih3	0.74	-1.24
Sned1	0.73	-0.62
Plekho2	0.72	-0.59
Cdc42ep1	0.72	-0.57
Itga7	0.72	-0.90
Tesc	0.70	-0.70
Mmp17	0.70	-0.43
Cgrrf1	0.68	-0.67
Tm6sf1	0.68	-0.45
Abcd2	0.67	-1.05
Ephb1	0.66	-0.62
Acsbg1	0.66	-0.61
Syt12	0.60	-0.77
Yjefn3	0.56	-0.48
Epha4	0.56	-0.40
Pcx	0.56	-0.53
Tst	0.55	-0.57
Angptl4	0.54	0.82
Lpar1	0.53	-0.79
Pde8b	0.53	0.14
Adarb1	0.52	-0.49
Gabrd	0.52	-0.65
Adamts2	0.51	-0.29
Chchd10	0.51	-0.54
Fam163a	0.51	0.59
Lrrc55	0.50	-0.76
Gpr146	0.49	-0.54
Rnf112	0.49	-0.44
Slc16a6	0.49	-0.96
Sla	0.49	0.45
Scrt2	0.47	-0.62
Padi2	0.47	-0.66
Klhdc7a	0.47	-1.28
Lingo3	0.46	-1.02
Lynx1	0.46	-0.79
Abcc4	0.45	-0.37
Ntsr2	0.45	-0.89
Cdh22	0.45	-0.57
Camk2n1	0.44	-0.45
E130114P18Rik	0.44	-0.39
Prss12	0.43	0.50
Slc4a4	0.43	-0.88
Hsd11b1	0.43	-0.71
Mfap3l	0.41	-0.50
Olig1	0.41	-0.47
Cacng2	0.40	-0.60
Ptchd2	0.40	-0.77
Hapln1	0.38	0.76

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid postnatal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo P21 Cxt [90] logFC
Pla2g7	0.38	-0.55
Cabp1	0.38	-0.63
Pltp	0.37	-0.97
Tlcd1	0.37	-0.51
Krt10	0.37	-0.87
Scrt1	0.36	-0.59
Megf10	0.36	-0.46
Lpl	0.36	-0.98
Acss2	0.35	-0.46
Ank1	0.35	-0.39
Paqr7	0.34	-0.46
Ier5	0.34	-1.41
Npy	0.33	-0.40
Gpc6	0.33	-0.17
Wbscr27	0.32	0.26
Tsc22d4	0.31	-0.27
Cxcr7	0.30	-0.80
Igsf9	0.30	-0.35
Mpp6	0.28	-0.52
Slc25a33	0.28	-0.60
Chst11	0.28	-0.47
Pfkfb3	0.28	-0.68
Slc4a8	0.27	0.52
Aqp4	0.27	-0.63
Ndufa1	0.27	-0.40
Kif1c	0.27	-0.33
Ckb	0.27	-0.45
Gstm1	0.24	0.45
Ppargc1a	0.24	-0.56
Klhl29	0.24	-0.51
Reln	0.23	0.93
Acsl6	0.22	-0.60
Prex1	0.21	-0.53
Ntm	0.20	-0.44
Rph3a	0.19	-0.44
Dpysl3	-0.15	0.71
Mtss1	-0.18	0.39
Mkl2	-0.19	-0.32
Apaf1	-0.19	0.73
Prrc1	-0.20	0.38
Rimklb	-0.20	0.60
Ntsr1	-0.21	0.93
Fam78b	-0.21	-0.51
Slc16a2	-0.22	-2.25
Creg2	-0.23	0.64
Pam	-0.23	0.42
Tspan6	-0.23	0.39
Myo5b	-0.25	0.60
Tox3	-0.25	0.43
Bbs9	-0.25	0.61

Differentially expressed genes in cerebrocortical cells after T3 treatment and hypothyroid postnatal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo P21 Cxt [90] logFC
Tspan2	-0.25	-0.37
Cntn5	-0.26	0.49
Smarca1	-0.26	0.54
Pnmal1	-0.27	0.49
Zbtb46	-0.29	0.25
Adrbk2	-0.29	0.50
Fat4	-0.29	0.45
Rsph9	-0.29	0.73
Casp3	-0.29	0.48
Cntnap4	-0.30	0.50
Gabra5	-0.32	0.41
Rasgrf1	-0.33	0.22
Ssbp2	-0.33	0.26
Pgap1	-0.34	0.34
Trim59	-0.34	-0.46
Trp53i11	-0.36	0.43
Slit3	-0.36	0.34
Ccdc39	-0.37	0.57
Efnb2	-0.39	0.39
Tmeff2	-0.39	-0.5
Lgals1	-0.40	0.54
Grp	-0.41	1.02
Slc24a4	-0.41	0.55
Adamts18	-0.42	1.01
Trip13	-0.42	0.49
Mcm6	-0.43	0.49
Unc5b	-0.44	-0.57
Zfp395	-0.44	0.77
Cadm1	-0.45	0.37
Npr3	-0.47	0.54
Fancd2	-0.47	0.62
Grin3a	-0.49	-0.29
Rfx3	-0.50	0.56
Plxnc1	-0.53	0.38
Ntf3	-0.54	1.16
Tac2	-0.54	1.00
Wdr66	-0.55	-1.02
Esr1	-0.55	1.10
Fank1	-0.55	0.35
Col23a1	-0.55	0.86
Syt17	-0.58	0.55
Cdkl1	-0.58	0.69
Gypc	-0.61	0.52
Nnmt	-0.61	0.67
Pygl	-0.70	0.85
Stbd1	-0.70	0.32
Gpc3	-0.86	1.09
Cmbl	-0.96	0.55
Slc38a4	-1.37	0.70
Col14a1	-1.57	0.76

Differentially expressed genes in cerebrocortical cells
after T3 treatment and hypothyroid postnatal cortex

Gene Name	T3 cerebrocortical cells logFC	Hypo P21 Cxt [90] logFC
Popdc2	-3.51	0.49

Annex IV. Overlap between the differentially expressed genes identified in primary cerebrocortical cells after T3 induction and differentially expressed genes in cerebral cortex in vivo at fetal [88,89] and postnatal stages [90] under different thyroid hormone conditions.