Inferring metabolic rewiring in embryonic neural development using single cell data

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Abstract

Metabolism is intricately linked with cell fate changes. Much of this understanding comes from detailed metabolomics studies averaged across a population of cells which may be composed of multiple cell types. Currently, there are no quantitative techniques sensitive enough to assess metabolomics broadly at the single cell level. Here we present *scMetNet*, a technique that interrogates metabolic rewiring at the single cell resolution and we apply it to murine embryonic development. Our method first confirms the key metabolic pathways, categorized into bioenergetic, epigenetic and biosynthetic, that change as embryonic neural stem cells differentiate and age. It then goes beyond to identify specific sub-networks, such as the cholesterol and mevalonate biosynthesis pathway, that drive the global metabolic changes during neural cortical development. Having such contextual information about metabolic rewiring provides putative mechanisms driving stem cell differentiation and identifies potential targets for regulating neural stem cell and neuronal biology.

Introduction

The rapid development of computational methods for single cell gene expression has led to new insights into developmental biology. These insights range from confirmation of known knowledge accumulated over decades from “one” experiment (Farrell et al., 2018; Karaikos et al., 2017), to discovering new cell types and their lineages (Kester & van Oudenaarden, 2018) and addressing tumor heterogeneity (Levitin, Yuan, & Sims, 2018). However, many challenges remain (Lähnemann et al., 2020) creating as many opportunities (Dasgupta, Bader, & Goyal, 2018).

One of the fundamental challenges is that while the cell types in developing tissues can be discerned the identity of key regulators behind the emergence of these cell types are hard to pinpoint. This is due to the low expression levels and often transient nature of transcription factors (Martin & Sung, 2018) that are thought to drive cell transitions. Another limitation of understanding key regulators from single cell analysis is not knowing the landscape of interactions between

proteins, genes, and small molecules. This is particularly challenging as these interactions change with biological context.

In addition to master gene regulators such as transcription factors, metabolic rewiring is known to accompany and regulate changes in cell state. For example, metabolism is being targeted in multiple disease states (Afonso, Santos, Longatto-Filho, & Baltazar, 2020; Huang & Perl, 2018; Luengo, Gui, & Vander Heiden, 2017). As a second example, metabolic rewiring accompanies early development (Intlekofer & Finley, 2019; Shyh-Chang & Ng, 2017) and external metabolites have been used to alter developmental output in organoids (Schell et al., 2017), suggesting a potential for effective *in vivo* metabolic interventions in stem cell biology.

Metabolism has been studied extensively within stem cells, with a focus on *in vitro* study of mouse embryonic stem cells (mESCs) and induced pluripotent stem cells (iPSCs) as biological models (Shyh-Chang & Ng, 2017). These studies have defined various metabolic pathways that govern stem cell fate decisions, proliferation and differentiation. Can single cell sequencing shed light on metabolic regulators of *in vivo* development? Prima facie, in the case of metabolism, two of the main challenges associated with understanding regulators of stem cell fate dynamics, low expression levels of transcription factors and lack of interaction map between the signaling proteins, may not be limiting. Genes that encode metabolic enzymes are often expressed at high levels, and a detailed understanding of metabolic pathways provides the required interaction landscape. Recently, metabolic changes in tumors have been addressed using single cell expression data (Xiao, Dai, & Locasale, 2019), thereby demonstrating the potential importance of such an approach at the therapeutic level.

Here we present *scMetNet*, a method that uses single cell gene expression data to create a map for metabolic rewiring between different cell types at a particular stage and for a particular cell type across developmental time. We have applied *scMetNet* to understand embryonic neural development, with a particular focus on the murine embryonic cortex from E13.5 to E17.5, a developing tissue that is comprised of radial glial precursors (RPs) that generate neurons either directly or via intermediate progenitors (IP), a neurogenic transit-amplifying cell (Yuzwa et al., 2017). Using this approach, we define unique metabolic states that are aligned significantly with RPs, IPs and newborn neurons. Given the significant concordance between cell types and their metabolism, we then inferred a metabolic network based on the known landscape of metabolic interactions from the Kegg database (Kanehisa & Goto, 2000). This connected network of genes and metabolites defined the metabolic re-wiring between RPs and neurons within each time point. As neurons increase in number to form the cortical networks that serve higher cognitive functions, RPs alter their metabolic character as they switch from generating neurons to transitioning to postnatal dormant forebrain neural stem cells. We document a similar metabolic signature using single cell gene expression from a second region of the brain where embryonic neural precursors persist to become dormant postnatal neural stem cells, the embryonic hippocampal dentate gyrus. Overall, *scMetNet* identified the precise metabolic rewiring that occurs as embryonic neural stem cells generate neurons, and provides potential molecular targets for regulating neural development.

Results

Here, we utilized scMetNet and analyzed single cell gene expression data from the murine embryonic cortex from E13.5 to E17.5, when there are three distinct cell types: radial glial precursors (RPs), a transit-amplifying intermediate progenitor cell called an intermediate progenitor (IP) and their newborn neuronal progeny (Figure 1A).

Metabolic states align with developmental lineages in embryonic neural development

To understand the metabolic changes that hallmark the neural stem cell to neuron transition during embryonic cortical development, we asked whether there are distinct metabolic states that accompany emerging cell types. To address this question, cells were clustered using only their metabolic gene expression profiles (Fig. 1B and Methods Section for details). These clusters defined the “metabolic states” of the cells in the developing cortex. Notably, by delineating the induced transcriptional metabolic pathway changes, three distinct metabolic states emerged that aligned well with the three embryonic cortical cell types (Fig. 1C). Can this alignment result from chance? To test this, we computed the overlap of cell types with clusters derived from random sets of non-metabolic genes with each set having the same number of genes as the metabolic genes. We found that there are no random sets that are better aligned with RPs than metabolic genes, while there may be some random sets that can show higher alignment with neurons and IPs. Overall, metabolic genes show significant overlaps ($p\text{value} < 10^{-3}$) across all the three cell types (Supplementary Fig. S1).

The metabolite specific alignment details a compelling picture of the temporal metabolic changes that accompany the transition between neural precursors and neurons. To determine the effect of number of metabolic states, we computed overlap of cell types with increasing numbers of metabolic clusters. We found that the largest cluster of cells annotated as neurons gets further segmented into different metabolic states (see Supplementary Fig. S2), consistent with multiple cell clusters observed earlier using all the variable genes (Yuzwa et al. 2017). Overall, these metabolic states were stably represented across the three developmental time points considered (see Supplementary Fig. S1). In particular at E15.5, the majority of cells identified as RPs map to metabolic state S_0 , most of the IPs are in metabolic state S_1 , and neurons are in S_1 and S_2 . The temporal change in the metabolic state of IPs is consistent with their transient nature; they first were almost equally split between metabolic states S_0 and S_1 at E13.5, then at E15.5 they were almost fully in state S_1 and then they were no longer present at E17.5. This is notable, as by E17.5 RPs are known to be generating very few neurons, hence may not be generating IPs (shown in Supplementary Fig. S1).

The metabolic changes between RPs and neurons reflect classic hallmarks of cellular differentiation

Given that the three cell types are largely in different metabolic states, we applied Gene Set Enrichment Analysis (GSEA) to ask what pathways separate the undifferentiated RPs from the differentiated neurons. GSEA leverages annotated pathway databases to identify “enriched” pathways by comparing the average gene expression between two cell types (Fig. 1D).

Consistent with the fact that RPs proliferate but neurons are post-mitotic, the most enriched gene sets in RPs are those involving DNA replication and the cell cycle. For neurons, the most significantly enriched are axon guidance, and neuronal migration-related genes.

When focusing on the metabolic subsets, GSEA confirmed important pathways previously seen during cellular differentiation in other systems. Many of these pathways fall into three functional categories often used to describe metabolic changes during tissue development (Shyh-Chang & Ng, 2017). First are bioenergetic pathways necessary to support rapid cell division in developing tissues. As such fatty acid oxidation and glycolysis were upregulated in RPs suggesting their role in meeting the energy requirements of these rapidly dividing cells. Second are epigenetic pathways involved in the large-scale epigenetic changes that drive cellular differentiation. Here, methionine metabolism was upregulated presumably to supply the methylation demands associated with modifying the epigenome of RPs differentiating into neurons (Shiraki et al., 2014). Third are biosynthetic pathways necessary for the rapid growth of biomass necessary to sculpt tissues. Here, we found glycine-serine and one carbon metabolism were the biosynthetic pathways driving synthesis of macromolecules, such as glycine and serine, essential for increasing neuron numbers during brain development. A restricted GSEA analysis only for metabolic pathways applied on the cells in the different metabolic states was consistent with the RP-vs-neurons GSEA analysis (Supplementary figure S3).

scMetNet reveals inter-connected metabolic modules

While the GSEA analysis identifies average changes that occur in different cell types during differentiation, it does not reveal specific genes that define these significant metabolic pathways, the specific reactions they catalyze, and/or the metabolites that are important. Having such a finer scale understanding is essential to altering the fate of cells and developing new potential therapeutics. We addressed these issues by adapting a network algorithm, CombiT (Jha et al., 2015), designed originally to utilize microarray and metabolite measurement data, into scMetNet pipeline to evaluate for metabolic rewiring that differentiates RPs from neurons. Specifically, CombiT algorithm allows scMetNet to identify the most connected network consisting of enzyme-metabolite reactions using the Kegg database that define the difference between the RPs and neurons. In addition to metabolic gene networks, scMetNet leverages the curated metabolic knowledge to infer the critical metabolites that form the metabolic backbone of the re-wired network. (Refer to methods)

scMetNet analysis of the E15.5 cortical cells (shown in Fig. 2) highlights differences in key genes and inferred metabolites that distinguish metabolism between RPs and neurons. As shown, the full metabolic network can be easily split into interconnected modules that align with the three functional categories of GSEA derived significant pathways, bioenergetic, epigenetic and biosynthetic.

With regard to bioenergetics, upregulation of both fatty acid and glycolysis modules in RPs support metabolism-driven oxidative-phosphorylation (OXPHOS) to fulfill the high-energy requirements of rapidly dividing RPs. Within the fatty acid module, surprisingly, both biosynthetic and catabolic genes were upregulated. In particular, upregulation of genes like *Acsbg1*, which is

involved in the synthesis of long chain fatty acids, suggest a biosynthetic role of fatty acid metabolism while upregulation of *Cpt1a*, which transports fatty acids to mitochondria, suggests a bioenergetic role utilizing glycolysis (Stephens, Constantin-teodosiu, & Greenhaff, 2007). More directed approaches will be required to distinguish futile metabolic cycling reactions versus activation of different subnetworks for some other process.

Although the OXPHOS module is not significantly different between RPs and neurons (Fig. 1D), its importance for RPs is suggested from the observed upregulation of *Psat1* and *Phgdh* which influence intracellular levels of alpha ketoglutarate (a-KG) (Hwang et al., 2016; Reid et al., 2018), an important component in the TCA cycle. The importance of the TCA cycle is also supported by upregulation of *Idh2*. Thus, RPs seem to utilize a mix of glycolysis and OXPHOS for their energy requirements. It is worth noting that while the GSEA did not identify OXPHOS as an enriched pathway, scMetNet pipeline revealed parts of OXPHOS pathway that are connected to other modules such as glycolysis, and hence might be important to the overall metabolic rewiring at this stage of development.

The second functional change involves epigenetic pathways, which center around the two key metabolites, alpha-ketoglutarate (a-KG) and methionine. In this regard, a-KG, a key cofactor for the TET family of DNA hydroxylases and Jumonji C-domain-containing histone demethylases, is linked with enzymes *Psat1* and *Phgdh*. Notably, these genes are thought to determine the timing of differentiation of embryonic stem cells by regulating a-KG levels (Hwang et al., 2016), and these findings suggest they may play a similar role in RPs. Methionine metabolism and methylation reactions are also apparently upregulated in RPs, as indicated by genes such as *Dnmt3a* and *Mettl1*, which likely play a role in maintaining SAM levels (Shiraki et al., 2014). The observed upregulation of specific genes controlling levels of a-KG and methionine metabolism suggest potential new targets for altering the neural stem cell to neuron transition at the epigenetic level.

With regard to the third functional category, biosynthesis, enzymes driving glycine/serine metabolism and one carbon metabolism provide rapid synthesis of macromolecules as they are required in fast dividing RPs. Specifically, the scMetNet network (Fig. 2) identifies upregulation of genes essential for anabolic reactions that are part of the Phosphate Pentose Pathway (PPP) such as *Tpi1* and *Tkt*, as well as for synthesis of a variety of macromolecules from nucleotides (*Shmt1*, *Paics*) to fatty acids (*Acsbg1*) (Stincone et al., 2015). It also identifies links between different modules, where one carbon metabolism influences methionine metabolism, and glycine and serine metabolism feed into one carbon metabolism by acting as a source for folate intermediates (Amelio, Cutruzzolá, Antonov, Agostini, & Melino, 2014). Specific enzymes, such as *Tpi1* and *Tkt* in PPP, *Mthfd1*, *Dhfr*, and *Tyms* in one carbon metabolism, and *Psph*, *Shmt1* and *Paics* in glycine-serine metabolism provide particular targets for altering RP neuronal output at this stage.

Cortical radial precursors shift their metabolic state as they transition from rapidly-proliferating to slowly-proliferating/quiescent during embryogenesis

While cortical RPs largely maintain their transcriptional identity over the developmental period from E13.5 to E17.5 (Yuzwa et al., 2017), they undergo a poorly-understood transition from rapidly-proliferating to slowly-proliferating as they switch to making glial cells and to populating the adult neural stem cell pool. We asked if this transition also involved metabolic changes, initially using differential GSEA. This analysis identified methionine, glycolysis and fatty acid metabolism as the most significantly changing pathways in RPs from E13.5 to E17.5 (Table S2 and S3). Methionine metabolism and glycolysis are downregulated at later developmental times, while fatty acid metabolism is significantly upregulated as shown by changes in gene enrichment scores for these pathways (Fig. 3A).

These temporal trends are reflected in gene expression of key markers for these pathways that are assembled by the network analysis at the three time points (Fig. 3B shows the genes that are assembled by scMetNet and networks for E13.5 and E17.5 are shown in see Supplementary Fig. S4 and S5). However, while there are changes from E13.5, there are twenty-nine common metabolic genes that remain significant across the different time points and thus form the core metabolism that define RPs (see highlighted genes in Fig. 3B). We also observe upregulation of fatty acid and lipid synthesis at E15.5 and E17.5 (see Supp Fig S6). The core metabolism genes in general correspond to glycine-serine and one carbon metabolism highlighting the importance of these pathways in neuronal development (Supp Fig S6 and Fig 3B).

Notably, over this timeframe, based on the increase in fatty acid metabolism, which we had suspected is feeding into TCA cycle, and decrease in glycolysis in RPs we hypothesize an apparent preference for OXPHOS over glycolysis, which may suggest an increasing role for ROS signaling in RP differentiation, as previously suggested (Madhavan, Ourednik, & Ourednik, 2006).

To examine these similarities and differences more closely, we performed the scMetNet analysis of RPs from E13.5 and E17.5 (see Fig. 4 for the metabolic network). Consistent with the global analysis, the metabolic rewiring network showed an upregulation of fatty acid metabolism that involved genes involved in both biosynthesis (*Acsbg1*) and beta oxidation (*Acadl*, *Acaa2*, *Hadh*). Notably, *Cpt1a*, which was identified as likely being important for fatty acid metabolism driving OXPHOS, is not picked up by the scMetNet pipeline, suggesting that there is no significant shift in metabolic rewiring around *Cpt1a*. Instead, the upregulation of fatty acid synthesis between E13.5 and E17.5 involves a significant upregulation of cholesterol and mevalonate biosynthesis pathways, with the mevalonate biosynthesis pathway feeding directly into cholesterol synthesis through squalene.

Notably, consistent with our hypothesis about the increasing role of ROS based on the global changes, scMetNet network (Fig. 4) shows glutathione S transferases and peroxidases (*Gstm1*, *Gpx7*) as being upregulated in later stage RPs. Taking a clue from the literature, this suggests that perturbing RPs ability to use peroxidases may increase neural differentiation and help alleviate pathologies related to delayed neural development (Savaskan, Borchert, Bräuer, & Kuhn, 2007). Here again CombiT revealed aspects of metabolic rewiring around glutathione that GSEA failed to highlight.

Finally, scMetNet also identified changes in metabolism around key epigenetic drivers between E13.5 and E17.5, which could be associated with the switch from making neurons to making glia and/or the switch to a quiescent postnatal neural stem cell state. These included genes associated with both Acetyl CoA production and methylation including downregulation of glycolysis associated genes such as *Ldha* and *Hk2*, and downregulation of methionine and SAM metabolism associated genes such as *Dnmt3a*, *Mat2a*, and *Ahcy*. Upregulation of *Pdha1* in conjunction with downregulation of glycolysis genes suggests diversion of Pyruvate to Acetyl CoA and hence increased OXPHOS. Consistent with the transition from a rapidly-dividing to slowly-proliferating/quiescent state, scMetNet also defined a downregulation of biosynthetic pathways such as PPP and nucleotide synthesis as signified by downregulation of enzymes like *Tkt*, *Tpi*, *Prps1* (PPP) and *Paics*.

Is metabolic rewiring consistent across different regions of the developing brain?

As discussed above we hypothesized that the changes in E17.5 cortical RPs might be part of their transition to a slowly-proliferating/quiescent state. To directly test this idea, and to ask whether this might be a common set of metabolic changes for stem cells, we compared the changes seen in E17.5 cortical RPs to E16.5 dentate gyrus RPs using previously published scRNA-seq data (Hochgerner, Zeisel, Lönnerberg, & Linnarsson, 2018). We found several striking similarities between the two regions (metabolic network shown in Fig. 5). Importantly, both the genes encoding metabolic enzymes and reactions common across the two regions were spread across the whole metabolic map; the common reactions are identified by dashed lines in Fig. 5, including the characteristic upregulation of fatty acid metabolism and the upregulation of mevalonate biosynthesis. Overall, we see thirteen out of the seventeen genes were present in the same metabolic modules. A notable difference is upregulation of Sam metabolism in dentate gyrus, which may partly be due to dentate gyrus data being from E16.5, as Sam upregulation was seen at E15.5 in cortical RPs.

Discussion

Single cell transcriptomics has fueled much of the recent rapid progress in understanding cell fate changes during development and tissue regeneration. Here we present scMetNet, a technique that leverages single-cell RNA sequencing data to infer global metabolic rewiring across emerging cell types expected in early developmental events. We first established the congruence between the cell states and metabolic states, both of which are identified using established clustering techniques developed to analyze single cell RNAseq data. To exploit the concordance of metabolic states with cell types we then applied a network-based analysis tool to infer metabolic wiring during early neurogenesis. Our analysis revealed important metabolic modules across the three important functional categories: *bioenergetics*, *epigenetics*, and *biosynthesis*. These modules went beyond the results from global analyses such as GSEA that stop at identifying differential pathways, to further pinpoint the underlying genetic and metabolic drivers of the epigenetic machinery responsible for maintaining pluripotency of stem-like cells (Fawal et al., 2018; Shiraki et al., 2014), the rate at which they differentiate, and of the bioenergetic pathways responsible for survival and proliferation this pool of stem-like cells (Hu et al., 2016). Many of

these have been suggested in different tissue contexts but have not been fully appreciated and tested in the context of embryonic neural development. One of the surprising findings our analysis uncovered was the upregulation of mevalonate and cholesterol and associated pathways in neural stem cells by stage E17.5. This coincides with a slowing down of RP-mediated neurogenesis. Other shifts such as increased OXPHOS and ROS also point towards the possibility of neural stem cells becoming more quiescent or dormant. High levels of cholesterol biosynthesis have been previously reported during neural development and in adult brains astrocytes/microglia. However, the role of cholesterol production by neural stem cells as their neuronal output slows down at E17.5 remains unclear. Previous work where alterations in cholesterol synthesis in stem cells led to neuron apoptosis rather than impaired neurogenesis (Saito et al., 2009) suggests a possible cell extrinsic role of cholesterol produced by the neural stem cells.

We would like to reiterate the subtle “signals” in metabolic rewiring, such as increased ROS activity at E17.5 and the associated genes and metabolites driving the increase remain hidden in global approaches such as GSEA. This is not surprising as GSEA relies on the a priori definitions of pathways, where each pathway is exhaustively defined across different biological contexts and hence have many more genes and metabolites that play out in any particular biological context. The output from the scMetNet analysis, on the other hand, is independent of such global pathway definitions and aims to build a network of connected metabolic reactions independent of the pathway they are associated with. Hence, scMetNet facilitates painting a holistic picture by directly providing interactions between the different pathways.

Now, armed with a detailed knowledge of metabolic rewiring at the level of individual genes and metabolites we explore here potential interventions to alter embryonic neural development with focus on epigenetic and bioenergetic modules. Our analysis identified twelve genes associated with epigenetic metabolites, acetyl-CoA and α -KG, one-carbon metabolism and methylation-demethylation associated genes. Out of the twelve, seven genes have been implicated in neuropathologies but have not yet been directly explored in their role in altering early neural development. For instance, deficiency of the two α -KG associated enzymes *Psat1* and *Phgdh*, which are upregulated in RPs, have been reported in microcephaly, psychomotor retardation and epilepsy (Acuna-Hidalgo et al., 2014; Sharma & Prasad, 2017; Yoshida et al., 2003). We hypothesize that a KO of *Phgdh/Psat1* will advance RP differentiation timing to an earlier developmental stage. In the same vein, we hypothesize that the perturbation of mitochondrial genes associated with acetyl-CoA (*Acadl*, *Acadm*, *Acss1*, *Hadh*, *Acaa2*) will lead to a smaller pool of RPs due to decreased energy production, while perturbing acetylation via *Hat1* will lead to early differentiation of RPs.

The coincidence of slowing down of RPs neural output at E17.5 and upregulation of mevalonate and cholesterol provide a set of potential targets for altering the RPs neuronal output. Our analysis suggests application of statins - inhibitors of *Hmgcr* - will promote neurogenesis. Previous work suggests this modulation may act through Wnt signaling (Robin et al., 2014), consistent with which we found upregulation of *Lfng* which is a part of Wnt signaling pathway in RPs at E17.5. Similarly, deficiency of *Dhcr7*, important for cholesterol synthesis, is known to cause Smith-Lemli-Opitz

syndrome (Liu et al., 2014), and we hypothesize perturbing its levels will alter the neuronal output in embryos.

More broadly, many of the genes that define the metabolic rewiring network have been associated with cancers, which is not surprising considering the connection between stemness and tumorigenesis. The genes *Psat1*, *Phgdh* and *Ptprz1* are known to be upregulated in glioblastoma and lower grade glioma. Both *Psat1* and *Phgdh*, which catalyze important biosynthetic reactions, have been extensively studied in many cancers (Amelio et al., 2014), and *Ptprz1* has been said to inhibit stem cell like properties of tumor cells (Fujikawa et al., 2017). Here we have highlighted a few examples of potential perturbations to alter embryonic neural development, please see the details for all the genes we find in the rewiring network and their potential effect in the Supplementary Table S4 and highlighted in Supplementary Fig S7.

Overall, in this paper we show that scMetNet can utilize single cell data to both identify key metabolic pathways and then go further and identify specific genes, metabolites, and reactions that drive those metabolic pathways. Here by applying our approach to early neural development we propose a specific set of perturbations that can be used to alter different aspects of neurogenesis. Our approach is general and can be carried over to single cell data from other tissues either during development or regeneration to discover novel therapeutic targets as discussed above in the context of neuropathologies.

Materials and Methods

The scMetNet pipeline combines single cell analysis (Butler, Hoffman, Smibert, Papalexi, & Satija, 2018; Stuart et al., 2019) with network analysis (Jha et al., 2015) into an integrated pipeline that can take single cell data and perform all the analysis detailed below. The pipeline is available to run with the data used in the paper at Polly (<https://polly.elucidata.io/>).

Data Processing and Differentially Expressed Genes Selection

Gene expression matrices of cortical cells at all three embryonic ages (E13.5, E15.5, E17.5) were used from the GEO accession: GSE107122. All of the analyses and plots in the paper have been done using R (version 3.5.2) and Seurat (V2) package (Butler et al., 2018). We used the following quality control steps to reproduce the analysis done by Yuzwa et al: (i) Cells that expressed less than 200 genes and cells that expressed more than 2500 genes were excluded from the analysis, lower than 200 genes is an indicator of lower quality while higher than 2500 genes indicates that the cell might be a potential doublet; (ii) Genes expressed by less than 3 cells were removed; (iii) Cells having more than 15% mitochondrial content (as denoted by genes derived from the mitochondrial genome) were removed. The data were normalized using the *NormalizeData* function keeping normalization method as *LogNormalize* and scale factor as 10000. Unwanted variation due to total RNA in cells and mitochondrial gene content were regressed out using the *ScaleData* function in Seurat. Highly variable genes were computed for the counts at each embryonic age using the *FindVariableGenes* method of Seurat, we used *ExpMean* as the mean function, *LogVMR* as the dispersion function, mean lower threshold as

0.0125, mean higher threshold as 8 and dispersion threshold as 0.05. Lower dispersion threshold was used to get a higher number of genes as scMetNet needs genes that show both high and low variability to ascertain the background signal and identify significant and connected subnetworks. Using this method, we got 5888, 6539 and 5265 highly variable genes for the embryonic ages E13.5, E15.5, E17.5 respectively.

PCA was done using the highly variable genes and the first 20 principal components for each embryonic age were used for clustering and TSNE implementation. Clustering was performed using the SNN-Cliq method implemented in Seurat with a resolution of 2.5 for all three embryonic ages. To visualize clusters, t-SNE projections were calculated on the principal components using the default perplexity of 30. Annotation of clusters was done using the expression of known marker genes for each population as mentioned in Supplementary Table S1.

Differential expression analysis was done between cell population clusters using the Wilcoxon Rank Sum test available in Seurat.

Combine Single-Cell Datasets

Gene expression matrices from E13.5 and E17.5 were combined using Canonical Correlation Analysis available in Seurat. A union of highly variable genes from the counts at the two embryonic ages was used and the number of canonical vectors to calculate was set to 20.

Metabolic state extraction

For finding metabolic states, the dataset was limited to the 5266 metabolic genes identified from KEGG before processing. Rest of the steps followed were identical to the procedure described above except at the clustering step the resolution parameter was chosen as 0.5, 0.3, 0.4 at the three embryonic ages E13.5, E15.5 and E17.5 respectively to get three clusters corresponding to the three metabolic states at E13.5, E15.5 and two clusters corresponding to two metabolic states at E17.5. For figure S1C, a set of 4000 random genes was sampled from all genes and the pipeline mentioned above was run for each random gene set. The random gene set sampling was done 1300 times. The overlap between cells for each cell type and corresponding metabolic state was found for each random gene set and compared to overlaps obtained for states obtained using only metabolic genes. The p-values for the cell identities assigned using metabolic genes clustering were calculated using the permutation test including all the permutations.

Network Analysis

Network analysis pipeline prescribed by Sergushichev et al (Sergushichev et al., 2016) was used to construct the maps depicted in all figures. The network analysis pipeline performs the integrated network analysis of transcriptional and metabolomic data to find the most changing

subnetworks in the KEGG database. The pipeline was run using Intomix which is a Polly (<https://polly.elucidata.io/>) software.

Intomix uses a similar pipeline as mentioned in Sergushichev et al. We used the latest kegg database as of 2019 (<https://www.genome.jp/kegg/>) to construct the global network using KEGG REACTION database. We downloaded enzymes, glycans, compounds and reactions from KEGG and made a global network by removing ubiquitous metabolites such as ATP and collapsing groups of anomeric metabolites into one metabolite. KEGG ENZYME database was used to make the enzyme-gene-organism mapping.

The analysis was performed as a two-step process -

1. Creating a network of reactions based on the scRNA differential expression data - The algorithm converts the differential expression of genes into the differential expression of reactions. Genes taking part in a specific reaction and coding for an enzyme are taken into account and the minimum pval represented within the gene set is selected as the p-value of the reaction. Reactions with no assigned p-values are dropped. The reactions are interpreted as a node for the purpose of network creation.
2. Finding a reaction module - After creating an optimal network, a connected reaction module representing significant changes between the cell clusters is identified. The optimal reaction module is found out based on the scoring of nodes and edges as per the assigned p-values. All modules are found using the Heinz solver, keeping the solving time to 4 minutes.

Metabolic genes

The set of metabolic genes used for analysis in Figure 1 was obtained from the KEGG pathway database.

Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) was done at each embryonic age using gene sets of metabolic pathways which were obtained from the KEGG database. The R package fgsea (Sergushichev, 2016) was used to perform this analysis. GSEA was done on pathways from the KEGG database which had more than 5 genes and less than 500 genes and the number of permutations to run was set to 100,000. All the GSEA results can be found in Supplementary Table S2 and S3.

Pathway over-representation analysis

Pathway over-representation analysis was done using the tool Enrichr (Kuleshov et al., 2016). All the genes picked by scMetNet were used to make a heatmap. The average expression of these genes for Neurons and RPs at the three embryonic stages were clustered according to embryonic days. Gene sets corresponding to each cluster were then used to find over-

represented pathways among Neurons and RPs. The heatmap thus obtained is shown in Supplementary Figure S6.

Code Availability

Complete code for the analysis done in this paper can be found at:

https://github.com/ElucidataInc/single_cell_metabolism_scripts

Acknowledgements: We would like to thank Richard Kibbey for critical readings of the manuscript and Gary Bader for many discussions. SG and FDM were supported by funding from the CFREF “Medicine by Design” program. FDM. is a Canada Research Chair and an HHMI Senior International Research Scholar.

Author contributions: SG and AJ conceived the study. SJ and SM analyzed data and implemented the scMetNet pipeline. SJ, SM, SG and FDM cowrote the manuscript.

References:

- Acuna-Hidalgo, R., Schanze, D., Kariminejad, A., Nordgren, A., Kariminejad, M. H., Conner, P., ... Zenker, M. (2014). Neu-laxova syndrome is a heterogeneous metabolic disorder caused by defects in enzymes of the l-serine biosynthesis pathway. *American Journal of Human Genetics*, 95(3), 285–293. <https://doi.org/10.1016/j.ajhg.2014.07.012>
- Afonso, J., Santos, L. L., Longatto-Filho, A., & Baltazar, F. (2020, February 1). Competitive glucose metabolism as a target to boost bladder cancer immunotherapy. *Nature Reviews Urology*. Nature Research. <https://doi.org/10.1038/s41585-019-0263-6>
- Amelio, I., Cutruzzolá, F., Antonov, A., Agostini, M., & Melino, G. (2014). Serine and glycine metabolism in cancer. *Trends in Biochemical Sciences*. Elsevier Ltd. <https://doi.org/10.1016/j.tibs.2014.02.004>
- Butler, A., Hoffman, P., Smibert, P., Papalexi, E., & Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature Biotechnology*, 36(5), 411–420. <https://doi.org/10.1038/nbt.4096>
- Dasgupta, S., Bader, G. D., & Goyal, S. (2018, August 7). Single-Cell RNA Sequencing: A New Window into Cell Scale Dynamics. *Biophysical Journal*. Biophysical Society. <https://doi.org/10.1016/j.bpj.2018.07.003>
- Farrell, J. A., Wang, Y., Riesenfeld, S. J., Shekhar, K., Regev, A., & Schier, A. F. (2018). Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis. *Science*, 360(6392). <https://doi.org/10.1126/science.aar3131>
- Fawal, M.-A., Jungas, T., Kischel, A., Audouard, C., Iacovoni, J. S., & Davy, A. (2018). Cross Talk between One-Carbon Metabolism, Eph Signaling, and Histone Methylation Promotes Neural Stem Cell Differentiation. *Cell Reports*, 23(10), 2864–2873.e7. <https://doi.org/10.1016/J.CELREP.2018.05.005>

- Fujikawa, A., Sugawara, H., Tanaka, T., Matsumoto, M., Kuboyama, K., Suzuki, R., ... Noda, M. (2017). Targeting PTPRZ inhibits stem cell-like properties and tumorigenicity in glioblastoma cells. *Scientific Reports*, 7(1), 5609. <https://doi.org/10.1038/s41598-017-05931-8>
- Hochgerner, H., Zeisel, A., Lönnerberg, P., & Linnarsson, S. (2018). Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing. *Nature Neuroscience*, 21(2), 290–299. <https://doi.org/10.1038/s41593-017-0056-2>
- Hu, C., Fan, L., Cen, P., Chen, E., Jiang, Z., Li, L., ... Li, L. (2016). Energy Metabolism Plays a Critical Role in Stem Cell Maintenance and Differentiation. *International Journal of Molecular Sciences*, 17(2), 253. <https://doi.org/10.3390/ijms17020253>
- Huang, N., & Perl, A. (2018, July 1). Metabolism as a Target for Modulation in Autoimmune Diseases. *Trends in Immunology*. Elsevier Ltd. <https://doi.org/10.1016/j.it.2018.04.006>
- Hwang, I.-Y., Kwak, S., Lee, S., Kim, H., Lee, S. E., Kim, J.-H., ... Youn, H.-D. (2016). Psat1-Dependent Fluctuations in α -Ketoglutarate Affect the Timing of ESC Differentiation. *Cell Metabolism*, 24(3), 494–501. <https://doi.org/10.1016/j.cmet.2016.06.014>
- Intlekofer, A. M., & Finley, L. W. S. (2019, February 1). Metabolic signatures of cancer cells and stem cells. *Nature Metabolism*. Nature Research. <https://doi.org/10.1038/s42255-019-0032-0>
- Jha, A. K., Huang, S. C. C., Sergushichev, A., Lampropoulou, V., Ivanova, Y., Loginicheva, E., ... Artyomov, M. N. (2015). Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity*, 42(3), 419–430. <https://doi.org/10.1016/j.immuni.2015.02.005>
- Kanehisa, M., & Goto, S. (2000). *KEGG: Kyoto Encyclopedia of Genes and Genomes*. *Nucleic Acids Research* (Vol. 28). Retrieved from <http://www.genome.ad.jp/kegg/>
- Karaiskos, N., Wahle, P., Alles, J., Boltengagen, A., Ayoub, S., Kipar, C., ... Zinzen, R. P. (2017). The Drosophila embryo at single-cell transcriptome resolution. *Science*, 358(6360), 194–199. <https://doi.org/10.1126/science.aan3235>
- Kester, L., & van Oudenaarden, A. (2018, August 2). Single-Cell Transcriptomics Meets Lineage Tracing. *Cell Stem Cell*. Cell Press. <https://doi.org/10.1016/j.stem.2018.04.014>
- Kuleshov, M. V, Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., ... Ma'ayan, A. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*, 44. <https://doi.org/10.1093/nar/gkw377>
- Lähnemann, D., Köster, J., Szczurek, E., McCarthy, D. J., Hicks, S. C., Robinson, M. D., ... Schönhuth, A. (2020, February 7). Eleven grand challenges in single-cell data science. *Genome Biology*. BioMed Central Ltd. <https://doi.org/10.1186/s13059-020-1926-6>
- Levitin, H. M., Yuan, J., & Sims, P. A. (2018, April 1). Single-Cell Transcriptomic Analysis of

- Tumor Heterogeneity. *Trends in Cancer*. Cell Press.
<https://doi.org/10.1016/j.trecan.2018.02.003>
- Liu, W., Xu, L., Lamberson, C., Haas, D., Korade, Z., & Porter, N. A. (2014). A highly sensitive method for analysis of 7-dehydrocholesterol for the study of Smith-Lemli-Opitz syndrome. *Journal of Lipid Research*, 55(2), 329–337. <https://doi.org/10.1194/jlr.D043877>
- Luengo, A., Gui, D. Y., & Vander Heiden, M. G. (2017, September 21). Targeting Metabolism for Cancer Therapy. *Cell Chemical Biology*. Elsevier Ltd.
<https://doi.org/10.1016/j.chembiol.2017.08.028>
- Madhavan, L., Ourednik, V., & Ourednik, J. (2006). Increased “Vigilance” of Antioxidant Mechanisms in Neural Stem Cells Potentiates Their Capability to Resist Oxidative Stress. *Stem Cells*, 24(9), 2110–2119. <https://doi.org/10.1634/stemcells.2006-0018>
- Martin, E., & Sung, M.-H. (2018). Challenges of Decoding Transcription Factor Dynamics in Terms of Gene Regulation. *Cells*, 7(9), 132. <https://doi.org/10.3390/cells7090132>
- Reid, M. A., Allen, A. E., Liu, S., Liberti, M. V., Liu, P., Liu, X., ... Locasale, J. W. (2018). Serine synthesis through PHGDH coordinates nucleotide levels by maintaining central carbon metabolism. *Nature Communications*, 9(1). <https://doi.org/10.1038/s41467-018-07868-6>
- Robin, N. C., Agoston, Z., Biechele, T. L., James, R. G., Berndt, J. D., & Moon, R. T. (2014). Simvastatin Promotes Adult Hippocampal Neurogenesis by Enhancing Wnt/ β -Catenin Signaling. *Stem Cell Reports*, 2(1), 9–17. <https://doi.org/10.1016/j.stemcr.2013.11.002>
- Saito, K., Dubreuil, V., Arai, Y., Wilsch-Bräuninger, M., Schwudke, D., Saher, G., ... Huttner, W. B. (2009). Ablation of cholesterol biosynthesis in neural stem cells increases their VEGF expression and angiogenesis but causes neuron apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*, 106(20), 8350–8355.
<https://doi.org/10.1073/pnas.0903541106>
- Savaskan, N. E., Borchert, A., Bräuer, A. U., & Kuhn, H. (2007). Role for glutathione peroxidase-4 in brain development and neuronal apoptosis: Specific induction of enzyme expression in reactive astrocytes following brain injury. *Free Radical Biology and Medicine*, 43(2), 191–201. <https://doi.org/10.1016/j.freeradbiomed.2007.03.033>
- Schell, J. C., Wisidagama, D. R., Bensard, C., Zhao, H., Wei, P., Tanner, J., ... Rutter, J. (2017). Control of intestinal stem cell function and proliferation by mitochondrial pyruvate metabolism. *Nature Cell Biology*, 19(9), 1027–1036. <https://doi.org/10.1038/ncb3593>
- Sergushichev, A. A. (2016). An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. *BioRxiv*, 060012. <https://doi.org/10.1101/060012>
- Sergushichev, A. A., Loboda, A. A., Jha, A. K., Vincent, E. E., Driggers, E. M., Jones, R. G., ... Artyomov, M. N. (2016). GAM: a web-service for integrated transcriptional and metabolic network analysis. *Nucleic Acids Research*, 44(W1), W194–W200.
<https://doi.org/10.1093/nar/gkw266>

- Sharma, S., & Prasad, A. (2017). Inborn Errors of Metabolism and Epilepsy: Current Understanding, Diagnosis, and Treatment Approaches. *International Journal of Molecular Sciences*, 18(7), 1384. <https://doi.org/10.3390/ijms18071384>
- Shiraki, N., Shiraki, Y., Tsuyama, T., Obata, F., Miura, M., Nagae, G., ... Kume, S. (2014). Methionine metabolism regulates maintenance and differentiation of human pluripotent stem cells. *Cell Metabolism*, 19(5), 780–794. <https://doi.org/10.1016/j.cmet.2014.03.017>
- Shyh-Chang, N., Daley, G. Q., & Cantley, L. C. (2013, June 15). Stem cell metabolism in tissue development and aging. *Development (Cambridge)*. <https://doi.org/10.1242/dev.091777>
- Shyh-Chang, N., & Ng, H.-H. (2017). The metabolic programming of stem cells. *Genes & Development*, 31(4), 336–346. <https://doi.org/10.1101/gad.293167.116>
- Stephens, F. B., Constantin-teodosiu, D., & Greenhaff, P. L. (2007, June 1). New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *Journal of Physiology*. Wiley-Blackwell. <https://doi.org/10.1113/jphysiol.2006.125799>
- Stincone, A., Prigione, A., Cramer, T., Wamelink, M. M. C., Campbell, K., Cheung, E., ... Ralser, M. (2015). The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biological Reviews*, 90(3), 927–963. <https://doi.org/10.1111/brv.12140>
- Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W. M., ... Satija, R. (2019). Comprehensive Integration of Single-Cell Data. *Cell*, 177(7), 1888-1902.e21. <https://doi.org/10.1016/j.cell.2019.05.031>
- Xiao, Z., Dai, Z., & Locasale, J. W. (2019). Metabolic landscape of the tumor microenvironment at single cell resolution. *Nature Communications*, 10(1), 1–12. <https://doi.org/10.1038/s41467-019-11738-0>
- Yoshida, K., Furuya, S., Osuka, S., Mitoma, J., Shinoda, Y., Watanabe, M., ... Hirabayashi, Y. (2003). Targeted Disruption of the Mouse 3-Phosphoglycerate Dehydrogenase Gene Causes Severe Neurodevelopmental Defects and Results in Embryonic Lethality*. <https://doi.org/10.1074/jbc.C300507200>
- Yuzwa, S. A., Borrett, M. J., Innes, B. T., Voronova, A., Ketela, T., Kaplan, D. R., ... Miller, F. D. (2017). Developmental Emergence of Adult Neural Stem Cells as Revealed by Single-Cell Transcriptional Profiling. *Cell Reports*, 21(13), 3970–3986. <https://doi.org/10.1016/J.CELREP.2017.12.017>

Figures

Figure 1: Cell types align with metabolic states. (A) t-distributed stochastic neighbour embedding (t-SNE) visualizations of cell types at E15.5. The clustering was done using highly variable genes, cell type-specific markers for Neurons, IPs and RPs were used to annotate cells. (B) t-SNE visualizations of metabolic states at E15.5. The clustering was done using only the metabolic genes. (C) The overlap of cell types and metabolic states at E15.5; here RPs are predominantly in metabolic state S₀ and all IPs are present in metabolic state S₁. (D) Gene Set Enrichment Analysis (GSEA) between RPs and Neurons shows the enriched pathways in the two cell types.

Figure 2: scMetNet network between RPs vs Neurons at E15.5. The circular nodes represent metabolites that connect the metabolic enzymes represented by square nodes; the size of square nodes around the enzymes denotes significance. Significant metabolic modules (in dashed boxes), and important metabolites (in yellow) are highlighted.

Figure 3: Metabolic ageing in radial precursors (RPs). (A) Global pathway changes between RPs and neurons using Gene Expression Enrichment Analysis (GSEA) at E13.5, E15.5 and E17.5 stages of embryonic development. (B) The core metabolic genes across E13.5, E15.5 and E17.5 and their associated pathways are identified.

Figure 4: scMetNet network between E17.5 and E13.5 RPs show how metabolism gets re-wired within RPs along developmental time; the circular nodes represent metabolites that connect the metabolic enzymes represented by square nodes; the size of square nodes around the enzymes denotes significance. The mevalonate biosynthesis, fatty acid and glutathione pathways are upregulated while the methionine metabolism is downregulated at E17.5.

Supplementary figures

Figure S1: (A) t-SNE visualization of cell clusters at E13.5, E15.5 and E17.5 using all the variable genes. (B) The overlap of cell types and metabolic states at E13.5, E15.5 and E17.5. (C) Cells were clustered using random sets of genes, the proportion of RPs in metabolic state S_0 (green), IPs in metabolic state S_1 (blue) and Neurons in metabolic state S_2 (red) were calculated. The p-values for the cell identities assigned using metabolic genes clustering calculated using the permutation test are $7.77e-4$, $7.44e-4$ and $7.33e-4$ for E13.5, E15.5 and E17.5 stages, respectively.

Figure S2: The proportion of cells from the clustering obtained using metabolic genes, present in the three cell type clusters with an increasing number of clusters at (A) E13.5 (B) E15.5 and (C) E17.5.

Figure S3: Gene Set Enrichment Analysis (GSEA) between S_0 and S_2

Figure S4: scMetNet network between RPs vs Neurons at E13.5; the circular nodes represent metabolites that connect the metabolic enzymes represented by square nodes; the size of square nodes around the enzymes denotes significance.

Figure S5: scMetNet network between RPs vs Neurons at E17.5; the circular nodes represent metabolites that connect the metabolic enzymes represented by square nodes; the size of square nodes around the enzymes denotes significance.

Figure S6: Heatmap of genes picked up by scMetNet for E13.5, E15.5, and E17.5 stages; same genes are shown in the Venn Diagram in main text Figure 3B. Enriched pathways are shown for each stage individually, pair of stages, and all the stages together. The number of genes considered are mentioned in parenthesis. Over-represented pathways for each day clusters along with p values are mentioned (see Methods for details).

Figure S7: Different modules/metabolites defining the epigenetic and bioenergetic landscape of RPs and their relationship to past metabolic studies, and new potential genetic targets to alter early brain development (also see Supplementary Table S4 for a comprehensive list).

Table S1: Marker Genes for the three time points

E13.5			E15.5			E17.5		
RP	IP	Neurons	RP	IP	Neurons	RP	IP	Neurons
Sox2	Eomes	Tbr1	Sox2	Eomes	Tbr1	Sox2	Eomes	Tbr1
Pax6	Gadd45g	Tubb3	Pax6	Gadd45g	Tubb3	Pax6	Sstr2	Tubb3
Hes1	Neurod1	Satb2	Hes1	Neurod1	Satb2	Hes1		Satb2
Hes5	Ngn1	Bhlhe22	Hes5	Ngn1	Bhlhe22	Hes5		Bhlhe22
Slc1a3	Slc17a6			Slc17a6	Unc5d	Slc1a3		
	Ngn2			Ngn2	Sema6d			
	Btg2			Btg2				
	Sstr2			Sstr2				
	Mfap4			Mfap4				

Table S2: RPs vs Neurons fgsea all days

Pathway	pval	padj	ES	NES	nMore Extm	size	Stage
KEGG_GLYCOLYSIS_GLUONEOGENESIS	0.00	0.001	0.707	2.169	1	25	E13.5
KEGG_CITRATE_CYCLE_TCA_CYCLE	0.50	0.696	0.325	0.968	24591	22	E13.5
KEGG_PENTOSE_PHOSPHATE_PATHWAY	0.00	0.014	0.71	1.915	53	15	E13.5
KEGG_FRUCTOSE_AND_MANNOSSE_METABOLISM	0.02	0.091	0.591	1.621	1156	16	E13.5
KEGG_GALACTOSE_METABOLISM	0.36	0.578	0.441	1.094	17713	11	E13.5
KEGG_FATTY_ACID_METABOLISM	0.03	0.103	0.526	1.566	1511	22	E13.5
KEGG_STEROID_BIOSYNTHESIS	0.96	0.983	0.231	0.537	48000	9	E13.5
KEGG_OXIDATIVE_PHOSPHORYLATION	0.93	0.976	0.187	0.752	43241	89	E13.5
KEGG_PURINE_METABOLISM	0.00	0.028	0.423	1.639	207	73	E13.5
KEGG_PYRIMIDINE_METABOLISM	0.00	0.002	0.527	1.97	3	60	E13.5
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	0.25	0.451	0.461	1.196	12327	13	E13.5
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	0.01	0.039	0.749	1.755	341	9	E13.5
KEGG_CYSTEINE_AND_METHIONINE_METABOLISM	0.03	0.103	0.563	1.592	1376	18	E13.5
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	0.00	0.021	0.564	1.812	143	30	E13.5
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_BIOSYNTHESIS	0.42	0.623	0.506	1.053	21231	6	E13.5
KEGG_LYSINE_DEGRADATION	0.10	0.261	0.427	1.348	5059	28	E13.5
KEGG_ARGININE_AND_PROLINE_METABOLISM	0.04	0.13	0.517	1.522	2061	21	E13.5
KEGG_HISTIDINE_METABOLISM	0.58	0.74	0.416	0.907	29216	7	E13.5
KEGG_TYROSINE_METABOLISM	0.17	0.354	0.524	1.299	8513	11	E13.5
KEGG_TRYPTOPHAN_METABOLISM	0.10	0.259	0.554	1.405	5036	12	E13.5
KEGG_BETA_ALANINE_METABOLISM	0.09	0.234	0.616	1.443	4358	9	E13.5
KEGG_SELENOAMINO_ACID_METABOLISM	0.98	0.994	0.201	0.511	48285	12	E13.5
KEGG_GLUTATHIONE_METABOLISM	0.05	0.136	0.5	1.504	2187	23	E13.5
KEGG_STARCH_AND_SUCROSE_METABOLISM	0.83	0.922	0.3	0.702	41229	9	E13.5
KEGG_N_GLYCAN_BIOSYNTHESIS	0.93	0.976	0.213	0.647	47640	26	E13.5
KEGG_OTHER_GLYCAN_DEGRADATION	1.00	0.998	0.204	0.401	49258	5	E13.5
KEGG_O_GLYCAN_BIOSYNTHESIS	0.76	0.867	0.385	0.76	38237	5	E13.5
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	0.84	0.922	0.246	0.733	40840	22	E13.5
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	0.90	0.958	0.312	0.616	45557	5	E13.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE	0.44	0.626	0.475	1.029	21784	7	E13.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_KERATAN_SULFATE	0.74	0.86	0.387	0.761	36697	5	E13.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_SULFATE	0.19	0.374	0.506	1.271	9662	12	E13.5

KEGG_GLYCEROLIPID_METABOLISM	0.40	0.622	0.37	1.048	19483	18	E13.5
KEGG_INOSITOL_PHOSPHATE_METABOLISM	0.85	0.93	0.244	0.715	43656	22	E13.5
KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_G PI_ANCHOR_BIOSYNTHESIS	0.99	0.998	0.178	0.441	49320	11	E13.5
KEGG_GLYCEROPHOSPHOLIPID_METABOLISM	0.86	0.93	0.229	0.734	41419	30	E13.5
KEGG_ETHER_LIPID_METABOLISM	0.31	0.527	0.508	1.15	15522	8	E13.5
KEGG_ARACHIDONIC_ACID_METABOLISM	0.88	0.951	0.306	0.634	43747	6	E13.5
KEGG_SPHINGOLIPID_METABOLISM	0.35	0.578	0.422	1.095	17242	13	E13.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_L ACTO_AND_NEOLACTO_SERIES	0.53	0.704	0.437	0.946	26406	7	E13.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_ GLOBO_SERIES	0.95	0.983	0.279	0.549	46630	5	E13.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_ GANGLIO_SERIES	0.56	0.736	0.468	0.921	27836	5	E13.5
KEGG_PYRUVATE_METABOLISM	0.00	0.02	0.656	1.856	127	18	E13.5
KEGG_GLYOXYLATE_AND_DICARBOXYLATE_M ETABOLISM	0.56	0.736	0.407	0.921	28211	8	E13.5
KEGG_PROPANOATE_METABOLISM	0.00	0.017	0.665	1.882	94	18	E13.5
KEGG_BUTANOATE_METABOLISM	0.09	0.242	0.534	1.415	4602	14	E13.5
KEGG_ONE_CARBON_POOL_BY_FOLATE	0.02	0.069	0.714	1.673	757	9	E13.5
KEGG_RIBOFLAVIN_METABOLISM	0.90	0.958	0.297	0.618	45290	6	E13.5
KEGG_NICOTINATE_AND_NICOTINAMIDE_MET ABOLISM	0.96	0.983	0.225	0.538	48199	10	E13.5
KEGG_PANTOTHENATE_AND_COA_BIOSYNTH ESIS	0.58	0.74	0.437	0.907	28595	6	E13.5
KEGG_FOLATE_BIOSYNTHESIS	0.08	0.226	0.694	1.445	4112	6	E13.5
KEGG_PORPHYRIN_AND_CHLOROPHYLL_MET ABOLISM	0.79	0.891	0.294	0.746	39003	12	E13.5
KEGG_TERPENOID_BACKBONE_BIOSYNTHESI S	0.23	0.421	0.512	1.234	11163	10	E13.5
KEGG_LIMONENE_AND_PINENE_DEGRADATIO N	0.11	0.261	0.706	1.393	5359	5	E13.5
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	0.21	0.396	0.375	1.204	10098	30	E13.5
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CY TOCHROME_P450	0.17	0.349	0.581	1.316	8239	8	E13.5
KEGG_DRUG_METABOLISM_CYTOCHROME_P4 50	0.17	0.349	0.581	1.316	8239	8	E13.5
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	0.60	0.751	0.358	0.888	29984	11	E13.5
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FA TTY_ACIDS	0.61	0.751	0.35	0.888	30099	12	E13.5
KEGG_ABC_TRANSPORTERS	0.99	0.998	0.222	-0.46	48978	6	E13.5
KEGG_RIBOSOME	0.00	0.001	0.669	2.579	0	71	E13.5
KEGG_RNA_DEGRADATION	0.00	0.015	0.5	1.8	70	50	E13.5
KEGG_RNA_POLYMERASE	0.09	0.234	0.483	1.404	4274	20	E13.5
KEGG_BASAL_TRANSCRIPTION_FACTORS	0.78	0.884	0.254	0.789	37734	26	E13.5
KEGG_DNA_REPLICATION	0.00	0.001	0.821	2.593	0	28	E13.5
KEGG_SPLICEOSOME	0.00	0.001	0.53	2.22	0	115	E13.5
KEGG_PROTEASOME	0.14	0.326	0.371	1.267	6729	39	E13.5
KEGG_PROTEIN_EXPORT	0.41	0.622	0.357	1.036	20087	20	E13.5
KEGG_PPAR_SIGNALING_PATHWAY	0.04	0.119	0.558	1.557	1868	17	E13.5
KEGG_BASE_EXCISION_REPAIR	0.00	0.021	0.655	1.853	135	18	E13.5

KEGG_NUCLEOTIDE_EXCISION_REPAIR	0.00	0.001	0.655	2.184	0	35	E13.5
KEGG_MISMATCH_REPAIR	0.00	0.001	0.764	2.188	0	19	E13.5
KEGG_HOMOLOGOUS_RECOMBINATION	0.00	0.015	0.677	1.917	62	18	E13.5
KEGG_NON_HOMOLOGOUS_END_JOINING	0.25	0.451	0.586	1.221	12502	6	E13.5
KEGG_MAPK_SIGNALING_PATHWAY	0.00	0.015	0.411	1.665	74	110	E13.5
KEGG_ERBB_SIGNALING_PATHWAY	0.02	0.08	0.449	-1.56	996	47	E13.5
KEGG_CALCIUM_SIGNALING_PATHWAY	0.04	0.112	0.443	1.494	1818	41	E13.5
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	0.84	0.922	0.259	0.722	42584	18	E13.5
KEGG_CHEMOKINE_SIGNALING_PATHWAY	0.03	0.103	0.399	1.473	1532	64	E13.5
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	0.04	0.112	0.495	1.534	1803	28	E13.5
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	0.00	0.026	0.614	1.818	192	23	E13.5
KEGG_CELL_CYCLE	0.00	0.001	0.537	2.139	0	85	E13.5
KEGG_OOCYTE_MEIOSIS	0.48	0.673	0.269	0.987	25032	62	E13.5
KEGG_P53_SIGNALING_PATHWAY	0.01	0.054	0.507	1.666	524	33	E13.5
KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	0.96	0.983	0.177	0.721	44449	96	E13.5
KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	0.03	0.103	-0.54	1.581	1474	22	E13.5
KEGG_REGULATION_OF_AUTOPHAGY	0.15	0.344	0.514	1.319	7779	13	E13.5
KEGG_LYSOSOME	0.53	0.704	0.269	0.956	27782	53	E13.5
KEGG_ENDOCYTOSIS	0.01	0.046	0.388	1.543	466	98	E13.5
KEGG_PEROXISOME	0.68	0.821	0.252	0.869	32594	41	E13.5
KEGG_MTOR_SIGNALING_PATHWAY	0.49	0.681	0.309	-0.98	25053	31	E13.5
KEGG_APOPTOSIS	0.02	0.08	0.493	1.598	967	34	E13.5
KEGG_CARDIAC_MUSCLE_CONTRACTION	0.72	0.852	0.255	0.835	37400	36	E13.5
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	0.02	0.086	0.475	1.568	1125	37	E13.5
KEGG_WNT_SIGNALING_PATHWAY	0.11	0.272	0.316	1.25	5347	81	E13.5
KEGG_DORSO_VENTRAL_AXIS_FORMATION	0.71	0.846	0.344	0.806	35249	9	E13.5
KEGG_NOTCH_SIGNALING_PATHWAY	0.02	0.081	0.504	1.605	957	29	E13.5
KEGG_HEDGEHOG_SIGNALING_PATHWAY	0.53	0.704	0.336	0.952	25702	18	E13.5
KEGG_TGF_BETA_SIGNALING_PATHWAY	0.28	0.496	0.336	1.125	13610	36	E13.5
KEGG_AXON_GUIDANCE	0.00	0.001	0.538	2.038	0	74	E13.5
KEGG_VEGF_SIGNALING_PATHWAY	0.03	0.103	0.486	1.542	1591	31	E13.5
KEGG_FOCAL_ADHESION	0.41	0.622	0.264	1.025	19010	74	E13.5
KEGG_ECM_RECEPTOR_INTERACTION	0.74	0.86	0.315	0.781	36902	11	E13.5
KEGG_CELL_ADHESION_MOLECULES_CAMS	0.01	0.038	0.547	1.723	339	30	E13.5
KEGG_ADHERENS_JUNCTION	0.72	0.852	0.245	0.844	37608	45	E13.5

KEGG_TIGHT_JUNCTION	0.41	0.622	-	-	21633	63	E13.5
KEGG_GAP_JUNCTION	0.01	0.038	-	-	330	38	E13.5
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0.36	0.578	0.377	1.079	17510	19	E13.5
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.18	0.367	-	-	9452	33	E13.5
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.68	0.821	0.292	0.847	34703	21	E13.5
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.60	0.751	-	-0.9	30882	24	E13.5
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	0.73	0.857	0.288	0.803	35882	17	E13.5
KEGG_JAK_STAT_SIGNALING_PATHWAY	0.43	0.626	0.306	1.014	20901	34	E13.5
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.00	0.014	-	-	52	33	E13.5
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.14	0.327	-0.36	1.257	7459	48	E13.5
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.19	0.374	-0.38	1.222	9987	33	E13.5
KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY	0.00	0.003	0.602	1.953	9	34	E13.5
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	0.06	0.171	-	-	3075	50	E13.5
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	0.18	0.363	0.353	1.219	8547	41	E13.5
KEGG_LONG_TERM_POTENTIATION	0.00	0.018	-	-1.8	112	38	E13.5
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	0.01	0.04	0.427	1.611	385	72	E13.5
KEGG_LONG_TERM_DEPRESSION	0.01	0.055	-	-	597	34	E13.5
KEGG_OLFACTORY_TRANSDUCTION	0.00	0.014	-	-1.91	47	11	E13.5
KEGG_TASTE_TRANSDUCTION	0.07	0.189	0.667	1.499	3310	8	E13.5
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	0.00	0.017	-	-	102	91	E13.5
KEGG_INSULIN_SIGNALING_PATHWAY	0.00	0.028	0.435	1.654	227	76	E13.5
KEGG_GNRH_SIGNALING_PATHWAY	0.02	0.092	-	-	1270	42	E13.5
KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	0.58	0.74	0.262	0.927	30339	52	E13.5
KEGG_MELANOGENESIS	0.17	0.354	-	-	8874	43	E13.5
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	0.21	0.396	0.408	-1.22	10599	24	E13.5
KEGG_TYPE_II_DIABETES_MELLITUS	0.00	0.017	-	-	89	21	E13.5
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	0.03	0.103	0.613	1.603	1548	14	E13.5
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	0.08	0.22	-0.45	1.405	4035	29	E13.5
KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION	0.79	0.891	-	-	39429	7	E13.5
KEGG_ALZHEIMERS_DISEASE	0.28	0.496	-0.27	1.092	15197	108	E13.5
KEGG_PARKINSONS_DISEASE	0.31	0.527	0.268	1.077	14401	89	E13.5

KEGG_AMYTROPHIC_LATERAL_SCLEROSIS_ALS	0.01	0.058	0.543	1.668	642	27	E13.5
KEGG_HUNTINGTONS_DISEASE	0.43	0.623	0.237	1.009	19482	127	E13.5
KEGG_PRION_DISEASES	0.12	0.272	0.498	1.365	5857	17	E13.5
KEGG_VIBRIO_CHOLERAЕ_INFECTION	0.02	0.08	0.523	1.622	930	28	E13.5
KEGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTION	0.09	0.242	0.413	1.355	4852	36	E13.5
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	0.01	0.046	0.523	1.684	469	33	E13.5
KEGG_LEISHMANIA_INFECTION	0.36	0.578	0.389	1.084	17507	17	E13.5
KEGG_PATHWAYS_IN_CANCER	0.38	0.602	0.239	1.033	17073	139	E13.5
KEGG_COLORECTAL_CANCER	0.30	0.513	0.319	1.109	15437	47	E13.5
KEGG_RENAL_CELL_CARCINOMA	0.34	0.573	0.315	1.077	17805	44	E13.5
KEGG_PANCREATIC_CANCER	0.42	0.623	0.301	1.021	21952	42	E13.5
KEGG_ENDOMETRIAL_CANCER	0.19	0.374	0.368	1.215	9998	37	E13.5
KEGG_GLIOMA	0.11	0.272	0.407	1.327	5798	35	E13.5
KEGG_PROSTATE_CANCER	0.52	0.699	0.266	0.964	24584	51	E13.5
KEGG_THYROID_CANCER	0.16	0.345	0.457	1.293	7732	18	E13.5
KEGG_BASAL_CELL_CARCINOMA	0.65	0.8	0.313	0.859	33213	17	E13.5
KEGG_MELANOMA	0.43	0.623	0.323	1.018	22094	30	E13.5
KEGG_BLADDER_CANCER	0.25	0.451	0.391	1.176	12072	23	E13.5
KEGG_CHRONIC_MYELOID_LEUKEMIA	0.51	0.696	-0.28	0.966	26517	46	E13.5
KEGG_ACUTE_MYELOID_LEUKEMIA	0.15	0.344	0.394	1.268	7970	33	E13.5
KEGG_SMALL_CELL_LUNG_CANCER	0.60	0.751	0.272	0.911	28809	36	E13.5
KEGG_NON_SMALL_CELL_LUNG_CANCER	0.39	0.611	0.331	1.049	19960	31	E13.5
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	0.05	0.158	0.513	1.489	2591	20	E13.5
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	0.25	0.451	0.397	1.176	12928	23	E13.5
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	0.50	0.696	0.319	-0.97	25804	26	E13.5
KEGG_DILATED_CARDIOMYOPATHY	0.16	0.345	0.433	1.281	8148	23	E13.5
KEGG_VIRAL_MYOCARDITIS	0.41	0.622	-0.38	1.042	20687	17	E13.5
KEGG_GLYCOLYSIS_GLUONEOGENESIS	0.00	0.033	0.597	1.808	173	28	E15.5
KEGG_CITRATE_CYCLE_TCA_CYCLE	0.80	0.93	0.263	0.766	41579	24	E15.5
KEGG_PENTOSE_PHOSPHATE_PATHWAY	0.01	0.07	0.647	1.676	730	15	E15.5
KEGG_FRUCTOSE_AND_MANNANOSE_METABOLISM	0.02	0.077	0.609	1.655	826	18	E15.5
KEGG_GALACTOSE_METABOLISM	0.86	0.94	0.286	0.684	44771	11	E15.5
KEGG_FATTY_ACID_METABOLISM	0.00	0.033	0.61	1.799	189	25	E15.5
KEGG_STEROID_BIOSYNTHESIS	0.86	0.94	0.291	0.678	44920	10	E15.5
KEGG_OXIDATIVE_PHOSPHORYLATION	0.88	0.951	0.205	0.786	42566	89	E15.5

KEGG_PURINE_METABOLISM	0.03	0.113	0.374	1.418	1587	86	E15.5
KEGG_PYRIMIDINE_METABOLISM	0.00	0.033	0.466	1.689	161	66	E15.5
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	0.88	0.951	0.269	0.659	46003	12	E15.5
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	0.00	0.035	0.716	1.79	215	13	E15.5
KEGG_CYSTEINE_AND_METHIONINE_METABOLISM	0.09	0.234	0.511	1.408	4564	19	E15.5
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	0.00	0.035	0.562	1.756	232	32	E15.5
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_BIOSYNTHESIS	0.44	0.661	0.514	1.038	23157	6	E15.5
KEGG_LYSINE_DEGRADATION	0.19	0.403	0.397	1.231	9628	31	E15.5
KEGG_ARGININE_AND_PROLINE_METABOLISM	0.20	0.424	0.42	1.224	10396	24	E15.5
KEGG_HISTIDINE_METABOLISM	0.42	0.66	0.46	1.04	22168	9	E15.5
KEGG_TYROSINE_METABOLISM	0.30	0.543	0.472	1.154	15437	12	E15.5
KEGG_PHENYLALANINE_METABOLISM	0.05	0.158	0.777	1.49	2455	5	E15.5
KEGG_TRYPTOPHAN_METABOLISM	0.18	0.403	0.5	1.273	9559	14	E15.5
KEGG_BETA_ALANINE_METABOLISM	0.21	0.433	0.537	1.251	11113	10	E15.5
KEGG_SELENOAMINO_ACID_METABOLISM	0.50	0.674	0.389	0.974	26050	13	E15.5
KEGG_GLUTATHIONE_METABOLISM	0.01	0.053	0.573	1.705	453	26	E15.5
KEGG_STARCH_AND_SUCROSE_METABOLISM	0.77	0.919	0.335	0.758	40185	9	E15.5
KEGG_N_GLYCAN_BIOSYNTHESIS	0.98	0.992	0.185	0.551	51004	26	E15.5
KEGG_OTHER_GLYCAN_DEGRADATION	0.52	0.689	0.457	0.965	27247	7	E15.5
KEGG_O_GLYCAN_BIOSYNTHESIS	0.86	0.94	0.331	0.644	41043	5	E15.5
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	0.99	0.992	0.17	0.496	51372	24	E15.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE	0.50	0.674	0.457	0.975	23519	7	E15.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_KERATAN_SULFATE	0.37	0.598	0.515	1.097	17359	7	E15.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_SULFATE	0.30	0.543	0.455	1.145	14315	13	E15.5
KEGG_GLYCEROLIPID_METABOLISM	0.17	0.383	0.463	1.275	8914	19	E15.5
KEGG_INOSITOL_PHOSPHATE_METABOLISM	0.84	0.94	0.245	-0.75	40178	29	E15.5
KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_BIOSYNTHESIS	0.99	0.992	0.188	0.452	47192	11	E15.5
KEGG_GLYCEROPHOSPHOLIPID_METABOLISM	0.83	0.94	0.243	0.758	43162	32	E15.5
KEGG_ETHER_LIPID_METABOLISM	0.49	0.674	0.435	0.984	25507	9	E15.5
KEGG_ARACHIDONIC_ACID_METABOLISM	0.97	0.992	0.234	0.498	46100	7	E15.5
KEGG_SPHINGOLIPID_METABOLISM	0.20	0.427	0.462	1.237	10636	17	E15.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	0.61	0.784	0.384	0.877	29180	9	E15.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GLOBO_SERIES	0.81	0.93	0.357	0.695	38549	5	E15.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GANGLIO_SERIES	0.38	0.619	0.563	1.097	18241	5	E15.5
KEGG_PYRUVATE_METABOLISM	0.02	0.092	0.558	1.594	1216	22	E15.5
KEGG_GLYOXYLATE_AND_DICARBOXYLATE_METABOLISM	0.32	0.555	0.486	1.132	16887	10	E15.5
KEGG_PROANOATE_METABOLISM	0.01	0.07	0.59	1.666	704	21	E15.5
KEGG_BUTANOATE_METABOLISM	0.12	0.295	0.5	1.358	6145	18	E15.5

KEGG_ONE_CARBON_POOL_BY_FOLATE	0.00	0.032	0.769	1.84	100	11	E15.5
KEGG_RIBOFLAVIN_METABOLISM	0.73	0.885	0.374	0.789	38311	7	E15.5
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	0.91	0.974	0.265	0.606	43369	9	E15.5
KEGG_PANTOTHENATE_AND_COA_BIOSYNTHESIS	0.54	0.715	0.5	0.959	28338	5	E15.5
KEGG_FOLATE_BIOSYNTHESIS	0.05	0.164	0.711	1.503	2828	7	E15.5
KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	0.96	0.992	0.221	0.563	49812	14	E15.5
KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS	0.44	0.661	0.428	1.024	22979	11	E15.5
KEGG_LIMONENE_AND_PINENE_DEGRADATION	0.14	0.317	0.667	1.347	7155	6	E15.5
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	0.65	0.817	0.281	0.877	33708	32	E15.5
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	0.08	0.215	0.644	1.456	4024	9	E15.5
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	0.07	0.204	0.63	1.469	3699	10	E15.5
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	0.30	0.543	0.481	1.151	15811	11	E15.5
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	0.33	0.569	0.445	1.113	17469	13	E15.5
KEGG_ABC_TRANSPORTERS	0.75	0.908	0.403	0.773	39438	5	E15.5
KEGG_RIBOSOME	0.00	0.001	0.589	2.189	0	76	E15.5
KEGG_RNA_DEGRADATION	0.01	0.038	0.493	1.692	262	50	E15.5
KEGG_RNA_POLYMERASE	0.57	0.746	0.318	0.919	29806	23	E15.5
KEGG_BASAL_TRANSCRIPTION_FACTORS	0.98	0.992	0.184	0.548	51045	26	E15.5
KEGG_DNA_REPLICATION	0.00	0.001	0.792	2.472	0	32	E15.5
KEGG_SPLICEOSOME	0.00	0.001	0.515	2.047	0	115	E15.5
KEGG_PROTEASOME	0.13	0.315	0.393	1.279	6920	39	E15.5
KEGG_PROTEIN_EXPORT	0.24	0.465	0.427	1.192	12433	20	E15.5
KEGG_PPAR_SIGNALING_PATHWAY	0.01	0.046	0.623	1.74	373	20	E15.5
KEGG_BASE_EXCISION_REPAIR	0.00	0.033	0.642	1.832	132	22	E15.5
KEGG_NUCLEOTIDE_EXCISION_REPAIR	0.00	0.005	0.633	2.038	8	37	E15.5
KEGG_MISMATCH_REPAIR	0.00	0.002	0.763	2.129	2	20	E15.5
KEGG_HOMOLOGOUS_RECOMBINATION	0.00	0.016	0.7	1.927	34	19	E15.5
KEGG_NON_HOMOLOGOUS_END_JOINING	0.34	0.57	0.558	1.128	17837	6	E15.5
KEGG_MAPK_SIGNALING_PATHWAY	0.00	0.033	0.393	1.577	158	119	E15.5
KEGG_ERBB_SIGNALING_PATHWAY	0.05	0.155	0.409	1.424	2167	53	E15.5
KEGG_CALCIIUM_SIGNALING_PATHWAY	0.11	0.274	0.388	1.313	5039	46	E15.5
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	0.78	0.928	0.275	0.776	40571	21	E15.5
KEGG_CHEMOKINE_SIGNALING_PATHWAY	0.04	0.151	0.387	1.407	2075	67	E15.5
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	0.03	0.103	0.481	1.537	1325	35	E15.5
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	0.01	0.055	0.557	1.693	467	28	E15.5
KEGG_CELL_CYCLE	0.00	0.001	0.552	2.124	0	93	E15.5
KEGG_OOCYTE_MEIOSIS	0.30	0.543	0.298	1.089	14625	69	E15.5
KEGG_P53_SIGNALING_PATHWAY	0.05	0.164	0.444	1.436	2717	38	E15.5

KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	0.99	0.992	0.172	0.672	51015	101	E15.5
KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	0.03	0.096	-	-	1204	27	E15.5
KEGG_REGULATION_OF_AUTOPHAGY	0.12	0.295	-0.56	1.381	5646	12	E15.5
KEGG_LYSOSOME	0.96	0.992	0.189	0.682	46136	64	E15.5
KEGG_ENDOCYTOSIS	0.00	0.033	0.406	1.597	160	105	E15.5
KEGG_PEROXISOME	0.01	0.058	0.48	1.619	556	46	E15.5
KEGG_MTOR_SIGNALING_PATHWAY	0.49	0.674	0.309	0.974	23638	33	E15.5
KEGG_APOPTOSIS	0.02	0.08	0.487	1.577	930	37	E15.5
KEGG_CARDIAC_MUSCLE_CONTRACTION	0.49	0.674	0.301	0.975	23761	37	E15.5
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	0.05	0.163	0.433	1.438	2405	42	E15.5
KEGG_WNT_SIGNALING_PATHWAY	0.25	0.465	0.295	1.122	12683	88	E15.5
KEGG_DORSO_VENTRAL_AXIS_FORMATION	0.48	0.674	0.437	0.989	25239	9	E15.5
KEGG_NOTCH_SIGNALING_PATHWAY	0.01	0.058	0.563	1.677	570	26	E15.5
KEGG_HEDGEHOG_SIGNALING_PATHWAY	0.49	0.674	0.366	0.979	25456	17	E15.5
KEGG_TGF_BETA_SIGNALING_PATHWAY	0.06	0.174	0.427	1.414	3013	42	E15.5
KEGG_AXON_GUIDANCE	0.00	0.026	0.463	1.727	66	77	E15.5
KEGG_VEGF_SIGNALING_PATHWAY	0.05	0.163	-0.46	1.452	2474	33	E15.5
KEGG_FOCAL_ADHESION	0.13	0.31	0.326	1.231	6670	84	E15.5
KEGG_ECM_RECEPTOR_INTERACTION	0.34	0.57	0.425	1.102	17790	15	E15.5
KEGG_CELL_ADHESION_MOLECULES_CAMS	0.05	0.163	0.454	-1.45	2432	35	E15.5
KEGG_ADHERENS_JUNCTION	0.45	0.672	0.292	1	23444	49	E15.5
KEGG_TIGHT_JUNCTION	0.68	0.846	0.243	0.886	35123	69	E15.5
KEGG_GAP_JUNCTION	0.01	0.055	0.491	1.641	468	43	E15.5
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0.14	0.319	0.472	1.318	7232	20	E15.5
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.72	0.885	0.261	0.839	37431	37	E15.5
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.85	0.94	0.247	0.728	44270	25	E15.5
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.80	0.93	-0.26	-0.77	38249	25	E15.5
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	0.79	0.928	0.285	0.762	41022	17	E15.5
KEGG_JAK_STAT_SIGNALING_PATHWAY	0.70	0.867	0.258	0.856	33632	42	E15.5
KEGG_HEMATOPOIETIC_CELL_LINEAGE	0.47	0.674	0.502	1.013	24614	6	E15.5
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.00	0.026	-0.58	1.866	60	36	E15.5
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.35	0.57	0.307	1.068	16587	53	E15.5
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.50	0.674	0.302	0.973	23922	36	E15.5
KEGG_FC_EPSILON_R1_SIGNALING_PATHWAY	0.00	0.033	0.556	1.788	127	36	E15.5
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	0.07	0.204	0.389	1.356	3434	54	E15.5

KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	0.01	0.041	0.506	1.692	295	44	E15.5
KEGG_CIRCADIEN_RHYTHM_MAMMAL	0.16	0.371	0.686	1.337	7821	5	E15.5
KEGG_LONG_TERM_POTENTIATION	0.00	0.033	-0.53	1.761	157	42	E15.5
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	0.04	0.151	-0.37	1.386	2053	79	E15.5
KEGG_LONG_TERM_DEPRESSION	0.02	0.078	0.488	1.589	864	38	E15.5
KEGG_OLFACTORY_TRANSDUCTION	0.02	0.092	0.682	1.645	1106	11	E15.5
KEGG_TASTE_TRANSDUCTION	0.31	0.543	0.522	1.154	14700	8	E15.5
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	0.02	0.078	0.374	1.456	863	99	E15.5
KEGG_INSULIN_SIGNALING_PATHWAY	0.00	0.035	0.436	1.629	215	78	E15.5
KEGG_GNRH_SIGNALING_PATHWAY	0.08	0.225	0.403	1.354	4015	44	E15.5
KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	0.96	0.992	0.193	0.679	45967	57	E15.5
KEGG_MELANOGENESIS	0.50	0.674	0.286	0.97	26053	47	E15.5
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	0.56	0.728	0.305	0.933	26713	29	E15.5
KEGG_TYPE_II_DIABETES_MELLITUS	0.01	0.045	0.598	1.757	327	24	E15.5
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	0.39	0.621	-0.37	1.051	18721	21	E15.5
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	0.02	0.08	0.519	1.603	922	30	E15.5
KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION	0.43	0.661	0.442	1.03	22671	10	E15.5
KEGG_ALZHEIMERS_DISEASE	0.02	0.078	0.368	-1.45	829	107	E15.5
KEGG_PARKINSONS_DISEASE	0.99	0.992	0.165	-0.63	47847	87	E15.5
KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS	0.01	0.044	0.571	1.737	309	28	E15.5
KEGG_HUNTINGTONS_DISEASE	0.99	0.992	0.167	0.676	47988	128	E15.5
KEGG_PRION_DISEASES	0.73	0.885	0.305	0.798	34987	15	E15.5
KEGG_VIBRIO_CHOLERAE_INFECTION	0.20	0.424	-0.38	1.207	9601	34	E15.5
KEGG_EPITHELIAL_CELL_SIGNALING_IN_Helicobacter_PYLORI_INFECTION	0.13	0.31	0.388	1.285	6173	41	E15.5
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	0.02	0.078	-0.5	1.599	829	35	E15.5
KEGG_LEISHMANIA_INFECTION	0.11	0.288	0.468	1.351	5793	23	E15.5
KEGG_PATHWAYS_IN_CANCER	0.06	0.176	0.31	1.281	3091	151	E15.5
KEGG_COLORECTAL_CANCER	0.50	0.674	0.286	0.974	25666	48	E15.5
KEGG_RENAL_CELL_CARCINOMA	0.40	0.628	-0.3	1.031	19222	50	E15.5
KEGG_PANCREATIC_CANCER	0.31	0.543	0.326	1.098	14845	45	E15.5
KEGG_ENDOMETRIAL_CANCER	0.31	0.544	0.342	-1.1	15106	36	E15.5

KEGG_GLIOMA	0.08	0.216	0.422	1.375	3785	38	E15.5
KEGG_PROSTATE_CANCER	0.24	0.465	0.329	1.148	12456	54	E15.5
KEGG_THYROID_CANCER	0.13	0.31	0.501	1.341	6748	17	E15.5
KEGG_BASAL_CELL_CARCIOMA	0.27	0.5	0.423	1.163	13862	19	E15.5
KEGG_MELANOMA	0.81	0.93	0.249	-0.77	39027	30	E15.5
KEGG_BLADDER_CANCER	0.49	0.674	0.339	0.98	25235	23	E15.5
KEGG_CHRONIC_MYELOID_LEUKEMIA	0.85	0.94	0.225	0.776	40656	50	E15.5
KEGG_ACUTE_MYELOID_LEUKEMIA	0.22	0.444	0.376	1.184	10694	33	E15.5
KEGG_SMALL_CELL_LUNG_CANCER	0.22	0.444	0.353	1.173	11574	43	E15.5
KEGG_NON_SMALL_CELL_LUNG_CANCER	0.65	0.817	0.279	0.881	31039	33	E15.5
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	0.25	0.465	0.414	1.181	12723	22	E15.5
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_H CM	0.43	0.661	0.343	1.017	20723	25	E15.5
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICUL AR_CARDIOMYOPATHY_ARVC	0.39	0.621	0.352	1.047	20392	26	E15.5
KEGG_DILATED_CARDIOMYOPATHY	0.61	0.784	-0.3	0.896	29374	26	E15.5
KEGG_VIRAL_MYOCARDITIS	0.21	0.433	0.449	1.228	10173	18	E15.5
KEGG_GLYCOLYSIS_GLUONEOGENESIS	0.00	0.04	0.609	1.832	159	26	E17.5
KEGG_CITRATE_CYCLE_TCA_CYCLE	0.36	0.659	0.363	1.073	19887	24	E17.5
KEGG_PENTOSE_PHOSPHATE_PATHWAY	0.03	0.144	0.637	1.612	1535	13	E17.5
KEGG_FRUCTOSE_AND_MANNOSSE_METABOLI SM	0.05	0.183	0.541	1.511	2829	19	E17.5
KEGG_GALACTOSE_METABOLISM	0.90	0.984	0.262	0.634	47403	11	E17.5
KEGG_FATTY_ACID_METABOLISM	0.00	0.015	0.693	2.028	13	23	E17.5
KEGG_STEROID_BIOSYNTHESIS	0.03	0.161	0.735	1.574	1784	7	E17.5
KEGG_STEROID_HORMONE_BIOSYNTHESIS	0.23	0.511	0.637	1.241	12080	5	E17.5
KEGG_OXIDATIVE_PHOSPHORYLATION	1.00	1	0.128	0.494	59208	89	E17.5
KEGG_PURINE_METABOLISM	0.39	0.66	0.275	1.037	22803	78	E17.5
KEGG_PYRIMIDINE_METABOLISM	0.15	0.391	0.34	1.232	8879	62	E17.5
KEGG_ALANINE_ASPARTATE_AND_GLUTAMAT E_METABOLISM	0.07	0.22	0.574	1.481	3618	14	E17.5
KEGG_GLYCINE_SERINE_AND_THREONINE_M ETABOLISM	0.01	0.069	0.701	1.773	365	13	E17.5
KEGG_CYSTEINE_AND_METHIONINE_METABO LISM	0.21	0.475	0.449	1.236	11232	18	E17.5
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DE GRADATION	0.00	0.016	0.615	1.959	32	33	E17.5
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_BI OSYNTHESIS	0.85	0.976	0.312	0.669	44643	7	E17.5
KEGG_LYSINE_DEGRADATION	0.33	0.627	0.366	1.101	18005	26	E17.5
KEGG_ARGININE_AND_PROLINE_METABOLISM	0.00	0.021	0.649	1.937	50	25	E17.5
KEGG_HISTIDINE_METABOLISM	0.45	0.667	0.458	1.017	23662	8	E17.5
KEGG_TYROSINE_METABOLISM	0.92	0.994	0.243	0.602	49050	12	E17.5
KEGG_PHENYLALANINE_METABOLISM	0.08	0.243	0.74	1.443	4128	5	E17.5
KEGG_TRYPTOPHAN_METABOLISM	0.04	0.172	0.632	1.565	2165	12	E17.5
KEGG_BETA_ALANINE_METABOLISM	0.05	0.183	0.62	1.537	2644	12	E17.5

KEGG_SELENOAMINO_ACID_METABOLISM	0.80	0.961	-	-	37621	12	E17.5
KEGG_GLUTATHIONE_METABOLISM	0.01	0.098	0.551	1.675	720	27	E17.5
KEGG_STARCH_AND_SUCROSE_METABOLISM	0.41	0.66	0.414	1.048	21595	13	E17.5
KEGG_N_GLYCAN_BIOSYNTHESIS	0.62	0.776	0.295	0.889	33950	26	E17.5
KEGG_OTHER_GLYCAN_DEGRADATION	0.45	0.667	0.475	1.017	23706	7	E17.5
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	0.54	0.737	0.31	0.942	29599	27	E17.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE	0.99	1	-	-	46985	8	E17.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_KERATAN_SULFATE	0.97	1	-	-	46749	5	E17.5
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_SULFATE	0.36	0.659	-	-	16789	12	E17.5
KEGG_GLYCEROLIPID_METABOLISM	0.03	0.144	0.593	1.61	1503	17	E17.5
KEGG_INOSITOL_PHOSPHATE_METABOLISM	0.92	0.994	0.222	0.65	50333	23	E17.5
KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_BIOSYNTHESIS	0.90	0.984	0.255	0.632	47910	12	E17.5
KEGG_GLYCEROPHOSPHOLIPID_METABOLISM	0.43	0.66	0.318	1.02	23898	34	E17.5
KEGG_ETHER_LIPID_METABOLISM	0.12	0.34	0.567	1.374	6548	11	E17.5
KEGG_ARACHIDONIC_ACID_METABOLISM	0.31	0.613	0.541	1.159	16014	7	E17.5
KEGG_SPHINGOLIPID_METABOLISM	0.29	0.603	0.422	1.144	15795	17	E17.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	0.14	0.362	-	-	6671	8	E17.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GLOBO_SERIES	0.87	0.976	0.332	0.647	45209	5	E17.5
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GANGLIO_SERIES	0.89	0.984	0.305	0.626	46510	6	E17.5
KEGG_PYRUVATE_METABOLISM	0.03	0.144	0.552	1.598	1537	22	E17.5
KEGG_GLYOXYLATE_AND_DICARBOXYLATE_METABOLISM	0.37	0.66	0.459	1.084	19639	10	E17.5
KEGG_PROANOATE_METABOLISM	0.00	0.025	0.651	1.905	73	23	E17.5
KEGG_BUTANOATE_METABOLISM	0.01	0.07	0.627	1.75	481	19	E17.5
KEGG_ONE_CARBON_POOL_BY_FOLATE	0.04	0.174	0.643	1.557	2273	11	E17.5
KEGG_RIBOFLAVIN_METABOLISM	0.61	0.775	0.399	0.886	31758	8	E17.5
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	0.84	0.976	-	-	39659	10	E17.5
KEGG_PANTOTHENATE_AND_COA_BIOSYNTHESIS	0.08	0.238	-	-	3599	5	E17.5
KEGG_FOLATE_BIOSYNTHESIS	0.05	0.183	0.77	1.501	2641	5	E17.5
KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	0.38	0.66	0.415	1.069	20117	14	E17.5
KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS	0.38	0.66	0.433	1.073	20214	12	E17.5
KEGG_LIMONENE_AND_PINENE_DEGRADATION	0.04	0.161	0.791	1.542	1876	5	E17.5
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	1.00	1	-	-	44385	29	E17.5
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	0.02	0.133	0.697	1.646	1152	10	E17.5
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	0.01	0.069	0.713	1.767	375	12	E17.5
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	0.77	0.935	0.313	0.757	40825	11	E17.5
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	0.07	0.22	0.564	1.482	3547	15	E17.5
KEGG_ABC_TRANSPORTERS	0.43	0.66	0.484	1.036	22657	7	E17.5

KEGG_RIBOSOME	0.00	0.021	0.487	1.808	59	71	E17.5
KEGG_RNA_DEGRADATION	0.95	1	0.193	0.669	53766	50	E17.5
KEGG_RNA_POLYMERASE	0.97	1	0.194	0.554	52847	21	E17.5
KEGG_BASAL_TRANSCRIPTION_FACTORS	0.78	0.94	0.264	0.773	42838	23	E17.5
KEGG_DNA_REPLICATION	0.00	0.025	0.618	1.894	83	28	E17.5
KEGG_SPLICEOSOME	1.00	1	0.129	0.521	60127	116	E17.5
KEGG_PROTEASOME	1.00	1	-	-	43548	37	E17.5
KEGG_PROTEIN_EXPORT	0.13	0.343	0.468	1.337	7010	21	E17.5
KEGG_PPAR_SIGNALING_PATHWAY	0.00	0.003	0.758	2.193	0	22	E17.5
KEGG_BASE_EXCISION_REPAIR	0.41	0.66	0.372	1.037	22239	19	E17.5
KEGG_NUCLEOTIDE_EXCISION_REPAIR	0.12	0.336	0.415	1.322	6710	33	E17.5
KEGG_MISMATCH_REPAIR	0.02	0.136	0.592	1.63	1294	18	E17.5
KEGG_HOMOLOGOUS_RECOMBINATION	0.03	0.158	0.606	1.592	1747	15	E17.5
KEGG_NON_HOMOLOGOUS_END_JOINING	0.16	0.395	0.646	1.325	8519	6	E17.5
KEGG_MAPK_SIGNALING_PATHWAY	0.04	0.161	0.313	1.347	1429	122	E17.5
KEGG_ERBB_SIGNALING_PATHWAY	0.26	0.553	-0.3	1.124	11177	56	E17.5
KEGG_CALCIUM_SIGNALING_PATHWAY	0.53	0.737	-	-	22784	53	E17.5
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	0.13	0.348	0.43	1.316	7343	28	E17.5
KEGG_CHEMOKINE_SIGNALING_PATHWAY	0.44	0.66	0.258	1.007	18460	70	E17.5
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	0.36	0.659	-0.32	1.059	16089	32	E17.5
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	0.18	0.411	0.365	1.226	7786	34	E17.5
KEGG_CELL_CYCLE	0.01	0.07	0.42	1.592	444	80	E17.5
KEGG_OOCYTE_MEIOSIS	0.05	0.174	0.363	1.395	1913	64	E17.5
KEGG_P53_SIGNALING_PATHWAY	0.04	0.172	0.473	1.505	2267	33	E17.5
KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	0.43	0.66	0.244	1.009	17482	96	E17.5
KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	0.39	0.66	0.333	1.048	17279	26	E17.5
KEGG_REGULATION_OF_AUTOPHAGY	0.47	0.67	0.378	0.992	21802	13	E17.5
KEGG_LYSOSOME	0.02	0.119	0.421	1.541	957	66	E17.5
KEGG_ENDOCYTOSIS	0.53	0.737	0.229	0.964	21463	107	E17.5
KEGG_PEROXISOME	0.00	0.015	0.577	1.97	14	46	E17.5
KEGG_MTOR_SIGNALING_PATHWAY	0.53	0.737	0.292	0.953	23336	30	E17.5
KEGG_APOPTOSIS	0.07	0.22	0.398	1.386	2960	40	E17.5
KEGG_CARDIAC_MUSCLE_CONTRACTION	0.61	0.776	0.274	0.899	34552	38	E17.5
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	0.32	0.622	-0.3	1.085	13868	47	E17.5
KEGG_WNT_SIGNALING_PATHWAY	0.35	0.659	0.277	1.058	20740	83	E17.5
KEGG_DORSO_VENTRAL_AXIS_FORMATION	0.57	0.756	0.37	0.916	30223	12	E17.5
KEGG_NOTCH_SIGNALING_PATHWAY	0.02	0.133	0.531	1.613	1203	27	E17.5
KEGG_HEDGEHOG_SIGNALING_PATHWAY	0.60	0.769	0.321	0.897	32400	19	E17.5

KEGG_TGF_BETA_SIGNALING_PATHWAY	0.01	0.069	0.524	1.722	378	38	E17.5
KEGG_AXON_GUIDANCE	0.00	0.016	-	-	20	80	E17.5
KEGG_VEGF_SIGNALING_PATHWAY	0.65	0.812	-	-	28805	34	E17.5
KEGG_FOCAL_ADHESION	0.01	0.07	0.398	1.556	515	95	E17.5
KEGG_ECM_RECEPTOR_INTERACTION	0.00	0.016	0.71	1.981	30	19	E17.5
KEGG_CELL_ADHESION_MOLECULES_CAMS	0.16	0.395	-	-	7045	36	E17.5
KEGG_ADHERENS_JUNCTION	0.46	0.669	0.292	0.997	25961	46	E17.5
KEGG_TIGHT_JUNCTION	0.64	0.799	0.245	0.901	37005	68	E17.5
KEGG_GAP_JUNCTION	0.06	0.2	-	-	2506	49	E17.5
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0.00	0.035	0.684	1.884	124	18	E17.5
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.41	0.66	0.313	1.033	22891	39	E17.5
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.55	0.738	0.315	0.932	30172	24	E17.5
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.97	1	-	-	43493	26	E17.5
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	0.47	0.67	0.365	0.989	25445	17	E17.5
KEGG_JAK_STAT_SIGNALING_PATHWAY	0.42	0.66	0.306	1.024	23568	42	E17.5
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.03	0.14	-	-	1117	37	E17.5
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.59	0.767	0.265	0.921	33326	50	E17.5
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.25	0.542	0.359	1.159	14192	35	E17.5
KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY	0.04	0.174	-	-	1935	37	E17.5
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	0.17	0.399	-	-	7182	53	E17.5
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	0.01	0.069	0.498	1.7	339	46	E17.5
KEGG_CIRCADIAN_RHYTHM_MAMMAL	0.84	0.976	-	-	40274	6	E17.5
KEGG_LONG_TERM_POTENTIATION	0.08	0.243	-	-	3507	46	E17.5
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	0.08	0.243	-	-	3336	79	E17.5
KEGG_LONG_TERM_DEPRESSION	0.13	0.343	-0.37	1.283	5533	39	E17.5
KEGG_OLFACTORY_TRANSDUCTION	0.02	0.133	-	-	1016	11	E17.5
KEGG_TASTE_TRANSDUCTION	0.09	0.259	-	-	4176	8	E17.5
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	0.16	0.391	0.301	1.197	9347	108	E17.5
KEGG_INSULIN_SIGNALING_PATHWAY	0.05	0.183	-	-	2154	78	E17.5
KEGG_GNRH_SIGNALING_PATHWAY	0.36	0.659	-	-	15330	52	E17.5
KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	0.47	0.67	-0.27	0.992	20044	51	E17.5
KEGG_MELANOGENESIS	0.67	0.83	0.256	0.867	38156	44	E17.5
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	0.83	0.976	-0.23	0.761	36804	32	E17.5

KEGG_TYPE_II_DIABETES_MELLITUS	0.01	0.07	-	-	402	20	E17.5
KEGG_TYPE_I_DIABETES_MELLITUS	0.25	0.539	0.628	1.225	12905	5	E17.5
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	0.31	0.613	0.409	1.126	16724	18	E17.5
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	0.17	0.406	0.381	1.243	7653	30	E17.5
KEGG_PROXIMAL_TUBULE_BICARBONATE_RECLAMATION	0.02	0.123	0.675	1.673	951	12	E17.5
KEGG_ALZHEIMERS_DISEASE	0.85	0.976	0.195	0.828	33920	112	E17.5
KEGG_PARKINSONS_DISEASE	0.97	1	0.177	0.681	57086	87	E17.5
KEGG_AMYTROPHIC_LATERAL_SCLEROSIS_ALS	0.02	0.133	0.477	1.578	994	32	E17.5
KEGG_HUNTINGTONS_DISEASE	1.00	1	0.147	0.6	60570	128	E17.5
KEGG_PRION_DISEASES	0.68	0.834	0.304	0.836	36964	18	E17.5
KEGG_VIBRIO_CHOLERAЕ_INFECTION	0.41	0.66	0.307	1.025	18259	33	E17.5
KEGG_EPITHELIAL_CELL_SIGNALING_IN_Helicobacter_Pylori_INFECTION	0.37	0.659	0.319	1.06	20744	40	E17.5
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	0.00	0.054	0.535	-1.77	188	32	E17.5
KEGG_LEISHMANIA_INFECTION	0.04	0.174	0.521	1.525	2412	23	E17.5
KEGG_PATHWAYS_IN_CANCER	0.11	0.305	0.294	1.229	6625	149	E17.5
KEGG_COLORECTAL_CANCER	0.44	0.66	0.296	1.009	24913	46	E17.5
KEGG_RENAL_CELL_CARCINOMA	0.88	0.976	0.213	0.739	49836	50	E17.5
KEGG_PANCREATIC_CANCER	0.87	0.976	0.213	-0.75	37791	42	E17.5
KEGG_ENDOMETRIAL_CANCER	0.88	0.976	0.216	0.738	38297	37	E17.5
KEGG_GLIOMA	0.57	0.756	0.266	0.931	24952	41	E17.5
KEGG_PROSTATE_CANCER	0.31	0.613	0.313	1.096	17773	52	E17.5
KEGG_THYROID_CANCER	0.42	0.66	0.386	1.031	22541	16	E17.5
KEGG_BASAL_CELL_CARCINOMA	0.54	0.737	0.341	0.937	29258	18	E17.5
KEGG_MELANOMA	0.42	0.66	0.327	1.028	23124	31	E17.5
KEGG_BLADDER_CANCER	0.31	0.613	0.382	1.117	17101	23	E17.5
KEGG_CHRONIC_MYELOID_LEUKEMIA	0.84	0.976	0.218	0.772	36719	43	E17.5
KEGG_ACUTE_MYELOID_LEUKEMIA	0.55	0.738	0.284	-0.94	24282	32	E17.5
KEGG_SMALL_CELL_LUNG_CANCER	0.11	0.304	0.401	1.331	5928	40	E17.5
KEGG_NON_SMALL_CELL_LUNG_CANCER	0.96	1	0.186	0.621	42505	33	E17.5
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	0.59	0.769	0.322	0.899	32235	19	E17.5
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	0.23	0.503	0.399	1.2	12412	26	E17.5
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	0.23	0.503	0.391	1.197	12486	28	E17.5
KEGG_DILATED_CARDIOMYOPATHY	0.29	0.595	0.371	1.136	15763	28	E17.5
KEGG_VIRAL_MYOCARDITIS	0.43	0.66	0.352	1.023	19445	19	E17.5

Table S3: E15.5 S₀ vs S₂ fgsea

pathway_name	pval	padj	ES	NES	nMoreE xtreme	size	logpadj	mark	Met. state
Glycolysis Gluconeogenesis	0.262	0.529	0.355	1.182	15877	18	0.277	1	S0
Citrate Cycle Tca Cycle	0.927	0.962	0.239	0.595	41286	6	0.017	0	S2
Pentose Phosphate Pathway	0.392	0.632	0.383	1.062	22632	10	0.199	0	S0
Fructose and Mannose Metabolism	0.333	0.58	0.435	1.122	18834	8	0.236	0	S0
Galactose Metabolism	0.419	0.646	0.445	1.031	18933	5	0.19	0	S2
Ascorbate and Aldarate Metabolism	0.378	0.63	0.592	1.093	19859	3	0.201	0	S0
Fatty Acid Metabolism	0.018	0.092	0.518	1.695	1112	17	1.035	1	S0
Steroid Biosynthesis	0.976	0.988	0.242	0.517	45032	4	0.005	0	S2
Oxidative Phosphorylation	0.029	0.121	-0.41	1.619	1112	23	0.918	1	S2
Purine Metabolism	0.186	0.427	0.304	1.243	12158	40	0.37	0	S0
Pyrimidine Metabolism	0.002	0.023	0.487	1.889	149	32	1.641	0	S0
Alanine Aspartate and Glutamate Metabolism	0.977	0.988	0.241	0.515	45090	4	0.005	0	S2
Glycine Serine and Threonine Metabolism	0.005	0.036	0.795	1.751	276	5	1.438	1	S0
Cysteine/Methionine Metabolism	0.581	0.779	0.366	0.904	32639	7	0.109	1	S0
Valine Leucine and Isoleucine Degradation	0.057	0.2	0.458	1.523	3456	18	0.699	0	S0
Lysine Degradation	0.248	0.529	0.422	1.206	14423	11	0.277	0	S0
Arginine and Proline Metabolism	0.619	0.804	0.296	0.872	36188	12	0.095	0	S0
Histidine Metabolism	0.669	0.843	0.382	0.842	36657	5	0.074	0	S0
Tyrosine Metabolism	0.412	0.646	0.577	1.064	21687	3	0.19	0	S0
Tryptophan Metabolism	0.157	0.392	0.514	1.326	8879	8	0.407	0	S0
Beta Alanine Metabolism	0.297	0.551	0.469	1.157	16708	7	0.259	0	S0
Taurine and Hypotaurine Metabolism	0.702	0.849	0.524	0.851	36073	2	0.071	0	S0
Selenoamino Acid Metabolism	0.956	0.979	0.261	0.556	44087	4	0.009	0	S2
Glutathione Metabolism	0.189	0.427	0.396	1.272	11321	16	0.369	0	S0
Starch and Sucrose Metabolism	0.863	0.937	0.353	0.678	40943	3	0.028	0	S2
N Glycan Biosynthesis	0.889	0.946	0.245	0.644	38927	7	0.024	0	S2
O Glycan Biosynthesis	0.926	0.962	0.374	0.622	45040	2	0.017	0	S2
Amino Sugar and Nucleotide Sugar Metabolism	0.585	0.779	0.322	0.891	25374	8	0.109	0	S2
Glycosaminoglycan Degradation	0.988	0.988	0.309	0.501	50810	2	0.005	0	S0
Glycosaminoglycan Biosynthesis Chondroitin Sulfate	0.089	0.263	0.818	1.359	4343	2	0.58	0	S2
Glycosaminoglycan Biosynthesis Keratan Sulfate	0.186	0.427	0.758	-1.26	9061	2	0.37	0	S2

Glycosaminoglycan Biosynthesis Heparan Sulfate	0.059	0.202	0.765	1.469	2797	3	0.695	0	S2
Glycerolipid Metabolism	0.366	0.623	0.392	1.086	21137	10	0.205	0	S0
Inositol Phosphate Metabolism	0.394	0.632	0.379	1.048	17104	8	0.199	0	S2
Glycosylphosphatidylinositol Gpi Anchor Biosynthesis	0.642	0.824	0.533	0.885	31237	2	0.084	0	S2
Glycerophospholipid Metabolism	0.582	0.779	0.315	0.901	33776	11	0.109	0	S0
Ether Lipid Metabolism	0.241	0.523	0.595	1.212	12977	4	0.282	0	S0
Sphingolipid Metabolism	0.316	0.57	0.516	1.137	17318	5	0.244	0	S0
Glycosphingolipid Biosynthesis Lacto and Neolacto Series	0.223	0.49	0.533	1.236	10066	5	0.31	0	S2
Glycosphingolipid Biosynthesis Globo Series	0.705	0.849	0.379	0.808	32508	4	0.071	0	S2
Glycosphingolipid Biosynthesis Ganglio Series	0.283	0.542	0.548	-1.17	13036	4	0.266	0	S2
Pyruvate Metabolism	0.263	0.529	0.416	1.189	15253	11	0.277	0	S0
Glyoxylate and Dicarboxylate Metabolism	0.644	0.824	0.391	0.862	35283	5	0.084	0	S0
Propanoate Metabolism	0.06	0.202	0.518	1.524	3506	12	0.695	0	S0
Butanoate Metabolism	0.565	0.779	0.32	0.915	32776	11	0.109	0	S0
One Carbon Metabolism	0.077	0.24	0.595	1.468	4344	7	0.62	1	S0
Riboflavin Metabolism	0.874	0.943	0.43	0.698	44936	2	0.026	0	S0
Nicotinate and Nicotinamide Metabolism	0.109	0.306	0.718	1.378	5184	3	0.515	0	S2
Folate Biosynthesis	0.107	0.306	0.813	1.319	5522	2	0.515	0	S0
Porphyrin and Chlorophyll Metabolism	0.69	0.849	0.384	0.819	31847	4	0.071	0	S2
Terpenoid Backbone Biosynthesis	0.881	0.944	0.36	0.664	46366	3	0.025	0	S0
Limonene and Pinene Degradation	0.164	0.399	0.593	1.306	9011	5	0.399	0	S0
Nitrogen Metabolism	0.27	0.535	0.712	1.184	13131	2	0.271	0	S2
Aminoacyl Trna Biosynthesis	0.717	0.851	0.278	0.795	41605	11	0.07	0	S0
Metabolism Of Xenobiotics By Cytochrome P450	0.322	0.57	0.482	1.129	17842	6	0.244	0	S0
Drug Metabolism Cytochrome P450	0.322	0.57	0.482	1.129	17842	6	0.244	0	S0
Drug Metabolism Other Enzymes	0.338	0.58	0.55	1.12	18198	4	0.236	0	S0
Biosynthesis Of Unsaturated Fatty Acids	0.511	0.74	0.371	0.958	28963	8	0.131	0	S0
Abc Transporters	0.902	0.948	0.308	0.628	48625	4	0.023	0	S0
Ribosome	0.087	0.26	0.401	1.429	5364	23	0.585	1	S0
Rna Degradation	0.181	0.427	0.344	1.265	11342	26	0.37	0	S0
Rna Polymerase	0.937	0.966	0.223	0.576	53052	8	0.015	0	S0
Basal Transcription Factors	0.149	0.378	0.542	1.339	8361	7	0.423	0	S0
DNA Replication	0.00E+00	0.001	0.727	2.751	0	29	2.885	1	S0
Spliceosome	0	0.009	0.431	1.965	26	66	2.034	1	S0

Proteasome	0.289	0.547	0.572	1.166	15558	4	0.262	0	S0
Protein Export	0.863	0.937	0.287	0.672	47871	6	0.028	0	S0
Ppar Signaling Pathway	0.079	0.24	0.515	1.472	4559	11	0.62	0	S0
Base Excision Repair	0.011	0.058	0.563	1.773	636	15	1.234	0	S0
Nucleotide Excision Repair	0.002	0.019	0.564	1.961	107	21	1.714	0	S0
Mismatch Repair	0	0.007	0.668	2.146	12	16	2.145	0	S0
Homologous Recombination	0.001	0.011	0.664	2.048	33	14	1.978	0	S0
Non Homologous End Joining	0.306	0.561	0.564	1.15	16480	4	0.251	0	S0
Mapk Signaling Pathway	0	0.009	0.417	2.039	11	51	2.034	0	S2
ErbB Signaling Pathway	0.011	0.058	0.449	1.772	416	23	1.234	0	S2
Calcium Signaling Pathway	0.092	0.267	0.362	-1.41	3534	22	0.573	0	S2
Cytokine Cytokine Receptor Interaction	0.762	0.861	0.288	0.758	33381	7	0.065	0	S2
Chemokine Signaling Pathway	0.029	0.121	0.373	1.592	1041	30	0.918	0	S2
Phosphatidylinositol Signaling System	0.007	0.045	-0.57	1.877	289	13	1.349	0	S2
Neuroactive Ligand Receptor Interaction	0.001	0.017	0.559	2.083	47	19	1.771	1	S2
Cell Cycle	0.00E+00	0.001	0.617	2.814	0	66	2.885	1	S0
Oocyte Meiosis	0.002	0.023	0.456	1.854	152	39	1.641	0	S0
P53 Signaling Pathway	0.003	0.026	0.543	1.91	172	22	1.59	0	S0
Ubiquitin Mediated Proteolysis	0.297	0.551	0.295	1.136	18866	31	0.259	0	S0
Snare Interactions In Vesicular Transport	0.048	0.176	0.452	-1.56	1928	15	0.756	0	S2
Regulation Of Autophagy	0.146	0.376	-0.58	1.344	6591	5	0.425	0	S2
Lysosome	0.724	0.854	0.196	0.821	26779	28	0.069	0	S2
Endocytosis	0.00E+00	0.003	0.453	2.172	1	47	2.482	1	S2
Peroxisome	0.176	0.421	0.38	1.285	10755	19	0.375	0	S0
Mtor Signaling Pathway	0.716	0.851	0.249	0.799	29751	12	0.07	0	S2
Apoptosis	0.004	0.036	0.539	1.938	164	17	1.442	0	S2
Cardiac Muscle Contraction	0.411	0.646	0.299	1.031	16581	15	0.19	0	S2
Vascular Smooth Muscle Contraction	0.051	0.184	0.384	1.519	1946	23	0.736	0	S2
Wnt Signaling Pathway	0.686	0.849	0.205	0.851	45201	43	0.071	0	S0
Dorso Ventral Axis Formation	0.338	0.58	0.55	1.12	18201	4	0.236	0	S0
Notch Signaling Pathway	0.009	0.052	0.57	1.796	529	15	1.281	0	S0
Hedgehog Signaling Pathway	0.511	0.74	0.357	0.957	29252	9	0.131	0	S0
Tgf Beta Signaling Pathway	0.116	0.319	0.395	1.374	7132	21	0.497	0	S0
Axon Guidance	0.005	0.036	0.359	-1.74	173	49	1.438	1	S2

Vegf Signaling Pathway	0.031	0.126	0.454	1.633	1241	17	0.9	0	S2
Focal Adhesion	0.76	0.861	0.192	0.8	50077	43	0.065	0	S0
Ecm Receptor Interaction	0.26	0.529	0.485	1.197	14626	7	0.277	0	S0
Cell Adhesion Molecules Cams	0.016	0.082	0.448	1.724	609	21	1.087	1	S2
Adherens Junction	0.443	0.671	0.259	1.009	16976	22	0.173	0	S2
Tight Junction	0.836	0.92	0.18	0.732	54588	39	0.036	0	S0
Gap Junction	0.024	0.11	0.412	1.648	908	24	0.958	0	S2
Antigen Processing and Presentation	0.81	0.897	0.308	0.721	44948	6	0.047	0	S0
Toll Like Receptor Signaling Pathway	0.556	0.777	0.273	0.923	22645	14	0.109	0	S2
Nod Like Receptor Signaling Pathway	0.747	0.861	0.269	0.769	43366	11	0.065	0	S0
Rig I Like Receptor Signaling Pathway	0.251	0.529	0.455	1.199	10977	7	0.277	0	S2
Cytosolic Dna Sensing Pathway	0.988	0.988	0.225	0.479	45605	4	0.005	0	S2
Jak Stat Signaling Pathway	0.61	0.799	0.266	0.886	37014	18	0.098	0	S0
Hematopoietic Cell Lineage	0.591	0.78	0.574	0.932	30366	2	0.108	0	S0
Natural Killer Cell Mediated Cytotoxicity	0.001	0.015	0.565	2.103	39	19	1.812	0	S2
T Cell Receptor Signaling Pathway	0.263	0.529	0.279	1.157	9764	27	0.277	0	S2
B Cell Receptor Signaling Pathway	0.45	0.675	0.269	1.004	17531	19	0.171	0	S2
Fc Epsilon R1 Signaling Pathway	0.001	0.011	0.599	2.154	20	17	1.978	0	S2
Fc Gamma R Mediated Phagocytosis	0.048	0.176	0.356	1.506	1758	29	0.756	0	S2
Leukocyte Transendothelial Migration	0.026	0.114	0.442	1.625	1604	26	0.942	0	S0
Long Term Potentiation	0.002	0.018	0.503	2.013	57	24	1.743	0	S2
Neurotrophin Signaling Pathway	0.011	0.058	0.395	1.729	380	33	1.234	0	S2
Long Term Depression	0.007	0.044	-0.51	1.865	264	18	1.352	0	S2
Olfactory Transduction	0.029	0.121	0.632	1.664	1257	7	0.918	0	S2
Taste Transduction	0.121	0.328	0.646	1.379	5587	4	0.485	0	S2
Regulation Of Actin Cytoskeleton	0.023	0.108	0.311	1.544	733	54	0.967	0	S2
Insulin Signaling Pathway	0.001	0.018	0.457	1.986	50	32	1.748	0	S2
Gnrh Signaling Pathway	0.023	0.108	0.433	1.665	880	21	0.967	0	S2
Progesterone Mediated Oocyte Maturation	0.004	0.036	0.486	1.838	278	29	1.439	0	S0
Melanogenesis	0.471	0.7	0.285	0.992	28996	21	0.155	0	S0
Adipocytokine Signaling Pathway	0.073	0.23	0.482	1.499	3046	11	0.637	0	S2

Type II Diabetes Mellitus	0	0.007	0.663	2.235	7	14	2.145	0	S2
Type I Diabetes Mellitus	0.697	0.849	0.507	0.843	33896	2	0.071	0	S2
Maturity Onset Diabetes Of The Young	0.001	0.011	0.985	1.598	34	2	1.95	0	S0
Aldosterone Regulated Sodium Reabsorption	0.272	0.535	0.388	1.168	11532	10	0.271	0	S2
Vasopressin Regulated Water Reabsorption	0.005	0.036	0.598	1.914	209	12	1.438	0	S2
Proximal Tubule Bicarbonate Reclamation	0.495	0.729	0.366	0.963	21664	7	0.138	0	S2
Alzheimers Disease	0.008	0.05	0.394	1.756	289	35	1.304	0	S2
Parkinsons Disease	0.389	0.632	0.258	1.048	14643	25	0.199	0	S2
Amyotrophic Lateral Sclerosis Als	0.005	0.036	0.583	1.918	217	13	1.438	0	S2
Huntingtons Disease	0.753	0.861	0.178	0.817	26151	39	0.065	0	S2
Prion Diseases	0.552	0.777	0.317	0.918	23622	9	0.109	0	S2
Vibrio Cholerae Infection	0.043	0.17	0.428	1.568	1702	18	0.769	0	S2
Epithelial Cell Signaling In Helicobacter Pylori Infection	0.065	0.214	0.386	1.486	2489	21	0.67	0	S2
Pathogenic Escherichia Coli Infection	0.163	0.399	-0.3	1.269	5994	29	0.399	0	S2
Leishmania Infection	0.388	0.632	0.362	1.064	22682	12	0.199	0	S0
Pathways In Cancer	0.22	0.49	0.255	1.182	15314	71	0.31	0	S0
Colorectal Cancer	0.55	0.777	0.252	0.935	34571	27	0.109	0	S0
Renal Cell Carcinoma	0.142	0.373	0.354	-1.32	5539	19	0.429	0	S2
Pancreatic Cancer	0.898	0.948	-0.16	0.664	33377	27	0.023	0	S2
Endometrial Cancer	0.281	0.542	0.314	1.149	11042	18	0.266	0	S2
Glioma	0.418	0.646	0.276	1.028	16265	19	0.19	0	S2
Prostate Cancer	0.428	0.654	0.309	1.028	25981	18	0.184	0	S0
Thyroid Cancer	0.378	0.63	0.436	1.076	21240	7	0.201	0	S0
Basal Cell Carcinoma	0.584	0.779	0.349	0.899	33097	8	0.109	0	S0
Melanoma	0.677	0.847	0.269	0.829	40141	14	0.072	0	S0
Bladder Cancer	0.546	0.777	0.325	0.93	31688	11	0.109	0	S0
Chronic Myeloid Leukemia	0.749	0.861	0.22	0.786	46409	23	0.065	0	S0
Acute Myeloid Leukemia	0.138	0.368	0.388	1.339	5587	15	0.434	0	S2
Small Cell Lung Cancer	0.045	0.172	0.447	1.555	2757	21	0.765	0	S0
Non Small Cell Lung Cancer	0.772	0.861	0.243	0.75	45747	14	0.065	0	S0
Systemic Lupus Erythematosus	0.069	0.225	0.473	1.491	4141	15	0.648	0	S0
Hypertrophic Cardiomyopathy Hcm	0.746	0.861	0.227	0.782	30147	15	0.065	0	S2

Arrhythmogenic Right Ventricular Cardiomyopathy Arvc	0.668	0.843	- 0.249	- -0.84	27227	14	0.074	0	S2
Dilated Cardiomyopathy	0.772	0.861	- 0.221	- 0.762	31187	15	0.065	0	S2
Viral Myocarditis	0.572	0.779	- 0.292	- 0.907	24003	11	0.109	0	S2

Table S4: Potential genetic targets for altering cell fate in early neural development

Module	Gene Symbol and reference	Description	Known in RPs or neural stem cell context	Summary
Acetyl-CoA module	Cpt1a (Carnitine Palmitoyltransferase 1A)	Catalyzes the transfer of the acyl group of long-chain fatty acid-CoA conjugates onto carnitine, an essential step for the mitochondrial uptake of long-chain fatty acids and their subsequent beta-oxidation in the mitochondrion. Plays an important role in triglyceride metabolism.	Yes	Required for stem cell maintenance and proper neurogenesis (Knobloch et al., 2017)
	Acadl (Acyl-CoA Dehydrogenase Long Chain)	The protein encoded by this gene belongs to the acyl-CoA dehydrogenase family, which is a family of mitochondrial flavoenzymes involved in fatty acid and branched chain amino-acid metabolism. This protein is one of the four enzymes that catalyze the initial step of mitochondrial beta-oxidation of straight-chain fatty acid. Defects in this gene are the cause of long-chain acyl-CoA dehydrogenase (LCAD) deficiency, leading to nonketotic hypoglycemia.	No	Expressed in SVZ and dentate gyrus (O'Leary et al., 2016)
	Hadh (Hydroxyacyl-CoA Dehydrogenase)	This gene is a member of the 3-hydroxyacyl-CoA dehydrogenase gene family. The encoded protein functions in the mitochondrial matrix to catalyze the oxidation of straight-chain 3-hydroxyacyl-CoAs as part of the beta-oxidation pathway. Its enzymatic activity is highest with medium-chain-length fatty acids. Mutations in this gene cause one form of familial hyperinsulinemic hypoglycemia. The human genome contains a related pseudogene of this gene on chromosome 15.	No	Affects brain development, clinically linked to mental retardation (Yang, He, & Schulz, 2005)
	Acaa2 (Acetyl-CoA Acyltransferase 2)	The encoded protein catalyzes the last step of the mitochondrial fatty acid beta-oxidation spiral. Unlike most mitochondrial matrix proteins, it contains a non-cleavable amino-terminal targeting signal.	No	Its deficiency leads to mental retardation (Fukao, Yamaguchi, Orii, & Hashimoto, 1995)
	Acadm (Acyl-CoA Dehydrogenase Medium Chain)	This gene encodes the medium-chain specific (C4 to C12 straight chain) acyl-Coenzyme A dehydrogenase. The homotetramer enzyme catalyzes the initial step of the mitochondrial fatty acid beta-oxidation pathway. Defects in this gene cause medium-chain acyl-CoA dehydrogenase deficiency, a disease characterized by hepatic dysfunction, fasting hypoglycemia, and encephalopathy, which can result in infantile death.	No	Its deficiency causes encephalopathy (O'Leary et al., 2016)
	Hat1 (Histone Acetyltransferase 1)	The protein encoded by this gene is a type B histone acetyltransferase (HAT) that is involved in the rapid acetylation of newly synthesized cytoplasmic histones, which are in turn imported into the nucleus for de novo deposition onto nascent DNA chains.	No	Maintains acetylation levels (O'Leary et al., 2016)
	Acss1 (Acyl-CoA Synthetase Short Chain Family Member 1)	This gene encodes a mitochondrial acetyl-CoA synthetase enzyme. A similar protein in mice plays an important role in the tricarboxylic acid cycle by catalyzing the conversion of acetate to acetyl CoA.	No	Helps in maintaining acetyl CoA levels (O'Leary et al., 2016)

Methylation module	Dnmt3a (DNA Methyltransferase 3 Alpha)	CpG methylation is an epigenetic modification that is important for embryonic development, imprinting, and X-chromosome inactivation. Studies in mice have demonstrated that DNA methylation is required for mammalian development. This gene encodes a DNA methyltransferase that is thought to function in de novo methylation, rather than maintenance methylation. The protein localizes to the cytoplasm and nucleus and its expression is developmentally regulated.	Yes	Deficiency associated with mental retardation (Z. Wu et al., 2012)
Glutathione	Gpx8 (Glutathione Peroxidase 8)	GPX8 (Glutathione Peroxidase 8 (Putative)) is a Protein Coding gene. Diseases associated with GPX8 include Anemia, Nonspherocytic Hemolytic, Due To G6pd Deficiency. Among its related pathways are Cellular Senescence (REACTOME) and Glutathione metabolism. Gene Ontology (GO) annotations related to this gene include oxidoreductase activity and peroxidase activity. An important paralog of this gene is GPX7.	Yes	Upregulated when NSCs are exposed to reactive oxidation species (Madhavan, Ourednik, & Ourednik, 2006)
Folate module	Dhfr (Dihydrofolate Reductase)	Dihydrofolate reductase converts dihydrofolate into tetrahydrofolate, a methyl group shuttle required for the de novo synthesis of purines, thymidylate, and certain amino acids. While the functional dihydrofolate reductase gene has been mapped to chromosome 5, multiple intronless processed pseudogenes or dihydrofolate reductase-like genes have been identified on separate chromosomes.	Yes	Inhibition leads to neural stem cell differentiation (Fawal et al., 2018)
	Mthfd1 (Methylenetetrahydrofolate Dehydrogenase, Cyclohydrolase And Formyltetrahydrofolate Synthetase 1)	This gene encodes a protein that possesses three distinct enzymatic activities, 5,10-methylenetetrahydrofolate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase and 10-formyltetrahydrofolate synthetase. Each of these activities catalyzes one of three sequential reactions in the interconversion of 1-carbon derivatives of tetrahydrofolate, which are substrates for methionine, thymidylate, and de novo purine syntheses.	No	Deficiency lead to neural tube defects (J. Wu et al., 2015)
	Tyms (Thymidylate Synthase)	Thymidylate synthase catalyzes the methylation of deoxyuridylate to deoxythymidylate using, 10-methylenetetrahydrofolate (methylene-THF) as a cofactor. This function maintains the dTMP (thymidine-5-prime monophosphate) pool critical for DNA replication and repair. The enzyme has been of interest as a target for cancer chemotherapeutic agents.	No	Deficiency lead to neural tube defects (Wang et al., 2018)
	Shmt1 (Serine Hydroxymethyltransferase 1)	This gene encodes the cytosolic form of serine hydroxymethyltransferase, a pyridoxal phosphate-containing enzyme that catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate. This reaction provides one-carbon units for synthesis of methionine, thymidylate, and purines in the cytoplasm. This gene is located within the Smith-Magenis syndrome region on chromosome 17.	No	Deficiency lead to neural tube defects (Beaudin et al., 2011)
a-KG module	Phgdh (Phosphoglycerate Dehydrogenase)	This gene encodes the enzyme which is involved in the early steps of L-serine synthesis in animal cells. L-serine is required for D-serine and other amino acid synthesis. The enzyme requires NAD/NADH as a cofactor and forms homotetramers for activity. Mutations in this gene have been found in a family with congenital microcephaly, psychomotor retardation and other symptoms.	Yes	Disruption leads to severe neurodevelopmental defects (Yoshida et al., 2003)
	Psat1 (Phosphoserine Aminotransferase 1)	This gene encodes a member of the class-V pyridoxal-phosphate-dependent aminotransferase family. The encoded protein is a phosphoserine aminotransferase and decreased expression may be associated with schizophrenia. Mutations in this gene are also associated with phosphoserine aminotransferase deficiency.	Yes	Deletion leads to increased stem cell differentiation (Hwang et al., 2016)

Cholesterol biosynthesis related	Fdft1 (Farnesyl-Diphosphate Farnesyltransferase 1)	This gene encodes a membrane-associated enzyme located at a branch point in the mevalonate pathway. The encoded protein is the first specific enzyme in cholesterol biosynthesis, catalyzing the dimerization of two molecules of farnesyl diphosphate in a two-step reaction to form squalene.	Yes	Deletion leads to reduced brain size (Coman et al., 2018)
	Hmgcr (3-Hydroxy-3-Methylglutaryl-CoA Reductase)	HMGCR (3-Hydroxy-3-Methylglutaryl-CoA Reductase) is a Protein Coding gene. Diseases associated with HMGCR include Cerebrotendinous Xanthomatosis and Familial Hyperlipidemia. Among its related pathways are Regulation of lipid metabolism by Peroxisome proliferator-activated receptor alpha (PPARalpha) and Statin Pathway.	Yes	Inhibition leads to neurogenesis (Robin et al., 2014)
	Dhcr7 (7-Dehydrocholesterol Reductase)	DHCR7 (7-Dehydrocholesterol Reductase) is a Protein Coding gene. Diseases associated with DHCR7 include Smith-Lemli-Opitz Syndrome and Holoprosencephaly. Among its related pathways are cholesterol biosynthesis I and Vitamin D Metabolism. Gene Ontology (GO) annotations related to this gene include oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor and 7-dehydrocholesterol reductase activity.	Yes	Causes Smith-Lemli-Opitz syndrome (Stelzer et al., 2016)
Cancer	Ptprz1 (Protein Tyrosine Phosphatase Receptor Type Z1)	This gene encodes a member of the receptor protein tyrosine phosphatase family. Expression of this gene is restricted to the central nervous system (CNS), and it may be involved in the regulation of specific developmental processes in the CNS.	Yes	Highly upregulated in glioblastoma (Fujikawa et al., 2017)

Beaudin, A. E., Abarinov, E. V, Noden, D. M., Perry, C. A., Chu, S., Stabler, S. P., ... Stover, P. J. (2011). Shmt1 and de novo thymidylate biosynthesis underlie folate-responsive neural tube defects in mice. *The American Journal of Clinical Nutrition*, 93(4), 789–798. <https://doi.org/10.3945/ajcn.110.002766>

Coman, D., Vissers, L. E. L. M., Riley, L. G., Kwint, M. P., Hauck, R., Koster, J., ... Pitt, J. (2018). Squalene Synthase Deficiency: Clinical, Biochemical, and Molecular Characterization of a Defect in Cholesterol Biosynthesis. *American Journal of Human Genetics*, 103(1), 125–130. <https://doi.org/10.1016/j.ajhg.2018.05.004>

Fawal, M.-A., Jungas, T., Kischel, A., Audouard, C., Iacovoni, J. S., & Davy, A. (2018). Cross Talk between One-Carbon Metabolism, Eph Signaling, and Histone Methylation Promotes Neural Stem Cell Differentiation. *Cell Reports*, 23(10), 2864-2873.e7. <https://doi.org/10.1016/J.CELREP.2018.05.005>

Fujikawa, A., Sugawara, H., Tanaka, T., Matsumoto, M., Kuboyama, K., Suzuki, R., ... Noda, M. (2017). Targeting PTPRZ inhibits stem cell-like properties and tumorigenicity in glioblastoma cells. *Scientific Reports*, 7(1), 5609. <https://doi.org/10.1038/s41598-017-05931-8>

Fukao, T., Yamaguchi, S., Orii, T., & Hashimoto, T. (1995). Molecular basis of β -ketothiolase deficiency: Mutations and polymorphisms in the human mitochondrial acetoacetyl-coenzyme a thiolase gene. *Human Mutation*, 5(2), 113–120. <https://doi.org/10.1002/humu.1380050203>

Hwang, I.-Y., Kwak, S., Lee, S., Kim, H., Lee, S. E., Kim, J.-H., ... Youn, H.-D. (2016). Psat1-Dependent Fluctuations in α -Ketoglutarate Affect the Timing of ESC Differentiation. *Cell Metabolism*, 24(3), 494–501. <https://doi.org/10.1016/j.cmet.2016.06.014>

- Knobloch, M., Pilz, G.-A., Ghesquière, B., Kovacs, W. J., Wegleiter, T., Moore, D. L., ... Jessberger, S. (2017). A Fatty Acid Oxidation-Dependent Metabolic Shift Regulates Adult Neural Stem Cell Activity. *Cell Reports*, 20(9), 2144–2155. <https://doi.org/10.1016/j.celrep.2017.08.029>
- Madhavan, L., Ourednik, V., & Ourednik, J. (2006). Increased “Vigilance” of Antioxidant Mechanisms in Neural Stem Cells Potentiates Their Capability to Resist Oxidative Stress. *Stem Cells*, 24(9), 2110–2119. <https://doi.org/10.1634/stemcells.2006-0018>
- O’Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., ... Pruitt, K. D. (2016). Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, 44(D1), D733–D745. <https://doi.org/10.1093/nar/gkv1189>
- Robin, N. C., Agoston, Z., Biechele, T. L., James, R. G., Berndt, J. D., & Moon, R. T. (2014). Simvastatin Promotes Adult Hippocampal Neurogenesis by Enhancing Wnt/ β -Catenin Signaling. *Stem Cell Reports*, 2(1), 9–17. <https://doi.org/10.1016/j.stemcr.2013.11.002>
- Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., ... Lancet, D. (2016). The GeneCards suite: From gene data mining to disease genome sequence analyses. *Current Protocols in Bioinformatics*, 2016(1), 1.30.1-1.30.33. <https://doi.org/10.1002/cpbi.5>
- Wang, X., Guan, Z., Dong, Y., Zhu, Z., Wang, J., & Niu, B. (2018). Inhibition of thymidylate synthase affects neural tube development in mice. *Reproductive Toxicology*, 76, 17–25. <https://doi.org/10.1016/j.reprotox.2017.12.007>
- Wu, J., Bao, Y., Lu, X., Wu, L., Zhang, T., Guo, J., & Yang, J. (2015). Polymorphisms in mthfd1 gene and susceptibility to neural tube defects: A case- control study in a chinese han population with relatively low folate levels. *Medical Science Monitor*, 21, 2630–2637. <https://doi.org/10.12659/MSM.895155>
- Wu, Z., Huang, K., Yu, J., Le, T., Namihira, M., Liu, Y., ... Fan, G. (2012). Dnmt3a regulates both proliferation and differentiation of mouse neural stem cells. *Journal of Neuroscience Research*, 90(10), 1883–1891. <https://doi.org/10.1002/jnr.23077>
- Yang, S.-Y., He, X.-Y., & Schulz, H. (2005). 3-Hydroxyacyl-CoA dehydrogenase and short chain 3-hydroxyacyl-CoA dehydrogenase in human health and disease. *FEBS Journal*, 272(19), 4874–4883. <https://doi.org/10.1111/j.1742-4658.2005.04911.x>
- Yoshida, K., Furuya, S., Osuka, S., Mitoma, J., Shinoda, Y., Watanabe, M., ... Hirabayashi, Y. (2003). Targeted Disruption of the Mouse 3-Phosphoglycerate Dehydrogenase Gene Causes Severe Neurodevelopmental Defects and Results in Embryonic Lethality*. <https://doi.org/10.1074/jbc.C300507200>