Transcriptional profiling defines unique subtypes of transit amplifying neural progenitors within the neonatal mouse subventricular zone

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Abstract

While significant progress has been made in understanding the heterogeneity of Neural Stem Cells (NSCs), our understanding of similar heterogeneity among the more abundant transit amplifying progenitors is lagging. Our work on the neural progenitors (NPs) of the neonatal subventricular zone (SVZ) began over a decade ago, when we used antibodies to the 4 antigens, CD133, LeX, CD140a, and NG2 to perform FACs to classify subsets of the neonatal SVZ as either multi-potential (MP1, MP2, MP3, MP4 and PFMPs), glial-restricted (GRP1, GRP2, and GRP3) or neuron-astrocyte restricted (BNAP). Using RNAseq, we have characterized the distinctive molecular fingerprints of 4 SVZ neural progenitors and compared their gene expression profiles to those of the NSCs. We performed bioinformatic analyses to provide insights into the unique interactome of each NP type and into the transcription factors regulating their development. Overall, we identified 1581 genes that were upregulated in at least one NP compared to the NSCs. Of these genes, 796 genes were upregulated in BNAP/GRP1 compared to NSCs; 653 in GRP2/MP3; 440 in GRP3; and 527 in PFMPs. One gene in particular that emerged from our analysis that can be used to distinguish the NPs from the NSCs is Etv1, also known as Er81. Also notable is that the neural stem cells downregulated cilia formation genes as they differentiated to become multipotential progenitors. Among the NPs, both PFMP and GRP3 subtypes differentially expressed genes related to neuron and oligodendrocyte development, including Matn4, Lhfpl3 and Olig2. GRP3s uniquely expressed Etv5, a transcription factor known to promote glial cell fate specification, while PFMPs uniquely expressed Lhx6, a transcription factor that regulates interneuron specification. PFMPs also expressed transcripts for olfactory receptors. Unlike the other NPs, the GRP1 and GRP2 NPs differentially expressed genes for proteins involved in immune function. The present work will serve as an important resource for investigators interested in further defining the transit amplifying progenitors of the mammalian SVZ.

Introduction

Mammalian neurodevelopment is a dynamic process that begins two weeks into pregnancy and progresses into adolescence. In early pregnancy, pyramidal neurons and astrocytes are generated from the ventricular zone (VZ). As neural development progresses, the VZ regresses and the subventricular zone (SVZ) expands to generate the bulk of the brain's interneurons and glia. There is increased interest in studying the SVZ as it remains active throughout the lifespan in rodents and through the first years of life in humans and can potentially be reprogrammed for neurogenesis to treat brain injury.

The subventricular zone of the adult has been extensively characterized. Neural stem cells (NSCs), often referred to as “Type B cells” (Doetsch, Garcia-Verdugo, and Alvarez-Buylla 1997; Doetsch et al. 1999), have ciliated processes that contact the cerebrospinal fluid, enabling them to respond to growth factors and cytokines that maintain their primitive state (Liu et al. 2021). NSCs arise in the SVZ during mid-embryonic development (Fuentealba et al. 2015) and divide symmetrically (Obernier et al. 2018) to produce intermediate progenitors (Transit Amplifying Progenitors; TAPs)(Ponti et al. 2013), originally called "Type C cells", which then become lineage-restricted to form terminally differentiated cells (Ponti et al. 2013). SVZ-born TAPs predominantly give rise to interneurons in the olfactory bulb and striatum, as well as to astrocytes and oligodendrocytes in the cortex, striatum, and white matter (Young et al. 2007; Merkle et al. 2004; Menn et al. 2006).

Increasingly, there is interest in characterizing the NSCs and TAPS of the fetal and neonatal brain, as SVZ-born progenitors are critical for proper neural development. Notably, the net number of neurons in the cerebral cortex doubles during the first two weeks of rodent postnatal life and the majority of glial cells are born postnatally(Bandeira, Lent, and Herculano-Houzel 2009; Lyck, Kroigard, and Finsen 2007). Subsequently, current evidence indicates that deficits in NSC proliferation and differentiation represent the major convergence point for neurodevelopmental disorders (NDDs), and mutations in genes that play a role in this process are implicated in the majority of NDDs(Ernst 2016; Xu et al. 2023). Correspondingly, evidence indicates that postnatal neurogenesis is profoundly impaired in NDDs, and manipulating neurogenesis can ameliorate phenotypes(Sheu et al. 2019).

There has been much debate in the neuroscience community about the definition of a “stem cell” vs an “intermediate progenitor”(Velloso, Shankar, et al. 2022). Many studies have characterized cells as NSCs based on their ability to form neurospheres or because they are tripotential, which is entirely inadequate given that many intermediate progenitors form neurospheres and are tripotential. However, unlike NSCs, intermediate progenitor cells do not exhibit self-renewal after several generations(Moore et al. 2014).

Therefore, understanding the differences between NSCs and the variety of neural progenitor subtypes is crucial to understand the transitions that occur during neurodevelopment.

Previous studies that aimed to investigate the diversity of cells generated postnatally in the SVZ have used scRNAseq on unsorted (Zywitza et al. 2018; Mizrak et al. 2019; Mizrak et al. 2020) and FACS-sorted cells(Dulken et al. 2017; Beckervordersandforth et al. 2010), and they used bioinformatics to classify the cells as NSCs, epithelial, neuronal, glial and immune lineages. Although these seminal studies describe some subtypes of TAPs, the emphasis of their analyses was to characterize the NSC subpopulations and terminally differentiated cells. Moreover, these studies classified adult SVZ cells where there variety of cell types being produced is restricted. Thus, the diversity and function of TAPs during early postnatal neurodevelopment in mice remain largely uncharacterized.

In 2007, David Panchision and co-workers combined antibodies against CD133, CD15, CD24, A2B5 and PSA-NCAM, to identify and enrich for 4 sets of neural precursors from the E13.5 and P2 mouse VZ/SVZ. They established that there were multipotent progenitors that could produce neurons, astrocytes and oligodendrocytes, that there were progenitors that were bipotential and produced either neurons and oligodendrocytes, or astrocytes and oligodendrocytes, and that there were progenitors that only produced neurons(Panchision et al. 2007a). Extending Panchision's studies, we combined CD133 and LeX and then added two intermediate progenitor antigens, CD140a and NG2(Hall, Giese, and Richardson 1996; Stallcup and Beasley 1987). With this strategy, 8 phenotypically defined subsets of neural progenitors could be identified within the periventricular region(Buono et al. 2012). To determine the developmental potentials of these 8 subpopulations, SVZ neurospheres were generated, dissociated into a single cell suspension, separated by FACS, plated onto laminin-coated chamber slides at low density and expanded with growth factors. The multipotential progenitors (MPs), included the NSCs, MP1, MP2, MP3, MP4 and the platelet-derived growth factor-fibroblast growth factor responsive MP cell (PFMP). There were 4 types of bipotential progenitors identified that included the bipotential neuronal-astrocytic progenitor (BNAP) and 3 glial-restricted progenitors (GRP1, GRP2 and GRP3). Building upon that work, here we have isolated the four largest populations of progenitors (BNAP/GRP1, GRP2/MP3, GRP3, and PFMP) using FACS and then subjected those cells to RNA sequencing. This approach allows us to analyze the transcriptome of early postnatal SVZ progenitors characterized by established antigenic profiles. In doing this, we can illuminate the stages of neurodevelopment from NSCs to mature neurons and macroglia.

Methods

Mice

Swiss Webster Mice from timed-pregnant females purchased from Charles River laboratories or generated in house were used for all studies. Each litter was analyzed collectively with both males and females. All experiments were performed in accordance with Protocol #999901108 approved by the IACUC committee of Rutgers University Biomedical Health Sciences.

SVZ Cell Dissociation

Mice were terminated via decapitation at postnatal day 5. The periventricular zone was micro-dissected from coronal sections on ice and into Flow Buffer (DMEM/F12 Media with 20mM HEPES, 1% BSA, and 1mM EDTA, pH 7.4). All pups from each litter were pooled for further experiments. Cells were minced then incubated in an enzyme solution (DMEM/F12 with 0.1Wu Liberase-DH, 10ug/mL DNase I, and 20mM HEPES) for 15 minutes with rocking at 37°C. 1mL FBS and 4mL of Flow Buffer was added and cells were centrifuged for 5 minutes at 300g. Cells were manually triturated using a P1000 tip until no visible debris remained, triturated using a 200uL pipette tip, and filtered through a 30 micron cell filter. Cells were then washed in a polystyrene tube to remove dead cells and debris.

Flow Cytometry and Cell Sorting

Flow cytometry was performed as described previously by our group (Buono et al. 2015b; Buono et al. 2012; Velloso, Kumari, et al. 2022a) using the following antibodies: Lewis-X (1/20; BD Biosciences), CD133-APC (1/50; eBioscience), CD140a (1/400; BioLegend), and NG2 chondroitin sulfate proteoglycan (1/50; Millipore). Goat anti-rabbit IgG Alexa Fluor 700 (1/100; Invitrogen) was used for NG2 and DAPI (1/50,000) was used for dead cell exclusion. Cells were collected into polypropylene tubes of lysis buffer using the FACS BD Aria II.

RNA Sequencing

RNA was isolated from SVZ neural progenitors (NP) using NucleoSpin® RNA XS from Macherey Nagel (Germany) according to the manufacturer instructions. The integrity of the RNA was analyzed on Bioanalyzer 2100 using a RNA Pico6000 Kit (Agilent Technologies). All RNA samples used in this study had an RNA integrity number (RIN) >7.0. PolyA plus RNAs were amplified and enriched using Ambion's messageAmp II aRNA amplification kit. The quality of amplified aRNA was verified using the Bioanalyzer. A cDNA library was constructed using Ilumina TrueSeq protocol.

The barcoded cDNA library was purified with AmpureXP beads and quantified using Qubit High sensitivity DNA kit and the quality of the library was determined by analyzing

on Agilent bioanalyzer. Libraries mixed at equimolar ratios were sequenced on a NextSeq500 (Illumina, San Diego, CA) for 75 cycles.

RNASeq Analysis

Data was processed using nf-core/rnaseq v3.10 (doi: https://doi.org/10.5281/zenodo.1400710) of the nf-core collection of workflows (Ewels et al. 2020) and executed with nextflow v22.10.11. Briefly, raw transcriptome reads were assessed for quality control (FASTQC v0.11.9) and trimmed for quality and adapter contaminant (cutadapt v3.4). Trimmed reads were aligned to the Mus musculus genome GRCm38 using STAR (v2.6.1), followed by transcript abundance calculation via Salmon (v1.9.0). 2 experiments were excluded because samples had low quality, and 4 experiments were analyzed further. Hit count normalization and differential gene expression group cross-comparisons were performed using DESeq2 (v1.26.0). Significant differentially expressed gene thresholds were set at FDR adjusted $p < 0.05$. Up- and down-regulated gene lists were generated for each NP subtype and composed of all genes with differential expression compared to NSCs ($p < 0.05$ adjusted). Each gene list was analyzed using Network Analyst to identify protein-protein interactions and transcription factor networks. Overrepresentation analysis was performed using the clusterProfiler package in R (version 4.16.0) to identify up- and down-regulated gene ontology terms in each population compared to NSCs.

Results

The cells of the subventricular zone differentially express surface antigens that identify unique subpopulations of neural progenitors.

Cells from the mouse P5 periventricular zone were dissociated using Liberase and manual trituration. This approach preserves surface antigens that are removed using other enzymes (Panchision et al. 2007b; Tsuji et al. 2017). The cells were stained for CD133/Prominin1, CD15/Lex/heat-stable antigen, CD140a/PDGFRα, and NG2/Chondroitin Sulfate and FACS-sorted according to antigenic profiles into five unique populations as described below. Single cells were identified using forward vs side-scatter to exclude debris and doublets, as shown in Figures 1A-1C. Dead cells were excluded using DAPI staining (Figure 1D). Live cells were then gated first according to CD140a and NG2 expression (Figure 1E) followed by a secondary gate of CD133 and CD15 (Figure 1F-1H). Neural Stem cells (NSCs), BNAP/GRP1s, GRP2/MP3s, GRP3s, and PFMPs were sorted according to antigen expression as described previously (Buono et al. 2012; Velloso, Kumari, et al. 2022a) and collected in lysis buffer for RNA extraction and further analysis.

Neural Progenitor populations have unique gene expression.

RNA sequencing was performed on each of the four cell populations, as well as on the NSCs. As each of the intermediate progenitor cells is a descendant of the NSCs, we sought to understand the genetic differences between each progenitor subtype and NSCs to understand the steps that occur in development. Therefore, subsequent analysis compared gene expression changes between NCSs and each progenitor cell type.

Overall, we identified 4647 genes with differential expression between at least one neural progenitor (NP) subtype and NSCs, of which 1581 are upregulated in at least one NP (Figure 2A), 3079 are downregulated (Figure 2B), and 13 have variable directionality between populations. 796 genes were upregulated in BNAP/GRP1 compared to NSCs; 653 in GRP2/MP3; 440 in GRP3; and 527 in PFMPs (Figure 2A). 1390 genes were downregulated in BNAP/GRP1 compared to NSCs; 1759 in GRP2/MP3; 1588 in GRP3; and 2149 in PFMPs (Figure 2B).

Surface markers that define NP populations correlate with gene expression.

As expected by surface antigen expression, expression of the NSC marker *Prom1* was down-regulated in all progenitor cell populations ($p < 0.01$ for all) compared to NSCs. Similarly, *Pdgfra* was upregulated in GRP3 and PFMP ($p = 6.5E-9$ and $p=6.5E-8$, respectively) compared to NSCs, but *Pdgfra* was not increased in BNAP/GRP1 and GRP2/MP3 ($p = 0.27$ and $p =1$) compared to NSCs. Interestingly, *Cspg4* expression was upregulated in GRP3 and PFMP compared to NSCs (GRP3 Fold Change: 9.64, p =

4.2E-3; PFMP Fold Change: 8.37, p = 6.4E-3) but not in BNAP/GRP1 and GRP2/MP3 (BNAP/GRP1 Fold Change: 0.90, p = 1; GRP2/MP3 Fold Change: 2.23, p = 0.78).

Each neural progenitor population upregulates unique and shared genes compared to neural stem cells.

Each NP population expressed, on average, 235 (100-366) uniquely upregulated genes compared to NSCs (Fig. 2A). The top 3 upregulated genes in each population were Igkv4-56 (fold change vs NSCs of 19.8), Cryba4 (fold change vs NSCs 19.2), and Gpr34 (fold change vs NSCs 18.7) for BNAP/GRP1; Gpr34 (fold change vs NSCs of 35.8), Gm29291 (fold change vs NSCs 32.2), and Higd1b (fold change vs NSCs 30.5) for GRP2/MP3; Lhfpl3 (fold change vs NSCs of 72.3), Dmrtb1 (fold change vs NSCs 45.9), and Matn4 (fold change vs NSCs 42.0) for GRP3; and Matn4 (fold change vs NSCs of 49.8), Tmem255b (fold change vs NSCs 49.4), and Brinp3 (fold change vs NSCs 46.7) for PFMPs (Fig. 2C-F).

BNAP/GRP1 progenitors share more genetic similarities with GRP2/MP3, while GRP3s are more similar to PFMPs.

There was significant overlap between upregulated genes vs NCSs in the progenitor populations, particularly between BNAP/GRP1 and GRP2/MP3 and between GRP3 and PFMP (Fig.2A). 16 of the top 50 upregulated genes were shared between BNAP/GRP1 and GRP2/MP3. For GRP3 and PFMP, 29 of the top 50 genes were shared. Overall, 237 upregulated genes were shared between BNAP/GRP1 and GRP2 and 157 between GRP3 and PFMP, while all other combinations of two or three NPs share <= 38 commonly upregulated genes. 51 genes were commonly upregulated in all NPs vs NSCs, of which the highest magnitude were Igf2bp3 (8.3x in BNAP/GRP1, 11.4x in GRP2/MP3, 8.7x in GRP3, and 3.9x in PFMP); Gpr155 (6.7x in BNAP/GRP1, 5.4x in GRP2/MP3, 4.5x in GRP3, and 4.5x in PFMP); and Gm3383 (5.4x in BNAP/GRP1, 5.9x in GRP2/MP3, 5.7x in GRP3, and 6.2x in PFMP).

Greater overlap was seen with downregulated genes, as 811 genes were downregulated in all four populations compared to NSCs (Fig. 2B). Of these, the highest magnitude were 1700012B09Rik (652.4x lower in BNAP/GRP1 than NSCs, 567.3x in GRP2/MP3, 894.3x in GRP3, and 983.3x in PFMP); Ccdc113 (829.1x lower in BNAP/GRP1 than NSCs, 372.9x in GRP2/MP3, 1041.8x in GRP3, and 661.1x in PFMP); and Fam183b (1058.7.1x lower in BNAP/GRP1 than NSCs, 366.5x in GRP2/MP3, 405.3x in GRP3, and 434.7x in PFMP). As with upregulated genes, the highest overlap between populations was seen between BNAP/GRP1 and GRP2/MP3 and between GRP3 and PFMP.

Each neural progenitor population expresses unique upregulated and downregulated genes.

Each of the NP populations expressed unique upregulated genes compared to neural stem cells. The greatest number was seen in BNAP/GRP1 with 366 uniquely upregulated genes while GRP2/MP3, GRP3, and PFMP had 270, 100, and 206 respectively (Figure 2A). The greatest number of uniquely downregulated genes was seen in PFMPs, with 697 genes uniquely downregulated compared to NSCs. BNAP/GRP1, GRP2/MP3, and GRP3 had 160, 300, and 153 unique downregulated genes respectively compared to NSCs. The top ten uniquely upregulated genes by magnitude for each population are shown in Figure 2B-E (BNAP/GRP1, GRP2/MP3, GRP3, and PFMP, respectively), and the 50 highest magnitude uniquely upregulated genes were plotted in the heatmap in Figure 2F.

Each neural progenitor has a unique signature of up and down-regulated transcription factors.

We were interested in analyzing differences in the expression of transcription factors and were particularly interested in identifying “hub” genes that would explain differences in gene expression between the NPs. Therefore, we compared the up and downregulated genes in each population to curated lists of mouse transcription factors (TFs) in AnimalTFDB3.0. We observed 21 transcription factors that were downregulated in all 4 NP populations compared to NSCs (top 10 listed in Table 1), including Foxj1, Zfp474, and Trp73. Each progenitor subtype had unique up- and down-regulated TFs, listed in Tables 2 and 3. Interestingly, no transcription factor was upregulated in all 4 NPs compared to NSCs. The only gene close to reaching significance in all four progenitors was Etv1, which was significantly upregulated in GRP2/MP3, GRP3, and PFMP compared to NSCs but just failed to reach significance for BNAP/GRP1 (p adjusted = 0.056).

Neural progenitor gene expression profiles indicate unique biological functions in neurodevelopment.

Neural stem cells downregulate expression of genes involved in cilia organization as they mature into neural progenitors.

We further analyzed the expression profiles in each population using over representation analysis which compared our gene lists to recognized Gene Ontology genesets. In each of the four NP populations, all 10 of the GO terms that were downregulated compared to NSCs were involved in ciliated cell function. The GO Term “Cilia Organization” (GO:0044782) was most significantly enriched with Fold Enrichment of NSCs compared to BNAP/GRP1 of 9.4-fold, 8.6 for GRP2/MP3, 9.2 for GRP3, 7.4 for PFMP) and the other 9 of the top-10 enriched GO terms had “Cilia Organization” as a parent term (Figure 3A).

Volcano plots of each cell type vs NSCs (Figures 3B-E for BNAP/GRP1, GRP2/MP3, GRP3, and PFMP, respectively) with cilia-related genes highlighted in purple clearly show that these genes are overrepresented in the downregulated genes in all four progenitor subtypes and make up some of the most statistically significant and high-magnitude downregulated genes. The genes with the largest change in this group were Ccdc113 (829.1-fold downregulation in BNAP/GRP1 compared to NSC, 372.9x in GRP2/MP3, 1041.7x in GRP3, and 661.1x in PFMP); Foxj1 (163.0x in BNAP/GRP1, 162.4x in GRP2/MP3, 141.4x in GRP3, and 620.5x in PFMP); and Tekt4 (54.3x in BNAP/GRP1, 315.7x in GRP2/MP3, 82.2x in GRP3, and 614.3x in PFMP).

BNAP/GRP1 and GRP2/MP3 express many immune-related genes and pathways.

The top GO terms associated with upregulated genes in BNAP/GRP1 and GRP2/MP3 emphasized immune signaling (GO:0002376, p =5.63e-4 for BNAP/GRP1 and p = 1.26e-15 for GRP2/MP3) (Figure 4A,B). In addition to Gene Ontology Terms, we also analyzed expression profiles using NetworkAnalyst to create upregulated protein-protein interaction networks by comparing upregulated genes in each NP population to known protein-protein interactions in the IMEx interactome. For both BNAP/GRP1 and GRP2/MP3, Sfpi1 and TNF formed central hubs of protein interactions. In GRP2/MP3, additional primary hubs included Irf8, Plcg2 and Syk (Figure 4A,B), which are uniquely upregulated in GRP2/MP3s).

GRP3 and PFMP upregulate genes related to neurogenesis and gliogenesis.

While interactome networks were smaller in GRP3 and PMFP than in BNAP/GRP1 and GRP2/MP3, the interactions highlighted are known to be involved in neurogenesis (Figure 4C,D), as reflected in the upregulated GO Terms that were related to neurogenesis and gliogenesis in both populations. The shared network (GRP3 Network and PFMP interactome Network 1; Figures 4G and 4I) include genes involved in cell fate commitment (GO:0045165, GRP3: p= 4.83e-3. PFMP: p= 5.85e-4), regulation of neurogenesis (GO:0050767, GRP3 p=2.56e-6, PFMP p=5.08e-3), and gliogenesis (GO:0042063. GRP3 p=2.81e-3, PFMP 5.21e-3). PFMPs also had an additional upregulated network (Figure 4J) involved in signaling pathways including import of proteins into the nucleus (GO:0006606, p = 0.023), cell differentiation (GO:0045595, p = 0.023), and cell proliferation (GO:0008283, p = 0.031).

Transcription factor analysis reveals common and unique hubs between neural progenitor subtypes.

Upregulated genes were analyzed to identify transcription factors likely driving differentiation using Network Analyst software and the TRRUST database. Primary networks were pruned by including only transcription factors with Betweenness

Centrality > 0.05 and results are shown in Figure 5. This analysis revealed 7 central transcription factors in BNAP/GRP1, 9 in GRP2/MP3, 6 in GRP3, and 5 in PFMP. Of these, only Sp1 was common to all populations. Twist1 was a hub transcription factor in BNAP/GRP1, GRP3 and PFMP but not in GRP2/MP3. Jun and Nfkb1 were expressed in BNAP/GRP1, GRP2/MP3, and GRP3 but not PFMP. Ets1 and Trp53 were expressed only in the BNAP/GRP1 and GRP2/MP3 networks while Ctnnb1b and Stat3 were expressed in the GRP3 and PFMP networks. E2f1 was unique to PFMPs, while GRP2/MP3s had four unique transcription factors: Gata4, Ppara, Cebpb, Foxo1.

Discussion

In this study, we characterized four categories of SVZ resident NPs. In comparing their gene expression profiles to those of the NSCs, we have begun to discern molecular changes that occur as cells change their identity from stem cells to early progenitors. Moreover, we have identified transcription factors that are uniquely expressed by particular NPs. We have performed bioinformatic analyses to provide insights into the transcription factor interactions that are likely regulating their development, as well as the functional consequences of these differences in gene expression.

While significant progress has been made in understanding genetic signatures of NSCs, our understanding of the heterogeneity among the more abundant transit amplifying progenitors is lagging. Thus, a major gap that this study addresses is to provide more granular information about the transcriptional profiles of the intermediate progenitors that reside in the neonatal SVZ. Over the past decade, we have characterized seven NP subpopulations based on expression of CD133, LeX, CD140a, and NG2 (Buono et al. 2015a; Buono et al. 2012; Velloso, Kumari, et al. 2022b). The present study expands our understanding of the molecular differences between these different subsets of NPs by including a comprehensive transcriptomic analysis of the individual NPs. Overall, we identified 1581 genes that were upregulated in at least one NP compared to the NSCs (Figure 2A). Of these, 796 genes were upregulated in BNAP/GRP1 compared to NSCs; 653 in GRP2/MP3; 440 in GRP3; 527 in PFMPs (Figure 2A). We also identified 51 genes that were upregulated in all NP populations compared to NSCs, the most significant of which was Igf2bp3. Igf2bp3 is involved in the proliferation of NSCs and progenitors(Samuels et al. 2020), which is consistent with progenitors that are rapidly dividing, in contrast with NSCs that are largely quiescent.

Shared TFs are expressed across NP subtypes.

We specifically examined differentially expressed transcription factors (TFs) and found that there were 21 transcription factors downregulated as NSCs become NPs. Many of these, including Foxj1 and Rfx2, function during ciliogenesis, so their loss is consistent with the loss of the NSC's cilia during differentiation. Meanwhile, other TFs such as Emx1, Id4, and HopX are known markers of NSCs and function to maintain a quiescent state.

While there were no transcription factors upregulated in all NPs, Etv1 was significant in three of the four NPs, with a very strong trend in BNAP/GRP1 ($p = 0.056$ adjusted). It is, therefore, possible that Etv1 activation is a hallmark of a neural stem cell’s differentiation into a neural progenitor. Etv1, also referred to as ER81, is a member of the Pea3 family of transcription factors and thus is involved in a wide variety of

developmental processes(Willardsen et al. 2014). It is upregulated by FGF signaling in the developing brain, as downregulation of FGF signaling significantly decreases Etv1 expression in neural development(Hasegawa et al. 2004).

In mouse embryos, Etv1 is expressed in cells of the Medial Ganglionic Eminence (MGE) and that become a subpopulation of striatal neurons. Other ER81+ cells are found in the dorsal-most Lateral ganglionic Eminence (LGE) and likely go on to form Etv1+ olfactory bulb neurons(Stenman, Toresson, and Campbell 2003). In postnatal and adult mice, Etv1 expression continues in the SVZ, rostral migratory stream, and in a layer-specific manner in the olfactory bulb (Shu et al. 2010; Saino-Saito et al. 2007) as well as in some cortical interneurons(Dehorter et al. 2015). However, infusion of AraC, which kills dividing cells but spares quiescent NSCs, eliminates Etv1 expression, which aligns with the observations in this study that it is expressed in the NPs and not the NSCs(Kohwi 2006). The function of ETV1 in the SVZ is unclear, but Etv1 is necessary for neuronal maturation in the Xenopus retina(Willardsen et al. 2014), cerebellum(Abe, Okazawa, and Nakanishi 2011; Ding et al. 2016), and spinal cord (Arber et al. 2000; Hippenmeyer et al. 2005) and for modulating neuronal activity in the striatum and cortex(Dehorter et al. 2015; Ahmed et al. 2021). The signaling pathways activated by ETV1 in NPs of the SVZ are also unknown.

NPs express unique transcription factors.

We found that NP subpopulations express unique TFs in the neonatal SVZ. In BNAP/GRP1s, the top transcription factors with unique upregulation were Zfp57, Hoxac6 and Zfp955a. Zfp57 is enriched in the SVZ and rostral migratory stream of the embryo and postnatal mouse (Ling et al. 2009) and downregulated in adulthood(Girard, Eichenberger, and Celio 2014). Zfp57 is a zinc finger transcriptional repressor that maintains DNA methylation, particularly at imprinting control regions during early development; however, its function in the SVZ during neural development is not known. Hoxac6 is generally considered a marker for cervical spinal cord neurons(Zeisel et al. 2018), and there is no prior evidence for its expression or function in the developing brain. Hoxc6 has also been shown to bind to and regulate the expression of NCAM(Jones et al. 1993), potentially implicating it in SVZ cell migration. In gliomas, Hoxc6 is highly expressed and associated with poor prognosis. Blocking Hoxc6 in a U87 cell line caused cell cycle arrest at G0/G1 and apoptosis by increasing WIF-1, an inhibitor of Wnt activation(Yan et al. 2018). Given the roles of Wnt in neural development(Adachi et al. 2007; Ille and Sommer 2005; Yu et al. 2006), Hoxc6 may act via the Wnt pathway to promote proliferation or alter the developmental fate of BNAP/GRP1 cells. Zfp955a is increased in neurons after seizure(Soon et al. 2023), but its expression in the developing brain has not been characterized. Of particular note, Neurod1 also is uniquely upregulated in BNAP/GRP1s but unchanged in the other populations. Interestingly, in the presence of Neurod1 signaling, NG2+ cells—all of the

progenitor populations in this study— are induced to form interneurons(Wei et al. 2023). This may represent a foundational signal for neurogenesis in the BNAP group, which pushes them towards a neuronal fate.

The top transcription factors with unique upregulation in GRP2/MP3 cells were Tbx2, Lyl1, and HeyL. Tbx2 is a member of the T-box family of transcription factors, which are known to play important roles in development and in situ hybridization studies have shown that Tbx2 is expressed in the P4 SVZ (Atlas 2006). While other T Box genes have been studied in the context of neural development, information on Tbx2 in this context is lacking. In the hypothalamus, Tbx2 has been shown to transcriptionally repress Shh, leading to increased differentiation and proliferation of a subset of hypothalamic NPs(Manning et al. 2006). Tbx2 also has been implicated in various cancers as a pro-proliferative factor and may play a similar role in GRP2/MP3(Decaesteker et al. 2018; Liu et al. 2019; McIntyre et al. 2022). Lyl1 is typically associated with hematopoietic stem cells and erythropoiesis (Souroullas et al. 2009) and also has been noted to have a role in the development of microglia(Wang et al. 2021). While there is no known role for Lyl1 in NPs, its expression does peak in the brain at P4, the period studied in this work, and the peak of SVZ cell output(Science 2004). HeyL is a known pro-neural transcription factor. When Jalali and colleagues grew NPs in culture, increasing Hey1 expression using a retroviral vector increased the proportion of neurons and decreased the proportion of astrocytes generated, while depleting HeyL reduced the numbers of neurons produced while promoting astrogliogenesis. Similarly, increasing Hey1 expression with a retroviral vector in E10.5 mouse embryos increased the proportions of neurons and decreased the proportion of astrocytes(Jalali et al. 2011).

The top transcription factors that were uniquely upregulated in GRP3s are Zfp157, Sox12 and Etv5. Zfp157 is expressed in the brain, but its role in neural development has not been established(Oliver, Nichols, and Watson 2013). Sox12 is a member of the SoxC subgroup of Sox genes along with Sox4 and Sox11. However, unlike Sox4 and Sox11, Sox12 null mice have no detectable phenotype(Hoser et al. 2008). Of note, past studies did not examine neural development nor behavior in these mice but it was noted that Sox12 null mice were grossly normal. It has been speculated that Sox12 may modulate the other members of the SoxC family, which, when induced in the neural tube of chicks, leads to expression of pro-neuronal markers(Bergsland et al. 2006).

Etv5 (also known as Erm) has been extensively studied for its role in neural development and has emerged as a crucial regulator of gliogenesis. In 2013, Li and colleagues showed that mice with a MEK deletion in NPs ($Mek1^{fl/fl}Mek2^{-/-}$NesCre) had significant impairments in gliogenesis while neurogenesis was preserved or increased(Li et al. 2012). The cells also showed a lack of response to CNTF (an astrocyte-stimulating signal) when differentiated *in vitro*. However, when they

overexpressed Etv5 by electroporation into dorsal cortical radial progenitors it dramatically increased gliogenesis. Furthermore, while MEK overexpression increased gliogenesis, this effect was ameliorated by a dominant negative Etv5, indicating that MEK signaling relies on Etv5 to promote gliogenesis. Liu et al., 2019 confirmed that Etv5 knockout in NSCs *in vitro* decreased self-renewal and glial-associated genes while promoting neuronal genes, and that this corresponded to the production of more neurons and fewer glia(Liu and Zhang 2019). They showed that this occurred due to Etv5 interacting with CoRest to bind to the promoter of Neurog2, a well-known pro-neuronal factor, and act as a repressor of Neurog2 transcription, thereby promoting a glial as opposed to a neuronal cell fate. This is particularly significant as GRP3 is the only population in this study that only produces glia, as the other two glial-restricted cell types are mixed with bipotential neuron-astrocyte or multipotential cells (BNAP/GRP1 and GRP2/MP3). Given its unique expression in GRP3s compared to all other cell types and the known correlation between ETV5 and gliogenesis, it is likely that this gene promotes the unique cell fate of the GRP3 subgroup.

The top transcription factors with unique upregulation in PFMPs are Rxrg, Glis1 and Lhx6. Retinoic acid receptors are involved in signaling throughout neural development. RXRγ is in the RXR family of receptors, which form dimers with RARs to affect gene expression (Kastner et al. 1997) and regulate various stages of neurogenesis and neural development, particularly promoting the differentiation of progenitors into neurons(Molotkova, Molotkov, and Duester 2007; Janesick, Wu, and Blumberg 2015). Rxrg is expressed at lower levels than Rxra and Rxrb in neurospheres(Wang, Zhang, and Parent 2005), and, therefore, its function in development is less studied. While Rxrg mutants are viable and grossly normal, they do show impaired long-term depression in the hippocampus(Krezel et al. 1996; Chiang et al. 1998). Rxrg is best studied for its role in oligodendrogliogenesis. NSCs isolated from Rxrg-null mice have impaired differentiation into oligodendrocytes and myelination. Instead of maturing, Rxrg-null cells remain in a primitive OPC-type state and they fail to exit the cell cycle(Baldassarro et al. 2019; de la Fuente et al. 2015).

Of the other two TFs upregulated in the PFMPs, Glis1 is a well-known transcription factor included in a panel to convert somatic cells to iPSCs(Maekawa et al. 2011). It may act by stimulating proliferation genes (Mycn and Mycl) and other genes involved in development, including the Wnt and Met pathways(Scoville, Kang, and Jetten 2017). Lhx6 is a strong predictor of interneuron fate during neural development(Liodis et al. 2007; Vogt et al. 2014). In the Lhx6 null mutants, the number of interneurons (GABA+) is normal, but their distribution is altered, with cells migrating preferentially to the upper or deeper layers while absent from the middle cerebral layers. Interneuron subpopulations, including PV, SST, and CR, are also reduced, indicating a role for Lhx6 in interneuron specification.(Liodis et al. 2007).

Protein Interaction Networks and Gene Ontology

GRP1 and GRP2s express immune signature genes.

Interestingly, the over-representation analysis revealed that the GRP1 and GRP2 NPs expressed many genes for proteins involved in immune cell function. This is not surprising, since many cytokines and their receptors, complement proteins and their receptors, and MHC molecules are expressed widely throughout the brain where they regulate cell proliferation, differentiation, migration, and synaptogenesis(Garay and McAllister 2010; Stolp 2013; Tran et al. 2007). Cytokines and chemokines also are well known regulators of neurogenesis and gliogenesis (Veerasammy et al. 2017). The expression of receptors for immune signaling molecules likely allow the BNAP/GRP1 and GRP2/MP3 cell groups to interact with cells in their milieu, notably microglia (Tanabe and Yamashita 2018). In addition to immune pathways, GRP1 and GRP2 NPs had upregulations in pathways related to gliogenesis ($p < 0.0001$ for both GRP1 and GRP2) and glial migration ($p < 0.0002$ for both GRP1 and GRP2), though many genes upregulated in these datasets are immune genes, including Cx3cr1, Ccl2, and Tnf.

Both GRP1 and GRP2 expressed Spfi1 (also known as Spi1) as a central hub in their protein-interaction networks, with GRP2 also having Irf8, Plcg2 and Syk as highly connected genes. Spi1, Irf8, and Syk are highly expressed in hematopoietic tissues and in microglial cells(Mócsai, Ruland, and Tybulewicz 2010; Wang et al. 2019; Kierdorf et al. 2013). Spi1 also is highly expressed in the SVZ, particularly during the period studied (Science 2004). However, data on their functions in NSCs or NPs are limited. Thus, this remains an area for further study. Plcg2, which was expressed in GRP2 cells, encodes phospholipase C gamma, a major intracellular signaling molecule, Plcg2 expression is increased by activation of the MEF2 in NSCs(Chan et al. 2015), but its role in SVZ neurogenesis or gliogenesis has not been explored. Tumor Necrosis Factor, which is highly expressed in both GRP1 and GRP2s (Figure 4), is known to be produced by NPs, where it is involved in cell survival and differentiation (Covacu et al. 2009; Lan et al. 2012; Liu, Lin, and Tzeng 2005).

GRP3 and PFMP express gene networks related to neurogenesis.

One protein pathway that is shared between GRP3 and PFMP cell types (Figure 4G and 4I), consists of Ascl1, Olig2, Sox8 and Tcf12. All of these genes are known to be involved in neurogenesis and gliogenesis (Wedel et al. 2020; Uittenbogaard and Chiaramello 2002; Wang et al. 2014). While Olig2 and Sox8 are typically associated with oligodendrogliogenesis (Wedel et al. 2020; Freudenstein et al. 2023), they also are involved in neurogenesis(Miyoshi et al. 2007; Merchan-Sala et al. 2017), astrogliogenesis (Cai et al. 2007; Tatsumi et al. 2018; Takouda et al. 2021),and the regulation of NP proliferation(Sun et al. 2011). Interestingly, del Águila et al., 2022 studied an Olig2-expressing subpopulation of cells in the SVZ in adolescent (P14) and

adult mice, likely a similar population to GRP3 and PFMPs, which we have previously shown to persist in the SVZ across the lifespan(Moore et al. 2013). Using a lineage tracing in Olig2-CreER mice, del Águila and colleagues showed that Olig2+ cells generate neurons in the adult olfactory bulb and that deleting Olig2 changes the proportion of neurons generated, specifically decreasing the genesis of calretinin+ interneurons(Del Águila et al. 2022). However, del Águila 2022 did not examine gliogenesis, a process that continues in the SVZ throughout the lifespan, and we know from other fate mapping studies that Olig2+ cells produce glia under physiological conditions as well as some glutamatergic neurons as well(Ono et al. 2008). Notably, the fate mapping was conducted in adult mice, so it is important to keep in mind the differences between the neonatal and adult brain, as the immature brain has much greater developmental potential.

The second network, expressed exclusively in PFMPs (Figure 4J), consists of signaling components that are less well-studied in the context of neurogenesis but likely play a role in the proliferation and migration of immature cells. For example, Ccnd1 (CyclinD1) is a known cell cycle inhibitor (Lange, Huttner, and Calegari 2009) and regulator of the balance between progenitor proliferation, migration, and neurogenesis(Lange, Huttner, and Calegari 2009; Lukaszewicz and Anderson 2011). Fyn regulates migration of NPs by reducing their cell-cell adhesion, potentially allowing the PFMP cells to leave the SVZ and migrate through the RMS or through the neocortex(Fujikake et al. 2018). Meanwhile, the expression of Pik3r1 and Prkcq, which interact to activate PIK3CA(Dahlin et al. 2018), is a major growth signaling pathway throughout the body and brain. In fact, deleting PIK3CA leads to smaller brain size in vivo and fewer neurospheres in vitro(Xie et al. 2021), indicating an important role in SVZ cell division. Taken altogether, this network likely promotes the proliferation and migration of the PFMP progenitor subtype.

Transcription Factor Networks

Our analysis of transcription factors (Figure 5) revealed a significant overlap between the transcription factors most likely to be influencing gene expression changes in the NP populations. If this is true, it's interesting because it indicates that the same transcription factors may have differential effects in NPs based on the cell type. The only TF present in all 4 transcription factor networks is Sp1, which is known to modulate neurogenesis by controlling both proliferation (Pauklin et al. 2016) and differentiation into neurons and glia(Billon et al. 1999; Mondanizadeh et al. 2015; Yeo et al. 2013; Cao et al. 2020; Yin, Lázaro, and Wang 2023). However, the genes downstream of Sp1 differed between all four NP populations studied, with no downstream genes shared between more than two populations. As Sp1 functions largely by interacting with other transcription factors (Opitz and Rustgi 2000; Palazuelos, Klingener, and Aguirre 2014; Melnikova et al. 2000) to exert its effects, the expression and availability of interactors may drive the differential

effects. Differences in downstream gene expression also may be due to differential chromatin availability for TF binding between the progenitor subtypes. This further emphasizes the point that the same stimulus, including transcription factors, can cause differential responses in each NP subtype.

Limitations

There are several opportunities to build upon our existing method. Specifically, this study relied on the manual dissection of the SVZ at a single time point with the sorting based on the binary expression of four antigens. This approach has it limitations. Notably, at least two mixed populations are present, with BNAP and GRP1, as well as MP3 and GRP2, sharing antigenic profiles while containing at least two subtypes of cells, according to their differentiation potentials (Buono et al. 2012). Thus far, we have been unable to separate these subtypes using surface antigens. Furthermore, there are likely intermediate steps between the differentiation of NSCs into each NP, which we are unable to capture here, but that are surely included in the NP subtypes we did not analyze (MP1, MP2, MP4).

Another limitation of this study is that we were unable to capture the location of the NPs. Ventral/dorsal patterning is key in neural development but we were unable to discern a clear pattern of dorsal and ventral signatures (Azim et al. 2015). Having identified genes that are uniquely expressed by each NP, our future studies should allow us to ascertain whether specific NPs are more dorsally vs. ventrally located.

There was also unavoidable contamination with cells from adjacent brain parenchyma, including pericytes, that are NG2+ (Ozerdem et al. 2001) and likely contained within the GRP2/MP3 cell population, which can explain why we see expression of pericyte-associated genes such as Rsg5 in the GRP2/MP3 subpopulation(Cho et al. 2003).

Our information on the developmental fate of our identified progenitors is also based on *in vitro* analysis, and future research should aim to track subtypes of progenitors *in vivo* across normal development to determine their developmental fates.

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Tables

	BNAP/GRP1		GRP2/MP3		GRP3		PFMP	
	Log2FC vs NSCs	P value	Log2FC vs NSCs	P value	Log2FC vs NSCs	P value	Log2FC vs NSCs	P value
Foxj1	-7.35	2.4E-15	-7.34	6.8E-14	-7.14	6.8E-14	-9.28	1.9E-22
Zfp474	-5.39	8.0E-05	-6.86	4.8E-07	-6.73	4.8E-07	-7.76	3.6E-09
Trp73	-4.36	8.8E-05	-5.38	2.8E-07	-5.98	2.8E-07	-5.61	7.1E-06
Emx1	-4.17	2.0E-05	-5.33	1.5E-05	-4.26	1.5E-05	-4.46	2.8E-06
Rfx2	-3.09	1.1E-03	-4.11	1.8E-07	-4.46	1.8E-07	-6.24	1.0E-12
Myb	-4.01	2.0E-09	-4.77	2.6E-10	-4.19	2.6E-10	-4.49	5.2E-12
Foxa2	-2.97	3.1E-02	-4.01	2.5E-03	-4.17	2.5E-03	-5.10	1.2E-04
Id4	-3.53	9.7E-05	-3.90	4.6E-08	-4.52	4.6E-08	-3.53	6.7E-05
Hopx	-3.17	3.8E-05	-3.77	7.0E-08	-3.88	7.0E-08	-3.54	9.3E-07
Pbx4	-3.34	2.9E-03	-5.09	6.0E-08	-3.34	1.2E-03	-2.42	4.2E-02

Table 1. Differentially expressed genes for each subtype were compared with mouse transcription factors on AnimalTFDB3.0. Differentially expressed transcription factors were defined as transcription factors with padj < 0.05. The table lists those transcription factors that were significantly downregulated in all NP populations (BNAP/GRP1, GRP2/MP3, GRP3, PFMP) compared to neural stem cells. Included are Log2 Fold Changes vs Neural Stem cells and FDR-adjusted p-values. No genes were upregulated in all NP populations compared to NSCs.

Table 2A. BNAP/GRP1 Downregulated

TF	Log2FC vs NSCs	P Value
Sox21	-2.23	0.041
Tsc22d1	-1.94	7.43E-03

Table 2B. GRP2/MP3 Downregulated

TF	Log2FC vs NSCs	P Value
Barx2	-3.48	6.41E-03
Vsx2	-3.26	0.015
Msx1	-3.01	0.019
Nr2e1	-3.01	2.71E-03
Smad9	-2.96	9.08E-03

Table 2. GRP3 Downregulated

TF	Log2FC vs NSCs	P Value
Tgif1	-2.27	0.046
Fosb	-1.98	0.047
Shox2	-1.45	0.043
Atf5	-1.24	0.033

Table 2D. PFMP Downregulated

TF	Log2FC vs NSCs	P Value
Lbx2	-4.70	4.67E-05
Nkx2-4	-4.57	2.72E-04
T	-3.91	8.99E-04
Hoxc11	-3.40	9.23E-03
Tbx1	-3.18	0.024

Table 2. Uniquely Downregulated Genes. Differentially expressed genes for each subtype were compared with mouse transcription factors on AnimalTFDB3.0. Differentially expressed transcription factors were defined as transcription factors with padj < 0.05. The table lists those transcription factors that were uniquely downregulated in each NP population subtype: **A: BNAP/GRP1; B: GRP2/MP3; C: GRP3; and D:**

PFMP compared to NSCs. Included are Log2 Fold Changes vs Neural Stem cells and FDR-adjusted p-values.

TF	Log2FC vs NSCs	P Value
Zfp57	3.77	6.25E-04
Hoxc6	3.21	0.015
Zfp955a	2.70	0.015
Lhx1	2.63	0.050
Gm13154	2.51	0.031

Table 3B. GRP2/MP3 Upregulated

TF	Log2FC vs NSCs	P Value
Tbx2	3.18	0.018
Lyl1	3.10	6.08E-03
Heyl	2.85	0.044
Rel	2.74	3.08E-03
Hmga1-rs1	2.51	0.017

Table 3C. GRP3 Upregulated

TF	Log2FC vs NSCs	P Value
Sox12	1.97	0.011
Etv5	1.94	8.93E-03
Zfp157	1.79	4.47E-03
Rfx7	1.13	0.047

Table 3D. PFMP Upregulated

TF	Log2FC vs NSCs	P Value
Rxrg	3.85	2.00E-04
Glis1	2.84	0.04
Lhx6	2.46	0.04
Zfp846	1.97	0.041
Zfp882	1.92	0.042

Table 3. Uniquely Upregulated Genes. Differentially expressed genes for each subtype were compared with mouse transcription factors on AnimalTFDB3.0. Differentially expressed transcription factors were defined as transcription factors with padj < 0.05. The table lists those transcription factors that were uniquely upregulated in each NP population subtype: **A: BNAP/GRP1; B: GRP2/MP3; C: GRP3; and D: PFMP** compared to NSCs. Included are Log2 Fold Change vs Neural Stem cells and FDR-adjusted p-values.

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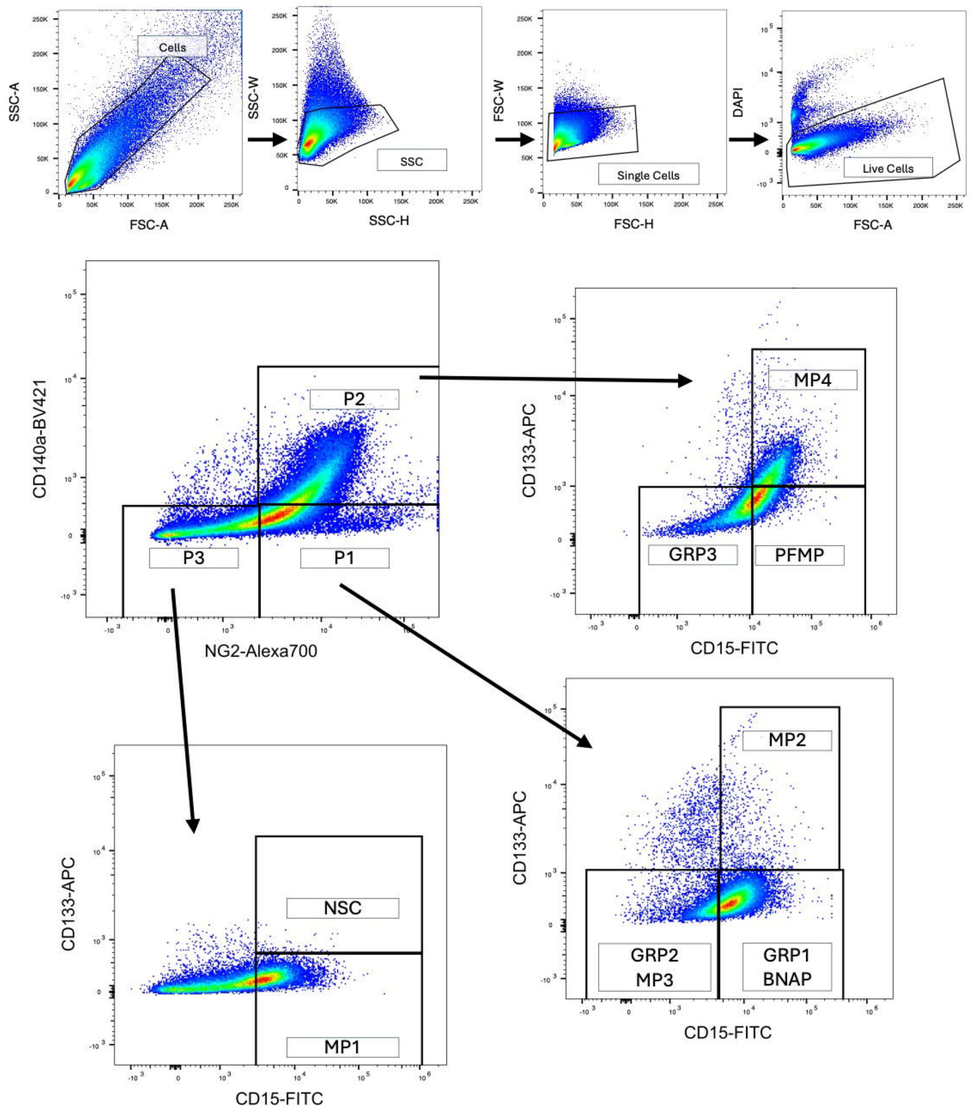
Cells
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FSC-A
SSC
SSC-W
SSC-H
Single Cells
FSC-W
FSC-H
Live Cells
DAPI
FSC-A
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P3
P1
CD140a-BV421
NG2-Alexa700
MP4
GRP3
PFMP
CD133-APC
CD15-FITC
NSC
MP1
CD133-APC
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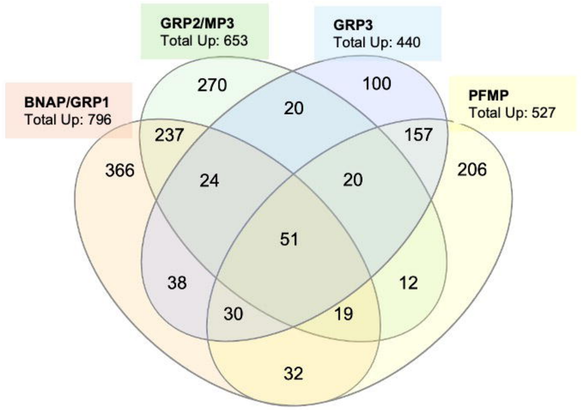
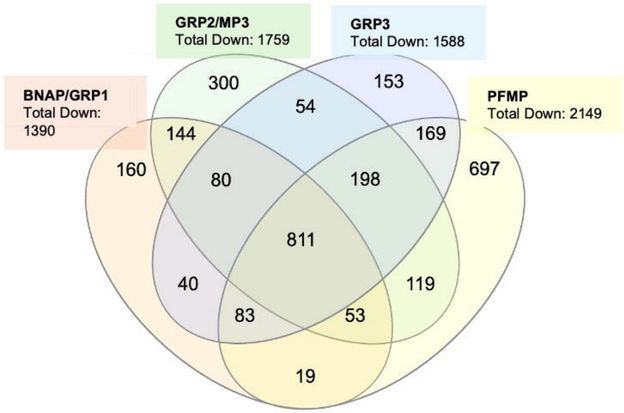
CD15-FITC

Gene Changes vs NSCs

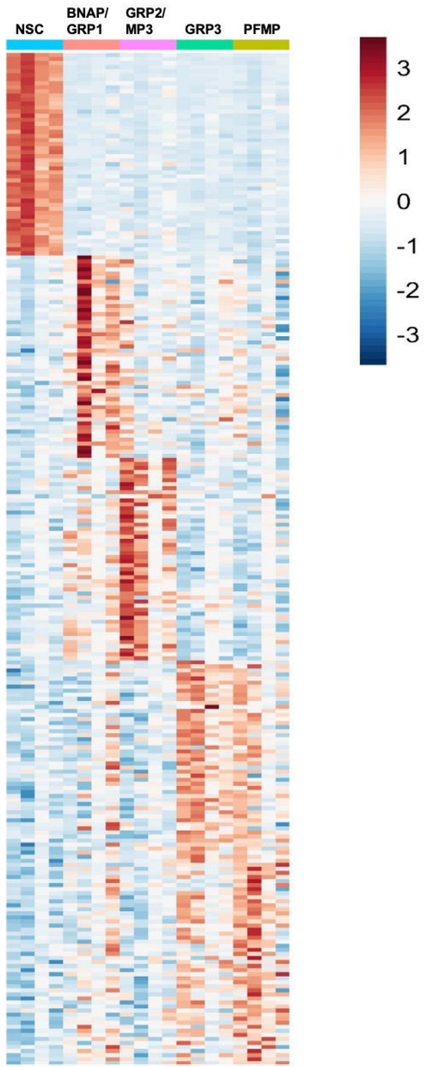
A) Upregulated Genes vs NSCs



B) Downregulated Genes vs NSCs



G) Uniquely Upregulated Genes Heatmap



Uniquely Upregulated Genes

C) BNAP/GRP1

Gene Name	Log2 Fold Change vs NSCs	P value
Igkv4-56	4.31	1.75E-03
2610528A11Rik	3.91	1.60E-02
Phxr2	3.91	3.29E-03
Iltifb	3.91	9.05E-03
Gm26807	3.87	1.14E-03
Gm9573	3.83	3.76E-03
Zfp57	3.77	6.25E-04
Gm16741	3.68	4.87E-03
RP23-358D12.4	3.63	8.55E-03
Gm5316	3.56	0.024

D) GRP2/MP3

Gene Name	Log2 Fold Change vs NSCs	P value
Higd1b	4.93	3.37E-04
Vtn	4.7	1.84E-05
Kcne4	4.47	1.15E-05
Adap2	4.23	4.05E-05
Arhgap9	4.1	7.42E-05
Rgs5	4.06	5.39E-04
Mgp	4.03	3.55E-04
Adap2os	3.98	2.80E-04
Il34	3.97	1.56E-03
Gm8325	3.95	5.89E-03

E) GRP3

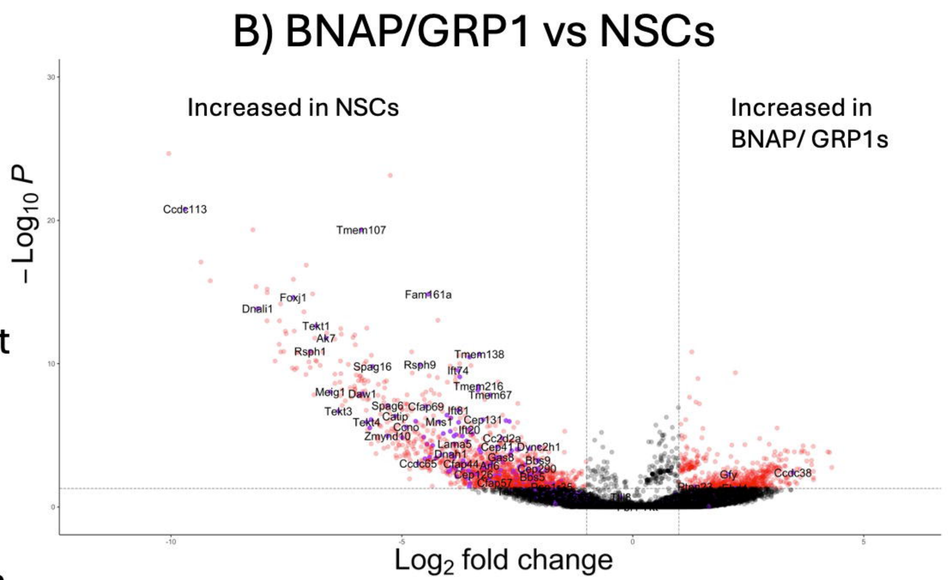
Gene Name	Log2 Fold Change vs NSCs	P value
Slc22a6	4.06	1.92E-03
Bricd5	4.03	1.29E-03
2010320O07Rik	3.93	1.12E-03
Ptchd2	3.53	3.00E-03
Aldh1a2	3.5	1.11E-03
Lnx1	3.48	3.91E-03
Gabrg2	3.35	3.33E-04
Spry4	3.33	0.014
Pcdha4	3.2	6.71E-04
RP24-82B9.1	3.17	0.021

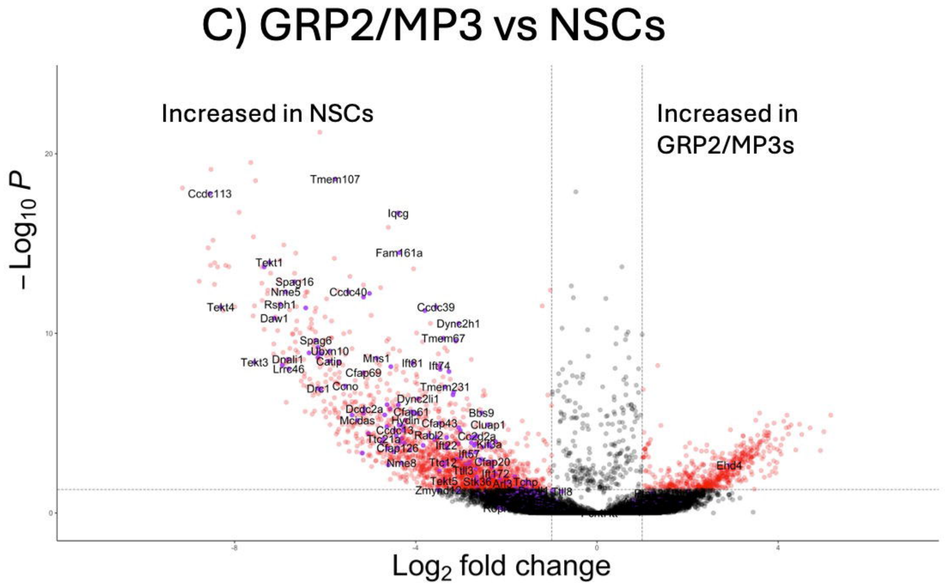
F) PFMP

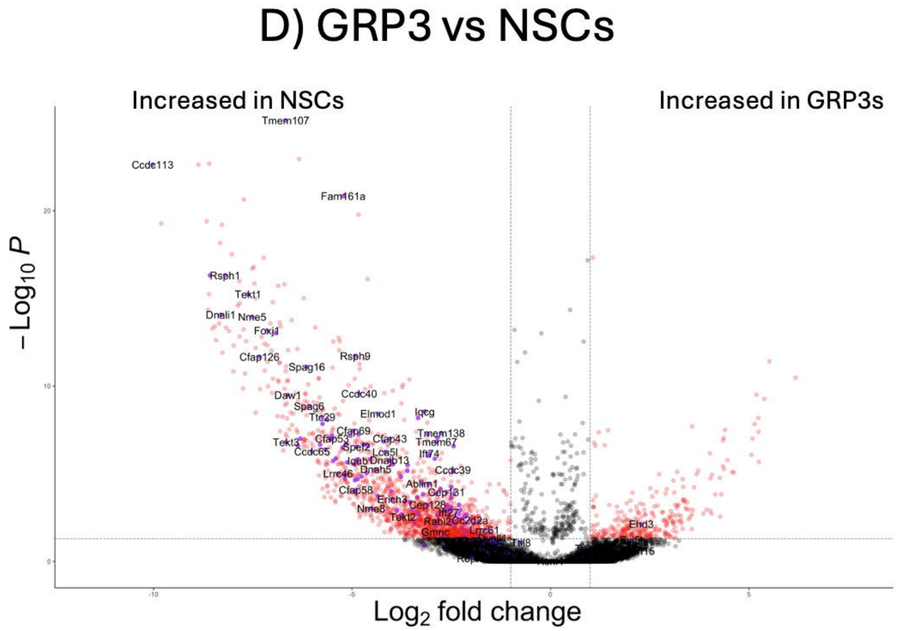
Gene Name	Log2 Fold Change vs NSCs	P value
Rxrg	3.85	2.00E-04
Gm37005	3.79	4.17E-04
Cacna2d3	3.6	8.76E-05
Dlgap3	3.55	1.44E-03
Atp2a1	3.51	4.45E-04
Wfdc18	3.46	3.15E-03
Olfr558	3.37	0.035
Mettl21c	3.36	4.53E-03
Moxd1	3.34	6.18E-03
Sstr1	3.3	0.011

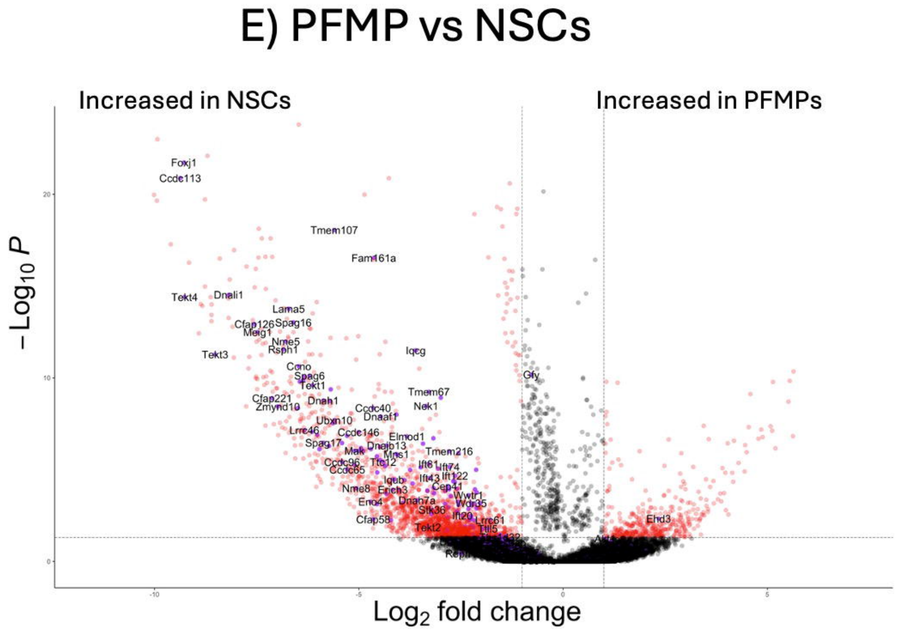
A) Top 8 Go Terms downregulated in NPs

1) cilium organization
2) cilium assembly
3) cilium movement
4) cilium or flagellum-dependent cell motility
5) sperm motility
6) flagellated sperm motility
7) axoneme assembly
8) microtubule bundle formation









Not Significant Significant Cilia Organization

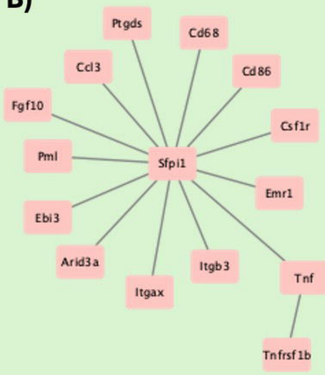
BNAP/GRP1

A) Top 10 G0 terms

Leukocyte activation involved in immune response
Cell activation involved in immune response
Myeloid leukocyte activation
leukocyte migration
Regulation of immune effector process
Myeloid cell activation involved in immune response
tumor necrosis factor production
tumor necrosis factor superfamily cytokine production
Regulation of tumor necrosis factor production
mast cell activation

Interactome

B)



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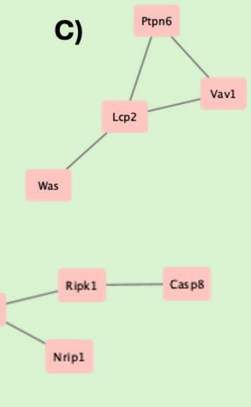
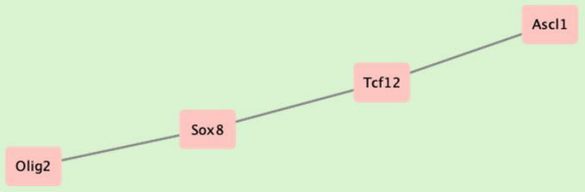


GRP3

F) Top 10 G0 terms

positive regulation of nervous system development
synapse assembly
gliogenesis
glial cell differentiation
positive regulation of synapse assembly
oligodendrocyte differentiation
regulation of neurogenesis
regulation of gliogenesis
myelination
positive regulation of cell junction assembly

G) Interactome

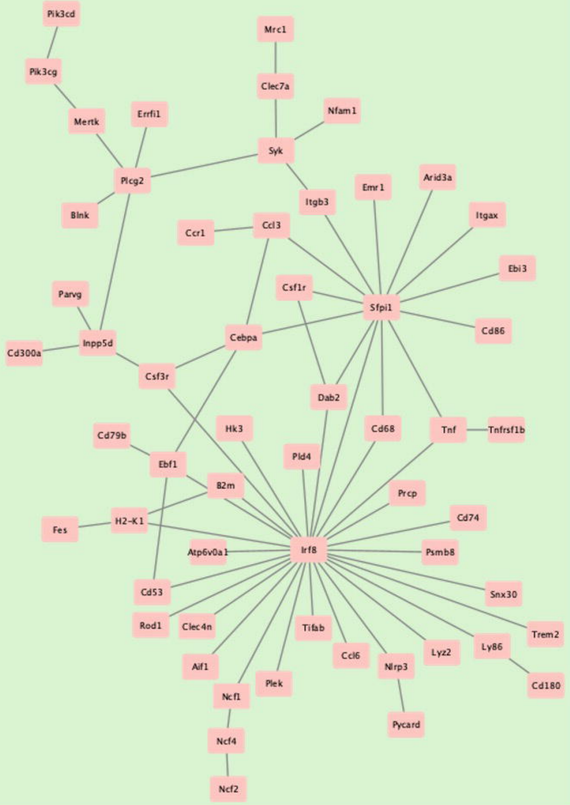


GRP2/MP3

D) Top 10 G0 terms

leukocyte activation involved in immune response
cell activation involved in immune response
regulation of immune effector process
leukocyte proliferation
myeloid leukocyte activation
leukocyte migration
B cell activation
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains
Chemotaxis

E) Interactome



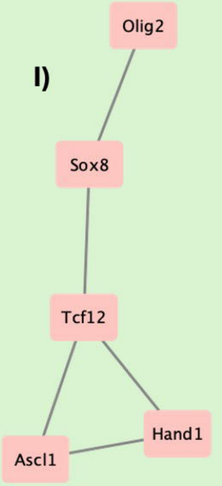
D) PFMP

H) Top 10 G0 terms

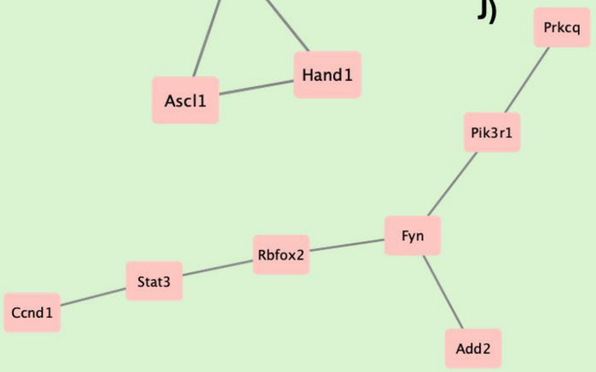
regulation of synapse organization
regulation of synapse structure or activity
synapse assembly
myelination
ensheathment of neurons
axon ensheathment
glial cell differentiation
positive regulation of nervous system development
gliogenesis
oligodendrocyte differentiation

Interactome

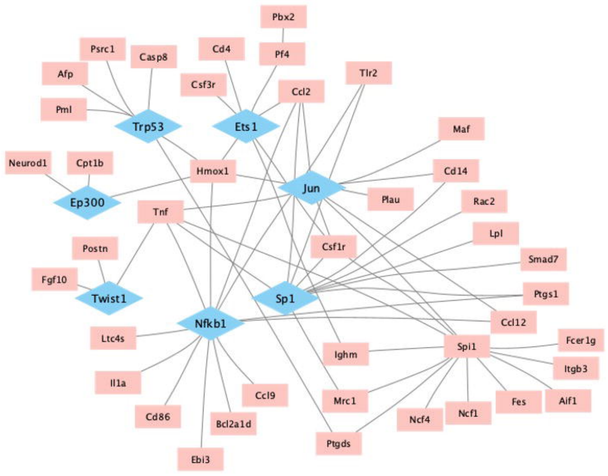
I)



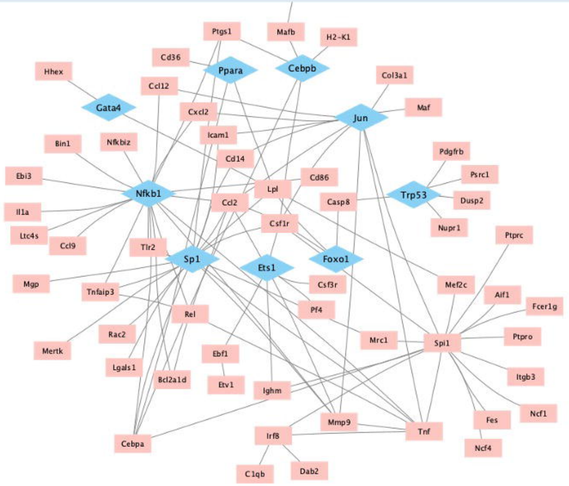
J)



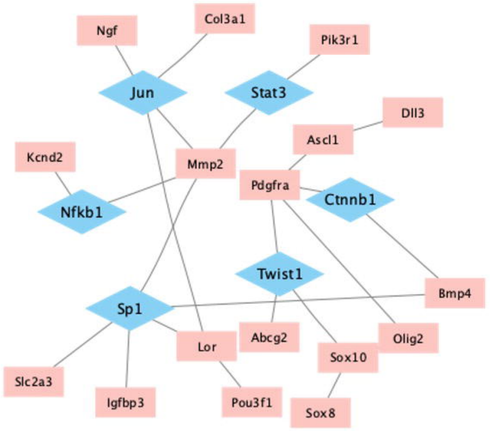
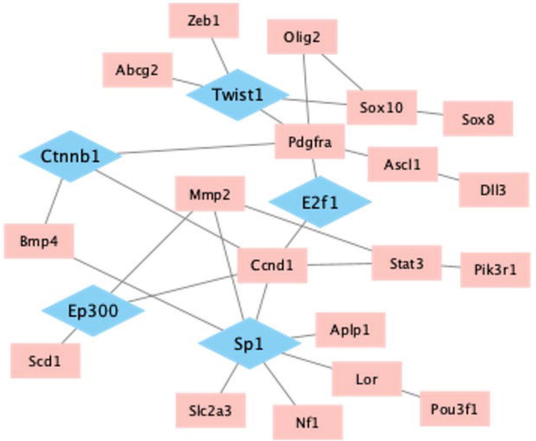
BNAP/GRP1



GRP2/MP3



GRP3



PFMP