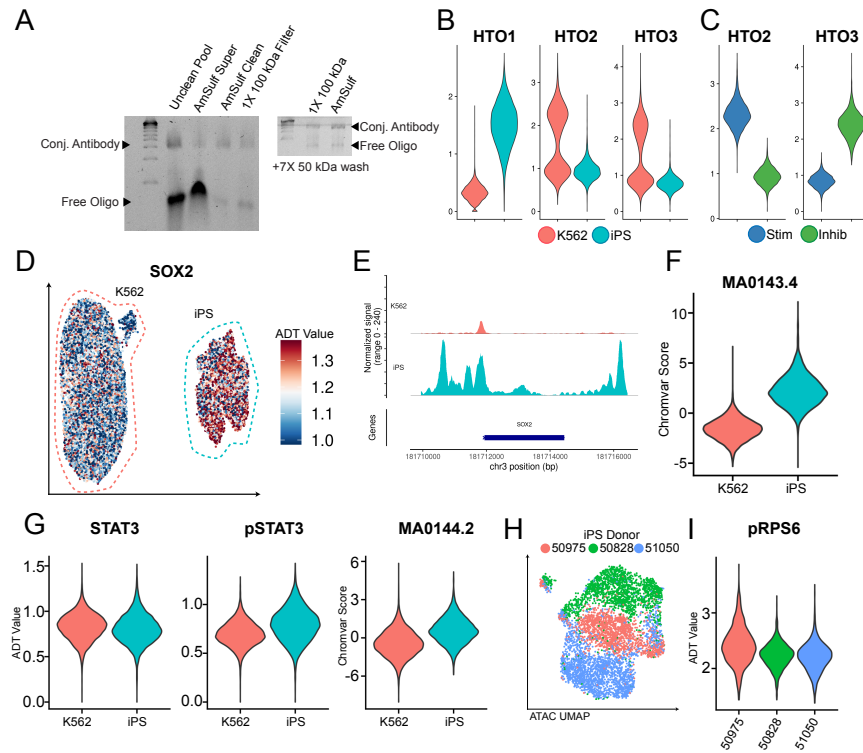
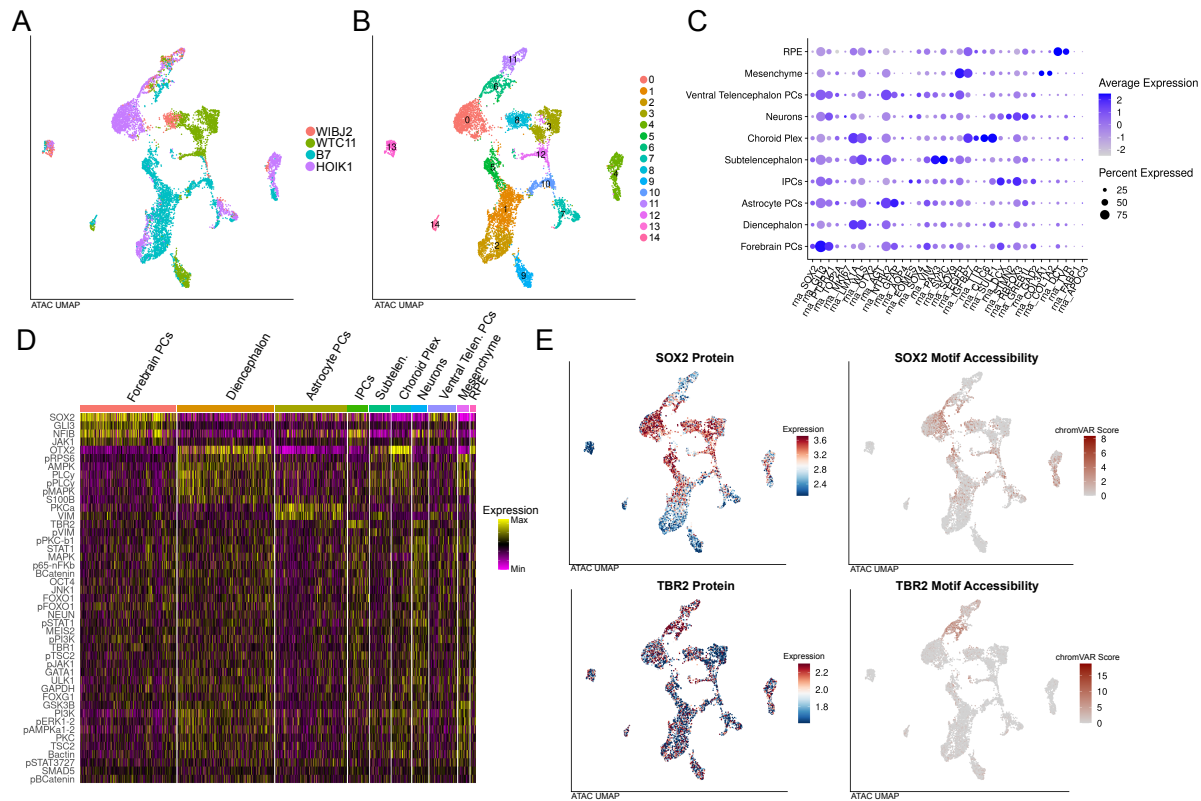


Figure S1



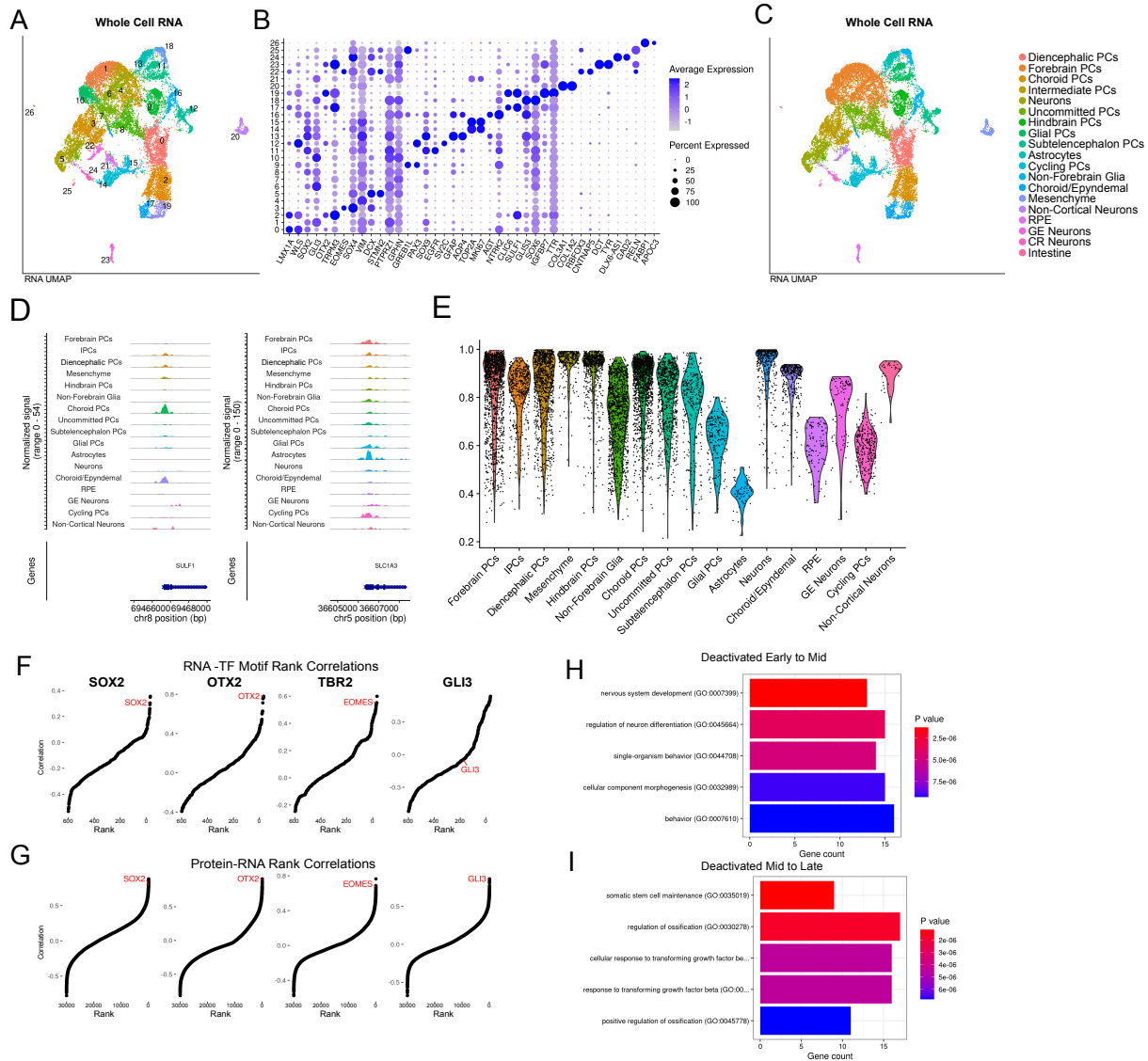
a) DNA electrophoresis gel of antibody pool at different indicated stages of clean-up showing the degree of remaining free oligo after filtration steps. **b)** Violin plot of HTO expression for each HTO in K562 and iPS cells. **c)** Violin plot of HTO expression for each HTO in K562 cells classified from stimulated or inhibited conditions. **d)** UMAP representation of K562 and iPS cells colored by normalized ADT values for SOX2. **e)** Coverage plot of chromatin accessibility of K562 and iPS cells at the SOX2 genomic locus. **f)** Violin plot of chromVAR scores for the SOX2 binding motif (MA0143.4) in K562 and iPS cells. **g)** Violin plots of normalized ADT values for STAT3 (left panel) pSTAT3 (middle panel) and STAT3 motif chromVAR scores (right panel) for K562 and iPS cells. **h)** UMAP representation of iPS cells colored by iPSC donor based on scATAC-sequencing data. **i)** Violin plot of normalized ADT values of pRPS6 split by iPSC donor identity.

Figure S2



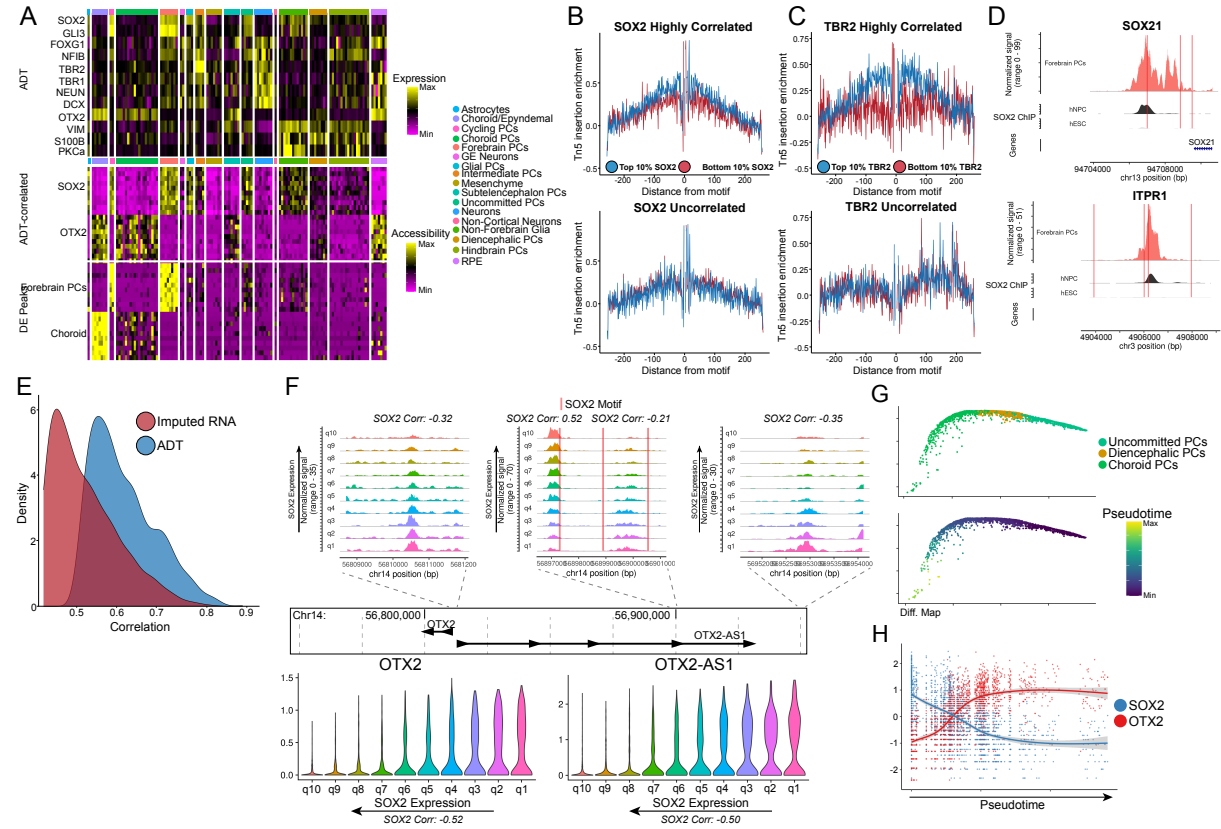
a) UMAP representation of cells colored by iPSC donor based on scATAC-Seq modality in Phospho-seq. **b)** UMAP representation of cells colored by unsupervised clustering based on scATAC-Seq modality in Phospho-seq. **c)** Dotplot showing the gene activity scores of marker genes and the percentage of cells they are expressed in from each assigned cell type identity in the Phospho-seq dataset. **d)** Heatmap showing scaled ADT expression for all differentially expressed ADTs organized by assigned cell type. **e)** UMAP representation of cells colored by normalized ADT values for SOX2 (upper left), chromVAR scores for SOX2 motif accessibility (upper right), normalized ADT values for TBR2 (lower left), chromVAR scores for TBR2 motif accessibility (lower right).

Figure S3



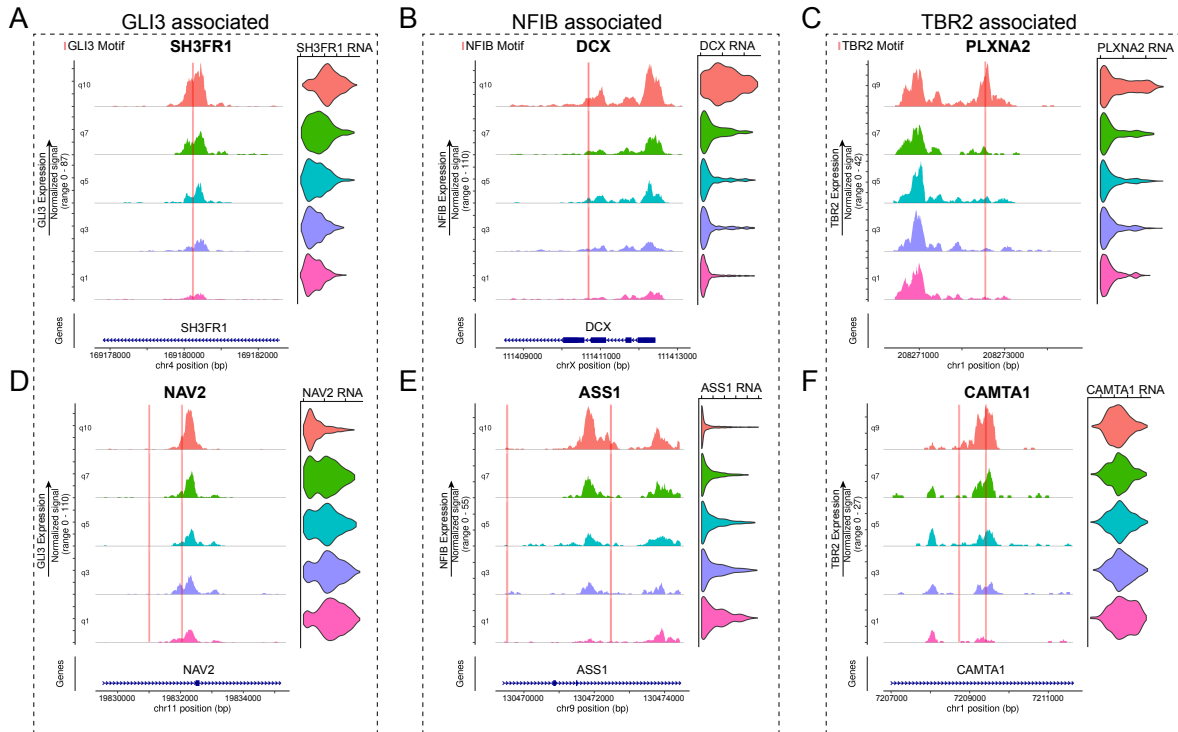
a) UMAP representation of cells colored by unsupervised cluster assignment from scRNA-Seq on whole unfixed cells. **b)** Dotplot showing the gene expression of marker genes and the percentage of cells they are expressed in from each cluster identity in whole, unfixed cells. **c)** UMAP representation of cells colored by cell type assignment from scRNA-Seq on whole unfixed cells. **d)** Coverage plots for SULF1 (left) and SLC1A3 (right) with transferred cell labels. **e)** Violin Plot of prediction scores per assigned cell type from bridge integration of whole cell RNA dataset with Phospho-seq dataset. **f)** Rank-correlation plots of imputed RNA vs TF-motif accessibility for each of the indicated genes. **g)** Rank-correlation plots of protein vs imputed RNA for each of the indicated proteins. **h)** Bar plot of the five most significant gene ontology categories associated with the top 500 genes decreasing from early to mid pseudotime bins across forebrain neuronal development. **i)** Bar plot of the five most significant gene ontology categories associated with the top 500 genes decreasing from mid to late pseudotime bins across forebrain neuronal development.

Figure S4



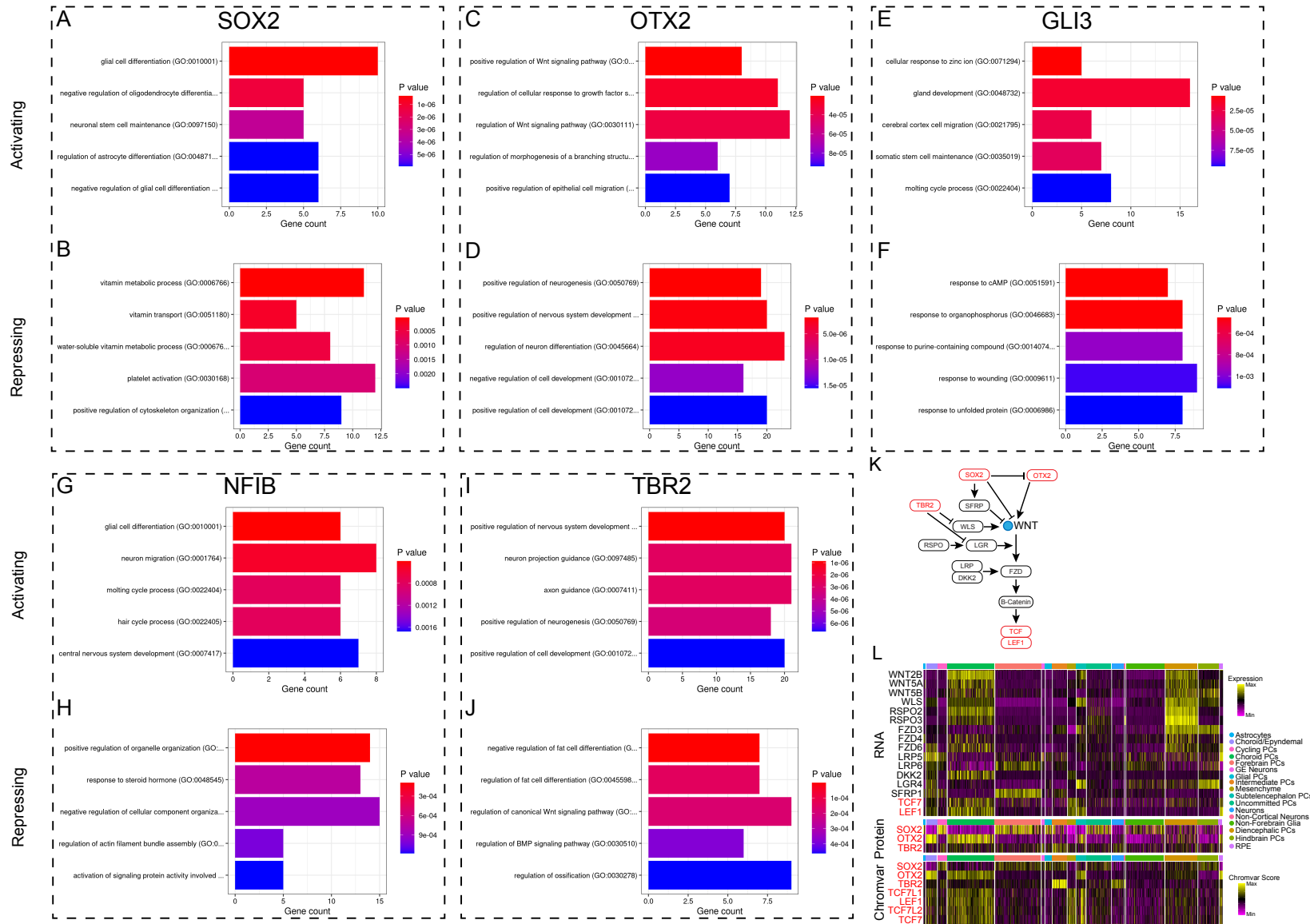
a) Heatmaps of metacelled data showing normalized marker ADT values (top panel), peaks correlated with SOX2 and OTX2 ADT expression (middle panel) and differentially accessible peaks for Forebrain and Choroid PCs determined from the non-metacelled data. **b)** Tn5 cut-site footprinting between cells with high SOX2 expression and low SOX2 expression in peaks that are highly correlated with SOX2 (top panel) and uncorrelated with SOX2 (bottom panel). **c)** Tn5 cut-site footprinting between cells with high TBR2 expression and low TBR2 expression in peaks that are highly correlated with TBR2 (top panel) and uncorrelated with TBR2 (bottom panel). **d)** Coverage plots of Forebrain PCs compared to peaks from a publicly SOX2 ChIP-seq dataset from human neural precursors and human hESCs. **e)** Density of the correlations of top 1000 SOX2 correlated peaks when using SOX2 imputed RNA vs. SOX2 ADT. **f)** Combined plot of accessibility in peaks proximal to the OTX2 locus and associated gene expression. Plots are organized by quantile ADT expression of SOX2 throughout the whole dataset. Red lines on coverage plots are indicative of SOX2 binding motifs. **g)** Diffusion map of cells differentiating from Uncommitted PCs to choroid PCs colored by cell type (top panel) and pseudotime as determined by monocle (bottom panel). **h)** Scatter plot showing scaled values of SOX2 and OTX2 Protein across pseudotime as determined in (g).

Figure S5



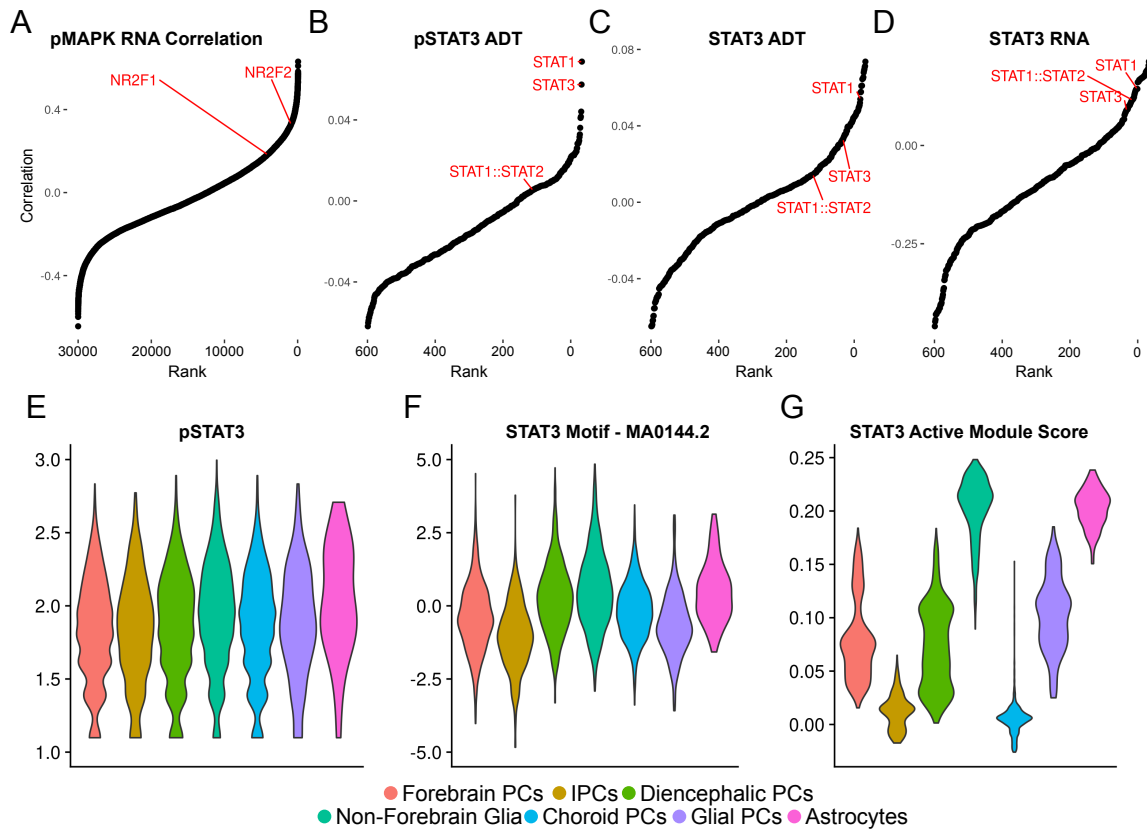
a) Example of an inferred transcription activating peak associated with GLI3. Coverage plots and violin plots are ordered by quantile ADT expression for GLI3. Red lines indicate the location of a GLI3 binding motif. **b)** Same as in **(a)** but for NFIB. **c)** same as in **(a)** but for TBR2. **d)** Example of an inferred transcription repression peak associated with GLI3. Coverage plots and violin plots are ordered by quantile ADT expression for GLI3. Red lines indicated the location of a GLI3 binding motif. **e)** Same as in **(d)** but for NFIB. **f)** same as in **(d)** but for TBR2.

Figure S6



- a)** Bar plot of the top 5 most significant gene ontology categories associated with the genes in the top 500 peak-gene links that are SOX2 activated or repressed **(b)**.
- c)** Bar plot of the top 5 most significant gene ontology categories associated with the genes in the top 500 peak-gene links that are OTX2 activated or repressed **(d)**.
- e)** Bar plot of the top 5 most significant gene ontology categories associated with the genes in the top 500 peak-gene links that are GLI3 activated or repressed **(f)**.
- g)** Bar plot of the top 5 most significant gene ontology categories associated with the genes in the top 500 peak-gene links that are NFIB activated or repressed **(h)**.
- i)** Bar plot of the top 5 most significant gene ontology categories associated with the genes in the top 500 peak-gene links that are TBR2 activated or repressed **(j)**.
- k)** Schematic of SOX2, OTX2 and TBR2 regulation of WNT signaling. **l)** Heatmap of WNT-signaling related gene expression (top panel), SOX2, OTX2 and TBR2 protein expression (middle panel) and chromVAR scores for WNT-signaling related transcription factors

Figure S7



a) Rank-correlation plot of pMAPK ADT signal vs. RNA expression with NR2F1 and NR2F2 highlighted **b)** Rank-correlation plot of pSTAT3 ADT signal vs. TF motif accessibility with the STAT1 and STAT3 motifs highlighted. **c)** Same as in **(a)** but STAT3 ADT vs. TF motif accessibility. **d)** Same as in **(a)** but STAT3 RNA vs. TF motif accessibility. **e)** Violin plot of normalized pSTAT3 ADT levels in a subset of progenitor and glial cells. **f)** Violin Plot of STAT3 motif chromVAR scores in a subset of progenitor and glial cells. **g)** Violin plot of STAT3 activated gene module scores in a subset of progenitor and glial cells.