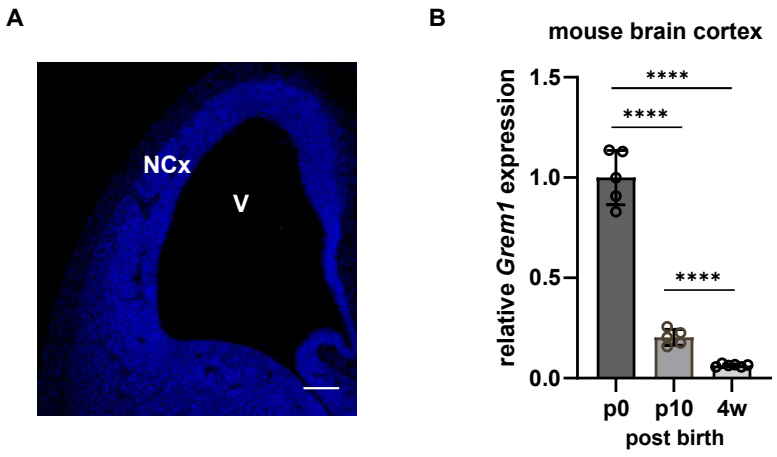
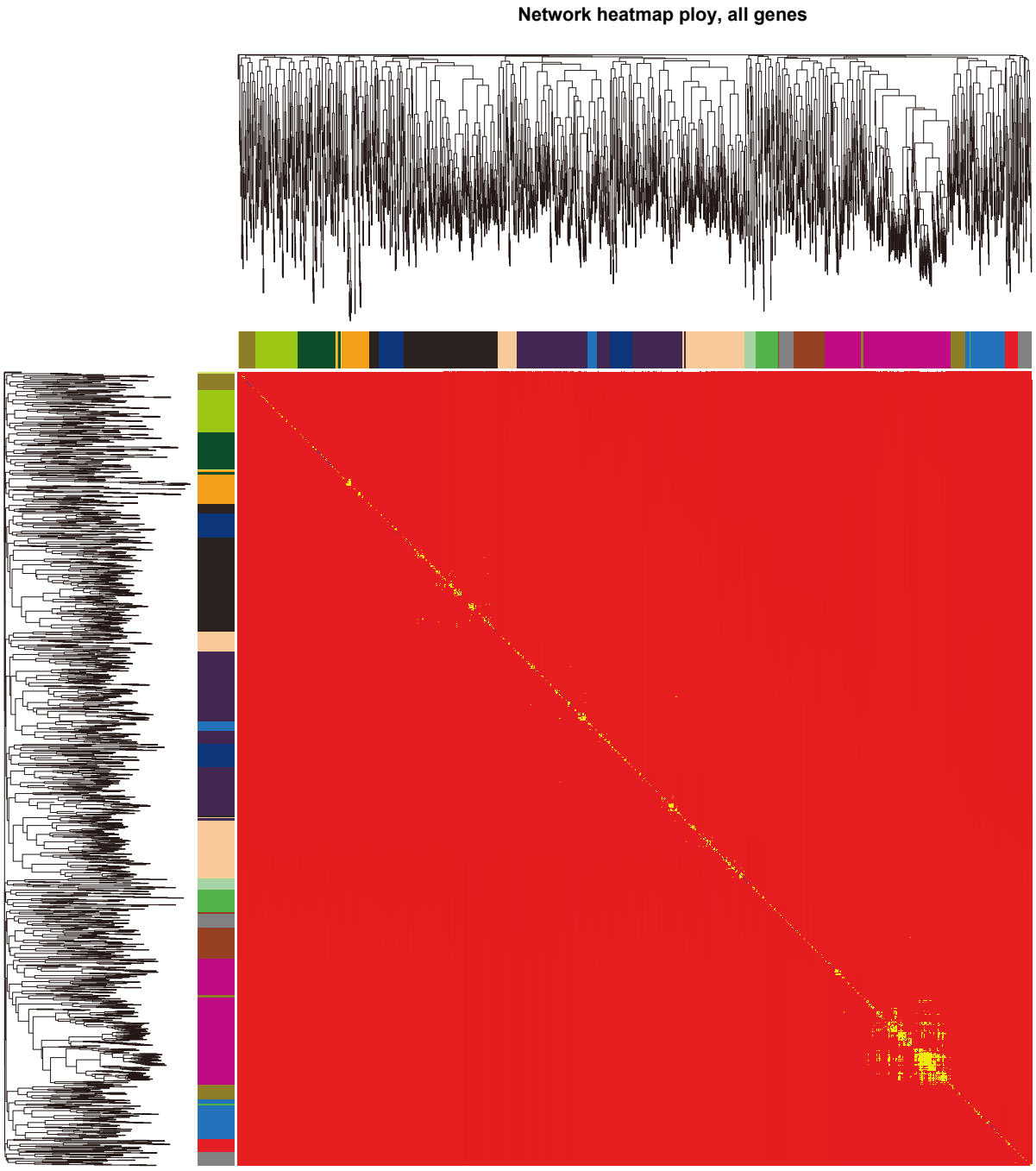
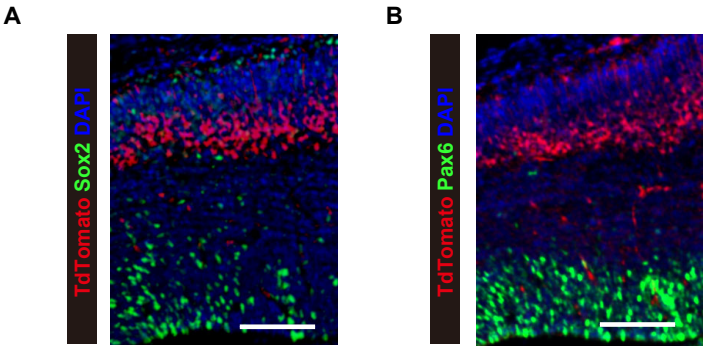


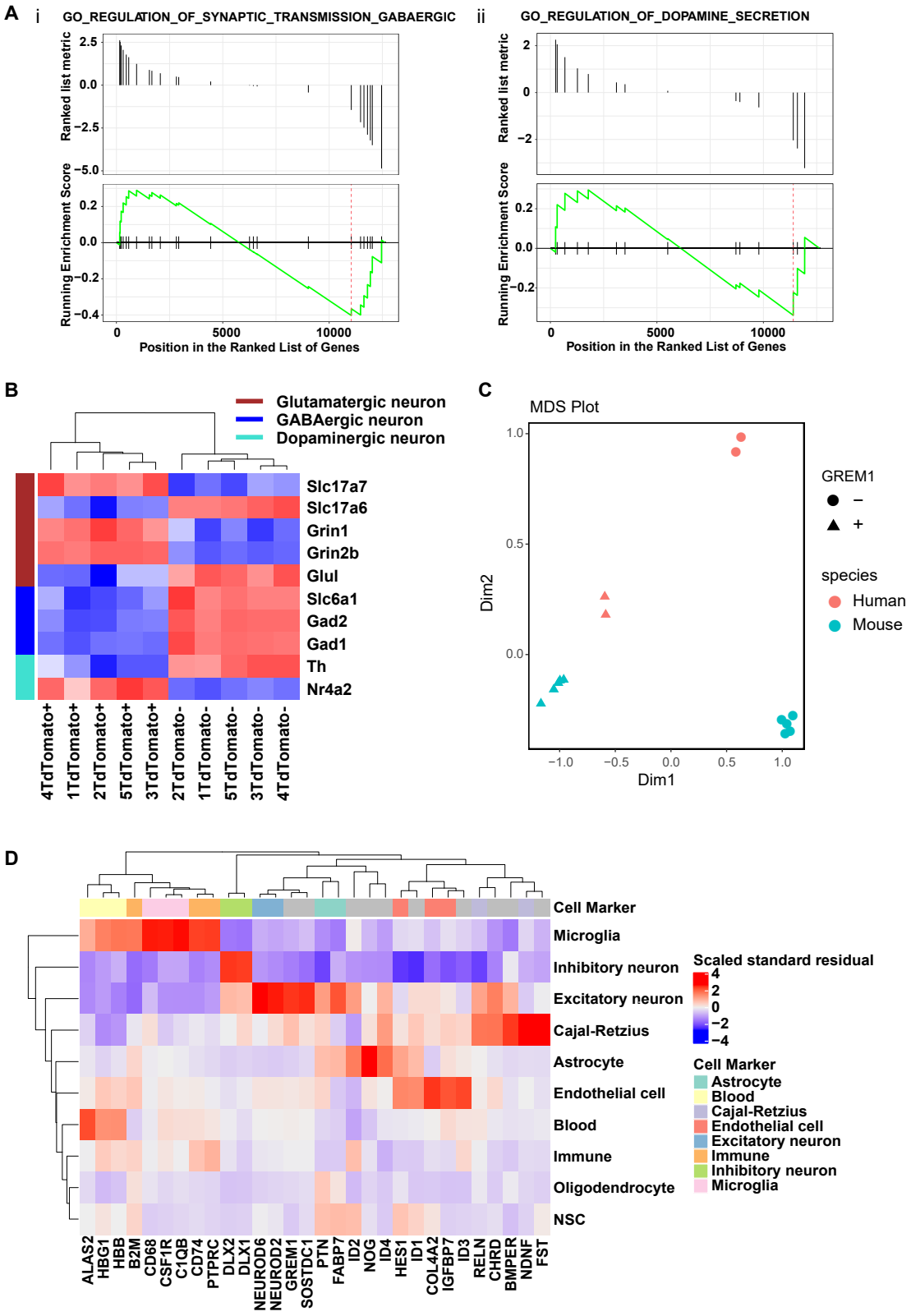
Supplmental Figure1. *Grem1* starts to express after 12.5 dpc and is dramatically decreased after birth. (A) Representative coronal images of immunofluorescence staining of 12.5 dpc telencephalon from *Grem1*-reporter (red) mice induced with tamoxifen at 11.5 dpc. Scale bar = 100 μm. LV, lateral ventricle ; NCx, neocortex (B) *Grem1* mRNA levels normalized to *Gapdh* were determined using real time qRT-PCR in mouse cortical brain samples collected at p0, p10 and 4 weeks post-birth (n=4-6 mice/group, PCR conducted in triplicate) Columns, mean; bars, SD. One way ANOVA with Tukey' s multiple test. ****p<0.0001



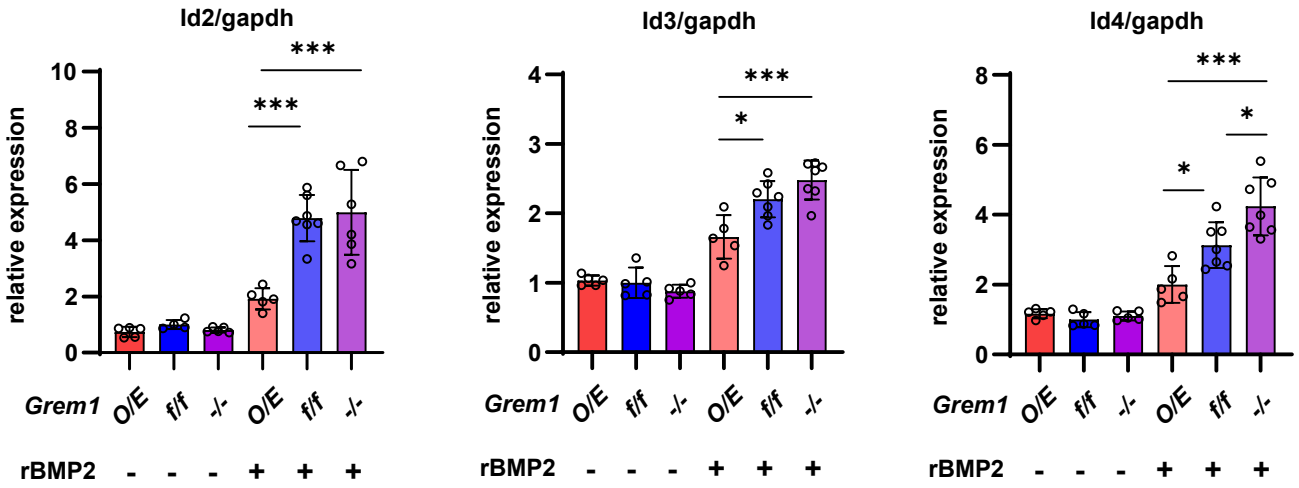
Supplmental Figure2. Clustering of differentially expressed genes. A heat map was generated to visualise the unsupervised hierarchical clustering of correlation scores for gene expression of DEG in TdTomato+ cells. Yellow colour indicates closely related and red not related. The color bar between the hierarchical clustering tree and plotting fields denotes the module membership of each gene.



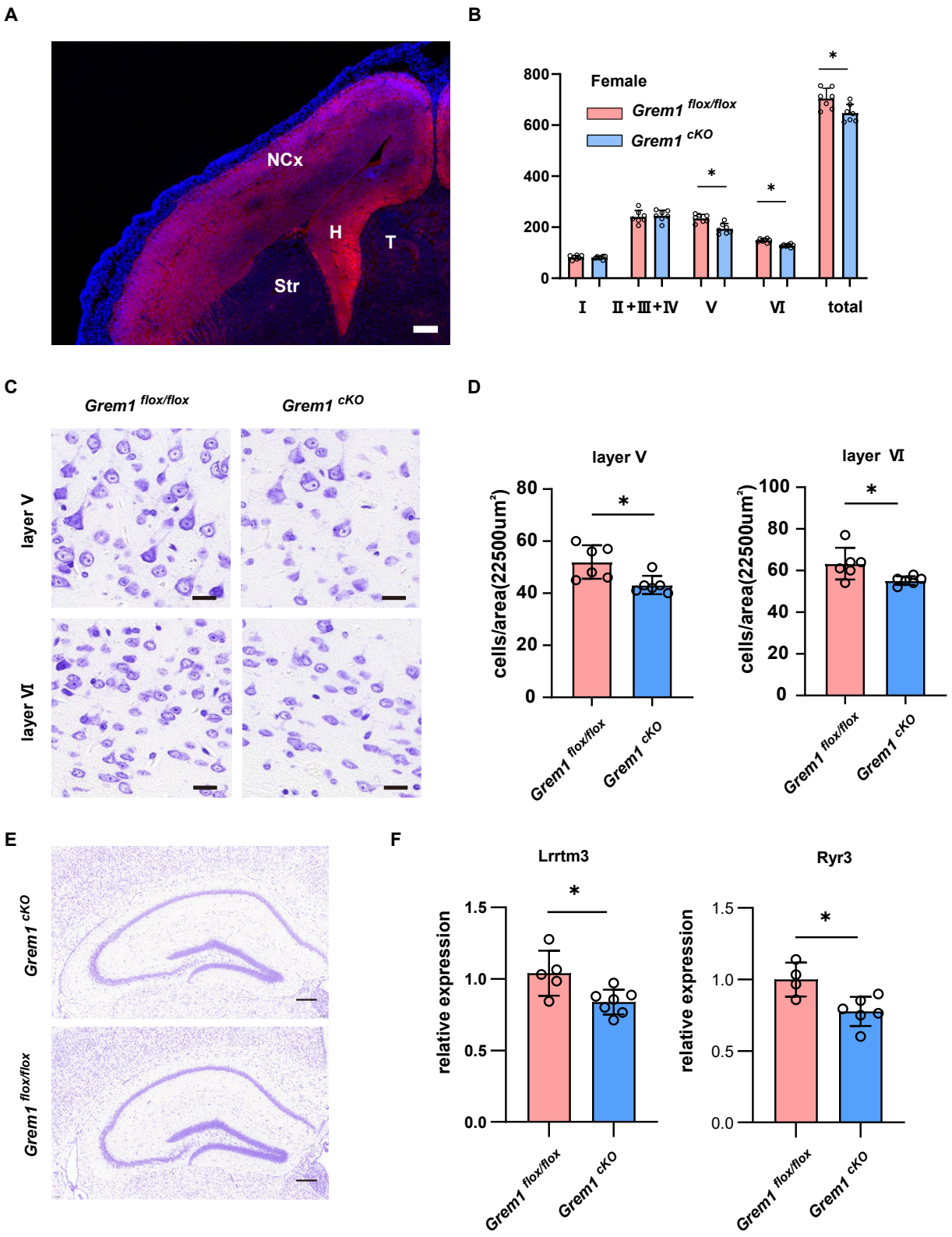
Supplmental Figure3. ***Grem1*****-expressing cells are radial glial stem cell markers,** Sox2 and Pax6 negative. Representative images of immunofluorescence staining of 14.5 dpc neocortex from Grem1-reporter (red) mice induced with tamoxifen at dpc13.5, (A) Sox2 (green), DAPI (blue). (B) Pax6 (green), DAPI (blue). Scale bar = 100 μm.



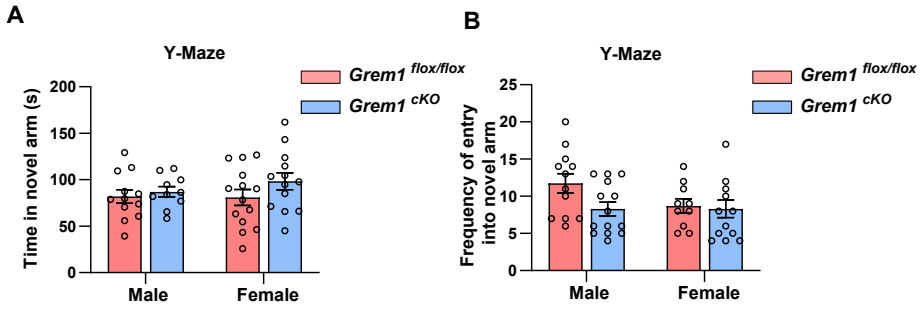
Supplmental Figure4. Extended transcriptome analysis of bulk mRNAseq data from *Grem1*-expressing TdTomato+ and TdTomato- telencephalon cells at 14.5dpc. (A) Gene set enrichment analysis (GSEA) for genes involved in the regulation of (i) GABAergic synaptic transmissions and (ii) dopamine secretion that were differentially regulated between *Grem1*-expressing TdTomato+ and TdTomato- cells. Normalised enrichment score (NES) = -0.93, p = 0.75 and NES = -0.69, p = 0.87. (B) Unsupervised clustering of *Grem1*-expressing TdTomato+ and TdTomato samples based on expression of representative markers for glutamatergic, GABAergic, and Dopaminergic neurons, in bulk mRNAseq data. Marker transcript expression is displayed as a heat map with high expression in red to low expression in blue. (C) Multidimensional scaling plot comparison of transcriptome of TdTomato+ and TdTomato- cells isolated from 14.5dpc *Grem1*-reporter mice induced with tamoxifen at dpc13.5 and *GREM1*+ and *GREM1*- cells from scRNAseq of human mid-gestational cortex. (D) *GREM1* expression is associated with the excitatory neuronal lineage in the developing human brain. Unsupervised clustering of human developmental brain cell populations from scRNAseq GSE103723 based on expression of lineage markers and transcripts encoding BMP antagonists (F*ST, NOG, CHRD, BMPER, GREM1, SOSTDC1*) and BMP target genes (*ID1-4*). Cell type is indicated by colour legend as per tSNE plot shown in Figure 4E. Chi-square testing was used to evaluate the level of each transcript in each cell population and the resulting standardized residual values are depicted in the heat map.



Supplmental Figure5. Grem1 acts to antagonize BMP signaling in NSPCs resulting in altered downstream transcript levels. Transcript levels of BMP target genes, *Id2*, *Id3* and *Id4*, normalized to *Gapdh* in *Grem1*$^{flox/flox}$, *Grem1*$^{-/-}$ and *Grem1*$^{O/E}$ NSPC treated with vehicle or rBMP2 for 24h. Results from 5 independent experiments performed in triplicate. Columns, mean; bars, SD. One way ANOVA with Tukey' s multiple test. *p<0.05, ***p<0.001



Supplmental Figure6. Extended morphological, immunostaining and transcript analysis of *Grem1*^{cKO} mice and littermate controls. (A) Representative coronal section of TdTomato+ (red) cells in the dorsal telencephalon at 14.5dpc in *Emx1-cre ; Rosa26LSLTdtomato* mouse brain. This cre-driver is broadly active across the telencephalon region in which *Grem1* is highly expressed. Scale bar = 100 µm. NCx, neocortex ; H, hippocampus ; Str, striatum ; T, thalamus (B) Quantification of cortical layer thickness compared between age and sex matched littermates at 10 weeks of age. Female, n=7 control and n=7 *Grem1*^{cKO},. t-test, *p<0.05. (C) Representative histological images of neocortical layer 5 and 6 from *Grem1*^{cKO} mice and *Grem1*$^{flox/flox}$ littermate controls at 10 weeks of age using Nissl. Scale bar = 20um. (D) Quantification of (C) showing the cell number per area of layer V and VI using Nissl in 2 HPF of 3 biological replicates. Columns, mean; bars, SD. t-test. *p<0.05. Scale bar = 20um. (E) Representative histological images of hippocampus from *Grem1*^{cKO} mice and *Grem1*$^{flox/flox}$ littermate controls at 10 weeks of age using Nissl. 8 pairs of males and 7 pairs of females were analyzed. Scale bar = 200 µm. (F) Transcripts from the Id1 associated gene cluster (Fig3E), *Ryr3* and *Lrrtm3* were evaluated in *Grem1*^{cKO} and *Grem1*$^{flox/flox}$ littermate control cortex at p10 using real time qRT-PCR. *Ryr3* and *Lrrtm3* levels were normalized to *Gapdh*. n=4-6 biological replicates. Columns, mean; bars, SD. t-test. *p<0.05, ****p<0.0001



Supplmental Figure7. Memory is not impaired in *Grem1*^{cKO} mice compared to *Grem1*$^{flox/flox}$ littermate controls. (A,B) Y-Maze test. Behavior was compared between age and sex matched *Grem1*^{cKO} mice and *Grem1*$^{flox/flox}$ littermate controls at 7-10 weeks of age. Male, n=12 control and n=10 *Grem1*^{cKO}, Female, n=14 control and n=13 *Grem1*^{cKO}. t-test. (A) Cumulative duration spent in new arm of Y-Mmaze. (B) The number of entries to new arm of the Y-Maze. Columns, mean; bars, SEM.