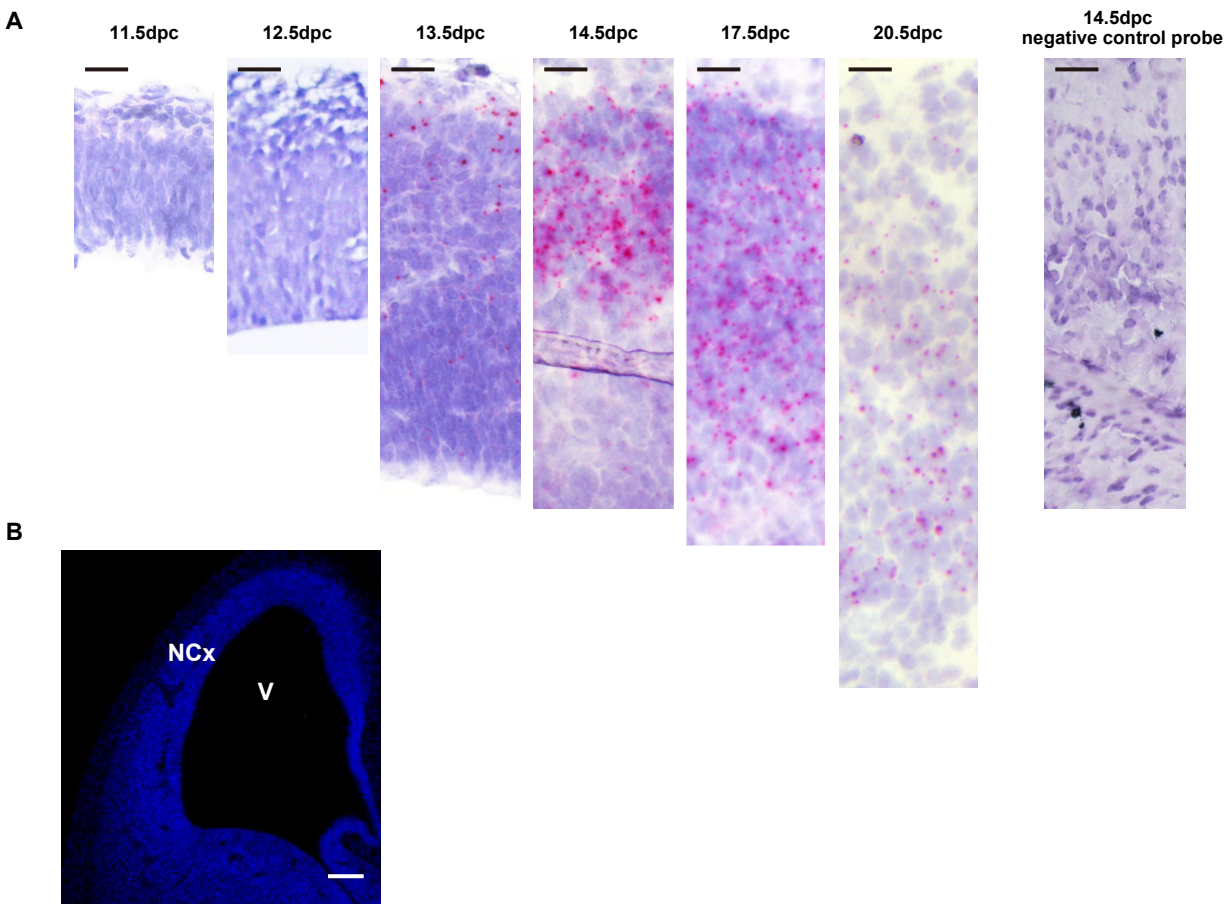


Fig. S1. *Grem1* starts to express at 13.5 dpc and is dramatically decreased after birth

(A) *Gremlin1* ISH of wild type mice cortex at stages spanning 11.5 to 20.5 dpc. No signal was detected with negative control probe at 14.5 dpc. Scale bar = 20 μm.
(B) 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
(C) Representative images of immunofluorescence staining of 20.5 dorsal telencephalon from *Grem1*-reporter (red) mice induced with tamoxifen at dpc13.5, DAPI (blue), and Ctip2 (green) for the left image, CDP (green) for the right image. Scale bar = 100 μm.
(D) *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
(E) Representative images of immunofluorescence staining of 14.5 dorsal telencephalon from *Grem1*-reporter (red) mice induced with tamoxifen at dpc13.5, Ki67 (green), DAPI (blue). Scale bar = 100 μm.

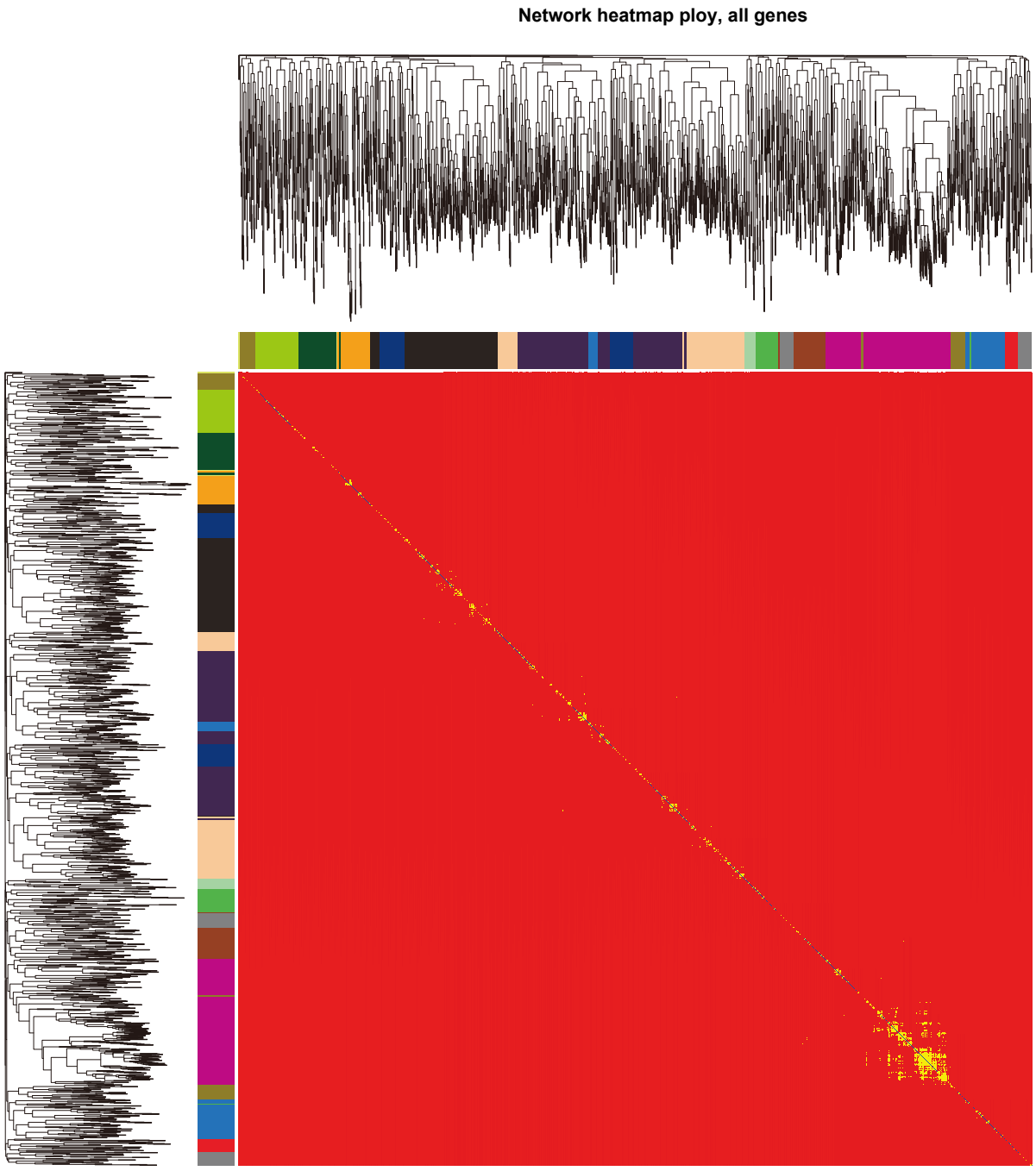


Fig. S2. Clustering of differentially expressed genes

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.

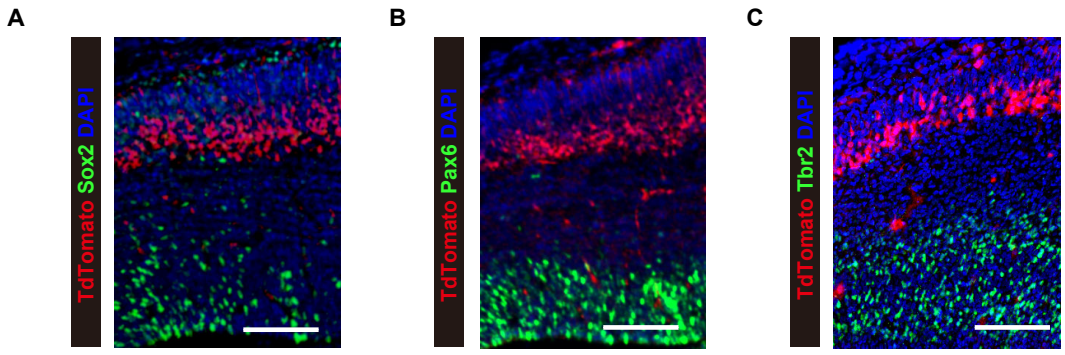


Fig. S3. Grem1-expressing cells are radial glial stem cell markers, Sox2 and Pax6 negative and an intermediate progenitor marker, Tbr2 negative

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). (C) Tbr2 (green), DAPI (blue). Scale bar = 100 μm.

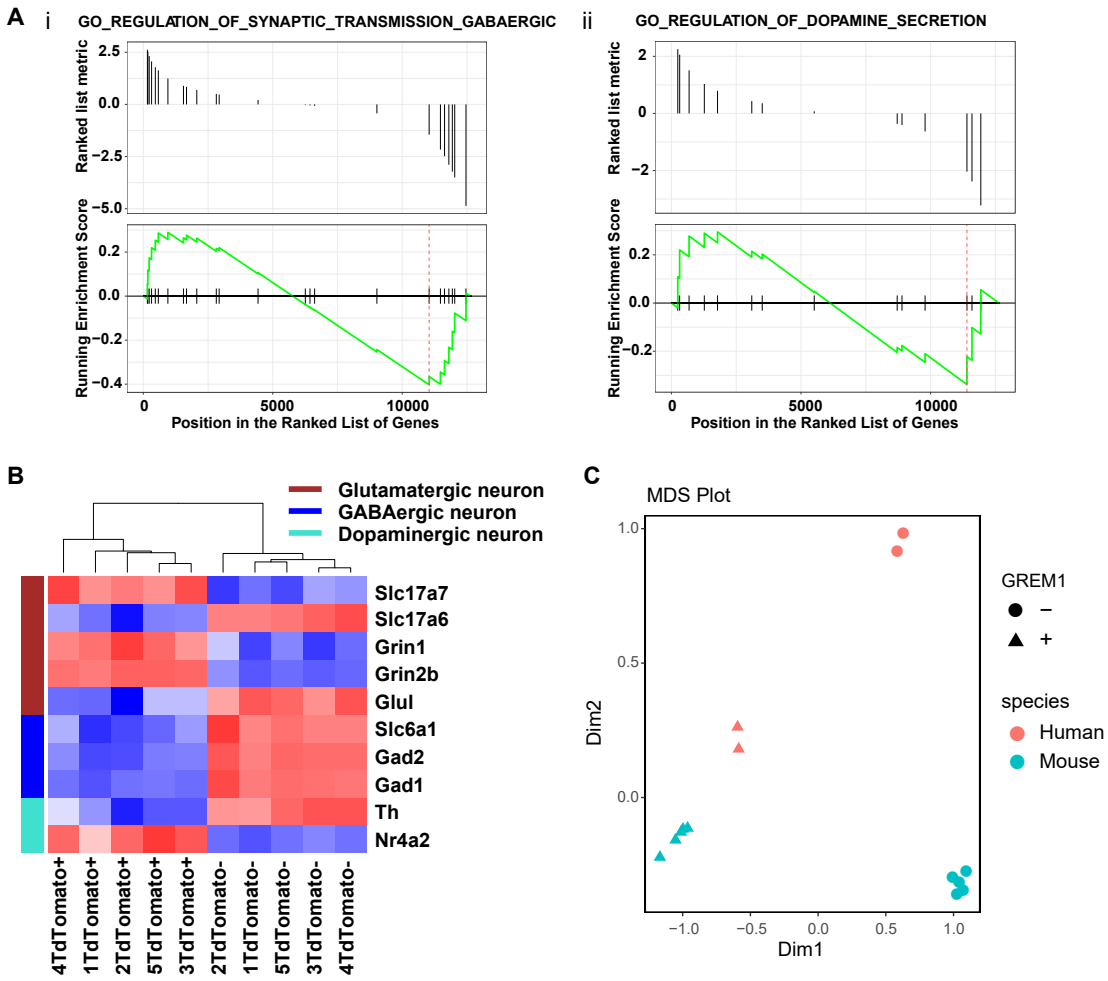
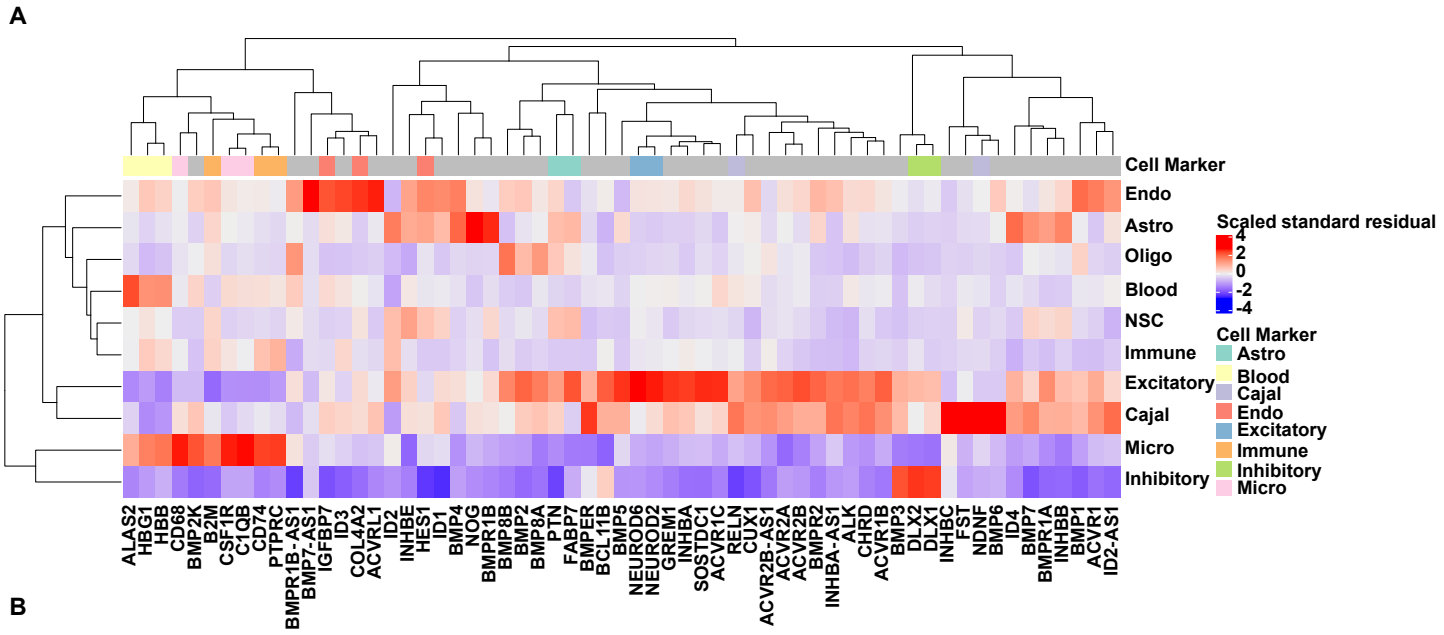


Fig. S4. Extended transcriptome analysis of bulk mRNAseq data from Grem1-expressing TdTomato+ and TdTomato- telencephalon cells at 14.5dpc

(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.


A
Cell Marker
Endo
Astro
Oligo
Blood
NSC
Immune
Excitatory
Cajal
Micro
Inhibitory
Scaled standard residual
4
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-4
Cell Marker
Astro
Blood
Cajal
Endo
Excitatory
Immune
Inhibitory
Micro
ALAS2 HBG1 HBB CD68 BMP2K B2M CSF1R C1QB CD74 PTPRC BMPR1B-AS1 BMP7-AS1 IGFBP7 ID3 COL4A2 ACVRL1 ID2 INHBE HES1 ID1 BMP4 NOG BMPR1B BMP8B BMP2 BMP8A PTN FABP7 BMPER BCL11B BMP5 NEUROD6 NEUROD2 GREM1 INHBA SOSTDC1 ACVR1C RELN CUX1 ACVR2B-AS1 ACVR2A ACVR2B BMPR2 INHBA-AS1 ALK CHRD ACVR1B BMP3 DLX2 DLX1 INHBC FST NDNF BMP6 ID4 BMP7 BMPR1A INHBB BMP1 ACVR1 ID2-AS1
B
BMP2
BMP4
BMP7
Inhibitory Micro NSC Oligo Immune Blood Endo Astro Cajal Excitatory
Scaled standard residual
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2
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C
GREM1 BMP2 BMP4 BMP7
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log2 (expression)
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ID1 ID2 ID3 ID4
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GREM1 expression
E
Endothelial cell
Layer Ⅱ-Ⅳ
Astrocyte
SMAD P
BMP target gene expression
BMP
BMP
Layer Ⅴ/Ⅵ
Grem1
SMAD
Neuron Apoptosis
BMP
Progenitor Proliferation
RGC
IP
D
Cell Marker
Apical precursor
Proliferative RGC
Non−proliferative RGC
Excitatory neuron
Newborn neuron
IP/Excitatory neuron
Apical IP
Basal IP
Ptprz1 Mfge8 Id3 Slc1a3 Vcam1 Id1 Bmp7 Sostdc1 Fst Acvr2b Cux1 Ung Id4 Pcna Bmp2k Prc1 Ube2c Cenpf Mki67 Bmpr1a Pax6 Dbi Nog Bmper Bmpr1b Hes1 Sox2 Hes5 Alk Inhbb Sema6d Unc5d Sema3c Sstr2 Pam Neurod1 Slc17a6 Eomes Mfap4 Gadd45g Btg2 Chrd Grem1 Tbr1 Bcl11b Acvr1b Tubb3 Bhlhe22 Id2 Satb2 Acvr1 Acvr1c Bmp3 Acvr2a Bmp1 Bmpr2 Bmp2 Foxp2 Reln
Cell Marker
Apical IP
Apical precursor
Basal IP
Excitatory neuron
goi
IP/Excitatory neuron
Newborn neuron
Non−proliferative RGC
Proliferative RGC
Scaled standard residual
2
1
0
−1
−2

Fig. S5. Grem1 suppresses intrinsic BMP signaling in developing human excitatory neurons, likely via antagonism of paracrine BMP ligands.
(A) *GREM1* expression is associated with the excitatory neuronal lineage in the developing human cortex. Unsupervised clustering of human developmental brain cell populations from scRNAseq GSE103723 based on expression of lineage markers and transcripts encoding BMP ligands, BMP receptors (*BMPRI1/1b/ACVR2b,ALK, ACVR1b/1/1c/2a/BMPR2*), BMP antagonists (*FST, 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.
(B) Heat map depicting expression of BMP ligands in human developmental brain cell populations from scRNAseq GSE103723. Cell types 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.
(C) *GREM1* expression inversely correlates with enrichment of *BMP2,7* and BMP target genes ID1, 3, 4 expression in human excitatory neurons in mid-gestation. Expression of transcripts encoding BMP ligands antagonised by *GREM1* and ID target genes in *GREM1*$^{+}$ and *GREM1*$^{-}$ cells is displayed from human scRNAseq GSE103723. One way ANOVA with Tukey's multiple test. ns p>0.05, *p<0.05, **p<0.01,****p<0.0001
(D) *Grem1* expression is associated with the excitatory neuronal lineage in the developing mouse cortex. Unsupervised clustering of mouse embryonic brain cell populations from scRNAseq GSE107122 based on expression of lineage markers and transcripts encoding BMP ligands, BMP receptors (*Bmpri1/1b/Acvr2b,Alk, Acvr1b/1/1c/2a/Bmpr2*), BMP antagonists (*Fst, Nog, Chrd, Bmper, Grem1, Sostdc1*), and BMP target genes (*Id1-4*). Cell type is indicated by colour legend. 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. RGC, radial glial cells ; IP, intermediate progenitors ; Str, striatum **(E)** Schematic depicting BMP signaling regulation by *GREM1* in the developing cortex. Our analyses of scRNA data suggest BMP ligands are expressed by different cell populations in the developing cortex to the excitatory neuron populations that express Grem1/GREM1. Expression of the BMP target genes, ID1,3,4 was inversely correlated with *GREM1*-expression in excitatory neurons suggesting *GREM1* acts locally to antagonize BMP signaling. Cortical deletion of *Grem1* resulted in increased apoptosis and phospho-SMAD1,5,8 in Ctip2+ layer V/VI neurons, but not upper cortical layers consistent with Grem1 acting locally in layer V/VI to antagonize BMP signaling and enhance neuron survival. It is possible that Grem1 also promotes proliferation of progenitors (dashed red line)

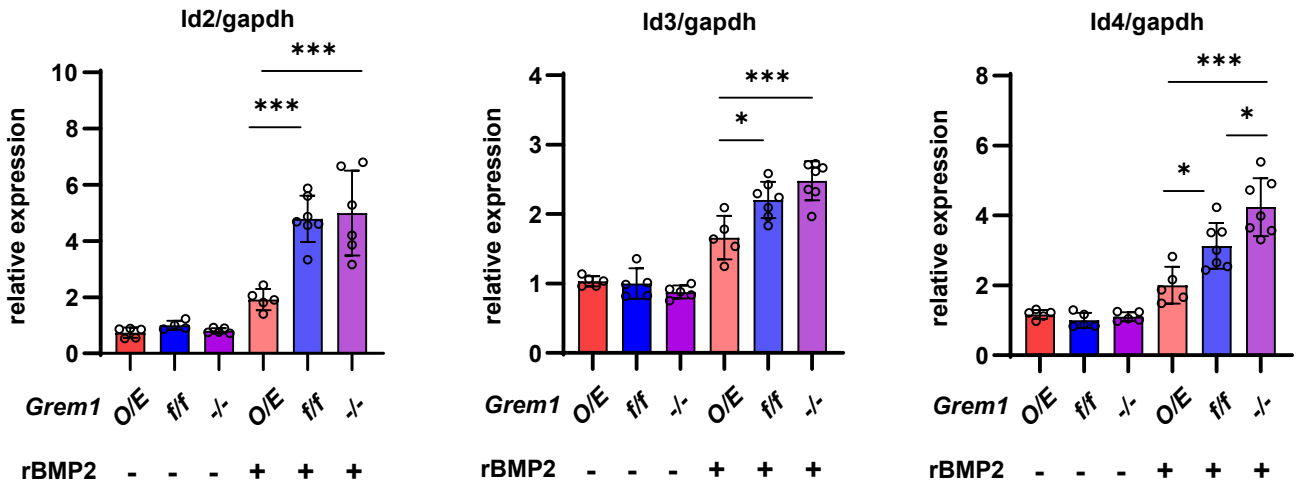


Fig. S6. Grem1 acts to antagonize BMP signaling in NSPCs resulting in altered downstream transcript levels

Transcript levels of BMP target genes, Id2, Id3 and Id4, normalized to Gapdh in Grem1flox/flox, Grem1-/- and Grem1O/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**

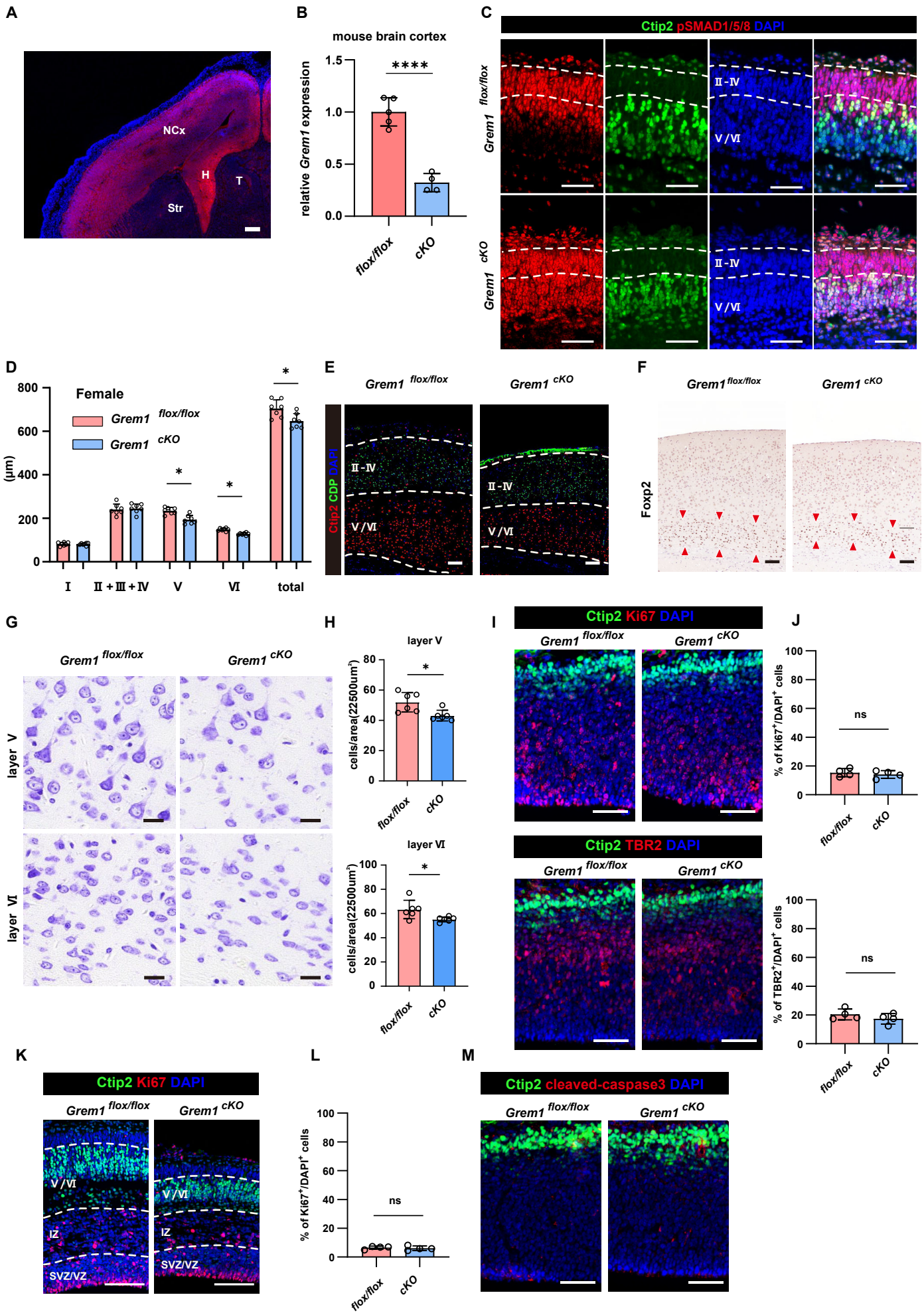


Fig. S7. Extended morphological, immunostaining and transcript analysis of *Grem1^{cKO}* mice and littermate controls

(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) *Grem1* mRNA levels normalized to *Gapdh* were determined using real time qRT-PCR in mouse cortical brain samples collected at postnatal day 0 from *Grem1^{cKO}* mice and littermate controls n=4 biological replicates. Columns, mean; bars, SD. t-test. ****p<0.0001

(C) Representative images of immunofluorescence staining of cortex of Grem1cKO mice and littermate controls at 20.5 dpc with layer Ⅴand Ⅵ with marker Ctip2 (green), phosphorylated smad1/5/8 (red), and DAPI (blue). n = 4 Scale bar = 100 μm.

(D) 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. **(E)** Representative images of immunofluorescence staining of cortex of *Grem1^{cKO}* mice and littermate controls at 10 weeks of age to visualise layer Ⅱ-Ⅳ with marker CDP (green), layer Ⅴand Ⅵ with marker Ctip2 (red), and DAPI (blue). n = 3 Scale bar = 100 μm.

(F) Representative images of immunohistochemical staining of cortex of *Grem1^{cKO}* mice and littermate controls at 10 weeks of age to visualise layer Ⅵ marker Foxp2 IHC (shown in red arrowheads). n = 3 Scale bar = 100 μm.

(G) Representative histological images of neocortical layer V and Ⅵ from *Grem1^{cKO}* mice and *Grem1$^{flox/flox}$* littermate controls at 10 weeks of age using Nissl. Scale bar = 20um.

(H) Quantification of (G) 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. **(I)** Representative images of immunofluorescence staining of cortex of *Grem1^{cKO}* mice and in 2 HPF of 3 biological replicates. Columns, mean; bars, SD. t-test. *p<0.05. Scale bar = 20um.

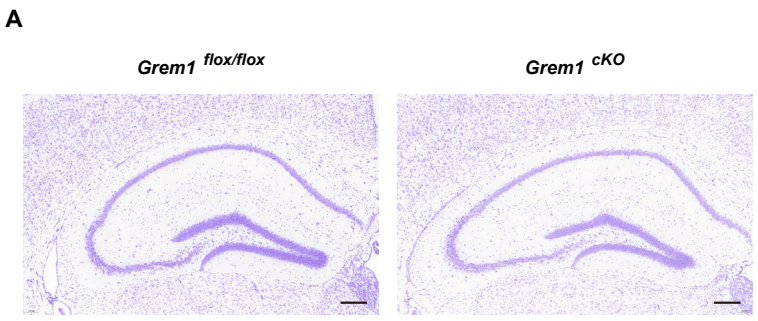
(I) Representative images of immunofluorescence staining of cortex of Grem1cKO mice and littermate controls with layer Ⅴand Ⅵ marker Ctip2 (green), Ki67 (red), and DAPI (blue) / Ctip2 (green), Tbr2 (red), and DAPI (blue) at 14.5 dpc. n = 4 Scale bar = 50 μm.

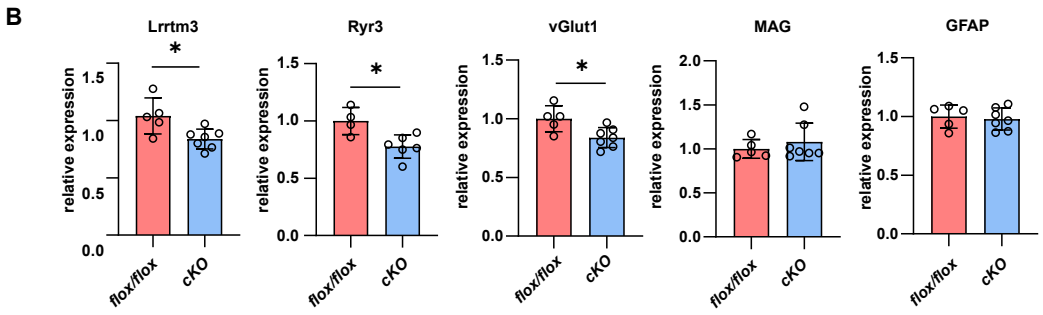
(J) Quantification of (I) showing the percentage of Ki67+ or Tbr2+cells that were also DAPI+ in 4 biological replicates. t-test. ns : not significant.

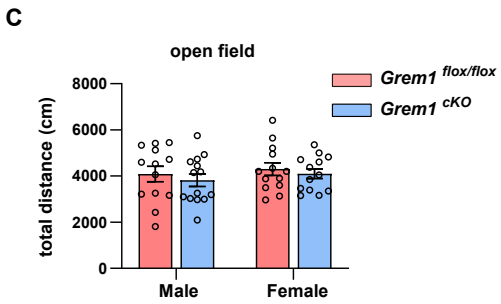
(K) Representative images of immunofluorescence staining of cortex of Grem1cKO mice and littermate controls with layer Ⅴand Ⅵ marker Ctip2 (green), Ki67 (red), and DAPI (blue) at 20.5 dpc. n = 4 Scale bar = 100 μm.

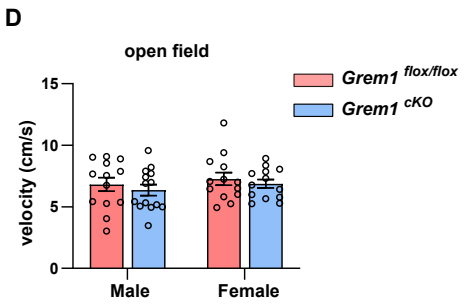
(L) Quantification of (K) showing the percentage of Ki67+ that were also DAPI+ in 4 biological replicates. t-test. ns : not significant.

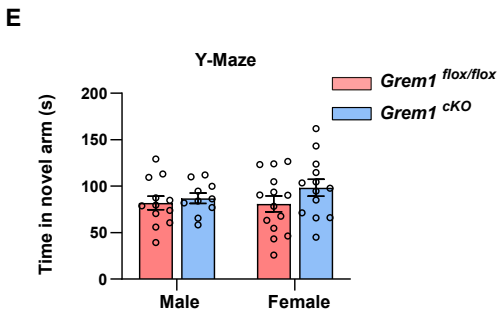
(M) Representative images of immunofluorescence staining of cortex of Grem1cKO mice and littermate controls with layer Ⅴand Ⅵ marker Ctip2 (green), cleaved caspase3 (red), and DAPI (blue) at 14.5 dpc. n = 4 Scale bar = 50 μm.











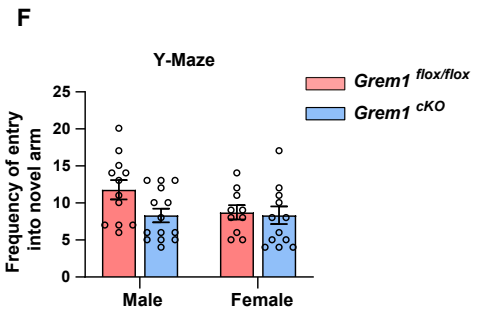


Fig.S8 Memory is not impaired in *Grem1*^{cKO} mice compared to *Grem1*$^{flox/flox}$ littermate ontrols

(A) 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.

(B) Transcripts from the *Id1* associated gene cluster (Fig2E), *Ryr3* and *Lrrtm3* , and differentiation markers, *vGlut1* for excitatory neurons, *GFAP* for astrocytes, and *MAG* for oligodendrocytes were evaluated in *Grem1*^{cKO} and *Grem1*$^{flox/flox}$ littermate control cortex at p10 using real time qRT-PCR. *Gene* levels were normalized to *Gapdh*. n=4-6 biological replicates. Columns, mean; bars, SD. unpaired t-test, two tailed. *p<0.05

(C,D) Open field 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}. unpaired t-test, two tailed. **(C)** The total distance moved. **(D)** The mean velocity. Columns, mean; bars, SEM.

(E,F) 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}. unpaired t-test, two tailed. **(E)** Cumulative duration spent in new arm of Y maze. **(F)** The number of entries to new arm of the Y maze. Columns, mean; bars, SEM.