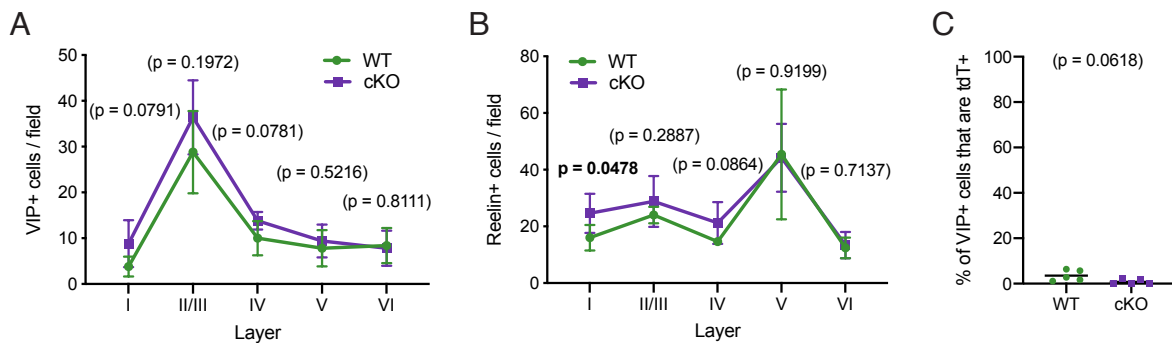


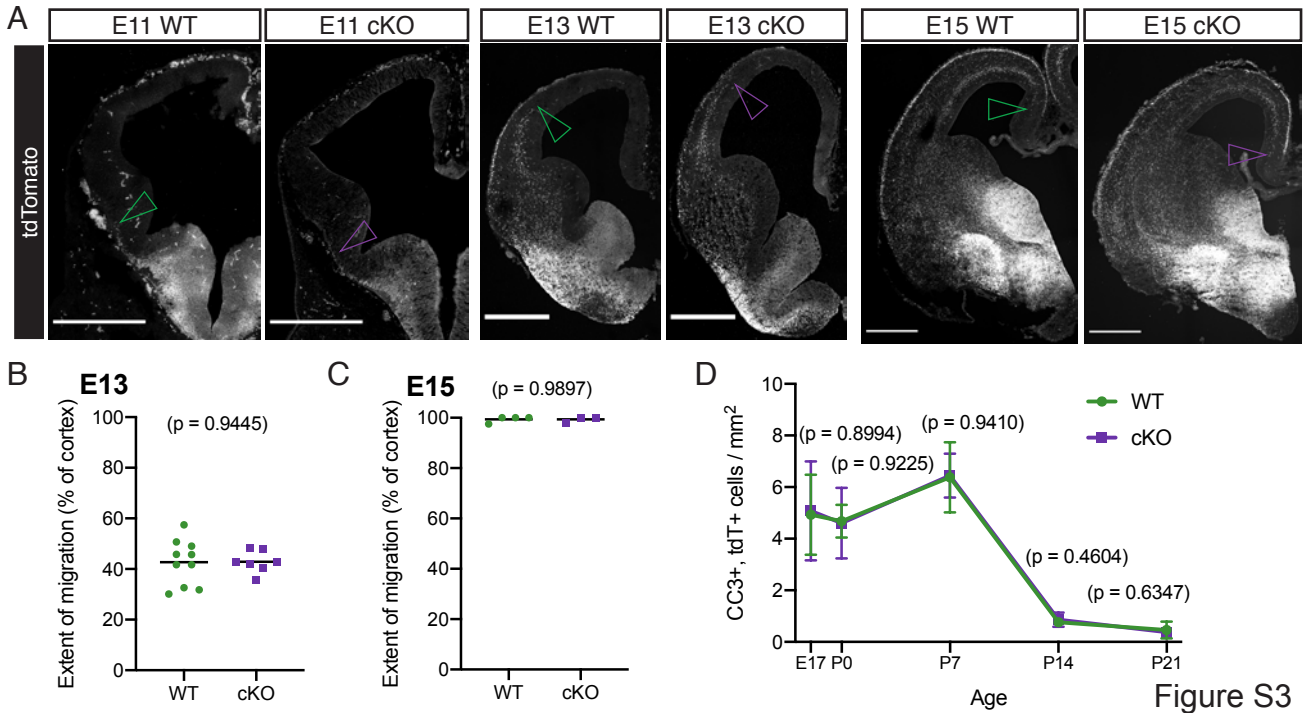
**Figure S1, related to Figure 1. Deletion of *Prdm16* in the *Nkx2.1* lineage causes loss of hippocampal interneurons. **A**) Representative images of the hippocampus of WT and cKO mice at P30, after immunofluorescence staining for tdTomato (red), counterstained with DAPI (blue). Scale bars, 500  $\mu$ m. Area labels (WT image): CA – *cornus Ammonis* (Ammon’s horn) regions 1, 2 and 3; DG – dentate gyrus; S – subiculum. Layer labels (cKO image): so – *stratum oriens*; sp – *stratum pyramidale*; sr – *stratum radiatum*; slm – *stratum lacunosum moleculare*; sl – *stratum lucidum*; ml – molecular layer; g – granule cell layer; h – hilus. **B**) Quantification of the number of tdTomato+ cells in the entire hippocampus of WT (green squares) and cKO (purple circles) mice (n = 10 for WT, n = 12 for cKO). **C**) Quantification of the total number of tdTomato+ cells across different areas of the entire hippocampus in WT and cKO mice at P30.**

**D)** Quantification of the number of tdTomato+ cells across different cellular layers in the entire hippocampus of WT and cKO mice at P30. **E)** Quantification of the total number of somatostatin+ cells in the entire hippocampus of P30 WT (n = 4) and cKO (n = 5) mice. **F)** Quantification of the total number of somatostatin+ cells in the entire hippocampus of P30 WT (n = 4) and cKO (n = 5) mice. **G, H)** Analysis of the distribution of tdTomato+ cells costained for SST (**G**) or PV (**H**) across the indicated layers of the hippocampus of P30 WT and cKO mice in areas CA1, CA2, CA3 and DG. **I, J)** Quantification of the number of tdTomato+ cells in the striatum (**I**) and nucleus accumbens (**J**) of P30 WT and cKO mice (n=3 mice per genotype). Means  $\pm$  S.D. are represented. Unpaired t-tests with Welch's correction (panels **B, E, F**) or multiple t-tests (panels **C, D**) were performed; p-values are indicated above the corresponding compared sets of data: those highlighted in bold represent significant differences ( $p < 0.05$ ).

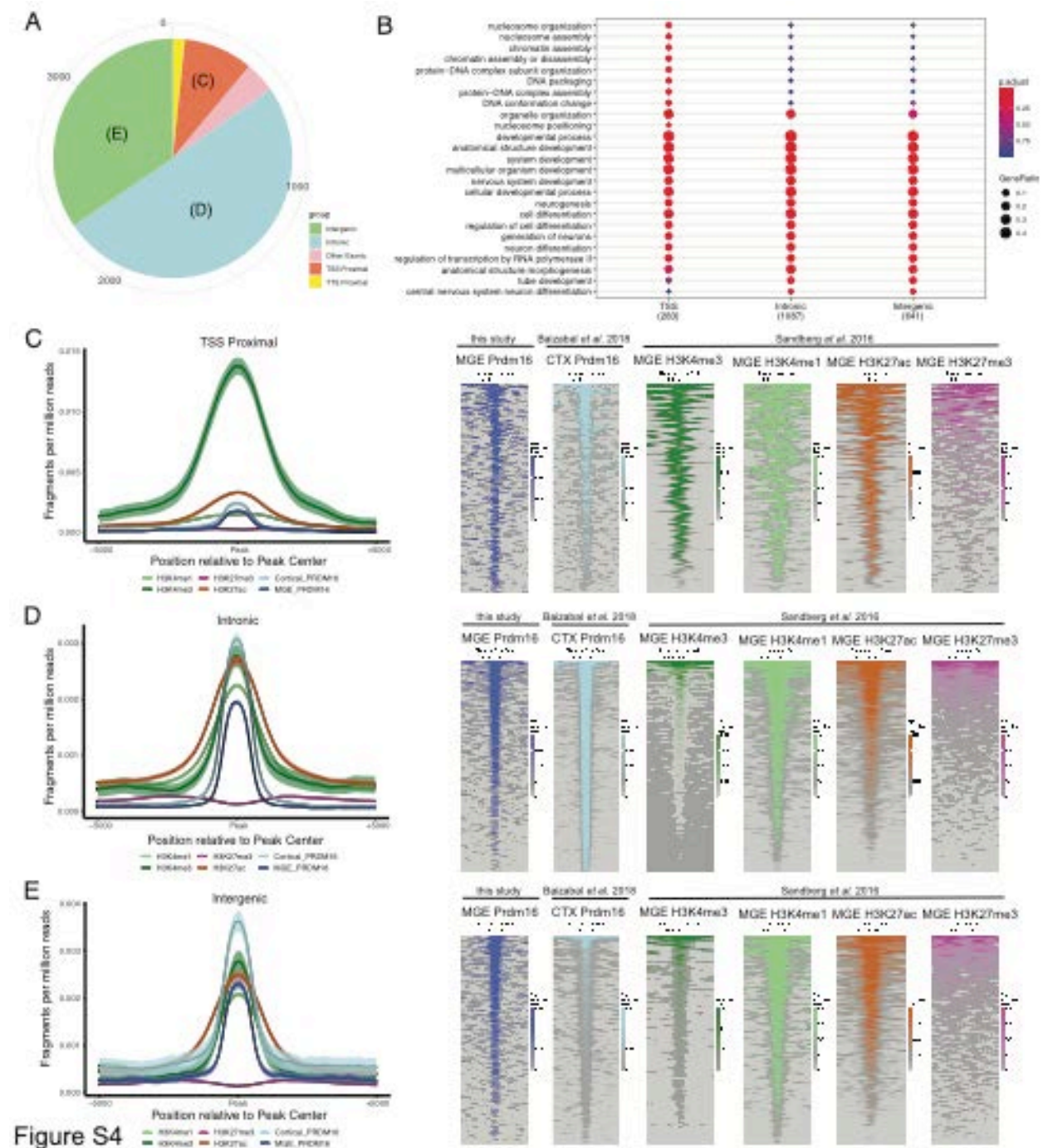
Figure S2



**Figure S2, related to Figure 2. Partial compensation of depleted MGE-derived interneurons is restricted to the reelin+ population in upper layers. A)** Number of VIP+ cells in each indicated cortical layer, per 1 mm-wide column. **B)** Number of reelin+ cells in each indicated cortical layer, per 1 mm-wide column. **C)** Percentage of VIP+ cells costained for tdTomato. Multiple t-tests (panels **A** and **B**) or unpaired t-tests with Welch's correction (panel **C**) were performed; p-values are indicated above the corresponding compared sets of data: those highlighted in bold represent significant differences ( $p < 0.05$ ).



**Figure S3, related to Figure 4. Loss of cortical interneurons is not due to defects in migration or increased cell death. A)** Overview of the migration of Nkx2.1-lineage cells (expressing tdTomato, white) within coronal hemisections of WT and cKO brains at E11, E13 and E15. Empty arrowheads indicate the extent of migration of MGE-derived interneurons into the cortex within each section. Scale bars, 500  $\mu$ m. **B, C)** Quantification of the extent of migration of Nkx2.1-lineage cells into the cortex, expressed as % of the length of the cortex occupied by tdTomato+ cells in WT (green circles) and cKO (purple squares) embryos at E13 (**B**) and E15 (**C**). **D)** Quantification of the number of cells costained with tdTomato (tdT+) and the apoptotic marker cleaved caspase 3 (CC3+) per  $\text{mm}^2$  in the cortex of WT (green circles) and cKO (purple squares) mice at the indicated developmental stages. Error bars represent S.D. Unpaired t-tests with Welch's correction (panels **B** and **C**) or multiple t-tests (panel **D**) were performed; p-values are indicated above the corresponding compared sets of data.



**Figure S4, related to Figure 6. Comparison of PRDM16 ChIP-Seq peaks between cortex and MGE. A)** Genome-wide distribution of E15 cortex PRDM16 ChIP-Seq peaks relative to gene annotations (TSS: transcription start site; TTS: transcription termination site). **B)** Top 30

gene ontology term enrichment in genes closest to PRDM16 ChIP-Seq peaks from E14 MGE (this study) and E15 cortex (Baizabal et al., 2018) experiments. Categories represent all PRDM16 peaks from both sets of experiments ('All'), common to both datasets ('Both'), or exclusive to either the cortex ('Cortical Only') or the MGE ('MGE Only'). **C-E** Read density in several embryonic ChIP-Seq experiments (aggregate line plots are shown on the left, and heatmaps are shown on the right). The peaks were grouped and analyzed by the location in either TSS (**C**), intronic (**D**) or intergenic (**E**) genomic regions. Data were generated for this study from E14 MGE (dark blue), or obtained from (Baizabal et al., 2018) (CTX, ChIP-Seq for PRDM16 in E15 cortex; light blue) and (Sandberg et al., 2016) (ChIP-Seq for each specified histone modification in E13 MGE; all other plots).

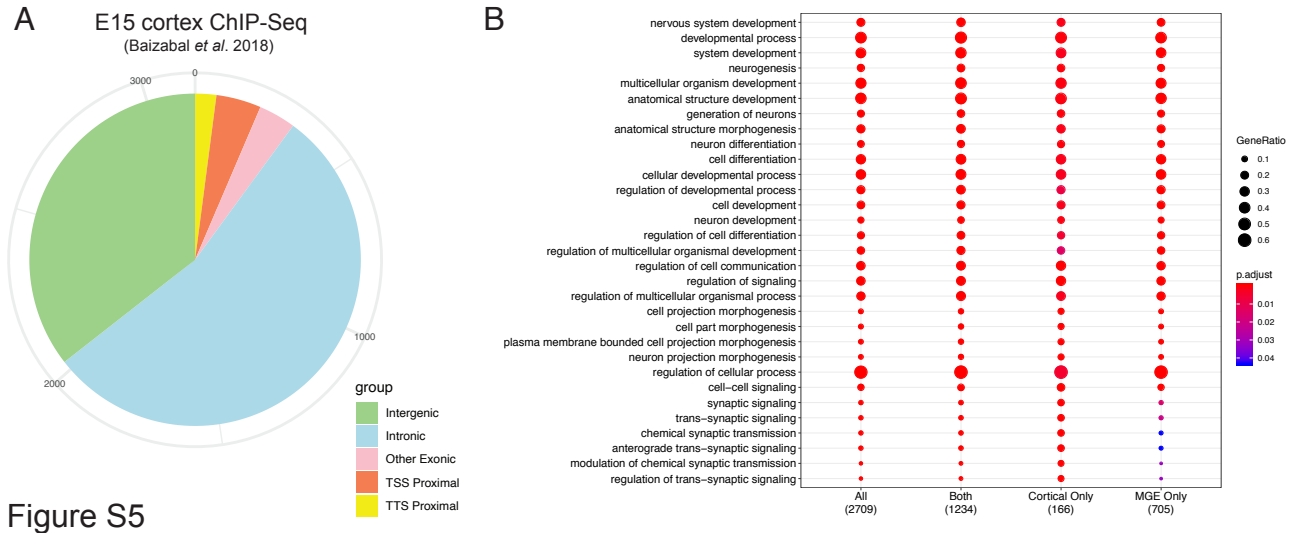


Figure S5

**Figure S5, related to Figure 6. Analysis of PRDM16 peaks relative to their genomic location. A)** Genome-wide distribution of E13 MGE PRDM16 ChIP-Seq peaks relative to gene annotations (TSS: transcription start site; TTS: transcription termination site) – note: this panel is the same data displayed as **Figure 6C**, and is included here just for clarity. **B)** Top 30 gene ontology term enrichment in genes closest to PRDM16 ChIP-Seq peaks located in TSS, intronic or intergenic locations, as indicated.

Table S1. Differentially regulated genes (RNA-Seq)

[Click here to Download Table S1](#)

Table S2. All PRDM16 MGE peaks

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Table S3. All PRDM16 peaks

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Table S4. PRDM16 peaks common to cortex and MGE

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Table S5. PRDM16 peaks exclusive to MGE

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Table S6. PRDM16 peaks exclusive to cortex

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Table S7. Overlapping PRDM16/ASCL1 peaks

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Table S8. Gene list comparisons

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Table S9. Reagents and resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Chicken polyclonal anti-GFP	Aves	Cat # GFP-1020
Rat monoclonal anti-pH3	Abcam	Cat # ab10543
Guinea pig polyclonal anti-PRDM3/PRDM16	Jeremy Dasen, NYU	N/A
Rabbit polyclonal anti-PRDM16	Patrick Seale, UPenn	N/A
Mouse monoclonal anti-Parvalbumin	Sigma	Cat # SAB4200545
Mouse monoclonal anti-Reelin	Millipore	Cat # MAB5364
Chicken polyclonal anti-RFP	Rockland	Cat # 600-901-379
Rabbit polyclonal anti-Somatostatin	Peninsula	Cat # T-4103
Rabbit polyclonal anti-VIP	Immunostar	Cat # 20077
Goat polyclonal anti-chicken Alexa 488	Thermo Fisher	Cat #A11039
Goat polyclonal anti-chicken Alexa 546	Thermo Fisher	Cat #A11040
Goat polyclonal anti-guinea pig Alexa 488	Thermo Fisher	Cat #A11073
Goat polyclonal anti-mouse Alexa 488	Thermo Fisher	Cat #A11001
Goat polyclonal anti-mouse Alexa 647	Thermo Fisher	Cat #A21236
Goat polyclonal anti-rabbit Alexa 488	Thermo Fisher	Cat #A11034
Goat polyclonal anti-rabbit Alexa 647	Thermo Fisher	Cat #A21245
Goat polyclonal anti-rat Alexa 488	Thermo Fisher	Cat #A11006
<b>Bacterial and Virus Strains</b>		
Replication-incompetent eGFP-expressing retrovirus	(Palmer et al., 1999)	N/A
<b>Critical Commercial Assays</b>		
RNAscope V2 Reagent Kit	Advanced Cell Diagnostics	Cat # 323100
<b>Deposited Data</b>		
<b>Experimental Models: Cell lines</b>		
HEK293 retrovirus packaging cell line	(Palmer et al., 1999)	293 gp NIT-GFP
<b>Experimental Models: Organisms/Strains</b>		
Mouse: CD-1	Charles River	Strain code 022
Mouse: C57BL/6J-Tg(Nkx2-1-cre)2Sand/J (Nkx2.1-Cre)	Jackson Laboratory	Stock no. 008661
Mouse: B6.129-Prdm16 <sup>tm1.1Brsp</sup> /J (Prdm16 <sup>fl</sup> )	Jackson Laboratory	Stock no. 024992
Mouse: B6.Cg-Gt(ROSA)26Sor <sup>tm14(CAG-tdTomato)Hze</sup> /J (Ai14)	Jackson Laboratory	Stock no. 007914
<b>RNAscope probes</b>		
<i>Lmo1</i>	Advanced Cell Diagnostics	Cat # 511211-C3
<i>Pdzrn3</i>	Advanced Cell Diagnostics	Cat # 517061
<i>tdTomato</i>	Advanced Cell Diagnostics	Cat # 317041
<b>Software and Algorithms</b>		
ImageJ 1.52o	(Schneider et al., 2012)	<a href="http://imagej.nih.gov/ij">http://imagej.nih.gov/ij</a>
Fiji 2.0.0-rc-68/1.52g	(Schindelin et al., 2012)	<a href="http://fiji.sc">http://fiji.sc</a>
Prism 7, Prism 8	GraphPad	
MATLAB	Mathworks	
Igor Pro	Wavemetrics	