SUPPLEMENTAL FIGURES, TABLES, AND EXPERIMENTAL PROCEDURES

Supplemental Figure S1 and figure legend, related to Figure 1

Supplemental Figure S2 and figure legend, related to Figure 3

Supplemental Figure S3 and figure legend, related to Figure 4

Supplemental Figure S4 and figure legend, related to Figure 5

Supplemental Figure S5 and figure legend, related to Figure 6

Supplemental Figure S6 and figure legend, related to Figure 7

Supplemental Figure S7 and figure legend, related to Figure 8

Supplemental Table S1, related to Figure 1

Supplemental Table S2, related to Figure 2

Supplemental Experimental Procedures

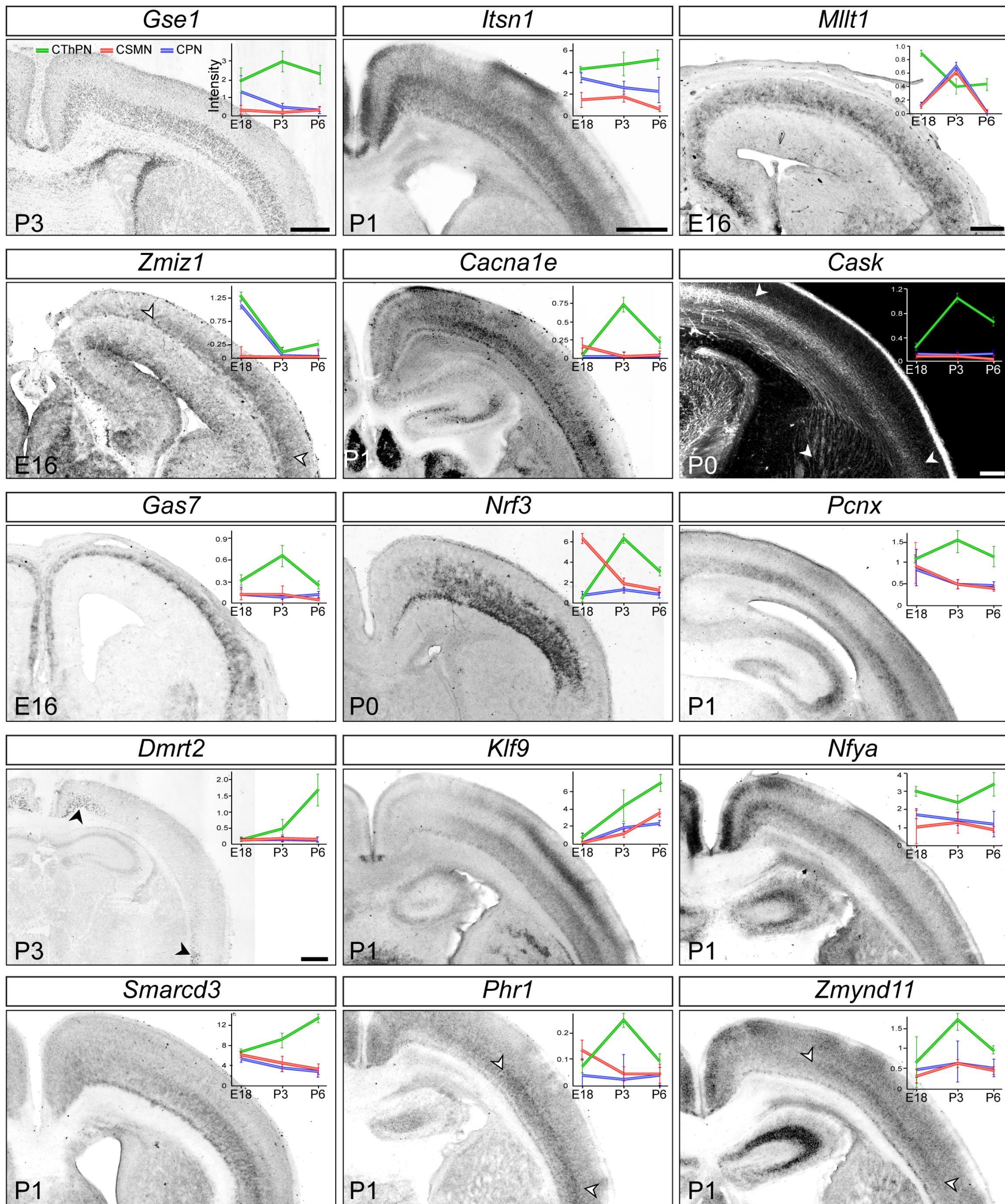

Figure S1. (related to Figure 1) Genes identified by microarray analysis are expressed in the cortical plate and layer VI.

In situ hybridization or immunocytochemistry showing expression in layer VI for all genes selected from Figure 1 (except genes shown in Figure 2). For each gene, the temporal course of gene expression from microarray analysis by CThPN (green), CSMN (red), and CPN (blue) are shown in the same panel. Expression is shown at E16 or postnatal

stages (P0, P1, or P3). For genes expressed by CThPN subpopulations (*Zmiz1, Cask, Dmrt2, Phr1, Zmynd11*), arrowheads indicate restricted expression. Immuncytochemistry for CASK detects CThPN in lower layer VI, and CThPN axons in the internal capsule (white arrowheads). Scale bars, 250 μm (E16), 150 μm (P0) and 500 μm (P1, P3).

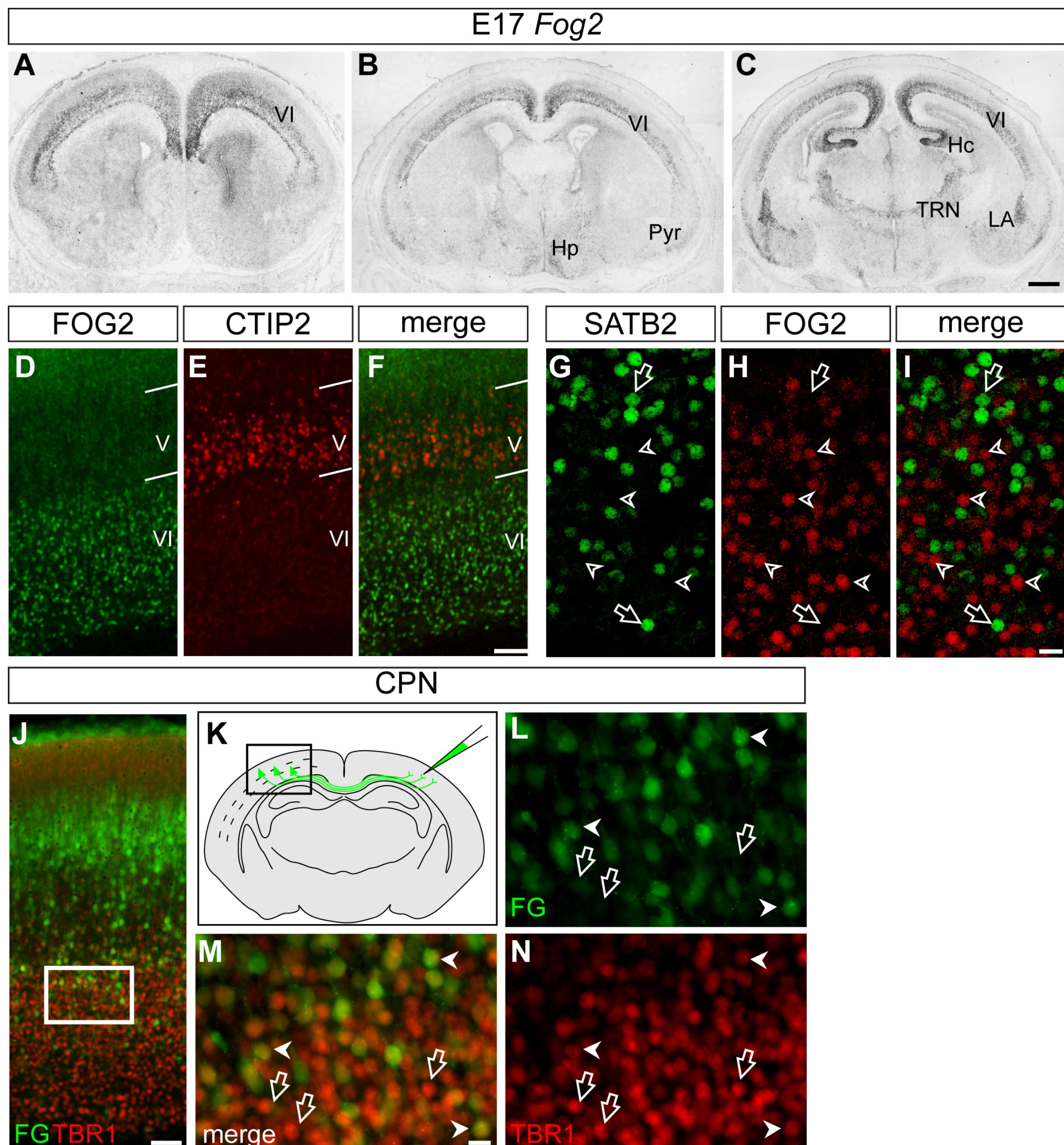

Figure S2. (related to Figure 3) *Fog2* is expressed by CThPN across cortical areas.

(A-C) *Fog2* is strongly expressed in layer VI by CThPN, and by other neuron subtypes in other forebrain regions. (D-I) In the cortex, FOG2 is excluded from high level CTIP2-expressing neurons (SCPN) (D-F), and SATB2-expressing neurons (CPN, arrows) (G-I). (J-N) FOG2 expression is CThPN specific, compared to TBR1, also expressed in layer VI CPN (white arrowheads in L-N). Scale bars, 500 μm (A-C), 100 μm (D-F, J), 20 μm (G-I) and 10 μm (L-N).

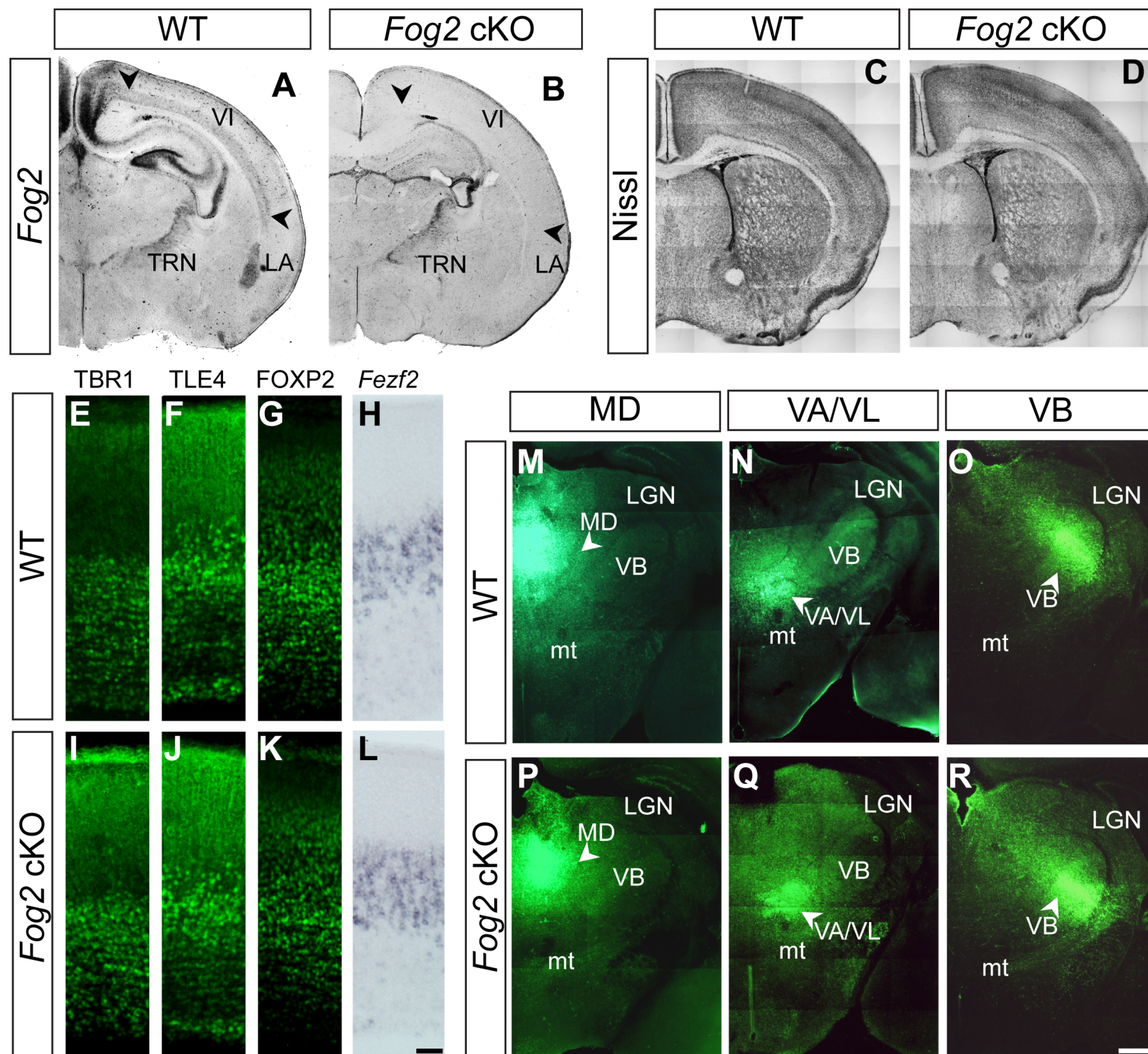

Figure S3. (related to Figure 4) Cortical morphology and expression of widely used layer VI markers are not affected by the absence of *Fog2* function in cortex. (A-B) *Fog2* cKO mice show absence of *Fog2* mRNA specifically in the neocortex (layer VI, black arrowheads), but show normal expression in other forebrain regions. (C-D) Nissl staining of cortex in wild-type (C) and *Fog2* cKO (D) mice at P7 reveals no differences in laminar patterning. (E-L) IHC for TBR1 (E, I), TLE4 (F, J), FOXP2 (G, K), and ISH for *Fezf2* (H, L) show no difference in expression of these markers between WT and *Fog2* cKO cortices. (M-R) FluoroGold injections in thalamic nuclei in WT and *Fog2* cKO brains: (M, P) Mediodorsal nucleus (MD); (N, Q) Ventral anterior/Ventro lateral nucleus (VA/VL); and (O, R) Ventrobasal complex (VB). Scale bar, 50 μm (E-K) and 500 μm (M-R).

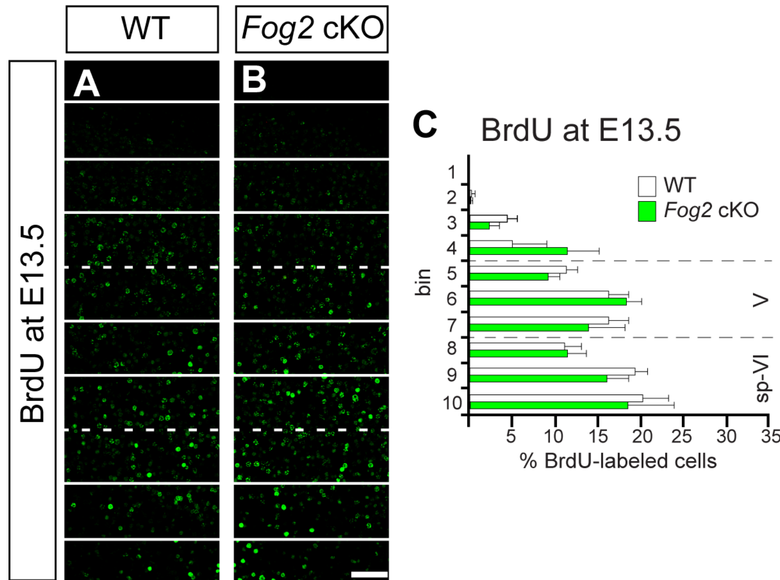

Figure S4. (related to Figure 5) In the absence of *Fog2* function, SCPN are born and migrate normally. (A-C) Quantification of E13.5 BrdU birthdated neurons at P6 across the thickness of motor cortex. There is no difference between WT and *Fog2* cKO cortex in the number or distribution of BrdU+ neurons at P6 that were labeled at E13.5, indicating that SCPN generation and migration are not altered in *Fog2* cKO mice. Scale bar, 100 μm (A-B). Data are represented as mean ± SEM.

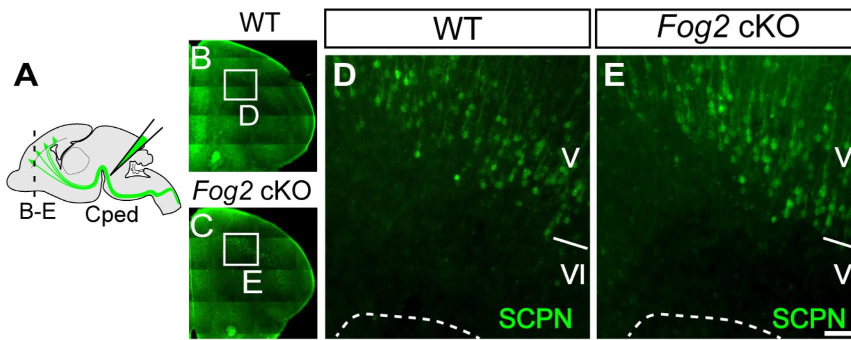

Figure S5. (related to Figure 6) Axonal projections to the cerebral peduncle are normal in the absence of *Fog2* function. (A-E) SCPN are correctly located in layer V, and not in layer VI, in motor cortex of *Fog2* cKO mice. (A) Schematic of experimental approach. (B, C) Low magnification images of the area analyzed. (D-E) High magnification of area boxed in B-C. Scale bar, 60 μm (D-E).

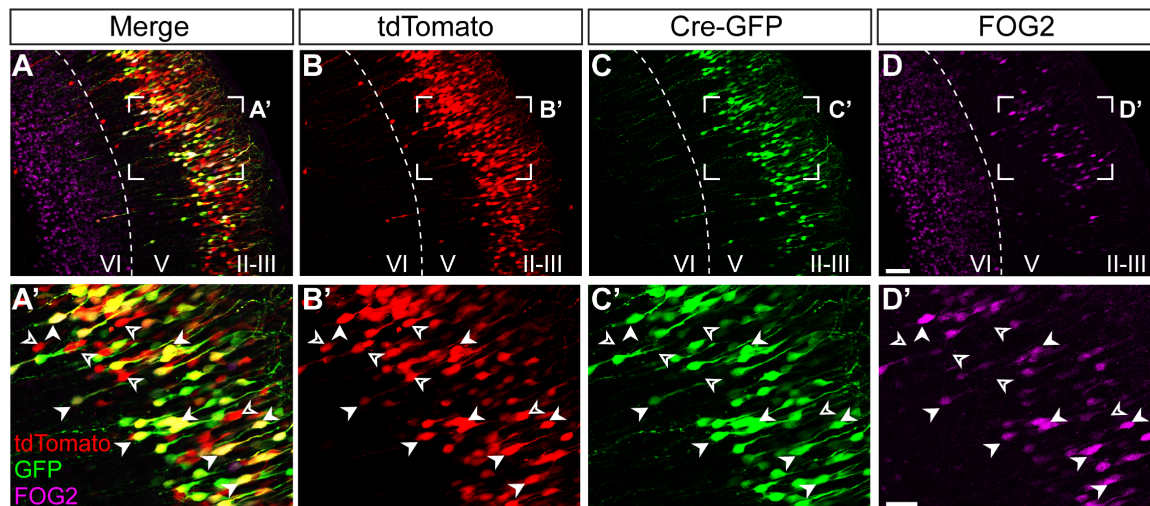

Figure S6. (related to Figure 7) *Fog2* is mis-expressed exclusively in Cre-electroporated neurons in CTV-*Fog2* embryos (CAG-floxed-Stop-*Fog2*-IRES-*Gfp*). (A-D') Cre and tdTomato co-electroporation in CTV-*Fog2* embryos at E13.5 results in *Fog2* mis-expression in layer V and upper-layers, only in Cre-recombined neurons (GFP$^+$, FOG2$^+$ detected by immunolabeling; white arrowheads in A'-D'). FOG2 is not expressed in tdTomato-only electroporated neurons (open arrowheads, A'-D'). (A'-D') High magnification of area boxed in A-D. Scale bars, 50 μm (A-D) and 25 μm (A'-D').

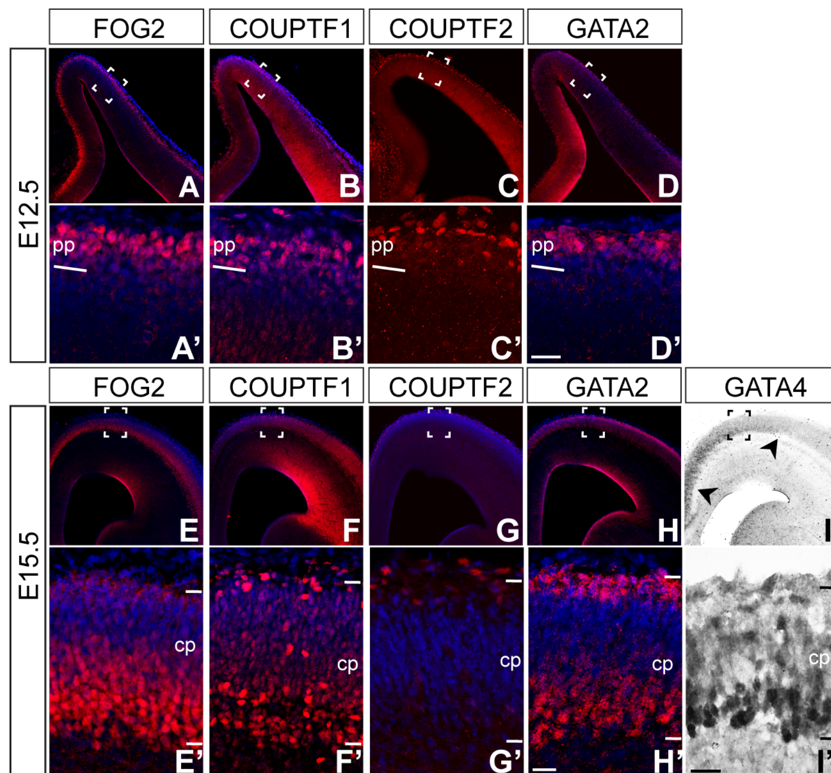

Figure S7. (related to Figure 8) COUPTF1, COUPTF2, GATA2, and GATA4 expression in the developing cortex. Immunocytochemistry for FOG2, COUPTF1, and GATA2 shows expression of these proteins in the preplate of developing cortex at E12.5 (A, B, D), and in the cortical plate at E15.5 (E, F, H). Higher magnification images of the boxed areas corresponding to developing motor cortex are shown in (A'- H'). Immuncytochemistry for COUPTF2 shows expression primarily by progenitors and meningeal cells, but not in the preplate at E12.5 (C-C'), nor in the dorsal cortical plate at E15.5 (G-G'). Immuncytochemistry for GATA4 shows expression at E15.5 in a medio-lateral gradient, with highest expression in the medial and dorsal cortical plate, but little or no expression the lateral cortical plate (I-I'). No expression was detected at earlier time points (not shown). Scale bars, 20 μm (A'-I').

	GO ID	Cluster frequency (% of genes in the input set)	Cluster frequency (% of genes in the genome	P-value	FDR %	False positive	Genes annotated to the term
cell differentiation	**GO:0030154**	29.10%	2807 of 25493 genes, 11.0%	0.0002	0.00	0.00	*Gas7, Gabbr1, Mycbp2, Ptk2b, Psen1, Kitl, Cxadr, Cntn2, Myo1e, Fog2, Nfib, Igfpb3, Tbr1, Sema5a, Pdpk1, Gabrb3, Smarcd3, Smap1, Stxbp1, Ror2, Antxr1, Bcl2, Socs2, Glis2, Gdf10, Tmod1, Ngfr, Drd1a, Neurod2, Shb, Ntrk3, Ank2*
regulation of developmental process	GO:0050793	20.00%	1631 of 25493 genes, 6.4%	0.002	0.06	0.02	*Gas7, Smap1, Ptk2b, Ror2, Psen1, Kitl,, Foxp2, Bcl2, Cxadr, Socs2, GDF10, Cntn2, Neurod2, Ngfr, Dmrt2, Shb, Fog2, Nfib, Igfpb3, Tbr1, Ntrk3, Smarcd3*
negative regulation of cellular process	GO:0048523	31.80%	2895 of 25493 genes, 11.4%	9.53E-06	0.00	0.00	*Gabbr1, Ptk2b, Psen1, Kitl, Cxadr, Tim3, Cntn2, Fog2, Nfib, Igfbp3, Tle4, Grik3, Mdm2, Pdk1, Rgs16, Gabrb3, Itsn1, Cdkn1a, Tbc1d14, Cask, Stxbp1, Ror2, Zmynd11, Foxp2, Bcl2, Socs2, Glis2, Ngfr, Drd1a, Neurod2, Shb, Cnot7, Ntrk3, Prkar2b, Mllt1*
positive regulation of cellular process	GO:0048522	30.00%	3133 of 25493 genes, 12.3%	0.0008	0.08	0.02	*Gabbr1,, EXTL3, Ptk2b, Psen1, Kitl, Nfia, Cntn2, Cxxc5, Ppp1r12a, Fog2, Nfib, Igfbp3, Tbr1, Mdm2, Pdpk1, Smarcd3, Itsn1, Cckn1a, Nfya, Cask, Smap1, Stxbp1, Ror1, Foxp2, Bcl2, Socs2, Glis2, Gdf10, Ngfr, Drd1a, Neurod2, Cnot7, Ntrk3*
negative regulation of cell death	**GO:0060548**	11.80%	631 of 25493 genes, 2.5%	0.004	0.04	0.02	*Itsn1, Fog2, Cdkn1a, Mdm2, Stxbp1, Ptk2b, Ntrk3, Pdpk1, Psen1, Kitl, Bcl2, Gabrb3, Ngfr*
cell adhesion	**GO:0007155**	15.50%	847 of 25493 genes, 3.3%	0.00016	0.00	0.00	*Tgfbl, Cdh9, Cask, Col5a1, Stxbp1, Ptk2b, Antxr1, Ror2, Psen1, Kitl, Bcl2, Cxadr, Cntn2, Ppp1r12a, Sdk1, Pdpk1, Dsg2S*
intracellular signal transduction	**GO:0035556**	22.7%	1825 of 25493 genes, 7.2%	0.00023	0.00	0.00	*tsn1, Tbc1d14, Cdkn1a, Spsb1, Smap1, Ptk2b, Ror2, Psen1, Kitl, Arf3, Zmynd11, Bcl2, Socs2, Neurod2, Drd1a, Ngfr, Cxxc5, Igfbp3, Plch1, Mdm, Ntrk3, Pdpk1, Srgap1, Ppp1r1b, Ank2*
response to stimulus	**GO:0050896**	51.80%	7403 of 25493 genes, 29.0%	0.00052	0.09	0.02	*Gabbr1, Mycbp2, Pnliprp2, Mme, Col5a1, Ptk2b, Psen1, Kitl, Akt3, Cxadr, Timp3, Cntn2, Myo1e, Arhgap25, Cxxc5, Pde9a, Nfib, Cygb, Rad21, Igfbp3, Plch1, Tle4, Grik3, Tbr1, Mdm2, Sema5a, Pdpk1, Srgap1, Rgs16, Gabrb3, Itsn1, Tbc1d14, Cdkn1a, Spsb1, Cask, Cacng8, Htr1f, Smap1, Stxbp1, Ror2, Arf3, Zmynd11, Foxp2, Bcl2, Tmod1, Glis2, Socs2, Neurod2, Ngfr, Drd1a, Plxna2, Marco, Ntrk3, Prkar2b, Cacna1e, Ppp1r1b, Ank2*
cognition	**GO:0050890**	7.30%	189 of 25493 genes, 0.7%	0.002	0.05	0.02	*Foxp2, Prkar2b, Cacna1e, Ppp1r1b, Cntn2, Neurod2, Drd1a, Psen1*
learning or memory	GO:0007611	7.30%	177 of 25493 genes, 0.7%	0.001	0.07	0.02	*Foxp2, Prkar2b, Cacna1e, Ppp1r1b, Cntn2, Neurod2, Drd1a, Psen1*
learning	GO:00	6.40%	104 of 25493 genes, 0.4%	0.00042	0.1	0.02	*Foxp2, Prkar2b, Cacna1e, Ppp1r1b, Cntn2, Neurod2, Drd1a*
neuron development	**GO:0048666**	13.6%	735 of 25493 genes, 2.9%	0.001	0.08	0.02	*Gas7, Mycbp2, Nfib, Tbr1, Stxbp1, Sema5a, Ptk2b, Ntrk3, Psen1, Bcl2, Gabrb3, Cntn2, Ngfr, Drd1a, Neurod2*
neurogenesis	GO:0022008	15.50%	1135 of 25493 genes, 4.5%	0.009	0.03	0.02	Gas7, Mycbp2, *Stxbp1, Ptk2b, Psen1, Bcl2, Socs2, Cntn2, Neurod2, Ngfr, Drd1a, Nfib, Tbr1, Sema5a, Ntrk3, Gabrb3, Smarcd3*
neuron projection morphogenesis	GO:0048812	10.00%	418 of 25493 genes, 1.6%	0.002	0.05	0.02	*Gas7, Mycbp2, Nfib, Tbr1, Stxbp1, Sema5a, Ntrk3, Psen1, Bcl2, Ngfr, Cntn2*
axon development	GO:0061564	9.10%	348 of 25493 genes, 1.4%	0.003	0.04	0.02	*Mycbp2, Nfib, Tbr1, Stxbp1, Sema5a, Ntrk3, Psen1, Bcl2, Ngfr, Cntn2*
neuron differentiation	GO:0030182	14.50%	963 of 25493 genes, 3.8%	0.005	0.04	0.02	*Gas7, Mycbp2, Stxbp1, Ptk2b, Psen1, Bcl2, Socs2, Cntn2, Neurod2, Ngfr, Drd1a, Nfib, Tbr1, Sema5a, Ntrk3, Gabrb3*
regulation of synaptic transmission	GO:0050804	7.30%	222 of 25493 genes, 0.9%	0.007	0.04	0.02	*Gabbr1, Grik3, Stxbp1, Psen1, Ngfr, Drd1a, Neurod2, Cntn2*

Supplemental Table 1. (related to Figure 1) Summary of Gene Ontology terms for biological functions over-represented among CThPN-genes.

Clone name	Source	Primers		Amplicon size	Plamid Vector
Fog2	RT-PCR	CGGACGACAGCATCTCCT	TTTTCCACTGCTGGGAGC	833	pCR II TOPO
Itsn1	RT-PCR	GAGTGGACACTTAACAGGTCCC	TCTTTTGAGGGTAAGAAGCGAG	1000	pCR II TOPO
Cacna1e	RT-PCR	CACAAAGGTGCCTGTAGCT	GATTAGGAATGACTCAGTGG	713	pCR II TOPO
Cxxc5	RT-PCR	CAGCAGCAGTAACACCAATAGC	GCACTGCTCACAGTTGATGC	775	pCR II TOPO
Dmrt2	RT-PCR	TCAAGCTGCTGCCTTATTCC	ACTTCTGGCTCTCCTTGACC	752	pCR II TOPO
Gse1	RT-PCR	TCTTCTCTCTGCCTGGAAGC	TCGTAGATGTAGGCTGGATC	760	pCR II TOPO
Mllt1	RT-PCR	CACTCTCTGCCTTCTCTGACC	GGTGTTCTCCTCTCCTGACG	749	pCR II TOPO
Nrf3	RT-PCR	CACGATTCTGTTGAGCTTGG	TAGCCTCGTGATCTTCTCG	629	pCR II TOPO
Pcnx	RT-PCR	TCATGTCCATCCTGTTCGC	AAGACTTGGAGCATGGAAGC	754	pCR II TOPO
Phr1	RT-PCR	CTCACCTGGTCTCAAACACC	ATGTCATCAGCATCCACAGC	748	pCR II TOPO
Rad21	RT-PCR	TCAGCAGATGCTTCATGGTC	GGCAGGTTTTCCTTTCAACA	578	pCR II TOPO
Zmiz1	RT-PCR	CCTCCACATCAAGGATGACC	TGAGTTCCGGCAGTAGATCC	617	pCR II TOPO
Ror2	RT-PCR	GGAGATGCCACTCATCAGC	CACGTCCTGGTTGGAGTACC	748	pCR II TOPO
Shb	RT-PCR	AGCTGATGACTACTCCGATCC	CTTCCTGGTGGTGTAGTAGTGG	694	pCR II TOPO
Gas7	RT-PCR	GGAACTGCTTTCCCCCTTAC	CTGAGCACAGGTTGGTGAGA	442	pCR II TOPO
Smarcd3	RT-PCR	CTATCAGCCTCCCCAGTTCA	GAGCAGGTCTTGGACGTAGC	502	pCR II TOPO
Nfya	RT-PCR	TGGCTGACAAACTGAAGTGG	CTAGCATGTGGGCAGACAGA	412	pCR II TOPO
Zmyd11	RT-PCR	GAGAGAGTGGTCCGTGAAGC	GTGCAAAATGGGAAGCAAAT	418	pCR II TOPO
Klf9	RT-PCR	GAGGTGACCAAGGAACACG	GATGGAACTCGGTGTGACG	735	pCR II TOPO
Tle4	RT-PCR	ATCGTCTTGGAAGAACATGG	GTTGCCCAAATAGACTCAAAGG	432	pCR II TOPO
Fezf2	Described in Arlotta et al., 2005				

Supplemental Table 2. (related to Figure 1 and Figure 2) cDNA clones for riboprobes.

Supplemental Experimental Procedures

Animals

Fog2^{floxed} mice were generated by Manuylov and colleagues (Manuylov et al., 2007, RRID:IMSR_JAX:007266). *Emx1*^{Cre} (RRID:IMSR_JAX:005628) mice were purchased from Jackson Laboratories. *Fog2* cKO animals are *Emx1-Cre*$^{+}$*;Fog2*$^{fx/fx}$ and WT are *Fog2*$^{fx/fx}$. CTV-*Fog2* mice (CAG-floxed-Stop-*Fog2*-IRES-*Gfp* cassette inserted in the Rosa26 locus) were the generous gift of Dr. L. Goodrich at Harvard Medical School. The date of vaginal plug detection was designated E0.5, and the day of birth as P0.

Purification of CThPN

CThPN in C57BL/6 mice were retrogradely labeled with green fluorescent microspheres (Lumafluor Corp., FL) injected into the thalamus of E17.5, P1, and P4 mice. All injections were performed using an ultrasound back-scatter microscopy and injection guidance system (Vevo 770, VisualSonics, Toronto). Cortex from E18.5, P3, and P6 mice was collected and dissociated; CThPN were purified by FACS as described in (Arlotta et al., 2005), using fluorescence, size, and side scatter to purify single retrogradely labeled CThPN to > 99.5 purity.

Affymetrix Microarrays

RNA was extracted using StrataPrep Total RNA Micro Prep Kits (Stratagene). RNA was amplified and biotinylated using a BioArray HighYield RNA Transcript Labeling Kit (Enzo). 6 RNA samples were hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 Array chips; GeneChips were processed using an Affymetrix Fluidics Station 450, and scanned with an Affymetrix Scanner 3000 7G. Data from microarrays were analyzed

as previously described (Arlotta et al., 2005). Microarray data have been deposited in the Gene Expression Omnibus database at NCBI (Accession GSE61711).

Immunocytochemistry and *in situ* hybridization

Brains were fixed and stained using standard methods (Molyneaux, et al., 2005). Primary antibodies and dilutions used: mouse anti-BrdU, 1:750 (RRID:AB_95024); rat anti-CTIP2, 1:500 (RRID:AB_2064130); rabbit anti-COUPTF1, 1:1000 (RRID:AB_1603458); rabbit anti-COUPTF2, 1:1000 (RRID:AB_1140738); rabbit anti-FOG2, 1:250 (RRID:AB_2218978); chicken anti-GFP, 1:200 (RRID:AB_10000240); rabbit anti-GFP, 1:500 (RRID:AB_2534134); rabbit anti-GATA2, 1:750 (RRID:AB_2294456); rabbit anti-GATA4, 1:200 (RRID:AB_2247396; RRID:AB_10670538); mouse anti-SATB2, 1:200 (RRID:AB_882455); rabbit anti-TBR1, 1:500 (RRID:AB_2200219); rabbit anti-FOXP2 1:1000 (RRID:AB_2107107); goat anti-TLE4, 1:200 (RRID:AB_2203853). Rat anti-BrdU requires antigen retrieval, as described in Magavi and Macklis, 2008. ISH was performed as previously described (Arlotta et al., 2005). cDNA clones for riboprobes are listed in Supplemental Table S2.

Retrograde Labeling

Projection neurons were retrograde labeled from their axon terminals by ultrasound guided injection of FluoroGold (FG, Molecular Probes) at P1 to P3. CThPN were labeled by injection into dorsal thalamus; CSMN were labeled by injection into upper cervical spinal cord; SCPN were labeled by injection into the cerebral peduncle; and CPN were labeled by injection into the contralateral hemisphere. For CThPN retrograde labeling from individual thalamic nuclei (MD, VA/VL, and VB), FG

microinjections were performed at P2, and tissue collected at P6. *Fog2* cKO and WT brains with matched injections (n=4 to 6, for each genotype, for each thalamic nucleus) were selected and processed for FG IHC. Labeled CThPN were systematically quantified in four coronal sections, 200 microns apart, in the area of maximum labeling. Neurons were counted by investigators blinded to genotype in a cortical area of equal size.

BrdU birthdating

Timed pregnant females were injected with bromodeoxyuridine (50 mg/kg, i.p) at E12.5 or E13.5. Brains were collected at P6, and processed for BrdU and Ctip2 IHC (n=5 each genotype). Quantification of BrdU-labeled cells, and their distribution within cortical layers, was analyzed using established methods (Molyneaux et al., 2005; Lai et al., 2008).

***In utero* electroporation**

Electroporation were performed as described in Molyneaux et al., 2005. For loss-of-function experiments, a *CMV/β-actin* promoter plasmid (derived from CBIG, gift of C. Lois) was used to drive expression of IRES-Gfp (control^{Gfp}) or Cre-IRES-Gfp (Cre^{Gfp}) in WT *Fog2$^{fx/fx}$* embryos at E11.5 (n=3 for each condition). For *Fog2* mis-expression experiments, different concentrations of two *CMV/β-actin* promoter plasmids driving expression of Cre and tdTomato (6 ng/μl and 1 μg/μl respectively) were co-electroporated in CTV-*Fog2* embryos at E13.5 and E14.5 (n=4 at E13.5; n=3 at E14.5).

Quantification and analysis in *Fog2* mis-expression studies

CThPN and CTh axons were quantified in 4 CTV-*Fog2* brains electroporated as previously described. Electroporated CThPN (tdTomato$^+$, and tdTomato$^+$/GFP$^+$ neurons,

located in layer VI) were quantified in every section across the electroporated area. CTh axons (tdTomato$^+$, and tdTomato$^+$/GFP$^+$ axons crossing from the internal capsule into the thalamus) were quantified in confocal images from serial sections containing the outer border of the thalamus.

The number of CTIP2$^+$/tdTomato$^+$ (control) and CTIP2$^+$/tdTomato$^+$/GFP$^+$ (experimental) neurons in layer V was quantified in confocal z-stacks at three levels across the electroporated area (4 brains). The average ratios of CTIP2$^+$/tdTomato$^+$ and CTIP2$^+$/tdTomato$^+$/GFP$^+$ were calculated. Data were normalized to the control ratio, and expressed as percentages. Subcerebrally projecting axons, both tdTomato$^+$ (control) and tdTomato$^+$/GFP$^+$ (experimental), were quantified in confocal z-stacks taken at three different rostro-caudal levels: caudal internal capsule, rostral cerebral peduncle, and caudal cerebral peduncle. At each level, the number of tdTomato$^+$ and tdTomato$^+$/GFP$^+$ axons were normalized to the corresponding number of tdTomato$^+$ and tdTomato$^+$/GFP$^+$ at the rostral-most level, the caudal internal capsule.

Co-Immunoprecipitation

Dissociated E15.5 cortical neurons were co-transfected with *HA-Fog2,* and either *Flag-Couptf1*, *Flag-Gata2*, or *Flag-Gata4* constructs using Amaxa nucleofection (Lonza). Protein IP was performed using Protein-A/G agarose beads (Pierce), following standard methods (Huggins et al., 2001). Mouse anti-HA (RRID:AB_2314672**)** and non-specific mouse IgG (4mg/ml; Invitrogen) were used for pull-down. Rabbit anti-Flag (1:2000, RRID:AB_439687) was used for immunoblotting.

Luciferase assays

The 8.4kb upstream of *Ctip2* was obtained from a BAC clone (RP24-201C18) and cloned into pGL3-Firefly luciferase upstream of the SV40 promoter (pGL3-luc; Promega), using a Gibson assembly kit (New England BioLabs). Dissociated neurons from dissected WT E15.5 cortices were nucleofected with pGL3-Ctip2-luc, Renilla luciferase vector, and either *Fog2, Couptf1, Couptf2, Gata2,* or *Gata4* expression plasmids, alone or in combination (5 to 7 independent replicates per condition). Luciferase activities were assayed 48 h later using the Dual-Glo system (Promega). Firefly/Renilla luciferase ratio was calculated for each condition, and referred to as the percentage relative to the baseline activity of pGL3-Ctip2-luc.