SUPPLEMENTAL FIGURES

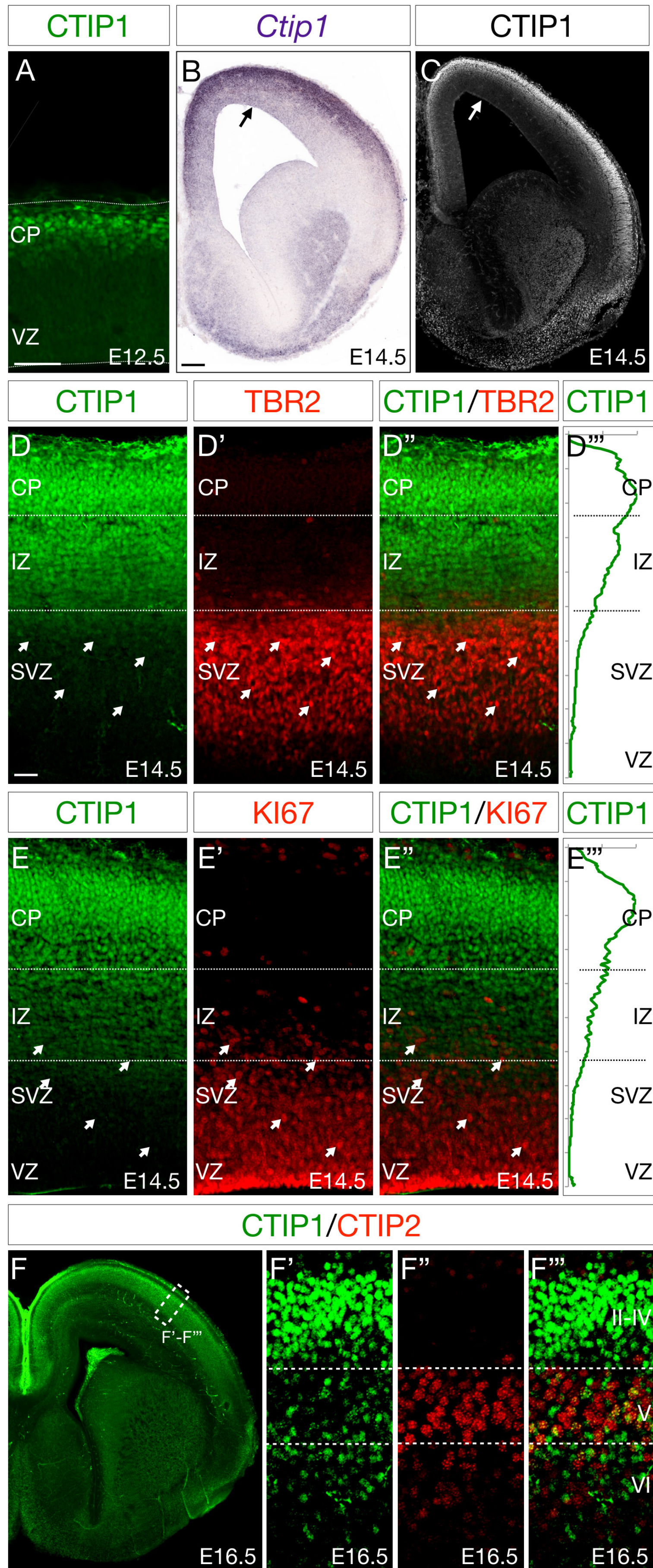

Figure S1. (related to Figure 1) CTIP1 is expressed by postmitotic neurons in the cortical plate.

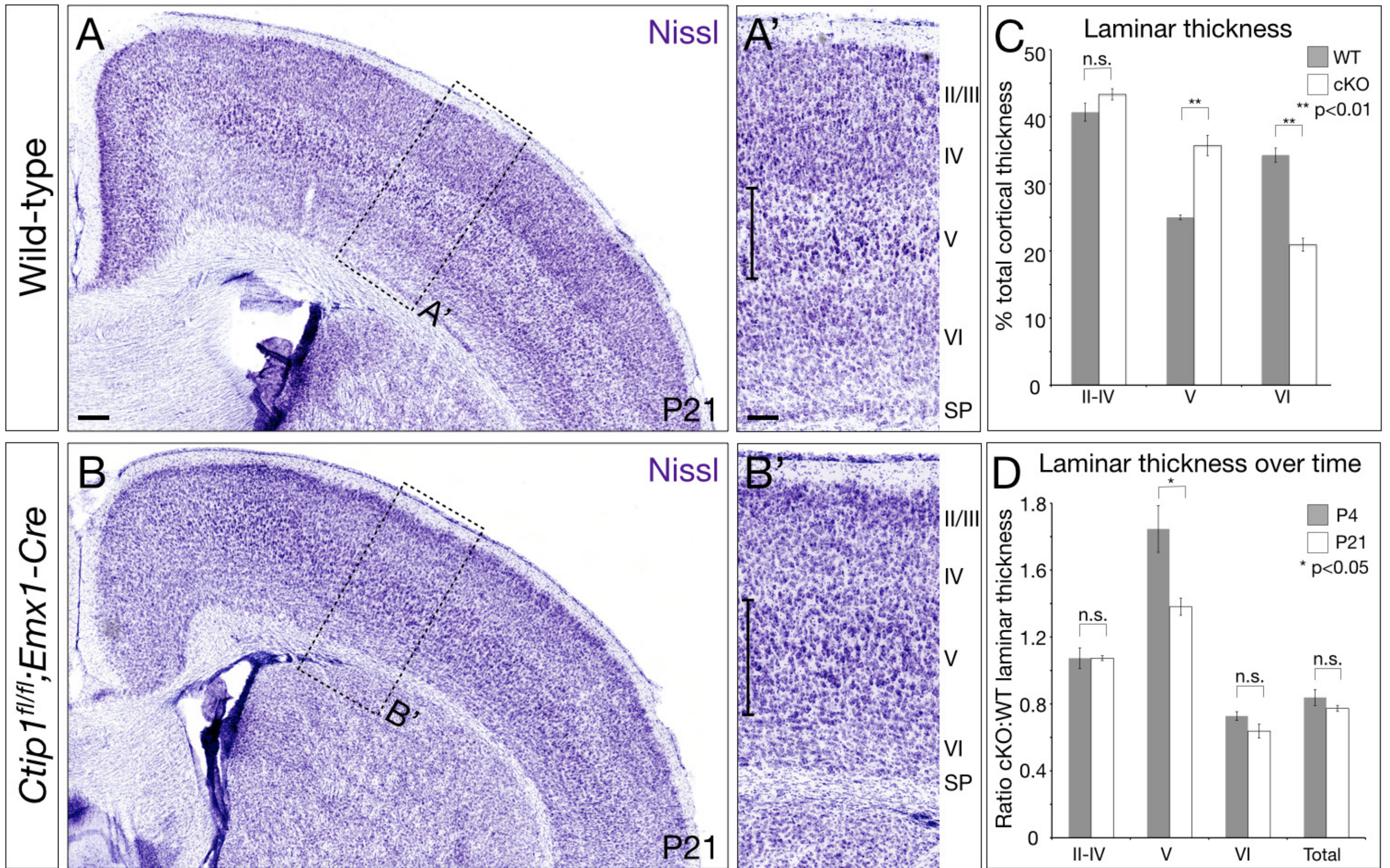

Figure S2. (related to Figure 2) Defects in lamination, migration, and neuronal positioning in *Ctip1$^{fl/fl}$;Emx1-Cre* mice persist until adulthood.

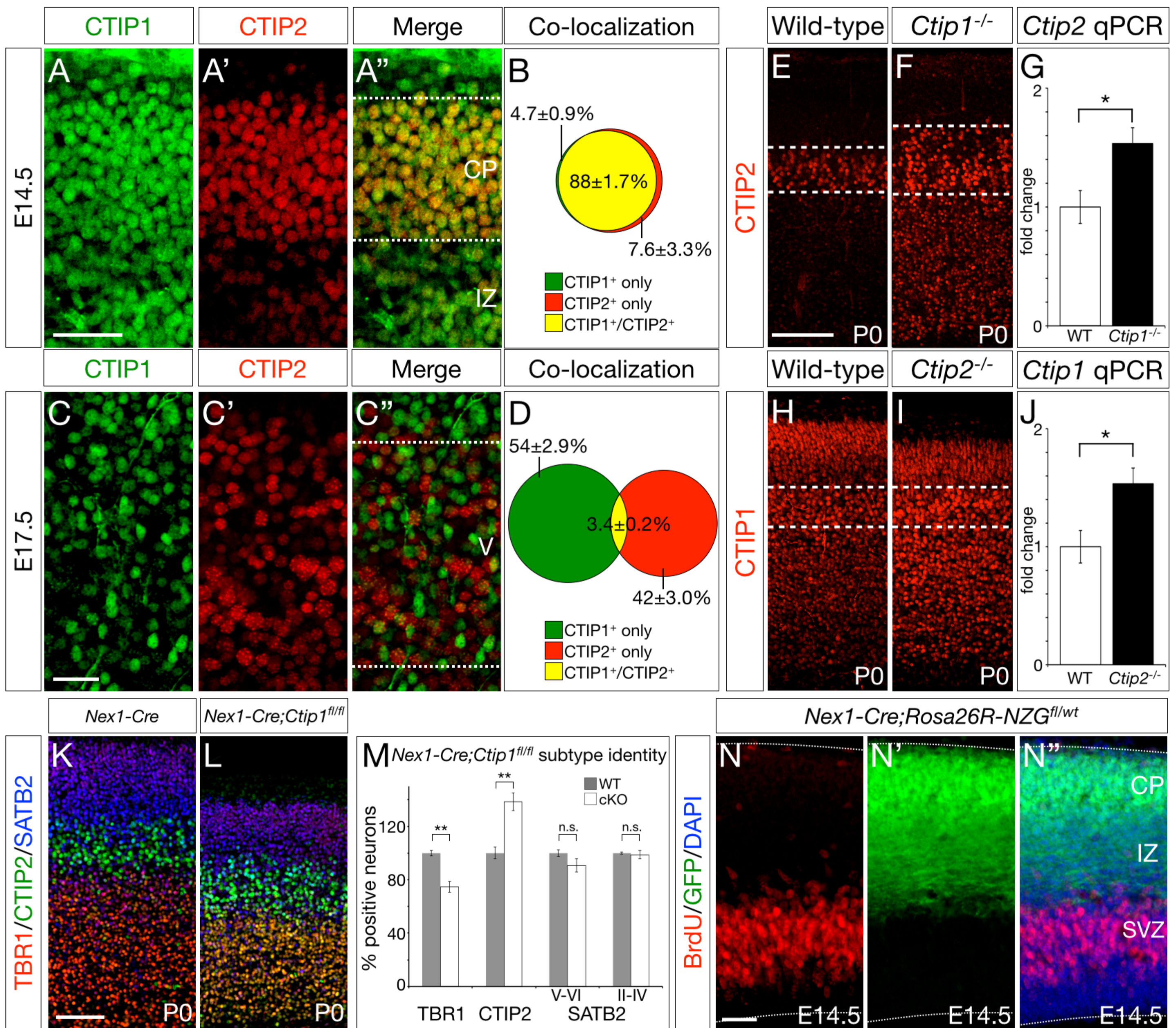

Figure S3. (related to Figure 3) *Ctip1* and *Ctip2* are genetically cross-repressive.

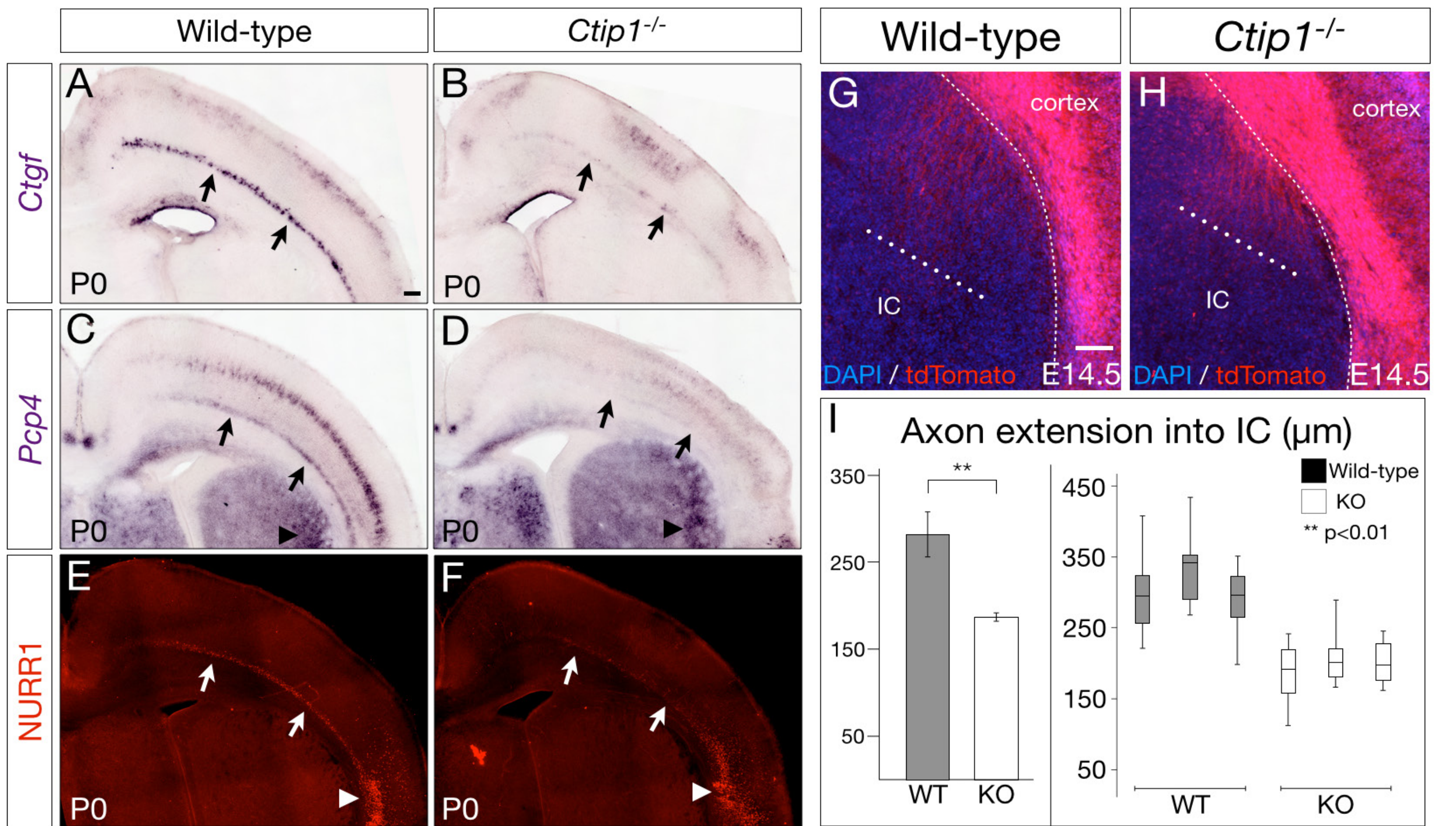

Figure S4. (related to Figure 4) Subplate neuron identity and projections are impaired in the global absence of *Ctip1* function.

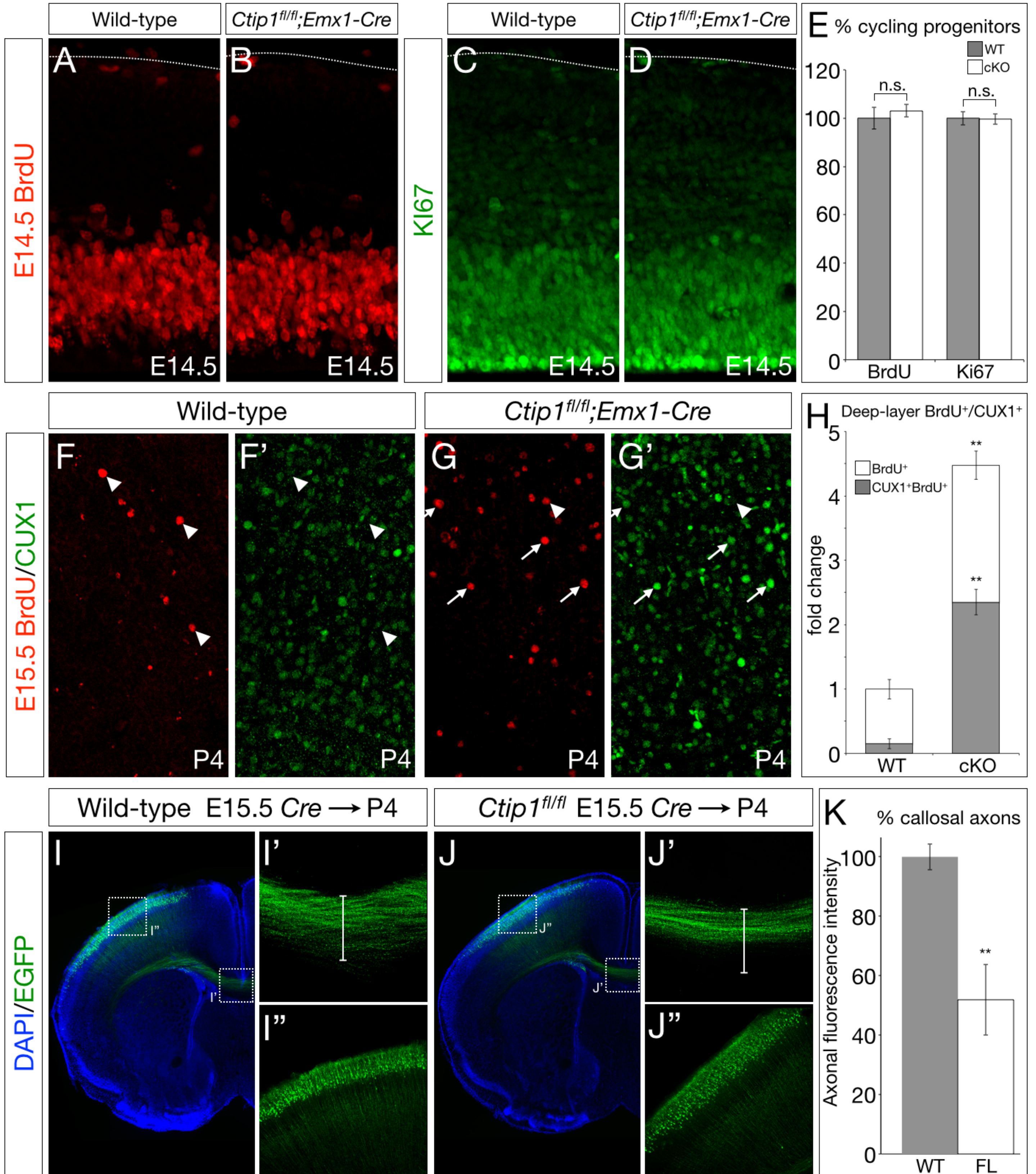

Figure S5. (related to Figure 5) Superficial-layer callosal projection neurons are produced in normal numbers in the absence of *Ctip1* function, but are impaired in migration, positioning, and projection.

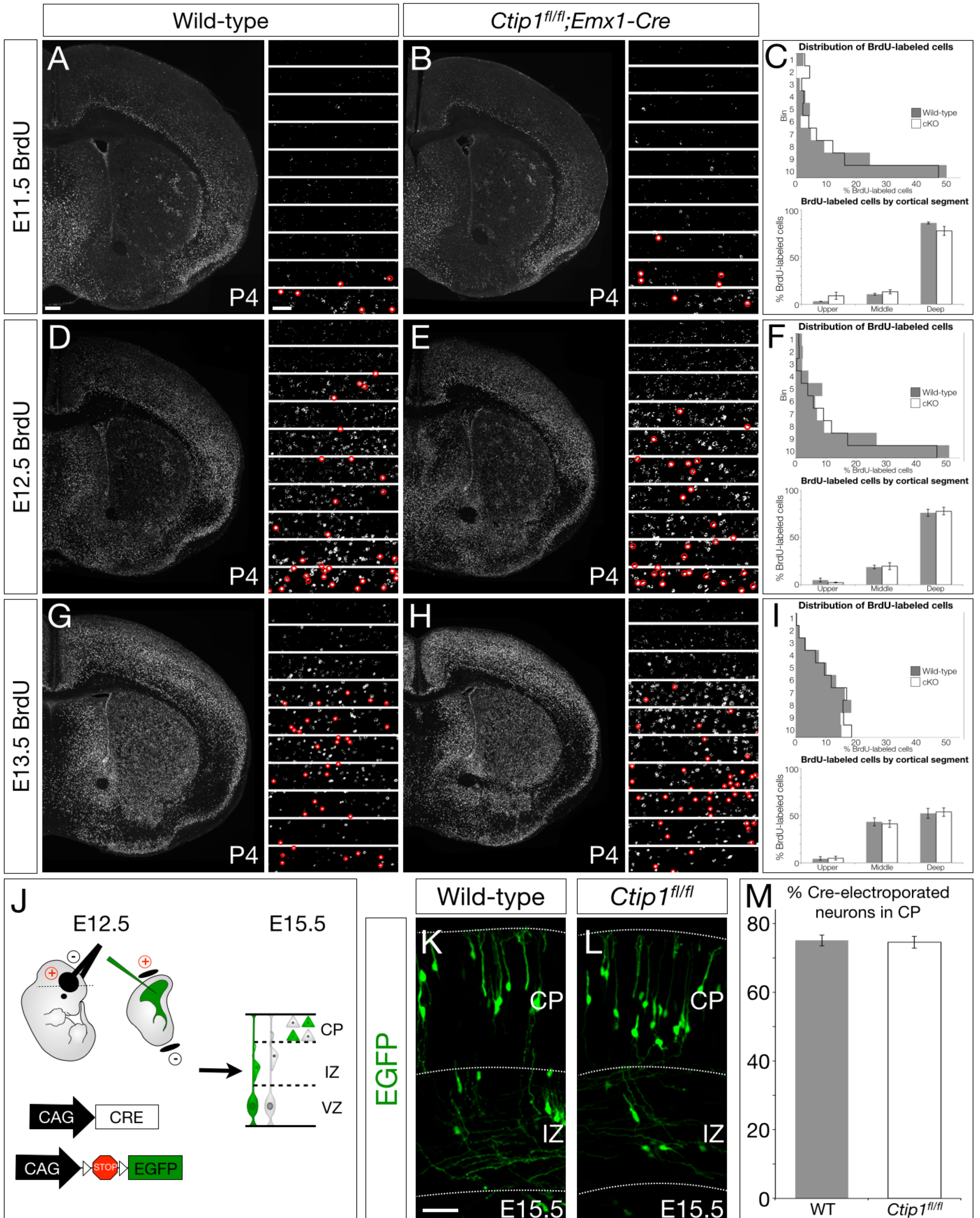

Figure S6. (related to Figure 6) Migration and laminar positioning of deep-layer neurons is not affected by the absence of *Ctip1* function.

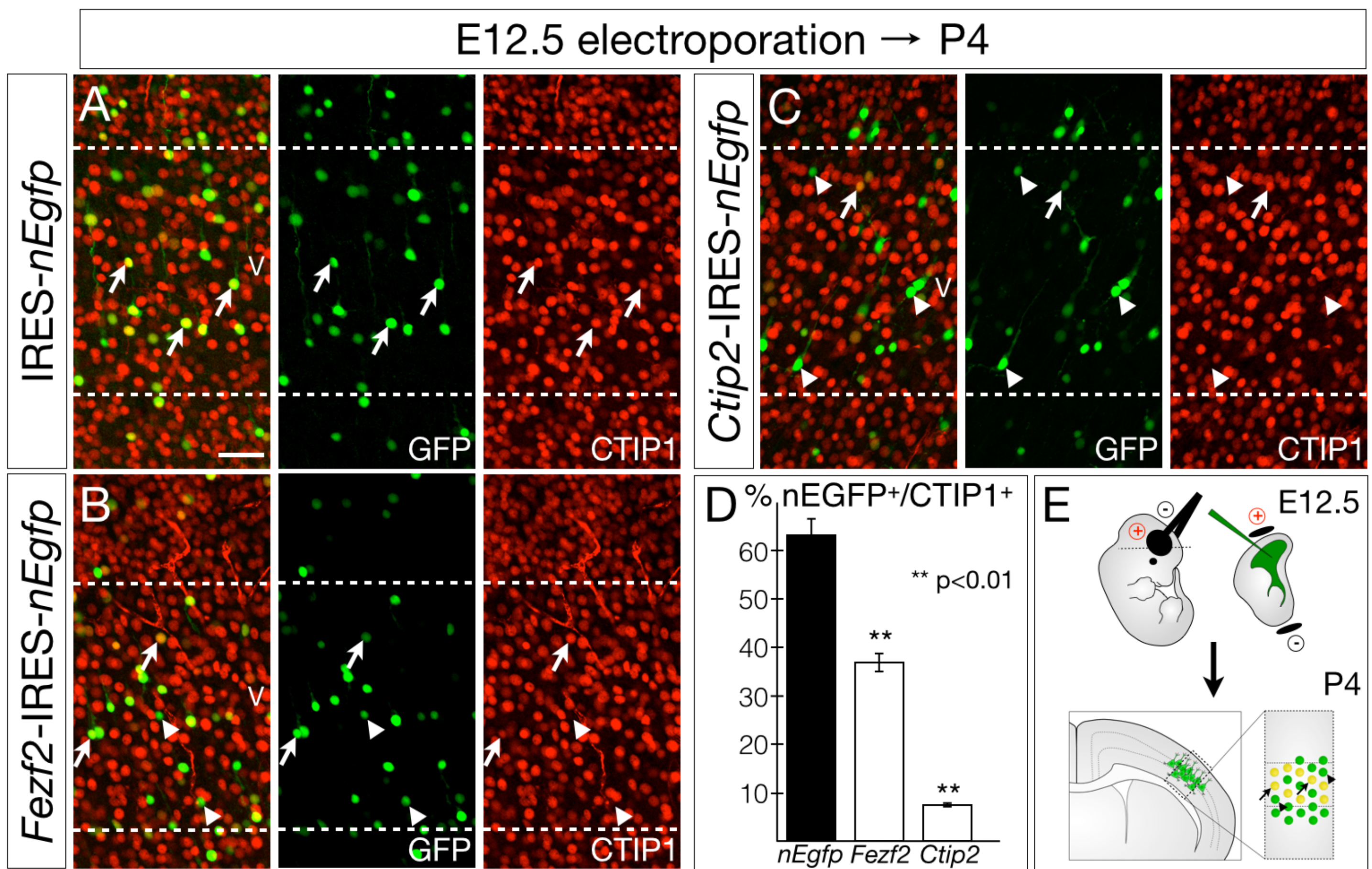

Figure S7. (related to Figure 7) Misexpression of either *Ctip2* or *Fezf2* at E12.5 represses expression of CTIP1.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. (related to Figure 1) CTIP1 is expressed by postmitotic neurons in the cortical plate.
(A) High-magnification confocal image from section shown in Figure 1A.
(B-C) *Ctip1* mRNA is expressed in proliferative zones at E14.5 (arrow in B), but CTIP1 protein is detected only in postmitotic neurons in an adjacent section (arrow in C).
(D-E) CTIP1 is not co-expressed with TBR2, which marks intermediate progenitors (D-D''), or with KI67, which marks actively proliferating progenitors (E-E''), indicating that it is exclusive to postmitotic neurons in cortex. Arrowheads mark cells expressing proliferative markers, but not CTIP1. CTIP1 expression increases in a graded fashion through the intermediate zone, and reaches highest levels in the cortical plate (D''', E'''). Fluorescence profiles are produced from images shown in D and E, with fluorescence intensity expressed in arbitrary units.
(F) CTIP1 and CTIP2 expression at E16.5. Panel F is reproduced from Figure 1B.
Scale bars: 200um (B-C), 50um (A, D-E).

Figure S2. (related to Figure 2) Defects in lamination, migration, and neuronal positioning in *Ctip1*$^{fl/fl}$*;Emx1-Cre* mice persist until adulthood.
Layer V remains expanded relative to layer VI in P21 *Ctip1*$^{fl/fl}$*;Emx1-Cre* mice (B) compared with wild-type (A) (quantification in C), and more large, darkly-stained pyramidal neurons are visible in conditional null brains (A'-B'). In addition, cortical layers remain less distinct from each other in conditional null mice, indicating that defects in subtype specification, migration, and neuronal positioning are not resolved later in development. Overall differences between wild-type and conditional null cortex are preserved between P4 and P21, although layer V is proportionally smaller, suggesting that some excess SCPN in conditional null cortex do not persist (D).
Scale bars: 200um (A-B), 100um (A'-B').
*, p<0.05; **, p<0.01; n.s., not significant; SP, subplate

Figure S3. (related to Figure 3) *Ctip1* and *Ctip2* are genetically cross-repressive.
(A-D) At E14.5, CTIP2 and CTIP1 are extensively co-expressed by newly postmitotic projection neurons (A, quantification in B). By E17.5, most neurons in layer V express either CTIP1 or CTIP2, and only 3.4% express both (C, quantification in D).
(E-J) CTIP2 expression is increased in layers V and VI of P0 *Ctip1*$^{-/-}$ cortex (E-F, qPCR quantification in G), and CTIP1 expression is increased in layers V and VI of P0 *Ctip2*$^{-/-}$ cortex (H-I, qPCR quantification in J).
(K-N) *Nex1-Cre;Ctip1*$^{fl/fl}$ conditional null mutants recapitulate subtype specification defects observed in *Emx1-Cre;Ctip1*$^{fl/fl}$ conditional null mutants, indicating that errors in subtype specification result from postmitotic deletion of *Ctip1*. TBR1 is expressed by fewer neurons in conditional null cortex, CTIP2 is expressed by more neurons, and SATB2 expression is not significantly changed in either deep or superficial layers (K-L, quantification in M). *Nex1-Cre* is expressed by postmitotic neurons starting in the intermediate zone, and expression does not overlap with cycling progenitors, labeled by acute BrdU at E14.5 (N-N'').
Scale bars: 25um (A, C), 100um (E-F, H-I, K-L), 50um (N)
*, p<0.05; **, p<0.01; n.s., not significant; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; V, layer V

Figure S4. (related to Figure 4) Subplate neuron identity and projections are impaired in the global absence of *Ctip1* function.
(A-F) Subplate neurons (arrows) normally express *Ctgf* (A), *Pcp4* (C), and NURR1 (E) at P0, but all three are strikingly reduced in *Ctip1* null subplate (B, D, F). Expression in other populations is unaffected (for example, arrowheads in C-D, E-F).
(G-H) Subplate projections do not pioneer as far into the internal capsule (IC) by E14.5 in *Ctip1*$^{-/-}$*;Rosa26R-tdTomato*$^{fl/wt}$*;Emx1-Cre* (null) mice (H) as in *Ctip1*$^{wt/wt}$*;Rosa26R-tdTomato*$^{fl/wt}$*;Emx1-Cre* (wild-type) (G). Dotted line indicates axon front.
(I) Quantification of G-H. Axons have extended significantly farther in wild-type than in *Ctip1* null internal capsule (left). "Box-and-whiskers" plot of the fifteen sections per brain, three pairs per genotype, used for quantification (right). Boxes extend from first to third quartile measurement, with median plotted as horizontal line within box. "Whiskers" extend from minimum to maximum measurement.
Scale bars: 100um

Figure S5. (related to Figure 5) Superficial-layer callosal projection neurons are produced in normal numbers in the absence of *Ctip1* function, but are impaired in migration, positioning, and projection.

(A-E) Progenitors cycle in normal numbers in *Ctip1$^{fl/fl}$;Emx1-Cre* cortex compared with wild-type at E14.5. Acute BrdU administration (A-B) and KI67 immunostaining (C-D) label the same number of neurons in wild-type and mutant cortex. Quantification (E).
(F-H) Late-born neurons mispositioned in deep layers express markers of superficial-layer neurons. Few wild-type deep-layer neurons labeled with BrdU at E15.5 express the superficial callosal projection neuron marker CUX1 (arrowheads, F-F'), but significantly more *Ctip1$^{fl/fl}$;Emx1-Cre* deep-layer neurons labeled with BrdU at E15.5 (arrows, G-G') do. Quantification (H).
(I-K) Fewer late-born neurons project across the corpus callosum in the cell-autonomous absence of *Ctip1*. *Cre* electroporation at E15.5 labels fewer *Ctip1$^{fl/fl}$* axons crossing the corpus callosum at P4 (J-J') than wild-type axons (I-I') (brackets in I' and J' are the same size), although electroporations are equivalent (I''-J''). Quantification of fluorescence intensity of axons crossing corpus callosum, corrected for fluorescence intensity of electroporation site (K).
n.s., not significant; **, $p<0.01$

Figure S6. (related to Figure 6) Migration and laminar positioning of deep-layer neurons is not affected by the absence of *Ctip1* function.
(A-I) When BrdU is administered to pregnant females at E11.5 (A-B), E12.5 (D-E), and E13.5 (G-H), BrdU-labeled neurons in *Ctip1$^{fl/fl}$;Emx1-Cre* cortex are equally likely to remain in deep layers compared with wild-type, and the overall laminar distribution of labeled neurons (marked in red) is normal (C, F, I). Comparisons between cortical segments are all non-significant.
(J-M) Sparse electroporation of *Cre* at E12.5 does not affect migration of *Ctip1$^{fl/fl}$* neurons at E15.5. Schematic of experimental approach (J). Wild-type neurons electroporated with *Cre* (K) are equally likely as electroporated *Ctip1$^{fl/fl}$* neurons (L) to have migrated into the cortical plate by E15.5 (quantification, M).
Scale bars: 200um (montages in A-H), 50um (confocal segments in A-H, K-L).

Figure S7. (related to Figure 7) Misexpression of either *Ctip2* or *Fezf2* at E12.5 represses expression of CTIP1.
(A-C) Cortical neurons in layer V electroporated at E12.5 with control *nEgfp* frequently express CTIP1 (arrows in A). Significantly fewer layer V neurons electroporated with *Fezf2*-IRES-*nEgfp* express CTIP1 (arrows in B), and most electroporated neurons express no CTIP1 (arrowheads in B). Almost no layer V neurons electroporated with *Ctip2*-IRES-*nEgfp* express CTIP1 (arrows in C), and virtually all electroporated neurons express no CTIP1 (arrowheads in C). In both B and C, electroporated neurons that express CTIP1 tend to be weakly electroporated.
(D) Quantification of A-C. Percentage of nEGFP-positive layer V neurons that are also CTIP1-positive. **, $p<0.01$.
(E) Schematic of experimental approach. Wild-type embryos were electroporated at E12.5, and brains were collected at P4 and analyzed for CTIP1 and nEGFP expression.
Scale bars: 50um.

Table S1. (related to Figures 1-S7) Raw values for quantitative measures reported in this study. Average and SEM are presented for each quantitative assessment in the study, organized by figure. Supplemental figure measures are presented with the main figure to which they refer.

SUPPLEMENTAL MATERIALS AND METHODS

Animals

Ctip1$^{fl/fl}$ mice were generated by Tucker and colleagues (RRID:MGI_4358088, Sankaran et al., 2009; Lee et al., 2013). *Ctip1$^{-/-}$* mice were generated by Copeland and colleagues (RRID:MGI_2663941, Liu et al., 2003), and were obtained from the RIKEN BioResource Center (stock number RBRC01190). *Ctip2$^{-/-}$* mice were generated by Kominami and colleagues (RRID:MGI_2663971, Wakabayashi et al., 2003; Arlotta et al., 2005). *Emx1-Cre* (RRID:MGI_4440744, stock number 005628), *Rosa26R-tdTomato-Ai9* (RRID: MGI_3809523, stock number 007909), and *Rosa26R-NZG* (RRID:MGI_3840211, stock number 012429) mice were purchased from Jackson Laboratories. *Ntsr1-Cre* mice (RRID:MGI_3836636, stock number 030648-UCD) and *Rbp4-Cre* mice (RRID: MGI_4367067, stock number 031125-UCD) were generated by the GENSAT project (Gong et al., 2007), and were purchased from the MMRRC. *Nex1-Cre* mice were generated by Nave and colleagues (RRID:MGI_2668659, Goebbels et al., 2006).

Immunocytochemistry

Primary antibodies and dilutions used: rat anti-BrdU, 1:500 (Accurate Chemical and Scientific Corporation

Cat# OBT-0030 RRID:AB_2341179); mouse anti-CTIP1 clone 14B5, 1:500 (Abcam Cat# ab19487, RRID:AB_444947), rabbit anti-CTIP2, 1:200 (Abcam Cat# ab28448, RRID:AB_1140055); rat anti-CTIP2, 1:200 (Abcam Cat# ab18465, RRID:AB_2064130); rabbit anti-CDP1/CUX1, 1:200 (Santa Cruz Biotechnology Cat# sc-13024, RRID:AB_2261231); rabbit anti-DARPP-32, 1:250 (Cell Signaling Technology Cat# 2306S, RRID:AB_823479); rabbit anti-FOG2, 1:250 (Santa Cruz Biotechnology Cat# sc-10755, RRID:AB_2218978); chicken anti-GFP, 1:200 (Aves Labs Cat# GFP-1020, RRID:AB_10000240); rabbit anti-GFP, 1:500 (Molecular Probes Cat# A11122, RRID:AB_221569); rabbit anti-KI67, 1:500 (Abcam Cat# ab15580, RRID:AB_443209); goat anti-LHX2, 1:200 (Santa Cruz Biotechnology Cat# sc-19344, RRID:AB_2135660); goat anti-NURR1, 1:100 (R and D Systems Cat# AF2156, RRID:AB_2153894); mouse anti-SATB2, 1:200 (Abcam Cat# ab51502, RRID:AB_882455); rabbit anti-TBR1, 1:200 (Abcam Cat# ab31940, RRID:AB_2200219); rabbit anti-TBR2, 1:500 (Abcam Cat# ab23345, RRID:AB_778267).

With the exception of rabbit/chicken anti-GFP, staining for all antibodies is improved by a 10-minute antigen retrieval at 95°C in 0.01M citric acid, pH 6.0; rat anti-BrdU requires a 90-minute antigen retrieval at room temperature in 2N HCl (Magavi et al., 2008). Tissue was incubated with primary antibody at 4°C overnight. Secondary antibodies were chosen from the Alexa series (Invitrogen), and used at a dilution of 1:500 for 4 hours at room temperature.

Non-radioactive *in situ* hybridization was performed as previously described (Arlotta et al., 2005). Probes for *Clim1*, *Ctgf*, *Dkk3*, *Fezf2*, *Lpl*, and *Pcp4* were previously described (Arlotta et al., 2005; Lai et al., 2008; Molyneaux et al., 2009). For Nissl staining, vibratome-sectioned tissue was mounted on gelatin-coated slides and stained with 0.25% cresyl violet, then dehydrated through a graded alcohol series and xylenes. Slides were mounted in DPX.

BrdU birthdating

Timed pregnant females were intraperitoneally injected with bromodeoxyuridine (50 mg/kg) at E11.5, E12.5, E13.5, E14.5, or E15.5. Littermate pairs of *Ctip1*$^{fl/fl}$*;Emx1-Cre* and *Ctip1*$^{wt/wt}$*;Emx1-Cre* pups were collected at P4 and processed for BrdU immunocytochemistry (Magavi et al., 2008). Six anatomically-matched sections from each mouse were selected and single confocal slices were imaged. Cells fully labeled with BrdU were counted by investigators blinded to genotype, dividing cortex into ten equal bins.

For acute BrdU administration, timed pregnant females were intraperitoneally injected with bromodeoxyuridine (75 mg/kg) at E14.5, and embryos were collected 30 minutes later and processed and quantified for BrdU immunocytochemistry, as above.

Quantitative RT-PCR

Total RNA was extracted from whole cortex of P0 *Ctip1*$^{-/-}$, *Ctip2*$^{-/-}$, and wild-type littermate control mice (n=3 of each genotype for each comparison), and cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen). qRT-PCR was performed using a LightCycler 1.5 system (Roche, Branford, CT). All values obtained were normalized with respect to mRNA expression levels of *Gapdh*.