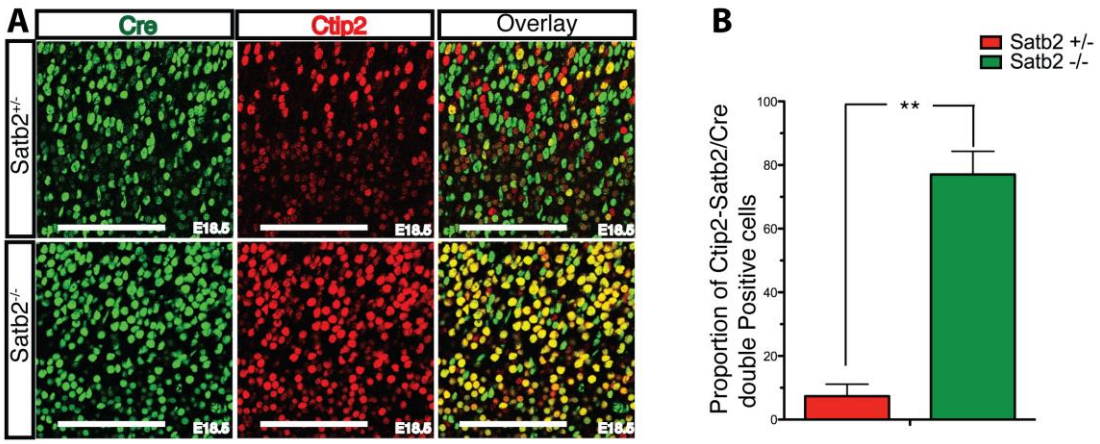
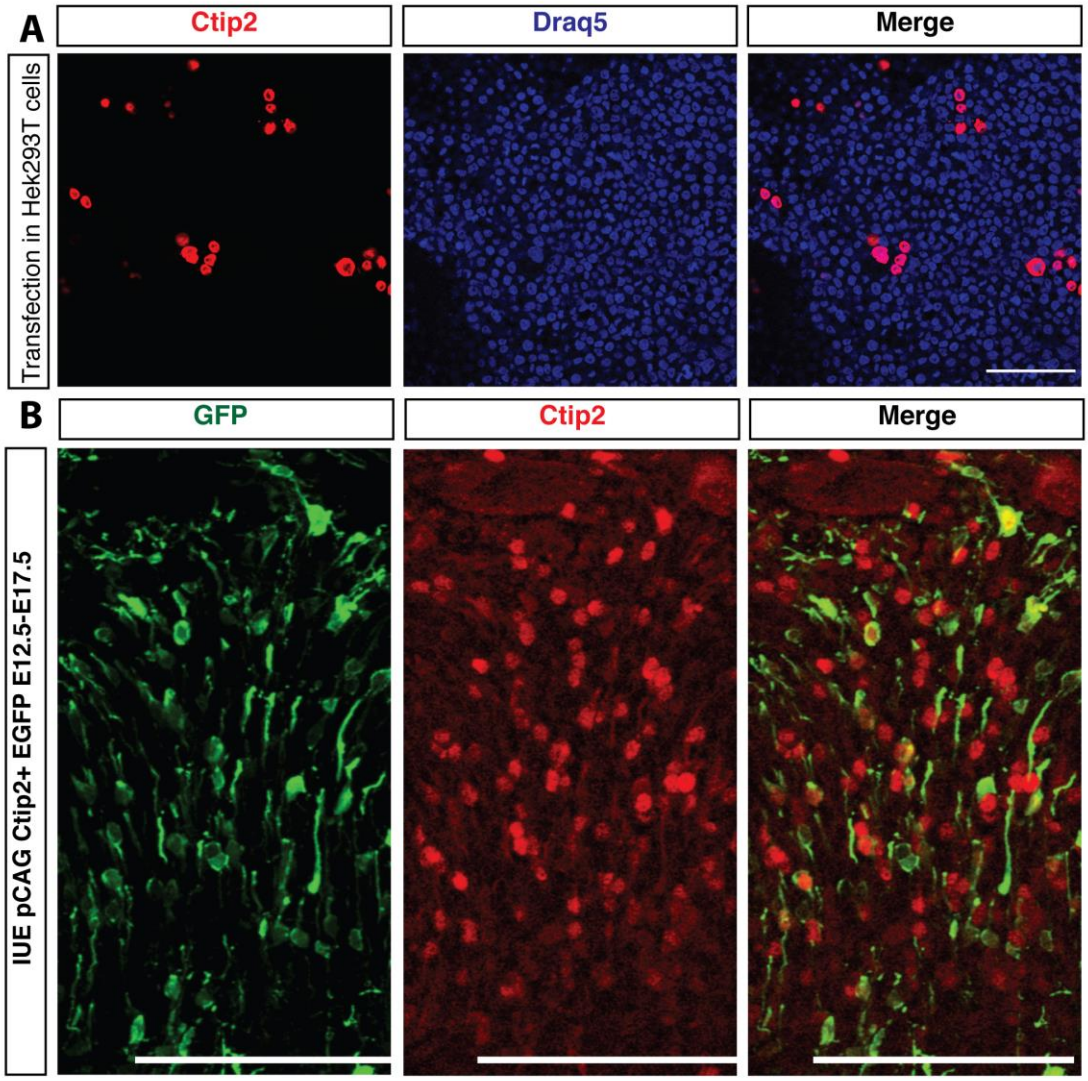


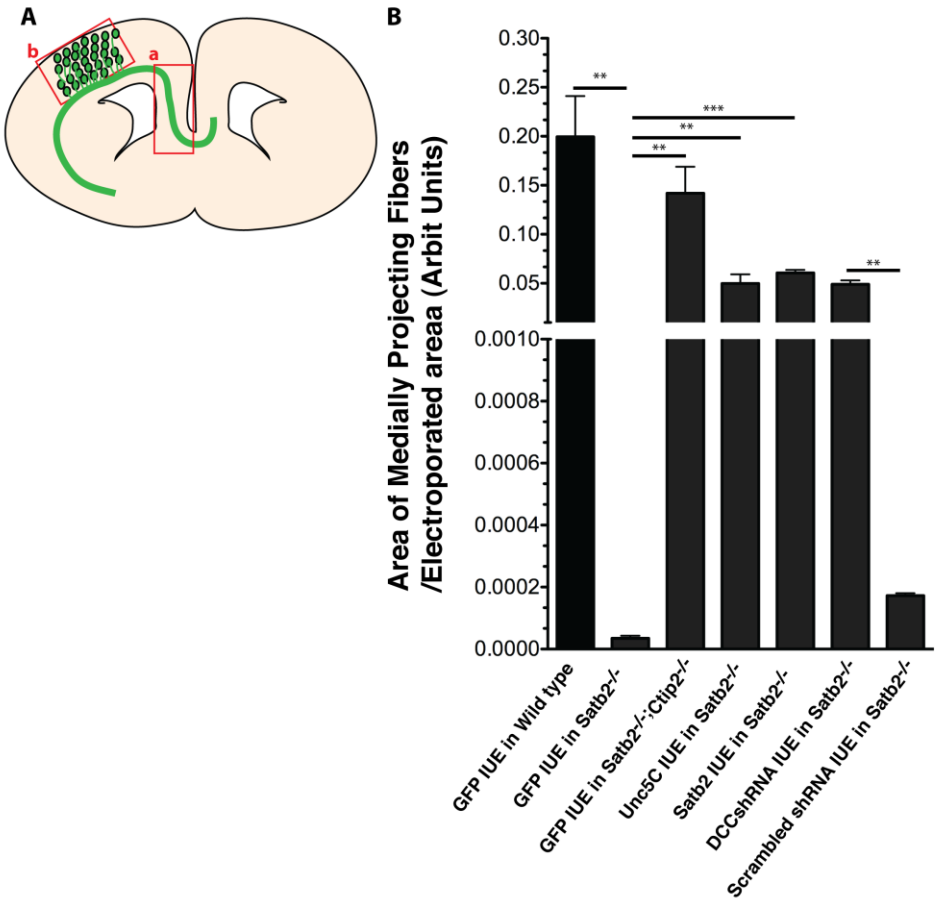
Supplementary Figure 1: Ctip2 is up-regulated in the deep layers of the cortex upon Satb2 deletion

(A) Immunohistochemical staining using anti-Cre and anti-Ctip2 antibody was performed in Satb2$^{+/-}$ and Satb2$^{-/-}$ brains. The proportion of deep layer Ctip2-positive cells that were also Satb2(Cre) double positive in Satb2$^{-/-}$ brains as compared to Satb2$^{+/-}$ was plotted. (**B**) An 8-fold increase was observed in the number of Ctip2-Cre double positive cells in Satb2$^{-/-}$ mutant when compared to the heterozygote (n=3, p-value=0.0006). Scale bar=100µm. Data are presented as average values ± standard deviation (SD).

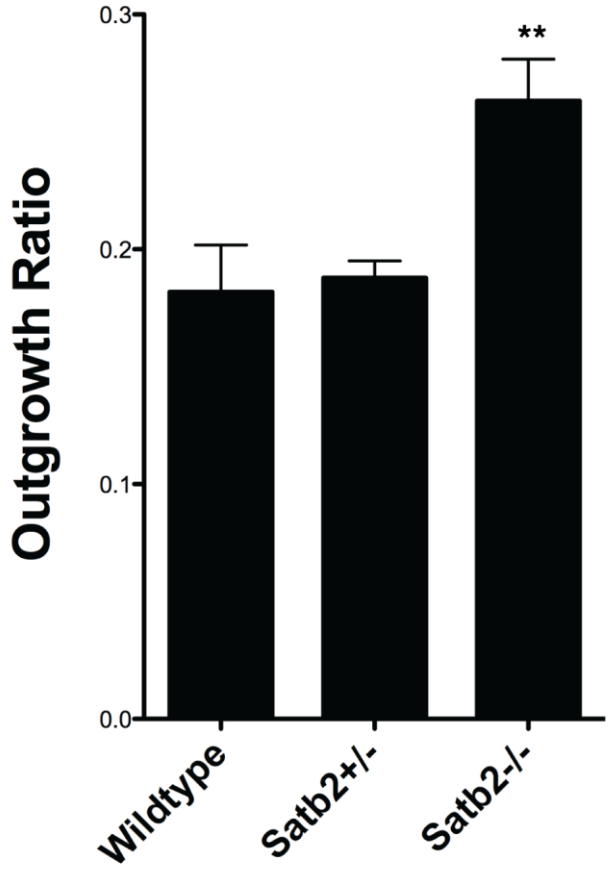


Supplementary Figure 2: Ctip2 expression in HEK cells and cortical plate

(**A**) Plasmid expressing Ctip2 under the ubiquitous CAG promoter was transfected into HEK cells. Immunohistochemistry of Ctip2 together with nuclear marker Draq5 confirmed the expression of Ctip2. (**B**) Plasmid expressing Ctip2 expressed in cortical neurons by *in-utero* electroporation at E12.5 and analyzed at E17.5. Scale bar=100µm.

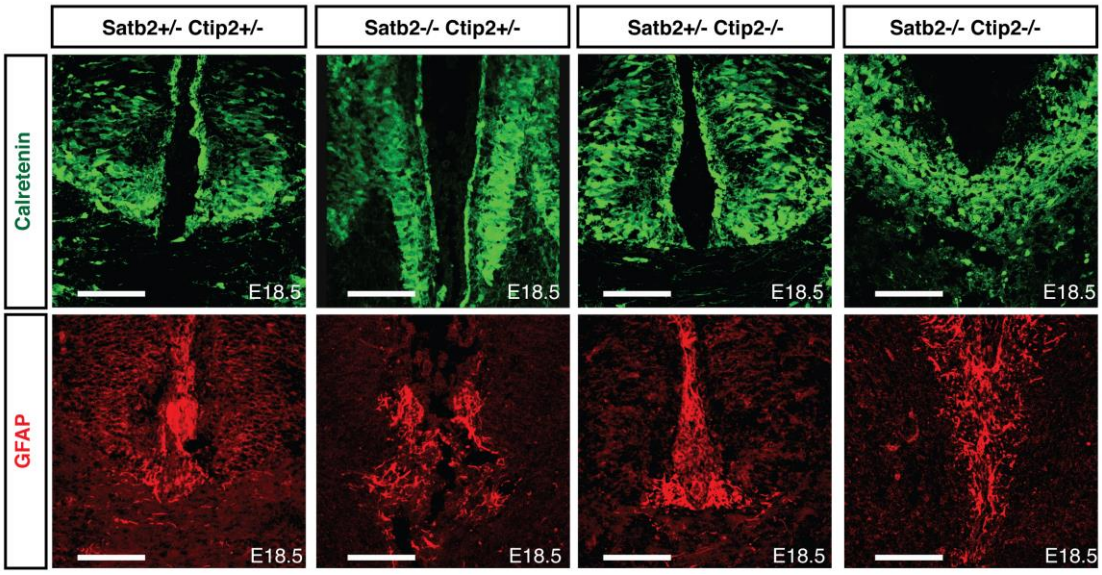


Supplementary Figure 3: Quantification of CC with respect to efficiency of electroporation in wild type and Satb2$^{-/-}$ mutant
(A) In order to quantify the extent of rescue of medial projections in the *in utero* experiments performed, ratio of the fluorescent area of the medially projecting fibers in the ipsilateral hemisphere (within box a) to the fluorescent area of the cells electroporated in the cortical plate (within box b) was measured. The size of box 'b' varied depending upon the extent of electroporation. The size of box 'a' was adjusted so as to enclose the extent of medially projecting fibers after they make the dorsal turn within the ipsilateral cortex till the midline. (B) The *in utero*-experiments with GFP in wild type, GFP in Satb2$^{-/-}$, GFP in Satb2$^{-/-}$;Ctip2$^{-/-}$, Unc5C in Satb2$^{-/-}$, Satb2 in Satb2$^{-/-}$, DCC shRNA in Satb2$^{-/-}$ and Scrambled shRNA in Satb2$^{-/-}$ were quantified. All values are represented as mean ± SEM. All p-values are calculated by student's t test. GFP in wild type (n=3) = 0.199±0.0415, GFP in Satb2$^{-/-}$ (n=3) = 0.00003±8.26x10^{-6}, p-value = 0.0086, GFP in Satb2$^{-/-}$;Ctip2$^{-/-}$ (n=2) = 0.1419±0.027, p-value = 0.0058, Unc5C over-expression in Satb2$^{-/-}$ (n=3) = 0.0498±0.009, p-value = 0.0058, Satb2 electroporation in Satb2$^{-/-}$ (n=2) = 0.0606±0.003, p-value = 0.00012, DCC shRNA in Satb2$^{-/-}$ (n=3) = 0.0489±0.003, compared to scrambled electroporation in the Satb2$^{-/-}$ (n=2) = 0.00017±7.83x10^{-6}, p-value = 0.00023).

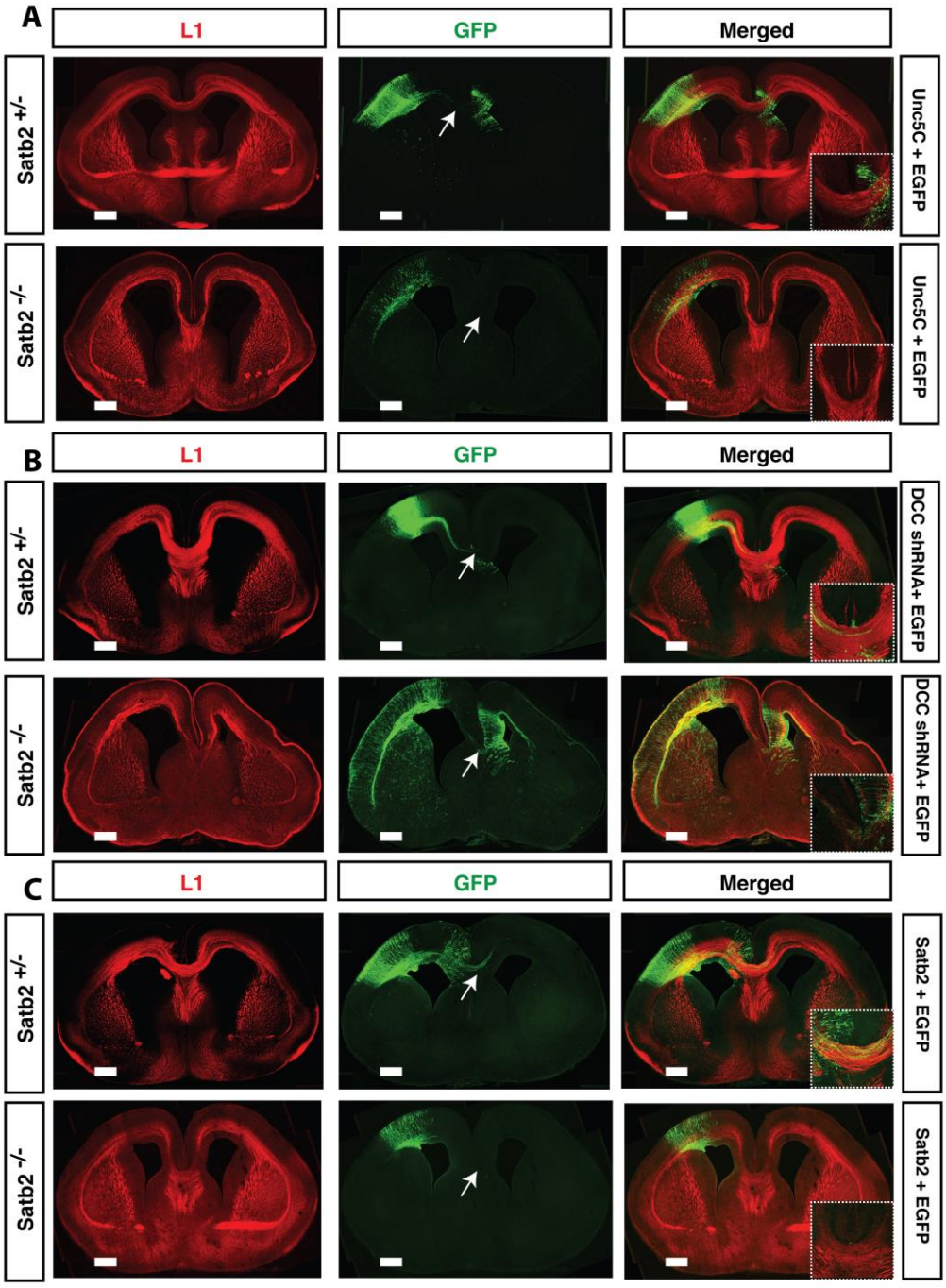


Supplementary Figure 4: Satb2$^{-/-}$ neurons do not show defects in neurite outgrowth

Outgrowth ratio of axons from cortical explants of wild type, Satb2$^{+/-}$ and Satb2$^{-/-}$ were compared. Satb2 deficient neurons did not show impairment in axonal growth, instead showed a slight increase in outgrowth ratio when compared to the controls (p-value= 0.0037 Student's t-test).

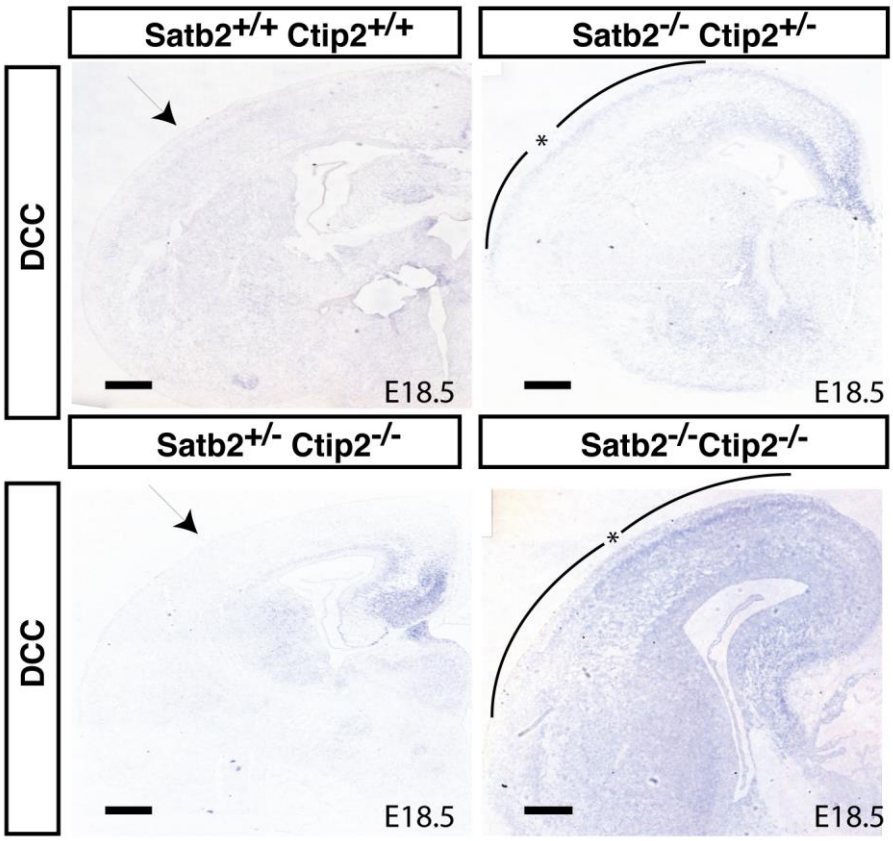


Supplementary Figure 5: Midline guidepost cells are not altered in Satb2$^{-/-}$, Ctip2$^{-/-}$ or Satb2$^{-/-}$Ctip2$^{-/-}$ mutants.
Midline guidepost cells were stained for using antibodies against Calretinin and GFAP in wild type, Satb2$^{-/-}$, Ctip2$^{-/-}$ and Satb2$^{-/-}$;Ctip2$^{-/-}$ brains, showing comparable expression patterns and levels in all four genotypes. Scale bar=100µm.

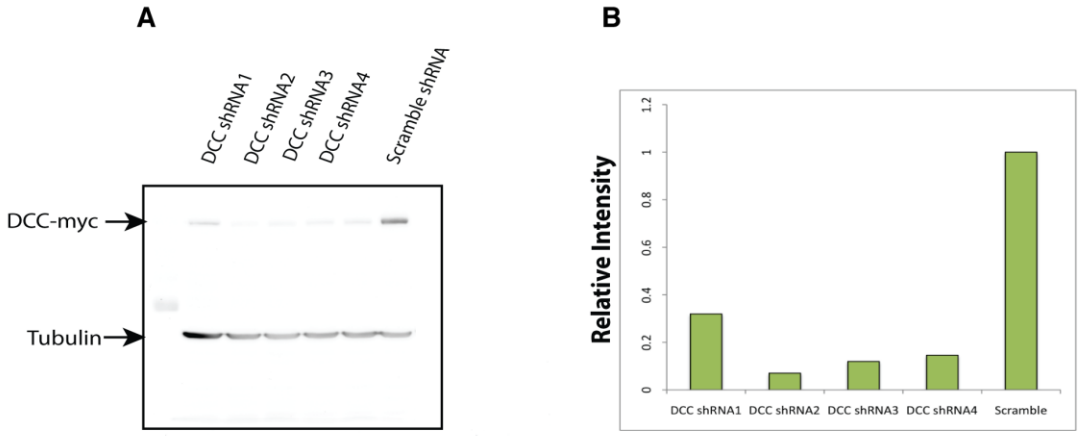


Supplementary Figure 6: Over-expression of Unc5C, Satb2 or down-regulation of DCC at E14.5 does not rescue the CC in Satb2$^{-/-}$ mutants

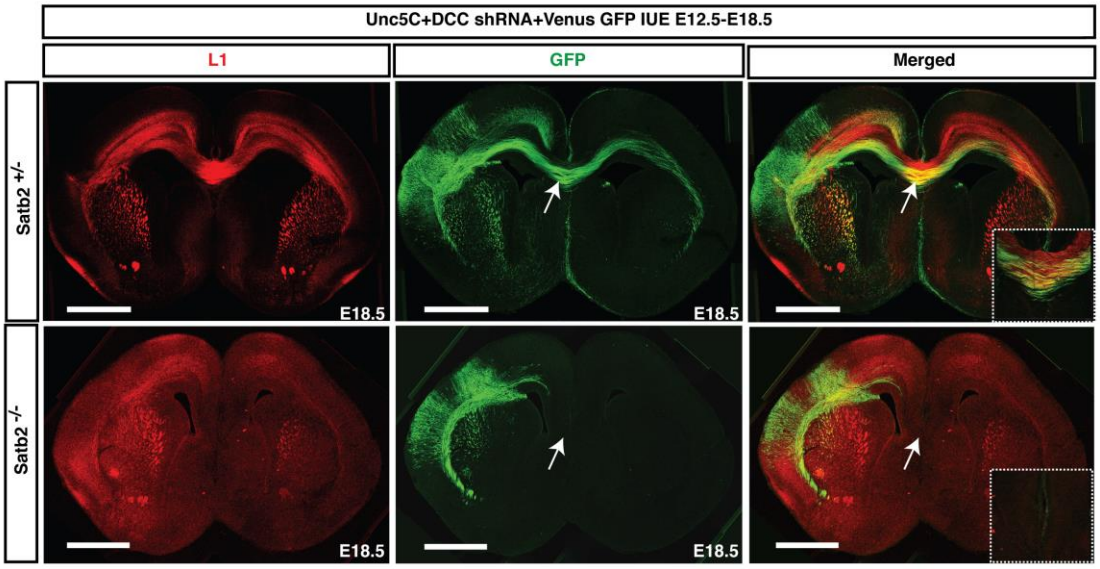
(A) Over-expression of Unc5C at E14.5 by *in utero* electroporation in Satb2$^{-/-}$ mutants does not rescue the CC, whereas wild type littermates show normal callosum. **(B)** Down regulation of DCC by shRNA electroporation at E14.5 does not rescue the CC in Satb2$^{-/-}$ mutants, whereas wild type littermates show normal callosum. (**C**) Over-expression of Satb2 at E14.5 does not rescue the CC in Satb2$^{-/-}$ mutants, whereas wild type littermates show normal CC. Inset images show higher magnification of the midline. Scale bar=450µm. Inset images show higher magnification of the midline.



Supplementary Figure 7: DCC is upregulated in Satb2$^{-/-}$ mutant and is unaltered in Ctip2$^{-/-}$ mutant
DCC *in situ* hybridization at E18.5 revealed an upregulation of DCC mRNA levels in Satb2$^{-/-}$ mutant when compared to the wild type, but the Ctip2$^{-/-}$ mutant did not show any alteration in DCC expression when compared to controls. Satb2$^{-/-}$;Ctip2$^{-/-}$ double mutant showed comparable expression to the Satb2$^{-/-}$ mutant. Scale bar=100µm.

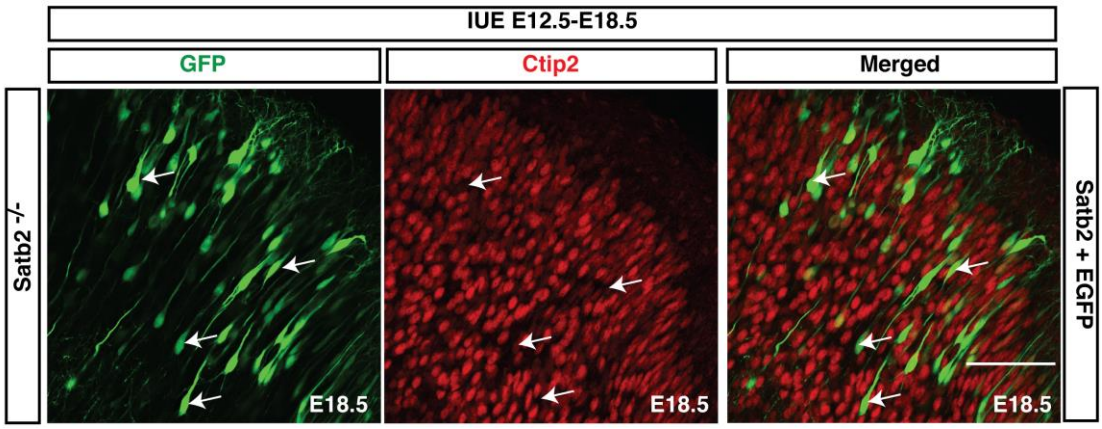


Supplementary Figure 8: Verification of DCC shRNA knock down efficiency in HEK cells.
(**A**) The efficiency of DCC knockdown was verified in HEK cells. We tested four different shRNAs for their efficiency in DCC knockdown. A scrambled shRNA was used as a control. Western blot analysis shows a clear reduction in DCC protein levels in all four shRNAs compared to the scrambled shRNA. (**B**) Quantification showing relative protein levels of DCC to tubulin. DCC shRNA 2 and 4 proved most efficient in knocking down DCC, and were thus used in the experiments.



Supplementary Figure 9: Over-expression of Unc5C with the simultaneous down-regulation of DCC does not rescue the Corpus Callosum.

Images show experiments, wherein Unc5C, DCC shRNA and Venus GFP were co-electroporated at E12.5 and the brains harvested and analyzed at E18.5. Wild type embryos display a normal CC at E18.5. The CC was not rescued when Unc5C and DCC shRNA were co-electroporated in Satb2$^{-/-}$ embryos. Inset images show higher magnification of the midline. Scale bar= 450µm.



Supplementary Figure 10: Satb2 over-expression in Satb2$^{-/-}$ embryos down-regulates Ctip2

In utero electroporation of Satb2 in Satb2$^{-/-}$ cortex results in a down regulation of Ctip2 expression in the electroporated cells, verifying the activity of Satb2 in these cells. Scale bar=100µm.

	Satb2-/-	Netrin1-/-	Unc5C-/-	DCC-/-
Corpus Callosum	Absent	Absent	Present	Absent
Probst Bundle	Absent	Present	-	Present
Medially Projecting Fibers	Absent	Present	Present	Present
Projection of Deep Layer CPN	Cortico-fugal	Cortico-fugal	Cortico-fugal	Not studied
Projection of Upper layer CPN	Intra-hemispheric	Medial	Medial	Not studied

Supplementary Table 1: Summary of Callosal phenotype of Satb2, Unc5C, Netrin1 and DCC mutants

The table summarizes the callosal phenotypes of all four mutants considered in this paper i.e. $Satb2^{-/-}$ $Unc5C^{-/-}$ $DCC^{-/-}$ and Netrin1 hypomorph. The fate of the callosal fibers, that are cell intrinsically dependent on Satb2 for making a medial projection choice, is also delineated in each of these mutants.