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Supplemental Information

Sonic Hedgehog Expression

in Corticofugal Projection Neurons

Directs Cortical Microcircuit Formation

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Figure S1: Sonic Hedgehog Protein is Secreted by Cortical Neurons and Glial Cells in the Cortex. (A) Coronal section of P15 non-mutant wild-type brain with cortical neurons labeled with NeuN (green) and Shh (red) antibodies. The general distribution of SHH expression appears consistent with the pattern observed in the ShhGFPCre;RYFP reporter brains, however there appears to be a small population of glial cells which also label with SHH antibody (arrows). (B) Shh protein appears to be distributed throughout the cell outlined with the dendritic microtubule marker MAP2 (green). (C) Immunohistochemistry of P28 brains of ShhcKO mice with Shh antibody (red) reveals a drastic reduction in secreted SHH protein. (D) Closer examination of cells with the SHH antibody against the N-terminus reveals that the nonfunctional SHH protein is primarily found in aggregates inside the cell body of neurons. Indicating that the primary source of secreted Shh appears to come from cortical neurons.

Figure S2: Cell death, Proliferation and Axon Guidance Appears Normal in ShhcKO Mice. (A and B) Coronal section of wild-type and ShhcKO mice in which fluorescent retrobeads were injected into the corticospinal tract. ShhcKO mice appear to have similar numbers of retrobead labeled cells located in layer V of the cortex. (C) The level of postnatal proliferation measured by BrdU labeling in the cortex and the spinal cord was not significantly different between ShhcKO and wild-type littermates. (D and E) The number of tunel positive cells in P6-15 brain and spinal cord was also not significantly different between conditional mutants and wild-type littermates. (C) n= 3 WT and 3 ShhcKO; (D and E) n= 3 animals per age group, for WT and ShhcKO.

Figure S3: Input Resistance is Significantly Increased in Layer V Neurons of ShhcKO Mice. (A) Recordings of mEPSCs from layer II/III and V neurons (B and C) Reveals a significant increase in the input resistance in layer V neurons of the conditional knockout ($190.4 \pm 14 M\Omega$) when compared to the control ($120.0 \pm 15.2 M\Omega$). (D and E) There is no significant change in mEPSC amplitude between in layer II/III or layer V of the two groups. N=3 animals per group and 9 cells per group. * $p < 0.01$, t-test.

Figure S4: Boc is Expressed in Layer II, III, IV and V of the Adult Cortex. (A) In situ hybridization with an antisense probe to Boc of a saggital section of cortex, showing signal (white) representing Boc mRNA in layers II/III, IV and Va. (B) Shh lineage cells in the cortex of adult ShhGFPCre;RYFP mice (green) are more concentrated in the deeper cortical layers, while (C) LacZ labeled cells from a Boc-LacZ-ires-PLAP mouse shows that Boc expression is more concentrated in layers II/III, IV and Va.

Figure S5: Boc is Expressed by Local and Callosal Projection Neurons. (A) Brain section located caudal and ipsilateral to the site of fluorogold injection in a Boc heterozygous mutant. Many LacZ positive neurons co-label with fluorogold located in layers II/III and Va. (B) PLAP staining of a saggital brain section from a Boc-LacZ-ires-PLAP mouse shows and absence of descending axon projections in the internal capsule.

Figure S6: Cell Migration, and Axon Guidance Appears Normal in Boc-shRNA Electroporated Neurons. (A) Coronal section of a P28 brain co-electroporated at E14 with a control or Boc-shRNA vector, pCAG-H2B-GFP-myr-Tdtomato and a synaptophysin-GFP plasmid. Nuclei of both control and Boc-shRNA electroporated cells (green) appear to migrate normally into layer III. Tdtomato labeled axons (red) also appear to grow have normal growth and guidance and are concentrated in cortical layer V. (B) Western blot of HEK293 cell transfected with a mBoc-EGFP expression vector and control or Boc-shRNA vectors. (C) Boc protein levels are substantially reduced in cells transfected with Boc-shRNA when compared with the control hairpin.

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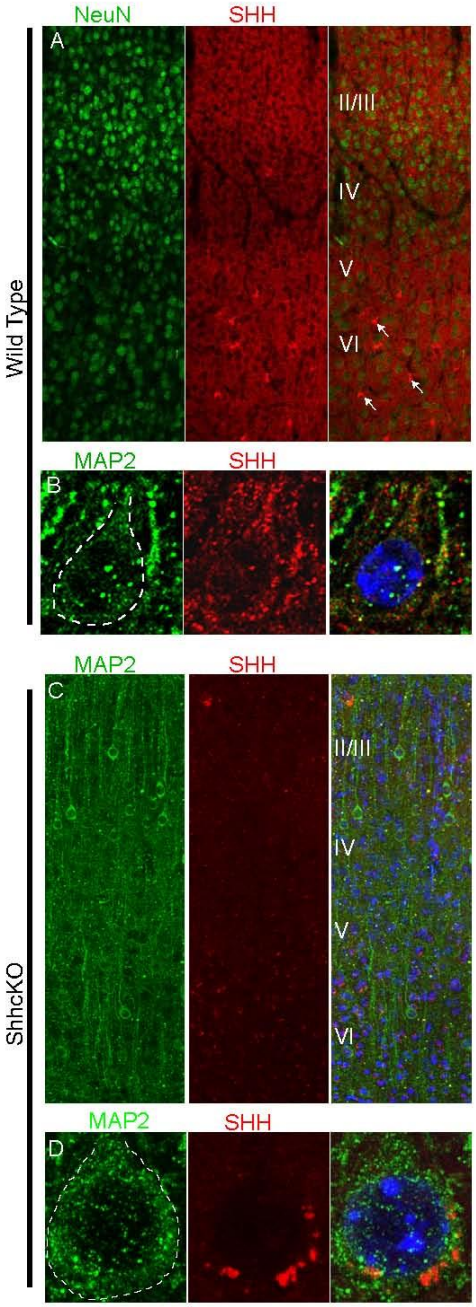

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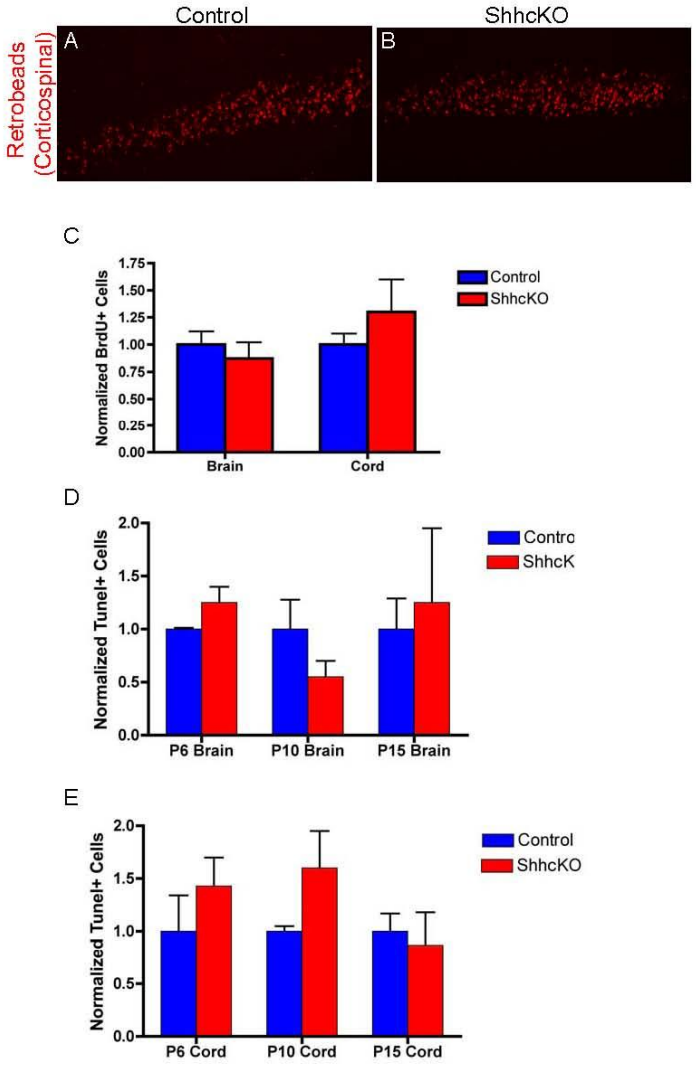

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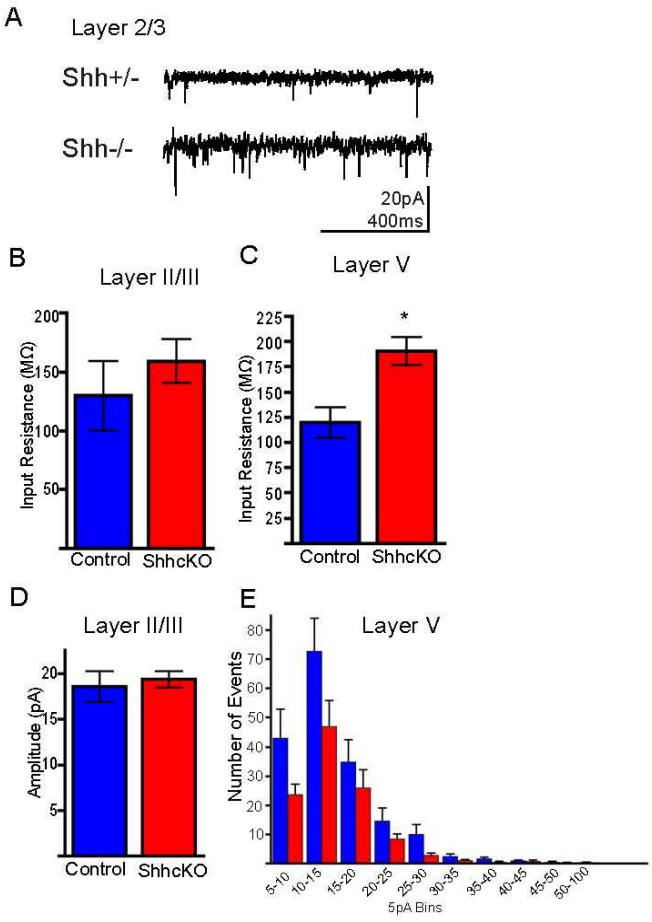

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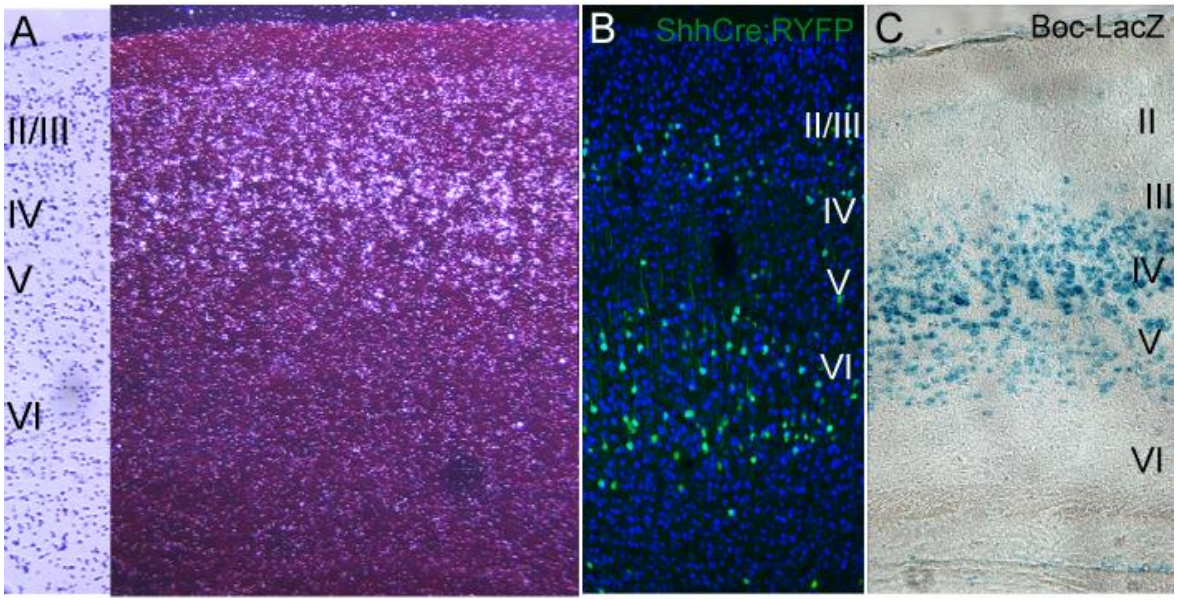

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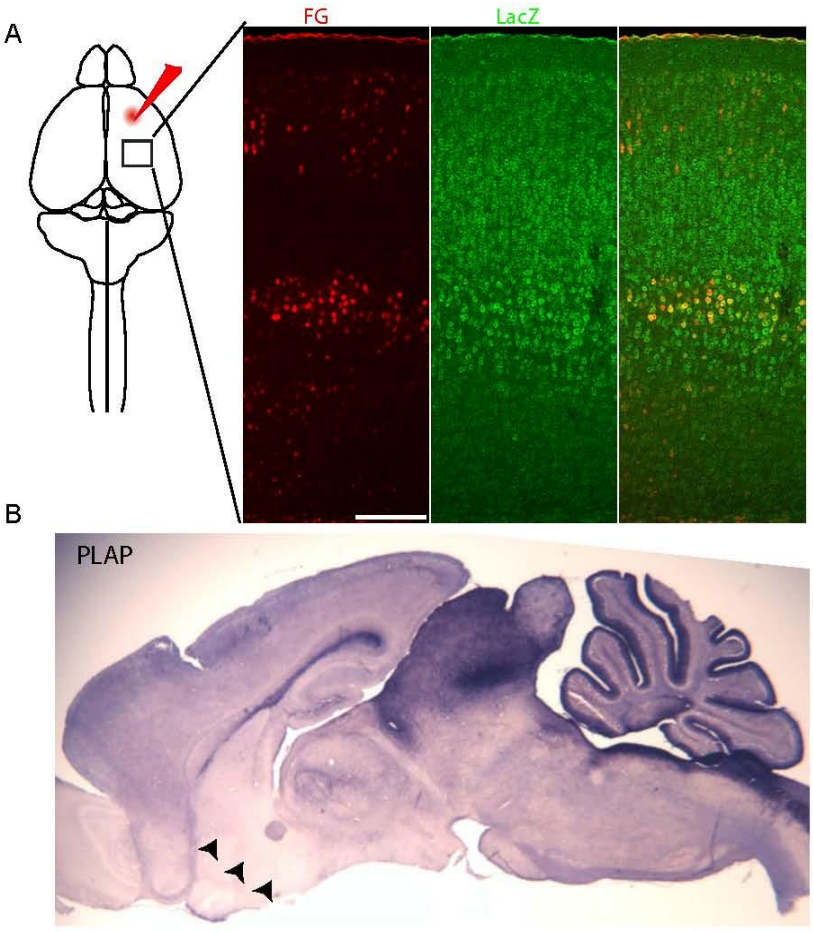

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