SUPPLEMENTAL FIGURES

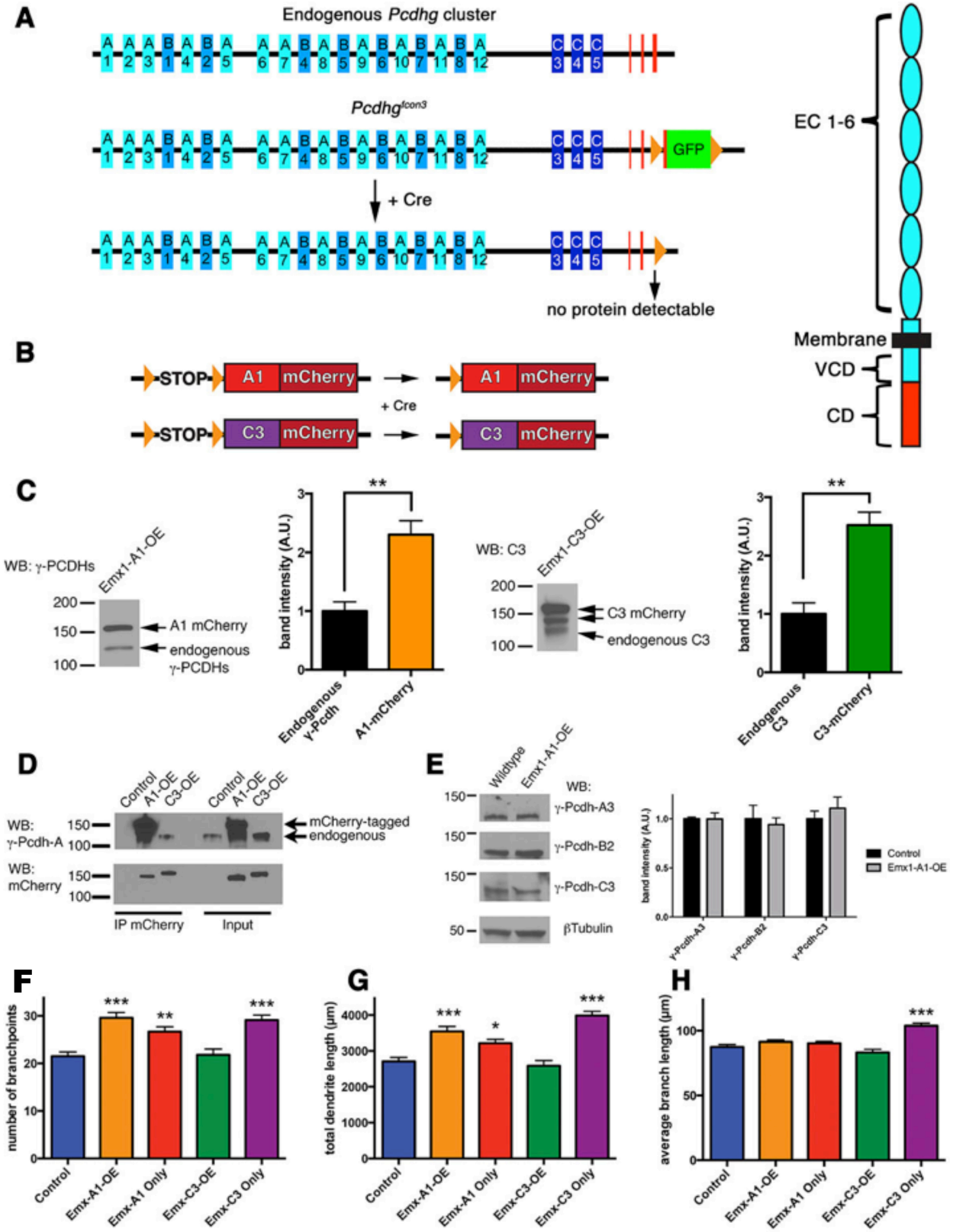

Figure S1 (Related to Figure 1). *Pcdhg* mouse lines used in these studies and measures of layer V dendritic arbors in Emx-KO, Emx-OE and Emx-Only cortex. A) Schematic of the endogenous mouse *Pcdhg* cluster on chromosome 18 showing A, B, and C subfamily variable exons (shades of blue) and the 3 constant exons (red). Each γ-Pcdh isoform is encoded by a transcript initiating upstream of one of the variable exons and containing that exon spliced to all 3 constant exons. The schematic (right) shows the contributions of these exons to the protein domains: Each variable exon encodes 6 extracellular cadherin (EC) repeats, a transmembrane domain, and a variable cytoplasmic domain (VCD), while the 3 constant exons encode a shared C-terminal domain (CD). In the $Pcdhg^{fcon3}$ allele, constant exon 3 is fused to a GFP sequence and flanked by loxP sites (orange triangles). Following Cre excision and deletion of constant exon 3, all *Pcdhg* transcripts are reduced, no protein is detectable, and phenotypes are identical to null mutant mice (Prasad et al., 2008). B) Schematics of the A1-mCherry and C3-mCherry inducible single-isoform transgenic mouse lines (previously described in Lefebvre et al., 2012). Transgenes are inserted into the ubiquitous Rosa locus and expressed from a Rosa-CAG promoter (not indicated). A floxed STOP cassette precedes the mCherry-tagged γ-Pcdh transgene; no expression is observed until Cre excision of the STOP. C) Western blot of cortical lysate from *Emx1-Cre;A1-mCherry* (left) or *Emx1-Cre;C3-mCherry* (right) mice with the indicated monoclonal antibodies (anti-γ-Pcdh constant domain, left; anti-C3 isoform, right). In each line, activation of the transgenic results in greater than 2-fold overexpression of the relevant isoform compared to wildtype levels of either all γ-Pcdhs (left) or endogenous γ-Pcdh-C3 (right); N=3. D) Exogenous mCherry-tagged A1 and C3 γ-Pcdh isoforms incorporate into multimers with endogenous γ-Pcdhs. Lysates from wildtype control, A1-OE, and C3-OE cortices were immunoprecipitated (IP) using an antibody against mCherry, western blotted, and probed using the same antibody or an antibody recognizing 11 of the 12 A subfamily γ-Pcdhs (γ-Pcdh-A). The mCherry antibody does not IP anything from control non-transgenic lysates, demonstrating specificity. In the A1-OE IP, a band of slightly over 150 kDa, representing the exogenous A1-mCherry and detected by both antibodies, is present, as well as other bands of ~120-140 kDa detected only with the γ-Pcdh-A antibody, representing endogenous A subfamily γ-Pcdhs, which vary in size. In the mCherry IP in C3-OE cortex, a band of ~170 kDa is detected only with the mCherry antibody, representing exogenous C3-mCherry, while a doublet of ~120-130 kDa is detected with the γ-Pcdh-A antibody, representing one or more endogenous A subfamily γ-Pcdhs. Together these results indicate that exogenous mCherry-tagged γ-Pcdhs can co-IP endogenous, untagged γ-Pcdhs, suggesting they incorporate into multimers with endogenous γ-Pcdhs *in vivo* as expected from prior *in vitro* studies (Schreiner and Weiner, 2010). E) Western blot of cortical lysates from wildtype and Emx-A1-OE mice demonstrating no change in the expression levels of three endogenous γ-Pcdhs for which validated monoclonal antibodies are available (see Lobas et al., 2012); quantification shows N=3 per genotype. G-I) Additional measures of layer V dendritic arbors in Emx-KO, Emx-OE and Emx-Only cortex. F) Number of branchpoints, calculated by taking the FIJI Sholl Analysis module's "endpoints" count and subtracting the number of primary dendrites; G) Total dendrite length; H) Average branch length, calculated by taking total dendrite length divided by the total number of branches; N=60 (control, A1-Only, C3-Only) or 120 (A1-OE, C3-OE) neurons per genotype; *$p<0.05$, **$p<0.01$, ***$p<0.001$.

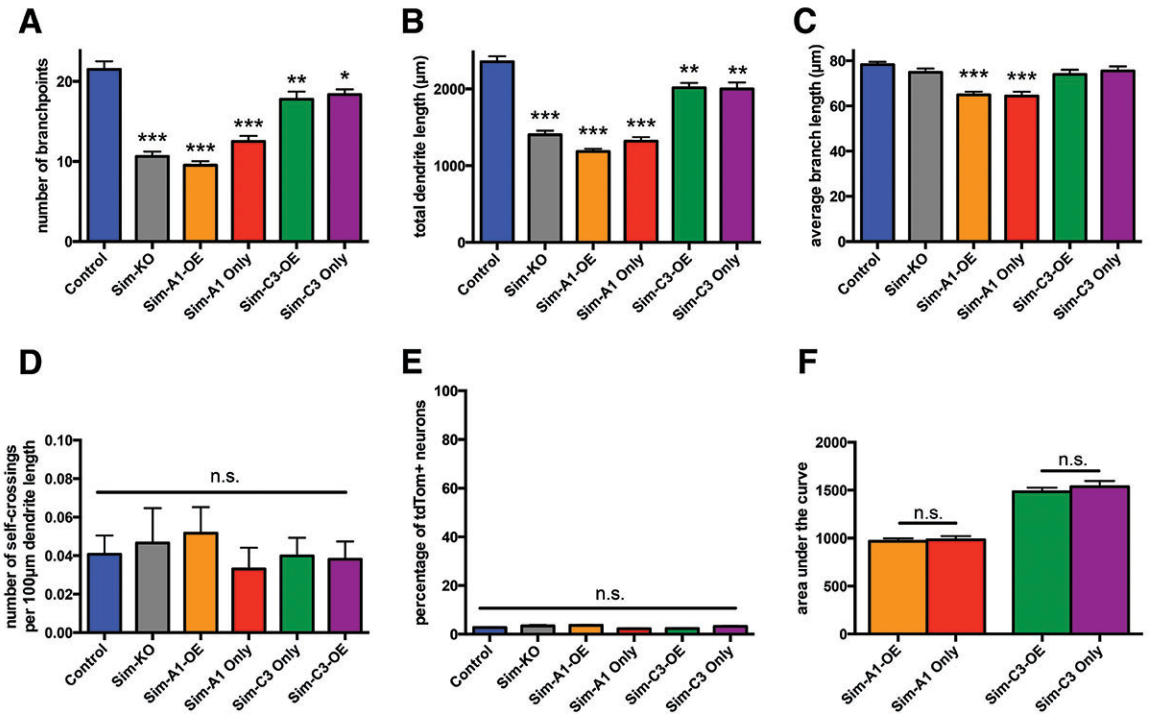

Figure S2 (Related to Figure 2). Measures of dendritic arbors in Sim-KO, Sim-OE and Sim-Only cortex. A-C) Number of branchpoints, total dendritic length, and average branch length were calculated as described in Figure S1. D) The number of self-crossings per 100 µm of dendrite length was determined for individual tdTomato+ layer V/VI neurons from 3D confocal stacks; E) The percentage of layer V/VI NeuN+ neurons that were tdTomato+ (and thus expressed Cre) in each field of view was quantified across all *Sim1-Cre* genotypes (<4% in all genotypes). F) Area under the curve graph separating the Sim-A1/C3–OE and Sim-A1/C3–Only mouse data that are presented combined in Figure 2, as no differences are observed. N=57-60 (control, Sim-KO, Sim-A1-OE) or 40 (Sim-A1-Only, Sim-C3-OE, and Sim-C3-Only) neurons per genotype; *p<0.05, **p<0.01, ***p<0.001; n.s., not significant.

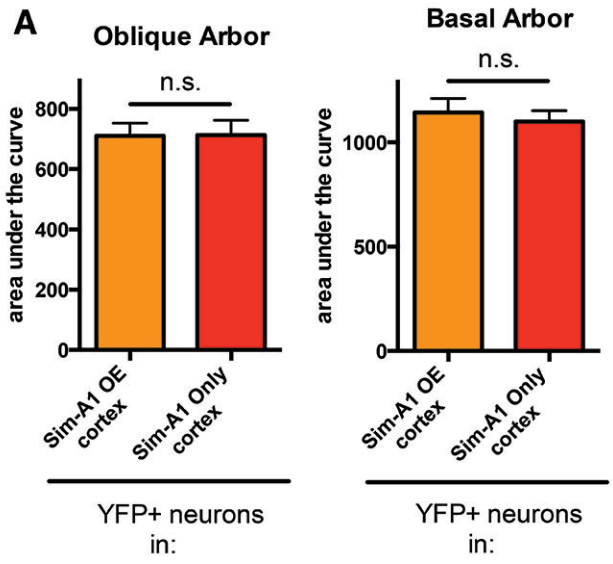

Figure S3 (Related to Figure 3). Comparing dendritic arbors between wildtype YFP+ layer V neurons in Sim-A1-OE and Sim-A1-Only cortex.

A,B) Area under the curve graphs of Sholl analysis from wildtype YFP+/tdTomato- layer V cortical neurons of either the oblique dendrite arbor elaborated within the dense tdTomato+ band in layer IV of the cortex (A) or the basal dendrite arbor elaborated within the tdTomato- upper layer V (B). These data are presented combined in the main Figure 3, as no differences are observed. N=20 neurons per arbor for the wildtype neurons in the Sim-A1-OE cortex and 10 neurons per arbor for the wildtype neurons in the Sim-A1-Only cortex. n.s., not significant.

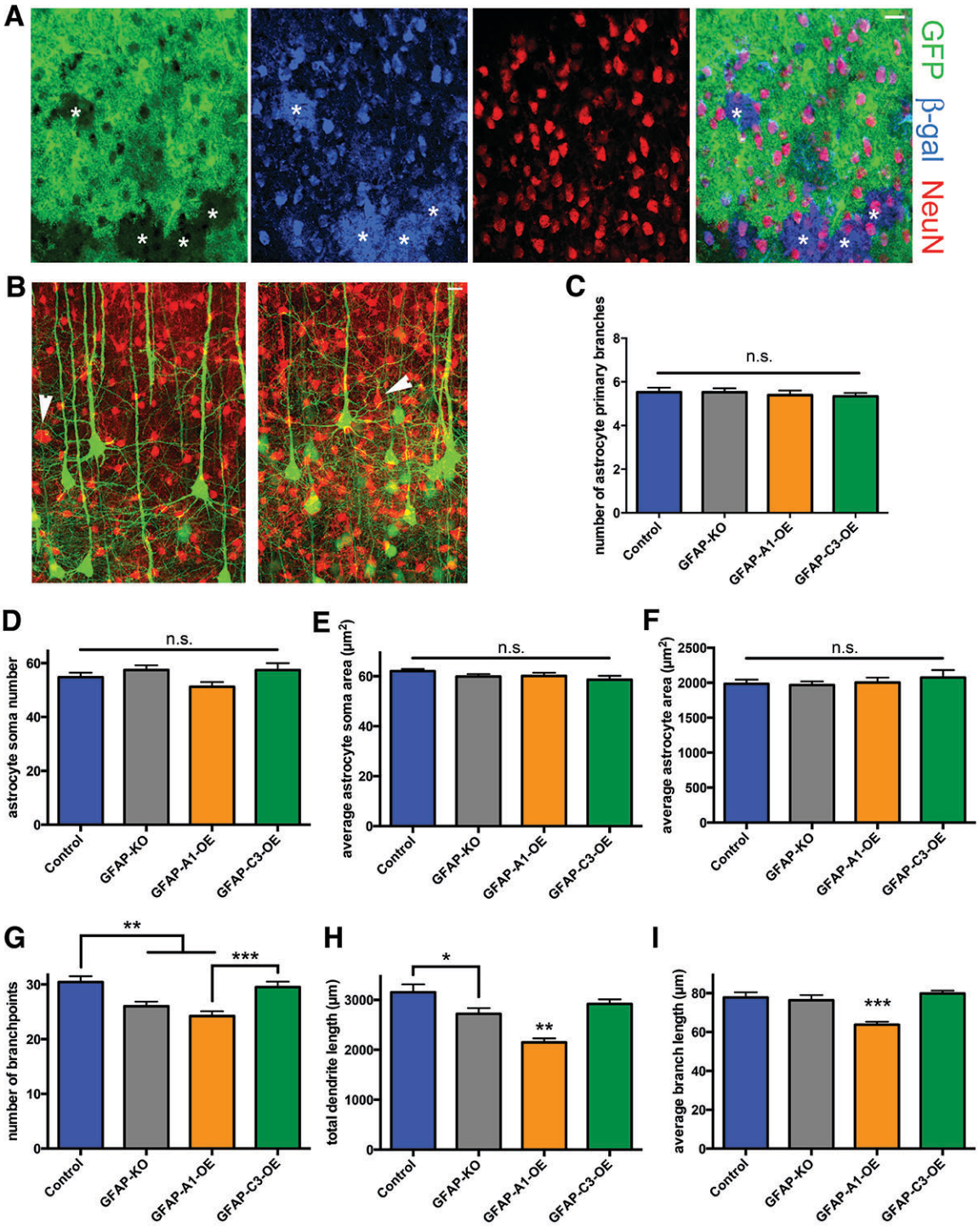

Figure S4 (Related to Figure 4). <u>Measures of YFP+ layer V neuron dendritic arbors and of astrocytes in GFAP-KO, GFAP-A1-OE and GFAP-A1-Only cortex.</u> A,B) *Gfap-Cre* line 77.6 restricts excision to astrocytes in the cerebral cortex. A) Cryosections of *Gfap-Cre;Z/EG* cortex. In the Z/EG reporter line, β-galactosidase is ubiquitously expressed until Cre excision deletes the *LacZ* gene and allows expression of EGFP. With *Gfap-Cre*, most protoplasmic astrocytes throughout the cortex are GFP+ (green); a few are still un-recombined and express β-galactosidase (blue, marked by asterisks). NeuN+ neurons (red) are also β-galactosidase-positive but GFP-negative.

B) Additional maximum projections of confocal stacks through the cortex of *Gfap-Cre;Thy1-YFPH;Ai-14-tdTomato* mice, similar to that shown in the main Figure 4C. Nearly all tdTomato+ cells have the morphology of astrocytes, and no YFP+ layer V neurons are tdTomato-filled (though several are contacted by tdTomato+ astrocytic processes). Very rarely, a tdTomato+ cell resembling a neuron can be found (arrowheads); all traced neurons were clearly YFP+/tdTomato-. C) Number of astrocyte primary branches. Maximum projections (30 μm depth around the center of the confocal stack's Z-axis) of tdTomato confocal stacks were thresholded to include only astrocyte somas and primary branches, and the primary branches were counted. D) Astrocyte soma number, measured from images prepared as in (C); only astrocyte somas were included and counts were made using FIJI's Analyze Particles module. E) Average astrocyte soma area, measured from images prepared as in (C); F) Average astrocyte area, taken by thresholding tdTomato confocal stacks to include all astrocyte somas and processes, measuring total area, and dividing by soma number. G-I) Number of branchpoints, total dendritic length, and average branch length were calculated as described in Figure S1. N=30 fields of view (C-F) or 60 neurons (G-I); *$p<0.05$, **$p<0.01$, ***$p<0.001$; n.s., not significant. Bar in A, B=20μm.

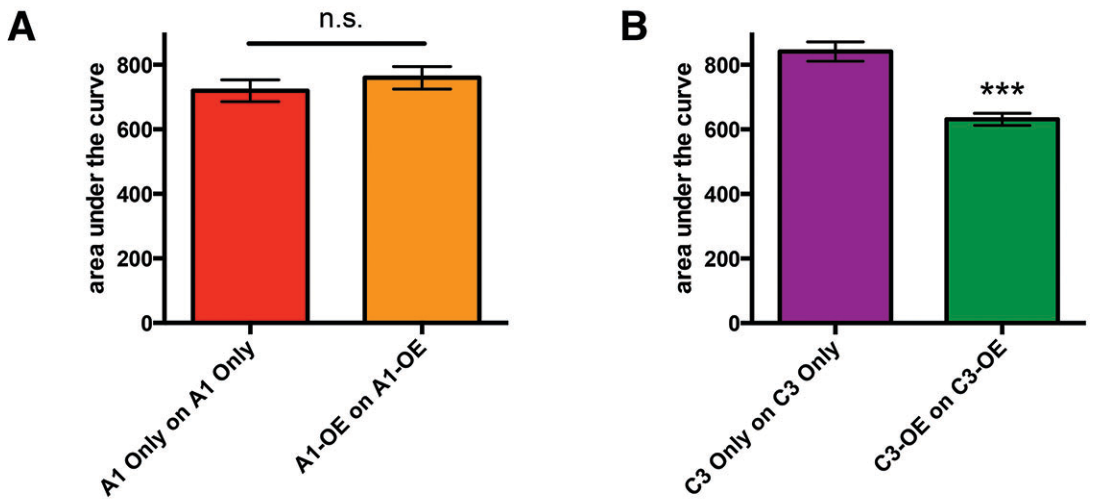

Figure S5 (Related to Figure 5). Measures of dendritic arbors in OE vs. Only neurons from *in vitro* co-culture experiments.

A,B) Area under the curve graphs of Sholl analysis from "A1-Only on A1-Only" vs. "A1-OE on A1-OE" and "C3-Only on C3-Only" vs. "C3-OE on C3-OE" cultures. As was seen in the *in vivo Emx1-Cre* results shown in Figure 1, the two A1 groups are identical (A), while dendrite arborization is significantly higher in neurons from "C3-Only on C3-Only" cultures than it is in neurons from "C3-OE on C3-OE" cultures (B). N=53-60 neurons per condition in (A,B); ***$p<0.001$; n.s., not significant.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mouse strains

The A1-mCherry and C3-mCherry transgenic lines, in which a single γ-Pcdh tagged at the C-terminus with mCherry is expressed from the Rosa locus following Cre excision of a STOP cassette, have been described previously (Lefebvre et al., 2012) and were the kind gift of Julie Lefebvre and Joshua Sanes (Harvard University). The *Pcdhg*^{fcon3} conditional mutant allele has been characterized previously (Prasad et al., 2008; Garrett and Weiner, 2009; Garrett et al., 2012a). The *Gfap-Cre 77.6* line (JAX stock #024098), *Emx1-Cre* line (JAX stock #005628), *Sim1-Cre* line (JAX stock #006451), *Thy1-YFPH* line (JAX stock #003782), *Z/EG* (JAX stock #004178) and *Ai14-TdTomato* (JAX stock #007914) were obtained from The Jackson Laboratory (Bar Harbor, ME). For each genotype presented in the main *in vivo* analyses, the indicated total number of neurons was traced from 3-6 animals at 5 weeks of age. Mice discussed as "OE" were compound transgenics harboring: 1) the indicated Cre allele; 2) 0 or 1 fcon3 allele; 3) the YFPH allele; 4) the A1- or C3-mCherry transgene. Mice discussed as "Only" were identical except they were homozygous for the fcon3 allele.

Antibodies

The following primary antibodies were used: chicken anti-GFP (Aves); rabbit anti-DsRed (Clontech); mouse anti-MAP2 (Sigma); mouse anti-γ-Pcdh-panA (Neuromab, clone N144/32); mouse anti-γ-Pcdh constant domain (Neuromab, clone N159/5); mouse anti-γ-Pcdh-C3 (Neuromab, clone N174B/27); rat anti-mCherry (Invitrogen); mouse anti-NeuN (Millipore). Isotype-specific fluorophore-tagged secondary antibodies were from Invitrogen.

Western blotting and co-immunoprecipitation

Cortical tissue was dissected and homogenized in lysis buffer [25mM Tris-HCl (pH 7.4), 50mM NaCl, 25mM NaF, 1mM EDTA, 1% Nonidet-P40 substitute, 5% Glycerol] complemented with protease inhibitors (Roche Mini Complete). Homogenized tissue was centrifuged at 2,000 x g for 15 minutes at 4˚C. Two micrograms of rabbit anti-DsRed was added to 0.5ml of lysate and incubated on a rotator at 4˚C overnight. The next day 25 µl of protein-A/G agarose (Pierce) was added and incubated for an additional 3 hours at 4˚C on a rotator. Subsequently beads were washed four times with lysis buffer. Beads were resuspended in 50 µl of 2X Laemmli buffer and analyzed by SDS/PAGE and western blot using standard methods as previously described (Schreiner and Weiner, 2010; Garrett et al, 2012a).

Cortical neuron cultures

Cultures were prepared from individual transgenic or knockout animals essentially as described (Garrett et al., 2012a) with some modifications. Briefly, cortices were dissected from postnatal day 0 animals, treated with enzyme solution (papain, 20 U/ml), and dissociated individually. For cortical co-cultures, cells harvested from individual animals were plated onto 12mm German glass coverslips (coated with Matrigel (Corning) diluted in Neurobasal medium (Invitrogen) at 1:50) at a density of 200,000 cells per coverslip in plating medium (BME, 5% fetal bovine serum, N2 supplements (Invitrogen), GlutaMAX (Invitrogen), and penicillin/streptomycin) Remaining cells were nucleofected with N1-EGFP plasmid using an Amaxa nucleofector and the Mouse Neuron Nucleofector Kit and plated at 2,000 cells per coverslip onto the previously-plated high-density cells from the same, or another, individual animal. After 2 hours, and every 3 days subsequent, media was changed to fresh Neurobasal media supplemented with GlutaMAX, B27 supplements (Invitrogen), and penicillin/streptomycin. Genotypes were determined from tail preps by PCR and desired co-cultures were kept for further analysis at 8 days *in vitro* (DIV). Nucleofection efficiency was ~50%. In other experiments, low efficiency (~1-5%) transfection using Lipofectamine 2000 (Invitrogen) was performed at 2 DIV. Each coverslip was incubated with 0.5µg total DNA and 1 µl Lipofectamine 2000, mixed in Neurobasal media according to manufacturer's instructions, for 2 hours before being changed back to complete Neurobasal medium. The GFP- and HA-tagged γ-Pcdh constructs were described previously (Schreiner and Weiner, 2010; Lobas et al., 2012). Cultures were fixed with 4% paraformaldehyde at 8 DIV, stained with anti-GFP and anti-DsRed (to enhance fluorescent staining), along with anti-MAP2 (to label all dendrites), and analyzed.

Image collection and analysis of dendrite arborization

For analysis of dendrite arborization *in vivo*, transgenic mice were perfused transcardially with 4% paraformaldehyde at 5 weeks of age. Following immersion in the same fixative overnight at 4˚C, brains were embedded in 2% agarose and cut into 200µm sections in the coronal plane using a Vibratome. Confocal images of YFP and tdTomato neurons and astrocytes were taken using a 20X objective on a Leica SP2 AOBS microscope or a 20X objective on a Leica TCS SPE microscope. YFP+ or tdTomato+ neurons were chosen for imaging only if the

majority of the basal and oblique arbors were near the middle of the 200 µm section, allowing for representative arbor analysis. Sixty µm Z-stack images were collected with a 0.5µm step size, as described (Garrett et al., 2012a). Z-stacks were imported into FIJI (NIH Image/J) and dendrites were traced in 3D by moving through the stack using the Simple Neurite Tracer plugin. Epifluorescence images of GFP+ transfected cultured neurons were taken using a 20X objective on a Leica TCS SPE microscope and traced in 2D using Simple Neurite Tracer. For Sholl analysis, concentric circles were spaced every 5 (for the *in vitro* cultures) or 10 (for the *in vivo* cortical neurons, respectively) µm from the cell body and dendrite crossings were counted using the Sholl Analysis function of Simple Neurite Tracer. For all *in vivo* and *in vitro* experiments the experimenter doing the tracing was blinded as to genotype or condition. Crossings were graphed in Prism (Graph Pad software) for display, and the area under the curve for each Sholl plot was calculated in Prism. Comparisons between genotypes or conditions were performed in Prism by one-way ANOVA, corrected for multiple comparisons with Tukey's test and reported multiplicity adjusted p values for each comparison, or by unpaired, two-tailed t-test when only comparing two samples using the area under the curve data. Asterisks in all figures consistently denote the following significance levels: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Analysis of oblique/basal dendrite complexity

To calculate the ratio of oblique/basal dendrite complexity in Figure 3, we separately traced the oblique arbor (branches coming off the apical shaft) and the basal arbor (branches coming off the cell body) for each YFP+, td-Tomato- layer V cortical neuron as detailed above. The complexity of these arbors was assessed using the Sholl Analysis function of Simple Neurite Tracer and presented in full in Figure 3C,D. Only a portion of the oblique tracings, from 30 to 200 µm, contained significant branches and lay within the td-Tomato+ layer IV band; the oblique area under the curve was thus calculated within this region (shaded red in Figure 3C). The values generated for the area under the curve of each Sholl plot (Figure 3C,D) were then compared by dividing the oblique value by the basal value for each individual neuron, thus generating a ratio. The oblique/basal ratio for control neurons was normalized to 1. Comparison between these conditions was performed in Prism by unpaired, two-tailed t-test.