Supplementary Figure 1. Effects of αE-catenin knockdown are cell-autonomous. Homozygous floxed αE-catenin embryos (*Ctnna1*$^{lox(Ex2)}$) were co-electroporated with an internal control expression plasmid (pCAG-mCherry) along with a GFP-expressing experimental construct (pCAG-Cre-IRES-GFP). The distribution of cells expressing the GFP-tagged construct (any GFP$^+$ cell) was compared with control cells expressing only mCherry. In this way, all experimental conditions (target tissue, surgical variability, electroporation location and effectiveness, timing, etc.) would be identical between cells expressing the experimental construct versus only the control mCherry. Analysis of internal controls (mCherry$^+$/Cre$^-$ electroporated cells in the same brains) showed that neighboring cells behaved similar to fluorescent-only controls where homozygous floxed embryos were electroporated with only fluorescent tagged proteins (images shown in Fig. 2). If non-autonomous effects were responsible for the phenotype that results following reduction in αE-catenin, then there would be a disparity between tissue-wide controls and internal controls. However, the striking similarity between the cortical distribution of fluorescent-only controls and internal controls demonstrates that the observed effects of αE-catenin knockdown are cell-autonomous. Images presented were electroporated at E13.5 and analyzed at E15.5. Cre: GFP signal (Cre expressing cells). mCherry: all cells expressing mCherry. Merge: Overlay image shows exclusively red (internal control), exclusively green, and co-labeled cells. Dots: Visual representation of the individual cells where all those electroporated with Cre-recombinase are labeled with green marks, while those that only received mCherry are labeled with red marks. The histogram compares the proportion of electroporated cells in each cortical zone between internal controls and fluorescent-only controls, showing that internal controls behaved normally and that loss of function effects were cell-autonomous. Data presented as mean ± s.e.m.; CP denotes cortical plate, IZ designates intermediate zone, and VZ represents the ventricular zone. Bar: 50 µm.

Supplementary Figure 2. Short-hairpin RNAs targeting αE-catenin can effectively reduce observed protein levels. Primary cortical cultures were induced with one of three different short hairpin RNA expression vectors (Open Biosystems, Huntsville, AL) targeting αE-catenin or pCAG-eGFP (control) and cultured for 48 hours, then their respective lysates were analyzed through western blot to determine the respective shRNA construct efficacies. Lysates from lane 1 were nucleofected with pSHAGM2-αEcatenin-2010-E-4 (clone ID: V2MM_67879); lane 2 was nucleofected with pSHAGM2-aEcatenin-2697-H-3 (clone ID: V2MM_67396); lane 3 was nucleofected with pSHAGM2-aEcatenin-2226-B-3 (clone ID: V2HS_165579); and lane 4 was nucleofected with pCAG-eGFP. Lysates were probed for αE-catenin to determine efficiency and actin as a loading control. Analysis demonstrated that protein levels of αE-catenin were significantly reduced in cells nucleofected with shRNA targeting this protein ($F_{[3,12]} = 85.310$, $p < 0.001$; the Tukey HSD post-hoc test determined that all treatments were significantly different from one another, except for the difference between constructs used in lanes 1 and 2). The relative intensity of αE-catenin expression in each lane was normalized using the corresponding intensity for actin expression. The construct employed in lane 1 was used in all other experiments detailed in this study. Data presented in the histogram as mean ± s.e.m..

Supplementary Figure 3. Cortical distribution of electroporated cells are consistent between control groups. A) Swiss-Webster embryos were electroporated on E13.5 with fluorescent protein constructs alone or with shRNA specific controls (shRNA targeting GFP or non-silencing shRNA) and analyzed 48 hours after surgery. The histogram compares the proportion of electroporated cells in each cortical zone between the two different control groups that have been compiled into one control group for comparison with the shRNA-mediated loss of αE-catenin function manipulation and shows that the groups do not significantly differ from one another ($\chi^2_{[2]} = 4.29$, $p = 0.117$). **B)** Homozygous floxed αE-catenin embryos (*Ctnna1*$^{lox(Ex2)}$) were electroporated (on E13.5 and analyzed 48 hours later) with fluorescent protein constructs only and wild-type mice of the same strain were electroporated with Cre-recombinase for control animals to compare to the Cre-recombinase experimental group. The histogram compares the proportion of electroporated cells observed in each cortical zone between the two control groups. No significant differences were observed between the two control groups for the Cre-mediated loss of αE-catenin ($\chi^2_{[2]} = 2.65$, $p = 0.266$), demonstrating that the Cre-recombinase construct did not have an additional effect on the observed results. Data presented as mean ± s.e.m.; CP denotes cortical plate, IZ designates intermediate zone, and VZ represents the ventricular zone. Bar: 50 µm.

Supplementary Figure 4. Additional examples that focal elimination of αE-catenin reduces β-catenin signaling transcriptional activity. β-catenin mediated Wnt signaling in the ventricular zone was examined through expression of destabilized GFP controlled by the TOP promoter. E13.5 embryos were electroporated with pTOP-dGFP and pCAG-mCherry in the Control treatment and analyzed at E14.5. Experimental loss of function treatments were electroporated with the TOP-dGFP reporter, mCherry, and pSHAGM2-αEcatenin-2010-E-4 (shRNA), or pCAG-Cre (Cre; into *Ctnna1*$^{lox(Ex2)}$ animals). Two examples separated by channel for each treatment are shown. Loss of αE-catenin function reduced the proportion of cells in the ventricular zone that display β-catenin mediated Wnt signaling. Bar: 50 μm.

Supplementary Figure 5: Additional examples that rescue of β-catenin signaling following elimination of αE-catenin function restores proliferation. Beta-catenin signaling was rescued by introducing a stabilized variant of the protein (Δ90-β-catenin) which lacks GSK3β phosphorylation sites that normally target the protein for degradation. **A**) E13.5 embryos were electroporated with pCAG-GFP alone (control), pCAG-Δ90-β-catenin-GFP (Δ90-β-catenin), pSHAGM2-αEcatenin-2010-E-4 and pCAG-GFP (shRNA), or pSHAGM2-αEcatenin-2010-E-4 and pCAG-Δ90-β-catenin-GFP (rescue). All treatments were immunostained for the proliferative marker Ki67 (shown in red). **B**) E13.5 homozygous floxed αE-catenin (*Ctnna1*$^{lox(Ex2)}$) embryos were electroporated with pCAG-GFP alone (control), pCAG-Δ90-β-catenin-GFP (Δ90-β-catenin), pCAG-Cre-IRES-GFP (Cre), or pCAG-Cre-IRES-GFP and pCAG-Δ90-β-catenin (rescue), then immunostained for Ki67 (red). Because the majority of GFP-tagged Δ90-β-catenin construct localizes to the cell membrane, visualization of targeted cells is often obscured. To ensure clarity of the presented approach, additional images of the Δ90-β-catenin treatments presented in both Fig. 8 and this supplemental figure have been included. These images also display the mCherry fluorescent construct that was co-electroporated into embryos. The images in (**C**) and (**E**) correspond to the Δ90-β-catenin images from (**A**) and Fig. 8A, respectively. The images in (**D**) and (**F**) correspond to the Δ90-β-catenin images from (**B**) and Fig. 8D, respectively. Additionally, a dot plot representation of cells expressing the Δ90-β-catenin construct (dots) has been included. All scale bars: 50 μm.

Supplementary Figure 6: Rescue of β-catenin signaling following elimination of αE-catenin function does not reduce migration of targeted cells to the cortical plate. Beta-catenin signaling was rescued by introducing a stabilized variant of the protein (Δ90-β-catenin) which lacks GSK3β phosphorylation sites that normally target the protein for degradation. **A**) E13.5 embryos were electroporated with pCAG-GFP alone (control), pCAG-Δ90-β-catenin-GFP (Δ90-β-catenin), pSHAGM2-αEcatenin-2010-E-4 and pCAG-GFP (shRNA), or pSHAGM2-αEcatenin-2010-E-4 and pCAG-Δ90-β-catenin-GFP (rescue). **B**) Visual representation of the individual cells where electroporated cells are labeled with white marks. **C**) Distribution indexes for Swiss-Webster treated animals, data presented as mean ± s.e.m (control n = 6 brains, 2213 cells; Δ90-β-catenin n = 6 brains,1967 cells; shRNA n = 3 brains, 1757 cells; rescue n = 3 brains, 474 cells). **D**) E13.5 homozygous floxed αE-catenin (*Ctnna1*$^{lox(Ex2)}$) embryos were electroporated with pCAG-GFP alone (control), pCAG-Δ90-β-catenin-GFP (Δ90-β-catenin), pCAG-Cre-IRES-GFP (Cre), or pCAG-Cre-IRES-GFP and pCAG-Δ90-β-catenin (rescue). **D**) Visual representation of the individual cells where electroporated cells are labeled with white marks. **F**) Distribution indexes for homozygous floxed αE-catenin treated animals, data presented as mean ± s.e.m (control n = 5 brains, 2341 cells; Δ90-β-catenin n = 6 brains,1967 cells; Cre n = 4 brains, 1847 cells; rescue n = 3 brains, 509 cells). Despite the partial restoration of proliferation following increased β-catenin signaling, the rescue treatments did not reduce the number of targeted cells that migrated to the cortical plate. Introduction of a stabilized form of β-catenin alone causes a slight increase in the fraction of cells remaining in the VZ (consistent with previous findings describing decreased neuronal differentiation following β-catenin overexpression). Although β-catenin also appears to restore the number of cells remaining in the VZ (in rescue experiments) compared to αE-catenin loss of function manipulations, the proportion of cells at the cortical plate remains unchanged. This observation that β-catenin does not rescue all of the alterations in cell positions caused by loss of αE-catenin is consistent with the idea that the adhesive function of αE-catenin may contribute to maintaining cells in the VZ. CP denotes cortical plate, IZ designates intermediate zone, and VZ represents the ventricular zone. All scale bars: 50 μm.

Supplementary Figure 7: No changes in adherens junction components following αE-catenin reduction. Homozygous floxed αE-catenin embryos (*Ctnna1*$^{lox(Ex2)}$) were electroporated (on E13.5 and analyzed 48 hours later) with either fluorescent protein constructs only (Control) or Cre-recombinase also expressing GFP (driven by IRES2) (Cre). **A)** Electroporated tissues were immunostained for several components of the adherens junction: filamentous actin, N-cadherin, and β-catenin and separated by channel. AJ: denotes the aforementioned adherens junction proteins; GFP: denotes either Cre-recombinase expressing GFP in Cre treatments or GFP in Control treatments; Merge: overlay of these images. Bar: 50 μm. **B)** Higher magnification of electroporated tissue displaying neighboring electroporated cells immunostained for the adherens junction components (AJ) N-cadherin or β-catenin separated by channel. AJ: denotes the aforementioned adherens junction proteins; GFP: denotes either Cre-recombinase expressing GFP in Cre treatments or GFP in Control treatments; Merge: overlay of these images. Arrows denote points of adhesion between adjacent electroporated cells. Bar: 10 μm. All images show no discernable changes in adhesion molecules between loss of function and control treatments.

Supplementary Figure 8: Increased β-catenin signaling rescues the reduction in proliferation caused by αE-catenin knockdown despite disruptions in tissue architecture and cell polarity. In some rescue brains we observed a disruption in epithelial integrity, suggesting that this approach caused a decrease in adhesion. Depicted is an image of tissue transfected with GFP-tagged Δ90-β-catenin and shRNA against αE-catenin immunostained for Ki67 demonstrating the increase in the proliferative fraction as well as non-autonomous adhesive deficits that occurred in some brains following introduction of the two experimental constructs. Scale bar: 50 μm.