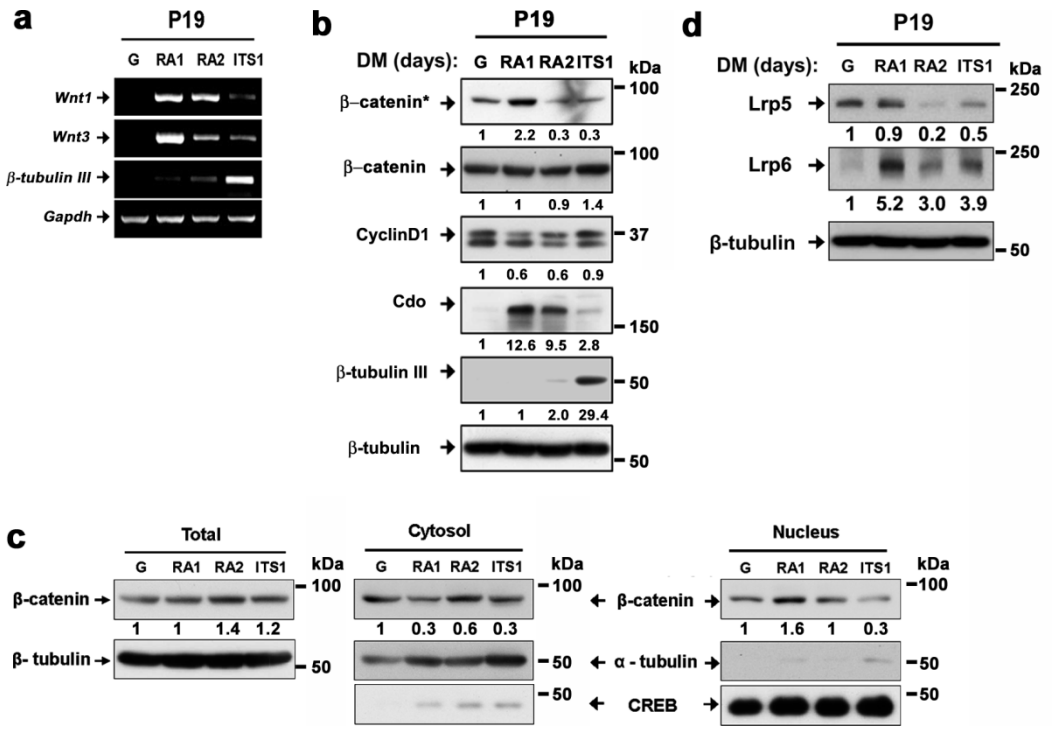
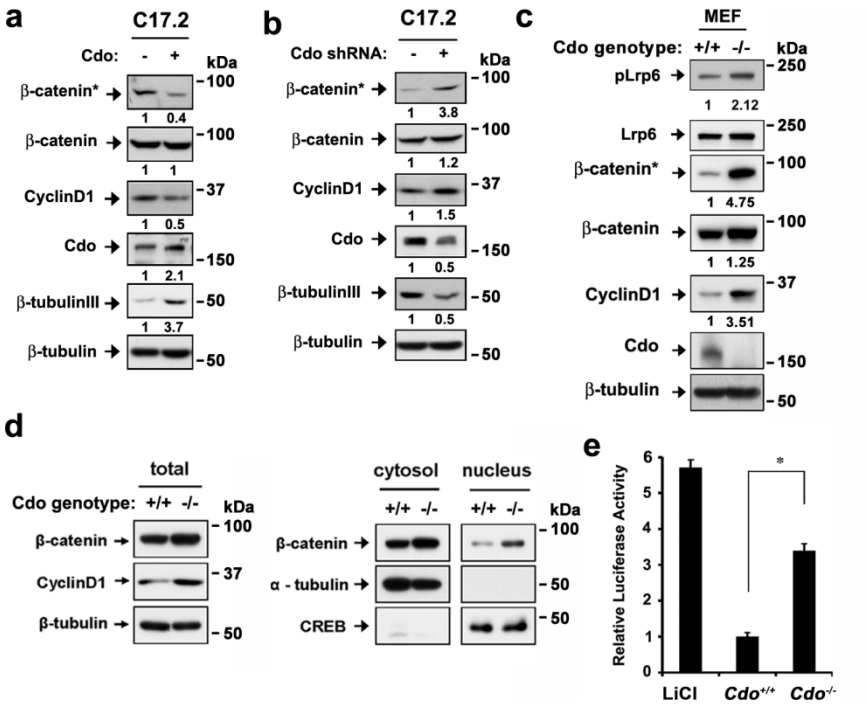


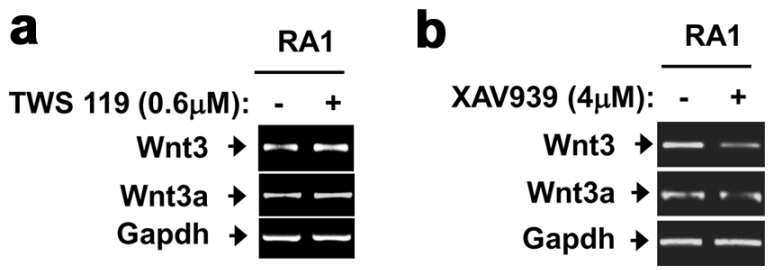
Supplementary Figure 1. Expression of Wnts and signaling activation in the initiation stages of neuronal differentiation.

(**a**) RNAs from P19 cells at the indicated time points were analysed by RT-PCR for Wnt1, 3, β-tubulin III and Gapdh as a control. (**b**) Immunoblotting analysis of P19 cell lysates from various differentiation time points with indicated antibodies. (**c**) Cellular fractionation and immunoblotting analysis of P19 cells from various differentiation time points. (**d**) Immunoblot analysis of P19 cell lysates of various differentiation stages with indicated antibodies.



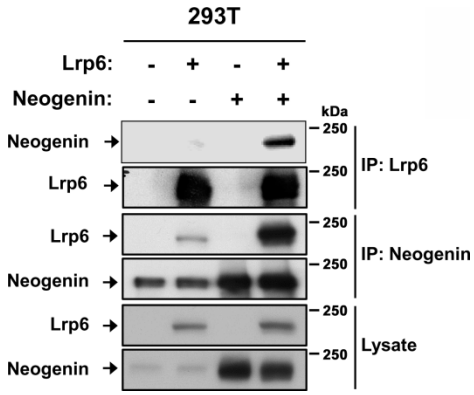
Supplementary Figure 2. Overexpression and knockdown of Cdo in C17.2 neural progenitor cells decreased and increased β-catenin activation, respectively.

(**a, b**) Lysates of control, Cdo or Cdo shRNA transfected C17.2 cells at differentiation day 1 were subjected to immunoblotting with indicated antibodies. (**c**) Lysates of *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ MEFs were analysed by immunoblotting with indicated antibodies. (**d**) *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ MEFs were subjected to cellular fractionation. The cytosol and nuclear fractions as well as total lyastes were analysed by immunoblotting. (**e**) The Top-flash reporter activity in *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ MEFs. Data are reported as the relative luciferase activity. Values are means ± SD from the determinants of triplicates, this experiment was repeated three times with similar results. **P*< 0.007. Statistical significance was tested by a Student *t*- test.

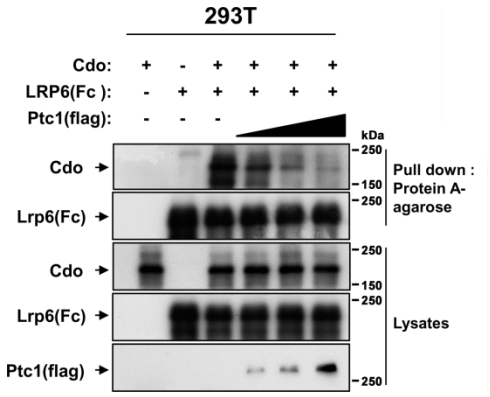


Supplementary Figure 3. The expression of Wnt3 and Wnt3a was dependent on Wnt signaling activity.

(**a**) RT-PCR of P19 cells treated with either the vehicle, DMSO (-) or TWS119 at RA1. (**b**) RT-PCR of P19 cells treated with either the vehicle, DMSO or XAV939 at RA1. All experiments were repeated at least three times with similar results.

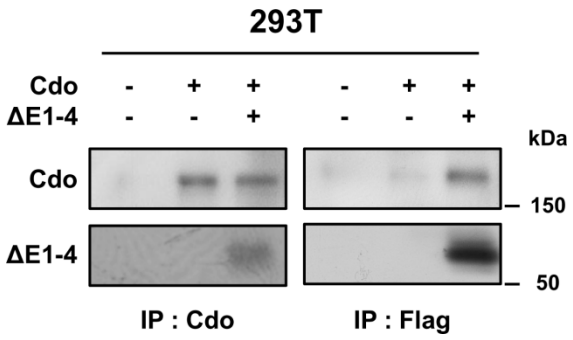


Supplementary Figure 4. The full-length Neogenin interacted with Lrp6. Coimmunoprecipitation of 293T cell lysates transfected with expression vectors for the full-length Lrp6 and Neogenin, followed by immunoblotting. This experiment has been repeated twice with similar results.

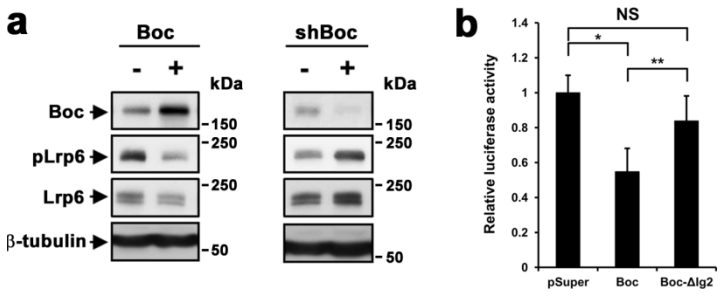


Supplementary Figure 5. Patched1 expression interfered with the interaction between Cdo and Lrp6.

Protein A-agarose pull-down assay with 293T cell lysates transfected with Lrp6(Fc), Cdo and increasing amounts of flag-tagged Patched 1, Ptc1(flag), followed by immunoblotting. This experiment has been repeated three times with similar results.

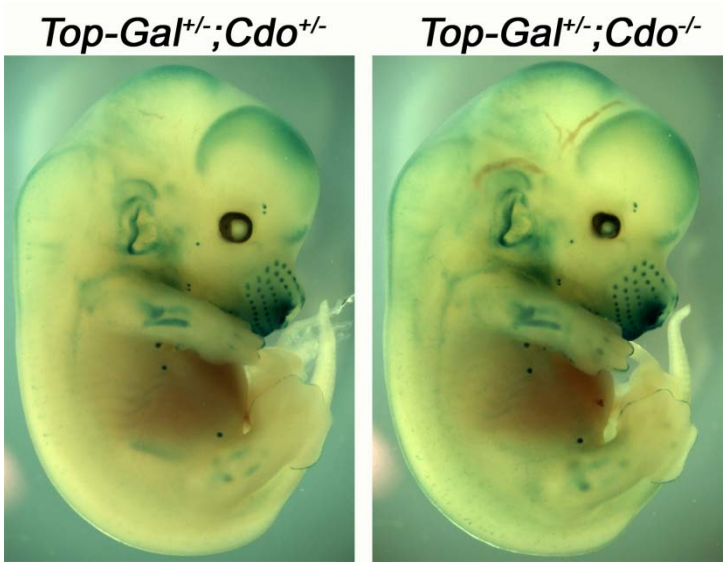


Supplementary Figure 6. Cdo interacted with EGF-like domains deletion mutant of Lrp6. Coimmunoprecipitation analysis of 293T cell lysates transfected with the combination of expression vectors for Cdo and flag tagged Lrp6 ΔE1-4 mutant. This experiment has been repeated three times with similar results.



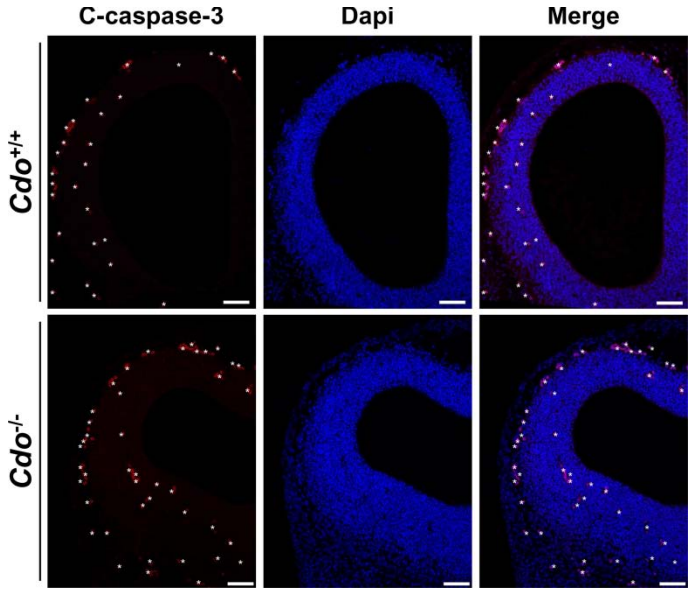
Supplementary Figure 7. Boc inhibited Wnt signaling.

(**a**) Immunoblotting analysis of control, Boc-overexpressing or knockdown P19 cell lysates at RA1. (**b**) The luciferase assay of 10T1/2 cells expressing control, Boc or ΔIg2 vectors. These values are the determinants of triplicates and this experiment was repeated three times with similar results. Values are means ±SD (n=3). **P*< 0.005, ***P*< 0.001. Statistical significance was tested by a Student *t*- test.



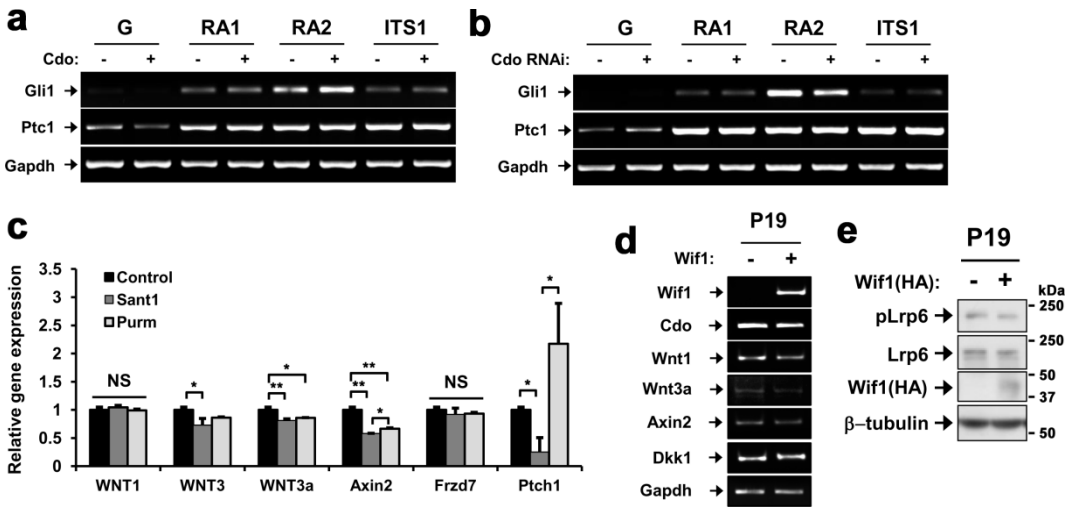
Supplementary Figure 8. The effects of Cdo deficiency on Top-gal reporter activity at E13.5 mouse embryos.

β-gal staining was carried with littermate E13.5 *Top-Gal*$^{+/-}$*;Cdo*$^{+/-}$ and *Top-Gal*$^{+/-}$*;Cdo*$^{-/-}$ embryos. The intensity of Top-gal staining between *Cdo*$^{+/-}$ and *Cdo*$^{-/-}$ embryos was not significantly different at mouse forebrain areas.



Supplementary Figure 9. Overt cell death was not detected in in *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ embryonic telencephalons.

Immunostaining of cleaved caspase-3 in *Cdo*$^{+/+}$ and *Cdo*$^{-/-}$ embryonic telencephalons at E11.5. Autofluorescence of red blood cells were marked with asterisks. Size bar = 100 μm. This experiment was three times with similar results.

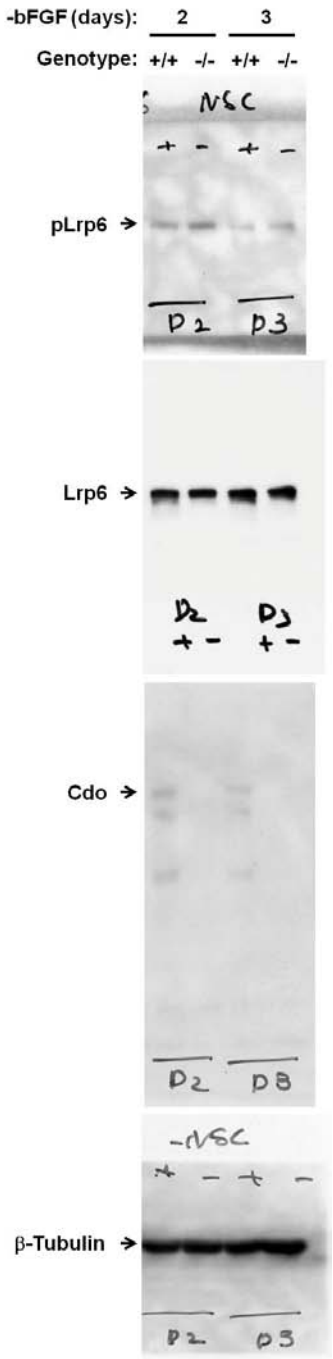


Supplementary Figure 10. Wnt signaling was independent of hedgehog signaling in P19 cells and Wif1 overexpression reduced Wnt signaling only slightly.

(a) Semi-quantitative RT-PCR analysis with RNAs isolated from control or Cdo-overexpressing P19 cells during neuronal differentiation. (b) Semi-quantitative RT-PCR analysis with RNAs isolated from control or Cdo-knockdown P19 cells during neuronal differentiation. (**c**) qPCR analysis with RNAs isolated from P19 cells treated with Shh antagonist, Sant1 (50 μM) or agonist purmorphamine (2μM) at RA1. Values are means ± SD from the determinants of triplicates. **P*< 0.05 ***P*< 0.01 Statistical significance was tested by a Student *t*- test. (**d)** RT-PCR and (**e**) Immunoblot analysis of P19 with Wif1 overexpression at RA1. All experiments were repeated at least three times with similar results.

Supplementary Note 1.

The uncropped scans of Figure 1g.



Supplementary Note 2.

The uncropped scans of Figure 3f.