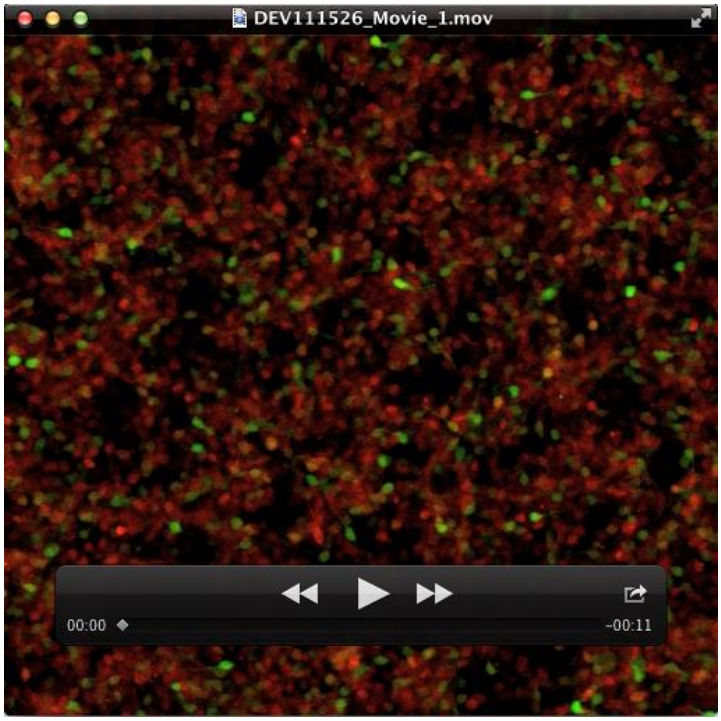
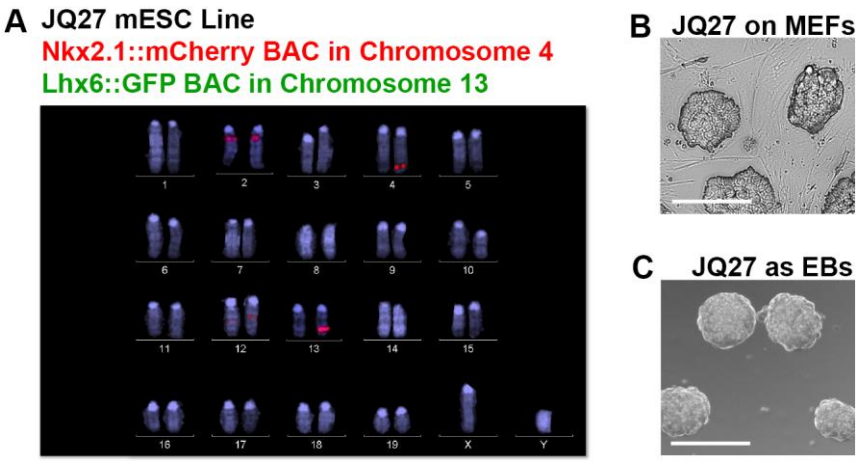
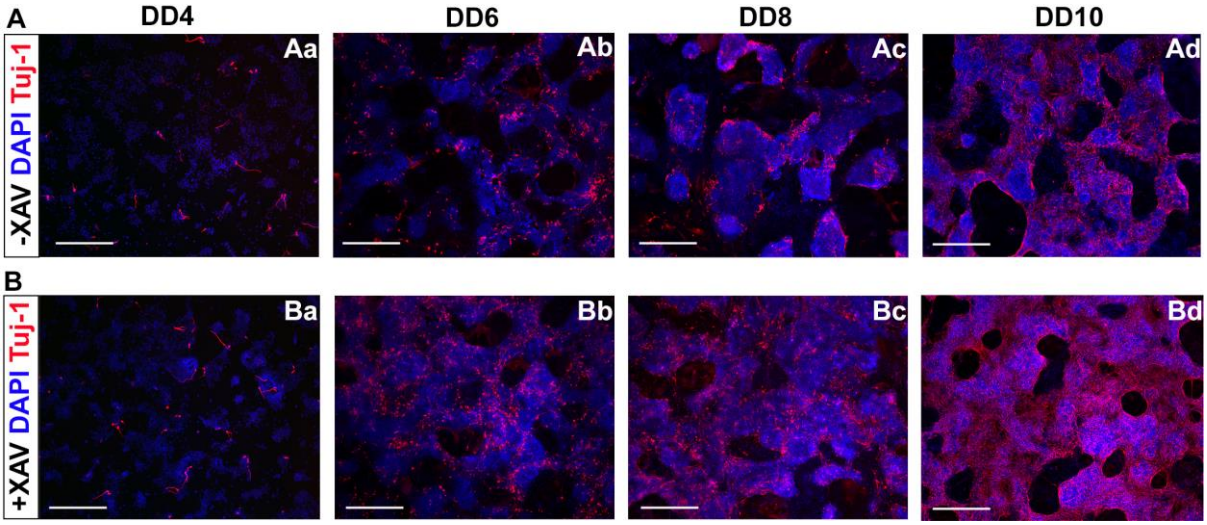


Movie 1. FACS sorted Nkx2.1::mCherry+ cells give rise to Lhx6::GFP+ putative interneurons

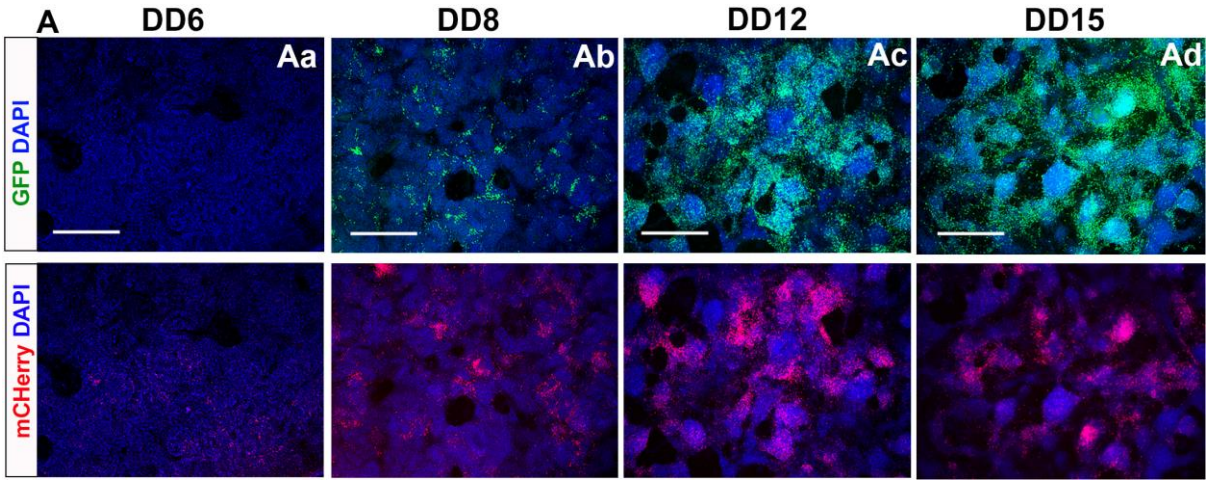
JQ27 mESCs were differentiated according to the protocol in Figure 1B, sorted for Nkx2.1::mCherry on DD12 and re-plated as a high density monolayer on matrigel. Time-lapse images were acquired at 10X magnification and at 10 min intervals for 8-14 hours using an Olympus FV10i confocal microscope, 37°C, 5% CO2. Over time, mCherry+ cells give rise to Lhx6::GFP+ putative mESC-derived interneuron precursors. Cells were plated, and left to settle for 2 hours post FACS to avoid significant z-drift during live imaging, then from t=0 images above represent 2hr intervals until +16hrs. Scale bar=175µm.



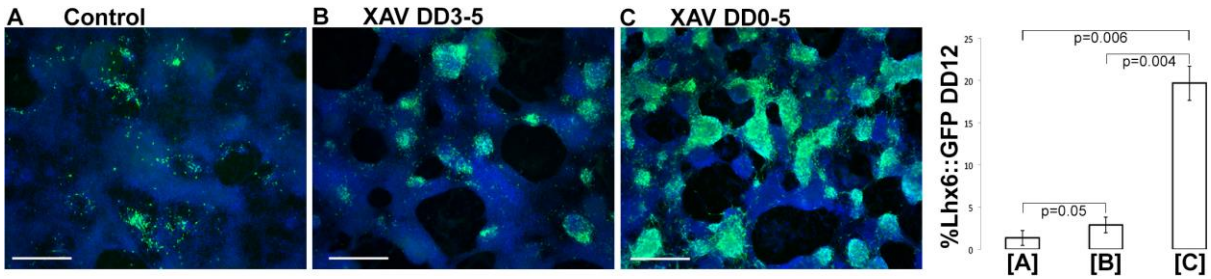
Supplementary Fig. 1. BAC integration in JQ27 and characteristic stem cell behavior
(A) Composite of BAC FISH chromosomal analysis of the JQ27 reporter line where Nkx2.1::mCherry has integrated once into chromosome 4, and Lhx6::GFP has integrated once into chromosome 13. Nkx2.1 genomic location can be seen on chromosome 12, and Lhx6 genomic location is detectable on chromosome 2. (B) Brightfield image of JQ27 mESCs plated on MEFs and allowed to expand for several days, showing classic pluripotent colony morphologies. (C) Brightfield image of JQ27 mESCs displaying typical embryoid body morphologies. Scale bar: 75µm



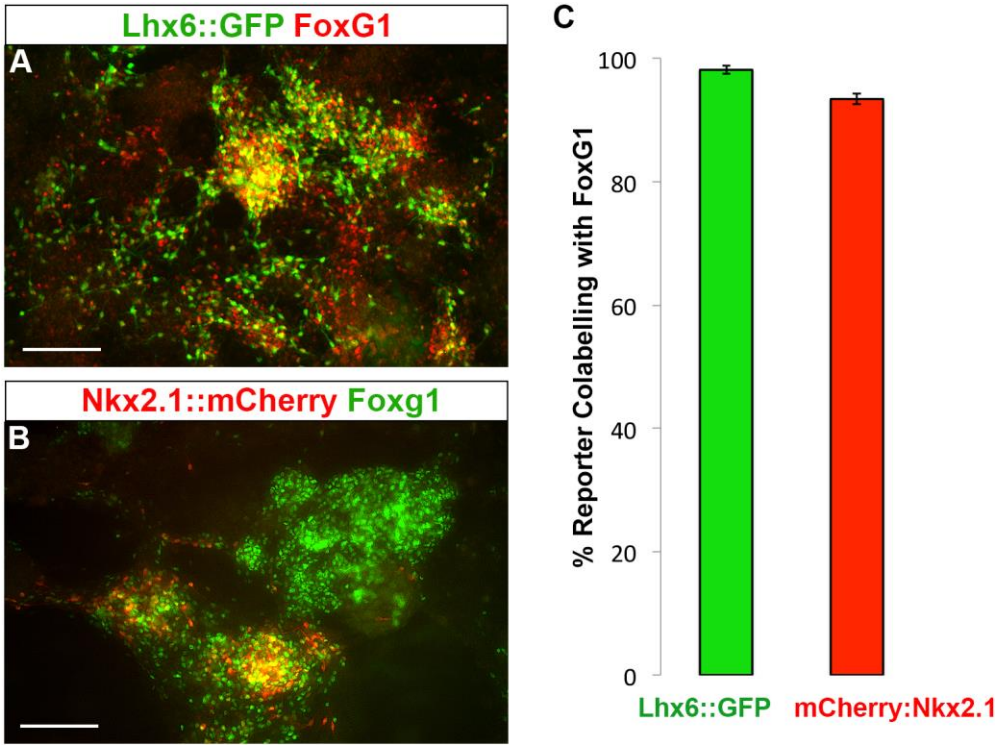
Supplementary Fig. 2. Early Wnt inhibition potentiates neural induction
(A-A4) Tuj1 (red, top panels) immunofluorescence on DD4, 6, 8 or 10 on mESCs that were differentiated without XAV939. (B-B4) Tuj1 (red, bottom panels) immunofluorescence on DD4, 6, 8 or 10 on mESCs which were differentiated towards a telencephalic fate with the addition of Wnt inhibitor XAV939 from DD0-5 in which floating embryoid bodies were dissociated and plated onto an adherent substrate as a low density monolayer at DD3. Blue signal shows the nuclear staining for DAPI in all panels. Scale bars: 500μm. DD= differentiation day.



Supplementary Fig. 3. Nkx2.1::mCherry and Lhx6::GFP expression timecourse
(A-A3) GFP (green, top panels) and mCherry (red, bottom panels) immunofluorescence on DD6, 8, 12 or 15 on mESCs that were differentiated towards a telencephalic fate with the addition of Wnt inhibitor XAV939 from DD0-5 in which floating embryoid bodies were dissociated and plated onto an adherent substrate as a low density monolayer at DD3. Blue signal shows the nuclear staining for DAPI in all panels. Scale bars: 500μm. DD= differentiation day.

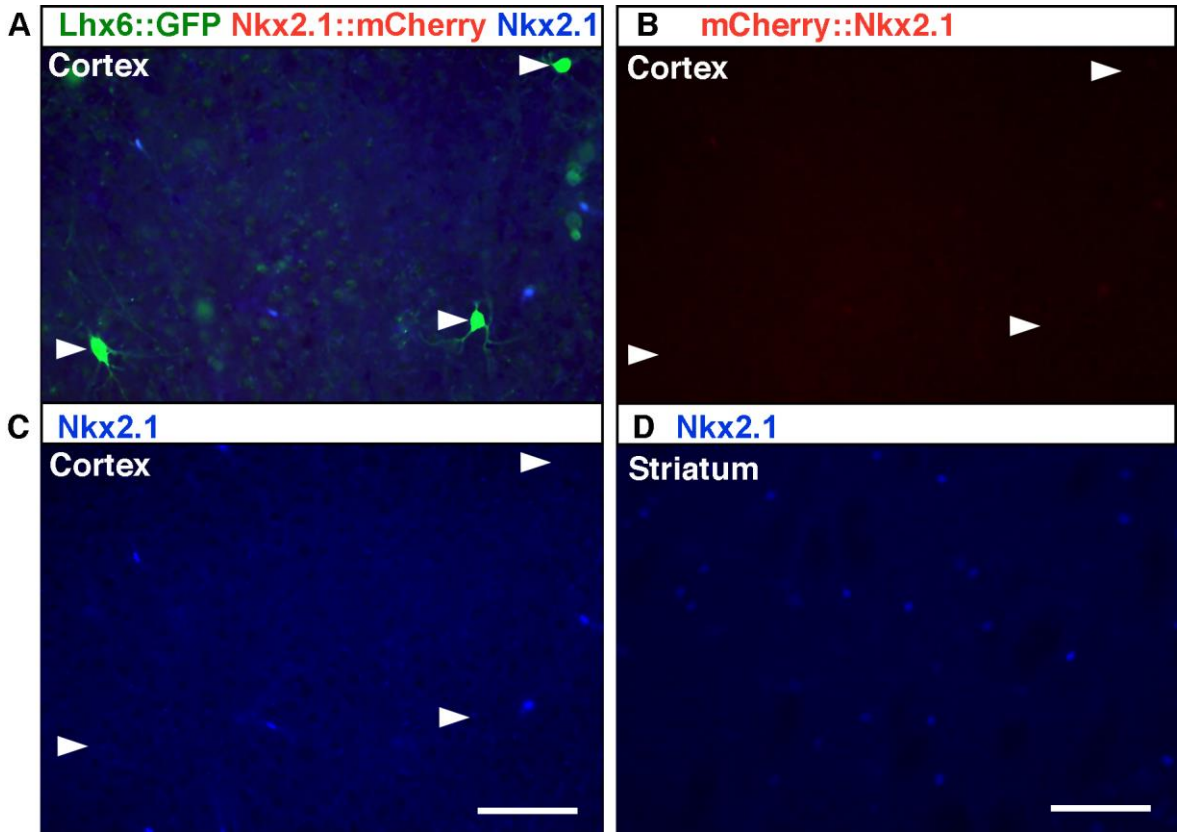


Supplementary Fig. 4. Potent Lhx6::GFP induction depends on early Wnt inhibition
(A) GFP (Green) immunofluorescence on DD12 on mESCs that were differentiated without XAV939. (B) GFP (Green) immunofluorescence on DD12 on mESCs that were differentiated with XAV939 from DD3-5. (C) GFP (Green) immunofluorescence on DD12 on mESCs that were differentiated with XAV939 from DD0-5. Blue signal shows the nuclear staining for DAPI in all panels. (D) Analysis of Lhx6::GFP induction at DD12 using FACS, demonstrated that early XAV treatment (DD0-5) increased Lhx6::GFP induction significantly over both no XAV or treating XAV from DD3-5. Scale bars: 500μm. DD= differentiation day.



Supplementary Fig. 5. Majority of Nkx2.1::mCherry and Lhx6::GFP mESCs express FoxG1

(A) Lhx6::GFP (green) and FoxG1 (red) immunofluorescence at DD12 on mESCs that were differentiated as described in (Fig. 1B). (B) Nkx2.1::mCherry (red) and Foxg1 (Green) immunofluorescence on DD12 on mESCs that were differentiated as described in (Fig. 1B). (C) Quantification of Lhx6::GFP co-labeling with Foxg1 (green bar) and quantification of Nkx2.1::mCherry co-labeling with Foxg1, error bars indicate mean values from three independent experiments ± SD. Scale bar 300μm. DD=differentiation day, DPT=days post transplant.



Supplementary Fig. 6. Neocortical transplants of mCherry+ cells do not express mCherry or Nkx2.1 30 days post transplant

Neocortical transplants of mCherry-expressing cells do not express mCherry or Nkx2.1 30 days post transplant. At differentiation day 12 mCherry+ cells were isolated by FACS, transplanted into neonatal neocortex, and evaluated in fixed sections prepared 30 days later. (A-C) Show the same view of neocortex, with immunolabeling for GFP (A), RFP (A and B; antibody recognizes mCherry), and Nkx2.1 (A and C; pseudocolored from Cy5). Arrows point to three GFP-expressing cells that do not express mCherry or Nkx2.1. Evaluation of over 100 such triple-labeled cells revealed no colabeling of GFP with either mCherry or Nkx2.1, consistent with the down regulation of Nkx2.1 in neocortical interneuron. (D) shows Nkx2.1 immunolabeling of striatum from the same section as in (A-C), and is included as a positive control. Scale bar=100µm.