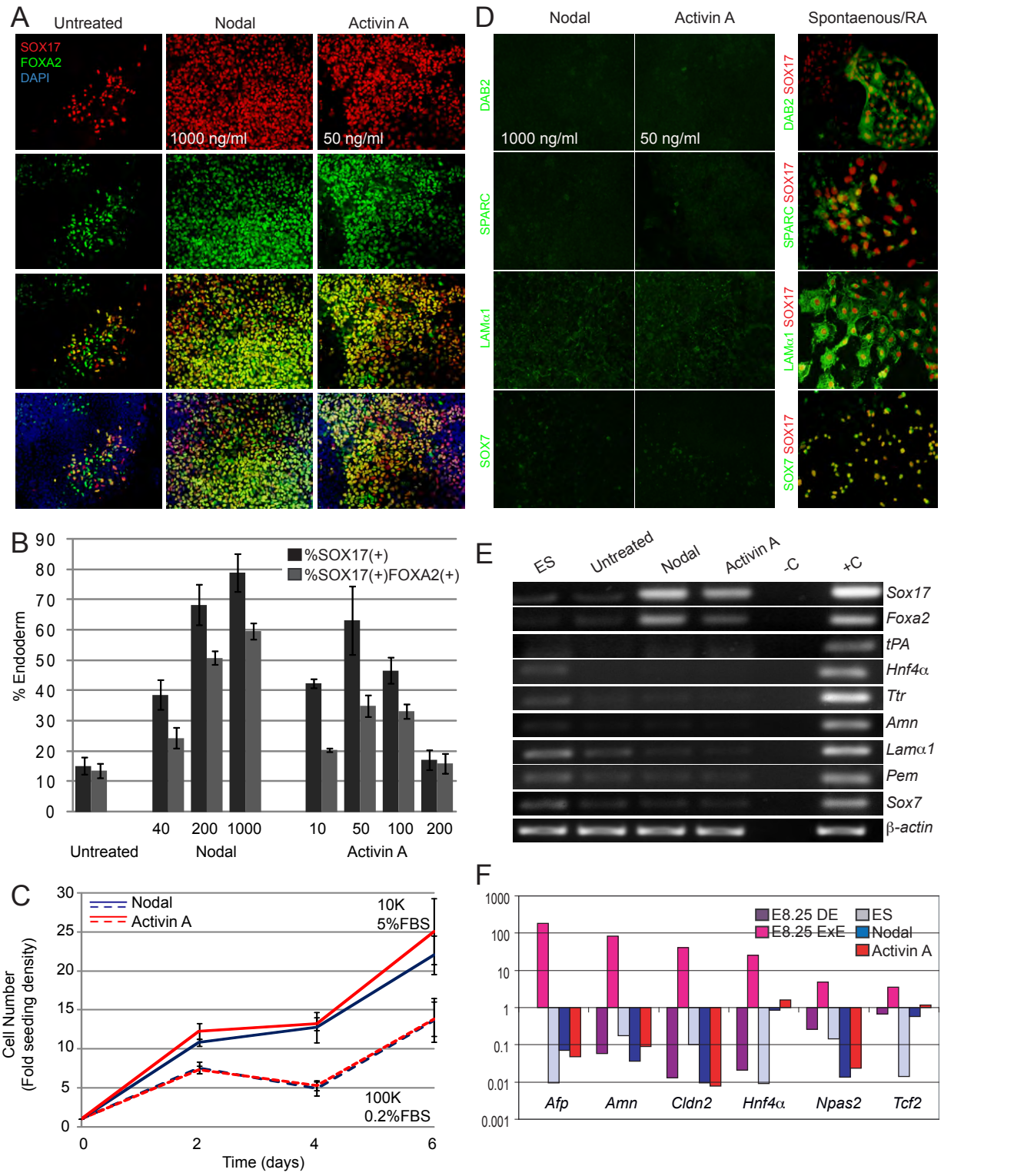

Fig. S1. Nodal and Activin preferentially induce definitive rather than extra-embryonic endoderm *in vitro*. (**A**) Immunofluorescence images of mouse AV3 ES cell-derived endoderm. Expression of pan-endoderm markers, Sox17 and Foxa2, in untreated, Nodal-treated and Activin-treated cells after 7 days of differentiation. Nuclei stained with DAPI. (**B**) Quantification of Sox17 and Foxa2 expression in differentiating mESCs cultured in the absence or presence of increasing concentrations (ng/ml) of Nodal or Activin for 1 week (n=4). Data represented as mean±s.e.m. (**C**) Proliferation rate of Nodal- and Activin-induced cultures at low and high seeding density (10,000 or 100,000) and serum content (0.2% or 5%) across 6 days of differentiation. Data represented as mean±s.e.m. (**D-F**) Nodal- and Activin-derived endoderm lack expression of ExEn markers (*Dab2*, *Sparc*, *Lama1*, *Sox7*, *tPA*, *Hnf4*, *Amn*, *Pem*, *Dpp4*, *Afp*, *Cldn2*, *Npas2*, *Tcf2*). (D) Immunofluorescence images of Nodal- and Activin-induced endoderm compared with spontaneous or RA-induced cultures. (E) RT-PCR analysis of endoderm gene expression in undifferentiated ES cells, untreated cells, and cells treated with Nodal or Activin. –C, no cDNA negative control; +C, E7.5 cDNA positive control. (F) Quantitative RT-PCR analysis of ExEn gene expression in undifferentiated Sox17-DsRed reporter mESCs, and purified DsRed(+) endoderm cells from cultures differentiated with Nodal or Activin. Embryonic DE and ExEn dissected from E8.25 mice are included as controls.

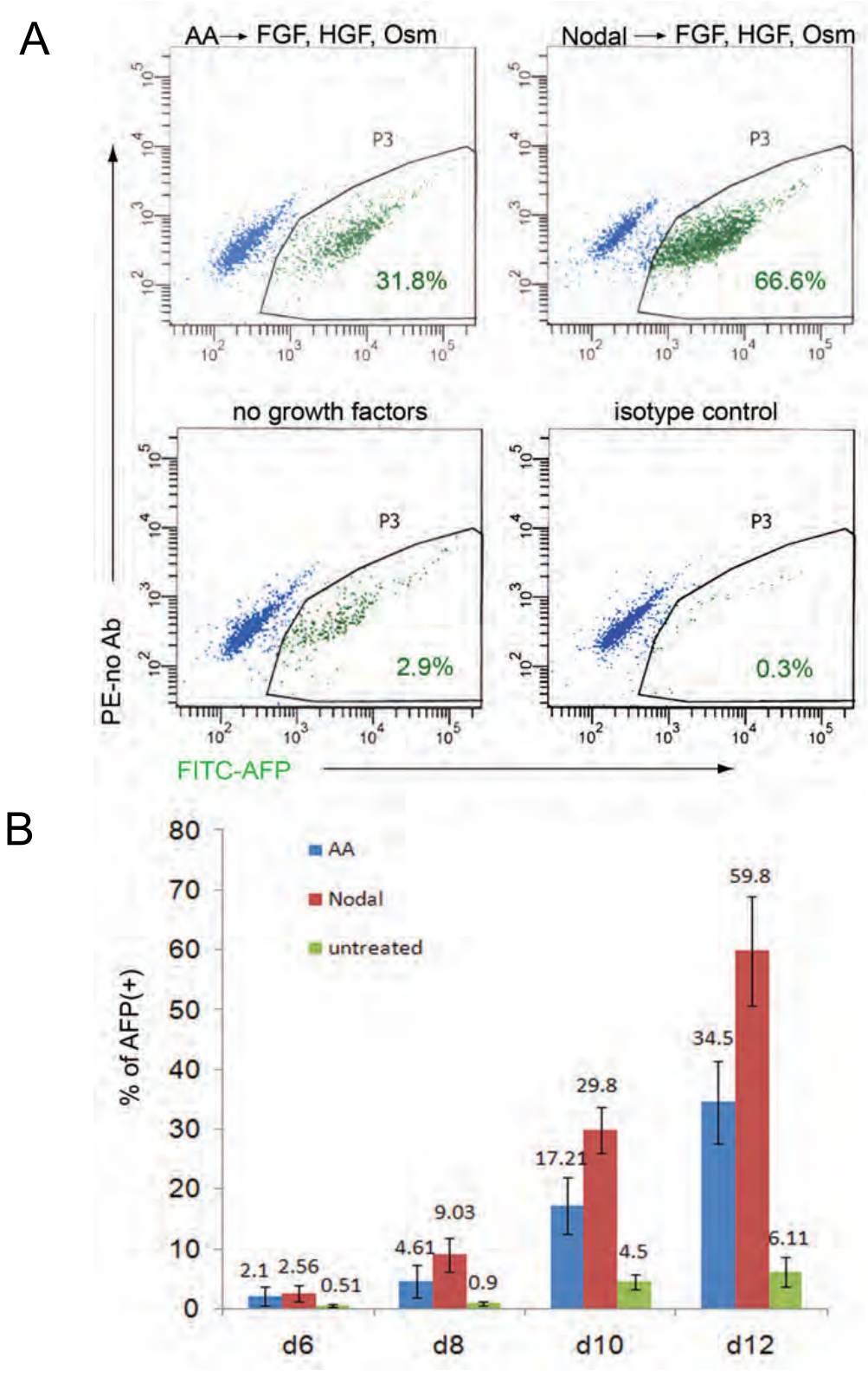

Fig. S2. Nodal- and Activin-derived endoderm differ in hepatic differentiation potential. (**A**) Quantification of alpha-fetoprotein (AFP) induction. AV3 mESCs treated/untreated with Nodal or Activin A for 6 days were then differentiated towards hepatic progenitors in the presence of FGF2, BMP4, HGF and Oncostatin M (Osm) for an additional 6 days (Gouon-Evans et al., 2006; Argwal et al., 2008). Samples were harvested on day (d) 12 for quantification by flow cytometry. Nodal-derived endoderm gives rise to twofold greater AFP(+) cells (66.6%) than does Activin-derived endoderm (31.8%). (**B**) Nodal-derived endoderm exhibits greater efficiency than Activin A in generating AFP(+) cells during the course of hepatic specification. Time course includes samples harvested prior to hepatic induction, d6, up to d12. Untreated samples included as controls. Data represented as mean±s.e.m.

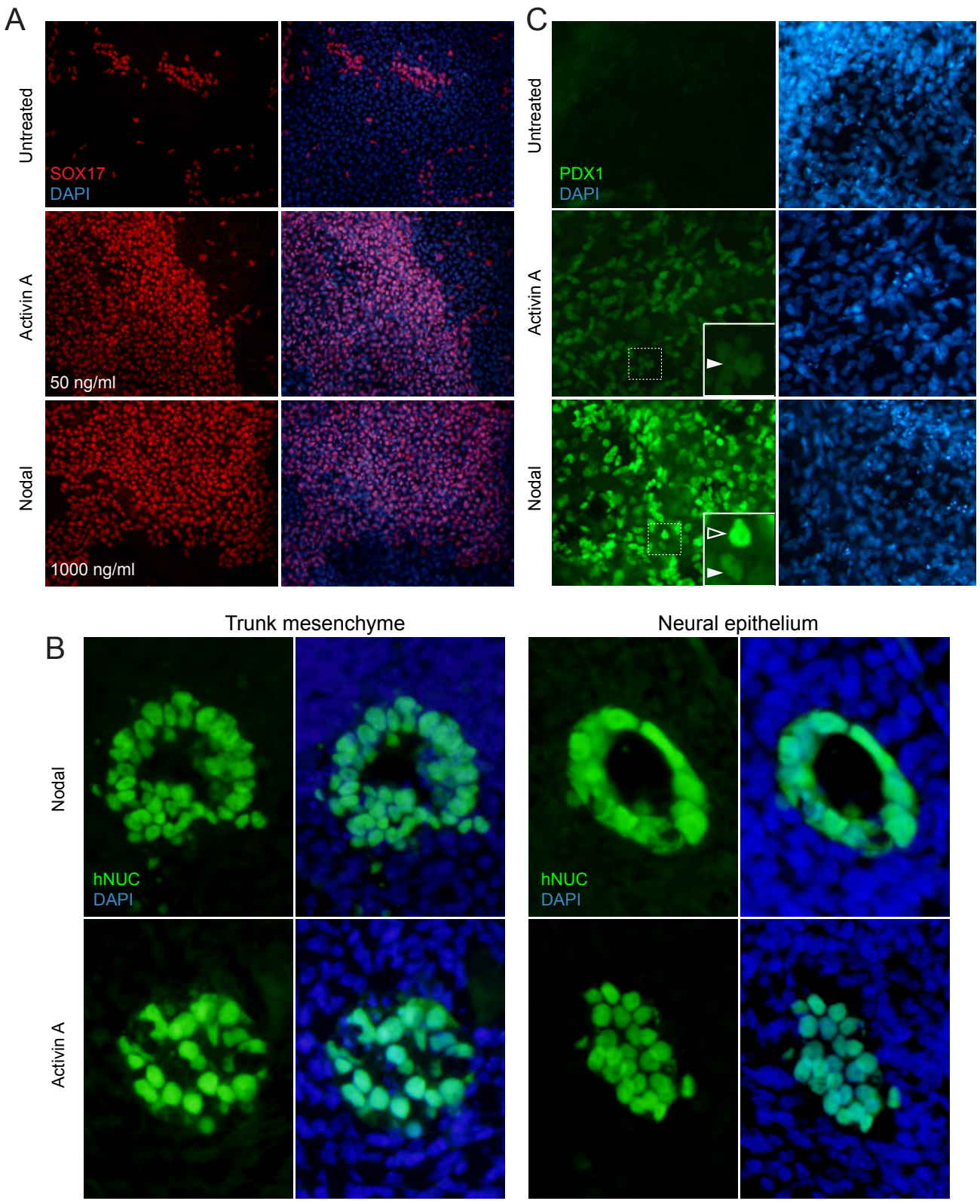

Fig. S3. Directed differentiation of human ES cells with Nodal and Activin results in functionally distinct endoderm populations. (**A**) Immunofluorescent images of Sox17 expression in untreated HUES8 control samples and those treated with Nodal (1000 ng/ml) or Activin (50 ng/ml). (**B**) Immunofluorescence analysis of sectioned embryos following injection of HUES8-derived endoderm into the trunk mesenchyme or neural epithelium of E8.75 mouse embryos and 24 hours of *ex vivo* embryo culture. Primitive gut tube-like structures of 50-75 μm in length with a prominent lumen and epithelial morphology formed from Nodal-treated cells. Activin-treated cells remain disorganized at the site of injection. hNuc, human nuclei. (**C**) HUES8 cells were treated/untreated with Nodal or Activin for 4 days then differentiated towards pancreatic endoderm in the presence of FGF10, RA, KAAD-Cyc and ILV. The majority of PDX-expressing cells in Activin-treated cultures were PDX^{low} (unfilled arrowhead), whereas those in Nodal-treated cultures contained PDX^{low} and PDX^{high} cells (filled arrowhead). Boxed regions are magnified in insets. Nuclei stained with DAPI.