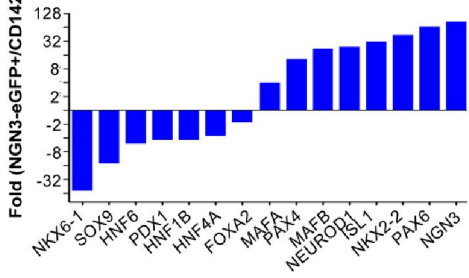
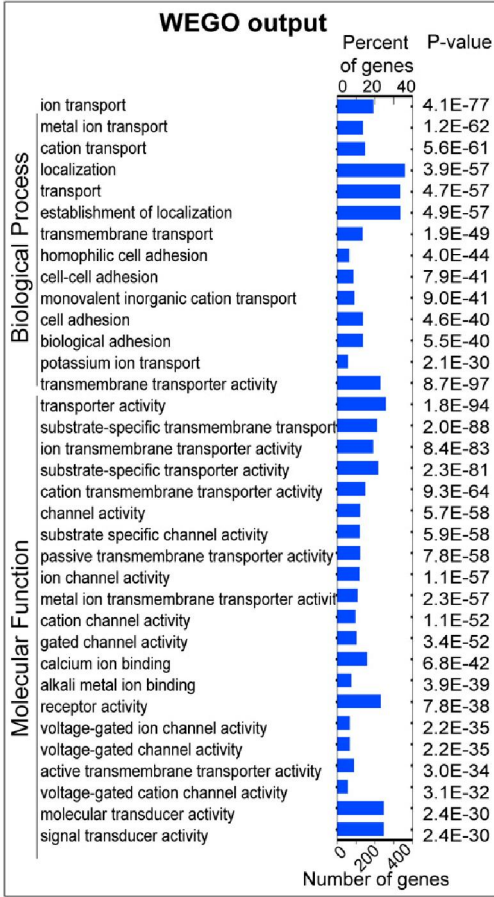


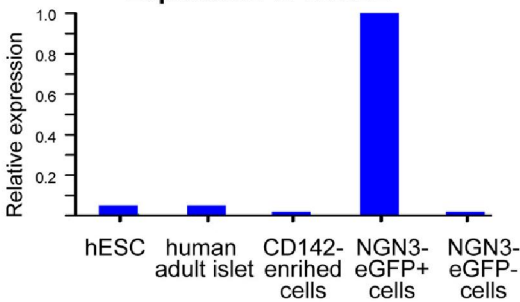
**A Gene expression analysis (NGN3-eGFP+ VS CD142-enriched cells)**



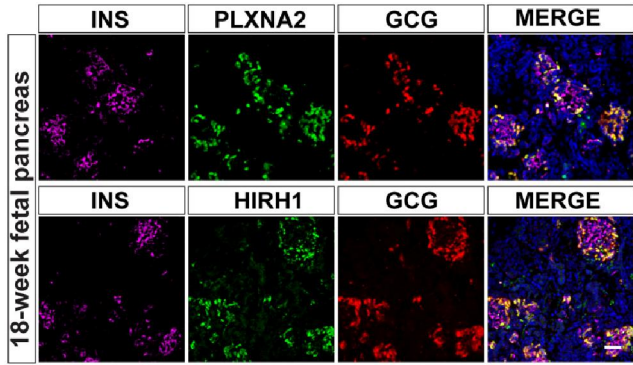
**B WEGO output**



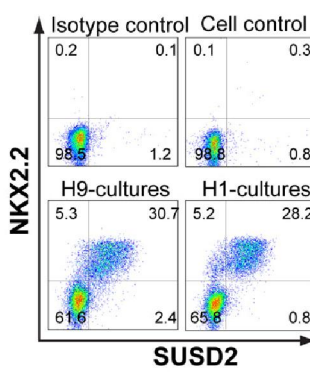
**C Expression of SUSD2**



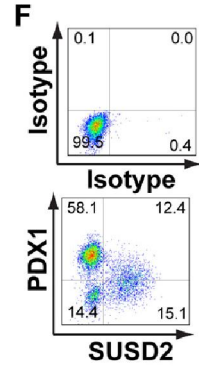
**D**



**E**

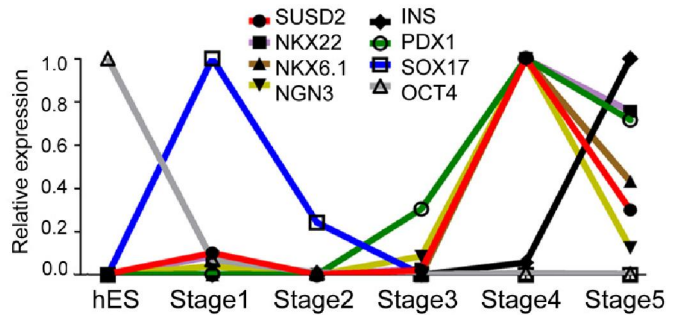


**F**

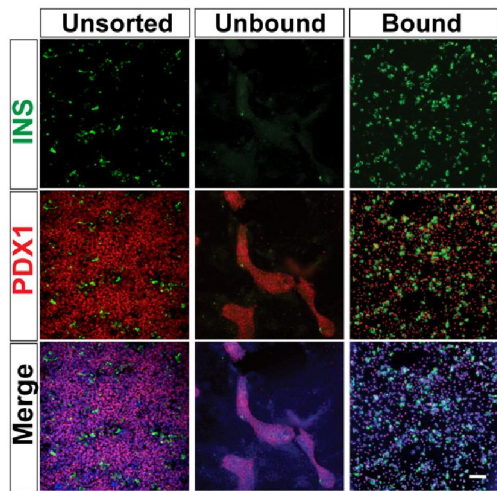


**G**

**Gene expression dynamics during hES differentiation**



**H**



**Supplementary information, Figure S4.** Gene ontology (GO) analysis of the differentially expressed transmembrane proteins and the characterization of NGN3-eGFP-enriched and SUSD2-enriched cells *in vitro*. (A) Transcript factors expressed in pancreatic progenitor cells are enriched in CD142-enriched pancreatic endoderm cells, and these expressed in the endocrine-associated cells are enriched in NGN3-eGFP<sup>+</sup> cells. RNA-seq data of CD142-enriched cells is from Gene Expression Omnibus (Kelly et al., 2011). (B) GO term analysis of 1,003 transmembrane proteins that are differentially expressed between NGN3-eGFP<sup>+</sup> and NGN3-eGFP<sup>-</sup> cells at stage 4. (C) A flow cytometric analysis of differentiating *NGN3-eGFP* cells at stage 4, day 3 demonstrated that SUSD2<sup>+</sup> cells exhibited weak or no PDX1 expression. (C) An analysis of *SUSD2* expression using available RNA-sequencing datasets for different cells and tissue types showed that *SUSD2* expression is highly detected in NGN3-eGFP<sup>+</sup> cells but weakly detected in NGN3-eGFP<sup>-</sup> cells, hESCs, CD142 enriched pancreatic endoderm cells and human islets. RNA-sequencing datasets were from Gene Expression Omnibus (Kelly et al., 2011, Xie et al., 2013). (D) PLXNA2 and HRH1 were specifically expressed in GCG-producing cells, but not INS-producing cells in 18-week human pancreas. (E) SUSD2 can highly enrich for NKX2.2<sup>+</sup> cells in wild type H1 and H9 cell cultures at the end of stage 4. (F) A flow cytometric analysis of differentiating NGN3-eGFP cells at stage 4 day 3 demonstrated that SUSD2<sup>+</sup> cells exhibited weak or no PDX1 expression. (G) Gene expression dynamics analysis of the entire hESC differentiation process demonstrated that the expression of *SUSD2* tightly follows that of endocrine progenitor-associated genes. (H) Stage 4 cells were magnetically sorted using an anti-SUSD2 antibody and further cultured before immunostaining analysis. The INS expression is enriched in the bound fraction-derived cells, while PDX1 expression is enriched in the unbound fraction-derived cells. Nuclear staining with DAPI (blue) is shown in the merged images. The scale bar represents 50  $\mu$ m. Abbreviation: INS (INSULIN).