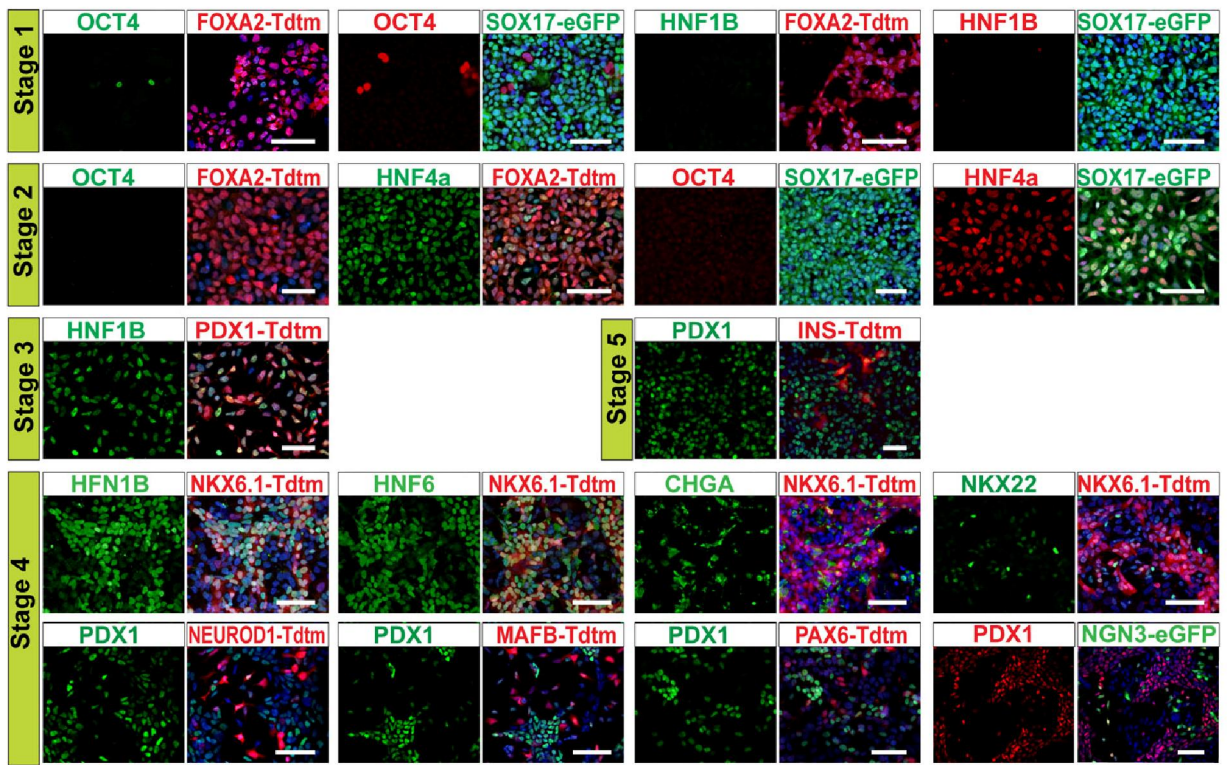
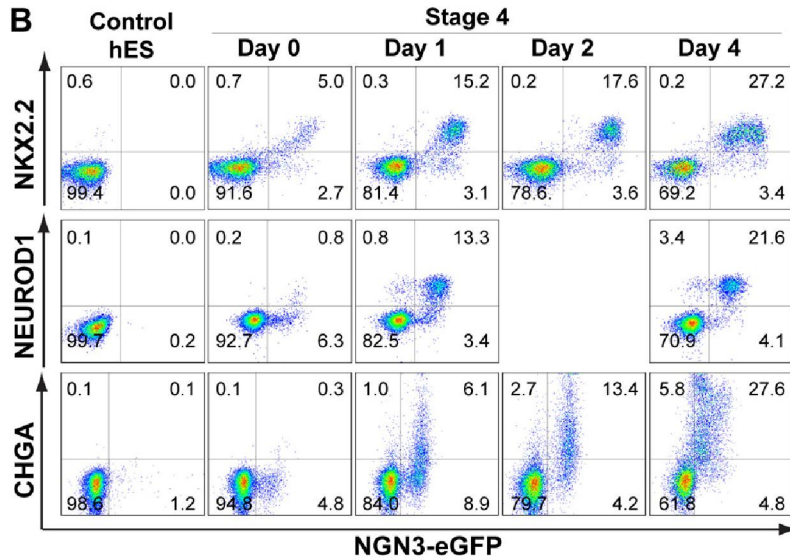


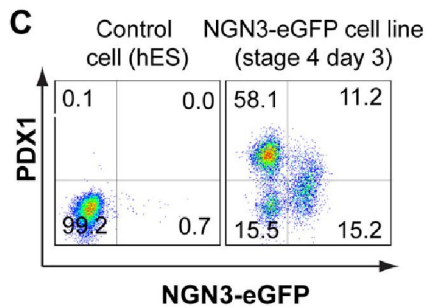
**A**



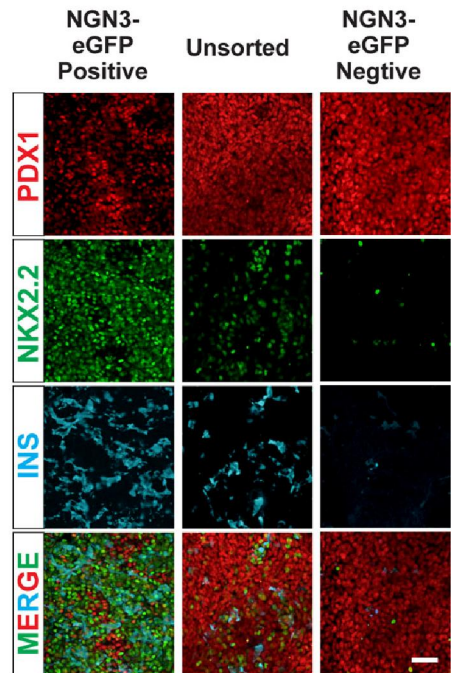
**B**



**C**



**D**



**Supplementary information, Figure S3.** Characterizing the different cell populations marked by individual gene reporters. (A) Immunostaining cell populations identified by individual gene reporters. At stage (S) 1, both FOXA2-Tdtm<sup>+</sup> cells and SOX17-eGFP<sup>+</sup> cells were negative for the pluripotency factor OCT4 and the primitive gut tube factor HNF1B or HNF4A. At S2, FOXA2-Tdtm<sup>+</sup> cells and SOX17-eGFP<sup>+</sup> cells express HNF4A but not OCT4. At S3, PDX1-Tdtm<sup>+</sup> cells expressed HNF1B. At stage 4, NKX6.1-Tdtm<sup>+</sup> cells expressed the pancreatic progenitor factors HNF1B and HNF6, but few cells expressed CHGA; a few of the NKX6.1-Tdtm<sup>+</sup> cells also expressed NKX2.2. NGN3-eGFP<sup>+</sup> cells, NEUROD1-Tdtm<sup>+</sup> cells, MAFB-Tdtm<sup>+</sup> cells and PAX6-Tdtm<sup>+</sup> cells expressed little or no PDX1, a pancreatic progenitor factor. The right panel of each pair of images is a merged image with DAPI. (B) A time-course flow cytometric analysis of the differentiating *NGN3-eGFP* cells throughout stage 4 demonstrated most of the eGFP<sup>+</sup> cells express NKX2.2 and NEUROD1 at this stage. Most of them expressed CHGA at the end of this stage. (C) Flow cytometric analysis of differentiating *NGN3-eGFP* cells at stage 4, day 3 demonstrated that the eGFP<sup>+</sup> cells exhibited weak or no PDX1 expression. (D) Cell fraction of *NGN3-eGFP* cell line at Stage 4 day 1-2 were obtained by FACS and further cultured before immunostaining analysis. The expression of INS and NKX2.2 were enriched in the eGFP<sup>+</sup> cells derived culture, while PDX1 expression was enriched in eGFP<sup>-</sup> cells derived culture. Nuclear staining with DAPI (blue) is shown in the merged images. The scale bar represents 50 μm. Abbreviations: INS (INSULIN); CHGA (CHROMOGRANIN A); Tdtm (TdTomato); SST (SOMATOSTATIN).