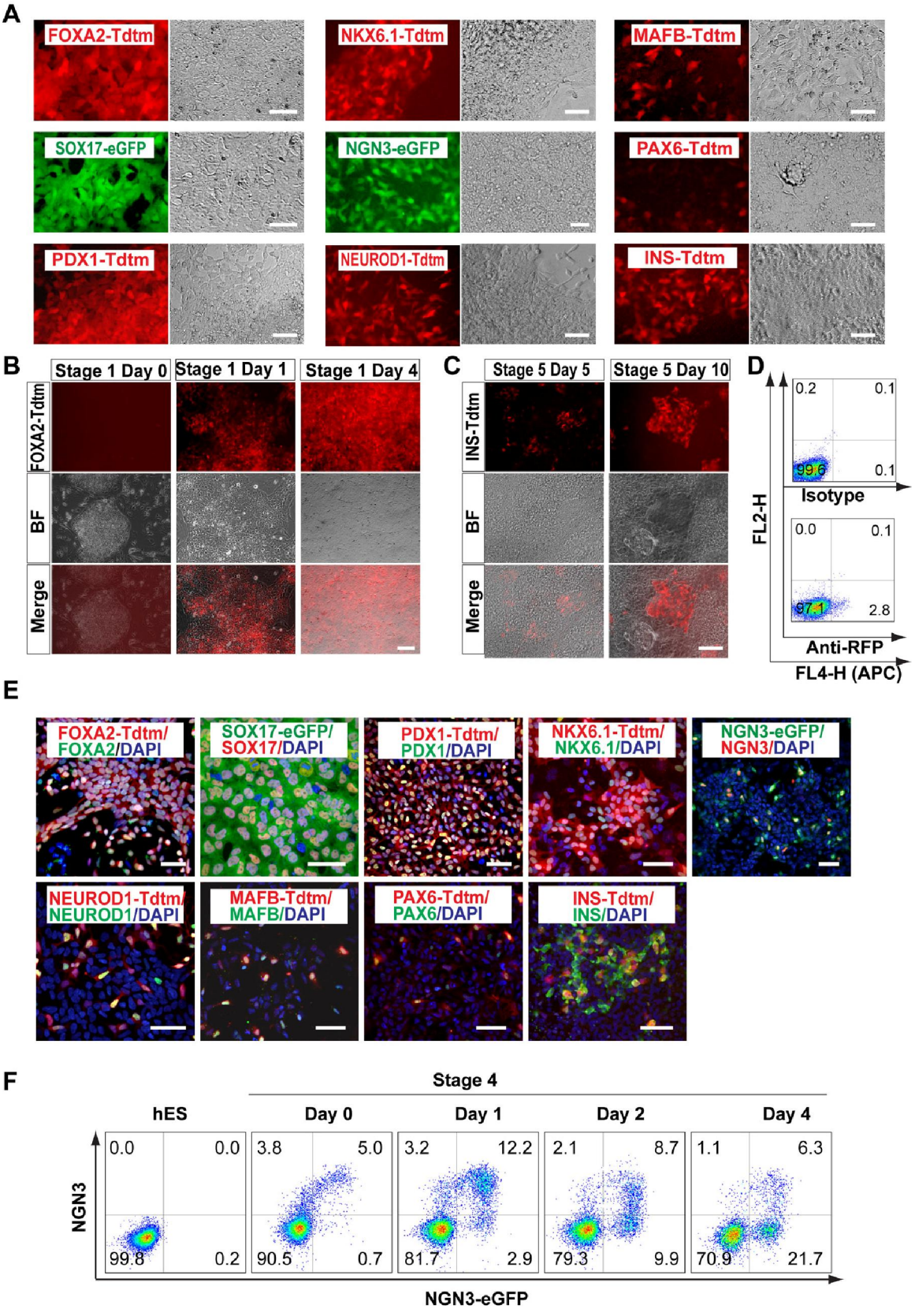

Supplementary information, Figure S2. The expression of fluorescent proteins during the

differentiation of reporter hESC lines into beta cells. (A) The expression of reporter genes from individual loci during hESC differentiation. The expression of *FOXA2-Tdtm* and *SOX17-eGFP* were evaluated at stage (S) 1, day (D) 4; *PDX1-Tdtm* was analyzed at S3, D4; *NKX6.1-Tdtm* and *NEUROD1-Tdtm* were analyzed at S4, D3; *NGN3-eGFP*, *MAFB-Tdtm* were analyzed at S4, D4; *PAX6-Tdtm* was analyzed at S4, D5; and *INS-Tdtm* was evaluated at S5, D5. (B) During the *in vitro* differentiation of the FOXA2-DR cell line, *Tdtm* expression could be detected 24 h after Activin-A treatment. (C) INS-Tdtm$^+$ cells showed a separated pattern at stage 5, day 4 but formed clusters after extended culture. (D) The expression of PAX6-Tdtm$^+$ cell fraction could not be clearly recognized by direct flow cytometric analysis. The amplification of the Tdtm signal by an anti-RFP antibody helped to recognized the PAX6-Tdtm+cells. The anti-RFP antibody was further stained with an APC-conjugated secondary antibody. (E) Co-staining reporter gene expression with endogenous gene expression. These are merged images for Fig 2B. Nuclear staining with DAPI (blue) is shown in the merged images. (F) Time-course flow cytometric analysis of differentiating *NGN3-eGFP* cells throughout stage 4 demonstrated that the agreement between eGFP$^+$ cells and NGN3$^+$ cells varied over time. On day 1, most of the eGFP$^+$ cells expressed NGN3 and *vice versa*. The percentage of NGN3$^+$ cells among the eGFP$^+$ cells gradually decreased, but the proportion of eGFP$^+$ cells among the NGN3$^+$ cells remained relatively stable. The scale bar represents 50 μm. Abbreviations: INS (INSULIN); Tdtm (TdTomato).