

Figure S1: Generation of INS^{EGFP} and INS^{EGFP} GCG^{mScarlet} reporter human embryonic stem cell (hESC) lines using CRISPR/Cas9.

(A) Genotyping PCR for 5' and 3' homology arm of the 2A-EGFP and 2A-mScarlet insertion for wildtype H1 parental, INS^{EGFP} clone #26, and three of the INS^{EGFP} GCG^{mScarlet} clones E7, C9 and D5. (B,C) Differentiated spheroids derived from the (B) INS^{EGFP} and (C) INS^{EGFP} GCG^{mScarlet} reporter cell lines expressing EGFP (green) and mScarlet (red) at different stages of pancreatic endocrine cell differentiation. Scale bars = 200 μ m.

(D) Brightfield images of differentiated spheroids derived from the INS^{EGFP} and INS^{EGFP} GCG^{mScarlet} reporter cell lines at different stages of pancreatic endocrine cell differentiation. Scale bars = 200 μ m.

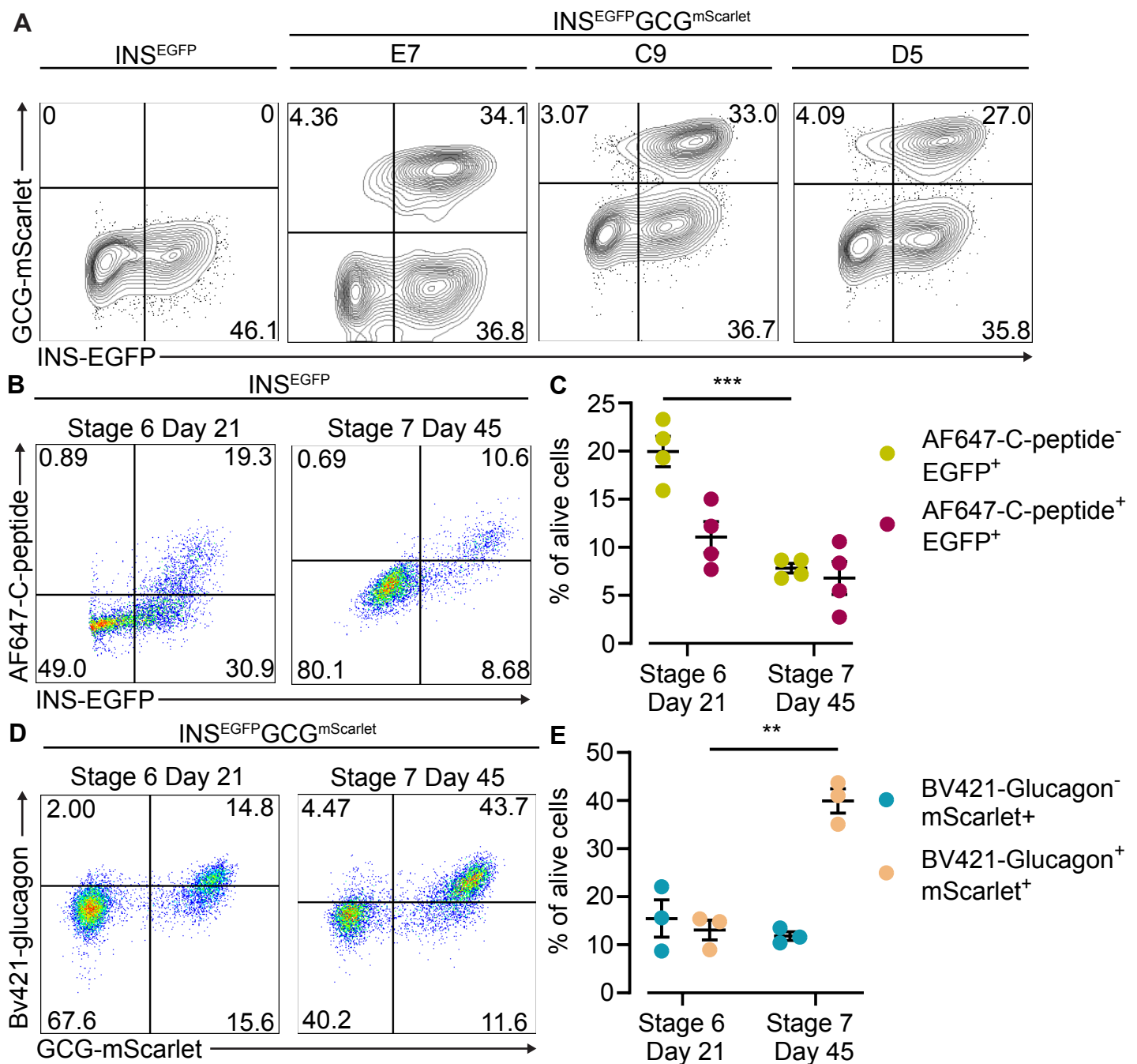


Figure S2: Majority of reporter-expressing cells co-express corresponding hormone at the mature endocrine cell stage.

(A) Representative flow cytometry plots showing the differentiation efficiencies (% of alive cells) of the INS^{EGFP} and INS^{EGFP}GCG^{mScarlet} Clones E7, C9, and D5 at the maturing endocrine cell stage (Stage 6 Day 21) (n=3).

(B,C) Representative flow cytometry plots and quantitative analysis illustrating the INS-EGFP reporter efficiency of maturing and mature endocrine cells derived from the INS^{EGFP} Clone #26 cell line. *** = $p \leq 0.0002$, n=4, two-way ANOVA, error bars \pm SEM.

(D,E) Representative flow cytometry plots and quantitative analysis illustrating the GCG-mScarlet reporter efficiency of maturing and mature endocrine cells derived from the INS^{EGFP}GCG^{mScarlet} Clone E7 reporter cell line. ** = $p \leq 0.0021$, n=3, two-way ANOVA, error bars \pm SEM.

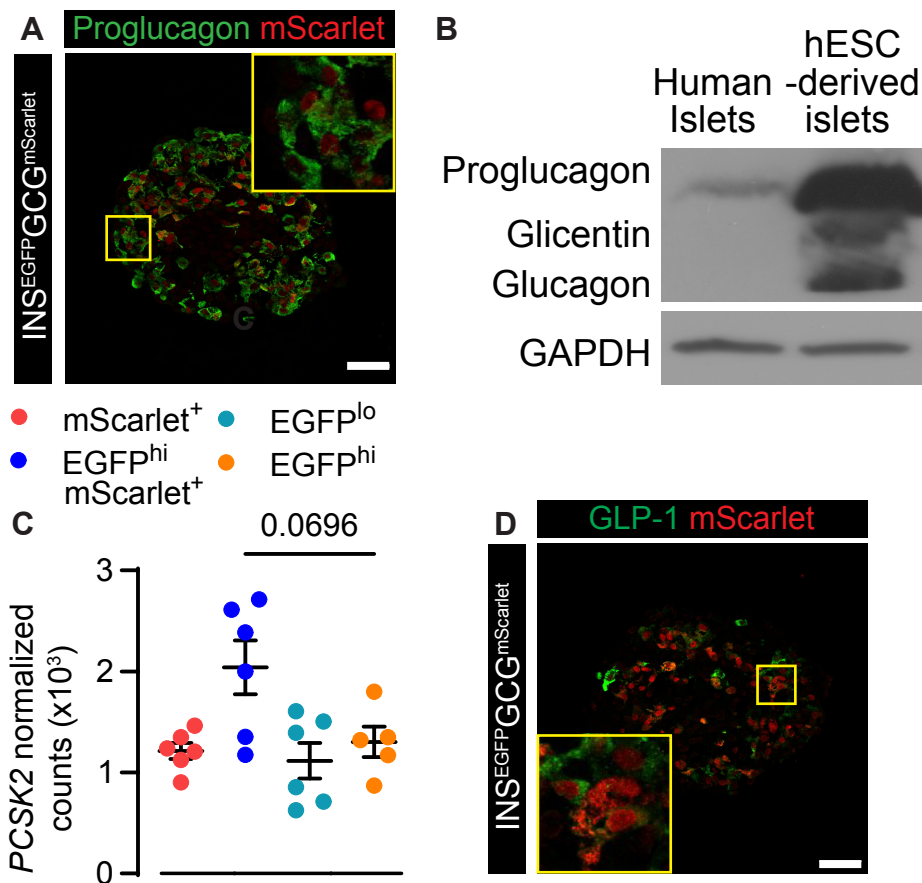


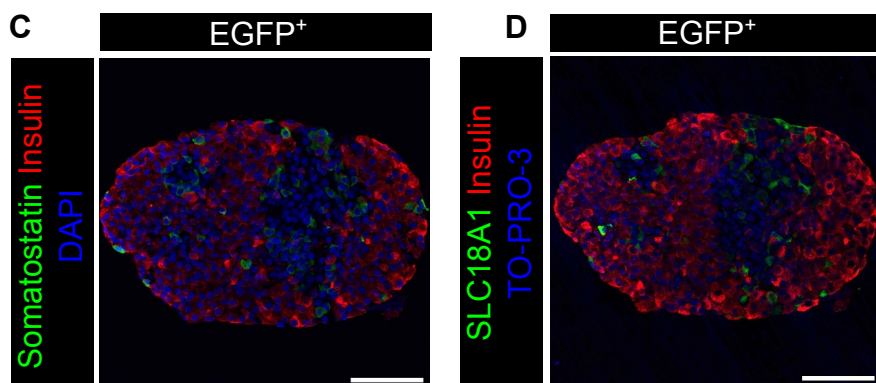
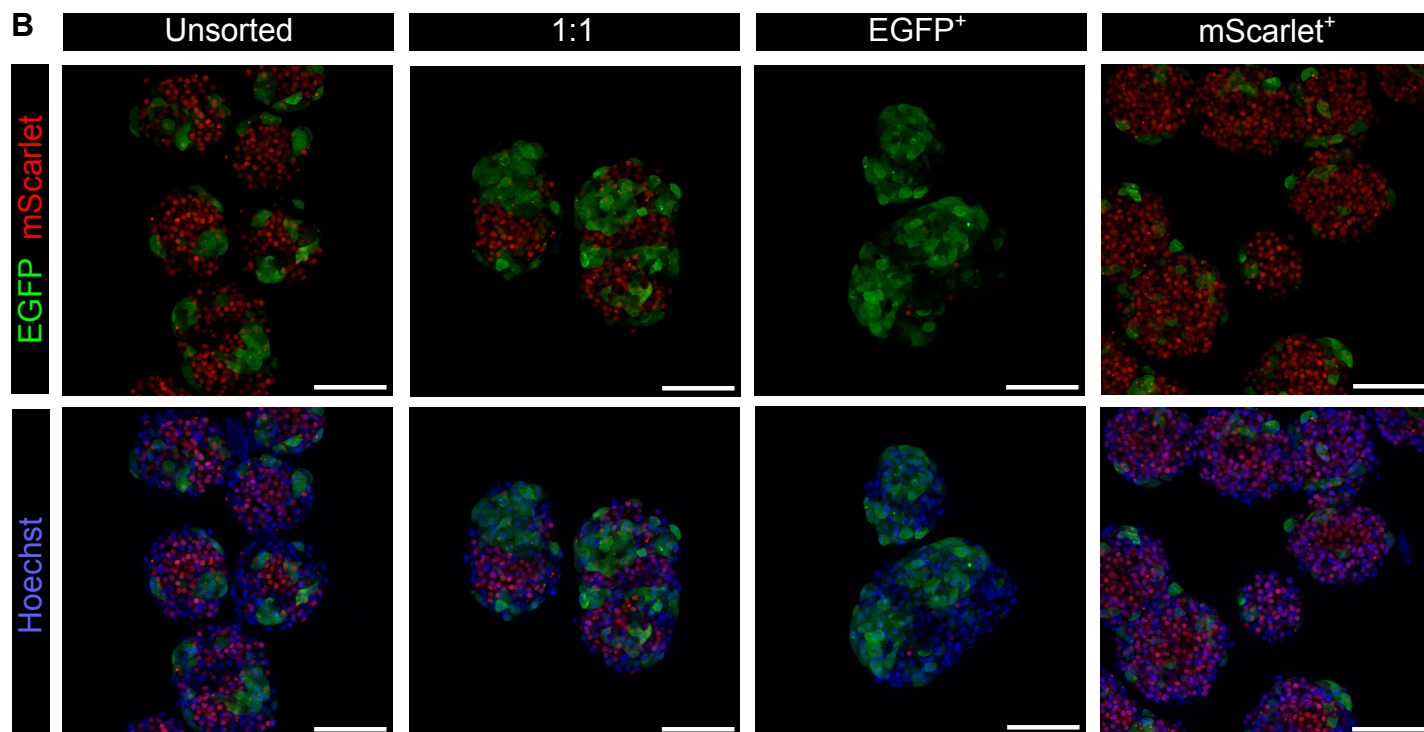
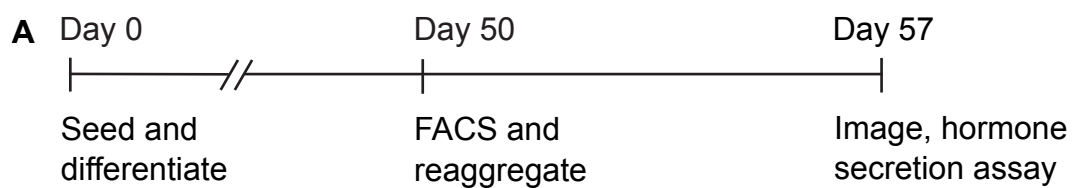
Figure S3: GCG^{mScarlet}-expressing cells co-express glucagon and its precursor proglucagon.

(A) Immunostaining of proglucagon (green) and mScarlet (red) of maturing pancreatic endocrine cells (Stage 6 Day 28) derived from INS^{EGFP}GCG^{mScarlet} reporter cell line. Images were obtained on a Leica SP8 confocal microscope. Scale bars = 50 μ m.

(B) Western blot of proglucagon, glicentin, and glucagon using human islets and H1-derived mature pancreatic endocrine cell (Stage 7 Day 36) lysates.

(C) Transcript expression of *PCSK2* in FACS-sorted maturing pancreatic endocrine cells (Stage 6 Day 25). n = 6, one-way ANOVA, error bars report \pm SEM.

(D) Immunostaining of GLP-1 (green) and mScarlet (red) of maturing pancreatic endocrine cells (Stage 6 Day 28) derived from INS^{EGFP}GCG^{mScarlet} reporter cell line. Images were obtained on a Leica SP8 confocal microscope. Scale bars = 50 μ m.



○ 2.8 mM Glucose ● 16 mM Glucose ● 16 mM Glucose + 30 mM KCl

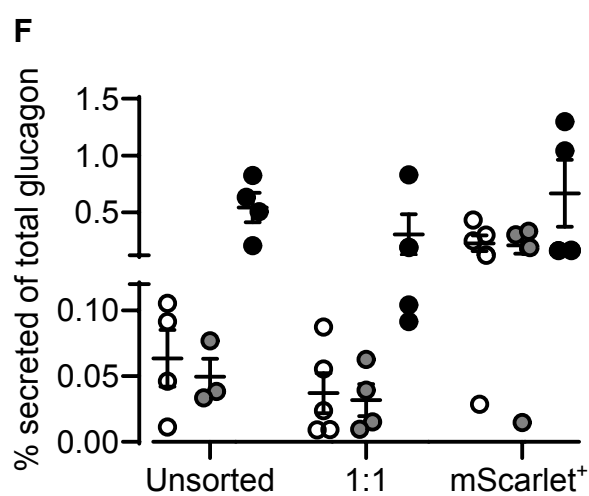
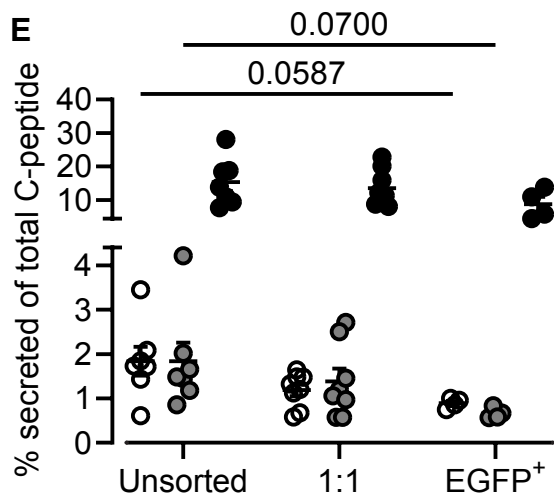


Figure S4: Reaggregates with a purer endocrine population does not exhibit improved glucose-mediated hormone secretion using current reaggregation protocol.

(A) Schematic of experimental methodology.

(B) Reaggregated unsorted, 1:1, GFP⁺, and mScarlet⁺ mixture of INS^{EGFP} and GCG^{mScarlet} mature pancreatic endocrine cells (Stage 7 Day 57). Images were obtained on a Leica SP8 confocal microscope. Scale bars = 100µm.

(C) Immunostaining of somatostatin (green), insulin (red), and DAPI (blue) of sorted and reaggregated GFP⁺ mature pancreatic endocrine cells (Stage 7 Day 57) derived from INS^{EGFP}GCG^{mScarlet} reporter cell line. Images were obtained on a Leica SP8. Scale bars = 100 µm.

(D) Immunostaining of SLC18A1 (green), insulin (red), and TO-PRO-3 (blue) of sorted and reaggregated GFP⁺ mature pancreatic endocrine cells (Stage 7 Day 57) derived from INS^{EGFP}GCG^{mScarlet} reporter cell line. Images were obtained on a Leica SP8. Scale bars = 100 µm.

(E) Glucose-stimulated insulin secretion assay using mature pancreatic endocrine cell reaggregates (Stage 7 Day 57) derived from INS^{EGFP}GCG^{mScarlet} reporter cell line. n = 4-8, two-way ANOVA, error bars report ±SEM.

(F) Glucose-mediated glucagon secretion assay using mature pancreatic endocrine cell reaggregates (Stage 7 Day 57) derived from INS^{EGFP}GCG^{mScarlet} reporter cell line. n = 4-5, error bars report ±SEM.