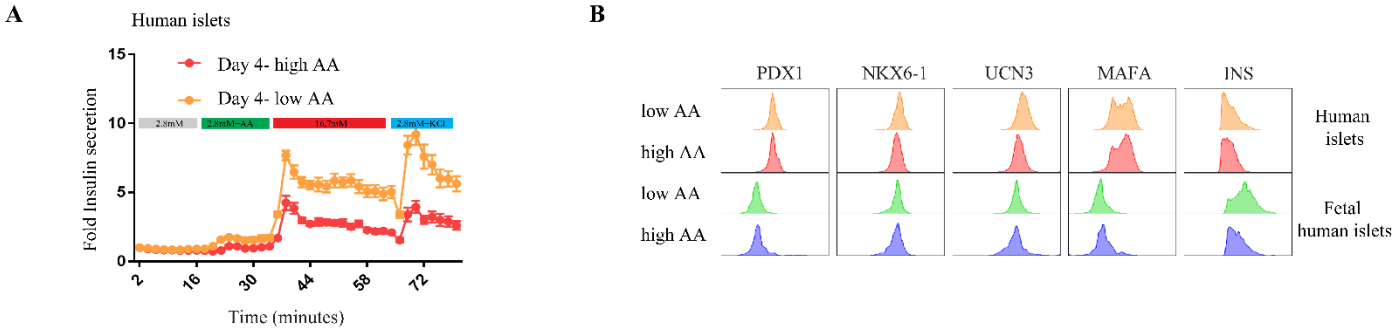
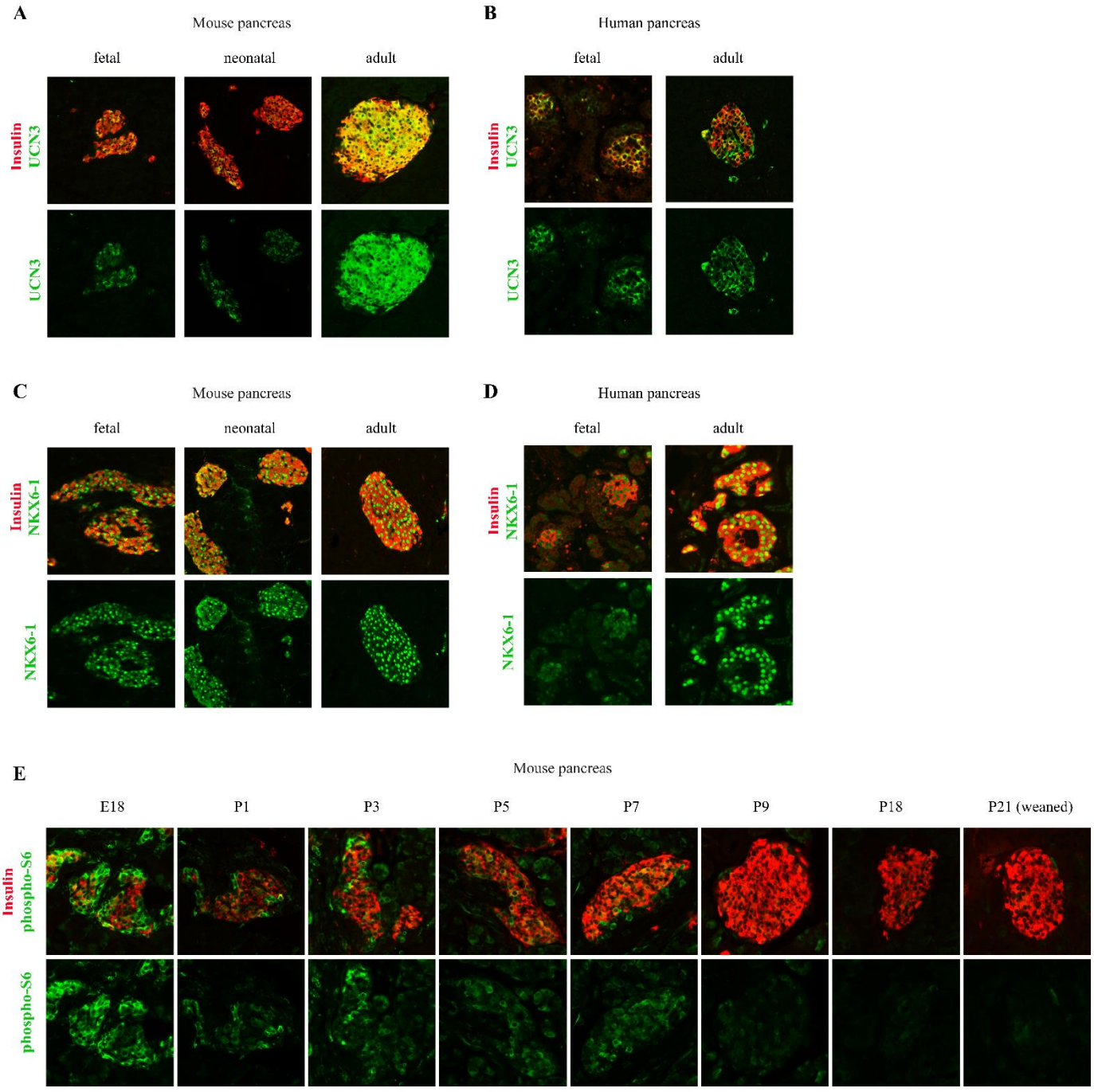
Figure S1. Related to Figure 1.



(A) Fold insulin levels secreted by mature human islets that were cultured *in vitro* in a fetal-like medium (red, 5.5mM glucose and high amino acid levels) and mature-like medium (orange, 5.5mM glucose and low amino acid levels). The result was repeated in three independent experiments. (B) Representative FACS staining histograms for PDX1, NKX6-1, UCN3, MAFA and INS of c-peptide+ cells from fetal (top) and mature (bottom) human islets that were cultured *in vitro* in a fetal-like medium and mature-like medium.

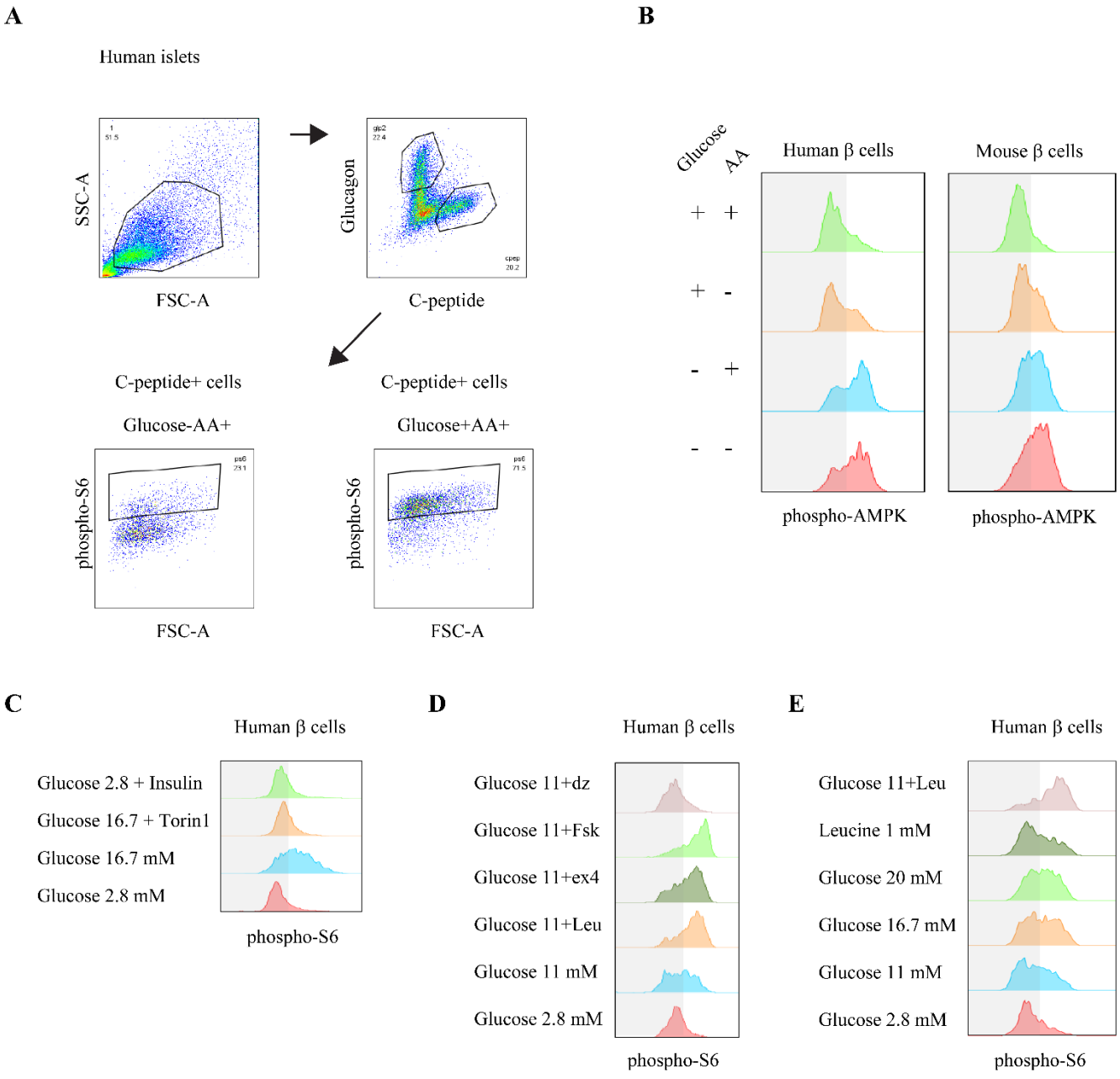
Figure S2. Related to Figure 2.



(A) Representative immunostainings of UCN3 (green) and insulin (labeling β cells, red), in pancreatic islet of E18 mouse embryo (left, n=3) P6 neonate (middle, n=3) and adult (P60, right, n=3) mice. (B) Representative immunostainings of UCN3 (green) and insulin (labeling β cells,

red), in pancreatic islet of human embryo (Day 120, left) and adult (right) subjects. (C) Representative immunostainings of NKX6-1 (green) and insulin (red), in pancreatic islet of E18 mouse embryo (left) P6 neonate (middle) and adult (P60, right) mice. (D) Representative immunostainings of NKX6-1 (green) and insulin (red), in pancreatic islet of human embryo (Day 120, left) and adult (right) subjects. Staining of human samples was repeated in 4 samples of fetal (gestational days 90-130) and 3 samples of adult pancreas. (E) Representative pancreatic sections from mice at indicated days after birth, immunostained for p-S6 (green) and insulin (red) (n=3 for each stage).

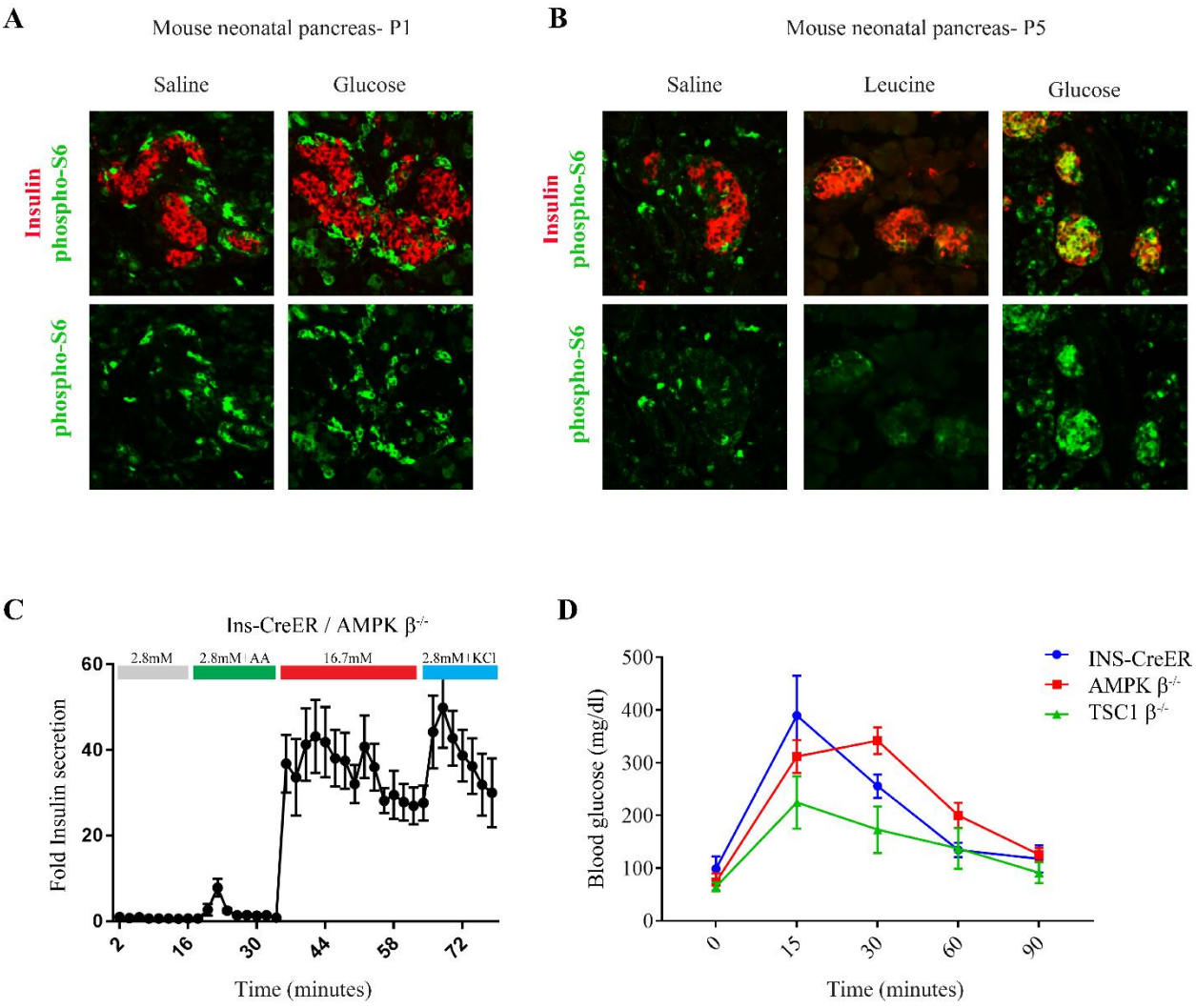
Figure S3. Related to Figure 3.



(A) FACS analysis strategy to detect mTORC1 activation in endocrine cells. Clusters are dispersed, fixed and stained for C-peptide (for human islets), Insulin (for mouse islets), Glucagon and p-S6, and the intensity of p-S6 and the percentages of p-S6+ cells in C-peptide+ and Glucagon+ cells in the different conditions is calculated. (B) Representative p-AMPK staining histograms of insulin+ cells from adult human (left) and mouse (right) islets, detected by FACS

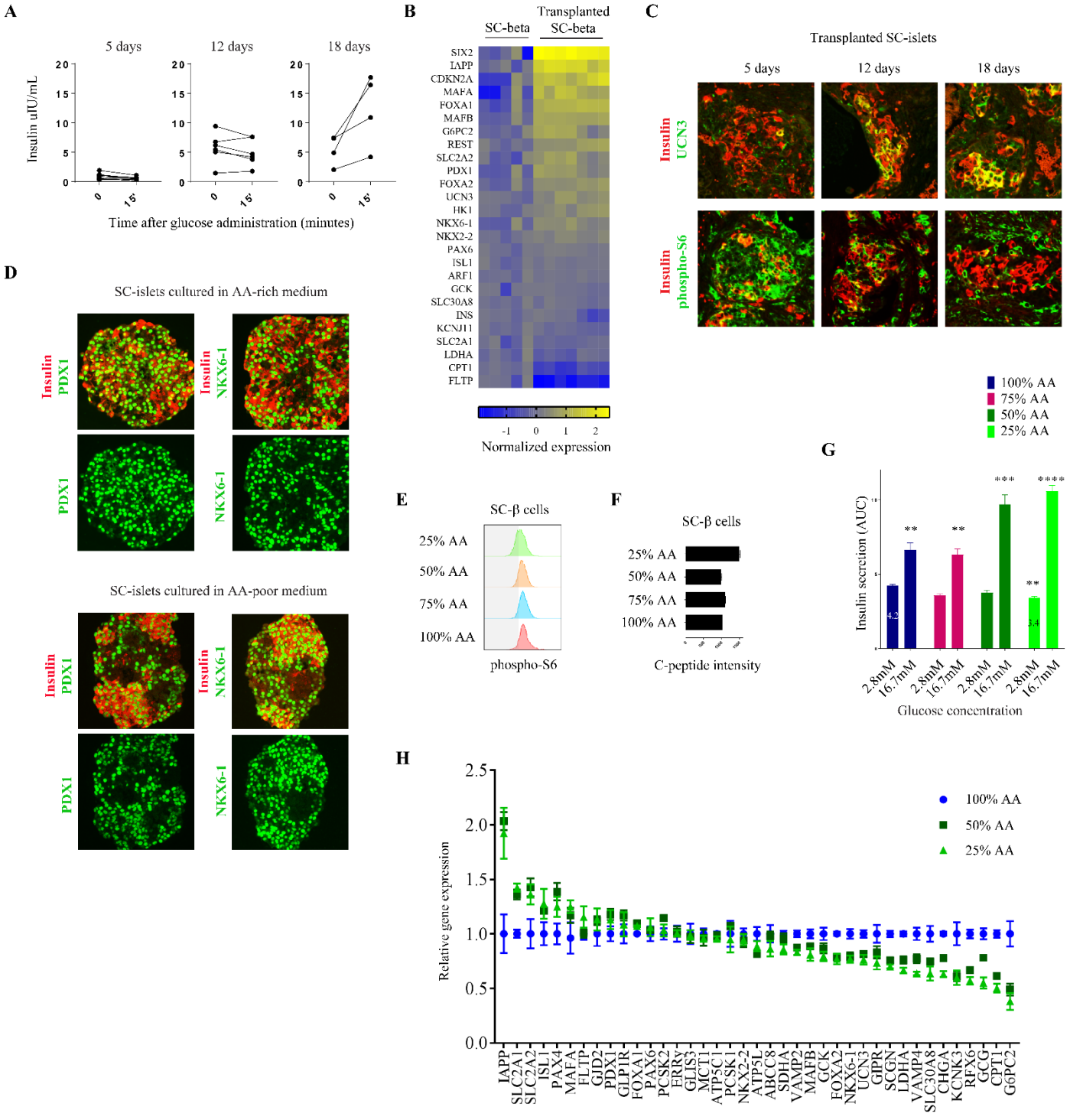
analysis compared to secondary antibodies only control. (C) Representative p-S6 staining histograms of c-peptide+ cells (human β cells) following 30 minutes incubation of human islets in RPMI with indicated nutrients. Note that phosphorylation of rpS6 in response to glucose stimulation is inhibited by Torin1 and is not mimicked by addition of insulin. (D) Representative p-S6 staining histograms of c-peptide+ cells (human β cells) following 30 minutes incubation of human islets in RPMI with glucose in the indicated concentrations and with the indicated compounds. Note that amplifiers of insulin secretion (forskolin, fsk and exendin4, ex4) strongly activate mTORC1 in β cells while blocking insulin secretion (with diazoxide, dz) inhibits mTORC1 activation. (E) Representative p-S6 staining histograms of c-peptide+ cells (human β cells) following 30 minutes incubation of human islets in RPMI with glucose in the indicated concentrations and with leucine. Note that addition of leucine amplifies the response to glucose in β cells.

Figure S4. Related to Figure 4.



(A) Representative immunostainings of p-S6 (green), and insulin (labeling β cells, red), in pancreatic islets of fasted P1 neonatal mice, injected with saline (n=3) or glucose (n=3) as indicated. (B) Representative immunostainings of p-S6 (green), and insulin (labeling β cells, red), in pancreatic islets of fasted P5 neonatal mice, injected with saline (n=3), leucine (n=3) or glucose (n=3) as indicated. Note that mTORC1 response to glucose is acquired by postnatal day 5. (C) Fold insulin levels secreted by isolated islets from AMPK knockout β cells (n=4), in a dynamic GSIS assay in low (2.8 mM, grey line), amino acids (green), high (16.7 mM, red) glucose concentrations and KCl (30 mM, blue). (D) Glucose tolerance test on Ins-CreER (blue, n=4), TSC (green, n=4) and AMPK (red, n=4) β cell-specific deficient mice.

Figure S5. Related to Figure 5.



(A) ELISA measurements of human insulin from the serum of mice transplanted with SC-β cells. Measurements were taken before (time 0) and 15 min after a glucose injection of mice at the indicated days post-transplantation. (B) Gene expression heat map of known regulators of β cell maturation and function four weeks after transplantation under the kidney capsule of mice, normalized to expression levels of the genes in SC-β cells before transplantation. SC-β cells were sorted from five independent differentiation flasks and seven transplanted mice. (C) Representative immunostainings of UCN3 (top panels, green), p-S6 (bottom panels, green) and c-peptide (labeling SC-β cells, red), in SC-β cells transplanted under the kidney capsule for indicated days. Transplanted mice were fasted overnight before transplant collection. Note the inhibition of mTORC1 signaling 12 days after transplantation. (D) Representative immunostainings of PDX1 or NKX6-1 (green) and c-peptide (labeling SC-β cells, red), in clusters of in-vitro differentiated stem cells grown in an amino acid-rich media (left panels) or acid-poor media (right panels). (E) Representative p-S6 staining histograms of c-peptide+ cells (SC-β cells) and percentages of p-S6 positive and negative SC-β cells grown in media with indicated amino acid levels compared to the amino acid-rich media (100% AA). (F) Staining intensity of c-peptide in SC-β cells grown in media with indicated amino acid levels (n=6 for each condition), compared to amino acid-rich culture media (100% AA). (G) Basal and stimulated insulin secretion levels as measured by the area under the curve (AUC) of the dynamic insulin secretion in 4I, for the indicated conditions. Data points represent mean ± SEM. P-values, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$, unpaired Student's t test. (H) A relative RNA expression ratio of indicated β cell function regulators in sorted TSQ-expressing cells from the indicated conditions (n=4 for each condition).