

## **Supplemental Methods**

The following protocols were used to generate supplemental figures.

### ***Western blotting***

Human pseudoislets (500 islet equivalents/condition) were collected after 5 days in culture and lysed in RIPA buffer + 1% HALT inhibitor by sonication. Lysates were then concentrated using the Microcon-30kDA centrifugal filter to a volume suitable for running within a single gel lane. Samples were then processed, run, and transferred according to the BioRad 4-15% Mini-PROTEAN TGX Precast Protein Gel manual. The blot was incubated in 1:20000 HRP-conjugated anti-beta-actin for one hour, or in 1:500 rabbit anti-RFX6 overnight at 4°C followed by a 1 hour incubation in HRP-conjugated donkey anti-rabbit secondary, and chemical development. The blot was then exposed for between 30sec – 1.5hrs on autoradiography film and developed using a Konica Minolta medical film processor.

### ***Immunohistochemistry***

Kidney grafts with transplanted human pseudoislets were recovered and fixed in 4% PFA overnight at 4°C. Untransplanted human pseudoislets were fixed with 4% PFA for 10mins at 4°C and embedded in collagen. Six µm-thick frozen sections were cut and immunostained for microscopy. Tissue sections were washed in PBS and permeabilized/blocked (1% bovine serum albumin, 0.2% non-fat milk, 0.5% Triton-X in PBS) for 1h. Primary antibodies were mixed with permeabilization/blocking buffer at appropriate concentrations and incubated on slides at 4°C overnight. The following primary antibodies were used: guinea pig anti-Glucagon (1:500), rat anti-Insulin (1:500), rabbit anti-Somatostatin (1:200), rabbit anti-FLAG (1:500). Slides were washed with PBS and incubated with Alexa Fluor secondary antibodies (1:500) at RT for 2h. Slides were then washed with PBS and incubated with Hoescht (1:2000) and washed again.

Following the final wash with PBS, slides were preserved with mounting medium containing DAPI. Images were obtained using a Leica SP8 confocal or ZEISS Axio Scope light microscope.