

## Expanded View Figures

### Figure EV1. Incorporation of MVF into $\text{MIN6}\beta\text{PI}$ , diameters, and insulin secretion of islets and islet organoids.

- A Fusion of MIN6 $\beta$ -cells without or with MVF into  $\text{MIN6}\beta\text{PI}$  and  $\text{MIN6}\beta\text{PI}$  + MVF on day -5, -3 and 0 by means of liquid overlay technique. Scale bar: 150  $\mu\text{m}$ .
- B Quantitative analysis of the diameter ( $\mu\text{m}$ ) of FI, CI, PI, and PI + MVF ( $n = 15$  each). Mean  $\pm$  SD. One-way ANOVA and Tukey's multiple comparisons *post hoc* test were used for statistical analysis. \* $P < 0.05$  vs. FI or hFI; # $P < 0.05$  vs. CI or hCI; + $P < 0.05$  vs. PI or hPI.
- C Neutral red and trypan blue staining of  $\text{MIN6}\beta\text{PI}$ ,  $\text{MIN6}\beta\text{PI}$  + MVF, and  $\text{MIN6}\beta\text{PI}$  + MVF incubated for 24 h in 0.2%  $\text{H}_2\text{O}_2$ . Scale bar: 100  $\mu\text{m}$ .
- D Length distribution of MVF (%) ( $n = 3$ ). Mean  $\pm$  SD.
- E Absolute insulin secretion ( $\mu\text{U/ml}$ ) of FI, CI, PI, and PI + MVF exposed to 1.1 and 16.5 mM glucose ( $n = 3$  each). Mean  $\pm$  SD. Unpaired Student's *t*-test was used for statistical analysis. \* $P < 0.05$  vs. insulin secretion at 1.1 mM glucose within each individual group.
- F Insulin SI of FI, CI, PI, and PI + MVF in response to 1.1 and 16.7 mM glucose ( $n = 3$  each). Mean  $\pm$  SD. One-way ANOVA and Tukey's multiple comparisons *post hoc* test were used for statistical analysis. \* $P < 0.05$  vs. FI; # $P < 0.05$  vs. CI.
- G Representative immunofluorescent stainings of insulin and CD31 in  $\text{MIN6}\beta\text{PI}$  and  $\text{MIN6}\beta\text{PI}$  + MVF. Cell nuclei are stained with Hoechst 33342 (blue). Scale bar: 100  $\mu\text{m}$ .
- H Quantitative analysis of insulin- ( $\beta$ -cells) and CD31-positive (endothelial) cells in  $\text{MIN6}\beta\text{PI}$  + MVF. Data are expressed in % of all cells ( $n = 20$ ). Mean  $\pm$  SD.
- I Western blot analysis of CD31 and  $\beta$ -actin in cell extracts of  $\text{MIN6}\beta\text{PI}$ ,  $\text{MIN6}\beta\text{PI}$  + MVF, and MVF.

Data information: Exact *P*-values are specified in Appendix Table S1.

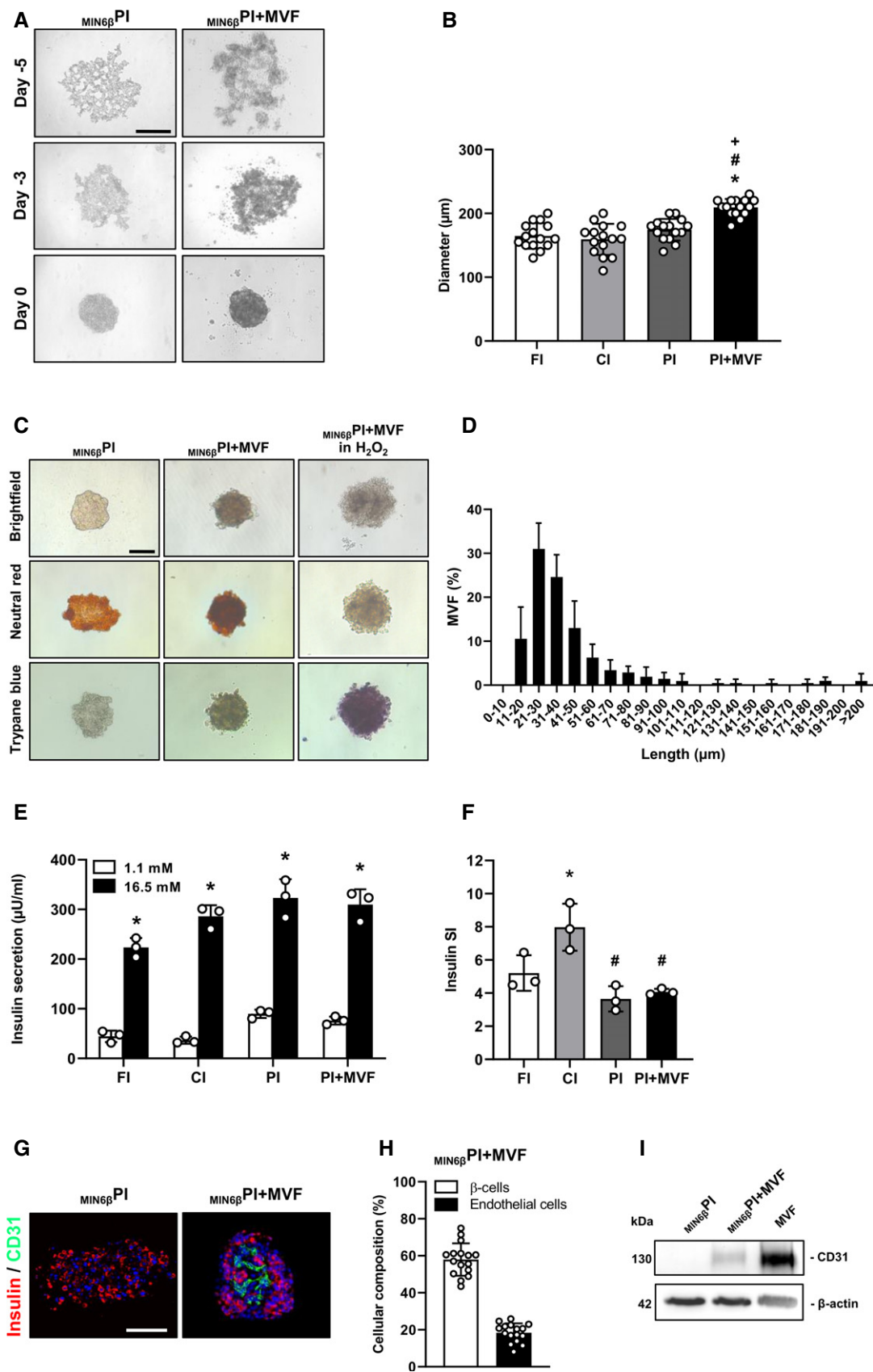
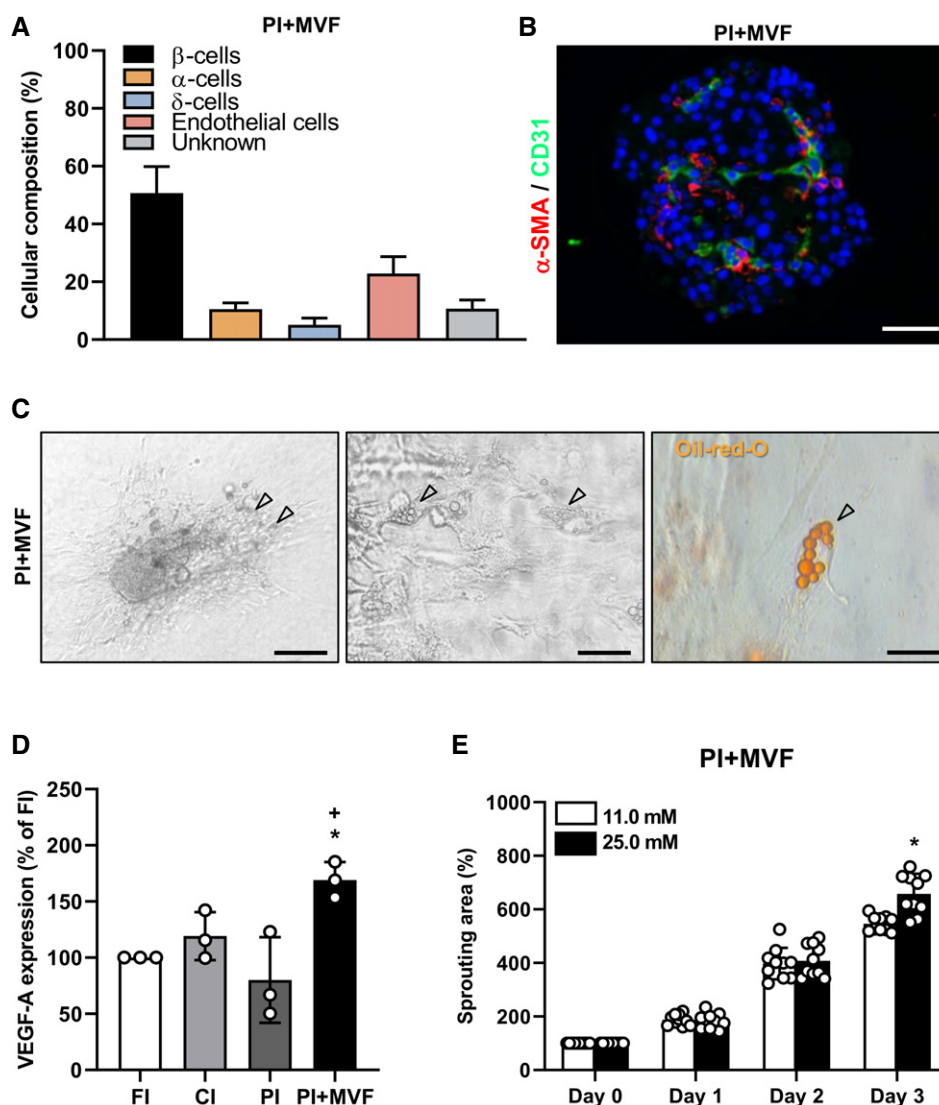


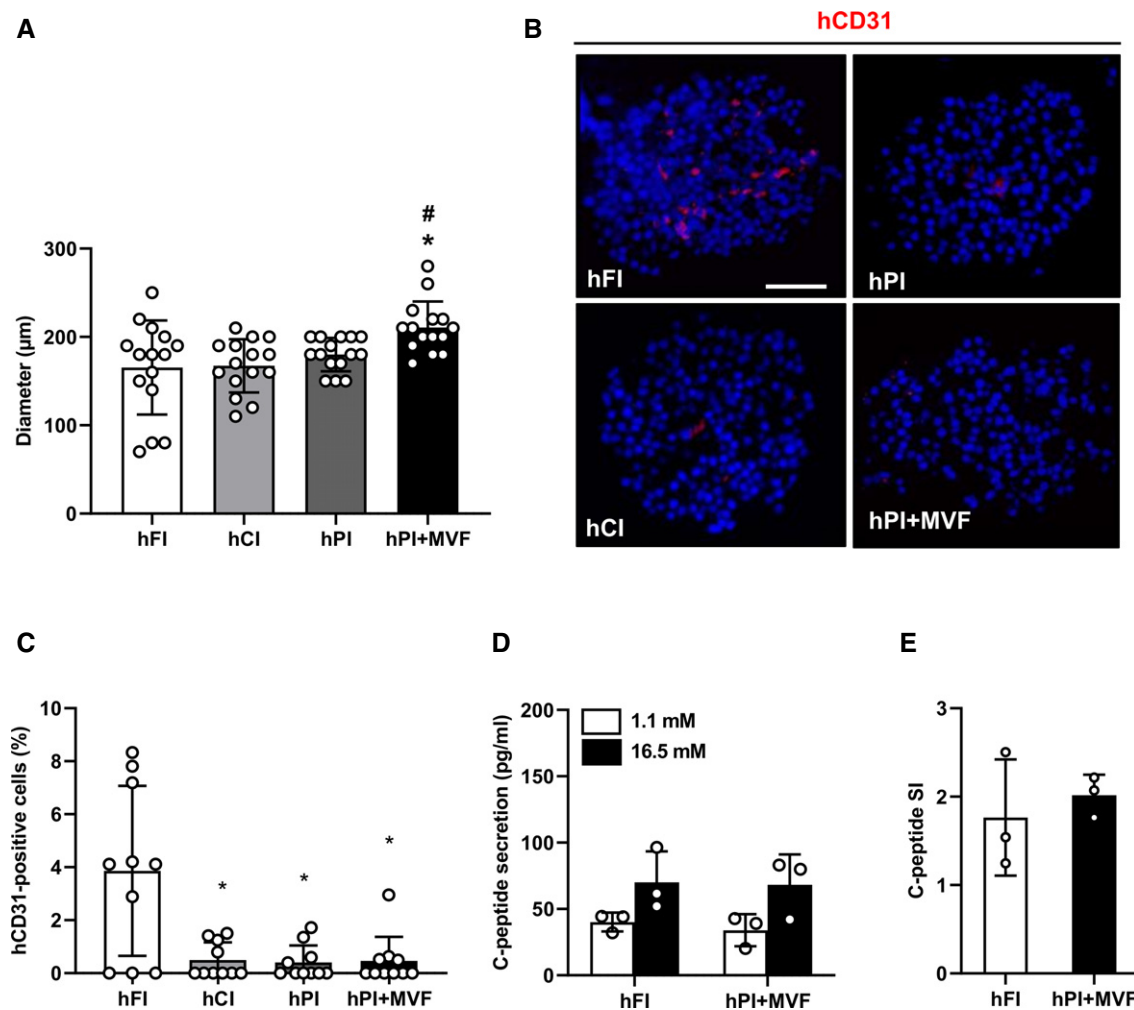
Figure EV1.



**Figure EV2. Cellular composition of PI + MVF, VEGF-A expression, and glucose-dependent sprouting.**

- A** Quantitative analysis of insulin- (β-cells), glucagon- (α-cells), somatostatin- (δ-cells), and CD31-positive (endothelial) cells in PI + MVF. Based on these findings,  $11 \pm 3\%$  of islet cells (gray bar) are not reliable. Data are expressed in % of total islet cells ( $n = 20$ ). Mean  $\pm$  SD.
- B** Representative immunofluorescent staining of α-SMA and CD31 in PI + MVF. Cell nuclei are stained with Hoechst 33342 (blue). Scale bar: 50 μm.
- C** Bright field images of PI + MVF seeded on culture dish and cultivated for 5 days under 2 μM insulin exposure. Preadipocytes in PI + MVF are marked by arrows. Scale bars: 150 μm (left image) and 30 μm (middle image). Right image shows Oil Red O staining of lipid droplets in preadipocytes of PI + MVF (marked by arrow). Scale bar: 10 μm.
- D** Quantitative analysis of VEGF-A mRNA-expression in FI, CI, PI, and PI + MVF. Data are expressed as % of FI ( $n = 3$  each). Mean  $\pm$  SD. One-way ANOVA and Tukey's multiple comparisons *post hoc* test were used for statistical analysis. \* $P < 0.05$  vs. FI; † $P < 0.05$  vs. PI.
- E** Quantitative analysis of sprouting areas of PI + MVF exposed to 11.0 mM glucose and 25.0 mM glucose. Data are expressed in % of initial size (day 0;  $n = 10$  each). Mean  $\pm$  SD. Unpaired Student's *t*-test was used for statistical analysis. \* $P < 0.05$  vs. 11.0 mM glucose.

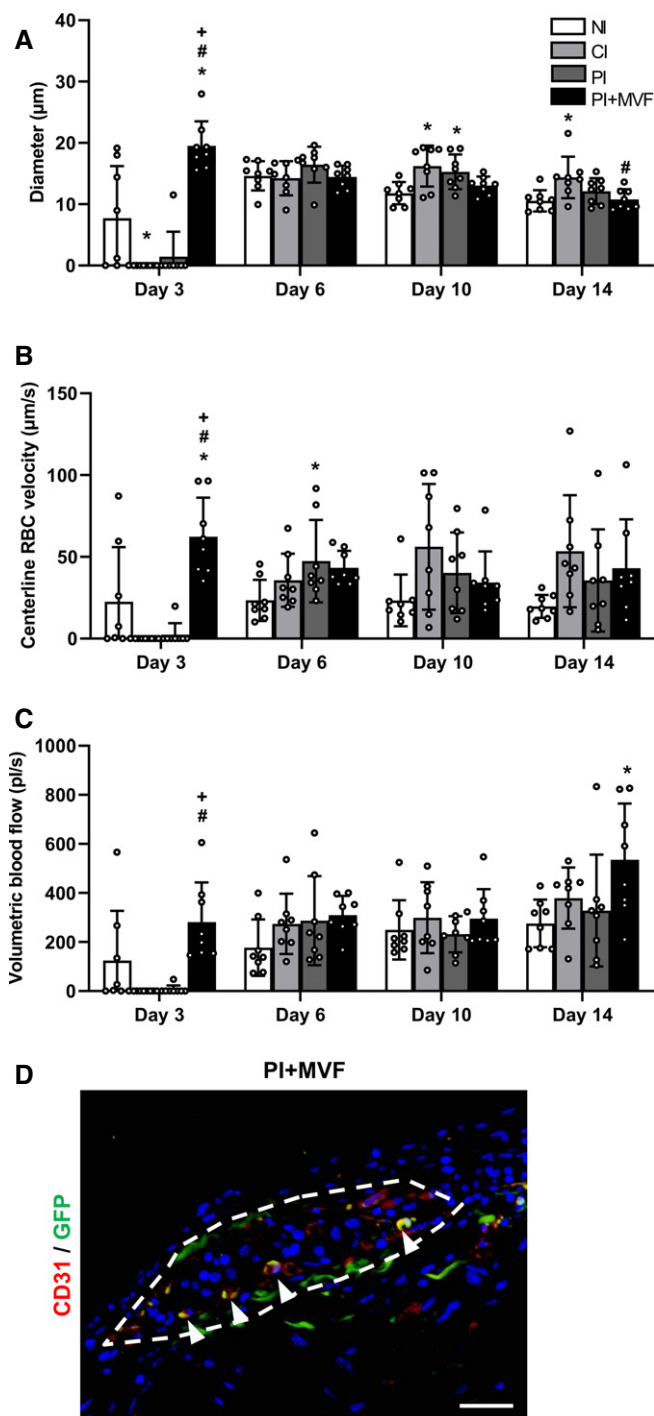
Data information: Exact *P*-values are specified in Appendix Table S1.



**Figure EV3. Diameters, insulin secretion, and CD31 expression of human islets and islet organoids.**

- A** Quantitative analysis of the diameter ( $\mu\text{m}$ ) of hFI, hCI, hPI, and hPI + MVF ( $n = 15$  each). Mean  $\pm$  SD. One-way ANOVA and Tukey's multiple comparisons *post hoc* test were used for statistical analysis. \* $P < 0.05$  vs. FI or hFI; # $P < 0.05$  vs. CI or hCI.
- B** Representative immunofluorescence stainings of human CD31 (hCD31) in hFI, hCI, hPI, and hPI + MVF. Cell nuclei are stained with Hoechst 33342 (blue). Scale bar: 50  $\mu\text{m}$ .
- C** Quantitative analysis of hCD31-positive cells in hFI, hCI, hPI, and hPI + MVF in % of all islet or organoid cells ( $n = 10$  each). Mean  $\pm$  SD. One-way ANOVA and Tukey's multiple comparisons *post hoc* test were used for statistical analysis. \* $P < 0.05$  vs. hFI.
- D** Absolute C-peptide secretion (pg/ml) of hFI and hPI + MVF exposed to 1.1 and 16.5 mM glucose ( $n = 3$  each). Mean  $\pm$  SD.
- E** C-peptide SI of hFI and hPI + MVF in response to 1.1 and 16.7 mM glucose ( $n = 3$  each). Mean  $\pm$  SD.

Data information: Exact  $P$ -values are specified in Appendix Table S1.



**Figure EV4. Microhemodynamic parameters of transplanted FI, CI, PI, and PI + MVF as well as detection of MVF-derived endothelial cells within PI + MVF.**

A–C Quantitative analysis of diameter ( $\mu\text{m}$ ) (A), centerline RBC velocity ( $\mu\text{m/s}$ ) (B) and volumetric blood flow (pl/s) (C) of newly formed microvessels in transplanted FI, CI, PI, and PI + MVF on day 3, 6, 10, and 14 ( $n = 8$  each). Mean  $\pm$  SD. One-way ANOVA and Tukey's multiple comparisons *post hoc* test were used for statistical analysis. \* $P < 0.05$  vs. FI; # $P < 0.05$  vs. CI; + $P < 0.05$  vs. PI.

D Representative immunofluorescent stainings of CD31/GFP in PI + MVF consisting of GFP-positive MVF and GFP-negative islet cells on day 14 after transplantation. Cell nuclei are stained with Hoechst 33342 (blue). The border of the PI + MVF is marked by broken line. Arrowheads mark double-positive cells. Scale bar: 40  $\mu\text{m}$ .

Data information: Exact  $P$ -values are specified in Appendix Table S1.

**Figure EV5. Identification of the critical islet number necessary for the restoration of normoglycemia in STZ-induced diabetic mice as well as proliferation and apoptosis in FI and PI + MVF after transplantation in diabetic mice.**

- A Immunofluorescence stainings of insulin in a mouse pancreas treated without and with STZ. Cell nuclei are stained with Hoechst 33342 (blue). Scale bar: 70  $\mu$ m.
- B Quantitative analysis of body weight (g) from day -8 to day 28 of diabetic mice without transplantation (sham group;  $n = 5$  each). Mean  $\pm$  SD.
- C Quantitative analysis of blood glucose level (mg/dl) from day -8 to day 28 of animal of the sham group ( $n = 5$  each). Mean  $\pm$  SD.
- D Quantitative analysis of blood glucose level (mg/dl) according to the IPGTT of sham-transplanted diabetic mice on day 0 (sham D0) and 28 (sham D28;  $n = 5$  each). Mean  $\pm$  SD. Unpaired Student's  $t$ -test was used for statistical analysis.  $*P < 0.05$  vs. sham D28.
- E AUC of IPGTT of sham-transplanted diabetic mice on day 0 (sham D0) and 28 (sham D28;  $n = 5$  each). Mean  $\pm$  SD. Unpaired Student's  $t$ -test was used for statistical analysis.  $*P < 0.05$  vs. sham D28.
- F Plasma insulin level ( $\mu$ U/ml) of sham-transplanted diabetic mice on day 0 (sham D0) and 28 (sham D28;  $n = 4$  each). Mean  $\pm$  SD.
- G Quantitative analysis of body weight (g) from day -8 to day 28 of diabetic mice transplanted with 200, 250, 300 or 400 FI on day 0 ( $n = 3$  each). Mean  $\pm$  SD.
- H Quantitative analysis of blood glucose level (mg/dl) from day -8 to day 28 of diabetic mice transplanted with 200, 250, 300, or 400 FI on day 0 ( $n = 3$  each). Mean  $\pm$  SD.
- I Representative stainings of Ki67 and casp-3 in FI and PI + MVF 3 days after transplantation in diabetic mice. Scale bar: 50  $\mu$ m.
- J, K Quantitative analysis of Ki67- (J) and casp-3-positive cells (K) in FI and PI + MVF on day 3 after transplantation in % of all islet or islet organoid cells ( $n = 13$  each). Mean  $\pm$  SD. Unpaired Student's  $t$ -test was used for statistical analysis.  $*P < 0.05$  vs. FI.

Data information: Exact  $P$ -values are specified in Appendix Table S1.

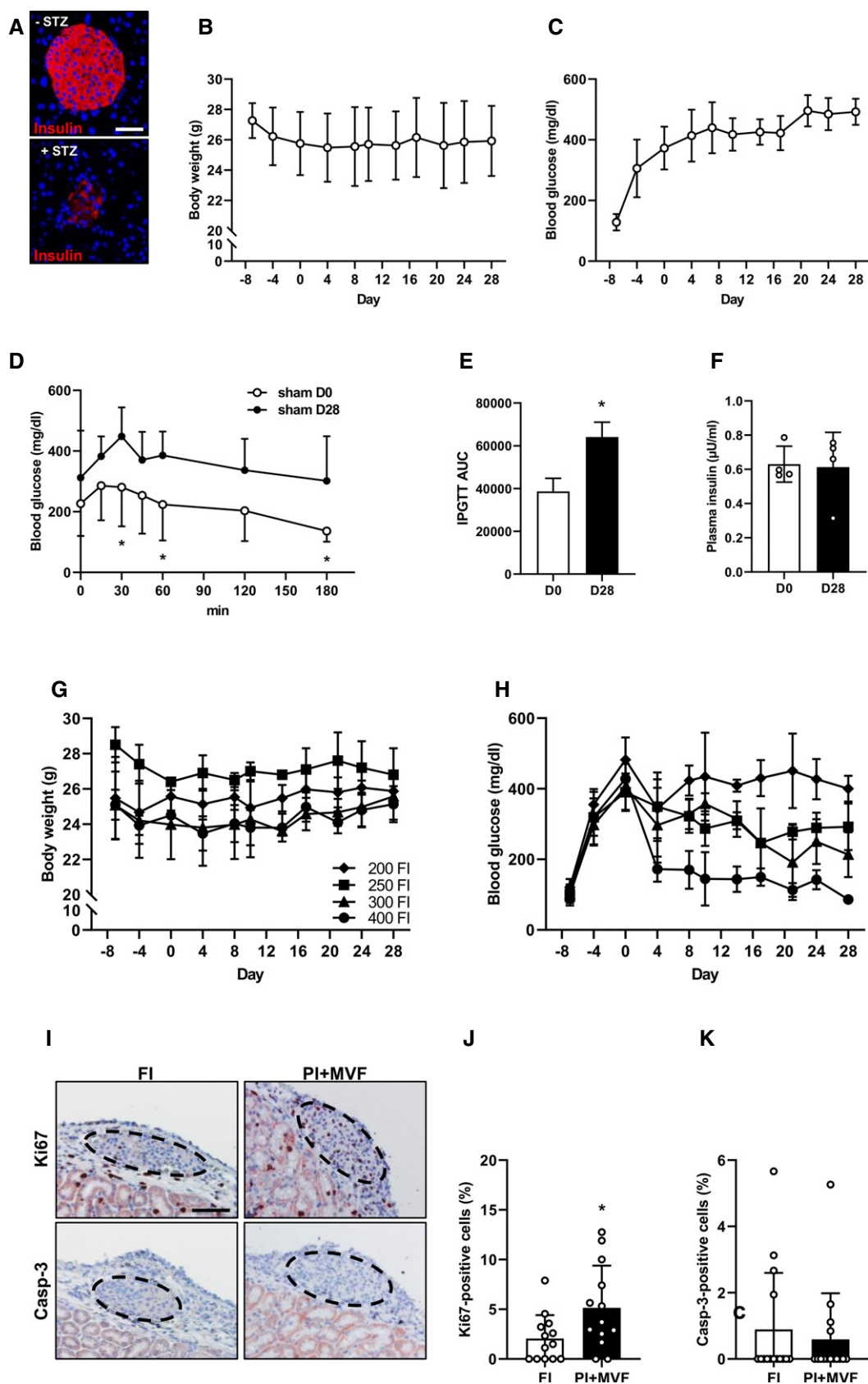


Figure EV5.