SUPPLEMENTARY DATA

Supplementary Table 1. Primers for quantitative real-time RT-PCR

Gene Symbol	TaqMan Assay ID
Gck	Mm00439129_m1
Glut2	Mm00446224_m1
Hif1a	Mm00468869_m1
Ins2	Mm00731595_gh
Kdr	Mm00440099_m1
Mafa	Mm00845209_s1
Pdx1	Mm00435565_m1
Rn18s	Hs99999901s1
Vegfa (custom-made *loxP* modification)	Mm00437306_m1
Vegfb	Mm00442102_ml

Supplementary Table 2. Sources and concentrations of primary antibodies

Antigen	Host Species	Cryosections	Whole Mount	Source
Amylase	rabbit	1:1000	-	Sigma
BrdU	rat	1:100	-	Abcam
Cpa1	goat	1:250	-	R&D Systems
E-cadherin	mouse	1:500	-	BD Biosciences
Glucagon	rabbit	1:100	-	Cell Signaling
Glucagon	guinea pig	-	1:10000	Linco
Hif-1α	rabbit	1:1000	-	Novus Biologicals
Insulin	guinea pig	1:200	-	Linco
Insulin	guinea pig	1:500	1:100	Dako
Ki67	rabbit	1:500	-	Abcam
MafA	rabbit	1:10000	-	R. Stein
Ngn3	goat	1:5000	-	G. Gu
PDX1	goat	1:10000	1:5000	C. V. E. Wright
PECAM1	rat	1:50	1:100	BD Pharmingen
pH3	rabbit	1:500	-	Upstate
Somatostatin	sheep	1:500	-	American Research Products
Sox9	rabbit	1:500	-	Millipore
VEGF-A	goat	1:100	-	R&D Systems

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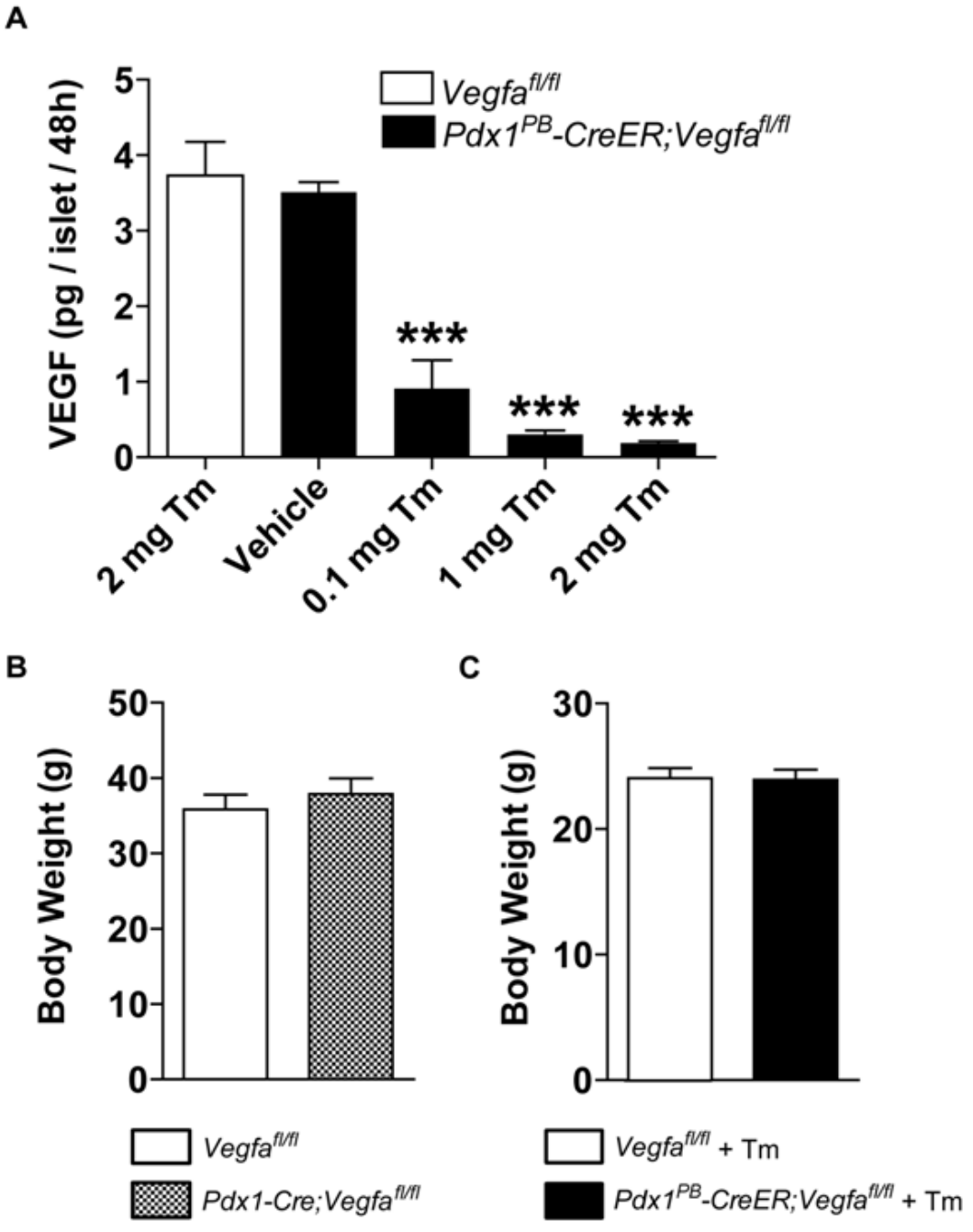
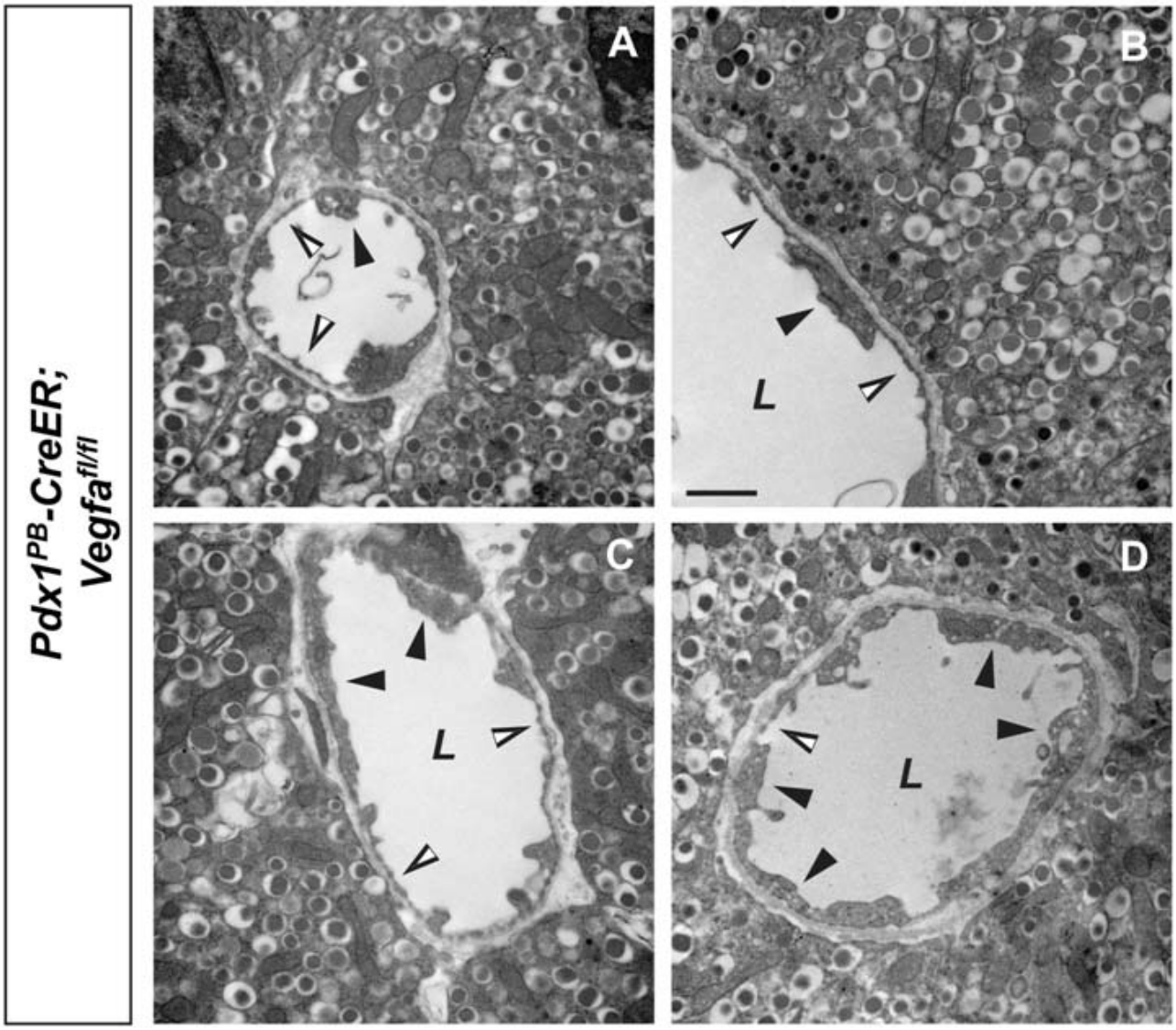
Supplementary Figure 1. Inactivation of VEGF-A in adult β-cells. (A) VEGF-A secretion by cultured islets was analyzed by ELISA one week after treating 4-month-old mice with Tm or Veh. Values are 3.51 ± 0.13 pg/islet/48hr in vehicle-treated *Pdx1^{PB}-CreER;Vegfa$^{fl/fl}$* islets, 3.73 ± 0.44 pg/islet/48hr in 3 x 2 mg Tm-treated *Vegfa$^{fl/fl}$* islets, 0.19 ± 0.03 pg/islet/48hr in 3 x 2 mg Tm-treated *Pdx1^{PB}-CreER;Vegfa$^{fl/fl}$* islets, 0.30 ± 0.07 pg/islet/48hr in 3 x 1 mg Tm-treated *Pdx1^{PB}-CreER;Vegfa$^{fl/fl}$* islets, and 0.91 ± 0.38 pg/islet/48hr in 3 x 0.1 mg Tm-treated *Pdx1^{PB}-CreER;Vegfa$^{fl/fl}$* islets. ***P < 0.001 vs. Tm-treated *Vegfa$^{fl/fl}$* islets and vs. vehicle-treated *Pdx1^{PB}-CreER;Vegfa$^{fl/fl}$* islets; n = 3 per group. (B) Body weight in 6-month-old *Vegfa$^{fl/fl}$* and *Pdx1-Cre;Vegfa$^{fl/fl}$* mice; 35.9 ± 2.0 g in *Vegfa$^{fl/fl}$* mice and 37.87 ± 2.079 g in *Pdx1-Cre;Vegfa$^{fl/fl}$* mice (n = 15-16/genotype; P > 0.05). (C) Body weight in 7-month-old Tm-treated *Vegfa$^{fl/fl}$* and *Pdx1^{PB}-CreER;Vegfa$^{fl/fl}$* mice; 24.0 ± 0.8 g in *Vegfa$^{fl/fl}$* mice and 23.9 ± 0.8 g in *Pdx1^{PB}-CreER;Vegfa$^{fl/fl}$* mice (n = 14-18/genotype; P > 0.05).



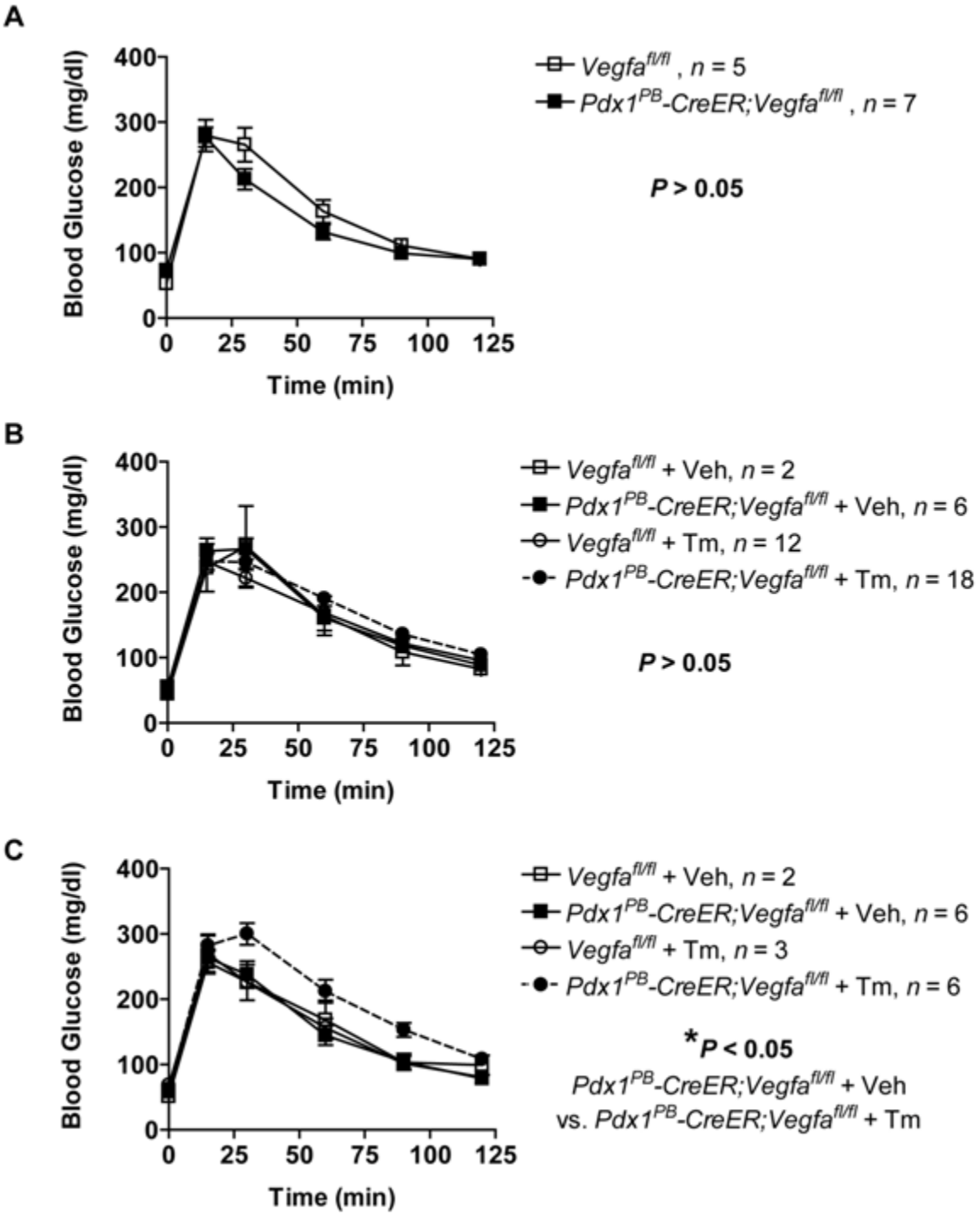
SUPPLEMENTARY DATA

Supplementary Figure 2. Islet VEGF-A inactivation reduces endothelial cell fenestrations. Transmission electron micrographs of intraislet capillaries in Tm-treated *Pdx1*^{PB}*-CreER;Vegfa*$^{fl/fl}$ mice. Images were acquired at 15000x. Scale bar in B is 100 nm, and applies to panels A, C, and D. *L*, capillary lumen; *N*, endothelial cell nucleus; open arrowheads denote fenestrations and closed arrowheads denote caveolae.



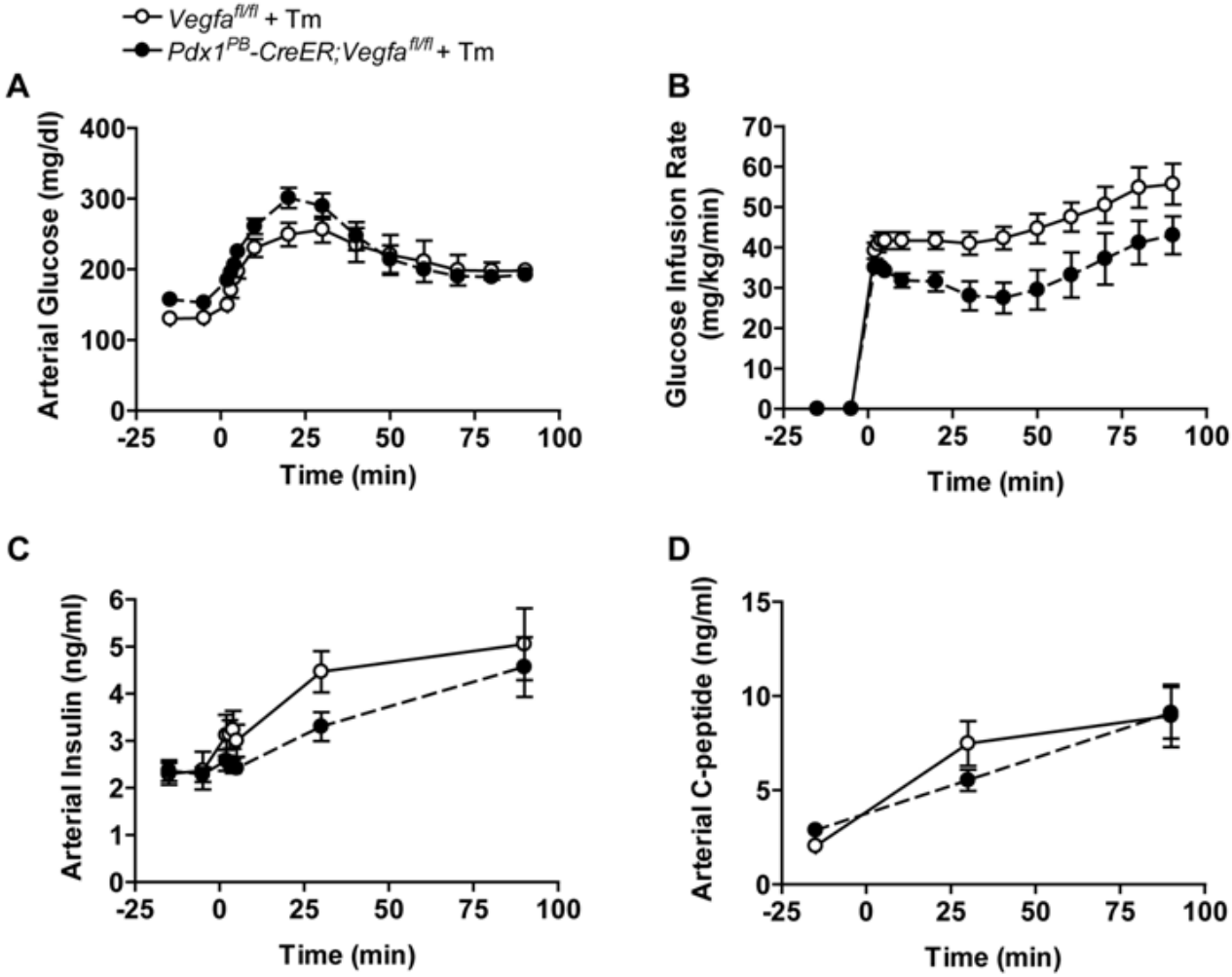
SUPPLEMENTARY DATA

Supplementary Figure 3. Impaired glucose tolerance develops more slowly in female *Pdx1*^{PB}-*CreER;Vegfa*$^{fl/fl}$ mice. Glucose tolerance testing was performed in female *Vegfa*$^{fl/fl}$ and *Pdx1*^{PB}-*CreER;Vegfa*$^{fl/fl}$ mice before tamoxifen (Tm) treatment (A), and one month (B) and three months (C) following Tm or Veh treatment. Mice were fasted for 16 hours before intraperitoneal injection of 2 g/kg glucose. Data were analyzed by one-way ANOVA of the area under the curve.



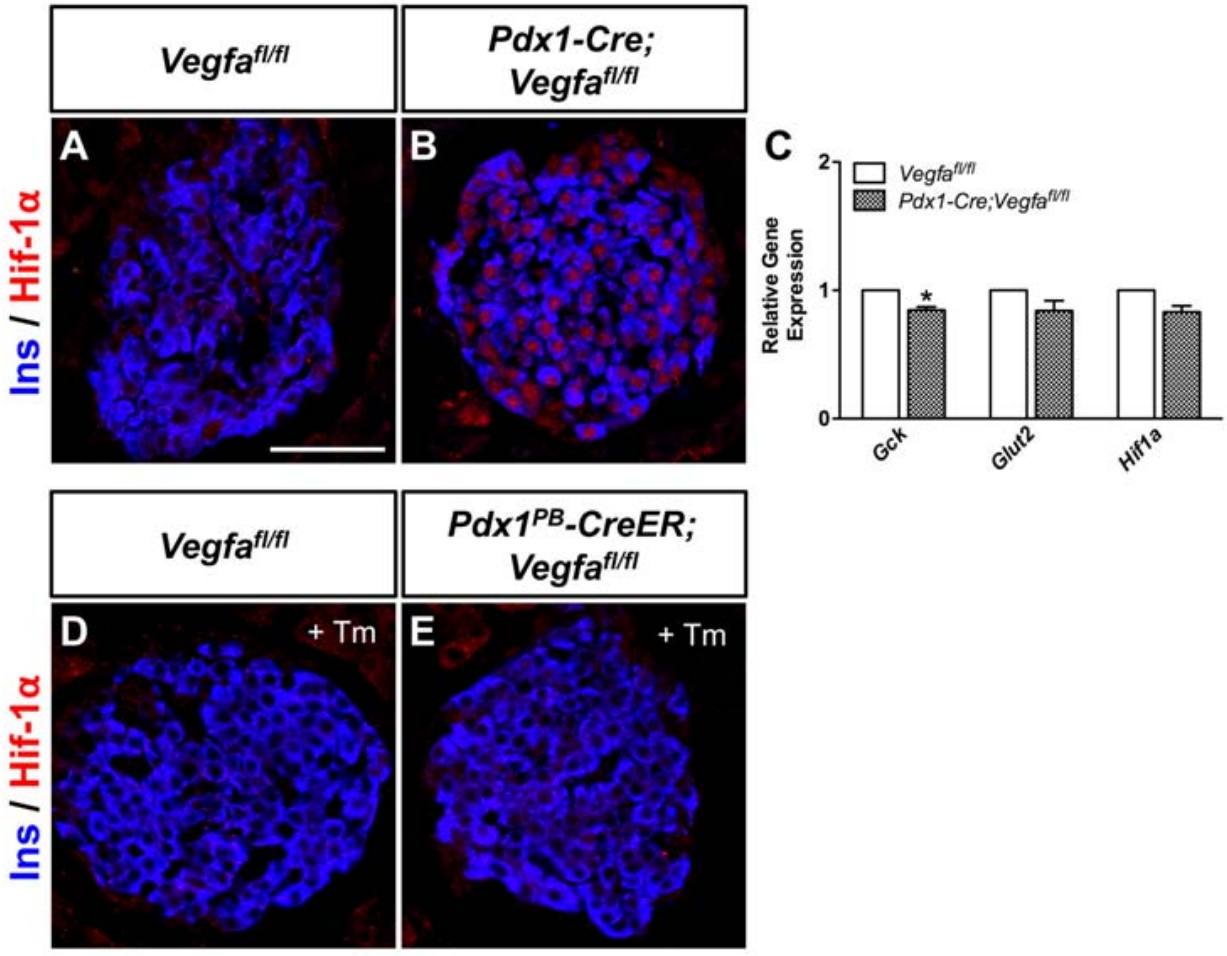
SUPPLEMENTARY DATA

Supplementary Figure 4. Insulin secretion is delayed but not reduced in *Pdx1*^{PB}*-CreER;Vegfa*$^{fl/fl}$ mice. A hyperglycemic clamp was performed on six-month-old Tm-treated male mice. One week after undergoing surgery to implant catheters in the carotid arteries and jugular veins, mice were fasted for 6 hours before infusing glucose to maintain a target blood glucose level of ~200 mg/dl. With the time of initial glucose infusion being t = 0', arterial blood samples were taken at t = -15', -5', 2', 3', 4', 5', 10', 20', 30', 40', 50', 60', 70', 80', and 90', and assessed for blood glucose, serum insulin and serum C-peptide levels. Data were analyzed by a two-way ANOVA with repeated measures, using Time and Genotype as factors. (A) Arterial blood glucose values. $P < 0.0001$ for Time, $P = 0.1689$ for Genotype, $P = 0.1864$ for Interaction. (B) Glucose infusion rate. $P < 0.0001$ for Time, $P = 0.0108$ for Genotype, $P = 0.0291$ for Interaction. (C) Serum insulin values. $P < 0.0001$ for Time, $P = 0.1701$ for Genotype, $P = 0.5523$ for Interaction. (D) Serum C-peptide values. $P < 0.0001$ for Time, $P = 0.7764$ for Genotype, $P = 0.2435$ for Interaction.



SUPPLEMENTARY DATA

Supplementary Figure 5. Hif1α expression in islets with reduced vascular supply. (A,B) Pancreatic cryosections from *Pdx1-Cre;Vegfa*$^{fl/fl}$ and their controls were labeled with antibodies to Hif1α (red) and insulin (Ins, blue). (C) Relative gene expression of *Gck*, *Glut2*, and *Hif1a* in islets from 6-month-old *Pdx1-Cre;Vegfa*$^{fl/fl}$ mice evaluated by quantitative RT-PCR. Values are 0.85 ± 0.02 for *Gck* (n = 3; *P = 0.0235 vs. control), 0.84 ± 0.08 for *Glut2* (n = 3; P = 0.1801 vs. control), and 0.83 ± 0.05 for *Hif1a* (n = 3; P = 0.0755 vs. control). (D,E) Pancreatic cryosections from 7-month-old Tm-treated *Pdx1*^{PB}*-CreER;Vegfa*$^{fl/fl}$ their controls were labeled with antibodies to Hif1α (red) and insulin (Ins, blue). Scale bar in A is 50 μm and corresponds also to B, D, and E.



SUPPLEMENTARY DATA

Supplementary Figure 6. β-cell proliferation is unchanged following VEGF-A inactivation in adult islets. β-cell proliferation in adult mice was measured three months after Tm treatment using the proliferation marker Ki67. β-cell proliferation was 0.1341% ± 0.1341% in *Vegfa*$^{fl/fl}$ mice and 0.06165% ± 0.02719% in *Pdx1*^{PB}*-CreER;Vegfa*$^{fl/fl}$ mice ($P > 0.05$, $n = 3$ mice, at least 8000 β-cells counted per genotype).