

Deletion of the von Hippel-Lindau gene in pancreatic beta cells impairs glucose homeostasis in mice.

Supplemental Information

Supplemental methods

Growth hormone assay

Pituitaries were dissected and homogenized in 1 ml of PBS. Aliquots were taken for assay of growth hormone using specific reagents kindly provided by Dr. A. L. Parlow and by the National Institute of Diabetes, Digestive and Kidney Disease, NIH, Bethesda¹.

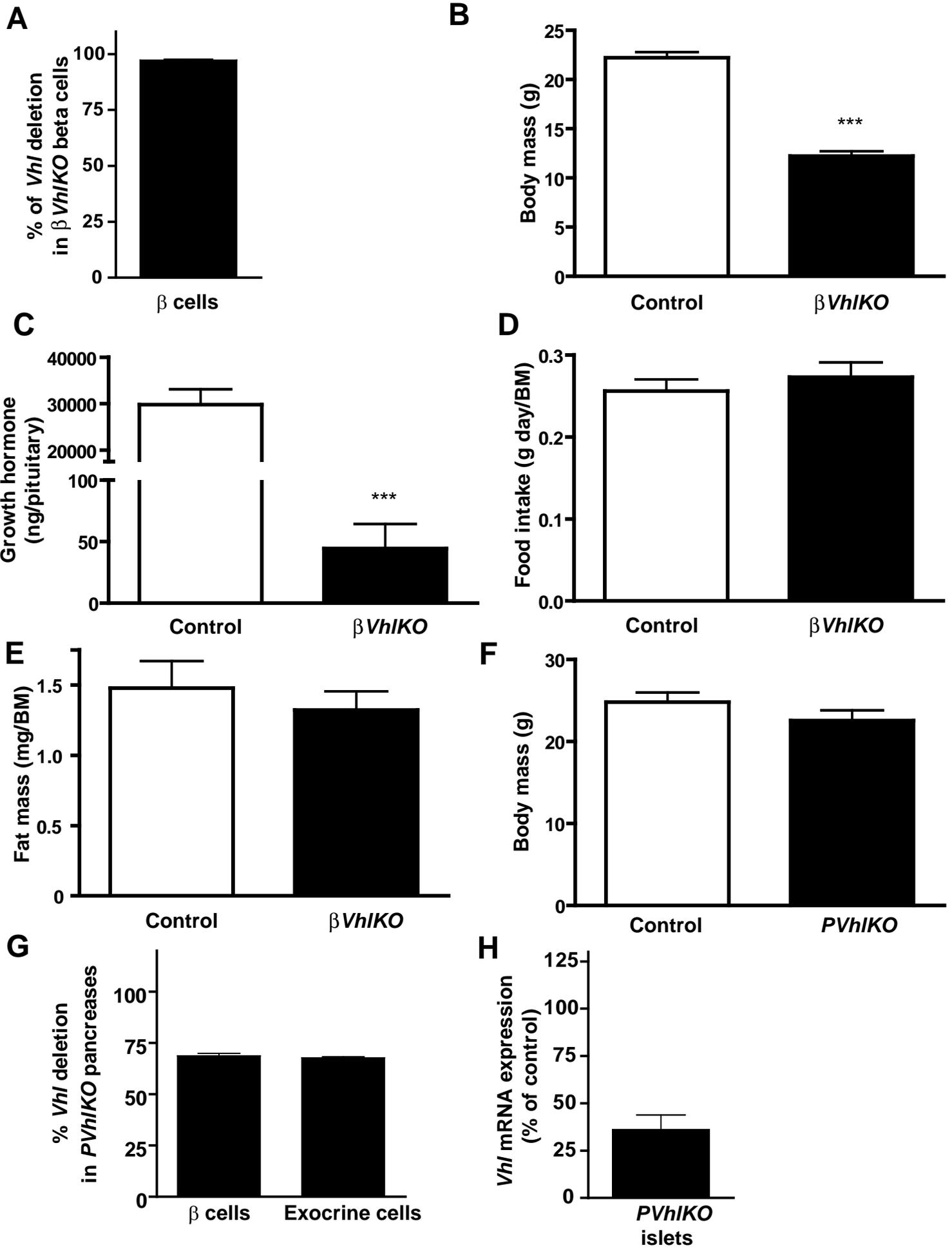
Primer sequences used for RTPCR assays

TaqMan® Gene Expression Assays (Applied Biosystems) were used as follows: *Glut1* Mm00441473_m1, *Glut2* Mm00446224_m1, *Gck* Mm00439129_m1, *Neurod1* Mm01280117_m1, *Ecad* Mm00486906_m1, *Nkx6.1* Mm00454962_m1, *Hnf3b* Mm00839704_mH, *Hnf1a* Mm00493434_m1, *Hnf4a* Mm00433964_m1, *Gapdh* Mm99999915_g1, *Aldo* Mm00523296_m1, *Pfk* Mm03053257_s1, *Pdk1* Mm01276566_m1, *Cd31* Mm00476702_m1, *Vhl* Mm00494136 and *Hprt* Mm00446968_m1 (used as a control in all assays).

Supplemental Reference

1. Carmignac, D.F. & Robinson, I.C. Growth hormone (GH) secretion in the dwarf rat: release, clearance and responsiveness to GH-releasing factor and somatostatin. *J Endocrinol* 127, 69-75 (1990).

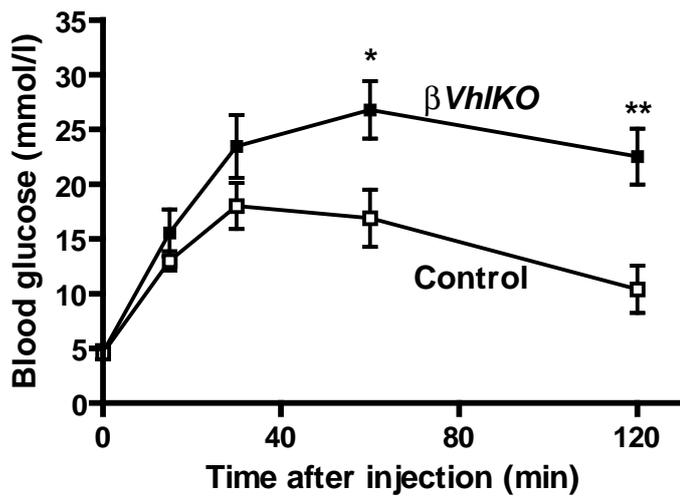
Supplemental Figure 1



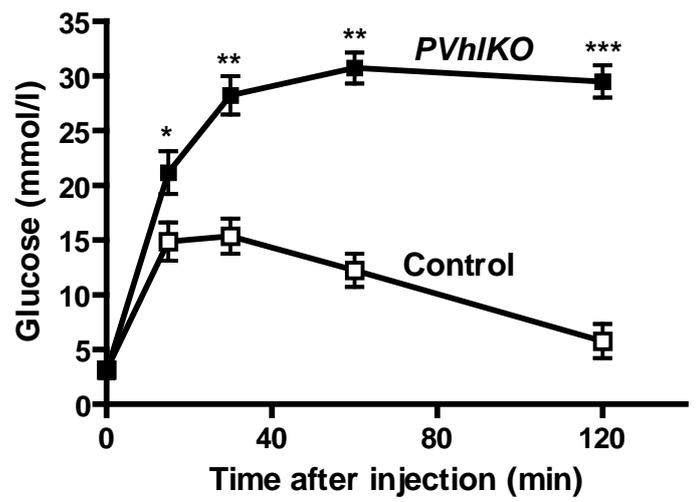
Supplemental Figure 1. Dwarf phenotype in $\beta VhIKO$ mice, normal body weight in $PVhIKO$ mice and deletion of Vhl in beta cells of $\beta VhIKO$ and $PVhIKO$ mice. (A) Percentage of Vhl deletion in beta cells assessed by Hif-1 α staining in pancreases from $\beta VhIKO$ mice. Hif-1 α immunoreactivity was examined in 2007 insulin-positive cells in the pancreases from 2 $\beta VhIKO$ mice. (B) Body mass in male 12 week old control and $\beta VhIKO$ mice, n = 8, *** p < 0.001. (C) Growth hormone levels in pituitary extracts from 12 week old male control and $\beta VhIKO$ mice, n = 5, *** p < 0.001. (D) Daily food intake expressed per body mass (BM) in 12 week old male control and $\beta VhIKO$ mice, n = 8. (E) Fat mass expressed per body mass (BM) in 12 week old male control and $\beta VhIKO$ mice, n = 8. (F) Body mass in male 12 week old control and $PVhIKO$ mice, n = 8. (G) Percentage of Vhl deletion in beta cells and exocrine cells as assessed by HIF1 α staining in pancreases from $PVhIKO$ mice. Hif-1 α immunoreactivity was examined in 3254 insulin-positive cells and in 4197 exocrine cells in the pancreases from 3 $PVhIKO$ mice. (H) $PVhIKO$ islet Vhl mRNA expression relative to control islets, n = 4.

Supplemental Figure 2

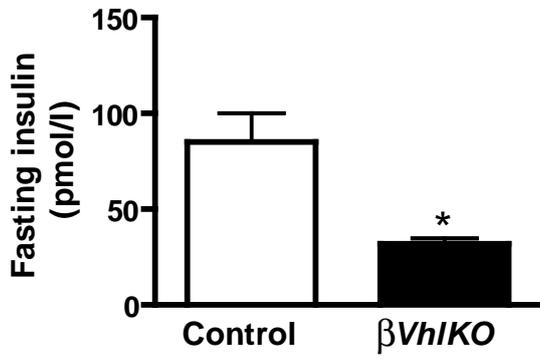
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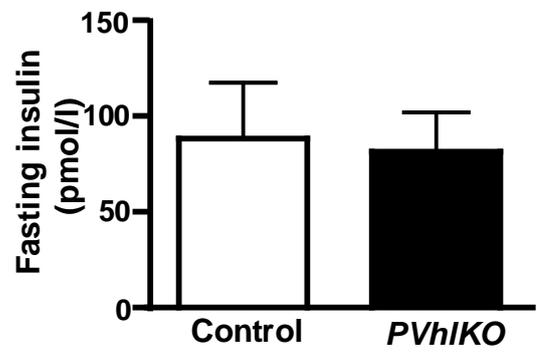
B



C

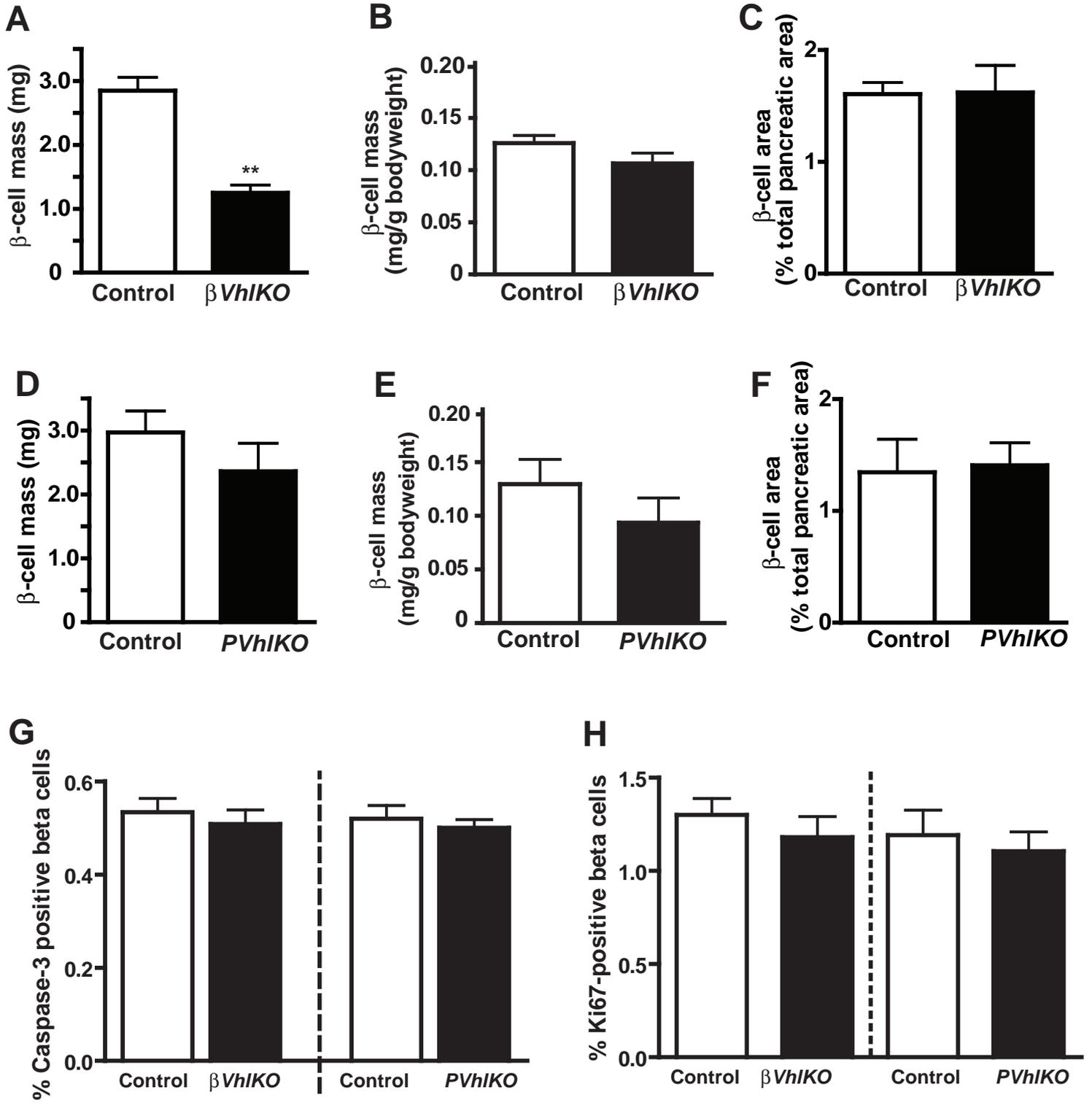


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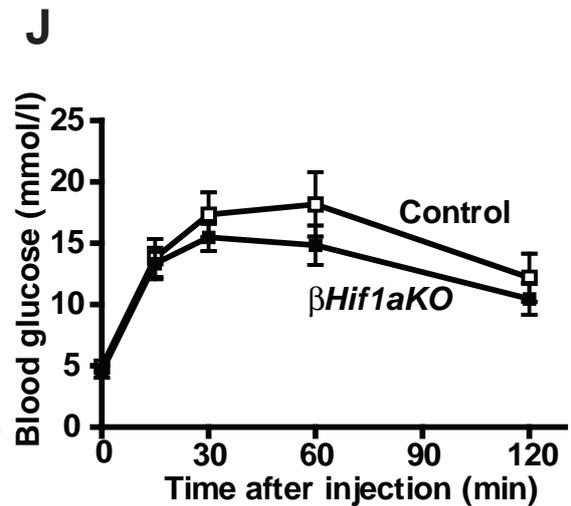
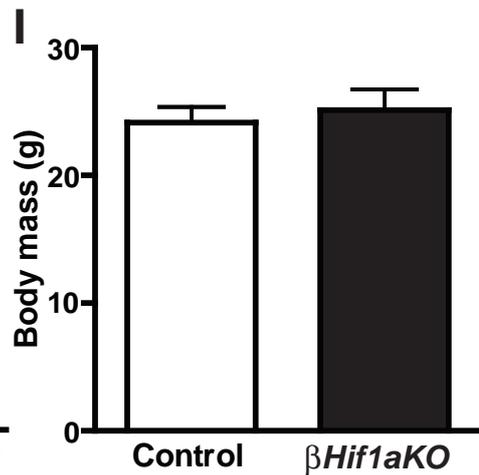
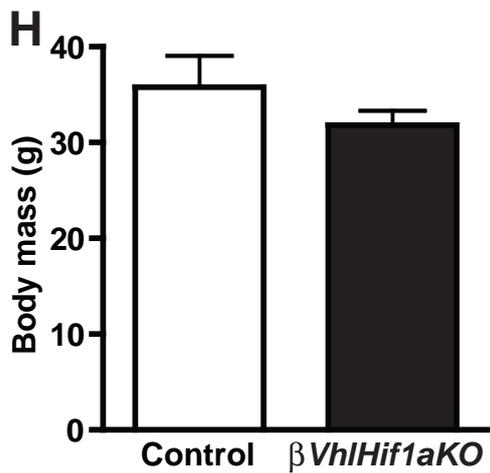
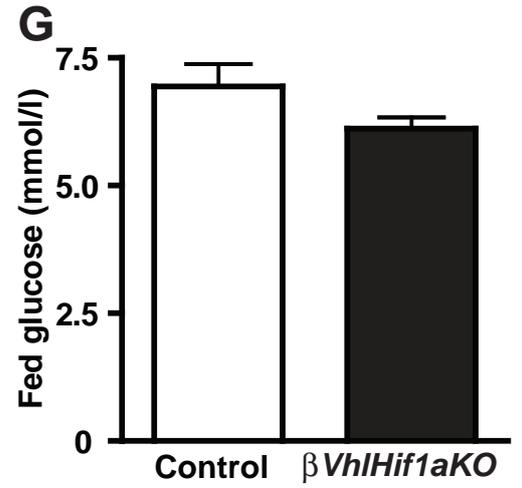
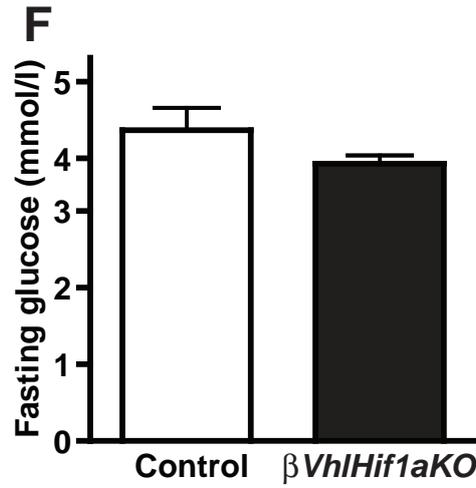
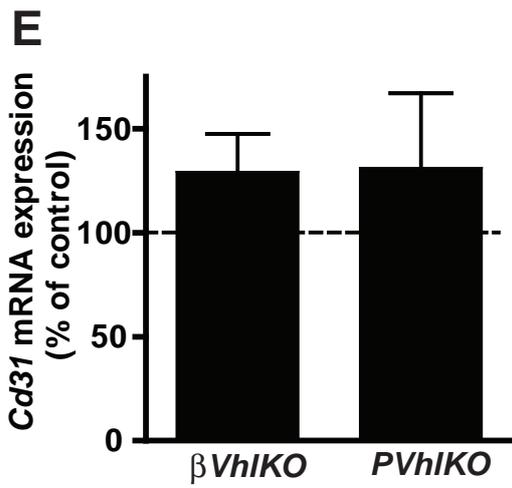
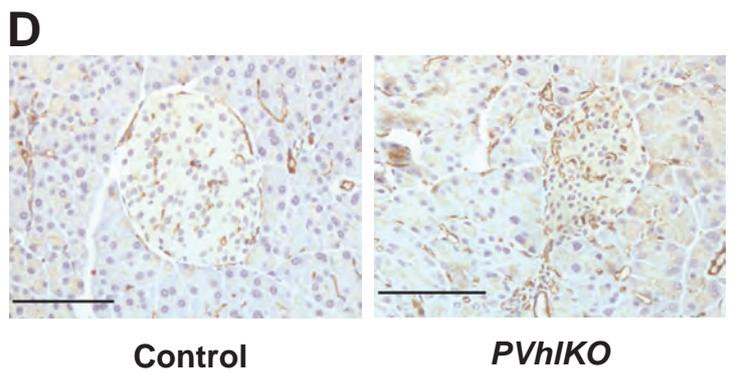
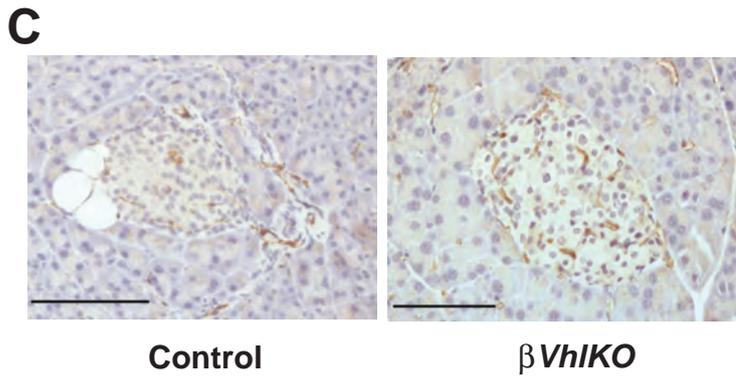
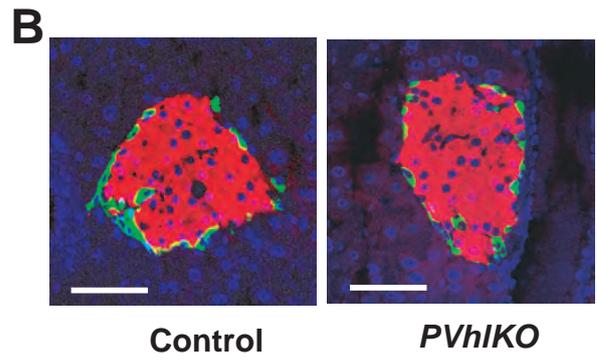
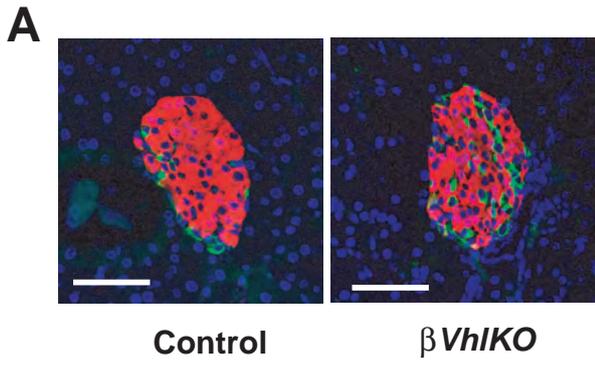
Supplemental Figure 2. Impaired glucose handling in male *β VhIKO* and *PVhIKO* mice. (A and B) Blood glucose after an intraperitoneal injection of glucose (2 g/kg bodyweight) in 12 week old male control, *β VhIKO* and *PVhIKO* mice, n = 8, * p < 0.05, ** p < 0.01, *** p < 0.001. (C and D) Fasted plasma insulin levels in 12 week old female control, *β VhIKO* and *PVhIKO* mice, n = 8, * p < 0.05.

Supplemental Figure 3



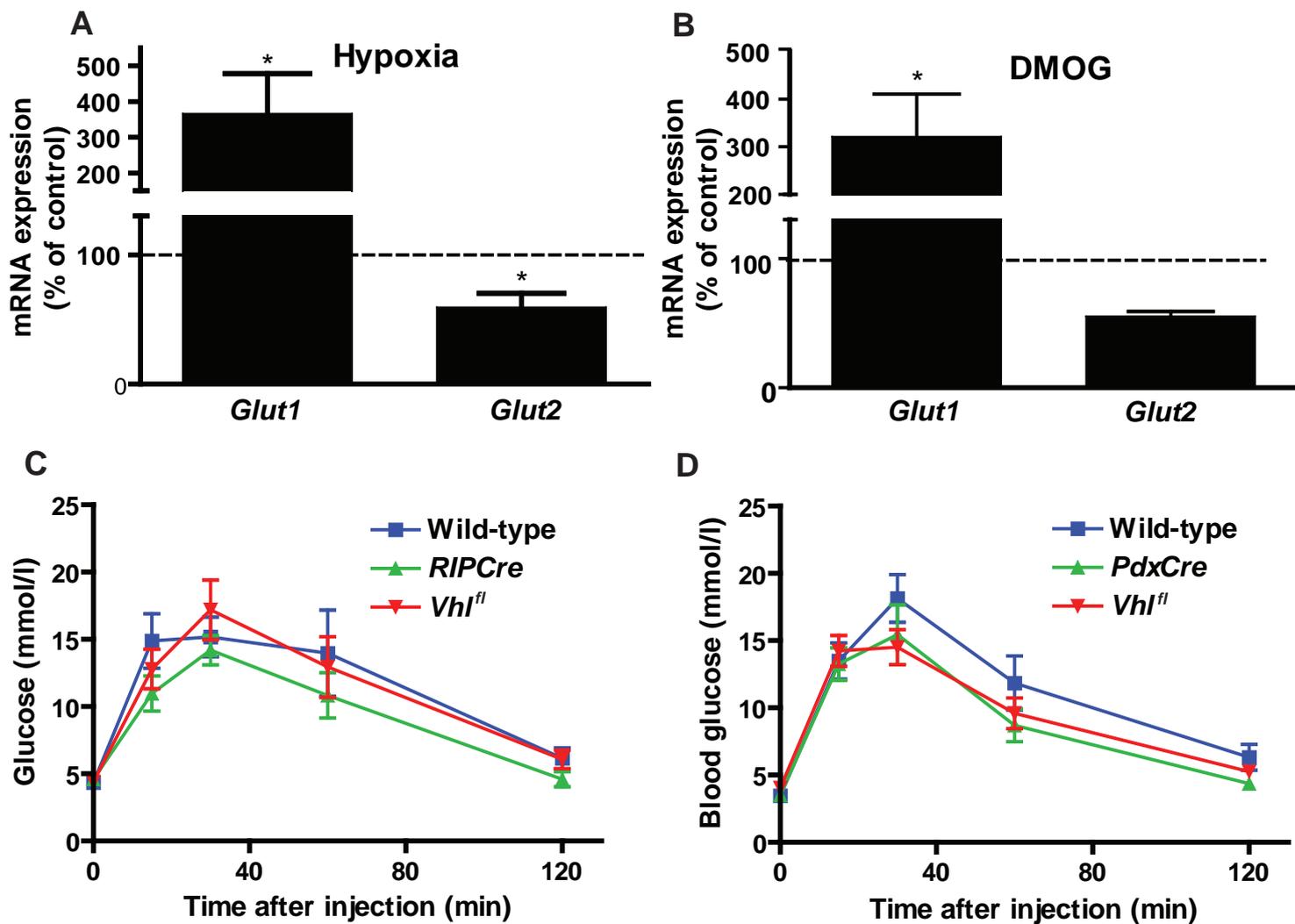
Supplemental Figure 3. Beta cell mass, proliferation and survival in $\beta VhIKO$ and $PVhIKO$ mice. (A, B and C) Absolute beta cell mass (A), beta cell mass expressed per bodyweight (B) and total beta cell area (C) in 12 week old control and $\beta VhIKO$ mice, n = 4, p = N.S. (D, E and F) Absolute beta cell mass (D), beta cell mass expressed per bodyweight (E) and total beta cell area (F) in 12 week old control and $PVhIKO$ mice, n = 4, p = N.S. (G and H) Beta cell apoptosis (determined by activated caspase-3 staining) and proliferation (determined by Ki67 staining) in $\beta VhIKO$ and $PVhIKO$ mice, n = 3-4 mice per genotype, p = N.S.

Supplemental Figure 4



Supplemental Figure 4. Islet morphology in *β VhIKO* and *PVhIKO* mice and glucose homeostasis in *β VhIHif1aKO* and *β Hif1aKO* mice. (A and B) Immunofluorescence staining for insulin (red) and glucagon (green) in 12 week old control, *β VhIKO* and *PVhIKO* mice. Nuclei are stained blue with DAPI. Representative sections are presented. Scale bars are 100 μ m. (C and D) Staining for Cd31 in 12 week old control, *β VhIKO* and *PVhIKO* mice. Representative sections are presented. Scale bars are 100 μ m. (E) *Cd31* mRNA expression in *β VhIKO* and *PVhIKO* islets relative to control islets, n = 6, p = N.S. (F and G) Fasting and fed blood glucose levels in 4-6 month old male *β VhIHif1aKO* mice, n = 8, p = N.S. (H) Bodyweight in 4-6 month old male *β VhIHif1aKO* mice, n = 8, p = N.S. (I) Bodyweight in 12 week old male *β Hif1aKO* mice, n = 8, p = N.S. (J) Blood glucose after an intraperitoneal injection (2 g/kg bodyweight) of glucose in 12 week old male control and *β Hif1aKO* mice, n = 8.

Supplemental Figure 5



Supplemental Figure 5. Altered gene expression in Min6 cells exposed to hypoxia or DMOG and normal glucose handling in wild-type, Cre transgenic and *Vhl*^{fl/fl} mice from *RIPCre* and *PdxCre* strains. (A) Expression of *Glut1* and *Glut2* mRNA in Min6 cells exposed to 1% oxygen for 16 hours relative to cells under normoxic conditions, n = 7, * p < 0.05. (B) Expression of *Glut1* and *Glut2* mRNA in Min6 cells exposed to 0.5 mmol/l DMOG for 16 hours relative to vehicle treated cells, n = 3,* p < 0.05. (C) Blood glucose after an intraperitoneal injection of glucose (2 g/kg bodyweight) in 12 week old female wild-type, *RIPCre* and *Vhl*^{fl/fl}, n = 8 per genotype. (D) Blood glucose after an intraperitoneal injection of glucose (2 g/kg bodyweight) in 12 week old female wild-type, *PdxCre* and *Vhl*^{fl/fl} mice, n = 3-4 per genotype.