Zehetner_Fig.S1.

A

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Arnt

- **total pancreas**
- **islets**

B

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<tr>
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<th>ARNT</th>
<th>Insulin</th>
<th>Merge</th>
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Scale bars: 100 μm
Supplemental Figures

**Supplemental Figure S1.** Expression of *Arnt* mRNA in pancreatic mouse islets.

(A) Real-time PCR analysis of *Arnt* mRNA expression in pancreatic islets and total pancreas of wild-type (wt) mice (n<3 mice). (B) Confocal images of wt pancreatic islets stained for ARNT and insulin (upper row). Negative controls represented in the lower row show staining of the secondary antibodies alone (scale bars=50µm, n=3 mice).

**Supplemental Figure S2.** 2D view of DAVID functional annotation clustering.

Cluster of genes being highly differentially regulated in control versus *Vhlh*−/− mice. 7 genes were annotated to glycolysis (P=1.3E⁻⁹, Benjamini correction: 1.1E⁻⁶).

**Supplemental Figure S3.** Voltage-dependent Ca²⁺-currents are not affected in *Vhlh*−/− animals.

Peak Ca²⁺-current (I) displayed against the voltage during the depolarization (V) of control (black dashed), *Vhlh*−/− (grey), and *Vhlh*−/−*Hif1α*−/− (black) animals. Mean values ± SE for 16 (control), 19 (*Vhlh*−/−), and 12 (*Vhlh*−/−*Hif1α*−/−) β-cells (from 2 mice).
Supplemental Material and Methods

Mouse genotyping

Genotyping was performed using tail DNA. The following primers were used for PCR-mediated amplification:

**Vhlh** forward: 5’CATGTGCCTGCAGAGACCAG3’

**Vhlh** reverse: 5’CACGCATCCACATCAGGTG3’

**Hif1α** forward: 5’GCAGTTAAGAGCACTAGTTG3’

**Hif1α** reverse: 5’GGAGCTATCTCTCTAGACC3’

**Rip2-Cre** forward: 5’CCTGTTTTTGACGTTTCAACG3’

**Rip2-Cre** reverse: 5’ATGCTTTCTGTCCGTGGCCG3’

To test for β-cell specific deletion, DNA isolated from pancreatic β-cell was compared to tail DNA using the following primers:

**Vhlh** forward 1: 5’CTGGTACCCACGAAACTGTC3’

**Vhlh** forward 2: 5’CTAGGCACCGAGCTTAGAGGTTTGCG3’

**Vhlh** reverse 1: 5’CTGACTTCCACTGATGCTTGTCACAG3’

**Hif1α** forward 1: 5’TGGGGATGAAAACATCTGCT3’

**Hif1α** reverse 1: 5’GTTGGGATGAAAACATCTGC3’

Morphometric measurements

Pancreata were excised, weighted, and fixed in 10% formalin overnight at 4°C. Consequently, paraffin sections of 5µm thickness were made. Sections were stained with Hematoxylin and Eosin (H&E) or by immunofluorescence with Insulin, Glucagon, and DAPI co-staining to visualize β- and α-cells as well as cell nuclei. For pancreatic area three H&E-stained sections, 300µm apart from each other were imaged on a CTR6000 microscope (Leica) using a motorized stage and a 5x objective. All three pancreatic sections were finally analyzed using
the imaging software Metamorph (Molecular Devices). For islet area, β-cell size and density
H&E adjacent sections were stained by immunofluorescence. Anti-insulin antibody (Linco,
#4011-01), anti-glucagon (Zymed, #18-0064), and DAPI for nuclei staining were used. Islet
number was determined for each section and images from each islet were taken with an
Axioplan 2 microscope (Zeiss) using a 20x objective. For insulin staining FITC (488nm), for
glucagon staining Texas Red (555nm) and for nuclei staining UV (405nm) were used as
filters. In Metamorph, images were thresholded, pixel area was measured and converted to
μm². For each animal, the mean of all three sections was calculated and used for further
calculation.

Functional clustering
Exclusively genes regulated more than 2-fold in a significant manner (P>0.05) were used for
As settings were chosen: Species: mus musculus, background: Mouse430_2 and a high
classification stringency.

cRNA preparation
The quality of the isolated RNA was determined with a NanoDrop ND 1000 (NanoDrop
Technologies) and a Bioanalyzer 2100 (Agilent). Only those samples with a 260nm/280nm
ratio between 1.8–2.1 and a 28S/18S ratio within 1.5–2 were further processed. Total RNA
samples (30ng) were reverse-transcribed into double-stranded cDNA with Two-Cycle cDNA
Synthesis Kit (Affymetrix Inc.). The double-stranded cDNA was purified using a Sample
Cleanup Module (Affymetrix Inc.). The purified double-stranded cDNA were in vitro
transcribed in presence of biotin-labeled nucleotides using an IVT Labeling Kit (Affymetrix
Inc.). The biotinylated cRNA was purified using a Sample Cleanup Module (Affymetrix Inc.)
and its quality and quantity was determined using NanoDrop ND 1000 and Bioanalyzer 2100.
Array hybridization

Biotin-labeled cRNA samples (15µg) were fragmented randomly to 35–200bp at 94°C in Fragmentation Buffer (Affymetrix Inc.) and were mixed in 300µl of hybridization buffer containing a hybridization Control cRNA and Control Oligo B2 control (Affymetrix Inc.), 0.1mg/ml herring sperm DNA and 0.5mg/ml acetylated bovine serum albumin in 2-(4-morpholino)-ethane sulfonic acid (MES) buffer, pH 6.7, before hybridization to GeneChip® Mouse Genome 430 2.0 arrays for 16 hours at 45°C. Arrays were then washed using an Affymetrix Fluidics Station 450 EukGE-WS2v5_450 protocol. An Affymetrix GeneChip Scanner 3000 was used to measure the fluorescent intensity emitted by the labeled target.

Statistical analysis

Raw data processing was performed using the Affymetrix GCOS 1.4 software. After hybridization and scanning, probe cell intensities were calculated and summarized for the respective probe sets by means of the MAS5 algorithm (Hubbell et al 2002). To compare the expression values of the genes from chip to chip, global scaling was performed, which resulted in the normalization of the trimmed mean of each chip to a target intensity (TGT value) of 500. Quality control measures were considered before performing the statistical analysis. These included adequate scaling factors (between 1 and 3 for all samples) and appropriate numbers of present calls calculated by application of a signed-rank call algorithm (Liu et al., 2002). The efficiency of the labeling reaction and the hybridization performance was controlled with the following parameters: Present calls and optimal 3'/5' hybridization ratios (around 1) for the housekeeping genes (GAPDH and ACO7), for the poly A spike in controls and the prokaryotic control (BIOB, BIOC, CREX, BIODN).
Real-time PCR primer sequences

The following primers were used for real-time PCR analysis:

18s (5'TGGTGTTGGCCCTTCCGTCAAT3', 5'GTTCCGACCATAAAGATGCC3')

Vhlh (5'GAGGGACCCGGTTCAATAAT3', 5'GGTTGCAAGAGATGACCTGAG3')

Hif1α (5'TGCTCATCAGTGCCACTTC3', 5'CGGCATCCAGAAGTTTTCTC3')

Arnt (5'CCAACCGTGGCTCAAATT3', 5'CTAGGTGCTTGTGCTTGTG3')

Slc2al (5'GCTTATGGGCTTCTCCCAAAT3', 5'GGTGACACCTCTCCACATAC3')

Ldha (5'TGTCTCCAGCAAAGACTACTGT3', 5'GACTGTACTTGCAAAATGTTGGGA3')

Pdk1 (5'GGACTTCGGGCCAGTGAAATG3', 5'TCCTGAGAGATGTCGGGGA3')

Gck (5'AGGAGGCCAGTGAAAGATG3', 5'CTCCAGTGCTAAGGAGAAAA3')

Gpi1 (5'TCAAGCTGCGGCGCTTCTCCACCAG3', 5'CTCCAGTCTCTCAGTACAG3')

Pfk1 (5'GGAGGCCAGAAGCATCAAGCC3', 5'CGGCCTTCCCTCGTAGTGA3')

Aldoa (5'CGTTGTAATCCCTCGCACTTG3', 5'CAGCCCCCTGGGATGTTC3')

Tpi1 (5'CCAGGAAGTTCTTCTGTTGGG3', 5'CAAGGTGTAGTGAAGGGG3')

Gapdh (5'CATCGTCCAGATGACTCCACTC3', 5'GGCCTCACCACCCTTTGATGT3')

PgL (5'ATGTCGGTTCTCACAAGCCTG3', 5'GCTCCATTGTGCAAGCAAT3')

Pgam1 (5'TCTGTGACAGAGAGGAGACATCC3', 5'CTGTCAGACCGCCATAGTGT3')

Enol (5'TGCGTCCACTGCACTC3', 5'CAAGCAGCAAGCGCAATAGT3')

Pklr (5'TCAAGGGCAGGATGAACATT3', 5'CACGGGTCTGATGCTGAGT3')