Electronic Supplementary Material

A mutation in *KCNJ11* causing human hyperinsulinism (Y12X) results in a glucose intolerant phenotype in the mouse.

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Methods

Insulin secretion assay

Islets were isolated by liberase digestion and handpicking (for a detailed protocol see [1]). Insulin secretion from isolated islets (5 islets/well) was measured during 1h static incubations in Krebs-Ringer Buffer (in mmol/l: 118.5 NaCl, 2.54 $CaCl_2$, 1.19 KH_2PO_4, 4.74 KCl, 25 $NaHCO_3$, 1.19 $MgSO_4$, 10 HEPES, pH 7.4, 0.2% BSA) containing either 0, 7, or 20mmol/l glucose or 0.1mM tolbutamide in the presence of 7mmol/l glucose or 5µmol/l GLP-1 or 10µmol/l GIP both in the presence of 20mmol/l glucose. Each assay was carried out in triplicate. Islets were exposed to low glucose (2mmol/l) solution for 1h prior to the experiment. Samples of the supernatant were assayed for insulin using a Rat/Mouse insulin ELISA kit (Millipore). Insulin content was extracted using 95:5 ethanol/acetic acid.

Immunoblotting

Western blots were carried out using Tris-HCl 18% resolving gels (Bio-Rad) and transferred onto Hybond-P membrane (GE Healthcare). Immunodetection was undertaken using rabbit anti-Insulin (H-86) (1:600, Santa Cruz) and the secondary antibody HRP-conjugated goat anti-rabbit IgG (1:1000, Bio-Rad) and ECL plus western blotting detection system (GE Healthcare). Membranes were re-probed with mouse anti-Actin (1:1000, Millipore) and the secondary antibody HRP-conjugated goat anti-mouse IgG (1:1000, Bio-Rad).

Antibodies unsuccessfully tested against KIR6.2 were as follows: Goat-anti KCNJ11/KIR6.2 Everest Biotech; rabbit anti-KIR6.2 (H-55), Santa-Cruz and rabbit anti-potassium channel KIR6.2, Sigma.

Immunohistochemistry

Animals were sacrificed at 13 weeks and pancreases dissected with the spleen attached (for orientation). Islet immunohistochemistry was carried out on pancreas paraffin sections using primary antibodies, rabbit anti-Cleaved Caspase-3 (1:200, Cell Signaling), rabbit anti-human Glucagon (1:40, AbD Serotec) and rabbit anti-Insulin (H-86) (1:50, Santa-Cruz) and an ABC staining system (Santa-Cruz) according to manufacturer's instructions. Sections were counterstained with hematoxylin. Analysis of sections was carried out using a BX51 microscope (Olympus, Essex, UK). Apoptosis was quantified by counting the number of apoptotic cells and islets per section.

Islet area

Ten H-E stained serial sections (separated by 20 sections) from each mouse pancreas were photographed completely using a BX51 microscope (Olympus). Islet area was expressed relative to total pancreas section area. Islet and total pancreas area was calculated using Cell^D software (Olympus).

Quantitative Reverse Transcription PCR (qRT-PCR)

cDNA was analyzed by qRT-PCR using the TaqMan system based on real-time detection of accumulated fluorescence (ABI Prism 7500, Applied Biosystems). *Kcnj11* gene expression was normalized relative to the expression of peptidylprolyl isomerase A (*Ppia*) also known as cyclophilin A (similar results were obtained with islet amyloid polypeptide (*Iapp*) or beta actin (*Actb*)) using mouse specific Taqman probes. Samples were tested in triplicate and results expressed relative to *Ppia* using $2^{-\Delta\Delta CT}$ (Applied Biosystems).

Supplementary Figure Legends

Figure S1 Sequence analysis of cDNA synthesised from total RNA to confirm the presence of the mutation in transcribed DNA.

Figure S2 Impaired intraperitoneal glucose tolerance and insulin secretion. Glucose tolerance (a, c) and insulin secretion (b, d) in homozygous (hom, red lines), heterozygotes (het, blue lines) and wildtype littermate (wt, black lines) backcross three *Kcnj11*^{Y12STOP} mice at 20 weeks of age. (a) Female mice, n=9 wt, n=14 het n=6 hom. (b) Female mice n=9 wt, n=14 het, n=6 hom. (c) Male mice n=9 wt, n=14 het, n=6hom. (d) male mice n=9 wt, n=14 het, n=6 hom. (a,b) Female mice, (c,d) Female mice. For (a),(b),(c) and (d) n=9 wt, n=14 het, n=6 hom. Bars represent mean±SD. * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$ for Student's t-test when comparing hom to wt.

Figure S3 Impaired oral glucose tolerance. Backcross 9 and 10 male homozygous (hom, red lines), heterozygous (het, blue lines) and wildtype littermate (wt, black lines) *Kcnj11*^{Y12STOP} mice at 12 weeks of age. (a) n=11 wt, n=13 het, n=7 hom. *** = $p<0.001$ for Student's t-test comparing wt to hom. (b) AUC comparison of OGTT (white bars) and IPGTT (black bars). Data represent mean±SEM.

Figure S4 Reduced insulin content in islets from homozygous 13-week old *Kcnj11*^{Y12STOP} mice. (a) Total insulin content measured by ELISA: n=54 each for wildtype (wt) and homozygous (hom) mice, with 5 islets from each mouse. *** = $p<0.001$ for Student's t-test comparing wt to hom. (b) Immunoblotting of islets prepared from wildtype (Wt), heterozygous (Het) or homozygous (Hom) littermates. Each lane represents an entire islet preparation from an individual mouse. Blots were first probed with an antibody against

mouse insulin (1:600, Santa Cruz) and then with an anti-actin antibody (1:1000, Millipore) as a control for the amount of protein loaded in each lane. Insulin = 12kDa, actin = 42kDa. (c,d) Relative expression of *Ins1* (c) and *Ins2* (d) measured by qRT-PCR in wildtype (white bars), heterozygous (grey bars) and homozygous (black bars) islet cDNA. Mean±SEM of 3 animals per genotype.

Figure S5 Immunohistochemistry of homozygous *Kcnj11*^{Y12STOP} islets and wildtype islets from 13-week old mice. Staining with insulin (x100 magnification), glucagon (x100 magnification) and cleaved Caspase-3 (x400 magnification). * Positive control for cleaved Caspase-3, thymus section.

Table S1 Number of apoptotic cells from immunohistochemistry analysis. Analysis of sections was carried out using a BX51 microscope (Olympus). Apoptosis was quantified by counting the number of apoptotic cells and islets per section.

Figure S1 Sequence analysis of cDNA synthesised from total RNA to confirm the presence of the mutation in transcribed DNA.

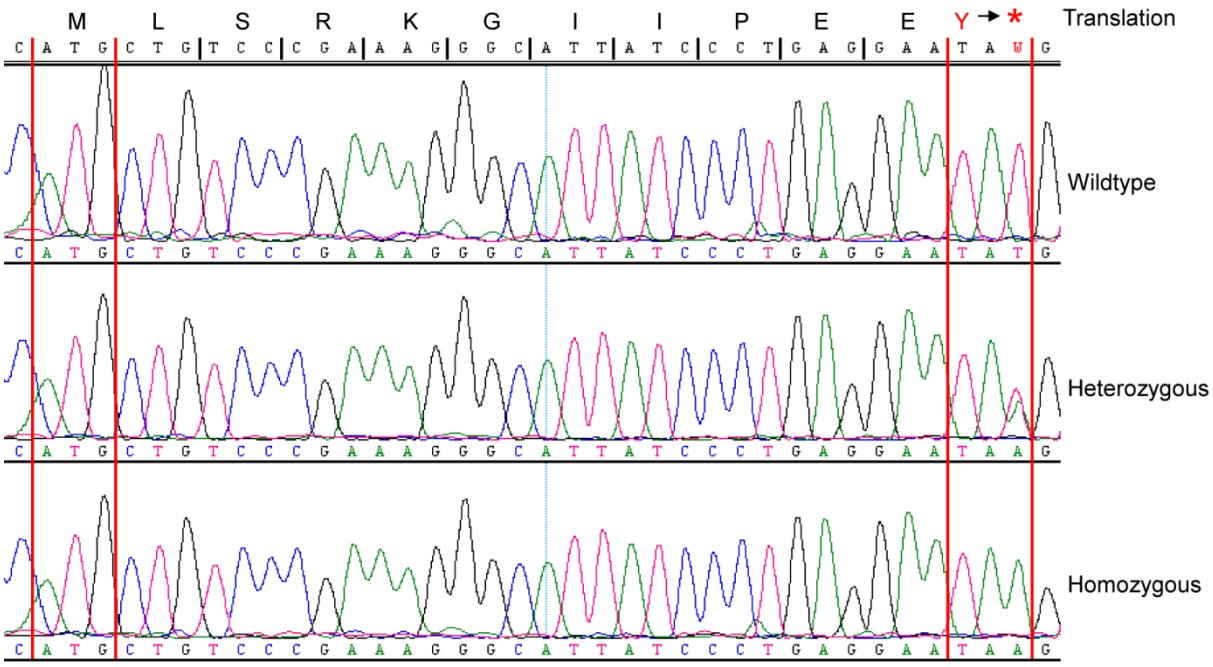

Figure S2 Impaired intraperitoneal glucose tolerance and insulin secretion at 20 weeks of age.

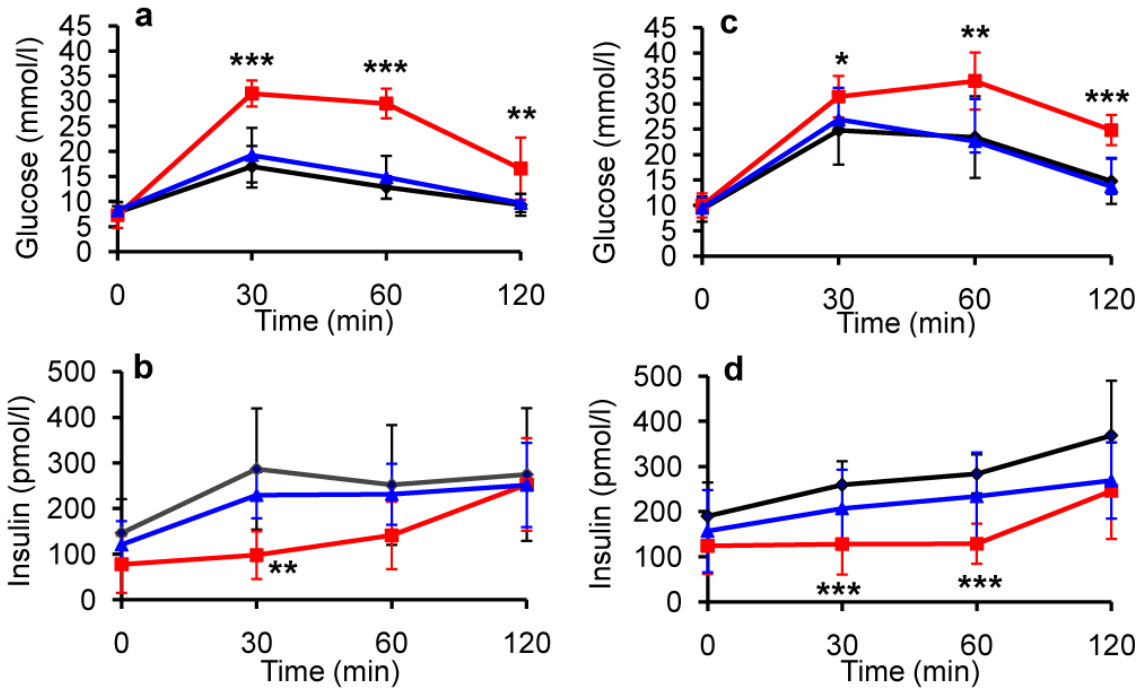

Figure S3 Impaired oral glucose tolerance.

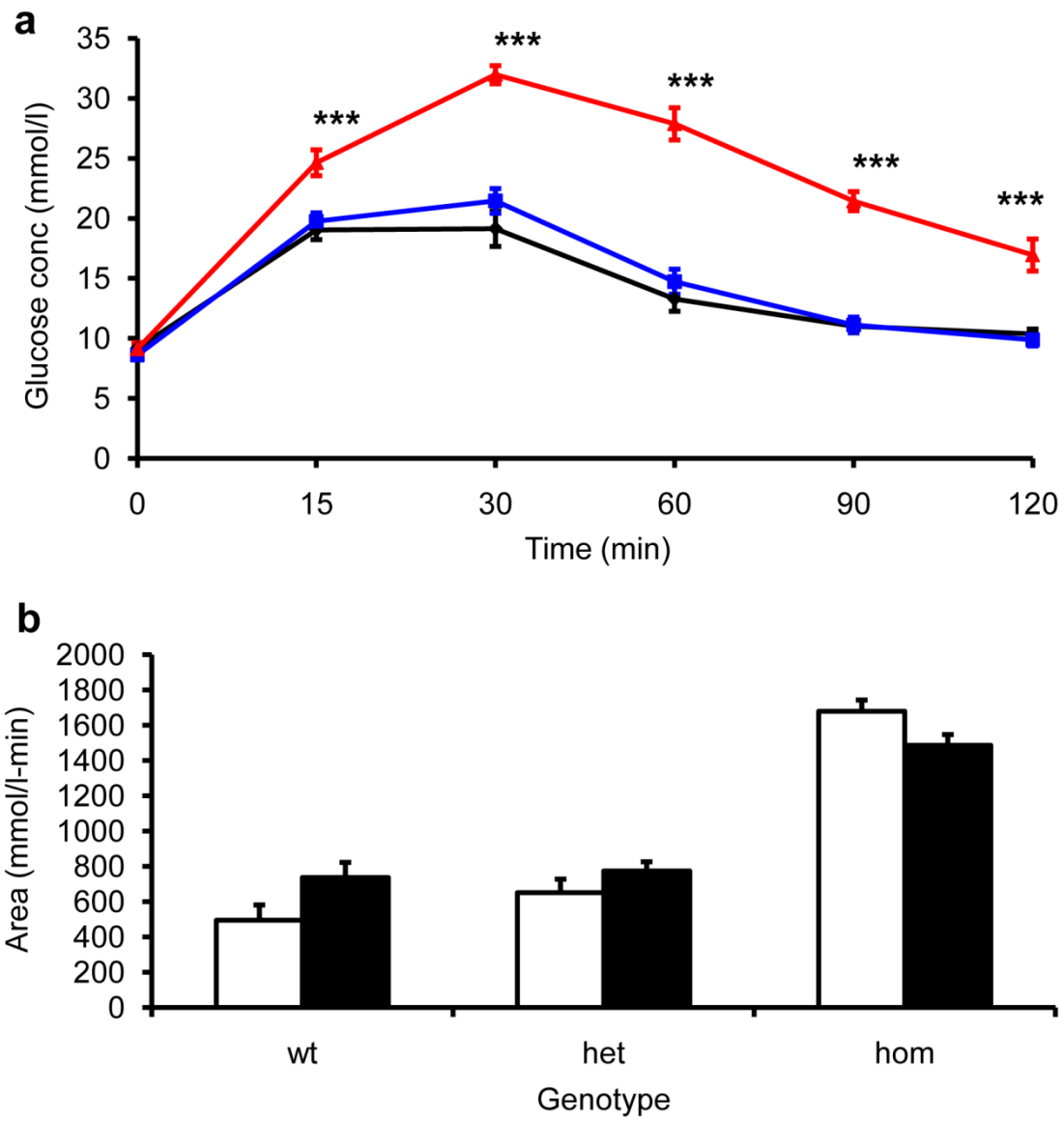

Figure S4 Reduced insulin content in islets from homozygous 13-week old mice

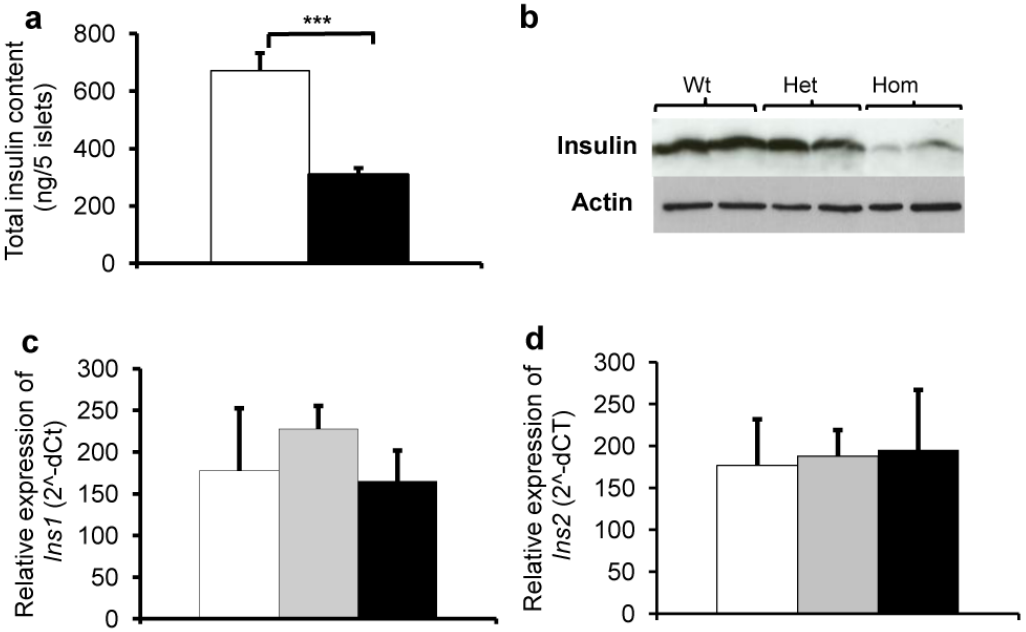

Figure S5 Immunohistochemistry of homozygous *Kcnj11*^{Y12STOP} islets and wildtype islets from 13-week old mice.

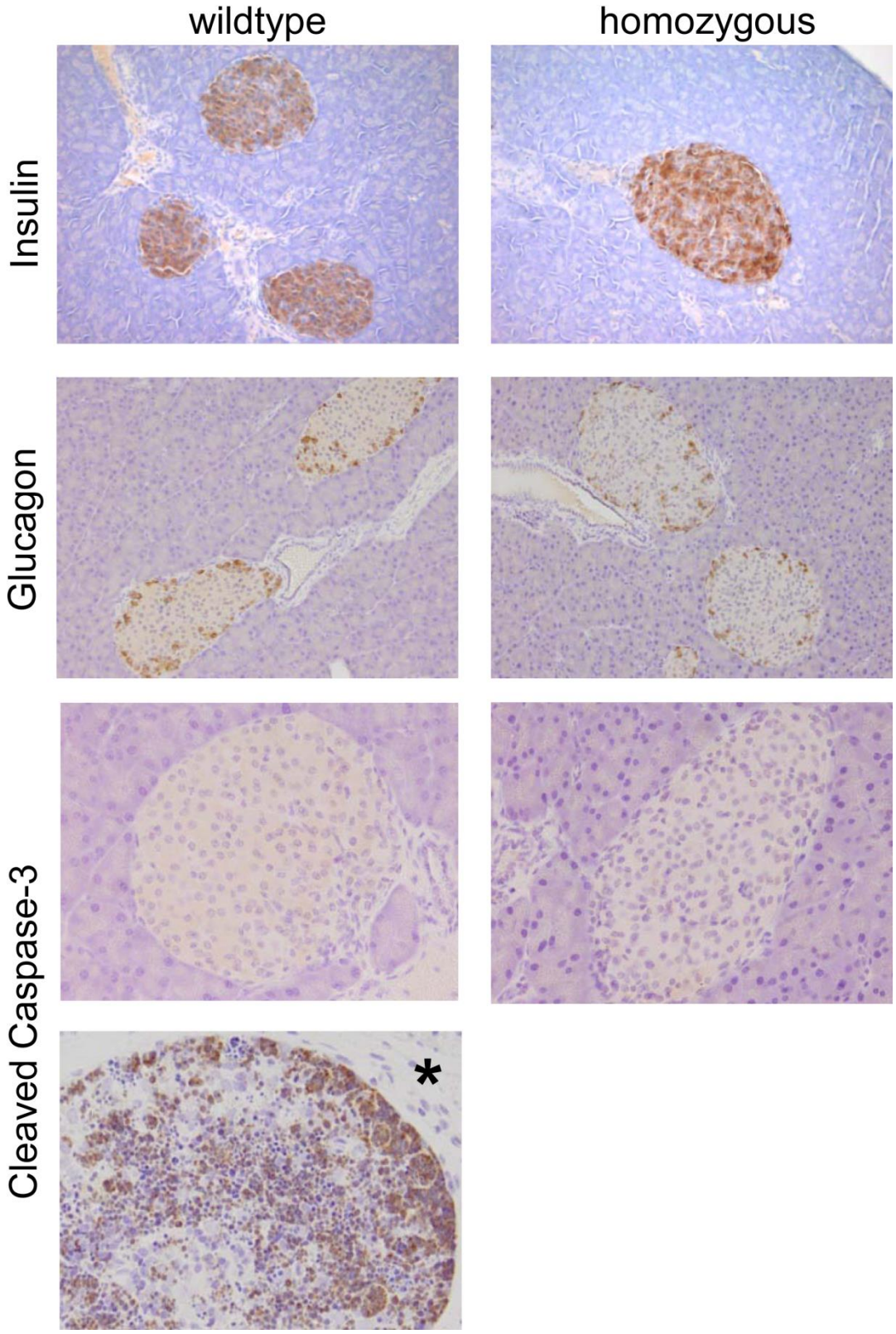

Table S1 Number of apoptotic cells from immunohistochemistry analysis

Genotype	No. of islets	No. of apoptotic cells
Wildtype	22	2
Wildtype	17	3
Wildtype	16	3
Wildtype	44	0
Wildtype	27	1
Total	**126**	**11**
Homozygous	29	2
Homozygous	16	0
Homozygous	20	0
Homozygous	27	0
Homozygous	18	1
Total	**110**	**2**

Supplementary References

[1] Shimomura K, Galvanovskis J, Goldsworthy M, et al. (2009) Insulin secretion from beta-cells is affected by deletion of nicotinamide nucleotide transhydrogenase. Methods Enzymol 457: 451-480