Supplemental Experimental Procedures

Supplemental antibodies

Rabbit anti-somatostatin antibody (A0566; DAKO) and AlexaFluor 594-conjugated chicken anti-rabbit antibody; anti-caspase-3 antibody (9661L; Cell Signaling Tech.) and AlexaFluor 594-conjugated chicken anti-rabbit antibody; sheep anti-BrdU antibody (B2850; US Biological) and cy3 labeled donkey anti sheep (713-165-147; Jackson ImmunoResearch).

Generation of transgenic RIP-Sec mice

The *mSec61a1* cDNA was cut out of pSport6-CMV (MGC clone #BC003707) with restriction endonuclease XmaI, which cuts 28 bp 5' of the insert and in the 3' UTR to yield a 2499 bp fragment. The XmaI fragment was cloned into the XmaI site of pBS-KS-RIP8 (Hanahan, 1985) downstream of the rat insulin2 promoter (RIP), first exon, first intron, and four bp of the second exon and upstream of a heterologous polyadenylation signal. Linearized plasmid was microinjected into C57Bl/6 pronucleus at the Scripps mouse genetics core facility. A single founder line was established, maintained on the B6 background, and bred to *Sec61a1*^{Y344H} mice.

Genotyping of transgenic animals

Genomic DNA was extracted from mouse tails. 35 cycles of PCR was carried out with Platinum Taq (Invitrogen), at an annealing temperature of 58º C, using primers that anneal to the transgene or to exons 9 and 10 of the endogenous gene spanning an intron of 239 bp. Primer sequences: Fwd: 5'-gtcatctcccagatgctgtcag-3' and Rev: 5'- gatgtacacgaccgcatggacag-3'.

GTT and ITT

Age- and sex-matched mice were fasted for 6 h prior to testing. Mice were then injected IP with 0.5 U/Kg of Novolin (Novo Nordisk) for ITT, or 1.5 g/Kg of glucose (Fisher Scientific) for GTT. Blood glucose was then monitored using a OneTouch Ultra handheld glucometer.

Proinsulin analysis

Islets were isolated from four week-old mice which had been weaned on high fat diet. After culturing for 24 hours in RPMI, extract was prepared and run on a 10-20% polyacrylamide, tricine gel under non-reducing conditions as described (13).

RT-PCR analysis of transgene expression

RNA was extracted from pancreatic islets using an Rneasy kit (Qiagen). 10 ng of template RNA was reverse transcribed using Superscript III enzyme (Invitrogen). 40 cycles of PCR were carried out at an annealing temperature of 58º C using the primers: Fwd: 5'-agccctaagtgaccagctac-3' and Rev: 5'-agaaatttgatcgccatggc-3'. β-actin was used as a positive control for amplification with the primers Fwd- tgggccgccctaggcacc and Rev-cggttggccttagggttcag.

TUNEL staining

Age- and sex-matched mice of the indicated age were sacrificed (n=1 per age per genotype) livers were removed and embedded in paraffin . Thin sections were made and stained using the DeadEnd Fluorometric TUNEL staining kit (Promega). Slides were mounted in Prolong GOLD +DAPI (Invitrogen) Sections were imaged using a Bio-Rad (Zeiss) Radiance 2100 Rainbow laser scanning confocal microscope and DAPI and TUNEL positive cells were counted using Image-Pro Plus software. Minimum of 4 sections per sample.

BrdU incorporation and staining

BrdU (Biotium, Inc.) was administered in the drinking water (1mg/mL) of age- and sex-matched mice of the indicated age (n=1 per age per genotype) for 7 days. Mice were then sacrificed and livers were embedded in paraffin and thin sectioned. Mice were stained with a

BrdU antibody and an appropriately labeled secondary. Sections were imaged using a Bio-Rad (Zeiss) Radiance 2100 Rainbow laser scanning confocal microscope and DAPI and TUNEL positive cells were counted using Image-Pro PLus software. Minimum of 4 sections per sample.

Supplemental Table 1: Analysis of Metabolic Parameters in 12-Week Old Male and Female Wildtype (*Sec61α1*$^{+/+}$), Heterozygous (*Sec61α1*$^{+/Y344H}$) and Mutant (*Sec61α1*$^{Y344H/Y344H}$) Mice, Fed Chow or High-Fat Diets.

		Chow			High-Fat Diet		
		Sec61α1$^{+/+}$	***Sec61α1***$^{+/Y344H}$	***Sec61α1***$^{Y344H/Y344H}$	***Sec61α1***$^{+/+}$	***Sec61α1***$^{+/Y344H}$	***Sec61α1***$^{Y344H/Y344H}$
		n=5	n=14	n=5	n=4	n=8	n=10
MALES	**Cholesterol (mg/dL)**	65.2±(2.3)	64.35±(2.8)	85.8±(18.1)	132.0±(38.2)	194.1±(20.5)	457.9±(109.4)*
	HDL-Cholesterol (mg/dL)	47.2±(1.8)	45.43±(2.6)	40.6±(11.6)	81.5±(20.9)	92.6±(11.0)	141.1±(13.9)
	Trigylceride (mg/dL)	83.8±(15.6)	69.71±(6.6)	77±(15.4)	82.3±(6.1)	92.0±(4.9)	140.7±(29.0)*
	Glucose (mg/dL)	139.2±(9.4)	145.6±(7.9)	226.6±(28.5)*	158.0±(32.7)	222.5±(13.3)	469.3±(46.5)***
	Insulin (ng/mL)	1.16±(0.1)	1.29±(0.1)	0.65±(0.1)**	5.2±(1.8)	4.0±(0.6)	0.4±(0.1)*
	Fat (g)	2.1±(0.2)	2.1±(0.1)	1.9±(0.2)*	5.4±(1.7)	6.4±(0.9)	1.9±(0.2)*
	Lean (g)	22.2±(0.9)	21.6±(0.4)	18.9±(1.0)*	23.6±(1.2)	23.0±(0.5)	18.4±(1.1)***
	Weight (g)	27.3±(1.2)	26.3±(0.4)	23.0±(1.0)*	31.8±(1.7)	32.4±(0.8)	23±(1.2)**

		Sec61α1$^{+/+}$	***Sec61α1***$^{+/Y344H}$	***Sec61α1***$^{Y344H/Y344H}$	***Sec61α1***$^{+/+}$	***Sec61α1***$^{+/Y344H}$	***Sec61α1***$^{Y344H/Y344H}$
		n=6	n=14	n=1	n=8	n=13	n=10
FEMALES	**Cholesterol (mg/dL)**	65.7±(6.0)	56.2±(1.6)	90.0	152.3±(13.3)	137.4±(8.9)	278.5±(47.9)**
	HDL-Cholesterol (mg/dL)	38.0±(1.5)	36.8±(0.8)	56.0	84.6±(3.2)	81.3±(4.0)	100.3±(11.5)*
	Trigylceride (mg/dL)	80.3±(15.2)	79.4±(11.3)	39.0	68.3±(5.3)	68.2±(3.8)	90.1±(10.4)
	Glucose (mg/dL)	134.3±(7.6)	140.1±(4.1)	299.0	175.8±(8.4)	163.7±(4.8)	593.0±(58.3)***
	Insulin (ng/mL)	0.8±(0.1)	0.7±(0.1)	0.5	2.9±(0.5)	1.3±(0.2)	0.5±(0.1)*
	Fat (g)	2.2±(0.3)	2.1±(0.1)	2.7	6.7±(1.4)	5.1±(0.5)	2.8±(0.2)
	Lean (g)	15.6±(0.5)	15.7±(0.2)	15.3	17.1±(0.5)	16.6±(0.2)	13.3±(0.6)**
	Weight (g)	20.0±(0.8)	19.9±(0.3)	19.8	26.3±(1.7)	24.1±(0.5)	18.1±(0.9)**

Data represents averages ± sem. Statistical significance was determined using a Students two-tailed T-Test assuming unequal variances (vs. wildtype values of same sex and treatment); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Supplemental figures

Figure S1

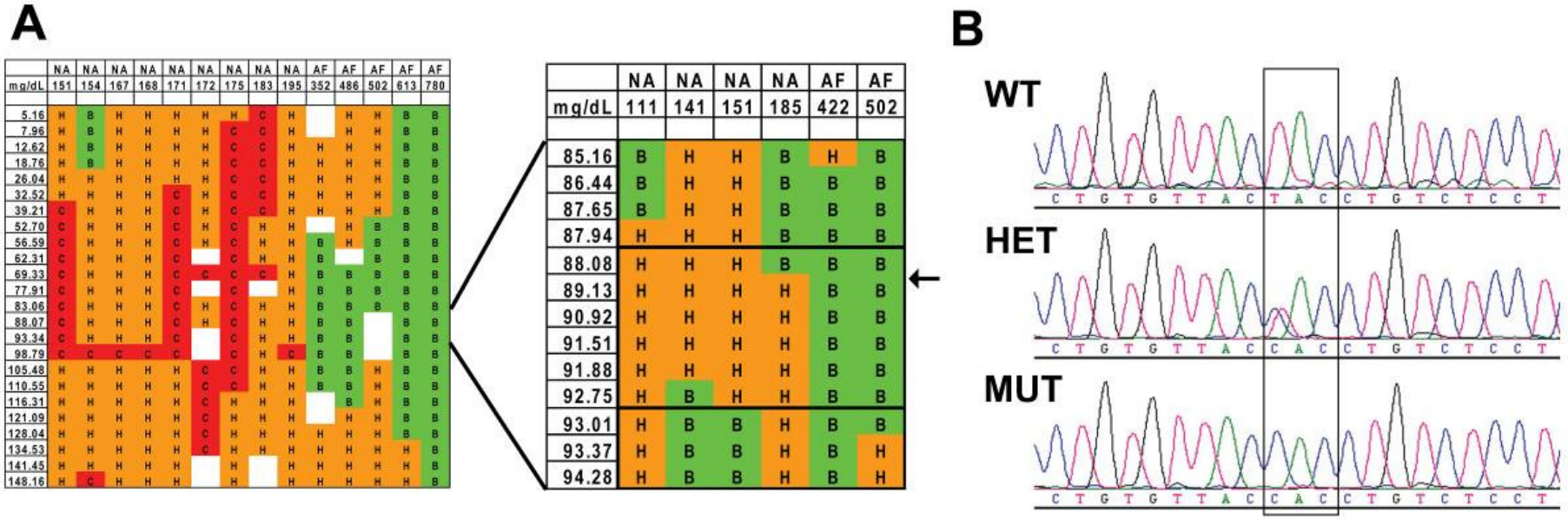


Figure S1. Identification of a mutation in *Sec61a1* associated with diabetes in a mutant family of mice.
(A) Coarse mapping (left) of the diabetic phenotype with SNP markers in five affected and nine unaffected animals from a diabetic family of mice. Further fine mapping (left) narrows the interval containing the causative mutation to a ~5 Mb interval on chromosome 6. The arrow indicates the location of *Sec61a1*. Each mouse is depicted as affected (AF) or not affected (NA) and their plasma glucose concentration (mg/ dl) is given.
(B) Sequencing of genes in the genomic interval enriched in pancreas revealed a C→T transition in *Sec61a1* that altered the native tyrosine codon at amino acid position 344 to histidine (Y344H).

Figure S2

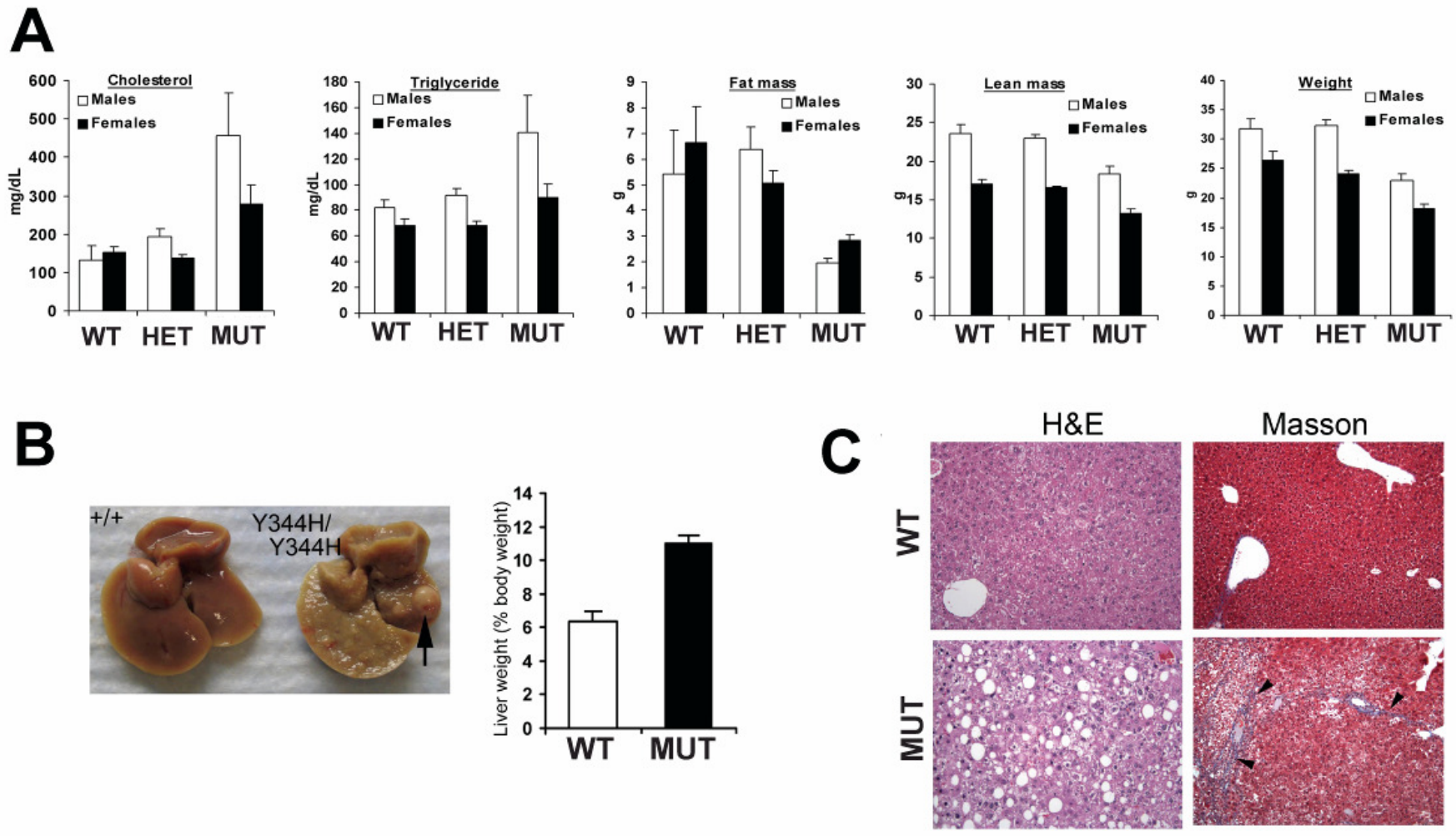


Figure S2. ***Sec61a1***$^{\mathbf{Y344H/Y344H}}$ **mice exhibit multiple metabolic and endocrinological phenotypes.** All data represent means ± sem.

(A) Plasma cholesterol and triglycerides, fat and muscle mass, and weight in the twelve-week-old, HFD-fed mice described in Figure 1B.

(B) Gross examination of the livers (left image) from a ten-month-old, HFD-fed *Sec61a1*$^{Y344H/Y344H}$ and *Sec61a1*$^{+/+}$ mouse and quantitative assessment of hepatomegaly in three *Sec61a1*$^{+/+}$ and three *Sec61a1*$^{Y344H/Y344H}$ six-month-old, HFD-fed mice (right graph). The wildtype liver was excised from a 28 g mouse whereas the mutant liver was removed from a 14 g mouse. Liver weight is expressed as a percentage of total body weight. Note the discoloration, hepatomegaly, and regeneration nodules (arrow) of the mutant liver.

(C) Left: H & E-stained sections of livers from six-month-old, HFD-fed *Sec61a1*$^{+/+}$ (WT) and three *Sec61a1*$^{Y344H/Y344H}$ (MUT) mice (×200 magnification). Note the gross steatosis (macro- and micro-vesicular) in the mutant liver, confirmed by Oil-Red O staining (data not shown). Right: Masson-Trichrome-stained sections of livers from ten-month-old, HFD-fed *Sec61a1*$^{+/+}$ (WT) and three *Sec61a1*$^{Y344H/Y344H}$ (MUT) (x 100 magnification). Note the cirrhosis revealed by extensive collagen deposition (blue stained areas, arrowheads).

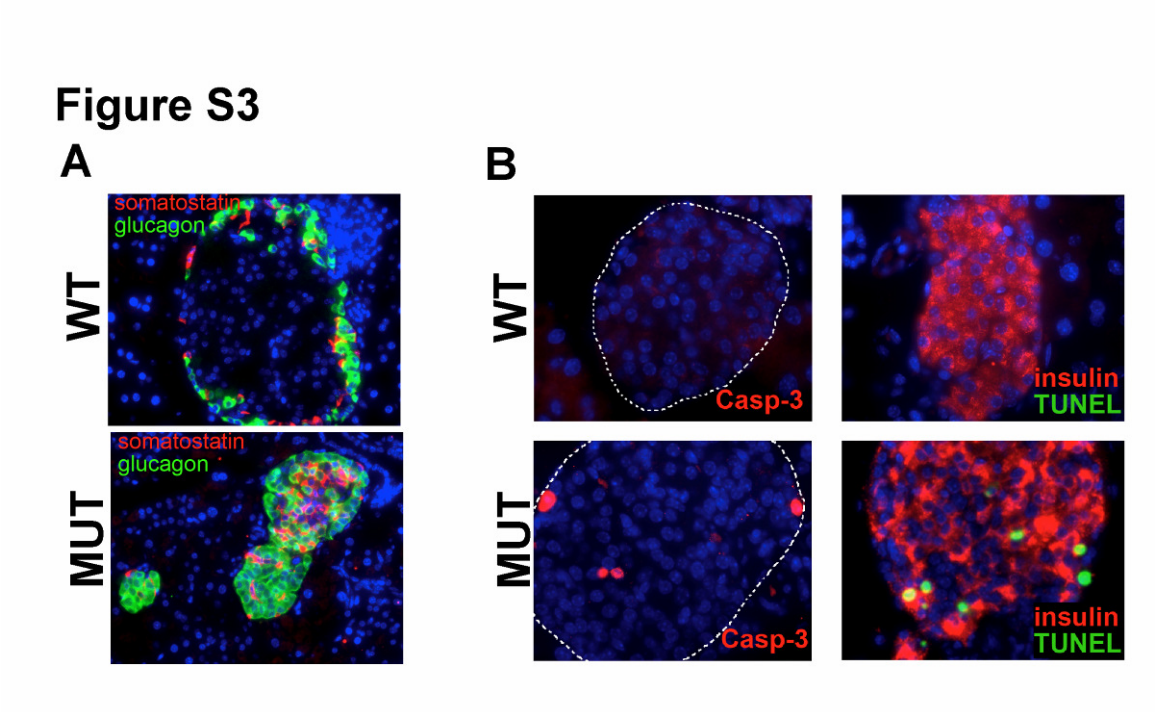


Figure S3. ***Sec61α1*** $^{Y344H/Y344H}$ **mice lose β-cells by apoptosis.**
(A) Islets from twelve-week-old, HFD-fed, *Sec61a1*$^{+/+}$ (WT) and *Sec61a1*$^{Y344H/Y344H}$ (MUT) mice stained for somatostatin (red), glucagon (green), or nuclei (DAPI-blue) (×200 magnification).
(B) Islets from four-week-old *Sec61a1*$^{+/+}$ (WT) and *Sec61a1*$^{Y344H/Y344H}$ (MUT) mice fed HFD for 1 week. Multiple TUNEL+ (green) β-cells (stained for insulin -red) were observed in the same islets (left two panels). Additionally, activated caspase-3 positive cells were commonly observed in islets (outlined by the white dotted line) from mutant but not from wildtype mice (left two panels). Slides were counterstained with DAPI (×600 magnification).

Figure S4

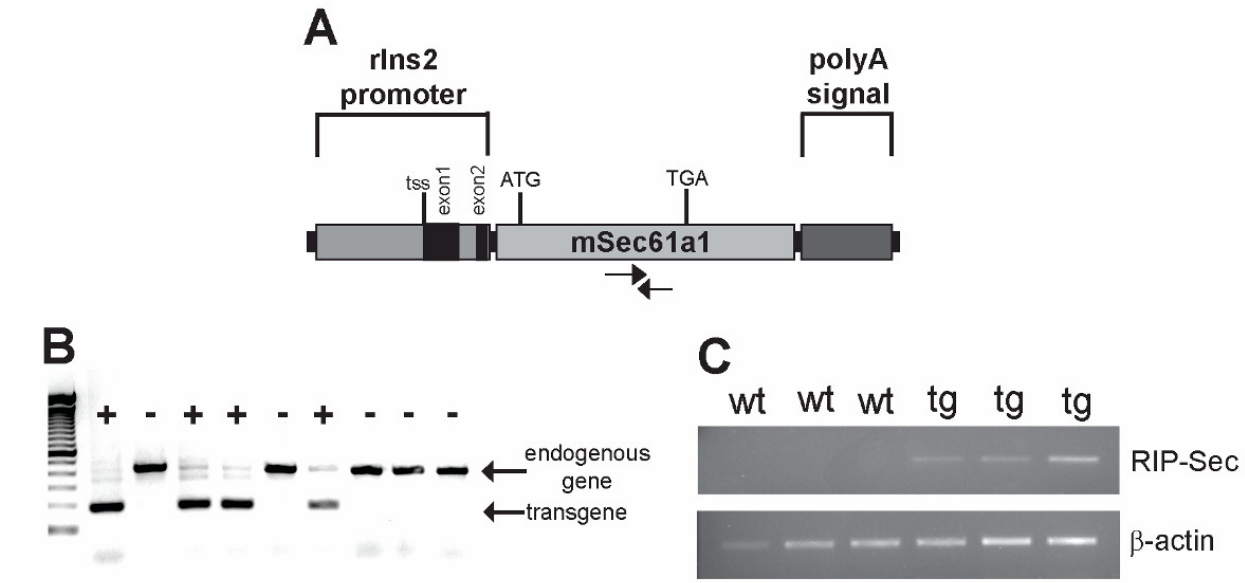


Figure S4. Generation, genotyping, and characterization of transgenic mice expressing m*Sec61a1* in β-cells.

(A) Schematic representation of the Rip-Sec construct. rIns2 promoter: -695 to +153 of rat *Ins2* gene; polyA signal: heterologous poly adenylation signal; arrows: location of primers used for genotyping.

(B) PCR genotyping of nine mice derived from the original transgenic founder with primers that anneal to the transgenic cDNA and yield a 189 bp PCR product or to exons 9 and 10 of the endogenous gene, which are separated by a 239 bp intron, and yield a 428 bp PCR product.

(C) RT-PCR of RNA from islets isolated from wildtype (wt) and Tg[RIP-Sec] (tg) mice.

Figure S5

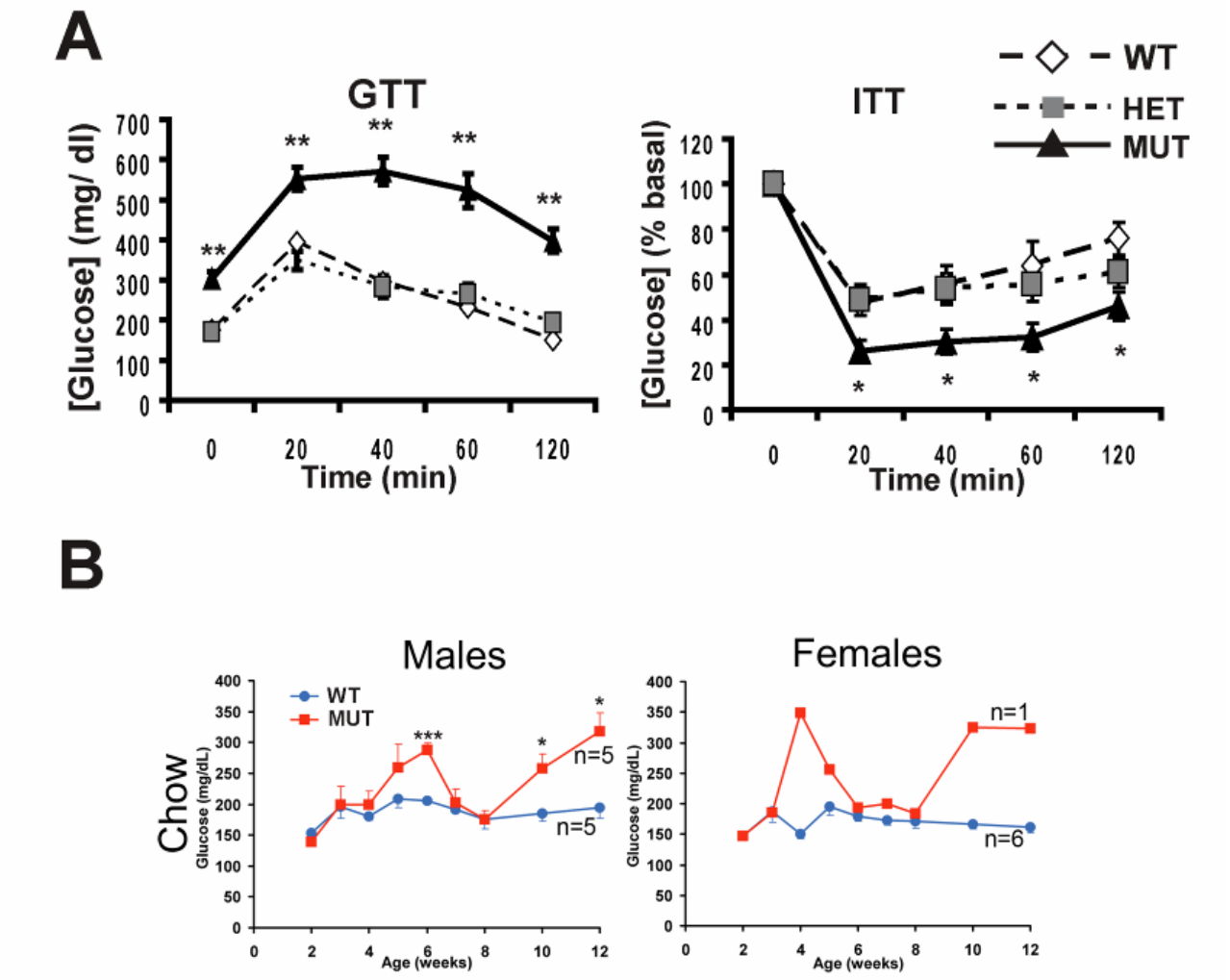


Figure S5. Y344H/Y344H mutant mice become diabetic on chow.
(A) GTT and ITT in male *Sec61a1*$^{+/+}$ (WT, n=10 and 6, respectively), *Sec6a1*$^{+/Y344H}$ (HET, n=7 and 9), and *Sec61a1*$^{Y344H/Y344H}$ (MUT, n=8 and 7) mice fed regular chow. Values shown are mean ± sem. Statistical assessments were made using a two-tailed Student's t-test assuming unequal variances (vs. wild-type littermates); * p<0.05, ** p<0.001.
(B) Glucose levels are elevated in *Sec61a1* $^{Y344H/Y344H}$ mice(MUT, red) compared to *Sec61a1*$^{+/+}$ (WT, blue) animals fed on a chow diet. Values shown are mean ± sem. Statistical assessments were made using a two-tailed Student's t-test assuming unequal variances (vs. wild-type littermates); * p<0.05, ** p<0.01, *** p<0.001.

Figure S6

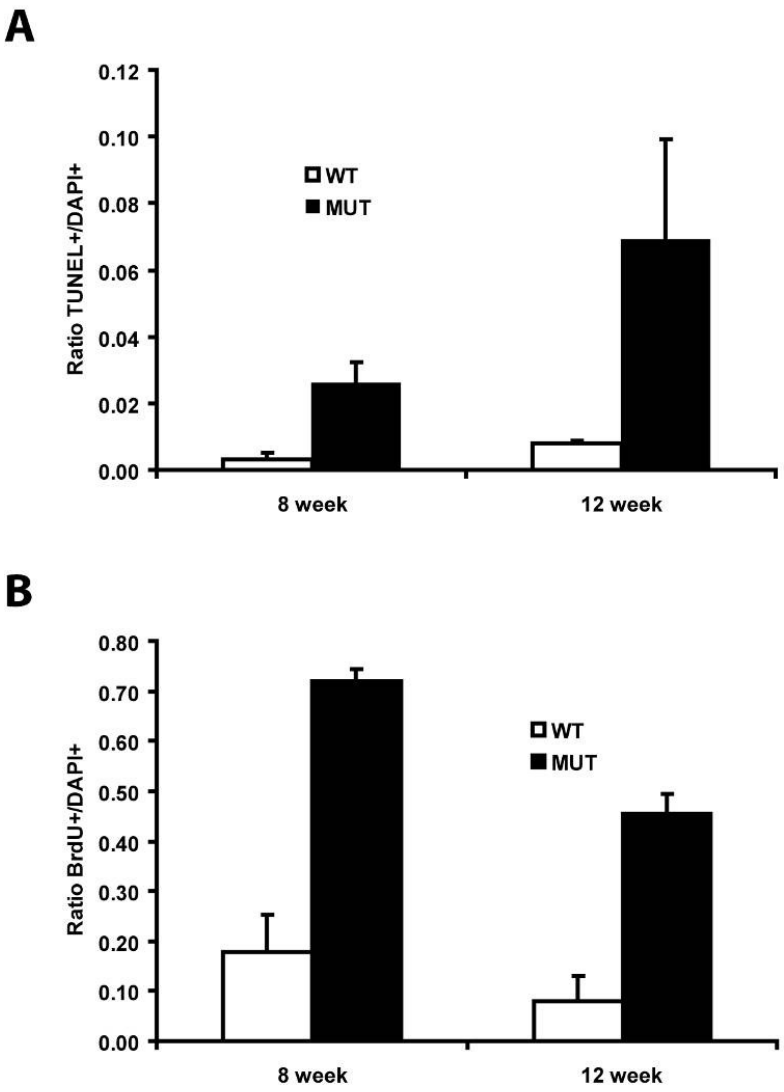
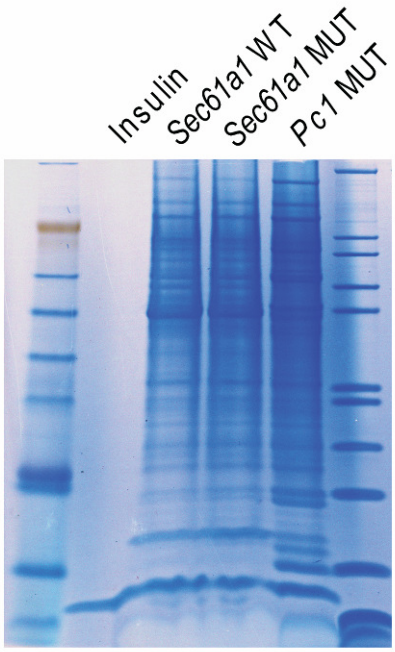


Figure S6. Increased apoptosis and proliferation in mutant livers. (A) Livers from mutant mice (black bars) show increased TUNEL staining at both 8 and 12 weeks of age when compared to wildtype mice (open bars). Error bars represent standard deviation of quantitation of 4 independent images taken from stained liver sections. (B) Livers from mutant mice (black bars) show increase BrdU incorporation at both 8 and 12 weeks of age when compared to wildtype mice (open bars). Error bars represent standard deviation of quantitation of 4 independent images taken from stained liver sections.

Figure S7



A) Figure S7. Insulin processing in Sec61α1 mutant mice. SDS-PAGE gel of fully processed insulin. Islets were cultured from mice of various genetic backgrounds as indicated. After culturing extracts were prepared and fractionated on a 10-20% polyacrylamide tricine gel under non-reducing conditions. Insulin – 1 µg purified, recombinant human insulin; +/+ - extract of islets from *Sec61a1*$^{+/+}$ mice; Y344H/Y344H - extract of islets from *Sec61a1*$^{Y344H/Y344H}$ mice; *Pc1* MUT - extract of islets from *Pc1*$^{N222D/N222D}$ mice (REF).

REFERENCES

Hanahan, D. (1985). Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. Nature *315*, 115-122.