SUPPLEMENTARY DATA

Supplementary experimental procedures

Glucose tolerance, insulin secretion and insulin tolerance tests

Glucose tolerance test (GTT) and glucose stimulated insulin secretion (GSIS) was measured in over-night fasted (15-17hrs) sedated (Hypnorm/Dormicum) mice following intraperitoneal (i.p.) injection of glucose (2g/kg body weight). When performing GTT in HFD treated mice, three of the mice in each group tested reached maximum levels (High > 33.3 mml/l) on the gluconometer and this value was used to calculate mean blood glucose concentrations at 30, 60, 90, and 120 minutes after i.p. glucose injections. Insulin tolerance test (ITT) was performed on non-fasted mice were human insulin (0.75U/kg body weight, Novo Nordisk) was i.p. injected. Glibenclamide, arginine and carbachole were injected i.p. in non-fasted mice at f.c. 5mg/kg, 1g/kg and 0.16µmol/kg, respectively. Blood glucose levels were measured using the One-Touch Ultra Glucometer (In-Vitro Diagnosticum) and plasma insulin levels were determined using the mouse insulin ELISA kit (Crystal Chem Inc.).

Cell counting

Cell counting analyses was performed by sectioning through the whole pancreas from 20 weeks old *Ide* WT and *Ide* KO mice and 40 weeks old *Rip/Snca* mice (n=3 of each genotype) and collecting 10µm thick sections with ~160µm intervals. Sections were subjected to immunoperoxidase staining and analysed using Image-J software.

In vitro culture of islets

For ATP/ADP ratio measurements, islets were distributed in 2ml eppendorf tubes (5 islets/tube, in total 6x5 islets/animal/condition), pre-incubated 1hr in UB-buffer + 2.8mM glucose (2), followed by incubation in UB-buffer containing either 2.8 or 16.8mM glucose for 10, 30 or 60 min. Islets were precipitated with 6% TCA, diethyleter extracted, and the pellet dissolved in 1xPBS. ATP/ADP ratio was determined using ATP-assay kit (SIGMA, FL-AA) and a FB12 Luminometer (Berthold Detection System). In the diazoxide experiments, 4 islets/well were incubated in 150□M diazoxide (SIGMA, D9035) and 30mM K^+. After a 1 minute incubation in the first well (= time point zero), islets were sequentially transferred to new wells at 3, 10, 20, 30 and 40 minutes in either 16.8mM or 2.8mM glucose, respectively. Islets were collected in 1 ml acidic ethanol and insulin content as well as insulin concentration in medium samples were analysed using the mouse insulin ELISA kit (Crystal Chem). For the latrunculin-B experiments, 5 islets/well were incubated for 1 hour in 10□M latrunculin-B (MERCK, 428020) at 16.8mM or 2.8mM glucose, respectively. Islets and medium samples were collected for insulin determination as described above. In the islet perifusion experiments, 30 islets/animal were recovered over-night in RPMI-1640 medium, equilibrated the next day in UB-buffer containing 0.1% BSA, 2.8 mM glucose for 40min followed by a 20min equilibration in the column in the same buffer. Medium was sampled at a 30 second interval and both islets and medium samples were collected for total protein and insulin determination, respectively, as described previously. The perifusion column was made from a 1mL syringe cut at 400µL, where islets were placed on a cotton-filled tip. A peristaltic pump (Pharmacia pump P-1) was used to control the column flow rate (700µL/min). These experiments were performed at 37^{o}C.

Western blot analyses

For WB analyses of LC3 and tubulin, ~ 30 isolated islets recovered over night, were incubated in rich RPMI-1640 medium (non-starved condition), RPMI-1640 medium + 16.8 mM glucose (stimulated condition), or in UB-buffer (starved condition) for 4 hrs, in absence or presence of f.c. 50µM chloroquine (SIGMA, C6628). In experiments using BafA1 (Calbiochem, #196000), f.c. 200nM BafA1 was added to the final 2hrs of the 4hrs islet incubation on isolated islets that were recovered for only 2hrs (Fig. 5 and 6). Fractionation of monomeric and polymeric tubulin and actin were performed as described previously (3).
Determination of IDE and α-synuclein levels were performed using freshly isolated mouse or frozen human islets. Used antibodies are listed in Table S1.

IDE and α-synuclein *in vitro* analyses

IDE and α-synuclein peptides were used at 60nM and 20nM, respectively, in experiments presented in Fig. 5*E* and *F* and in Supplementary Fig.5 *E-G* unless otherwise noted. Incubations were performed in phosphate buffer (pH7.4) at 37°C and analysed by western blot. Degradation of α-synuclein (4µM) and insulin (600µM) by IDE

(0.6μM) assays were performed as described above and analysed by Coomassie staining. Used antibodies are listed in Table S1.

Supplementary Table 1. Primary and secondary antibodies

Antigen	Species	Supplier	Applications
Insulin	guinea pig	Dako #A0564	IHC
Glucagon	rabbit	Euro-Diagnostica #B31-1	IHC
Somatostatin	rat	AbD Serotec #8330-0009	IHC
PP	rabbit	Linco Research #4041-01	IHC
Glut2	rabbit	own	IHC
IPF1/PDX1	rabbit	own	IHC
Isl	rabbit	own	IHC
Caspase-3	rabbit	Cell Signalling #9661	IHC
LC3	rabbit	Cell Signalling #2775	WB
LC3	rabbit	Novus Biologicals #NB100-2220	IHC, WB
p62	guinea pig	Progen #GP62-C	IHC
Ubiquitin	rabbit	Abcam #ab7780-500	IHC
Tubulin	mouse	Sigma-Aldrich #T5168	WB
Actin	mouse	Cell Signalling #4967	WB
α-synuclein	rabbit	Santa Cruz #sc-7011-R	IHC, WB
IDE	mouse	Ref. (1)	WB
IDE	rabbit	Calbiochem #PC730	WB
GAPDH	rabbit	Cell Signalling #2118	WB
Alexa 488	anti-guinea pig	Invitrogen #A11073	IHC
Alexa 488	anti-rabbit	Jackson Laboratories #111-546-047	IHC
DyLight 594	anti-rabbit	Jackson Laboratories # 711-515-152	IHC
Cy3	anti-rat	Jackson Laboratories #112-165-008	IHC
Phalloidin-FITC	Actin-binding toxin	Sigma-Aldrich #P5282	IHC
HRP	anti-mouse	Jackson Laboratories #115-035-003	WB
HRP	anti-rabbit	Jackson Laboratories #111-035-003	WB
HRP	anti-guinea pig	Jackson Laboratories # 106-035-003	WB

SUPPLEMENTARY DATA

Supplementary Table 2. Oligonucleotide sequences

Gene	Forward	Reverse
TBP	5′-GAATTGTACCGCAGCTTCAAAA-3′	5′-AGTGCAATGGTCTTTAGGTCAAGTT-3′
B2M	5′-GCTATCCAGAAAACCCCTCAAA-3′	5′-CTGTGTTACGTAGCAGTTCAGTATGTTC-3′
Isl1	5′-GCCTTGCAAAAGCGACATAGAT-3′	5′-GAATTAGAGCCTGGTCCTCCTTCT-3′
Ipf1	5′-GACACATCAAAATCTGGTTCCAAA-3′	5′-GGTCCCGCTACTACGTTTCTTATC-3′
Nkx6.1	5′-GCACGCTTGGCCTATTCTCT-3′	5′-TCTCGGCTGCGTGCTTCT-3′
NeuroD	5′-AGGCAGCCCTTTTGCTAAGAT-3′	5′-TTCCAAAGGCAGTAACGACAATAA-3′
MafA	5′-GCTGCTGCGGCCTATGAG-3′	5′-TGGCCGGCACCCATGT-3′
Hhex (mouse)	5′-GTTTCAGAATCGCCGAGCTAA-3′	5′-CAAACTGTCCAACGCATCCTT-3′
Hhex (human)	5′-GGAGACTAAAACAGGAGAACCCTCA-3′	5′-CAGGAACTGTCCAAACTTTCCAGT-3′
Kif11	5′-GCCAGCAAGGAGACCAGTCA-3′	5′-CTCCAGAGCCTCCCTCTCTTC-3′
Glut2	5′-TCCTCGTGGCGCTGATG-3′	5′-CTGGTTGAATAGTAAAATATCCCATTGAT-3′
GK	5′-GGAAGAGGCTCTTGGAGGTTGT-3′	5′-GCAAGCTCTAGGTGAACTGGAA-3′
SUR1	5′-CTGCGCTGGATCCTTACCTT-3′	5′-GGATTCTGTCACCCCATCAGA-3′
Kir6.2	5′-GGACCTCCGAAAGAGCATGA-3′	5′-GCGCACCACCTGCATGT-3′
Rab3a	5′-CCACCTGGGCTTTGAGTTCTT-3′	5′-ATCACGTCCACCAGACGTTCA-3′
Rab3b	5′-CAGGACTGGGCTACTCAGATTAAGAC-3′	5′-ACTCTTTCCTCTTCCATGTCACACT-3′
Rab3c	5′-ATGGAAGATGAGCGGGTTGT-3′	5′-AACTCAAACCCGAGCTGTTCTC-3′
Rab27a	5′-GGAGGCCCGGGAACTTG-3′	5′-TCTCAATCGCGTGGCTTATG-3′
Vamp2	5′-ATGAGGTGGTGGACATCATGAG-3′	5′-ATCCAGCTCCGACAACTTCTG-3′
Snap25	5′-GTGGATGAACGGGAGCAGAT-3′	5′-TCCCGGGCATCATTTGTTAC-3′
Stx1a	5′-AAGGCGCGCAGGAAGAA-3′	5′-CCTCAGGGAGACCCATCCA-3′
Stx4	5′-CCGGACGACGAGTTCTTCCA-3′	5′-TGCCGAATTGTCTGCACCTTC-3′
Syt7	5′-ATTCCTTGGAGACGGTGGGC-3′	5′-CCCTCCTGCAGGCAACTTGA-3′
Syt9	5′-CAACACCATCAGCTCGGCAT-3′	5′-TCCGGGTTCGACAGGTTGAG-3′
Atg5	5′-AGTCTGTCCTTCCGCAGTCG-3′	5′-CACCTGGCTCCTCTTCTCTCC-3′
Atg6/Beclin	5′-GGACAAGCTCAAGAAAACCAA-3′	5′-TGTCCGCTGTGCCAGATGT-3′
Atg7	5′-GGTGGCTTCCTACTGTTATTGC-3′	5′-CCAAGGCAGCGTTGATGAC-3′
Atg8/LC3	5′-TCGCCGACCGCTGTAAG-3′	5′-CTCGATGATCACCGGGATCT-3′
Xbp1[40]	5′-CTGAGTCCGAATCAGGTGCAG-3′	5′-GTCCATGGGAAGATGTTCTGG-3′
	5′-CAGCACTACGACTATGTGCA-3′	
Atf4	5′-GGAATGGCCGGCTATGG-3′	5′-TCCCGGAAAAGGCATCCT-3′
Chop	5′-CAGAAGGAACTGCATCTTCATAC-3′	5′-CACTGCCACGTGGACCAGGTT-3′
BiP/Grp78	5′-CAGAAGGAACTGCATCTTCATAC-3′	5′-CACTGCCACGTGGACCAGGTT-3′

SUPPLEMENTARY DATA

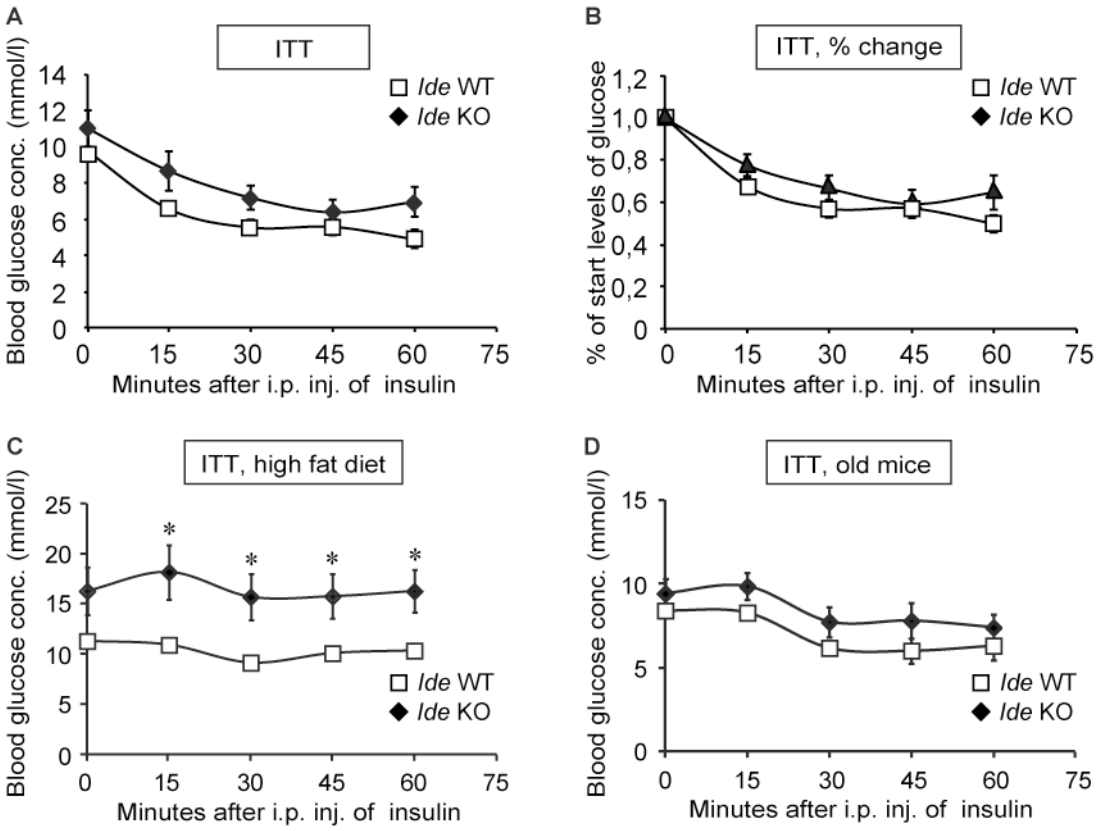

Fig. S1 Insulin resistance in young, old or HFD treated Ide KO mice. (*A*) ITT in 15 weeks old *Ide* KO (n=5) and *Ide* WT (n=8) mice. (*B*) Percentage rates of glucose removal during the ITT in (*A*). (*C*) ITT in *Ide* KO (n=9) and *Ide* WT (n=16) mice kept on HFD diet for 13 weeks. (*D*) ITT in 12 to 16 months old *Ide* KO (n=4) and *Ide* WT (n=3) mice. Data are presented as mean +/- s.e.m., where *P<0.05 (Students t-test)

SUPPLEMENTARY DATA

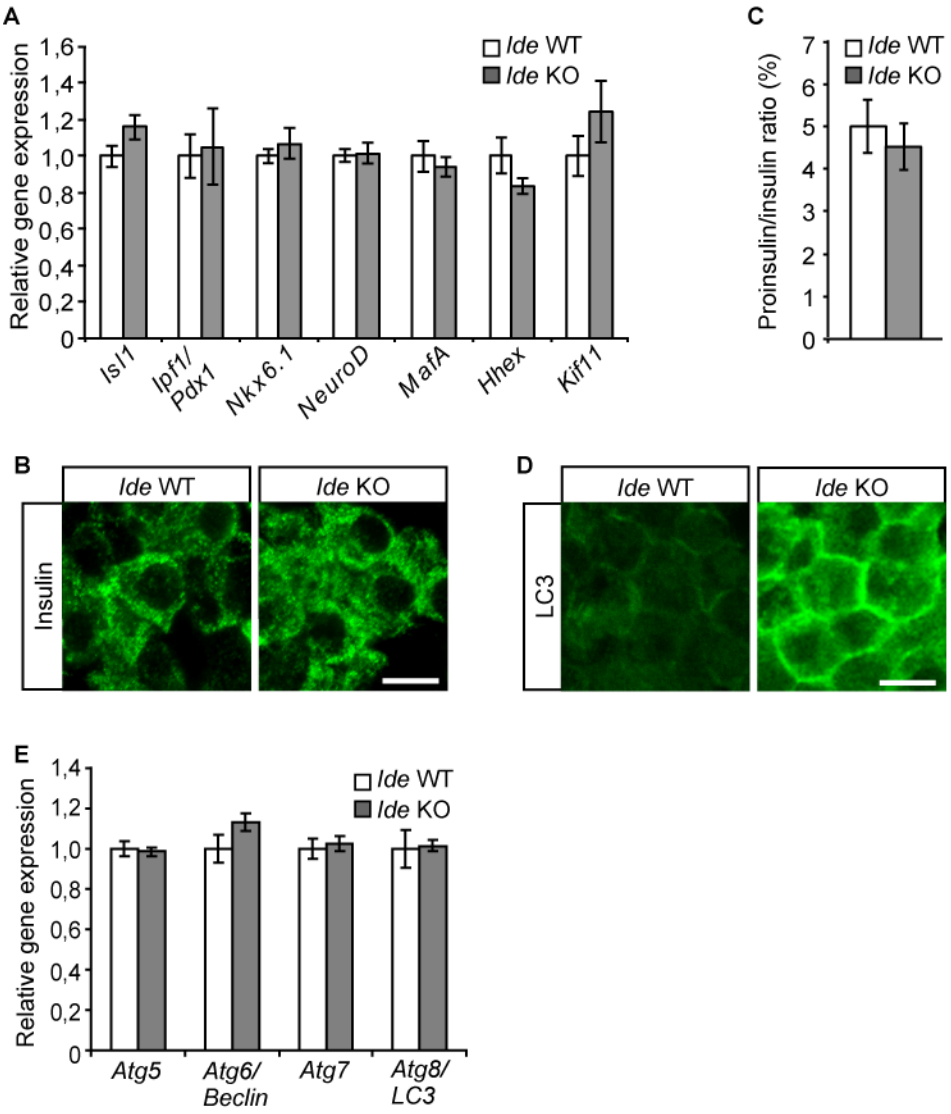

Fig. S2. Expression of genes necessary for ß-cell identity and function in *Ide* KO mice. (*A*) Real-time PCR analyses of indicated transcription factors and kinesin motor protein in *Ide* KO (n=3 mice) and *Ide* WT (n=3 mice) islets. (*B*) Representative immunohistochemical staining of pancreatic sections showing insulin vesicles in green (n=3 mice per genotype). Scale bar is 10µm. (*C*) Proinsulin/insulin ratio determined from pancreas extracts isolated from *Ide* KO (n=4) and *Ide* WT (n=4) mice. (*D*) Representative immunohistochemical LC3 staining in 24 hrs starved mice (n=3 mice per genotype). Scale bar is 10 µm. (*E*) Real-time PCR analyses of indicated autophagic genes in *Ide* KO and *Ide* WT islets (n=3 mice per genotype). Data are presented in (*A*, *B* and *E*) as mean +/- s.e.m..

SUPPLEMENTARY DATA

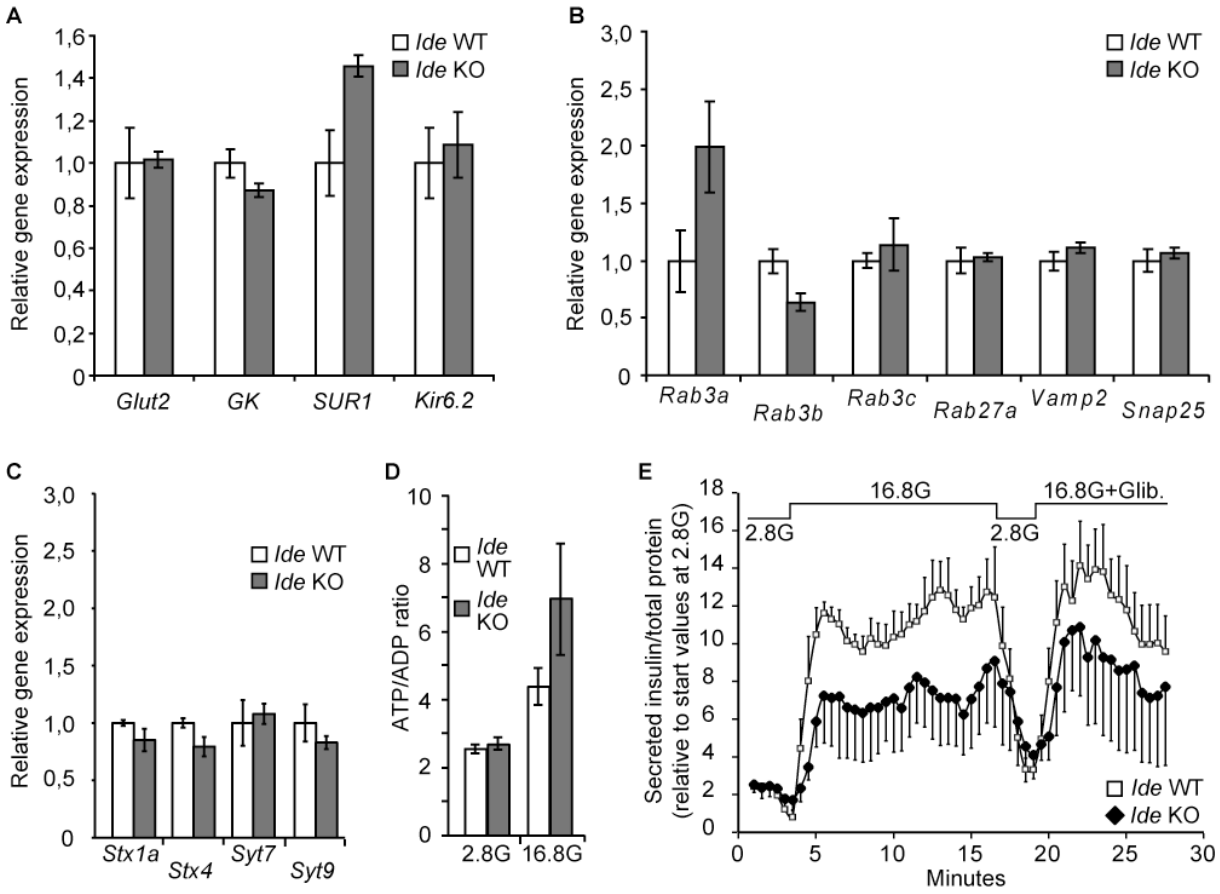

Fig. S3. Real-time RT-PCR analyses on genes involved in (*A*) glucose uptake, glucose metabolism and membrane depolarization and (*B* and *C*) granule exocytosis in islets isolated from *Ide* WT (n=3) and *Ide* KO (n=3) mice. (*D*) ATP/ADP ratio in response to glucose stimulation in islets isolated from *Ide* WT (n=3) and *Ide* KO (n=3) mice. (*E*) Insulin secretion profiles of perifused *Ide* WT (n=4 mice) and *Ide* KO (n=4 mice) islets stimulated with 16.8 mM glucose for 12 minutes, followed by 2.8 mM glucose for 3 minutes, and then stimulated with 16.8 mM glucose plus 100 nM glibenclamide for an additional 10 minutes. Data are presented as mean +/- s.e.m..

SUPPLEMENTARY DATA

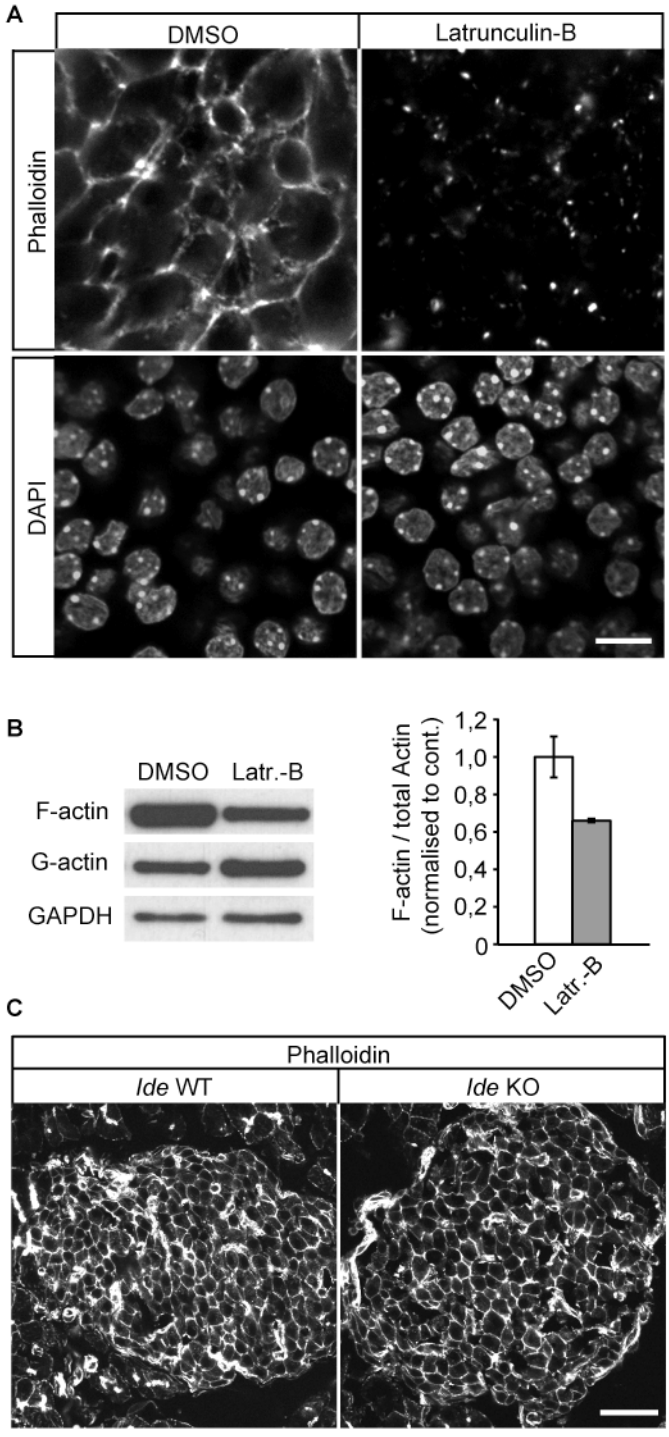

Fig. S4. Effect of the actin depolymerization drug latrunculin-B.
(*A*) Isolated wild type islets incubated with 10 µM latrunculin-B for 2 hours were stained with phalloidin and to visualize actin filaments and nuclei, respectively. (*B*) Immunoblot and quantification analyses of cytosolic (G-actin) and membrane fractions (F-actin) of isolated islets incubated as in (*A*). (*C*) Actin visualized by phalloidin staining in islets from *Ide* WT and *Ide* KO pancreas. Scale bar in (*A*) is 20µm and in (C) 40 µm. Data in (B) are represented as mean +/- s.e.m.

SUPPLEMENTARY DATA

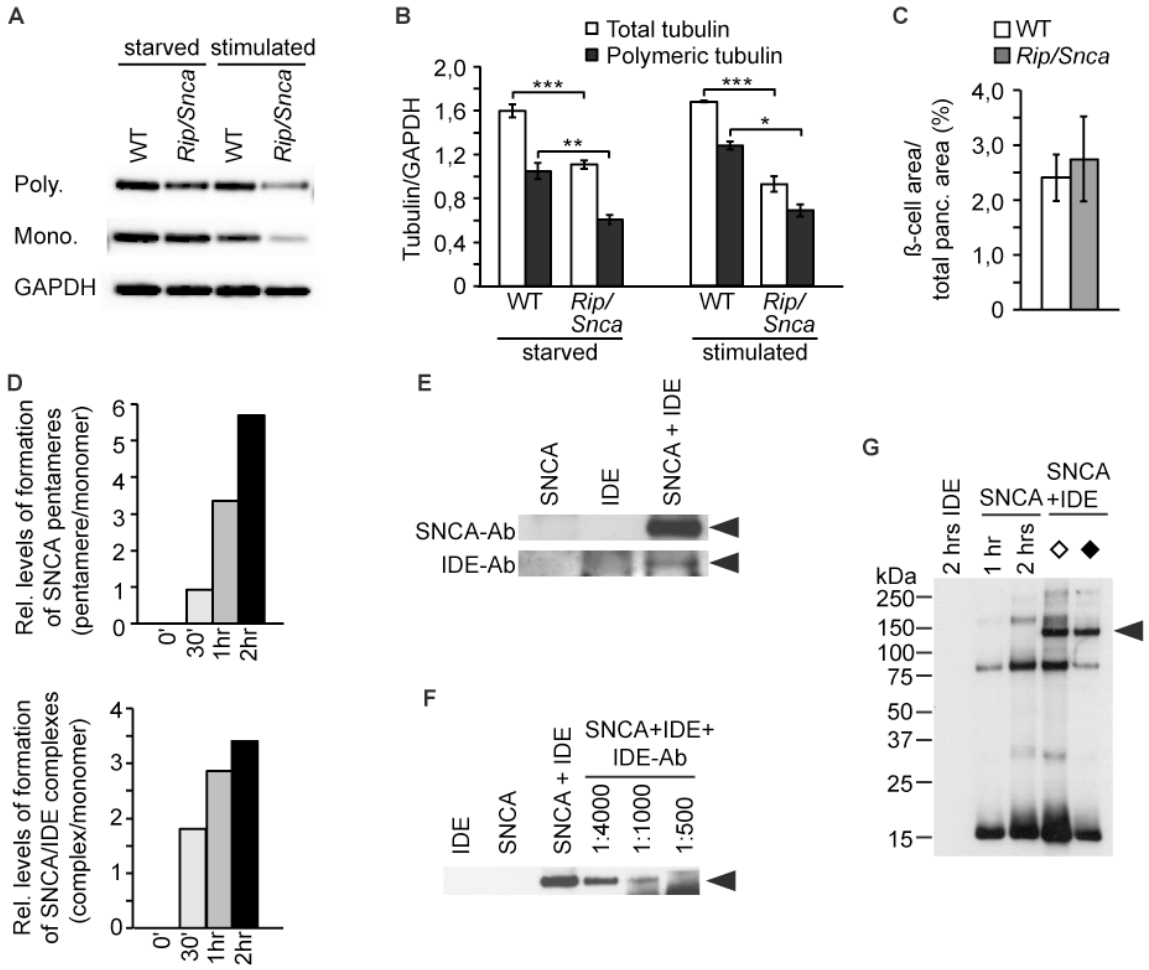

Fig. S5. Reduced tubulin levels in *Rip/Snca* islets and SNCA/IDE interaction *in vitro*. (*A*) Representative immunoblot and (*B*) quantification analyses of tubulin levels in WT (n=6 mice) and *Rip/Snca* (n=6 mice) islets. (*C*) Quantification of ß-cell area in WT (n=3 mice) and *Rip/Snca* (n=3 mice) pancreas. (*D*) Quantification of levels of SNCA pentamer and SNCA/IDE complex formation of immunoblot shown in Fig. 5*E*. (*E*) Immunoblot of *in vitro* SNCA/IDE complexes detected by SNCA and IDE antibodies (arrowheads). (*F*) *In vitro* competition assay where increasing amounts of IDE antibody suppress formation of SNCA/IDE complexes (arrowhead). (*G*) Immunoblot of SNCA oligomers where IDE has been added to the incubation from start (black diamond) or after 1 hour (open diamond). Arrowhead indicates SNCA/IDE complex. Data in (*B* and *C*) are presented as mean values +/- s.e.m. where in (B) *P<0.05; **P<0.01; ***P<0.001 (Students t-test).

SUPPLEMENTARY DATA

Supplementary references

1. Delledonne A, Kouri N, Reinstatler L, Sahara T, Li L, Zhao J, Dickson DW, Ertekin-Taner N, Leissring MA: Development of monoclonal antibodies and quantitative ELISAs targeting insulin-degrading enzyme. Mol Neurodegener 2009;4:39

2. Steneberg P, Rubins N, Bartoov-Shifman R, Walker MD, Edlund H: The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. Cell Metab 2005;1:245-258

3. Hu JY, Chu ZG, Han J, Dang YM, Yan H, Zhang Q, Liang GP, Huang YS: The p38/MAPK pathway regulates microtubule polymerization through phosphorylation of MAP4 and Op18 in hypoxic cells. Cell Mol Life Sci 2010;67:321-333