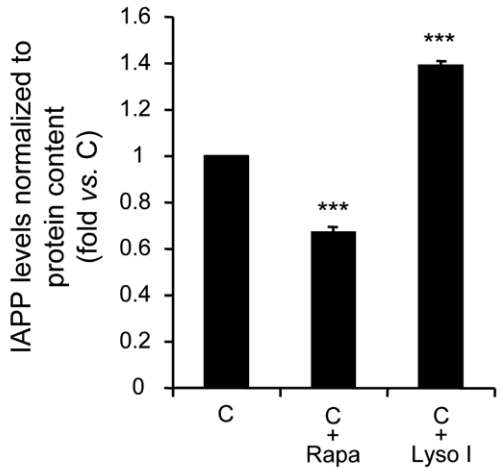
SUPPLEMENTAL TABLE

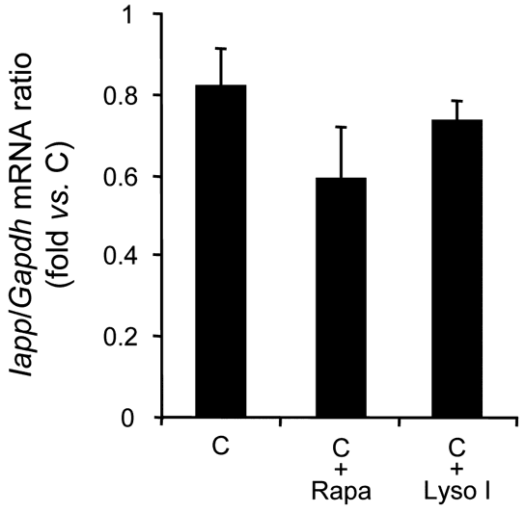
Age (weeks)	6-7	8-9	10-11	12-13	14-15
Control	15	15	14	10	6
h-IAPP$^{+/-}$	15	15	13	12	8
Atg7$^{\Delta\beta cell}$	7	7	3	3	3
h-IAPP$^{+/-}$:Atg7$^{\Delta\beta cell}$	7	7	7	4	3

Supplemental Table 1. Number of mice followed per group at given age. Mice used for measurements of fasting blood glucose (Figure 6A).

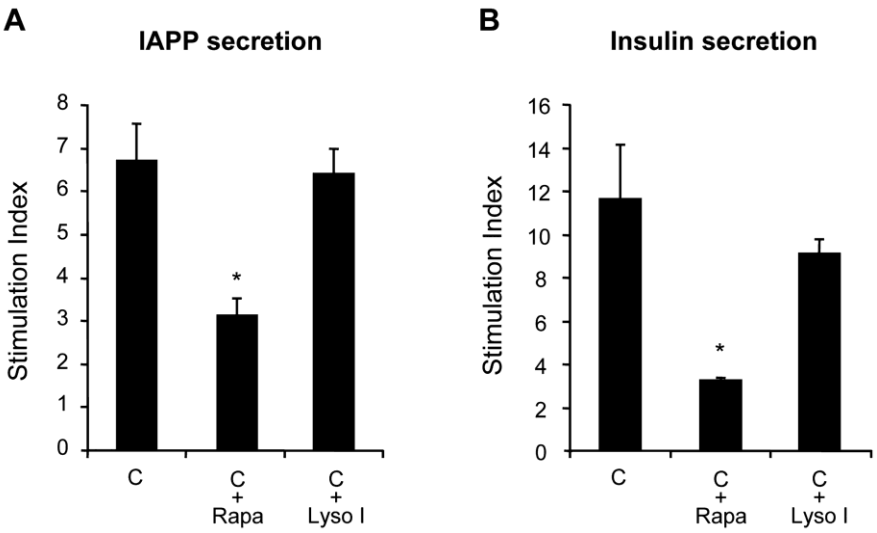
SUPPLEMENTAL FIGURES



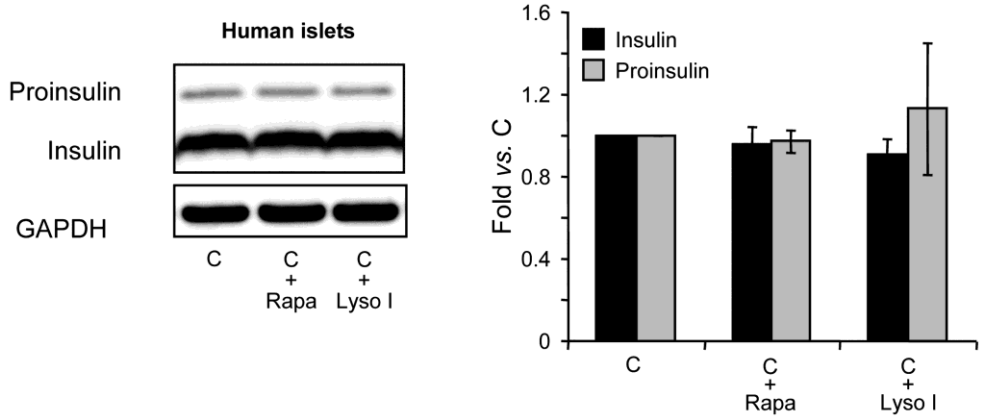
<u>Supplemental Figure 1.</u> Intracellular IAPP content in INS 832/13 cells treated with regulators of autophagy. INS 832/13 cells were treated with rapamycin (Rapa, 10 nM) for 40 h, or lysosomal inhibitors (Lyso I) (E-64-d, 10 μg/ml and pepstatin A, 10 μg/ml) for 24 h, or left untreated (C). Using cell lysates, levels of IAPP were assessed by enzyme immunoassay (rat/mouse amylin EIA kit, Phoenix Pharmaceuticals, EK-017-11) and normalized to protein content (n=3). Data are expressed as mean ± S.E.M.; ***P<0.001, significant differences *versus* C.



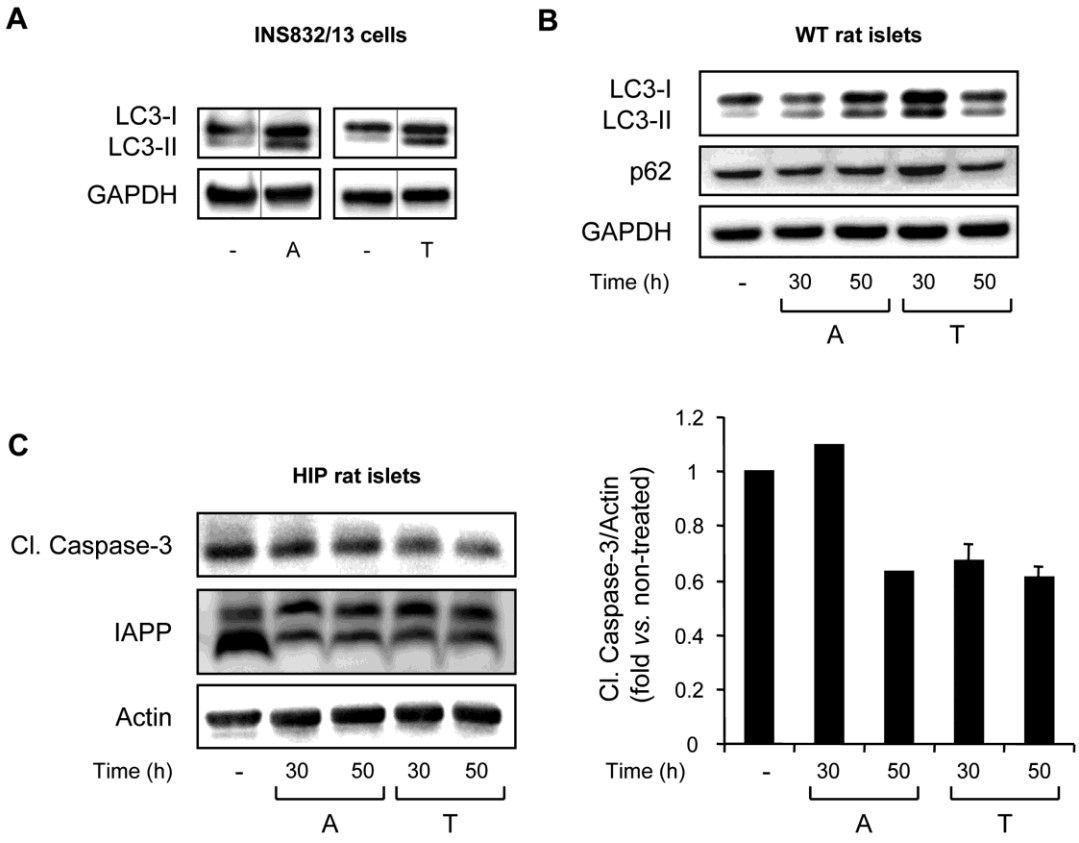
<u>Supplemental Figure 2.</u> *Iapp* mRNA levels in beta-cells treated with regulators of autophagy. Levels of *Iapp* mRNA were evaluated by RT-qPCR in INS 832/13 cells treated with rapamycin (Rapa, 10 nM) for 40 h, or lysosomal inhibitors (Lyso I) (E-64-d, 10 μg/ml and pepstatin A, 10 μg/ml) for 24 h, or left untreated (C). *Gapdh* was used as housekeeping gene. Data are expressed as mean ± S.E.M (n=3).



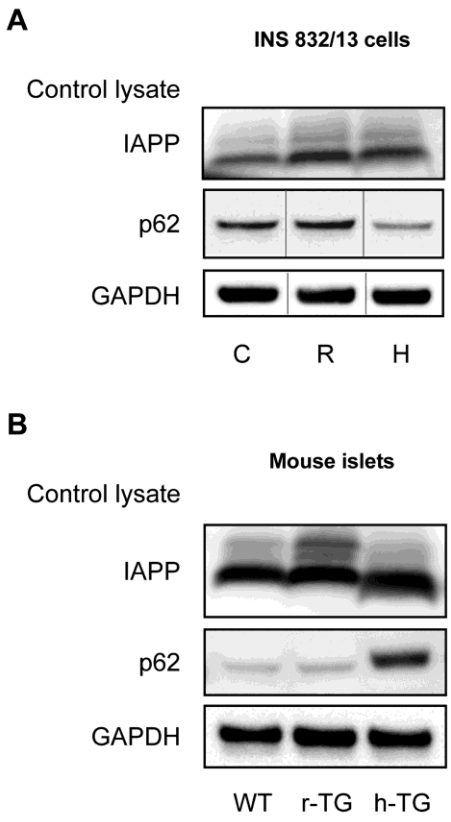
Supplemental Figure 3. IAPP and insulin secretion in beta-cells treated with regulators of autophagy. INS 832/13 cells were treated with rapamycin (Rapa, 10 nM) for 40 h, or lysosomal inhibitors (Lyso I) (E-64-d, 10 μg/ml and pepstatin A, 10 μg/ml) for 24 h, or left untreated (C). To assess glucose-stimulated IAPP and insulin secretion, cells were preincubated for 2 h in Krebs Ringer Buffer (KRB) containing 0.5% BSA and 2.8 mmol/l glucose, and subjected to two successive 1-h incubations with 2.8 (basal) and 16.7 (stimulation) mmol/l glucose. IAPP was measured using an enzyme immunoassay (rat/mouse amylin EIA kit, Phoenix Pharmaceuticals, EK-017-11) **(A)**. Insulin was measured using competitive colorimetric enzyme-linked immunosorbent assay (rat insulin ELISA, Alpco, 80-INSRT-E01) (**B**). The stimulation index was defined as the ratio of stimulated to basal secretion (n=3). Data are expressed as mean ± S.E.M.; **P*<0.05.



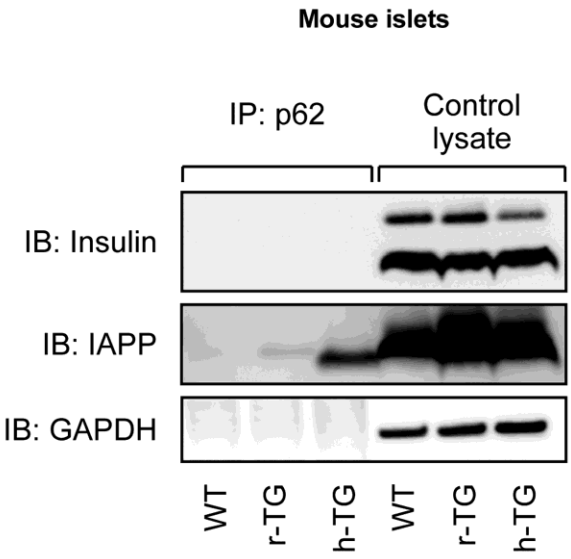
<u>Supplemental Figure 4.</u> Intracellular insulin content in human islets treated with regulators of autophagy. Human islets were treated with rapamycin (Rapa, 10 nM), or lysosomal inhibitors (Lyso I) (E-64-d, 10 μg/ml and pepstatin A, 10 μg/ml) for 30 h, or left untreated (C). Intracellular protein levels of insulin were assessed by western blot. GAPDH was used as loading control. The graph represents the quantification of insulin protein levels (n=3 for LI, n=2 for Rapa). Data are expressed as mean ± S.E.M.



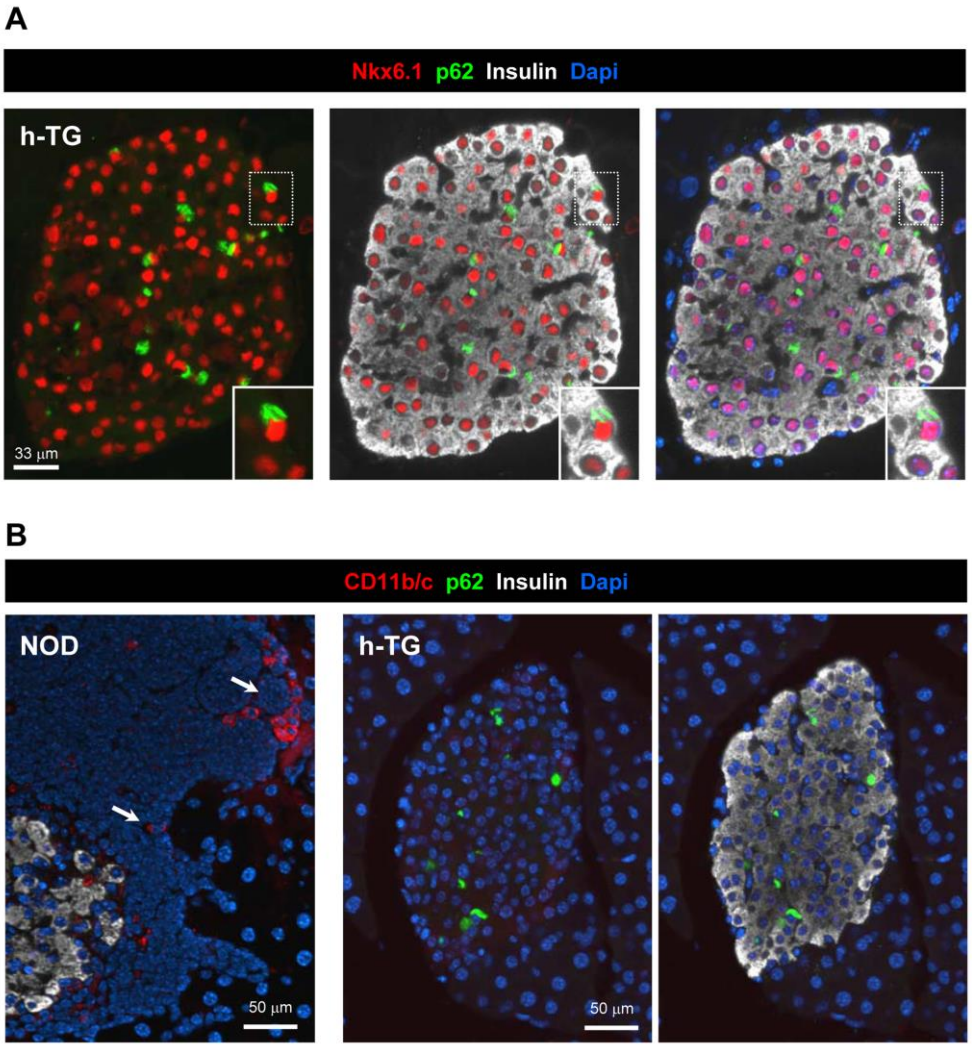
<u>Supplemental Figure 5.</u> Treatment with enhancers of lysosomal degradation decreases IAPP levels and cleaved caspase-3 in HIP rat islets. (**A**) INS 832/13 cells were treated with amiodarone (A, 5 µM) or trifluoperazine (T, 5 µM) for 42 h. Levels of LC3-II were assessed by western blot. GAPDH was used as loading control. For LC3 and GAPDH images, lanes were run on the same gel but were noncontiguous. (**B**) Islets were isolated from 4 to 6 month-old control rats and treated or not with amiodarone (A, 5 µM) or trifluoperazine (T, 5 µM) for the indicated time. Levels of LC3-II and p62 were assessed by western blot. GAPDH was used as loading control. (**C**) Islets were isolated from 4 to 6 month-old HIP rats and treated or not with amiodarone (A, 5 µM) or trifluoperazine (T, 5 µM) for the indicated time. Levels of cleaved caspase-3 (Cl. Caspase-3) were assessed by western blot. Actin was used as loading control. The graph represents the quantification of Cl. Caspase-3 protein levels (n=2). Data are expressed as mean ± S.E.M.



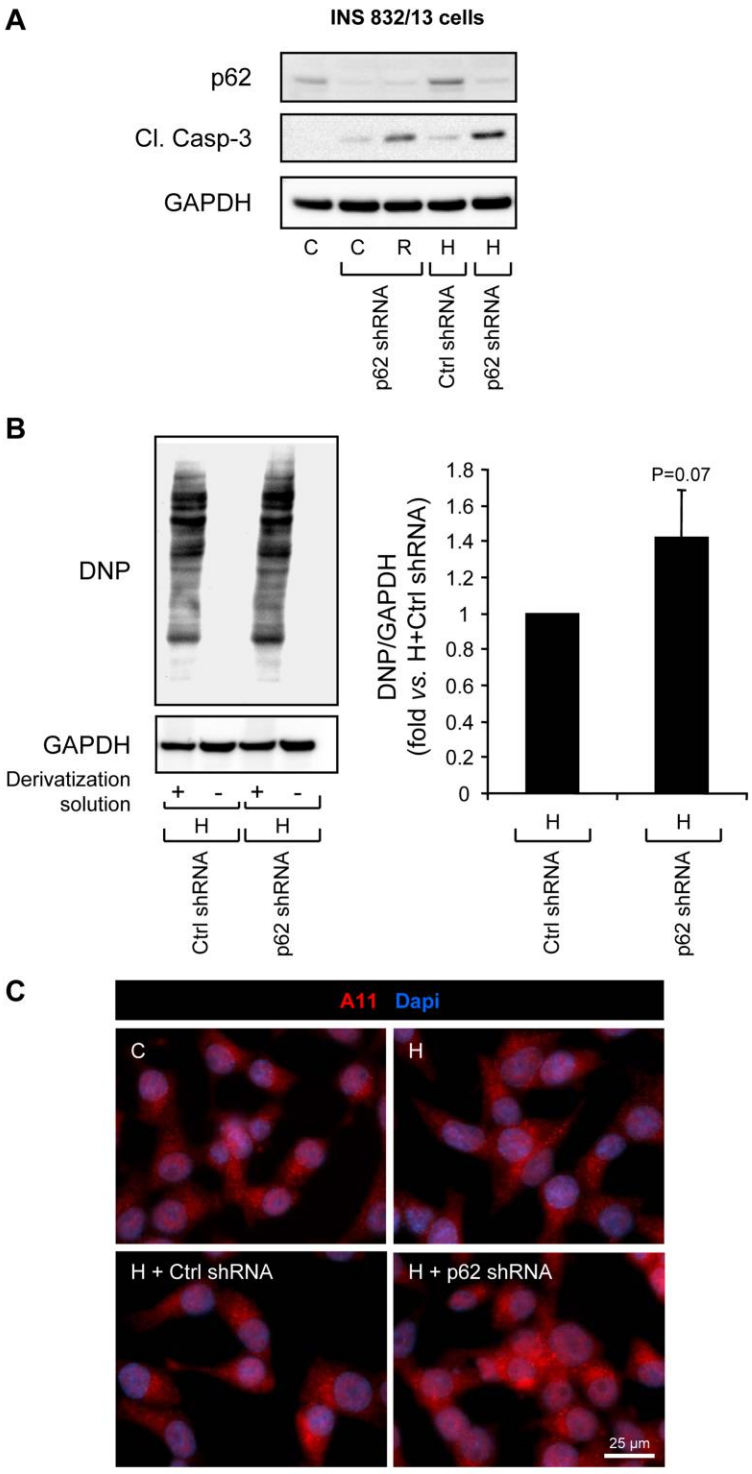
<u>Supplemental Figure 6.</u> IAPP and p62 protein levels in cell lysates used for immunoprecipitation. (**A**) INS 832/13 cells were transduced at 400 MOI for 36 h with r-IAPP (R) or h-IAPP (H) adenoviruses (C, non-transduced cells). Protein levels of IAPP and p62 were analyzed by western blot. GAPDH was used as loading control. For p62 and GAPDH images, lanes were run on the same gel but were noncontiguous. (**B**) Islets were isolated from 9 to 10 week-old wild type (WT), r-IAPP (r-TG) and homozygous h-IAPP transgenic mice (h-TG). Protein levels of IAPP and p62 were analyzed by western blot. GAPDH was used as loading control.



Supplemental Figure 7. IAPP specifically interacts with p62 in beta-cells. Islets were isolated from 9 to 10 week-old wild type (WT), r-IAPP (r-TG) and homozygous h-IAPP transgenic mice (h-TG) and lysed with NP-40 lysis buffer. Islet lysates were subjected to immunoprecipitation (IP) with anti-p62 antibody. Immunoprecipitated proteins and control lysates were resolved by SDS-PAGE and immunoblotted (IB) with anti-IAPP, anti-insulin and anti-GAPDH antibodies.

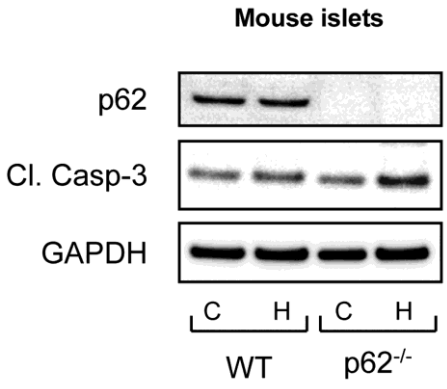


<u>Supplemental Figure 8.</u> p62-positive cells are beta-cells. (A) Nkx6.1, p62 and insulin levels were assessed by immunofluorescence in pancreatic sections from h-TG mice (Nrf2, red; p62, green; insulin, white; nuclei, blue). Insert in image represents a higher magnification of boxed area. **(B)** CD11b/c, p62 and insulin levels were assessed by immunofluorescence in pancreatic sections from non-obese diabetic (NOD) mice (left panel) and h-TG mice (right panels) (CD11b/c, red; p62, green; insulin, white; nuclei, blue). Arrows indicate macrophages. The spleen from NOD and h-TG mice served as a positive control for CD11b/c staining (data not shown).

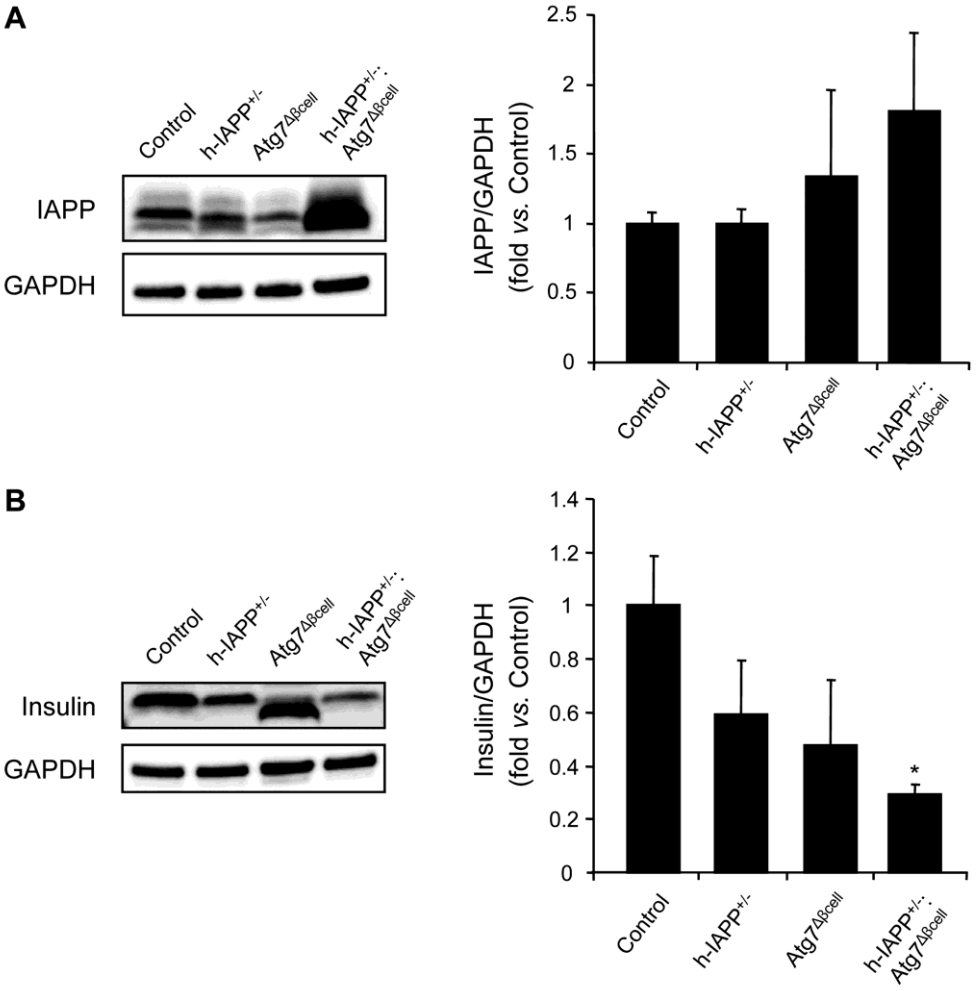


<u>Supplemental Figure 9</u>. p62 knockdown exacerbates h-IAPP toxicity by increasing apoptosis, oxidative stress and accumulation of toxic oligomers in INS 832/13 cells. INS 832/13 cells were transduced for 48 h with control shRNA or p62 shRNA lentivirus (0.1 µg/ml) and transduced at 400 MOI with r-IAPP (R) or h-IAPP (H) adenovirus for the last 27 h as indicated (C, non-transduced cells). Levels of p62 and cleaved caspase-3 (Cl. Casp-3) were

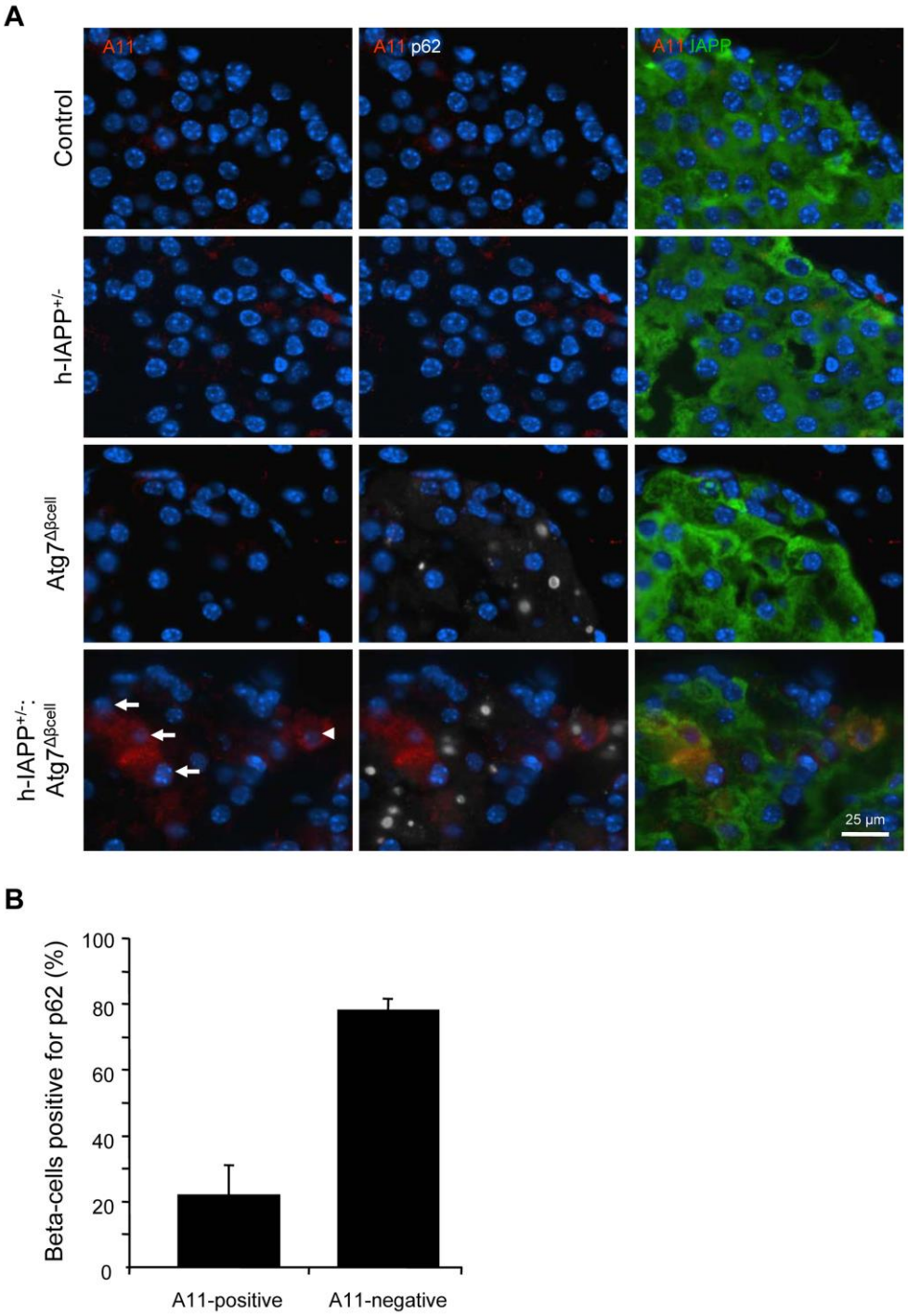
analyzed by western blot. GAPDH was used as loading control (**A**). Cell lysates were treated with dinitrophenylhydrazine (DNPH, derivatization solution (+)) to derivatize carbonyl groups to DNP-hydrazone, or control solution (-). Lysates were resolved by SDS-PAGE and immunoblotted with anti-DNP antibody and anti-GAPDH antibody for loading control. The graph represents the quantification of the DNP signal/smear. Data are expressed as mean ± S.E.M (n=4) (**B**). Oligomer presence was assessed by immunofluorescence using A11 antibody (A11, red; nuclei, blue) (**C**).



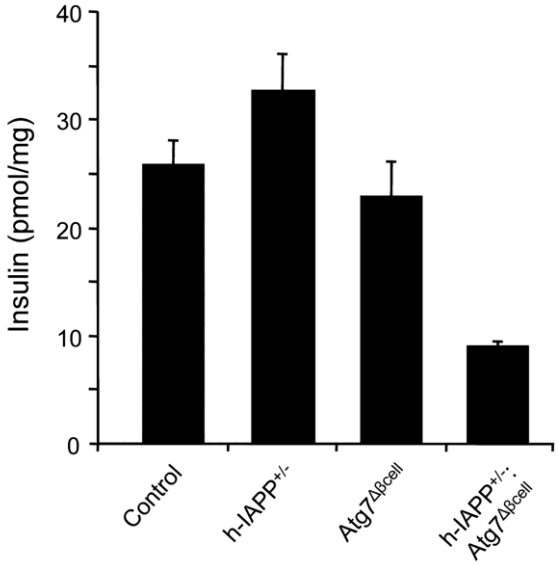
<u>Supplemental Figure 10.</u> Knockdown of p62 exacerbates h-IAPP-induced apoptosis in mouse islets. Islets isolated from 5 week-old wild type (WT) and $p62^{-/-}$ mice were transduced at 400 MOI for 72 h with h-IAPP (H) adenovirus (C, non-transduced islets). Levels of cleaved caspase-3 (Cl. Casp-3) and p62 were assessed by western blot. GAPDH was used as loading control.



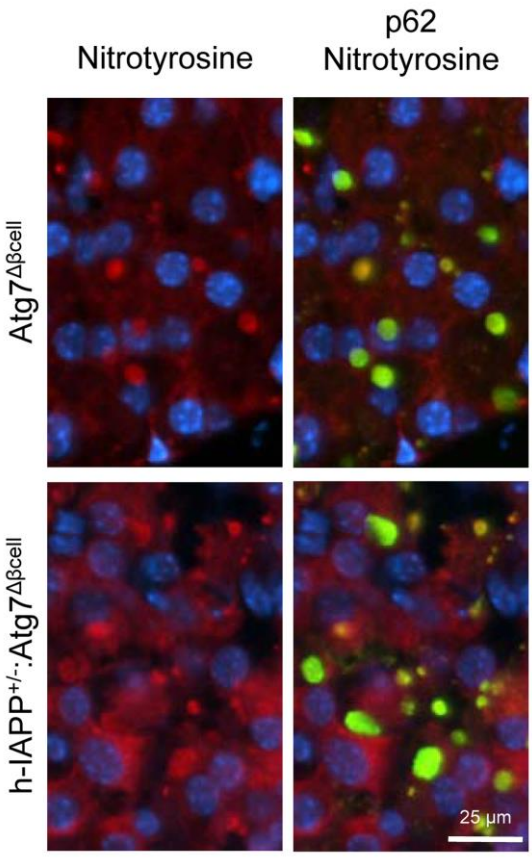
<u>Supplemental Figure 11.</u> Insulin and IAPP content in islets from autophagy-deficient mice expressing h-IAPP. (**A**) Protein levels of IAPP were assessed by western blot using islet lysates obtained from Control (9 weeks, n=6), h-IAPP$^{+/-}$ (9 weeks, n=5), Atg7$^{\Delta\beta cell}$ (9 weeks, n=3) and h-IAPP$^{+/-}$:Atg7$^{\Delta\beta cell}$ (9 weeks, n=6). GAPDH was used as loading control. (**B**) Protein levels of insulin were assessed by western blot using islet lysates obtained from Control (9 weeks, n=3), h-IAPP$^{+/-}$ (9 weeks, n=3), Atg7$^{\Delta\beta cell}$ (9 weeks, n=3) and h-IAPP$^{+/-}$:Atg7$^{\Delta\beta cell}$ (9 weeks, n=3). GAPDH was used as loading control. Data are expressed as mean ± S.E.M.; **P*<0.05, significant differences *versus* Control mice.



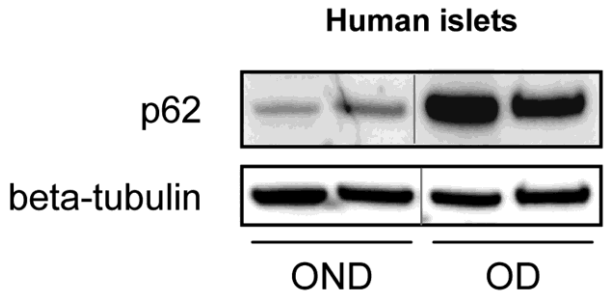
<u>Supplemental Figure 12.</u> Beta-cells positive for p62 aggregates are mostly negative for toxic oligomers. (**A)** Oligomer staining was assessed by immunofluorescence using A11 antibody in pancreatic sections from Control, h-IAPP$^{+/-}$, Atg7$^{\Delta\beta cell}$ and h-IAPP$^{+/-}$:Atg7$^{\Delta\beta cell}$ mice (A11, red; p62, white; IAPP, green; nuclei, blue). Images were acquired with a fluorescent microscope. Arrows depict cells positive for A11 and IAPP but not p62. Arrowhead points to a cell positive for A11, IAPP and p62. (**B**) Quantification of A11-positive and A11-negative beta-cells amongst all p62-labeled beta-cells in 15 week-old h-IAPP$^{+/-}$:Atg7$^{\Delta\beta cell}$ mice. Data are expressed as mean ± S.E.M.



<u>Supplemental Figure 13.</u> Pancreatic insulin content is decreased in autophagy-deficient mice expressing h-IAPP. Pancreatic insulin content of Control (9 weeks, n=3), h-IAPP$^{+/-}$ (9 weeks, n=3), Atg7$^{\Delta\beta cell}$ (9 weeks, n=2) and h-IAPP$^{+/-}$:Atg7$^{\Delta\beta cell}$ mice (9 weeks, n=2) assessed by mouse insulin ELISA. Values are given in picomoles per milligram of total tissue protein content. Data are expressed as mean ± S.E.M.



<u>Supplemental Figure 14.</u> p62 inclusions are positive for nitrotyrosine. Nitrotyrosine and p62 levels were assessed by immunofluorescence in pancreatic tissue from $Atg7^{\Delta\beta cell}$ and h-$IAPP^{+/-}$:$Atg7^{\Delta\beta cell}$ mice (nitrotyrosine, red; p62, green; nuclei, blue).



<u>Supplemental Figure 15.</u> p62 protein levels are increased in islets from obese subjects with T2D. p62 protein levels were analyzed by western blot in islets isolated from obese non-diabetic subjects (OND, n=2) and obese subjects with T2D (OD, n=2). Beta-tubulin was used as loading control. For p62 and beta-tubulin images, lanes were run on the same gel but were noncontiguous.

SUPPLEMENTAL METHODS

Detection of oxidatively-modified proteins. 5 µl of cell lysates (~15 µg of protein) obtained as previously described were mixed with 5 µl of 12% sodium dodecyl sulfate (SDS). Samples were then treated with 10 µl of 2,4-Dinitrophenylhydrazine (DNP) solution or 10 µl of control derivatization solution and incubated at room temperature for 15 min, after which 7.5 µl of neutralization solution was added. Proteins were then separated on a 4-12% Bis-Tris NuPAGE gel and transferred onto a polyvinylidene fluoride (PVDF) membrane (FluoroTrans; VWR), membrane was blocked in 1% bovine serum albumin (BSA) for 1 h. Membrane was incubated overnight with primary antibody (Rabbit anti-DNP, 1:150 in 1% BSA, Millipore), and then for 1 h at room temperature with Horseradish peroxidase (HRP)-conjugated secondary antibody (goat anti-rabbit, 1:300 in 1% BSA, Millipore). Proteins were visualized by enhanced chemiluminescence (Millipore) and protein expression levels were quantified using the Labworks software (UVP) using GAPDH (anti-GAPDH antibody, 1:1000, Cell Signaling) as loading control.