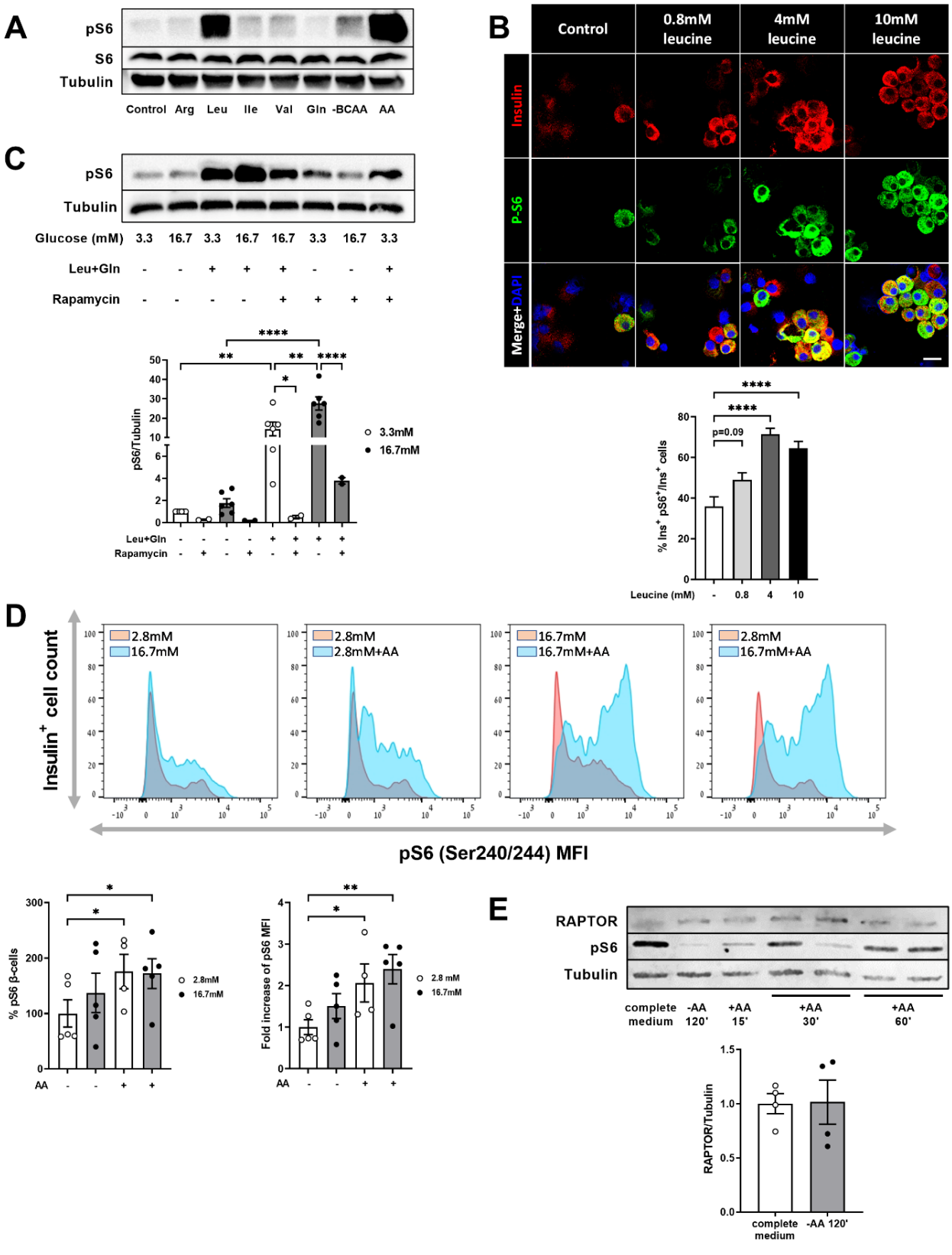
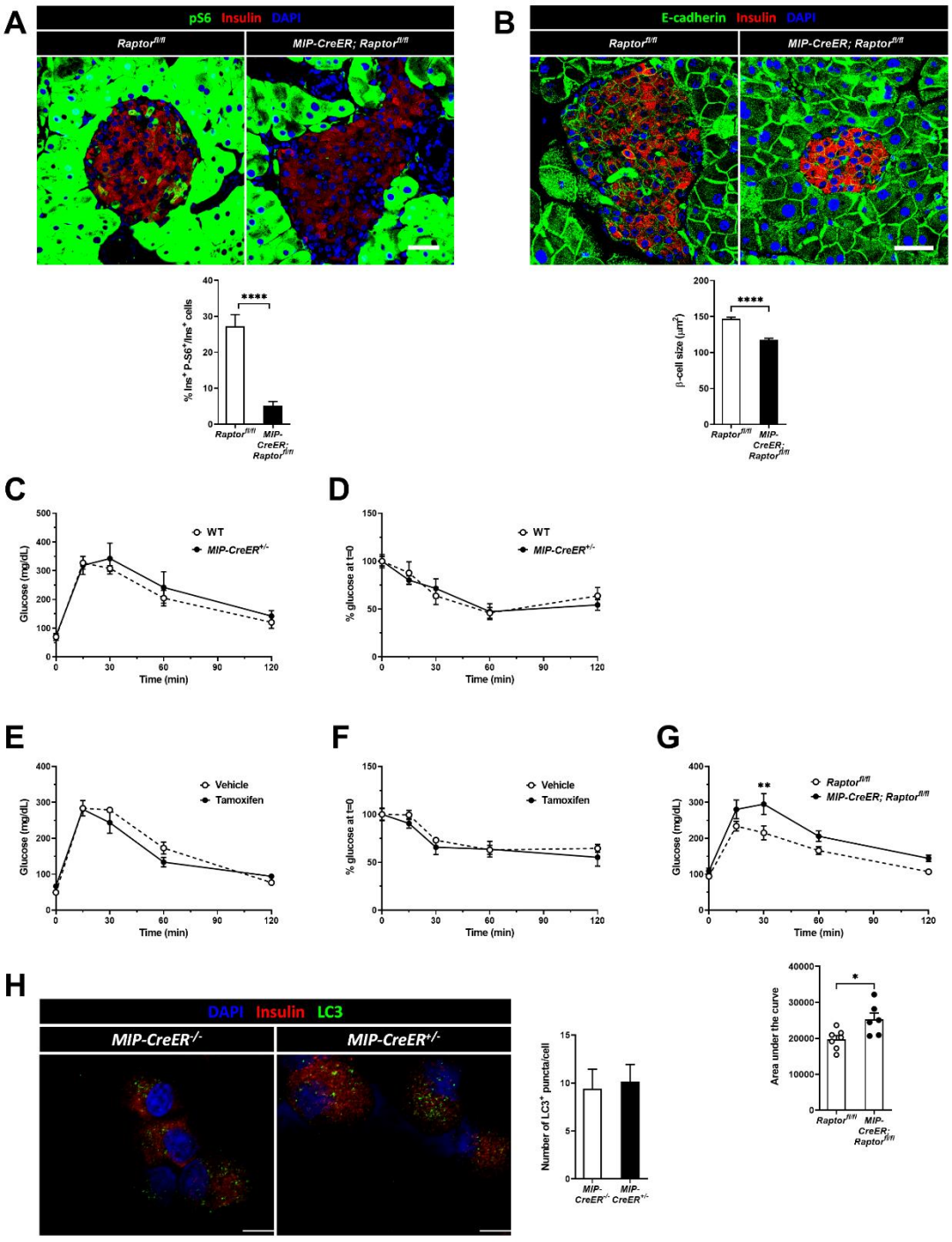
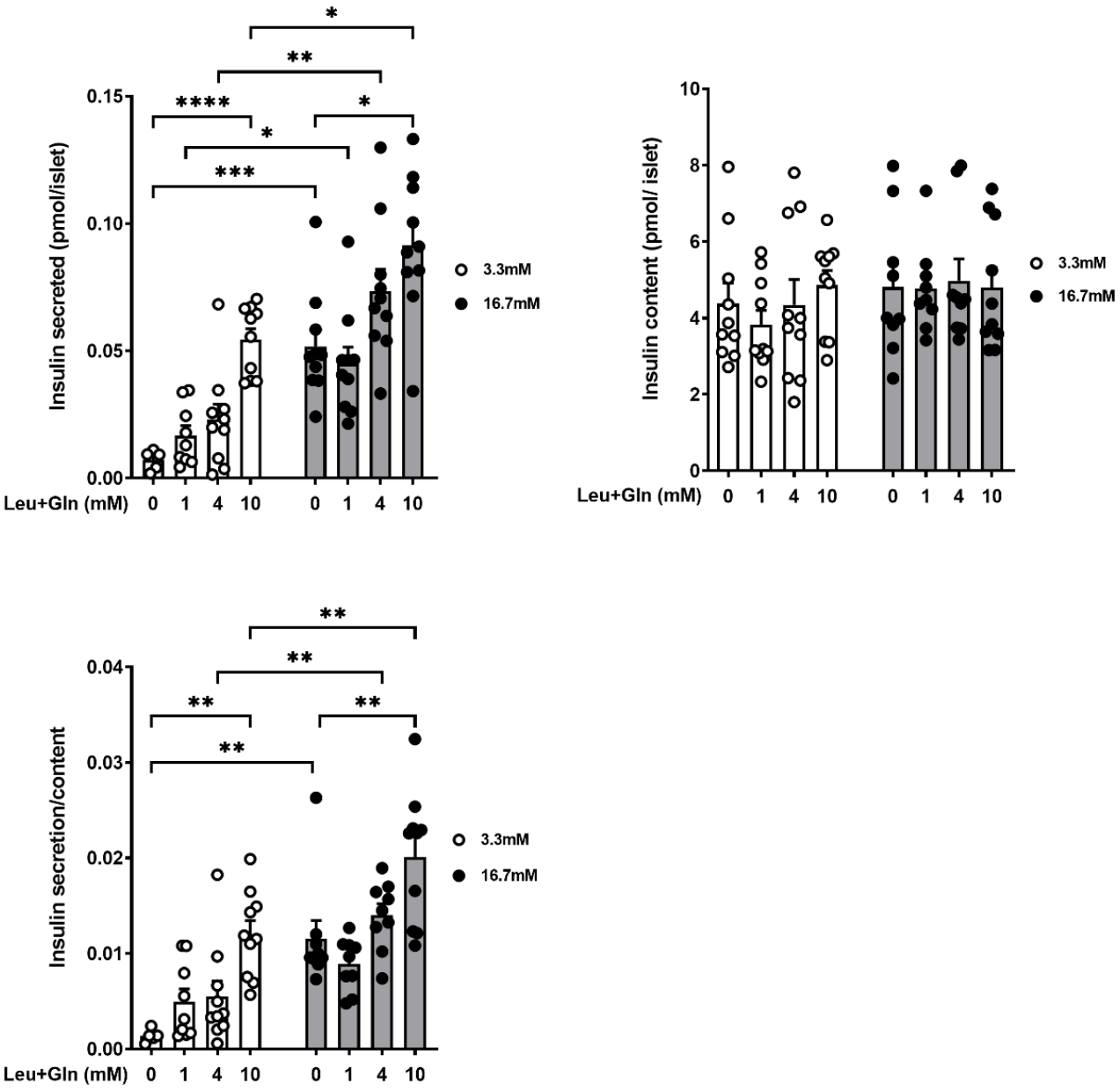

A
pS6
S6
Tubulin
Control Arg Leu Ile Val Gln -BCAA AA
B
Control
0.8mM leucine
4mM leucine
10mM leucine
Insulin
P-S6
Merge+DAPI
% Ins+ pS6+/Ins+ cells
p=0.09
Leucine (mM) - 0.8 4 10
C
pS6
Tubulin
Glucose (mM) 3.3 16.7 3.3 16.7 16.7 3.3 16.7 3.3
Leu+Gln
Rapamycin
pS6/Tubulin
3.3mM
16.7mM
D
Insulin+ cell count
2.8mM
16.7mM
2.8mM+AA
16.7mM+AA
pS6 (Ser240/244) MFI
% pS6 β-cells
Fold increase of pS6 MFI
AA
E
RAPTOR
pS6
Tubulin
complete medium
-AA 120'
+AA 15'
+AA 30'
+AA 60'
RAPTOR/Tubulin

Supplemental Figure 1: Regulation of mTORC1 by amino acids in INS-1 β-cells and islets. (**A**) INS-1 cells were preincubated for 30 min in AA-free RPMI at 3.3 mmol/l glucose without serum and then treated without or with the AAs arginine, leucine, isoleucine, valine, glutamine (10 mmol/l each), or in complete RPMI medium containing a mixture of all amino acids with or without BCAAs for 30 min followed by Western blotting for pS6, (Ser240/244), S6 and tubulin (n=2). (**B**) Islets were isolated from C57BL/6 mice, preincubated in AA-free RPMI with 3.3 mmol/l glucose with 10% FCS for 40 min and then treated with different concentrations of leucine for 30 min. Islets were dispersed and immunostained for insulin and pS6. Quantification of pS6 activity in β-cells is expressed as percentage of $pS6^+$/$insulin^+$ cells. Scale bar: 20 μm. n=4 separate experiments. (**C**) INS-1 cells were incubated at 3.3 or 16.7 mmol/l glucose with or without leucine + glutamine (10 mmol/l each) and rapamycin (100 nmol/l) for 1 h. pS6 (Ser240/244) was analyzed by Western blotting. Quantification is shown at the bottom; n=6. (**D**) Islets were treated at 2.8 or 16.7 mmol/l glucose with or without a mixture of all AAs for 30 min. mTORC1 activity, evident by S6 phosphorylation, was analyzed by flow cytometry, n=4. (**E**) Western blotting for RAPTOR and pS6 in mouse islets that were cultured in presence or absence of AAs for 2 h; AAs were added to starved cells for different periods of time; n=4.

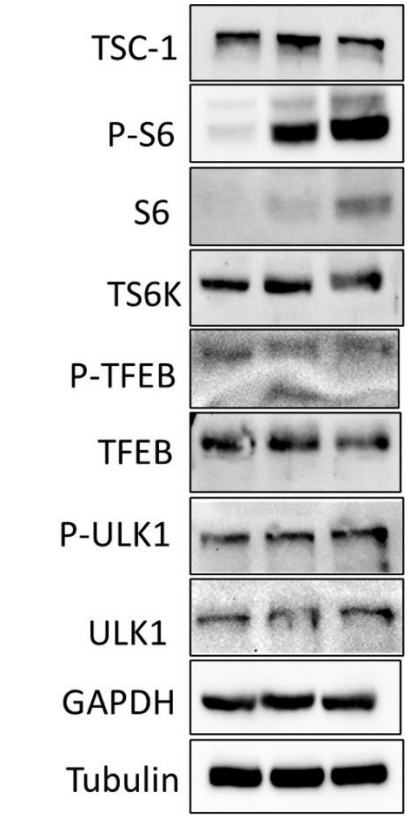


Supplemental Figure 2: Cre recombinase and tamoxifen did not affect glucose tolerance and insulin sensitivity. Tamoxifen-induced *Raptor* KO in β-cells leads to mild glucose intolerance. (**A-B**) Control (*Raptor*$^{fl/fl}$) and *MIP-CreER; Raptor*$^{fl/fl}$ mice were injected with tamoxifen. Two weeks later,

pancreases were harvested, fixed and immunostained for insulin and pS6 (**A**) or E-cadherin (**B**). (**A**) n=2 separate experiments, 1400-3500 cells per group were counted; (**B**) n=550 cells per group. Scale bar: 40 μm. (**C-D**) Wildtype (WT) and *MIP-CreER* mice were injected with tamoxifen, followed by IPGTT (C; 2 g/kg) and insulin tolerance test (D; 1 IU/kg, ITT) two weeks later, n=3 in each group. (**E-F**) *MIP-CreER* mice were injected with corn oil (vehicle) or tamoxifen, followed by IPGTT (**E**), and ITT (**F**), n=3 mice in each group. (**G**) Control *(Raptor*$^{fl/fl}$) mice and *MIP-CreER*; *Raptor*$^{fl/fl}$ mice were injected with tamoxifen, followed by IPGTT two weeks later, n=6-10 mice in each group. (**H**) Effects of the MIP-CreER construct on β-cell autophagy. Wildtype and MIP-CreER islets were incubated at 3.3 mmol/l glucose and 1% serum with bafilomycin A1 for 2 h and stained for LC3. n=3 mice, 430 to 530 cells were counted in each group, Scale bar: 10 μm

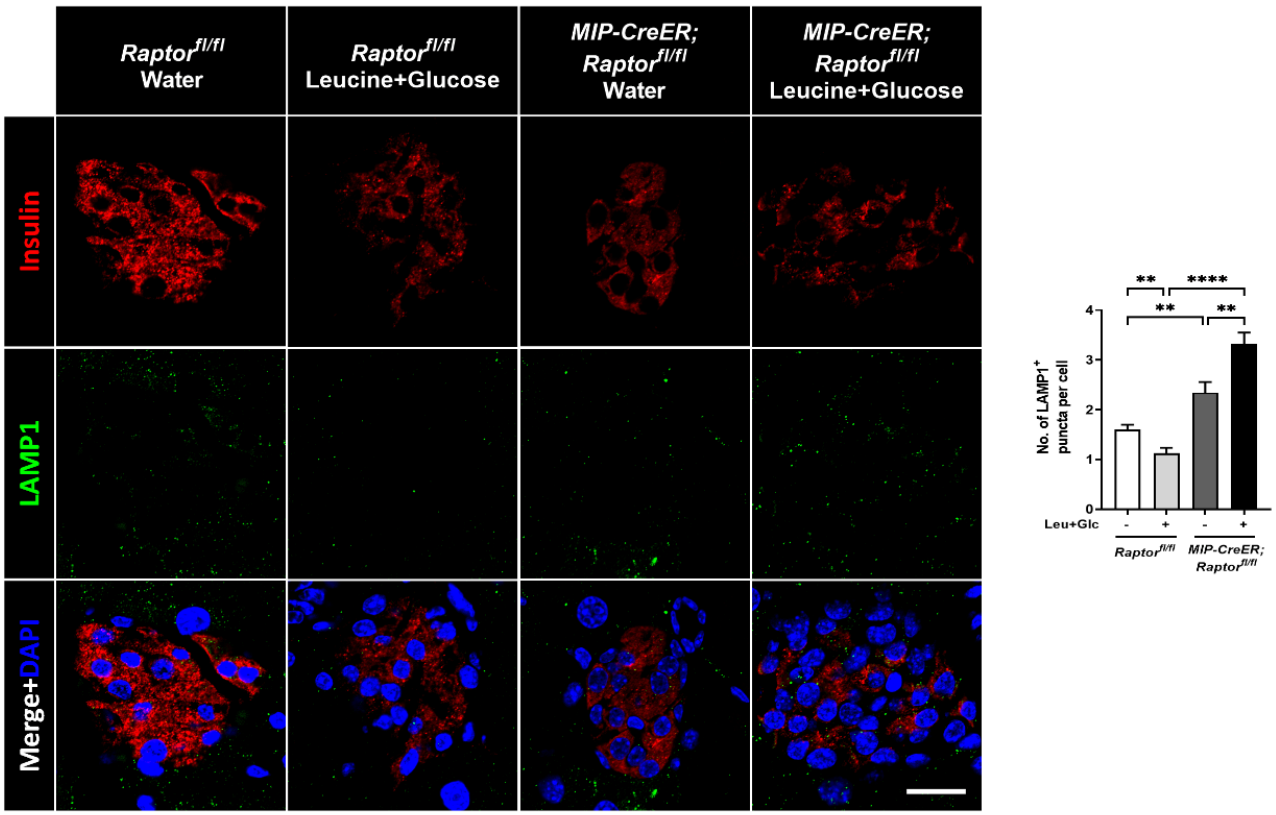


Supplemental Figure 3. Concentration-dependent amplification of insulin secretion by amino acids. Islets were preincubated in KRB medium and then treated with different concentrations of leucine and glutamine at 3.3 and 16.7 mmol/l glucose for 1 h. Insulin secretion and content were analyzed by ELISA. Results are means ± SEM of 3 separate experiments in quadruplicates.

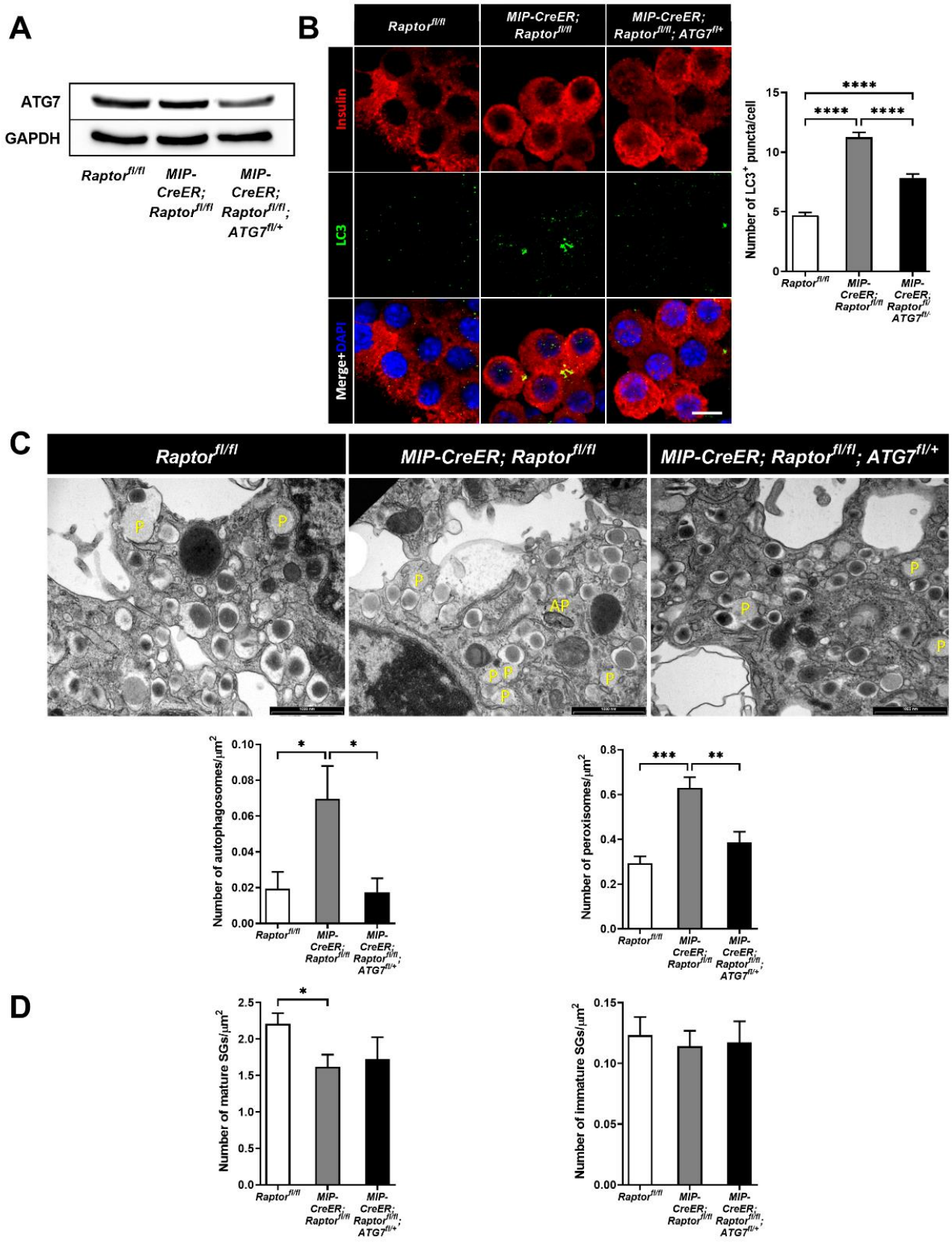


P-S6/Tubulin	1	6.35	13.29
S6/Tubulin	1	1.65	1.98
P-S6/S6	1	3.83	6.71
P-TFEB/GAPDH	1	0.89	0.92
TFEB/GAPDH	1	0.92	0.77
P-TFEB/TFEB	1	0.87	1.02
P-ULK1/GAPDH	1	1.2	1.94
ULK1/GAPDH	1	1.27	1.01
P-ULK1/ULK1	1	0.84	1.65

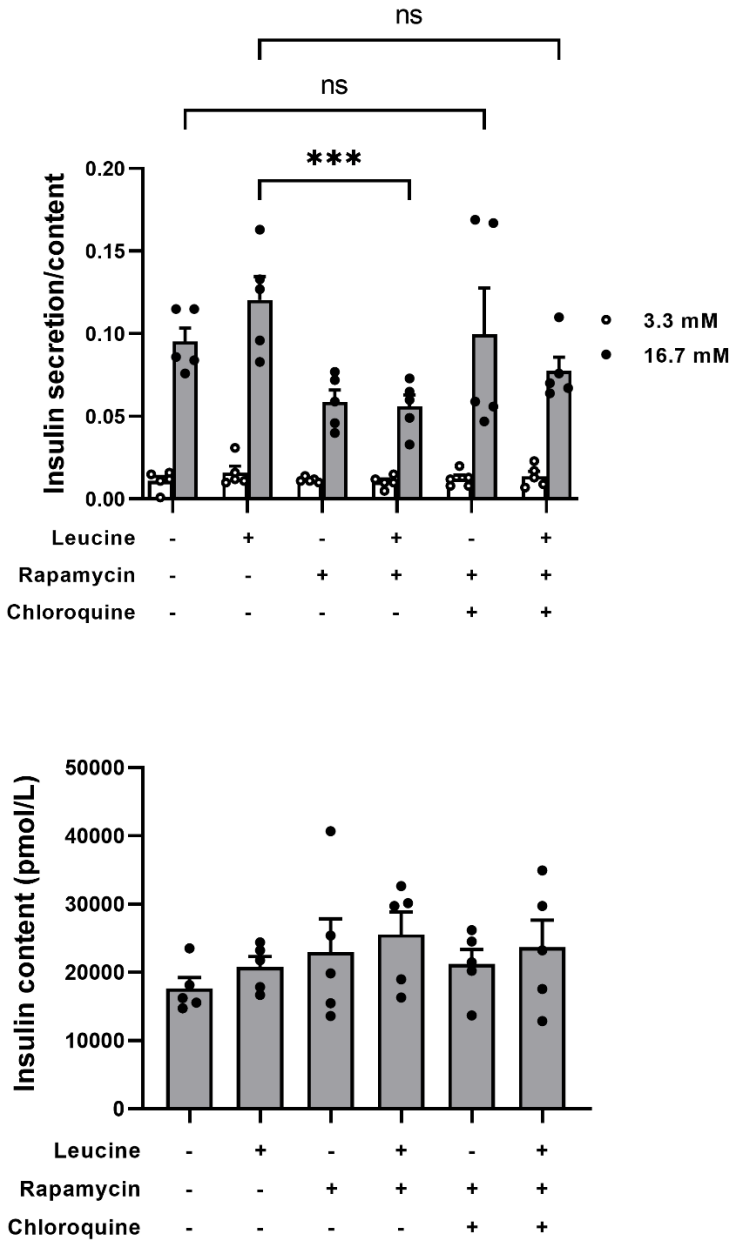
Supplemental Figure 4. Effects of leucine and glucose on the activation of mTORC1 and of autophagy regulators. Islets from 18 mice were isolated, pooled and incubated overnight in complete RPMI. Islets were pre-incubated at 3.3 mmol/l glucose in absence of AAs and serum and then incubated in medium containing 1% serum and 3.3 mmol/l glucose, 16.7 mmol/l glucose or 10 mmol/l leucine for 1 h. Islet extracts were analyzed by Western blotting for pULK1 (Ser757), ULK1, pTFEB (Ser122), TFEB, pS6 (Ser240/244), S6, S6K1, TSC1, GAPDH and tubulin. Quantifications of the blots are shown.



Supplemental Figure 5. Effects of nutrients and mTORC1 on lysosome number in β-cells. Control and β*Raptor* KO mice were fasted overnight and administered water or 2 g/kg glucose + 0.39 g/kg leucine by gavage, followed by removal of the pancreas 1 h later. Pancreatic sections were immunostained for insulin and LAMP1. The number of LAMP1$^+$ puncta in β-cells was quantified. Scale bar: 20 μm. n=2 separate experiments, 210-375 cells per treatment were counted.


A
ATG7
GAPDH
Raptor fl/fl
MIP-CreER; Raptor fl/fl
MIP-CreER; Raptor fl/fl; ATG7 fl/+
B
Raptor fl/fl
MIP-CreER; Raptor fl/fl
MIP-CreER; Raptor fl/fl; ATG7 fl/+
Insulin
LC3
Merge+DAPI
Number of LC3+ puncta/cell
****
****
****
C
Raptor fl/fl
MIP-CreER; Raptor fl/fl
MIP-CreER; Raptor fl/fl; ATG7 fl/+
P
AP
Number of autophagosomes/μm2
*
*
Number of peroxisomes/μm2
***
**
D
Number of mature SGs/μm2
*
Number of immature SGs/μm2

Supplemental Figure 6: Effects of inhibiting mTORC1 and of heterozygous *Atg7* KO on autophagy and β-cell ultrastructure. Islets were isolated from control (*MIP-CreER*), *βRaptor*$^{-}$ KO (*MIP-CreER; Raptor*$^{fl/fl}$) and *βRaptor* KO, *Atg7*$^{+/-}$ mice (*MIP-CreER; Raptor*$^{fl/fl}$*; ATG7*$^{fl/+}$). (**A**) Western blotting for ATG7 and GAPDH (n=2). (**B**) Dispersed islets were stained for insulin and LC3B followed by confocal microscope analysis and quantification of the number of LC3^{+} puncta in β-cells. Scale bar: 10 μm. n=3 separate experiments, 350-1050 cells per group were counted. (**C-D**) TEM analysis on islets from control (*Raptor*$^{fl/fl}$), *MIP-CreER; Raptor*$^{fl/fl}$ and *MIP-CreER; Raptor*$^{fl/fl}$*; Atg7*$^{fl/+}$ mice. Scale bar: 1000 nm (**C**). Quantifications of the number of autophagosomes and peroxisomes per cell and of mature and young secretory granules (**D**). n= 7-19 cells per pancreas from three mice. AP, autophagosome, P, peroxisome.



Supplemental Figure 7: Effects of acute exposure to nutrients, rapamycin and chloroquine (CQ) on insulin secretion in human islets. Islets were pre-incubated at 3.3 mmol/l glucose for 1 h and then incubated at 3.3 mmol/l glucose for 45 min followed by 16.7 mmol/l glucose with or without 10 mmol/l leucine, 100 nmol/l rapamycin and/or 10 μmol/l CQ for 45 min. Insulin secretion normalized to content is shown above and insulin content below.