

Supplementary Figures and Figure Legends, Burns et al.

A

Predicted Cleavage Site



WT INS-1

actctctcagactgagctATGG

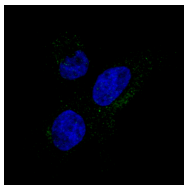
VPS41 KO

actctctcagactgagctATTTGG

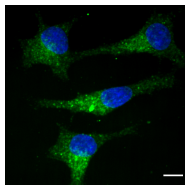
actctctcagactgagctAGTTGG

B

VPS41 KO



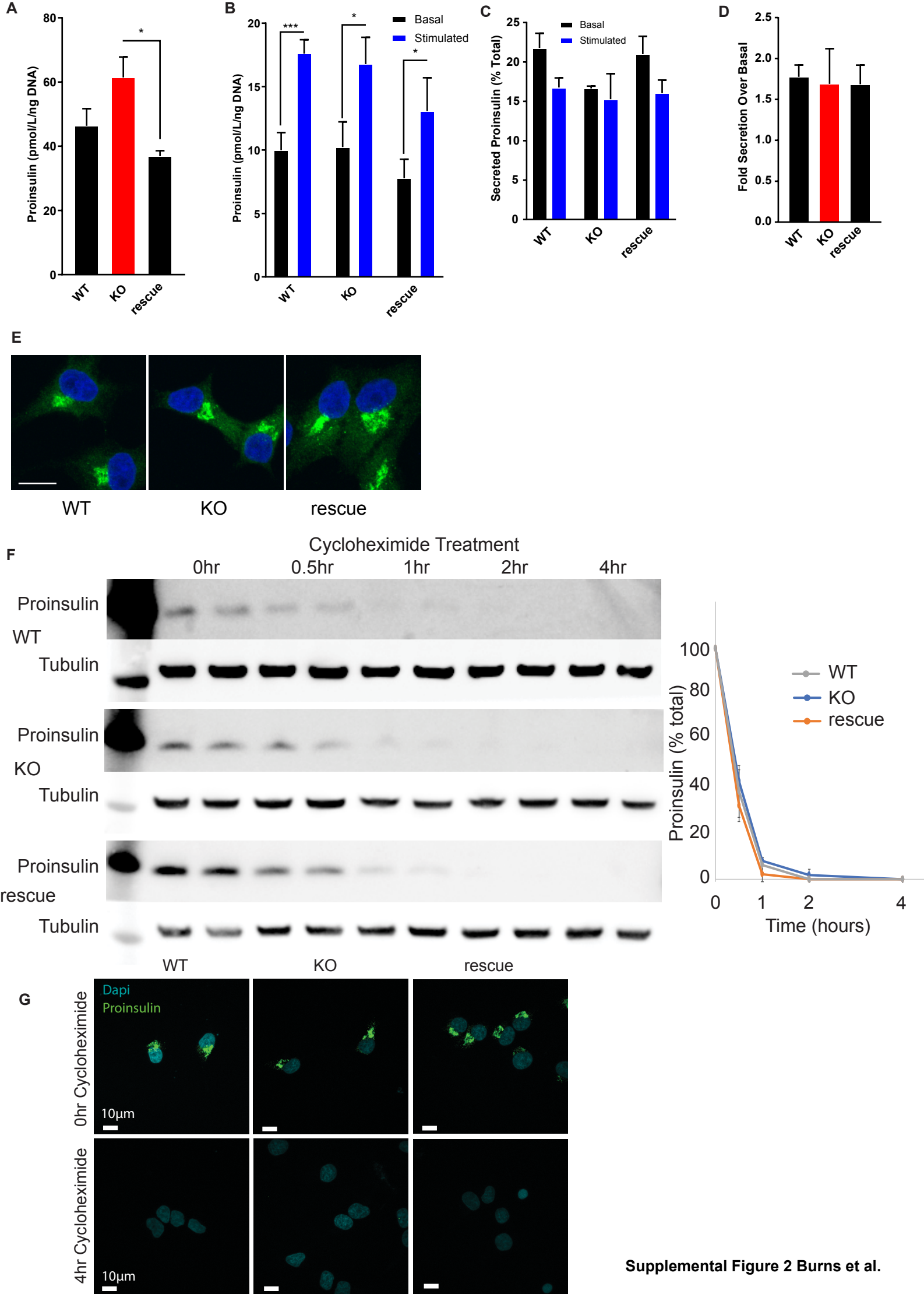
HA-VPS41



IF: HA

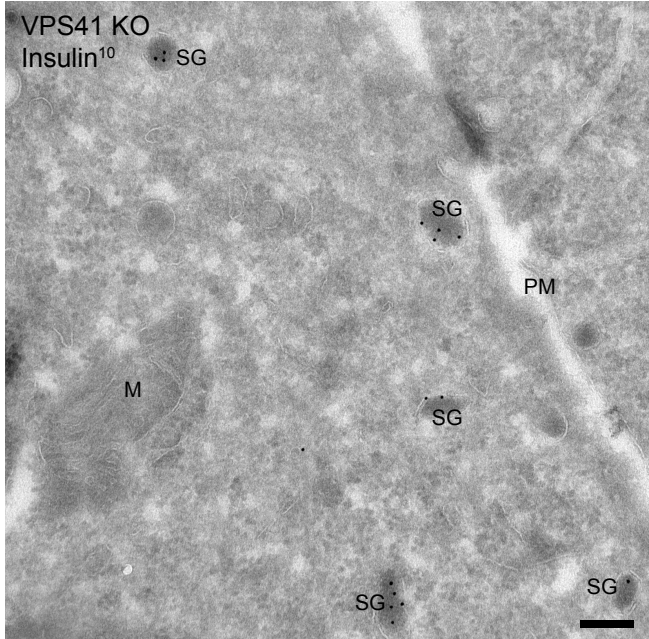
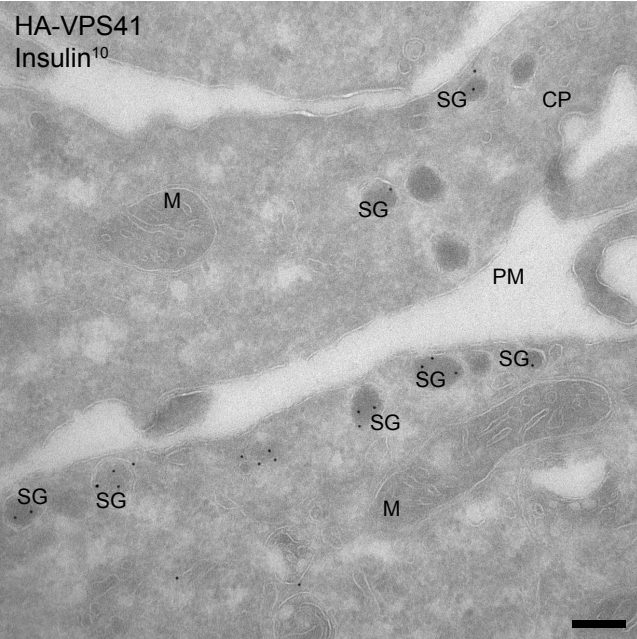
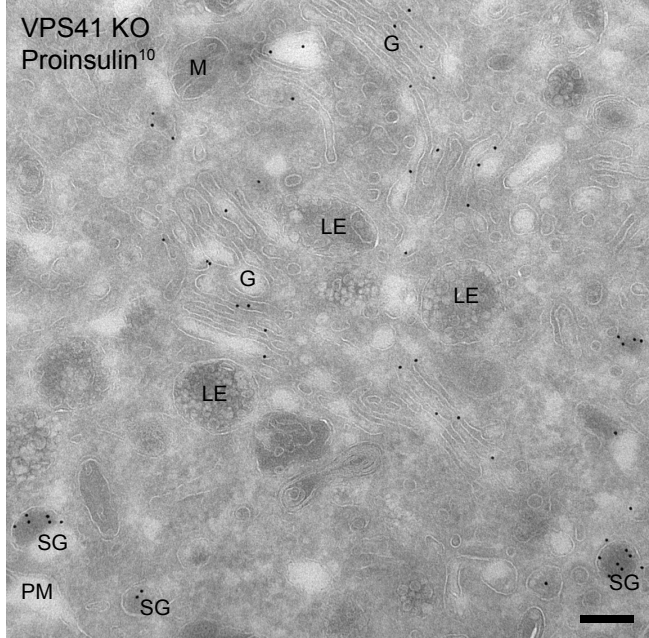
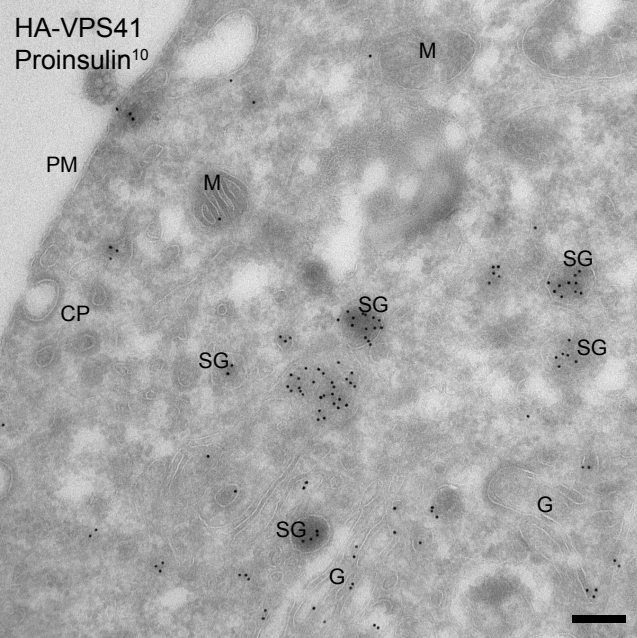
Supplementary Figure 1

(A) Predicted cleavage site for Cas9. Indels, both single base pair insertions, are shown in red in sequence disrupting the initiator ATG in Exon 1. (B) Representative images of VPS41 KO and HA-VPS41 rescue INS-1 cells immunostained for HA. Scale bar indicates 10 μ m.



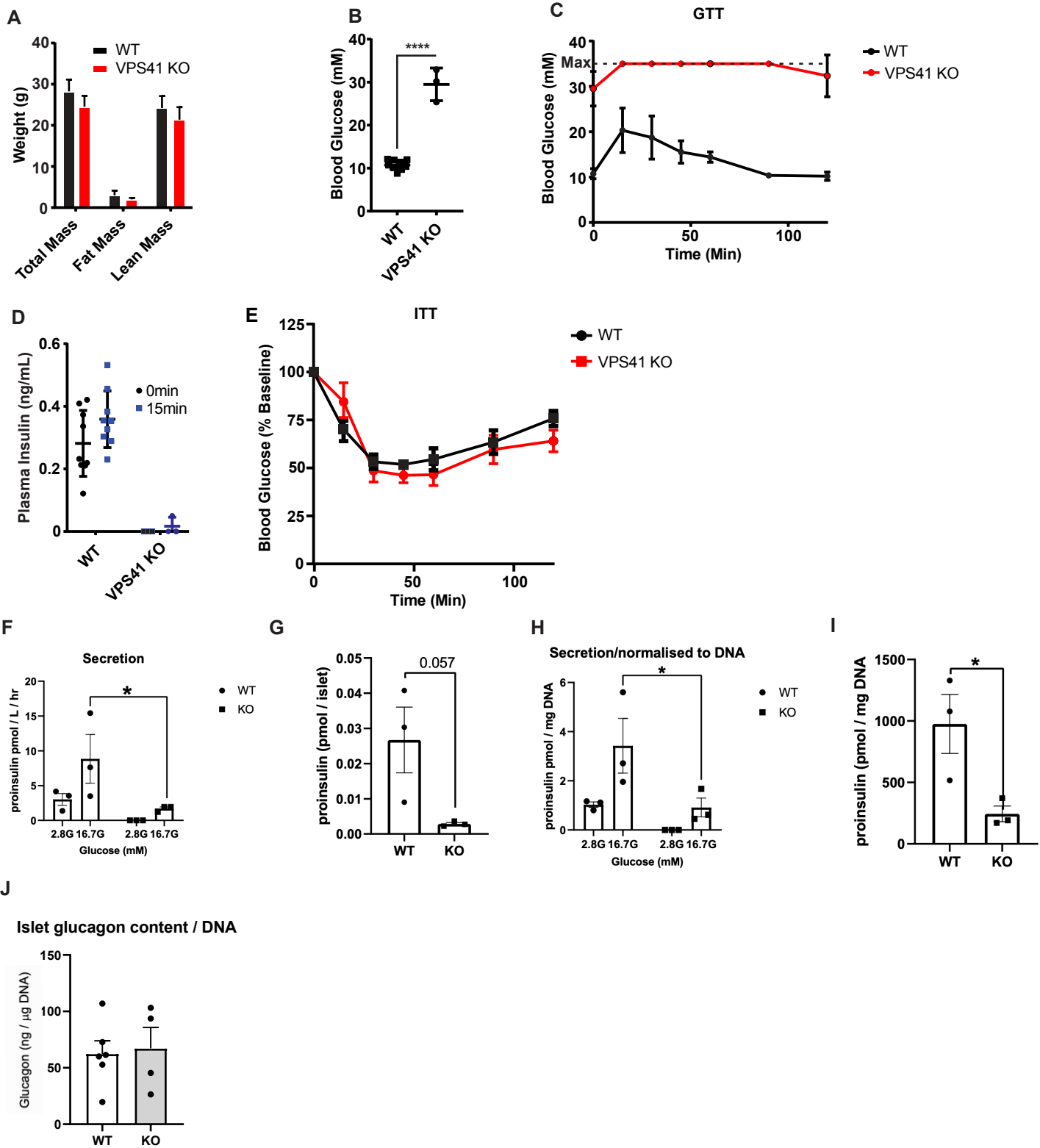
Supplementary Figure 2

(A) Total cellular proinsulin pool under basal conditions from VPS41 KO and rescue INS-1 was determined by ELISA. (B) Basal and stimulated proinsulin secreted from VPS41 KO and rescue INS-1 was determined by ELISA and analyzed by ANOVA. (C) Basal and stimulated proinsulin release from VPS41 KO and rescue INS-1 normalized to total cellular stores, analyzed by ANOVA. (D) Proinsulin secretion, fold stimulated release over basal release. Proinsulin cellular and secreted by ELISA. Data indicate mean \pm s.e.m.; $n=3$ independent experiments *: $p < 0.05$. ***: $p < 0.001$ analyzed by one way ANOVA. (E) Representative images of WT, VPS41 KO and rescue INS-1 cells stained for proinsulin (green) and DAPI (blue) by immunofluorescence. Scale bar indicates $10\mu\text{m}$. (F,G) Proinsulin levels at indicated time points after cycloheximide treatment were determined by western blot (F) and immunofluorescence (G). Western blot values for proinsulin were normalized to tubulin and expressed as percent of time = 0. The experiments were performed twice in duplicate.



Supplementary Figure 3

Immuno-electron microscopy of VPS41KO or HA-VPS41 expressing INS-1 cells. Proinsulin (top) and insulin (bottom) localize to the lumen of secretory granules in both VPS41 KO and rescue cells expressing HA-VPS41 indicating that depletion of VPS41 does not result in missorting of (pro)insulin. CP= Coated pit, G= Golgi, LE= Late endosome, M= Mitochondria, PM= Plasma membrane, SG= Secretory granule. Scale bars: 200nm.



Supplementary Figure 4

(A) Fat and lean mass measurements of age matched 15-week-old WT and VPS41 KO mice. (B) Blood glucose measurement of mice fasted for 8hrs. (C) Blood glucose measurements during glucose tolerance test (GTT). The dotted lines indicate the maximum value of the glucometer. (D) Circulating blood insulin levels before and 15 min after glucose injection. Data indicate mean \pm s.e.m.; KO n=3, WT n=9 ****: $p < 0.0001$ secretion data analyzed by one-way ANOVA. (E) Blood glucose measurements during glucose tolerance test (ITT), values are normalized to the starting glycemia. (F) Stimulated proinsulin secretion from isolated islets from WT and VPS41 KO mice under basal or high glucose conditions. (G) Islet proinsulin content normalized to DNA content, analyzed by t-test. Proinsulin secretion (H) and content (I) from isolated islets normalized to DNA. Data indicate mean \pm s.e.m.; n=3 *: $p < 0.05$, secretion data analyzed by one-way ANOVA. (J) Glucagon content from isolated islets normalized to DNA. Data indicate mean \pm s.e.m.; n=4, 6 *: $p < 0.05$, secretion data analyzed by one-way ANOVA.

Supplementary Movie 1

3D array tomography of VPS41 KO INS-1 cells.

Supplementary Movie 2

3D array tomography of VPS41 KO INS-1 rescue cells.

Supplementary Movie 3

Representative movie of NPY-GFP RUSH in VPS41 KO cells. Scale bar indicated 10 μm .

Supplementary Movie 4

Representative movie of NPY-GFP RUSH in HA-VPS41 rescue cells. Scale bar indicated 10 μm .

Supplementary Movie 5

Representative movie showing basal and stimulated exocytosis of NPY-pHluorin in VPS41 KO INS-1 cells. Stimulation indicated by “High K^+ ”. Scale bar indicates 10 μm .

Supplementary Movie 6

Representative movie showing basal and stimulated exocytosis of NPY-pHluorin in HA-VPS41 rescue INS-1 cells. Stimulation indicated by “High K^+ ”. Scale bar indicates 10 μm .