SUPPLEMENT

Supplement Figure legends

Supplement Fig. S1. **A)** A portion of Figure 2J is reproduced (above); nonreducing SDS-PAGE and anti-proinsulin immunoblotting is shown below, highlighting the presence of aberrant disulfide-linked proinsulin complexes. **B)** Quantitation of islet proinsulin and insulin protein levels (each point an independent male animal) by Western blotting as in panel A (mean ± s.d.; * $p < 0.05$; ** $p < 0.01$).

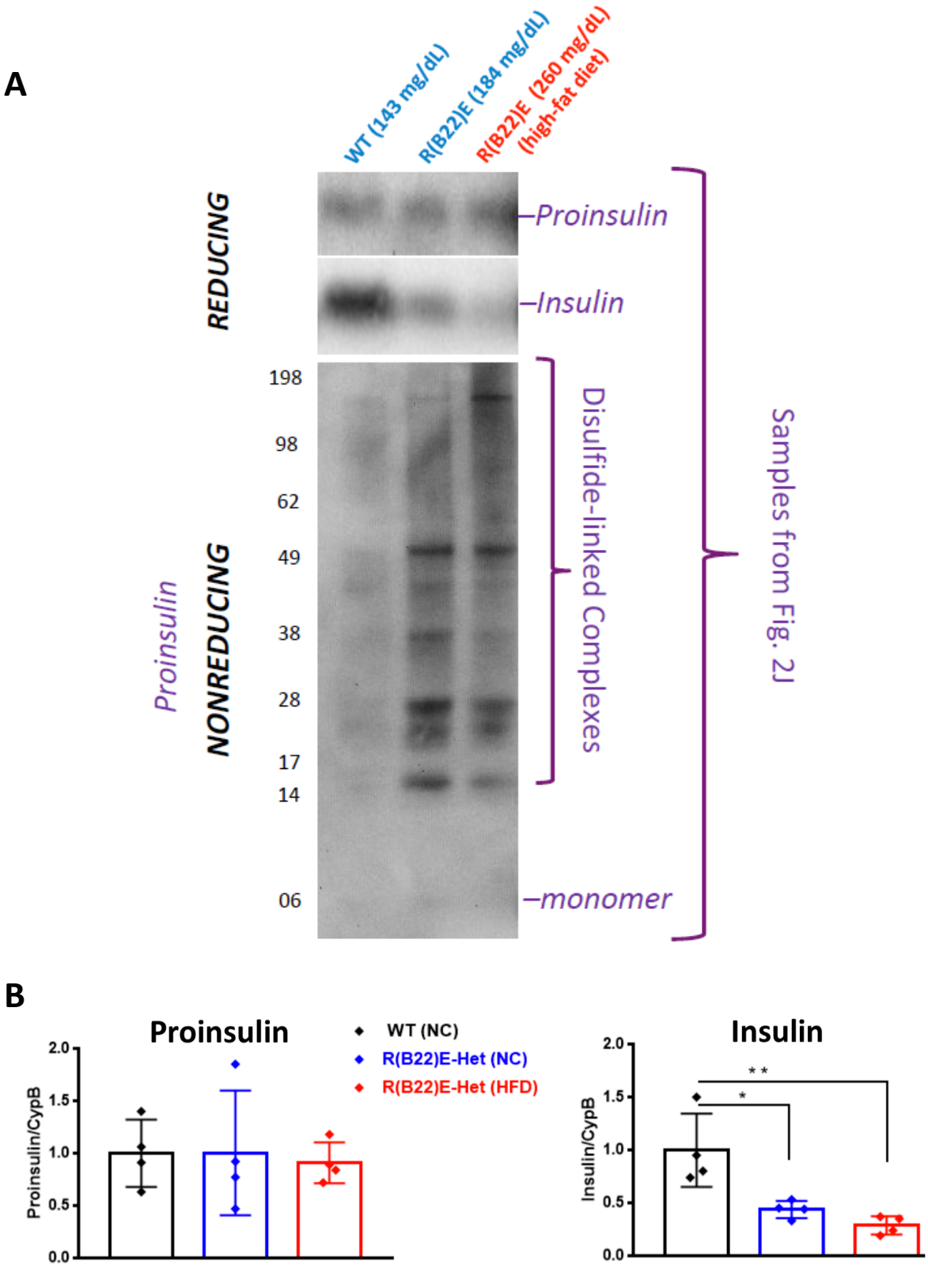
Supplement Fig. S2. Circulating proinsulin, and proinsulin-to-insulin ratio, from 11.5 week old HFD-fed *Ins2*-proinsulin-R(B22)E heterozygous males (pink symbols), superimposed onto the data reproduced from Figs 2G and H. Horizontal asterisks represent statistical differences (mean ± s.d.) along the X-axis (blood glucose); vertical asterisks indicate statistical differences along the Y-axis (proinsulin level, or proinsulin-to-insulin ratio).

Supplement Fig. S3. Transmission electron micrographs of islets cells from the genotypes (and diet) included in this study. Random blood glucose at the time of euthanasia is indicated on each figure. Scale bars are shown on all figures. **A)** Typical β-cell from male WT mouse and *Ins2*-proinsulin-R(B22)E heterozygote on normal chow diet (age 4 weeks). **B)** Another typical β-cell from the animals analyzed in panel A, at higher magnification to highlight ER and secretory granule morphology. **C)** β-cell from WT mouse fed a HFD for 6 weeks with organelles labeled on the figure. ISG = immature secretory granule. VTCs are a pre-Golgi compartment. **D)** *Upper left image*: low-power of islet from HFD-fed male *Ins2*-proinsulin-R(B22)E heterozygote (age 6 weeks). "Cell A and B" are β-cells; "Cell C" is an α-cell. Note that Cell B is poorly granulated. *Right*: A magnified image from the white-boxed region of the upper left micrograph, showing that

Cell B has expanded ER and numerous small and under-filled insulin secretory granules with low electron-density contents. *Lower left*: A magnified image from the green-boxed region of the upper left micrograph, showing a neighboring portion of the cytoplasm of Cell B with numerous small and under-filled insulin secretory granules. **E)** *Ins2*-proinsulin-R(B22)E male homozygote (age 4 weeks) highlighting a β-cell essentially lacking all secretory granules but with markedly expanded ER. A fragment of the cytoplasm of a neighboring cell bearing secretory granules is visible in the lower left corner. **F)** A portion of the cytoplasm of two further β-cells from the animal in panel E: the upper cell shows a profusion of ER with very few micro-granules; the lower cell shows a profusion of under-filled micro-granules (i.e., abnormally small size and lower-than-normal electron density of contents). **G)** Several further β-cells from the animal in panel E; a portion of the cytoplasm of an α-cell is shown at the bottom right. The main β-cell captured almost in its entirety, albeit uncommon, highlights expanded ER and under-filled secretory pathway organelles; the cytoplasm of other β-cells in the lower left corner or upper right corner of the same image do show sparse insulin granules. *Boxed image at right*: A magnified image from the red-boxed region of the left micrograph, highlighting under-filled secretory pathway organelles with low electron-density contents.

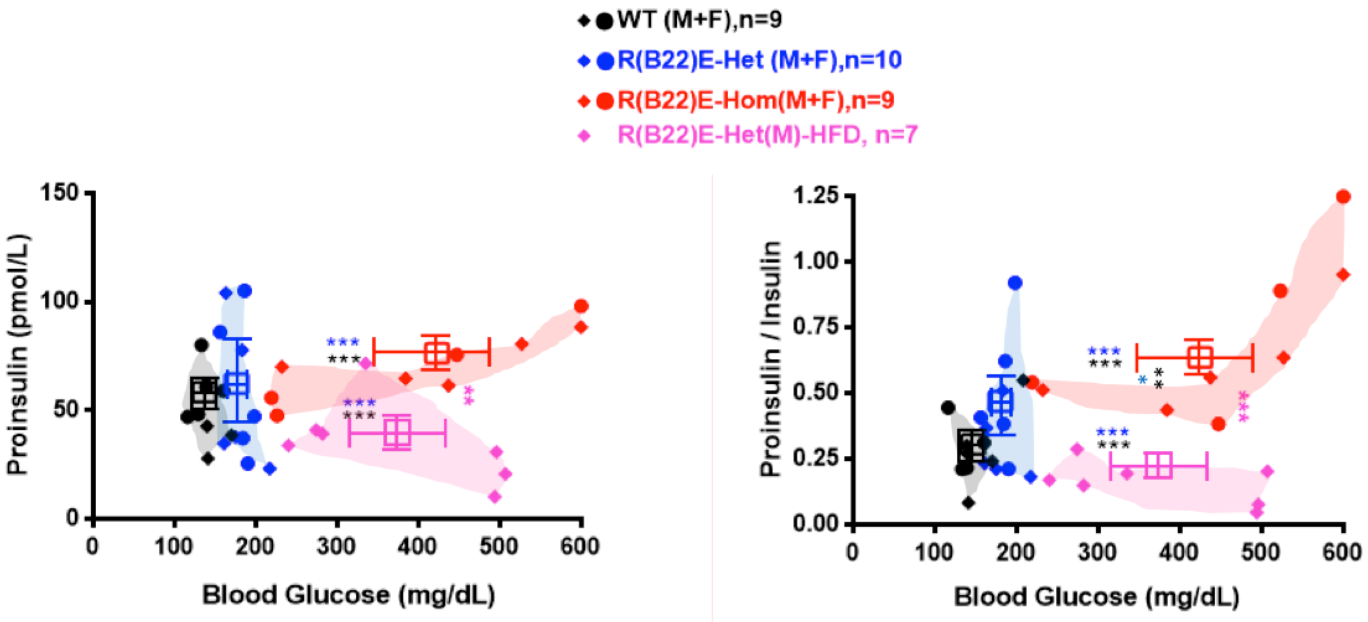
Supplement Fig. S4. From multiple independent WT or normal chow-fed nondiabetic heterozygous *Ins2* proinsulin-R(B22)E islet images of total glucagon-positive cells plus insulin- and/or proinsulin-positive (with this total defined as 1.0) compared to normal chow-fed homozygous *Ins2* proinsulin-R(B22)E islet images (mean random glucose 313 mg/dL) — i.e., images like those shown in Fig. 6) — the fraction of glucagon-positive cells was quantified (mean ± s.d.; $p = 0.002$).

Supplement Fig. S5. **A)** ER resident proteins (anti-KDEL, blue) in subpopulations of proinsulin-enriched cells (red) and insulin-enriched cells (green), as identified by triple immunofluorescence (merged images shown). The genotypes and random blood glucose at the time of euthanasia is noted on each image. Note that KDEL proteins are strongly expressed in the exocrine pancreas (blue), which lacks proinsulin or insulin. A purple merged image derives from the sum of proinsulin (red) and KDEL proteins (blue). The animal genotypes are shown on the figure (females, age 5-6 weeks). **B)** In control islets of WT mice, the fraction of β-cells with immunofluorescent KDEL proteins above threshold is approximately zero (first group shown). The fraction of β-cells with immunofluorescent KDEL protein was also quantified in the *Ins2*-R(B22)E heterozygous and homozygous condition, respectively (mean ± s.d.; $p = 0.0078$ for the homozygous condition).



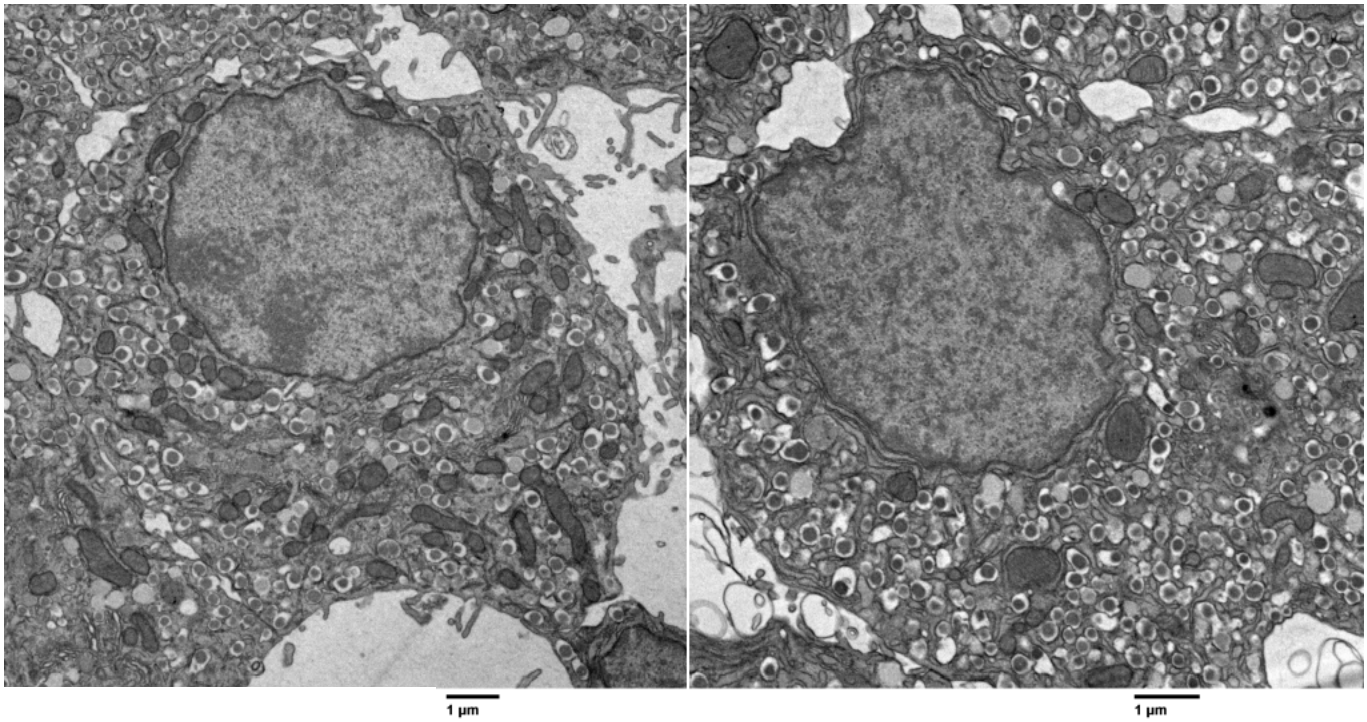
Maroof et al., Fig. S1

R(B22)E-Het (Male)HFD mice included in these graphs



Maroof et al., Fig. S2

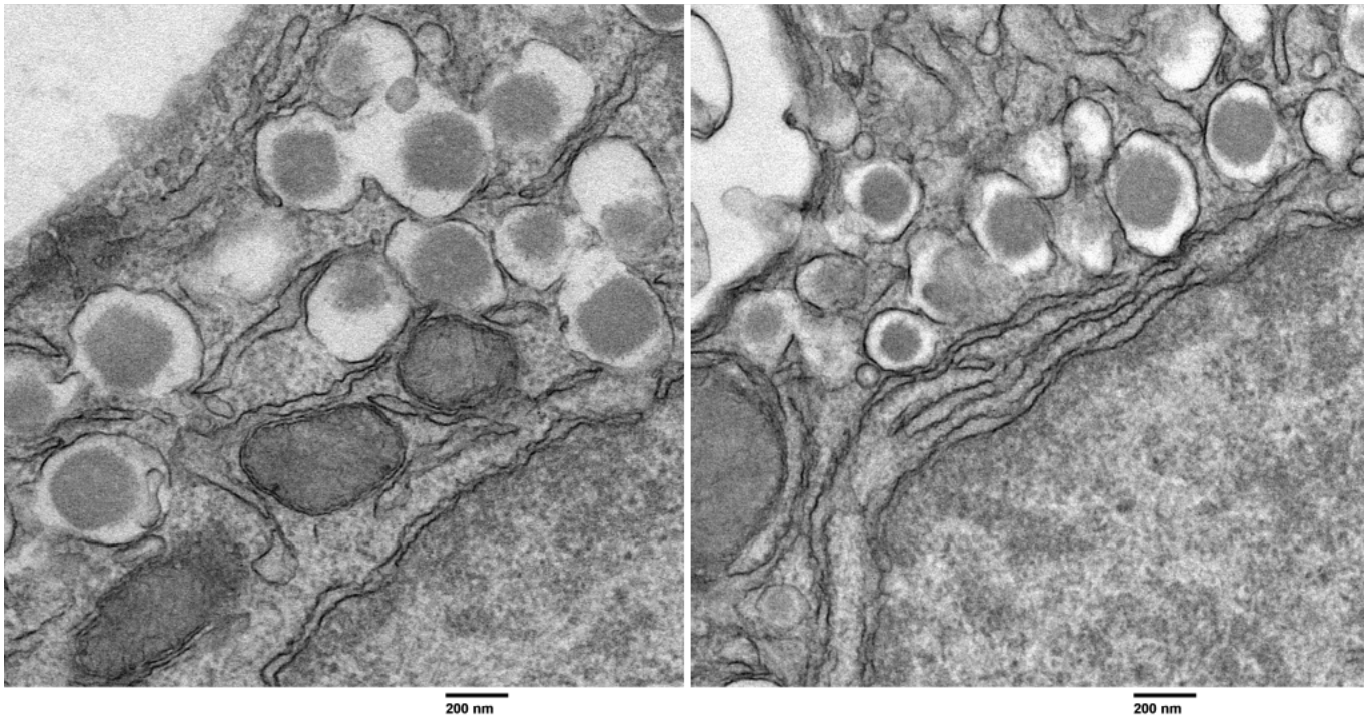
A



WT (random glucose 121 mg/dL) R(B22)E Het (random glucose 112 mg/dL)

Normal Chow Diet

B

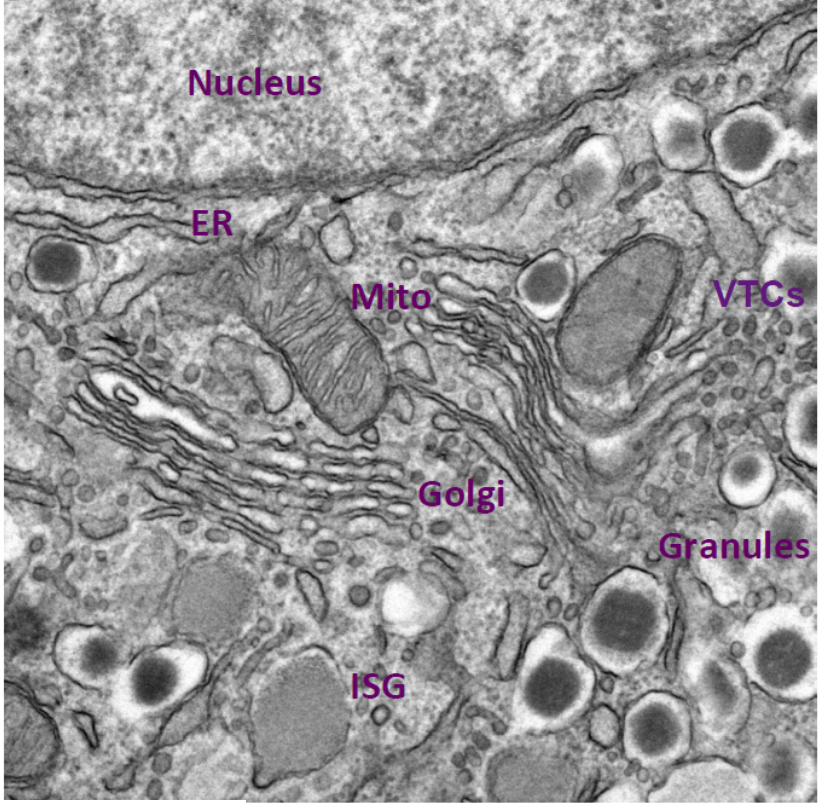


WT (random glucose 121 mg/dL) R(B22)E Het (random glucose 112 mg/dL)

Normal Chow Diet

Maroof et al., Fig. S3

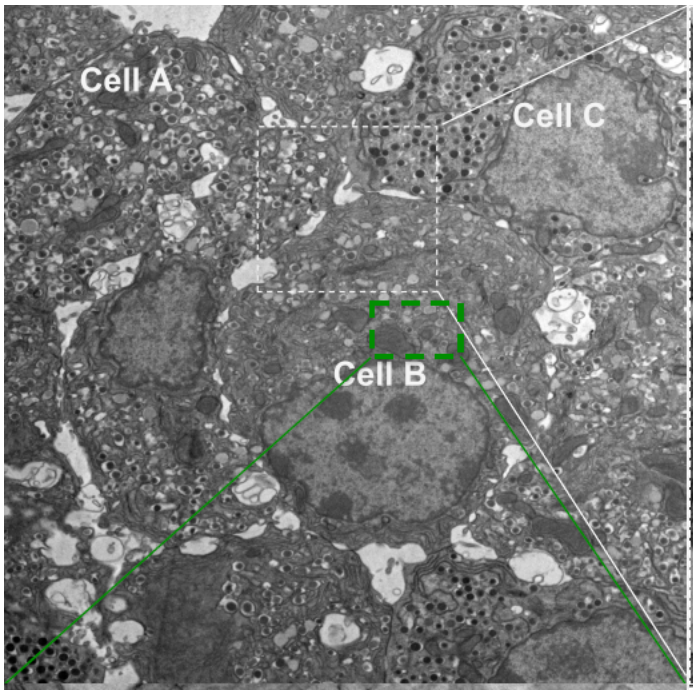
C

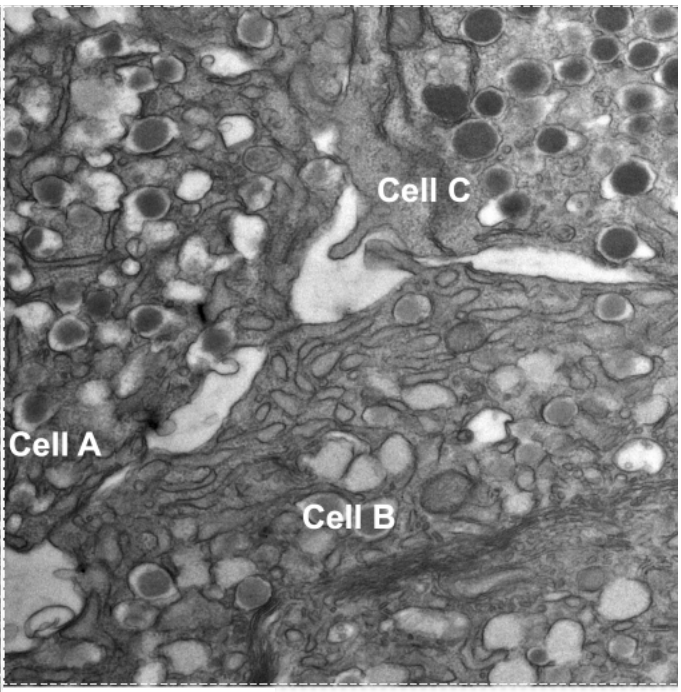


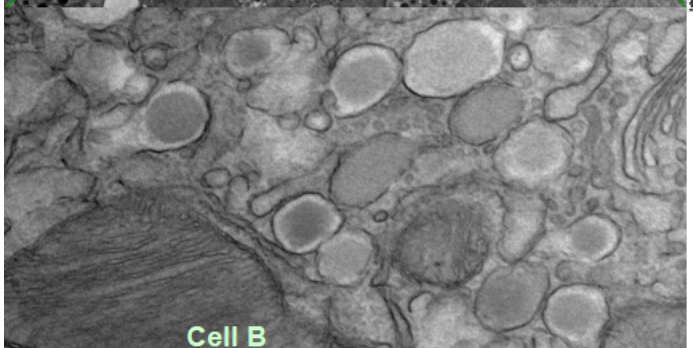
WT (random glucose 220 mg/dL) 400 nm High Fat Diet

Maroof et al., Fig. S3

D

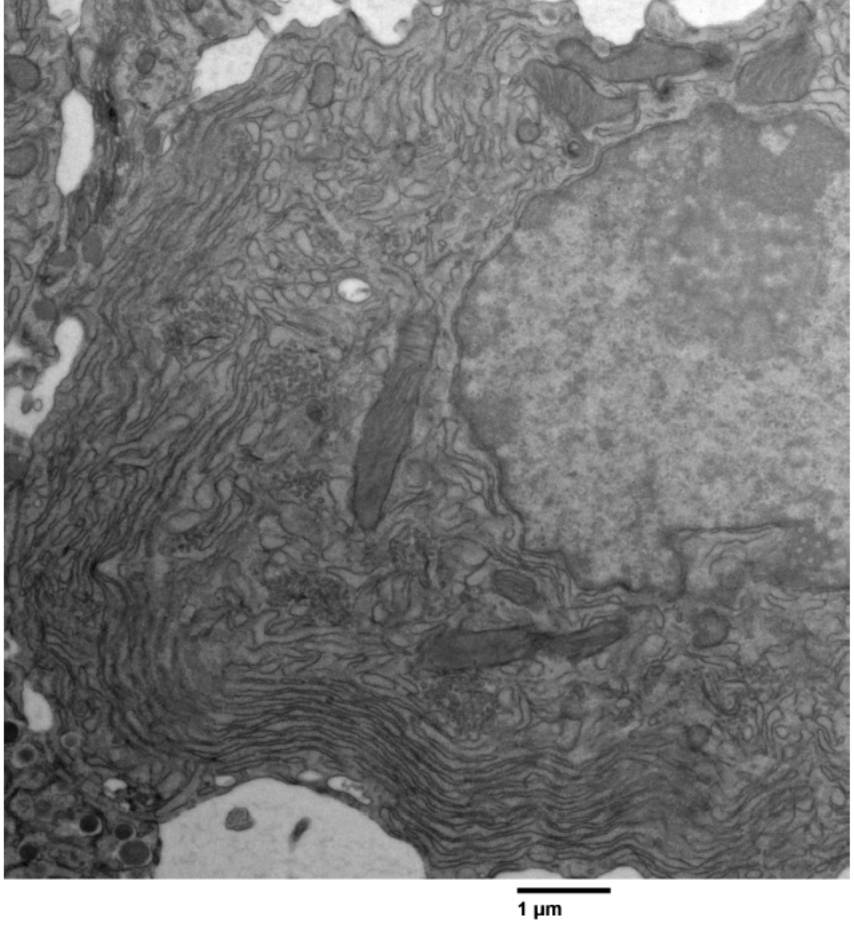






R(B22)E Het
(random glucose 260 mg/dL)
High Fat Diet

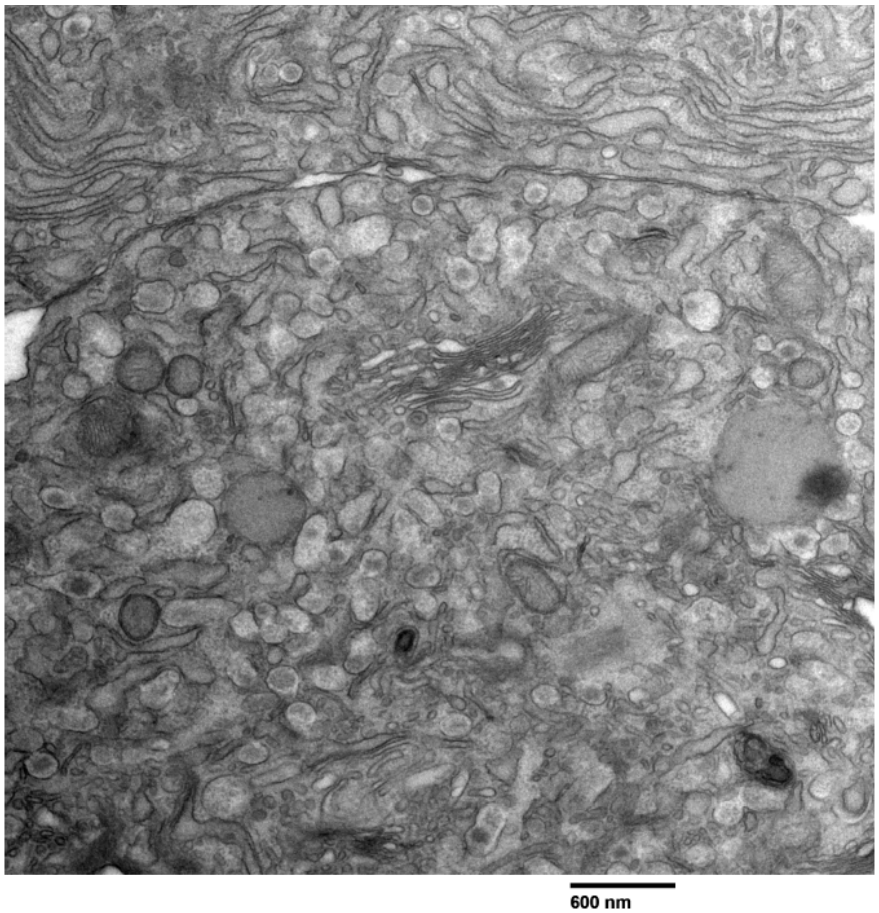
E



R(B22)E Hom
(random glucose =
300 mg/dL)

Normal Chow

F

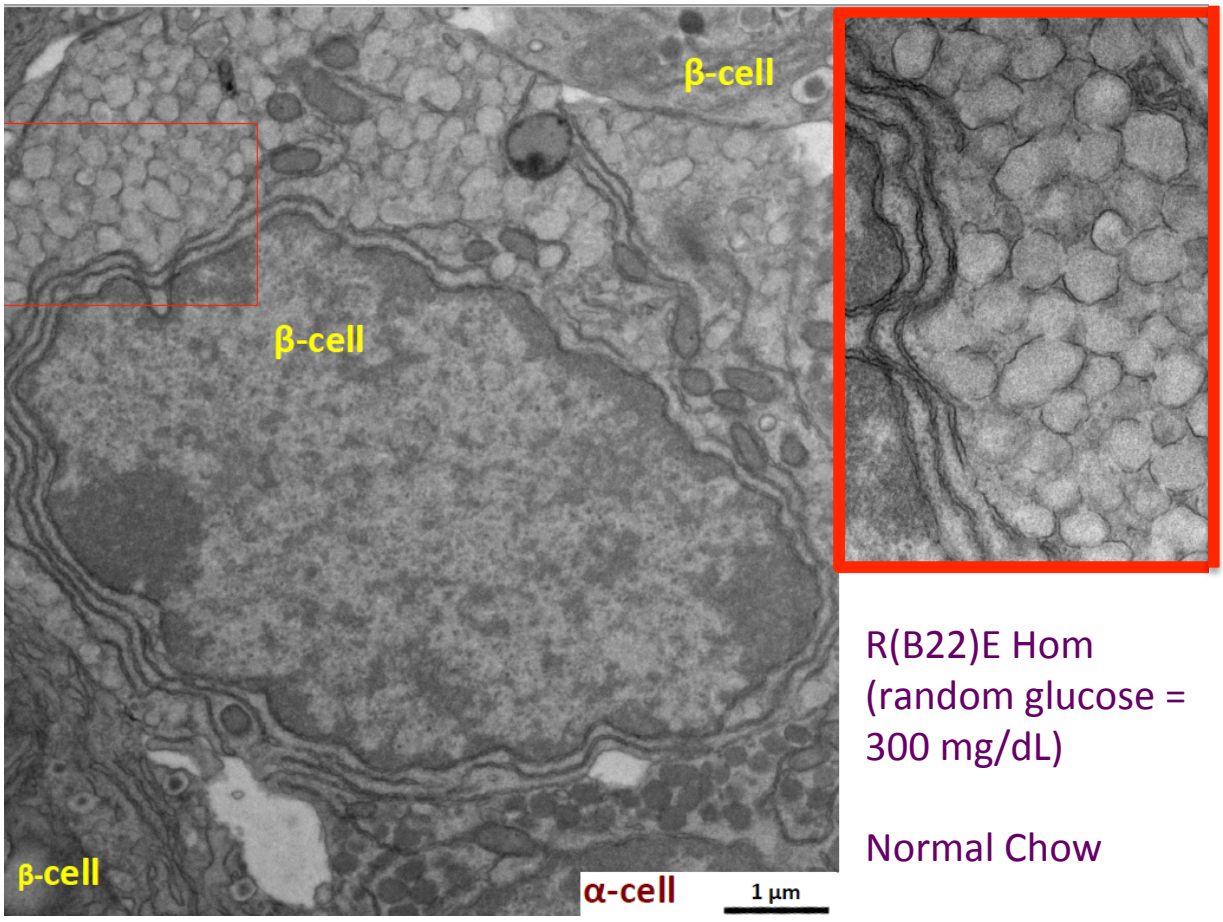


R(B22)E Hom
(random glucose =
300 mg/dL)

Normal Chow

Maroof et al., Fig. S3

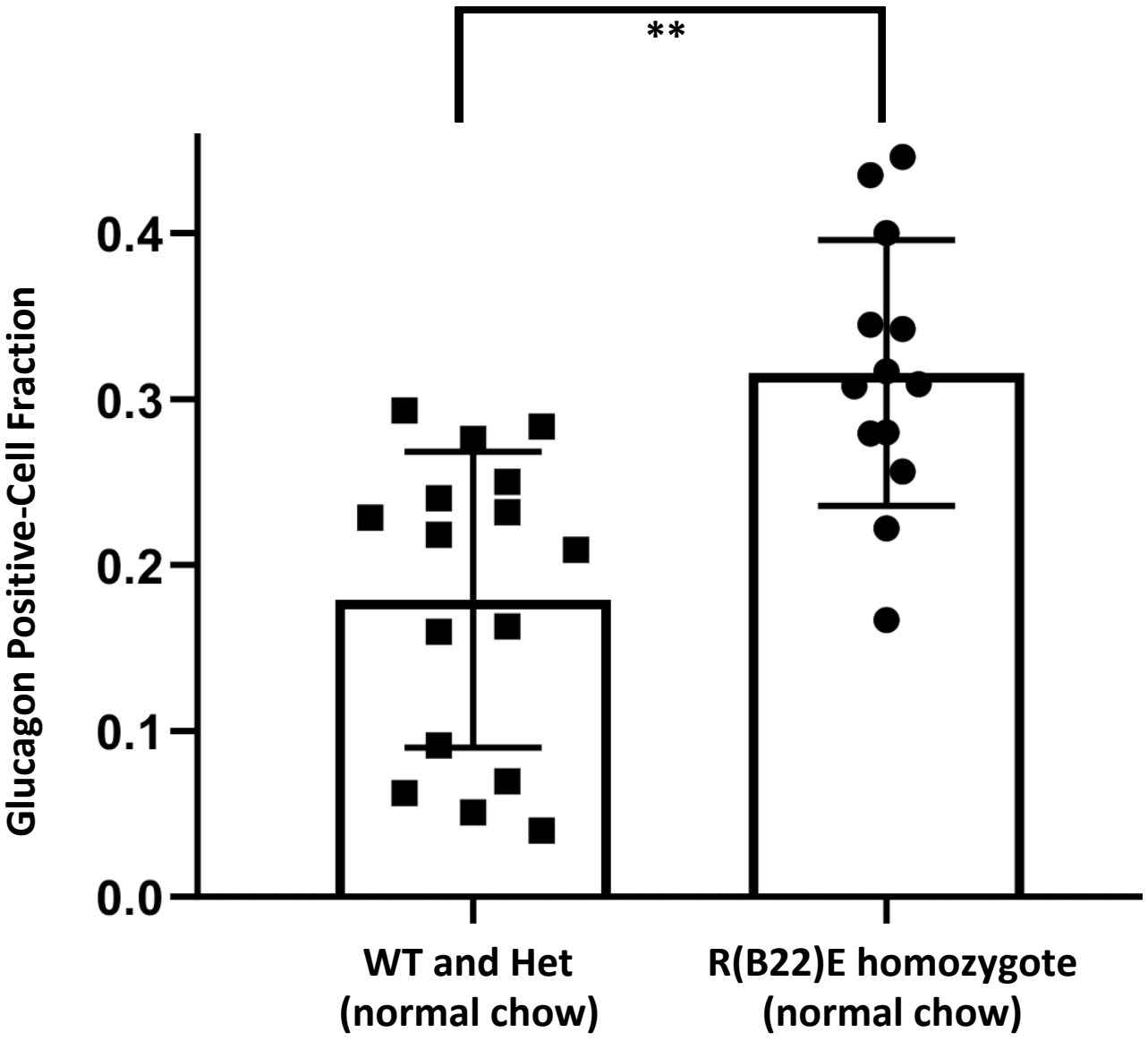
G



R(B22)E Hom
(random glucose =
300 mg/dL)

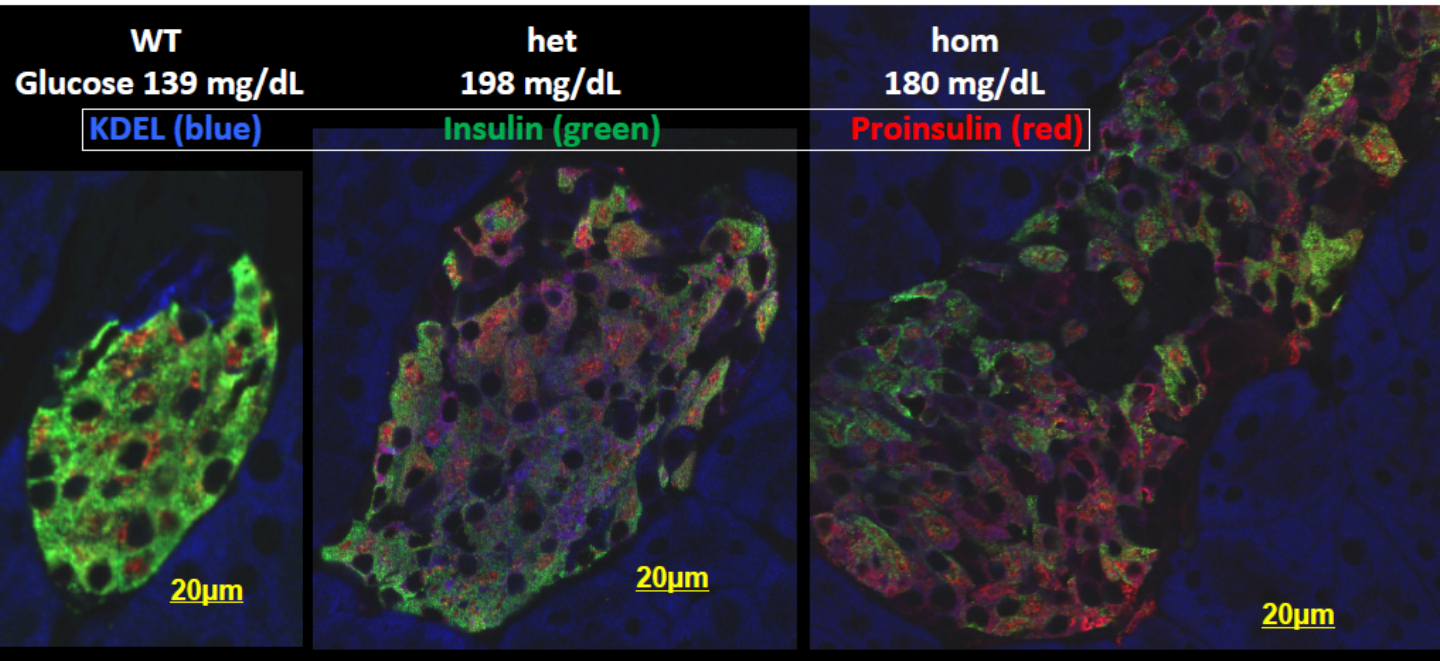
Normal Chow

Maroof et al., Fig. S3



Maroof et al., Fig. S4

A



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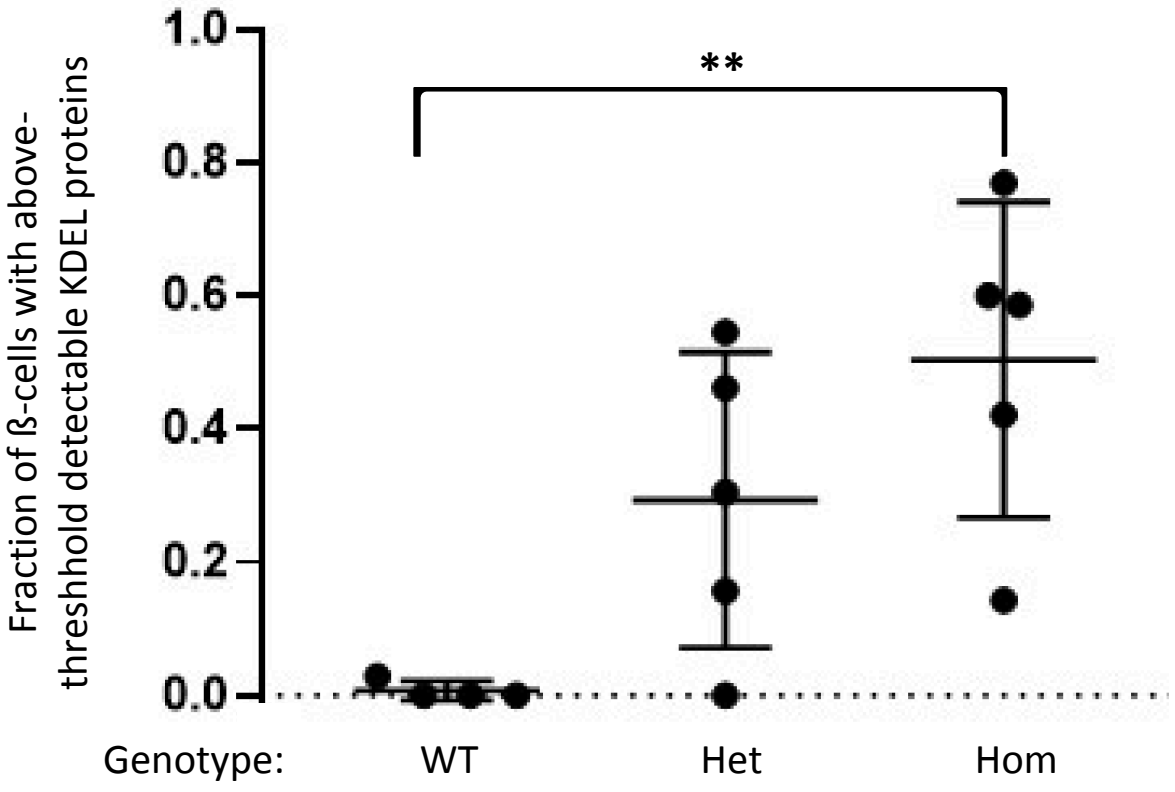

Maroof et al., Fig. S5