ONLINE APPENDIX

Supplemental Fig. S1. An acute ablation of Perk function leads to an ER to Golgi anterograde trafficking defect. Immunohistochemistry in HepG2 cells (A-C), AD293 cells (D-E). ts045 VSVG-GFP was co-transfected with an empty vector into HepG2 cells. Cells were incubated overnight at the restrictive temperature (40°C) to allow the cargo to accumulate in the ER. Cells were then shifted to the permissive temperature (32°C) for 15 minutes to allow the protein to traffic to the Golgi. Dashed line indicates ts045 VSVG-GFP (green, A) transfected cell. Staining for the Golgi marker GM130 is shown in red (B) and the merged image is seen in C. Similarly, successful trafficking of the VSVG cargo (green) to the Golgi is seen in empty vector co-transfected AD293 cells (D). However, an ablation of Perk function in ts045 VSVG-GFP (green) and cmyc-tagged DNPerk (red) co-transfected AD293 cells shows a large number of impacted-ER-like cells (E).
Supplemental Fig. S2. HA tagged S2P redistributes to the ER (A-D). Immunohistochemistry in AD293 cells (A-D). Cells were cultured and prepared for IHC as described in Fig 2. **A:** Normal Golgi localization of HA tagged S2P (red). Nuclear staining with DAPI is shown in blue. **B-D:** c-myc-tagged DNPerk is shown in green (B) and the merged image with HA-S2P (red, C) is shown in D. Arrows show normal Golgi localization of HA-S2P and the arrowheads indicate the ER redistribution of HA-S2P in DNPerk co-transfected cells. The results of these experiments need to be confirmed by ablating Perk mRNA expression by use of siRNA knockdown.
Supplemental Fig. S3. Loss of PERK function leads to alterations in ER and Golgi sterol composition. Representative data from two independent experiments involving subcellular fractionation in Perk-ablated AD293 cells is shown. SCF was carried out as described in Fig. 3. Fractions were extracted with an equal volume of chloroform:methanol (9:1) followed by evaporation of the organic phase. The residue was resuspended in the appropriate buffer for the cholesterol assay, which was carried out using the Biovision Cholesterol Quantitation Kit. A-B: Cholesterol content of membrane fractions in Perk-ablated cells (solid line) and non-ablated vector controls (dashed line). The ER fractions (enriched in fractions 6-10) and the Golgi (enriched in fractions 2-5) were analyzed for total (A) and esterified cholesterol (B) following the instructions provided in the kit. Acute ablation of Perk resulted in a large increase in the total cholesterol content in the ER of Perk-ablated AD293 cells and a corresponding decrease in the Golgi amplifying the disparity in cholesterol content between them (A). Further analysis revealed that esterified cholesterol accounted for 74.3% of the total Golgi cholesterol (fraction 4) and 2.6% of the ER cholesterol (fraction 9) in the control cells (B). In contrast, esterified cholesterol accounted for 21.2% of the total ER cholesterol content (fraction 9) in Perk-deficient cells (B).
Supplemental Fig. S4. Impaired ERAD in Perk-deficient cells. Immunohistochemistry in AD293 cells (A-B). Cells were transfected with either V5 epitope tagged wild-type proinsulin that showed a punctate, perinuclear Golgi localization (arrow, red, A) or V5 epitope tagged wild-type proinsulin-KDEL that showed ER-localization (arrowhead, red, B). Staining for the ER marker Calnexin is shown in green (A, B). C: Proinsulin synthesis rates in AD293 cells cultured in 11.1mM glucose. These cells were cotransfected with Ins$^{s}$-KDEL with either empty vector or DNPerk. Cells were then pulsed with S$^{35}$ labeled cysteine and methionine for 30 minutes followed by an immunoprecipitation of the V5-tagged proinsulin. Quantification of autoradiogram signals is shown at the bottom of the panel and represents proinsulin synthesis independent of secretion. D: Increased ER-retention of V5-tagged Ins$^{s}$-KDEL in Perk-ablated AD293 cells. Ins$^{s}$-KDEL was co-transfected with either empty vector or DNPerk and separated into cytosolic (C) and ER (ER) fractions and immunoblotted for calnexin (ER marker), tubulin (cytosolic marker) and the V5 tag. Increased ER-retention of ubiquitylated species is seen in Perk-ablated cells. E: AD293 cells were transfected with V5 tagged wild-type (Ins$^{s}$) or mutant (Ins$^{M}$) proinsulin (the latter bearing a C(B19)A point mutation that effectively leaves the A20 cysteine unpaired (Liu et al. 2005 J. Biol. Chem 280:13209.) following transfection with empty vector (Vector) or DNPerk. Lysates were immunoblotted with the anti-V5 antibody. Arrowhead indicates V5-tagged proinsulin. Multiple higher MW bands (arrow) are ubiquitylated proinsulin species as seen in Fig. 5D. Quantification of ubiquitylated bands indicates a 62% increase in ubiquitylated proinsulin (normalized to total proinsulin) in Perk-ablated cells over empty vector controls.
Supplemental Fig S5. Proinsulin and insulin content is reduced in the islets of Akita mutants and increasing the Perk gene dosage accelerates the progression to diabetes in the \textit{Ins}^{2+/}\textit{Akita} mutants. A&D: Islet immunohistochemistry for 21-day-old \textit{Ins}^{+/+} B&E: \textit{Ins}^{2+/Akita} & C&F: \textit{Ins}^{2\text{Akita/Akita}} littermates. Red=proinsulin; Blue=glucagon; Green=insulin. The signal intensity for glucagon (blue) has been kept uniform for all the panels and the relative proinsulin (red, A-C) and insulin (green, D-F) signal is shown.

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