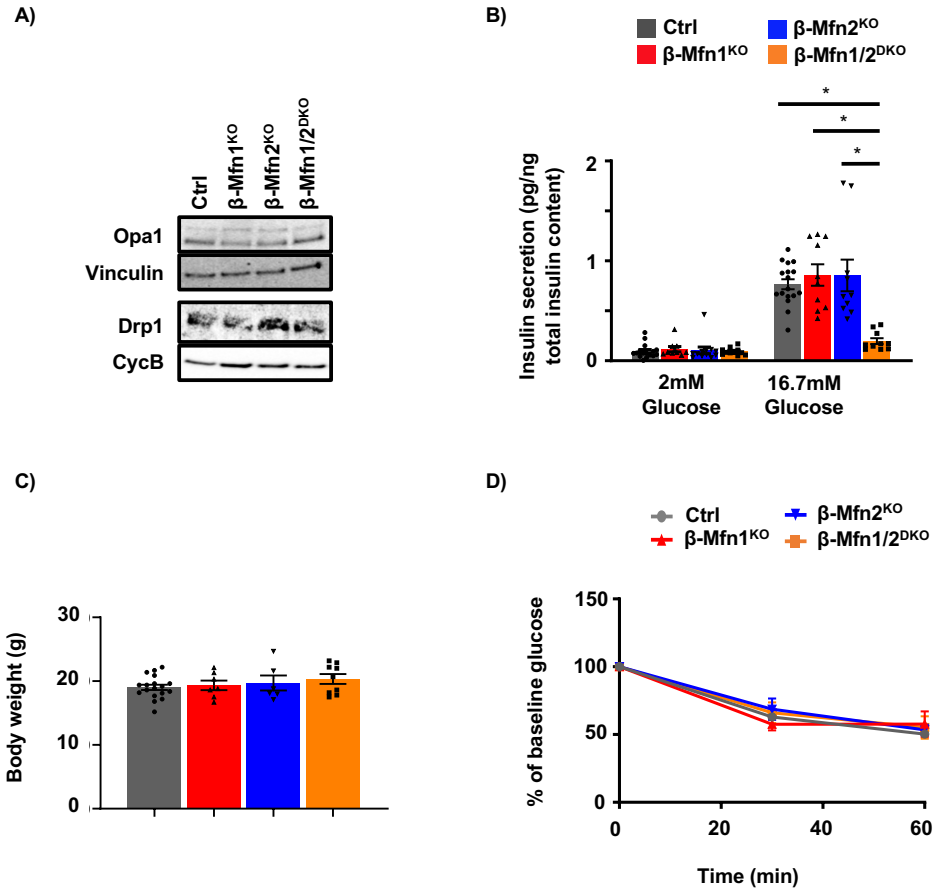

Figure S1. Body weight or insulin sensitivity are unchanged following loss of Mfn1 and/or Mfn2 in β-cells.

(A) Expression of Opa1 and Drp1 by Western blot (WB) in islets isolated from 8-10-week-old Ctrl, β-Mfn1^{KO}, β-Mfn2^{KO} and β-Mfn1/2^{DKO} mice. Vinculin and cyclophilin B serve as loading controls. Representative of 4 independent mice/group. **(B)** Glucose-stimulated insulin secretion (normalized to total islet insulin content) following static incubations in 2mM and 16.7 mM glucose performed in isolated islets of 8-week-old Ctrl, β-Mfn1^{KO}, β-Mfn2^{KO} and β-Mfn1/2^{DKO} littermates. n=8-16/group. *p<0.05 by ANOVA. **(C)** Total body weight measured in 10-week-old Ctrl, β-Mfn1^{KO}, β-Mfn2^{KO} and β-Mfn1/2^{DKO} mice. n=6-18/group. **(D)** Blood glucose concentrations (presented as % of baseline glucose) measured during insulin tolerance testing (ITT) of 8-week-old Ctrl, β-Mfn1^{KO}, β-Mfn2^{KO} and β-Mfn1/2^{DKO} littermates. n=5-19/group.

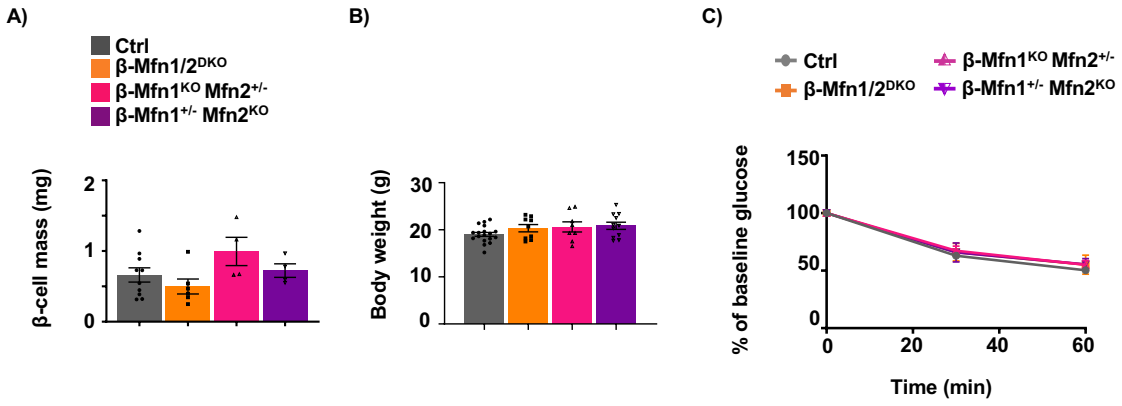

Figure S2. Defects in β-cell mass, body weight, or insulin sensitivity are not observed in mice bearing only a single allele of *Mfn1* or *Mfn2* in β-cells.

(A) Pancreatic β-cell mass (n=4-10/group) measured in 10-week-old Ctrl, β-Mfn1/2^{DKO}, β-Mfn1$^{+/-}$Mfn2^{KO}, and β-Mfn1^{KO}Mfn2$^{+/-}$ littermates. **(B)** Total body weight measured in 10-week-old Ctrl, β-Mfn1/2^{DKO}, β-Mfn1$^{+/-}$Mfn2^{KO}, and β-Mfn1^{KO}Mfn2$^{+/-}$ littermates. n=8-18/group. **(C)** Blood glucose concentrations (presented as % of baseline glucose) measured during insulin tolerance testing (ITT) of 8-week-old Ctrl, β-Mfn1/2^{DKO}, β-Mfn1$^{+/-}$Mfn2^{KO}, and β-Mfn1^{KO}Mfn2$^{+/-}$ littermates. n=12-19/group. Of note, studies in Ctrl and β-Mfn1/2^{DKO} mice were performed together alongside all β-Mfn1^{KO}, β-Mfn2^{KO}, β-Mfn1$^{+/-}$Mfn2^{KO}, and β-Mfn1^{KO}Mfn2$^{+/-}$ littermates and thus appear twice for purposes of relevant comparisons (in Figures 1F, S1C, S1D and again in Figures S2A-C).

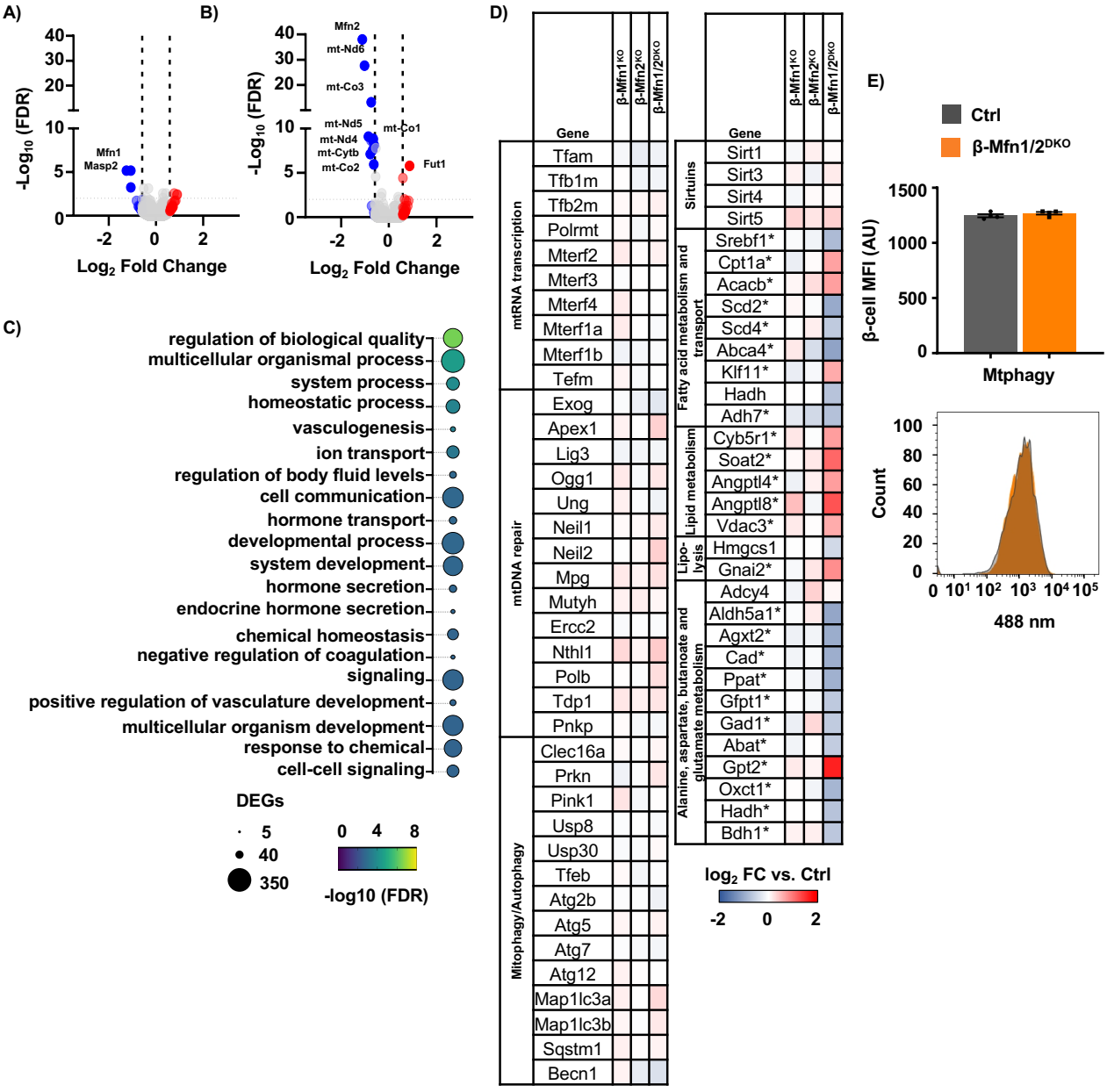

Figure S3. Transcriptomic profiling suggests defects in metabolic pathways, but not in mtDNA repair or mitophagy in Mfn1/2-deficient islets.

(A) Volcano plot depicting differential RNA expression in islets of β-Mfn1^{KO} mice compared to littermate Ctrl mice. Significantly differentially expressed genes demarcated by -log10 FDR > or < 2 and log2 fold change (FC) > or < 0.6. n=4-6/group. **(B)** Volcano plot depicting differential RNA expression in islets of β-Mfn2^{KO} mice compared to littermate Ctrl mice. Significantly differentially expressed genes demarcated by -log10 FDR > or < 2 and log2 fold change (FC) > or < 0.6. n=4-6/group. **(C)** Gene ontology (GO) enrichment analysis of top differentially regulated GO terms in β-Mfn1/2^{DKO} islets compared to littermate Ctrl islets, demarcated by both FDR and the number of significantly DEGs. n=4-6/group. **(D)** Differential RNA expression heatmap of selected genes from islets of β-Mfn1^{KO}, β-Mfn2^{KO} and β-Mfn1/2^{DKO} mice compared to littermate Ctrl mice. * Benjamini-Hochberg FDR (Padj) <0.05 comparing β-Mfn1/2^{DKO}(*) to Ctrl. n=4-6/group. **(E)** Assessment of mitophagy by flow cytometric quantification of Mtphagy dye mean fluorescence intensity (MFI - top) and a histogram plot of intensity following excitation at 488 mm in β-cells of 6-week-old Ctrl and β-Mfn1/2^{DKO} islets following 3 hour incubation with 100 nM Mtphagy dye. n=4/group.

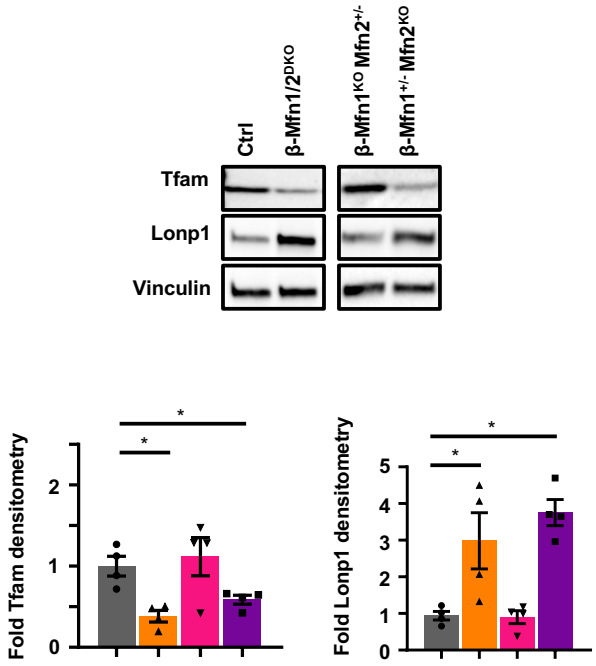

Figure S4. Expression of Tfam and Lonp1 are preserved in mice bearing a single allele of *Mfn2*, but not *Mfn1*, in β-cells.

Expression of Tfam and Lonp1 by Western blot (WB) in islets isolated from 11-week-old Ctrl, β-Mfn1/2^{DKO}, β-Mfn1$^{+/-}$Mfn2^{KO}, and β-Mfn1^{KO}Mfn2$^{+/-}$ littermates. Representative of 4 independent mice per group. Tfam (left) and Lonp1 (right) densitometry, normalized to vinculin. n=4/group; *p<0.05 by ANOVA. Of note, studies in Ctrl and β-Mfn1/2^{DKO} mice were performed together alongside β-Mfn1^{KO}, β-Mfn2^{KO}, β-Mfn1$^{+/-}$Mfn2^{KO}, and β-Mfn1^{KO}Mfn2$^{+/-}$ littermates and thus densitometry appears twice for purposes of relevant comparisons (in Figure 7B and again in Figure S4).

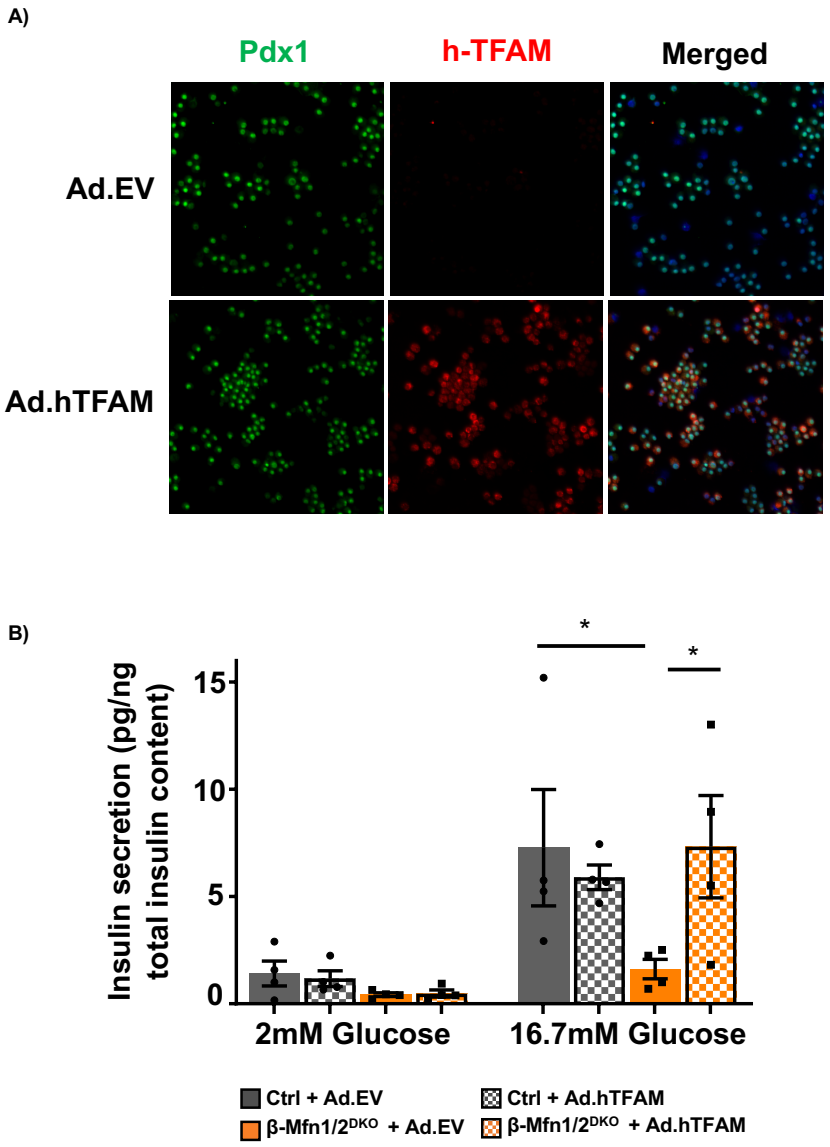

Figure S5. Overexpression of human-specific TFAM in primary mouse islets.
(A) Immunofluorescence image at 20X magnification of mouse islets following transduction with Ad.EV or Ad.hTFAM adenoviral particles and stained for Pdx1 (green), human-specific TFAM (red), and DAPI (blue). Representative of 3 independent experiments. **(B)** Glucose-stimulated insulin secretion (normalized to total islet insulin content) following static incubations in 2mM and 16.7 mM glucose, performed in isolated Ctrl and β-Mfn1/2^{DKO} islets following transduction with Ad.EV or Ad.hTFAM adenoviral particles. n=4/group. *p<0.05 by ANOVA.