**Supplemental figure legends.**

**Figure S1. Insulin tolerance measurements of wild type (WT) and *Abcc8*knockout (KO) mice on HFD and HFD+verapamil. A)** Intraperitoneal insulin tolerance test (ITT) results comparing regular chow (RC) and HFD (HFD) fed mice at 8-9 weeks. KO mice have higher insulin sensitivity than WT mice on a regular chow as well as on HFD. n=14-16 (\*\*\*\* p ≤ 0.0001; \*\* p≤0.01; \*p≤0.05: HFD-WT vs HFD-KO; ## p≤0.01: RC-WT vs RC-KO; ^^^^ p≤0.05: HFD-KO vs RC-KO). **B)** ITT results comparing HFD and HFD+verapamil (HFD+ver) fed mice at 8-9 weeks. Verapamil improves insulin sensitivity of WT mice on HFD. n=14-16 (\*\*p≤0.01; HFD-WT vs HFD-WT+ver; ## p≤0.01 HFD-KO vs HFD-KO+ver). **C)** ITT area under the curve measurements. n=14-16 (7-8 males and 7-8 females) for each condition. Error bars: ± SEM. \*\*\*\* p ≤ 0.0001; \*\*\*p≤0.001; \*\*p≤0.01; \*p≤0.05; NS, not significant. p-values were determined by ANOVA.

**Figure S2. RNA-seq profiling of purified -cells under different stress conditions.** 31 RNA-seq datasets were obtained by whole transcriptome sequencing of RNA isolated from FACS-purified β-cells using *Ins2Apple* fluorescent reporter. Datasets represent different -cell stress conditions determined by mouse genotype (*Abcc8* knockout (KO) or wild type (WT)) and diet (regular chow (RC) or high fat diet (HFD) for 5 weeks). RNA-Seq was performed on FACS-purified pancreatic β-cells isolated from postnatal day (P) 60 males and females (N=3-5 for each sex). Principal component analysis (PCA) plot (**A**) and sample transcriptome clustering (**B**) for 31 RNAseq datasets shows clear separation of *Abcc8* KO and WT samples.

**Figure S3. Differences in results of *Abcc8* knockout (KO) vs wild type (WT) differential expression analysis based on the use of the MIP-GFP transgene or *Ins2Apple* allele fluorescent reporters. A**) Venn diagram showing overlap between numbers of genes dysregulated in *Abcc8* KO -cells purified using either *Ins2Apple* allele or MIP-GFP transgene. 2501 dysregulated genes are common for both comparisons. **B**) Correlation scatter plot for Log2 fold change (Log2FC) values for 2,501 genes dysregulated in *Abcc8* KO β-cells in both MIP-GFP marked and *Ins2Apple*-marked -cells where R is Pearson correlation.

**Figure S4. -cell genes that are up-regulated in response to excitotoxicity and HFD.** Differential expression of select up-regulated genes common for both excitotoxicity and HFD stress responses.Genes are grouped by functional associations indicated by at the bottom. Log2FC: Log2 Fold Change. padj<0.05 for all presented genes in HFD-WT vs RC-WT; RC-KO vs RC-WT and HFD-KO vs RC-WT comparisons.

**Figure S5. -cell genes that are down-regulated in response to excitotoxicity and HFD.** Differential expression levels of select DRGs common for excitotoxicity and HFD stress responses.Genes are grouped by functional associations indicated by at the bottom. Log2FC: Log2 Fold Change. padj<0.05 for all presented genes in HFD-WT vs RC-WT; RC-KO vs RC-WT and HFD-KO vs RC-WT comparisons.

**Figure S6. -cell long non-coding RNAs that are down-regulated in response to excitotoxicity and HFD.** Transcripts are grouped by mouse genome informatics (MGI) biotype indicated under gene lists. Antisense, lincRNA (long interspersed ncRNA) and TEC (To be Experimentally Confirmed) transcripts, as defined by GENECODE reference annotation. Log2FC: Log2 Fold Change. padj<0.05 for all presented genes in HFD-WT vs RC-WT; RC-KO vs RC-WT and HFD-KO vs RC-WT comparisons.

**Figure S7.** **-cell genes dysregulated in response to both excitotoxicity and overnutrition. A**) Functional enrichment analysis of up-regulated (URGs) and down-regulated (DRGs) genes. Select top enriched pathways are shown. **B**)Differential expression of select top URGs (top) and DRGs (bottom). Colors indicate gene functional associations. Log2FC: Log2 Fold Change. padj<0.05 for all presented genes in HFD-KO vs RC-WT comparison.

**Figure S8. Effect of sex on weight gain and blood glucose concentration measurements of wild type (WT) and *Abcc8* knockout (KO) mice on HFD and HFD+verapamil.** Weight gain on HFD (**A**) and on HFD+verapamil (**B**) for 5 weeks. (\*\*\*p≤0.001; \*\*p≤0.01; \*p≤0.05 WT male vs WT female). WT males gain more weight than females on a HFD or HFD+verapamil. Fasting (**C**) and fed (**D**) blood glucose measurements at 8-9 weeks. KO males have higher fasting and fed blood glucose on a HFD in comparison to females. Verapamil improved blood glucose level on HFD more efficiently in males than in females. Regular chow (RC) or high fat diet (HFD). n=7-8 for each sex and condition. Error bars: ± SEM. \*\*\*\* p ≤ 0.0001; \*\*\*p≤0.001; \*\*p≤0.01; \*p≤0.05. p-values were determined by ANOVA.

**Figure S9. Sex differences in glucose tolerance of wild type (WT) and *Abcc8* knockout (KO) mice on HFD and HFD+verapamil. A**) Intraperitoneal glucose tolerance test (GTT) results comparing male and female WT or KO mice fed regular chow (RC) at 8-9 weeks. RC-WT females have improved glucose tolerance in comparison to RC-WT males. (\*\*p≤0.01; \*p≤0.05: WT males vs females; # p≤0.05: KO males vs females). **B**) GTT results comparing HFD fed (HFD-) WT and KO male and female mice at 8-9 weeks. HFD-WT and HFD-KO females have improved glucose tolerance in comparison to HFD-WT and HFD-KO males, respectively (\*\*\*\* p ≤ 0.0001; \*\*p≤0.01: WT males vs females; #### p≤0.0001; ### p≤0.001: KO males vs females). **E**) GTT results comparing HFD+verapamil (HFD+ver) fed WT and KO male and female mice at 8-9 weeks. There was no statistically significant difference between males and females in glucose tolerance on HFD+ver. **F**) GTT area under the curve measurements, n=7-8 (\*\* p≤0.01; \*p≤0.05). n=7-8 for each sex and condition. Error bars: ± SEM. \*\*\*\* p ≤ 0.0001; \*\*\*p≤0.001; \*\*p≤0.01; \*p≤0.05. p-values were determined by ANOVA.

**Figure S10. Sex differences in insulin tolerance of wild type (WT) and *Abcc8* knockout (KO) mice on HFD and HFD+verapamil. A)** Intraperitoneal insulin tolerance test (ITT) results comparing male and female WT or KO mice fed regular chow (RC) at 8-9 weeks. **B)** ITT results comparing HFD-fed WT or KO male and female mice at 8-9 weeks. n=7-8 (\*\* p≤0.01; \*p≤0.05: WT males vs females). WT females have improved insulin sensitivity in comparison to WT males at 15 and 30 minutes time points. (\*\* p≤0.01; \*p≤0.05: WT males vs females; ## p≤0.01: KO males vs females). **C**) ITT results comparing HFD+verapamil (HFD+ver)-fed WT or KO male and female mice at 8-9 weeks. **D**). ITT area under the curve measurements. n=7-8 for each sex and condition. Error bars: ± SEM. \*\*p≤0.01; \*p≤0.05. p-values were determined by ANOVA.

**Figure S11. Sex differences in -cell transcriptome in response to excitotoxicity and overnutrition.** **A**) Venn diagrams indicating overlap between genes differentially expressed (padj<0.05) between females and males in -cells from WT (RC-WT females vs males), KO (RC-KO females vs males), HFD (HFD-WT females vs males), and HFD-KO (HFD-KO females vs males) comparisons. 7 core sex-enriched genes overlapping between all 4 comparisons are presented in red font (female-enriched) and blue font (male enriched). **B)** HFD-WT males vs females comparison: KEGG pathway functional enrichment analysis of up-regulated (increased in females on HFD) and down-regulated (increased in males on HFD) genes. Select top enriched pathways are shown. **C)** Differential expression levels of select top URGs (top) and DRGs (bottom) with colors indicating gene functional associations. Log2FC: Log2 Fold Change HFD-WT females vs HFD-WT male comparison.

**Figure S12. Excitotoxicity and overnutrition alters mitochondrial respiration, biogenesis and fatty acid (FA) -oxidation and contributes to metabolic inflexibility and loss of function in -cells.** **A)** Increased intracellular Ca2+ (excitotoxicity) activates mitochondrial respiration and mitochondrial biogenesis programs in -cells.In muscle, increased [Ca2+]i activates calcineurin (CaN), MAPK- and CaMK- kinases that, in turn, activate transcription factors *Mef2c* and *Pparggc1a* (1). Our results suggest that in pancreatic β-cells, anincrease in [Ca2+]i and, at a lesser extent, overnutrition activate a similar gene cascade, causing an increase in mitochondrial respiration, biogenesis, fatty acid -oxidation and ROS detoxification. **B**) In muscle tissues, activation of FA -oxidation leads to repression of glucose metabolism by resulting metabolites, a process known as glucose sparing (2). Our results show that in -cells expression of FA -oxidation genes (e.g. rate-limiting enzyme *Acadvl*) is increased in response to high fat diet (overnutrition) and strongly increased in response to increased intracellular Ca2+ (excitotoxicity). These later changes contributed to decrease in islet glucose-stimulated mitochondrial respiration. **C**) The combination of excitotoxicity and overnutrition, in addition to FA -oxidation genes, leads to an increase in expression of genes controlling pyruvate entrance into TCA cycle (*Pdk4*) and utilization of ketones as metabolic fuel (*Hmgs2*). These changes suggest a rise in metabolic inflexibility, or inability to utilize glucose as metabolic fuel for mitochondrial ATP production, that prevents glucose-stimulated metabolic coupling and contributes to the loss of -cell function. Red font and arrows indicate observed increases in gene expression in response to excitotoxicity and/or overnutrition.

**Supplemental table legends.**

**Table S1. Primers used in this study.** The table summarizes genotyping and RT-qPCR primers used in this study.

**Table S2. Summary of RNAseq datasets obtained in this study.**

The table summarizes -cell RNA-seq datasets obtained in this study. Characteristics and treatment conditions of mice used for -cell isolations were as follows. All mice were 8-9 weeks of age at the time of β-cell isolation. A high fat diet (HFD) was fed to wild type (WT) or *Abcc8-/-* (KO) mice for 5 weeks beginning at the time of weaning. β-cells were purified by FACS based on the red fluorescence of the *Ins2Apple* allele present in all animals. A total of 31 different datasets were generated. N=3-5 for each group.

**Table S3. Summary of pairwise comparisons of RNAseq datasets performed in this study.** Summary of pairwise comparisons of RNAseq datasets performed in this study. Using the 31 RNA-seq datasets collected in this study, we performed a total of seven different pairwise analyses. All mice contained a single *Ins2Apple* allele. The number of affected genes based on a padj.< 0.05. HFD, high fat diet. RC, regular chow. WT, wild type. KO, knockout.

**Table S4, supplemental Excel file. Transcriptional response of -cells under different stress conditions. Results of pairwise comparisons of RNAseq datasets performed in this study (in a separate Excel file).** Differential expression analysis of RNA-seq datasets was done using DEseq2. Expression levels of genes are presented as normalized counts. Each tab corresponds to each of the following pairwise comparisons: RC-KO vs RC-WT (excitotoxicity stress response), HFD-WT vs RC-WT (overnutrition stress response), HFD-KO vs RC-WT (overnutrition and excitotoxicity combined), RC-WT females vs males (sex differences in normal -cells), HFD-WT females vs males (sex differences in overnutrition stress response), RC-KO Females vs Males (sex differences in excitotoxicity stress response), HFD-KO females vs males (sex differences in overnutrition stress response in the presence of excitotoxicity).

**Table S5, supplemental Excel file. Functional enrichment analysis of dysregulated genes.** Gene ontology and signaling pathway functional enrichment analysis of dysregulated genes was done using Metascape. Each tab corresponds to functional enrichment data for genes dysregulated in the following pairwise comparisons: RC-KO vs RC-WT (excitotoxicity stress response), HFD-WT vs RC-WT (overnutrition stress response), HFD-KO vs RC-WT (overnutrition and excitotoxicity combined), overlapping stress genes (up- or down-regulated genes that are common between RC-KO vs RC-WT, HFD-WT vs RC-WT and HFD-KO vs RC-WT comparisons), HFD-KO vs RC-WT only (genes that were up-or down-regulated only in HFD-KO vs RC-WT comparison), HFD-KO females vs males HFD-WT (sex differences in response to overnutrition).

**Supplemental references.**

1. Lira VA, Benton CR, Yan Z, Bonen A: PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. Am J Physiol Endocrinol Metab 2010;299:E145-161

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