Supplementary Materials for

A common genetic variant of a mitochondrial RNA processing enzyme predisposes to insulin resistance

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Figs. S1 to S10
Figure S1. Related to Figure 1. The effects of \textit{N434S} variant on MRPP3 levels and body weight. (A) Immunoblot of MRPP3 in cells expressing the \textit{wt} or \textit{N434S} variant. (A) Weekly weight measurements of \textit{wt} and \textit{N434S} mice on NCD or HFD compared to their respective controls (n=5 per diet and genotype).
Figure S2. Related to Figure 2. Differences between *wt* and *N434S* skeletal muscle and white adipose tissue. Skeletal muscle and white adipose tissue (WAT) from 20-week old *wt* and *N434S* mice fed on NCD or HFD were stained with Haematoxylin and Eosin (H&E) and visualized at 20x magnification. (A) In the skeletal muscle of the *N434S* mice fed on a NCD there was a slight increase in the endomysium of the connective tissue and a mild presence of necrotic fibers, while in the HFD fed *N434S* mice there was centralization of nuclei (white arrow). (B) The WAT from the *N434S* mice fed a NCD showed signs of inflammation (black arrows) while the WAT from the *N434S* mice fed a HFD had increased adipocytes size compared to *wt* mice on the respective diets. (C) Skeletal muscle lysates (30 µg) from 20 week-old *N434S* mice and *wt* mice fed a HFD following insulin exposure were immunoblotted against antibodies to investigate the endogenous levels of Akt, its
phosphorylated form (Ser\textsuperscript{473}) and GLUT4. GAPDH was used as a loading control. Relative abundance of proteins was measured using Image Studio Lite and normalized to GAPDH. The data are representative of results obtained from 3 mice from each genotype. Error bars * $p < 0.05$, ** $p < 0.01$ Student’s $t$ test. (D) \textit{In vitro} basal and insulin-stimulated glucose uptake was measured in skeletal muscle from control and \textit{N434S} mice fed a HFD (n=3 per genotype).
Figure S3. Related to Figures 3. Glucose oxidation is favoured over fatty acid oxidation in N434S liver mitochondria for energy production, causing lipid accumulation in the liver.

Oxygen consumption for Leak State (L), OXPHOS capacity (P) and ET-capacity (ET) was measured in mitochondria isolated from liver of 20-week old N434S and wt mice either fed a NCD (A) or HFD (B). Oxygen levels were measured with an OROBOROS oxygen electrode using Succinate (S pathway control state) as substrate in presents of inhibitors and the uncoupler FCCP. The data represented is from at least 4 mice from each genotype per each diet. Error bars are SEM *p < 0.05, Student’s t test.
FAO controlled pathway oxygen consumption was measured in mitochondria isolated from liver of 20-week old N434S and wt mice either fed a NCD (C) or HFD (D). Oxygen levels were measured with an OROBOROS oxygen electrode using palmitoyl carnitine as substrate in the presence of inhibitors to measure FAO Leak State (FL), FAO controlled OXPHOS Capacity (FP), FAO controlled OXPHOS Capacity in the presence of succinate (FSp) and FAO ET-capacity in the presence of succinate and FCCP (FSE). The data represented is from at least 4 mice from each genotype per each diet. Error bars are SEM *p < 0.05, p < 0.01, Student’s t test.

(E) The levels of the mitochondrial located proteins CPTII were measured by immunoblotting in isolated mitochondria from liver and pancreas of 20-week old N434S and wt mice either fed a NCD or HFD. Porin was used as a loading control. The data are representative of results obtained from at least 6 mice from each genotype.
Figure S4. Related to Figure 4. The effects of the N434S variant on mitochondrial RNA metabolism and biogenesis in liver.

(A) Quantitative RT-PCR was used to measure mitochondrial RNA junctions in livers from 20-week old N434S and wt mice either fed a NCD or HFD. The data were normalized to 18S rRNA and are representative of results obtained from at least 3 mice from each genotype per diet.

(B) Northern blotting was used to measure the relative mtRNA abundance in livers from 20-week old N434S and wt mice either fed a NCD or HFD. The data were normalized to 18S rRNA and are representative of results obtained from at least 3 mice from each genotype per diet. Error bars indicate SEM; *p < 0.05, **p < 0.01, Student’s t test.

(C) Liver mitochondrial proteins (30 µg) were isolated from 20-week old N434S mice and wt mice fed a NCD or HFD and immunoblotted against antibodies to investigate the steady-state levels of nuclear-encoded and mitochondrially-encoded OXPHOS proteins. SDHA was used as loading control. Relative abundance of proteins was measured using Image Studio Lite and normalized to SDHA. The data are representative of results obtained from at least 6 mice from each genotype. Error bars indicate SEM.
Figure S5. Related to Figure 4. The effects of the N434S variant on mitochondrial biogenesis in the heart.
(A) Heart mitochondrial proteins (30 µg) were isolated from 20-week old N434S mice and wt mice fed a NCD or HFD and immunoblotted against antibodies to investigate the steady-state levels of nuclear-encoded and mitochondrially-encoded OXPHOS proteins. SDHA was used as loading control. Relative abundance of proteins was measured using Image Studio Lite and normalized to SDHA. The data are representative of results obtained from at least 6 mice from each genotype. Error bars indicate SEM.

(B) Immunoblotting was used to measure the levels of MRPP2 and CPTII in heart mitochondria (30 µg) isolated from 20-week old N434S mice and wt mice fed a NCD or HFD. Porin was used as loading control. Relative abundance of proteins was measured using Image Studio Lite and normalized to porin. The data are representative of results obtained from at least 6 mice from each genotype. Error bars indicate SEM.
Figure S6. Related to Figure 4. The effects of the N434S variant on mitochondrial biogenesis in skeletal muscle.

(A) Skeletal muscle mitochondrial proteins (30 µg) were isolated from 20-week old N434S mice and wt mice fed a NCD or HFD and immunoblotted against antibodies to investigate the steady-state levels of nuclear-encoded and mitochondrially-encoded OXPHOS proteins. SDHA was used as loading control. Relative abundance of proteins was measured using Image Studio Lite and normalized to SDHA. The data are representative of results obtained from at least 6 mice from each genotype. Error bars indicate SEM.

(B) Immunoblotting was used to measure the levels of MRPP2 and CPTII in skeletal muscle mitochondria (30 µg) isolated from 20-week old N434S mice and wt mice fed a NCD or HFD. Porin was used as loading control. Relative abundance of proteins was measured using Image Studio Lite and normalized to porin. The data are representative of results obtained from at least 6 mice from each genotype. Error bars indicate SEM.

(C) Immunoblotting was used to measure the levels of GLUT4 in skeletal muscle lysates (30 µg) isolated from 20-week old N434S mice and wt mice fed a NCD or HFD. GAPDH was used as loading control. Relative abundance of proteins was measured using Image Studio Lite and normalized to GAPDH. The data are representative of results obtained from at least 6 mice from each genotype. Error bars indicate SEM.
Figure S7. Related to Figure 5. Targeting the wt and N434S MRPP3 proteins to mitochondria.

143B cells were transiently transfected with pMRPP3-EGFP (A, B) or pMRPP3-N434S-EGFP (C, D) to confirm the correct expression and the mitochondrial localisation of the wt and N434S MRPP3 variant protein, respectively. Cells were incubated with 50 nM Mitotracker Orange and fixed. The overlay image (yellow) show the co-localisation for both MRPP3-EGFP and MRPP3-N434S-EGFP (green) within mitochondria (red). (E) The empty plasmid was used as a negative control.

(F) Cell lysates were immunoblotted against MRPP3 and HA tag to confirm expression. Non transfected cells (lane 1) and cells transfected with the empty plasmid (lane 3) were used as negative controls. Porin was used as a loading control.
Figure S8. Related to Figure 5. The effects of the N434S variant on mitochondrial DNA- and RNA-binding proteins.

(A) The levels of MRPP2 and LETM1 were measured by immunoblotting of pancreatic mitochondria isolated from 20-week old N434S mice and wt mice fed a NCD or HFD. (B) Liver mitochondrial proteins (30 µg) were isolated from 20-week old N434S mice and wt mice fed a NCD or HFD and immunoblotted for LETM1 and MRPP2. Pancreas (C) and liver (D) mitochondrial proteins (30 µg) were isolated from 20-week old N434S mice and wt mice fed a NCD or HFD and immunoblotted for LRPPRC and TFAM. (E) Pancreas mitochondrial proteins (30 µg) were isolated from 20-week old N434S mice and wt mice fed a NCD or HFD and immunoblotted for MCU. Relative abundance of proteins was measured using Image Studio Lite and normalized to porin. The data are representative of results obtained from at least 3 mice from each genotype and diet. Error bars indicate SEM; *p < 0.05, **p < 0.01, Student’s t test.
Figure S9. Related to Figure 5. The effects of the N434S variant on amino acid metabolism.

Amino acid content was measured by reversed-phase high-speed quantitative ultra-performance liquid chromatography - tandem mass spectrometry in pancreata from 20-week old N434S mice and wt mice fed a NCD or HFD. The data are representative of results obtained from at least 6 mice from each genotype and diet. Error bars are SEM, **p<0.05, Student’s t-test.
Figure S10. Related to Figure 6. The N434S variant does not lead to pancreatic inflammation.

(A) JC-1 was used to measure mitochondrial membrane potential in pancreatic islet cells isolated from 20-week old N434S mice and wt mice fed a NCD or HFD. Data are expressed as percent of control and error bars are SEM *** p < 0.001, Student’s t test.
(B) Liver and pancreata homogenates (30 µg) were prepared from 20-week old N434S mice and wt mice fed a NCD or HFD and immunoblotted for SAPK/JNK and phopho-SAPK/JNK to investigate changes in inflammation. (C) Immunoblotting was used to measure LC3A/B levels in pancreata (30 µg) isolated from 20-week old N434S mice and wt mice fed a NCD or HFD. Relative abundance of proteins was measured using Image Studio Lite and normalized to GAPDH. The data are representative of results obtained from at least 6 mice from each genotype. Error bars are SEM *** p < 0.001, Student’s t test.