

## Supplemental Information

### Exercise and metformin counteract altered mitochondrial function in the insulin resistant brain

Gregory N. Ruegsegger<sup>1</sup>, Patrick M. Vanderboom<sup>1</sup>, Surendra Dasari<sup>2</sup>, Katherine A. Klaus<sup>1</sup>, Parijat Kabiraj<sup>3</sup>, Christina B McCarthy<sup>3</sup>, Claudia F. Lucchinetti<sup>3,4</sup>, K. Sreekumaran Nair<sup>1\*</sup>

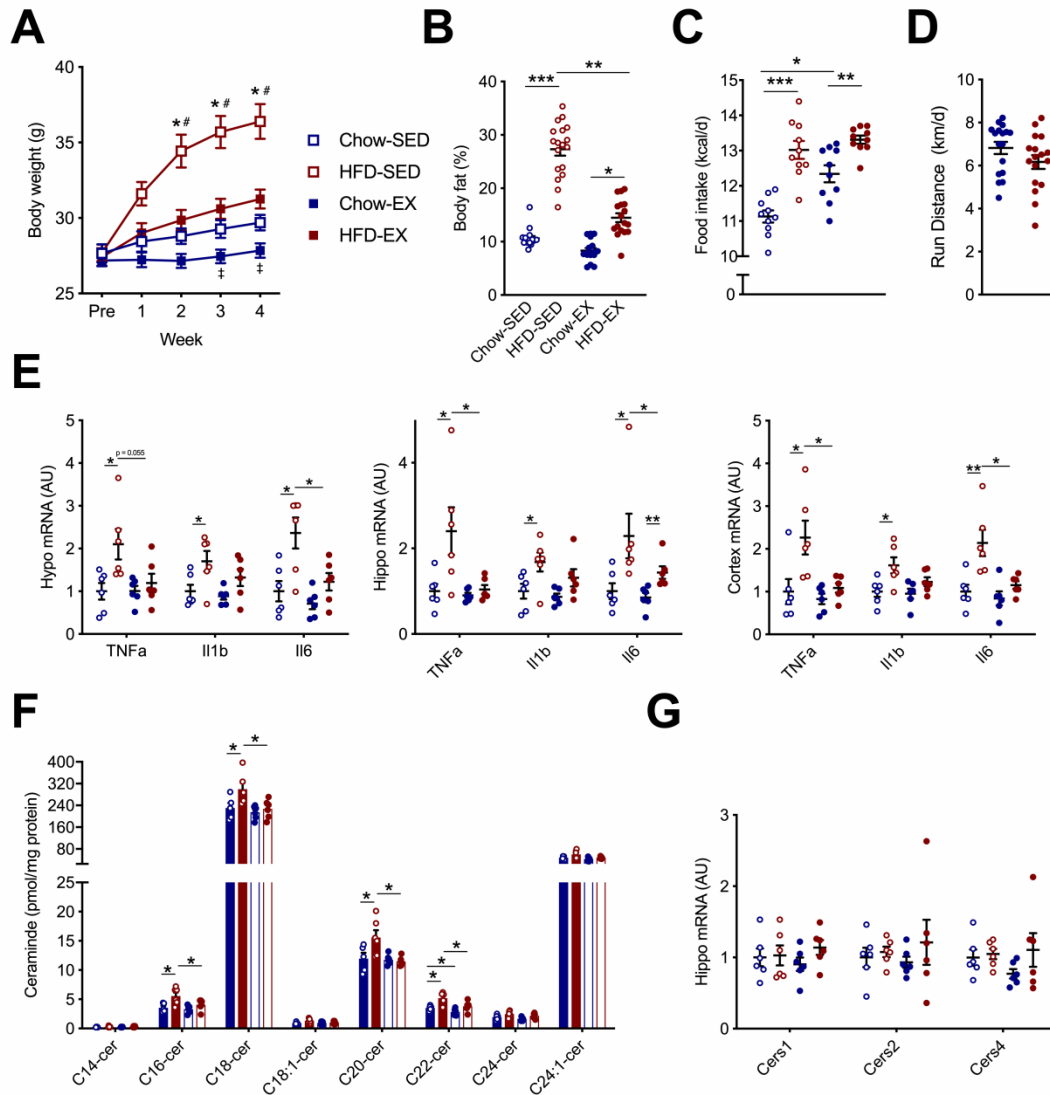
#### *Affiliations*

<sup>1</sup> Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

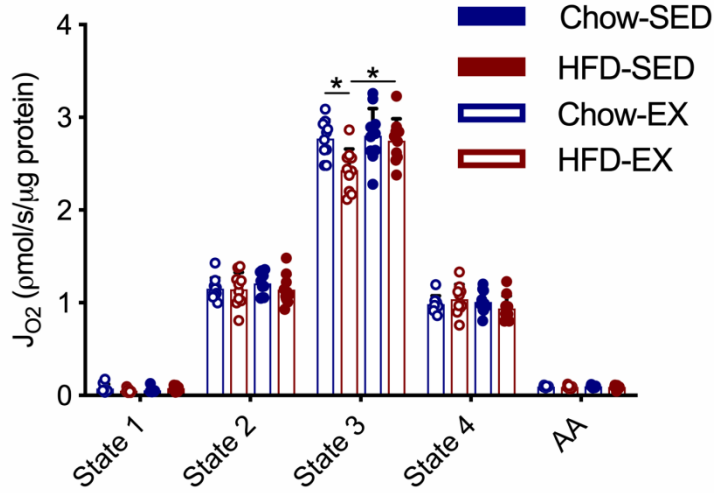
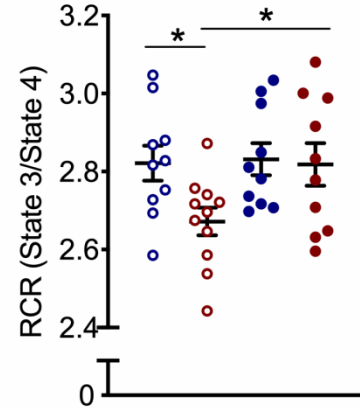
<sup>2</sup> Division of Biomedical Statistics and Informatics, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

<sup>3</sup> Department of Neurology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

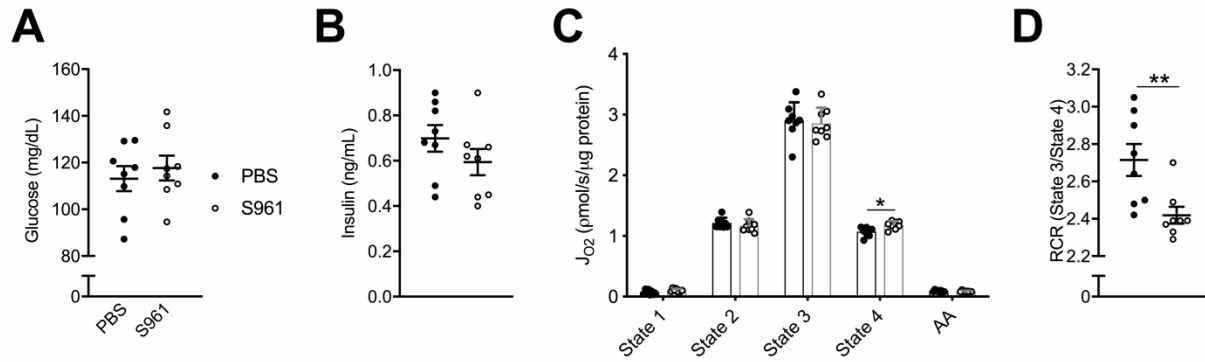
<sup>4</sup> Center for Multiple Sclerosis and Autoimmune Neurology, Mayo Clinic, Rochester, MN, USA.



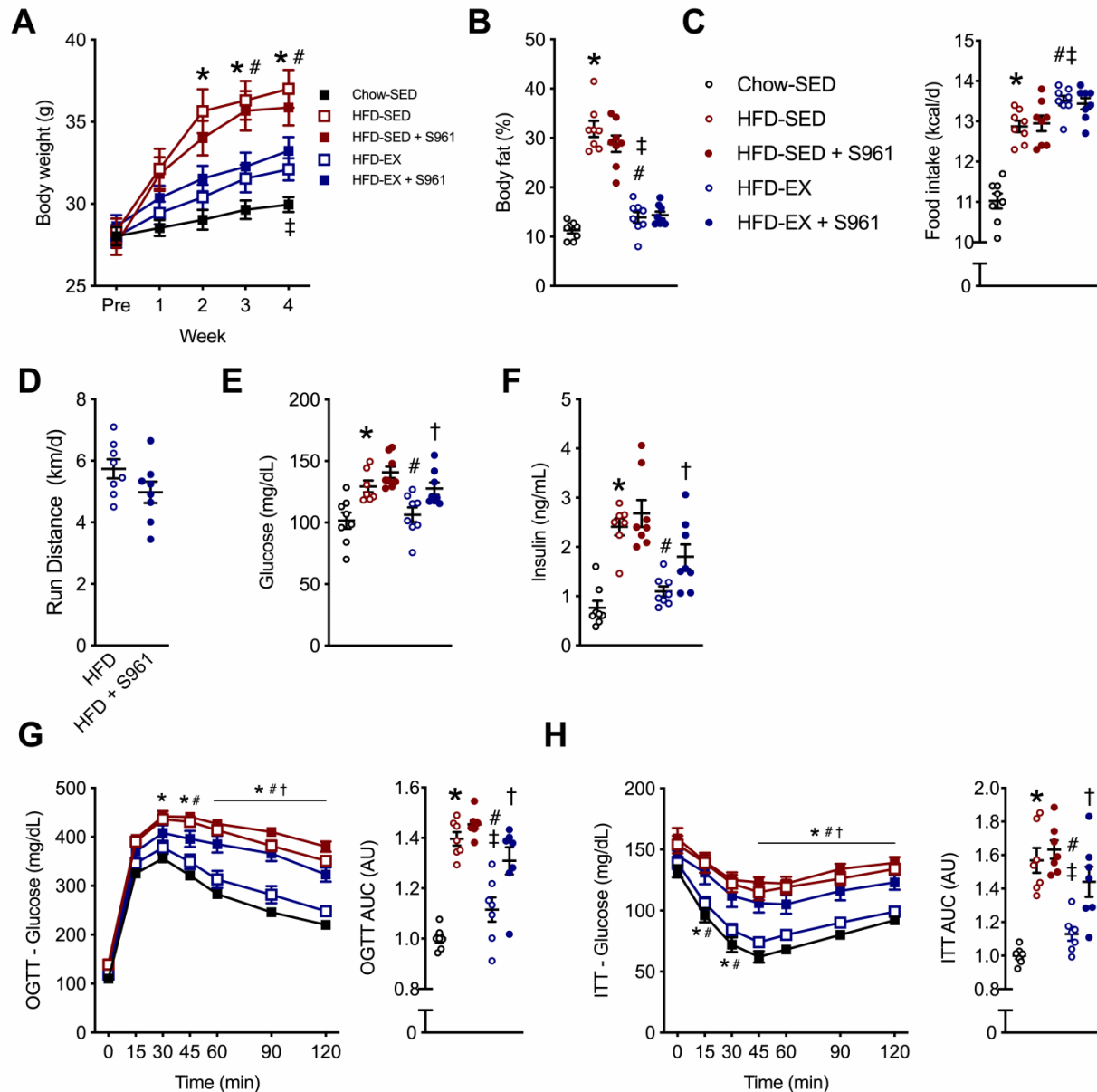
**Figure S1. Aerobic exercise attenuates weight gain, inflammation, and increased ceramide content accompanying HFD.** (A-B) 4 weeks of HFD led to greater body weight (A) and body fat (B), which was corrected in HFD-EX (n = 16-17) [for panel A: \*p < 0.05: HFD-SED vs. Chow-SED; # p < 0.05: HFD-EX vs. HFD-SED; ‡ p < 0.05: Chow-EX vs. Chow-SED]. (C) Food intake was greater in HFD-fed mice independent of exercise status, while exercise increased food intake in Chow-fed mice (n = 10). (D) HFD did not influence running distance (n = 17). (E) *Tnfα*, *Il1b*, and *Il6* mRNA expression was higher in the hypothalamus (hypo), hippocampus (hippo), and cortex of HFD-SED mice, which was partially attenuated in HFD-EX (n = 6). (F) C16:0, C18:0, C20:0, and C22:0 ceramides were greater in the hippocampus of HFD-SED, which was corrected in HFD-EX (n = 6). (G) *Cer1*, *Cer2*, *Cer4* mRNA expression in the hippocampus did not differ between groups (n = 6) Data represent means ± SEM and were analyzed with two-way ANOVA followed by Tukey's multiple comparisons post hoc test (A-G); \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

**A****B**

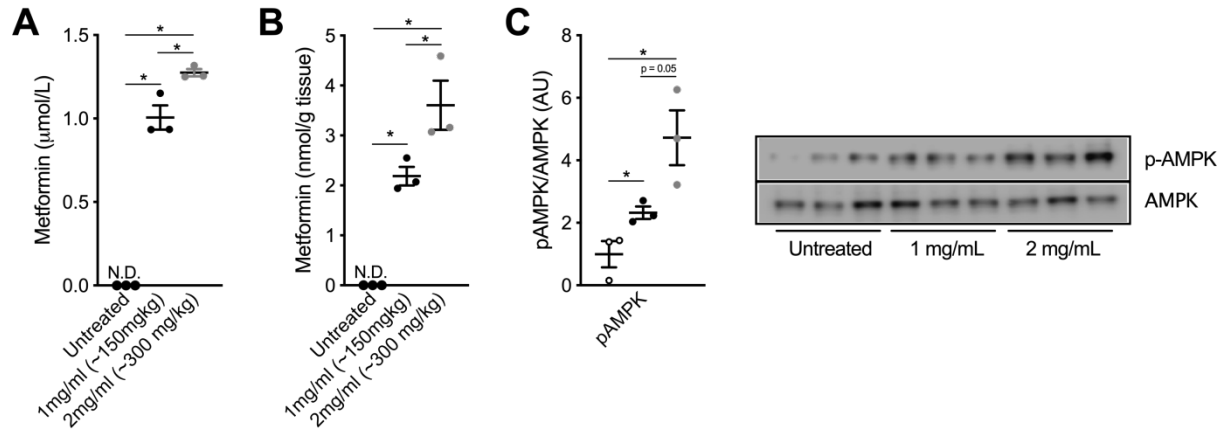
**Figure S2. Aerobic exercise corrects altered mitochondrial respiration accompanying HFD. (A)** State 3 mitochondrial respiration in isolated cerebral mitochondria was lower in HFD-SED, which was corrected in HFD-EX (n = 10-11). **(B)** The respiratory control ratio (RCR), which is indicative of mitochondrial coupling, was lower in HFD-SED, and normalized in HFD-EX (n = 10-11). Data represent means  $\pm$  SEM and were analyzed with two-way ANOVA followed by Tukey's multiple comparisons post hoc test; \* $p < 0.05$ .



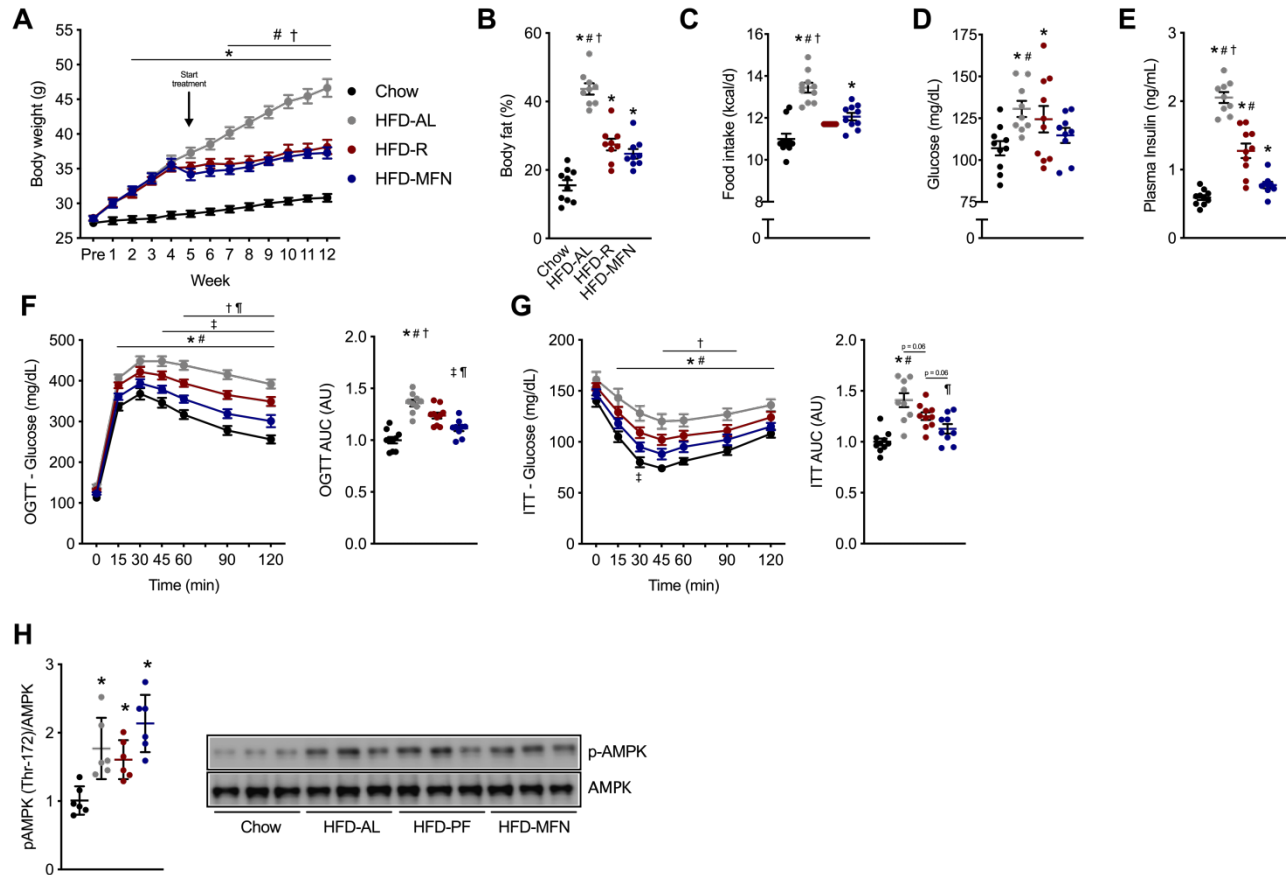
**Figure S3. Intranasal insulin receptor antagonism influences mitochondrial respiration.** (A-B) Daily intranasal injection of the insulin receptor antagonist S961 for 7 days did not influence plasma glucose (A) and insulin (B) concentrations (n = 8). (C) S961 increased state 4 mitochondrial respiration in isolated cerebral mitochondria (n = 8). (D) S961 decreased the respiratory control ratio (RCR), indicative of uncoupled mitochondrial respiration (n = 8). Data represent means  $\pm$  SEM and were analyzed with two-tailed Student's t-test; \*\*p < 0.01.



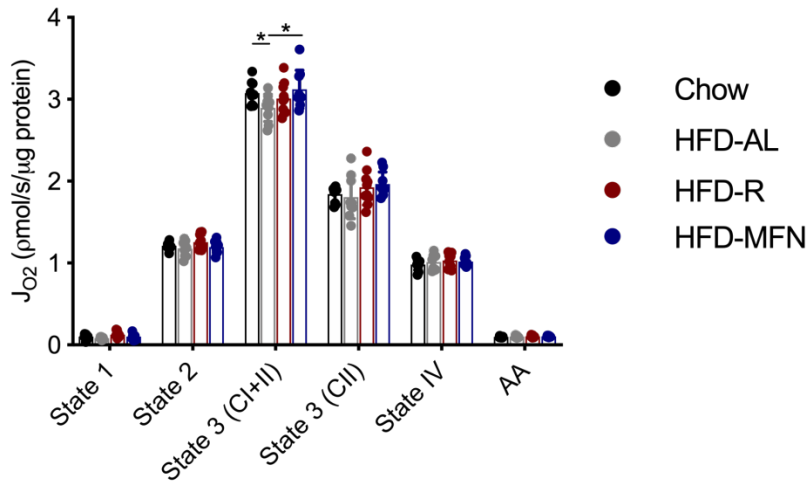
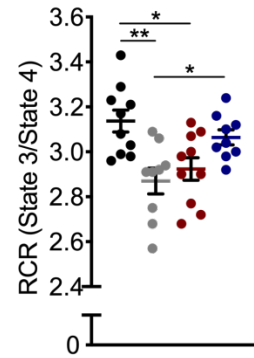
**Figure S4. Intranasal S961 blocks exercised-induced improvements in glucose and insulin tolerance. (A-B)** Exercise attenuated gains in body weight (**A**) and body fat (**B**) independent of S961 treatment ( $n = 8$ ). (**C**) HFD and exercise independently increased food intake independent of S961 treatment ( $n = 8$ ). (**D**) S961 did not alter voluntary running distance ( $n = 8$ ). (**E-F**) S961 prevented exercise-induced improvements in fasting plasma glucose (**E**) and insulin (**F**) in HFD-fed mice ( $n = 7$ ). (**G-H**) S961 prevented exercise-induced improvements in oral glucose tolerance (**G**) and intraperitoneal insulin tolerance (**H**) in HFD-fed mice ( $n = 7$ ). Data represent means  $\pm$  SEM and were analyzed with Mixed Model Analysis and Student's *t*-test post hoc comparisons; \* $p < 0.05$ : HFD-SED vs. Chow-SED; #  $p < 0.05$ : HFD-EX vs. HFD-SED; †  $p < 0.05$ : HFD-EX vs. HFD-EX + S961; ‡  $p < 0.05$ : HFD-EX vs. Chow-SED.



**Figure S5. Metformin crosses the blood-brain barrier.** (A-B) Metformin administration in drinking water dose-dependently increased metformin concentration in plasma (A) and hippocampal tissue (B) (n = 3). (C) Metformin administration in drinking water dose-dependently increased AMPK phosphorylation at Thr-172 in the hippocampus (n = 3). Data represent means  $\pm$  SEM and were analyzed with two-tailed Student's t-test; \*p < 0.05.



**Figure S6. Metformin improves whole-body glucose and insulin tolerance.** (A) Following 4-weeks of HFD, mice were provided ad libitum (HFD-AL), HFD provided ad libitum while receiving metformin (2mg/mL; ~300mg/kg/day) in the drinking water (HFD-MFN), or HFD restricted to 85% (HFD-PF) for 8 additional weeks. HFD-PF and HFD-MFN prevented further gains in body weight compared to HFD-AL ( $n = 9-10$ ). (B) HFD-MFN and HFD-PF attenuated gains in body fat compared to HFD-AL ( $n = 9-10$ ). (C) HFD-AL lead to greater food intake, which was partially attenuated in HFD-MFN ( $n = 9-10$ ). (D-E) HFD-AL increased fasting plasma glucose (D) and insulin (E) which were partially attenuated in HFD-AL and further reduced in HFD-MFN ( $n = 9-10$ ). (F-G) HFD-AL increased oral glucose tolerance (F) and intraperitoneal insulin tolerance (G) which were partially attenuated in HFD-AL and further reduced in HFD-MFN ( $n = 9-10$ ) (H) AMPK phosphorylation at Thr-172 in the hippocampus was increased by HFD independent of treatment ( $n = 6$ ). Data represent means  $\pm$  SEM and were analyzed with Mixed Model Analysis and Student's t-test post hoc comparisons. Symbols in panels A-E, H: \* $p < 0.05$  compared to Chow; #  $p < 0.05$  compared to HFD-MFN; †  $p < 0.05$  compared to HFD-AL. Symbols in panels F, G: \* $p < 0.05$ : Chow vs. HFD-AL; #  $p < 0.05$ : HFD-AL vs. HFD-MFN; †  $p < 0.05$ : HFD-AL vs. HFD-PF; ‡  $p < 0.05$ : HFD-PF vs. HFD-MFN; ¶  $p < 0.05$ : Chow vs. HFD-MFN.

**A****B**

**Figure S7. Metformin corrects altered mitochondrial respiration accompanying HFD. (A)** State 3 mitochondrial respiration in isolated cerebral mitochondria was lower in HFD-SED, which was corrected in HFD-MFN (n = 9-10). **(B)** The respiratory control ratio (RCR) was lower in HFD-SED and HFD-PF, and normalized in HFD-MFN (n = 9-10). Data represent means  $\pm$  SEM and were analyzed with Mixed Model Analysis and Student's t-test post hoc comparisons; \*p < 0.05, \*\* p < 0.01.



**Table S1. Individual protein differential expression data from the 159 significantly affected proteins shown in the Figure 3A volcano plot (Chow-SED vs. HFD-SED comparison).** Table lists protein symbols, and names as given by UniProt database, together with their expression ratio (in log<sub>2</sub> scale), FDR (in log<sub>2</sub> scale) and protein affiliate group as shown in Figure 3A (Group). Protein group was determined by cross-reference against the MitoCarta database from protein affiliation. Proteins are listed by log<sub>2</sub> fold-change from most up-regulated to most-downregulated in HFD-SED compared to Chow-SED.

<b>Gene Symbol</b>	<b>Log2 (HFD-SED/Chow-SED)</b>	<b>Log2FDR</b>	<b>Group</b>
H2-M10.3	29.81	7.32	
Ptpn12	28.68	7.38	
Ccdc175	27.29	7.07	
Mrpl2	27.08	7.32	Mitochondrial
Cwf19l2	26.92	4.60	
Thg1l	25.97	8.76	Mitochondrial
Mrpl53	25.89	8.14	Mitochondrial
Rundc3b	25.89	7.38	
Ccdc170	25.56	7.38	
Tifa	25.13	18.12	
Tor2a	24.48	5.71	
S100a1	24.39	7.37	
Ranbp17	23.44	5.77	
Cacna1h	21.85	7.28	
Ppig	20.82	4.94	
Unc5a	3.49	5.82	
Apoa4	3.15	8.55	
Ndufs6	2.97	4.76	Mitochondrial
Dnal1	2.73	5.86	
Vimp	2.61	5.70	
Lrrc20	2.21	19.55	
Ccdc115	2.13	5.87	
Fhl2	2.06	11.59	
Commd8	2.02	7.38	
Prrt1	1.97	11.39	
Fkbp1a	1.88	4.60	
H1fx	1.71	12.56	
Erc1	1.69	26.76	
Nrp2	1.66	7.20	
Pcsk2	1.62	7.47	
Ociad1	1.58	6.11	Mitochondrial
MacroD2	1.58	7.47	
Fmnl2	1.46	9.94	

Ddn	1.45	5.10	
Tceal6	1.44	6.68	
Lpin2	1.44	7.26	
Pdzd2	1.41	5.55	
Rwdd4	1.40	10.88	
Txn	1.38	7.87	Mitochondrial
Pycard	1.38	8.35	
Jph3	1.37	5.96	
Rpl18a	1.30	9.29	
Dbi	1.30	6.33	
Nek9	1.28	5.42	
Mif	1.26	10.40	
Ppp1cb	1.25	4.65	
Kiaa1671	1.23	6.24	
Cox5a	1.22	16.76	Mitochondrial
Tceal5	1.20	6.46	
Tmem263	1.19	4.39	
Fibp	1.16	4.52	
Pnck	1.12	4.94	
Crym	1.05	4.60	
Naip6	1.05	5.18	
Hpca	1.04	5.49	
Gm10020	1.02	10.15	
Nebi	1.02	17.19	
Synpr	1.02	7.79	
Trappc1	0.98	4.60	
Ptprs	0.96	18.21	
Arpc2	0.91	18.12	
Mtmr12	0.91	5.10	
Ccdc43	0.90	7.55	
Kbtbd11	0.89	5.42	
Asphd2	0.88	9.75	
Dgkb	0.88	6.33	
Ube2g2	0.87	7.38	
Capzb	0.87	4.68	
Hebp1	0.86	7.26	
Ncs1	0.82	6.33	
Ccsap	0.80	5.07	
Eif3d	0.80	6.56	
Frrs1l	0.80	6.74	
Kctd12	0.78	7.29	

Fdx1l	0.77	4.52	Mitochondrial
Begain	0.75	6.20	
Cpeb4	0.74	5.12	
Letmd1	0.74	7.38	Mitochondrial
Rogdi	0.73	7.22	
Anks1b	0.72	11.39	
Coq3	0.71	6.93	Mitochondrial
Srr	0.71	5.09	
Nfu1	0.71	5.77	Mitochondrial
Mcu	0.71	33.49	Mitochondrial
Faah	0.69	6.94	
Stxbp6	0.68	9.29	
Dcakd	0.66	4.52	Mitochondrial
Grb2	0.65	12.30	
Csnk2b	0.63	5.16	
Adprh	0.62	6.96	
Cttnbp2	0.60	12.80	
Cenpv	0.60	7.37	
Chchd6	0.59	5.96	Mitochondrial
Nptxr	0.59	5.30	
Mta1	0.58	5.42	
Map6d1	0.57	6.68	
Pebp1	0.55	10.01	
Mgll	0.54	7.26	
Sdcbp	0.53	6.25	
Ak4	0.53	7.59	Mitochondrial
Src	0.52	8.09	
Acy1	-0.51	19.63	
Gap43	-0.51	6.95	
Mettl7a1	-0.52	6.24	
Atg2b	-0.56	7.29	
Vat1	-0.60	5.73	
Acaa2	-0.61	4.42	Mitochondrial
Osbpl1a	-0.64	5.78	
Suc1g2	-0.65	4.65	Mitochondrial
Trim23	-0.65	5.98	
Aplp2	-0.67	7.78	
Enpp6	-0.67	5.86	
Mag	-0.69	8.74	
Syt2	-0.70	4.52	
Tpp1	-0.76	6.09	

9030624J02Rik	-0.78	4.76	
Btbd17	-0.79	12.30	
Eml1	-0.80	9.29	
Atpaf1	-0.89	4.46	Mitochondrial
Arf2	-0.93	6.71	
Pygm	-0.97	7.80	
Xdh	-1.04	6.89	
Sccpdh	-1.05	12.30	Mitochondrial
Fntb	-1.06	4.73	
Astn2	-1.08	7.38	
Esyt1	-1.12	15.34	
Ptgfm	-1.19	9.46	
Trdn	-1.21	20.27	
Limk1	-1.24	7.60	
Ctnna3	-1.36	5.46	
Serpinh1	-1.40	7.75	
Itih5	-1.47	4.69	
Cbln1	-1.72	5.47	
Elovl5	-1.75	8.57	
Hbs1l	-1.78	8.26	
C3	-1.85	12.30	
Kcnj10	-1.97	4.82	
Rplp2	-2.32	5.27	
Krt79	-2.69	5.71	
Krt13	-2.74	5.86	
Zmat4	-2.76	7.85	
Pcdha9	-2.88	11.56	
Fxyd1	-3.28	6.10	
Faxc	-3.29	5.96	
Lsm3	-3.88	5.42	
Rbx1	-3.98	8.44	
mt-Nd3	-23.72	7.38	
P2rx5	-24.08	7.38	
Gm5814	-24.67	7.38	
Nexn	-25.05	7.38	
Uaca	-25.15	6.47	
Ccdc12	-25.16	7.09	
Fbxo45	-25.53	7.29	
Spag1	-25.77	7.38	
Trpv3	-27.74	6.93	
Wdr93	-28.12	7.38	

Timm10b	-28.68	10.06
Ddx18	-29.67	7.20
Efcab5	-30.77	7.06

---

**Table S2. Individual protein differential expression data from the 80 significantly affected proteins shown in the Figure 3B volcano plot (HFD-EX vs. HFD-SED comparison).** Table lists protein symbols, and names as given by UniProt database, together with their expression ratio (in log2 scale), FDR (in log2 scale) and protein affiliate group as shown in Figure 3A (Group). Protein group was determined by cross-reference against the MitoCarta database from protein affiliation. Proteins are listed by log2 fold-change from most up-regulated to most-downregulated in HFD-EX compared to HFD-SED.

<b>Gene Symbol</b>	<b>Log2 (HFD-EX/HFD-SED)</b>	<b>Log2FDR</b>	<b>Group</b>
Chst2	30.57928945	10.3499198	
Wdr93	27.87785762	6.13060635	
Fndc3a	27.56561228	5.85679016	
Arx	27.38796895	6.00818213	
Uaca	26.85925458	6.13060635	
Gripap1	26.18921249	6.13060635	
Dnm3	25.9136367	6.08532642	
Pan3	25.67784477	5.96363029	
Pus7	25.08679061	5.42132934	
Gbp7	24.86270322	6.13060635	
Fam84a	24.78288049	5.89018752	
Ces1d	24.386462	4.99602708	
Olf1366	3.939230252	6.13060635	
Itpr3	2.949655009	5.31161532	
Hbs1l	2.413692778	7.51419831	
Lpar1	2.17448437	5.41401313	
Atp6v0a2	2.000232684	6.8173756	
Slc38a3	1.254305173	5.85374218	
Ppip5k2	1.246130073	6.8173756	
Otud7b	1.063255236	6.13060635	
Ptgfn	1.025359494	10.4622151	
Mettl7a1	0.663729263	6.4515734	
Prodh	-0.587383818	4.790666921	Mitochondrial
Ccdc43	-0.634767352	5.2344249	
Crat	-0.67250904	12.4479769	Mitochondrial
Gpsm2	-0.699883875	6.8743411	
Ptprs	-0.768926624	7.1885077	
Ttyh3	-0.860426922	5.45204551	
Cox6c	-0.920196554	6.86229361	Mitochondrial
Ankrd34a	-1.056863136	4.50658769	
Atp5l	-1.094192618	8.86166319	Mitochondrial
Stmn2	-1.154278813	14.8007901	
Camk2b	-1.154793661	5.61282458	

Cox5a	-1.183274643	5.41401313	Mitochondrial
Metap1	-1.192342594	11.1492491	
Stxbp3	-1.217905691	5.83935327	
Tceal5	-1.250398282	5.96363029	
Mrpl43	-1.342351215	5.42132934	Mitochondrial
Ube2g2	-1.370964244	15.525809	
Mapkap1	-1.37353765	6.13060635	
Dyrk1a	-1.375855975	7.42105644	
Pdzd2	-1.875528048	10.4622151	
Ppp1r16b	-1.96748281	10.4622151	
Ppcdc	-1.985164093	7.42105644	
Amdhd2	-2.142626617	6.13060635	
Clpx	-2.36855178	5.61282458	Mitochondrial
Cdkn2aip	-3.077933467	10.3499198	
Dennd5a	-3.421933907	15.2782696	
Mtmr14	-3.9753476	5.94862	
C12orf65	-4.130136709	6.70971713	Mitochondrial
Utp3	-4.253815001	6.13060635	
Nmnat1	-25.70029648	6.10109022	
Dnajc27	-25.72784327	6.9412687	
Ostc	-26.19482399	6.13060635	
Nt5e	-26.21538337	6.21402166	
Trmt44	-26.80912546	7.36611593	
Atxn3	-29.47760787	6.08532642	
Gm17415	-6.501950404	6.83648462	
Ebp	-3.312758189	10.7824257	
Ccdc134	-1.957238948	4.9939141	
Kcnj10	-1.907217163	5.4409846	
Wrb	-1.126584825	6.09831279	
Slc30a3	-0.813709429	6.64611542	
Arhgap25	-0.779785274	6.23952888	
Rasgrp1	-0.77316913	6.37323827	
Rap1a	0.538033207	5.12125095	
Mtss1l	0.553037652	7.87523597	
Cdc16	0.553836409	10.2657849	
Cd34	0.660868114	6.64315909	
Dnajc7	0.77476249	6.68089697	
Dnajc24	0.906220253	5.88190255	
Ddx50	1.023922559	6.08516303	
Fam103a1	1.080369402	6.00704841	
Ppp1r2	1.129974926	6.74563102	

Papss2	1.155776423	5.83332444
Tceal6	1.186501871	6.89431781
Clstn3	0.981779744	9.4032018
Rnf14	0.908344442	7.3389257
Ncs1	-0.641759063	7.59865927
Atad3	-0.691043267	6.58289573

---



**Table S3. Individual protein oxidation data from the 244 significantly affected proteins shown in the Figure 3A volcano plot (Chow-SED vs. HFD-SED comparison).** Table lists protein symbols, and names as given by UniProt database, together with their expression ratio (in log<sub>2</sub> scale), p-value (in -log scale) and protein affiliate group as shown in Figure 3C (Group). Protein group was determined by cross-reference against the MitoCarta database from protein affiliation. Proteins are listed by log<sub>2</sub> fold-change from most up-regulated to most-downregulated in HFD-SED compared to Chow-SED.

Gene Symbol	Log <sub>2</sub> (HFD-SED/Chow-SED)	-Log (P-value)	Group
PPP3CB	23.94	1.40	
ATP6AP2	22.72	1.40	
FYN	22.64	1.40	
DCXR	22.47	2.40	
MRPL15	22.42	1.40	Mitochondrial
VIMP	22.29	1.40	
BNIP1	21.54	1.40	
SLC25A19	21.52	1.40	Mitochondrial
2810459M11Rik	21.35	2.40	
ctsl	21.12	2.40	
CCDC170	21.11	2.40	
DPCD	21.11	1.40	
WDYHV1	20.92	2.40	
TTLL12	20.85	1.40	
RGMA	20.56	1.40	
RNASE4	20.48	1.40	
TRIP12	20.38	1.40	
RCC2	19.95	1.40	
MYO9A	18.72	1.40	
MTA1	17.39	1.40	
ppp1cb	3.95	2.64	
EIF2A	2.18	1.67	
AK4	1.85	1.43	
GMFB	1.85	2.76	
STXBP1	1.82	2.41	
NCAN	1.77	1.60	
ITPA	1.62	2.29	
LRRC20	1.59	1.79	
RAP1B	1.54	2.81	
RAP1A	1.46	1.52	
CPNE6	1.45	2.89	
CAB39	1.43	1.57	
RGS14	1.42	2.16	

PGAM2	1.41	1.40	
Hbb-b1	1.39	1.32	
PRDX2	1.25	1.66	Mitochondrial
CPLX2	1.24	1.35	
PHYHIPL	1.24	3.25	Mitochondrial
ATAD3A	1.19	1.44	Mitochondrial
WASF2	1.15	3.88	
pcsk2	1.14	1.54	
PPIG	1.14	1.53	
RPS19	1.10	1.55	
epha6	1.09	1.53	
METAP1	1.09	1.53	
HPCAL1	1.06	2.38	
MLST8	1.04	1.53	
COMMD10	1.01	2.55	
SHISA6	1.00	1.66	
HPCA	0.98	3.10	
2900026A02Rik	0.98	1.97	
AK1	0.97	2.96	
WDR61	0.96	1.42	
PRRT1	0.96	2.62	
APOA1	0.94	1.49	
RAP2C	0.92	1.79	
BID	0.92	1.51	Mitochondrial
NUDT11	0.91	1.44	
Cox5b	0.90	1.86	Mitochondrial
CAPZB	0.89	2.25	
TAGLN3	0.89	2.11	
Cdk3-ps	0.88	2.04	
TCEAL6	0.88	1.86	
CALM1	0.87	1.40	
VPS37B	0.86	2.05	
NIPSNAP3B	0.82	2.24	Mitochondrial
EVA1A	0.82	2.29	
RGS7	0.81	1.68	
C1QA	0.81	2.52	
ITPKA	0.80	2.90	
ARPC5	0.79	2.13	
Gm5160	0.79	1.47	
RBBP9	0.78	1.39	
HPCAL4	0.77	1.65	

TIMM23	0.77	2.51	Mitochondrial
MRPS25	0.76	1.73	Mitochondrial
SNRPB	0.75	2.52	
EIF3D	0.75	1.49	
TRAPPC1	0.75	1.49	
UBE2I	0.74	1.64	
PYCR2	0.74	1.45	
CAMK2B	0.74	1.35	
GRB2	0.73	1.49	
DHX38	0.72	1.31	
NDUFB6	0.70	1.39	Mitochondrial
Csl	0.70	1.33	
ACADVL	0.69	2.08	Mitochondrial
HEBP1	0.69	2.03	
ARPC2	0.69	3.63	
NEBL	0.68	2.18	
COQ9	0.68	3.30	Mitochondrial
U2AF1	0.67	1.58	
ANKS1B	0.67	2.16	
SAR1B	0.65	2.44	
SNX4	0.65	1.57	
PRKCE	0.64	1.79	
SCN2B	0.64	2.37	
MIF	0.63	1.89	
TMED4	0.63	1.33	
SLC25A22	0.62	3.11	Mitochondrial
RAC3	0.62	1.33	
ARAF	0.62	2.48	
CTTN	0.62	1.56	
CPPED1	0.62	2.49	
2610301B20Rik	0.62	1.91	
CAMKV	0.60	2.15	
YWHAQ	0.60	2.00	
JPH3	0.59	1.50	
ADPRH	0.59	2.19	
PSMB7	0.59	2.92	
ACOT13	0.57	1.36	
PLCB4	0.57	2.30	
BCAP29	0.56	1.33	
TOMM34	0.56	2.78	Mitochondrial
GNB4	0.55	2.87	

DBT	0.55	1.76	Mitochondrial
DHDH	0.55	1.66	
Gstp3	0.54	1.45	
prkcg	0.54	1.81	
YWHAB	0.54	1.59	
NUMBL	0.54	1.32	
IFT22	0.53	1.33	
NECAP1	0.53	1.94	
PPP6C	0.53	1.41	
SRSF1	0.53	1.51	
PRPS2	0.53	1.34	
NDUFB11	0.53	2.21	Mitochondrial
SYN2	0.52	1.43	
MECR	0.52	1.40	Mitochondrial
GSTP1	0.51	1.37	
LRRTM1	0.51	1.34	
LYPLA2	0.51	1.38	
ISCU	0.51	1.46	Mitochondrial
CNRIP1	0.50	2.23	
ARMCX3	-0.51	1.34	
HADHB	-0.51	3.43	Mitochondrial
TMED2	-0.52	1.32	
SERPING1	-0.53	2.64	
Gm11214	-0.54	2.24	
TMX3	-0.55	1.33	
TUBA1B	-0.55	1.95	
NDRG4	-0.56	2.31	
SDK2	-0.56	2.47	
PYGM	-0.57	2.35	
ERP44	-0.61	1.47	
ATP1B1	-0.62	3.33	
HSP90AA1	-0.62	1.32	
h2afy	-0.62	2.10	
TACC2	-0.62	1.48	
PZP	-0.64	1.98	
ATG7	-0.68	1.88	
cyp46a1	-0.69	1.32	
Fam177a	-0.70	1.51	
UBA5	-0.71	1.71	
ENPP6	-0.73	1.51	
EFTUD2	-0.76	1.50	

TLN1	-0.77	1.41	
DNAJA2	-0.78	1.88	
BAG6	-0.79	1.61	
CMAS	-0.80	2.28	
GNAS	-0.80	1.39	
PRKAR2A	-0.80	1.39	
TNPO3	-0.80	1.41	
ADSL	-0.81	1.40	Mitochondrial
G6pdx	-0.82	1.37	
VARs2	-0.82	2.28	
OSBPL1A	-0.83	1.81	
CTNND1	-0.84	1.84	
NUP160	-0.86	1.80	
PIP4K2A	-0.88	1.79	
cpt2	-0.89	1.91	Mitochondrial
EEF1A1	-0.93	1.37	
TROVE2	-0.96	1.40	
PRKRA	-0.98	2.54	
BTBD17	-0.99	1.62	
SYMPK	-1.00	1.63	
NDUFA3	-1.01	1.31	Mitochondrial
FAM169A	-1.01	1.62	
IPO4	-1.01	1.60	
PGM1	-1.02	1.90	
YLPM1	-1.02	1.42	
CHERP	-1.05	2.54	
FAM151B	-1.08	1.53	
KIF13B	-1.08	1.51	
ATPAF1	-1.14	1.57	Mitochondrial
Srp54a	-1.15	1.38	
UBAP2	-1.18	1.36	
Pakap	-1.20	1.37	
CORO2A	-1.22	2.31	
USP8	-1.23	1.42	
CCDC85B	-1.25	1.56	
OSBPL8	-1.27	1.58	
TSC22D1	-1.40	2.30	
ARID1A	-1.49	1.35	
NT5C	-1.49	1.32	Mitochondrial
atg2b	-1.57	1.46	
FABP3	-1.61	1.66	

Supt16	-1.68	1.55	
abca2	-1.69	1.88	
SLC25A13	-1.69	2.63	Mitochondrial
RPLP2	-1.72	1.82	
RBX1	-2.15	2.42	
RGS17	-2.34	2.06	
PVALB	-2.37	1.67	
VAT1L	-2.43	1.94	
AHNAK2	-2.91	1.33	
MYH8	-18.49	1.39	
WDR36	-18.70	1.40	
TMEM165	-19.13	1.40	
CBLN1	-19.14	1.39	
HCN4	-19.18	2.40	
ADCYAP1R1	-19.48	1.40	
CNEP1R1	-19.55	1.40	
RHOG	-19.74	1.40	
P2RX5	-19.86	2.40	
PSEN1	-20.01	1.40	
PPM1L	-20.05	1.39	
LRRC40	-20.13	2.40	
FAM65A	-20.27	1.40	
FUBP3	-20.31	1.40	
SNX25	-20.55	1.40	
RDH11	-20.56	1.40	
PRKAG1	-20.58	2.40	
PBDC1	-20.66	1.40	
KRT16	-20.69	1.40	
FMN2	-20.77	1.39	
ADAR	-20.90	1.40	
GPRASP1	-21.01	1.40	
RPRD1B	-21.13	1.40	
ANXA4	-21.56	1.40	
NHP2L1	-21.62	2.40	
PHKB	-21.70	1.40	
CFAP221	-21.79	1.39	
asic1	-22.03	2.40	
TIMM10B	-22.24	11.04	
MOB4	-22.30	2.40	
CDC42EP4	-22.30	1.40	
WASH1	-22.52	2.40	

CSTB	-22.66	1.40
SELENBP1	-22.85	1.40
TUBA8	-22.91	1.40
SNRNP70	-23.43	1.40
RBMXL1	-24.08	2.40
WDR93	-24.41	2.40

---

**Table S4. Individual protein oxidation data from the 244 significantly affected proteins shown in the Figure 3D volcano plot (HFD-EX vs. HFD-SED comparison).** Table lists protein symbols, and names as given by UniProt database, together with their expression ratio (in log2 scale), p-value (in -log scale) and protein affiliate group as shown in Figure 3C (Group). Protein group was determined by cross-reference against the MitoCarta database from protein affiliation. Proteins are listed by log2 fold-change from most up-regulated to most-downregulated in HFD-EX compared to HFD-SED.

<b>Gene Symbol</b>	<b>Log2 (HFD-EX/HFD-SED)</b>	<b>-Log (P-value)</b>	<b>Group</b>
WDR93	24.23	2.40	
TBC1D10A	22.45	1.40	
EPHX1	22.44	1.40	
MOB4	22.43	1.39	
CFAP221	22.32	13.08	
SNX17	22.07	1.40	
QTRTD1	22.04	1.40	
WASH1	21.77	2.40	
VDAC2	21.58	1.40	Mitochondrial
NPTX1	21.52	1.39	
RPRD1B	21.42	1.40	
MAGEE1	21.33	1.40	
GUF1	21.32	2.40	Mitochondrial
LRRFIP1	21.29	1.39	
PPM1L	21.13	1.40	
FUBP3	21.12	1.40	
PBDC1	21.10	2.40	
PYGL	20.65	2.40	
MATN4	20.59	1.40	
PSMG1	20.43	2.40	
TOP1	20.33	1.40	
KRT16	20.02	13.37	
FAM65A	19.92	15.06	
RDH11	19.84	1.40	
ADCYAP1R1	19.65	1.40	
ANKRD17	19.64	11.97	
CAV2	19.43	2.40	
SLC22A4	18.64	1.40	Mitochondrial
AGO3	4.43	1.45	
TRHDE	3.76	3.02	
WIPI2	1.85	1.32	
ZMPSTE24	1.78	1.58	
SLC6A11	1.66	2.74	



Pakap	1.58	1.50	
WRB	1.57	1.47	
LPCAT3	1.56	1.61	
atg2b	1.52	1.47	
CNTN5	1.34	2.59	
SMARCA4	1.32	2.44	
ITPA	1.29	2.08	
NEDD4L	1.22	1.78	
BRCC3	1.17	2.01	
ADCY2	1.12	1.47	
EIF3C	1.10	1.60	
PRKCD	1.06	1.56	
HSP90AA1	1.06	2.64	
ABCB8	1.01	1.72	Mitochondrial
SYMPK	0.98	1.56	
TNPO3	0.94	1.64	
HECW1	0.93	1.37	
NCAM1	0.90	1.35	
DHX36	0.86	2.19	
DIAPH1	0.85	1.38	
SLC1A4	0.80	1.63	
PZP	0.78	1.73	
CAPRIN1	0.71	1.58	
ARCN1	0.68	1.33	
MAP4K5	0.67	1.48	
PSMC1	0.65	1.49	
VPS52	0.65	1.49	
CMAS	0.63	1.65	
UBA5	0.62	1.45	
TSC22D3	0.62	1.62	
DNAJA2	0.62	1.69	
kif1bp	0.59	1.85	
NCOA7	0.58	1.46	
ATP1B1	0.57	2.45	
TUBA1B	0.55	1.94	
STRIP1	0.55	1.38	
IGSF21	0.53	1.64	
XRCC5	0.53	2.48	
QARS	0.50	1.57	
ATAD3A	-0.50	2.57	Mitochondrial
2610301B20Rik	-0.50	1.67	

MRPL50	-0.50	1.45	Mitochondrial
SUMO1	-0.51	1.80	
YWHAB	-0.52	1.69	
MRPS10	-0.53	2.46	Mitochondrial
ACOT13	-0.54	1.41	
map4k4	-0.57	2.31	
Zfp593	-0.59	1.32	
NECAP1	-0.60	1.40	
CALM1	-0.61	1.43	
SLC25A27	-0.61	1.38	Mitochondrial
FKBP5	-0.61	2.32	
MOGS	-0.62	2.31	
STXBP3	-0.63	1.64	
SNX5	-0.63	1.44	
PPIL1	-0.64	1.48	
RAC3	-0.64	1.61	
MIF	-0.65	1.98	
EEF1E1	-0.65	1.53	
SNRPC	-0.67	1.68	
OPALIN	-0.68	2.13	
UBE2I	-0.70	1.39	
FARSB	-0.72	1.94	
UBE2N	-0.73	1.97	
TCEAL6	-0.73	1.86	
PFDN5	-0.73	1.61	
COQ10A	-0.74	1.71	Mitochondrial
CPPED1	-0.74	1.76	
POLR2H	-0.81	1.34	
PLCB4	-0.82	2.29	
PDZD2	-0.83	3.01	
CRAT	-0.84	1.59	Mitochondrial
ACADVL	-0.87	2.42	Mitochondrial
SNX1	-0.87	1.54	
PRODH	-0.89	2.84	Mitochondrial
S100A16	-0.92	1.32	
EIF3D	-0.92	2.02	
Gm5160	-0.96	1.76	
AHCYL1	-0.98	1.43	
PHYHIPL	-0.98	2.73	Mitochondrial
HEXB	-1.06	1.78	
SCN2B	-1.07	1.61	

Car4	-1.18	1.83	
APOO	-1.41	1.76	
ENTPD2	-1.43	1.68	
STXBP1	-1.43	1.49	
TCEAL5	-2.00	1.64	
SLC25A19	-2.52	1.40	Mitochondrial
AKT3	-22.17	1.39	

---

**Table S5. Relevant primary and secondary antibodies.**

Protein	Source	Identifier
pAKT-Ser473	Cell Signaling Technology	Cat. # 9271
AKT	Cell Signaling Technology	Cat. # 9272
pGSK3B-Ser9	Cell Signaling Technology	Cat. # 9336
GSK3B	Cell Signaling Technology	Cat. # 9315
Insulin receptor	Cell Signaling Technology	Cat. # 3025S
IDE	Abcam	Cat. # 32216
MFN1	ThermoFisher Scientific	Cat. # PA5-67905
MFN2	ThermoFisher Scientific	Cat. # PA5-42171
OPA1	Novus Biologicals	Cat. # 59770
pDRP1-Ser616	Cell Signaling Technology	Cat. # 3455S
DRP1	Santa Cruz Biotechnology	Cat. # 101270
pAMPK-Thr172	Santa Cruz Biotechnology	Cat. # 33524
AMPK	Santa Cruz Biotechnology	Cat. # 74461
Ubiquitin	Cell Signaling Technology	Cat. # 3933S
VDAC	Cell Signaling Technology	Cat. # 4866S
B-actin	LI-COR Biosciences	Cat. # 926-42212
Anti-rabbit IgG	Cell Signaling Technology	Cat. # 7074
Anti-mouse IgG	Cell Signaling Technology	Cat. # 7076

**Table S6. qPCR primers used to assess mtDNA copy number.**

Gene	Forward (5'- 3')	Reverse (5'- 3')
28S	TGGGAATGCAGCCCAAAG	CCTTACGGTACTTGTTGACTATCG
Nd1	AAGGAGAATCAGAATTAGTATCAGG GTT	TAGTACTCTGCTATAAAGAATAACGCGA AT
Nd4	TCCAACACTACGAACGGATCCA	AAGTGGGAAGACCATTTGAAGTC

**Table S7. qRT-PCR primers for gene expression analysis.**

Gene	Forward (5'- 3')	Reverse (5'- 3')
Gapdh	ACCCTTAAGAGGGATGCTGC	G TTCACACCGACCTTCACCA
mt-Nd1	ATTACTTCTGCCAGCCTGACC	GGCCCGGTTTGTCTGCTA
mt-Cyb	GGCTACGTCCTTCCATGAGG	TGGGATGGCTGATAGGAGGT
mt-Co1	AGAAGCAGGAGCAGGAACAG	CTGTGTTATGGCTGGGGTT
mt-Atp6	GTGGAAGGAAGTGGGCAAGTGAGC	GCCATTCCACTATGAGCTGGAGCC
Ndufa9	TTCGGGTTGTTAGAGCGCTT	TGTGACCCCATTCGTCCAAG
Sdhb	CAGGAGGGCAAGCAACAGTA	CAGGCGTTCCTCTGTGAAGT
Uqcrc1	ACATTGGTCGCAGTCTCCTG	CGGATCCGGTTGTAGTCTGG
Cox4i1	CAGAAGGCGCTGAAGGAGAA	ATCAGAACGAGCGCAGTGAA
Atp5a	TCCGAGAAGCTGCAAGGATG	TCAACAGACGTGTCAGCTCC
Tfam	TCCGCCCTATAAGCATCTTG	CCGAGGTGGTTTTTCATCTGT
Pgc1a1	GGACATGTGCAGCCAAGACTCT	CACTTCAATCCACCCAGAAAGCT
Pgc1a2	CCACCAGAATGAGTGACATGGA	G TTCAGCAAGATCTGGGCAA
Pgc1a4	TCACACCAAACCCACAGAAA	CTGGAAGATATGGCACAT
Tnfa	ATGAGCACAGAAAGCATGA	AGTAGACAGAAGAGCGTGGT
Il1b	GTGCTGTCGGACCCATATGA	AGGCCACAGGTATTTTGTCTGT
Il6	CCTCTGGTCTTCTGGAGTACC	ACTCCTTCTGTGACTCCAGC
CerS1	GCCACCACACACATCTTTTCGG	GGAGCAGGTAAGCGCAGTAG
CerS2	AGAGTGGGCTCTCTGGACG	CCAGGGTTTATCCACAGTGAC
Cers4	CTGTGGTACTGTTGTTGCATGAC	GCGCGTGTAGAAGAAGACTAAG