

Supplementary Material

Manuscript title:

Haplotype variability in mitochondrial rRNA predisposes to metabolic syndrome

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Contents:

Supplementary Tables 1-3

Supplementary Figures 1-9

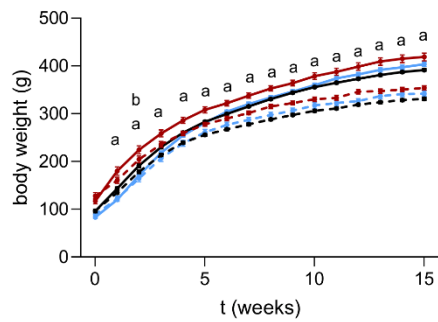
Supplementary table 1. Amino acid (AA) substitutions caused by mitochondrial DNA variants in mtSHR, mtF344 and mtBN strains.

OXPPOS gene	AA No.	mtSHR	mtF344	mtBN
<i>mt-Nd2</i>	18	Ala	Val	Val
	150	Ser	Asn	Asn
	244	Ala	Ala	Thr
	265	Thr	Ala	Ala
	304	Met	Thr	Met
	316	His	1AA del	His
<i>mt-Nd4</i>	23	Thr	Ile	Ile
	356	Thr	Ala	Ala
	393	Ile	Met	Ile
	401	Ile	Val	Val
	405	Met	Met	Ile
<i>mt-Nd6</i>	30	Phe	Phe	Leu
	139	Val	Ile	Ile
<i>mt-Cytb</i>	214	Asn	Asp	Asp
	334	Val	Val	Ile
<i>mt-Co1</i>	2	Phe	Phe	Leu
	406	Asn	Asp	Asn
<i>mt-Co2</i>	165	Val	Ile	Val
<i>mt-Atp6</i>	35	Lys	Glu	Glu
<i>mt-Atp8</i>	58	Ile	Ile	Thr

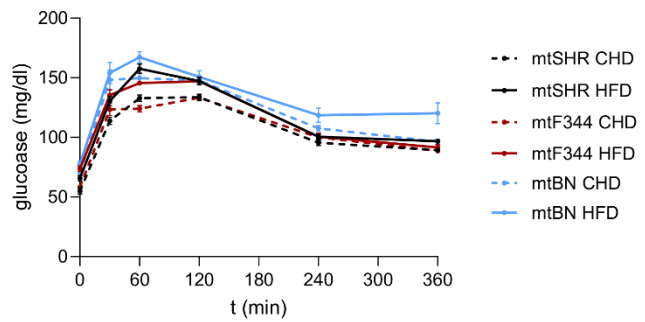
Supplementary table 2. Mitochondrial DNA variants in transfer and ribosomal RNA genes in mtF344 and mtBN strains compared to mtSHR. Nucleotide No.: position in mtSHR (genome numbering). NC – no change in sequence with respect to mtSHR sequence.

	gene	Nucleotide No.	mtF344	mtBN
mt-tRNA	<i>Phe</i>	m.32A	del A	del A
		m.52C	ins A	ins A
	<i>Cys</i>	m.5198G	G>A	G>A
		m.5200G	G>A	G>A
	<i>Tyr</i>	m.5235A	A>T	A>T
		m.5267C	C>G	C>G
	<i>Asp</i>	m.6976A	A>G	A>G
	<i>His</i>	m.11540A	NC	A>G
	<i>Thr</i>	m.15331A	A>G	NC
	<i>Pro</i>	m.15398C	NC	C>T
mt-rRNA	<i>mt-Rnr1</i>	m.648C	NC	C>A
		m.935G	G>A	NC
		m.942T	T>C	NC
	<i>mt-Rnr2</i>	m.1099C	NC	C>T
		m.1132C	del C	NC
		m.1137C	C>A	C>A
		m.1223G	G>A	NC
		m.1248T	T>C	NC
		m.1521A	A>G	A>G
		m.1585C	C>T	C>T
		m.1653T	ins AC	NC
		m.1693T	NC	T>C
		m.1716C	C>T	C>T
		m.1832A	A>G	NC
		m.1918A	NC	G>A
		m.2170C	C>T	NC
m.2647T	NC	del T		

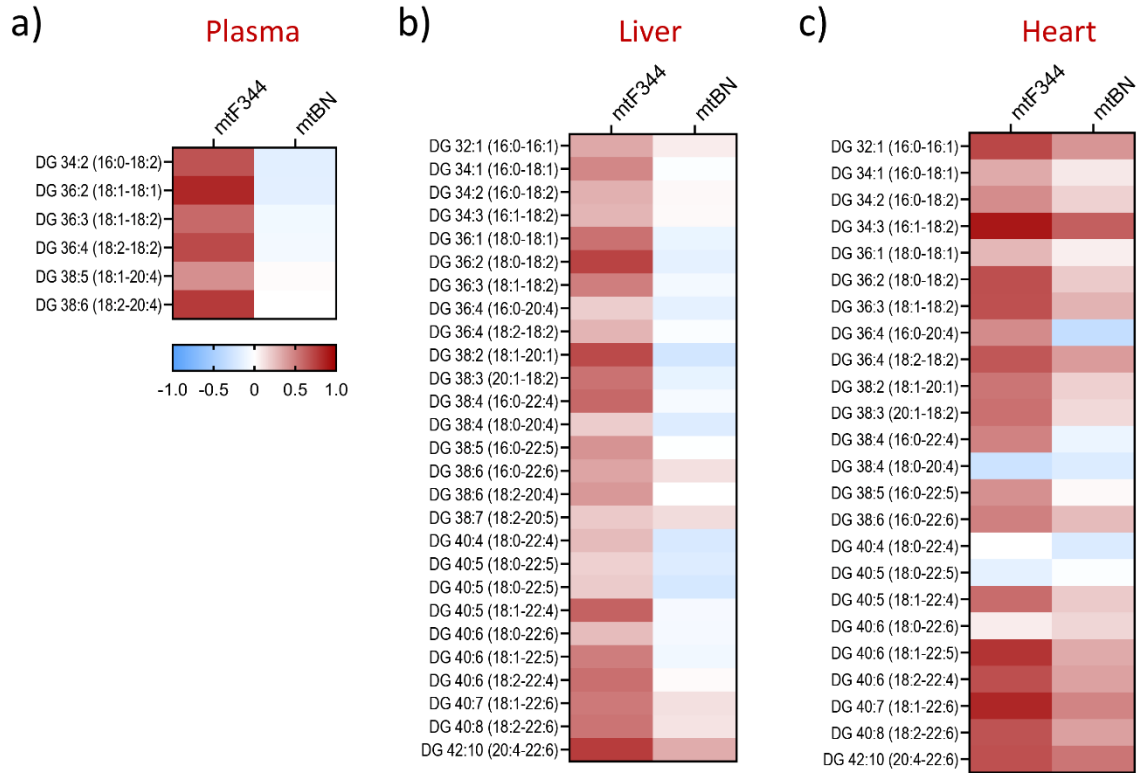
a)



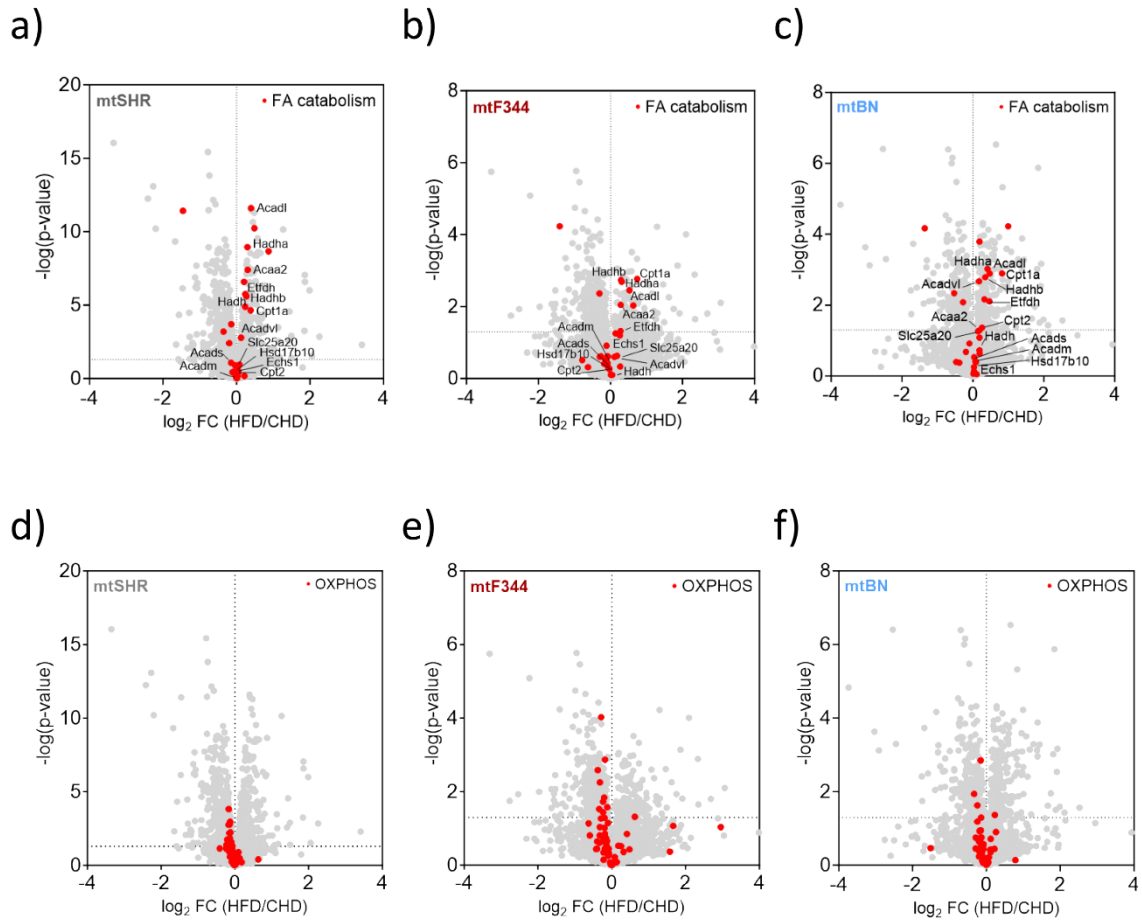
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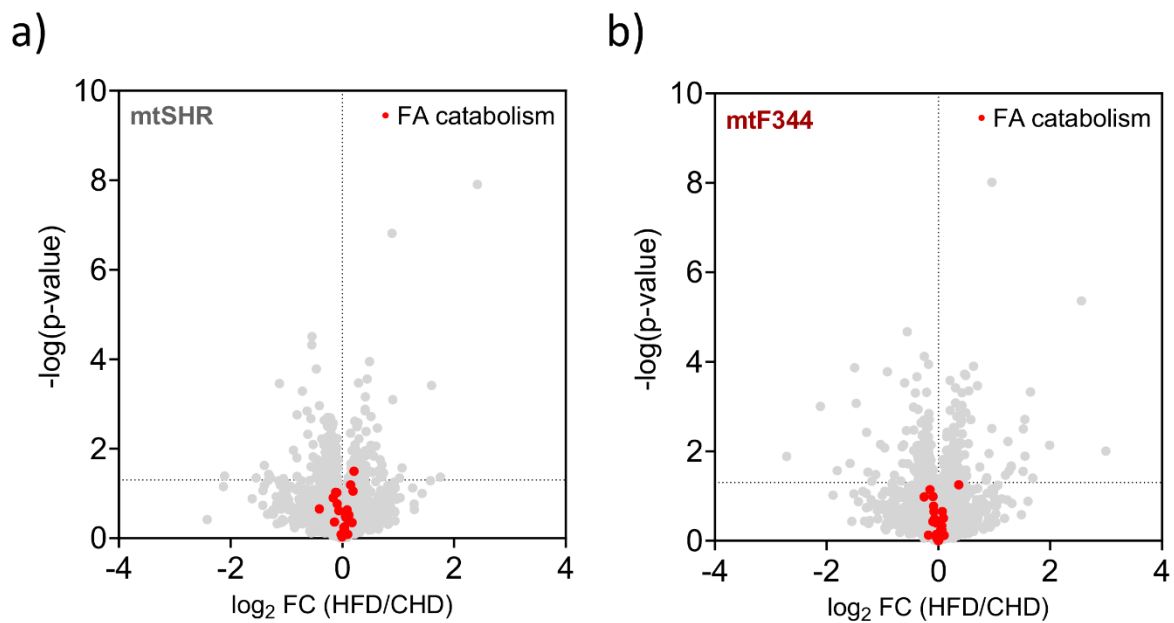
Supplementary figure 1: Metabolic phenotype of the conplastic strains. (a) Body weights of mtSHR, mtBN and mtF344 animals during 15 weeks on chow (CHD) or high-fat (HFD) diet. The significant change between mtF344 and mtSHR on HFD diet is depicted as *a* and between mtBN and mtSHR as *b* (p -value < 0.05) (b) Time course of glycemia during oral glucose tolerance test (OGTT). Data represents means \pm S.D. from at least 10 animals.



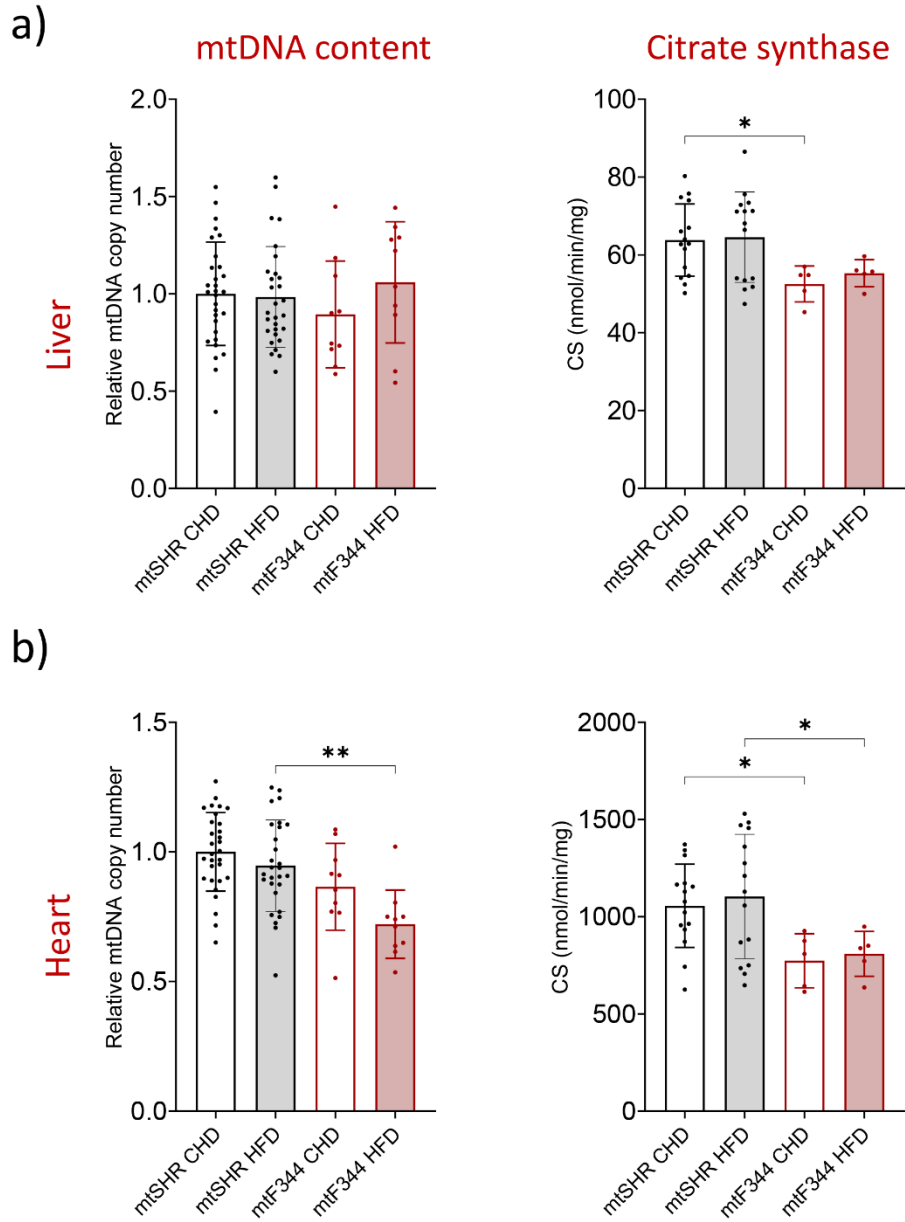
Supplementary figure 2: LC-MS analysis of individual diacylglycerols in the conplastic strains on HFD. The heatmap represents average log₂ fold change of individual diacylglycerols compared to mtSHR control in plasma (a), liver (b), and heart (c) of mtF344 and mtBN strains (n = 6).



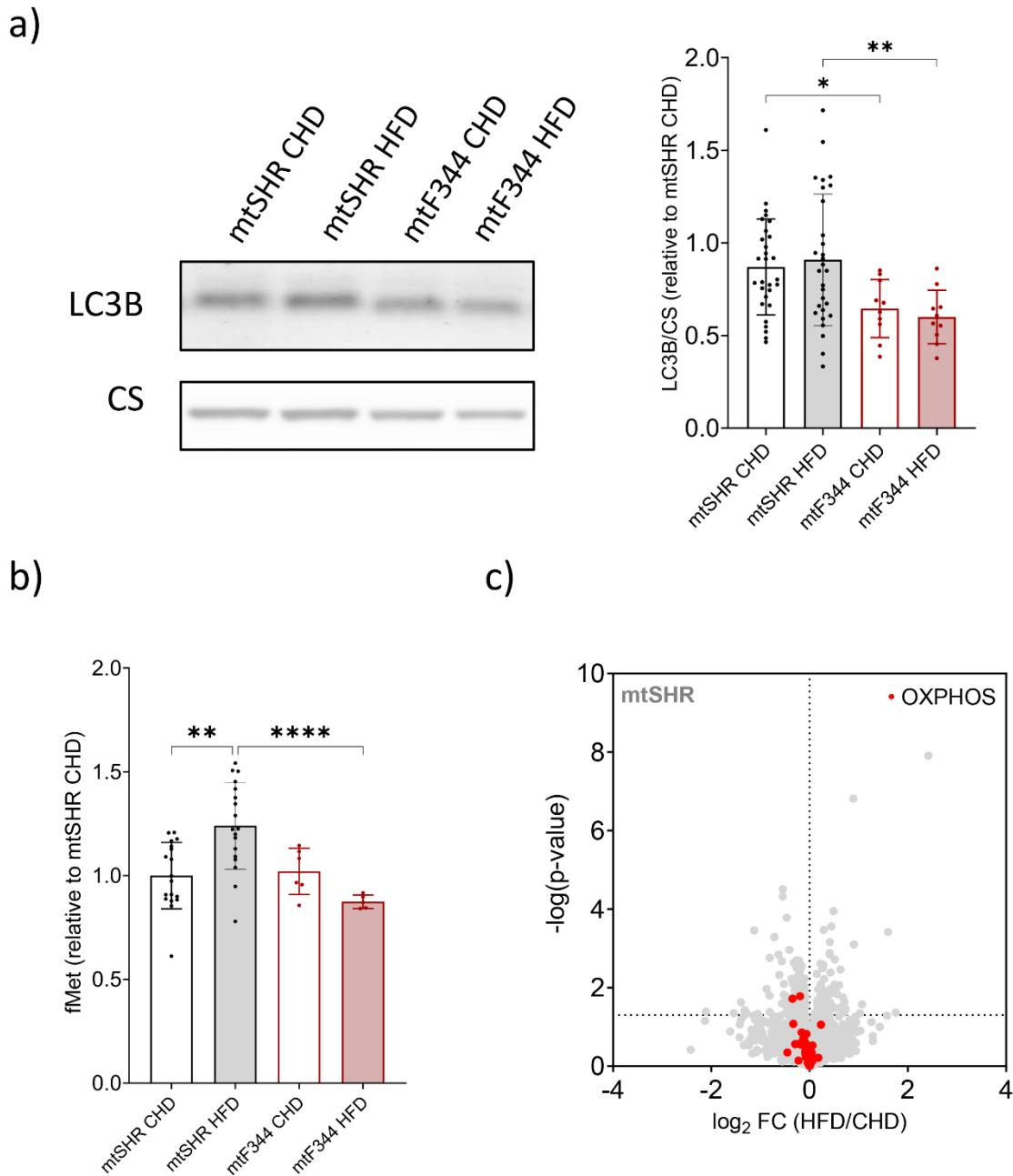
Supplementary figure 3: Label-free quantification mass spectrometry analysis (LFQ-MS) of liver tissue. Volcano plots depict differential contents of all proteins in between HFD and CHD in mtSHR (a, d), mtF344 (b, e) and mtBN (c, f). Red circles represent proteins involved in fatty acid (FA) degradation (a-c) and OXPHOS (d-f) according to KEGG pathways, enzymes of mitochondrial β-oxidation are labeled. Each point represents the data from 6 animals.



Supplementary figure 4: Label-free quantification mass spectrometry analysis (LFQ-MS) of heart tissue. Volcano plots depict differential contents of all proteins in between HFD and CHD in mtSHR (a) and mtF344 (b) strains. Red circles represent proteins involved in fatty acid (FA) degradation (according to KEGG pathways), enzymes of mitochondrial β -oxidation are labeled. Each point represents the data from 6 animals.

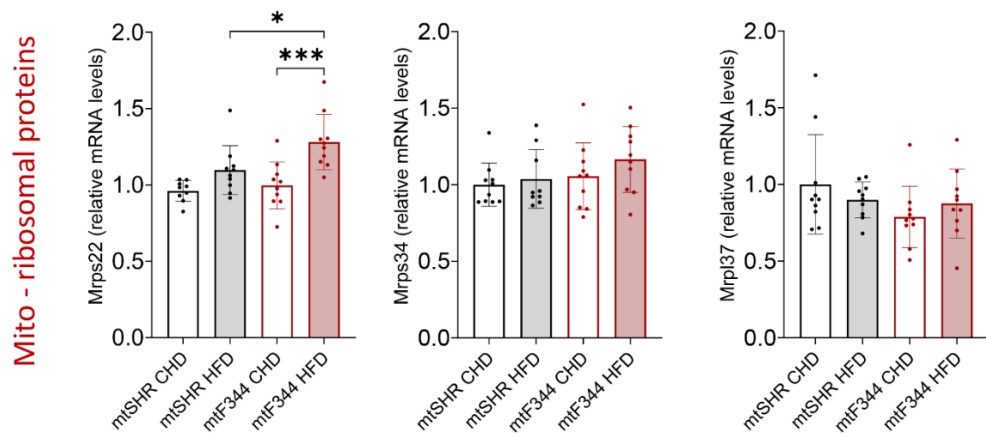


Supplementary figure 5: Content of mitochondria in mtSHR and mtF344 animals. Mitochondrial DNA copy number was measured by qPCR and citrate synthase activity (TCA cycle enzyme) was measured spectrophotometrically in liver (a) and heart (b) tissues. The data are expressed as means \pm S.D. from at least 5 animals.

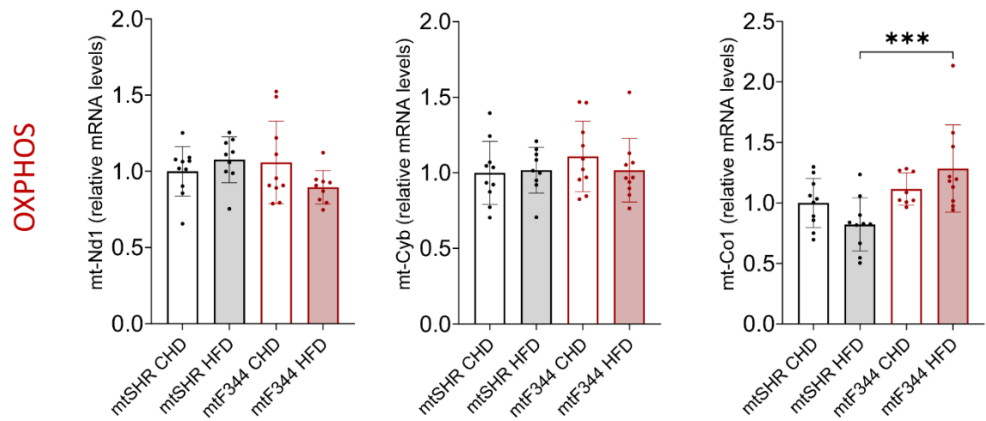


Supplementary figure 6: Analysis of autophagy and mitochondrial translation initiation in heart tissue of mtSHR and mtF344 animals. (a) Representative Western blot and quantification of LC3B protein in heart homogenates of at least 10 animals. Graph represents quantification of LC3B normalized to citrate synthase (CS) that was used as a marker of mitochondrial content. (b) The level of N-formylmethionine (fMet) in heart tissue measured by LC-MS metabolomics. The data are expressed as means \pm S.D. from at least 5 animals. (c) Label-free quantification mass spectrometry analysis (LFQ-MS) of mtSHR heart tissue. Volcano plots depict differential contents of all proteins in between HFD and CHD. Red circles represent subunits of OXPHOS complexes. The data are expressed as means \pm S.D. and each point represents the data from 6 animals.

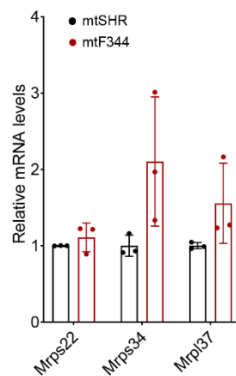
a)



b)



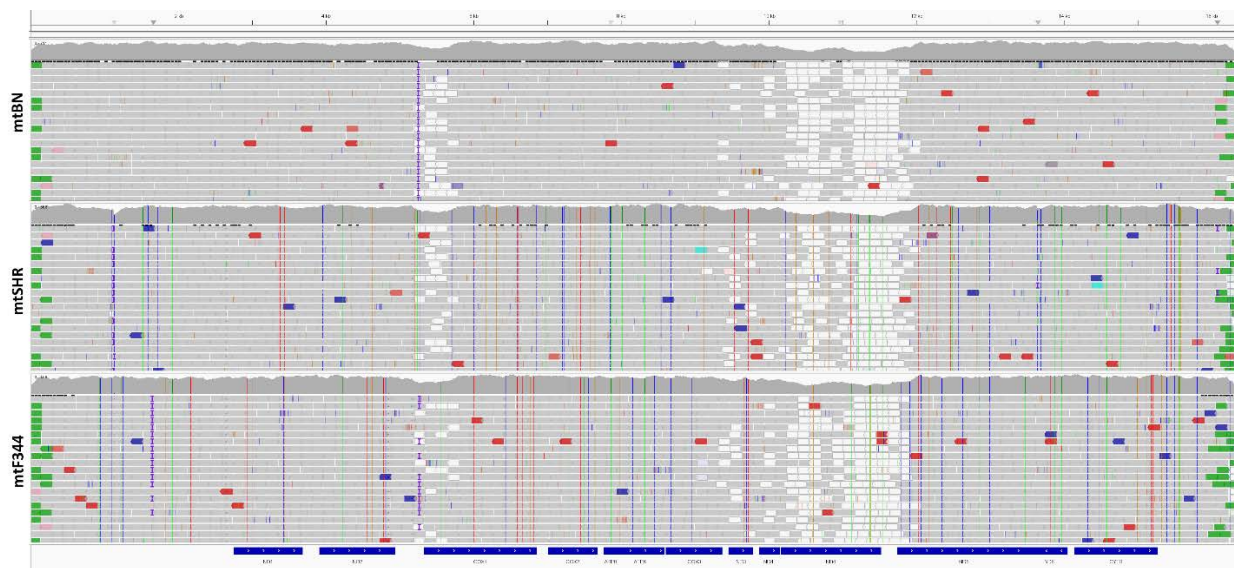
c)



Supplementary figure 7: Relative mRNA levels of mitochondrial encoded subunits and mitochondrial ribosomal proteins. Relative mRNA levels of (a) mitochondrial ribosomal proteins encoded by *Mrps22* and *Mrps34* (small mitochondrial ribosomal subunit) and *Mrpl37* (large mitochondrial ribosomal subunit) genes and (b) OXPHOS subunits encoded by *mt-Nd1*, *mt-Cyb* and *mt-Co1* genes in heart of mtF344 and mtSHR on CHD or HFD. Data represents means \pm S.D. from at least 8 animals. (c) Relative mRNA levels of mitochondrial ribosomal proteins (as in a) in cultured skin fibroblasts derived from mtF344 and mtSHR. Data represents means \pm S.D. from 3 independent experiments. Asterisks represent p-value: * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.

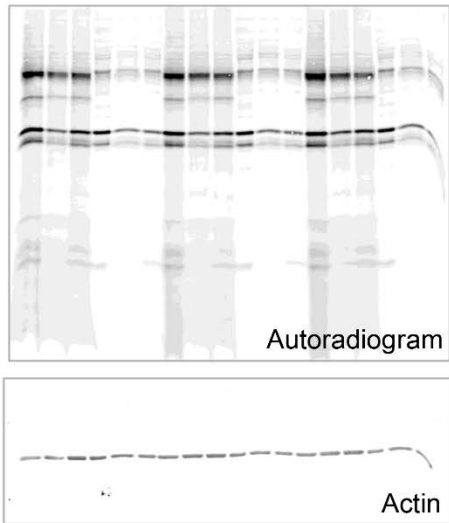
Supplementary table 3: SNP variants unique for SHR strain - indicative of potential heteroplasmic contamination from the founder strain (N – nucleotide).

position No.		mtSHR			mtF344			mtBN		
SHR mtDNA	RN7 reference	N	coverage (reads)	identity (%)	N	coverage (reads)	identity (%)	N	coverage (reads)	identity (%)
1137	1137	C	239	100	A	384	100	A	570	100
1521	1521	A	478	99	G	357	100	G	627	100
1585	1585	C	519	100	T	367	100	T	668	100
1716	1716	C	463	100	T	340	100	T	556	100
4351	4352	G	499	100	A	390	100	A	544	100
4695	4696	A	443	100	G	387	100	G	590	100
5198	5200	G	412	100	A	288	100	A	508	100
5200	5202	G	407	100	A	289	100	A	502	100
5235	5237	A	390	99	T	258	100	T	486	100
5267	5269	C	386	100	G	243	100	G	499	100
6976	6978	A	495	100	G	408	100	G	616	100
8019	8021	A	501	100	G	387	100	G	591	100
10225	10227	C	360	100	T	295	100	T	418	100
11223	11225	A	259	100	G	216	100	G	302	100
11358	11360	A	235	100	G	197	100	G	318	100
14773	14775	A	453	100	G	386	100	G	599	100

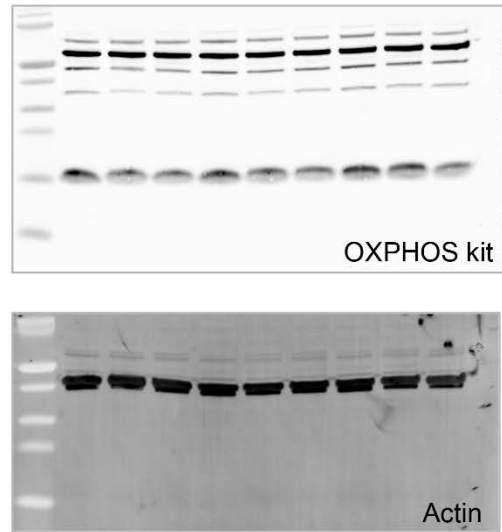


Supplementary figure 8: Mapping of individual reads for mtDNA against rn6 reference genome (BN strain based).

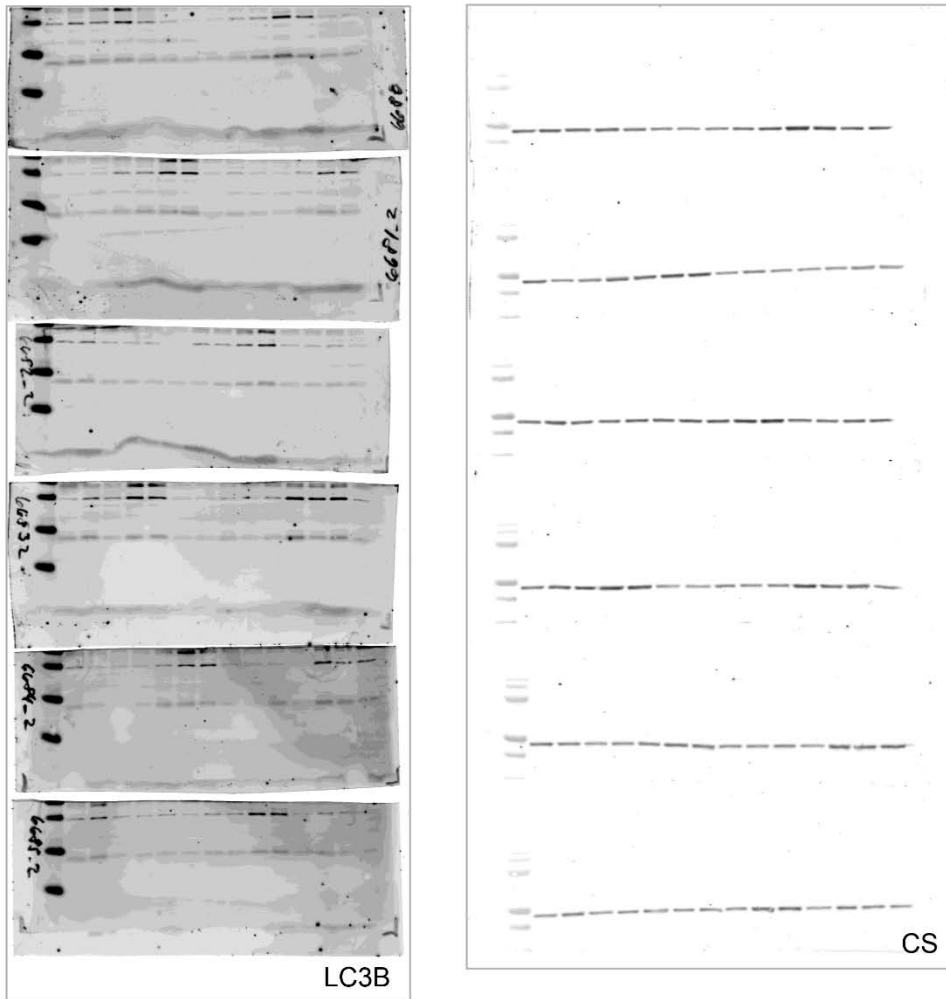
a)



b)



c)



Supplementary figure 9: Uncropped and unedited Western blots images. The images were used for analyses in Figure 7a (a), 7c (b) and Supplementary figure 6a (c).