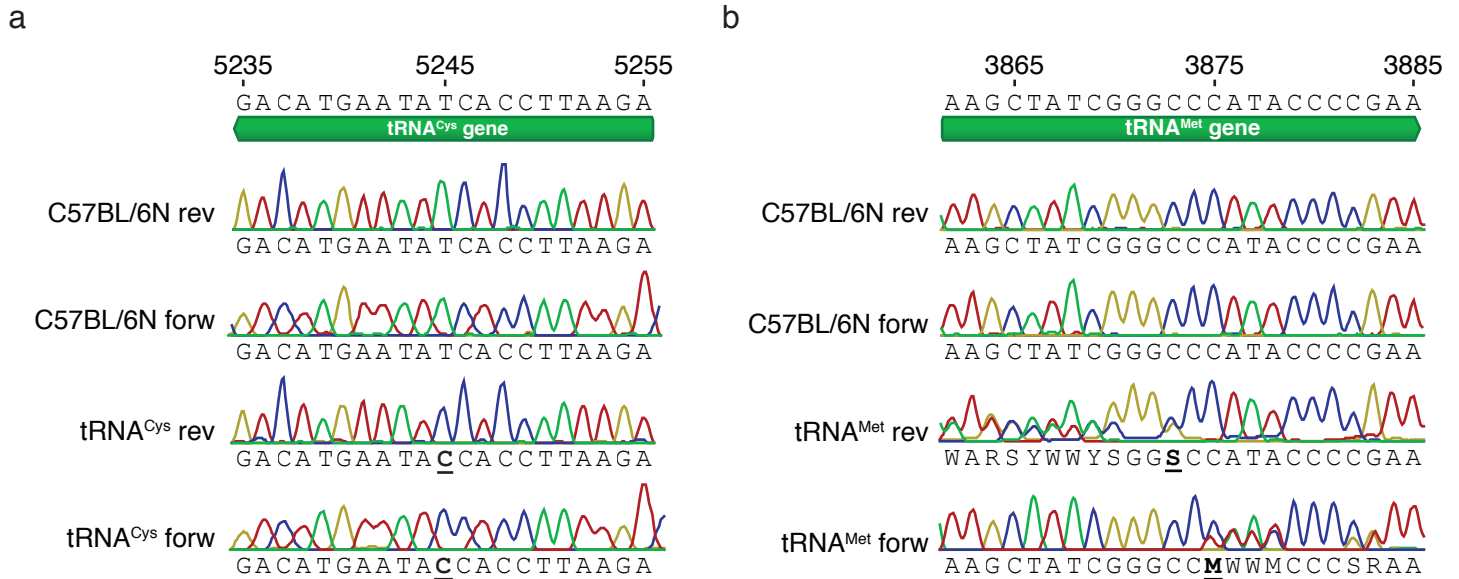


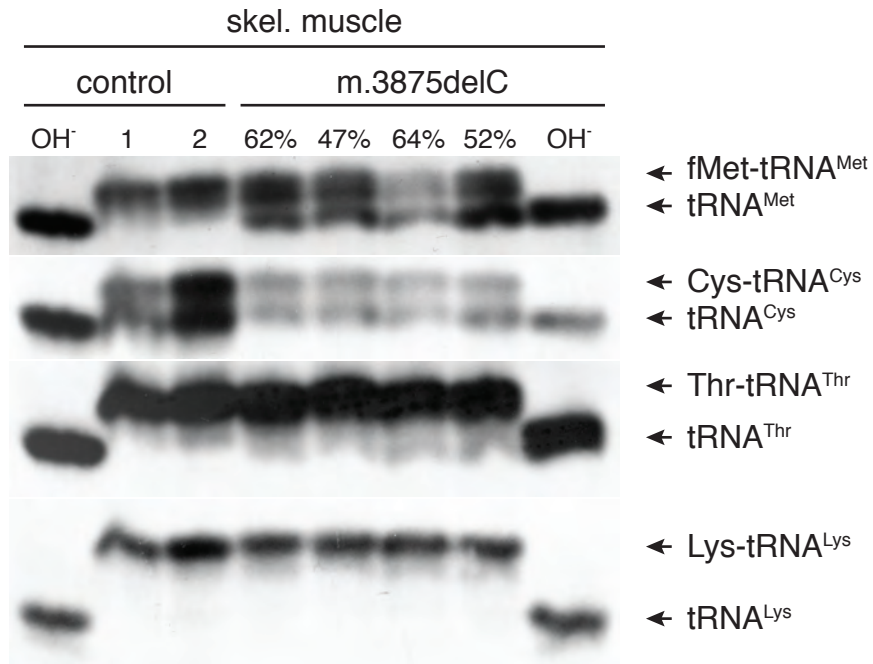
Variation in germ line mtDNA heteroplasmy is determined prenatally but modified during subsequent transmission

Christoph Freyer, Lynsey M. Cree, Arnaud Mourier, James B. Stewart, Camilla Koolmeister, Dusanka Milenkovic, Timothy Wai, Vasileios I. Floros, Erik Hagström, Emmanouella E. Chatzidaki, Rudolph Wiesner, David C Samuels, Nils-Göran Larsson, Patrick F. Chinnery

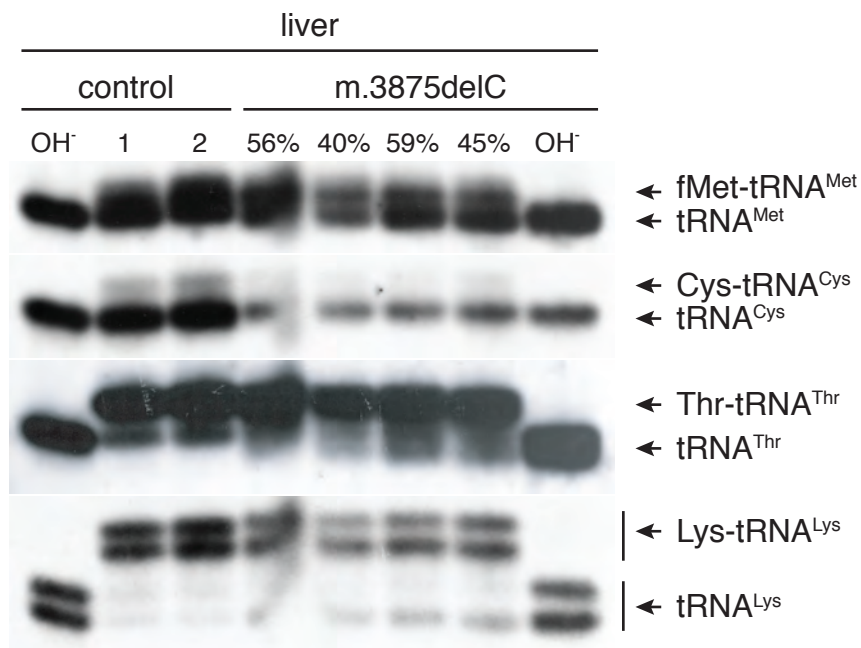


Supplementary Figure 1. Electropherogram of the (a) m.5245T>C mutation in tRNA^{Cys} and (b) m.3875delC in tRNA^{Met} mutation from tail clip DNA.

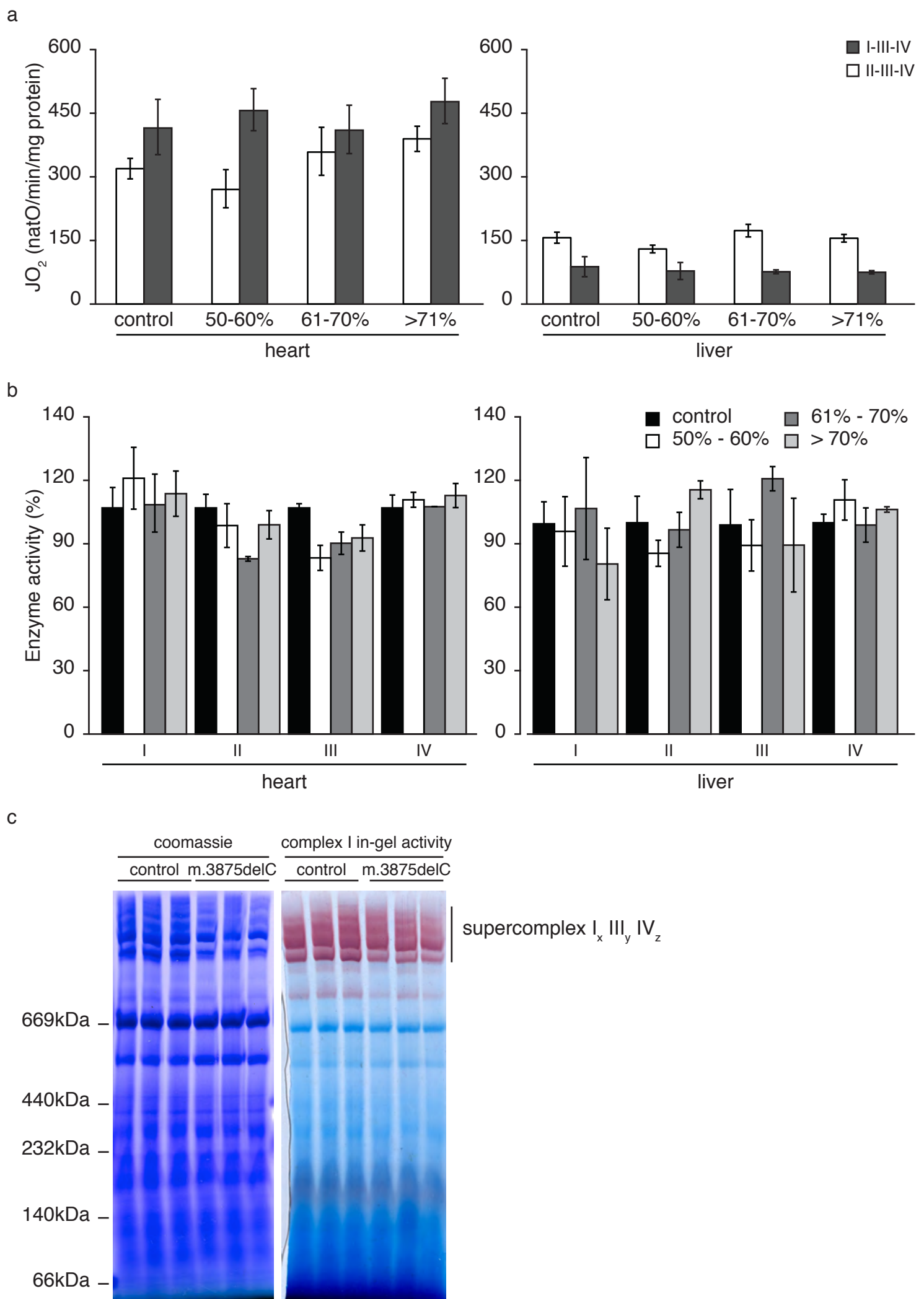
a



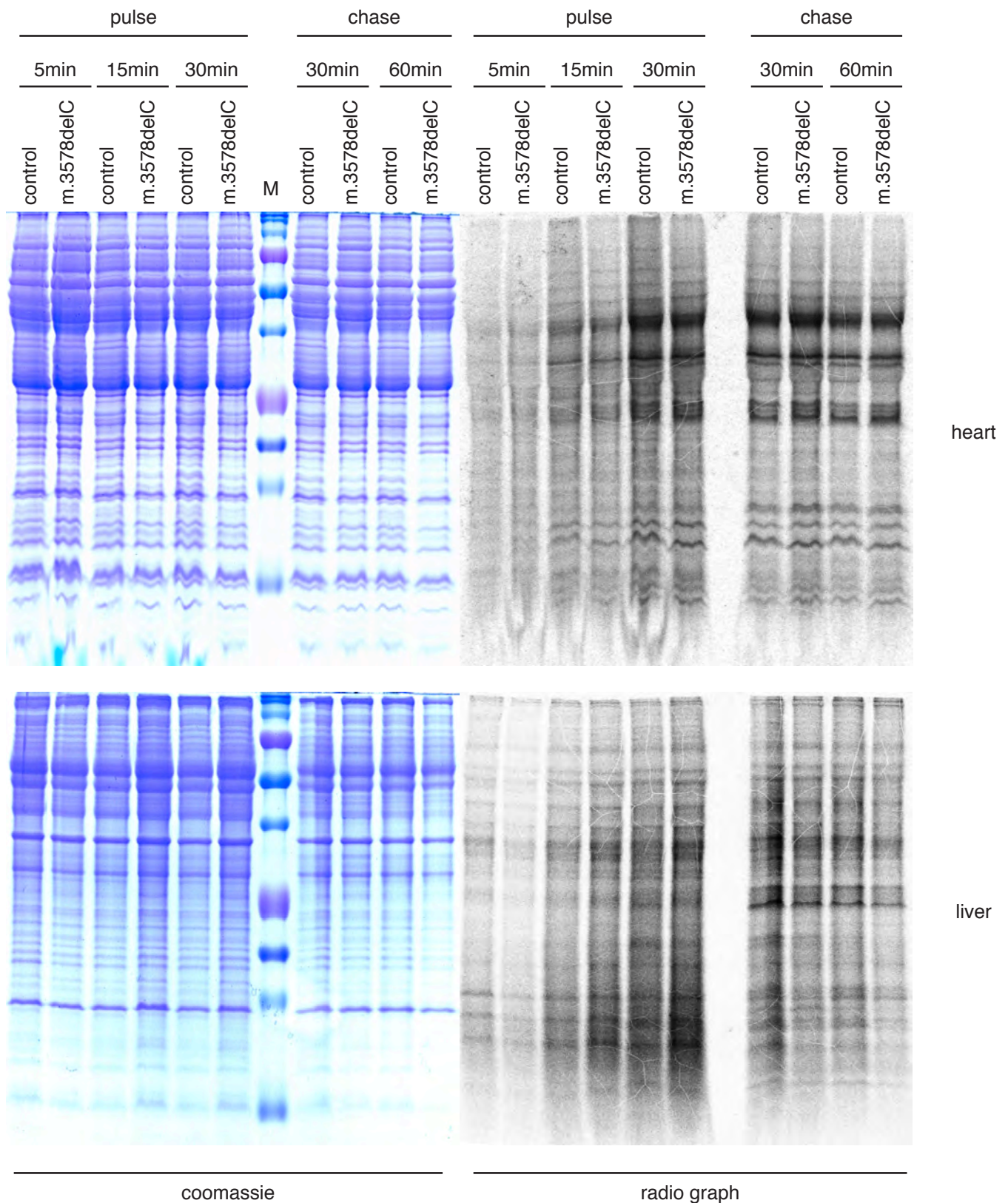
b



Supplementary Figure 2. Aminoacylation status of four mtDNA-encoded tRNAs as determined by separation on 6% acid-urea PAGE, followed by Northern blot analysis against mitochondrial tRNAs in (a) skeletal muscle and (b) liver samples from 25-week-old animals carrying the m.3875delC mtDNA mutation at the indicated level of heteroplasmy.



Supplementary Figure 3. Biochemical characterization of heart samples from 25-30 week-old mice carrying the m.3875delC mutation at varying heteroplasmy levels and C57BL/6N control animals. (a) Mitochondrial uncoupled respiration measured on freshly isolated mitochondria from heart or liver samples from control (livers N=4, or hearts N=5) of mice carrying the m.3875delC mutation at 50-60% (livers N=3, or hearts N=3), 61-70% (livers N=3, or hearts N=8) or >70% (livers N=5, or hearts N=10) heteroplasmy levels (Respiration was measured using either PGM (grey) or succinate (white) as substrate). (b) Isolated mitochondrial respiratory chain complex activities in isolated mitochondria from heart or liver samples from control (black, N=5) or mice carrying the m.3875delC mutation at either 40-60% (white, N=3), 61-70% (dark grey, N=3) or >70% (light grey, N=2) heteroplasmy levels. Complex I to IV activities were measured using the appropriate substrates. (c) BN-PAGE analysis of mitochondrial protein extracts from 20-week-old heart samples followed by coomassie staining (left panel) and in-gel activity for complex I (right panel).



Supplementary Figure 4. In organello translation experiment in isolated heart (upper panel) and liver (lower panel) mitochondria from 25-week-old C57BL/6N control animals and animals carrying the m.3875delC mtDNA mutation at 80% heteroplasmy. Mitochondria were incubated for 5min, 15min, or 30min in the presence of [³⁵S]-methionine, before being washed and chased with unlabeled methionine for 30min or 60min. Mitochondrial protein preparations were then separated by SDS PAGE, stained with coomassie, and [³⁵S]-methionine-labeled proteins were visualized by autoradiography.

Supplementary Table 1. mtDNA mutations found in tail clip DNA of ten m.3875delC animals. The complete mtDNA sequence was determined by Sanger sequencing five sibling sets carrying the m.3875delC mutation at either high or low mutation levels, as determined by last-flourescent cycle RFLP. Heteroplasmies and substitutions were called using SeqScape V2.5 software (ABI) and compared to our C57Bl/6 mtDNA reference sequence. The software identified heteroplasmy or substitutions at $\geq 25\%$ signal intensity on both strands of DNA sequence. All heteroplasmies were confirmed by eye. All mutation sites are potentially heteroplasmic in this analysis, due to detection thresholds in sequencing technology²⁴. Thus, presented is the read-out of the sequencing reactions.

#	Position	gene	WT	codon	AA	Sibling set 1		Sibling set 2		Sibling set 3		Sibling set 4		Sibling set 5	
						30%	76%	50%	79%	21%	68%	57%	78%	32%	75%
1	190	12S RNA	T	-	-	T/C	T/C	T/C	T/C	C	T/C	T/C	T/C	T/C	T/C
2	604	12S RNA	-	-	-	-/insT	-/insT	-/insT	-/insT	-/insT	-/insT	-/insT	-/insT	-/insT	-/insT
3	1188	16S RNA	A	-	-	A/G	A/G	A/G	A/G	A/G	A/G	G	G	A/G	A/G
4	1511	16S RNA	T	-	-	T/C	T/C	T	T	T	T	T	T	T	T
5	1808	16S RNA	C	-	-	T	T	C/T	T	T	T	T	T	T	T
6	2243	16S RNA	A	-	-	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G
7	2659	16S RNA	C	-	-	T	T	C/T	T	T	T	T	T	T	T
8	2903	ND1	C	3	D→D	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T
9	3014	ND1	C	3	P→P	T	T	T	T	T	T	T	T	T	T
10	3555	ND1	A	1	T→S	A/T	A/T	A/T	A/T	T	A/T	A/T	A/T	A/T	A/T
11	3646	ND1	C	2	A→V	T	T	C/T	T	T	T	T	T	T	T
12	3875	tMet	C	-	-	C/delC	C/delC	C/delC	C/delC	C/delC	C/delC	C/delC	C/delC	C/delC	C/delC
13	4909	ND2	A	3	M→I	n/a	A/T	A/T	A/T	A/T	A/T	A/T	A/T	A/T	A/T
14	4963	tTrp	A	-	-	n/a	A/T	A/T	A/T	A/T	A/T	A/T	A/T	A/T	A/T
15	5105	tAsn	T	-	-	n/a	A	A	A	T/A	A	T/A	A	T/A	T/A
16	5245	tCys	T	-	-	n/a	C	C	C	C	C	C	C	C	C
17	5360	COX1	T	3	N→N	n/a	C	C	C	C	C	C	C	C	C
18	6481	COX1	C	2	A→V	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T
19	6722	COX1	T	3	V→V	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C
20	7255	COX2	A	3	L→L	A/T	A/T	A/T	A/T	T	A/T	A/T	A/T	A/T	A/T
21	7292	COX2	G	1	V→M	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A
22	7708	tLys	A	-	-	G	G	G	G	G	G	G	G	G	G
23	8145	ATP6	T	3	V→V	T/C	T/C	T	T	T	T	T	T	T	T
24	8692	COX3	C	2	S→L	T	T	T	T	T	T	T	T	T	T
25	8755	COX3	A	2	N→S	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G
26	8870	COX3	C	3	V→V	C/T	C/T	C/T	T	C/T	C/T	C/T	C/T	C/T	C/T
27	9305	COX3	C	3	F→K	T	T	T	C/T	T	T	T	T	T	T
28	9677	ND3	T	3	L→Q	G	G	G	G	G	G	G	G	G	G
29	9762	ND3	C	1	L→M	A	A	C/A	A	A	A	A	A	A	A
30	11363	ND4	A	2	N→S	A	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G
31	12077	ND5	C	2	P→L	n/a	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T
32	12534	ND5	C	3	H→H	C/T	C/T	C/T	C/T	T	C/T	C/T	C/T	C/T	C/T
33	12975	ND5	T	3	I→I	T	T	T	T	T	T	T	T	T	T
34	13886	ND6	C	3	G→G	C	C/G	C/G	C/G	C/G	C/G	C/G	C/G	C/G	C/G
35	14276	CytB	A	2	Q→L	n/a	A/T	A/T	A/T	T	A/T	A/T	A/T	A/T	A/T

Supplementary Table 2. Comparison of the m.5245T>C and m.3875delC mutations in nine tissue samples from 20 adult animals as determined by last-fluorescent-cycle RFLP.

animal	ear	SM	heart	m.3875delC						m.5245T>C							
				liver	spleen	kidney	blood	testis/ ovary	brain	SM	heart	liver	spleen	kidney	blood	testis/ ovary	brain
C57BL/6N-1	0%	-	1%	4%	-	-	-	-	1%	-	0%	0%	-	-	-	-	0%
C57BL/6N-2	0%	0%	0%	1%	0%	0%	-	-	-	0%	0%	0%	0%	0%	-	-	-
C57BL/6N-3	0%	0%	0%	1%	0%	0%	-	-	-	0%	0%	0%	0%	0%	-	-	-
3875delC-1	34%	47%	-	-	44%	46%	-	45%	-	100%	100%	-	100%	100%	-	100%	-
3875delC-2	41%	46%	45%	46%	44%	47%	44%	-	-	100%	100%	100%	100%	100%	100%	-	-
3875delC-3	60%	60%	-	65%	-	61%	-	-	-	100%	-	100%	-	100%	-	-	-
3875delC-4	61%	70%	70%	70%	65%	67%	62%	-	-	100%	-	100%	100%	100%	100%	-	-
3875delC-5	65%	74%	70%	70%	-	71%	-	73%	71%	100%	100%	100%	-	100%	-	100%	100%
3875delC-6	65%	68%	69%	69%	63%	70%	69%	-	-	100%	100%	100%	100%	100%	100%	-	-
3875delC-7	66%	62%	69%	70%	61%	69%	-	65%	68%	100%	100%	100%	100%	100%	-	100%	100%
3875delC-8	66%	63%	67%	65%	59%	66%	64%	-	-	100%	-	100%	100%	100%	100%	-	-
3875delC-9	66%	70%	72%	74%	67%	-	66%	-	-	100%	100%	100%	100%	-	100%	-	-
3875delC-10	67%	70%	-	71%	69%	76%	64%	-	-	100%	-	100%	100%	100%	100%	-	-
3875delC-11	68%	71%	72%	74%	66%	-	-	-	-	100%	100%	100%	100%	-	-	-	-
3875delC-12	70%	63%	69%	65%	65%	-	-	-	-	100%	100%	100%	100%	-	-	-	-
3875delC-13	71%	64%	63%	58%	56%	-	-	-	-	100%	100%	100%	100%	-	-	-	-
3875delC-14	73%	67%	69%	68%	65%	-	-	-	-	100%	100%	100%	100%	-	-	-	-
3875delC-15	73%	78%	74%	79%	73%	80%	-	78%	77%	100%	100%	100%	100%	100%	-	100%	100%
3875delC-16	76%	77%	78%	79%	73%	77%	-	-	79%	100%	100%	100%	-	100%	-	-	-
3875delC-17	80%	78%	76%	70%	72%	76%	-	76%	76%	100%	100%	100%	-	100%	-	100%	100%

Supplementary Table 3. m.3875delC mutation levels in seven tissues from 14 3.5d neonates, born to three females

animal	Mother %	Sex	tail	SM	heart	liver	spleen	kidney	brain
3875delC-1-nn1	49.42%	♀	66.43%	66.97%	68.71%	-	67.75%	70.12%	68.50%
3875delC-1-nn2	49.42%	♂	49.37%	52.91%	51.47%	53.39%	51.07%	51.37%	51.11%
3875delC-1-nn3	49.42%	♂	69.02%	70.96%	72.01%	70.28%	68.85%	71.36%	70.52%
3875delC-2-nn1	68.22%	♂	69.58%	66.62%	70.41%	67.34%	66.24%	67.85%	68.88%
3875delC-2-nn2	68.22%	♂	69.00%	68.33%	69.72%	69.80%	67.30%	70.09%	70.29%
3875delC-2-nn3	68.22%	♀	63.03%	63.78%	64.68%	66.17%	63.34%	63.34%	65.60%
3875delC-2-nn4	68.22%	♂	69.59%	66.57%	68.28%	68.11%	65.71%	67.23%	67.25%
3875delC-2-nn5	68.22%	♀	72.85%	70.98%	70.54%	69.85%	70.10%	72.11%	71.07%
3875delC-3-nn1	70.81%	♂	74.41%	72.37%	72.43%	71.75%	71.51%	73.19%	74.57%
3875delC-3-nn2	70.81%	♀	52.35%	51.79%	55.04%	54.60%	52.40%	55.56%	53.38%
3875delC-3-nn3	70.81%	♂	78.66%	74.94%	77.08%	76.79%	74.80%	78.08%	79.02%
3875delC-3-nn4	70.81%	♂	76.88%	73.97%	75.77%	75.09%	71.82%	76.74%	76.09%
3875delC-3-nn5	70.81%	♂	70.89%	67.56%	72.13%	70.68%	67.09%	70.07%	71.29%
3875delC-3-nn6	70.81%	♀	76.80%	74.94%	76.04%	76.43%	74.34%	77.49%	75.86%

Supplementary Table 5: Primer sequences for last-fluorescent RFLP mtDNA mutation level quantification. Primers for the m.5245C-T and m.3875delC were designed with 5' terminal M13 tag sequences (underlined) and the relevant restriction sites as internal digestion control (bold). A single nucleotide mismatch was incorporated into the m.3875delC primer to generate an NlaIV restriction site (bold/underlined).

m.5245C-T assay

forward TGT AAA ACG ACG GCC AGT AAG CTA AGA CCT CAA CTA GAT TGG CAG

reverse CAG GAA ACA GCT ATG ACC **GGT GAG** GGT TCC GAT ATC TTT GTG ATT GGT TG

m.3875delC assay

forward TGT AAA ACG ACG GCC AGT TCT CCG TGC TAC CTA AAC ACC

reverse CAG GAA ACA GCT ATG ACC TAG GTT **GGT GCC** GGA TAT TGT G