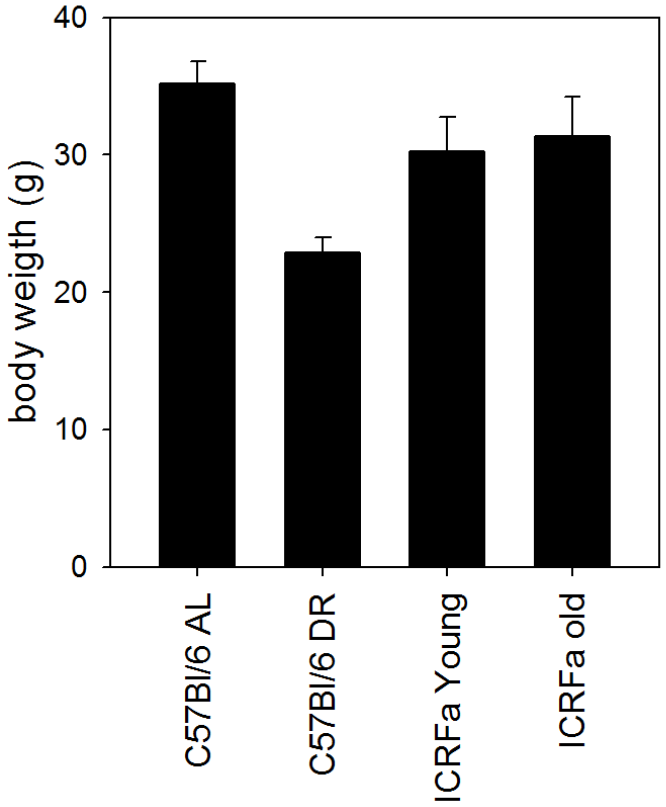
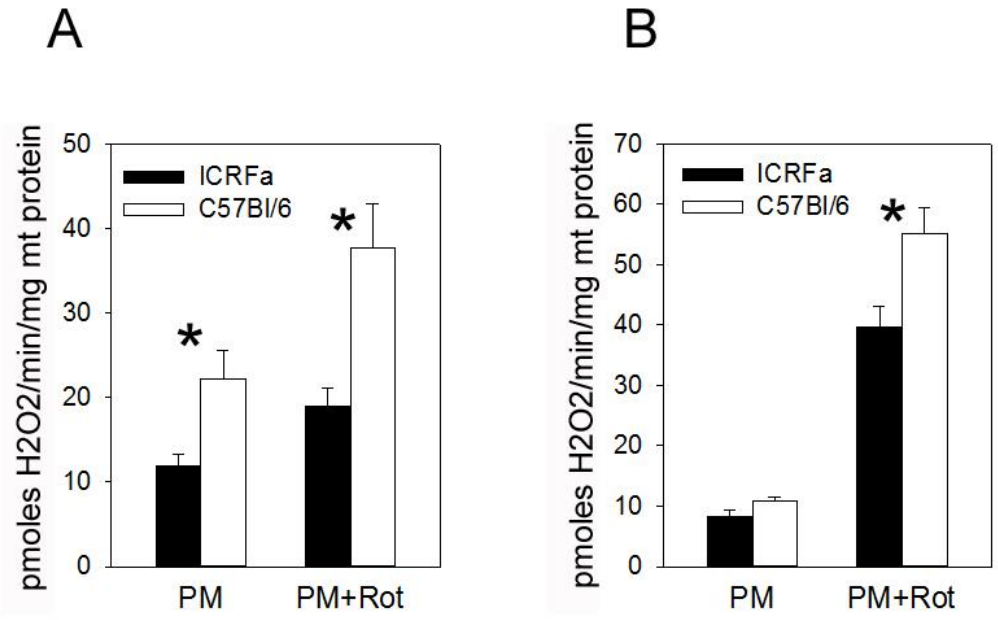
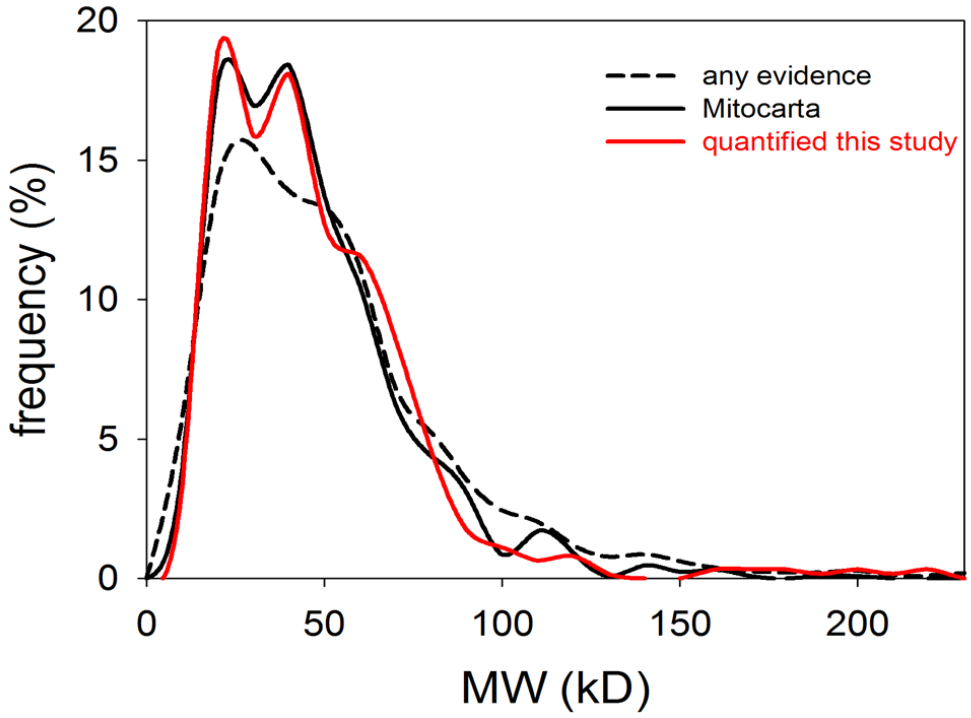
Supplementary Figures



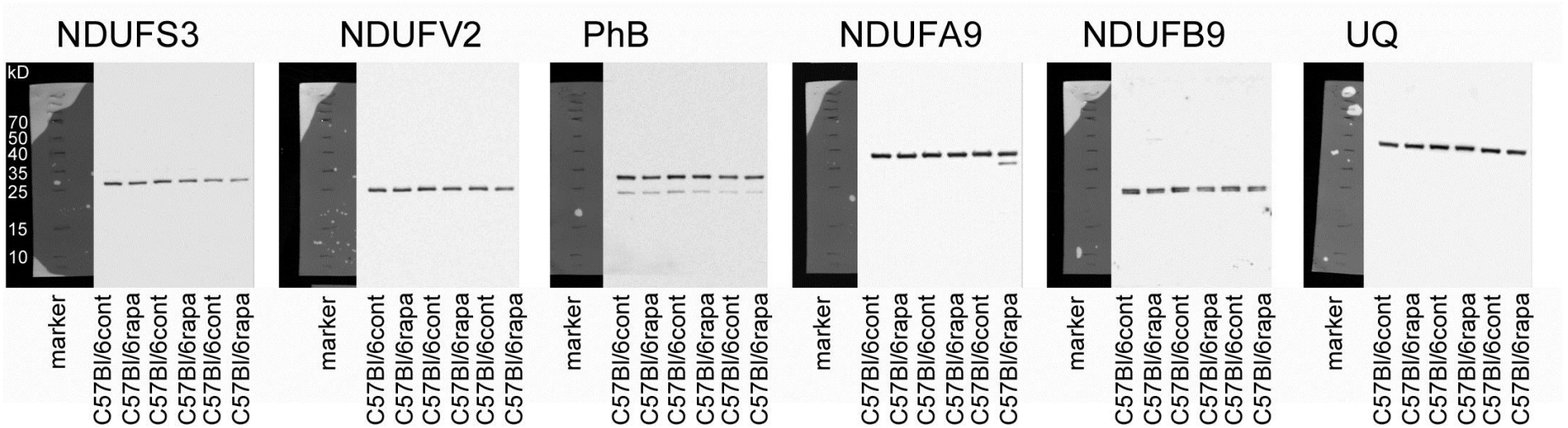
Supplementary Fig. 1: Body weights of the animals used for mitochondrial proteomics. Data are mean ± SD, n=3. DR animals weigh less ($p<0.05$, ANOVA), while weights of all other animals are not different.



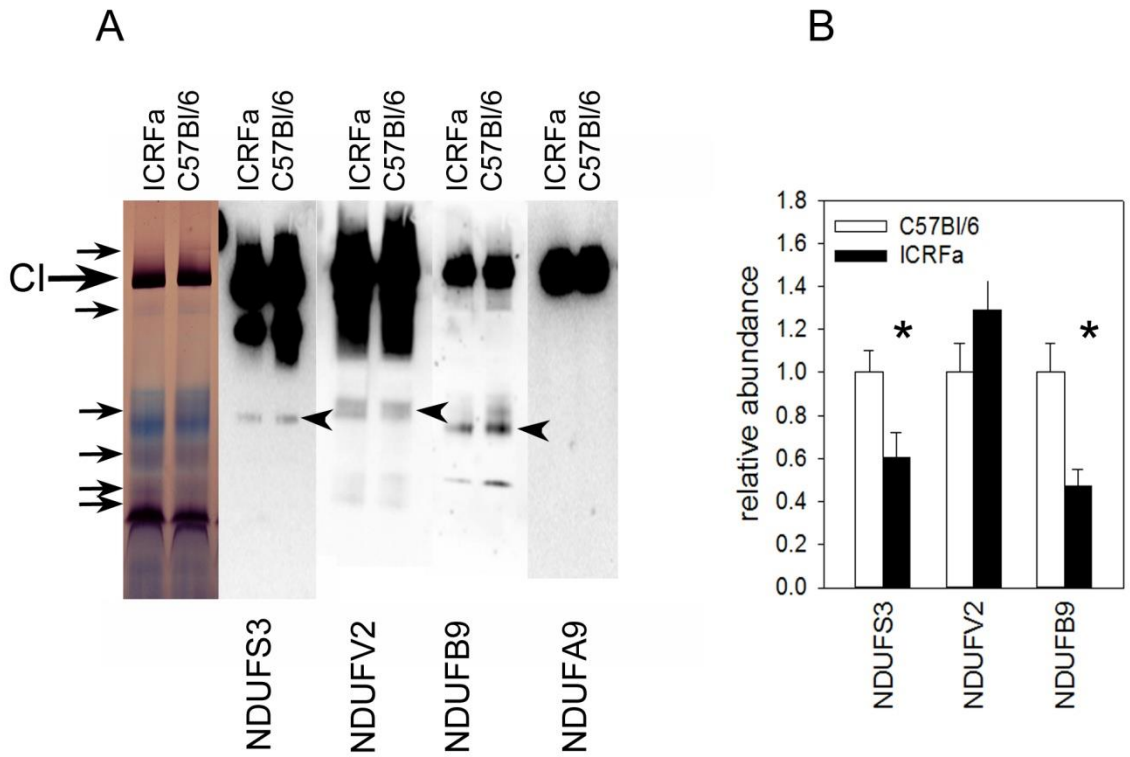
Supplementary Fig. 2: Lower ROS production from complex I in long-lived ICRFa mice. H_2O_2 release from purified liver (A) and brain (B) mitochondria from ICRFa and C57Bl/6 mice at 8-10 months of age. Data are mean ± SEM, n= 5 (liver) or 4 (brain). * indicates $p<0.05$, t test. PM: basal conditions (pyruvate+malate), PM+Rot: Pyruvate/malate plus rotenone, indicating maximum ROS production from complex I.



Supplementary Fig. 3: Molecular weight distributions of mitochondrial proteins. Red: proteins quantified in this study, black: all mouse proteins in MitoCarta, broken line: all mouse proteins with any evidence for mitochondrial localisation in MitoMiner.

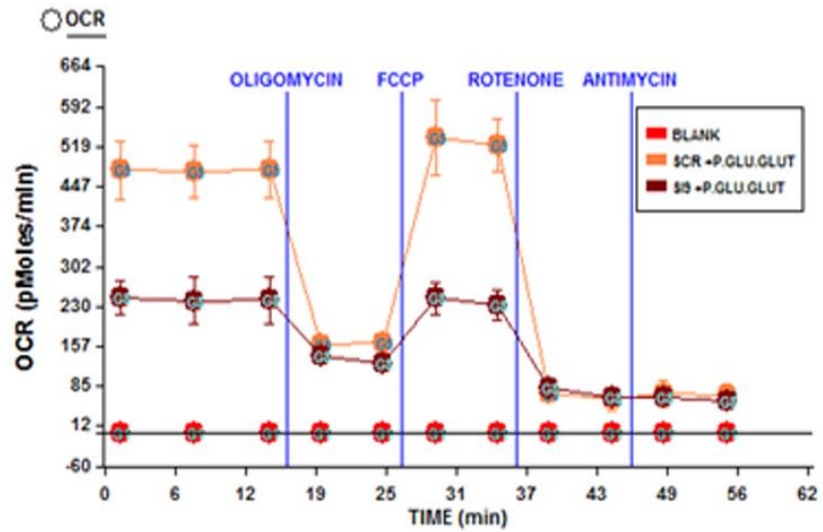


Supplementary Fig. 4: Full SDS-PAGE Western blots of complex I matrix arm subunits NDUFS3 and NDUFV2, prohibitin (PhB), membrane arm subunits NDUFA9 and NDUFB9 and UQCRC2 (UQ) in liver mitochondria from control and rapamycin-treated C57Bl/6 mice. Marker positions are shown on the left.

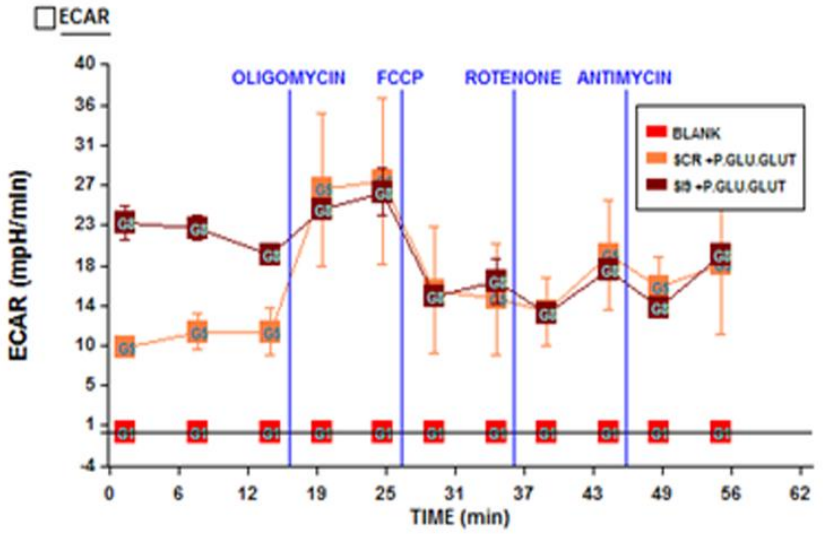


Supplementary Fig. 5: Assembly of complex I is more complete in long-lived mice. A) Complex I activity and blue native gels/Western blots of the indicated subunits of complex I in mice liver mitochondria from 8 months old ICRFa and C57Bl/6 mice. Same blots as in Fig. 5C but over-exposed. Arrowheads mark the position of the bands quantified in Fig. S4B. **B)** Ratio of lower/holocomplex band intensity of the indicated complex I subunits on blue native gel Westerns. Data are mean ± SEM, 3 animals/group. * indicates $p<0.05$, t test.

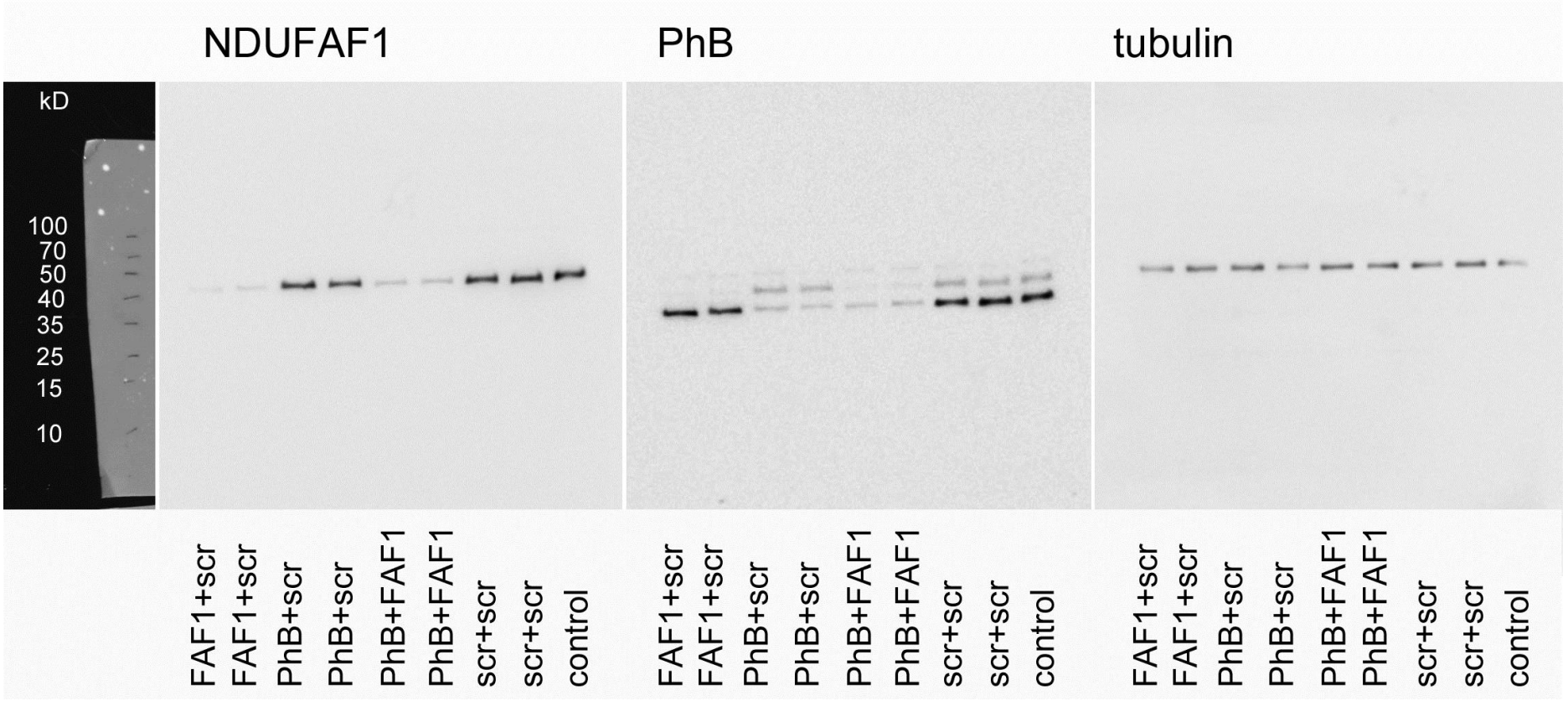
A



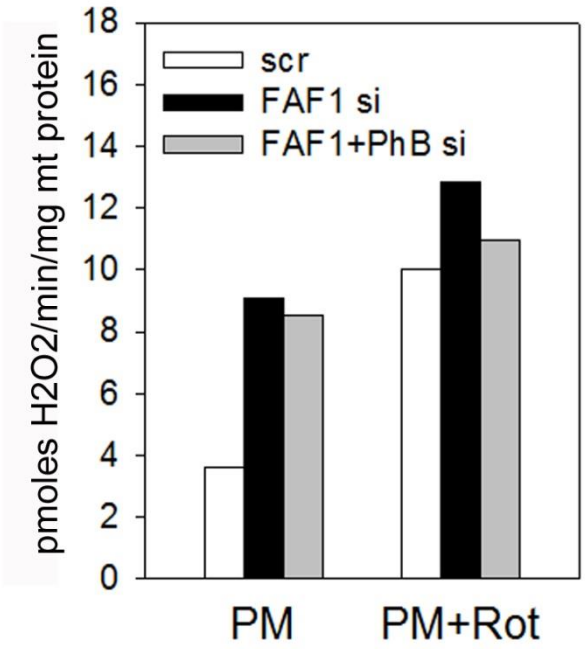
B



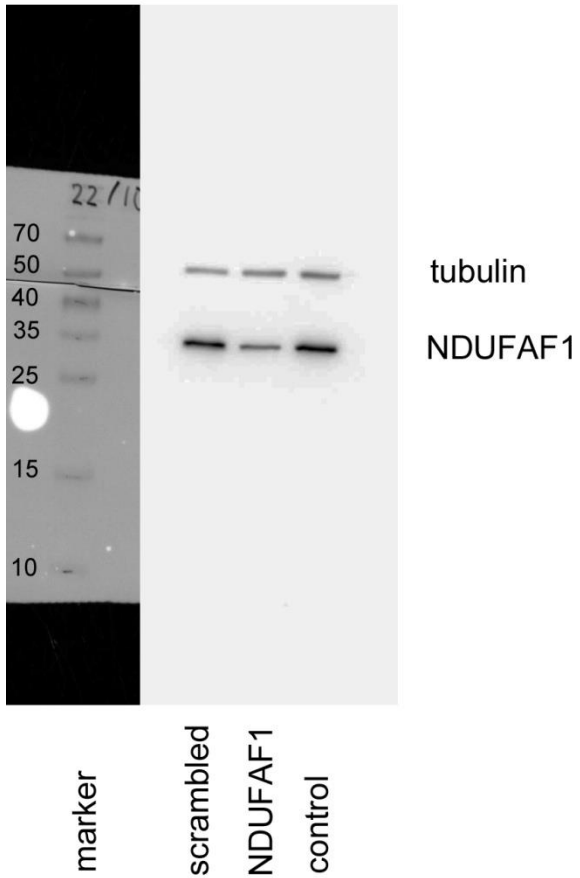
Supplementary Fig. 6: Oxygen consumption (A) and medium acidification (B) by HeLa cells transfected with anti-NDUFAF1 siRNA (brown) or scrambled control siRNA (orange). Data are mean ± SD from 4 wells per condition with 35,000 cells each. Red: blank.



Supplementary Fig. 7: Full SDS-PAGE Western blots of complex I assembly factor NDUFAF1, prohibitin (phB) and tubulin following siRNA-mediated knock-down of the indicated mRNAs in HeLa cells.



Supplementary Fig. 8: Knock down of the mitochondrial chaperone prohibitin reduces hydrogen peroxide release triggered by suppression of the complex I assembly factor NDUFAF1. H_2O_2 release under pyruvate+malate (native conditions) and under PM + rotenone (CI max) in isolated Hela cell mitochondria following transfection with the indicated siRNAs. Data are from a single experiment.



Supplementary Fig. 9: Full SDS-PAGE Western blots of complex I assembly factor NDUFAF1 and tubulin following siRNA-mediated knock-down of the indicated mRNAs in MRC5 fibroblasts.

Supplementary Table 1. Proteins significantly changed in all three comparisons

			DR -AL		Old -Young		ICRFa –C57Bl/6	
Uniprot ID	Gene names	Protein names	β	Fold change	β	Fold change	β	Fold change
O35129	Phb2	Prohibitin-2 (B-cell receptor-associated protein BAP37)	1	-2.47	1	1.84	1	-1.73
P12787	Cox5a	Cytochrome c oxidase subunit 5A, mitochondrial (Cytochrome c oxidase polypeptide Va)	1	-3.17	1	2.11	1	-2.19
P51881	Slc25a5	ADP/ATP translocase 2 (Solute carrier family 25 member 5)	1	-1.84	1	2.25	1	-1.52
P67778	Phb	Prohibitin (B-cell receptor-associated protein 32)	1	-2.80	1	1.76	1	-1.76
Q6PF96	Etfdh	Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	1	-2.31	1	1.95	1	-1.79
Q9CR68	Uqcrfs1	Cytochrome b-c1 complex subunit Rieske, mitochondrial (Complex III subunit 5) (Rieske iron-sulfur protein)	1	-1.96	1	2.43	1	-2.34
B1ASE2	Atp5h	ATP synthase subunit d, mitochondrial	1	-2.64	0.9998	2.08	0.646	-1.45
P56480	Atp5b	ATP synthase subunit beta,	1	-1.22	0.9922	1.19	1	-1.93

		mitochondrial						
Q9CZ13	Uqcrc1	Cytochrome b-c1 complex subunit 1, mitochondrial (Complex III subunit 1)	1	-2.39	0.9034	1.49	1	-1.59
Q9DCT2	Ndufs3	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial (Complex I-30kD)	1	-2.29	0.7084	1.32	0.982	-1.46
Q62425	Ndufa4	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 4	0.998	-2.06	0.8794	1.78	0.82	-1.55
Q99J99	Mpst	3-mercaptopyruvate sulfurtransferase (MST) (EC 2.8.1.2)	0.9676	1.34	1	1.66	1	-1.54
Q8BYC3	Acox1	Peroxisomal acyl-coenzyme A oxidase 1 (Palmitoyl-CoA oxidase)	0.9655	-1.32	0.9994	-1.51	0.805	-1.29

Supplementary Tab 2. Mitochondria specific KEGG pathway enrichment analysis: Pathways significantly (Q<0.05) enriched based on proteins that are more abundant in young C57Bl/6 under DR as compared to AL controls (more in DR). Q values for enrichment of the same pathways based on proteins more abundant in long-lived young ICRFa (centre) and on proteins less abundant in old ICRFa mice (right) are shown for comparison. No pathway (except the most general mmu01100) is consistently enriched in all three conditions.

KEGG Metabolic pathway	Q-Values		
	more in DR	more in young ICRFa	less in old ICRFa
mmu01100 Metabolism	**0**	**0**	**0.041249**
mmu00280 Valine, leucine and isoleucine degradation	**8.21E-22**	NA	NS0.137044
mmu00410 beta-Alanine metabolism	**1.43E-17**	NA	NS 0.112764
mmu00640 Propanoate metabolism	**1.49E-16**	NA	NS 0.136363
mmu00020 Citrate (TCA) cycle	**3.64E-13**	NA	NA
mmu00071 Fatty acid metabolism	**6.14E-11**	NA	**0.046149**
mmu00650 Butanoate metabolism	**8.40E-11**	NA	NS 0.124042
mmu00330 Arginine and proline metabolism	**1.09E-10**	**0.000348**	NA
mmu00250 Alanine, aspartate	**4.84E-10**	**5.54E-05**	NA

and glutamate metabolism			
mmu00310 Lysine degradation	**6.26E-10**	NA	NS0.145341
mmu00620 Pyruvate metabolism	**4.68E-08**	NA	NA
mmu00010 Glycolysis / Gluconeogenesis	**3.32E-07**	NA	NS 0.160339
mmu00380 Tryptophan metabolism	**3.83E-06**	NA	NS 0.169911
mmu00910 Nitrogen metabolism	**8.29E-06**	**0.001127**	NA
mmu00340 Histidine metabolism	**8.22E-05**	NA	NA
mmu00561 Glycerolipid metabolism	**8.90E-05**	NA	NA
mmu00471 D-Glutamine and D-glutamate metabolism	**0.000104**	NA	NA
mmu00630 Glyoxylate and dicarboxylate metabolism	**0.000168**	NA	NA
mmu00053 Ascorbate and aldarate metabolism	**0.00018**	NA	NA
mmu00062 Fatty acid	**0.000238**	**0.044435**	NA

elongation			
mmu03320 PPAR signaling pathway	**0.002648**	NA	NA
mmu00300 Lysine biosynthesis	**0.003505**	**0.03399**	NA
mmu00040 Pentose and glucuronate interconversions	**0.004877**	NA	NA
mmu00260 Glycine, serine and threonine metabolism	**0.009143**	NA	NA
mmu00290 Valine, leucine and isoleucine biosynthesis	**0.009433**	NA	NA
mmu04964 Proximal tubule bicarbonate reclamation	**0.013225**	NA	NA
mmu04146 Peroxisome	**0.019191**	NA	NA
mmu00480 Glutathione metabolism	**0.042182**	NS 0.077449	NS 0.156108
mmu01040 Biosynthesis of unsaturated fatty acids	**0.048593**	NA	NS 0.122241

NA: no proteins in the pathway, NS: not significant

Supplementary Table 3. Mitochondria specific KEGG pathway enrichment analysis: Pathways significantly (Q<0.05) enriched based on proteins that are less abundant in young C57Bl/6 under DR (left). Q values for enrichment of the same pathways based on proteins less abundant in long-lived young ICRFa mice (centre) and on proteins more abundant in old ICRFa mice (right) are also shown. Multiple pathways are enriched in all three conditions.

KEGG Metabolic pathway	Q-Values		
	less in DR	less in young ICRFa	more in old ICRFa
mmu01100 Metabolism	**0**	**0**	**0**
mmu00190 Oxphos	**1.55E-22**	**3.34E-12**	**1.18E-11**
mmu05012 Parkinson's disease	**2.76E-18**	**3.67E-12**	**3.62E-13**
mmu05016 Huntington's disease	**1.44E-17**	**1.05E-12**	**8.12E-13**
mmu05010 Alzheimer's disease	**4.07E-17**	**4.24E-12**	**1.89E-10**
mmu04146 Peroxisome	**0.000815**	**0.009966**	NA
mmu04141 Protein processing in endoplasmic reticulum	**0.001745**	NA	NA
mmu00120 Primary bile acid biosynthesis	**0.010878**	NA	NA
mmu00360 Phenylalanine metabolism	**0.029513**	NS 0.131602	NS 0.114578
mmu03320 PPAR signaling pathway	**0.043446**	NA	NS 0.225012

NA: no proteins in the pathway, NS: not significant

Supplementary table 4. Complex I profiling in C57Bl/6 AL liver mitochondria (15 months of age). Data are normalized peptide counts for all detected complex I proteins (plus NDUFA4 and prohibitins) in 12 BN gel slices as shown in Fig. 4E. Data are means from 3 mice run in separate gel lanes (± SEM in slice 1).

	Slice 1 M±SEM	Slice2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	Slice 8	Slice 9	Slice 10	Slice 11	Slice 12
MT-ND1	535±101											
MT-ND2	195 ± 41											
MT-ND3	113 ± 19	16										
MT-ND4	371±138											
MT-ND5	484±123											
NDUFA1	97 ± 7											
NDUFA10	949±169										250	169
NDUFA11	351 ± 19											
NDUFA12	298 ± 14							19			71	86
NDUFA13	1549±33	51	33									
NDUFA2	737±125											
NDUFA3	339 ± 59											
NDUFA4	134 ± 57	60	107	66	33	108	73	55		48	160	188
NDUFA5	397 ± 84											
NDUFA6	893±109	27										
NDUFA7	123 ± 20							19		16	194	242
NDUFA8	347 ± 48	16										
NDUFA9	2293±345	97										
NDUFB11	715 ± 80											
NDUFB3	343 ±58	27										
NDUFB4	435±145											
NDUFB5	273 ± 30											
NDUFB6	660 ± 41	44	17									

NDUFB7	328 ± 63	16										
NDUFB8	779±181											
NDUFB9	808 ± 15											
NDUFC2	441 ± 75											
NDUFS1	5160±455	200										
NDUFS2	1983±160	92										
NDUFS3	1451±206	60										
NDUFS4	263 ± 31											
NDUFS5	79 ± 11											
NDUFS7	896 ± 41											
NDUFS8	451 ± 65											
NDUFV1	1739±214	81										
NDUFV2	290 ± 26											
PHB	277 ± 56											
PHB2	413 ± 16										43	

Supplementary table 5. Complex I profiling in C57Bl/6 DR liver mitochondria (15 months of age, 12 months DR). Data are normalized peptide counts for all detected complex I proteins (plus NDUFA4 and prohibitins) in 12 BN gel slices as shown in Fig. 4E. Data are means from 3 mice run in separate gel lanes (± SEM in slice 1).

	Slice 1 M±SEM	Slice2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	Slice 8	Slice 9	Slice 10	Slice 11	Slice 12
MT-ND1	344 ± 48											
MT-ND2	190 ± 57											
MT-ND3	124 ± 17		14									
MT-ND4	242 ± 54											
MT-ND5	311 ± 35											
NDUFA1	59 ± 37											
NDUFA10	642 ± 73										209	223
NDUFA11	264 ± 64											34
NDUFA12	187 ± 9							20			93	80
NDUFA13	1111± 89	64	30									
NDUFA2	293 ± 22											
NDUFA3	179 ± 74		20									
NDUFA4	64 ± 19	31	60	76	75	94	66	109	123	62	156	175
NDUFA5	308 ± 7											
NDUFA6	393±122											
NDUFA7	62 ± 16						31	61	45	52	110	174
NDUFA8	327 ± 17											
NDUFA9	1655±155	17										
NDUFAB1	92 ± 64											
NDUFB11	328 ± 39	16										
NDUFB3	332 ± 70	19	30									
NDUFB4	373 ± 51											

NDUFB5	294 ± 7	17										
NDUFB6	407 ± 51	33	30									
NDUFB7	231 ± 15		13									
NDUFB8	571 ± 12											
NDUFB9	679 ± 60											
NDUFC2	243 ±48											
NDUFS1	3722±390	17				19						
NDUFS2	1650±128											
NDUFS3	1158±128	17					16					
NDUFS4	191 ± 53											
NDUFS5	78 ± 16											
NDUFS7	599 ± 73											
NDUFS8	353±132											
NDUFV1	1566±193	34										
NDUFV2	250 ± 28											
PHB	140 ± 7											
PHB2	254 ± 67							18			29	

Supplementary table 6. Complex I profiling in C57Bl/6 liver mitochondria (8 months of age). Data are normalized peptide counts for all detected complex I proteins (plus NDUFA4 and prohibitins) in 12 BN gel slices as shown in Fig. 4E. Data are means from 3 mice run in separate gel lanes (± SEM in slice 1).

	Slice 1 M±SEM	Slice2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	Slice 8	Slice 9	Slice 10	Slice 11	Slice 12
MT-ND1	575 ± 17											
MT-ND2	232 ± 90											
MT-ND3	233 ± 9											
MT-ND4	329 ± 66											
MT-ND5	412 ± 71	20										
NDUFA1	154 ± 5											
NDUFA10	1104±127										391	87
NDUFA11	447 ± 55											36
NDUFA12	323 ± 26									19	145	245
NDUFA13	1528± 75	39	73									
NDUFA2	531±110											
NDUFA3	235 ± 21											
NDUFA4	126 ± 37	87	131		71	46	51	19	95	83	265	268
NDUFA5	578±131											
NDUFA6	884 ± 45											
NDUFA7	123 ± 42							18	29	50	178	447
NDUFA8	681±254											
NDUFA9	2725±176											
NDUFB11	546 ± 73	20	36									
NDUFB3	630 ± 17	50	38									
NDUFB4	791 ± 18											
NDUFB5	420 ± 83		18									
NDUFB6	394 ± 24											

NDUFB7	576±100											
NDUFB8	814±100											20
NDUFB9	1178±115											
NDUFC2	502 ± 35											
NDUFS1	5332±152		55									
NDUFS2	2387±166	17					15					
NDUFS3	1799± 92											
NDUFS4	328 ± 74											
NDUFS5	254 ± 55											
NDUFS7	917 ± 55											
NDUFS8	589±155											
NDUFV1	2405± 30											
NDUFV2	308 ± 68											
PHB	367±107											47
PHB2	654±120	36	38								30	

Supplementary table 7. Complex I profiling in ICRFa liver mitochondria (8 months of age). Data are normalized peptide counts for all detected complex I proteins (plus NDUFA4 and prohibitins) in 12 BN gel slices as shown in Fig. 4E. Data are means from 3 mice run in separate gel lanes (± SEM in slice 1).

	Slice 1 M±SEM	Slice2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	Slice 8	Slice 9	Slice 10	Slice 11	Slice 12
MT-ND1	905±183											
MT-ND2	433 ± 15											
MT-ND3	229 ± 29											
MT-ND4	836 ± 56											
MT-ND5	723 ± 80											
NDUFA1	180 ± 39											
NDUFA10	2458± 77									149	63	
NDUFA11	493 ± 48											
NDUFA12	415 ± 75									34	42	
NDUFA13	2640±233											
NDUFA2	908 ± 69											
NDUFA3	497 ± 69											
NDUFA4	248 ± 58	80	117	82	31	14	47	20	16	79	75	97
NDUFA5	769 ± 44											
NDUFA6	1532± 23											
NDUFA7	263 ± 50						15	57	32	156	105	
NDUFA8	1181± 52											
NDUFA9	4551±287											
NDUFAB1	30 ± 30											
NDUFB11	1129± 27											
NDUFB3	826 ± 83											14
NDUFB4	868±120											
NDUFB5	532 ± 44											

NDUFB6	1142± 14											
NDUFB7	841 ± 42											
NDUFB8	851 ± 69											
NDUFB9	1368±122											
NDUFC2	863 ± 74											
NDUFS1	8528±190		17									
NDUFS2	3571± 50											
NDUFS3	3039±176											
NDUFS4	565 ± 58											
NDUFS5	503 ± 32											
NDUFS7	1424±131											
NDUFS8	932±104											
NDUFV1	3836±102											
NDUFV2	490 ± 20											
PHB	810 ± 28											
PHB2	918 ± 84	47	46								30	28