

Supplementary Materials for

**A conditional knockout rat resource of mitochondrial protein-coding genes  
via a DdCBE-induced premature stop codon**

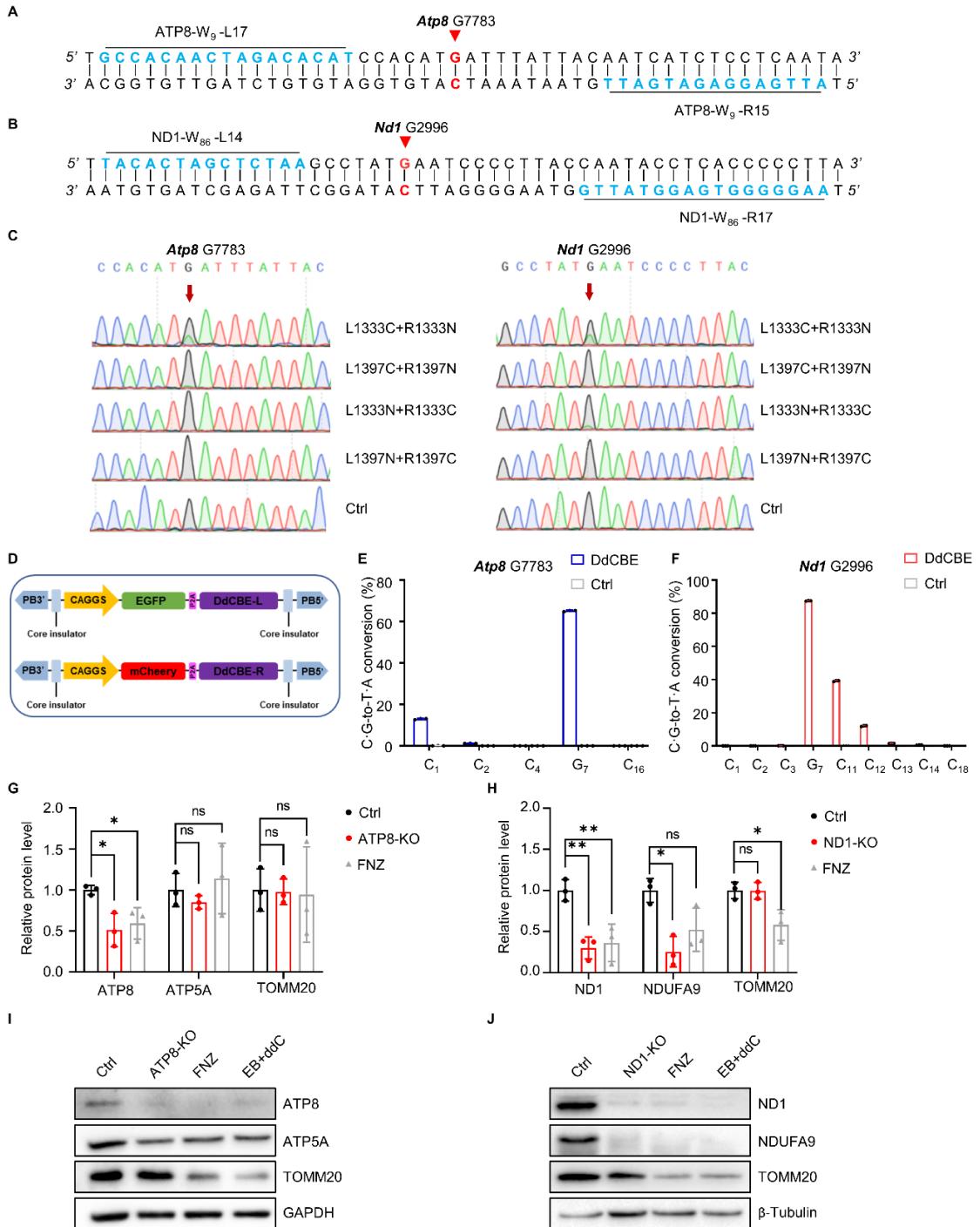
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**This PDF file includes:**

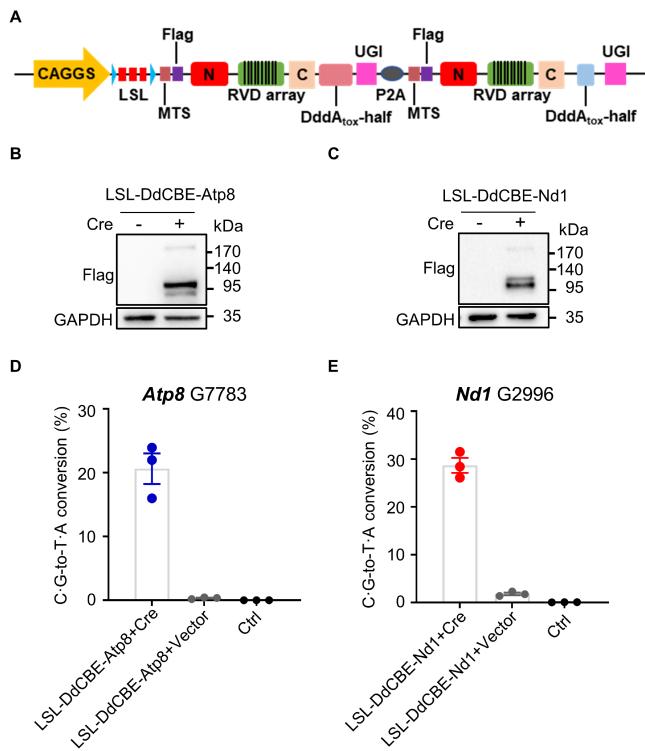
Figs. S1 to S12  
Tables S1 to S3  
Sequences S1 and S2



## **Fig. S1.** Screening efficient DdCBE pair for *Atp8* and *Ndl* targeting.

**(A and B)** Design overview of TALEs targeting rat *Atp8* G7783 site (A) and *Nd1* G2996 site (B). **(C)** The editing efficiency of four DdCBE pairs (L1333C + R1333N, L1397C + R1397N, L1333N + R1333C, and L1397N + R1397C) for *Atp8* G7783 site and *Nd1* G2996 site were analyzed by Sanger sequencing. **(D)** DdCBE pair with best performance was cloned into PB

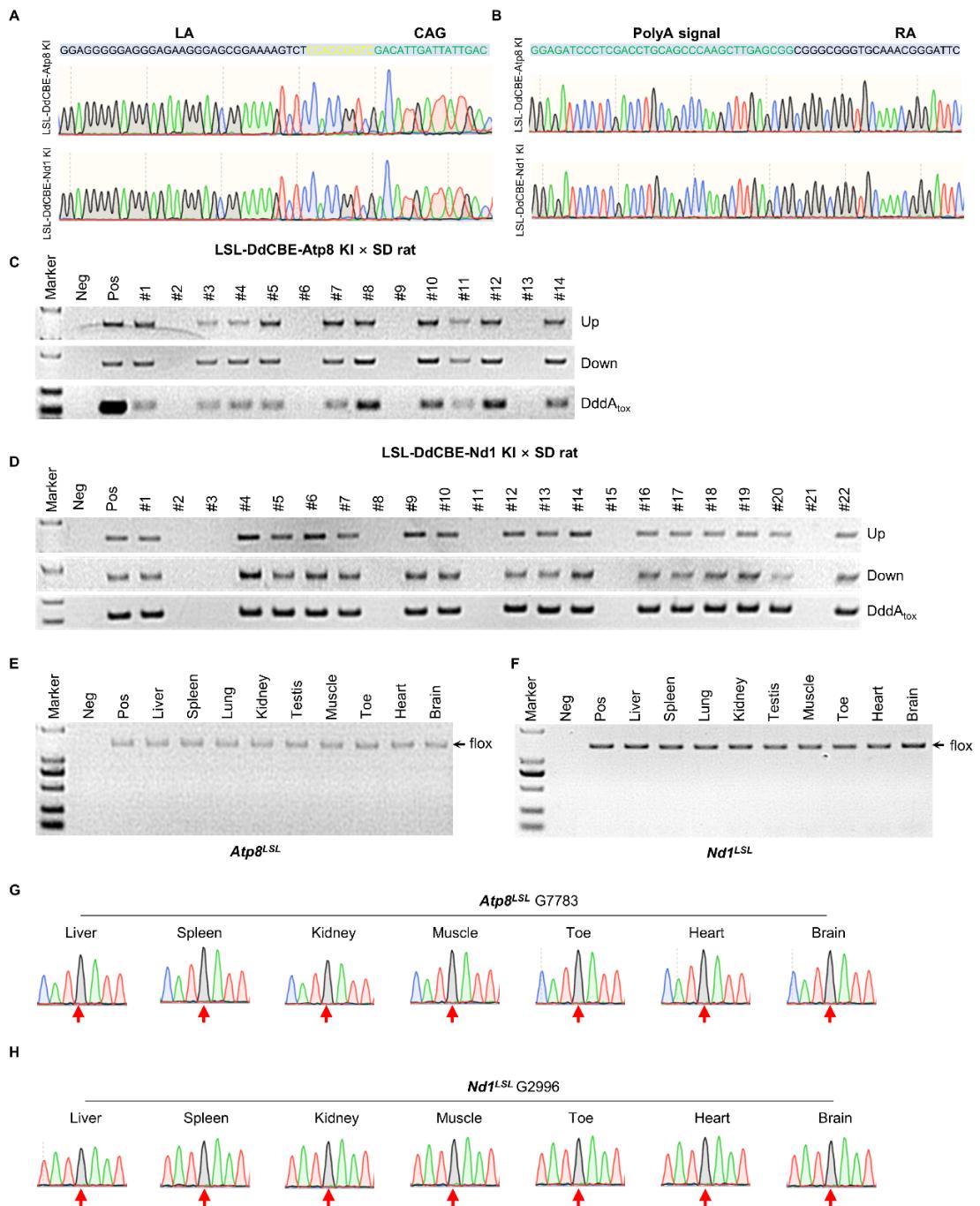
vector with EGFP or mCherry tags. **(E and F)** The frequencies of C•G to T•A conversion at *Atp8* G7783 site (E) and *Nd1* G2996 site (F) were analyzed by deep sequencing. n = 3 technical replicates. Data were presented as means  $\pm$  SD. **(G and H)** The qualitative analysis of protein expression in treated C6 cells from Figure 1I and Figure 1J. n = 3 technical replicates. Data were presented as means  $\pm$  SD. ns, not significant, \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$  by one-way ANOVA test paired with a Tukey's Honest Significant Difference. **(I and J)** The protein level was detected by western blot in C6 cells treated with DdCBE, FNZ, or EB + ddC.



**Fig. S2.**

**Construct a Cre-inducible DdCBE system in C6 cells.**

(A) Schematic overview of Cre-inducible DdCBE system. The inducible DdCBE plasmid (LSL-DdCBE) contains a CAG promoter, two *loxP* sites flanking a triple repeat of poly(A) sequence, and a DdCBE pair linked by P2A peptides. (B and C) Expression of DdCBE for *Atp8* G7783 site (B) and *Nd1* G2996 site (C) were analyzed by western blot after 48h co-transfection of LSL-DdCBE plasmid with or without *Cre* plasmid. (D and E) The frequency of DdCBE-mediated C•G to T•A conversion at *Atp8* G7783 site (D) and *Nd1* G2996 site (E) were analyzed by deep sequencing. n = 3 biological replicates for each group, biological replicates. Data were presented as means ± SD.

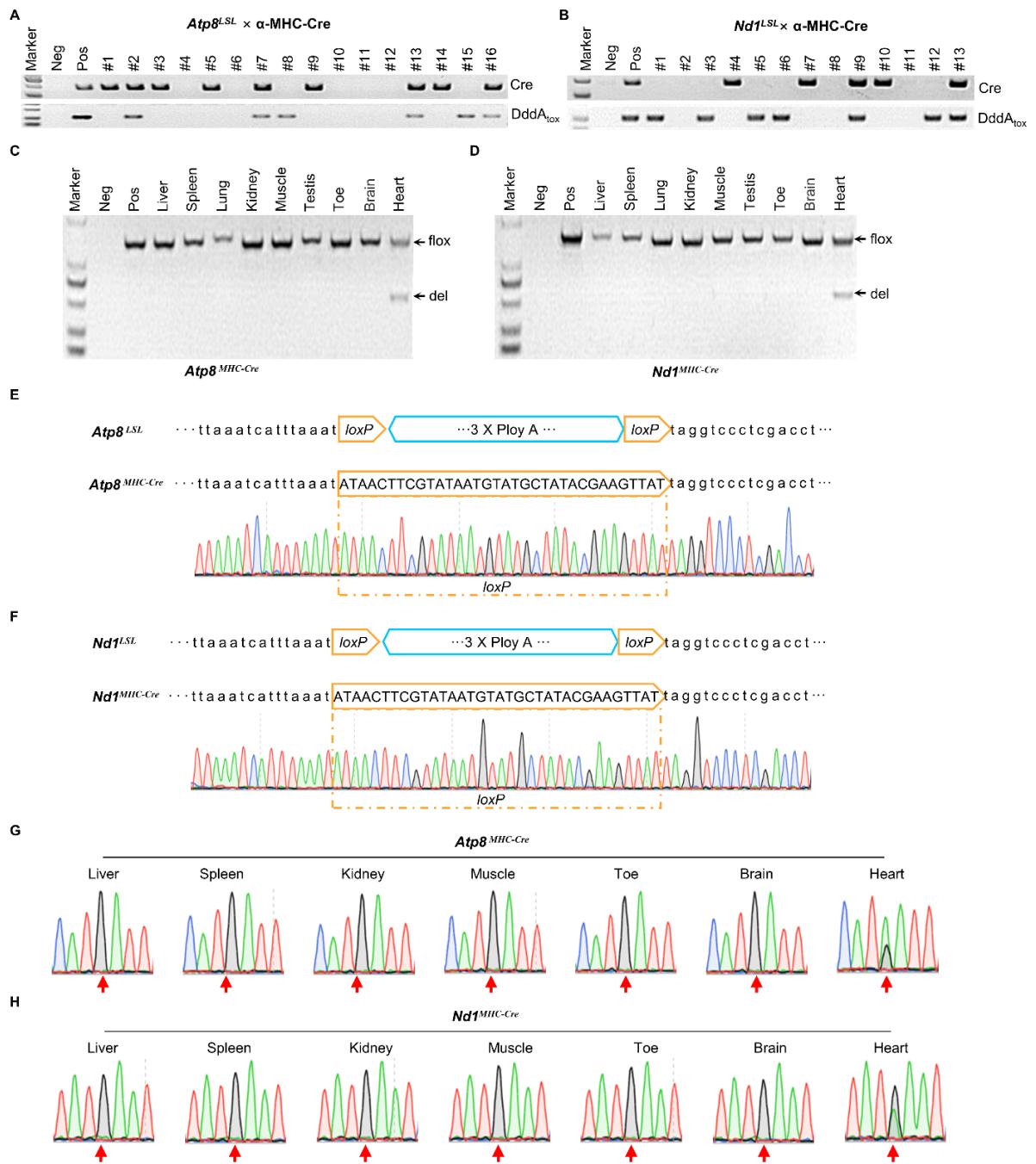


**Fig. S3.**

**The germline transmission of LSL-DdCBE KI rats.**

**(A and B)** The correct recombination of LSL-DdCBE cassettes were confirmed by Sanger sequencing for LA (A) and RA (B) in LSL-DdCBE-Atp8 KI F<sub>0</sub> rats and LSL-DdCBE-Nd1 KI F<sub>0</sub> rats. **(C and D)** Genotyping of LSL-DdCBE-Atp8 KI F<sub>1</sub> rats (C) and LSL-DdCBE-Nd1 KI F<sub>1</sub> rats (D) were analyzed using PCR with Up, Down, and DddA<sub>tox</sub> primer pairs. **(E and F)** The LSL element in various tissues of LSL-DdCBE-Atp8 KI F<sub>1</sub> rat (E) and LSL-DdCBE-Nd1 KI F<sub>1</sub> rat (F) were analyzed using PCR with the loxP primer pair. **(G and H)** Sanger sequencing for Atp8 G7783

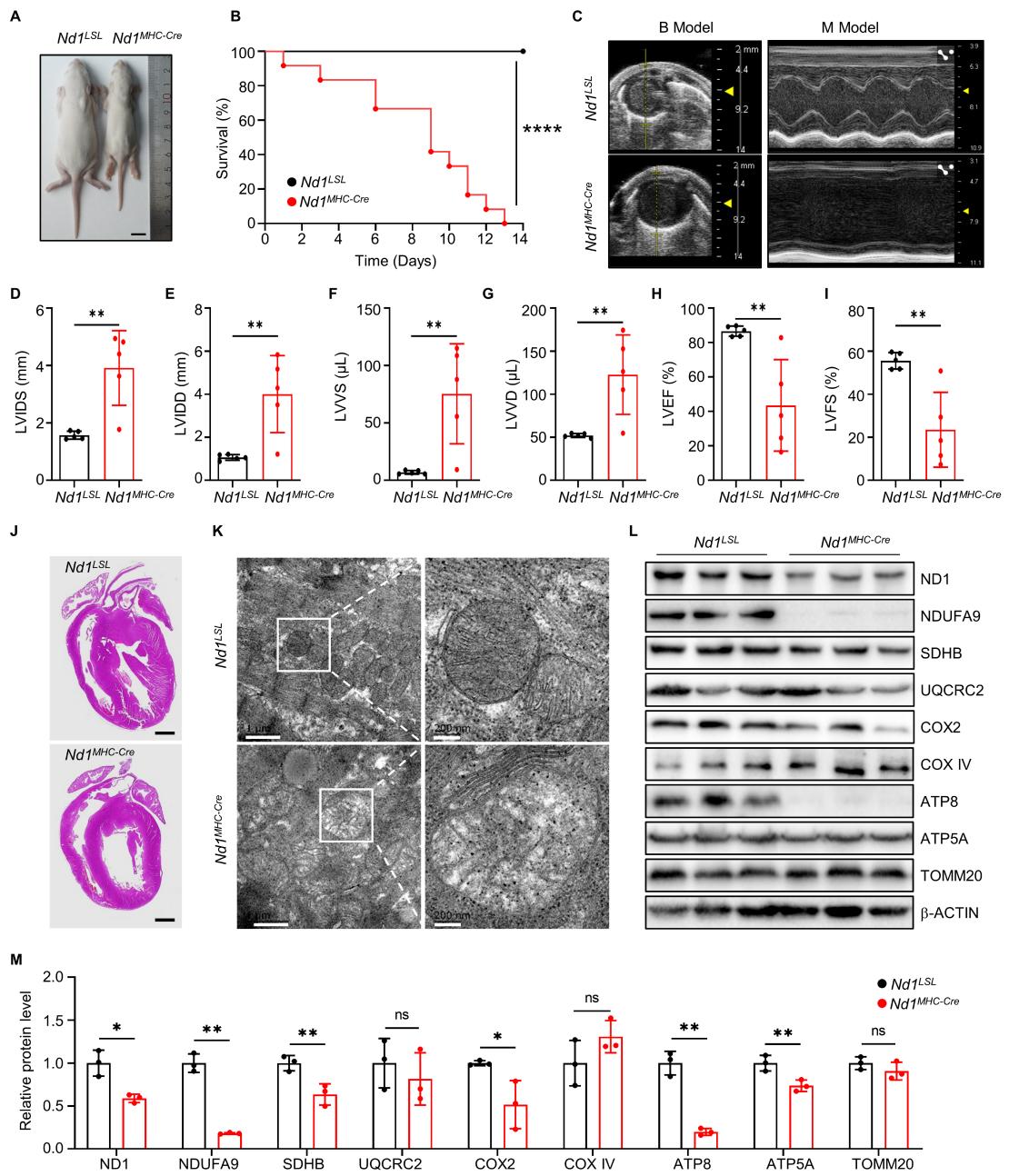
site (G) and *Nd1* G2996 site (H) in various tissues of LSL-DdCBE-Atp8 KI F<sub>1</sub> rat and LSL-DdCBE-Nd1 KI F<sub>1</sub> rat.



**Fig. S4.**

#### Heart-specific depletion of ATP8/ND1 using $\alpha$ -MHC-Cre rats.

**(A and B)** Genotyping of the offspring produced from  $Atp8^{LSL} \times \alpha$ -MHC-Cre rats (A) and  $Nd1^{LSL} \times \alpha$ -MHC-Cre rats (B) using Cre and DddA<sub>tox</sub> primer pairs. **(C and D)** The LSL element in various tissues of  $Atp8^{MHC-Cre}$  rat (C) and  $Nd1^{MHC-Cre}$  rat (D) was analyzed using the loxP primer pair. **(E and F)** Cre-mediated LSL deletion in heart tissues of  $Atp8^{MHC-Cre}$  rat (E) and  $Nd1^{MHC-Cre}$  rat (F) were confirmed by Sanger sequencing. **(G and H)** Sanger sequencing for  $Atp8$  G7783 site (G) and  $Nd1$  G2996 site (H) in various tissues of  $Atp8^{MHC-Cre}$  and  $Nd1^{MHC-Cre}$  rats.

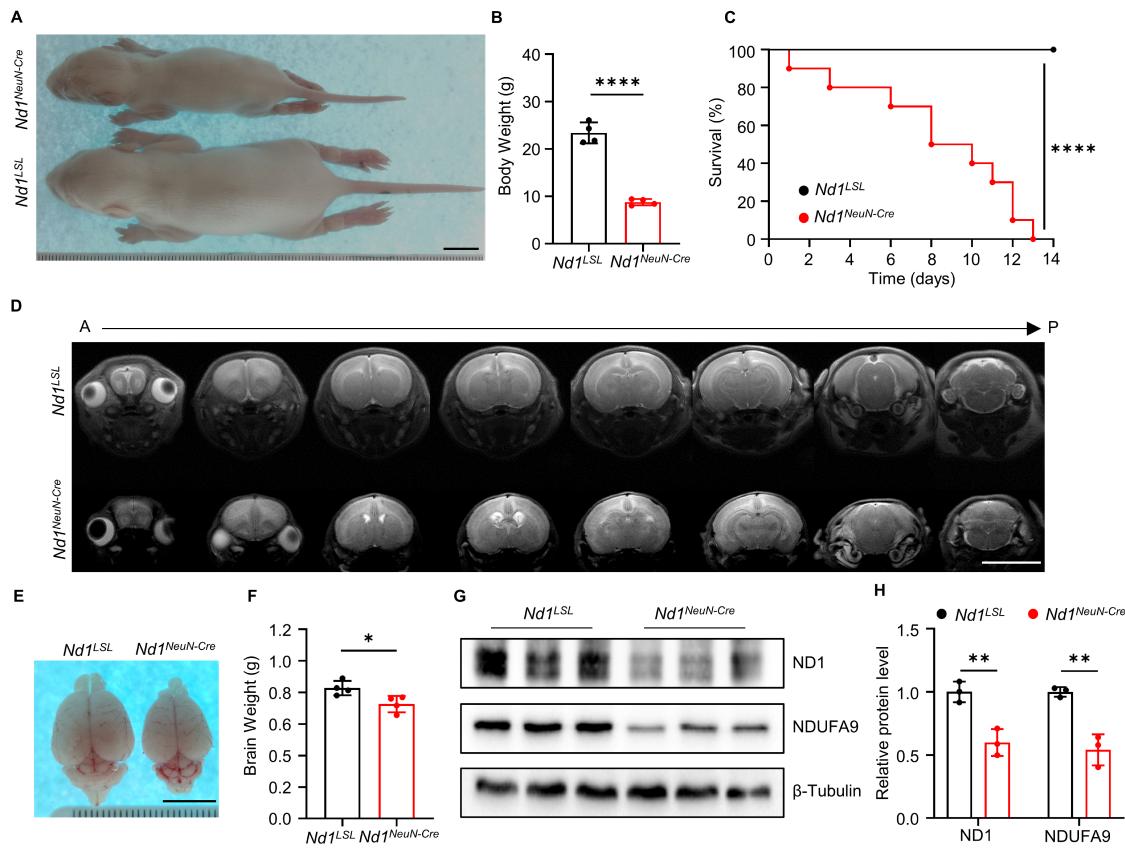


**Fig. S5.**

### Heart-specific depletion of ND1 causes heart failure in rats.

(A) The whole-body image of ten-day-age *Nd1<sup>LSL</sup>* and *Nd1<sup>MHC-Cre</sup>* pups. Scale bar, 1 cm. (B) The survival curve of *Nd1<sup>LSL</sup>* and *Nd1<sup>MHC-Cre</sup>* rats. n = 10 biological replicates for each group. \*\*\*\*P ≤ 0.0001 by Log-rank test. (C) The snap shot of echocardiography for *Nd1<sup>LSL</sup>* and *Nd1<sup>MHC-Cre</sup>* rats. (D to I) The cardiac structure and function parameters of *Nd1<sup>LSL</sup>* and *Nd1<sup>MHC-Cre</sup>* rats calculated from echocardiography in (C). Left ventricular (LV) diameter at end of systole (LVDS) (D) and diastole (LVIDD) (E); LV volume at end of systole (LVVS) (F) and diastole (LVVD) (G); LV ejection fraction (LVEF) (H); LV percentage fractional shortening (LVFS) (I). n = 5 biological replicates for each group. Data were presented as means ± SD. \*\*P ≤ 0.01 by Student's unpaired

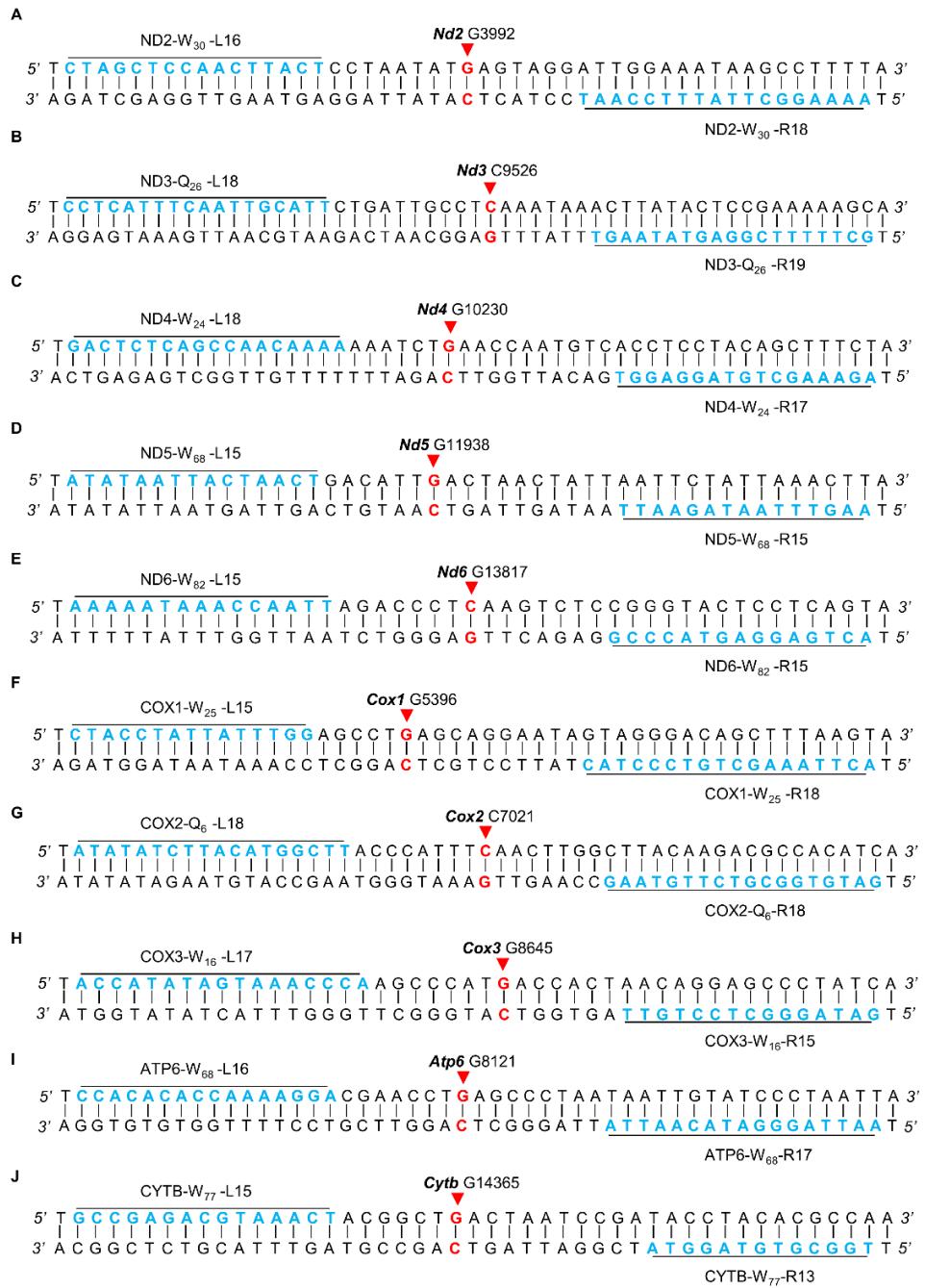
two-tailed *t*-test. **(J)** The H&E image of heart tissue from *Nd1<sup>LSL</sup>* and *Nd1<sup>MHC-Cre</sup>* rats. Scale bar, 1 mm. **(K)** The TEM image of mitochondria in heart tissue of *Nd1<sup>LSL</sup>* and *Nd1<sup>MHC-Cre</sup>* rats. **(L and M)** The mitochondrial protein level in heart tissues of *Nd1<sup>LSL</sup>* and *Nd1<sup>MHC-Cre</sup>* rats were analyzed by western blot (L) and quantification (M). n = 3 biological replicates for each group. Data were presented as means ± SD. ns, not significant, \**P* ≤ 0.05, \*\**P* ≤ 0.01 by Student's unpaired two-tailed *t*-test.



**Fig. S6.**

**Neuron-specific knockout ND1 results in postnatal death in rats.**

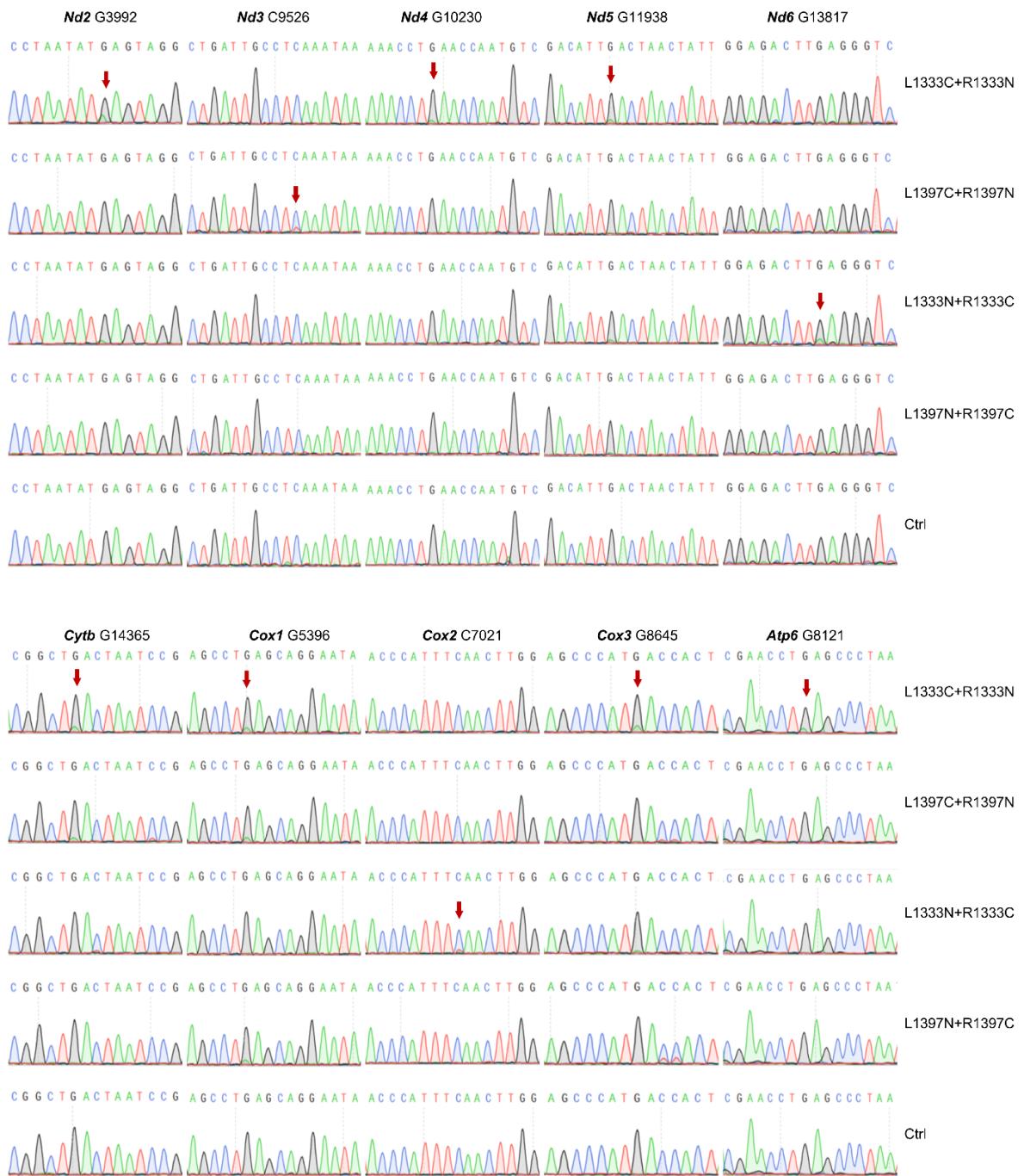
**(A)** The whole-body image of *Nd1<sup>LSL</sup>* and *Nd1<sup>NeuN-Cre</sup>* rats. Scale bar, 1 cm. **(B)** the body weight of *Nd1<sup>LSL</sup>* and *Nd1<sup>NeuN-Cre</sup>* rats. n = 4 biological replicates for each group. \*\*\*\*P ≤ 0.0001 by Student's unpaired two-tailed t-test. **(C)** The survival curve of *Nd1<sup>LSL</sup>* and *Nd1<sup>NeuN-Cre</sup>* rats. n = 10 biological replicates for each group. \*\*\*\*P ≤ 0.0001 by Log-rank test. **(D)** The brain MRI image of *Nd1<sup>LSL</sup>* and *Nd1<sup>NeuN-Cre</sup>* rats from anterior (A) to posterior (P). Scale bar, 1 cm. **(E)** The brain image of *Nd1<sup>LSL</sup>* and *Nd1<sup>NeuN-Cre</sup>* rats. Scale bar, 1 cm. **(F)** The brain weight of *Nd1<sup>LSL</sup>* and *Nd1<sup>NeuN-Cre</sup>* rats. n = 4 biological replicates for each group. \*\*\*\*P ≤ 0.0001 by Student's unpaired two-tailed t-test. **(G and H)** The protein level of ND1 and NDUFA9 in brain tissue of *Nd1<sup>LSL</sup>* and *Nd1<sup>NeuN-Cre</sup>* rats were analyzed by western blot (G) and quantification (H). n = 3 biological replicates for each group. Data were presented as means ± SD. \*\*P ≤ 0.01 by Student's unpaired two-tailed t-test.



**Fig. S7.**

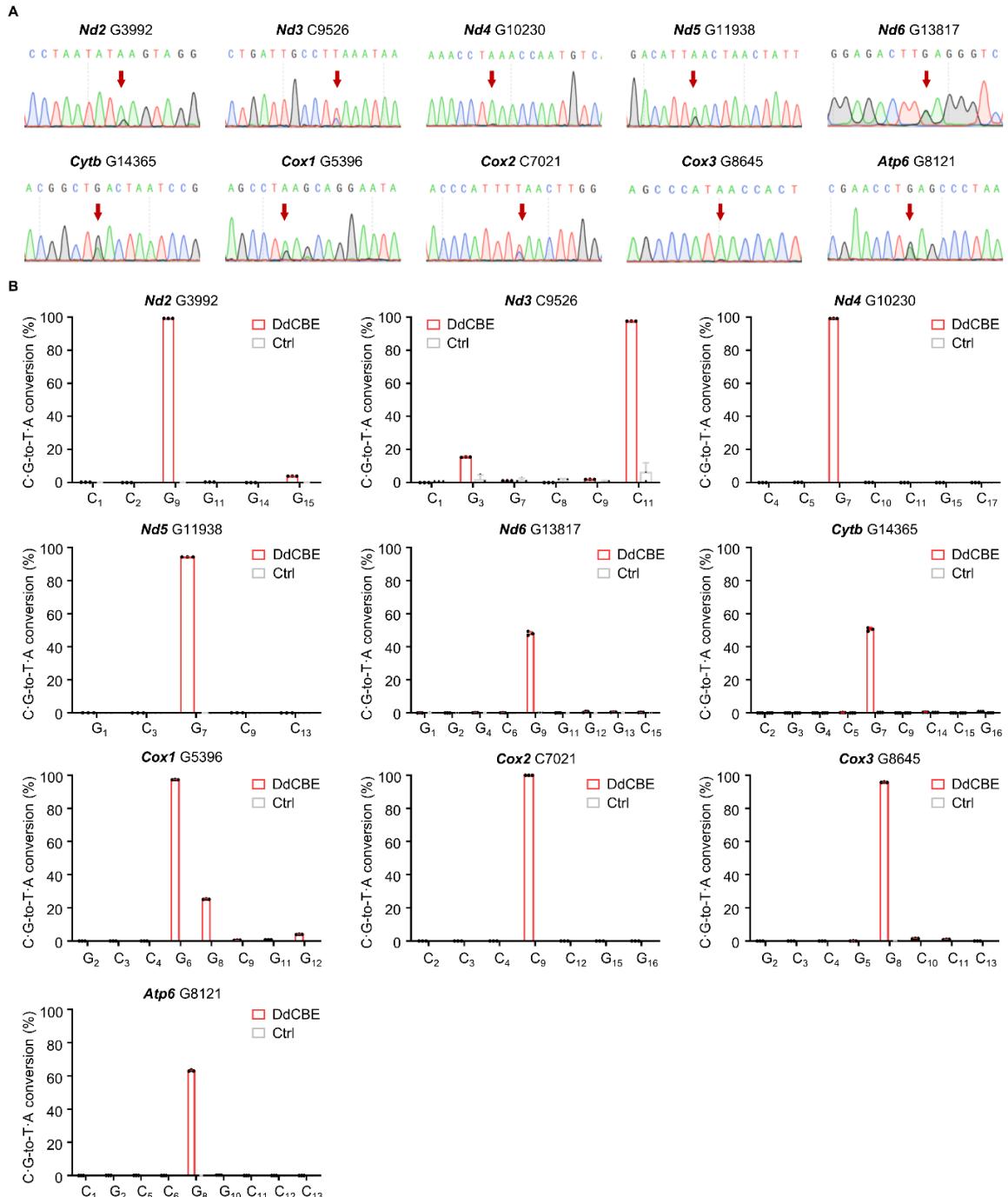
## **Design of knockout all rat mtDNA protein-coding genes.**

**(A to J)** Design overview of DdCBEs for *Nd2* G3992 (A), *Nd3* C9526 (B), *Nd4* G10230 (C), *Nd5* G11938 (D), *Nd6* G13817 (E), *Cytb* G14365 (F), *Cox1* G5396 (G), *Cox2* C7021 (H), *Cox3* G8645 (I), and *Atp6* G8121 (J) sites.



**Fig. S8.**

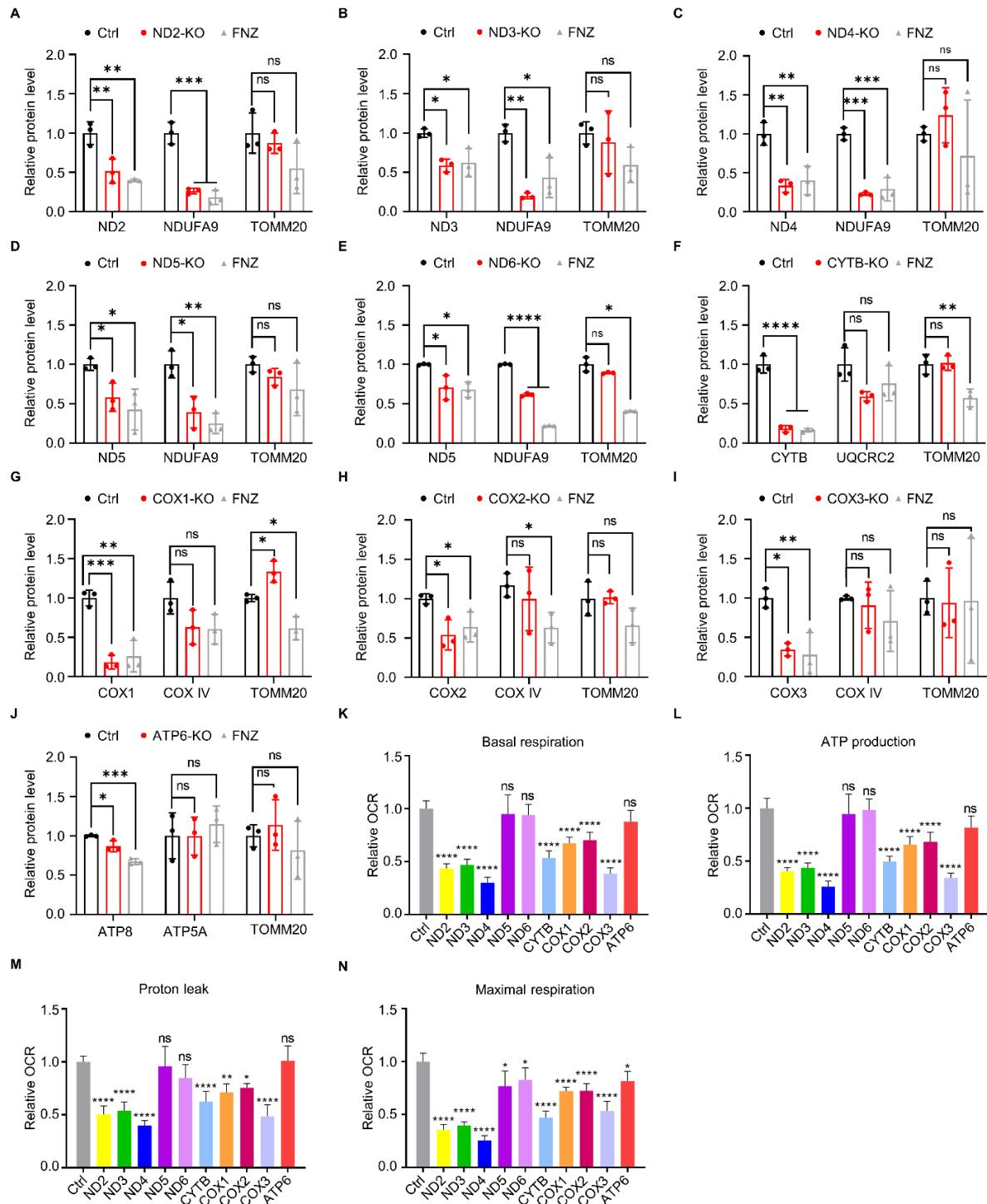
**Screening of efficient DdCBE pairs for targeting mtDNA protein-coding genes in C6 cells.**  
 Sanger sequencing for *Nd2* G3992, *Nd3* C9526, *Nd4* G10230, *Nd5* G11938, *Nd6* G13817, *Cytb* G14365, *Cox1* G5396, *Cox2* C7021, *Cox3* G8645, and *Atp6* G8121 sites in C6 cells transfected with four DdCBE pairs for each target site.



**Fig. S9.**

### The editing efficiency of DdCBE for rat mtDNA protein-coding genes in C6 cells.

(A) Sanger sequencing for *Nd2* G3992, *Nd3* C9526, *Nd4* G10230, *Nd5* G11938, *Nd6* G13817, *Cytb* G14365, *Cox1* G5396, *Cox2* C7021, *Cox3* G8645, and *Atp6* G8121 sites in PB-DdCBE plasmids transfected C6 cells. (B) The frequency of C•G to T•A conversion in the spacer region of *Nd2* G3992, *Nd3* C9526, *Nd4* G10230, *Nd5* G11938, *Nd6* G13817, *Cytb* G14365, *Cox1* G5396, *Cox2* C7021, *Cox3* G8645, and *Atp6* G8121 sites was analyzed by deep sequencing. n = 3 technical replicates. Data were presented as means ± SD.

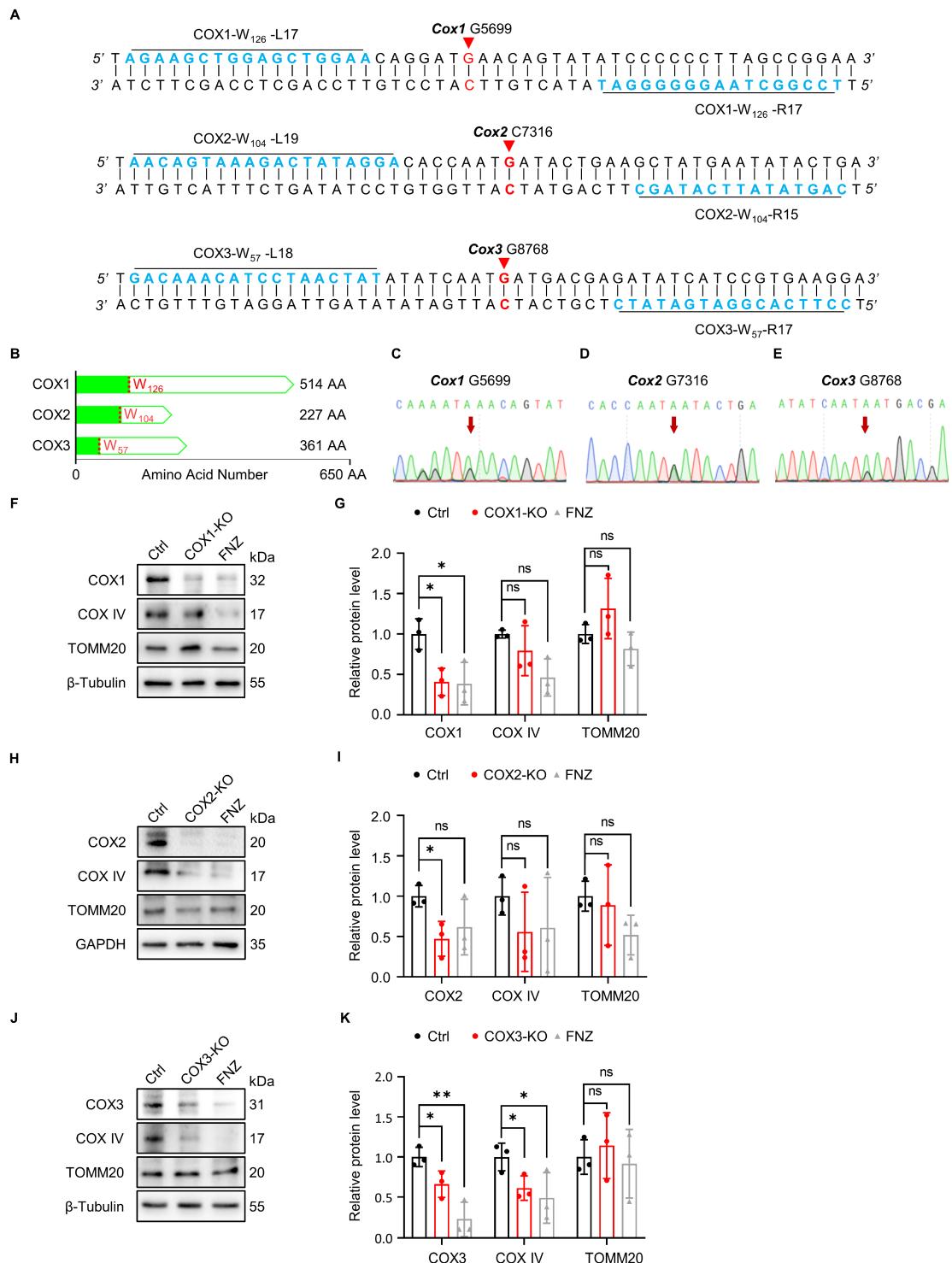


**Fig. S10.**

### Depletion of mtDNA-encoded proteins in C6 cells.

(A to J) The quantitative analysis of protein expression in ND2-KO (A), ND3-KO (B), ND4-KO (C), ND5-KO (D), ND6-KO (E), CYTB-KO (F), COX1-KO (G), COX2-KO (H), COX3-KO (I), and ATP6-KO (J) C6 cells from Figure 5I to 5L. n = 3 technical replicates. Data were presented

as means  $\pm$  SD. ns, not significant,  $*P \leq 0.05$ ,  $**P \leq 0.01$ ,  $***P \leq 0.001$ ,  $****P \leq 0.0001$  by one-way ANOVA test paired with a Tukey's Honest Significant Difference. **(K to N)** Relative values of OXPHOX parameters from Figure 5C to 5L. n = 5 technical replicates for Ctrl and n = 6 technical replicates for each KO cell line. Data were presented as means  $\pm$  SD. ns, not significant,  $*P \leq 0.05$ ,  $**P \leq 0.01$ ,  $***P \leq 0.001$ , and  $****P \leq 0.0001$  by one-way ANOVA test paired with a Tukey's Honest Significant Difference.

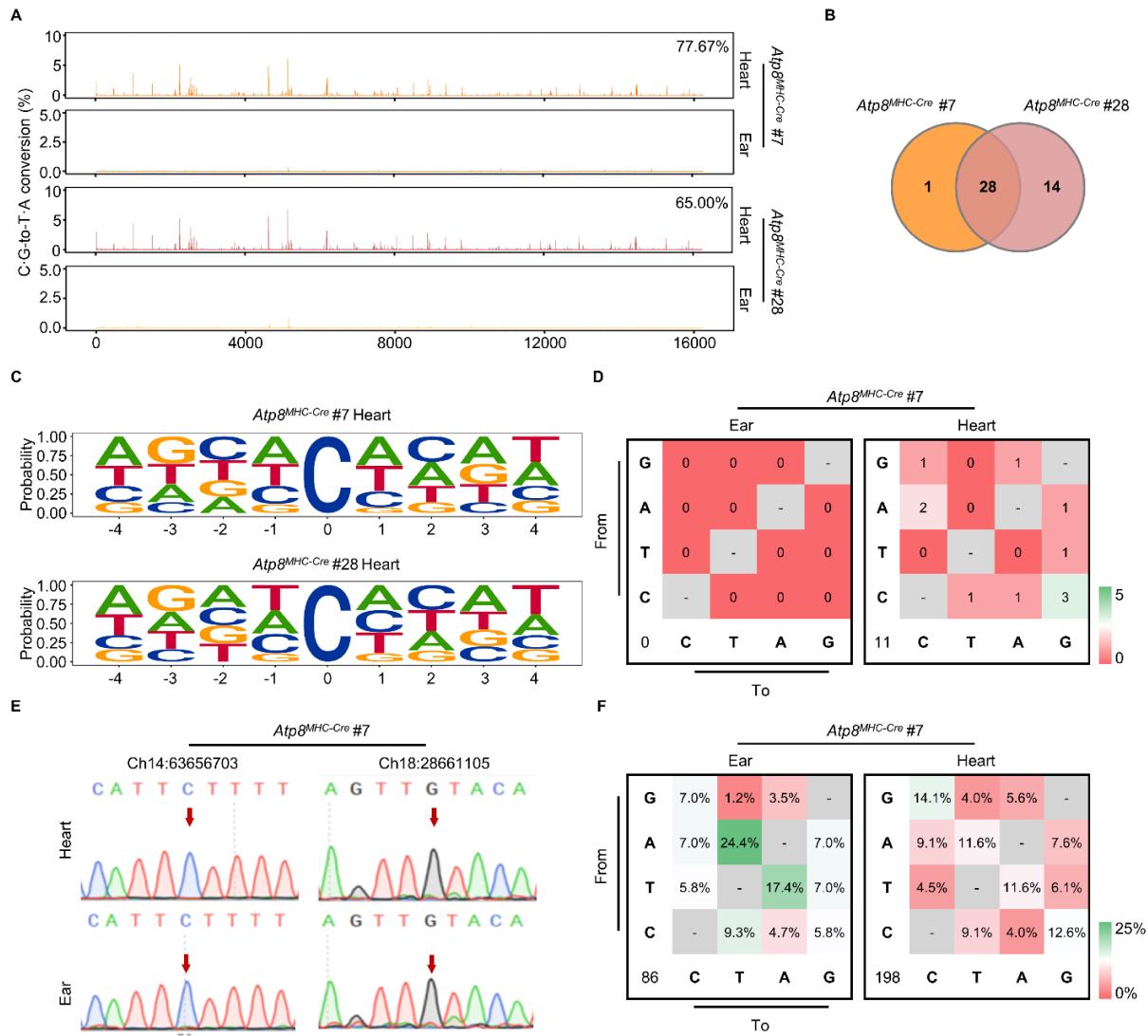


**Fig. S11.**

## **Depletion of mtDNA-encoded COXs in C6 cells.**

**(A)** Design overview of DdCBEs for *Cox1* G5699, *Cox2* G7316, and *Cox3* G8768 sites. **(B)** The location of DdCBE-introduced stop codon at COX1, COX2, and COX3. **(C to E)** Sanger

sequencing for *Cox1* G5699 (C), *Cox2* G7316 (D), and *Cox3* G9438 (E) sites in PB-DdCBE plasmids transfected C6 cells. **(F to K)** The protein level of COX1 (F and G), COX2 (H and I), and COX3 (J and K) in C6 cells treated with FNZ or targeted by corresponding DdCBEs were analyzed by western blot and quantification. n = 3 technical replicates. Data were presented as means  $\pm$  SD. ns, not significant, \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$  by one-way ANOVA test paired with a Tukey's Honest Significant Difference.



**Fig. S12.**

### Off-target analysis on whole mitochondrial genome and nuclear genome.

(A) The frequencies of C·G-to-T·A conversion at off-target sites in the whole mitochondrial genome of *Atp8<sup>MHC-Cre</sup>* rats. The editing frequency of on-target was detected by Deep sequencing and marked in the upper right corner. (B) Venn diagram showing the overlap of off-target sites between *Atp8<sup>MHC-Cre</sup>* #7 rat and *ATP8<sup>MHC-Cre</sup>* #28 rat. (C) The motif analysis for non-target sites in heart tissue of *Atp8<sup>MHC-Cre</sup>* #7 and *ATP8<sup>MHC-Cre</sup>* #28 rats. (D) The distribution of base conversion type in the nuclear genome of *Atp8<sup>MHC-Cre</sup>* #7 rat. The numbers in each cell indicated the number of a certain mutation type. The number of detected SNVs was marked in the bottom left cell. (E) Sanger sequencing for putative off-target sites in *Atp8<sup>MHC-Cre</sup>*#7. (F) The distribution of base conversion type in the nuclear genome of *Atp8<sup>MHC-Cre</sup>* #7 rat. The numbers in cell indicated the proportion of a certain mutation type.

**Table S1.****Injection summary of CRISPR/Cas9-mediated LSL-DdCBE knockin (KI) rats.**

Target gene	gRNA Con. (ng/µL)	Donor Con. (ng/µL)	Cas9 (ng/µL)	No. of injected zygotes	No. of transplanted zygotes	No. of pups	No. of KI pups
<i>Atp8</i>	12 + 12	7	30	75	44	10	5
<i>Nd1</i>	12 + 12	4	30	77	44	16	14
<i>Cytb</i>	12 + 12	8	30	79	43	8	8
<i>Cox3</i>	12 + 12	7	30	70	46	20	7
<i>Nd2</i>	12 + 12	4	30	60	41	10	6
<i>Nd5</i>	12 + 12	4	30	143	90	8	5

**Table S2.****Primers used in this study.**

	Primers	Sequence (5'-3')
Primers for RVD	RVD seq Fwd	TGACCGCAGTGGAGGCAGTG
	RVD seq Rev	GCATCCAGCGCAGGAC
Primers for genotyping	Rat-Up-Fwd	GGGTGGCGAAGGTAATGTCT
	Rat-Up-Rev	GCTATGAACTAATGACCCGTAATT
	Rat-Down-Fwd	GCATCTGACTCTGGCTAATAAAG
	Rat-Down-Rev	CATTAACAGGAAATGGCTCAGTTATAAATG
	Rat-G2966-Fwd	GAAAATCCTAGGCTACATACAACTAGC
	Rat-G2966-Rev	GAGGATAATGGCTATTGTGACTT
	Rat-G7783- Fwd	TGCTCTGAAATTGCGGCTC
	Rat-G7783-Rev	AGTCGGTTGCTGATTAGGCG
	Rat-loxP-Fwd	TCTGCTAACCATGTTCATGCC
	Rat-loxP-Rev	GCTACCTTATCGTCGTACCT
Primers for DddA <sub>tox</sub> halves	Halves Fwd	TTGCGGAGACTCACCCCTT
	Halves Rev	TGTAAACCCATGGCCGAC
Primers for on-target sanger-sequence	rND1KO-seq-Fwd	ATGGCCTCCTCACCCCTAGT
	rND1KO-seq-Rev	AATAGGGCGAATGGTCTGC
	rND2KO-seq-Fwd	GGCCCATACCCCGAAAATGT
	rND2KO-seq-Rev	TCCTTGGGTGACTTCGGGTA
	rCOX1KO-seq-Fwd	AGGCAGGGAGAACGCCTAGTA
	rCOX1KO-seq-Rev	AGATAGAACACCCCCGGCT
	rCOX2KO-seq-Fwd	AGCCTTCGCACTAAACGAG
	rCOX2KO-seq-Rev	TAGGTGATGTGGCGTCTTGT
	rATP8KO-seq-Fwd	CCCGCCCCATCTAACATCTC
	rATP8KO-seq-Rev	AGAACGCGTGTAGGGTTGCT
	rATP6KO-seq-Fwd	TCCC AACCTTCCTGCACC
	rATP6KO-seq-Rev	TCCTTGCCTAAAGAAGTGGG
	rCOX3KO-seq-Fwd	TTCTTACCGCAAGGAACCCC
	rCOX3KO-seq-Rev	TGGTGGCCTTGGTATGTTCC
	rND3KO-seq-Fwd	CGCAGCATGATACTGACACT
	rND3KO-seq-Rev	GTGCAGAACTTGTGGGTG
	rND4KO-seq-Fwd	TGCGAAGCAGCAGTAGGTTA
	rND4KO-seq-Rev	AAGCGTTCTGTTGGTTGCC
	rND5KO-seq-Fwd	ACCTTGGTCAACTCCAAAT
	rND5KO-seq-Rev	CGGTTAATGTGGGGATCAGAGT
	rND6KO-seq-Fwd	GCATCCTAGCAGGCTTCCTT
	rND6KO-seq-Rev	GGTTGTCTAGGGTTGGCGTT
	rCYTBKO-seq-Fwd	CCCGCCCCATCTAACATCTC
	rCYTBKO-seq-Rev	AGAACGCGTGTAGGGTTGCT
	rCOX1KO-2-seq-Fwd	AGGCAGGGAGAACGCCTAGTA
	rCOX1KO-2-seq-Rev	AGATAGAACACCCCCGGCT
	rCOX2KO-2-seq-Fwd	CTTGGCTACAAGACGCCAC
	rCOX2KO-2-seq-Rev	CCTAGTGAAGGGACGGCTCA
	rCOX3KO-2-seq-Fwd	TTCTTACCGCAAGGAACCCC
	rCOX3KO-2-seq-Rev	TGGTGGCCTTGGTATGTTCC
Barcoded Primers for on-target Deep-sequence	i5-rND1KO-Fwd	ACACTCTTCCTACACGACGCTTCCGATC
	i7-rND1KO-Rev	TTGCGAACCCATAACGCCCTAAC
	i5-rND2KO-Fwd	GTGACTGGAGTTCAGACGTGTGCTCTCCGA
	i7-rND2KO-Rev	TCTACCGCTCGTAGGGCTCCGAATAG
		ACACTCTTCCTACACGACGCTTCCGATC
		TTGCTGTTGGCTAAACCCCTCCG
		GTGACTGGAGTTCAGACGTGTGCTCTCCGA
		TCTACCTTGAGGCTGTAGCTGGGTT

i5-rCOX1KO-Fwd	ACACTTTCCCTACACGACGCTTCCGATC
i7-rCOX1KO-Rev	TTGACTGGAGTTCAGACGTGTGCTTCCGA
i5-rCOX2KO-Fwd	TCTACCTACGAATGCATGGGCTGTGA
i7-rCOX2KO-Rev	ACACTTTCCCTACACGACGCTTCCGATC
i5-rATP8KO-Fwd	TTGCGGAAGGATTCAACCCCCCTACA
i7-rATP8KO-Rev	GTGACTGGAGTTCAGACGTGTGCTTCCGA
i5-rATP6KO-Fwd	TCTACCTGGGGGTCTATTGTGCTTGT
i7-rATP6KO-Rev	ACACTTTCCCTACACGACGCTTCCGATC
i5-rCOX3KO-Fwd	TTGCAACTCATTGCGAAGCTTAGAGCG
i7-rCOX3KO-Rev	GTGACTGGAGTTCAGACGTGTGCTTCCGA
i5-rND3KO-Fwd	TCTACCTGCTCATAGGGGGATGGCTA
i7-rND3KO-Rev	ACACTTTCCCTACACGACGCTTCCGATC
i5-rND4KO-Fwd	TTGCACTTGAATTGCCGTAGCCT
i7-rND4KO-Rev	GTGACTGGAGTTCAGACGTGTGCTTCCGA
i5-rND5KO-Fwd	TCTACCGAGATGTTGTCAGGAGGCC
i7-rND5KO-Rev	ACACTTTCCCTACACGACGCTTCCGATC
i5-rCYTBKO-Fwd	TTGCGATGAGGATCCTACTCCCTAGT
i7-rCYTBKO-Rev	GTGACTGGAGTTCAGACGTGTGCTTCCGA
i5-rCOX1KO-2-Fwd	TCTACCGTGCAGAACTGTTGGGTG
i7-rCOX1KO-2-Rev	ACACTTTCCCTACACGACGCTTCCGATC
i5-rCOX2KO-2-Fwd	TTGCCTGCGAACAGCAGTAGGTT
i7-rCOX2KO-2-Rev	GTGACTGGAGTTCAGACGTGTGCTTCCGA
i5-rCOX3KO-2-Fwd	TCTACCTGGGGTGATAATGGATCGG
i7-rCOX3KO-2-Rev	ACACTTTCCCTACACGACGCTTCCGATC
Primers for long-range PCR	rat-mt F1 Fwd rat-mt F1 Rev rat-mt F2 Fwd rat-mt F2 Rev
	TTGCTCCTCCTAGCCTTACCT
	GTGACTGGAGTTCAGACGTGTGCTTCCGA
	TCTACCGGTTAATGTGGGGATCAGAG
	ACACTTTCCCTACACGACGCTTCCGATC
	TTGCCAACTATAAATAGCCCAACCCC
	GTGACTGGAGTTCAGACGTGTGCTTCCGA
	TCTACCTATTGGGGGGATGTTGGTTG
	ACACTTTCCCTACACGACGCTTCCGATC
	TTGCTCCTCACAGGCTTATTCTAGC
	GTGACTGGAGTTCAGACGTGTGCTTCCGA
	TCTACCTAGTCTCGTCCCACATGGA
	ACACTTTCCCTACACGACGCTTCCGATC
	TTGCAGGCTCGGGAACTGACTTG
	GTGACTGGAGTTCAGACGTGTGCTTCCGA
	TCTACCAGATAGAAGACACCCCGCT
	ACACTTTCCCTACACGACGCTTCCGATC
	TTGCCACAAGACAATAGACGCC
	GTGACTGGAGTTCAGACGTGTGCTTCCGA
	TCTACCACGAAGTTCACCTGGTTAGGT
	ACACTTTCCCTACACGACGCTTCCGATC
	TTGCACCCAAGCCCATGACCACTA
	GTGACTGGAGTTCAGACGTGTGCTTCCGA
	TCTACCATTCCGTATCGGAGGCCTTT

**Table S3.****The detailed information of the six cKO rat strains in Rat Resource Database.**

Accession No.	Strain Name	Gene	Type	Description	Links
GC00215	SD. Rosa26(tm-CAG-LSL-MT-ND1(G2996A))-GC/ILAS	MT- <i>Nd1</i> (G2996)	Knock in and condition knockout	Knockout mitochondrial protein-coding gene <i>Nd1</i> by using a DdCBE-induced premature stop codon at G2996 locus in the presence of Cre recombinase.	<a href="https://www.ratresource.com/detail/12/1670.html">https://www.ratresource.com/detail/12/1670.html</a>
GC00216	SD. Rosa26(tm-CAG-LSL-MT-ND2(G3992A))-GC/ILAS	MT- <i>Nd2</i> (G3992)	Knock in and condition knockout	Knockout mitochondrial protein-coding gene <i>Nd2</i> by using a DdCBE-induced premature stop codon at G3992 locus in the presence of Cre recombinase.	<a href="https://www.ratresource.com/detail/12/1671.html">https://www.ratresource.com/detail/12/1671.html</a>
GC00217	SD. Rosa26(tm-CAG-LSL-MT-ND5(G11938A))-GC/ILAS	MT- <i>Nd5</i> (G11938)	Knock in and condition knockout	Knockout mitochondrial protein-coding gene <i>Nd5</i> by using a DdCBE-induced premature stop codon at G11938 locus in the presence of Cre recombinase.	<a href="https://www.ratresource.com/detail/12/1672.html">https://www.ratresource.com/detail/12/1672.html</a>
GC00218	SD. Rosa26(tm-CAG-LSL-MT-CYTB(G14365A))-GC/ILAS	MT- <i>Cytb</i> (G14365)	Knock in and condition knockout	Knockout mitochondrial protein-coding gene <i>Cytb</i> by using a DdCBE-induced premature stop codon at G14365 locus in the presence of Cre recombinase.	<a href="https://www.ratresource.com/detail/12/1673.html">https://www.ratresource.com/detail/12/1673.html</a>
GC00219	SD. Rosa26(tm-CAG-LSL-MT-COX3(G8645A))-GC/ILAS	MT- <i>Cox3</i> (G8645)	Knock in and condition knockout	Knockout mitochondrial protein-coding gene <i>Cox3</i> by using a DdCBE-induced premature stop codon at G8645 locus in the presence of Cre recombinase.	<a href="https://www.ratresource.com/detail/12/1674.html">https://www.ratresource.com/detail/12/1674.html</a>

GC00220	SD. Rosa26(tm-CAG-LSL-MT-ATP8(G7783A))-GC/ILAS	MT- <i>Atp8</i> (G7783)	Knock in and condition knockout	Knockout mitochondrial protein-coding gene <i>Atp8</i> by using a DdCBE-induced premature stop codon at G7783 locus in the presence of Cre recombinase.	<a href="https://www.ratresource.com/detail/12/1675.html">https://www.ratresource.com/detail/12/1675.html</a>
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### Sequence S1.

#### Amino acid sequences for DdCBEs.

Amino acid sequences of DdCBE-Nd1 for *Nd1* G2996 site targeting were annotated as: red for MTS, italics for linker, yellow for flag tag, green for N&C-terminal domain, underlined for RVDs, purple for split DddA<sub>tox</sub> halves, and cyan for UGI. Other targeting DdCBE only showed RVDs sequences.

#### ND1-W<sub>86</sub>-L14 TALE-G1333C

MLGFVGRVAAPASGALRRLTPSASLPPAQLLRRAAPTAVHPVRDYAAQTSESGGGGSP  
*GAAADYKDDDDKGS*VDLRTLGYSQQQQE**KIKPKVRSTVAQHHEALVGHGFTAHIVAL**  
SQHPAALGTVAVKYQDMAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPLQ  
**LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNNGGKQALETVQR**  
LLPVLCQAHG**LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQVVIAIASHDGG**  
K**QALETVQRLLPVLCQAHG**LT**PAQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQV**  
**VAIASHDGGKQALETVQRLLPVLCQAHG**LT**PAQVVAIASNNGGKQALETVQRLLPVLC**  
Q**AHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQVVAIASNNNGGKQALET**  
V**QRLLPVLCQAHG**LT**PEQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PAQVVAIAS**  
**NNGGKQALETVQRLLPVLCQAHG**LT**PEQVVAIASNIGGKQALETVQRLLPVLCQAHG**  
**TPAQVVAIASNNGGKQALETVQRLLPVLCQAHG**LT**PDQVVAIASNIGGKQALETVQRLL**  
PVLCQAHG**LT****PDQVVAIASNIGGRPALE**SIVAQLSRPD**PAALTNDHLVALACLGGRP**  
**ALDAVKKGLGGS**PTPYPNYANAGHVEGQSALFMRDNGISEGLVFHNNSGGSTNLSDIIEK  
**TETLLPENAKMTVVPPEGAIPVKRGATGETKVFTGNSNSPKSPTKGCG**SGGSTNLSDIIIEK  
ETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLSDAPEYKPWAL  
VIQDSNGENKIKML

#### ND1-W<sub>86</sub>-L14 TALE-G1397C

MLGFVGRVAAPASGALRRLTPSASLPPAQLLRRAAPTAVHPVRDYAAQTSESGGGGSP  
*GAAADYKDDDDKGS*VDLRTLGYSQQQQE**KIKPKVRSTVAQHHEALVGHGFTAHIVAL**  
SQHPAALGTVAVKYQDMAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPLQ  
**LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNNGGKQALETVQR**  
LLPVLCQAHG**LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQVVIAIASHDGG**  
K**QALETVQRLLPVLCQAHG**LT**PAQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQV**  
**VAIASHDGGKQALETVQRLLPVLCQAHG**LT**PAQVVAIASNNGGKQALETVQRLLPVLC**  
Q**AHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQVVAIASNNNGGKQALET**  
V**QRLLPVLCQAHG**LT**PEQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PAQVVAIAS**  
**NNGGKQALETVQRLLPVLCQAHG**LT**PEQVVAIASNIGGKQALETVQRLLPVLCQAHG**  
**TPAQVVAIASNNGGKQALETVQRLLPVLCQAHG**LT**PDQVVAIASNIGGKQALETVQRLL**  
PVLCQAHG**LT****PDQVVAIASNIGGRPALE**SIVAQLSRPD**PAALTNDHLVALACLGGRP**  
**ALDAVKKGLGGS**AIPVKRGATGETKVFTGNSNSPKSPTKGCG**SGGSTNLSDIIIEKETGKQ**  
LVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLSDAPEYKPWALVIQDS  
NGENKIKML

#### ND1-W<sub>86</sub>-L14 TALE-G1333N

MLGFVGRVAAPASGALRRLTPSASLPPAQLLRRAAPTAVHPVRDYAAQTSESGGGGSP  
*GAAADYKDDDDKGS*VDLRTLGYSQQQQE**KIKPKVRSTVAQHHEALVGHGFTAHIVAL**  
SQHPAALGTVAVKYQDMAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPLQ  
**LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNNGGKQALETVQR**  
LLPVLCQAHG**LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQVVIAIASHDGG**  
K**QALETVQRLLPVLCQAHG**LT**PAQVVAIASNIGGKQALETVQRLLPVLCQAHG**LT**PDQV**

ND1-W<sub>86</sub>-L14 TALE-G1397N

MLGFVGRVAAAPASGALRRLTPSASLPPAQQLLRAAPTAHPVRDYAAQTSESGGGGSP  
GAAADYKDDDDKGSDLRTLGYSQQQQEIKPKVRSTVAQHHEALVGHGFTAHIVAL  
SQHPAALGTAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQ  
LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGG  
KQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPDQV  
VAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPVLC  
QAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALET  
VQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIAS  
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGL  
TPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  
PVLCQAHGLTPDQVVAIASNIGGRPALESIVAQLSRPDPAALTNDHLVALACLGGRP  
ALDAVKKGLGGSYSYALGPYQISAPQLPAYNGQTVGTFYYVNDAGGLESKVFSSGGPT  
PYPNYANAGHVEGQSALFMRDNGISEGLVFHNNEPGTCGFCVNMTETLLPENAKMTVV  
PPEGSGGSTNLSDIIEKETGKQLVIQESILMPEEEVEEVIGNKPESDILVHTAYDESTDENV  
MLLTSDAPEYKPWALVIODSNGENKJML

ND1-W<sub>86</sub>-R17 TALE-G1333C

MLGFVGRVAAAPASGALRRLTPSASLPPAQQLLRAAPTAHPVRDYAAQTESGGGGSP  
GAAA**DYKDDDDKGSDLRTLGYSQQQQEIKPKVRSTVAQHHEALVGHGFTAHIVAL**  
**SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQ**  
**LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNIGGKQALETVQR**  
LLPVLCQAHGTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGTPDQVVAIASNNGG  
KQALETVQRLLPVLCQAHGTLPEEQVVAIASNNGGKQALETVQRLLPVLCQAHGTLPEQ  
VVAIASNNGGKQALETVQRLLPVLCQAHGTLPEEQVVAIASNNGGKQALETVQRLLPV  
CQAHGTLPEEQVVAIASNNGGKQALETVQRLLPVLCQAHGTLPEEQVVAIASNNGGKQAL  
ETVQRLLPVLCQAHGTLPEEQVVAIASNNGGKQALETVQRLLPVLCQAHGTLPEEQVVAIA  
SNIGGKQALETVQRLLPVLCQAHGTLPEEQVVAIASNNGGKQALETVQRLLPVLCQAHG  
LTPEEQVVAIASNNGGKQALETVQRLLPVLCQAHGTLPEEQVVAIASNNGGKQALETVQR  
LLPVLCQAHGTLPEEQVVAIASNIGGKQALETVQRLLPVLCQAHGTLPEEQVVAIASNGGG  
KQALETVQRLLPVLCQAHGTLPEEQVVAIASNGGGKQALETVQRLLPVLCQAHGTLPEQ  
VVAIASNNGGPALE**SIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKG**GGSP  
TPYPNYANAGHVEGQSALFMRDNGISEGLVFHNNEPGTCGFCVNMTETLLPENAKMTV  
VPPEGAIPVKRGATGETKVFTGNSNSPKSPTKGGCSGGSTNLSDIIEKETGKQLVIQESIL  
MLPSEEVEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIK  
MI

ND1-W<sub>86</sub>-R17 TALE-G1397C

MLGFVGRVAAPASGALRRLTPSASLPPAQLLRRAAPTAVHPVRDYAAQTSESGGGGSP  
GAAA**DYKDDDDDKGSVDLRTLGYQQQQEIKPKVRSTVAQHHEALVGHGFTAHIVAL**  
**SQHPAALGTVAVKYQDMIAALPEATHEAIVVGKQWSGARALEALLTVAGELRGPLQ**  
**LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNIGGKQALETVQR**  
**LLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGG**  
**KQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ**

VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ

CQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQAL

ETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA

SNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHG

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR

LLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGG

KQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ

VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ

**IPVKRGATGETKVFTGNSNSPKSPTKGCGSGNSTNLSDIIEKETGKQLVIQESILMLPEEVE**

**EVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKWPWALVIQDSNGENKIKML**

#### ND1-W<sub>86</sub>-R17 TALE-G1333N

MLGFVGRVAAPASGALRRLTPSASLPPAQLLRRAAPTAVHPVRDYAAQTSESGGGGSP  
GAAA**DYKDDDDDKGSVDLRTLGYQQQQEIKPKVRSTVAQHHEALVGHGFTAHIVAL**  
**SQHPAALGTVAVKYQDMIAALPEATHEAIVVGKQWSGARALEALLTVAGELRGPLQ**  
**LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNIGGKQALETVQR**  
**LLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGG**  
**KQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ**

VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ

CQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQAL

ETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA

SNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHG

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR

LLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGG

KQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ

VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ

**SIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGGS**

**SYALGPYQISAPQLPAYNGQTGVTFYYVNNDAGGLESKVSSGGNSTLSDIIEKETGK**

**QLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKWPWALVIQD**

**SNGENKIKML**

#### ND1-W<sub>86</sub>-R17 TALE-G1397N

MLGFVGRVAAPASGALRRLTPSASLPPAQLLRRAAPTAVHPVRDYAAQTSESGGGGSP  
GAAA**DYKDDDDDKGSVDLRTLGYQQQQEIKPKVRSTVAQHHEALVGHGFTAHIVAL**  
**SQHPAALGTVAVKYQDMIAALPEATHEAIVVGKQWSGARALEALLTVAGELRGPLQ**  
**LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNIGGKQALETVQR**  
**LLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGG**  
**KQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ**

VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ

CQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQAL

ETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA

SNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHG

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR

LLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNGG  
KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGKQALETVQRLLPVLCQAHGLTPEQ  
VVAIASNNGGRPALESISVAQLSRPD<sup>P</sup>ALAALTNDHLVALACLGGRPALDAVKKGLGG  
SYALGPYQISAPQLPAYNGQTVGTFYYVNDAGGLESKVFS<sup>S</sup>GGPTP<sup>P</sup>NYANAGHVEG  
QSALFMRDNGISEGLVFHN<sup>N</sup>PEGTCGF<sup>C</sup>VNM<sup>T</sup>ETLLPENAKMTVVP<sup>P</sup>PEGSGGSTNLSDII  
EKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVM<sup>L</sup>LTSDAPEYKPW  
ALVIQDSNGEN<sup>K</sup>IKML

#### ATP8-W<sub>9</sub>-L17

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQR  
LLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIG  
KQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASHDGGKQALETVQRLLPVLCQAHGLTPAQ  
VVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNIGGKQALETVQRLLPVLC  
QAHGLTPDQVV<sup>A</sup>IASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALE  
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IAS  
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGL  
TPDQVV<sup>A</sup>IASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLL  
PVLCQAHGLTPDQVV<sup>A</sup>IASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIG  
QALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNGGGRPALESISVAQLSRPD<sup>P</sup>AL

#### ATP8-W<sub>9</sub>-R15

LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNGGKQALETVQR  
LPVLCQAHGLTPEQVVAIASNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNN  
KQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQV  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLC  
QAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNNGGKQALE  
VQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASN  
GGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT  
PEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNGGKQALETVQRLL  
PVLCQAHGLTPEQVVAIASNGGGRPALESISVAQLSRPD<sup>P</sup>AL

#### ND2-W<sub>30</sub>-L16

LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNN  
KQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPV  
CQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQAL  
ETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IA  
HDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHG  
LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLL  
PVLCQAHGLTPDQVV<sup>A</sup>IASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNG  
G<sup>P</sup>RPALESISVAQLSRPD<sup>P</sup>AL

#### ND2-W<sub>30</sub>-R18

LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNIGGKQALETVQR  
LPVLCQAHGLTPDQVV<sup>A</sup>IASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNIG  
KQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASNNGGKQALETVQRLLPVLCQAHGLTPEQVV  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ  
AHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALE  
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV<sup>A</sup>IASN

GGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGRPALESIVAQLSRPDPAL

ND3-Q<sub>26</sub>-L18

**ND3-Q<sub>26</sub>-R19**

LTPEQQVVAIASNNGGKQALETVQRLLPVLCQAHGTLTPEQVVAIASHDGGKQALETVQR  
LLPVLCQAHGGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHGGLTPEQVVAIASNNGG  
GKQALETVQRLLPVLCQAHGGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGGLTPE  
QVVAIASNNGGKQALETVQRLLPVLCQAHGGLTPEQVVAIASNNGGKQALETVQRLLPV  
LCQAHGGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGGLTPAQVVAIASNNGGKQAE  
LETVQRLLPVLCQAHGGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGGLTPEQVVAI  
ASNIGGKQALETVQRLLPVLCQAHGGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAH  
GLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGGLTPEQVVAIASNIGGKQALETVQR  
LLPVLCQAHGGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGGLTPEQVVAIASNIGG  
KQALETVQRLLPVLCQAHGGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGGLTPDQV  
VAIASNNGGKQALETVQRLLPVLCQAHGGLTPEQVVAIASNNGGRPALESIVAQLSRPD  
AL.

ND4-W<sub>34</sub>-L18

NDT-W24-L16  
LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHG LTPEQVVAIASNIGGKQALETVQRL  
LPVLCQAHG LTPDQVVAIASHDGGKQALETVQRLLPVLCQAHG LTPA QVVAIASNGGG  
KQALETVQRLLPVLCQAHG LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG LTPA Q  
VVAIASNGGGKQALETVQRLLPVLCQAHG LTPEQVVAIASHDGGKQALETVQRLLPV  
CQAHG LTPA QVVAIASNIGGKQALETVQRLLPVLCQAHG LTPDQVVAIASNNGGKQAL  
ETVQRLLPVLCQAHG LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG LTPA QVVAIA  
SHDGGKQALETVQRLLPVLCQAHG LTPA QVVAIASNIGGKQALETVQRLLPVLCQAHG  
LTPDQVVAIASNIGGKQALETVQRLLPVLCQAHG LTPDQVVAIASHDGGKQALETVQRL  
LPVLCQAHG LTPA QVVAIASNIGGKQALETVQRLLPVLCQAHG LTPDQVVAIASNIGGK  
QALETVQRLLPVLCQAHG LTPDQVVAIASNIGGKQALETVQRLLPVLCQAHG LTPDQV  
VVAIASNIGGRPALESIVAQLSRPDPAL

ND4-W<sub>24</sub>-R17

ND5-W<sub>68</sub>-L15

ND5-W<sub>68</sub>-R15

LTPEQQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLL  
PVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAISNGGGK  
QALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQV  
VAISNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQ  
AHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQALET  
VQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASN  
NGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTP  
DQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQALETVQRLLPV  
LCQAHGLTPEQVVAIASNGGGRPALESIVAQLSRPDPAL

EQUATIONS

ND6-W<sub>82</sub>-L15

NDU\_W2\_L15  
LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~DQVV~~V~~AIASNIGGKQALETVQRLL  
PVLCQAHG~~L~~T~~P~~DQVV~~V~~AIASNIGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~DQVV~~V~~AIASNIGGKQ  
ALETVQRLLPVLCQAHG~~L~~T~~P~~DQVV~~V~~AIASNIGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~DQVV~~V~~  
IASNGGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~EQVV~~V~~AIASNIGGKQALETVQRLLPVLCQAH  
GLT~~P~~DQVV~~V~~AIASNIGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~DQVV~~V~~AIASNIGGKQALETVQR  
LLPVLCQAHG~~L~~T~~P~~DQVV~~V~~AIASHHDGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~AQVV~~V~~AIASHHDG  
GKQ~~A~~LETVQRLLPVLCQAHG~~L~~T~~P~~AQVV~~V~~AIASNIGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~DQ  
V~~V~~AIASNIGGKQALETVQRLLPVLCQAHG~~L~~T~~P~~DQVV~~V~~AIASNGGGKQALETVQRLLPVLC  
QAHG~~L~~T~~P~~EQVV~~V~~AIASNGGGRPALESIVAQLSRPDPAL

ND6-W<sub>82</sub>-R15

LTPEQQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRL  
LPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGG  
KQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQV  
VAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLC  
QAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALET  
VQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASN  
IGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTP

AQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLP  
VLCQAHGLTPAQVVAIASNNGGPALESIVQLSRPDPAL

**COX1-W<sub>25</sub>-L15**

LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGG  
KQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLC  
QAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALE  
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASN  
NGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGL  
TPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  
LPVLCQAHGLTPEQVVAIASNNGGPALESIVQLSRPDPAL

**COX1-W<sub>25</sub>-R18**

LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGG  
KQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLC  
QAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALE  
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASN  
NGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGL  
TPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  
LPVLCQAHGLTPEQVVAIASNNGGPALESIVQLSRPDPAL

**COX2-Q<sub>6</sub>-L18**

LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQR  
LPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGK  
QALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQV  
VAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC  
QAHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALE  
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASH  
DGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTP  
DQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLL  
PVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQ  
ALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVV  
VAIASNNGGPALESIVQLSRPDPAL

**COX2-Q<sub>6</sub>-R18**

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQR  
LPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGG  
KQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPV  
LCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQAL  
ETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPDQVVAIASH  
SHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHG  
LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIG  
KQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQV  
VAIASNNGGPALESIVQLSRPDPAL

COX3-W<sub>16</sub>-L17

LTPEQQVVAIASNIGGKQALETVQRLLPVLCQAHG~~L~~TPDQVVAIASHDGGKQALETVQRL  
LPVLCQAHG~~L~~TPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~L~~TPAQVVAIASNIGGK  
QALETVQRLLPVLCQAHG~~L~~TPDQVVAIASNGGGKQALETVQRLLPVLCQAHG~~L~~TPEQV  
VAIASNIGGKQALETVQRLLPVLCQAHG~~L~~TPDQVVAIASNGGGKQALETVQRLLPVLCQ  
AHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG~~L~~TPDQVVAIASNGGGKQALETV  
QRLLPVLCQAHG~~L~~TPEQVVAIASNGGGKQALETVQRLLPVLCQAHG~~L~~TPEQVVAIASNI  
GGKQALETVQRLLPVLCQAHG~~L~~TPDQVVAIASNIGGKQALETVQRLLPVLCQAHG~~L~~TPD  
QVVAMASNIGGKQALETVQRLLPVLCQAHG~~L~~TPDQVVAIASHDGGKQALETVQRLLPV  
LCQAHG~~L~~TPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~L~~TPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~L~~TPAQVVAIASNIGGRPALESIVAQLSRPDPAL

COX3-W<sub>16</sub>-R15

LTPEQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQQVVAIASNIGGKQALETVQRL  
LPVLCQAHG~~L~~TDQVVAIASNGGKQALETVQRLLPVLCQAHG~~L~~TPEQQVVAIASNIGGK  
QALETVQRLLPVLCQAHG~~L~~TDQVVAIASNGGKQALETVQRLLPVLCQAHG~~L~~TPEQV  
VAIASNGGKQALETVQRLLPVLCQAHG~~L~~TPEQQVVAIASNGGKQALETVQRLLPVLC  
QAHG~~L~~TPEQQVVAIASHDGGKQALETVQRLLPVLCQAHG~~L~~TPAQVVAIASNGGKQALE  
TVQRLLPVLCQAHG~~L~~TPEQQVVAIASHDGGKQALETVQRLLPVLCQAHG~~L~~TPAQVVAIAS  
HDGGKQALETVQRLLPVLCQAHG~~L~~TPAQVVAIASNGGKQALETVQRLLPVLCQAHG  
TPEQQVVAIASNGGKQALETVQRLLPVLCQAHG~~L~~TPEQQVVAIASNGGKQALETVQRL  
LPVLCQAHG~~L~~TPEQQVVAIASNGGGRPALESIVAQLSRPDPAL

ATP6-W<sub>68</sub>-L16

ATP6-W<sub>68</sub>-R17

LTPEQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGG  
KQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQV  
VAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC

## TRANSGENIC **CYTB-W<sub>77</sub>-L15**

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQR  
LLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNG

GKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQ  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLC  
QAHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALE

TVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIAS  
NIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLT

PDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLP  
VLCQAHGLTPAQVVAIASNNGGRPALESIVAQLSRPDAL

#### **CYT-B-W<sub>77</sub>-R13**

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASASHDG

KQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ  
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPV

CQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALE

TVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIAS  
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGL

TPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGRPALESIVAQLS

RPDPAL

#### **COX1-W<sub>126</sub>-L17**

LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQR  
LPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGK

QALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQV  
VAIASASHDGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPV

CQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALE

TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIAS  
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGL

TPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASASHDGKQALETVQRLLPVLCQAHGL

TPAQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR  
LPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGK

QALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGK

#### **COX1-W<sub>126</sub>-R17**

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASASHDGKQALETVQR  
LLPVLCQAHGLTPAQVVAIASASHDGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNG

GKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPE  
QVVAIASASHDGKQALETVQRLLPVLCQAHGLTPAQVVAIASNNGGKQALETVQRLLPV

LCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQAL

ETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA  
SNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHG

LTP EQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR  
LLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGG

KQALETVQRLLPVLCQAHGLTPDQVVAIASNNGGRPALESIVAQLSRPDAL

#### **COX2-W<sub>104</sub>-L19**

LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLL  
PVLCQAHGLTPDQVVAIASASHDGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGK

QALETVQRLLPVLCQAHGLTPDQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQV  
VAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQ

COX2-W<sub>104</sub>-R15

LTPEQQVVAIASHDGGKQALETVQRLLPVLCQAHGTLPAQVVAIASNIGGKQALETVQRL  
LPVLCQAHGTLPDQVVAIASNNGGKQALETVQRLLPVLCQAHGTLPEQVVAIASNGGG  
KQALETVQRLLPVLCQAHGTLPEQVVAIASNIGGKQALETVQRLLPVLCQAHGTLPDQV  
VAIASNGGGKQALETVQRLLPVLCQAHGTLPEQVVAIASNIGGKQALETVQRLLPVLCQ  
AHGLTPDQVVAIASNGGGKQALETVQRLLPVLCQAHGTLPEQVVAIASNGGGKQALET  
VQRLLPVLCQAHGTLPEQVVAIASHDGGKQALETVQRLLPVLCQAHGTLPAQVVAIAS  
NIGGKQALETVQRLLPVLCQAHGTLPDQVVAIASNGGGKQALETVQRLLPVLCQAHG  
LPEQVVAIASNIGGKQALETVQRLLPVLCQAHGTLPDQVVAIASNGGGKQALETVQRLL  
PVLCQAHGTLPEQVVAIASHDGGKQALETVQRLLPVLCQAHGTLPAQVVAIASNGGGR  
PALESIVQLSRPDPAL

COX3-W<sub>57</sub>-L18

LTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRL  
LPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGK  
QALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVV  
AIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQA  
HGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQALETV  
QRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASH  
DGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLT  
PEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLP  
VLCQAHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQ  
ALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQ  
ALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQ

## **COX3-W<sub>57</sub>-R17**

LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
LLPVLCQAHG~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
QVVAIANIGGKQALETVQRLLPVLCQAHG~~LTPDQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
CQAHG~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
ETVQRLLPVLCQAHG~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~~~LTPDQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
SNGGGKQALETVQRLLPVLCQAHG~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
LTPEQVVAIANIGGKQALETVQRLLPVLCQAHG~~LTPDQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
LPVLCQAHG~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~~~LTPDQVVAIASHDGGKQALETVQRLLPVLCQAHG~~  
QALETVQRLLPVLCQAHG~~LTPAQVVAIASHDGGKQALETVQRLLPVLCQAHG~~~~LTPDQVVAIASHDGGKQALETVQRLLPVLCQAHG~~

## Sequence S2.

### Amino acid sequences of ND1-W<sub>86</sub>-L-EGFP/R-mCherry-DdCBE.

Amino acid sequences of ND1-W<sub>86</sub>-L-EGFP/R-mCherry-DdCBE using for FASC are annotated as: green for EGFP, red for mCherry, italics for linker, pink for P2A, and purple for DdCBE.

#### ND1-W<sub>86</sub>-EGFP-L-DdCBE

MVSKGEELFTGVVPILVELGDGVNGHKFSVSGEGERGATYGLTLKFIITGKLPVPWP  
TLVTTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEG  
DTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKNGIKVNFKIRHNIEDGSV  
QLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLFVTAAGITLGMD  
ELYK*GSG*ATNFSLLKQAGDVEENPGPMLGFVGRVAAAPASGALRRLTPSASLPPAQ**LLL**  
RAAPTAVHPVRDYAAQTSES~~GGGGSPGAAADYK~~DDDDKGSVDLRTLGYSQQQKEKIK  
PKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEATHEAIVG  
VGKQWSGARALEALLTVAGELRG~~PPLQLDTG~~Q~~LKIAKRG~~GT~~V~~AVEAVHAWRNALTG  
APLNLTPEQVVAIASNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNIGGKQ~~A~~LET  
VQR~~L~~LPVLCQAHGLT~~P~~DQVVAIASHDGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLT  
TPAQVVAIASNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNIGGKQ~~A~~LET~~V~~QR~~L~~  
PVLCQAHGLT~~P~~DQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASHDGGK  
Q~~A~~LET~~V~~QR~~L~~LPVLCQAHGLT~~P~~AQVVAIASNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQV  
VAIASHDGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLT~~P~~AQVVAIASNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLC  
QAHGLTPEQVVAIASNIGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLT~~P~~DQVVAIASNIGGRPALESI  
VAQLSRPDPALAALTNDHLVALACLGG~~R~~GPALDAVKKGLGGSPTPYPNYANAGHVEGQ  
SALFMRDNGISEGLVFHN~~N~~PEGTCGFCVNMTETLLPENAKMTVV~~P~~PEGAIPVKRGATGE  
TKVFTGNSNSPKSPTKGGCSGGSTNLSDII~~E~~KETGKQLVIQESILMLPEEEV~~E~~VIGNKPESD  
ILVHTAYDESTDENVMLLTS~~A~~PEYKP~~W~~ALVIQDSNGENKIKML

#### ND1-W<sub>86</sub>-mCherry-R-DdCBE

MVSKGEEDNMAI~~I~~KEFMRFKVHMEGSVNGHEFEIEGEGERPYEGTQTAKLKVTKG~~GP~~  
LPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVT~~T~~QDS  
SLQDG~~E~~FIYKV~~K~~LRTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKD  
GGHYDAEVK~~T~~TYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRH~~S~~TGGMD  
ELYK*GSG*ATNFSLLKQAGDVEENPGPMLGFVGRVAAAPASGALRRLTPSASLPPAQ**LLL**  
RAAPTAVHPVRDYAAQTSES~~GGGGSPGAAADYK~~DDDDKGSVDLRTLGYSQQQKEKIK  
PKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEATHEAIVG  
VGKQWSGARALEALLTVAGELRG~~PPLQLDTG~~Q~~LKIAKRG~~GT~~V~~AVEAVHAWRNALTG  
APLNLTPEQVVAIASNIGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLT~~P~~DQVVAIASNIGGKQ~~A~~LET~~V~~  
QR~~L~~LPVLCQAHGLT~~P~~DQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASN  
NGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLT  
PEQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~  
LPVLCQAHGLTPEQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNNGGGKQ  
ALET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNIGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLT~~P~~DQVVA  
IASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAH  
GLTPEQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNIGGKQ~~A~~LET~~V~~  
RLLPVL~~C~~QAHGLT~~P~~DQVVAIASNNGGGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNG  
GGKQ~~A~~LET~~V~~QR~~L~~LPVLCQAHGLTPEQVVAIASNNGGRPALESIVAQLSRPDPALAALT  
DHLVALACLGG~~R~~GPALDAVKKGLGGSASPTPYPNYANAGHVEGQ~~S~~ALFMRDNGISEGLV  
FHN~~N~~PEGTCGFCVNMTETLLPENAKMTVV~~P~~PEGAIPVKRGATGETK~~V~~FTGNSNSPKSPT

KGGCSGGSTNLSDIIEKETGKQLVIQESILMLPEEVVEEVIGNKPESDILVHTAYDESTDEN  
VMLLTSDAPEYKPWALVIQDSNGENKIKML