SUPPLEMENTARY MATERIAL

LEGENDS TO FIGURES

**Supplementary Figure 1.** Transgene expression levels in TFAM- and Twinkle-mice, and analysis of deletions and nucleoid protein ATAD3. (A) mtDNA quantification by Southern blot of 10-week-old mice gave essentially the same result as qPCR. The mtDNA signal was corrected for loading against the nuclear 18S rDNA gene. (B) Western blots for hTFAM revealed equal levels of the protein in mitochondrial enrichments from muscle and heart of hTFAM-mice and bitransgenic mice. (C) The murine Twinkle protein was detectable in mitochondria-enriched tissue fractions only upon overexpression. The levels of Twinkle protein were the highest in the homozygous Twinkle+/- mice. In the heart, the Twinkle protein levels of heterozygous Twinkle-mice were comparable to those of bitransgenic mice. (D) RNA levels of endogenous mouse TFAM (mTFAM) were measured by qPCR. Neither HTFAM nor Twinkle overexpression affected the endogenous mTFAM mRNA levels. (E) Long-range PCR from 10-week-old wild-type (WT), Twinkle- (Tw), hTFAM- (TF) or bitransgenic (Tw+TF) mice revealed no deleted molecules; the full-length mtDNA was effectively amplified (arrow). (F) The levels of ATAD3 protein were unaltered in the transgenic mice compared to the wild-type mouse. CI=RC complex II 70 kDa subunit. Error bars indicate SEM.

**Supplementary Figure 2.** Mitochondrial RNA transcript levels. (A) Light strand encoded transcripts were quantified by qPCR from muscle and heart of all genotypes (N=3-11). The positions of the transcripts are schematically shown in (B). (C) The level of the HS-encoded tRNA-His transcript was decreased in the heart of bitransgenic mice. WT=wild-type, Tw+/-
Supplementary Figure 3. Measurement of respiratory chain complexes. Blue native polyacrylamide gel electrophoresis and immunoblotting were used to measure the relative abundance of respiratory chain complexes I-IV (CI-CIV) in muscle (A) and heart (B) of wild-type and transgenic mice. Densitometric quantification and normalization against the CII signal revealed depletion of complexes in the muscle (C) of TFAM-mice, and a more severe depletion in bitransgenic Twinkle+TFAM mice. In the heart (D) quantification revealed modest depletion of CIV in the bitransgenic mice. N=3-5. *p<0.05, **p<0.01, ***p<0.001; Student’s t-test compared to wild-type. Error bars indicate SEM.