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SUPPLEMENTARY INFORMATION

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MitoTALEN reduces mutant mtDNA load and restores $tRNA^{Ala}$ levels in a mouse model of heteroplasmic mtDNA mutation

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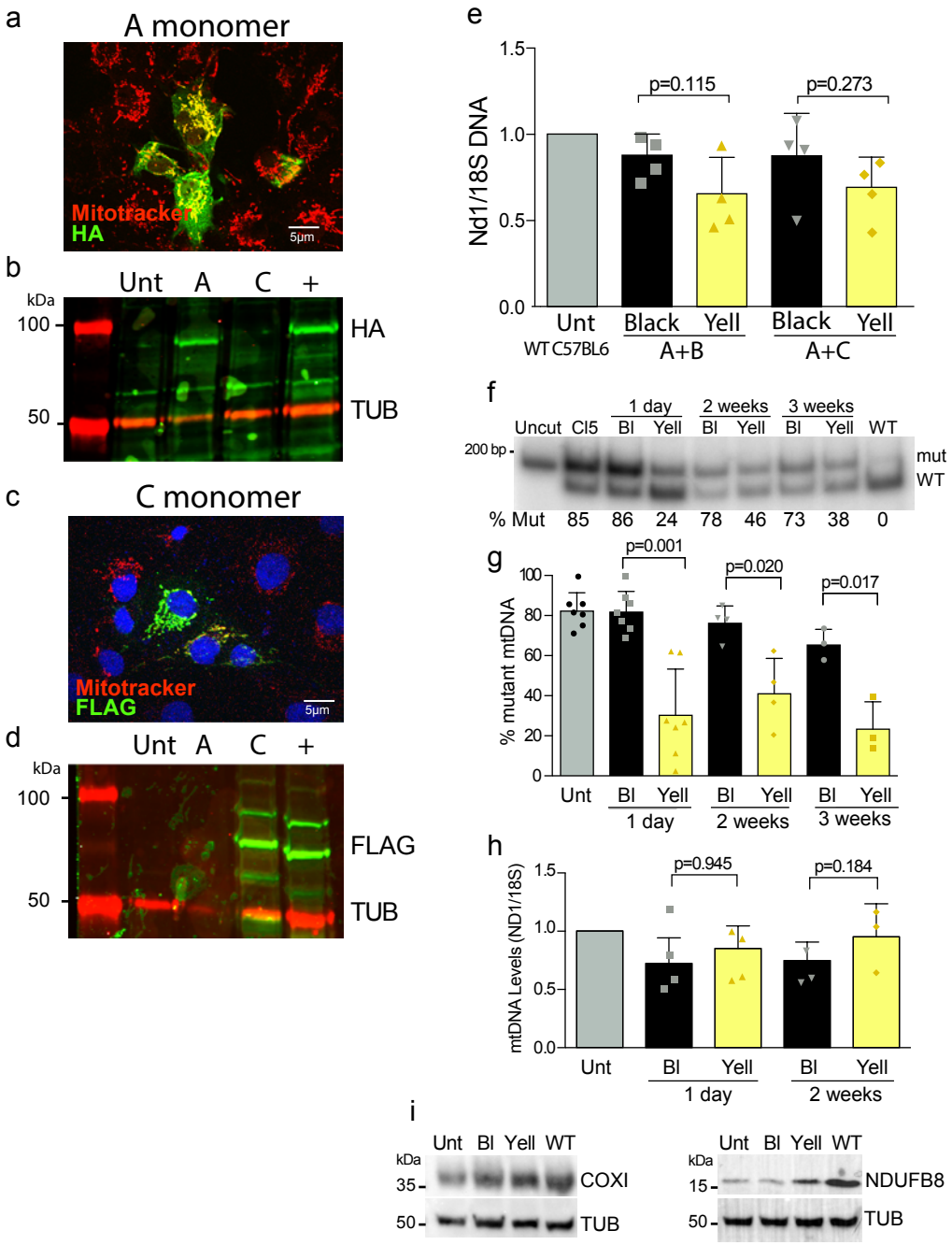
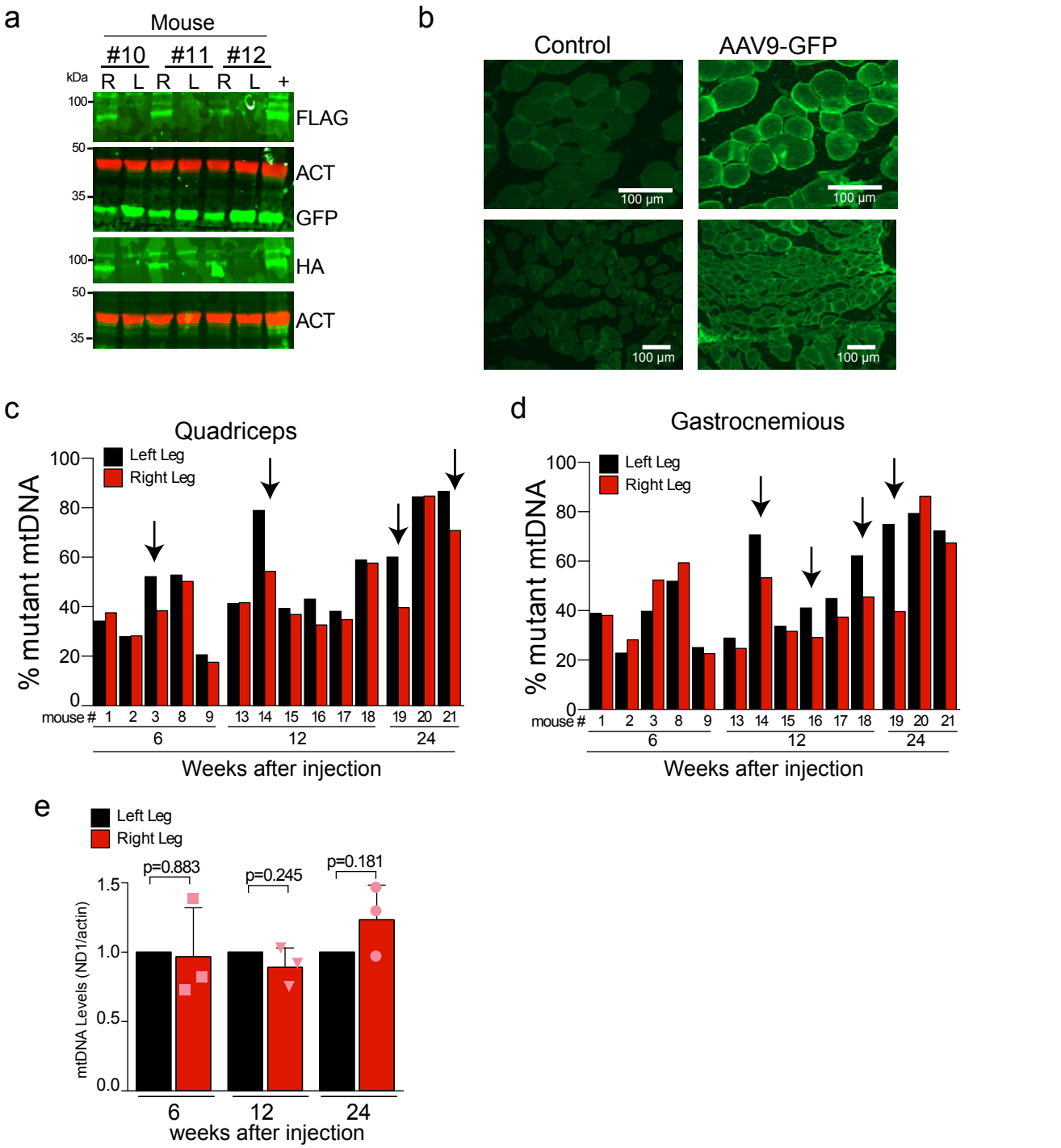
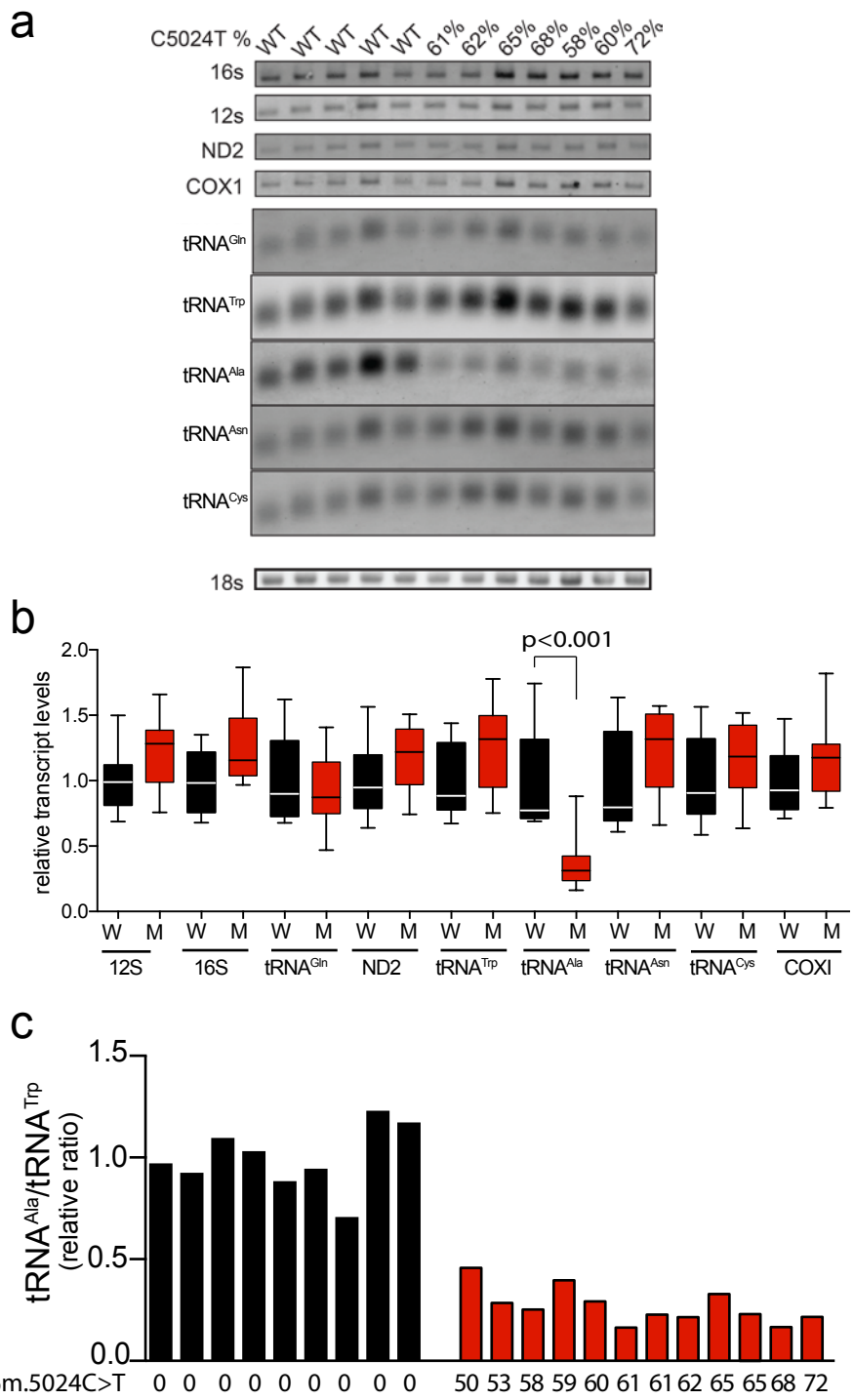


Supplementary Fig. 1. Characterization of a mitoTALEN specific for the mouse mtDNA m.5024C>T mutation. a) COS7 cells were transfected with monomers A and C, encoding a mitoTALEN. After 24 hours cells were incubated with mitotracker red, fixed and immunostained for HA (panel a) or FLAG (panel c). Panel c also shows DAPI staining in blue. Cell extracts were analyzed by western blot and the proteins were detected at the expected molecular weight with anti-HA (panel b) or anti-FLAG (panel d) antibodies. (+): positive controls are homogenates of HeLa cells transiently transfected with each monomer. Scale bar on panels a and c=5 μm. Panel e shows the total mtDNA levels in sorted (Black "Bl" and Yellow "Yell") wild-type C57Bl/6 fibroblasts 48 hours after transfection with mitoTALEN (n=4). (panels f) PCR/RFLP of sorted cells after transfection of Cl5 with mitoTALEN. This experiment was repeated five times with similar results. (panel g) Quantification of PCR/RFLP experiments showing a decrease in mutant mtDNA. (1 day, n=6; 2 weeks, n=4; 3 weeks, n=3). (panel h) Quantification of mtDNA relative levels (1 day, n=4; 2 weeks, n=3). (panel i) Western blots of sorted populations for COXI and NDUFB8. WT=wild-type MEF. This experiment was repeated twice with similar results. All error bars represent +1 SD of the mean. Student t-test (two tailed unpaired) was used for all comparisons.

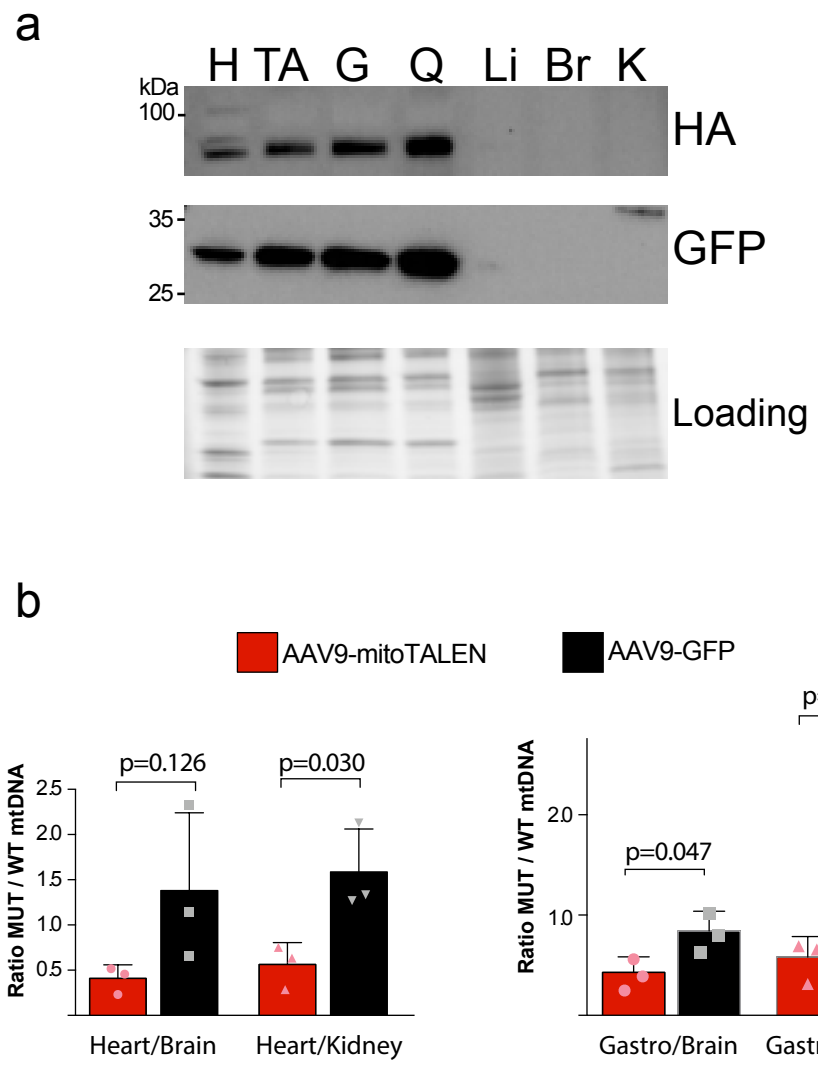
Cell sorting stategy: Cell sorting of transfected cells was performed by gating on Forward Scatter (FSC) and Side Scatter (SSC), then doing a double discrimination for single cells using FSC-Area vs Width and SSC-Area vs Width. From that serial gating we then identify green vs. red cells and use quadrants to separate single positives (green and red), double positives (yellow) and double negatives (black).



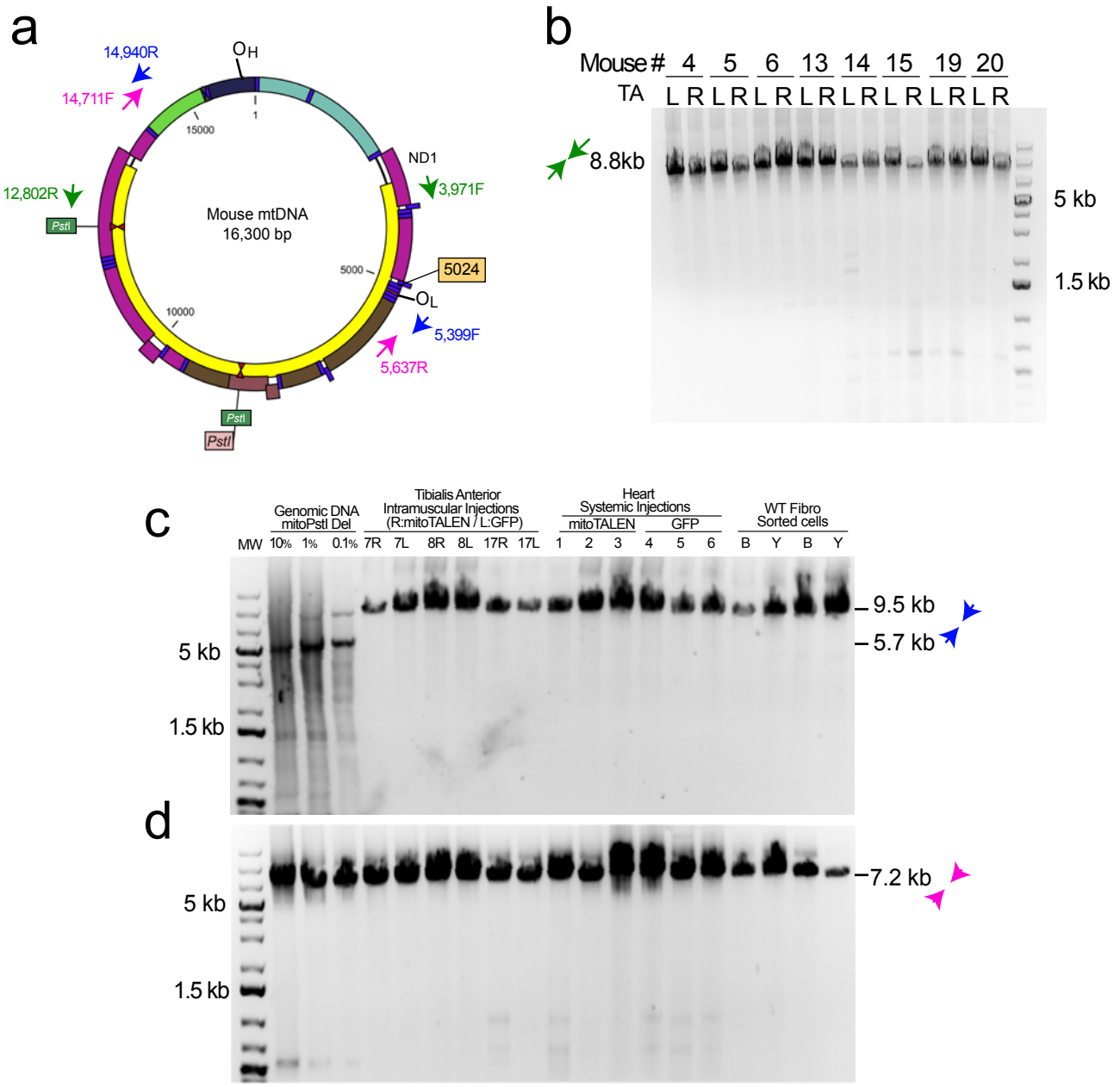
Supplementary Figure 2. AAV9-mitoTALEN expression in injected TA and adjacent muscles. Panel a) Western blots of TA homogenates (10 weeks post-injection) using antibodies against FLAG and HA. Positive FLAG and HA expression was observed in AAV9-mitoTALENs injected right-TA, while GFP expression was observed in both right and left skeletal muscle after IM injection. (+): positive control is an homogenate of HeLa cells transiently transfected with each monomer. ACT=actin. This experiment was repeated for all injected animals with similar results. Panel b) Immunocytochemistry analysis of TA 24 weeks post-injection of the mitoTALEN AAV9-GFP. Muscle sections showed positive GFP expression with a monoclonal GFP antibody in the left TA. Scale bar= 100µm. Panels c,d) Muscles adjacent to the TA, i.e. quadriceps (panel c) and gastrocnemius (panel d) were also analyzed for mtDNA heteroplasmy change. Heteroplasmy change could be detected in a few samples (arrows), suggesting that the AAV9-mitoTALEN was able to transduce neighboring muscles in some cases. Panel e) Total mtDNA levels were not significantly altered at different times after intramuscular injection (n=3). Student t-test (two-tailed, unpaired) was used to test for significance.



Supplementary Figure 3. tRNA^{Ala} levels in m.5024C>T mutant mice's skeletal muscle. a) Representative northern blot showing transcripts expression levels in quadriceps from controls and m.5024C>T mutant mice. The transcripts evaluated were: 16s, 12s, ND2, COX1, and tRNA: glutamine, tryptophan, alanine, asparagine and cysteine. b) Transcripts expression (relative to nuclear 18S rRNA) in quadriceps harboring between 50% to 72% m.5024C>T mtDNA (mutant, M, red; n=12) and wild-type skeletal muscle (W, black; n=9). The northern analyses was repeated twice with similar results. The box plots quartiles with the line inside representing the median. Whiskers represent minima and maxima. c) tRNA^{Ala} /tRNA^{Trp} ratios in skeletal muscle from controls (black bars) and from mice with higher that 50% mutant mtDNA (m.5024C>T, red bars).



Supplementary Figure 4. MtDNA heteroplasmy change after IP injection. a) Western blot for HA and GFP from various tissues 6 weeks after intraperitoneal injection (IP) in neonates (P3). AAV9-mitoTALENs were expressed in cardiac (H) and skeletal tissues (TA, G, Q), with low expression in liver (L), and undetectable expresion in brain (Br), and kidney (K). This experiment was repeated three times with similar results. b) Heteroplasmy determination 6 weeks after IP injection with AAV9-mitoTALEN. The mutant load in heart and gastrocnemious was graphed as a ratio to non-expressing tissues (brain or kidney). Some of the statistical comparisons did not reach significance (unpaired two-tail student t-test), likely because of the different initial heteroplasmy in individual mice in this small group (n=3). Error bars are +1 SD of the mean.



Supplementary Figure 5. Tissues and cells treated with AAV9-mitoTALEN show no detectable recombinant molecules. Long-range PCR was employed to detect mtDNA with large deletions, which are preferentially amplified over the full-length amplicon. Panel a shows a representation of the mouse mtDNA indicating the presence of the primer pairs used for amplifications (green, blue and pink arrows) as well as the location of *Pst*I endonuclease sites. Panel b shows long-range PCR amplifications (green arrows) of DNA from right (AAV9-mitoTALEN injected) and left (AAV9-GFP injected) TA muscles after 6, 12 or 24 weeks. This experiment was repeated three times with similar results. Panel c shows amplifications (blue arrows) of TA and heart 6 and 12 weeks after systemic injections. In addition, we analyzed C57BL/6J fibroblasts containing only the wild-type m.5024C allele 48 hours after transfection with the mitoTALENs (Black and Yellow populations after sorting). Panel d shows amplifications of the same samples as panel c, with different primers (pink arrows). In panels c and d we included a positive control (mouse cortex DNA expressing mito*Pst*I) showing the presence of the *Pst*I-mediated mtDNA deletion at 10%, 1% and 0.1%. Mouse number described in Fig. 2e (TA); Fig. 3 (Systemic injections); Supplementary Fig. 1 (Fibroblasts). Experiments in c and d were repeated twice with similar results.

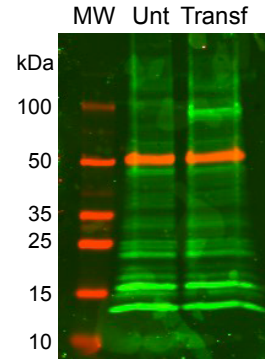


Fig. 1c: HA+TUB

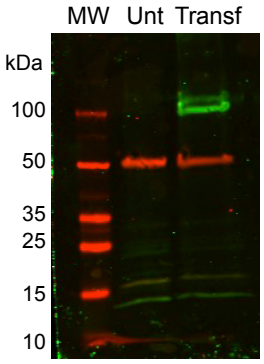


Fig. 1d: FLAG+TUB

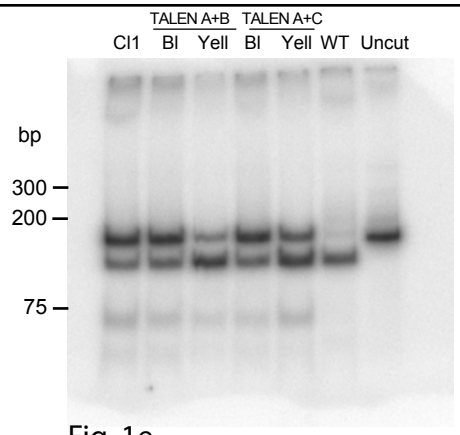
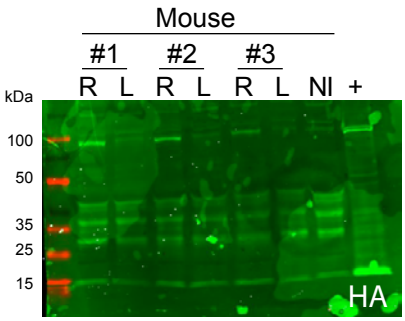


Fig. 1e



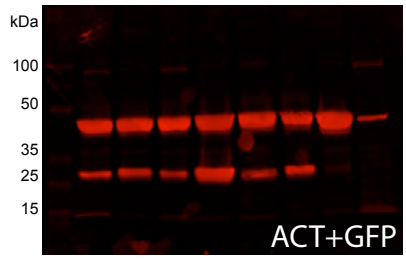
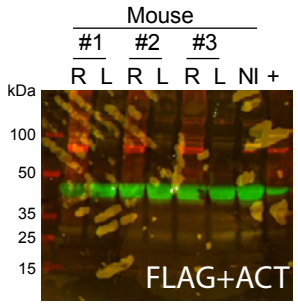


Fig. 2a



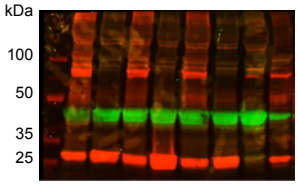


Fig. 2b FLAG+GFP+ACT

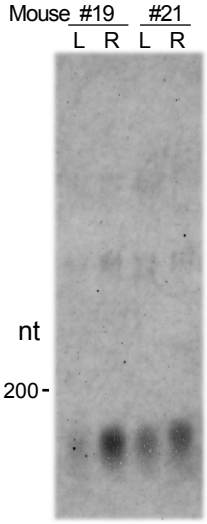


Fig. 2g: tRNA Ala

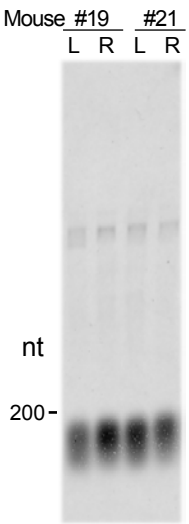


Fig. 2g: tRNA Trp

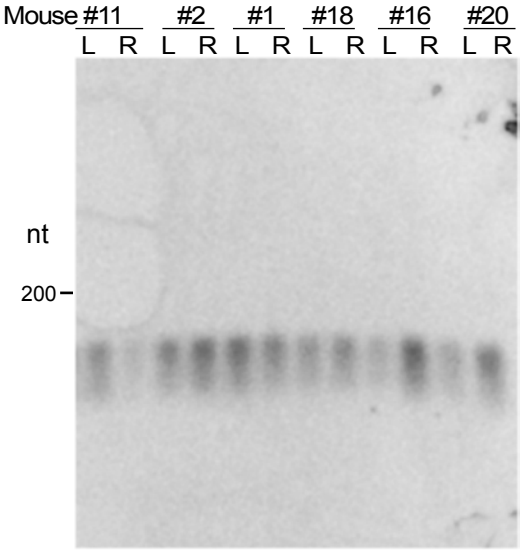


Fig. 2h: tRNA Ala

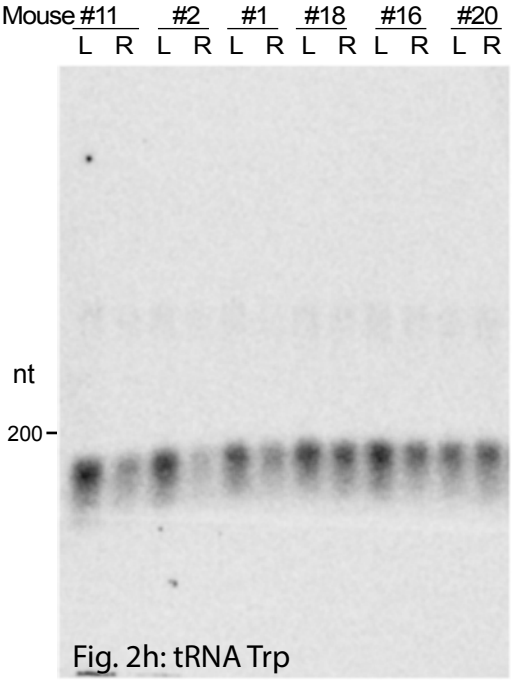
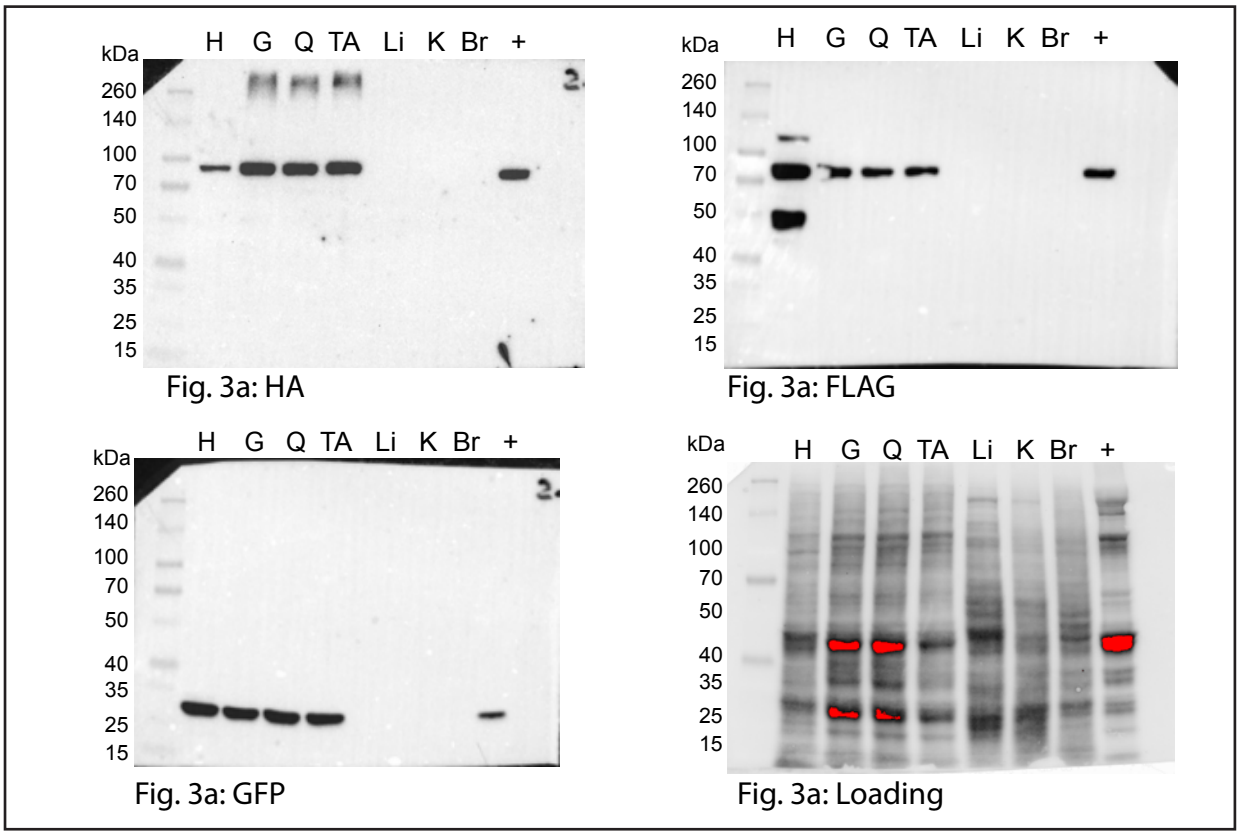
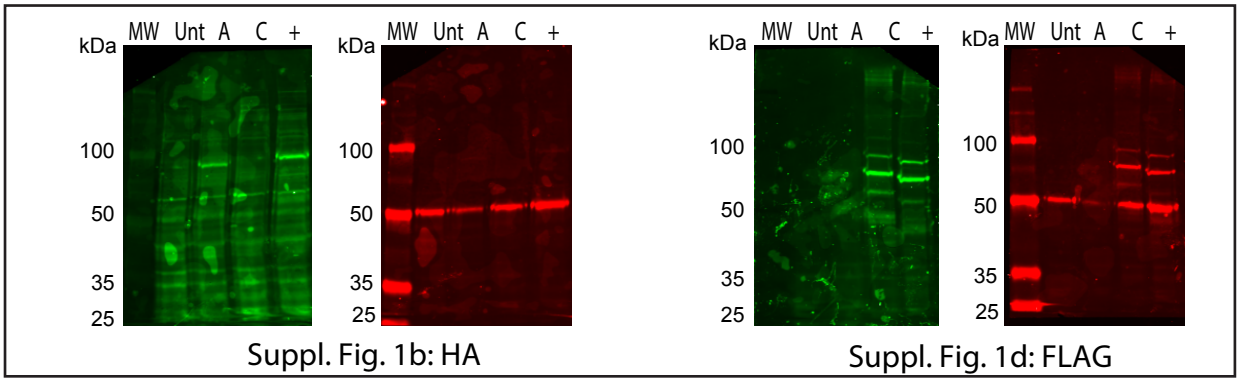
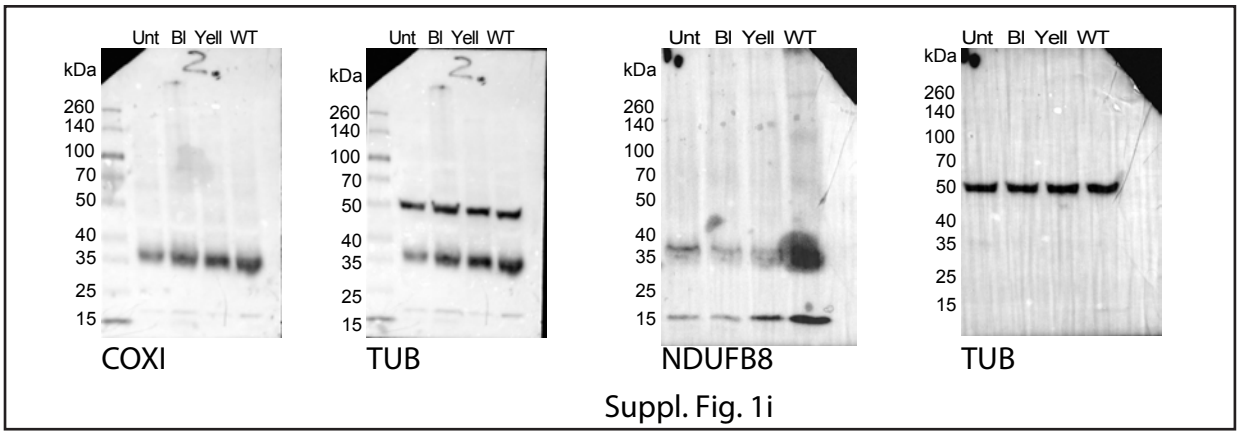


Fig. 2h: tRNA Trp

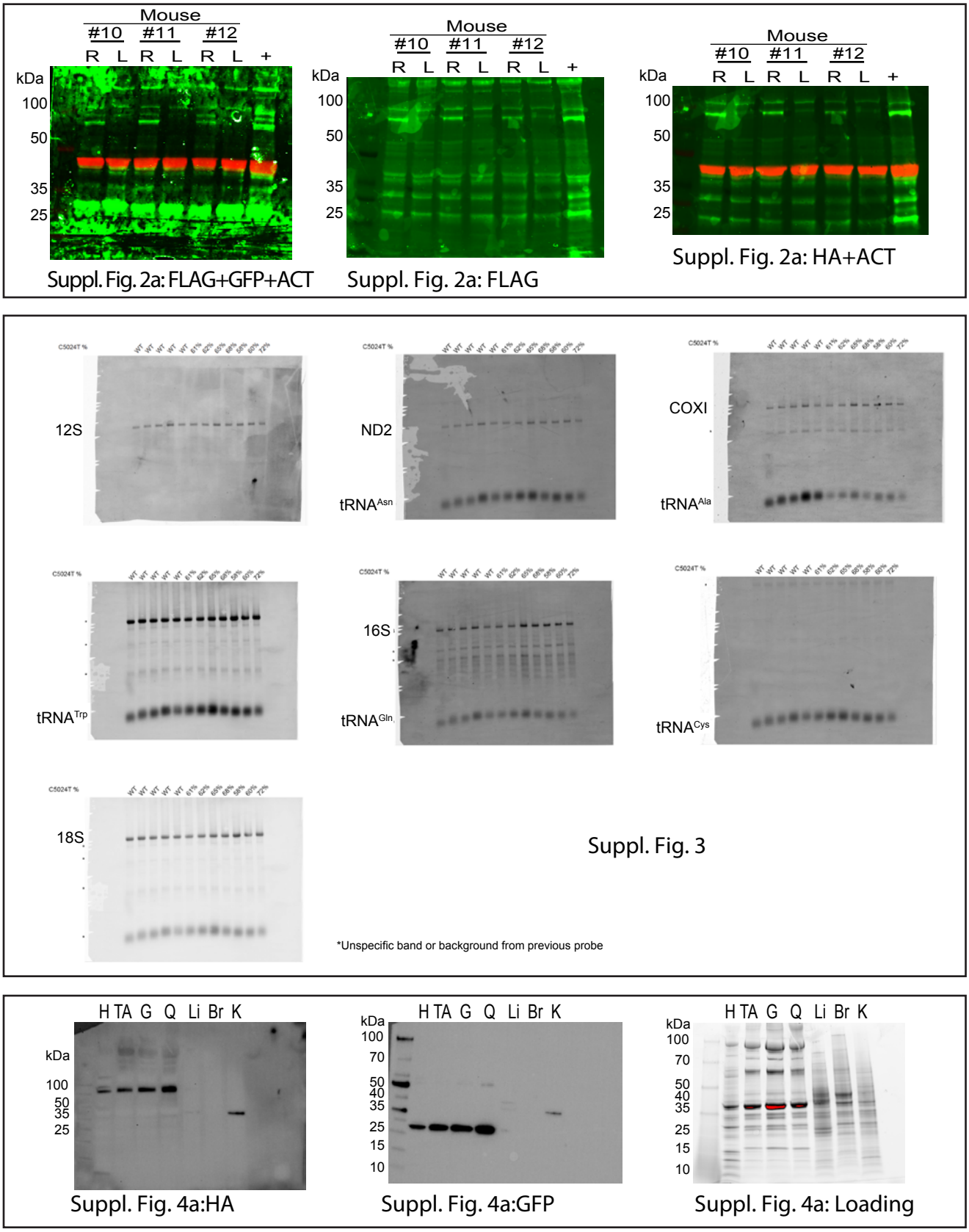
Supplementary Figure 6. Uncropped blots for figures 1 and 2.







Supplementary Figure 7. Uncropped blots for figure 3 and supplementary figure 1.



Supplementary Figure 8. Uncropped blots for supplementary figures 2, 3 and 4.