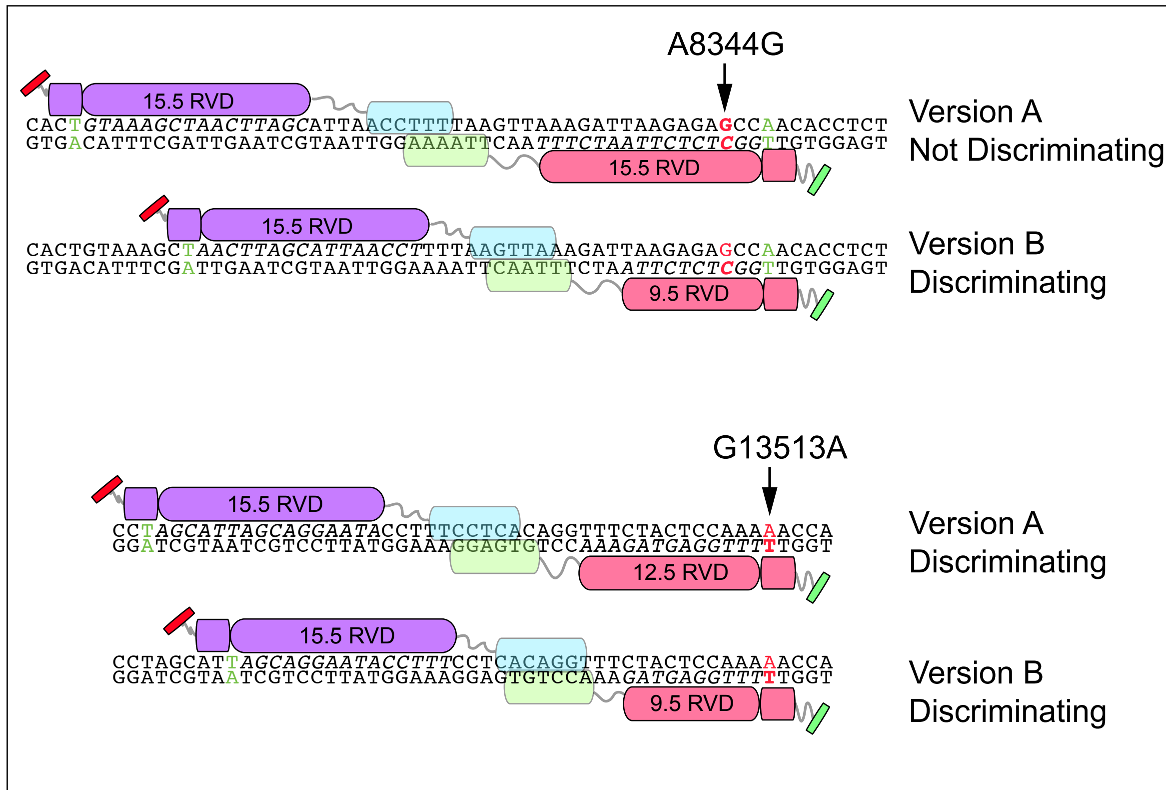
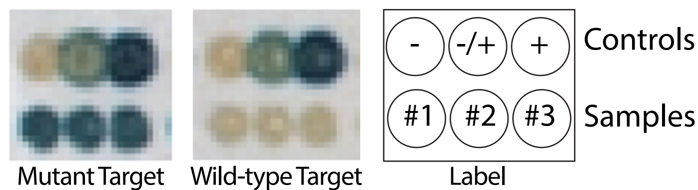


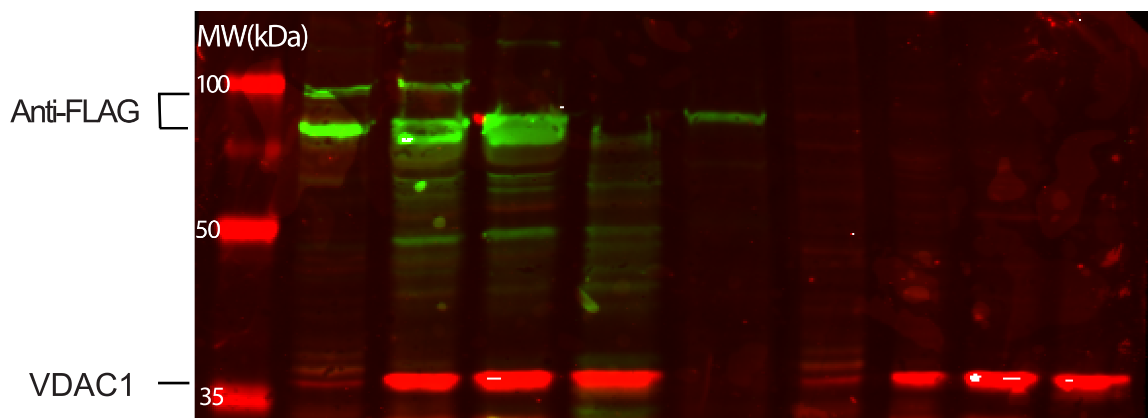
Supplementary Figure S1. Design and binding of mitoTALENs against two different pathogenic mtDNA mutations. A) mitoTALENs against the MERRF m.8344A>G mutation. B) mitoTALENs against the Leigh Syndrome m.13513G>A mutation. Version A of m.8344A>G mitoTALEN was found to be non-discriminating by SSA assays (supplementary figure S2). Base pair at position T0 is indicated in green and the mutated base pair in red (when they coincide, they are shown in red).

m.8344A>G TALEN Version B

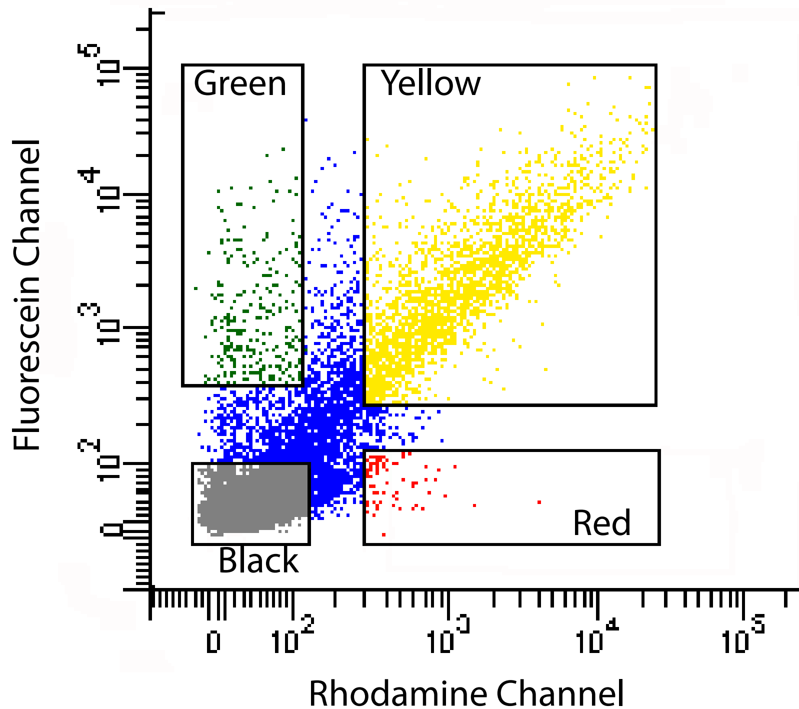


TALEN	%Digest MUT	%Digest WT
A8344G Version A	78	76
A8344G Version B	82	40

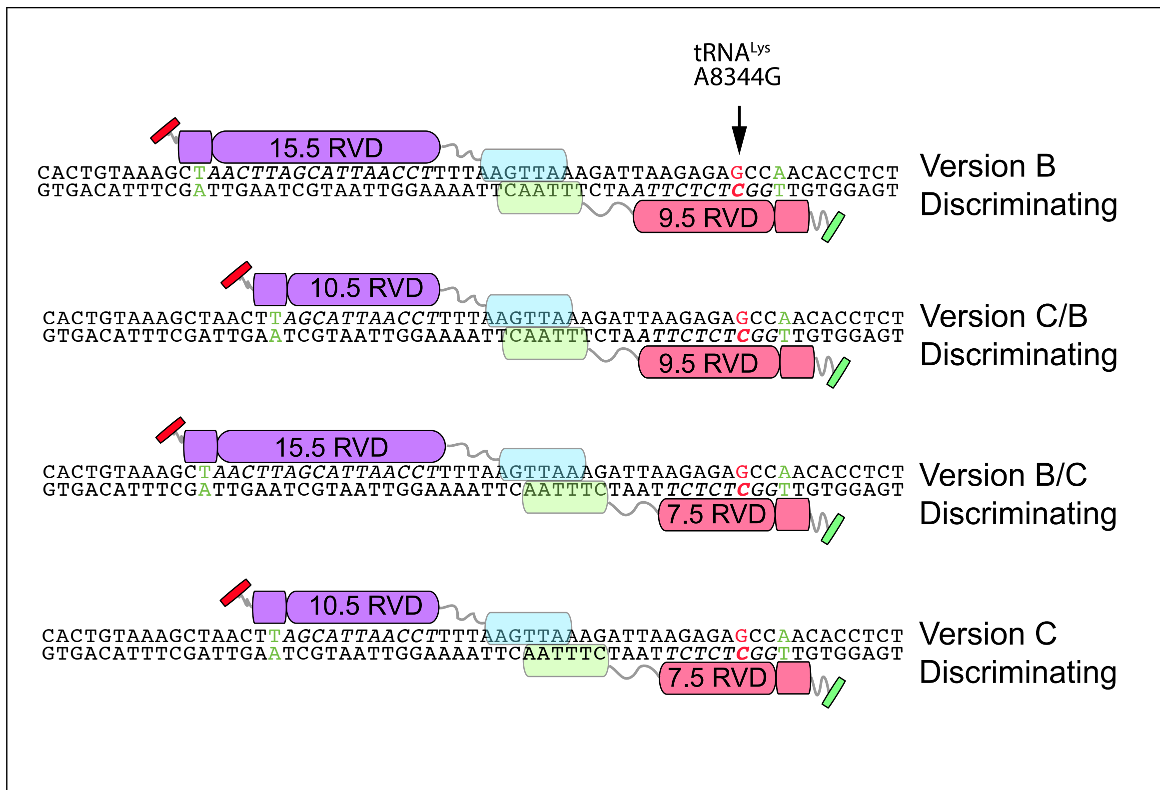
Supplementary Figure S2. Single Strand Annealing assay of m.8344A>G-TALENs. TALENs were expressed in yeast in a system whereas a Double Strand Break triggers a recombination event that can restore LacZ expression. The upper panel shows an example of the raw data with the m.8344A>G Version B, which displayed good specificity towards the mutant DNA sequence. The label indicates the position of the negative, intermediate and positive controls, as well as the triplicates of the test (#1-#3).



Supplementary Figure S3. A small fraction of mitoTALEN monomer remains attached to the outside of the mitochondria. HEK293T cells were transfected with the plasmids coding for one of the m.8344A>G shortened mitoTALEN monomers (version C monomer with 7.5 RVDs and the C8S9 mitochondrial targeting sequence). After 24 hours, mitochondria was isolated and treated with proteinase K as shown in the figure. Samples were analyzed by western blot with antibodies against FLAG. We also used an antibody against VDAC1, which is a mitochondrial protein known to be resistant to proteinase K.

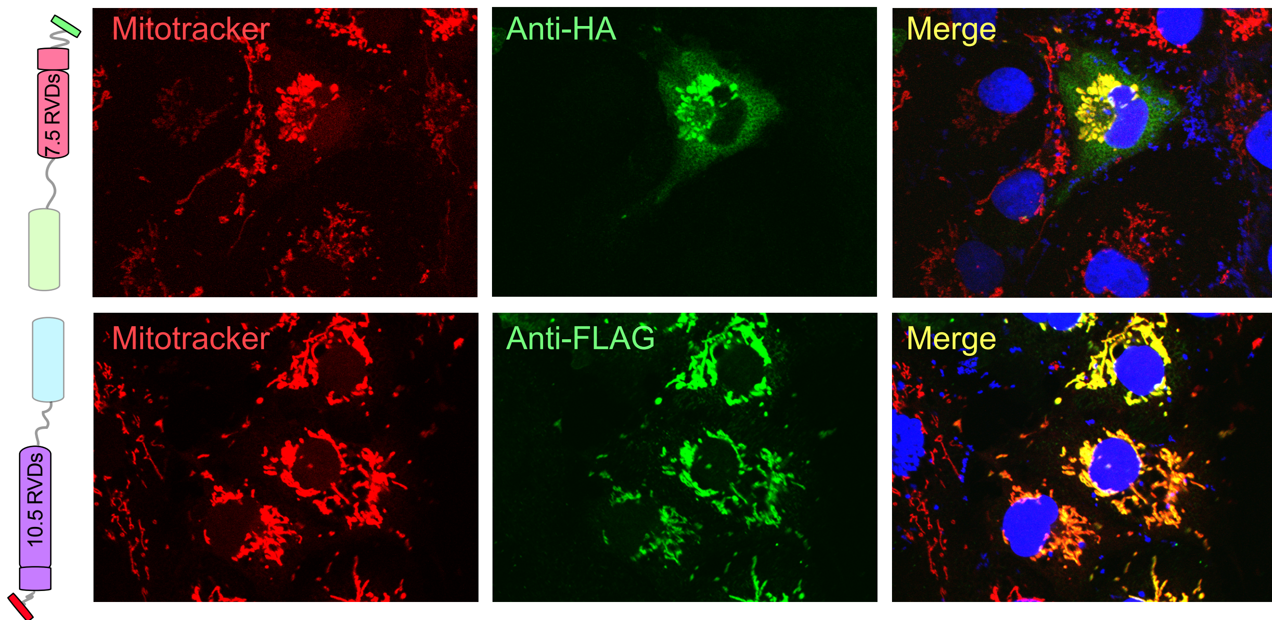


Supplementary Figure S4. Sorting of cells transfected with mitoTALEN. Transmitochondrial osteosarcoma cybrids harboring the heteroplasmic levels of the m.8344A>G mutation were transfected with two plasmids expressing the two mitoTALEN monomers. Cells were sorted by the fluorescent markers eGFP and mCherry. Cells expressing both were termed “Yellow”. Cells expressing none were termed “Black”.

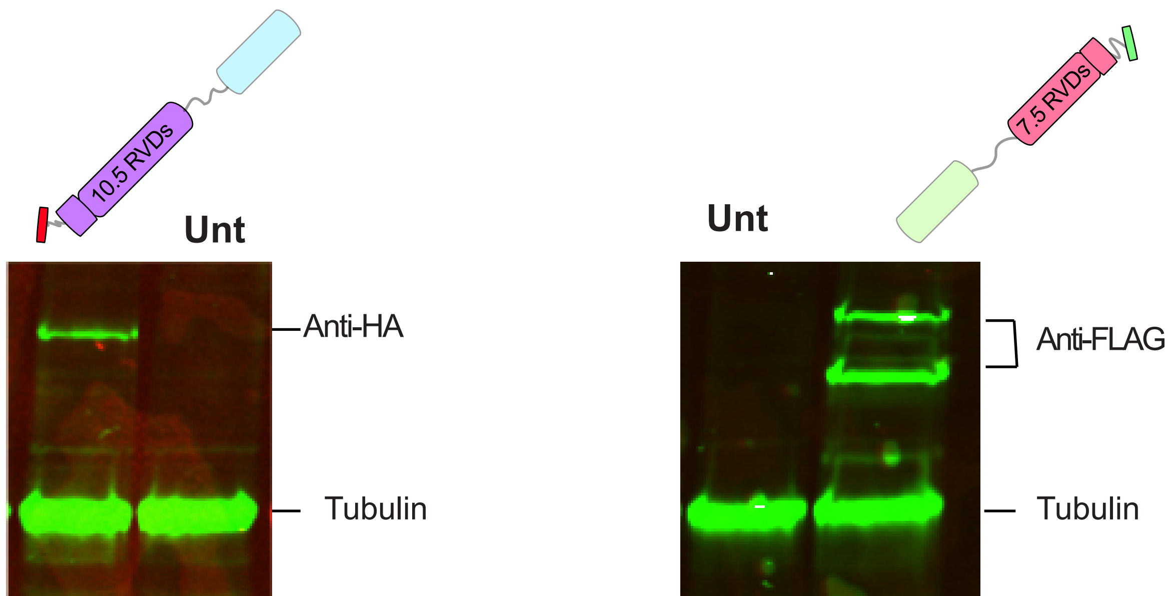


Supplementary Figure S5. Design and binding of short mitoTALENs against the m.8344A>G mtDNA mutation. The design and DNA binding domains are shown for the different combinations of sense and antisense monomers harboring the original (15.5 and 9.5) or reduced (10.5 and 7.5) RVDs. The combined shortened version was named “version C”. Base pair at position T0 is indicated in green and the mutated base pair in red.

a



b



Supplementary Figure S6. Shortened mitoTALEN monomers are expressed and localized in mitochondria. (a) COS7 cells were transfected with the respective plasmids coding for the m.8344A>G shortened mitoTALEN monomers (version C). After 48 hours, we immunostained the cells with antibodies against FLAG or HA. Cells were incubated with mitotracker before fixation. Western blots (b) showed the presence of specific proteins. Double bands may indicate incomplete removal of the MLS.

Supplementary Text

Production of cybrids with the ND5 m.13513G>A mutation.

A skin biopsy was performed on a 5.8-year-old Caucasian girl with Leigh syndrome caused by the *ND5* m.13513G>A mutation who is followed in the Mitochondrial-Genetics Clinic at The Children's Hospital of Philadelphia, following informed consent per Children's Hospital of Philadelphia Institutional Review Board (IRB) approved study #08-6177 (M.J.F., PI). Her major clinical manifestations include Leigh syndrome involving progressive mid-brain and basal ganglia lesions with white matter signal abnormalities, severely disabling chorea and dystonia, truncal hypotonia and appendicular hypertonia, metabolic encephalopathy, primary lactic acidosis, global developmental delay with regression and complete loss of ability to stand unassisted, walk or speak, heat intolerance, intermittent ptosis with heat, progressive ophthalmoplegia and strabismus, bilateral optic atrophy, progressive fatigue, anxiety, swallowing dysfunction and failure to thrive requiring gastrostomy feeds, chronic constipation, wolff-parkinson-white arrhythmia, renal tubular acidosis, and tracheostomy with intermittent ventilator support for respiratory failure and central sleep apnea. Her fibroblast cell line was established in the Children's Hospital of Philadelphia Clinical CytoGenomics Laboratory. Clinical diagnostic testing demonstrated variable heteroplasmy load in her different tissues and over time, including in blood (14% at 2.5 years, 46% at 4.9 years), urine (43% at 2.7 years), buccal (32% at 2.7 years), and skin fibroblasts (67% at 5.8 years).

Cybrid clones for the point-mutation m.13513G>A in *ND5* were produced by fusing her dermal fibroblasts treated with 1 µg/ml actinomycin D (A-9415; Sigma-Aldrich) with 143B/206 Rho Zero cells [24]. Individual colonies were manually picked and screened for heteroplasmy levels of the m.8344A>G and m.13513G>A mtDNA mutations respectively. The m.13513G>A point mutation has been reported and characterized in other patients [16, 17].