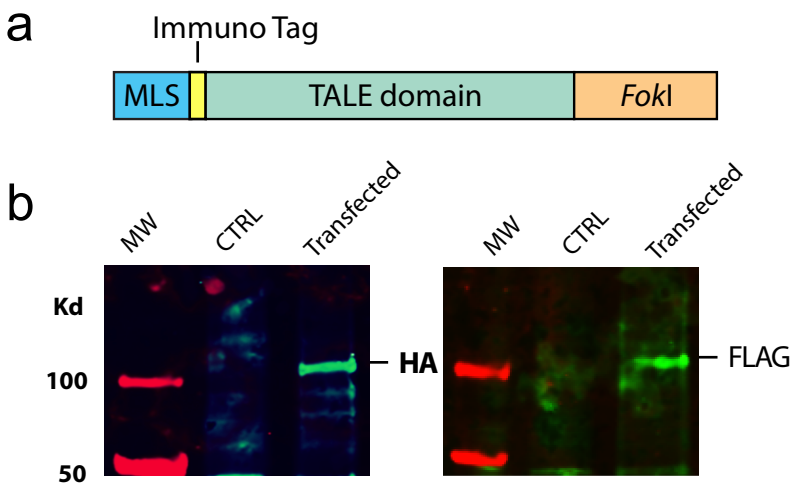
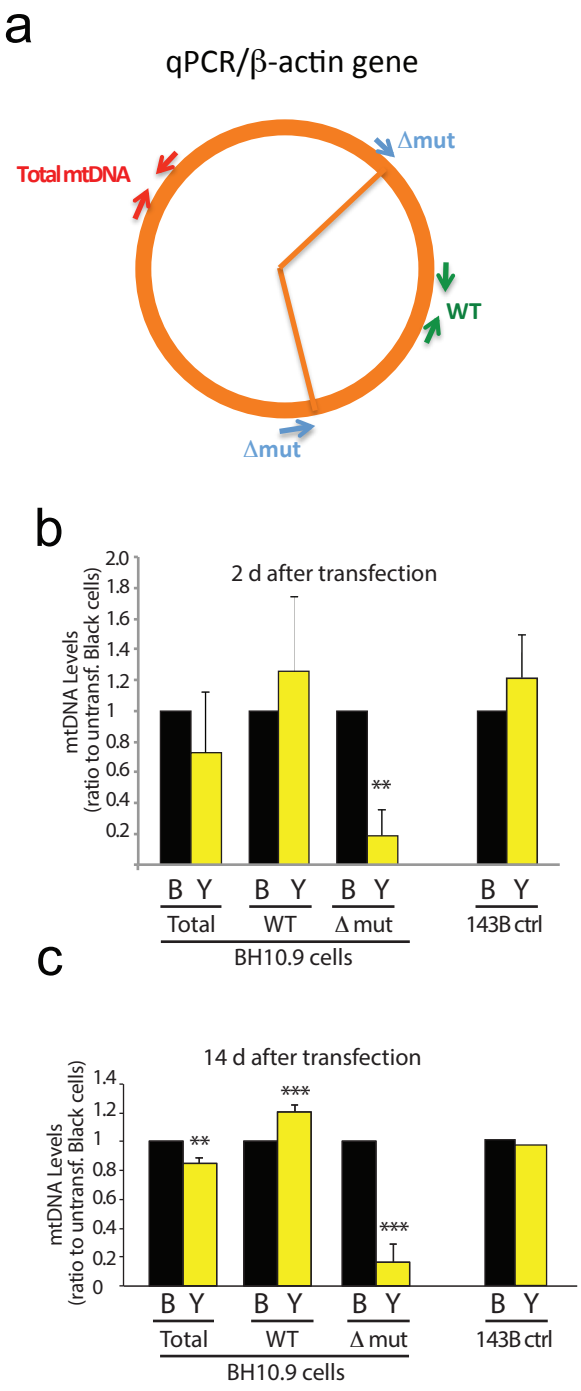
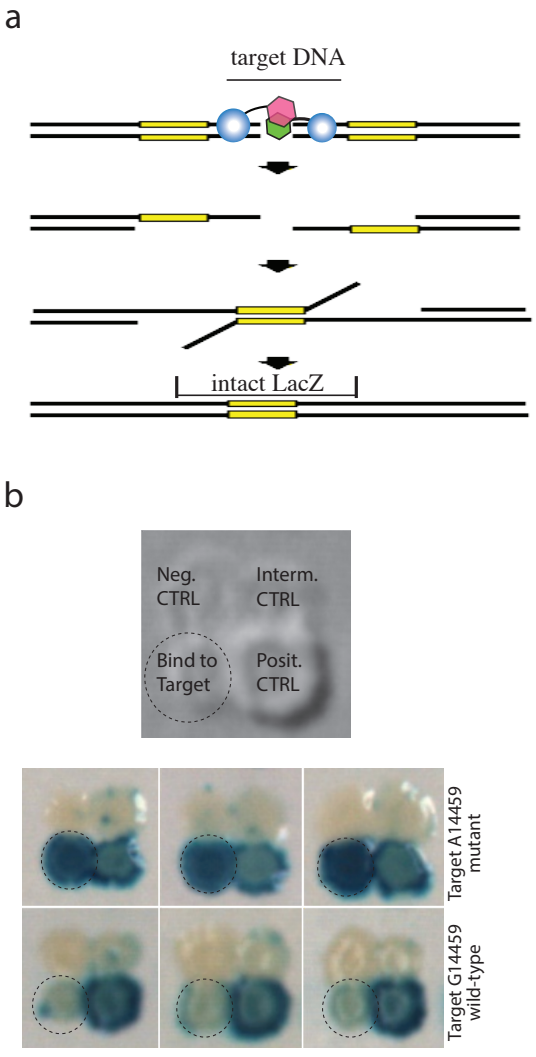


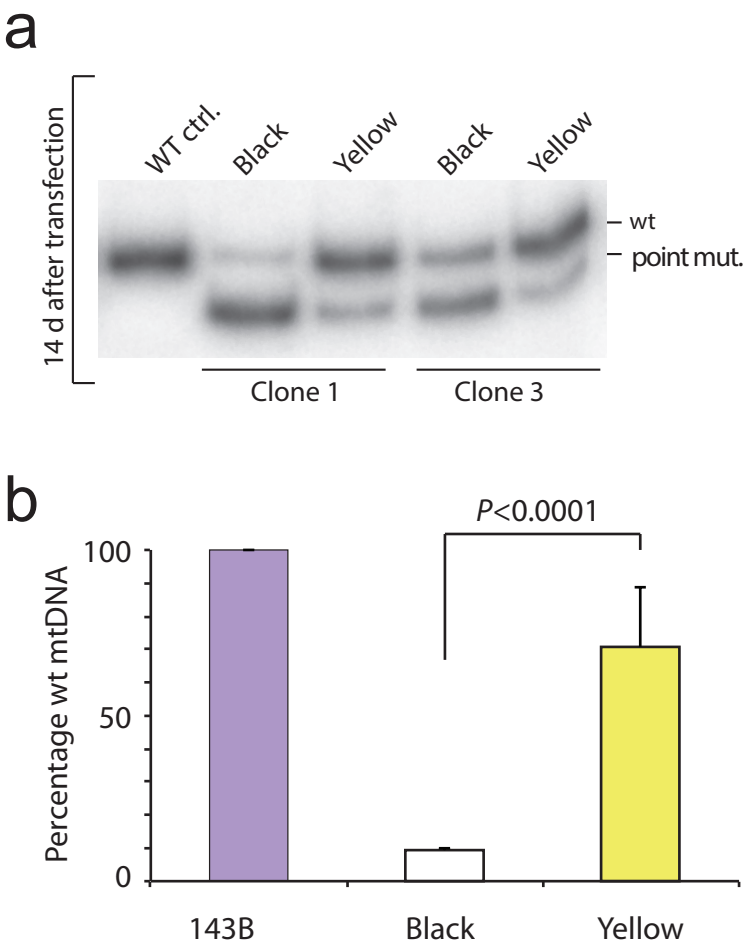
Supplementary Figure 1. General composition of mitoTALEN proteins. (**a**), Schematic drawing of the re-engineered mitoTALEN polypeptide monomer containing a mitochondrial localization signal in the N-terminus (MLS), an immunological epitope tag (HA or FLAG), the specific TALE domain and an obligate heterodimeric *Fok*I domain (FokI Left/Right). (**b**) Western blot of homogenates of COS7 cells transfected with plasmids coding for each of the two Δ5-mitoTALEN monomers. Anti-HA or anti-FLAG antibodies were used for infrared western blot detection.



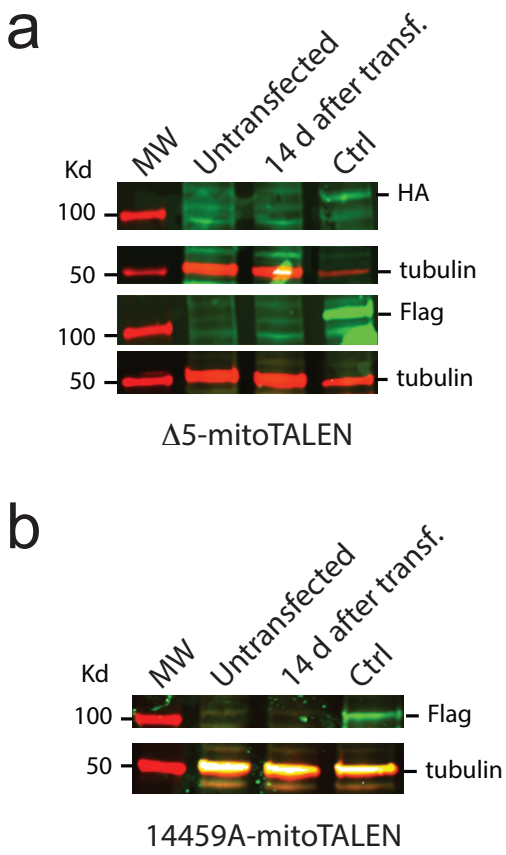
Supplementary Figure 2. Change in heteroplasmy is due to elimination of deleted mtDNA. Analyses of mtDNA levels of mitoTALEN transfected BH10.9 cells by qPCR. Approximate location of qPCR primers in the mtDNA is depicted in (**a**). qPCR data was normalized to the β-actin gene. We analyzed the levels of these mtDNA regions in FACS sorted cells black(B) and yellow (Y). (**b**) Results 2 days after transfection. (**c**) Results 14 days after transfection. The parental cell 143B, harboring wild-type mtDNA was also analyzed. ***P* = <0.003; ****P* = <0.001. t-Test unpaired comparisons between “black” and “yellow” cells.



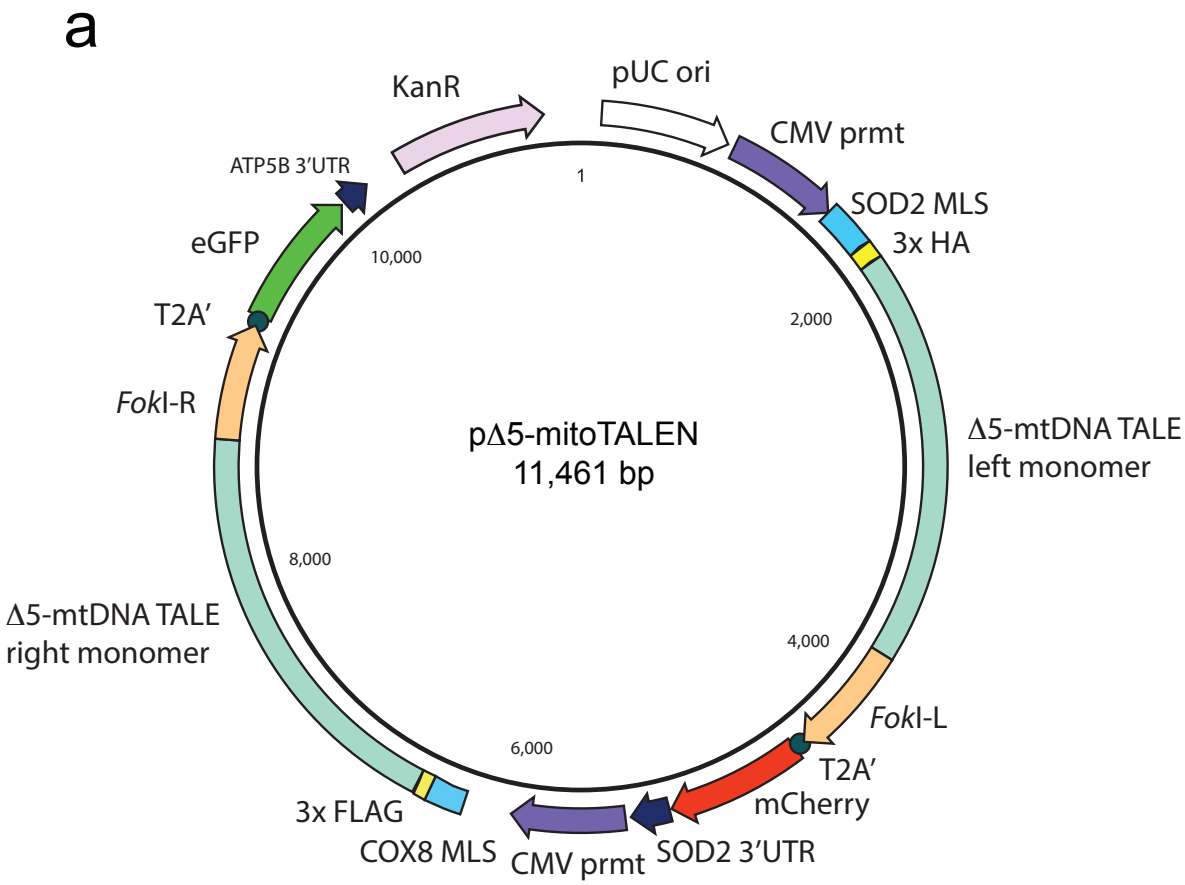
Supplementary Figure 3. Yeast Single-Strand Annealing (SSA) assay to determine binding to sites differing by a single nucleotide. SSA measures the ability of the TALEN to cleave a target sequence and restore Lac-Z activity by promoting homologous recombination between two halves of the LacZ gene. (**a**) Cartoon illustrating the system. If the target DNA is cleaved, the LacZ gene product is restrored and expressed creating blue colonies under appropriate conditions. (**Upper part of b**) shows the identity of the four colonies, three of which are controls. The experiment was performed in triplicate (**Lower part b**). The binding of this specific TALEN pair to the m.14459A mutant was approximately 0.8 Relative Units whereas the binding to the wt m.14459G was less than 0.4 Relative Units.



Supplementary Figure 4. Change in m.14459A mtDNA heteroplasmy is stable after mitoTALEN treatment. (**a**) RFLP analysis of the mutation load in the "black" and "yellow" cells 14 days after transfection. Cells were FACS sorted at 48 hours as in Fig. 1d and cultured for 13 additional days. (**b**) Quantitation of 3 independent experiments.



Supplementary Figure 5. Transiently expressed mitoTALENs are not detectable after 14 days. Human cybrid cells were transfected with plasmids expressing the two mitoTALEN monomers for the common deletion (Δ5-mitoTALEN, panel **a**) and the *MT-ND6* point mutation (14459A-mitoTALEN, panel **b**). Western blots from "yellow" cells grown for 14 days did not show the presence of the tagged mitoTALEN monomers (HA or FLAG). Only FLAG was tested for the 14459A-mitoTALEN. Ctrl were cells harvested 48 hours after transfection with the respective mitoTALEN.



Supplementary Figure 6. Combined plasmid for co-expression of mitoTALEN monomers. To obtain higher levels of double-transfecte cells, both mitoTALEN and markers were combined in a single 11,461 bp plasmid. Besides the mitochndrial targeting sequences, the constructs also had 3' UTRs from mitochondrial genes. Fluorescent markers were translated independently from the same transcriptional unit as the TALEN monomers with a help of a T2A' picornavirus sequence.

Supplementary Table 1. Search for mtDNA targets in the nuclear genome. We searched for similar sequences in the nuclear genome using BLAST and found none. Although there are known to be many mtDNA pseudogenes, we could not find segments comprising the entire mitoTALEN recognition sites for the deletion breakpoint nor the *MT–ND6* gene sequence harboring the G->A mutation in the nucleus (HG19 nuclear chromosome reference sequences). Motif searches for individual TALEN monomer identified isolated nuclear recognition sites for each monomer. See Online methods for search approach.

TALEN Monomer (length)	Δ5 L (17 bp)	Δ5 R (14 bp)	14459A L (11 bp)	14459A R (12 bp)
Hits per nuclear genome	1	28	1232	339
Hits per mtDNA	1	1	1	1(in mutant)
Nuclear Genome hits per 16.6 Kbp	5.35×10^{-6}	1.50×10^{-4}	6.59×10^{-3}	1.81×10^{-3}