SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Induction of heteroplasmy shift in NZB/BALB tail tip fibroblasts and oocytes, Related to Figure 1

(A) Mitochondrial co-localization of mito-ApaLI with Mitotracker. Scale bars, 10μm.

(B) RFLP analysis of mtDNA heteroplasmy in NZB/BALB cells transfected with mito-GFP, ApaLI and mito-ApaLI after 72 h.

(C) Quantification of mtDNA levels by qPCR in NZB/BALB cells transfected with mito-GFP, ApaLI and mito-ApaLI after 72 h.

(D) RFLP analysis and quantification of mtDNA heteroplasmy in control and mito-ApaLI injected oocytes after 48 h at polymorphic HindIII site (Control *n*=8; mito-ApaLI *n*=7). Representative gel.

(E) Quantification of mtDNA copy number by qPCR in control and mito-ApaLI injected oocytes after 48 h at polymorphic HindIII site (Control *n*=4; mito-ApaLI *n*=8).

(F) Analysis of mito-ApaLI specificity in BALB and NZB single haplotype oocytes. Quantification of mtDNA copy number by qPCR in BALB (Control *n*=14; mito-ApaLI *n*=12) and NZB (Control *n*=8; mito-ApaLI *n*=5) MII oocytes 48 h after injection of mito-ApaLI mRNA.

Error bars represent ± SEM. ***p*<0.01. *****p*=0.0001. ns.

Figure S2. Analysis of heteroplasmy shift in NZB/BALB embryos Related to Figure 2

(A) RFLP analysis and quantification of mtDNA heteroplasmy in control and mito-ApaLI injected embryos at polymorphic HindIII site (Control *n*=7; mito-ApaLI *n*=7). Representative gel.

(B) Quantification of mtDNA copy number by qPCR in control and mito-ApaLI injected embryos at polymorphic HindIII site (Control *n*=5; mito-ApaLI *n*=6).

Error bars represent ± SEM. ***p*<0.01. *****p*<0.0001.

Figure S3. Analysis of F1 mito-ApaLI mice, Related to Figure 3

(A) RFLP analysis of mtDNA heteroplasmy in tail tip biopsies of F1 mito-ApaLI pups at polymorphic HindIII site. (mito-ApaLI *n*=9).

(B) RFLP analysis of mtDNA heteroplasmy in tail, brain, muscle, heart and liver of F1 mito-ApaLI mice at polymorphic HindIII site.

(C) Array comparative genomic hybridization (array CGH) in mito-ApaLI mice for genome wide detection of copy number variants (CNVs), duplications/deletions, unbalanced translocations and aneuploidies. Gains are drawn on the right and losses on the left side of the diagram.

Figure S4. Analysis of F2 mito-ApaLI mice, Related to Figure 4

RFLP analysis of mtDNA heteroplasmy in tail tip biopsies of F2 mito-ApaLI pups at the polymorphic HindIII site. (F2 mito-ApaLI *n*=12).

Figure S5. Induction of heteroplasmy shift in NZB/BALB tail tip fibroblasts and oocytes using NZB mito-TALEN, Related to Figure 5

(A) Diagram illustrating targeting sequences of NZB TALEN.

(B) Schematic representation of Luciferase-based NZB targeting specificity assay.

(C) NZB targeting specificity of NZB TALEN collection. Blue square indicates NZB TALEN with highest specificity.

(D) Mitochondrial co-localization of NZB TALEN monomers with Mitotracker. Scale bars, 10μm.

(E) RFLP analysis of mtDNA heteroplasmy in NZB/BALB cells transfected with mito-GFP and NZB TALEN after 72 h.

(F) Quantification of mtDNA levels by qPCR in NZB/BALB cells transfected with mito-GFP and NZB TALEN after 72 h.

(G) RFLP analysis and quantification of heteroplasmy shift in NZB/BALB in control and NZB TALEN injected MII oocytes after 48 h at the polymorphic HindIII site (Control *n*=4; mito-ApaLI *n*=4). Representative gel.

Error bars represent ± SEM. **p*<0.05.

Figure S6. Induction of heteroplasmy shift using patient specific TALENS, Related to Figure 6

(A) PCR analysis of human mtDNA region containing the LHON m.14459G>A mutation in fused MII oocytes.

(B) Expression of fluorescent reporters of LHOND TALEN monomer in fused MII oocytes.

(C) Diagram illustrating targeting sequences of NARP TALEN.

(D) Schematic representation of Luciferase-based NARP targeting specificity assay.

(E) NARP targeting specificity of NARP TALEN collection. Blue square indicates NARP TALEN with highest specificity.

(F) Mitochondrial co-localization of NARP mito-TALEN monomers with Mitotracker. Scale bars, 10μm.

(G) RFLP analysis of mtDNA heteroplasmy in NARP patient cells transfected with mito-GFP and NARP TALEN after 72 h.

(H) Quantification of mtDNA levels by qPCR in NARP patient cells transfected with mito-GFP and NARP TALEN after 72 h.

(I) Expression of fluorescent reporters of NARP TALEN monomer in fused MII oocytes.

Error bars represent ± SEM.

Table S1. Blood analysis, Related to Figure 4

Analysis of mito-ApaLI animals demonstrated normal complete blood count.

Hematology	Normal Range	mito-ApaLI mice
White blood cell count (K/µl)	7.84 (±2.25)	6.18 (±0.8)
Neutrophils (%)	20.65 (±4.74)	15.24 (±4.16)
Lymphocytes (%)	74.71 (±5.27)	76.28 (±5.7)
Monocytes (%)	3.87 (±1.54)	7.73 (±1.87)
Eosinophils (%)	0.65 (±0.34)	0.56 (±0.59)
Basophils (%)	0.12 (±0.08)	0.16 (±0.28)
Red blood cell count (M/µl)	9.86 (±0.59)	10.03 (±0.22)
Haemoglobin (g/dL)	14.1 (±1)	15.01 (±0.52)
HCT (%)	45.5 (±4.6)	42.77 (±1.76)
Mean Corpuscular volume (fL)	46.1 (±3.7)	42.65 (±1.65)
Mean Corpuscular Haemoglobin (pg)	14.3 (±0.8)	14.96 (±0.58)
Mean Corpuscular Haemoglobin Concentration (g/dL)	31.1 (±1.5)	35.12 (±0.75)
RDW (%)	18.1 (±1.1)	17.03 (±0.43)
Platelets (K/µl)	992 (±145)	838 (±204)
MPV (fL)	4.55 (±0.46)	4.67 (±0.24)

Table S2. Primer Sets Used in This Study, Related to Experimental Procedures Section

Primer sets used for Last-cycle hot' PCR and quantification of mtDNA copy number.

'Last-cycle hot' PCR primers*

		Primer Sequence (5´- 3´)	Restriction enzyme
NZB/BALB	F_5184	GGCGGTAGAAGTCTTAGT	ApaLI
	R_5646	GGAGAAGGAGAAATGATGG	
NZB/BALB	F_8811	GGCCACCACACTCCTATTGT	HindIII
	R_9305	ATGCTGCGGCTTCAAATCCG	
LHON	F_14425	CCCCCATGCCTCAGGATACTCCTCAATAG**TG**ATC	BclI
	R_14552	TGATTGTTAGCGGTGTGGTCGGGTGTGT	
NARP	F_8904	CCACTTCTTACCACAAGGCACACCTACACC	BsrI
	R_9319	AGGCCTAGTATGAGGAGCGTTATGGAGT	

*5´ Fluorescein amidite (FAM) labeled. Mismatch codons are marked in bold.

Primers for quantification of mtDNA copy number

		Primer Sequence (5´- 3´)	Restriction enzyme
NZB/BALB	F_5184	GGCGGTAGAAGTCTTAGT	Standard curve
	R_5646	GGAGAAGGAGAAATGATGG	(ApaLI site)
NZB/BALB	F	GAGCGGGAATAGTGGGTACTG	qPCR -
	R	ACAAAAGCATGGGCAGTTACG	ApaLI site
NZB/BALB	F_8811	GGCCACCACACTCCTATTGT	Standard curve
	R_9305	ATGCTGCGGCTTCAAATCCG	(HindIII site)
NZB/BALB	F	CAAGCCCTACTAATTACCATTATAC	qPCR -
	R	AGTCCATGGAATCCAGTAGCC	HindIII site
LHON	F_14425	CCCCCGAGCAATCTCAATTA	Standard curve
	R_14552	TGATTGTTAGCGGTGTGGTCGGGTGTGT	
LHON	F	CCCCCATGCCTCAGGATACTCCTCAATAG	qPCR
	R	TGATTGTTAGCGGTGTGGTCGGGTGTGT	
NARP	F_8904	CCACTTCTTACCACAAGGCACACCTACACC	Standard curve
	R_9319	AGGCCTAGTATGAGGAGCGTTATGGAGT	
NARP	F	CTGACTATCCTAGAAATCGC	qPCR
	R	GATTGGTGGGTCATTATGTG	

EXTENDED EXPERIMENTAL PROCEDURES

Single strand annealing (SSA) reporter assay

The pGL4-SSA reporter construct was purchased from Addgene (#42962). The construction of TALEN target sequence followed previous reports (Targeted mutagenesis in the sea urchin embryo using zinc-finger nucleases). NZB target sequence is amplified from mitochondria DNA of NZB/BALB cells using the following primers (ApaL_luc-F: TGGACTAGGGTCTCGCGGGAATAGTGGGTACTGC and ApaL_luc-R: CGCTCCTAGGTCTCATTATAACAAAAGCATGGGCAGTTACG). The PCR products were subcloned into BsaI site of pGL4-SSA and NZB target reporter (pGL4SSA-NZB) plasmids were selected by sequencing. WT and NARP target mitochondrial DNA sequences were amplified from healthy donor or NARP patient cells with the following primers (NAPR_luc-F: TGGACTAGGGTCTCTATCCTAGAAATCGC TGTCGCC and NAPR_luc-R: CGCTCCTAGGTCTCATAGGCATGTGATTGGT GGGTC). The PCR products were subcloned into BsaI site of pGL4-SSA, and constructed WT (pGL4SSA-WT) and NARP target reporter (pGL4SSA-NARP) plasmids. To detect TALEN activity for the target site, SSA assay was done with SSA reporter plasmids (pGL4SSA-NZB, pGL4SSA-WT or pGL4SSA-NARP) following previous reports (Targeted mutagenesis in the sea urchin embryo using zinc-finger nucleases). To determine TALEN specificity for the NZB or NARP target site, the detected luciferase activities for the target sites (i.e. NZB or NARP) were normalized to the non-target sites (i.e. WT).

Blood and plasma parameters

Blood collection was performed by sub-mandibular bleeding. Blood was allowed to drip into EDTA-containing polypropylene microtubes (Becton Dickinson). Blood in the tube was immediately mixed well by tapping and inverting tube five times to ensure proper anticoagulation. Samples were kept at room temperature until analysis (within four hours). Whole EDTA blood samples were analyzed in duplicates for Complete Blood Count (CBC) with leukocyte differential and platelet count on a Hemavet 950FS Multi Species Hematology System (Drew Scientific) programmed with mouse hematology settings.

For glucose and lactate analysis, blood was allowed to drip into heparin-containing polypropylene microtubes (Becton Dickinson). Blood in the tube was immediately mixed well by tapping and inverting tube five times to ensure proper anticoagulation. To obtain plasma, blood was immediately centrifuged after collection at 3000g at 4°C. Plasma glucose concentration was

determined using the Glucose (GO) Assay Kit (Sigma) according to the manufacturer's instructions. Plasma lactate concentration was determined using the Lactate Assay Kit (Sigma) according to the manufacturer's instructions.

Behavioral analysis

Behavioral testing was carried out at the Salk Institute for Biological Studies Behavioral Testing Core. Basic sensorimotor function was assessed in the Open Field Test, Rotarod, Grip Strength and Neurological Screen. Tests were carried out in the order above and separated by a 24-hours rest period to reduce carry-over effects.

Locomotor Activity. The Open Field test measure baseline levels of locomotor activity in freely moving mice. Mice are individually placed into clear Plexiglas boxes (40.6 x 40.6 x 38.1 cm) surrounded by multiple bands of photo beams and optical sensors that measure horizontal (ambulatory) and vertical (rearing) activity (Med Associates, USA). Their movement is detected as breaks within the beam matrices and automatically recorded for 60 minutes.

Motor Coordination. The Rotarod test measures locomotor coordination on a gradually accelerating spinning rod. Mice are placed on an elevated spinning rod and the latency and speed at which a fall occurs is automatically recorded (San Diego Instruments, USA. The test is conducted in two phases beginning with a 60-sec training session at a constant speed of 3 RPM followed by 4 trials using an accelerating ramp profile of 0-30 RPM in 300 seconds. To examine potential differences in motor learning, the test is repeated 24-hours later.

Neuromuscular Function. The Grip Strength test (GS) measures grip force in the forelimbs. The apparatus consists of an acrylic platform with 2 horizontally mounted digital gauges connected to each end of the platform (San Diego Instruments, USA). The digital gauges record both compression and tension using the displacement force exerted onto a metal bar attached to each gauge. When determining forelimb strength, the animal is lowered toward the metal bar until it grips the bar with its forepaws and is gently tugged away in a horizontal motion. The maximum tension force is recorded upon release. This procedure is repeated 4 times and the average force in grams is calculated for each animal.

Neurological Screen. Gross sensory function is assessed using a subset of tests from the primary SHIRPA protocol (Rogers et al., 1997). This assessment includes screening the presence or absence of the righting response, corneal reflex (eye blink response to direct air puff stimulus), pinna reflex (ear twitch), visual acuity (pupillary light reflex and reaching), olfactory response to aversive stimuli, and the startle response.

Array comparative genomic hybridization (aCGH)

aCGH was performed following Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis (Agilent Technologies, Santa Clara, CA) protocol version 7.3. Labeled DNA was hybridized to the 4x180k SurePrint G3 Mouse CGH Microarray with the design based on NCBI37/mm9 and an overall median probe spacing of 10.9kb (9.1kb in Refseq genes). The microarray was scanned with the Agilent C Scanner (Agilent Technologies). Normalization was performed with Agilent Feature Extraction software and analyzed with Agilent Genomic Workbench (Agilent Technologies).

Exome capture and high-throughput sequencing

Exome capture was performed on pooled libraries generated from tail DNA of one animal of each inbred parental strain and from two male and two female mito-ApaLI offspring, using the SeqCap EZ Mouse Exome Design probe pool (54 Mb, NimbleGen) according to the manufacturer's protocol. Capture libraries were sequenced for 84 cycles on an Illumina HiSeq 2500. Exome coverage was 18x and 70x for the parentals and 61x to 92x for the offspring. Sequencing reads were aligned to the mm9 reference genome using bwa, then potential variants were detected using samtools, considering only sites where C1 and C4 matched the reference sequence and genotype quality met a Q30 threshold.

SUPPLEMENTAL REFERENCE

Rogers, D.C., Fisher, E.M., Brown, S.D., Peters, J., Hunter, A.J., and Martin, J.E. (1997). Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mamm. Genome *8*, 711–713.