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Supplemental Information

Preclinical Efficacy and Safety Evaluation of Hematopoietic Stem Cell Gene Therapy in a Mouse Model of MNGIE

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Supplemental information

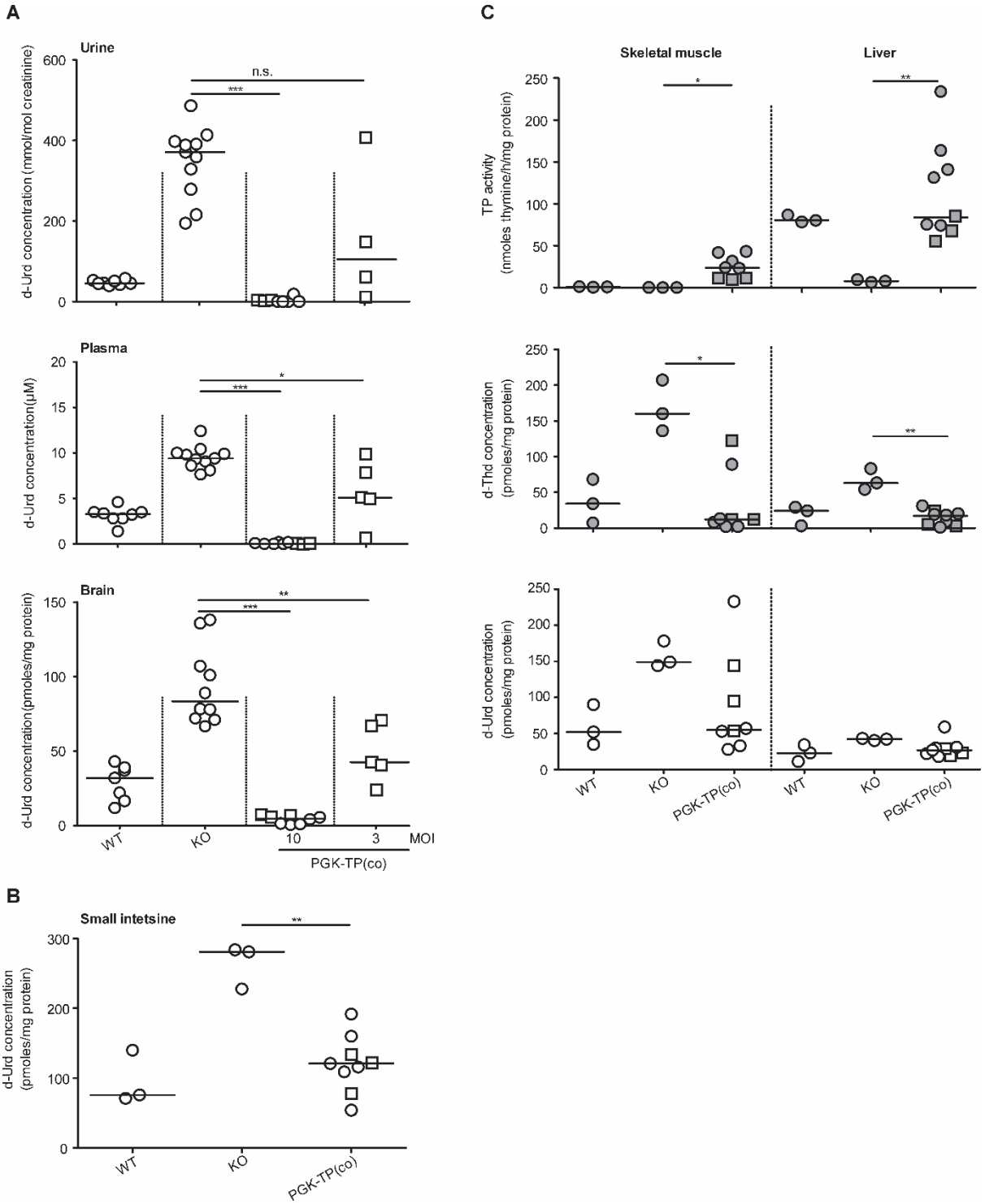


Figure S1: Biochemical correction in transplanted MNGIE mice. (A) Quantifications of deoxyuridine (d-Urd) in urine, plasma of blood and brain tissues 8-11 months after transplantation of 5×10^5 LV transduced Lin- cells (MOI 10, 3), *n*= 4-11 mice /group and **(B)** in intestines 11 months after transplantation of 5×10^5 LV transduced Lin- cells (MOI10), *n*= 3-9 mice /group. **(C)** Biochemical correction in skeletal muscle and liver tissues 11 months after transplantation of 5×10^5Lin- (MOI 10), *n*= 3-9 mice /group. The horizontal line represents the median. **P* <0.05, ***P* <0.01 and ****P* <0.001, n.s. = not significant. Mice in the PGK-TPco treatment group are identified (square symbols).

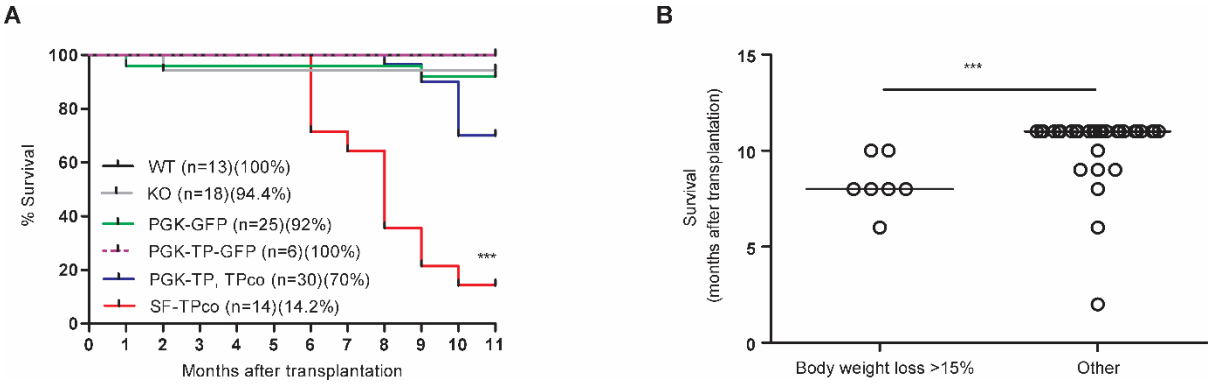
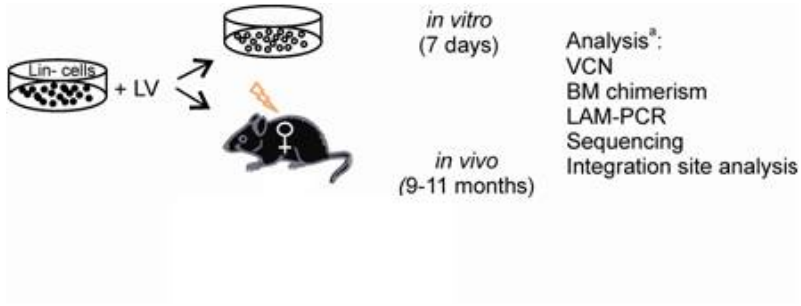


Figure S2: Reduced survival and severe body weight loss in primary recipients of SF-TPco. (A) The numbers of animals per group at start of the experiment and % survival are shown between the parenthesis in the survival plot. Log-rank test for comparison of median survival: ****P*< 0.0001 SF-TPco versus KO, WT, PGK-GFP or PGK-TP, TPco. PGK-GFP includes recipients of KO or WT PGK-GFP transduced Lin-. **(B)** PGK-TP, PGK-TPco and SF-TPco recipients and untreated KO mice were divided into two groups based on severe body weight loss at the time of death (>15% weight loss occurred over a time span of 1 to 2 months, SF-TPco, median body weight 15.7g (range, 14.3-17, *n*= 6) and a recipient of PGK-TP (14 g) and the other group are mice which gained or did not remarkably lose weight until termination (*n*= 39), plotted on the y-axis the survival of animals in these 2 groups. The horizontal line represents the median, ****P* <0.0001.

A



B

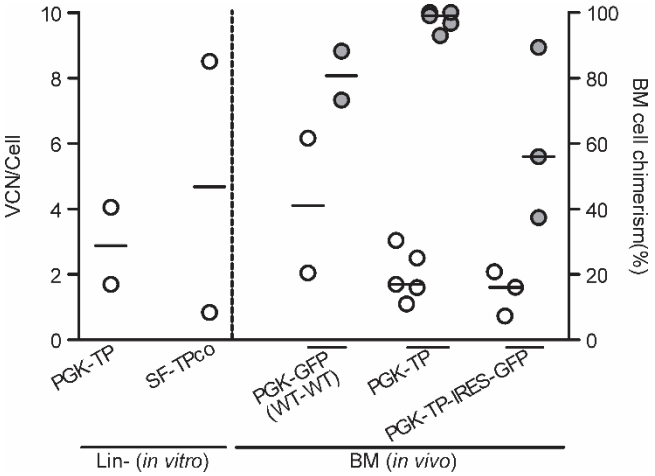


Figure S3: LV integration site analysis. (A) Schematic representation of in vitro and in vivo experiments for identification of LV integration sites. Bone marrow Lin- cells were transduced (MOI 10) overnight and were either cultured for seven day, or transplanted into KO or WT recipient mice. [a] Integration site analysis was performed on the in vitro cultured Lin- cells and BM of primary recipients. The vector copy number (VCN/ cell) measured in Lin- cells, *n*= 2 experiments, and VCN/ cell and BM chimerism measured in bone marrow cells, *n*= 2 -5 mice/group, is illustrated in **(B)**. The horizontal line represents the median.

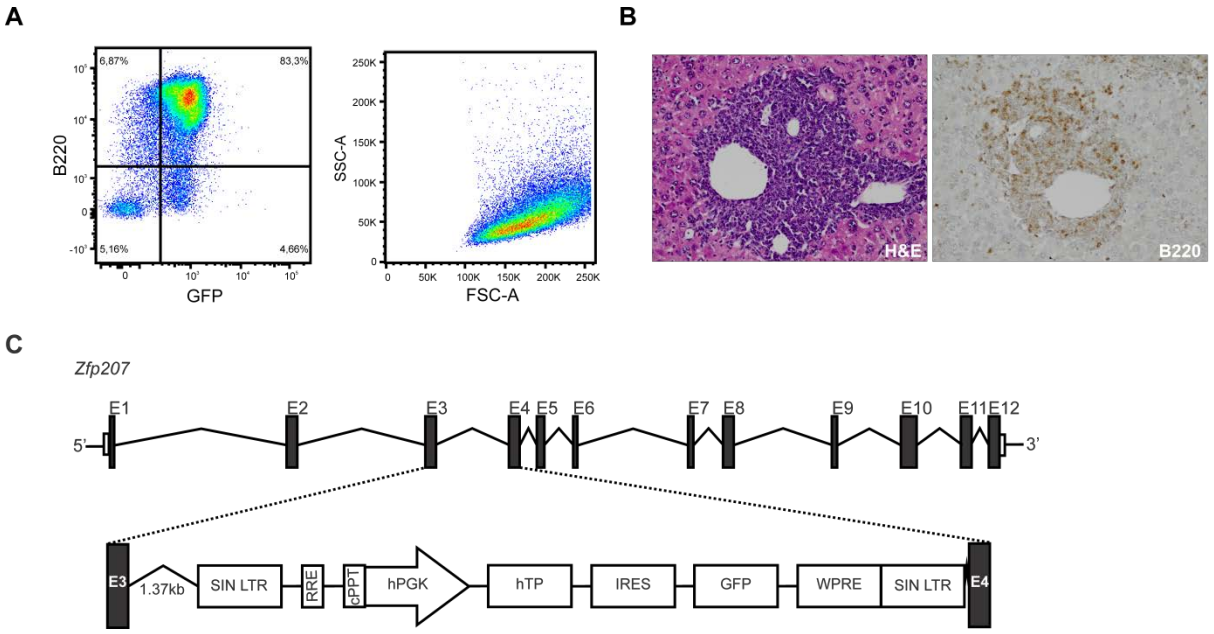


Figure S4: Characterization of LV vector positive B-cell lymphoma. (A) FACS blot of BM cells stained for B220+ and demonstration of the size (FSC) and granularity (SSC) of the B220+GFP+ population**. (B)** Histology and immunohistochemistry for large B-cell lymphoma infiltration in liver. H&E and B220 staining (200x) **(C)** Overview of the PGK-TP-GFP insertion into the third intron of *Zfp207* gene as identified by LAM-PCR and sequencing analysis of BM**.**

Table S1. *In vitro* analysis of TP enzyme activity.

	Lin-		CFU-GM		293T
LV	**Exp. 1**	**Exp. 2**	**Exp. 1**	**Exp. 2**	
KO	und.	2.2	n.a.	1.0	und (293T cells)
WT	und.	78.3	n.a.	29.0	
PGK-TP-GFP	1429.8	n.a.	n.a.	n.a.	4599.96
PGK-TP	4804.8	53.8	844.3	1139.4	6057.3
PGK-TPco	1787.5	143.3	506.5	817.4	1308.9

In two experiments BM Lin- cells were transduced (MOI 10) overnight then cultured in liquid medium or seeded in semisolid medium. Seven days after culture, TP enzyme activity (nmol/h/mg protein) was measured in Lin- cells or granulocyte-monocyte progenitors (CFU-GM), as well as in 293T cells (MOI 10). und. undetectable; n.a. not available.

Table S2. Molecular analysis and GFP expression in BM Lin- cells transduced and cultured for 7 days, and BM cells from primary recipients of $5x10^5$ transduced BM Lin- cells. MOI, multiplicity of infection; n.a. not available.

#	Construct (LV)	MOI	Transduction efficiency (VCN/ cell, Lin- cells)	% transduced cells (% GFP, Lin- cells)	VCN/ cell (BM)	% Chimerism (BM)	%transduced cells following engraftment (% GFP, BM)
1	PGK-TP	10	n.a.	Not applicable	1.3	77.4	Not applicable
2					3.0	87.1	
3					1.7	100.0	
4					1.5	44.9	
5					1.1	71.1	
6					3.6	50.7	
7			4.1		1.0	100.0	
8					3.0	96.8	
9					1.8	100.0	
10					2.6	99.1	
11					1.6	93.0	
12					1.2	100.0	
13	PGK-TPco	10	n.a		0.7	100.0	
14					0.2	21.0	
15					1.3	84.9	
16			2.9		1.3	80.0	
17					1.1	99.3	
18					1.0	85.4	
19					1.4	100.0	
20					1.3	95.7	
21					0.5	16.4	
22	PGK-TPco	3	0.5		0.3	47.2	
23					0.8	46.9	
24					0.1	66.8	
25					0.5	76.0	
26					0.1	58.4	
27	PGK-TP-GFP	10	n.a.	50.5	0.6	79.3	48
28					1.6	56.0	29.2
29					0.8	40.5	33.1
30					2.1	89.4	53.6
31					0.7	81.9	31.2
32					0.7	37.4	22.8
33	PGK-GFP (KO-KO)	10	n.a.	80.4	4.3	72.1	77.2
34					9.8	88.8	93.5
35					6.3	52.6	77.8
36					4.5	26.8	92
37					7.2	64.6	92.6
38			5.1	88.4	1.8	97.5	80.3
39					4.1	84.0	81.2
40					3.5	89.9	89.5
41					2.4	95.2	45.3

Table S3. Complete blood counts (CBC) of gene therapy treated primary recipient mice and control groups[a]

LV vector	WBC ($x10^3/\mu l$)	RBC ($x10^6/\mu l$)	HGB ($x10^5$)	HCT ($x10^5$)	PLT ($x10^9/l$)	MCV	MCH	MCHC
KO (*n*=7)	6.5 (2.7-11.4)	9.8 (9.3-10.6)	9.1 (8.3-9.6)	0.5 (0.45-0.52)	1196 (974-1529)	50 (48-51)	0.b93 (0.89-0.95)	18.4 (17.80-18.90)
WT (*n*=6)	4.4 (3-5.7)	9.1 (8.5-10.6)	8.4 (7.8-9.6)	0.46 (0.4-0.5)	901 (473-1489)	50 (48-52)	0.91 (0.89-0.98)	18.2 (17.9-19.1)
PGK-TP (*n*=5)	6.2 (5.2-7.2)	8.9 (8-10.2)	8.1 (7.6-9.1)	0.46 (0.4-0.5)	1277 (931-1705)	50 (49-52)	0.93 (0.88-0.95)	18.2 (17.1-19)
PGK-TPco (*n*=6)	5.1 (3.9-9.3)	7.7 (3.4-9.5)	7.2 (4-9)	0.39 (0.21-0.48)	861 (240-1531)	51.5 (50-63)	0.96 (0.90-1.1)	18.3 (17.6-18.7)
SF-TPco (*n*=4)	4 (1.9-5.4)	5 (3-9.2)	4.8 (3-8.3)	0.26 (0.1-0.4)	1145 (773-1198)	52 (49-54)	0.94 (0.9-0.96)	18 (17.3-18.5)
PGK-GFP (KO-KO) (*n*=4)	5.8 (3-7)	9.6 (9.4-9.9)	8.7 (8.6-9)	0.48 (0.46-0.5)	1086 (609-1236)	50 (48-54)	0.90 (0.88-0.92)	18 (17.2-18.4)
PGK-GFP (WT-WT) (*n*=4)	3.7 (3-4)	8.1 (8-9.4)	7.8 (7.2-9.2)	0.41 (0.41-0.48)	665 (590-793)	51.5 (51-52)	0.97 (0.88-0.97)	18.8 (17-19)

[a] WBC :white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, Hematocrit ;PLT, platelets; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, Mean cellular hemoglobin concentration. Data represent median (range)

Table S4. A. *In vitro* analysis of TP enzyme activity (LV-SF-TPco).

	Lin-		CFU-GM		293T
LV	**Exp. 1**	**Exp. 2**	**Exp. 1**	**Exp. 2**	
SF-TPco	19830.8	501.5	2153.3	2220.0	18071.2

In two experiments BM Lin- cells were transduced (MOI 10) overnight then cultured in liquid medium or seeded in semisolid medium. Seven days after culture, TP enzyme activity (nmol/h/mg protein) was measured in Lin- cells or granulocyte-monocyte progenitors (CFU-GM), as well as in 293T cells (MOI 10).

B. *In vivo* biochemical and molecular data of LV-SF-TPco treatment group

Blood TP activity (nmoles thymine/h/mg protein)	**Urine Thd (mmol/mol creatinine)**	**Urnie d-Urd (mmol/mol creatinine)**	**Plasma d-Thd (µM)**	**Plasma d-Urd (µM)**	**Brain TP activity (nmoles thymine/h/mg protein)**	**Brain d-Thd (pmoles/mg protein)**	**Brain d-Urd (pmoles/mg protein)**	**VCN/ cell**	**BM cell chimerism (%)**
462.7 (351-521.8) (*n*= 6)	0 (*n*= 2)	0 (*n*= 2)	0 (0-0.1) (*n*= 3)	0.1 (0-0.1) (*n*= 3)	240 (133-501) (*n*= 3)	0.6 (0.2-1.6) (*n*= 3)	1.6 (0.1-3) (*n*= 3)	1.8 (0.3-11) (*n*= 7)	98 (75-100) (*n*= 6)

Quantification of thymidine phosphorylase (TP) enzyme activity, thymidine (d-Thd) and deoxyuridine (d-Urd) 6-11 months after transplantation of 5×10^5 LV-SF-TPco transduced Lin- cells (MOI 10). Data represent median (range).

Table S5. Incidence of hematological abnormalities in secondary recipients of LV transduced cells

LV vector	Number of donor mice	Number of secondary recipients	VCN/cell [a]	BM cell chimerism (%) [b]	Number of vector-positive hematological aberrations [c]	Number of non-vector-positive hematological aberrations [d]	Number of prematurely dead mice or mice sacrificed with high discomfort scores [e]
PGK-GFP [f]	16	32	0.09 (0.03-0.12)	0.25 (0.16-0.4)	0	1 B220+ B cells (86%)	5 (found dead) 5 (wasting) 1 (malocclusion, wasting)
PGK-TP-GFP	6	12	0.52 (0.21-0.86)	30.4 (3-67.3)	2 B220+GFP+ B-cell lymphoma (83%, 85%)	0	1 (found dead) 3 (wasting)
PGK-TP	6	12	0.22 (0.04-16)	2.8 (0.77-100)	0	1 CD4+CD8+ T cells (52%)	2 (malocclusion, wasting) 1 (wasting)
PGK-TPco	6	12	0.12 (0.01-4.4)	2 (0.10-100)	0	1 B220+ B cell- leukemia (85%,WBC=53×10^3/µl)	1 (reactive pathology/ hepatic steatosis observed at autopsy) 2 (wasting) 1 (passive)
SF-TPco	10	20	0.01 (0.0-0.5)	1 (0.1-47.2)	0	6 B220+ B cell- leukemia (*n*=1; 64%, WBC= 57×10^3/µl) CD4+CD8+ T cells (*n*=3; 40%, 68%, 7%) Gr-1+CD11b+ Myeloid cells (*n*=2; 57%, 35%)	1 (found dead) 2 (wasting) 1 (diarrhea, wasting)

Mice were transplanted with LV transduced 5×10^5 BM Lin- cells (MOI 10 or 12) and followed for 8-11 months after transplantation. Two secondary recipients were transplanted with 2×10^5 BM Lin- cells isolated from one primary recipient mouse. [a, b] Vector copy number per cell (VCN/cell) and bone marrow chimerism of all the secondary recipients in each treatment group are presented as median (range), *n*=3-16/ group. [c, d] Hematological aberrations and clonal expansion of transduced cells were assessed based on altered FACS phenotypes and/or elevated WBCs (> 25.0 x 10^3 cells/µl= leukemia), and /or enlarged spleen, thymus or lymph nodes of mice with high discomfort scores. The percentage of the abnormal BM phenotypic cells are indicated between parenthesis. The hematological aberration is vector-positive if both donor cells and VCN were prominent in the recipient's BM. [e] The right column includes mice that were found dead or mice sacrificed prematurely during the experiment due to high discomfort scores and hematological aberrations could not be identified (details are specified between parenthesis). [f] PGK-GFP group include recipient mice of KO or WT Lin- cells transduced by PGK-GFP.

Table S6. Primers used in quantitative PCR (qPCR)

Abbreviation	Forward primer (5'>3')	Reverse primer (5'>3')	Employed for
HIV-1 (U3-Psi)	CTGGAAGGGCTAATTCACTC	GGTTTCCCTTTCGCTTTCAG	Detection of integrated viral copies (VCN/cell)
SRY	CATCGGAGGGCTAAAGTGTCAC	TGGCATGTGGGTTCCTGTCC	Detection of male chromosome (Donor cell chimerism)
Mouse GAPDH	ACGGCAAATTCAACGGCACAG	ACACCAGTAGACTCCACGACATAC	Internal control
WPRE	GAGGAGTTGTGGCCCGTTGT	TGACAGGTGGTGGCAATGCC	WPRE mRNA expression
Mouse mtDNA1978F, 2086R	TGCCTGCCCAGTGACTAAG	GACCCTCGTTTAGCCGTTCA	Quantify mtDNA copynumber
Mouse NDUFV1	ATCCSAGGATCCCACAGAGCT	CCTTTCCAGCAGATGTGGGT	
Mouse mtDNA probe	FAM-TGACCGTGCAAAGGTAGCAT-MGB		
Mouse NDUFV1 probe	VIC-GAGCCTTAGGGAAGAGGCAG-MGB		

Table S7. Antibodies used in this study

Anti-	Clone	Host organism	Vendor	Dilution	WB*/ IHC*
Human TP, 55kDa	P-GF.44C	Mouse	Calbiochem-Merck, GF40	1:250	WB
GAPDH, 36 kDa	6C5	Mouse	Applied Biosystems, AM4300	1:250	WB
Mouse IgG H&L	Polyclonal	Goat	Abcam, Ab97023	1:1000	WB
Mouse PLP	Plpc1	Mouse	AbD Serotec, MCA839G	1:3000	IHC
Mouse MBP	Not specified by vendor	Mouse	Millipore, MAB387	1:50	IHC
Mouse GFAP	Polyclonal	Chicken	Millipore, Ab5541	1:1000	IHC
Mouse B220	RA3.6B2	Rat	Epirus Biopharmaceuticals	1:50	IHC
Mouse IgG	Polyclonal	Goat	Dako, K4000	1:1000	IHC
Rat IgG H&L	Polyclonal	Rabbit	Vector labs, BA-4000	1:150	IHC

*WB, Western blot. *IHC, Immunohistochemistry.