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Supplemental Information

Matching Mitochondrial DNA Haplotypes for Circumventing Tissue-Specific Segregation Bias

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Supplemental Figures and Legends

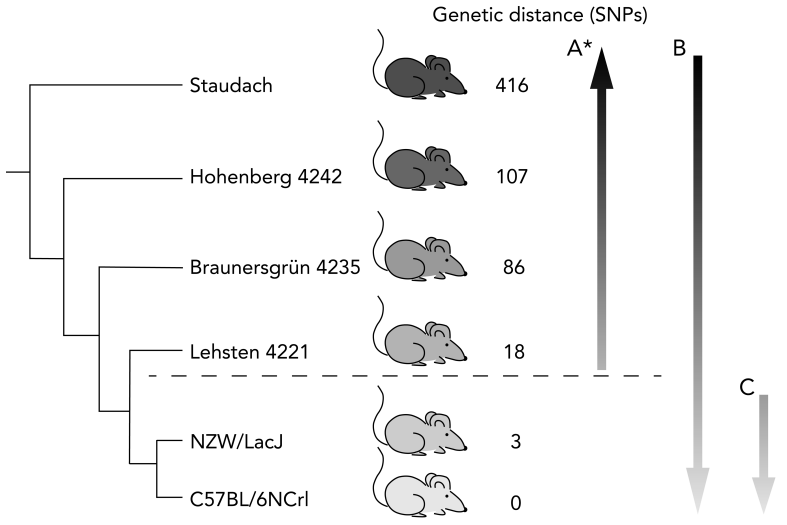

Figure S1. Schematic charts of this study hypothesis: Phylogenetic tree hypothesizes the correlation between tissue segregation of mtDNA and mitochondrial genetic distance, Related to Figure 1.

(A) Segregation increases with the genetic distance between "donor" and "recipient" mtDNA haplotypes. * Referred to Burgstaller et al. Cell Reports 7, 2031–2041, 2014. (B) Hypothesis of this study: shortening genetic distance between "donor" and "recipient" can circumvent the segregation of pathogenic maternal mtDNA. (C) Experiments of this study.

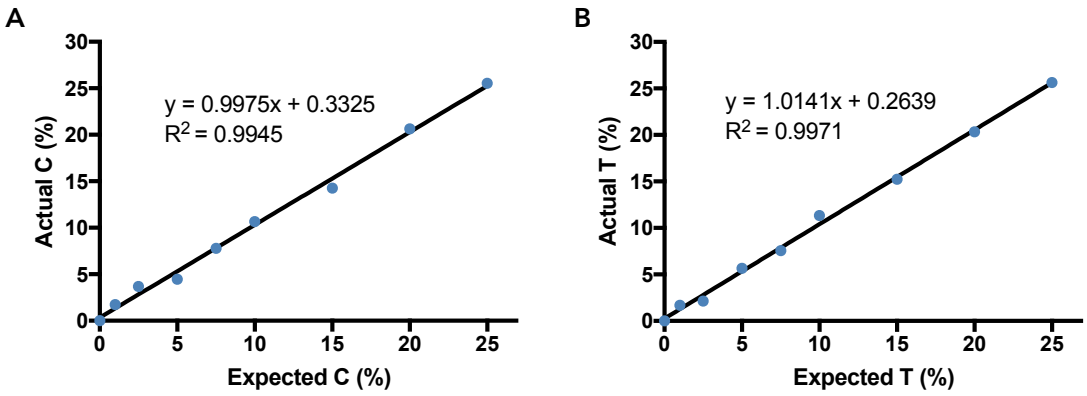

Figure S2. Standard curves for the pyrosequencing assay, Related to Figures 1, 2 and Transparent Methods

(A) Linear relationship between the actual heteroplasmy values and expected heteroplasmy values of SNP "C". (B) Linear relationship between the actual heteroplasmy values and expected heteroplasmy values of SNP "T". The lowest reliable level of heteroplasmy detection is 1%. Each data point indicates the mean of triplicate samples.

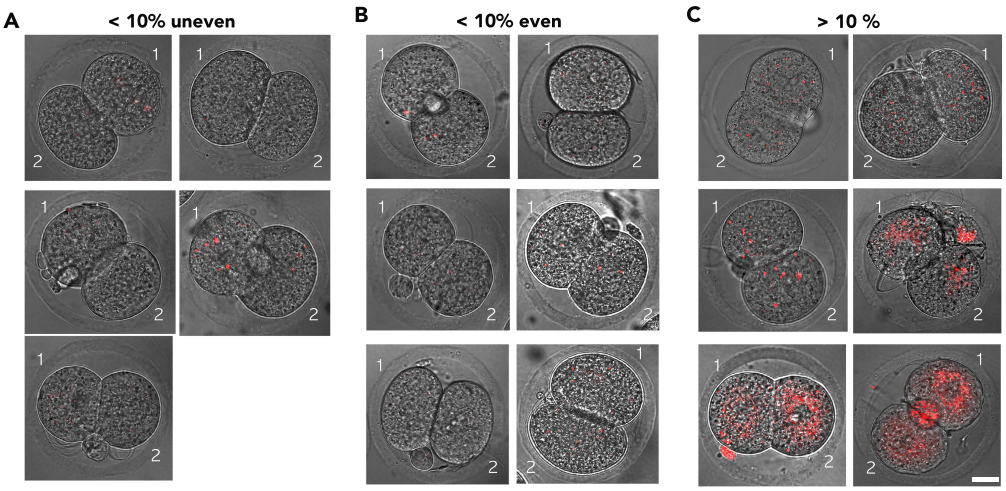

Figure S3. Tracking donor mtDNA distribution in embryos at 2-cell stage, Related to Figure 4
(A) Uneven distribution of donor mtDNA in embryos at 2-cell stage from heteroplsmic oocytes with <10% donor mtDNA. (B) Even distribution of donor mtDNA in embryos at 2-cell stage from heteroplsmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in embryos at 2-cell stage from heteroplsmic oocytes with >10% donor mtDNA. The fourth image in (A) and the third image in (C) (count by row) was also presented in Figure 4B to demonstrate the living cell staining experiment. Scale bar, 40 μm.

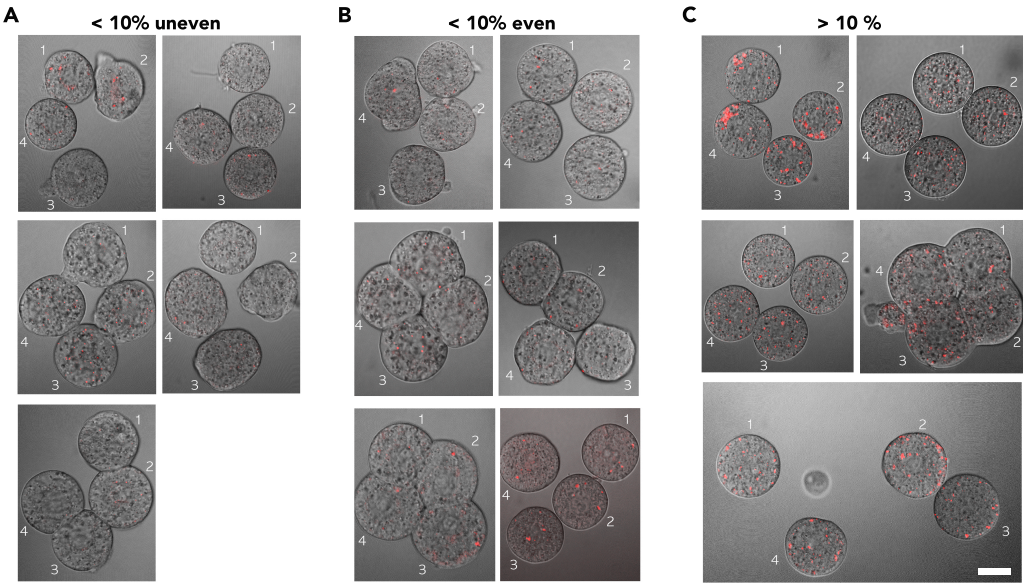

Figure S4. Tracking donor mtDNA distribution in embryos at 4-cell stage, Related to Figure 4 (A) Uneven distribution of donor mtDNA in embryos at 4-cell stage from heteroplsmic oocytes with <10% donor mtDNA. (B) Even distribution of donor mtDNA in embryos at 4-cell stage from heteroplsmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in embryos at 4-cell stage from heteroplsmic oocytes with >10% donor mtDNA. Scale bar, 40 μm.

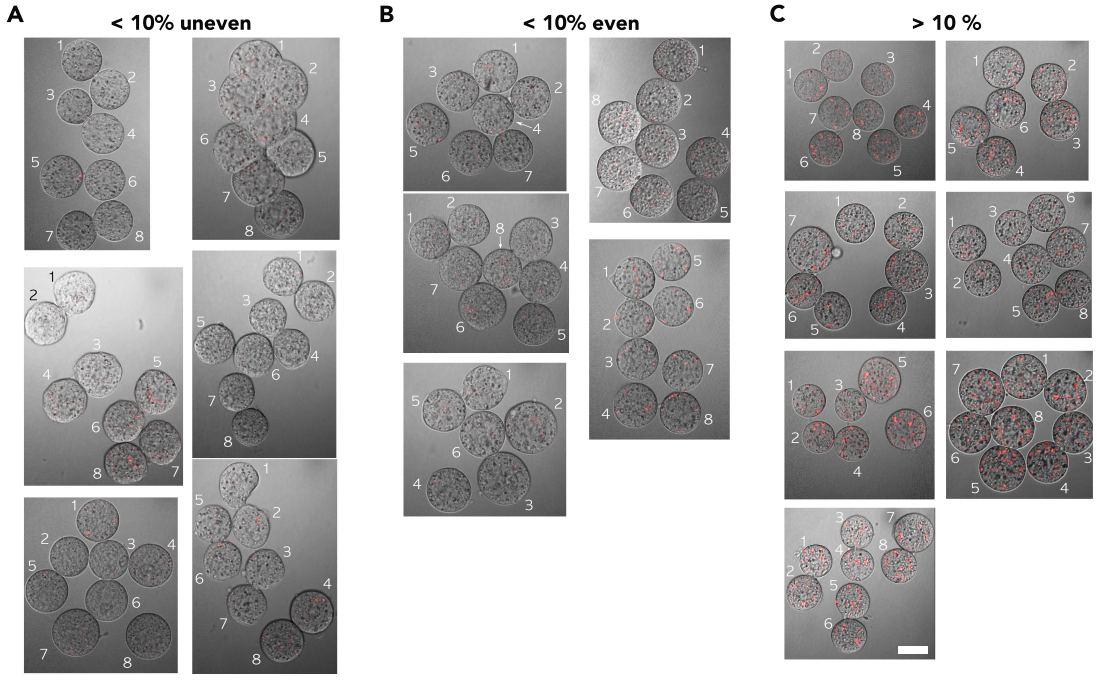

Figure S5. Tracking donor mtDNA distribution in embryos at 8-cell stage, Related to Figure 4
(A) Uneven distribution of donor mtDNA in embryos at 8-cell stage from heteroplsmic oocytes with <10% donor mtDNA. (B) Even distribution of donor mtDNA in embryos at 8-cell stage from heteroplsmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in embryos at 8-cell stage from heteroplsmic oocytes with >10% donor mtDNA. Scale bar, 40 μm.

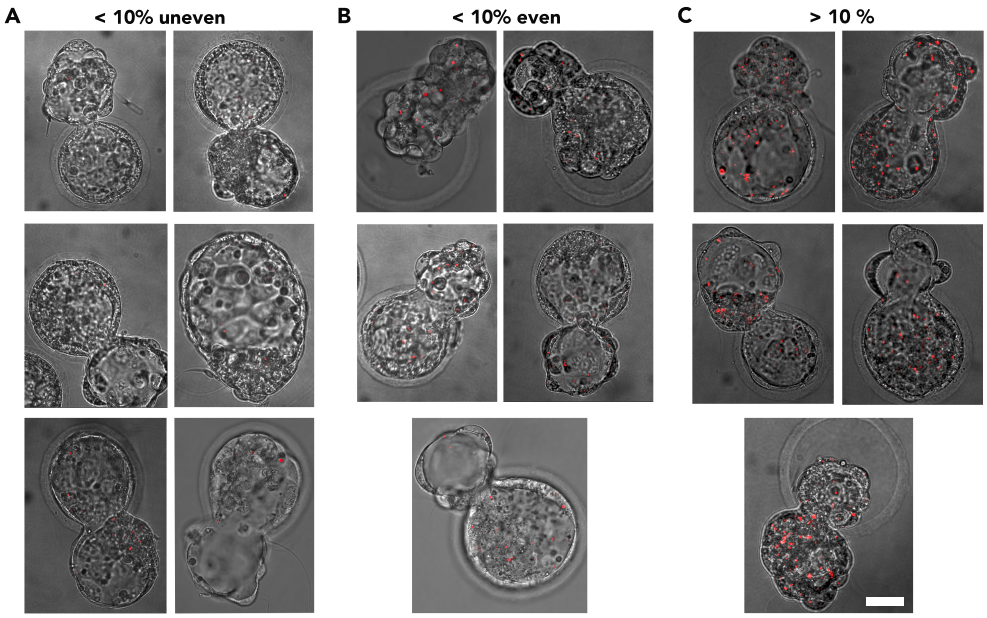

Figure S6. Tracking donor mtDNA distribution in blastocysts, Related to Figure 4

(A) Uneven distribution of donor mtDNA in blastocysts from heteroplsmic oocytes with <10% donor mtDNA. (B) Even distribution of donor mtDNA in blastocysts from heteroplsmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in blastocysts from heteroplsmic oocytes with >10% donor mtDNA. The fourth image in (A) and the second image in (C) (count by row) was also presented in Figure 4B to demonstrate the living cell staining experiment. Scale bar, 40 μm.

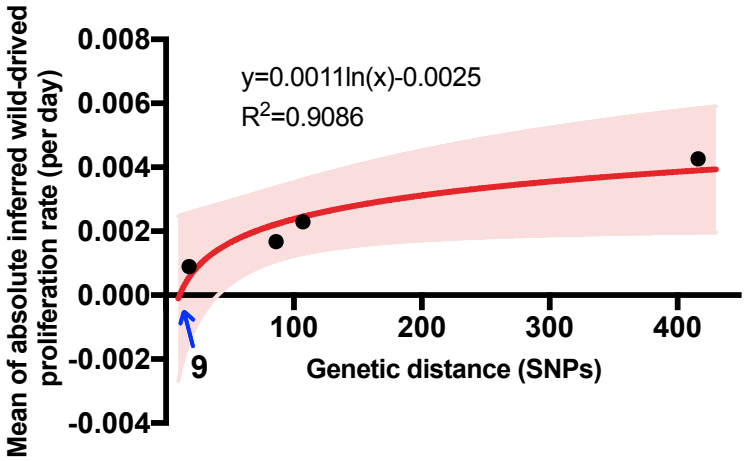

Figure S7. Correlation of proliferation rate of wild-derived mtDNA and genetic distance, Related to Figures 1-4.

The proliferation rate increases as genetic distance of haplotypes rises based on past data (Burgstaller et al., 2014). When the genetic distance is less than 9 SNPs，the anticipated mean of proliferation rate equals to 0. Regression curve and shaded region (red) show curve model fit and 95% confidence intervals.

Supplemental Tables

Table S1. Nucleotide differences between B6D2F1(C57/BL6×DBA) and NZW/Lac J mitochondrial DNA, Related to Figures 1, 2 and Transparent Methods.

Genes	Polymorphism	Sequences
ND3	C9461T	Query 9421 TCCAATTAGTAGATTCTGAATAAACCCAGAAGAGAGTAATCAACCTGTACACTGTTATCT 9480 Sbjct 9421 TCCAATTAGTAGATTCTGAATAAACCCAGAAGAGAGTAATTAACCTGTACACTGTTATCT 9480
tRNA-Arg	A9821-	Query 9781 AAAAAGGATTAGAATGAACAGAGTAAATGGTAATTAGTTTAAAAAAAAATTAATGATTTC 9840 Sbjct 9781 AAAAAGGATTAGAATGAACAGAGTAAATGGTAATTAGTTT-AAAAAAAATTAATGATTTC 9839
ND5	C13053T	Query 13021 ATTTACTTCGTAACAATAACAAAACCGCGTTTCCCCCCCCTAATCTCCATTAACGAAAAT 13080 Sbjct 13020 ATTTACTTCGTAACAATAACAAAACCGCGTTTTCCCCCCCTAATCTCCATTAACGAAAAT 13079

Table S2. Primer sequences and conditions of PCR for mitochondria genome (nucleotide position, 9201-11102) amplification, Related to Figures 1, 2 and Transparent Methods.

Primary PCR	5′-ATGGCTACTGGATTCCATGG-3′ 3′- GCTCCTATGAAGCTTCATGG-5′
Conditions	95°C for 3 min; 40 cycles with denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and elongation at 72°C for 1 min; 1 cycle at 72°C for 7 min; hold at 4°C
Second round PCR	5′-TTTGAAGCCGCAGCATGA-3′ 3′-ATTTATTTGGGGGAGTCAGAATGC-5′
Conditions	95°C for 3 min, 40 cycles with denaturation at 94°C for 30 s, annealing at 53°C for 30 s, and elongation at 72°C for 1 min; 1 cycle at 72°C for 7 min; hold at 4°C

Table S3. Heteroplasmy in adult tissues, Related to Figures 1and 3.

Mouse	Donor mtDNA (%) in adult tissues																Mean (%)	S.D.	V'(h)	Range (%)
	Brain	Heart	Lung	Liver	Kidney	Stomach	Intestine	Spleen	Muscle	Adipose	Skeleton	Bladder	Gonad	Hypoth-alamus	Optic Nerve	Skin				
1	0	2.71	0	3.15	0	3.66	0	0	0	3.81	0	2.2	2.88	0	0	11.29	1.86	0.03	0.0478	11.29
2	6.21	0	6.49	0	0	0	7.16	4.45	2.9	5.66	0	6.36	0	6.96	0	6.13	3.27	0.03	0.0311	7.16
3	0	6.6	4.43	6.13	4.62	6.15	4.32	5.23	4.77	6.6	4.57	5.91	5.44	5.13	6.26	0	4.76	0.02	0.0089	6.60
4	7.67	0	7.58	0	7.69	0	7.29	5.63	4.95	7.05	6.73	7.33	4.94	7.4	5.68	5.96	5.37	0.03	0.0156	7.69
5	7.32	7.02	0	0	8.02	6.97	9.17	5.43	8.49	8.11	7.68	7.22	5.17	7.81	5.28	7.11	6.30	0.03	0.0124	9.17
6	5.58	6.28	4.62	6.43	7.56	7.05	6.12	6.3	6.6	6.14	7.04	5.89	6.06	6.32	6.13	6.78	6.31	0.01	0.0008	2.94
7	8.67	0	8.13	0	8.52	5.48	8.46	6.29	6.53	8.33	6.29	9.16	6.04	9.17	6.48	7.1	6.54	0.03	0.0130	9.17
8	6.85	6.65	6.16	6.28	6.89	5.48	6.18	7.58	5.91	7.28	6.77	7.19	6.73	6.58	8.19	6.87	6.72	0.01	0.0007	2.71
9	5.62	8.73	6.6	7.61	7.4	7.16	0	0	9.31	6.29	9.08	7.05	9.71	8.53	7.94	7.32	6.77	0.03	0.0131	9.71
10	9.07	0	9.23	8.58	8.46	0	8.53	5.8	9.21	8.35	6.27	8.13	6.95	8.93	6.72	7.58	6.99	0.03	0.0132	9.23
11	9.37	5.94	8.8	5.91	8.77	5.49	6.89	6.2	7.43	7.48	6.67	8.69	4.88	7.91	5.93	8.35	7.17	0.01	0.0028	4.49
12	6.53	7.89	5.81	8.56	7.21	8.63	5.63	8.51	7.94	11.04	7.2	8.11	8.73	7.21	7.76	7.19	7.75	0.01	0.0023	5.41
13	7.58	9.7	6.43	7.9	8.68	6.52	8.74	6.35	9.68	6.29	9.02	8.42	8.77	8.31	8.41	10.18	8.19	0.01	0.0021	3.89
14	8.82	10.28	9.08	9.48	8.26	11.47	8.22	10.76	8.54	10.89	9.28	10.19	10.56	9.34	10.28	10.6	9.75	0.01	0.0012	3.25
15	8.43	9.96	9.71	10.82	9.89	12.35	12.96	10.85	10.3	11.35	11.71	7.87	9.74	9.48	10.14	1.94	9.84	0.02	0.0070	11.02
16	10.58	9.81	10.89	8.75	9.33	9.99	9.32	10.08	10.77	10.85	9.32	10.16	9.09	9.26	10.62	10.65	9.97	0.01	0.0006	2.14
17	11.18	9.47	10.08	9.77	11.23	7.82	12.68	9.84	9.12	11.67	11.06	12.06	7.85	11.26	8.82	13.08	10.44	0.02	0.0027	5.26
18	10.54	13.33	11.6	13.38	12.17	10.86	14.34	11.78	13.41	11.34	12.04	11.63	12.7	11.65	12.5	11.17	12.15	0.01	0.0010	3.80
19	12.06	12.18	11.75	14.36	12.68	11.25	14.81	12.56	12.96	12.88	14.03	11.03	15.03	14.6	13.02	10.21	12.84	0.01	0.0018	4.82

20	14.99	11.9	15.12	10.83	13.84	10.48	14.02	11.85	13.96	15.61	12.38	15.12	12.24	14.04	11.67	11.81	13.12	0.02	0.0024	5.13
21	15.19	14.63	13.61	10.79	14.4	9.61	13.89	12.5	13.28	12.68	12.6	13.14	12.45	14.42	13.62	16.36	13.32	0.02	0.0023	6.75
22	13.71	13.37	14.69	12.46	15.19	11.97	14.43	13.72	11.95	13.86	12.29	15.03	11.18	15.74	11.94	14.39	13.50	0.01	0.0016	4.56
23	15.68	14.45	15.66	11.47	15.34	13.22	15.58	14.37	16.69	15.71	14.79	16.49	15.4	16.45	13.24	17.09	15.10	0.01	0.0017	5.62
24	14.23	16	15	16.38	14.87	13.33	14.71	12.37	15.47	13.16	16.76	15.46	17.76	15.68	15.51	16.67	15.21	0.01	0.0016	5.39
25	15.32	15.9	14.48	14	14.42	17.34	14.56	15.57	13.92	15.38	15.45	16.69	16.9	14.66	15.21	15.33	15.32	0.01	0.0008	3.42
26	14.93	16.68	14.36	13.73	16.79	15.9	15.03	15.74	17.14	14.6	16.85	15.46	16.89	15.89	17.32	15.06	15.77	0.01	0.0009	3.59
27	16.62	19.69	17.74	16.46	17.53	17.68	18.92	17.33	17.25	17.58	19.03	18.98	19.02	19.93	18.37	16.93	18.07	0.01	0.0008	3.47
28	21.24	20.76	23.06	17.67	20.71	21.88	23.19	19.06	19.14	20.73	20.38	19.76	18.42	21.38	18.55	21.9	20.49	0.02	0.0016	5.52
29	19.91	21.99	21.29	23.08	23.94	18.38	25.25	20.39	21.26	19.3	22.02	21.65	22.76	21.75	23.28	15.81	21.38	0.02	0.0031	9.44
30	21.58	21.38	22.58	20.83	20.44	21.63	21.66	22.1	21.87	23.86	21.4	22.56	22.55	19.13	21.87	21.94	21.71	0.01	0.0006	4.73
31	23.64	25.23	24.62	22.35	25.8	24.68	24.42	23.84	26.53	24.86	21.92	23	24	21.78	24.7	17.87	23.70	0.02	0.0023	8.66
32	24.14	24.22	26.75	22.79	27.03	23.91	24.93	22.59	25.19	23.52	23.06	23.22	21.92	24.1	22.02	25.26	24.04	0.02	0.0012	5.11
33	24.3	27.65	25.87	26.5	26.72	23.97	24.95	24.48	23.23	24.86	24.11	24.01	24.44	21.89	23.68	22.48	24.57	0.02	0.0013	5.76
34	27.33	27.69	26.11	25.77	29.92	28.17	27.99	26.05	27.02	25.81	25.35	26.32	23.94	28.21	26.55	26.04	26.77	0.01	0.0010	5.98
35	32.54	33.41	31.53	34.13	32.01	34	32.58	33.91	32.94	28.67	33.46	33.89	33.49	32.36	33.82	35.58	33.02	0.02	0.0010	6.91
36	35.71	33.56	33.57	32.39	36.79	32.72	36.72	32.23	36.15	32.31	36.23	36.85	35.31	34.27	36.31	34.81	34.75	0.02	0.0013	4.62
37	39.3	40	37.37	40.72	35.73	41.9	36.39	39.04	33.79	37.16	36.58	39.05	39.28	39.01	38.91	35.91	38.13	0.02	0.0019	8.11
Mean	14.39	14.19	14.35	13.50	14.83	13.71	14.87	13.80	14.48	14.62	14.31	14.90	14.30	14.77	14.24	14.34	14.35	0.02	0.0056	6.01

S.D., standard deviation; V'(h), normalised variance; Range, differences between maximum and minimum values among 16 adult tissues.

Table S4. Heteroplasmy in blastomeres of 2 cell embryos, Related to Figures 2and 3.

Embryo	Donor mtDNA (%) in blastomeres		Mean (%)	S.D.	V'(h)	Range (%)
	1	2				
1	3.89	0.00	1.95	0.02	0.0198	3.89
2	5.86	0.00	2.93	0.03	0.0302	5.86
3	6.04	0.00	3.02	0.03	0.0311	6.04
4	2.23	4.71	3.47	0.01	0.0046	2.48
5	4.16	4.92	4.54	0.00	0.0003	0.76
6	8.74	6.08	7.41	0.01	0.0026	2.66
7	5.58	9.97	7.78	0.02	0.0067	4.39
8	9.52	10.05	9.79	0.00	0.0001	0.53
9	10.93	8.79	9.86	0.01	0.0013	2.14
10	8.79	12.53	10.66	0.02	0.0037	3.74
11	15.39	15.39	15.39	0.00	0.0000	0.00
12	14.88	16.09	15.49	0.01	0.0003	1.21
13	15.24	15.91	15.58	0.00	0.0001	0.67
14	16.85	14.45	15.65	0.01	0.0011	2.40
15	23.41	8.91	16.16	0.07	0.0388	14.50
16	18.66	16.38	17.52	0.01	0.0009	2.28
17	18.99	16.74	17.87	0.01	0.0009	2.25
18	19.88	20.58	20.23	0.00	0.0001	0.70
19	23.05	20.56	21.81	0.01	0.0009	2.49
20	23.05	20.56	21.81	0.01	0.0009	2.49
21	21.80	26.14	23.97	0.02	0.0026	4.34
22	26.86	24.23	25.55	0.01	0.0009	2.63
23	25.22	27.33	26.28	0.01	0.0006	2.11
24	27.84	28.06	27.95	0.00	0.0000	0.22
25	29.10	28.52	28.81	0.00	0.0000	0.58
26	37.43	41.82	39.63	0.02	0.0020	4.39

S.D., standard deviation; V'(h), normalised variance; Range, differences between maximum and minimum values in daughter blastomeres.

Table S5. Heteroplasmy in blastomeres of 3-4 cell embryos, Related to Figures 2 and 3.

Embryo	Donor mtDNA (%) in blastomeres				Mean (%)	S.D.	V'(h)	Range (%)
	1	2	3	4				
1	0.00	3.71	0.00	3.51	1.81	0.02	0.0184	3.71
2	3.99	0.00	3.24	0.00	1.81	0.02	0.0188	3.99
3	0.00	3.85	0.00	3.53	1.85	0.02	0.0189	3.85
4	3.12	0.00	4.86	0.00	2.00	0.02	0.0223	4.86
5	4.86	0.00	3.77	0.00	2.16	0.02	0.0228	4.86
6	5.69	0.00	0.00	4.63	2.58	0.03	0.0270	5.69
7	9.21	0.00	0.00	2.14	2.84	0.04	0.0519	9.21
8	6.17	0.00	5.55	0.00	2.93	0.03	0.0304	6.17
9	3.88	3.16	3.55	3.20	3.45	0.00	0.0003	0.72
10	5.51	4.77	5.08	0.00	3.84	0.02	0.0135	5.51
11	5.21	0.00	3.93	6.60	3.94	0.02	0.0160	6.60
12	4.51	4.18	4.06	5.50	4.56	0.01	0.0007	1.44
13	7.41	5.60	6.74	0.00	4.94	0.03	0.0182	7.41
14	6.95	6.91	7.88	5.21	6.74	0.01	0.0015	2.67
15	4.42	12.00	5.24	8.14	7.45	0.03	0.0128	7.58
16	11.46	9.68	12.70	0.00	8.46	0.05	0.0323	12.70
17	8.60	9.67	6.65	9.55	8.62	0.01	0.0019	3.02
18	9.83	7.41	9.66	8.82	8.93	0.01	0.0011	2.42
19	8.39	10.72	7.60	11.50	9.55	0.02	0.0030	3.90
20	11.25	10.20	8.54	8.50	9.62	0.01	0.0016	2.75
21	12.13	7.29	10.46	9.81	9.92	0.02	0.0034	4.84
22	10.52	8.07	11.72	10.98	10.32	0.01	0.0020	3.65
23	11.52	8.90	12.15	9.32	10.47	0.01	0.0021	3.25
24	12.65	14.00	9.52	9.75	11.48	0.02	0.0036	4.48
25	10.99	12.21	12.29	11.44	11.73	0.01	0.0003	1.30
26	13.98	13.94	11.81	NA	13.24	0.01	0.0009	2.17
27	14.95	10.79	14.14	14.86	13.69	0.02	0.0024	4.16
28	13.14	13.76	15.16	15.08	14.29	0.01	0.0006	2.02
29	14.56	14.03	14.42	14.72	14.43	0.00	0.0001	0.69
30	14.51	14.77	14.88	NA	14.72	0.00	0.0000	0.37
31	9.94	16.07	17.84	15.91	14.94	0.03	0.0070	7.90

32	15.94	17.26	14.24	NA	15.81	0.01	0.0011	3.02
33	15.35	16.68	16.33	16.81	16.29	0.01	0.0002	1.46
34	19.22	16.09	15.85	18.64	17.45	0.01	0.0016	3.37
35	17.19	20.44	17.33	NA	18.32	0.02	0.0015	3.25
36	21.89	22.20	19.28	21.59	21.24	0.01	0.0008	2.92
37	21.59	23.76	20.86	24.63	22.71	0.02	0.0013	3.77
38	22.40	24.94	23.24	22.01	23.15	0.01	0.0007	2.93
39	27.86	24.13	25.89	29.14	26.76	0.02	0.0019	5.01
40	22.63	33.66	33.71	NA	30.00	0.05	0.0129	11.08
41	56.28	50.94	54.14	53.23	53.65	0.02	0.0015	5.34
42	54.39	50.12	55.90	56.53	54.24	0.02	0.0025	6.41

S.D, standard deviation; CV, coefficient of variation; Range, differences between maximum and minimum values in daughter blastomeres; NA, none applicable.

Table S6. Heteroplasmy in blastomeres of 6-8 cell embryos, Related to Figures 2 and 3.

Embryo	Donor mtDNA (%) in blastomeres								Mean (%)	S.D.	V'(h)	Range (%)
	1	2	3	4	5	6	7	8				
1	4.25	0	4.79	0	3.5	0	3.29	4.7	2.57	0.02	0.0167	4.79
2	4.54	8.83	0.00	6.90	0.00	3.21	0.00	1.90	3.17	0.03	0.0324	8.83
3	3.85	7.67	6.68	3.57	3.66	0	2.79	1.76	3.75	0.02	0.0149	7.67
4	0	0	0	3.81	7.46	8.7	5.59	5.08	3.83	0.03	0.0290	8.70
5	21.04	0	3.8	0	0	0	NA	NA	4.14	0.08	0.1488	21.04
6	0	0.00	0.00	2.97	0.00	3.10	3.17	39.83	6.13	0.13	0.2853	39.83
7	7.42	5.69	6.59	5.27	6.08	5.93	7.12	5.24	6.17	0.01	0.0010	2.18
8	10.92	0	7.34	0	11.78	5.69	8.24	8.19	6.52	0.04	0.0285	11.78
9	6.75	7.80	6.34	7.06	9.10	6.92	10.02	NA	7.71	0.01	0.0022	3.68
10	8.96	9.59	7.28	10.6	7.33	9.37	7.47	NA	8.66	0.01	0.0019	3.32
11	5.75	7.73	6.33	8.76	12.54	11.08	14.15	7.28	9.20	0.03	0.0098	8.40
12	7.11	9.21	12.77	12.97	5.15	11.98	NA	NA	9.87	0.03	0.0099	7.82
13	15.49	8.13	8.63	16.99	7.61	5.49	7.27	NA	9.94	0.04	0.0188	11.50
14	8.69	8.36	11.03	8.96	11.65	9.81	10.72	11.37	10.07	0.01	0.0016	3.29
15	18.62	20.95	11.41	6.55	5.59	5.11	NA	NA	11.37	0.06	0.0397	15.84
16	8.13	11.01	6.58	9.26	3.97	12.68	22.66	25.32	12.45	0.07	0.0467	21.35
17	13.95	12.35	12.82	13.84	13.76	12.99	12.32	12.34	13.05	0.01	0.0004	1.63
18	16.93	8.46	12.81	13.43	14.14	14.71	11.98	14.17	13.33	0.02	0.0045	8.47
19	12.84	15.03	17.72	16.17	14.37	16.07	15.32	21.30	16.10	0.02	0.0042	8.46
20	11.42	12.90	37.15	11.67	14.84	13.27	12.91	NA	16.31	0.09	0.0538	25.73
21	20.79	19.75	19.19	18.59	14.96	15.09	15.37	12.94	17.09	0.03	0.0050	7.85
22	17.46	15.58	16.81	16.18	17.81	19.19	NA	NA	17.17	0.01	0.0010	3.61
23	18.18	21.37	18.56	16.3	18.6	17.07	15.92	NA	18	0.02	0.0019	5.45
24	12.06	29.32	19.8	14.68	22.81	22.12	24.61	15.88	20.16	0.05	0.0178	17.26
25	21.16	27.53	20.11	22.74	23.09	22.51	20.3	28.19	23.20	0.03	0.0047	8.08
26	29.51	32.13	27.42	28.24	26.86	28.11	25.35	NA	28.23	0.02	0.0020	6.78
27	29.25	33.07	27.62	29.24	27.64	31.90	29.92	19.12	28.47	0.04	0.0077	13.95
28	33.84	38.34	34.65	34.17	36.28	34.54	35.97	37.34	35.64	0.02	0.0010	4.50
29	36.33	47.29	37.67	33.27	34	33.25	NA	NA	36.97	0.05	0.0103	14.04
30	35.13	34.98	37.57	36.67	39.36	37.55	38.19	NA	37.06	0.01	0.0009	4.38
31	47.09	37.87	41.8	38.35	42.21	44.99	39.29	NA	41.66	0.03	0.0042	9.22

32	49.7	41.29	50.21	44.04	46.5	44.37	45.76	NA	45.98	0.03	0.0035	8.92
33	51.34	50.6	51.45	51.56	52.15	54.36	NA	NA	51.91	0.01	0.0006	3.76

S.D, standard deviation; CV, coefficient of variation; Range, differences between maximum and minimum values in daughter blastomeres; NA, none applicable.

Table S7. Fluorescence intensity in blastomeres of 2 cell embryos, Related to Figure 4.

Proportion of mitotracker	Embryo	Fluorescence intensity in blastomeres		Proportion		Variance
		1	2	1	2	
<10% uneven	1	44770	7297	0.86	0.14	0.0198
	2	7302	3887	0.65	0.35	0.0302
	3	27181	0	1.00	0.00	0.0311
	4	170043	32531	0.84	0.16	0.0046
	5	76730	16188	0.83	0.17	0.0003
<10% even	1	35819	33211	0.52	0.48	0.0026
	2	29913	14320	0.68	0.32	0.0067
	3	17629	29768	0.37	0.63	0.0001
	4	54076	49367	0.52	0.48	0.0013
	5	30674	33145	0.48	0.52	0.0037
	6	92391	104795	0.47	0.53	0.0000
>10%	1	147731	106206	0.58	0.42	0.0003
	2	233691	174094	0.57	0.43	0.0001
	3	190303	260991	0.42	0.58	0.0011
	4	592428	338429	0.64	0.36	0.0388
	5	1422731	2361465	0.38	0.62	0.0009
	6	1359749	672487	0.67	0.33	0.0009

Proportion, the ratio of each blastomere fluorescence intensity to total intensity; variance, variance of proportion.

Table S8. Fluorescence intensity in blastomeres of 4 cell embryos, Related to Figure 4.

Proportion of mitotracker	Embryo	Fluorescence intensity in blastomeres				Proportion				Variance
		1	2	3	4	1	2	3	4	
<10% uneven	1	150041	128995	0	70537	0.43	0.37	0.00	0.20	0.0278
	2	8448	2662	66156	44323	0.07	0.02	0.54	0.36	0.0461
	3	25738	153750	181180	94722	0.06	0.34	0.40	0.21	0.0172
	4	4180	0	26384	41607	0.06	0.00	0.37	0.58	0.0548
	5	13082	57861	4806	3752	0.16	0.73	0.06	0.05	0.0782
<10% even	1	24502	38983	10516	69138	0.17	0.27	0.07	0.48	0.0230
	2	66231	44487	28675	41905	0.37	0.25	0.16	0.23	0.0055
	3	114208	44697	102938	39832	0.38	0.15	0.34	0.13	0.0123
	4	33237	46103	33116	42960	0.21	0.30	0.21	0.28	0.0014
	5	28904	48390	113791	39221	0.13	0.21	0.49	0.17	0.0208
	6	88670	107586	111489	95982	0.22	0.27	0.28	0.24	0.0005
>10%	1	590333	638842	579461	806561	0.23	0.24	0.22	0.31	0.0012
	2	335749	290522	267510	231713	0.30	0.26	0.24	0.21	0.0011
	3	232264	263602	290182	312649	0.21	0.24	0.26	0.28	0.0007
	4	185487	151531	414783	391565	0.16	0.13	0.36	0.34	0.0107
	5	648333	1015563	368786	522352	0.25	0.40	0.14	0.20	0.0088

Proportion, the ratio of each blastomere fluorescence intensity to total intensity; variance, variance of proportion.

Table S9. Fluorescence intensity in blastomeres of 6-8 cell embryos, Related to Figure 4.

Proportion of mitotracker	Embryo	Fluorescence intensity in blastomeres								Proportion								Variance
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	
<10% uneven	1	3039	3698	1537	9627	25819	8369	4377	1355	0.05	0.06	0.03	0.17	0.45	0.14	0.08	0.02	0.0171
	2	24950	1618	2611	28760	0	6518	1116	5436	0.35	0.02	0.04	0.41	0.00	0.09	0.02	0.08	0.0224
	3	0	0	10006	14533	73739	56436	7347	77209	0.00	0.00	0.04	0.06	0.31	0.24	0.03	0.32	0.0170
	4	14540	19066	7499	22147	13967	13924	0	0	0.16	0.21	0.08	0.24	0.15	0.15	0.00	0.00	0.0071
	5	40895	1619	3880	22006	6828	2726	11442	0	0.46	0.02	0.04	0.25	0.08	0.03	0.13	0.00	0.0213
	6	14468	50243	10369	39879	26394	45449	6960	24044	0.01	0.22	0.03	0.23	0.17	0.04	0.23	0.07	0.0082
<10% even	1	1636	38848	5745	40990	31144	6836	41016	12283	0.31	0.11	0.03	0.13	0.30	0.02	0.10	NA	0.0122
	2	34253	22860	44841	45996	11659	54296	20628	72583	0.11	0.07	0.15	0.15	0.04	0.18	0.07	0.24	0.0037
	3	36344	12701	3702	15010	34763	2107	10986	NA	0.16	0.11	0.09	0.04	0.08	0.17	0.12	0.22	0.0028
	4	34253	22860	44841	45996	11659	54296	20628	72583	0.14	0.36	0.07	0.12	0.16	0.16	NA	NA	0.0081
	5	32328	22743	18416	8944	16369	34701	23669	44728	0.16	0.10	0.12	0.10	0.20	0.11	0.11	0.11	0.0012
>10%	1	14012	35680	6909	12183	15539	15757	NA	NA	0.17	0.09	0.10	0.14	0.11	0.16	0.11	0.11	0.0007
	2	115035	68640	83185	75013	144522	77619	76613	75835	0.15	0.13	0.18	0.18	0.21	0.14	NA	NA	0.0009
	3	136937	76765	86675	115616	91588	134712	91084	94228	0.12	0.17	0.14	0.09	0.08	0.18	0.23	NA	0.0024
	4	178010	148108	210702	214750	249067	161807	NA	NA	0.14	0.08	0.11	0.13	0.14	0.11	0.18	0.09	0.0009
	5	133526	181106	153246	92104	81988	189512	241635	NA	0.13	0.11	0.08	0.10	0.32	0.26	NA	NA	0.0083
	6	101428	56838	80342	95025	97152	79724	128508	64966	0.11	0.14	0.10	0.09	0.17	0.07	0.17	0.14	0.0014
	7	118481	107027	75974	92444	305334	245952	NA	NA	0.13	0.15	0.09	0.11	0.12	0.09	0.13	0.19	0.0009

Proportion, the ratio of each blastomere fluorescence intensity to total intensity; variance, variance of proportion; NA, none applicable.

Table S10. Fluorescence intensity in regions of blastocyst, Related to Figure 4.

Proportion of mitotracker	embryo	Fluorescence intensity in regions of blastocyst										Proportion										Variance
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	
<10% uneven	1	0	3419	0	0	0	0	0	0	0	0	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0900
	2	0	5555	0	0	0	0	0	4592	4828	8172	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.20	0.21	0.35	0.0165
	3	0	0	0	0	0	2447	7167	0	0	0	0.00	0.00	0.00	0.00	0.00	0.25	0.75	0.00	0.00	0.00	0.0521
	4	0	0	7753	0	0	1188	0	0	9451	0	0.00	0.00	0.42	0.00	0.00	0.06	0.00	0.00	0.51	0.00	0.0346
	5	7223	5301	0	0	0	0	0	5918	22981	0	0.17	0.13	0.00	0.00	0.00	0.00	0.00	0.14	0.55	0.00	0.0275
	6	7756	0	0	3556	0	23785	0	0	0	0	0.22	0.00	0.00	0.10	0.00	0.68	0.00	0.00	0.00	0.00	0.0418
<10% even	1	12377	24569	44108	19765	2648	2444	5591	393	7186	0	0.10	0.21	0.37	0.17	0.02	0.02	0.05	0.00	0.06	0.00	0.0125
	2	7303	10915	16585	22295	6898	9332	6742	3854	0	0	0.09	0.13	0.20	0.27	0.08	0.11	0.08	0.05	0.00	0.00	0.0062
	3	23954	18478	0	10546	0	0	10900	7836	23400	2398	0.25	0.19	0.00	0.11	0.00	0.00	0.11	0.08	0.24	0.02	0.0085
	4	0	14054	9787	21325	3070	7415	7877	11165	6195	8866	0.00	0.16	0.11	0.24	0.03	0.08	0.09	0.12	0.07	0.10	0.0039
	5	0	0	20183	46521	1807	9458	0	6461	26690	21930	0.00	0.00	0.15	0.35	0.01	0.07	0.00	0.05	0.20	0.16	0.0120
>10%	1	14657	11743	27500	67664	124327	19723	22254	23700	14729	51855	0.04	0.03	0.07	0.18	0.33	0.05	0.06	0.06	0.04	0.14	0.0078
	2	90847	1925	26203	63079	54377	61617	74529	31476	62918	34358	0.18	0.00	0.05	0.13	0.11	0.12	0.15	0.06	0.13	0.07	0.0025
	3	32887	21227	24335	14613	40121	20612	69636	15484	46936	15639	0.11	0.07	0.08	0.05	0.13	0.07	0.23	0.05	0.16	0.05	0.0031
	4	19699	42001	6231	3064	13044	20212	8052	0	20484	2093	0.15	0.31	0.05	0.02	0.10	0.15	0.06	0.00	0.15	0.02	0.0080
	5	23139	20390	173049	186452	23889	1991	55685	18418	82425	59600	0.04	0.03	0.27	0.29	0.04	0.00	0.09	0.03	0.13	0.09	0.0092

Proportion, the ratio of each blastomere fluorescence intensity to total intensity; variance, variance of proportion.

Transparent Methods

Animals breeding scheme and ethics statement

Mitochondria replacement founders were generated from our lab between female NZW/LacJ (NZW) and female BDF1 from C57/BL6×DBA (C57) during the past study (Wang et al., 2014). Then the female founders were mated with male mice (BDF1) to reproduce heteroplasmic mice for this study. All mice used in this study were maintained in accordance with the guidelines of the Laboratory Animal Service, Fudan University (research license 20160225-103).

Generation of heteroplasmic standard samples

Whole genomic DNA of C57 and NZW were extracted from liver. Then the region of mitochondria genome (nucleotide position, 9201-11102) was amplified using primers of primary PCR and condition in Table S2. PCR products were cloned using the pMD18-T Vector System (Takara) according to the manufacturer's instructions. Plasmid DNA was isolated using QIAGEN Plasmid Kit. The DNA concentration was determined by Quantitative real-time PCR using ViiA 7 Real-Time PCR System (Applied Biosystems) with primers of primary PCR and condition in Table S2. Equimolar concentrations of mtDNA with C57 and NZW genotypes were combined in varying ratios to generate gradient standard samples, ranging from 0 to 25%.

Genotyping of donor mtDNA Heteroplasmy Level of embryonic blastomeres and adult tissues

Zona pellucida of embryos at 2-cell, 4-cell, and 8-cell stage was digested by brief exposure to 0.5% of pronase (Roche, 70229227), 37°C, 5min. Then blastomeres of the denuded embryos were disaggregated by brief exposure to trypsin-EDTA, 37°C, 5min. The single blastomere was lysised into a 0.2 ml PCR tube containing 4 μl of PBS. Add 3 μl buffer D2 and incubate at 65 °C for 10 min, followed by adding 3 μl stop solution. Then whole genomic DNA from single blastomere was amplified using REPLI-g Single Cell Kit (QIAGEN, 150345).

Whole genomic DNA of heteroplasmic mice (6~8 months old) were isolated using DNA extracted from brain, heart, lung, liver, spleen, bone, bladder, gonad, pituitary, skin, optic nerve, stomach, intestine, fat, muscle, and kidney using Genomic DNA Kit (Tiangen).

To determine the distribution of donor mtDNA in pre-implanted embryos and adult tissues, the region of mice mitochondria genome (nucleotide position, 9201-11102) was amplified from total genome of single blastomere and tissue using the primary PCR. The primer sequences and conditions were seen in Table S2. PCR was performed using ABI cycler. The SNP used for detecting heteroplasmy is m.9461C>T (Table S1) and confirmed by pyrosequencing. Detailed methods for pyrosequencing were processed according to the previously described methods (Wang et al., 2014). The second round PCR sequences and conditions were seen in Table S2. Single-stranded biotinylated PCR products were prepared for sequencing by Pyrosequencing Vacuum Prep Tool (Biotage AB) according the protocol of PyroMark Q96 ID platform (Qiagen). Primer used for pyrosequencing was 5′-GAATAAACCCAGAAGAGAGT-3′. Quantification of the donor mtDNA heteroplasmy level of variant m.9461C>T was performed using allele frequency quantification (AQ) function in PyroQ-AQ software (Wang et al., 2014). A standard curve was generated by linking expected heteroplasmy values and actual heteroplasmy values for the gradient standard samples.

Mathematical analysis to predict the genetic distance related to segregation

The mathematical model was created to describe the relationship between the proliferation rate

of donor mtDNA (calculated as mean of absolute inferred wild-derived proliferation rate) and genetic distance based upon past data (Burgstaller et al., 2014a). Logarithmic fitting on their 4 sets of data was performed to get the following expression:

$$r(d)=\begin{cases} 0 & 0\le d\le 9 \\ 0.00111\ln(d)-0.0025 & d\ge 10 \end{cases}$$

r was defined as the mean proliferation rate of donor mtDNA and d as the genetic distance (SNPs) of mtDNA between haplotypes. This model is used to find the maximum genetic distance before segregation bias takes effect and predict the proliferation rate at a certain genetic distance (Figure S7).

Heteroplasmic oocytes construction by spindle-chromosome complex transfer (spindle transfer)

The lyophilized MitoTracker Red CMXRos (M7512, Life Technology) was dissolved in high-quality, anhydrous dimethylsulfoxide (DMSO) to a final concentration of 1 mM to prepare a stock solution. Then stock solution was diluted to 250nM concentration (working solution) in G1 medium. Donor mouse oocytes were dyed with 250nM MitoTracker. Then Spindle-chromosome complex with different amount of red mitochondria were transferred into enucleated oocyte containing unstained mitochondria (Wang et al., 2014). All manipulations were performed on a 37 °C -heated stage (Tokai Hit) of a Nikon TE 2000S inverted microscope equipped with Narishige micromanipulators, a laser objective and an Oosight™ Imaging System. Stained oocytes and unstained oocytes were placed into manipulation droplets of G-gamete containing CB in a glass-bottom dish.

An unstained oocyte was suctioned with the holding pipette, so that the spindle was located in the 3 o'clock position. The zona pellucida close to the spindle was drilled with a laser, and an enucleation pipette was then inserted through the hole in the zona pellucida. The spindle was enucleated with a minimal amount of red mitochondria, and the enucleated oocyte was released into manipulation medium. Then the spindle-chromosome complex of stained oocyte was enucleated as the same to the unstained oocyte with a diameter of 12 μm pipette and then transferred to the HVJ-E (inactivated Hemagglutinating Virus of Japan envelope, GenomeOne, Cosmo Bio) drop for brief exposure. The enucleated spindle-chromosome complex (red) was slowly moved to the end of the pipette and a suction force was made to take up a small amount of HVJ-E into the pipette. The recipient oocyte was immobilized so that the drilled hole of the zona was at 3 o'clock position. The red spindle-chromosome complex was gently released from the pipette and transferred into the enucleated recipient oocyte. After that, the reconstructed oocytes were briefly left in the manipulation drop to allow the fusion of the red spindle-chromosome complex and the recipient oocyte for 10-20 min. After fusion, the reconstructed oocytes were washed several times and placed in HTF medium for recovery and *in vitro* fertilization. See also Video S1.

***In Vitro* Fertilization and culture after spindle transfer**

Male mice (BDF1, 10–15 weeks) were sacrificed by cervical dislocation. After dissections, sperms were incubated for sperm capacitation in HTF medium at 37.5°C under 5% CO_2, 5% O_2, incubation for 1 h. Then 2 × 10^6 sperms/ml were added into the HTF drops containing the heteroplasmic oocytes after spindle transfer. The sperms and heteroplasmic oocytes were co-cultured at 37.5°C for at least 4~6 hrs. Then heteroplasmic oocytes were washed three times in G1 medium. Oocytes with two visible pronuclei (2PN) were considered fertilized and transferred into G1 medium (100 μl drop) and cultured up to 72 hours at 37.5 °C under 5% CO_2,

5% O_2, and 90% N_2 incubation.

Living cell imaging of the whole embryos and its single blastomere developed from heteroplasmic oocytes

To visualize the distribution of foreign mitochondria upon division, embryos developed from heteroplasmy oocytes were transferred to a 35mm glass bottom dish for mitoTracker analysis (Leica Microsystems, Inc.). Fluorescent images were obtained at 5-μm Z-axis intervals under confocal microscope.

To quantify and explore the distribution of stained mitochondria in daughter blastomeres, blastomeres of the reconstructed embryos were disaggregated by brief exposure to 0.5% pronase and 0.05% trypsin-EDTA. Then all blastomeres of a whole embryo were transferred to a 35mm glass bottom dish for analyzing distribution of stained mitochondria. Fluorescent images were obtained at 2-μm Z-axis intervals under confocal microscope. The relative fluorescent signals of blastomeres were determined using the Leica Application Suite-Advanced Fluorescence software.

Statistical Analysis

Statistical analysis of mtDNA segregation was performed using GraphPad Prism 7. The normalised measure of heteroplasmy variance (V'(h)) was used to compare heteroplasmy variance among samples with different mean heteroplasmy, taking the form

$$V'(h) = \frac{V(h)}{E(h)\,(1 - E(h))}$$

where V(h) is the variance of a set of samples and E(h) is its mean (Johnston et al., 2015, Johnston and Jones, 2016). To access donor mtDNA heteroplasmy in different tissues, Friedman test was used, where the significance was set at $p < 0.05$. For heteroplasmy level of three germ layers, Mann-Whitney test was performed, where the significance was set at $p < 0.05$. For correlation between heteroplasmy value and V'(h), Spearman correlation test was used, where the significance was set at $p < 0.05$. For spread range of heteroplasmy value, Mann-Whitney test was employed, where the significance was set at $p < 0.05$. To access V'(h) of different development stages, Mann-Whitney test was performed, where the significance was set at $p < 0.05$. For heteroplasmy level of different development stages, Mann-Whitney test was used, where the significance was set at $p < 0.05$. For heteroplasmy value distribution, Kolmogorov-Smirnov test was performed, where the significance was set at $p < 0.05$. To access variance of fluorescence intensity in blastomeres, Mann-Whitney test was employed, where the significance was set at $p < 0.05$. Asterisks indicate statistical significance ($*$ denotes a p value of < 0.05 and $**$ denotes a p value of < 0.01).