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HIGHLIGHTS

Matching mitochondrial DNA haplotypes make the nucleus treat different mtDNA the same.

Similar mtDNA haplotypes prevents tissue-specific segregation bias.

Low level of mtDNA heteroplasmy results in uneven inheritance rather than segregation.

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Matching Mitochondrial DNA Haplotypes for Circumventing Tissue-Specific Segregation Bias

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SUMMARY
Mitochondrial DNA (mtDNA) segregation associated with donor-recipient mtDNA mismatch in mitochondria replacement therapy leads to unknown risks. Here, to explore whether matching mtDNA haplotypes contributes to ameliorating segregation, we reproduced various degrees of heteroplasmic mice with three single nucleotide polymorphisms to monitor segregation severity. “Segregation” presented in tissues of heteroplasmic mice containing low-level donor mtDNA heteroplasmy, and disappeared as donor mtDNA heteroplasmy levels ascended. Meanwhile, we found that distribution of donor mtDNA among the blastomeres of preimplantation embryos from the heteroplasmic mice shared the same tendency as that in adult tissues. Statistical analysis showed that no selective replication of donor mtDNA occurred during lifespan. Tracking donor mtDNA distribution showed that uneven distribution of donor mtDNA among embryonic blastomeres gradually became even as donor mtDNA heteroplasmy increased, indicating that the “segregation” in tissues was inherited from the uneven distribution. Our finding suggested that donor-recipient mtDNA matching could circumvent segregation in mitochondria replacement therapy.

INTRODUCTION
Mitochondrial replacement has the potential to reduce the transmission of inherited mitochondrial diseases (Wang et al., 2014; Paull et al., 2013; Graven et al., 2010; Tachibana et al., 2009). However, mitochondrial replacement will inevitably result in trace levels of heteroplasmy (Paull et al., 2013; Graven et al., 2010; Tachibana et al., 2009). Although such trace levels of heteroplasmy do not exceed a pathogenic threshold, if the pathogenic maternal mitochondrial DNA (mtDNA) is given a selective advantage, it is possible that it achieves dominance and manifests a pathogenic phenotype through the process of segregation, a common phenomenon in tissues of patients with mitochondrial disease (Frederiksen et al., 2006; Nishizuka et al., 1998). Recent studies of human mitochondrial replacement show sharp drifts of pathogenic mtDNA haplotype on a cellular level (Hyslop et al., 2016; Kang et al., 2016; Yamada et al., 2016), indicating that the nuclear genome preferentially regulates the replication and segregation of the native pathogenic mitochondria. Thus the requirement of a functional match between donor and recipient mtDNA, as well as between the mtDNA and nuclear genome, is of the utmost importance in clinical applications of mitochondria replacement (Latorre-Pellicer et al., 2016; Reinhardt et al., 2013; Sharpley et al., 2012; St John and Campbell, 2010). Previous study suggested that the segregation of donor mtDNA in mitochondria replacement could be alleviated if the donor mtDNA haplotype matches with the recipient mtDNA haplotype (Latorre-Pellicer et al., 2016; Reyvik et al., 2016).

Obviously, the optimal recipient mitochondria would have the same haplotype as the donor. However, it is impossible to have two identical haplotypes of mtDNA in humans (He et al., 2010). However, the shorter the genetic distance between haplotypes, the less pronounced is the segregation bias. Therefore, exploring haplotype matching between donor and recipient mtDNA is particularly important for eliminating segregation of donor mtDNA. As tissue-specific segregation of different mtDNA genotypes is also common in heteroplasmic mice created from ooplasm or nuclear transfer, various heteroplasmic mice models were used to elucidate the mechanisms for controlling segregation of different mtDNA genotypes (Jokinen et al., 2015; Neupane et al., 2015; Sato et al., 2007; Battersby et al., 2005; Takeda et al., 2000; Jenuth et al., 1997). Among these models, heteroplasmic mice constituted by NZB and C57BL/6N were the dominant heteroplasmic models with 106 single nucleotide polymorphism (SNP) differences. In addition, Burgstaller et al. found highly significant positive correlation between individual tissue-specific segregation and mtDNA genetic distance in specific heteroplasmic mice generated using wild mice in Europe.
and C57BL/6N, where the wild mice display a spectrum of genetic distance (18, 86, 107, and 416 SNP differences at mtDNA) with C57BL/6N (Burgstaller et al., 2014a). Common segregation present in those heteroplasmic mice indicated that none of the models above underwent haplotype matching, leading to the absence of related progress in segregation.

Here we therefore intend to shorten the distance between the donor and recipient mtDNA haplotypes to explore whether matching mtDNA haplotypes between donor and recipient mtDNA can circumvent the segregation bias toward donor mtDNA in tissues of mitochondria replacement mice (Figures S1A and S1B). We had established a specific model of heteroplasmic mice from NZW/Lac J and B6D2F1 (C57/BL6×DBA) using mitochondria replacement from our past study (Wang et al., 2014). The mtDNA genotypes of NZW strain differ from those of C57 strain at only three SNPs, making them ideal models for matching mtDNA haplotypes. To study the effect of matching mtDNA haplotypes on the tissue segregation, we reared offspring of the heteroplasmic mice to test whether matching mtDNA haplotypes between “donor” and “recipient” can circumvent the segregation of donor mtDNA (Figure S1C).

RESULTS
Tissue-Specific “Segregation” Gradually Disappeared with the Increase of Donor mtDNA Mean Heteroplasmy in Adult Mice
To explore whether matching mtDNA haplotypes can circumvent the segregation bias, we first sought to test whether the segregation occurred in different tissues from heteroplasmic mice with 3 SNP difference (Table S1), which was derived as described using mitochondria replacement technique (Wang et al., 2014). To avoid the potential impact of heteroplasmy level and trauma from mitochondria replacement manipulation on segregation behavior, heteroplasmic mice from the mitochondria replacement founder were used, which possessed naturally inherited levels of heteroplasmy. Pyrosequencing, which has a 1% detection threshold, 100% sensitivity, and 100% specificity (Hyslop et al., 2016; Wang et al., 2014; Blakely et al., 2013; White et al., 2005) (Figure S2), was adopted to measure the level of donor mtDNA heteroplasmy with primary and second primers (Table S2, see Transparent Methods). The donor mtDNA heteroplasmy was measured with pyrosequencing in 16 tissues from 37 heteroplasmic mice that were sacrificed as adults (6–8 months old) (Figure 1A). The mean heteroplasmy level of each adult displaces the natural range (from 1.86% to 38.13%) (Figure 1B and Table S3). The heteroplasmic value of 16 tissues showed similar regional distribution (p > 0.05) (Figure 1C), which initially indicated that segregation of donor mtDNA does not appear in different tissues of the heteroplasmic mice with 3 SNP difference. After incorporation of different tissues into the corresponding germ layer, it was found that there was no significant difference in donor mtDNA heteroplasmy between the three germ layers (p > 0.05) (Figure 1D). Then we compared the distribution of donor mtDNA in individual tissues of each adult mouse using normalized variance (V(h)) calculation as a way to measure the dispersion of donor mtDNA heteroplasmy. A drastic dispersion of donor mtDNA presented in individual tissues of adult mice with lesser than 10% donor mtDNA, evidenced by the greater V(h) (r = −0.53, p < 0.001) (Figure 1E). However, a much narrower distribution with few deviations appeared in individual tissues of adult mice with 10%–20%, or greater than 20%, donor mtDNA, supported by the low V(h) (Figure 1E).

Distribution of Donor mtDNA in the Blastomeres of Preimplantation Embryos Shared the Same Tendency as that in Adult Tissues
It is known that no net replication of mtDNA takes place before embryo implantation. Therefore each subsequent cell division reduces the amount of mtDNA within the daughter cells by about 50% (Carling et al., 2011). Next, to explore whether “segregation” actually occurs at lower levels of heteroplasmy in adult tissues, we observed the distribution of the two different mtDNA genotypes in each blastomere of embryos at 2-cell, 4-cell, and 8-cell stages from the heteroplasmic mice. As the adult tissues of the heteroplasmic mice, embryos at 2-cell, 4-cell, and 8-cell stages maintained a natural distribution value, ranging from 1.95 to 39.63, 1.81 to 54.24, and 2.57 to 51.91, respectively (Figures 2A–2C, Tables S4–S6). Similar to adult tissues, we observed that distribution of donor mtDNA heteroplasmy in each blastomere became less diverse as the mean levels of donor mtDNA heteroplasmy gradually increased in 2-, 4-, and 8-cell embryos, respectively. It was witnessed that V(h) values present a negative correlation with the mean heteroplasmy of embryos at 2-, 4-, and 8-cell stages (r = −0.45, p < 0.05 for 2-cell stage; r = −0.58, p < 0.0001 for 4-cell stage; r = −0.48, p < 0.005 for 8-cell stage) (Figure 2D).
No Selective Replication of Donor mtDNA Took Place during the Progressive Cleavage across Developmental Stages

To relate the similar distribution trend of donor mtDNA seen in adult tissues with those in blastomeres of cleaving embryos, we compared the dispersion of donor mtDNA from 2-cell with that from adult stage using several statistical comparison methods. We first observed the spread trends from 2-cell to adult stage. The spread of donor mtDNA heteroplasm between daughter blastomeres within each embryo, calculated as the heteroplasmic range and $V(h)$ values, increased gradually from the 2-cell through the 4-cell to 8-cell groups ($p < 0.05$) (Figures 3A–3D). These phenomena are consistent with recent studies’ findings, which showed increasing cell-to-cell heteroplasmy variability through early embryonic cleavages (Lee et al., 2012; Johnston et al., 2015; Johnston and Jones, 2015, 2016). However, the spread trend returned to the original level as that of 2-cell embryos as development progresses to adult stage (Figures 3A–3D). Then we further found that adult tissues share a similar distribution of heteroplasmy with early embryos at 2-, 4-, and 8-cell stages ($p > 0.05$), calculated as mean heteroplasmy (Figure 3E), frequency distribution (Figure 3F), and cumulative probability distribution (Figure 3G). Thus, from the spread trends and the distribution, it can be deduced that the distribution of donor mtDNA in adult tissues depends on the distribution present in early embryos, suggesting that no selective replication of donor mtDNA took place during the progressive cleavage from the 2- to 4- to 8-cell stages extending to adult.

Figure 1. Tissue-Specific Segregation Disappeared with the Increase of Donor mtDNA Mean Heteroplasmy in Adult Mice

(A) Schematic model of heteroplasmic mice and dissected tissues in this study.
(B) Heteroplasmy of donor mtDNA in individual tissues from heteroplasmic mice with different heteroplasmic levels.
(C) Comparison of heteroplasmy distribution in 16 tissues from 37 mice ($p > 0.05$, Friedman test). Data are represented as scatterplot with mean ± SD.
(D) Comparison of mean heteroplasmy levels in the ectoderm, endoderm, and mesoderm ($p > 0.05$, Mann-Whitney test). Data are represented as mean ± SD.
(E) Negative correlation between heteroplasmic levels (green) and $V(h)$ (red) in 16 tissues of each mouse ($r = -0.53$, $p < 0.001$, Spearman correlation test). Error bars indicate SD, with the mean value.
See also Table S3.
Tracking Donor mtDNA Distribution Exhibited that Low Level of Donor mtDNA Heteroplasmy Resulted in Its Uneven Inheritance during Early Embryonic Cleavage

To explore why donor mtDNA deviation occur in tissues and blastomeres in the <10% group, we generated heteroplasmic oocytes to observe the distribution of donor mtDNA via spindle-chromosome complex transfer (spindle transfer) (Figure 4A) (Wang et al., 2014). Briefly, the donor mitochondria were labeled with 250 nM MitoTracker Red. Then spindle transfer was performed between the stained oocytes (donor) and unstained oocytes (recipient) (Figures 4A and 4B and Video S1). Differing amounts of donor mtDNA were fused into an enucleated recipient oocyte, resulting in varying levels of heteroplasmy (<10% and >10%; here we only use >10% as past results showed no significant difference between the 10% to 20% and the >20% groups). The levels of heteroplasmy were calculated from the volume ratio (on average 10%:90%) by measuring the diameters of karyoplasts (carrying donor mtDNA). After the oocytes were fertilized in vitro and development proceeded, red mitochondria

Figure 2. Distribution of Donor mtDNA in the Blastomeres of Pre-implantation Embryos Shared the Same Tendency as that in Adult Tissues

(A) Schematic model of embryos and dissected blastomeres of embryos at 2-cell stage. Heteroplasmy distribution of donor mtDNA in blastomeres of embryos at 2-cell stage from heteroplasmic mice.

(B) Schematic model of embryos and dissected blastomeres of embryos at 4-cell stage. Heteroplasmy distribution of donor mtDNA in blastomeres of embryos at 4-cell stage from heteroplasmic mice.

(C) Schematic model of embryos and dissected blastomeres of embryos at 8-cell stage. Heteroplasmy distribution of donor mtDNA in blastomeres of embryos at 8-cell stage from heteroplasmic mice.

(D) Negative correlation between donor mtDNA heteroplasmy (green) and $V^\text{h}$ (red) values in blastomeres of embryos at 2, 4, and 8-cell stage ($r = -0.45$, $p < 0.05$ for 2-cell stage; $r = -0.58$, $p < 0.0001$ for 4-cell stage; $r = -0.48$, $p < 0.005$ for 8-cell stage. Spearman correlation test).

Error bars indicate SD, with the mean value. See also Tables S4–S6.
distribution was monitored in individual blastomeres of embryos at the 2-, 4-, and 8-cell stages and the blastocyst. A distinct correlation was observed between the distribution of donor mitochondria and the level of heteroplasmy. For <10% group, uneven and even configurations of red mitochondria distribution were found in the preimplantation embryos from 2-cell to blastocyst stage. In the uneven group, the number of red mitochondria in each blastomere varied significantly under confocal microscope, with no red mitochondria in several of the blastomeres (Figures 4Ba and S3–S6). By contrast, almost equal numbers of red granules were distributed in each blastomere in the even group (Figures 4B and S3–S6). However, in cells with higher levels of heteroplasmy (>10%), we observed only even distribution of stained mitochondria, with cells portraying close to equal levels of heteroplasmy.

Figure 3. No selective replication of donor mtDNA took place during the progressive cleavage across developmental stages

(A and B) (A) Spread of donor mtDNA heteroplasmy from embryonic blastomere to adult tissue expressed by heteroplasmy range (maximum or minimum heteroplasmy value minus the mean median of heteroplasmy value). The mean median is defined as half of the sum of maximum and minimum values. Light bar = maximum – median = maximum – minimum = maximum – minimum = maximum – minimum; dark bar = minimum – median = minimum – maximum = minimum – maximum = minimum – maximum (B) V(h) values of embryonic blastomeres and adult tissue.

(C) Comparison of the spread ranges among embryos at 2-, 4-, and 8-cell stage and adults. Data are represented as mean ± SD.

(D) Comparison of V(h) values among embryos at 2-, 4-, and 8-cell stage and adults. Different letters indicate p values < 0.05 (Mann-Whitney test); error bars indicate SD. See also Tables S3–S6.

(E) Comparison of mean heteroplasmy values of embryos at 2-, 4-, and 8-cell stage and adult tissues (p > 0.05, Mann-Whitney test). Data are represented as scatterplot with mean ± SD.

(F) Frequency histogram of the donor mtDNA heteroplasmy of embryos at 2-, 4-, and 8-cell stage and adult tissues.

(G) Cumulative probability distribution functions for the heteroplasmy of embryos at 2-, 4-, and 8-cell stage and adult tissues. (p > 0.05, Kolmogorov-Smirnov test).

See also Tables S3–S6.
and S3–S6). Furthermore, statistical comparison found that there were significant differences between <10% uneven and <10% even or >10% groups for donor mtDNA distribution in each blastomere of embryos at 2-, 4-, and 8-cell stages and blastocysts (Figures 4C and S3–S6 and Tables S7–S10). On the contrary, there were no obvious differences between <10% even and >10% groups for the distribution in each blastomere of embryos at 2-, 4-, and 8-cell stages and blastocysts (Figures 4C and S3–S6 and Tables S7–S10). This suggests that disproportionate variance increase can arise from partitioning noise with low mitochondrial volumes.

**DISCUSSION**

As we know, segregation of mutant mtDNA is a universal event during individual development (Burgstaller et al., 2014b). The coexistence of two kinds of mitochondria and its mtDNA may have fatal consequences for the development of offspring (Schon et al., 2012). Mitochondrial replacement technology will inevitably lead to the coexistence of two kinds of mitochondria and mtDNA, so the public has been worried about the potential safety risks since its conception. This study demonstrated that matching the haplotypes of the donor and recipient mtDNA has the potential to circumvent segregation bias and prevent the occurrence of mitochondrial diseases.
When heteroplasmic values are close to the detection limit, technical variability will likely be mixed in with the biological variability and may contribute to “segregation” as well. However, the spread trend of mtDNA heteroplasm in preimplantation embryo was consistent with recent studies (Johnston et al., 2015; Lee et al., 2012), suggesting that heteroplasmic values from pyrosequencing are reliable in our study. Our results showed that uneven inheritance donor mtDNA in embryonic blastomeres rather than selective replication of donor mtDNA causes the “segregation” in adult tissues of heteroplasmic mice with low level of heteroplasm. The spread trend of donor mtDNA heteroplasmy and \( V(h) \) values increases from 2-cell, through 3- to 4-cell, to 6- to 8-cell blastomeres, evidenced by how the spread trend of 8-cell is greater in turn than that of 4-cell and 2-cell blastomeres. This phenomenon is consistent with the results of Johnston et al. and Lee et al. studies (Johnston et al., 2015; Lee et al., 2012). Recent studies showed that mitochondrial concentration is controlled and 2-cell blastomeres. This phenomenon is consistent with the results of Johnston et al. and Lee et al. studies to 6- to 8-cell blastomeres, evidenced by how the spread trend of 8-cell is greater in turn than that of 4-cell and 2-cell blastomeres. This phenomenon is consistent with the results of Johnston et al. and Lee et al. studies (Johnston et al., 2015; Lee et al., 2012). Recent studies showed that mitochondrial concentration is controlled in the stochastic partition between cell division (Das Neves et al., 2010; Jajoo et al., 2016). Thus the spread trend could be attributed to how donor mitochondria are randomly assigned into two daughter blastomeres during mitosis of preimplantation embryos, resulting in much higher uneven inheritance of mtDNA in each blastomere when cleavage frequency increased, due to how they are stochastically partitioned during cell division. However, the spread trend in adult tissues almost resumes the initial spread range of the first embryonic cleavage in this study (Figures 3A–3D). As development progressed to adult stage, each tissue may be developed from more than one blastomere of an embryo at 8-cell stage, thus leading to the spread trend initialization, which indicated that no selective replication of donor mtDNA took place during the progressive cleavage across developmental stages.

Burgstaller et al. found that mtDNA segregation in heteroplasmic tissues is common in vivo and may be modulated by haplotype differences (Burgstaller et al., 2014a). Based upon their data (See Mathematical Analysis section), we created a mathematical model and formula that describes the relationship between the proliferation rate of donor mtDNA and genetic distance. From the formula we can deduce that when genetic distance \( d \) of haplotype differences is equal to or less than 9 SNPs, the expected level of segregation, albeit with substantial uncertainty, drops to zero (Figure S7). Thus, in our study, no segregation is present in tissue of heteroplasmic mice containing two mtDNA genotypes of NZW strain and C57 strain, as the mtDNA genotypes differ at only three SNPs. As mtDNA segregation is controlled by the nuclear genome (Agaronyan et al., 2015; Battersby et al., 2003), our results suggest that haplotype matching matches foreign mitochondrial and nuclear DNA as well, so that the nucleus treats similar mtDNA sequence the same, thus preventing segregation.

As we know, mtDNA point mutation causes a variety of different phenotypes in humans. The effect of point mutations on segregation in different tissues during lifetime is still enigmatic. Segregation of some mtDNA mutations, such as 8993T > G, yield no tissue segregation (White et al., 1999), whereas for others, such as 3243A > G, segregation varies drastically among tissues (Frederiksen et al., 2006). The latter seems to violate our results and speculation from Burgstaller’s data, in which differences less than 9 SNPs could circumvent segregation of mutant mitochondria (Figure S7). Owing to a rapid mutation rate over the human lifetime, a number of novel mtDNA mutations, which constitutes mtDNA polymorphisms, were detected in both pathogenic mtDNA carriers’ and healthy donors’ oocytes (Kang et al., 2016). Owing to how genetic distance between two random selected people will differ at 100 SNPs (Rayvik et al., 2016), previous studies found that polymorphisms can grant a replicative advantage (Burgstaller et al., 2014b; Kang et al., 2016). It has been demonstrated that some mtDNA point mutations, such as 3394C variant, may either be deleterious or beneficial depending on its haplogroup and environmental context (Ji et al., 2012), suggesting the segregation of point mutations associated with the polymorphisms. We hypothesize that original polymorphisms and novel variations may interact with pathogenic point mutation to constitute a network with nuclear DNA to regulate mutant and non-mutant mtDNA proliferation. Thus, multiple factors should be combined and taken into account for the point mutations as well as other mutants on segregation in further study.

In summary, mitochondrial segregation inevitably occurs in offspring from mitochondrial replacement manipulation if no haplotype matching has been conducted. This study indicates that genetic similarity between donor and recipient mtDNA has the potential to circumvent the segregation bias toward pathogenic maternal mtDNA in tissues of mitochondria replacement offspring. Thus our results recommend that mtDNA haplotype matching should be undertaken between the donor and recipient, as it could “fool” the nucleus into treating the donated mtDNA and the native pathogenic mtDNA the same, thereby eliminating any proliferative advantage, and circumvent any segregation bias and prevent the onset of mitochondrial diseases.
Limitations of the Study

Although this study has demonstrated that matching mtDNA haplotypes could circumvent the tissue segregation of mutant mitochondria in heteroplasmic mice with 3 SNP difference, more spectra (such as 3–18 SNPs) of mitochondrial genetic differences between two kinds of mice should be conducted to clearly address the minimum distance that can circumvent tissue segregation. Furthermore, screening key SNP loci or regulatory networks, which is associated with mtDNA replication and proliferation in nuclear and mtDNA sequences, may make it easier to find a suitable recipient donation and prevent the occurrence of mitochondrial diseases.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.03.002.

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AUTHOR CONTRIBUTIONS

H.S. and X.D. supervised and designed the experiments. H.S. and J.P. manipulated mitochondrial donation. J.P. and C.L. performed staining and confocal analysis. L.W. detected heteroplasmy level of samples. Y.Z. did data statistics. Z.M. supervised mice. H.S., C.L., and J.P. prepared the figures and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Matching Mitochondrial DNA Haplotypes for Circumventing Tissue-Specific Segregation Bias

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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 heteroplasmic oocytes with <10% donor mtDNA. (B) Even distribution of donor mtDNA in embryos at 2-cell stage from heteroplasmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in embryos at 2-cell stage from heteroplasmic 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 heteroplasmic oocytes
with <10% donor mtDNA. (B) Even distribution of donor mtDNA in embryos at 4-cell stage from
heteroplasmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in
embryos at 4-cell stage from heteroplasmic 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 heteroplasmic oocytes
with <10% donor mtDNA. (B) Even distribution of donor mtDNA in embryos at 8-cell stage from
heteroplasmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in
embryos at 8-cell stage from heteroplasmic 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 heteroplasmic oocytes with <10% donor mtDNA. (B) Even distribution of donor mtDNA in blastocysts from heteroplasmic oocytes with <10% donor mtDNA. (C) Even distribution of donor mtDNA in blastocysts from heteroplasmic 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 TCCAATTAGTAGATTCTGAATAAAACCCCAGAGAGAGTAATGAAACCTGTACACTGTATCT 9480 Sbjct 9421 TCCAATTAGTAGATTCTGAATAAAACCCCAGAGAGAGTAATGAAACCTGTACACTGTATCT 9480 |
| tRNA-Arg | A9821-      | Query 9781 AAAAGGATTAGAATGAACAGAGTAATGTTATAGTTTAAAAAATTATGGTTT 9840 Sbjct 9781 AAAAGGATTAGAATGAACAGAGTAATGTTATAGTTTAAAAAATTATGGTTT 9839 |
| ND5   | C13053T      | Query 13021 ATTTACTTCGTAACAATAAAACCGCGTTTCCCCCTAATCTCATTAAACGAAAAT 13080 Sbjct 13020 ATTTACTTCGTAACAATAAAACCGCGTTTCCCCCTAATCTCATTAAACGAAAAT 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.

| PCR Type          | Primer Sequences                                  | Conditions                                                                 |
|-------------------|----------------------------------------------------|-----------------------------------------------------------------------------|
| Primary PCR       | 5'-ATGGCTACTGGATTCCATGG-3' 3'-GCTCCTATGAAGCTTCATGG-5' | 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’-ATTTATTGGGGAGTCAGAATGC-5’ | 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 |
| Mouse | Brain | Heart | Lung | Liver | Kidney | Stomach | Intestine | Spleen | Muscle | Adipose | Skeleton | Bladder | Gonad | Hypothalamus | Optic | Nerve | Skin |
|-------|-------|-------|------|-------|--------|---------|-----------|--------|--------|---------|----------|---------|-------|-------------|-------|-------|------|
| 1     | 0     | 2.71  | 0    | 3.15  | 0      | 3.66    | 0         | 0      | 0      | 0       | 0        | 2.2     | 2.88  | 0           | 0     | 11.29 | 0.01 |
| 2     | 6.21  | 0     | 6.49 | 0     | 4       | 6.15    | 6.32      | 5.32   | 4.77   | 6.6     | 4.57     | 5.91    | 5.44  | 5.13         | 6.26  | 0     | 4.76 |
| 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 |
| 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  | 3.7 |
| 5     | 7.32  | 7.02  | 0    | 8     | 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     | 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 |
| 7     | 8.67  | 0     | 8.13 | 0     | 8.52    | 5.48    | 8.46      | 6.29   | 6.53   | 6.29    | 9.16     | 6.04    | 9.17  | 6.48         | 7.1    | 6.54  | 0.03 |
| 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 |
| 9     | 5.62  | 8.73  | 6.6  | 7.61  | 7.4     | 7.16    | 0         | 0.91   | 6.29   | 9.08    | 7.05     | 9.71    | 8.53  | 7.94         | 7.32  | 6.77  | 0.03 |
| 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  | 6.99  | 0.03 |
| 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 |
| 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  | 0.01 |
| 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 | 0.01 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 | 12.84 | 0.01 |

Table S3. Heteroplasmy in adult tissues, Related to Figures 1 and 3.
| 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 | 23.7 | 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 2 and 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      | 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 |
|    | S.D | CV  | Range | NA | NA | S.D | CV  | Range |
|----|-----|-----|-------|----|----|-----|-----|-------|
| 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 |
|--------------------------|--------|----------------------------------------|------------|----------|
| <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 |
|--------------------------|--------|--------------------------------------|------------|----------|
| <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 1006 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 | Proportion | Variance |
|--------------------------|--------|------------|----------|
| <10% uneven               |        |            |          |
| 1                        | 0 3419 | 0.00       | 0.0900   |
| 2                        | 0 5555 | 0.00       | 0.0165   |
| 3                        | 0 0    | 0.00       | 0.0521   |
| 4                        | 0 7753 | 0.00       | 0.0346   |
| 5                        | 7223   | 0.17       | 0.0275   |
| 6                        | 7756   | 0.22       | 0.0418   |
| >10% even                 |        |            |          |
| 1                        | 12377  | 0.10       | 0.0125   |
| 2                        | 7303   | 0.09       | 0.0062   |
| 3                        | 23954  | 0.25       | 0.0085   |
| 4                        | 0 14054| 0.00       | 0.0039   |
| 5                        | 0 20183| 0.00       | 0.0120   |
| >10%                      |        |            |          |
| 1                        | 14657  | 0.04       | 0.0078   |
| 2                        | 90847  | 0.18       | 0.0025   |
| 3                        | 32887  | 0.11       | 0.0031   |
| 4                        | 19699  | 0.15       | 0.0080   |
| 5                        | 23139  | 0.04       | 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 lysed 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:

\[ r(d) = \begin{cases} 
0 & 0 \leq d < 9 \\
0.0011 \ln(d) - 0.0025 & d \geq 10
\end{cases} \]

\( r \) 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 dimethyl sulfoxide (DMSO) to a final concentration of 1 mM to prepare a stock solution. Then stock solution was diluted to 250 nM concentration (working solution) in G1 medium. Donor mouse oocytes were dyed with 250 nM 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% CO₂, 5% O₂, incubation for 1 h. Then \( 2 \times 10^6 \) 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% CO₂,
5% O₂ and 90% N₂ 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 heteroplasmic 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 heteroplasmcy variance (V'(h)) was used to compare heteroplasmcy variance among samples with different mean heteroplasmcy, 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 heteroplasmcy in different tissues, Friedman test was used, where the significance was set at p < 0.05. For heteroplasmcy level of three germ layers, Mann-Whitney test was performed, where the significance was set at p < 0.05. For correlation between heteroplasmcy value and V'(h), Spearman correlation test was used, where the significance was set at p < 0.05. For heteroplasmcy level of different development stages, Mann-Whitney test was performed, where the significance was set at p < 0.05. For heteroplasmcy value distribution, Kolmogorov-Smirnov test was performed, 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 heteroplasmcy value distribution, Kolmogorov-Smirnov test was performed, 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).