Supporting Information

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SI Text

Calculating Hybridization Energies. The mitochondrial DNA (mtDNA) sequences of *Macaca mulatta* and *Rattus norvegicus* were downloaded from GenBank (accession no. X14848 and AY612638), and 100-bp segments (i.e., 5,700–5,800; 5,800–5,900;. . ., etc.) covering the major arc were extracted using a Python program. To calculate the free energy ΔG for all pairwise combinations, we used the hybrid-min program of the software package UNAFold 3.8 (1) under default parameters. This assumes a temperature of 37 °C and ion concentrations of 1 M Na^+ and 0 M Mg^{2+}. We performed several tests to assess the influence of different sodium concentrations and found that whereas the absolute values of free energy depend strongly on the ion concentration, the relative ranking is affected hardly at all. We therefore decided to use the standard values. Each hybridization of two double-stranded DNA segments has two potential structures: (*i*) hybridization of the 5′-3′ L strand with the 3′-5′ H strand and (*ii*) hybridization of the 3′-5′ L strand with the 5′-3′ H strand. Normally both duplexes result in different ΔG values and, as in Guo et al. (2), we selected the structure with the minimal free energy.

For the calculation of the free energies of the observed mitochondrial deletions, we assumed that they too resulted from a 100-bp hybridization. However, as shown in Fig. S1 there is an ambiguity as to where exactly the hybridization occurs. Because the cut point that resolves the hybridization structure can be at different places, there are a total of 100 different possible locations for the hybridization. We calculated them all and then used the mean value.

mtDNA Deletions in Mice. The most appropriate data sets to support our hypothesis are single-cell studies studying COX-negative cells. This ensures that the observed deletions are definitely capable of accumulating to such high levels inside cells that they cause physiological consequences (COX deficiency). However, to emphasize that the phenomenon of a shared deletion area seemingly extends to a fourth mammalian species, we also analyzed the data set of Chen et al. (3). They applied high-throughput sequencing to muscle tissue of mitofusin knockout mice that suffered from a high mutational load. They could identify nine different deletions; Fig. S2 shows that eight of these deletions share a common area, which again affects the genes ND4 and ND5.

1. Markham NR, Zuker M (2008) UNAFold: Software for nucleic acid folding and hybridization. *Methods Mol Biol* 453:3–31.
2. Guo X, et al. (2010) Repeats, longevity and the sources of mtDNA deletions: Evidence from 'deletional spectra'. *Trends Genet* 26(8):340–343.
3. Chen H, et al. (2010) Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell* 141(2):280–289.

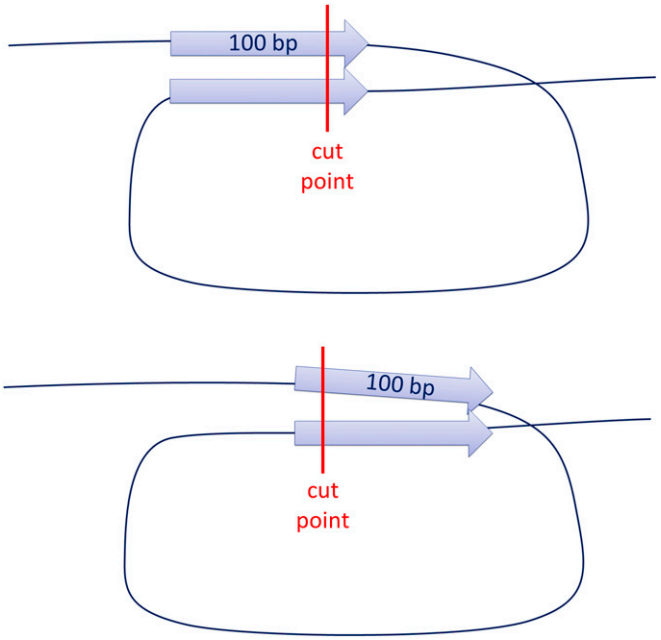

Fig. S1. Ambiguity of the exact location of the hybridization area that results in a certain deletion. There are multiple possible positions of the hybridization area relative to the cut point. For this study we took the mean value of the free energy resulting from all 100 possible locations.

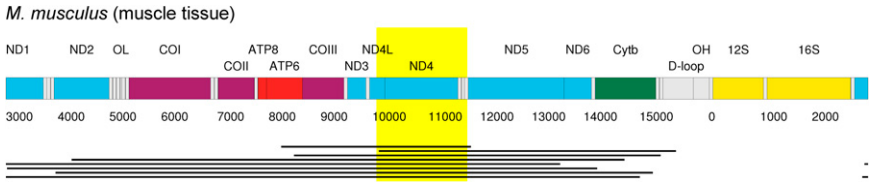

Fig. S2. Locations of mitochondrial deletions found in mice (3). The highlighted area indicates a stretch of mtDNA that is common to eight of nine deletions in mouse muscle tissue. The mtDNA map is based on GenBank entry 7770098.