Supplementary Note 1

We hypothesize that knocking down and overexpressing Mitofusin and Drp1 affect selection by altering mitochondrial connectivity. Functions other than fusion and fission have been proposed for both Mitofusin and Drp1. For example, Mitofusin modulates the tethering of mitochondria to the ER and is essential for the trafficking of mitochondria along axons (reviewed in Filadi et al. 2018). However, to our knowledge, only for their role in mediating mitochondrial connectivity via fusion and fission do Mitofusin and Drp1 have opposite functions. To further confirm that our manipulations of Mitofusin and Drp1 are affecting selection via their effects on mitochondrial connectivity, we overexpressed Mitofusin or Drp1 in ovaries of wildtype homoplasmic *Drosophila melanogaster* flies and measured mtDNA copy number (as an approximation for mtDNA replication), ATP levels (as an indicator of mitochondrial function), and mitochondrial motility. If the effects we observed on selection are due to changes in replication, mitochondrial function or motility we would expect overexpression of Mitofusin or Drp1 to oppositely affect these parameters, similar to their effect on selection. This is not what we find. The overexpression of Mitofusin and Drp1 had similar effects on mtDNA copy number (Extended Data Fig. 6f), the overexpression of Mitofusin or Drp1 had no significant effect on ATP levels (Extended Data Fig. 6g), and finally, within the limits of our assay overexpression of Mitofusin or Drp1 did not significantly affect mitochondrial motility (Extended Data Fig. 6h). In none of these experiments, did we observe the opposing effects upon Mitofusin and Drp1 overexpression that would be expected given the observed effects on selection. Therefore, our results cannot be explained by effects on copy number (mtDNA replication), ATP generation (mitochondrial function) or mitochondrial motility, and are best interpreted as being a consequence of altered mitochondrial connectivity.

Supplementary Note 2

References for Supplementary Note 1 and Supplementary Table 1

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