SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Generating linear mtDNA fragments from paused replication intermediates. Replication has traversed two-thirds of a mtDNA molecule and is paused at O_H and O_L, nascent strands are shown in blue. Nicking at both ends of one branch of the paused replication intermediate (A); or fork reversal and Holliday junction resolution (B) can both yield a 16kb species (circle) and an ~ 11 kb linear fragment. Although scheme A requires breakage at both ends on the same branch to release the linear species of ~ 11 kb, one, three and four breaks may also occur. Four breaks must be rare as it would yield one fragment of ~ 5 kb for every two fragments of ~ 11 kb, and no such 5 kb fragment was detected (data not shown). Limited fork reversal in mutator mouse but not wild-type mouse (B) could potentially explain the difference in the fragment ends within the NCR (Fig. 3A, 3B), as the reversed fork would be sensitive to single-strand nuclease digestion; however, this explanation cannot be applied to the mutator mouse fragment ends near O_L, as they fall either side of the ends found in wild-type littermates (Fig. 3C, 3D).

Supplementary Figure 2. In mouse liver mtDNA prominent free 5' ends of DNA are concentrated in the tRNA^{Cys} gene, immediately adjacent to O_L. Extraction of mouse liver mtDNA and identification of free 5' ends of DNA by ligation mediated-PCR were as previously described (36). The products were separated by PAGE, on a 6% gel, visualized by autoradiography. The signals were quantified using a phosphorimager to determine the relative intensities of the different 5' ends of DNA, which were represented as vertical blue lines in Fig. 3C. The nucleotide numbers of the most prominent free 5' ends are indicated in black, the numbers of less prominent

ends are coloured gray.

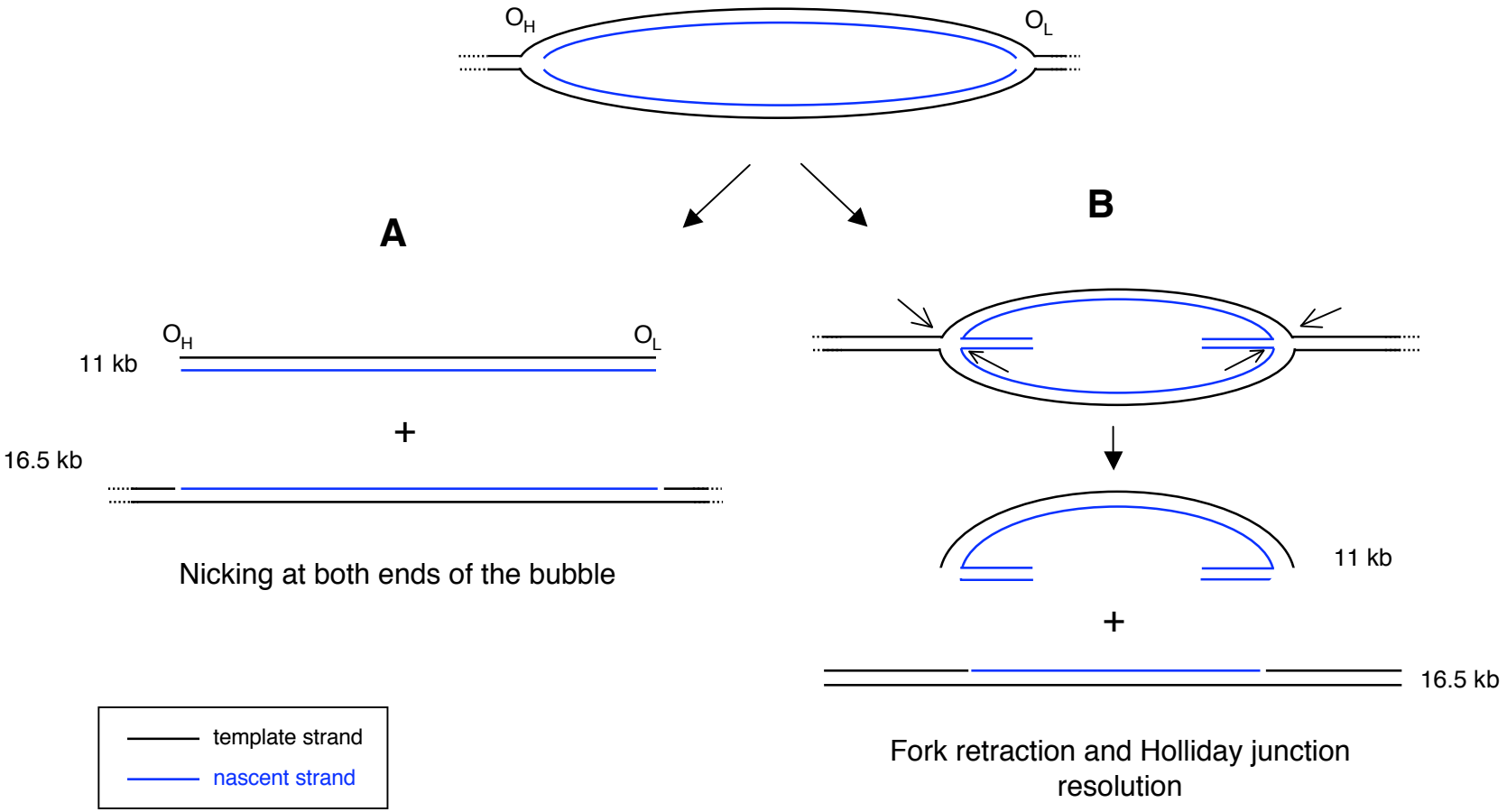
Supplementary Figure 3. Some fragments of high abundance in mutator mouse result from point mutations rather than replication pausing. The increased abundance of some fragments (e.g. Fig. 2D, species f) was inconsistent with pausing at O_H and O_L and could therefore be indicative of elevated pausing at other positions of the mouse mitochondrial genome. Comparison of several different digests failed to map any of these species (data not shown). Gel-extraction of *Sac*I/S1 treated fragments (~ 8 kb) yielded clones with an unmodified *Sac*I site (i.e. S1 nuclease had failed to remove the overhanging single-stranded part of the restriction site). Re-cloning without an S1 nuclease treatment gave a greater yield of PCR product; nine clones were sequenced and all were found to contain a *Sac*I site that is absent from wild-type mtDNA. In 8 of 9 clones a single point mutation accounted for the site gain, in the ninth two point mutations had occurred. The bases that were mutated are indicated in blue text. Vertical blue bars mark the position of the site gains on a schematic map of part of the mouse mitochondrial genome. The shortest blue bars were unique clones, whereas site gains at 1,344 and 1,430 occurred twice and three times, respectively, among the nine clones sequenced. *Sac*I site gains were not distributed evenly throughout the mitochondrial genome but were concentrated in the 16S rDNA gene; inset at the base of the figure are the positions where a single point mutation could create a new *Sac*I site in mouse mtDNA, those in red represent observed site gains, whereas black diamonds are theoretical site gains that were not seen. The one yellow diamond represents the clone where the *Sac*I site gain was the result of two mutations. Hence, *Sac*I site gains were concentrated in the 16S rDNA gene, suggesting that more errors are made in this region of mtDNA by POLG1 than

elsewhere; this appears to be a truism as the same bands were present in wild-type mice (black vertical lines), albeit at lower abundance.

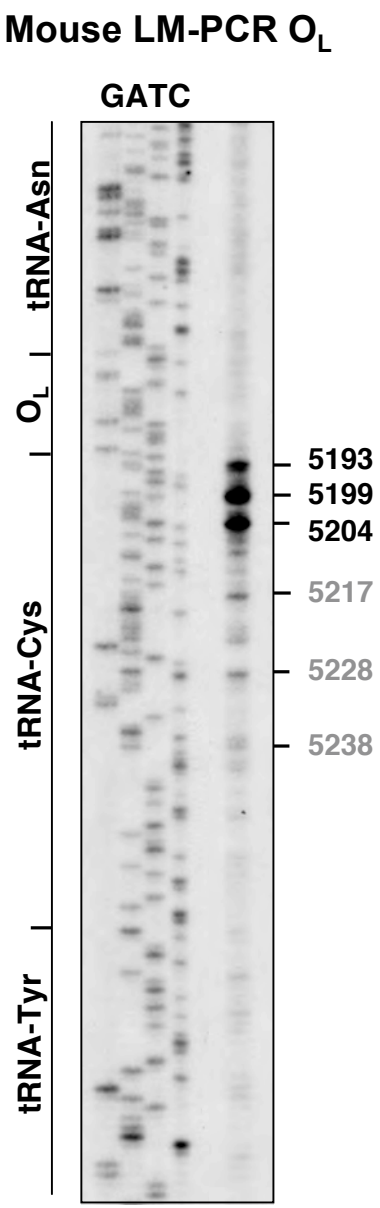
Reference

1. Yasukawa, T., Reyes, A., Cluett, T. J., Yang, M. Y., Bowmaker, M., Jacobs, H. T., and Holt, I. J. (2006) *EMBO J* **25**, 5358-5371

Supplementary Figure 1, Bailey et al.



Supplementary Figure 2, Bailey et al.



Supplementary Figure 3, Bailey et al.