Supplementary Material

Model behavior

The amount of cell death in our model for the pathogenesis of mtDNA mutations depends on five parameters:

1. The mutation rate, which affects the generation of lethal mutations with age.
2. The ratio of lethal to benign mutations, a measure of the target size for lethal mutations in the mitochondrial genome.
3. The number of lethal mutations needed to kill a cell, which we interpret as reflecting the sensitivity of a cell to apoptosis caused by lethal mutations.
4. The rounds of replication, a measure of age.
5. The number of mtDNA molecules per cell, a consequence of the hypothesis that lethal mutations are phenotypically dominant.

To explore how age-related levels of cell death depend on those parameters in the heart, we performed simulations where either the mutation rate or the ratio of lethal to benign mutations varied up to fourfold (both up and down) from the values presented in the Methods section. Each simulation was analyzed for cell death where the number of lethal mutations needed to kill a cell ranged from 1 to 20. Since in the adult heart the number of mitochondrial DNA (mtDNA) molecules per cardiomyocytes does not significantly change with age,[22] we did not perform simulations varying that parameter. We note, however, that the number

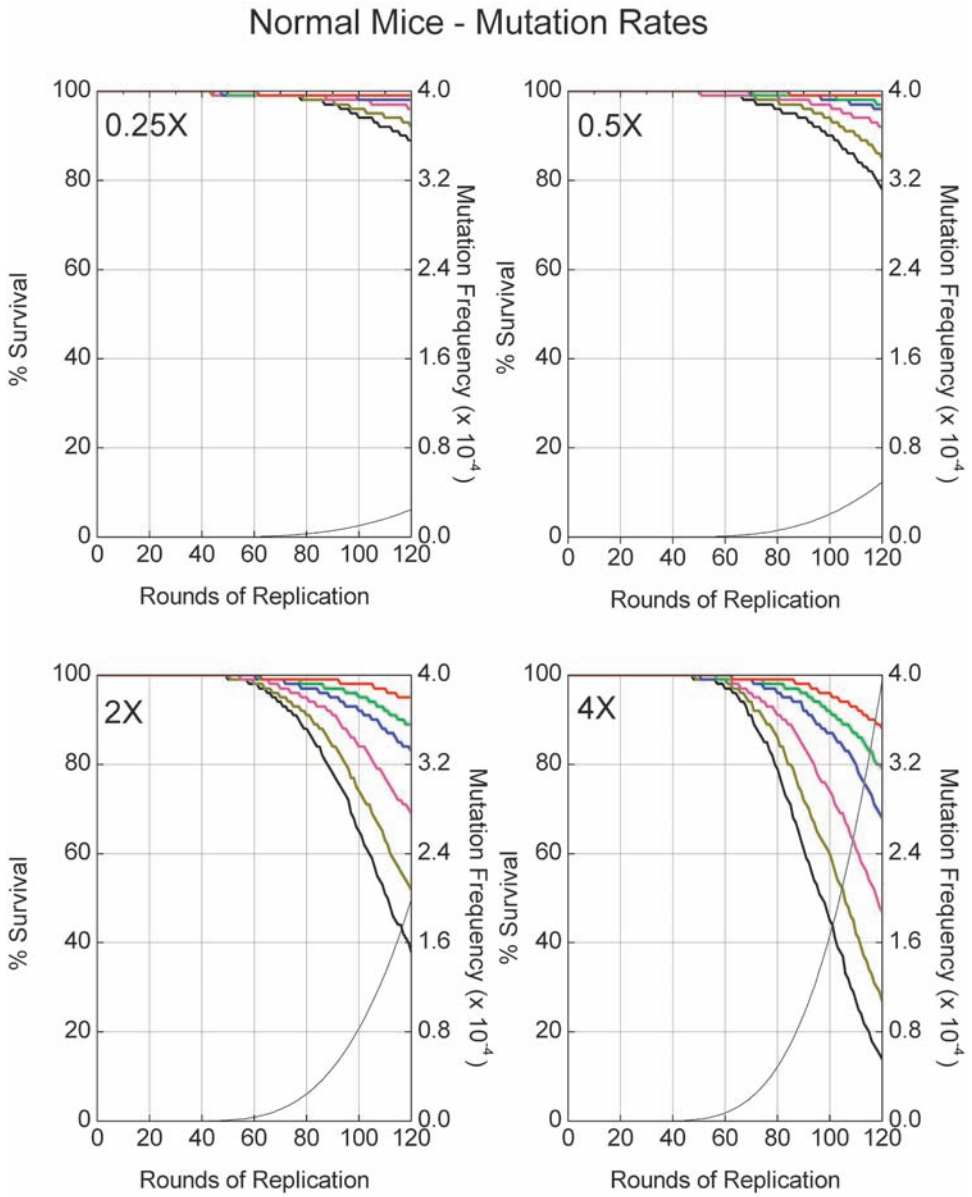

FIG. S1. Effect of variations in mutation rates on cell survival in normal mice. The standard mutation rate (1×) used for the simulation presented in the main text is 1.2×10^{-7} mutations/base. Panels show simulations where the mutation rate is varied from 0.25× to 4× the standard rate. In each panel the family of curves from bottom to top represent 1, 2, 4, 8, 12, and 20 lethal mutations needed to kill a cell. The rising black curve gives the mutation frequency (right axis).

of lethal mutations per cell will vary nearly identically with changes in either the mutation rate or the number of mtDNA molecules per cell since their product determines the number of total mutations per cell, each of which has a specified probability (i.e., the ratio of lethal to benign mutations) of being a lethal mutation.

Figures S1 and S2 show how age-related cell death in normal mice varies with mutation rate and the ratio of lethal to benign mutations, respectively. As can be seen, the model is well behaved in that the age-related percent survival of cardiomyocytes changes monotonically as the values of each parameter are varied. As expected, survival decreases as either mutation rates or the lethal to benign ratios increase, reflecting that the probability of a lethal mutation occurring rises with increases in either of those parameters. In each simulation, age-related survival decreases as the number of lethal mutations needed to kill a cell decreases.

The relationship between percent survival at three years of age and either mutation rates or lethal:benign ratios is shown in Figure S3. For the most part, cell death is approximately linearly proportional to each parameter – doubling of either parameter doubles cell death. It is primarily in situations where cell death is extensive (1 or 2 lethal mutations needed to kill a cell) where increases in either parameter decrease percent survival proportionately less. Fig. S3 also shows that increasing the mutation rate has nearly an identical effect on percent cell survival as the same relative increase in the ratio of lethal to benign mutations (the solid to the dotted curves, mutation rate verus lethal:benign ratio, respectively).

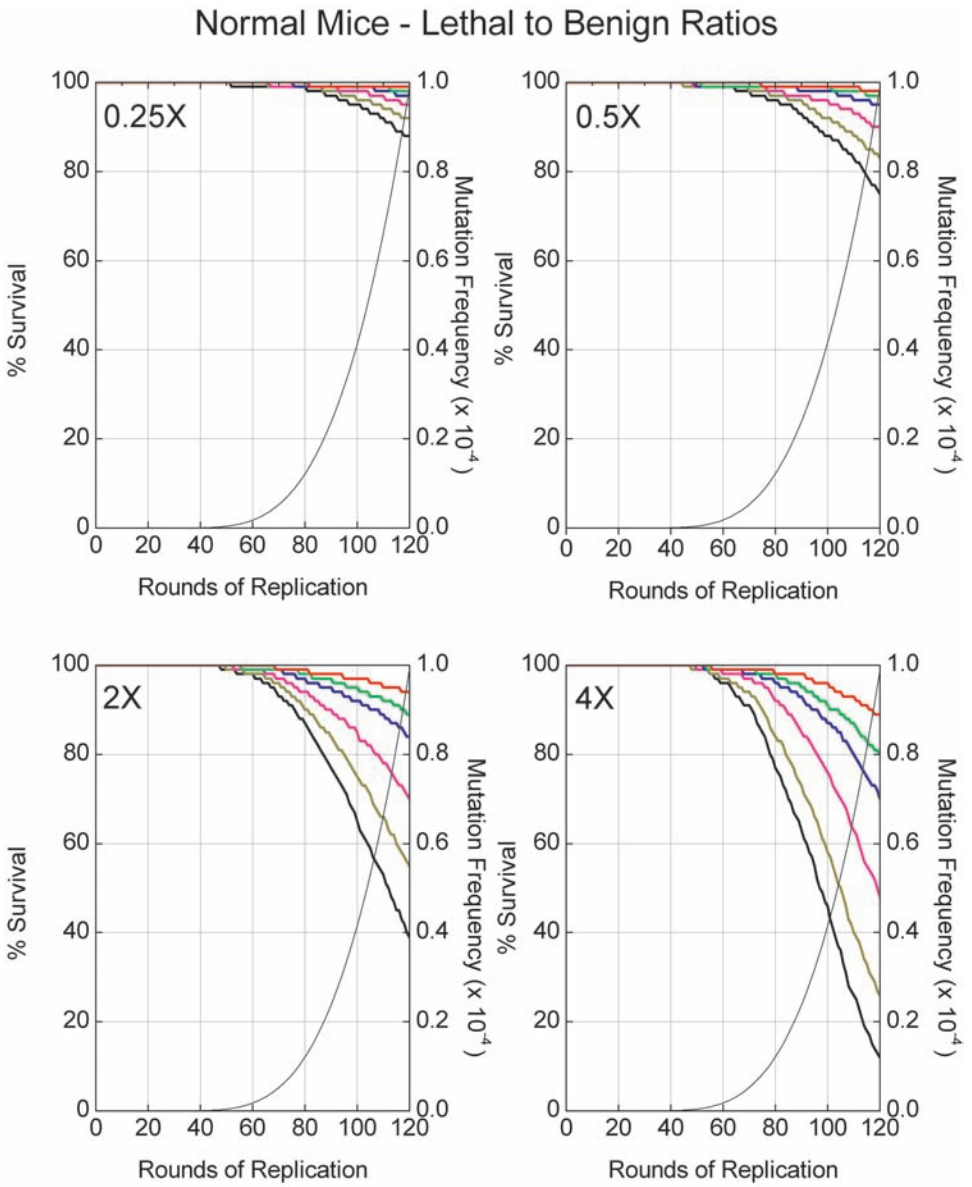

FIG. S2. Effect of variation in the ratio of lethal to benign mutations on cell survival in normal mice. The standard ratio of lethal to benign mutations used for the simulation presented in the main text is 1:16,000. Panels show simulations where that ratio is varied from 0.25× to 4× the standard ratio. In each panel the family of curves from bottom to top represent 1, 2, 4, 8, 12, and 20 lethal mutations needed to kill a cell. The rising black curve gives the mutation frequency (right axis).

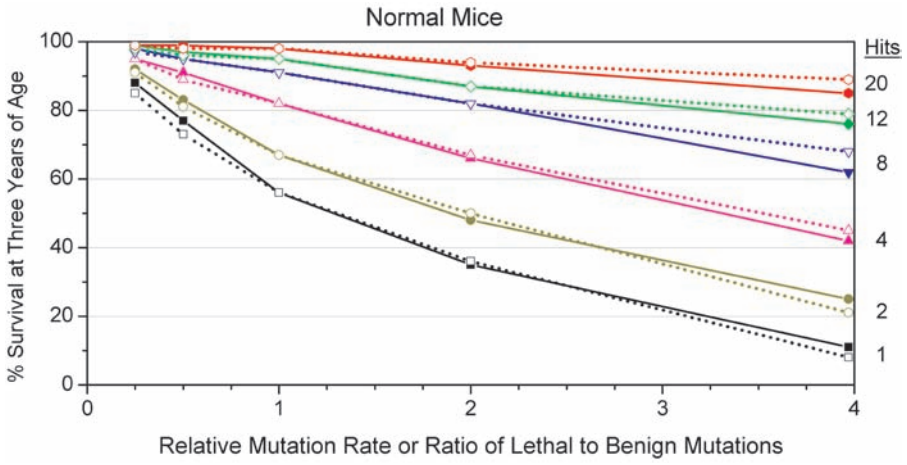

FIG. S3. Effect on cell survival in normal mice at three years of age of variations in mutation rates and ratios of lethal to benign mutations. Data was obtained from Figures S1 and S2. Solid lines show results from varying mutation rates, whereas dotted lines show results from varying the ration of lethal to benign mutations. Hits (the number of lethal mutations needed to kill a cell) are identified on the right for the family of curves.

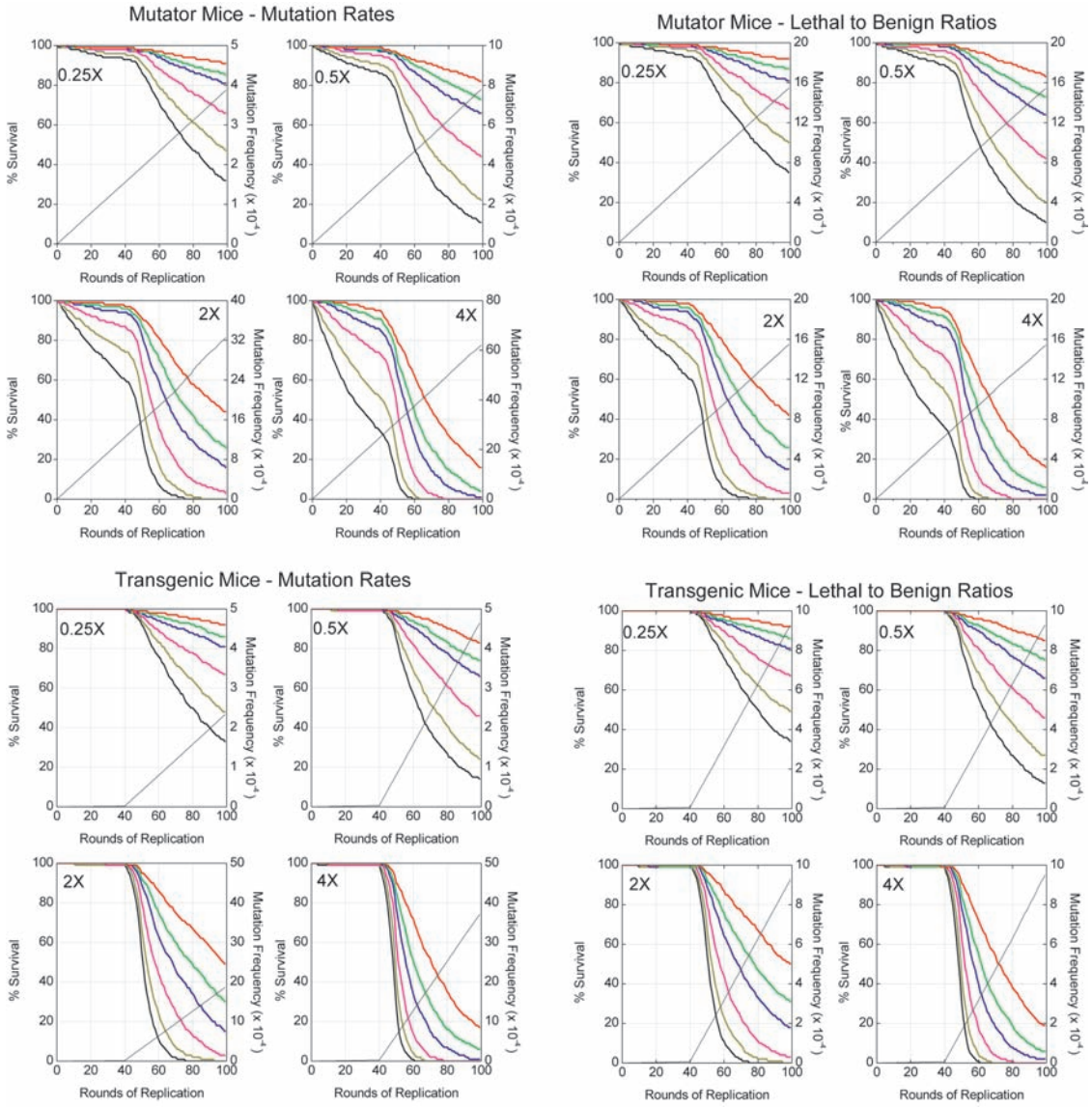

FIG. S4. Effect of variations in mutation rates and ratios of lethal to benign mutations on cell survival in mutator and transgenic mice. Conditions as described in the legends to Figures S1 and S2 except that the mutation rate for error-prone replication is 1.2×10^{-5} mutations per base.

Figure S4 present simulations where mutation rates and the ratios of lethal to benign mutations are varied in models for mutator and transgenic mice. Again, results indicate that the models are well behaved and that percent survival is approximately inversely proportional to each parameter until cell death climbs to high levels.

Source Code

The code, written in Python, for each model (mutator, transgenic, and normal mice) is included in Notepad files (see supplemental online video at www.liebertonline.com/rej).