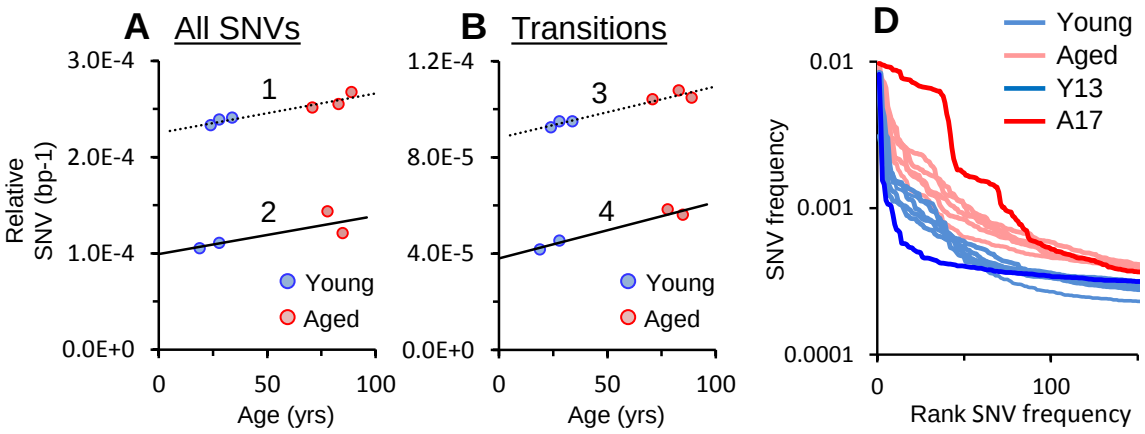
Figure S5



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Plot	Data	A-Y	ΔFrequency/yr	R^2	Intercept	Run
1	All SNVs	2.03e-5	4.09e-7	0.91	2.25e-4	2
2	All SNVs	1.30e-5	3.95e-7	0.62	9.93e-5	1
3	Transitions	1.15e-5	2.14e-7	0.94	8.80e-5	2
4	Transitions	1.37e-5	2.34e-7	0.96	3.80e-5	1

Figure S5 Commentary.

Quality based SNV filtering, as implemented in this study, is a robust means of removing uncertain SNV calls caused by errors during sequencing runs. However, it cannot address the full spectrum of potential errors that occur throughout an NGS workflow and is incapable of removing errors that do not influence call quality, such as base incorporation errors during library preparation. The extent of NGS errors that cannot be removed using simple quality filtering was recently revealed using duplex consensus sequence filtering [27]. We determined that the high coverage and absence of significance filtering in our study would provide consistent un-filterable error rates across all samples. While this would reduce the confidence in the absolute mean SNV frequency of a given sample, the differences between samples within an NGS workflow would still be valid. Our data (Fig. S5) support this assumption given the reproducible A-Y values, the near parallel ΔFrequency/yr gradients and high R^2 values, yet differing intercept values for each sequencing run. In line with transitions having low false call rates [27] and being the most prevalent form of mtDNA variant, the data is stronger for transitions than for total SNVs (transitions plus transversions). In our view this allows for normalization the SNV data from the two sequencing runs and validates findings derived from A-Y values and ΔFrequency/yr gradients. All sequencing data was handled identically and bioinformatic variables can be ruled out as a basis for the difference between sequencing run SNV frequencies.