Supplemental Figure Legends:

Figure S1. Mutation spectra become more distinct after permissive filtering. (A) Total number of unique variants for all biological replicates before (Pre-filter; upper panel) and after (Post-filter; lower panel) application of the permissive filter. **(B)** The sum of all variant frequencies for all biological replicates before (Pre-filter; upper panel) and after (Post-filter; lower panel) application of the permissive filter. Both plots exhibited changes post-permissive filtering, but they are more pronounced in unique variants (**A**).

Figure S2. Mutation spectra for all biological replicates. (**A**) The SNV mutation spectra based on the total of unique variants and (**B**) the sum of frequencies from each of the 6 classes observed in each biological replicate. (**C**) The deletion spectra based on the unique variants and (**D**) the sum of frequencies. (**E**) Insertion spectra based on unique variants and (**F**) the sum of frequencies.

Figure S3. The average variant frequency across *CAN1* by genotype. Low variant frequencies (<5%) and distribution for (**A)** *RNR1*, (**B**) *rnr1D57N*, (**C**) *rnr1Y285F pGAL-RNR1*, (**D)** *rnr1Y285F, (***E)** *rnr1Y285A pGAL-RNR1*, **(F)** and *rnr1Y285A*. The *rnr1Y285A* genotypes exhibited a lower unique variant frequency

Figure S4. High frequency variants across *CAN1*. Variants that occurred at greater than 5% average frequency within a genotype are plotted across *CAN1*.

Figure S5. Correlation analysis on SNVs in triplet context. Spearman correlation performed on the average number of each SNV as it occurs in unique triplet nucleotide context. Correlation values are displayed in **Table S7**.