Supplementary Figure Legends

Figure S1. Absolute variant frequency is consistent with selection at *CAN1.* (A) The absolute variant frequency was calculated by taking the sum of variant frequencies in each genotype and averaging for all biological replicates sequenced in a genotype. Error bars represent the standard deviation between biological replicates within a genotype. The number of total biological replicates sequenced varied by genotype; numbers are displayed in Table S3. Samples grown under permissive conditions, from four genotypes, are graphed in peach. All other samples grown under selection are graphed in turquoise. **(B)** The average absolute variant frequency before (red) and after (turquoise) the permissive variant filter was applied.

Figure S2. Hierarchical cluster analysis using cosine similarity score analysis on all individual samples in our study. Boxed in black are notable clusters where biological replicates from the same genotypes cluster together. Throughout, biological replicates tended to cluster. Similarly, *rnr1Y285F pGAL-RNR1* and *rnr1Y285A pGAL-RNR1* strains clustered with *rnr1Y285F* and *rnr1Y285A* strain, respectively.

Figure S3. Mutation spectra comparison between rnr1 alleles and *rnr1-pGAL-RNR1* counterparts. **(A)** The SNV spectra normalized out of total SNVs and **(B)** total variants. **(C)** The deletion spectra normalized out of total deletions and **(D)** total variants. **(E)** The insertion spectra normalized out of total insertions and **(F)** total variants. To compare the individual frequencies between genotypes, we took the average frequency and calculated the standard error of the mean (SEM) (**Fig. S4**). Error bars are not shown here for ease of viewing the data.

Figure S4. Average variant frequency of each variant type as a function of genotype. The average variant frequency for each genotype is shown. At least 4 independent biological replicates were sequenced for each genotype. The error bars represent the standard error of the mean (SEM). (**A**) G/C 1 bp deletions (top) and insertions (bottom), (**B**) A/T 1 bp deletions (top) and insertions (bottom), (**C**) >1 bp deletions (top) and insertions (bottom), (**D**) mononucleotide variant (top) and replacements (bottom), (**E**) single nucleotide variants: C/G>A/T, C/G>G/C, C/G>T/A (left) and T/A>AT, T/A>C/G and T/A>G/C (right).

Figure S5. Hierarchical cluster analysis of mutation signatures from this study compared with COSMIC SBS signatures. The single base substitutions (SBS) COSMIC signatures from GRCh38 (v3.2-March 2021, https://cancer.sanger.ac.uk/signatures/downloads/) were combined with the normalized SNVs in trinucleotide context (**Fig. 4).** Our samples formed a distinct cluster, which included COSMIC signature SBS32, a mutation profile associated with azathioprine treatment (**box I**). A second cluster (**box II**) correlated well with several of our samples (**Table S20**). Clustering was performed based on cosine similarity scores.