Supporting information

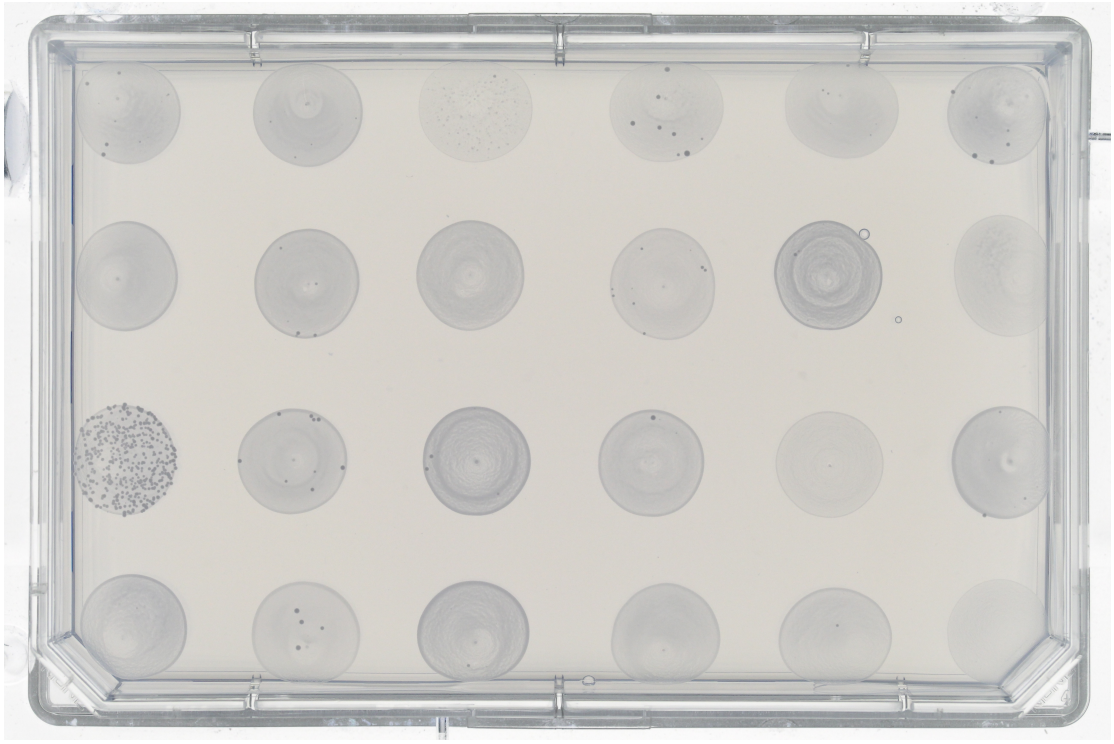

Figure S1. An example of the fluctuation analysis canavanine plate.

Saturated yeast cultures were spot-plated onto canavanine plates in a four by six configuration using the automated workstation. Plates with spot-plated yeast culture were dried and incubated at 30ºC for ~48 hours. Images of the plates were taken by an imaging robot. The above plate was spot-plated with 24 independent yeast cultures. The little dark dots in the spot are the canavanine resistant colonies. The number of observed resistant colonies varies between different cultures.

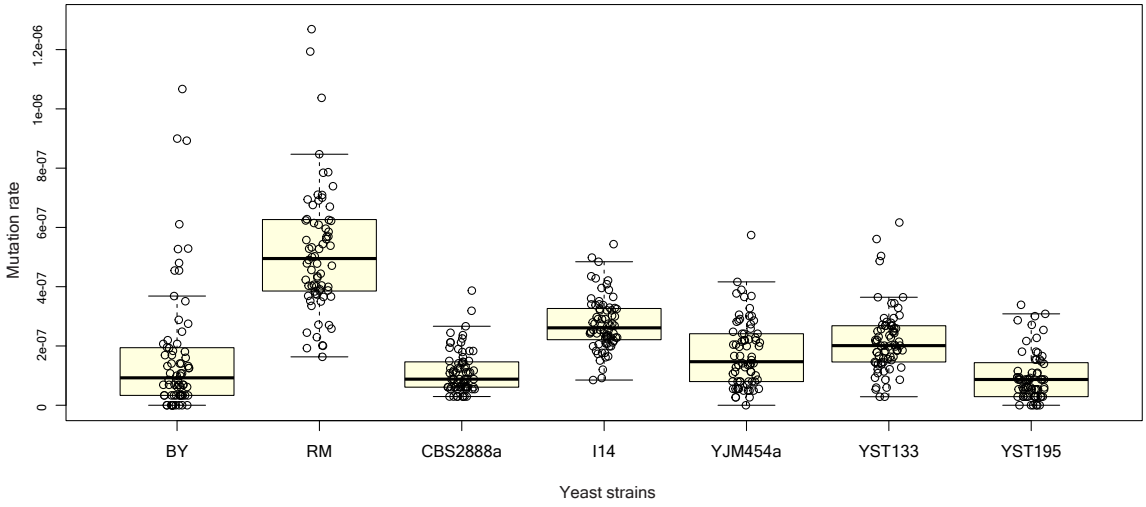

Figure S2. Mutation rate differs between seven natural yeast strains.

Ninety-six measure of mutation rate was performed for each strain. Means of the mutation rate are plotted as the line. Boxes show the 25%-75% percentile.

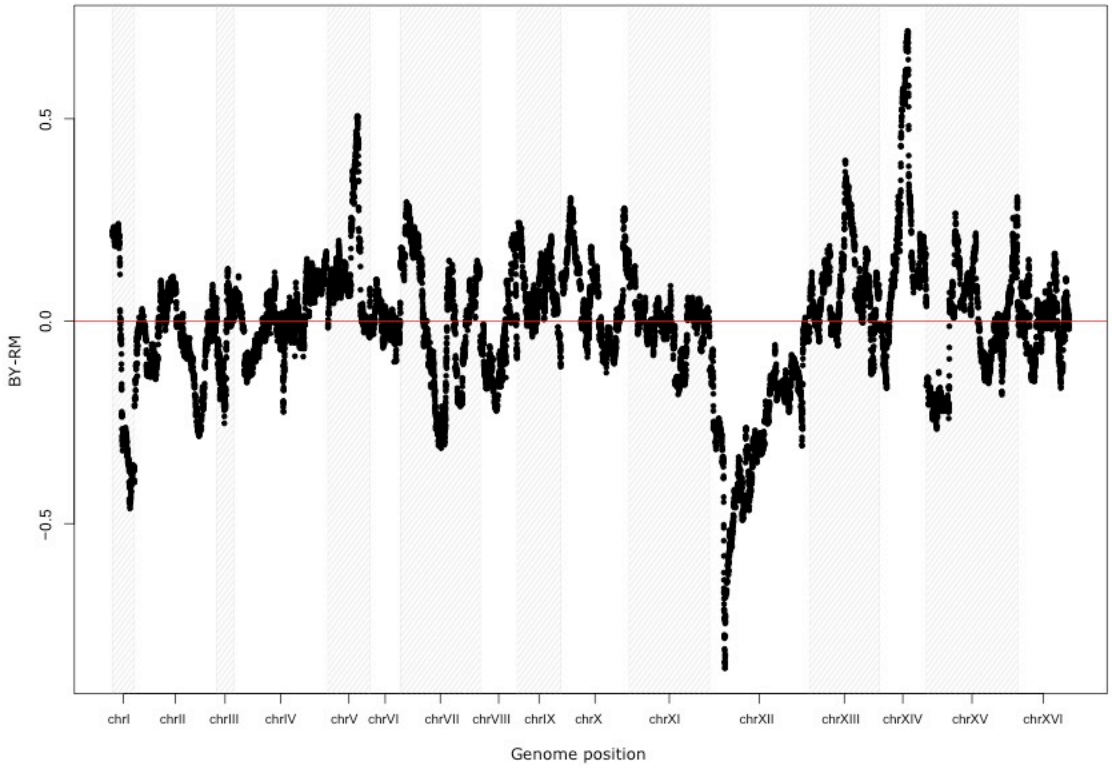

Figure S3. Loci on chromosome XII and XIV have large effects on mutation rate. Effect size of genetic markers along the genome shows the BY alleles on chromosome XIV and V increase the mutation rate, while the RM alleles on chromosome XII and I increase the mutation rate.

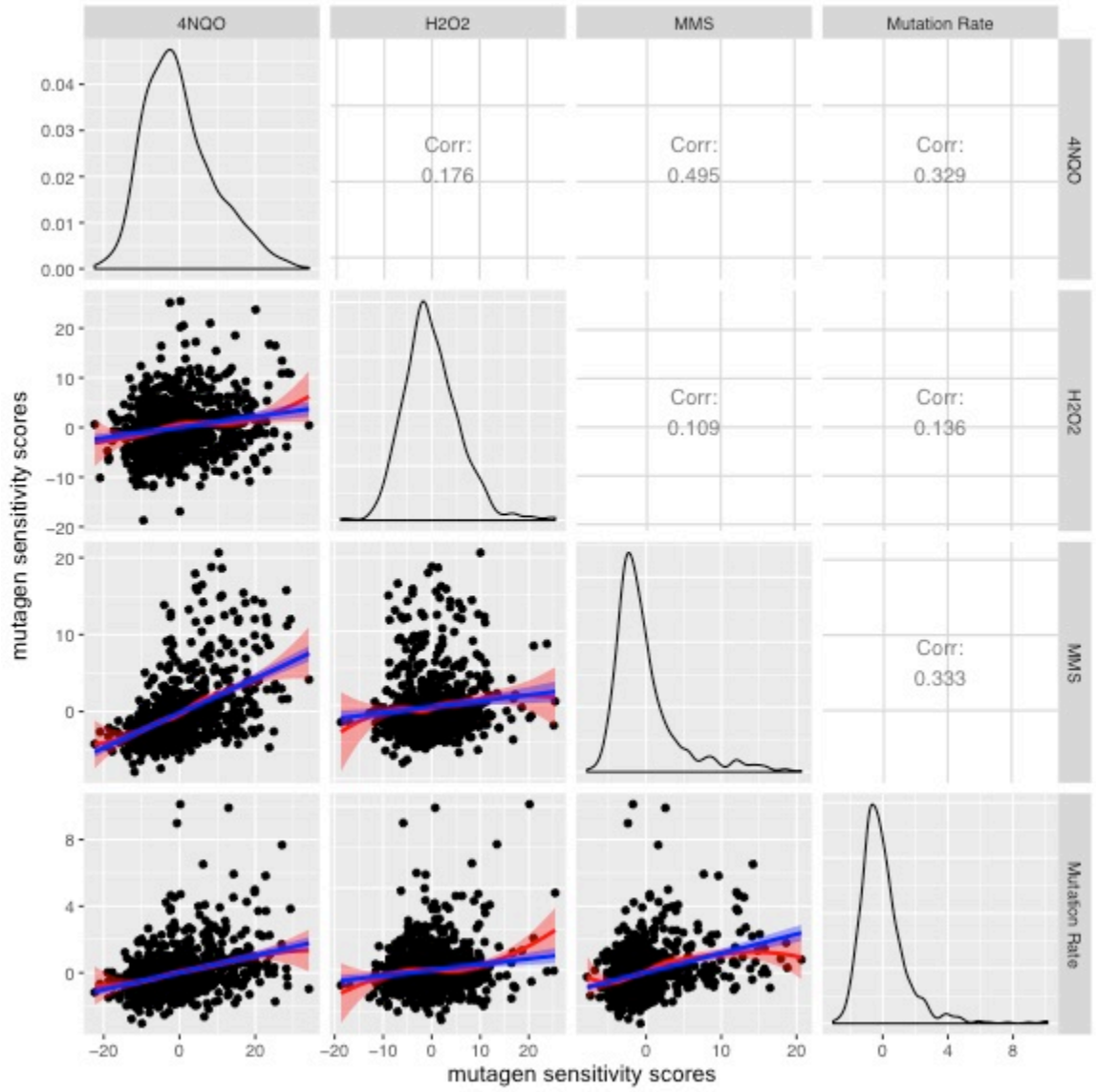

Figure S4. Mutation rate is positively correlated with 4NQO, MMS and H_2O_2 sensitivity in the segregant panel.

Trait values for mutagens are proxy measurements for mutagen resistance. As shown in the last row, mutation rate is negatively correlated with 4NQO, MMS and H_2O_2 resistances, meaning mutation rate is positively correlated with the sensitivity of these mutagens. Data are displayed in the lower triangle and the linear Pearson correlation values are shown in the upper triangle. The

blue lines show the linear regression fit for the points. The red lines show the locally weighted scatterplot smoothing (LOWESS) fit for the points. The slopes of the lines indicate the correlation between the sensitivity of different mutagens and mutation rate.

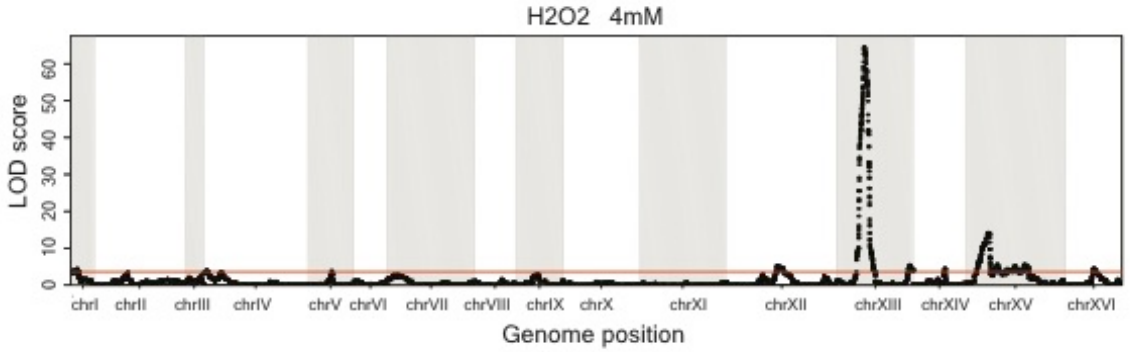

Figure S5. Loci underlie the H_2O_2 sensitivity.

LOD scores of sensitivity for H_2O_2 (4mM) are plotted against the genetic map. The red line indicates the significant threshold (3.64) from 1000 permutations.

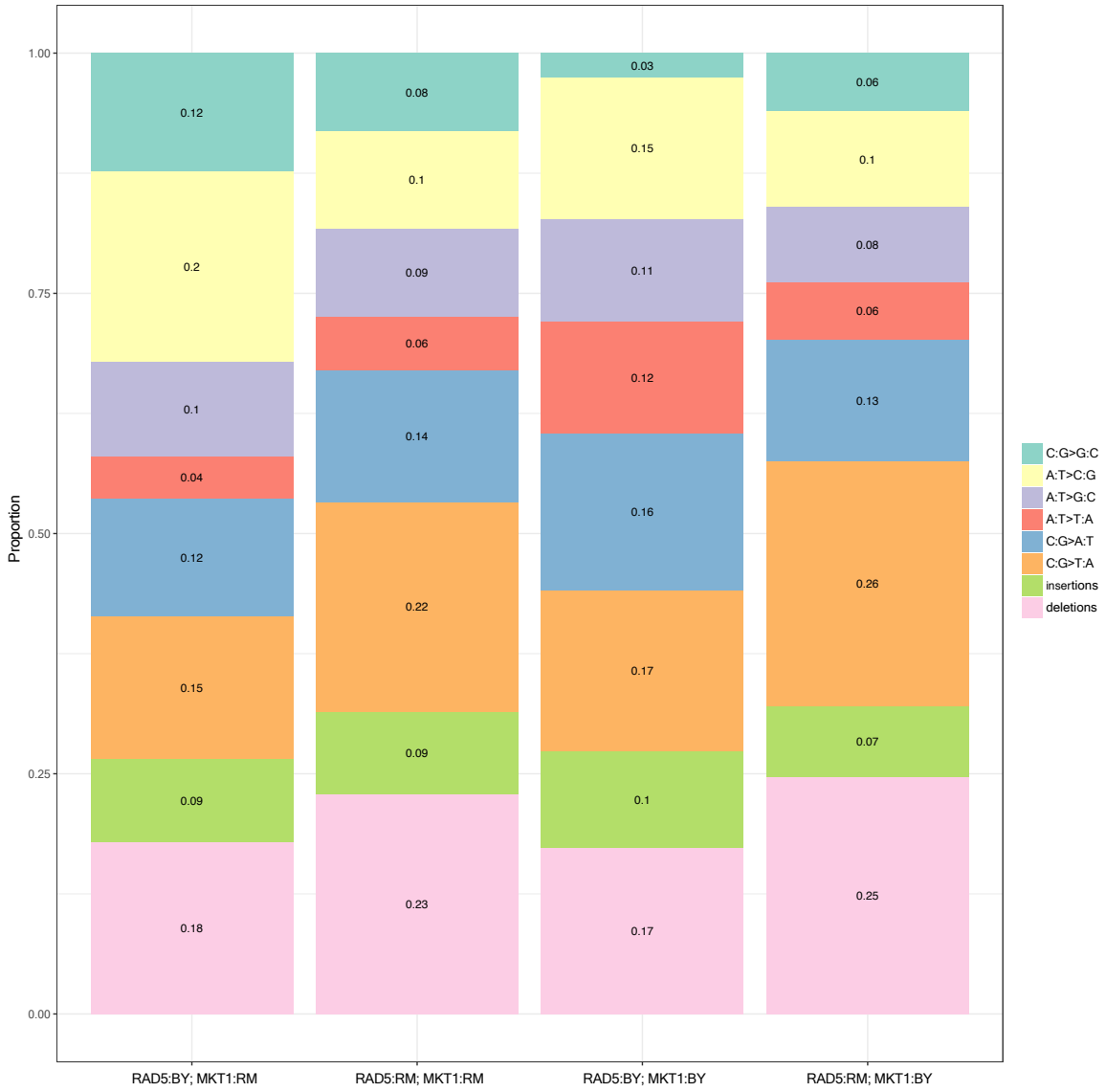

Figure S6. The proportions of the possible base pair substitution types and indels in different segregant groups.

The color represents six different types of base pair substitutions, and the proportion of each substitution type or indels is labeled on the figure.

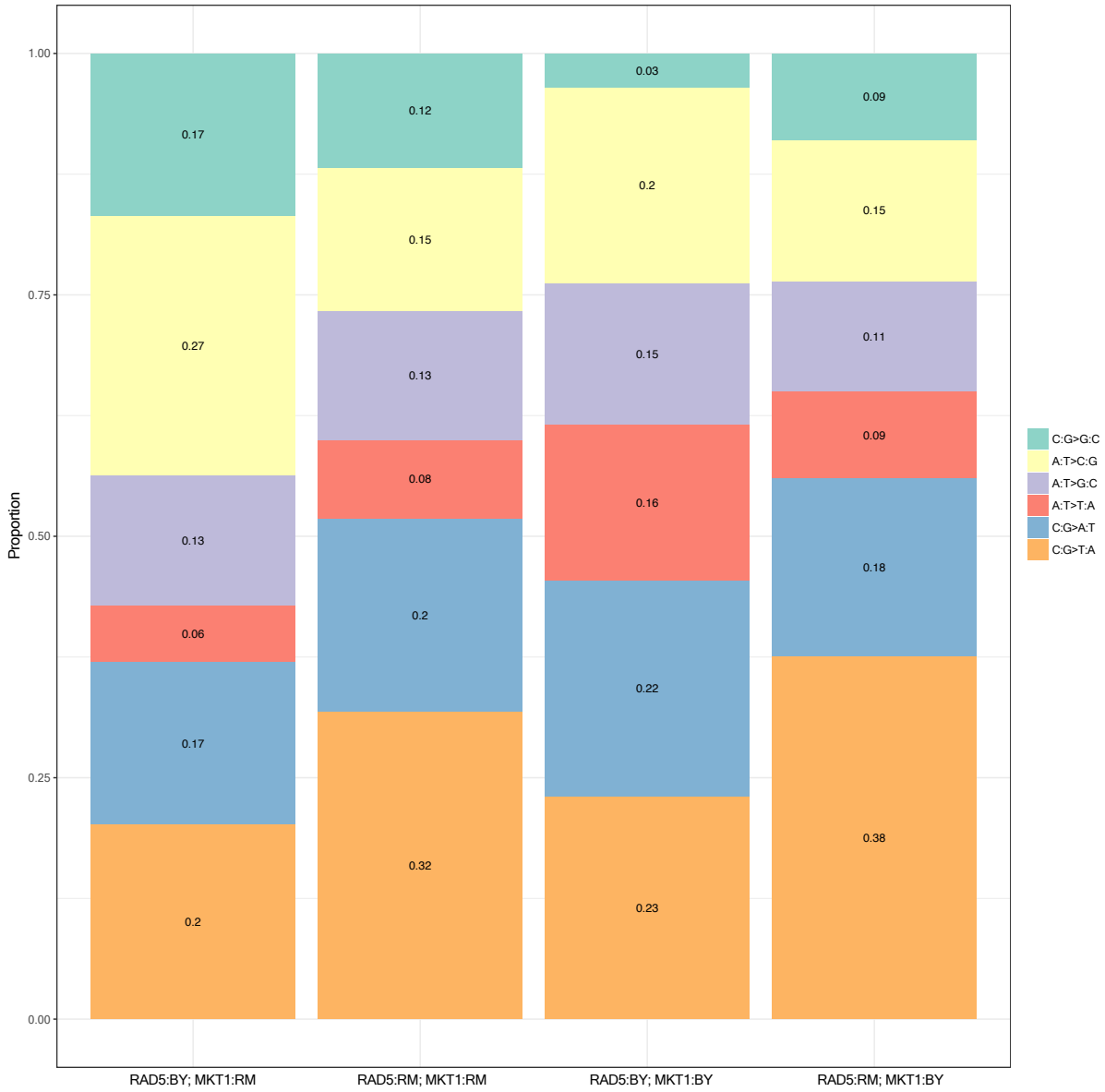

Figure S7. The proportions of the possible base pair substitution types in different segregant groups.

The color represents six different types of base pair substitutions, and the proportion of each type is labeled on the figure.

Table S1. The mutation rate of seven natural yeast strains.

Strain	Mutation rate	Std. Error
BY4724	1.7×10^{-7}	2.2×10^{-7}
RM11-1a	5.8×10^{-7}	4.0×10^{-7}
CBS2888a	1.1×10^{-7}	7.2×10^{-8}
I14	2.8×10^{-7}	9.0×10^{-8}
YJM454a	1.7×10^{-7}	1.1×10^{-7}
YST133	2.2×10^{-7}	1.1×10^{-7}
YST195	1.7×10^{-7}	5.3×10^{-7}

Mutation rate shown in the table is the mean of ninety-six replicates.

Table S2. The number of segregants and the allele at gene *RAD5* and *MKT1* of each group.

Allele at *RAD5*	RM	RM	BY	BY
Allele at *MKT1*	RM	BY	RM	BY
Number of segregants	281	230	252	277

We divided 1040 segregants into four groups based on their genotypes at *RAD5* and *MKT1*. The genotype and the number of segregants within each group is shown in the above table.

Table S3. The *CAN1* region amplicon sequencing read counts of segregants in four groups.

Allele at *RAD5*	RM	RM	BY	BY
Allele at *MKT1*	RM	BY	RM	BY
Original read counts	91722	248508	190548	160182
Adjusted read counts	91722	92433	88963	89090

Segregants were assigned into four groups based on their alleles at gene *RAD5* and *MKT1* (Table S2). The *CAN1* coding region of the segregants in each group was amplified and sequenced. The number of the original aligned read counts and the adjusted read counts for each library is shown in the table. The read counts were adjusted by a down-sampling process that is described in the methods section.

Table S4. The mutation spectra of the four groups.

Type of mutation	Number of mutations detected			
Allele				
RAD5	RM	RM	BY	BY
MKT1	RM	BY	RM	BY
Transition				
C:G → T:A	43	59	24	33
A:T → G:C	18	18	16	21
Transitions total	61	77	40	54
Transversion				
C:G → A:T	27	29	20	32
C:G → G:C	16	14	20	5
A:T → T:A	11	14	7	23
A:T → C:G	20	23	32	29
Transversions total	74	80	79	89
One base pair indels				
Insertions	17	17	14	20
Deletions	45	57	29	34
Indels total	62	74	43	54
Total	197	231	162	197

File S1. The mutation rate score for the segregants.

We performed fluctuation analysis on 1040 segregants between BY and RM. We control the quality of measure by removing 197 individuals with poor measure. 843 segregants were left and used for linkage mapping. This file contains the mutation rate score (the residue of m) and the label for each segregants. The labels corresponds to the labels in the genotype data, which is available at https://github.com/gouliangke/Mutation-rate/tree/master/genotype.

File S2. The estimated mutation rate for allele replacement strains.

The file contains the mutation rate for six strains (BY, RM, BY:*RAD5*, RM:*MKT1*, BY:*RAD5*-I791S, BY:*RAD5*-E783D), and for each strain 96 replicates were performed.

File S3. The primers used for amplifying the *CAN1* gene region.

Eight primers were used to amplify the coding region of gene *CAN1*, each primer has the linked MiSeq adapter sequence.