Supplementary Figure 1

A.



B.



**Determining fitness effect of *msh2*𝛥 A.** In a head-to-head competition we found a determinantal fitness effect associated with the maintenance of the plasmid, which should insure maintenance of the plasmid through out the experiment. There was also found to be a small, 1.5%/ generation fitness effect of being a *msh2∆* mutant.  **B.** Chemostats were inoculated with the stated genotype and the number of canavanine resistant per 107 cells were tracked over the course of ~50 generations.

Supplementary Fig 2

A.



B.



**Scatter plot of WT barcode read count by mutation rate.** Red line indicates cut off for minimum read number to be included in mutation rate assessment. Cut off was determined by highest reduction in standard deviation in mutation rate calculations.

Supplementary Figure 3

A.



B.



**Determining filters for fitness effects. A.** Blue lines indicate 1 standard deviation from the median of all barcodes. Barcodes outside 1 std deviation were not used in the analysis. **B.** Spearman correlation of fitness values of WT barcodes between chemostat replicates.

Supplementary Figure 4

A.



B.



**Comparing results across biological replicates A**. Comparison of fold change calculations by barcode across all 4 chemostat replicates **B.** Comparison of fold change across 4 replicates by variant, correlation is spearman correlation.

Supplementary Figure 5



**Replicate PCRS for 3 time points.** For 3 time points, two separate PCRS were done on the same plasmid prep and compared. þ = Spearman’s correlation between replicate PCRS of the same plasmid prep.

Supplementary Figure 6

A.



B.



**Fold change in mutation rate by competitive fitness, broken down by barcode A.** Barcodes that were used in mutation rate assessment compared to the competitive fitness calculations for that barcode. **B.** A, Zoomed in to 10 fold over WT on the y axis.

Supplementary Figure 7



**Barcode coverage of variants after quality filters** A histogram of the number of barcodes per variant.

Supplementary Figure 8

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**Comparison of Jia *et al* deep mutational scan and scores found in this study.**  A scatter plot comparing scores found in pooled barcoded assay (Log2 (Fold Change over WT)) to the loss-of-function score in Jia *et al.* 2021*.*

| **SUPPLEMENTARY TABLE 1** | | | | | |
| --- | --- | --- | --- | --- | --- |
| Yeast Msh2 Variant | Counta | Median Mutation Rate  Unbarcodedb | IQRc | Gammie Mutation Rated | Gammie Phenotypee |
| S318Y | 3 | 1.70E-07 | 2.95E-07 | 9.90E-07 | Wild Type Like |
| Q852E | 4 | 3.63E-07 | 4.00E-07 | 8.30E-07 | Wild Type Like |
| K873E | 4 | 3.80E-07 | 3.03E-07 | 3.10E-06 | Loss of Function |
| C67Y | 4 | 5.20E-07 | 5.64E-07 | NA | Loss of Function |
| S318C | 4 | 5.29E-07 | 2.54E-07 | 1.10E-06 | Wild Type Like |
| L402F | 4 | 5.47E-07 | 3.85E-07 | 7.30E-07 | Wild Type Like |
| G692S | 4 | 5.81E-07 | 4.85E-07 | 6.70E-07 | Wild Type Like |
| R352H | 4 | 5.86E-07 | 3.18E-07 | 5.10E-07 | Wild Type Like |
| G317D | 4 | 5.97E-07 | 4.17E-07 | 6.70E-07 | Wild Type Like |
| A892G | 4 | 6.19E-07 | 4.71E-07 | 7.70E-07 | Wild Type Like |
| I141M | 4 | 6.31E-07 | 2.45E-07 | 7.60E-07 | Wild Type Like |
| A733V | 4 | 6.53E-07 | 3.15E-07 | 7.00E-07 | Wild Type Like |
| T347I | 4 | 7.31E-07 | 4.25E-07 | 3.80E-06 | Wild Type Like |
| E912G | 4 | 7.97E-07 | 1.60E-07 | 1.10E-06 | Wild Type Like |
| Q61P | 4 | 8.33E-07 | 9.06E-07 | NA | Wild Type Like |
| E580V | 4 | 9.85E-07 | 3.97E-07 | 5.40E-07 | Wild Type Like |
| M707I | 4 | 9.92E-07 | 3.92E-07 | 2.10E-06 | Loss of Function |
| T44M | 2 | 1.09E-06 | 7.29E-07 | 5.50E-07 | Wild Type Like |
| G122S | 4 | 1.18E-06 | 8.12E-07 | 1.10E-06 | Wild Type Like |
| Y104C | 4 | 1.58E-06 | 1.93E-07 | 4.40E-07 | Wild Type Like |
| G711R | 4 | 2.00E-06 | 4.67E-07 | NA | Loss of Function |
| T743I | 4 | 2.42E-06 | 2.94E-06 | NA | Loss of Function |
| A618V | 4 | 2.49E-06 | 2.44E-06 | NA | Loss of Function |
| L521P | 4 | 3.20E-06 | 3.64E-06 | 1.40E-05 | Loss of Function |
| L457P | 4 | 3.39E-06 | 3.20E-06 | NA | Loss of Function |
| G693D | 4 | 3.68E-06 | 1.24E-06 | NA | Loss of Function |
| C345R | 4 | 3.76E-06 | 1.26E-06 | NA | Loss of Function |
| C345Y | 4 | 4.03E-06 | 1.32E-06 | NA | Loss of Function |
| D524Y | 4 | 4.04E-06 | 2.39E-06 | NA | Loss of Function |
| R371S | 4 | 4.11E-06 | 2.87E-06 | NA | Loss of Function |
| G772D | 4 | 4.25E-06 | 1.47E-06 | NA | Loss of Function |
| P640L | 4 | 4.38E-06 | 5.25E-07 | NA | Loss of Function |
| D621N | 4 | 4.54E-06 | 2.60E-06 | NA | Loss of Function |
| G772E | 4 | 4.65E-06 | 2.20E-06 | NA | Loss of Function |
| S695N | 4 | 4.68E-06 | 1.94E-06 | NA | Loss of Function |
| C716R | 4 | 4.97E-06 | 5.30E-06 | NA | Loss of Function |
| G350R | 4 | 5.05E-06 | 5.17E-06 | 1.50E-05 | Loss of Function |
| S742L | 4 | 5.21E-06 | 4.10E-06 | 1.80E-05 | Loss of Function |
| S762Y | 4 | 5.79E-06 | 5.79E-06 | NA | Loss of Function |
| G711D | 4 | 5.82E-06 | 1.93E-06 | NA | Loss of Function |
| C195R | 4 | 5.87E-06 | 3.04E-06 | NA | Loss of Function |
| G770R | 4 | 6.51E-06 | 6.18E-06 | NA | Loss of Function |
| G688D | 4 | 6.77E-06 | 5.42E-06 | NA | Loss of Function |
| H658R | 4 | 7.28E-06 | 1.65E-06 | NA | Loss of Function |
| E747K | 4 | 8.84E-06 | 4.30E-06 | NA | Loss of Function |
| G693D-S695T | 4 | 1.05E-05 | 2.44E-06 | NA | Loss of Function |

a. Number of chemostat replicates.

b. Median mutation rate (mutants per cell division) found using the unbarcoded assay on previously published variants. Data is sorted by this value from least to most.

c. Interquartile range of the mutation rate calculations in the unbarcoded assay.

d. Mutation Rate (mutants per cell division) found in previous Gammie *et al.* 2007 study using traditional fluctuation assays. N/A represents mutation rate data was not calculated in the previous study.

e. The repair phenotype found in previous Gammie *et al.* 2007 study as determined by fluctuation assays, qualitative patch assays, and yeast two hybrid experiments assaying the interaction between Msh2 and subunit partners Msh3 and Msh6.

| **SUPPLEMENTARY TABLE 2** | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Yeast Msh2 Genotypea | Human Msh2 Genotypeb | AlleleIDc | countd | Fold Induction CANe | IQRf | Scoreg | 95% CIh | Sigi | ClinVarj | Star Ratingk |
| P27L | P27L | 181892 | 7 | 0.48 | 0.80 | -1.06 | 0.87 | ns | VUS | 2 |
| P27R | P27R | 558125 | 7 | 1.86 | 1.28 | 0.90 | 0.53 | ns | VUS | 1 |
| P27T | P27T | 238804 | 6 | 0.89 | 0.65 | -0.18 | 0.50 | ns | VUS | 1 |
| T33P | T33P | 96743 | 5 | 2.52 | 2.50 | 1.34 | 0.81 | ns | VUS | 2 |
| Y43D | Y43D | 551834 | 7 | 4.31 | 2.16 | 2.11 | 0.47 | \*\*\* | VUS | 2 |
| Y43D\_Y606\* | - | - | 4 | 6.36 | 5.83 | 2.67 | 0.91 | \*\* | - | - |
| Y43F | Y43F | 405752 | 10 | 1.16 | 1.00 | 0.21 | 0.71 | ns | VUS | 2 |
| Y43H | Y43H | 181897 | 15 | 1.58 | 1.15 | 0.66 | 0.44 | ns | VUS | 1 |
| A54S | A54S | 181900 | 16 | 0.92 | 0.40 | -0.11 | 0.34 | ns | VUS | 2 |
| A54T | A54T | 232513 | 24 | 1.22 | 0.84 | 0.29 | 0.43 | ns | VUS | 2 |
| A54T\_E798V | A54T\_E768V | 232513 | 4 | 0.70 | 0.21 | -0.52 | 0.62 | ns | VUS | 2 |
| A54V | A54V | 472743 | 20 | 1.10 | 0.58 | 0.14 | 0.37 | ns | VUS | 1 |
| V84I | V78I | 238809 | 8 | 1.28 | 1.07 | 0.36 | 0.70 | ns | VUS | 2 |
| L91F | - | - | 4 | 1.37 | 0.41 | 0.45 | 0.38 | ns | - | - |
| G103E\_G317V | - | - | 4 | 1.27 | 0.60 | 0.34 | 0.49 | ns | - | - |
| N123T | N127T | 558488 | 4 | 0.98 | 0.53 | -0.02 | 0.79 | ns | VUS | 1 |
| G158A | G162A | 96581 | 30 | 0.96 | 0.97 | -0.05 | 0.34 | ns | VUS | 2 |
| G158R | G162R | 96580 | 19 | 2.14 | 2.15 | 1.10 | 0.51 | \*\* | P | 3 |
| G158T | - | - | 4 | 3.06 | 2.97 | 1.62 | 1.14 | \* | - | - |
| V159A | V163A | 232532 | 7 | 1.39 | 1.44 | 0.47 | 0.93 | ns | VUS | 1 |
| D163H | D167H | 96587 | 8 | 1.28 | 0.25 | 0.35 | 0.26 | ns | B | 3 |
| L183F | L187F | 472789 | 14 | 1.19 | 1.31 | 0.25 | 0.52 | ns | VUS | 2 |
| L183I | L187I | 472788 | 16 | 1.63 | 3.75 | 0.71 | 0.84 | ns | VUS | 1 |
| L183P | L187P | 96609 | 21 | 6.74 | 5.88 | 2.75 | 0.61 | \*\*\*\* | P | 3 |
| L183R | L187R | 96610 | 15 | 6.10 | 5.09 | 2.61 | 0.65 | \*\*\*\* | P | 3 |
| I188M | I192M | 561579 | 4 | 0.61 | 0.95 | -0.72 | 1.48 | ns | VUS | 1 |
| E194G | E198G | 96620 | 12 | 0.94 | 1.27 | -0.09 | 0.78 | ns | B | 3 |
| G221W\_M702V | - | - | 4 | 2.26 | 1.20 | 1.17 | 0.88 | ns | - | - |
| L226F\_T754A | - | - | 4 | 2.27 | 0.61 | 1.18 | 0.55 | ns | - | - |
| A267V | A272V | 50090 | 18 | 1.14 | 0.99 | 0.19 | 0.48 | ns | B | 3 |
| L305P | L310P | 96720 | 17 | 7.39 | 7.24 | 2.89 | 0.66 | \*\*\*\* | P | 3 |
| L305R | L310R | 96721 | 14 | 9.50 | 13.27 | 3.25 | 0.74 | \*\*\*\* | CIP | 1 |
| L305V | L310V | 232559 | 47 | 1.14 | 2.01 | 0.19 | 0.35 | ns | VUS | 1 |
| G317D | G322D | 16801 | 8 | 1.10 | 0.65 | 0.14 | 0.38 | ns | B | 3 |
| G317S | G322S | 181927 | 29 | 0.95 | 0.72 | -0.07 | 0.32 | ns | VUS | 2 |
| G317V | G322V | 152230 | 24 | 1.30 | 0.53 | 0.38 | 0.51 | ns | VUS | 2 |
| N331D\_P361L | - | - | 4 | 16.22 | 6.44 | 4.02 | 0.47 | \*\* | - | - |
| C345R | C333R | 96747 | 7 | 8.96 | 5.86 | 3.16 | 0.85 | \*\*\* | LP | 2 |
| G350E | G338E | 95979 | 11 | 9.74 | 8.43 | 3.28 | 0.79 | \*\*\*\* | P | 3 |
| G350V | G338V | 560675 | 14 | 7.98 | 15.14 | 3.00 | 0.76 | \*\*\*\* | VUS | 1 |
| P361A | P349A | 95987 | 61 | 1.79 | 2.03 | 0.84 | 0.30 | \*\*\* | CIP | 1 |
| P361L | P349L | 95989 | 30 | 5.80 | 4.69 | 2.54 | 0.39 | \*\*\*\* | P | 3 |
| P361R | P349R | 95988 | 53 | 5.06 | 8.95 | 2.34 | 0.42 | \*\*\*\* | P | 3 |
| P361R\_E761\* | - | - | 4 | 3.70 | 2.19 | 1.89 | 1.22 | ns | - | - |
| P361S | P349S | 482373 | 47 | 7.48 | 8.01 | 2.90 | 0.43 | \*\*\*\* | VUS | 2 |
| P361T | P349T | 405779 | 30 | 4.71 | 7.79 | 2.24 | 0.54 | \*\*\*\* | LP | 1 |
| R371K | R359K | 472774 | 7 | 2.25 | 1.26 | 1.17 | 0.41 | \* | VUS | 2 |
| R371S | R359S | 96014 | 4 | 6.18 | 8.38 | 2.63 | 1.36 | \* | P | 3 |
| Q386H | Q374H | 133083 | 31 | 1.06 | 0.85 | 0.09 | 0.29 | ns | VUS | 2 |
| Q386R | Q374R | 232572 | 47 | 1.15 | 1.29 | 0.20 | 0.42 | ns | CIP | 1 |
| Q386R\_A627V | Q374R\_A609V | 232572 | 4 | 0.68 | 0.41 | -0.56 | 0.67 | ns | CIP | 1 |
| L402F | L390F | 50080 | 25 | 0.85 | 0.88 | -0.23 | 0.35 | ns | B | 3 |
| L402V | L390V | 482375 | 29 | 1.02 | 1.68 | 0.03 | 0.53 | ns | VUS | 1 |
| L457F | L440F | 472876 | 28 | 0.97 | 0.85 | -0.05 | 0.34 | ns | VUS | 2 |
| L457P | L440P | 96100 | 12 | 4.63 | 7.57 | 2.21 | 0.93 | \*\*\* | P | 3 |
| K466N | K449N | 150578 | 10 | 0.56 | 0.28 | -0.84 | 0.44 | \*\* | VUS | 2 |
| M470K | M453K | 96113 | 5 | 1.98 | 1.50 | 0.99 | 0.64 | ns | P | 3 |
| F523L\_V725L | - | - | 4 | 4.74 | 3.08 | 2.25 | 0.82 | \* | - | - |
| D526G | - | - | 7 | 0.82 | 0.54 | -0.29 | 0.46 | ns | - | - |
| R542C | R524C | 405792 | 13 | 3.61 | 7.10 | 1.85 | 0.89 | \*\*\* | VUS | 2 |
| R542H | R524H | 180015 | 9 | 3.49 | 5.85 | 1.80 | 1.05 | \* | VUS | 2 |
| R542L | R524L | 96176 | 12 | 4.18 | 2.22 | 2.06 | 0.85 | \*\*\* | VUS | 2 |
| D547E | - | - | 4 | 1.24 | 1.85 | 0.31 | 1.47 | ns | - | - |
| T571N | - | - | 4 | 1.05 | 0.72 | 0.06 | 0.70 | ns | - | - |
| Q573R | - | - | 8 | 0.74 | 0.20 | -0.44 | 0.66 | ns | - | - |
| L617S | L599S | 226299 | 16 | 1.26 | 0.93 | 0.33 | 0.39 | ns | VUS | 2 |
| A627P | A609P | 427153 | 12 | 2.56 | 3.36 | 1.36 | 0.73 | \*\* | VUS | 1 |
| A627S | A609S | 232602 | 23 | 1.08 | 1.75 | 0.11 | 0.58 | ns | VUS | 2 |
| A627V | A609V | 96270 | 19 | 1.40 | 0.58 | 0.49 | 0.40 | ns | VUS | - |
| A627V\_Q748E | A609V\_Q718E | 96270 | 4 | 1.14 | 1.38 | 0.19 | 1.12 | ns | VUS | - |
| P634R | P616R | 133093 | 11 | 1.13 | 0.56 | 0.17 | 0.52 | ns | VUS | 2 |
| P634S | P616S | 152379 | 6 | 2.31 | 1.66 | 1.21 | 1.00 | ns | VUS | 2 |
| R639P | R621P | 473027 | 13 | 9.28 | 10.78 | 3.21 | 0.74 | \*\*\*\* | VUS | 2 |
| R639Q | R621Q | 451338 | 4 | 8.29 | 2.07 | 3.05 | 0.46 | \*\* | VUS | 2 |
| P640A | P622A | 181966 | 9 | 4.12 | 3.17 | 2.04 | 0.61 | \*\*\* | VUS | 1 |
| P640Q | P622Q | 416960 | 5 | 3.95 | 2.00 | 1.98 | 0.53 | \*\* | VUS | 1 |
| P640T | P622T | 96280 | 7 | 6.96 | 4.45 | 2.80 | 0.88 | \*\* | LP | 2 |
| K641R | - | - | 4 | 2.47 | 2.15 | 1.30 | 1.41 | ns | - | - |
| E673K | E643K | 171076 | 24 | 4.41 | 5.92 | 2.14 | 0.46 | \*\*\*\* | VUS | 2 |
| Q675E | Q645E | 96297 | 27 | 0.93 | 0.83 | -0.11 | 0.37 | ns | VUS | 2 |
| Q675H | Q645H | 139567 | 29 | 1.30 | 1.29 | 0.38 | 0.40 | ns | VUS | 2 |
| D676A | D646A | 238851 | 22 | 1.36 | 1.36 | 0.44 | 0.55 | ns | VUS | 2 |
| D676G | D646G | 427155 | 17 | 0.97 | 0.65 | -0.04 | 0.45 | ns | VUS | 2 |
| I678N | I648N | 232607 | 43 | 0.87 | 0.95 | -0.21 | 0.32 | ns | VUS | 2 |
| I681V | I651V | 238853 | 70 | 1.07 | 1.14 | 0.09 | 0.28 | ns | VUS | 2 |
| D684E | D654E | 232610 | 53 | 1.04 | 1.10 | 0.05 | 0.29 | ns | VUS | 1 |
| V685I | V655I | 133094 | 18 | 1.15 | 0.91 | 0.21 | 0.38 | ns | VUS | 3 |
| E688G | E658G | 180019 | 71 | 1.10 | 1.11 | 0.13 | 0.28 | ns | VUS | 2 |
| K691E | K661E | 518338 | 9 | 1.08 | 1.12 | 0.10 | 0.53 | ns | VUS | 1 |
| K691R | K661R | 558514 | 11 | 0.88 | 1.50 | -0.18 | 0.63 | ns | VUS | 1 |
| F694L | F664L | 472943 | 16 | 0.97 | 0.68 | -0.05 | 0.50 | ns | VUS | 1 |
| I697V | I667V | 232613 | 8 | 0.81 | 0.39 | -0.30 | 0.94 | ns | VUS | 1 |
| T698P | T668P | 405811 | 17 | 5.03 | 11.27 | 2.33 | 0.85 | \*\*\*\* | VUS | 1 |
| G699A | G669A | 96328 | 32 | 0.96 | 0.72 | -0.07 | 0.34 | ns | VUS | - |
| G699C | G669C | 473032 | 12 | 8.08 | 5.35 | 3.01 | 0.51 | \*\*\*\* | CIP | 1 |
| G699D | G669D | 96327 | 18 | 6.63 | 11.83 | 2.73 | 0.67 | \*\*\*\* | VUS | 1 |
| G699D\_Frame\_Shift | G669D | 96327 | 5 | 7.35 | 8.09 | 2.88 | 0.71 | \*\* | VUS | 1 |
| G699R | G669R | 96319 | 13 | 4.90 | 2.62 | 2.29 | 0.57 | \*\*\*\* | VUS | 3 |
| G699V | G669V | 96329 | 35 | 8.77 | 15.24 | 3.13 | 0.54 | \*\*\*\* | P | 3 |
| P700H | P670H | 244390 | 10 | 1.56 | 1.53 | 0.64 | 0.80 | ns | VUS | 2 |
| P700L | P670L | 96330 | 8 | 1.23 | 0.89 | 0.29 | 0.65 | ns | VUS | 2 |
| P700R | P670R | 451379 | 6 | 6.31 | 6.23 | 2.66 | 0.80 | \*\* | VUS | 2 |
| P700S | P670S | 561652 | 8 | 2.18 | 2.96 | 1.12 | 0.93 | ns | VUS | 2 |
| N701D | N671D | 232615 | 13 | 8.61 | 6.56 | 3.11 | 0.53 | \*\*\*\* | VUS | 1 |
| N701K | N671K | 96333 | 4 | 2.50 | 1.40 | 1.32 | 0.57 | ns | LP | 1 |
| N701S | N671S | 575673 | 6 | 0.87 | 1.40 | -0.20 | 1.02 | ns | VUS | 2 |
| N701Y | N671Y | 96332 | 6 | 4.58 | 11.53 | 2.20 | 1.80 | ns | VUS | - |
| M702R | M672R | 181973 | 32 | 1.61 | 3.37 | 0.69 | 0.52 | \* | VUS | 2 |
| M702V | M672V | 432518 | 37 | 2.27 | 2.37 | 1.18 | 0.41 | \*\*\*\* | VUS | 1 |
| G704D | G674D | 96336 | 9 | 7.84 | 5.84 | 2.97 | 1.16 | \*\* | LP | 3 |
| G704R | G674R | 96335 | 6 | 9.94 | 8.29 | 3.31 | 1.18 | \*\*\* | LP | 3 |
| G704R\_Frame\_Shift | G674R | 96335 | 4 | 5.34 | 5.47 | 2.42 | 0.93 | \* | LP | 3 |
| K705E | K675E | 393142 | 8 | 3.66 | 7.49 | 1.87 | 1.04 | \*\*\* | VUS | 1 |
| S706L | S676L | 427156 | 5 | 4.18 | 1.43 | 2.06 | 0.70 | \*\* | VUS | 1 |
| S706N | S676N | - | 5 | 6.58 | 2.51 | 2.72 | 0.54 | \*\* | - | - |
| S706P | S676P | 96339 | 8 | 2.48 | 4.55 | 1.31 | 0.84 | \* | VUS | - |
| T707A | T677A | 482416 | 22 | 1.50 | 1.94 | 0.59 | 0.57 | ns | VUS | 2 |
| T707R | T677R | 232616 | 13 | 7.41 | 4.94 | 2.89 | 0.49 | \*\*\*\* | VUS | 2 |
| Y708C | Y678C | 482393 | 64 | 1.04 | 1.02 | 0.06 | 0.27 | ns | VUS | 2 |
| Y708H | Y678H | 232617 | 25 | 1.09 | 1.04 | 0.13 | 0.39 | ns | VUS | 2 |
| R710G | R680G | 181976 | 19 | 1.09 | 1.92 | 0.13 | 0.59 | ns | VUS | 2 |
| R710P | R680P | 472861 | 9 | 13.00 | 17.19 | 3.70 | 1.15 | \*\*\* | VUS | 1 |
| R710Q | R680Q | 561662 | 27 | 1.51 | 1.71 | 0.59 | 0.45 | ns | VUS | 2 |
| Q711E | Q681E | 180020 | 20 | 2.09 | 2.69 | 1.06 | 0.48 | \*\* | VUS | 2 |
| Q711H | Q681H | 180021 | 5 | 1.30 | 0.70 | 0.38 | 0.69 | ns | VUS | 2 |
| G713E | G683E | 482418 | 54 | 2.88 | 3.62 | 1.53 | 0.35 | \*\*\*\* | VUS | 2 |
| G713R | G683R | 96343 | 17 | 5.35 | 5.22 | 2.42 | 0.68 | \*\*\*\* | P | 3 |
| G713V | G683V | 212198 | 68 | 1.14 | 1.01 | 0.19 | 0.28 | ns | VUS | 2 |
| G713W | G683W | 96344 | 18 | 5.81 | 6.00 | 2.54 | 0.59 | \*\*\*\* | VUS | - |
| V714L | V684L | 392793 | 8 | 0.99 | 0.79 | -0.02 | 0.56 | ns | VUS | 1 |
| I715M | I685M | 518347 | 8 | 1.38 | 1.26 | 0.46 | 0.80 | ns | VUS | 2 |
| I715V | I685V | 389527 | 6 | 1.20 | 1.28 | 0.27 | 0.88 | ns | VUS | 2 |
| L717P | L687P | 96348 | 4 | 6.13 | 14.40 | 2.62 | 2.82 | ns | CIP | 1 |
| M718R | M688R | 96349 | 9 | 8.98 | 7.41 | 3.17 | 1.17 | \*\*\* | P | 3 |
| A719P | A689P | 419393 | 9 | 6.27 | 5.14 | 2.65 | 0.90 | \*\* | VUS | 1 |
| A719V | A689V | 393145 | 11 | 3.17 | 2.58 | 1.66 | 0.58 | \*\* | VUS | 1 |
| Q720E | Q690E | 96351 | 15 | 6.03 | 8.01 | 2.59 | 0.64 | \*\*\*\* | VUS | - |
| G722D | - | - | 6 | 5.04 | 2.43 | 2.33 | 0.54 | \*\* | - | - |
| G722E | G692E | 259741 | 14 | 5.04 | 8.82 | 2.33 | 0.52 | \*\*\*\* | CIP | 1 |
| G722R | G692R | 96353 | 11 | 4.82 | 4.60 | 2.27 | 0.92 | \*\*\* | LP | 3 |
| G722V | G692V | 96355 | 16 | 5.37 | 3.09 | 2.42 | 0.58 | \*\*\*\* | LP | 3 |
| G722W | G692W | 419395 | 23 | 6.33 | 6.60 | 2.66 | 0.61 | \*\*\*\* | CIP | 1 |
| C723R | C693R | 561664 | 13 | 5.00 | 10.95 | 2.32 | 0.80 | \*\*\*\* | VUS | 2 |
| C723Y | C693Y | 380277 | 4 | 9.70 | 3.71 | 3.28 | 0.90 | \*\* | VUS | 1 |
| F724S | F694S | 419396 | 6 | 4.98 | 4.31 | 2.32 | 1.37 | \*\*\* | LP | 1 |
| F724S\_A972D | - | - | 4 | 18.83 | 23.31 | 4.24 | 2.50 | ns | - | - |
| V725L | V695L | 551836 | 12 | 2.08 | 1.77 | 1.06 | 0.57 | \* | VUS | 2 |
| P726L | P696L | 96356 | 10 | 3.21 | 11.41 | 1.68 | 1.06 | \*\*\* | P | 3 |
| P726S | P696S | 472955 | 5 | 3.27 | 1.66 | 1.71 | 0.98 | \* | VUS | 2 |
| C727F | C697F | 96358 | 4 | 4.59 | 8.06 | 2.20 | 1.79 | \* | P | 3 |
| C727R | C697R | 96357 | 13 | 8.62 | 12.63 | 3.11 | 0.93 | \*\*\*\* | P | 3 |
| C727Y | C697Y | 181980 | 16 | 7.27 | 12.14 | 2.86 | 0.74 | \*\*\*\* | LP | 3 |
| A730E | A700E | 232619 | 9 | 2.78 | 3.79 | 1.48 | 1.16 | \*\* | VUS | 1 |
| E731A | E701A | 232621 | 10 | 1.08 | 1.01 | 0.11 | 0.97 | ns | VUS | 2 |
| I734T | I704T | 232622 | 11 | 1.32 | 0.85 | 0.40 | 0.58 | ns | VUS | 2 |
| I734V | I704V | 180022 | 19 | 0.99 | 0.75 | -0.01 | 0.33 | ns | VUS | 2 |
| V735L | V705L | 451166 | 19 | 0.93 | 0.95 | -0.10 | 0.43 | ns | VUS | 1 |
| V735M | V705M | 558518 | 33 | 0.81 | 1.32 | -0.31 | 0.38 | ns | VUS | 1 |
| D736E | D706E | 232623 | 20 | 0.80 | 0.66 | -0.31 | 0.35 | ns | VUS | 1 |
| I738T | I708T | 473041 | 4 | 1.05 | 1.80 | 0.07 | 1.48 | ns | VUS | 2 |
| I738V | I708V | 392902 | 9 | 1.12 | 2.41 | 0.16 | 0.97 | ns | VUS | 2 |
| L739V | L709V | 392905 | 16 | 1.47 | 1.43 | 0.55 | 0.55 | ns | VUS | 1 |
| R741L | R711L | 518447 | 11 | 1.43 | 2.03 | 0.52 | 0.80 | ns | VUS | 2 |
| R741Q | R711Q | 472967 | 25 | 1.62 | 2.63 | 0.69 | 0.55 | \* | VUS | 2 |
| A744V | A714V | 96382 | 12 | 1.01 | 0.91 | 0.01 | 0.79 | ns | VUS | 2 |
| K750E | K720E | 232625 | 6 | 1.69 | 0.77 | 0.75 | 0.70 | ns | VUS | 2 |
| S753C | S723C | 472863 | 16 | 1.38 | 1.51 | 0.47 | 0.51 | ns | VUS | 1 |
| T754A | T724A | 244392 | 35 | 1.13 | 1.06 | 0.18 | 0.33 | ns | VUS | 2 |
| T754I | - | - | 24 | 4.34 | 5.17 | 2.12 | 0.60 | \*\*\*\* | - | - |
| T754M | T724M | 180024 | 34 | 3.83 | 3.51 | 1.94 | 0.42 | \*\*\*\* | VUS | 2 |
| T754R | T724R | 472868 | 18 | 5.76 | 6.85 | 2.53 | 0.68 | \*\*\*\* | VUS | 1 |
| M756I | M726I | 152055 | 29 | 1.09 | 1.19 | 0.13 | 0.43 | ns | VUS | 2 |
| M756L | M726L | 558522 | 36 | 1.19 | 1.13 | 0.25 | 0.39 | ns | VUS | 1 |
| M756V | M726V | 451363 | 36 | 0.83 | 0.81 | -0.26 | 0.32 | ns | VUS | 2 |
| T762I | T732I | 483367 | 13 | 1.08 | 0.60 | 0.11 | 0.60 | ns | VUS | 1 |
| T762S | T732S | 180025 | 8 | 1.17 | 0.52 | 0.23 | 0.75 | ns | VUS | 1 |
| T762S\_A870E | - | - | 4 | 6.13 | 0.60 | 2.62 | 0.34 | \*\* | - | - |
| A763T | A733T | 212200 | 8 | 1.08 | 1.10 | 0.11 | 0.93 | ns | VUS | 2 |
| S764C | S734C | 451371 | 33 | 1.20 | 0.74 | 0.26 | 0.40 | ns | VUS | 2 |
| I765V | I735V | 171077 | 8 | 0.95 | 0.37 | -0.07 | 0.54 | ns | VUS | 2 |
| L766F | L736F | 232628 | 52 | 1.05 | 1.06 | 0.07 | 0.36 | ns | VUS | 1 |
| A769P | A739P | 483395 | 4 | 0.65 | 0.41 | -0.63 | 0.92 | ns | VUS | 1 |
| S773Y | - | - | 6 | 2.16 | 10.90 | 1.11 | 2.01 | ns | - | - |
| D778H | - | - | 4 | 4.46 | 6.04 | 2.16 | 2.27 | ns | - | - |
| E779K | E749K | 96417 | 10 | 8.92 | 12.42 | 3.16 | 0.92 | \*\*\*\* | LP | 3 |

a. The yeast *MSH2* genotype. Frame\_Shift means that at the position there was a frame shift mutation. \* means that there is a stop codon at that position. Data is sorted in amino acid order.

b. The corresponding human *MSH2* genotype. – indicates no homologous site in the human protein.

c. Allele ID for Clinvar. A – indicates that there is no Allele ID.

d. Count, the number of times a variant was assayed in total

e. For stated genotype, variant mutation rate (mutations per cell division) was compared WT. Barcode and chemostat replicates were combined to calculate the median.

f. Interquartile range of all barcode and chemostat replicates

g. For stated genotype, variant mutation rate (mutations per cell division) was compared WT. Barcode and chemostat replicates were combined to calculate the median and the score was Log2 transformed.

h. 95% confidence interval calculated on log2 transformed fold change values

i. Significance is calculated by a Wilcoxian Ranksum test with the Benjamini-Hochberg correction for multiple hypothesis testing. \*< 0.05   \*\* < 0.01  \*\*\*< 0.001 \*\*\*\*< 0.0001

j. Initial clinical classification as stated in ClinVar. B = Benign, CIP = Conflicting Interpretations of pathogenicity, LP = Likely Pathogenic, P = Pathogenic, VUS = Variants of uncertain significance

k. ClinVar star rating 1= Single submitter - criteria provided, 2 = criteria provided, multiple submitters, no conflicts, 3 = reviewed by expert panel, NA = Variant not in Clinvar

**SUPPLEMENTARY TABLE 3**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Yeast Msh2 Genotype | No Barcode  Mutation  Ratea | Barcoded Mutation  Rateb | Fold Induction CAN Barcodedc | Fold Induction CAN  Gammied | Gammie Phenotypee | Significantly different from WT  Barcodedf |
| A733V | 7.08E-07 | 4.99E-06 | 1.00 | 1 | Wild Type Like | ns |
| C345R | 4.13E-06 | 1.52E-05 | 8.96 | NA | Loss of Function | \*\*\* |
| C716R | 6.67E-06 | 2.26E-05 | 8.62 | NA | Loss of Function | \*\*\*\* |
| G317D | 7.91E-07 | 2.58E-06 | 1.10 | 1 | Wild Type Like | ns |
| G688D | 7.26E-06 | 2.16E-05 | 7.35 | NA | Loss of Function | \*\*\*\* |
| G688D | 7.26E-06 | 2.16E-05 | 7.35 | NA | Loss of Function | \*\* |
| G693D | 4.12E-06 | 1.93E-05 | 7.84 | NA | Loss of Function | \*\* |
| G711D | 6.47E-06 | 1.10E-05 | 5.04 | NA | Loss of Function | \*\* |
| G711R | 2.32E-06 | 2.09E-05 | 4.82 | NA | Loss of Function | \*\*\* |
| L402F | 5.34E-07 | 2.74E-06 | 0.85 | 2 | Wild Type Like | ns |
| L457P | 4.68E-06 | 1.22E-05 | 4.63 | NA | Loss of Function | \*\*\* |
| R371S | 4.01E-06 | 1.30E-05 | 6.18 | NA | Loss of Function | \* |
| S695N | 4.19E-06 | 1.18E-05 | 6.57 | NA | Loss of Function | \*\* |
| S762Y | 7.31E-06 | 1.43E-05 | 2.16 | NA | Loss of Function | ns |
| T743I | 3.55E-06 | 1.60E-05 | 4.34 | NA | Loss of Function | \*\*\*\* |

a. Mutation rate (mutants per cell division) found using the non-barcoded assay on previously reported variants

b. Mutation rate (mutants per cell division) found using the barcoded assay on variants generated during the study

c. In the barcoded assay, for stated genotype, variant mutation rate (mutants per cell division) was compared WT. Barcode and chemostat replicates were combined to calculate the median.

d. Fold increase over WT found in previous Gammie *et al.* 2007 study.

e. The repair phenotype found in previous Gammie *et al.* 2007 study as determined by fluctuation assays, qualitative patch assays, and yeast two hybrid experiments assaying the interaction between Msh2 and subunit partners Msh3 and Msh6.

f. In the barcoded experiment, significance is calculated by a Wilcoxian Ranksum test with the Benjamini-Hochberg correction for multiple hypothesis testing. \*< 0.05   \*\* < 0.01  \*\*\*< 0.001 \*\*\*\*< 0.0001. We would expect loss of function mutants to have a significantly increased mutation rate compared to WT.

**SUPPLEMENTARY TABLE 4**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Yeast Genotype | Human Genotypea | Luria-Delbrück Mutation Rateb | Mean Fold Induction CAN  Luria-Delbrückc | Lea-Coulson Mutation Rated | Mean Fold Induction CAN Lea-Coulsone | Median Fold Induction CAN Pooledf | Sigg |
| *MSH2* | WT | 4.26 X 107 0.45 | 1 | 3.16 X 107 .52 | 1 | N/A | N/A |
| *msh2∆* [*MSH2*] | WT | 3.50 X 107  0.67 | 0.82 | 3.14 X 107 .53 | 0.85 | 1.00 | N/A |
| *msh2∆* | Deletion | 1.28 X 105 | 30.13 | 8.97 X 106 | 28.4 | N/A | N/A |
| *msh2∆* [*MSH2-K446R*] | K449R | 3.58 X 107 | 0.84 | 3.12 X 107 | 0.85 | N/A | N/A |
| *msh2∆* [*MSH2-K446N*] | K449N | 3.89 X 107 | 0.91 | 3.79 X 107 | 1.03 | 0.56 | \*\* |
| *msh2∆* [*MSH2-P689S*] | P670S | 1.42 X 106 0.11 | 3.32 | 1.10 X 106 .78 | 2.98 | 2.178 | ns |
| *msh2∆* [*MSH2-P689R*] | P670R | 5.06 X 106 0.94 | 11.87 | 3.87 X 106  0.11 | 10.52 | 6.31 | \*\* |
| *msh2∆* [*MSH2-R699Q*] | R680Q | 2.77 X 107 | 0.65 | 2.95 X 107 | 0.80 | 1.51 | ns |
| *msh2∆* [*MSH2-R699P*] | R680P | 4.95 X 107 | 11.62 | 5.47 X 106 | 14.88 | 13.00 | \*\*\* |
| *msh2∆* [*MSH2-G780V*] | G761V | 2.50 X 107 | 5.86 | 1.42 X 106 | 3.87 | 2.71 | \*\* |
| *msh2∆* [*MSH2-G780R*] | G761R | 9.80 X 107 | 2.30 | 1.31 X 106 | 3.55 | 1.39 | \* |
| *msh2∆* [*MSH2-G780A*] | G761A | 2.89 X 107 | 0.68 | 2.57 X 106 | 0.70 | 0.81 | ns |

a. The yeast genotype mapped back to the human variant. For variants, all are on plasmids within the *msh2∆* background.

b. Mutation rate (mutations per cell division) estimated using traditional Luria-Delbrück -ln(P0)/N method. For genotypes *MSH2, msh2∆* [*MSH2-P689S*], and *msh2∆* [*MSH2-P689R*] stated mutation rate is the average of two replicates, for *msh2∆* [*MSH2*] it is the average of 6 replicates, with standard error calculated from those replicate values.

c. For stated genotype, variant mutation rate (mutations per cell division) was compared to the *MSH2* strain using the Luria-Delbrück -ln(P0)/N method.

d. Mutation rate estimated using Lea-Coulson maximum likelihood method as implemented in rSalvador. . For genotypes *MSH2, msh2∆* [*MSH2-P689S*], and *msh2∆* [*MSH2-P689R*] stated mutation rate is the average of two replicates, for *msh2∆* [*MSH2*] it is the average of 6 replicates, with standard error calculated from those replicate values.

e. For stated genotype, variant mutation rate (mutations per cell division) was compared to the *MSH2* strain using the Lea-Coulson maximum likelihood method as implemented in rSalvador.

f. For stated genotype, variant mutation rate (mutations per cell division) was compared WT using the pooled barcoded assay. Median of all values was calculated and presented.

g. Significance is calculated by a Wilcoxian Ranksum test with the Benjamini-Hochberg correction for multiple hypothesis testing. \*< 0.05   \*\* < 0.01  \*\*\*< 0.001 \*\*\*\*< 0.0001 using the pooled barcoded assay.