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|--------------------|---|--|
| ADE1 target region | | ---ATGTCAATTACGAAGACTGAACT <u>TGG</u> AC |
| ADE1 guide | | -----GTCAATTACGAAGACTGAACT----- |
| White (S2L) | [| ATAATGTTAATTACGAAGACTGAACTGGAC |
| | | ATAATGTTAATTACGAAGACTGAACTGGAC |
| | | ATAATGTTAATTACGAAGACTGAACTGGAC |
| | | ATAATGTTAATTACGAAGACTGAACTGGAC |
| | | ATAATGTTAATTACGAAGACTGAACTGGAC |
| | | ATAATGTTAATTACGAAGACTGAACTGGAC |
| | | ATAATGTTAATTACGAAGACTGAACTGGAC |
| | | ATAATGTTAATTACGAAGACTGAACTGGAC |
| Red (S2*) | [| ATAATGTGAATTACGAAGACTGAACTGGAC |
| | | ATAATGTGAATTACGAAGACTGAACTGGAC |
| | | ATAATGTGAATTACGAAGACTGAACTGGAC |
| | | ATAATGTAAATTACGAAGACTGAACTGGAC |
| | | ATAATGTGAATTACGAAGACTGAACTGGAC |
| | | ATAATGTGAATTACGAAGACTGAACTGGAC |

Figure S1 Different Target-AID mutations result in different phenotypes. The target site within ADE1 was sequenced for colonies from 4 independent mutagenesis experiments. As expected, only the mutations creating a premature stop codon result in LOF, while the S2L substitution does not affect function in a detectable manner.