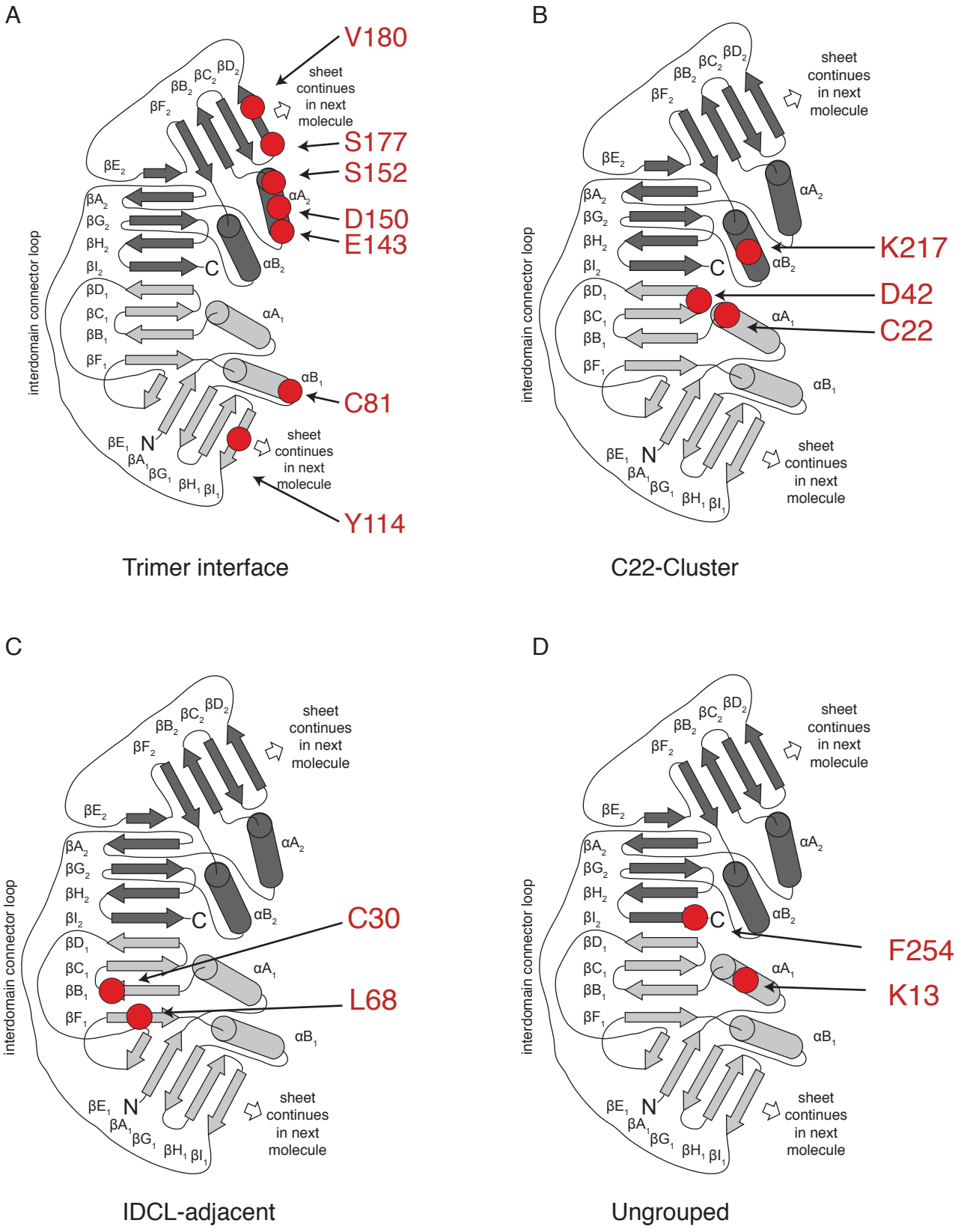
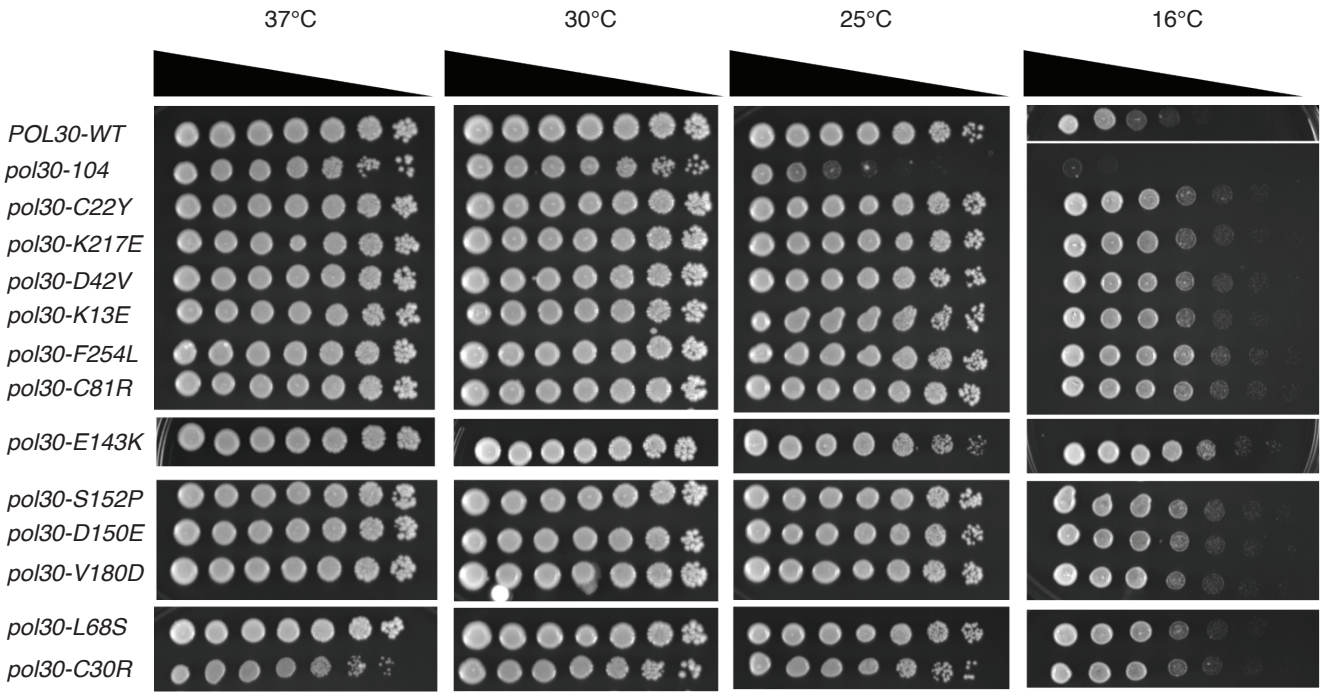
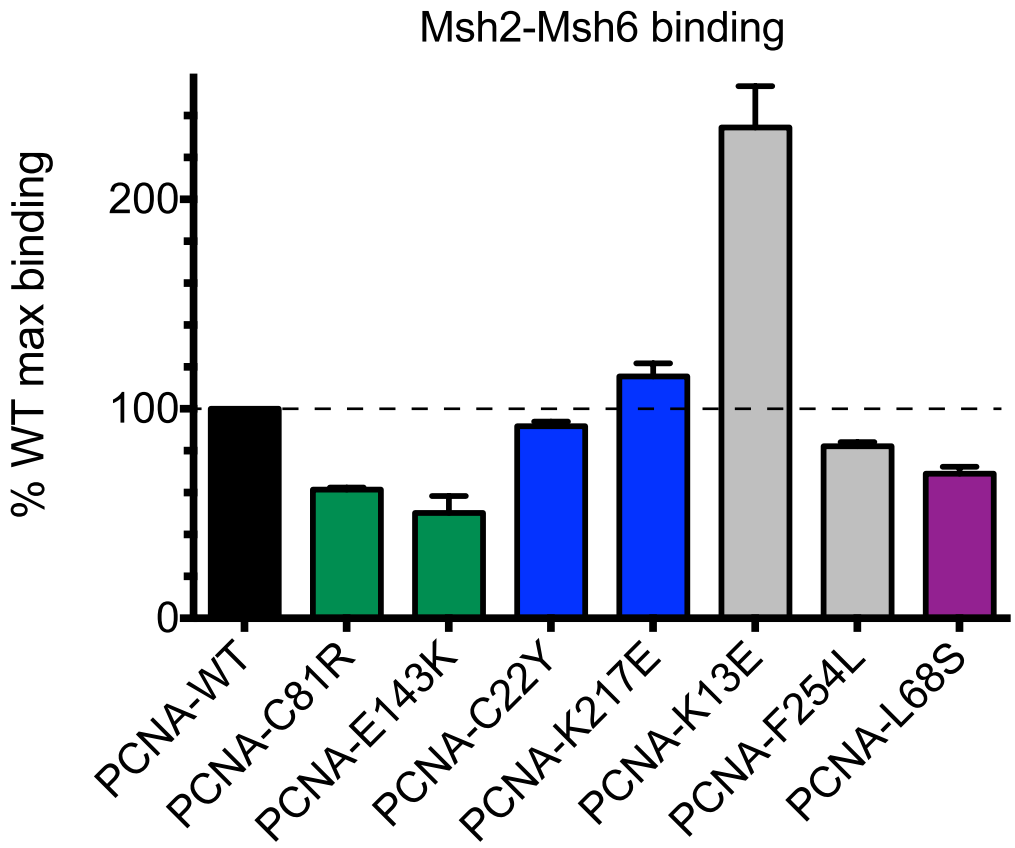
## Supplemental Figure 1



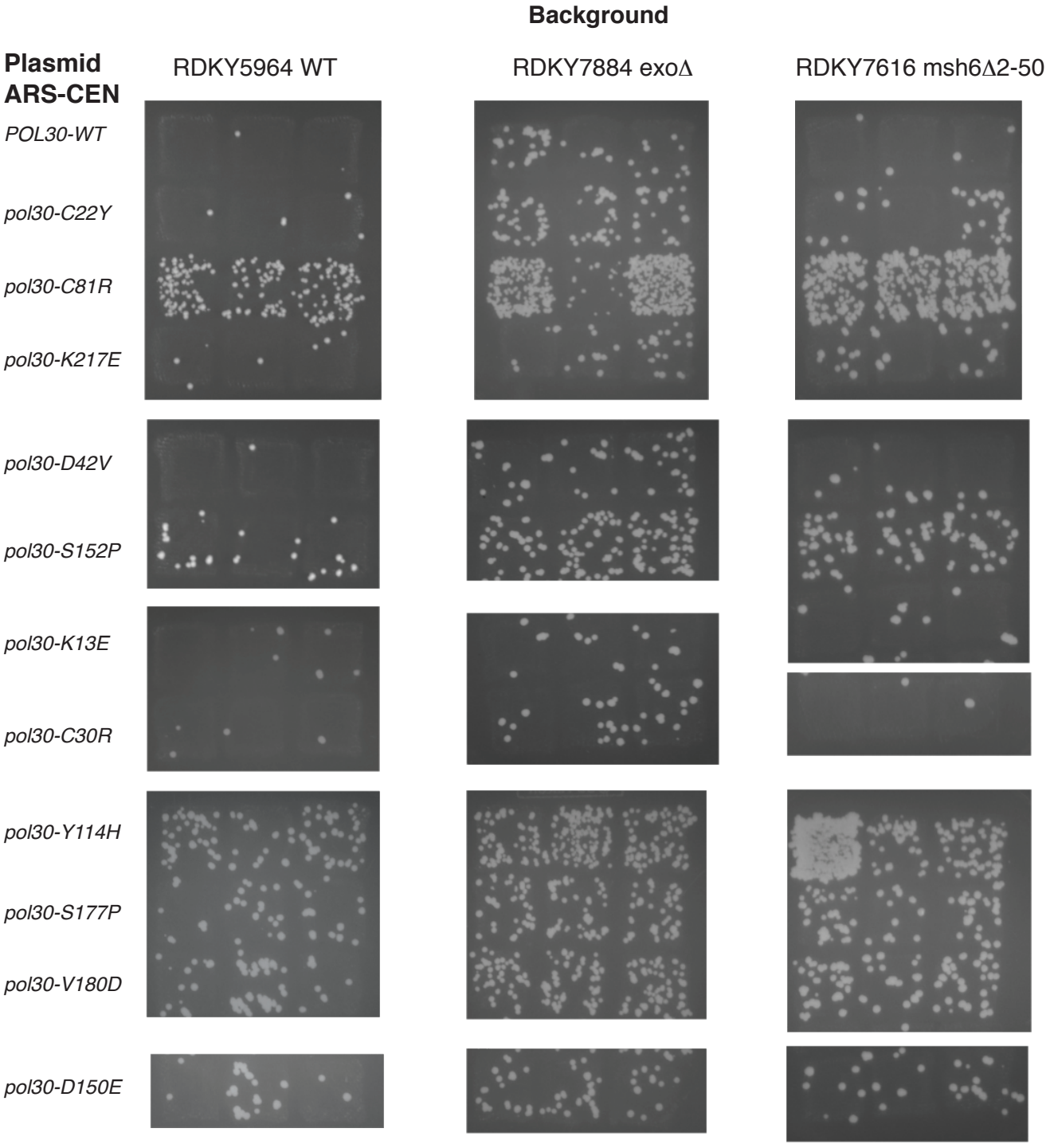
## Supplemental Figure 2



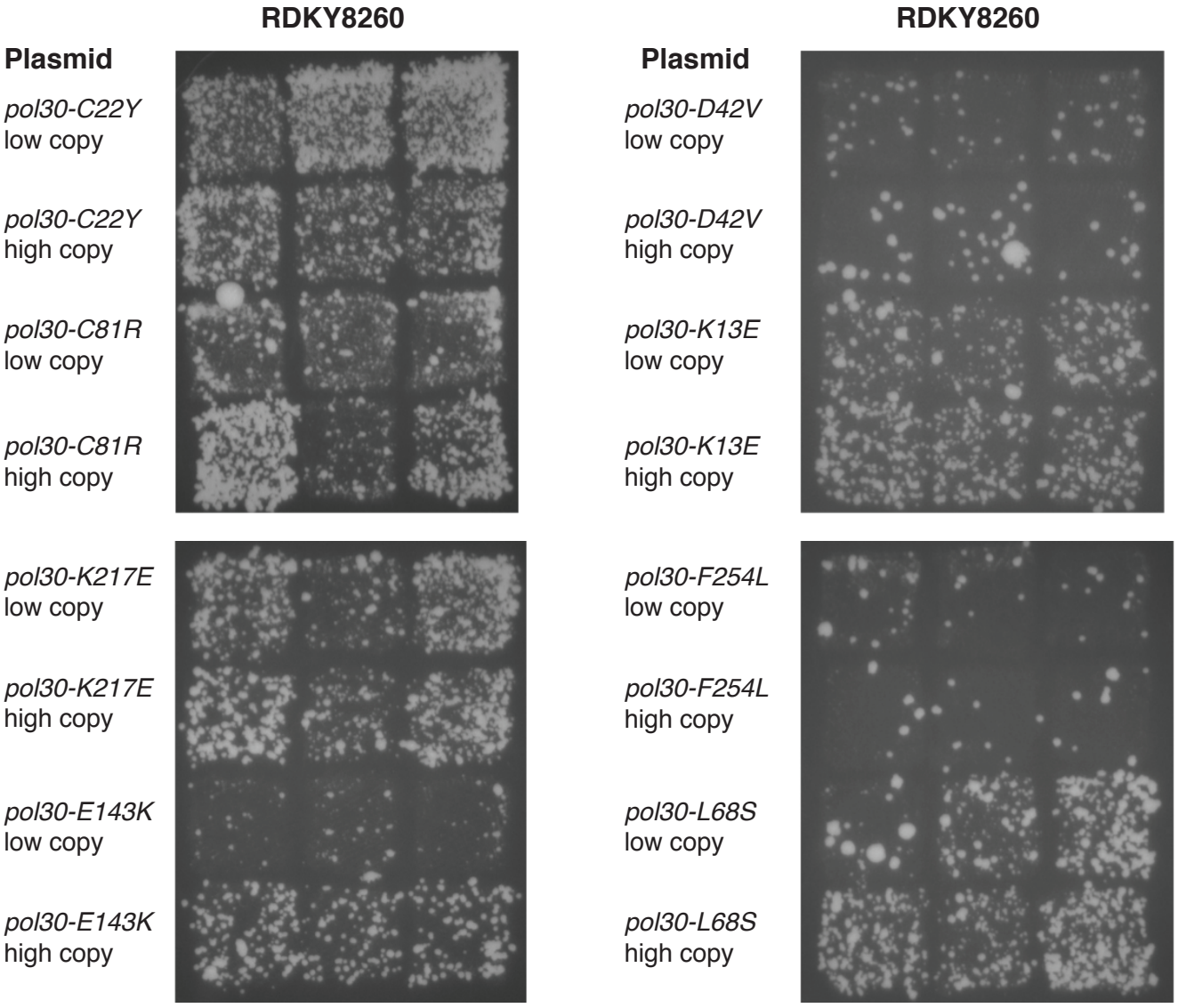
**Supplemental Figure 3**



## Supplemental Figure 4



## Supplemental Figure 5



**Supplemental Figure legends**

**Figure S1. *pol30* mutations that disrupt Exo1-independent MMR cause amino acid changes that form clusters on the PCNA crystal structure.**

Altered amino acids for the trimer interface mutations (A), the C22-cluster mutations (B), the IDCL-adjacent mutations (C) and the unclustered mutations (D) are depicted as red circles on a diagram of the secondary structure of a PCNA monomer (modified from (Krishna et al., 1994)). Relates to Figure 1.

**Figure S2. Temperature sensitivity of *pol30* mutation containing strains.**

A series of 5 fold serial dilutions for strains containing the indicated *pol30* mutation integrated at the genomic locus is shown. Plates were incubated for 3-6 days at the either 37°C, 30°C, 25°C or 16°C. *pol30-104*, shown here for comparison, was isolated previously and shown to be a cold sensitive allele with cell-cycle defects (Lau et al., 2002). Relates to Table 1.

**Figure S3. PCNA mutants show altered interactions with Msh2-Msh6.**

The binding of Msh2-Msh6 to bound wild-type and mutant PCNAs was evaluated by surface plasmon resonance in at least 3 independent experiments as shown in Figure 2. The extent of maximal binding to each mutant PCNA (binding at 100 s post Msh2-Msh6 injection) was normalized to binding to wild-type PCNA (set at 100%) observed in the same experiment using the same chip and the average binding (+/- standard deviation) is presented. Mutants are color-coded according to their grouping on the PCNA crystal structure (blue- C22Y-cluster, green-trimer interface, purple- IDCL-adjacent, grey- ungrouped). Relates to Figure 2.

**Figure S4. *pol30* trimer interface mutations result in a dominant mutator phenotype when present on a low copy plasmid**

Patch tests of the *lys2::InsE-A10* frameshift reversion assay for *pol30* mutations introduced on a low copy ARS-CEN plasmid into the indicated strain backgrounds, all containing a wild-type copy of *POL30* at the genomic locus. Relates to Table 2.

**Figure S5. Overexpression of *pol30* mutations on a high copy plasmid does not rescue the mutator phenotype.**

Patch tests for the *lys2::InsE-A10* frameshift reversion assay for RDKY8260 in which the wild-type copy of *POL30* was eliminated by plasmid shuffle and the indicated *pol30* mutation was introduced on either a low copy ARS-CEN plasmid or a high copy 2μ plasmid. Relates to Table 2.

**Table S1**. *S. cerevisiae* strains used in this study. Relates to Table 1.

| ***Name*** | ***Genotype*** | ***Reference*** |
|---|---|---|
| RDKY4080 | *MATa ura3-52 leu2Δ1 trpΔ63 his3Δ200 hom3-10 lys2ΔBgl ade2Δ1 ade8 pol30-104::LEU2* | (Lau et al., 2002) |
| RDKY7616 | *MATa ura3-52 leu2Δ1 trpΔ63 his3Δ200 hom3-10 lys2::InsE-A10 msh3::HIS3 msh6Δ2-50* | This study |
| RDKY5964 | *MATa ura3-52 leu2Δ1 trpΔ63 his3Δ200 hom3-10 lys2::InsE-A10* | (Hombauer et al., 2011) |
| RDKY7884 | RDKY5964 *exo1::hphNT1* | This study |
| RDKY8260 | RDKY5964 *exo1::hphNT1 pol30::HIS3* [pRDK900, *POL30*] | This study |
| RDKY8071 | RDKY5964 *pol30-201::LEU2* | This study |
| RDKY8073 | RDKY5964 *pol30-201::LEU2 exo1::hphNT1* | This study |
| RDKY8075 | RDKY5964 *pol30-K217E::LEU2* | This study |
| RDKY8077 | RDKY5964 *pol30-K217E::LEU2 exo1::hphNT1* | This study |
| RDKY8079 | RDKY5964 *pol30-D42V::LEU2* | This study |
| RDKY8081 | RDKY5964 *pol30-D42V::LEU2 exo1::hphNT1* | This study |
| RDKY8083 | RDKY5964 *pol30-K13E::LEU2* | This study |
| RDKY8085 | RDKY5964 *pol30-K13E::LEU2 exo1::hphNT1* | This study |
| RDKY8087 | RDKY5964 *pol30-F254L::LEU2* | This study |
| RDKY8089 | RDKY5964 *pol30-F254L::LEU2 exo1::hphNT1* | This study |
| RDKY8091 | RDKY5964 *pol30-204::LEU2* | This study |
| RDKY8093 | RDKY5964 *pol30-204::LEU2 exo1::hphNT1* | This study |
| RDKY8095 | RDKY5964 *pol30-E143K::LEU2* | This study |
| RDKY8097 | RDKY5964 *pol30-E143K::LEU2 exo1::hphNT1* | This study |
| RDKY8099 | RDKY5964 *pol30-S152P::LEU2* | This study |
| RDKY8101 | RDKY5964 *pol30-S152P::LEU2 exo1::hphNT1* | This study |
| RDKY8103 | RDKY5964 *pol30-D150E::LEU2* | This study |
| RDKY8105 | RDKY5964 *pol30-D150E::LEU2 exo1::hphNT1* | This study |
| RDKY8107 | RDKY5964 *pol30-V180D::LEU2* | This study |
| RDKY8109 | RDKY5964 *pol30-V180D::LEU2 exo1::hphNT1* | This study |
| RDKY8111 | RDKY5964 *pol30-L68S::LEU2* | This study |
| RDKY8113 | RDKY5964 *pol30-L68S::LEU2 exo1::hphNT1* | This study |
| RDKY8115 | RDKY5964 *pol30-C30R::LEU2* | This study |
| RDKY8117 | RDKY5964 *pol30-C30R::LEU2 exo1::hphNT1* | This study |
| RDKY7588 | RDKY5964 *PMS1-4GFP::KanMX6* | (Hombauer et al., 2011) |
| RDKY7936 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-201::LEU2* | This study |
| RDKY8234 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-K217E::LEU2* | This study |
| RDKY8236 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-D42V::LEU2* | This study |
| RDKY8238 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-K13E::LEU2* | This study |
| RDKY8240 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-F254L::LEU2* | This study |
| RDKY7938 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-204::LEU2* | This study |
| RDKY7940 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-E143K::LEU2* | This study |
| RDKY8246 | RDKY5964 *PMS1-4GFP::KanMX6 pol30-L68S::LEU2* | This study |
| RKDY8248 | RDKY5964 *msh3::HIS3 msh6Δ2-50* | This study |
| RDKY8249 | RDKY5964 *msh3::HIS3 msh6Δ2-50 pol30-K217E::LEU2* | This study |
| RDKY8251 | RDKY5964 *msh3::HIS3 msh6Δ2-50 pol30-K13E::LEU2* | This study |
| RDKY8253 | RDKY5964 *msh3::HIS3 msh6Δ2-50 pol30-E143K::LEU2* | This study |
| RDKY8255 | RDKY5964 *msh3::HIS3 msh6Δ2-50 pol30-F254L::LEU2* | This study |
| RDKY8256 | RDKY5964 *msh3::HIS3 msh6Δ2-50 pol30-L68S::LEU2* | This study |
| RDKY8258 | RDKY5964 *msh3::HIS3 msh6Δ2-50 pol30-S152P::LEU2* | This study |
| RDKY8263 | RDKY5964 *msh2::HIS3* | This study |
| RDKY8265 | RDKY5964 *exo1::hphNT1 msh2::HIS3* | This study |
| RDKY8267 | RDKY5964 *pol30-K217E::LEU2 msh2::HIS3* | This study |

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|---|---|---|
| RDKY8269 | RDKY5964 *pol30-K13E::LEU2 msh2::HIS3* | This study |
| RDKY8271 | RDKY5964 *pol30-E143K::LEU2 msh2::HIS3* | This study |
| RDKY8273 | RDKY5964 *pol30-F254L::LEU2 msh2::HIS3* | This study |
| RDKY8275 | RDKY5964 *pol30-L68S::LEU2 msh2::HIS3* | This study |
| RDKY8277 | RDKY5964 *pol30-S152P::LEU2 msh2::HIS3* | This study |
| RDKY8279 | RDKY5964 *pol30-D42V::LEU2 msh2::HIS3* | This study |

**Table S2**. Plasmids used in this study. Relates to Table 1.

| *Name* | *Relevant Genotype* | *Reference* |
|---|---|---|
| pRDK833 | *amp$^r$ ori 2u TRP POL30* | (Lau et al., 2002) |
| pRDK837 | *amp$^r$ CEN6 ARSH4 TRP POL30* | (Lau et al., 2002) |
| pRDK900 | *amp$^r$ CEN6 ARSH4 URA3 POL30* | (Lau et al., 2002) |
| pRDK902 | *amp$^r$ CEN6 ARSH4 TRP POL30::LEU2* | (Lau et al., 2002) |
| pRDK930 | *amp$^r$ PT7 POL30* | (Lau et al., 2002) |
| pRS315 | *amp$^r$ CEN6 ARSH4 LEU2* | (Sikorski and Hieter, 1989) |
| pRDK1020 | *amp$^r$ CEN6 ARSH4 LEU2 EXO1* | (Wei et al., 2003) |
| pRDK1710 | *amp$^r$ CEN6 ARSH4 TRP pol30-K13E, L50S, F144L* | This study |
| pRDK1711 | *amp$^r$ CEN6 ARSH4 TRP pol30-K13E* | This study |
| pRDK1712 | *amp$^r$ CEN6 ARSH4 TRP pol30-C22Y, D93G* | This study |
| pRDK906 | *amp$^r$ CEN6 ARSH4 TRP pol30-C22Y* | (Lau et al., 2002) |
| pRDK1714 | *amp$^r$ CEN6 ARSH4 TRP pol30-V23A* | This study |
| pRDK1715 | *amp$^r$ CEN6 ARSH4 TRP pol30-C30R* | This study |
| pRDK1716 | *amp$^r$ CEN6 ARSH4 TRP pol30-D42V* | This study |
| pRDK1717 | *amp$^r$ CEN6 ARSH4 TRP pol30-S49P* | This study |
| pRDK1718 | *amp$^r$ CEN6 ARSH4 TRP pol30-L50S* | This study |
| pRDK1719 | *amp$^r$ CEN6 ARSH4 TRP pol30-L68S, S145P* | This study |
| pRDK1720 | *amp$^r$ CEN6 ARSH4 TRP pol30-L68S* | This study |
| pRDK1721 | *amp$^r$ CEN6 ARSH4 TRP pol30-T73I, V180D* | This study |
| pRDK1722 | *amp$^r$ CEN6 ARSH4 TRP pol30-T73I* | This study |
| pRDK909 | *amp$^r$ CEN6 ARSH4 TRP pol30-C81R* | (Lau et al., 2002) |
| pRDK1724 | *amp$^r$ CEN6 ARSH4 TRP pol30-D93G* | This study |
| pRDK1725 | *amp$^r$ CEN6 ARSH4 TRP pol30-Y114H* | This study |
| pRDK1726 | *amp$^r$ CEN6 ARSH4 TRP pol30-D120V, K217E* | This study |
| pRDK1727 | *amp$^r$ CEN6 ARSH4 TRP pol30-D120V* | This study |
| pRDK1728 | *amp$^r$ CEN6 ARSH4 TRP pol30-D134G, D150E* | This study |
| pRDK1729 | *amp$^r$ CEN6 ARSH4 TRP pol30-D134G* | This study |
| pRDK1730 | *amp$^r$ CEN6 ARSH4 TRP pol30-E143K* | This study |
| pRDK1731 | *amp$^r$ CEN6 ARSH4 TRP pol30-F144L* | This study |
| pRDK1732 | *amp$^r$ CEN6 ARSH4 TRP pol30-S145P* | This study |
| pRDK1733 | *amp$^r$ CEN6 ARSH4 TRP pol30-D150E* | This study |
| pRDK1734 | *amp$^r$ CEN6 ARSH4 TRP pol30-S152P* | This study |
| pRDK1735 | *amp$^r$ CEN6 ARSH4 TRP pol30-S177P* | This study |
| pRDK1736 | *amp$^r$ CEN6 ARSH4 TRP pol30-V180D* | This study |
| pRDK1737 | *amp$^r$ CEN6 ARSH4 TRP pol30-I181V, F254L* | This study |
| pRDK1738 | *amp$^r$ CEN6 ARSH4 TRP pol30-I181V* | This study |
| pRDK1739 | *amp$^r$ CEN6 ARSH4 TRP pol30-F207S, K217E* | This study |
| pRDK1740 | *amp$^r$ CEN6 ARSH4 TRP pol30-K217E* | This study |
| pRDK1741 | *amp$^r$ CEN6 ARSH4 TRP pol30-S222P* | This study |
| pRDK1742 | *amp$^r$ CEN6 ARSH4 TRP pol30-F254L* | This study |
| pRDK925 | *amp$^r$ CEN6 ARSH4 TRP pol30-C22Y::LEU2* | (Lau et al., 2002) |
| pRDK1744 | *amp$^r$ CEN6 ARSH4 TRP pol30-K217E::LEU2* | This study |
| pRDK1745 | *amp$^r$ CEN6 ARSH4 TRP pol30-D42V::LEU2* | This study |
| pRDK1746 | *amp$^r$ CEN6 ARSH4 TRP pol30-K13E::LEU2* | This study |
| pRDK1747 | *amp$^r$ CEN6 ARSH4 TRP pol30-F254L::LEU2* | This study |
| pRDK926 | *amp$^r$ CEN6 ARSH4 TRP pol30-C81R::LEU2* | (Lau et al., 2002) |
| pRDK1749 | *amp$^r$ CEN6 ARSH4 TRP pol30-E143K::LEU2* | This study |
| pRDK1750 | *amp$^r$ CEN6 ARSH4 TRP pol30-S152P::LEU2* | This study |
| pRDK1751 | *amp$^r$ CEN6 ARSH4 TRP pol30-D150E::LEU2* | This study |
| pRDK1752 | *amp$^r$ CEN6 ARSH4 TRP pol30-V180D::LEU2* | This study |

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|---|---|---|
| pRDK1753 | *amp$^r$ CEN6 ARSH4 TRP pol30-L68S::LEU2* | This study |
| pRDK1754 | *amp$^r$ CEN6 ARSH4 TRP pol30-C30R::LEU2* | This study |
| pRDK1755 | *amp$^r$ ori 2μ TRP pol30-C22Y* | This study |
| pRDK1756 | *amp$^r$ ori 2μ TRP pol30-C81R* | This study |
| pRDK1757 | *amp$^r$ ori 2μ TRP pol30-K13E* | This study |
| pRDK1758 | *amp$^r$ ori 2μ TRP pol30-D42V* | This study |
| pRDK1759 | *amp$^r$ ori 2μ TRP pol30-E143K* | This study |
| pRDK1760 | *amp$^r$ ori 2μ TRP pol30-F254L* | This study |
| pRDK1761 | *amp$^r$ ori 2μ TRP pol30-L68S* | This study |
| pRDK1762 | *amp$^r$ ori 2μ TRP pol30-K217E* | This study |
| pRDK931 | *amp$^r$ PT7 pol30-C22Y* | (Lau et al., 2002) |
| pRDK932 | *amp$^r$ PT7 pol30-C81R* | (Lau et al., 2002) |
| pRDK1763 | *amp$^r$ PT7 pol30-K217E* | This study |
| pRDK1764 | *amp$^r$ PT7 pol30-E143K* | This study |
| pRDK1765 | *amp$^r$ PT7 pol30-K13E* | This study |
| pRDK1766 | *amp$^r$ PT7 pol30-L68S* | This study |
| pRDK1767 | *amp$^r$ PT7 pol30-F254L* | This study |