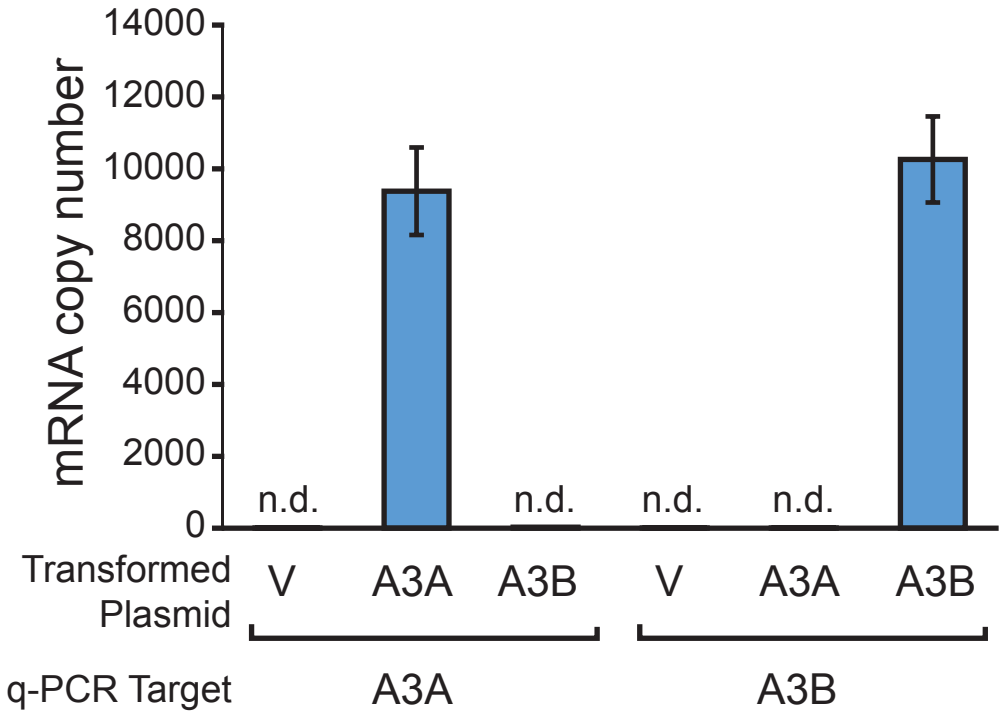

Figure S1, related to Figure 1. mRNA levels of A3A and A3B in the haploid yeast transformed with A3A or A3B expression constructs. The absolute quantities A3A and A3B mRNA transcripts in strains carrying an empty vector (V; i.e. plasmid pySR419), pySR419-A3A (A3A), or pySR419-A3B (A3B) were quantified with Q-rtPCR by comparison to a standard curves using linear regression. The mRNA copy number is per 105 ng of total yeast RNA and was normalized based on Q-rtPCR data for a reference gene, *TAF10*. Error bars indicate standard deviation of copy number among three replicate samples. n.d. denotes none dectected.

Supplemental Experimental Procedures

Detailed description of yeast strain construction

Novel *ung1*Δ yeast strains in the CG379 genetic background were produced by transformation with PCR fragments containing at nourseothricin resistance cartridge (NAT-R) flanked by sequence homologous to *UNG1* and replacement of the wild type gene with the NAT-R sequence by homologous recombination. Homozygous diploid *ung1*Δ strains expressing chromosomally integrated A3A or A3B were constructed by transforming haploid versions on these strains (construction described in (Chan et al., 2015)) with YEpHO(Leu). This plasmid ectopically expresses the HO endonuclease, causing mating type switching in a subset of yeast cells. Diploid strains resulting from the mating of a and α haploid yeast were selected on SC-leucine media and confirmed by tetrad dissection. The *rfa1-t33* allele was generated by targeted editing of *RFA1* with CRISPR/Cas9 as in (Laughery et al., 2015). Briefly, a plasmid containing a *LEU2* selectable marker and expressing both yeast codon-optimized Cas9 and the guide RNA, GGTTCTGTTGCTGCCATTAAAGG, was co-transformed into yeast along with annealed repair oligos 5′-ATGGAATCAGCAAGCCCTTGATTTCAACCTTCCTGAAGGTCCTGTTGCTGCCATTAAAGGTGTTCGTGT GACGGATTTTGG and 5′-CCAAAATCCGTCACACGAACACCTTTAATGGCAGCAACAGGACCTTCAGGAAGGTTGAAATCAAGGG CTTGCTGATTCCAT, which contain sequence that encodes the serine to proline substitution and destroys the guide RNA binding site. Transformants were first selected on media lacking leucine followed by propagation without selection to lose the Cas9 plasmid. The *RFA1* gene of several independent transformants was amplified by PCR and sequenced with the primers, 5′-CGACAAAAGACGCGTGAACT, 5′-GCTGAACCGCCCTTCAAAAA, 5′-AGCAAGAGTTTCCTACAAGGGA, and 5′-GGCTCGGATTTCTCCAGAGG to verify introduction of the S373P mutation. Construction of all other strains is described in (Chan et al., 2015, Roberts et al., 2012).

Q-rtPCR

Quantification of A3A and A3B mRNA levels in derivatives of yeast strain ySR_128 that contained plasmid pYSR419, pYSR419-A3A, or pYSR419-A3B was done as follows. Total yeast RNA was extracted from yeast spheroblasts produced by zymolyase treatment using the cultured cell RNA purification kit (IBI, Peosta IA). Purified RNA was treated with DNase I, which was subsequently inactivated prior to cDNA synthesis. cDNA was produced using the ProtoScript First Strand cDNA Synthesis Kit (NEB, Ipswich MA) using an oligo d(T)23 primer following the manufacturer protocol. Absolute quantification of A3A and A3B mRNA was performed on the Applied Biosystems 7500 fast real-time PCR system using the Power SYBR green PCR Master Mix (Life technologies). The oligonucleotides used for qPCR are listed in Table S4. The standard curve for A3A and A3B quantification was templated using gel purified PCR products produced with primers 5′-GTTTCTTAGACGTCAGGTGGCACTTT and 5′-GCCAGAAAATGTTGGTGATGCGC from plasmids pYSR419-A3A, or pYSR419-A3B, respectively. An 8-fold, 1:10 serial dilution starting at 5×10^8 copies of each PCR product served as the standard curve. Input cDNA was quantified using qPCR by amplification of the reference gene, *TAF10,* using oligos listed in Table S4 and normalized for A3A and A3B quantification.

Supplemental References

LAUGHERY, M. F., HUNTER, T., BROWN, A., HOOPES, J., OSTBYE, T., SHUMAKER, T. & WYRICK, J. J. 2015. New vectors for simple and streamlined CRISPR-Cas9 genome editing in Saccharomyces cerevisiae. *Yeast*.