Supplemental Materials for

Control of 3' Splice Site Selection in *S. cerevisiae* by a Highly Conserved Amino Acid within the Prp8 α-finger Domain

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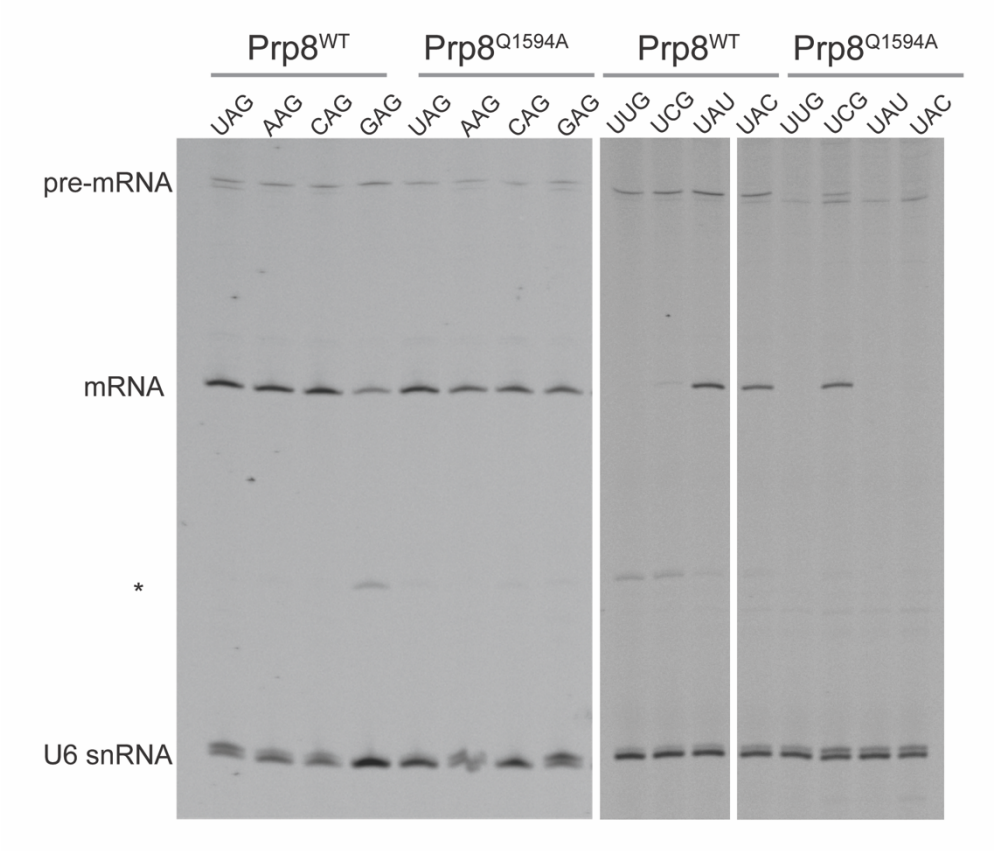
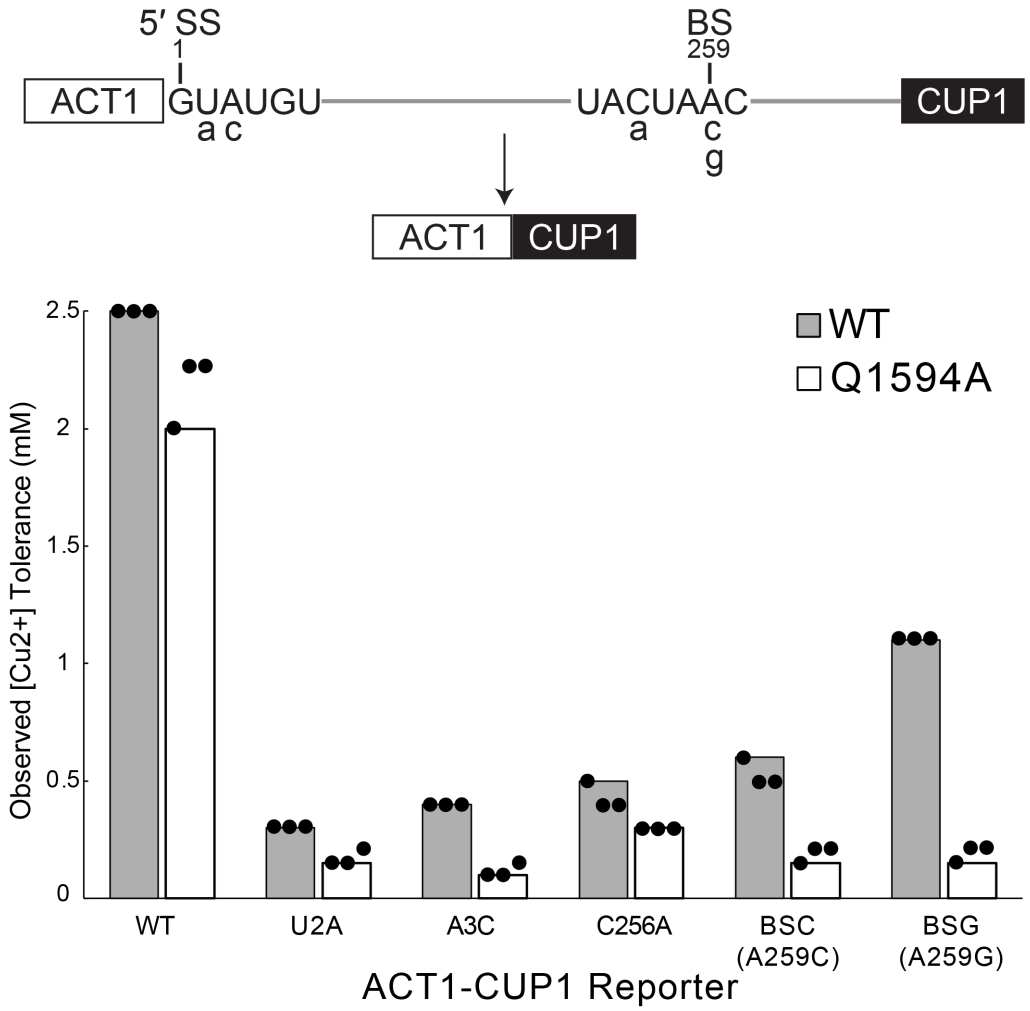
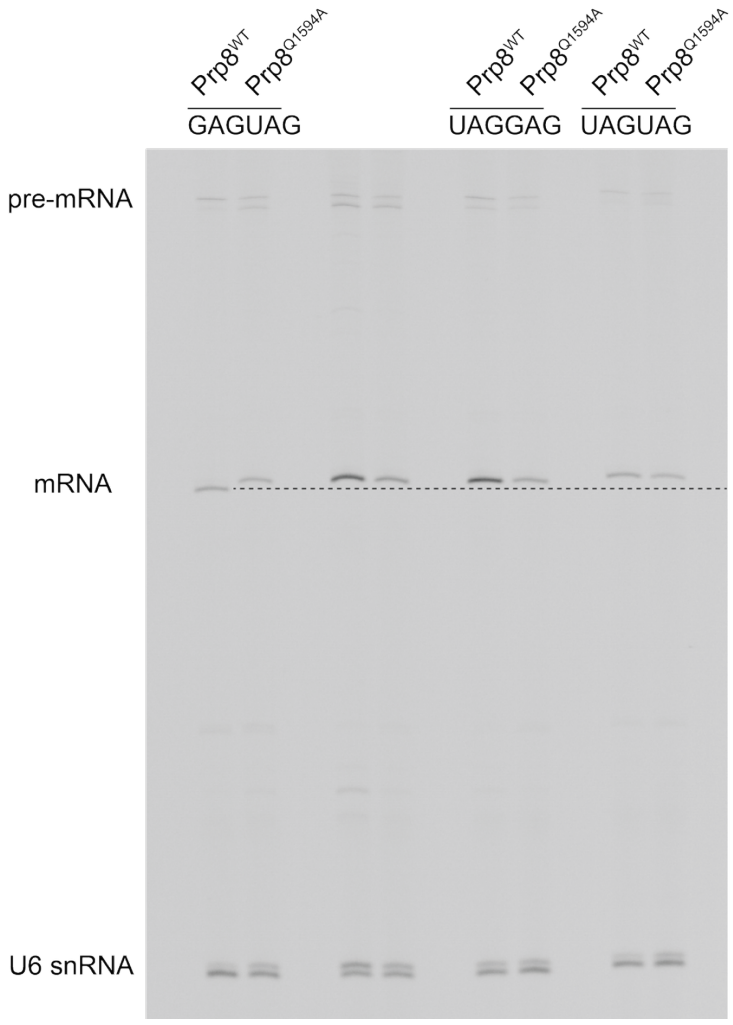
Supplementary Figure S1. Multiple sequence alignment of the Prp8 α-finger region from different species. The red box marks yeast Q1594. Sequences were aligned and visualized using Clustal Omega (Madeira et al. 2024) and Boxshade (Albà 2000).



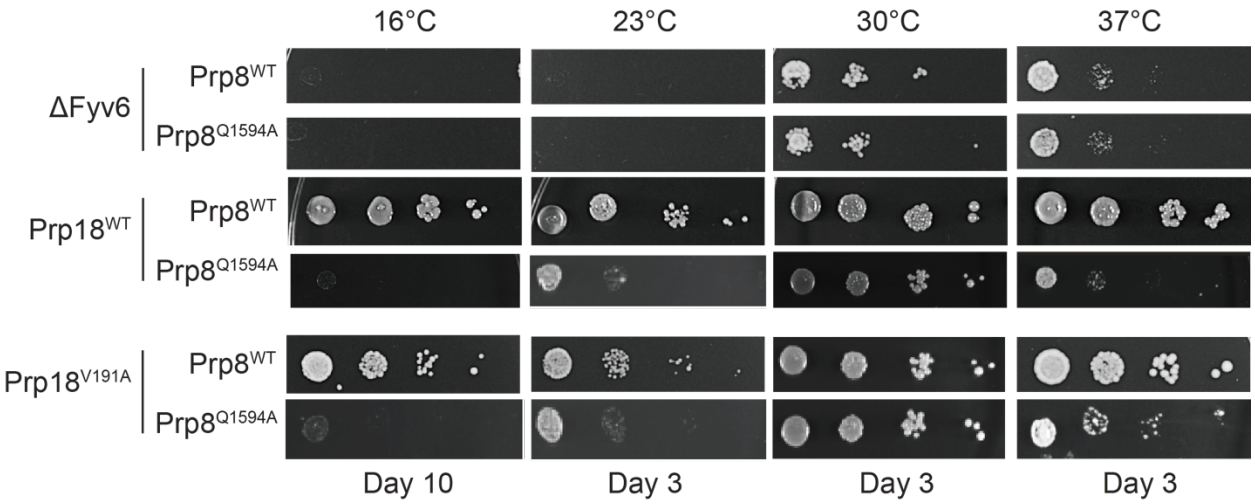
Supplementary Figure S2. Primer extension analysis of ACT1-CUP1 reporter RNAs in the presence of Prp8^{WT} or Prp8^{Q1594A}. White bars indicate that the experiments were run on different gels or intervening lanes removed to generate this figure. Note that bands corresponding to mRNAs are observed for Prp8^{WT} with the UAU and UAC reporters despite the low copper tolerances observed (see **Fig. 2**). This is likely due to splicing at a flanking "AG" site (UAU/AG; UAC/AG) created by the substitution at the normal 3'SS and a shift in the protein reading frame. Also note that these mRNAs are not observed if the same reporters are present with Prp8^{Q1594A}. The asterisk represents an unknown band the sometimes appears as a primer extension product. We did not observe the presence of the intron lariat or lariat intermediate in these assays.



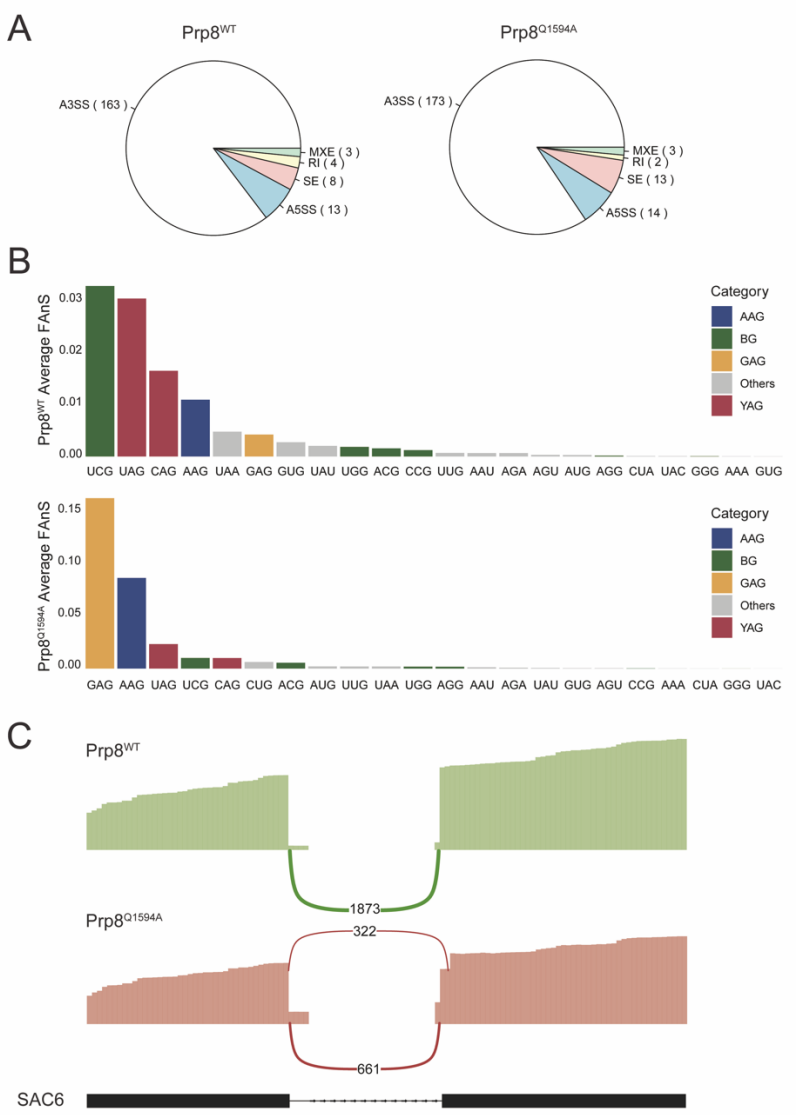
Supplementary Figure S3. ACT1-CUP1 assays for Prp8^{WT} and Prp8^{Q1594A} with 5'SS and BPS substitution reporters. (Top) Schematic of the ACT1-CUP1 splicing reporter assay, with tested variants noted. (Bottom) Observed Cu^{2+} tolerances. Bar heights represent the values from a single biological replicate. Circles represent values obtained from three biological replicates.



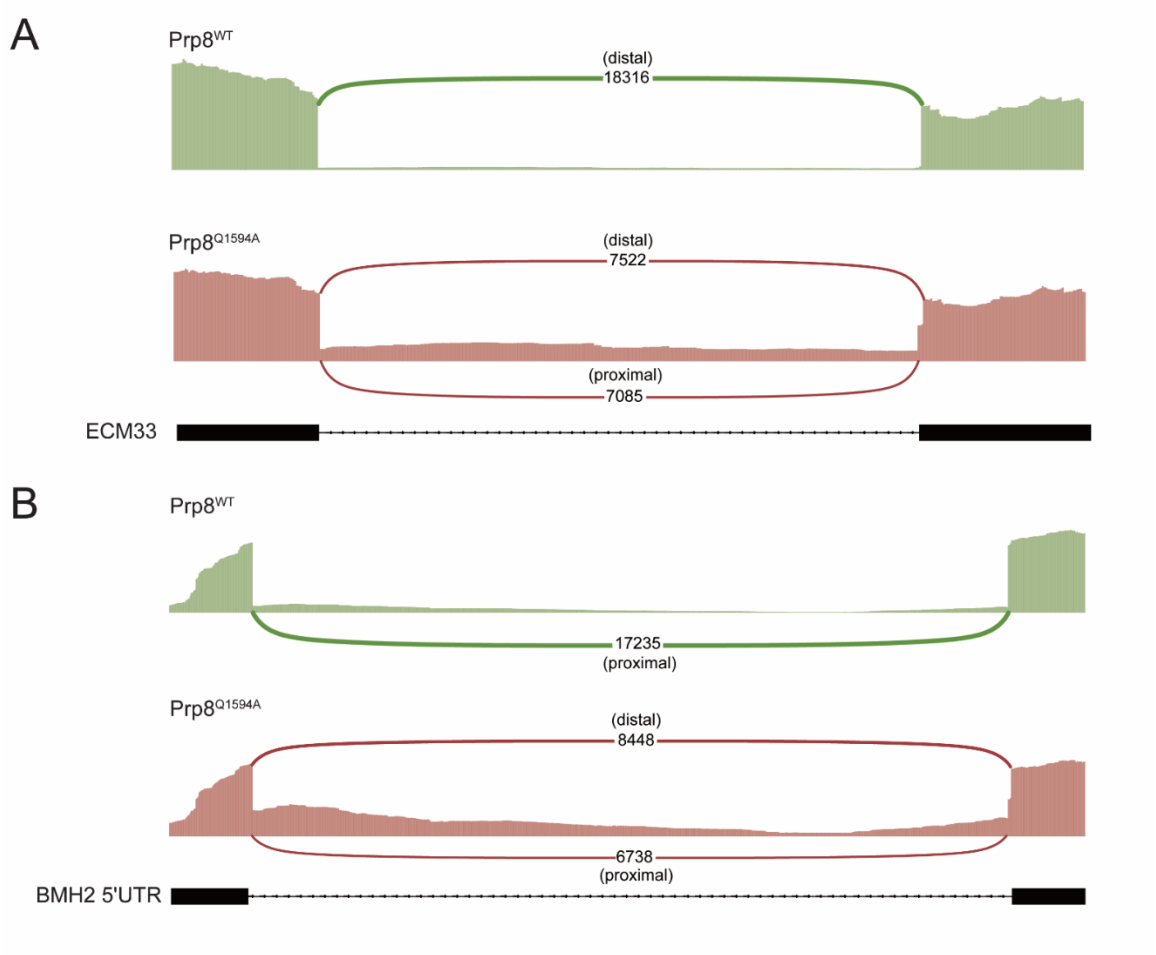
Supplementary Figure S4. Primer extension analysis of ACT1-CUP1 reporters with competing 3'SS. This is the full image corresponding to the cropped region shown in **Fig. 3B**. A dashed line has been added at the position of the mRNA corresponding to distal site usage. The lanes between the GAGUAG and UAGGAG samples represent an experiment not discussed. Note that the intensity of the mRNA band for the UAGGAG reporter in the Prp8^{WT} sample is higher than others, despite similar levels of U6. We do not know the origin of this and cannot exclude the possibility it is related to the allele of Prp8 present.



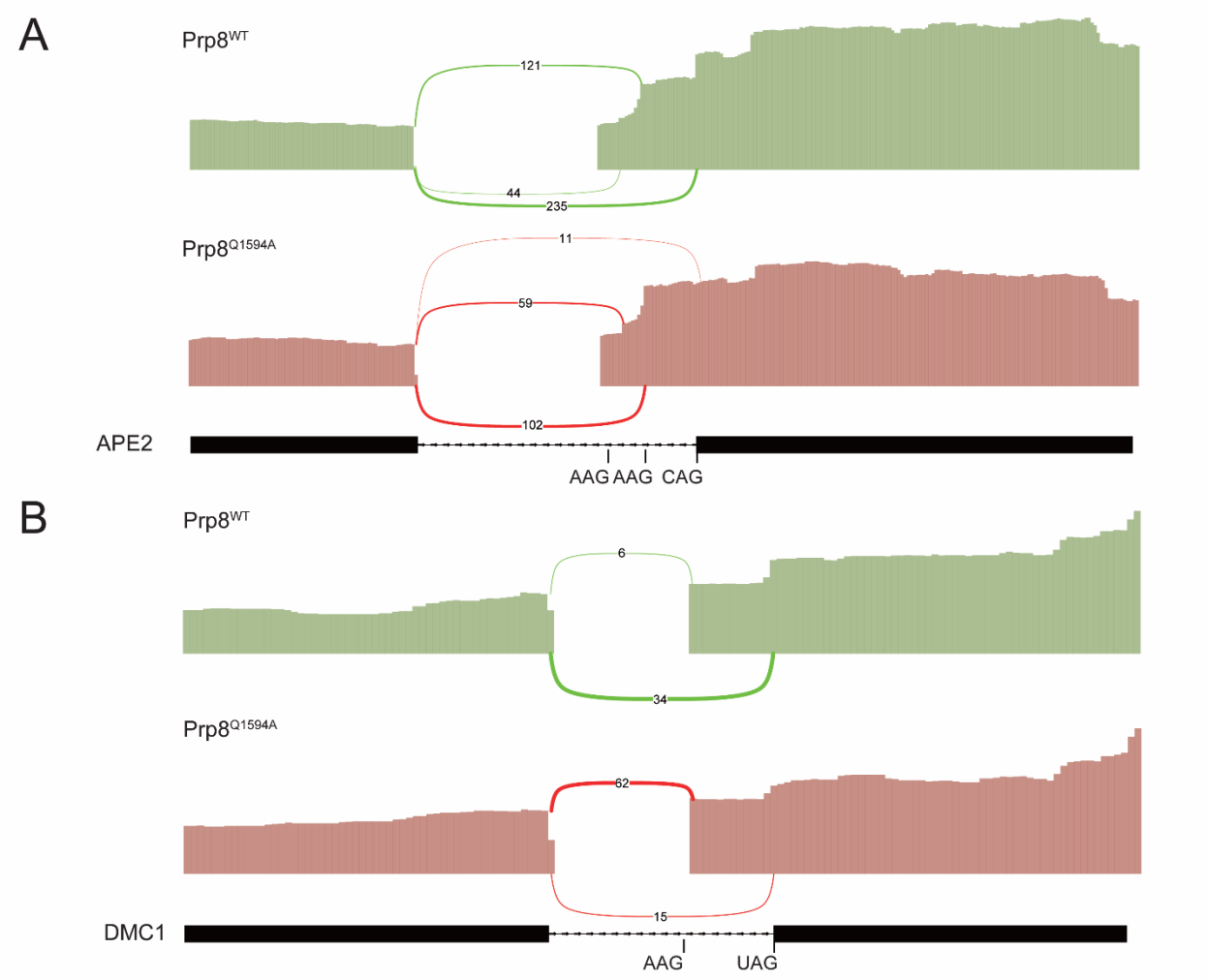
Supplementary Figure S5. Yeast growth assays in Fyv6 deletion or Prp18 mutation backgrounds. Growth assays comparing WT Prp8 and the Q1594A mutant at different temperatures in the absence of Fyv6 (ΔFyv6, YPD plates) or in the presence of WT or a dominant negative mutant of Prp18 ($Prp18^{V191A}$;-URA plates). Plates were incubated at the given temperatures and images collected on the noted days.



Supplementary Figure S6. RNA seq analysis of Prp8^{WT} or Prp8^{Q1594A} yeast strains. **(A)** Pie charts showing the numbers and types of alternative splicing events detected in the Prp8^{WT} and Prp8^{Q1594A} strains. **(B)** Bar graphs showing the average FAnS values for each 3'SS trinucleotide motif in Prp8^{WT} (top) and Prp8^{Q1594A} (bottom) for alternate 3'SS. Note that the Y axis is from 0.00-0.03 for Prp8^{WT} but from 0.00-0.15 for Prp8^{Q1594A}. **(C)** Sashimi plot showing alternative 3'SS usage in the SAC6 gene. In the Prp8^{WT} strain (green), the canonical distal 3'SS is predominantly used. In contrast, the Prp8^{Q1594A} strain (red) shows increased usage of a proximal, cryptic 3'SS (~1:2 ratio of the cryptic to canonical site based on read counts).



Supplementary Figure S7. Sashimi plots showing alternative 3'SS usage in the ECM33 and BMH2 genes. In the Prp8^{WT} strain (green), the canonical 3'SS are predominantly used (distal in panel A and proximal in panel B). In contrast, the Prp8^{Q1594A} strain (red) shows increased usage of cryptic 3'SS located proximal (panel A) and distal (panel B) to the BS.



Supplementary Figure S8. Sashimi plots showing alternative 3'SS usage in the APE2 and DMC1 genes. (A) When Prp8^{WT} is present, the canonical CAG site is most frequently used in the *APE2* RNA relative to two other AAG sites at a ratio of read counts of ~ 1:2.8:5.3 (going from most proximal to the BS to the most distal). When Prp8^{Q1594A} is present, this ratio changes to 1:1.7:0.19. (**B**) For the *DMC1* RNA, we observed more read counts for usage of the canonical UAG site relative to the cryptic AAG site (ratio of 1:5.7) with WT Prp8. This ratio is nearly flipped when the Prp8^{Q1594A} mutant is present (ratio of 1:0.25). Note that for both *APE2* and *DMC1* RNAs, we observed relatively few read counts that spanned exon-exon junctions.

Supplementary Table S1. Yeast strains used in this study.

Strain	Genotype	Description	Reference
yAAH0117	ade2, cup1delta:ra3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8(URA)	Prp8WT strain	Umen and Guthrie, Genetics, 1996
yAAH3853	yAAH0117 + pAAH1440	pJU169:PRP8_WT(TRP)	This work
yAAH3527	yAAH0117 + pAAH1627	pJU169:PRP8_Q1594A(TRP)	This work
yAAH3817	ade2, cup1delta:ra3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8(URA)UPF1::NATR	PRP8(URA) UPF1 deletion	This work
yAAH3605	yAAH3817 + pAAH1440	PRP8_WT(TRP) UPF1 deletion	This work
yAAH3818	yAAH3817 + pAAH1627	PRP8_Q1594A(TRP) UPF1 deletion	This work
yAAH3532	yAAH3853 + pAAH0470	PRP8_WT(TRP)ACT1-CUP1 WT	This work
yAAH3533	yAAH3853 + pAAH0526	PRP8_WT(TRP)ACT1-CUP1 U301G(3'ssGAG)	This work
yAAH3534	yAAH3853 + pAAH1644	PRP8_WT(TRP)ACT1-CUP1 U301A(3'ssAAG)	This work
yAAH3535	yAAH3853 + pAAH1645	PRP8_WT(TRP)ACT1-CUP1 U301C(3'ssCAG)	This work
yAAH3814	yAAH3853 + pAAH1684	PRP8_WT(TRP)ACT1-CUP1 3'ssGUGnew	This work
yAAH3544	yAAH3527 + pAAH0470	PRP8_Q1594A(TRP)ACT1-CUP1 WT	This work
yAAH3545	yAAH3527 + pAAH0526	PRP8_Q1594A(TRP)ACT1-CUP1 U301G(3'ssGAG)	This work
yAAH3546	yAAH3527 + pAAH1644	PRP8_Q1594A(TRP)ACT1-CUP1 U301A(3'ssAAG)	This work
yAAH3547	yAAH3527 + pAAH1645	PRP8_Q1594A(TRP)ACT1-CUP1 U301C(3'ssCAG)	This work
yAAH3816	yAAH3527 + pAAH1684	PRP8_Q1594A(TRP)ACT1-CUP1 3'ssGUGnew	This work
yAAH3541	yAAH3853 + pAAH1752	PRP8_WT(TRP)ACT1-CUP1 A302U(3'ssUUG)	This work
yAAH3542	yAAH3853 + pAAH1753	PRP8_WT(TRP)ACT1-CUP1 A302C(3'ssUCG)	This work
yAAH3543	yAAH3853 + pAAH1754	PRP8_WT(TRP)ACT1-CUP1 G303U(3'ssUAU)	This work
yAAH3548	yAAH3853 + pAAH1755	PRP8_WT(TRP)ACT1-CUP1 G303C(3'ssUAC)	This work
yAAH3549	yAAH3527 + pAAH1752	PRP8_Q1594A(TRP)ACT1-CUP1 A302U(3'ssUUG)	This work

yAAH3550	yAAH3527 + pAAH1753	PRP8_Q1594A(TRP)ACT1-CUP1 A302C(3'ssUCG)	This work
yAAH3551	yAAH3527 + pAAH1754	PRP8_Q1594A(TRP)ACT1-CUP1 G303U(3'ssUAU)	This work
yAAH3552	yAAH3527 + pAAH1755	PRP8_Q1594A(TRP)ACT1-CUP1 G303C(3'ssUAC)	This work
yAAH3530	yAAH3853 + pAAH1729	Prp8_WT(TRP) ACT1-CUP1 3'ss GAGUAG	This work
yAAH3529	yAAH3527 + pAAH1729	Prp8_Q1594A(TRP) ACT1-CUP1 3'ss GAGUAG	This work
yAAH3913	yAAH3853 + pAAH1761	Prp8_WT(TRP) ACT1-CUP1 3'ss UAGUAG	This work
yAAH3916	yAAH3527 + pAAH1761	Prp8_Q1594A(TRP) ACT1-CUP1 3'ss UAGUAG	This work
yAAH3914	yAAH3853 + pAAH1762	Prp8_WT(TRP) ACT1-CUP1 3'ss UAGGAG	This work
yAAH3917	yAAH3527 + pAAH1762	Prp8_Q1594A(TRP) ACT1-CUP1 3'ss UAGGAG	This work
yAAH3972	ade2, cup1delta:ra3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8(TRP) Fyv6::KAN	PRP8_WT(TRP) FYV6 deletion	This work
yAAH3973	ade2, cup1delta:ra3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8_Q1594A(TRP) Fyv6::KAN	PRP8_Q1594A(TRP) Fyv6 deletion	This work
yAAH3956	yAAH3972 + pAAH0470	PRP8_WT(TRP) FYV6_del ACT1-CUP1 WT	This work
yAAH3957	yAAH3972 + pAAH0526	PRP8_WT(TRP) FYV6_del ACT1-CUP1 U301G(3'ssGAG)	This work
yAAH3958	yAAH3972 + pAAH1644	PRP8_WT(TRP) FYV6_del ACT1-CUP1 U301A(3'ssAAG)	This work
yAAH3959	yAAH3972 + pAAH1645	PRP8_WT(TRP) FYV6_del ACT1-CUP1 U301C(3'ssCAG)	This work
yAAH3960	yAAH3972 + pAAH1752	PRP8_WT(TRP) FYV6_del ACT1-CUP1 A302U(3'ssUUG)	This work
yAAH3961	yAAH3972 + pAAH1753	PRP8_WT(TRP) FYV6_del ACT1-CUP1 A302C(3'ssUCG)	This work
yAAH3962	yAAH3972 + pAAH1754	PRP8_WT(TRP) FYV6_del ACT1-CUP1 G303U(3'ssUAU)	This work

yAAH3963	yAAH3972 + pAAH1755	PRP8_WT(TRP) FYV6_del ACT1-CUP1 G303C(3'ssUAC)	This work
yAAH3964	yAAH3973 + pAAH0470	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 WT	This work
yAAH3965	yAAH3973 + pAAH0526	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 U301G(3'ssGAG)	This work
yAAH3966	yAAH3973 + pAAH1644	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 U301A(3'ssAAG)	This work
yAAH3967	yAAH3973 + pAAH1645	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 U301C(3'ssCAG)	This work
yAAH3968	yAAH3973 + pAAH1752	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 A302U(3'ssUUG)	This work
yAAH3969	yAAH3973 + pAAH1753	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 A302C(3'ssUCG)	This work
yAAH3970	yAAH3973 + pAAH1754	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 G303U(3'ssUAU)	This work
yAAH3971	yAAH3973 + pAAH1755	PRP8_Q1594A(TRP) FYV6_del ACT1-CUP1 G303C(3'ssUAC)	This work
yAAH3929	ade2, cup1delta:ra3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8(TRP) Fyv6::KAN	PRP8_WT(TRP) Prp18_V191A	This work
yAAH3930	ade2, cup1delta:ra3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8_Q1594A(TRP) Fyv6::KAN	PRP8_Q1594A(TRP) Prp18_V191A	This work
yAAH3948	yAAH3929 + pAAH0470	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 WT	This work
yAAH3949	yAAH3929 + pAAH0526	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 U301G(3'ssGAG)	This work
yAAH3950	yAAH3929 + pAAH1644	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 U301A(3'ssAAG)	This work
yAAH3951	yAAH3929 + pAAH1645	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 U301C(3'ssCAG)	This work

yAAH3952	yAAH3929 + pAAH1752	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 A302U(3'ssUUG)	This work
yAAH3953	yAAH3929 + pAAH1753	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 A302C(3'ssUCG)	This work
yAAH3954	yAAH3929 + pAAH1754	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 G303U(3'ssUAU)	This work
yAAH3955	yAAH3929 + pAAH1755	PRP8_WT(TRP) Prp18_V191A ACT1-CUP1 G303C(3'ssUAC)	This work
yAAH3940	yAAH3930 + pAAH0470	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 WT	This work
yAAH3941	yAAH3930 + pAAH0526	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 U301G(3'ssGAG)	This work
yAAH3942	yAAH3930 + pAAH1644	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 U301A(3'ssAAG)	This work
yAAH3943	yAAH3930 + pAAH1645	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 U301C(3'ssCAG)	This work
yAAH3944	yAAH3930 + pAAH1752	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 A302U(3'ssUUG)	This work
yAAH3945	yAAH3930 + pAAH1753	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 A302C(3'ssUCG)	This work
yAAH3946	yAAH3930 + pAAH1754	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 G303U(3'ssUAU)	This work
yAAH3947	yAAH3930 + pAAH1755	PRP8_Q1594A(TRP) Prp18_V191A ACT1-CUP1 G303C(3'ssUAC)	This work
yAAH3933	ade2, cup1delta:ura3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8_WT(TRP) Prp22::HygR WT Prp22 (URA3/CEN)	PRP8_WT(TRP)WT Prp22 (URA/CEN)	This work
yAAH3935	ade2, cup1delta:ura3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8_Q1594A(TRP) Prp22::HygR WT Prp22 (URA3/CEN)	PRP8_Q1594A(TRP)WT Prp22 (URA/CEN)	This work

yAAH3974	ade2, cup1delta:ura3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8_WT(TRP) Prp22::HygR WT Prp22 (HIS3/CEN)	PRP8_WT(TRP)WT Prp22 (HIS/CEN)	This work
yAAH3975	ade2, cup1delta:ura3 his3 leu2 lys2 prp8delta:lys2 trp1 pJU169:PRP8_Q1594A(TRP) Prp22::HygR WT Prp22 (HIS3/CEN)	PRP8_Q1594A(TRP)WT Prp22 (HIS/CEN)	This work
yAAH3989	yAAH 3933 + pAAH1793	PRP8_WT(TRP)WT Prp22 (URA/CEN) Prp22 S635A (HIS/CEN)	This work
yAAH3992	yAAH 3933 + pAAH1796	PRP8_WT(TRP)WT Prp22 (URA/CEN) Prp22 G810A (HIS/CEN)	This work
yAAH3993	yAAH 3933 + pAAH1797	PRP8_WT(TRP)WT Prp22 (URA/CEN) Prp22 I1133R (HIS/CEN)	This work
yAAH3987	yAAH 3935 + pAAH1793	PRP8_Q1594A(TRP)WT Prp22 (URA/CEN) Prp22 S635A (HIS/CEN)	This work
yAAH3994	yAAH 3935 + pAAH1796	PRP8_Q1594A(TRP)WT Prp22 (URA/CEN) Prp22 G810A (HIS/CEN)	This work
yAAH3995	yAAH 3935 + pAAH1797	PRP8_Q1594A(TRP)WT Prp22 (URA/CEN) Prp22 I1133R (HIS/CEN)	This work
yAAH4000	yAAH 3933 + pAAH1799	PRP8_WT(TRP)WT Prp22 (URA/CEN) Prp22 G810A I1133R (HIS/CEN)	This work
yAAH4001	yAAH 3935 + pAAH1799	PRP8_Q1594A(TRP)WT Prp22 (URA/CEN) Prp22 G810A I1133R (HIS/CEN)	This work

Supplementary Table S2. Plasmids used in this study

Stock ID	Plasmid name	Reference
pAAH1440	pRS424 Prp8 WT TRP1	This work
pAAH1626	pRS424 Prp8_Q1594N TRP1	This work
pAAH1627	pRS424 Prp8_Q1594A TRP1	This work
pAAH1628	pRS424 Prp8_Q1594R TRP1	This work
pAAH1629	pRS424 Prp8_Q1594E TRP1	This work
pAAH1630	pRS424 Prp8_Q1594G TRP1	This work
pAAH0470	pGAC24-ACT1-CUP1 wild type	(Steinmetz and Brow 2003); gift from Dave Brow
pAAH1644	pGAC24-ACT1-CUP1 U301A(3'ssAUG)	This work
pAAH1645	pGAC24-ACT1-CUP1 U301C(3'ssCUG)	This work
pAAH1684	pGAC24-ACT1-CUP1 U301G(3'ssGUG)	This work
pAAH1729	pGAC24-ACT1-CUP1 3'ssGAGUAG	This work
pAAH1752	pGAC24-ACT1-CUP1 A302U(3'ssUUG)	This work
pAAH1753	pGAC24-ACT1-CUP1 A302C(3'ssUCG)	This work
pAAH1754	pGAC24-ACT1-CUP1 G303U(3'ssUAU)	This work
pAAH1755	pGAC24-ACT1-CUP1 G303C(3'ssUAC)	This work
pAAH1761	pGAC24-ACT1-CUP1 3'ssUAGUAG	This work
pAAH1762	pGAC24-ACT1-CUP1 3'ssUAGGAG	This work
pAAH0278	pAG25-NATMX	(Goldstein and McCusker 1999); Euroscarf
pAAH0093	pUG6-KANMX	(Goldstein and McCusker 1999); Euroscarf
pAAH1603	p360-Prp18-11(Prp18V191A)	(Aronova et al. 2007); gift from Beate Schwer
pAAH1791	pRS314-WT Prp22 His	This work
pAAH1793	pRS314-Prp22 S635A His	This work
pAAH1796	pRS314-Prp22 G810A His	This work
pAAH1797	pRS314-Prp22 I1133R His	This work
pAAH1799	pRS314-Prp22_G810A I1133R His	This work

SUPPLEMENTAL REFERENCES

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