Supplementary Information

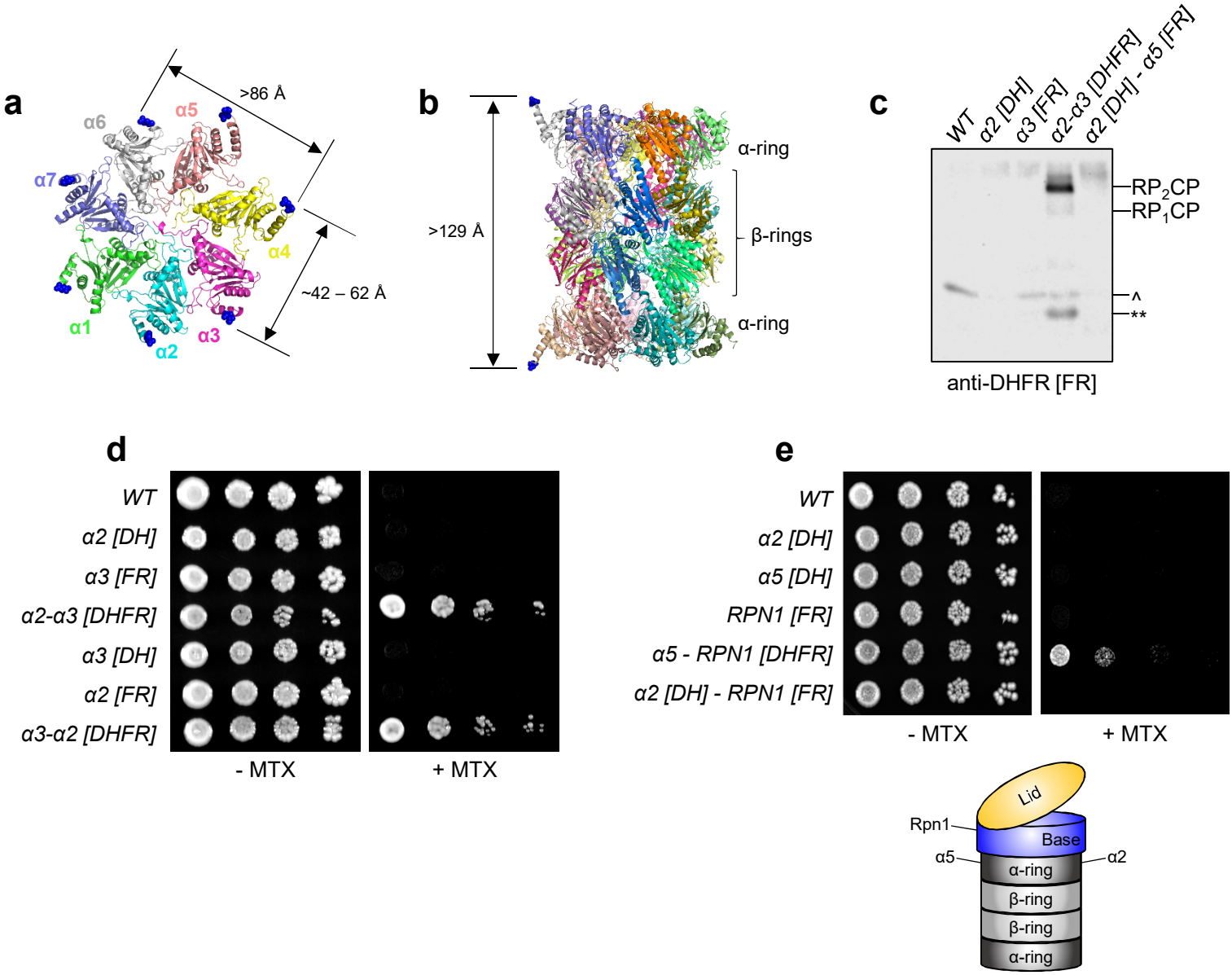
Proteasome subunit α1 overexpression preferentially drives canonical proteasome biogenesis and enhances stress tolerance in yeast

Lauren A. Howell, Anna K. Peterson, and Robert J. Tomko Jr.*

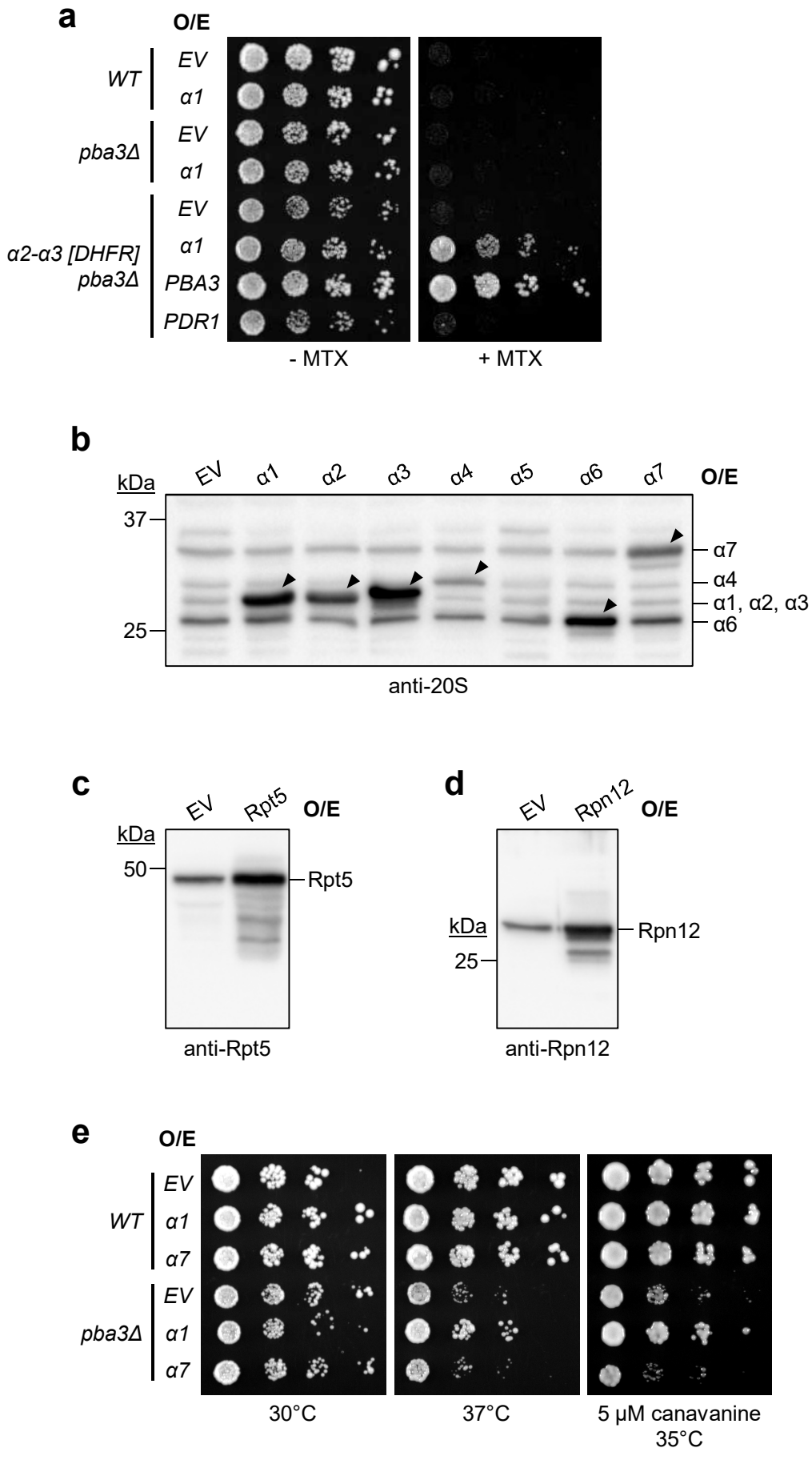
Department of Biomedical Sciences, Florida State University College of Medicine, Tallahassee, Florida 32306, USA

*Correspondence and requests for materials should be addressed to R. J. T., Jr. (email: robert.tomko@med.fsu.edu)

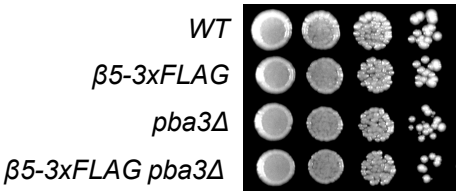
Howell *et al*, Supplementary Figure S1
Data related to Figure 1 in main text



Howell *et al*, Supplementary Figure S2
Data related to Figure 2 in main text

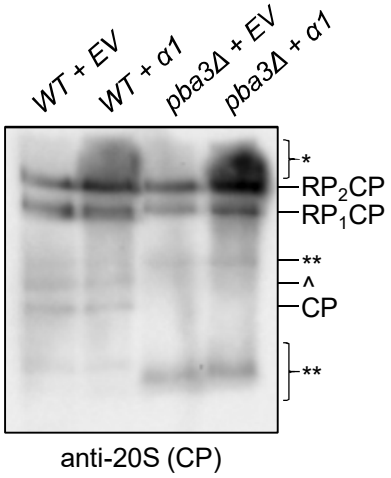


Howell *et al*, Supplementary Figure S3
Data related to Figure 3 in main text

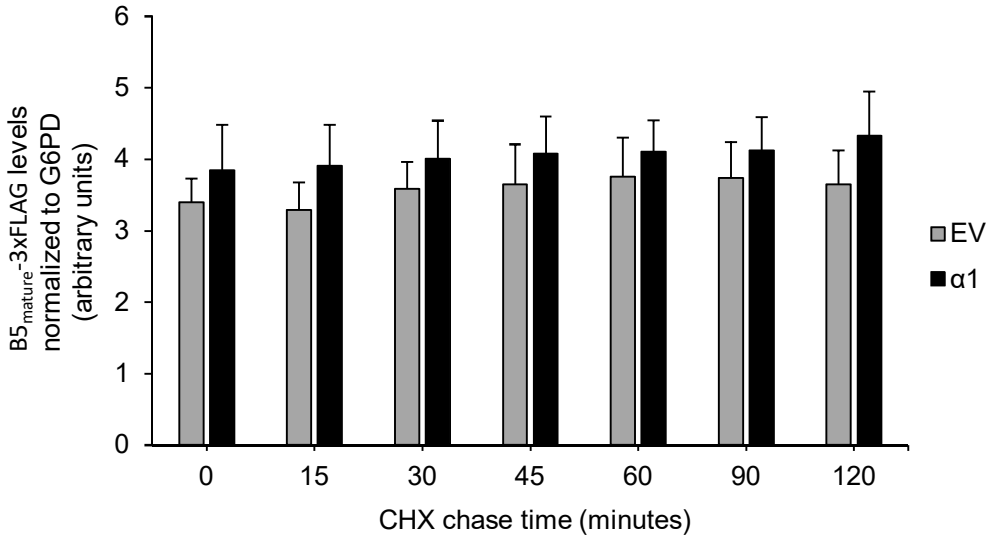


YPD, 30°C

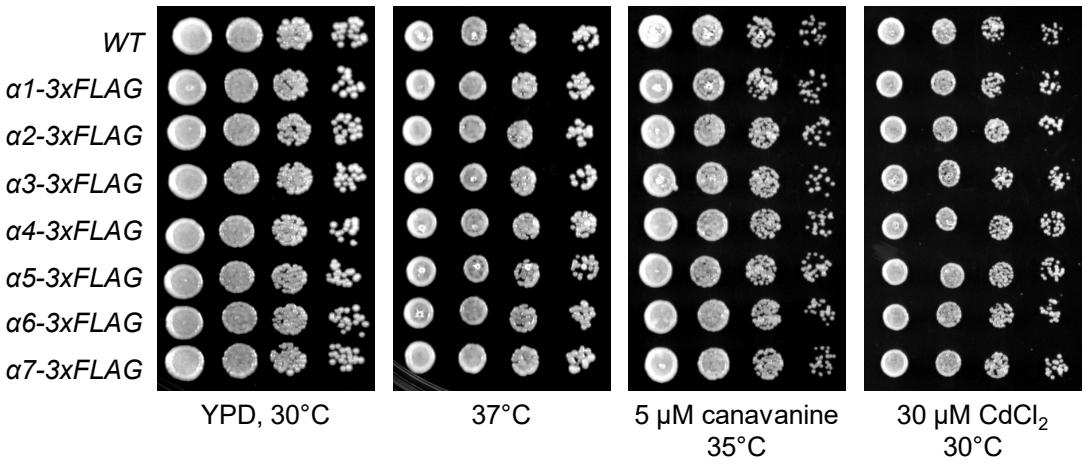
Howell *et al*, Supplementary Figure S4
Data related to Figure 5 in main text



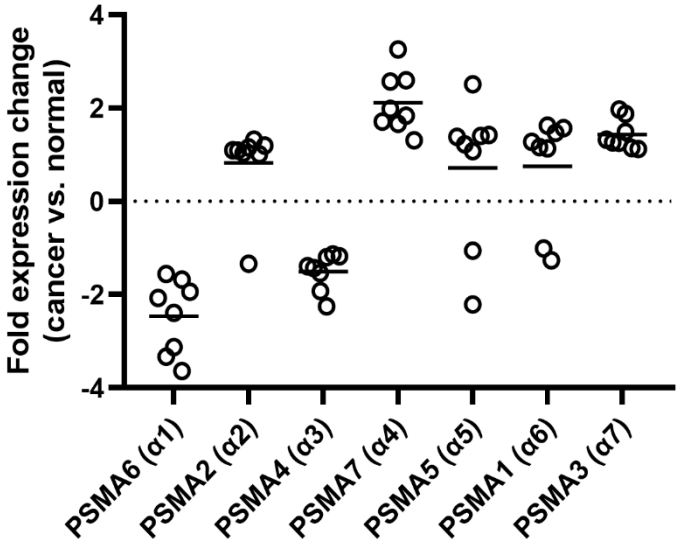
Howell *et al*, Supplementary Figure S5
Data related to Figure 6 in main text



Howell *et al*, Supplementary Figure S6
Data related to Figure 7 in main text



Howell *et al*, Supplementary Figure S7
Data related to Discussion in main text



Howell *et al*, Supplementary Figure S8
Full-length blots/gels for main text figures

Fig. 1d - 20S

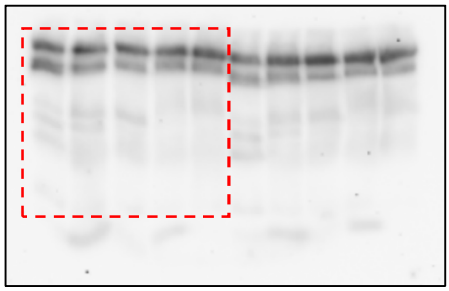


Fig. 1d - Rpn12

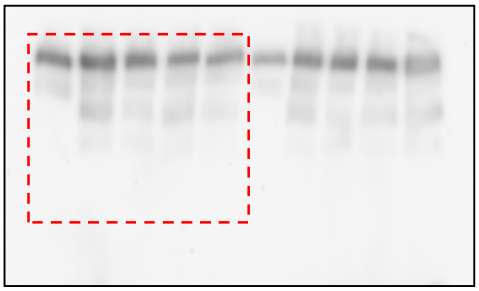


Fig. 3a

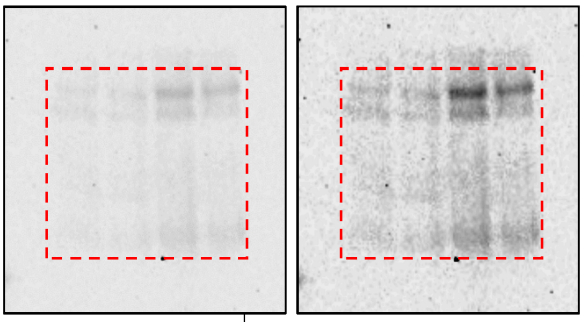


Fig. 3b

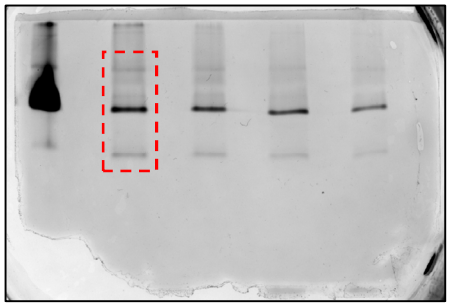


Fig. 4a

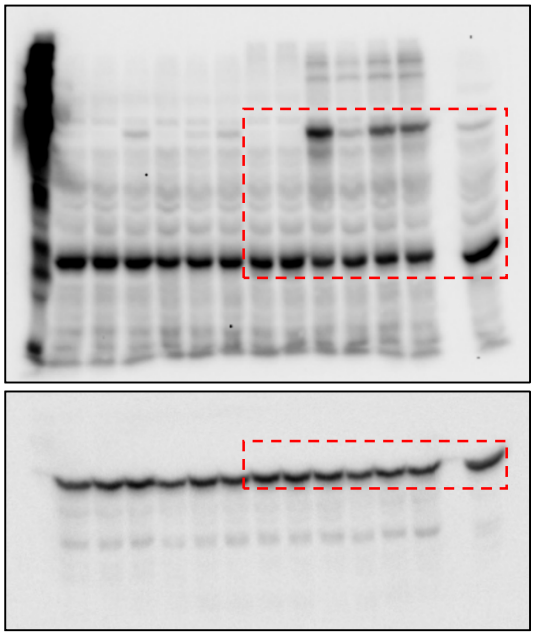


Fig. 4c

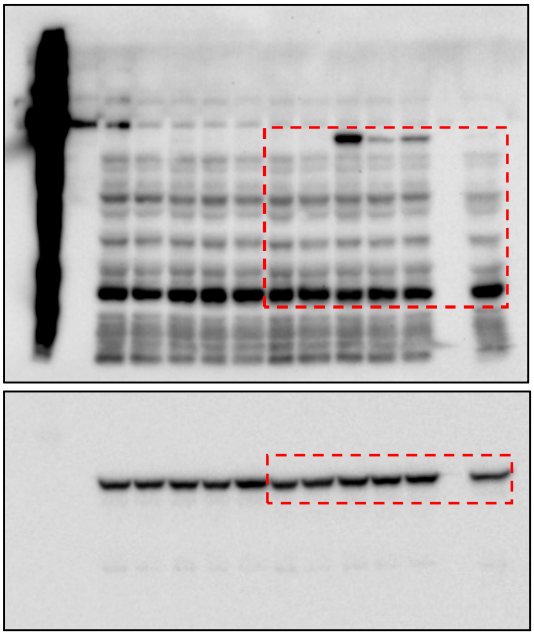


Fig. 5d

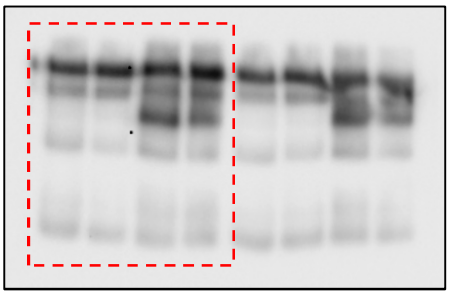


Fig. 6

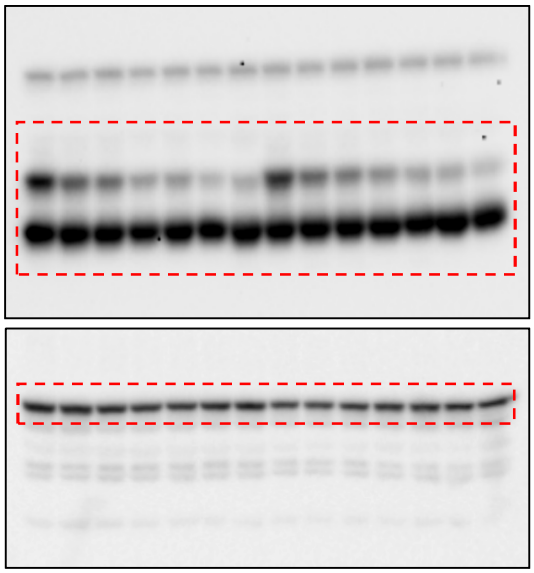


Fig. 7a

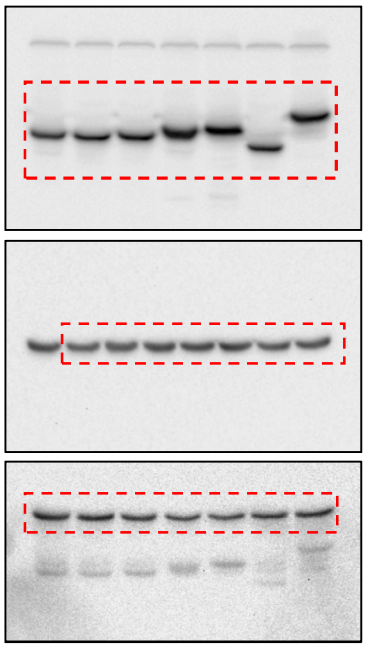


Fig. 7b

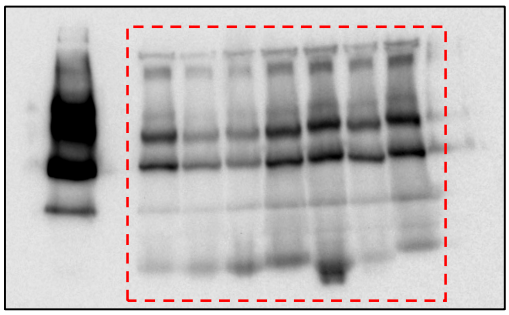
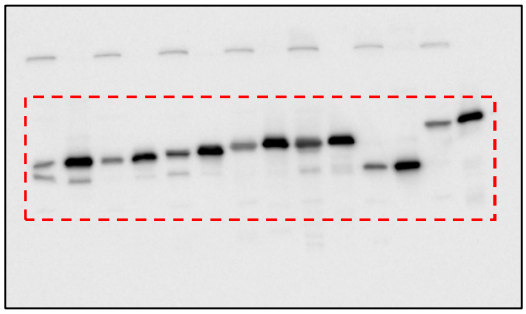
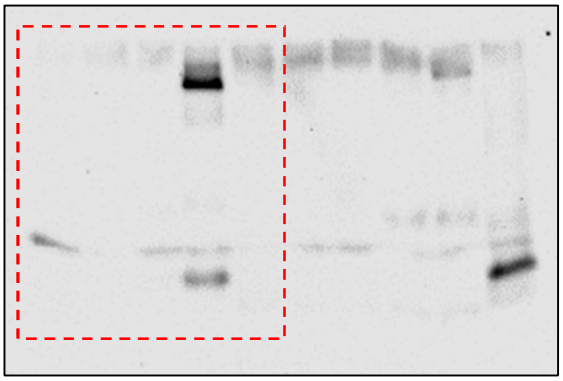


Fig. 7c

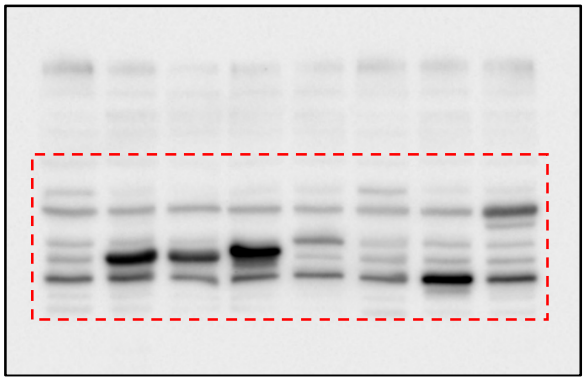


Howell *et al*, Supplementary Figure S9
Full-length blots for Supplementary Figures

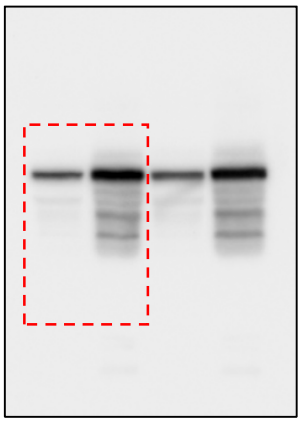
Supplementary Fig. S1c



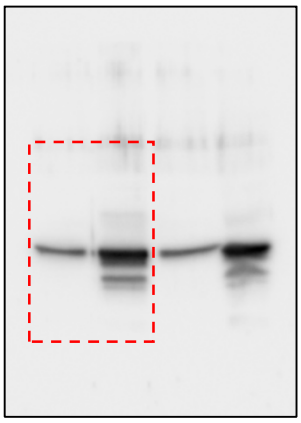
Supplementary Fig. S2b



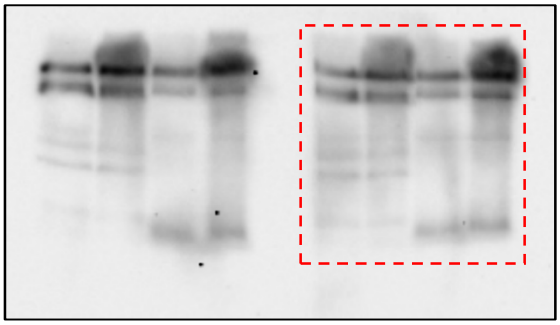
Supplementary Fig. S2c



Supplementary Fig. S2d



Supplementary Fig. S4



Supplementary Figure Legends

Supplementary Figure S1 related to Figure 1 in main text. (**a**) Estimation of intra-ring distance constraints for design of split-DHFR reporter system. A top view of the α-ring (PDB 1RYP) is shown, with the C-terminal amino acids of each subunit displayed as blue spheres. Example measurements of the distance between the C-terminal carboxylate carbons of nearest-adjacent neighbor and nearest-nonadjacent neighbor subunits are shown. Nearest-adjacent measurements spanned ~42-62 Å, whereas all nearest-nonadjacent measurements were greater than 86 Å. (**b**) Estimation of inter-ring distance constraints for design of split-DHFR reporter system. A side view of the α-ring (PDB 1RYP) is shown, with the C-terminal amino acids of opposing α-subunits displayed as blue spheres. An example measurement of the distance between the nearest inter-ring α-subunits is shown. All such measurements exceeded 129 Å. (**c**) Cell extracts from yeast strains expressing α-subunit fusions to N- or C-terminal DHFR fragments (designated [DH] or [FR], respectively) from their chromosomal loci were separated by non-denaturing PAGE and immunoblotted with antibodies against the C-terminal DHFR fragment. The positions of doubly-capped CP (RP_2CP) and singly-capped CP (RP_1CP) are shown. Under non-denaturing conditions, the [FR] fragment appears to be cleaved from subunits, but is stabilized upon complementation by an adjacent α-subunit [DH] fusion. **, possible CP assembly intermediate; ^, cross-reactive species. Full-length blot is presented in Supplementary Fig. S9. (**d,e**) Equal numbers of cells from the indicated yeast strains were spotted in six-fold serial dilutions on synthetic complete plates lacking or containing MTX and incubated for three days at 30°C. The illustration of the 26S proteasome accompanying (**e**) depicts the location of the RP base subunit Rpn1 relative to the α-subunits α2 and α5.

Supplementary Figure S2 related to Figure 2 in main text. (**a**) Overexpression of *PDR1* does not confer MTX resistance. The gene encoding *PDR1*, a transcription factor that regulates the pleiotropic drug response, was also encoded by the genomic fragment containing *α1*. To test whether the enhanced growth on MTX could be due to Pdr1-dependent efflux of MTX from cells, *the α2-α3 [DHFR] pba3Δ* reporter strain was transformed with a high-copy plasmid encoding *PDR1*. Transformants were spotted in six-fold serial dilutions onto synthetic complete plates lacking or containing MTX and incubated for three days at 30°C. Overexpression of Pdr1 did not confer growth on MTX. The growth assay shown here is an uncropped form of the image shown in **Fig. 2b in main text**. EV, empty vector. (**b-d**) Whole cell extracts were prepared from *pba3*Δ cells transformed with high-copy plasmids encoding the indicated proteins (**from Fig. 2c in main text**), followed by SDS-PAGE and immunoblotting with antibodies against the 20S CP (**b**), the RP base subunit Rpt5 (**c**), or the RP lid subunit Rpn12 (**d**). The 20S antibody does not recognize the α5 subunit. EV, empty vector. Full-length blots are presented in Supplementary Fig. S9. (**e**) Equal numbers of cells from the indicated yeast strains expressing empty vector (EV), α1, or α7 from a high-copy plasmid were spotted in six-fold serial dilutions on the indicated media and incubated as specified for three days.

Supplementary Figure S3 related to Figure 3 in main text. Equal numbers of cells from the indicated yeast strains were spotted in six-fold serial dilutions on YPD plates and incubated for two days at 30°C.

Supplementary Figure S4 related to Figure 5 in main text. Cell extracts of WT or *pba3Δ* yeast expressing empty vector (EV) or α1 from a high-copy plasmid were separated by non-denaturing PAGE and immunoblotted with antibodies against the 20S CP. The positions of doubly-capped CP (RP_2CP), singly-capped CP (RP_1CP), and CP are shown. **, CP assembly intermediates; ^, Blm10-CP; *, cross-reactive species that forms upon exogenous expression of α1. Full-length blot is presented in Supplementary Fig. S9.

Supplementary Figure S5 related to Figure 6 in main text. Quantification of mature β5 levels throughout the course of the cycloheximide (CHX) chase shown in **Fig. 6 in main text**. Bands corresponding to the mature species ($\beta5_{mature}$-3xFLAG) were normalized to the G6PD loading control (*n* = 8; error bars = s.e.m.).

Supplementary Figure S6 related to Figure 7 in main text. Equal numbers of cells from the indicated yeast strains were spotted in six-fold serial dilutions on the indicated media and incubated for two days as specified.

Supplementary Figure S7 related to Discussion in main text. Differential mRNA expression of proteasome α-subunits was examined in eight published testicular cancer datasets exhibiting elevated α4 mRNA levels (Korkola et al., 2006 (mixed germ cell tumor, embryonal carcinoma, seminoma, yolk sac tumor, and teratoma); Skotheim et al., 2005 (embryonal carcinoma, yolk sac tumor, and teratoma)) using Oncomine (www.oncomine.org). Data points represent the fold change in expression of a given gene for each cancer subtype, calculated via "cancer vs. normal" analysis (Rhodes et al., 2007). The means for each α-subunit is shown as a horizontal bar

Supplementary Figure S8: Full-length blots/gels for main text figures. Red boxes indicate the cropped portion of the blot/gel used in the figure.

Supplementary Figure S9: Full-length blots for Supplementary Figures. Red boxes indicate the cropped portion of the blot used in the figure.

Supplementary Table S1: Yeast strains used in this study

Name	Genotype	Source
RTY1	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1* (alias MHY500)	Chen et al., 1993
RTY2	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1* (alias MHY501)	Chen et al., 1993
RTY7	*MATa ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63 lys2-801 ade2-101* (alias YPH499)	Sikorski and Hieter, 1989
RTY25	*MATa ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63 lys2-801 ade2-101 ump1Δ::HIS3*	This study
RTY361	*MATα ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63 lys2-801 ade2-101 RPN1-GFP(S65T):kanMX6*	This study
RTY978	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE6-yEGFP:kanMX6*	Nemec et al., 2017
RTY1008	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pba3Δ::kanMX4* (alias MHY4386)	Kusmierczyk et al., 2008
RTY1010	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pba4Δ::hphMX4* (alias MHY4298)	Kusmierczyk et al., 2008
RTY1064	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre9Δ::natMX4*	This study
RTY1244	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4*	This study
RTY1249	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE9-DHFR[1,2]:natMX4*	This study
RTY1255	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE9-DHFR[3]:hphMX4*	This study
RTY1257	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[3]:hphMX4*	This study
RTY1263	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 PRE9-DHFR[3]:hphMX4*	This study

RTY1274	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE9-DHFR[1,2]:natMX4 PRE8-DHFR[3]:hphMX4*	This study
RTY1304	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 PRE9-DHFR[3]:hphMX4 pba3Δ::kanMX4*	This study
RTY1310	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 PRE9-DHFR[3]:hphMX4 nas2Δ::HIS3*	This study
RTY1337	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre6Δ::HIS3 [pRS317-pre6-N79C-6His] pre9Δ::HIS3* (alias MHY2896)	Velichutina et al., 2004
RTY1338	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre6Δ::HIS3 [pRS317-pre6-I155C-6His] pre9Δ::HIS3* (alias MHY2897)	Velichutina et al., 2004
RTY1339	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre6Δ::HIS3 [pRS317-pre6-N79C,I155C-6His]* (alias MHY2900)	Velichutina et al., 2004
RTY1340	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre6Δ::HIS3 [pRS317-pre6-N79C,I155C-6His] pre9Δ::HIS3* (alias MHY2901)	Velichutina et al., 2004
RTY1347	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 PRE9-DHFR[3]:hphMX4 pba1Δ::kanMX4*	This study
RTY1434	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre6Δ::HIS3 [pRS316-PRE6] pre9Δ::HIS3*	This study
RTY1446	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PUP2-DHFR[1,2]:natMX4*	This study
RTY1454	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PUP2-DHFR[3]:hphMX4*	This study
RTY1479	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PUP2-DHFR[1,2]:natMX4 PRE9-DHFR[3]:hphMX4*	This study
RTY1637	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre6Δ::HIS3 [pRS316-PRE6]*	This study
RTY1680	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE9-DHFR[1,2]:natMX4 pre6Δ::HIS3 [pRS316-PRE6]*	This study
RTY1686	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PUP2-DHFR[3]:hphMX4 pre6Δ::HIS3 [pRS316-PRE6]*	This study
RTY1693	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 SCL1-DHFR[1,2]:natMX4 PRE8-DHFR[3]:hphMX4*	This study

RTY1734	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 RPN1-DHFR[1,2]:natMX4*	This study
RTY1768	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 RPN1-DHFR[1,2]:natMX4 PUP2-DHFR[3]:hphMX4*	This study
RTY1884	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 RPN1-DHFR[3]:hphMX4*	This study
RTY2002	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 SCL1-DHFR[1,2]:natMX4 pre6Δ::HIS3 [pRS316-PRE6]*	This study
RTY2004	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 PUP2-DHFR[3]:hphMX4*	This study
RTY2116	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 PRE9-DHFR[3]:hphMX4 PRE6-yEGFP:kanMX6*	This study
RTY2117	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-DHFR[1,2]:natMX4 PRE9-DHFR[3]:hphMX4 RPN1-GFP(S65T):kanMX6*	This study
RTY2205	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pba3Δ::kanMX4 pre6Δ::HIS3 [pRS316-PRE6]*	This study
RTY2206	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre6Δ::HIS3 [pRS317-pre6-N79C,I155C-6His] pba3Δ::kanMX4*	This study
RTY2213	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre8-K160C-HF::URA3 pre9Δ::HIS3 [pRS315-pre9-L56C-T7] pba3Δ::kanMX4*	This study
RTY2214	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre8-K160C-HF::URA3 pre9Δ::HIS3 [pRS315-PRE9-T7]* (alias MHY1838)	Velichutina et al., 2004
RTY2215	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-HF:URA3 pre9Δ::HIS3 [pRS315-pre9-L56C-T7]* (alias MHY1850)	Velichutina et al., 2004
RTY2216	*MATα his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 pre8-K160C-HF::URA3 pre9Δ::HIS3 [pRS315-pre9-L56C-T7]* (alias MHY1839)	Velichutina et al., 2004
RTY2231	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE2-6xGly-3xFLAG:HIS3MX6*	This study
RTY2263	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE1-6xGly-3xFLAG:kanMX6 pba3Δ::kanMX4*	This study
RTY2320	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 SCL1-6xGly-3xFLAG:HIS3MX6*	This study

RTY2322	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE8-6xGly-3xFLAG:HIS3MX6*	This study
RTY2324	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE9-6xGly-3xFLAG:HIS3MX6*	This study
RTY2326	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE6-6xGly-3xFLAG:HIS3MX6*	This study
RTY2328	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PUP2-6xGly-3xFLAG:HIS3MX6*	This study
RTY2330	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE5-6xGly-3xFLAG:HIS3MX6*	This study
RTY2332	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE10-6xGly-3xFLAG:HIS3MX6*	This study
RTY2510	*MATa his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 PRE6-6xGly-3xFLAG:HIS3MX6 pba3Δ::kanMX4*	This study

Supplementary Table S2: Plasmids used in this study

Name	Genotype	Source
pRT474	pRS424	Christianson et al., 1992
pRT711	pRS424-PRE8	This study
pRT845	pRS424-RPN12	This study
pRT1449*	pAG25-DHFR[1,2] (natMX4)	Tarassov et al., 2008
pRT1450*	pAG32-DHFR[3] (hphMX4)	Tarassov et al., 2008
pRT1513	pRS314-PRE6	This study
pRT1514	pRS315-PRE6	This study
pRT1515	pRS424-PRE9	This study
pRT1516	pRS424-PRE6	This study
pRT1517	pRS424-PRE10	This study
pRT1526	pRS314-PRE6-DHFR[1,2]	This study
pRT1527	pRS315-PRE6-DHFR[3]	This study
pRT1885	pRS424-RPT5	This study
pRT1964	pRS424-PBA3	This study

pRT1976	pRS424-SCL1	This study
pRT2202	pRS424-PDR1	This study
pRT2203	pRS424-DOA5	This study
pRT2204	pRS424-PRE5	This study

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* The linker sequence for pRT1449 and pRT1450 is as follows: GGCGGTGGCGGATCAGGAG GCGGTGGGTCT, encoding amino acids GGGGSGGGGS.

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