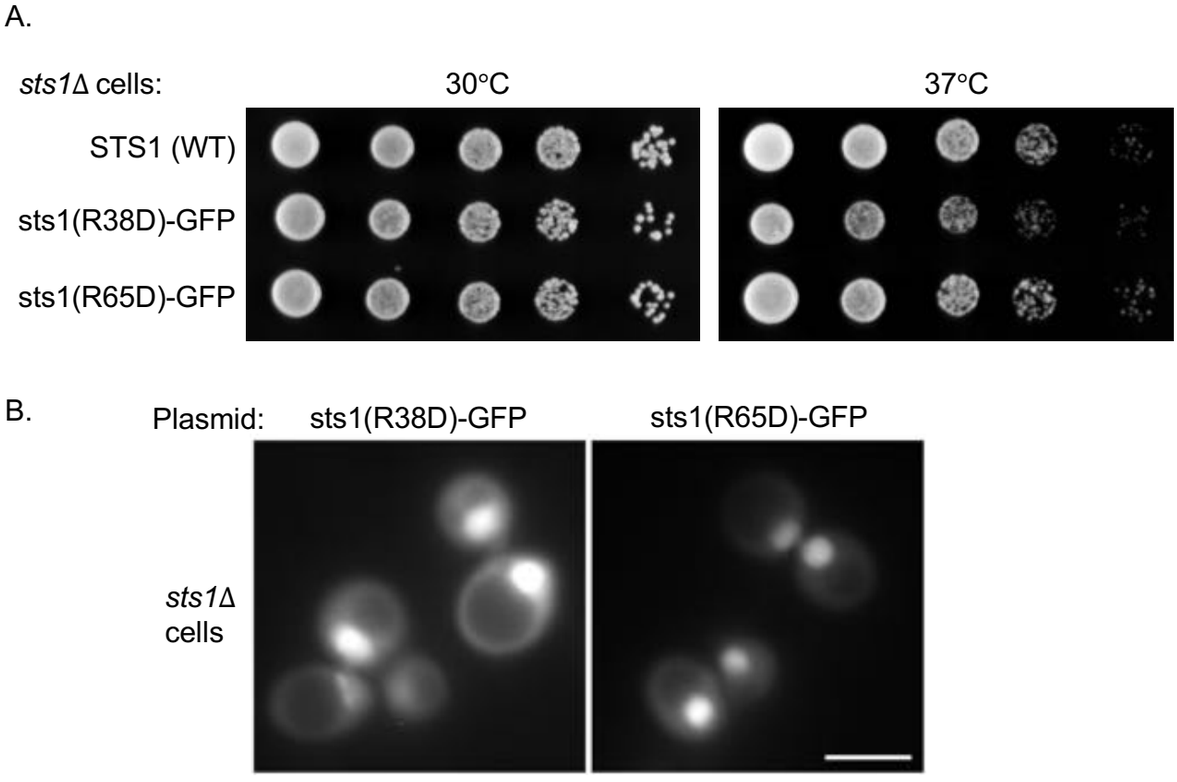
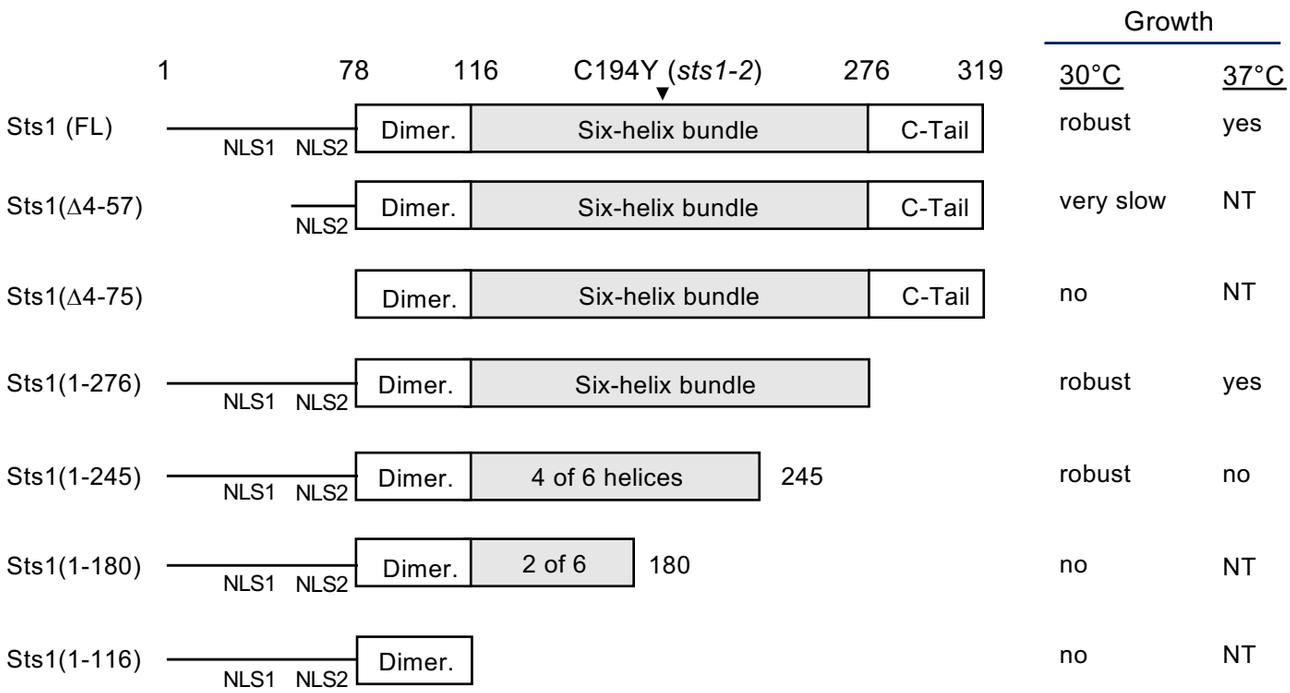
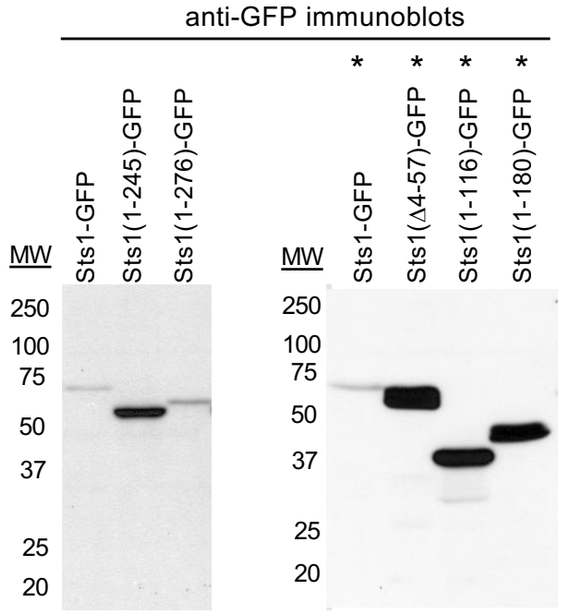


Figure S1. The *sts1-R38D* mutant has minor growth and localization defects.
(A) MHY9580 (*sts1Δ*/pRS316-STS1) was transformed with p415MET25-STS1-GFP, p415MET25-sts1R38D-GFP, or p415MET25-sts1R65D-GFP. These transformants were struck on 5-FOA plates to select against pRS316-STS1. The resulting strains were spotted in 6-fold serial dilutions on SD complete plates and grown at 30°C or 37°C for 2 days. (B) The *sts1* NLS single-mutant GFP fusions were expressed from p415GPD vectors at 30°C and visualized by fluorescence microscopy. Scale bar, 5 um. Greater defects due to mutation of the N-terminal basic segment is typical of bipartite NLSs.

A.



B.



C.

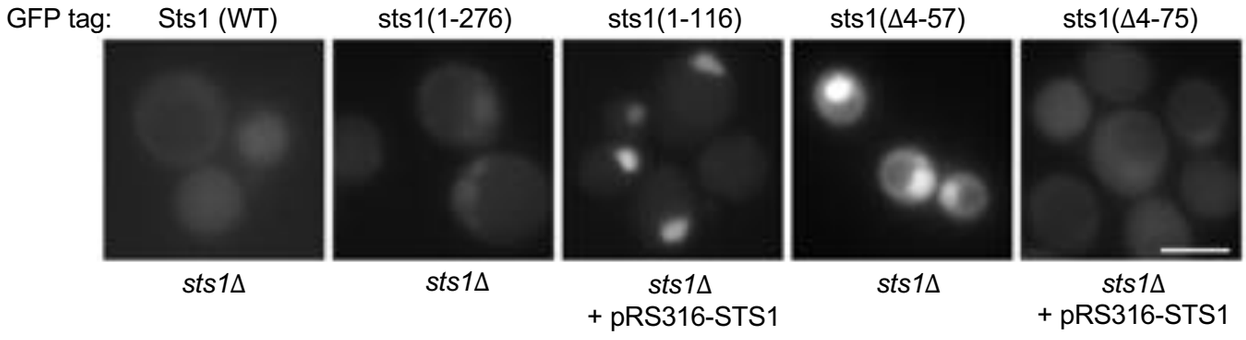


Figure S2. Truncated forms of Sts1 with impaired function are more abundant in cells. (A) Plasmids bearing the *MET25* promoter and coding for the indicated regions of the Sts1 protein, all bearing a C-terminal GFP extension, were transformed into a strain (MHY9579) deleted for *STS1* on the chromosome and carrying pRS316-STS1. Two resulting transformants for each plasmid were struck on media containing 5-FOA to eject the pRS316-STS1 plasmid. Growth of these transformants at 30°C on media containing 5-FOA is indicated in the left column. For strains that grew well at 30°C, growth on YPD at 37°C was tested, and the results are shown. NT = not tested. (B) Equal amounts of cells (assayed by optical density measurement) were lysed, and the cell extracts were subjected to immunoblot analysis for the various Sts1-GFP proteins expressed in part A. For plasmids that allowed growth of the *sts1*Δ strain on 5-FOA, expression was tested in a strain expressing the Sts1-GFP protein as the only form of Sts1. For plasmids that did not allow replacement of pRS316-STS1 (as indicated by asterisks), extracts of WT cells expressing these proteins were used. The expected size for full-length Sts1-GFP is ~65 kD. (C) Localization of select GFP-tagged Sts1 proteins expressed with the *MET25* promoter on a low-copy plasmid. Scale bar, 5 μm.

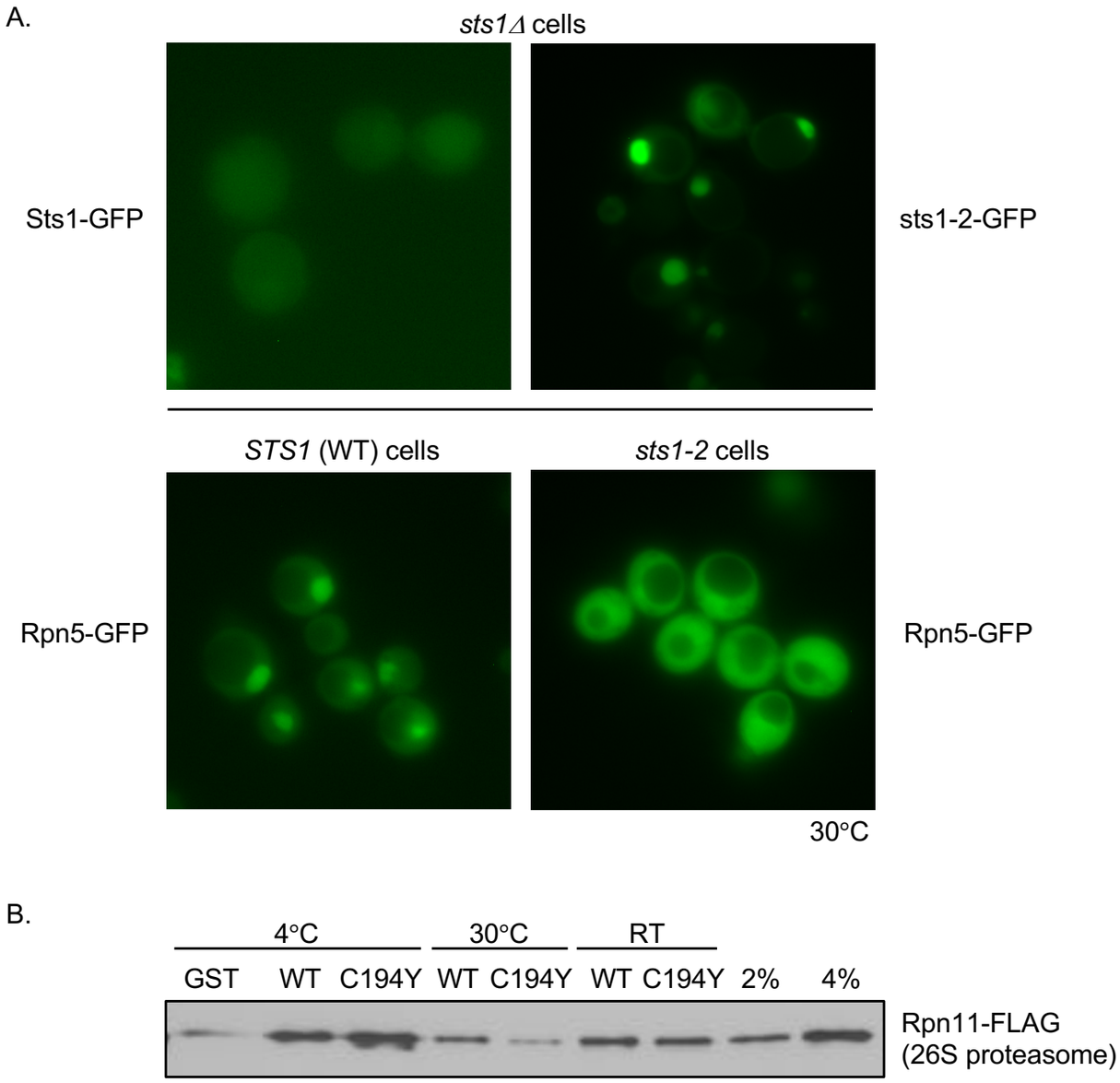


Figure S3. The sts1-2 mutation (C194Y) allows Sts1 nuclear concentration but impairs proteasome binding and nuclear localization.

(A) To localize Sts1 derivatives (top row), MHY9579 (*sts1*Δ [pRS316STS1]) cells were transformed with pRS415MET25 plasmids expressing the indicated GFP fusions, and the original plasmid was evicted on 5-FOA. Only low levels of WT Sts1 accumulate under these expression condition. For proteasome localization, a low-copy plasmid expressing Rpn5-GFP (native promotor) was transformed into WT and *sts1-2* mutant cells and visualized by fluorescence microscopy of live cells at 30°C. (B) Binding of 26S proteasomes to Sts1-Srp1. Proteasomes (1 μM) affinity purified from yeast were mixed with GST-Srp1/Sts1-6His (1 μM) at the indicated temperatures and loaded on GSH resin. Eluted proteins were examined by anti-Flag immunoblotting. The sts1-C194Y exhibited temperature-sensitive proteasome binding. First lane, GST only control; proteasome inputs shown at right.

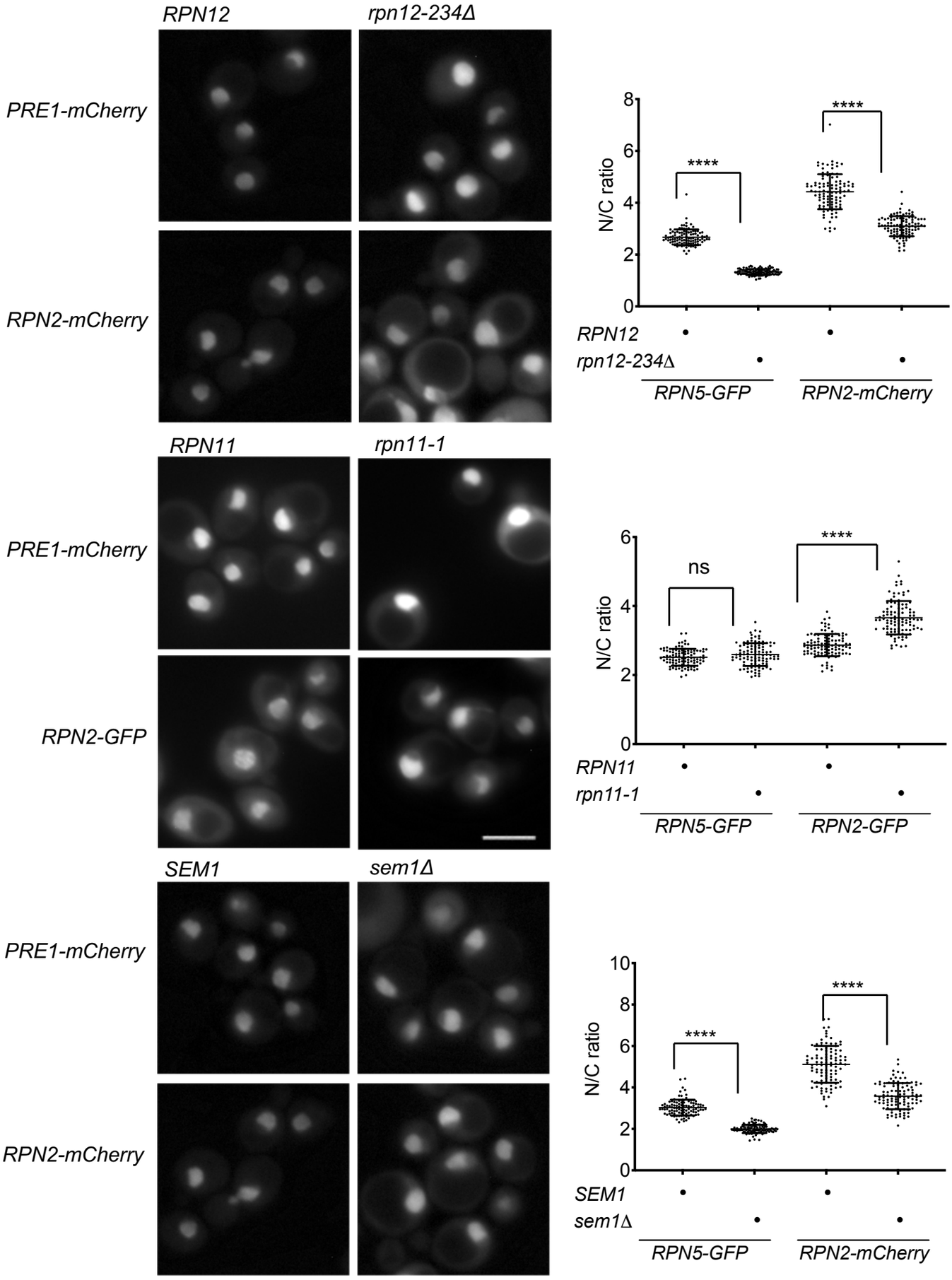


Figure S4. Localization of base and CP in lid mutants. Proteasome assembly mutants were grown in SD-complete media to log phase and imaged by fluorescence microscopy; three replicates of at least 100 cells each were counted. ANOVA was used to determine statistical significance of differences (**** $p<0.0001$). Scale bar is 5 μm. Yeast strains used were MHY6938, MHY6946, MHY6954, MHY6956, MHY6964, MHY6966, MHY9172, MHY9174, MHY9573, and MHY9581.

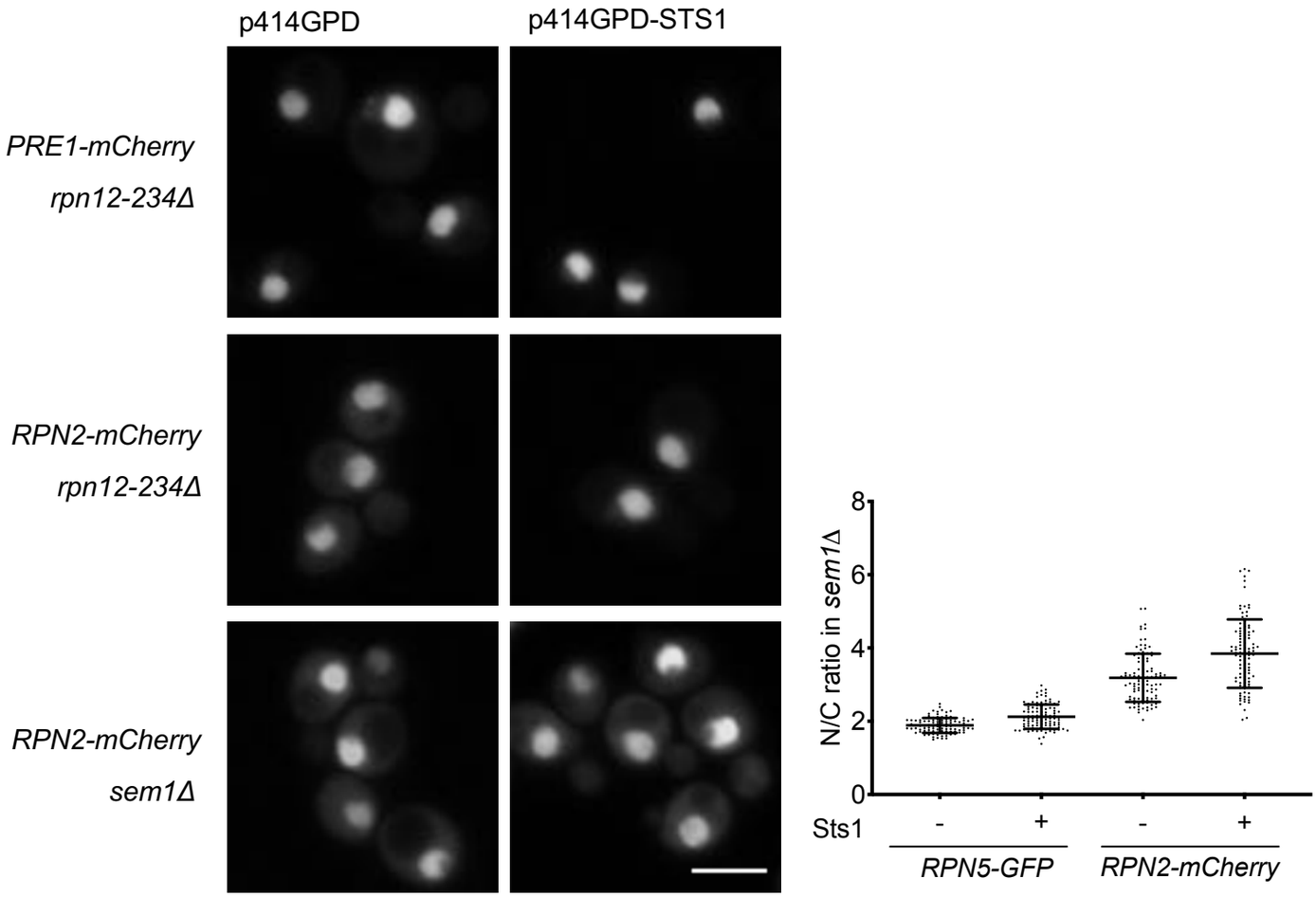


Figure S5. Effect of *STS1* overexpression on base and CP localization in lid mutants.
In the *rpn12-234Δ* mutant, both base and CP subunits show increased nuclear localization upon Sts1 overexpression. The very low cytoplasmic signals precluded accurate measurement of N/C signal ratios. Microscopy was performed as in Fig. 5 with 3 replicates of at least 100 cells per replicate counted. Yeast strains used were MHY6954, MHY6964, and MHY9174.

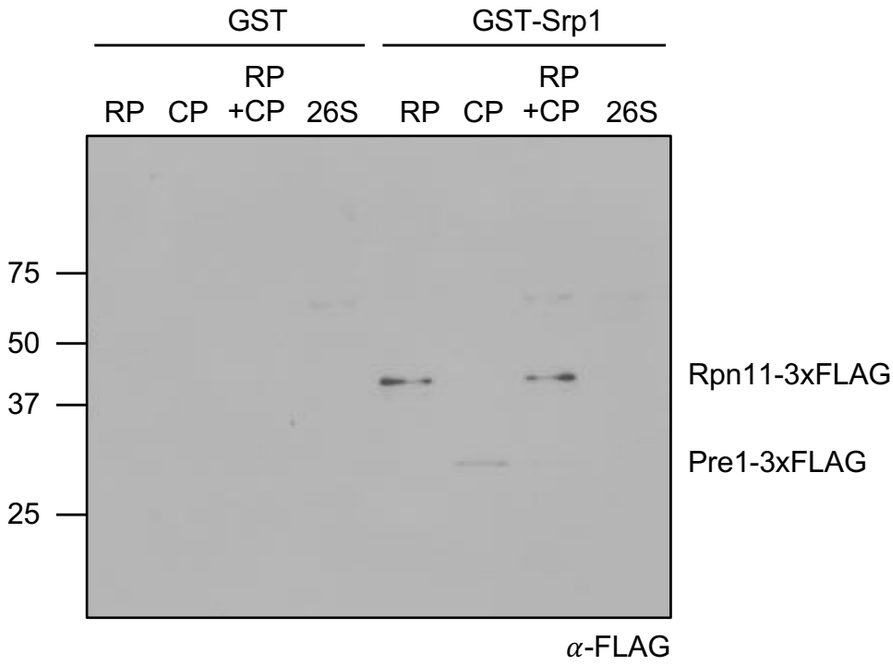


Figure S6. GST and GST-Srp1 control pulldowns of proteasome species.

Recombinant GST-Srp1/Sts1-6His complex or GST alone was immobilized on glutathione (GSH) resin and incubated with purified CP, RP, reconstituted CP+RP, or 26S proteasome. Anti-Flag immunoblot of tagged subunits.

Table S1. Yeast Strains Used in This Study

Strain	*Genotype*	Source
MHY500	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2*	(Chen et al. 1993)
MHY5748	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 rpn12-234Δ:hphMX4*	(Tomko, 2011)
MHY5841	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN11-6xGly-3xFLAG:kanMX6*	(Li et al., 2015)
MHY6952	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 PRE1-6xGly-3xFLAG:kanMX6*	(Li et al., 2015)
MHY6377	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN5-GFP(S65T):HIS3MX6*	R. Tomko, MH lab strain
MHY6824	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 rpn12-234Δ:hphMX4 RPN5-GFP(S65T):HIS3MX6*	R. Tomko, MH lab strain
MHY6938	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 PRE1-mCherry:NAT*	R. Tomko, MH lab strain
MHY6946	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN2-GFP(S65T):kanMX6*	R. Tomko, MH lab strain
MHY6954	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN5-GFP(S65T):HIS3MX6 PRE1-mCherry:NAT rpn12-234Δ:hphMX4*	R. Tomko, MH lab strain
MHY6956	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN5-GFP(S65T):HIS3MX6 PRE1-mCherry:NAT*	R. Tomko, MH lab strain
MHY6964	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN5-GFP(S65T):HIS3MX6 RPN2-mCherry:NAT rpn12-234Δ:hphMX4*	R. Tomko, MH lab strain
MHY6966	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN5-GFP(S65T):HIS3MX6 RPN2-mCherry:NAT*	R. Tomko, MH lab strain
MHY7257	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN8-GFP(S65T):HIS3MX6*	R. Tomko, MH lab strain

MHY8602	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 NUP49-GFP:HIS5 RPN5-mCherry:natMX*	R. Tomko, MH lab strain
MHY8748	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN8-GFP(S65T):HIS3MX6 rpn12-234Δ:hphMX4*	R. Tomko, MH lab strain
MHY9039	*MATa his3 ura3 leu2-3,112*. This strain is a hybrid between MHY500 and W303, has some combination of *trp1-1, lys2-801, ade2-1, can1-100 NUP49-GFP:HIS5 RPN5-mCherry:natMX sem1Δ::kanMX6*	This study
MHY9059	*MATa his3 ura3 leu2-3,112*. This strain is a hybrid between MHY500 and W303, has some combination of *trp1-1, lys2-801, ade2-1, can1-100 NUP49-GFP:HIS5 RPN5-mCherry:natMX rpn11-1*	This study
MHY9134	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sem1Δ::kanMX6*	This study
MHY9172	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sem1Δ::kanMx6 RPN5-GFP(S65T):HIS3MX6 PRE1-mCherry:NAT*	This study
MHY9174	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN5-GFP(S65T):HIS3MX6 RPN2-mCherry:NAT sem1Δ::kanMx6*	This study
MHY9509	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 rpn11-1/pRS426-RPN11*	This study
MHY9573	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN2-GFP(S65T):kanMX6 rpn11-1/*pRS316-*RPN11*	This study
MHY9579	*MATalpha his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sts1Δ::hphMX4/*pRS316-*STS1*	This study
MHY9580	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sts1Δ::hphMX4/*pRS316-*STS1*	This study
MHY9581	*MATa his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 PRE1-mCherry:NAT rpn11-1/*pRS426-*RPN11*	This study

MHY9583	*MATalpha his3Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 RPN5-GFP(S65T):HIS3MX6 rpn11-1/*pRS426-*RPN11*	This study
MHY9690	*MATalpha ade2-1 his3-11,15 leu2-3,112 trp1 ura3-1 can1-100 STS1 (W303)*	This study
MHY9691	*MATalpha ade2-1 his3-11,15 leu2-3,112 trp1 ura3-1 can1-100 sts1-2 (W303)*	This study
MHY9692	*MATa ade2-1 his3-11,15 leu2-3,112 trp1 ura3-1 can1-100 STS1 (W303)*	This study
MHY9693	*MATa ade2-1 his3-11,15 leu2-3,112 trp1 ura3-1 can1-100 sts1-2 (W303)*	This study

Table S2: Plasmids Used in this Study

Plasmid	*Description*	*Source*
2-8-6	pRS314	(Sikorski et al. 1989)
16-6-3	p414GPD	(Mumberg et al. 1995)
57-4-2	pRS316-RPN5-GFP-FLAG	(Nemec et al. 2019)
59-4-1	P415GPD	(Mumberg et al. 1995)
61-7-6	pGEX-2TK-Sts1	(Chen et al. 2011)
71-3-8	pET42b-Rpn3:Sem1:HA-Rpn7	(R. Tomko, MH lab)
72-5-4	pET42b-Rpn3:Sem1:HA-Rpn7:Rpn12	(R. Tomko, MH lab)
73-4-5	pCDF42b-6His-Rpn6:Rpn9:Rpn11:Rpn5:Rpn8	(Tomko et al. 2015)
80-3-7	pGEX6P1-Srp1	(J. Ronau, MH lab)
90-9-6	pRS316-STS1	This study
91-2-5	p415MET25-STS1-GFP	This study
91-2-6	p415GPD-STS1-GFP	This study
91-3-7	p415MET25-sts1R38D-GFP	This study
91-3-8	p415MET25-sts1C194Y-GFP	This study
91-3-9	p415GPD-sts1R38D-GFP	This study
91-4-3	pRS314-STS1	This study
91-4-4	pRS314-sts1R38D,R65D	This study
91-4-5	pGPD414-STS1	This study
91-4-6	pGPD414-sts1R38D,R65D	This study
91-6-4	pET42b-STS1-6His	This study
91-7-9	pRS314-sts1R38D	This study
91-8-5	p415GPD-sts1R38D,R65D-GFP	This study
91-9-8	p415GPD-sts1-R65D-GFP	This study
92-1-1	p415MET25-sts1(Δ4-75)-GFP	This study
94-3-1	p415MET25-sts1(Δ4-57)-GFP	This study
94-3-2	p415MET25-sts1(1-116)-GFP	This study
94-3-3	p415MET25-sts1(1-180)-GFP	This study
94-3-4	p415MET25-sts1(1-245)-GFP	This study
94-3-5	p415MET25-sts1(1-276)-GFP	This study

94-5-3	p415MET25-sts1R38D,R65D-GFP	This study
94-5-4	p415MET25-sts1R65D-GFP	This study
95-5-1	pET42b-sts1(C194Y)-6His	This study
95-6-6	pET42-sts1R38D, R65D-6His	This study
96-2-2	p415GPD-SV40-STS1-GFP	This study
96-2-3	p415GPD-SV40-sts1R38D,R65D-GFP	This study
98-7-1	pRS314-sts1-R65D	This study
98-7-8	p415MET25-SV40-STS1-GFP	This study
98-8-1	p415MET25-SV40-sts1R38D,R65D-GFP	This study