Funk et al., http://www.jcb.org/cgi/content/full/jcb.201310018/DC1

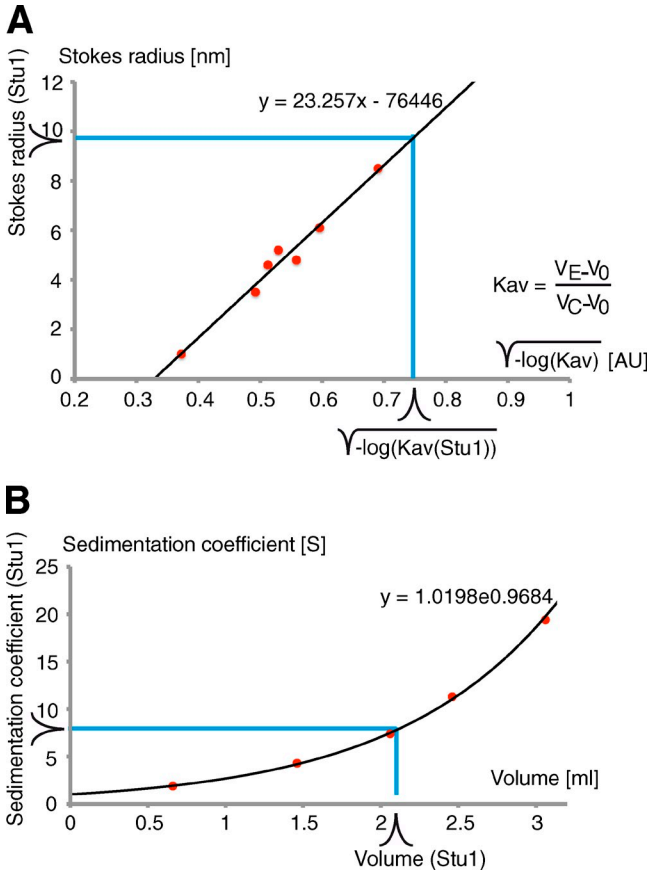
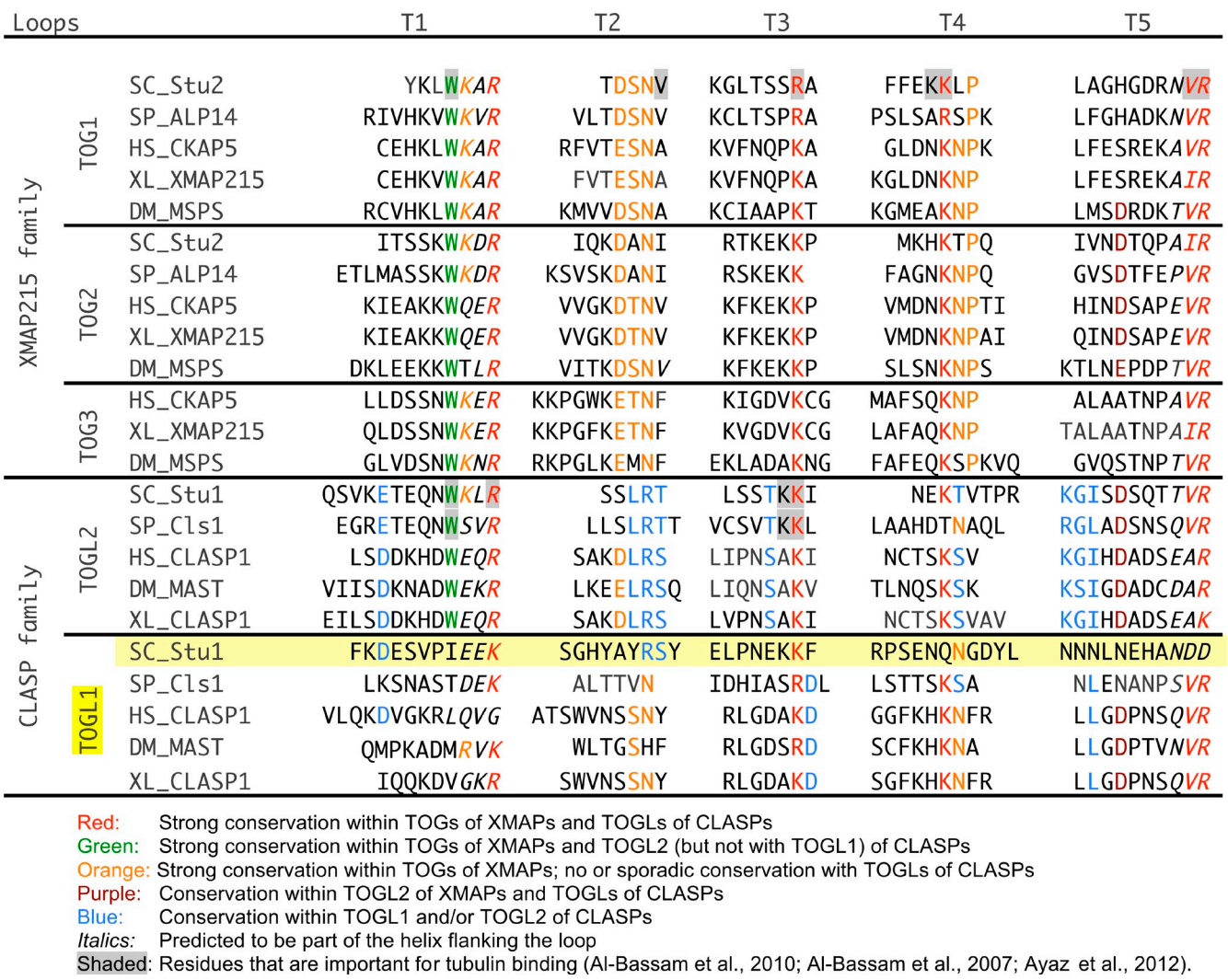


Figure S1. **Calibration curves for hydrodynamic protein analyses.** (A) Calibration curve of the Superose 6 10/300 column used to determine the Stokes radius of Stu1 (Fig. 1 A). Kav is the gel-phase distribution coefficient, V_E is the elution volume, V_C is the column volume, and V_O is the void volume. The data shown are from a single representative experiment out of two analyses. AU, arbitrary unit. (B) Sucrose gradient calibration curve used to determine the sedimentation coefficient of Stu1 (Fig. 1). The data shown are from a single representative experiment out of two analyses.



Red: Strong conservation within TOGs of XMAPs and TOGLs of CLASPs
Green: Strong conservation within TOGs of XMAPs and TOGL2 (but not with TOGL1) of CLASPs
Orange: Strong conservation within TOGs of XMAPs; no or sporadic conservation with TOGLs of CLASPs
Purple: Conservation within TOGL2 of XMAPs and TOGLs of CLASPs
Blue: Conservation within TOGL1 and/or TOGL2 of CLASPs
Italics: Predicted to be part of the helix flanking the loop
Shaded: Residues that are important for tubulin binding (Al-Bassam et al., 2010; Al-Bassam et al., 2007; Ayaz et al., 2012).

Figure S2. **Sequence alignment of the intra-HEAT repeat loops of the XMAP215 and CLASP family TOG domains.** The intra-HEAT repeat loops were identified using crystal structure information (Ayaz et al., 2012; De la Mora-Rey et al., 2013; Leano et al., 2013) or via secondary structure prediction (PredictProtein) and aligned manually.

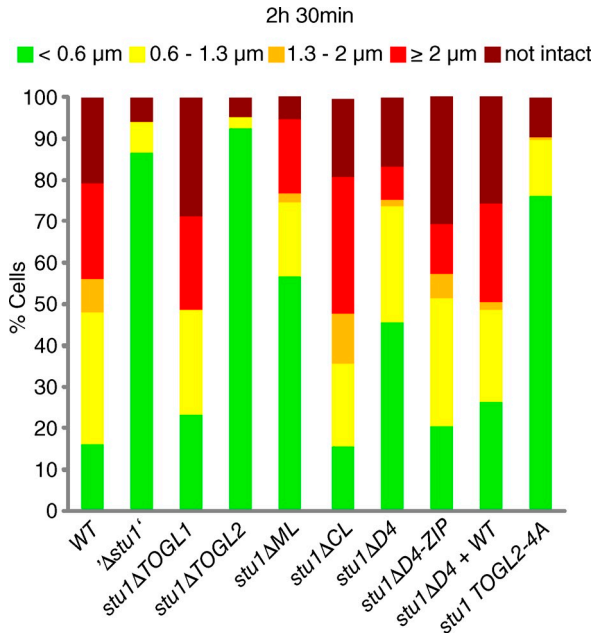


Figure S3. **Spindle phenotypes of Stu1 mutants.** Cells were treated as described in Fig. 6 (A–E). Spindle length and spindle phenotypes were quantified as in Fig. 6 F but 2.5 h after the G1 release.

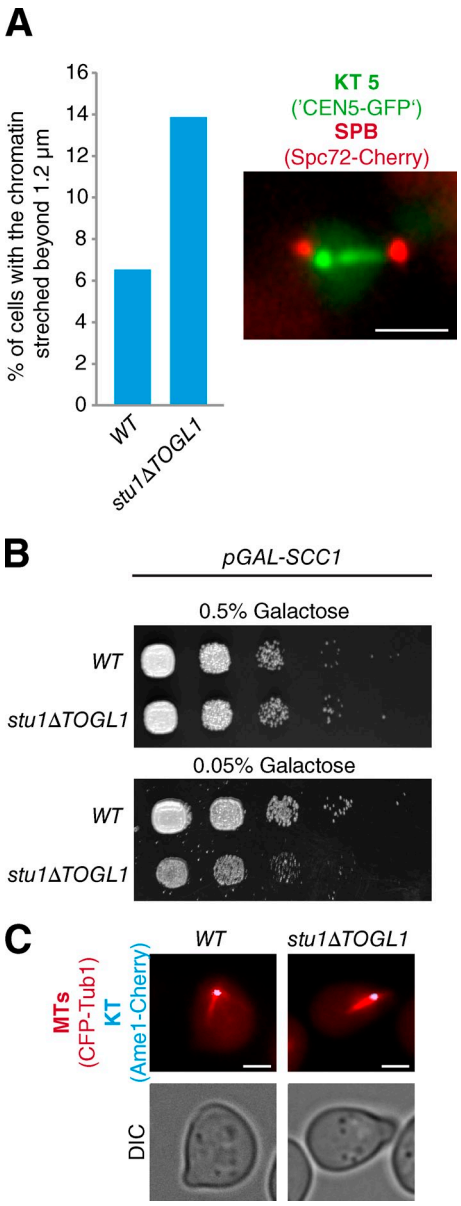


Figure S4. **Deletion of TOGL1 compromises specifically kMTs.** (A and B) The tension at metaphase KTs is increased in *stu1ΔTOGL1* cells. (A) The chromatin in the vicinity of CEN5 is on average more stretched in *stu1ΔTOGL1* cells. Cells were arrested in metaphase by Cdc20 depletion for 5 h. Stretched chromatin in the vicinity of CEN5, labeled by a tetO array, and visualized by tetR-GFP was quantified as indicated. $n > 130$. Bar, 2 µm. (B) *stu1ΔTOGL1* cells are more sensitive to reduced amounts of cohesion. Dilution series of *STU1* and *stu1ΔTOGL1* cells with *SCC1* expressed under the control of the *pGAL* promoter. (C) Deletion of TOGL1 has, if anything, a stabilizing effect on non-kMTs in G1. Cells were synchronized in G1 by the addition of α factor. DIC, differential interference contrast. Bars, 2 µm.

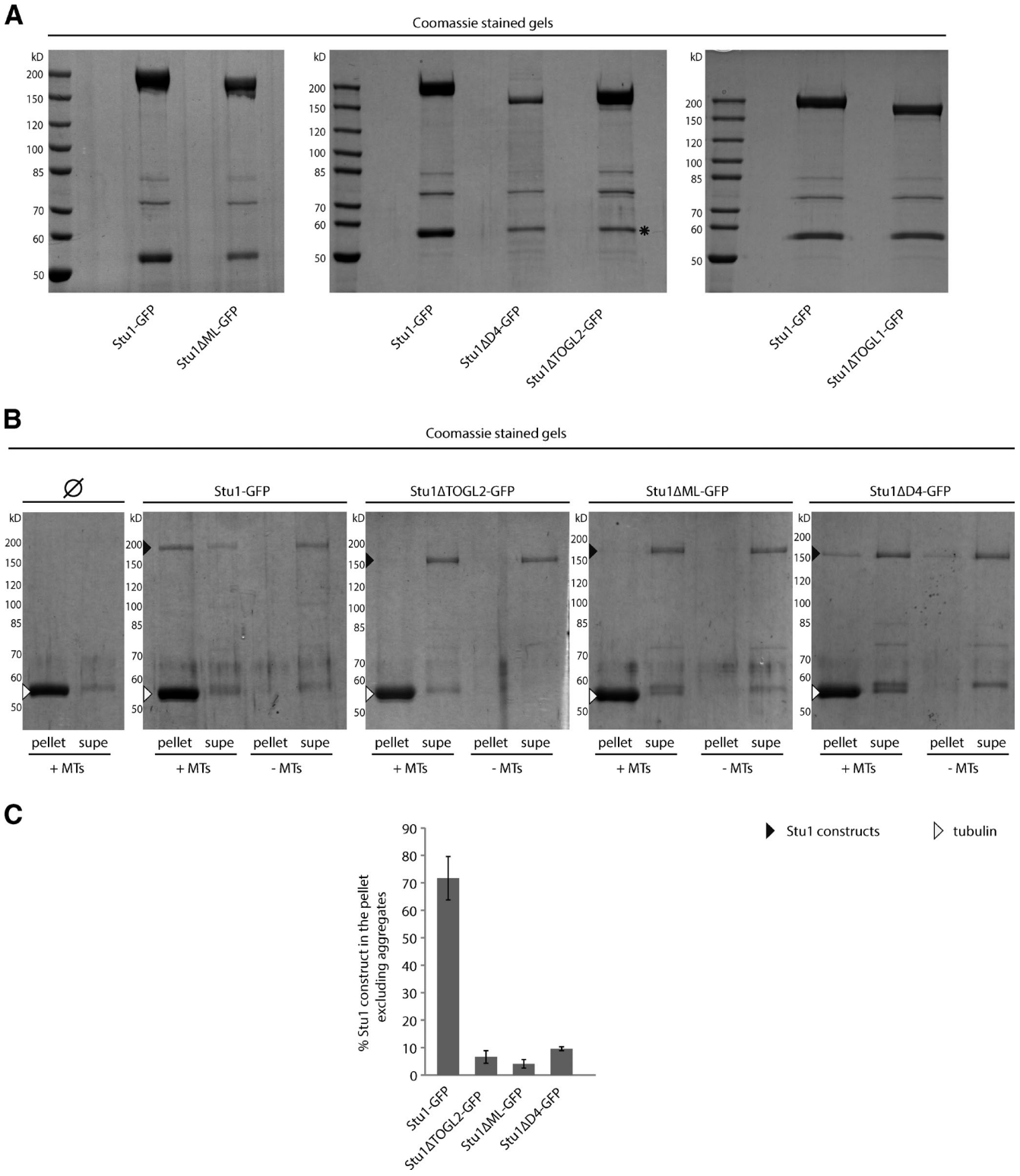


Figure S5. **Stu1 constructs used for in vitro MT binding experiments and MT sedimentation assay.** (A) Indicated Stu1 constructs were purified from yeast as described in the Materials and methods section. The asterisk shows IgG (mass spectrometry). (B) MT sedimentation assays. Stu1 constructs (100 nM) were incubated with and without taxol-stabilized MTs (310 nM polymerized tubulin) and centrifuged through a 40% glycerol cushion. 50% of the supernatant (supe) or resuspended pellet was analyzed. Ø indicates that no Stu1 construct was added. (C) Quantification of the MT pelleting assay. The intensity of the Stu1 bands was measured in each lane. The percentage of the Stu1 constructs present in the pellet of the control (no MTs) was subtracted from the percentage of Stu1 in the pellet containing MTs. Error bars represent the SDs of two experiments.

Table S1. **Yeast strains used in this study**

Strain name	Relevant genotype	Experiment/figure
YPH499	*MATa ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre*	
YMS231	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre*	
YCF2177	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 cyh2R [CF: CEN6 URA3 SUP11 CYH2S] lys2-801ambre::pSTU1-FLAG-STU1-NLS-GFP::LYS2 Δstu1::HIS3MX6*	T1
YSK633	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre cyh2R [CF: CEN6 URA3 SUP11 CYH2S] Δmad2::klTRP1*	T1
YCF2174	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 cyh2R [CF: CEN6 URA3 CYH2S SUP11] lys2-801ambre::pSTU1-FLAG-stu1Δ(aa1–260)-NLS::LYS2 Δstu1::HIS3MX6*	T1
YVS1772	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 stu1Δ(aa301–569) -EGFP::klTRP1 lys2-801ambre::CFP-TUB1::LYS2 AME1-mCherry::hphNT1 + pCF1137*	T1
YVS2222	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 cyh2R [CF: CEN6 URA3 CYH2S SUP11] lys2-801ambre::pSTU1-FLAG-stu1Δ(aa570–716)-NLS-GFP:: LYS2 Δstu1::HIS3MX6*	T1
YVS2220	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 cyh2R [CF: CEN6 URA3 CYH2S SUP11] lys2-801ambre::pSTU1-FLAG-stu1Δ(aa717–996)-NLS-GFP:: LYS2 Δstu1::HIS3MX6*	T1
YVS2201	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 cyh2R [CF: CEN6 URA3 CYH2S SUP11] lys2-801ambre::pSTU1-FLAG-stu1Δ(aa995–1,180)-NLS-GFP::LYS2 stu1Δ::HIS3MX6*	T1
YCF2175	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 cyh2R [CF: CEN6 URA3 CYH2S SUP11] lys2-801ambre::pSTU1-FLAG-stu1Δ(aa1,182–1,513)-NLS-GFP::LYS2 Δstu1:: HIS3MX6*	T1
YCF2176	*MATα ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 cyh2R [CF: CEN6 URA3 CYH2S SUP11] lys2-801ambre::pSTU1-FLAG-stu1Δ(aa1,182–1,513)-Zipper-NLS-GFP:: LYS2 Δstu1::HIS3MX6*	T1
YMK1905	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre:: pGAL1-FLAG-STU1::LYS2*	1 (B and C) and S1 (A and B)
YCF1964	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1 lys2-801ambre::Stu1 promotor-FLAG-stu1(aa1–762)-NLS-GFP::LYS2*	1 E
YCF1965	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1 lys2-801ambre::Stu1 promotor-FLAG-stu1(aa717–1,513)-NLS-GFP::LYS2*	1 E
YCF2178	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1 lys2::Stu1 promotor-stu1(aa717–996)-NLS-GFP::LYS2*	1 E
YCF2179	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1 lys2::Stu1 promotor-stu1Δ(aa717–994)-NLS-GFP::Lys2*	1 E
YCF2182	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1, lys2::Stu1 promotor-stu1(aa996-1,181)-NLS-GFP::LYS2*	1 E
YCF2237	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1 lys2::Stu1 promotor-stu1Δ(aa995–1,180)-NLS-GFP::LYS2*	1 E
YCF2180	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1 lys2::Stu1 promotor-stu1(aa1,181–1,513)-NLS-GFP::LYS2*	1 E
YCF2181	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Stu1-6Ha::klTRP1 lys2::Stu1 promotor-stu1Δ(aa1,182–1,513)-NLS-GFP::LYS2*	1 E
YVS1718	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 ura3-52::CFP-TUB1::URA3 AME1-mCherry::hphNT1 lys2-801ambre::pGAL-STU1-NLS-GFP::LYS2*	2 (A, B, and D), 6 H, 7 (B and C), and S5
YJO1164	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre STU1-ProA-7HIS::HIS3MX6*	2 C
YCF1970	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 STU1-ProA-7HIS:: HIS3MX6 lys2-801ambre::pGAL1-FLAG-stu1Δ(aa1–260)-NLS-GFP::LYS2*	2 C
YCF1969	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 STU1-ProA-7HIS:: HIS3MX6 lys2-801ambre::pGAL1-FLAG-stu1(aa1–260)-NLS-GFP::LYS2*	2 C
YVS1972	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 STU1-ProA-7HIS:: HIS3MX6 lys2-801ambre::pGAL1-FLAG-stu1Δ(aa301–560)-NLS-GFP::LYS2*	2 C
YVS1971	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 STU1-ProA-7HIS:: HIS3MX6 lys2-801ambre::pGAL1-FLAG-stu1(aa261–569)-NLS-GFP::LYS2*	2 C
YVS1919	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 STU1-ProA-7HIS:: HIS3MX6 lys2-801ambre::pGAL1-FLAG-stu1(W339A, R342A, K428A, K429A) -NLS-GFP::LYS2*	2 D
YVS1408	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3 STU1-NLS-EGFP::klTRP1 lys2-801ambre::CFP-TUB1::LYS2 AME1-mCherry::hphNT1*	2 (E and F), 3 A, 6 (A and F), 8 (A and D), and S3

Table S1. **Yeast strains used in this study** *(Continued)*

Strain name	Relevant genotype	Experiment/figure
YVS1820	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3 stu1Δ(aa1–260)-EGFP::klTRP1 lys2-801ambre::CFP-TUB1::LYS2 AME1-mCherry::hphNT1*	2 (E and F), 6 (B and F), S3, and S4 C
YVS2232	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1 KANMX6::pGAL1-UbiR-STU1 lys2-801ambre::pSTU1-FLAG-stu1Δ(aa301–569)-NLS-GFP::LYS2*	2 (E and F) and S3
YVS2230	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1 KANMX6::pGAL-UbiR-STU1*	2 (E and F) and S3
YVS2231	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1 KANMX6::pGAL-UbiR-STU1 lys2-801ambre::pSTU1-FLAG-stu1(W339A, R342A, K428A, K429A)-NLS-GFP::LYS2*	2 (E and F) and S3
YCF2272	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3 Δstu1::HIS3MX6 lys2-801ambre::CFP-TUB1::LYS2 AME1-mCherry::hphNT1 lys2-801ambre::pSTU1-stu1Δ(aa1,182–1,513)-NLS-GFP::LYS2*	3 B, 6 (D and F), 8 (B and D), and S3
YCF2274	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1 KANMX6::pGAL-UbiR-STU1 lys2-801ambre::pSTU1-FLAG-stu1(aa1,181–1,513)-NLS-GFP::LYS2*	3 B
YCF2273	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3 Δstu1::HIS3MX6 lys2-801ambre::CFP-TUB1::LYS2 AME1-mCherry::hphNT1 lys2-801ambre::pSTU1-stu1Δ(aa1,182–1,513)-Zipper-NLS-GFP::LYS2*	3 B, 6 (E and F), 8 (C and D), and S3
YVS2028	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre STU1-CFP::KANMX6 SPC72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3*	3 C and 5 C
YCF2069	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa1–260)-NLS-CFP::KANMX6 ade2::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 Spc72-mCherry::hphNT*	3 C and 5 C
YCF2165	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Δstu1::stu1Δ(aa1–260, aa995–1,180)-GFP::TRP1 ade1::pURA3-TetR-3×CFP-HPH1 CEN5-tetO2 × 112::URA3 SPC72-3mCherry::natNT2*	3 C
YCF2167	*MATa Δsst1 trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Δstu1::stu1Δ(aa1–260, aa570–716)-CFP::KANMX6 Spc72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3*	3 C
YVS2029	*MATa Δsst1 trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1 Δ(aa995–1,180)-CFP::KANMX6 Spc72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3*	3 (C and D) and 5 C
YVS2152	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa995–1,180)-EGFP::klTRP1 ade1::pURA3-TetR-3×CFP::hphNT1 SPC72-mCherry::natNT2 CEN5-tetO2 × 112::URA3*	3 (C and D)
YVS2154	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa570–716, aa995–1,180)-EGFP::klTRP1 ade1::pURA3-TetR-3×CFP::hphNT1 SPC72-mCherry::natNT2 CEN5-tetO2 × 112::URA3*	3 (C and D)
YCF2271	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1 KANMX6::pGAL-UbiR-STU1 pSTU1-FLAG-stu1(aa1–260, aa1,181–1,513)-NLS-GFP::LYS2*	3 E
YCF2270	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1 KANMX6::pGAL-UbiR-STU1 pSTU1-FLAG-stu1(aa1–260)-Zipper-NLS-GFP::LYS2*	3 E
YCF1690	*Mat a Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre STU1-CFP::KANMX6 SPC72-mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 ChrV-tetO2 × 112-URA3-ChrV spc105::TRP1-pGal-UbiR-SPC105*	3 F
YCF2226	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 Δstu1::HIS3MX6 lys2-801ambre::pSTU1-FLAG-STU1-NLS-GFP::LYS2 cdc20::LEU2-pMET25-CDC20*	4 (A–C)
YCF2228	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 Δstu1::HIS3MX6 lys2-801ambre::pSTU1-FLAG-stu1Δ(aa1–260)-NLS-GFP::LYS2 cdc20::LEU2-pMET25-CDC20*	4 (A and B)
YVS2266	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 Δstu1::HIS3MX6 lys2-801ambre::pSTU1-FLAG-stu1Δ(aa570–716)-NLS-GFP::LYS2 cdc20::LEU2-pMET25-CDC20*	4 (A and B)
YVS2236	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 Δstu1::HIS3MX6 lys2-801ambre::pSTU1-FLAG-stu1Δ(aa995–1,180)-NLS-GFP::LYS2 cdc20::LEU2-pMET25-CDC20*	4 (A and B)
YCF2227	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 Δstu1::HIS3MX6 lys2-801ambre::pSTU1-FLAG-stu1Δ(aa1,182–1,513)-NLS-GFP::LYS2 cdc20::LEU2-pMET25-CDC20*	4 (A and B)

Table S1. **Yeast strains used in this study** *(Continued)*

Strain name	Relevant genotype	Experiment/figure
YCF2229	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 Δstu1::HIS3MX6 lys2-801ambre::pSTU1-FLAG-stu1Δ(aa1,182–1,513)-Zipper-NLS-GFP::LYS2 cdc20::LEU2-pMET25-CDC20*	4 (A and B)
YJO2349	*MATa ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre sst1::loxP cdc15-1 STU1-yEGFP::hphNT1*	4 C
YCF2170	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre STU1-CFP::KANMX6 SPC72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 cdc20::HIS3MX6-pMET25-CDC20*	5 (A, D, F, and G), 6 G, and S4 A
YCF2172	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa1–260)-NLS-CFP::KANMX6 ade2::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 SPC72-mCherry::hphNT1 cdc20::HIS3MX6-pMET25-CDC20*	5 (A, D, F, and G), 6 G, and S4 A
YVS2104	*MATa Δsst1 trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa995–1,180) -CFP::KANMX6 SPC72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 cdc20::LEU2-pMET25-CDC20*	5 (A, F, and G) and 6 G
YCF2284	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre Δstu1::stu1Δ(aa1–260, aa995–1,180)-GFP::TRP1 ade1::pURA3-TetR-3×CFP-HPH1 CEN5-tetO2 × 112::HIS3MX6 SPC72-3mCherry::natNT2 cdc20::LEU2-pMET25-CDC20*	5 (A and G)
YJO1334	*MATa Δsst1 trp1-Δ63 leu2-Δ1::LEU2 ura3-52 his3-Δ200 lys2-801ambre ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 SPC72-3mCherry::hphNT1 klTRP1::pGAL-UbiR-STU1-CFP::KANMX4*	5 C
YVS2078	*MATa Δsst1 ade2-101ochre leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre ade1::pURA3-TetR-3×CFP-hphNT1 CEN5-tetO2 × 112::URA3 SPC72-mCherry::natNT2 trp1-Δ63::GFP-TUB1::TRP1 KANMX6-pGAL-UbiR-STU1*	5 C
YCF2314	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa1–260)-NLS-CFP::kanMX6 ade2::tetR-GFP:ADE2 1.4kb left of CEN5-tetO2 × 112::URA3 Spc72-mCherry::hphNT cdc20::HIS3MX6-pMET25-CDC20 mtw1::MTW1-stu1Δ(aa1–260)-natNT2*	5 D
YCF2315	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa1–260)-NLS-CFP::KANMX6 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 SPC72-mCherry::hphNT cdc20::HIS3MX6-pMET25-CDC20 LYS2::pSTU1-stu1Δ(aa1–260)-NLS*	5 D
YBK2242	*MATa Δsst1 trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre STU1-CFP::KANMX6 SPC72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 cdc20::HIS3MX6-pMET25-CDC20 Δcin8::natNT2*	5 (E and H)
YBK2241	*MATa Δsst1 trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa995–1,180)-CFP::KANMX6 SPC72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 cdc20::HIS3MX6-pMET25-CDC20 Δcin8::natNT2*	5 (E and H)
YBK2243	*MATa Δsst1 trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa1–260) -CFP::KANMX6 SPC72-3mCherry::hphNT1 ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 cdc20::HIS3MX6-pMET25-CDC20 Δcin8::natNT2*	5 (E and H)
YVS2001	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3 stu1Δ(aa570–716)-EGFP::klTRP1 lys2-801ambre::CFP-TUB1::LYS2 AME1-mCherry::hphNT1*	6 (C and F) and S3
YCF2285	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1 lys2-801ambre::pSTU1-stu1Δ(aa1,182–1,513)-NLS-GFP::LYS2*	6 F, 8 E, and S3
YVS1651	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3 stu1Δ(aa995–1,180)-EGFP::klTRP1 lys2-801ambre::CFP-TUB1::LYS2 AME1-mCherry::hphNT1*	6 F and S3
YVS2209	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 hi-Δ200 lys2-801ambre stu1Δ(aa570–716)-EGFP::klTRP1 ade1::pURA3-TetR-3xCFP::hphNT1 SPC72-mCherry::natNT2 CEN5-tetO2 × 112::URA3 cdc20::LEU2-pMET25-CDC20*	6 G
YCF2283	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 Δstu1::HIS3MX6 ade1::pURA3-TetR-3×CFP::hphNT1 CEN5-tetO2 × 112:URA3 SPC72-mCherry::natNT2 lys2-801ambre::pSTU1-stu1Δ(aa1,182–1,513)-NLS-GFP::LYS2 cdc20::LEU2-pMET25-CDC20*	6 G
YCF2280	*MATa Δsst1 trp1-Δ63 leu2-Δ1::LEU2 ura3-52 his3-Δ200 lys2-801ambre ade2-101ochre::tetR-GFP::ADE2 CEN5-tetO2 × 112::URA3 SPC72-3mCherry::hphNT1 stu1Δ(aa1,182–1,513)-Zipper-ECFP::KANMX4 cdc20::HIS3MX6-pMET25-CDC20*	6 G
YCF2351	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52:TUBI-CFP::URA3 AME1-mCherry::hphNT1 lys2-801ambre::pGAL1-FLAG-stu1Δ(aa570–716)-NLS-GFP::LYS2*	6 (H–J), 7 (B and C), and S5
YCF1975	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52:TUBI-CFP::URA3 AME1-mCherry::hphNT1 lys2-801ambre::pGal1-FLAG-Stu1Δ(aa1–260)-NLS-GFP::LYS2*	6 H, 7 (B and C), and S5

Table S1. **Yeast strains used in this study** *(Continued)*

Strain name	Relevant genotype	Experiment/figure
YCF2355	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52:TUBI-CFP::URA3 AME1-mCherry::hphNT1 lys2-801ambre:: pGal-FLAG-stu1Δ(aa1,182–1,513)-NLS-GFP::LYS2*	6 H, 7 (B and C), and S5
YCF2356	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52:TUBI-CFP::URA3 AME1-mCherry::hphNT1 lys2-801ambre:: pGal1-FLAG-stu1Δ(aa301–560)-NLS-GFP::LYS2*	6 (H and I), 7 C, and S5
YVS2311	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52::CFP-TUB1::URA3 STU1-GFP::HIS3MX6 ASE1-mCherry::hphNT1 cdc20::LEU2-pMET25-CDC20*	7 A
YVS2308	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre STU1-CFP::KANMX6 GFP-TUB1::TRP1 cdc20:: LEU2-pMET25-CDC20 Δase1::HIS3MX6*	7 A
YVS1678	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52:TUBI-CFP::URA3 AME1-mCherry::hphNT1*	7 C
YJO1998	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre:: pGAL1-FLAG-STU1::LYS2 Δmad2::TRP1 ura3-52:CFP-TUBI:URA3 MTW1-mCherry::hphNT1*	7 C
YCF2278	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre:: pSTU1-stu1(aa1,181–1,513)-NLS-GFP stu1Δ(aa1,182–1,513)-Zipper-ECFP:: KANMX4 ura3-52::CFP-TUB1::URA3 AME1-3mCherry::hphNT1*	8 F
YCF2357	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52::TUBI-CFP::URA3 stu1Δ(aa1,181–1,513)-NLS-TEV-ProtA-7HIS-KANMX6*	8 G
YCF2358	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52::TUBI-CFP::URA3 stu1Δ(aa1,181–1,513)-NLS-TEV-ProtA-7HIS-KANMX6 lys2-801ambre::Stu1 promotor-stu1(aa1,181–1,513)-NLS-GFP::LYS2*	8 G
YCF2359	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 his3-Δ200 lys2-801ambre ura3-52::TUBI-CFP::URA3 stu1Δ(aa1,181–1,513)-NLS-TEV-ProtA-7HIS-KANMX6 lys2-801ambre::Stu1 promotor-stu1(aa261–569, aa1,181–1,513)-NLS-GFP::LYS2*	8 G
YCF2185	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre PDS1-9MYC::klTRP1 scc1::HIS3MX6-pGAL-3HA-SCC1 SLK19-6HA::natNT2*	S4 B
YCF2184	*MATa Δsst1 ade2-101ochre trp1-Δ63 leu2-Δ1 ura3-52 his3-Δ200 lys2-801ambre stu1Δ(aa–260)-NLS-CFP::KANMX6 scc1::TRP1-pGal1-3HA-SCC1 PDS1-9myc::hphNT1 SLK19-6HA::natNT2*	S4 B

Table S2. **Plasmids used in this study**

Plasmid name	Description and markers
YDpK	*LYS2, ampR*
pBSII/SK	*ampR*
pJO607	*mad2::TRP1, ampR*
pBK1503	*cin8::natNT2, ampR*
pCF1137	*pSTU1-STU1::URA3* in *YDpK*
pCF1419	*pGAL1-FLAG-stu1Δ(aa1–260)-NLS-GFP* in *YDpK*
pCF1420	*pGAL1-FLAG-stu1(aa1–260)-NLS-GFP* in *YDpK*
pCF1421	*pGAL1-FLAG-stu1(aa261–569)-NLS* in *YDpK*
pCF1441	*pSTU1-FLAG-stu1(aa1–762)-NLS-GFP* in *YDpK*
pCF1442	*pSTU1-FLAG-stu1(aa717–1,513)-NLS-GFP* in *YDpK*
pCF1473	*pSTU1-FLAG-stu1(aa717–996)-NLS-GFP* in *YDpK*
pCF1474	*pSTU1-FLAG-stu1Δ(aa717–994)-NLS-GFP* in *YDpK*
pCF1475	*pSTU1-FLAG-stu1(aa996-1,181)-NLS-GFP* in *YDpK*
pCF1498	*pSTU1-FLAG-stu1Δ(aa995–1,180)-NLS-GFP* in *YDpK*
pCF1477	*pSTU1-FLAG-stu1(aa1,181–1,513)-NLS-GFP* in *YDpK*
pCF1478	*pSTU1-FLAG-stu1Δ(aa1,182–1,513)-NLS-GFP* in *YDpK*
pCF1479	*pSTU1-FLAG-stu1Δ(aa1,182–1,513)-Zipper-NLS-GFP* in *YDpK*
pCF1480	*pSTU1-FLAG-stu1(aa1–260)-Zipper-NLS-GFP* in *YDpK*
pCF1481	*pSTU1-FLAG-stu1(aa1–260, aa1,181–1,513)-NLS-GFP* in *YDpK*
pCF1515	*S3-stu1Δ(aa1–260)-natNT2-S2, ampR*
pCF1523	*pGal-FLAG-stu1Δ(aa570–716)-NLS-GFP* in *YDpK*
pCF1524	*pGal-FLAG-stu1Δ(aa1,182–1,513)-NLS-GFP* in *YDpK*
pCF1526	*pSTU1-FLAG-stu1(aa261–569, aa1,181–1,513)-NLS-GFP* in *YDpK*
pCF1527	*pSTU1-FLAG-stu1Δ(aa1–260)-NLS-GFP* in *YDpK*
pCF1482	*pSTU1-FLAG-stu1Δ(aa1–260)-NLS* in *YDpK*
pVS1410	*pGal1-FLAG-stu1Δ(aa301–560)-NLS-GFP* in *YDpK*
pVS1412	*pGal1-FLAG-stu1(W339A, R342A, K428A, K429A)-NLS-GFP* in *YDpK*
pVS1325	*pGal1-FLAG-STU1-GFP* in *YDpK*
pVS1414	*pSTU1-stu1Δ(aa1–260)-NLS-CFP::KANMX6* in *pBSII/SK*
pVS1362	*pSTU1-stu1Δ (aa301–569)-EGFP::klTRP1* in *pBSII/SK*
pVS1444	*pSTU1-stu1Δ(aa570–716)-EGFP::klTRP1* in *pBSII/SK*
pVS1309	*pSTU1-stu1Δ (aa995–1,180)-EGFP::klTRP1* in *pBSII/SK*
pCF1496	*pSTU1-stu1Δ(aa1–260, aa995–1,180)-GFP::klTRP1* in *pBSII/SK*
pCF1483	*pSTU1-stu1Δ(aa1–260, aa570–716)-CFP::KANMX6* in *pBSII/SK*
pVS1328	*pSTU1-stu1Δ(aa416–716) Δ(aa995–1,180)-EGFP::klTRP1* in *pBSII/SK*
pCF1511	*pSTU1-FLAG-stu1Δ(aa1,182–1,513)-Zipper-NLS-ECFP::KANMX4, ampR*
pVS1381	*pSTU1-stu1Δ(aa1–260)-NLS-EGFP::klTRP1* in *pBSII/SK*
pVS1472	*pSTU1-FLAG-stu1Δ(aa301–569)-NLS* in *YDpK*
pVS1471	*pSTU1-FLAG-stu1(W339A, R342A, K428A,K429A)-NLS* in *YDpK*
pVS1346	*pSTU1-stu1Δ(aa995–1,180)-CFP::KANMX6* in *pBSII/SK*

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