Figure S1

The tetratricopeptide repeats of the ChAPs family are located in conserved regions

(A) BLASTP alignment of *S. cerevisiae* Chs6p against ChAPs proteins in other fungi. Colours display the degree of conservation, as indicated by the scale below. Several regions appeared highly conserved, including TPR3-4 and TPR5 but also other parts such as stretch of about 114 amino acids at the N-terminus whose function is unknown. (B) Sequence alignment of the *S. cerevisiae* ChAPs. Dark grey bars indicate the degree of sequence conservation, red boxes the approximate position of the tetratricopeptide repeats.

Figure S2

TPR1-4 is required for co-precipitation of Chs6p with Bud7p

Co-immunoprecipitation was performed as in Figure 5. Interaction of Chs6(ΔTPR1-4) with Bud7p was entirely abolished, while Chs6(ΔTPR5) only showed a mild reduction in binding, suggesting that TPR1-4 is generally required for co-precipitation of the ChAPs family members. Two different exposures were cropped together because of the strong signal of the precipitated myc-tagged constructs.

Figure S3

Chs6p requires an intact TPR fold for function

(A) Primary sequence of TPR5 in Chs6p. Residues, which were considered part of the conserved TPR backbone are highlighted in red. Chs6p bearing a double point mutation in two neighboring TPR backbone residues (L619G/G620W) was non-functional, as judged by mis-localization of Chs3p (B) and calcofluor resistance (C). Scale bar: 5 µm (D) Cargo interaction and Chs5p binding by Chs6p can be decoupled. Deletion of TPR1-4 or the last 13 amino acids in Chs6p abolishes Chs5p binding but does not influence the binding of Chs6(ΔTPR1-4) and Chs6(ΔC13) to Chs3p. Cargo interaction was assessed by

precipitating Chs3p from DSP-cross linked lysates with anti-Chs3p antibodies and probing precipitates for different Chs6p constructs

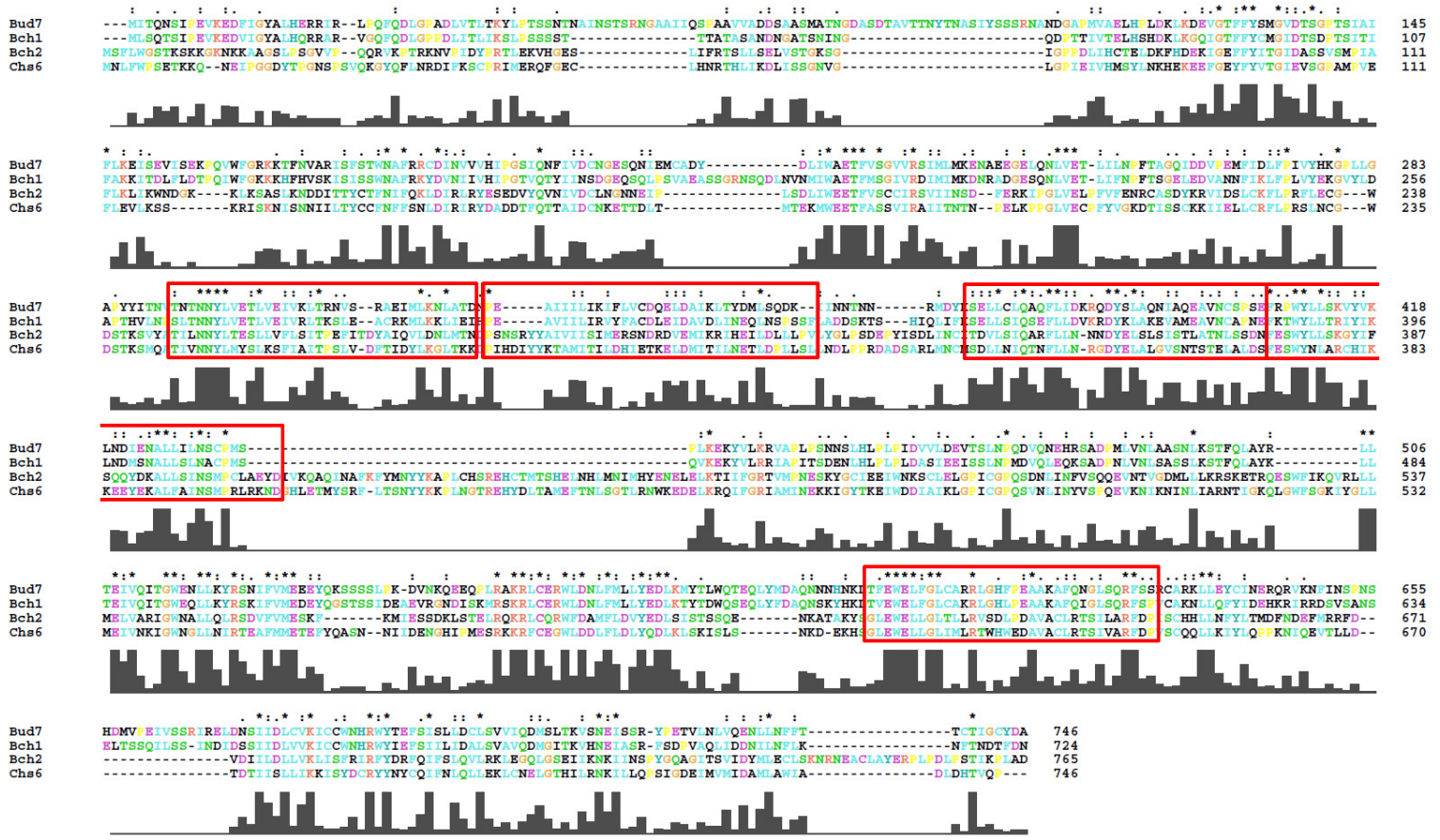
Figure S4

Bch2p-Chs6p chromosomally generated chimera constructs used in the study. Numbers in brackets indicate amino acid sequences of Chs6p and Bch2p domains in each construct. In bold italic: Chs6p domains replaced with corresponding domains of Bch2p.

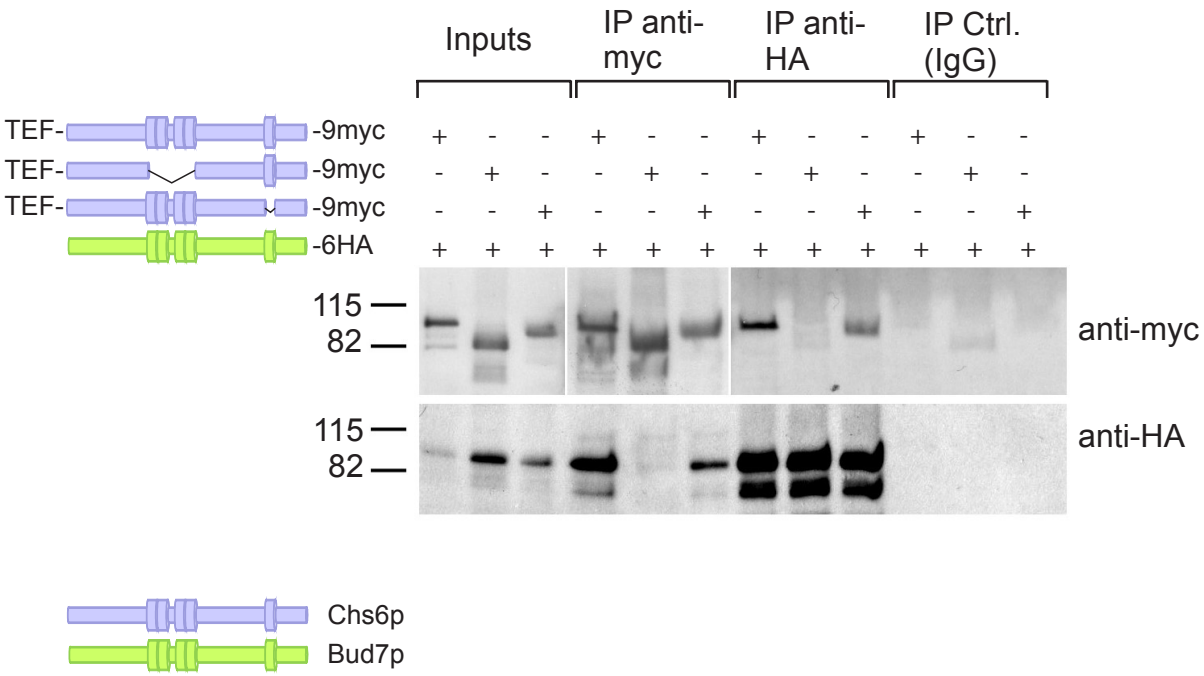
A


TPR 1-4
TPR5
aa 0
100
200
300
400
500
600
700
Neg. P exponent:
<10
10-50
50-100
100-200

B


Bud7
Bch1
Bch2
Chs6

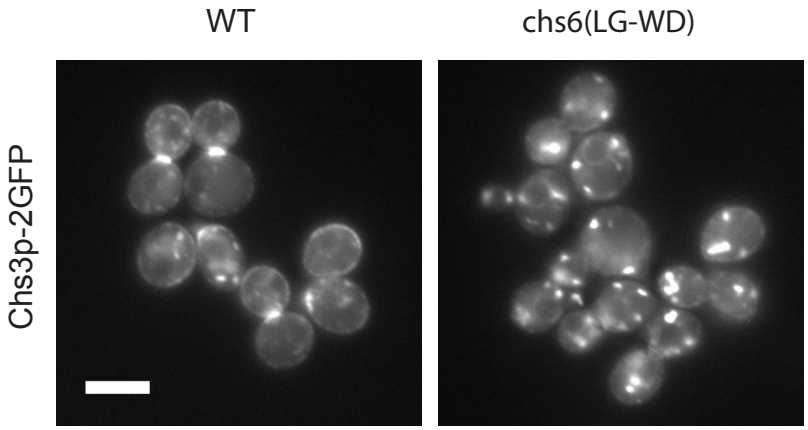
Rockenbauch et al., Figure S2



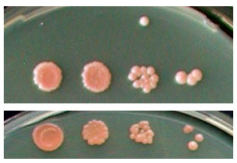
Rockenbauch et al., Figure S3

A

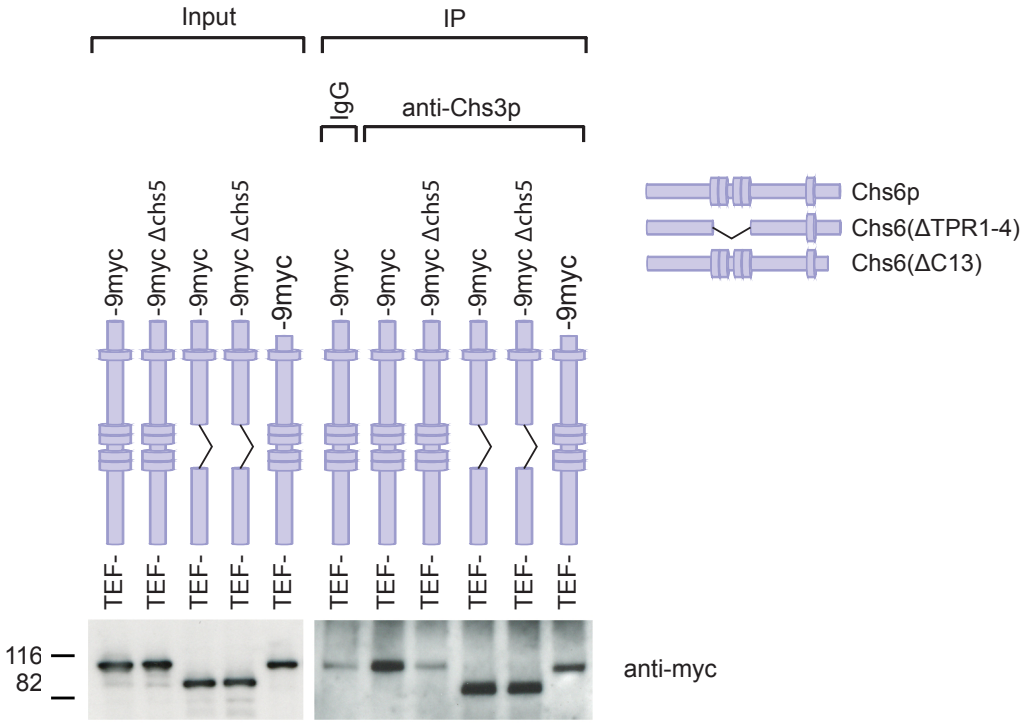
B



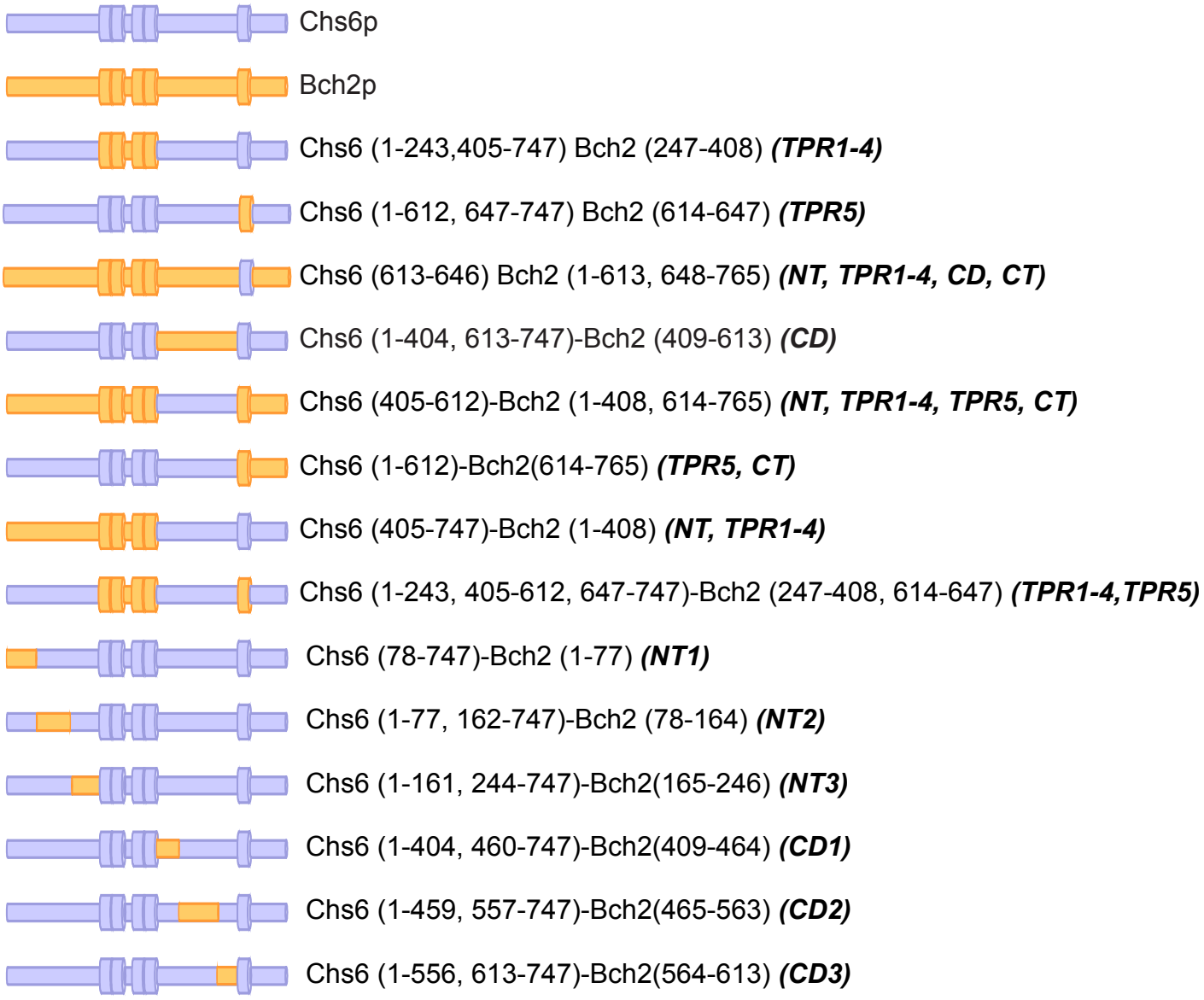
C



D



Rockenbauch et al., Figure S4



Supplementary Table 1. Strains used in this study

Name	Genotype	Reference
YPH499	*MAT a ade2 his3 leu2 lys2 trp1 ura3*	Sikorski and Hieter, 1989
YAS328	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::CHS6-9myc (KI TRP1)*	Trautwein et al., 2006
YAS2413	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(Δ730-1212)-9myc (HIS3MX6)*	This study
YAS2414	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(Δ1837-1938)-9myc (HIS3MX6)*	This study
YAS2506	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::TEF(natNT2)-CHS6-9myc (KI TRP1)*	This study
YAS3206	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6-9myc (HIS3MX6)*	This study
YAS3290	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6-9myc (HIS3MX6) CHS5::LEU2*	This study
YAS2475	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6(Δ730-1212)-9myc (HIS3MX6)*	This study
YAS2712	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6(Δ730-1212)-9myc (HIS3MX6) CHS5::LEU2*	This study
YAS2476	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6(Δ1837-1938)-9myc (HIS3MX6)*	This study
YAS563-2a	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::URA3*	Trautwein et al., 2006
YAS2855	*MAT a/α ade2/ade2 his3/his3 leu2/leu2 lys2/lys2 trp1/trp1 ura3/ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(Δ730-1212)-9myc (HIS3MX6) CHS6::CHS6(Δ1837-1938)*	This study
YAS563-4A	*MAT a ade2 his3 leu2 lys2 trp1 ura3 BCH2::KAN (Tn903)*	Trautwein et al., 2006
YAS525	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::URA3 BCH2::KAN (Tn903)*	Trautwein et al., 2006
YAS2938	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::LEU2 BCH2::BCH2(Δ739-1224)*	This study
YAS2939	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::LEU2 BCH2::BCH2(Δ1840-1941)*	This study
YAS563-5a	*MAT a ade2 his3 leu2 lys2 trp1 ura3 BCH1::HIS5 (S. pombe)*	Trautwein et al., 2006
YAS2852	*MAT a ade2 his3 leu2 lys2 trp1 ura3 BCH1::BCH1(Δ793-1251)*	This study
YAS2853	*MAT a ade2 his3 leu2 lys2 trp1 ura3 BCH1::BCH1(Δ1723-1824)*	This study
YAS2923	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::CHS6-3GFP (KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS2924	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3 (URA3)* CHS6::CHS6(Δ730-1212)-3GFP (KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS2925	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3 (URA3)* CHS6::MET25(natNT2)-CHS6(Δ1837-1938)-3GFP (KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS2700	*MAT a ade2 his3 leu2 lys2 trp1 ura3 SEC7-dsRed (LEU2)*	This study
YAS2561	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::TEF(natNT2)-CHS6-9myc (URA3) BCH1::BCH1-6HA (KanMX4)*	This study
YAS2570	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(Δ730-1212)-9myc (HIS3MX6) BCH1::BCH1-6HA (KanMX4)*	This study
YAS2508	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(Δ1837-1938)-9myc (HIS3MX6) BCH1::BCH1-6HA (KanMX4)*	This study
YAS2854	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6(L619W/G620D)-9myc (HIS3MX6)*	This study
YAS2851	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6-BCH2(739-1224)*	This study
YAS2850	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6-BCH2(1840-1941)*	This study
YAS3083	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::LEU2 BCH2::BCH2-CHS6(1837-1938)*	This study
YAS2927	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6-BCH2(1225-1839)-9myc (HIS3MX6)*	This study
YAS3084	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) BCH2::BCH2-CHS6(1213-1836)-9myc (HIS3MX6) CHS6::LEU2*	This study
YAS3021	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) BCH2::CHS6 CHS6::LEU2*	This study
YAS3019	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) BCH2::BCH2-CHS6(1-1836)-9myc (HIS3MX6) CHS6::LEU2*	This study
YAS3085	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) BCH2::BCH2-CHS6(1213-2241)-9myc (HIS3MX6) CHS6::LEU2*	This study
YAS3087	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) BCH2::BCH2-CHS6(1-729,1213-1836, 1939-2241) CHS6::LEU2*	This study
YAS3265	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(232-2241)-BCH2(1-231)-9myc (KanMx4) CHS6::LEU2*	This study
YAS3266	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6(1-231,484-2241)-BCH2(231-492)-9myc (KanMx4) CHS6::LEU2*	This study
YAS3267	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6(1-483,730-2241)-BCH2(493-738)-9myc (KanMx4) CHS6::LEU2*	This study
YAS3270	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::GPD(natNT2)-CHS6(1-1377,1669-2241)-BCH2(1225-1392)-9myc (KanMx4) CHS6::LEU2*	This study
YAS3271	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::TEF(natNT2)-CHS6(1-1377,1669-2241)-BCH2(1393-1689)-9myc (KanMx4) CHS6::LEU2*	This study
YAS3272	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(1-1668,1837-2241)-BCH2(1690-1839)-9myc (KanMx4) CHS6::LEU2*	This study
YAS3091	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3(Δ3433-3498)-3GFP (KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS3093	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3(Δ3385-3498)-3GFP (KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS1516	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS3::LEU2*	This study
YAS3077	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::TEF(natNT2)-CHS6-9myc (KI TRP1) CHS3::LEU2*	This study
YAS2632	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::TEF(natNT2)-CHS6-9myc (KI TRP1) CHS5::LEU2*	This study
YAS2562	*MAT a ade2 his3 leu2 lys2 trp1 ura3 CHS6::TEF(natNT2)-CHS6-9myc (URA3) BUD7::BUD7-6HA (KanMX4)*	This study
YAS2571	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(Δ730-1212)-9myc (HIS3MX6) BUD7::BUD7-6HA (KanMX4)*	This study
YAS2510	*MAT α ade2 his3 leu2 lys2 trp1 ura3 CHS3::CHS3-2GFP (KI TRP1) CHS6::CHS6(Δ1837-1938)-9myc (HIS3MX6) BUD7::BUD7-6HA (KanMX4)*	This study
YAS3257	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2-GFP(KI TRP1)*	This study
YAS3286	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2-GFP(KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS3258	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-GFP(KI TRP1)*	This study
YAS3288	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-GFP(KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS3274	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-GFP(KI TRP1) END3::URA3*	This study
YAS3273	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-GFP(KI TRP1) CHS5::URA3*	This study
YAS3259	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-CHS3(3331-3495)-GFP(KI TRP1)*	This study
YAS3289	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-CHS3(3331-3495)-GFP(KI TRP1) SEC7-dsRed (LEU2)*	This study
YAS3276	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-CHS3(3331-3495)-GFP(KI TRP1) END3::URA3*	This study
YAS3275	*MAT a ade2 his3 leu2 lys2 trp1 ura3 KEX2::KEX2(Δ2098-2442)-CHS3(3331-3495)-GFP(KI TRP1) CHS5::URA3*	This study

* A 3xGFP-tag was inserted into the *CHS3* locus and later replaced with a *URA3* cassette, thus removing the tag and restoring WT Chs3p expression.