Neller et al., http://www.jcb.org/cgi/content/full/jcb.201407126/DC1



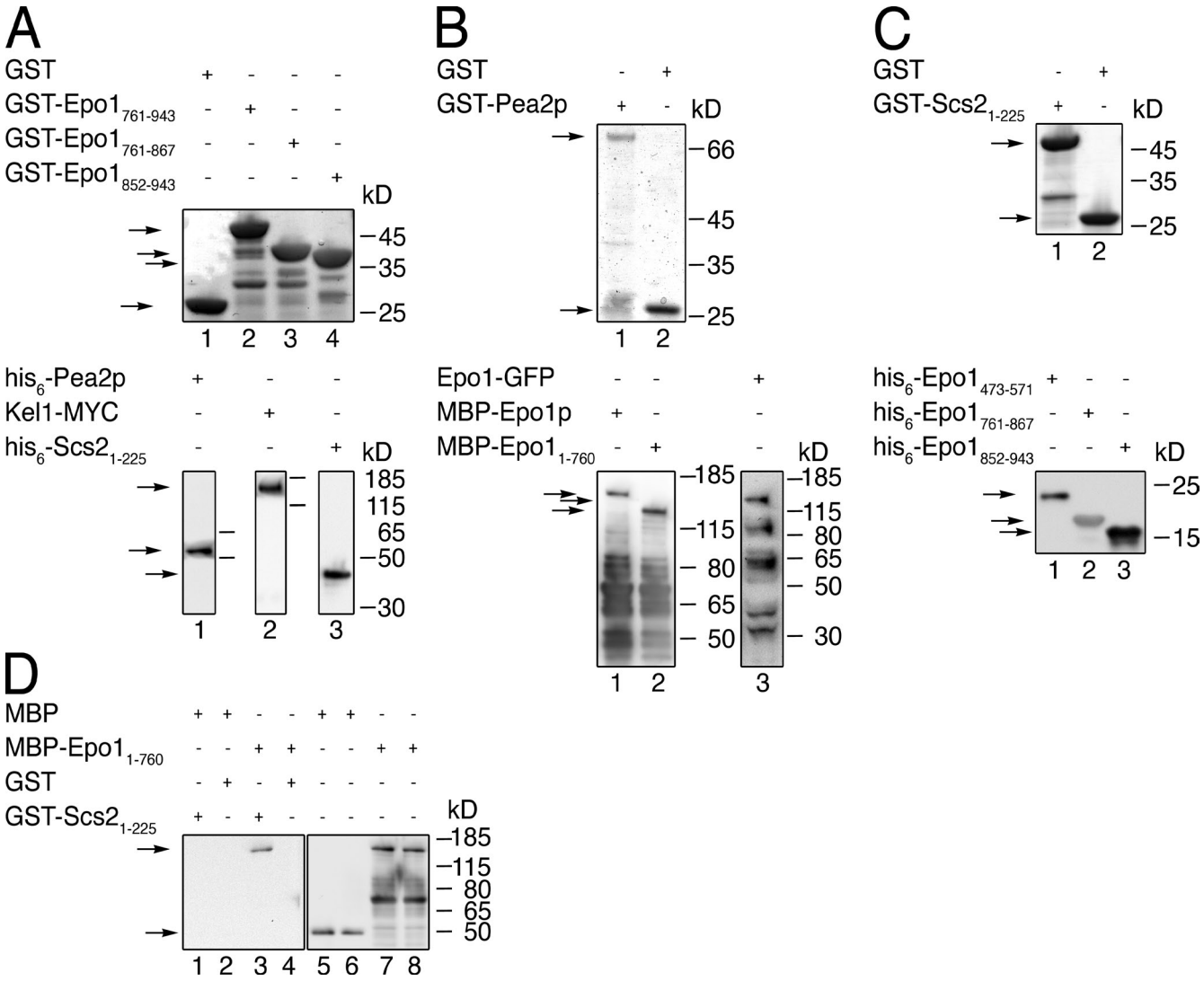



Figure S1. **Expression of fusion proteins used for pull-down analysis.** (A, top) SDS-PAGE of glutathione eluates of glutathione-coupled Sepharose beads incubated with extracts of bacterial cells expressing GST (lane 1), GST-Epo1$_{761–943}$ (lane 2), GST-Epo1$_{761–867}$ (lane 3), or GST-Epo1$_{852–943}$ (lane 4). The proteins were stained with Coomassie. (bottom) SDS-PAGE of yeast (lane 2) or *E. coli* extracts (lanes 1 and 3) containing his$_6$-Pea2p (lane 1), Kel1-MYC (lane 2), or his$_6$-Scs2$_{1–225}$ (lane 3). After transfer, the fusion proteins were detected by Western blotting using anti-His (lanes 1 and 3) or anti-MYC (lane 2) antibodies. (B, top) As in A (top), but with extracts from *E. coli* expressing GST-Pea2p (lane 1) or GST (lane 2). (bottom) SDS-PAGE of *E. coli* (lanes 1 and 2) of yeast extracts (lane 3) containing MBP-Epo1p (lane 1), MBP-Epo1$_{1–760}$ (lane 2), or Epo1-GFP (lane 3). After transfer, the fusion proteins were detected by Western blotting using anti-MBP (lanes 1 and 2) or anti-GFP (lane 3) antibodies. (C, top) As in A (top), but with extracts from *E. coli* expressing GST-Scs2$_{1–225}$ (lane 1) or GST (lane 2). (bottom) SDS-PAGE of *E. coli* extracts containing his$_6$-Epo1$_{473–571}$ (lane 1), his$_6$-Epo1$_{761–867}$ (lane 2), or his$_6$-Epo1$_{852–943}$ (lane 3). After transfer, the fusion proteins were detected by Western blotting using anti-His antibodies. (D) Eluates of Sepharose bead-coupled GST-Scs2$_{1–225}$ (lanes 1 and 3) or GST (lanes 2 and 4) incubated with *E. coli* extracts containing MBP (lanes 1, 2, 5, and 6) or MBP-Epo1$_{1–760}$ (3, 4, 7, and 8) were separated by SDS-PAGE. Western blots were probed with anti-MBP antibodies. Lanes 5–8 show the unbound fractions of the extracts. The arrows indicate the running positions of the fusion proteins on the gel.

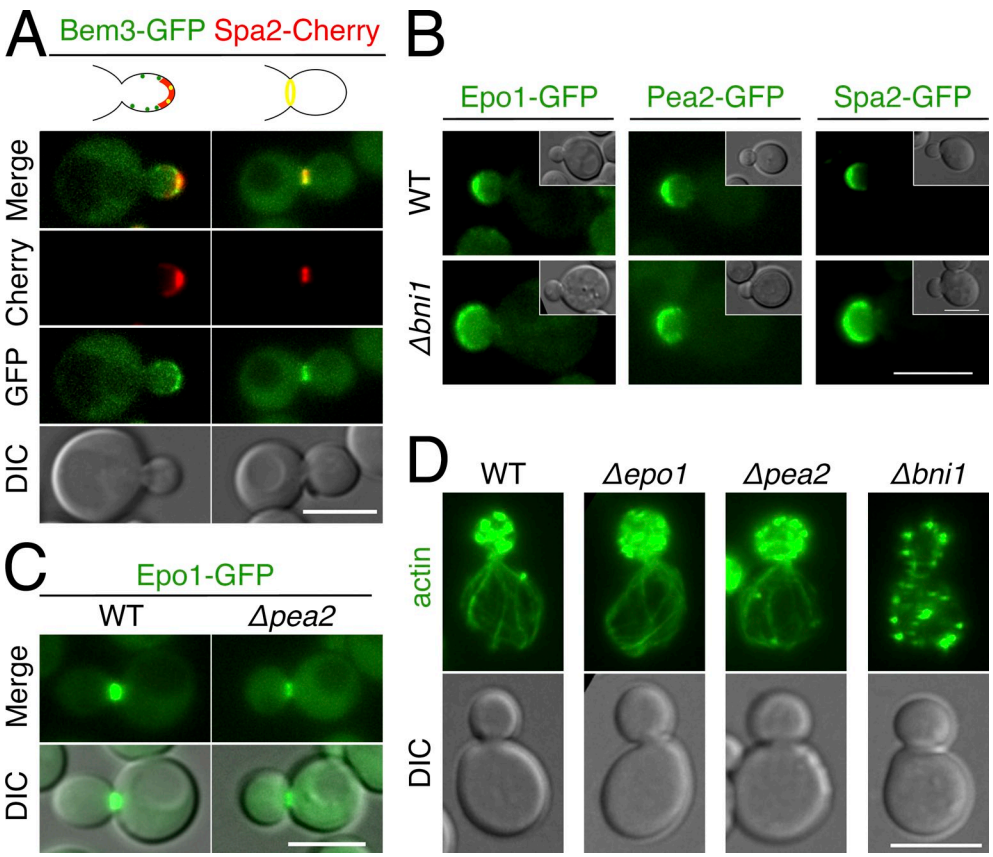



Figure S2. **Localizations of Bem3-GFP in WT cells and of Epo1-, Pea2-, and Spa2-GFP in *Δbni1* or *Δpea2* cells and the actin staining of WT and of *Δepo1* cells.** (A) Bud tip and neck staining of Bem3-GFP. Two-channel microscopy of cells coexpressing Bem3-GFP and Spa2-Cherry. Shown is one representative cell during tip growth or mitosis. (B) Epo1-, Pea2- , and Spa2-GFP lose their focused distribution upon deletion of *BNI1*. Shown are the GFP images of one representative cell each during tip growth in WT (top) or *Δbni1* cells (bottom). Insets show a reduction of the DIC image of the same cell. (C) Representative images of WT and *Δpea2* cells displaying Epo1-GFP at the bud neck. (D) Alexa Fluor 488 Phalloidin staining of WT cells and cells lacking *EPO1*, *PEA2*, or *BNI1*. Note the WT-like actin cytoskeleton in *Δpea2* and *Δepo1* cells as compared with *Δbni1* cells. Bars, 5 µm.

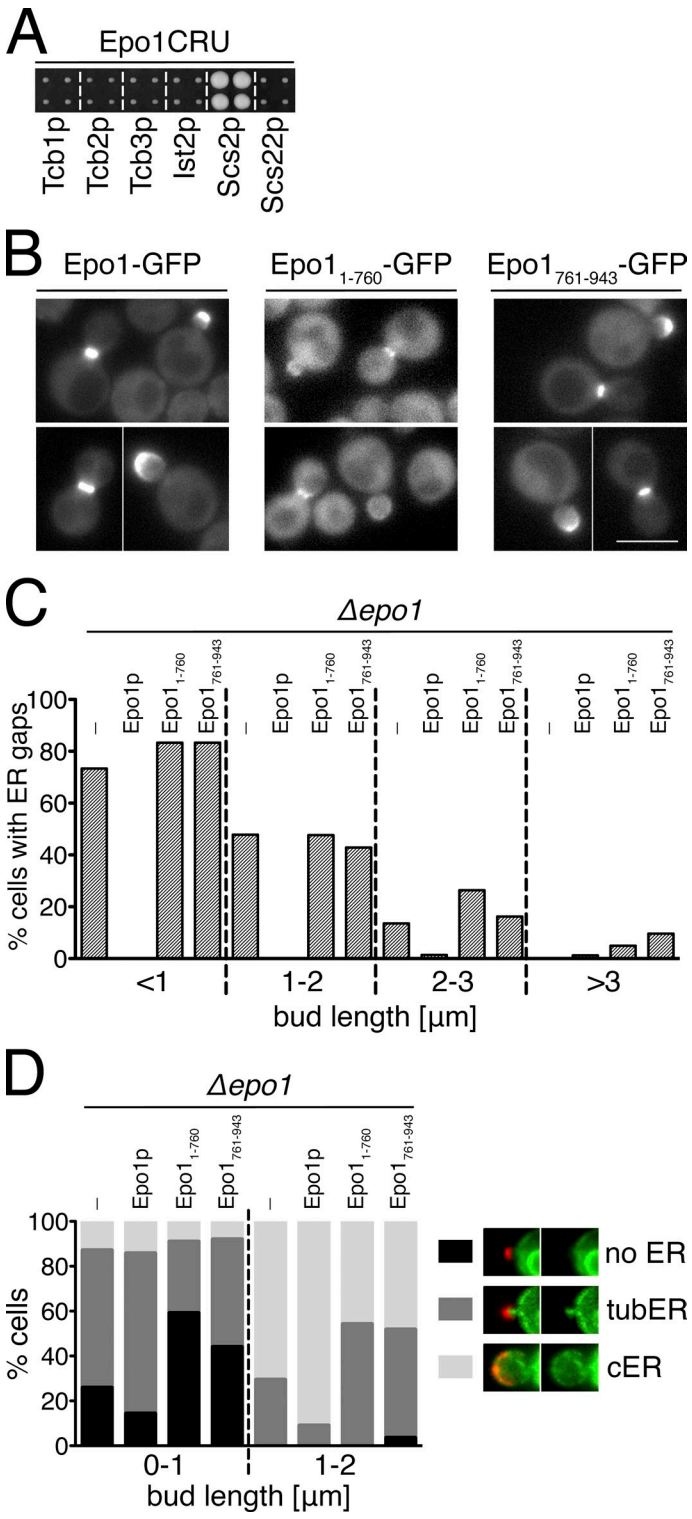



Figure S3. **Array-based Split-Ub interaction assay between Epo1CRU and the $N_{ub}$ fusions of all ER–PM tethers and the complementation of *Δepo1* cells by the expression of Epo1p fragments.** (A) Epo1p interacts exclusively with the ER–PM tether protein Scs2p. Split-Ub interaction assay of six yeast strains each coexpressing Epo1CRU with either $N_{ub}$-fused Tcb1p, Tcb2p, Tcb3p, Ist2p, Scs2p, or Scs22p. Shown are quadruplets of each strain after 4 d of growth on medium containing 5-FOA. Dashed lines separate the yeast strains expressing the different $N_{ub}$ fusion proteins. (B) The isolated Scs2p binding sites on Epo1p do not complement the loss of *EPO1*. Fluorescence images of cells expressing Epo1-GFP (left) or Epo1$_{1–760}$-GFP (middle), both from the native promoter, or Epo1$_{761–943}$-GFP (right) expressed from the $P_{MET17}$ promoter. Bar, 5 µm. (C) *Δepo1* cells expressing GFP-Sec62p and an empty plasmid (−) or a plasmid carrying the Cherry fusion to Epo1p, Epo1$_{1–760}$, or Epo1$_{761–943}$ were quantified for gaps of ER staining at the bud tip as in Fig. 7 B ($42 < n < 300$). (D) *Δepo1* cells expressing GFP-Sec62p and an empty plasmid (−) or a plasmid carrying the Cherry-fusion to Epo1p, Epo1$_{1–760}$, or Epo1$_{761–943}$ were scored for the three stages of cER inheritance (no, tubular [tubER], or cER). The same representative images were shown previously in Fig. 7 C. Quantification was as described in Fig. 7 C ($105 < n < 225$).

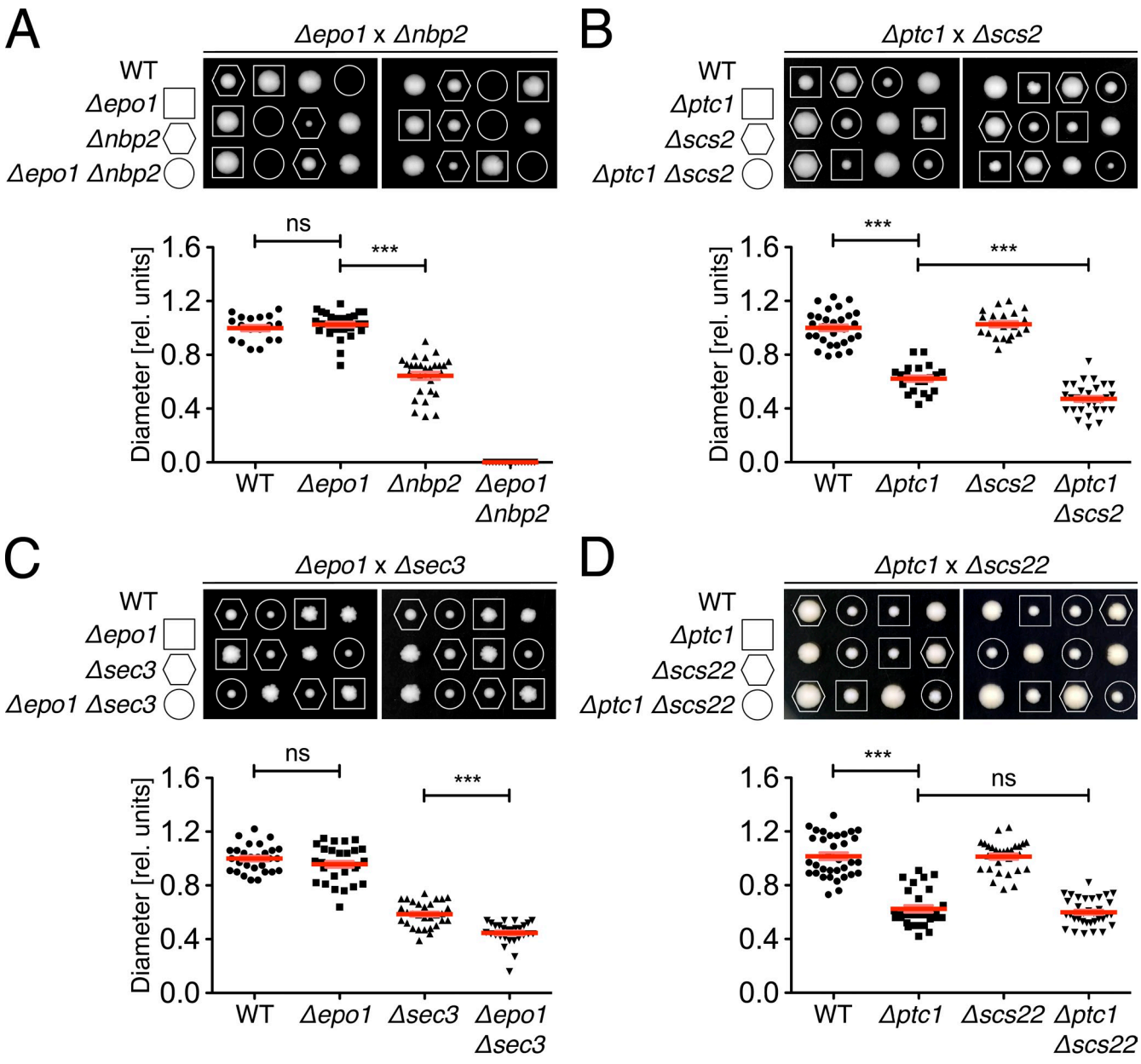



Figure S4. **Tetrad analyses of heterozygous double-deletion strains after sporulation.** (A, top) Tetrad analysis of the meiotic progeny derived from diploid cells heterozygous for *Δepo1* and *Δnbp2*. (bottom) Quantification of the diameters of the spores ($17 < n < 31$; error bars show SEM). ***, $P < 0.001$. (B) As in A, but with *Δptc1/PTC1; Δscs2/SCS2* cells. (C) As in A, but with *Δepo1/EPO1; Δsec3/SEC3* cells. (D) As in A, but with *Δptc1/PTC1; Δscs22/SCS22* cells. Spores in A, B, and D were grown at 30°C for 4 d and, in C, at 23°C for 6 d. rel., relative.

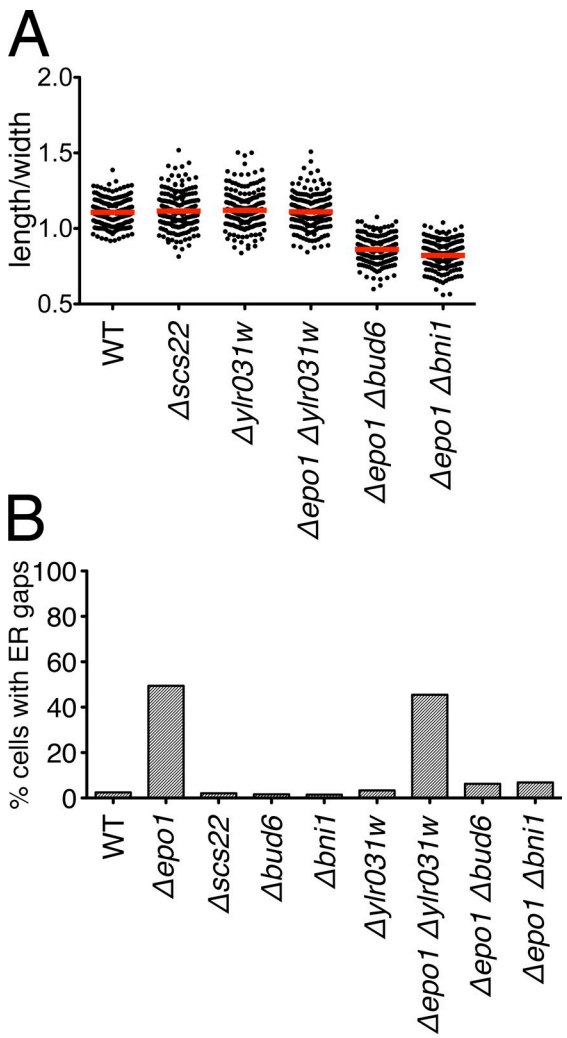



Figure S5. **Quantification of length/width ratios and ER gaps.** Quantification of length/width ratios (200 < *n* < 206, error bars show SEM; the value for the WT is taken from Fig. 3 D) and ER gaps (80 < *n* < 139) of different yeast deletion strains. Analysis was performed as in Fig. 8 (A and B).

Table S1. **List of all tested $N_{ub}$ fusions**

| | | | | | | |
|---|---|---|---|---|---|---|
| ABP1 | BUD20 | EDE1 | KEL1 | PAN1 | SEC4 | STE11 |
| ACF2 | CAP1 | ELG1 | KEL2 | PBS2 | SEC5 | STE20 |
| ACT1(P) | CBK1 | ELM1 | KIC1 | PDE1 | SEC6 | STE50 |
| ACT1$^{V152G;T318I}$ | CDC3 | ELO1 | KIN1 | PDS1 | SEC8 | STT4 |
| AFR1 | CDC5 | END3 | KIN2 | PEA2 | SEC9 | STU1 |
| AIP1 | CDC6 | ENT2 | KIN4 | PEX4(P) | SEC10 | STU2 |
| AIR1 | CDC10 | EPO1 | KIP2 | PEX19(P) | SEC14 | SVL3 |
| AMN1 | CDC11 | ESP1 | KIP3 | PFK1 | SEC15 | SWE1 |
| APC2 | CDC12 | EXO70 | KOG1 | PFY1 | SEC62(P) | SWI4 |
| APG17 | CDC14 | EXO84 | KSS1 | PHO85 | SFH1 | SWI6 |
| APP1 | CDC20 | FAB1 | LAC1 | PIK1 | SFH2 | SYP1 |
| ARC1 | CDC24 | FAR1 | LAG1 | PIL1 | SFH3 | TAO3 |
| ARC40 | CDC25 | FKH1 | LAS17 | PKC1 | SFH4 | TCB1 |
| ARP1 | CDC28 | FKH2 | LCB4 | PKH1 | SFH5 | TCB2 |
| ARP6 | CDC37 | FKS1 | LCB5 | PKH2 | SFK1 | TCB3 |
| ASE1 | CDC42(P) | FKS2 | LDB17 | PKH3 | SHE4 | TCO89 |
| ASK10 | CHK1 | FUS2 | LRE1 | PLC1 | SHO1(P) | TDH1 |
| AUR1 | CHS1 | FUS3 | LRG1 | PNC1 | SHO1$^{Cterm}$ | TEF1 |
| AVO1 | CHS2 | GIC1 | LSP1 | PPH3 | SHS1 | TEL1 |
| AVO2(P) | CHS3 | GIM4 | LST8 | PRK1 | SIT4 | TEM1(P) |
| AXL2$^{Cterm}$ | CIK1 | GIN4 | LTE1 | PSY4 | SIZ1 | TLG2(P) |
| BBC1 | CIN1 | GLG1 | MAD2 | PTC1 | SKG6 | TOK1 |
| BCK1 | CIN8 | GPA2 | MAD3 | PTC2 | SKM1 | TOR1 |
| BCK2 | CLA4 | GPB1 | MCD1 | PUF3 | SKN7 | TOR2 |
| BCY1 | CLB1 | GPB2 | MCK1 | RAD9 | SKO1 | TOS2 |
| BEM1 | CLB2 | GSY1 | MEC1 | RAS1 | SKT5 | TPK1 |
| BEM2 | CLB3 | GUK1(P) | MID2$^{Cterm}$ | RAS2 | SLA1 | TPM1 |
| BEM3 | CLB4 | GUS1 | MKK1 | RAX1 | SLA2 | TPM2 |
| BEM4 | CLN1 | HCM1 | MLC1(P) | RGA2 | SLI15 | TSC11 |
| BFA1 | CLN2 | HLR1 | MLC2 | RGD1 | SLM1 | TSC13 |
| BIK1 | CLN3 | HOF1 | MOB1 | RGS2 | SLT2 | TUS1 |
| BIM1 | CMD1 | HOF1$^{98–699}$ | MPS1 | RHO1 | SMC1 | UBC6(P) |
| BIR1 | CMK1 | HOG1 | MSB1 | RHO2 | SMI1 | UBC9 |
| BIT61 | CMP2 | HOT1 | MSB3 | RHO4 | SMP1 | UBP7 |
| BMH1 | CNA1 | HSK3 | MSB4 | RHO5 | SMT3(P) | VAC8 |
| BMH2 | CNB1 | HSL1 | MSN1 | RIM21 | SMY1 | VAC17 |
| BNI1 | CPR6 | HSL7 | MSN2 | RLM1 | SNC1(P) | VHS1 |
| BNI1$^{Cterm}$ | CRN1 | HSP82 | MSN4 | ROM1 | SOG2 | VHS2 |
| BNI4 | CRZ1 | HUA1 | MSO1 | ROM2 | SPA2 | VMA13 |
| BNI5 | CSE4 | HYM1 | MSS4 | RSR1 | SPC110 | VRP1 |
| BNR1 | CYK3 | IME2 | MYO1 | RVS161 | SPC19 | WSC1$^{Cterm}$ |
| BNR1$^{Cterm}$ | CYK3$^{69–885}$ | INN1 | MYO2 | RVS167 | SPC24 | YBR053C |
| BOI1 | CYK3$^{196–885}$ | INP51 | MYO5 | SAC1 | SPC34 | YBR238C |
| BOI1$^{1–95}$(P) | CYR1 | INP52 | NAP1 | SAC6 | SPC72 | YDL173W |
| BOI1$^{758–980}$(P) | DAD1 | IPL1 | NAS2 | SAC7 | SPH1 | YDL203C |
| BOI2 | DAD2 | IPP1 | NBA1 | SAK1 | SPR28 | YDR348C |
| BUB2 | DAD3 | IQG1 | NBP2 | SCC2 | SPT5 | YEL023C |
| BUB3 | DAM1 | IQG1$^{Cterm}$ | NET1 | SCC4 | SRO7 | YGR058W |
| BUD2 | DBF2 | IRA1 | NHA1 | SCP1 | SRO77 | YKL161C |
| BUD3 | DBF20 | IRA2 | NIP100 | SCP160 | SRV2 | YOR304C-A |
| BUD4 | DOP1 | IRR1 | NIS1 | SCS2 | SSD1 | YPD1 |
| BUD5 | DSE1 | IRS4 | NUM1 | SCS22 | SSK1 | YPK1 |
| BUD6 | DUN1 | IST2 | ORC6 | SDP1 | SSK2 | ZDS1 |
| BUD8 | DUO1 | KAR3 | PAC1 | SEC1 | SSO1 | ZDS2 |
| BUD9 | DYN1 | KAR9 | PAC10 | SEC2 | STE5 | ZEO1 |
| BUD14 | ECM25 | KCC4 | PAM1 | SEC3 | STE7 | |

(P), plasmid encoded; Cterm, $N_{ub}$ fused to the C terminus of the protein.

Table S2. **Identities and positions of the $N_{ub}$ fusions on the $N_{ub}$ array shown in Fig. 1 A**

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aip1 | Bem1 | Bem2 | Bem3 | Bem4 | Boi1 | Boi2 | Scs2 | Scs22 | Bud6 | Bud14 | Cdc42 | Ist2 | Exo70 | Exo84 | Hof1 | Kel1 | Kel2 | Kin1 | Kin2 | Mso1 | Tcb1 | Nbp2 | Pea2 |
| Ptc1 | Rga2 | Rgd1 | Rho1 | Rho4 | Sac6 | Sec1 | Sec2 | Sec3 | Sec4 | Sec5 | Sec6 | Sec8 | Sec9 | Sec10 | Sec15 | Snc1 | Spa2 | Sro7 | Sro77 | Sso1 | Epo1 | Yor304c | $N_{ub}^{-}$ |

Table S3. **List of constructs used and created in this study**

| Name | Description | Origin |
|---|---|---|
| Epo1-CRU pRS303 | *EPO1-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| Pea2-CRU pRS303 | *PEA2-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| Ist2-CRU pRS303 | *IST2-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| Tcb1-CRU pRS303 | *TCB1-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| Tcb2-CRU pRS303 | *TCB2-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| Tcb3-CRU pRS303 | *TCB3-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| $P_{MET17}$-Kel1-CRU pRS313 | *$P_{MET17}$-KEL1-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| $P_{MET17}$-Epo1-CRU pRS313 | *$P_{MET17}$-EPO1-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| $P_{MET17}$-Epo1$_{761–943}$-CRU pRS313 | *$P_{MET17}$-EPO1$_{761–943}$-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| Epo1$_{1–760}$-CRU pRS303 | *EPO1$_{1–760}$-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| $P_{MET17}$-Epo1$_{1–876}$-CRU pRS313 | *$P_{MET17}$-EPO1$_{1–876}$-$C_{ub}$-R-URA3, $Amp^R$, HIS3* | This work |
| $P_{CUP1}$-Nub-HA kanMX4 | *$P_{CUP1}$-$N_{ub}$-HA, $Amp^R$, kanMX4* | Dünkler et al., 2012 |
| $P_{CUP1}$-$N_{ub}$-HA-kanMX4 CEN | *$P_{CUP1}$-$N_{ub}$-HA kanMX4 CEN, $Amp^R$* | Dünkler et al., 2012 |
| $P_{CUP1}$-$N_{ub}$-HA-Guk1 kanMX4 CEN | *$P_{CUP1}$-$N_{ub}$-HA-GUK1 kanMX4 CEN, $Amp^R$* | This work |
| $P_{CUP1}$-$N_{ub}$-HA-Bem3$_{1–633}$ kanMX4 CEN | *$P_{CUP1}$-$N_{ub}$-HA-BEM3$_{1–633}$ kanMX4 CEN, $Amp^R$* | This work |
| $P_{CUP1}$-$N_{ub}$-HA-Bem3$_{1–300}$ kanMX4 CEN | *$P_{CUP1}$-$N_{ub}$-HA-BEM3$_{1–300}$ kanMX4 CEN, $Amp^R$* | This work |
| $P_{CUP1}$-$N_{ub}$-HA-Bem3$_{1–100}$ kanMX4 CEN | *$P_{CUP1}$-$N_{ub}$-HA-BEM3$_{1–100}$ kanMX4 CEN, $Amp^R$* | This work |
| $P_{CUP1}$-$N_{ub}$-HA-Bem3$_{300–633}$ kanMX4 CEN | *$P_{CUP1}$-$N_{ub}$-HA-BEM3$_{300–633}$ kanMX4 CEN, $Amp^R$* | This work |
| $P_{CUP1}$-$N_{ub}$-HA-Scs2$_{1–225}$ kanMX4 CEN | *$P_{CUP1}$-$N_{ub}$-HA-SCS2$_{1–225}$ kanMX4 CEN, $Amp^R$* | This work |
| Epo1-CCG pRS306 | *EPO1-Cherry-$C_{ub}$-R-GFP, $Amp^R$, URA3* | This work |
| Bem3-GFP pRS304 | *BEM3-GFP, $Amp^R$, TRP1* | This work |
| Pea2-GFP pRS304 | *PEA2-GFP, $Amp^R$, TRP1* | This work |
| Spa2-GFP pRS304 | *SPA2-GFP, $Amp^R$, TRP1* | This work |
| Epo1-GFP pRS304 | *EPO1-GFP, $Amp^R$, TRP1* | This work |
| pRH475 | *HMG1-GFP, $Amp^R$, URA3* | Wilhovsky et al., 2000 |
| $P_{MET17}$-GFP-Sec62 pRS313 | *$P_{MET17}$-GFP-SEC62, $Amp^R$, HIS3* | This work |
| $P_{MET17}$-GFP-Scs2$_{1–225}$ pRS313 | *$P_{MET17}$-GFP-SCS2$_{1–225}$, $Amp^R$, HIS3* | This work |
| $P_{MET17}$-Epo1$_{1–760}$-GFP pRS304 | *$P_{MET17}$- EPO1$_{1–760}$-GFP, $Amp^R$, TRP1* | This work |
| $P_{MET17}$-Epo1$_{761–943}$-GFP pRS313 | *$P_{MET17}$- EPO1$_{761–943}$-GFP, $Amp^R$, HIS3* | This work |
| Spa2-Cherry pRS305 | *SPA2-Cherry, $Amp^R$, LEU2* | This work |
| Spa2-Cherry pRS306 | *SPA2-Cherry, $Amp^R$, URA3* | This work |
| Kel1-Cherry pRS305 | *KEL1-Cherry, $Amp^R$, LEU2* | This work |
| Shs1-Cherry pRS306 | *SHS1-Cherry, $Amp^R$, URA3* | Schneider et al., 2013 |
| $P_{MET17}$-Cherry pRS315 | *$P_{MET17}$-Cherry, $Amp^R$, LEU2* | This work |
| $P_{MET17}$-Hhf2-Cherry pRS315 | *$P_{MET17}$-HHF2-Cherry, $Amp^R$, LEU2* | This work |
| $P_{MET17}$-Epo1-Cherry pRS315 | *$P_{MET17}$-EPO1-Cherry, $Amp^R$, LEU2* | This work |
| $P_{MET17}$-Epo1$_{1–760}$-Cherry pRS315 | *$P_{MET17}$-EPO1$_{1–760}$-Cherry, $Amp^R$, LEU2* | This work |
| $P_{MET17}$-Epo1$_{761–943}$-Cherry pRS315 | *$P_{MET17}$-EPO1$_{761–943}$-Cherry, $Amp^R$, LEU2* | This work |
| Kel1-9×Myc pRS304 | *KEL1-9×MYC, $Amp^R$, TRP1* | This work |
| pGEX-2T | *$P_{tac}$-GST, $Amp^R$* | GE Healthcare |
| Pea2 pGEX-2T | *$P_{tac}$-GST-PEA2, $Amp^R$* | This work |
| Epo1$_{761–943}$ pGEX-2T | *$P_{tac}$-GST-EPO1$_{761–943}$, $Amp^R$* | This work |
| Epo1$_{761–867}$ pGEX-2T | *$P_{tac}$-GST-EPO1$_{761–867}$, $Amp^R$* | This work |
| Epo1$_{852–943}$ pGEX-2T | *$P_{tac}$-GST-EPO1$_{852–943}$, $Amp^R$* | This work |
| Scs2$_{1–225}$ pGEX-2T | *$P_{tac}$-GST-SCS2$_{1–225}$, $Amp^R$* | This work |
| Bem3$_{1–100}$ pGEX-2T | *$P_{tac}$-GST-BEM3$_{1–100}$, $Amp^R$* | This work |
| pAC | *$P_{T7}$-6HIS, $kanMX^R$* | Iffland et al., 2000 |
| Pea2 pAC | *$P_{T7}$-6HIS-PEA2, $kanMX^R$* | This work |
| Epo1$_{473–571}$ pAC | *$P_{T7}$-6HIS-EPO1$_{473–571}$, $kanMX^R$* | This work |
| Epo1$_{761–867}$ pAC | *$P_{T7}$-6HIS-EPO1$_{761–867}$, $kanMX^R$* | This work |
| Epo1$_{852–943}$ pAC | *$P_{T7}$-6HIS-EPO1$_{852–943}$, $kanMX^R$* | This work |
| Scs2$_{1–225}$ pAC | *$P_{T7}$-6HIS-SCS2$_{1–225}$, $kanMX^R$* | This work |
| pAGT-Xpress | *$P_{T7}$-6HIS-AGT, $Amp^R$* | This work |
| Spa2$_{1–488}$ pAGT-Xpress | *$P_{T7}$-6HIS-Spa2$_{1–488}$-AGT, $kanMX^R$* | This work |
| Spa2$_{1–535}$ pAGT-Xpress | *$P_{T7}$-6HIS-Spa2$_{1–535}$-AGT, $kanMX^R$* | This work |
| pMal-c5x | *$P_{tac}$-MBP, $Amp^R$* | New England Biolabs, Inc. |
| Epo1 pMal-c5x | *$P_{tac}$-MBP-EPO1, $Amp^R$* | This work |
| Epo1$_{1–760}$ pMal-c5x | *$P_{tac}$-MBP-EPO1$_{1–760}$, $Amp^R$* | This work |

Table S3. **List of constructs used and created in this study** *(Continued)*

| Name | Description | Origin |
|---|---|---|
| pFA6a-natNT2 | $P_{AgTEF}$-*natNT2, Amp*$^R$ | Janke et al., 2004 |
| pFA6a-hphNT1 | $P_{AgTEF}$-*hphNT1, Amp*$^R$ | Janke et al., 2004 |
| pFA6a-kanMX6 | $P_{AgTEF}$-*kanMX6, Amp*$^R$ | Bähler et al., 1998 |
| pFA6a-CmLEU2 | $P_{CmLEU2}$-*CmLEU2, Amp*$^R$ | Schaub et al., 2006 |

Table S4. **List of yeast strains used and created in this study**

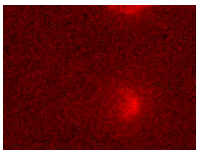
| Strain | Name | Relevant genotype | Origin |
|---|---|---|---|
| JD47 | JD47 | MATa, *his3-Δ200, leu2-3, 112 lys2-801, trp1-Δ63, ura3-52* | Dohmen et al., 1995 |
| JD53 | JD53 | MATα, *his3-Δ200, leu2-3, 112 lys2-801, trp1-Δ63, ura3-52* | Dohmen et al., 1995 |
| JD51 | JD51 | MATa/α, *his3-Δ200/his3-Δ200, leu2-3/leu2-3, 112 lys2-801/112 lys2-801, trp1-Δ63/trp1-Δ63, ura3-52/ura3-52* | Dohmen et al., 1995 |
| YAD1222 | Δbni1 | JD47, *BNI1::hphNT1* | This work |
| YAD1294 | Δbud6 | JD47, *BUD6::natNT2* | This work |
| YAD249 | Δpea2 | JD47, *PEA2::hphNT1* | This work |
| YAD248 | Δspa2 | JD47, *SPA2::hphNT1* | This work |
| YAD250 | Δkel1 | JD47, *KEL1::hphNT1* | This work |
| YAD251 | Δbem3 | JD47, *BEM3::hphNT1* | This work |
| YAD1384 | Δptc1 | JD53, *PTC1::kanMX6* | This work |
| AHY140 | Δnbp2 | JD47, *NBP2::natNT2* | Hruby et al., 2011 |
| YWY473 | Δsec3 | JD53, *SEC3::hphNT1* | This work |
| YAD1772 | Δscs22 | JD47, *SCS22::hphNT1* | This work |
| YAD253 | Δylr031w | JD47, *YLR031W::hphNT1* | This work |
| YAD398a | Δepo1_hph | JD47, *EPO1::hphNT1* | This work |
| YAD400a | Δepo1_kan | JD53, *EPO1::kanMX6* | This work |
| YAD399a | Δepo1_nat | JD47, *EPO1::natNT2* | This work |
| YAD1153 | Δscs2_hph | JD47, *SCS2::hphNT1* | This work |
| YAD1385 | Δscs2_kan | JD53, *SCS2::kanMX6* | This work |
| YAD1405 | Δepo1 Δscs2 | *EPO1::hphNT1 SCS2::kanMX6* | This work |
| YAD1407 | Δepo1 Δptc1 | *EPO1::hphNT1 PTC1::kanMX6* | This work |
| YAD1476 | Δepo1 Δnbp2 | *EPO1::kanMX6 NBP2::natNT2* | This work |
| YAD1482 | Δepo1 Δsec3 | *EPO1::natNT2 SEC3::hphNT1* | This work |
| YAD1478 | Δscs2 Δptc1 | *SCS2::hphNT1 PTC1::kanMX6* | This work |
| YAD1801 | Δscs22 Δptc1 | *SCS2::hphNT1 PTC1::kanMX6* | This work |
| YAD302.1 | Δepo1 Δpea2 | *PEA2::hphNT1 EPO1::kanMX6* | This work |
| YAD301.1 | Δepo1 Δspa2 | *SPA2::hphNT1 EPO1::kanMX6* | This work |
| YAD417.1 | Δepo1 Δbem3 | *BEM3::hphNT1 EPO1::kanMX6* | This work |
| YAD285 | Δepo1 Δylr031w | *YLR031W::hphNT1 EPO1::kanMX6* | This work |
| YAD1794 | Δepo1 Δbud6 | *EPO1::hphNT1 BUD6::natNT2* | This work |
| YAD1790 | Δepo1 Δbni1 | *EPO1::natNT2 BNI1::hphNT1* | This work |
| YAD416.1 | Δpea2 Δbem3 | *BEM3::hphNT1 PEA2::kanMX6* | This work |
| YAD213.1 | Pea2-CRU | JD47, *PEA2::PEA2-Cub-R-URA3 HIS3* | This work |
| YAD347 | Pea2-CRU Δepo1 | JD47, *PEA2::PEA2-Cub-R-URA3 HIS3 EPO1::CmLEU2* | This work |
| YAD346.1 | Epo1-CRU | JD47, *EPO1::EPO1-Cub-R-URA3 HIS3* | This work |
| YAD288 | $P_{MET17}$-Epo1-CRU | JD47, $P_{MET17}$-*EPO1-*$C_{ub}$*-R-URA3 HIS3 pRS313* | This work |
| YAD967 | $P_{MET17}$-Epo1-CRU Δpea2 | JD47, $P_{MET17}$-*EPO1-*$C_{ub}$*-R-URA3 pRS313 PEA2::CmLEU2* | This work |
| YAD363 | $P_{MET17}$-Epo1-CRU Δspa2 | JD47, $P_{MET17}$-*EPO1-*$C_{ub}$*-R-URA3 pRS313 SPA2::CmLEU2* | This work |
| YAD341 | $P_{MET17}$-Epo1-CRU Δkel1 | JD47, $P_{MET17}$-*EPO1-*$C_{ub}$*-R-URA3 pRS313 KEL1::hphNT1* | This work |
| YAD410 | $P_{MET17}$-Epo1-CRU Δbem3 | JD47, $P_{MET17}$-*EPO1-*$C_{ub}$*-R-URA3 pRS313 BEM3::hphNT1* | This work |
| YAD1394 | $P_{MET17}$-Epo1$_{761–943}$-CRU | JD47, $P_{MET17}$-*EPO1*$_{761–943}$-$C_{ub}$*-R-URA3 pRS313* | This work |
| YAD233 | $P_{MET17}$-Epo1$_{1–760}$-CRU | JD47, $P_{MET17}$-*EPO1*$_{1–760}$-$C_{ub}$*-R-URA3 pRS313* | This work |
| YAD580 | $P_{MET17}$-Epo1$_{1–876}$-CRU | JD47, $P_{MET17}$-*EPO1*$_{1–876}$-$C_{ub}$*-R-URA3 pRS313* | This work |
| YAD329 | $P_{MET17}$-Kel1-CRU | JD47, $P_{MET17}$-*KEL1-*$C_{ub}$*-R-URA3 HIS3 pRS313* | This work |
| YAD1175 | Ist2-CRU | JD47, *IST2::IST2-Cub-R-URA3 HIS3* | This work |
| YAD1176 | Tcb1-CRU | JD47, *TCB1::TCB1-Cub-R-URA3 HIS3* | This work |
| YAD1177 | Tcb2-CRU | JD47, *TCB2::TCB2-Cub-R-URA3 HIS3* | This work |
| YAD1178 | Tcb3-CRU | JD47, *TCB3::TCB3-Cub-R-URA3 HIS3* | This work |
| YAD305 | $N_{ub}$-Spa2 Δpea2 | JD53, $P_{SPA2}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA PEA2::CmLEU2* | This work |
| YAD307 | $N_{ub}$-Kel1 Δpea2 | JD53, $P_{KEL1}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA PEA2::CmLEU2* | This work |
| YAD311 | $N_{ub}$-Bem3 Δpea2 | JD53, $P_{BEM3}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA PEA2::CmLEU2* | This work |
| YAD306 | $N_{ub}$ Δpea2 | JD53, $P_{CUP1}N_{ub}$*-HA kanMX4-CEN PEA2::CmLEU2* | This work |
| YAD310 | $N_{ub}$-Bud6 Δpea2 | JD53, $P_{BUD6}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA PEA2::CmLEU2* | This work |
| YAD1358 | $N_{ub}$-Scs2 Δpea2 | JD53, $P_{SCS2}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA PEA2::CmLEU2* | This work |
| YAD384 | $N_{ub}$-Pea2 Δspa2 | JD53, $P_{PEA2}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA SPA2::natNT2* | This work |
| YAD380 | $N_{ub}$-Kel1 Δspa2 | JD53, $P_{KEL1}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA SPA2::natNT2* | This work |
| YAD365 | $N_{ub}$-Bem3 Δspa2 | JD53, $P_{BEM3}$*::kanMX6* $P_{CUP1}N_{ub}$*-HA SPA2::natNT2* | This work |

Table S4. **List of yeast strains used and created in this study** *(Continued)*

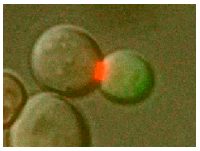
| Strain | Name | Relevant genotype | Origin |
|---|---|---|---|
| YAD382 | $N_{ub}$ Δspa2 | JD53, *$P_{CUP1}N_{ub}$-HA kanMX4-CEN SPA2::natNT2* | This work |
| YAD351 | $N_{ub}$-Pea2 Δkel1 | JD53, *$P_{PEA2}$::kanMX6 $P_{CUP1}N_{ub}$-HA KEL1::CmLEU2* | This work |
| YAD344 | $N_{ub}$-Spa2 Δkel1 | JD53, *$P_{SPA2}$::kanMX6 $P_{CUP1}N_{ub}$-HA KEL1::CmLEU2* | This work |
| YAD352 | $N_{ub}$-Bem3 Δkel1 | JD53, *$P_{BEM3}$::kanMX6 $P_{CUP1}N_{ub}$-HA KEL1::CmLEU2* | This work |
| YAD346 | $N_{ub}$ Δkel1 | JD53, *$P_{CUP1}N_{ub}$-HA kanMX4-CEN KEL1::CmLEU2* | This work |
| YAD356 | $N_{ub}$-Bud6 Δkel1 | JD53, *$P_{BUD6}$::kanMX6 $P_{CUP1}N_{ub}$-HA KEL1::CmLEU2* | This work |
| YAD402 | $N_{ub}$-Pea2 Δbem3 | JD53, *$P_{PEA2}$::kanMX6 $P_{CUP1}N_{ub}$-HA BEM3::natNT2* | This work |
| YAD401 | $N_{ub}$-Spa2 Δbem3 | JD53, *$P_{SPA2}$::kanMX6 $P_{CUP1}N_{ub}$-HA BEM3::natNT2* | This work |
| YAD403 | $N_{ub}$-Kel1 Δbem3 | JD53, *$P_{KEL1}$::kanMX6 $P_{CUP1}N_{ub}$-HA BEM3::natNT2* | This work |
| YAD408 | $N_{ub}$ Δbem3 | JD53, *$P_{CUP1}N_{ub}$-HA kanMX4-CEN BEM3::natNT2* | This work |
| YAD312 | $N_{ub}$-Spa2 Δepo1 | JD53, *$P_{SPA2}$::kanMX6 $P_{CUP1}N_{ub}$-HA EPO1::natNT2* | This work |
| YAD313 | $N_{ub}$-Kel1 Δepo1 | JD53, *$P_{KEL1}$::kanMX6 $P_{CUP1}N_{ub}$-HA EPO1::natNT2* | This work |
| YAD316 | $N_{ub}$-Bem3 Δepo1 | JD53, *$P_{BEM3}$::kanMX6 $P_{CUP1}N_{ub}$-HA EPO1::natNT2* | This work |
| YAD315 | $N_{ub}$ Δepo1 | JD53, *$P_{CUP1}N_{ub}$-HA kanMX4-CEN EPO1::natNT2* | This work |
| YAD1446 | $N_{ub}$-Scs2 Δepo1 | JD53, *$P_{SCS2}$::kanMX6 $P_{CUP1}N_{ub}$-HA EPO1::natNT2* | This work |
| YAD1111 | p$N_{ub}$-Scs2$_{1–225}$ | JD53, *$P_{CUP1}N_{ub}$-HA-SCS2$_{1–225}$ kanMX4-CEN* | This work |
| YAD1814 | p$N_{ub}$-Bem3$_{1–633}$ | JD53, *$P_{CUP1}N_{ub}$-HA-BEM3$_{1–633}$ kanMX4-CEN* | This work |
| YAD1815 | p$N_{ub}$-Bem3$_{1–300}$ | JD53, *$P_{CUP1}N_{ub}$-HA-BEM3$_{1–300}$ kanMX4-CEN* | This work |
| YAD1816 | p$N_{ub}$-Bem3$_{1–100}$ | JD53, *$P_{CUP1}N_{ub}$-HA-BEM3$_{1–100}$ kanMX4-CEN* | This work |
| YAD1817 | p$N_{ub}$-Bem3$_{300–633}$ | JD53, *$P_{CUP1}N_{ub}$-HA-BEM3$_{300–633}$ kanMX4-CEN* | This work |
| YAD974 | $P_{MET17}$-EPO1-CCG | JD47, *$P_{EPO1}$::$P_{MET17}$ natNT2 EPO1::EPO1-Cherry-$C_{ub}$-R-GFP URA3* | This work |
| YAD225 | Epo1-GFP | JD47, *EPO1::EPO1-GFP TRP1* | This work |
| YAD239 | Epo1$_{1–760}$-GFP | JD47, *EPO1::EPO1$_{1–760}$-GFP TRP1* | This work |
| YAD1321 | $P_{MET17}$-Epo1$_{761–943}$-GFP | JD47, *$P_{MET17}$-EPO1$_{761–944}$-GFP HIS3 pRS313* | This work |
| YAD1195 | Epo1-GFP Δpea2 | JD47, *EPO1::EPO1-GFP TRP1 PEA2::hphNT1* | This work |
| YAD1223 | Epo1-GFP Δbni1 | JD47, *EPO1::EPO1-GFP TRP1 BNI1::hphNT1* | This work |
| YAD241 | Pea2-GFP | JD47, *PEA2::PEA2-GFP TRP1* | This work |
| YAD1288 | Pea2-GFP Δepo1 | JD47, *PEA2::PEA2-GFP TRP1 EPO1::hphNT1* | This work |
| YAD1292 | Pea2-GFP Δbni1 | JD47, *PEA2::PEA2-GFP TRP1 BNI1::hphNT1* | This work |
| YAD959 | Spa2-GFP | JD47, *SPA2::SPA2-GFP TRP1* | This work |
| YAD1293 | Spa2-GFP Δbni1 | JD47, *SPA2::SPA2-GFP TRP1 BNI1::hphNT1* | This work |
| YAD1112 | $P_{MET17}$-GFP-Scs2$_{1–225}$ | JD47, *$P_{MET17}$-GFP-SCS2$_{1–225}$ HIS3 pRS313* | This work |
| YAD1137 | $P_{MET17}$-GFP-Scs2$_{1–225}$ Δepo1 | JD47, *$P_{MET17}$-GFP-SCS2$_{1–225}$ HIS3 pRS313 EPO1::hphNT1* | This work |
| YAD1135 | $P_{MET17}$-GFP-Scs2$_{1–225}$ Δpea2 | JD47, *$P_{MET17}$-GFP-SCS2$_{1–225}$ HIS3 pRS313 PEA2::hphNT1* | This work |
| YAD1136 | $P_{MET17}$-GFP-Scs2$_{1–225}$ Δspa2 | JD47, *$P_{MET17}$-GFP-SCS2$_{1–225}$ HIS3 pRS313 SPA2::hphNT1* | This work |
| YAD1134 | $P_{MET17}$-GFP-Scs2$_{1–225}$ Δkel1 | JD47, *$P_{MET17}$-GFP-SCS2$_{1–225}$ HIS3 pRS313 KEL1::hphNT1* | This work |
| YAD1133 | $P_{MET17}$-GFP-Scs2$_{1–225}$ Δbem3 | JD47, *$P_{MET17}$-GFP-SCS2$_{1–225}$ HIS3 pRS313 BEM3::hphNT1* | This work |
| YAD519 | $P_{MET17}$-GFP-Sec62 | JD47, *$P_{MET17}$-GFP-SEC62 HIS3 pRS313* | This work |
| YAD520 | $P_{MET17}$-GFP-Sec62 Δepo1 | JD47, *$P_{MET17}$-GFP-SEC62 HIS3 pRS313 EPO1::hphNT1* | This work |
| YAD1297 | Spa2-GFP Kel1-Cherry | JD47, *SPA2::SPA2-GFP TRP1 KEL1::KEL1-Cherry LEU2* | This work |
| YAD1309 | Spa2-GFP Kel1-Cherry | JD53, *SPA2::SPA2-GFP TRP1 KEL1::KEL1-Cherry LEU2* | This work |
| YAD816 | Epo1-GFP Kel1-Cherry | JD47, *EPO1::EPO1-GFP TRP1 KEL1::KEL1-Cherry LEU2* | This work |
| YAD1311 | Epo1-GFP Kel1-Cherry | JD53, *EPO1::EPO1-GFP TRP1 KEL1::KEL1-Cherry LEU2* | This work |
| YAD230 | Epo1-GFP Spa2-Cherry | JD47, *EPO1::EPO1-GFP TRP1 SPA2::SPA2-Cherry URA3* | This work |
| YAD1313 | Epo1-GFP Spa2-Cherry | JD53, *EPO1::EPO1-GFP TRP1 SPA2::SPA2-Cherry LEU2* | This work |
| YAD794 | Bem3-GFP Spa2-Cherry306 47 | JD47, *BEM3::BEM3-GFP TRP1 SPA2::SPA2-Cherry URA3* | This work |
| YAD1312 | Bem3-GFP304 Spa2-Cherry | JD53, *BEM3::BEM3-GFP TRP1 SPA2::SPA2-Cherry LEU2* | This work |
| YAD1536 | Spa2-GFP Shs1-Cherry | JD47, *SPA2::SPA2-GFP TRP1 SHS1::SHS1-Cherry URA3* | This work |
| YAD1766 | Epo1-GFP Shs1-Cherry | JD47, *EPO1::EPO1-GFP TRP1 SHS1::SHS1-Cherry URA3* | This work |
| YAD966 | $P_{MET17}$-Hhf2-Cherry Spa2-GFP | JD47, *$P_{MET17}$-HHF2-Cherry LEU2 pRS315 SPA2::SPA2-GFP TRP1* | This work |
| YAD1199 | $P_{MET17}$-GFP-Sec62 Spa2-Cherry | JD47, *$P_{MET17}$-GFP-SEC62 HIS3 pRS313 SPA2::SPA2-Cherry LEU2* | This work |
| YAD1200 | $P_{MET17}$-GFP-Sec62 Spa2-Cherry Δepo1 | JD47, *$P_{MET17}$-GFP-SEC62 HIS3 pRS313 SPA2::SPA2-Cherry LEU2 EPO1::hphNT1* | This work |
| YAD1457 | $P_{MET17}$-Cherry $P_{MET17}$-GFP-Sec62 Δepo1 | JD47, *$P_{MET17}$-Cherry LEU2 pRS315 $P_{MET17}$-GFP-SEC62 HIS3 pRS313 EPO1::hphNT1* | This work |
| YAD1323 | $P_{MET17}$-Epo1-Cherry $P_{MET17}$-GFP-Sec62 Δepo1 | JD47, *$P_{MET17}$-EPO1-Cherry LEU2 pRS315 $P_{MET17}$-GFP-SEC62 HIS3 pRS313 EPO1::hphNT1* | This work |
| YAD1324 | $P_{MET17}$-Epo1$_{1–760}$-Cherry $P_{MET17}$-GFP-Sec62 Δepo1 | JD47, *$P_{MET17}$-EPO1$_{1–760}$-Cherry LEU2 pRS315 $P_{MET17}$-GFP-SEC62 HIS3 pRS313 EPO1::hphNT1* | This work |

Table S4. **List of yeast strains used and created in this study** *(Continued)*

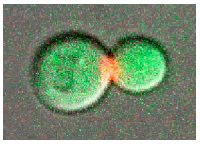
| Strain | Name | Relevant genotype | Origin |
|---|---|---|---|
| YAD1325 | $P_{MET17}$-Epo1$_{761–943}$-Cherry $P_{MET17}$-GFP-Sec62 Δepo1 | JD47, *$P_{MET17}$-EPO1$_{761–943}$-Cherry LEU2 pRS315 $P_{MET17}$-GFP-SEC62 HIS3 pRS313 EPO1::hphNT1* | This work |
| YAD1282 | Hmg1-GFP | JD47, *URA3::HMG1-GFP URA3* | This work |
| YAD1283 | Hmg1-GFP Δepo1 | JD47, *URA3::HMG1-GFP URA3 EPO1::hphNT1* | This work |
| YAD1449 | Hmg1-GFP Δscs2 | JD47, *URA3::HMG1-GFP URA3 SCS2::hphNT1* | This work |
| YAD1448 | Hmg1-GFP Δptc1 | JD53, *URA3::HMG1-GFP URA3 PTC1::kanMX6* | This work |
| YAD1547 | Hmg1-GFP Δbem3 | JD47, *URA3::HMG1-GFP URA3 BEM3::hphNT1* | This work |
| YAD1545 | Hmg1-GFP Δpea2 | JD47, *URA3::HMG1-GFP URA3 PEA2::hphNT1* | This work |
| YAD1546 | Hmg1-GFP Δspa2 | JD47, *URA3::HMG1-GFP URA3 SPA2::hphNT1* | This work |
| YAD1805 | Hmg1-GFP Δscs22 | JD47, *URA3::HMG1-GFP URA3 SCS22::hphNT1* | This work |
| YAD1770 | Hmg1-GFP Δylr031w | JD47, *URA3::HMG1-GFP URA3 YLR031W::hphNT1* | This work |
| YAD1798 | Hmg1-GFP Δbud6 | JD47, *URA3::HMG1-GFP URA3 BUD6::natNT2* | This work |
| YAD1797 | Hmg1-GFP Δbni1 | JD47, *URA3::HMG1-GFP URA3 BNI1::natNT2* | This work |
| YAD1451 | Hmg1-GFP Δepo1 Δscs2 | *URA3::HMG1-GFP URA3 EPO1::kanMX6 SCS2::hphNT1* | This work |
| YAD1453 | Hmg1-GFP Δepo1 Δptc1 | *URA3::HMG1-GFP URA3 PTC1::kanMX6 EPO1::hphNT1* | This work |
| YAD1548 | Hmg1-GFP Δepo1 Δpea2 | *URA3::HMG1-GFP URA3 EPO1::kanMX6 PEA2::hphNT1* | This work |
| YAD1549 | Hmg1-GFP Δepo1 Δspa2 | *URA3::HMG1-GFP URA3 EPO1::kanMX6 SPA2::hphNT1* | This work |
| YAD1551 | Hmg1-GFP Δepo1 Δbem3 | *URA3::HMG1-GFP URA3 EPO1::kanMX6 BEM3::hphNT1* | This work |
| YAD1771 | Hmg1-GFP Δepo1 Δylr031w | *URA3::HMG1-GFP URA3 EPO1::kanMX6 YLR031W::hphNT1* | This work |
| YAD1800 | Hmg1-GFP Δepo1 Δbud6 | *URA3::HMG1-GFP URA3 EPO1::hphNT1 BUD6::natNT2* | This work |
| YAD1799 | Hmg1-GFP Δepo1 Δbni1 | *URA3::HMG1-GFP URA3 EPO1::natNT2 BNI1::hphNT1* | This work |
| YAD1550 | Hmg1-GFP Δpea2 Δbem3 | *URA3::HMG1-GFP URA3 PEA2::kanMX6 BEM3::hphNT1* | This work |
| YAD512 | Kel1-MYC | JD47, *KEL1::KEL1-9×MYC TRP1* | This work |



Video 1. **Time-lapse analysis of yeast cells coexpressing Spa2-Cherry with Epo1-GFP.** Cells were incubated at 30°C on SD complete. Frames were taken every 3 min for 120 min using a DeltaVision life cell imaging unit (Applied Precision). Selected stills are shown in Fig. 2 B.



Video 2. **Time-lapse analysis of yeast cells coexpressing Shs1-Cherry with Spa2-GFP.** Cells were incubated at 30°C on SD complete. Frames were taken every minute for 85 min using a DeltaVision life cell imaging unit. Selected stills are shown in Fig. 2 C.



Video 3. **Time-lapse analysis of yeast cells coexpressing Shs1-Cherry with Epo1-GFP.** Cells were incubated at 30°C on SD complete. Frames were taken every minute for 65 min using a DeltaVision life cell imaging unit. Selected stills are shown in Fig. 2 C.

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