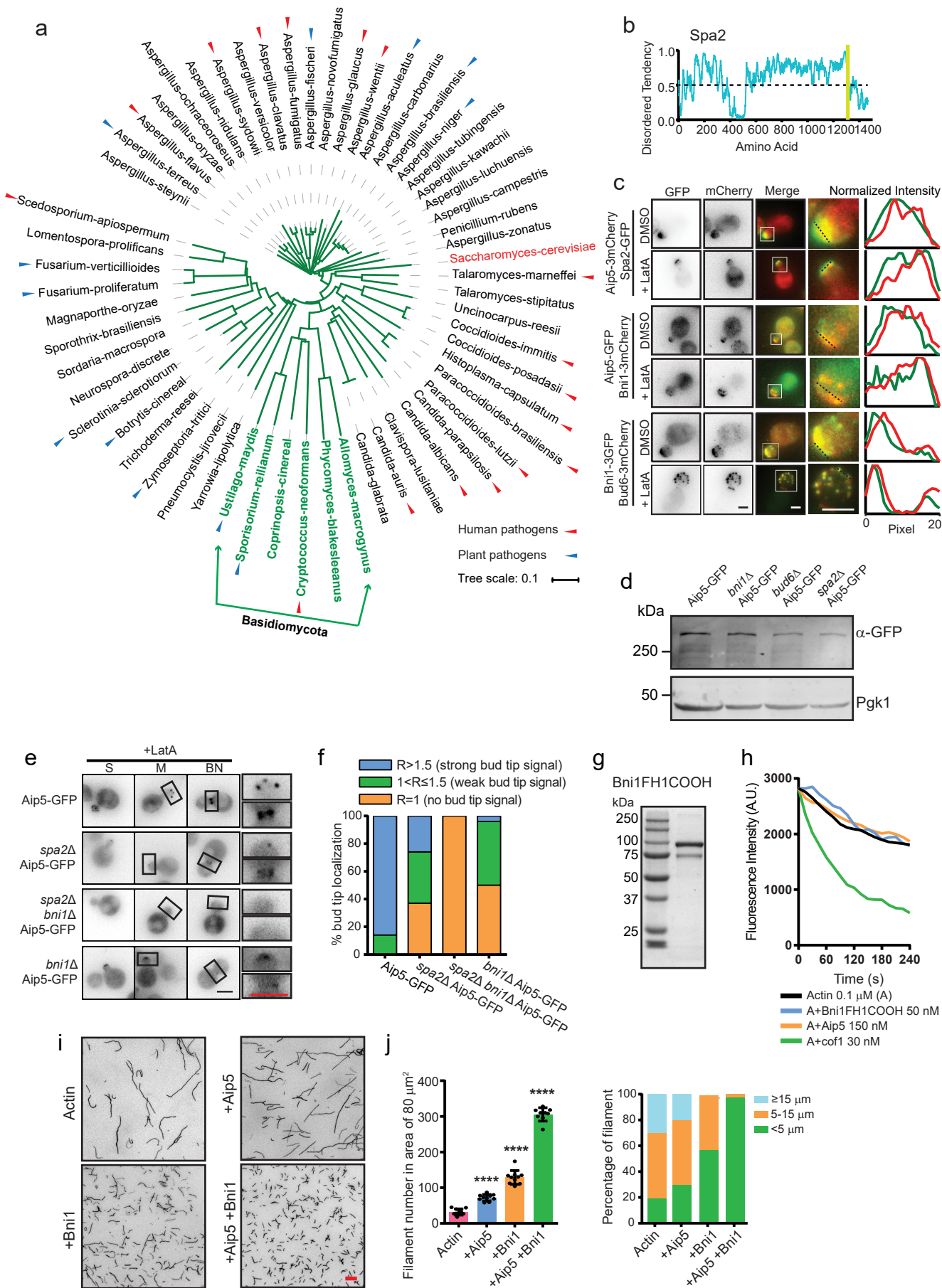


## **Supplementary Information**

### **Polarisome scaffold Spa2-mediated macromolecular condensation of Aip5 for actin polymerization**

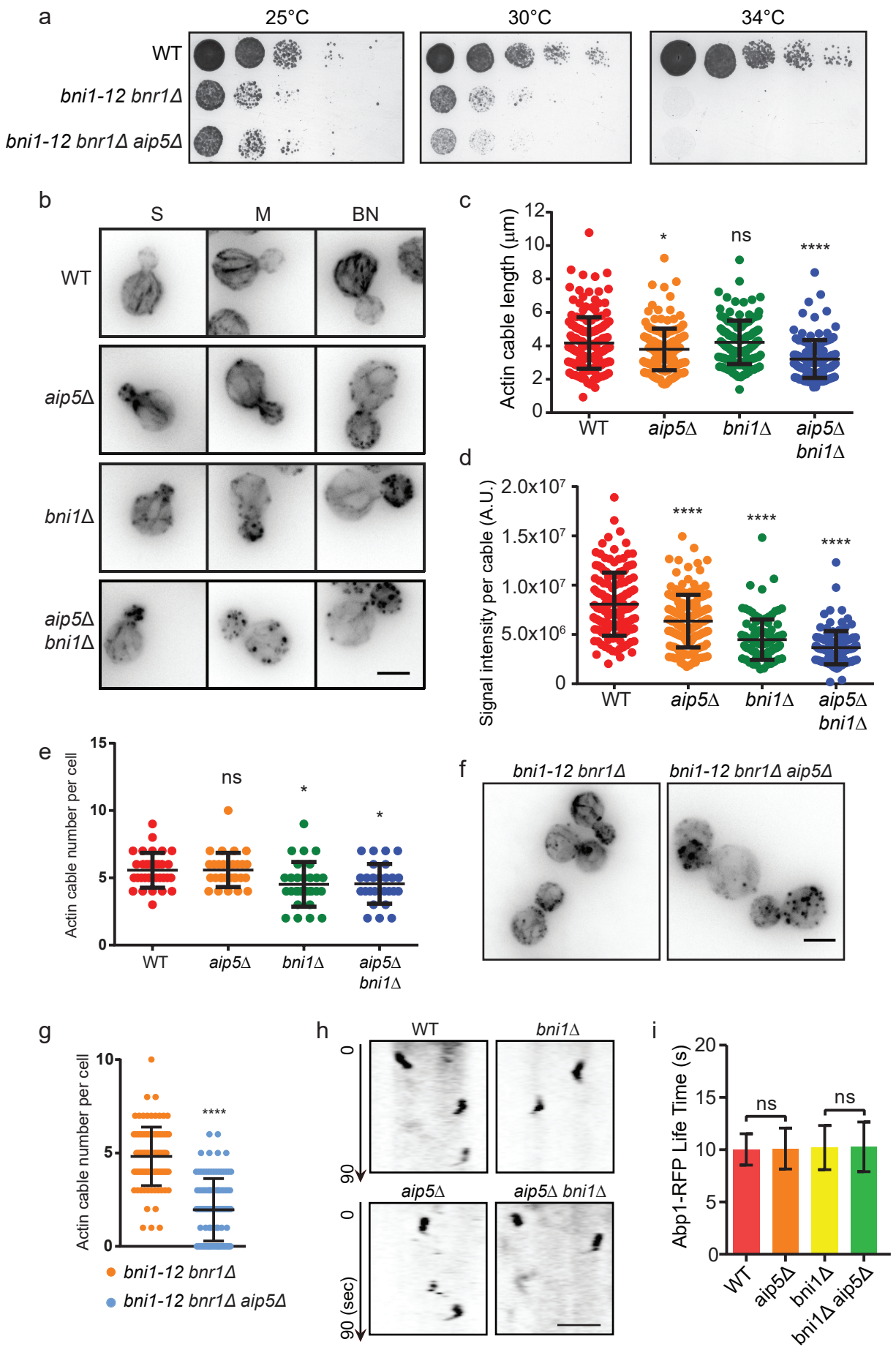
*(Ying et al.,)*





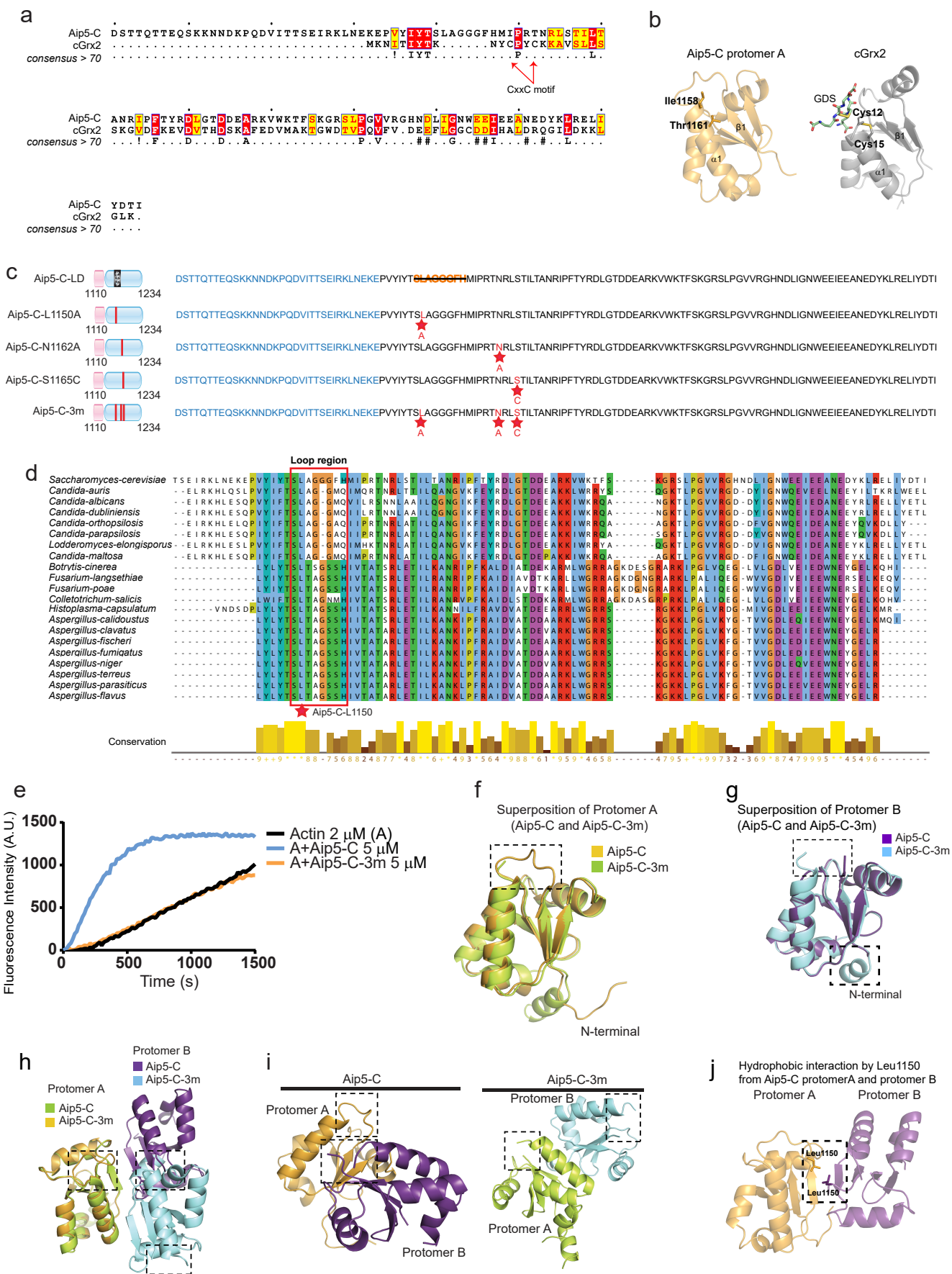
**Supplementary figure 1. Aip5 protein analysis and *in vivo* localization.**

**(a)** Aip5 protein sequence conservation analysis among species, the highlighted species in green color belongs to phylum Basidiomycota, the rest of the species belongs to phylum Ascomycota. **(b)** Predicted intrinsic disorder profile of Spa2 by ANCHOR. **(c)** Representative maximum Z-projection images of fluorescent fusions of polarisome proteins at the bud tip, in the absence or presence of 10  $\mu$ M LatA for 30 min. Lines crossing 20 pixels were drawn at the bud tip in determining the colocalized fluorescence signals of GFP and mCherry. Scale bars represent 2  $\mu$ m. (S, small-budded cell; M, medium size-budded cell; BN, bud neck.) **(d)** Western blot detection of endogenously expressed Aip5-GFP protein under a native promoter in WT and polarisome mutants. (Pgk1 was used as a loading control.) **(e)** Representative maximum Z-projection fluorescence images of Aip5-GFP at the bud tip of WT and polarisome protein mutants, with a 10  $\mu$ M LatA treatment for 30 min before imaging. Scale bars represent 3  $\mu$ m. **(f)** Relative ratio (R) quantification of the signal intensity of Aip5-GFP at medium-sized bud tip in the conditions of (e). Aip5-GFP, n=49 cells; *spa2* $\Delta$  Aip5-GFP, n=35 cells; *spa2* $\Delta$  *bni1* $\Delta$  Aip5-GFP, n=19 cells; *bni1* $\Delta$  Aip5-GFP, n=26 cells. **(g)** SDS-PAGE of purified Bni1FH1COOH. **(h)** Time course of pyrene actin de-polymerization in the presence of full-length Aip5, Bni1FH1COOH, and Cof1, at the indicated protein concentration. **(i)** Representative fluorescence images of Acti-stain 488 phalloidin labeled-actin filaments that are polymerized from 2  $\mu$ M actin, with the addition of 2  $\mu$ M Aip5 for 30 min in the presence or absence of 0.5  $\mu$ M Bni1FH1COOH. Scale bar represents 5  $\mu$ m. **(j)** Quantification of the number and length of the actin filament in an area size of 80  $\mu$ m<sup>2</sup>. (control, 318 filaments from 10 fields; +Aip5, 389 filaments from 10 fields; +Bni1FH1COOH, 438 filaments from 10 fields; +Aip5+Bni1FH1COOH, 328 filaments from 9 fields) Source data are provided as a Source Data file.



**Supplementary figure 2. Aip5 and Bni1 regulate *in vivo* actin cable assembly.**

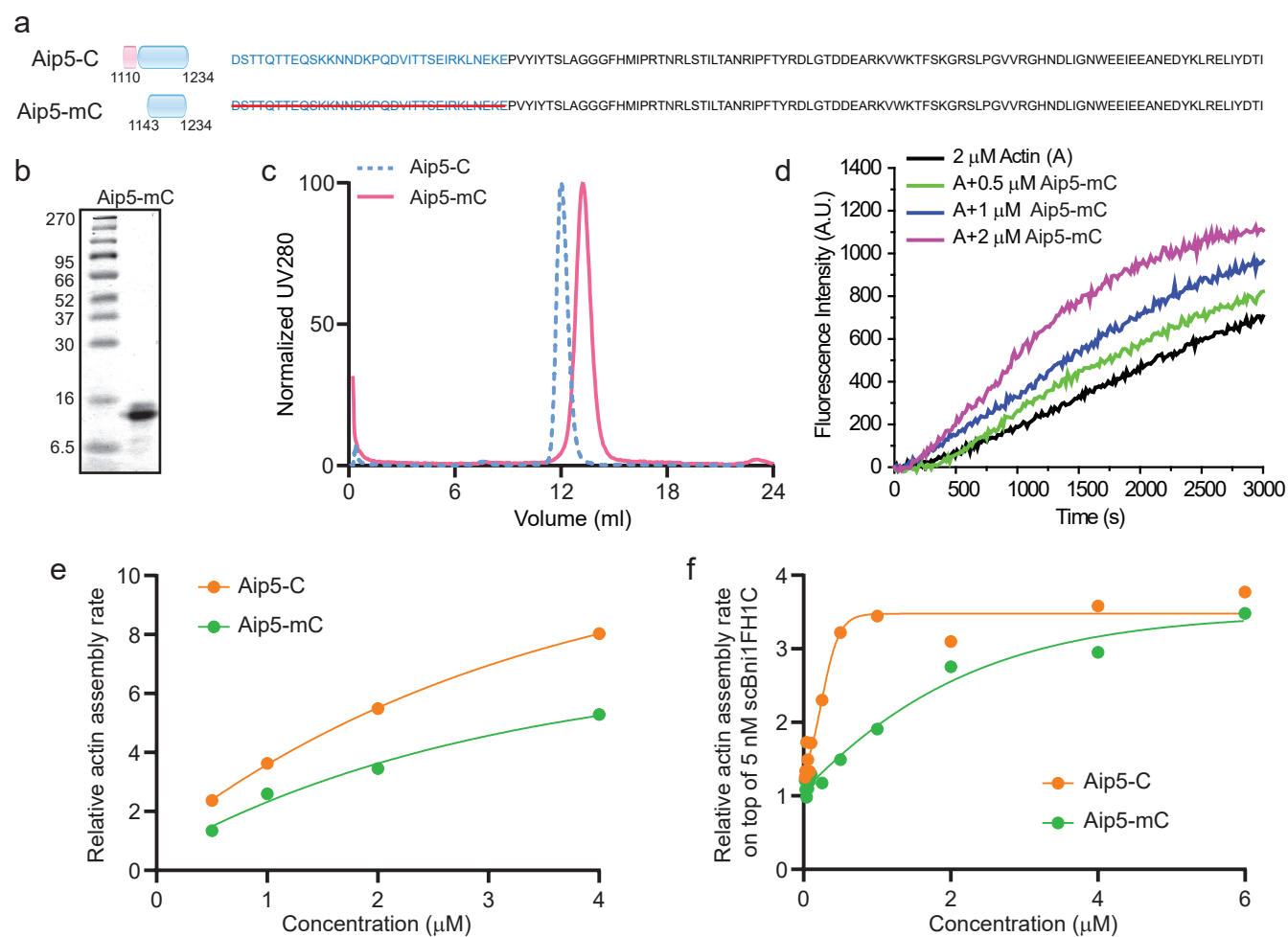
**(a)** Genetic interaction of *AIP5* with *BNI1* was shown by spotting assay at the indicated temperatures after 36 hours growth. **(b)** Representative maximum Z-projection fluorescence images of Abp140-3GFP marked actin cables of the indicated strains. S, small-budded cell; M, medium size-budded cell; BN, bud neck. **(c, d)** Quantification of the length and total fluorescent intensity of Abp140-3GFP labelled actin cables (length: n=178 actin cable for wild type (WT); n=162 actin cable for *aip5* $\Delta$ ; n=131 actin cable for *bni1* $\Delta$ ; n=123 actin cable for *aip5* $\Delta$  *bni1* $\Delta$ . For actin cable intensity: n=149 actin cable for WT; n=153 actin cable for *aip5* $\Delta$ ; n=106 actin cable for *bni1* $\Delta$ ; n=112 actin cable for *aip5* $\Delta$  *bni1* $\Delta$ .) **(e)** Quantification of actin cable number labeled by Abp140-3GFP, as in (b). (WT, n=32 cells; *aip5* $\Delta$ , n=29 cells; *bni1* $\Delta$ , n=29 cells; *aip5* $\Delta$  *bni1* $\Delta$ , n=27 cells.) **(f)** Representative maximum Z-projection fluorescence images of Abp140-3GFP marked actin cables of the indicated strains at 25°C. **(g)** Quantification of detectable of actin cable, as in (f). (*bni1-12 bnr1* $\Delta$ , n=93 cells; *bni1-12 bnr1* $\Delta$  *aip5* $\Delta$ , n=102 cells) **(h)** Kymograph of Abp1-RFP lifetime from a movie of 90 seconds (1 frame/s), in the indicated strains. **(i)** Quantification of the Abp1-RFP lifetime, as in (h). (n=75 patches for each strain). All scale bars represent 3  $\mu$ m. P-values in (c), (d), and (e) were determined by the one-way ANOVA, P-value in (g) was determined by the two-tailed Student's *t*-test assuming equal variances, \*\*\*\*p < 0.0001; \*p < 0.05; ns = not significant. All the graphs represent mean  $\pm$  S.D. Source data are provided as a Source Data file.



**Supplementary figure 3. The homologous analysis of Aip5-C and Aip5-C mutant structure.**

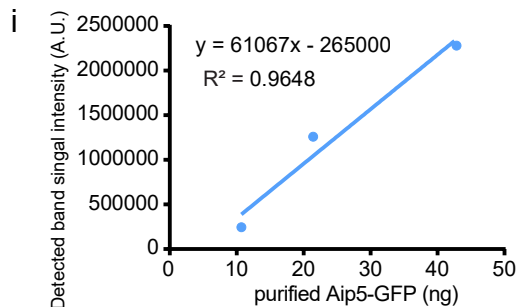
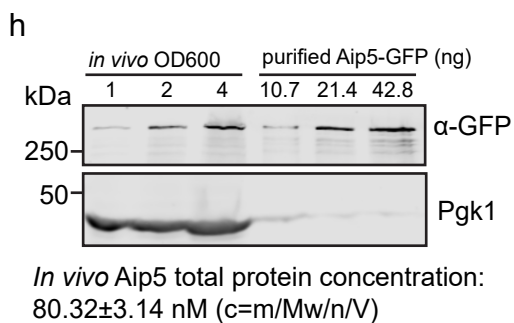
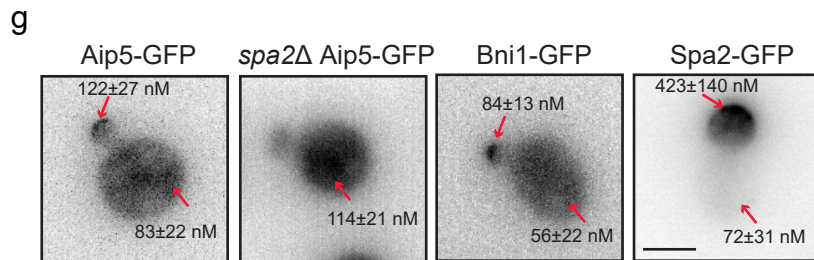
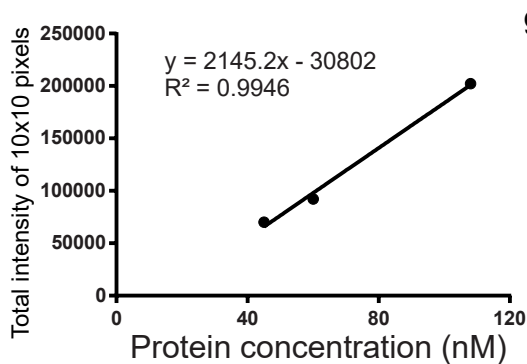
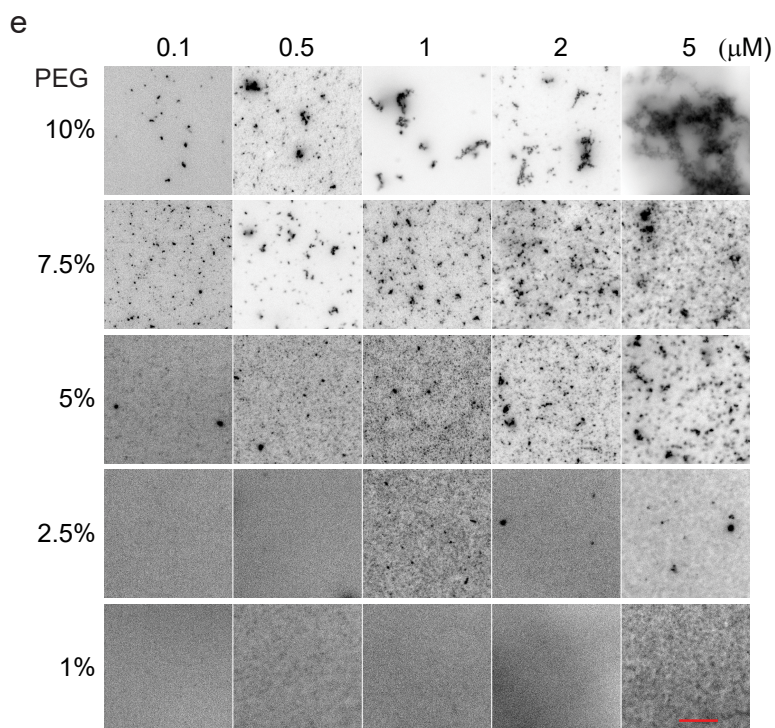
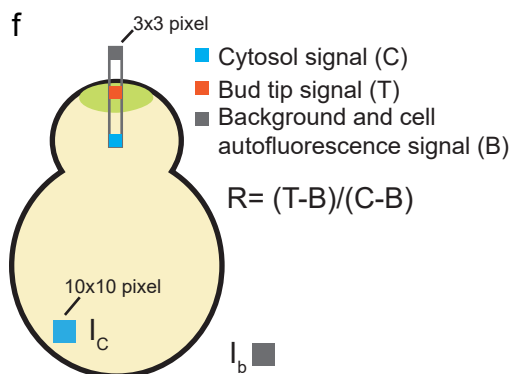
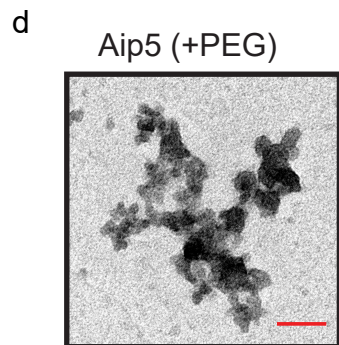
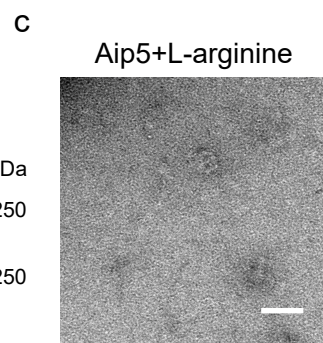
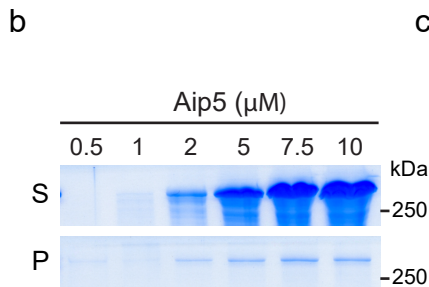
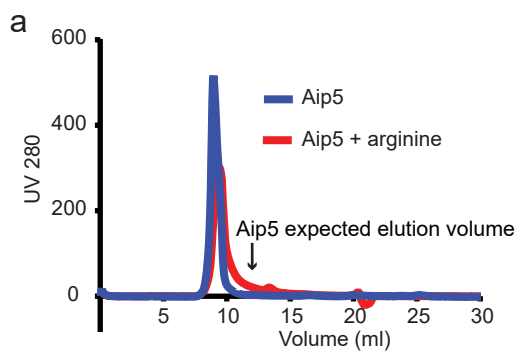
**(a)** Sequence alignment of Aip5-C with the structural homolog cGrx2, in which the CxxC motif for redox activity is absent in Aip5. **(b)** Structural comparison of Aip5-C with cGrx2 at the corresponding CxxC motif. **(c)** Domain schematics of Aip5-C mutation variants and the amino acid sequences. The deleted loop region and the sites of single mutations were highlighted. **(d)** Sequence alignment of Aip5-C among pathogenic filamentous fungus, illustrated by Jalview using the Clustal Omega. **(e)** Pyrene actin polymerization reaction with 5  $\mu$ M Aip5-C or Aip5-C-3m. **(f)** Superposition of protomer A of Aip5-C and that of Aip5-C-3m. **(g)** Superposition of protomer B from Aip5-C and Aip5-C-3m. **(h)** Superposition of Aip5-C and Aip5-C-3m. A significant conformational change was observed upon mutations. Loop region that consists of L1150A mutation is highlighted in the black squared box. **(i)** The orientation of the loop region in Aip5-C and Aip5-C-3m. **(j)** Leu1150 in the loop connecting  $\beta$ 1 and  $\alpha$ 1 in protomer A of Aip5-C forms hydrophobic interaction with Leu1150 from protomer B, stabilizing the dimer formation. Such establishment disappeared in Aip5-C-3m due to its conformational change upon mutations.





**Supplementary figure 4. Monomeric Aip5-mC activities in actin polymerization.**

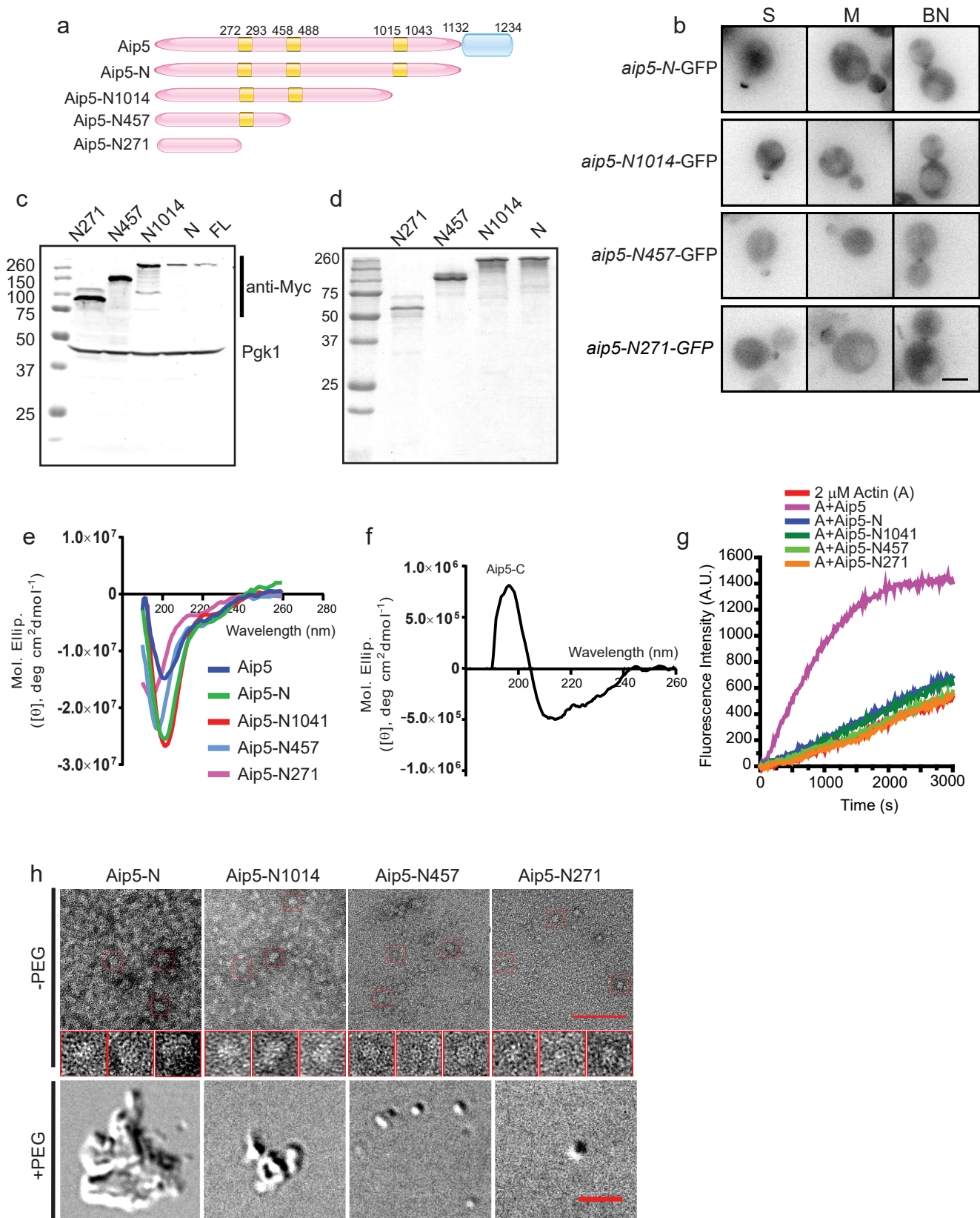
**(a)** Domain schematics of dimer Aip5-C and monomeric version Aip5-mC in budding yeast, where the 33 residues at the N terminus were truncated and resulted in a monomer form of Aip5-C. **(b)** SDS-PAGE of purified monomeric Aip5-C protein in budding yeast (Aip5-mC). **(c)** The elution profile of Aip5-C and Aip5-mC proteins from calibration column Superdex 75 10/300 GL. **(d)** Pyrene assay with increasing concentrations of Aip5-mC. **(e)** Relative actin assembly rate of Aip5-C and Aip5-mC at the indicated concentrations by normalizing to the control of 2  $\mu$ M actin. **(f)** Relative actin assembly rate of Aip5-C and Aip5-mC at the indicated concentrations by normalizing to 5 nM Bni1FH1COOH. The plots in (e) and (f), respectively, show the mean value from two biological replicates. Source data are provided as a Source Data file.





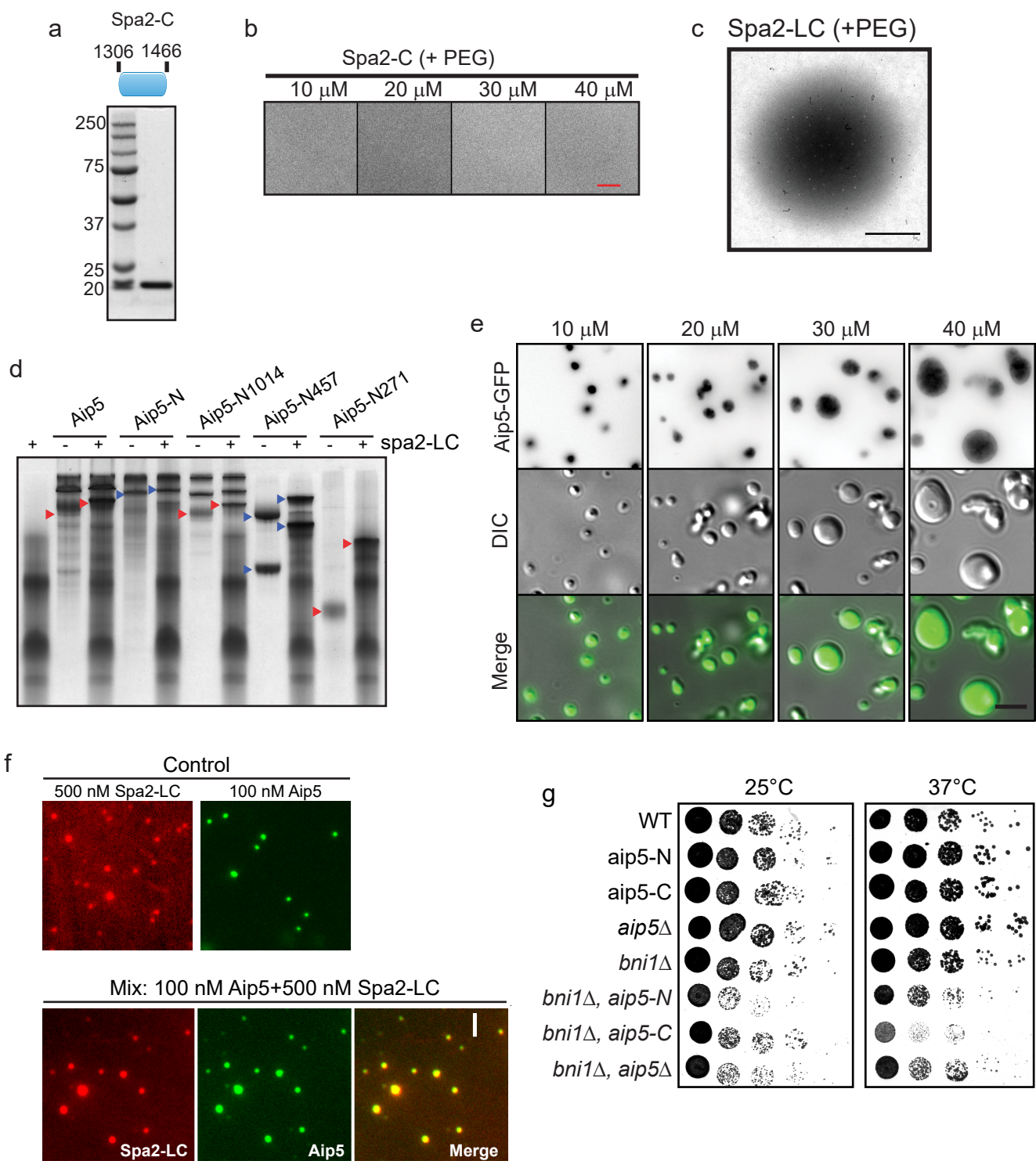
**Supplementary figure 5. *In vitro* molecular assembly of Aip5 and *in vivo* Aip5 concentration.**

**(a)** The elution profile of Aip5 from superdex 200 increase calibration column, with or without a pre-incubation with 500 nM of L-arginine for 15 min, where the Aip5 predicted dimer elution volume was indicated. **(b)** Soluble Aip5 protein, at a concentration in a range from 0.5 to 10  $\mu$ M, from high-speed centrifugation for 30 min at 100,000g before subjecting to SDS-PAGE gel and staining. S: supernatant protein fraction; P: pellet protein fraction. **(c)** Representative TEM images of *in vitro* 1  $\mu$ M full-length Aip5 incubating with 500 nM L-arginine on ice for 15 min before loading onto the grid. Scale bar represents 100 nm. **(d)** Representative TEM images of Aip5 assemblies *in vitro* at 1  $\mu$ M formed at 5 min after incubating with 10% PEG3,350. The scale bar represents 100 nm. **(e)** Representative fluorescence images of Aip5 assemblies (10% Aip5-GFP) formed *in vitro* at the indicated concentrations, which were incubated with the indicated concentrations of PEG 3,350 for 5 min before imaging. The scale bar represents 5  $\mu$ m. **(f)** *In vivo* concentration of polarisome proteins using signal intensity measurements, which were compared with reference proteins (see Methods). The signal intensities of the cytosol ( $I_c$ ), background and auto-fluorescence intensity from cells ( $I_b$ ), and bud tip (T) of medium size-budded cells were measured. The protein concentrations standard curve was generated. **(g)** The calculated polarisome proteins concentrations at bud tip and cytosol regions were indicated. The scale bar represents 3  $\mu$ m. **(h)** Western blot detection of endogenously expressed Aip5-GFP protein under a native promoter in wild-type yeast and the purified recombinant protein Aip5-GFP. Pgk1 was used as a loading control. **(i)** The standard curve was generated by plotting Aip5-GFP protein mass against the protein signal intensity from western-blot, where the calculated total Aip5-GFP protein concentration *in vivo* was  $80.32 \pm 3.14$  nM. The value was mean and S.D calculated from three independent loading samples. Source data are provided as a Source Data file.



**Supplementary figure 6. N-terminal IDR determines the molecular condensation of Aip5.**

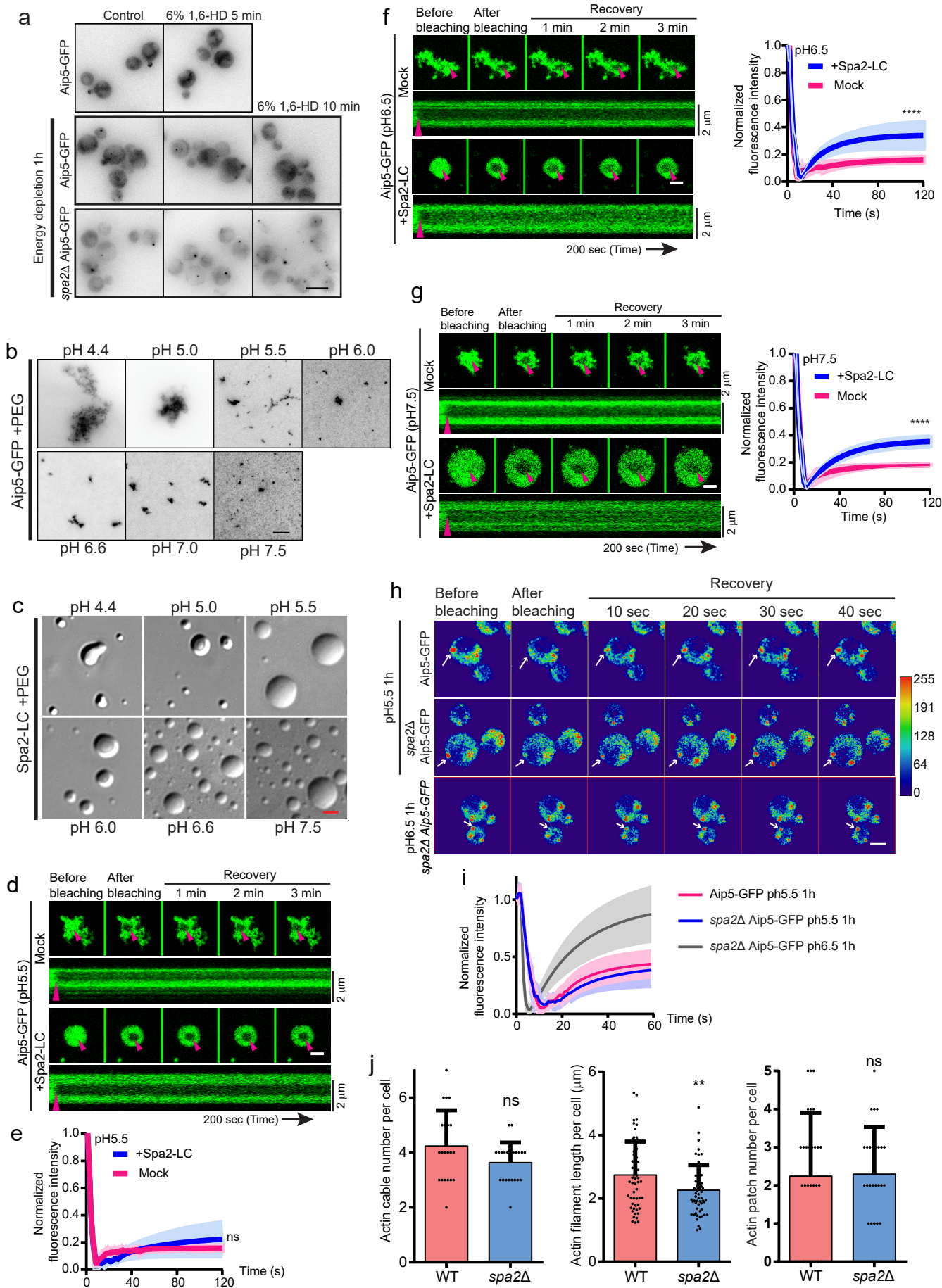
**(a)** Domain schematics of Aip5. The Aip5 N terminal truncation proteins were designed based on the predicted coiled-coil domains using Pfam. **(b)** Representative maximum Z-projection images of endogenous GFP tagged Aip5 N terminal truncation proteins localization in yeast. S, small-budded cell; M, medium-size budded cell; BN, bud neck. Scale bar represents 3  $\mu\text{m}$ . **(c)** Endogenously expressed Aip5 variants with the C-terminal Myc-tags that were detected by anti-Myc antibodies. (Pgk1 was used as the loading control.) **(d)** The SDS-PAGE gel of purified Aip5-N variants. **(e, f)** Circular dichroism spectra of Aip5 truncating variants at a concentration of 0.5 mg/ml. **(g)** Spontaneous actin polymerization by 2  $\mu\text{M}$  Aip5 N-terminal truncations. **(h)** Representative TEM images of *in vitro*-formed condensates of Aip5 variants under non-PEG environments (upper panel). Insets showed an enlarged view of Aip5 oligomers. Differential interference contrast (DIC) images showed the protein condensates formed under 10% PEG 3,350 at 5 min (2  $\mu\text{M}$ , lower panel). The scale bars represent 100 nm and 3  $\mu\text{m}$ , respectively, for upper and lower panel.



**Supplementary figure 7. Liquid-liquid phase separation of Spa2 relies on its IDR region.**

**(a)** Domain schematic and SDS-PAGE gel of recombinant Spa2-C protein. **(b)** Representative DIC images of *in vitro* formation of Spa2-C assemblies in the presence of 10% PEG3,350 after 5 min of incubation at room temperature. Scale bar: 5  $\mu$ m. **(c)** Representative TEM images of *in vitro*-formed Spa2-LC droplet (40  $\mu$ M), which was induced by 10% PEG3,350 after 5 min of incubation at room temperature. Scale bar, 500 nm. **(d)** Native-PAGE gel of different protein combinations as indicated, where 4  $\mu$ g of Spa2-LC was incubated with 2  $\mu$ g of truncating Aip5 variants at room temperature for 30 min prior to electrophoresis. Arrows indicated the shifted bands of Aip5 variants in the presence of Spa2-LC. Native gel marker was not used. **(e)** Representative fluorescence images of *in vitro* formed droplets of Aip5 (500 nM, 10% Aip5-GFP) by mixing with the indicated concentrations of Spa2-LC for 5 min in 10% PEG3,350. The scale bar represents 5  $\mu$ m. **(f)** Representative fluorescence images of 100 nM Aip5 (10% Aip5-GFP) and 500 nM Spa2-LC (10% Spa2-LC labelled by dye alexa568) formed protein assemblies under 20% peg10,000 at room temperature for 5 min. The scale bar represents 2  $\mu$ m. **(g)** Yeast growth in spotting assay after 36 hours of incubation at 25°C and 37°C, respectively.

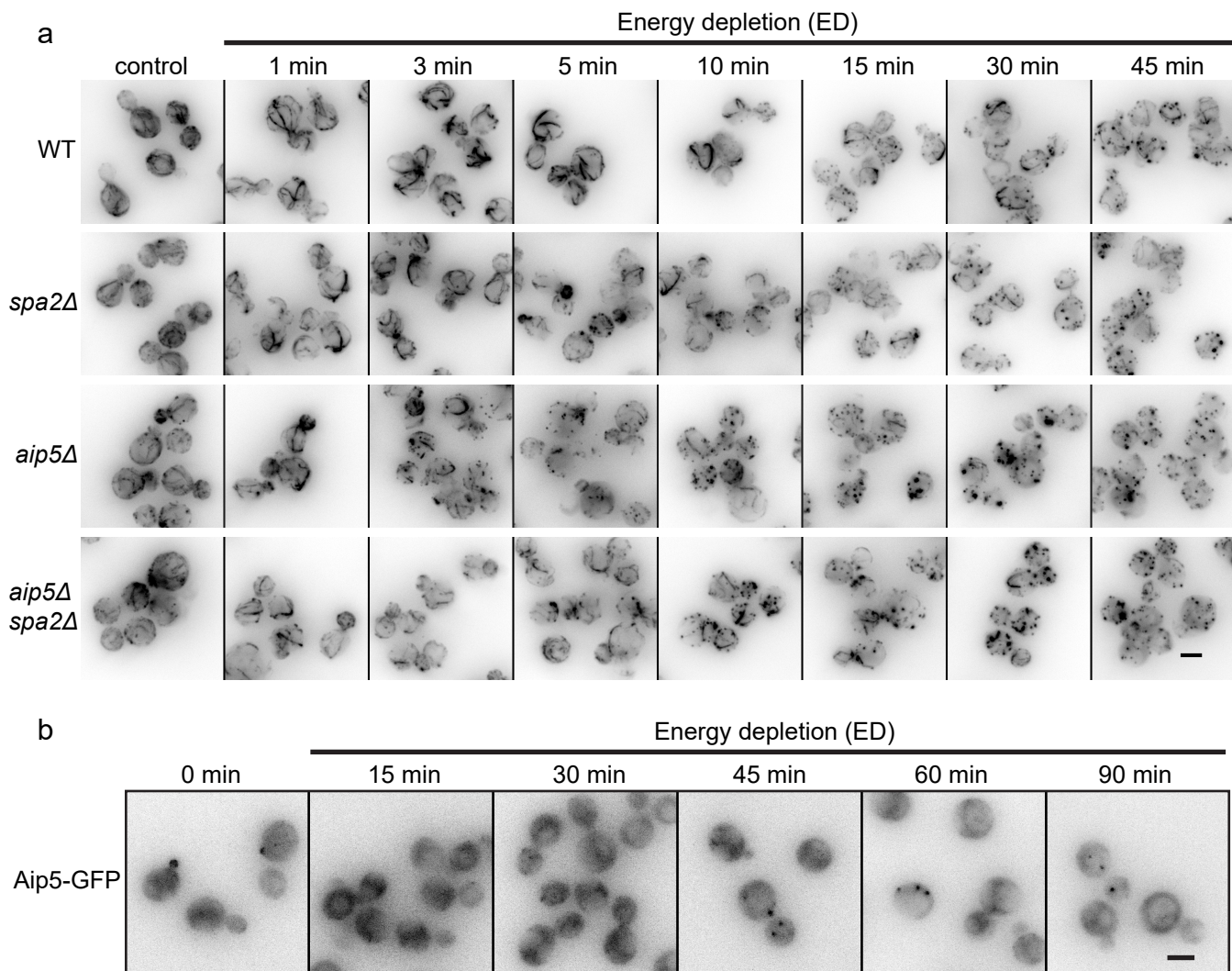




**Supplementary figure 8. Spa2-mediated Aip5 condensates *in vivo* under stressed conditions.**

**(a)** Representative fluorescence images of Aip5-GFP signal in normal growth or 1-hour energy depletion condition treated by 6% 1, 6 hexanediol for 5 and 10 min. The scale bar represents 5  $\mu$ m. **(b)** Representative fluorescence images of *in vitro* Aip5 (2  $\mu$ M, 10% Aip5-GFP) assemblies that were formed in the indicated pH condition with 10% PEG3,350 for 5 min before imaging. The scale bar represents 5  $\mu$ m. **(c)** Representative DIC images of *in vitro* Spa2-LC (30  $\mu$ M) droplets formed in the indicated pH condition with 10% PEG3,350 for 5 min before imaging. The scale bar represents 2  $\mu$ m. **(d-g)** Fluorescence recovery after photobleaching (FRAP) assay for *in vitro* Aip5 assemblies, in the absence or presence of Spa2-LC in three pH buffer (5.5, 6.5 and 7.5). ROIs used for generating kymographs were indicated by the arrowhead. 500 nM Aip5 (10% Aip5-GFP) was mixed with or without 30  $\mu$ M Spa2-LC in the presence of 10% PEG 3,350. The scale bars represent 2  $\mu$ m. Quantification of fluorescent signals of ROIs in FRAP experiments: n=7 for Aip5-GFP pH5.5; n=8 for Aip5-GFP+Spa2-LC pH5.5; n=8 for Aip5-GFP pH6.5; n=9 for Aip5-GFP+Spa2-LC pH6.5; n=8 for Aip5-GFP pH7.5; n=9 for Aip5-GFP+Spa2-LC pH7.5. **(h)** *In vivo* FRAP analysis of Aip5 condensates in wild-type and *spa2* $\Delta$  cells that were stressed by different pH buffer for 1-hour. Representative deconvoluted-images of Aip5 condensates, indicated by the white arrow, were shown. The colour key indicates signal intensity. The scale bar represents 5  $\mu$ m. **(i)** Quantification of fluorescent signals of FRAP experiments, as in (h). n=12 ROIs for Aip5-GFP pH5.5 treatment; n=13 for *spa2* $\Delta$  Aip5-GFP pH5.5 treatment; n=15 for *spa2* $\Delta$  Aip5-GFP pH5.5 treatment. **(j)** Quantification of the Abp140-3GFP marked actin cables and actin patches under normal growth condition in Fig. 6b. Actin cable number: n=21 cells for WT; n=22 cells for *spa2* $\Delta$ ; Actin filament length: n=57 cells for WT and *spa2* $\Delta$ ; Actin patch number: n=29 cells for WT and n=27 cells for *spa2* $\Delta$ . P-values were determined by the two-tailed Student's *t*-test assuming equal variances, \*\*\*\*p < 0.0001, \*\*p < 0.01. ns=not significant. All the bar graphs represent mean with an error bar, S.D. Source data are provided as a Source Data file.





**Supplementary figure 9. Time course phenotype of actin cable and Aip5 condensates under energy depletion condition.**

**(a)** Representative fluorescence images of actin cable phenotypes revealed by Abp140-3GFP in the indicated yeast strains at different time points with energy depletion treatment (SD medium without glucose and supplement with 20 mM 2-Deoxy-D-glucose, 10  $\mu$ M antimycin A). **(b)** Representative fluorescence images of Aip5-GFP aggregates formation in wild type at the indicated time points under energy depletion treatment. WT: wild type. Both scale bars represent 3  $\mu$ m.

**Supplementary figure 10.**

Western blot and staining gel images displayed in the main and other supplementary figures. Red boxes indicate areas used for the figures.

Fig. 1c

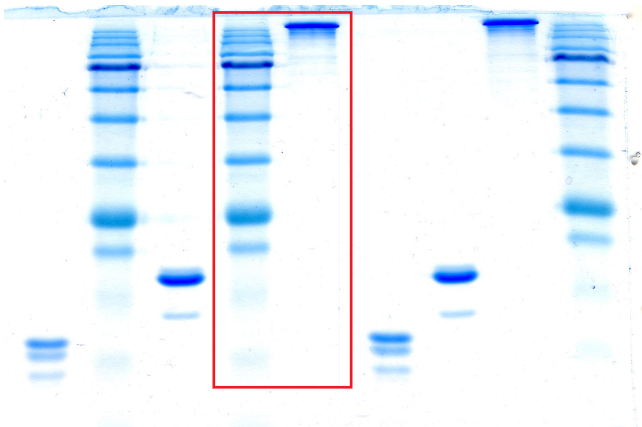


Fig. 2c

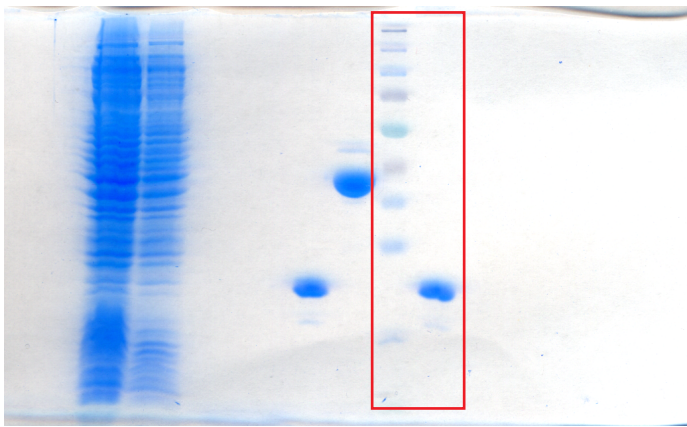


Fig. 2k

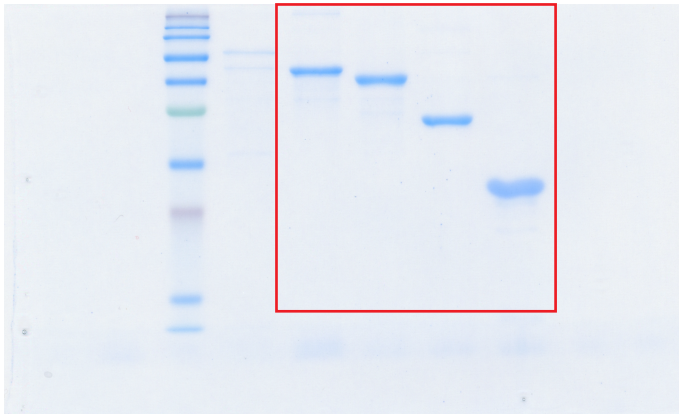
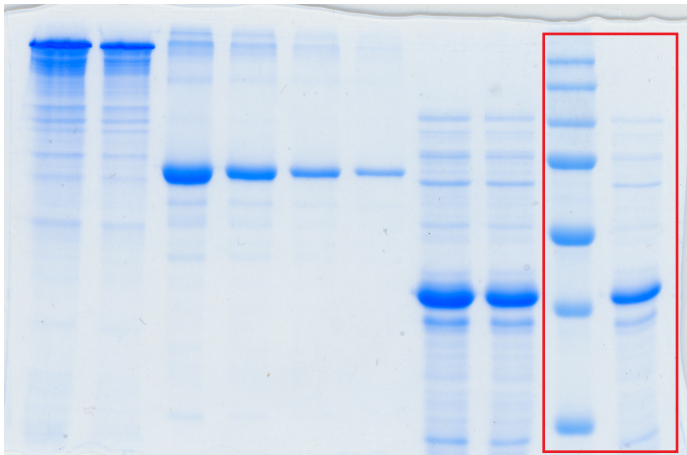
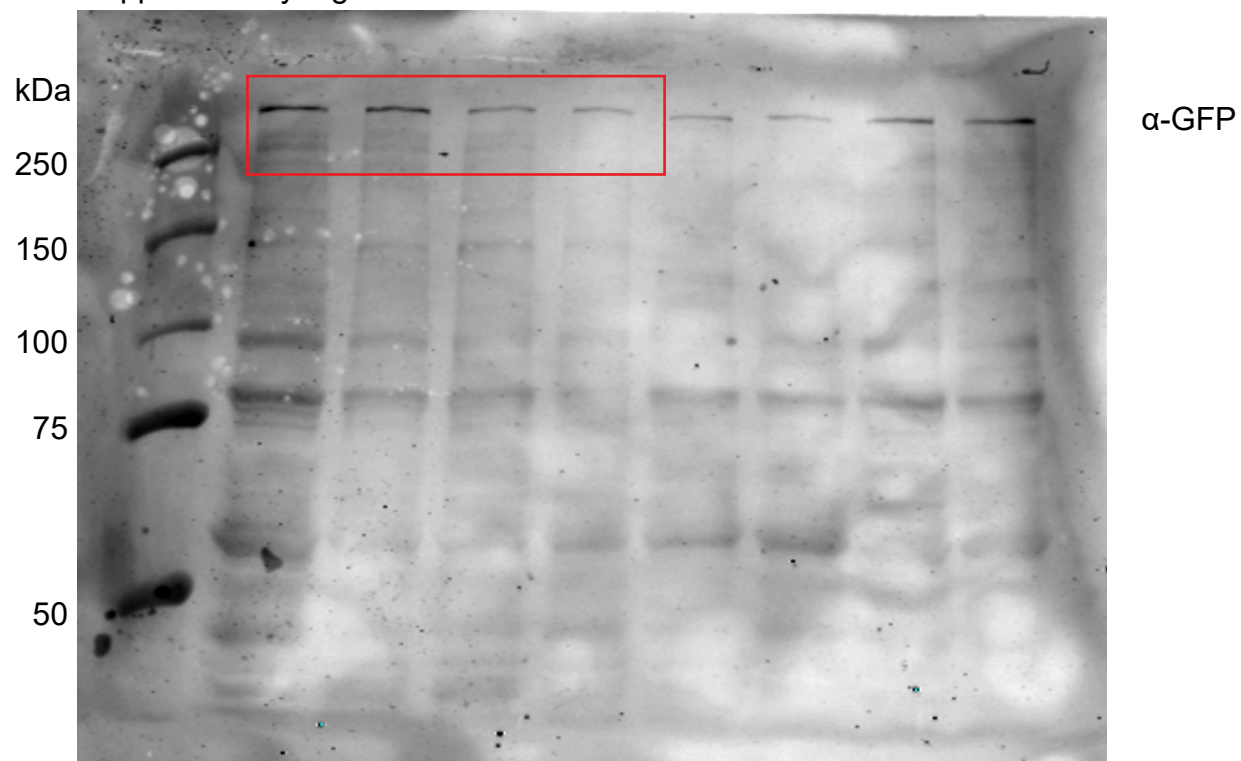


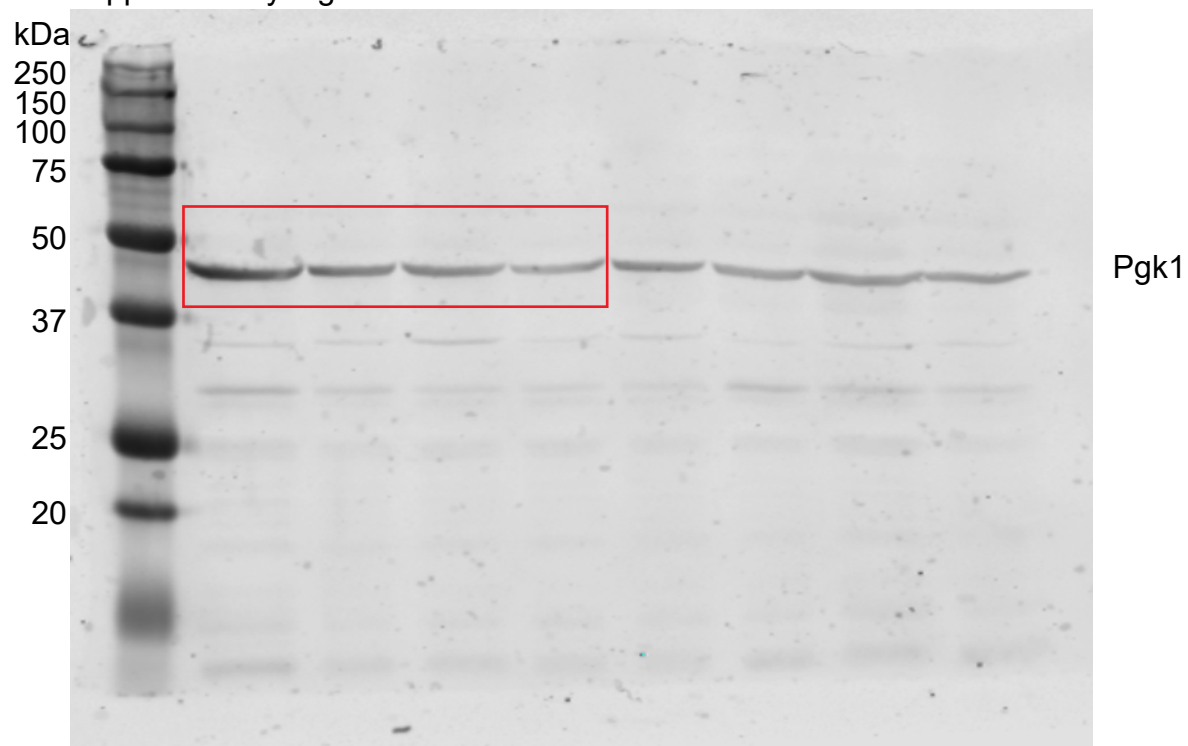
Fig. 4g



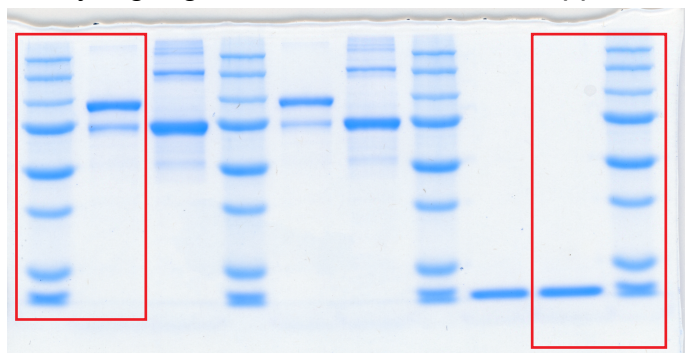
Supplementary Fig. 1d



Supplementary Fig. 1d



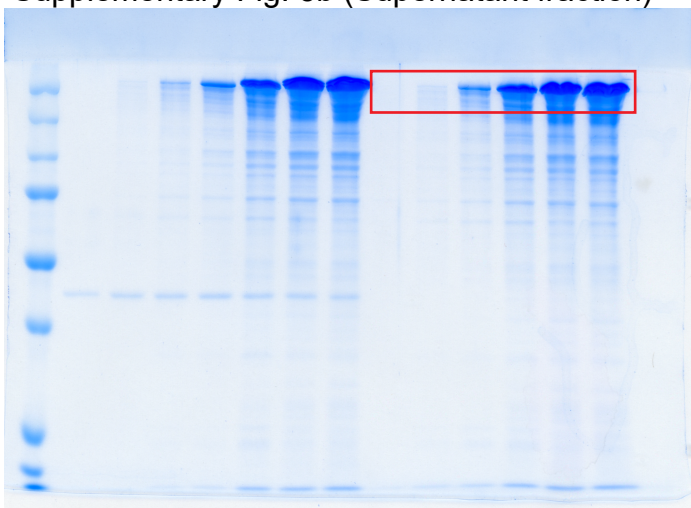
Supplementary Fig. 1g



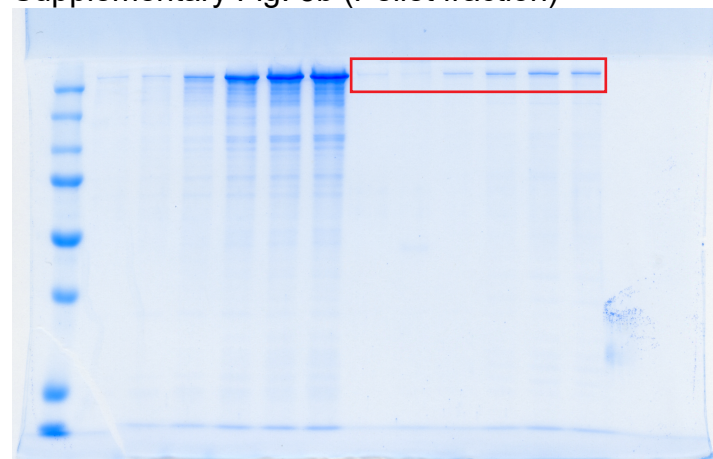
Supplementary Fig. 7a



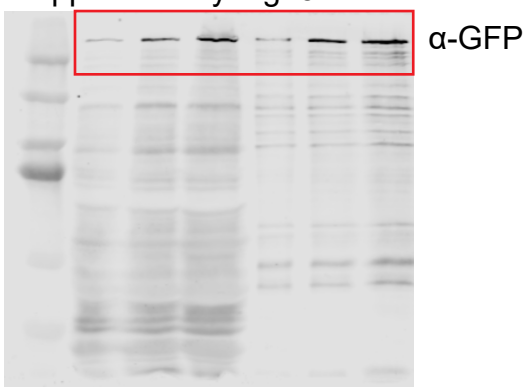
Supplementary Fig. 5b (Supernatant fraction)



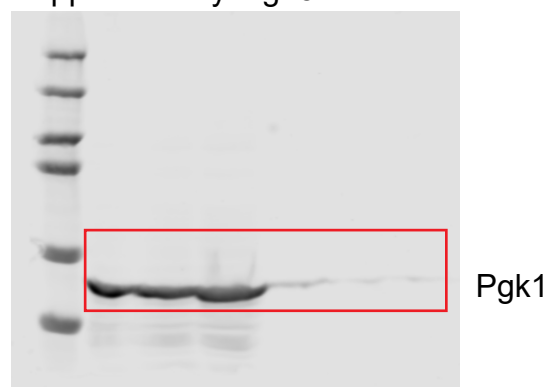
Supplementary Fig. 5b (Pellet fraction)



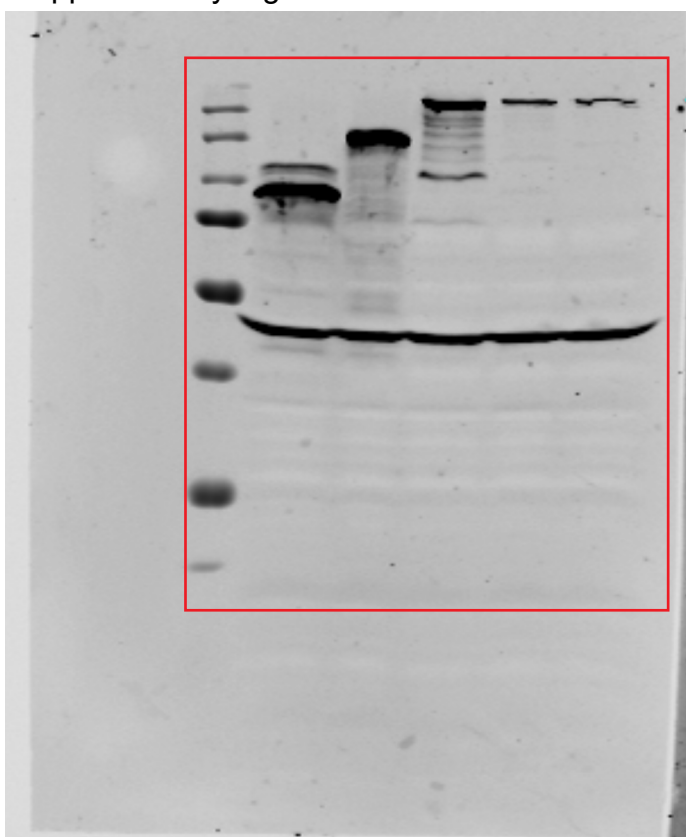
Supplementary Fig. 5h



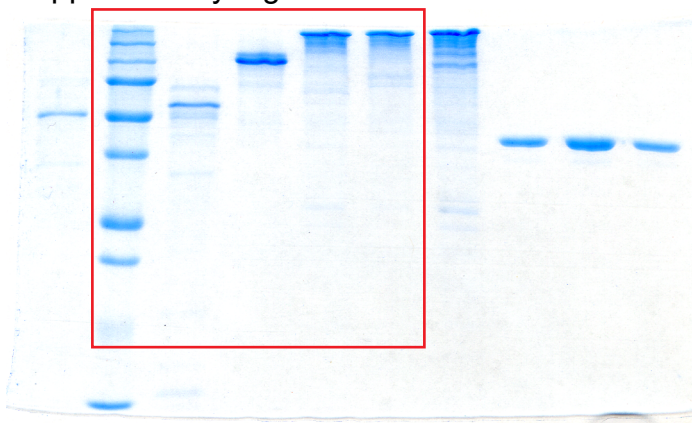
Supplementary Fig. 5h



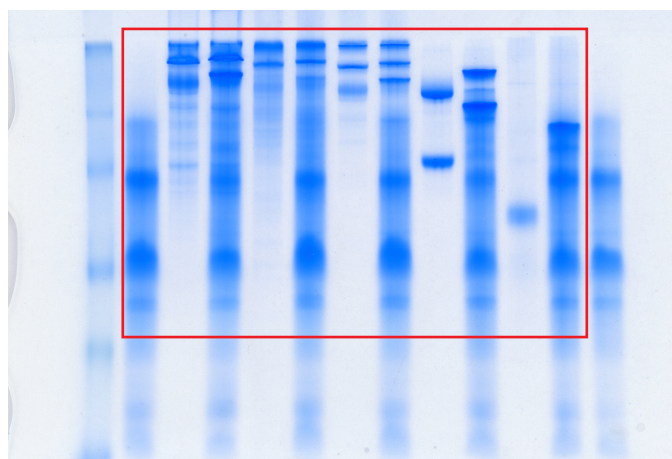
Supplementary Fig. 6c



Supplementary Fig. 6d



Supplementary Fig. 7d



Supplementary Table 1. Yeast strains used in the study.

| Strain name | Genotype                                                                                                                                                              | Source     |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| YMY2011     | MAT $\alpha$ /MAT $\alpha$ <i>his3-<math>\Delta</math>200/his3-<math>\Delta</math>200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 lys2-801/LYS2 ade2-1/ADE2</i>             | 1          |
| YMY2012     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52</i>                                                                                                  | 1          |
| YMY2013     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Aip5-GFP::KanMX</i>                                                                                 | This study |
| YMY2014     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, spa2<math>\Delta</math>::Leu2, Aip5-GFP::KanMX</i>                                                  | This study |
| YMY2015     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, bni1<math>\Delta</math>::Leu2, Aip5-GFP::KanMX</i>                                                  | This study |
| YMY2016     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, bud6<math>\Delta</math>::Ura3, Aip5-GFP::KanMX</i>                                                  | This study |
| YMY2017     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, spa2<math>\Delta</math>::Leu2, bni1<math>\Delta</math>::His3, Aip5-GFP::KanMX</i>                   | This study |
| YMY2018     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Spa2-GFP::His3</i>                                                                                  | This study |
| YMY2019     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Spa2<math>\Delta</math>C-GFP::KanMX4</i>                                                            | This study |
| YMY2020     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Spa2<math>\Delta</math>C-GFP::KanMX4, Aip5-3mCherry::URA3</i>                                       | This study |
| YMY2021     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Spa2-GFP::His3, Aip5-3mCherry::URA3</i>                                                             | This study |
| YMY2022     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Aip5-GFP::KanMX, Bni1-3mCherry::His3</i>                                                            | This study |
| YMY2023     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Aip5-GFP::KanMX, Bud6-3mCherry::His3</i>                                                            | This study |
| YMY2024     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Bni1-3GFP::His3 Bud6-3mCherry::His3 cdk1-as1::NAT</i>                                               | This study |
| YMY2025     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Abp1-RFP::His3 Abp140-3GFP::His3</i>                                                                | 1          |
| YMY2026     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5<math>\Delta</math>::Ura3, Abp1-RFP::His3 Abp140-3GFP::His3</i>                                 | This study |
| YMY2027     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, bni1<math>\Delta</math>::Ura3, Abp1-RFP::His3 Abp140-3GFP::His3</i>                                 | 1          |
| YMY2028     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5<math>\Delta</math>::Ura3, bni1<math>\Delta</math>::Ura3, Abp1-RFP::His3 Abp140-3GFP::His3</i>  | This study |
| YMY2029     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, bnr1<math>\Delta</math>::KanMX, Abp140-3GFP::His3, Abp1-RFP::His3</i>                               | This study |
| YMY2030     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5<math>\Delta</math>::Ura3 bnr1<math>\Delta</math>::KanMX, Abp140-3GFP::His3, Abp1-RFP::His3</i> | This study |
| YMY2031     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5-N-GFP::Ura3</i>                                                                                | This study |
| YMY2032     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5<math>\Delta</math>::Ura3, leu2-3:: aip5-C-GFP::Leu2</i>                                        | This study |
| YMY2033     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5<math>\Delta</math>::Ura3, leu2-3:: aip5-C-GFP::Leu2, spa2<math>\Delta</math>::Leu2</i>         | This study |
| YMY2034     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5-N-GFP::Ura3, spa2<math>\Delta</math>::Leu2</i>                                                 | This study |
| YMY2035     | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, spa2<math>\Delta</math>::Leu2, Abp140-3GFP::His3</i>                                                | This study |



|         |                                                                                                                                                                                              |              |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| YMY2036 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5-N-GFP::Ura3, bni1<math>\Delta</math>::Leu2, Abp140-3GFP::His3, Abp1-RFP::His3</i>                                     | This study   |
| YMY2037 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5<math>\Delta</math>::Ura3, bni1<math>\Delta</math>::Ura3, leu2-3:: aip5-C::Leu2, Abp140-3GFP::His3, Abp1-RFP::His3</i> | This study   |
| YMY2038 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, Aip5-13Myc::KanMX</i>                                                                                                      | This study   |
| YMY2039 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5-N-13Myc::KanMX</i>                                                                                                    | This study   |
| YMY2040 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5-N1014-13Myc::KanMX</i>                                                                                                | This study   |
| YMY2041 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5-N457-13Myc::KanMX</i>                                                                                                 | This study   |
| YMY2042 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5-N271-13Myc::KanMX</i>                                                                                                 | This study   |
| YMY2043 | Mata <i>ade2, leu2, his,3 trp1, ura3, lys2::Pgal1:GAL4::Lys2, pep4::His3, bar1::HisG</i>                                                                                                     | <sup>1</sup> |
| YMY2044 | MAT $\alpha$ <i>his3-<math>\Delta</math>200 leu2-3, 112, ura3-52, aip5<math>\Delta</math>::Ura3</i>                                                                                          | This study   |

Supplementary Table 2 Oligonucleotide primers used in the study.

|                    |                                                                             |
|--------------------|-----------------------------------------------------------------------------|
| Aip5-GFP/13Myc-F   | AAGAAGCTAACGAAGACTACAAATTGCGGGAACCTCATTTAC<br>GATACTATTCGGATCCCCGGGTAAATTAA |
| Aip5-GFP/13Myc-R   | ACATGTGCTGTCAAGAGATTATGTTAGTTACGTAGCGCGCTT<br>AAGCTTTCGAATTCGAGCTCGTTTAAAC  |
| Aip5-N-GFP/13Myc-F | CTACTGAGCAATCCAAGAAAAACAATGACAAGCCACAAGAC<br>GTAATAACGCGGATCCCCGGGTAAATTAA  |
| Spa2-C-GFP-F       | AAGTGAAGATGACGATGAAGAGGAAGAAGACAGTGATTTTG<br>ATCGGATCCCCGGGTAAATTAA         |
| Spa2-C-GFP-R       | CTTTGTCTTCCTTTTCTTTCTCCTCTAGATACTACTAACTGAA<br>TTCGAGCTCGTTTAAAC            |
| P634-Aip5-F        | TTAATTAATGGTGCAACCG                                                         |
| P634-Aip5N-R       | CGTTATTACGTCTTGTGG                                                          |
| P634-Aip5N1014-R   | AGGAAGAGAAGTTTCTTTCTC                                                       |
| P634-Aip5N457-R    | ACCGTGTTGTAGATTTTTTTC                                                       |
| P634-Aip5N271-R    | GTTAGGCACATCTATGTC                                                          |
| Aip5-N1014-13Myc-F | ATTGGAAGTAGAAGAAACAGAGAAAGAACTTCTCTTCCTC<br>GGATCCCCGGGTAAATTAA             |
| Aip5-N1014-13Myc-R | CATTCTTTTCTCCGTGATGTTTTCTTCCACTACAAGATCGAA<br>TTCGAGCTCGTTTAAAC             |
| Aip5-N457-13Myc-F  | AAAGCGCATTAGTGACAATGAAAAAATCTACAACACGGTC<br>GGATCCCCGGGTAAATTAA             |
| Aip5-N457-13Myc-R  | CTTCTTCTTCTTTTCAACTTCTACAGATATATCGTTAGTGAA<br>TTCGAGCTCGTTTAAAC             |
| Aip5-N271-13Myc-F  | TGAATCTAGTGACAAAACCTTCGACATAGATGTGCCTAACCG<br>GATCCCCGGGTAAATTAA            |
| Aip5-N271-13Myc-R  | TGTTCTCGGACTTTGAGGAAGTTTCGTCAACATTATCTTTGA<br>ATTCGAGCTCGTTTAAAC            |
| Bni1FH2C-F         | TTTGAAAAGTATCCTCGTCCACAC                                                    |
| Bni1FH2C-R         | CATGGATTGGAAGTACAGGTTCTC                                                    |
| Spa2-LC-Vec-F      | TGACAGTAAAGGTGGATACGG                                                       |
| Spa2-LC-Vec-R      | CATGGATTGGAAGTACAGGTTCTC                                                    |

|                  |                                                   |
|------------------|---------------------------------------------------|
| Spa2-LC-Insert-F | CTGTACTTCCAATCCATGACTGGTGCATATACGAAAAC            |
| Spa2-LC-Insert-R | CGTATCCACCTTTACTGTCACCTTCAACTTCGAATTCAAATAA<br>TT |

## References

1. Miao, Y. *et al.* Fimbrin phosphorylation by metaphase Cdk1 regulates actin cable dynamics in budding yeast. *Nature communications* **7**, 11265 (2016).