Supplementary information

Yeast strains, media and plasmids

Yeast strains used in this study are listed in Table I. Knock-out of *SMP2*, *INO2* and *IRE1* in the RS453 strain were done by homologous recombination using the appropriate *smp2*::*TRP1*, *ino2*::*KanMX4* (kindly provided by Prof. H.J. Schueller), or *ire1::HIS3* (kindly provided by F. Reggiori) constructs. The Smp2-PtA and Sec63-GFP fusions were constructed by inserting the TEV cleavage site-Protein A or GFP fragment to the C-terminus of *SMP2* or *SEC63* respectively. In both cases expression was driven by the endogenous *SMP2* or *SEC63* promoters and the low copy Ycplac111 vector. The *NEM1*^{D257A} point mutant was generated by site-directed mutagenesis. The high copy library used for the suppressor screen was from ATCC (No 37323). To co-overexpress *NEM1-PtA* and *SPO7*, both genes were placed under the control of the inducible *GAL1/10* promoter and cloned in the Ycplac111-*LEU2* and Ycplac33-*URA3* centromeric plasmids respectively. To overexpress *SMP2* or *OPI1*, the open reading frames were fused to the *GAL1/10* promoter and cloned into Yeplac181 or Ycplac111 vectors, respectively. *GAL1/10*-dependent overexpression was done by changing the carbon source of early log phase cells from 2% raffinose to 2% galactose. *Matα smp2Δ* cells expressing *SMP2PtA* were arrested with 5μg/ml alpha-factor (Sigma) for 2 h at 30˚C.

High copy number suppressor screen with a *nup84Δ spo7Δ* mutant

The *nup84Δ spo7Δ* double knock-out strain complemented with a pRS316-*NUP84* plasmid was transformed with a high copy (2μ *LEU2*) yeast genomic library (Nasmyth and Reed, 1980). Approximately 10,000 transformants were pooled from the original transformation plates and various dilutions were spotted onto 5-fluoro-orotic acid (5-FOA)-containing plates. Library plasmids were recovered from 8 colonies that could grow on 5-FOA plates and then retransformed in the original synthetic lethal strain to verify the complementation/suppression. Out of these, four contained genomic fragments of *NUP84* and three of *SPO7*. Sequencing of the remaining library plasmid followed by

subcloning of various open reading frames found within it showed that the *SMP2* gene rescues the synthetic lethality of the *nup84Δ spo7Δ* mutant on 5-FOA plates.

Affinity purifications of Protein A fusions and phosphatase assays

The Nem1PtA-Spo7 complex was purified from extracts of strains overexpressing the *NEM1*-PtA and *SPO7* from the inducible *GAL1/10* promoter as follows. Cells were shifted in the appropriate selective medium containing 2% galactose for 10h and spheroplasted. Affinity purification of Nem1-PtA fusions by IgG-Sepharose chromatography was performed as described previously (Siniossoglou *et al*, 2000). For phosphatase assays using as a substrate p-nitrophenyl-phosphate (pNPP), IgG-Sepharose beads with 0.5μg bound Protein A fusions were washed with 20 ml of phosphatase buffer (50 mM Tris-HCl pH 7.1, 10 mM potassium acetate, 10 mM magnesium chloride, 0.5 mM DTT, 0.01 Triton X-100) at room temperature. Beads were then incubated with 200 μl of phosphatase buffer containing 5 mg/ml *p*-NPP (Sigma) for 45 min at 30 °C. The supernatant was collected by centrifugation and its absorbance was measured at 410 nm. To affinity purify native hyperphosphorylated Smp2, *nem1*Δ *spo7*Δ *smp2*Δ cells expressing a Smp2-PtA fusion from the endogenous *SMP2* promoter were grown to OD_{600} 1.0, washed once in water and frozen into liquid nitrogen. Cell pellets were grinded in the presence of liquid nitrogen and the powder was resuspended in lysis buffer (20 mM Tris-HCl pH 8.0, 150 mM potassium chloride, 5mM magnesium chloride, 1% Triton X-100) in the presence of phosphatase (Sigma, cat no P5726) and protease inhibitors (Roche, cat no 1873580). Cell extracts were centrifuged at 100,000 g for 30 min at 4˚C and Smp2PtA was affinity purified from the supernatant as above. The PtA tag was then removed by TEV protease digestion. For in *vitro* dephosphorylation reactions, 2μg of Smp2 in 50 mM Tris-HCl pH 7.1, 150 mM NaCl, 10 mM sodium acetate, 10 mM magnesium chloride, 1 mM DTT, 0.01% Triton X-100 was incubated with Nem1PtA-Spo7 beads for 30 min at 30˚C. Supernatants were collected by centrifugation, boiled in sample buffer and analyzed by SDS-PAGE followed by Coomassie staining.

Antibodies

The antibody used to detect protein A fusions was from DAKO (cat no Z0113). The anti-myc polyclonal antibody (cat no sc-789) and the anti-Clb2 were from Santa- Cruz. The anti-Pgk1 and MPM2 monoclonals were from Molecular Probes (cat A-6457) and Upstate (cat 05-368) respectively.