APPENDIX

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Appendix Supplementary Materials and Methods

Materials

We obtained Edinburgh minimal medium from MP Biomedicals; yeast extract, peptone, agar from Becton Dickson; oligonucleotides from Integrated DNA Technologies; alkaline phosphatase, complete EDTA-free protease inhibitor from Roche; cobalt (II) chloride ($CoCl_2$), amino acids, anti-FLAG M2 magnetic beads, sodium ascorbate, Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) from Sigma-Aldrich; Invivo$_2$ 400 Hypoxic Workstation from Biotrace, Inc. (Cincinnati, OH); Prestained Protein Standards from Bio-Rad; Biotin-phenol from Iris Biotech; MagnetGST beads from Promega, Streptavidin magnetic beads from Pierce; n-Dodecyl β-D-maltoside (DDM) from Acros Organics; Gibson Assembly Master Mix from NEB BioLabs; Bortezomib from LC laboratories.

Antibodies

We obtained anti-FLAG M2 from Sigma. Antisera to Sre1 (aa 1-260) and Sre2 (aa 1-426) polyclonal IgG generated against the cytosolic N terminus of fission yeast Sre1 and Sre2 have been described previously (Hughes et al, 2005; Stewart et al, 2011). HRP-conjugated Dsc5 antibody has been described previously (Raychaudhuri & Espenshade, 2015). IRDye800CW Goat anti-Mouse IgG and Rabbit IgG, IRDye680RD Goat anti-Mouse IgG and Rabbit IgG, IRDye800CW Streptavidin from LI-COR, Mouse anti-GFP monoclonal antibody from Roche, Mouse anti-HA monoclonal antibody from abcam, and Mouse anti-ubiquitin monoclonal P4D1 IgG from Jackson ImmunoResearch Laboratories. Antiserum to Cdc48 was the kind gift of R. Hartmann-Petersen (University of Copenhagen).

Yeast strains and media

Wild-type haploid *S. pombe* and derived strains were grown to exponential phase at 30°C in YES medium (5 g/L yeast extract plus 30 g/L glucose supplemented with 225 mg/L each of adenine, uracil, leucine, histidine, and lysine) or Edinburgh minimal medium (EMM) plus supplements, unless otherwise noted (Moreno et al, 1991). $CoCl_2$, when used, was dissolved in H_2O and used at 1.6 mM in YES medium. *S. pombe* strains were generated using homologous recombination via standard molecular biology and genetic techniques (Bahler et al, 1998). Geneticin (100 µg/L) and clonNAT (100 µg/L) were used to select for the *kanMX* and *natMX* marker genes, respectively. Transformants were screened for homologous integration using PCR. A complete list of strains is described in Appendix Table S1.

Cloning and mutagenesis

Gene and protein sequence for *rbd2* were derived from *S. pombe* GeneDB (http://old.genedb.org/genedb/pombe/). *rbd2* plasmids in this study were engineered using the upstream ATG and code for a protein of 251 amino acids. All primers used for cloning are described in Appendix Table S2. An *rbd2* insert flanked by 600 bp of upstream genomic sequence and 600 bp downstream genomic sequence were generated via PCR with primers oDJ53 and oDJ54, and inserted into a *his3*$^+$ locus integrating plasmid (pES205) to generate pDJ28. The *rbd2-3xFlag*-integrating plasmid was generated by introducing synthesized 3xFlag

sequences (two annealed oligomers, oJH01 and oJH02; Integrated DNA technologies) into NotI-digested pDJ28. The *rbd2-3xFlag*-integrating plasmid was subjected to site-directed mutagenesis to generate plasmids for *rbd2-S130A-3xFlag* (with primers oJH09 and oJH10), *rbd2-H182A-3xFlag* (oJH11 and oJH12), *rbd2-G244R-3xFlag* (oHG05 and oHG06) and *rbd2-G246R-3xFlag* (oHG11 and oHG12). Plasmids containing *rbd2-SHP-3xFlag* and *rbd2-SHP*-3xFlag* were generated via PCR amplification of the *rbd2-3xFlag*-integrating plasmid with primers oJH055 and oJH056 or oJH055 and oJH057, respectively, followed by blunt-end ligation. A plasmid containing *rbd2-Flag-APEX2* under control of *S. pombe* adh1^{+} promoter (Lum et al, 1996) was generated via Gibson Assembly protocol (NEB BioLabs) by assembling three PCR-amplified fragments of adh1^{+} promoter-driven vector pJB114 (by primers oJH41 and oJH42), *rbd2* from the *rbd2-3xFlag*-integrating plasmid (oJH43 and oJH44) and *Flag-APEX2* (oJH39 and oJH40) from pcDNA3 APEX2-NES (Plasmid #49386, Addgene). The *rbd2-Flag-APEX2* was subjected to site-directed mutagenesis to generate plasmids of *rbd2-G246R-Flag-APEX2* (with primers oHG11 and oHG12), *rbd2-S130A-Flag-APEX2* (oJH09 and oJH10) and *rbd2-G246R/S130A-Flag-APEX2*. The *rbd2-6xmCherry* plasmid was generated by ligating PCR-amplified *rbd2* into a *6xmCherry* tagging plasmid (pES210). The *3xFlag-sre2MS* and *sre1N* constructs were previously generated (Cheung & Espenshade, 2013; Stewart et al, 2011). The *3xFlag-sre2MS* mutant constructs were generated by site-directed mutagenesis. Bacterial expression plasmids GST-Rbd2$^{(aa200-251)}$, GST-Rbd2$^{(aa200-225)}$, GST-Rbd2$^{(aa200-240)}$, GST-Rbd2$^{(aa225-251)}$, GST-Dsc2$^{UBA(aa298-372)}$ GST-Dsc5$^{UBX(aa323-425)}$, GST-Af RbdB$^{(aa211-272)}$, and GST-Sc Rbd2$^{(aa192-262)}$ were generated by ligating a designated PCR-amplified fragment into pGEX-KG vector (ATCC 77103). The GST-Rbd2$^{(aa200-251)}$ plasmid was subjected to site-directed mutagenesis to generate the plasmids GST-Rbd2^{G244R} and GST-Rbd2^{G246R}.

Rhomboid expression constructs

The *S. pombe* (Sp) *rbd2* ORF was subcloned into pcDNA3.1(+) for expression in mammalian cells. Recoded Sp *rbd2* gene was synthesized by GeneArt AG (Regensburg, Germany). Sequence of the synthetic DNA encoding Sp Rbd2 was deposited in GenBank (accession #KU375575). *P. falciparum* ROM4, *D. melanogaster* Rho1, human RHBDL2, GFP-Spitz, GFP-EBA175, and GFP-TatA-Flag were described previously (Baker & Urban, 2015; Baker et al, 2006). TatA point mutants were generated using QuikChange site-directed PCR mutagenesis.

Bioinformatic analysis

Protein folding and three-dimensional structure predictions in Fig 1B were obtained using Phyre2 (Kelley et al, 2015). The predicted structure of Rbd2 was aligned to *E. coli* GlpG (PDB code: 3b45) using PyMOL. Clustal Omega server was used to generate multiple sequence alignment for protein sequences of *E. coli* GlpG (ec, P09391), human RHBDL4 (hs, Q8TEB9), *S. pombe* Rbd2 (sp, O74926), and *S. cerevisiae* Rbd2 (sc, Q12270).

Rhomboid activity analysis in mammalian cells

Mammalian transfection was performed as previously described (Baker & Urban, 2015). Briefly, HEK293 cells were transiently transfected using X-tremeGENE HP (Roche) for the expression of GFP-tagged substrate and 3xHA-tagged rhomboid proteases. For the TatA substrate, we

used a GFP-TatA-Flag construct. 24 hr post transfection, serum-free media was conditioned for an addition 18-24 hr. Media and cell samples were analyzed by western blotting.

Mass spectrometry

C-terminal cleavage products were purified from transfected cells by anti-Flag immunoaffinity isolation with the M2 resin (Sigma) and analyzed by MALDI-TOF mass spectrometry using sinapinic acid matrix as described previously (Moin & Urban, 2012).

Low oxygen cell culture

Sre1 hypoxic cleavage assays were performed as described previously (Hughes et al, 2005). Briefly, cells grown in log phase were centrifuged to remove oxygenated medium and resuspended in deoxygenated medium under anaerobic conditions inside an Invivo$_2$ 400 hypoxic workstation (Biotrace, Inc.). Cells were cultured inside the workstation for 0.5-4 hr depending on the particular experiment, before being removed, centrifuged, washed with water, and flash-frozen as cell pellets. Cell pellets were then stored at -80°C or subjected to cell lysis.

SREBP cleavage assay

For Sre1 and Sre2 cleavage assays, whole cell lysates were prepared for western blotting analysis as previously described (Cheung & Espenshade, 2013; Hughes et al, 2005). Briefly, cells were lysed by addition of 27 mM NaOH, 1% (v/v) 2-mercaptoethanol for 10 min on ice. Total protein was precipitated with trichloroacetic acid followed by acetone wash. Proteins were solubilized in 100 µl lysis buffer (50 mM Tris pH8.0, 150 mM NaCl, 1% SDS) containing Complete Protease Inhibitor EDTA free (Roche) by sonication. For phosphatase treatment, protein were first denatured by heating at 75°C for 15 min and treated with alkaline phosphatase (0.05U/µl) for 1 hr at 37°C.

Western blotting

Equal amounts of protein (30-50 µg) from each sample were loaded on an SDS-PAGE gel. Gels were transferred to nitrocellulose using Trans-Blot Turbo Transfer system (Bio-Rad), blocked using a solution of 5% milk-PBST (PBS with 0.05% Tween 20), and probed with primary antibody followed by mouse or rabbit IgG (IRDye800CW or IRDye680RD from LI-COR) or IRDye800CW Streptavidin. Blots were scanned through channel 700 or 800 using an Odyssey CLx imager from LI-COR. Where indicated in figure legends, western blots were developed using a chemiluminescence kit (Pierce).

Immunoprecipitation

Immunoprecipitation assay was performed as described previously (Stewart et al, 2011) with a minor change. Briefly, cells (6×10^8) from exponentially growing wild-type, *rbd2-3xFlag* or *rbd2-G246R-3xFlag* mutant cells were collected and lysed using glass beads in 1% dodecyl maltoside (DDM) lysis buffer (25 mM Tris, pH 7.4, 1% DDM, 150 mM NaCl, supplemented with protease inhibitors). Cellular debris was removed by centrifugation at 20,000 × g for 15 min at 4°C followed by the addition of anti-FLAG M2 magnetic beads. Binding reaction was rotated for

4 hr at 4°C, and unbound proteins were removed by four washes with 1 ml of wash buffer (25 mM Tris, pH 7.4, 0.1% DDM, 150 mM NaCl). The bound fraction was eluted by heating at 75°C 10 min in SDS-lysis buffer (10 mM Tris-HCl, pH 6.8, 100 mM NaCl, 1% SDS and 1 mM EDTA) and analyzed by western blotting.

Linkage analysis

The linkage analysis was performed as previously described (Stewart et al, 2012). Briefly, to assay linkage, each strain from our previous collection of mutant strains was mated to *rbd2*Δ cells (Stewart et al, 2012). A suspension of haploid spores from the mating was treated with glusulase (PerkinElmer) and plated on YES and YES containing 1.6 mM $CoCl_2$. Mating was scored as positive (+) or negative (-) based on growth on YES, and linkage was scored (+ or -) based on growth on YES+$CoCl_2$. The inability to recover $CoCl_2$-resistant spores indicated linkage between the mutations. Matings that failed to grow on YES were not scored and indicated a mating defect.

Proteasome inhibition

Bortezomib (Bz) was used at a concentration of 1 mM for 2-3 hr to inhibit the proteasome *in vivo*. In strains with an *mts3-1* proteasome mutation, the proteasome was inhibited by growing cells at non-permissive temperature (36°C) for 3 hr.

***In vitro* binding assay**

The *in vitro* GST binding assay was performed as previously described (Lloyd et al, 2013). Briefly, GST-fusion proteins were expressed in *E. coli*, lysed by sonication in PBS plus protease inhibitors, incubated for 90 min with 1% (w/v) Triton X-100, and cleared by centrifugation at 15,000 x g for 20 min at 4°C. Bacterial lysates were then incubated with MagnetGST beads (25 µl) for 1 hr at 4°C. The beads were washed three times with PBS and once with binding buffer (20 mM HEPES, pH7.2, 150 mM KOAc, 5 mM MgOAc, 250 mM sorbitol, 0.2% Nonidet P-40, protease inhibitors) before *S. pombe* cytosol (0.7 mg) was added to the reaction. After three washes with binding buffer, bound proteins were eluted by boiling in SDS-PAGE loading buffer.

Microscopy and image processing

Confocal fluorescence microscopy was performed as previously described (Raychaudhuri & Espenshade, 2015). Briefly, *dsc2-6xGFP rbd2-6xmCherry or sec72-6xGFP rbd2-6xmCherry* cells were immobilized on rich medium containing 2% agarose as described previously (Tran et al, 2004) and imaged using a 3i Marianis/Yokogawa Spinning Disk Confocal microscope equipped with Axio Observer (Zeiss) and Photometrics Cascade II EM-CCD camera (Roper Scientific). Images were converted to TIFF format and analyzed using ImageJ, including the Colocalization Finder Plug-in. Minimum and maximum pixel intensity values for each channel were adjusted until background was negligible, then these values were applied to all images from the same imaging session. To determine the outlined boarders of cells as indicated in Figure 1, the background was increased until cell outlines were again visible, then lines were hand-drawn to indicate the location of individual cells, and the outlines were superimposed on the low-background images.

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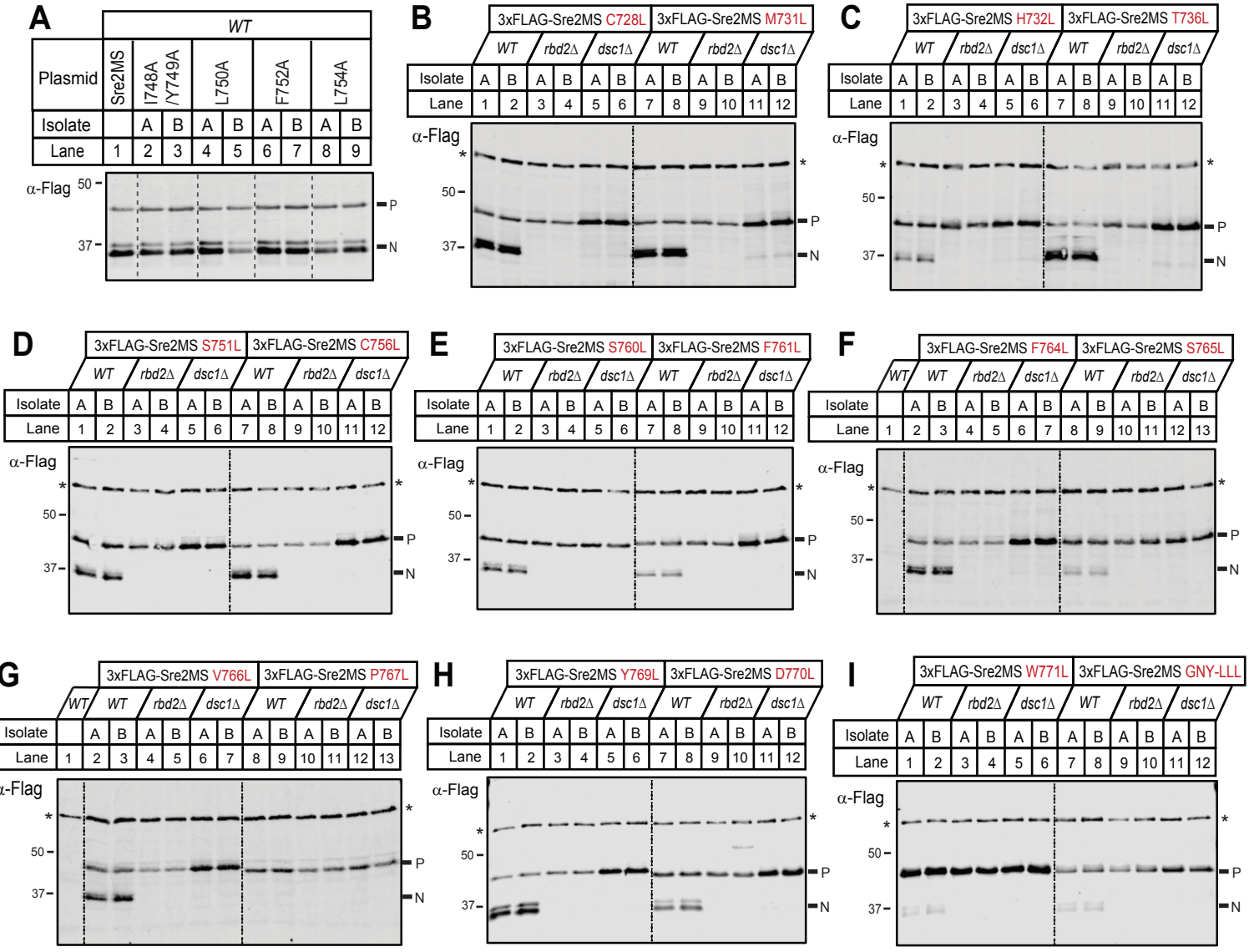
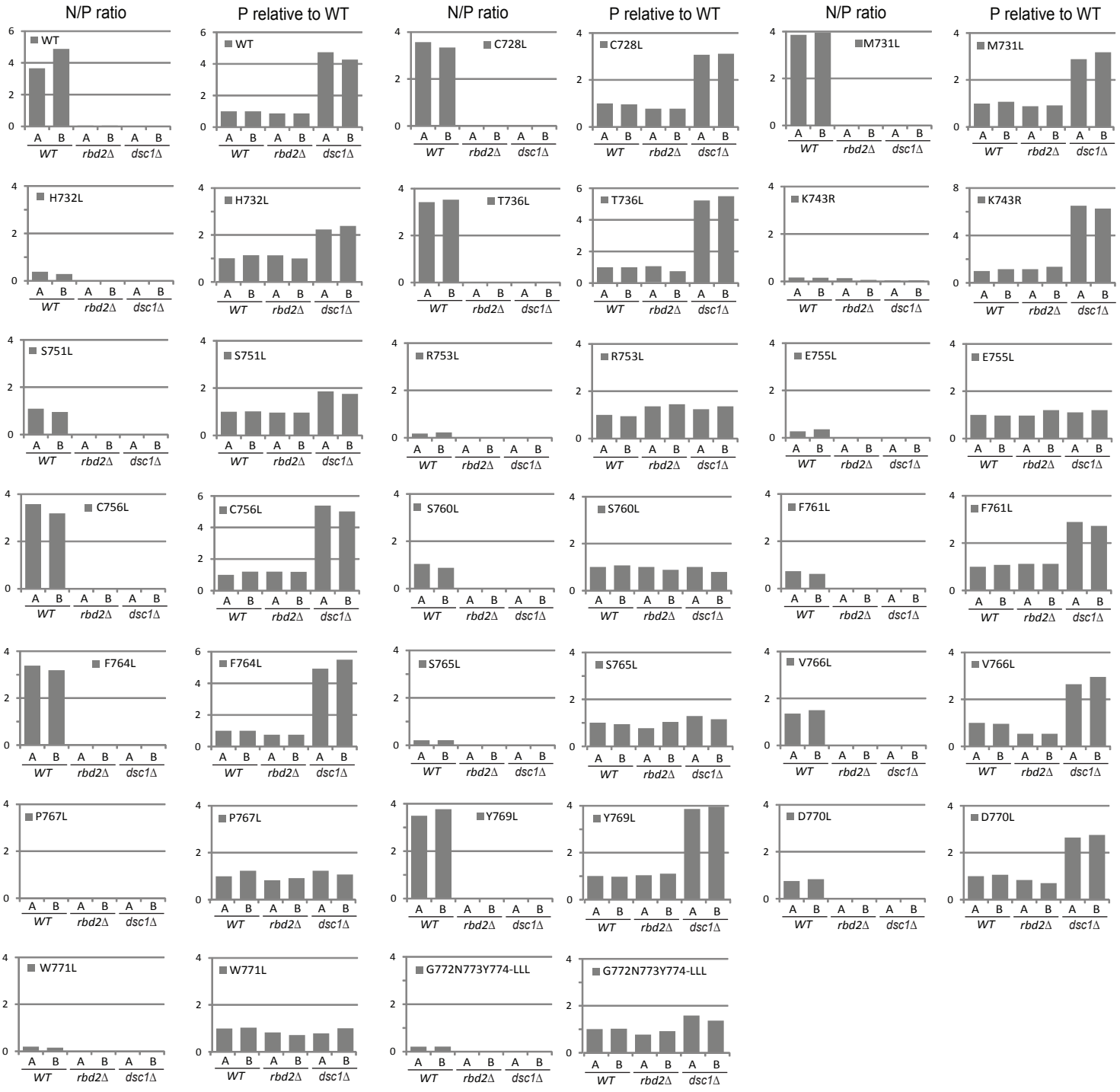


Appendix Figure S1 – Mutagenesis of Sre2MS.

A-I. Western blot was probed with anti-Flag IgG of whole cell lysates from yeast strains carrying plasmid expressing 3xFlag-Sre2MS WT (Sre2MS) or indicated mutants in wild-type (WT), *rbd2Δ* or *dsc1Δ* background. P and N denote Sre2 precursor and cleaved forms, respectively. Asterisks indicate non-specific, loading control bands, which were used to normalize the intensities of P and N in Appendix Figure S2. Two independent isolates, A and B, are shown for each strain.



Appendix Figure S2 – Quantification of Sre2MS cleavage.
Intensities of individual P and N bands from western blots in Figure 5 and S1 were quantified using LI-COR software. Relative intensity of P and N to corresponding non-specific band (*) in each lane was normalized to that of P level in isolate A of WT strain for each mutant. The relative P level or ratio of N to P was presented in bar graphs.

Table S1. *S. pombe* strain list

Strains	*S.pombe Strains*	Genotype	Reference	Figure
KGY425	*WT*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1*	ATCC	Fig 2,3,5,7,8,9,EV1-3,S1
PEY522	*sre1Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δsre1-D1::kanMX6*	Hughes et al., 2005	Fig 2,8,9,EV1
PEY553	*sre2Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δsre2-D1::kanMX6*	Hughes et al., 2005	Fig 2,3,8,EV1
PEY1448	*dsc1Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc1-D1::kanMX6*	Stewart et al., 2011	Fig 2,3,5,8,EV1-3,S1
PEY1547	*dsc2Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc2-D1::kanMX6*	Stewart et al., 2011	Fig 3
PEY1546	*dsc3Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc3-D1::kanMX6*	Stewart et al., 2011	Fig 3
PEY1548	*dsc4Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc4-D1::kanMX6*	Stewart et al., 2011	Fig 3
PEY1681	*rbd2Δ*	*h+ leu1-32 ura4-D18 ade6-M210 his3-D1 Δrbd2-D1::natR*	This study	Fig 2,3,5,7,8,9,EV1-3,S1
PEY1685	*dsc1Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc1-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig 3,EV1
PEY1686	*dsc2Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc2-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig 3
PEY1687	*dsc3Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc3-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig 3
PEY1688	*dsc4Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc4-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig 3
ESY58-107	*rbd2 G128V*	*h- leu1-32 ade6-M210 ura4D18::7xSRE-ura4 kanR, his3-D1::7xSRE-lacZ rbd2 G128V*	This study	Fig 2
ESY58-4	*rbd2 A127D*	*h- leu1-32 ade6-M210 ura4D18::7xSRE-ura4 kanR, his3-D1::7xSRE-lacZ rbd2 A127D*	This study	Fig 2
ESY58-122	*rbd2 A186T*	*h- leu1-32 ade6-M210 ura4D18::7xSRE-ura4 kanR, his3-D1::7xSRE-lacZ rbd2 A186T*	This study	Fig 2
DJY454	*dsc2-6xGFP dsc2Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1::dsc2-6xGFP-his3$^+$ Δdsc2-D1::kanMX6 Δrbd2::natR*	This study	Fig 1
DJY450	*sec72-6xGFP sec72Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1 sec72-GFP::ura4$^+$ Δrbd2::natR*	This study	Fig 1
JHY6	*rbd2-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 2,8,9,EV3
JHY12	*rbd2 S130A-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-S130A-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 2,8,9
JHY13	*rbd2 H182A-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-H182A-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 2
PEY1642	*dsc1Δsre2Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δdsc1-D1::kanMX6 Δsre2-D1::kanMX6*	Cheung and Espenshade, 2013	Fig 2
SRY193	*rbd2Δsre2Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δsre2-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig 2
PEY874	*mts3-1*	*h- leu1-32 ura4-D18 mts3-1 ts strain*	Hughes and Espenshade, 2008	Fig 3
SRY140	*mts3-1 rbd2Δ*	*h- leu1-32 ura4-D18 mts3-1 Δrbd2-D1::natR ts strain*	This study	Fig 3
PEY1693	*dsc1 C634A rbd2Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::dsc1-C634A-his3$^+$ Δdsc1-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig 3
JHY7	*rbd2 G244R-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-G244R-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 8
JHY8	*rbd2 G246R-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-G246R-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 8,9,EV3
JHY51	*rbd2-SHP-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-SHP(wild-type)-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 8
JHY52	*rbd2-SHP*-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-SHP(mutant)-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 8
JHY53	*rbd2 G246R-SHP-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-G246R-SHP(wild-type)-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 8
JHY54	*rbd2 G246R-SHP*-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-G246R-SHP(mutant)-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig 8
JHY36	*rbd2-UBX-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-UBX-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig EV3
JHY37	*rbd2 G246R-UBX-Flag*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1::rbd2-G246R-UBX-3xFlag-his3$^+$ Δrbd2-D1::natR*	This study	Fig EV3
PEY893	*hrd1Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δhrd1-D1::kanMX6*	Hughes and Espenshade, 2008	Fig EV1
JHY270	*hrd1Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1 Δhrd1-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig EV1
PEY894	*doa10Δ*	*h- leu1-32 ura4-D18 ade6-M210 his3-D1 Δdoa10-D1::kanMX6*	Hughes and Espenshade, 2008	Fig EV1
JHY271	*doa10Δrbd2Δ*	*h? leu1-32 ura4-D18 ade6-M210 his3-D1 Δdoa10-D1::kanMX6 Δrbd2-D1::natR*	This study	Fig EV1
JHY385	*WT*	*h+ leu1-32 ura4-D18 ade6-M210*	This study	Fig EV4
JHY386	*dsc1Δ*	*h+ leu1-32 ura4-D18 ade6-M210 Δdsc1-D1::kanMX6*	This study	Fig EV4
JHY387	*rbd2Δ*	*h+ leu1-32 ura4-D18 ade6-M210 Δrbd2-D1::kanMX6*	This study	Fig EV4
JHY388	*ypf1Δ*	*h+ leu1-32 ura4-D18 ade6-M210 Δypf1-D1::kanMX6*	This study	Fig EV4
JHY389	*yps1Δ*	*h+ leu1-32 ura4-D18 ade6-M210 Δyps1-D1::kanMX6*	This study	Fig EV4
JHY390	*sxa1Δ*	*h+ leu1-32 ura4-D18 ade6-M210 Δsxa1-D1::kanMX6*	This study	Fig EV4

Table S2. List of primer sequences

Names	For / Rev	Primer sequences
oDJ53	F	GAAATAGCTCACACTCCACACG
oDJ54	R	CTAATTGTCCCTAACTCCGTGC
oHG05	F	CCTTTTCTTCTTTCCCCAGAAAAGGAACCCGTCTT
oHG06	R	AAGACGGGTTCCTTTTCTGGGGAAAGAAGAAAAGG
oHG11	F	CTTCTTTCCCCGGAAAAAGAACCCGTCTTGGG
oHG12	R	CCCAAGACGGGTTCTTTTTCCGGGGAAAGAAG
oJH01	F	GGCCGCAGACTACAAGGACGACGATGACAAGGATTACAAAGATGACGACGATAAGGACTATAAGGACGATGATGATAAATAAGC
oJH02	R	GGCCGCTTATTTATCATCATCGTCCTTATAGTCCTTATCGTCGTCATCTTTGTAATCCTTGTCATCGTCGTCCTTGTAGTCTGC
oJH09	F	CGTTTCTATTGCTG GACTTGCTGGATGGGCTTTTGCTTTTATCT
oJH10	R	AGATAAAAGCAAAAGCCCATCCAGCAAGTCCAGCAATAGAAACG
oJH11	F	CCAAAAGCTAGCTTTATCGGGGCTGCATCCGGAGCTGTTATG
oJH12	R	CATAACAGCTCCGGATGCAGCCCCGATAAAGCTAGCTTTTGG
oJH039	F	CTAGGGCCATTAATGGACTACAAGGATGACGAC
oJH040	R	ATGGATCGGCCCTTAGTCCAGGGTCAGGCG
oJH041	F	CTGGACTAAGGGCCGATCCATTCTT
oJH042	R	GTAGTCCATTAATGGCCCTAGAGTCGACCT
oJH043	F	GTGGAGCTGGCGGCCGCAGACTACAA
oJH044	R	CATCTTCAGATCCCCCAAGACGGGT
oJH055	F	AAAAAGTTAGGCACTGCGGCCGCAGACTAC
oJH056	R	GCCCTTCCCAATGAATCCCCCAAGACGGGT
oJH057	R	CCTCTTCCCAATGAATCCCCCAAGACGGGT