SUPPLEMENTAL MATERIAL

Identification and functional testing of novel interacting protein partners for the stress sensors Wsc1p and Mid2p of *Saccharomyces cerevisiae*.

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Table S1. Strains used in this study.

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Figure S1. NubG/I test for integrated C-tagged Wsc1p and Mid2p.

Figure S2. Localization of Wsc1p and Mid2p.

Figure S3. Validation of MYTH-tagged Mid2p and Wsc1p expression by Western blot.

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Figure S5. Growth curve analysis of single and double mutants exposed at different concentrations of Hydrogen Peroxide.

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Figure S7. Western blot analysis showing the phosphorylation of Slt2p in single and double mutant strains treated with 1mM hydrogen peroxide (H_2O_2) for 1 hr at 27°.

Figure S8. Western blot analysis showing the phosphorylation of Slt2p in single and double mutant strains treated with 75ng/ml Caspofungin for 1 hr at 27°.

Figure S9. Network graph of the Wsc1p and Mid2p interactome identified by iMYTH screen at 37°.

Table S3. Percentages of positive interactors for Wsc1p and Mid2p identified by two independent iMYTH screens performed at 37°.

Figure S10. A representative drop dilution assay of sensor and interactor null mutants exposed to stress conditions.

Table S1. Strains used in this study

Strain	Genotype	Source
Wsc1 THY L2	*MATa leu2 ura3 trp1::(lexAop)-lacZ (lexAop)-HIS3 (lexAop)-ADE2 WSC1::(Cub-lexA-VP16-KanMX)*	This study
Wsc1 L40 L3	*MATa trp1 leu2 his3 ade2 LYS2::lexA-HIS3 URA3::lexA-lacZ WSC1::(Cub-YFP-lexA-VP16-KanMX)*	This study
Mid2 THY L3	*MATa leu2 ura3 trp1::(lexAop)-lacZ (lexAop)-HIS3 (lexAop)-ADE2 MID2::(Cub-YFP-lexA-VP16-KanMX)*	This study
Mid2 L40 L3	*MATa trp1 leu2 his3 ade2 LYS2::lexA-HIS3 URA3::lexA-lacZ MID2::(Cub-YFP-lexA-VP16-KanMX)*	This study
THY.AP4	*MATa leu2 ura3 trp1::(lexAop)-lacZ (lexAop)-HIS3 (lexAop)-ADE2*	Paumi *et al.* 2007
L40	*MATa trp1 leu2 his3 ade2 LYS2::lexA-HIS3 URA3::lexA-lacZ*	Paumi *et al.* 2007
Artificial Bait in THY (A0286) - control	*MATa leu2 ura3 trp1::(lexAop)-lacZ (lexAop)-HIS3 (lexAop)-ADE2 Matα-CD4(TM)::(Cub-YFP-lexA-VP16-KanMX)*	Snider *et al.* 2013
Artificial Bait in L40 (A0287) - control	*MATa his3-200 trp1-901 leu2-3 112 ade2 LYS2::(lexAop)$_4$-HIS3 URA3::(lexAop)$_8$-lacZ GAL4 Matα-CD4(TM)::(Cub-YFP-lexA-VP16-KanMX)*	Snider *et al.* 2013
BY4742 (wild type α, wt-α)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	ATCC
YOR008C (*wsc1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 wsc1Δ::KanMX4*	Open Biosystems
YLR332W (*mid2Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 mid2Δ::KanMX4*	Open Biosystems
YLR332W (*mid2Δ*)	*MATa his3Δ1 leu2Δ0 met15Δ0 mid2Δ::URA3*	This study
YCR088W (*abp1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 abp1Δ::KanMX4*	Open Biosystems
YNL259C (*atx1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 atx1Δ::KanMX4*	Open Biosystems
YGR282C (*bgl2Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 bgl2Δ::KanMX4*	Open Biosystems
YLR429W(*crn1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 crn1Δ::KanMX4*	Open Biosystems
YPL037C (*egd1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 egd1Δ::KanMX4*	Open Biosystems
YHR193C (*egd2Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 egd2Δ::KanMX4*	Open Biosystems
YNL135C (*fpr1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 fpr1Δ::KanMX4*	Open Biosystems
YCL035C (*grx1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 grx1Δ::KanMX4*	Open Biosystems
YIR038C (*gtt1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 gtt1Δ::KanMX4*	Open Biosystems
YDR097C (*msh6Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 msh6Δ::KanMX4*	Open Biosystems
YDL173W (*par32Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 par32Δ::KanMX4*	Open Biosystems

YLR044C (*pdc1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pdc1Δ::KanMX4*	Open Biosystems
YDR032C (*pst2Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pst2Δ::KanMX4*	Open Biosystems
YNL098C (*ras2Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ras2Δ::KanMX4*	Open Biosystems
YGL031C (*rpl24aΔ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rpl24aΔ::KanMX4*	Open Biosystems
YIL148W (*rpl40aΔ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rpl40aΔ::KanMX4*	Open Biosystems
YLR048W (*rps0bΔ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rps0bΔ::KanMX4*	Open Biosystems
YOR007C (*sgt2Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 sgt2Δ::KanMX4*	Open Biosystems
YMR183C (*sso2Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 sso2Δ::KanMX4*	Open Biosystems
YDL229W (*ssb1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ssb1Δ::KanMX4*	Open Biosystems
YHR135C (*yck1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 yck1Δ::KanMX4*	Open Biosystems
YPL199C (*ypl199cΔ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ypl199cΔ::KanMX4*	Open Biosystems
YOL109W (*zeo1Δ*)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 zeo1Δ::KanMX4*	Open Biosystems
BY4741 (wild type a, wt-a)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	ATCC
Wsc1-TAP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 WSC1-TAP::HIS3MX6*	Dharmacon
Mid2-TAP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MID2-TAP::HIS3MX6*	Dharmacon
Wsc1-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 WSC1-GFP::HIS3MX6*	Thermo Fisher
Mid2-GFP	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MID2-GFP::HIS3MX6*	Thermo Fisher

Table S2. Oligonucleotides used in this study

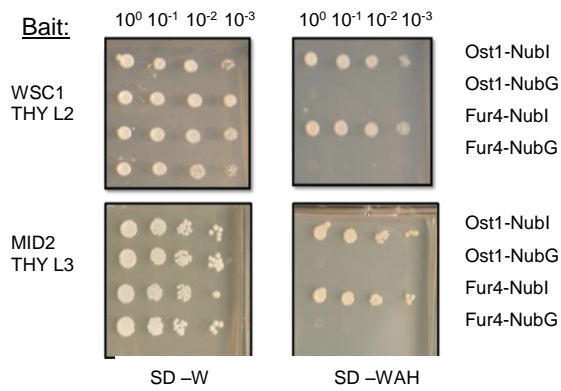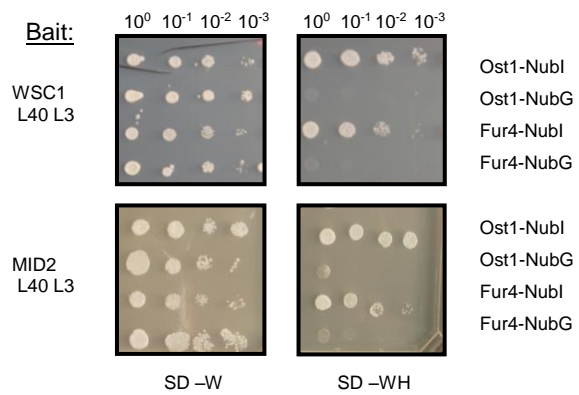| Oligonucleotides name	Sequence (5'→3')
Rev. KanMX	GAGCGTTTCCCTGCTCGCAG
Wsc1 MYTH 5'	GGG AAA AAC AAC GTT TTA ACA GTG GTC AAT CCA GAC GAA GCT GAT ATG TCG GGG GGG ATC CCT CC
Wsc1 MYTH 3'	GCT CTC CAG CTC TCA AAT TCG AAA TGT ATG AAT TTT TAG AGG ATC ACT ATA GGG AGA CCG GCA G
Wsc1 int chk 5'	CGA GGA GGA GCA CAC CAA AG
Fwd Mid2 - MYTH	AAATTCTATGATGAACAAGGTAACGAATTATCACCACGAAATT AT ATG TCG GGG GGG ATC CCT CC
Rev Mid2 - MYTH	GAA GGT GAT AAT ATT TGT AAA AAT ACT AAT GAAGTCCACCTACTC ACTATAGGGAGACCGGCAG
Int. Fwd Mid2	CCA ACA CCG CCG ACC ATA AT
Fwd NubG	CCGATACCATCGACAACGTTAAGTCG
Fw- Abp1	GCT GCT AGT CCA CTC ATC
Fw-Atx1	GCC GAC ACT CCC CTG CGA AG
Fw-Bgl2	CCC TGC TTC CGT ACC CCA GGG
Fw-Crn1	GCC AGA AAT GTG GGA GGA CC
Fw-Egd1	AAGCAGTATGGCGAAGAACG
Fw-Egd2	GAC TGC CAG CTT TCC CGT CCG
Fw-Fpr1	GCA CGG GAG GAA TTC TGG CG
Fw-Grx1	GAG CAG CCG CAA CGG CGT T
Fw-Gtt1	TTGGGGGAGGACATATTACG
Fw-Mek1	TGC AAG AGG GGT CCT GTG C
Fw-Msh6	TGG GGA GTT TCA AGG CGG T
Fw-Par32	TCG AGG GAG GTG GTC GGA GA
Fw-Pdc1	CGT GTG ATG AGG CTC GTG G
Fw-Pst2	AAG GTG CGT GCG CCT GGC
Fw-Rpl24a	TGG CGA CCG AGG GCA GCG TT
Fw-Rpl40a	TCC GCC TGG ACA TAG GCG GA
Fw-Rps0b	GAG GGT CCC GCT GGA GGT TG
Fw-SGT2	CGT GGC CCA ATG GTC ACG GCG
Fw-SSB1	CAG AGG AGT ACA CAC GGG AC
FW-SSO2	ACG AGC CGG GTA CCA AGC GG
Fw-Yck1	TAC CCC GCC TGT GGG CTC GT
Fw-YPL199C	GTG CGC GTG TGG GTA CCT CAA CTG
Fw-Zeo1	CCC GAC GGT CCG GGT GCC TA
Fw-Ras2	CCCCTGCGCCATGTCTCTGC
R Kan B	CTGCAGCGAGGAGCCGTAAT
Fwd Wsc1-TAP	AGCTCTCAATCACAGGACGC
Fwd Mid2TAP	CGTACACACCATCAGCAACC
Rev TAP Primer	AACCCGGGGATCCGTCGACC

A)

B)

Figure S1. NubG/I test for integrated C-tagged Wsc1p and Mid2p. A) THY.AP4 and B) L40 background strains. Yeast cells expressing endogenous Wsc1-Cub-LexA-VP16 and Mid2-Cub-LexA-VP16 were transformed with control prey-Nub constructs. Transformations were verified on minimal SD medium depleted of Trp (SD-W). Interaction between the bait and prey was indicated by growth on minimal SD medium lacking Trp, Ade and His (SD-WAH) or Trp and His (SD-WH). Transformants were spotted in serial dilutions. Growth on selective plates with the positive control (Ost1-NubI and Fur4-NubI) and not with the negative control (Ost1-NubG and Fur4-NubG) indicates correct bait expression and lack of self-activation.

A)

B)

C)

DIC

YFP

Wsc1 L40 L3   Mid2 L40 L3   Mid2 THY L3

Figure S2. Localization of Wsc1p (A) and Mid2p (B and C) at the emerging bud (red arrows) and yeast plasma membrane respectively. Images were acquired with a Leica DMI 6000B Inverted Confocal Microscope. YFP= Yellow Fluorescent Protein, DIC= Differential Interference Contrast
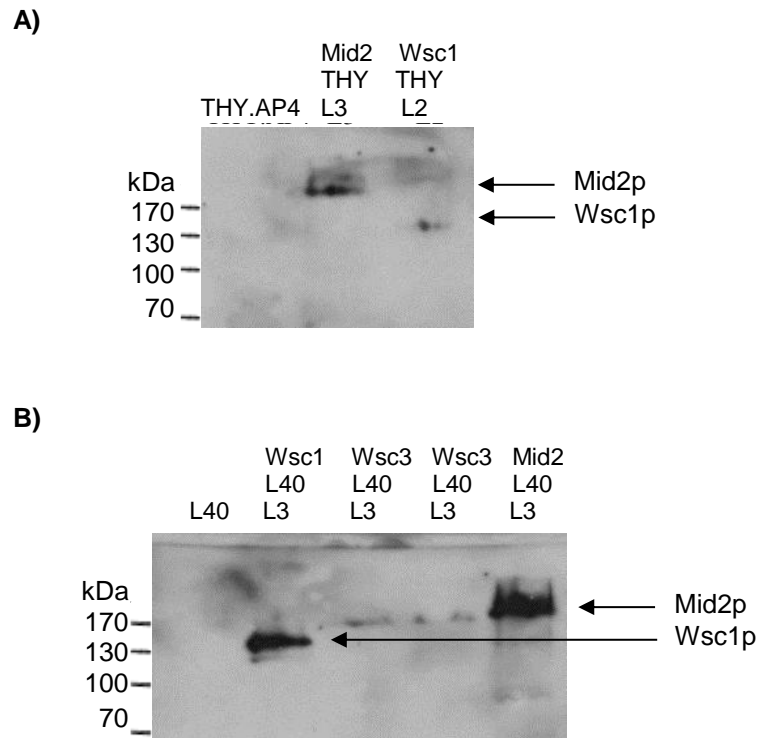
A)

B)

Figure S3. Validation of MYTH-tagged Mid2p and Wsc1p expression by Western blot. A) THY.AP4 and B) L40 background strains. Total cell extract of sensor bait constructs were examined using antibody against the VP16 component of the MYTH tag. THY.AP4 or L40 cells were used as negative control.

Figure S4. WSC1 and MID2 - iMYTH interactome showing only interactors identified in the iMYTH screen at 30°. The 14 novel Wsc1p and 31 Mid2p interactors are shown as color-coded geometric shapes according to the gene ontology annotation of biological process and localization. See Figure 3 for details. Black edge represents physical protein-protein interactions.
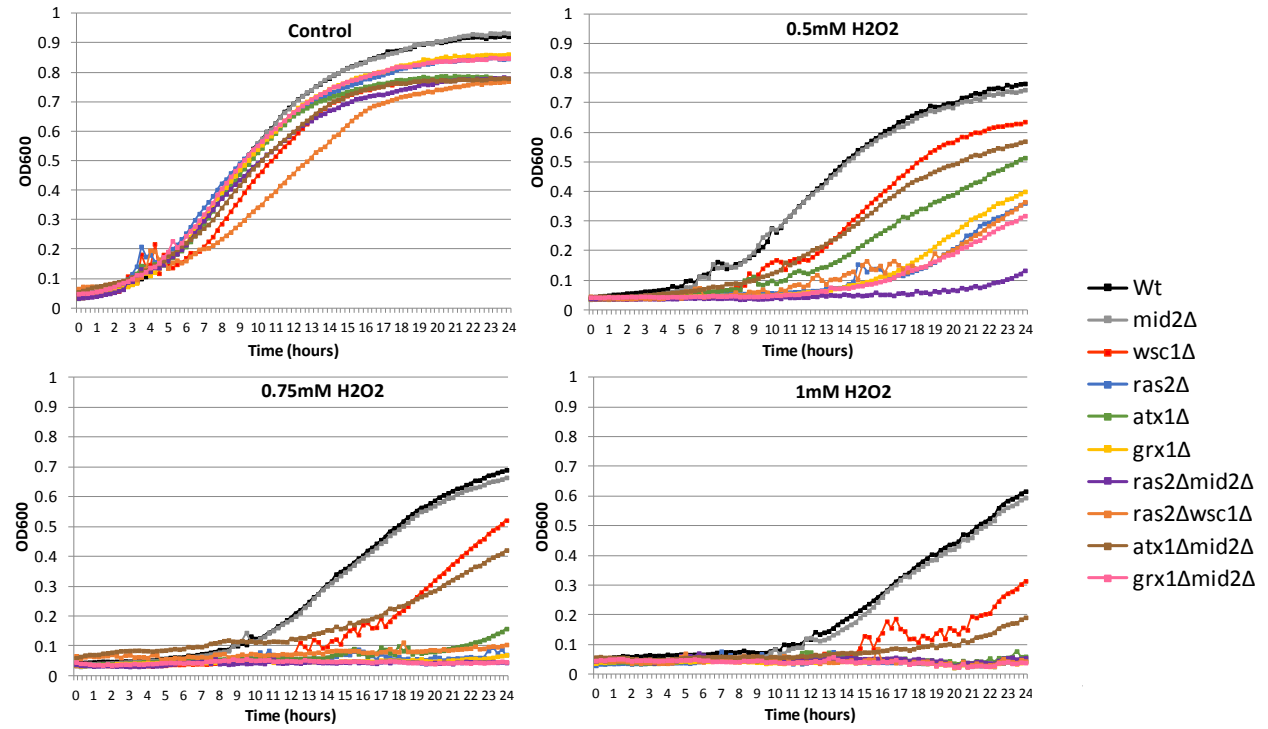
Figure S5. Growth curve analysis of single and double mutants exposed at different concentrations of Hydrogen Peroxide. The plates were incubated at 27° and OD_{600} measurements were taken every 15 min for up to 24 hrs.
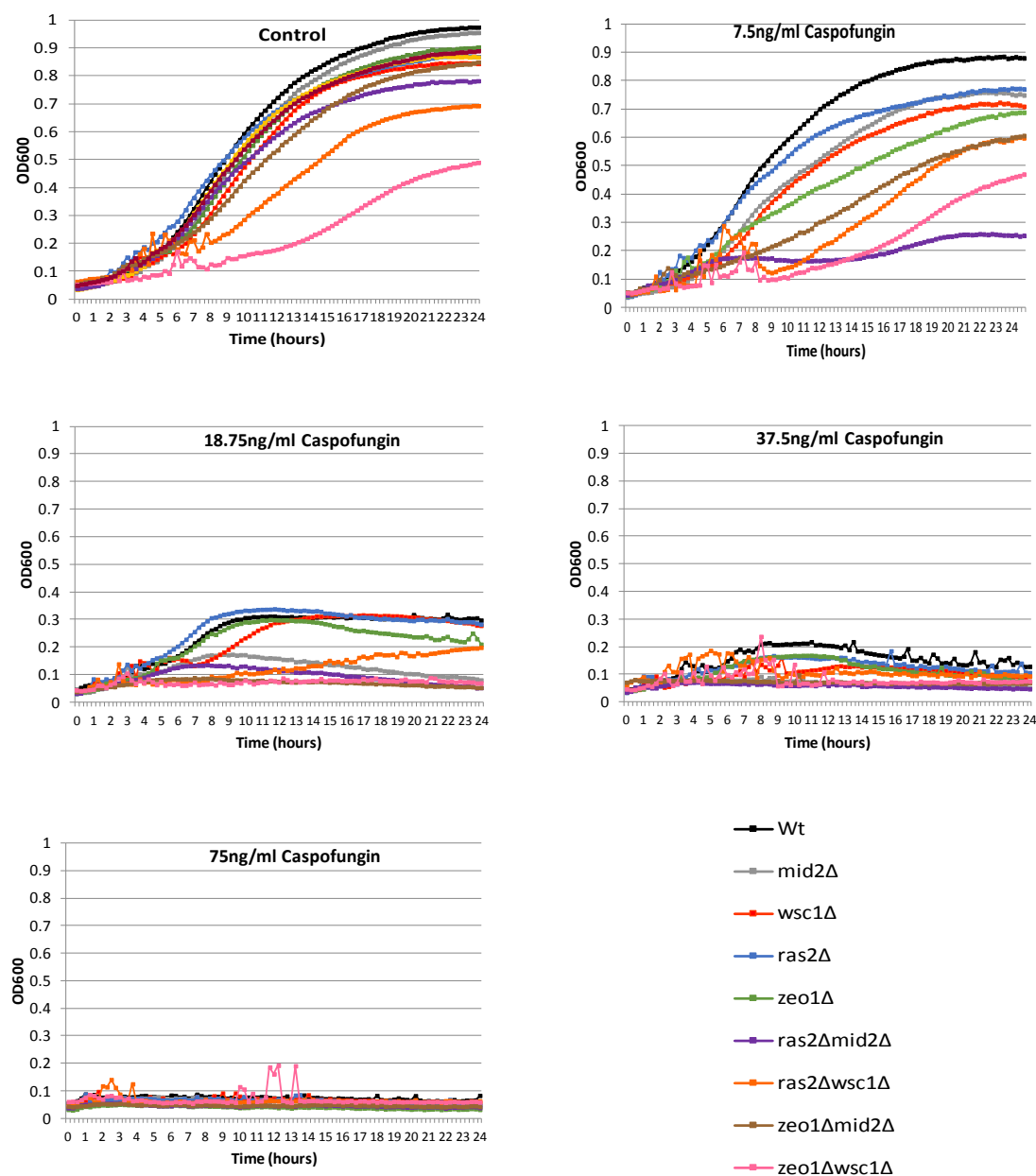
Figure S6. Growth curve analysis of single and double mutants exposed at different concentrations of Caspofungin. The plates were incubated at 27° and OD_{600} measurements were taken every 15 min for up to 24 hrs. The 7.5ng/ml Caspofungin graph (average of two replicates). Control, 18.75ng/ml, 37.5ng/ml, 75ng/ml Caspofungin graphs (average of three or more replicates).
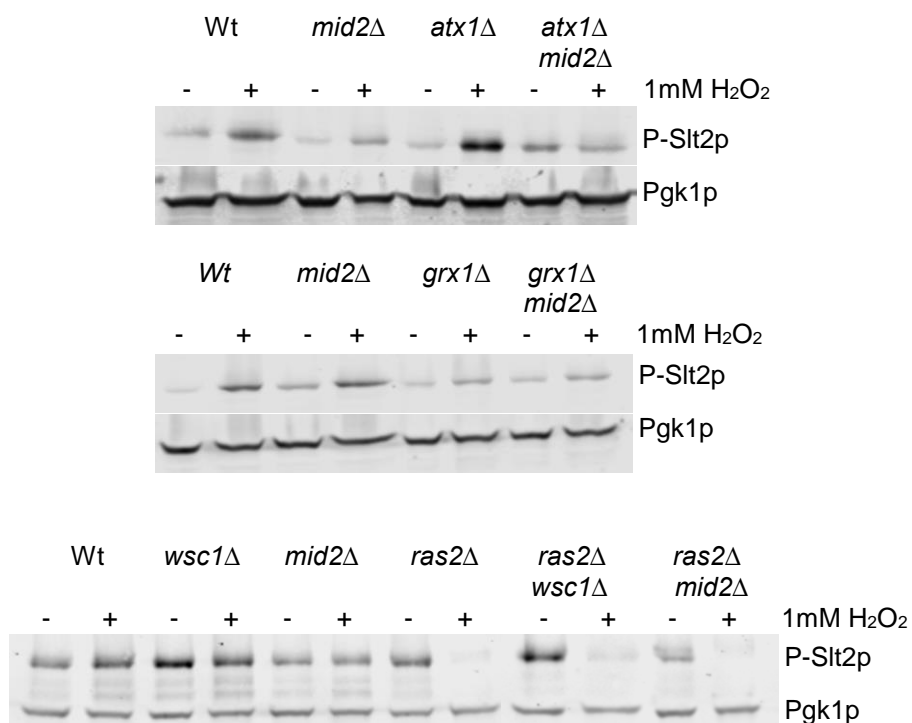
Figure S7. Western blot analysis showing the phosphorylation of Slt2p in single and double mutant strains treated with 1mM hydrogen peroxide (H_2O_2) for 1 hr at 27°. Blots shown are representative images from 3 biological replicates. The loading control used was Pgk1p.
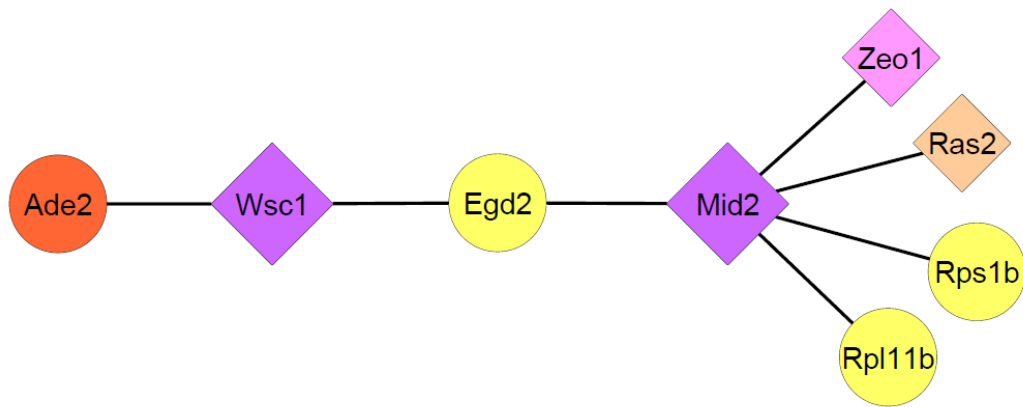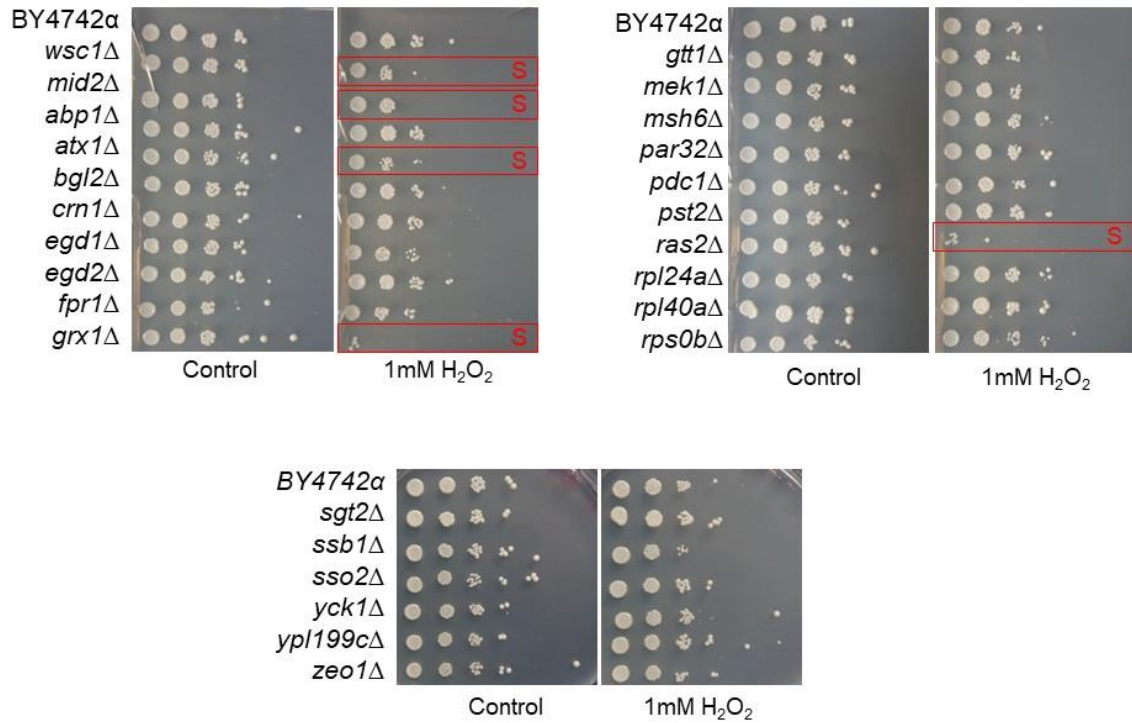
Figure S8. Western blot analysis showing the phosphorylation of Slt2p in single and double mutant strains treated with 75ng/ml Caspofungin for 1 hr at 27°. Blots shown are representative images from 3 biological replicates. The loading control used was Pgk1p.

Figure S9. Network graph of the Wsc1p and Mid2p interactome identified by iMYTH screen at 37°. At 37° the predominant interactor between Wsc1p and Mid2p was Egd2p identified by a yellow circle.
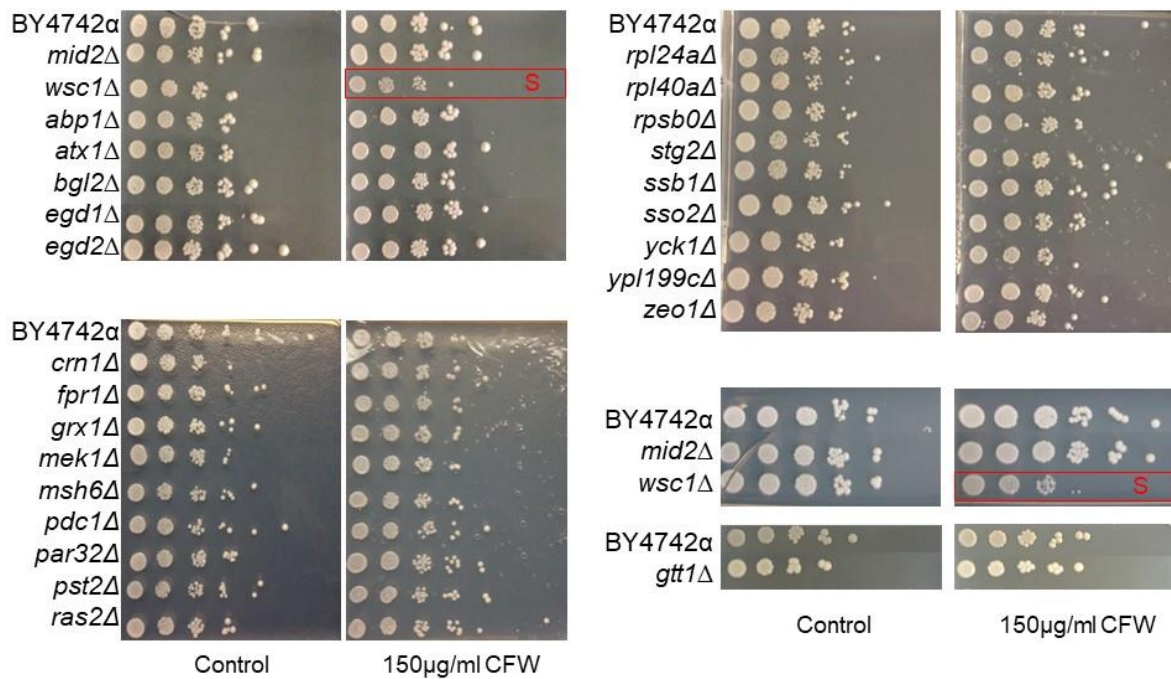
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Table S3. Percentages of positive interactors for Wsc1p and Mid2p identified by two independent iMYTH screens performed at 37º.

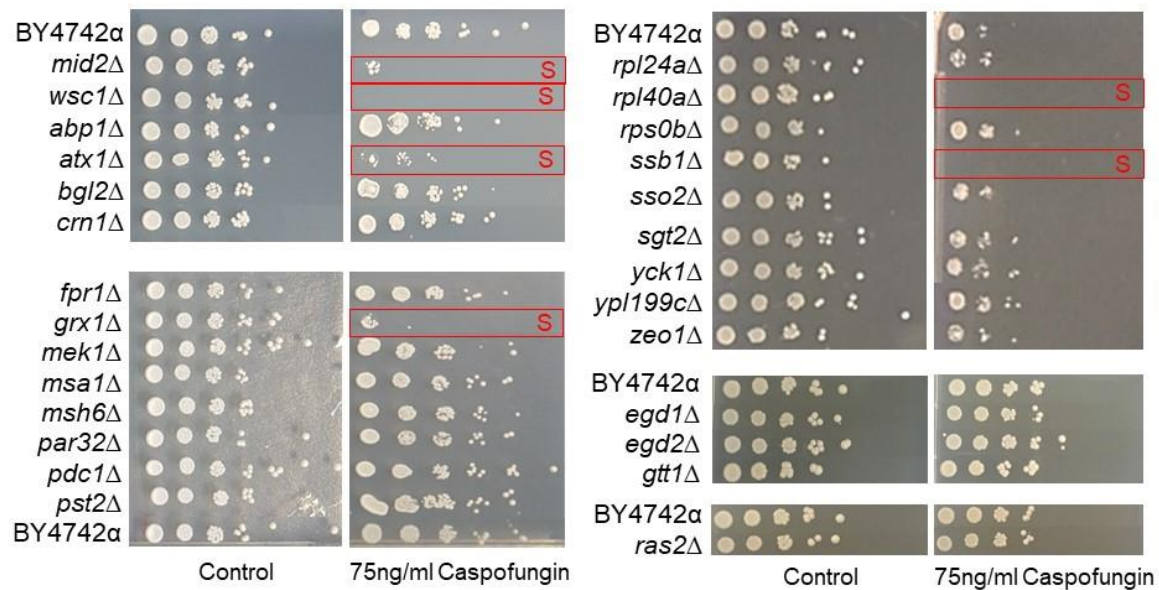| Interactor name	Percentage of hits for Mid2 bait protein	Percentage of hits for Wsc1 bait protein
Egd2	90	83
Rps1b	1.08	0
Ras2	2.17	0
Zeo1	1.08	0
Rpl11b	1.08	0
Ade2	0	5.5

A)
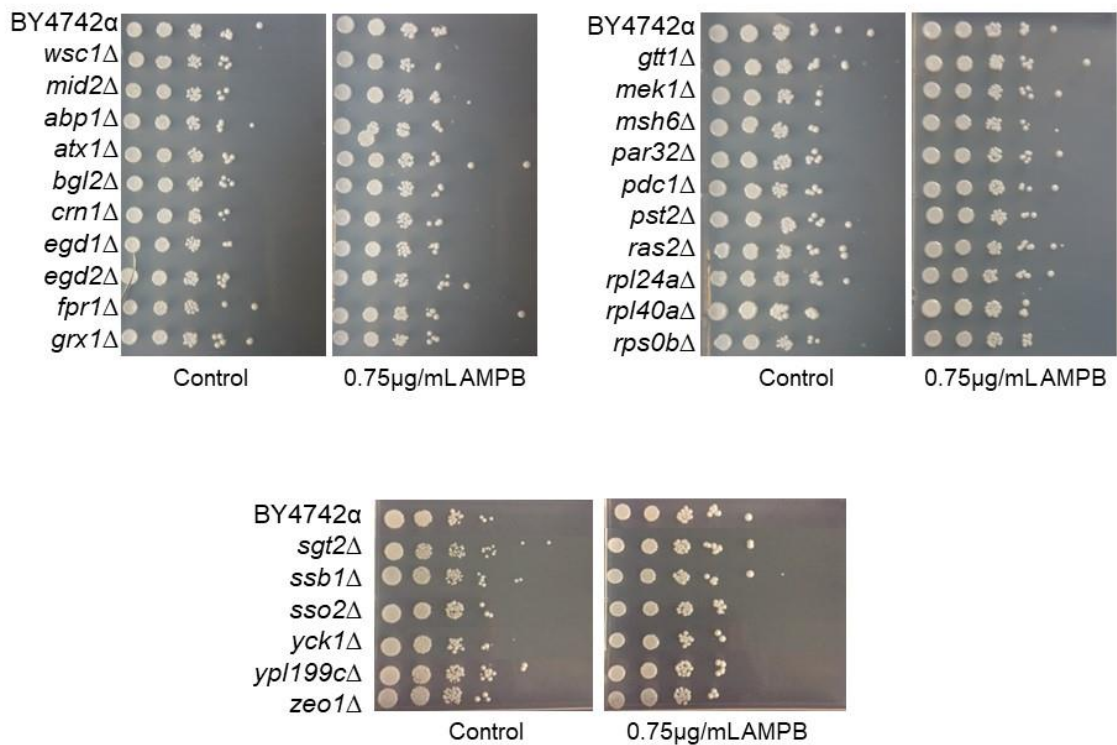

B)

C)

D)

Figure S10. A representative drop dilution assay of sensor and interactor null mutants exposed to stress conditions. Identical volumes of 10-fold serial dilutions of wild type (BY4724α) and single mutants were spotted onto CSM plates and incubated at 30°; A)1mM hydrogen peroxide (H_2O_2, oxidative stress); B) 150 µg/ml Calcofluor white (CFW); C) 75 ng/ml Caspofungin (CSP) and D) 0.75 µg/ml Amphotericin B (AMPB). The plates were inspected after three days of incubation.