SUPPLEMENTAL MATERIAL

Protein Interactions of the Mechanosensory Proteins Wsc2 and Wsc3 for Stress Resistance in *Saccharomyces cerevisiae*.

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Figure S2. Western blot analysis of phospho-Slt2p (P-Slt2p) levels in null mutant strains of Wsc2p and Yck2p treated with 75 ng/ml CS.

Table S1. Double mutant strains used in this study.

Strain	Genotype	Source
bmh1Δwsc3Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 bmh1Δ::kanMX4 wsc3Δ::URA3*	Lab Strain
cpr1Δwsc3Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 cpr1Δ::kanMX4 wsc3Δ::URA3*	Lab Strain
egd1Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 egd1Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
egd2Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 egd2Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
egd2Δwsc3Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 egd2Δ::kanMX4 wsc3Δ::URA3*	Lab Strain
gtt1Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 gtt1Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
msa1Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 msa1Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
par32Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 par32Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
ras2Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 ras2Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
tma7Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 tma7Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
wsc3Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 wsc3Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
yck1Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 yck1Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
yck1Δwsc3Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 yck1Δ::kanMX4 wsc3Δ::URA3*	Lab Strain
yck2Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 yck2Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
yke2Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 yke2Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
ypl199cΔwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 ypl199cΔ::kanMX4 wsc2Δ::URA3*	Lab Strain
wsc2Δwsc3Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 wsc2Δ::kanMX4 wsc3Δ::URA3*	Lab Strain
zeo1Δwsc2Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 zeo1Δ::kanMX4 wsc2Δ::URA3*	Lab Strain
zeo1Δwsc3Δ	*MAT α his3Δ1 leu2Δ0 lys2Δ0 zeo1Δ::kanMX4 wsc3Δ::URA3*	Lab Strain

Table S2. Primers used in this study.

Primer name	Sequence 5'→3'
Rev-KanMX-iMYTH	GAGCGTTTCCCTGCTCGCAG
Fwd-Wsc2-iMYTH	GAT GAT TAC GAT GAT GCA AAG GAT TCC AAT AAT AGT TCT TTG CGC ATG TCG GGG GGG ATC CCT CC
Rev-Wsc2-iMYTH	CGT GCA ATA ATC GTC GGC TTT AAT AAT ATT ATA CAT ACA AAG GGA CTA TAG GGA GAC CGG CAG
Wsc2 int chk 5'	CCT CGG TAG TGT ACG AGG AG
Fwd-Wsc3-iMYTH	AAATTCTATGATGAACAAGGTAACGAATTATCACCACGAAATTAT ATG TCG GGG GGG ATC CCT CC
Rev-Wsc3-iMYTH	CAG TTG AAG TGC CAA AAG CAG TAA ATT GTT TAA ATA TTA GGG TAT ACT ATA GGG AGA CCG GCA G
Wsc3 int chk 5'	CGA CAT GAT GAT GAG CGG ACC
Fwd-NubG	CCGATACCATCGACAACGTTAAGTCG
Fwd-Bmh1	TCG GTA GAT CGA AAA CAC AG
Fwd-Cpr1	TCT GCC TTA CCG ATG AGA CC
Fwd-Egd1	AAG CAG TAT GGC GAA GAA CG
Fwd-Egd2	GAC TGC CAG CTT TCC CGT CCG
Fwd-Gtt1	TT.G GGG GAG GAC ATA TTA CG
Fwd-Msa1	GAC ACA ATC GTT GAG GAT GC
Fwd-Par32	GAG AGT GGG ATG CGG GCG TG
Fwd-Ras2	CCC CTG CGC CAT GTC TCT GC
Fwd-Tma7	TAA GCG GAT ACA AGA TCA CC
Fwd-Yck1	GGG AGA ACG ACT GTG TCA TTG
Fwd-Yck2	ATT CCC GTC TTG ATG CTC TG
Fwd-Yke2	ACG GTC GTC GAA TAT TGA GG
Fwd-Ypl199c	GTG CGC GTG TGG GTA CCT CAA CTG
Fwd-Zeo1	CCC GAC GGT CCG GGT GCC TA
R Kan B	CTG CAG CGA GGA GCC GTA AT
Fwd-Wsc2-TAP	GAG CTC GTC CAA GAG CAA AG
Fwd-Wsc3-TAP	GCT GCC GCC TCA AAT AAT AG
Rev TAP Primer	AACCCGGGGATCCGTCGACC
Fwd-Wsc2	CGA AGA AAT AAA GAG GAG AAA CCC
Rev-Wsc2	GGT TCA AAT CCG ATT GGA AGC
Fwd-Wsc3	CTG CAG ACC AGG ACC ATT GT
Rev-Wsc3	GGT AAG GCG CCA CAC TAG

Table S3. Susceptibility profiles of single mutant strains of Wsc2p interactors.

Strains	Susceptibility		
	CS 75ng/ml	FCZ 100µM	H_2O_2 1mM
BY4742 α	NP	NP	NP
wsc2Δ	NP	S	NP
egd1Δ	NP	NP	
egd2Δ	NP	NP	
gtt1Δ	NP	S	NP
msa1Δ	NP	S	
ras2Δ	NP	NP	S
tma7Δ	NP	NP	
yck1Δ	NP	NP	
yck2Δ	NP	NP	
ypl199cΔ	S*	NP	
zeo1Δ	NP	NP	

NP- no phenotype; S- sensitive; S*- displayed a general reduction in growth and viability in untreated cultures.

Table S4. Susceptibility profiles of double mutant strains of Wsc2p and interactors.

Strains	Susceptibility		
	CS 75ng/ml	FCZ 100µM	H_2O_2 1mM
BY4742 α	NP	NP	NP
egd1Δwsc2Δ	NP	NP	
egd2Δwsc2Δ	NP	R	
gtt1Δwsc2Δ	NP	S	NP
msa1Δwsc2Δ	S	NP	
ras2Δwsc2Δ	NP	NP	S
tma7Δwsc2Δ	NP	NP	
yck1Δwsc2Δ	NP	NP	
yck2Δwsc2Δ	S*	R	
ypl199cΔwsc2Δ	S*	NP	
zeo1Δwsc2Δ	NP	NP	

NP- no phenotype; S- sensitive; S*- displayed a general reduction in growth and viability in untreated cultures.

Table S5. Susceptibility profiles of single mutant strains of Wsc3p interactors.

Strains	Susceptibility	
	CS 75ng/ml	FCZ 100µM
BY4742 α	NP	NP
wsc3Δ	NP	S
bmh1Δ	NP	S
cpr1Δ	NP	NP
egd2Δ	NP	NP
yck1Δ	NP	NP
zeo1Δ	NP	NP

NP- no phenotype; S- sensitive

Table S6. Susceptibility profiles of double mutant strains of Wsc3p and interactors.

Strains	Susceptibility	
	CS 75ng/ml	FCZ 100µM
BY4742 α	NP	NP
bmh1Δwsc3Δ	NP	NP
cpr1Δwsc3Δ	NP	NP
egd2Δwsc3Δ	NP	R
yck1Δwsc3Δ	NP	NP
zeo1Δwsc3Δ	NP	NP

NP- no phenotype; R- resistant

Table S7. Colony Forming Units (CFUs) assay of hypersensitive mutant strains of Wsc2p, Wsc3p, and their interactors exposed to 2% DMSO vehicle and 100 µM Fluconazole (FCZ). N=3 for all experiments.

Strain ID	CFU count				% growth inhibition	Comments
	2% DMSO vehicle	Average	100 µM FCZ	Average		
BY4742 α	23, 24, 21	22.7	4, 6, 9	6.3	72% (NP)	Very small colonies (< 1 mm) FCZ plate, Reference value for (NP)
wsc2	35, 33, 35	34.3	0, 0, 0	0	100% (S)	Consistent with spotting test result (S)
wsc3	3, 6, 13	7.3	0, 0, 0	0	100% (S)	Very small colonies (< 1 mm) FCZ plate, Consistent with spotting test result (S)
bmh1	22, 26, 26	24.7	1, 0, 0	0.33	99% (S)	Consistent with spotting test result (S)
gtt1	34, 29, 28	30.3	1, 0, 0	0.33	99% (S)	Consistent with spotting test result (S)
msa1	17, 20, 14	17	1, 0, 1	0.67	96% (S)	Consistent with spotting test result (S)
gtt1wsc2	458, 378, 388	408	91, 84, 88	87.7	79% (S)	Very small colonies (< 1 mm) FCZ plate, Consistent with spotting test result (S)

Overnight cell cultures were diluted to 100 cells and each was spread onto agar plates containing 2% DMSO vehicle or 100µM Fluconazole (FCZ) in triplicate. After culturing for 2 days at 30°, CFUs were counted for each strain and the mean was calculated from an n=3. Percent of growth inhibition was calculated as [1 – (mean of 100µM FCZ colonies / mean of 2% DMSO colonies)] X 100. (**NP- no phenotype; S- sensitive**).

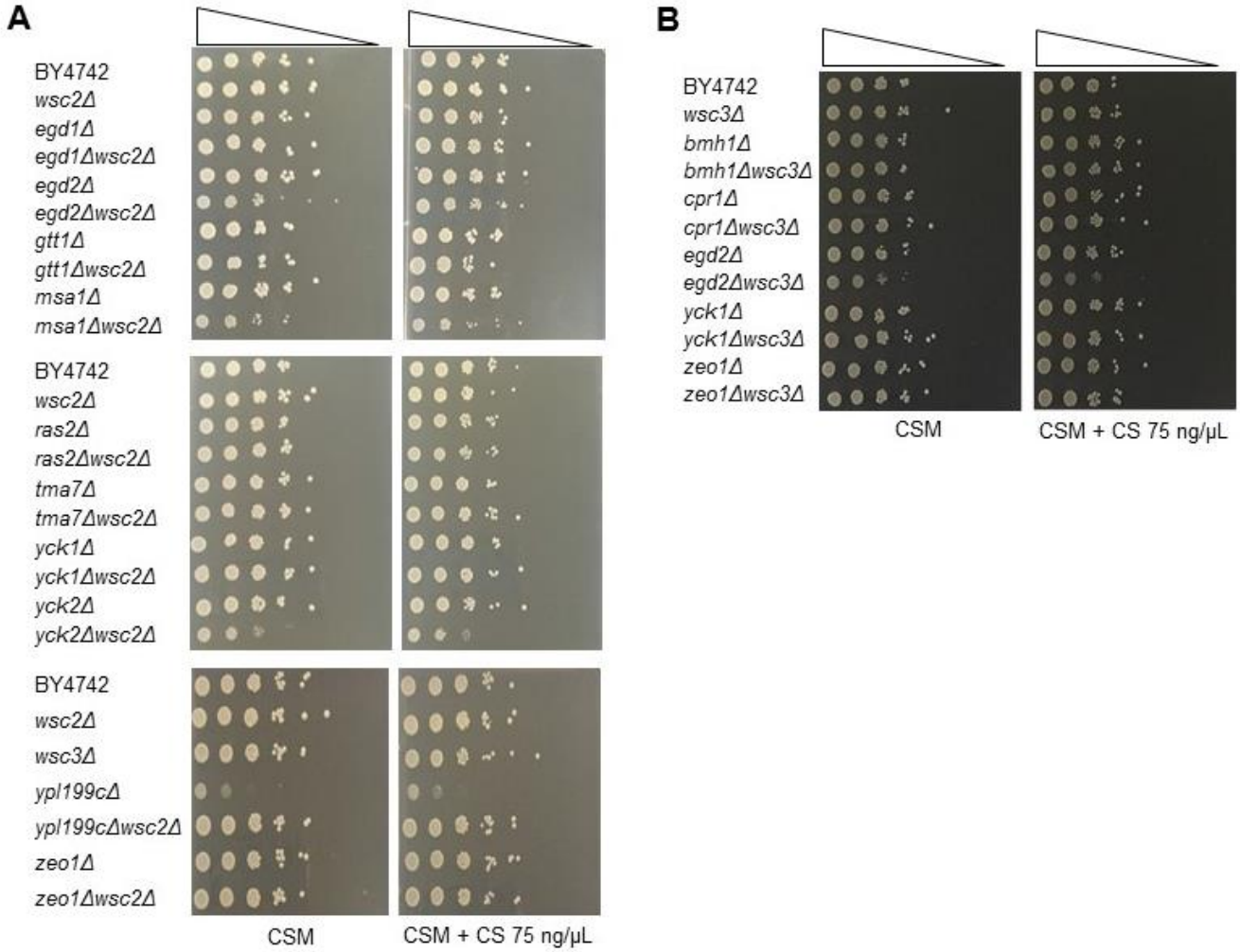

Figure S1. A representative spotting test for growth of null mutant strains of Wsc2p, Wsc3p, and their interactors treated with 75 ng/ml CS. Identical volumes representing 10-fold serial dilutions (10^8-10^2) of wild type (BY4742α), single, and double mutants, were spotted onto CSM plates with or without treatments and incubated at 30°. From left to right, A: *wsc2Δ* interactors with no treatment (CSM), treated with 75 ng/ml CS; and B: *wsc3Δ* interactors with no treatment (CSM), treated with 75 ng/ml CS. Three replicates were done for each experiment.

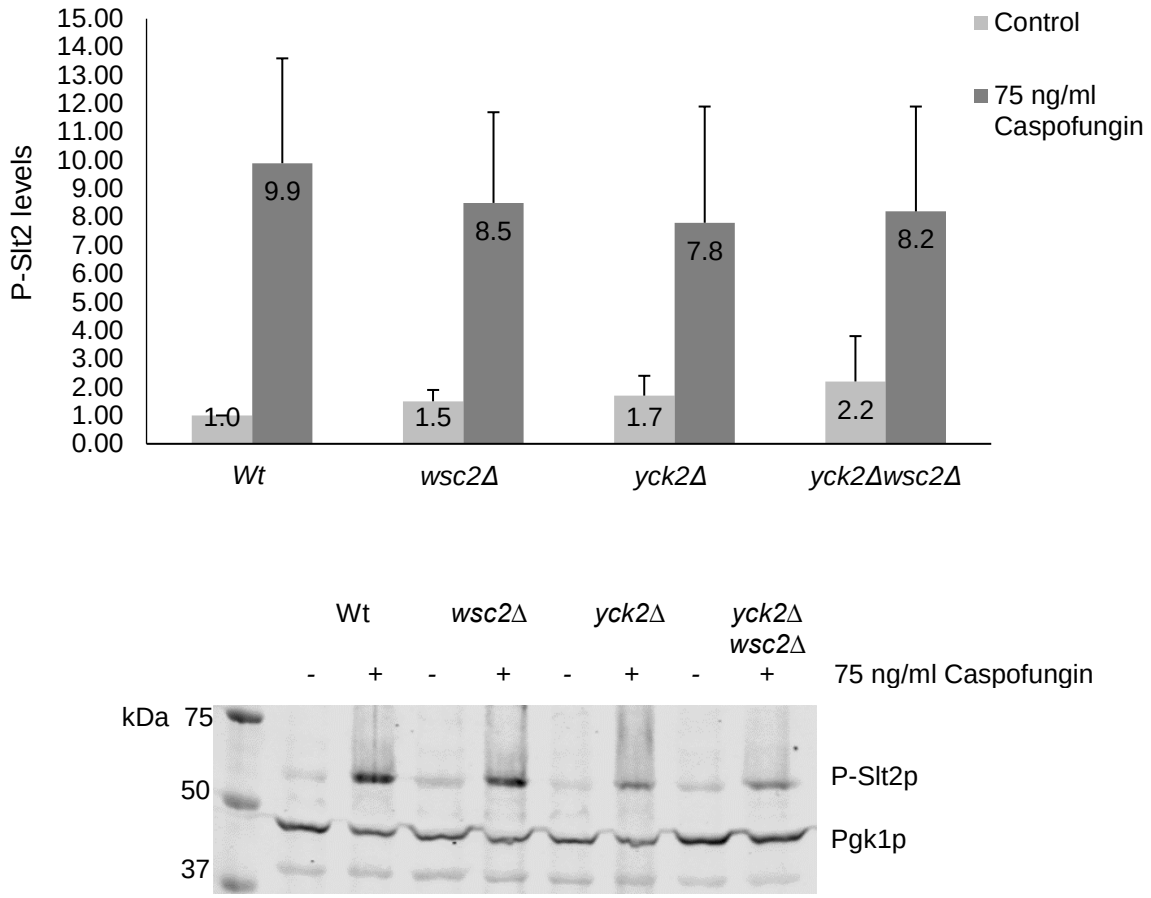

Figure S2. Western blot analysis of phospho-Slt2p (P-Slt2p) levels in null mutant strains of Wsc2p and Yck2p treated with 75 ng/ml CS. Total protein extracts derived from treated and control cultures reacted with anti-phospho p44/42 MAPK (P-Slt2p) and anti phosphoglycerate kinase (Pgk1p) antibodies by Western blot analysis as described in the Materials and Methods. Numerical values shown within each histogram represent fold-change of the P-Slt2p intensity in each sample adjusted to the P-Slt2p intensity of wild type control (WT) and normalized against Pgk1p. Each data point shown represents the mean $\pm$ SEM of $n \geq 3$.