Supplemental Table 1: Genomic hits from high throughput CHI screens with *vma13Δ* and *vma3Δ* mutants.

***vma13*△ CHI Hits**

System Name	Standard Name	Description
YJL210W	*PEX2*	RING-finger peroxin and E3 ubiquitin ligase, peroxisomal membrane protein with a C-terminal zinc-binding RING domain, forms translocation subcomplex with Pex10p and Pex12p which functions in peroxisomal matrix protein import
YLR006C	*SSK1*	Cytoplasmic response regulator, part of a two-component signal transducer that mediates osmosensing via a phosphorelay mechanism; dephosphorylated form is degraded by the ubiquitin-proteasome system; potential Cdc28p substrate
YMR299C	*DYN3*	Dynein light intermediate chain (LIC); localizes with dynein, null mutant is defective in nuclear migration
YOR008C	*SLG1*	Sensor-transducer of the stress-activated PKC1-MPK1 kinase pathway involved in maintenance of cell wall integrity; involved in organization of the actin cytoskeleton; secretory pathway Wsc1p is required for the arrest of secretion response
YOL025W	*LAG2*	Protein that negatively regulates the SCF E3-ubiquitin ligase by interacting with and preventing neddyation of the cullin subunit, Cdc53p; longevity determinant that is preferentially expressed in young cells; similar to mammalian Cand1
YBL015W	*ACH1*	Protein with CoA transferase activity, particularly for CoASH transfer from succinyl-CoA to acetate; has minor acetyl-CoA-hydrolase activity; phosphorylated; required for acetate utilization and for diploid pseudohyphal growth
YDR130C	*FIN1*	Spindle pole body-related intermediate filament protein; forms cell cycle-specific filaments between spindle pole bodies in mother and daughter cells; localization cell-cycle dependent; involved in Glc7p localization and regulation
YOR208W	*PTP2*	Phosphotyrosine-specific protein phosphatase involved in the inactivation of mitogen-activated protein kinase (MAPK) during osmolarity sensing; dephosporylates Hog1p MAPK and regulates its localization; localized to the nucleus
YIL017C	*VID28*	Protein involved in proteasome-dependent catabolite degradation of fructose-1,6-bisphosphatase (FBPase); localized to the nucleus and the cytoplasm
YBR019C	*GAL10*	UDP-glucose-4-epimerase, catalyzes the interconversion of UDP-galactose and UDP-D-glucose in galactose metabolism; also catalyzes the conversion of alpha-D-glucose or alpha-D-galactose to their beta-anomers
YBR212W	*NGR1*	RNA binding protein that negatively regulates growth rate; interacts with the 3' UTR of the mitochondrial porin (POR1) mRNA and enhances its degradation; overexpression impairs mitochondrial function; expressed in stationary phase

YPR164W	*MMS1*	Subunit of an E3 ubiquitin ligase complex involved in resolving replication intermediates or preventing the damage caused by blocked replication forks; regulates Ty1 transposition; involved with Rtt101p in nonfunctional rRNA decay
YDR003W	*RCR2*	Vacuolar protein that presumably functions within the endosomal-vacuolar trafficking pathway, affecting events that determine whether plasma membrane proteins are degraded or routed to the plasma membrane; similar to Rcr1p
YBR119W	*MUD1*	U1 snRNP A protein, homolog of human U1-A; involved in nuclear mRNA splicing
YAL002W	*VPS8*	Membrane-associated protein that interacts with Vps21p to facilitate soluble vacuolar protein localization; component of the CORVET complex; required for localization and trafficking of the CPY sorting receptor; contains RING finger motif. Decreased ionic stress resistance in null. Increased PMA1 mRNA accumulation; Increased inositol excretion
YJL140W	*RPB4*	RNA polymerase II subunit B32; forms two subunit dissociable complex with Rpb7p; involved in recruitment of 3'-end processing factors to transcribing RNA polymerase II complex and in export of mRNA to cytoplasm under stress conditions. Decreased invasive growth in null. Abnormal cell shape. Decreasd oxidative stress resistance
YMR214W	*SCJ1*	One of several homologs of bacterial chaperone DnaJ, located in the ER lumen where it cooperates with Kar2p to mediate maturation of proteins. Increased glycogen accumulation
YPL050C	*MNN9*	Subunit of Golgi mannosyltransferase complex also containing Anp1p, Mnn10p, Mnn11p, and Hoc1p that mediates elongation of the polysaccharide mannan backbone; forms a separate complex with Van1p that is also involved in backbone elongation. Increased glycogen accumulation.
YBR173C	*UMP1*	Short-lived chaperone required for correct maturation of the 20S proteasome; may inhibit premature dimerization of proteasome half-mers; degraded by proteasome upon completion of its assembly.
YDL090C	*RAM1*	Beta subunit of the CAAX farnesyltransferase (FTase) that prenylates the a-factor mating pheromone and Ras proteins; required for the membrane localization of Ras proteins and a-factor; abnormal polyphosphate accumulation, decreased hyperosmotic stress resistance in null.

YGL012W	*ERG4*	C-24(28) sterol reductase, catalyzes the final step in ergosterol biosynthesis; mutants are viable, but lack ergosterol. Null has increased invasive growth, abnormal Ste20 distribution, decreased acid pH resistance, decresed endocytosis, decreased hyperosmotic stress resistance, decreased ionic stress resistance.
YGL038C	*OCH1*	Mannosyltransferase of the cis-Golgi apparatus, initiates the polymannose outer chain elongation of N-linked oligosaccharides of glycoproteins
YGR229C	*SMI1*	Protein involved in the regulation of cell wall synthesis; proposed to be involved in coordinating cell cycle progression with cell wall integrity. Abnormal polyphosphate accumulation, decreased resistance to calcium dichloride,
YER044C	*ERG28*	Endoplasmic reticulum membrane protein, may facilitate protein-protein interactions between the Erg26p dehydrogenase and the Erg27p 3-ketoreductase and/or tether these enzymes to the ER, also interacts with Erg6p. Null: increased inositol excretion, abnormal mitochondrial morphology
YDL067C	*COX9*	Subunit VIIa of cytochrome c oxidase, which is the terminal member of the mitochondrial inner membrane electron transport chain. Decreased acid pH resistance, decreased glycogen accumulation

***vma3*∆ CHI Hits**

System Name	Standard Name	Description
YJR103W	*URA8*	Minor CTP synthase isozyme (see also URA7), catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to UTP, forming CTP, the final step in de novo biosynthesis of pyrimidines; involved in phospholipid biosynthesis. Decreased acid resistance in null.
YEL027W	*VMA3*	V-ATPase subunit [same as CHI bait, so homozygous diploid was identified.]
YIL049W	*DFG10*	unknown function; involved in filamentous growth. Decreased endocytosis and no pseudohyphal growth in null.
YDL025C	*RTK1*	Putative protein kinase, potentially phosphorylated by Cdc28p; interacts with ribosome biogenesis factors, Cka2, Gus1 and Arc1. Null has increased glycogen accumulation.
YGR003W	*CUL3*	Ubiquitin-protein ligase, member of the cullin family with similarity to Cdc53p and human CUL3; required for ubiquitin-dependent degredation of the RNA Polymerase II subunit RPO21

YGR023W	*MTL1*	putative plasma membrane sensor, involved in cell integrity signaling and stress response during glucose starvation and oxidative stress. Structural and functional similarity to Mid2. Decreased glycogen accumulation.
YJL128C	*PBS2*	MAP kinase kinase that plays a pivotal role in the osmosensing signal-transduction pathway, activated under severe osmotic stress; decreased hyperosmotic stress resistance, increased invasive growth, and abnormal cell shape in null.
YJL160C		Putative protein of unknown function; member of the PIR (proteins with internal repeats) family of cell wall proteins;
YDL194W	*SNF3*	Plasma membrane low glucose sensor that regulates glucose transport; contains 12 predicted transmembrane segments and a long C-terminal tail required for induction of hexose transporters; also senses fructose and mannose; similar to Rgt2p
YNL069C	*RPL16B*	N-terminally acetylated protein component of the large (60S) ribosomal subunit, binds to 5.8 S rRNA; has similarity to Rpl16Ap, E. coli L13 and rat L13a ribosomal proteins; transcriptionally regulated by Rap1p. Haploinsufficient. Decreased endocytosis.
YDR538W	*PAD1*	phenylacrylic acid decarboxylase, decarboxylates aromatic carboxylic acids to corresponding vinyl derivatives, has mRNA binding activity
YER121W		Putative protein of unknown function; may be involved in phosphatase regulation and/or generation of precursor metabolites and energy
YLR034C	*SMF3*	Putative divalent metal ion transporter involved in iron homeostasis; transcriptionally regulated by metal ions; member of the Nramp family of metal transport proteins. Localizes to vacuole
YKL205W	*LOS1*	Nuclear pore protein involved in nuclear export of pre-tRNA and in re-export of mature tRNAs after their retrograde import from the cytoplasm
YML055W	*SPC2*	Subunit of signal peptidase complex (Spc1p, Spc2p, Spc3p, Sec11p), which catalyzes cleavage of N-terminal signal sequences of proteins targeted to the secretory pathway;

Three separate sets of three parallel, independent screens were performed, two sets with *vma13Δ* and one set with *vma3Δ* as described in Materials and Methods. Mutants that were scored as having CHI interactions in two of the three screens in any of the three sets are listed. Scoring was done manually, by comparing growth on -FOA plates (where the plasmid is maintained, masking the *vma13Δ* or *vma3Δ* mutation) to growth on +FOA plates (where the plasmid is lost, unmasking the complex heterozygosity). Plates were scored after 2 days of growth at 30°C.

The screen is far from saturating because different mutations were identified in the first and second set of *vma13Δ* CHI screens. Limited overlap between screens along with high false positive and false negative rates have been noted previously for high-throughput CHI screens (Haarer *et al.*, 2007). Nevertheless, these high throughput screens have proven to be valuable in combination with manual validation of interactions ((Haarer *et al.*, 2007); see Fig. 2 and Supp. Fig. 1).

Supplemental Table 2: FUNSPEC analysis of combined hits from CHI screens

MIPS Functional Classification

Category	p-value	Genes in category from screens
Protein Binding	4.995×10^{-5}	*UMP1 FIN1 CUL3 PBS2 PEX2 LOS1 SSK1 SLG1 SPC2 SCJ1*
Osmosensing and Response	0.001014	*PBS2 SSK1 PTP2*
Cell Wall	0.001222	*SNF3 MTL1 SMI1 LAG2 SLG1 MNN9*
N-directed Glycosylation, Deglycosylation	0.001852	*OCH1 SCJ1 MNN9*

MIPS Phenotypes

Osmotic Sensitivity	0.001852	*PBS2 SLG1 MNN9*

GO Biological Process

ER-Nucleus Signaling Pathway	0.0007639	*MTL1 SMI1 SLG1 PTP2*
Intracellular Protein Kinase Cascade	0.00141	*SSK1 PTP2*
Osmosensory Signaling Pathway	0.001717	*PBS2 PTP2*

GO Molecular Function

alpha-1,6-mannosyltransferase Activity	0.00141	*OCH1 MNN9*

Yeast Fitness Category

Slow Growers	0.001967	*UMP1 RAM1 ERG28 OCH1 VID28 DFG10 RPL16B SLG1 MNN9 MMS1*

The combined lists of genes from Supplemental Table 1 were submitted to FUNSPEC (Toronto) (Robinson *et al.*, 2002). All items from each category that had a p value < 0.002 are shown.

Supplemental Table 3. Osmotic-related mRNA transcripts that are significantly induced in a *vma2*Δ mutant (Milgrom *et al.*, 2007)

ORF name	Gene name	Fold overexpression in *vma2*Δ	Description from Saccharomyces Genome Database
YPL223C	*GRE1*	3.46	Hydrophilin of unknown function; stress induced (osmotic, ionic, oxidative, heat shock and heavy metals); regulated by the HOG pathway.
YOL059W	*GPD2*	2.73	NAD-dependent glycerol 3-phosphate dehydrogenase, homolog of Gpd1p, expression is controlled by an oxygen-independent signaling pathway required to regulate metabolism under anoxic conditions; located in cytosol and mitochondria. (Hog1-dependent transcription of Gpd2 is essential for resistance to long-term salt stress, particularly in the absence of Gpd1; Westfall et al, 2008)
YML131W	---	2.28	Putative protein of unknown function with similarity to oxidoreductases; mRNA expression is increased in a HOG1 and SKO1-dependent manner after osmotic shock; GFP-fusion protein localizes to the cytoplasm
YOR208W	*PTP2*	2.28	Phosphotyrosine-specific protein phosphatase involved in the inactivation of mitogen-activated protein kinase (MAPK) during osmolarity sensing; dephosphorylates Hog1p MAPK and regulates its localization
YMR175W	*SIP18*	2.94	Phospholipid-binding protein; expression is induced by osmotic stress.
YLL052C	*AQY2*	2.17	Water channel that mediates the transport of water across cell membranes, only expressed in proliferating cells, controlled by osmotic signals.

Supplemental Table 4. Transcripts negatively regulated by Hog1p (Rep *et al.*, 2000) that are signficantly downregulated in a *vma2Δ* mutant (Milgrom *et al.*, 2007)

ORF Name	Gene Name	Relative transcript level *vma2Δ/wt*	Description from SGD
YBR040w	*FIG1*	0.241	Integral membrane protein required for efficient mating; may participate in or regulate the low affinity Ca2+ influx system, which affects intracellular signaling and cell-cell fusion during mating
YDR461w	*MFA1*	0.337	Mating pheromone a-factor, made by a cells
YGL032c	*AGA2*	0.339	Adhesion subunit of a-agglutinin of a-cells
YCL027w	*FUS1*	0.344	Membrane protein localized to the shmoo tip, required for cell fusion; expression regulated by mating pheromone; proposed to coordinate signaling, fusion, and polarization events required for fusion
YNR044w	*AGA1*	0.361	Anchorage subunit of a-agglutinin of a-cells; linked to adhesion subunit Aga2p via two disulfide bonds
YBR083w	*TEC1*	0.409	Transcription factor required for full Ty1 expression, Ty1-mediated gene activation, and haploid invasive and diploid pseudohyphal growth
YFL170c	*ASG7*	0.426	Protein that regulates signaling from a G protein beta subunit Ste4p and its relocalization within the cell; specific to a-cells and induced by alpha-factor
YNL279w	*PRM1*	0.441	Pheromone-regulated multispanning membrane protein involved in membrane fusion during mating; predicted to have 5 transmembrane segments and a coiled coil domain; localizes to the shmoo tip; regulated by Ste12p

YIL015w	*BAR1*	0.451	Aspartyl protease secreted into the periplasmic space of mating type a cells, helps cells find mating partners, cleaves and inactivates alpha factor allowing cells to recover from alpha-factor-induced cell cycle arrest

References for Supplementary Tables:

Haarer, B., Viggiano, S., Hibbs, M.A., Troyanskaya, O.G., and Amberg, D.C. (2007). Modeling complex genetic interactions in a simple eukaryotic genome: actin displays a rich spectrum of complex haploinsufficiencies. Genes Dev *21*, 148-159.
Milgrom, E., Diab, H., Middleton, F., and Kane, P.M. (2007). Loss of vacuolar proton-translocating ATPase activity in yeast results in chronic oxidative stress. J Biol Chem *282*, 7125-7136.
Rep, M., Krantz, M., Thevelein, J.M., and Hohmann, S. (2000). The transcriptional response of Saccharomyces cerevisiae to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high osmolarity glycerol pathway-dependent genes. J Biol Chem *275*, 8290-8300.
Robinson, M.D., Grigull, J., Mohammad, N., and Hughes, T.R. (2002). FunSpec: a web-based cluster interpreter for yeast. BMC Bioinformatics *3*, 35.

Supplemental Figure Legends

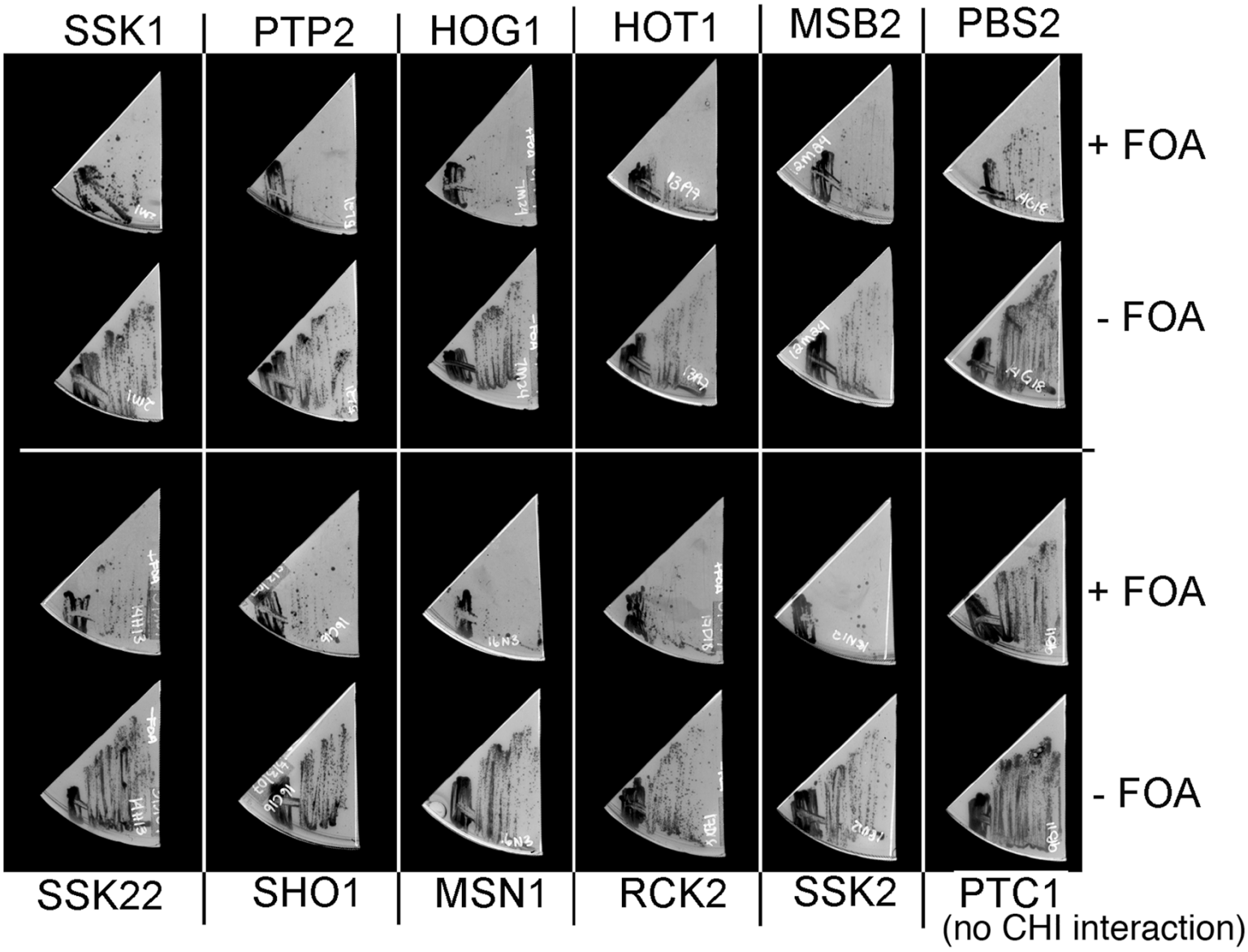
Figure S1. CHI interactions between *vma3*Δ and HOG pathway members.

Members of the HOG MAPK pathway (see Fig.2b) were re-tested for CHI interactions with *vma13*Δ and *vma3*Δ. The *vma13*Δ*::Nat*^{R} strain (S1a) or the *vma3*Δ::Nat^R strain (S1b) was mated to different HOG pathway deletion mutants from the Euroscarf library. "+FOA" and "-FOA" plates were incubated at 30 °C for 48 hours before scoring. Poor growth on –FOA relative to growth on +FOA medium indicates a CHI interaction. No CHI interaction was observed with *PTC1* (S1a) or *PAM1* (S1b), so these are shown as controls. (Note that a subset of the *vma13*Δ CHI interactions is shown in Fig. 2a, but these are included with a larger set of Hog pathway deletion mutants here for comparison.)

Figure S2. Growth curves of haploid wildtype, *vma3*Δ, *pbs2*Δ and *vma3*Δ*pbs2*Δ mutants in liquid synthetic complete media containing increasing concentrations of salt (A – 0 M NaCl, B – 0.25 M NaCl, C – 0.5 M NaCl). Growth was observed over the span of 24 hours at 30 °C.

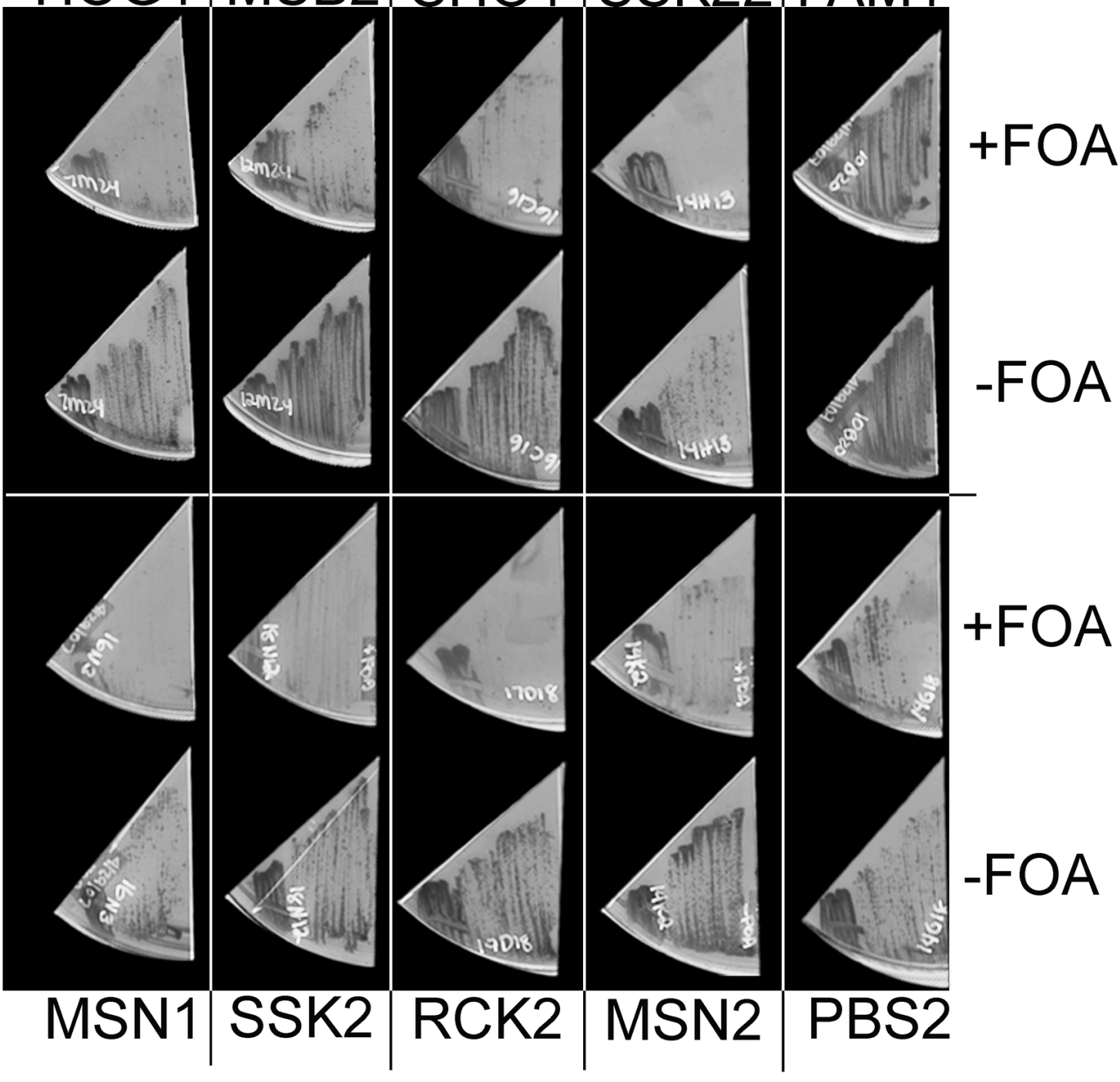
Supplemental Figure 1a:

Phenotypes of *vma13Δ* compound heterozygotes

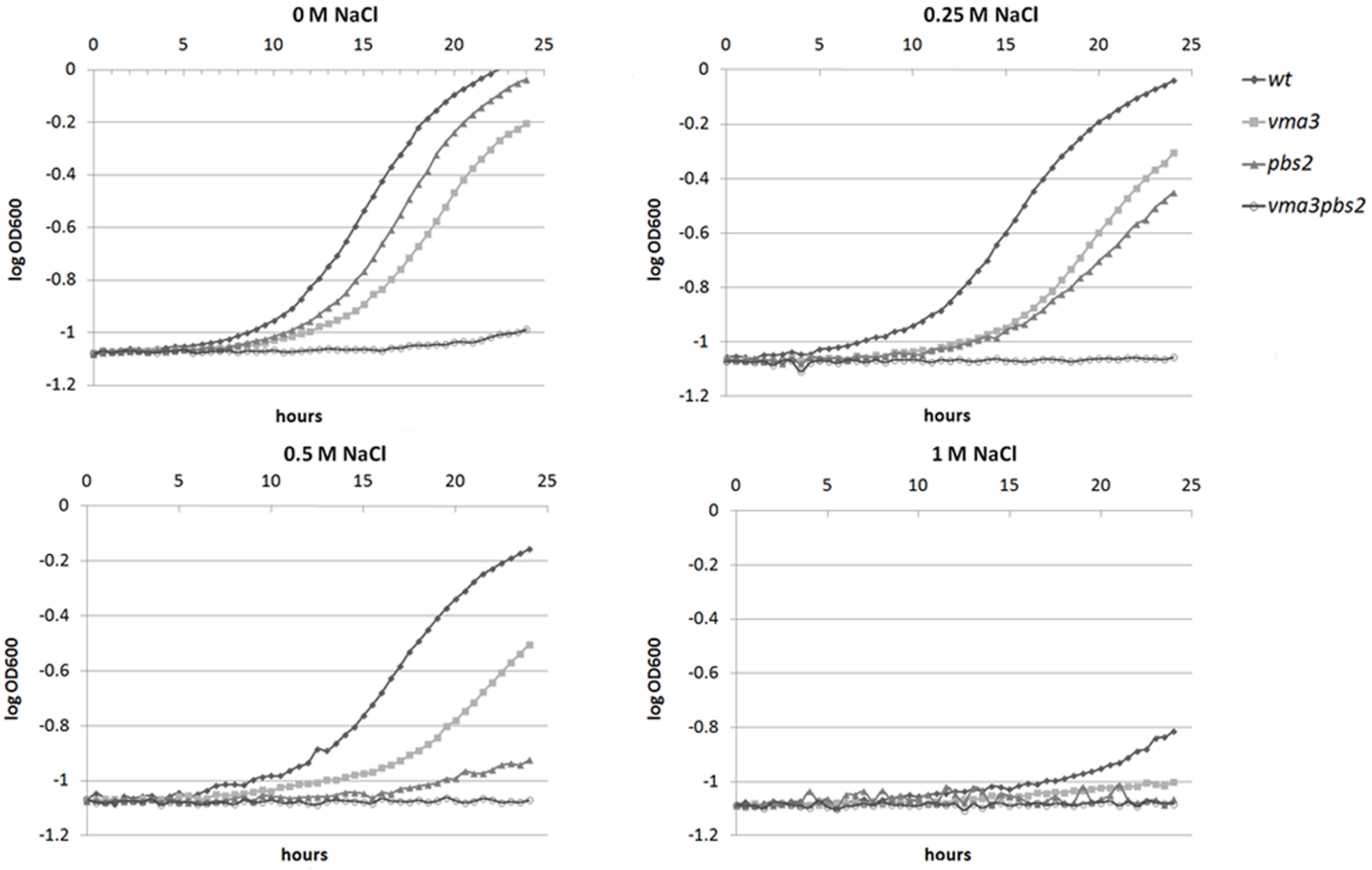


Supplemental Fig. 1b:

Phenotypes of *vma3Δ* compound heterozygotes (no CHI interaction)



Supp. Fig. 2


0 M NaCl
0.25 M NaCl
0.5 M NaCl
1 M NaCl
log OD600
hours
0
5
10
15
20
25
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
wt
vma3
pbs2
vma3pbs2