Supplementary Data

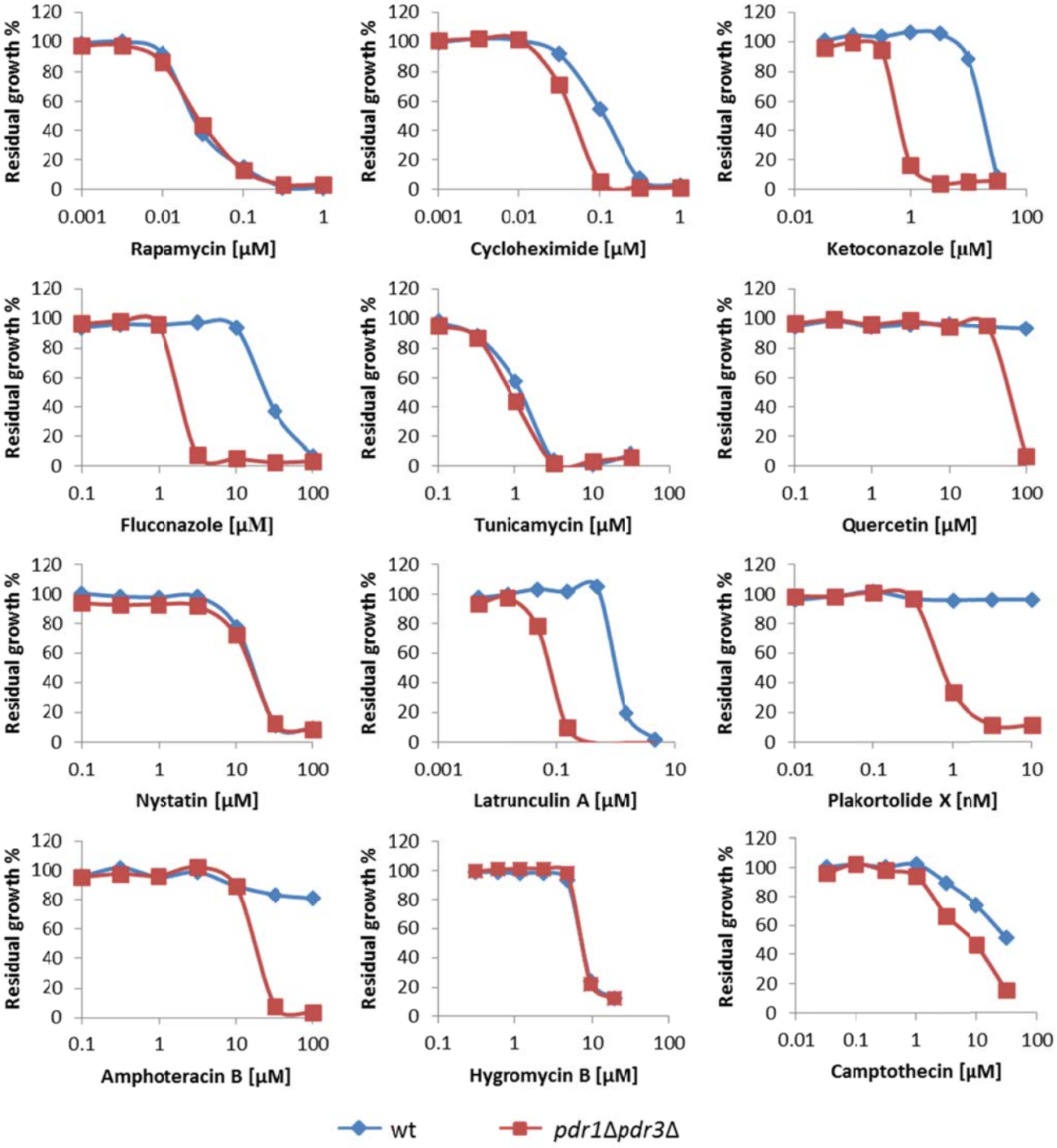

Figure S1 - The sensitivity of wild type (PDR replete) and *pdr1Δpdr3Δ* strains to xenobiotics. 10 out of the 12 xenobiotics screened were more growth inhibitory in the *pdr1Δpdr3Δ* double mutant, while the growth inhibition of rapamycin and hygromycin B were comparable to wild type. The growth assays were performed in SC media treated with the indicated

concentrations for 17 h in triplicate experiments. Residual growths were determined by carrier solvent (DMSO) control averages from each experiment.

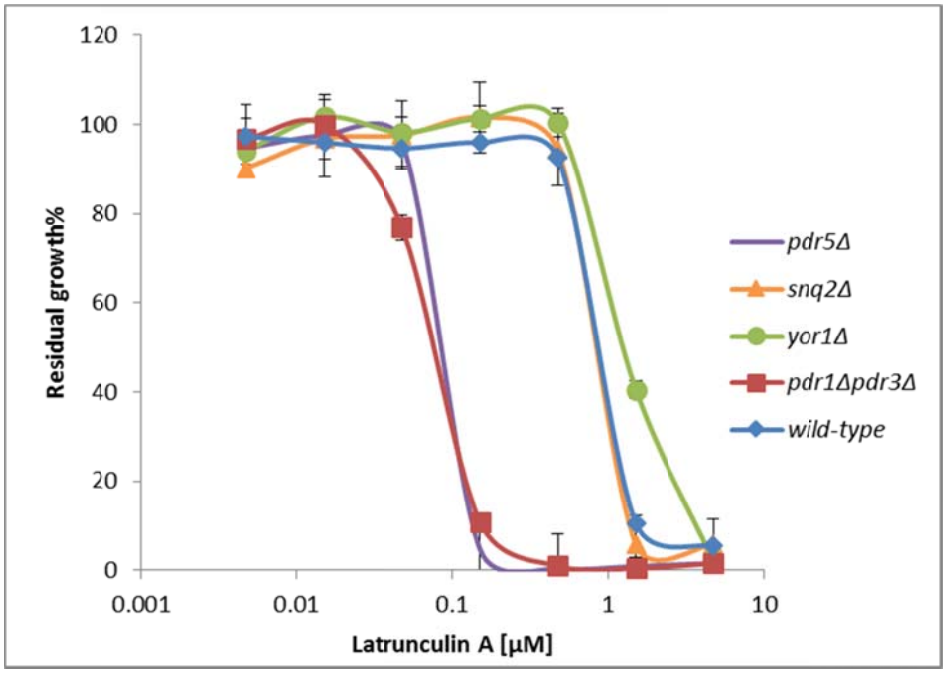

Figure S2 - Latrunculin A is a Pdr5p substrate. The sensitivity of *pdr5*Δ mutant to latrunculin A was comparable to the *pdr1*Δ*pdr3*Δ double mutant strain and was different from wild type. The *snq2*Δ strain had similar sensitivity to the wild type, while *yor1*Δ conferred resistance to latrunculin A compared to the wild type control.

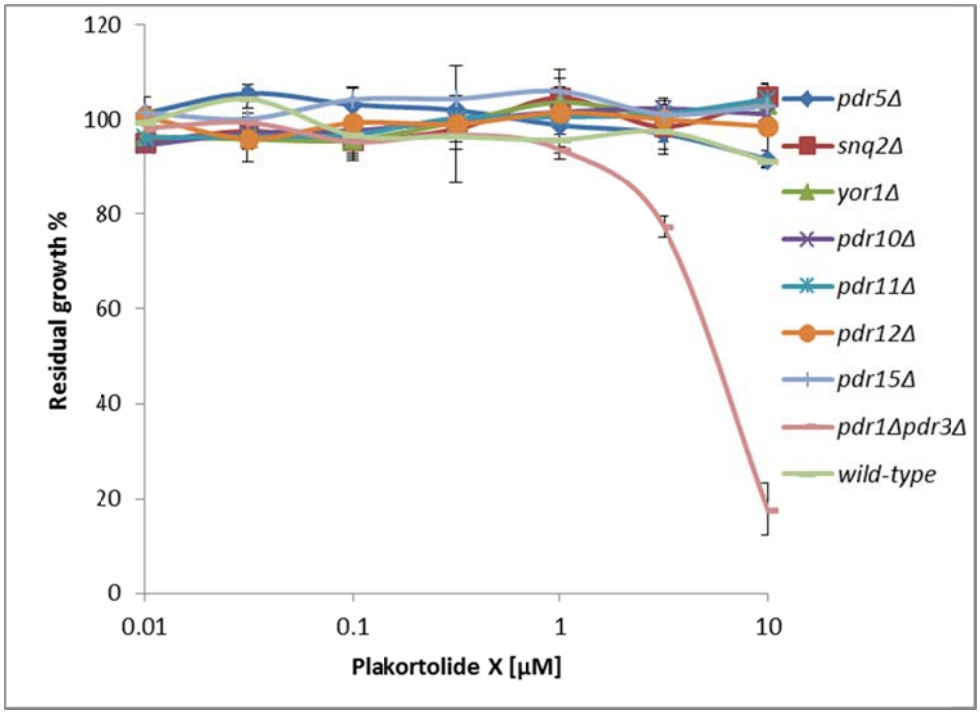

Figure S3 - Resistance to plakortolide X is not mediated by a single PDR drug efflux transporter. The PDR drug efflux transporter mutants *pdr5*Δ, *snq2*Δ, *yor1*Δ, *pdr10*Δ, *pdr11*Δ, *pdr12*Δ, *pdr15*Δ and the wild type control strain had similar sensitivities to plakortolide X.

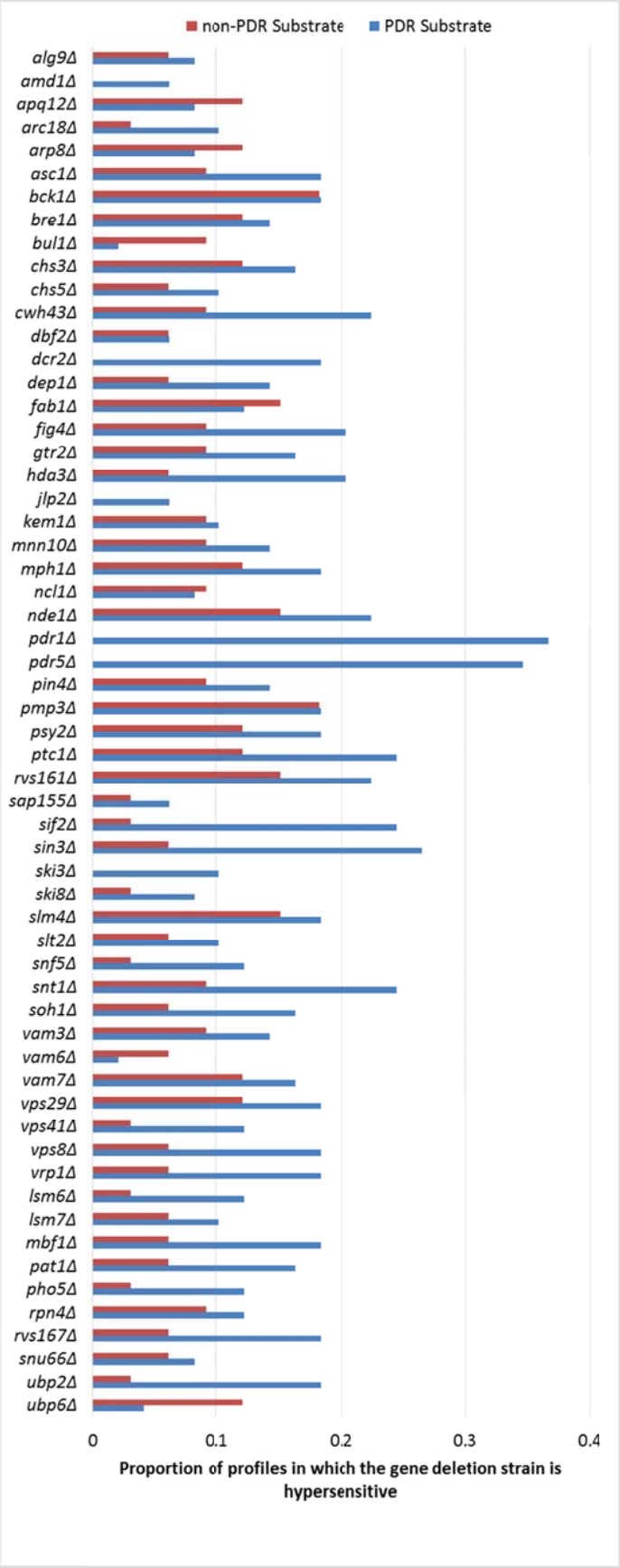

Figure S4 - Cycloheximide hypersensitive gene deletion strains annotated by sensitivity to PDR verses non-PDR substrates from Parsons *et al*. 2006.[1] Compounds screened by Parsons *et al*. were divided into two groups: PDR-substrates or non-PDR substrates. A compound was deemed a PDR-substrate if one of the following gene deletion strains displayed hypersensitivity to it: *PDR1*, *PDR3*, *PDR5*, *PDR10*, *PDR12*, *PDR15*, *YOR1* or *SNQ2*. The number of PDR-substrates and non-PDR substrates that a CHX-hypersensitive gene deletion strain was hypersensitive to was calculated and represented as a proportion of the total number of compounds in each category. CHX hypersensitive deletions strains *gim3Δ, opi9Δ, ygl024wΔ*, *ygl214wΔ*, *ymr031w-aΔ*, *ynl324wΔ*, and *ypl182cΔ*, were absent in the Parsons *et al.* data set and therefore were not included the above analysis.

	pH ~ 4.5		pH ~ 8	
Compound name	***pdr1Δpdr3Δ***	**Wild type**	***pdr1Δpdr3Δ***	**Wild type**
Wortmannin from Penicillium funiculosum	-10.83	-11.47	-0.31	-0.46
Dequalinium analog, C-14 linker	-10.44	-11.29	-9.33	-10.39
Calcimycin	-10.14	-0.15	-4.55	0.14
Clotrimazole	-9.49	-4.68	-7.92	-7.98
Ketoconazole	-9.14	0.06	-6.62	-5.52
(R,R)-cis-Diethyl tetrahydro-2,8-chrysenediol	-7.79	-1.04	-1.08	-0.47
Tyrphostin AG 879	-7.54	-1.23	-4.38	-1.13
Z-L-Phe chloromethyl ketone	-6.66	-1.98	-7.02	-2.32
Tyrphostin A9	-6.54	-1.34	0.47	0.25
U0126	-6.48	-1.45	-0.02	-0.42
Bay 11-7085	-6.16	-6.57	-6.17	-7.13
2-(alpha-Naphthoyl) ethyltrimethylammonium iodide	-6.14	-3.74	-3.09	-3.93
N-p-Tosyl-L-phenylalanine chloromethyl ketone	-6.09	-0.21	-6.70	-1.10
Meclofenamic acid sodium	-5.94	-0.55	-0.15	0.27
Dequalinium dichloride	-5.89	-9.33	-9.11	-10.39
Cantharidic Acid	-5.68	-3.68	0.20	0.36
Cantharidin	-5.41	-3.76	-0.11	0.66
3,4-Dichloroisocoumarin	-4.61	-4.64	-3.89	-3.79
Ammonium pyrrolidinedithiocarbamate	-2.89	-3.11	0.31	-0.19
Sanguinarine chloride	-2.59	-1.40	-0.18	-0.28
Methotrexate	-2.58	-3.87	0.02	-0.57
MRS 1845	-2.49	0.03	-0.20	-0.10
Ebselen	-2.35	-0.87	-2.35	-1.87
ZM 39923 hydrochloride	-2.25	-2.19	-4.45	-5.50
SP600125	-2.24	-2.00	-5.25	-6.29
(Z)-Gugglesterone	-2.18	-0.20	-1.70	0.13
Ruthenium red	-2.11	-2.84	0.15	0.37
6-Nitroso-1,2-benzopyrone	-2.03	-2.02	0.20	-0.04
(S)-(+)-Camptothecin	-2.02	-0.70	-1.13	-0.12
Diphenyleneiodonium chloride	-0.79	-0.88	-6.14	-7.38
Emodin	-0.61	-0.29	-3.16	-1.45

Capsazepine	-0.53	-0.83	-3.61	-4.51
Cinnarizine	-0.39	0.16	-5.60	0.23
S-(p-Azidophenacyl)glutathione	-0.15	-0.26	-9.43	-2.22
(±)-Verapamil hydrochloride	-0.04	0.01	-6.70	-1.10
4-Imidazoleacrylic acid	0.09	0.01	-4.38	-1.13
Amiodarone hydrochloride	0.12	-0.67	-4.38	-0.62
Ro 41-1049 hydrochloride	0.14	0.29	-3.00	-2.92
Ellipticine	0.21	-0.05	-6.70	-5.90
T-1032	0.24	0.20	-1.98	-3.06
SIB 1757	0.35	0.04	-2.01	-3.06
(±)-Sulpiride	0.42	0.20	-3.09	-1.23
Total	**29**	**15**	**26**	**17**

Table S1 - Growth inhibition screen of LOPAC library with the *pdr1*Δ*pdr3*Δ and wild type (PDR-replete) yeast strains. The LOPAC library was screened at a concentration of 10 µM in SC media (pH ~4.5) or SC media buffered with HEPES (pH ~ 8). Presented are the Z-scores less than -2 from triplicate experiments. The z-scores were calculated using the carrier solvent (DMSO) control averages from each plate.

DTT [µM]	**0**	**0.13**	**0.25**	**0.5**	**1**	**2**	**4**
***pdr1*Δ*pdr3*Δ UPRE-GFP (RFU)**	496.8	522.2	607.4	809.3	1283.2	1725.1	1725.6
Wild type UPRE-GFP (RFU)	440.7	500.0	561.0	852.7	1517.8	1947.7	1811.0

Table S2 - Dose-dependent induction of UPRE-GFP in the *pdr1*Δ*pdr3*Δ and wild type strains following exposure to dithiothreitol (DTT) at the indicated concentrations. Absolute fluorescence was measured for each cell using the OPERA high-throughput confocal microscope. Presented are average GFP intensities from duplicate experiments.

Tunicamycin [µM]	**0**	**0.38**	**0.75**	**1.5**	**3**
***pdr1*Δ*pdr3*Δ UPRE-GFP (RFU)**	694.3	658.2	627.0	1049.6	1429.0
Wild type UPRE-GFP (RFU)	542.6	484.7	618.5	978.5	1292.3

Table S3 - Dose-dependent induction of UPRE-GFP in the *pdr1*Δ*pdr3*Δ and isogenic wild type strains following exposure to tunicamycin at the indicated concentrations. Absolute fluorescence was measured for each cell using the OPERA high throughput confocal microscope. Presented are average GFP intensities from duplicate experiments.

ORF	**Gene**	**Description**

YJR077C	*MIR1*	Mitochondrial phosphate carrier
YGL136C	*MRM2*	Mitochondrial 2' O-ribose methyltransferase
YMR201C	*RAD14*	Protein that recognises and binds damaged DNA during nucleotide excision repair
YGL147C	*RPL9A*	Protein component of the large (60S) ribosomal subunit
YCL037C	*SRO9*	RNA-binding protein that associates with translating ribosomes
YIL012W	*YIL012W*	Dubious ORF but close to YIL013C/PDR11
YLR346C	*YLR346C*	Putative protein of unknown function found in mitochondria
YPR126C	*YPR126C*	Dubious ORF but overlaps YPR125W/YLH47
YMR243C	*ZRC1*	Vacuolar membrane zinc transporter, transports zinc from the cytosol
YJL157C	*FAR1*	Cyclin-dependent kinase inhibitor
YDR461W	*MFA1*	Mating pheromone a-factor
YHL007C	*STE20*	Cdc42p-activated signal transducing kinase of the PAK family

Table S4 - Putative negative genetic interactions from the *pdr1*Δ*pdr3*Δ SGA. Strains *mfa1*Δ, *far1*Δ and *ste20*Δ are known to be low efficiency mating strains and were excluded from further analysis. Genes were annotated according to SGD.[36]

Triple mutants	**Growth on diploid selection**	**Number of colonies on MATa haploid selection (sporulation efficiency)**	**Number of colonies on MATa triple mutants**	**SL or SS interactions**
*mir1*Δ*pdr1*Δ*pdr3*Δ	normal	465	501	No
*mrm2*Δ*pdr1*Δ*pdr3*Δ	normal	0	ND	ND
*rad14*Δ*pdr1*Δ*pdr3*Δ	normal	2	ND	ND
*rpl9a*Δ*pdr1*Δ*pdr3*Δ	reduced	0	ND	ND
*sor9*Δ*pdr1*Δ*pdr3*Δ	normal	1	ND	ND
*yil012w*Δ*pdr1*Δ*pdr3*Δ	reduced	2	ND	ND
*ylr346c*Δ*pdr1*Δ*pdr3*Δ	normal	412	387	No
*ypr126c*Δ*pdr1*Δ*pdr3*Δ	reduced	1	ND	ND
*zrc1*Δ*pdr1*Δ*pdr3*Δ	normal	361	499	No
*his3*Δ*pdr1*Δ*pdr3*Δ (wild type control)	normal	474	503	No

Table S5 - No synthetic lethal (SL) or synthetic sick (SS) interactions were identified with *pdr1*Δ*pdr3*Δ in the triple mutants. The sporulation efficiency of *mir1*Δ*pdr1*Δ*pdr3*Δ*, ylr346c*Δ*pdr1*Δ*pdr3*Δ *and zrc1*Δ*pdr1*Δ*pdr3*Δ triple mutants were comparable to the wild type control (*his3*Δ*pdr1*Δ*pdr3*Δ). *mrm2*Δ*pdr1*Δ*pdr3*Δ, *rad14*Δ*pdr1*Δ*pdr3*Δ, *rpl9a*Δ*pdr1*Δ*pdr3*Δ, *sor9*Δ*pdr1*Δ*pdr3*Δ, *yil012w*Δ*pdr1*Δ*pdr3*Δ and *ypr126c*Δ*pdr1*Δ*pdr3*Δ triple deletion mutants had reduced sporulation efficiencies in comparison to the wild type control. The sporulation efficiencies were calculated by the number of colonies formed on *MAT***a** haploid selection media from 20 µL of spores. The numbers of triple mutants were determined by the number of colonies formed on *MAT***a** triple mutant selection media from a 180 µL volume of spores. Gene deletion mutants were determined to be SL if triple mutants were absent while sporulation efficiencies were similar to the wild type control. SS phenotype was determined by reduced colony size of the triple mutants.

Cycloheximide hypersensitive single gene deletion strains from the agar based screen

ORF	Gene	Description
YNL219C	*ALG9*	Mannosyltransferase, involved in N-linked glycosylation
YML035C	*AMD1*	AMP deaminase
YIL040W	*APQ12*	Protein required for nuclear envelope morphology
YLR370C	*ARC18*	Subunit of the ARP2/3 complex
YOR141C	*ARP8*	Nuclear actin-related protein involved in chromatin remodeling
YMR116C	*ASC1*	G-protein beta subunit and guanine dissociation inhibitor for Gpa2p
YJL095W	*BCK1*	MAPKKK acting in the protein kinase C signaling pathway
YDL074C	*BRE1*	E3 ubiquitin ligase
YMR275C	*BUL1*	Ubiquitin-binding component of the Rsp5p E3-ubiquitin ligase complex
YBR023C	*CHS3*	Chitin synthase III
YLR330W	*CHS5*	Component of the exomer complex
YCR017C	*CWH43*	Putative sensor/transporter protein involved in cell wall biogenesis
YGR092W	*DBF2*	Ser/Thr kinase involved in transcription and stress response
YLR361C	*DCR2*	Phosphoesterase
YAL013W	*DEP1*	Component of the Rpd3L histone deacetylase complex
YFR019W	*FAB1*	1-phosphatidylinositol-3-phosphate 5-kinase
YNL325C	*FIG4*	Phosphatidylinositol 3,5-bisphosphate (PtdIns[3,5]P) phosphatase
YNL153C	*GIM3*	Subunit of the heterohexameric cochaperone prefoldin complex
YGR163W	*GTR2*	Putative GTP binding protein
YPR179C	*HDA3*	Subunit of the HDA1 histone deacetylase complex
YMR132C	*JLP2*	Protein of unknown function
YDR378C	*LSM6*	Lsm (Like Sm) protein
YNL147W	*LSM7*	Lsm (Like Sm) protein
YOR298C-A	*MBF1*	Transcriptional coactivator
YDR245W	*MNN10*	Subunit of a Golgi mannosyltransferase complex
YIR002C	*MPH1*	3'-5' DNA helicase involved in error-free bypass of DNA lesions
YBL024W	*NCL1*	S-adenosyl-L-methionine-dependent tRNA: m5C-methyltransferase
YMR145C	*NDE1*	Mitochondrial external NADH dehydrogenase
YLR338W	*OPI9*	Dubious open reading frame
YCR077C	*PAT1*	Deadenylation-dependent mRNA-decapping factor
YGL013C	*PDR1*	Transcription factor that regulates the pleiotropic drug response
YOR153W	*PDR5*	Plasma membrane ATP-binding cassette (ABC) transporter
YBR093C	*PHO5*	Repressible acid phosphatase
YBL051C	*PIN4*	Protein involved in G2/M phase progression and response to DNA damage
YDR276C	*PMP3*	Small plasma membrane protein
YNL201C	*PSY2*	Subunit of protein phosphatase PP4 complex
YDL006W	*PTC1*	Type 2C protein phosphatase (PP2C)
YDL020C	*RPN4*	Transcription factor that stimulates expression of proteasome genes
YCR009C	*RVS161*	Amphiphysin-like lipid raft protein

YDR388W	*RVS167*	Actin-associated protein with roles in endocytosis and exocytosis
YFR040W	*SAP155*	Protein required for function of the Sit4p protein phosphatase
YBR103W	*SIF2*	WD40 repeat-containing subunit of Set3C histone deacetylase complex
YOL004W	*SIN3*	Component of both the Rpd3S and Rpd3L histone deacetylase complexes
YPR189W	*SKI3*	Ski complex component and TPR protein
YGL213C	*SKI8*	Ski complex component and WD-repeat protein
YBR077C	*SLM4*	Component of the EGO and GSE complexes
YHR030C	*SLT2*	Serine/threonine MAP kinase
YBR289W	*SNF5*	Subunit of the SWI/SNF chromatin remodeling complex
YCR033W	*SNT1*	Subunit of the Set3C deacetylase complex
YOR308C	*SNU66*	Component of the U4/U6.U5 snRNP complex
YGL127C	*SOH1*	Subunit of the RNA polymerase II mediator complex
YOR124C	*UBP2*	Ubiquitin-specific protease
YFR010W	*UBP6*	Ubiquitin-specific protease
YOR106W	*VAM3*	Syntaxin-like vacuolar t-SNARE
YDL077C	*VAM6*	Vacuolar protein involved in vacuolar membrane fusion tethering
YGL212W	*VAM7*	Vacuolar SNARE protein
YHR012W	*VPS29*	Subunit of the membrane-associated retromer complex
YDR080W	*VPS41*	Vacuolar membrane protein that is a subunit of the HOPS complex
YAL002W	*VPS8*	Membrane-binding component of the CORVET complex
YLR337C	*VRP1*	Proline-rich actin-associated protein
YGL173C	*XRN1*	Evolutionarily-conserved 5'-3' exonuclease
YGL024W	*YGL024W*	Dubious open reading frame
YGL214W	*YGL214W*	Dubious open reading frame
YMR031W-A	*YMR031W-A*	Dubious open reading frame
YNL324W	*YNL324W*	Dubious open reading frame
YPL182C	*YPL182C*	Dubious open reading frame

Table S6 - Agar-plate based parental *MAT***a** DMA library chemogenomic screen results against 380 nM CHX. The chemogenomic screen was performed according to the method in Parsons *et al*. 2004 [2]. Genes were annotated according to SGD.[36]

GO term	**p-value**	**Genes**
Autophagy	2.10E-03	*VAM3, BCK1, VAM7, SLM4, VAM6, GTR2, SLT2, VPS41*
Histone deacetylation	2.10E-03	*HDA3, SNT1, SIF2, SIN3, DEP1*
RNA metabolic process	5.27E-03	*PAT1, SKI3, BRE1, LSM6, SKI8, LSM7, ARP8, DEP1, MBF1, SNF5, NCL1, HDA3, SOH1, PDR1, SLT2, SNU66, PTC1, SIN3, RPN4, XRN1*
Phosphatidylinositol metabolic process	1.41E-02	*CWH43, FIG4, ALG9, FAB1*
Regulation of MAPK cascade	1.66E-02	*SNT1, PTC1, SIF2*
Actin cortical patch localisation	1.66E-02	*RVS161, VRP1, RVS167*

Endoplasmic reticulum unfolded protein response	1.86E-02	*BCK1, SLT2, DCR2*
Response to stress	2.44E-02	*RVS161, VAM3, PSY2, BRE1, ARP8, DCR2, PIN4, DEP1, SNF5, BCK1, SOH1, PHO5, SLT2, MPH1, PTC1, SIN3, RPN4*
Mitochondrion inheritance	2.72E-02	*PTC1, ARC18, BUL1*
Chromatin organisation	3.05E-02	*SNF5, HDA3, BRE1, ARP8, SNT1, SIF2, SIN3, DEP1*
Cell cycle	3.22E-02	*PAT1, DBF2, VRP1, PSY2, BRE1, SKI8, DCR2, PIN4, CHS5, SOH1, SAP155, SIN3, XRN1*
DNA recombination	3.41E-02	*SNF5, SKI8, BRE1, ARP8, SOH1, MPH1*
Cytokinesis	3.41E-02	*MNN10, VRP1, SLT2, CHS3, CHS5*
Regulation of gene expression	3.65E-02	*PAT1, BRE1, ARP8, ASC1, CHS5, DEP1, MBF1, SNF5, HDA3, SOH1, PDR1, GTR2, SNT1, SIF2, SIN3, RPN4*
Cell wall macromolecule metabolic process	4.32E-02	*MNN10, CHS3, CHS5*
Vesicle-mediated transport	4.37E-02	*VPS29, RVS161, VAM3, VRP1, VAM7, VAM6, RVS167, VPS41, CHS5, VPS8*
Glycerophospholipid metabolic process	4.77E-02	*CWH43, FIG4, ALG9, FAB1*
Response to DNA damage stimulus	4.86E-02	*SNF5, BRE1, PSY2, ARP8, SOH1, MPH1, PIN4, SIN3*
Barrier septum assembly	4.88E-02	*MNN10, SLT2*
U4/U6 x U5 tri-snrnp complex	2.31E-04	*LSM6, LSM7, SNU66*
Histone deacetylase complex	3.89E-03	*HDA3, SNT1, SIF2, SIN3, DEP1*
Fungal-type vacuole membrane	3.89E-03	*VAM3, VAM7, SLM4, VAM6, GTR2, VPS41, FIG4, FAB1*
Rpd3L-Expanded complex	3.89E-03	*SNT1, SIF2, SIN3, DEP1*
Nuclear chromosome	4.64E-03	*SNF5, HDA3, SKI8, ARP8, GTR2, SNT1, SIF2, SIN3, DEP1*
HOPS complex	5.28E-03	*VAM6, VPS41*
EGO complex	5.28E-03	*SLM4, GTR2*
Ski complex	5.28E-03	*SKI3, SKI8*
Small nuclear ribonucleoprotein complex	5.28E-03	*LSM6, LSM7, SNU66*
Endosome	6.43E-03	*VPS29, SLM4, GTR2, SLT2, VPS41, FAB1, VPS8*
GSE complex	8.34E-03	*SLM4, GTR2*
Vacuolar membrane	9.78E-03	*VAM3, VAM7, SLM4, VAM6, GTR2, VPS41, FIG4, FAB1*
Endosomal part	1.36E-02	*SLM4, GTR2, SLT2, FAB1, VPS8*
Cytoplasmic mRNA processing body	1.36E-02	*PAT1, LSM6, XRN1*
PAS complex	1.63E-02	*FIG4, FAB1*
Set3 complex	2.14E-02	*SNT1, SIF2*
Cytoskeleton	2.40E-02	*DBF2, RVS161, VRP1, ARP8, RVS167, XRN1, ARC18*
SNARE complex	4.91E-02	*VAM3, VAM7*
Golgi stack	4.91E-02	*MNN10, VPS8*

Table S7 - GO term enrichment of the parental *MAT***a** DMA library CHX chemogenomic screen hits. The BiNGO[32] plugin in Cytoscape[35] was used to calculate enrichments and P-values using the *MAT***a** DMA library as a reference set and default settings.

ORF	Gene	Description
YBR194W	*AIM4*	Protein proposed to be associated with the nuclear pore complex
YBL069W	*AST1*	Lipid raft associated protein
YPL178W	*CBC2*	Small subunit of the heterodimeric cap binding complex with Sto1p
YGR157W	*CHO2*	Phosphatidylethanolamine methyltransferase (PEMT)
YMR202W	*ERG2*	C-8 sterol isomerase
YBR047W	*FMP23*	Putative protein of unknown function
YDR378C	*LSM6*	Lsm (Like Sm) protein
YNL147W	*LSM7*	Lsm (Like Sm) protein
YOR298C-A	*MBF1*	Transcriptional coactivator
YCR077C	*PAT1*	Deadenylation-dependent mRNA-decapping factor
YBR093C	*PHO5*	Repressible acid phosphatase
YGR135W	*PRE9*	Alpha 3 subunit of the 20S proteasome
YBL057C	*PTH2*	One of two mitochondrially-localised peptidyl-tRNA hydrolases
YMR247C	*RKR1*	RING domain E3 ubiquitin ligase
YFR032C-A	*RPL29*	Ribosomal 60S subunit protein L29
YLR448W	*RPL6B*	Ribosomal 60S subunit protein L6B
YDL020C	*RPN4*	Transcription factor that stimulates expression of proteasome genes
YNL302C	*RPS19B*	Protein component of the small (40S) ribosomal subunit
YHR021C	*RPS27B*	Protein component of the small (40S) ribosomal subunit
YDR388W	*RVS167*	Actin-associated protein with roles in endocytosis and exocytosis
YBL066C	*SEF1*	Putative transcription factor
YOR308C	*SNU66*	Component of the U4/U6.U5 snRNP complex
YKL081W	*TEF4*	Gamma subunit of translational elongation factor eEF1B
YPL157W	*TGS1*	Trimethyl guanosine synthase, conserved nucleolar methyl transferase
YHR025W	*THR1*	Homoserine kinase
YDL122W	*UBP1*	Ubiquitin-specific protease
YBL067C	*UBP13*	Ubiquitin-specific protease that cleaves Ub-protein fusions
YOR124C	*UBP2*	Ubiquitin-specific protease
YFR010W	*UBP6*	Ubiquitin-specific protease
YNR006W	*VPS27*	Endosomal protein that forms a complex with Hse1p
YPR173C	*VPS4*	AAA-ATPase involved in multivesicular body (MVB) protein sorting
YNL197C	*WHI3*	RNA binding protein that sequesters CLN3 mRNA in cytoplasmic foci
YCL046W	*YCL046W*	Dubious open reading frame
YLR184W	*YLR184W*	Dubious open reading frame

Table S8 - Agar-plate based *MAT***a** PA-DMA library chemogenomic screen results against 100 nM CHX. The chemogenomic screen was performed according to the method in Parsons *et al*. 2004.[2] Genes were annotated according to SGD.[36]

GO term	p-value	Genes
Cellular macromolecule catabolic process	5.42E-03	*PAT1, UBP6, CBC2, UBP13, LSM6, LSM7, PRE9, RKR1, UBP2, UBP1*
Ribonucleoprotein complex biogenesis	1.07E-02	*PAT1, RPS27B, LSM6, LSM7, SNU66, RPL6B, RPS19B*
Regulation of proteasomal ubiquitin-dependent protein catabolic process	1.07E-02	*PTH2, RPN4*
RNA splicing, via transesterification reactions	1.21E-02	*CBC2, LSM6, LSM7, SNU66*
Ribosome biogenesis	1.43E-02	*RPS27B, LSM6, LSM7, SNU66, RPL6B, RPS19B*
mRNA processing	1.57E-02	*PAT1, CBC2, LSM6, LSM7, SNU66*
Regulation of proteasomal protein catabolic process	1.87E-02	*PTH2, RPN4*
Ribosomal small subunit biogenesis	1.87E-02	*RPS27B, LSM6, LSM7, RPS19B*
Ubiquitin-dependent protein catabolic process	1.87E-02	*UBP6, UBP13, PRE9, RKR1, UBP2, UBP1*
RNA splicing	1.87E-02	*CBC2, LSM6, LSM7, SNU66*
Protein deubiquitination	1.87E-02	*UBP6, UBP2, UBP1*
Cellular component biogenesis	1.97E-02	*PAT1, RPS27B, LSM6, LSM7, RVS167, PRE9, SNU66, RPL6B, VPS4, TGS1, RPS19B*
Modification-dependent macromolecule catabolic process	1.97E-02	*UBP6, UBP13, PRE9, RKR1, UBP2, UBP1*
mRNA catabolic process	1.97E-02	*PAT1, CBC2, LSM6, LSM7*
Cellular protein catabolic process	2.65E-02	*UBP6, UBP13, PRE9, RKR1, UBP2, UBP1*
Maintenance of protein location in cell	2.65E-02	*WHI3, VPS4, VPS27*
RNA processing	2.65E-02	*PAT1, RPS27B, CBC2, LSM6, LSM7, SNU66, TGS1*
Protein modification by small protein removal	2.65E-02	*UBP6, UBP2, UBP1*
Gene expression	2.65E-02	*PAT1, CBC2, LSM6, LSM7, RPL29, MBF1, RPS27B, TEF4, SNU66, SEF1, RPL6B, TGS1, RPN4, RPS19B*
Protein catabolic process	2.65E-02	*UBP6, UBP13, PRE9, RKR1, UBP2, UBP1*
Maintenance of protein location	2.87E-02	*WHI3, VPS4, VPS27*
Regulation of protein catabolic process	3.01E-02	*PTH2, RPN4*
rRNA processing	3.31E-02	*RPS27B, LSM6, LSM7, SNU66*
RNA catabolic process	3.31E-02	*PAT1, CBC2, LSM6, LSM7*
mRNA metabolic process	3.31E-02	*PAT1, CBC2, LSM6, LSM7, SNU66*
Protein retention in Golgi apparatus	3.31E-02	*VPS4, VPS27*
Regulation of proteolysis	3.31E-02	*PTH2, RPN4*
Maturation of SSU-rRNA	3.70E-02	*RPS27B, LSM6, LSM7*
Maintenance of location	3.70E-02	*WHI3, VPS4, VPS27*
rRNA metabolic process	4.54E-02	*RPS27B, LSM6, LSM7, SNU66*
U4/U6 x U5 tri-snrnp complex	1.09E-04	*LSM6, LSM7, SNU66*
Ribonucleoprotein complex	1.80E-03	*PAT1, RPS27B, CBC2, TEF4, LSM6, LSM7, SNU66, RPL6B, RPL29, RPS19B*
U6 snrnp	3.84E-03	*LSM6, LSM7*

Small nuclear ribonucleoprotein complex	5.63E-03	*LSM6, LSM7, SNU66*
Ribosome	2.64E-02	*PAT1, RPS27B, TEF4, RPL6B, RPL29, RPS19B*
Cytosolic ribosome	4.20E-02	*PAT1, RPS27B, RPL6B, RPL29, RPS19B*

Table S9 - GO term enrichment of the agar-plate based *MAT***a** PA-DMA library CHX chemogenomic screen hits. The BiNGO[32] plugin in Cytoscape[35] was used to calculate enrichments and P -values using the *MAT***a** PA-DMA library as a reference set and default settings.

ORF	**Gene**	**Description**
YHR129C	*ARP1*	Actin-related protein of the dynactin complex
YMR119W	*ASI1*	Putative integral membrane E3 ubiquitin ligase
YJL020C	*BBC1*	Protein possibly involved in assembly of actin patches
YIL159W	*BNR1*	Formin
YER048C	*CAJ1*	Nuclear type II J heat shock protein of the E. coli dnaJ family
YKL007W	*CAP1*	Alpha subunit of the capping protein (CP) heterodimer
YIL034C	*CAP2*	Beta subunit of the capping protein (CP) heterodimer
YMR198W	*CIK1*	Kinesin-associated protein
YIL035C	*CKA1*	Alpha catalytic subunit of casein kinase 2 (CK2)
YOR039W	*CKB2*	Beta' regulatory subunit of casein kinase 2 (CK2)
YIL157C	*COA1*	Mitochondrial membrane protein required for assembly of complex IV
YJR048W	*CYC1*	Cytochrome c
YAL026C	*DRS2*	Trans-golgi network aminophospholipid translocase (flippase)
YMR202W	*ERG2*	C-8 sterol isomerase
YCR034W	*FEN1*	Fatty acid elongase
YHL031C	*GOS1*	v-SNARE protein involved in Golgi transport
YNL281W	*HCH1*	Heat shock protein regulator that binds to Hsp90p
YIL116W	*HIS5*	Histidinol-phosphate aminotransferase
YGL168W	*HUR1*	Protein of unknown function
YDR123C	*INO2*	Component of the Ino2p/Ino4p transcription activator
YGL216W	*KIP3*	Kinesin-related motor protein involved in mitotic spindle positioning
YLL049W	*LDB18*	Component of the dynactin complex
YNL323W	*LEM3*	Membrane protein of the plasma membrane and ER
YOR231W	*MKK1*	MAPKK involved in the protein kinase C signaling pathway
YNL076W	*MKS1*	Pleiotropic negative transcriptional regulator
YNL099C	*OCA1*	Putative protein tyrosine phosphatase
YLR338W	*OPI9*	Dubious open reading frame unlikely to encode a functional protein
YGR078C	*PAC10*	Part of the heteromeric co-chaperone GimC/prefoldin complex
YOR153W	*PDR5*	Plasma membrane ATP-binding cassette (ABC) transporter
YGL045W	*RIM8*	Protein involved in proteolytic activation of Rim101p
YOL039W	*RPP2A*	Ribosomal protein P2 alpha

YDL225W	*SHS1*	Component of the septin ring that is required for cytokinesis
YDR011W	*SNQ2*	Plasma membrane ATP-binding cassette (ABC) transporter
YIL073C	*SPO22*	Meiosis-specific protein essential for chromosome synapsis
YDR463W	*STP1*	Transcription factor
YER111C	*SWI4*	DNA binding component of the SBF complex (Swi4p-Swi6p)
YJL004C	*SYS1*	Integral membrane protein of the Golgi required for targeting Arl3p
YBL054W	*TOD6*	PAC motif binding protein involved in rRNA and ribosome biogenesis
YNL079C	*TPM1*	Major isoform of tropomyosin
YDR074W	*TPS2*	Phosphatase subunit of the trehalose-6-phosphate synthase complex
YNL054W	*VAC7*	Integral vacuolar membrane protein involved in vacuole organisation
YDR247W	*VHS1*	Cytoplasmic serine/threonine protein kinase
YKR001C	*VPS1*	Dynamin-like GTPase required for vacuolar sorting
YLR200W	*YKE2*	Subunit of the heterohexameric Gim/prefoldin protein complex
YBR178W	*YBR178W*	Dubious open reading frame unlikely to encode a functional protein
YFR016C	*YFR016C*	Putative protein of unknown function
YGL042C	*YGL042C*	Dubious open reading frame unlikely to encode a functional protein
YIR042C	*YIR042C*	Putative protein of unknown function
YMR316C-A	*YMR316C-A*	Protein of unknown function
YNL120C	*YNL120C*	Dubious open reading frame unlikely to encode a functional protein
YOR019W	*YOR019W*	Protein of unknown function

Table S10 - The complete list of gene deletions hypersensitive to 28 nM latrunculin A in the PA-DMA barcode microarray. 51 latrunculin A hypersensitive strains were identified from the *pdr1*Δ*pdr3*Δ*xxx*Δ deletion library in a DNA barcode microarray experiment. Genes were annotated according to SGD.[36]

Primer	**Sequence 5' to 3'**
PDR1 deletion primer forward	CATCTCAGCCAAGAATATACAGAAAAGAATCCAAGAAACTGGAAGACATGGAGGCCCAGAATACCCT
PDR1 deletion primer reverse	AGGAAGGAAGTTTTTGAGAACTTTTATCTATACAAACGTATACGTCAGTATAGCGACCAGCATTCAC
PDR3 deletion primer forward	ACTGCATCAGCAGTTTTATTAATTTTTTCTTATTGCGTGACCGCAACATGGAGGCCCAGAATACCCT
PDR3 deletion primer reverse	CCATTTACTATGGTTATGCTCTGCTTCCCTATTTCTTTTGCGTTTCAGTATAGCGACCAGCATTCAC

Table S11 - Deletion PCR primers for *pdr1*Δ*pdr3*Δ double mutant construction. The deletion primers were designed with 50 bp of homology to the 5' or 3' flanking regions of *PDR1* or *PDR3* loci and 18 bp with homology to *natR* or *URA3* cassette according to C. Janke, M. M. Magiera, N. Rathfelder, C. Taxis, S. Reber, H. Maekawa, A. Moreno-Borchart, G. Doenges, E. Schwob, E. Schiebel and M. Knop, *Yeast*, 2004, **21**, 947–962.