Supporting Information for : A biosensor-based approach reveals links between efflux pump expression and cell cycle regulation in pleiotropic drug resistance of yeast

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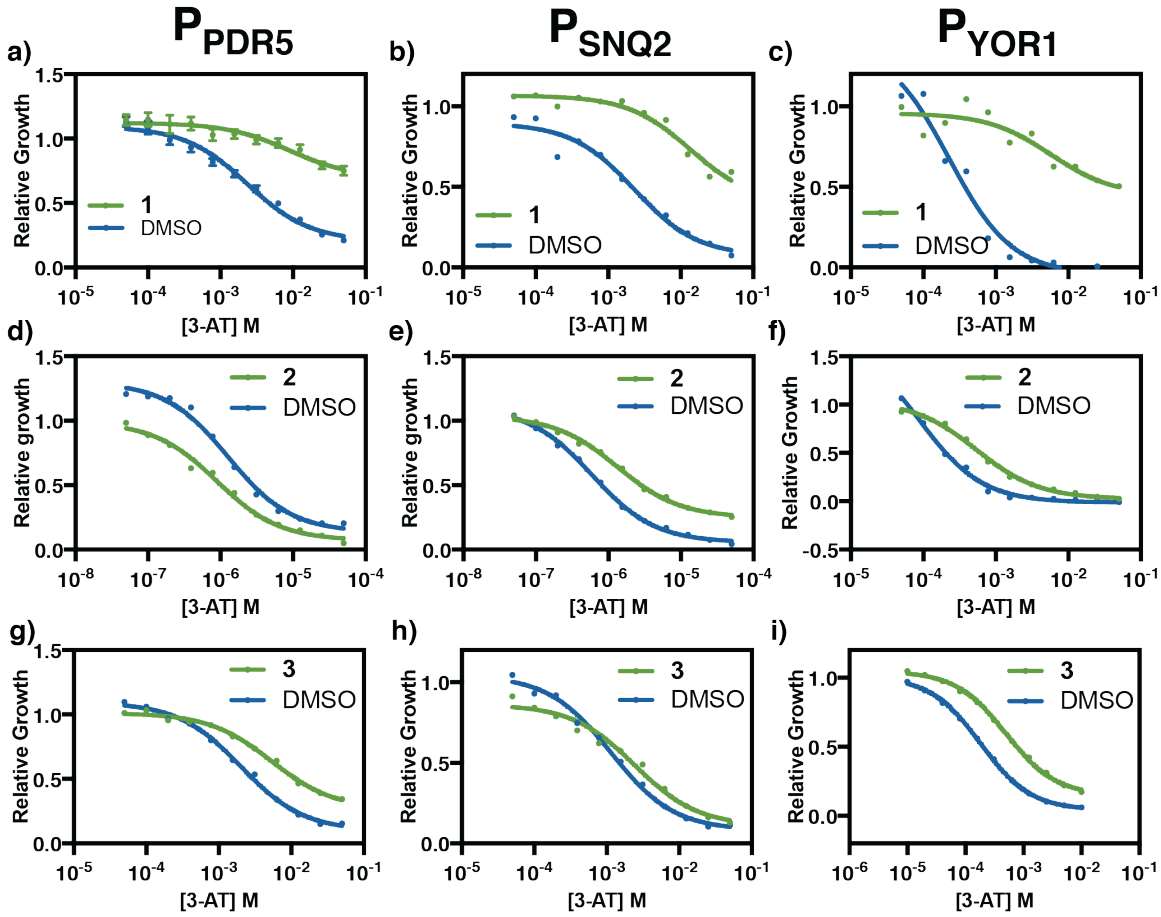

Figure S1: Does dependent response of strains JLY31-33 to 3-AT in the presence of compounds 1-3. All strains were grown in YNB media with 10 µM drug or an equivalent amount of DMSO**.** Line represents best fit dose-response curve, and error bars represent SD (n=4). **a)** JLY31 treated with **1** (reused from Figure 1c and included here for context). **b)** JLY32 treated with **1**. **c**) JLY33 treated with **1**. **d)** JLY31 treated with **2**. **e)** JLY32 treated with **2**. **f**) JLY33 treated with **2**. **g)** JLY31 treated with **3**. **h)** JLY32 treated with **3**. **i**) JLY33 treated with **3**.

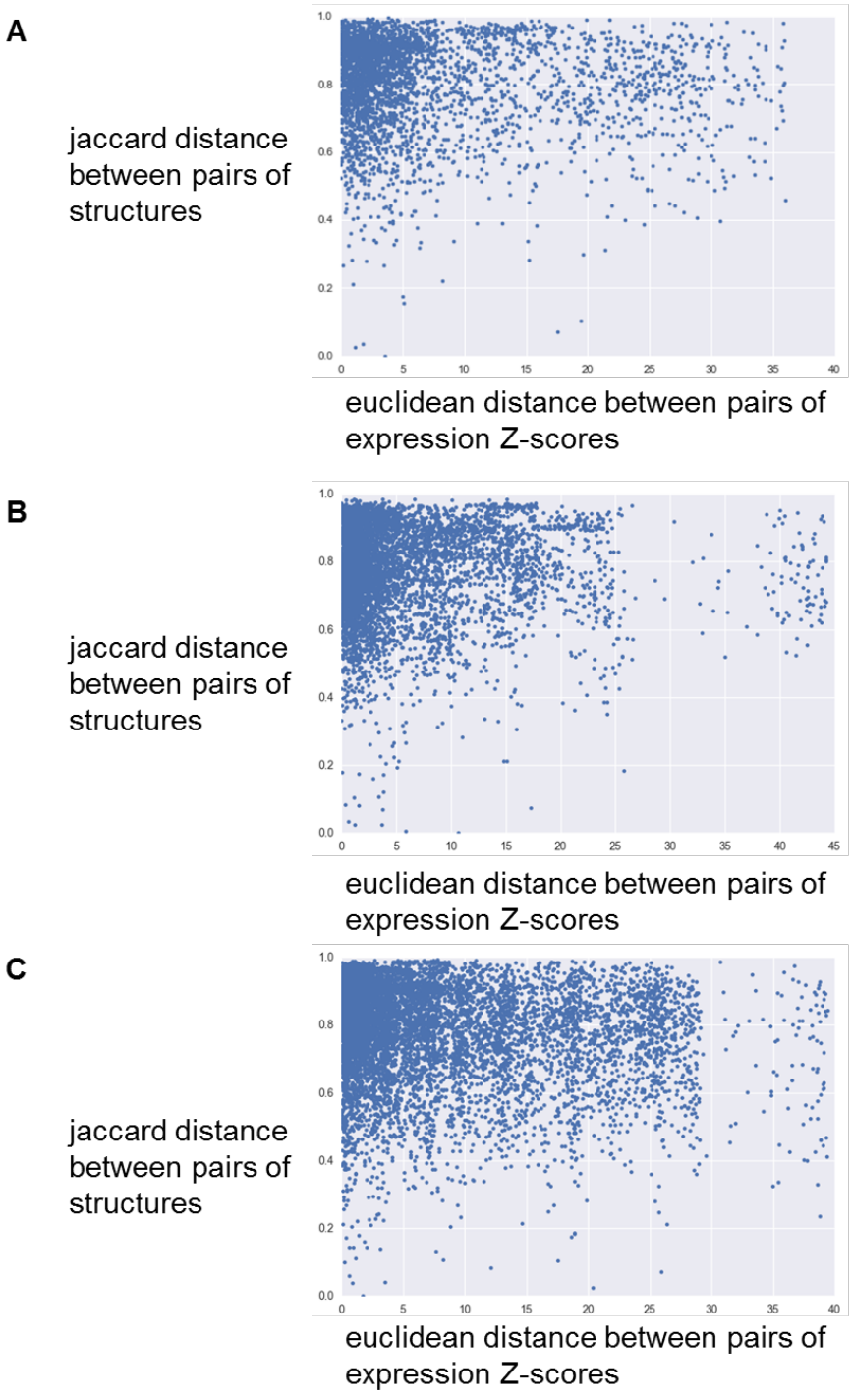

Figure S2: Similarity in structure of microsource natural products does not correlate with similarities in transporter activations. For drugs that had a Z-score >3 for each promoter, jaccard distance were calculated between pairs of the structures, and euclidean distance were calculated between the difference in Z-scores. **a)** JLY31 induction. **b)** JLY32 induction. **c)** JLY33 induction.

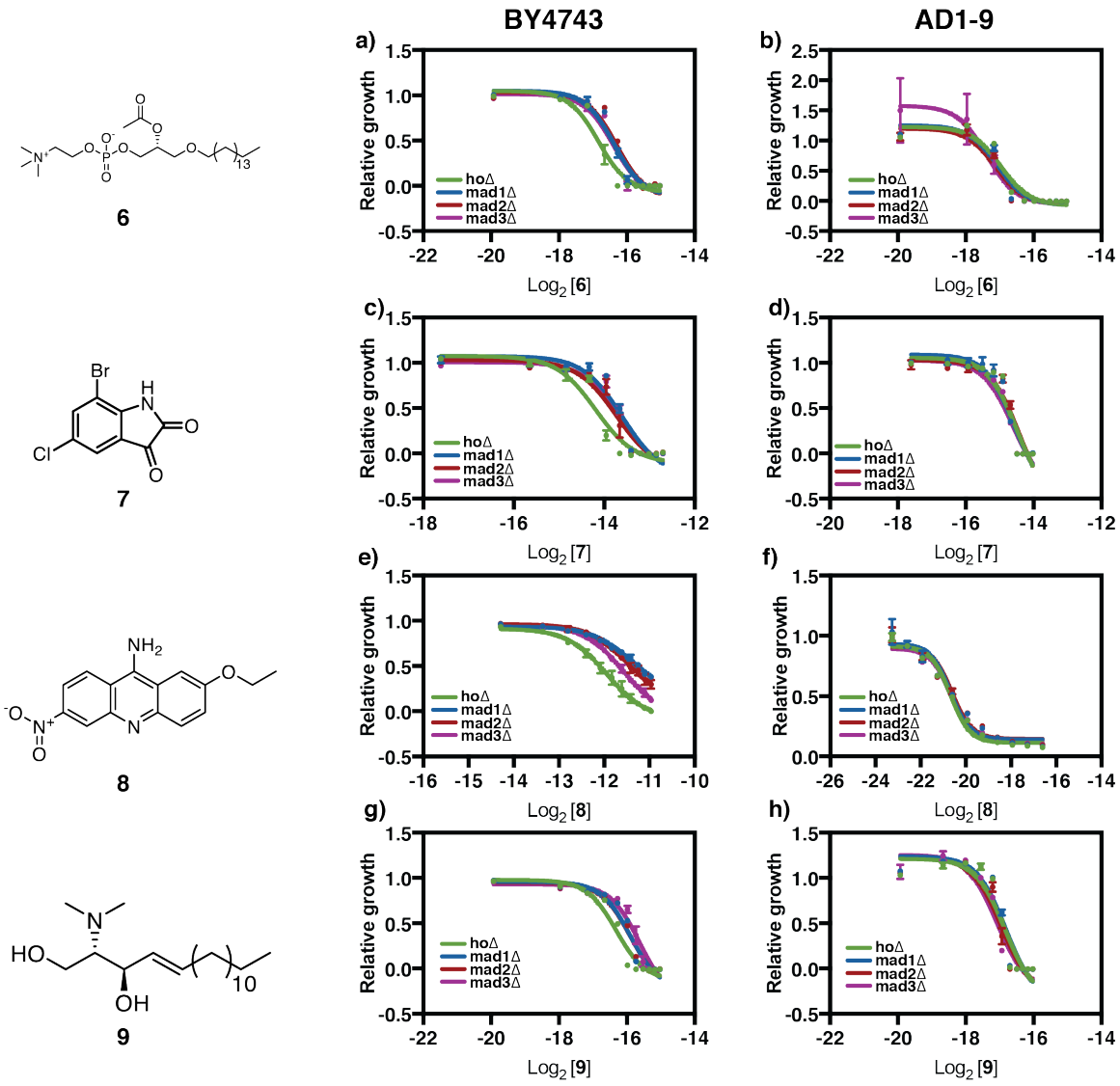

Figure S3. A diverse group of chemicals that show enhanced mad deletion resistance when transporters are present. The *hoΔ* and *mad* deletion strains in BY4743 background and AD1-9 PDR null background were treated with **6-9**, similar to methods described in Figure 4. **a**), **c**), **e**) and **g**) are BY4743 background stains, and **b**), **d**), **f**) and **h**) are AD1-9 background stains. Growth relative to that in the absence of **drug** is indicated by the *y* axis and was calculated as follows: Relative growth = $AUC_{drug}/AUC_{no\ drug}$. All wells contain the same DMSO concentration. AUC calculation was performed with baseline corrected growth curve. Line represents best fitted dose-response curve, and error bar represents SD (n=3).

Supplementary Tables

Table S1: Sequences of the promoters used in this study

Promoter	Sequence
P_{PDR5}	CGTTAACGTAAATATGTCTTCCTCTTTGATTCCAACAATGATATTATAAGGAAATTATCGTCACA ATCTAATCAACAAGGACAAAGAAAAGAAAAAGTTGAGAGAGAGAACAAAAGTTCAAATCAAAG AAAAAAAAGAACATCAAATTACCTATTACAATAAACAATTAAGCCATACTCGCAACAATTTGCC ATAATAGAAAGCAGCACCTCGTTGGCGCAGTCCCTTACATAGTACACAACATTTATCACTTCAC ACAATCAGGAGTGGAACTCAATGGAAAACAACACCACACGTATGATCTTACTAATAAAAGAACA TGAACGTTCCTCAGCGCGAACGTTCGCATTCTGCGCCTTCGAGCACAGGATAAGTTGCAGGAAG CCATCACATCTATGCAACGATTATCACGACACAACCTTGCCGCCGAGAAAACGTCCGTGGAGAA CCATTCGGTCGATTGCTTCCCACGGAACGAGTGGACTGAAACTTAAGACTGCCCCTCTCTTTCC GCGGAATCGCTCATGCCGCGGTGCCACAACATTTTCAGATTTACTAAGACTCCGGTGAGTGTGG GCTCACCCGCGGGTCGTGATCACGATTCAGCACCCTTTGGACTCGTGATTCCGTGGAAAGGTCA GATCTGTATTCCTACTTATGGTAATGTGCTAAAAAAAGAGAAATGTCTCCGCGGAACTCTTCTAC GCCGTGGTACGATATCTGTTGAACGTAATCTGAGCAATACAAACAAGGCCTCTCCTATACATAT ATAATTGTGATGTGCATAACCTTATGGCTGTTCGCTTTTATTATCATACCTTAGAATGAAATCCAA AAGAAAAAAGTCACGCAAAGTTGCAAACATATAACAACTGTGTTAGTTATCACTCGACTTTGTTA TTCTAATTATAAATAAATTGGCAACTAGGAACTTTCGAAAAAGAAATTAAAGACCCTTTTAAGTT TTCGTATCCGCTCGTTCGAAAGACTTTAGACAAAA
P_{SNQ2}	GATCTCACAGATGAAGAACTAAAAGACGCAATTATAGTTTCGAAGCCTGCCTTGGGTACTTGTTT GTACGAACAATAACACTAAACACCTTCTTGGAACAACCTTTAAATATTAAAAGTTTACTCATACC TCTTACACTCTATAAAGAAACAAGTTGGTGAGTCCAATAGAACCCATTCCAGCATCCGATGCAA CCATAGCCCGGTTGATGCGAGTCAGAGTGGCTCCGCACACAGAGGTTATGTGACCATGGTAACC GTACACATTTTTCCACGGCAAGGAAGTGGCGCGAAAGAAGCACCGCCCTCCACGGATCACCCC ATTTGGAAAAGAACGGAGACATAAAAAAAAATTACCTTCACGCCAGACTATGTATGACTCGTAG AAATCTAGCATCCGCGGAGCTATTTTAAGTTTCCGCGGATGCCTTTCGATCCTTTCCCAGTCGTC TGGTTCCGCGCACATATGACTAAATGTCCGCCCATTTCCGTTTAAATCCGTTTTCTATTTTCTTTA GGTCTTTCCATTGTATTCCCTGCAACCCCGCTGGGTATTGGTATTACTAATAGTGACTACGCAAC ATCAACATATAAAACAAATGATGAGTAGTGCCCCTCCCTCACCGCTATCGCCTCACCACTTTTT ATGCTTGTATATGCTTGTAGATAAATAAATACCTAACGAAAACAATATATACGAACCTAGTGTAT TTGTATCTTTTGTTTTTCAAGTTGAAGTGTTGCGAGGTCAAAAAAAAAAAAAGCTCACTGTAGGAT ATAGGAGTTACTATATCACCAGTGCATTACATTCTCAGTGCATCCATCGTCTTCAACATTGATTA TTTTCTCCTTCCATTGATTAGAGTTCAAGCTCCCTGAGAAAACGAAGGTATAGCGGACGTACCCG CAGAGACATAAAAAAAGAAAAACTATATCGAAGACCGAAAGCAGTAAAAAAGTGGATAGAATA ACACAGCTACCAAAATACGTAAAGAGAATTCA
P_{YOR1}	TTCCTTTTCTTTTTTTTTTTTCTTTGACTGTTACCCGGTTGTTTATATTTGTAGGAAAACAACAAC GACAGAGAAAATATCCTTGCAGTGGCGGCTAATTTGTTAGTTGACTGATTGATCACCTTCACTTA TTAAAGTAAAATCAGCATACAAGAGATCAGAAGGGAGAAAGAGAGTGGGCAAGGCTATAGTACT TTGAAGAAAGCATCTTTGAACCGACCAGTTCTCTTCACAAGCAAAATCTATATGACTAACCGCA AGGGGCAAAGGGTTGTGAGAGGGCCCGTCTTTCTCCCGCTATAGCCGTCACTGGTATCCCTCCT GGCTGCACAAATCCGATAGAAAGGGGAAGAAGGAAGTTTAGTGCCACCTTATAGCACGCAGTTA CTGTTTACGCTAAGGAGAGGCATACTCAATTTTTATTAGTCGCCTTCTTTAGTTGCTGCGTTTTTA TCCACGGTTCTCTACTAAATGCTTGCGATAAGCGCTTCTATTTTCCTCCCCACCGCGAGGCGGAA ATGGCACATTTTTTTTCTTTTGCTTCTGTGCTTTTGTTGTAATTTTTGGCATGTGCTATTGTATGAA GATAACGCGTGGTTCCGTGGAAATAGCCGGAAATTTTGCCGGGAATATGACGGACATGATTTAA CACCCGTGGAAATGAAAAAAGCCAAGGTAAGAAAGTGGCAATATTTTTCCTACAAATAGATCTG CTGTCCCTTAAATGATTACCATACATATATATATTTATTACACATCTGTCAGAGGTAGCTAGCGA AGGTGTCACTGAAATATTTTTTGTTCCAGTTAGTATAAATACGGAGGTAGAACAGCTCTCCGCGT GTATATCTTTTTTTGCGCTATACAAGAACAGGAAGAACGCATTTCCATACCTTTTTCTCCTTACA GGTGCCCTCTGAGTAGTGTCACGAACGAGGAAAAAGATTAATATTACTGTTTTTATATTCAAAAA GAGTAAAGCCGTTGCTATATACGAAT

Table S2: GO Enrichment analysis of screen hits

Treatment Condition	GO Term	Description	P-value
3	GO:0048583	regulation of response to stimulus	1.33E-04
	GO:0010646	regulation of cell communication	3.38E-04
	GO:0042147	retrograde transport, endosome to Golgi	3.45E-04
	GO:0010647	positive regulation of cell communication	4.67E-04
2	GO:0010468	regulation of gene expression	4.00E-05
	GO:0044774	mitotic DNA integrity checkpoint	6.25E-05
	GO:0031326	regulation of cellular biosynthetic process	1.28E-04
	GO:0009889	regulation of biosynthetic process	1.31E-04
	GO:0031570	DNA integrity checkpoint	1.35E-04
	GO:2000112	regulation of cellular macromolecule biosynthetic process	1.67E-04
	GO:0010556	regulation of macromolecule biosynthetic process	1.97E-04
	GO:0050789	regulation of biological process	2.09E-04
	GO:0019354	siroheme biosynthetic process	2.74E-04
	GO:0046156	siroheme metabolic process	2.74E-04
	GO:0050794	regulation of cellular process	2.81E-04
	GO:1901991	negative regulation of mitotic cell cycle phase transition	2.81E-04
	GO:0065007	biological regulation	3.00E-04
	GO:1901988	negative regulation of cell cycle phase transition	3.65E-04
	GO:0051171	regulation of nitrogen compound metabolic process	4.37E-04
	GO:0000075	cell cycle checkpoint	6.59E-04
	GO:0000103	sulfate assimilation	6.65E-04
	GO:0007093	mitotic cell cycle checkpoint	7.31E-04
	GO:2000765	regulation of cytoplasmic translation	8.12E-04
1	GO:0061188	negative regulation of chromatin silencing at rDNA	3.82E-06
	GO:0061187	regulation of chromatin silencing at rDNA	7.53E-06
	GO:0061186	negative regulation of chromatin silencing at silent mating-type cassette	2.19E-05
	GO:0090054	regulation of chromatin silencing at silent mating-type cassette	5.03E-05
	GO:0031939	negative regulation of chromatin silencing at telomere	7.16E-05
	GO:0031938	regulation of chromatin silencing at telomere	2.85E-04
	GO:2000217	regulation of invasive growth in response to glucose limitation	3.56E-04
	GO:0031936	negative regulation of chromatin silencing	3.56E-04
	GO:0045815	positive regulation of gene expression, epigenetic	4.39E-04
	GO:0060969	negative regulation of gene silencing	5.35E-04
	GO:0061408	positive regulation of transcription from RNA polymerase II promoter in response to heat stress	6.89E-04
	GO:0010646	regulation of cell communication	9.28E-04
	GO:2000219	positive regulation of invasive growth in response to glucose limitation	9.35E-04
Overall	GO:0065007	biological regulation	7.36E-07
	GO:0050794	regulation of cellular process	1.10E-06
	GO:0050789	regulation of biological process	2.09E-06
	GO:0031326	regulation of cellular biosynthetic process	2.42E-06
	GO:0009889	regulation of biosynthetic process	2.54E-06
	GO:0080135	regulation of cellular response to stress	2.72E-06
	GO:0010556	regulation of macromolecule biosynthetic process	3.36E-06

	GO:0080134	regulation of response to stress	3.57E-06
	GO:0010468	regulation of gene expression	6.25E-06
	GO:2000112	regulation of cellular macromolecule biosynthetic process	1.17E-05
	GO:0048583	regulation of response to stimulus	1.37E-05
	GO:0051171	regulation of nitrogen compound metabolic process	1.75E-05
	GO:0080090	regulation of primary metabolic process	3.56E-05
	GO:0060255	regulation of macromolecule metabolic process	4.06E-05
	GO:0019222	regulation of metabolic process	4.43E-05
	GO:0031323	regulation of cellular metabolic process	4.87E-05
	GO:0010646	regulation of cell communication	5.85E-05
	GO:0016569	covalent chromatin modification	5.99E-05
	GO:0006357	regulation of transcription from RNA polymerase II promoter	8.41E-05
	GO:0010647	positive regulation of cell communication	1.24E-04
	GO:0048522	positive regulation of cellular process	1.41E-04
	GO:0061188	negative regulation of chromatin silencing at rDNA	1.63E-04
	GO:0048523	negative regulation of cellular process	1.63E-04
	GO:0006355	regulation of transcription, DNA-templated	1.83E-04
	GO:2001141	regulation of RNA biosynthetic process	1.83E-04
	GO:1903506	regulation of nucleic acid-templated transcription	1.83E-04
	GO:0031936	negative regulation of chromatin silencing	1.86E-04
	GO:0048518	positive regulation of biological process	2.08E-04
	GO:0048519	negative regulation of biological process	2.19E-04
	GO:0031939	negative regulation of chromatin silencing at telomere	2.31E-04
	GO:0045815	positive regulation of gene expression, epigenetic	2.55E-04
	GO:0061187	regulation of chromatin silencing at rDNA	3.14E-04
	GO:0051252	regulation of RNA metabolic process	3.20E-04
	GO:0060969	negative regulation of gene silencing	3.42E-04
	GO:0009966	regulation of signal transduction	3.93E-04
	GO:0023051	regulation of signaling	3.93E-04
	GO:0006325	chromatin organization	7.69E-04
	GO:0061186	negative regulation of chromatin silencing at silent mating-type cassette	8.72E-04
	GO:0048584	positive regulation of response to stimulus	9.98E-04

Table S3: qPCR validation of PDR transcriptional regulators

Drug	Gene deleted	Systematic name	Brief Description	PDR5		SNQ2		YOR1		Screen Result
				fc	p	fc	p	fc	p	
1	*hoΔ*	YDL227C	Site-specific endonuclease	15.61		6.90		5.68		
	pdr1Δ	YGL013C	Transcription factor that regulates the pleiotropic drug response	1.40	***0.000***	1.91	***0.001***	1.17	***0.000***	Upregulator
	nbp2Δ	YDR162C	Protein involved in the HOG (high osmolarity glycerol) pathway	8.91	***0.012***	6.03	0.152	4.38	0.095	Upregulator
	cho2Δ	YGR157W	Phosphatidylethanolamine methyltransferase (PEMT)	8.21	***0.005***	4.10	***0.007***	4.98	0.138	Upregulator
	ypt6Δ	YLR262C	Rab family GTPase	8.20	***0.037***	4.33	***0.024***	4.00	0.069	Upregulator
	gyp1Δ	YOR070C	Cis-golgi GTPase-activating protein (GAP) for yeast Rabs	9.56	***0.021***	4.03	0.072	3.99	0.064	Upregulator
	gcn2Δ	YDR283C	Protein kinase	22.90	***0.025***	11.94	***0.005***	8.26	***0.015***	Downregulator
	mad1Δ	YGL086W	Coiled-coil protein involved in spindle-assembly checkpoint	22.46	0.123	11.83	***0.022***	9.85	***0.014***	Downregulator
	fyv10Δ	YIL097W	Subunit of GID complex	17.98	0.148	11.64	***0.005***	6.05	0.324	Downregulator
	sap30Δ	YMR263W	Component of Rpd3L histone deacetylase complex	18.07	0.144	12.20	***0.005***	8.45	***0.013***	Downregulator
	tom7Δ	YNL070W	Component of the TOM (translocase of outer membrane) complex	22.65	***0.023***	13.77	***0.002***	9.00	***0.009***	Downregulator
2	*hoΔ*	YDL227C	Site-specific endonuclease	1.52		36.15		26.59		
	pdr1Δ	YGL013C	Transcription factor that regulates the pleiotropic drug response	1.37	0.381	20.14	***0.021***	23.76	0.303	Upregulator
	yrr1Δ	YOR162C	Zn2-Cys6 zinc-finger transcription factor	2.72	0.092	11.87	***0.002***	15.10	***0.041***	Upregulator
	ssn2Δ	YDR443C	Subunit of the RNA polymerase II mediator complex	2.91	0.073	20.28	***0.014***	15.79	***0.032***	Upregulator
	met1Δ	YKR069W	S-adenosyl-L-methionine uroporphyrinogen III transmethylase	1.46	0.454	21.07	***0.016***	10.58	***0.006***	Upregulator
	met8Δ	YBR213W	Bifunctional dehydrogenase and ferrochelatase	1.61	0.438	18.22	***0.010***	9.42	***0.006***	Upregulator
	cho2Δ	YGR157W	Phosphatidylethanolamine methyltransferase (PEMT)	1.66	0.412	20.42	***0.046***	14.92	***0.026***	Upregulator
	spf1Δ	YEL031W	P-type ATPase, ion transporter of the ER membrane	3.29	0.051	73.58	***0.016***	45.53	***0.033***	Downregulator
	alg6Δ	YOR002W	Alpha 1,3 glucosyltransferase	2.98	0.070	62.82	***0.048***	32.90	0.211	Downregulator
	mad2Δ	YJL030W	Component of the spindle-assembly checkpoint complex	2.46	0.119	56.78	***0.026***	30.75	0.233	Downregulator
3	*hoΔ*	YDL227C	Site-specific endonuclease	9.53		3.27		4.04		
	pdr1Δ	YGL013C	Transcription factor that regulates the pleiotropic drug response	1.83	***0.000***	1.42	***0.000***	1.46	***0.000***	Upregulator
	uga2Δ	YBR006W	Succinate semialdehyde dehydrogenase	8.31	0.079	2.69	***0.008***	3.29	***0.027***	Upregulator
	cho2Δ	YGR157W	Phosphatidylethanolamine methyltransferase (PEMT)	5.70	***0.001***	3.75	0.056	4.58	0.141	Upregulator
	ykl053wΔ	YLK053W	Dubious ORF	8.38	***0.021***	3.37	0.314	4.44	0.238	Downregulator
	dph1Δ	YIL103W	Protein required for synthesis of diphthamide	14.73	***0.005***	3.81	0.205	6.80	0.052	Downregulator
	sif2Δ	YBR103W	WD40 repeat-containing subunit of Set3C histone deacetylase complex	10.28	0.261	6.05	***0.000***	6.45	***0.001***	Downregulator

Table S4: qPCR on *mad*Δ strains

Drug	[Drug](µM)	Strain	PDR5		SNQ2		YOR1	
			FC	P	FC	P	FC	P
5	50	*hoΔ*	22.81		1.80		22.36	
		mad1Δ	37.83	***0.014***	2.94	***0.001***	46.05	***0.033***
		mad2Δ	34.27	0.058	2.41	***0.022***	43.01	***0.041***
		mad3Δ	26.60	0.083	2.57	***0.009***	36.52	0.068
6	10	*hoΔ*	2.51		1.24		3.26	
		mad1Δ	3.94	***0.019***	1.40	0.220	6.71	***0.001***
		mad2Δ	4.29	***0.007***	1.21	0.449	7.08	***0.001***
		mad3Δ	4.06	***0.010***	0.97	0.087	6.41	***0.007***
7	50	*hoΔ*	1.01		2.88		0.91	
		mad1Δ	1.28	0.079	4.90	***0.002***	1.26	***0.010***
		mad2Δ	0.93	0.127	5.70	***0.003***	0.84	0.268
		mad3Δ	1.17	***0.005***	3.74	***0.000***	1.17	***0.001***
8	50	*hoΔ*	3.11		1.70		2.03	
		mad1Δ	6.09	***0.001***	1.67	0.446	4.46	***0.004***
		mad2Δ	5.98	***0.003***	1.83	0.325	4.43	***0.003***
		mad3Δ	5.10	0.094	1.97	0.195	4.02	***0.014***
9	25	*hoΔ*	1.46		0.95		3.15	
		mad1Δ	2.22	***0.025***	0.98	0.357	5.75	***0.009***
		mad2Δ	1.79	0.153	1.05	0.297	5.01	***0.001***
		mad3Δ	1.47	0.469	0.97	0.414	4.07	***0.019***

Table S5: Description of all genes tested in qPCR experiments*

Gene	Systematic Name	Gene name	Brief Description	Description
hoΔ	YDL227C	HOmothallic switching endonuclease	Site-specific endonuclease	Site-specific endonuclease; required for gene conversion at the MAT locus (homothallic switching) through the generation of a ds DNA break; expression restricted to mother cells in late G1 as controlled by Swi4p-Swi6p, Swi5p, and Ash1p
pdr1Δ	YGL013C	Pleiotropic Drug Resistance	Transcription factor that regulates the pleiotropic drug response	Transcription factor that regulates the pleiotropic drug response; zinc cluster protein that is a master regulator involved in recruiting other zinc cluster proteins to pleiotropic drug response elements (PDREs) to fine tune the regulation of multidrug resistance genes; relocalizes to the cytosol in response to hypoxia; PDR1 has a paralog, PDR3, that arose from the whole genome duplication
yrr1Δ	YOR162C	Yeast Reveromycin-A Resistant	Zn2-Cys6 zinc-finger transcription factor	Zn2-Cys6 zinc-finger transcription factor; activates genes involved in multidrug resistance; paralog of Yrm1p, acting on an overlapping set of target genes; YRR1 has a paralog, PDR8, that arose from the whole genome duplication
ssn2Δ	YDR443C	Suppressor of SNf1	Subunit of the RNA polymerase II mediator complex	Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; required for stable association of Srb10p-Srb11p kinase; essential for transcriptional regulation

met1Δ	YKR069W	METhionine requiring	S-adenosyl-L-methionine uroporphyrinogen III transmethylase	S-adenosyl-L-methionine uroporphyrinogen III transmethylase; involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase; required for sulfate assimilation and methionine biosynthesis
met8Δ	YBR213W	METhionine requiring	Bifunctional dehydrogenase and ferrochelatase	Bifunctional dehydrogenase and ferrochelatase; involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase; required for sulfate assimilation and methionine biosynthesis
cho2Δ	YGR157W	CHOline requiring	Phosphatidylethanolamine methyltransferase (PEMT)	Phosphatidylethanolamine methyltransferase (PEMT); catalyzes the first step in the conversion of phosphatidylethanolamine to phosphatidylcholine during the methylation pathway of phosphatidylcholine biosynthesis
spf1Δ	YEL031W	Sensitivity to Pichia Farinosa killer toxin	P-type ATPase, ion transporter of the ER membrane	P-type ATPase, ion transporter of the ER membrane; required to maintain normal lipid composition of intracellular compartments and proper targeting of mitochondrial outer membrane tail-anchored proteins; involved in ER function and Ca2+ homeostasis; required for regulating Hmg2p degradation; confers sensitivity to a killer toxin (SMKT) produced by Pichia farinosa KK1

alg6Δ	YOR002W	Asparagine-Linked Glycosylation	Alpha 1,3 glucosyltransferase	Alpha 1,3 glucosyltransferase; involved in transfer of oligosaccharides from dolichyl pyrophosphate to asparagine residues of proteins during N-linked protein glycosylation; C998T transition in human ortholog ALG6 causes carbohydrate-deficient glycoprotein syndrome type-Ic; wild-type human ortholog ALG6 can partially complement yeast alg6 mutant
mad2Δ	YJL030W	Mitotic Arrest-Deficient	Component of the spindle-assembly checkpoint complex	Component of the spindle-assembly checkpoint complex; delays onset of anaphase in cells with defects in mitotic spindle assembly; forms a complex with Mad1p; regulates APC/C activity during prometaphase and metaphase of meiosis I; gene dosage imbalance between MAD1 and MAD2 leads to chromosome instability
nbp2Δ	YDR162C	Nap1 Binding Protein	Protein involved in the HOG (high osmolarity glycerol) pathway	Protein involved in the HOG (high osmolarity glycerol) pathway; negatively regulates Hog1p by recruitment of phosphatase Ptc1p the Pbs2p-Hog1p complex; interacts with Bck1p and down regulates the cell wall integrity pathway; found in the nucleus and cytoplasm, contains an SH3 domain and a Ptc1p binding domain (PBM)

ypt6Δ	YLR262C	Yeast Protein Two	Rab family GTPase	Rab family GTPase; Ras-like GTP binding protein involved in the secretory pathway, required for fusion of endosome-derived vesicles with the late Golgi, maturation of the vacuolar carboxypeptidase Y; resides temporarily at the Golgi, dissociates into cytosol upon arrival of the Rab GTPaseYpt32p, which also functions in the late Golgi; Golgi-localized form is bound to GTP, while cytosolic form is GDP-bound; homolog of the mammalian Rab6
gyp1Δ	YOR070C	Gtpase-activating protein for YPt1p	Cis-golgi GTPase-activating protein (GAP) for yeast Rabs	Cis-golgi GTPase-activating protein (GAP) for yeast Rabs; the Rab family members are Ypt1p (in vivo) and for Ypt1p, Sec4p, Ypt7p, and Ypt51p (in vitro); involved in vesicle docking and fusion
gcn2Δ	YDR283C	General Control Nonderepressible	Protein kinase	Protein kinase; phosphorylates the alpha-subunit of translation initiation factor eIF2 (Sui2p) in response to starvation; activated by uncharged tRNAs and the Gcn1p-Gcn20p complex; contributes to DNA damage checkpoint control

mad1Δ	YGL086W	Mitotic Arrest-Deficient	Coiled-coil protein involved in spindle-assembly checkpoint	Coiled-coil protein involved in spindle-assembly checkpoint; required for inhibition of karyopherin/importin Pse1p (aka Kap121p) upon spindle assembly checkpoint arrest; phosphorylated by Mps1p upon checkpoint activation which leads to inhibition of anaphase promoting complex activity; forms a complex with Mad2p; gene dosage imbalance between MAD1 and MAD2 leads to chromosome instability
fyv10Δ	YIL097W	Function required for Yeast Viability	Subunit of GID complex	Subunit of GID complex; involved in proteasome-dependent catabolite inactivation of gluconeogenic enzymes FBPase, PEPCK, and c-MDH; forms dimer with Rmd5p that is then recruited to GID Complex by Gid8p; contains a degenerate RING finger motif needed for GID complex ubiquitin ligase activity in vivo, as well as CTLH and CRA domains; plays role in anti-apoptosis; required for survival upon exposure to K1 killer toxin
sap30Δ	YMR263W	Sin3-Associated Polypeptide	Component of Rpd3L histone deacetylase complex	Component of Rpd3L histone deacetylase complex; involved in silencing at telomeres, rDNA, and silent mating-type loci; involved in telomere maintenance

tom7Δ	YNL070W	Translocase of the Outer Mitochondrial membrane	Component of the TOM (translocase of outer membrane) complex	Component of the TOM (translocase of outer membrane) complex; responsible for recognition and initial import steps for all mitochondrially directed proteins; promotes assembly and stability of the TOM complex
uga2Δ	YBR006W	Utilization of GAba	Succinate semialdehyde dehydrogenase	Succinate semialdehyde dehydrogenase; involved in the utilization of gamma-aminobutyrate (GABA) as a nitrogen source; part of the 4-aminobutyrate and glutamate degradation pathways; localized to the cytoplasm
ykl053wΔ	YLK053W		Dubious ORF	
dph1Δ	YIL103W	DiPHthamide biosynthesis	Protein required for synthesis of diphthamide	Protein required for synthesis of diphthamide; required along with Dph2p, Kti11p, Jjj3p, and Dph5p; diphthamide is a modified histidine residue of translation elongation factor 2 (Eft1p or Eft2p); may act in a complex with Dph2p and Kti11p
sif2Δ	YBR103W	Sir4p-Interacting Factor	WD40 repeat-containing subunit of Set3C histone deacetylase complex	WD40 repeat-containing subunit of Set3C histone deacetylase complex; complex represses early/middle sporulation genes; antagonizes telomeric silencing; binds specifically to the Sir4p N-terminus

***:** All descriptions are directly adapted from Saccharomyces Genome Database (1).

Table S6: Plasmids used in this study

Name	**Description**
pCH81	pRS25_P*PDR5*-HIS3-T*CYC1*
pCH82	pRS25_P*SNQ2*-HIS3-T*CYC1*
pCH83	pRS25_P*YOR1*-HIS3-T*CYC1*

Table S7: Strains used in this study

Strain	Description	Source	Notes
JLY30	*MAT*a/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0 Δho::KanMX4/Δho::KanMX4*	Yeast deletion collection (2)	Aka *hoΔ*; *hoΔ* BY4743
JLY31	JLY30 transformed with pCH81	This study	
JLY32	JLY30 transformed with pCH82	This study	
JLY33	JLY30 transformed with pCH83	This study	
JLY34	MATα, pdr1-3, ura3, his1, Δyor1::hisG, Δsnq2::hisG, pdr5-Δ2::hisG, Δpdr10::hisG, Δpdr11::hisG, Δycf1::hisG, pdr3-Δ2::hisG, Δpdr15::hisG, pdr1-Δ3::hisG, *Δho::KanMX4*	This study	Aka *hoΔ* AD1-9
JLY35	MATα, pdr1-3, ura3, his1, Δyor1::hisG, Δsnq2::hisG, pdr5-Δ2::hisG, Δpdr10::hisG, Δpdr11::hisG, Δycf1::hisG, pdr3-Δ2::hisG, Δpdr15::hisG, pdr1-Δ3::hisG, *Δmad1::KanMX4*	This study	Aka *mad1Δ* AD1-9
JLY36	MATα, pdr1-3, ura3, his1, Δyor1::hisG, Δsnq2::hisG, pdr5-Δ2::hisG, Δpdr10::hisG, Δpdr11::hisG, Δycf1::hisG, pdr3-Δ2::hisG, Δpdr15::hisG, pdr1-Δ3::hisG, *Δmad2::KanMX4*	This study	Aka *mad2Δ* AD1-9
JLY37	MATα, pdr1-3, ura3, his1, Δyor1::hisG, Δsnq2::hisG, pdr5-Δ2::hisG, Δpdr10::hisG, Δpdr11::hisG, Δycf1::hisG, pdr3-Δ2::hisG, Δpdr15::hisG, pdr1-Δ3::hisG, *Δmad3::KanMX4*	This study	Aka *mad3Δ* AD1-9

All other deletions strains used in this study is retrieved from the yeast deletion collection.

Supplementary Datasets:

Dataset 1. Z-scores for each drug under with each biosensor construct in MSNP collection.

Dataset 2. Identity, fold change and significance of all samples that pass significance threshold in screenings

Reference

1. Cherry JM*, et al.* (2012) Saccharomyces Genome Database: the genomics resource of budding yeast. *Nucleic acids research* 40(Database issue):D700-705.
2. Giaever G*, et al.* (2002) Functional profiling of the Saccharomyces cerevisiae genome. *Nature* 418(6896):387-391.