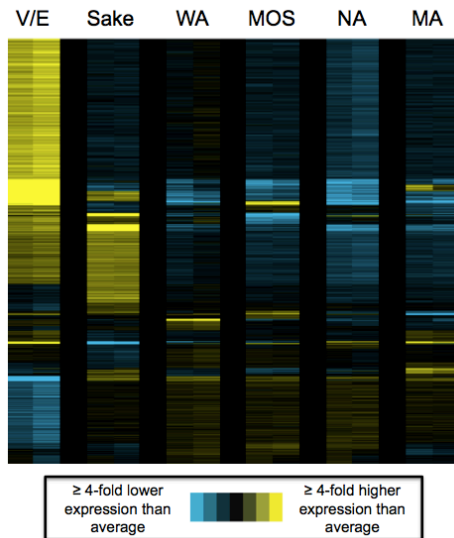


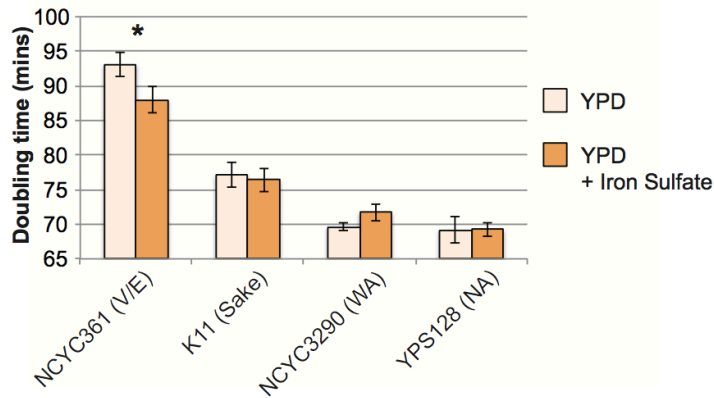
SUPPLEMENTARY FIGURES:

Figure S1.



Supplementary Figure S1. Transcriptional differences in YPD are mainly driven by sensitive strain NCYC361. Shown is the clustering analysis of log₂ RPKM mean center data for 4,523 genes whose expression was significant different (FDR < 1%) among our six strains; NCYC361 (V/Y), K11 (sake), NCYC3290 (WA), Y7568 (MOS), YPS128 (NA), and UWO.SO5.22-7 (MA). Clustering revealed that most of the differences are driven the sensitive strain NCYC3290 (V/Y).

Figure S2.



Supplementary Figure S2. Iron supplementation benefits NCYC361. Shown are the average and standard deviation of the doubling times measured for each strain in YPD and in YPD supplemented with 100 mg/L of iron sulfate. The doubling time was faster in NCYC361 when medium was supplemented with iron ($p < 0.0008$, T-test) but not significantly different for any other strain. Data represent three biological replicates.

Figure S3.

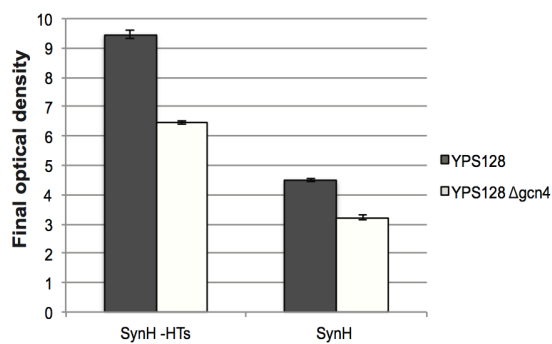


Figure S3. Deleting *GCN4* reduces growth in both SynH -HTs and SynH.

Final cell density after 24 hour growth in each medium was measured for HT-resistant strain YPS128 and YPS128 *gcn4D::KanMX*. Shown is the average and standard deviation of two biological replicates in SynH -HTs and SynH.

Figure S4.

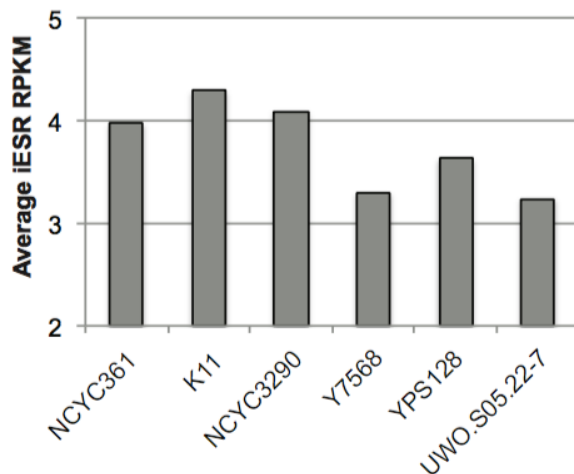


Figure S4. Sensitive strains showed higher induction of ESR genes.

The average transcript abundance ($\log_2(\text{RPKM})$ values) of genes induced in the ESR are shown for listed strains.

Figure S5.

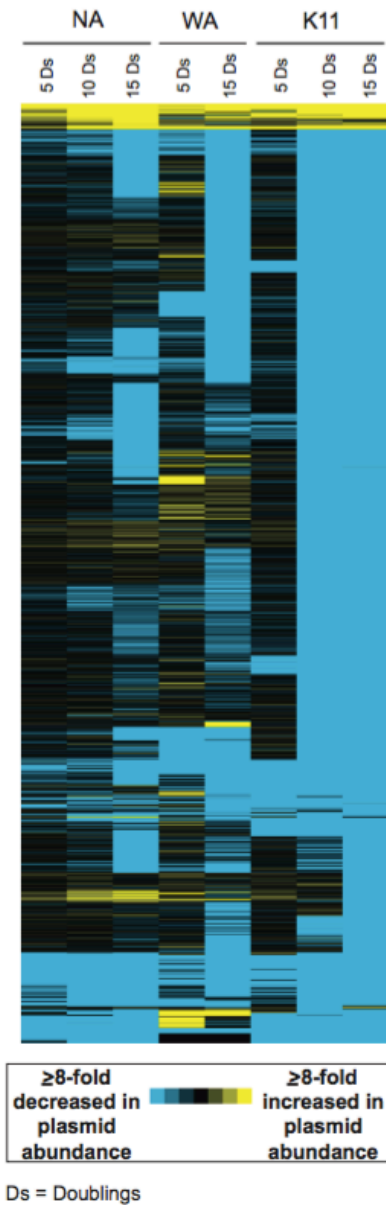


Figure S5. Strain-specific responses to gene over-expression. Shown is the combined set of genes that when over expressed in NA (YPS128), WA (NCYC3290), and K11 caused a significant ($FDR < 0.01$) fitness effect. In blue

are gene-plasmids that decreased in abundance compared to the initial unselected pool, while in yellow are gene-plasmids that increased in abundance overtime compared to the initial pool.

SUPPLEMENTARY TABLES:

Table S1. *Saccharomyces cerevisiae* strains

Strain	Strain source category	Location of isolation	HT relative growth rate
NCY3455	Clinical	Newcastle, UK	5
K9	Sake	Japan	10
UC5	Sake	Kurashi, Japan	15
Y2189	Natural Isolate	San Jacinto, California, USA	24
K10	Sake	Japan	26
NCYC361	Other Fermentation	NA	29
YJM269	Other Fermentation	NA	30
YB4082	Natural Isolate	Philippines	31
Y1	Natural Isolate	NA	32
YJM320	Clinical	USA	34
K11	Sake	Japan	34
K1	Sake	Japan	35
SK1	Lab	USA	37
YJM428	Clinical	USA	38
322134S	Clinical	Newcastle, UK	38
SB	Natural Isolate	Indonesia	40

YS2	Bakery	Australia	42
NCY3290	Other Fermentation	Indonesia	44
L-1528	Wine fermentation	Maule Region, Chile	47
DBVPG6765	Natural Isolate	Indonesia	48
DCM6	Natural Isolate	Wisconsin, USA	49
Y389	Natural Isolate	NA	49
CLIB324	Bakery	Vietnam	49
273614N	Clinical	Newcastle, UK	50
YPS1009	Oak	Mettlers Woods, NJ, USA	50
CLIB382	Other Fermentation	Ireland	51
WE372	Wine fermentation	Cape Town, South Africa	52
YJM308	Clinical	USA	53
M22	Vineyard	Italy	54
T73	Wine fermentation	Alicante, Spain	54
378604X	Clinical	Newcastle, UK	54
DBVPG6044	Other Fermentation	West Africa	54
DBVPG1853	Unknown	Ethiopia	57
YJM326	Clinical	USA	58
CBS7960	Industrial fermentation (sugar cane)	Sau Paulo, Brazil	58
YJM978	Clinical	Bergamo, Italy	58
YPS606	Natural Isolate	Woodland, PA	59
Y6	Natural Isolate	French Guiana	59
DBVPG1373	Natural Isolate	The Netherlands	59
YJM981	Clinical	Bergamo, Italy	60
DBVPG1788	Natural Isolate	Turku, Finland	60
YPS163	Oak	Pennsylvania, USA	60

YB210	Natural Isolate	Costa Rica	60
YJM454	Clinical	USA	60
YPS1000	Oak	Mettlers Woods, NJ, USA	60
YIIC17_E5	Wine fermentation	Sauternes, France	61
YJM1129	Other Fermentation	NA	61
DBVPG1106	Natural Isolate	Australia	61
Y2	Other Fermentation	Trinidad	61
YJM975	Clinical	Bergamo, Italy	62
UWOPS83-787.3	Natural Isolate	Bahamas	62
BC187	Other Fermentation	Napa Valley, USA	63
I14	Vineyard	Petina, Italy	63
CLIB215	Bakery	New Zealand	63
YS9	Bakery	Singapore	63
DCM16	Natural Isolate	Wisconsin, USA	63
Y3	Other Fermentation	Africa	64
YS4	Bakery	The Netherlands	64
YJM339	Clinical	USA	64
IL-01	NA	Cahokia, IL	64
YJM421	Clinical	USA	64
NCYC110	Other Fermentation	West Africa	65
Y55	Lab	NA	65
L-1374	Wine fermentation	Maule Region, Chile	65
YPS128	Natural Isolate	Pennsylvania, USA	66
YJM440	Clinical	USA	66
NC-02	Other Fermentation	Smoky Mountains, NC	67
UWOPS87-2421	Natural isolate	Hawaii	68
Y7568	Natural Isolate	Philippines	68

FL100	Lab	NA	69
PW5	Other Fermentation	Aba, Nigeria	69
PE-2	Other Fermentation	Brazil	69
EthanolRed	Other Fermentation	NA	70
YJM653	Clinical	NA	71
Y2209	Natural Isolate	San Jacinto, California, USA	72
YJM451	Clinical	Europe	73
UWOPS05-227.2	Natural Isolate	Trigona, Malaysia	74
UWOPS05-217.3	Natural Isolate	Malaysia	75
UWOPS03-461.4	Natural Isolate	Malaysia	78

Saccharomyces cerevisiae strains used in this study, with their niche, location of isolation, and hydrolysate tolerance (HT) score.

Table S2. Toxic cocktail components.

HT groups		
AMIDES	ACIDS	ALDEHYDES
Feruloyl amide	p-Coumaric acid	Vanillin
Coumaroyl amide	Ferulic acid	Syringaldehyde
	Benzoic acid	4-Hydroxybenzaldehyde
	Syringic acid	4-Hydroxyacetophenone
	Cinnamic acid	Hydroxymethyl furfural
	Vanillic acid	
	Caffeic acid	

Toxins present in the hydrolysate are divided in three inhibitory groups; amides, acids, and aldehydes.

Table S3. Genes responding to HT

PROCESS	GENES
Oxidation-reductase activity	
Quinone reductase	ZTA1, YLR460c
Aldehyde reductase	ARI1
Alcohol dehydrogenase	ADH7, ADH5
Alpha keto amide reductase	YDL124W
Aryl alcohol dehydrogenase	AAD4, AAD6, AAD16

Nitric oxide oxidoreductase	YHB1
Oxidation of thiols	FMO1
Nitroreductase	FRM2
Fatty-acyl coenzyme A oxidase	POX1
NADPH oxidoreductase	OYE3
NADPH regeneration	YMR315W, PYC1, ZWF1
de-novo NAD biosynthesis from tryptophan	BNA1, BNA5
Lyase activity	
Decarboxylase	FDC1
Plasma Membrane Transporter	
ABC Transporter	SNQ2, PDR12
Multidrug transporter	FLR1

Genes with significantly increased expression when toxins are present were involved in processes that included oxidation-reduction, lyase activity, and plasma membrane transporters.

Table S4. Composition of the Synthetic Hydrolysate Media.**SynH base media**

Chemical Catalog # (Vendor)	Component	mM Final Concentration
P386-500 (Fisher)	KH ₂ PO ₄	8.76
P288-500 (Fisher)	K ₂ HPO ₄	16.725
7783-20-2 (MP or Acros)	(NH ₄) ₂ SO ₄	45
P217-500 (Fisher)	KCl	55.2
S271-500 (Fisher)	NaCl	1.95
C79-500 (Fisher)	CaCl ₂ ·2H ₂ O	8.25
M33-500 (Fisher)	MgCl ₂ ·6H ₂ O	18.75
05129-25G (Fluka)	L-Alanine	1.758
11039 (Sigma)	L-Arginine.HCl	0.216
11149-25G-F (Sigma)	L-Asparagine	0.342
A2025-100G (Sigma)	DL-Aspartic acid.K	0.891
30129-25G (Sigma)	L-Cysteine.HCl	0.075
49419-25G (Sigma)	L-glutamine	0.3885
G1501-500G (Sigma)	L-Glutamic acid.K	0.9105
410225-250G (Sigma)	Glycine	0.567
71-00-1 (Acros)	L-Histidine	0.0561
58879 (Sigma)	L-Isoleucine	0.393
61819-25G (Sigma)	L-Leucine	0.5565

L5626-100G (Sigma)	L-Lysine.HCl	0.2625
64319-25G-F (Sigma)	L-Methionine	0.15
P5482-25G (Sigma)	L-Phenylalanine	0.423
81709-25G (Sigma)	L-Proline	0.984
84959-100G (Sigma)	L-Serine	0.5535
89179-10G (Sigma) or 72-19-5 (Acros)	L-Threonine	0.465
93659-10G (Sigma)	L-Tryptophan	0.075
72-18-4 (Acros)	L-Valine	0.636
93829-25G (Sigma)	L-Tyrosine	0.303
1152 (Calbiochem)	Adenine	0.075
71-30-7 (Alfa Aesar)	Cytosine	0.075
6630 (Calbiochem)	Uracil	0.075
73-40-5 (Alfa Aesar)	Guanine	0.075
MP103028 or Sigma BP892-100	Thiamine HCl	0.0006
Sigma C8731-25G	Calcium Pantothenate	0.0045
96469 (Fluka)	ZnCl ₂	30
M87-500 (Fisher)	MnCl ₂ ·4H ₂ O	136.5
307483-100G (Sigma)	CuCl ₂ ·2H ₂ O	2.85
60820 (Fluka)	CoCl ₂ ·6H ₂ O	0.045
B6768-500G (Sigma)	H ₃ BO ₄	34.65
09878 (Fluka)	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.465
10025-77-1 (Acros)	FeCl ₃ ·6H ₂ O	30

S648-500 (Fisher)	Sodium formate	4.2
S343-500 (Fisher)	Sodium nitrate	1.65
S413-500 (Fisher)	Sodium succinate	0.75
G33-500 (Fisher), D=1.261 g/ml	Glycerol	6.15
14300 (Fluka)	Betaine.H2O	1.05
67-48-1 (Acros)	Choline Chloride	0.45
461-05-2 (Acros)	DL-Carnitine	0.45
00160 (Fluka)	Acetamide	120
BP333-500 (Fisher)	Sodium acetate	48
	L-lactatic acid (90%)	6
3458-28-4 (Acros)	D-Mannose	1.8
87-72-9 (Acros)	L-Arabinose	30
L96-500 (Fisher)	D-Fructose	36
G0750-500G (Sigma)	D-Galactose	4.35
D16-1 (Fisher)	D-Glucose	90g/l (500mM)
141000025 (Acros)	D(+)Xylose	45g/l (300 mM)
BP267750 (Fisher)	Pyridoxine.HCl	3.21 µM
1282950000 (Acros)	Nicotinic Acid	40.17 µM
B4501-10G (Sigma)	Biotin	0.15 µM
57570-25G (Fluka) myo-Inositol	Inositol	0.084 mM
59924-1KG-F (Fluka)	Polysorbate 80 (Tween 80)	1.5 ml/l
E6510-10G (Sigma)	Ergosterol	15 mg/l

HT cocktail

Chemical Catalog # (Vendor)	Toxin	mM
Home made	Feruloyl amide	8.25
Home made	Coumaroyl amide	8.25
H40807-5G (Aldrich)	5-Hydroxymethyl-2-furadehyde	1.65
C9008-25G (Sigma)	<i>p</i> -Coumaric acid	3.15
W518301-100G (Sigma)	Ferulic acid	1.065
242381-100G (Sigma)	Benzoic acid	0.73
S6881-25G (Sigma)	Syringic acid	0.11
C80857-250G (Aldrich)	Cinnamic acid	0.14
H36001-25G (Aldrich)	Vanillic acid	0.13
C0625-5G (Sigma)	Caffeic acid	0.02
V1104-100G (Sigma)	Vanillin	0.20
S7602-25G (Aldrich)	Syringaldehyde	0.24
144088-50G (Aldrich)	4-Hydroxybenzaldehyde	0.30
278564-100G (Aldrich)	4-Hydroxyacetophenone	0.04