

Supplemental Information

Figure S1. Conservation of mutated residues in yeast, humans and *E. coli*. Sequence of yeast, human and *E. coli* Hsp90 were aligned using Clustal Omega. Residues mutated in this study are shown in red. The location of epitope used for anti-Hsc82 antisera and the site of deletion in Hsc82 Δ 34 are shown.

Figure S2. Further analysis of growth of *hsc82* mutants in presence of *utp21-S602F* mutation. Cells that grew on 5-FOA plates in Fig. 4 were grown overnight, serially diluted 10-fold, plated on rich media and grown for 2 d at the indicated temperature.

Figure S3. Further analysis of growth of *hsc82* mutants in presence of the *ssl2-I690N* mutation. **A.** A comparison of growth defects of cells expressing *utp21-S602F* or *ssl2-I690N*. Cells were grown on rich media for 2 d at the indicated temperature. **B.** Cells that grew on 5-FOA plates in Fig. 4 were grown overnight, serially diluted 10-fold, plated on rich media and grown for 2 d at the indicated temperature.

Figure S4. Raw 2D-DIGE images from each dataset. **A.** WT Hsc82, R46G and G309S. **B.** S25P, R46G and K102E. **C.** WT Hsc82, R46G and G424D. Spots that are of similar abundance in the 2D gel are shown in yellow. Spots that are more abundant in one sample than the other are shown in red or green.

Table S1. Protein level changes. Scanned images were analyzed by Image QuantTL software and subjected to in-gel analysis and cross-gel analysis using DeCyder software. The ratio change of the protein differential expression was obtained from in-gel DeCyder software analysis. The identity of chosen spots is shown. Proteins identified by mass spectrometry in more than one dataset are shown shaded in gray. **A.** WT Hsc82, R46G and G309S. **B.** S25P, R46G and K102E. **C.** WT Hsc82, R46G and G424D.

Table S2. Table of spots identified by mass spectrometry. The spots of interest were picked based on the in-gel analysis. Trypsin-digested peptides were subject to MALDI-TOF (MS) and TOF/TOF (tandem MS/MS). The resulting peptide mass and the associated fragmentation spectra were submitted to the MASCOT search engine to search protein sequence databases. The quantified protein levels for each spot shown in Figure S4 is shown. **A.** WT Hsc82, R46G and G309S. **B.** S25P, R46G and K102E. **C.** WT Hsc82, R46G and G424D.

Table S3. Comparison of 2D-DIGE hits with other studies that identified Hsc82/Hsp82 interactors.

Table S4. GO analysis of 2D-DIGE hits.

Supplemental Figure 1. Conservation of mutated amino acids.

		S25	R46
HSC82	-----MAGETFEFQAEITQLMSLIINTVY	SNKEIFLRELISNASDALDKI	RYQALSDPKQLETEPDLFIRITPKPEEKV
HSP82	-----MASETFEFQAEITQLMSLIINTVY	SNKEIFLRELISNASDALDKI	RYKSLSDPKQLETEPDLFIRITPKPEQKV
HSP90α	MPEETQTQDQPMEEEEVETFAFQAEIAQLMSLIINTFY	SNKEIFLRELISNSSDALDKI	RYESLTDPSKLDGSGKELHINLIIPNKQDRT
HSP90β	MPEE-----VHHGEEEVETFAFQAEIAQLMSLIINTFY	SNKEIFLRELISNASDALDKI	RYESLTDPSKLDGSGKELKIDIIIPNPQERT
HTPG	-----MKGQETRGRGFQSEVQQLHLMLHSLY	SNKEIFLRELISNASDAADKL	RFRALSNPDLYEGDGLRVRVSPDKDKRT
	K102		
HSC82	LEIRDSGIGMT	KAEILNNLGTIAKSGTKAFMEALSA--GADVSMIGQFGVGFYSLFLVADRQVVISKNN--	EDEQYIWESNAGGSFT
HSP82	LEIRDSGIGMT	KAEILNNLGTIAKSGTKAFMEALSA--GADVSMIGQFGVGFYSLFLVADRQVVISKSN--	DDEQYIWESNAGGSFT
HSP90α	LTIVDTGIGMT	KADLNNLGTIAKSGTKAFMEALQA--GADISMGQFGVGFYSAYLVAEKVTVITKHN--	DDEQYAWESSAGGSFT
HSP90β	LTIVDTGIGMT	KADLNNLGTIAKSGTKAFMEALQA--GADISMGQFGVGFYSAYLVAEKVVVITKHN--	DDEQYAWESSAGGSFT
HTPG	LTISDNVGMTRDEVIDHLGTIAKSGTKSFLES	LSGSDQAKDSQLIGQFGVGFYSAFIVADKVTVRTRAAGEKPENGVFWESAGEGYT	
HSC82	VTLDENVNERIGRGTVLRLFLKDDQLEYLEEKRIKEVIKRHSEFVAYPIQLLVTKVEKEVPI	PEEEKKDE-----	EKKDEDDKKPKL
HSP82	VTLDENVNERIGRGTILRLFLKDDQLEYLEEKRIKEVIKRHSEFVAYPIQLLVTKVEKEVPI	PEEEKKDE--	EKKDEEKDDDDKKPKL
HSP90α	VRTD--TGEPMGRGTKVILHLKEDQTEYLEERRIKEIVKKHSQFIGYPITLFEKERDKEVSDDEA	EKEEKDEKEEKEKEEKESEDKPEI	
HSP90β	VRAD--HGEPIGRGTKVILHLKEDQTEYLEERRVKEVVKHSQFIGYPITLFEKEREKEISDDEA	EEEKG---	EKEEEDKDDDEKPKI
HTPG	VADI---TKEDRGTEITLHLREGEDFLDDWRVRSIIISKYSDHIALPVEIEKRE-----		
		W296	G309
HSC82	EEVDEEEE-----EKKPKTKKVKEEVQ	LEELNKTTPWTRNPSDITQEEYN	AFYKSI
HSP82	EEVDEE-E-----EKKPKTKKVKEEVQ	LEELNKTTPWTRNPSDITQEEYN	AFYKSI
HSP90α	EDVGSDEEEKKDGDKKKKKIKKEYIDQEEELNKT	KPIWTRNPDITNEEYGEFYKSLTND	WEDHLAVKHFSVEGQLEFRALLFVPRR
HSP90β	EDVGSDEEDDSGDKKKKKKKIKKEYIDQEEELNKT	KPIWTRNPDITQEEYGEFYKSLTND	WEDHLAVKHFSVEGQLEFRALLFIPRR
HTPG	-----EKDGETVISWEKINKAQAALWTRNKSEIT	DEEYKEFYKHIAHDFNDPLTWSHNRVE	GKQEYTSLLYIPSQ
		Q380	K394
HSC82	APFDLFESKKKKNNIKLYVRRVFI	TDEAEDLIPWLSFVKGVVDS	EDLPLNLSREMLQQNKIMKVI
HSP82	APFDLFESKKKKNNIKLYVRRVFI	TDEAEDLIPWLSFVKGVVDS	EDLPLNLSREMLQQNKIMKVI
HSP90α	APFDLFENRKKKKNNIKLYVRRVFI	MDNCEELIPEYLN	FIRGVVDS
HSP90β	APFDLFENRKKKKNNIKLYVRRVFI	IMDSDELIPYLN	FIRGVVDS
HTPG	APWDMWNR--DHKGLGLYVQRVFI	IMDDAEQFMPNYLR	FVRGLIDSSDLPLNV
		G424	S481
HSC82	FDKFYSAFAKNIKL	GVHEDTQNRAALAKLLRYNSTK--SVDELTS	LDYVTRMPEHQKNIYYITGESLKAVEK
HSP82	FEKFYSAFSKNIKL	GVHEDTQNRAALAKLLRYNSTK--SVDELTS	LDYVTRMPEHQKNIYYITGESLKAVEK
HSP90α	YKFFYEQFSKNIKL	GIHEDSQNRKKLSELLRYTSA--SGDEMVS	LKDYCTRMMENQKHIIYYITGETKDQVAN
HSP90β	YKFFYEAFSKNIKL	GIHEDSTNRRRLSELLRYHTSQ--SGDEMTS	LSEYVSRMKETQKSIYYITGESKEQVAN
HTPG	YQTFWQQFGLVLKE	GPAEDFANQEAIAKLRF	FASTHTDSSAQTVSLEDYVSRMKEGQE
		T521	
HSC82	FLTDPIDIEYAFTQLKEFEGKTLVDI	TKD--FELEETDEEKA--EREKEI	KEYEPLTKALKDILGDQVEKVVVSYKLLDAPAAIRTGQFGW
HSP82	FLTDPIDIEYAFTQLKEFEGKTLVDI	TKD--FELEETDEEKA--EREKEI	KEYEPLTKALKEILGDQVEKVVVSYKLLDAPAAIRTGQFGW
HSP90α	YMIETIDIEYCVQQLKEFEGKTLVSV	TKEGLELPEDEEEKK--QKEEKKTKFENLCKIMKD	ILEKKVEKVVVSNRLVTSPPCIVTSTYGW
HSP90β	YMTEPIDIEYCVQQLKEFDGKSLVSV	TKEGLELPEDEEEKK--KMEESKAKFENLCKLMKE	ILDKKVEKVTISNRLVSSPCCIVTSTYGW
HTPG	LLSDRIDEWMMNYLT	EFDGKPFQSVSKVDESLEKLAD	EVDES
		A583	
HSC82	SANMERIMKAQALRDSSMSYSSSKKT	FEISPKSPIIKELKKRVDEGGAQDKTVKDLTNL	FETALLTSGFSLEEPTSFASRINRLIS
HSP82	SANMERIMKAQALRDSSMSYSSSKKT	FEISPKSPIIKELKKRVDEGGAQDKTVKDLTKLLY	ETALLTSGFSLEDEPTSFASRINRLIS
HSP90α	TANMERIMKAQALRDNSTMGYMAAKKHLE	INPDHSIIETLRQKA--EADKNDKSVKDLVILLY	ETALLSSGFSLEDPQTHANRIYRMIK
HSP90β	TANMERIMKAQALRDNSTMGYMAAKKHLE	INPDHIVETLRQKA--EADKNDKAVKDLVLL	FETALLSSGFSLEDPQTHSNRIYRMIK
HTPG	STQMAKLFAAAGK-----VPEVYI	FELNPDHVLVKRAADTED----	EAKFSEWVELLLDQALLAERG
		α-Hsc82 Epitope	
		Deleted in A34	
HSC82	LG	LNIDEDEETETAPEASTEA---	PVEEVPADTEMEEVD
HSP82	LG	LNIDEDEETETAPEASTAA---	PVEEVPADTEMEEVD
HSP90α	LG	LGIDEDDPTADDTSAAVTEEMP	LEGDDDSRMEEVD
HSP90β	LG	LGIDEDEVAEEPNAAVPDEI	PPLEGDEDDASRMEEVD
HTPG	S-	-----	

Sequences were aligned with Clustal Omega. Accession numbers *S. cerevisiae* HSC82 (KZV09036.1), *S. cerevisiae* HSP82 (NP_015084.1), *Homo sapiens* Hsp90 alpha isoform (AAI21063.1), *Homo sapiens* Hsp90 beta isoform (NP_031381.2), *E. coli* HtpG (NP_415006.1).

Fig S2. Effect of combination of *hsc82* mutations and *utp21-S602F* mutation

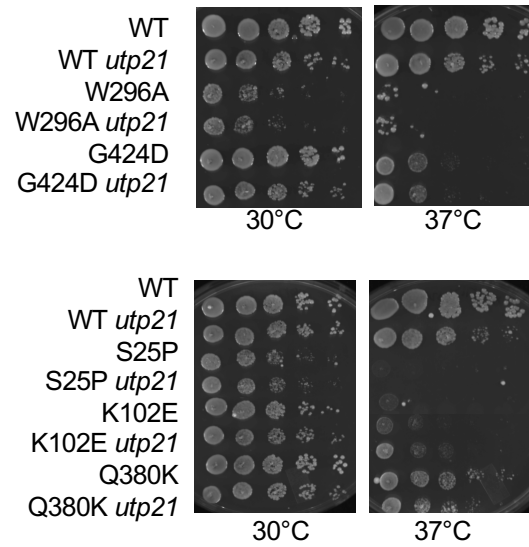
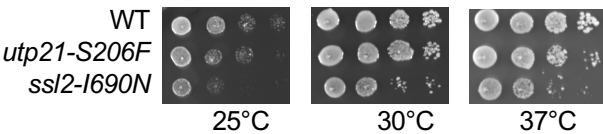


Fig S3. Effect of combination of *hsc82* mutations and *ssl2-l690N* mutation

A.



B.

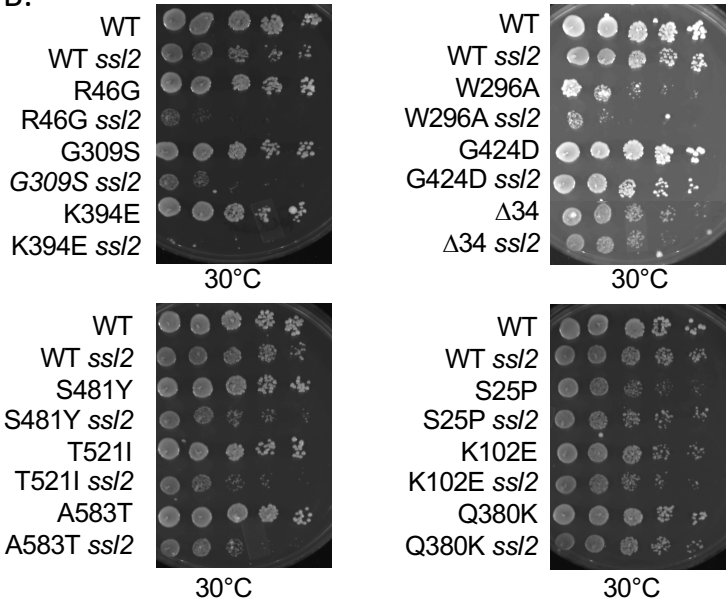
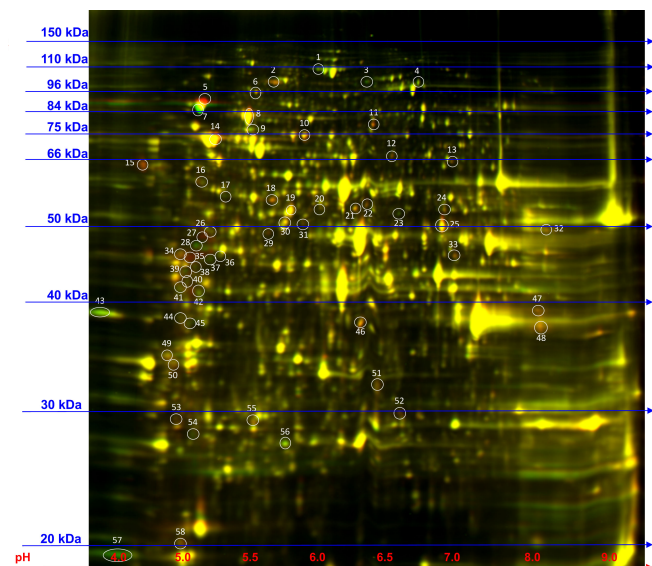
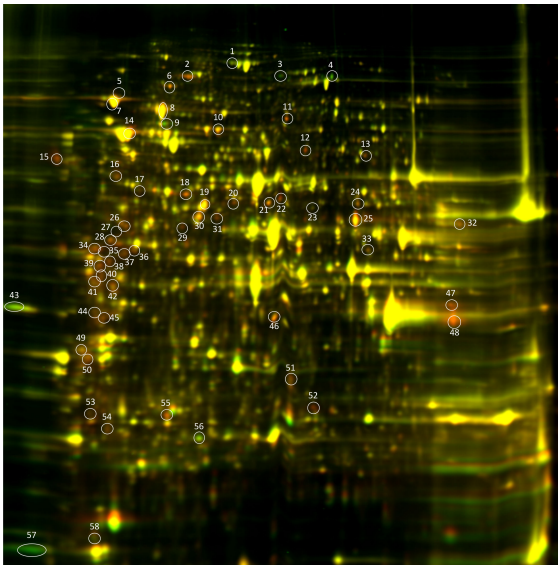


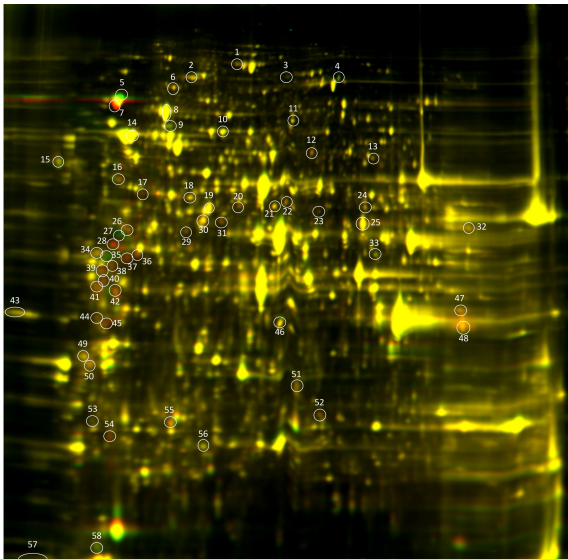
Fig. S4A: WT, R46G, G309S



Green WT
Red R46G

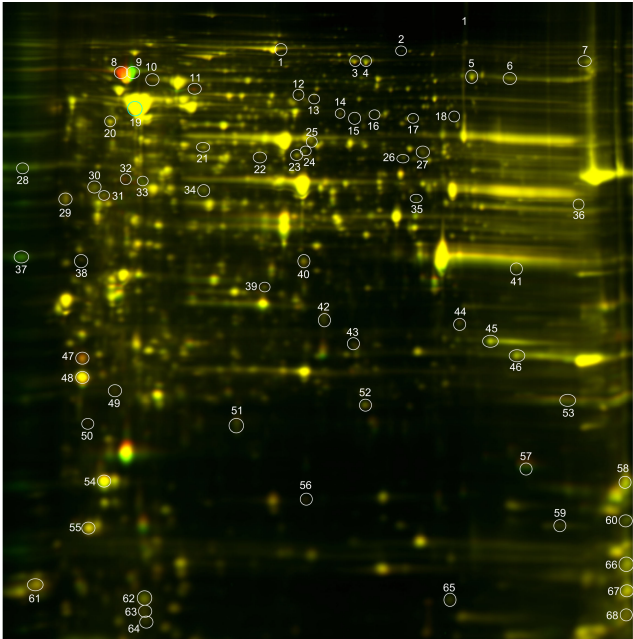


Green WT
Red G309S

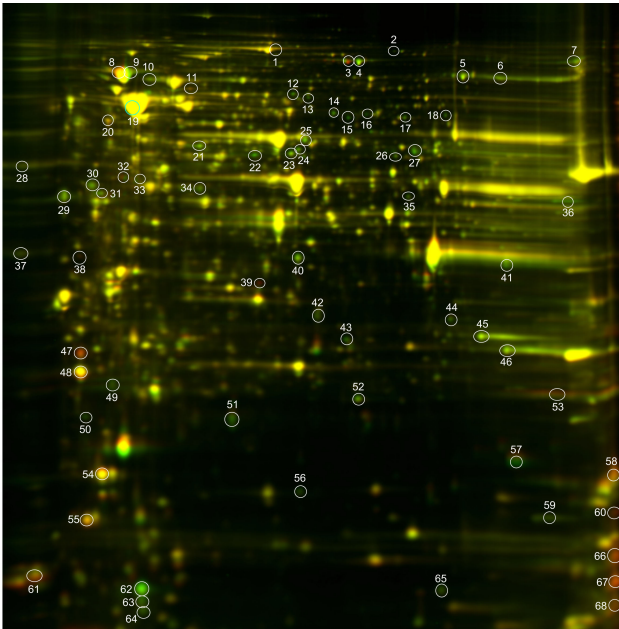


Green R46G
Red G309S

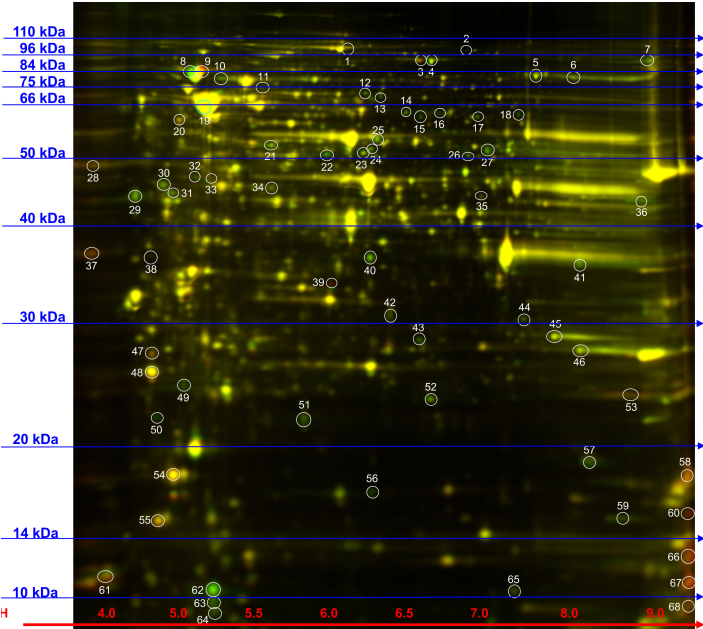
Fig. S4B. S25P, R46G, K102E



Green S25P
Red K102E

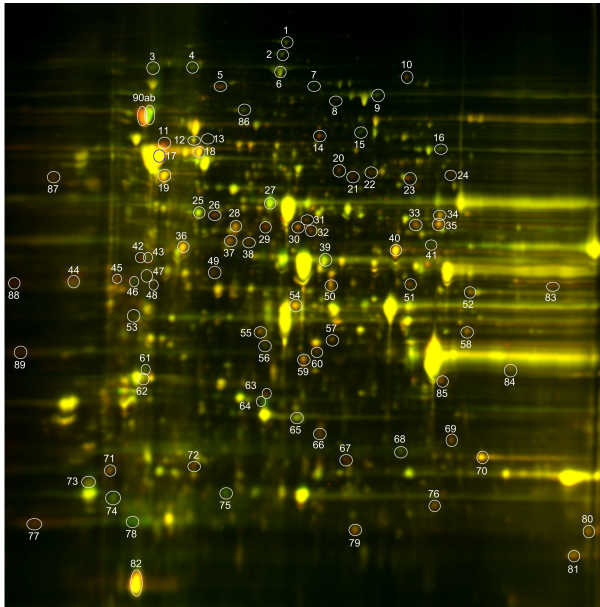


Green R46G
Red K102E

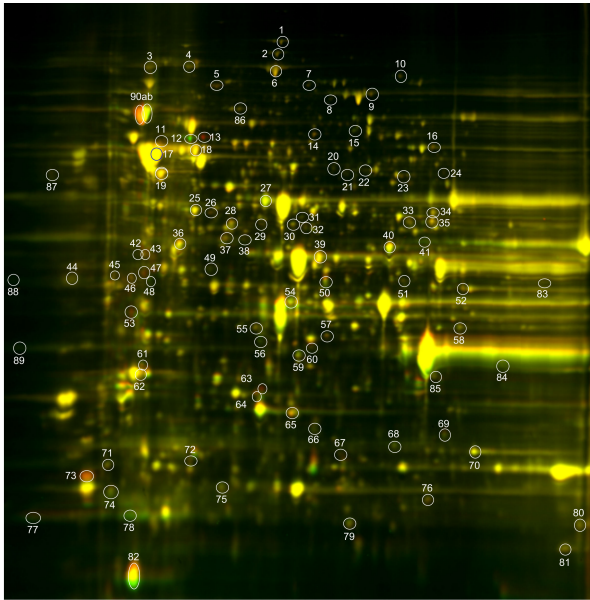


Green R46G
Red S25P

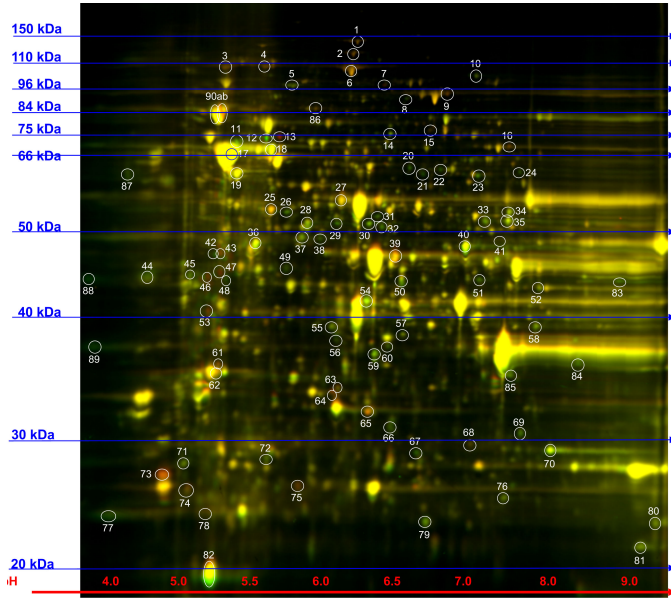
Fig. S4C. WT, R46G, G424D



Green WT
Red R46G



Green WT
Red G424D



Green R46G
Red G424D

Table S1A. WT, R46G, G309S

positive: up-regulation
negative: down-regulation

Assigned spot#	R46G / WT	G309S / WT	G309S / R46G	Mss Spec ID°
1	-1.7	-1.47	1.14	YEP3
2	2.05	2.35	1.14	HSP104
3	-1.7	-1.88	-1.11	SEC24
4	-1.36	-2.44	-1.8	EFT1/PFK2
5	3.54	1.71	-2.08	
6	1.32	1.88	1.43	HIS4
7	-2.77	1.9	5.25	
8	1.7	1.68	-1.01	
9	-1.24	-2.04	-1.64	NOP56
10	1.72	1.7	-1.02	
11	2.39	2.49	1.04	HSP78
12	1.39	2.02	1.45	PAB1/PGM2
13	1.89	2.24	1.18	ACH1
14	2.55	2.52	-1.01	SSA1
15	3.4	3.07	-1.11	DDR48
16	1.06	1.93	1.82	
17	-1.41	1.32	1.85	
18	2.23	2.6	1.16	HXK1
19	1.48	1.72	1.16	
20	1.24	2.18	1.75	GDH1
21	1.66	1.91	1.15	
22	1.87	2.87	1.53	APE1/GDH1
23	-1.53	-1.01	1.5	
24	1.44	1.72	1.19	
25	1.75	2.52	1.44	
26	1.38	2.2	1.59	
27	7.82	2.43	-3.22	
28	-1.5	2.16	3.24	
29	1.53	1.72	1.12	
30	1.28	1.74	1.36	
31	1.54	2.37	1.54	ARG1
32	1.79	2.3	1.28	ENO1
33	1.83	1.55	-1.19	
34	2.18	2.58	1.18	
35	9.15	2.37	-3.88	
36	-1.05	1.92	2.02	
37	-1.08	1.98	2.13	
38	1.51	2.05	1.36	
39	-1.1	1.94	2.14	
40	1.16	2.57	2.2	
41	1.23	2.16	1.75	
42	-1.06	1.76	1.87	
43	-1.95	-1.94	1	STM1/TDH3
44	1.42	1.92	1.34	
45	-1.03	2.29	2.36	
46	1.89	2.05	1.08	YDL124W
47	1.79	2.74	1.53	TDH3
48	1.53	2.75	1.79	TDH2/SIS1
49	1.48	1.71	1.16	
50	1.37	1.76	1.28	
51	1.76	2.06	1.17	RPL2B/GPP1
52	1.54	2.31	1.49	SSA2/URA3
53	1.69	1.8	1.06	
54	-1.08	1.58	1.7	
55	1.45	2.13	1.47	SEC53
56	-1.55	-1.75	-1.13	FUR1
57	-3.13	-3.94	-1.26	HYP2
58	1.75	1.18	-1.48	

*In some cases, multiple proteins were identified in a spot. See table S2 for clarification
Proteins identified by mass spec in more than one dataset are shaded in gray.

Table S1B. S25P, R46G, K102E

positive: up-regulation negative: down-regulation				
Assigned spot#	S25P/R46G	K102E / R46G	K102E / S25P	Mss Spec ID°
1	1.28	1.51	1.17	
2	-1.52	-1.54	-1.02	
3	1.34	1.45	1.07	EFT1
4	-1.82	-1.95	-1.08	EFT1
5	-1.41	-1.5	-1.07	
6	-1.43	-1.55	-1.09	
7	-1.66	-1.73	-1.05	
8	-1.29	1.28	1.64	
9	1.35	-1.29	-1.76	
10	-1.66	-1.34	1.23	
11	-1.01	1.58	1.59	APE3
12	-2.26	-1.94	1.15	TKL2
13	-1.69	-1.5	1.12	
14	-1.94	-1.9	1.01	PGM2
15	-2.83	-3.35	-1.19	CTT1
16	-2.11	-2.38	-1.14	ADE16
17	-1.95	-2.09	-1.09	ACH1/YDR3
18	-2.41	-2.35	1.01	GUT2
19	1.43	1.52	1.05	
20	1.38	1.51	1.08	
21	-1.97	-1.78	1.1	
22	-3.52	-3.03	1.15	ALD3/GDH1
23	-1.88	-1.83	1.02	
24	-1.79	-1.96	-1.11	GLK1
25	-1.65	-1.57	1.04	
26	-1.61	-1.66	-1.04	
27	-2.76	-2.52	1.08	MSC1/ATP1
28	1.56	-1.25	-1.97	TEF1
29	-1.99	-1.7	1.16	PEP4
30	-1.6	-1.5	1.05	
31	-1.87	-1.53	1.21	LSP1
32	-1.68	1.17	1.95	HSC82
33	1.32	-1.26	-1.68	
34	-1.62	-1.76	-1.1	
35	-1.37	-1.71	-1.26	
36	-2.24	-2.36	-1.06	OM45
37	2.34	-1.22	-2.87	TDH3/STM1
38	1.46	1.53	1.04	
39	3.4	3.61	1.05	GPP1/SEC13/ASC1
40	-2.17	-1.91	1.12	YDL124W
41	-1.68	-2.06	-1.24	TDH1/SIS1
42	-1.57	-1.59	-1.02	
43	-2.04	-1.77	1.14	TFS1
44	-1.72	-1.62	1.05	
45	-1.33	-1.55	-1.18	
46	-1.31	-1.52	-1.17	
47	1.47	3.05	2.05	TIF6/GSP2/RPS5
48	1.2	1.52	1.25	
49	-2.3	-1.71	1.33	
50	-3.65	-2.36	1.53	GRE1RPS9B
51	-2.09	-1.94	1.07	PST2
52	-1.96	-1.89	1.02	SOD2
53	1.49	1.73	1.15	
54	1.58	1.61	1.01	
55	1.52	1.72	1.12	
56	-2.85	-1.97	1.44	EGD1
57	-1.74	-1.3	1.32	
58	1.99	1.94	-1.04	RPL11B/RPL12B
59	-1.52	-1.61	-1.07	
60	2.68	2.34	-1.16	MBF1
61	1.33	1.62	1.2	
62	-3.61	-3.86	-1.08	HSP12
63	-2.78	-2.65	1.04	HSP12
64	-1.99	-1.93	1.02	
65	-1.65	-1.73	-1.06	
66	2.94	2.81	-1.06	RPS14A/RPL23B/RPS22B
67	2.87	2.89	-1	RPS22A
68	2.03	2.23	1.09	RPL30/RPS10A

*In some cases, multiple proteins were identified in a spot. See table S2 for clarification
Proteins identified by mass spec in more than one dataset are shaded in gray.

Table S1C. WT, R46G, G424D

Assigned spot#	R46G / WT	G424D / WT	positive: up-regulation	
			negative: down-regulation	
			G424D / R46G	Mass Spec ID*
1	-1.3	1.15	1.5	
2	-1.35	1.14	1.55	
3	-1.33	1.16	1.55	
4	-1.44	1.12	1.61	
5	2.14	1.42	-1.5	
6	-1.24	1.26	1.58	
7	-1.4	1.12	1.57	
8	1.53	1.13	-1.35	
9	-1.41	1.18	1.67	
10	1.56	1.18	-1.32	
11	2.1	1.41	-1.48	
12	1.06	-2.37	-2.5	LB-C virus Gag-Pol
13	1.11	3.79	3.44	LB-C virus Gag-Pol
14	2.39	1.41	-1.69	HSP78
15	-1.53	-1.25	1.23	
16	-1.31	1.14	1.5	TKL1
17	2.24	1.53	-1.45	SSA1
18	1.69	1.34	-1.25	
19	1.64	1.44	-1.13	
20	1.81	1.11	-1.62	
21	1.98	1.08	-1.83	
22	1.77	1.06	-1.66	
23	2.59	1.24	-2.08	ACH1
24	1.81	1.05	-1.71	
25	-1.3	1.09	1.42	
26	3.51	1.6	-2.18	HXK1
27	-1.48	-1.23	1.21	
28	1.68	1.12	-1.5	
29	2.64	1.11	-2.36	GDH1/ALD3
30	1.9	-1	-1.89	
31	1.76	1	-1.75	
32	2.52	1.14	-2.21	
33	1.69	1.11	-1.52	
34	1.62	1.19	-1.36	
35	1.84	1.3	-1.41	
36	1.79	1.19	-1.5	
37	1.77	1.15	-1.53	
38	1.81	1.07	-1.68	
39	-1.19	1.29	1.54	
40	1.7	-1.03	-1.74	
41	-1.36	-1.54	-1.13	GLR1/TEF1
42	4.79	1.43	-3.34	HSC82
43	-2.01	1.84	3.72	HSC82
44	1.64	1.08	-1.51	
45	2.61	1.41	-1.85	
46	-2.14	1.67	3.59	HSC82
47	-1.43	2.6	3.74	HSC82
48	1.23	-1.3	-1.6	
49	1.56	-1.22	-1.9	
50	1.59	1.13	-1.4	
51	1.91	1.02	-1.87	
52	1.67	1.31	-1.26	
53	-1.28	1.73	2.23	
54	1.49	-1.17	-1.74	
55	1.51	1.12	-1.35	
56	1.07	-1.61	-1.72	
57	1.69	1.24	-1.36	
58	1.6	-1.02	-1.64	
59	1.66	-1.11	-1.84	
60	2.13	1.54	-1.38	ARA1
61	-1.34	1.32	1.78	
62	-1.1	1.39	1.53	
63	1.99	2.59	1.31	ASC1/GPP1
64	-1.28	1.52	1.96	ASC1
65	-1.14	1.26	1.44	
66	1.55	-1.01	-1.55	
67	2.69	1.29	-2.07	
68	-1.57	-1.28	1.23	
69	1.94	1.08	-1.79	
70	1.59	-1.05	-1.67	
71	1.61	1.08	-1.48	
72	1.56	1.07	-1.45	
73	-1.19	2.18	2.61	TIF6
74	-1.68	-1.31	1.28	
75	-1.9	-1.08	1.77	FUR1
76	1.79	1.17	-1.52	
77	2.08	1.19	-1.74	
78	-2.04	-1.84	1.11	TSA1
79	1.58	-1.04	-1.64	
80	1.59	1.07	-1.48	
81	1.62	1.13	-1.42	
82	1.75	1.03	-1.69	
83	4.31	1.23	-3.48	OM45
84	1.09	-1.8	-1.95	TDH1/SIS1
85	1.53	-1.23	-1.88	
86	-1.33	1.12	1.5	
87	2.2	1.21	-1.81	
88	2.75	-1.34	-3.66	SBP1
89	1.93	-1.51	-2.91	STM1
90ab	-1.06	1.39	1.48	

*In some cases, multiple proteins were identified in a spot. See table S2 for clarification
 Proteins identified by mass spec in more than one dataset are shaded in gray.

Table S2A: WT, R46G, G309S

Spot number	MALDI well number	Top Ranked Protein Name [Species]	Accession No.	Protein MW	Protein PI	Pep.Count	Protein Score	Protein Score C.I.%	Total Ion Score	Total Ion C.I.%	Comments / Other Possible Hits
1	E1	Elongation factor 3A	YEF3	115,872	5.73	31	1150	100	961	100	
2	E2	Heat shock protein 104	HSP104	101,972	5.31	29	462	100	290	100	
3	E3	Protein transport protein	SEC24	103,569	5.83	8	53	96	40	100	
4	E4	Elongation factor 2 *	EFT1	93,230	5.92	21	445	100	342	100	
		ATP-dependent 6-phosphofructokinase subunit beta	PFK2	104552	6.23	21	244	100	155	100	
6	E5	Histidine biosynthesis trifunctional protein	HIS4	87,666	5.17	18	440	100	359	100	
9	E6	Nucleolar protein 56 GN=NOP56	NOP56	56,829	8.96	12	56	98			
11	E7	Heat shock protein 78, mitochondrial	HSP78	91,280	8.17	28	678	100	521	100	
12	E8	Polyadenylate-binding protein, cytoplasmic and nuclear	PAB1	64,304	5.71	4	163	100	155	100	
		Phosphoglucomutase 2*	PGM2	63,049	6.18	8	64	100	41	100	
13	E9	Acetyl-CoA hydrolase	ACH1	58,675	6.30	14	418	100	347	100	
14	E10	Heat shock protein SSA1	SSA1	69,615	5.00	28	1360	100	1147	100	
15	E11	Stress protein DDR48	DDR48	46,207	4.22	8	369	100	334	100	
18	E12	Hexokinase-1	HXK1	53,705	5.28	19	664	100	533	100	
20	E13	NADP-specific glutamate dehydrogenase 1	GDH1	49,539	5.56	9	418	100	380	100	
22	E14	Vacuolar aminopeptidase 1	APE1	57,057	5.55	13	372	100	307	100	
		NADP-specific glutamate dehydrogenase 1	GDH1	49,539	5.56	12	125	100	66	100	
31	E15	Argininosuccinate synthase	ARG1	46,898	5.45	18	606	100	487	100	
32	E16	Enolase 1	ENO1	46,787	6.16	17	939	100	823	100	
43	E17	Suppressor protein STM1	STM1	29,977	9.66	14	573	100	466	100	
		Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	TDH3	35724.4	6.46	5	130	100	110	110	
46	E18	NADPH-dependent alpha-keto amide reductase	YDL124W	35,538	5.84	16	815	100	690	100	
47	E19	Glyceraldehyde-3-phosphate dehydrogenase 3	TDH3	35,724	6.46	9	317	100	267	100	
48	E20	Glyceraldehyde-3-phosphate dehydrogenase 2	TDH2	35,824	6.46	12	540	100	463	100	
		Protein SIS1	SIS1	37566.7	9.02	8	122	100	86	100	
51	E21	60S ribosomal protein L2-B	RPL2B	27,392	11.10	10	257	100	191	100	
		Glycerol-1-phosphate phosphohydrolase 1*	GPP1	27929.3	5.35	5	220	100	198	100	or GPP2
52	E22	Heat shock protein SSA2	SSA2	69,428	4.95	12	451	100	406	100	or SSA1
		Orotidine 5'-phosphate decarboxylase*	URA3	29221.1	6.91	9	170	100	126	100	
55	E23	Phosphomannomutase	SEC53	29,045	5.14	7	223	100	189	100	
56	E24	Uracil phosphoribosyltransferase	FUR1	24,579	5.58	16	762	100	627	100	
57	F1	Eukaryotic translation initiation factor 5A-1*	HYP2	17103.3	4.81	4	112	100	93	100	

Table S2B: S25P, R46G, K102E

Spot number	MALDI well number	Top Ranked Protein Name [Species]	Gene Name	Protein MW	Protein PI	Pep.Count	Protein Score	Protein Score C.I.%	Total Ion Score	Total Ion C.I.%	Comments / Other Possible Hits
3	G1	Elongation factor 2	EFT1	93,230	5.92	34	1,280	100	1,011	100	
4	A9	Elongation factor 2	EFT1	93230.2	5.92	38	1,490	100	1160	100	
11	A10	Aminopeptidase Y	APE3	60099.8	5.13	6	116	100	101	100	
12	G2	Transketolase 2	TKL2	74,983	5.75	19	655	100	548	100	
14	A11	Phosphoglucomutase 2	PGM2	63048.9	6.18	16	441	100	355	100	
15	G3	Catalase T	CTT1	64,527	6.09	17	404	100	310	100	
16	G4	Bifunctional purine biosynthesis protein A	ADE16	65,242	6.12	13	257	100	200	100	
17	A12	Acetyl-CoA hydrolase*	ACH1	58675	6.3	18	666	100	552	100	
		Arginine--tRNA ligase	YDR3	69480.9	6.32	23	441	100	304	100	
18	G5	Glycerol-3-phosphate dehydrogenase, r	GUT2	72,343	7.96	29	694	100	480	100	
22	G6	Aldehyde dehydrogenase [NAD(P)+] 2 *	ALD3	55,350	5.56	21	632	100	487	100	or ALD2
		NADP-specific glutamate dehydrogenas	GDH1	49539.0	5.56	13	305	100	236	100	
23	A13	Alpha, alpha-trehalose-phosphate syntha	TPS1	56112.5	5.71	20	1,170	100	1024	100	
24	A14	Glucokinase-1	GLK1	55342.3	5.8	23	1,580	100	1395	100	
27	G7	Meiotic sister chromatid recombination p	MSC1	59552.0	7.06	15	421	100	339	100	
		ATP synthase subunit alpha, mitochondr	ATP1	58,572	9.06	19	440	100	324	100	
28	A15	Elongation factor 1	TEF1	50001.2	9.14	16	728	100	630	100	
29	A16	Saccharopepsin	PEP4	44471.1	4.7	16	986	100	877	100	
31	A17	Sphingolipid long chain base-responsive	LSP1	38048	4.62	12	451	100	377	100	
32	A18	Hsc82	HSC82	80849.7	4.78	14	158	100	102	100	
36	G8	Mitochondrial outer membrane protein O	OM45	44,554	8.55	24	1,050	100	853	100	
37	G9	Glyceraldehyde-3-phosphate dehydroge	TDH3	35724.0	6.46	5	108	100	90	100	or G3P2
		Suppressor protein STM1	STM1	29,977	9.66	14	874	100	766	100	
39	G10	Glycerol-1-phosphate phosphohydrolas	GPP1	27,929	5.35	12	411	100	324	100	or GPP2
		Protein transport protein SEC13	SEC13	33023.0	5.45	7	232	100	199	100	
		Guanine nucleotide-binding protein subu	ASC1	34783.6	5.8	8	124	100	83	100	
40	G11	NADPH-dependent alpha-keto amide rec	YDL124	35,538	5.84	18	1,040	100	889	100	
41	G12	Glyceraldehyde-3-phosphate dehydroge	TDH1	35,728	8.29	21	950	100	757	100	or G3P2, G3P3
		Protein SIS1	SIS1	37566.7	9.02	6	106	100	84	100	
43	G13	Carboxypeptidase Y inhibitor	TFS1	24,342	6.07	9	745	100	681	100	
47	G14	Eukaryotic translation initiation factor 6*	TIF6	26,441	4.54	6	448	100	418	100	
		GTP-binding nuclear protein GSP2/CNR	GSP2	24974.7	6.22	6	159	100	128	100	
		40S ribosomal protein S5	RPS5	25023.3	8.63	4	154	100	138	100	
50	G15	Protein GRE1 *	GRE1	19014.5	4.55	4	136	100	115	100	
		40S ribosomal protein S9-B	RPS9B	22,285	10.09	10	171	100	118	100	or RPS9A
51	G16	Protoplast secreted protein 2	PST2	20,953	5.46	8	1,100	100	1,038	100	
52	A19	Superoxide dismutase [Mn], mitochondri	SOD2	25758.2	8.49	7	716	100	674	100	
56	G17	Nascent polypeptide-associated comple	EGD1	17,010	6.10	3	201	100	186	100	
58	A20	60S ribosomal protein L11-B	RL11B	19737.4	9.92	13	489	100	382	100	or RPL11A
		60S ribosomal protein L12-B	RL12B	17811.7	9.43	7	196	100	147	100	
60	G18	Multiprotein-bridging factor 1	MBF1	16,394	10.61	8	174	100	117	100	
62	G19	12 kDa heat shock protein	HSP12	11,686	5.22	9	664	100	579	100	
63	G20	12 kDa heat shock protein	HSP12	11,686	5.22	7	424	100	364	100	
66	G21	40S ribosomal protein S14-A	RPS14	14,528	10.73	6	305	100	264	100	
		60S ribosomal protein L23-B	RPL23B	14463.6	10.33	10	303	100	231	100	
		40S ribosomal protein S22-B	RPS22B	14617.0	9.94	8	200	100	138	100	
67	G22	40S ribosomal protein S22-A	RPS22A	14,617	9.94	9	698	100	621	100	or RPS22B
68	G23	60S ribosomal protein L30	RPL30	11,408	9.80	7	388	100	330	100	
		40S ribosomal protein S10-A	RPS10A	12732.0	8.73	5	274	100	236	100	or RPS10B

Table S2C: WT, R46G, G424D

Spot number	MALDI well number	Top Ranked Protein Name	Gene Name	Protein MW	Protein PI	Pep Count	Protein Score	Protein Score C.I.%	Total Ion Score	Total Ion C.I.%	Comments / Other Possible Hits
12	C1	Probable RNA-directed RNA polymerase L-BC virus	LB-C virus Gag-Pol	78,316	5.20	27	393	100	297	100	
13	C2	Probable RNA-directed RNA polymerase L-BC virus	LB-C virus Gag-Pol	78,316	5.20	25	257	100	178	100	
14	A1	Heat shock protein 78, mitochondrial	HSP78	91,280	8.17	28	902	100	730	100	
16	A2	Transketolase 1	TKL1	73,760	6.51	25	1,200	100	1,026	100	
17	A3	Heat shock protein SSA1	SSA1	69,615	5.00	29	1,490	100	1,256	100	Ssa2, 3 or 4
23	C3	Acetyl-CoA hydrolase	ACH1	58,675	6.30	13	482	100	417	100	
26	C4	Hexokinase-1	HXK1	53,705	5.28	26	1,010	100	779	100	
29	C5	NADP-specific glutamate dehydrogenase 1	GDH1	49,539	5.56	12	281	100	220	100	
		Aldehyde dehydrogenase [NAD(P)+] 2	ALD3	55350.0	5.56	12	198	100	143	100	
41	A4	Glutathione reductase*	GLR1	53,408	7.67	18	588	100	470	100	
		Elongation factor 1-alpha	TEF1	50001.2	9.14	9	286	100	251	100	
42	C6	ATP-dependent molecular chaperone HSC82	HSC82	80,850	4.78	15	498	100	435	100	
43	C7	ATP-dependent molecular chaperone HSC82	HSC82	80,850	4.78	16	482	100	415	100	
46	C8	ATP-dependent molecular chaperone HSC82	HSC82	80,850	4.78	20	530	100	424	100	
47	C9	ATP-dependent molecular chaperone HSC82	HSC82	80,850	4.78	17	591	100	511	100	
60	A6	D-arabinose dehydrogenase [NAD(P)+] heavy chain	ARA1	38,859	5.65	16	659	100	540	100	
63	C10	Guanine nucleotide-binding protein subunit beta-like protein	ASC1	34,784	5.80	11	675	100	604	100	
		Glycerol-1-phosphate phosphohydrolase 1	GPP1	27929.3	5.35	13	285	100	188	100	
64	A7	Guanine nucleotide-binding protein subunit beta-like protein	ASC1	34,784	5.80	15	1,030	100	916	100	
73	C11	Eukaryotic translation initiation factor 6*	TIF6	26,441	4.54	5	344	100	322	100	
75	C12	Uracil phosphoribosyltransferase	FUR1	24,579	5.58	16	822	100	683	100	
78	C13	Peroxisome protein TSA1*	TSA1	21576.2	5.03	7	334	100	289	100	
83	C14	Mitochondrial outer membrane protein OM45	OM45	44,554	8.55	10	408	100	360	100	
84	A8	Glyceraldehyde-3-phosphate dehydrogenase 1	TDH1	35,728	8.29	18	623	100	473	100	TDH2 or TDH3
		Protein SIS1	SIS1	37566.7	9.02	9	133	100	91	100	
88	C15	Single stranded nucleic acid binding protein	SBP1	33,969	5.48	17	991	100	842	100	
89	C16	Suppressor protein STM1	STM1	29,977	9.66	11	754	100	680	100	or TDH3

Table S3. Comparison of 2D-DIGE hits to other analyses of Hsp90 interactors

	RNA or protein level by alteration of Hsp90 activity			Genome-wide screens		Hsp90 complexes
	Gopinath	Echeverria	Flom	Franzoza	McClellan	Girstmar
ACH1	yes		yes			
ADE16						
ALD3	yes		yes			yes
APE1	yes					
APE3						
ARA1						yes
ARG1		yes				
ASC1					yes	yes
ATP1	yes				yes	yes
CTT1	yes		yes			yes
DDR48		yes				
EFT1	yes					yes
EGD1						
ENO1	yes					yes
FUR1	yes	yes				
GDH1						yes
GLK1						yes
GLR1						
GPP1	yes				yes	yes
GRE1			yes			
GUT2	yes		yes			
HIS4						
HSP104	yes					yes
HSP12	yes	yes				yes
HSP78	yes	yes				yes
HXK1	yes					yes
HYP2						yes
LSP1			yes			
MBF1						yes
MSC1	yes		yes			
NOP56				yes		
OM45	yes		yes	yes		yes
PAB1						yes
PEP4	yes			yes		yes
PGM2	yes		yes			yes
PST2	yes					yes
RPL11B						
RPL14A				yes		yes
RPL2B						
RPL30					yes	yes
RPS22A						
SBP1					yes	
SEC13						yes
SEC24						yes
SEC53						yes
SOD2	yes					
SSA1	yes					yes
SSA2						yes
TDH1	yes				yes	yes
TDH2						
TDH3						yes
TEF1						yes
TFS1	yes		yes			
TIF6	yes					
TKL1						yes
TKL2						
TPS1	yes					yes
TSA1						yes
YDL124W	yes					
YEF3						yes
YLR150W/STM1						
YMR205C/PF	yes					yes

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Table S4. GO analysis of 2D-DIGE hits

GO Terms from the biological process Ontology

carbohydrate metabolic process (GO:0005975)	ARA1, ENO1, GLK1, GPP1, GUT2, HXK1, PGM2, SEC53, TDH1, TDH2, TDH3, TPS1, TSA1, YMR205C	14 of 62 genes, 22.58%	248 of 6439 annotated genes, 3.85%
response to chemical (GO:0042221)	APE1, ASC1, CTT1, GLR1, HSP104, HSP12, HSP78, SEC13, SOD2, SSA1, SSA2, TPS1, TSA1, YDL124W	14 of 62 genes, 22.58%	552 of 6439 annotated genes, 8.57%
nucleobase-containing small molecule metabolic process (GO:0055086)	ADE16, ATP1, ENO1, FUR1, GLK1, HXK1, PGM2, SEC53, TDH1, TDH2, TDH3, YMR205C	12 of 62 genes, 19.35%	219 of 6439 annotated genes, 3.40%
generation of precursor metabolites and energy (GO:0006091)	ENO1, GLK1, HXK1, PGM2, TDH1, TDH2, TDH3, TKL1, TKL2, YMR205C	10 of 62 genes, 16.13%	136 of 6439 annotated genes, 2.11%
monocarboxylic acid metabolic process (GO:0032787)	ACH1, ALD3, ENO1, GLK1, HXK1, TDH1, TDH2, TDH3, YMR205C	9 of 62 genes, 14.52%	174 of 6439 annotated genes, 2.70%
response to oxidative stress (GO:0006979)	CTT1, GLR1, HSP104, HSP12, SOD2, TPS1, TSA1, YDL124W	8 of 62 genes, 12.90%	131 of 6439 annotated genes, 2.03%
response to osmotic stress (GO:0006970)	APE1, GPP1, HSP104, HSP12, SSA1, SSA2, TPS1, TSA1	8 of 62 genes, 12.90%	88 of 6439 annotated genes, 1.37%
cytoplasmic translation (GO:0002181)	HYP2, RPL11B, RPL14A, RPL2B, RPL30, RPS22A, SSA1	7 of 62 genes, 11.29%	206 of 6439 annotated genes, 3.20%
translational elongation (GO:0006414)	ASC1, EFT1, HYP2, TEF1, YEF3, YLR150W	6 of 62 genes, 9.68%	339 of 6439 annotated genes, 5.26%
regulation of translation (GO:0006417)	ASC1, EFT1, HYP2, PAB1, SBP1, YLR150W	6 of 62 genes, 9.68%	196 of 6439 annotated genes, 3.04%
protein targeting (GO:0006605)	APE1, EGD1, PEP4, SEC53, SSA1, SSA2	6 of 62 genes, 9.68%	253 of 6439 annotated genes, 3.93%
transmembrane transport (GO:0055085)	ATP1, GLK1, HXK1, SSA1, SSA2	5 of 62 genes, 8.06%	459 of 6439 annotated genes, 7.13%
protein folding (GO:0006457)	HSP104, HSP78, SSA1, SSA2, TSA1	5 of 62 genes, 8.06%	120 of 6439 annotated genes, 1.86%
response to heat (GO:0009408)	HSP104, HSP12, HSP78, LSP1, TPS1	5 of 62 genes, 8.06%	67 of 6439 annotated genes, 1.04%
translational initiation (GO:0006413)	HYP2, PAB1, TIF6, YLR150W	4 of 62 genes, 6.45%	74 of 6439 annotated genes, 1.15%