Experiment Procedures (Supplemental Information)

Yeast strains, plasmids, and growth conditions

ΔPLD82a of the W303 background was a kind gift from Susan Lindquist (Nathan and Lindquist, 1995) and has the following genotype: *MAT**a** can1-100 ade2-1 his3-11,15 leu2-3,12 trp1-1 ura3-1 HSC82 Δhsp82::LEU2.* YKR314 was constructed by transforming ΔPLD82a sequentially with a *GAL-Hsp82*-CEN-ARS-*URA3* plasmid (pJV522; (Picard et al., 1990)) and a *Δhsc82::KanMX* DNA fragment obtained by amplifying the genomic DNA from strain RG771 (see below) with the following primers: 5' GAT GAG AAG AAG TGC CGT ACA CTT GAT ACA TG and 5' CAG GCG GAA TGG GTA TGC TAT TCT TGC AGA. Knockout of the *HSC82* gene was confirmed by PCR analysis and the inability to grow on 5-FOA media. All of the strains carrying alleles of human *HSP90* were generated by transforming YKR314 with *HSP90* plasmids carrying either the *HIS3* or *TRP1* marker and selecting for either HIS+/5-FOA+/ura- or TRP+/5-FOA+/ura- colonies. RG771 of the S288c background was purchased from Open Biosystems and has the following genotype: *MAT**a** ura3 leu2 his3 met15 hsc82::kanMX.* YKR315 was constructed by transforming RG771 sequentially with pJV522 and a *Δhsp82::LEU2* DNA fragment obtained by amplifying the genomic DNA from strain ΔPLD82a with the following primers: 5' CAC GCG CAT GGT TTT ATG AGC GGT TAA TTC TC and 5' CAA CAT ACC CTT TAT CTT CGC AGT AAA GTC CAT. Knockout of the *HSP82* gene was confirmed as described above. Strains carrying mutant alleles of yeast *HSP82* were generated by transforming YKR315 with *HSP82* plasmids carrying the *MET15* marker and selecting for MET+/5-FOA+/ura- colonies.

The following primers were used to amplify the *ADH1* promoter in the 2-micron based plasmids: 5' TCG GTT AGA GCG GAT CTT AGC TAG CCG CGG TAC CAA GCT ATC CTT TTG TTG TTT CCG GGT GTA C and 5' GAT CTT ATC GTC GTC ATC CTT GTA ATC CAT

TGT ATA TGA GAT AGT TGA TTG TAT GC. These primers include bases at their 5' ends that are homologous to sequences adjacent to the Gal promoter in the pESC vector. A *his3* yeast strain was co-transformed with the *ADH1* promoter PCR product as well as pESC-*ura3*::*HIS3* linearized by EcoRI and HIS+ colonies were selected for. The plasmid pESC-*ura3*::*HIS3*-*ADH1*p was rescued from these colonies as previously described (Robzyk and Kassir, 1992) and was used as a vector to express human Hsp90 beta and the various alleles of human Hsp90 alpha. The following primers were used to amplify beta: 5' AGC TAT ACC AAG CAT ACA ATC AAC TAT CTC ATA TAC AAT G CCT GAG GAA GTG CAC CAT GGA G and 5' CTT ATT TAA TAA TAA AAA TCA TAA ATC ATA AGA AAT TCG CT CT AAT CGA CTT CTT CCA TGC GAG AC. ResGen clone # RG000363 carries a copy of the beta cDNA and was used as template. The following primers were used to amplify the alpha alleles: 5' AGC TAT ACC AAG CAT ACA ATC AAC TAT CTC ATA TAC AAT G CCTGAG GAA ACC CAG ACC CAA G and 5' CTT ATT TAA TAA TAA AAA TCA TAA ATC ATA AGA AAT TCG CTT AGT CTA CTT CTT CCA TGC GTG ATG. Clones carrying the various alleles of alpha as described above were used as templates. Plasmid pESC-*URA3*::*HIS3*-*ADH1*p was linearized with Sac1 and co-transformed along with the various amplified alleles of human Hsp90 into strain YKR314.

For site-directed mutagenesis of K274 in *HSP82*, the following primers were used: 5' ATG GCT AGT GAA ACT TTT GAA TTT CAA GCT G and either 5' GGT TTC TAG TCC ACA AAG GCC TAG TCT TGT TTA GTT CTT CTA TCT CTT G (K274R) or 5' GGT TTC TAG TCC ACA AAG GTT GAG TCT TGT TGA GCT CTT CTA TCT CTT GAA CTT CTT C (K274Q) to amplify the upstream portion of *HSP82*; 5' CCT TCA ATT GAG TGA AGG CGT ATT CAT CAA TTG G and either 5' GAA GAA CTA AAC AAG ACT AGG CCT TTG TGG ACT AGA AAC C (K274R) or 5' GAA GAG CTC AAC AAG ACT CAA CCT TTG TGG ACT AGA AAC C (K274Q) to amplify the downstream portion of *HSP82*. p*GPD1*pro-*HSP82*-CEN-ARS-*TRP1* was digested with Bgl II and BsrG I and co-transformed with a matched pair of PCR

products generated using these primers into YKR314. Following selection for TRP+/5-FOA+/ura- colonies, the desired products of recombination were rescued and verified by restriction digest and sequencing of both strands of those sequences that had been amplified by PCR. Verified clones were then converted into to MET15 versions of the pRS series of vectors by digesting with Bsu36 I and co-transforming with PRS411 cut with ScaI and Pst I into YKR315. Plasmids were rescued from MET+/5-FOA+/ura- colonies and analyzed by restriction digests.

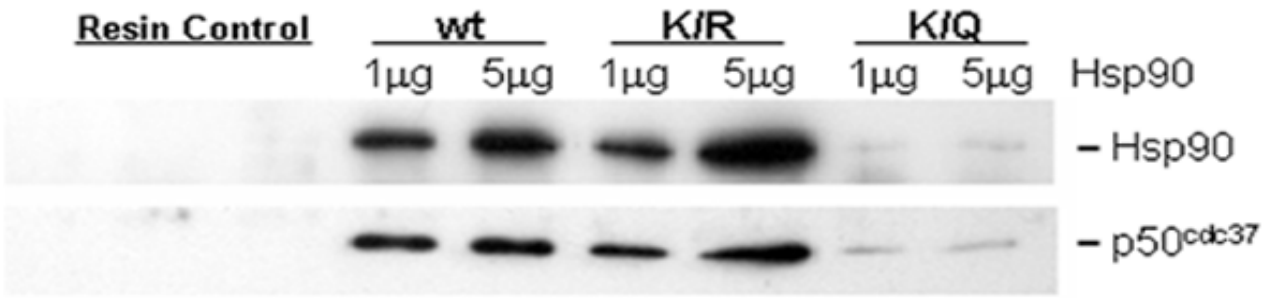
Table 1 (Supplemental Information)

Strain	Genotype	Source
ΔPLD82a	*MAT**a** can1 ade2 his3 leu2 trp1 ura3 ⊿hsp82::LEU2*	S. Lindquist
YKR314	ΔPLD82a + *⊿hsc82::KanMX* (pRS316-*GAL1*pro-*HSP82-URA3*)	This study
RG771	*MAT**a** ura3 leu2 his3 met15 hsc82::kanMX*	Open Biosystems
YKR315	RG771 + *⊿hsp82::LEU2* (pRS316-*GAL1*pro-*HSP82-URA3*)	This study
	Human *HSP90*	
YKR403	YKR314+[pESC-*ADH1*pro-*HSP90*beta-*HIS3*]	This study
YKR404	YKR314+[pESC-*ADH1*pro-*hsp90*alpha(K294Q)-*HIS3*]	This study
YKR405	YKR314+[pESC-*ADH1*pro-hsp*90*alpha(K294A)-*HIS3*]	This study
YKR407	YKR314+pRS414-*ADH1*pro-*HSP90*alpha-*TRP1*	This study
YKR408	YKR314+pRS414-*ADH1*pro-*HSP90*beta-*TRP1*	This study
YKR409	YKR314+pRS414-*ADH1*pro-hsp*90*alpha(K294Q)-*TRP1*	This study
YKR410	YKR314+pRS414-*ADH1*pro-hsp*90*alpha(K294A)-*TRP1*	This study
	Yeast *HSP90*	
YKR428	YKR315+pRS411-*GPD1*pro-*HSP82-MET15*	This study
YKR430	YKR315+pRS411-*GPD1*pro-*hsp82(K274R)-MET15*	This study
YKR432	YKR315+pRS411-*GPD1*pro-*hsp82(K274Q)—MET15*	This study

Supplemental Figure Legends

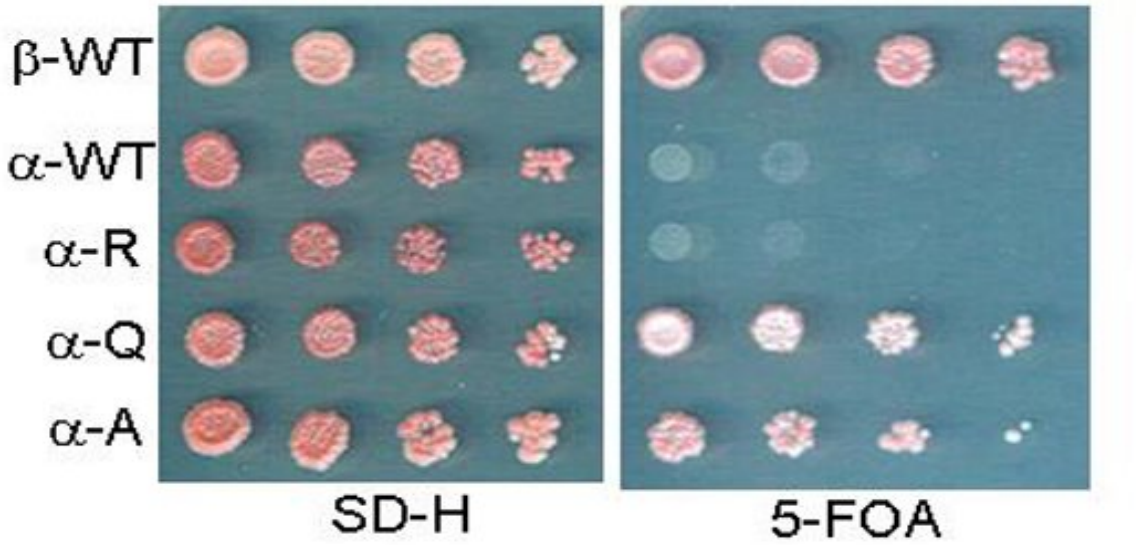
Supplemental Figure 1: Acetylation Status of K294 Affects Ability of High Copy Hsp90α Proteins to Complement Yeast Growth and Viability

An *S. cerevisiae* strain (YKR314) deleted for both genomic copies of yeast *HSP90* but carrying both wild type yeast *HSP82* (on a *URA3* marked plasmid) and human *HSP90* constructs (on high copy, 2μ-*HIS3* marked plasmids) was grown at 30°C to mid-log phase in liquid SD-HIS media . Cultures were adjusted to the same cell density before 5-fold serial dilutions were spotted onto SD-HIS plates either with or without 5-FOA and incubated at 30°C for 3 days before being photographed.



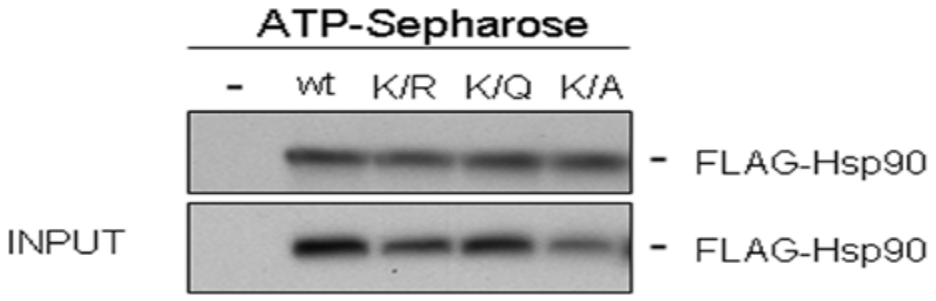
Supplemental Figure 2: Purified WT and Mutant Hsp90α Proteins Interact Differently with Chk1 Kinase *In Vitro*

Resin-bound GST-Chk1 (amino acids 1-265) was reconstituted with purified WT or mutant Hsp90α (1 or 5 μg) and other proteins as in Fig. 4A. After washing and incubation with substrate protein, the Chk1-chaperone complexes and kinase reaction products were separated by SDS-PAGE and transferred to Immobilon-P membrane. Bound chaperones were detected by immunoblotting with anti-Hsp90 or anti-$p50^{cdc37}$ antibodies (see Arlander et al., 2006).



Supplemental Figure 3: ATP Binding and Dimerization of Hsp90α Are Not Affected by K294 Mutation

COS7 cells were transfected with the indicated FLAG-Hsp90 construct. Cells were lysed and mixed with ATP-agarose. Bound proteins were separated by SDS-PAGE and Hsp90 binding was detected by immunoblotting with anti-FLAG antibody.



Supplemental References

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