Supplemental information

SI 1. Thermal aggregation of citrate synthase was performed as described previously (1). Citrate synthase (0.2 μM) was incubated with indicated concentration of yHsp90 wild-type (Wt), yHsp90 Pf-CL (Pf-CL) or yHsp90 GSS95 (GSS95) protein at 43 ºC for 15min. Light scattering was measured at an optical density of 490 nm in a BioTek Instruments Inc ELx808IU spectrophotometer. Light scattering (proportional to aggregation) of heated citrate synthase in the absence of yHsp90 was set at 1 and the relative impact on aggregation of increasing concentrations of yHsp90 proteins is displayed.

SI 2. Wild type cystic fibrosis transmembrane conductance regulator protein (CFTR) was expressed by transient transfection in HEK-293 cells, in the presence or absence ('pc') of Flag-tagged wild type (Wt) hHSP90, hHSP90-Sc CL, or hHSP90-Pf CL. Mature (band 'C') and immature (band 'B') CFTR protein expression was visualized by western blotting with anti-CFTR antibody. Comparable expression of transfected HSP90 proteins was confirmed by immunoblotting with anti-Flag antibody.

SI 3. Hsp90 deuteron incorporation kinetics were obtained for yHsp90-GSS7 and yHsp90-GSS56 proteins as described in the legend to fig 4.

SI 4. Yeast cells expressing indicated N-terminal His-tagged yHsp90 protein were lysed and proteins were precipitated by Ni-NTA. Precipitates were digested with TPCK-treated trypsin and detected by immunoblotting with anti-His-tag antibody. Molecular weight

(kDa) and schematic diagram of domain structure of Hsp90 are shown on the left. Arrows indicate trypsin cleavage sites in yHsp90.

SI 5. Yeast cells expressing indicated N-terminal His-tagged yHsp90 protein were lysed and proteins were precipitated by Ni-NTA. Associating co-chaperones, Aha1, $Sti1^{Hop}$, $Cdc37^{p50}$ and $Sba1^{p23}$ were detected by immunoblotting (A). COS7 cells were transfected with wild-type or mutant Flag-hHsp90 constructs. hHsp90-client protein complexes were immunoprecipitated (IP) using Flag-antibody-conjugated agarose. Co-precipitating proteins were detected by immunoblotting (B).

SI 6. Yeast Aha1 stimulates the ATPase activities of purified yeast wild type Hsp90 and of yHsp90 Pf-CL proteins. yHsp90 ATPase activities were determined in the presence of indicated concentrations of Aha1 protein as described in the main text.

SI 7. Ste11ΔN (A) was transformed into cells that did or did not express exogenous Aha1 (as indicated) and also expressed either wild type (Wt) or indicated mutant yHsp90 constructs. Ste11ΔN protein expression was detected by immunoblotting (A). GR activity was assessed in the same strains after co-expression of GR and a GR-responsive *lacZ* reporter gene (B). Data are expressed as a percentage of the GR activity observed in Wt cells, and are depicted as the mean ± standard deviation derived from three independent experiments. Aha1 over-expression was confirmed by immunoblotting with anti-Flag antibody (A).

SI 8. Yeast expressing either wild type yHsp90 or yHsp90-Pf-CL were transduced either with empty plasmid or with plasmid containing yAha1, and subjected to temperature stress as described in the legend to fig. 1. Over-expression of yAha1 rescues the temperature sensitive phenotype of yeast expressing yHsp90-PF-CL (A). *S. cerevisiae* strains that do or do not express exogenous Aha1 (as indicated) were spread onto SD-TRP agar plates either with or without 5-FOA at 25ºC for 4 days. Excess Aha1 is not able to rescue yeast harboring I205A-yHsp90 (B).

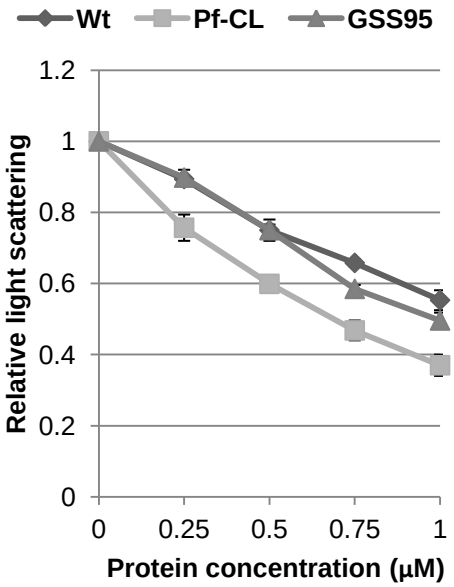
SI 9. Overall charge in Hsp90 constructs used in this study. The predicted net charge of the protein at pH7 was determined based on amino acid composition using the program SEDNTERP.

SI 10. A summary of the characteristics and activities of the primary Hsp90 proteins used in this study (A). The negative impact of charged linker mutation on Hsp90 ATPase activity and function is counterbalanced by Aha1. Our data suggest that motif(s) within the Hsp90 charged linker cooperate with the co-chaperone Aha1 to regulate Hsp90 ATPase activity and consequently chaperone function *in vivo* (B).

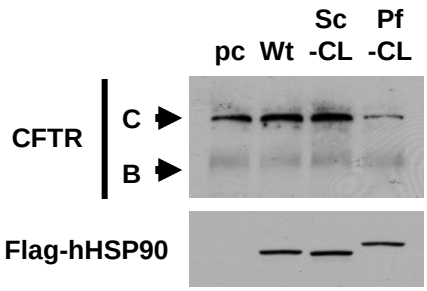
Supplemental reference

1. Johnson BD, *et al.* (2000) Hsp90 chaperone activity requires the full-length protein and interaction among its multiple domains. *J Biol Chem* 275(42):32499-32507.

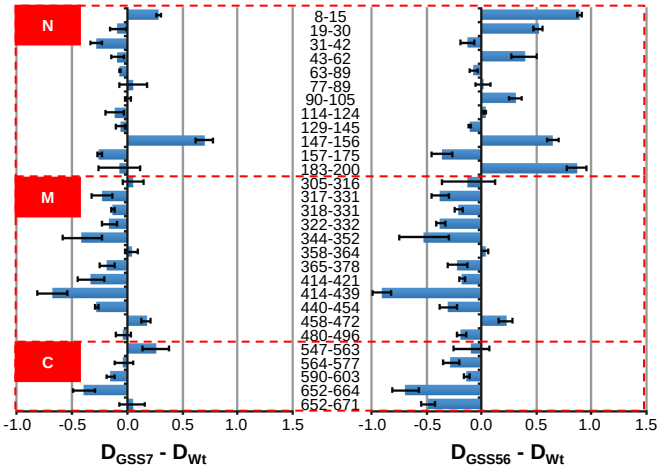
Tsutsumi *et al*., SI 1



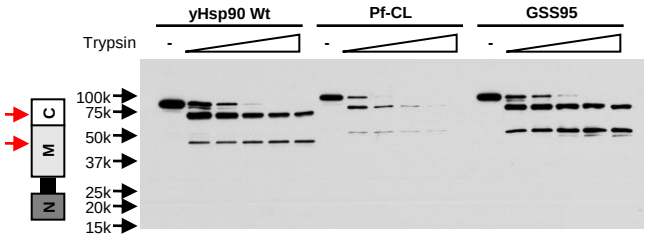
Tsutsumi *et al*., SI 2



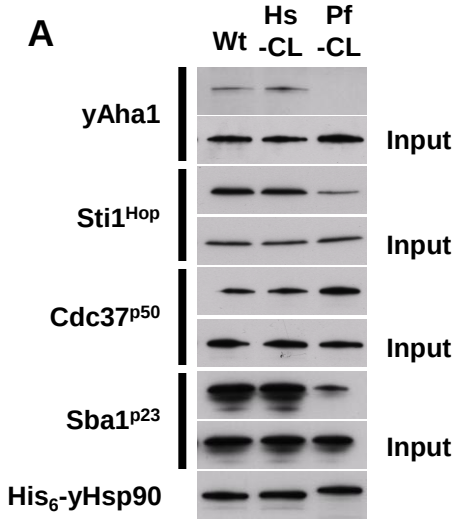
Tsutsumi *et al*., SI 3

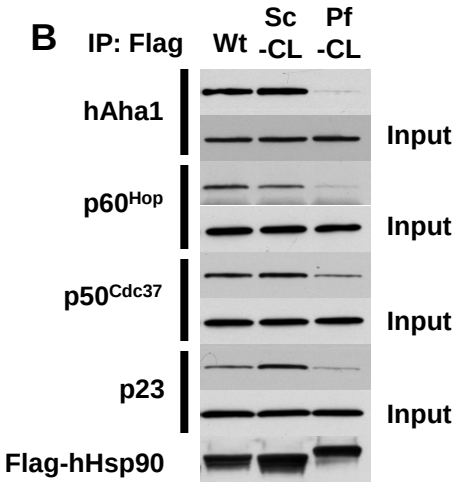


Tsutsumi *et al*., SI 4

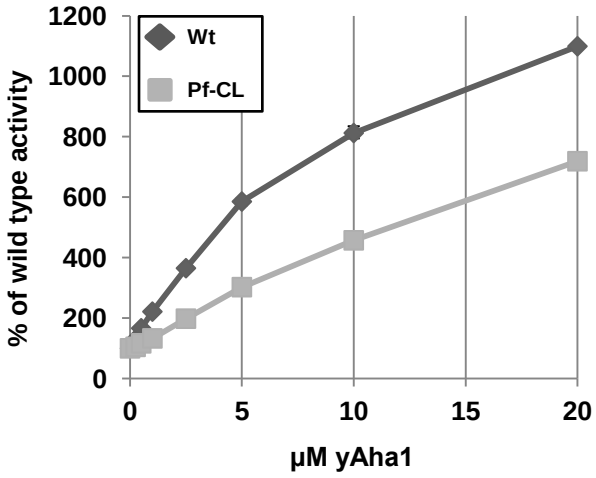


Tsutsumi *et al*., SI 5

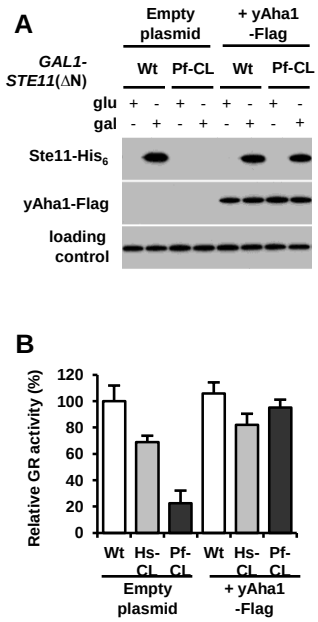




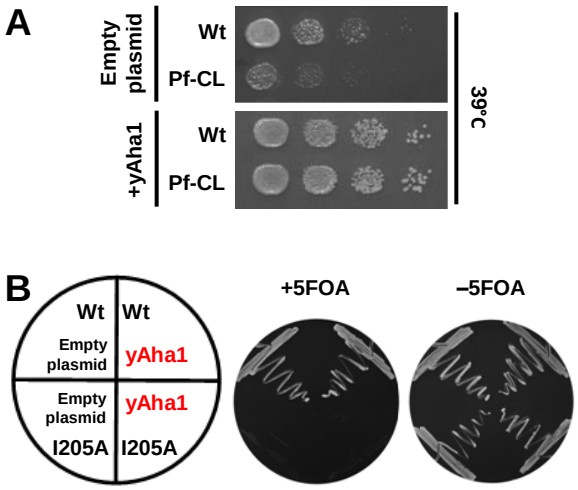
Tsutsumi *et al*., SI 6



Tsutsumi *et al*., SI 7



Tsutsumi *et al*., SI 8



Tsutsumi *et al*., SI 9

Hsp90 construct	net charge
yHsp90 wt	-37
yHsp90 Δ210-265	-29
yHsp90 Hs-CL	-41
yHsp90 Pf-CL	-48
yHsp90 GSS7	-29
yHsp90 GSS56	-29
yHsp90 GSS63	-29
yHsp90 GSS95	-29
hHsp90 wt	-39
hHsp90 Δ223-285	-27
hHsp90 Sc-CL	-35
hHsp90 Pf-CL	-46
hHsp90 GSS63	-27
hHsp90 GSS95	-27

Tsutsumi *et al*., SI 10

A

Charged-linker			Growth		ATPase	Chaperone function	Aha1 association
	Length	Charge	25°C	39°C	(%)		
Wt	56	-37	+	+	100	+	+
Hs-CL	63	-41	+	+	65	+	+
Pf-CL	95	-48	+	-	9	-	-
GSS7	7	-29	+	+	114	+	n.d.
GSS56	56	-29	+	+	39	+	+
GSS63	63	-29	+	+	49	+	n.d.
GSS95	95	-29	+	+	27	+	+

B