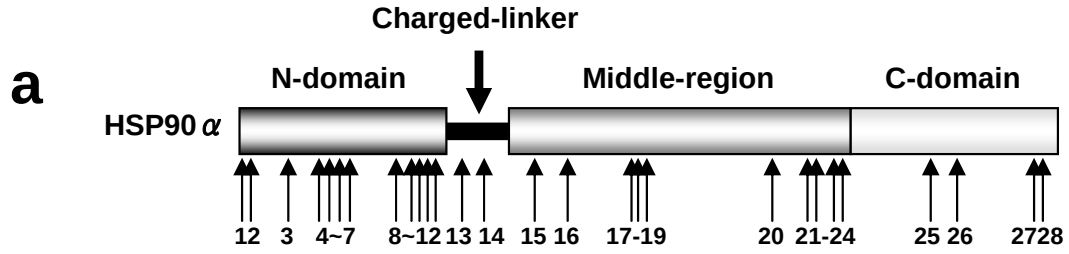
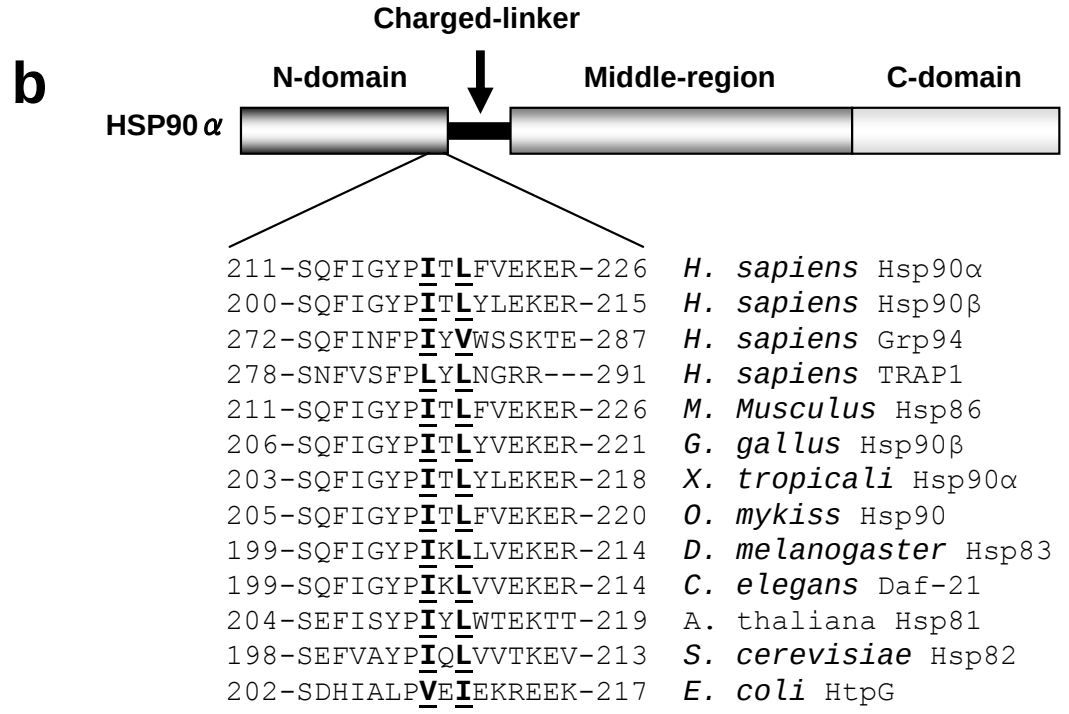
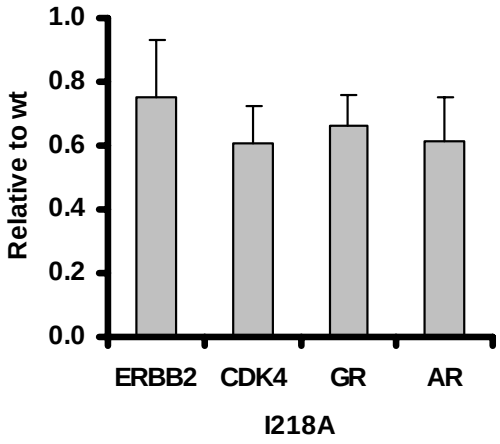
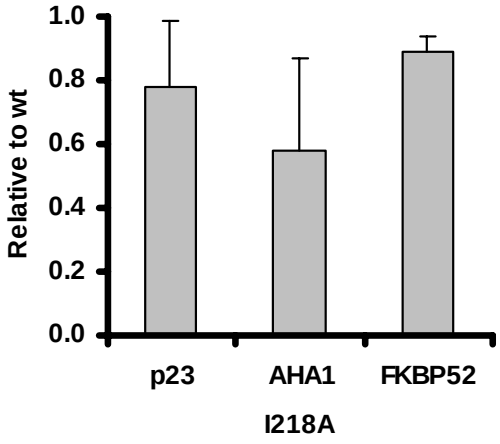


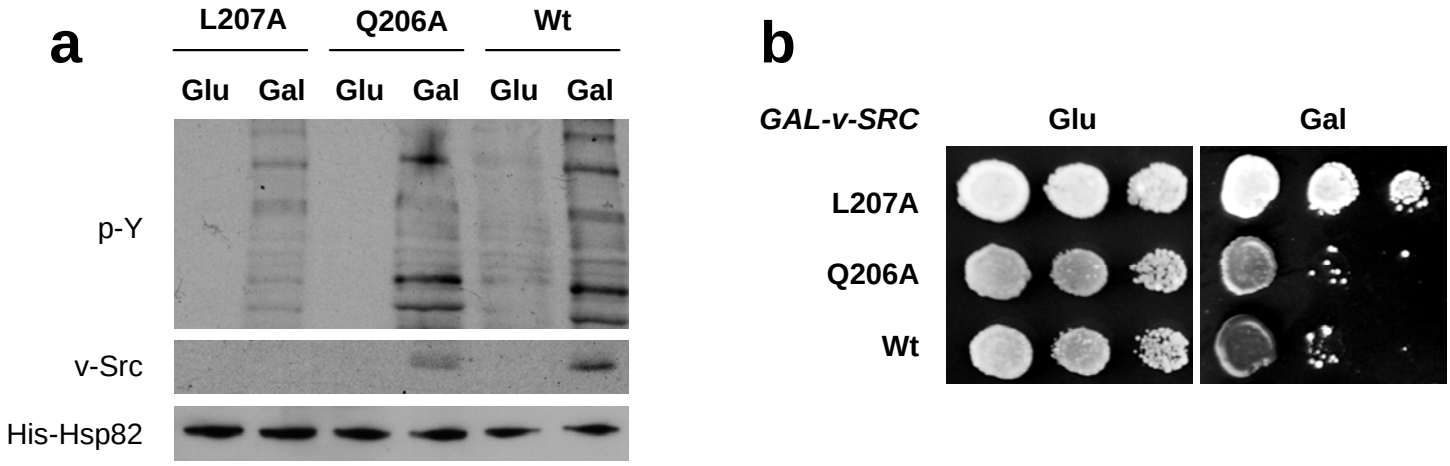


Supplementary Figure 1. Identification of hydrophobic IxL motif in the N-domain of Hsp90. a. A domain map of Hsp90 showing locations (arrows) of the 28 point mutations that were assessed for their impact on Hsp90 secretion. The mutated residues are as follows (refer to the number under each arrow): 1:T5, 2:T7, 3:T36, 4:T88, 5:T90, 6:D93, 7:T115, 8:Y197, 9:P217, 10:I218, 11:T219, 12:L220, 13:S231, 14:S263, 15:K294, 16:Y313, 17:V368, 18:F369, 19:I370, 20:Y492, 21:I522, 22:I525, 23:L549, 24:L551, 25:Y604, 26:Y626, 27:L694, 28:L696. **b.** Representation of Hsp90 domains and an alignment of the sequence surrounding the IxL motif in β strand 8 of the N domain (containing I218 and L220) from different organisms and isoforms of Hsp90. I218/L220 or corresponding residues in other organisms/isoforms are underlined and bolded.

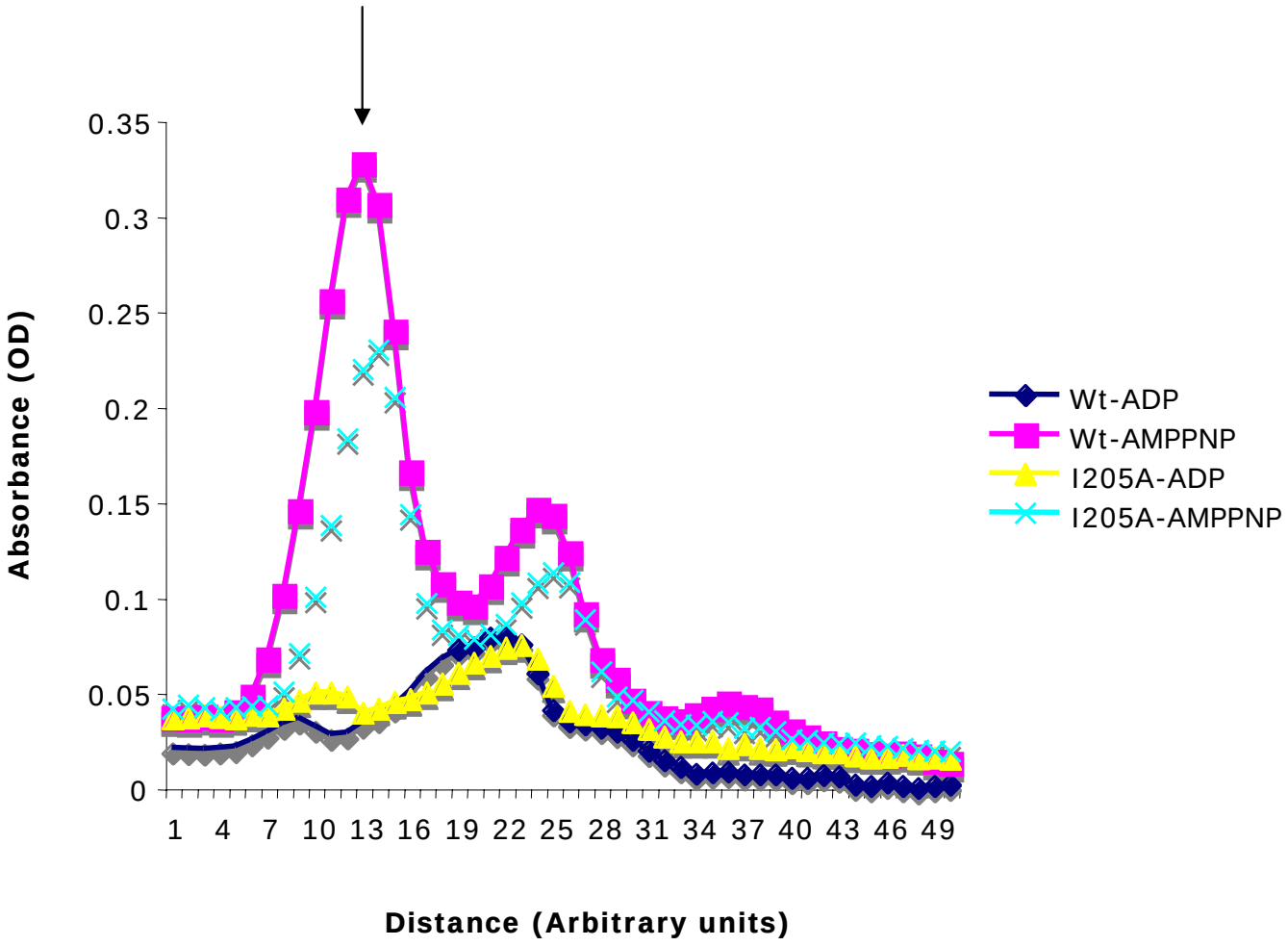




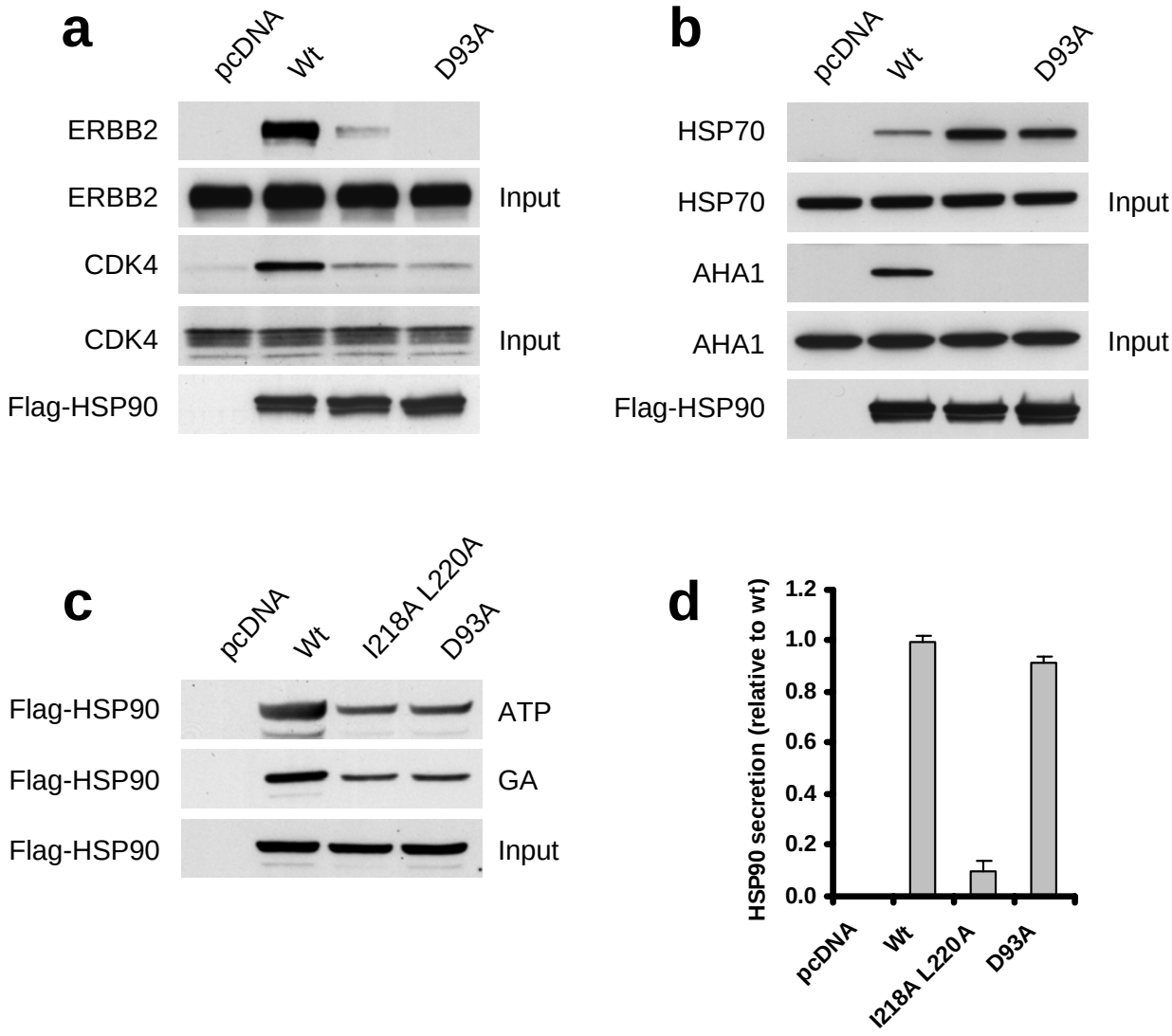
Supplementary Figure 2. a. Impact of I218A mutation on Hsp90 interaction with client proteins. COS7 cells were transfected with the Flag-Hsp90 I218A together with one of the Hsp90 clients ERBB2, CDK4, GR, or AR After Flag immunoprecipitation, association of each client with Hsp90 I218A was determined by Western blotting using specific antibodies. Each experiment was performed three times and data are expressed as the mean optical density of each client band (relative to that obtained for association with wild type Hsp90) +/- S.D. **b. Impact of I218A mutation on Hsp90 interaction with selected co-chaperones.** Similar experiments were performed in triplicate as those described above, except association of endogenous selected client proteins with Flag - Hsp90 I218A (compared to wild type Hsp90) was quantified. Data are expressed as the mean optical density of each co-chaperone band (normalized to that obtained for wild type Hsp90) +/- S.D.



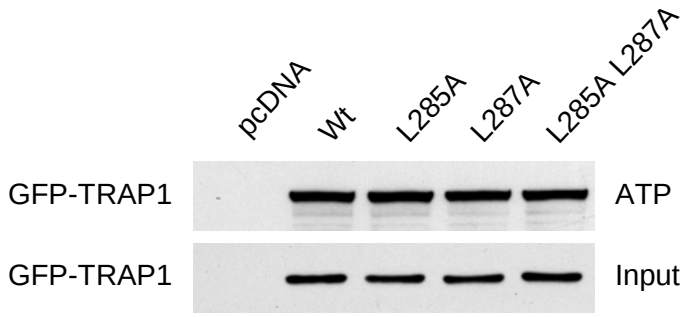
Supplementary Figure 3. L207 is an important determinant of Hsp90 chaperone function. a-b. v-Src under the control of the GAL1 promoter was transformed into the same yeast cells expressing wild type, mutant Q206A or L207A Hsp82. Growth of these cells in the presence of glucose (Glu) represses v-Src expression, which is induced with galactose (Gal). *In vitro* v-Src activity and protein expression level were detected by immunoblotting (a), and *in vivo* v-Src activity was determined by its effect on cell growth (b).



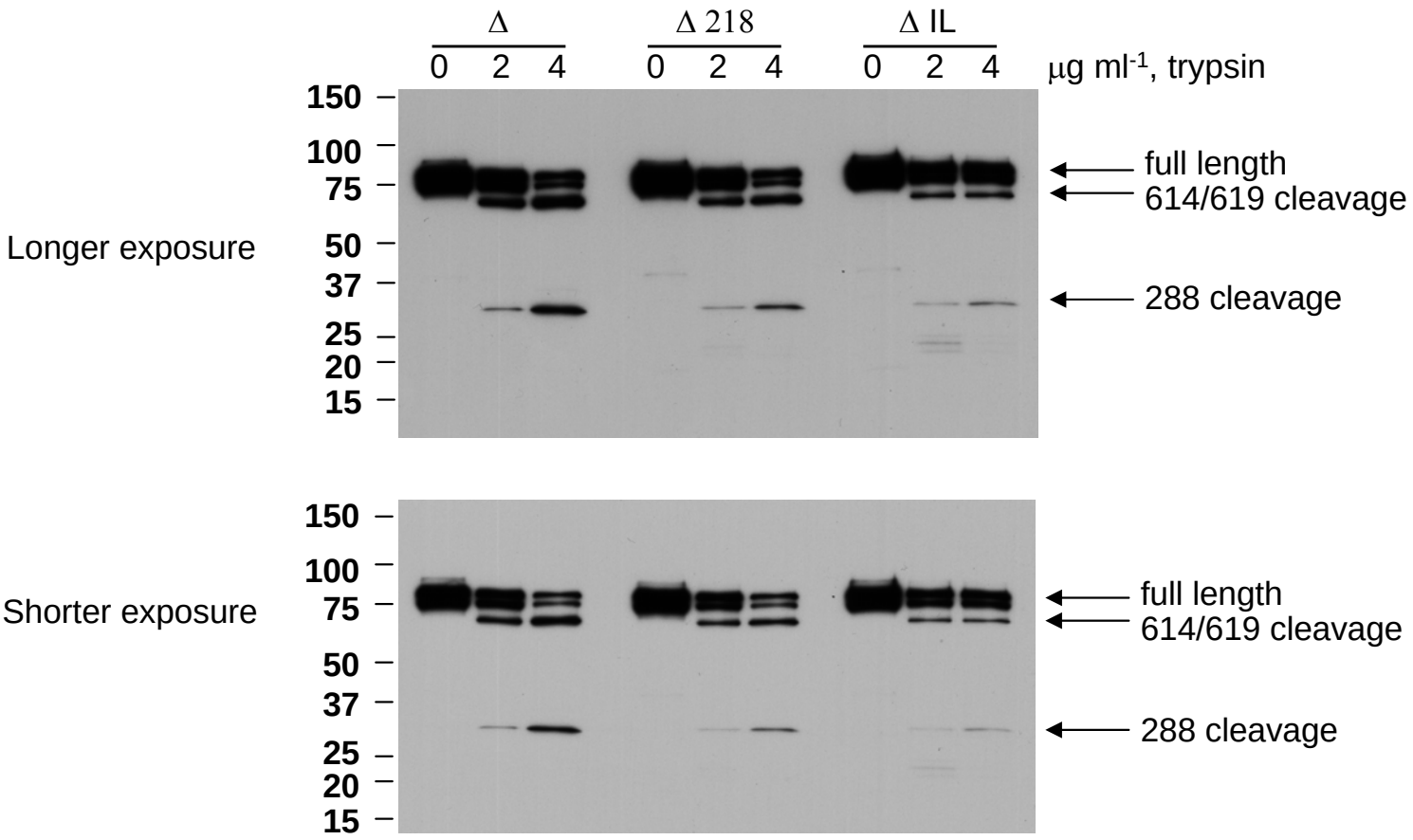
Supplementary Figure 4. Quantification of AMPPNP-dependent N-domain dimerization of yeast Hsp90 I205A compared to wild type Hsp90. N-domain dimerization wild type and I205A yeast Hsp90 proteins was assessed as described in the text. For quantification, gels were scanned and where necessary normalized against total intensity. The peak representing the slowest migrating band visible on the gels is shown by the black arrow.



Supplementary Figure 5. IxL motif in β strand 8 independently affects Hsp90 secretion and nucleotide-dependent chaperone cycling. COS7 cells were transfected with empty vector (pcDNA) or indicated Flag-Hsp90 constructs, either alone (b, c) or together with ERBB2 (a). **a-b.** After incubation, cells were lysed, and proteins were immunoprecipitated by Flag antibody-conjugated agarose, and separated by SDS-PAGE. Indicated co-precipitating proteins were detected by immunoblotting. **c.** Cells were lysed, and proteins were precipitated by ATP- or GA-conjugated agarose. Precipitated proteins were separated by SDS-PAGE and Hsp90 was detected by immunoblotting. **d.** COS7 cells were transfected with indicated Flag-Hsp90 constructs. Conditioned medium was collected and secreted Hsp90 proteins were detected by ELISA.



Supplementary Figure 6. β strand 8 is dispensable for ATP-Sepharose binding of the Hsp90 paralog TRAP1. COS7 cells were transfected with indicated GFP-TRAP1 constructs. Cells were lysed, and proteins were precipitated by ATP-conjugated agarose. Precipitated proteins were separated by SDS-PAGE, and GFP-TRAP1 was detected by immunoblotting.



Supplementary Figure 7. Impact of charged linker truncation on Hsp90 trypsin sensitivity. Flag - Hsp90 with the charged linker truncated (Δ), Flag-Hsp90 I218A Δ (Δ218), and Flag - Hsp90 I218A L220A Δ (ΔIL) constructs were transfected into ŒOS7 cells. After lysis in low detergent lysis buffer, exogenously expressed Hsp90 was isolated using anti-Flag - agarose. Immune pellets were salt stripped in 500 mM NaCl for 2 hours sat 4°C, and then digested with TPCK-treated trypsin at the indicated concentrations (ug ml^{-1}) for 6 minutes on ice. Samples were separated on 4-20% (w/v) SDS-PAGE gels and immunoblotted with an N-terminal Hsp90 antibody (PA3-013). Two film exposures are presented.

Supplementary methods

ATP- and geldanamycin (GA)-binding assay

COS7 cells were transfected as indicated. Cell lysates were incubated with 20 ul of pre-equilibrated ATP- or GA-agarose, and incubated at 4ºC for 2 hr. Pellets were washed 4 times with fresh lysis buffer and eluted with 2X Laemmli buffer. Precipitated proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Precipitated FLAG-Hsp90 and GFP-TRAP1 were detected by immunoblotting with anti-FLAG or anti-GFP antibody, respectively.