Supporting Information

Karagöz et al. 10.1073/pnas.1011867108

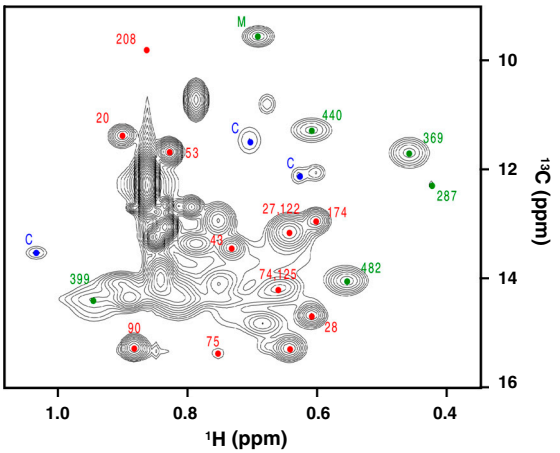

Fig. S1. Assignment of isoleucine residues in ^{1}H-^{13}C-Ile methyl-transverse-relaxation optimized spectroscopy. ^{1}H-^{13}C-Ile methyl-TROSY cross-peaks of full-length Hsp90. For the isoleucines that were unambiguously assigned the residue number is indicated. Allocation to an individual domain is represented by a colored dot (Hsp90-N, red; Hsp90-M, green; Hsp90-C, blue).

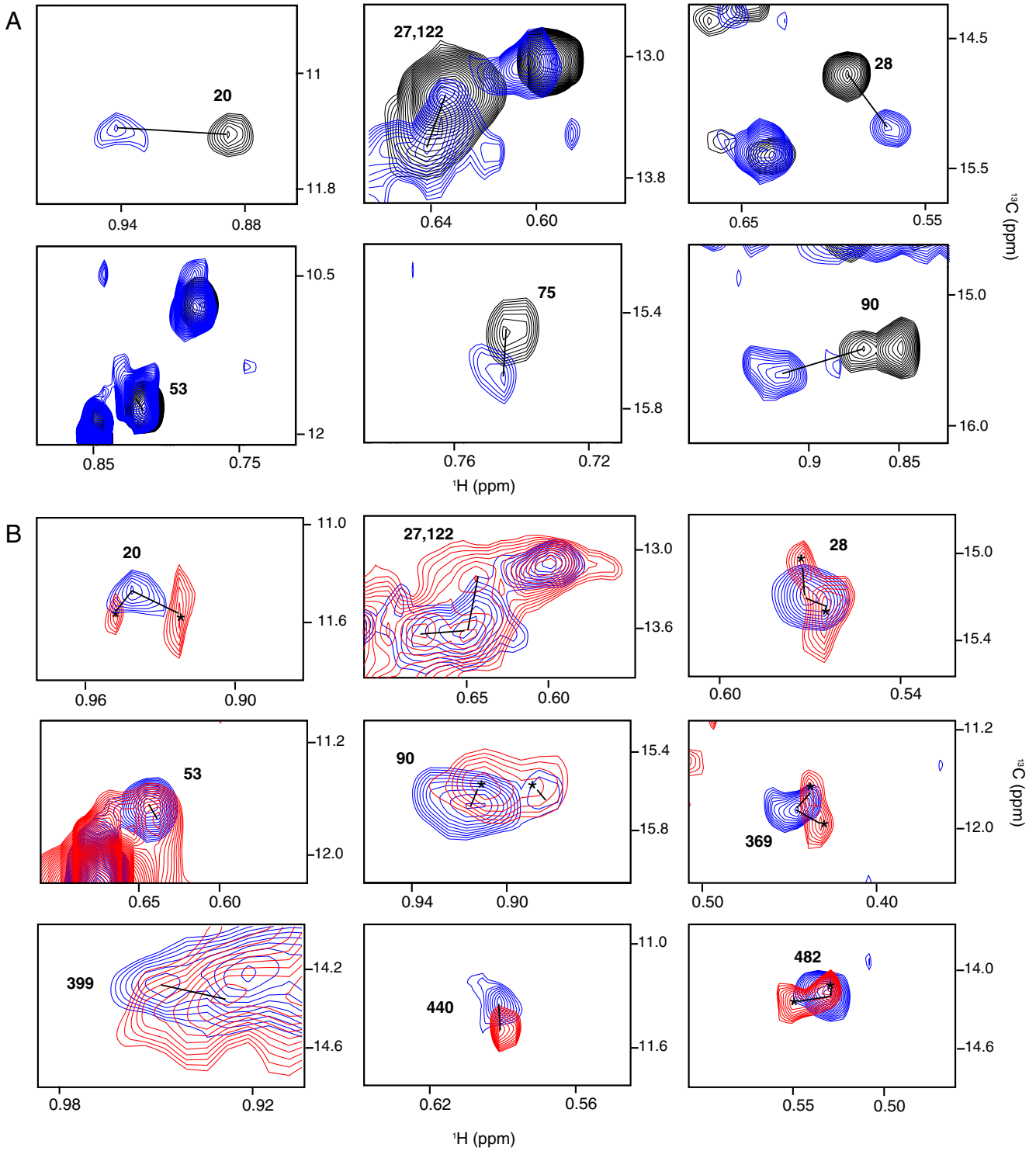

Fig. S2. Changes in the spectra of full-length Hsp90 in presence of ATP and p23. Closeup of the ^{1}H-^{13}C-Ile methyl-TROSY for the isoleucine residues that shifted upon addition of ATP (Fig. 2*A*) and by the addition of p23 (Fig. 2*B*). (*A*) Hsp90 in black, Hsp90 + ATP in blue. (*B*) Hsp90 + ATP in blue and Hsp90 + ATP + p23 in red. The extent of each shift is indicated by a line. For the Hsp90 + ATP + p23 spectrum the peaks that split into two peaks are marked with asterisks.

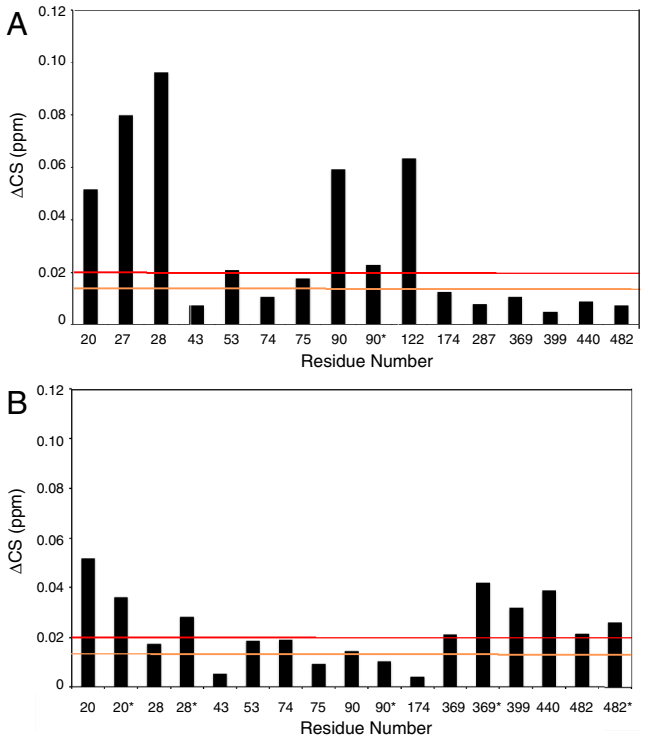

Fig. S3. Chemical shift perturbation analysis of ATP and p23 binding to Hsp90. The combined chemical shift perturbations of the isoleucines are calculated as $\Delta\nu = ((0.25\Delta\nu_C)^2 + \Delta\nu_H{}^2)^{1/2}$. Red line and orange line indicate shifts $\Delta\nu > 0.020$ and $\Delta\nu > 0.015$, respectively, consistent with the color code in Figs. 2*C* and 3*C*. (*A*) The change in the chemical shifts upon ATP binding to Hsp90. (*B*) The change in the chemical shifts upon p23 binding to Hsp90 + ATP. Peaks that split upon binding of ATP and/or p23 are indicated by asterisks.

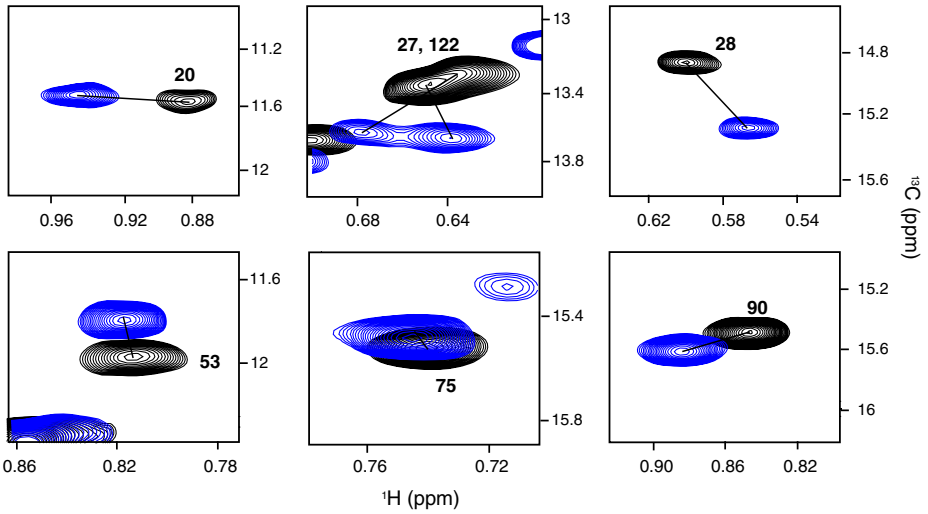

Fig. S4. Isoleucine chemical shifts on the isolated N-terminal domain of Hsp90 upon ATP binding. Closeup of the isoleucine residues shifting upon the addition of ATP to the N-terminal domain; Hsp90-N is depicted in black and Hsp90-N + ATP in blue. The extent of each shift is indicated by a line.

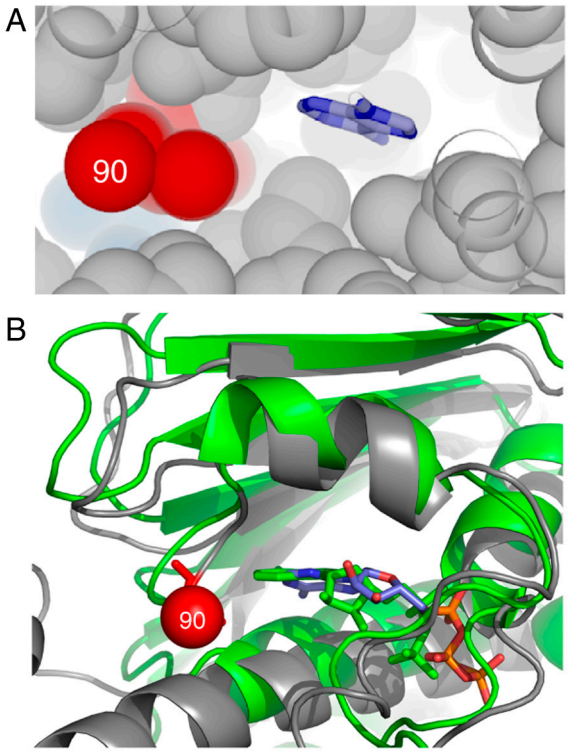

Fig. S5. ATP and p23-dependent effects at the adenine end of the nucleotide binding pocket. (*A*) Closeup of Hsp90's nucleotide binding pocket around Ile90 and the adenine ring of ATP. Hsp90 is shown as homology model of human Hsp90β based on the crystal structure of the complex of yeast Hsp90 bound to an ATP analogue and $Sba1^{p23}$ (1). Hsp90, gray space fillings, Ile90 side chain, red space fillings; ATP, sticks, natural colors. (*B*) Overlay of the ATPase domains and bound nucleotide of human Hsp90 (Hsp90, gray ribbons; Ile90, red sticks; Ile90 δ-methyl group, red space filling; ATP, natural colors) and MutL (PDB ID code 1B62; protein and nucleotide, green ribbons and sticks) (1, 2).

1. Ali MM, et al (2006) Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. *Nature* 440:1013–1017.
2. Ban C, Junop M, Yang W (1999) Transformation of MutL by ATP binding and hydrolysis: A switch in DNA mismatch repair. *Cell* 97:85–97.

Table S1. Distribution of Ile side chains in Hsp90

	Number of Ile residues					Peaks shifts	
		Per domain					
Domains	Total*	Detected†	Assigned‡	Allocated§	Assigned in full length¶	+ATP	+ATP+p23
Hsp90-N	20	20	20	13	12	7	7
Hsp90-M	17	17	10	6	5	0	4
Hsp90-C	10	8	0	3	0	0	0
Full length‖	48	45	31	23	18	7	11

*Isoleucine residues in each individual domain.
†Detected in spectrum of individual domains.
‡Assigned in spectrum of individual domains.
§Allocated to individual domains in the full-length Hsp90 spectra.
¶Assigned in full-length Hsp90 by transport of assignments of individual domains.
‖Sum of Iles in Hsp90-N, Hsp90-M, Hsp90-C and the flexible linker.