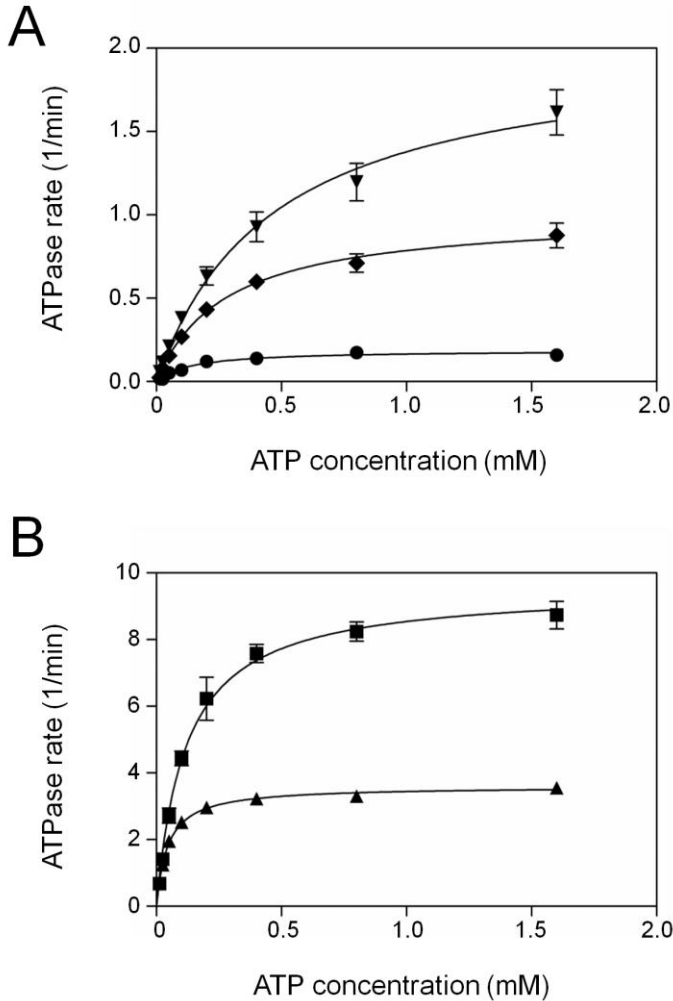


Supplementary information

The conserved NxNNWHW Motif in Aha-type co-chaperones modulates the kinetics of Hsp90
ATPase stimulation

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Supplementary figure 1



Supplementary Figure 1. The NxNNWHW motif in Aha1p and Hch1p modulates the apparent K_M for ATP of Hsp90. A. Kinetic analysis was carried out for Hsp82p alone (circles) and in the presence of Hch1p (down triangles) or Hch1p^{Δ11} (diamonds). B. Kinetic analysis was carried out for Hsp82p in the presence of Aha1p (squares) or Aha1p^{Δ11} (up triangles). ATPase reactions were carried out with increasing concentrations of ATP (12.5, 25, 50, 100, 200, 400, 800, 1600 μ M) and ATPase rates were analyzed with the Michaelis-Menten non-linear regression function in GraphPad Prism. The curve fits all had R^2 values greater than 0.9 ($n=3$; $n=4$ for Hsp82p alone). 2 μ M of Hsp82p was added to reactions containing 20 μ M of either Aha1p or Aha1p^{Δ11} or 4 μ M of Hsp82p was added to reactions containing 40 μ M Hch1p or Hch1p^{Δ11}. 4 μ M of Hsp82p was used in reactions containing Hsp82p alone.

Supplementary figure 2

Primer #	Primer name	Primer sequence
13	senseScAha1 BamHI	gagagaggatccatggtcgtgaataacccaaataactg
16	antisenseScAha1mycXhoI	gagagactcgagtcactacaaatcttctcagaaatcaatttttgttcaatacggcaccaagccgaatg
17	senseScHch1 BamHI	gagagaggatccatggtgtcttgaatccaaataactg
20	antisenseScHch1 mycXhoI	gagagactcgagtcactacaaatcttctcagaaatcaatttttgttcaactgtatatcctttgagtg
27	CScHch1 BamHI	gagagaggatccctcactaaactgtatatcctttgagtg
29	CScAha1 BamHI	gagagaggatccctcactataatacggcaccaagccg
90	antisenseHch1mycSacI	gagagagagctctcactacaaatcttctcagaaatcaatttttgttccataactgtatatcctttgagtg
126	antisensemycAHA1NotI	gagagagcgccgctcactacaaatcttctcagaaatcaatttttgttccattaatacggcaccaagccg
571	senseBamHIHch1del11	gagagaggatccatgGTGGATAAAAACACCTTACCTTGGTC
572	senseNdelAha1del11	gagagacatatgGTCGATAAGAACTGCATC
573	senseBamHIAha1del11	gagagaggatccatgGTCGATAAGAACTGCATC
601	senseNdelHch1del11	gagagacatatgGTGGATAAAAACACCTTACCTTGGTC
648	senseBamHIHch1Aha1del11	gagagaggatccatgggccatcaccatcaccatcacatgGTCGATAAGAACTGCATC
649	senseBamHIHch1Aha1	gagagaggatccatgggccatcaccatcaccatcacatggtcgtgaataacccaaataactg
654	SenseNco1Hch1	gagagaccatgGTTGTCTTGAATCCAAATAACTGGCATTG
655	SenseNco1Del11Hch1	gagagaccatgGTGGATAAAAACACCTTACCTTGGTCTAAG
656	AntisenseBamH1CtermHisTagHch1	gagagaggatccctcactaATGATGATGATGATGATGaatgtatatcctttgagtg
657	SenseXba1Aha1	gagagaTCTAGAAataatttttgttaactttaagaaggagatataccATGgtcgtgaataacccaaataactggcactgggtcg
658	SenseXba1Del11Aha1	gagagaTCTAGAAataatttttgttaactttaagaaggagatataccATGgtcgataagaactgcacggatgggccaaggag
659	AntisenseBamH1CtermHisTagAha1	gagagaggatccctcactaATGATGATGATGATGATGaatcggcaccaagccgaatg
664	aScAha1NtermBamHICtermHistag	GAGAGAGGATCCTCACTAATGATGATGATGATGATGacacctgaatgtcattac
672	sQCHsp82S25PEcoRVremoval	caacaccgtctatcctaacaaggaaattttcttgagagaactgatctctaatgcc
673	aQCHsp82S25PEcoRVremoval	ggcattagagatcagttctctcaagaaatttcctgttaggatagacgggtgtg

Supplementary Figure 2. List of primers used in this study.