Supplementary Information

Binding of a Small Molecule at a Protein-Protein Interface Regulates the Chaperone Activity of Hsp70-Hsp40

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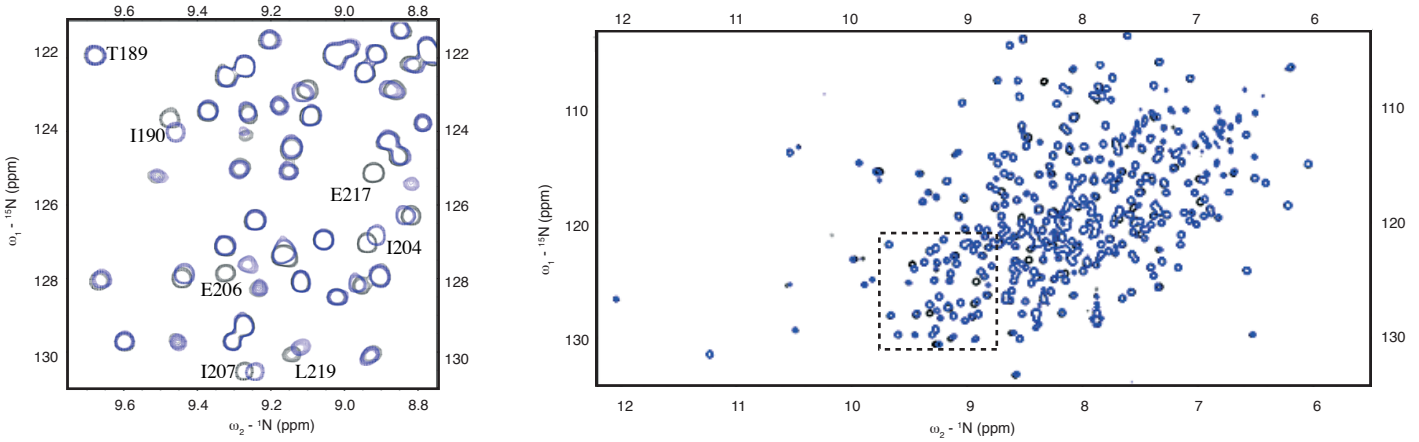
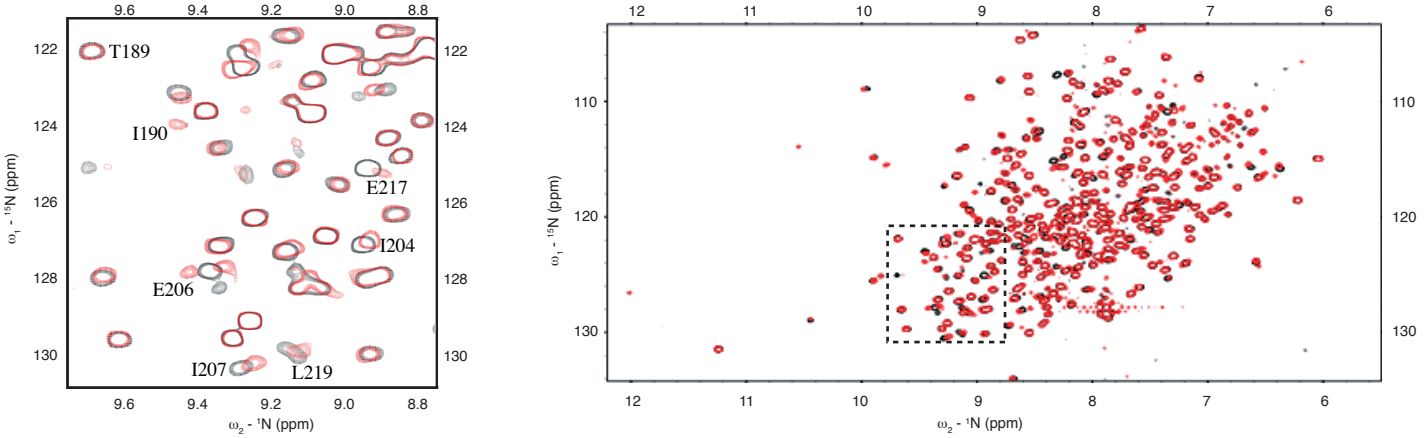
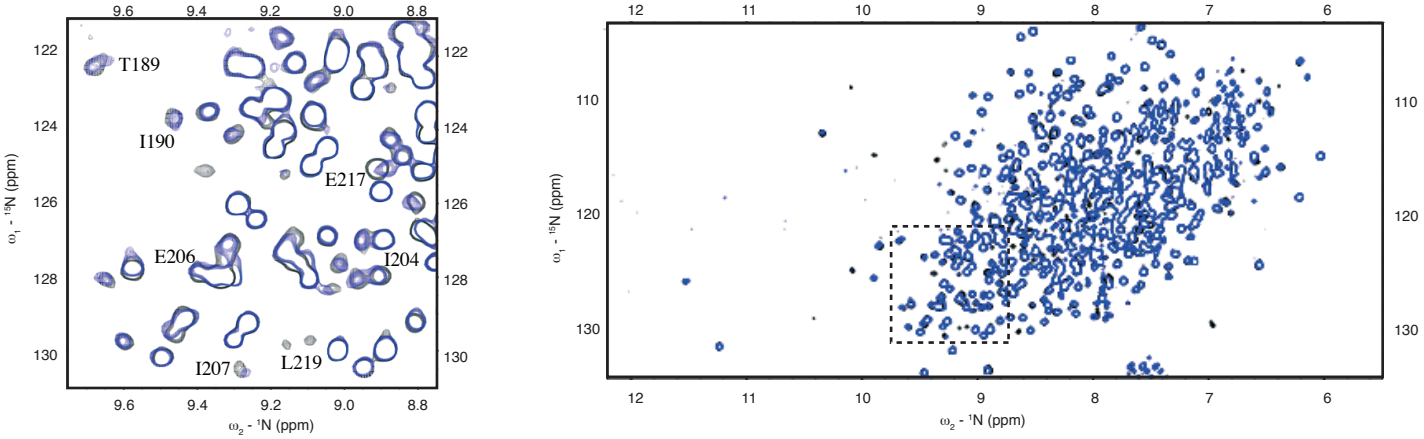
(A) $DnaK_{NBD}$ (5 mM ADP, 10 mM KPO_4)
$DnaK_{NBD}$ (5 mM ADP, 10 mM KPO_4, 1 mM 115-7c)



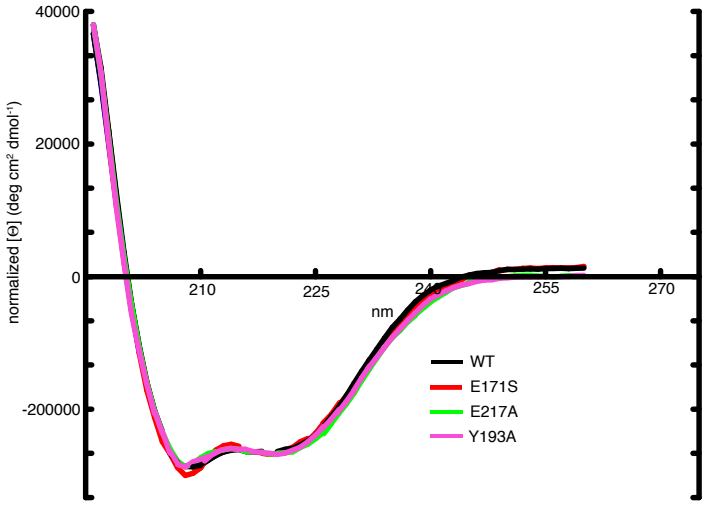
(B) $DnaK_{NBD}$ (5 mM ATP, 10 mM KPO_4)
$DnaK_{NBD}$ (5 mM ATP, 10 mM KPO_4, 1 mM 115-7c)



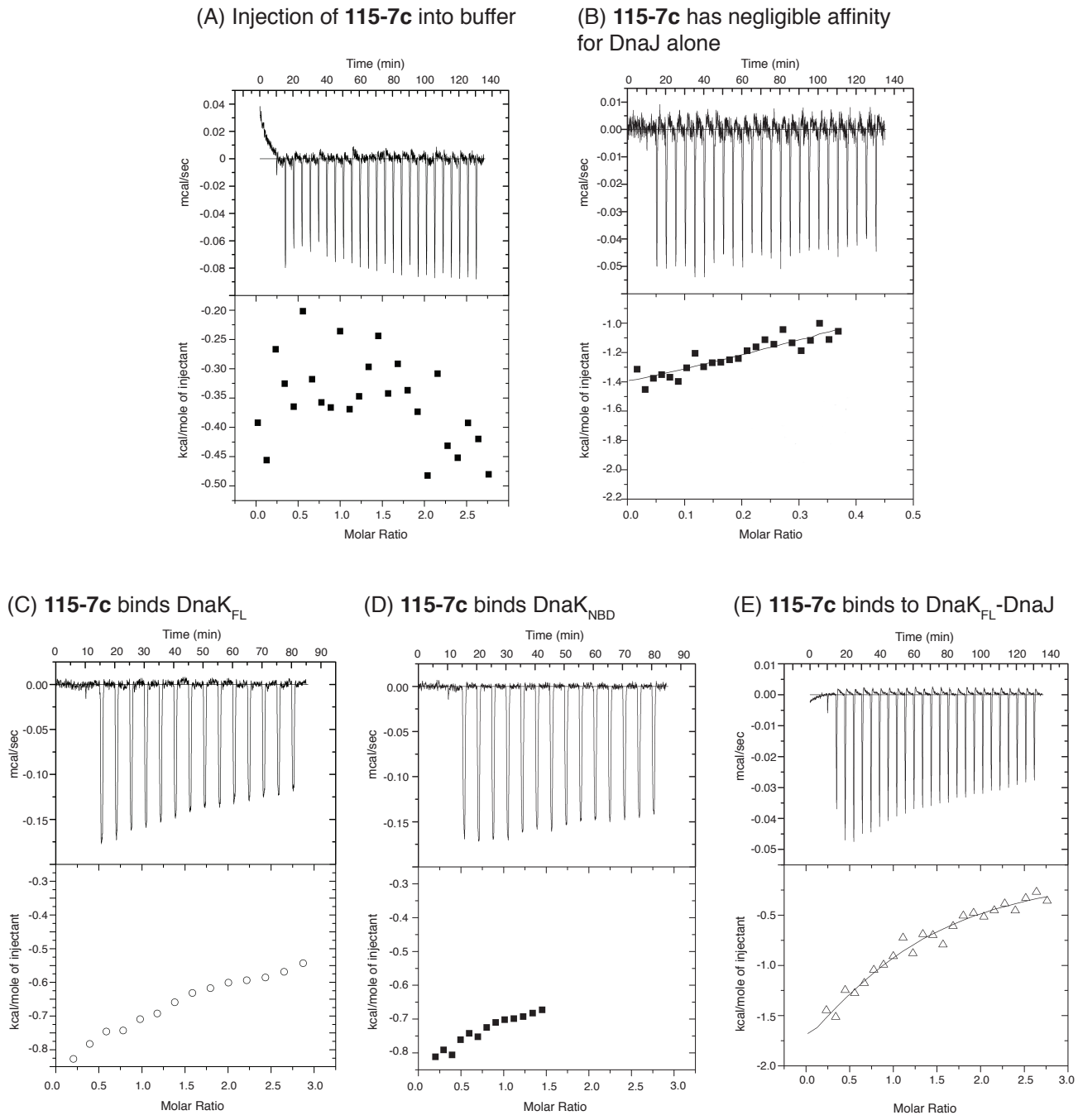
(C) $DnaK_{FL}$ (5 mM ADP, 10 mM KPO_4, 2.5 mM substrate peptide)
$DnaK_{FL}$ (5 mM ADP, 10 mM KPO_4, 2.5 mM substrate peptide, 1 mM 115-7c)



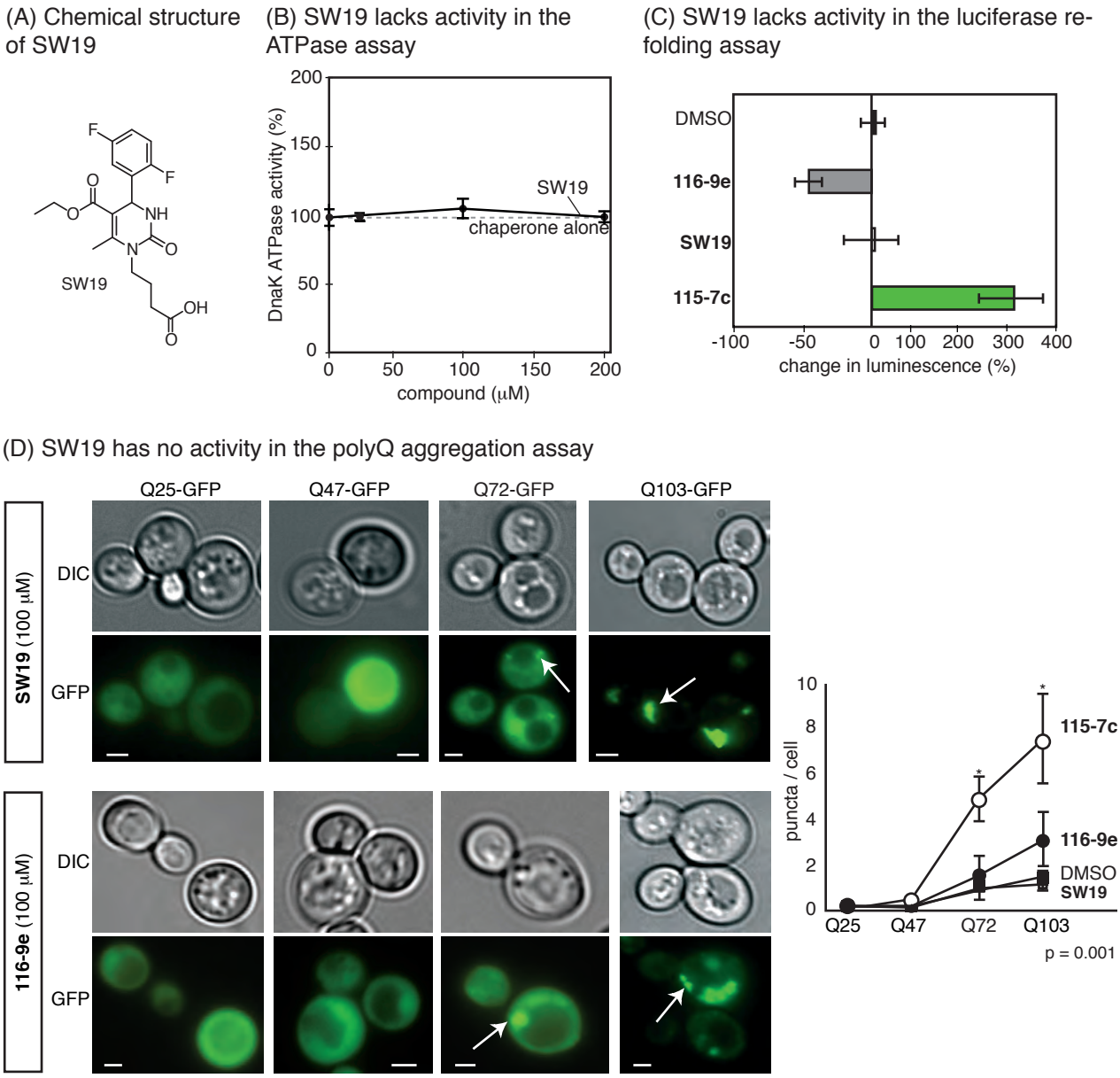
Supplemental Figure 1. NMR results for binding of **115-7c** to either the (A) ADP- or (B) ATP-bound form of $DnaK_{NBD}$ and binding of **115-7c** to the (C) ADP- and substrate-bound form $DnaK_{FL}$. The final DMSO concentration was ~ 1%.



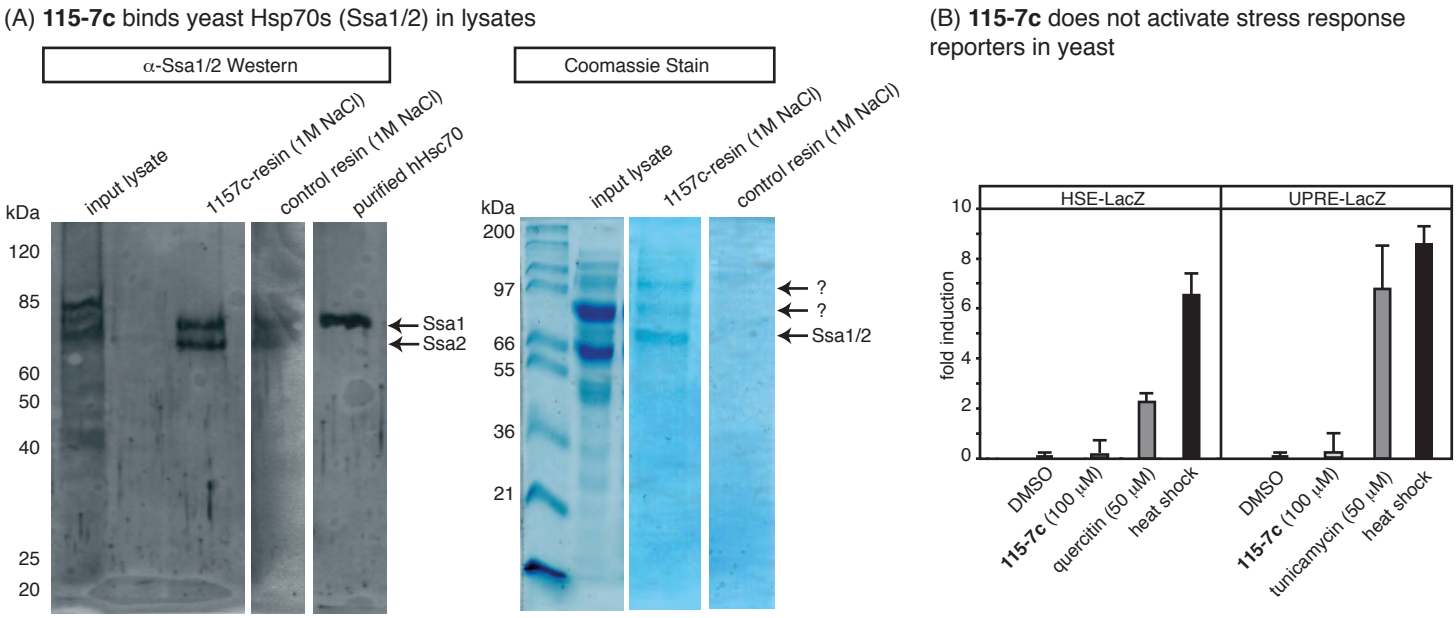
Supplemental Figure 2. Point mutants in DnaK$_{FL}$ do not drastically alter its structure. CD spectra of purified proteins (10 μM) were collected in Tris-buffered saline pH 7.2.



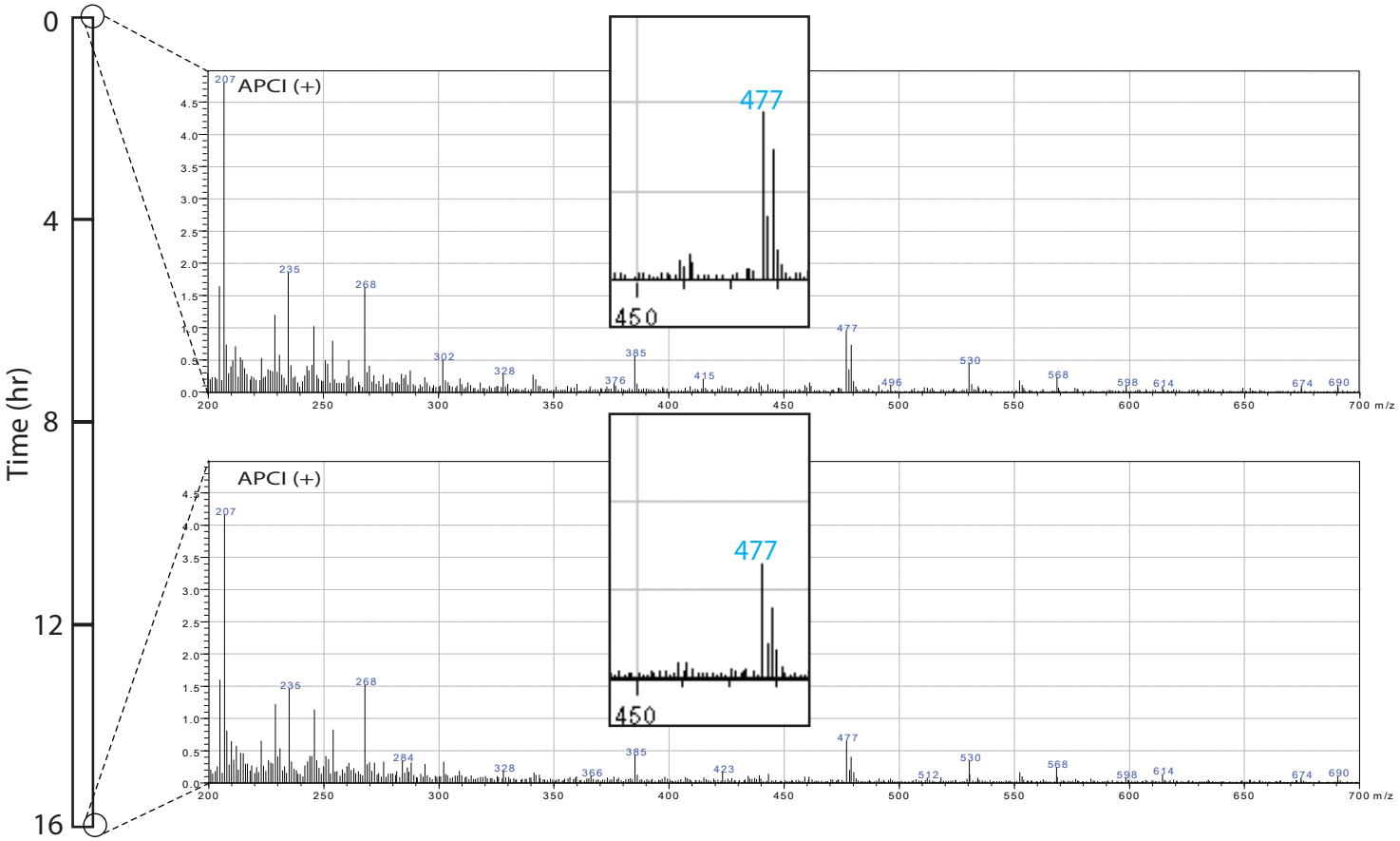
Supplemental Figure 3. Compound **115-7c** binds DnaK and the DnaK-DnaJ complex. Results are representative of at least duplicate trials.



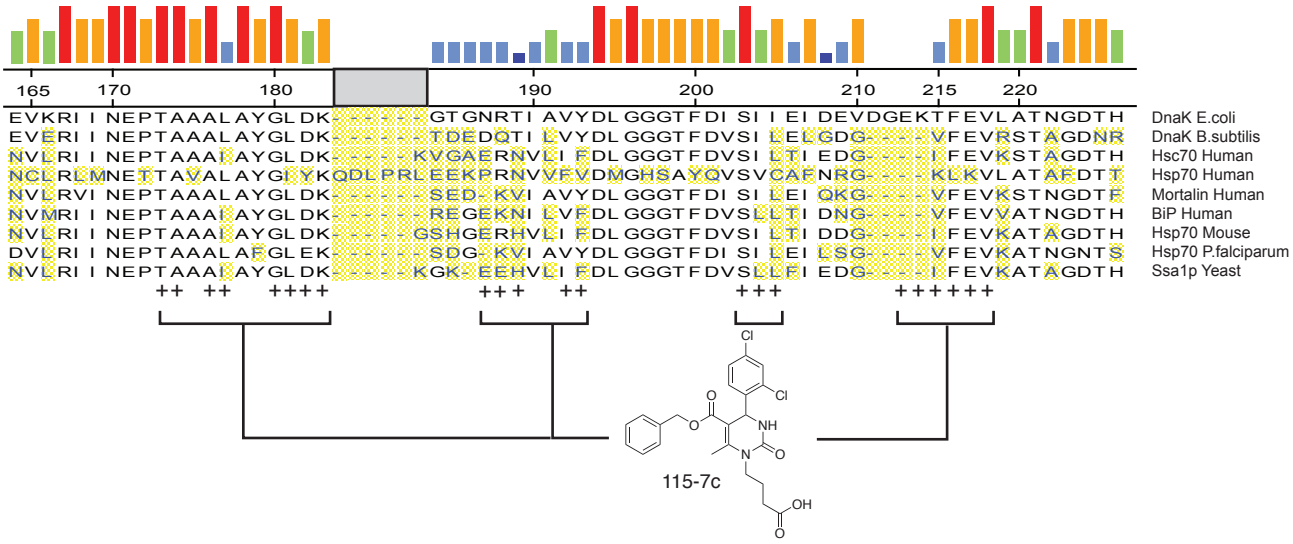
Supplemental Figure 4. An isosteric control, SW19, has no effect on biological activity in the ATPase, luciferase re-folding or yeast assays. (A) Chemical structure of SW19, which shares some features in common with 115-7c and 116-9e. (B) ATPase assay using DnaK (1 μM), which shows that SW19 does not stimulate or inhibit enzymatic activity. Results are the average of triplicates and the error bars are standard deviations. (C) Results of luciferase re-folding assays in which DnaK, DnaJ and GrpE (1 μM, 1 μM, 0.5 μM) was used to re-fold chemically denatured firefly luciferase. The results are shown relative to an untreated control (see Figure 1). Refolding was carried out for 60 min. at 37 °C and the luminescence signal recorded. Compounds or vehicle (DMSO) were added to 100 μM at the start of the refolding process. Results are the average of quadruplicates and the error is standard deviation. (D) The inhibitor, 116-9e, or the inactive control, SW19, were added to yeast expressing polyQ constructs (see the main text for details). Compounds were added at 100 μM and treatment was for 24 hours. Images were quantified as outlined in the Methods. Results were analyzed sing Student's T test (p = 0.001).



Supplemental Figure 5. The activator, **115-7c**, binds Hsp70s in yeast and does not induce a stress response. (A) Immobilized **115-7c** binds Hsp70s in yeastextracts. **115-7c** was immobilized on CnBr resin, via an enthylene diamine linker to produce the functionalized beads. As a control, ethanolamine was immobilized. Lysates of yeast were incubated with the resin and the washes were separated by SDS-PAGE and subject to either anti-Ssa1/2 Western blots (left) or Coomassie stains (right). The functionalized resin retained more Ssa1/2 than the control resin. In addition two higher molecular mass bands were retained, but the identity of these proteins is currently unknown. (B) Pharmacological modifiers of Hsp70 do not stimulate the stress response. Yeast cells (strain W303) expressing a LacZ reporter fused to either a heat shock element (HSE) or an unfolded protein response element (UPRE) were treated with compounds or heat (30 min, 37 °C). The cells were lysed and the LacZ signal measured. Results are the average of triplicates and the error bars represent standard deviations.



Supplemental Figure 6. 115-7c is stable in active yeast growth media (complete YPD) for 16 hours at 30 °C. Cultures (10 mL; YPD) from overnight stocks were diluted to ~0.2 and **115-7c** was added to a final concentration of 100 μM (less than 1% DMSO). At each indicated time, a 500 μL aliquot was centrifuged at 3,000xg for two minutes, the pellet was washed and cells lysed using glass beads. Each sample was then vigorously vortexed for 30 seconds with 500 μL of ethyl acetate (ThermoFisher). The organic fractions were separated at 13,000g for three minutes. Samples were stored at -80°C before analyzing on a Shimadzu LC-MS 2010 system (Columbia, MD) in positive mode APCI using acetonitrile (with 0.1% acetic acid) as the solvent. An external standard was generated using identical methods. Error was approximately 15%, as judged by triplicate experiments. Similar results were seen in both the supernatant and cell samples. At least 50% of the compound remained after 16 hours.



Supplemental Figure 7. Residues that interact with 115-7c are largely conserved. The variance in amino acid identity at positions relevant to compound binding were analyzed by Clustal W. The color and height of the bars indicate the shared identity. The yellow boxes indicate residues that are not conserved in relation to E. coli DnaK. The "+" are residues that display strong chemical shifts in response to addition of 115-7c in the NMR experiments (see Figure 3 and Supplemental Figure 1). These results demonstrate that much of the core binding region is conserved amongst different Hsp70s, especially between residues 173-183, 203-205 and 215-218.