Supplemental Data

The unique peptide substrate binding properties of 110 kDa heat-shock protein (Hsp110) determines its distinct chaperone activity

Xinping Xu*, Sarbeng Evans Boateng*, Christina Vorvis*, Divya Prasanna Kumar, Lei Zhou and Qinglian Liu

Department of Physiology and Biophysics, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298

* These authors contributed equally to this work.

Address correspondence to:
Qinglian Liu
Department of Physiology and Biophysics
School of Medicine
Virginia Commonwealth University
Richmond, VA 23298
Email: qliu3@vcu.edu
Phone: 804-628-4851
Fax: 804-828-9492

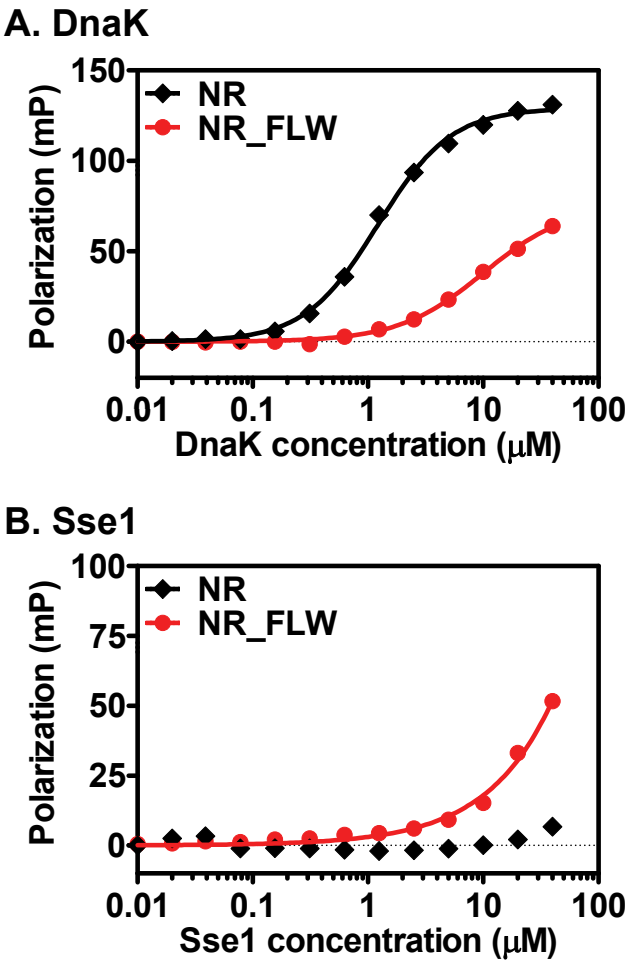

Figure S1. Comparison of the peptide NR and NR_FLW binding to DnaK (A) and Sse1 (B). The sequence of the NR_FLW peptide is: NRFLWTG. Fluorescence anisotropy assays were used to measure the binding of the NR peptide (black) and NR_FLW peptide (red).

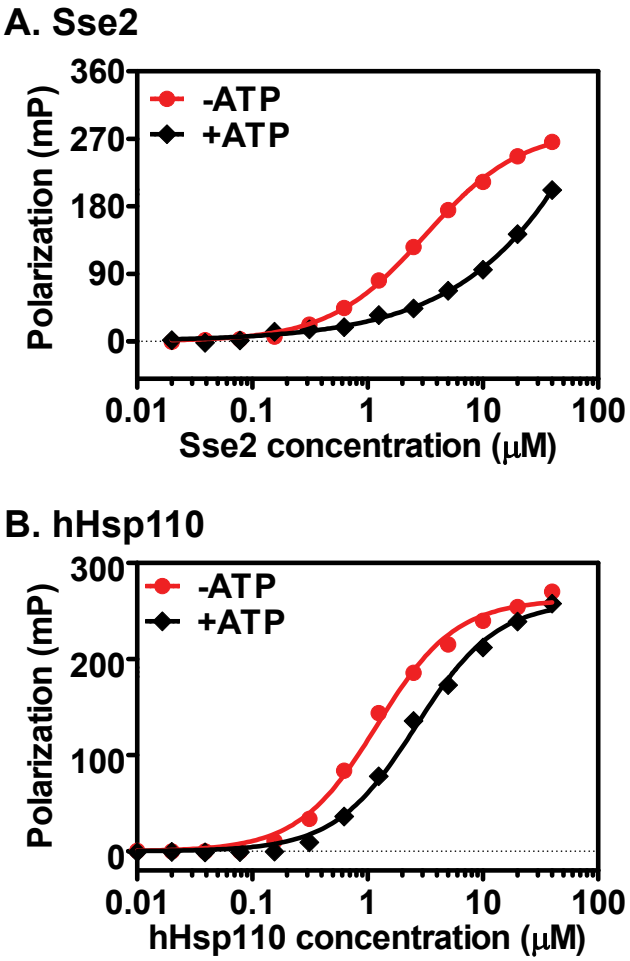

Figure S2. Peptide substrate binding in Hsp110s is ATP-sensitive. (A) Sse2 binding to the TRP2 peptide. (B) hHsp110 binding to the TRP2_181 peptide. The TRP2_181 peptide was used due to its higher affinity. The ATP sensitivity in hHsp110 is less pronounced than Sse1 and Sse2. This could be due to the fusion protein of hHsp110 used in our assay.

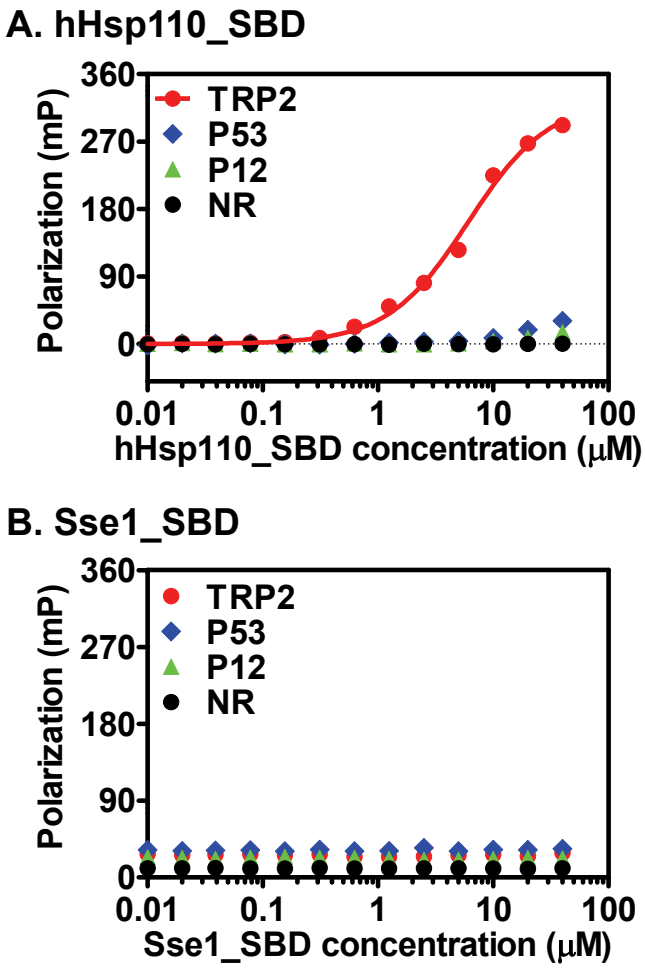

Figure S3. Peptide substrate binding to the isolated SBDs from hHsp110 (A) and Sse1 (B). Four peptides were tested: TRP2 (red), P53 (blue), P12 (green) and NR (black).

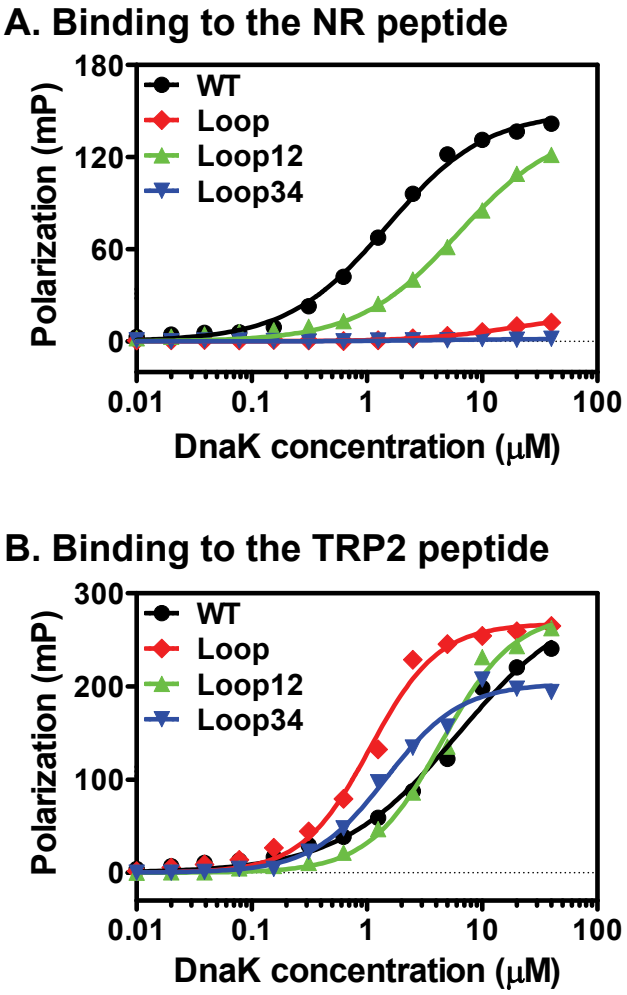

Figure S4. Peptide binding activities of the mutant DnaK proteins. (A) binding to the NR peptide. (B) binding to the TRP2 peptide. DnaK_Loop, DnaK_Loop12, and DnaK_Loop34 are labeled as Loop, Loop12 and Loop34.

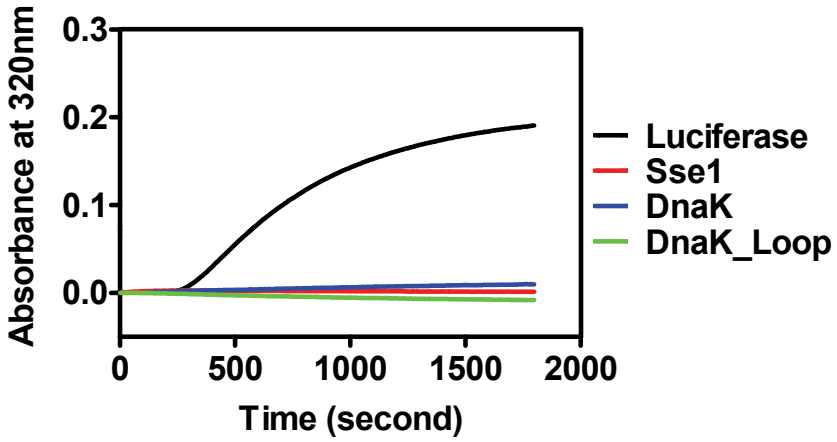

Figure S5. The chaperone proteins Sse1 and DnaK did not show aggregation when incubated at 41°C. The assay was done as described for Figure 6C. 800 nM of each chaperone protein was used, and luciferase alone was used as a control.

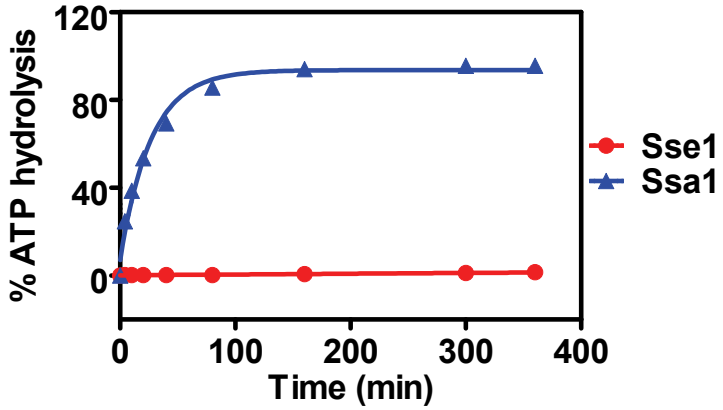

Figure S6. Sse1 does not exhibit ATPase activity. Single-turnover ATPase assays with purified Sse1 (red circle) and Ssa1 (blue triangle) were performed as described previously (52). Briefly, radio-labeled ATP was first incubated with either Sse1 or Ssa1 to form chaperone-ATP complex. Then, the complex was isolated using a spin column at 4°C, and the conversion of ATP to ADP, the ATP hydrolysis, in the complex was assayed over time at 30°C. The determined catalytic constant, k_{cat}, of Ssa1 is 0.0374±0.0053 /min, whereas there was no significant ATP hydrolysis for Sse1.

REFERENCES

52. Liu, Q., Krzewska, J., Liberek, K. & Craig, E.A. Mitochondrial Hsp70 Ssc1: role in protein folding. *J Biol Chem* **276**, 6112-8 (2001).