Supporting Information

Kravats et al. 10.1073/pnas.1719969115

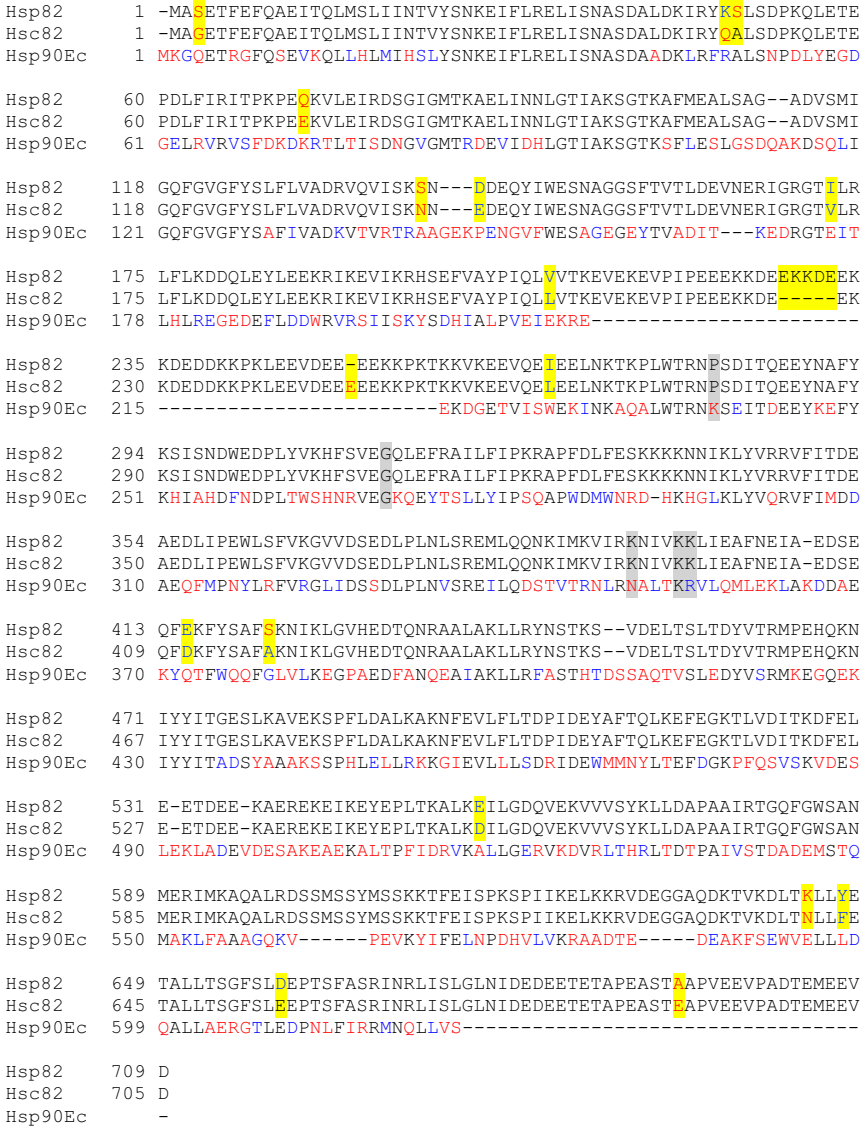

Fig. S1. Sequence alignment of Hsp82, Hsc82, and $Hsp90_{Ec}$. Protein sequences were obtained from UniProt and aligned by T-COFFEE (tcoffee.crg.cat/) (1) using default parameters. The figure was produced using the BOXSHADE program (https://www.ch.embnet.org/software/BOX_form.html). The differences between Hsp82 and Hsc82 are highlighted in yellow, involving 16 amino acid substitutions and two gaps within the 709 amino acids of Hsp82. The residues focused on in this study are shaded in gray and are conserved between Hsp82 and Hsc82. Additionally, three of the five residues in this study are conserved from *E. coli* to yeast. Identical residues are in black type, similar residues are in blue, and dissimilar residues are red.

1. Notredame C, Higgins DG, Heringa J (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* 302:205–217.

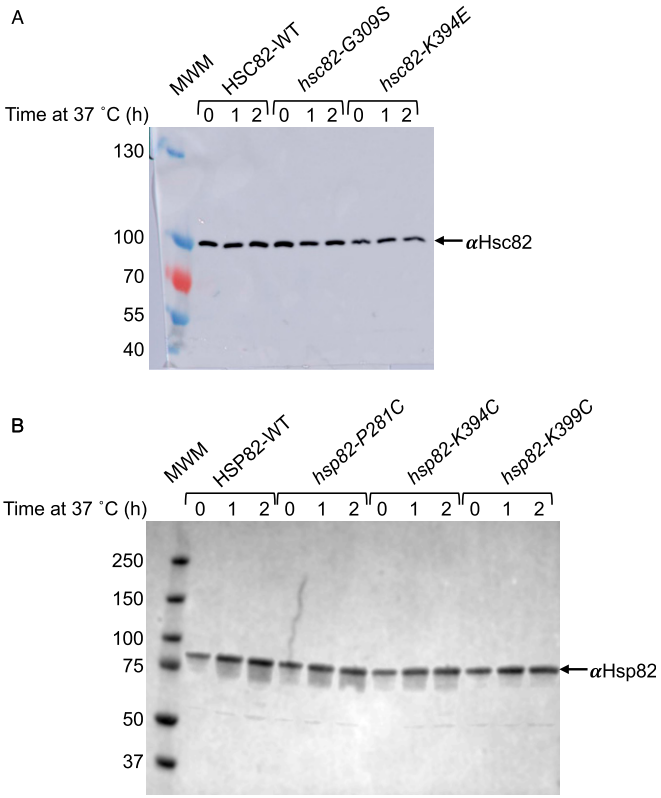

Fig. S2. Expression levels of Hsc82 and Hsp82 mutant proteins. (*A*) Overnight cultures of strains expressing wild-type Hsc82 or mutant proteins were diluted to a starting $OD_{600} = 0.3$ and were grown at 30 °C until $OD_{600} = 1.0$. A sample of each culture (30 °C sample) was taken at $t = 0$. Each culture was then transferred to a 37 °C shaking incubator. Additional samples were taken at $t = 1$ h and $t = 2$ h. The cells were lysed in PBS containing 1% SDS and 0.1% Triton X-100 using glass beads. SDS PAGE loading dye was added, and samples were boiled and run on a 7.5% gel followed by Western blot analysis using antibody specific to Hsc82. (*B*) Overnight cultures of strains expressing wild-type Hsp82 or mutant proteins were diluted to a starting $OD_{600} = 0.3$ and grown at 30 °C until $OD_{600} = 1.0$. A sample of each culture (30 °C sample) was taken at $t = 0$. Each culture was then transferred to a prewarmed tube in a 37 °C water bath. Additional samples were taken at $t = 1$ h and $t = 2$ h. The cells were lysed in 50 mM Hepes (pH 7.3), 100 mM NaCl, 2 mM $MgCl_2$, and 0.1% Tween 20 with protease inhibitors using glass beads. Lysates were spun at 5,000 × *g* for 5 min, SDS PAGE loading dye was added, and samples were boiled and run on a 4–20% Criterion gel followed by Western blot analysis using antibody specific to Hsp82 (SPA-840).

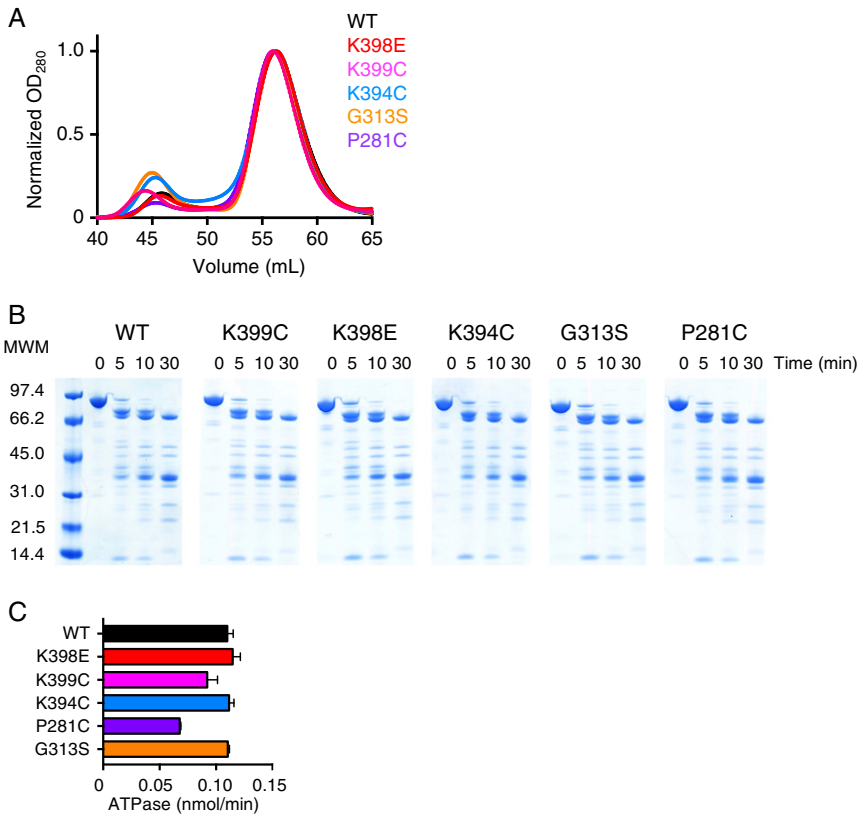

Fig. S3. Properties of Hsp82 mutant proteins. (*A*) Size-exclusion chromatography of Hsp82 mutants. Gel-filtration analyses of purified wild-type Hsp82 or mutants were performed with a HiLoad 16/60 Superdex 200 column (GE Healthcare) equilibrated with Tris·HCl buffer (50 mM, pH 7.5) containing 100 mM KCl, 10% glycerol, and 1 mM DTT. (*B*) Partial protease digestion patterns of Hsp82 mutant proteins compared with the wild type. Reaction mixtures (50 μL) containing wild-type or mutant Hsp82 (15 μg), 25 mM Hepes (pH 7.5), 50 mM KCl, and 2 mM DTT were made, and 10 μL of each reaction was removed ($t = 0$). Trypsin (10 ng) was added to the remaining reaction mixtures, which were incubated at 37 °C for the indicated times. Proteolysis was terminated with LDS sample buffer. The samples were incubated at 85 °C for 5 min and analyzed by Coomassie staining following SDS/PAGE. (*C*) ATP hydrolysis by Hsp82 mutant proteins compared with the wild type. Assays were carried out as described in *Materials and Methods*, and data are presented as the mean ± SEM ($n = 3$).

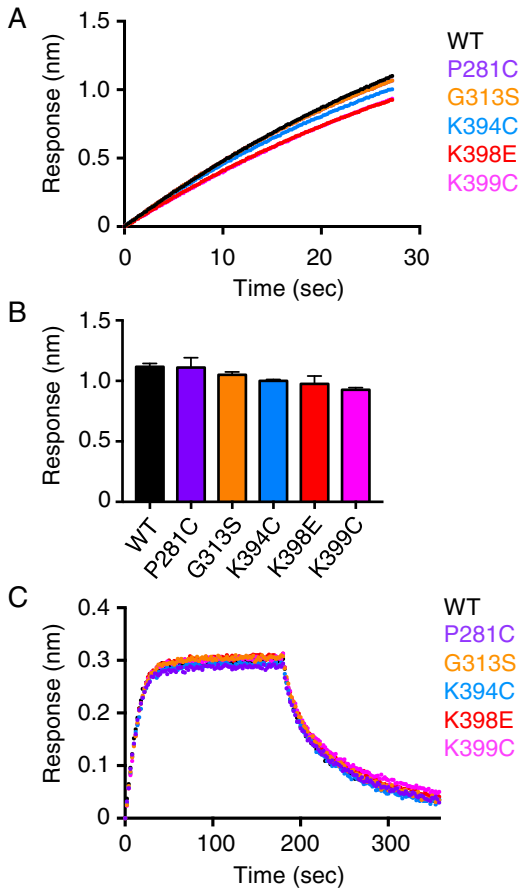

Fig. S4. Properties of BLI assay. (*A*) A representative trace of the loading of biotinylated wild-type Hsp82 and mutants onto the streptavidin-coated biosensor as a function of time (see *Materials and Methods*). (*B*) Similar amounts of wild-type or mutant Hsp82 are bound to the biosensors at the conclusion of the loading phase in *A*. The maximum response value reached during the loading phase is plotted for biotinylated wild-type or mutant Hsp82. Data from three or more replicates are presented as mean ± SD. (*C*) Biotinylated wild-type Hsp82 and mutants interact similarly with Sti1. Shown are association and dissociation curves of Sti1 (0.1 μM) binding to biotinylated wild-type or mutant Hsp82. Wild-type or mutant Hsp82 was bound to the streptavidin-coated biosensors to a response of ∼1.1 nm. Data were collected from three or more experiments, and representative curves for wild-type Hsp82 and each mutant are shown.

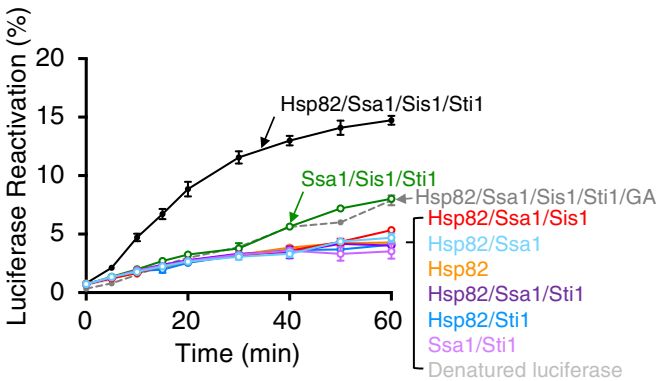

Fig. S5. Properties of the luciferase reactivation assay. Reactivation of heat-inactivated luciferase was measured as described in *Materials and Methods* with the indicated components. Data from three or more replicates are presented as mean ± SEM.

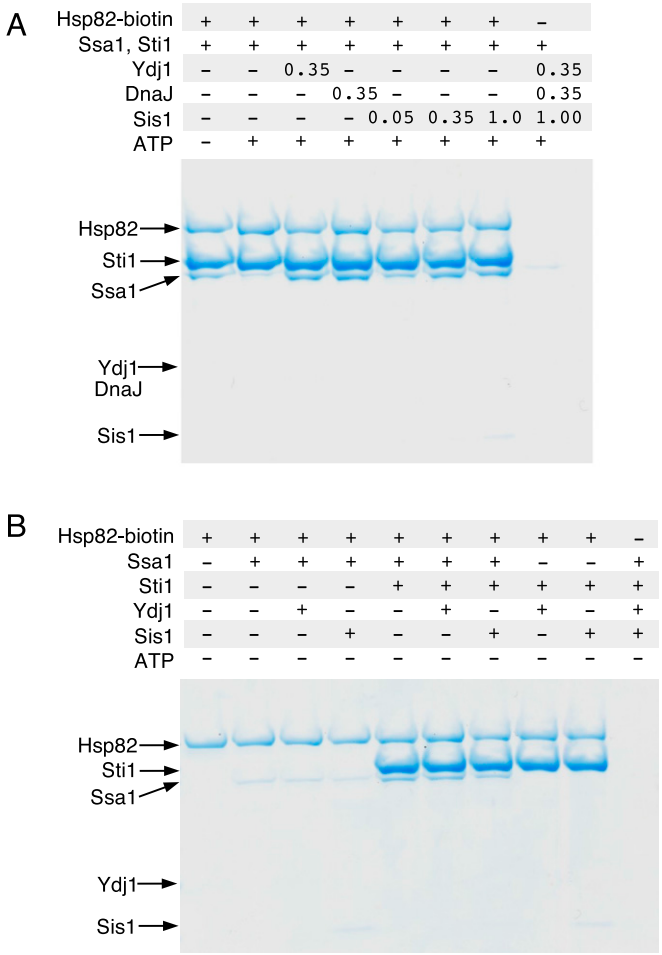

Fig. S6. Effect of Ydj1 or Sis1 on Ssa1 and Sti1 binding to wild-type Hsp82 using a pull-down assay. (*A*) The interaction between biotin-labeled wild-type Hsp82 (1 μM), Ssa1 (6 μM), and Sti1 (1 μM) was monitored in the presence of Ydj1 (0.35 μM), DnaJ (0.35 μM), or Sis1 (0.05, 0.35, or 1.0 μM) in the presence of ATP, as described in *Materials and Methods*. (*B*) Interaction between biotin-labeled wild-type Hsp82 (1 μM) and Ssa1 (6 μM), with or without 1 μM Sti1 and with 0.35 μM Ydj1 or 0.35 μM Sis1 where indicated, as described in *Materials and Methods*. Representative gels of three or more experiments are shown.

Table S1. In vivo effects of Hsp82 and Hsc82 amino acid substitutions on growth and activity

Hsp82/Hsc82 wild type or substitution mutant	Growth at 37 °C	Growth in *sti1Δ*	GR activation	In vivo interaction
Hsp82 WT/Hsc82 WT	+	+	+	+
Hsp82-P281C/Hsc82-P277	+	+	+	+
Hsp82-G313S/**Hsc82-G309S**	–*,†	–*,‡	–†	+/–*
Hsp82-K394C/Hsc82-K390	+	+/–	+/–	+/–
Hsp82-K398E/**Hsc82-K394E**	–	–	ND	–
Hsp82-K399C/Hsc82-K395	–	–	–	+/–

Bold indicates proteins tested in these assays in this study. ND, no data.
*Previously published results and results shown here for Hsc82-G309S (1).
†Previously published results using Hsp82-G313S/N (2, 3).
‡Previously published results using Hsp82-G313S (4).

1. Flom G, Behal RH, Rosen L, Cole DG, Johnson JL (2007) Definition of the minimal fragments of Sti1 required for dimerization, interaction with Hsp70 and Hsp90 and in vivo functions. *Biochem J* 404:159–167.
2. Bohen SP, Yamamoto KR (1993) Isolation of Hsp90 mutants by screening for decreased steroid receptor function. *Proc Natl Acad Sci USA* 90:11424–11428.
3. Nathan DF, Lindquist S (1995) Mutational analysis of Hsp90 function: Interactions with a steroid receptor and a protein kinase. *Mol Cell Biol* 15:3917–3925.
4. Chang HC, Nathan DF, Lindquist S (1997) In vivo analysis of the Hsp90 cochaperone Sti1 (p60). *Mol Cell Biol* 17:318–325.

Table S2. In vitro effects of Hsp82 amino acid substitutions on protein–protein interactions and activity

Hsp82 wild type or substitution mutant	Hsp82–Ssa1 interaction (BLI)	Hsp82–Ssa1 pulldown (binary)	Hsp82–Ssa1 pulldown + Sti1	Hsp82–Ssa1 pulldown + Sti1 and Ydj1	Luciferase refolding	Hsp82–Sti1 interaction (BLI and pulldown)	ATP hydrolysis	Inhibition of ATP hydrolysis by Sti1
Hsp82 WT	+++	+++	+++	+++	+++	+++	+++	+++
Hsp82-P281C	++	+++	+++	+++	+++	+++	++	+++
Hsp82-G313S	–	+	++	++	–	+++	+++	+++
Hsp82-K394C	–	–	+	+	–	+++	+++	+++
Hsp82-K398E	–	–	–	+	–	+++	+++	+++
Hsp82-K399C	–	+	+	+	–	+++	+++	+++

Each plus sign indicates ~33% activity.