SUPPLEMENTAL INFORMATION

Interplay between *E. coli* DnaK, ClpB and GrpE during protein disaggregation

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Supplemental Methods and Results

Trimer docking. $ClpB_{Ec}$ trimer was based on the existing $ClpB_{Tth}$ trimer found in the protein databank (PDB ID 1QVR). The model of the $ClpB_{Ec}$ trimer was generated using the GalaxyGemini server (http://galaxy.seoklab.org/) [1] using $ClpB_{Ec}$ subunit A as a template. To preserve the trimeric structure with distinct orientations of both N- and M-domains found in $ClpB_{Tth}$, models of individual $ClpB_{Ec}$ subunits were superimposed onto the predicted $ClpB_{Ec}$ trimer. To this end, we used a best-fitting approach to minimize the backbone root-mean-square deviation (RMSD) of $ClpB_{Ec}\,\Delta$ N fragments of each monomer with respect to the target structure. RMSD values of $ClpB_{Ec}\,\Delta$ N fragments and full-length subunits are in agreement with those found in $ClpB_{Tth}$. The docking method can be found in Methods in the main text.

In all decoys, we observed binding between one DnaK monomer and two adjacent ClpB subunits (Supplemental Fig. S4). The top docking decoy indicates subdomains IB and IIB of DnaK participate in interactions with ClpB, as shown by large changes in SASA between 50% and 95% for the residues important for collaboration with ClpB, including R56, V59, T60, L257, R261, N282, P284 and Y285 (Supplemental Table S2). Predicted interactions between ClpB and DnaK involved 13 contacts with ClpB chain A region 1 (between residues 413 and 447) and 14 contacts in ClpB chain B region 2 (between residues 483 and 508). Further interactions were observed in ClpB NBD1, forming 10 contacts with DnaK. Supplemental Figure S4 highlights the interactions between DnaK and ClpB in the top docking decoy of the ClpB trimer and DnaK.

Supplemental Table S1. Residues within 6 Å in a GrpE[a] and DnaK complex or a ClpB hexamer[b] and DnaK complex

GrpE	DnaK[c]	ClpB chain E	ClpB chain F
	45	221, 222, 225	
	46	225, 226, 227	
	47	225	
	52	495	
112	53	495	
116	55		
113, 116, 148	56	495, 499	
112	57	490	
117, 119	59		
	63		423
	87		445, 449
	90		427
	92		452
	94		456
	95		456
45	130		
42	131		
41	132		
	234		433, 435
	237		433
	238		433
	253		433, 434
	256		428, 431
118, 119, 135	257		
	259		431
118	260		
150	261	503	
71, 136	284		
74, 135	285	508, 511	
	290	518	
	292	511, 514, 515, 518	
	293	515	
74, 75	295		

[a] Residues identified by the DnaK-GrpE crystal structure (1DKG) [2]

[b] Residues identified by modeling the DnaK monomer with the ClpB hexamer in this study

[c] Blue indicates DnaK residues within 6 Å of GrpE residues in the crystal structure, black indicates residues within 6 Å of ClpB residues as identified in this study and red indicates DnaK residues within 6 Å of either GrpE or ClpB residues in the DnaK-GrpE structure or DnaK-ClpB model

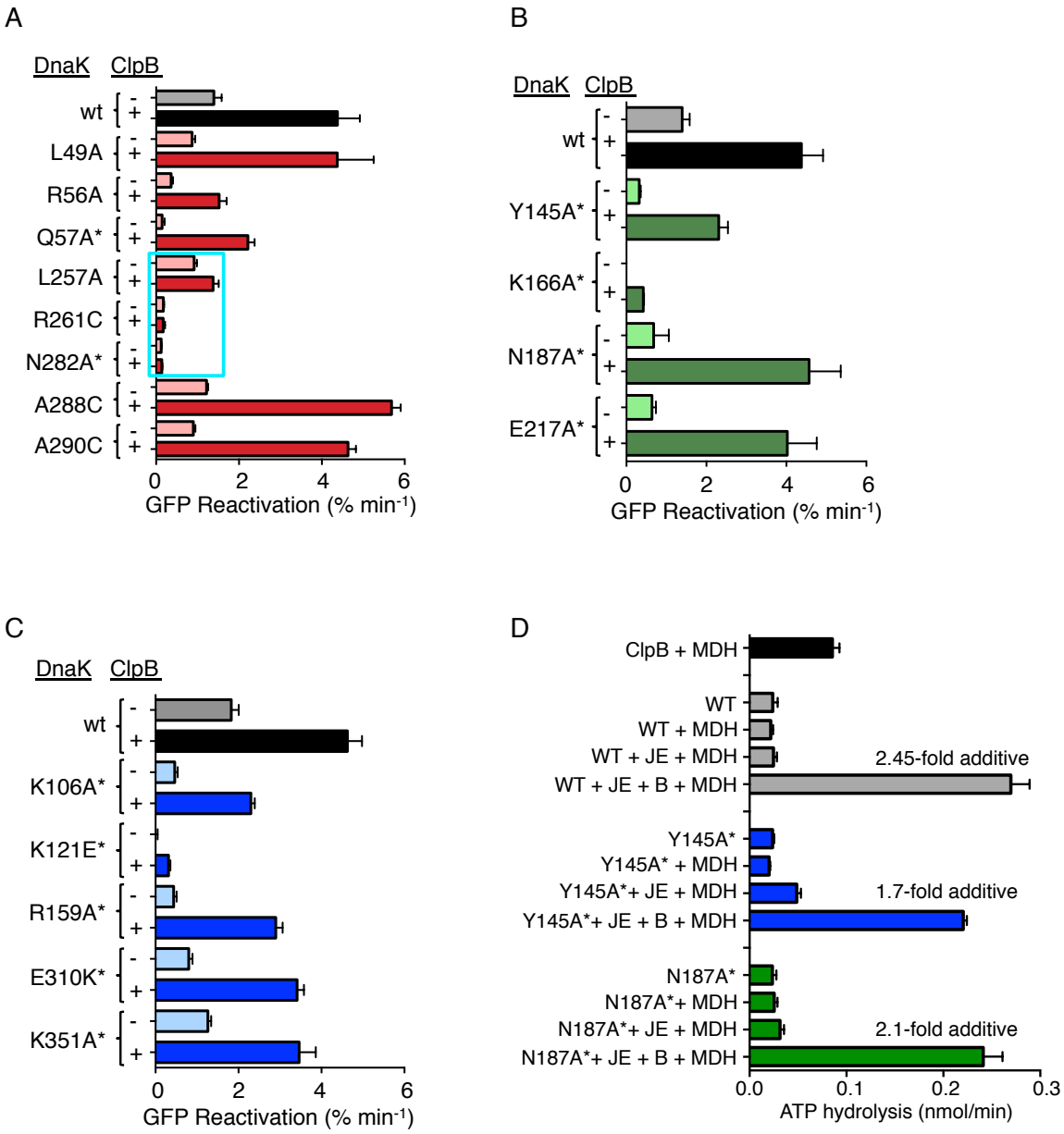
Supplemental Table S2. Residues involved in DnaK-ClpB interaction

Residue in DnaK	SASA[a]	DnaK	DnaK-ClpB trimer complex	DnaK-GrpE complex
R56	Absolute SASA (Å)^2	154.2	9.0	10.7
	Relative[b] SASA	0.6	0	0
V59	Absolute SASA (Å)^2	87.4	44.1	13.8
	Relative SASA	0.5	0.3	0.1
T60	Absolute SASA (Å)^2	101.7	39.3	25.6
	Relative SASA	0.7	0.3	0.2
L257	Absolute SASA (Å)^2	115.2	13.9	8.6
	Relative SASA	0.6	0.1	0
R261	Absolute SASA (Å)^2	100.6	13.1	0
	Relative SASA	0.4	0.1	0
N282	Absolute SASA (Å)^2	73.2	36.2	35.4
	Relative SASA	0.5	0.2	0.2
P284	Absolute SASA (Å)^2	81.1	22.1	0.3
	Relative SASA	0.6	0.2	0
Y285	Absolute SASA (Å)^2	179.7	11.0	70.1
	Relative SASA	0.8	0.0	0.3

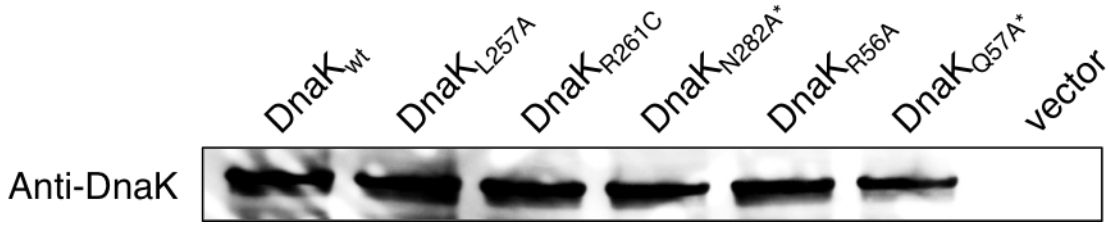
[a] SASA = solvent accessible surface area (see Methods)
[b] Relative SASA values of ~1 suggest the residue is located on the surface of the protein

Supplemental Table S3. Plasmids

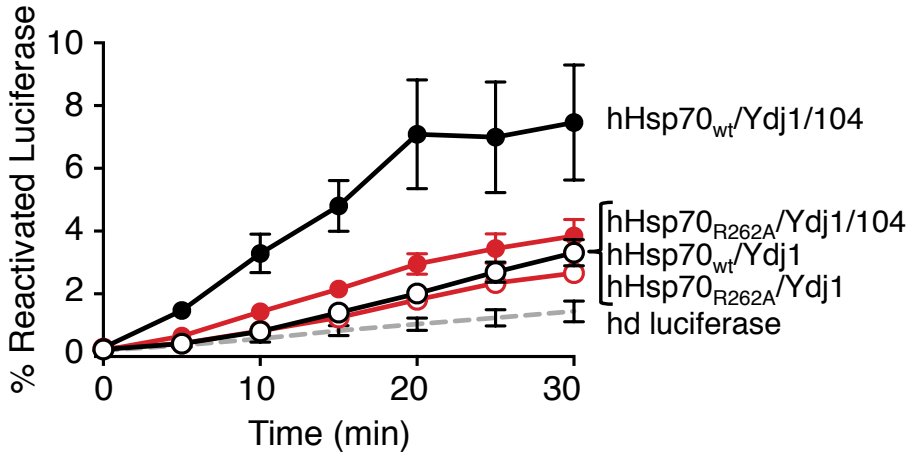
Plasmid	Relevant features	Reference or source
pET24b	Kan^r, T7 promoter	Novagen
pET24b-DnaK	*dnaK* gene in pET24b	This study
pET24b-Ssa1	*SSA1* gene in pET24b	This study
pET23c-*HSPA1A*	*Human Hsp70 gene (HSPA1A) in pET23c*	Gift of Len Neckers
pEB355	pUT18C derivative, Amp^r, Col E1 ori, Plac, T18 domain	[3]
pEB354	pKT25 derivative, Kan^r, p15A, Plac, T25 domain	[3]
p18link-ClpB	*clpB* gene in pEB355	[4]
p25link-DnaK	*dnaK* gene in pEB354	[4]



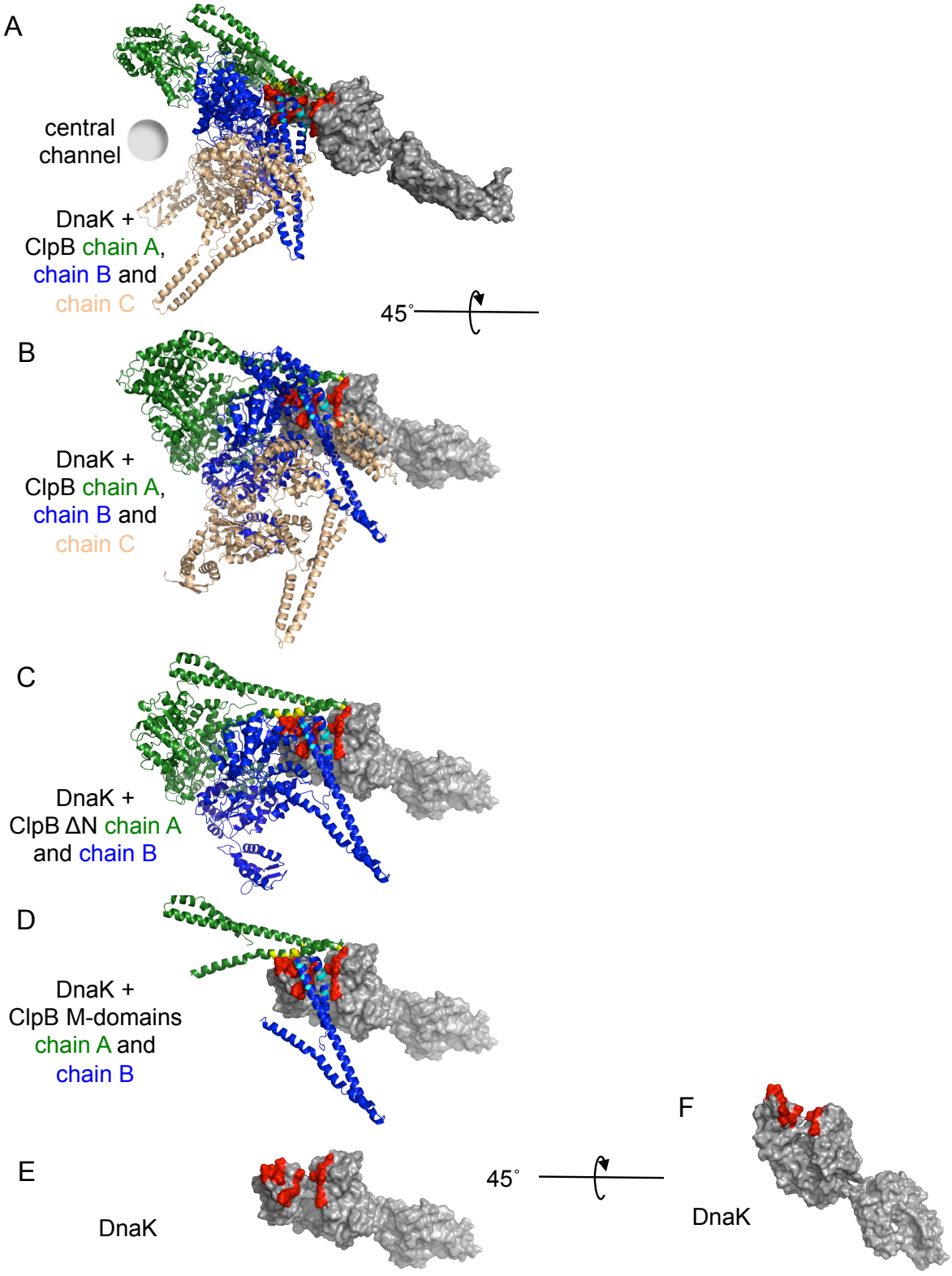
Supplemental Fig. S1. Substitution mutants in DnaK NBD subdomain IIB are defective for collaboration with ClpB in protein remodeling. A-C. The DnaK system (DnaK wild-type or mutant, DnaJ and GrpE) was incubated with heat-denatured GFP (hdGFP), ATP and ClpB as indicated. The increase in fluorescence intensity was monitored over time. Rates of reactivation of aggregated GFP were determined as described in Methods. DnaK mutants in the GrpE binding region (**A**) in the DnaJ binding region (**B**) or in regions with no known interactions (**C**) were tested. **D.** Steady-state ATP hydrolysis was measured in reactions containing DnaK wild type or mutant, ATP, DnaJ, GrpE, ClpB and heat-denatured malate dehydrogenase (MDH) as indicated and described in Methods. The fold above additive is calculated by dividing the rate of ATP hydrolysis for DnaK/JEB/MDH by the sum of the rates for DnaK/JE/MDH and ClpB/MDH. **A**-**D** are means ± s.e.m (n=3).



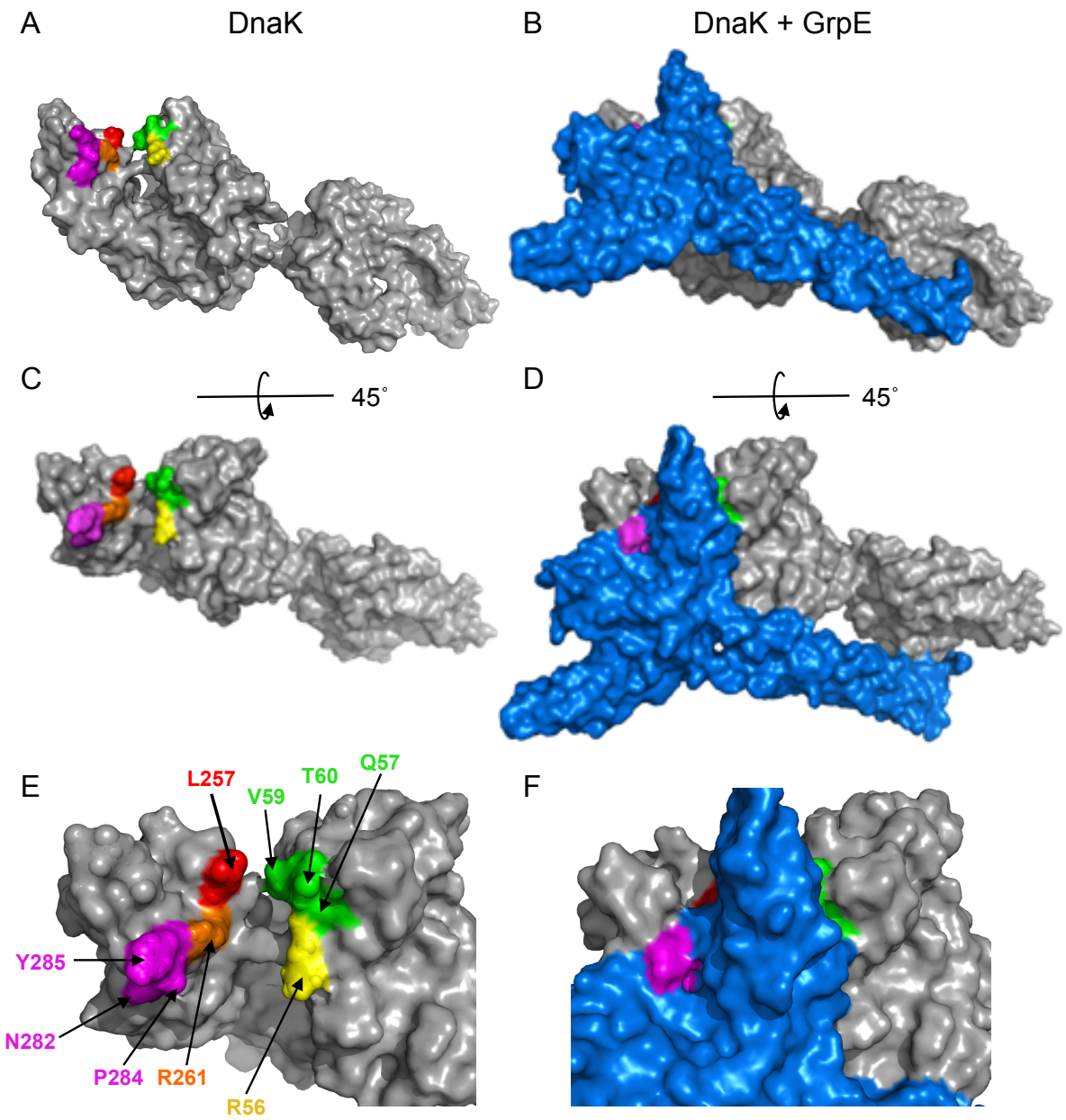
Supplemental Fig. S2. Bacterial two-hybrid fusion protein expression. T25 fusion proteins were expressed in *E. coli* MG1655 under the same conditions used for the β-galactosidase experiments and protein levels were determined by Western blot analysis using polyclonal DnaK antisera.



Supplemental Fig. S3. Human Hsp70 (hHsp70) mutant in subdomain IIB is defective for collaboration with Hsp104. Reactivation of heat-denatured luciferase was measured over time as described in Methods. Heat-denatured luciferase was incubated with hHsp70 wild type (0.95 μM) or mutant, Ydj1 (0.17 μM) and ATP in the presence or absence of Hsp104 (0.45 μM), as indicated. Data are means ± s.e.m (n=3).


A
central channel
DnaK + ClpB chain A, chain B and chain C
45˚
B
DnaK + ClpB chain A, chain B and chain C
C
DnaK + ClpB ΔN chain A and chain B
D
DnaK + ClpB M-domains chain A and chain B
E
DnaK
45˚
F
DnaK

Supplemental Fig. S4. Computational model of DnaK and a ClpB trimer indicates that one DnaK interacts with two ClpB protomers. A. Model of full-length ClpB trimer shown bound to full-length DnaK (gray). The two ClpB protomers that interact with DnaK are shown in green (chain A) and blue (chain B). Residues on DnaK identified from modeling performed in this study as interacting with the ClpB trimer are shown in red. DnaK interaction sites on ClpB chain A (yellow) and chain B (cyan) as identified from the trimer model are shown. Location of the ClpB central channel is indicated to aid in orienting the model. **B**. DnaK and ClpB trimer as in (**A**) rotated by 45˚ to visualize the region of ClpB interaction on DnaK. **C**. DnaK as in (**B**) shown bound to ClpB with chain C and the N-terminal domains (ΔN) removed to aid in visualizing the interaction site on DnaK. **D**. DnaK as in (**B**) shown with the interacting ClpB M-domains (residues 386-526) from chain A (green) and chain B (blue). **E.** DnaK alone as shown in (**B**). **F**. DnaK alone but rotated 45 ˚ from panel (**E**). Models were obtained as described in Methods and images in A-F were made using PYMOL (www.pymol.org) [5].



Supplemental Fig. S5. Residues involved in interactions with ClpB also interact with GrpE. **A,C,E**. Model of the solution NMR structure of DnaK in the ADP bound conformation (pdb:2KHO) [6]. Residues identified in biochemical studies as interacting with ClpB are shown: R56 (yellow), Q57, V59 and T60 (green), L257 (red), R261 (orange), N282, P284 and Y285 (magenta). Model in (**A**) is rotated 45˚ for the model in (**C**) and (**E**). **E** is a close up view of the model in (**C**). **B,D,F**. Model of the crystal structure of DnaK (gray) in complex with GrpE (blue) (pdb:1DKG) [2]. Orientations of DnaK in **B**, **D** and **F** correspond with the DnaK alone models in **A**, **C** and **E**, respectively. GrpE interacts with residues identified above, and are therefore masked by the GrpE structure. Images were created using PYMOL (www.pymol.org) [5].

Supplemental References

1. Lee, H., Park, H., Ko, J. & Seok, C. GalaxyGemini: a web server for protein homo-oligomer structure prediction based on similarity. *Bioinformatics* **29**, 1078-80 (2013).
2. Harrison, C.J., Hayer-Hartl, M., Di Liberto, M., Hartl, F. & Kuriyan, J. Crystal structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK. *Science* **276**, 431-5 (1997).
3. Battesti, A. & Bouveret, E. Acyl carrier protein/SpoT interaction, the switch linking SpoT-dependent stress response to fatty acid metabolism. *Mol Microbiol* **62**, 1048-63 (2006).
4. Miot, M. et al. Species-specific collaboration of heat shock proteins (Hsp) 70 and 100 in thermotolerance and protein disaggregation. *Proc Natl Acad Sci U S A* **108**, 6915-20 (2011).
5. PyMOL. in The PyMOL Molecular Graphics System (Schrodinger, LLC, 2006).
6. Bertelsen, E.B., Chang, L., Gestwicki, J.E. & Zuiderweg, E.R. Solution conformation of wild-type E. coli Hsp70 (DnaK) chaperone complexed with ADP and substrate. *Proc Natl Acad Sci U S A* **106**, 8471-6 (2009).