1 Supporting Information Lee et al. 10.1073/pnas.1712051115

2 Supporting Figures and Figure legends



3

Fig. S1 Phylogenetic analysis of ClpG/ClpG_{GI} **proteins.** ClpG/ClpG_{GI} proteins form a distinct group within the Clp protein family distant from ClpB, ClpC and other class I Clp family members (ClpA, B, C, D, E and V). The ClpG_{GI} group members located on the TLPQC-1 like genomic islands form a specific sub-group. Protein sequences were aligned with ClustalX2 using standard parameters and a neighbor-joining tree was built using MEGA 7.0. Branch lengths correspond to substitutions per site and branch support values are indicated in %.

	10	20	30	40	50	60	70	80	90	100
ClpG_PAER ClpG_BMUL ClpG_ILIM ClpG_L_ANAE ClpG_L_SG17M ClpG_L_RMAN ClpG_L_PSTU ClpK_KPNE ClpG_BUB CLPB_ECOLI CLPB_ECOLI CLPB_SG17M_PPA4542	MAE	MAQBLCAICH MPALCDICH MPQKLCDICQ RDAGALCDICH MARKQCQVCG MARKQCQVCG MARKQCQVCG	IERPAVARVSLU JARPATVAVTVLI JARPATVRVTVLI IRRPAAVRVTVS - OPATVRVEAN - OPATVRVEAN - OPATVRVEAN - OPATVRVEAN M	ONGORRELAL ODCERKTISI RDGORROLDW LNGRHSTMLL LNGRHSTMLL LNGRNSTMLL LNGRNSTMLL NGRHSTMLL NGRHSTMLL	CBLHYRQLMR CDYHPRQLMR CDYHYAQIAR CDYHYAQIAR CBQDYARLQA CDDHYRQLVR CDDHYRQLVR CDDHYRQLVR CDDHYRQLVR	00-RMRSPLE HO-SMLNPPD HO-RYVSPLE 00KRTVSPLE 00KRTVSPLE 00KRTVSPLE 00KRTVSPLE	SLPGGGS SLLGGGP ALVRGGM SLPGGGL ALPGSRSGL ALPGSRSGL ALPGSRSGL	PPDBIPSGPG SSLPGCLD LEDFPGQAOP FCDDIMGCLP FEDFLGSDFP FEDFLGSDFP FEDFLGSDFP FEDFLGSDFP	G- RIGDDAPSMA RIGDDAPSMA RIGEDATPVA RIGDDATPVA	ADTDE ADTDE ADTDD ADTDD ADTDD
ClpG_PAER ClpG_BMUL ClpG_ILIM ClpG _{uL_} SANAB ClpG _{uL_} SG17M ClpG _{uL} SG17M ClpG _{uL} SG17M ClpG _{uL} PMAN ClpG _{uL} PSTU ClpG_BSUB CLPB_ECOLI CLPB_SG17M_PPA4542	EQSPVTPV EQSPVTPV RASPLARE VGMPRRGP -EBCLAPRGVGRQR VVDASPGEPAPAGT VVDASPGEPAPAGT VVDASPGEPAAGS	IZU RAREPEAVDIA IPR-BSVDIT PRPBHAGVDLQ PRPBHAGVDLQ GTARRGSGLA GAPRRGSGLA GAPRRGSGLA MRL MRI	LIGO EYPSKOTTEYL DAFSEQTELL ISHFSEQAKEML ISHFSEQAKEML ISRISEQSEALL SRISEQSEALL SRISEQSEALL SRISEQSEALL ISRISEQSEALL DRLTSKLOLAL DRLTSKLOLAL	140 ORAAQVAAEP ORAAEKAHELI ORAAERAVOR OCAARAAVOR OEAARHAAEP OEAARHAAEP OEAAKHAAEP OEAAKHAAEP ALAQEBALRLI ADAQSLALGH SDAQSLAVGH	ISU CKREVDTEHL RRNELDTEHL GARDVDTEHL GRADVDTEHL GRAEVDTEHL GRSEVDTEHL GRSEVDTEHL GINNIGTEHI DNQPIEPLHI DHPAIEPVHL	Leo LYALA DAD LYALA DTD LHELS BSB LIALS DSD LLALS DSD LLALS DSD LLALA DSD	VVQAVLKOP WCAALLKEL WVQAILSRP WVQAILSRP WVKTILGOP WVKTILGOP WVKTILGOP STAKALQAL SIKPLLMQV	LISU GLSPADLKQY KLSPQDIKAY KLSPBDLKAY KLSPBDLKAQ KIKVDDLKRQ KIKVDDLKRQ KIKVDDLKRQ GLSSEKIQKE GINAGQLRTD GFDIAALRSG	IEANAVRG-A IDEHAHTCNA IDENAHTCNA IDENSPREA IESEAKRGDK IESEAKRGDK IESEAKRGDK VESLIGRGGE INOALNRLPQ LNKELDALPK	SKGEA DPDQS RVG-P KEG-R PFE PFE PFE PFE VEG-T IQS-P
	210	220	230	240	250	260	270	280	290	300
ClpG_PAER ClpG_BMUL ClpG_ILIM ClpG_LANAE ClpG_L_SANAE ClpG_L_RMAN ClpK_KPNE ClpG_L_PSTU ClpG_BSUB CLPB_ECOLI CLPB_SG17M_PPA4542	SEDMT ISPRVKSAL LEKLSISPRVKAAV RTE IGVSPRVKGAL -GE IGVSPRVKGAL -GE IGVSPRVKDAL -GE IGVSPRVKDAL -GE IGVSPRVKDAL -TIHYTPRAKKVI GGDVQPSQDLVRVL TGDVNLSQDLARLL	OHAPALSRBLC OYAPQASRDLG DOAFLISRQLG BRAPHVSRDLG SRAPVASNBLG SRAPVASNBLG SRAPVASNBLG SRAPVASNBLG BLSMDRARKLG NLCDKLAQKRG NQADRLAQQKG	HSYVGPEHLLI HSYVGPEHLLI HSYVGPEHLLI HSYVGPEHPLI HSYVGPEHPLI HSYVGPEHPLI HSYVGPEHPLV HSYVGPEHILL DDNFISSELFVL DQPISSELVLL	GLAAVPDS FA GLASVPDS IA GLAEVPDS FA GLAEVPS FA GLAEEGEGLA GLAEEGEGLA GLAEEGEGLA GLAEEGEGLA GLIREGEGVA AALESRGTLA AAMDENTRLG	GTLLKKYGLT GTLLKKYGLT GDLLRRYGLT ANLLRRYGLT ANLLRYGLT ANLLRRYGLT ANLLRRYGLT ARVINNLGVS - DILKAAGAT - KLLLGOGVS	BOALROKVX PBALROKVXX POALROQTVR POALROQTVR POALROQVSK POALROQVSK POALROQVSK POALROQVSK INKAROQVLO TANITQAIBO RKALBNAVAN	VVGKGA VVGKGA VVGKGA VVGKGA VVGKGA VVGKGA VVGKGA LLLGSNBTGS MRGGB ILRGGB	EDGRVDGPSN EDGRVDTPTG EEGRVAGPSN EEGRLEEKSD EDGRAETPTN EDGRAETPTN EDGRAETPTN SAAGTNSNAN SVNDQGAEDQ AVNDPNVEES	TPOLDKFSRD TPNLDKPGRD TPNLDKYSRD TPNLDKYSRD TPBLDKYSRD TPBLDKYSRD TPBLDKYSRD TPBLDKYSRD TPTLDSLARD RQALKKYTID RQALDKYTVD	LTRLA LTRLA LTLLA LTLLA LTKMA LTKMA LTKMA LTKMA LTKMA LTKMA LTKMA
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	710	720	730	740	750	760	770	780	790	800
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ClpG_PAER ClpG_EMUL ClpG_ILIM ClpGu_ANAE ClpGu_SG17M ClpGu_RMAN ClpK_KPNE ClpG_BSUB ClpG_BSUB CLPB_ECOLI CLPB_ECOLI	1010 AAPKPAARKKSGAGI ABAKDAKTAKQAGNA AKPWEGKAAPARRAI AAAAAPSADR PAKPAEPEKPD PAKPAEPEKPD POTPAEPEOPD PAKPAEPEOPD	1020 STPKCRAT SSAKCNGSAAA KSAKGGRT 	1030 AARKPAAKK ADGRPDGEA PAKRAAAKA RAAKBAGEP IVABTPPSDA CATETPHGDA CATETPHGDA	1040 II. GAAAKGKADK GASDGAPAEA AKTAKTTKRS AKTAKTTKRS SKPARKKKSA SKPARKKKSA GKDSSKKKSA	1050 PR-AK PPPAKKSGKKS PP-ARPKG PCRHR GGES GGES SDAS SDAS	1060 SSGARKDAP				

Fig. S2 Sequence alignment of selected AAA+ Hsp100 family proteins. Alignment of 14 selected ClpG/ClpG_{GI} homolog in comparison with *B. subtilis* ClpC and ClpB of *E. coli* and 15 P. aeruginosa SG17M. ClpG and ClpG_{GI} homolog contain an N-terminal extension with a 16 conserved 3Cys-His motif (red letters). Conserved domains present in all proteins were boxed: 17 Clp N domain (pfam02861, gray background), AAA+ ATPase domains (AAA-1, AAA-2) 18 (cd00009, yellow background) and M-domains (green background). WalkerA/B motifs are in 19 green letters; the pore loop motifs in AAA-1 and AAA-2 are in gray letters with the 20 conserved tyrosine residues (Y) in brown, sensor 1 motifs in AAA-1 and AAA-2 are in gray 21 22 letters with the conserved threonine (T) and asparagine (N) in AAA-1 and AAA-2, respectively, in orange and sensor 2 motif at C-terminal region after AAA-2 is in orange 23

letter. The VGF ClpP interaction loop of ClpC is in turquoise. ClpC, P37571.1 (*Bacillus subtilis*); ClpG_BMUL, KGC07149.1 (*Burkholderia multivorans*), ClpG_ILIM,
WP_026873947.1 (*Inquilinus limosus*); ClpG_ANAE, ABS27505.1 (*Anaeromyxobacter* sp.
Fw109-5); ClpG_{GI}_RMAN, WP_045786171.1, (*Ralstonia mannitolilytica*); ClpK_KPNE,
AFV70479.1 (*Klebsiella pneumoniae*); ClpG_{GI}_PSTU, F2MZ57 (*Pseudomonas stutzeri*).



Fig. S3 Comparative phylogenetic analysis of ClpG, ClpG_{GI} and ClpB proteins. (A) 31 Phylogenetic neighbor-joining tree of ClpG_{GI} homologs compared to the ClpB and 16S RNA 32 phylogenetic tree. (B) Unique position of ClpG between the major facilitator superfamily 33 (MFS) transporter PA0458 and a DUF2780 superfamily protein (PA0460) in P. aeruginosa. 34 The corresponding genomic region of Pseudomonas fluorescens SBW25, which is lacking 35 ClpG, is given. Genomic organizations of ClpG homologs from P. fluorescens NCIMB 36 11764, Pseudomonas mandelii JR-1 and Pseudonomas citronellolis P3B5 are different and 37 shown for comparison. Also in E. coli KTE154, the ClpG homolog is found on a putative 38 genomic island as compared with E. coli K-12 MG1655. 39



Time at 50°C (min)

Fig. S4 Complementation of *P. aeruginosa* SG17M $\triangle clpG \triangle clpG_{GI}$ -double and $\triangle clpB$ 42

 $\Delta clpG \Delta clpG_{GI}$ -triple disaggregase mutants by arabinose-induced expression of ClpG 43

and $ClpG_{GI}$. P. aeruginosa SG17M wild type and indicated mutant cells expressing clpG or 44

 $clpG_{GI}$ from the *araC* promoter (ParaC) at the chromosomal Tn7 site (*att*Tn7) were grown to 45

- stationary phase at 20°C and heat shocked at 50°C for 30 and 60 min. Cellular viabilities 46 were determined by spotting serial dilutions $(10^{-1} - 10^{-7})$ of cells on LB plates followed by
- 47 incubation at 37°C for 18 h. 48



Time at 50°C (min)

Fig. S5 ClpG/ClpG_{GI} function independent from ClpP in heat tolerance. *P. aeruginosa* SG17M wild type and indicated mutant cells expressing *clpG* or *clpG_{GI}* from the *araC* promoter (P*araC*) at the chromosomal Tn7 site (*att*Tn7) were grown to stationary phase at 20°C and heat shocked at 50°C for 30 and 60 min. Cellular viabilities were determined by spotting serial dilutions ($10^{-1} - 10^{-7}$) of cells on LB plates followed by incubation at 37°C for 18 h.





60 Fig. S6 Total protein of *P. aeruginosa* SG17M cells lacking ClpB and ClpG/ClpG_{GI}. Total protein content loading controls for estimation of isolated protein aggregates in P. 61 aeruginosa strains (Fig. 2) analyzed by SDS-PAGE. (A) P. aeruginosa SG17M and mutants 62 were cultured at 42°C for 24 h. (B) P. aeruginosa SG17M and mutants were grown at 30°C 63 overnight (1), incubated at 42°C for 60 min (2), and then shifted to 30°C for 60 (3) and 120 64 (4) min for recovery. 65



Fig. S7 Oligomerization of ClpG, ClpG_{GI} and respective $\Delta N2$ deletion mutants. 70 Oligomerization studies of 10 µM ClpG and ClpG_{GI} wild type and indicated deletion mutants 71 were conducted using a Superdex 200 10/300 GL size exclusion column. Individual runs 72 were performed in the absence or presence of nucleotide (2 mM ATP) in assay buffer (50 73 mM Tris pH 7.5, 150 mM KCl, 20 mM MgCl₂, 2 mM DTT) at room temperature. For 74 visualization the fractions were collected and analyzed by SDS-PAGE and subsequent 75 SYPRO Red staining. A molecular weight marker is shown and the elution volumes of 76 77 standard proteins are indicated.

78



Fig. S8 Analysis of ClpG_{GI} particles by transmission electron microscopy (TEM). (A) 81 Oligomerization of E. coli K-12 ClpB and P. aeruginosa SG17M ClpB and ClpG_{GI}. An 82 oligomeric protein structure for ClpB and ClpG_{GI} was observed by TEM. E. coli ClpB 83 showed a shift from monomer to apparent hexamer formation in the presence of 2 mM ATP. 84 Formation of hexamers by *P. aeruginosa* ClpB is induced by ATP with hexamers already 85 present before the addition of ATP. Oligomerization of ClpG_{GI} is hardly induced upon 86 addition of ATP under the same condition. Samples were incubated with 2 mM ATP at room 87 temperature for 10 min and subsequently subjected to TEM. (B) Upon incubation with 88 ATPyS, monodispersed molecules with roughly hexagonal top-views (circles) and bipartite 89 side-views (arrows) can be recognized in the negatively stained sample by TEM. However, a 90 significant background of low-mass proteins exists. The diameter of top-view molecules 91 92 ranges from 12.2 nm to 18.6 nm with a mean of 14.6 nm \pm 1.4 nm (N=56). The height, as measured from side-view projections reaches a mean of 9.75 nm \pm 0.86 nm (N=89) within a 93 range from 7.03 nm to 11.82 nm. (C) The appearance of class averages (left) and views of 94 their back projections (right) sufficiently coincide at the base of the resolution reached. For 95

- 3D-reconstruction of ClpG_{GI} molecules 1897 particles have been chosen and a first reference-
- 97 free class average was calculated.



Fig. S9 Modeling of the ClpG_{GI} hexameric structure. (A/B/C) Top, side and bottom view of the electron density model of ClpG_{GI}. The top-view shows a closed surface, while the bottom-view with its C-termini shows an open molecule. The model diameter is 13.6 nm versus 10.3 nm as model height, given a height/diameter ratio of 0.76 and thus appears smaller relative to direct measurements on negatively stained molecules (see Fig. S8). Assessment of the ClpG_{GI} density model by semi-automatically fitting of X-ray data of homologous *E. coli* ClpB (PDB code: 4D2Q) into the model.



Fig. S10 KJE-dependent disaggregating activities of *P. aeruginosa* and *E. coli* ClpB and 109 stand-alone disaggregating activity of P. aeruginosa ClpG. (A) ClpB of P. aeruginosa 110 SG17M and E. coli K-12 show KJE-dependent disaggregation activity. E. coli DnaK (EcKJE) 111 or P. aeruginosa DnaK (PaK-EcJE) together with E. coli DnaJ and GrpE cooperate with E. 112 coli ClpB (EcClpB) and P. aeruginosa ClpB (PaClpB) in the refolding of heat-aggregated 113 Malate Dehydrogenase (MDH). Negative: no addition of disaggregating chaperones. All 114 reactions were performed in the presence of GroES/EL. (B) ClpG exhibits comparable 115 disaggregating activity to ClpG_{GI}. Refolding of heat-aggregated luciferase was monitored in 116 117 presence of ClpG or ClpG_{GI}. After 90 min of luciferase disaggregation ClpG activity corresponds to 90% of the activity of ClpG_{GI}. (C) Refolding of aggregated luciferase was 118 119 monitored in absence or presence of PaDnaK-EcDnaJE(PaK-JE) at 0 and 0.5 µM of ClpG_{GI}.

- 120 (D) Total intensities of protein bands below $ClpG_{GI}$ were quantified by ImageJ for each lane
- 121 of the SDS-PAGE (see Fig. 3D). *, p < 0.05; **, p < 0.01.



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Fig. S11 Soluble fraction of *E. coli dnaK103* mutant cells containing an empty vector (p) or expressing $clpG_{GI}$ (p $clpG_{GI}$). Cells were grown at 30°C in the presence of 250 µM IPTG and shifted to 45°C for 20 min followed by a recovery period at 30°C. Soluble protein fractions (Fig. 4E) were isolated at the indicated time points and analyzed by SDS-PAGE. p=pUHE21; p $clpG_{GI}$ = $clpG_{GI}$ cloned in pUHE21.



Fig. S12 Effect of guanidine hydrochloride (GuHCl) on Hsp104, ClpG and ClpG_{GI} and 132 the prion phenotype in Saccharomyces cerevisiae upon expression of ClpG_{GI}. (A) 133 Disaggregation and refolding of urea-denatured luciferase was determined for Hsp104 (in 134 presence of cooperating yeast Hsp70 system: Ssa1/Ydj1/Sse1) and ClpG/ClpG_{GI} in absence 135 and presence of 5 mM GuHCl. Disaggregation activities of Hsp104, ClpG and ClpG_{GI} 136 determined in absence of GuHCl were set at 100% (***, p<0.001). (B) S. cerevisiae 779-6A 137 and 1075 cells (without or with a ClpG_{GI}-expression plasmid), reporting on [PSI+] and 138 [URE3] prion phenotypes, respectively, were grown in 3 mM guanidine hydrochloride to 139 inhibit Hsp104 activity. The prion states after Hsp104 inhibition were monitored on plates 140 with limiting adenine medium (Lim. Ade) and on adenine depletion medium (-Ade). 141 142



Fig. S13 Basal ATPase activities of *P. aeruginosa* SG17M ClpG_{GI} and its double Walker
B mutant. ClpG_{GI} exhibits similar basal ATPase activity as ClpG, while the double Walker B

mutant ClpG_{GI E383A E723A} has lost the ATPase activity (**, p<0.01; ***, p<0.001; compared to ClpG_{GI}).

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Fig. S14 Expression levels of ClpB, ClpG and ClpG_{GI} and respective N-terminal deletion variants. P. aeruginosa SG17M $\triangle clpB \ \triangle clpG \ \triangle clpG_{GI}$ mutant cells containing pJN105 plasmid derivatives expressing full-length or N-terminally shortened ClpB, ClpG, ClpG_{GI} were subjected to SDS-PAGE analysis. Full-length and truncated ClpB, ClpG and $ClpG_{GI}$ are marked by arrows. p=pJN105.



Fig. S15 Secondary structure analysis of ClpG, Δ N2-ClpG, ClpG_{GI} and Δ N2-ClpG_{GI}. Circular Dichroism Spectra were recorded from 250-190 nm at a protein concentration of 2.5 μ M in sodium phosphate buffer (50 mM, pH 7.5). The buffer control spectrum is also given and shown in black. ClpG/ Δ N2-ClpG are shown in brown/red and ClpG_{GI}/ Δ N2-ClpG_{GI} are shown in green/blue.



166 Fig. S16 MDH disaggregation activities of ClpG/ClpG_{GI} and respective $\Delta N2$ deletion

167 mutants. Refolding of heat-aggregated MDH was monitored in the presence of indicated

168 chaperones. The activity of respective full-length proteins was set as 100% (***, p < 0.001).



Fig. S17 Solubilities of ClpG, Δ N2-ClpG, ClpG_{GI} and Δ N2-ClpG_{GI} and ClpB. Indicated Hsp100 proteins were incubated with 2 mM ATP γ S in the absence of heat-aggregated

173 luciferase and soluble (Sup) and insoluble (Pellet) fractions were isolated and analyzed by

174 SDS-PAGE.

176 Supporting Tables

Table S1. Identification of *in vivo* aggregation-enhanced proteins in *P. aeruginosa*SG17M

Name	Size	Genbank accession number	Function	Strain background where
	(kDa)			the aggregate was observed
HtpG	90	WP_004349034.1	Hsp90 family chaperone	$\Delta clpB \Delta clpG \Delta clpG_{GI}$
NuoCD	70	WP_003090458.1	NADH-ubiquinone oxidoreductase, subunit CD	$\Delta clpG_{GI}$
IbpA	15	WP_003091405.1	Small heat shock protein 20	$\Delta clpB \Delta clpG \Delta clpG_{GI}$
LasI	23	WP_024082598.1	Quorum sensing protein	$\Delta clpB$

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181	Table S2. B	Bacterial st	trains use	d in	this	study
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Strain	Genotype	Source or reference
P. aeruginosa SG17M an	d derivatives	
SG17M	Wild type, Environment, river water	(1, 2)
SG17M002	SG17M Δdna -shsp20 _{GF} clpG _{GI} (previously SG17M Δdna -shsp20c-clpBc)	(3)
SG17M008	SG17M $\triangle clpG$	This study
SG17M009	SG17M $\Delta clpG_{Gl}$	This study
SG17M010	SG17M $\Delta clpB$	This study
SG17M011	SG17M $\triangle clpB \ \triangle clpG$	This study
SG17M012	SG17M $\triangle clpB \ \triangle clpG_{GI}$	This study
SG17M013	SG17M $\triangle clpB \ \triangle clpG \ \triangle clpG_{Gl}$	This study
SG17M014	SG17M attTn7	This study
SG17M015	SG17M $\Delta clpG \ \Delta clpG_{GI} attTn7::araC-clpG$	This study
SG17M016	SG17M $\triangle clpG \ \triangle clpG_{GI} attTn7::araC-clpG_{GI}$	This study
SG17M017	SG17M $\triangle clpB$ $\triangle clpG$ $\triangle clpG_{Gl}$ attTn7::araC-clpG	This study
SG17M018	SG17M $\triangle clpB$ $\triangle clpG$ $\triangle clpG_{Gl}$ attTn7::araC-clpG _{Gl}	This study
SG17M019	SG17M $\triangle clpP$	This study
SG17M020	SG17M $\triangle clpG \ \triangle clpG_{GI} \ \triangle clpP$	This study
SG17M021	SG17M $\triangle clpP$ attTn7	This study
SG17M022	SG17M $\triangle clpG \ \triangle clpG_{GI} \ \triangle clpP \ attTn7::araC-clpG$	This study
SG17M023	SG17M $\triangle clpG \ \triangle clpG_{GI} \ \triangle clpP \ attTn7::araC-clpG_{GI}$	This study
Other P. aeruginosa stra	ins	1
PAO1	Wild type, genetic reference strain, burn wound isolate, Australia, 1953	(4)
PAO1-001	PAO1 \(\Delta clpB\)	This study
PAO1-002	PAO1 $\Delta clpG$	This study
PAO1-003	PAO1 $\triangle clpB \ \triangle clpG$	This study
E. coli K-12 derivatives		
TOP10	Strain for cloning and harboring plasmids	Invitrogen
HB101 pRK2013	Helper strain for conjugation, Km ^R	(5)
DH5α λpir	Cloning and harboring plasmids of R6K origin of replication	Lab collection
DH5α λpir pTNS2	Helper strain for chromosomal integration of mini- <i>Tn7</i> element, Ap ^R	(6)
MC4100	In vivo heat shock and chaperone assay, MC4100 placIq pHSG-YFP-Luciferase	(7)
MC4100 $\triangle clpB$	In vivo heat shock and chaperone assay, MC4100 $\triangle clpB$::Km placIq pHSG-YFP-Luciferase	(7)
MC4100 dnaK103	In vivo heat shock, temperature-sensitive dnaK mutant	(8)
Yeast		
779-6A	$MAT\alpha$, kar1-1, SUQ5, ade2-1, his3 Δ 202, leu2 Δ 1, trp1 Δ 63, ura3-52	(9)
1075	$MAT\alpha$, kar1-1, P_{DALS} :: ADE2, his3 $\Delta 202$, leu2 $\Delta 1$, trp1 $\Delta 63$, ura3-52	(9)

JG26	Saccharomyces cerevisiae InvSc1strain for yeast cloning	Invitrogen

Table S3. Plasmids used in this study

Plasmid	Description	Source or reference
pEX18Tc	Gene replacement vector; ColE1 origin of replication, Tc ^R , <i>oriT</i> ⁺ , <i>sacB</i> ⁺	(10)
pSG001	pEX18Tc containing FRT-Gm ⁸ -FRT cassette cloned in <i>Bam</i> HI and <i>Sal</i> I site	(3)
pSG010	pSG001-\(\Delta clpB\), construct for clpB deletion with fragment up and downstream of clpB cloned in SacI/BamHI and PstI/HindIII	This study
pSG011	pSG001- $\Delta clpG_{Gl}$, construct for $clpG_{Gl}$ deletion with fragment up- and downstream of $clpG_{Gl}$ cloned in SacI/BamHI and	This study
	Sall/HindIII	
pMQ150	Vector for yeast cloning and gene replacement; Km ^R (selection in <i>E. coli</i>), URA3 (selection in yeast)	(11)
pSG013	pMQ150- $\Delta clpP$, construct for $clpP$ deletion, Gm cassette flanked by fragment up and downstream of $clpP$	This study
pSG014	pMQ150- $\Delta clpG$, construct for $clpG$ deletion, Gm cassette flanked by fragment up and downstream of $clpG$	This study
pMR116	Expression vector in yeast, Ap ^R , TRP1 (selection in yeast)	(12)
pSG015	pMR116 with C-terminal 6xHis tag fusion to $clpG_{GI}$ cloned in NheI and XbaI sites	This study
pUC18T-mini-	Genome insertion vector; ColE1 origin of replication, Ap ^R , Gm ^R	(6)
Tn7T-GmTn7		
pTNS2	Helper plasmid for chromosomal integration of mini-Tn7 element; Ap ^R	(6)
pSG016	pUC18T-mini-Tn7T-Gm with araC-clpG cloned in EcoRI and Kpnl sites	This study
pSG017	pUC18T-mini-Tn7T-Gm with araC-clpG _{GI} cloned in SpeI and KpnI sites	This study
pJN105	A broad-host range vector with arabinose inducible <i>araBAD</i> promoter; pBBR1origin of replication; Gm ^R	(13)
pSG018	ClpB -6xHis cloned in pJN105 in NheI and XbaI sites	This study
pSG019	ClpG -6xHis cloned in pJN105 NheI and Xbal sites	This study
pSG020	$ClpG_{Gr}$ -6xHis cloned in pJN105 in <i>Nhe</i> l and <i>Xba</i> l sites	This study
pSG021	P. aeruginosa dnaK -6xHis cloned in pJN105 in NheI and XbaI sites	This study
pSG022	P. aeruginosa 6xHis-ibpA cloned in pJN105 in NheI and XbaI sites	This study
pSG007	6xHis-shsp20 _{GI} cloned in pJN105 in NheI and XbaI sites	(3)
pSG023	$shsp20_{GI} clpG_{GI}$ -6xHis cloned in pJN105 in <i>Nhe</i> I and <i>Xba</i> I sites	This study
pSG027	$\Delta N(V149M)$ clpB-6xHis cloned in NheI and XbaI sites	This study
pSG028	Δ <i>N</i> (<i>V</i> 83 <i>M</i>) <i>clpG</i> -6xHis cloned in pJN105 in <i>Nhe</i> I and <i>Xba</i> I sites	This study
pSG029	$\Delta N(R234M)$ clpG-6xHis cloned in pJN105 in Nhel and XbaI sites	This study
pSG030	$\Delta N(S106M) \ clpG_{Gr}$ 6xHis cloned in pJN105 in <i>NheI</i> and <i>XbaI</i> sites	This study
pSG031	$\Delta N(R255M)$ clpG _{GP} 6xHis cloned in pJN105 in NheI and XbaI sites	This study
pSG032	pJN105 with C-terminal 6xHis fusion to N-terminal region of <i>clpB</i> (M1 to V149) cloned in <i>Nhe</i> I and <i>Xba</i> I sites	This study
pSG033	pJN105 with C-terminal 6xHis fusion N-terminal region of <i>clpG</i> (M1 to A82) cloned in <i>Nhe</i> I and <i>Xba</i> I sites	This study
pSG034	pJN105 with C-terminal 6xHis fusion N-terminal region of clpG (M1 to R234) cloned in Nhel and XbaI sites	This study
pSG035	pJN105 with C-terminal 6xHis fusion N-terminal region of clpG _{GI} (M1 to G105) cloned in NheI and XbaI sites	This study
pSG036	pJN105 with C-terminal 6xHis fusion N-terminal region of clpG _{GI} (M1 to R255) cloned in Nhel and Xbal sites	This study
pSG037	pJN105 $clpG_{GI E383A E723A}$ 6xHis cloned in <i>Nhel</i> and <i>Xbal</i> sites	This study
pFLP2	FRT cassette excision vector, Tc ^R	(14)
pRK2013	Helper plasmid for mobilization of non-self-transmissible plasmids, Km ^R	(5)
pUHE21	Vector for IPTG-inducible gene expression in E. coli	(8)
pUHE21-dnaK	Vector for IPTG-inducible expression of <i>dnaK</i> in <i>E. coli</i>	(8)
pUHE21-clpG _{GI}	Vector for IPTG-inducible expression of $clpG_{GI}$ in <i>E. coli</i> , cloned into <i>BamH</i> I and <i>Xba</i> I sites	This study
pET24a-clpG _{GI}	Vector for IPTG-inducible expression of $clpG_{Gl}$ in <i>E. coli</i> BL21 cloned into <i>Nde</i> I and <i>Xho</i> I sites	This study
pET24a-clpG	Vector for IPTG-inducible expression of <i>clpG</i> in <i>E. coli</i> BL21 cloned into <i>Nde</i> I and <i>Xho</i> I sites	This study
рЕТ24а- <i>ДN2</i> -	Vector for IPTG-inducible expression of $\Delta N2$ -clpG _{GI} in E. coli BL21 cloned into NdeI and XhoI sites	This study
$clpG_{GI}$		

рЕТ24а- <i>ДN2</i> -	Vector for IPTG-inducible expression of $\Delta N2$ -clpG in E. coli BL21 cloned into NdeI and XhoI sites	This study
clpG		

187 Table S4. Primers used in this study

Primer	Sequence ¹	Purpose	Source or reference
clpB Up F SacI	5' G <u>GAGCTC</u> GACTGAGCTAACGACCCAAG 3'	Construction of a <i>clpB</i> -deletion mutant (upstream region)	This study
clpB Up R BamHI	5' CGC <u>GGATCC</u> GTTCGGCACAGATCCAGGAGCTG 3'	Construction of a <i>clpB</i> -deletion mutant (upstream region)	This study
clpB Dn F PstI	5' GCG <u>CTGCAG</u> AGCTTGCTGGTCAAACGGTCTATTC 3'	Construction of a <i>clpB</i> -deletion mutant (downstream region)	This study
clpB Dn R HindIII	5' CGC <u>AAGCTT</u> CTGCTCAGCCTGTTGCGCCAGG 3'	Construction of a <i>clpB</i> -deletion mutant (downstream region)	This study
$clpG_{GI}$ Up F SacI	5' G <u>GAGCTC</u> CTTGCAAGAACTTCCTCAGG 3'	Construction of a $clpG_{Gl}$ -deletion mutant (upstream region)	This study
clpG _{GI} Up R BamHI	5' CGC <u>GGATCC</u> AGACTTGGCATTGTTTTCTGGC 3'	Construction of a $clpG_{Gl}$ -deletion mutant (upstream region)	This study
$clpG_{GI}$ Dn F SalI	5' GCG <u>GTCGAC</u> AAGTCAGCGGGCGGCGAATCT 3'	Construction of a $clpG_{Gl}$ -deletion mutant (downstream region)	This study
$clpG_{GI}$ Dn R HindIII	5' CGC <u>AAGCTT</u> CACGAAGTAAGGTGTCCAGATG 3'	Construction of a $clpG_{Gl}$ -deletion mutant (downstream region)	This study
ClpP Up F	5' GTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGCTGGAAGACATCAAGGTCGAG 3'	Construction of a <i>clpP</i> -deletion mutant (yeast cloning)	This study
ClpP Up R	5' AAGAGTTGCGAGACATGTCTTG 3'	Construction of a <i>clpP</i> -deletion mutant (yeast cloning)	This study
ClpP Gm F	5' TCTGTTAGGG AGTGATCGCA AGACATGTCT CGCAACTCTTCGAATTAGCTTCAAAAGCGCTCTGA 3'	Construction of a <i>clpP</i> -deletion mutant (yeast cloning)	This study
ClpP Gm R	5' CTTGGTCGGGTCACTTGGCCTTCGGCTTGTCGGCCTTGCCCGAATTGGGGATCTTGAAGTTCCT 3'	Construction of a <i>clpP</i> -deletion mutant (yeast cloning)	This study
ClpP Dn F	5' GACCTGGCCGTCTAAGGCTC 3'	Construction of a <i>clpP</i> -deletion mutant (yeast cloning)	This study
ClpP Dn R	5' CAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGCACTTCGGCATTGAAACCGATG 3'	Construction of a <i>clpP</i> -deletion mutant (yeast cloning)	This study
ClpG Up F	5' GTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGCTTCCAGGGCGATGGAGATCAG 3'	Construction of a <i>clpG</i> -deletion mutant (yeast cloning)	This study
ClpG Up R	5' AGATAGCGCAAAGTTCCTGGGC 3'	Construction of a <i>clpG</i> -deletion mutant (yeast cloning)	This study
ClpG Gm F	5' GA AACAGGAGTCCTCCCATGGCCCAGGAACTTTGCGCTATCTCGAATTAGCTTCAAAAGCGCTCTGA 3'	Construction of a <i>clpG</i> -deletion mutant (yeast cloning)	This study
ClpG Gm R	5' CTTGGTCGGGTCACTTGGCCTTCGGCTTGTCGGCCTTGCCCGAATTGGGGATCTTGAAGTTCCT 3'	Construction of a <i>clpG</i> -deletion mutant (yeast cloning)	This study
ClpG Dn F	5' GGCAAGGCCGACAAGCCGAAG 3'	Construction of a <i>clpG</i> -deletion mutant (yeast cloning)	This study
ClpG Dn R	5' CAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGTGATCGTCGACATCCAGAC 3'	Construction of a <i>clpG</i> -deletion mutant (yeast cloning)	This study
pMQ150 F	5' CTGGCGAAAGGGGGATGTG 3'	Confirmation of positive clones of pMQ105	This study
pMQ150 R	5' GTTGTGTGGAATTGTGAGCGG 3'	Confirmation of positive clones of pMQ105	This study
$\Delta ClpB$ confirm F	5' GCCAACTGCTCTACCGACTG 3'	Confirmation of the <i>clpB</i> -deletion mutant	This study
∆ClpB confirm R	5' CACATGAGCCATATCGGCTATC 3'	Confirmation of the <i>clpB</i> -deletion mutant	This study
ΔClpG confirm F	5' GAACAGGTTGCCGAGGATGC 3'	Confirmation of the <i>clpG</i> -deletion mutant	This study
∆ClpG confirm R	5' CAGTCGCCCTATCGCCACCTG 3'	Confirmation of the <i>clpG</i> -deletion mutant	This study
$\Delta ClpG_{GI}confirmF$	5' GACTTTCGGCATGTTAGGCG 3'	Confirmation of the $clpG_{GF}$ deletion mutant	This study
$\Delta ClpG_{GI} \text{ confirm } R$	5' CGAAGTAAGGTGTCCAGATG 3'	Confirmation of the $clpG_{G\Gamma}$ deletion mutant	This study

ΔClpP confirm F	5' CGATGAGTGTCATTCGTCAG 3'	Confirmation of the <i>clpP</i> -deletion mutant	This study
ΔClpP confirm R	5' TCCGGACGGAATTCGAGATC 3'	Confirmation of the <i>clpP</i> -deletion mutant	This study
PglmS-up	5' CTGTGCGACTGCTGGAGCTGA 3'	Confirmation of the genome integration	(15)
PglmS-down	5' GCACATCGGCGACGTGCTCTC 3'	Confirmation of the genome integration	(15)
ClpB pJN F NheI	5' GCA <u>GCTAGC</u> CTATAGAAGGAAGGACGACC 3'	Cloning <i>clpB</i> -6xHis for expression	This study
ClpB-His pJN R XbaI	5' CGC <u>TCTAGA</u> TCAGTGATGATGATGATGATGGGGCGAAGACGATCTCGTCGC 3'	Cloning <i>clpB</i> -6xHis for expression	This study
ClpG pJN F NheI	5' GCA <u>GCTAGC</u> CCTTCCGTGGAAACAGGAGTC 3'	Cloning <i>clpG</i> -6xHis for expression	This study
ClpG-His pJN R XbaI	5' CGC <u>TCTAGA</u> TCAGTGATGATGATGATGATGCTTGGCCTTCGGCTTGTCG 3'	Cloning <i>clpG</i> -6xHis for expression	This study
sHsp20 _{GI} pJN F NheI	5' GCA <u>GCTAGC</u> CTTCTGGAGGTGTGACATGGA 3'	Cloning $shsp20_{GF}clpG_{GF}$ 6xHis for expression	This study
ClpG _{GI} pJN F NheI	5' CGCG <u>GCTAGC</u> CTTTACAGGAGCATCAGCATGGCCA 3'	Cloning $clpG_{G}$ -6xHis for expression	This study
ClpG _{GI} -His pJN R XbaI	5' GCA <u>TCTAGA</u> TCAGTGATGATGATGATGATGAGATTCGCCGCCCGCTGACTTC 3'	Cloning $clpG_{GI}$ -His/shsp20 _{GI} -clpG _{GI} -6xHis for expression	This study
DnaK pJN F NheI	5' GCA <u>GCTAGC</u> CAAAGTTTCTGGAGAGTGAAT 3'	Cloning dnaK-6xHis for expression	This study
DnaK-His pJN R XbaI	5' CGC <u>TCTAGA</u> TTAGTGATGATGATGATGATGCTTGTTGTCCTTGACCTCTTC 3	Cloning dnaK-6xHis for expression	This study
ClpB V149M pJN F NheI	5' GCA <u>GCTAGC</u> CTATAGAAGGAAGGACGACCCATGAACGACCCGAACGTCGAGGAGTC 3'	Cloning ΔN - <i>clpB</i> for expression	This study
ClpG R234M pJN F NheI	5' GCA <u>GCTAGC</u> CCTTCCGTGGAAACAGGAGTCCTCCCATGGTGGACGGCCCGAGCAACACTC 3'	Cloning $\Delta N1$ - <i>clpG</i> for expression	This study
ClpG V83M pJN F NheI	5' GCA <u>GCTAGC</u> CCTTCCGTGGAAACAGGAGTCCTCCCATGGACATCGCCGAGTACTTCAG 3'	Cloning $\Delta N2$ - <i>clpG</i> for expression	This study
ClpG _{GI} R255M pJN F NheI	5' CGCG <u>GCTAGC</u> CTTTACAGGAGCATCAGCATGGCCGAGACGCCGACCAACAC 3'	Cloning $\Delta N1$ - <i>clpG_{GI}</i> for expression	This study
ClpG _{GI} S106M pJN F NheI	5' CGCG <u>GCTAGC</u> CTTTACAGGAGCATCAGCATGGGGGCTCGCCAGCCGTATCAG 3'	Cloning $\Delta N2$ - <i>clpG_{GI}</i> for expression	This study
ClpB V149-His pJN R XbaI	5' CGC <u>TCTAGA</u> TCAGTGATGATGATGATGATGCACCGCTTCGCCGCCACGCAG 3'	Cloning N-term of <i>clpB</i> for expression	This study
ClpG R234-His pJN R XbaI	5' CGC <u>TCTAGA</u> TCAGTGATGATGATGATGATGGCGGCCGTCCTCGGCGCCCT 3'	Cloning N-term of <i>clpG</i> for expression	This study
ClpG _{GI} R255-His pJN R XbaI	5' CGC <u>TCTAGA</u> TCAGTGATGATGATGATGATGGCGGCCATCCTCGGCCCCTT 3'	Cloning N-term of $clpG_{Gl}$ for expression	This study
araC pJN F EcoRI	5' GCG <u>AAGCTT</u> GATTCGTTACCAATTATGAC 3'	Amplifying <i>araC</i> and <i>araBAD</i> promoter	This study
araC pJN F SpeI	5' GCGC <u>ACTAGT</u> GATTCGTTACCAATTATGAC 3'	Amplifying <i>araC</i> and <i>araBAD</i> promoter	This study
ClpG-His mini R KpnI	5' CGC <u>GGTACC</u> TCAGTGATGATGATGATGATGCTTGGCCTTCGGCTTGTCG 3'	Cloning araC-clpG-6xHis for single copy complementation	This study
ClpG _{GI} -His mini R KpnI	5' CGC <u>GGTACC</u> TTAGTGATGATGATGATGATGATGAGATTCGCCGCCCGCTGACTTC 3'	Cloning araC-clpG _{GF} 6xHis for single copy complementation	This study
ClpG _{GI} pMR F EcoRI	5' TATATT <u>GAATTC</u> ATGGCCAGAAAACAATGCCAAG 3'	Cloning <i>clpG_G</i> -6xHis into pMR116 vector for yeast	This study
ClpG _{GI} -His pMR R XhoI	5' GCGC <u>CTCGAG</u> TTAGTGATGATGATGATGATGAGATTCGCCGCCGCCGCTGACTTC 3'	Cloning <i>clpG_{GF}</i> 6xHis into pMR116 vector for yeast	This study
clpG _{GI} -BamHI-A	5' GGCCAT <u>GGATCC</u> ATGGCCAGAAAACAATGCCAAGTCTGC 3'	Cloning $clpG_{GI}$ into pUHE21 for expression in <i>E. coli</i>	This study
clpG _{GI} -XbaI-B	5' GGCCAT <u>TCTAGA</u> TCAAGATTCGCCGCCCGCTGACTTCTTC 3'	Cloning $clpG_{GI}$ into pUHE21 for expression in <i>E. coli</i>	This study
ClpG _{GI} E383A F	5' GATTCTCTTCATCGACGCCGTGCACACCATCGTCG 3'	Amino acid replacement E383A in ClpG _{GI}	This study

ClpG _{GI} E383A R	5' CGACGATGGTGTGCACGGCGTCGATGAAGAGAATC 3'	Amino acid replacement E383A in ClpG _{GI}	This study
ClpG _{GI} E723A F	5' CGTGTTGCTGCTGGACGCCATCGAGAAGGCTCACC 3'	Amino acid replacement E723A in ClpG _{GI}	This study
ClpG _{GI} E723A R	5' GGTGAGCCTTCTCGATGGCGTCCAGCAGCAACACG 3'	Amino acid replacement E723A in ClpG _{GI}	This study

¹Restriction sites are underlined

189 Supporting experimental procedures

190 Strains, plasmids and growth condition

- 191 The aquatic isolate *P. aeruginosa* SG17M was selected as the representative clone C strain.
- All strains, plasmids and primers used in this study are listed in tables S2–S4. If not otherwise specified, cells were grown in LB medium at 37°C containing appropriate antibiotics with
- specified, cells were grown in LB medium at 37° C containing appropriate antibiotics with agitating speed 200 rpm. For *P. aeruginosa* SG17M, gentamicin (Gm) was used at 30 µg
- ml^{-1} and tetracycline (Tc) at 70 µg ml⁻¹. *Escherichia coli* TOP10 was used for cloning and
- retaining of plasmids requiring Gm at 30 μ g ml⁻¹, Tc at 20 μ g ml⁻¹, kanamycin (Km) at 25
- $\mu g m l^{-1}$ and ampicillin (Ap) at 100 $\mu g m l^{-1}$ for plasmid propagation.
- 198

199 Mutant construction

- 200 All primers are described in supplemental table S4. Briefly, in-frame-deletion mutants were
- 201 constructed in *E. coli* TOP10 by cloning flanking regions of the target genes into pSG001
- (Table S3), a derivative of pEX18Tc harboring a FLP-excisable Gm cassette (3). Constructed
 vectors were introduced into SG17M by triparental mating aided by *E. coli* HB101 pRK2013.
- vectors were introduced into SG17M by triparental mating aided by *E. coli* HB101 pRK2013.
 Selection was on citrated-based Vogel-Bonner minimal medium (VBMM) containing Gm
- 205 (16).
- The gene replacement vectors to construct clpP and clpG in-frame-deletion mutants were assembled by yeast cloning. The flanking regions of target genes and the Gm cassette were
- amplified by specific primers (Table S4) which overlap with vector pMQ150 and each other
- for subsequent recombination in yeast strain JG26 (Table S2) (10, 11). The suicide vector
- 210 pMQ150 was digested by BamHI and SalI. Restricted vector and all three amplified
- fragments were transformed into JG26 using the lithium acetate method (17). Positive
- colonies were screened by colony PCR with primers pMQ150 F and pMQ150 R (Table S4).
 Recombinant plasmids from positive colonies were isolated by ZymoprepTM Yeast Plasmid
- 213 Recombinant plasmids from positive colonies were isolated by ZymoprepTM Yeast Plasmid 214 Miniprep II kit (Zymo Research), transformed into DH5 α λ pir, which was subsequently used
- as the conjugal donor strain. The final constructs were verified by sequencing.
- as the conjugal donor strain. The final constructs were verified by sequencing. The final constructs were verified by sequencing.
- To identify double crossover events, Tc^{S} and Gm^{R} conjugants were selected and streaked for
- single colony. The pFLP3 plasmid was used for Flp-mediated excision of the Gm marker
 following established procedures (16). Deletion mutants were confirmed by PCR using
 primers outside of the region of potential homologous recombination.
- 220

221 **Bioinformatic analysis**

- Protein sequences of ClpG (acc. no.: EWH25562), ClpG_{GI} (acc. no.: EWH27925) and ClpB
 (acc. no.: EWH24017) of *P. aeruginosa* SG17M (3) were used as queries to search ClpG,
 ClpG_{GI} and ClpB homologs in NCBI databases by BLASTP using standard parameters. *Cupriavidus necator* (ClpG, WP_013952998; ClpB, WP_013957113; 16s rRNA, AF191737), *Ralstonia* sp. (ClpG, WP_010809302; ClpB, SDO57215; 16s rRNA, AY741342), *Paraburkholderia fungorum* (ClpG, WP_028194728; ClpB, WP_074763873; 16s rRNA,
 AF215705), *Burkholderia territorii* (ClpG, KWA28617; ClpB, WP_060105492; 16s rRNA,
- 229 LK023503), P. citronellolis (ClpG, AMO76619; ClpB, WP 061560717; 16s rRNA, Z76659),
- 230 P. fluorescens (ClpG, WP_031318856; ClpB, KTC62816; 16s rRNA, D84013), P. mandelii

(ClpG, WP 042932128, ClpB, SDU43686; 16s rRNA, AF058286), E. coli (ClpG, 231 WP 063856138; ClpB, ESE24594; 16s rRNA, X80725), Serratia sp. (ClpG, WP 006327187; 232 ClpB, WP 006323719; 16s rRNA, AJ233431), P. aeruginosa (16s rRNA, HE978271), I. 233 limosus (ClpG, WP 026873947; ClpB, WP 026872601; 16s rRNA, AY043374), Paracoccus 234 versutus (ClpG, WP 036750696; ClpB, WP 036753552; 16s rRNA, AY014174), 235 Chthonomonas calidirosea (ClpG, CEK12415; ClpB, WP 016481690; 16s rRNA, 236 AM749780), Candidatus Acetothermus autotrophicum (ClpG, BAL59480; ClpB, BAL58994; 237 16s rRNA, AP011801), Dictyoglomus turgidum (ClpG, WP 012583264; ClpB, 238 WP 012583832; 16s rRNA, NR 074885), Anaeromyxobacter sp. (ClpG, ABS27505; ClpB, 239 WP 041448603; 16s rRNA, NR 074927) are the Acc. No. of protein sequences used in Fig. 240 1C. The CDART database was searched for proteins with identical domain structure. Proteins 241 homologous over the entire length of the sequence (>93%) were considered. These database 242 searches retrieved ClpG homologs (including ClpG_{GI} members) with an identity/homology 243 down to 63/80%. There was a clear distinction to the next class of proteins, which showed 244 identity of 53 % or less over <90% of protein length. Within their subgroup, ClpG_{GI} proteins 245 were as low as 92/94% identical/homologous. On most circumstances, not more than one 246 protein homolog from each genus was selected and most distant members were considered. 247 Proteins were aligned using ClustalX2 using standard parameters (18). Representative protein 248 sequences of experimentally characterized class I Clp proteins, ClpV, ClpA, ClpL, ClpD, 249 ClpE and ClpC were included. The aligned sequences were subjected to phylogenetic 250 analysis by applying neighbor-joining, maximum likelihood and maximum parsimony 251 method in MEGA7.0 (19) with essentially the same results. The protein distance matrices 252 were calculated by using the PROTDIST program from the PHYLIP package, with the 253 Dayhoff PAM matrices/Poisson as amino acid replacement models. The tree was drawn with 254 the Treeview program. The robustness of the phylogenetic tree topologies was evaluated by 255 bootstrap analysis with 1000 replications in MEGA7.0. 256

257

258 Plasmid construction and single-copy complementation

Genes were cloned into pJN105 with a 6xHis tag at the C terminal end (Table S3, 4) (13). 259 Subsequently, *clpG* open reading frames including *araC* and *araC*-promoter were amplified 260 (primers described in Table S4) and cloned into the *Bam*HI and *Kpn*I site of the multiple 261 cloning site of the pUC18T-mini-Tn7T-Gm vector for single copy complementation on the 262 chromosome. The vector and pTNS2 helper plasmid encoding the TnsABCD transposase 263 subunits were co-conjugated into SG17M aided by E. coli HB101 containing pRK2013 264 (Table S2). Gm^R conjugants were selected and chromosomal integration was confirmed by 265 PCR with primers PglmS-up and PglmS-down (Table S4). Excision of the Gm marker was 266 obtained with the pLFP3 plasmid (16). The double Walker B mutant ClpG_{GLE383A E723A} was 267 constructed by site-directed mutagenesis (Table S2, S4). The double Walker B mutant of the 268 ClpB protein binds to ATP and forms hexamers but does not hydrolyze ATP (20). 269

270

271 Heat shock tolerance assay

P. aeruginosa cells incubated in LB broth with shaking at 200 rpm at 20°C and 37°C were harvested from logarithmic ($OD_{600} = 0.7$) and stationary ($OD_{600} = 2.5$) phase. For

examination of the impact of the N-terminal region of ClpB and ClpG proteins, cells 274 harboring the cloned gene on pJN105 were grown at 37°C for 24 h in LB broth and 0.1% L-275 arabinose was added at 6 h. E. coli K-12 strains were grown at 30°C and 0.1% L-arabinose or 276 100 μ M IPTG was added at OD₆₀₀ = 0.2 with heat treatment at OD₆₀₀ = 0.6. A cell 277 suspension of 500 µl was exposed to 50°C for 10, 30 and 60 min and a control cell 278 279 suspension was kept on ice. Cell viability after exposure to the lethal heat shock was determined by the spotting assay. 5 µl of 10-fold dilutions were spotted onto a LB plate. 280 Growth was assessed after 12–14 h of incubation at 37°C or 24 h at 30°C. 281

282

283 **Production of antisera**

Polyclonal antibodies were raised against purified *P. aeruginosa* ClpB, ClpG and ClpG_{GI} in rabbits by Davids Biotechnologie GmbH.

286

287 Western blot analysis

As for heat shock analysis, P. aeruginosa cells incubated in LB broth with shaking at 20°C 288 were harvested from logarithmic ($OD_{600} = 0.7$) and stationary ($OD_{600} = 2.5$) phase. Cell 289 lysates with equal protein content, as assessed by Coomassie brilliant blue staining of protein 290 gels, were subjected to SDS-PAGE (4% stacking and 10% separating gel) and subsequently 291 292 blotted onto polyvinylidene difluoride membrane (Millipore). As loading control, the membrane was stained with Ponceau S and then incubated in blocking solution (1 × Tris-293 buffered saline (TBS) pH 7.4, 0.01% Tween 20, 5% skim milk powder) overnight at 4°C. 294 After washing, ClpG, ClpG_{GI} and ClpB were detected with anti-ClpG, anti-ClpG_{GI} and anti-295 296 ClpB antiserum at 1:500 000, 1:1 000 000 and 1:2 000 000 dilution, respectively, and with 1:4000 goat anti-rabbit secondary antibody (Jackson Immuno Research, UK). Bound 297 antibody was visualized with enhanced chemiluminescence (ECL) detection reagent (Roche). 298 Purified proteins were used as a concentration control. Detection of protein production was 299 performed at least twice in independent experiments. 300

301

302 Protein purification

Full length C-terminal 6xHis-tagged ClpB, DnaK, ClpG, ClpG_{GI} and deletion variants of 303 ClpB, ClpG and ClpG_{GI} were expressed from pJN105 (Table S3) (13) in P. aeruginosa 304 SG17M derivatives or in E. coli BL21 cells. SG17M derivatives or E. coli BL21 were grown 305 to mid-exponential phase (OD₆₀₀ = 0.5) in LB broth at 37°C (exceptional, ClpG harboring) 306 strain was grown at 42°C). Protein expression was induced by adding 0.1% L-arabinose or 307 500 µM IPTG (in case of E. coli BL21). Cells were grown for an additional 4 h, harvested 308 and resuspended in binding buffer (20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM 309 NaCl). After disruption by sonication or French press (BL21), cell debris was removed by 310 centrifugation at 15 000 g for 30 min and the protein was purified according to standard 311 procedures using Ni-NTA columns (Qiagen) or Ni-IDA (Macherey-Nagel). ClpG proteins 312 purified from E. coli were additionally subjected to Superdex S200 size exclusion 313 chromotography in assay buffer (50 mM Tris-Cl pH 7.5, 150 mM KCl, 20 mM MgCl₂, 2 mM 314 DTT). The protein concentration was determined by using Bradford reagent, and purity was 315 assessed by SDS-PAGE. E. coli ClpB, DnaK, DnaJ, GrpE, GroEL and GroES were purified 316

- 317 as described (21, 22).
- 318

319 Transmission electron microscopy (TEM)

Protein samples of ClpB and ClpG_{GI} were prepared for energy-filtered transmission electron 320 microscopic (EF-TEM) analysis (Libra 120 plus, Zeiss, Oberkochen, Germany) and 3D-321 reconstruction was performed (23). 20 µl (0.5 mg/ml) of E. coli/P. aeruginosa ClpB proteins 322 and $ClpG_{GI}$ was incubated with 2 mM ATP/ATP γ S at ambient temperature for 10 min. 180 µl 323 of assay buffer were added to result in 50 µg/ml final protein concentration and the sample 324 was immediately assessed (Fig. S8 A). ClpG_{GI} in the assay buffer with MnCl₂ replacing 325 MgCl₂ was incubated on ice for 24 h (Fig. S8 B,C). Electron micrographs were recorded with 326 327 a bottom-mount, cooled 2048x2048 CCD frame transfer camera (SharpEye; Tröndle, Wiesenmoor, Germany) at close to low dose conditions in the elastic bright field mode as 328 Zero-loss images (nominal magnification: 40,000x; slit width: 10 eV; 0.5 mrad illumination 329 aperture, 60 µm objective aperture, beam current: 1 µA), close to the Gaussian focus, i.e. 330 about 0 to 100 nm underfocus. 331

332

333 3D-reconstruction of ClpG_{GI} molecules

For ClpG_{GI} 3D-reconstruction a data set of 1897 particles was extracted from raw data, using 334 'boxer' of the EMAN2 program (24), setting the box-size to 100 pixel, which leads to a final 335 sampling size of 0.29 nm at the probe level. Reference-free classification of the data set, 336 based on the box size and the molecular protein mass, was done and refinement calculations 337 based on C1 and finally C6-symmetries were performed, running into conversion within 12 338 cycles, which led to the final 3D-density model of $ClpG_{GI}$. With the aid of Chimera-1.8 (25), 339 340 X-ray model data, i.e. a highly analogous E. coli ClpB mutant E432A (PDB code: 4D2Q; (26)), were semi-automatically fitted into the electron microscopic density map, which was 341 median-filtered with two iterations and was set to a level of 0.0283 within a range of -0.044 342 to 0.0446, using voxel size 0.33 nm. Further modeling and analytical work were done 343 accordingly. 344

345

346 Size exclusion chromatography

To estimate the molecular mass and oligomeric state, ClpG_{GI}, ClpG and N-terminal deletion 347 variants were subjected to size exclusion chromatography (SEC) using a Superdex 200 348 10/300 GL (GE Healthcare) equilibrated with assay buffer. 10 µM of proteins was 349 preincubated +/- 2 mM ATP for 5 min at room temperature and column-applied in assay 350 buffer +/- 2 mM ATP at room temperature, respectively, as a running buffer. Since ATP in 351 the running buffer interferes with protein detection, eluted fractions were analyzed by SDS-352 PAGE. Blue-dextran, thyroglobulin, ferritin, aldolase, conalbumin and ovalbumin were used 353 as molecular mass standards. 354

355

356 Circular Dichroism (CD)

CD measurements were performed in 50 mM Na phosphate (pH 7.5) at 20°C by using a Jasco

- $_{358}$ J-175 Spectropolarimeter using 2.5 μ M ClpG/ClpG_{GI} and N-terminal deletion mutants.
- 359

360 In vitro disaggregating activity assay

Disaggregating activity was determined as described with minor modification (27). Malate 361 dehydrogenase (MDH; Roche) and luciferase (Roche) were used as model substrates. 1 µM 362 of MDH and 0.1 µM of luciferase in assay buffer were heat-denatured at 47°C for 30 min and 363 at 45°C for 15 min, respectively, and incubated at room temperature for 5 min. 50 µl of 364 denatured substrate was mixed with 50 µl assay buffer containing 1 µM disaggregating 365 chaperone and the ATP-generating system (2 mM ATP, 3 mM phosphoenolpyruvate and 20 366 ng/µl pyruvatekinase final concentration in 100 µl of reaction volume) in common and, 367 optionally, accessory proteins, 1 µM DnaK-0.2 µM DnaJ-0.1 µM GrpE (KJE) (for ClpB) and 368 1 µM each GroEL/ES (for refolding of MDH by ClpB and ClpG/ClpG_{GI}). Samples were 369 incubated at 30°C for disaggregation and refolding and samples were removed at certain time 370 points after incubation, To detect recovered MDH activity, consumption of NADH was 371 measured by monitoring decrease of absorbance at 340 nm using UV spectrophotometer 372 (Novaspec Plus, Amersham). 10 µl of sample was added into 690 µl of MDH assay buffer 373 (150 mM potassium phosphate pH 7.6, 1 mM DTT, 0.5 mM oxaloacetate, 0.28 mM NADH). 374 To assess luciferase activity, 2 µl of sample was mixed with 125 µl 2X luciferase assay buffer 375 (25 mM glycylglycin, 12.5 mM MgSO₄, 5 mM ATP) and 125 µl 25 mM luciferin (Gold 376 Biotech.) solution and activity measured by Berthold Lumat LB 9507. 100% activity 377 corresponds to the activity before heat denaturation. 378

379

380 In vivo chaperone assay

A single colony of the *E. coli* MC4100 $\Delta clpB$ strain expressing YFP-luciferase and the 381 respective chaperone (Table S2) was cultured in LB-medium at 30°C overnight, re-inoculated 382 into fresh LB medium containing 0.1% L-arabinose and 0.25 mM isopropyl-β-d-383 thiogalactopyranoside (IPTG). Cells were grown to $OD_{600} = 0.6$ and 800 µg/ml of 384 erythromycin was added to cease further translation. To heat shock, cells were incubated at 385 non-lethal 45°C for 20 min in the water bath. Afterwards, the cells were incubated at 30°C 386 for recovery and 200 µl cell suspension at 30, 60 and 90 min was used to measure luciferase 387 activity. 200 µl of 25 mM luciferin was added to monitor luciferase activity in a Berthold 388 Lumat LB 9507. 100% of luciferase activity corresponds to the activity before heat shock. 389

390

391 ATPase assay

ATPase activity was determined by a coupled ADP monitoring/recycling reaction of pyruvate 392 kinase (PK) and lactate dehydrogenase (LDH). The targeted ATPase converts the substrate 393 ATP to produce ADP. PK converts phosphoenolpyruvate (PEP) into pyruvate with ADP to 394 vield ATP. LDH converts pyruvate into lactate by oxidizing NADH to NAD⁺ which is 395 monitored by decrease of absorbance at 340 nm. ATPase activity was calculated by assuming 396 that one oxidized NAD⁺ corresponds to the production of one molecule of ADP. 1 μ M 397 ATPase was examined in 100 µl assay buffer with 5 mM NADH, 10 mM PEP (Sigma) and 398 PK/LDH (Sigma) in a 96-well plate (TPP) format. 50 µM B1 peptide (28) or 0.1 mg/ml 399 casein was added to assess stimulation of ATPase activity. 100 µl of 4 mM ATP was added to 400 each sample to start the reaction. Decrease of absorbance at 340 nm was measured by 401 FLUOstar-Omega (BMG-Labtech). 402

403 **Prion propagation test**

Propagation of [PSI⁺] and [URE3] was monitored by their ability to promote expression of 404 Ade2p using yeast strains 779-6A (*MATa*, kar1-1, SUQ5, ade2-1, his $3\Delta 202$, leu $2\Delta 1$, trp $1\Delta 63$, 405 ura3-52) and 1075 (*MATa*, kar1-1, P_{DAL5} :: *ADE2*, $his3\Delta 202$, $leu2\Delta 1$, $trp1\Delta 63$, ura3-52) (29). 406 Cells deficient in Ade2p (Ade⁻) display red on adenine-limited medium due to accumulation 407 of a metabolite of adenine biosynthesis and cannot grow on medium without adenine. Sup35p 408 catalyzes translation termination at stop codons, thus preventing translation of ade2-1 mRNA 409 containing amber mutation in 779-6A strain. Integration of Sup35p into [PSI+] prion 410 aggregates depletes Sup35p to cause transcription of full-length ade2-1 mRNA and 411 restoration of adenine production. Ure2p is a transcriptional repressor of DAL5 gene. In 1075 412 strain, the ADE2 gene is fused to the DAL5 promoter. Integration of soluble Ure2p into 413 [URE3] prion aggregates relieves Ure2p-mediated transcriptional repression and cells express 414 Ade2p to restore adenine-related phenotypes. Yeast strains containing pMR116 empty vector 415 and pMR116 $clpG_{GI}$ were cured from prions in the presence of 3 mM guanidine 416 hydrochloride on Synthetic Dextrose (SD)-Trp (Sunrise Science Products) containing limited 417 concentration of adenine (8 mg/l) (29) (Table S3). Subsequently, the cured colonies were 418 streaked on SD-TRP-limited adenine and SD-TRP-ADE plates and incubated for 3-4 days at 419 30°C. 420

421

422 **Refolding of urea-denatured luciferase**

Urea-denatured luciferase was used as substrate when determining the impact of guanidine hydrochloride (GuHCl) on Hsp104, $ClpG_{GI}$ and ClpG disaggregation activities. 4 M luciferase was denatured in 6 M urea at 30°C for 30 min and 80-fold diluted in buffer (50 mM HEPES pH 7.5, 150 mM KCl, 20 mM MgCl₂, 2 mM DTT) containing chaperones (1 M Hsp104/2 M Ssa1/1 M Ydj1/0.1 M Sse1; 1 M ClpG_{GI}; 1 M ClpG) and an ATP regenerating system in the absence or presence of 5 mM GuHCl. Luciferase activities were determined as described before.

430

431 Disaggregation of proteins in heat-denatured crude extracts

Disaggregation of proteins in heat denatured crude extracts was performed with minor 432 modifications (30). Bacterial cells were cultured at 30°C for 24 h, 10 ml cell suspension 433 collected and resuspended in 1 ml breakage buffer (50 mM HEPES pH 7.6, 150 mM KCl, 20 434 mM MgCl₂, 10 mM DTT). Cells were disrupted by sonication and the cell lysate centrifuged 435 at 15,000 g for 15 min at 4°C to acquire soluble proteins. Proteins (approx. 3 µg/µl) were 436 incubated at 42°C for 15 min in a water bath and formed aggregates isolated by 437 centrifugation at 15,000 g for 15 min at 4°C. 100 µl of aggregates were supplemented with 5 438 μ M ClpG_{GI} and 20 mM ATP in the breakage buffer at a final volume of 200 μ l. The samples 439 were incubated at 30°C for 60 min for disaggregation and refolding. After incubation, soluble 440 and insoluble proteins were separated by centrifugation at 15,000 g for 15 min at 4°C and 441 visualized with Coomassie staining after SDS-PAGE separation. 442

443

444 Isolation of *in vivo* protein aggregates

445 To assess protein aggregation *in vivo* (31), cells were cultured at 30°C for 24 h in LB medium.

Subsequently, cells were heat shocked at 42°C for 1 h and recovered at 30°C for 1 and 2 h.
Cells were collected before, during and after heat shock at various time points.

To identify substrates of disaggregating chaperones, cells were grown at 42°C for 24 h and the aggregated protein content was analyzed. 2 ml cell culture was harvested and collected

the aggregated protein content was analyzed. 2 ml cell culture was harvested and collected
 cell pellet was resuspended in 40 μl buffer A (10 mM potassium phosphate buffer pH 6.5, 1

451 mM EDTA, 20% sucrose, 1 mg/ml lysozyme) and incubated on ice for 30 min. 360 μl buffer

- B (10 mM potassium phosphate buffer pH 6.5, 1 mM EDTA) was added and cells disrupted
- by sonication. Intact cells were separated by mild centrifugation at 2,000 g for 15 min at 4°C.
 Subsequently, soluble protein was separated by centrifugation at 15,000 g for 30 min at 4°C
- and aggregated protein in the pellet was separated from the membrane associated proteins by washing twice with 400 µl buffer B containing 2% NP40. Lastly, aggregated proteins were washed with 400 µl buffer B and resuspended in 50 µl of buffer B. Total crude extract, soluble and insoluble aggregated proteins were analyzed by Coomassie staining after SDS-
- 459 PAGE.
- 460

461 *In vitro* substrate binding assay

To examine the interaction between aggregated luciferase or aggregated MDH and Clp 462 protein variants, luciferase and MDH were heat-denatured at 45°C for 15 min or 47°C for 30 463 min in assay buffer. The samples were cooled down to 25°C for 10 min. 2 µM of aggregated 464 luciferase or 4 µM of aggregated MDH were mixed with 1.5 µM ClpB/ClpG_{GI}/ClpG protein 465 or N2 subdomain deletion variants with 2 mM of ATPyS in 100 µl assay buffer. Samples 466 were incubated at 25°C for 10 min. Soluble and insoluble fractions were separated by 467 centrifugation at 13,000 rpm for 25 min at 4°C. The pellet fraction was washed once with 150 468 µl assay buffer and centrifuged again at 13,000 rpm for 10 min at 4°C. Binding assays were 469 performed in Low binding micro tubes (Sarstedt). Supernatant and pellet fractions were 470 mixed with protein sample buffer and analyzed by Coomassie staining after SDS-PAGE (8-471 16% gradient gels). Each assay was repeated three times independently. As control purified 472 Clp proteins without aggregated proteins were subjected to the same protocol. Band 473 intensities were analyzed using ImageJ. 474

475

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