SUPPLEMENTAL MATERIALS, METHODS, DATA, AND FIGURES

SUPPLEMENTAL METHODS

Bacterial cultures and protein expression

Strains and Culture Conditions

E. coli strain BL21 star (DE3) was used to express recombinant proteins. Unless otherwise stated, LB broth (5 g yeast extract, 10 g tryptone, and 5 g NaCl per liter) was supplemented with 100 µg/mL ampicillin, 100 µg/mL carbenicillin, 50 µg/mL kanamycin, or 25 µg/mL chloramphenicol. Cells were cultured at 37°C with shaking at 250 rpm. The *Yersinia* KIM5$^-$ strain was cultured in heart infusion broth (10 g beef heart infusion, 10 g tryptose, and 5 g NaCl per liter) containing the desired antibiotics. P1 transduction was used to generate the *lon::kan* disruption in the *dnaA508 tnaA*::Tn*10* parental strain.

Protein Expression and Purification

The pET28b-lon plasmid was used to overexpress *Yersinia* Lon in *E. coli* strain BL21 star (DE3). Cells were cultured in LB containing 50 µg/mL kanamycin at 37°C to optical density at 600 nm (OD_{600}) of 0.5. Protein expression was induced by 1 mM IPTG. Protein overexpression was carried out for 16 hours at 16°C. Cells were harvested by centrifugation at 3,700 xg and resuspended in buffer A (50 mM $KHPO_4$ pH 7, 1mM EDTA, 1mM DTT, and 10% glycerol). After sonication, cleared cell lysate was prepared by centrifugation at 30,000 xg. Activated and buffer A equilibrated P11-cellulose resin was added to the cleared cell lysate to allow Lon binding. The column was washed with buffer A to remove unbound proteins, and bound Lon protein was eluted in 10 mL of elution buffer B (400 mM $KHPO_4$ pH 7, 1mM EDTA, 1mM DTT, and 10% glycerol). Lon protein was further purified on a Source 15Q ion-exchange column using buffer C (50 mM Tris pH 7.5, 50 mM KCl, and 1 mM DTT). Bound Lon was eluted using a 20 column-volume linear-gradient (0-100%) of buffer D (50 mM Tris pH 7.5, 1 M KCl, and 1 mM DTT). Lon eluted at 200 mM KCl. Fractions containing Lon protease were pooled, concentrated, and loaded on S300 gel filtration column in buffer E (50 mM Tris pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 1mM DTT, 20% glycerol). Aliquots of purified Lon were flash-frozen and stored at -80°C.

HspQ and its variants were purified using a combination of Ni-NTA affinity, ion exchange, and size exclusion chromatography steps. BL21 star (DE3) harboring pET28b-hspQ plasmid was grown in LB containing 50 µg/mL kanamycin, and protein expression was induced with 1 mM IPTG at OD_{600} of 0.5. Cultures were allowed to grow for 3 h while shaking. Harvested cells were resuspended in lysis buffer (50 mM Tris pH 8, 1M NH_4Cl, 2 mM beta-mercaptoethanol (β-ME), and 10 mM imidazole). After sonication, buffer-equilibrated Ni-NTA beads were added to cleared cell lysates. After 1 h end-to-end rocking at 4°C, unbound proteins were removed and the beads were washed extensively, and the bound proteins were eluted using a step elution with lysis buffer containing 250 mM imidazole. HspQ protein containing fractions were combined, buffer exchanged to buffer F (50 mM Tris pH 8, 50 mM KCl, 2 mM β-ME), loaded on a Source15Q column, and eluted using a 20 column-volume linear-gradient (0-100%) of buffer G (50 mM Tris pH 8, 1M KCl, 2 mM β-ME). HspQ eluted at 250 mM KCl. Fractions containing HspQ were pooled, concentrated and loaded on a Superdex 75 column equilibrated in buffer H (50 mM Tris pH 8, 50 mM KCl, 2 mM β-ME). Protein aliquots containing 10% glycerol were flash-frozen and stored at -80°C.

Biochemical Assays

In vitro Proteolysis Assay

Each *in vitro* proteolysis assay reaction was carried out in Lon activity buffer (50 mM Tris-HCl pH 8, 100 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, and 10% Glycerol), ATP regeneration system (16 mM creatine phosphate, 0.32 mg/mL creatine kinase, and 4 mM ATP). Substrate and Lon hexamer (Lon_6) concentrations were as specified in figure legends. All reaction components except ATP regeneration system were mixed and incubated at 37°C. Warm ATP regeneration system was added to initiate the reaction. Aliquots at specific time were mixed with 2X-SDS sample buffer to terminate the reaction. Reaction products were resolved by electrophoresis on 15% Tris-Tricine gels and scanned using a Li-COR Odyssey scanner and quantified using the Image Studio software. Fraction of substrate remaining was estimated and the data was normalized to creatine kinase as loading control. These data were fit to a straight line and the slope was extracted to calculate the rate of degradation. For GFP assays, the circularly permuted GFP-CP6 variant (Wohlever et al., 2013) (herein referred to as GFP) was used to construct the GFP^{Y2853}

reporter, carrying the Y2853 C-terminal 20 residues, and the GFP^{sul20} reporter, carrying the SulA C-terminal 20 residues. These GFP variants were subjected to proteolysis by Lon, and the GFP protein levels were evaluated by fluorescence measurements obtained using a SpectraMax M5e microplate reader at excitation and emission wavelengths of 480 nm and 510 nm, respectively. All reaction components were pre-warmed to 37°C. Degradation rates for a range of substrate concentrations were fit to a modified Hill Equation $Y=Vmax*X^H/(K_{0.5}{}^H + X^H)$ where X is the substrate concentration, Y is degradation rate, and H is the Hill constant.

***In vitro* ATP-Hydrolysis Assay**

Coupled ATP hydrolysis assay was carried out in Lon activity buffer (50 mM Tris-HCl pH 8, 100 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, and 10% Glycerol). Reactions contained 100 nM Lon_6, 1 mM NADH, 10 U/mL lactate dehydrogenase, 20 mM phosphoenol pyruvate, 10 U/mL pyruvate kinase, and 2 mM ATP. Lon and other reaction components were warmed separately at 30°C. Reactions were initiated by adding Lon, and NADH disappearance was monitored at 340 nm.

In vivo HspQ protein detection

To monitor HspQ protein level in *Yersinia pestis* under heat-shock (45˚C), $KIM5^-$ cells were grown overnight at 25°C in heart infusion (HI) broth containing 50 µg/mL ampicillin. Cells were sub-cultured in HI to a starting OD_{600} of 0.1, and grown to OD_{600} of 0.6 at 25°C. At this point, the zero-minute sample was collected–by adding 1X SDS sample buffer to cell pellets–and the remaining culture was placed in a shaking water bath at 45°C. Aliquots containing equal number of cell were taken at indicated time points. To monitor HspQ level under T3SS inducing condition, overnight $KIM5^-$ cells were sub-cultured in T3SS permissive (HI with 20 mM $MgCl_2$, 20 mM NaOxalate) and T3SS non-permissive (HI with 2.5 mM $CaCl_2$) media at 25°C. Both cultures were grown to OD_{600} of 0.6 at 25°C. T3SS permissive cultures were switched to 37°C at the zero-minute time. To monitor HspQ protein levels, equal number of cells was removed and prepared at specified time-points. All samples were resolved by electrophoresis on 15% Tris-Tricine gels, proteins were transferred to PVDF membrane, and Western hybridization was performed using anti-HspQ serum. Blots were scanned using a Li-COR Odyssey scanner and quantified using the

Image Studio software. Data were normalized to Coomassie stained gels as loading controls. Fold change in HspQ protein level was calculated by normalizing data to the zero time-point.

In vivo proteolysis assays

In vivo HspQ stability

To monitor HspQ degradation *in vivo*, KIM5^{-} 3001 *lon*$^{+}$ and *lon*$^{-}$ strains were grown overnight at 25°C in HI media. Cells were sub-cultured in HI/amp to a starting OD_{600} of 0.1, and grown to OD_{600} of 0.6. At this point, cultures were shifted to 37°C, allowed to grow for an additional ten minutes, and 200 µg/mL chloramphenicol was added to inhibit protein synthesis. To monitor HspQ, equal number of cells was removed and samples were prepared at specified time-points. All samples were resolved on 15% Tris-Tricine gels and subjected to Western blot analysis using anti-HspQ serum. Blots were scanned using a Li-COR Odyssey scanner.

In vivo DnaA stability

To monitor DnaA508 degradation *in vivo*, *E. coli dnaA508*, its *hspQ*$^{-}$ (*dnaA508*/*hspQ*$^{-}$*),* and *lon*$^{-}$ (*dnaA508*/*lon*$^{-}$) derivatives were grown overnight at 30°C on LB/Tet plates. Cells were sub-cultured in LB/Tet and grown to OD_{600} of 0.5. Chloramphenicol at 200 µg/mL was added to stop new rounds of protein synthesis. The cultures were immediately transferred to 41˚C and equal number of cells were harvested at the indicated time points, resolved by electrophoresis on 10% Tris-Tricine gels, and subjected to Western blot analysis using DnaA antiserum. DnaA protein bands were quantified and plotted. Blots were scanned using a Li-COR Odyssey scanner.

CD Measurements

Applied Photophysics Chirascan circular dichroism spectrometer was used to perform CD wavelength scans. Measurements were performed in a 1 mm path length quarts cuvette from 190 to 260 nm in buffer containing 10 mM potassium phosphate pH 8.0, and 50 mM NaF. Three scans were averaged to record the final scans. Molar ellipticity for each protein against wavelength is presented.

Plots and statistical evaluation

GraphPad Prism software was used for data analysis. Mean and standard error of mean (SEM) was calculated by performing column statistics. Paired or unpaired one-tailed *t*-test analysis was performed and p-values were calculated from relevant data sets ($n \geq 3$). Statistical significance is reported as n.s. = not statistically significant, * significant at $p < 0.05$, ** significant at $p < 0.005$, *** significant at $p < 0.001$.

CONTACT FOR REAGENT AND RESOURCE SHARING

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SUPPLEMENTAL TABLES:

Table S1: Kinetic parameters for degradation of substrates by Lon.

Substrate	Additional HspQ	$K_{0.5}$ (μM)	Hill Constant	k_{deg} (min^{-1} Lon_6^{-1})	Catalytic Efficiency $k_{deg}/K_{0.5}$ (μM^{-1} min^{-1} Lon_6^{-1})
HspQ	-	16.6 ± 1.6	1.3 ± 0.1	10.8 ± 0.4	-
YmoA	No	29.1 ± 8.2	1.1 ± 0.1	1.3 ± 0.2	0.04 ± 0.01
YmoA	Yes	3.6 ± 0.2	2.6 ± 0.4	11.4 ± 0.3	3.2 ± 0.2

Values calculated from at least three independent experiments.
Errors in catalytic efficiency were calculated as $[1/(n-1) \sum (\text{value} - \text{mean})^2]^{1/2}$ based on (Farrell et al., 2007).

Table S2: Degradation rates of Lon substrates in absence and presence of HspQ.

Substrate	Degradation Rate (min^{-1} Lon_6^{-1}) Without HspQ	Degradation Rate (min^{-1} Lon_6^{-1}) With HspQ	Lon Specificity Enhancement With HspQ [(a)]
YmoA	0.4 ± 0.1	11.2 ± 0.6	29.5
RsuA	2.1 ± 0.1	13.4 ± 0.9	6.2
Y0390	1.2 ± 0.1	3.5 ± 0.1	2.9
Fur	0.9 ± 0.1	4.0 ± 0.1	4.6
Y2853	5.2 ± 0.1	5.9 ± 0.5	1.1
CRP	0.4 ± 0.1	0.4 ± 0.1	1.0

(a), ratio of substrate degradation rate in the presence of HspQ divided by substrate degradation rate in the presence of HspQ.

Table S3: HspQ degradation rate in the presence of Lon substrates.

Lon Substrate	HspQ Degradation Rate (min^{-1} Lon_6^{-1})
None	3.6 ± 0.2
YmoA	3.4 ± 0.5
RsuA	3.1 ± 0.1
Y0390	3.2 ± 0.2
Fur	3.0 ± 0.2
Y2853 (phase 1)	0.6 ± 0.1
Y2853 (phase 2)	3.2 ± 0.5

Values calculated from at least three independent experiments.

Table S4: Substrate stimulated Lon ATPase rates.

Lon Substrate	Lon ATPase rate (ATP min^{-1} Lon_6^{-1})
None	62.7 ± 8.1
HspQ	252.9 ± 20.7
Y2853	346.7 ± 11.4
RsuA	172.1 ± 14.2
Y0390	172.9 ± 12.5
CRP	174.9 ± 7.5
Fur	148.3 ± 11.8
YmoA	112.1 ± 8.6
HspQ + YmoA	280.1 ± 20.7

Values calculated from at least three independent experiments.

Table S5: Degradation parameters for HspQ variants.

HspQ Protein	HspQ degradation rate (min^{-1} Lon_6^{-1})	Lon ATPase rate (min^{-1} Lon_6^{-1})	YmoA degradation rate (min^{-1} Lon_6^{-1})
$HspQ^{WT}$	3.9 ± 0.2	252.9 ± 20.7	11.2 ± 0.6
$HspQ^{95}$	0.37 ± 0.04	95.3 ± 7.2	0.20 ± 0.03
$HspQ^{DD}$	0.30 ± 0.01	99.0 ± 8.6	0.20 ± 0.02
$HspQ^{K5D}$	12.02 ± 0.07	n.d.	1.38 ± 0.34
$HspQ^{K5E}$	10.09 ± 0.31	281.3 ± 14.4	n.d.
$HspQ^{K5R}$	6.20 ± 0.22	n.d.	n.d.
$HspQ^{D25N}$	3.12 ± 0.11	244.7 ± 6.2	2.23 ± 0.26
$HspQ^{D27N}$	4.41 ± 0.11	259.7 ± 11.9	1.04 ± 0.09
$HspQ^{Y10}$	5.65 ± 0.71	253.9 ± 11.4	0.21 ± 0.01
$HspQ^{Y20}$	8.25 ± 0.23	n.d.	n.d.
$HspQ^{ext}$	1.22 ± 0.05	197.8 ± 3.9	0.30 ± 0.07

Values calculated from at least three independent experiments.

Table S6: Kinetic parameters for Lon degradation of GFP with SulA and Y2853 degrons.

Substrate	$K_{0.5}$ (μM)	Hill Constant	k_{deg} (min^{-1} Lon_6^{-1})
GFP-Y20	12.4 ± 1.4	1.3 ± 0.1	3.2 ± 0.2
GFP-Sul20	13.5 ± 2.5	1.6 ± 0.3	2.9 ± 0.3

Values calculated from at least three independent experiments.

REFERENCES:

Farrell, C.M., Baker, T.A., and Sauer, R.T. (2007). Altered specificity of a AAA+ protease. Mol Cell *25*, 161-166.

SUPPLEMENTAL FIGURE LEGENDS:

Figure S1: HspQ structure and sequence conservation. (A) Multiple sequence alignment of HspQ sequences from select bacterial species was generated using Clustal Omega. Secondary structure elements are indicated above the sequence. The highlighted rectangles indicate the position of conserved residues K5, D25, D27, S93, and R95, H96, and Q97. (B) *E. coli* HspQ structure (1VBV) is shown, with the modifications indicating the position of the missing internal loop (light blue dashed line), the unstructured C-terminal tail (red dashed line), and the conserved residues K5, D25 and D27 highlighted in red. (C) *Yersinia pestis* HspQ protein levels do not change under normal growth conditions at 25˚C. Total cellular proteins, from equal number of cells, at indicated time point, were examined by Western blot analysis to detect changes in HspQ protein levels, using polyclonal anti-HspQ antibodies. Representative Western blot is shown, and the data presented in the graph are from three independent experiments (mean ± SEM).

Figure S2: *In vitro* proteolysis of YmoA by Lon is non-cooperative in the absence of HspQ. (A) *In vitro* proteolysis of YmoA by Lon is non-cooperative. YmoA degradation rates by 200 nM Lon_6, at a range of YmoA concentrations (2-40 μM), were determined in the absence of HspQ. Reactions were carried out at 37°C in 1X Lon activity buffer in the presence of an ATP regeneration system. Data presented are from three independent experiments (mean ± SEM). (B) HspQ forms stable complex with Lon. HspQ was mixed with an ATPase deficient variant of Lon, Lon^{E424Q}, at a molar ratio similar to that used in degradation assays (Fig 1D), in a buffer containing 50 mM Tris pH 8, 75 mM KCl, 10 mM $MgCl_2$, 10 % Glycerol, 2 mM β-ME and 1 mM ATP. The mixture was incubated at 4˚C for 1h and loaded on Superdex-200 gel-filtration column. Collected fractions were resolved by electrophoresis on 15% Tris-Tricine gels. Column profile and Coomassie Brilliant Blue stained gel are shown. (C) YmoA does not form a complex with HspQ. HspQ was mixed with YmoA at molar ratio of 4:1 (HspQ: YmoA) in a buffer containing 50 mM Tris pH 8, 75 mM KCl, 10 mM $MgCl_2$, 10 % Glycerol, and 2 mM β-ME. The mixture was incubated at 4˚C for 1h and loaded on a Superdex-75 gel-filtration column. Collected fractions were resolved by electrophoresis on Tris-Tricine gels. Column profile and Coomassie Brilliant

Blue stained gel are shown. (D) HspQ dependent Lon activation is conserved in *E. coli*. *In vitro* proteolysis of 200 µg/mL β-casein by 200 nM *E. coli* Lon_6 was carried out in absence and presence of 10 µM *E. coli* HspQ. Reactions were carried out at 37°C in 1X Lon activity buffer in the presence of an ATP regeneration system. Aliquots at specified time points were mixed with 2X-SDS sample buffer, resolved by electrophoresis on 15% Tris-Tricine gels, stained with Coomassie Brilliant Blue, scanned using a Li-COR Odyssey scanner, and quantified using the Image Studio software. Representative Coomassie Brilliant Blue stained gels are shown.

Figure S3: HspQ enhances Lon protease mediated degradation of the DnaA508 ts mutant. (A) The *E. coli dna508* temperature-sensitive mutant and its *hspQ* deletion variant (*dna508/hspQ*$^{-}$) were grown in LB/Tet medium to OD_{600} of 0.5. Equal number of cells in 5 µL of 10-fold serial dilution of the cultures were spotted on duplicate LB/Tet plates and grown at 30˚C or 41˚C. Loss of HspQ rescues the ts phenotype of the *dna508* mutant strain. (B) The parental *dna508* mutant and its *dna508/lon*$^{-}$ variant were grown to OD_{600} of 0.5, harvested and resuspended in 1/10 the original volume of LB to increase the cell number by ten fold. Equal cell numbers in 5 µL, of 3-fold serial dilution of the cultures, were spotted on three LB/Tet plates and grown at 30˚C, 39˚C, or 41˚C. Loss of Lon rescues the ts phenotype of the *dna508* mutant strain at 39˚C. (C) DnaA508 protein is substantially stabilized in the Lon deficient strain. The parental *dna508* mutant and its *dna508/lon*$^{-}$ variant were grown to OD_{600} of 0.5, and chloramphenicol at 200 µg/mL was added to stop new rounds of protein synthesis. The cultures were immediately transferred to 41˚C and equal number of cells for each culture were harvested at the indicated time points, resolved by electrophoresis on 10% Tris-Tricine gels and subjected to Western blot analysis using DnaA antiserum. DnaA protein bands were quantified and plotted, N=3 independent experiments.

Figure S4: HspQ has a C-terminal Lon recognition degron. HspQ with an N-terminal His6-tag (^{H6}HspQ) is fully capable of activating Lon. The effect of HspQ N-terminal modification (^{H6}HspQ) on allosteric activation of Lon for enhanced YmoA proteolysis was examined and compared to native (untagged) HspQ. 10 µM YmoA was subjected to proteolysis by 200 nM Lon_6 in the presence of 10 µM of HspQ or ^{H6}HspQ. Reactions were

carried out at 37°C in 1X Lon activity buffer in the presence of an ATP regeneration system. Representative Coomassie Brilliant Blue stained gels are shown.

Figure S5: HspQ has a C-terminal Lon recognition degron. (A) 10 μM of each reporter protein, the untagged λ cI N-terminal domain (λ) and the λ-Q20 variant carrying the C-terminal 20 residues of HspQ were examined in proteolysis assays with 200 nM Lon_6 to assess whether the HspQ degron can enhance degradation of the reporter protein. The λ reporter carrying a strong Lon degron (Ge and Karzai, 2009), the *Mycoplasma pneumonia* ssrA degron (λ-ssrA^{MP}), was used as a positive control in these assays. (B) To assess whether the HspQ degron attached to the λ reporter (λ-Q20) was sufficient for enhancing YmoA degradation by Lon, the effect of the λ-Q20 construct on YmoA degradation rate was examined using 10 μM YmoA in absence and presence of equimolar λ-Q20. (C) Using data from these experiments, the effect of YmoA on the degradation rate of the λ-Q20 was also measured and plotted. Degradation rates for YmoA and λ-Q20 reporter are presented. (D) The partial structural model of *E. coli* HspQ (1VBV) was modified to show the 8-amino acid insertion (GSTGSTGS) in the HspQ^{ext} construct. Representative Coomassie Brilliant Blue stained gels are shown, and the data presented in graphs are from three independent experiments (mean ± SEM).

Figure S6: Y2853 interacts with Lon and The C-terminal Histidine (H161) of the Y2853 degron constitutes a Lon recognition signal. (A) Y2853 forms a stable complex with Lon. Y2853 was mixed with Lon^{E424Q} hexamers at molar ratio of 18:1(Y2853: Lon hexamer) in a buffer containing 50 mM Tris pH 8, 75 mM KCl, 10 mM $MgCl_2$, 10 % Glycerol, 2 mM β-ME and 1 mM ATP. The mixture was incubated at 4˚C for 1h and loaded on Superdex 200 gel-filtration column. Collected fractions were resolved on Tris-Tricine gels. Column profile and Coomassie Brilliant Blue stained gel are shown. (B) Y2853^{DD} does not affect HspQ degradation by Lon. *In vitro* Lon proteolysis of 10 μM HspQ was carried out in absence or presence of equimolar Y2853 ^{DD} or Y2853 ^{WT}. Reactions were carried out in 1X Lon activity buffer and 200 nM Lon_6 at 37°C. Fraction full-length HspQ protein remaining from proteolysis reactions are plotted over time. Data are normalized to protein amount at the zero time point for each degradation assay. The data presented in graph are from three independent experiments (mean ± SEM). (C) To elucidate Lon

recognition signals in the Y2853 degron, the C-terminal histidine residue was mutated to alanine in the context of GFP^{Y20}, generating the $GFP^{Y20(H161A)}$ reporter protein. The proteolytic propensity of $GFP^{Y20(WT)}$ and $GFP^{Y20(H161A)}$ were compared in a proteolysis assay, using 10 µM each substrate and 200 nM Lon_6. Reactions were carried out in 1X Lon activity buffer in the presence of an ATP regeneration system at 37°C. Aliquots at specified time points were mixed with 2X-SDS sample buffer, resolved by electrophoresis on 15% Tris-Tricine gels, stained with Coomassie Brilliant Blue, scanned using a Li-COR Odyssey scanner, and quantified using the Image Studio software. Data presented are from three independent experiments (mean ± SEM). It is noteworthy that the degradation rate of 10 µM GFP^{Y20} measured by the gel-based degradation assay (1.1 ± 0.09), shown here, is virtually indistinguishable from that measured by the fluorescent based degradation assay (1.2 ± 0.04), shown in Figure 6D and Table 6.

Figure S7: Contributions of conserved residues to HspQ function and stability. (A) *In vitro* proteolysis reactions were carried out at 37°C using 10 µM concentration of each HspQ K5 variant and 200 nM Lon_6 in 1X Lon activity buffer in the presence of an ATP regeneration system. Substitutions at K5 lead to enhanced HspQ degradation by Lon. (B) YmoA degradation reactions were performed and the YmoA fraction remaining at each time point was determined in absence and presence of each HspQ K5 variant. (C) CD-spectra of $HspQ^{WT}$ and $HspQ^{K5D}$ at 25°C with a 20 µM protein concentration. (D) *In vitro* proteolysis of YmoA without and with HspQ variants. HspQ C-terminal helix variants, $HspQ^{S93A}$ and $HspQ^{RHQ/AAA}$, were tested for Lon activation with 10 µM YmoA under conditions described in (A). Representative Coomassie Brilliant Blue stained gels are shown, and the data presented in graphs are from three independent experiments (mean ± SEM).

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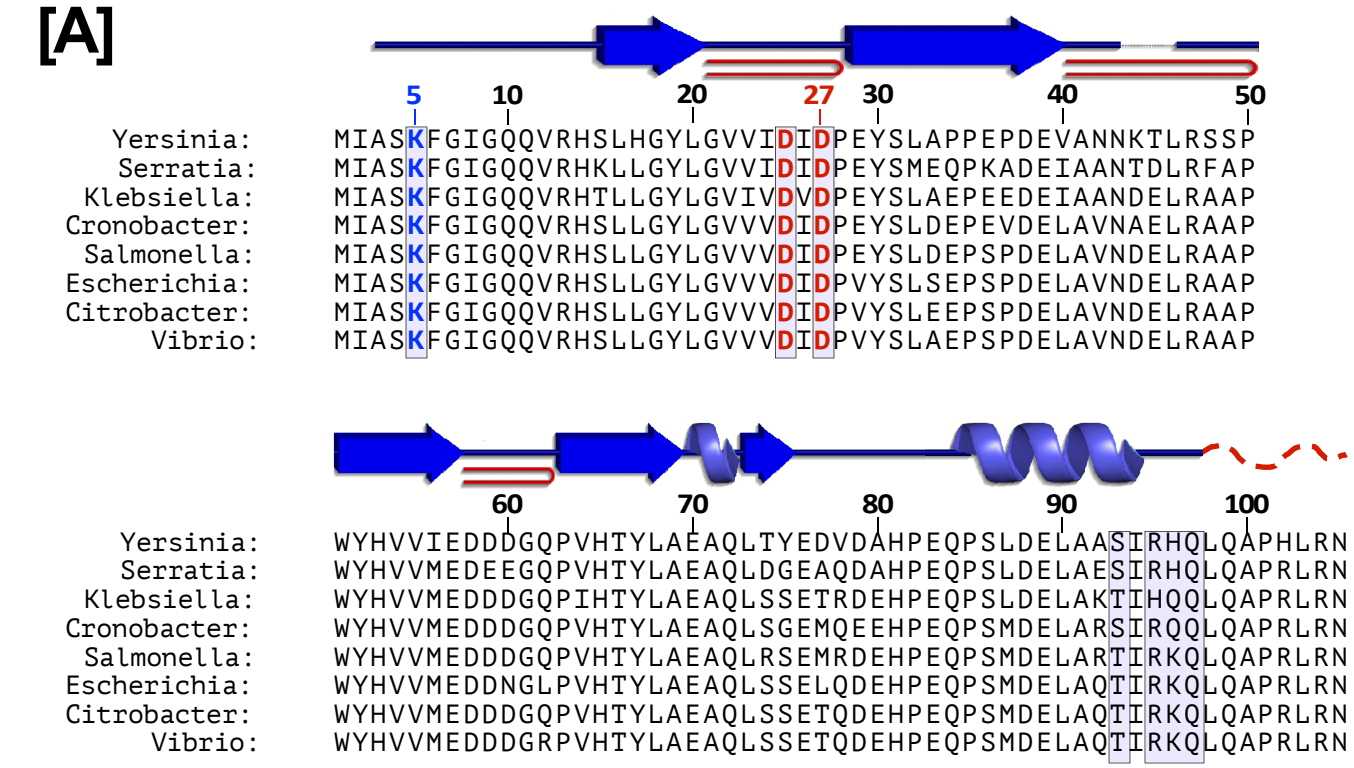
Ge, Z., and Karzai, A.W. (2009). Co-evolution of multipartite interactions between an extended tmRNA tag and a robust Lon protease in Mycoplasma. Mol Microbiol *74*, 1083-1099.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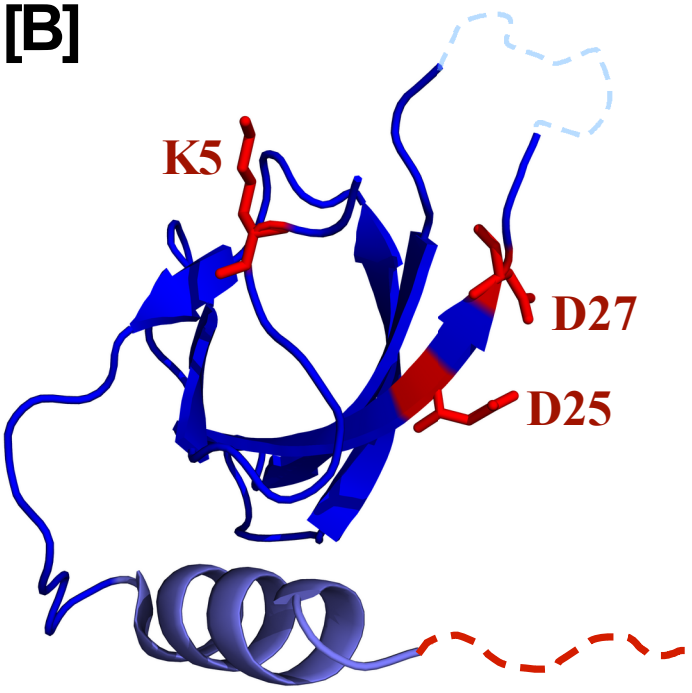
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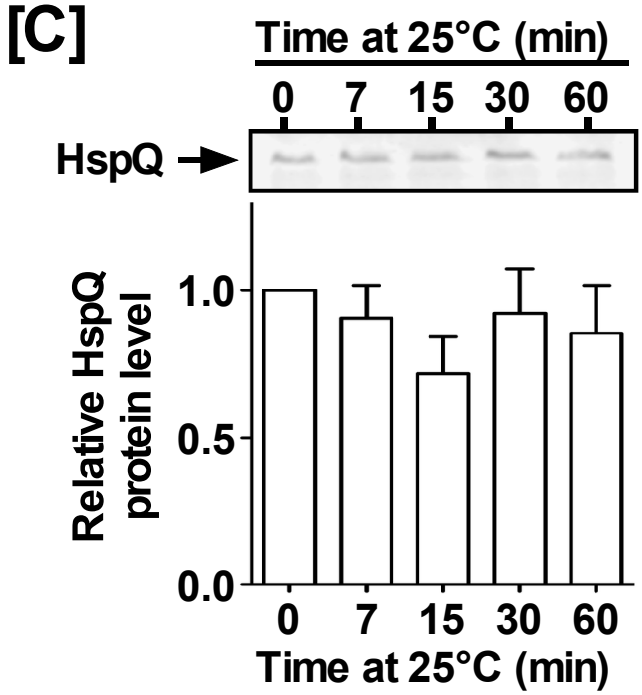
D25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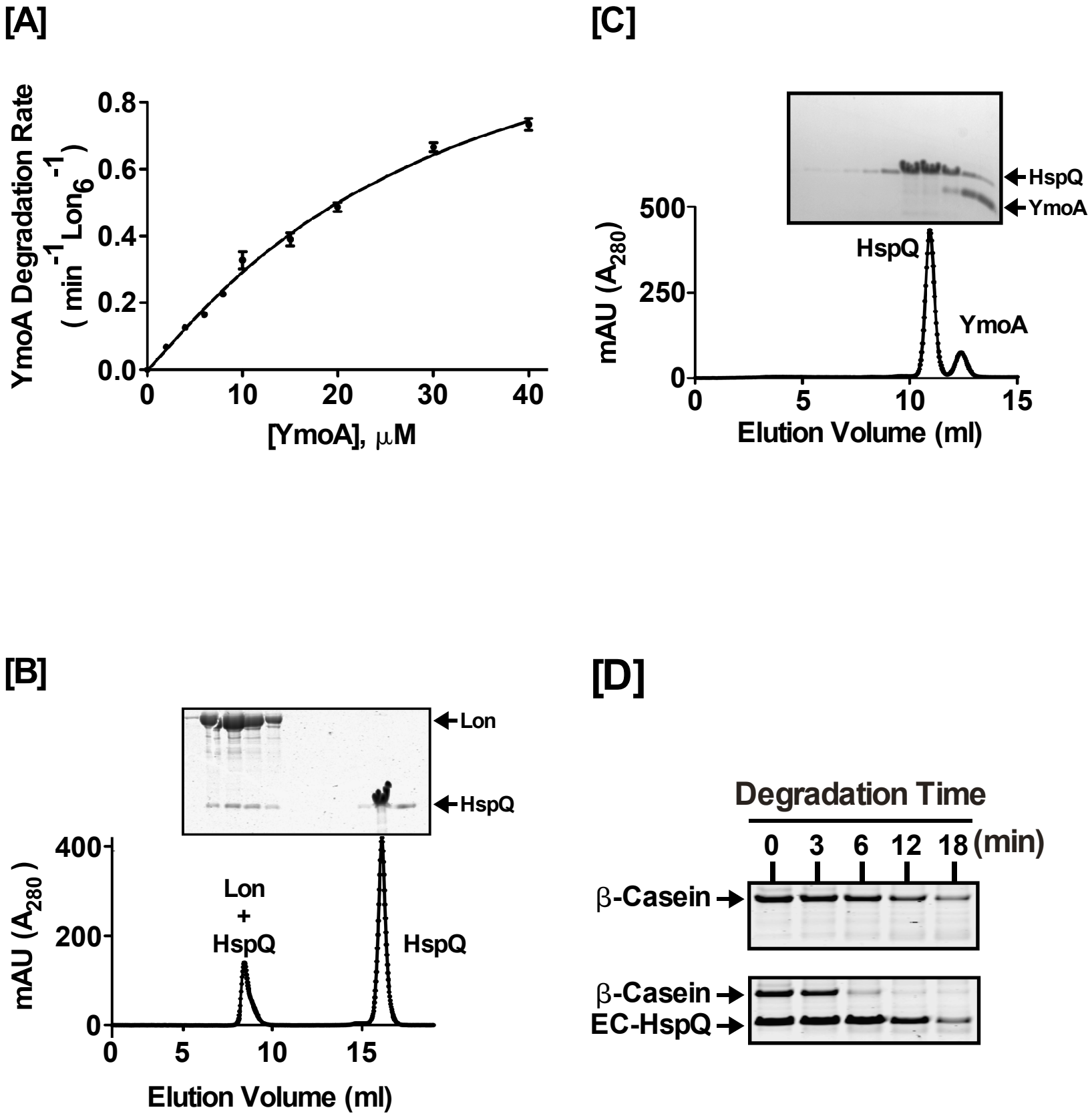
7
15
30
60
Time at 25°C (min)

Fig. S1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30
40
[YmoA], μM
[C]
HspQ
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0
mAU (A280)
HspQ
YmoA
0
5
10
15
Elution Volume (ml)
[B]
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HspQ
400
200
0
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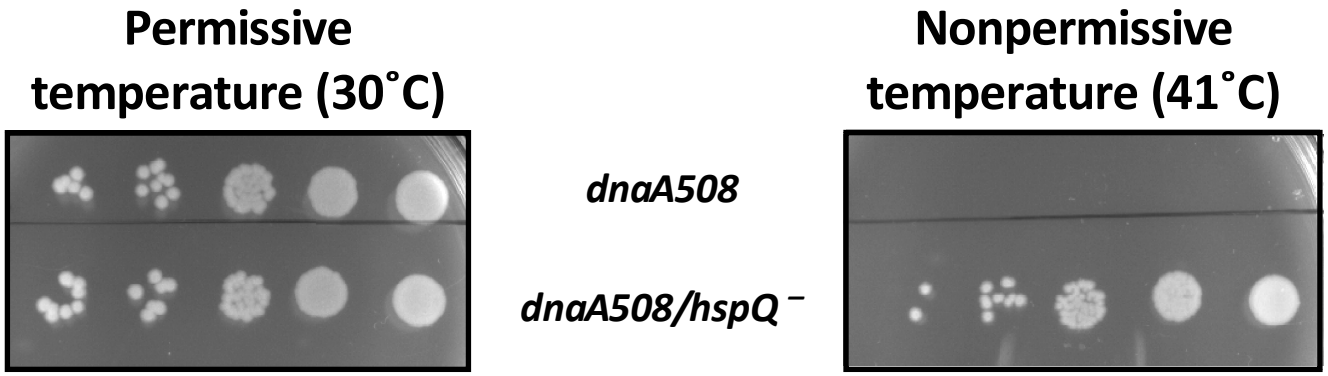
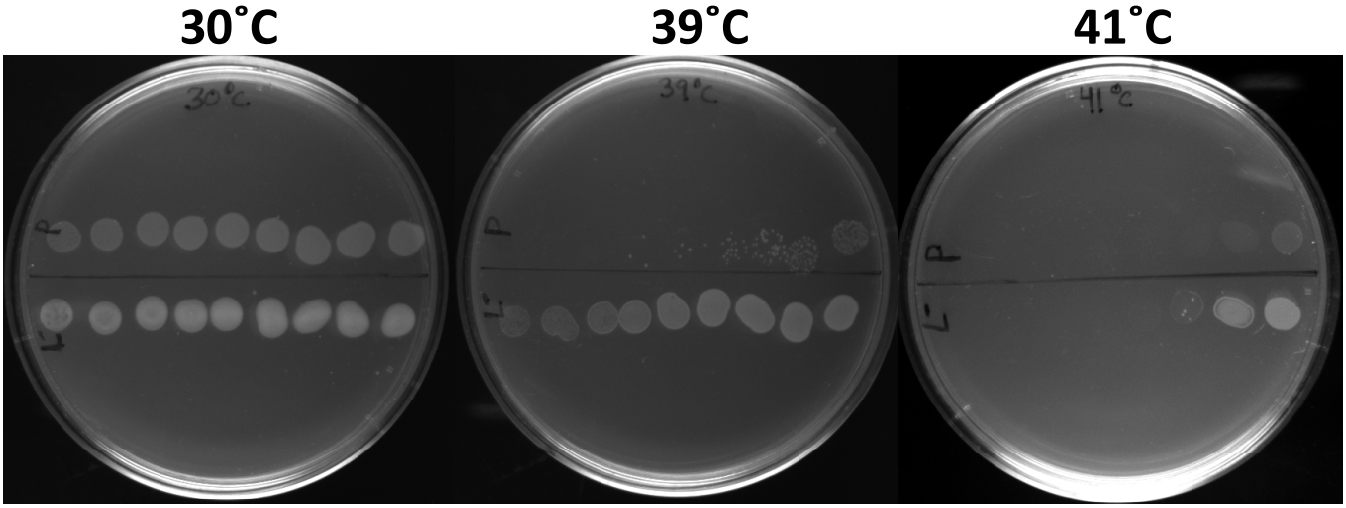
Lon
+
HspQ
HspQ
0
5
10
15
Elution Volume (ml)
[D]
Degradation Time
0
3
6
12
18
(min)
β-Casein
β-Casein
EC-HspQ

Fig. S2

[A]


Permissive temperature (30˚C)
Nonpermissive temperature (41˚C)
dnaA508
dnaA508/hspQ⁻

[B]


30˚C
39˚C
41˚C
dnaA508
dnaA508/lon⁻

[C]

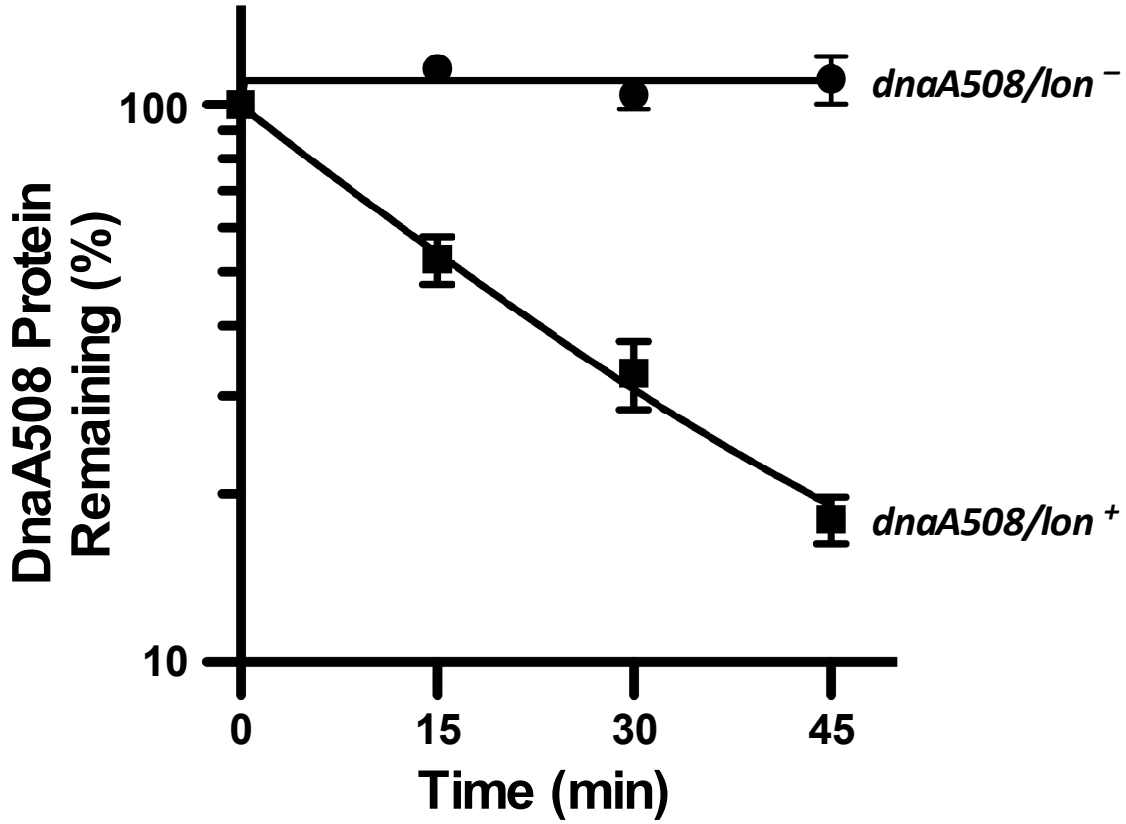

dnaA508/lon⁻
dnaA508/lon⁺
100
10
DnaA508 Protein Remaining (%)
0
15
30
45
Time (min)

Fig. S3

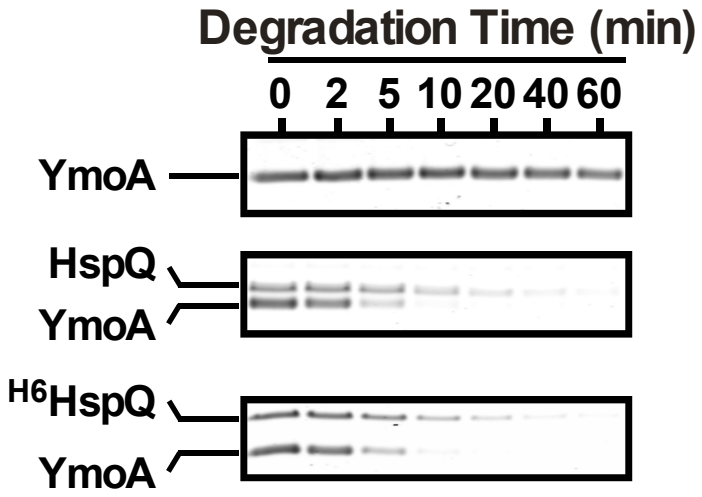
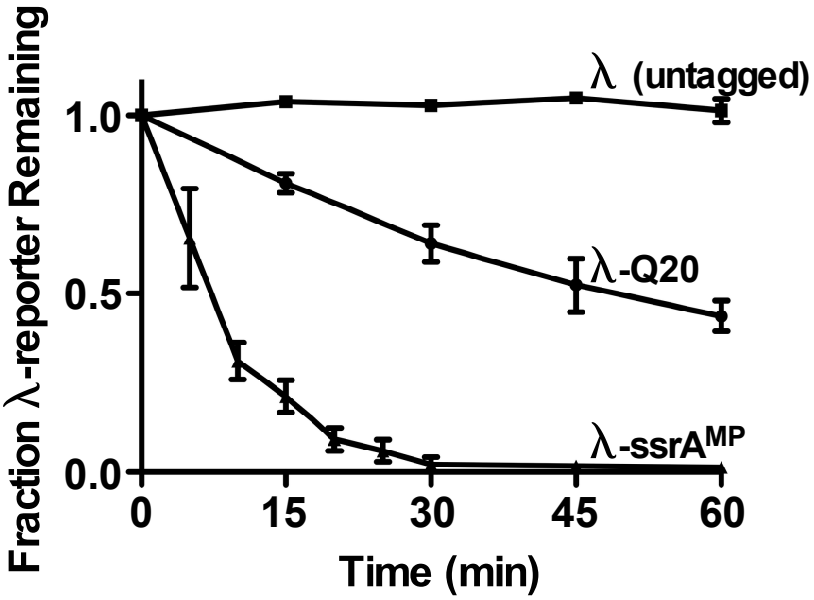
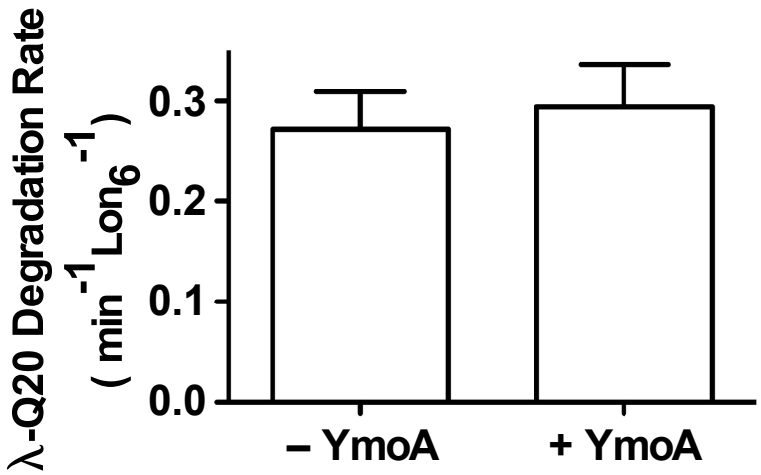

Degradation Time (min)
0 2 5 10 20 40 60
YmoA
HspQ
YmoA
H6HspQ
YmoA

Fig. S4

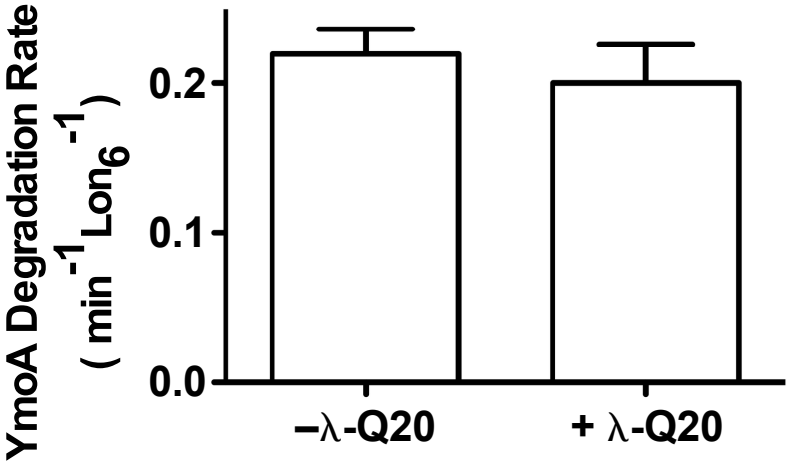
[A]


Fraction λ-reporter Remaining
1.0
0.5
0.0
0
15
30
45
60
Time (min)
λ (untagged)
λ-Q20
λ-ssrA^MP

[C]


λ-Q20 Degradation Rate
(min^-1 Lon6^-1)
0.3
0.2
0.1
0.0
– YmoA
+ YmoA

[B]


YmoA Degradation Rate
(min^-1 Lon6^-1)
0.2
0.1
0.0
–λ-Q20
+ λ-Q20

[D]

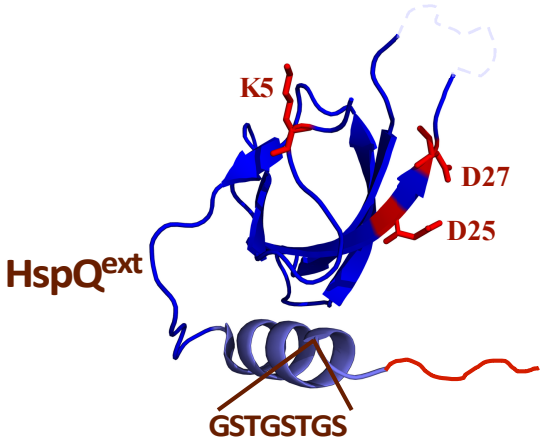

K5
D27
D25
HspQ^ext
GSTGSTGS

Fig. S5

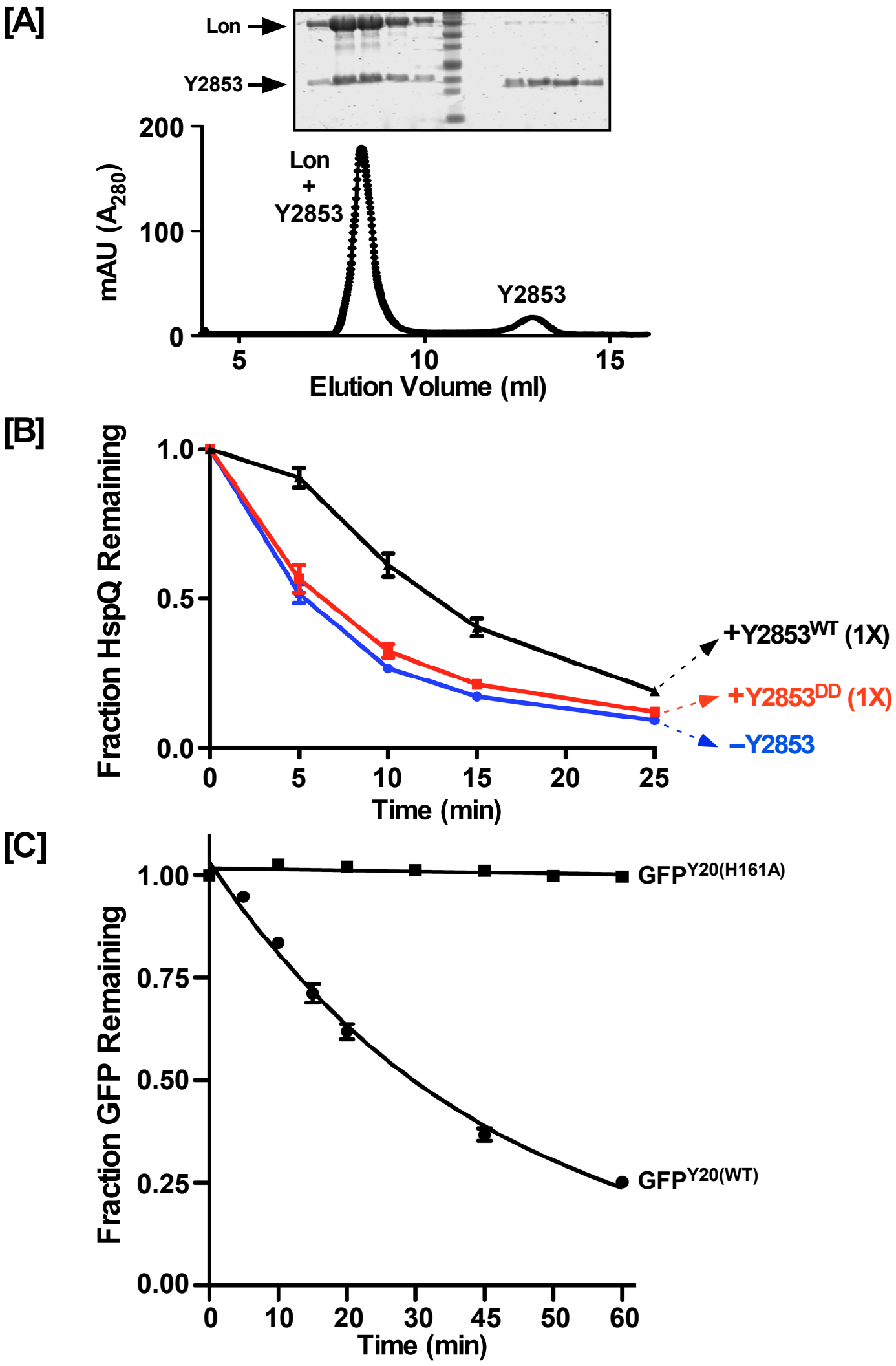

[A]
Lon
Y2853
200
100
0
mAU (A280)
Lon
+
Y2853
Y2853
5
10
15
Elution Volume (ml)
[B]
1.0
0.5
0.0
Fraction HspQ Remaining
0
5
10
15
20
25
Time (min)
+Y2853WT (1X)
+Y2853DD (1X)
–Y2853
[C]
1.00
0.75
0.50
0.25
0.00
Fraction GFP Remaining
0
10
20
30
45
50
60
Time (min)
GFPY20(H161A)
GFPY20(WT)

Fig. S6

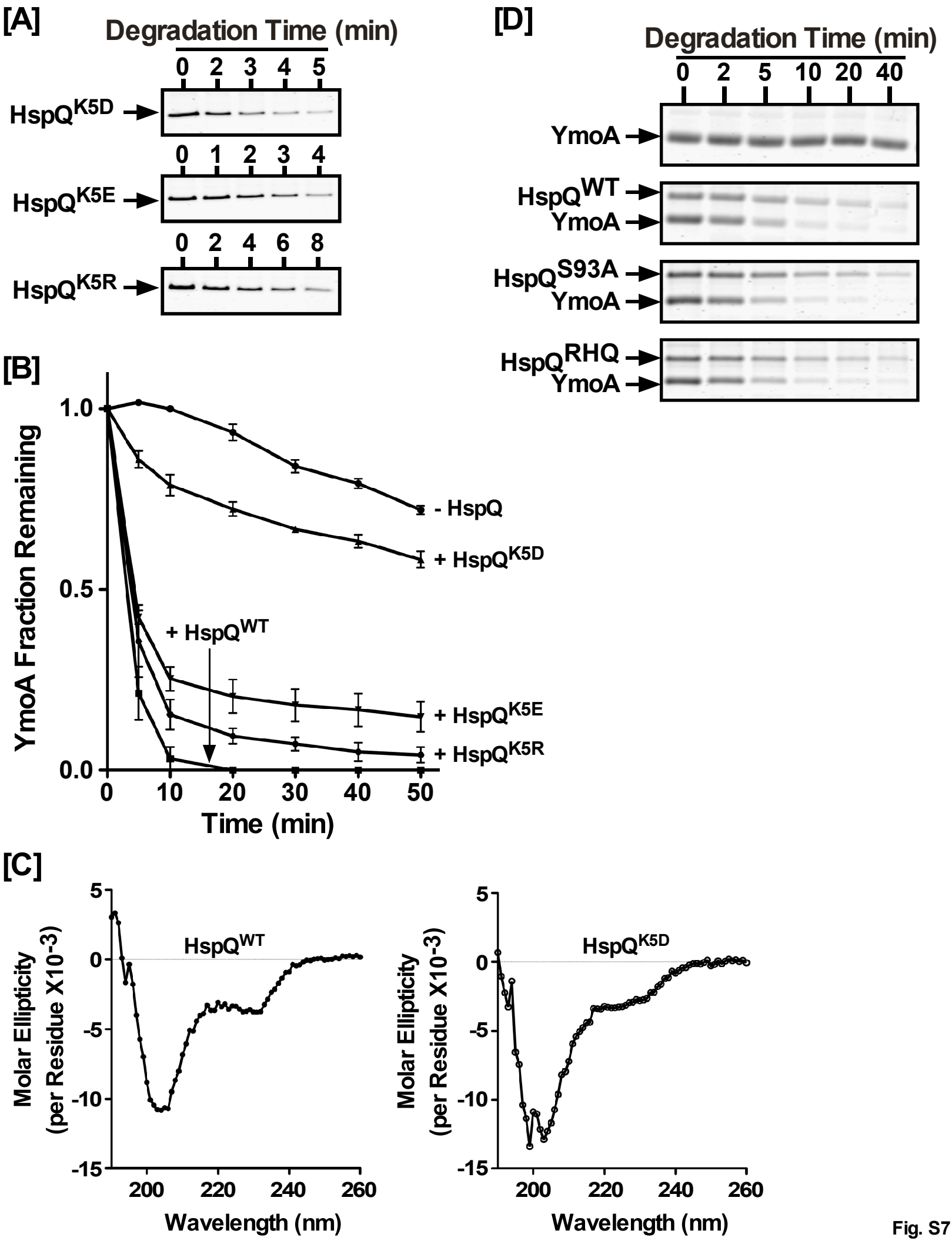

[A]
Degradation Time (min)
0 2 3 4 5
HspQ^K5D
0 1 2 3 4
HspQ^K5E
0 2 4 6 8
HspQ^K5R
[D]
Degradation Time (min)
0 2 5 10 20 40
YmoA
HspQ^WT
YmoA
HspQ^S93A
YmoA
HspQ^RHQ
YmoA
[B]
YmoA Fraction Remaining
1.0
0.5
0.0
- HspQ
+ HspQ^K5D
+ HspQ^WT
+ HspQ^K5E
+ HspQ^K5R
0 10 20 30 40 50
Time (min)
[C]
Molar Ellipticity (per Residue X10^-3)
HspQ^WT
5 0 -5 -10 -15
200 220 240 260
Wavelength (nm)
Molar Ellipticity (per Residue X10^-3)
HspQ^K5D
5 0 -5 -10 -15
200 220 240 260
Wavelength (nm)

Fig. S7