Supporting Information

Structural basis for DNA-mediated allosteric regulation facilitated by AAA$^+$ module of Lon protease

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[1,2,3] A-YL, Y-DC and Y-YC made equal contributions to this manuscript and can be considered co-first authors.

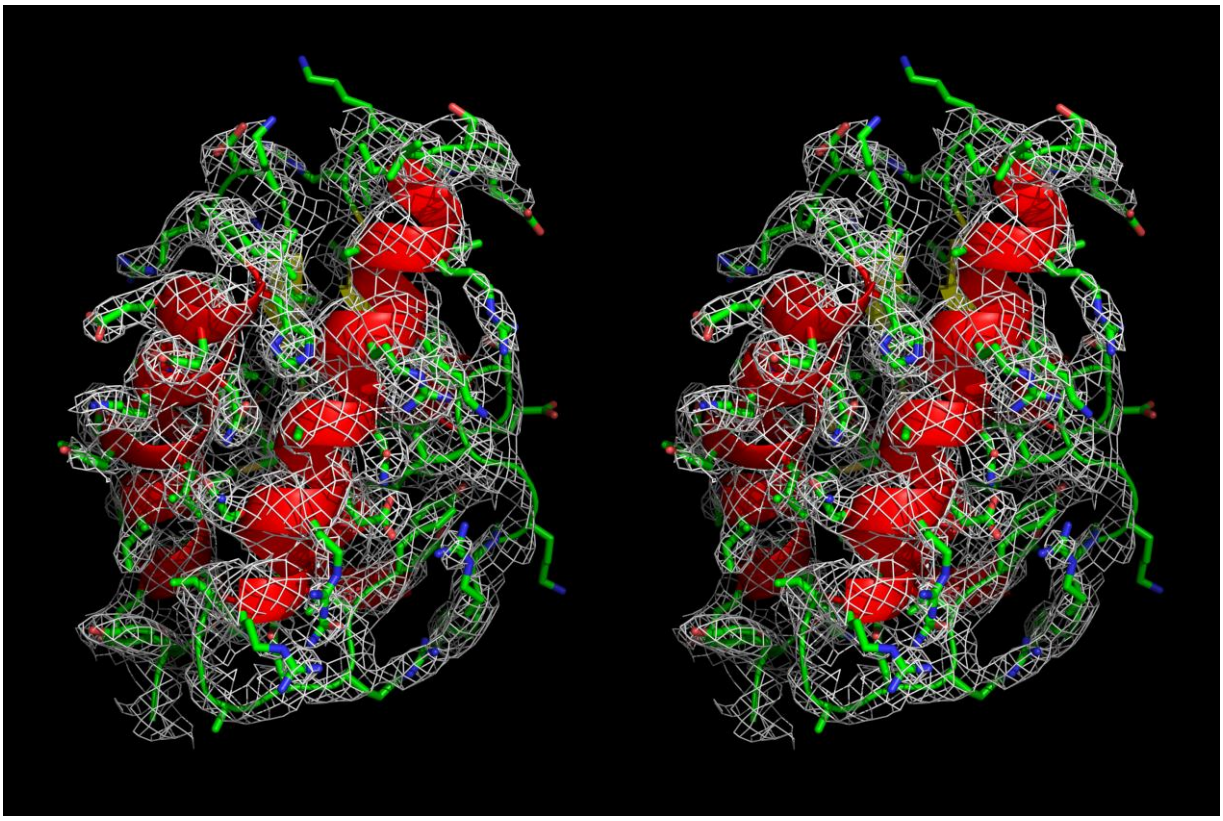

Figure S1. Stereoview of an electron-density map ($2F_o$-F_c) covering α sub-domain shown in ribbon diagram with side-chain in stick drawing is presented.

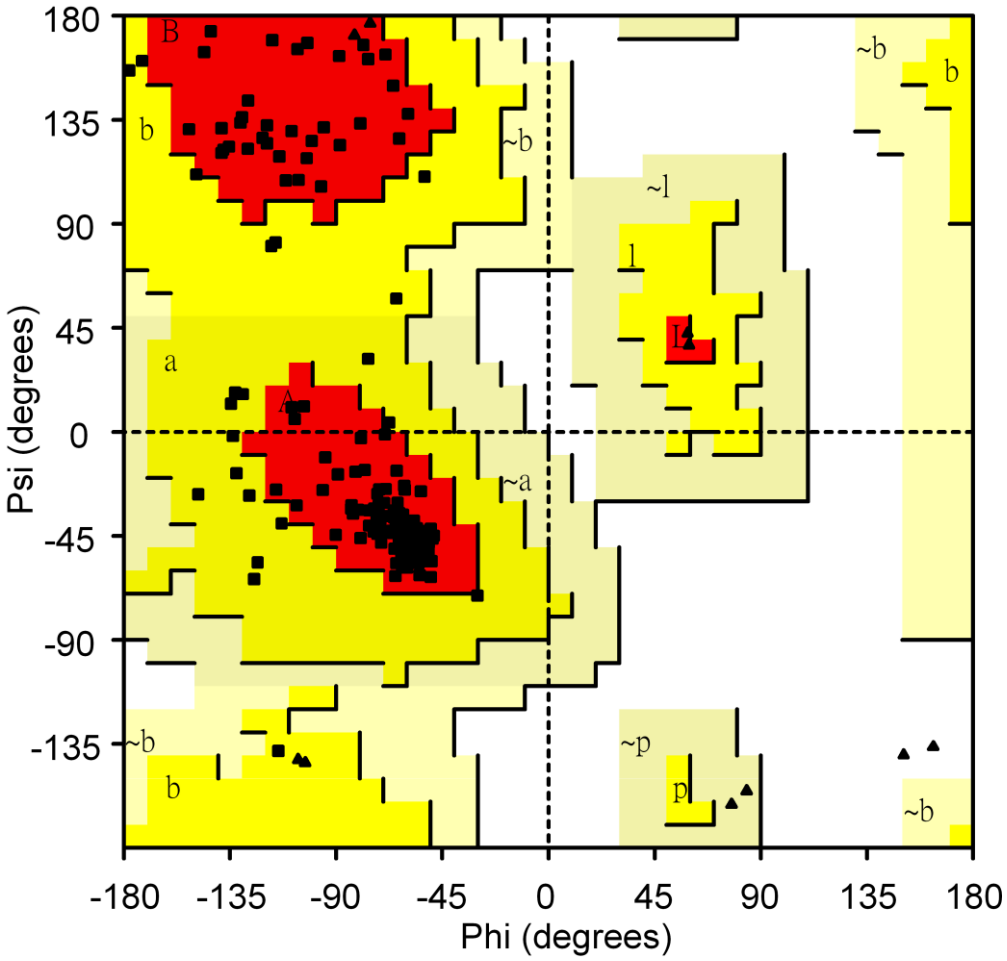

Figure S2. Ramachandran plot of ψ and φ dihedral angles for crystal structure of Bt-Lon α sub-domain.

The plot for α sub-domain generated using PROCHECK program revealed that 88.4% and 11.6% of the residues lie in the most favored and additional allowed regions, respectively. Triangles in the plot represent the angles for glycine residues. The regions are labeled as follows: A (Core alpha), a (Allowed alpha), ~a (Generous alpha), B (Core beta), b (Allowed beta), ~b (Generous beta), L (Core left-handed alpha), l (Allowed left-handed alpha), ~l (Generous left-handed alpha), p (Allowed epsilon) and ~p (Generous epsilon).

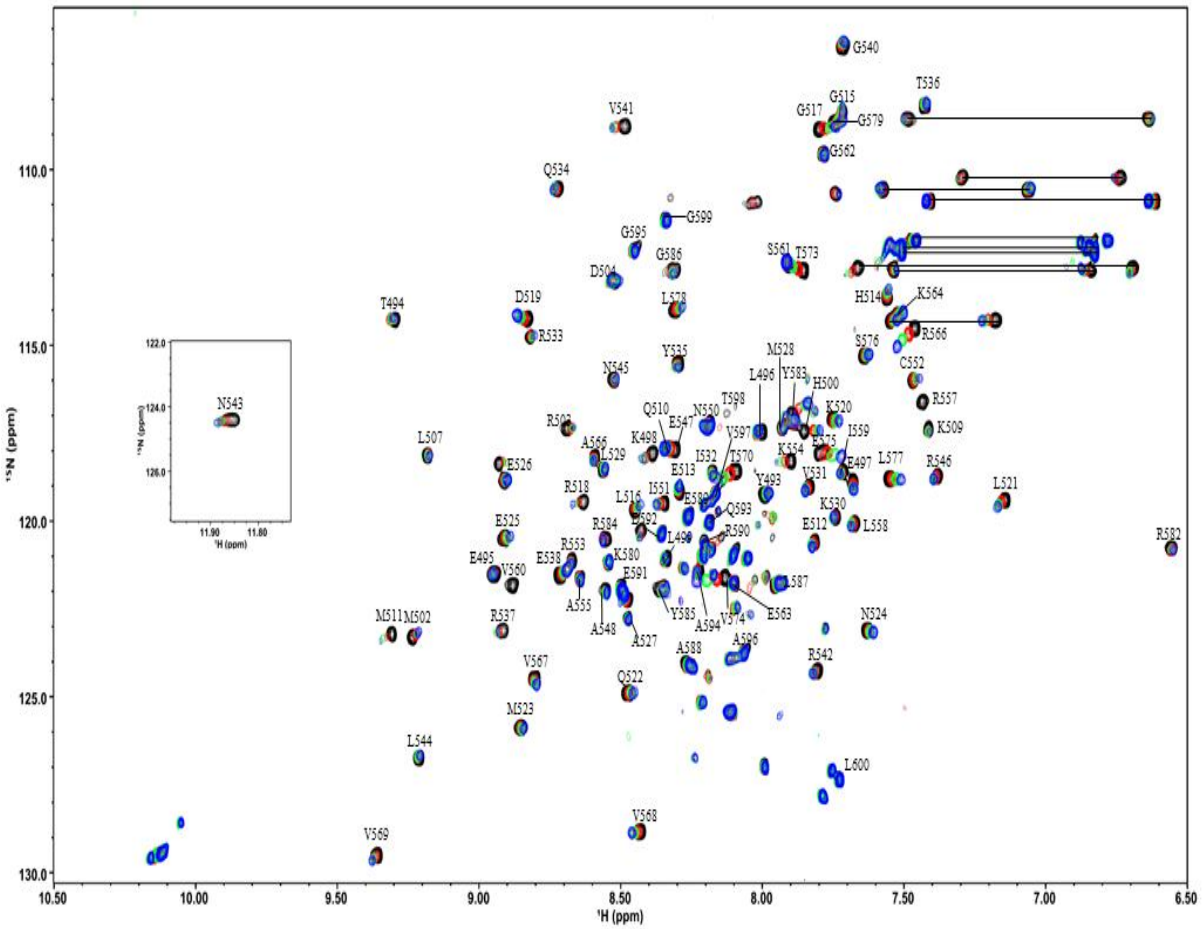

Figure S3. HSQC spectra of Bt-Lon α sub-domain.

^{1}H-^{15}N-HSQC spectra shows free Bt-Lon α sub-domain (black) titrated with 0.5 (red), 1 (green), 1.5 (blue) molar equivalents of ds-ms2 DNA, respectively. All backbone resonances are labeled with a one-letter code.

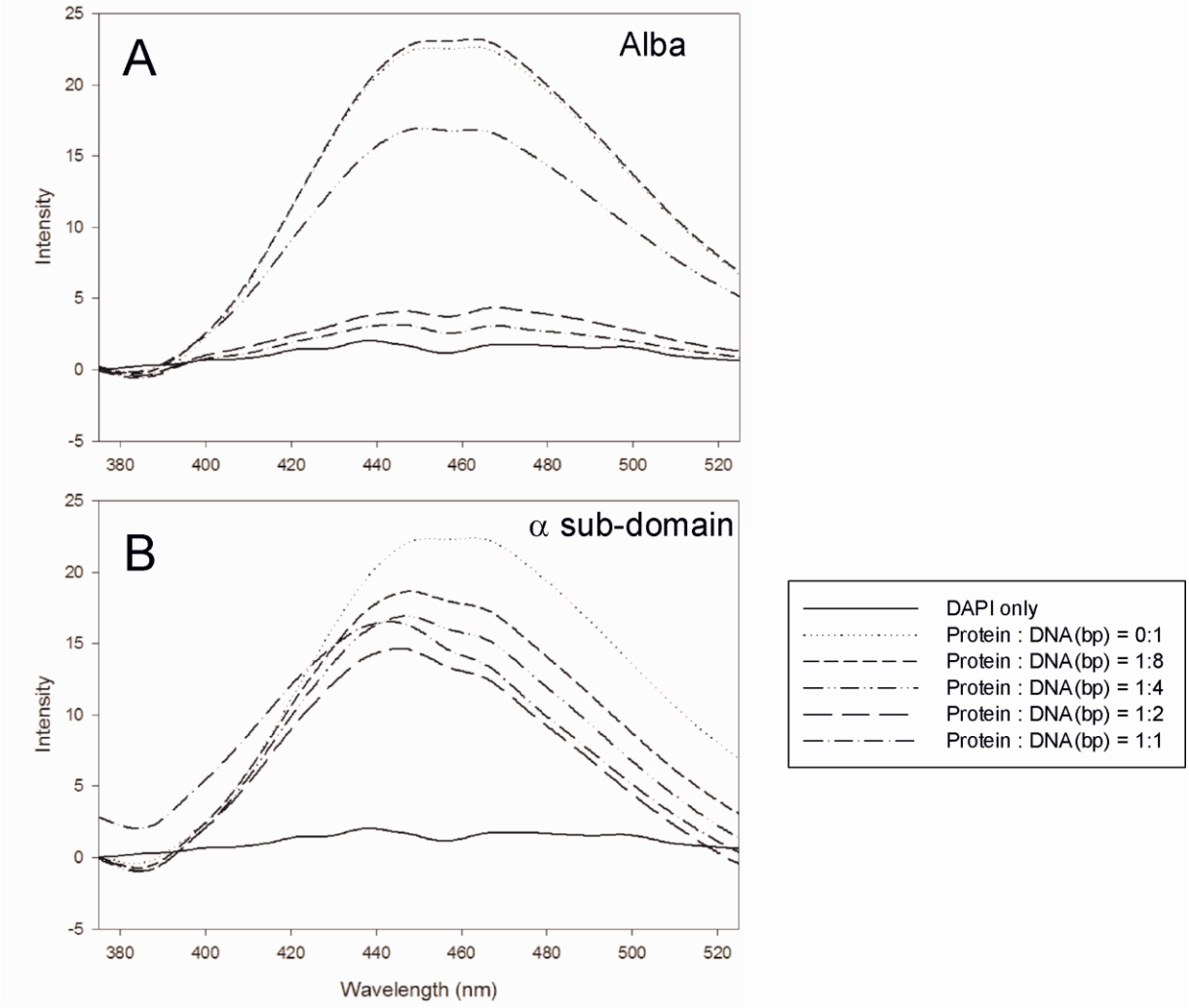

Figure S4. DAPI dye displacement assay.

pET-28a plasmid (1 nM), saturated with the fluorescent dye DAPI, a DNA minor groove binding molecule was incubated in binding buffer with increasing concentrations of recombinant Alba protein (A) and Bt-Lon α sub-domain (B). Alba was demonstrated as a DNA minor groove binding protein. The decrease in fluorescence intensity due to displacement of the DAPI was monitored as described in Materials and methods. Compared with Alba, Bt-Lon α sub-domain showing no significant fluorescence intensity decreasing indicates the protein bound to DNA should be in a major groove binding manner.

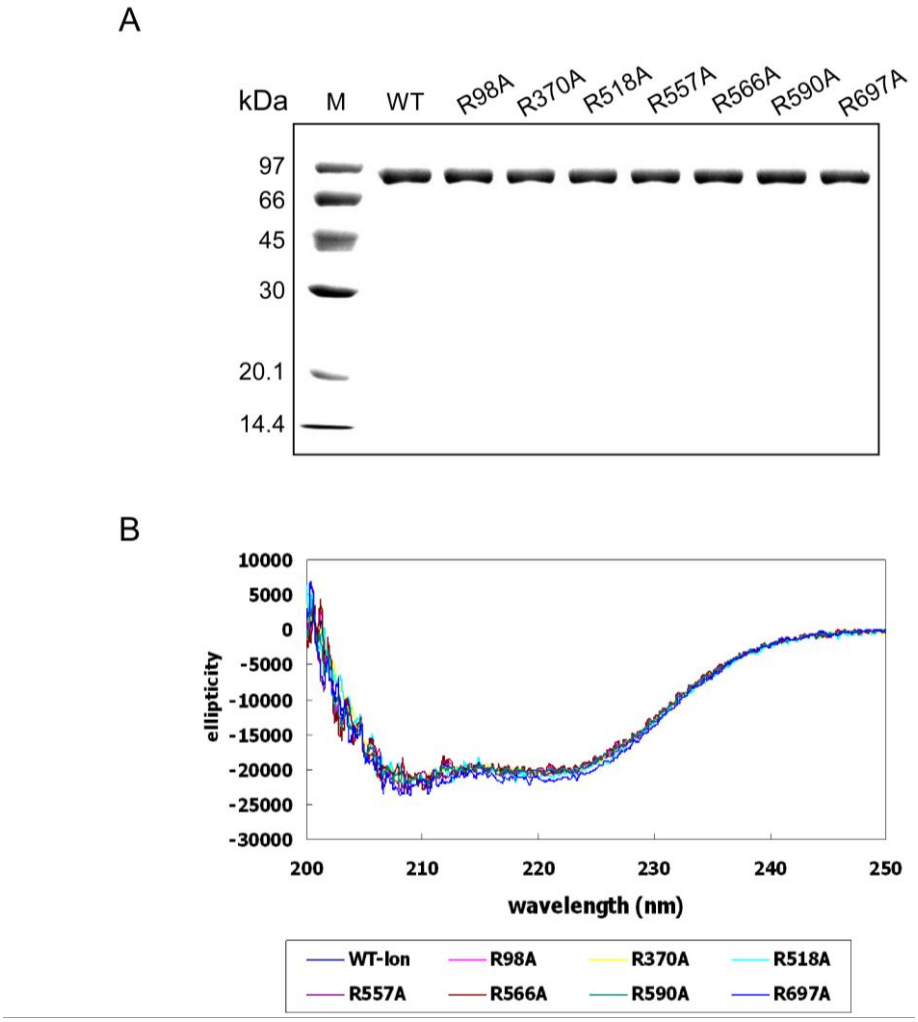

Figure S5. Characterization of the wild-type and arginine mutants of Bt-Lon.

(A) SDS-PAGE of the recombinant Bt-Lon variants. The recombinant Bt-Lon proteins with His-tag were purified by Ni-NTA affinity column and samples were examined by a 10% SDS-polyacrylamide gel electrophoresis. The Bt-Lon variants are labeled as indicated above the gel. M: molecular markers, 97, 66, 45, 30, 20.1, and 14.4 kDa; WT: wild-type Bt-Lon. **(B)** Far-UV circular dichroism spectra of the recombinant Bt-Lon variants. The circular dichroism spectra of the wild-type Bt-Lon and its mutants were obtained using a Jasco J-810 spectropolarimeter. The protein sample (0.5-0.6 mg/mL) was scanned from 200 to 250 nm at 25 °C. The Bt-Lon variants are labeled as indicated. Data are presented as molar ellipticity [θ] (deg cm^2 $dmol^{-1}$).

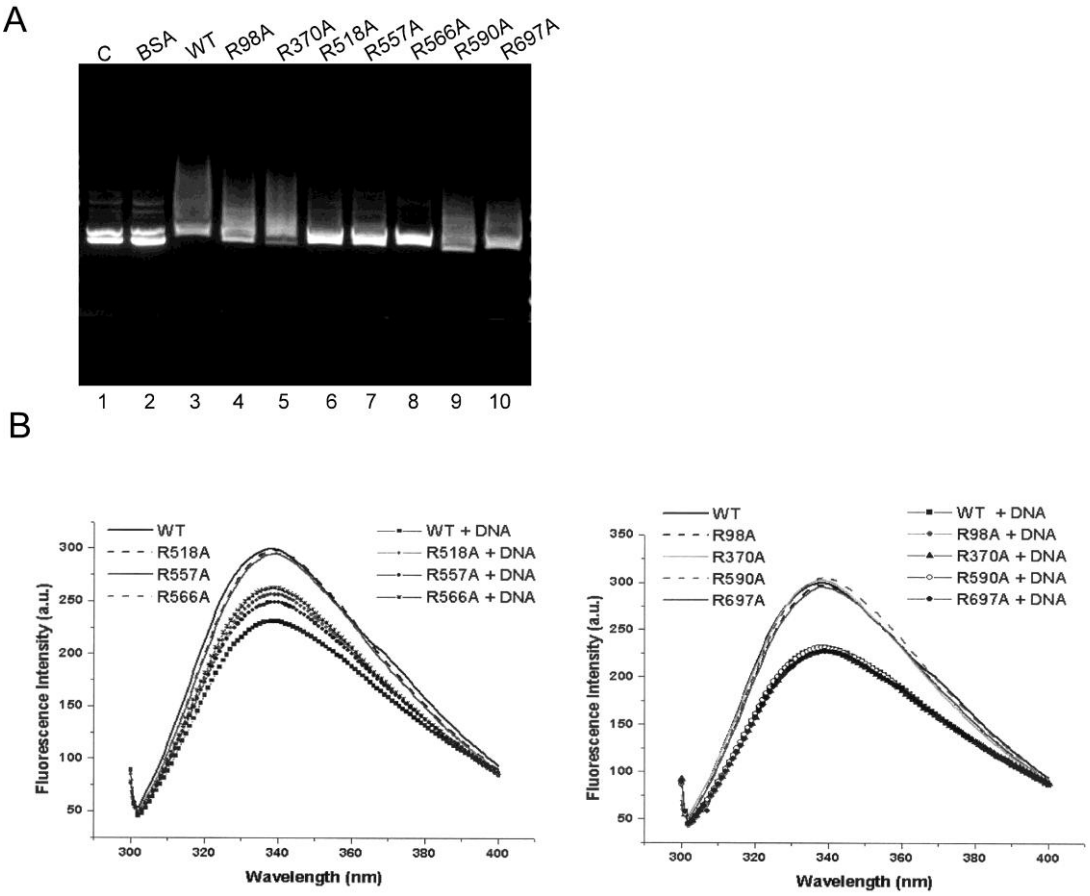

Figure S6. Electrostatic interactions contributed by arginine residues in the α sub-domain are important to DNA-binding activity of Bt-Lon.

(A) Effect of NaCl on the DNA-binding ability of Bt-Lon. Affinity-purified Bt-Lon (4 μg) incubated with 500 ng of plasmid DNA was mixed with indicated concentration of NaCl then subjected to EMSA. Total volume is 25 μl. In the control ("C"), the reaction mixture was identical except that NaCl was omitted. (B) DNA-binding activity of Bt-Lon arginine mutants. A mixture (25 μl) containing 4 μg of the affinity-purified Bt-Lon or its mutants and 500 ng of plasmid DNA was incubated for 10 min, then subjected to EMSA. The mutant proteins assayed are indicated above the gel. *Lane C*, Control with no protein; *lane BSA*, 30 μg of bovine serum albumin plus DNA; *WT*, wild-type. (C) DNA-binding activity of Bt-Lon arginine mutants analyzed by the intrinsic tryptophan fluorescence. Emission spectra of a solution (0.35 mg/ml) of Bt-Lon or the arginine mutants in the presence or absence of 200 μg/ml of plasmid DNA were recorded. *a.u.*, arbitary units.

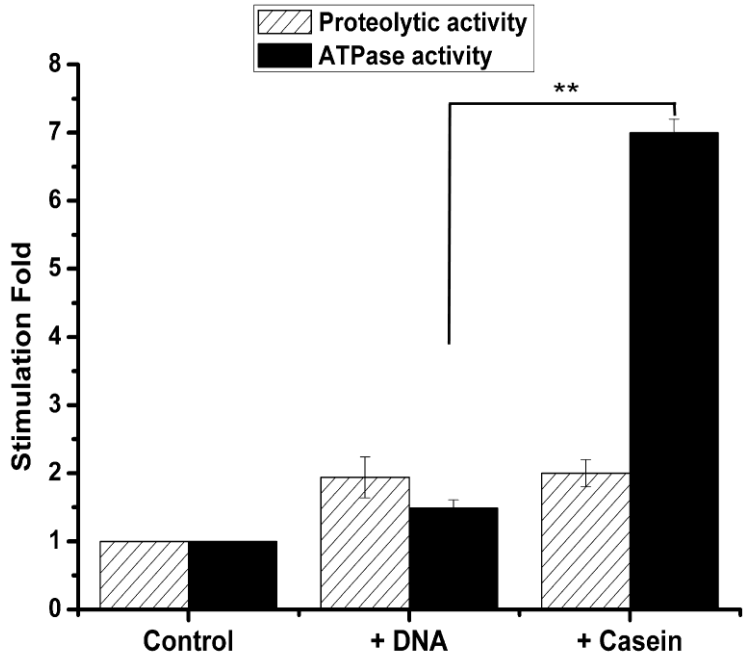

Figure S7. Stimulation of enzymatic activities of Bt-Lon by DNA and α-casein.

Stimulation of peptidase and ATPase activity by DNA and α-casein was performed as described as in Fig.4 and 5. The fold stimulation of the activities in the absence of DNA or α-casein was regarded as control and set as 1.