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*Supplementary Data contains four figures and two tables*

**Binding-induced folding of prokaryotic ubiquitin-like protein on the *Mycobacterium*  
proteasomal ATPase targets substrates for degradation**

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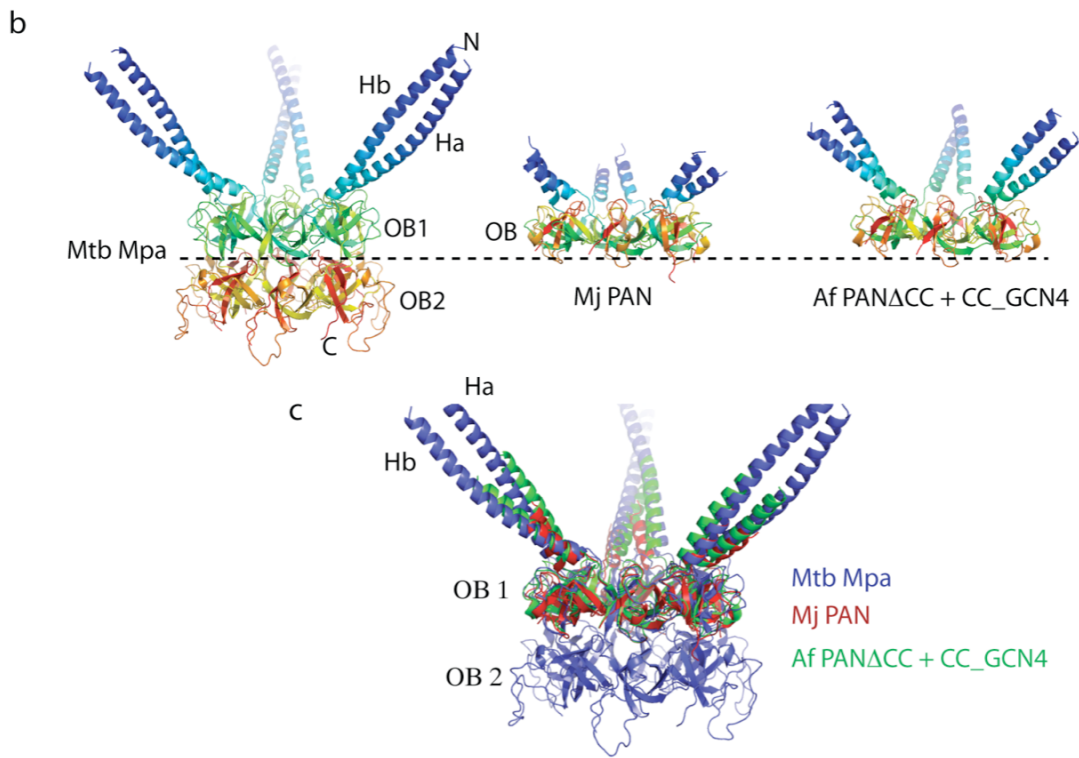
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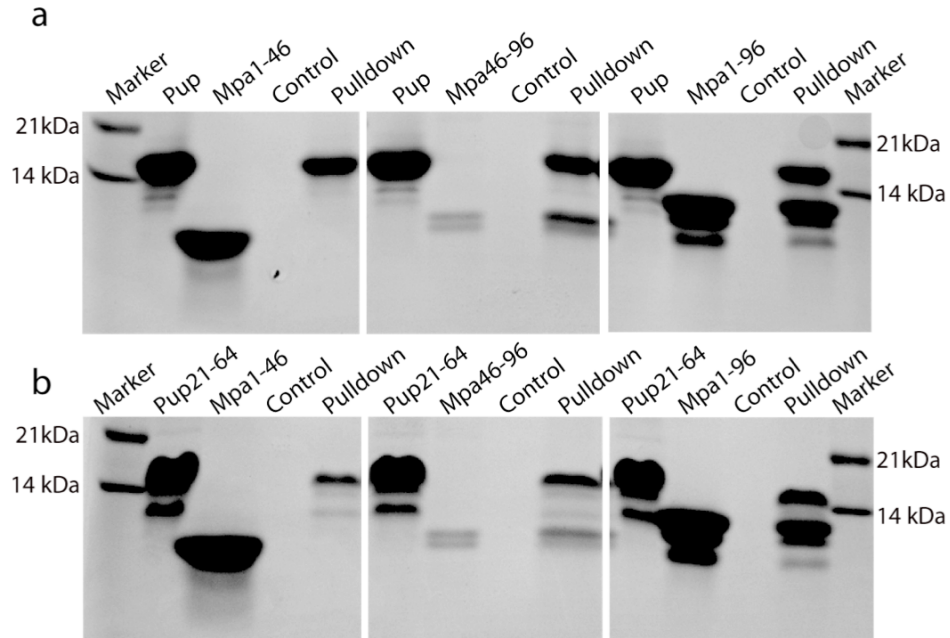
**a**

|     | gi  113700393 gb ABI36485.1      |  | 1   | 10     | 20       |
|-----|----------------------------------|--|---|--------|----------|
| Mpa | gi  113700393 gb ABI36485.1      |  | .....   | .....  | .....    |
| ARC | gi  226306666 ref YP_002766626.1 |  | .....   | .....  | .....    |
|     | gi  111017868 ref YP_700840.1    |  | .....   | .....  | .....    |
|     | gi  54025149 ref YP_119391.1     |  | .....   | .....  | .....    |
|     | gi  254819647 ref ZP_05224648.1  |  | .....   | .....  | .....    |
|     | gi  118465707 ref YP_881600.1    |  | MTKILTRFRGYLTTHINRQSPVALKYPPRQFGVGVKERRKMGSSERSEAFGT..... | PRESDM | SGDEALEL |
|     | gi  183983090 ref YP_001851381.1 |  | .....   | .....  | .....    |
|     | gi  15827684 ref NP_301947.1     |  | .....   | .....  | .....    |
|     | gi  118470801 ref YP_888192.1    |  | .....   | .....  | .....    |
|     | gi  257056211 ref YP_003134043.1 |  | .....   | .....  | .....    |
|     | gi  3676746 gb AAC64282.1        |  | .....   | .....  | .....    |
|     | gi  72162208 ref YP_289865.1     |  | .....   | .....  | .....    |
|     | gi  260903701 ref ZP_05912023.1  |  | .....   | .....  | .....    |
|     | gi  62390379 ref YP_225781.1     |  | .....   | .....  | .....    |
| PAN | gi  15669365 ref NP_248170.1     |  | .....   | .....  | .....    |

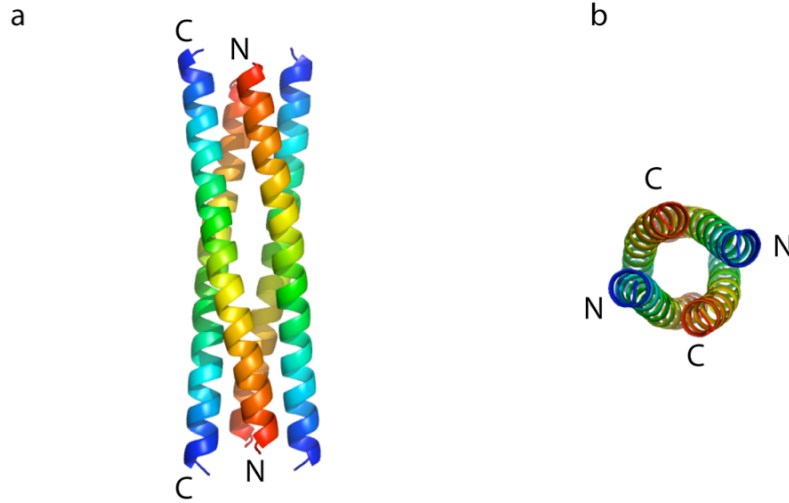
|  | gi  113700393 gb ABI36485.1      |  | 30       | 40      | 50      | 60      | 70      | 80      | 90      |
|--|----------------------------------|--|----------|---------|---------|---------|---------|---------|---------|
|  | gi  113700393 gb ABI36485.1      |  | RREAAVLR | EQLENAV | GSHAPTR | SARDIHQ | LEARIDS | LAARNSK | ME      |
|  | gi  226306666 ref YP_002766626.1 |  | RVEACVLR | RRQLAQ  | .....   | SPEQVRE | LESKVDS | LSIRNSK | MD      |
|  | gi  111017868 ref YP_700840.1    |  | RAEASVLR | RRQLAE  | .....   | SPEQLRE | MESRVDS | LSIRNTK | MD      |
|  | gi  54025149 ref YP_119391.1     |  | RAEAAVLR | RRQLAD  | .....   | SPDRTR  | LEARIDS | LTIRNTK | MD      |
|  | gi  254819647 ref ZP_05224648.1  |  | RREAAVLR | EQLEH   | TVGAQGG | GARSARD | VHQLEAR | IDS     | LAARNSK |
|  | gi  118465707 ref YP_881600.1    |  | RREAAVLR | EQLEH   | AVGSHGG | ARSARD  | VHQLEAR | IDS     | LAARNSK |
|  | gi  183983090 ref YP_001851381.1 |  | RREAAVLR | EQLEH   | AVGPGGT | ARSARD  | VHQLEAR | IDS     | LAARNSK |
|  | gi  15827684 ref NP_301947.1     |  | RREIVVLR | EQLEH   | AVGPHGS | VSARD   | VHQLEAR | IDS     | LTARNSK |
|  | gi  118470801 ref YP_888192.1    |  | RREAAVLR | EQLENAV | QSGLSR  | SARD    | VHQLEAR | IDS     | LAARNAK |
|  | gi  257056211 ref YP_003134043.1 |  | EEEVAL   | LRRLTDS | .....   | PRQNRV  | LEQLAE  | ANERVSQ | LTERNNK |
|  | gi  3676746 gb AAC64282.1        |  | EQEIAV   | LRRLAES | .....   | PRHRTLE | ERIVELQ | TNLAV   | SAQNER  |
|  | gi  72162208 ref YP_289865.1     |  | EKELSL   | LRRLADS | .....   | PRHVRLL | EDRLQEA | QTALAA  | ANAKNER |
|  | gi  260903701 ref ZP_05912023.1  |  | REDTAV   | LRRLQ   | LFSS    | .....   | .....   | .....   | AGKRNEA |
|  | gi  62390379 ref YP_225781.1     |  | SNSFSD   | FNREEQ  | SR      | .....   | .....   | .....   | LGARNAK |
|  | gi  15669365 ref NP_248170.1     |  | KEEKEI   | NDNSNL  | KNDLLKE | ELQEKAR | IAELES  | RILK    | LELEKKE |



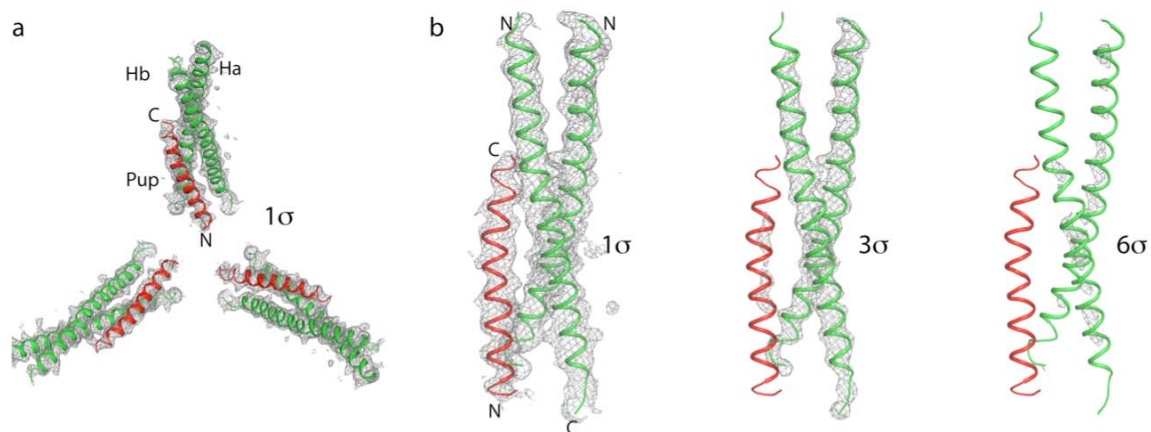
**Supplementary Fig. 1 Comparison of Mpa with archaeal proteasome ATPases.** (a) Sequence alignment of Mpa. Note the conserved Asn70, Pro97, and Pro98 in Mpa. (b) Comparison of the crystal structure of Mpa with the archaeal proteasomal ATPase PAN. The structures of Mpa1-234, *Archaeoglobus fulgidus* PAN- $\Delta$ CC<sub>GCIN4</sub> hybrid (PDB ID 2WG5), and the *Methanocaldococcus jannaschii* PAN (PDB ID 3H43) are aligned and displayed individually (b) and superimposed (c). The similar position and orientation of the coiled-coils may indicate they are relatively rigid.



**Supplementary Fig. 2. Pup21-64 interacts with Mpa coiled coil.** (a) Full-length Pup-His<sub>6</sub> retained Mpa1-96 and Mpa 46-96, but not Mpa1-46, on the Ni-NTA resin, indicating that Mpa1-46 is not involved in recognizing Pup. (b) Pup21-64 bound to Mpa peptides (Mpa1-46, Mpa46-96, Mpa1-96) essentially as well as to full-length Pup, indicating that the Pup1-21 is not involved in binding the coiled-coil region of Mpa. Samples in (a) and (b) were separated by SDS-PAGE and stained with Coomassie brilliant blue. Pup migrates in SDS-PAGE gels with an apparent molecular mass of ~14 kDa, most likely due to the relatively small number of hydrophobic residues that reduces binding to SDS.



**Supplementary Fig. 3.** Crystal structure cartoons of Mpa46-96 from the side (a) and top (b) views. In the crystal, the Mpa46-96 helix formed a non-physiological anti-parallel, four-helix coiled-coil bundle. Thus the formation of the parallel two-helix bundle in the presence of Pup would require disruption of this *in vitro*, non-native four-helix bundle.



**Supplementary Fig. 4. The  $2F_o-F_c$  electron density map of Pup:Mpa1-234 complex.** (a) Top view along the three-fold axis of the Mpa hexamer. Only the three coiled-coils of Mpa and Pup are shown. The hexameric OB fold region was omitted for clarity. The cartoon view of Mpa coiled-coil structure is superimposed in green, and the Pup helix in red. The electron density is shown at  $1\sigma$  level. (b) Zoomed side view at one coiled-coil region showing the electron density at  $1\sigma$  (Left),  $3\sigma$  (middle), and  $6\sigma$  (right) threshold levels, respectively. At the  $3\sigma$  display level, the Pup helix density almost disappeared, while the Mpa coiled-coil density remained robust.

## Supplementary Table 1. Plasmids and strains used in this study

| For recombinant protein production   |  |  |
|--|--|--|
| Protein (residues)   | Plasmid/Host   | Genotype/Characterization  |
| Mpa1-234   | pET24b(+)/ER2566   | Kan <sup>r</sup> , NdeI/NotI, C terminal His <sub>6</sub> -tag with thrombin site  |
| Mpa23-234  | pET24b(+)/ER2566   | Kan <sup>r</sup> , NdeI/NotI, C terminal His <sub>6</sub> -tag with thrombin site  |
| Mpa46-234  | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Mpa1-46  | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Mpa46-96   | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Mpa1-96  | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Mpa20-96   | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Mpa98-609  | pET24b(+)/ER2566   | Kan <sup>r</sup> , NdeI/NotI, C terminal His <sub>6</sub> -tag with thrombin site  |
| Mpa97-245  | pET24b(+)/ER2566   | Kan <sup>r</sup> , NdeI/NotI, C terminal His <sub>6</sub> -tag with thrombin site  |
| Mpa1-609<br>C-tag  | pET24b(+)/ER2566   | Kan <sup>r</sup> , NdeI/NotI, C terminal His <sub>6</sub> -tag, non-cleavable      |
| Mpa1-609<br>Wild type  | pET24b(+)/ER2566   | Kan <sup>r</sup> , NdeI/NotI, wild type, native protein                            |
| Pup1-64-GGE<br>N-tag   | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Pup1-64-GGQ<br>N-tag   | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Pup21-64-GGE<br>N-tag  | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Pup21-64-GGQ<br>N-tag  | pET15b/BL21(DE3)   | Amp <sup>r</sup> , NdeI/BamHI, N-terminal His <sub>6</sub> -tag with thrombin site |
| Pup1-64-GGQ<br>C-tag   | pET24b(+)/ER2566   | Kan <sup>r</sup> , NdeI/NotI, C terminal His <sub>6</sub> -tag, non-cleavable      |
| <b>Primers for site-directed mutagenesis of <i>pup-zur-his<sub>6</sub></i></b> |  |  |
| <b>R28AR29A</b>  | F : gggccaggagGcTGCgaaaagctgac<br>R: gtcagcttttcgGCaGCctcctggccc |  |
| <b>L39SL40S</b>  | F: gaccgacgatAGTAGcgacgaaatcga<br>R: tcgatttcgtcCTACTatcgtcggtc  |  |
| <b>V46SL47S</b>  | F: aatcgacgacAGcAGcgaggagaacg<br>R: cgttctcctcgCTgCTgctcgtcgatt  |  |

**Supplementary Table 2. Crystallization conditions**

| <b>Crystal</b>    | <b>Crystallization mother liquor</b>   | <b>Cryo-Protectant</b>                            | <b>Sample preparation</b>   |
|-------------------|--|---|---|
| Mpa1-234          | 30% v/v Tacsimate (1.8305 M Malonic acid, 0.25 M Ammonium citrate tribasic, 0.12 M Succinic acid, 0.3 M DL-Malic acid, 0.4 M Sodium acetate trihydrate, 0.5 M Sodium formate, 0.16 M Ammonium tartrate dibasic), pH 7.0, 21 °C | 30% w/v sucrose, 5% v/v glycerol in mother liquor | ~15 mg ml <sup>-1</sup> Mpa1-234 in buffer: 5 mM HEPES [pH 7.5], 50 mM NaCl, 4mM MgCl <sub>2</sub> , 2 mM β-ME<br>2 μl sample with 2 μl mother liquor   |
| Mpa1-234:Pup1-64  | 30% v/v Tacsimate (1.8305 M Malonic acid, 0.25 M Ammonium citrate tribasic, 0.12 M Succinic acid, 0.3 M DL-Malic acid, 0.4 M Sodium acetate trihydrate, 0.5 M Sodium formate, 0.16 M Ammonium tartrate dibasic), pH 7.0, 21 °C | 30% w/v sucrose, 5% v/v glycerol in mother liquor | Mpa1-234 saturated with overloaded Pup1-64-GGE. ~20 mg ml <sup>-1</sup> in buffer: 5 mM HEPES [pH 7.5], 50 mM NaCl, 4mM MgCl <sub>2</sub> , 2 mM β-ME<br>2 μl sample with 2 μl mother liquor  |
| Mpa46-96          | 0.1 M sodium citrate, 2.1 M ammonium sulfate, 240 mM sodium/potassium tartrate, 250 mM sodium chloride, pH 5.5, 21 °C  | 40% w/v sucrose in mother liquor                  | ~24 mg ml <sup>-1</sup> Mpa46-96 in buffer: 5 mM HEPES [pH 7.5], 250 mM NaCl, 4 mM MgCl <sub>2</sub> , 2 mM β-ME<br>2 μl sample with 2 μl mother liquor   |
| Mpa46-96:Pup21-64 | 0.1M sodium acetate 16% v/v 1,4-dioxane, 250mM sodium chloride, 2mM magnesium chloride with 1X protease inhibitor cocktail (Roche), pH 4.5, 21 °C  | 20% v/v glycerol in mother liquor                 | Mpa46-96 saturated with overloaded Pup21-64-GGE. ~13 mg ml <sup>-1</sup> in buffer: 5 mM HEPES [pH 7.5], 400 mM NaCl, 4 mM MgCl <sub>2</sub> , 2 mM β-ME, 2X protease inhibitor cocktail (Roche)<br>2 μl sample with 2 μl mother liquor |