Supporting Information (SI)

Insights into the mechanism and regulation of the CbbQO-type Rubisco activase, a MoxR AAA+ ATPase

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Figs. S1 to S5

Tables S1 to S2

Other supplementary materials for this manuscript include the following:

Dataset S1

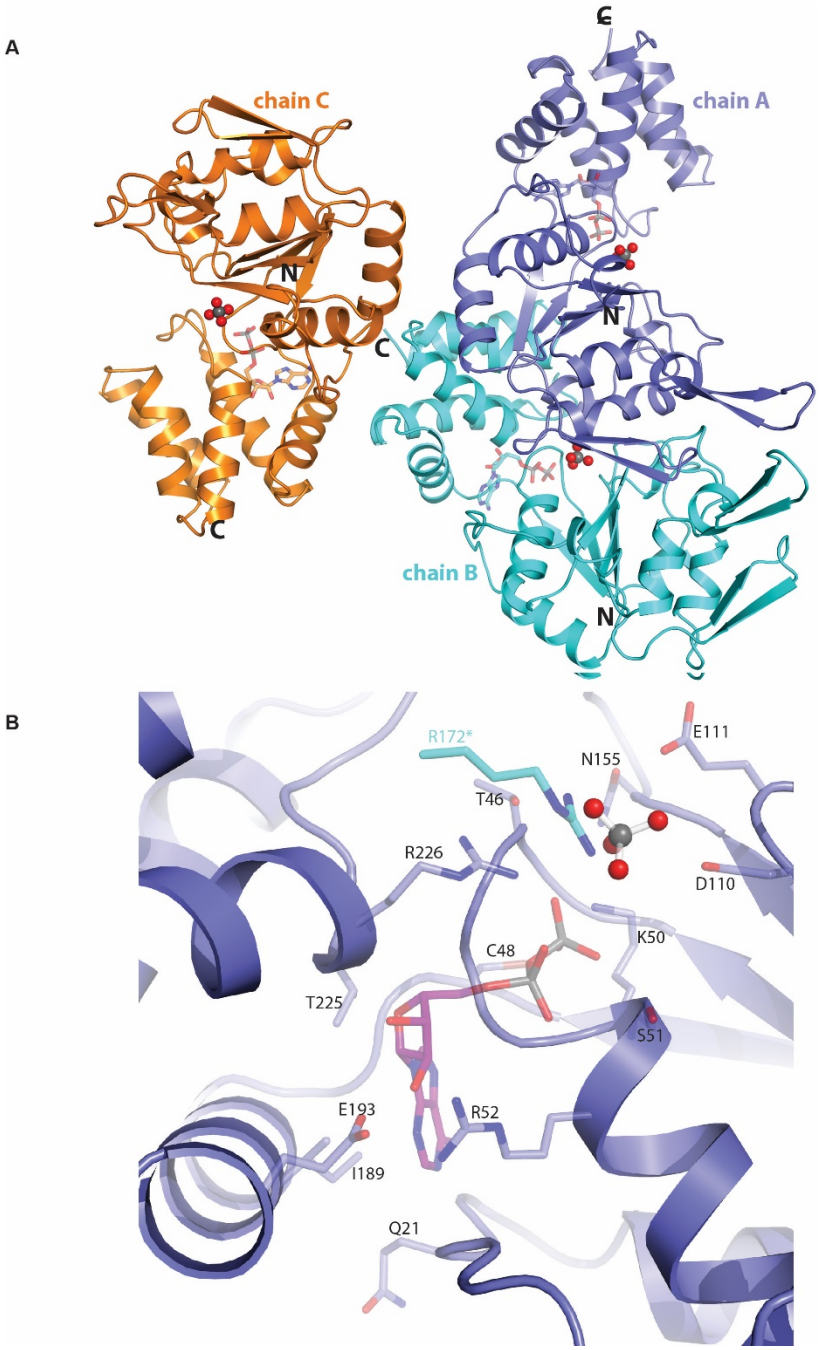

Fig. S1. The crystal structure of CbbQ2. (A) Three subunits (Chains A, B, C are colored slate, cyan and orange) in one ASU. ADP was colored in accordance with its subunit and shown as stick, Pi was shown as sphere. The atoms oxygen, nitrogen and phosphorus are displayed as red, blue and grey, respectively. **(B)** The active site of CbbQ2. Binding pocket of ADP-Pi, the residues interacting with ADP-Pi are shown as sticks, Pi is shown as ball-and-stick. R172 from the adjacent subunit was colored cyan. The atoms of nitrogen, oxygen and phosphorus are colored blue, red and grey, respectively.

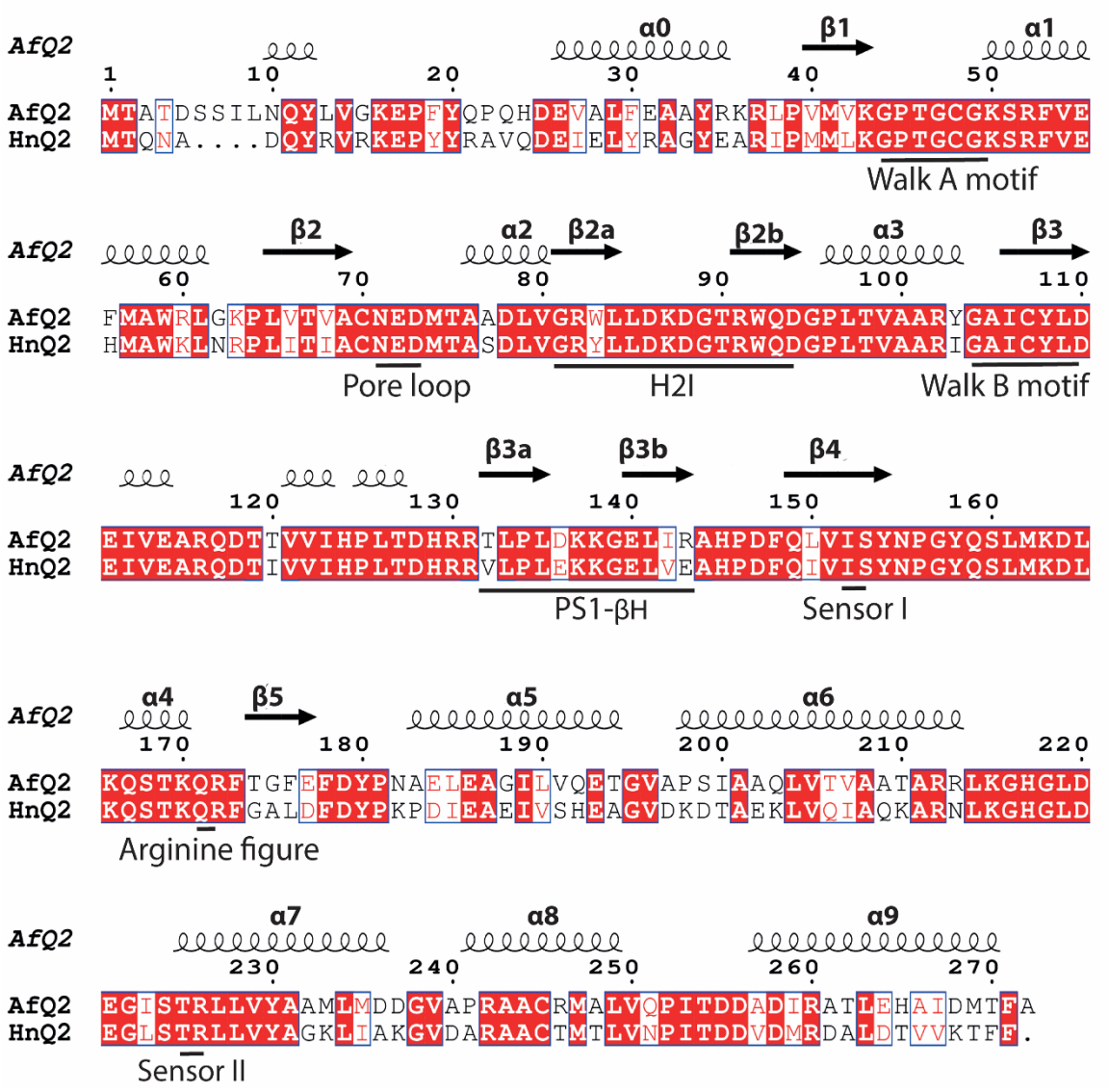

Fig. S2. Structure-based sequence alignment of CbbQ2 with *Hn* CbbQ (PDB code 5C3C) from *Halothiobacillus neapolitanus*.

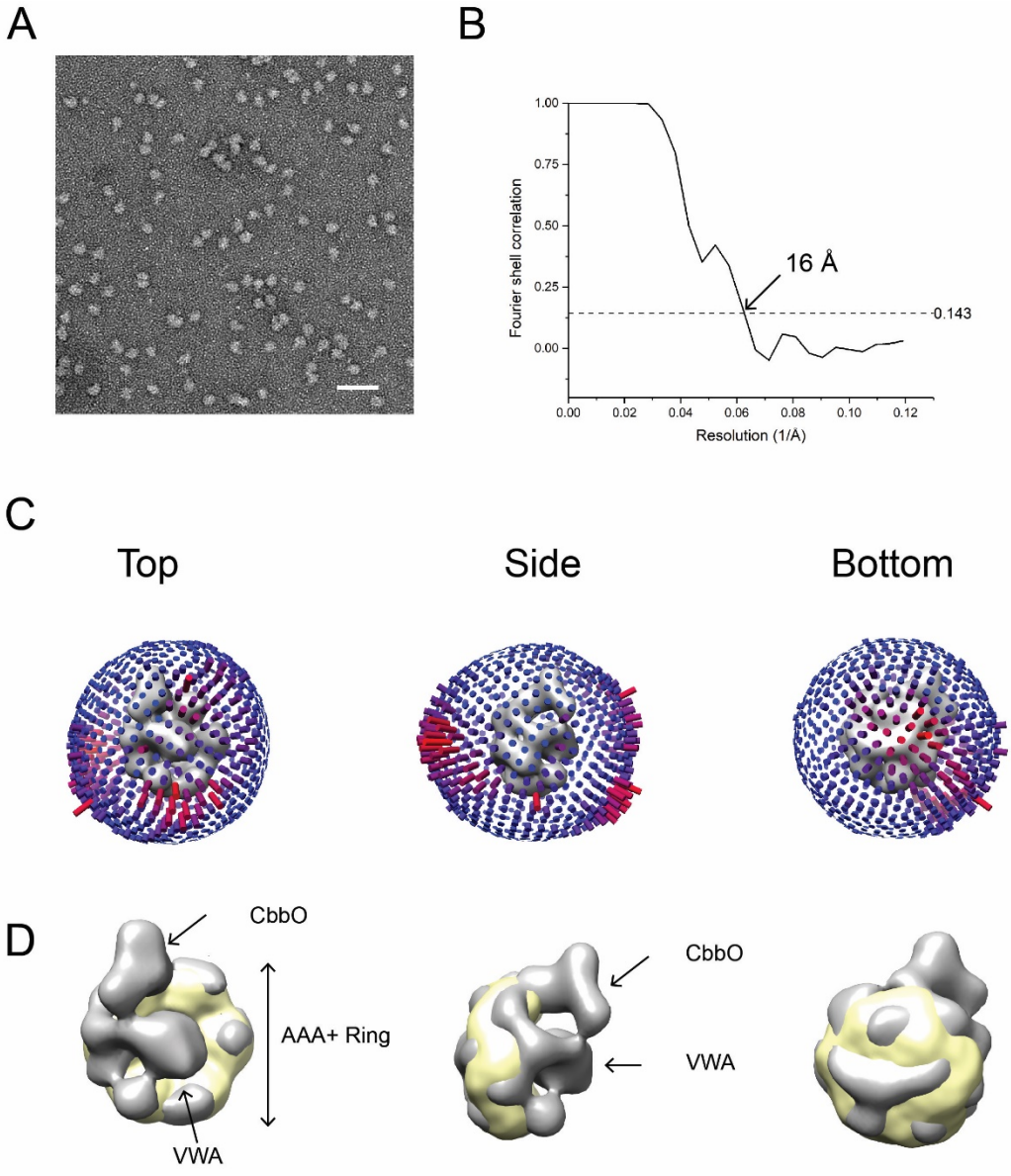

Fig. S3. Electron microscopy of AfQ2O2. (A) Representative micrograph of AfQ2O2 (0.05 mg ml^{-1}) in APO state. Scale bar, 50 nm. **(B)** Fourier shell correlation (FSC) curves of AfQ2O2 as determined by gold standard FSC procedure in Relion-3. **(C)** Angular distribution of the dataset. Each cylinder represents one view, and the height is scaled to the number of particles in that view. **(D)** 3D reconstruction of Q2O2 fit with the EM reconstruction of AfCbbQ2 in yellow (EMDB: EMD-6477), showing additional density on the concave face of the AAA+ ring attributed to CbbO2.

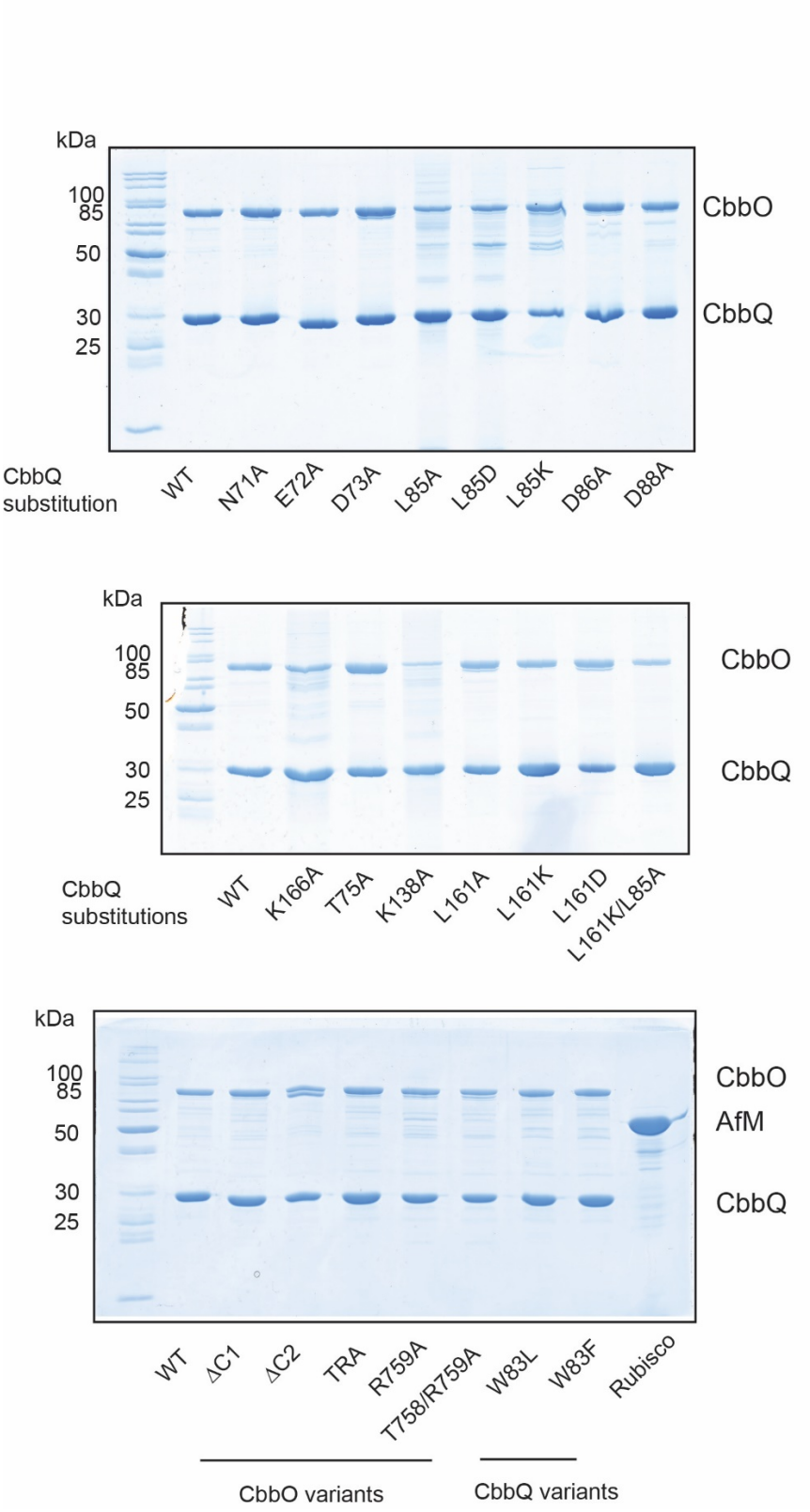

Fig. S4. SDS-PAGE analysis of proteins used in this study. 4 µg of protein was loaded per lane.

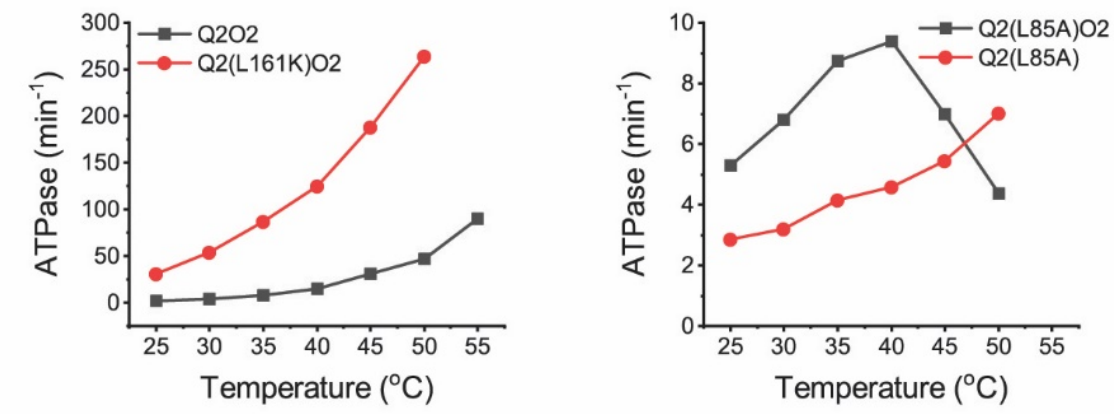

Fig. S5. Temperature activity profiles of selected CbbQ and CbbQO proteins. ATPase activity of the indicated complexes (0.27 μM oligomer) was measured at different temperatures. The protein was preincubated at the target temperature for 10 minutes prior to assay.

Table S1. Data collection for X-Ray crystallographic data.

Synchrotron beamline	NSRRC 13B1
Data collection	
Space group	P6
Unit cell dimensions	
a,b,c (Å)	167.75, 167.75, 48.29
α,β,γ (°)	90, 90, 120
Resolution (Å)	30-2.2 (2.27-2.2)
R_{merge} (%)	9.7 (36.4)
R_{pim}(%)	4.6 (17.8)
$CC_{1/2}$(%)	99.9(95.0)
I/σI	16.1 (5.1)
Completeness (%)	99.9 (99.5)
Redundancy	10.5 (9.7)
Refinement	
Resolution (Å)	30-2.2
R_{work}/R_{free} (%)	19.4/23.8
Average B factor (Å^2)	
Protein	38.0
Ligand	30.6
PO_4^{3-}	42.3
Water	34.9
R.m.s.deviations	
Bond length (Å)	0.005
Bond angle (°)	0.945
Ramachandran plot	
Favored regions (%)	97.36
Allowed regions (%)	2.51
Outlier (%)	0.13

* Numbers in parenthesis refer to outer resolution shell

Table S2. Mutagenic primers used in this study.

Primer name	Primer sequence (5’ to 3’)
AfQ2N71A_For	ACCGTAGCCTGTGCAGAGGACATGACC
AfQ2N71A_Rev	GGTCATGTCCTCTGCACAGGCTACGGT
AfQ2E72A_For	TAGCCTGTAACGCGGACATGACCGC
AfQ2E72A _Rev	GCGGTCATGTCCGCGTTACAGGCTA
AfQ2D73A_For	CCTGTAACGAGGCCATGACCGCCGC
AfQ2D73A_Rev	GCGGCGGTCATGGCCTCGTTACAGG
AfQ2L85A_For	GCCGCTGGCTGGCCGACAAGGACG
AfQ2L85A_Rev	CGTCCTTGTCGGCCAGCCAGCGGC
AfQ2L85D_For	GCCGCTGGCTGGATGACAAGGACGGT
AfQ2L85D_Rev	ACCGTCCTTGTCATCCAGCCAGCGGC
AfQ2L85K_For	GCCGCTGGCTGAAAGACAAGGACGGT
AfQ2L85K_Rev	ACCGTCCTTGTCTTTCAGCCAGCGGC
AfQ2D86A_For	GCTGGCTGCTCGCCAAGGACGGTAC
AfQ2D86A_Rev	GTACCGTCCTTGGCGAGCAGCCAGC
AfQ2D88A_For	TGCTCGACAAGGCAGGTACCCGTTGG
AfQ2D88A_Rev	CCAACGGGTACCTGCCTTGTCGAGCA
AfQ2K166A_For	CTGATGAAGGATCTCGCGCAATCCACCAAGCA
AfQ2K166A_Rev	TGCTTGGTGGATTGCGCGAGATCCTTCATCAG
AfQ2T75A_For	CTAACGAGGACATGGCCGCCGCCGAT
AfQ2T75A_Rev	ATCGGCGGCGGCCATGTCCTCGTTAG
AfQ2K138A_For	CCGCTGGACAAGGCGGGCGAACTGATC
AfQ2K138A_Rev	GATCAGTTCGCCCGCCTTGTCCAGCGG
AfQ2L161A_For	GCTATCAGTCGGCGATGAAGGATCT
AfQ2L161A_Rev	AGATCCTTCATCGCCGACTGATAGC
AfQ2L161K_For	GGCTATCAGTCGAAAATGAAGGATCTC
AfQ2L161K_Rev	GAGATCCTTCATTTTCGACTGATAGCC
AfQ2L161D_For	GCTATCAGTCGGATATGAAGGATCT
AfQ2L161D_Rev	AGATCCTTCATATCCGACTGATAGC
AfO2ΔC1_For	ATCGCCCTGACCTAATAAAGCTTGGCT
AfO2ΔC1_Rev	AGCCAAGCTTTATTAGGTCAGGGCGAT
AfO2ΔC2_For	TTCATCGCCCTGTAACGGTAAAGCTTG
AfO2ΔC2_Rev	CAAGCTTTACCGTTACAGGGCGATGAA
AfO2TRA_For	GCCCTGACCCGGGCATAAAGCTTGGCT
AfO2TRA_Rev	AGCCAAGCTTTATGCCCGGGTCAGGGC
AfO2R759A_For	ATCGCCCTGACCGCATAAAGCTTGGCT
AfO2R759A_Rev	AGCCAAGCTTTATGCGGTCAGGGCGAT
AfO2T758A/R759A_For	TTCATCGCCCTGGCAGCATAAAGCTTGGCT
AfO2T758A/R759A_Rev	AGCCAAGCTTTATGCTGCCAGGGCGATGAA