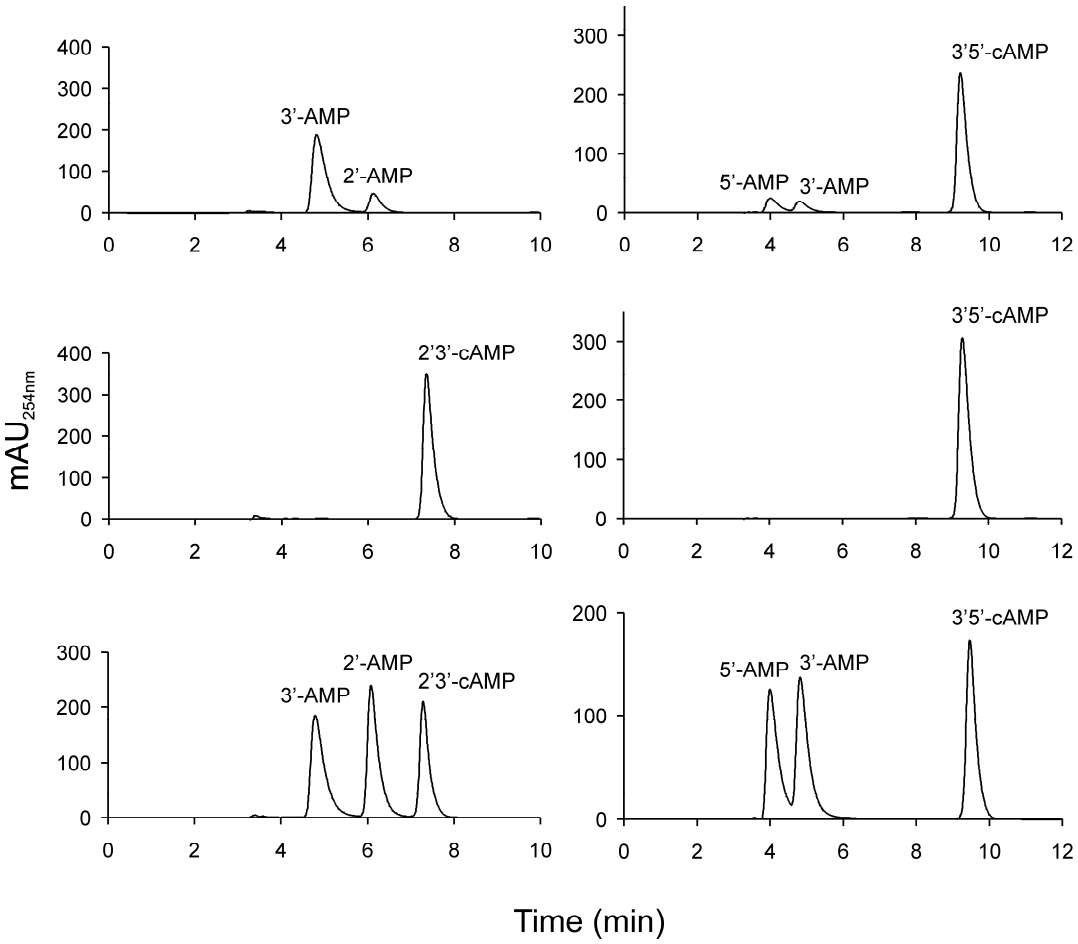
Supplementary Table 1: Oligonucleotides used in this study

Primer Name	Primer sequence (5'-3')
0805H98A	GCGCCGAGCTGGTCTGGGTGATGGGTAACGCCGACGACCGGGCC
0805H140A	GCGGATTTCGCCGTGAGCATGCCCGGGTACCGAGGTATC
0805H209A	CATTTTGGCCGGGCACCTGGCCTACAGTACTAATGCACCTTCGTCGG
0805Y229A	CGACTTGCGCCACCCAGGACCTGACCG

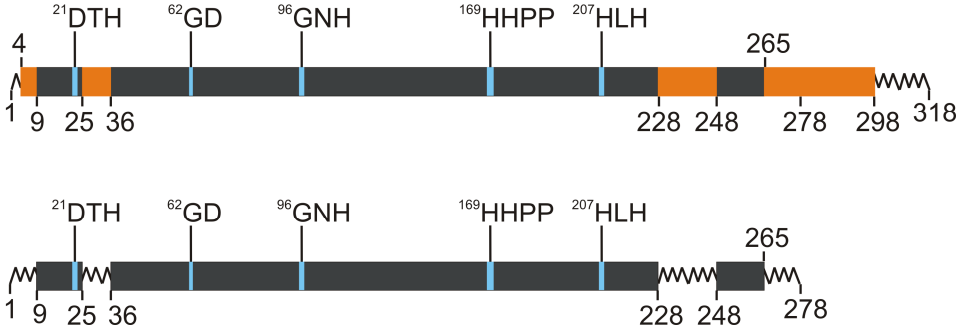
Supplementary Methods

Rv0805$^{1-318}$ protein (1 μM) was incubated with 5 mM of 2'3'-cAMP or 3'5'-cAMP in 50 mM Tris pH 8.5, 10 mM NaCl, 200 μM $MnCl_2$ and 5 mM 2-mercaptoethanol in a volume of 25 μl, at 37°C. The reaction was terminated after 2 hours of incubation by addition of 50 μl of 20mM $(NH_4)H_2PO_4$ pH 6.2. As a control, an assay mixture without any protein added was used.

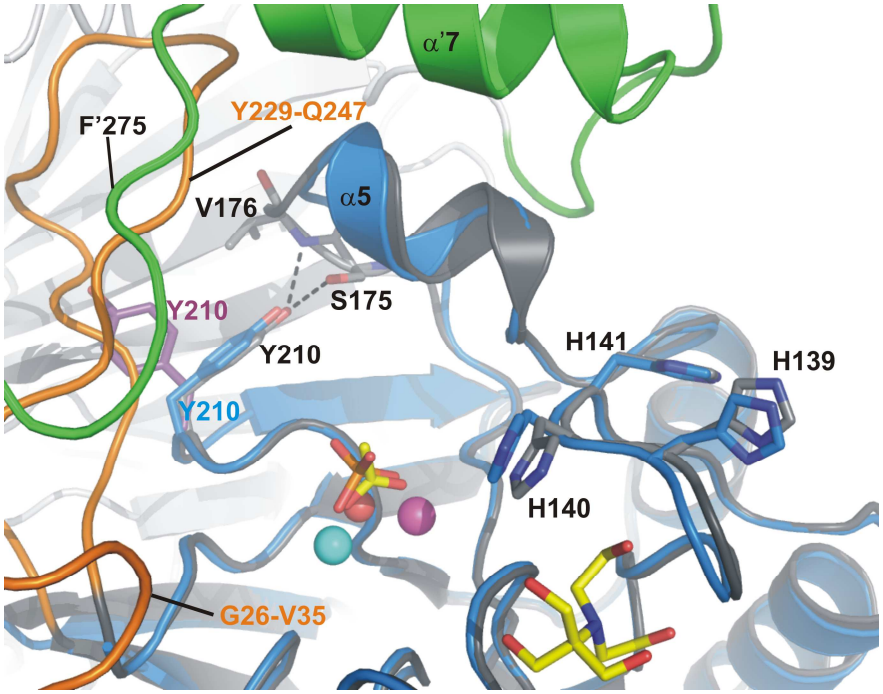
Samples were analyzed by HPLC system (Agilent 1100 Series, Agilent Interface 35900E, USA) using SUPELCOSIL™ LC-8 HPLC Column (SUPELCO, USA: 5 μm particle size, 25 cm × 4.6 mm), and a Guard column: SUPELCOSIL ™ LC-8 Supelguard™ Cartridge (SUPELCO, USA: 5 μm, 2 cm × 4.6 mm). Buffer system: buffer A: 20 mM $(NH_4)H_2PO_4$ pH 6.2; buffer B: 20 mM $(NH_4)H_2PO_4$ pH 6.2 + 20% acetonitrile. Buffers A and B were mixed to give a final mixture of 20 mM $(NH_4)H_2PO_4$ pH 6.2 + 13% methanol, which was used for equilibration of the column and separation of components at a flow rate of 1 ml/min, and maximal column pressure 120 bar. Samples were injected in volumes of 5 μL. Retention times of nucleotides (2'3'-cAMP, 3'5'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP) were determined by separation of standard 10 mM nucleotide solutions in 10 μL injections, individually as well as in mixture.



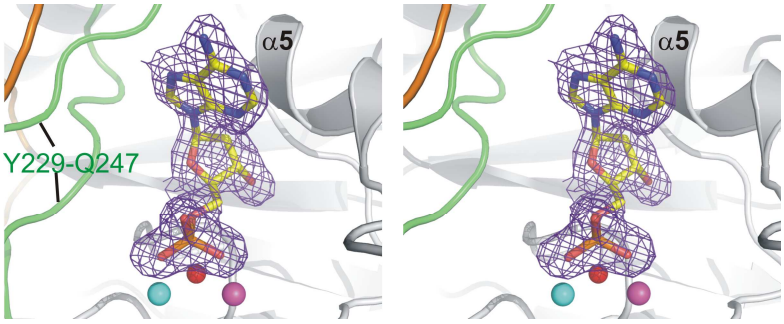
Supplemental Figure 1. *In vitro* assay of Rv0805$^{1-318}$ with cyclic nucleotides, analyzed by HPLC. **a,** 2'3'-cAMP, **b,** 3'5'-cAMP. The incubation time was 2 h at 37°C. Upper panels show the analysis of reaction assay in the presence of Rv0805$^{1-318}$. Middle panels: control without enzyme. Bottom panels: standard mixture of nucleotides.



Supplemental Figure 2. Schematic representation of structural elements determined in **a,** Rv0805$^{1\text{-}318}$ and **b,** Rv0805$^{1\text{-}278\ 13}$. Rectangular blocks show the parts of the structures that were defined in the electron density. Zig-zag line represents the unstructured regions, although present in the protein polypeptide chain in the crystal. The highly conserved amino acid residues are marked (blue lines), as well as numbers of some other residues, for clarity. The new parts of structure defined in Rv0805$^{1\text{-}318}$ are marked in orange blocks. For simplicity, 12 residues on the N-terminus that are from the vector and remain after the TEV-protease cleavage, are not shown.



Supplemental Figure 3. Superposition of Rv0805$^{1-278}$ onto Rv0805$^{1-318}$. Main chain of Rv0805$^{1-278}$ is in light blue and Rv0805$^{1-318}$ is colored as in the main text figures. Two equally occupied alternative conformations of Y210 in Rv0805$^{1-278}$ (PDB-ID: 2HY1) are shown (light blue and magenta side chains (colors of carbon); oxygen: red). In addition side chains of three histidine from the loop V136-G142 are shown (Rv0805$^{1-278}$: carbon: light blue; nitrogen: blue; Rv0805$^{1-318}$: carbon: dark-gray; nitrogen; blue). Acetate and bis-Tris bound to Rv0805$^{1-318}$ are shown as sticks (carbon: yellow; nitrogen: blue; oxygen: red), as well as the phosphate from Rv0805$^{1-278}$ (phosphorus: orange; oxygen: red). Hydrogen bonds between Y210 in Rv0805$^{1-318}$ and S175 and V176 of the same Rv0805$^{1-318}$ protomer are marked as black dashed lines. Fe^{3+}: cyan spehere, Mn^{2+}: magenta sphere; planar active site water: red sphere. Residues belonging to light-gray/green protomer of Rv0805$^{1-318}$ are marked by a prime (') and of dark-gray/orange are non-primed. The two structures were superimposed on CA atoms of residues R40-P220.



Supplemental Figure 4. Stereo representation of a difference electron density map (Fo-Fc; magenta dash) at 2σ before 5'-AMP was included into the refinement. Rv0805$^{1-318}$ is colored as in Figure 1. 5'-AMP is shown in sticks (carbon yellow, oxygen: red, nitrogen: blue; phosphorus: orange). Fe^{3+}: cyan spehere, Mn^{2+}: magenta sphere; planar water: red sphere.