

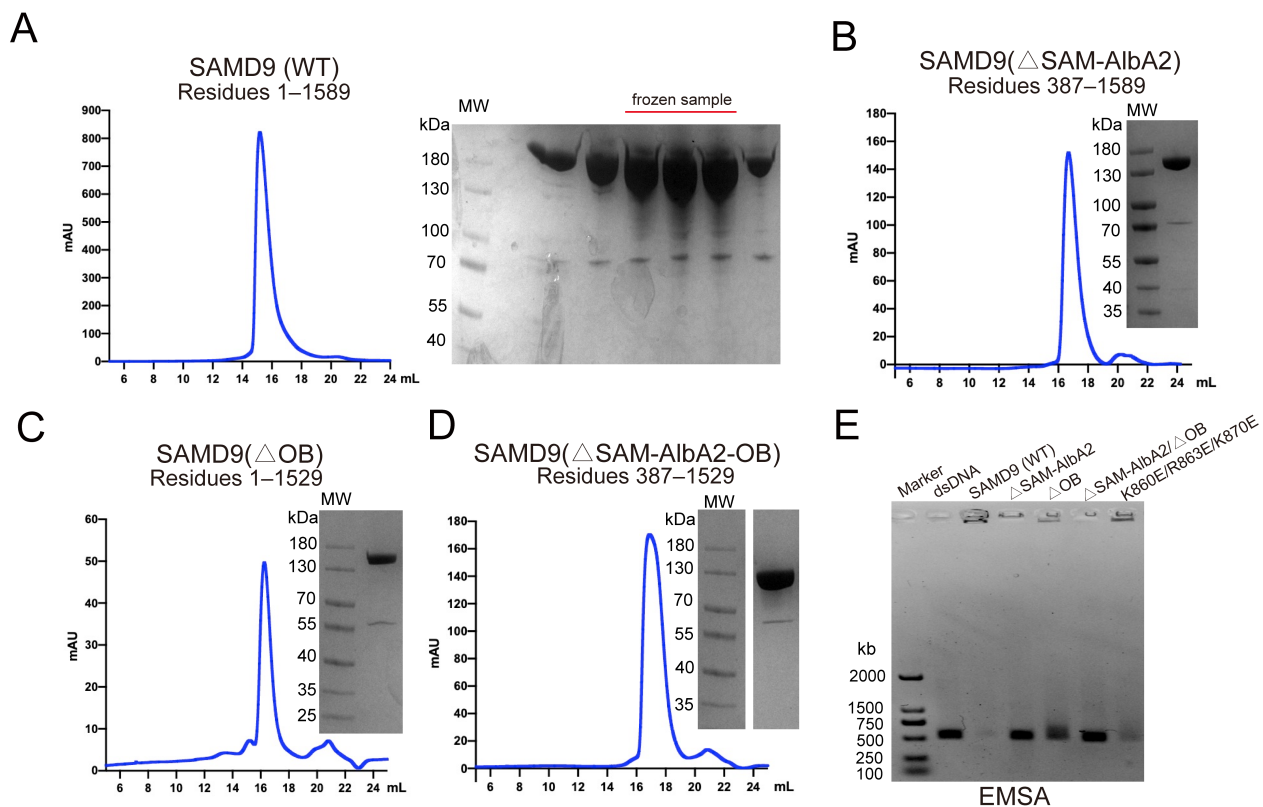
Supplementary Information for

The structural basis of human SAMD9 and the functional separation of its dual immune activities

Authors

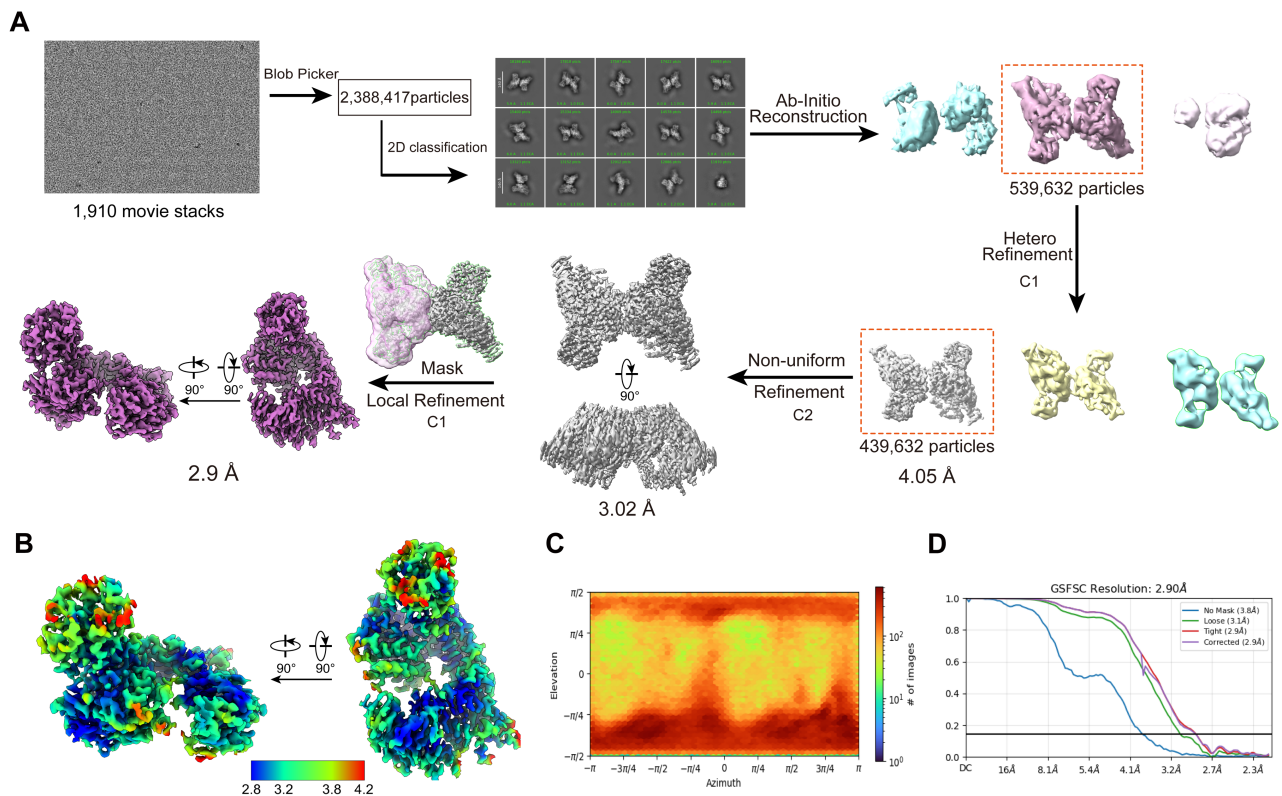
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This file contains Extended Data Figure 1-6 and Table 1.



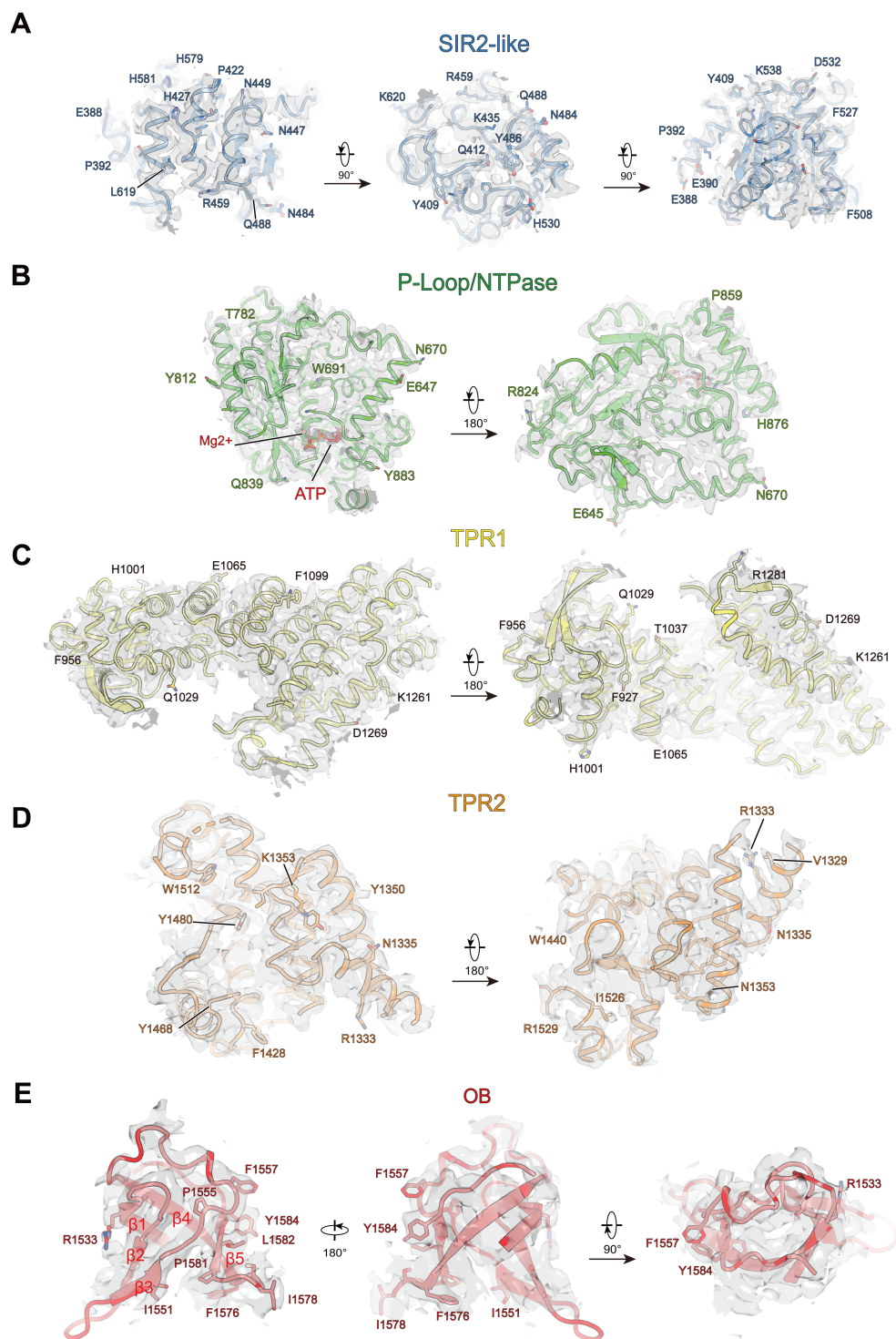
Extended Data Figure 1. Purification and nucleic acid-binding analysis of SAMD9 and domain-deletion variants.

A–D, Representative gel filtration profiles and SDS-PAGE analyses of purified SAMD9 WT (**A**), SAMD9ΔSAM-AlbA2 (**B**), SAMD9ΔOB (**C**), and SAMD9ΔSAM-AlbA2/ΔOB (**D**). **E**, EMSA analysis of dsDNA binding by SAMD9 WT, domain-deletion variants, and the dimerization-deficient mutant K860E/R863E/K870E. n = 3 independent experiments.



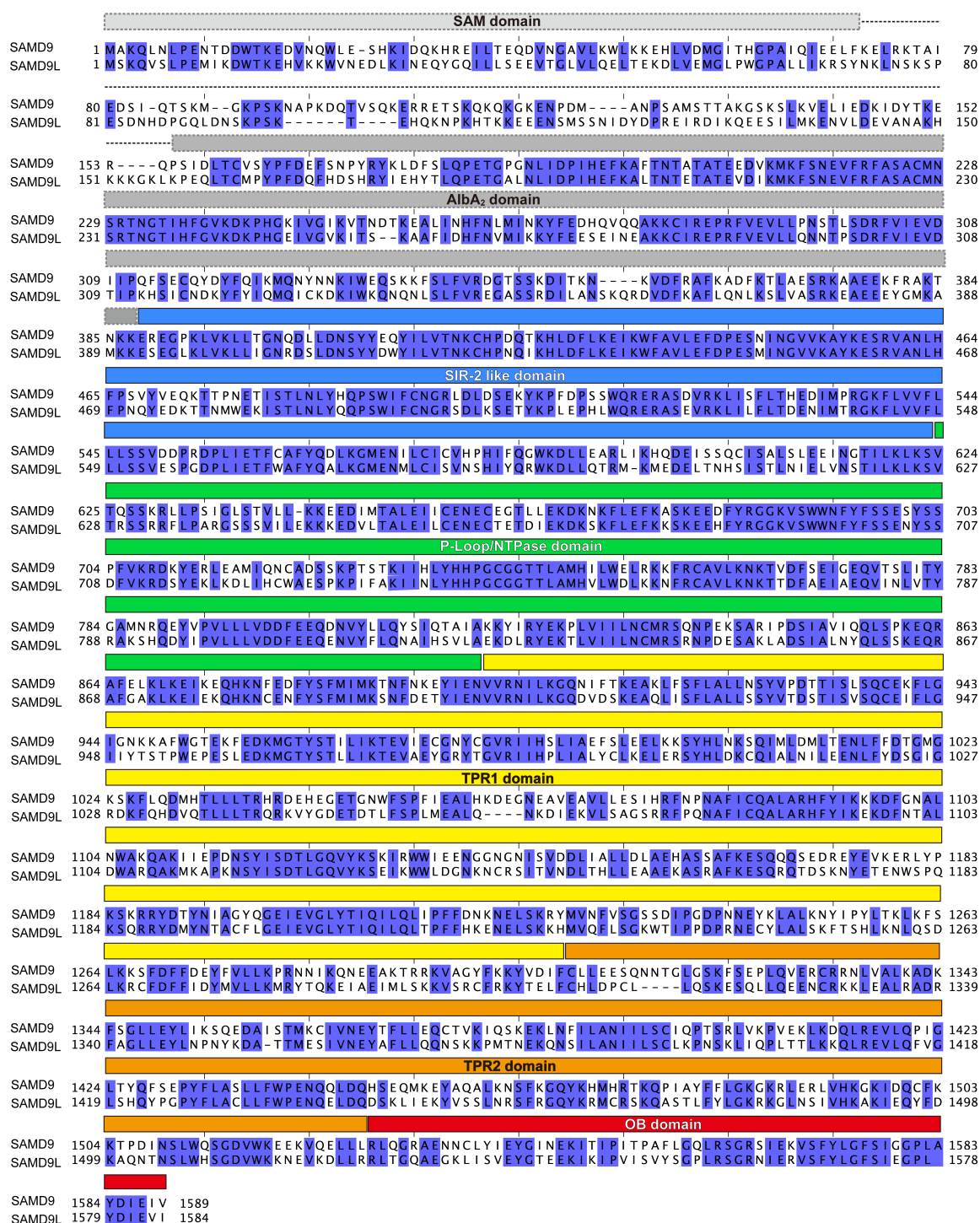
Extended Data Figure 2. Cryo-EM data processing of human SAMD9

A, Flowchart of cryo-EM data processing for human SAMD9. Particle picking and 2D classification were performed to remove bad particles, followed by ab initio reconstruction, heterogeneous refinement, and non-uniform refinement. The resulting particles were further subjected to local refinement using a focused mask. The final EM map was reconstructed at 2.9 Å resolution. **B**, Local resolution distribution of the final map of hSAMD9. **C**, Angular distribution of cryo-EM reconstruction of hSAMD9 used for the final refinement. **D**, Fourier shell correlation (FSC) curves of the final hSAMD9 map. The resolution of the final map was determined at FSC = 0.143.



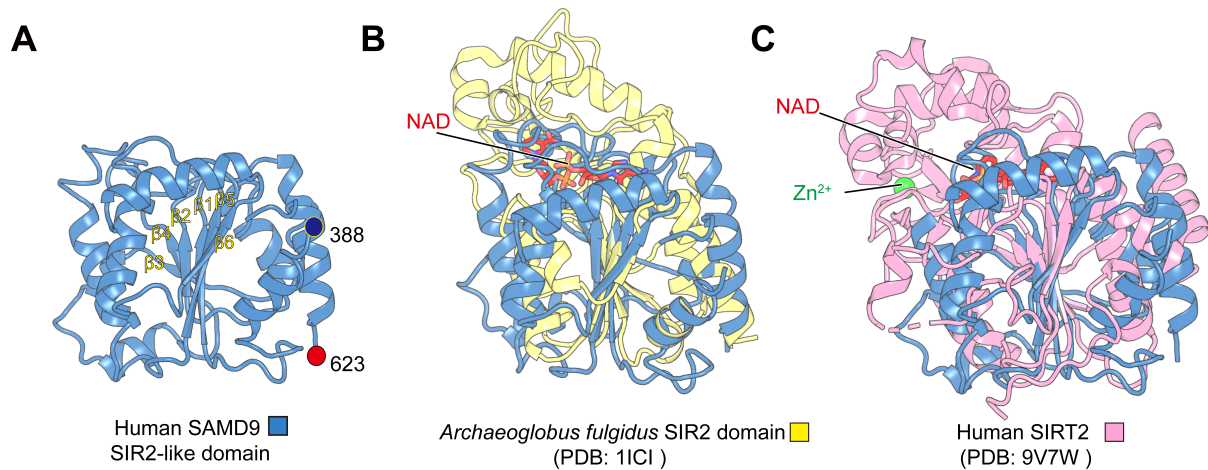
Extended Data Figure 3. Representative EM densities of SAMD9

A–E, Representative EM densities of the SIR2-like (A), P-loop/NTPase (B), TPR1 (C), TPR2 (D), and OB (E) domains of SAMD9 are shown as gray surfaces. The corresponding atomic models are shown in cartoon representation, with representative side chains shown as sticks.



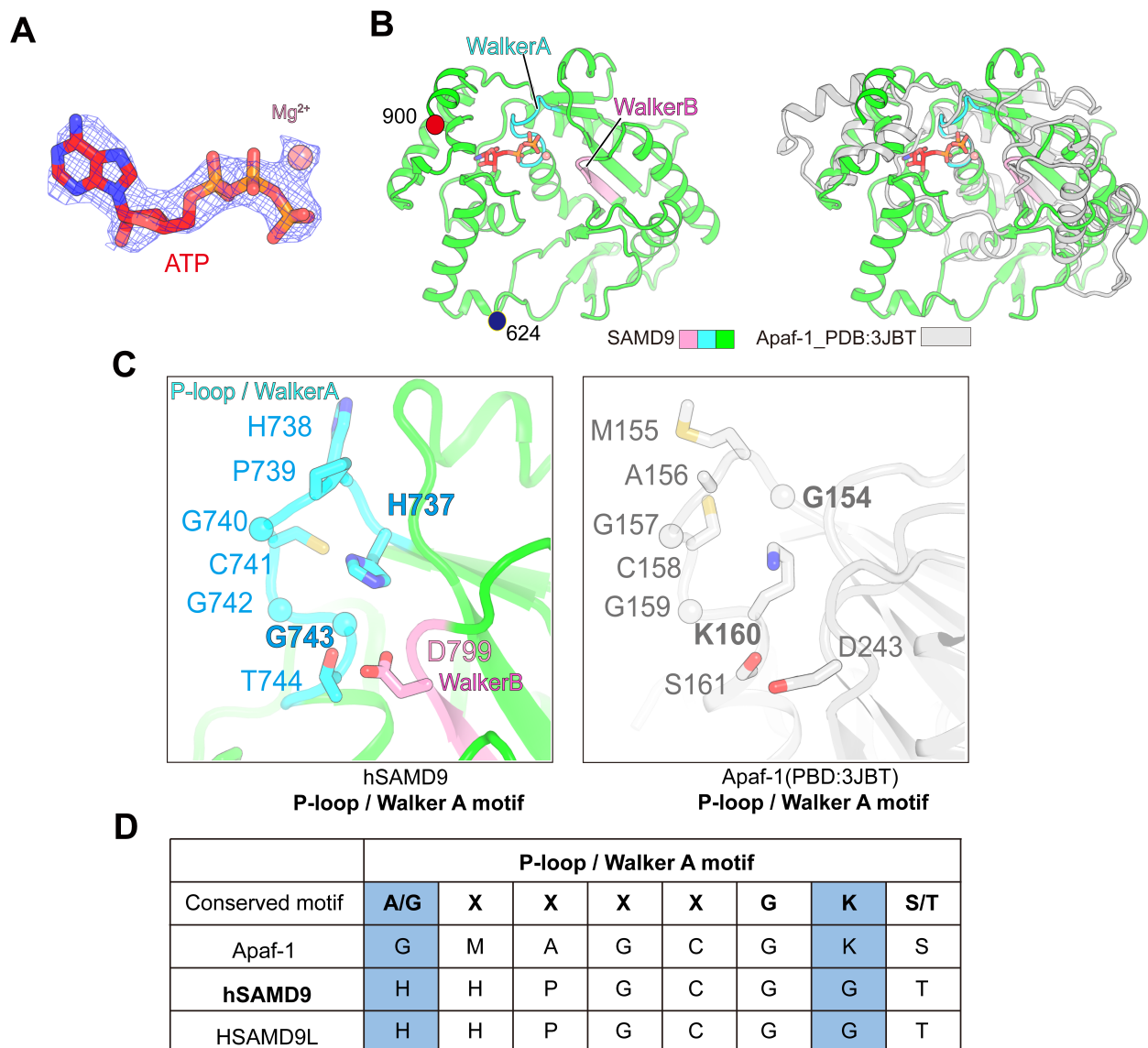
Extended Data Figure 4. Sequence alignment of SAMD9 and SAMD9L

Sequence alignment of human SAMD9 and SAMD9L. Conserved residues are highlighted in blue. The SAM, AlbA₂, SIR2-like, P-loop/NTPase, TPR1, TPR2, and OB domains are indicated by gray, gray, cyan, green, yellow, orange, and red bars, respectively. Sequence alignment was generated using Jalview8.3.



Extended Data Figure 5. Structural comparison of the SAMD9 SIR2-like domain with representative sirtuins

A, Overall structure of the human SAMD9 SIR2-like domain. The N- and C-terminal residues are indicated. **B**, Structural superposition of the SAMD9 SIR2-like domain with the SIR2 domain from *Archaeoglobus fulgidus* (PDB: 1ICI). Bound NAD is shown in red. **C**, Structural superposition of the SAMD9 SIR2-like domain with human SIRT2 (PDB: 9V7W). Bound NAD and Zn²⁺ are indicated.



Extended Data Figure 6. Structural comparison of the SAMD9 P-loop/NTPase domain with Apaf-1

A, Cryo-EM density of ATP and the coordinated Mg^{2+} ion bound within the P-loop/NTPase domain of SAMD9. **B**, Overall structure of the SAMD9 P-loop/NTPase domain and structural superposition with the nucleotide-binding domain of Apaf-1 (PDB: 3JBT). The Walker A and Walker B motifs are indicated. **C**, Close-up comparison of the P-loop/Walker A and Walker B regions of SAMD9 and Apaf-1, highlighting the noncanonical Gly743 residue in SAMD9 corresponding to Lys160 in Apaf-1. **D**, Sequence comparison of the P-loop/Walker A motif in Apaf-1, SAMD9, and SAMD9L, showing the conserved substitution of the canonical lysine by glycine in SAMD9/9L.

SAMD9 (EMDB-83565) (PDB: 8HIP)	
Data collection and processing	
Magnification	130,000 ×
Voltage (kV)	300
Electron exposure (e ⁻ /Å ²)	50
Defocus range (μm)	-1.2 ~ -2.0
Pixel size (Å)	1.0825
Symmetry imposed	C1
Initial particle images (no.)	2,388,417
Final particle images (no.)	439,632
Map resolution (Å)	2.9
FSC threshold	0.143
Map resolution range (Å)	2.8 ~ 4.2
Refinement	
Initial model used (PDB code)	AF3 model
Model resolution (Å)	2.9
FSC threshold	0.5
Map sharpening <i>B</i> factor (Å ²)	-87.7
Model composition	
Non-hydrogen atoms	9,177
Protein residues	1,114
Ligands	2
<i>B</i> factors (Å ²)	
Protein	86.9
Ligand	37.4
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.670
Validation	
MolProbity score	2.15
Clashscore	12
Ramachandran plot	
Favored (%)	95.89
Allowed (%)	3.93
Disallowed (%)	0.18