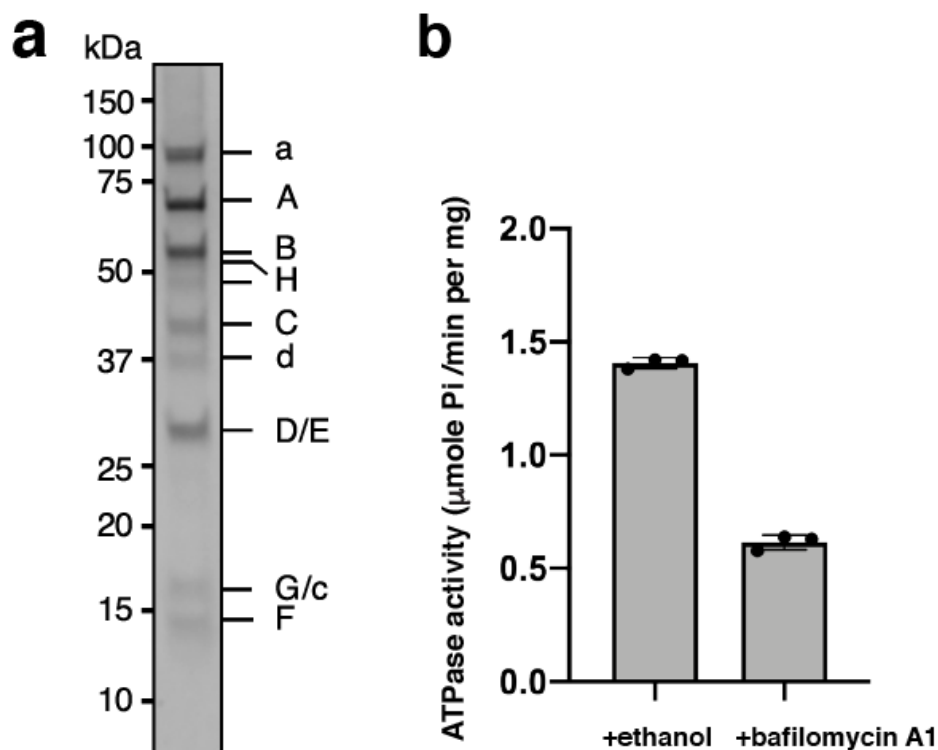


## Cryo-EM structures of intact V-ATPase from bovine brain

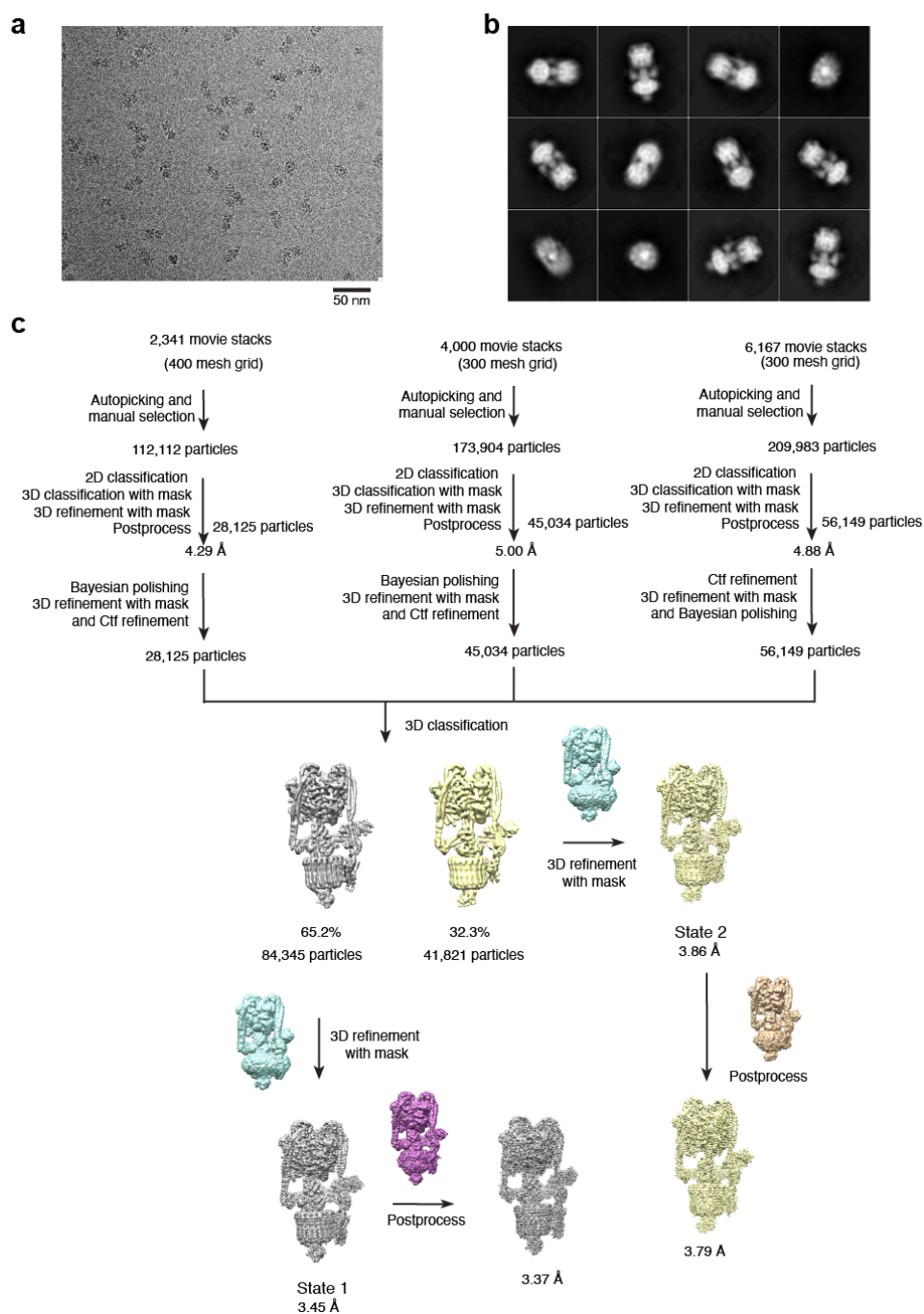
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**Supplementary Fig. 1 V-ATPase activity assay.**

**a.** SDS-PAGE for V-ATPase from endogenous bovine brain. **b.** The purified bovine V-ATPase in 20 mM Hepes pH 7.5, 150 mM NaCl, 0.1% CHAPS, 0.004% glyco-diosgenin was used for V-ATPase activity assay, and the activity was measured as liberation of  $^{32}\text{Pi}$  from  $[\gamma\text{-}^{32}\text{P}]$  ATP as described in methods. The addition of bafilomycin A1 at 1  $\mu\text{M}$  decreased the V-ATPase activity comparing with WT. Each bar represents the mean of triplicate determination of the experiment repeated at least twice. Data are mean  $\pm$  s.d. Source data are provided as a source data file.



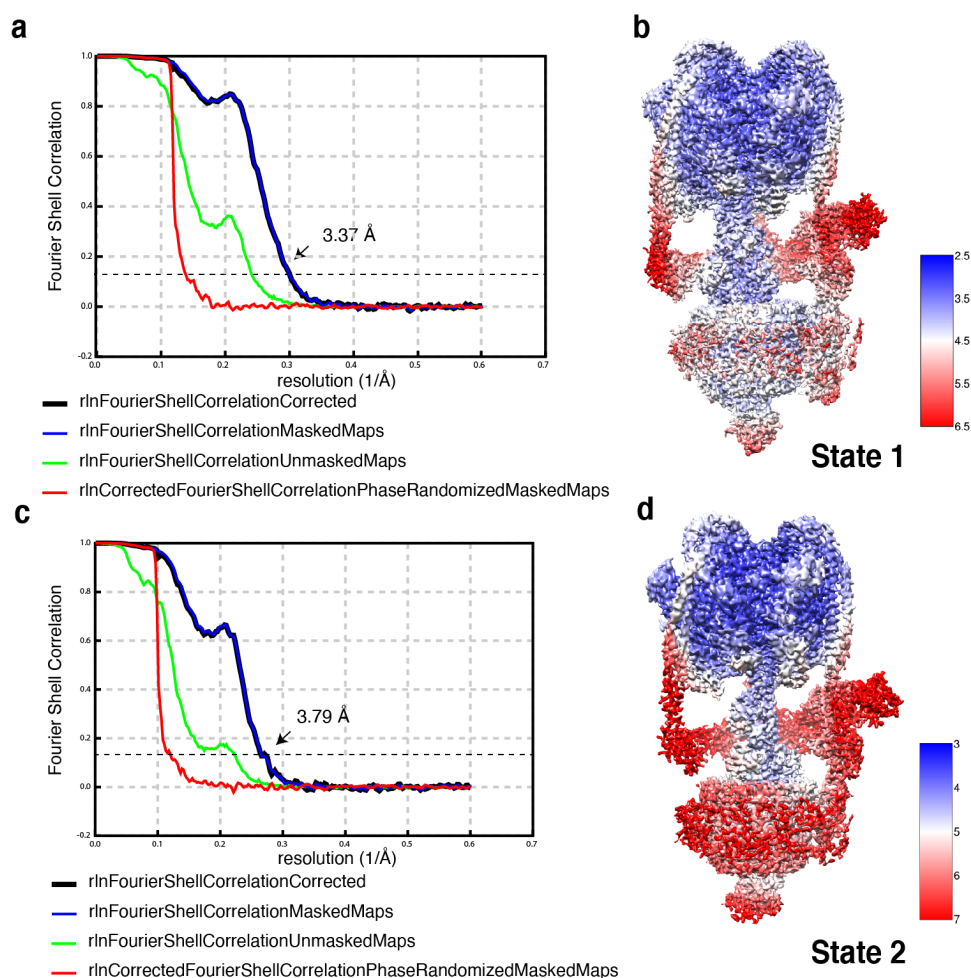
### Supplementary Fig. 2 Data processing.

**a.** A representative electron micrograph at  $-2.0\ \mu\text{m}$  defocus. **b.** The cryo-EM 2D classification from RELION is shown. **c.** The data processing work-flow.



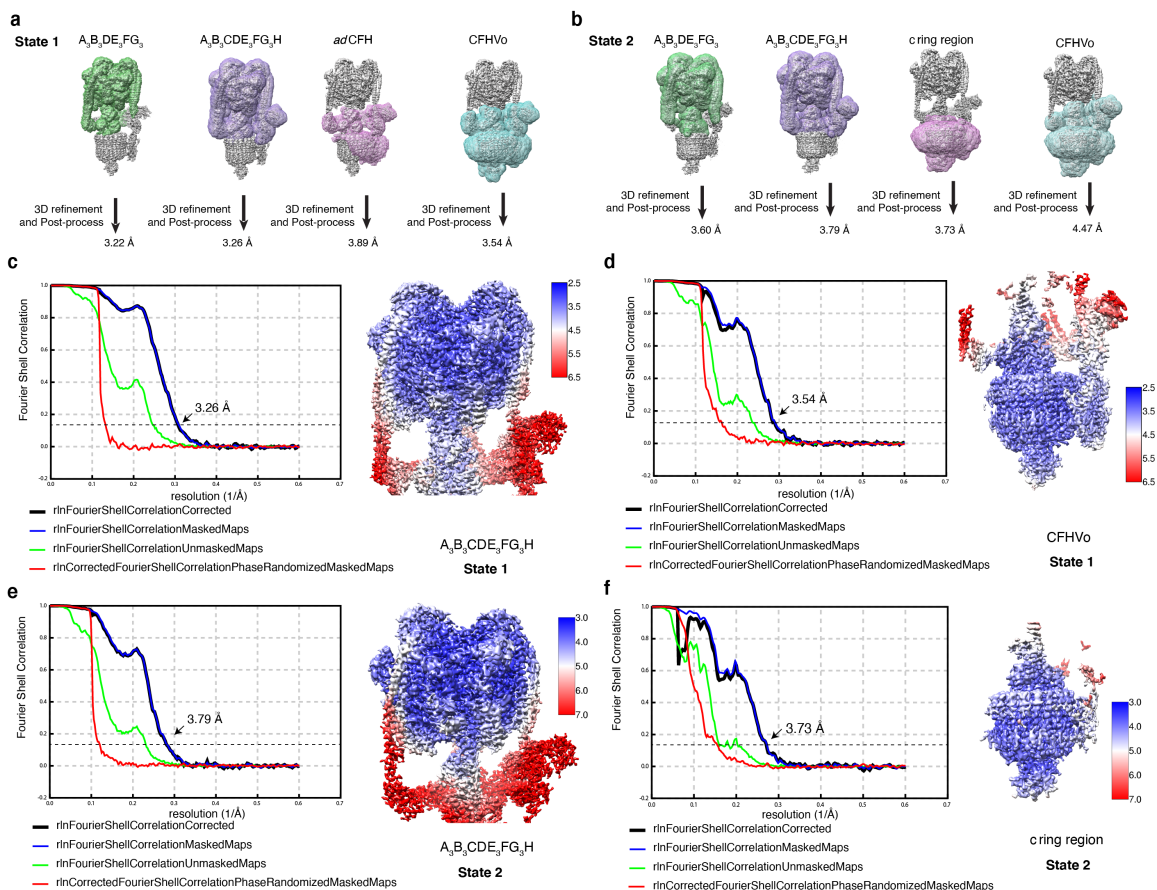
**Supplementary Fig. 3 Sequence alignment of models of individual subunits of bovine V-ATPase that generated from the MODELLER software by Esript 3.0.**

The first sequence is the model we refer to for each subunit modeling as described in method, the second sequence is our bovine sequence, and the third sequence is an alternative model from other species with lower rank. Eh, Bt, Tt, Sc represent *Enterococcus hirae*, *Bos taurus*, *Thermus thermophilus*, *Saccharomyces cerevisiae*, respectively. For subunit A and B, the Eh model is from PDB-5ZE9, the Tt model is from PDB-3GQB; For subunit C, the Sc model is from PDB-1U7L, the Tt model is from PDB-6R0W; For subunit D, the Sc model is from PDB-4RND, the Eh model is from PDB-3AON; For subunit E and G, the Sc model is from PDB-4EFA, the Tt model is from PDB-3V6I; For subunit F, the Sc model is from PDB-4IX9, the Tt model is from PDB-2D00; For subunit H, the Sc model is from PDB-1HO8; For subunit *a*, the Sc model is from PDB-6O7U, the Tt model is from PDB-6R0W; For subunit *c* and *c'*, the Sc model is from PDB-6O7U, the Eh model is from PDB-2BL2; For subunit *d*, the Sc model is from PDB-6O7U, the Tt model is from PDB-1V9M; For subunit *e*, the Sc model is from PDB-6O7U. Strictly conserved residues are highlighted in shaded red boxes and conserved residues in open red boxes. The secondary structures of the model are placed on the top of the alignments.



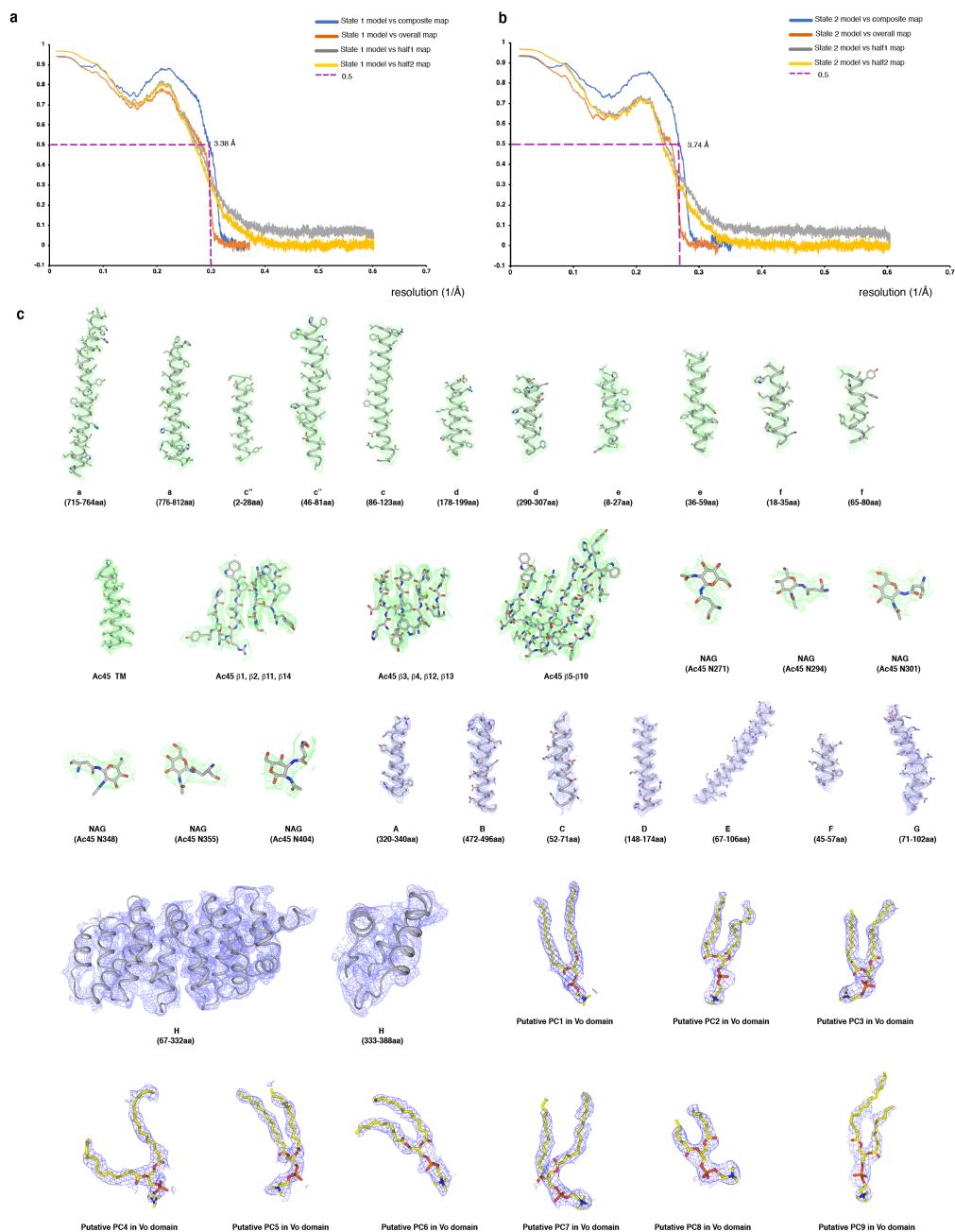
**Supplementary Fig. 4 FSC curve and local resolution estimation of the intact V-ATPase in the two states.**

**a.** Fourier shell correlation (FSC) curve as a function of resolution of state 1 using RELION-3 output. **b.** Density maps of state 1 structure colored by local resolution estimation using RELION-3. **c.** Fourier shell correlation (FSC) curve as a function of resolution of the state 2 using RELION-3 output. **d.** Density maps of state 2 structure colored by local resolution estimation using RELION-3.



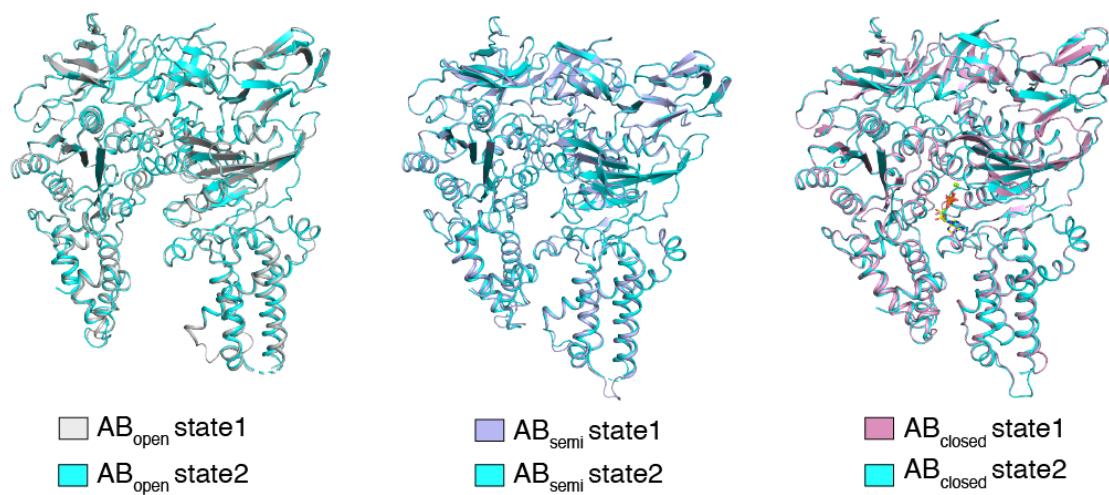
**Supplementary Fig. 5 The focused maps and their FSC curves, local resolution estimation of the focused map of V1 and Vo domains in the two states.**

**a.** and **b.** The masked regions for focused refinement. **c.** and **d.** Fourier shell correlation (FSC) curve as a function of resolution of the V1 of the state 1 (**c**) and the Vo of the state 1 (**d**) using RELION-3 output. Density maps of the V1 of the state 1 (**c**) and the Vo of the state 1 (**d**) are colored by local resolution estimation using RELION-3. **e.** and **f.** Fourier shell correlation (FSC) curve as a function of resolution of the V1 of the state 2 (**e**) and the Vo of the state 2 (**f**) using RELION-3 output. Density maps of the V1 of the state 2 (**e**) and the Vo of the state 2 (**f**) are colored by local resolution estimation using RELION-3.



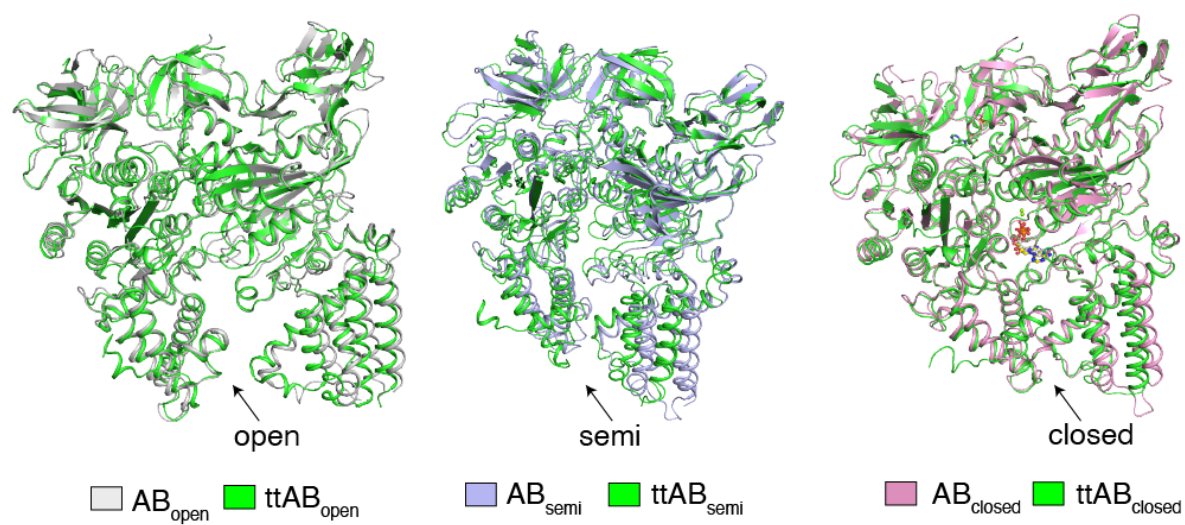
**Supplementary Fig. 6 Cryo-EM map of structural elements.**

**a.** and **b.** The FSC curves of state 1 (**a**) and state 2 (**b**) calculated between the refined structure model and the composite map (blue) used for refinement, the overall half map (gray), the other half map (yellow) and the overall full map (orange). **c.** The structural elements of the state 1 with cryo-EM map. The maps are shown at  $5\sigma$  level.



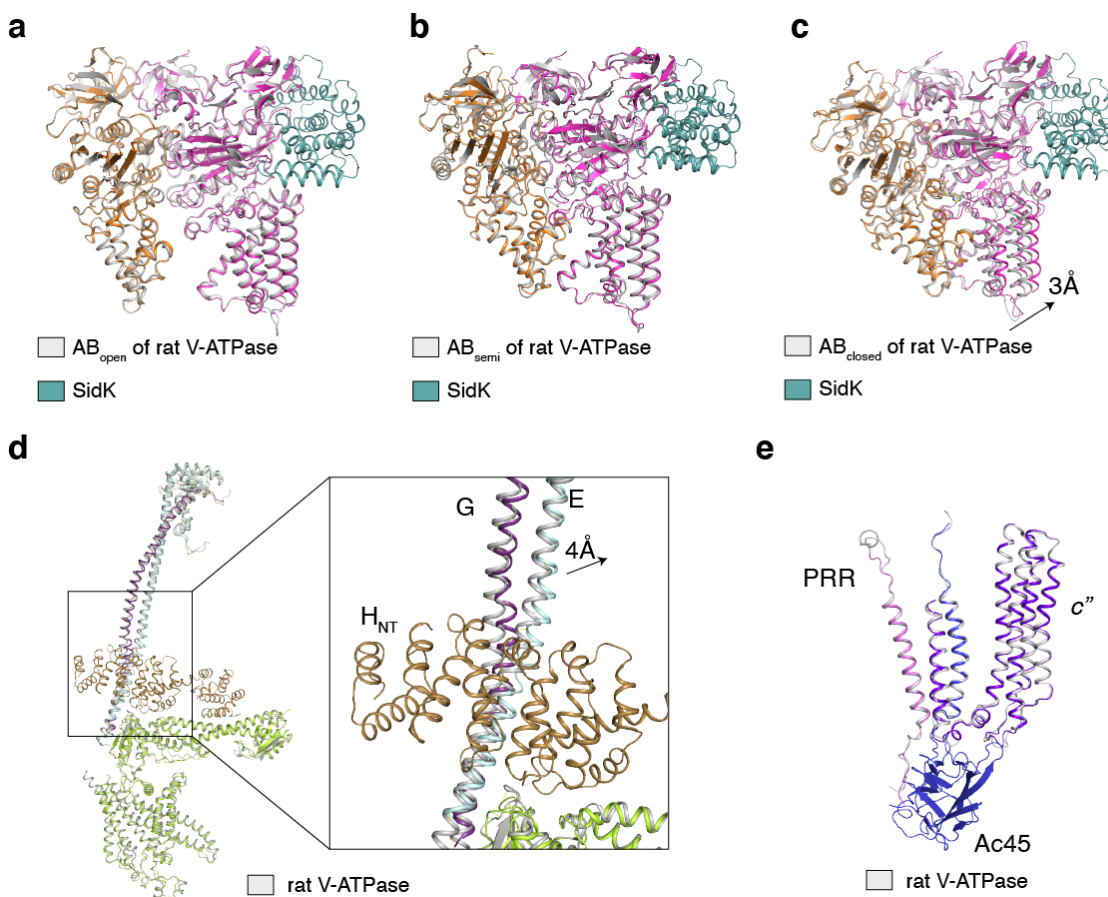
**Supplementary Fig. 7 Structural comparison of AB heterodimers in two states.**

The AB heterodimers in the state 2 are colored in cyan.



**Supplementary Fig. 8 Structural comparison of AB heterodimers with ttV/A-ATPase.**

The AB heterodimers in ttV/A-ATPase are colored in green.



**Supplementary Fig. 9 Structural comparison of bovine V-ATPase with rat V-ATPase (pdb code: 6VQ6).**

**a-c.** Structural comparison of AB heterodimers. **d.** The conformations of subunits E, G and H. **e.** The conformations of subunits PRR, *c''* and Ac45. The subunits in rat V-ATPase are colored in gray and SidK is colored in teal.

**Supplementary Table 1 Summary of each subunit of state1 model.**

| Subunit | Chain | Total Residues Built | Remove Side Chains | Unmodelled                        | Mass Spectrometry Accession | Coverage of Peptides (%) |
|---------|-------|----------------------|--------------------|-----------------------------------|-----------------------------|--------------------------|
| A       | A     | 16-612               |                    | 1-15, 558-565, 613-617            | P31404                      | 65.48                    |
| A       | B     | 16-612               |                    | 1-15, 251-257, 613-617            | P31404                      | 65.48                    |
| A       | C     | 16-612               |                    | 1-15, 613-617                     | P31404                      | 65.48                    |
| B       | D     | 38-506               |                    | 1-37, 217-224, 507-511            | P31408                      | 90.61                    |
| B       | E     | 38-506               |                    | 1-37, 214-224, 507-511            | P31408                      | 90.61                    |
| B       | F     | 38-506               |                    | 1-37, 215-224, 507-511            | P31408                      | 90.61                    |
| C       | G     | 2-379                |                    | 1, 12-17, 81-85, 345-365, 380-382 | P21282                      | 52.36                    |
| D       | H     | 6-216                |                    | 1-5, 217-247                      | A0A3Q1M4W9                  | 42.91                    |
| E       | I     | 6-223                | 6-53               | 1-5, 224-226                      | P11019                      | 71.24                    |
| E       | J     | 6-223                | 6-50               | 1-5, 224-226                      | P11019                      | 71.24                    |
| E       | K     | 4-223                | 4-59               | 1-3, 224-226                      | P11019                      | 71.24                    |
| F       | L     | 5-107                |                    | 1-4, 108-119                      | Q28029                      | 53.78                    |
| G       | M     | 6-113                | 6-70               | 1-5, 114-118                      | Q0VVCV6                     | 51.69                    |
| G       | N     | 6-113                | 6-70               | 1-5, 114-118                      | Q0VVCV6                     | 51.69                    |
| G       | O     | 6-113                | 6-70               | 1-5, 114-118                      | Q0VVCV6                     | 51.69                    |
| H       | P     | 66-445               | 72-107, 120-132    | 1-65, 108-119, 446-476            | F1MZL6                      | 55.91                    |
| a       | a     | 4-834                |                    | 1-3, 141-166, 659-713, 835-838    | F1MJV0                      | 35.44                    |
| c''     | b     | 2-205                |                    | 1                                 | ND                          |                          |
| d       | d     | 4-351                |                    | 1-3                               | P61420                      | 35.9                     |
| e       | e     | 8-80                 |                    | 1-7, 81                           | ND                          |                          |
| f       | f     | 16-87                |                    | 1-15, 37-63, 88-98                | Q3ZC23                      | 6                        |
| c       | c     | 6-155                |                    | 1-5                               | P23956                      | 36.13                    |
| c       | g     | 6-155                |                    | 1-5                               | P23956                      | 36.13                    |
| c       | k     | 6-155                |                    | 1-5                               | P23956                      | 36.13                    |
| c       | l     | 6-155                |                    | 1-5                               | P23956                      | 36.13                    |
| c       | m     | 6-155                |                    | 1-5                               | P23956                      | 36.13                    |
| c       | n     | 6-155                |                    | 1-5                               | P23956                      | 36.13                    |
| c       | o     | 5-155                |                    | 1-4                               | P23956                      | 36.13                    |
| c       | p     | 5-155                |                    | 1-4                               | P23956                      | 36.13                    |
| c       | q     | 5-155                |                    | 1-4                               | P23956                      | 36.13                    |
| PRR     | r     | 293-338              |                    | 1-292, 339-351                    | P81134                      | 20.51                    |
| Ac45    | s     | 251-455              |                    | 1-250, 456-468                    | P40682                      | 13.03                    |

**Supplementary Table 2 Cryo-EM data collection, refinement and validation statistics**

|                                                  | State 1<br>(EMDB-EMD-22121)<br>(PDB-6XBW)    | State 2<br>(EMDB-EMD-22122)<br>(PDB-6XBY) |
|--------------------------------------------------|----------------------------------------------|-------------------------------------------|
| <b>Data collection and processing</b>            |                                              |                                           |
| Magnification                                    |                                              | 60024                                     |
| Voltage (kV)                                     |                                              | 300                                       |
| Electron exposure (e-/Å <sup>2</sup> )           |                                              | 60                                        |
| Defocus range (μm)                               |                                              | -1.0 to -2.0                              |
| Pixel size (Å)                                   |                                              | 0.833                                     |
| Symmetry imposed                                 |                                              | C1                                        |
| Initial particle images (no.)                    |                                              | 495,999                                   |
| Final particle images (no.)                      | 84,345                                       | 41,821                                    |
| Overall map resolution (Å)                       | 3.37                                         | 3.79                                      |
| FSC threshold                                    | 0.143                                        | 0.143                                     |
| <b>Refinement</b>                                |                                              |                                           |
| Initial model used (PDB code)                    | 5ZE9, 1U7L, 4RND<br>4EFA, 4IX9, 1HO8<br>6O7U | State 1                                   |
| Model resolution (Å)                             | 3.38                                         | 3.74                                      |
| FSC threshold                                    | 0.5                                          | 0.5                                       |
| Map sharpening <i>B</i> factor (Å <sup>2</sup> ) | -79.3                                        | -85.8                                     |
| Model composition                                |                                              |                                           |
| Non-hydrogen atoms                               | 63090                                        | 57518                                     |
| Protein residues                                 | 8186                                         | 7765                                      |
| Ligands                                          | 18                                           | 15                                        |
| <i>B</i> factors (Å <sup>2</sup> )               |                                              |                                           |
| Protein                                          | 70.07                                        | 74.75                                     |
| Ligand                                           | 51.57                                        | 62.56                                     |
| R.m.s. deviations                                |                                              |                                           |
| Bond lengths (Å)                                 | 0.010                                        | 0.006                                     |
| Bond angles (°)                                  | 1.259                                        | 1.039                                     |
| Validation                                       |                                              |                                           |
| MolProbity score                                 | 1.68                                         | 1.76                                      |
| Clashscore                                       | 8.21                                         | 6.94                                      |
| Poor rotamers (%)                                | 0.60                                         | 0.41                                      |
| Ramachandran plot                                |                                              |                                           |
| Favored (%)                                      | 96.46                                        | 94.38                                     |
| Allowed (%)                                      | 3.27                                         | 5.46                                      |
| Disallowed (%)                                   | 0.27                                         | 0.16                                      |