

## Expanded View Figures

**Figure EV1. Packing of SERCA2a E2–AlF<sub>4</sub><sup>-</sup>-CPA and [Ca<sub>2</sub>]E1-AMPPCP crystals.**

- A Parallel packing of the SERCA2a structure in the E2–AlF<sub>4</sub><sup>-</sup>-CPA conformational state. SERCA2a forms contact points between the A-domains and N-domains (Met1-Asn3, His15 to Glu458-Lys460) and between the A-domain (Arg134, Arg139, Lys141) and the P-domain (Glu659, Glu667, Ser663), as well as N-domain (Thr506-Ser509, Asp567-Arg571) and L7/8 (Asp859-Arg863, Lys876-Gly884).
- B Parallel packing of the SERCA2a structure in the E2–AlF<sub>4</sub><sup>-</sup>-CPA conformational state. Contact points are within the A-domain (Arg35-Glu40) and P-domain (Asp658-Ser663). A-, P-, and N-domains are colored in yellow, blue, and red, respectively. M1-M2 are colored in wheat, M3-M4 in brown, M5-M6 in dark gray, and M7-M10 in light gray.

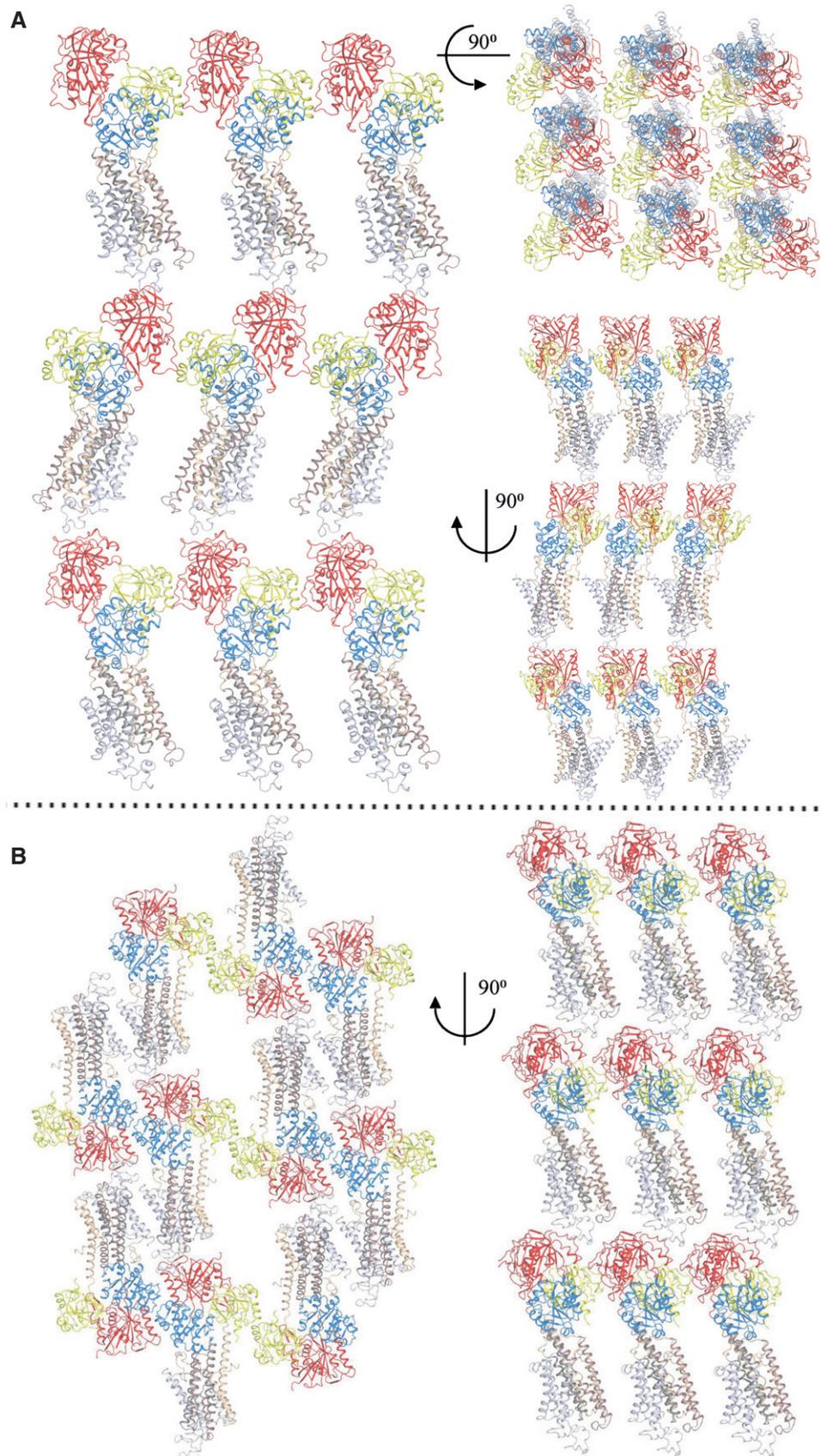
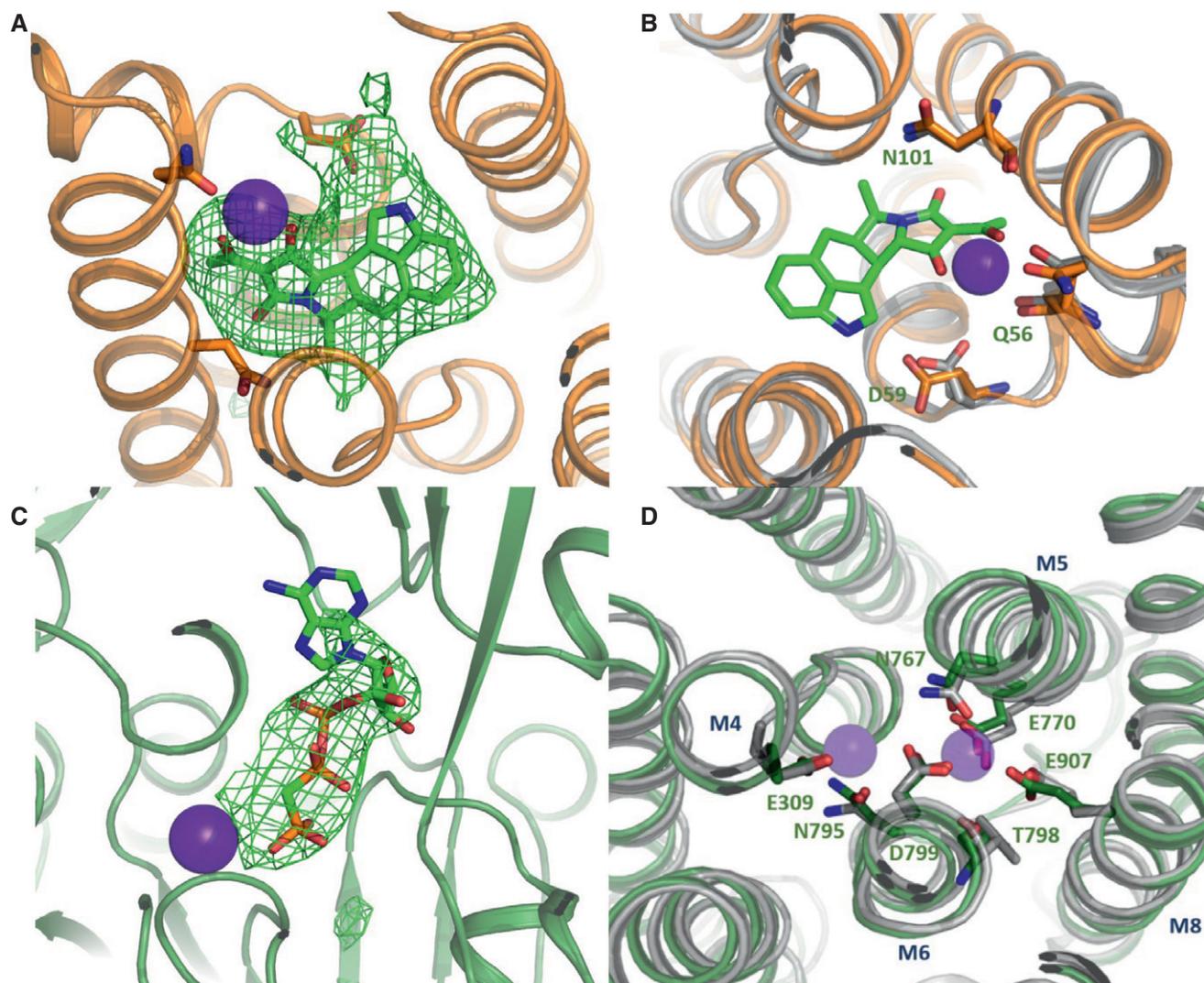


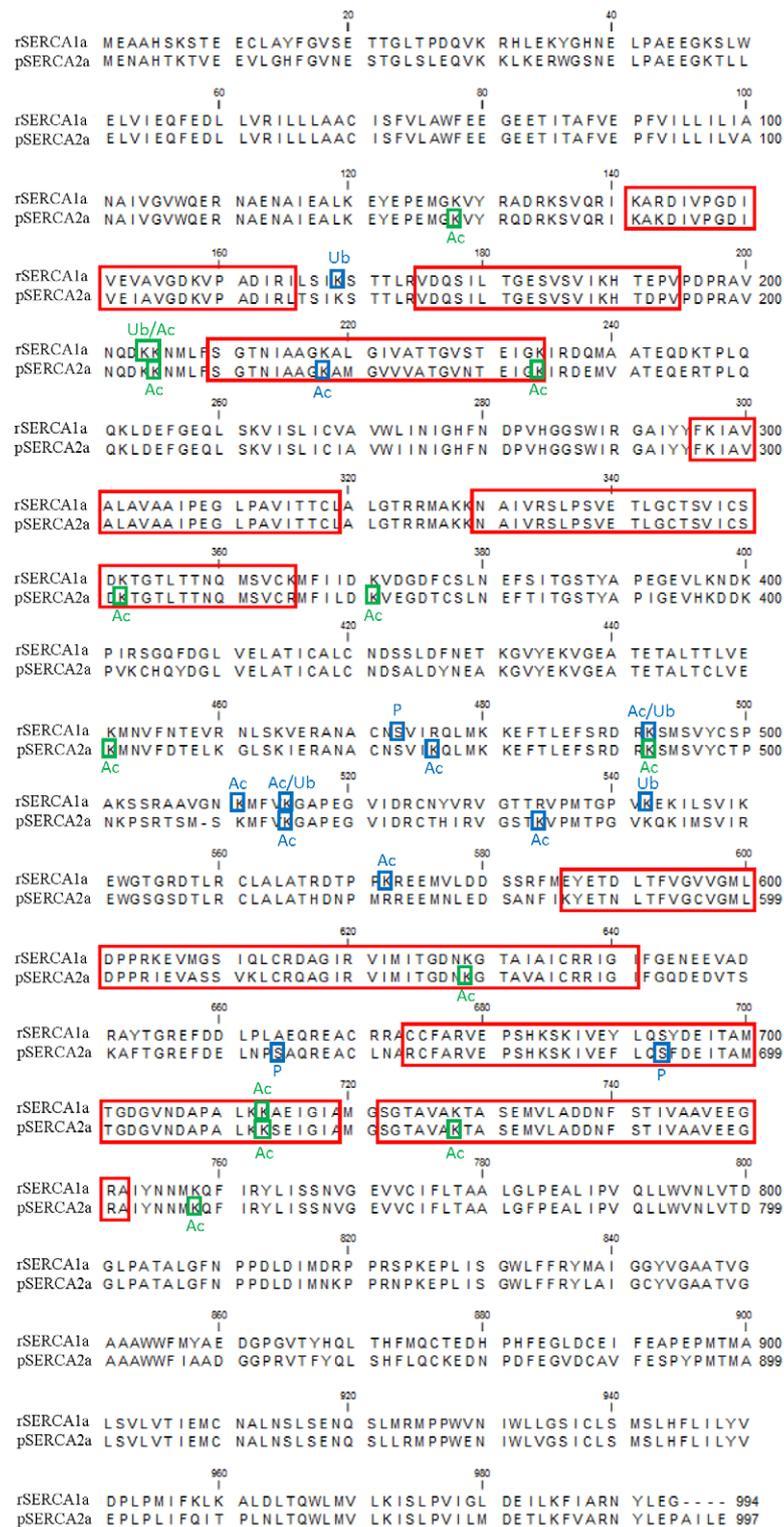
Figure EV1.



**Figure EV2. Coordination of ions and molecules in the SERCA2a structures.**

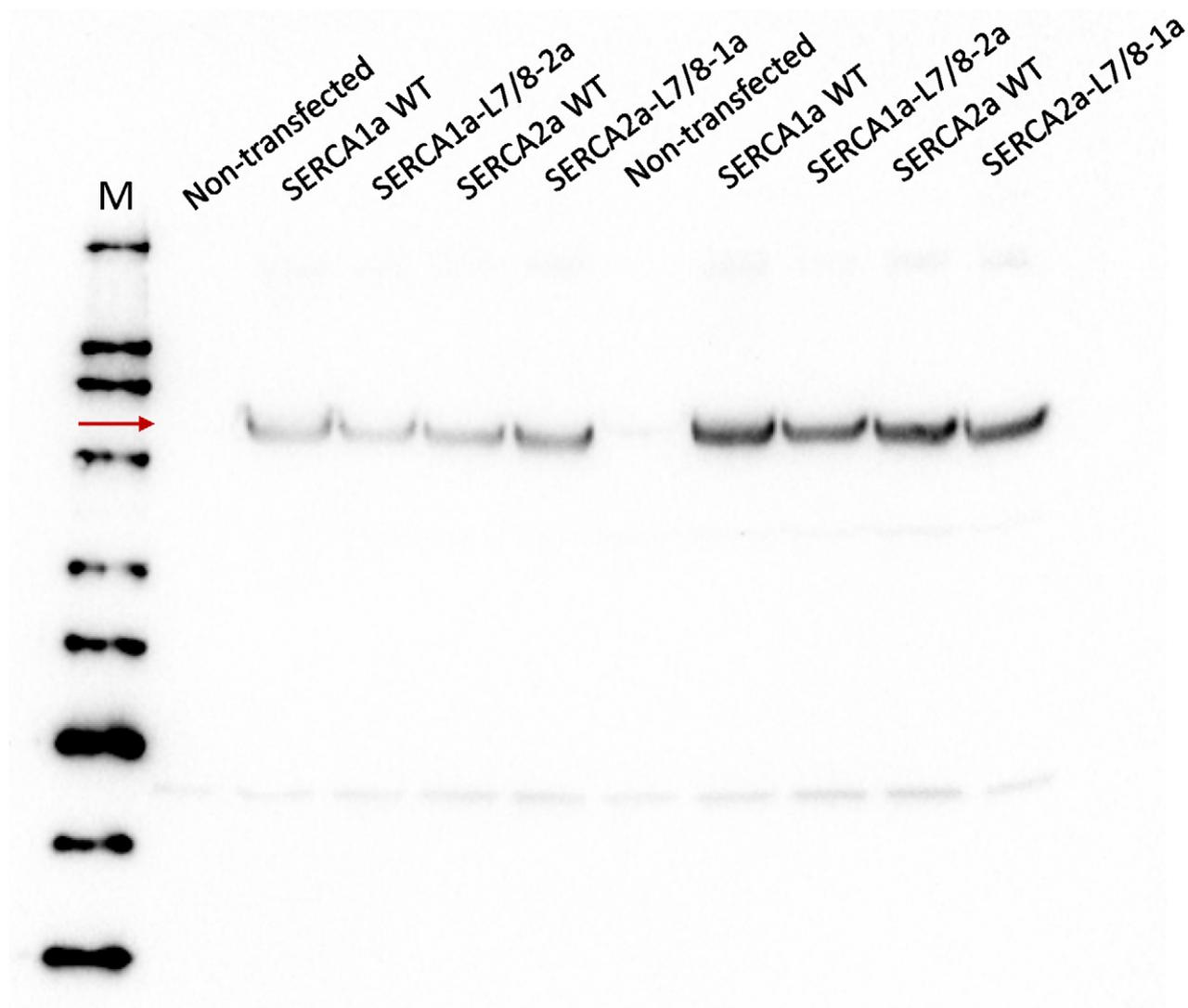
A–C  $\text{Ca}^{2+}$  ions are shown in purple spheres. Omit maps for the CPA (A) and the AMPPCP (C) molecules contoured at  $3\sigma$ . (B) CPA coordination in  $[\text{H}_{2-3}\text{E}2-\text{AlF}_4^-]\text{-CPA}$  state of SERCA. SERCA1a is depicted in gray and SERCA2a in orange.

D Coordination of  $\text{Ca}^{2+}$  ions in the  $[\text{Ca}_2]\text{E1-AMPPCP}$  state. SERCA1a is shown in gray and SERCA2a in green. Residues are marked according to the SERCA2a numbering.



**Figure EV3. Sequence alignment of SERCA1a and SERCA2a.**

Alignment of rabbit SERCA1a and pig SERCA2a protein sequences indicating the eight conserved core sequence regions in P-type ATPases (red squares) (Axelsen & Palmgren, 1998). The post-translational modifications found by MS in this study are marked by blue (reported before) and green (identified in this study) squares.



**Figure EV4. Expression levels of SERCA1a and SERCA2a constructs.**

Immunoblot with a non-isoform-specific SERCA TRY2 antibody (Mountian *et al*, 2001) depicting similar protein levels of the WT and chimera constructs of SERCA2a and SERCA1a following transient expression in COS cells.