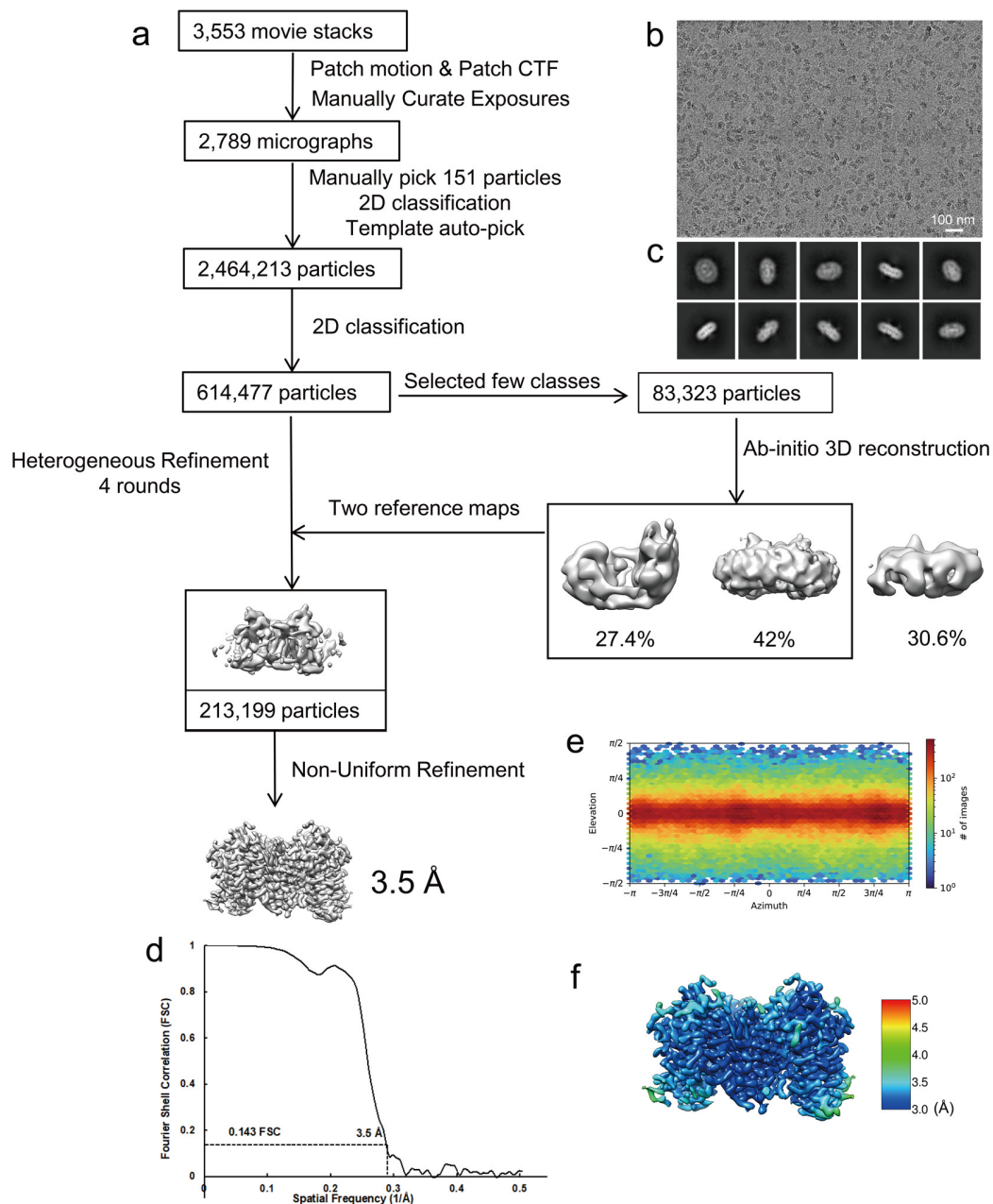
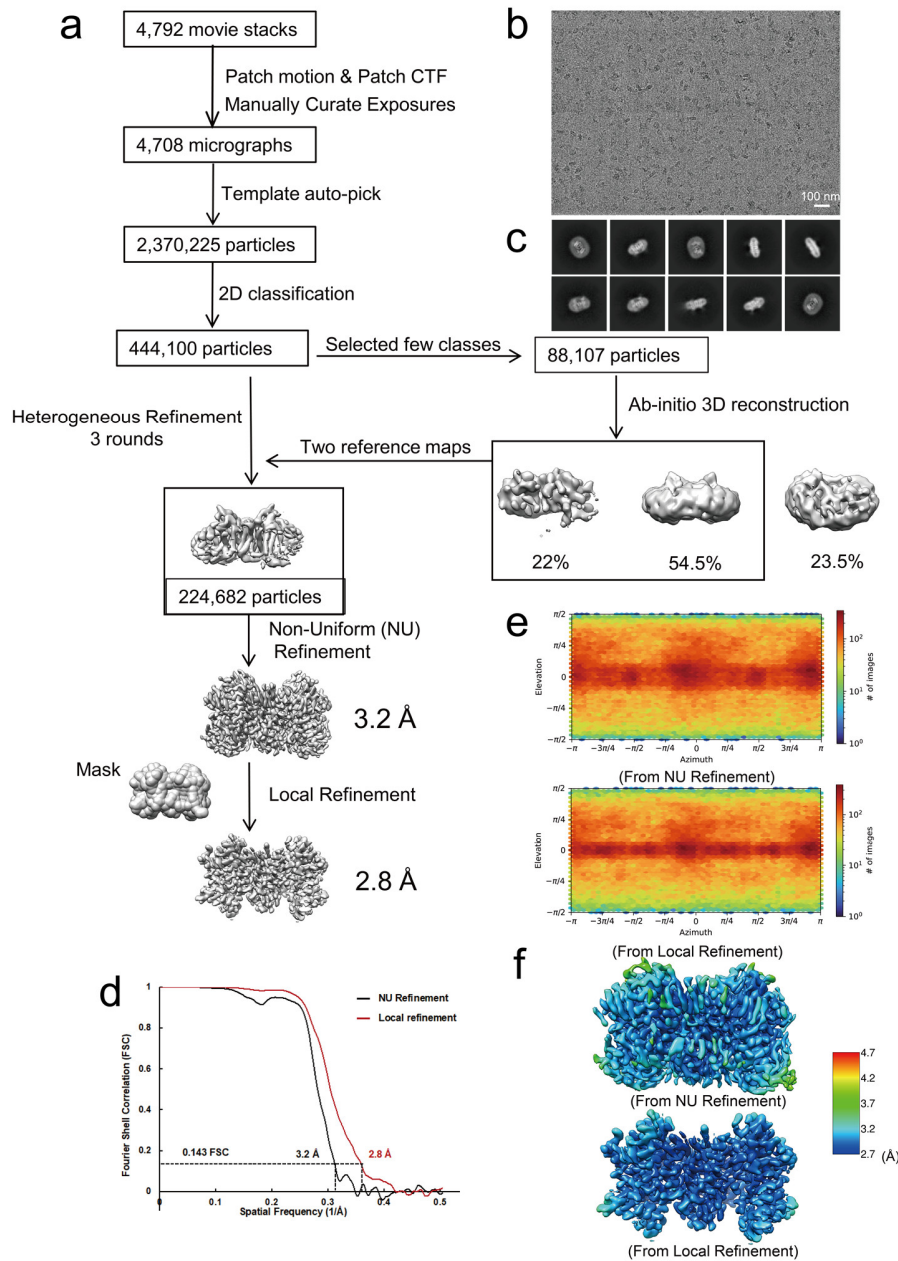




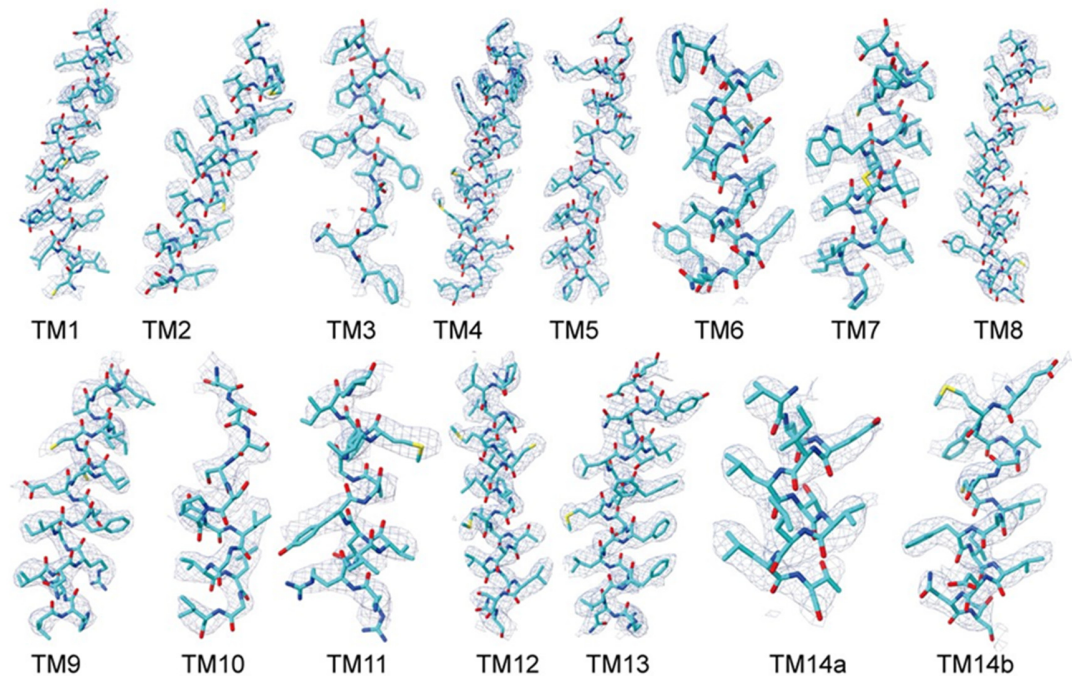


**Supplementary Figure 2 | Single-particle cryo-EM data processing of apo MmSVCT1.** **a**, Workflow of the data processing. **b**, Representative electron micrograph of apo MmSVCT1. **c**, Reference-free 2D class averages of computationally extracted particles. **d**, Gold standard FSC plot for the final 3D reconstruction. **e**, Euler angle distribution of the particle images. **f**, Resolution map for the 3D reconstruction calculated in cryoSPARC.

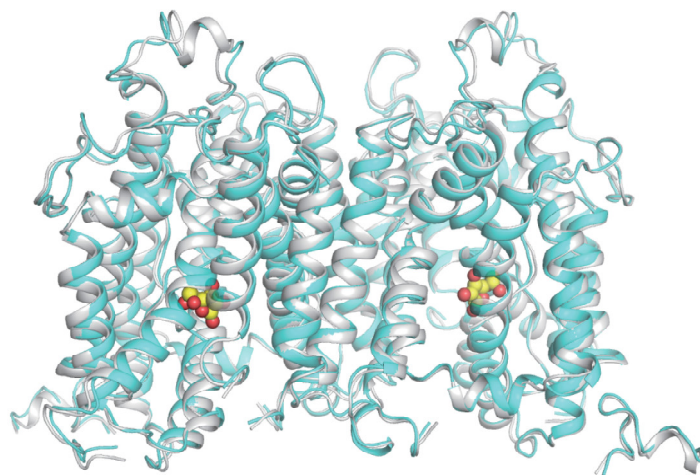


**Supplementary Figure 3 | Single-particle cryo-EM data processing of substrate-bound MmSVCT1.** **a**, Workflow of the data processing. **b**, Representative electron micrograph of substrate-bound MmSVCT1. **c**, Reference-free 2D class averages of computationally extracted particles. **d**, Gold standard FSC plots for the final 3D reconstruction. **e**, Euler angle distribution of the particle images for the 3D reconstructions from NU refinement (upper) and local refinement

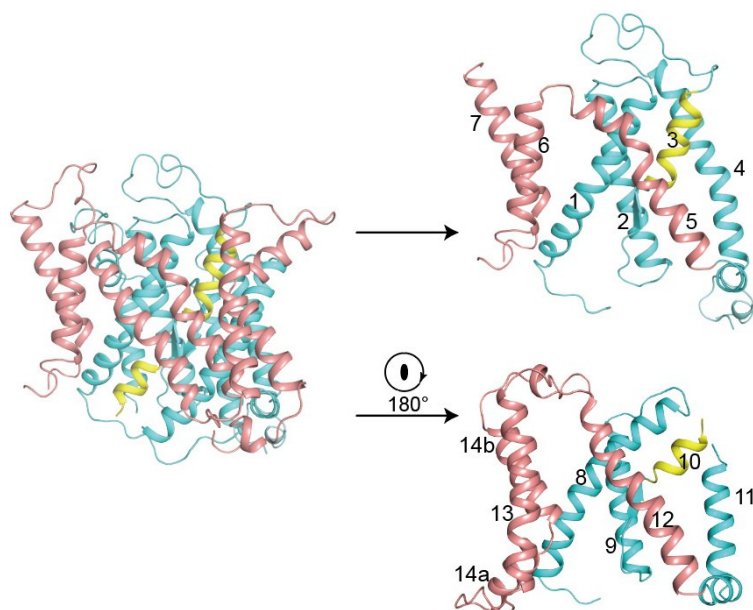
(lower). **f**, Resolution map for the 3D reconstructions from NU refinement (upper) and local refinement (lower).



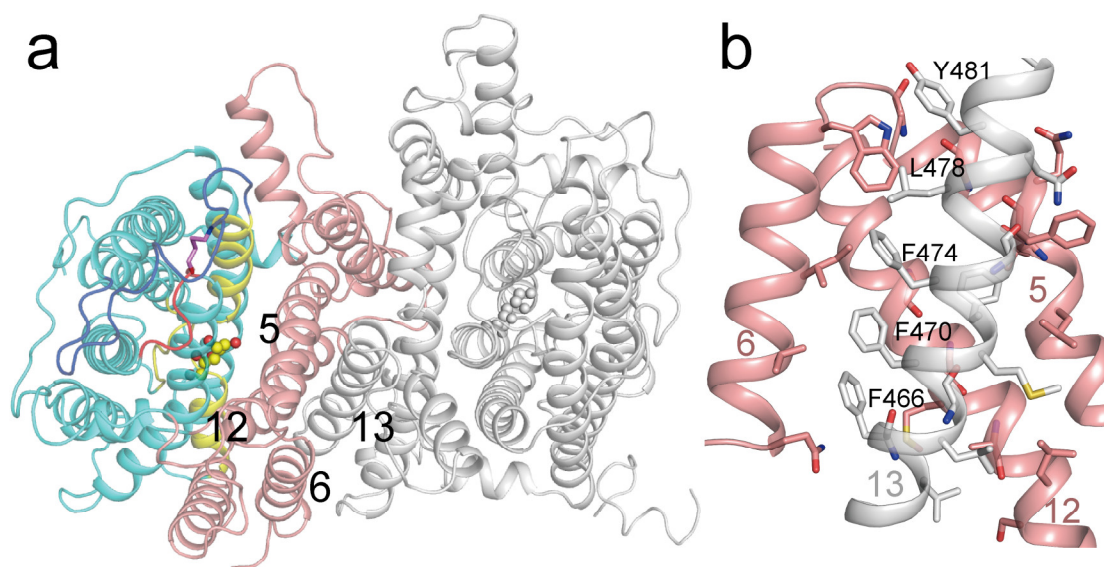
**Supplementary Figure 4 | Sample electron microscopy density maps (grey mesh) for substrate-bound MmSVCT1.** Refined coordinates are shown as sticks.



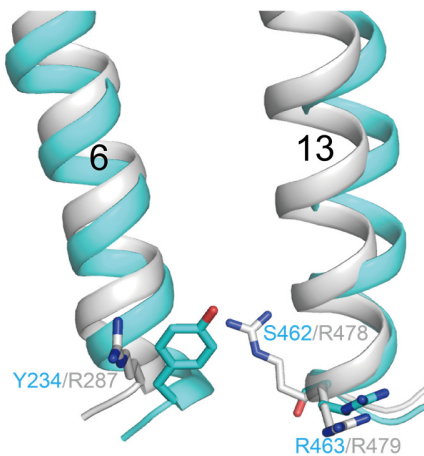
**Supplementary Figure 5 | Structure of MmSVCT1.** Superposition of the overall structures of apo MmSVCT1 (grey) and substrate-bound MmSVCT1 (cyan). Vitamin C is shown as spheres.



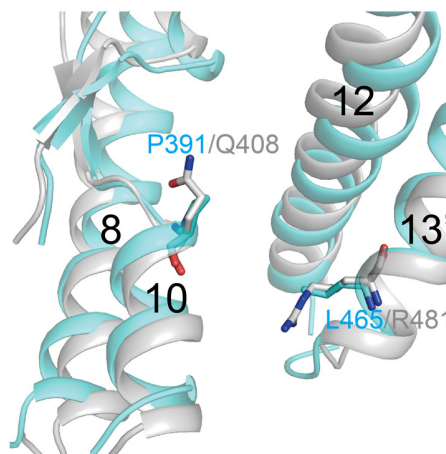
**Supplementary Figure 6 | Structural repeats (TM1-7 and TM8-14) in MmSVCT1.** TM8-14 (bottom right) is shown after an  $\sim 180^\circ$  rotation around a pseudo two-fold axis parallel to the membrane.



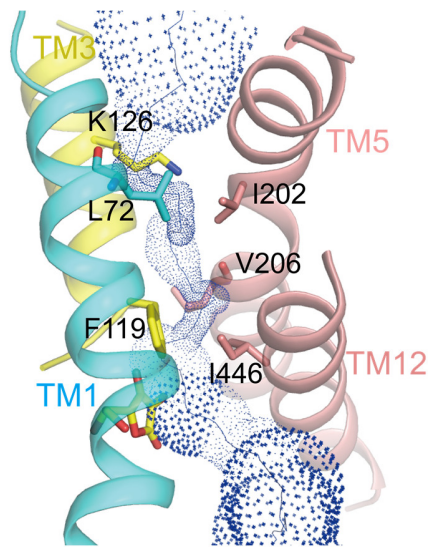
**Supplementary Figure 7 | Dimeric interface of MmSVCT1.** **a**, Dimeric MmSVCT1 viewed from the extracellular side. **b**, Residues involved in the dimeric interface are shown as sticks.



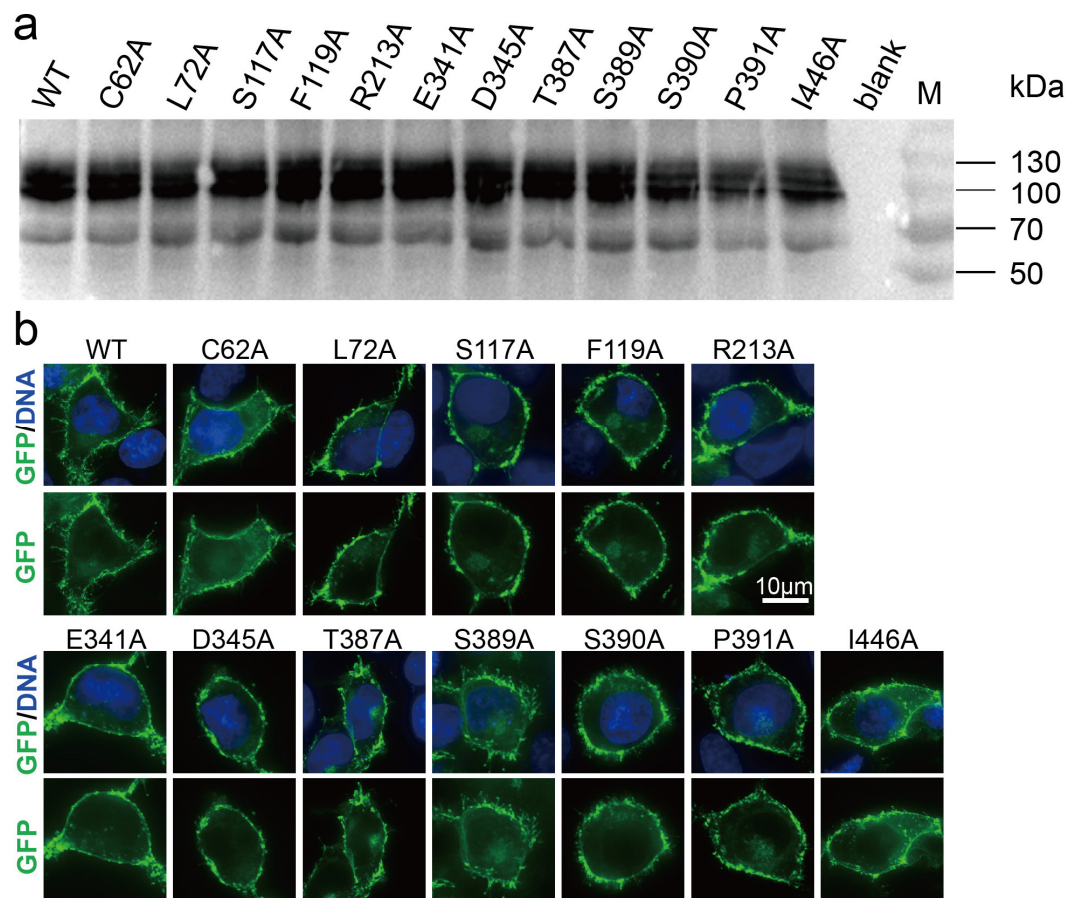
**Supplementary Figure 8 | Structural alignment of MmSVCT1 (cyan) and UapA (grey) around the lipid binding site of UapA.** Arg287, Arg478 and Arg479 form a lipid binding site in UapA.



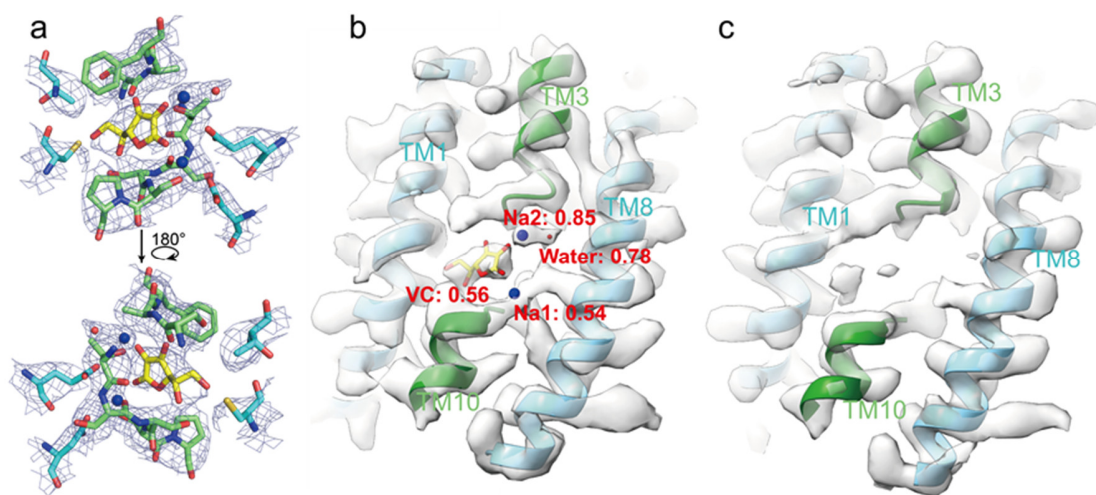
**Supplementary Figure 9 | Structural alignment of the substrate release channel of MmSVCT1 (cyan) and UapA (grey).** TM13' is from the opposing subunit.



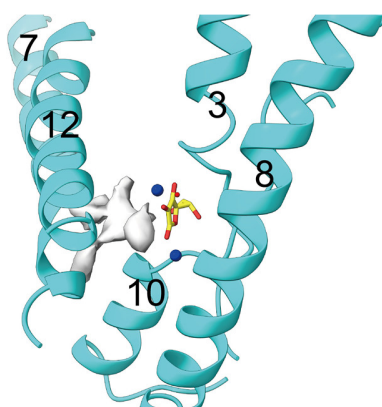
**Supplementary Figure 10 | Central pore of MmSVCT1.** The central pore is shown as a dotted mesh. Residues forming the narrowest constriction sites are shown as sticks.



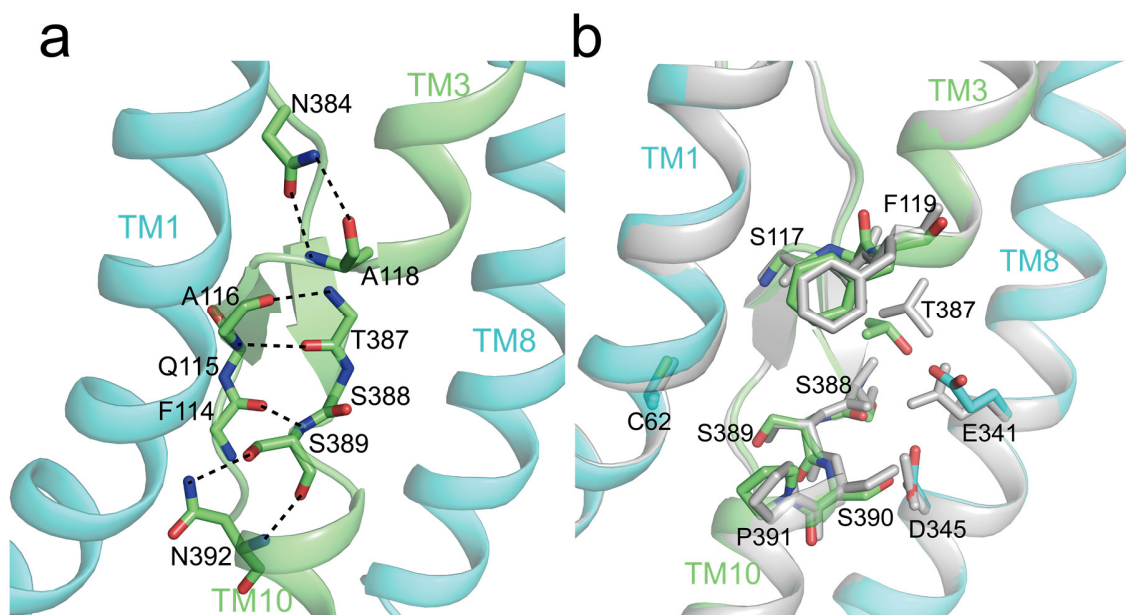
**Supplementary Figure 11 | The expression and localization of WT and MmSVCT1 mutants.** **a**, The expression levels of MmSVCT1 variants examined by western blot. Source data are provided as a Source Data file. **b**, Representative immunofluorescence images of HEK293T cells expressing GFP-tagged WT and MmSVCT1 mutants. DNA was stained with DAPI. The experiments were repeated three times independently with similar results.



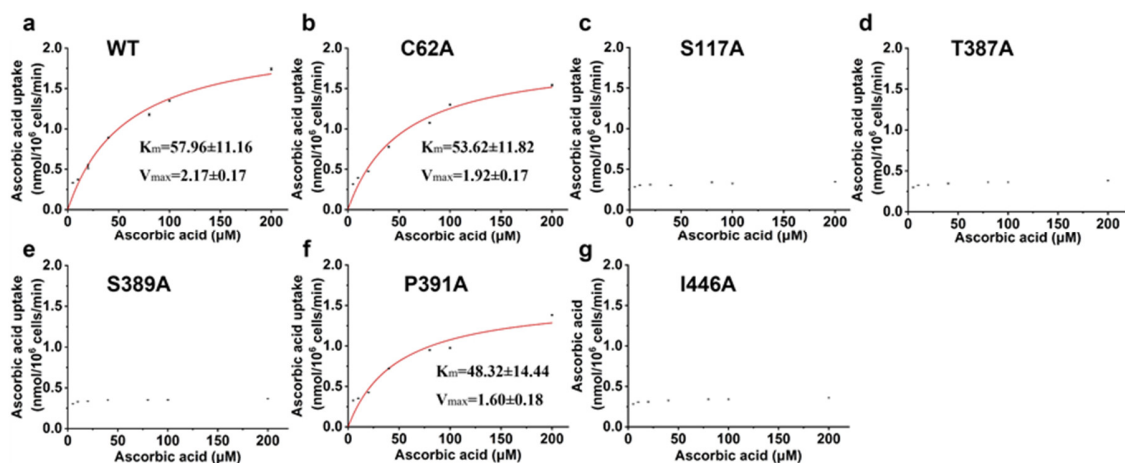
**Supplementary Figure 12 | Electron microscopy density maps for the substrate binding site.** **a**, Two views of density maps of the substrate binding site generated by PyMol from the substrate-bound map. Vitamin C (yellow sticks), sodium ions (blue spheres), and water (a red sphere) are shown. **b**, **c**, Density maps of the substrate binding site from the substrate-bound map (**b**) and apo map (**c**) generated by ChimeraX. Vitamin C (VC), sodium ions and water are displayed with Q-scores indicated.



**Supplementary Figure 13 | The nonprotein densities (grey) outside the binding pocket between the core and gate domains.**



**Supplementary Figure 14 | Vitamin C binding site.** **a**, Hydrogen bond network (dashed lines) in the unwound regions of TM3 and TM10. The side chains of some residues are omitted for clarity. **b**, Structural comparison at the vitamin C binding site between the substrate-bound (cyan or green) and apo (grey) states.



**Supplementary Figure 15 | The initial rate of ascorbic acid uptake versus concentrations of WT and MmSVCT1 mutants.** The data were fitted by the Michaelis–Menten equation.  $K_m$ , the Michaelis constant in  $\mu\text{M}$ ;  $V_{max}$ , the maximum uptake rate in nmol/10<sup>6</sup> cells/min. The data for the S117A, T387A, S389A, and

I446A mutants were not fitted due to the weak signals. Data points are the mean  $\pm$  s.e.m. (n=3 independent experiments). Source data are provided as a Source Data file.

## Supplementary information Table 1

Cryo-EM data acquisition, reconstruction and model refinement statistics.

|                                                     | Vitamin C bound<br>MmSVCT1<br>(EMDB-EMD-34094)<br>(PDB-7YTW) | Apo MmSVCT1<br>(EMDB-EMD-34095)<br>(PDB-7YTY) |
|-----------------------------------------------------|--------------------------------------------------------------|-----------------------------------------------|
| <b>Data collection and processing</b>               |                                                              |                                               |
| Microscope/Detector                                 | K3                                                           | K3                                            |
| Voltage (kV)                                        | 300                                                          | 300                                           |
| Electron exposure (e <sup>-</sup> /Å <sup>2</sup> ) | 55                                                           | 55                                            |
| Defocus range (μm)                                  | -1.5 to -2.7                                                 | -1.5 to -2.7                                  |
| Pixel size (Å)                                      | 0.82                                                         | 0.82                                          |
| Symmetry imposed                                    | C2                                                           | C2                                            |
| Initial particle images                             | 2,370,225                                                    | 2,464,213                                     |
| Final particle images                               | 224,682                                                      | 213,199                                       |
| Map resolution (Å)                                  | 3.2                                                          | 3.5                                           |
| FSC threshold                                       | 0.143                                                        | 0.143                                         |
| <b>Refinement</b>                                   |                                                              |                                               |
| Initial model used                                  | AlphaFold: AF-Q9Z2J0-F1                                      | AlphaFold: AF-Q9Z2J0-F1                       |
| Model resolution (Å)                                | 3.3                                                          | 3.7                                           |
| FSC threshold                                       | 0.5                                                          | 0.5                                           |
| Model composition                                   |                                                              |                                               |
| Non-Hydrogen atoms                                  | 7,744                                                        | 7,608                                         |
| Protein residues                                    | 1014                                                         | 1002                                          |
| B factors (Å <sup>2</sup> )                         |                                                              |                                               |
| Protein                                             | 47.37                                                        | 56.27                                         |
| Ligand                                              | 31.62                                                        | ---                                           |
| R.m.s. deviations                                   |                                                              |                                               |
| Bond lengths (Å)                                    | 0.008                                                        | 0.009                                         |
| Bond angles (°)                                     | 1.112                                                        | 1.172                                         |
| Validation                                          |                                                              |                                               |
| MolProbity score                                    | 1.80                                                         | 1.69                                          |
| Clashscore                                          | 8.32                                                         | 7.48                                          |
| Poor Rotamers (%)                                   | 0.00                                                         | 0.00                                          |
| Ramachandran plot                                   |                                                              |                                               |
| Favored (%)                                         | 95.01                                                        | 95.96                                         |
| Allowed (%)                                         | 4.99                                                         | 4.04                                          |
| Disallowed (%)                                      | 0.00                                                         | 0.00                                          |