

**Supplementary figure 1 Expression and purification of TRPA1 protein.**

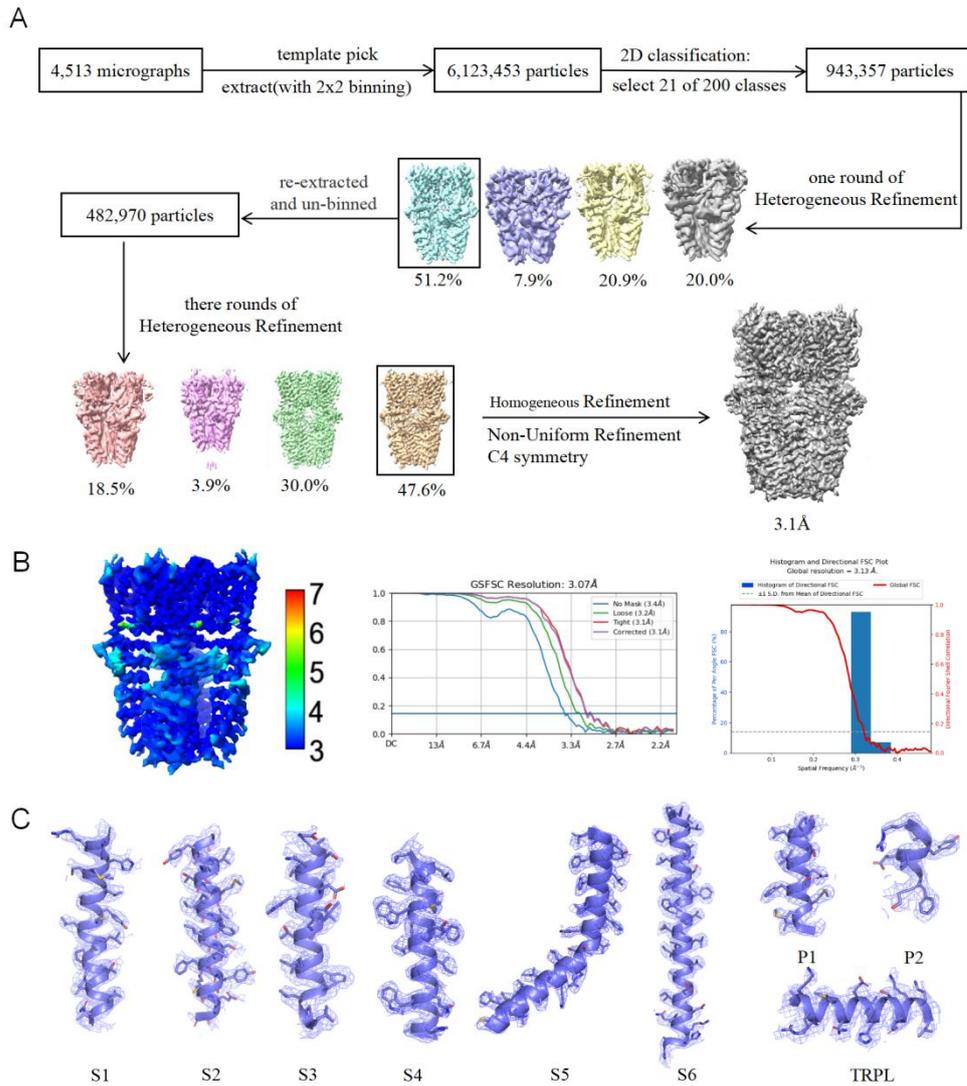
(A) The ratios of the currents of TRPA1 to the control currents when 100 nM cro, 10  $\mu$ M AITC, and 30  $\mu$ M IA were applied respectively (n = 9, +120 mV). p-values are denoted as follows: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, and ns not significant.

(B) The size exclusion chromatography curve (Superose 6 increase 10/300 GL) of Full-length human TRPA1 protein purified by Strep-Tactin XT resin affinity chromatography.

(C) SDS-PAGE of the TRPA1 protein following its purification by size-exclusion chromatography.

(D) Representative cryo-EM micrographs of TRPA1.

(E) 2D classification averages of TRPA1.

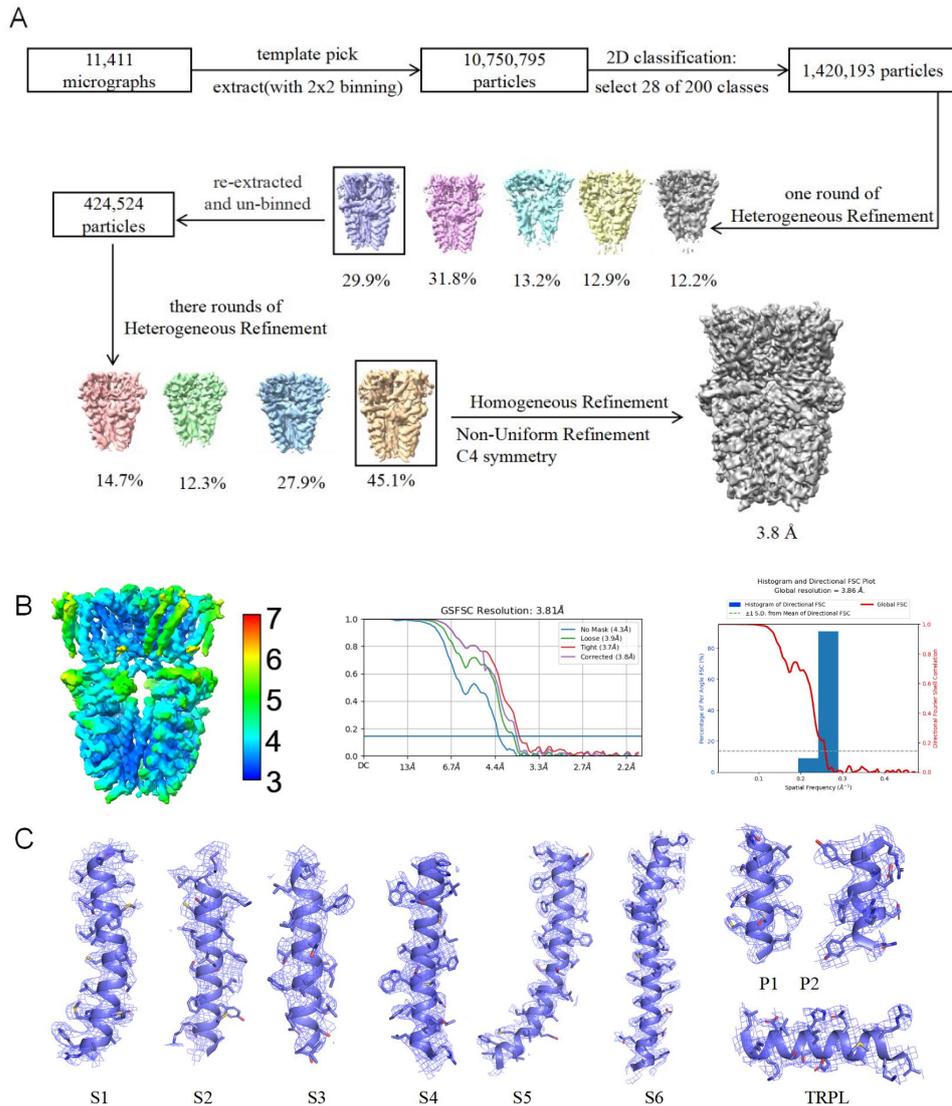


**Supplementary figure 2 Structure determination of the ligand-free TRPA1.**

(A) The flow chart of cryo-EM data processing of the ligand-free TRPA1. The overall resolution of the ligand-free TRPA1 was determined at 3.1 Å.

(B) Local resolution, GSFSC curve and 3D FSC curve of ligand-free TRPA1.

(C) Local EM density of transmembrane helices and P1, P2, TRPL helices of the ligand-free TRPA1.



**Supplementary figure 3 Structure determination of TRPA1 in complex with crotalphine.**

(A) The flow chart of cryo-EM data processing of TRPA1 in complex with crotalphine. The overall resolution of the TRPA1 in complex with crotalphine was determined at 3.1 Å.

(B) Local resolution, GSFSC curve and 3D FSC curve of TRPA1 in complex with crotalphine.

(C) EM density of transmembrane helices and P1, P2, TRPL helices of the TRPA1 in complex with crotalphine.