

Expanded View Figures

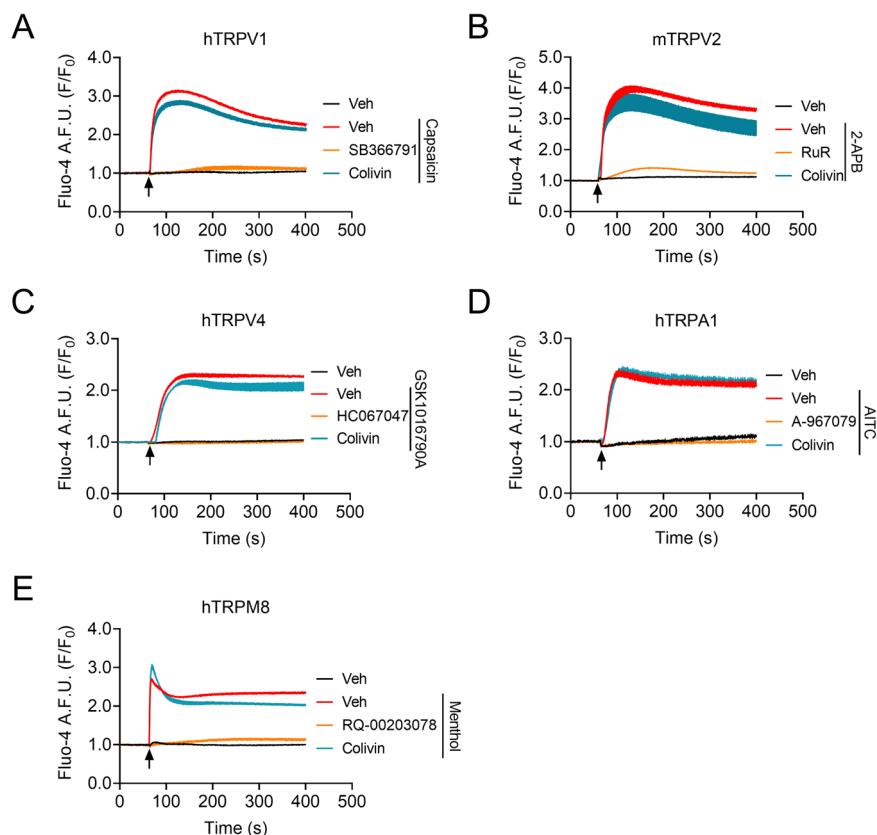


Figure EV1. Effects of Colivin on thermosensitive TRP channels.

Time courses of Ca^{2+} influx induced by capsaicin (1 μM), 2-APB (100 μM), GSK1016790A (10 nM), AITC (30 μM), and menthol (300 μM) in HEK-293 cells expressing hTRPV1 (A), mTRPV2 (B), hTRPV4 (C), hTRPA1 (D), and hTRPM8 (E), respectively. Colivin (10 μM), along with the corresponding inhibitors: SB366791 (1 μM), ruthenium red (30 μM), HC067047 (1 μM), A-967079 (1 μM), and RQ-00203078 (1 μM), was incubated for 5 min, followed by the addition of agonists at 60 s (black arrow). Each data point represents mean $\pm$ SEM ($N = 3$ wells). Each experiment was repeated in three independent cultures with similar results. Related to Fig. 1. Source data are available online for this figure.

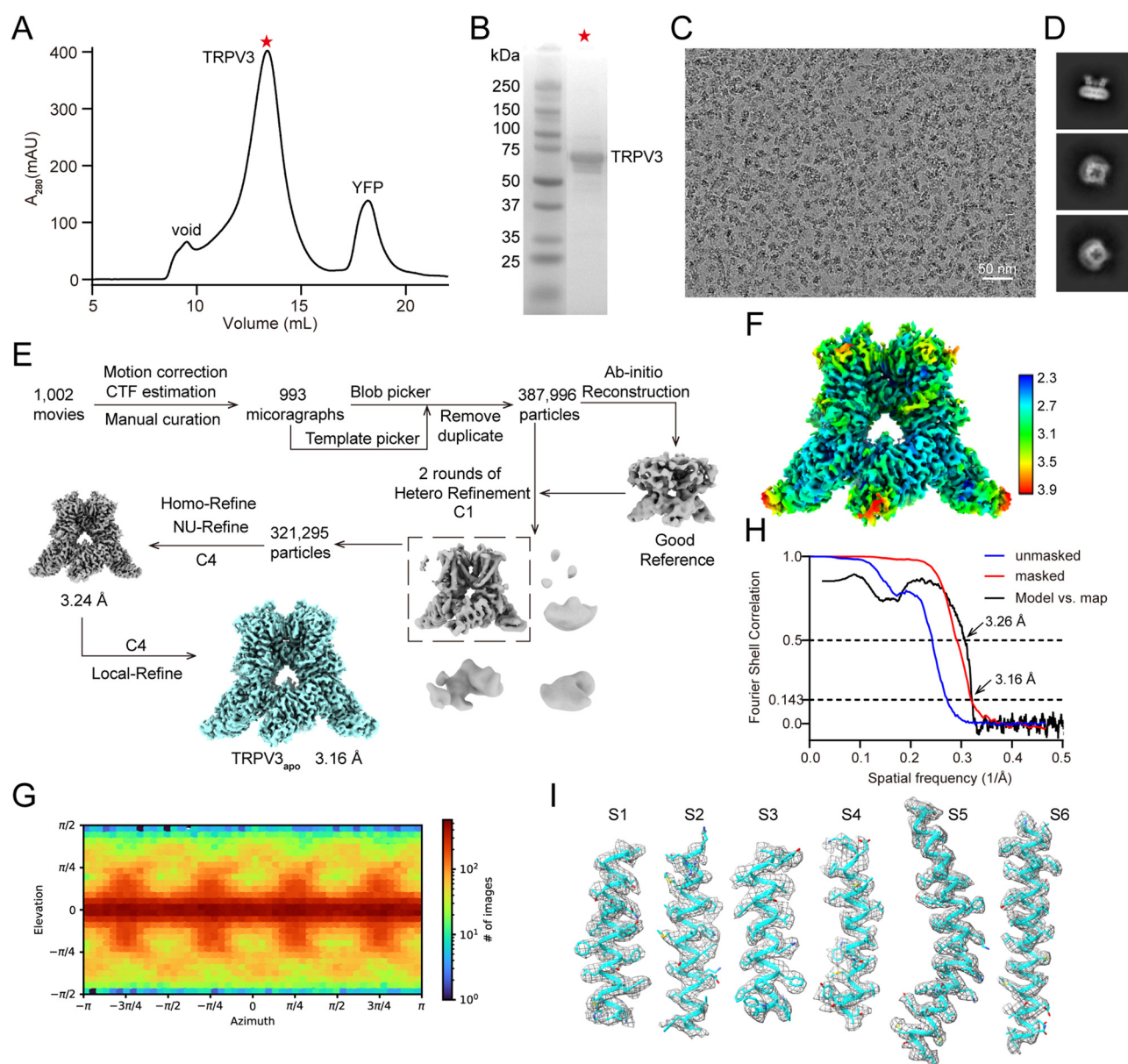


Figure EV2. Protein purification and Cryo-EM reconstruction of TRPV3_{apo}.

(A) Size-exclusion chromatography of purified human TRPV3. The fractions were pooled for cryo-EM sample preparation. (B) SDS-PAGE gel of purified human TRPV3. (C) A representative motion-corrected micrograph of the dataset. Scale bar = 50 nm. (D) Representative 2D class averages generated from particles for the TRPV3_{apo} map. (E) Flow chart of cryo-EM data processing of TRPV3_{apo}. Further details can be found in Methods. The contour level of the final map is 0.25. (F) Local resolution map of TRPV3_{apo}, colored according to the estimated values ranging from 2.3 to 3.9 Å. (G) Particle angular distribution calculated in cryoSPARC for the final reconstruction. (H) Gold standard Fourier Shell Correlations (FSC) curves of unmasked map (blue), masked map (red), and model versus map (black), marked with resolutions corresponding to FSC = 0.5 and 0.143. (I) Representative cryo-EM densities of transmembrane helices (S1-S6) of TRPV3_{apo}, contoured at 0.25. Related to Fig. 2.

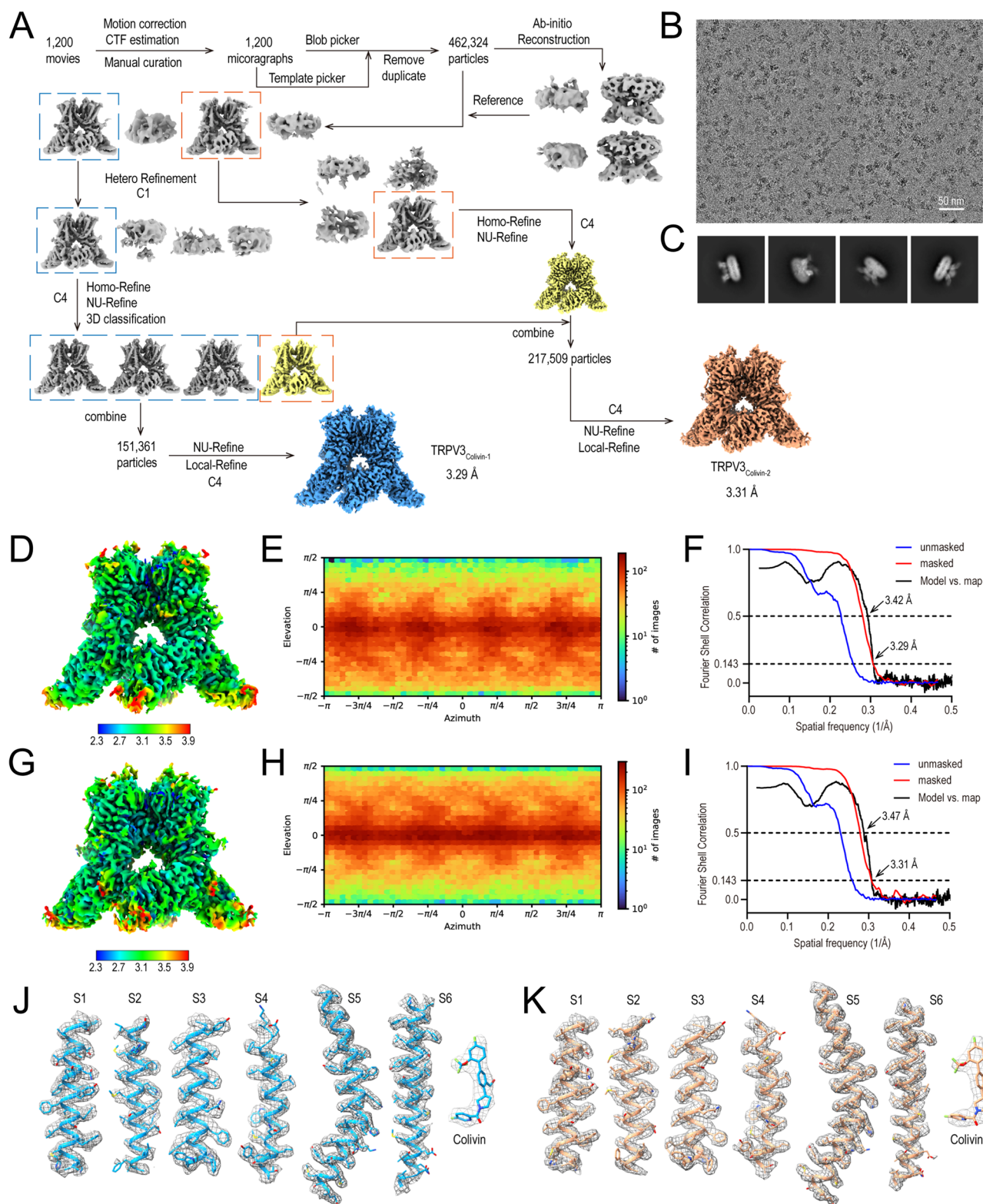


Figure EV3. Cryo-EM reconstruction of TRPV3_{Colivin}

(A) Flow chart of cryo-EM data processing of TRPV3_{Colivin}. Further details can be found in "Methods". The contour level of the final maps is 0.3. (B) A representative motion-corrected micrograph of the dataset. Scale bar = 50 nm. (C) Representative 2D class averages generated from particles. (D, G) Local resolution map of TRPV3_{Colivin-1} (D) and TRPV3_{Colivin-2} (G), colored according to the estimated values ranging from 2.3 to 3.9 Å. (E, H) Particle angular distributions calculated in cryoSPARC for the final reconstruction of TRPV3_{Colivin-1} (E) and TRPV3_{Colivin-2} (H). (F, I) FSC curves of unmasked map (blue), masked map (red), and model versus map (black) for the final map of TRPV3_{Colivin-1} (F) and TRPV3_{Colivin-2} (I), marked with resolutions corresponding to FSC = 0.5 and 0.143. (J, K) Representative cryo-EM densities of transmembrane helices (S1-S6) and ligands for TRPV3_{Colivin-1} (J) and TRPV3_{Colivin-2} (K), contoured at 0.3. Related to Fig. 2.

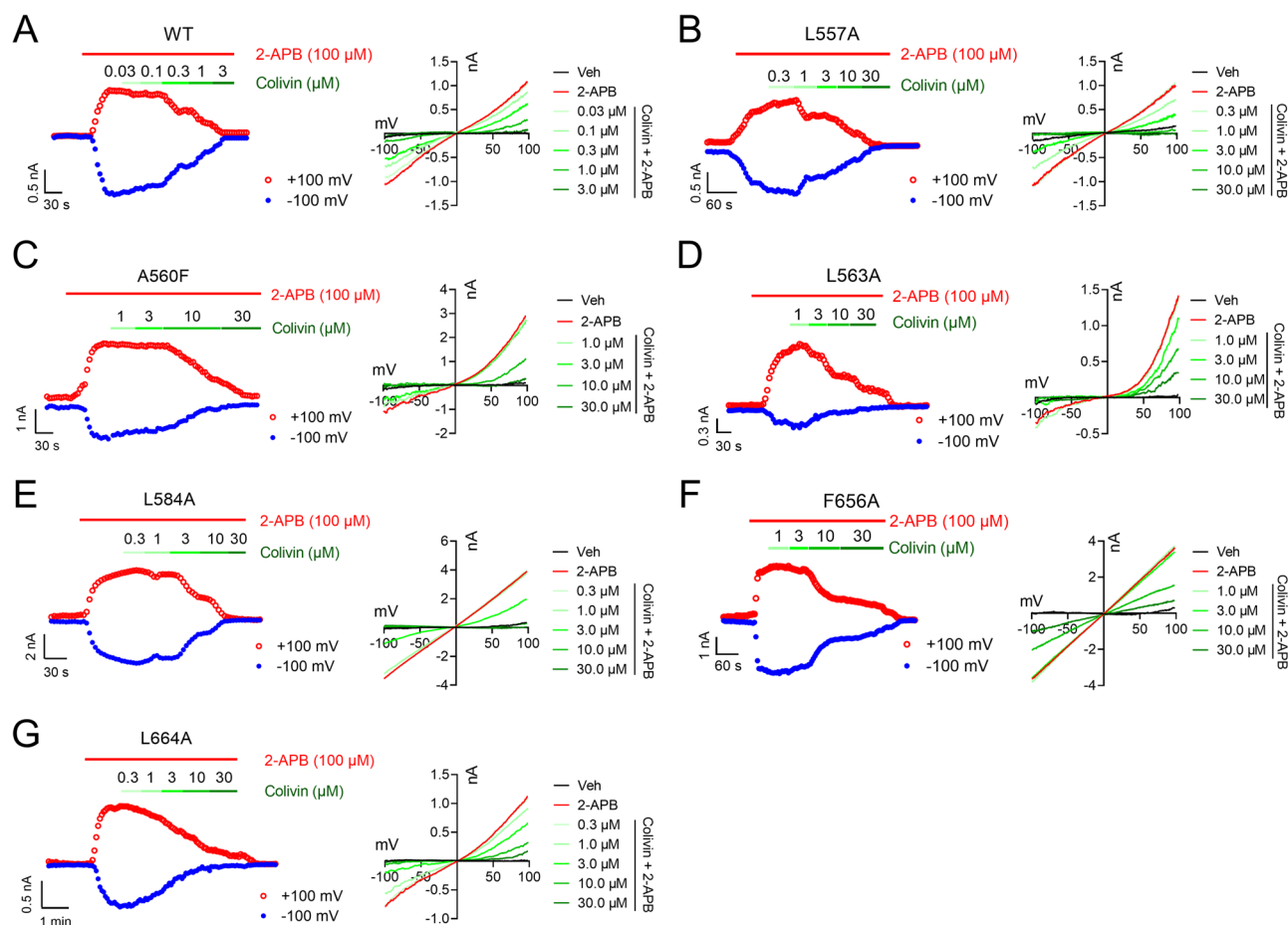


Figure EV4. Mutations in the vanilloid site reduced the apparent affinity of Colivin on hTRPV3.

Effects of different concentrations of Colivin on 2-APB (100 μ M)-evoked whole-cell currents at ± 100 mV in HEK-293 cells transiently expressing wild-type (WT) hTRPV3 (A) and L557A (B), A560F (C), L563A (D), L584A (E), F656A (F), and L664A (G) mutants of hTRPV3. Also shown are representative I-V curves of WT hTRPV3 and its mutants, derived from the different time points of drug applications. Related to Fig. 2. Source data are available online for this figure.

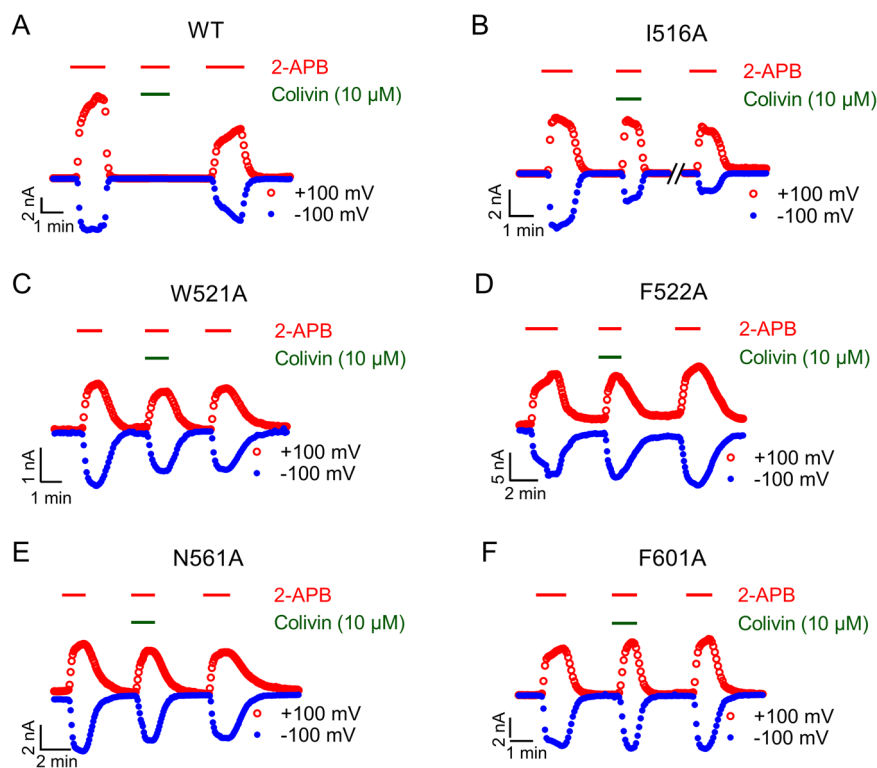


Figure EV5. Colivin inhibition of hTRPV3 was abolished by mutations at critical residues.

Representative whole-cell current traces at ± 100 mV with bath applications of 2-APB (100 μ M) and Colivin (10 μ M) as indicated in HEK-293 cells transiently expressing wild-type (WT) hTRPV3 (A) and mutants: I516A (B), W521A (C), F522A (D), N561A (E), and F601A (F). Related to Fig. 2. Source data are available online for this figure.