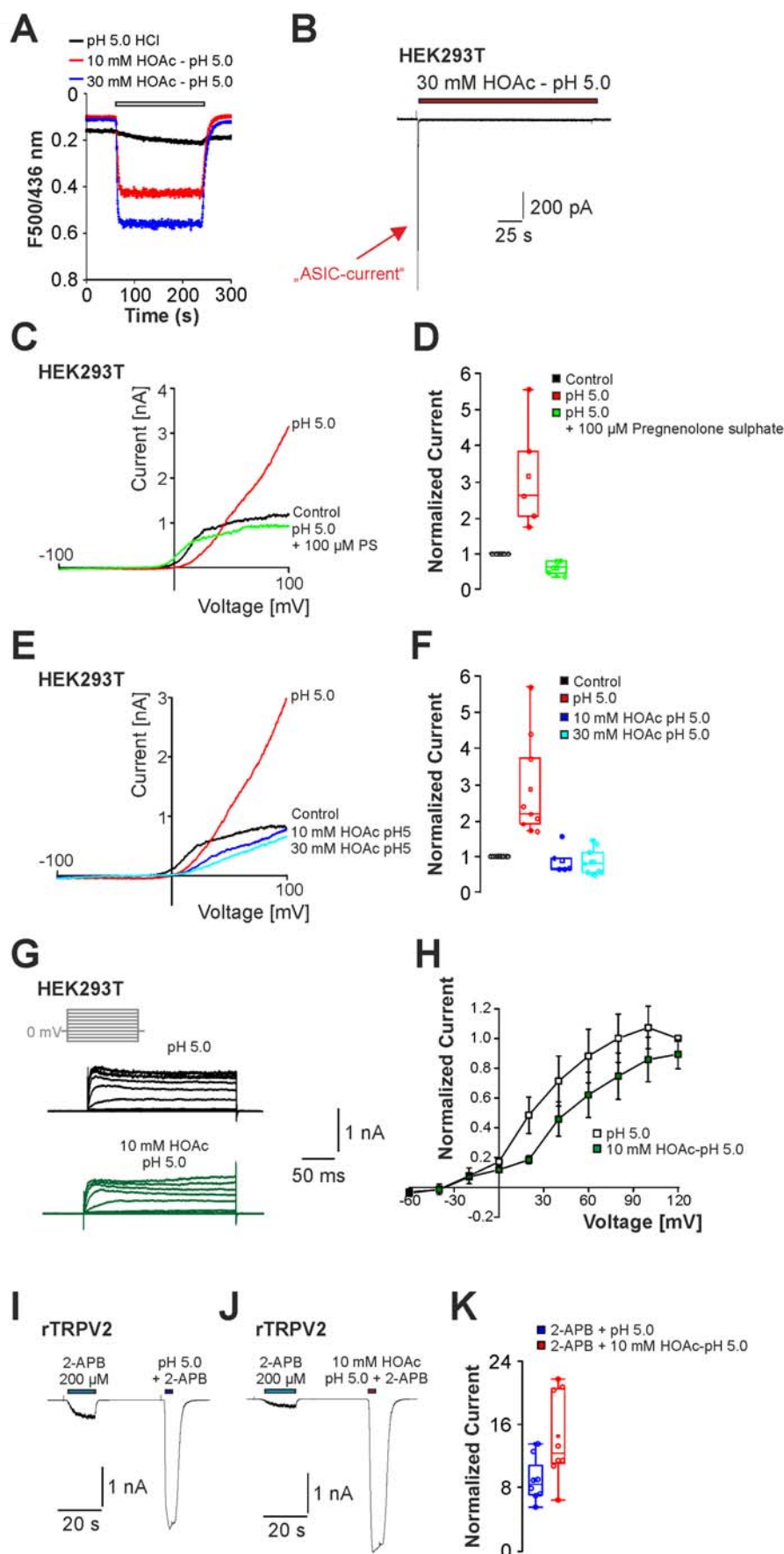
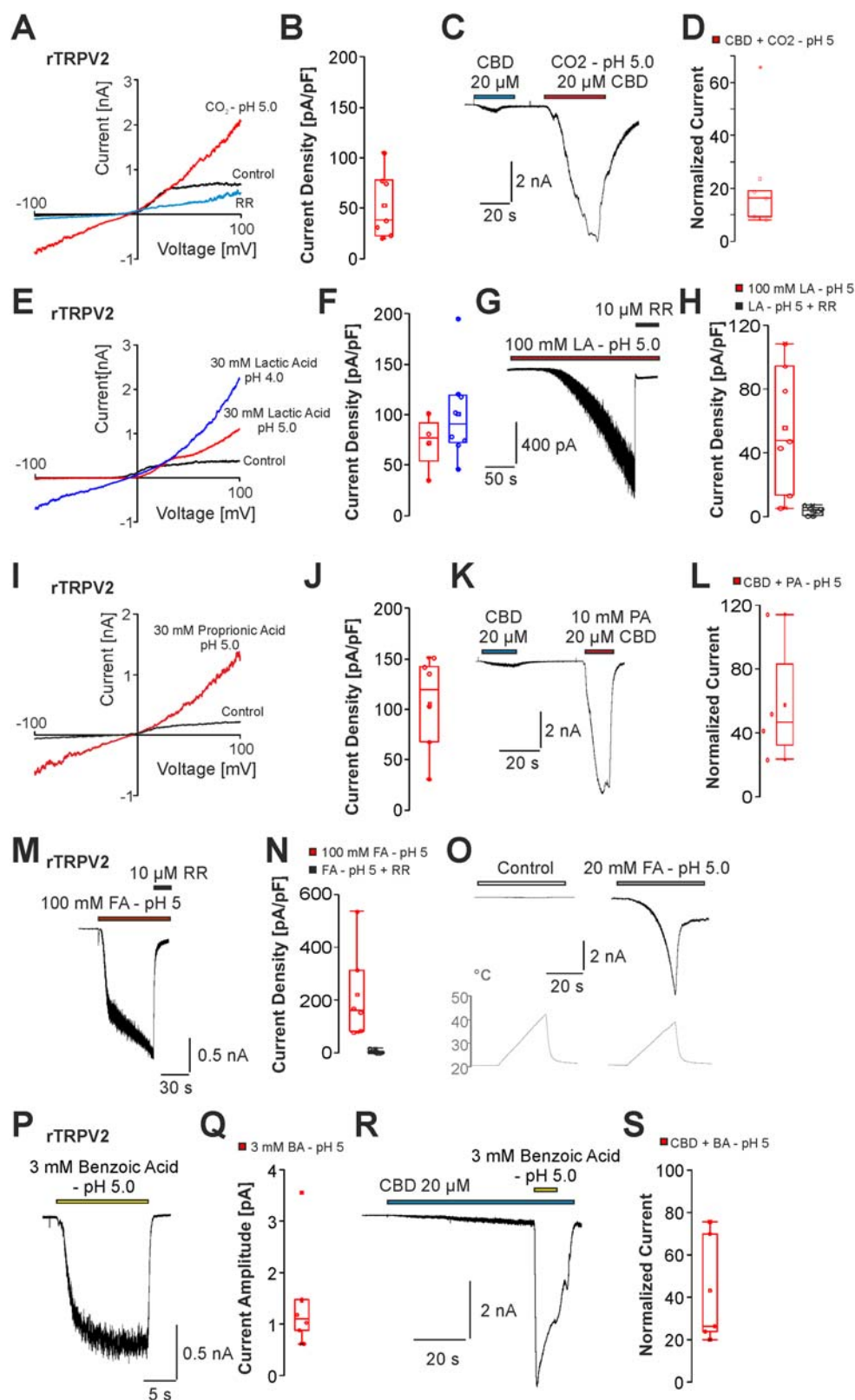


Expanded View Figures

Figure EV1. Weak acids inhibit endogenous PAC-mediated currents in HEK293T cells.

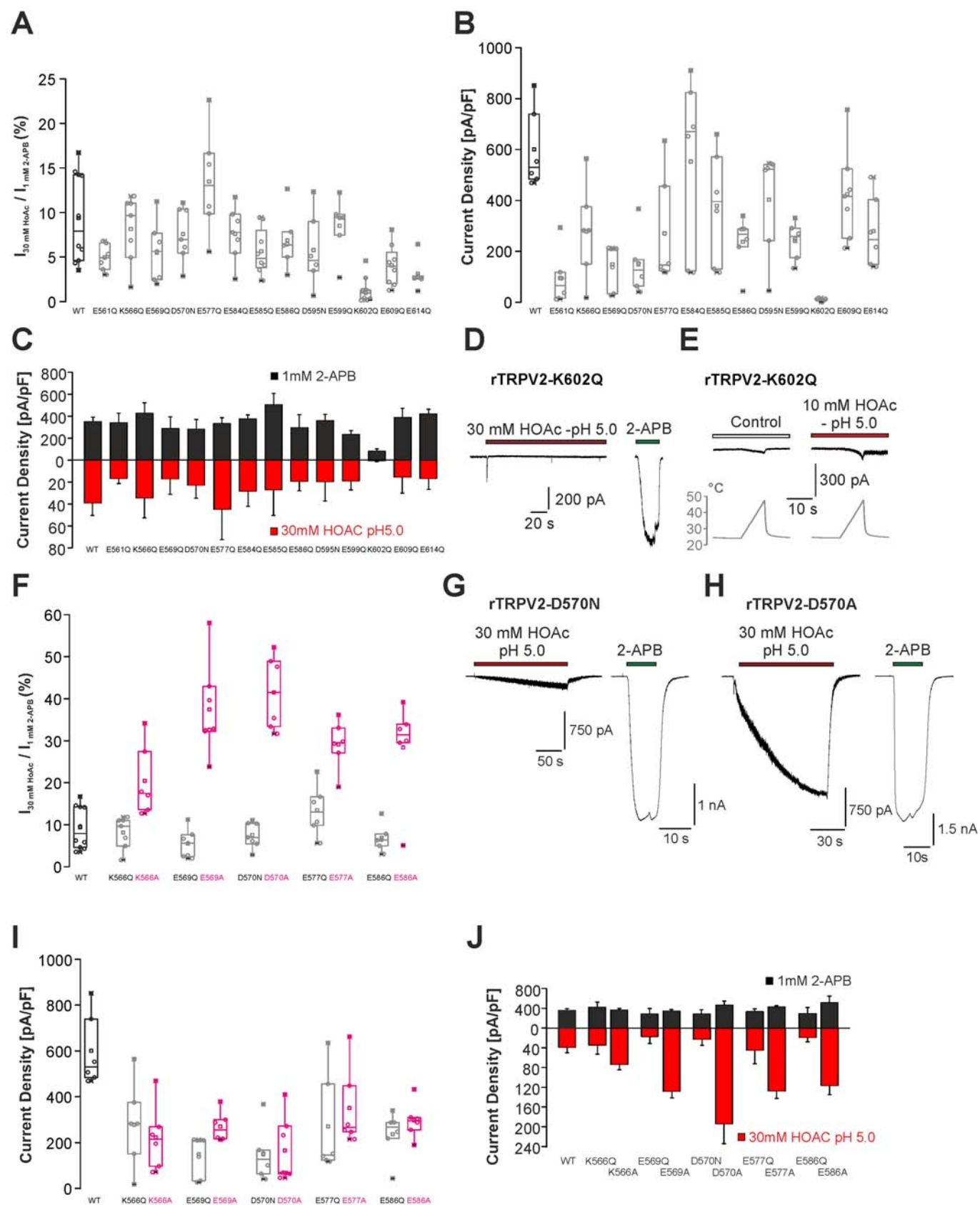
(A) BCECF-based ratiometric imaging on non-transfected HEK293T cells treated with pH 5.0 titrated with HCl, or 10 or 30 mM HOAc at pH 5.0. Application of HOAc, and to a lesser degree pH 5.0 induced a reversible decrease in fluorescence ratio, correlating with an intracellular acidosis. (B) Representative current trace from a non-transfected HEK293T cell demonstrating that 30 mM HOAc at pH 5.0 fails to evoke slowly activating currents without TRPV2 in the cell. Note the very rapidly inactivating currents emerging at the beginning of the recordings, demonstrating proton-evoked activation of endogenous acid-sensitive ion channels (ASIC1a) in some HEK293T cells. (C) Membrane currents in non-transfected HEK293T cells challenged with pH 5.0 or the combination of pH 5.0 and 100 μ M pregnenolone sulfate. (D) Box diagrams with dot plots displaying normalized currents at +100 mV from cells described under C ($n = 5$). (E) Membrane currents in non-transfected HEK293T cells challenged with pH 5.0 or the combination of pH 5.0 ($n = 9$) and 10 ($n = 5$) or 30 mM ($n = 9$) HOAc. (F) Box diagrams with dot plots displaying normalized currents at +100 mV from cells described under (E). (C, E, G) Currents were monitored during a 500 ms long voltage ramp ranging from -100 mV to $+100$ mV. (G) Voltage-dependent membrane currents in a non-transfected HEK293T cell evoked by 200 ms long pulses from -60 to 120 mV applied in steps of 20 mV. Currents were evoked in control solution and in 10 mM HOAc at p 5.0. (H) Current-voltage plots of experiments performed in (G) ($n = 4$). Peak current amplitudes were normalized to the amplitude evoked at 120 mV. (I, J) Current traces on rTRPV2-expressing cells with two consecutive applications of 200μ M 2-APB. For the 2nd application, 2-APB was combined with pH 5.0 with HCl (I) or with 10 mM HOAc at pH 5.0 (J). (K) Box diagrams with dot plots displaying normalized current amplitudes of currents in induced by 2-APB (set as 1), 2-APB + pH 5.0 ($n = 8$) or 2-APB + HOAc ($n = 8$). (D, F, K) The box denotes the 50th percentile (median) as well as the 25th and 75th percentile. The whiskers mark the 5th and 95 percentiles. Data points beyond the whiskers are outliers. Source data are available online for this figure.





◀ **Figure EV2. Several weak acids activate TRPV2.**

(A) Membrane currents observed in cells expressing rTRPV2 challenged with CO₂ at pH 5.0. (B) Box diagrams with dot plots displaying the mean current densities at +100 mV induced by CO₂ ($n = 7$). (C) Current trace from a rTRPV2-expressing cell generating inward currents induced by CBD or CBD in combination with CO₂. (D) Box diagrams with dot plots displaying the degree of potentiation induced by CO₂ ($n = 5$). The CO₂-solution was achieved by bubbling the extracellular solution with CO₂ using a commercially available soda streamer. (E) Membrane currents induced by 30 mM lactic acid at pH 5.0 ($n = 4$) and 4.0 ($n = 8$). (F) Box diagrams with dot plots displaying the mean current densities at +100 mV induced by lactate. (G) Activation of an inward current in a rTRPV2-expressing cell exposed to 100 mM lactate at pH 5.0. The inward current was blocked by co-application with 10 μ M ruthenium red (RR). (H) Box diagrams with dot plots displaying the current densities of lactate-induced inward currents in rTRPV2-expressing cells ($n = 7$). (I) Membrane currents induced by 30 mM propionic acid at pH 5.0 on rTRPV2. (J) Box diagram with dot plots displaying the mean current densities at +100 mV induced by propionic acid ($n = 6$). (K) Current trace from a rTRPV2-expressing cell generating inward currents induced by CBD or CBD in combination with propionic acid at pH 5.0. (L) Box diagram with dot plots displaying the degree of potentiation induced by propionic acid. (M) Inward current induced by 100 mM formic acid (FA) at pH 5.0 on rTRPV2 ($n = 4$). The inward current was blocked by 10 μ M ruthenium red (RR). (N) Box diagrams with dot plots displaying the current densities of formic acid-induced inward currents in rTRPV2-expressing cells ($n = 6$). (O) Representative traces displaying heat-evoked currents induced by 20 mM formic acid at pH 5.0 on a rTRPV2-expressing cell. (P) Inward current induced by 3 mM benzoic acid at pH 5.0 on rTRPV2. (Q) Box diagrams with dot plots displaying the current densities of benzoic acid-induced inward currents ($n = 6$). (R) Original trace from a rTRPV2-expressing cell generating inward currents induced by CBD or CBD in combination with 3 mM benzoic acid at pH 5.0. (S) Box diagram with dot plots displaying the degree of potentiation induced by benzoic acid ($n = 5$). In (B, D, F, H, J, L, N, Q, S), the box denotes the 50th percentile (median) as well as the 25th and 75th percentile. The whiskers mark the 5th and 95 percentiles. Data points beyond the whiskers are outliers. Source data are available online for this figure.



◀ **Figure EV3. The role of pore residues for weak acid-sensitivity of TRPV2.**

(A) Box diagrams with dot plots displaying the relative magnitudes of inward currents induced by 30 mM HOAc at pH 5.0 normalized with the responses evoked by 1 mM 2-APB in the corresponding cells ($n = 5-10$ for each mutant). (B) Box diagrams with dot plots displaying the current densities of heat-evoked currents provoked by 10 mM HOAc at pH 5.0 ($n = 6-7$ for each mutant). (C) Bar diagrams displaying mean ($\pm$ SEM) current densities for currents evoked by 30 mM HOAc at pH 5.0 or 1 mM 2-APB ($n = 5-8$ for each mutant). (A-C) Data are demonstrated for rTRPV2-WT, -E561Q, -K566Q, -E569Q, -D570N, -E577Q, -E584Q, -E585Q, -E586Q, -D595N, -E599Q, -K602Q, -E609Q and -614Q. (D) Example of a current trace displaying that 30 mM HOAc at pH 5.0 fails to activate rTRPV2-K602Q. Note that the inward current evoked by 1 mM 2-APB is relatively small. (E) Heat-evoked currents in a cell expressing rTRPV2-K602Q. Note that 10 mM HOAc at pH 5.0 only provokes a minimal heat-evoked current. (F) Box diagrams with dot plots displaying the relative magnitudes of HOAc-evoked inward currents normalized with the responses evoked by 1 mM 2-APB ($n = 6-7$ for each mutant). Data are displayed for rTRPV2-WT, -K566Q, -K566A, -E569Q, -E569A, -D570N, -D570A -E577Q, -E577A, -E586Q and -E586A. (G, H) Representative current traces from HEK293T cells expressing rTRPV2-D570N (G) and rTRPV2-D570A (H). 30 mM HOAc at pH 5.0 evoked large currents in cells expressing rTRPV2-D570A. Functionality and expression was validated by application of 1 mM 2-APB. (I) Box diagrams with dot plots displaying the current densities of heat-evoked currents provoked by 10 mM HOAc at pH 5.0 in cells expressing rTRPV2-WT, -K566Q, -K566A, -E569Q, -E569A, -D570N, -D570A -E577Q, -E577A, -E586Q and -E586A ($n = 6-7$ for each mutant). (J) Bar diagrams displaying mean ($\pm$ SEM) current densities for currents evoked by 30 mM HOAc at pH 5.0 or 1 mM 2-APB in cells expressing rTRPV2-WT, -K566Q, -K566A, -E569Q, -E569A, -D570N, -D570A -E577Q, -E577A, -E586Q and -E586A ($n = 6-7$ for each mutant). (A, B, F, I) The box denotes the 50th percentile (median) as well as the 25th and 75th percentile. The whiskers mark the 5th and 95 percentiles. Data points beyond the whiskers are outliers. Source data are available online for this figure.

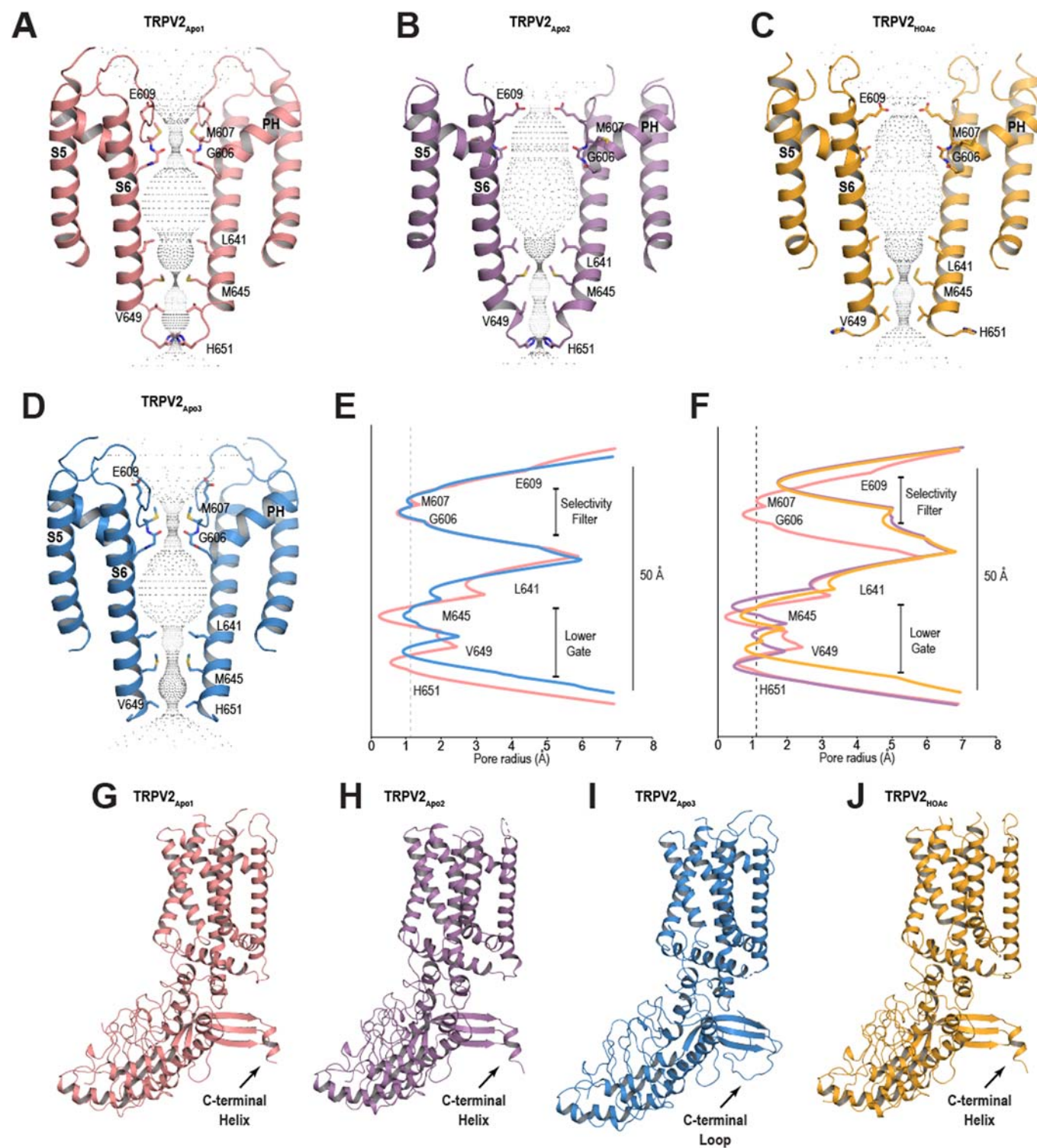


Figure EV4. The effect of HOAc on the pore profile of rTRPV2.

(A–D) Pore profiles of TRPV2_{Apo1} (A), TRPV2_{Apo2} (B), TRPV2_{HOAc} (C), and TRPV2_{Apo3} (D). (E, F) A graphical representation comparing the pore diameter of TRPV2_{Apo3} (blue) to TRPV2_{Apo1} (salmon) (E) or TRPV2_{HOAc} (orange) to TRPV2_{Apo2} (purple) and TRPV2_{Apo1} (salmon) (F). The dotted line marks the radius of a dehydrated calcium ion. (G–J) Side view of a single monomer of TRPV2_{Apo1} (G), TRPV2_{Apo2} (H), TRPV2_{Apo3} (I), and TRPV2_{HOAc} (J). Arrows indicate C-terminus.

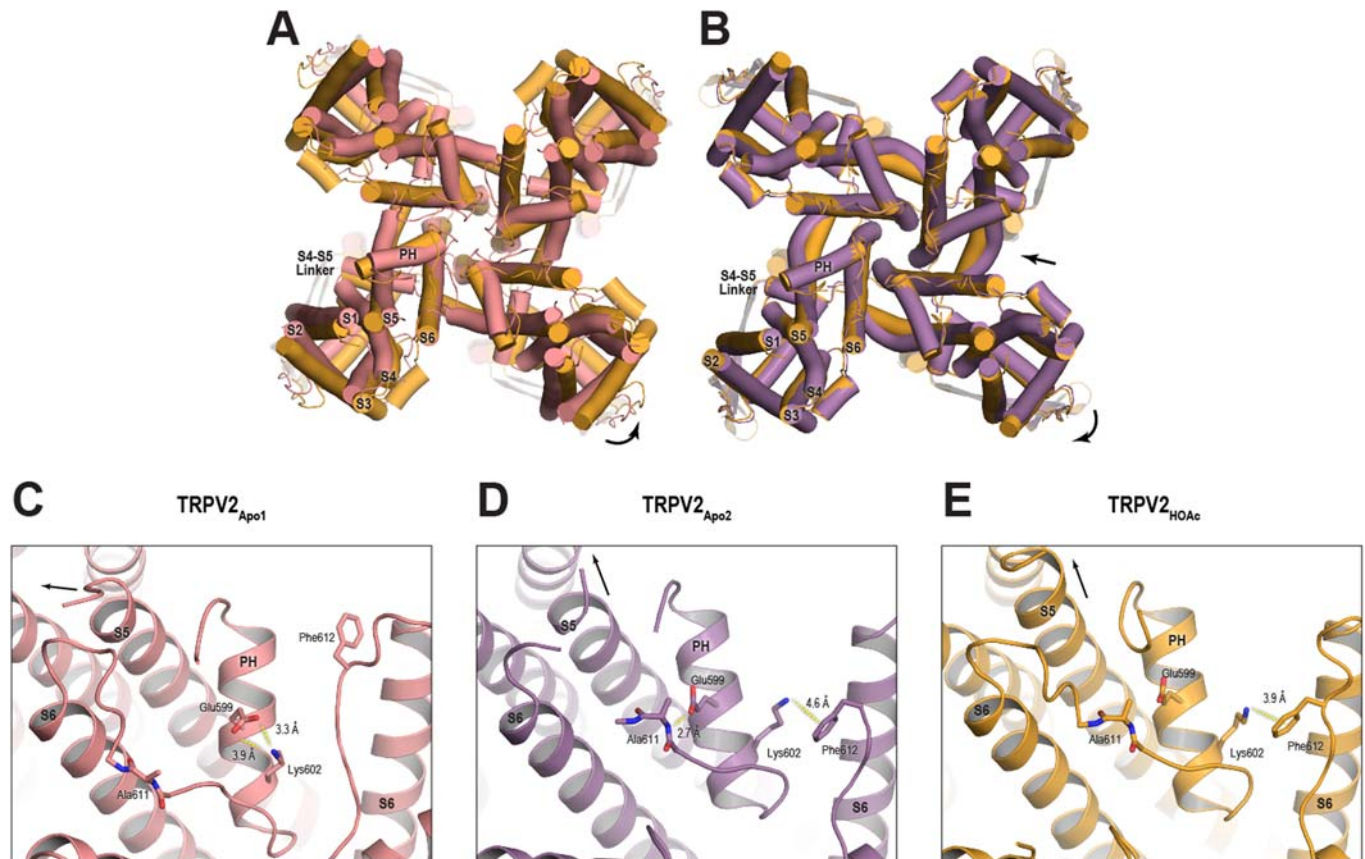


Figure EV5. HOAc-induced movements of rTRPV2.

(A, B). Alignments of TRPV2_{Apo1} (salmon) vs. TRPV2_{HOAc} (orange) (A) or TRPV2_{Apo2} (purple) vs. TRPV2_{HOAc} (orange) (B) at the extracellular face of the channel. Arrows indicate directions of movement in the transition from the apo states to TRPV2_{HOAc}. (C-E) View the pore helix in TRPV2_{Apo1} (C), TRPV2_{Apo2} (D), and TRPV2_{HOAc} (E). Bonds between residues are indicated by a yellow dashed line. Arrows indicate trajectory of the top of S5.