Mullins et al., http://www.jgp.org/cgi/content/full/jgp.201511437/DC1

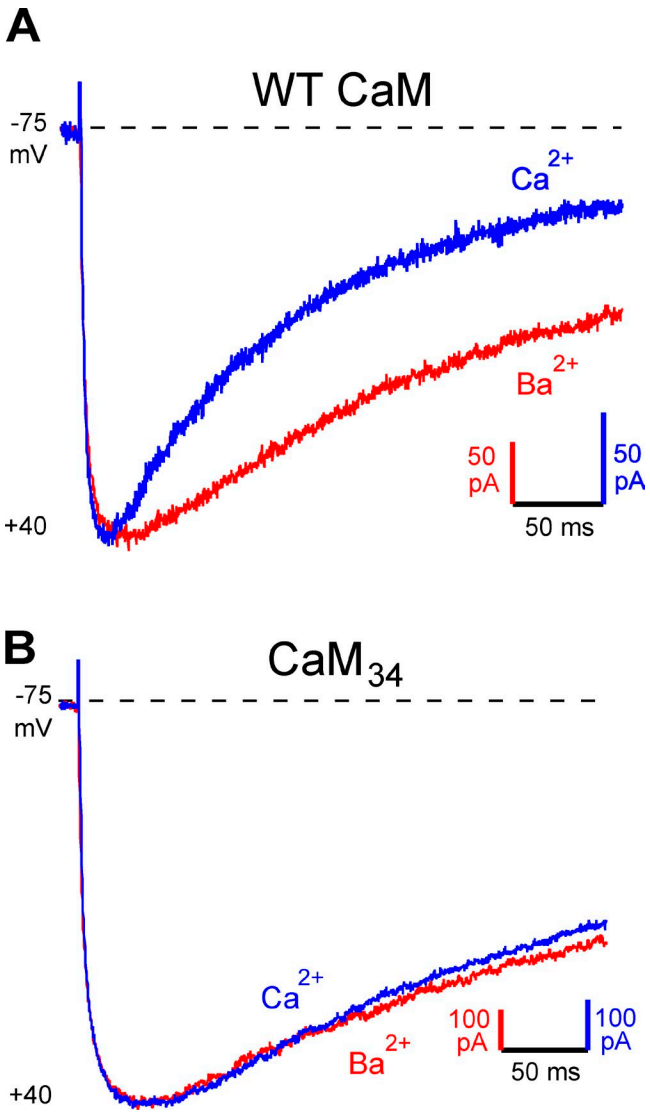

Figure S1. CDI of $Ca_v1.2$ currents is suppressed by CaM_{34}. HEK293H cells were transfected with $Ca_v1.2$, β1b, α2δ1, and CaM-IRES-GFP and recorded during 300-ms depolarizing steps from a holding potential of −75 mV (see Materials and methods). (A) Representative scaled currents in 20 mM Ca^{2+} (blue) and 20 mM Ba^{2+} (red) recorded from a single cell transfected with WT CaM and stepped to +40 mV. The difference between the blue and red traces is a measure of CDI, and the remaining inactivation in Ba^{2+} is by definition voltage-dependent. (B) Representative scaled currents in 20 mM Ca^{2+} (blue) and 20 mM Ba^{2+} (red) recorded from a single cell transfected with CaM_{34} and stepped to +40 mV. The similarity of the scaled traces in Ca^{2+} and Ba^{2+} is consistent with almost complete suppression of CDI by CaM_{34}. The current remaining at the end of the pulse was divided by the peak current to obtain a normalized measure of inactivation for each cell in both Ca^{2+} and Ba^{2+} (R_{300} for Ca^{2+} and for Ba^{2+}). For summary and statistical comparisons, the difference between the R_{300} values in Ca^{2+} vs. Ba^{2+} (ΔR_{300}, an index of CDI) was determined for each cell at several potentials, and the mean ± SEM for $n = 4–5$ cells from each condition are plotted in Fig. 2 A (bottom).

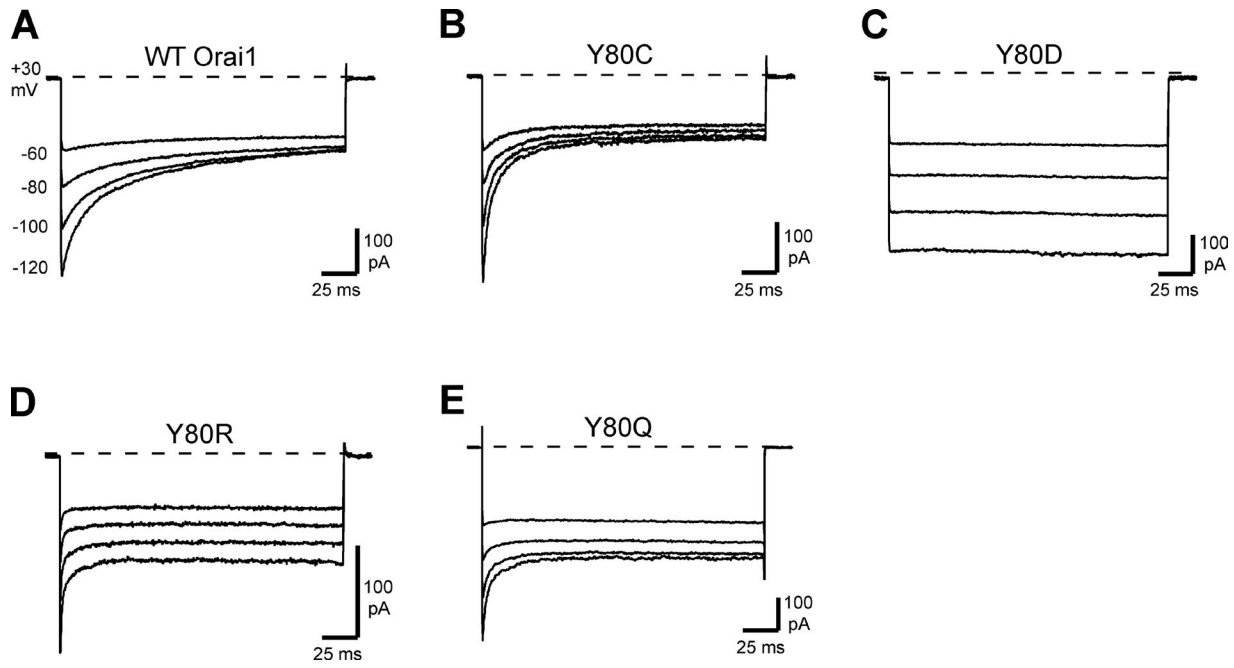

Figure S2. Representative currents for additional mutations at Orai1 position Y80. All currents were recorded and analyzed as described for Fig. 3. Representative currents are shown for WT Orai1 (A, same traces shown in Fig. 3 A) and the Orai1 point mutants Y80C (B), Y80D (C), Y80R (D), and Y80Q (E). Extents of CDI for these mutations are summarized in Fig. 3 G. Fast and slow time constants from bi-exponential fits are plotted against test potential in Fig. 3 (H and I) and fast and slow component amplitudes are summarized in Fig. S3 (A and B).

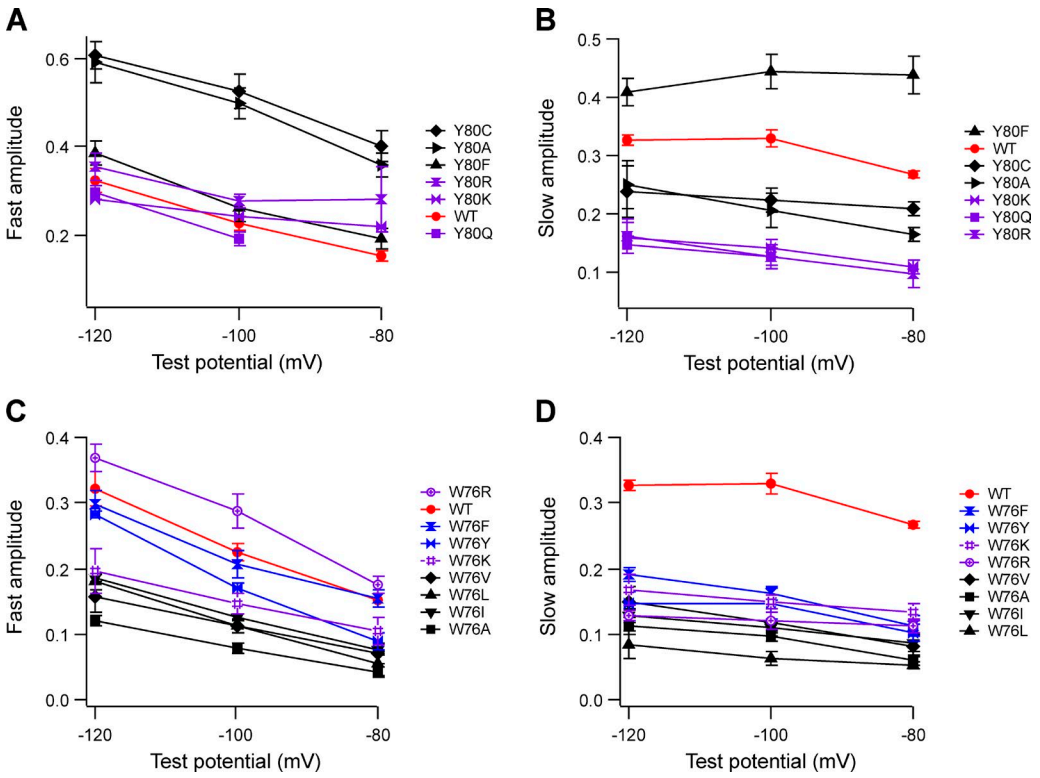

Figure S3. Fast and slow contributions to CDI for Orai1 mutations at the Y80 and W76 positions. (A–D) Current traces collected in 20 mM $Ca^{2+}{}_o$, were fitted by the biexponential function $I = I_o + A_1e^{-t/\tau 1} + A_2e^{-t/\tau 2}$, where τ1 and τ2 represent fast and slow time constants. Time constants from these fits are included in Figs. 3 and 5. Each point represents the mean ± SEM for $n = 4–7$ cells. Fast (A) and slow (B) amplitudes for Y80 mutations are plotted against test potential. Fast (C) and slow (D) amplitudes for W76 mutations are plotted against test potential.

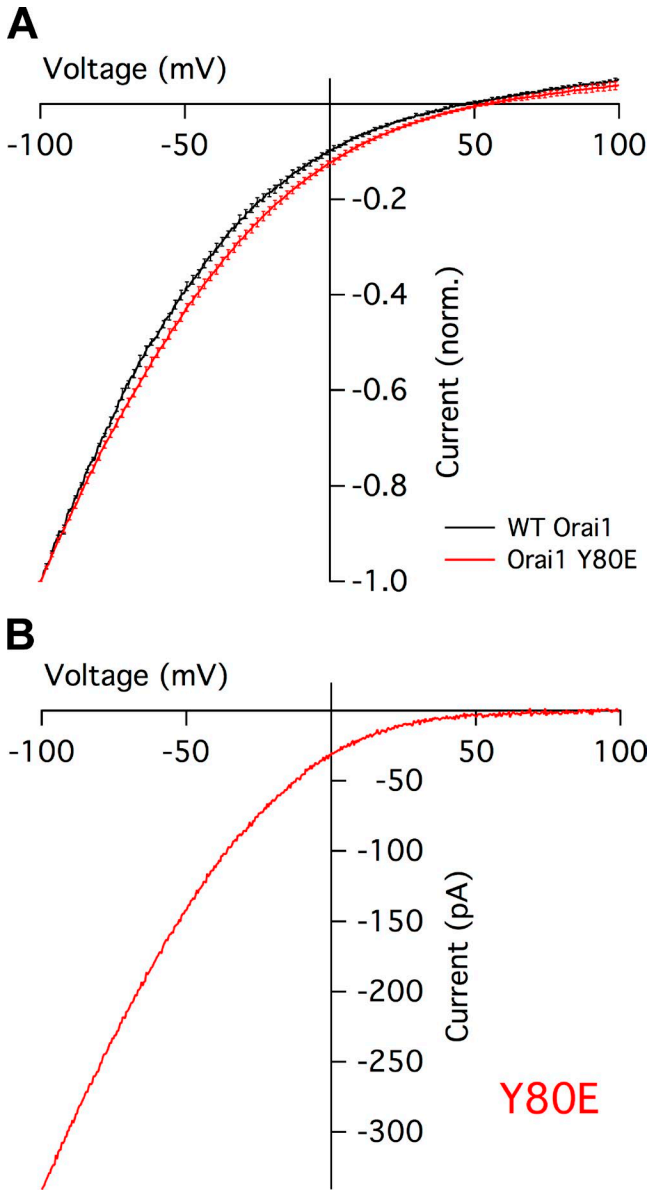

Figure S4. Selectivity of the Orai1 Y80E mutant channels as assessed by voltage ramps. (A) WT and Orai1 Y80E mutant currents were measured in DVF external solution during 100-ms hyperpolarizing steps to −100 mV (not depicted) followed by 100-ms ramps from −100 mV to + 100 mV. Data were leak-subtracted and normalized to the current at −100 mV. Normalized mean ± SEM (with SEM plotted for every fifth point) is shown for $n = 5$ cells. (B) Orai1 Y80E current evoked in 20 mM Ca^{2+} Ringer's solution by the step-ramp protocol described in A (mean of five consecutive leak-subtracted ramp currents from a representative cell).

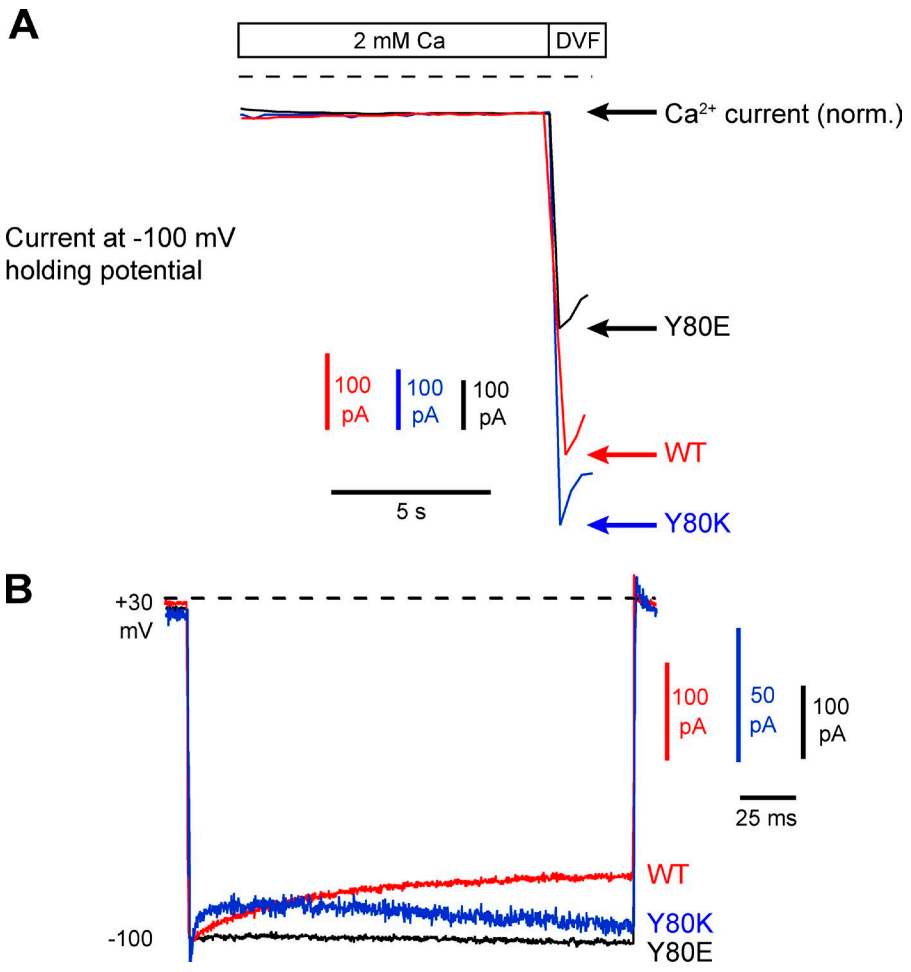

Figure S5. A method for estimating i_{Ca} from i_{Na}. We estimated the unitary Ca^{2+} current (i_{Ca}) using the following equation (see Materials methods):

$$i_{Ca} = i_{Na} \frac{I_{Ca}}{I_{Na}} \frac{1}{(1-CDI)},$$

where I_{Ca} is the Ca^{2+}-CRAC current measured in 2 mM Ca^{2+}, I_{Na} is the Na^{+}-CRAC current measured immediately after switching to DVF extracellular solution, and *CDI* is the extent of inactivation in 2 mM Ca^{2+} at −100 mV. Dashed lines indicate zero current level. (A) Measuring I_{Ca} and I_{Na}. Scaled representative currents are shown for WT (red), Y80K (blue), and Y80E (black) Orai1 channels recorded at −100 mV holding potential starting in 2 mM Ca^{2+} (top arrow), and the color-coded arrows mark the peak currents attained upon switching to DVF. Mean I_{Ca}/I_{Na} values were 0.096 ± 0.005 (WT, $n = 3$), 0.082 ± 0.009 (Y80K, $n = 4$), and 0.156 ± 0.007 (Y80E, $n = 4$). (B) Measuring the CDI correction factor. Scaled representative currents from WT (red), Y80K (blue), and Y80E (black) channels are shown during 200-ms pulses from +30 to −100 mV in 2 mM Ca^{2+}. The current remaining at 195 ms was divided by the peak current 1–3 ms after the beginning of the pulse. For Y80K channels, which showed slow potentiation in 2 mM Ca^{2+}, the minimum current during the pulse was used. The values of remaining current were averaged from four cells, and the inverse (1/(1−CDI)) was used as a CDI correction factor to compensate for the partial inactivation of Ca^{2+} current that is immediately lost upon exposure to DVF. The CDI correction factors were 1.27 (WT), 1.11 (Y80K), and 0.99 (Y80E).

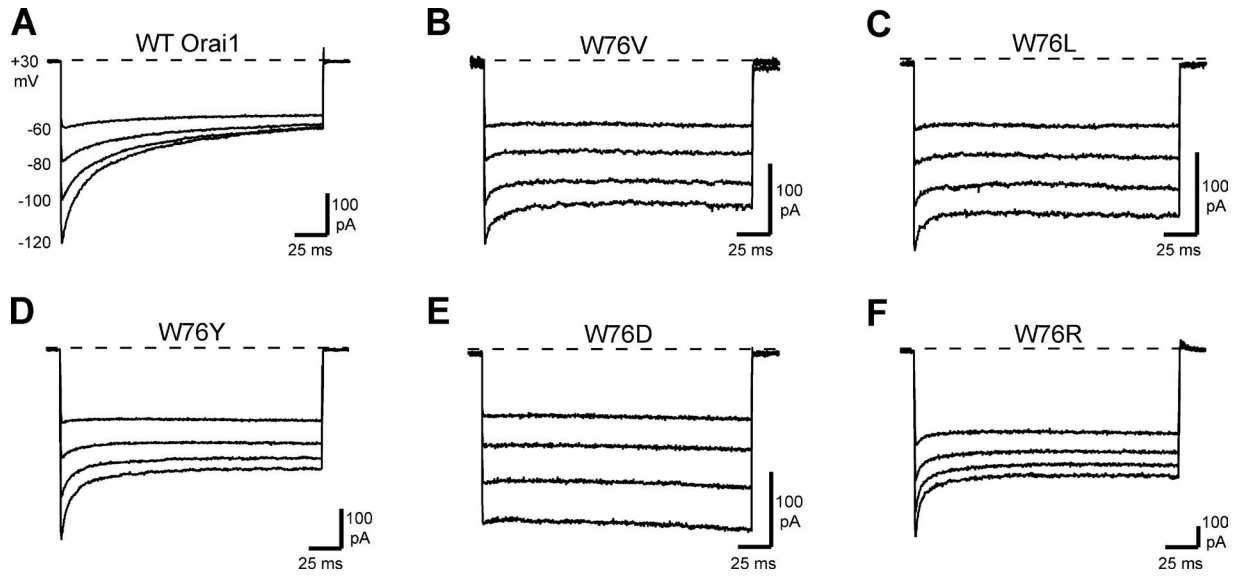

Figure S6. Representative currents for additional mutations at Orai1 position W76. All currents were recorded and analyzed as described for Fig. 3. Representative currents are shown for WT Orai1 (A, same traces shown in Fig. 3 A) and Orai1 point mutants W76V (B), W76L (C), W76Y (D), W76D (E), and W76R (F). Extents of CDI for these mutations are summarized in Fig. 5 G. Fast and slow time constants from biexponential fits are plotted against test potential in Fig. 5 H and I. Corresponding amplitudes of fast and slow components are summarized in Fig. S3 (C and D).

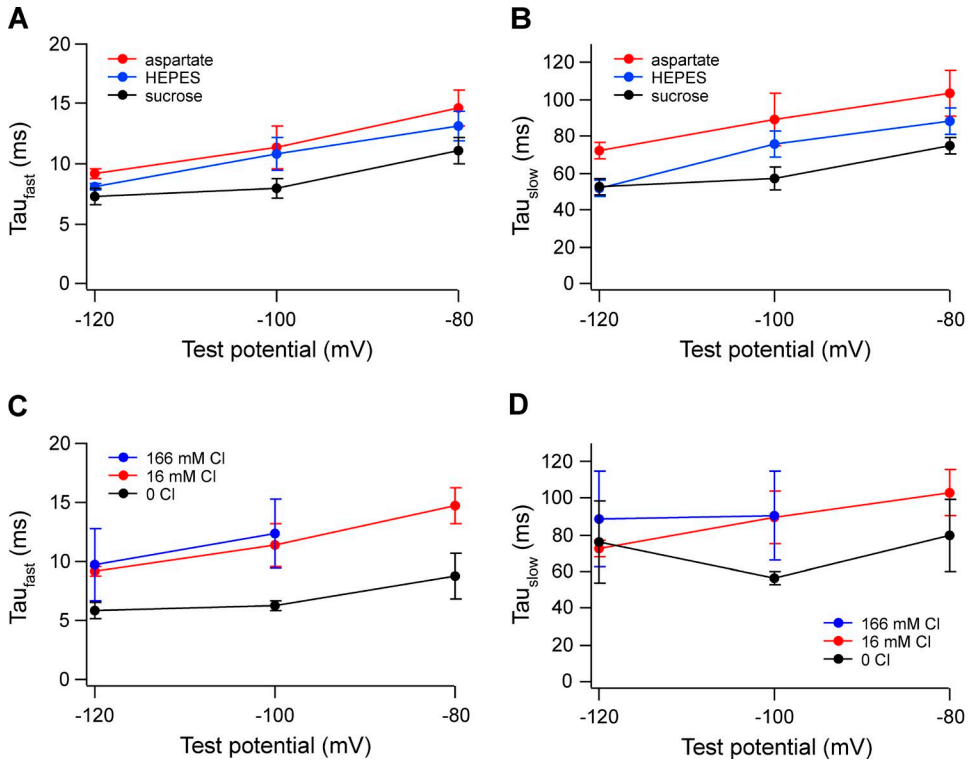

Figure S7. Inactivation kinetics for WT Orai1 with a range of internal anions. (A–D) Current traces like those shown in Fig. 7 were fitted by the biexponential function $I = I_o + A_1e^{-t/\tau 1} + A_2e^{-t/\tau 2}$, where τ1 and τ2 represent fast and slow time constants of inactivation. Each point represents the mean ± SEM for $n = 5–6$ cells. Fast (A) and slow (B) time constants of inactivation for aspartate, HEPES, and sucrose are plotted against test potential. Fast (C) and slow (D) time constants of inactivation for three chloride concentrations are plotted against test potential.