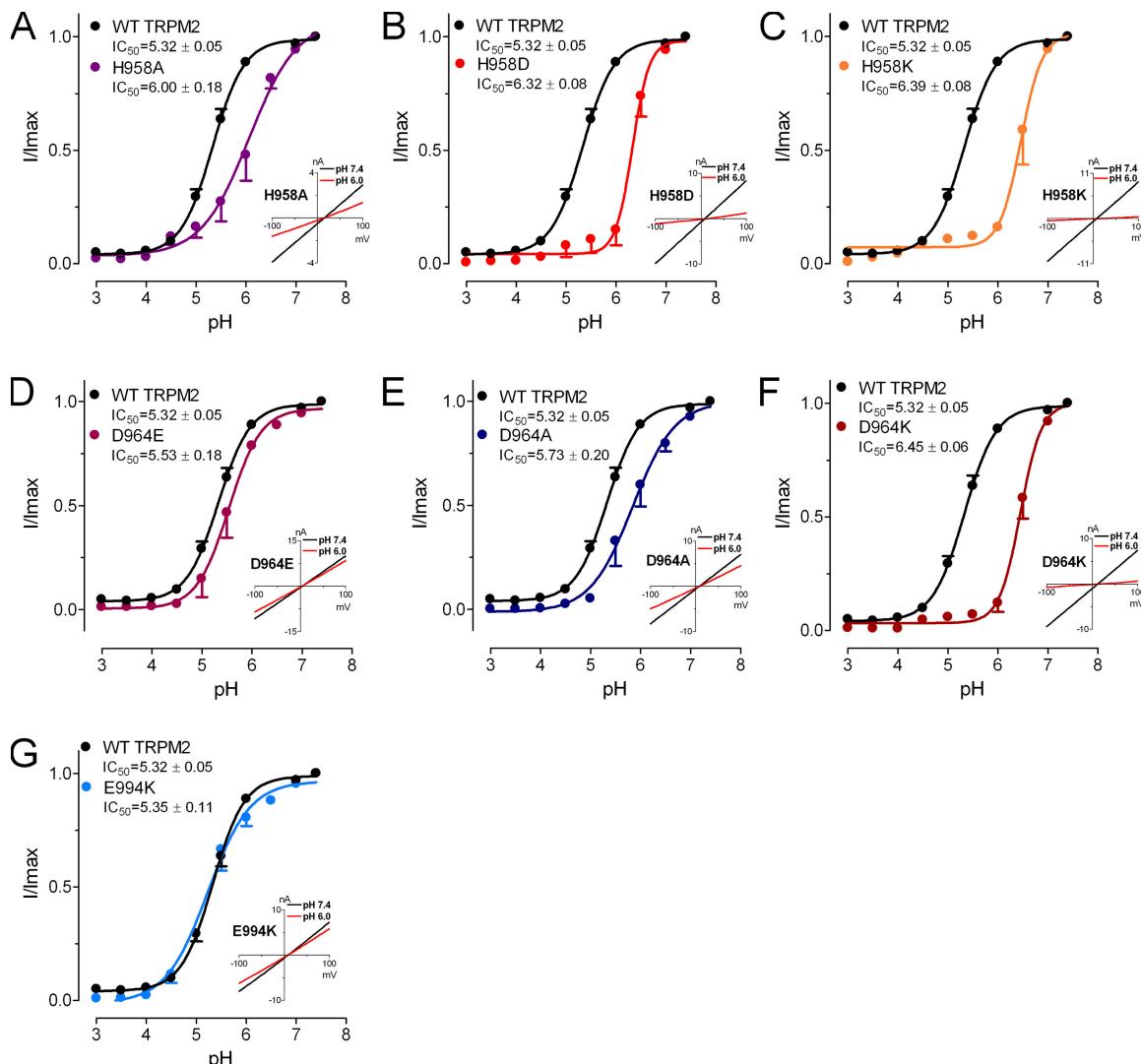
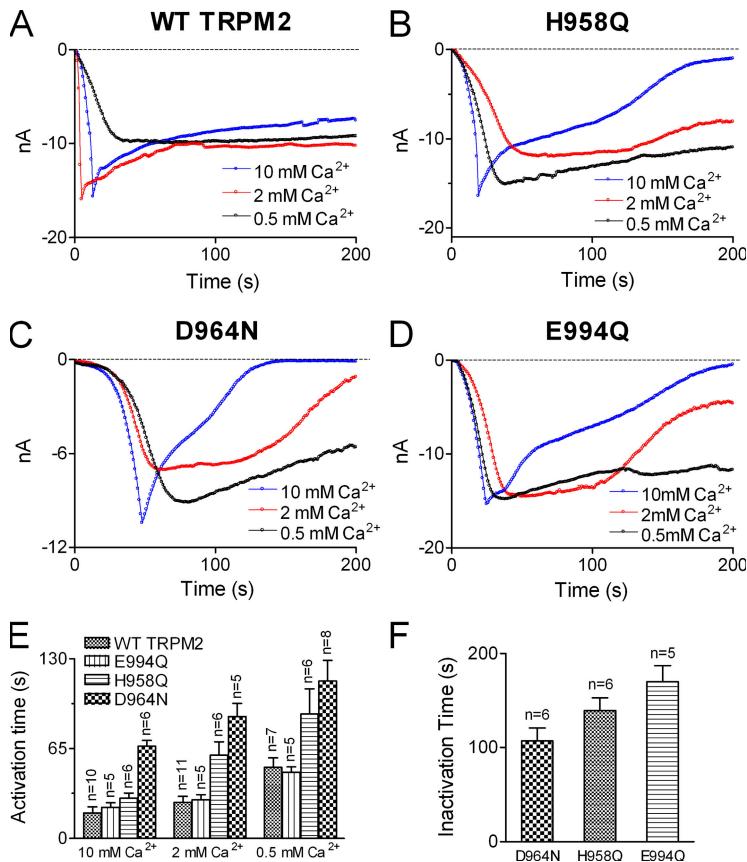


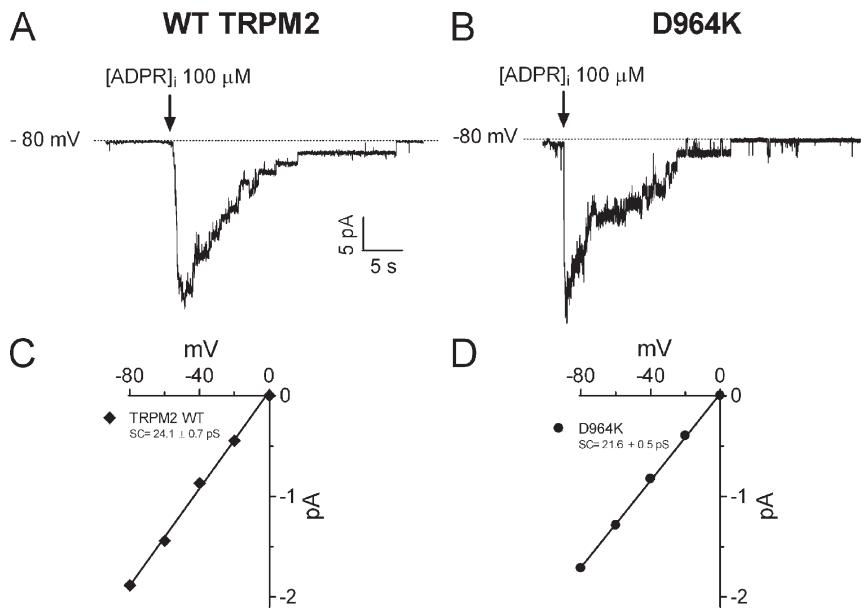
Du et al., <http://www.jgp.org/cgi/content/full/jgp.200910254/DC1>



**Figure S1.** Changes in  $pH_o$  sensitivity in the mutants at positions H958, D964, and E994. Current amplitude at the indicated  $pH$  was normalized to maximal current at  $pH$  7.4. Inset panels illustrate the original recordings of WT TRPM2 and the mutants at  $pH$  7.4 and 6.0. (A–C) Dose–response curves of the effects of acidic  $pH_o$  on mutants H958A, H958D, and H958K. Best fit of the dose–response curves yielded  $IC_{50}$ s (pH units) of  $6.0 \pm 0.18$  ( $n = 8$ ) for H958A,  $6.3 \pm 0.08$  ( $n = 6$ ) for H958D, and  $6.4 \pm 0.08$  ( $n = 8$ ) for H958K. The Hill coefficient was  $n_H = 0.9 \pm 0.2$  for H958A,  $n_H = 2.6 \pm 0.5$  for H958D, and  $n_H = 2.0 \pm 0.5$  for H958K. (D–F) Dose-dependent effects of acidic  $pH_o$  on D964E, D964A, and D964K. The  $IC_{50}$ s (pH units) were  $5.5 \pm 0.2$  ( $n = 8$ ) for D964E,  $5.7 \pm 0.2$  ( $n = 7$ ) for D964A, and  $6.5 \pm 0.06$  ( $n = 10$ ) for D964K. The Hill coefficient was  $n_H = 1.3 \pm 0.2$  for D964E,  $n_H = 1.0 \pm 0.2$  for D964A, and  $n_H = 2.0 \pm 0.3$  for D964K, respectively. (G) Effects of acidic  $pH$  on E994K. The  $IC_{50}$  was  $5.35 \pm 0.11$  pH units and  $n_H = 1.0 \pm 0.1$  ( $n = 8$ ).



**Figure S2.** Time-dependent activation and inactivation of H958Q, D964N, and E994Q. (A) WT TRPM2 inward current at the indicated  $[\text{Ca}^{2+}]_o$  concentrations. (B) H958Q displayed time-dependent inactivation and completely inactivated at 10 mM  $[\text{Ca}^{2+}]_o$ . (C) Time-dependent inactivation of D964N at the indicated  $[\text{Ca}^{2+}]_o$ . (D) Inactivation of E994Q at the indicated  $[\text{Ca}^{2+}]_o$ . (E) Average time courses for activation of WT TRPM2, H958Q, D964N, and E994Q at the indicated external  $\text{Ca}^{2+}$  concentrations. Activation of D964N was much slower than WT TRPM2 at various  $\text{Ca}^{2+}$  concentrations. The activation of H958Q was significantly slower than WT TRPM2 at 2 and 0.5 mM  $\text{Ca}^{2+}$ . (F) The inactivation time courses of H958Q, D964N, and E994Q at 10 mM  $\text{Ca}^{2+}$ . H958Q, D964N, and E994Q completely inactivated in  $139.2 \pm 13.7$  ms ( $n = 6$ ),  $107.2 \pm 13.7$  ms ( $n = 6$ ), and  $170.0 \pm 17.1$  ms ( $n = 5$ ), respectively.



**Figure S3.** Single-channel currents of WT TRPM2 and D964K carried by  $\text{Ca}^{2+}$ . (A and B) Representative currents of WT TRPM2 and D964K recorded in inside-out patches in the internal (bath) solution containing 100  $\mu\text{M}$  ADPR and nominally free  $\text{Ca}^{2+}$  and external (pipette) solution containing isotonic  $\text{Ca}^{2+}$  solution. Currents were recorded at  $-80$  mV. (C and D) Single-channel currents carried by  $\text{Ca}^{2+}$  at various voltages. Linear regression fit of the current amplitudes yielded single-channel conductances of  $24.1 \pm 0.7$  pS ( $n = 10$ ) for WT TRPM2 and  $21.6 \pm 0.5$  pS for D964K ( $n = 10$ ;  $P > 0.05$ ).