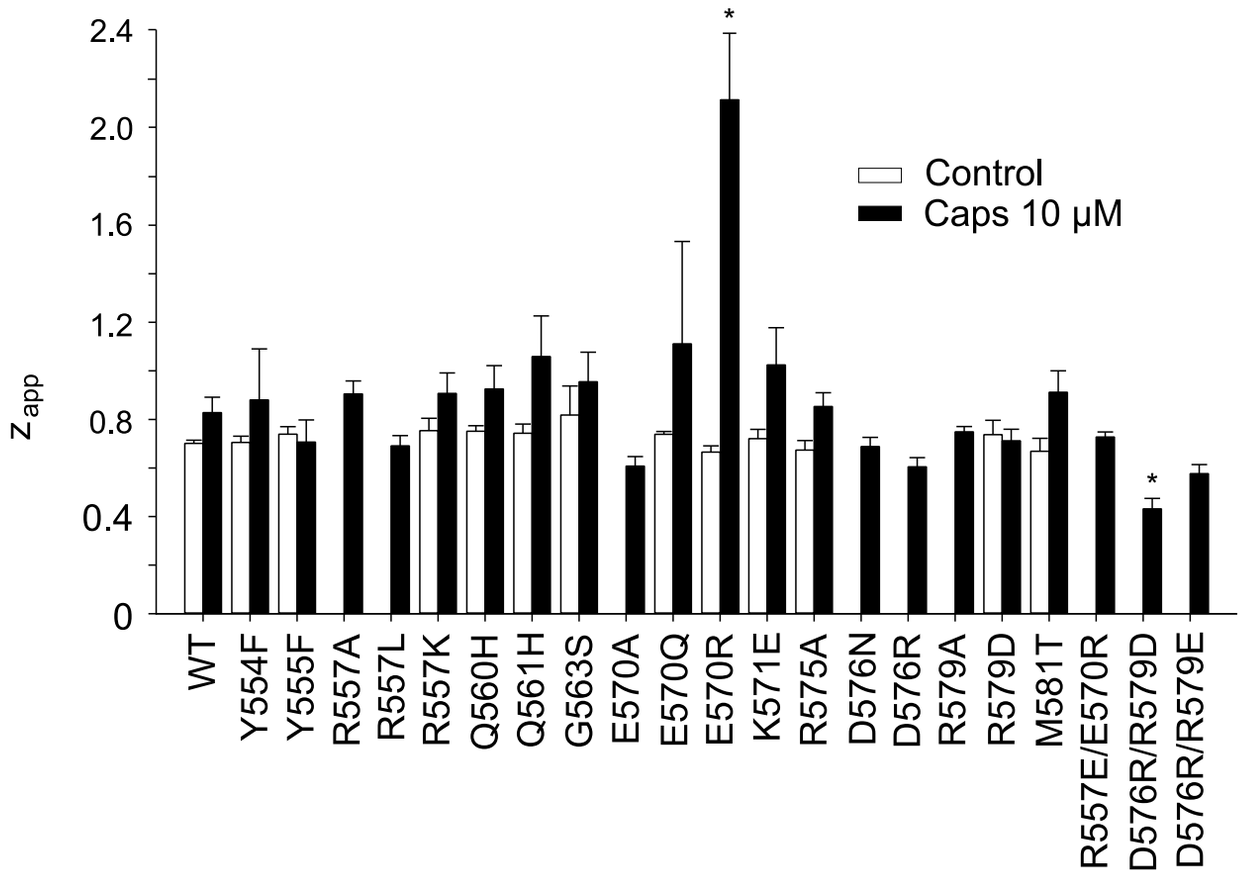
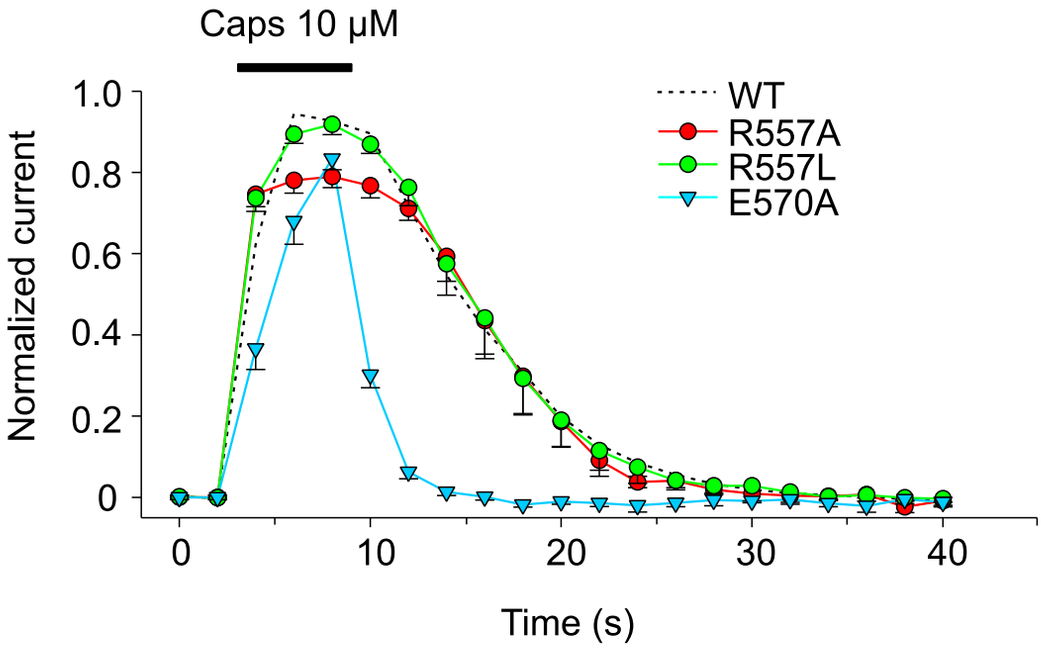
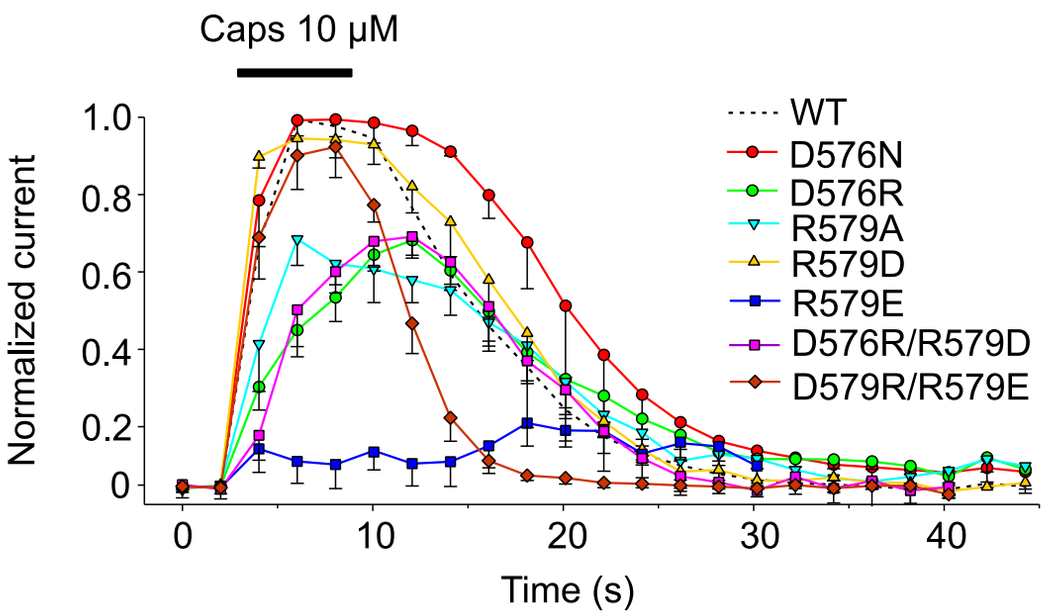
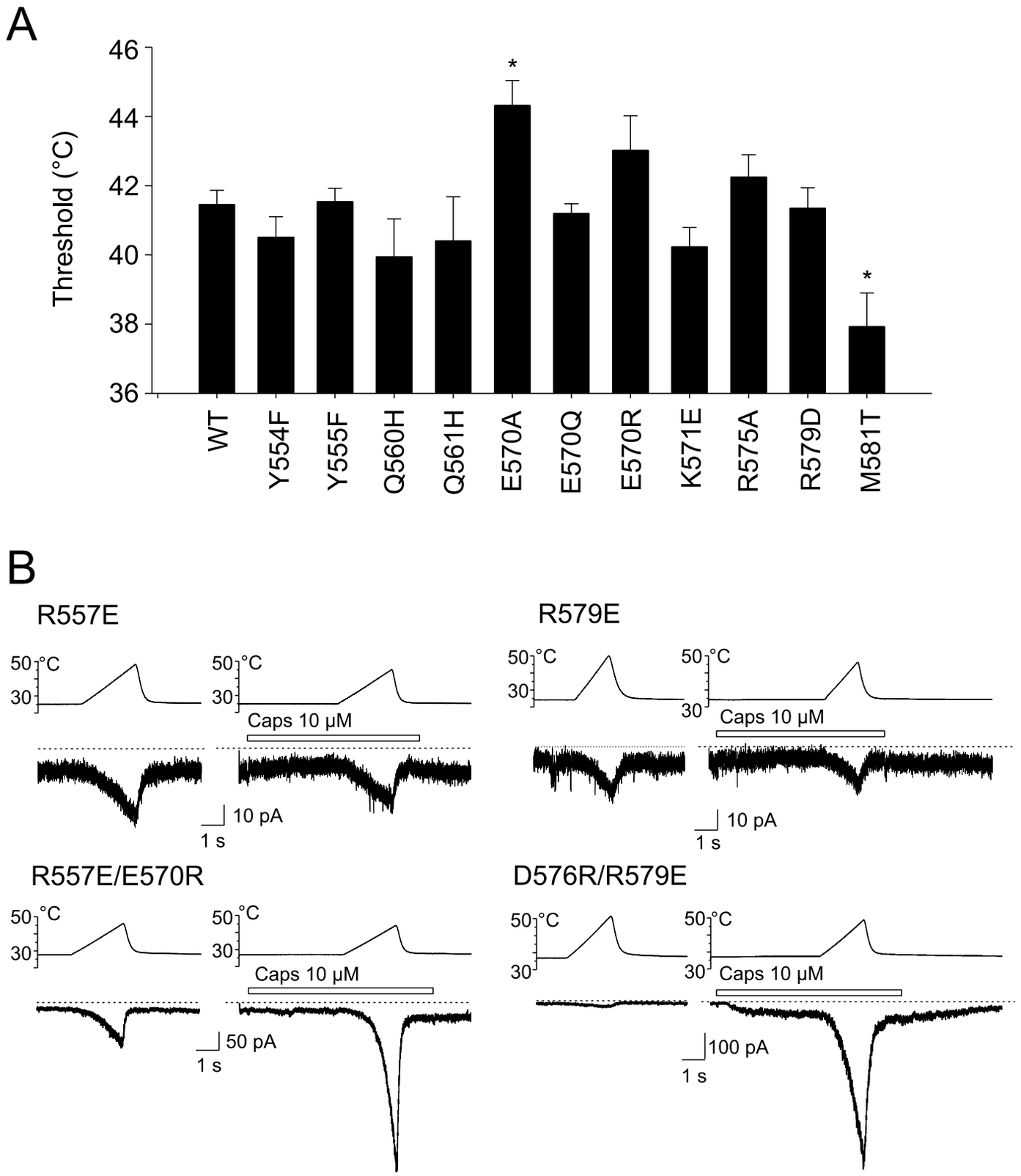


Supplemental Figure 1

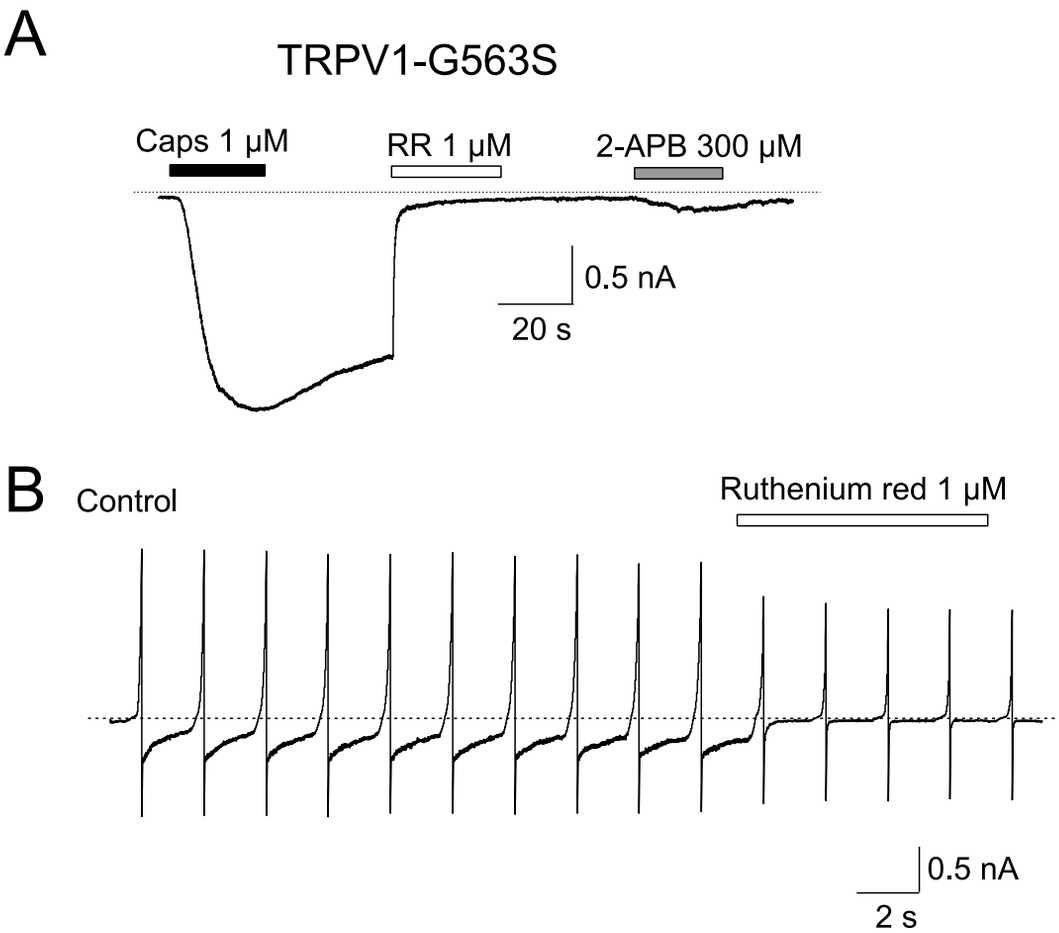




Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

Fig. S1. Voltage dependence of TRPV1 mutants. Summary of the apparent number of gating charges (*z*) for wild-type TRPV1 and indicated mutants in the absence (*open bars*) or presence of 10 μM capsaicin (*filled bars*). Each bar is the mean ± s.e.m.; *n* = 60 for wild type and 4-17 for mutants.

Fig. S2. Mutations in S4 and the S4-S5 linker alter kinetics of capsaicin-induced whole cell currents. (*A*) Averaged wole-cell currents measured at +90 mV before, during 6-s exposure to and after washout of 10 μM capsaicin. A voltage protocol consisted of a 500-ms ramp from –70 mV to +100 mV applied every 2 s. Averaged currents were constructed from 3-5 independent recordings such as shown in Fig. 3*C*. The time courses of the capsaicin-induced (depolarization-modulated) whole-cell currents through E570A and (*B*) R576R/R579E exhibited a faster offset of capsaicin-dependent responses than in wild type TRPV1. Superimposed *dotted line* indicates the averaged currents of wild type. Note the rescue of nonfunctional R579E mutant by the charge-swapping mutation D579R/R579E.

Fig. S3. Mutations in S4 and S4-S5 linker altering temperature sensitivity in TRPV1. (*A*) Effects of mutations on the threshold for temperature activation in wild-type TRPV1 (*n* = 30) and indicated mutants (*n* = 3 - 19). Each bar is the mean ± s.e.m.. (*B*) Representative recordings of whole-cell current responses to application of 25-48°C heat ramp (10°C/s) measured from nonfunctional R557E and R579E and the rescued double-mutants R557E/E570R and D576R/R579E in the absence or presence of 10 μM capsaicin. Holding potential -70 mV.

Fig. S4. TRPV1 mutation G563S stabilizes the open conformation and leads to overactive channels. (*A*) Whole-cell current recordings from HEK293T cell expressing TRPV1-G563S mutant. Holding potential –70 mV. (*B*) Whole cell currents in response to a voltage protocol consisting of a 500-ms ramp from –70 mV to +100 mV applied every 2 s in the control extracellular solution.