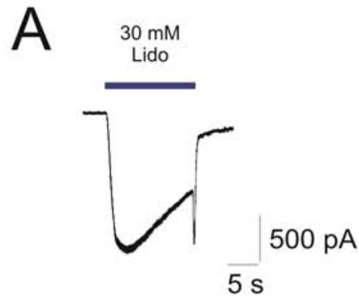
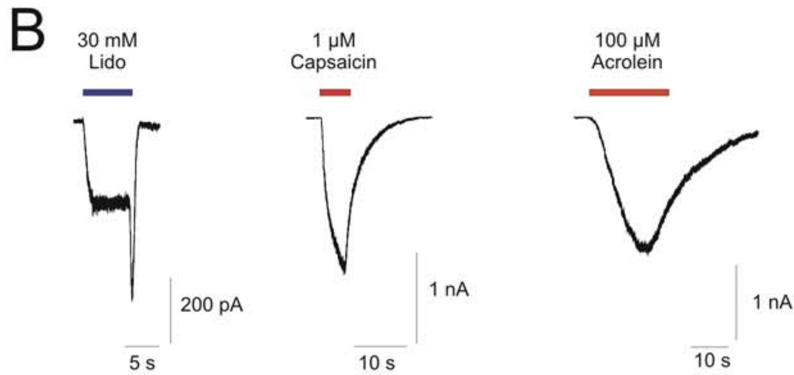
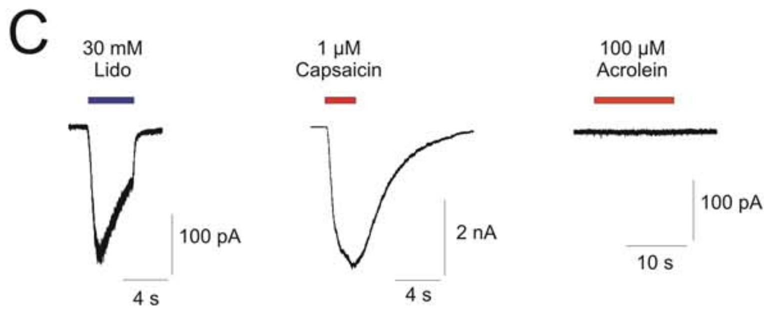
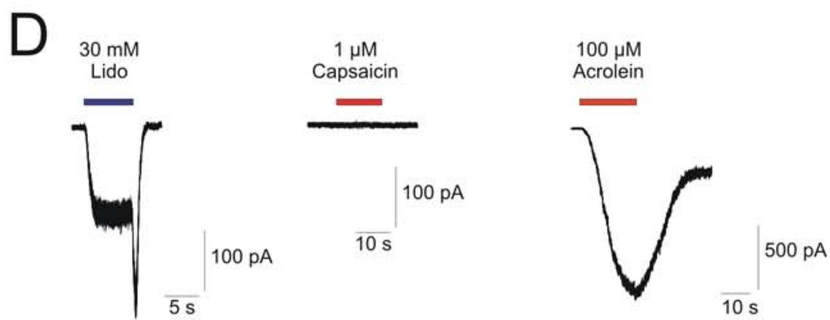
rTRPA1 - HEK293t



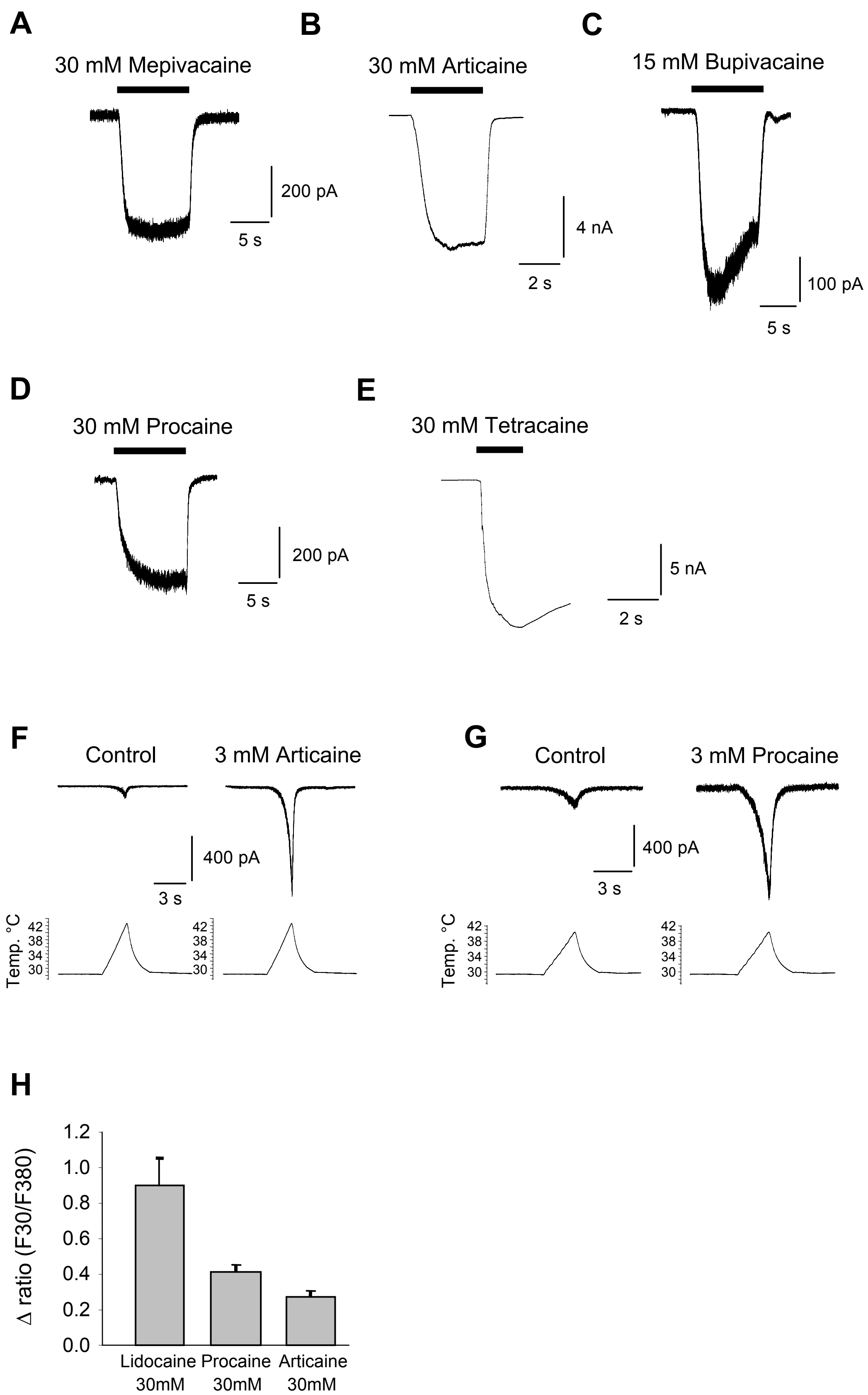
Mouse DRG Neuron



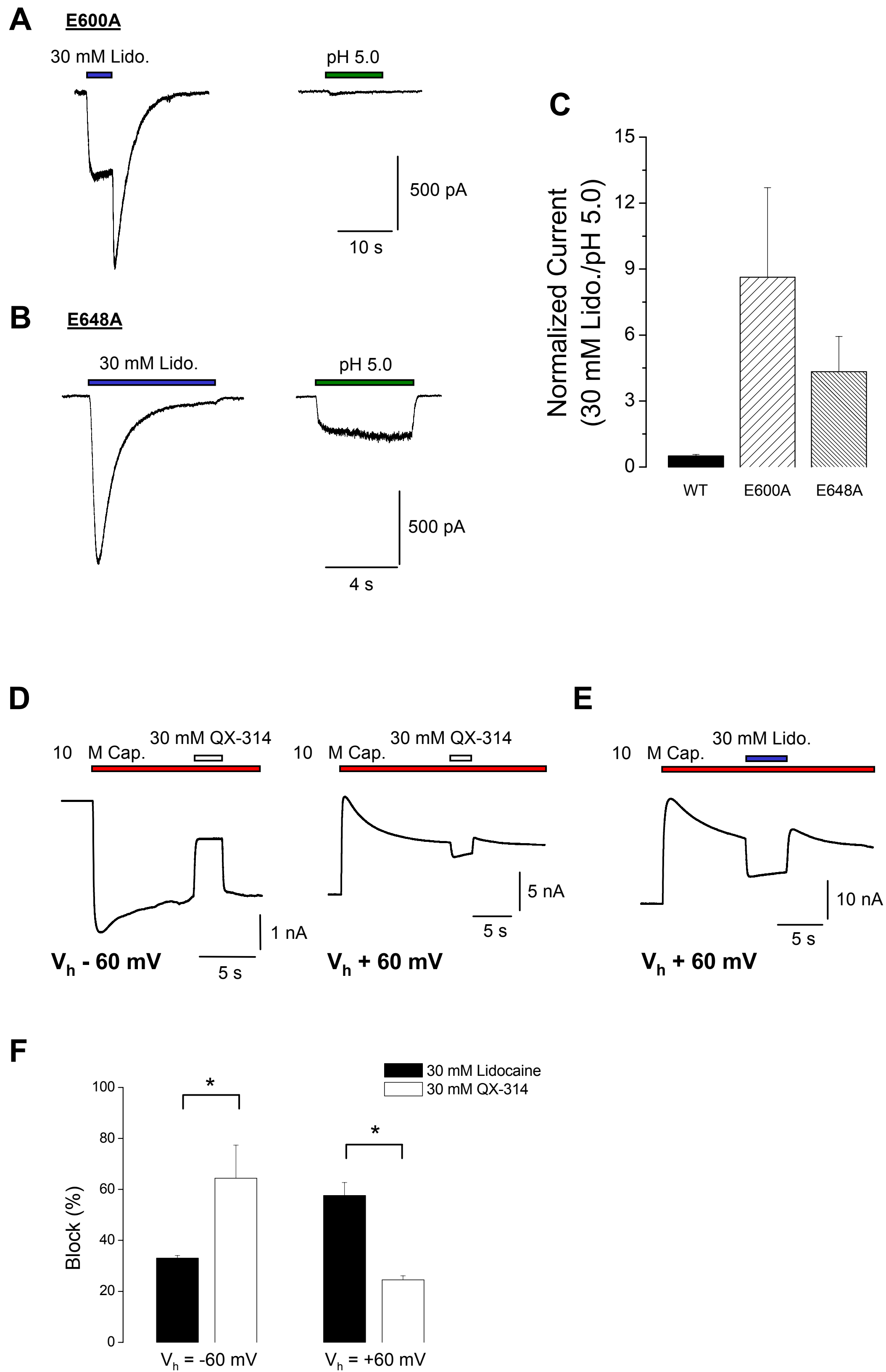




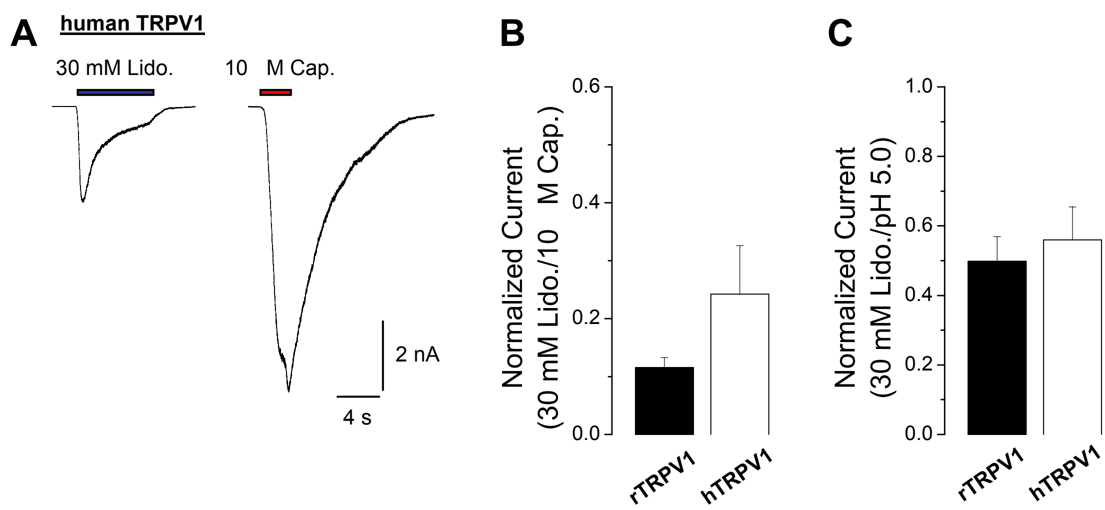
Supplementary Figure 1



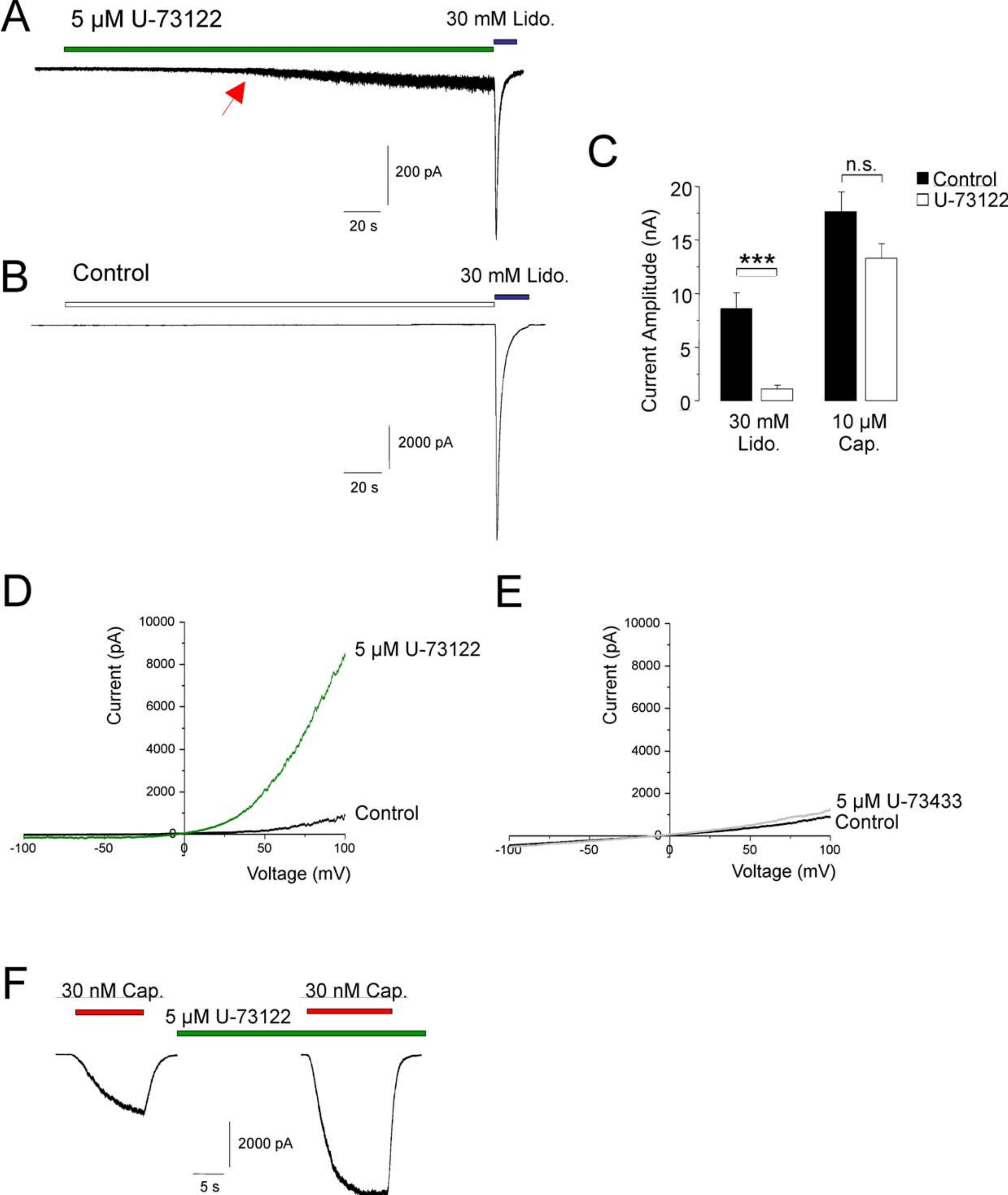
Supplementary Figure 2



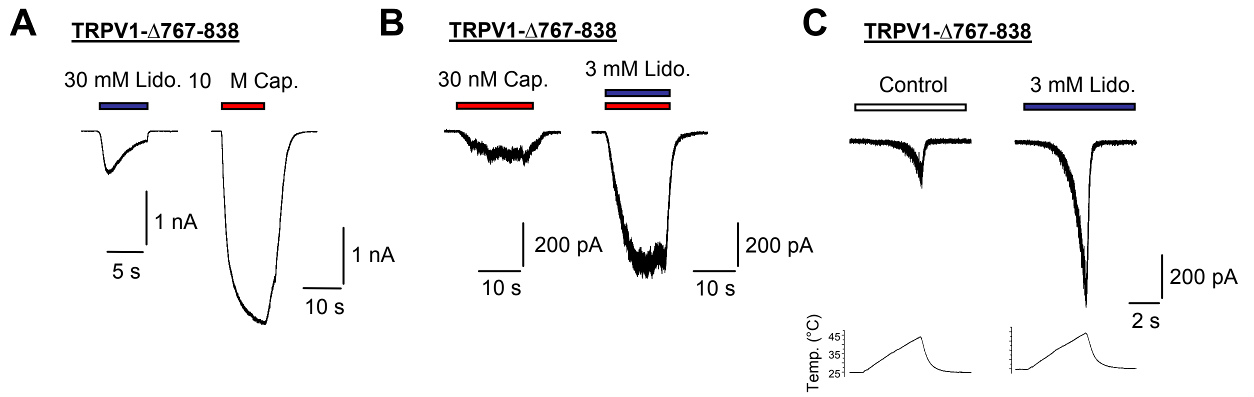
Supplementary Figure 3



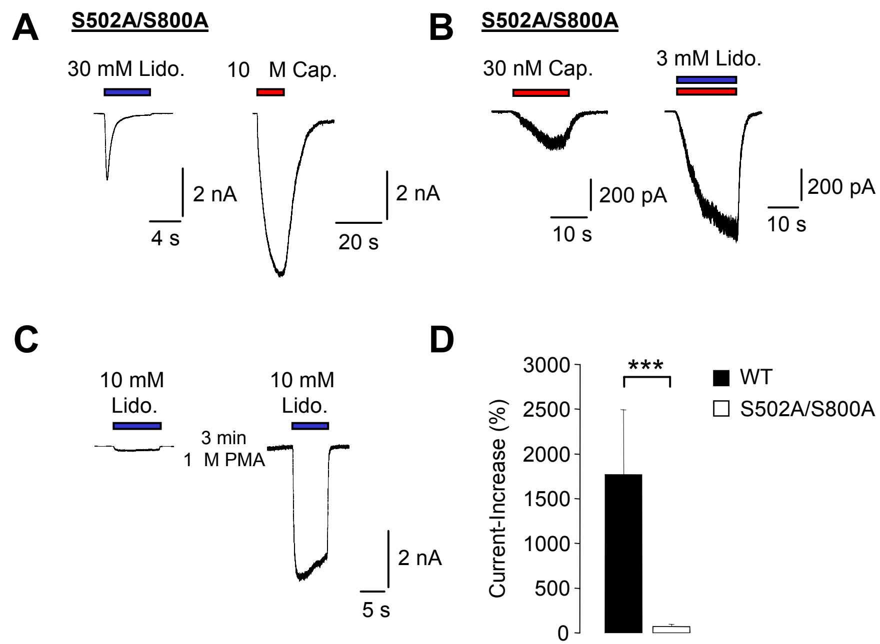
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7

Supplementary Figure 1. Lidocaine activates recombinant rTRPA1 expressed in HEK293t cells and native TRPA1 in mouse DRG neurons. **A.** Typical current trace of rTRPA1 expressed in HEK293t cells activated by 30 mM lidocaine. The holding potential (V_h) was -60 mV and lidocaine was applied for about 12 s. **B – D.** Representative current traces of lidocaine-sensitive mouse DRG neurons also displaying inward currents activated by (B) both the TRPV1 agonist capsaicin and the TRPA1 agonist acrolein, (C) only by capsaicin, or (D) only by acrolein. In intervals of 2 min, lidocaine (30 mM) and capsaicin (1 μM) were applied for about 5 – 10 s, acrolein (100 μM) for about 10 – 20 s. V_h = -60 mV.

Supplementary Figure 2. Both amide and ester local anaesthetics activate and sensitize recombinant TRPV1 expressed in HEK293t cells. **A – C.** TRPV1-currents activated by the amides mepivacaine (A), articaine (B) and bupivacaine (C). **D – E.** TRPV1-currents activated by the esters procaine (D) and tetracaine (E). The holding potential (V_h) was -60 mV. Current traces are representative samples out of 3- 4 cells examined for each LA. **F, G.** Representative heat-evoked inward currents of TRPV1 in control solution and in the presence of (F) 3 mM articaine (increase by 7.0 ± 1.2-fold, n = 7) and (G) 3 mM procine (increase by 5.3 ± 0.4-fold, n = 6). **H.** Mean changes in fluorescence ratios (Δratio (F340/F380)) induced by 30 mM lidocaine, procaine, or articaine in rat DRG neurons.

Supplementary Figure 3. The extracellular proton-binding sites E600 and E648 are not crucial for the lidocaine-sensitivity of TRPV1. **A, B.** Representative current traces of E600A (A) and E648A (B) activated by 30 mM lidocaine and pH 5.0. **C.** Mean ratios of lidocaine- (30 mM) to proton-activated (pH 5.0) currents of TRPV1-WT, TRPV1-E600A (n= 5) and TRPV1-E648A (n= 5). **D. – F.** The membrane impermeable lidocaine-

derivative QX-314 blocks capsaicine-evoked inward and outward currents. **D, E.** TRPV1 currents evoked by 10 µM capsaicin in cells held at -60 mV or + 60 mV. 30 mM QX-314 (D) or 30 mM lidocaine (E) were co-applied with 10 µM capsaicin after currents were activated by 10 µM capsaicin alone. Experiments were performed in Ca^{2+}-free extracellular solution to avoid Ca^{2+}-dependent desensiziation. **F.** Mean block ± SEM of capsaicin-evoked inward (V_h = -60 mV) and outward currents (V_h = +60 mV) by 30 mM QX-314 and 30 mM lidocaine (unpaired Student's *t*-test).

Supplementary Figure 4. Human TRPV1 is activated by lidocaine. **A.** Representative current traces of human TRPV1 activated by 30 mM lidocaine and 10 µM capsaicin. **B.** Mean ratios of lidocaine- (30 mM) to capsaicin-evoked (10 µm) currents of TRPV1-WT and human TRPV1 (n= 6). **C.** Mean ratios of lidocaine- (30 mM) to proton-activated (pH 5.0) currents of TRPV1-WT and human TRPV1 (n= 7).

Supplementary Figure 5. The PLC-blocker U73122 reduces lidocaine-sensitivity and is able to activate and sensitize TRPV1. **A, B.** Lidocaine-evoked inward currents activated in control solution (B) or after a 3 min-long treatment with the PLC-blocker U73122 (A). Note the small inward currents activated by U73122 indicated by the arrow **C.** Mean current amplitudes ± SEM measured in experiments described in A and B with 30 mM lidocaine (n= 9) or with 10 µM capsaicin (n= 9) (unpaired Student's *t*-test). **D, E.** Representative ramp currents of TRPV1 in control solution and in 5 µM U73122 (D, n= 12) or in 5 µM U73433 (E, n= 4). Cells were held at -60 mV and currents were measured during 500 ms long voltage-ramps from -100 to +100 mV. Note the typical outward-rectification of TRPV1 with U73122. **F.** Representative currents demonstrating the sensitizing effect of U73122 on TRPV1-currents activated by 30 nM capsaicin (n= 5). In

A- F, experiments were performed in Ca^{2+}-free extracellular solution and cells were held at -60 mV.

Supplementary Figure 6. The putative $PI(4,5)P_2$-binding site within the distal C terminus of TRPV1 is not required for lidocaine-sensitivity. **A.** Representative current traces of TRPV1-ΔE767-738 activated by 30 mM lidocaine and 10 µm capsaicin. **B, C.** Typical effect of 3 mM lidocaine on capsaicin-evoked (B) and heat-evoked (C) currents of TRPV1. In A- C, experiments were performed as described under Fig. 1 and 2.

Supplementary Figure 7. Phosphorylation by PKC is not required for lidocaine-evoked activation and sensitization of TRPV1 but sensitizes TRPV1 to lidocaine. **A.** Representative currents of TRPV1-S502A/S800A activated by 30 mM lidocaine and 10 µM capsaicin. **B.** Effect of 3 mM lidocaine on capsaicin-evoked TRPV1- S502A/S800A-currents. **C.** Currents of TRPV1 activated by 10 mM lidocaine before and after treatment with the PKC-activator PMA. Currents were activated every 3 min in Ca^{2+}-free extracellular solution. **D.** Mean increase of current amplitudes ± SEM of lidocaine-activated TRPV1-WT (18.7 ± 7.3-fold, n= 6) and TRPV1-S502A/S800A (1.7 ± 0.2-fold, n= 11) after treatment with 1 µM PMA. Current amplitudes were normalized to the value obtained with the first application of lidocaine (unpaired Student´s *t*-test).