SUPPLEMENTAL MATERIAL

Nörenberg et al., http://www.jgp.org/cgi/content/full/jgp.201611595/DC1

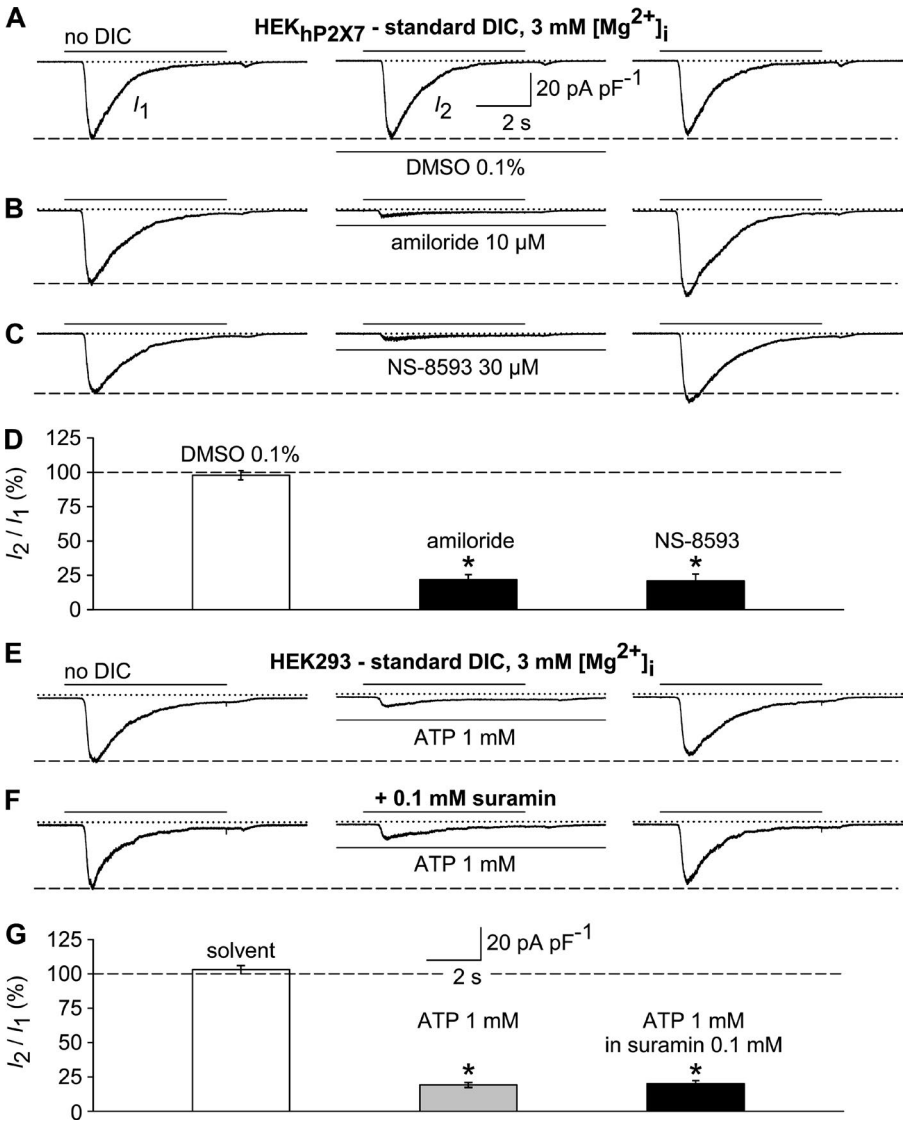

Figure S1. **Transient currents evoked by DIC-free bath solutions (no DIC) are amiloride sensitive and inhibited by ATP.** (A–G) Phasic currents in HEK$_{hP2X7}$ cells were recorded with a 3 mM Mg^{2+}–containing pipette solution to block TRPM7 activity. (A–C) Inward currents at −60 mV in HEK$_{hP2X7}$ cells were elicited by no-DIC solutions either under control conditions (left, I_1) or 6 min after the addition of 0.1% DMSO, the ASIC blocker amiloride (10 µM), or the TRPM7 modulator NS-8593 (30 µM; middle, I_2). (right) No-DIC–induced inward currents 6 min after removal of the test compounds. (D) Statistical analysis of several experiments performed as shown in A–C (n = 7–9 each). ASIC1a-like peak currents in the presence of test compounds (I_2) are depicted as percentage of the respective pre-application peak current (I_1). *, P < 0.001, significant difference to the control condition (DMSO). (E–G) Experiments similar to those in A–D but with 1 mM ATP as test compound were performed in parental HEK293 parental cells to exclude P2X7 receptor activation. (G) Statistical analysis of current inhibition measured in several experiments performed as in E and F. *, P < 0.001, significant difference from the control condition (no DIC; n = 9 each). Note that inhibition of ASIC1a-like currents by 1 mM ATP is unchanged in the continuous presence of the broad-spectrum P2 receptor antagonist suramin (F and G). In electrophysiological figures, dotted lines indicate the zero current level. Error bars indicate SEM.

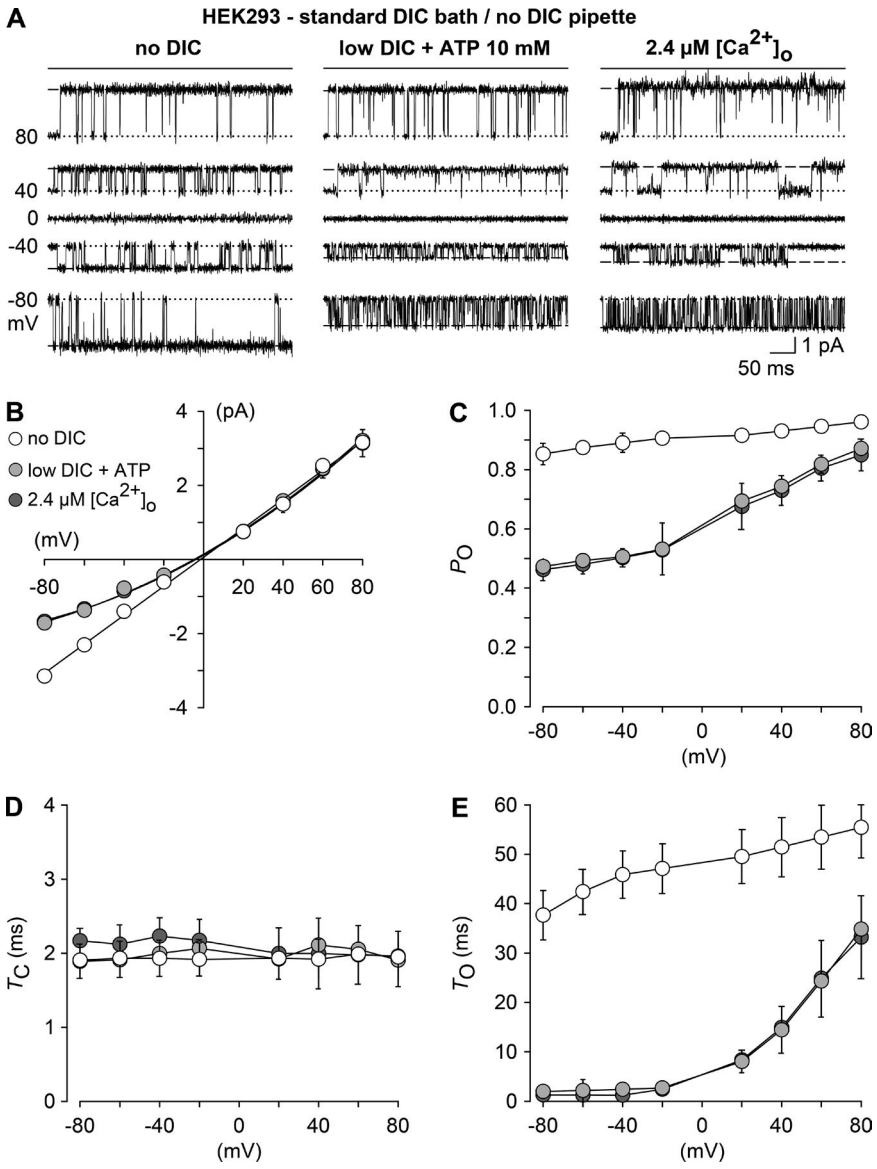

Figure S2. **Voltage dependence of TRPM7 block by ATP in low DIC and in the presence of 2.4 µM $[Ca^{2+}]_o$.** (A) Representative current traces obtained at the indicated patch potentials in outside-out patches from HEK293 cells in bath solutions containing no DIC, 10 mM ATP in low DIC (2.4 µM calculated free $[Ca^{2+}]_o$), or 2.4 µM $[Ca^{2+}]_o$ buffered with EGTA and EDTA. (B–E) Plot of unitary current amplitudes (B), open probability (P_O; C), mean closed time (T_C; D), and mean open time (T_O; E) versus patch potential in the presence of no DIC (open circles), 10 mM ATP in low DIC (light gray circles), and 2.4 µM $[Ca^{2+}]_o$ (dark gray circles) from n = 6–7 experiments similar to those in A. In electrophysiological figures, dotted lines indicate the zero current level. Error bars indicate SEM.

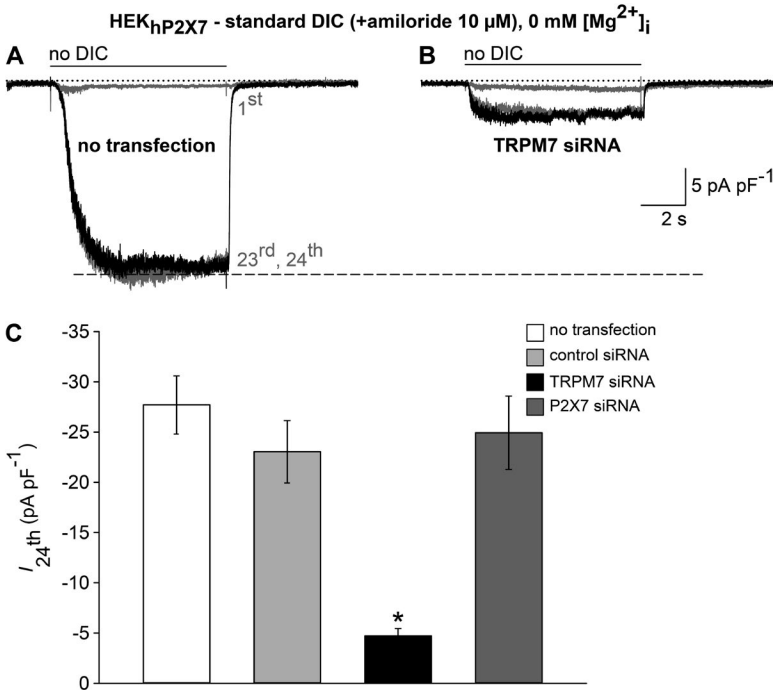

Figure S3. **Knockdown of TRPM7, but not P2X7, suppresses sustained no-DIC–induced current in HEK$_{hP2X7}$ cells.** HEK$_{hP2X7}$ cells were transfected with pools of three (30 nM each) duplex siRNAs (Sigma-Aldrich) targeting TRPM7 (SASI_Hs01_00120022, SASI_Hs01_00120023, SASI_Hs01_00120024) or P2X7 (SASI_Hs01_00127827, SASI_Hs01_00127828, SASI_Hs01_00127829) or with an unrelated control sequence (SIC001; 90 nM) using Lipofectamine 2000 (Invitrogen). After 5 h, the transfection mix was removed by medium change. 2 d after transfection, cells were seeded onto 35-mm dishes and subjected to patch-clamp experiments within 12–24 h. Noninactivating currents in HEK$_{hP2X7}$ cells were recorded with a Mg^{2+}-free pipette solution and in the presence of 10 µM amiloride to block phasic current activity. (A and B) Shown are superimposed membrane currents in response to a DIC-free bath solution (no DIC) that were recorded at a holding potential of −60 mV from a nontransfected (A) or a TRPM7 siRNA–transfected HEK$_{hP2X7}$ cell (B). Cells were stimulated 24 times and at 30-s intervals with 8-s-long no-DIC pulses to induce full current run-up. Note that only the responses to the 1st, 23rd, and 24th challenge are shown. (C) Statistical analysis of experiments similar to those in A and B (n = 7 each). Bars represent peak current densities in response to the 24th no-DIC pulse that were obtained under control conditions (no transfection) or after cells had been transfected with control siRNA, TRPM7 siRNA, or P2X7 siRNA. *, $P < 0.001$, significant difference from the currents in nontransfected HEK$_{hP2X7}$ cells. In electrophysiological figures, dotted lines indicate the zero current level. Error bars indicate SEM.

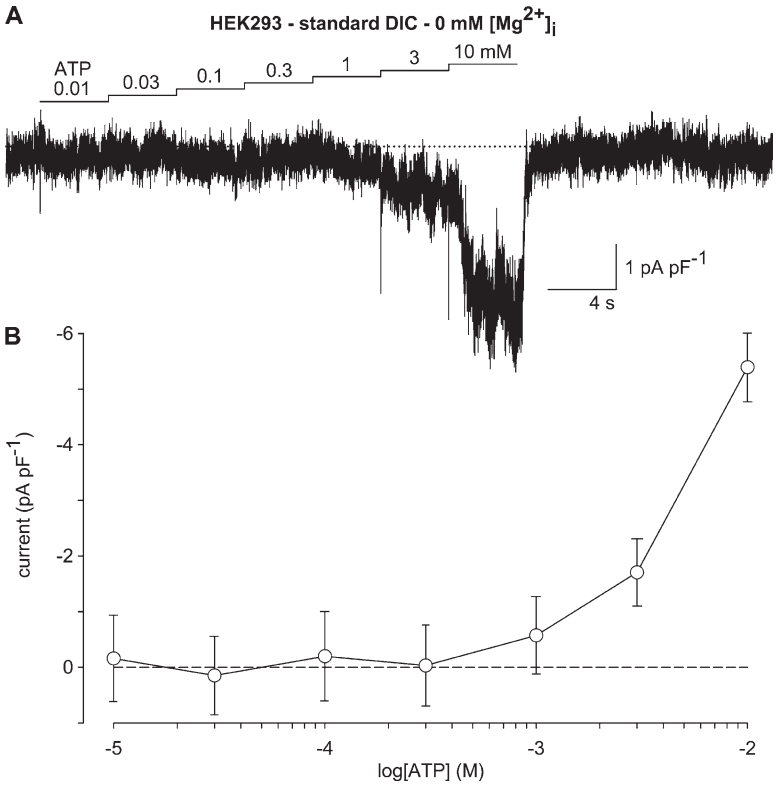

Figure S4. **ATP evokes TRPM7-like currents in the presence of standard extracellular DIC concentrations.** (A) Whole-cell currents (at −60 mV) in parental HEK293 cells in the presence of increasing ATP concentrations. (B) Statistical evaluation of seven experiments. Recordings were performed with a pipette solution containing no Mg^{2+}, and cells were preconditioned with repetitive applications of 10 mM ATP (for 4 s at 120-s intervals) until current run-up in response to 10 mM ATP had been completed (not depicted). In electrophysiological figures, dotted lines indicate the zero current level. Error bars indicate SEM.

Transient ASIC1a-like currents are induced by low $[Ca^{2+}]_o$ in HEK_{hP2X7} cells

In HEK_{hP2X7} cells, a transient inward current developed during $[Ca^{2+}]_o$ removal but not during the application of 1–10 mM ATP. These rapidly inactivating inward currents were reminiscent of ASIC1a channels known to be expressed in HEK293 cells (Gunthorpe et al., 2001). Because the pH dependence of ASIC1a is strongly modulated by $[Ca^{2+}]_o$ (Babini et al., 2002; Sherwood et al., 2012), lowering $[Ca^{2+}]_o$ to subphysiological levels may shift the threshold for channel activation to higher pH values, thereby allowing the channel to gate at the pH 7.3, given by the bath solution. Consistent with this assumption, transient inward currents induced by 6-s pulses of a no-DIC bath solution were almost abrogated by preincubating HEK_{hP2X7} cells with 10 µM amiloride (Fig. S1, A–D). To clarify why similar currents were not seen in HEK_{hP2X7} or in HEK293 cells exposed to 1–10 mM ATP, we tested the impact of 1 mM ATP on no-DIC–induced ASIC1a-like currents in HEK293 cells. In the presence of 1 mM ATP, transient inward currents induced by DIC-free media were inhibited by ∼80% (Fig. S1, E–G), an effect which largely remained in the presence of 0.1 mM of the broad-spectrum P2 receptor inhibitor suramin (von Kügelgen and Harden, 2011), indicating that the inhibitory effect of ATP is not caused via P2Y or P2X receptors. Thus, lowering the $[Ca^{2+}]_o$ by millimolar ATP concentrations masks the activation of ASIC1a-like currents, presumably because of direct inhibition by ATP. As an incidental finding, we observed that 30 µM NS-8593 almost completely blocked the ASIC1a-like currents (Fig. S1, B and D).

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