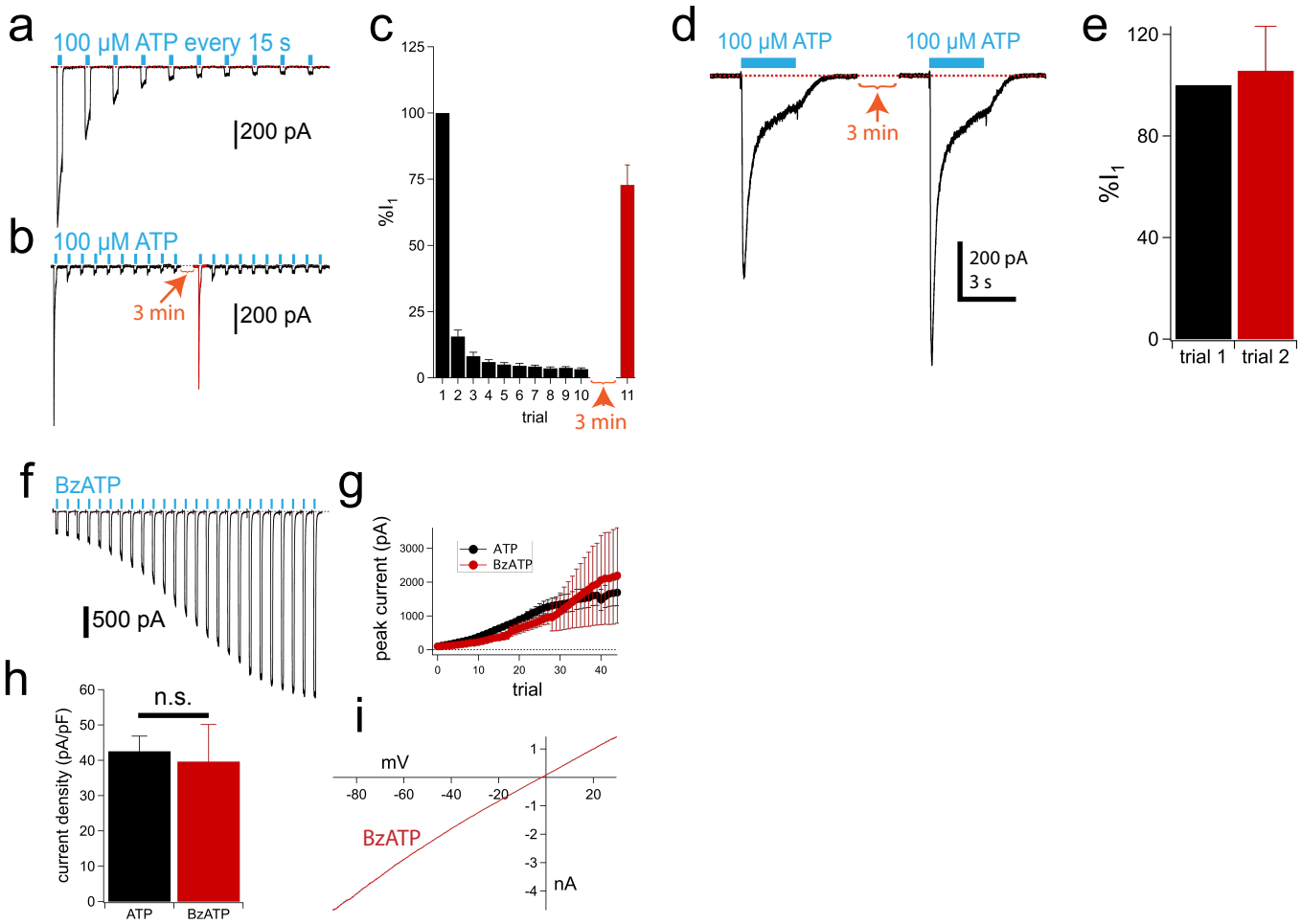
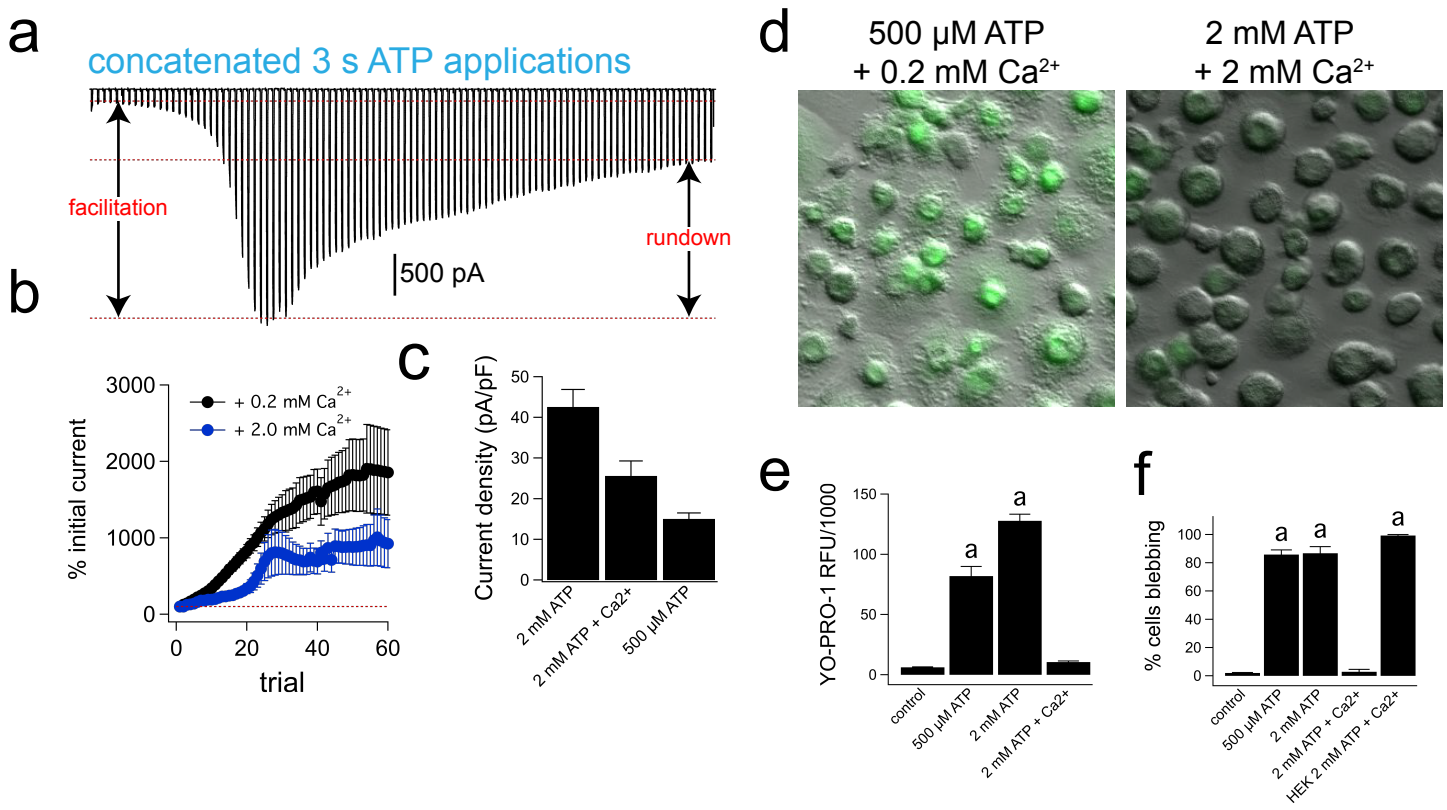
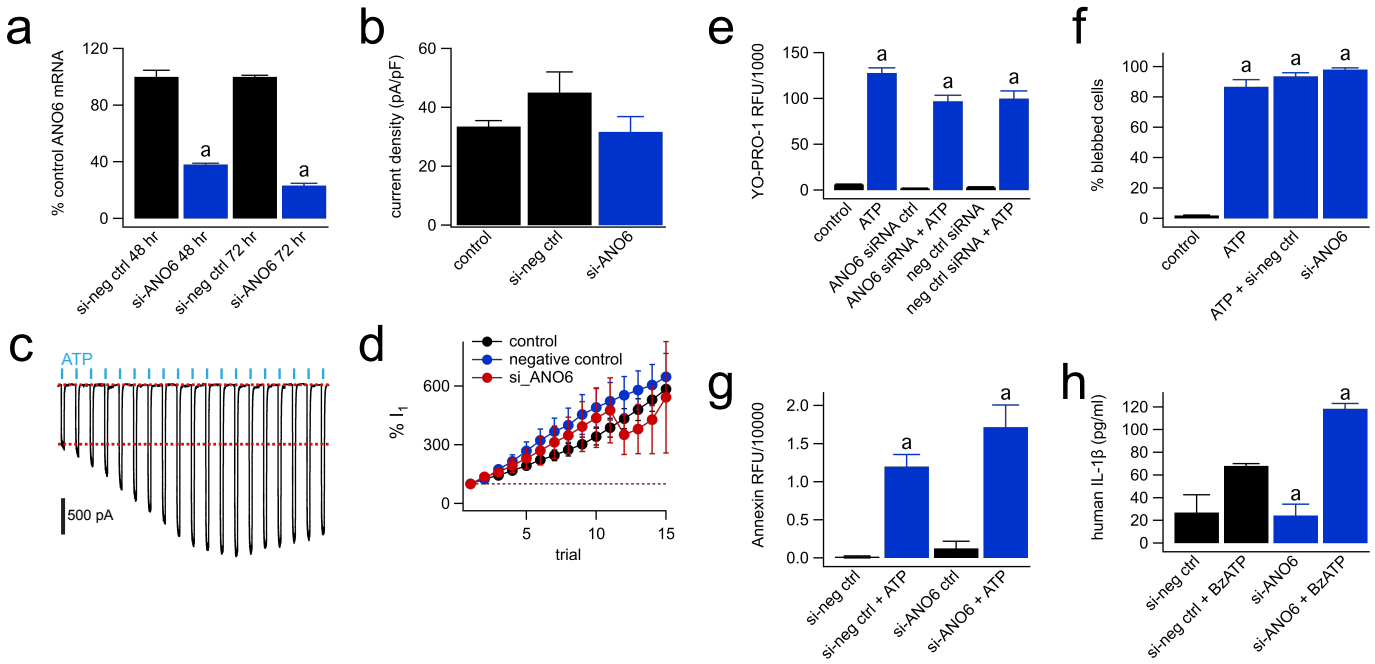


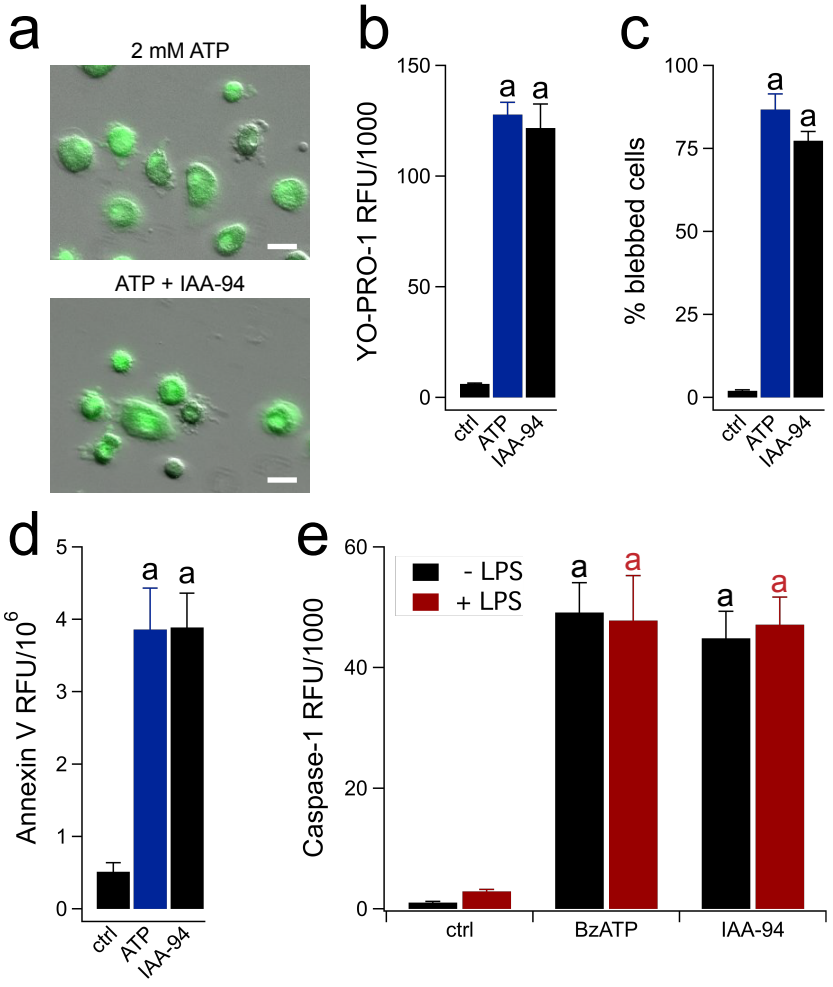
Supplemental Figure 1. Macrophages respond to low concentrations of ATP and high concentrations of BzATP (a) ATP (100 µM) was applied for 3 s every 15 s. Traces are truncated for clarity as described in Fig 1. The current shows immediate and significant desensitization. **(b)** Currents were desensitized by repeated applications of 100 µM ATP applied every 15 s. Applications were halted for 3 min, and then restarted. The 3 min gap resulted in near complete recovery of the ATP-gated current. **(c)** Percentage of initial current (n = 25 cells) after repetitive pulses of ATP (100 µM) and recovery (73%, n=9 cells) after 3 min rest period (orange bar). **(d)** A single volley of ATP was applied for 3 s, followed by a second volley 3 min later. **(e)** Quantification of data like that of Panel "d". Current recovers by 106% after 3 min rest period (n=15 cells). **(f)** BzATP (300 µM) was applied for 3 s every 15 s. **(g)** Facilitating currents induced by repetitive BzATP applications grew to 1941% (n = 6 cells). **(h)** Current density (pA/pF) stimulated by 2 mM ATP (42.6 pA/pF, n = 31 cells) was not significantly different than that of 300 µM BzATP (39.6 pA/pF, n = 5 cells). **(i)** Current-voltage relationship from macrophage held at -60 mV and stimulated with voltage ramp from -90 mV to +30 mV reveals E_{rev} is -1.51 ± 0.58 mV (n = 5 cells).



Supplemental Figure 2. P2X7Rs display run-down upon stimulation with high extracellular Ca^{2+}. (a) Representative tracing of current run-down recorded from a macrophage stimulated with successive applications (3s) of 2 mM ATP in ECS containing 2 mM Ca^{2+} separated by 15 s intervals, from a holding potential of −60 mV. Current first facilitates then subsequently runs-down. **(b)** Quantification of percentage of initial current after repetitive ATP (2 mM) pulses in normal low Ca^{2+} ECS (black, n=31 cells) or high Ca^{2+} ECS (blue, n=7 cells). **(c)** The ATP (2 mM) current density is reduced (25.6 pA/pF; n=7 cells) under high Ca^{2+} ECS vs. normal ECS (42.6 pA/pF; n=31 cells) and 500 µM ATP under normal Ca^{2+} ECS (0.2 mM) generates lowest current density (15.0 pA/pF; n=14 cells). (**d** and **e)** YO-PRO-1 is taken up after stimulation with 500 µM ATP under normal Ca^{2+} ECS (0.2 mM) but not after stimulation with ATP (2 mM) with high Ca^{2+} ECS (2 mM) at 37°C ("a": significantly different from control, $p < 0.0001$ unpaired t-test, n= 3 experiments). **(f)** 500 µM ATP under normal Ca^{2+} ECS (0.2 mM) stimulates macrophage blebbing, however, ATP (2 mM) with high Ca^{2+} ECS (2 mM) does not cause blebbing in human macrophages at 37°C. HEK293 cells stably expressing P2X7Rs rapidly bleb after treatment with ATP (2 mM) under high Ca^{2+} ECS (2 mM) ("a": significantly different from control, $p < 0.0001$ unpaired t-test, n= 3 experiments). Scale bars: 20 µm.



Supplemental Figure 3. Ano6 is not required for P2X7R-driven functions in human macrophages. (a) RT-qPCR on total RNA isolated from human macrophages after treatment with Ano6 or negative control siRNA for 48 or 72 hrs. The expression of Ano6 was significantly reduced by 48 hrs (61.8%; n=4 experiments) and 72 hrs (76.7%, n = 4 experiments) siRNA treatment. The housekeeping gene β-actin (ACT) was used as an internal control. ("a": significantly different from control, $p < 0.0001$ unpaired t-test) **(b)** Current density was not inhibited by Ano6 siRNA (31.7 pA/pF; n=5 cells) or negative control siRNA (45 pA/pF; n = 4 cells) treatment. **(c)** Representative tracing of whole-cell currents from Ano6 siRNA treated cells activated by 3 s applications of 2 mM ATP every 15 s. **(d)** Current growth upon successive 3 s ATP applications is not different from untreated macrophages (black; n = 31 cells) after Ano6 (red; n = 5 cells) or negative control (blue; n = 6 cells) siRNA treatment. ATP-induced (2 mM) YO-PRO-1 RFU **(e)**, blebbing **(f)**, and Annexin V RFU **(g)** at 37°C is not impacted after Ano6 or neg control siRNA treatment ("a": significantly different from control, $p < 0.0001$ unpaired t-test, n= 4 experiments for each). **(h)** BzATP-induced (300 μM) IL-1β release is not inhibited by Ano6 or neg control siRNA treatment at 37°C ("a": significantly different from control, $p < 0.0001$ unpaired t-test, n= 3 experiments).



Supplemental Figure 4. CLICs are not required for P2X7R functions in human macrophages. (a and **b)** ATP (2 mM) induced YO-PRO-1 uptake is not inhibited by 30 min preincubation with IAA-94 (150 µM) at 37°C. Neither blebbing **(c)** nor Annexin V RFU **(d)** (2 mM ATP stimulated) is inhibited by IAA-94 (150 µM) preincubation at 37°C ("a": significantly different from control, $p < 0.0001$ unpaired t-test, n= 4 experiments). **(e)** Caspase-1 activation stimulated by BzATP (300 µM) at 37°C is not inhibited by IAA-94 (150 µM) preincubation. ("a": significantly different from control, $p < 0.0001$ unpaired t-test, n= 3 experiments). Scale bars: 20 µm.