K^+ efflux agonists induce NLRP3 inflammasome activation independently of Ca^{2+} signaling
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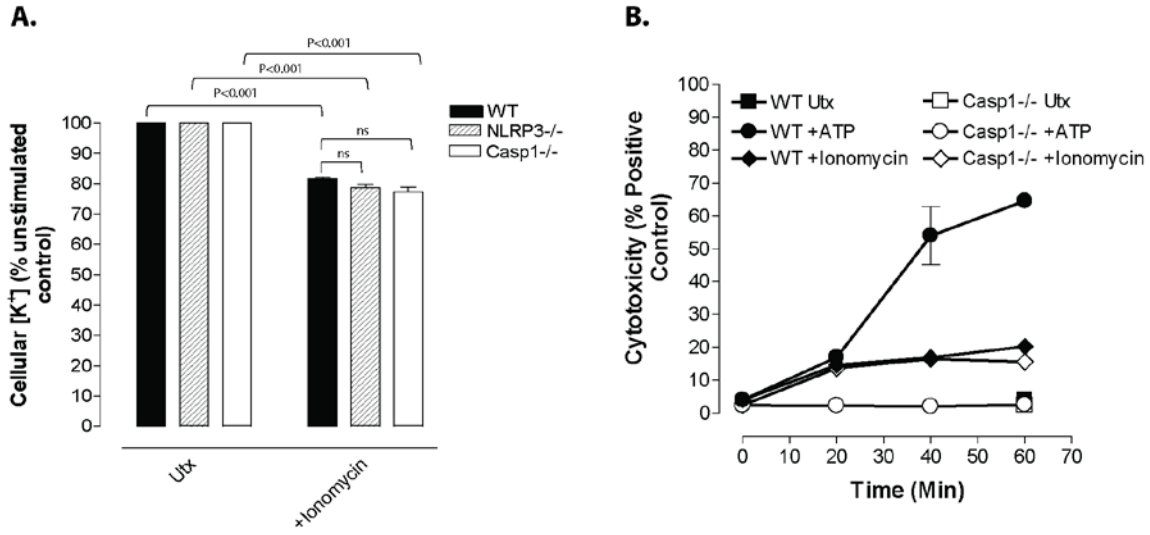
Supplemental Data

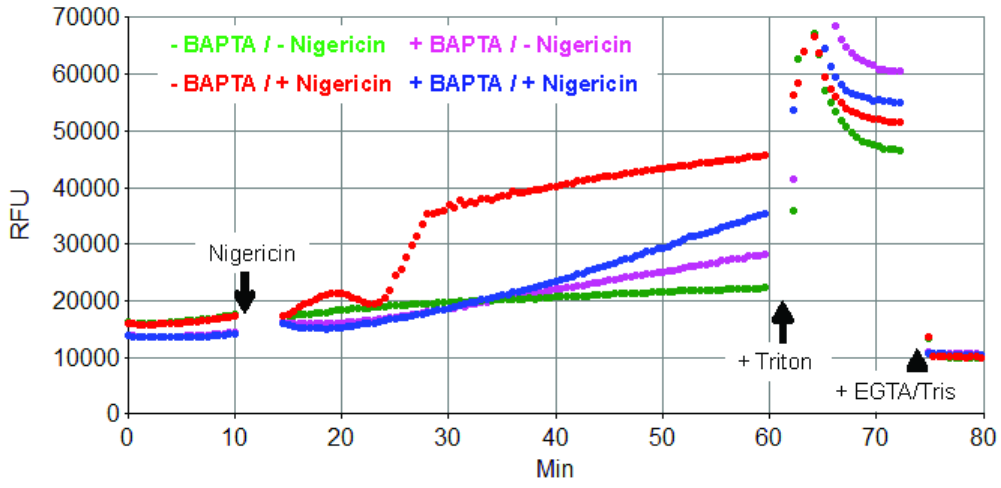
Supplemental Figure 1



<u>Supplemental Figure 1</u>. Effects of ionomycin on K^+ efflux and cell death in wildtype, *Casp1/11*$^{-/-}$, or *Nlrp3*$^{-/-}$ BMDC

A, LPS-primed wildtype, *Casp1/11*$^{-/-}$, or *Nlrp3*$^{-/-}$ BMDC were treated with 3μM ionomycin for 30 min. Cells were lysed with 10% nitric acid and lysates were analyzed by atomic absorption spectroscopy to measure cellular [K^+]. Data represent mean of triplicate determinations from a single experiment. **B**, LPS-primed wildtype or *Casp1/11*$^{-/-}$ BMDC were treated with 5 mM ATP or 3 μM ionomycin for the indicated times prior to collection of the extracellular medium for analysis of LDH release. The LDH release was normalized to the total LDH content measured in triton lysates. Data represent mean of triplicate determinations from a single experiment.

Supplemental Figure 2



<u>Supplemental Figure 2</u>. Effects of BAPTA on nigericin-induced increases in cytosolic [Ca^{2+}] in BMDC incubated in Ca^{2+}-containing media.

LPS-primed BMDC were loaded with 1µg/ml fluo-4 AM +/- 25µM BAPTA-AM for 45 min. Each well was briefly washed with PBS prior to the addition of 0.5 ml 1.5mM $CaCl_2$-containing BSS supplemented with 2.5mM probenecid. Baseline fluorescence (485 nm excitation → 528 nm emission) was recorded for 10 min at 30 sec intervals. BMDC in each well were then stimulated by addition of 10µM nigericin or no agonist (arrow) and the fluorescence recorded for 50 min at 30 sec intervals. Assays were terminated by permeabilization of the cells with 1% triton X-100 for maximum Ca2+-dependent fluorescence followed by 15mM EGTA/50mM Tris to chelate Ca^{2+} for minimum Ca^{2+}-independent fluorescence. Y-axis shows the changes in relative fluorescence units (RFU). Data are representative of results from three similar experiments.