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Supplemental Information

Intercellular Calcium Signaling Induced by ATP Potentiates Macrophage Phagocytosis

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SUPPLEMENTAL FIGURE 01

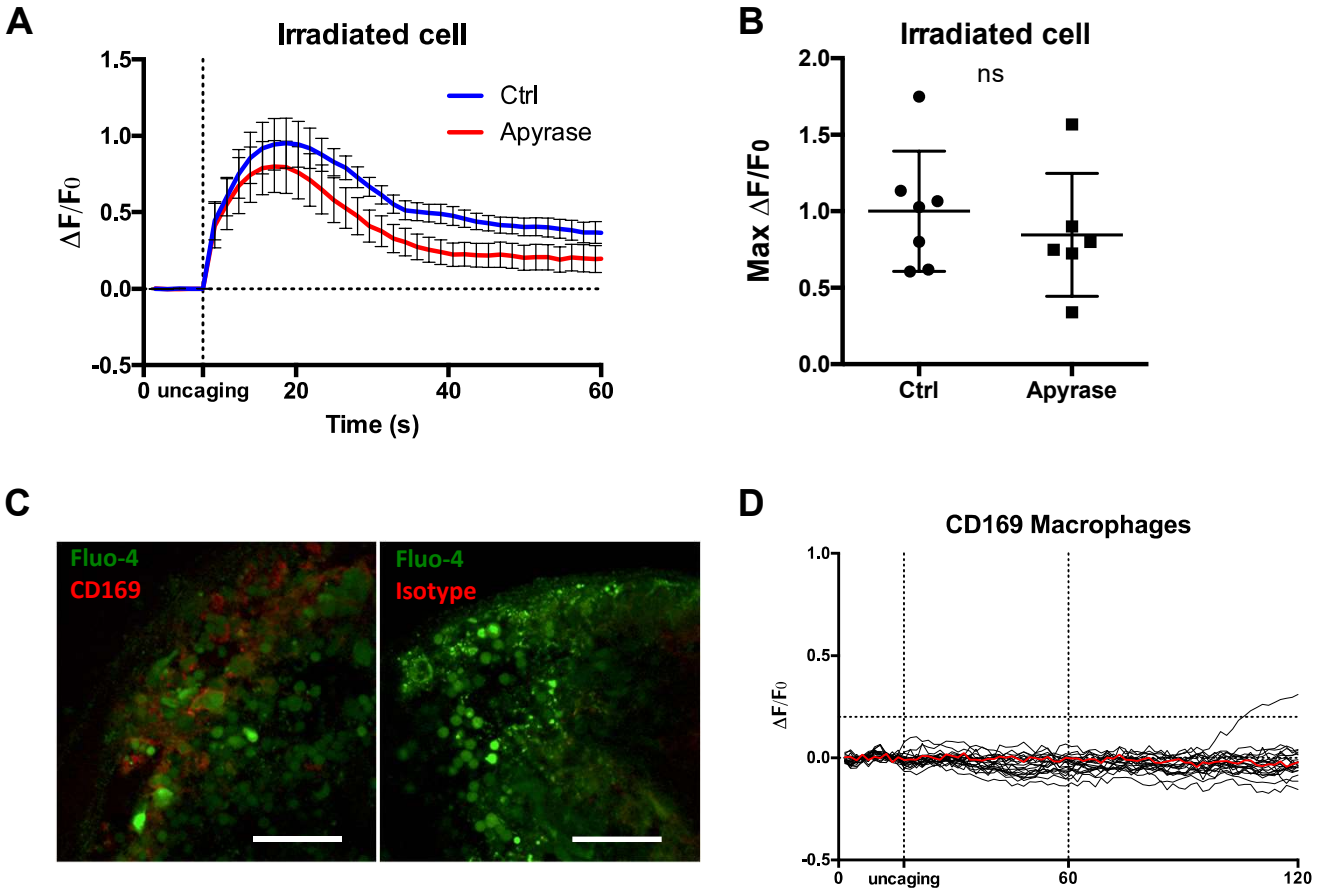


Figure S1. Propagation of calcium signals by macrophages. Related to Figure 1.
A-B) The Fluo4 fluorescence variation of the irradiated cell (origin cell) is compared in the control (Ctrl) and apyrase conditions. The $\Delta F/F_0$ is similar in the two conditions, as shown by the average traces (A) or the maximal $\Delta F/F_0$ calculated for repeated experiments (B). n=6 cells. Error bars represent s.e.m. For data analysis, Student t-test was used (ns = non significant).
C) Mice were subcutaneously injected with a fluorescently-labelled anti CD-169 (shown in red - left) or the appropriate isotype control (right). 1 hour later, fresh murine popliteal lymph nodes were enclosed in 4% agarose gel, cut into 200 µm-slices and loaded Fluo4-AM (shown in green). Scalebar: 50 µm.
D) Representative traces of the control live calcium imaging experiment performed with lymph nodes loaded with Fluo4-AM only, showing the fluorescence variation after the UV-irradiation of the origin cell (red) and the bystander macrophages (black).

SUPPLEMENTAL FIGURE 02

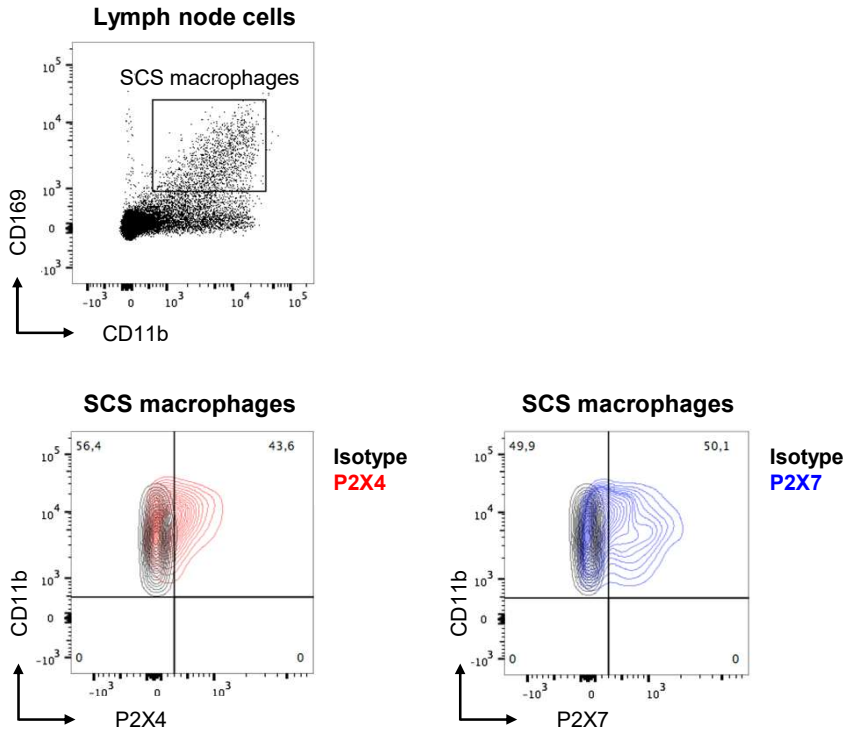


Figure S2. P24XR and P2X7R mediate ATP-dependent calcium signal propagation. Related to Fig. 2.
To identify subcasular (SCS) macrophages, lymph node cell suspensions were stained with anti-CD11b and anti-CD169 antibodies (top panel). The expression of P2X4R and P2X7R was then assessed in the $CD11b^{+}$ $CD169^{+}$ population with the P2X4 or P2X7-specific antibody or their isotype controls.

SUPPLEMENTAL FIGURE 03

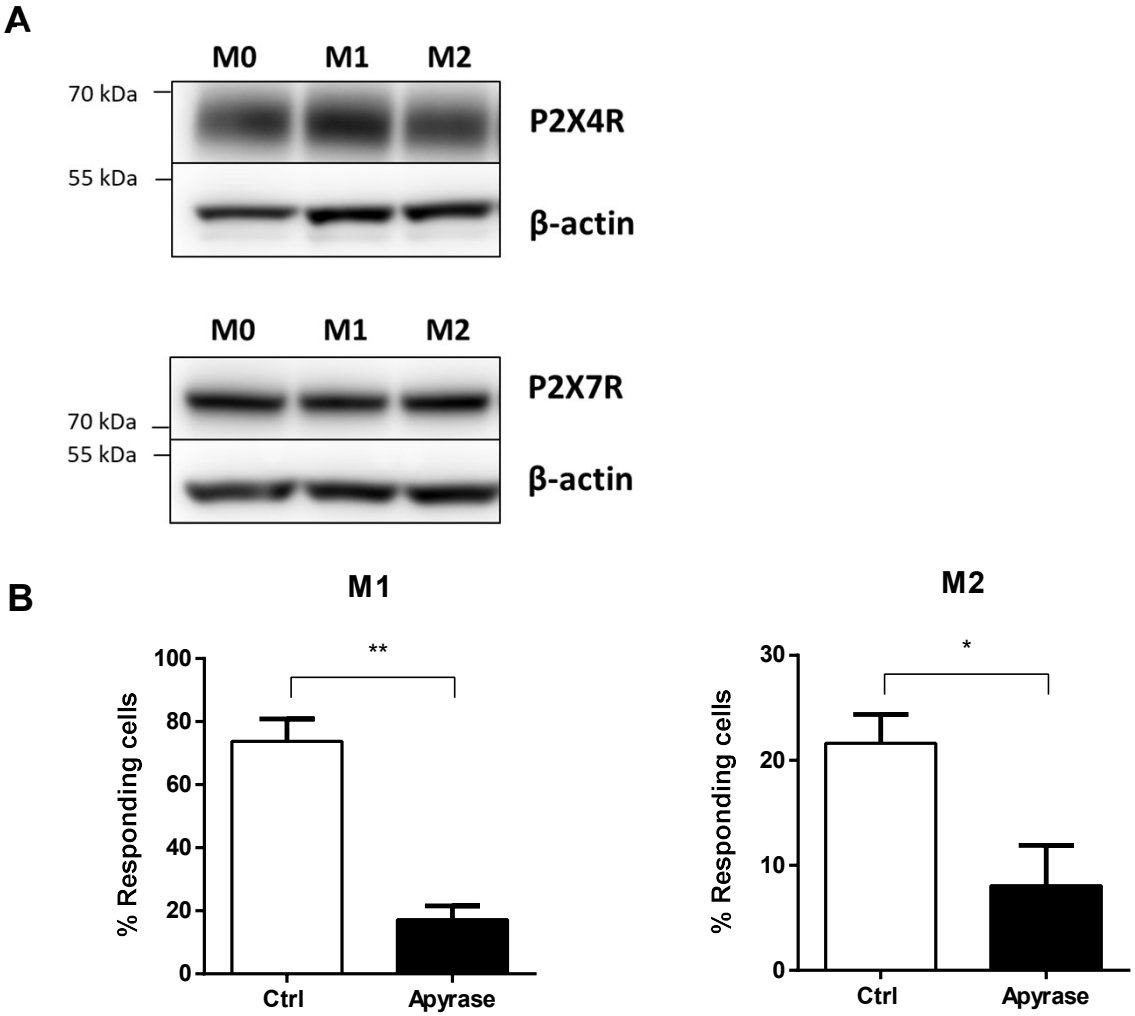
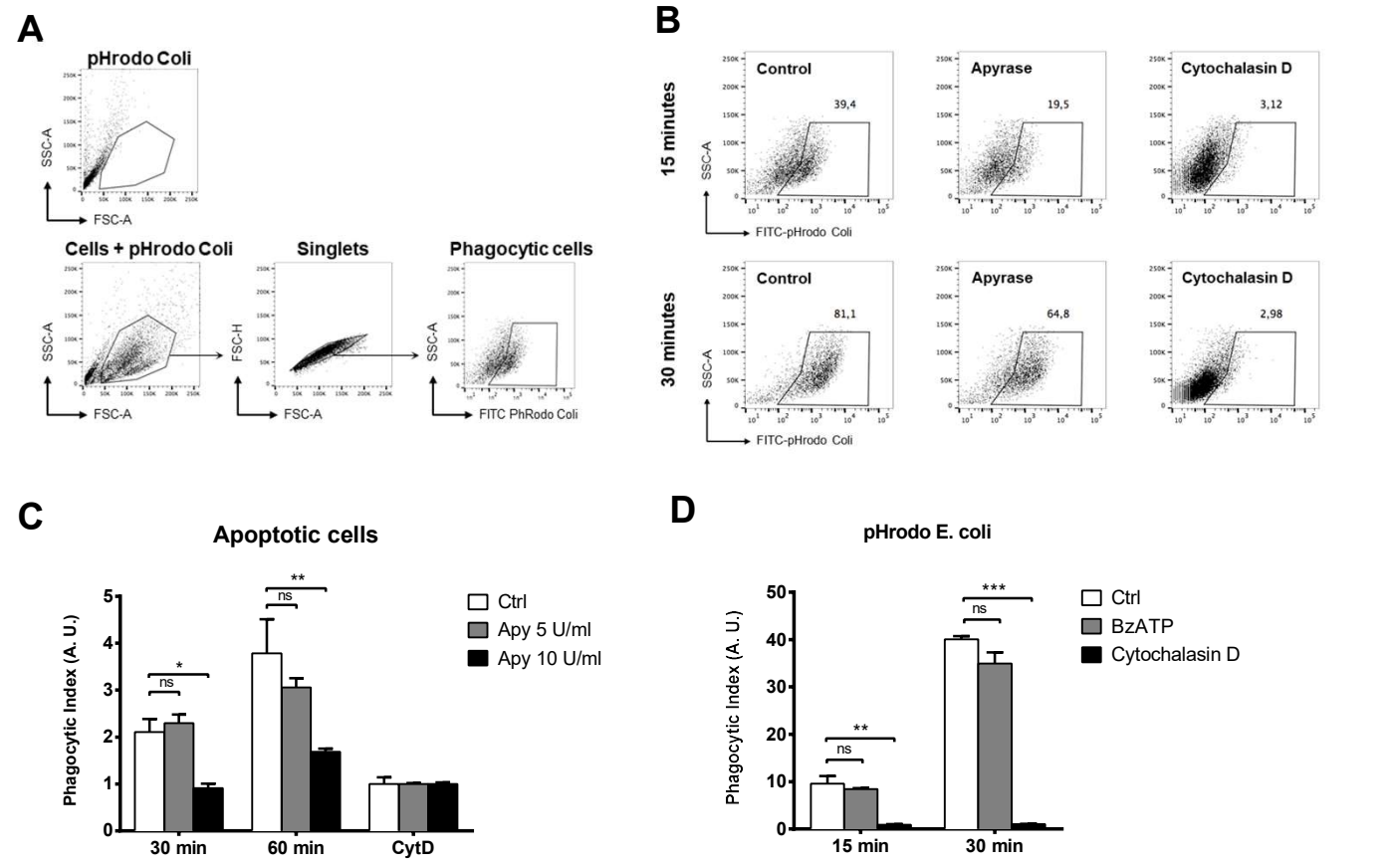


Figure S3. Macrophage polarization status affects calcium signal propagation. Related to Fig. 3.
A) The total expression of P2X4R (top) and P2X7R (bottom) was analysed by flow cytometry in resting (M0), IFNγ-treated (M1) or IL4-treated (M2) macrophages and analysed by western blot on total cell lysates.
B) Quantification of 3 repeated live calcium imaging experiments performed with M1 (left) or M2 (right) macrophages, treated or not with 5 U/ml apyrase. Error bars represent s.e.m. For data analysis, Student t-test was used (*=$p>0.05$; **= $p<0.01$).

SUPPLEMENTAL FIGURE 04



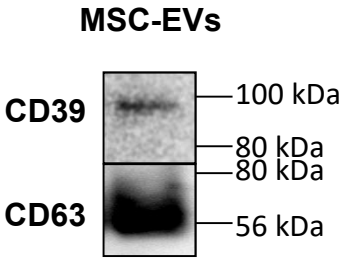


Figure S4. Extracellular ATP is required for efficient phagocytosis. Related to Fig. 4.
A-B) Gating strategy (A) and representative dot plots (B) of *in vitro* phagocytosis assay of PhRodo-Coli fluorescent bioparticles. Cytochalasin D was used as negative control.
C) BMDM were incubated with apoptotic B16F10 cells, in presence or absence of apyrase. Phagocytosis was monitored after 30 and 60 minutes by flow cytometry. Macrophage treated with 20 µ M Cytochalasin D were used as negative reference. The graph is representative of 3 repeated experiments. Error bars represent s.e.m.. For Data Analysis, Two-way ANOVA followed by Tukey's multiple comparisons test was used (ns= non significant; *= p<0,05; **= p<0,01).
D) Primary BMDM were incubated with PhRodo® E. coli fluorescent bioparticles in presence or absence of 100 µM BzATP. Phagocytosis was monitored by flow cytometry. Macrophage treated with 20 µ M Cytochalasin D were used as negative reference. The graph is representative of 3 repeated experiments. Error bars represent s.e.m.. For Data Analysis, Two-way ANOVA followed by Tukey's multiple comparisons test was used (ns= non significant; **= p<0,01; ***= p<0,001).
E) Western blot analysis of CD39 and of CD63 expression by MSC-derived EVs. CD63 is a typical marker of extracellular vesicles.