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Supplementary Materials for

Autocrine Purinergic Receptor Signaling Is Essential for Macrophage Chemotaxis

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/3/132/ra55/DC1)

Movie S9 (.mov format). *P2ry2*$^{-/-}$ macrophages in a C5a chemotactic gradient with "cocktail block."
Movie S10 (.mov format). *P2ry2*$^{-/-}$ macrophages in a C5a chemotactic gradient with "triple block."
Movie S11 (.mov format). *Panx1*$^{-/-}$ macrophages in a C5a chemotactic gradient.
Movie S12 (.mov format). ATP-induced lamellipodial membrane protrusions.
Movie S13 (.mov format). Lack of ATP-induced lamellipodial formation in *P2ry2*$^{-/-}$ macrophages.

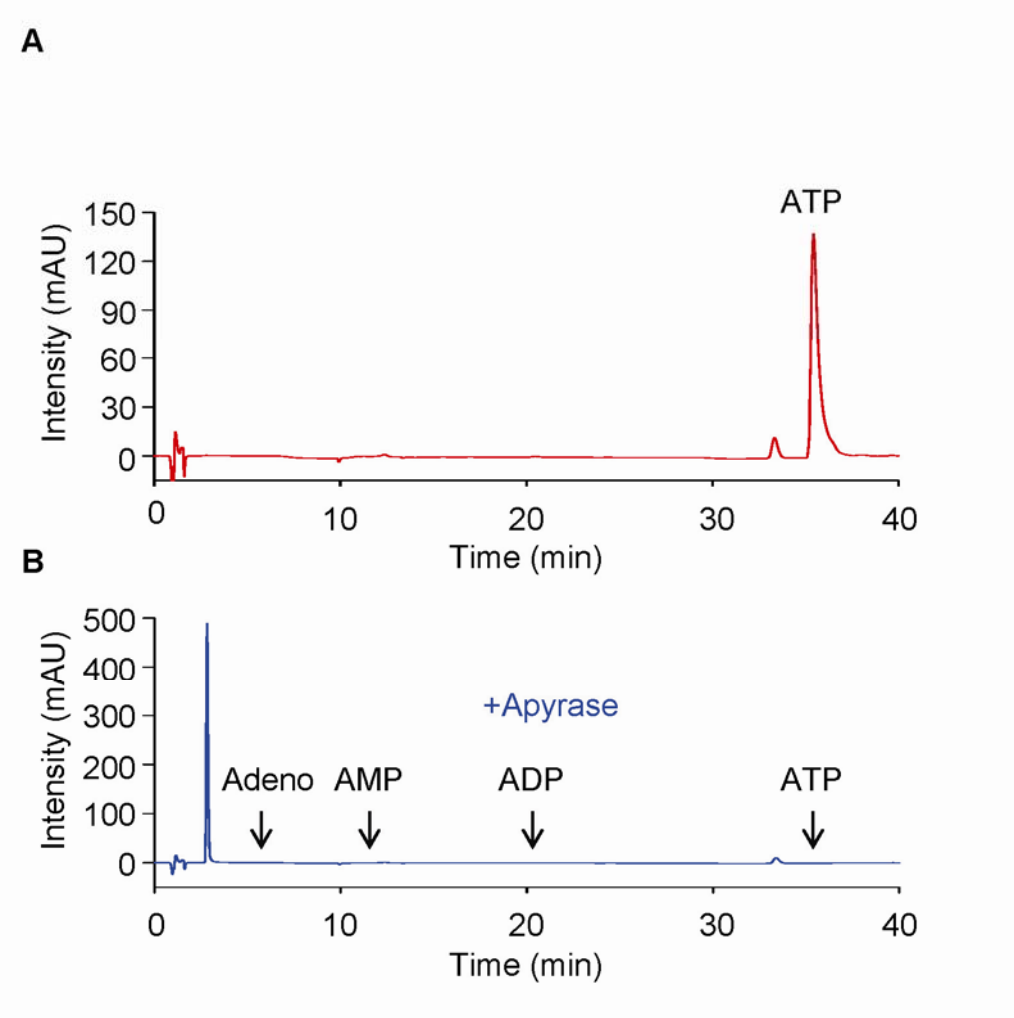

Fig. S1. Reversed-phase HPLC analysis of ATP degradation by potato apyrase. Absorbance was detected at 260 nm. (**A**) Chromatogram of the ATP standard. (**B**) Addition of apyrase (40 U/ml for 2 hours) completely degraded ATP, and an unidentified product was detected. The retention times of ATP, ADP, AMP, and adenosine are indicated by arrows. The chromatograms are representative of two independent experiments.

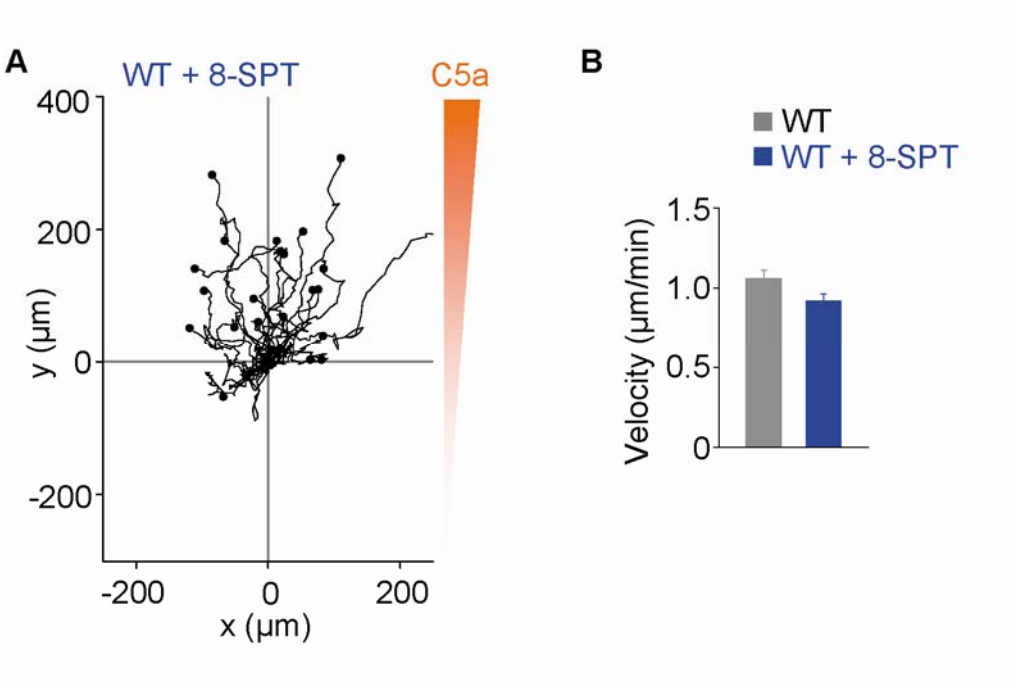

Fig. S2. Inhibition of the adenosine feedback loop with 8-SPT. (**A**) Migration plots of macrophages in a gradient of C5a after blocking adenosine receptors with the nonselective antagonist 8-SPT. The start point of each track was normalized to the position x = 0 and y = 0, and positive y-axis values represent movement in the direction of the source of chemoattractant. (**B**) Plot of the mean velocities of wild-type (WT) macrophages in the absence and presence of 100 µM 8-SPT (n = 75 cells, representative of three independent experiments). Velocity data were analyzed with the Kruskal-Wallis test. For a plot of chemotaxis index, see Fig. 3D.

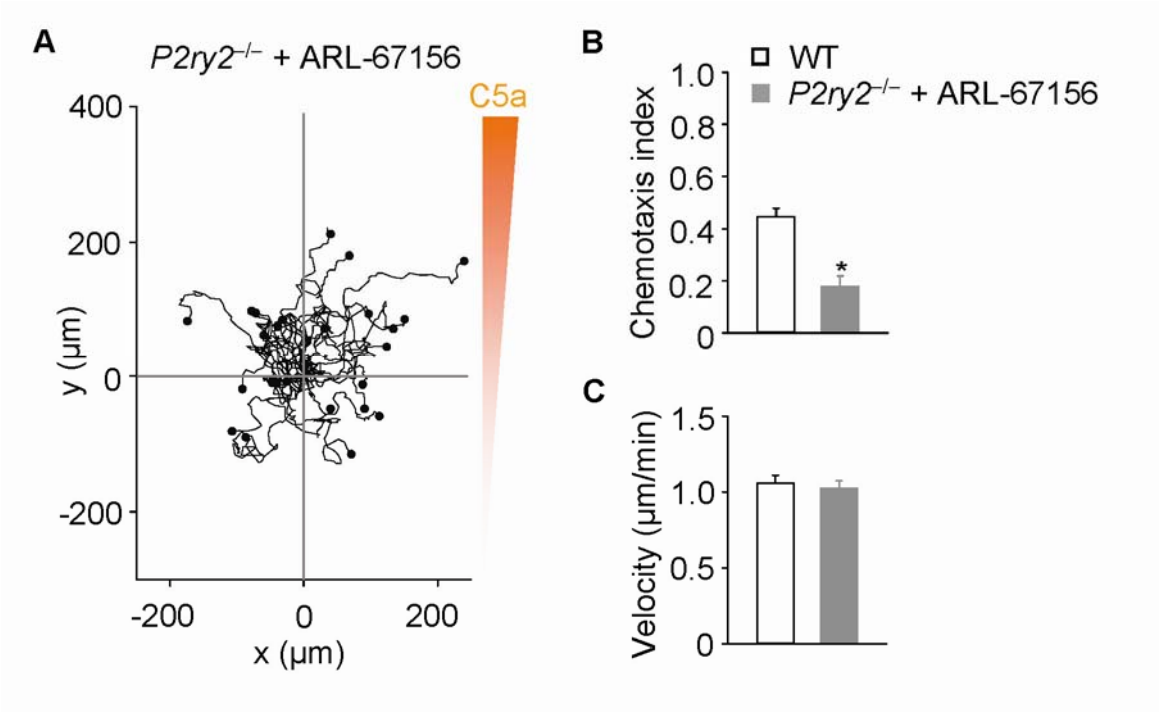

Fig. S3. Chemotaxis is impaired by blocking ecto-ATPase in *P2ry2*-deficient macrophages. (**A**) Migration plot of *P2ry2*$^{-/-}$ macrophages in the presence of the ecto-ATPase inhibitor ARL-67156 (100 µM). The start point of each track was normalized to the position x = 0 and y = 0, and positive y-axis values represent movement in the direction of the source of chemoattractant. (**B**) Mean chemotaxis index of WT macrophages (n = 100 cells, four independent experiments; see Fig. 1) and *P2ry2*$^{-/-}$ macrophages treated with ARL-67156 (n = 75 cells, three independent experiments). *, $P < 0.05$ (by ANOVA). (**C**) Plot of the mean velocities of WT macrophages and *P2ry2*$^{-/-}$ macrophages treated with ARL-67156 (n = 75 cells, three independent experiments). Velocity data were analyzed with the Kruskal-Wallis test.

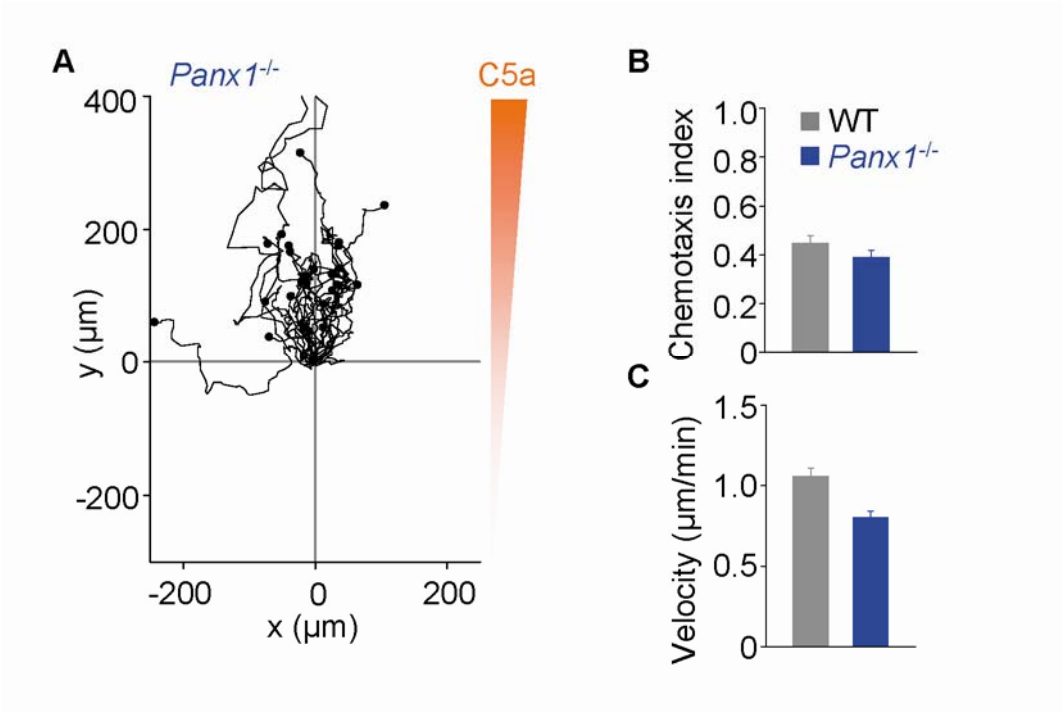

Fig. S4. Chemotaxis is not impaired in *Panx1*-deficient macrophages. (**A**) Migration plot of *Panx1*$^{-/-}$ macrophages in a gradient of C5a. The start point of each track was normalized to the position x = 0 and y = 0, and positive y-axis values represent movement in the direction of the source of chemoattractant. (**B**) Mean chemotaxis index of WT macrophages (n = 100 cells, four independent experiments; see Fig. 1) and *Panx1*$^{-/-}$ macrophages (n = 125 cells, five independent experiments). (**C**) Plot of the mean velocities of WT and *Panx1*$^{-/-}$ macrophages (n = 125 cells). Velocity data were analyzed with the Kruskal-Wallis test.

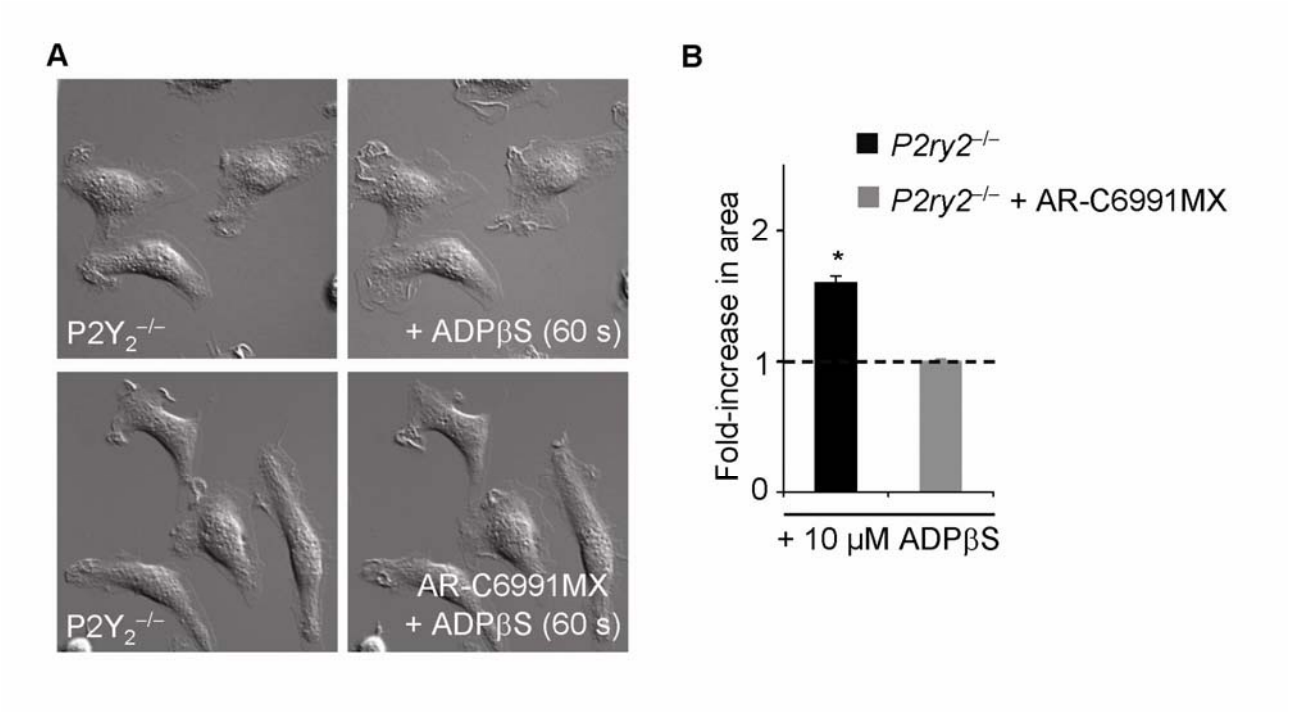

Fig. S5. The $P2Y_{12}$ receptor antagonist AR-C69931MX blocks ADPβ-S-induced lamellipodial membrane protrusions. (**A**) Phase-contrast images of *P2ry2*$^{-/-}$ macrophages before and 60 s after the application of ADPβS (10 μM) in the presence of hexokinase. (**B**) Summary of the effect of ADPβS on the projected (two-dimensional) surface area of a macrophage. The cell surface area ~180 s after the application of nucleotide was divided by the initial area to obtain the fold increase. *, $P < 0.05$, when comparing conditions before and after the application of ADPβS.

Movie descriptions

Movie S1. Wild-type macrophages in a C5a chemotactic gradient. Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S2. Wild-type macrophages in a C5a chemotactic gradient with apyrase (40 U/ml). Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S3. *P2ry2*$^{-/-}$ macrophages in a C5a chemotactic gradient. Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S4. *P2ry12*$^{-/-}$ macrophages in a C5a chemotactic gradient. Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S5. *P2ry2*$^{-/-}$ macrophages in a C5a chemotactic gradient with AR-C69931MX (10 μM). Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S6. *P2ry2*$^{-/-}$ macrophages in a C5a chemotactic gradient with 8-SPT (100 μM). Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S7. *P2ry12*$^{-/-}$ macrophages in a C5a chemotactic gradient with 8-SPT (100 μM). Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S8. Wild-type macrophages in a C5a chemotactic gradient with 8-SPT (100 μM). Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S9. *P2ry2*$^{-/-}$ macrophages in a C5a chemotactic gradient with "cocktail block". *P2ry2*$^{-/-}$ macrophages were treated with the $P2X_1$ and $P2X_4$ inhibitor NF449 (10 μM), the $P2Y_{12}$ inhibitor AR-C69931MX (10 μM), the non-selective adenosine receptor inhibitor 8-SPT (100 μM), and the $P2Y_1$ inhibitor MRS-2179 (100 μM). Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S10. *P2ry2*$^{-/-}$ macrophages in a C5a chemotactic gradient with "triple block". *P2ry2*$^{-/-}$ cells were treated with the $P2Y_{12}$ inhibitor AR-C69931MX (10 μM) and the nonselective adenosine receptor inhibitor 8-SPT (100 μM). Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S11. *Panx1*$^{-/-}$ macrophages in a C5a chemotactic gradient. Time-lapse images are 500 x 700 μm and span a time period of 10 hours.

Movie S12. ATP-induced lamellipodial membrane protrusions. Time-lapse, phase-contrast images of macrophages after the application of ATP (100 μM). The images (110 x 110 μm) were acquired every 15 s, and the movie spans a time period of 20 min.

Movie S13. Lack of ATP-induced lamellipodial formation in *P2ry2*$^{-/-}$ macrophages. Time-lapse, phase-contrast images of macrophages after the application of ATP (100 μM). The images (110 x 110 μm) were acquired every 15 s and the movie spans a time period of 20 min.