Appendix Data

A1 is induced by pathogen ligands to limit myeloid cell death and NLRP3 inflammasome activation

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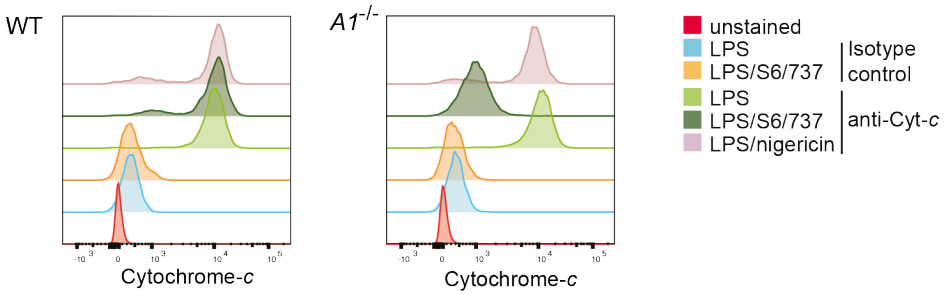
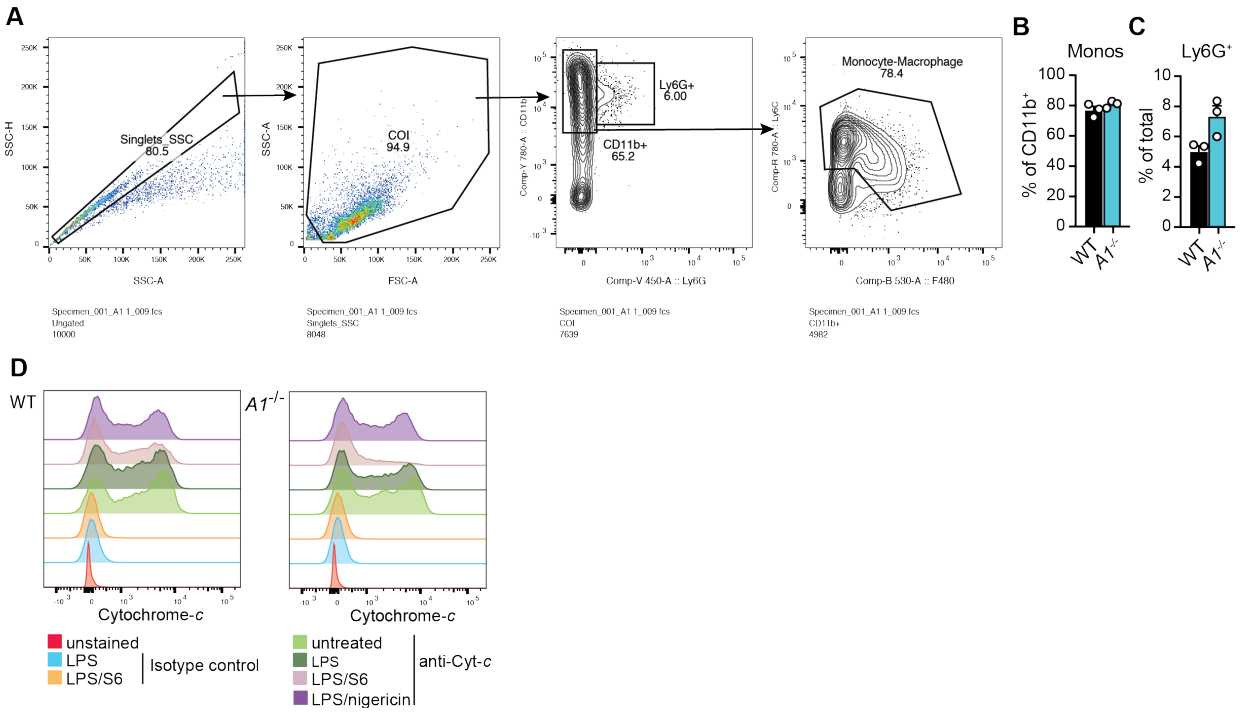


Appendix Figure S1. A1 deficiency leads to the rapid loss of cytochrome-*c* in LPS-primed macrophages upon BCL-XL and MCL-1 targeting. WT and A1-deficient (*A1*$^{-/-}$) BMDMs were primed with B5 LPS (50 ng/ml) for 3 h, prior to the addition of S63845 (S6; 10 μM) and ABT-737 (737; 500 nM) for 2-3 h. Alternatively, LPS-primed cells were treated with Nigericin (10 μM) for 1 h. MOMP-dependent loss of cytochrome-*c* staining was analysed by flow cytometry. Data are representative of two biological experiments.

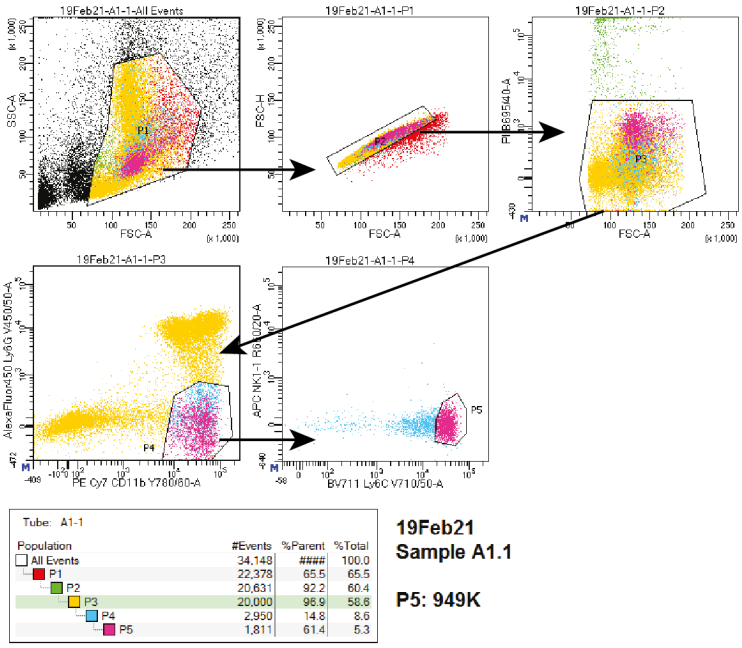


Appendix Figure S2. LPS-primed A1-deficient Ly6C^{hi} BMMo display accelerated cytochrome-*c* loss upon MCL-1 targeting

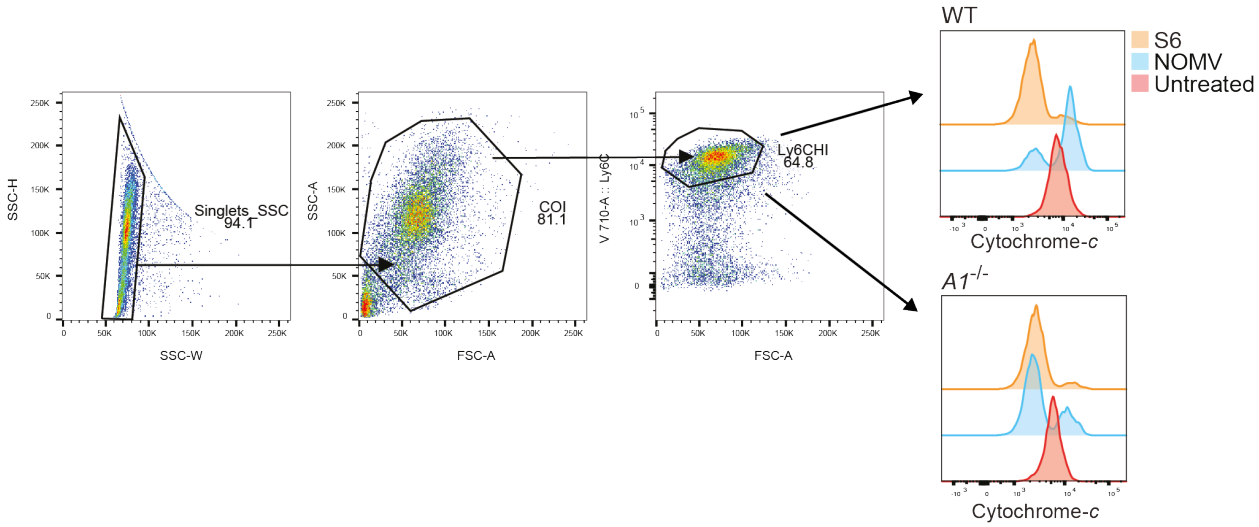
A-C Purity of WT and A1-deficient (*A1*$^{-/-}$) BMMo cultures after 4 days of LCCM culture was analysed by flow cytometry. **(A)** Gating strategy: Doublets and cell debris were removed and monocytes/immature macrophage-like cells were analysed as CD11b^{+}Ly6C^{+} (and CD11b^{+}Ly6C^{int}F4/80$^{lo\text{-}int}$) cells. **(B)** % of CD11b^{+} cells that were monocyte/immature macrophage-like cells. **(C)** % of total cells that were Ly6G^{+} neutrophils.

D WT and A1-deficient (*A1*$^{-/-}$) BMMo were primed with B5 LPS (50 ng/ml) for 3 h and treated with S63845 (S6; 10 μM) or nigericin (10 μM) for 2 h or 1 h, respectively. MOMP was evaluated by flow cytometric analysis of loss of cytochrome-*c* retention from monocyte/immature macrophage-like cells.

Data information: Each dot represents an individual biological replicate (B, C). Data are representative of at least two independent biological experiments and presented (B, C) as the mean + SEM.



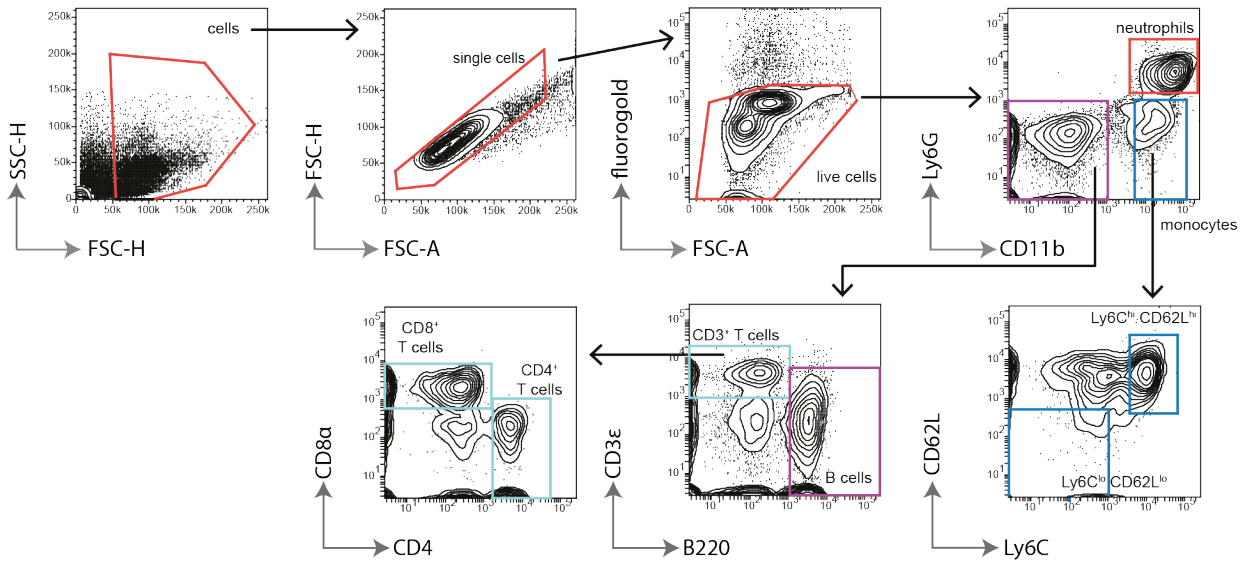
Appendix Figure S3. Gating strategy for sorting primary Ly6C^{hi} inflammatory monocytes from the bone marrow. Bone marrow was isolated from WT and A1-deficient mice and stained with fluorescently labelled anti-mouse mAb against CD11b, Ly6G, NK1.1 and Ly6C. Shown is the flow cytometry gating strategy used to isolate Ly6C^{hi} monocytes from the bone marrow.



Appendix Figure S4. *Neisseria Gonorrhoeae*-derived OMVs trigger rapid loss of cytochrome-*c* in primary bone marrow Ly6C^{hi} monocytes lacking A1

Monocytes were isolated from WT and A1-deficient (*A1*$^{-/-}$) bone marrow using magnetic separation and stimulated with NOMVs (50 µg/ml) or S63845 (S6; 10 µM) for 3 h. Flow cytometry gating strategy showing the proportion of Ly6C^{hi} cell that were then analysed for MOMP-induced loss of cytochrome-*c* staining.

Data information: Data are representative of at least two independent biological experiments.



Appendix Figure S5. A1 deficiency does not perturb peripheral blood immune cell responses upon intraperitoneal NOMV injection. WT and A1-deficient mice were injected intraperitoneally with 100 µg of NOMVs (or PBS) and peripheral blood and peritoneal lavage fluid harvested after 6 h. Flow cytometry gating strategy for identifying immune cell populations in peripheral blood of PBS or NOMV treated mice.