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Expanded View Figures

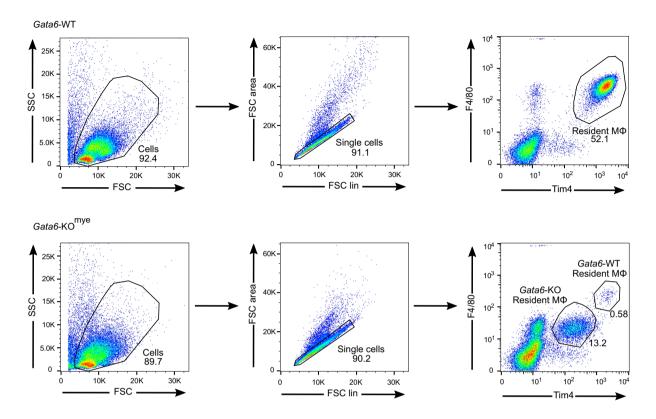


Figure EV1. Representative gating strategy of pM Φ from Gata6-WT and Gata6-KO mye mice.

First, dead cells are excluded based on FSC and SSC value; then, single cells are gated based on FCS area and FCS linear value. $pM\Phi$ are then identified as F4/80^{hi}Tim4⁺ (Gata6-WT) or F4/80⁺Tim4⁺ (Gata6-KO^{mye}).

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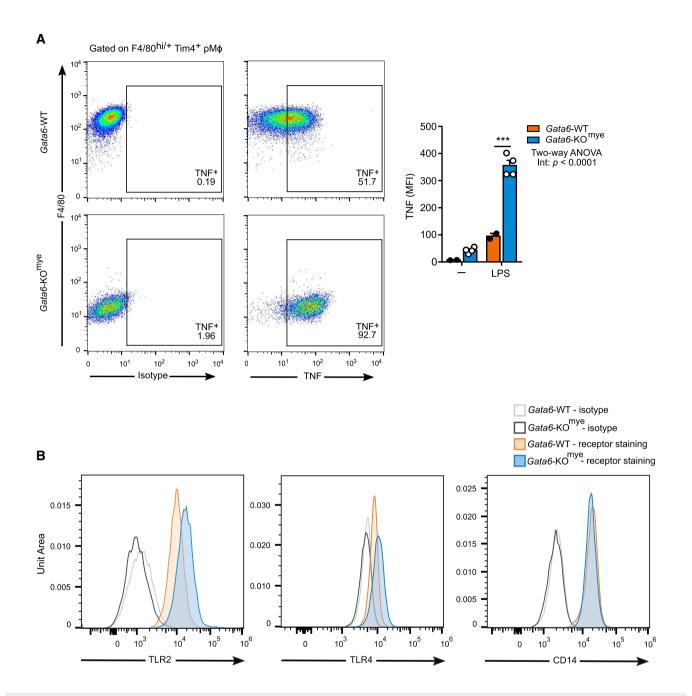


Figure EV2. Flow cytometry analysis of intracellular TNF and extracellular TLR2, TLR4 and CD14 expression.

A Representative flow cytometry analysis of TNF expression in Gata6-WT and Gata6-KO^{mye} pM Φ stimulated for 3 h with 100 ng/ml LPS and 0.1 % (v/v) GolgiBlock. Data shown are representative of at least three independent experiments. Data are expressed as mean \pm SEM. Two-way ANOVA analysis followed by Tukey's multiple comparison post-test was performed. ***P < 0.001.

В Histogram overlays of flow cytometry analysis of cell surface expression of TLR2, TLR4 and CD14 of Gata6-WT and Gata6-KO^{mye} pMФ.

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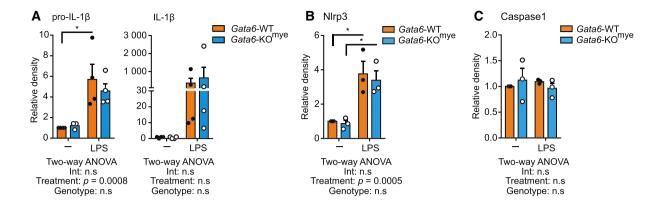


Figure EV3. Western blot quantification of the immature and mature IL-1ß and the canonical inflammasome machinery.

Western blot quantification of pro-IL-1 β and mature IL-1 β (A), NIrp3 (B) and caspase1 (C) of Gata6-WT and Gata6-KO^{mye} pM Φ , unstimulated or stimulated overnight with 100 ng/ml LPS. Results showed are pooled from three independent experiments, normalized to Gata6-WT unstimulated sample and are expressed as mean \pm SEM. Two-way ANOVA analysis followed by Tukey's multiple comparison post-test was performed. *P < 0.05.

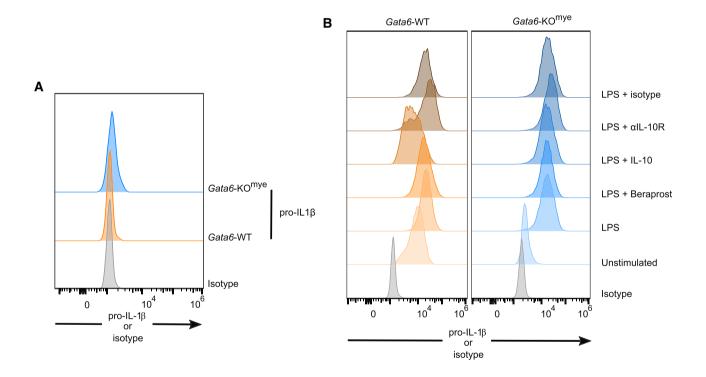


Figure EV4. Flow cytometry analysis of intracellular pro-IL-1 β .

EV3

Flow cytometry analysis of intracellular pro-IL-1 β content in Gata6-WT and Gata6-KO^{mye} pM Φ freshly isolated (A) or after 16 h incubation unstimulated or stimulated with 100 ng/ml ultrapure LPS, 10 μ M beraprost, 10 ng/ml IL-10, 5 μ g/ml α IL-10R or 5 μ g/ml isotype (B). Data shown are representative of n=6–13 individual mice.

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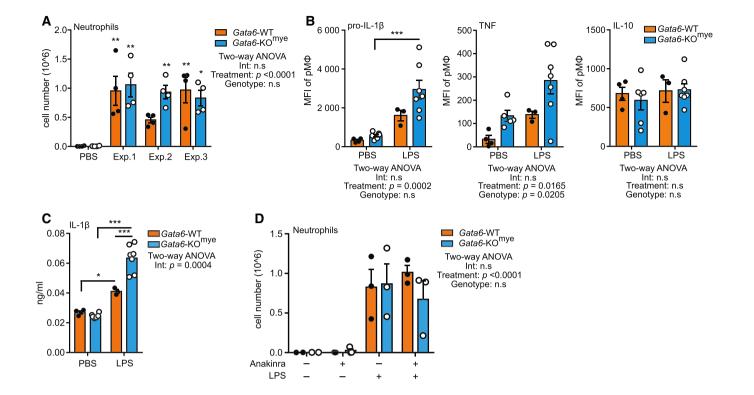


Figure EV5. Flow cytometry analysis of LPS-induced peritonitis in Gata6-WT and Gata6-KO^{mye} mice.

- A Flow cytomerty analysis of neutrophil (Ly6G* CD11b*) number recruited to the peritoneal cavity of Gata6-WT and Gata6-KO^{mye} mice 4 h after i.p injection of 1 ng ultra-pure LPS. Three independent experiments are shown, n = 4 mice per group. Significance are indicated by * over the bar, compared to the corresponding control (Gata6-WT or Gata6-KO^{mye} PBS injected).
- B Flow cytometry analysis of intracellular cytokines in Gata6-WT and Gata6-KO^{mye} pMΦ, 3 h after i.p injection of PBS or 1 ng ultra-pure LPS.
- C ELISA analysis of IL-1β contained in the peritoneal fluid of Gata6-WT and Gata6-KO^{mye} mice, 3 h after i.p injection of PBS or 1 ng ultra-pure LPS. n = 3–6 mice per group.
- D Flow cytomerty analysis of neutrophil (Ly6G* CD11b*) number recruited to the peritoneal cavity of Gata6-WT and Gata6-KO^{mye} mice 4 h after i.p injection of 1 ng ultra-pure LPS combined or not with 50 mg/ml Anakinra, n=2-3 mice per group. Results are expressed as mean \pm SEM. Two-way ANOVA analysis followed by Tukey's multiple comparison post-test was performed. *P<0.05, **P<0.01, ***P<0.001.

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