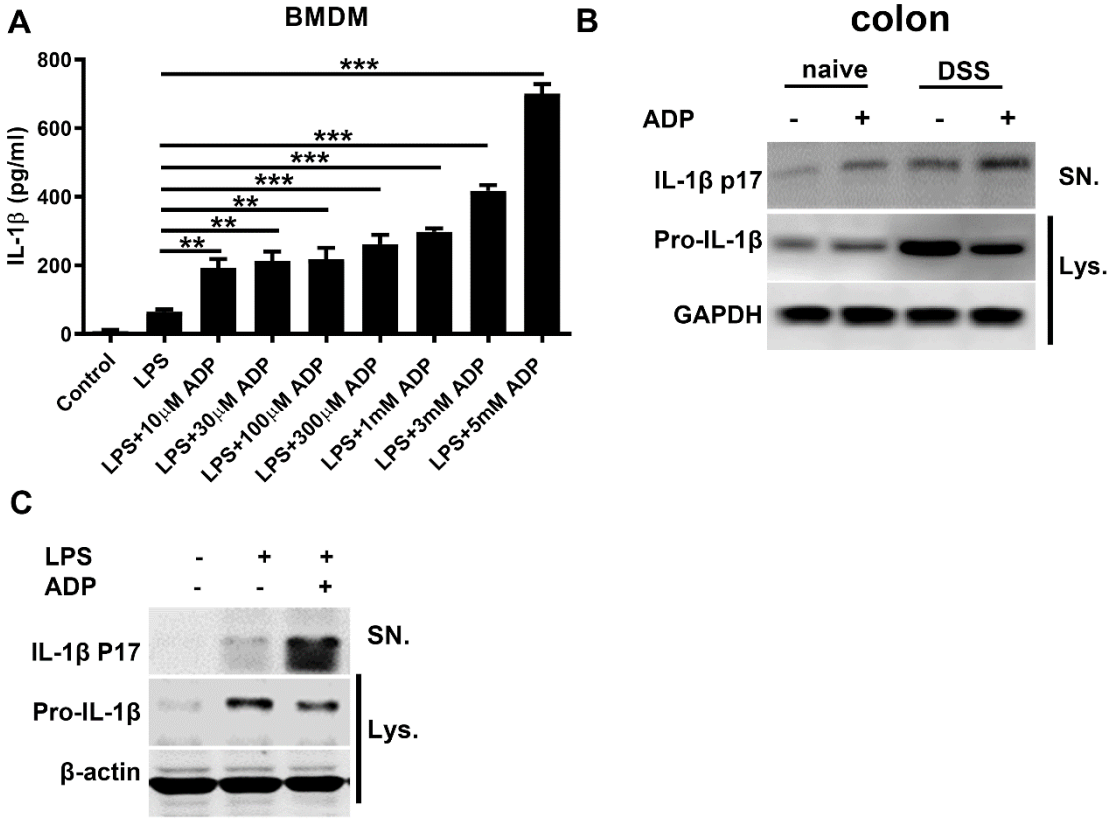
Supplementary Figures:

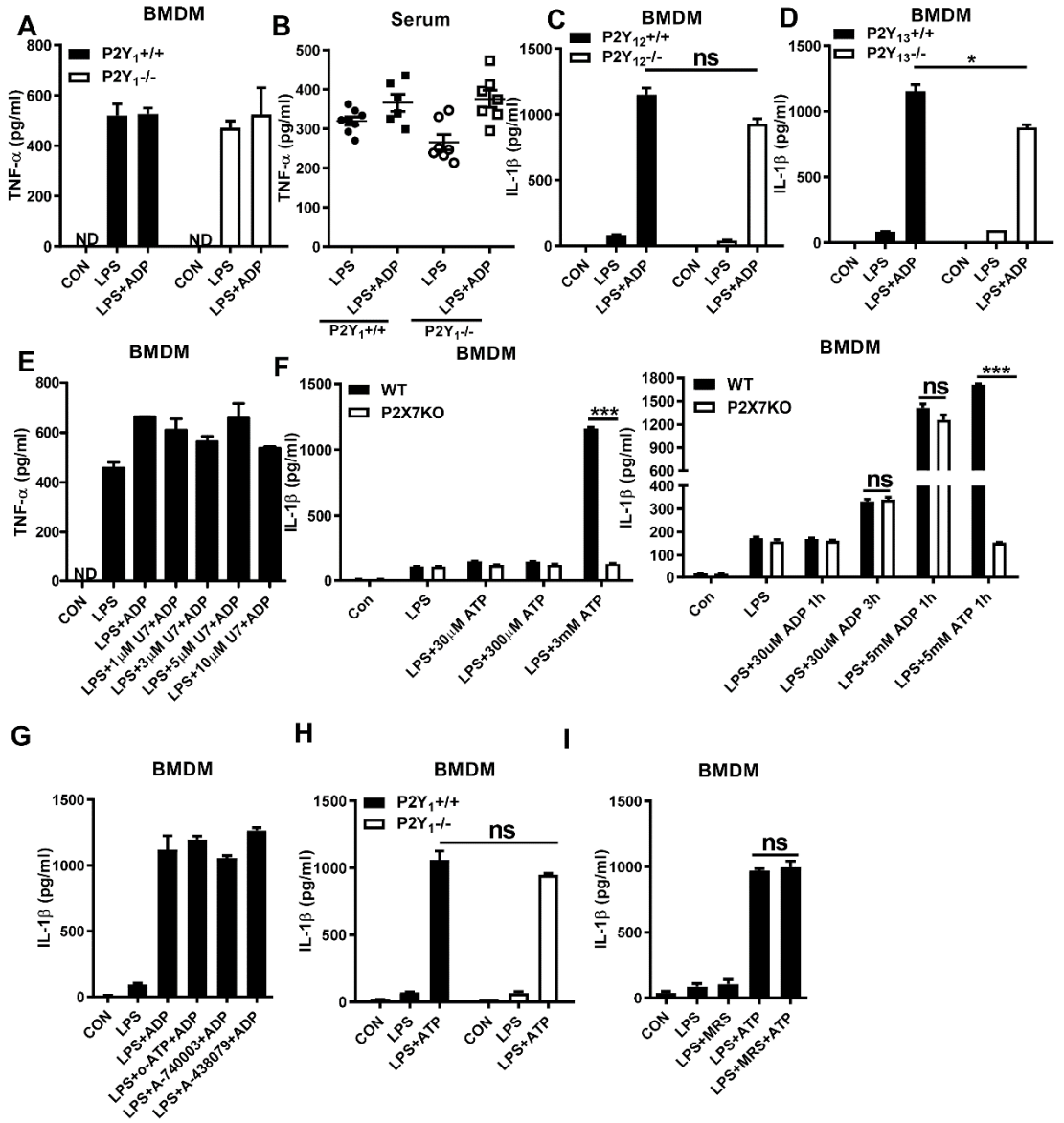
Supplementary Figure 1



Supplementary Figure 1. ADP promotes the mature of IL-1β but not the expression of pro- IL-1β, related to Figure 2

(A) LPS-primed BMDM cells were stimulated with indicated concentrations of ADP for 3 h, then production of IL-1β was measured by ELISA. (B) WT mice were treated with 2.5% DSS for 7 days and euthanized at day 7, 0.3 g colon sections were cultured in DMEM for 6 h and then stimulated with ADP (5 mM) for 2 h. The expression of pro-IL-1β in tissue lysates and mature IL-1β in culture supernatants (SN) were analyzed by immunoblotting. (C) LPS-primed BMDM cells were stimulated with ADP (5 mM) for 80 min, the expression of pro-IL-1β in cell lysates and mature IL-1β in culture supernatants (SN) were analyzed by immunoblotting. Data are shown as mean ±SEM. $^{**}p<0.01$; $^{***}p<0.001$.

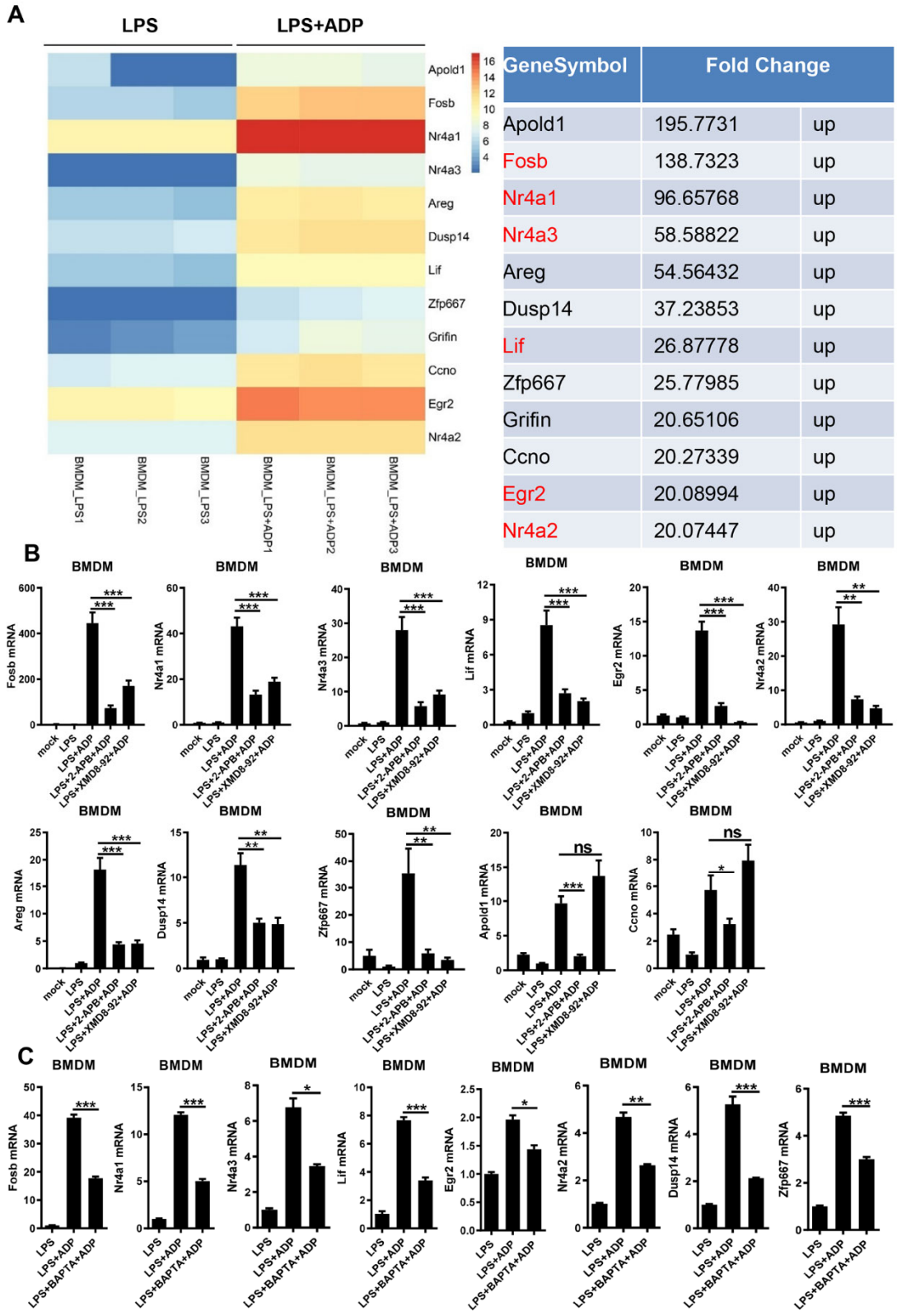
Supplementary Figure 2



Supplementary Figure 2. $P2Y_{12}$ and $P2Y_{13}$ deficiency have little influence on ADP activation of the NLRP3 inflammasome, related to Figure 4

(A) LPS-primed $P2Y_1^{+/+}$ and $P2Y_1^{-/-}$ BMDM cells were treated with ADP (5 mM) for 1 h and production of TNF-α was measured by ELISA. (B) $P2Y_1^{+/+}$ and $P2Y_1^{-/-}$ mice were intraperitoneally treated with 20 mg/kg LPS for 6h and then intraperitoneally challenged with ADP (300mg/kg) for 1 h. The production of TNF-α in serum was measured by ELISA. (C) LPS-primed $P2Y_{12}^{+/+}$ and $P2Y_{12}^{-/-}$ BMDM cells were treated with ADP (5 mM) for 1 h and the production of IL-1β was measured by ELISA. (D) LPS-primed $P2Y_{13}^{+/+}$ and $P2Y_{13}^{-/-}$ BMDM cells were treated with ADP (5 mM) for 1 h and the production of IL-1β was measured by ELISA. (E) LPS-primed BMDM cells were pretreated with indicated concentrations of U73122 for 1 h and then stimulated with ADP (5 mM) for 1 h. The production of TNF-α was measured by ELISA. (F) LPS-primed $P2X7^{+/+}$ and $P2X7^{-/-}$ BMDM cells were stimulated with indicated concentrations of ATP for 1 h or indicated concentrations of ADP for the indicated time; the production of IL-1β was measured by ELISA. (G) LPS-primed BMDM cells were pretreated with P2X7 inhibitors (100 μM oATP, 5 μM A-740003, 5 μM A-438079) for 1 h and then stimulated with ADP (5 mM) for 1 h, the production of IL-1β was measured by ELISA. (H) LPS-primed $P2Y_1^{+/+}$ and $P2Y_1^{-/-}$ BMDM cells were treated with ATP (5 mM) for 1 h and the production of IL-1β was measured by ELISA. (I) LPS-primed BMDM cells were pretreated with MRS2179 (10 μM) or not for 1 h and then stimulated with ATP (5 mM) for 1 h, production of IL-1β was detected by ELISA. Data are shown as mean ±SEM. *p<0.05; ***p<0.001; ns, not significant; ND, not detected.

Supplementary Figure 3

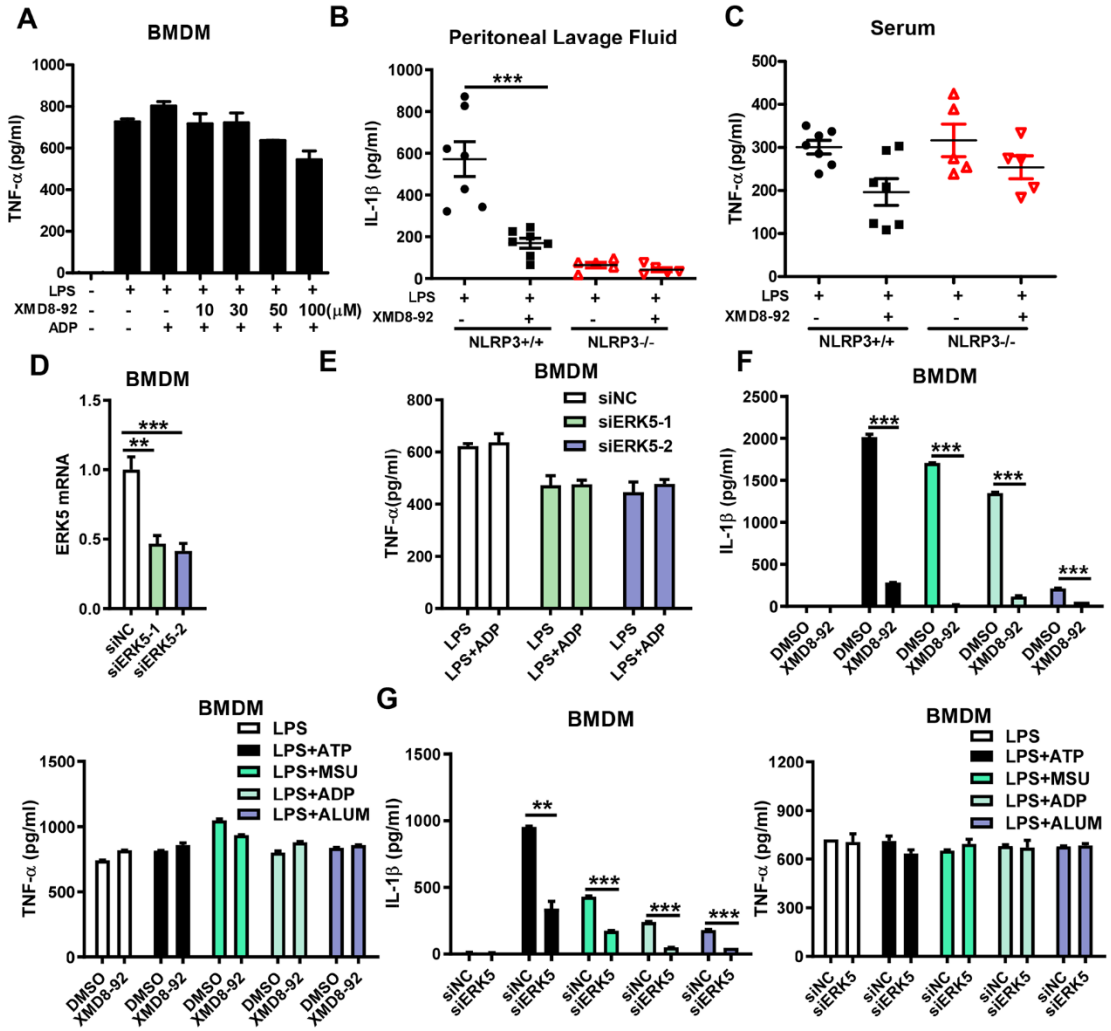


GeneSymbol	Fold Change	
Apold1	195.7731	up
Fosb	138.7323	up
Nr4a1	96.65768	up
Nr4a3	58.58822	up
Areg	54.56432	up
Dusp14	37.23853	up
Lif	26.87778	up
Zfp667	25.77985	up
Grifin	20.65106	up
Ccno	20.27339	up
Egr2	20.08994	up
Nr4a2	20.07447	up

Supplementary Figure 3. ERK5 pathway is involved in ADP activation of the NLRP3 inflammasome, related to Figure 6

(A) LPS-primed BMDM cells were stimulated with ADP (5 mM) for 1 h, then mRNA levels were analyzed by microarray. Shown is the heatmap of strongly upregulated genes (Left) and genes upregulated >20-fold change were listed (right). (B) LPS-primed BMDM cells were pretreated with 2-APB (100 μM) or XMD8-92 (100 μM) for 1 h and then stimulated with ADP (5 mM) for 1 h. The expression of Fosb, Nr4a1, Nr4a2, Nr4a3, Lif, Egr2, Areg, Dusp14, Zfp667, Apold1 and Ccno mRNA were analyzed by QPCR. (C) LPS-primed BMDM cells were pretreated with 50 μM BAPTA-AM for 1 h and then stimulated with ADP (5 mM) for 1 h. The expression of Fosb, Nr4a1, Nr4a2, Nr4a3, Lif, Egr2, Dusp14, Zfp667 mRNA were analyzed by QPCR. Data are shown as mean ±SEM. *p<0.05; **p<0.01; ***p<0.001; ns, not significant.

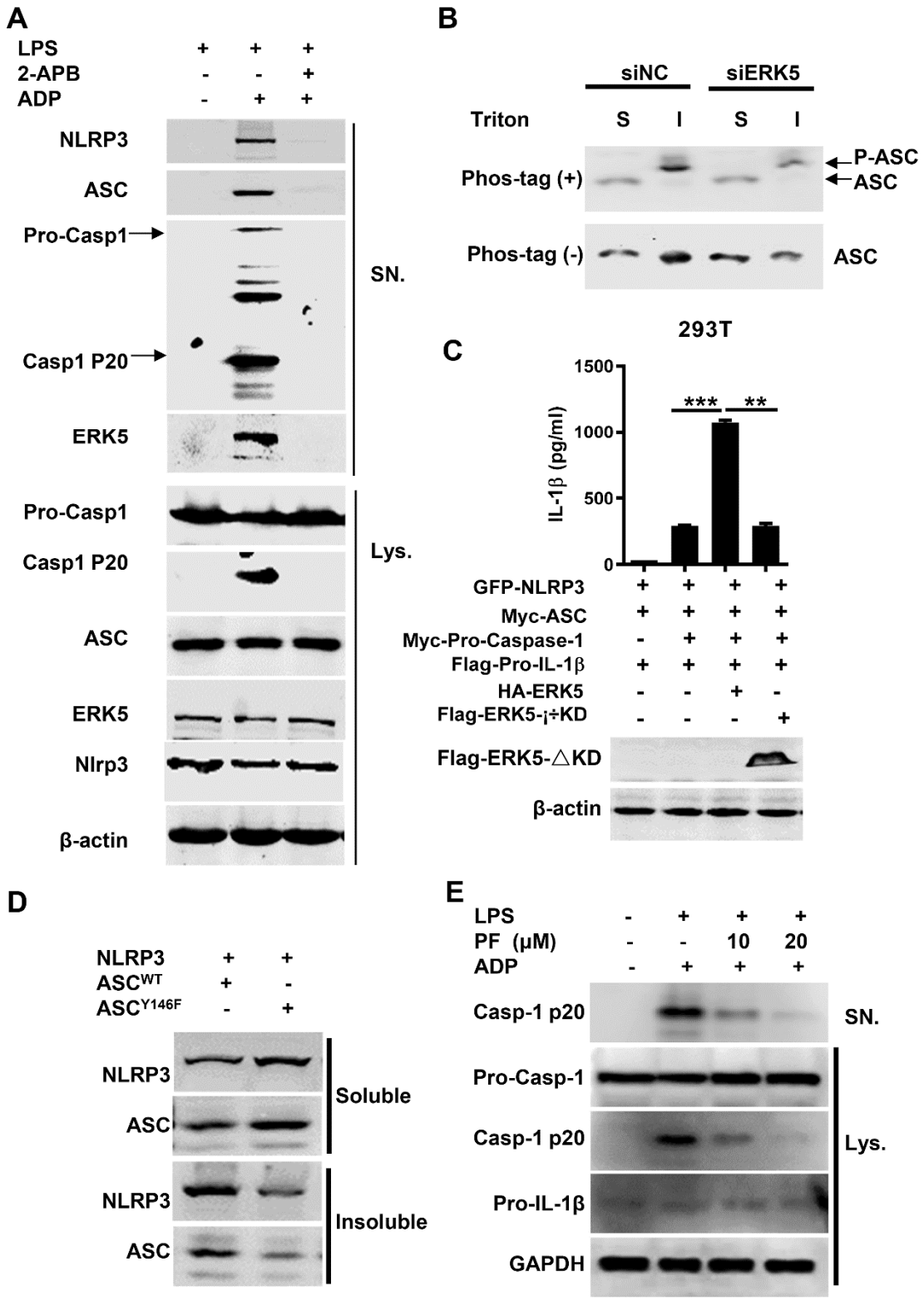
Supplementary Figure 4



Supplementary Figure 4. Blocking ERK5 attenuated NLRP3 inflammasome activity, related to Figure 6

(A) LPS-primed BMDM cells were pretreated with indicated concentrations of XMD8-92 for 1 h and then stimulated with ADP (5 mM) for 1 h. The production of TNF-α was detected by ELISA. (B-C) $NLRP3^{+/+}$ and $NLRP3^{-/-}$ mice were intraperitoneally treated with XMD8-92 (50 mg/kg) and LPS (20 mg/kg) for 6h, and levels of IL-1β in peritoneal lavage fluid and TNF-α in serum were measured by ELISA. (D) BMDM cells were transfected with negative control siRNA or ERK5-specific siRNA for 30 h, and expression of ERK5 mRNA was analyzed by QPCR. (E) BMDM cells were transfected with negative control siRNA or ERK5-specific siRNA for 36 h and then primed with LPS (500 ng/ml) for 6h. The cells were then treated with 5 mM ADP for 1 h, and production of TNF-α was detected by ELISA. (F) LPS-primed BMDM cells were pretreated with XMD8-92 (100 μM) for 1 h and then stimulated with indicated NLRP3 inflammasome inducers. The production of IL-1β and TNF-α was measured by ELISA. (G) BMDM cells were transfected with negative control siRNA or ERK5-specific siRNA for 36 h and then primed with LPS (500 ng/ml) for 6h. The cells then stimulated with indicated NLRP3 inflammasome inducers, and production of IL-1β and TNF-α was measured by ELISA. Data are shown as mean ±SEM. $^{**}p<0.01$; $^{***}p<0.001$.

Supplementary Figure 5



Supplementary Figure 5. ERK5 is released from cells along with the NLRP3 inflammasome, related to Figure 7

(A) LPS-primed BMDM cells were pretreated with 2-APB (100 μM) for 1 h and then stimulated with ADP (5 mM) for 1 h. NLRP3, ASC, Pro-caspase-1 and ERK5 levels in cell culture supernatants (SN) or in cell lysates were analyzed by immunoblotting. (B) PEMs were transfected with negative control siRNA or ERK5-specific siRNA for 36 h and then primed with LPS (500 ng/ml) for 6h. The cells were then treated with 5 mM ADP for 1 h, and phosphorylated and total ASC in the Triton X-soluble and Triton X-insoluble fractions was analyzed by SDS-PAGE with (+) or without (–) Phos-tag. (C) The NLRP3 inflammasome was reconstituted in ERK5$^{-/-}$ 293T cells by transfection of HA-ERK5 or Flag-ERK5-△KD, GFP-NLRP3, Myc-ASC, Myc-pro-caspase-1 and Flag-Pro-IL-1β for 30 h. The production of IL-1β was detected by ELISA; expression of Flag-ERK5-△KD was assessed by immunoblotting. (D) The NLRP3 inflammasome was reconstituted in 293T cells by transfection of GFP-NLRP3, Myc-ASC^{WT} and Myc-ASC^{Y146F} for 30 h, and ASC in Triton X-soluble and Triton X-insoluble fractions was analyzed by immunoblotting. (E) LPS-primed BMDM cells were pretreated with 10 or 20 μM PF-431396 for 1 h, then stimulated with ADP (5 mM) for 80 min. Cleaved caspase-1 (p20) in culture supernatants (SN) or in cell lysates and pro-caspase-1, pro-IL-1β in cell lysates were assessed by immunoblotting. Data are shown as mean ±SEM. $^{**}p<0.01$; $^{***}p<0.001$.

Supplementary table 1. Primers used for Q-PCR

Gene name	Primers (5'-3')
β-actin	Forward: GTACGCCAACACAGTGCTG
	Reverse: CGTCATACTCCTGCTTGCTG
Fosb	Forward: CTCCAGCTGTTGACCCTTAT
	Reverse: GGGTAAGTGTCTCTTCTCGG
Nr4a1	Forward: CCGGTGACGTGCAACAATTT
	Reverse: CGGGTTTAGATCGGTATGCCA
Nr4a3	Forward: TATGGCTCGGAATACACCACA
	Reverse: GCCCTCCATGAAGGTACTGAA
Lif	Forward: AACCAGATCAAGAATCAACTGGC
	Reverse: TGTTAGGCGCACATAGCTTTT
Egr2	Forward: GCAAATGATGACCGCCAAGG
	Reverse: GTTGATCATGCCATCTCCCG
Nr4a2	Forward: TCGGTTTACTACAAGCCCTCT
	Reverse: GGGGCGACTGCTTAAAGGA
Areg	Forward: GGGGACTACGACTACTCAGAG
	Reverse: TCTTGGGCTTAATCACCTGTTC
Dusp14	Forward: TTGCTCAGATCACCTCCTCTC
	Reverse: AGTACAGTCTAATGGGGGCAT
Zfp667	Forward: TCCAAGGCACCAGTGACTTTT
	Reverse: GGCAAGACCAACTGAGACTAGA
Apold1	Forward: GAAAGCCACCCGAAGAGTCA
	Reverse: AGGTGGCTAAGTCACACGAC
Ccno	Forward: CTACGACTTCCGAAAGGCG
	Reverse: TCCAGAGTATTCACGGTCAGAC
ERK5	Forward: CCGGTCGAGGCGGAA
	Reverse: CGTCAAAGGTCACGTCGAA