Supplementary Figures

Pnpt1 mediates NLRP3 inflammasome activation by MAVS and metabolic reprogramming in macrophages

Running title: Pnpt1 mediates inflammation in macrophages

Supplementary Fig. 1 Pnpt1 deletion has minimal effects on IL-6 and MCP-1 production. Ten-week-old male $Pnpt1^{m-/-}$ and WT mice were sacrificed 6 h after i.p. injection with 40 mg/kg of LPS. The whole blood samples were collected and peritoneal cavities were washed with PBS. (A) MCP-1 and (B) IL-6 in peritoneal lavage fluid; (C) MCP-1 and (D) IL-6 in plasma (n=4-5 mice per group). Statistics in B-E were performed using a 2-way ANOVA and Bonferroni's post hoc test. *P<0.05 between LPS- $Pnpt1^{WT}$ and LPS-$Pnpt1^{m-/-}$ groups. Bars represent mean ± SEM. Statistics in B-C were performed using a two-way ANOVA and Bonferroni's post hoc test. Bars represent mean ± SEM. *p <0.05.

Supplementary Figure S2. Pnpt1 deletion increases GSDMD cleavage after inflammasome activation in vivo. Pnpt1^{WT} and Pnpt1$^{m-/-}$ mice were injected i.p. with 10 mg/kg of LPS for 3 hr followed by 30 min ATP i.p. injection. Peritoneal exudate cells were collected for protein extraction. (A) GSDMD expression (B) Quantification results of cleaved GSDMD. N=4 mice from each group). Bars represent mean ± SEM. ****p <0.001.

Supplementary Figure S3. Pnpt1 deletion worsens septic survival. $Pnpt1^{WT}$ and $Pnpt1^{m-/-}$ mice were challenged with 10 mg /kg LPS for 3 hr followed by ATP (100 mM in 100 µl, pH 7.4) i.p. injection. Survival rate was measured until 60 hr after ATP injection. (n=10 from each group). Statistical analysis was performed using the log-rank (Mantel–Cox) test. **p <0.001.

Supplementary Figure S4. Pnpt1 deletion enhances GSDMD cleavage and pyroptosis in vitro macrophages. Peritoneal macrophages from Pnpt1^{WT} and Pnpt1$^{m-/-}$ mice were stimulated with or without LPS (100 ng/mL) for 3 hr and followed by 2 μM nigericin (Nig) for 30 min (A) GSDMD expression was analyzed by western blot, and images were representative of three independent experiments.(B) Quantification of cleaved GSDMD. (C) Macrophages were stimulated with or without LPS (100 ng/mL) for 3 hr and followed by 2 μM nigericin (Nig) for 2 hr. Medium was used for LDH assay. Statistics in B and C were performed using a 2-way ANOVA and Bonferroni's post hoc test. **P<0.01, ***P<0.001, between Pnpt1m-/- and WT groups after stimulation. Bars represent mean ± SEM.

Supplementary Figure S5. Pnpt1 enhances NLRP3 inflammasome activation via a transcription-independent mechanism. Pnpt1^{WT} and Pnpt1$^{m-/-}$ macrophages were stimulated with nigericin (Nig, 2 μM) after 10 min LPS (100 ng/mL) priming. Western blots are representative of three independent experiments.

Supplementary Figure S6. Pnpt1 deletion has no effect on NLRC4 and AIM2 activation.

BMDMs from Pnpt1^{WT} and Pnpt1$^{m-/-}$ mice were stimulated with LPS (100 ng/mL) for 3 hr followed by (A) flagellin (2 µg/mL) or (B) Poly(dA:dT) (2 µg/mL) transfection for 3 hr. IL-1β was measured by ELISA. Statistics in A & B were performed using a 2-way ANOVA and Bonferroni's post hoc test. $P<0.05$ between Pnpt1^{WT} and Pnpt1 $^{m-/-}$ groups. Bars represent mean ± SEM.

Supplementary Figure S7. Pnpt1 enhances lactate production after NLRP3 inflammasome activation. Pnpt1^{WT} and Pnpt1$^{m-/-}$ BMDMs were primed with LPS for 3 hr. The medium was removed and washed twice before Nig (2 μM) stimulation. After 1 hr, Lactate concentration in the medium was measured. Bars represent mean ± SEM. *p <0.05.

Supplementary Fig. 8. Pnpt1 deletion increases oxygen consumption. Untreated (Con) or LPS-activated (3 hr) peritoneal macrophages from $Pnpt1^{m-/-}$ and $Pnpt1^{WT}$ mice were subjected to a mitochondrial stress test using a Seahorse XF-analyzer. (A) A representative seahorse plot of the mitochondrial stress test assessed by oxygen consumption rate (OCR) , an index of glycolysis after injection of oligomycin (1 μg/ml), carbonyl cyanide-4 (trifluoromethoxy)phenylhydrazone (FCCP, 1 μM), and rotenone (1 μM) plus antimycin (1 μM). (B) Basal OCR (C) Maximal OCR. Bars represent mean ± SEM. Statistics in B-C were performed using a two-way ANOVA and Bonferroni's post hoc test. Bars represent mean ± SEM. *p <0.05.

Supplementary Figure S9. Exogenous heptelidic acid reverses Pnpt1-mediated inflammasome activation. BMDMs were incubated with DMSO or 3 μM heptelidic acid (HA) and stimulated with LPS (100 ng/mL) for 3 hr, then followed by 2 μM nigericin (Nig) for 1 hr. IL-1β in the medium was measured by ELISA. Statistics were performed using a 2-way ANOVA and Bonferroni's post hoc test. Bars represent mean ± SEM. ****p <0.001.

Supplementary Figure S10. Exogenous 2-DG treatment has no effect on Pnpt1-mediated caspase-1 cleavage. Peritoneal macrophages were stimulated with LPS (100 ng/mL), and co-incubated with PBS or 10 mM 2-Deoxy-D-glucose (2-DG) for 3 hr, then followed by 2 μM nigericin (Nig) for 30 min. Western blots are representative of three independent experiments.

Supplementary Figure S11. MAVS is required for Pnpt1 regulated NLRP3 inflammasome activation in human macrophages. THP-1 macrophages were treated with scrambled, Pnpt1 or MAVS siRNA for 48 hr., and stimulated with LPS (100 ng/mL) for 3 hr. followed by (E) nigericin (Nig, 2 μM) for 1 hr. The amount of IL-1β in the medium was measured. (Con: control, KD: knockdown). Statistics was performed using a one-way ANOVA and Bonferroni's post hoc test. N=4 experiments. Bars represent mean ± SEM. ****p <0.001.

Supplementary Figure S12. MAVS inhibition by exogenous lactate reverses Pnpt1-mediated inflammasome activation. BMDMs from Pnpt1$^{m-/-}$ and Pnpt1^{WT} mice were stimulated with LPS (100 ng/mL) for 3 hr, co-incubated with PBS or 10 mM Lactate, then followed by 2 μM nigericin (Nig) for 1 hr. IL-1β in the medium was measured by ELISA. Statistics was performed using a 2-way ANOVA and Bonferroni's post hoc test. Bars represent mean ± SEM. ****p <0.001.

Supplementary Figure S13. Pnpt1 deletion in macrophages induces MAVS oligomerization. BMDMs were stimulated with or without LPS (100 ng/mL) for 3 hr and followed by 2 μM nigericin (Nig) for 1 hr. MAVS oligomerization was analyzed after cross-linking by western blots. MAVS and β-actin from whole cell lysate (WCL) were measured by western blots.