***Slc6a13* deficiency attenuates *Pasteurella multocida* infection**-**induced hyperinflammation *via* glycine-inflammasome signaling**

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**The legends of Supplementary Figures**

**Supplementary Fig. 1. Slc6a13 deficiency has little effect on the levels of GABA. A:** Genotyping PCR to detect WT (+/+), and Slc6a13 homozygous (–/–) knockout mice (n=6). **B:** The mRNA expression of major virulence factor of *P. multocida* in WT and Slc6a13–/– mice (n=6, multiple t-test). **C:** The levels of GABA in the lungs of WT and Slc6a13–/– mice infected or uninfected by *P. multocida* (n=6, multiple t-test). All data were expressed as means ±SD.

**Supplementary Fig. 2. The data analysis of transcriptome sequencing in WT and KO mice infected or uninfected by *P. multocida*.**

**A:** The clustering heat-map of DEGs in Illumina sequencing for KO vs KO-PmCQ2 (n=3). **B:** Numbers of up/down-regulated DEGs for KO vs KO-PmCQ2 (n = 3). **C:** The shared results of upregulated DEGS between WT vs. WT-PmCQ2 and KO vs. KO-PmCQ2 mice. **D:** The shared results of downregulated DEGS between WT vs. WT-PmCQ2 and KO vs. KO-PmCQ2 mice. **E:** The shared results of upregulated DEGS between WT vs. KO and WT-PmCQ2 vs. KO-PmCQ2 mice. **F:** The shared results of downregulated DEGS between WT vs. KO and WT-PmCQ2 vs. KO-PmCQ2 mice.

**Supplementary Fig. 3. The levels of amino acids and the expression of GlyT and GlyR in the lungs of WT and Slc6a13–/– mice infected or uninfected by *P. multocida*.**

**A:** The levels of amino acids in the lungs of WT and Slc6a13–/– mice infected or uninfected by *P. multocida* (n=6, one-way ANOVA). **B:** The mRNA expression of GlyT in the lungs of WT and Slc6a13–/– mice infected or uninfected by *P. multocida* (n=6, one-way ANOVA). **C:** The mRNA expression of GlyR in the lungs of WT and Slc6a13–/– mice infected or uninfected by *P. multocida* (n=6, one-way ANOVA).GlyR: glycine receptors, GlyT: glycine transporters. All data were expressed as means ±SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

**Supplementary Fig. 4. The levels of amino acids and the expression of GlyT and GlyR in the WT and Slc6a13–/– macrophages infected or uninfected by *P. multocida*.**

**A:** The levels of amino acids in the WT and Slc6a13–/– macrophages infected or uninfected by *P. multocida*(n=6, one-way ANOVA). **B:** The mRNA expression of GlyT in the WT and Slc6a13–/– macrophages infected or uninfected by *P. multocida* (n=6, one-way ANOVA)**.** **C:** The mRNA expression of GlyR in the WT and Slc6a13–/– macrophages infected or uninfected by *P. multocida* (n=6, one-way ANOVA).GlyR: glycine receptors, GlyT: glycine transporters. All data were expressed as means ±SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

**Supplementary Fig. 5. Effect of glycine on macrophage mediated inflammation. A:** The cell viability of macrophages with or without glycine supplementation (1 mM, 5 mM, and 10 mM) (n=6, One way ANOVA). **B:** The production of NO from macrophages infected by *P. multocida* with or without glycine (10 mM) treatment (n=6, unpaired, two-tailed Student’s t-test). **C-D:** The protein abundance of IL-1β, TNF-α, IFN-γ, IL-6, and IL-12 in PEMs **(C)** and ANA-1 **(D)** cells infected by *P. multocida* at 12 h with or without glycine (10 mM) treatment (n=6 or 7, one-way ANOVA). All data were expressed as means ±SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

**Supplementary Fig. 6. The influence of exogenous 10 mM glycine on mTOR, HIF-1α, and NF-κB pathway in macrophages. A-B:** The activation of mTOR, HIF-1α, and NF-κB in WT+PmCQ2, KO+PmCQ2, KO+PmCQ2+glycine macrophages infected by *P. multocida* (n=4, one-way ANOVA). All data were expressed as means ±SD. \*P < 0.05, \*\*\*\*P < 0.0001.