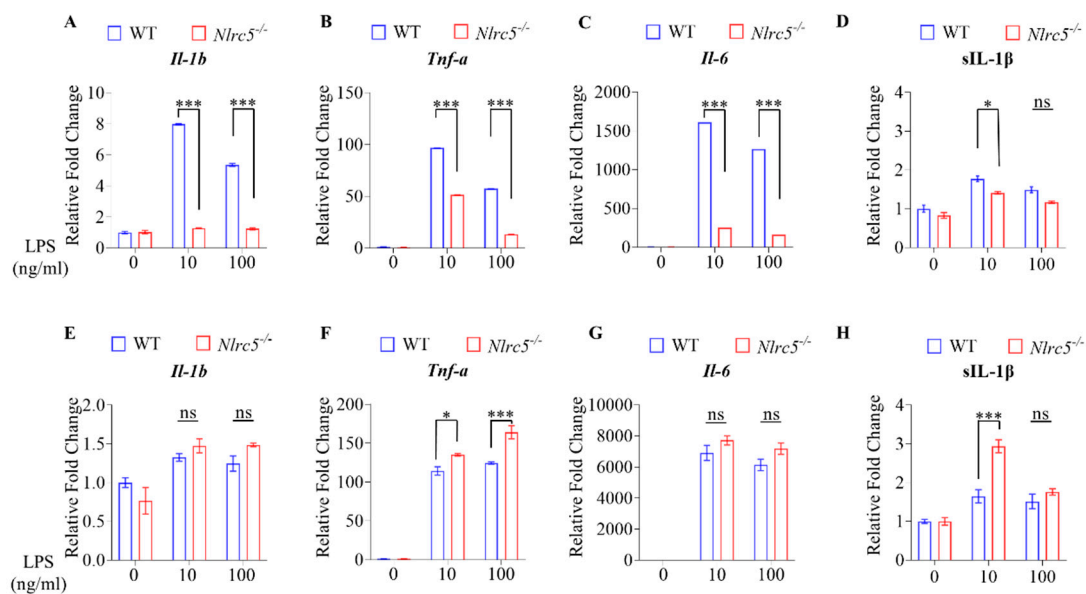


### Supplementary Figure S1. Identification of primary cultured microglia.

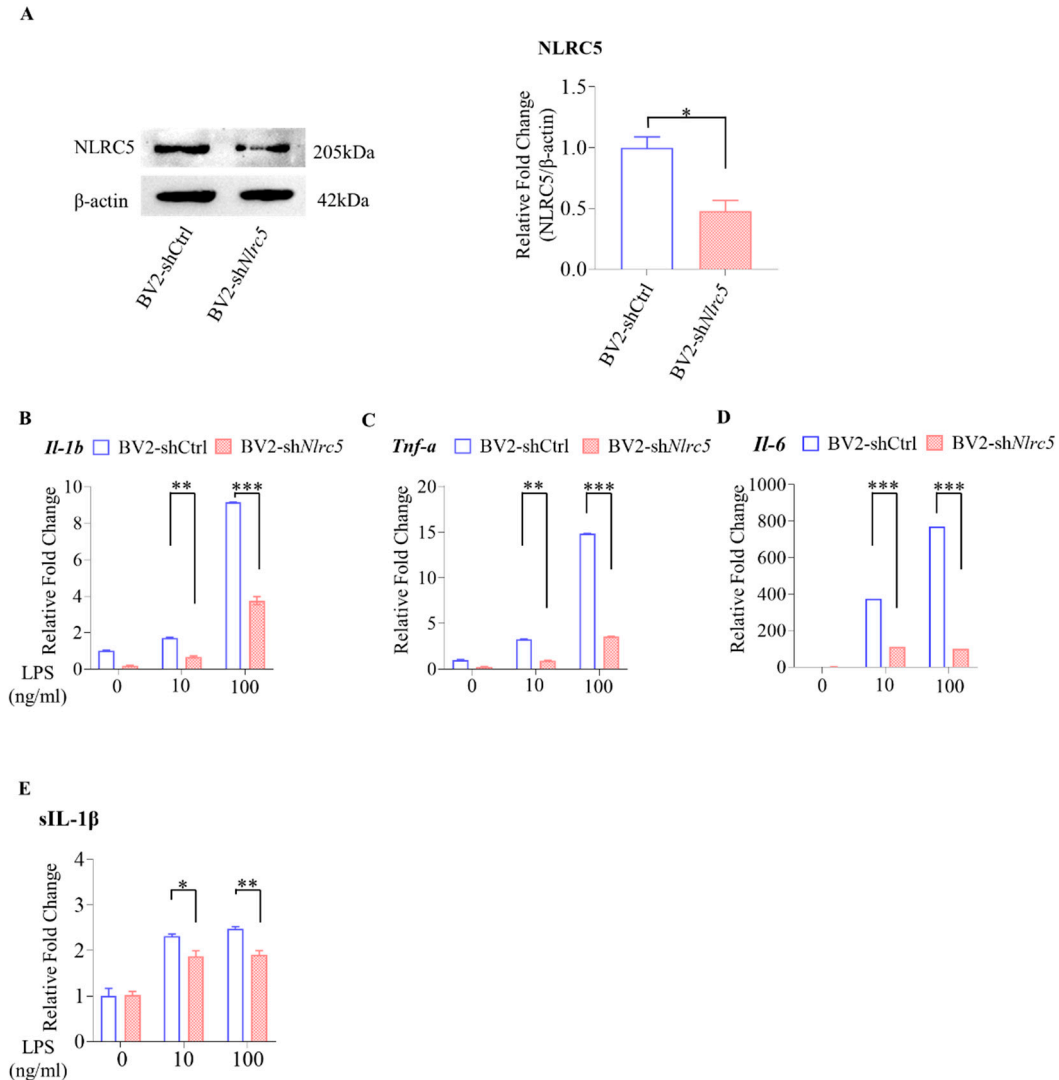
Representative immunofluorescence images of primary cultured microglia (Blue: DAPI, Red: Iba-1, Scale bar: 100  $\mu$ m).



### Supplementary Figure S2. *Nlr5* knockout differently regulates cytokine production in primary cultured microglia and BMDM after treated with LPS for 8h.

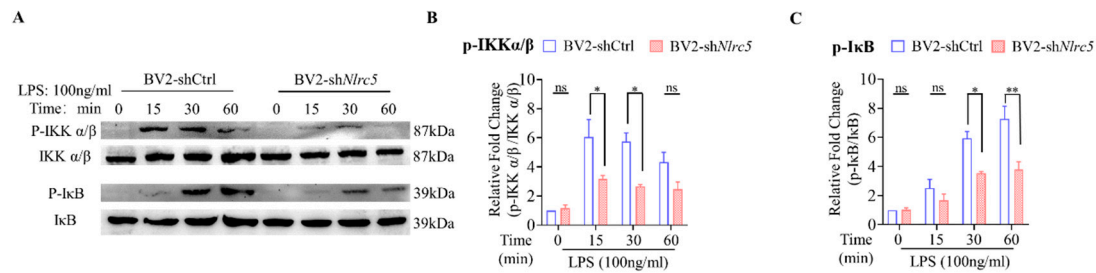
**(A-D)** Effect of *Nlr5* knockout on cytokine production in primary cultured microglia. The mRNA of *Il-1b* **(A)**, *Tnf-a* **(B)** and *Il-6* **(C)** of microglia treated with different concentration of LPS for 8 hours was detected by RT-qPCR and secreted IL-1β **(D)** in the supernatant was detected by ELISA (n=3 for each group). **(E-H)** Effect of *Nlr5* knockout on cytokine production in BMDM. The mRNA of *Il-1b* **(E)**, *Tnf-a* **(F)** and *Il-6* **(G)** in BMDM treated with different concentration of LPS for 8 hours was detected by RT-qPCR and IL-1β secretion **(H)** in the supernatant was detected by ELISA (n=3 for each group). All data were presented as mean  $\pm$  SEM. Two-way ANOVA was used to

determine the statistical significance between two group. \* $p \leq 0.05$ , \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ ; ns: no significant difference. BMDM: bone marrow derived macrophages.



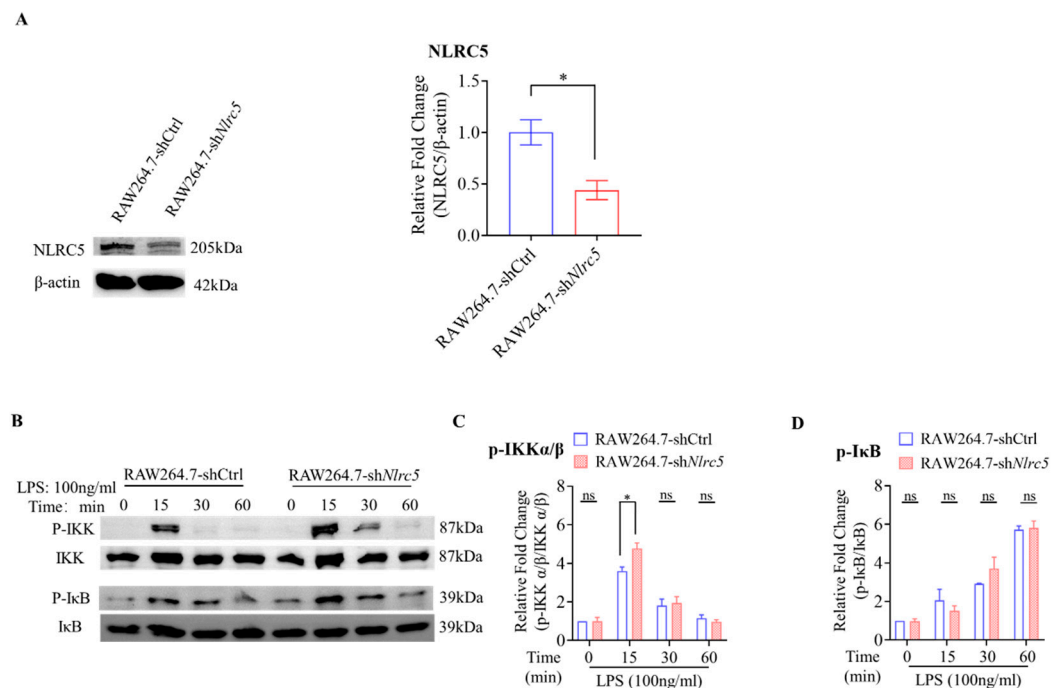
**Supplementary Figure S3. Knockdown of *Nlr5* reduced LPS-induced cytokines production in BV2.**

**(A)** Confirmation the knockdown of *Nlr5* by Western blot in BV2 cells. T test was used to determine the statistical significance between two groups. \* $p \leq 0.05$ . The mRNA of *Il-1b* **(B)**, *Tnf-α* **(C)** and *Il-6* **(D)** of BV2-shCtrl and BV2-shNlr5 treated with different concentration of LPS for 24 hours was detected by RT-qPCR and secreted IL-1β **(E)** in the supernatant was detected by ELISA (n=3 for each group). All data were presented as mean  $\pm$  SEM. Two-way ANOVA was used to determine the statistical significance between two group. \* $p \leq 0.05$ , \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ .



**Supplementary Figure S4. Knockdown of *Nlr5* inhibits IKK and IκB phosphorylation in BV2 cells.**

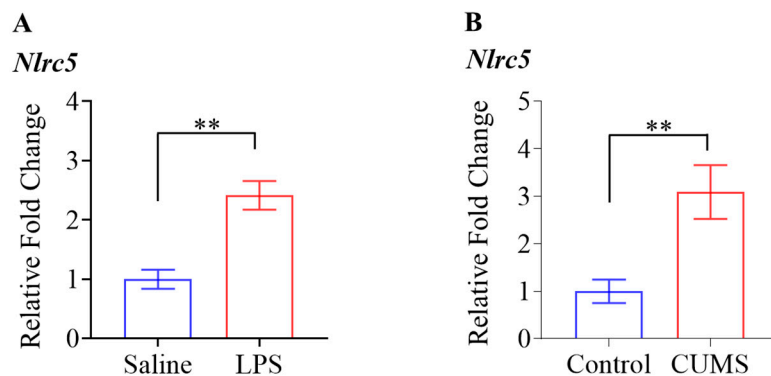
**(A)** Representative Western blot image of p-IKKα/β, IKKα/β, p-IκB and IκB in BV2-shCtrl and BV2-sh*Nlr5* cells treated with LPS (100 ng/mL) for different time. **(B,C)** The ratio of relative expression of p-IKKα/β to IKKα/β **(B)** and p-IκB to IκB **(C)** in LPS treated to untreated groups was quantified (n=4 for each group). All data were presented as mean ± SEM. Two-way ANOVA was used to determine the statistical significance between two group. \* $p \leq 0.05$ , \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ . ns: no significant difference.



**Supplementary Figure S5. Knockdown of *Nlr5* promotes IKK and IκB phosphorylation in RAW264.7 macrophages.**

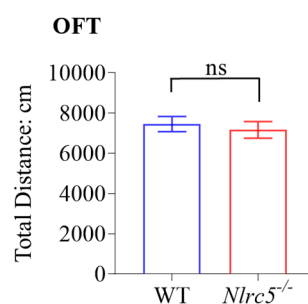
**(A)** Confirmation the knockdown of *Nlr5* by Western blot in RAW264.7 macrophages. **(B)** Representative Western blot image of p-IKKα/β, IKKα/β, p-IκB and IκB in RAW264.7-shCtrl and RAW264.7-sh*Nlr5* cells treated with LPS (100 ng/mL) for

different time. **(C, D)** The ratio of relative expression of p-IKK $\alpha$ / $\beta$  to IKK $\alpha$ / $\beta$  **(C)** and p-I $\kappa$ B to I $\kappa$ B **(D)** in LPS treated to untreated groups was quantified (n=4 for each group). All data were presented as mean  $\pm$  SEM. Two-way ANOVA was used to determine the statistical significance between two group. \* $p \leq 0.05$ , \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ . ns: no significant difference.



**Supplementary Figure S6. *Nlrc5* was up-regulated in hippocampus tissue of mouse model of depression.**

Expression of *Nlrc5* was assessed in hippocampus tissues of LPS **(A)** and CUMS **(B)** mice model by RT-qPCR (n=5 for each group). All data were presented as mean  $\pm$  SEM. T test was used to determine the statistical significance between two groups. \*\* $p \leq 0.01$ . ns: no significant difference.



**Supplementary Figure S7. Assessment of spontaneous locomotor ability in OPT.**

Total travel distance of WT and *Nlrc5*<sup>-/-</sup> mice in open field test (n=12 for each group). All data were presented as mean  $\pm$  SEM. T test was used to determine the statistical significance between two groups. ns: no significant difference.