C5aR2 contributes to NLRP3 inflammasome activation and HMGB1 release via promoting PKR expression in macrophages

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This supporting file contains revised supplementary figures 1-5 and legends as follows.

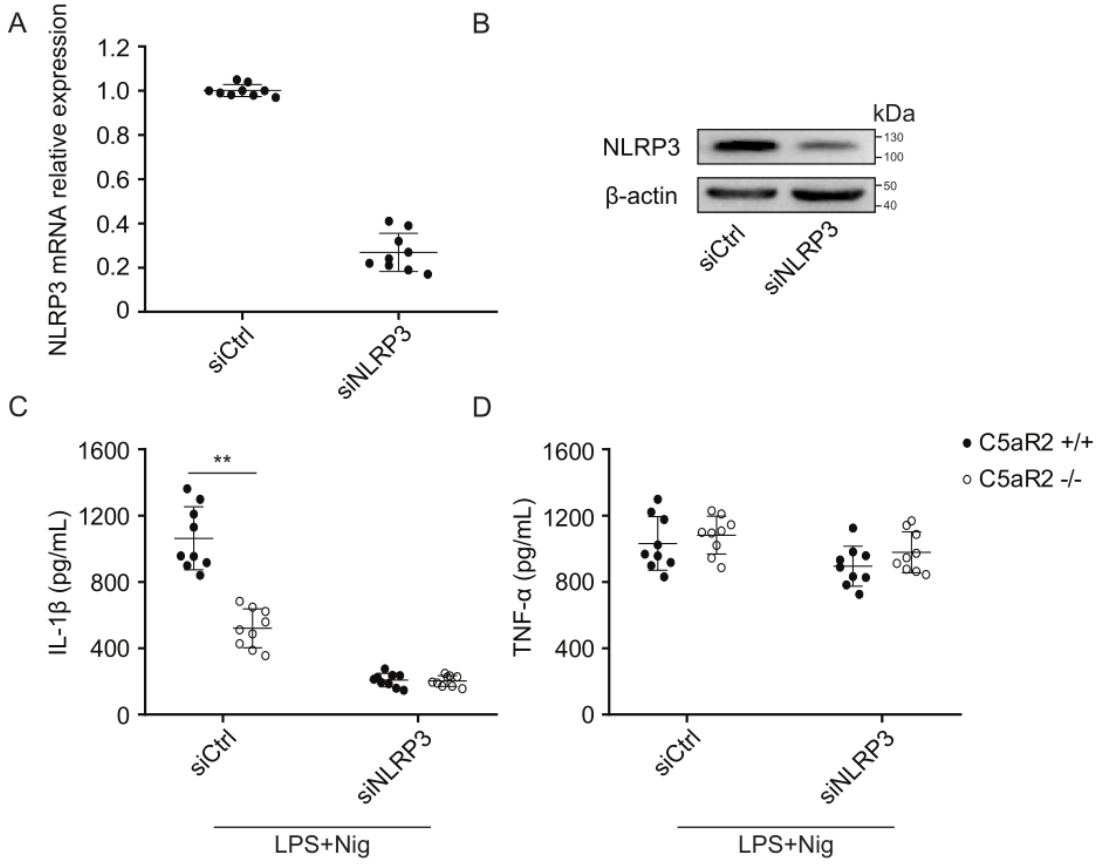

Figure S1. NLRP3 is required for C5aR2-mediated NLRP3 inflammasome activation. Peritoneal macrophages collected from *C5aR2+/+* and *C5aR2-/-* mice were transfected with siNLRP3 or siCtrl 48 h before priming with LPS for 3 h and stimulation with Nigericin for 1 h. Total RNA (A) and protein (B) were extracted and subjected to Q-PCR and Western blot analysis of NLRP3 expression. Cell supernatants were analyzed by ELISA for IL-1β (C) and TNF-α (D). siCtrl: the scrambled negative control siRNA. The results represent the mean ± S.D. of three independent experiments performed in triplicate (A, C, D). Two-tailed Student's *t* test was used (C). For Western blot analysis, data are representative of three independent experiments (B).

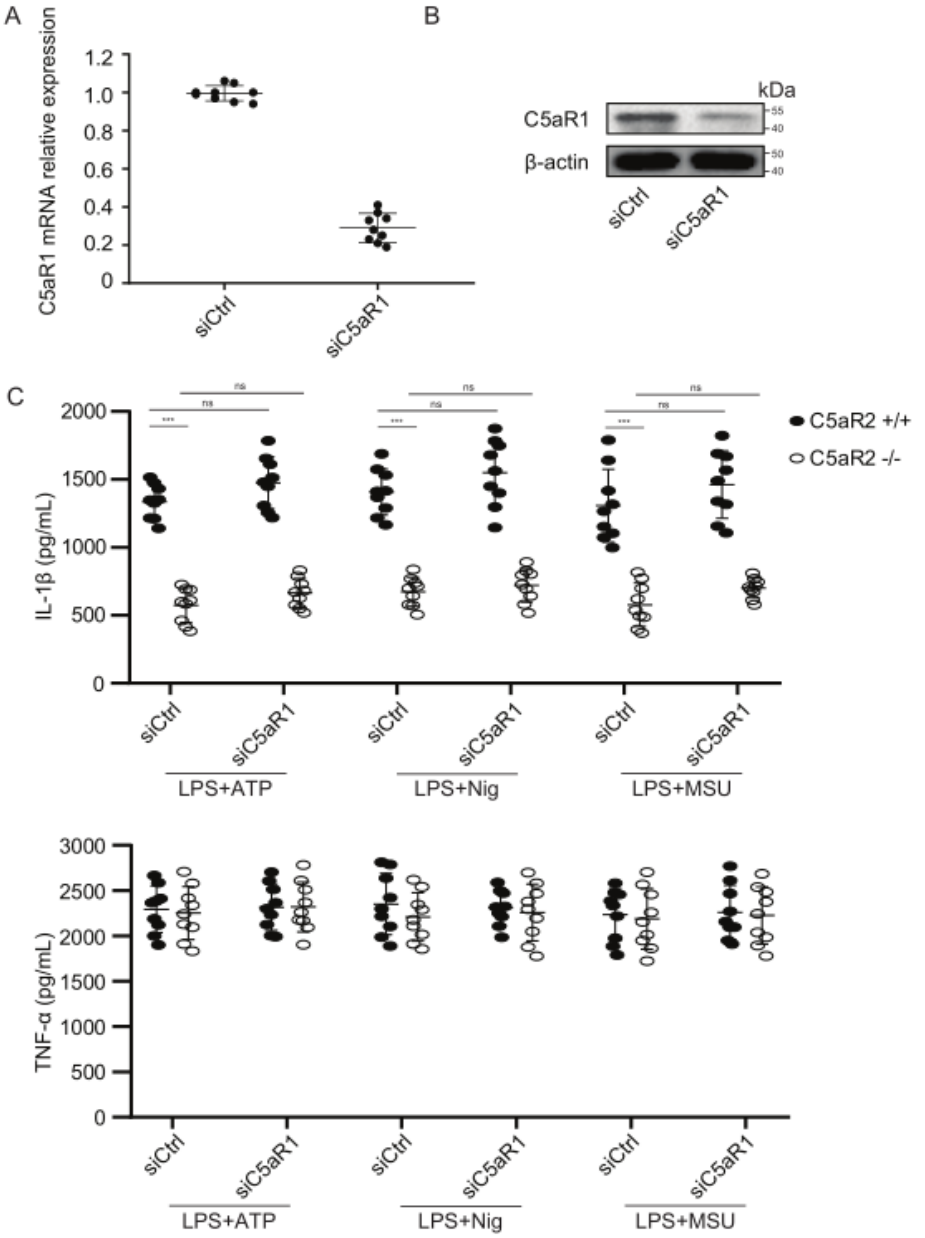

Figure S2. Comparison of C5aR1 and C5aR2's role in NLRP3 inflammasome activation. Peritoneal macrophages collected from *C5aR2+/+* and *C5aR2-/-* mice were transfected with siC5aR1 or siCtrl 48 h before priming with LPS for 3 h and stimulation with ATP or Nigericin for 1 h or MSU for 6 h. Total RNA (A) and protein (B) were extracted and subjected to Q-PCR and Western blot analysis of C5aR1 expression. Cell supernatants were analyzed by ELISA for IL-1β and TNF-α (C). siCtrl: the scrambled negative control siRNA. The results represent the mean ± S.D. of three independent experiments performed in triplicate (A, C). Two-tailed Student's *t* test was used (C); ns, not significant. For Western blot analysis, data are representative of three independent experiments (B).

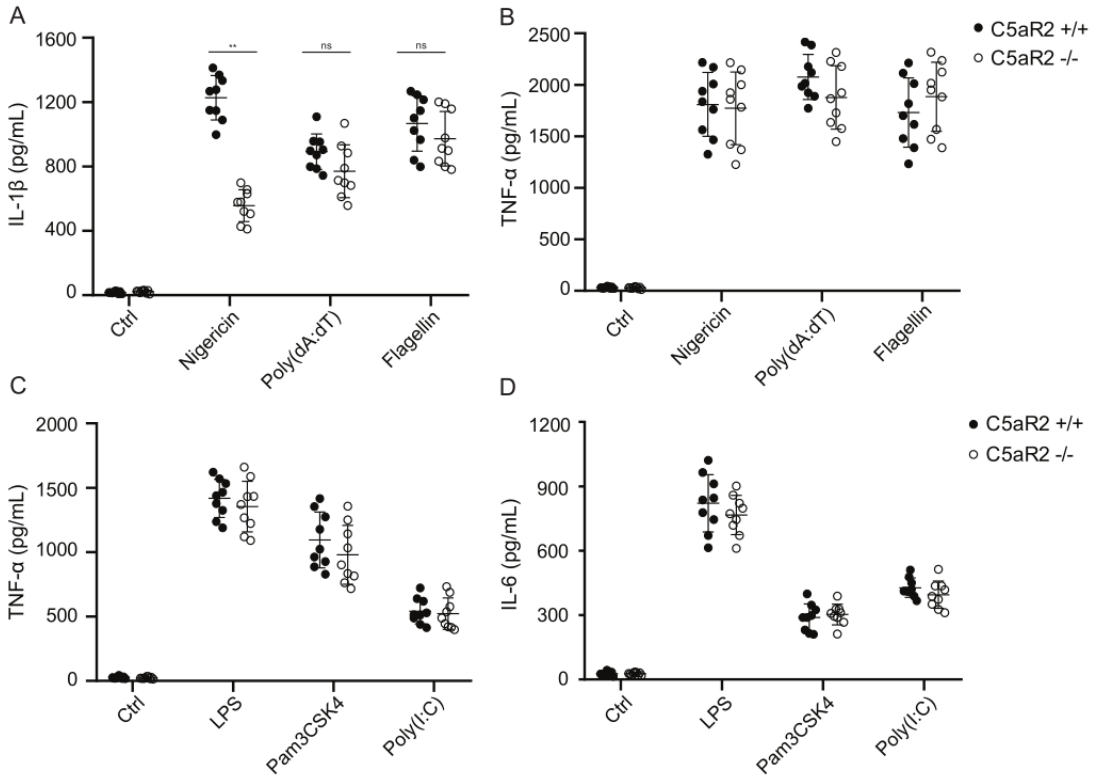

Figure S3. Comparison of C5aR2's role in various inflammasomes and TLRs signaling. (A, B) The peritoneal macrophages from *C5aR2*+/+ and *C5aR2*-/- mice were primed with LPS and stimulated with NLRP3 agonist (Nigericin), AIM2 agonist (Poly (dA:dT)) and NLRC4 agonist (Flagellin) respectively. Cell supernatants were analyzed by ELISA for IL-1β (A) and TNF-α (B). (C, D) The peritoneal macrophages from *C5aR2*+/+ and *C5aR2*-/- mice were treated with LPS, Pam3CSK4 and Poly (I:C) to activate TLR4, TLR2 and TLR3 signaling, respectively. Cell supernatants were analyzed by ELISA for TNF-α (C) and IL-6 (D). The results represent the mean ± S.D. of three independent experiments performed in triplicate (A-D). Two-tailed Student's *t* test was used (A); ns, not significant.

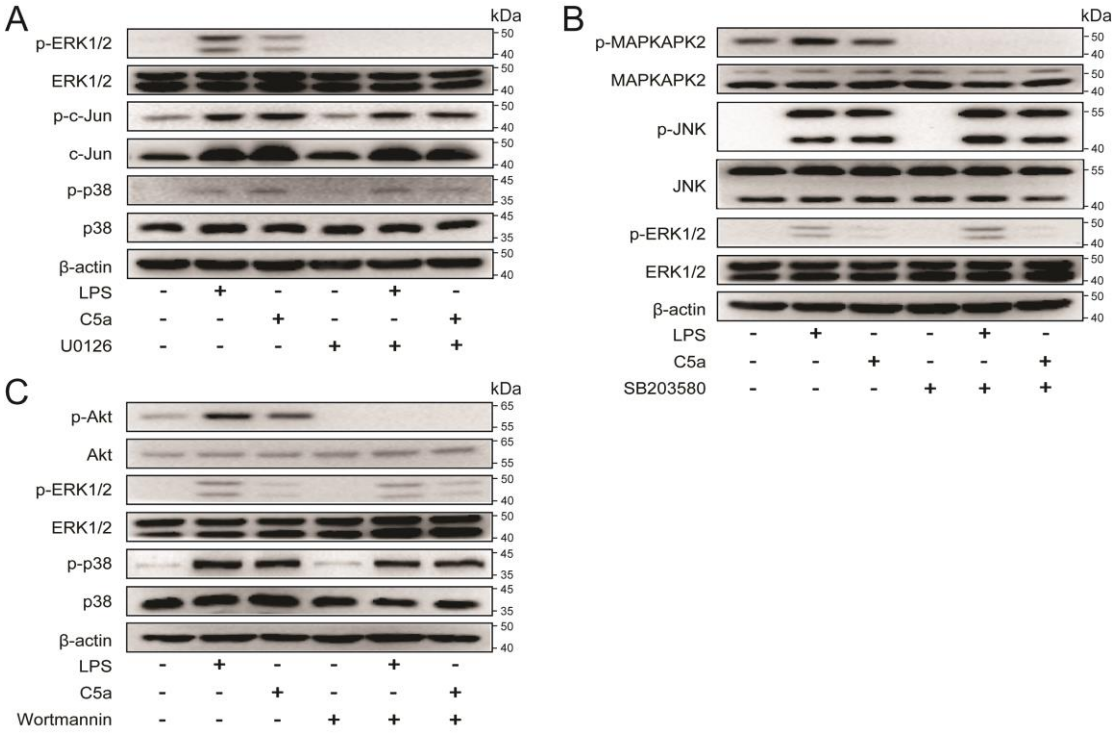

Figure S4. Determination of the specificity of U0126, SB203580 and Wortmannin.
Peritoneal macrophages from WT mice were treated with U0126, SB203580 or Wortmannin 1 h before stimulation with LPS or C5a for 3 h. Total protein were extracted and subjected to Western blot analysis of the phosphorylation of target and none-target proteins. The MEK/ERK inbibitor U0126 could directly suppress the phosphorylation of ERK1/2 without affecting the activation of c-Jun and p38 (A). The p38 MAPK inbibitor SB203580 could directly inhibit the phosphorylation of MAPKAPK2 without influencing the activation of JNK and ERK1/2 (B). The PI3K/Akt inbibitor Wortmannin could directly suppress the phosphorylation of Akt without interfering the activation of ERK1/2 and p38 (C). Data are representative of three independent experiments.

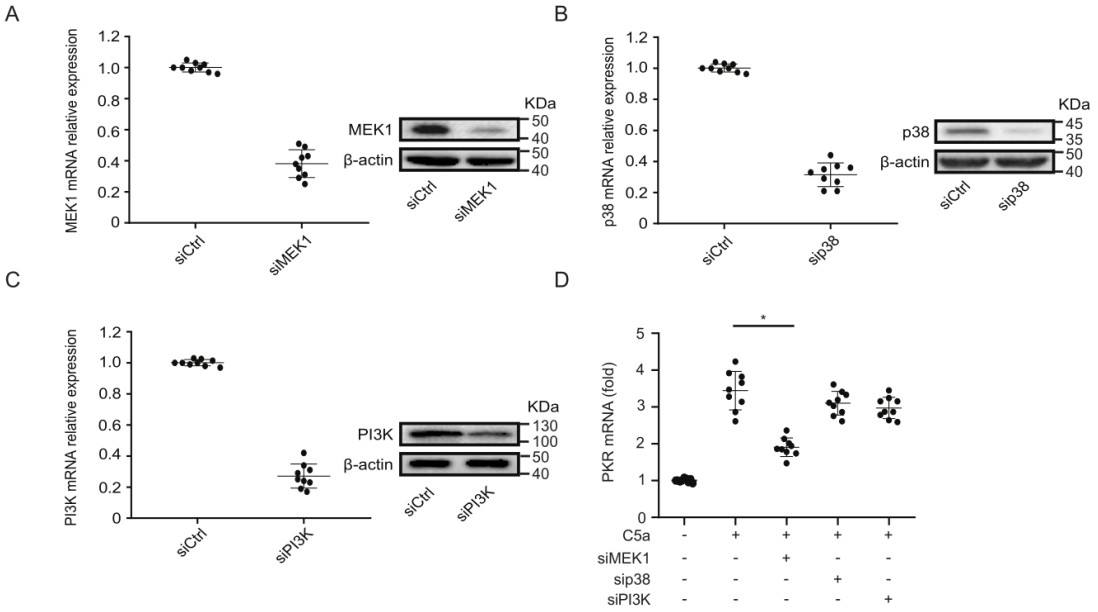

Figure S5. Knockdown of MEK1 reduces the expression of PKR following C5a treatment. Peritoneal macrophages from WT mice were transfected with siMEK1, sip38, siPI3K or siCtrl 48 h before challenge of C5a (1 μg/mL) for 3 h. Total RNA and protein were extracted and subjected to Q-PCR and Western blot analysis of MEK1 (A), p38 (B), PI3K (C) and PKR (D) expression. siCtrl: the scrambled negative control siRNA. The results represent the mean ± S.D. of three independent experiments performed in triplicate (A-D). Two-tailed Student's *t* test was used (D). For Western blot analysis, data are representative of three independent experiments (A-C).