The brake mode for RKIP on inflammasome activation and inflammasome-dependent diseases.

Supplementary Figures and legends

Supplementary Figure 1 , related to Figure 1; Supplementary Figure 2 , related to Figure 5;

Supplementary Figure 3 , related to Figure 6.

Supplemental Figure 1

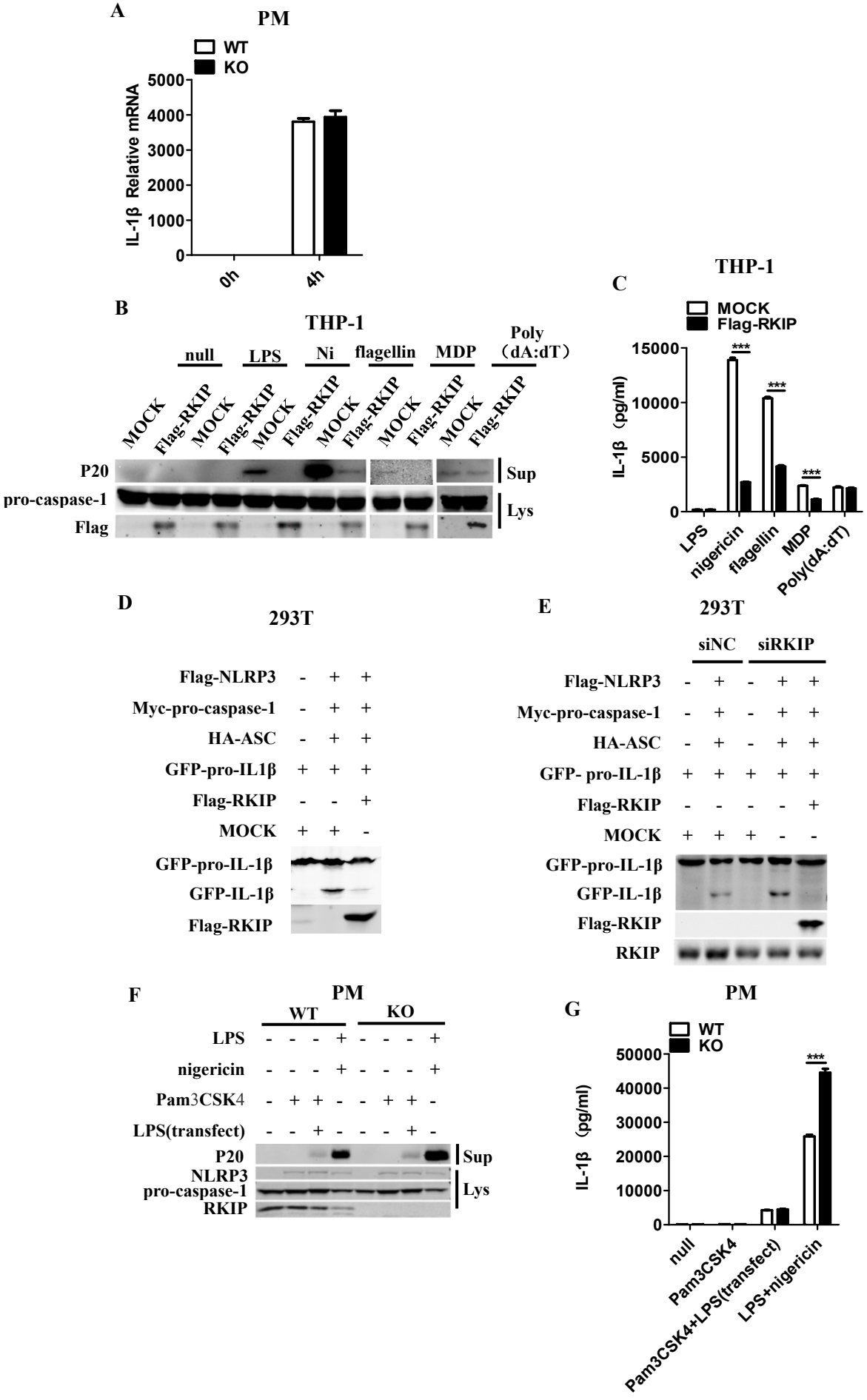

Figure S1 RKIP inhibits NLRP1, NLRP3 and NLRC4 inflammasomes activation *in vitro*.

(**A**) 1 × 10^6 wild-type (WT) or Rkip-knockout (KO) peritoneal macrophages (PMs) were stimulated with LPS (1 μg/ml) for 4 hours. Cell extracts were analyzed by quantitative PCR for mRNA expression of pro-IL-1β.

(**B** and **C**) 1 × 10^6 MOCK or THP1 stable transfectants were differentiated with 100 nM PMA overnight in 12-well plates, then were primed with LPS and treated with nigericin (Ni), flagellin , Poly(dA:dT) and MDP for indicated times. Immunoblotting analysis of the supernatants (Sup) and cell extracts (Lys) (**B**), and ELISA analysis of the supernatants (Sup) for IL-1β release (**C**).

(**D**) Mock or Flag-RKIP was co-transfected with Flag-NLRP3, Myc-pro-caspase-1, HA-ASC and GFP-pro-IL-1β into 293T cells (2 × 10^5), immunoblotting analysis of the mature IL-1β after 24 hours.

(**E**) The expression of RKIP was knockdown in 293T cells (1 × 10^5) by using a specific siRNA, and then the cells were co-transfected with Mock or Flag-RKIP and Flag-NLRP3, Myc-pro-caspase-1, HA-ASC and GFP-pro-IL-1β, immunoblotting analysis of the mature IL-1β after 24 hours.

(**F** and **G**) 1 × 10^6 WT or Rkip-KO PMs were primed with Pam3CSK4 and transfected with LPS (2 μg/ml) or primed with LPS and treated with nigericin (Ni) (20 μM) for indicated times. Immunoblotting analysis of the supernatants (Sup) and cell extracts (Lys) (**F**), and ELISA analysis of the supernatants (Sup) for IL-1β release (**G**).

Data are mean ± SEM (**A**,**C** and **G**) and representative of three independent experiments. Student's t test was used for statistical calculation. * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Supplemental Figure 2

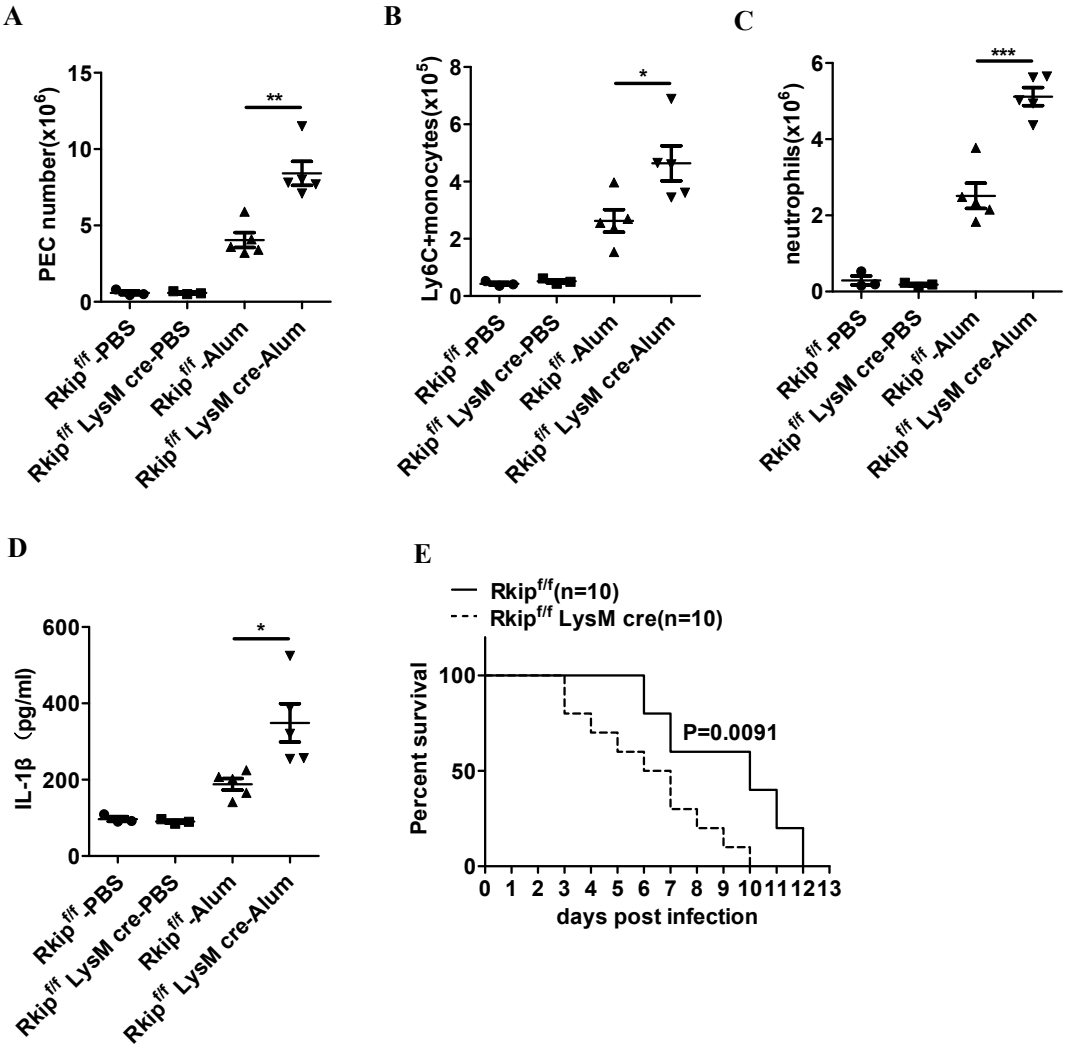

Figure S2 Rkip myeloid-knockout promotes NLRP3 and NLRC4 inflammasome activation *in vivo*.

(**A**-**D**) Rkip$^{f/f}$ and Rkip$^{f/f}$ LysM Cre mice were intraperitoneally injected of with Alum (50 mg/kg body weight) to induce peritonitis. After 12 hours, the mice were sacrificed and the peritoneal cavities were washed with 10 ml ice-cold PBS. FACS analysis of peritoneal exudates cells (PECs) (**A**) , Ly6C^{+} monocytes (**B**) or neutrophils (**C**) and ELISA analysis of IL-1β (**D**) in the peritoneal lavage fluid (PLF) (n=3-5 mice per group).

(**E**) Survival of Rkip$^{f/f}$ and Rkip$^{f/f}$ LysM Cre mice infected intraperitoneally with 5x10^{2} CFU

log-phase S. typhimurium (n=10 mice per group).

Data are mean ± SEM (**A-D**) and representative of two independent experiments. Student's t test was used for statistical calculation. * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Supplemental Figure 3

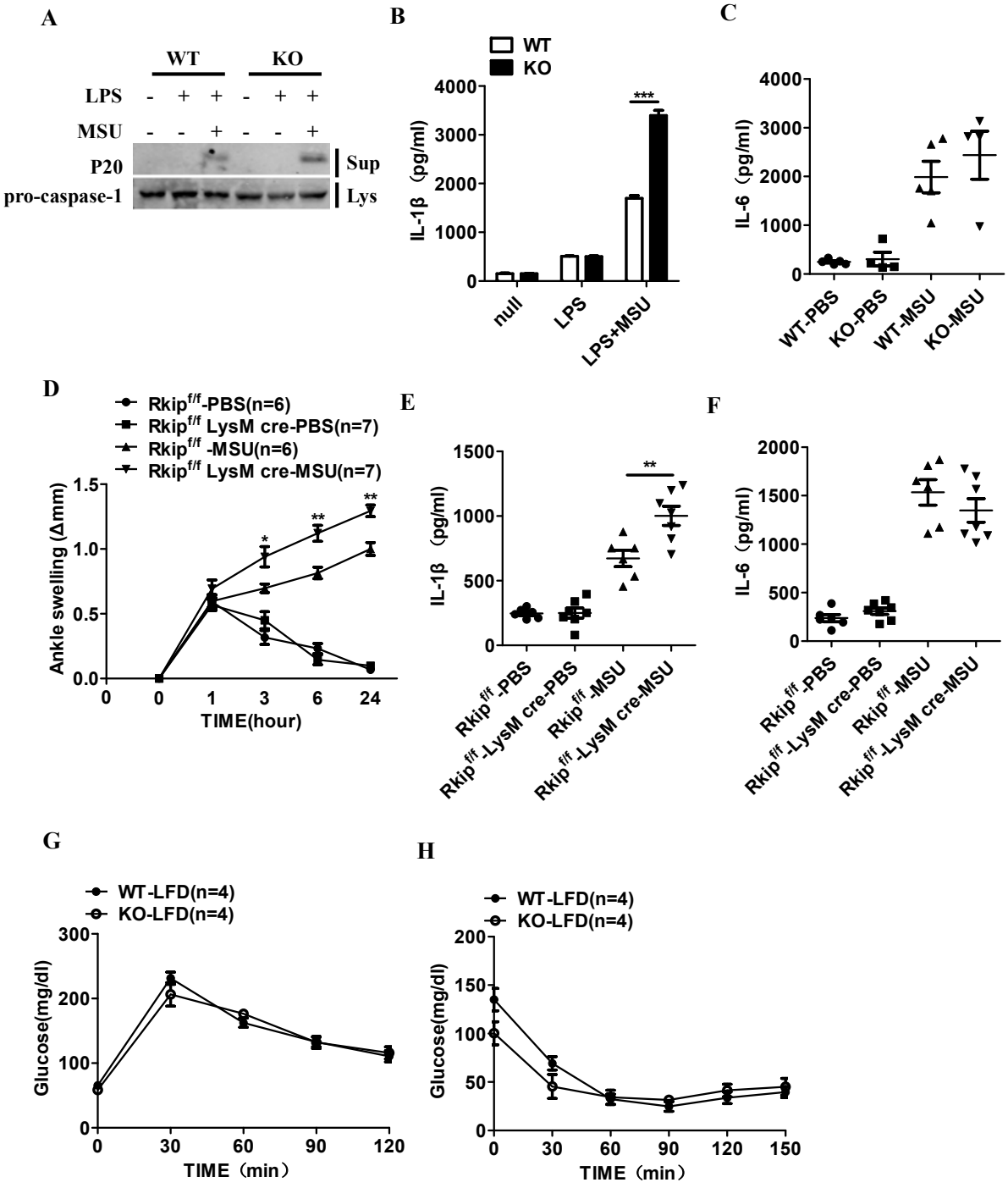

Figure S3 Rkip myeloid-knockout mice are more sensitive to NLRP3 inflammasome-related diseases.

(**A** and **B**) 1×10^6 WT or Rkip-KO PMs were primed with LPS and treated with MSU (300 μg/ml) for 12 hours, immunoblotting analysis of the supernatants (Sup) and cell extracts (Lys) (**A**), and ELISA analysis of the supernatants (Sup) for IL-1β release (**B**).

(C) WT and Rkip-KO mice were injected with MSU (0.5 mg) in the ankle to induce inflammation,

and ELISA analysis of IL-6 (24 hours later) in ankle culture from WT and Rkip-KO mice (n=4-5 mice per group).

(**D**-**F**) $Rkip^{f/f}$ and $Rkip^{f/f}$ LysM Cre mice were injected with MSU (0.5 mg) in the ankle to induce inflammation. Time course of changes in MSU induced ankle swelling of $Rkip^{f/f}$ and $Rkip^{f/f}$ LysM Cre mice (**D**), and ELISA analysis of IL-1β (**E**) and IL-6 (**F**) (24 hours later) in ankle culture from $Rkip^{f/f}$ and $Rkip^{f/f}$ LysM Cre mice (n=6-7 mice per group).

(**G** and **H**) GTTs (**G**) and ITTs (**H**) in WT and Rkip-KO mice after LFD feeding for 24 weeks (n= 4 mice per group).

Error bars represent SEM for **D**, **G** and **H**, data are mean ± SEM (**B**,**C**,**E** and **F**), and are representative of two independent experiments. Student's t test was used for statistical calculation. * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.