Cell Reports, Volume *26*

Supplemental Information

Macrophage β2-Integrins Regulate IL-22 by ILC3s and Protect from Lethal *Citrobacter rodentium*-Induced Colitis

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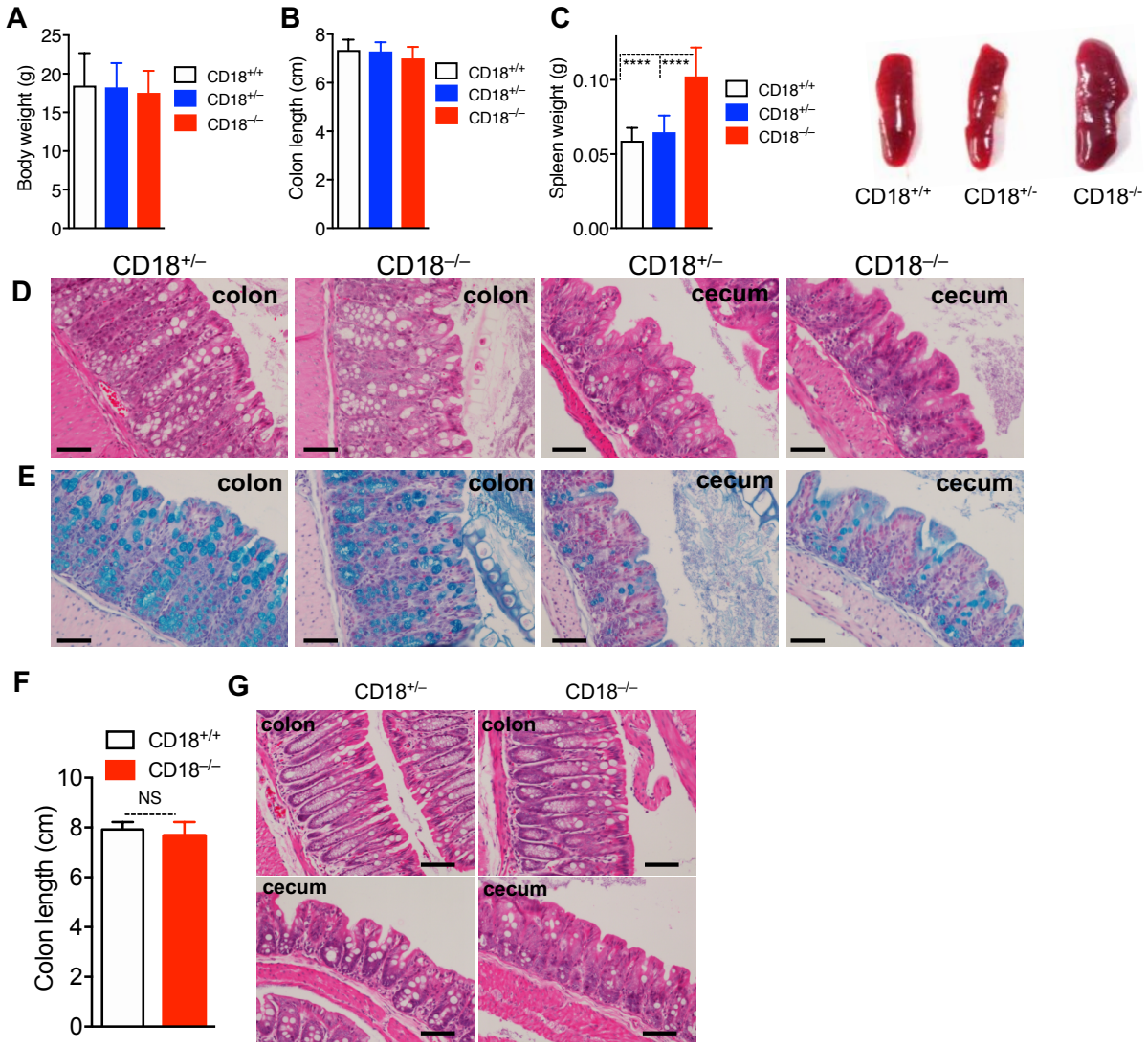

Figure S1: CD18-deficient mice do not exhibit intestinal pathology at steady state. Related to Figure 1.
(A-C) Eight-week-old CD18$^{-/-}$, CD18$^{+/-}$ and CD18$^{+/+}$ littermates were compared in terms of body weight (A), colon length (B), and spleen weight (left) and gross appearance of spleen (right) (C).
(D,E) Representative histological images of colon and cecum of the indicated mouse genotypes stained with H&E (D) or with Alcian blue which stains mucins in goblet cells (E). Scale bars, 50μm.
(F,G) Eighteen-week-old CD18$^{-/-}$ and CD18$^{+/+}$ littermates were compared for colon length (f) and histological appearance of colon and cecum tissue (G). Scale bars, 100μm.
Numerical data are means ± SD and are pooled from three independent experiments with three mice per group in two experiments and four mice per group in another experiment, for a total of ten mice per group (A-C,F). Images are representative of two independent experiments with three mice per group in each experiment (D,E,G). ****$P <$ 0.001 (one-way ANOVA with Tukey's multiple-comparisons test (A-C) or two-tailed Student's *t*-test (F)). NS, non-significant.

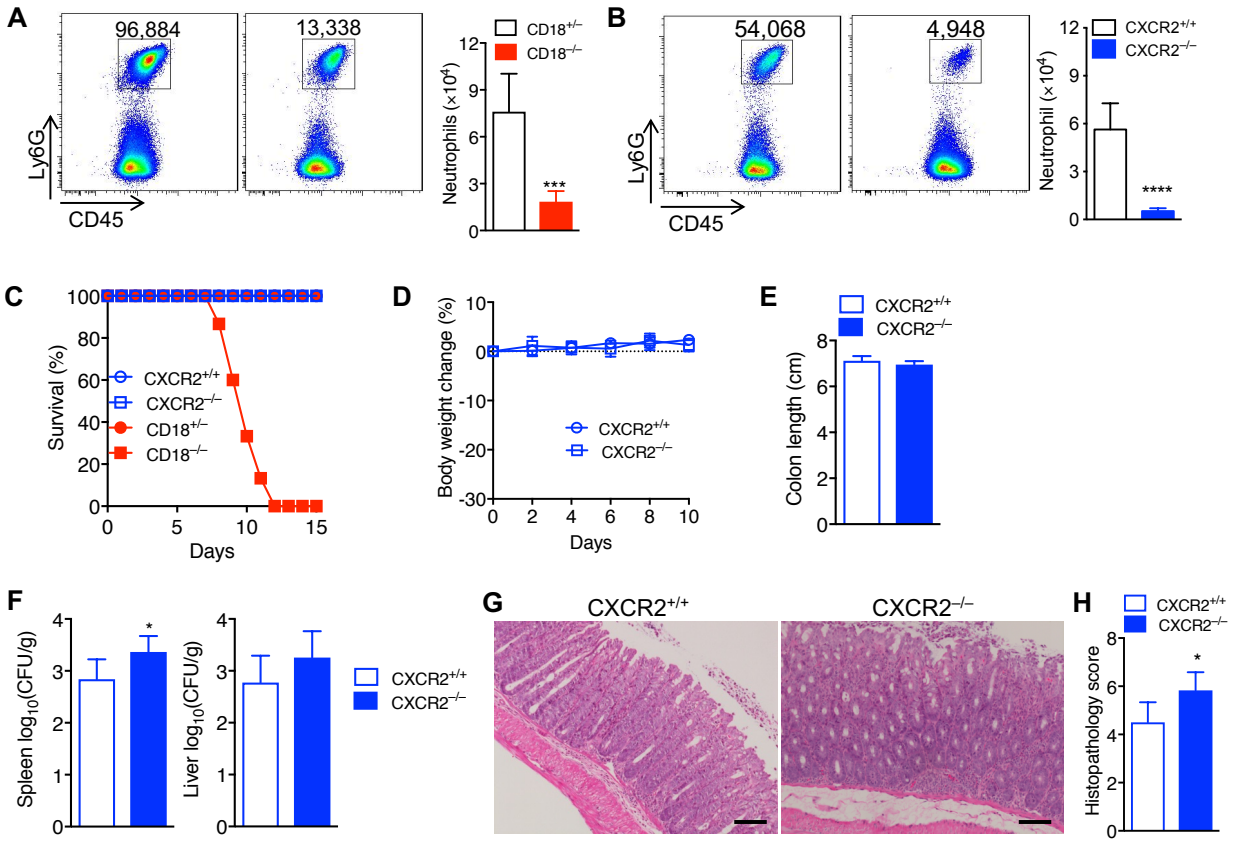

Figure S2: CXCR2 deficiency does not lead to acute lethality in *C. rodentium* infection. Related to Figure 1.
(A,B) Eight-week-old CD18$^{-/-}$ mice and CD18$^{+/-}$ littermate controls (A), or eight-week-old CXCR2$^{-/-}$ mice and CXCR2$^{+/+}$ littermate controls (B), were orally inoculated with *C. rodentium*. On day 8 post-infection, colonic LP cells were isolated and analyzed by flow cytometry for numbers of neutrophils (gated on CD45^{+}CD3^{-}B220^{-}F4/80^{-} Ly6G^{+}); FACS plots (left) and bar graph (right) showing absolute numbers of neutrophils.
(C-H) Eight-week-old CXCR2$^{-/-}$ and CXCR2$^{+/+}$ littermate controls, as well as CD18$^{-/-}$and CD18$^{+/-}$ littermate controls, were orally inoculated with *C. rodentium*. (C) Survival rates and (D) average weight changes at the indicated time points. At day 8 after inoculation, tissues were harvested and assayed for (E) colon length and (F) $\log_{10}$CFU of bacteria in spleen and liver. (G) Histological analysis of H&E-stained colon (scale bars, 100μm) and (H) histopathology scores of colon sections at day 8 post-infection.
Numerical data are means ± SD and are pooled from three independent experiments with two mice per group in each experiment, for a total of 6 mice per group (A,B); are from three independent experiments with five mice per group in each experiment, for a total of 15 mice per group (C); are from two independent experiments with three mice per group in each experiment, for a total of six mice per group (D,E); or are from three independent experiments with two mice per group in each experiment, for a total of six mice per group (F). Images are representative of three independent experiments with two mice per group in each experiment (G,H). *$P < 0.05$; ***$P < 0.001$ (two-tailed Student's *t*-test).

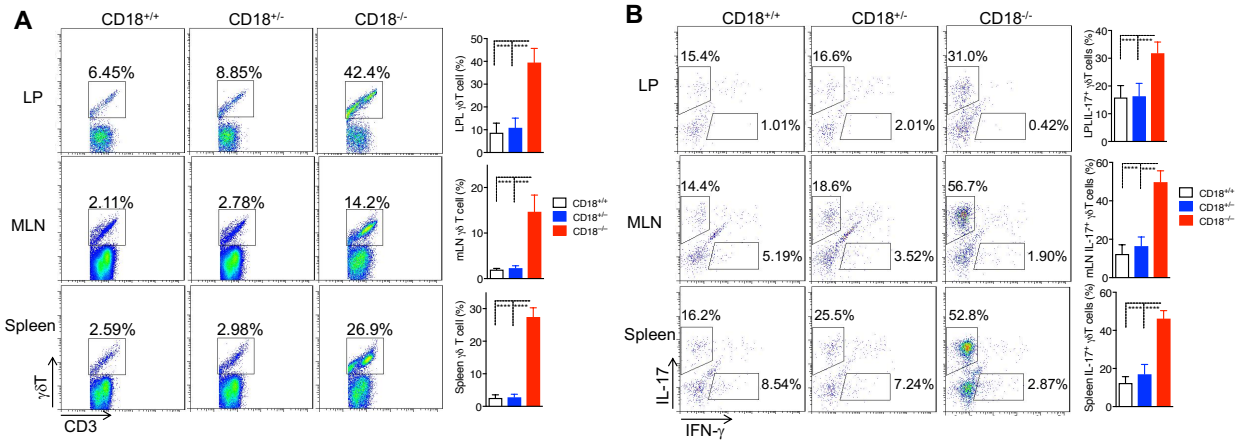

Figure S3: Elevated IL-17$^+$γδ T cells in CD18$^{-/-}$mice at steady state and after *C. rodentium* infection. Related to Figures 2 and 3.

(A,B) Eight-week-old CD18$^{-/-}$, CD18$^{+/-}$ and CD18$^{+/+}$ littermates were orally inoculated with *C. rodentium*. At day 8 post-inoculation, large intestine, MLN, and spleen were harvested and processed for flow cytometric analysis of frequency of γδT cells (gated on CD45$^+$CD3$^+$ cells) (A) and frequency of IL-17A$^+$ cells in γδ T cell population (B). (C,D) Large intestinal lamina propria (LP), MLN, and spleen were harvested from eight-week-old naïve CD18$^{-/-}$, CD18$^{+/-}$ and CD18$^{+/+}$ littermates and processed for flow cytometric analysis of frequency of γδT cells (gated on CD45$^+$CD3$^+$ cells) (C) and frequency of IL-17A$^+$ cells in γδ T cell population (D).

Numerical data are means ± SD. Data are representative of two independent experiments with three mice per group in one experiment and four mice in the other, for a total of seven mice per group (A,D) or are representative of two independent experiments with three or four mice per group, for a total of six to eight mice per group (B,C). ****P < 0.0001 (one-way ANOVA with Tukey's multiple-comparisons test).

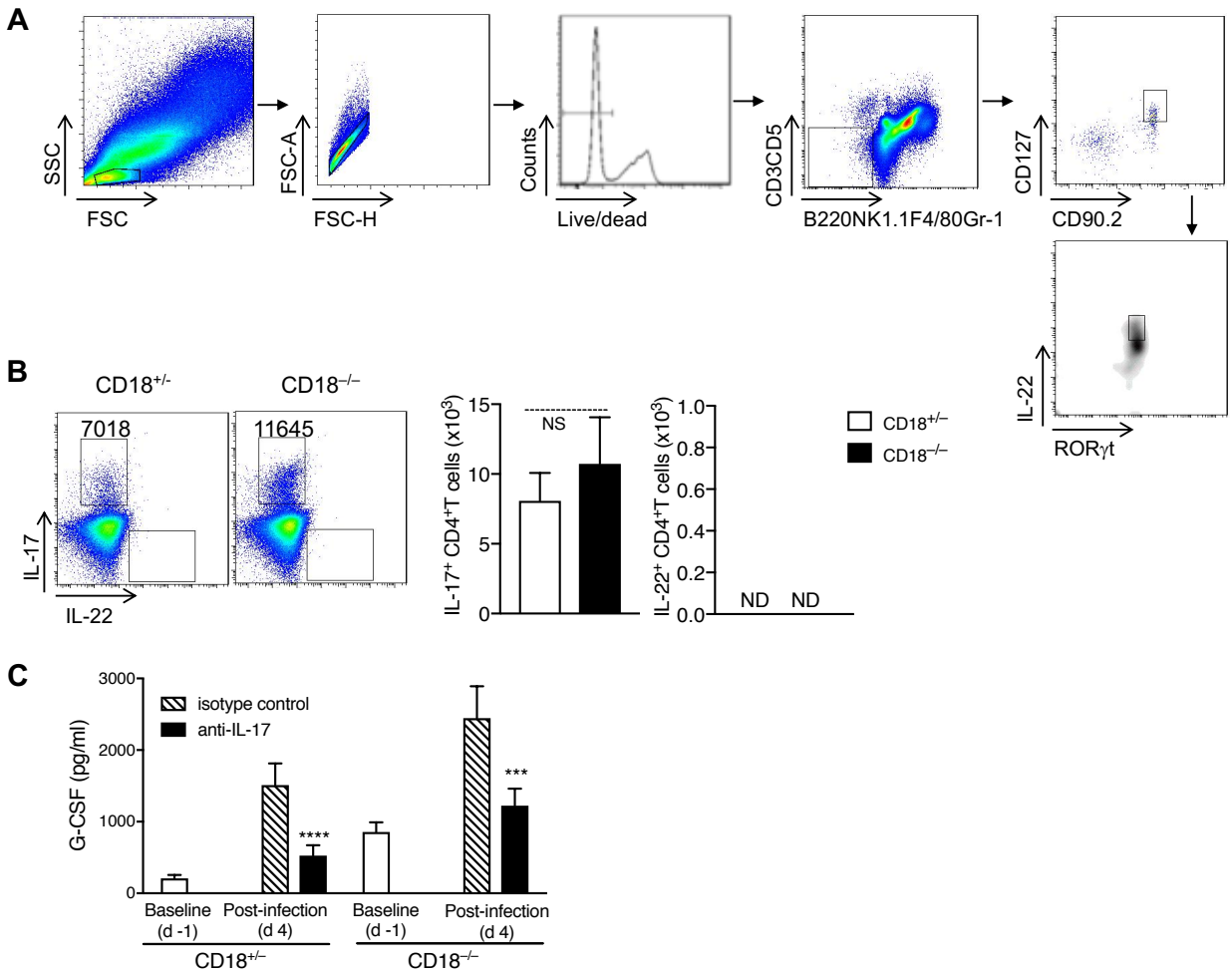

Figure S4: (A) Gating strategy for IL-22⁺ ILC3 detection. Related to Figures 2C, 3B, and 3D.
(B) Comparable numbers of intestinal IL-22⁺CD4⁺T cells in CD18$^{-/-}$ and CD18$^{+/-}$ mice. Related to Figure 2.
Eight-week-old CD18$^{-/-}$ mice and CD18$^{+/-}$ littermate controls were orally inoculated with *C. rodentium*. On day 8 post-infection, LPMCs were isolated from the large intestine and analyzed by flow cytometry; shown are FACS plots (left) and absolute numbers (right) of IL-22⁺CD4⁺T cells (gated on CD3⁺CD4⁺). Data are means ± SD and are pooled from two independent experiments with three mice per group in one experiment and two mice per group in the other experiment, for a total of five mice per group. NS, non-significant (two-tailed Student's *t*-test). ND, not detectable.
(C) Anti-IL-17 causes a decrease in the G-CSF levels in the serum of *C. rodentium*-infected mice. Related to Figure 3. Eight-week-old CD18$^{-/-}$ or CD18$^{+/+}$ littermates were orally inoculated with *C. rodentium*. Anti-IL-17 antibody was given on a daily basis starting 1 day before infection (d -1; baseline). Serum samples were obtained at baseline and day 4 post-infection (d 4) and serum G-CSF levels were measured by ELISA.
Numerical data represent means ± SD and are from two independent experiments each with three mice per group, for a total of six mice per group. ***$P < 0.001$; ****$P < 0.0001$ (two-tailed Student's *t*-test).

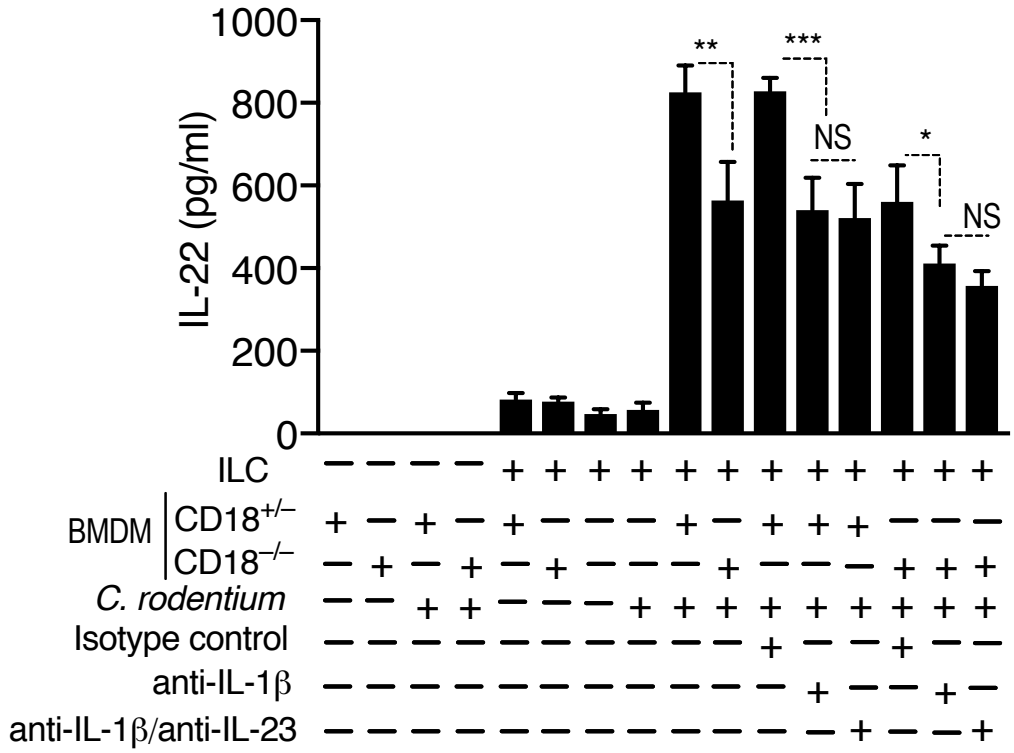

Figure S5: Importance of IL-1β and IL-23 for induction of IL-22 in *C. rodentium*-stimulated BMDM-ILC co-culture system. Related to Figure 5.

ILCs ($CD3^-CD5^-B220^-NK1.1^-F4/80^-Gr\text{-}1^-CD90^+CD127^+CD25^+KLRG1^-$) were sort-purified from the intestine of naïve $CD18^{-/-}$ mice. Sorted ILCs and BMDMs from naïve $CD18^{+/-}$ or $CD18^{-/-}$ mice were cultured alone or co-cultured, with or without stimulation with *C. rodentium* (MOI 20:1) for 24h, in the presence of anti-IL-1β, anti-IL-1β and anti-IL-23, or isotype control (IC). IL-22 was measured by ELISA of culture supernatants. Data are means ± SD and are pooled from two independent experiments performed in duplicate, for a total of four replicates (co-cultures) per group. ** $P < 0.01$ (two-tailed Student's *t*-test). NS, non-significant.

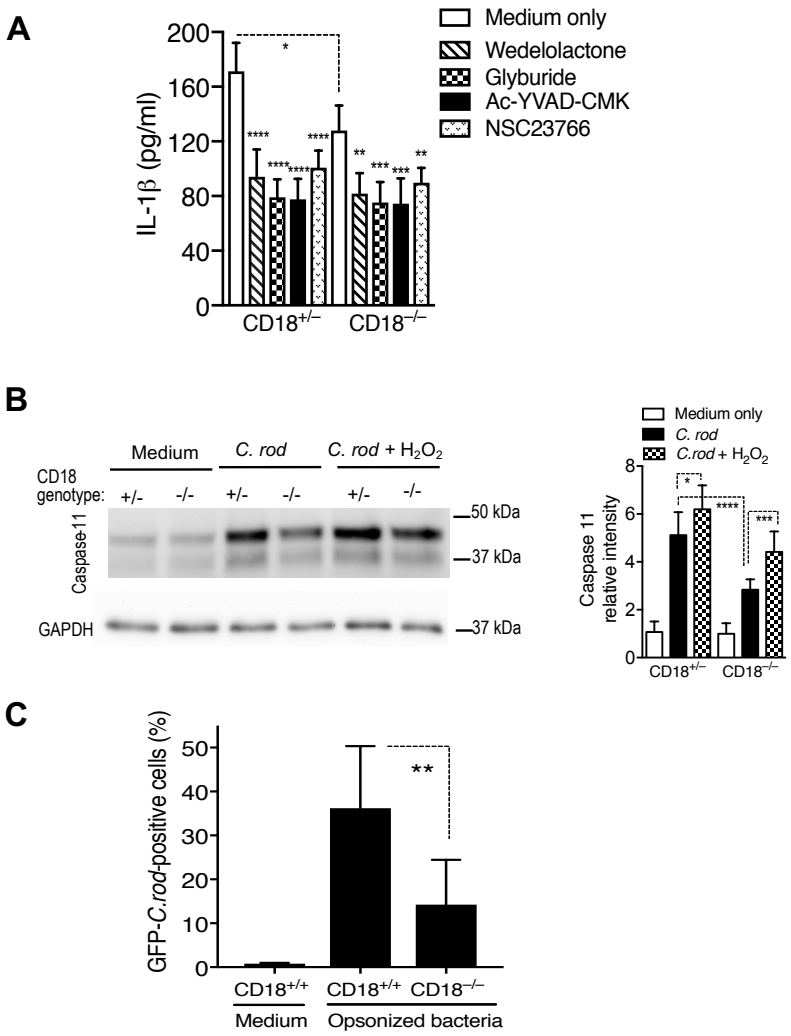

Figure S6: (A) IL-1β production by *C. rodentium*-stimulated intestinal macrophages is regulated by CD18, Rac1, and the inflammasome pathway. Related to Figure 6. $CD18^{+/-}$ and $CD18^{-/-}$ intestinal macrophages ($CD45^{+}Lin^{-}$ (CD3NK1.1B220Ly6G)$F4/80^{+}CX3CR1^{+}$) were pre-treated for 1h with the indicated inhibitors and then infected with *C. rodentium* for 24h. Supernatants were harvested and analyzed for IL-1β release by ELISA. Data are means ± SD and are pooled from two independent experiments performed in duplicate, for a total of four replicates (cell cultures) per group. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$ (one-way ANOVA with Dunnett's multiple comparisons test or two-tailed Student's *t*-test for comparing untreated $CD18^{+/-}$ and $CD18^{-/-}$ macrophages).
(B) H_2O_2 treatment restores caspase-11 expression in $CD18^{-/-}$ BMDMs. Related to Figure 6. $CD18^{+/-}$ and $CD18^{-/-}$ BMDMs were infected with *C. rodentium* at a MOI of 20:1 and after 6h were treated with 50 μM of H_2O_2 as a ROS source. After 2.5h, cell lysates were harvested and analyzed by immunoblotting with anti-mouse-caspase-11 antibody (left) and densitometry (right). Numerical data are means ± SD and are pooled from four independent experiments with two replicates per group for a total of eight replicates per group. $*P < 0.05$; $***P < 0.001$; $****P < 0.0001$ (two-tailed Student's *t*-test).
(C) Phagocytosis of *C. rodentium* by $CD18^{-/-}$ and $CD18^{+/+}$ BMDMs. Related to Figure 6. BMDMs were exposed to serum-opsonized GFP-*C. rodentium* (MOI 20:1) at 37°C for 30 mins to allow uptake of bacteria by the BMDMs. After BMDMs were treated with gentamycin to kill non-phagocytosed bacteria, they were stained with anti-mouse F4/80 antibody and phagocytic activity (% cells positive for GFP-*C. rodentium* [GFP-*C. rod*]) was assessed by flow cytometric analysis. Non-infected BMDMs were used as negative controls. Data are means ± SD and are pooled from three independent experiments for a total of seven to nine biological replicates per group. $**P < 0.01$ (two-tailed Student's *t*-test).