

#### Supplementary Figure 1. RegIII $\beta/\gamma$ induction is regulated by recruitment of CCR2<sup>+</sup> monocytes.

Colonic epithelial cells were isolated from C. rodentium infected (8 dpi) and uninfected  $Ccr2^{+/+}$  and  $Ccr2^{-/-}$  mice. Expression of Reg3 $\beta$  and Reg3 $\gamma$  mRNA was analyzed by qPCR. Expression of target genes was normalized to Gapdh. Data are given as mean ± s.d. (n=3). \*\*\*p<0.001 by Student's t test.

#### Small intestine



# Supplementary Figure 2. Analysis of subsets of mononuclear phagocytes in the small intestine.

Total LP cells were isolated from the small intestine of uninfected CD115<sup>GFP</sup> reporter mice. CD45<sup>+</sup>MHC-II<sup>+</sup> mononuclear phagocytes from CD115<sup>GFP</sup> mice were further analyzed by flow cytometry.



### Supplementary Figure 3. Selective depletion of MP1 subset in CCR2<sup>DTR</sup> mice after single and multiple injections of DT.

Uninfected CCR2<sup>DTR</sup> mice were injected with diphtheria toxin (DT; 10 ng g<sup>-1</sup> body weight) 4 times in 2 day intervals. Colonic LP cells were isolated pre-injection, on day 1 post injection (single injection), and on day 7 (post 4 injections), and colonic macrophage and DC subsets were analyzed. Results are representative of at least 2 individual mice.



## Supplementary Figure 4. Transferred monocytes do not migrate to extra-intestinal lymphoid tissues.

 $Ccr^{2-/-}$  mice were infected with *C. rodentium*. On day 4 post-infection, CD11b<sup>+</sup>Ly6C<sup>hi</sup> bonemarrow monocytes were isolated from CD115-GFP mice and transferred into  $Ccr^{2-/-}$  recipient mice as described in Figure 2d. The presence of GFP<sup>+</sup> cells in spleen and MLN (day 10 post transfer) was assessed. Results are representative of 2 independent experiments.



#### Supplementary Figure 5. ROR $\gamma$ t<sup>+</sup> ILC3s produce robust amounts of IL-22 and IFN- $\gamma$ in response to IL-23 and IL-1 $\beta$ .

(a) LPMCs were isolated from ROR $\gamma$ t<sup>GFP</sup> reporter mice and CD3<sup>+</sup>RORgt<sup>+</sup> (Th17) and CD3<sup>-</sup>ROR $\gamma$ t<sup>+</sup> (ILC) cells were further stained for NKp46. (b) Purified intestinal CD3<sup>+</sup>CD4<sup>+</sup>ROR $\gamma$ t<sup>+</sup> Th17 cells and CD3<sup>-</sup>RORgt<sup>+</sup> ILCs (1 x 10<sup>6</sup> cells ml<sup>-1</sup>) were stimulated with recombinant IL-23 (20 ng ml<sup>-1</sup>), IL-1 $\beta$  (20 ng ml<sup>-1</sup>), or IL-23 plus IL-1 $\beta$  for 24 hrs. Cytokines in culture supernatants were analyzed by ELISA. Data are given as mean ± s.d. (n=4). \*p<0.05; \*\*p<0.01;\*\*\*p<0.001; N.S., not significant by Bonfferoni test.



## Supplementary Figure 6. $CCR2^{WT}/II1\beta^{-/-}$ mixed chimeric mice produce detectable but impaired level of IL-1 $\beta$ compared to non-chimeric control mice.

(a) Peripheral blood were isolated from WT, CCR2<sup>WT</sup>/*II1β*-/- (IL-1β<sup>WT</sup>) and CCR2<sup>DTR</sup>/*II1β*-/- (IL-1β<sup>ΔMo/MP</sup>) chimeric mice. Expression of CCR2-CFP was analyzed on CD45<sup>+</sup>CD11b <sup>+</sup>Ly6C<sup>hi</sup> monocytes. (b) WT, *Ccr2<sup>WT</sup>/II1β*-/- (IL-1β<sup>WT</sup>) and *Ccr2<sup>DTR</sup>/II1β*-/- (IL-1β<sup>ΔMo/MP</sup>) mice were infected with *C. rodentium*, and CCR2<sup>+</sup> monocytes and monocyte-derived MP1 cells were depleted by DT injection (10 ng/g body weight) on days 5 and 7 post-infection. On day 8 post infection, colonic LPMCs were isolated. 2 x 10<sup>6</sup> cells ml<sup>-1</sup> LPMCs were cultured in the presence or absence of heat-killed *C. rodentium* (MOI=10) for 24 hrs. Cytokines in culture supernatants were analyzed by ELISA. Data are given as mean ± s.d. (n=3).



### Supplementary Figure 7. Analysis of CCR2<sup>DTR</sup>/*II1b*-/- mixed chimeric mice colonic macrophages.

 $CCR2^{WT}/II1b^{-/-}$  (IL-1 $\beta^{WT}$ ) or  $CCR2^{DTR}/II1\beta^{-/-}$  (IL-1 $\beta^{\Delta Mo/MP}$ ) mice were infected with *C. rodentium*, and  $CCR2^+$  monocytes and monocyte-derived MP1 cells were depleted by DT injection (10 ng g<sup>-1</sup> body weight) on days 5 and 7 post-infection. On day 8 post infection, colonic LPMCs were isolated, and colonic macrophage and DC subsets were analyzed. The percentage of colonic macrophages and DCs were comparable between IL-1 $\beta^{WT}$  and IL-1 $\beta^{\Delta Mo/MP}$  mice.



#### Supplementary Figure 8. Inflammasome activation in MP1 cells.

MP1 and DC1 subsets were isolated from uninfected and *C. rodentium*-infected CD115-GFP animals. Cytokine mRNA expression was analyzed by qPCR. Data are given as mean  $\pm$  s.d. (n=5-7).



#### Supplementary Figure 9. Ler-dependent virulence factors in *C.* rodentium is required for caspase-11-mediated IL-1 $\beta$ induction.

(a) BMDMs from WT and *NIrp3*<sup>-/-</sup> mice were stimulated with WT *C. rodentium* or isogenic mutants (MOI=25) 1 hr without antibiotics and then cultured additional 17 hrs in the presence of 100  $\mu$ g ml<sup>-1</sup> gentamicin. Cytokines in the culture supernatant were analyzed by ELISA. Data are given as mean ± s.d. (n=3). Results are representative of 3 independent experiments. \*\*\* p<0.001 by Dunnett's test (compared to WT). (b) WT and  $\Delta$ *ler* mutant *C. rodentium* were cultured in DMEM in cell culture incubator for 16 hrs. Bacterial culture supernatants were harvested and live bacteria were removed by passing 0.45  $\mu$ m syringe filter. One third volume of sterile bacterial culture sups were added in BMDMs from WT and *Casp11*<sup>-/-</sup> mice, and incubated for 18 hrs. Cytokines in the culture supernatant were analyzed by ELISA. mean ± s.d. (n=3). Results are representative of 3 independent experiments. \*\*p<0.001; \*\*\*p<0.001; N.S., not significant by Bonfferoni test.



### Supplementary Figure 10. $CX_3CR1$ expression in MP1 and CD103<sup>+</sup> DC subsets.

MP1 and DC1 subsets were isolated from uninfected and *C.* rodentium-infected CD115<sup>gfp</sup> animals. Expression of CX<sub>3</sub>CR1 was analyzed by qPCR. Data are given as mean  $\pm$  s.d. (n=5-7). \*p<0.05; by Mann-Whitney *U* test.



### Supplementary Figure 11. MyD88 signaling is required for IL-1 $\beta$ production and ILC activation by MP1.

(a) WT and *Myd88<sup>-/-</sup>* mice were infected with *C. rodentium*. On day 8 post infection, colonic LPMCs were isolated. 2 x 10<sup>6</sup> cells ml<sup>-1</sup> LPMCs were cultured in the presence or absence of heat-killed *C. rodentium* (MOI=10) for 24 hrs. Cytokines in culture supernatants were analyzed by ELISA. Data are given as mean ± s.d. (n=3). Results are representative of 3 independent experiments. \*\*\* p<0.001 by Dunn's test. (b) CD3<sup>-</sup> ROR<sub>Y</sub>t<sup>+</sup> ILCs from uninfected ROR<sub>Y</sub>t<sup>GFP/+</sup> reporter mice and MP1 cells from *C. rodentium*-infected (day 8) WT and *Myd88<sup>-/-</sup>* mice were isolated. ILCs and MP1 cells (1 x 10<sup>6</sup> cells ml<sup>-1</sup>) were cultured alone or co-cultured with heat-killed *C. rodentium* (MOI=10) for 24 hrs. Data are given as mean ± s.d. of 3 independent experiment.



Supplementary Figure 12. The original images for the main figures. (a) Fig. 5e; Caspase-11. (b) Fig. 5e;  $\beta$ -actin

Genes	Forward	Reverse
IL-1β	5'-CAACCAACAAGTGATATTCTCCATG-3'	5'-GATCCACACTCTCCAGCTGCA-3'
IL-23p19	5'-TCCCTACTAGGACTCAGCCAAC-3'	5'-GCTGCCACTGCTGACTAGAA-3'
IL-12p35	5'-CCAGGTGTCTTAGCCAGTCC-3'	5'-GCAGTGCAGGAATAATGTTTCA-3'
IL-12/23p40	5'-CCTGAAGTGTGAAGCACCAAATTAC-3'	5'-GAACTTCAAGTCCATGTTTCTTTGC-3'
IL-6	5'-GAGGATACCACTCCCAACAGACC-3'	5'-AAGTGCATCATCGTTGTTCATACA-3'
TNF	5'-GCCTCCCTCTCATCAGTTCT-3'	5'-CACTTGGTGGTTTGCTACGA-3'
IL-10	5'-CCCTTTGCTATGGTGTCCTT-3'	5'-TGGTTTCTCTTCCCAAGACC-3'
TGF-b1	5'-TGACGTCACTGGAGTTGTACGG-3'	5'-GGTTCATGTCATGGATGGTGC-3'
IL-22	5'-TTTCCTGACCAAACTCAGCA-3'	5'-TCTGGATGTTCTGGTCGTCA-3'
NLRP3	5'-ATGGTATGCCAGGAGGACAG-3'	5'-ATGCTCCTTGACCAGTTGGA-3'
NLRC4	5'-AGAAGGGCTCAGCGGCCTGCAA-3'	5'-TTCACCCAGGGGGTAGAAGTTCA-3'
Caspase-1	5'-GCCCACTGCTGATAGGGTGA-3'	5'-CCCGGGAAGAGGTAGAAACG-3'
Caspase-11	5'-TGTCATCTCTTTGATATATTCCTGAAG-3'	5'-CAAGGTTGCCCGATCAAT-3'
RegIIIβ	5'-CTCTCCTGCCTGATGCTCTT-3'	5'- GTAGGAGCCATAAGCCTGGG-3'
RegIIIγ	5'- TCAGGTGCAAGGTGAAGTTG-3'	5'-GGCCACTGTTACCACTGCTT-3',
CX <sub>3</sub> CR1	5'-AAGTTCCCTTCCCATCTGCT-3'	5'-CAAAATTCTCTAGATCCAGTTCAGG-3'
β-actin	5'-AAGTGTGACGTTGACATCCG-3'	5'-GATCCACATCTGCTGGAAGG-3'

#### Supplementary Table 1. Primer sequences used in this study.