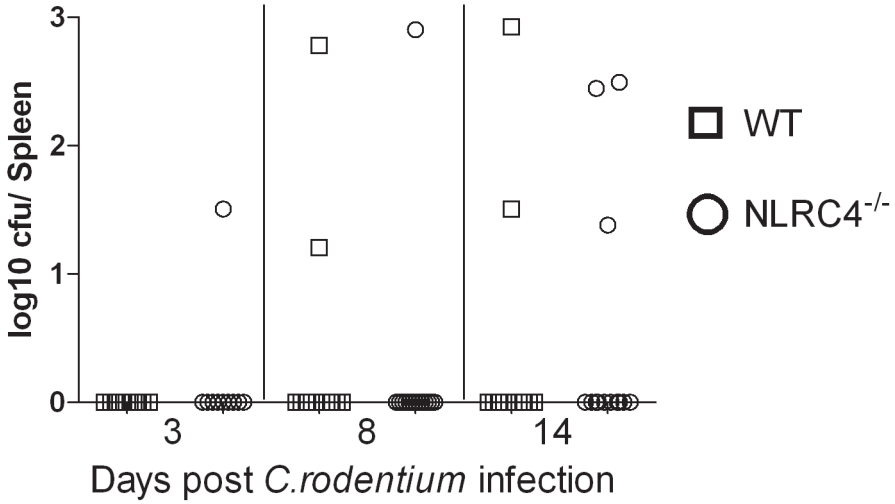

Supplementary figure 1

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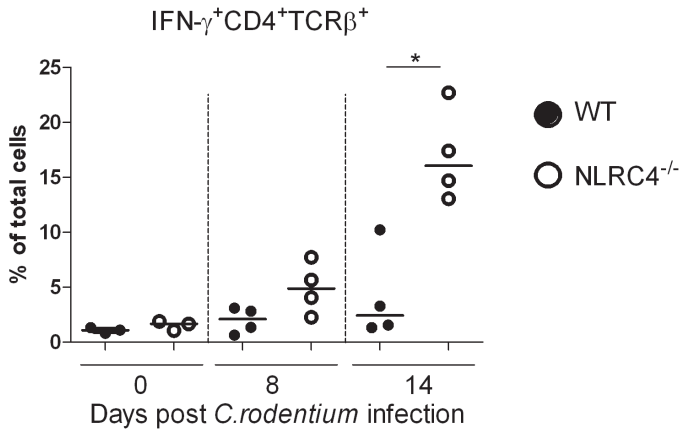

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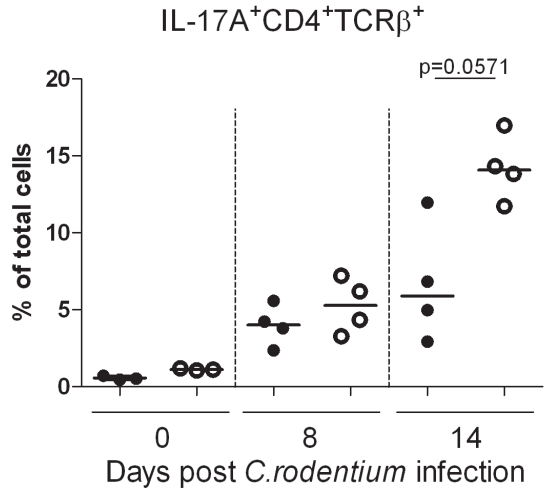
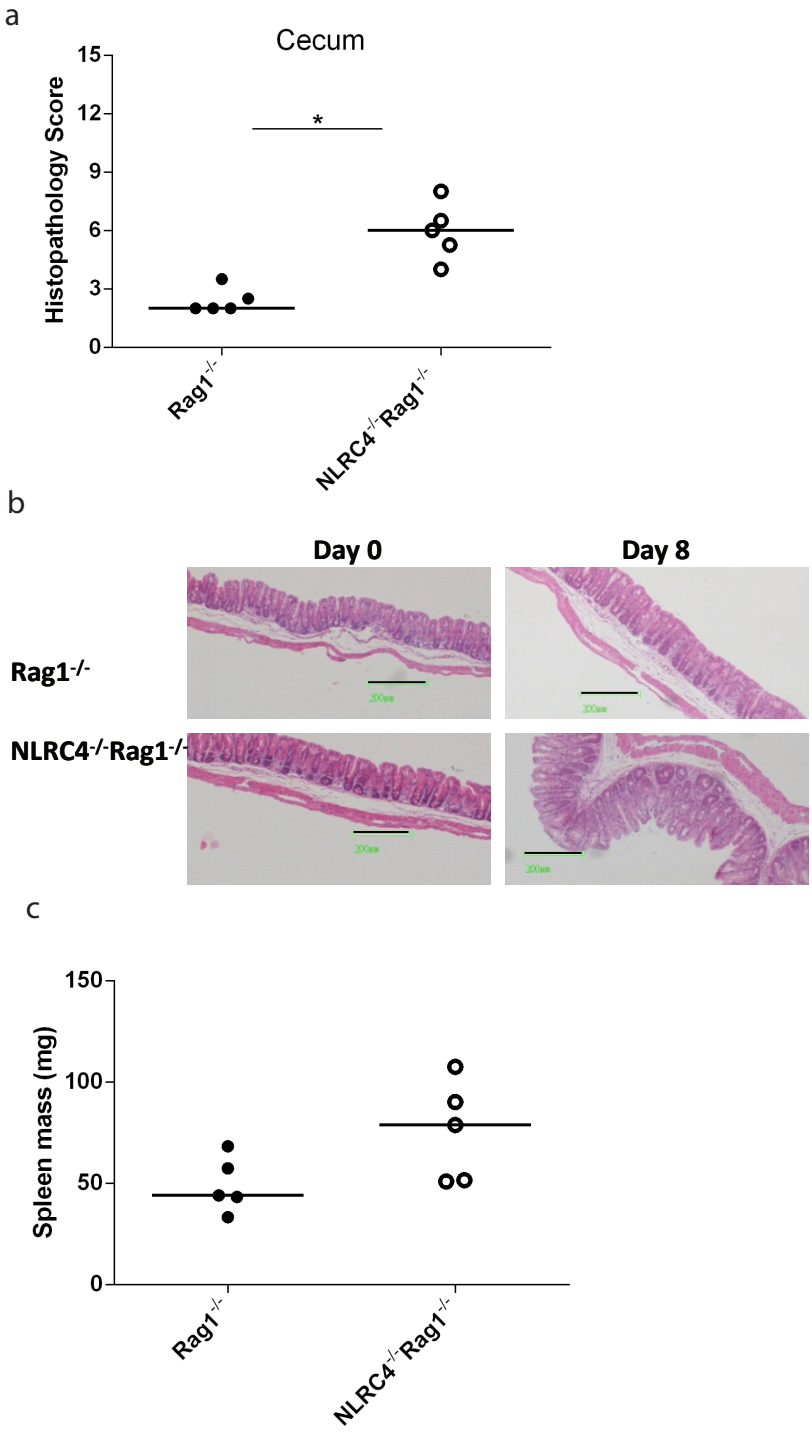


Supplementary figure 2



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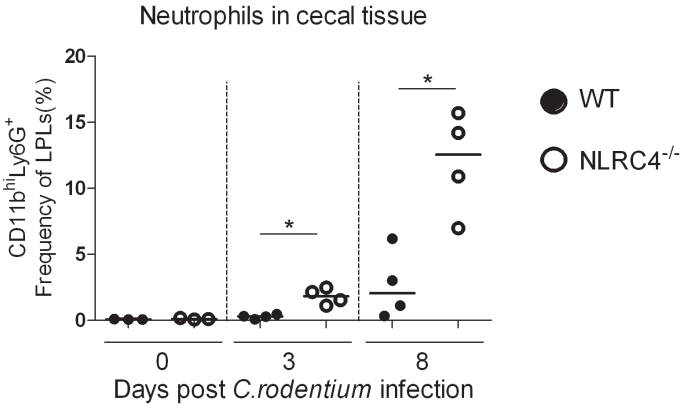
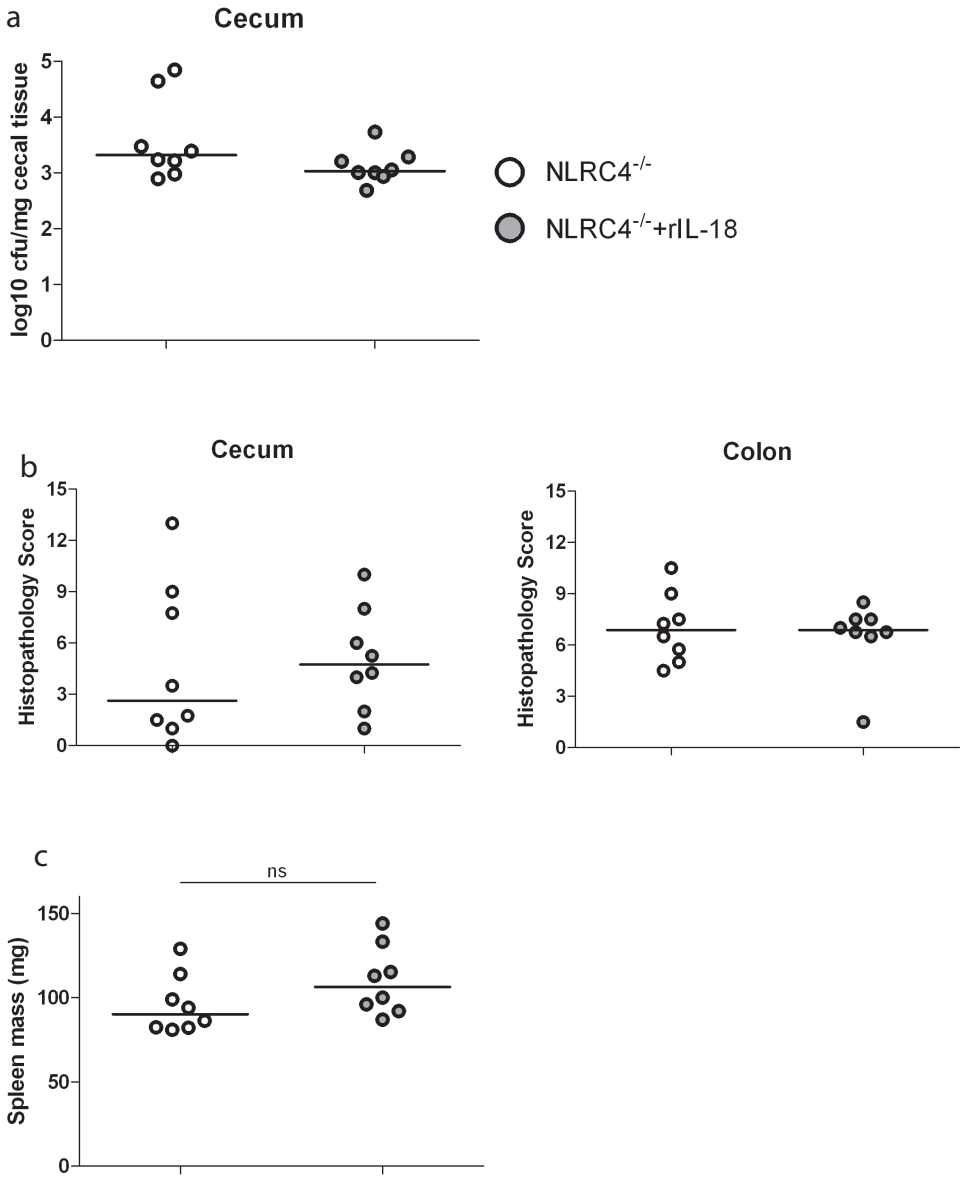
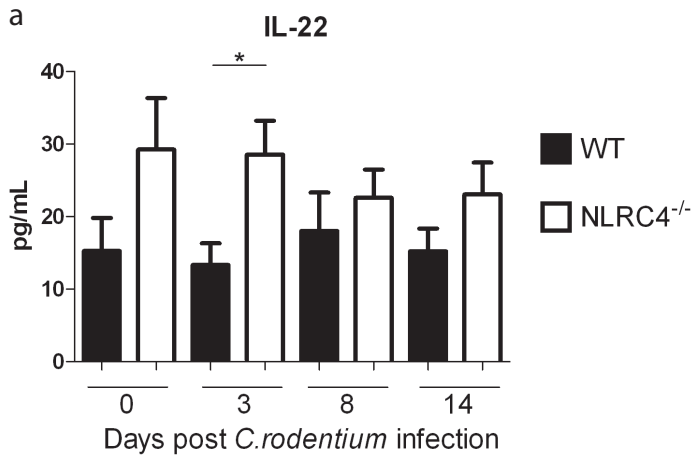
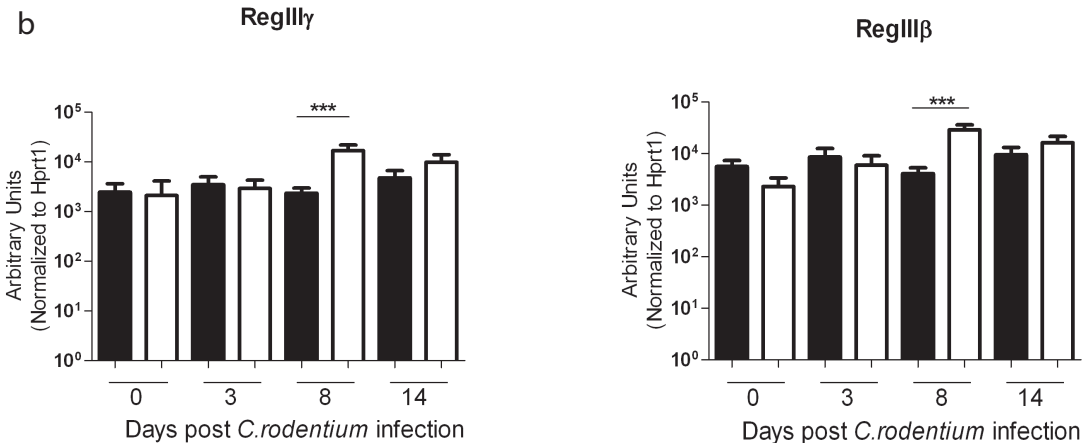


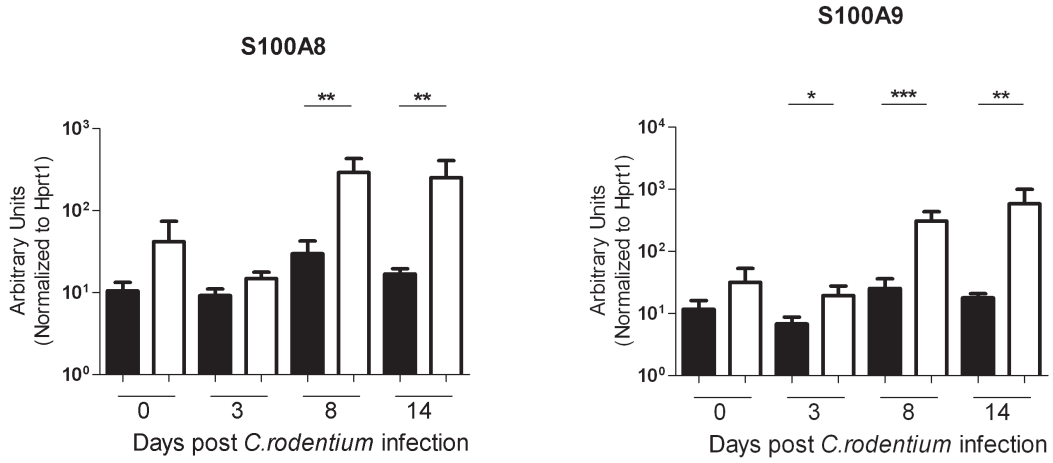
Supplementary figure 4



Supplementary figure 5

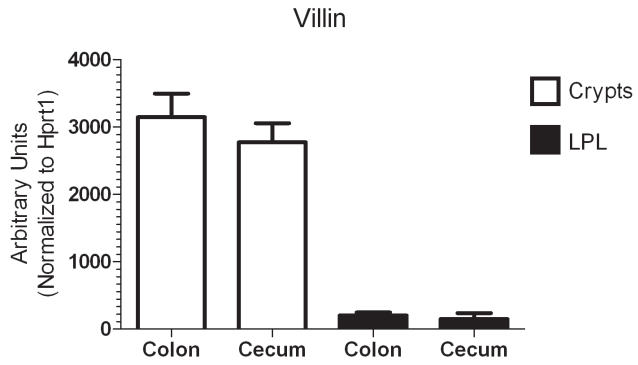






Supplementary figure 6

a



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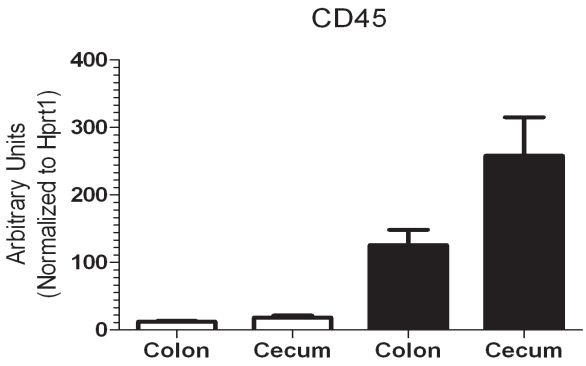

Supplementary figure 7

Supplementary figure 1: *Nlrc4*$^{-/-}$ mice do not exhibit increased systemic translocation of *C. rodentium.* C57BL/6 wild-type and *Nlrc4*$^{-/-}$ mice were infected with *C. rodentium*. Spleens were obtained after 3, 8 and 14 days of infection, homogenized and cultured on selective media. Colony forming units were determined. Data are pooled from a minimum of 2 independent experiments (n= 10-14 animals per group) per time point.

Supplementary figure 2: *Nlrc4*$^{-/-}$ mice develop elevated Th1 and Th17 responses during *C. rodentium* infection. C57BL/6 wild-type and *Nlrc4*$^{-/-}$ mice were infected with *C. rodentium*. Cecal tissue was obtained after 0, 8 and 14 days of infection. Lamina propria leukocytes were isolated from cecal tissue and restimulated with PMA (0.1µg/ml), ionomycin (1 µg/ml) and Brefeldin A (10 µg/ml). Cells were stained for Live/Dead, CD4 and TCRβ, permeabilized and stained for IL-17A and IFN-γ. (A) Th1 cells and (B) Th17 cells. Frequencies are expressed as percentages of total cells. Data are from 1 experiment (n=3-4 animals per group). Horizontal lines are group median values and the non-parametric Mann-Whitney test was used for statistical analysis, * $p<0.05$.

Supplementary figure 3: *Nlrc4*$^{-/-}$*Rag1*$^{-/-}$ mice develop exacerbated *C.rodentium* mediated disease. *Rag1*$^{-/-}$ and *Nlrc4*$^{-/-}$*Rag1*$^{-/-}$ mice were infected with *C. rodentium* and sacrificed after 8 days of infection. Typhlitis scores and representative micrographs of cecum are shown in A and B respectively. Black bars are equivalent to 200µm. Spleen weights are depicted in C and results are from 1 experiment, n=5 per genotype. The non-parametric Mann-Whitney test was used for statistical analysis, * $p<0.05$.

Supplementary figure 4: NLRC4 deficiency leads to increased neutrophil recruitment to the lamina propria. C57BL/6 wild-type and *Nlrc4*$^{-/-}$ mice were infected with *C. rodentium*. Lamina propria leukocytes (LPLs) were isolated from cecal tissue after 0, 3, and 8 days of infection. Cells were stained for surface markers CD45, CD11b and Ly6G. Each symbol represents an individual animal and horizontal bars represent group median values. Data are from 1 experiment, n=3-4 animals/group. The non-parametric Mann-Whitney test was used for statistical analysis, * $p<0.05$.

Supplementary figure 5: Administration of recombinant IL-18 does not reduce susceptibility in *Nlrc4*$^{-/-}$ mice. Mice were infected with *C. rodentium* and sacrificed after 8 days of infection. *Nlrc4*$^{-/-}$ (n=8) mice were IP injected with recombinant IL-18 (rIL-18, 0.5µg) daily starting 2 days prior to infection and for 6 days post-infection. As a control group *Nlrc4*$^{-/-}$ mice (n=8) were infected. Colonization levels in cecal tissue, pathology scores and spleen weights are shown in (A), (B) and (C) respectively. Data are pooled from 2 independent experiments.

Supplementary figure 6: NLRC4 deficiency does not result in reduced IL-22 mediated responses. C57BL/6 wild-type and *Nlrc4*$^{-/-}$ mice were infected with *C. rodentium*. (A) Cecal explant culture supernatants were assayed for the protective cytokine IL-22. (n=7-8 per group, pooled from 2 independent experiments.) (B) Cecal mRNA levels for IL-22 induced anti-microbial peptides (AMPs). RNA was extracted from snap-frozen cecal tissue. Expression of RegIIIγ, RegIIIβ, S100A8 and S100A9 was assessed by Quantitative Real-Time PCR in uninfected WT and *Nlrc4*$^{-/-}$ mice and after 3, 8 and 14 days of *C. rodentium* infection. Bars represent group means ± SEM (n=13-16 per group for Reg3γ at d0 and d3 pooled from 3 independent experiments. For the remaining time points and AMPs n = 7-10 per group pooled from 2 independent experiments.) The non-parametric Mann-Whitney test was used for statistical analysis. * $p<0.05$, **$P<0.01$, ***$p<0.001$.

Supplementary figure 7: Expression levels of villin and CD45 in crypt and LPL isolates. Expression levels of villin1 and CD45 in cecum and colon tissue fractions enriched for intestinal epithelial cells (Crypts) and lamina propria leukocytes (LPLs) respectively were measured by qPCR. Data represent arbitrary units of villin1 and CD45+ expression, normalised to expression of Hprt. Bar graphs represent means +/- SEM. Samples were isolated from 4-6 individual mice per group.