Figure S1. Kamada N. et al.

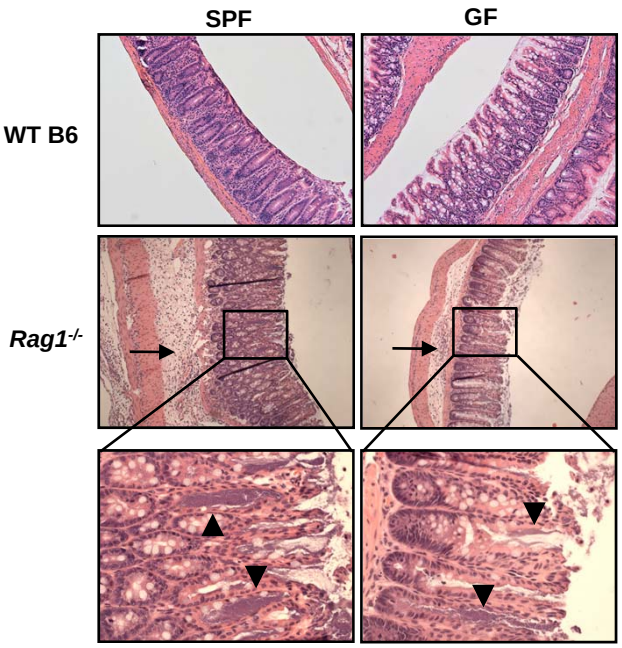

Fig. S1, related to Fig. 1. Ler-regulated virulence factors are required for pathogen association near the epithelial surface.
Histological analysis of the distal colon of SPF and GF WT or *Rag1*$^{-/-}$ mice infected with *C. rodentium* on day 14 post-infection. Arrows denote marked submucosal edema and infiltration of acute inflammatory cells (magnification 250X). Arrowheads in high power fields (magnification 1000X) show bacterial colonies that had invaded the mucosa.

Figure S2. Kamada N. et al.

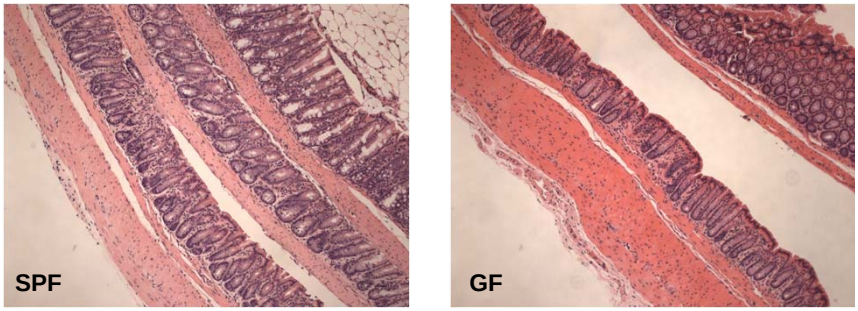

Fig. S2, related to Fig. 2. . Avirulent *C. rodentium* does not induce intestinal inflammation in immunodeficient mice
Histological analysis of the distal colon of SPF and GF *Rag1*$^{-/-}$ mice infected with *ler* mutant (*⊿ler*) *C. rodentium* on day 21 post-infection (magnification 250X).

Figure S3. Kamada N. et al.

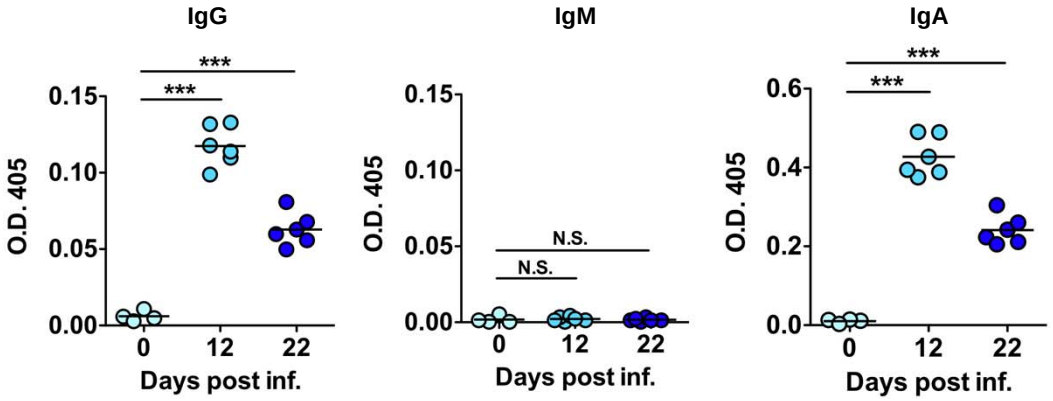

Fig. S3, related to Fig. 3. Pathogen-specific antibodies are found in the intestinal lumen of *C. rodentium*-infected mice.
GF WT mice were infected orally with $1x10^9$ cfu of *C. rodentium*. Production of total IgG, IgM and IgA against *C. rodentium* in the luminal content of GF mice before (d0) and after (day 12 and 22) oral infection with *C. rodentium* was analyzed. Dots represent individual mice. ***, $p<0.001$; N.S., not significant by Dunnett' test.

Figure S4. Kamada N. et al.

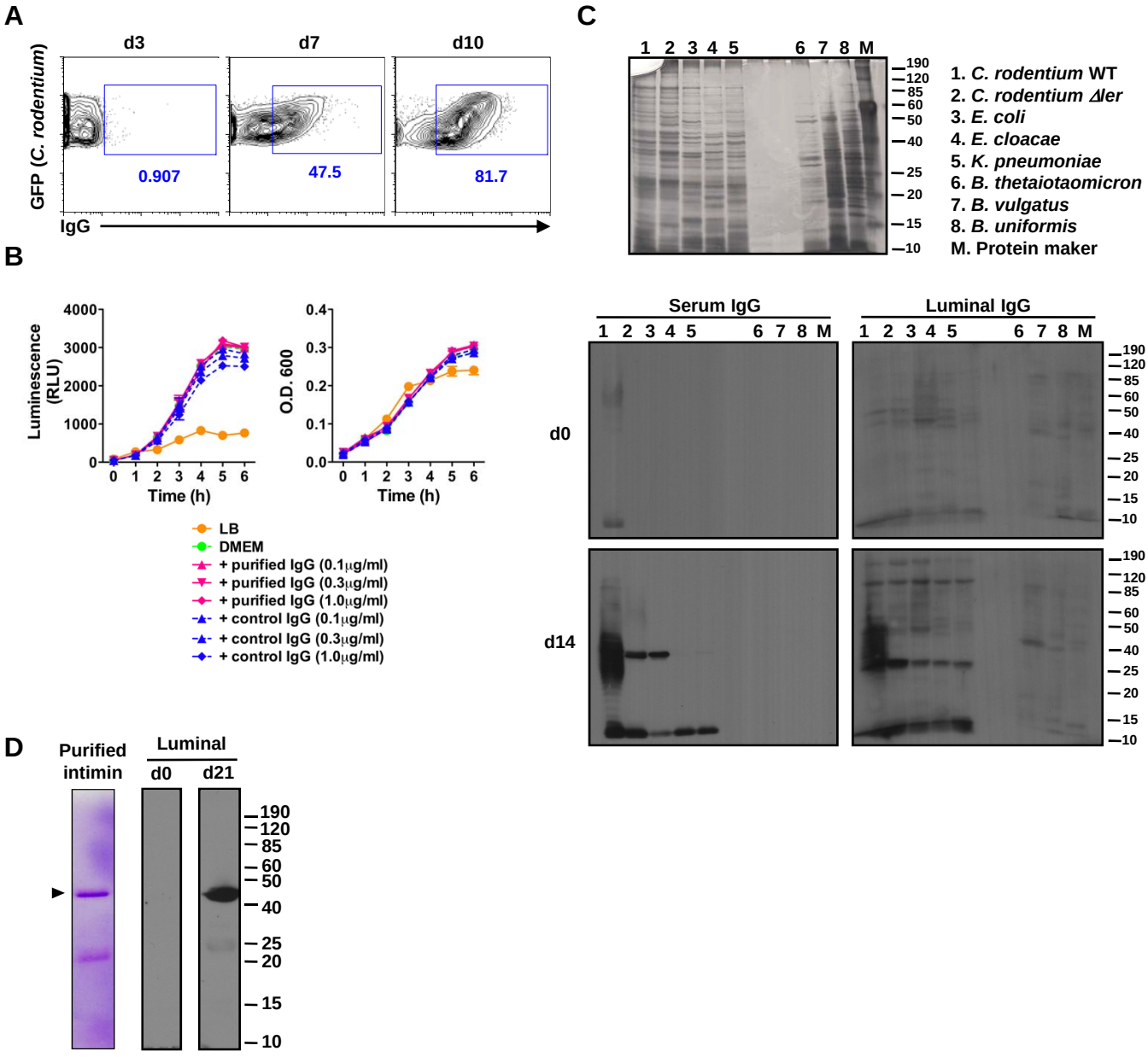

Fig. S4, related to Fig. 4. *C. rodentium* infection induces Ler-regulated virulence factor-specific IgG. **A,** GF WT mice were infected with GFP-expressing *C. rodentium.* Cecal bacteria were harvested at indicated days post-infection and binding of IgG was analyzed by flow cytometry. Results are representative of 3 experiments. **B,** Purified IgG from the sera of *C. rodentium*-infected mice (day 42 post-infection), or control IgG was added to the reporter *ler-lux C. rodentium* strain in DMEM medium and expression of *ler* overtime was determined by luminescence (left panel). RLU, relative light unit. Bacteria grown in DMEM (positive control) or LB medium (negative control) are shown for comparison. Bacterial growth in DMEM or LB medium was assessed in parallel (right panel). Data are representative of 2 independent experiments in triplicate cultures (mean ± SD). **C**, Bacterial lysates of WT and Δler mutant *C. rodentium* were loaded with SDS-PAGE. Serum or luminal content were obtained from naïve (d0) and *C. rodentium*-infected (d21) SPF mice, and used as primary antibodies. *C. rodentium*-specific IgG was detected by anti-mouse IgG secondary Ab. **D**, Purified intimin protein was loaded with SDS-PAGE (left), and blotted with luminal content obtained from naïve (d0) and *C. rodentium*-infected (d21) GF mice. *C. rodentium*-specific IgG was detected by anti-mouse IgG secondary Ab. Results are representative of 2 independent experiments.

Figure S5. Kamada N. et al.

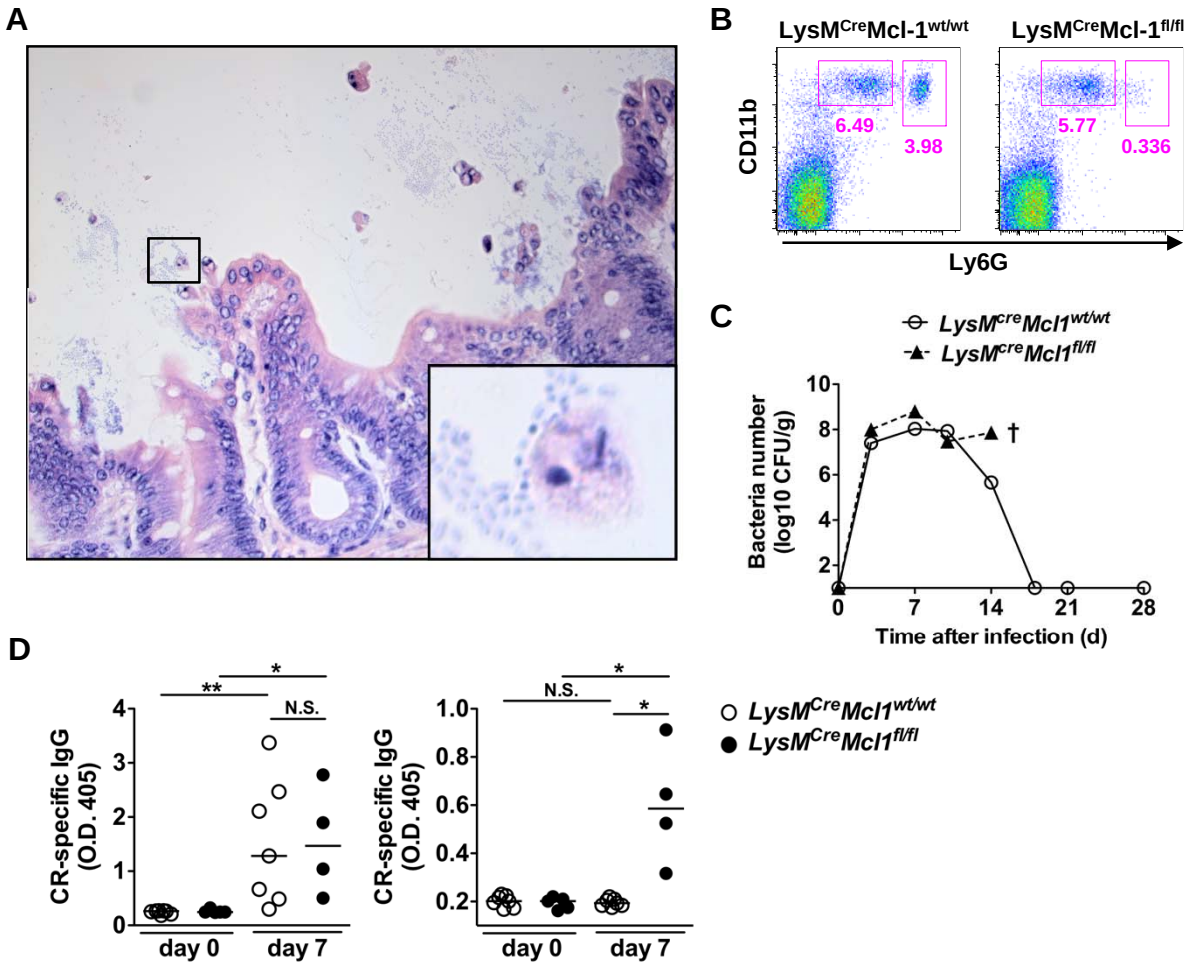

Fig. S5, related to Fig. 6. Impaired eradication of *C. rodentium* in *LysM^{Cre}Mcl1$^{fl/fl}$* chimeric mice.

A, GF WT mice were infected with *C. rodentium*. At day 7 post infection, cecal tissues were harvested and fixed with Carnoy's to preserve mucus layer. Luminal neutrophils were assessed by H&E staining. Inset denotes intraluminal neutrophil surrounded by *C. rodentium*. **B**, Confirmation of neutrophils depletion in *LysM^{Cre}Mcl1$^{fl/fl}$* chimeric mice. Cells were isolated from the peripheral blood of *LysM^{Cre}Mcl1$^{wt/wt}$* or *LysM^{Cre}Mcl1$^{fl/fl}$* chimeric mice. Isolated cells were stained with CD45, CD11b, Ly6G and DAPI, and analyzed by flow cytometry. Percentage of neutrophils ($CD45^+CD11b^+Ly6G^{hi}$) and monocytes ($CD45^+CD11b^+Ly6G^{lo}$) is indicated. Data are representative of 4 individual mice. **C**, *LysM^{Cre}Mcl1$^{wt/wt}$* or *LysM^{Cre}Mcl1$^{fl/fl}$* chimeric mice (n= 12; *Mcl1$^{wt/wt}$*, n=9; *Mcl1$^{fl/fl}$*) were infected orally with $1x10^9$ cfu of *C. rodentium* and pathogen load in feces was determined over the indicated time. Data points are given as median. † denotes bacterial loads could not be determined beyond this time due to mouse lethality. **D,** *LysM^{Cre}Mcl1$^{wt/wt}$* or *LysM^{Cre}Mcl1$^{fl/fl}$* chimeric mice were infected orally with $1x10^9$ cfu of *C. rodentium*. Production of *C. rodentium*-specific IgG in the serum and the luminal content of GF mice was analyzed before (d0) and after (day 7) infection. Dots represent individual mice. *, $p<0.05$; **, $p<0.01$; N.S., not significant by Dunn's test.