

Supplementary:

Method S1: Sample preparation for flow cytometric analysis

Half of each spleen was cut into small pieces, digested in 2.4 mL of 1x Buffer S (Spleen Dissociation Kit, Miltenyi Biotech) prepared in sterile water (Sigma-Aldrich) with the addition of 50 μ L of enzyme D (Spleen Dissociation Kit, Miltenyi Biotech) and 15 μ L of enzyme A (Spleen Dissociation Kit, Miltenyi Biotech), incubated at 37 ° C for 15 min with rotation, and finally homogenised into a single-cell suspension through a 40 μ m nylon mesh (Corning). The single-cell suspension was centrifuged at 300 rcf for 10 min (Eppendorf 5810R, A-4-62) at 4 ° C and resuspended in 5 mL of 1x RBC lysis buffer (eBioscience) for 8 min and washed twice in sterile supplemented HBSS buffer (Gibco) containing 2 % foetal bovine serum (FBS) (ThermoFisher) and 2 mM EDTA (ThermoFisher).

Live spleen cells were counted by Trypan blue (Gibco) exclusion using a haemocytometer. Fc receptors (FcR) were blocked with FcR blocking agent (Miltenyi Biotech) in 1 % heat-inactivated mouse serum (Dako) at 4 ° C for 5 min. The cells were then stained with either isotype control or specific antibodies listed in Table S1 (all purchased from Miltenyi Biotech, used at the concentration suggested by the manufacturer) for 15 min at 4 ° C. The stained cells were washed by resuspending in supplemented HBSS buffer and centrifuged at 300 rcf for 10 min at 4 ° C. The stained cells were then resuspended in 1 mL of supplemented HBSS buffer and filtered through a 30 μ m filter (Celltrics).

Compensation controls were prepared using compensation beads (anti-REA and anti-mouse Igk, Miltenyi Biotech), by incubating antibody conjugated to fluorophore with the beads in 100 μ L of the supplemented HBSS buffer at room temperature for 10 min. After the incubation, the beads were resuspended to 1 mL with supplemented HBSS buffer. The GFP compensation control was prepared by infecting RAW264.7 cells at a multiplicity of infection (MOI) of 10 using the *S. Typhimurium* SL1344 *sifB::gfp* strain for 1 h.

Samples were analysed using a Becton Dickinson AriaIII using DIVA v8.1 and FloJo V10 software.

Method S2: Testing the efficacy of clodronate liposomes

We tested whether clodronate or PBS liposome treatment affected bacterial numbers *in vivo* in the absence of ciprofloxacin treatment. We infected mice with 10³ cfu i.v. and allowed the infection to progress until day 3, when liposomes were administered i.p. and the infection was allowed to progress for one more day. On day 4 p.i. the mice were killed, and spleens were collected for enumeration of viable *Salmonella*. No significant difference in bacterial counts in the spleen (data not shown) were induced by treatment of infected mice with clodronate liposomes in comparison to control PBS liposomes.

Method S3: Counting viable bacterial load in the spleen

For enumeration of viable *Salmonella* in spleen, half of each spleen was individually homogenised in 5 mL sterile water (Sigma) using a Stomacher-80 (Seward) (the other half of the spleen was used for flow cytometric analysis, see Material and Methods and Method S2). The homogenates were serially diluted in sterile water and plated in LB agar (Sigma). The

plates were then incubated at 37 ° C overnight and colony forming units (cfu) counted the next day.

Table S1. Cell markers and antibody fluorophores for the multicolour flow cytometry panel.

Cell marker	Antibody fluorophore	Cell Types
CD11b	PE	expressed on macrophage, dendritic cells and neutrophils
CD19	VIOBLUE	predominantly expressed on B-cells
CD11c	APC	expressed on macrophage and dendritic cell populations.
CD45	VIOGREEN	Pan leukocyte marker
Ly6G	APC-VIO 770	Marker expressed on neutrophils
NK1.1	PE-VIO 770	Marker expressed on Natural Killer cells
CD3	PE-CY5.5	CD marker expressed on T-cell populations

Table S2. Depletion of CD11b⁺ CD11c^{hi} and CD11b⁺ CD11c^{lo} cells after clodronate liposome treatment

Group	Mean cell numbers*	
	CD11b ⁺ CD11c ^{hi}	CD11b ⁺ CD11c ^{lo}
PBS liposome treated	2575	2785
Clodronate liposome treated	1746	770

*Day 7 p.i.

Table S3. Depletion of Anti-Ly6G⁺ CD11b⁺ neutrophils after anti-Gr-1 treatment

Group	Mean cell numbers*
Control IgG treated	2041
Anti-Gr-1 antibody treated	227

*Day 7 p.i.

Table S4. Depletion of CD19⁺ B cells after anti-CD20 treatment

Group	Mean cell numbers*
Control IgG treated	21614
Anti-CD20 antibody treated	218

*Day 7 p.i.

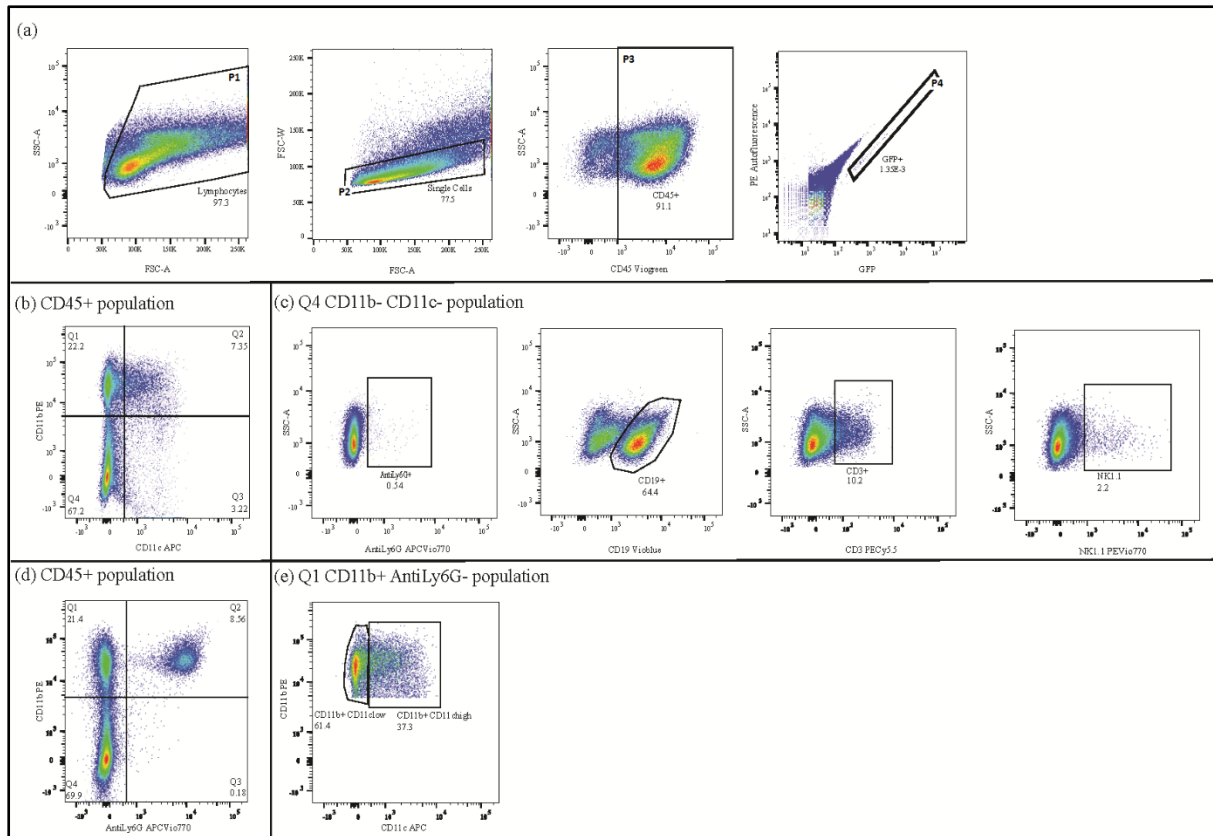


Fig. S1. Gating strategy for multicolour flow cytometric analysis of splenic cells. Spleen cells from infected mice which had undergone different treatments were analysed using the following gating strategy. **(a)** Scatter graphs depicting the sequential gating of splenocytes to yield CD45⁺ single cell populations are shown. Cells were first gated on FSC-area/SSC-area (P1) and then FSC-area/FSC-width to select single cells (P2). Single cell staining for the CD45 marker is shown against SSC (P3) and CD45⁺ cells could then be selected using GFP and PE-autofluorescence signals to gate the GFP⁺ cells (P4). To determine the overall population of haemopoietic cells in the spleen, CD45⁺ single cell populations were analysed for expression of other cell markers. To determine bacteria-associated cell populations, GFP⁺ cells were analysed for expression of the other cell markers. **(b)** CD45⁺ cells (as in (a)) were gated and stained for CD11c (APC) and CD11b (PE). The CD11b⁻CD11c⁻ population was gated in Q4. **(c)** The CD11b⁻CD11c⁻ population from (b) was then utilised to identify different cell populations such as CD19⁺, NK1.1⁺, Ly6G⁺ and CD3⁺ cells. **(d)** The CD45⁺ single cell population from (a) was also used to show staining for Ly6G (APCVio770) and CD11b (PE). CD11b⁺Ly6G⁺ cells are seen in Q2 and CD11b⁺Ly6G⁻ populations in Q1, respectively. **(e)** The CD11b⁺Ly6G⁻ population in Q1 of (d) was further gated to show CD11b⁺CD11c^{lo} and CD11b⁺CD11c^{hi} populations.

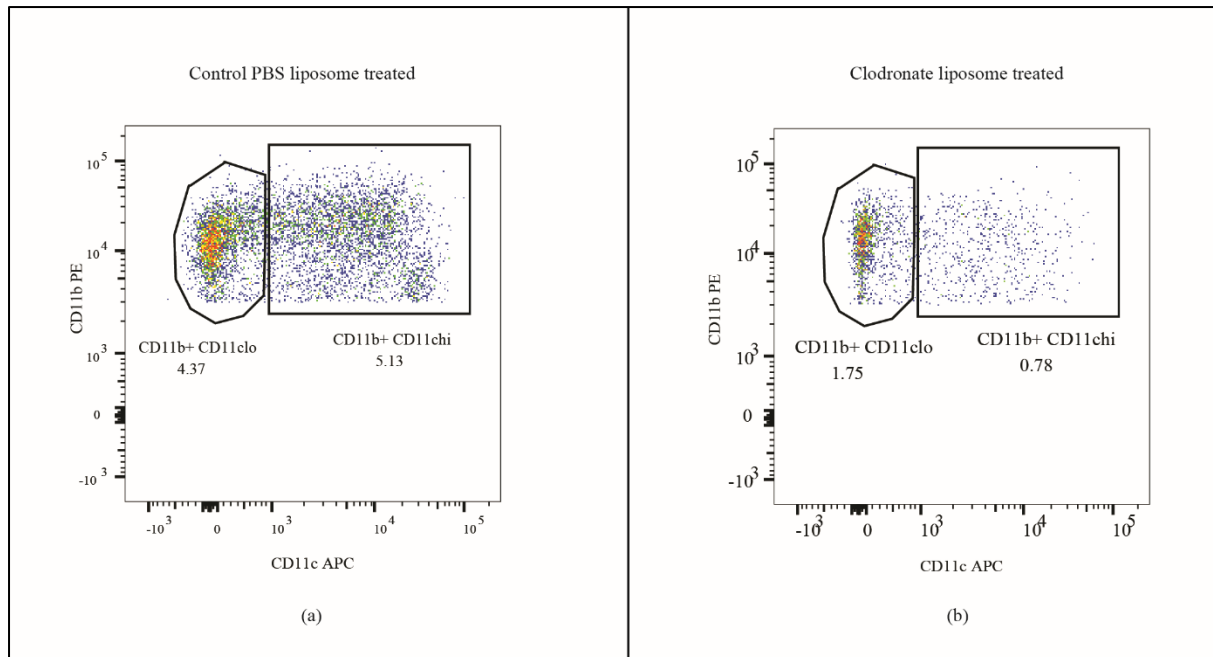


Fig. S2. Depletion of CD11b⁺CD11c⁺ populations using clodronate liposomes. The gating strategy in Fig. S1 (e) was used to obtain the scatter graphs of CD11b⁺ cells with CD11c low and high staining populations in the spleen shown here. Spleens collected on day 7 p.i. from *Salmonella*-infected, antimicrobial-treated mice injected with (a) control PBS liposomes and (b) clodronate liposomes, from day 3 to day 6 p.i..

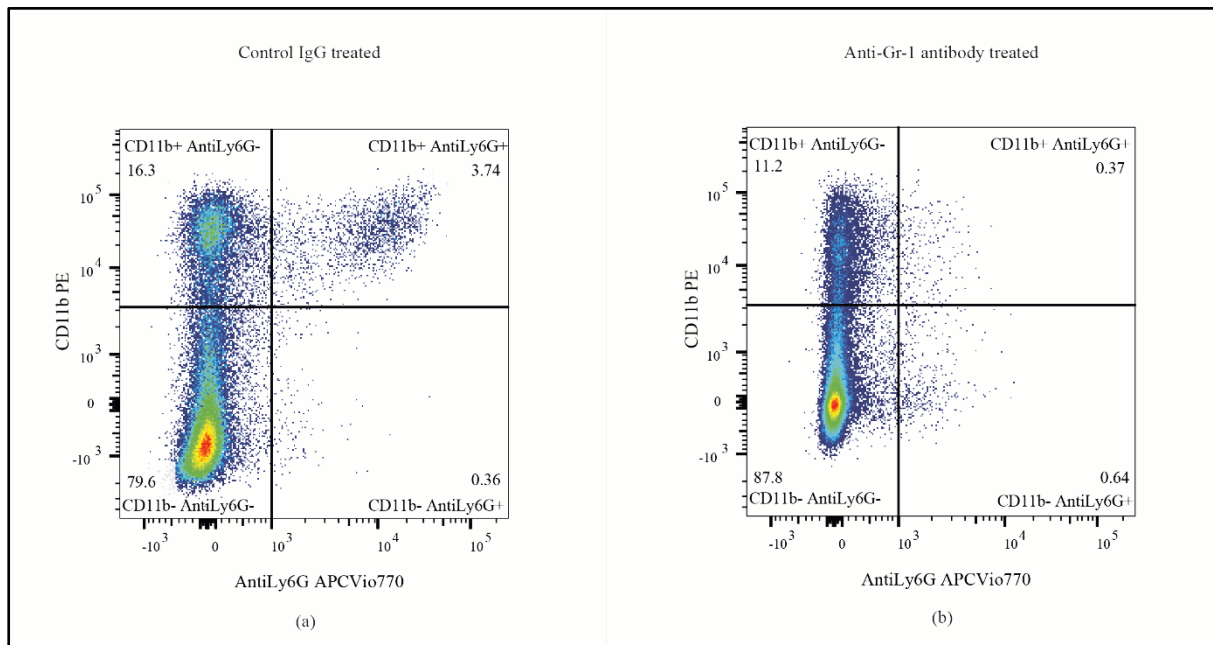


Fig. S3. Depletion of polymorphonuclear populations using anti-Gr-1 antibody. The gating strategy in Fig. S1 (d) was used to obtain the scatter graphs of Ly6G and CD11b staining shown here. Spleens taken on day 7 p.i. from *Salmonella*-infected, antimicrobial-treated mice injected with (a) control IgG and (b) anti-Gr-1 antibody, on day 3 p.i..

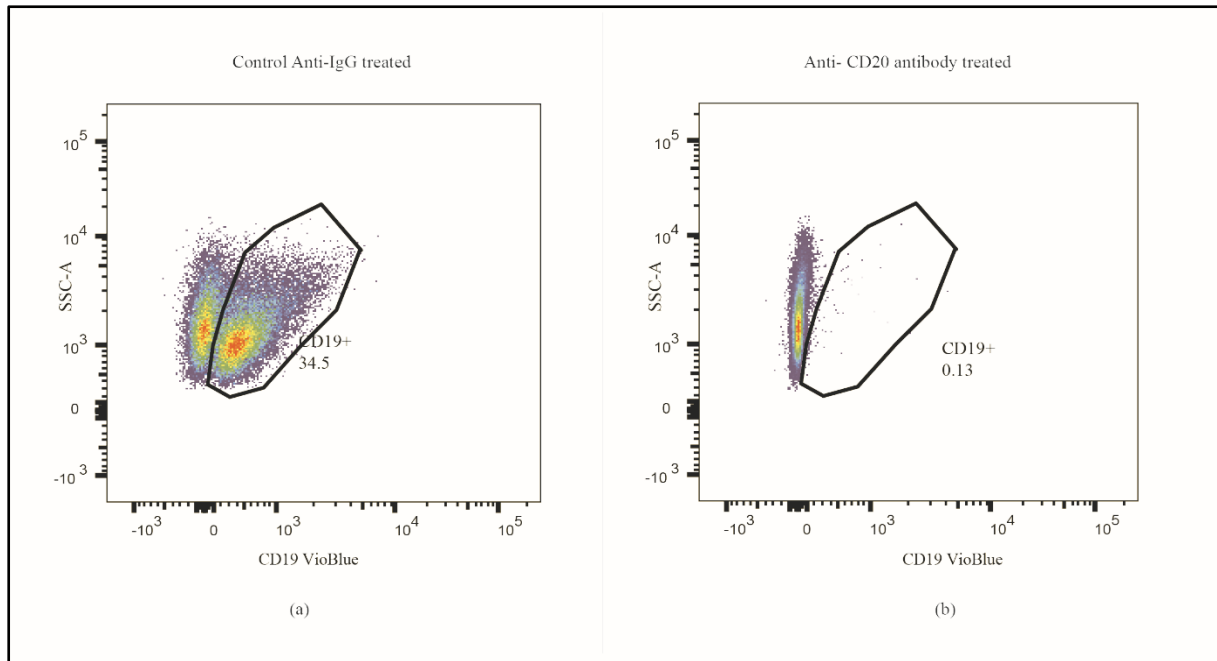


Fig. S4. Depletion of B-cells using anti-CD20 antibodies. The gating strategy in Fig. S1 (c) was used to obtain the scatter graphs of CD19 staining against SSC profile shown here. Spleens taken on day 7 p.i. from *Salmonella*-infected, antimicrobial-treated mice injected with (a) control IgG and (b) anti-CD20 antibody, on day 3 p.i..

Statistics and data analysis. The distributions of cell markers within samples were analysed using generalised linear models with quasi-likelihood distributions to account for over-dispersion. To analyse variations in the proportions of specific cell types between groups, we used quasi-binomial distributions. To analyse differences in the polymorphonuclear distributions of cell types between *Salmonella*-associated cells and overall cells within mice, we used quasi-Poisson distributions. Statistical analyses were performed using the glm function in R version 3.6.