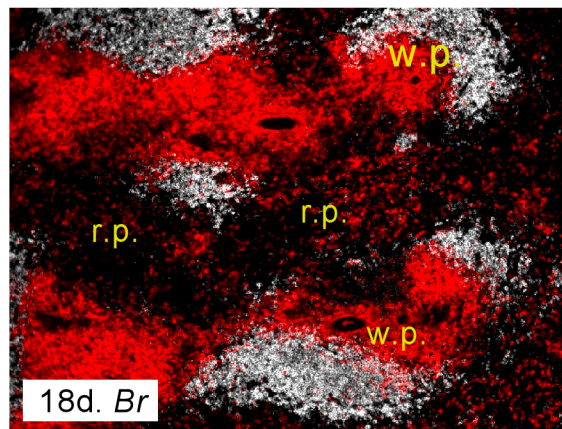
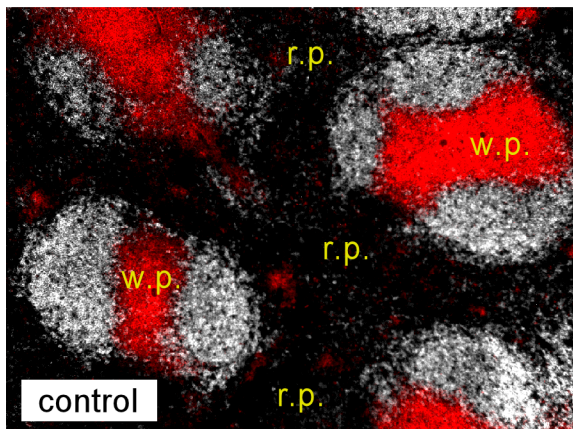


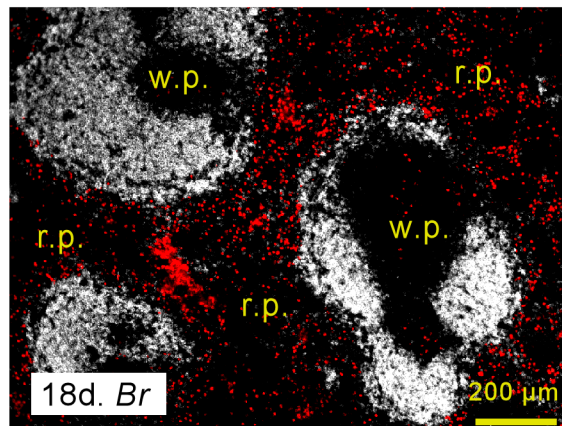
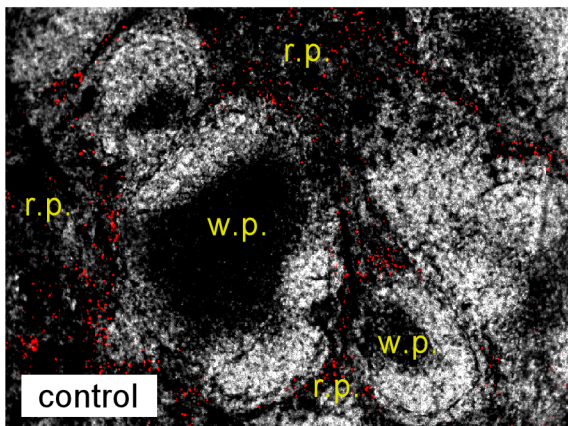
A

CD90 (T cells)



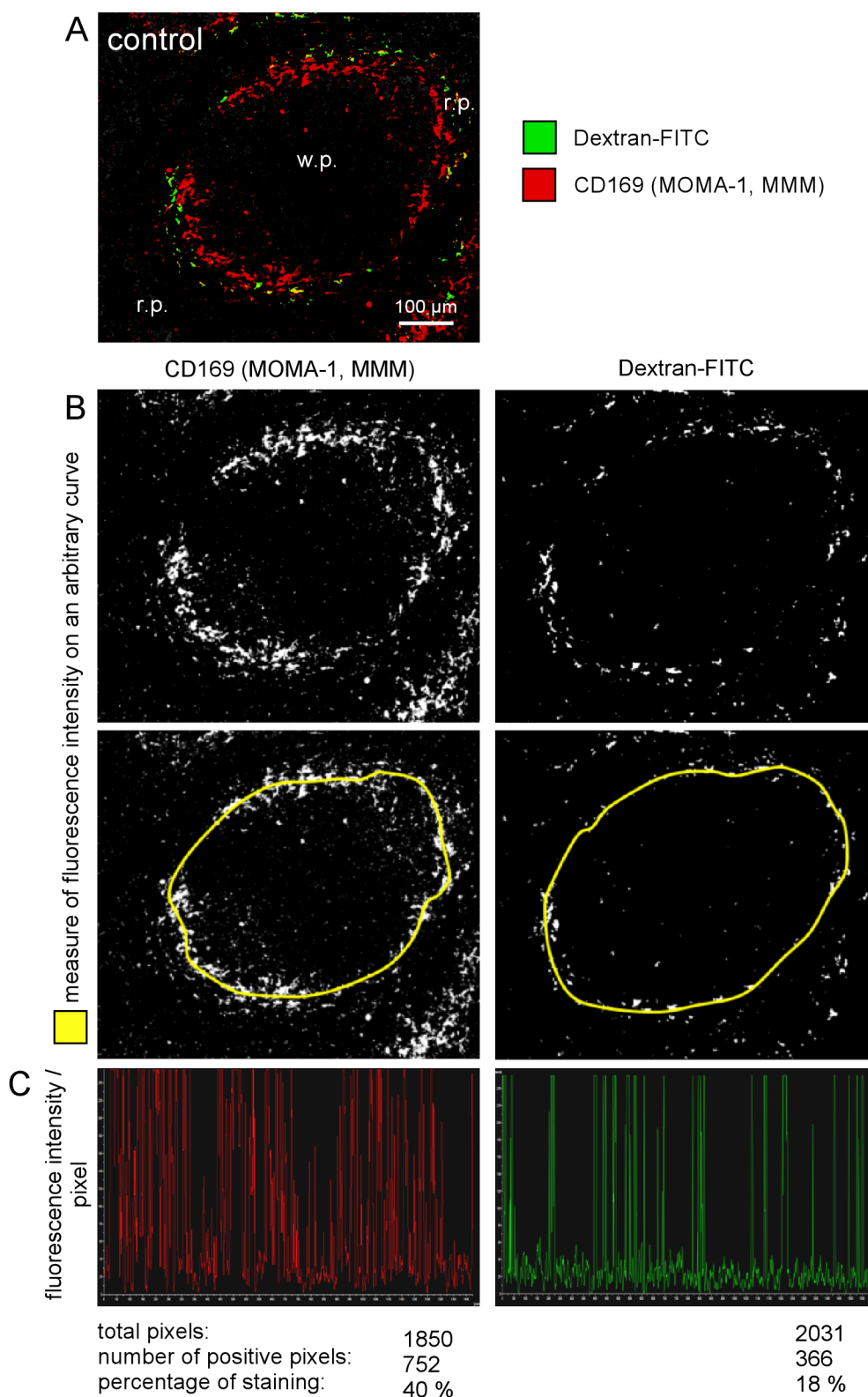
B

GR1

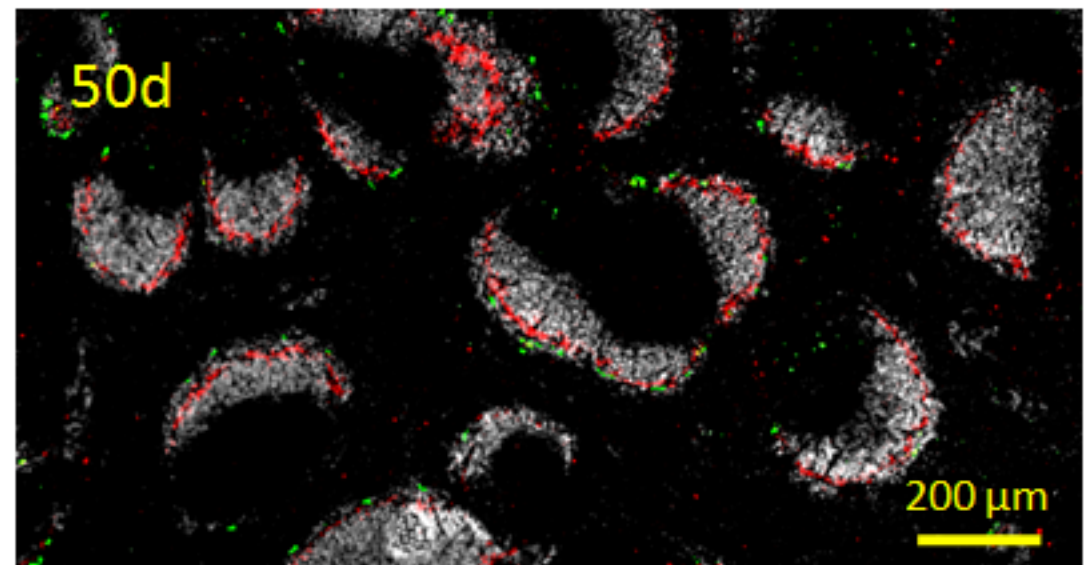
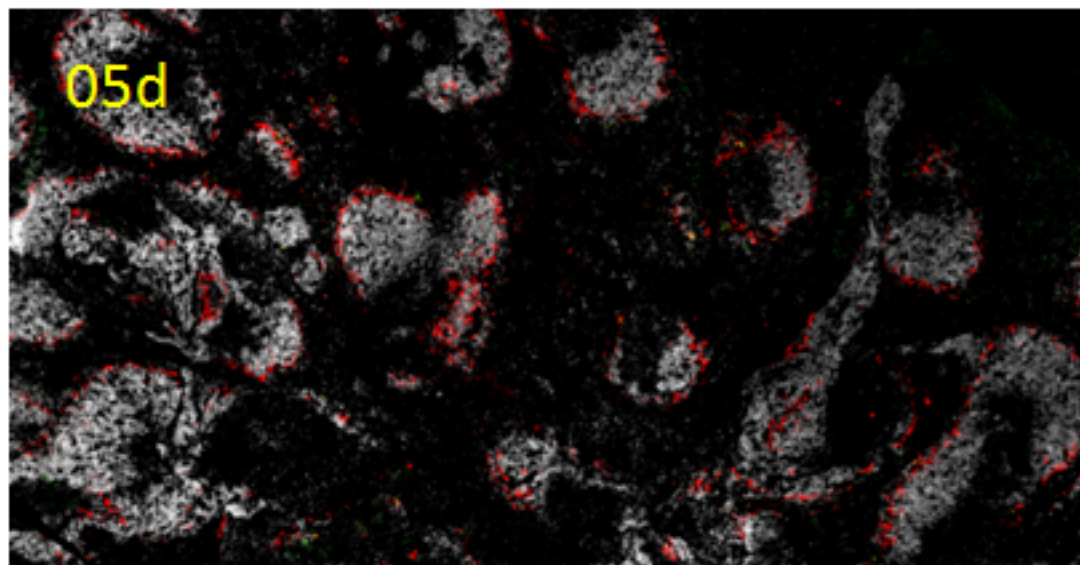
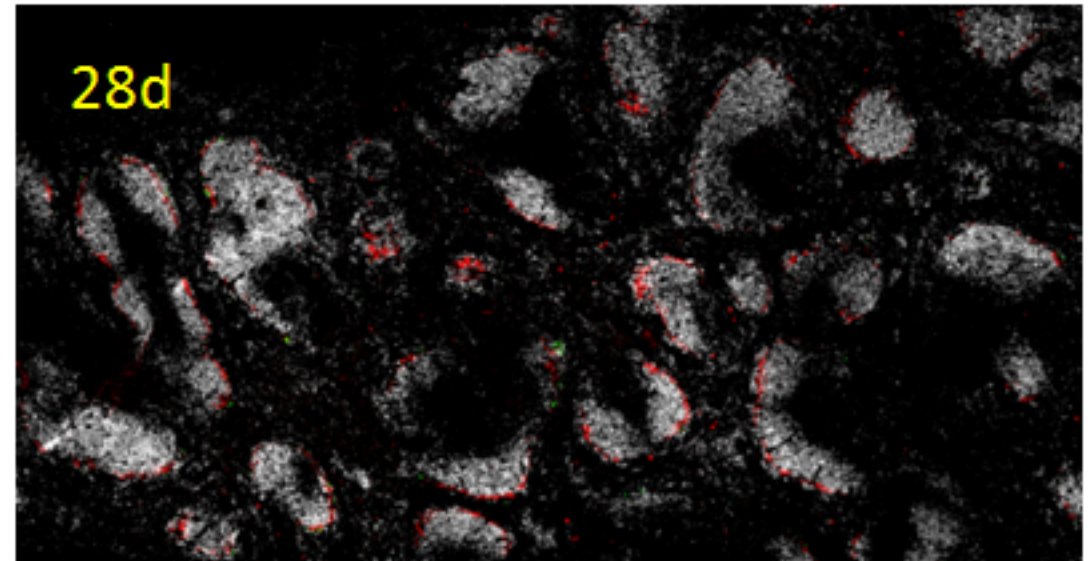
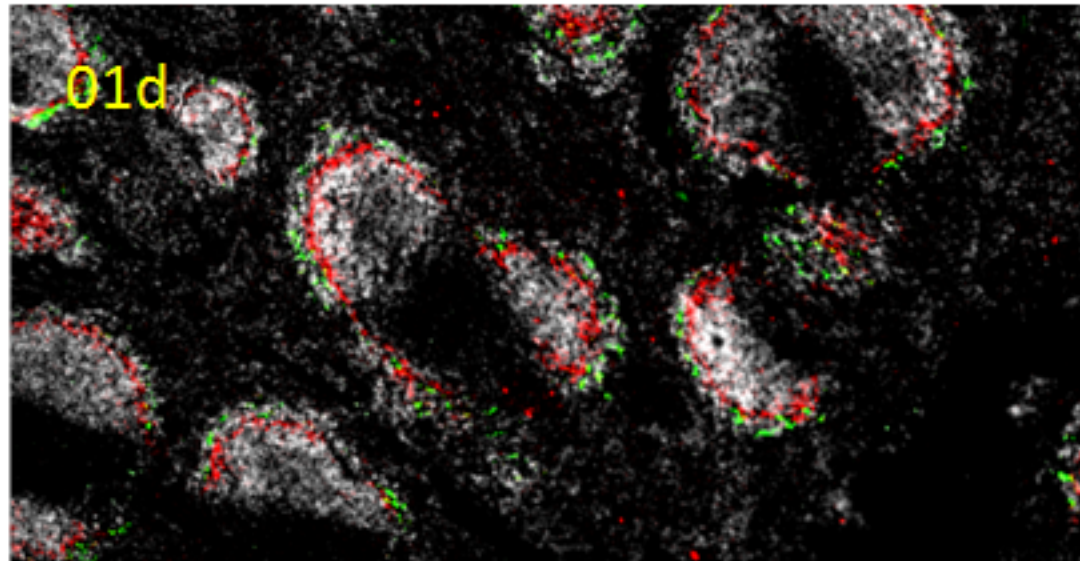
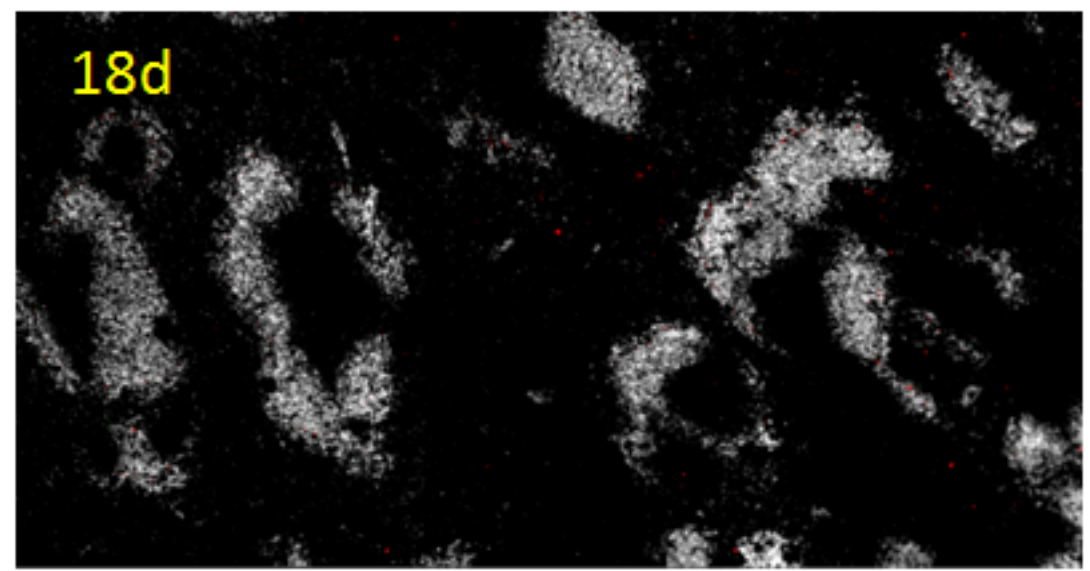
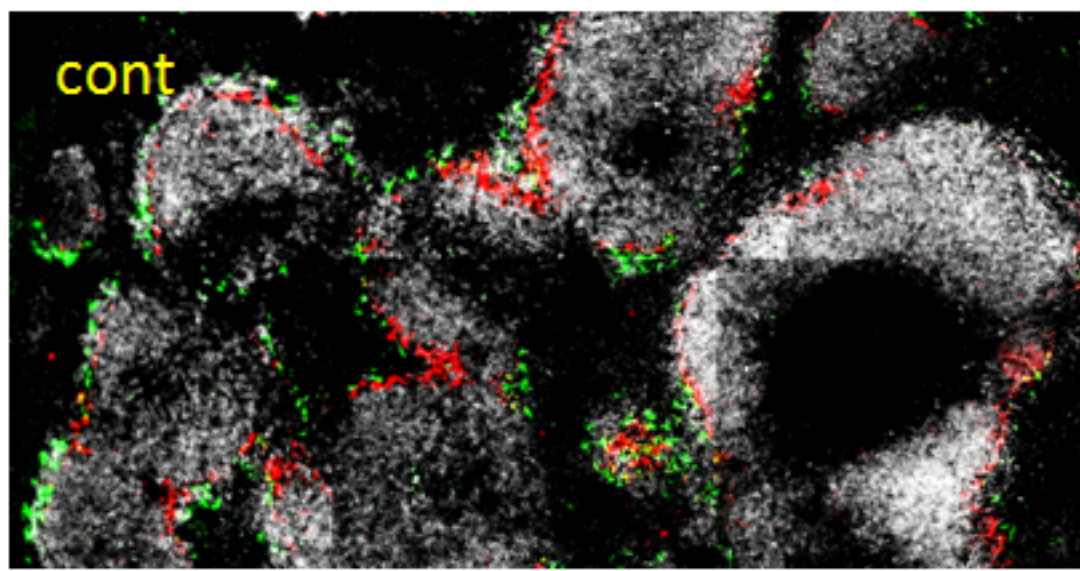


□ CD45R (B220, B cells)

**Supplementary Figure S1: Location of B cells, T cells and GR1<sup>+</sup> cells in the spleen during chronic *Brucella melitensis* infection.** Wild-type C57BL/6 mice were infected i.p. with a dose of  $10^5$  CFU of *B. melitensis* and sacrificed at 18 days post-infection. The panels represent the localization by immunofluorescence of CD45R/B200+ B cells and (A) CD90<sup>+</sup> T cells, (B) GR1<sup>+</sup> cells in the spleens of naive (control) and infected mice. The panels are color-coded with the text for the antigen examined. Scale bar = 200  $\mu$ m, as indicated. r.p.: red pulp; w.p.: white pulp. The data are representative of at least two independent experiments (n=5).

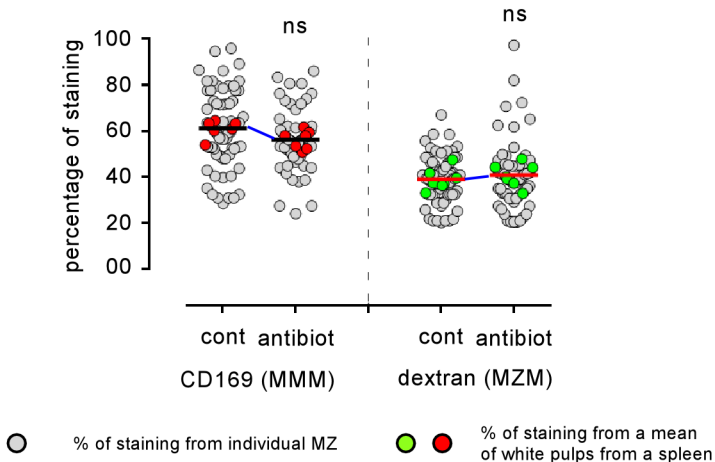


**Supplementary Figure S2: Quantification of marginal zone macrophage populations around white pulp follicles.** The panels represent (A) the localization by immunofluorescence of CD169<sup>+</sup> and Dextran-FITC<sup>+</sup> cells in the spleens of naive (control) wild-type C57BL/6 mice. The panels are color-coded with the text for the antigen examined. Scale bar = 100 μm, as indicated. r.p.: red pulp; w.p.: white pulp. B, C. Example of quantification of marginal zone macrophage populations around a white pulp area. The experimenter defined an arbitrary line covering the marginal area (B), then the images were processed using the NIS-Elements program (Nikon Instruments) (C) to measure the percentage of positive staining per white pulp area.

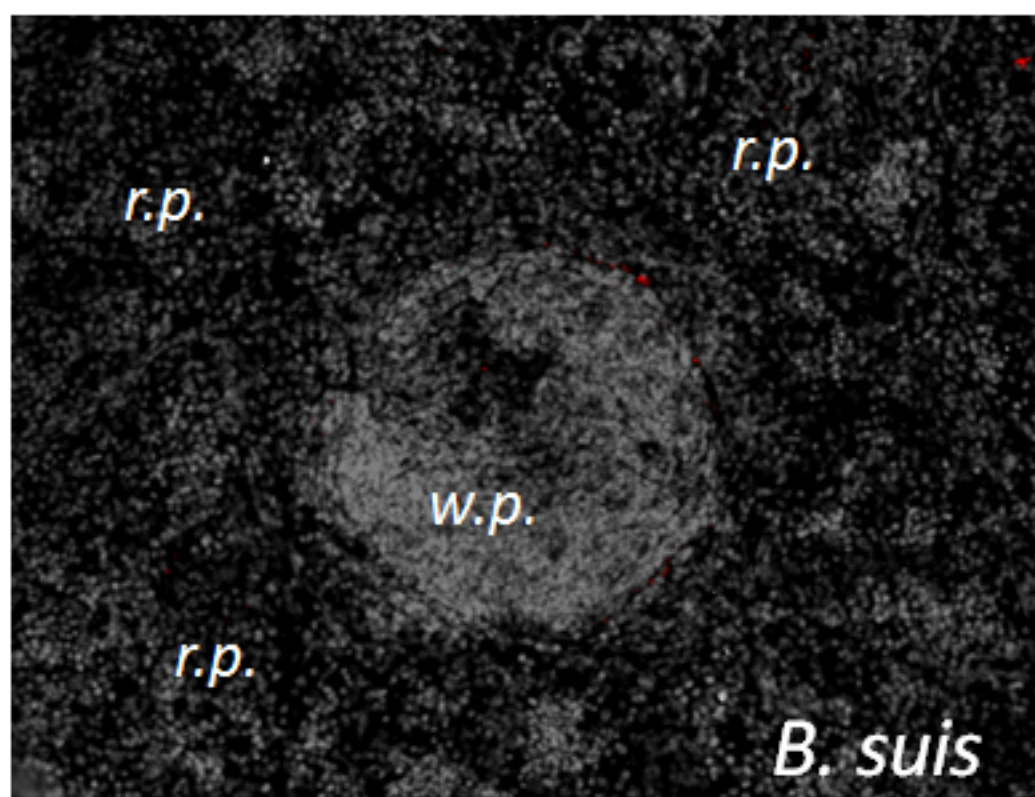
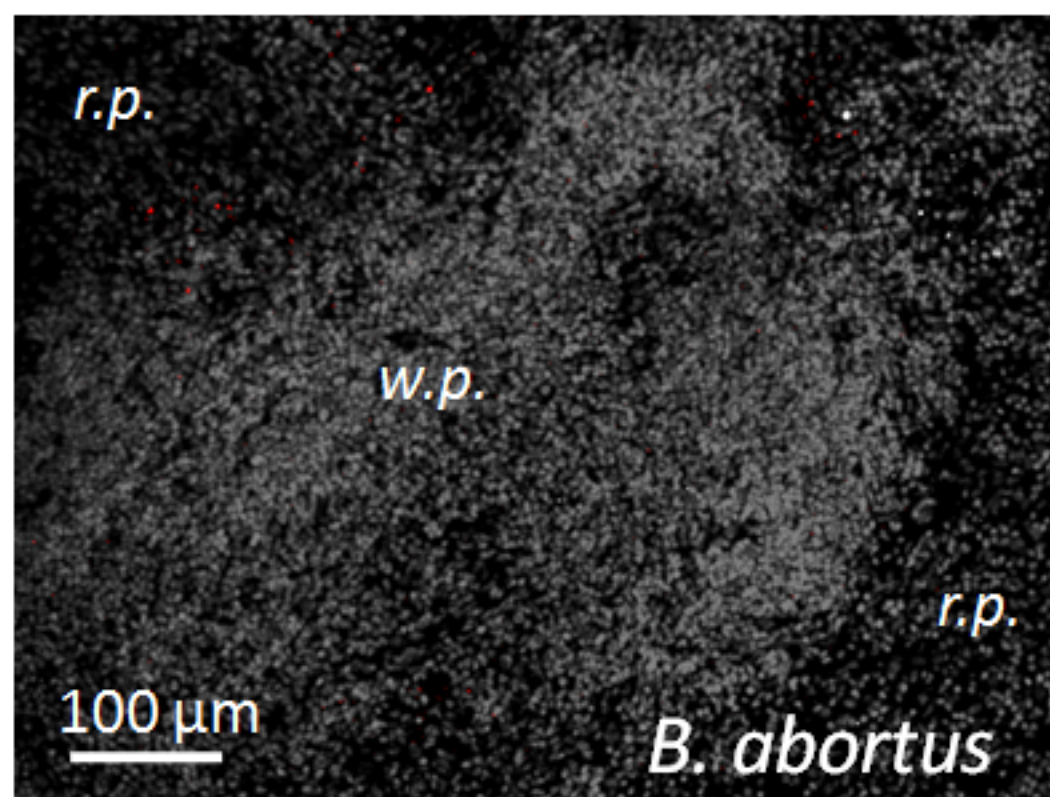
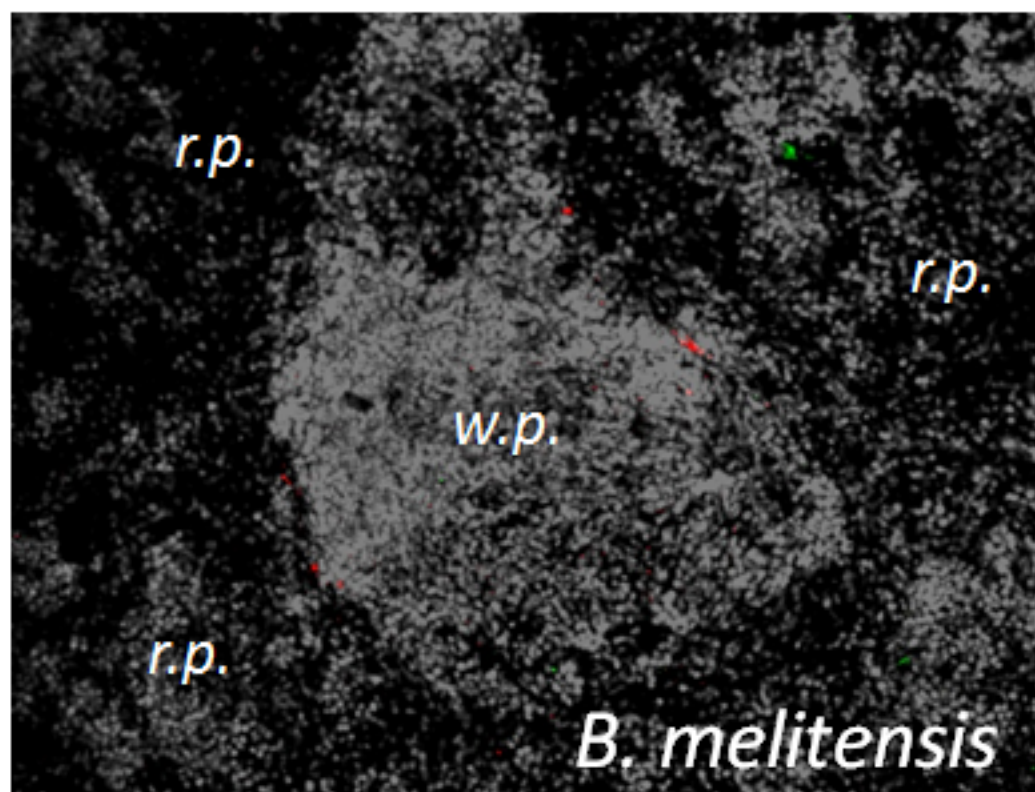
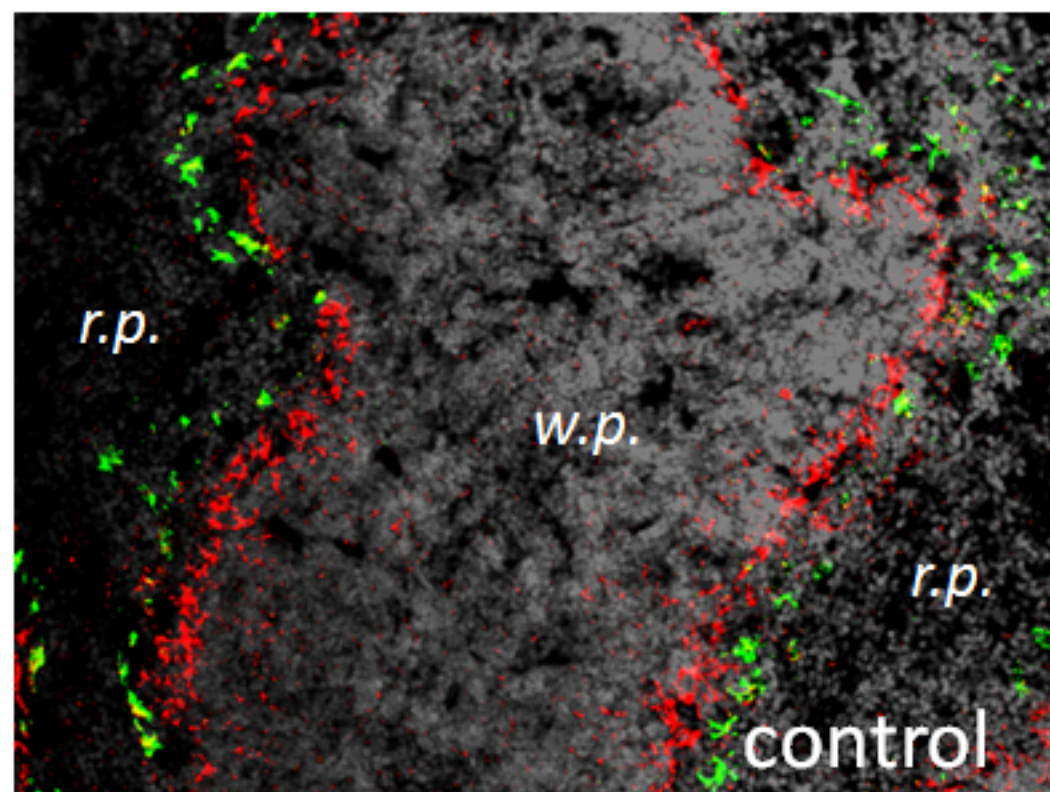


□ CD45R (B220, B cells)    ■ dextran (MZMs)    ■ CD169 (MMMs)

**Supplementary Figure S3: Representative image from control and 1, 5, 18, 28, and 50 days infected wild type C57BL/6 mice.** Mice were infected with  $10^5$  CFU of *Brucella melitensis* and the spleen were harvested at the indicated time for microscopic analysis. The panels represent the localization by immunofluorescence of CD45R/B220<sup>+</sup> cells, dextran<sup>+</sup> cells and CD169<sup>+</sup> cells in control and infected mice. The panels are color-coded with the text for the antigen examined. Scale bar = 200  $\mu$ m, as indicated. The data are representative of at least two independent experiments.

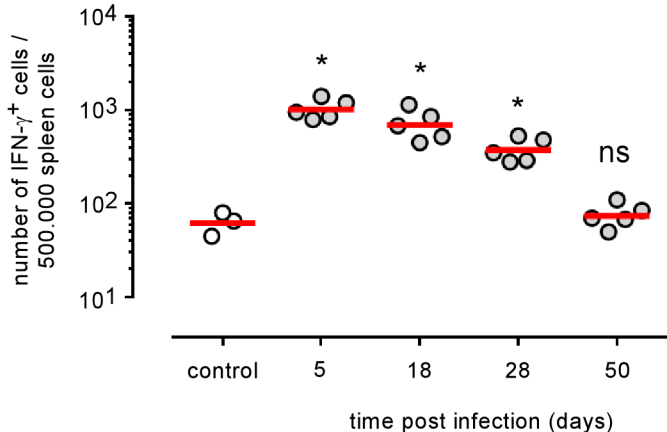


**Supplementary Figure S4: Antibiotic treatment does not affect MMMs and MZMs.** Wild-type C57BL/6 mice were treated or not (control group), as indicated in the Materials and Methods, with antibiotic. Mice were sacrificed after 2 weeks of antibiotic treatment. Spleens were harvested, fixed, frozen and stained as indicated in the Materials and Methods section to detect MZM and MMM populations. The panels represent a semi-quantitative estimation of the presence of MZMs and MMMs in the spleens of control and infected mice. Grey circles show the percentage of positive staining of individual white pulp areas. Green and red circles show the mean of positive staining of at least ten white pulp areas from one frozen spleen section and constitute the mean of positive staining per spleen. For each condition, at least seven frozen spleen sections from three individual mice were analyzed. The horizontal bar indicates the global median. The statistical analysis was performed on the mean of positive staining per spleen. These results are representative of at least two independent experiments (n=8).



■ nucleus ■ dextran ■ CD169 (MMMs)

**Supplementary Figure S5: Loss of marginal zone macrophage populations following *Brucella melitensis*, *Brucella abortus* and *Brucella suis* infection.** Wild-type C57BL/6 mice were infected i.p. with a dose of  $10^5$  CFU of *B. melitensis*, *B. abortus* or *B. Suis* and sacrificed at 18 days post-infection. 30 minutes before sacrifice, mice were injected i.p. with FITC-coupled dextran, as indicated in the Materials and Methods section. The panels represent the localization by immunofluorescence of CD169<sup>+</sup> and FITC-coupled Dextran<sup>+</sup> cells in the spleens of naive (control) and infected mice. The panels are color-coded with the text for the antigen examined. Scale bar = 100  $\mu$ m, as indicated. r.p.: red pulp; w.p.: white pulp. The data are representative of at least two independent experiments with n=5.



**Supplementary Figure S6: IFN- $\gamma$  production in the spleen of wild type C57BL/6 mice during the course of *B. melitensis* infection.**

Mice were infected i.p. with  $10^5$  CFU of *B. melitensis* and sacrificed at the indicated time points. Spleen cells were collected and analyzed by flow cytometry. Cells were gated according to the size and scatter to exclude dead cells and debris for analysis. The panel represents the number of IFN- $\gamma$ <sup>+</sup> cells per 500.000 spleen cells acquired at different times post infection. These results are representative of at least two independent experiments.

**Supplemental Table 1:**

Gene	PCR Primer
$\beta$ -actin	forward: 5'- ACGGCCAGGTCATCACTATTGG-3' reverse: 5'- GTTTCATGGATGCCACAGGATTCC-3'
CD169	forward: 5'- CCAAGAACTGACCCAGTTGAAGG -3' reverse: 5'- ACTTCTGGGCCACAGAGAAGAAG -3'
CD209	forward: 5'- TGTGGACTCTGGAACTACCACTG -3' reverse: 5'- GAGCTCAGGTGATGGTGAAACC -3'
CCL21a	forward: 5'-ACAGACACAGCCCTCAA-3' reverse: 5'-CATGAGGTGGCTGCTTT-3'
CXCL13	forward: 5'-GAACAGGCATTTAGTGACAAC-3' reverse: 5'-TTTTGGAAGCCTGCGTTTT-3'
CCL19	forward: 5'-CTGCCTCAGATTATCTGCCAT-3' reverse: 5'-TCATAAGCACCCCCAGAGT-3'