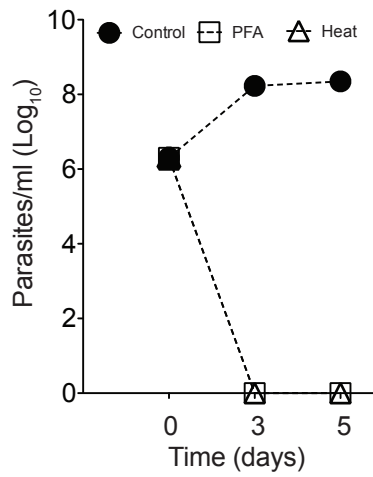
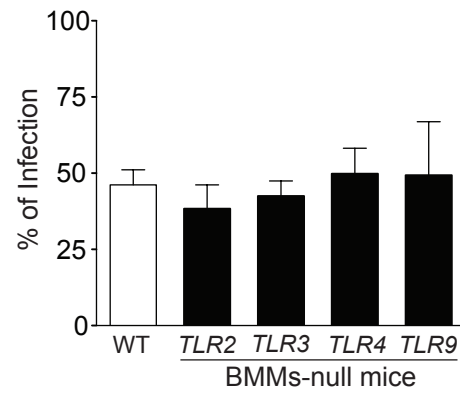


## Supplementary figure 1



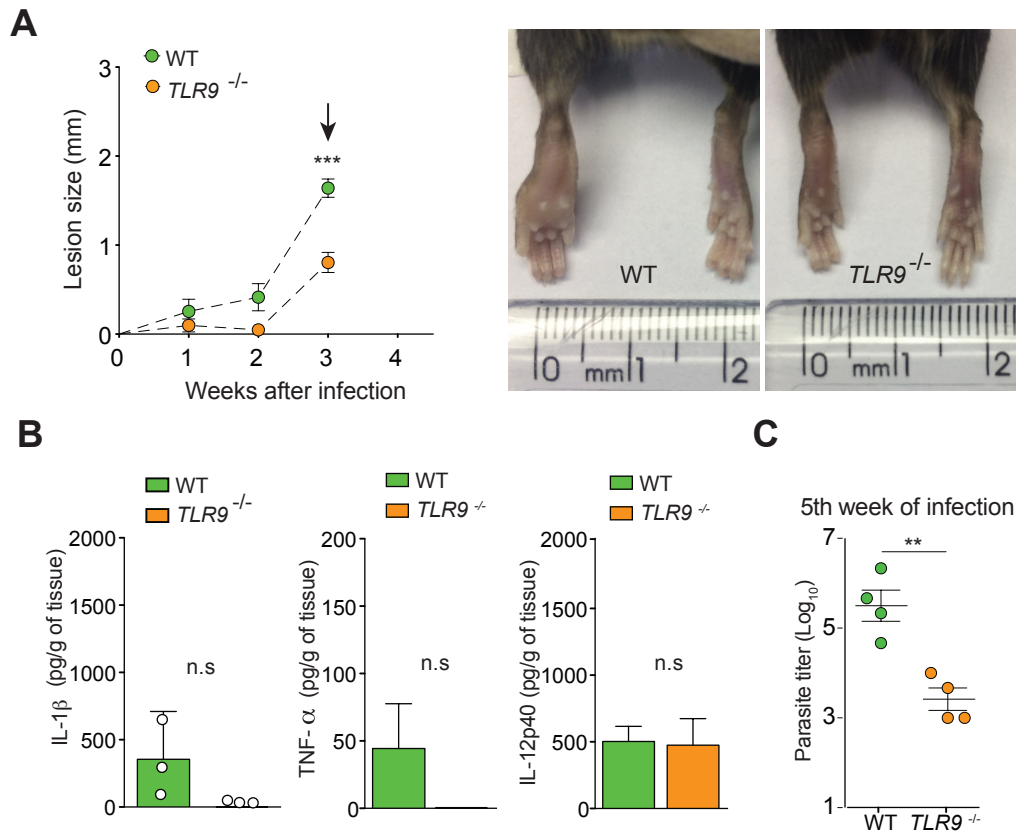
**Supplementary figure 1. Viability of *L. amazonensis* amastigotes after PFA or heat inactivation.** Axenic amastigotes of *L. amazonensis* were treated as described in figure 1D. After washes, the samples were incubated in complete promastigote media at 26° C and counted on the 3<sup>rd</sup> and 5<sup>th</sup> day of culture. Graph corresponds to the number of promastigotes per ml as a representative result of 3 independent assays.

## Supplementary figure 2



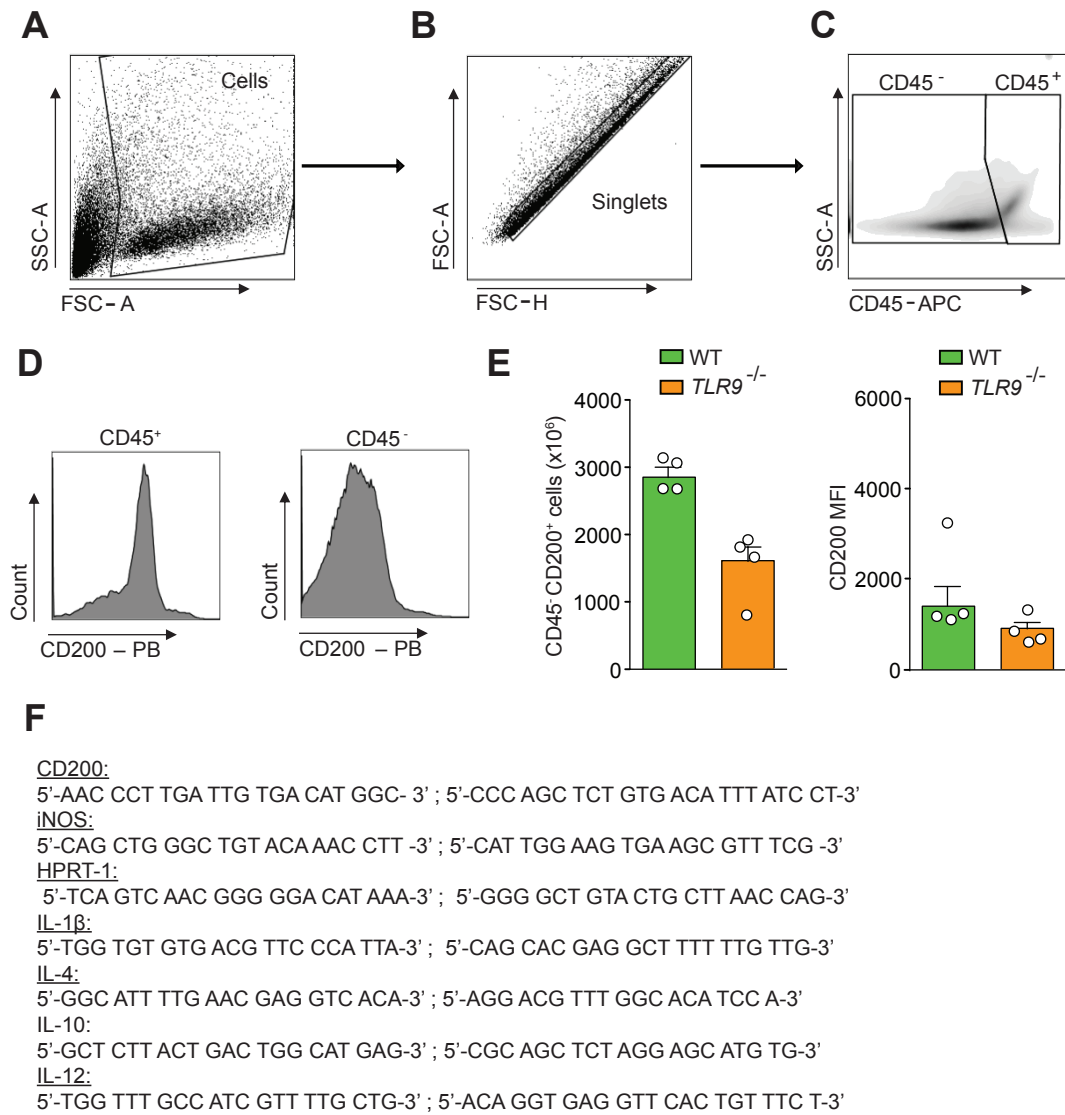
**Supplementary figure 2. *L. amazonensis* infection in BMMs from WT or *TLR*<sup>-/-</sup> mice.** Percentage of infection for the experiment in Figure 2B. Data correspond to the mean  $\pm$  SD of triplicates. Results represent 3 independent experiments. No statistical differences were observed by using the Student *t*-test.

## Supplementary figure 3



**Supplementary figure 3. Progression of *L. amazonensis* lesions in WT and *TLR9*<sup>-/-</sup> mice.** (A) Lesion size (mm) expressed as the mean  $\pm$  SD from at least n=5 mice per group. The arrow indicates the week of sacrifice of mice for parasite load determination (shown in Fig. 4C). Representative image of the lesion from WT or *TLR9*<sup>-/-</sup> mice infected by *L. amazonensis* on the left footpad compared to the non-infected footpad (right). (B) Detection of IL-1 $\beta$ , TNF- $\alpha$  and IL-12p40 in the footpad homogenates at the third week of *Leishmania* infection and quantified by ELISA. Results correspond to the mean  $\pm$  SD (n=4). n.s: not significant; (Student's *t*-test). (C) Parasite load in the footpads of WT and *TLR9*<sup>-/-</sup> mice infected with *L. amazonensis* at the fifth week of infection. Results correspond to mean  $\pm$  SD (n=4). \*\*p= 0.0028 (Student's *t*-test).

## Supplementary figure 4



**Supplementary figure 4. Gating strategy for macrophage immunophenotyping in the lesion of *L. amazonensis*-infected mice.** Lesions were processed as described in the Methods and the resulting cells were evaluated by flow cytometry. **(A)** Debris exclusion based on forward versus side scatter parameters (FSC and SSC). **(B)** Single cell events (singlets) were selected based on FSC-H versus FSC-A parameters. **(C)** CD45<sup>+</sup> and CD45<sup>-</sup> cells were gated for evaluation of CD200<sup>+</sup> in the entire population **(D)**. **(E)** CD45-CD200<sup>+</sup> cells were obtained from the total number of cells (absolute population), analyzing the median fluorescence intensity (MFI). **(F)** List of oligonucleotide primers used for the qPCR.

## Supplemental Table 1

### Antibodies used in the study:

rabbit anti-mouse iNOS mAb	Abcam, ab136918
rabbit anti-mouse actin mAb	Imuny-VBP Biotecnologia Ltda, IM-0075
goat anti-mouse CD200	R&D Systems, AF2554
Anti-mouse CD200	BD Biosciences, clone OX-90
Anti-mouse CD45	Biolegend clone, 30-F11
Anti-mouse CD11b	BD Biosciences clone, M1/70
Anti-mouse CD11c	Biolegend, clone N418
Anti-mouse MHC class II	BD Biosciences, clone 2G9
Anti-mouse F4/80	eBiosciences, clone BM8
Anti-rabbit IgG conjugated Alexa Fluor 488	Thermo Fischer Scientific, A11070
Anti-IgG rabbit peroxidase-conjugated	Imuny-VBP Biotecnologia Ltda, IC-3R01
Anti-IgG mouse peroxidase-conjugated	Imuny-VBP Biotecnologia Ltda, IC-1G01