Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary material

Table 1. Primers used for parasite burden quantification.

	Sense primer (5´-3´)	Antisense primer (5´-3´)
ß-actin	CGCGTCCACCCGCGAG	CCTGGTGCCTAGGGCG
Leish 18S	CCAAAGTGTGGAGATCGAAG	GGCCGGTAAAGGCCGAATAG
Leish kDNA	CCTATTTTACACCAACCCCCAGT	GGGTAGGGGCGTTCTGCGAAA

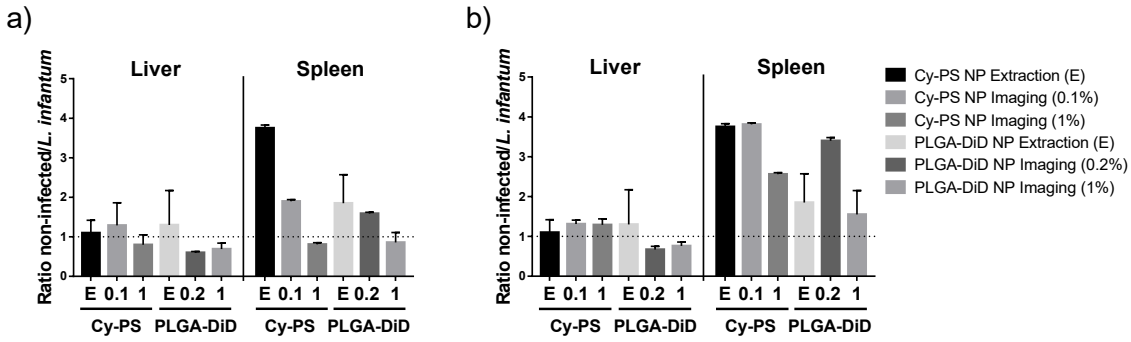

Figure 1. Dye quantification ratios (non-infected/*L. infantum*-infected) comparing results obtained by dye extraction and quantification (NP of low dye loading), and PhotonImager™ ex vivo fluorescence intensity (low and high dye loadings). a) Imaging ratios expressed as ph/cm2. b) Imaging ratios expressed as ph/g organ. Data are expressed as mean ± SD (n = 4).

Table 2**.** Percentage of the different cell subsets in blood of a) non-infected and b) *L. infantum*-infected mice before and after 4 h i.v. administration of PS NP at 0.2 mg/kg of Cy5.5 or PLGA NP at 0.5 mg/kg of DiD. Data are presented as mean ± SD (n = at least 4). Data were analysed using a non-parametric Mann Whitney test. ****$p < 0.0001$.

a)	non-infected	non-infected + PS NP	non-infected + PLGA NP
B cells	11.3 ± 3.8	12.1 ± 4.0	11.5 ± 3.6
PMN	30.5 ± 3.0	58.9 ± 4.2****	48.4 ± 5.6****
Ly6C- monocytes	1.7 ± 0.4	5.8 ± 1.8	2.3 ± 0.6
Ly6C+ monocytes	6.0 ± 1.0	3.3 ± 0.5	6.4 ± 1.5

b)	*L. infantum*	*L. infantum* + PS NP	*L. infantum* + PLGA NP
B cells	5.4 ± 1.3	5.9 ± 1.3	10.9 ± 5.7
PMN	23.7 ± 8.2	46.7 ± 14.7****	51.5 ± 6.9****
Ly6C- monocytes	3.1 ± 1.3	1.6 ± 0.9	1.8 ± 1.0
Ly6C+ monocytes	5.2 ± 1.6	7.2 ± 3.4	6.8 ± 2.7

Table 3. Percentage of the different cell subsets in the spleen of a) non-infected and b) *L. infantum*-infected mice before and after 4 h i.v. administration of PS NP at 0.2 mg/kg of Cy5.5 or PLGA NP at 0.5 mg/kg of DiD. Data are presented as mean ± SD (n = at least 4).

a)	non-infected	non-infected + PS NP	non-infected + PLGA NP
B cells	42.3 ± 4.9	43.8 ± 5.8	45.3 ± 3.5
PMN	7.1 ± 3.8	7.5 ± 3.0	7.3 ± 1.6
Ly6C- monocytes	2.0 ± 0.6	2.8 ± 0.8	2.1 ± 0.4
Ly6C+ monocytes	2.1 ± 0.5	4.0 ± 1.2	2.3 ± 0.5
RPM	0.6 ± 0.3	0.7 ± 0.3	0.7 ± 0.3
pDC	0.5 ± 0.2	0.2 ± 0.1	0.7 ± 0.2
mDC	1.2 ± 0.4	0.8 ± 0.2	0.8 ± 0.1

b)	*L. infantum*	*L. infantum* + PS NP	*L. infantum* + PLGA NP
B cells	51.6± 6.1	54.9 ± 6.0	55.0 ± 8.8
PMN	5.1 ± 1.0	8.3 ± 3.2	3.7 ± 1.6
Ly6C- monocytes	1.8 ± 0.4	1.5 ± 0.7	1.4 ± 0.4
Ly6C+ monocytes	3.5 ± 0.7	2.5 ± 0.7	2.6 ± 0.3
RPM	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
pDC	0.6 ± 0.2	0.7 ± 0.2	1.5 ± 0.5
mDC	1.3 ± 0.3	1.5 ± 0.2	2.1 ± 0.2

Table 4. Percentage of the different cell subsets in BM of non-infected and *L. infantum*-infected mice before and after 4 h i.v. administration of PS NP at 0.2 mg/kg of Cy5.5 or PLGA NP at 0.5 mg/kg of DiD. Data are presented as mean ± SD (n = at least 4). Data were analysed using a non-parametric Mann Whitney test. ****$p < 0.0001$.

a)	non-infected	non-infected + PS NP	non-infected + PLGA NP
B cells	34.3 ± 5.9	30.3 ± 2.7	35.8 ± 4.1
PMN	40.9 ± 7.2	40.9 ± 4.6	36.6 ± 2.7
Ly6C- monocytes	5.4 ± 1.2	5.2 ± 1.5	4.1 ± 0.5
Ly6C+ monocytes	4.7 ± 0.9	7.3 ± 2.4	4.7 ± 0.7
RPM	6.1 ± 2.4	4.3 ± 0.6	4.3 ± 0.6
pDC	1.9 ± 0.5	1.3 ± 0.3	1.7 ± 0.1

b)	*L. infantum*	*L. infantum* + PS NP	*L. infantum* + PLGA NP
B cells	27.6 ± 4.2	25.2 ± 3.7	26.8 ± 5.4
PMN	31.6 ± 6.7	40.8 ± 2.4****	34.5 ± 7.4
Ly6C- monocytes	4.7 ± 1.7	4.4 ± 1.6	3.0 ± 0.6
Ly6C+ monocytes	7.6 ± 1.0	8.5 ± 1.6	8.2 ± 1.5
RPM	1.5 ± 1.5	5.1 ± 1.8	4.1 ± 0.8

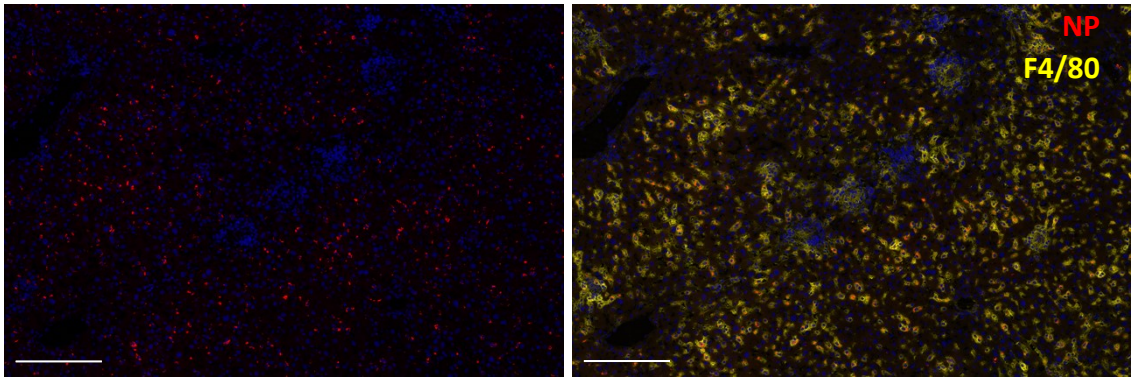

Figure 2. Immunofluorescence images of livers stained with F4/80 (in yellow), showing the formation of granulomas in *L. infantum*-infected mice and the inability of PS NP (in red) to reach its core after 24 h i.v. administration. Scale bar 200 µm.

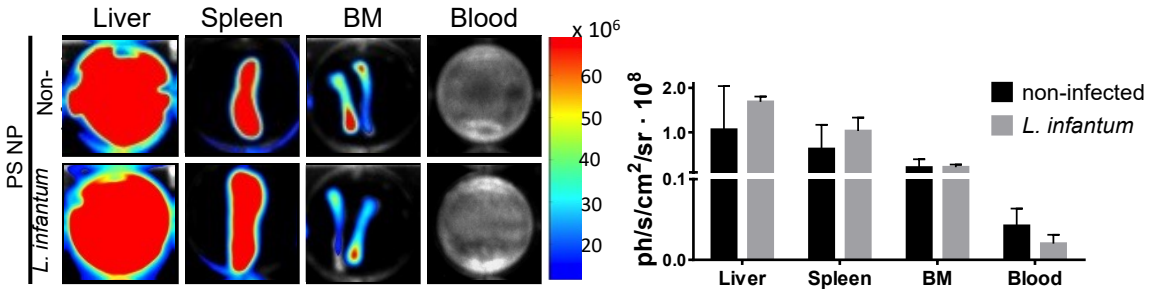

Figure 3. *In vivo* fluorescence of PS NP after 4 h i.v. injection of 2 mg/kg Cy5.5 in non-infected and *L. infantum*-infected organs. Scale expressed in ph/s/cm2/sr · 10^6. Data are expressed as mean ± SD (n = 6).

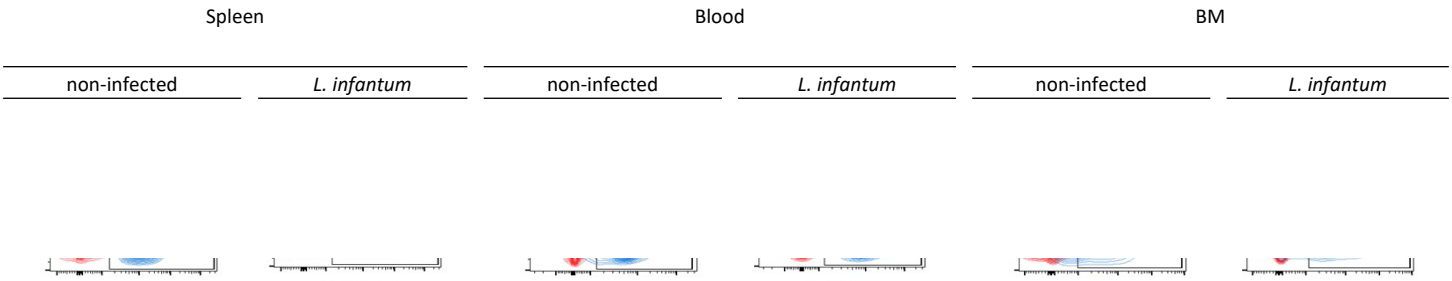

Figure 4. *In vivo* NP uptake in Spleen, Blood and BM of non-infected and *L. infantum*-infected mice after 4 h NP i.v. administration, at 2 mg/kg PS NP. Representative contour plots of each group (n = 6) of NP+ cells (in blue) vs their control (in red) are presented.

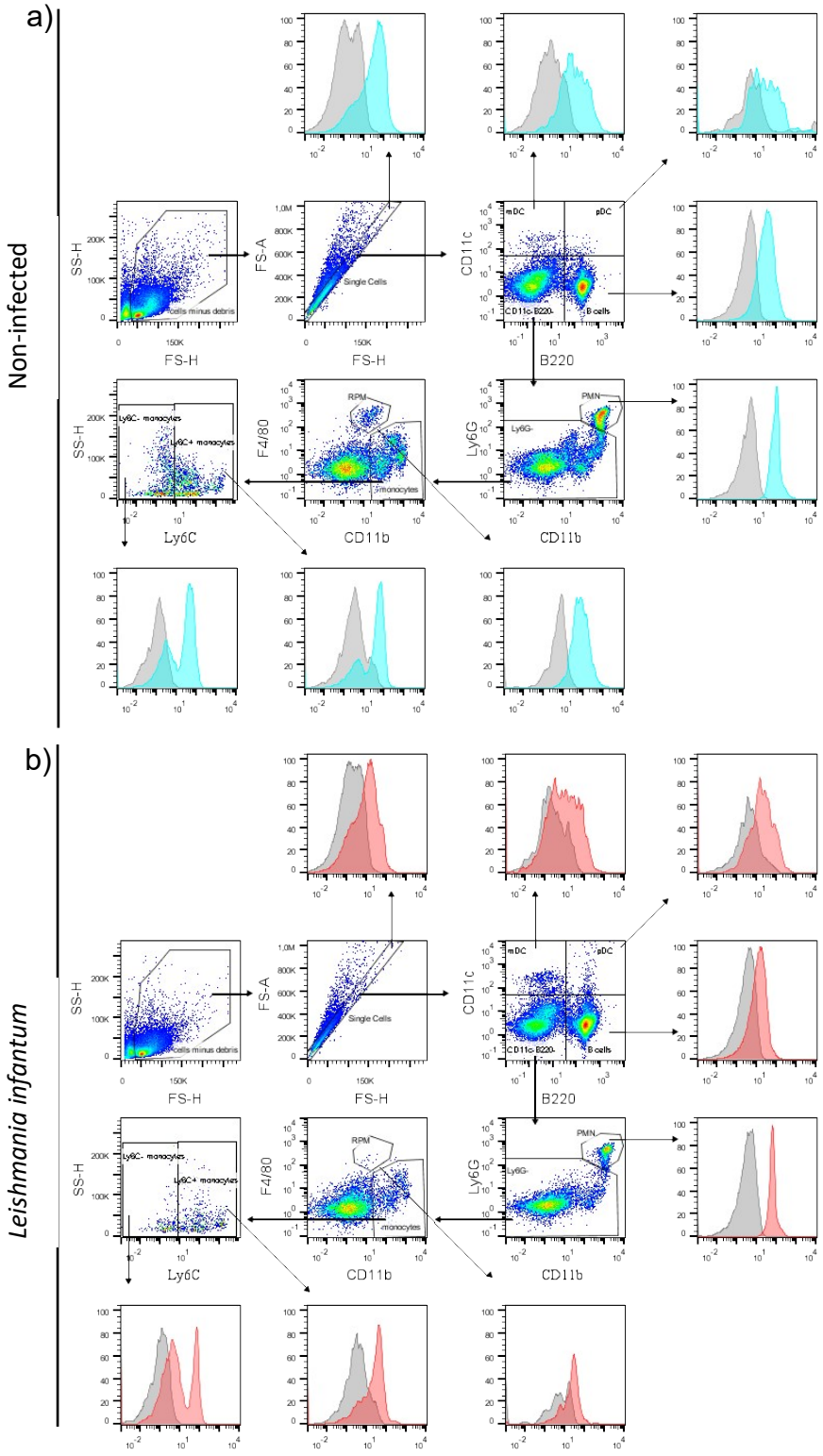

Figure 5. Immunophenotyping cells from the monocyte-phagocytic system in mouse spleen of a) non-infected and b) *L. infantum* infected mice treated with 0.2 mg/kg PS NP. Total mouse splenocytes from BALB/c mice were prepared as described in material and methods. Debris (SS-H vs FS-H) and doublets (FS-A vs FS-H) were excluded from mouse splenocytes. After gating out CD11c+ cells (B220- (mDC) and B220+ (pDC)) and B220+ cells (B cells), Ly6G+ (PMN) were gated out and the remaining Ly6G- cells were divided into two populations (F4/80 vs CD11b): RPM (F4/80+, CD11b^{int}) and monocytes (CD11b+). Monocytes subpopulation was further divided into Ly6C+ and Ly6C- monocytes. The MFI of each population for AF700 of the control (grey histogram) and NP+ (a) blue histogram in non-infected and b) red histogram in *L. infantum* infected mice) is represented.

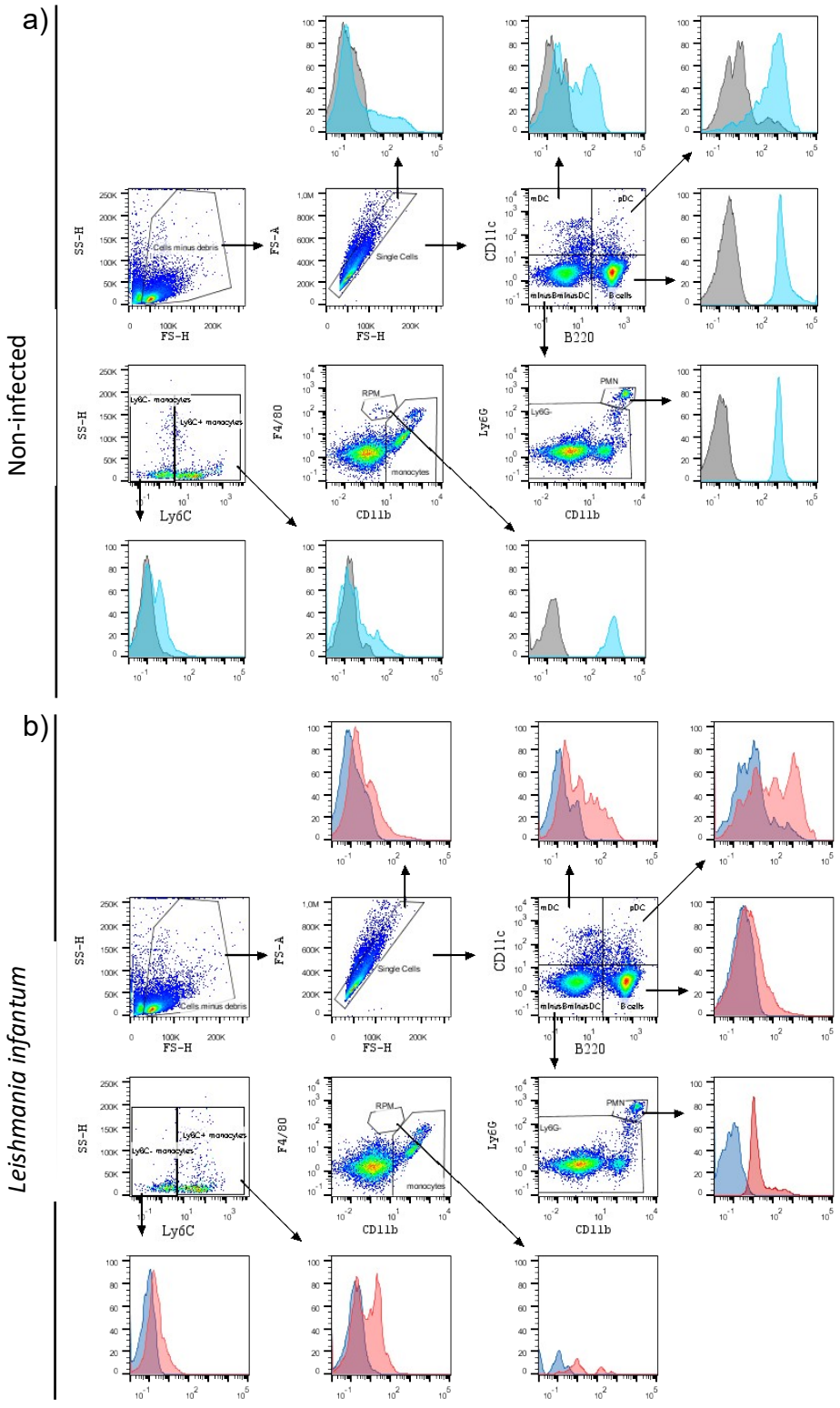

Figure 6. Immunophenotyping cells from the monocyte-phagocytic system in mouse spleen of a) non-infected and b) *L. infantum* infected mice treated with 0.5 mg/kg PLGA NP. Total mouse splenocytes from BALB/c mice were prepared as described in material and methods. Debris (SS-H vs FS-H) and doublets (FS-A vs FS-H) were excluded from mouse splenocytes. After gating out CD11c+ cells (B220- (mDC) and B220+ (pDC)) and B220+ cells (B cells), Ly6G+ (PMN) were gated out and the remaining Ly6G- cells were divided into two populations (F4/80 vs CD11b): RPM (F4/80+, $CD11b^{int}$) and monocytes (CD11b+). Monocytes subpopulation was further divided into Ly6C+ and Ly6C- monocytes. The MFI of each population for APC of the control (grey histogram in non-infected and blue histogram in L. infantum infected mice) and NP+ (blue and red histograms for non infected and infected mice, respectively) is represented.

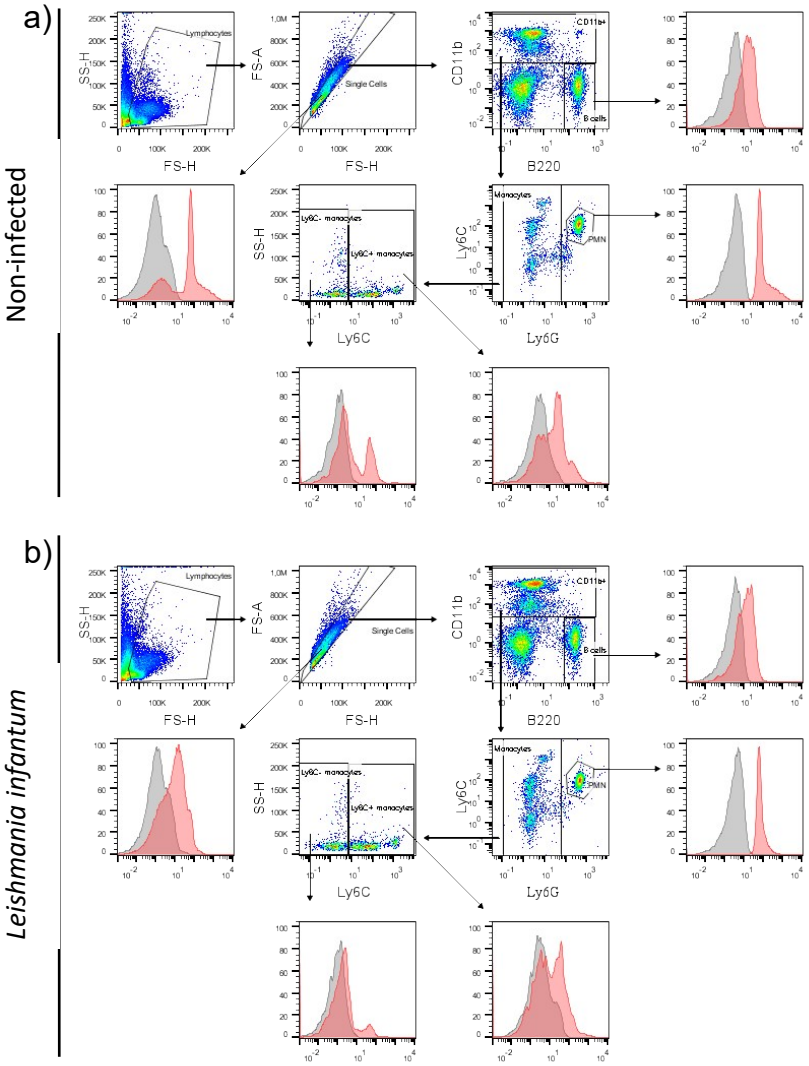

Figure 7. Immunophenotyping cells from the monocyte-phagocytic system in mouse blood of a) non-infected b) *L. infantum* infected mice at 0.2 mg/kg PS NP. Blood cells were prepared as described in material and methods. Debris (SS-H vs FS-H) and doublets (FS-A vs FS-H) were excluded from mouse splenocytes. After gating out B220+ cells (B cells), CD11b+ cells were divided into two populations (Ly6C vs Ly6G): Ly6G+ (PMN) and monocytes (Ly6G-). This last population was further divided into Ly6C+ and Ly6C- monocytes. The MFI of each population for AF700 of the control (grey histogram) and NP+ (a) red histogram is represented.

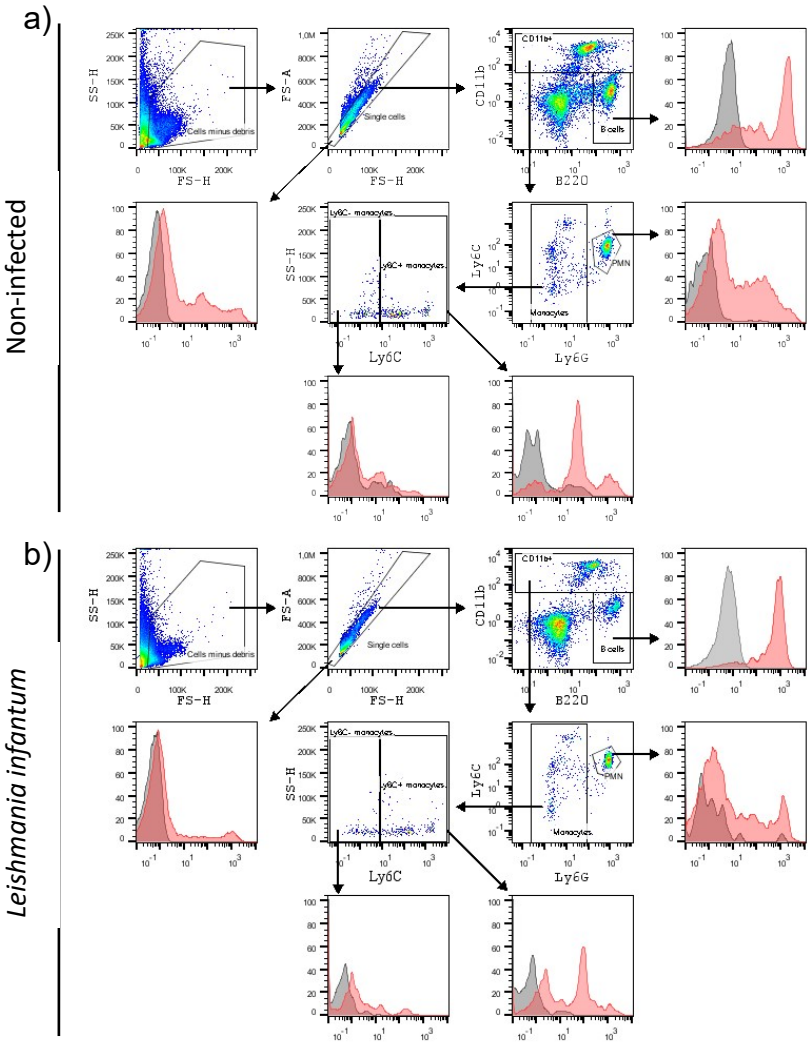

Figure 8. Immunophenotyping cells from the monocyte-phagocytic system in mouse blood of a) non-infected b) *L. infantum* infected mice at 0.5 mg/kg PGA-DiD NP. Blood cells were prepared as described in material and methods. Debris (SS-H vs FS-H) and doublets (FS-A vs FS-H) were excluded from mouse splenocytes. After gating out B220+ cells (B cells), CD11b+ cells were divided into two populations (Ly6C vs Ly6G): Ly6G+ (PMN) and monocytes (Ly6G-). This last population was further divided into Ly6C+ and Ly6C- monocytes. The MFI of each population for APC of the control (grey histogram) and NP+ (a) red histogram is represented.

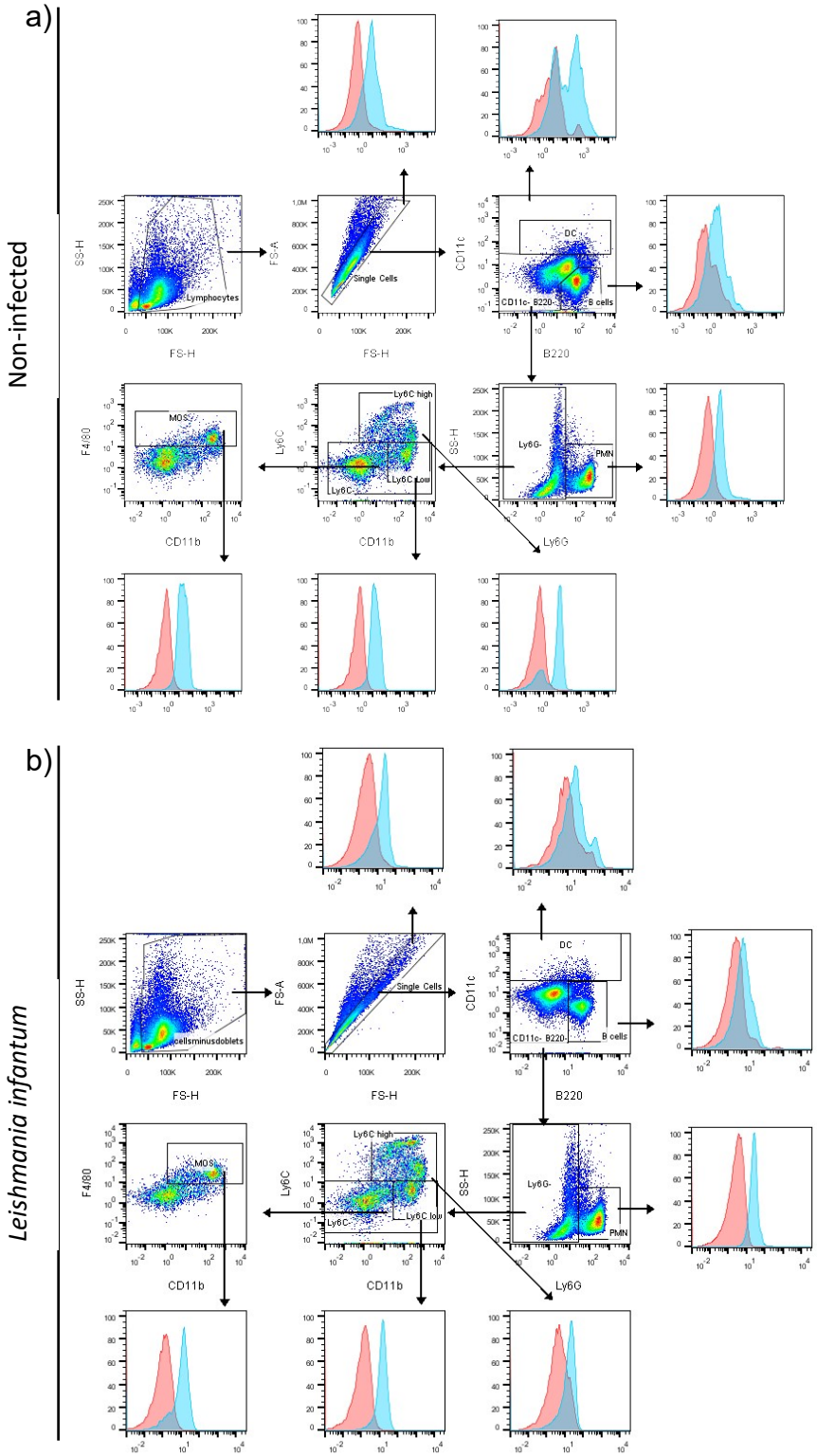

Figure 9. Immunophenotyping cells from the monocyte-phagocytic system in mouse BM of a) non-infected b*) L. infantum* infected mice at 0.2 mg/kg PS NP. Blood cells were prepared as described in material and methods. Debris (SS-H vs FS-H) and doublets (FS-A vs FS-H) were excluded from mouse splenocytes. After gating out B220+ cells (B cells) and CD11b+ cells (DC), Ly6G+ (PMN) were gated out and the remaining Ly6G- cells were divided into three populations (Ly6C vs CD11b): Ly6C^{high}, Ly6C^{low} and Ly6C-. Finally, MOS subset (F4/80+) was gated from Ly6C- (F4/80 vs CD11b). The MFI of each population for AF700 of the control (red histogram) and NP+ (a) blue histogram is represented.

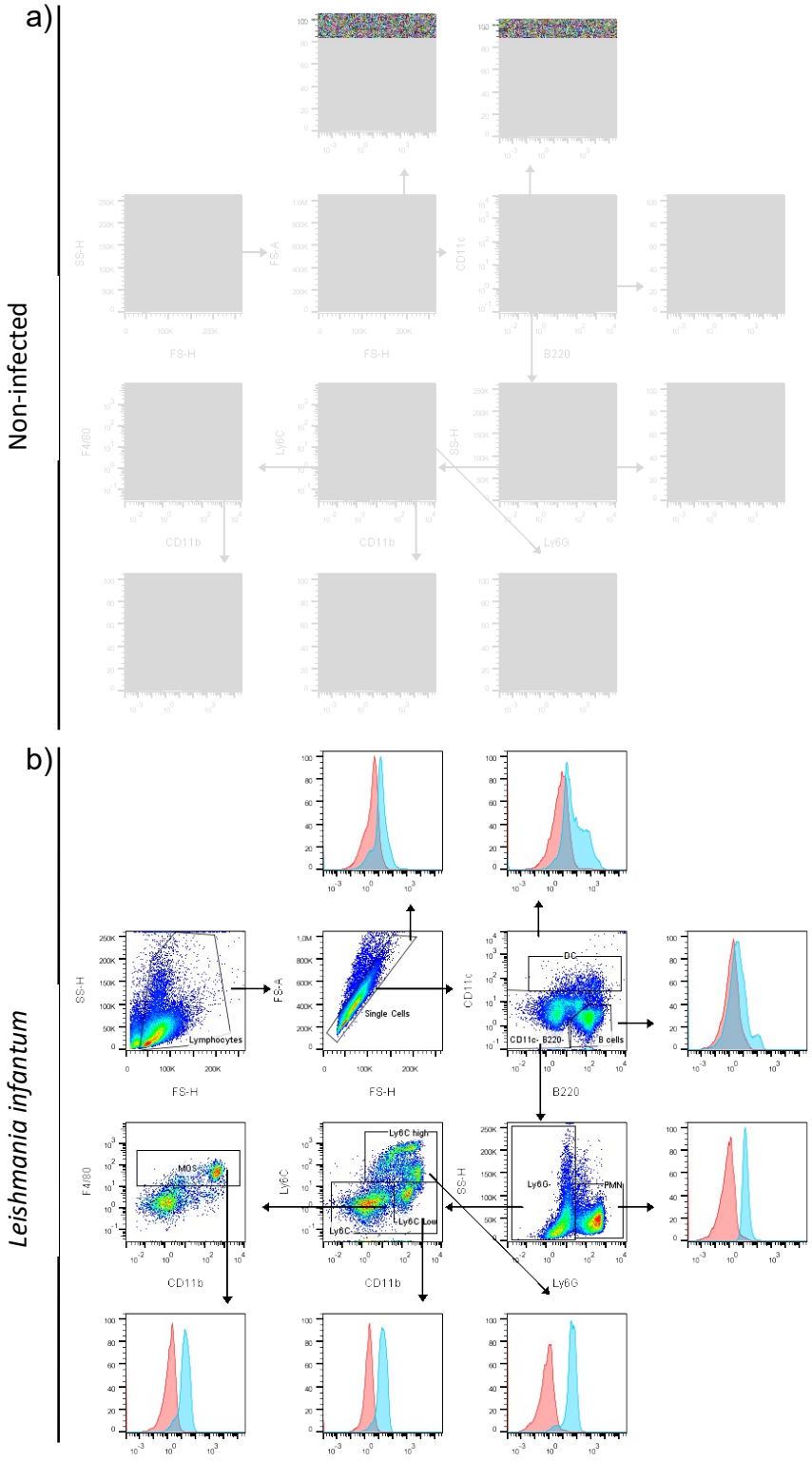

Figure 10. Immunophenotyping cells from the monocyte-phagocytic system in mouse BM of a) non-infected b) *L. infantum* infected mice at 0.5 mg/kg PLGA NP. Blood cells were prepared as described in material and methods. Debris (SS-H vs FS-H) and doublets (FS-A vs FS-H) were excluded from mouse splenocytes. After gating out B220+ cells (B cells) and CD11b+ cells (DC), Ly6G+ (PMN) were gated out and the remaining Ly6G- cells were divided into three populations (Ly6C vs CD11b): Ly6C^{high}, Ly6C^{low} and Ly6C-. Finally, MOS subset (F4/80+) was gated from Ly6C- (F4/80 vs CD11b). The MFI of each population for AF700 of the control (red histogram) and NP+ (a) blue histogram is represented.