Immunotherapy using anti-PD-1 and anti-PD-L1 in *Leishmania amazonensis*-infected BALB/c mice reduce parasite load.

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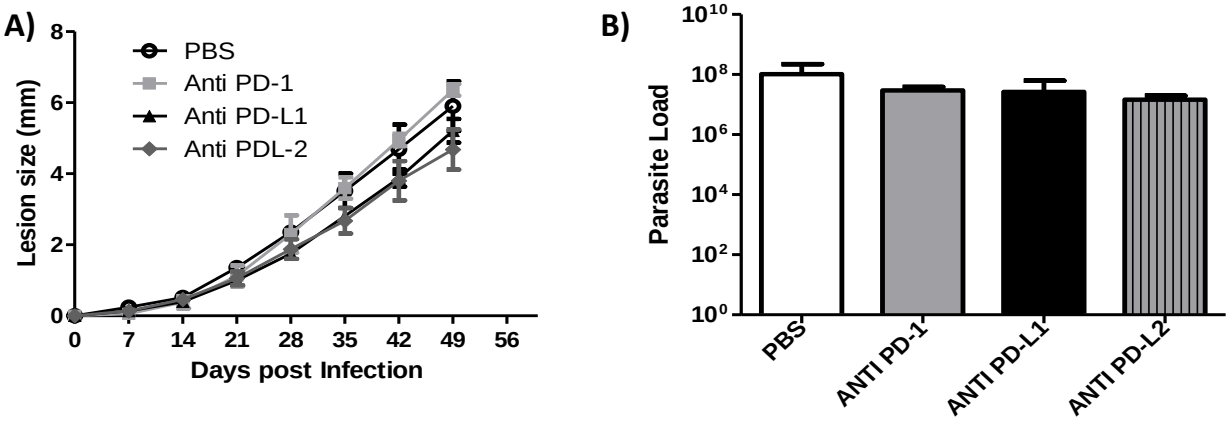
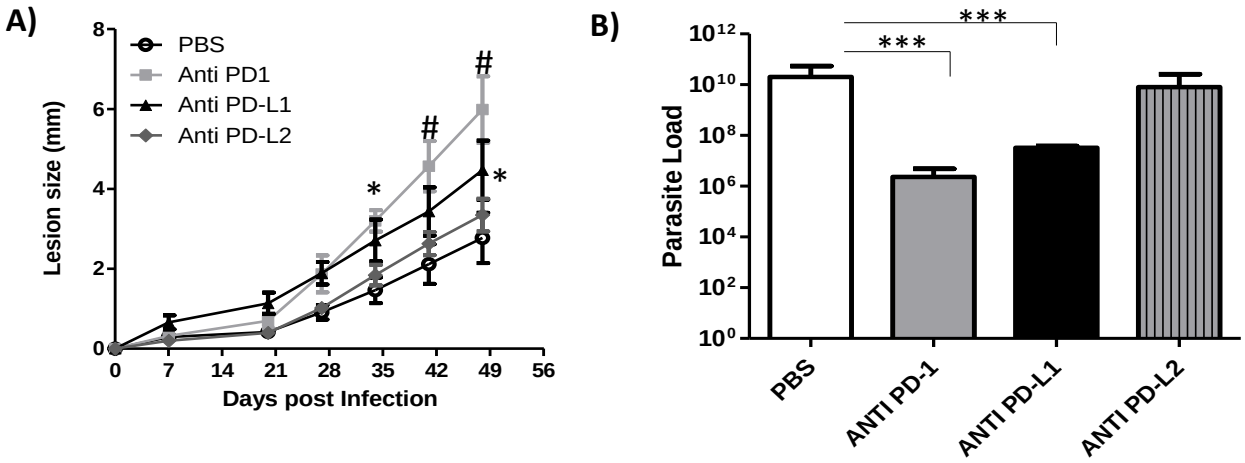
Supplementary Information:

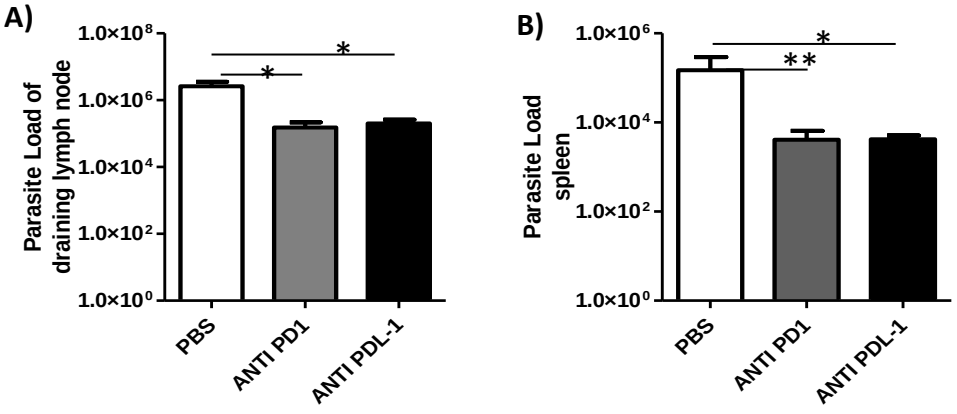
Suppl. Fig. 1: Ineffectiveness of treatment with a weekly dose of specific antibodies. Mice were infected in the footpad with *L. amazonensis* promastigotes ($2x10^6$) and treated with anti-PD-1, anti-PD-L1 or anti-PD-L2, all at a 100 µg/dose, administered once a week intraperitoneally beginning at 7 days post-infection. (A) Progression of the lesion. (B) Parasite load. Data ± SEM of individually mice (5 mice/group) .



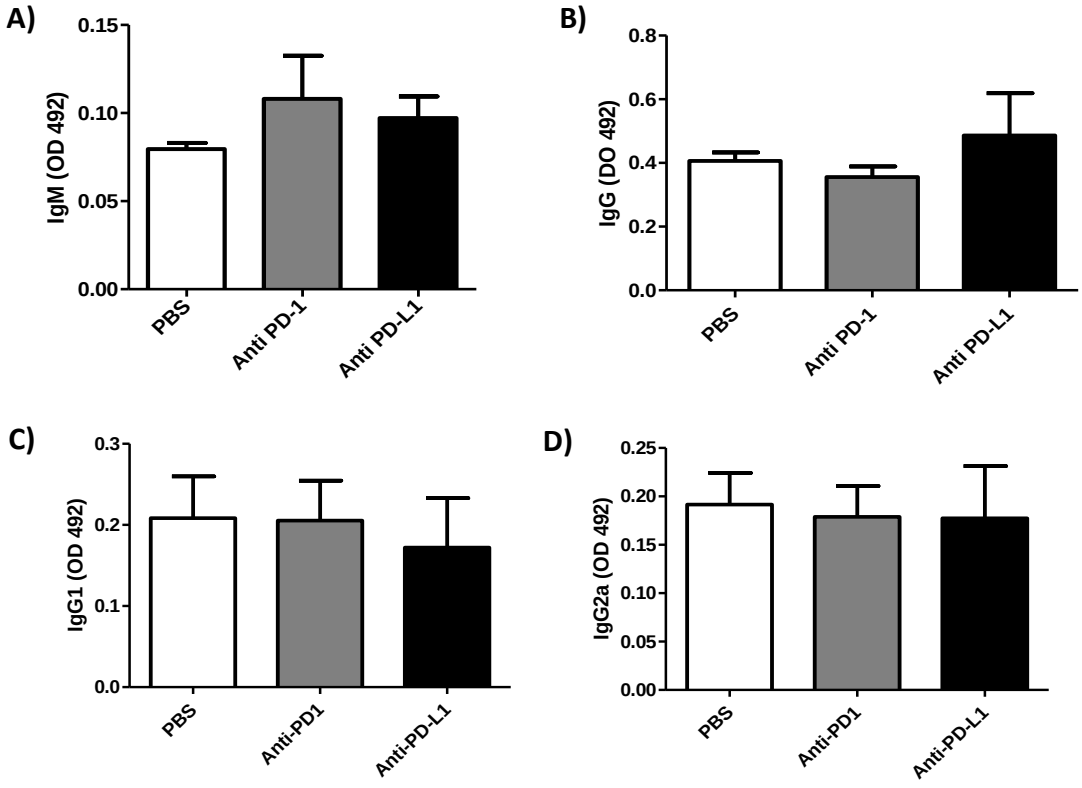
Suppl. Fig. 2: Unaltered lesion growth and control of parasite load from two weekly doses of specific antibodies. Mice were infected in the footpad with *L. amazonensis* promastigotes ($2x10^6$) and treated with anti-PD-1, anti-PD- L1 or anti-PD-L2, all at a 100 µg/dose, administered twice per week intraperitoneally beginning at 7 days post-infection. (A) Progression of the lesion. (B) Parasite load of paw analyzed by limiting dilution. Data ± SEM of individually mice (5 mice/group). ***p <0.0001 (T Test).



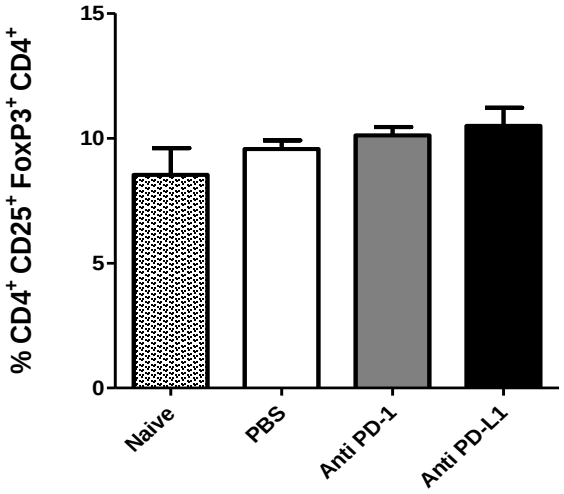
Suppl. Fig. 3: Control of parasite load from two weekly doses of specific antibodies. Mice were infected in the footpad with *L. amazonensis* promastigotes ($2x10^6$) and treated with anti-PD-1, anti-PD-L1, all at a 100 µg/dose, administered twice per week intraperitoneally beginning at 7 days post-infection. (A) Parasite load of draining lymph node. (B) Parasite load of spleen. Data ± SEM of individually mice (5 mice/group). *p<0.05, **p<0.0375. (T test (A) and Mann Whitney test. (B)).



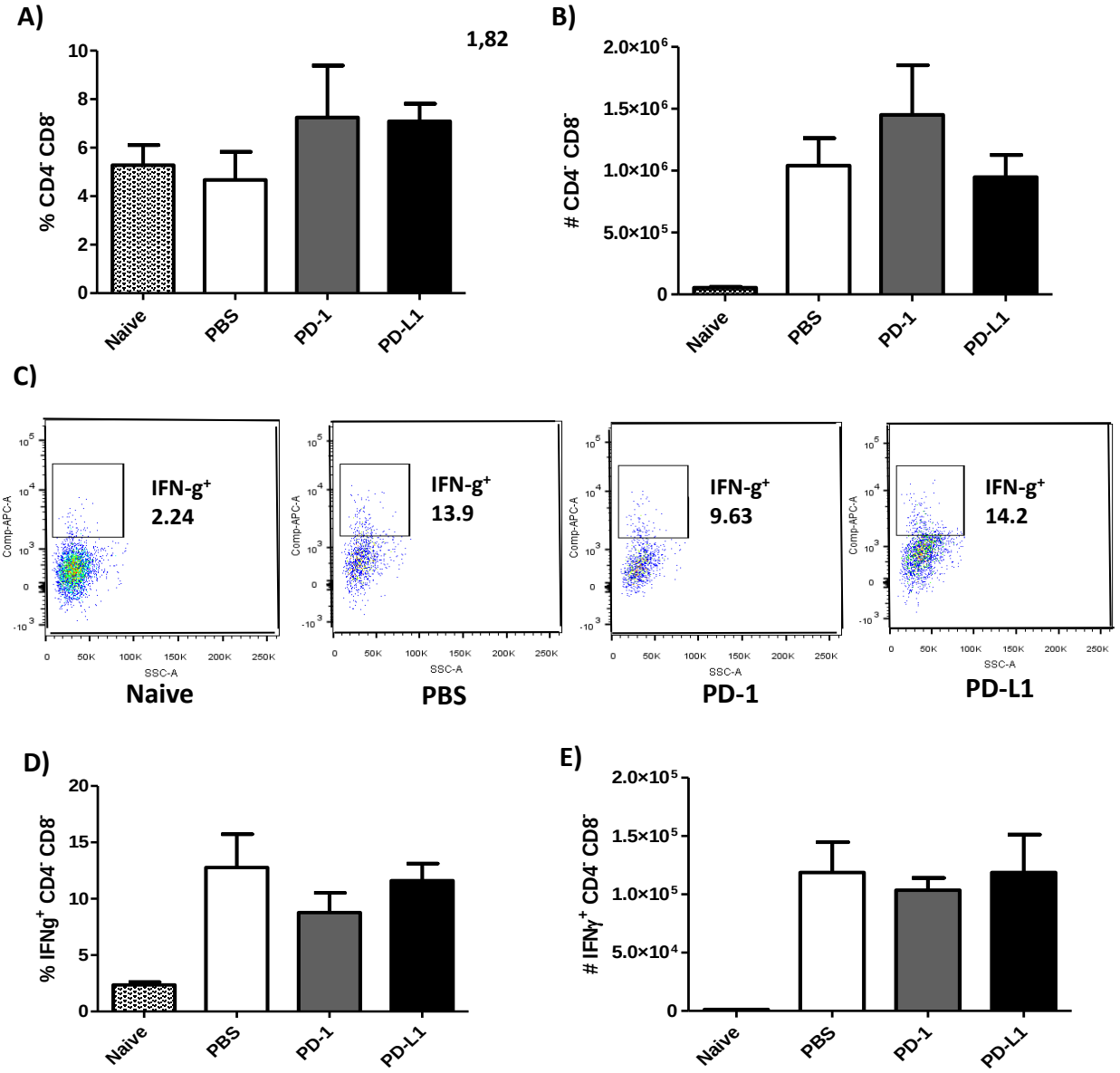
Suppl. Fig. 4: Effects of MoAb treatment on the specific anti-*Leishmania* immunoglobulins. (A) Specific IgM. (B) IgG. (C) IgG1. (D) IgG2a. Antibodies were detected in the serum (IgM, IgG and IgG1- diluted 1:500; IgG2a- diluted 1:40) of *L. amazonensis*-infected mice through ELISA using total *L. amazonensis* antigens (1 µg/well). Animals were treated for 49 days with antibodies administered twice a week intraperitoneally, starting at 7 days post- infection. Data ± SEM of individually mice (5 mice/group) are representative of two independent experiments producing the same result profile.



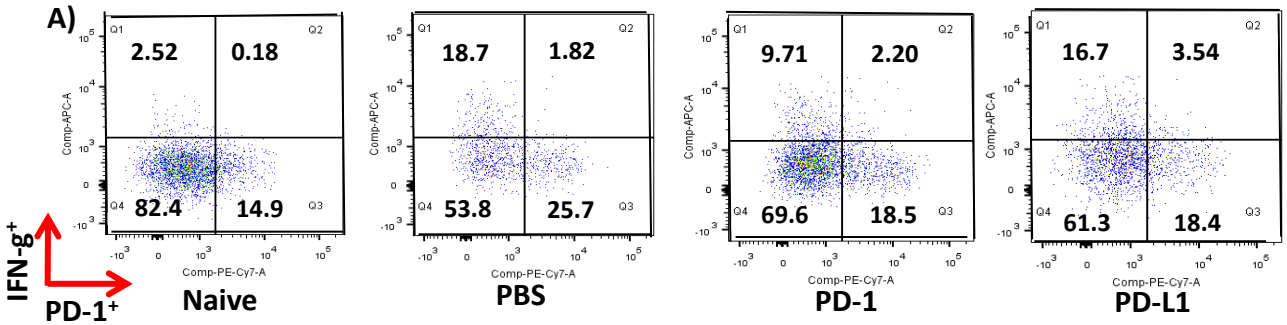
Suppl. Fig. 5: Treg cells in *L. amazonensis*-infected BALB/c mice. Mice were infected in the footpad with *L. amazonensis* promastigotes ($2x10^6$) and treated with anti-PD-1, anti-PD-L1 or anti-PD-L2, all at a 100 µg/dose administered twice per week intraperitoneally beginning at 7 days post-infection. Naive mice were used as control. Percentage of Treg cells. Data ± SEM of individually mice (5 mice/group) are representative of two independent experiments producing the same result profile.

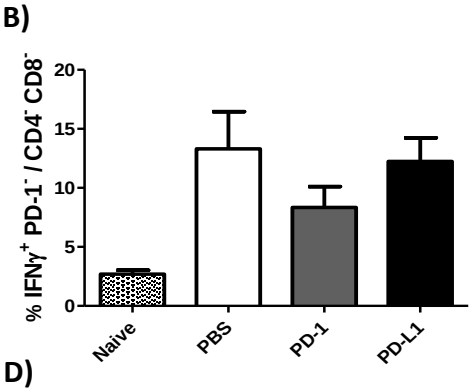


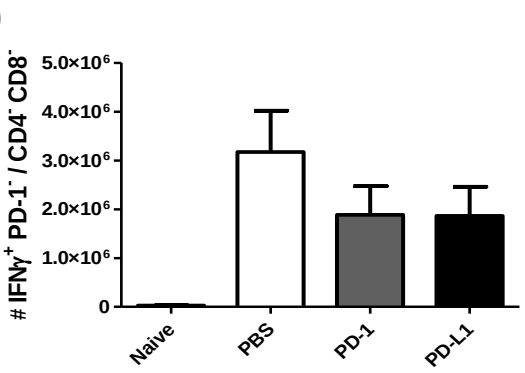
Suppl. Fig. 6: Analyse of IFN-ɣ production on $CD3^+CD4^-$ $CD8^-$. Lymphocytes were collected from the popliteal lymph node of an *L. amazonensis*-infected paw after approximately 2 months of treatment with anti-PD-1 or anti-PD-L1, administered twice a week intraperitoneally beginning after 7 days of infection.
(A) Percentage of $CD4^-$ $CD8^-$ T cells. (B) Number of $CD4^-$ $CD8^-$ T cells. (C) Dot plot of IFN-ɣ expression (APC-IFN-ɣ, SSC-A) . (D) Percentage of IFN-$ɣ^+$ $CD4^-$ $CD8^-$ T cells. (E) Number of IFN-$ɣ^+$ $CD4^-$ $CD8^-$ T cells. Naive = mice without infection and therapy, PBS = infected mice injected with PBS on treatment days, PD-1 = mice infected and treated with anti-PD-1 (100 µg/dose), PD-L1 = mice infected and treated with anti-PD-L1 (100 µg/dose). Data ± SEM of individually mice (5 mice/group) are representative of two independent experiments producing the same result profile.

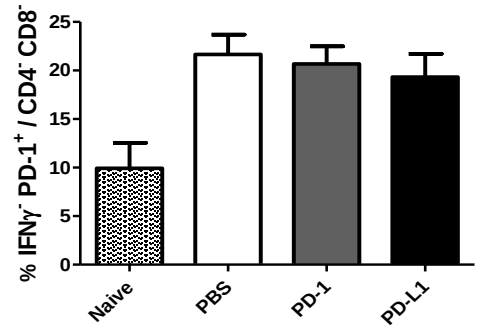


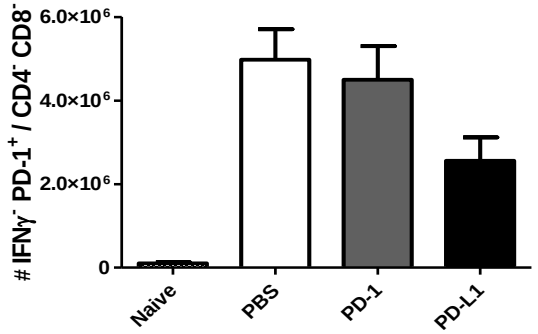
Suppl. Fig. 7: Analyse of IFN-ɣ$^+$PD-1$^+$ CD4$^-$ CD8$^-$ T cells after anti-PD-1 and anti-PD-L1 MoAb treatment. Lymphocytes were collected from the popliteal lymph node of an *L. amazonensis*-infected paw after approximately 2 months of treatment with anti-PD-1 or anti-PD-L1, administered twice a week intraperitoneally beginning after 7 days of infection. (A) Dot plot of IFN-γ and PD-1 expression (APC-IFN-ɣ, PE-Cy7-PD-1). (B) Percentage of IFN-ɣ$^+$ PD-1$^-$ CD4$^-$ CD8$^-$ T cells. (C) Number of IFN-ɣ$^+$PD-1$^-$CD4$^-$ CD8$^-$ T cells. (D) Percentage of IFN-ɣ$^-$PD-1$^+$ CD4$^-$ CD8$^-$ T cells. (E) Number of IFN-ɣ$^-$ PD-1$^+$ CD4$^-$ CD8$^-$ T cells. (F) Percentage of IFN-ɣ$^+$PD-1$^+$ CD4$^-$ CD8$^-$ T cells. (G) Number of IFN-ɣ$^+$PD-1$^+$ CD4$^-$ CD8$^-$ T cells. Naive = mice without infection and therapy, PBS = infected mice injected with PBS on treatment days, PD-1= mice infected and treated with anti-PD-1 (100 µg/dose), PD-L1= mice infected and treated with anti-PD-L1 (100 µg/dose). Data ± SEM of individually mice (5 mice/group) are representative of two independent experiments producing the same result profile.

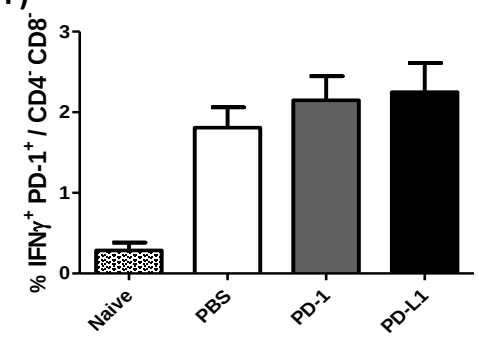


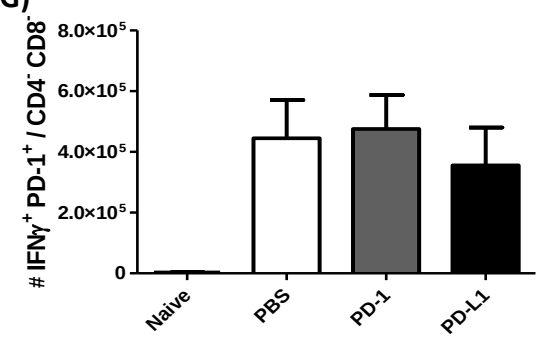




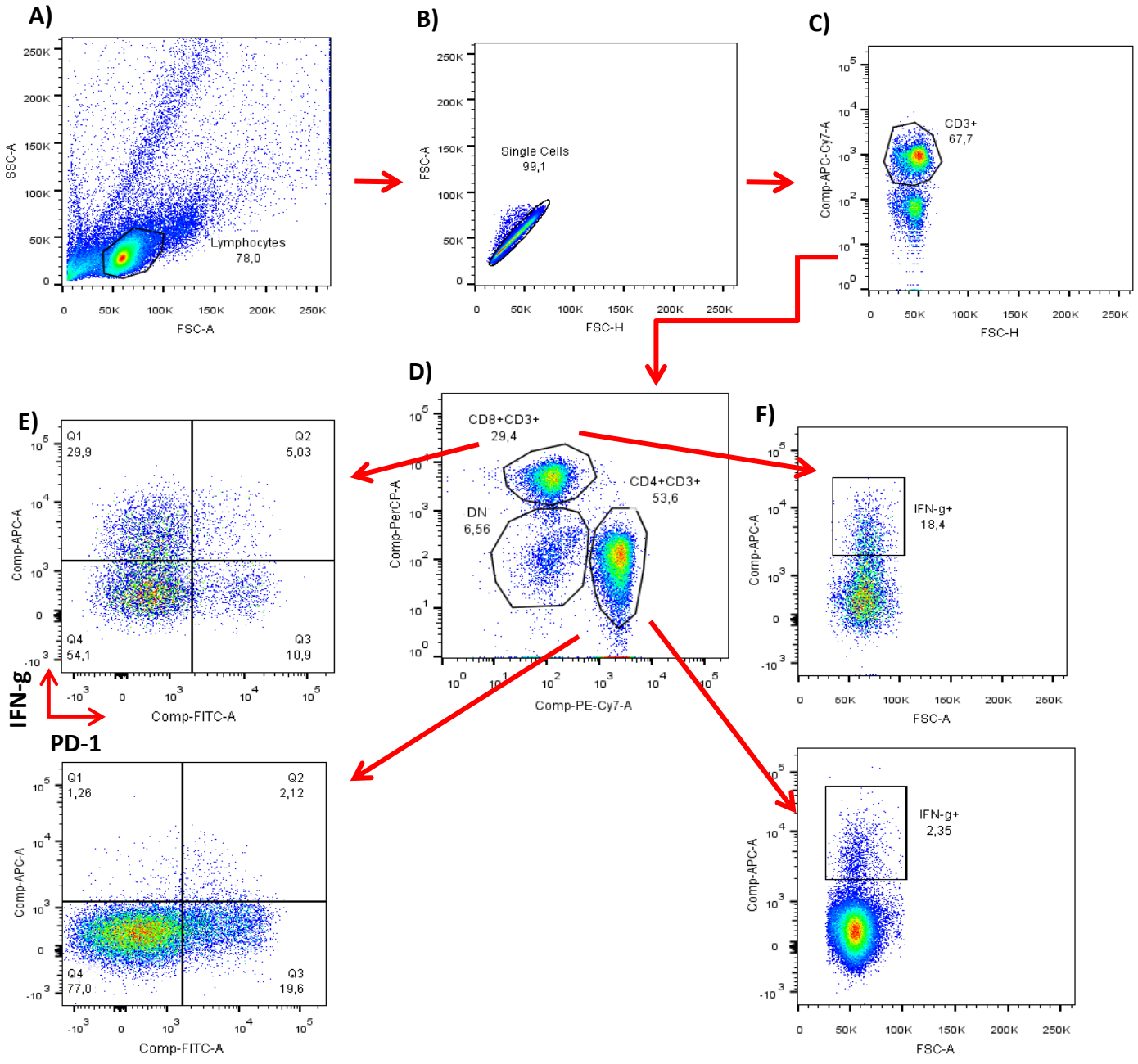








Suppl. Fig. 8: Strategy of gate of CD4 and CD8 T cells. (A) Lymphocytes- FSC x SSC. (B) Single cells- FSC-A x FSC-H. (c) $CD3^+$-APC cy7 x FSC-H. (D) $CD8^+CD3^+$-PerCP x $CD4^+CD3^+$-PE cy7 x DN- double negative. (E) IFN-ɣ- APC x PD-1- FITC . (F) IFN-ɣ- APC x FSC-A.



Suppl. Fig. 9: Strategy of gate of PD-L1^{+} CD11c^{+}. (A) Cells- FSC x SSC. (B) Single cells- FSC-A x FSC-H. (c) CD11c^{+}- PerCP x SSC-H. (D) Histogram of PD-L1 expression. Red= negative of PD-L1, Blue= naïve and Orange= PD-L1^{+} (APC-PD-L1).