

Pro-cellular exhaustion markers are associated with splenic microarchitecture disorganization and parasite load in dogs with visceral leishmaniasis

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Supplementary Table 1

Target genes and their sequences used in quantification of the parasite load. S, sense; AS, antisense, bp, base pairs

Target Gene	Access number or reference		Sequence (5'-3')	Base pair size (bp)
HPRT Canine target	NW_003726126. 1	S	AAAACAATGCAGACTTTGCT	58
		AS	CCTTGACCATCTTTGGATTA	
SsrRNA Parasite target	Prina et al. (2007)	S	TACTGGGGCGTCAGAG	153
<i>Leishmania infantum</i>	(L).	AS	GGGTGTCATCGTTTGC	

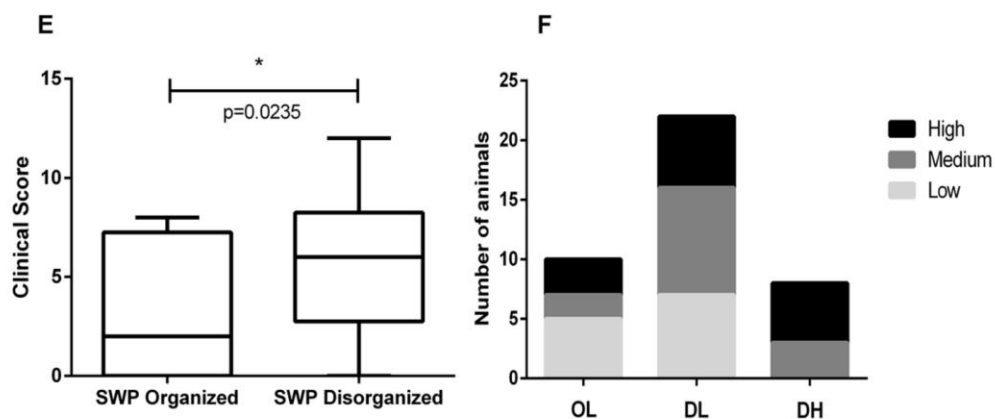
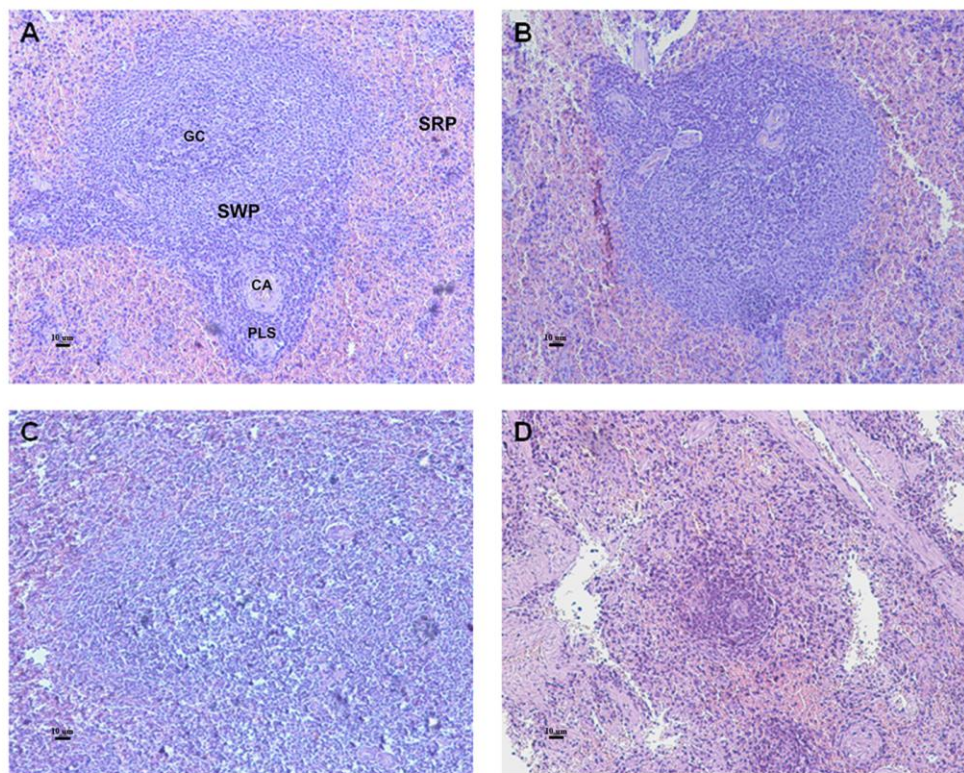
Supplementary Table 2

Target genes and primer design for cDNA. S, sense; AS, antisense, bp, base pairs

Target gene	Access number		Sequence (5'-3')	Base pair size (bp)
HPRT	NW_003726126.1	S	AAAACAATGCAGACTTTGCT	58
		AS	CCTTGACCATCTTTGGATTA	
GAPDH	XM_003434387.2	S	ATGCCCAACATTGGTTATGG	103
		AS	CTCTTTCCACGATGGCTTTG	
PD-1	XM_022409577.1	S	TCCACGAACACTCAGAAGCC	136
		AS	TTCCTACTCCCCTTCAGGCA	
PD-L1	AB_850883.1	S	CCGCCAGCAGGTCACTTC	139
		AS	CCATTGTCACATTGCCACCA	
PD-L2	XM_014117874.2	S	CCTTGAAGTGGTGTGGCAGA	132
		AS	TGCACTCCCAGGCTCAAAT	
LAG-3	XM_005637438.2	S	AGTCATCACAGTGAAGTCCCA	132
		AS	GAACCTCGCCAAGACTGCTT	

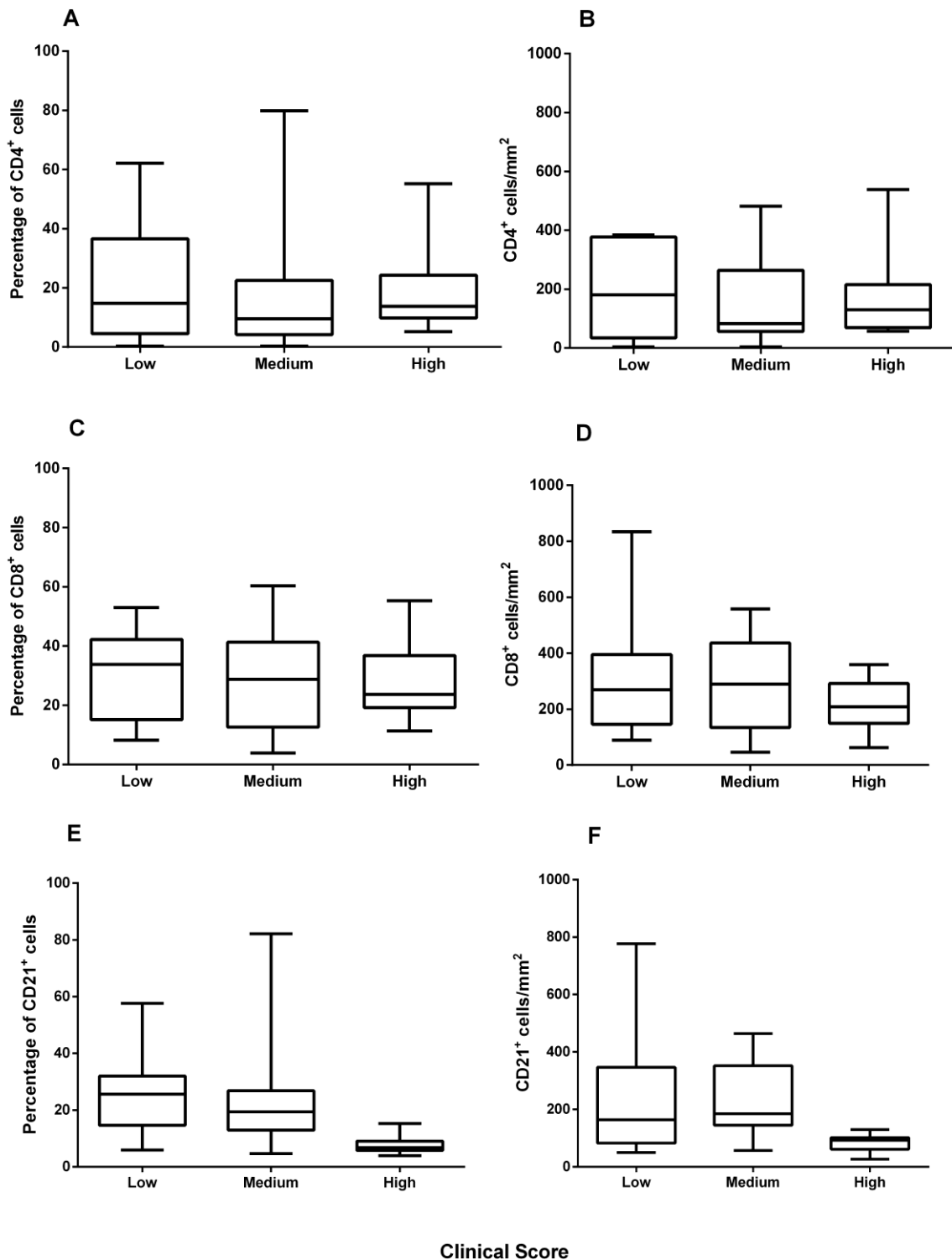
Supplementary Figure S1

Representative images of the splenic white pulp (SWP) of dogs naturally infected with *L. infantum*. (A) Organized SWP, (B) slightly disorganized SWP, (C), SWP with medium disorganization, and (D), intensely disorganized SWP. Scale bar 10 μ m. SWP = Splenic White Pulp; GC = Germinal Centre; CA = Central Artery; PLS = Periarteriolar Lymphatic Sheath; and SRP = Splenic Red Pulp. (E) Clinical score observed according to the degree of organization of the SWP. Mann-Whitney test: * $p=0,0235$. (F) Quantitative analysis of the dogs in the different groups according to the clinical score and splenic disorganization/parasite load. Organized SWP: Animals with SWP organized to slightly disorganized. SWP disorganized: animals with medium to intense disorganization of the SWP.



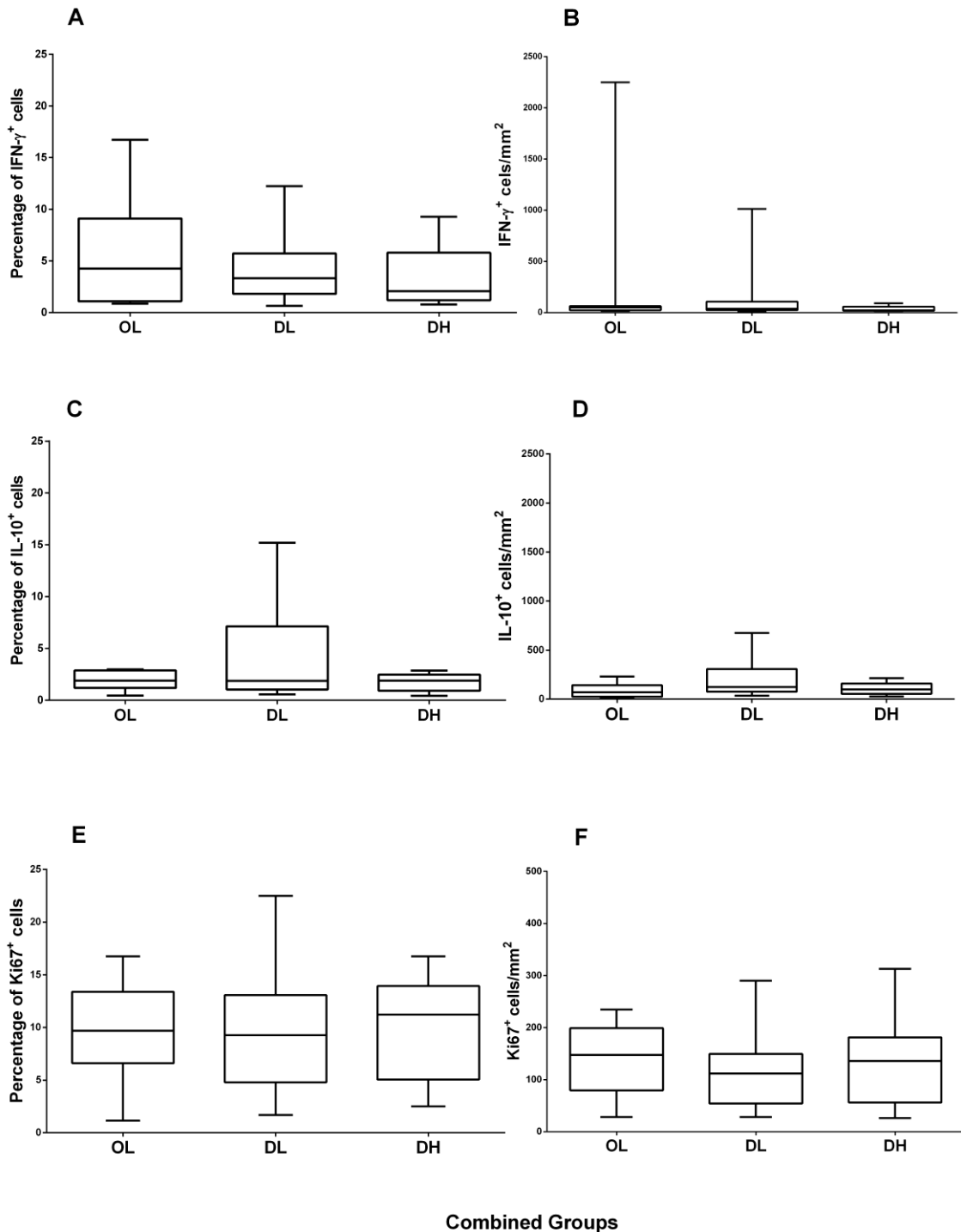
Supplementary Figure S2

Cell profile observed in the spleen of dogs naturally infected with *L. infantum* according to the clinical score: (A) percentage of CD4⁺ cells, (B) CD4⁺ cells/mm², (C) percentage of CD8⁺ cells, (D) CD8⁺ cells/mm², (E) percentage of CD21⁺ cells and (F) CD21⁺ cells/mm². Mann-Whitney and Kruskal-Wallis tests: $p > 0.05$.



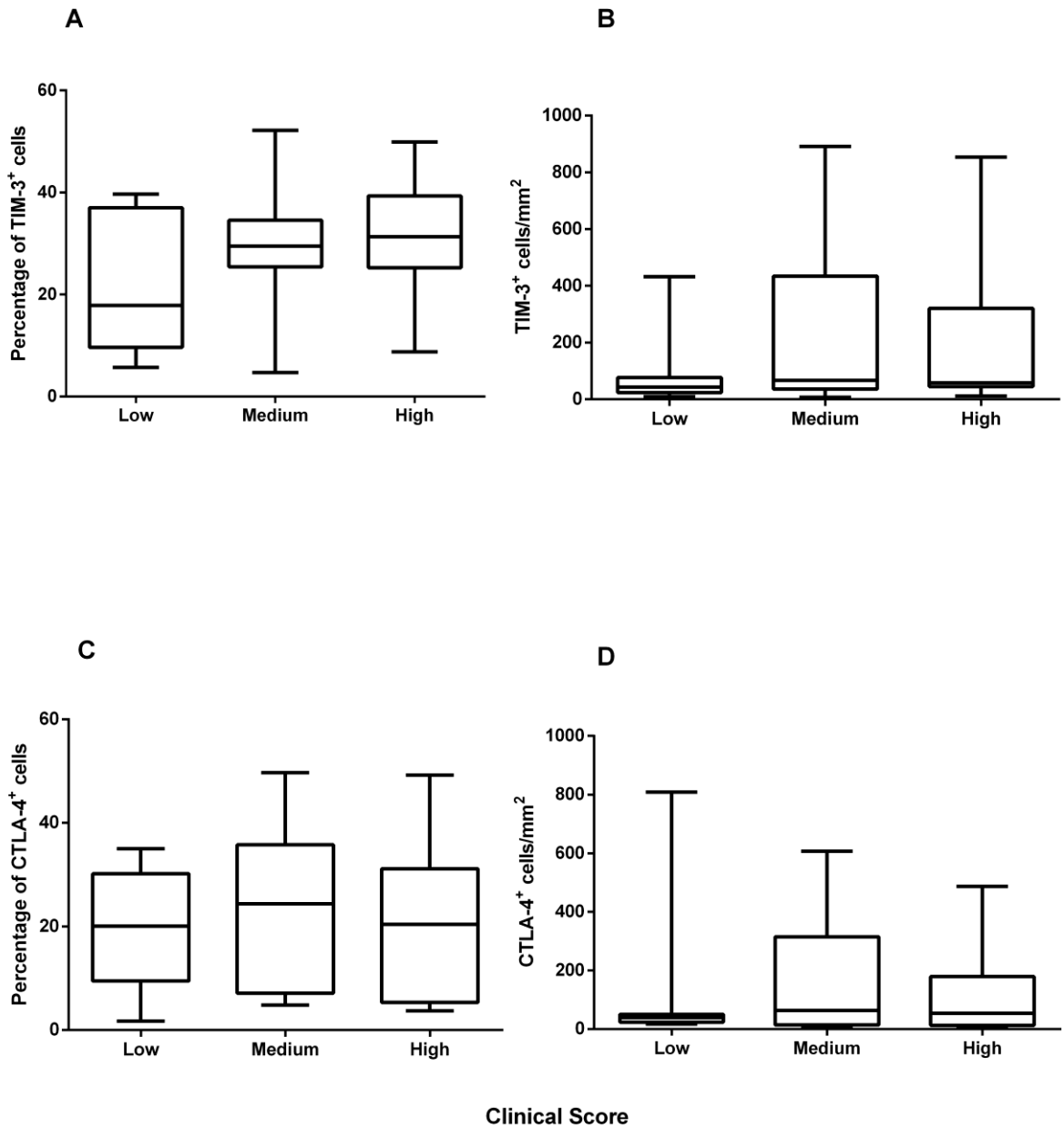
Supplementary Figure S3

Cell profile observed in the spleen of dogs naturally infected by *L. infantum* grouped according to the disorganization of the white splenic pulp and the parasite load: (A) percentage of IFN- γ ⁺ cells, (B) IFN- γ ⁺/mm² cells, (C) percentage of IL-10⁺ cells, (D) IL-10⁺ cells/mm², (E) percentage of Ki-67⁺ cells, and (F) Ki-67⁺ cells/mm². Mann-Whitney and Kruskal-Wallis tests: $p > 0.05$.



Supplementary Figure S4

Cellular exhaustion profile observed in the spleen of dogs naturally infected with *L. infantum* according to the clinical score: (A) percentage of TIM-3⁺ cells, (B) TIM-3⁺/mm², (C) percentage of CTLA-4⁺ cells, and (D) CTLA-4⁺ cells/mm². Mann-Whitney and Kruskal-Wallis tests: p>0.05.



Supplementary Figure S5

Quantitative analysis by flow cytometry of CD4⁺ and CD8⁺ subpopulations expressing the exhaustion receptors TIM-3 and CTLA-4. (A) Representative images of lymphocyte gates, histograms of CTLA-4- and TIM-3-positive cells. **(B)** Percentage of CD4⁺ cells, **(C)** CD4⁺CTLA-4⁺ cells, **(D)** CD4⁺TIM-3⁺ cells, **(E)** CD8⁺ cells, **(F)** CD8⁺CTLA-4⁺ cells, **(G)** CD8⁺TIM-3⁺ cells among groups. OL: organized SWP and low parasite load; DL: disorganized SWP and low parasite load; DH: disorganized SWP and high parasite load.

