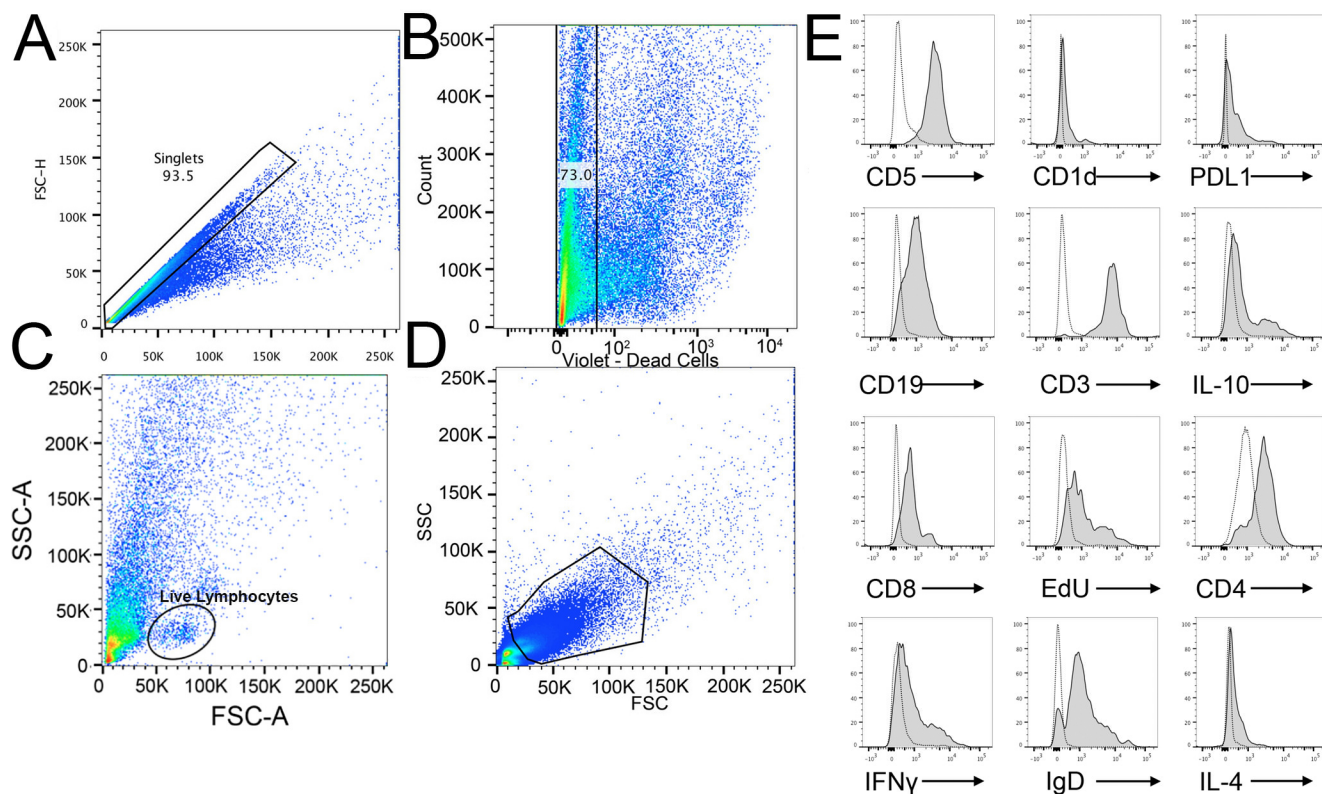


Supplemental Table 1. Canine History of PCR and Serological Status

Sex	Group	Test 1 Titer	Test 1 PCR	Test 1 DPP	Test 2 Titer	Test 2 PCR
F	Oligosymptomatic	512	Positive	N/A	256	Positive
M	Asymptomatic	128	Negative	Negative	32	Negative
M	Oligosymptomatic	128	Positive	Positive	512	Positive
F	Negative	64	Negative	Positive	16	Negative
M	Asymptomatic	128	Negative	Positive	64	Negative
F	Oligosymptomatic	128	Negative	Positive	256	Borderline
F	Oligosymptomatic	256	Negative	Negative	256	Positive
F	Oligosymptomatic	256	Negative	Positive	512	Borderline
M	Asymptomatic	0	Negative	Negative	128	Negative
F	Oligosymptomatic	128	Positive	N/A	512	Borderline
F	Asymptomatic	0	Negative	Negative	32	Negative
F	Asymptomatic	0	Negative	Negative	32	Borderline
M	Negative	32	Negative	Negative	16	Negative
F	Negative	16	Negative	Negative	0	Negative
F	Asymptomatic	0	Negative	Negative	32	Negative
F	Asymptomatic	0	Negative	Negative	32	Borderline
F	Negative	0	Negative	Negative	0	Negative
F	Negative	0	Negative	Negative	0	Positive
M	Oligosymptomatic	0	Positive	Positive	512	Positive
F	Asymptomatic	128	Negative	Positive	0	Positive
M	Asymptomatic	64	Negative	Negative	64	Negative

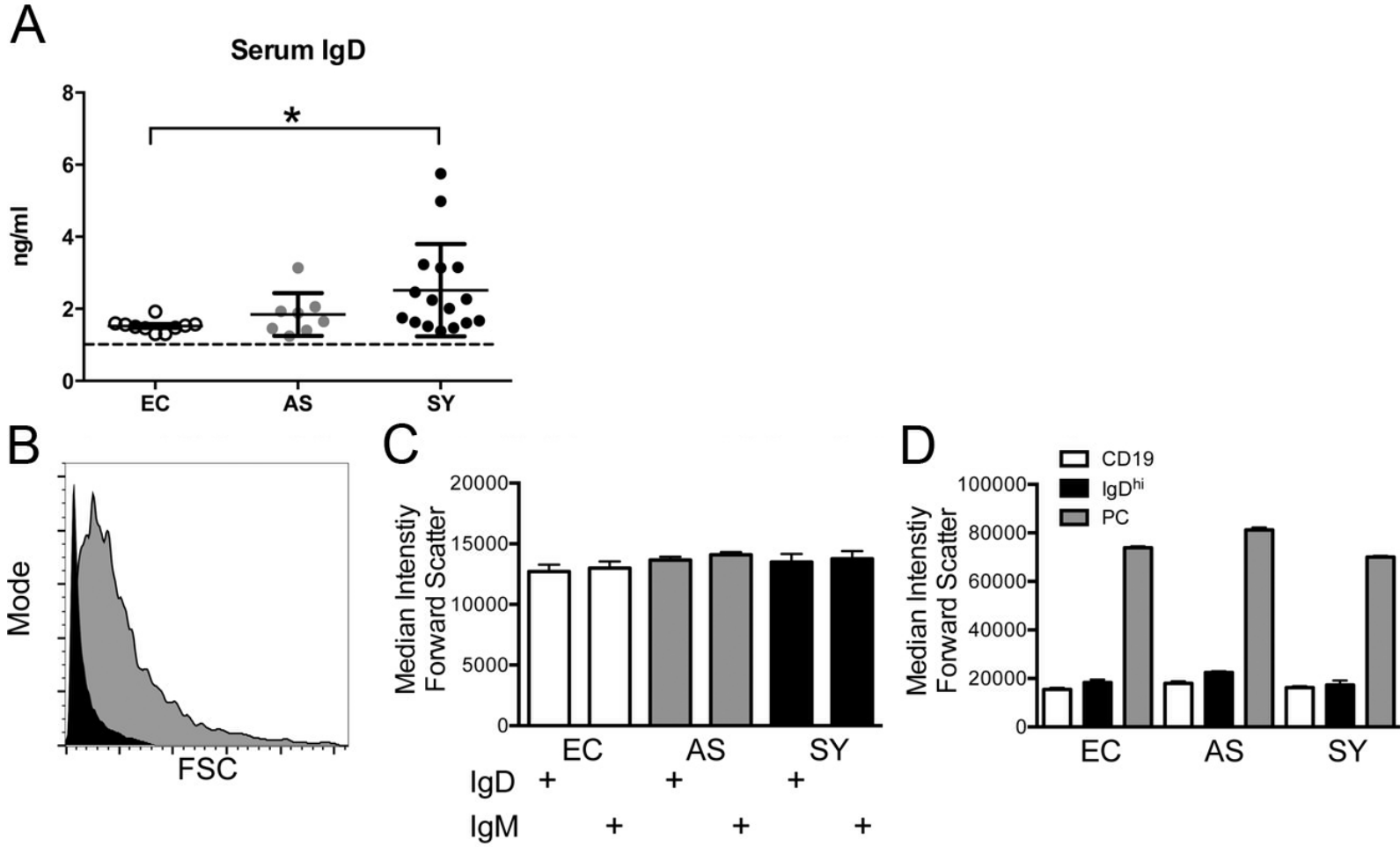
PCR Negative = 0/3 wells amplified, Borderline = 1/3 wells amplified, Positive 2/3 or 3/3 amplified. DPP (Dual Path Platform) = test which utilizes an immobilized antibody to detect Leishmania K39 antigen.

Supplemental Figure 1. Gating strategy.

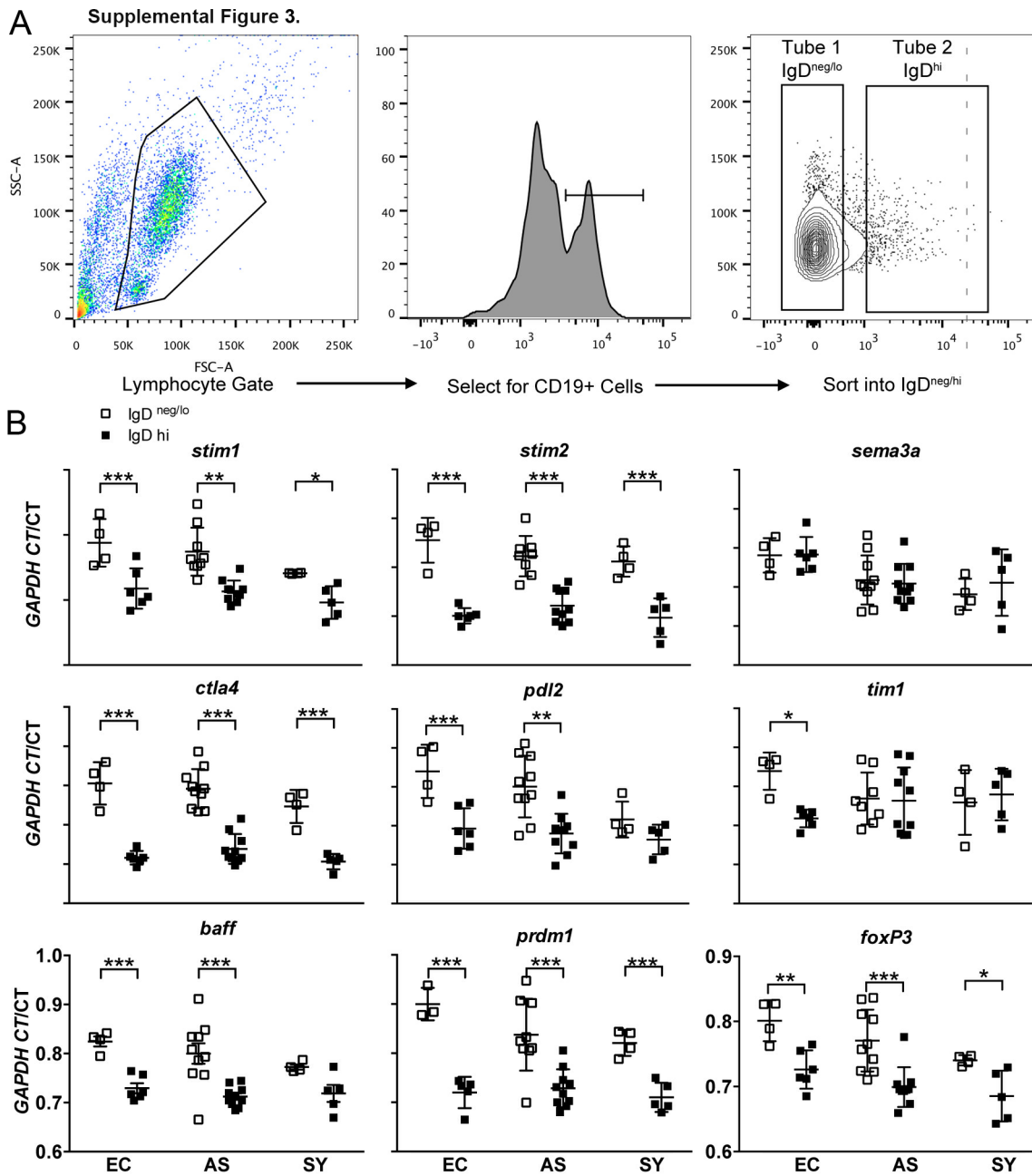


Supplemental Figure 1. Gating scheme and size analysis of IgD^{hi} B cells. (a) Exclusion of doublets by forward-scatter height analysis. (b) Exclusion of dead cells through dye incorporation. (c) Sample live lymphocyte gate. (d) Exclusion of debris by side-scatter, forward-scatter gate location. (e) Isotype control (dashed) compared to lymphocytes positive control (solid, grey) fluorescent labeling.

Supplemental Figure 2



Supplemental Figure 2. Serum levels of IgD increase as leishmaniasis progresses and IgD^{hi} B cells are not activated or increased in size. (A) Serum samples were assayed for IgD by ELISA in duplicate from endemic control, asymptomatic (AS) or symptomatic (SY) dogs. Standard curve was generated using a manufacturer's standard with known concentration of human IgD. Dots represent individual dogs. Bars are \pm SEM. N=10 for EC, 10 for AS, and 16 for SY. * $p < 0.05$, statistical analysis performed using one way-ANOVA with multiple comparisons of the mean. (B) IgD^{hi} (black) size as determined by forward light scatter (FSC) compared to CD19⁺ B cells which have been treated with TLR7/8 agonists (grey). (C) Comparison between IgM and IgD expressing B cells from endemic controls (EC), asymptomatic (AS) and symptomatic (SY) dogs. (D) Comparison of median intensity forward light scatter between CD19⁺ B cells, IgD⁺ B cells and CD19⁺ TLR7/8 stimulated-positive control (PC) B cells. Graphs are representative of n=7 per group and 2 experiments.



Supplemental Figure 3. IgD^{hi} B cells had differential targeted transcript expression compared to IgD^{lo} B cells consistent across clinical groups. (a) Gating scheme for isolation of IgD^{lo/neg} or IgD^{hi} B cells via fluorescent cell sorting. (b) Analysis of targeted B cell transcription factors, immune exhaustion-associated targets and regulatory B cell-associated molecules across experimental groups by IgD expression. Unpaired Student's t-test with Welch's correction was used for analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, N=7 per group.