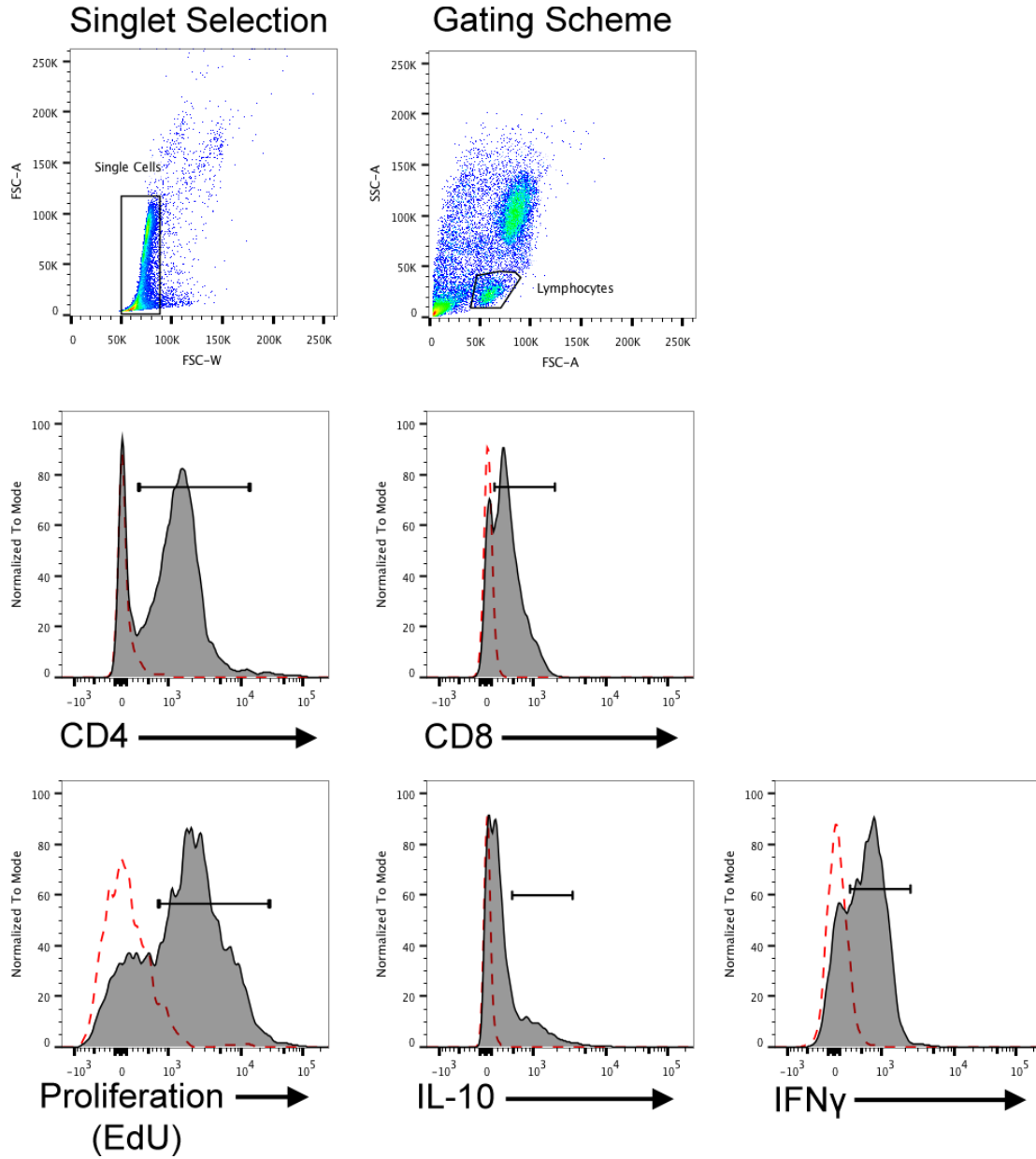


1 **Supplementary Materials for “Dampened vaccine antigen-specific T cell responses during**
2 ***Leishmania infantum* infection recovered by addition of TLR-agonist adjuvant”**

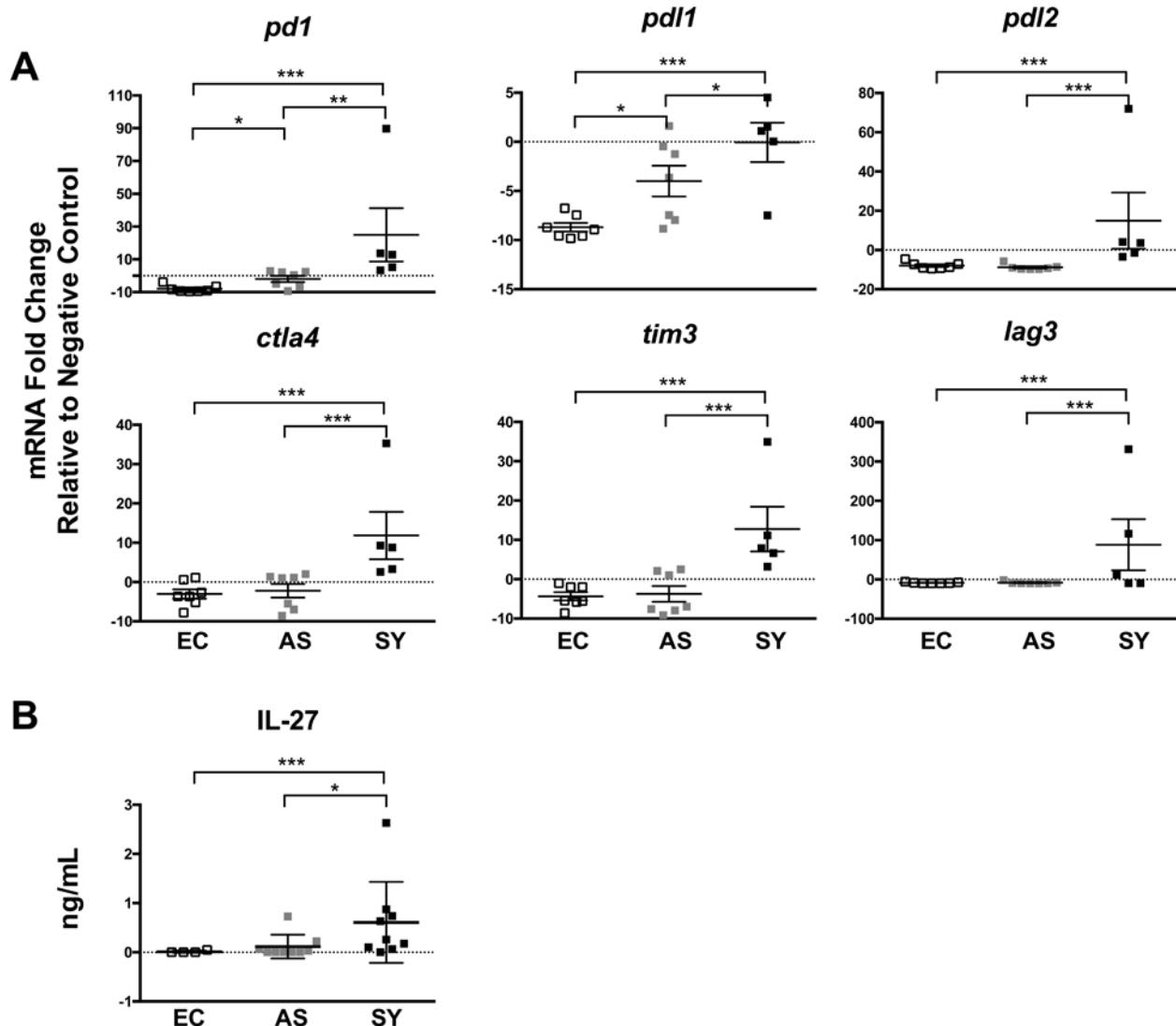
3



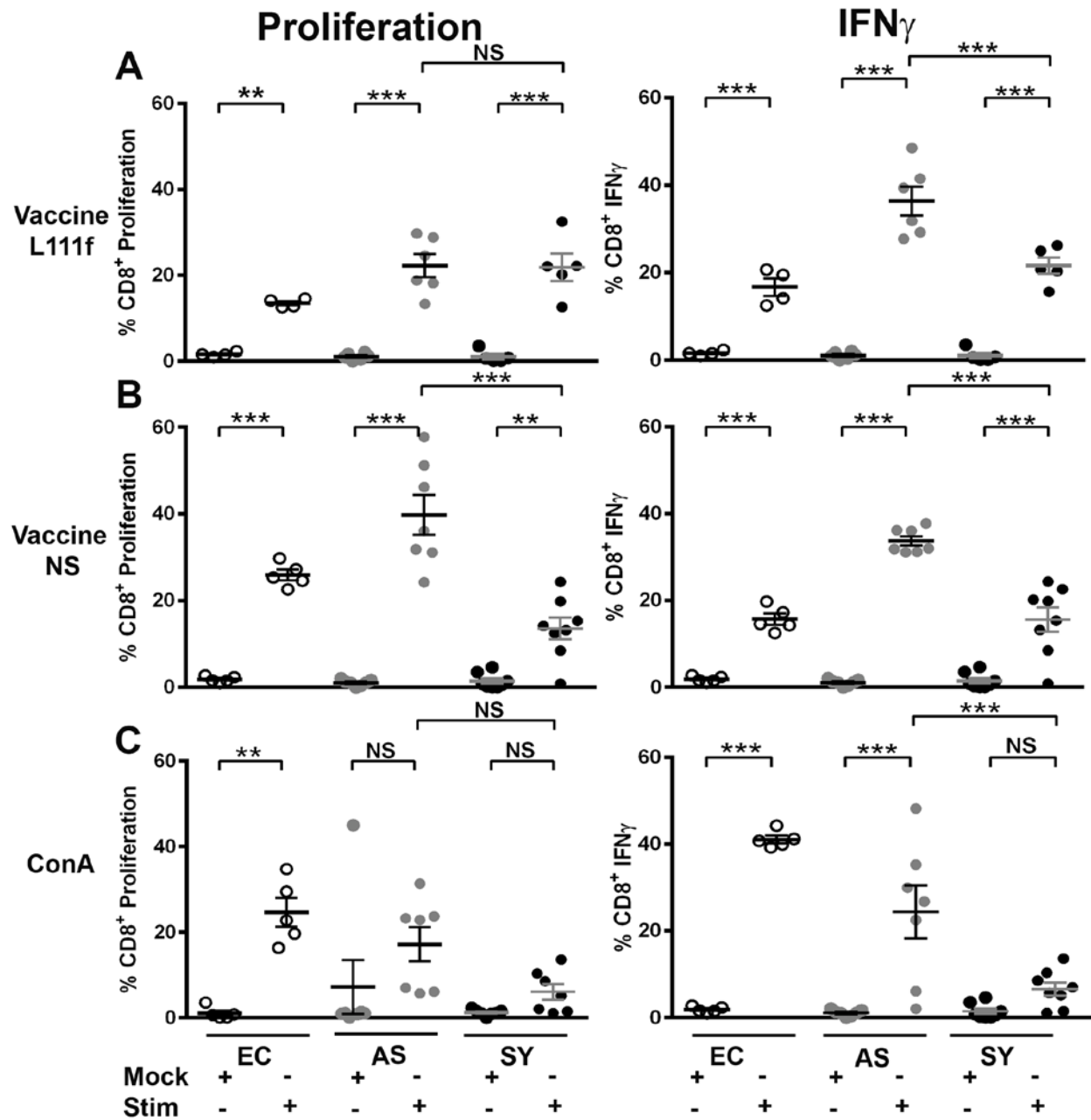
4

5

6 **Fig. S1. Gating scheme for flow cytometry.** Representative plots for single cell selection,
7 lymphocyte gate and gating of markers. Red dashed line is isotype control. Grey filled
8 histogram indicates labeled cells. Positive selection representative of ConA-treated endemic
9 control (EC) dogs demonstrated by representative histogram gate.



10
 11 **Fig. S2. Inhibitory receptor and ligand expression increased with VL progression in PBMC**
 12 **from same animals used for ex vivo vaccine immunogenicity studies.** (A) Differential
 13 expression of surface markers across infected groups. Healthy donor dogs from the local
 14 community were utilized as negative control for relative gene expression. qPCR data was
 15 generated utilizing primer sets and SYBR green chemistry. (B) IL-27 protein secretion supports
 16 exhausted cytokine environment in cells cultured from *L. infantum*- symptomatic dogs. Cell
 17 supernatants were harvested from unstimulated whole blood in culture at 7 days. 50 μ L of cell
 18 supernatant (1:2 dilution and 1:10 dilution) was added in duplicate to an anti-human IL-27
 19 specific coated high-binding ELISA plate. THP-1 cells were stimulated with 200 ng *E. coli* LPS
 20 and used as a positive control (not shown). EC = endemic control (open symbols), AS =
 21 asymptomatic, *L. infantum* positive (grey symbols), SY = symptomatic, *L. infantum* positive
 22 (black symbols). Error bars are \pm SEM. N=6-8 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
 23 Statistical analysis was performed via one way-ANOVA.



24

25 **Fig. S3. CD8⁺ T cell data corresponding to Fig. 2.** Whole blood was stimulated (“+” for

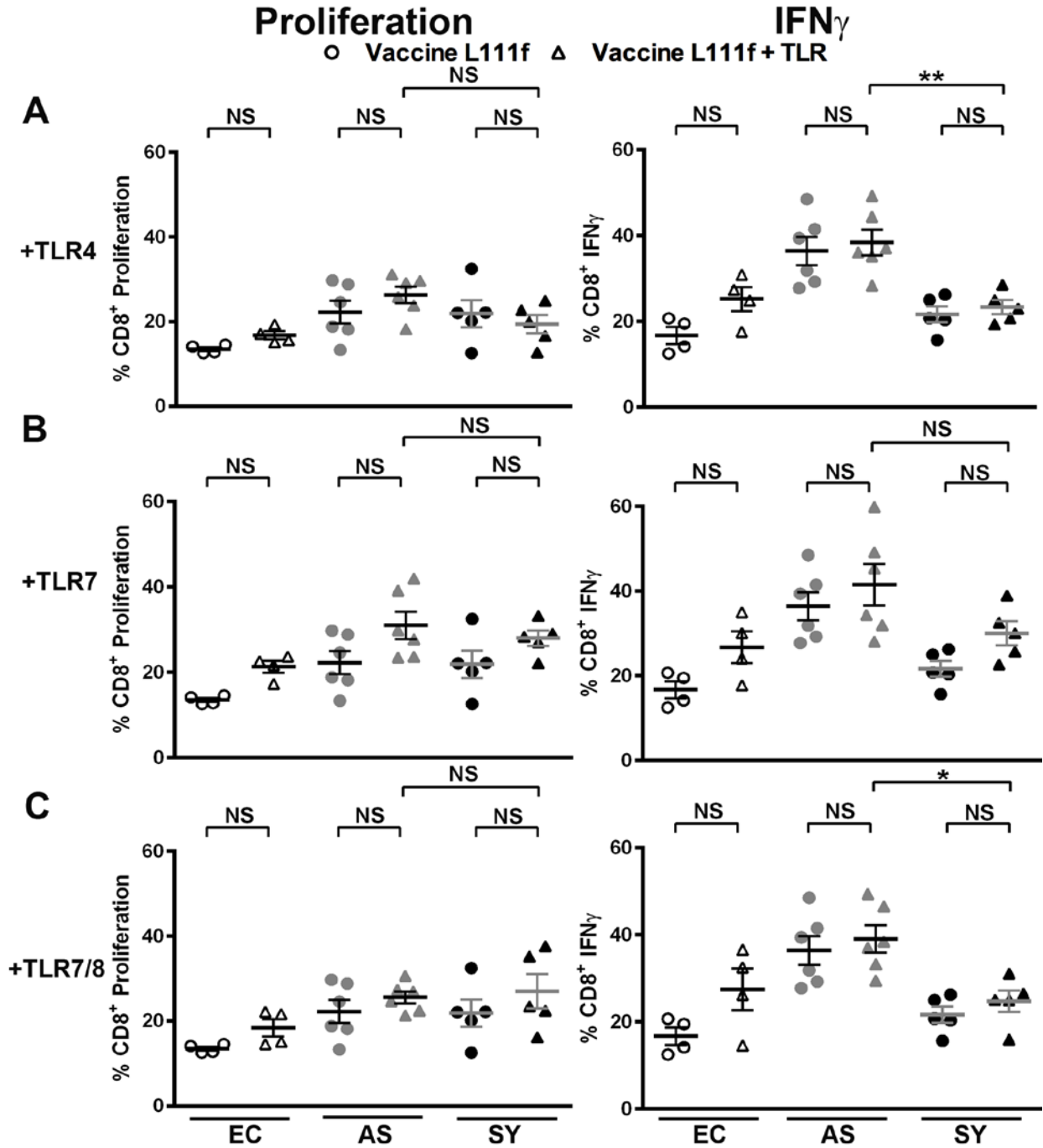
26 stimulated) with 1 μg vaccine L111f (A), or 1 μg vaccine NS (B) for 7 days, or 1 μg ConA for 4

27 days (C), or left untreated/mock (“-” for mock). Each dot represents an individual dog. EC =

28 endemic control (open circles), AS = asymptomatic, *L. infantum* positive (grey circles), SY =

29 symptomatic, *L. infantum* positive (black circles). Error bars are ± SEM. N=4-8 per group. *

30 $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical analysis was performed via one way-ANOVA.



31

32 **Fig. S4. CD8⁺ T cell data corresponding to Fig. 3.** Whole blood was stimulated with either 1

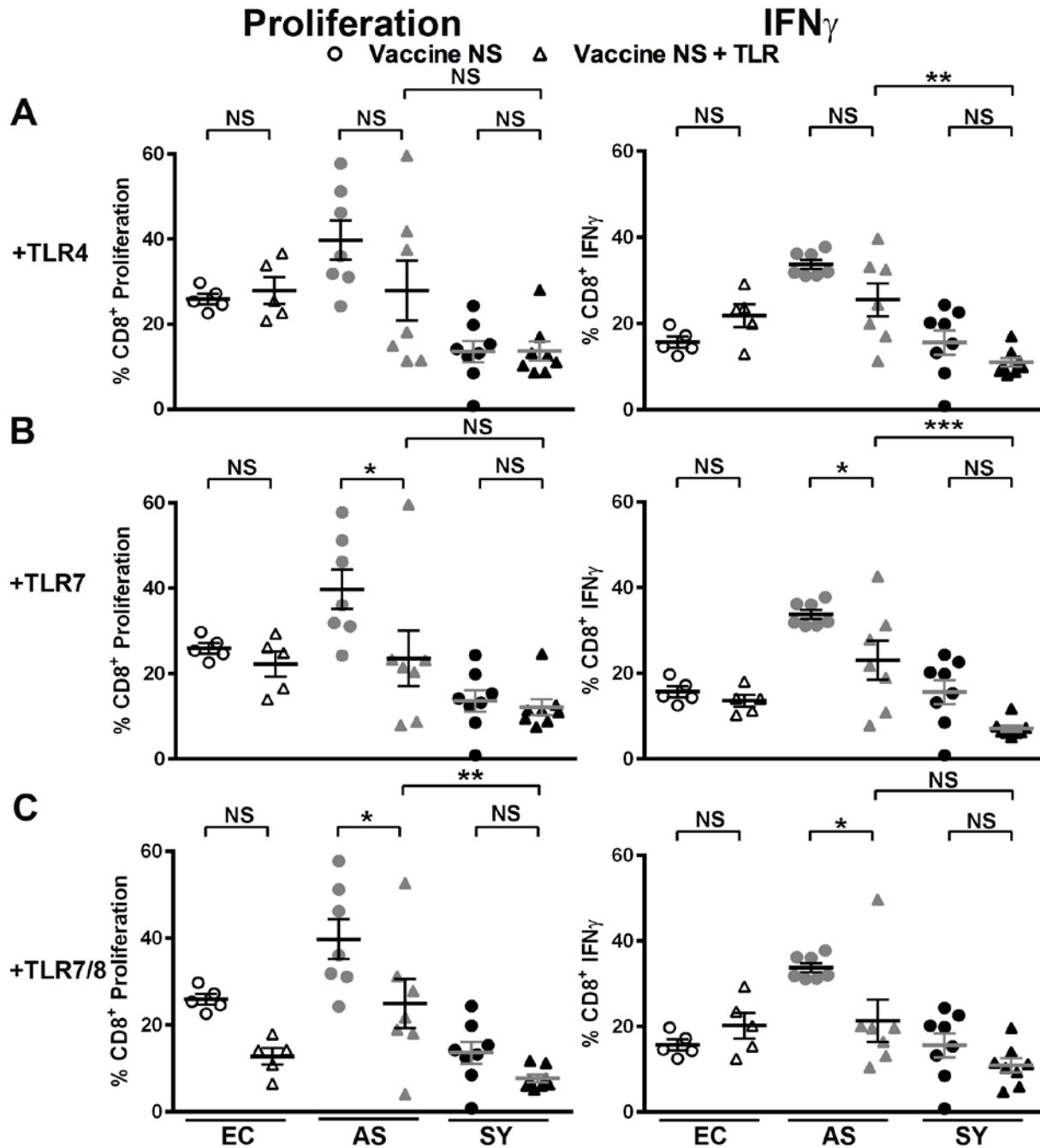
33 μ g vaccine L111f (circles) or vaccine L111f and TLR agonists (triangles) for 7 days. TLR

34 agonists were added at 1 μ g for Glucopyranosyl Lipid Adjuvant [TLR4] (A), 0.1 μ g for

35 imiquimod [TLR7] (B) or 10 μ g for resiquimod [TLR7/8] (C). Each dot represent an individual

36 dog. EC = endemic control (open), AS = asymptomatic, *L. infantum* positive (grey), SY =
37 symptomatic, *L. infantum* positive (black). Error bars are \pm SEM. N=4-6 per group. * $p < 0.05$,
38 ** $p < 0.01$, *** $p < 0.001$. Statistical analysis was performed via one way-ANOVA.

39



40

41 **Fig. S5. CD8⁺ T cell data corresponding to Fig.4.** Whole blood was stimulated with either 1

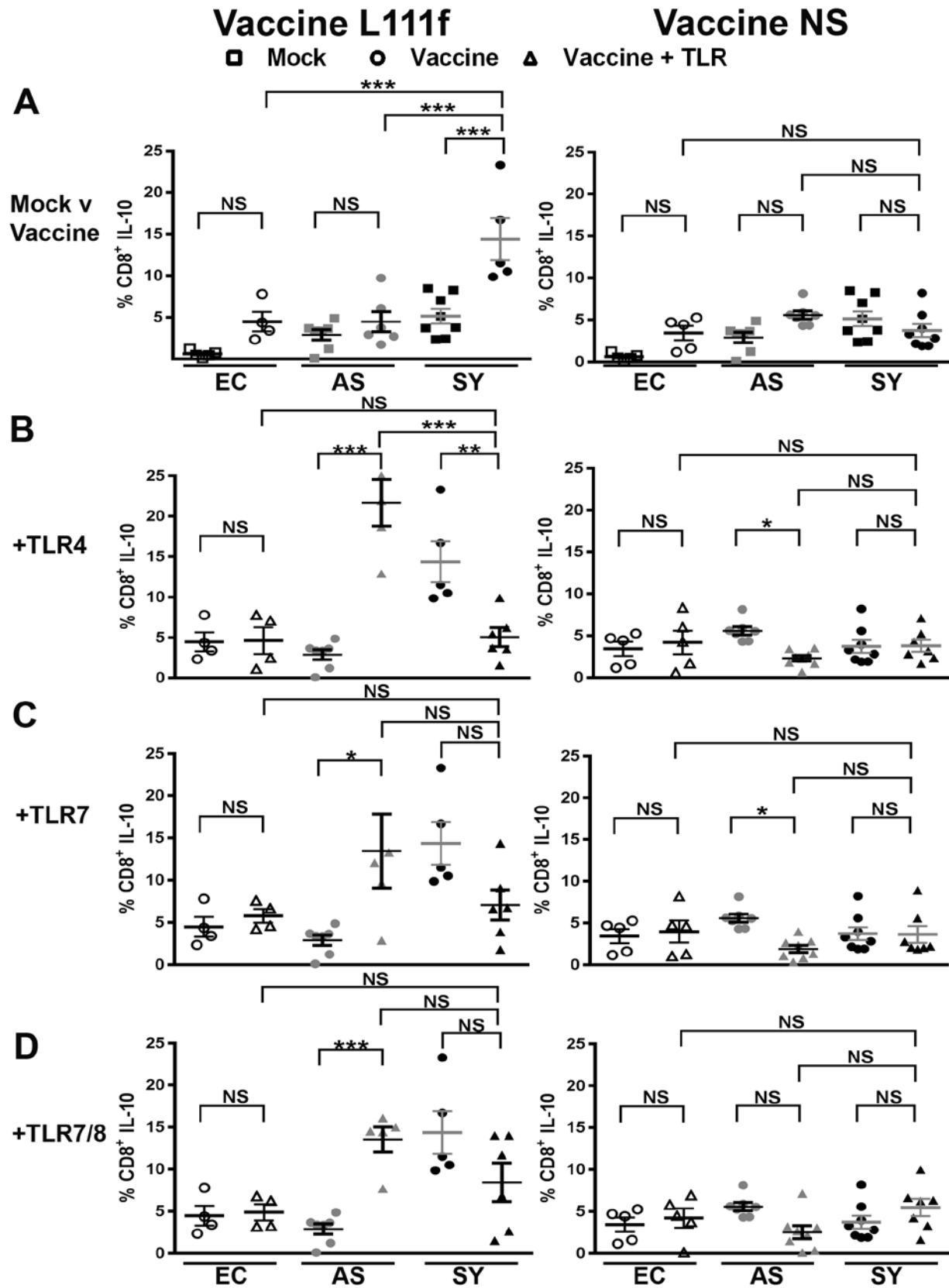
42 μ g vaccine NS (circles) or vaccine NS and TLR agonists (triangles) for 7 days. TLR agonists

43 were added at 1 μ g for TLR4 (A), 0.1 μ g for TLR7 (B) or 10 μ g for TLR7/8 (C). Each dot

44 represent an individual dog. EC = endemic control (open), AS = asymptomatic, *L. infantum*

45 positive (grey), SY = symptomatic, *L. infantum* positive (black). Error bars are \pm SEM. N=5-8

46 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical analysis was performed via one way-
47 ANOVA.



49 **Fig. S6. CD8⁺ T cell data corresponding to Fig.5.** Whole blood was stimulated (A) 1 μ g
50 vaccine (circles), or left untreated “mock” (squares) for 7 days. Vaccine, L111f or NS, used for
51 stimulation is indicated in each column. In B to D whole blood was stimulated with either 1 μ g
52 vaccine L111f or NS alone (circles) or vaccine with TLR agonists (triangles) for 7 days. TLR
53 agonists were added at 1 μ g for TLR4 (B), 0.1 μ g for TLR7 (C) or 10 μ g for TLR7/8 (D). Each
54 dot represents an individual dog. EC = endemic control (open circles), SY = symptomatic, *L.*
55 *infantum* positive (black circles). Error bars are \pm SEM. N= 5-8 for L111f and N = 4-8 for NS.
56 * p <0.05, ** p <0.01, *** p <0.001. Statistical analysis was performed via one way-ANOVA
57