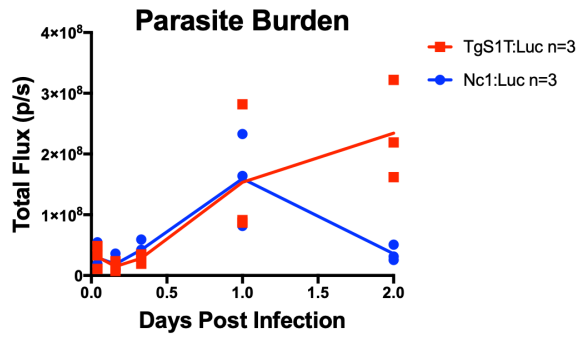
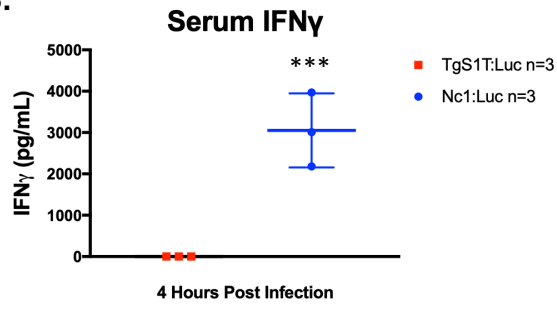


A.



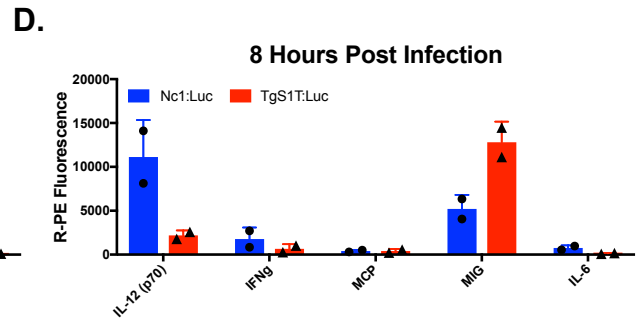
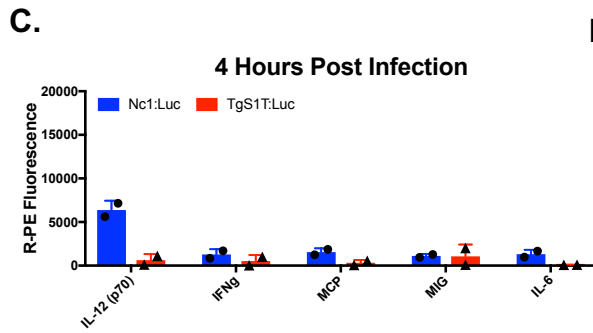
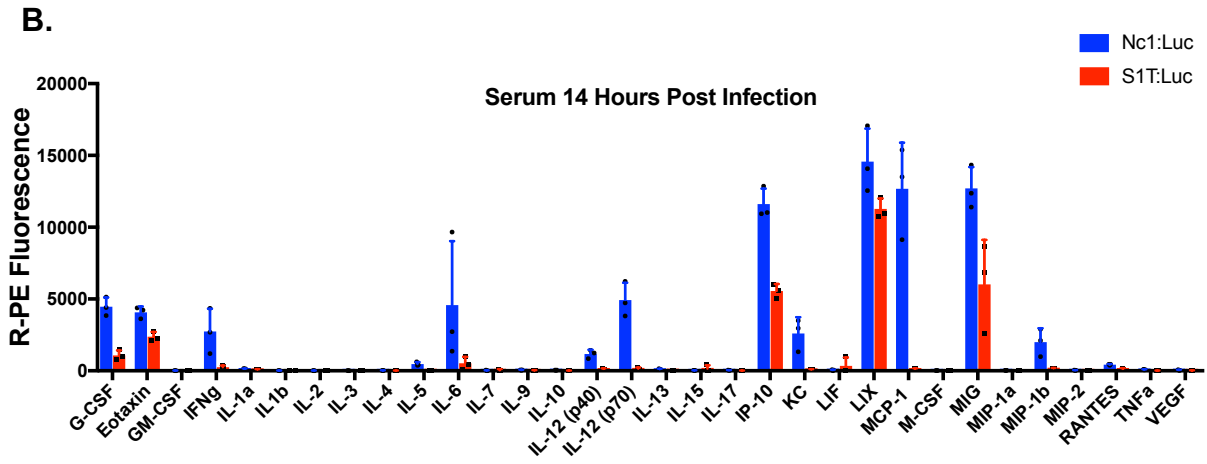
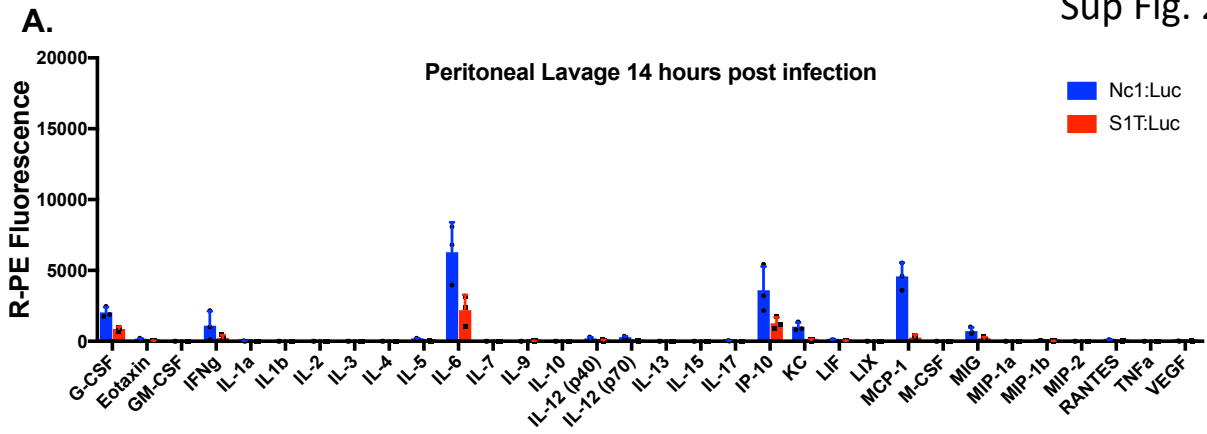
B.



2 **Fig S1. Parasite burden and early cytokine levels in mice infected with *N. caninum***  
3 **(Nc-1) or *T. gondii* (S1T).** Six week old BALB/c mice were IP injected with either  $10^6$   
4 TgS1T:Luc (red) or Nc1:Luc (Blue) tachyzoites. Bioluminescence imaging was used to  
5 monitor parasite burden throughout the infection. Serum was collected and analyzed for  
6 IFN $\gamma$  by ELISA. **A)** Quantification of bioluminescence during *in vivo* infections in BALB/c  
7 mice (n=3 per parasite species) **B)** Cytokine quantification using ELISA for mouse serum  
8 IFN $\gamma$  (n=3 per parasite species). All imaging data were log transformed and then a two-  
9 way repeated measure ANOVA (alpha=0.05) with Sidak's multiple comparisons test was  
10 performed. Cytokines were analyzed by a two-way repeated measure ANOVA  
11 (alpha=0.05) with Sidak's multiple comparisons test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

12

Sup Fig. 2

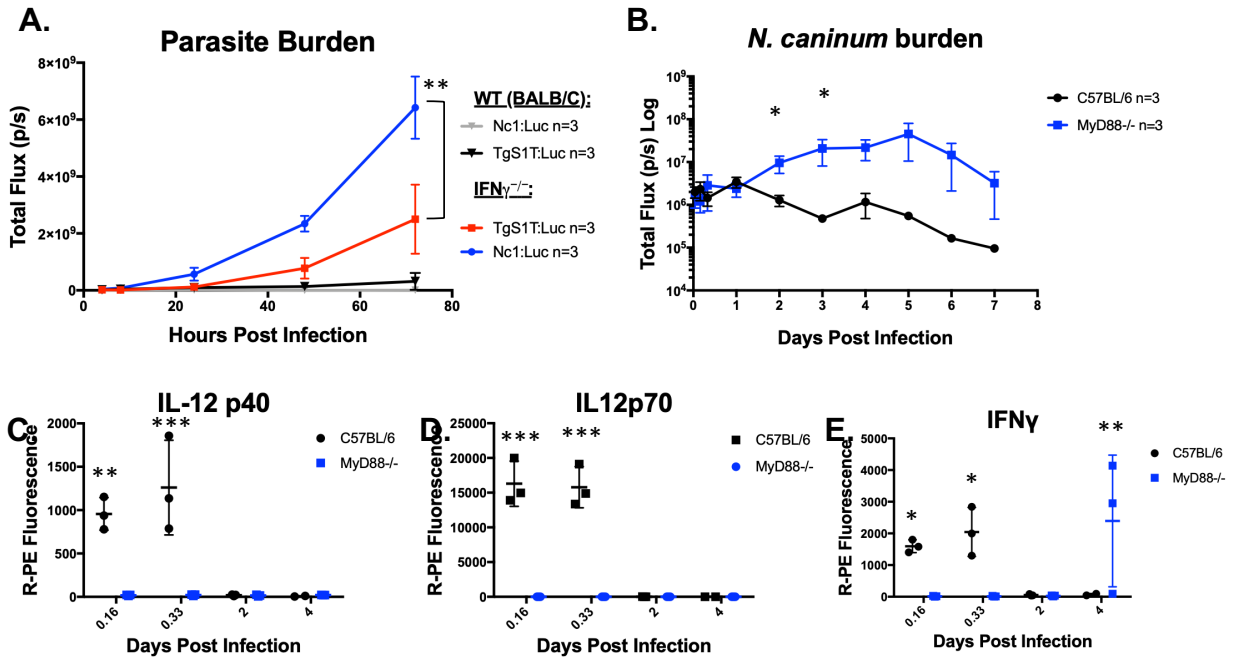


14 **Fig S2. Early cytokine levels in mice infected with *N. caninum* or *T. gondii*.** Six-week  
15 old BALB/C mice injected IP with either  $10^6$  TgS1T:Luc (red) or Nc1:Luc (Blue)  
16 tachyzoites. **A)** Peritoneal lavage samples were collected at 14 hours after injection and  
17 supernatant was analyzed for multiple cytokines; n=3 per parasite species. **B-D)** Blood  
18 samples were collected at 14 (**B**, n=3 per parasite species), 4 (**C**, n=2 per parasite  
19 species), or 8 (**D**, n=2 per parasite species) hours post-infection. Blood samples and  
20 lavage supernatant were analyzed using a mouse cytokine/chemokine multiplex assay  
21 (Luminex) and 32 mouse analytes were tested.

22

23

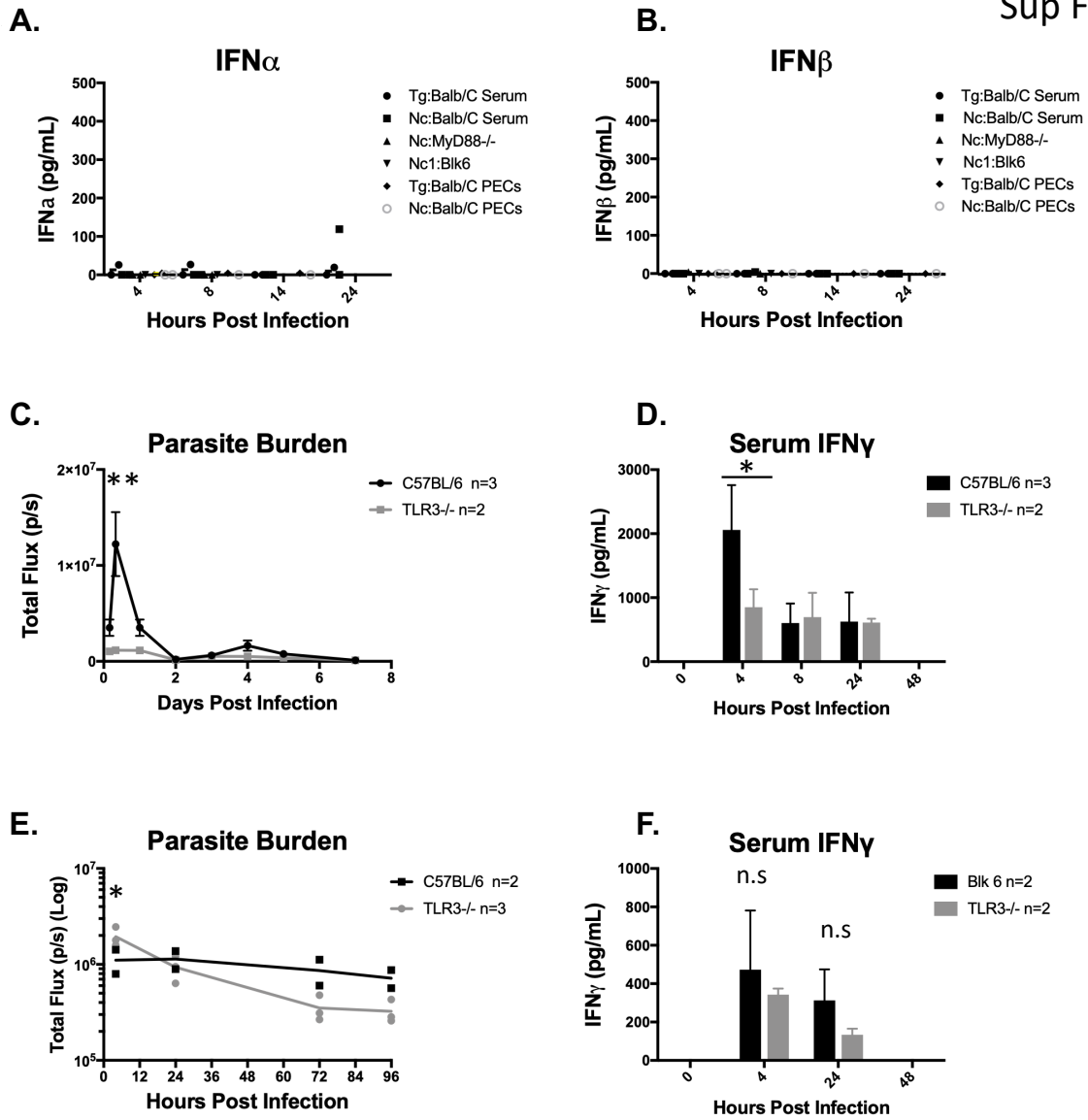
Sup Fig. 3



25

26 **Fig S3 Parasite burden and early cytokine levels in IFN $\gamma$  or MyD88 knockout mice**  
27 **infected with *N. caninum* (Nc-1) or *T. gondii* (S1T).** Six week old BALB/c or IFN $\gamma$   
28 knockout (IFN $\gamma$ <sup>-/-</sup>) mice or six week old C57BL/6 or MyD88 knockout (MyD88<sup>-/-</sup>) mice were  
29 injected IP with 10<sup>6</sup> Nc1:Luc or TgS1T:Luc tachyzoites. **A)** BALB/c or IFN $\gamma$ <sup>-/-</sup> mice:  
30 quantification of bioluminescence imaging days 0-7 post-infection (n=3 per mouse strain  
31 per parasite). **B)** C57BL/6 or MyD88<sup>-/-</sup> mice: quantification of bioluminescence imaging of  
32 MyD88<sup>-/-</sup> (n=3) or C57BL/6 (n=3) mice infected with 10<sup>6</sup> Nc1:Luc tachyzoites. All BLI data  
33 were log transformed and two-way repeated measure ANOVA (alpha=0.05) with Dunnett  
34 multiple comparisons test was performed. **(C-E)** Serum samples were collected at 4, 8,  
35 48, and 96 hpi (0.16, 0.33, 2, and 4 dpi) and analyzed using a 32 plex Luminex cytokine  
36 response panel. Data shown is for **C)** IL-12p40, **D)** IL-12p70 and **E)** IFN $\gamma$  (n=3 per mouse  
37 strain). Cytokines were analyzed by a two-way repeated measure ANOVA (alpha=0.05)  
38 with Sidak's multiple comparisons test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

39



41 **Fig S4. Bioluminescent imaging and IFN $\gamma$  concentration in *N. caninum* infections**  
42 **in TLR3 knockout mice and IFN $\alpha/\beta$  concentration in multiple infections. A)** Interferon  
43 alpha (IFN $\alpha$ ) or **B)** Interferon beta (IFN $\beta$ ) concentration in serum or peritoneal lavage  
44 supernatant (PECs) from multiple mouse strains infected with either Nc1:Luc (NC)  
45 or TgS1T:Luc (Tg). Sample size was either 3 for each mouse strain/parasite species  
46 combination except for MyD88 $^{-/-}$  and C57BL/6, where the sample size was n=2. Samples  
47 were analyzed with a multiplex assay (Luminex) and concentration was calculated using  
48 a standard curve. **C)** Six to eight week old TLR3 knockout mice (TLR3 $^{-/-}$ , gray n=2) or WT  
49 C57BL/6 (black n=3) mice were infected IP with 10<sup>6</sup> Nc1:Luc tachyzoites. Quantification  
50 of bioluminescence imaging was conducted for 7 days of infection (photons/s). Data were  
51 log transformed and two-way repeated measure ANOVA (alpha=0.05) with Dunnett  
52 multiple comparisons test was performed. TLR3 $^{-/-}$  infections had significantly less parasite  
53 burden compared to control infections at 8 hpi ( $p < 0.01$ ). **D)** Serum IFN $\gamma$  (pg/mL) during  
54 the first 48 hours of infection, analyzed by ELISA. Data were analyzed by a two-way  
55 repeated measure ANOVA (alpha=0.05) with Sidak's multiple comparisons test. TLR3 $^{-/-}$   
56 mice had significantly less ( $p < 0.05$ ) than control mice at 4 hpi. **E)** As in C, 6-8 week old  
57 TLR3 knockout mice (TLR3 $^{-/-}$ , gray n=3) or WT C57BL/6 (black n=2) mice were injected  
58 IP with 10<sup>6</sup> Nc1:Luc tachyzoites. Quantification of bioluminescence imaging was  
59 performed through 96 hours of infection (photons/s). Data were log transformed and two-  
60 way repeated measure ANOVA (alpha=0.05) with Dunnett multiple comparisons test was  
61 performed. TLR3 $^{-/-}$  infections had significantly less parasite burden when compared to  
62 control infections at 4 hpi ( $P < 0.01$ ). **F)** Serum IFN $\gamma$  (pg/mL) during the first 48 hours of  
63 infection, analyzed by ELISA. Data were analyzed by a two-way repeated measure



64 ANOVA ( $\alpha=0.05$ ) with Sidak's multiple comparisons test. No statistically significant  
65 differences in IFN $\gamma$  levels were observed. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$