Supplemental Material

Supplemental Figure Legends

Figure S1. Frequency of Treg cells in *T. gondii*-infected TLR11-deficient mice.

TLR11-/- mice (five animals per group) were infected intraperitoneally (IP) or orally (ORAL) with an average of 20 *T. gondii* strain ME49 cysts per mouse, and the frequency of Treg cells was analyzed in the (A) spleen and (B) mesenteric lymph nodes at the indicated number of days post-infection. (C) Average frequency of Foxp3+ cells in the spleens and mLNs of TLR11-/- mice infected intraperitoneally (blue circles) or orally (red circles). The data shown are representative of five independent experiments.

Figure S2. Analysis of Treg cell proliferation

(A) WT mice (five animals per group) were infected intraperitoneally (IP) with an average of 20 *T. gondii* strain ME49 cysts per mouse. Analysis of Treg cell proliferation was performed by intracellular staining for the nuclear antigen Ki-67 at the indicated number of days post-infection. The numbers were calculated as percentages of all CD4+ cells shown on the plots. The data shown are representative of four independent experiments. (B) WT mice (five animals per group) were infected intravenously with *L. monocytogenes* (10^4 CFU per mouse) or (C) vaccinia virus (10^6 PFU per mouse). Analysis of Treg cell proliferation was performed by intracellular staining for the nuclear antigen Ki-67 at the indicated number of days post-infection. The numbers were calculated as percentages of all CD4+ cells shown on the plots. The data shown are representative of four independent experiments.

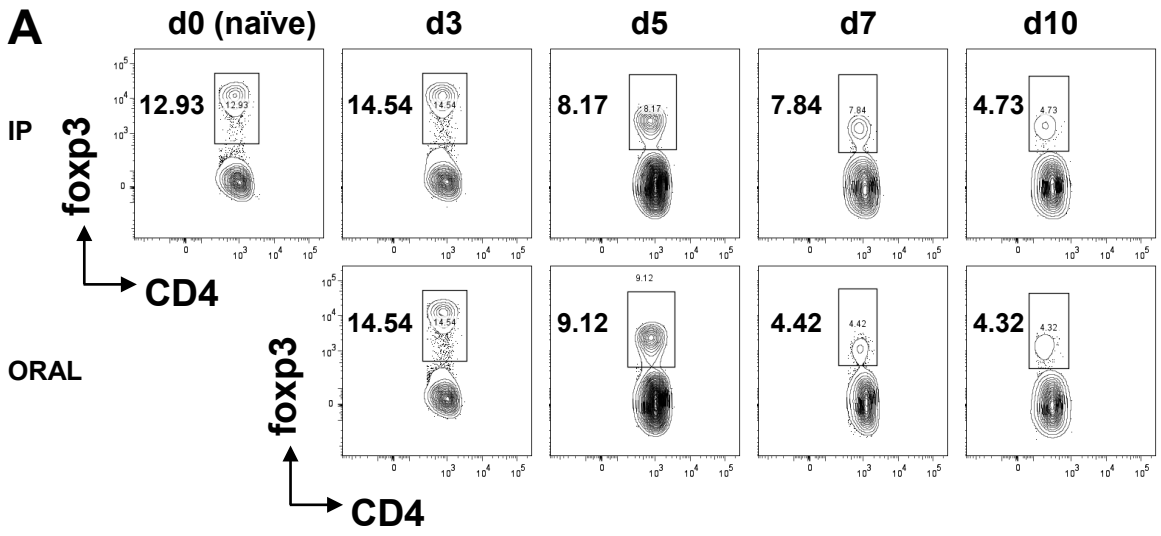
Figure S3 *T. gondii*-specific Th1 CD4+ T cells produce limited amounts of IL-2

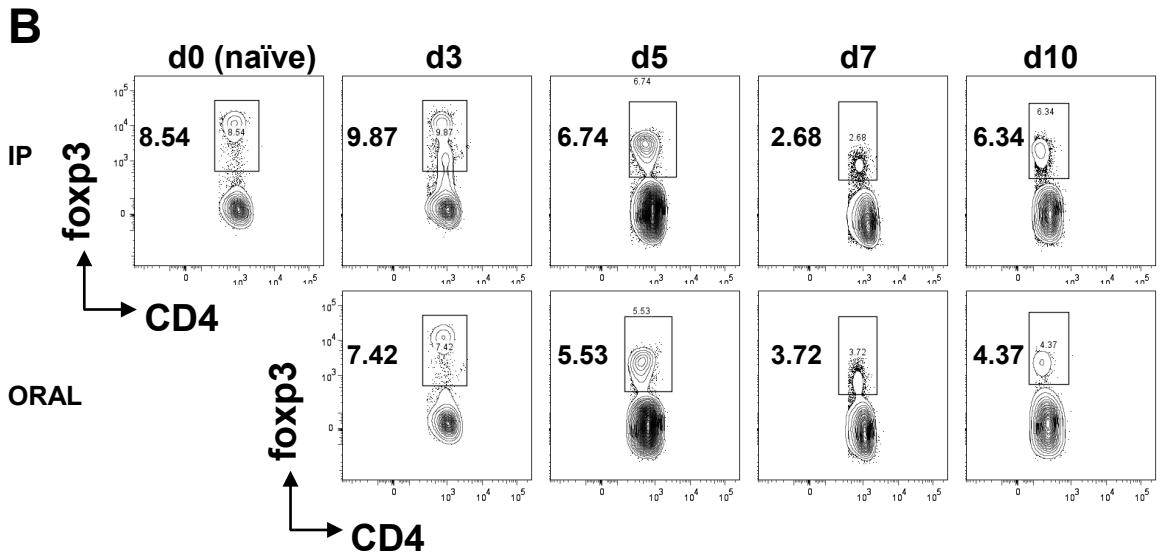
WT animals were (A) left untreated or were infected with *T. gondii* (B) intraperitoneally or (C) orally, and seven days later, the ability of CD4+ T cells isolated from the mesenteric lymph nodes to produce IFN-γ, TNF, and IL-2 was analyzed. The data shown are representative of four independent experiments. (D) WT animals were either left untreated (d0) or infected with *T. gondii* intraperitoneally and at the indicated time points post infection the ability of splenic CD4+ T cells to produce IL-2 and IFN-γ in response to *T. gondii* extract STAg (10 ug/ml) were analyzed. The data shown are representative of three independent experiments. (E) WT mice (five animals per group) were infected intraperitoneally with an average of 20 *T. gondii* strain ME49 cysts. CD25 expression was analyzed in splenic CD4+Foxp3- and CD4+Foxp3+ T cells isolated from naïve or *T. gondii* infected mice as shown in Figure 3G.

Figure S4. The appearance of ALDH+ cells in response to *T. gondii* infection.

Splenocytes from naïve and infected mice were incubated in the dark for 35 minutes at 37°C in the ALDEFLUOR assay buffer containing the activated ALDEFLUOR substrate, with or without the ALDH inhibitor diethylaminobenzaldehyde (DEAB). Cells were subsequently stained using the CD11b-, CD11c-, and Gr1-specific antibodies (BD Biosciences) and the appearance of ALDH+ cells in response to *T. gondii* infection was analyzed by flow cytometry on days 0, 7, 10, 14, 21, and 28 post-infection. The data shown are representative of three experiments.

Figure S1


A
d0 (naïve)
d3
d5
d7
d10
IP
ORAL
foxp3
CD4
12.93
14.54
8.17
7.84
4.73
14.54
9.12
4.42
4.32


B
d0 (naïve)
d3
d5
d7
d10
IP
ORAL
foxp3
CD4
8.54
9.87
6.74
2.68
6.34
7.42
5.53
3.72
4.37

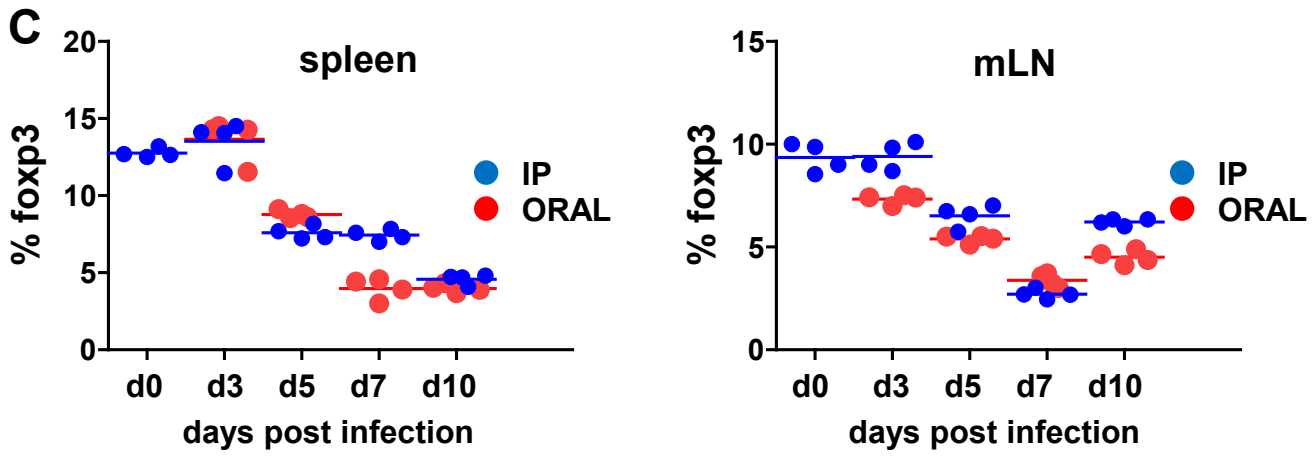

C
spleen
mLN
% foxp3
days post infection
d0
d3
d5
d7
d10
IP
ORAL
0
5
10
15
20

Figure S2

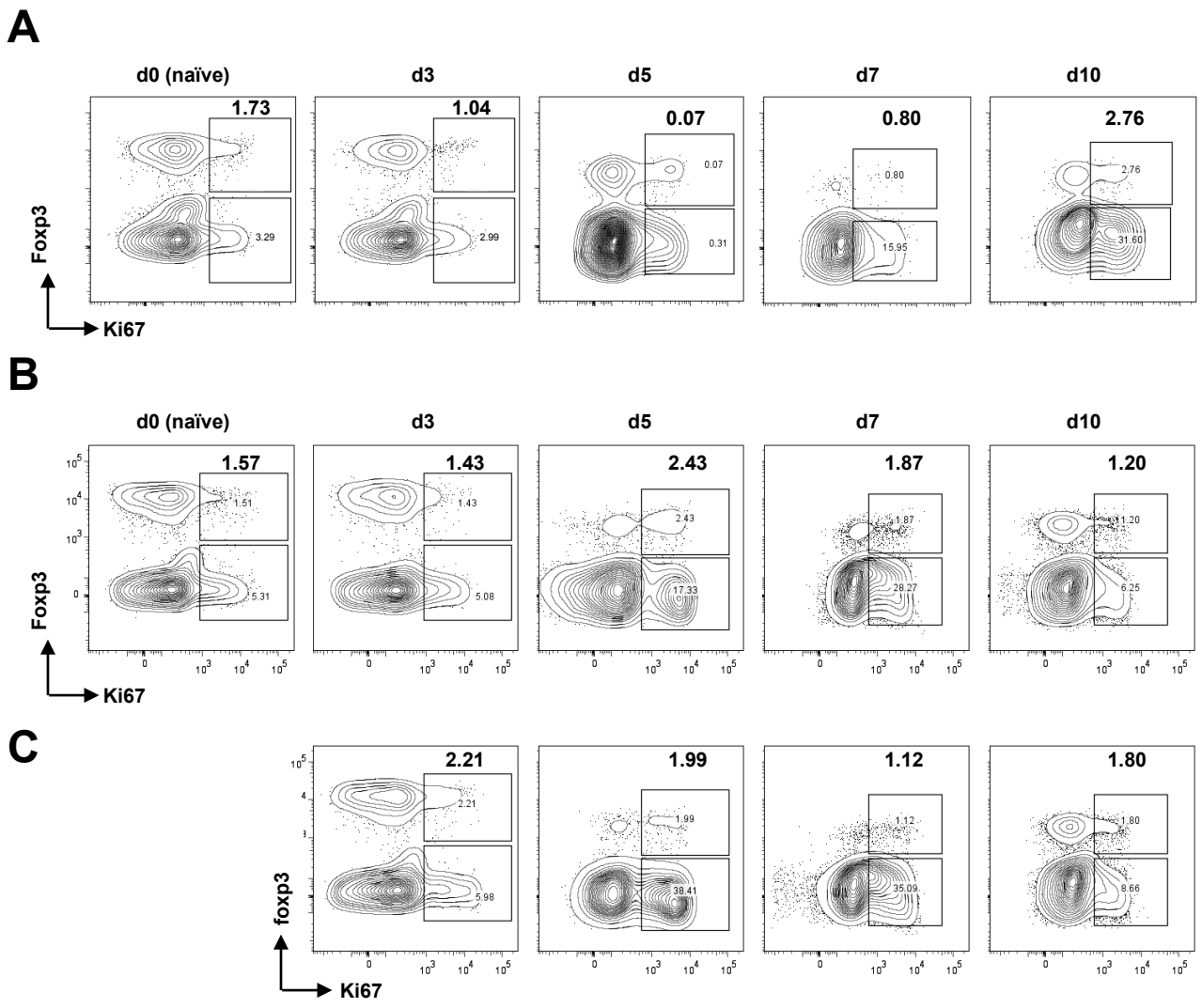

A
d0 (naïve)
d3
d5
d7
d10
1.73
3.29
1.04
2.99
0.07
0.07
0.31
0.80
0.80
15.95
2.76
2.76
31.60
Foxp3
Ki67
B
d0 (naïve)
d3
d5
d7
d10
1.57
1.51
5.31
1.43
1.43
5.08
2.43
2.43
17.33
1.87
1.87
28.27
1.20
1.20
6.25
Foxp3
Ki67
C
2.21
2.21
5.98
1.99
1.99
38.41
1.12
1.12
35.09
1.80
1.80
8.66
foxp3
Ki67

Figure S3

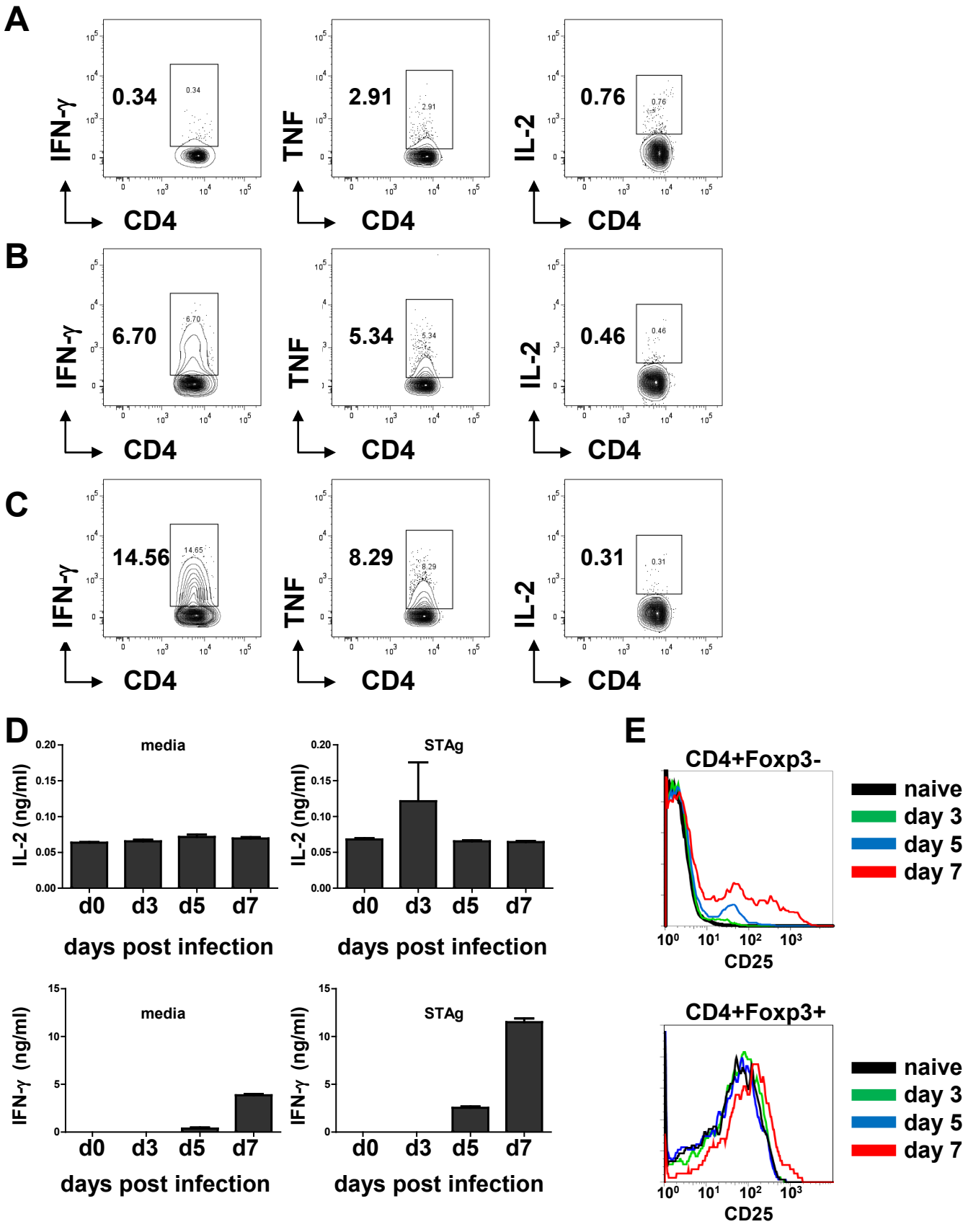
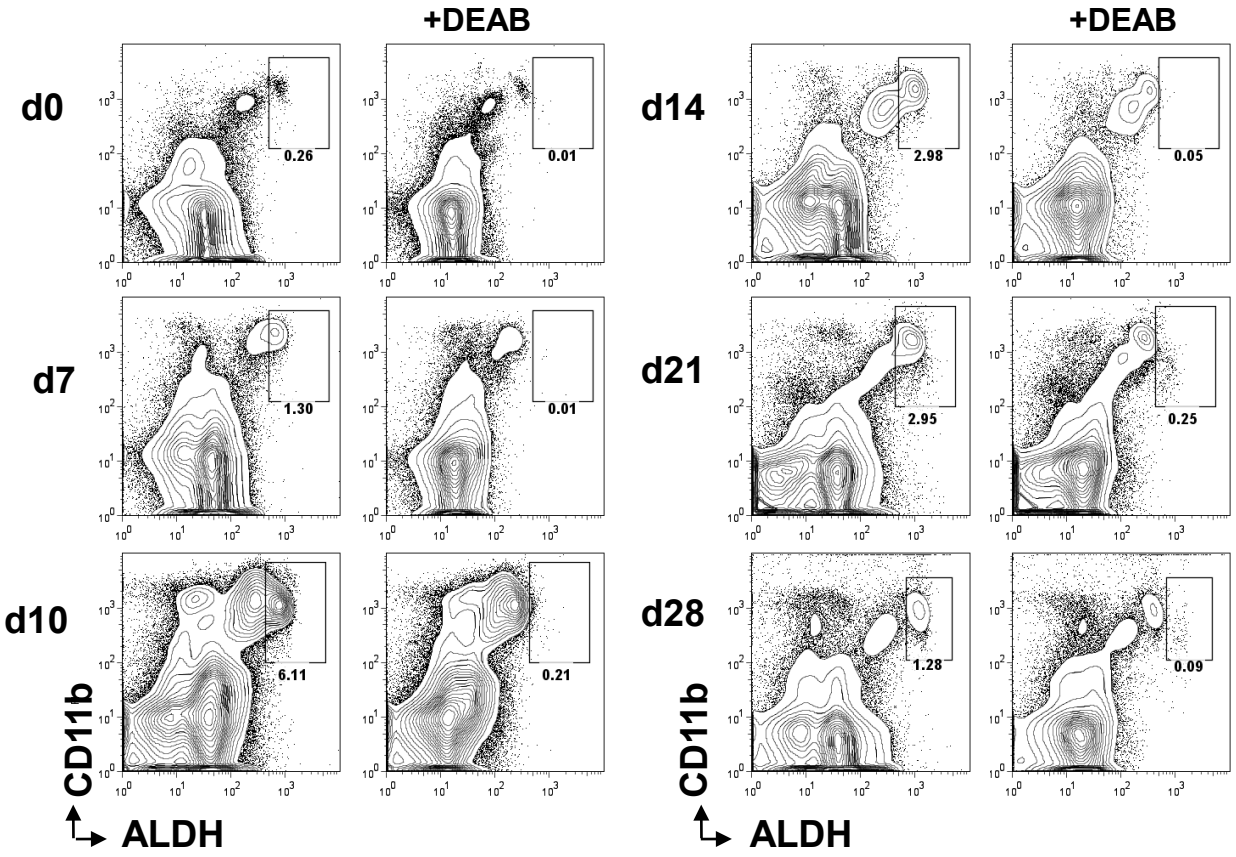

A
IFN-γ
0.34
CD4
TNF
2.91
CD4
IL-2
0.76
CD4
B
IFN-γ
6.70
CD4
TNF
5.34
CD4
IL-2
0.46
CD4
C
IFN-γ
14.56
CD4
TNF
8.29
CD4
IL-2
0.31
CD4
D
media
IL-2 (ng/ml)
d0 d3 d5 d7
days post infection
STAg
IL-2 (ng/ml)
d0 d3 d5 d7
days post infection
media
IFN-γ (ng/ml)
d0 d3 d5 d7
days post infection
STAg
IFN-γ (ng/ml)
d0 d3 d5 d7
days post infection
E
CD4+Foxp3-
CD25
naive
day 3
day 5
day 7
CD4+Foxp3+
CD25
naive
day 3
day 5
day 7

Figure S4


+DEAB
+DEAB
d0
d7
d10
d14
d21
d28
0.26
0.01
1.30
0.01
6.11
0.21
2.98
0.05
2.95
0.25
1.28
0.09
CD11b
ALDH
CD11b
ALDH