Supplementary figure legends

Figure S1 - DTX treatment depletes CD11+ F4/80+ macrophages in CD11b-DTR mice

(a) Thioglycollate-elicited peritoneal cells from wild-type and CD11b-DTR (n=3/group) mice were cultured for 24 hours in the presence or absence of DTX (500ng/ml). *In vitro* DTX treatment had no effect on wild-type cells but reduced cell viability in peritoneal cells isolated from CD11b-DTR mice. **(b)** Thioglycollate-elicited peritoneal cells were harvested from untreated control or CD11b-DTR mice treated with DTX (n=3/group) (25ng/g IP) 24 hours prior to harvest. The majority of cells from control mice were F4/80+ Gr-1- macrophages with a much smaller population of F4/80- Gr-1+ neutrophils. *In vivo* DTX treatment eliminated the majority of F4/80+ Gr-1- macrophages but did not deplete F4/80- Gr-1+ neutrophils. **(c)** Diff-Quick stained cytospins of thioglycollate-elicited peritoneal cells from control and DTX treated (n=3/group) CD11b-DTR mice highlighted the loss of macrophages but not neutrophils. Results represent four independent experiments.

Figure S2 - Macrophage depletion impaired both primary lung granuloma formation and maintenance in *Schistosoma mansoni* egg challenged mice

Naïve CD11b-DTR mice were challenged by intravenous *S. mansoni* eggs on D0. Half of the mice were treated with DTX (25ng/g) when indicated in Fig.2a and analyzed on D4 (control n=5; DTX n=8), D7 (control n=15; DTX n=18), or D14 (control n=15; DTX n=19). Representative images of Masson's Trichrome stained lung tissue show granulomatous inflammation and collagen (blue). DTX treatment reduced primary lung granuloma volumes at all time points assessed. Results represent two independent experiments.

Figure S3 – Macrophage depletion weakened Th2-induced gene expression in mice with primary *Schistosoma mansoni* egg-induced granulomas

Relative gene expression in lung tissue from naïve (n=10), egg challenged (D4 n=7; D7 n=9, D10 n=7), and egg challenged, DTX treated (D4 n=8; D7 n=8, D10 n=10) CD11b-DTR mice.

Results were normalized to RPLP2 and scaled to naïve mice. All Th2-induced genes were reduced following DTX treatment. Data are presented as mean ± s.e.m. Statistical significance was calculated using unpaired two-tailed Student's *t* test. * $p<0.05$, ** $p<0.01$. Results represent two independent experiments.

Figure S4 - DTX treatment did not alter cytokine production per CD4 T cell in mediastinal lymph node in mice with secondary *Schistosoma mansoni* egg-induced lung granulomas

Mediastinal lymph nodes isolated from naïve (n=20), egg primed and challenged (D4 n=8; D7 n=6, D14 n=6), and primed, challenged, DTX treated (D4 n=8; D7 n=4, D14 n=7) CD11b-DTR mice were restimulated with PMA plus Ionomycin and stained under normalized conditions for flow cytometry to compare per-cell cytokine production by CD4 T lymphocytes. No difference in the intensity of cytokine staining in effector CD4 T cells was observed in the lymph nodes. Data are presented as median and statistical significance calculated using Mann-Whitney U test. *$p<0.05$. Results represent two independent experiments.

Figure S5 - Macrophage depletion did not reduce the frequency and caused little changes in the intensity of cytokine production in the mediastinal lymph node effector CD4 T cell populations of mice with primary *Schistosoma* mansoni egg-induced lung granulomas

CD11b-DTR mice were challenged by intravenous *S. mansoni* eggs on D0. Half of the mice were treated with DTX (25ng/g) when indicated in Figure 2 and analyzed on D4, 7, or 14. Mediastinal lymph nodes isolated from naïve (n=5), egg challenged (D4 n=7; D7 n=8, D14 n=8), and egg challenged, DTX treated (D4 n=8; D7 n=10, D14 n=9) CD11b-DTR mice were restimulated with PMA plus Ionomycin and stained to compare cytokine production by CD4 T lymphocytes. Macrophage depletion **(a)** increased the frequencies of cytokine+ CD4 T lymphocytes on D7 and **(b)** marginally decreased IL-13 and IL-4 production per cell on D14. Data are presented as median and statistical significance calculated using Mann-Whitney U test. *$p<0.05$, ** $p<0.01$, *** $p<0.001$. Results represent two independent experiments.

Figure S6 – Direct and cumulative effects of DTX treatment in lung-draining mediastinal lymph nodes

The lung-draining mediastinal lymph node leukocyte populations were analysed by flow cytometry as in Figure 1a. DTX treatment cumulatively, but not directly, decreased the numbers of total cells, CD11b+ DCs, and CD11b- DCs in the mediastinal lymph nodes. Statistical significance was calculated using unpaired two-tailed Student's *t* test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Results represent three independent experiments.

Figure S7 – DTX treatment during airway HDM allergen challenge of pre-immunized CD11b-DTR mice reduced the number of effector CD4 T lymphocytes in the lungs without impacting the response in lymph nodes

CD11b-DTR mice were primed by intraperitoneal injection of house dust mite (HDM, 200µg) on D0 and 7 prior to intratracheal challenge with HDM (50µg) on D14 and D16. Half of the mice were treated with DTX (25ng/g) at D13 and 15 and all mice were harvested on D17. **(a)** Mediastinal lymph node and **(b)** lung leukocytes isolated from naïve (n=8), HDM primed and challenged (HDM n=12), and primed, challenged, DTX treated (HDM DTX n=10) CD11b-DTR mice were restimulated with PMA plus Ionomycin and stained to compare cytokine production by CD4 T lymphocytes by flow cytometry. **(c)** Total numbers of cytokine-producing inflammatory CD4 T lymphocytes in the lungs. No significant differences were observed in the lung-draining lymph node. In the lung, by contrast, although per-cell cytokine production did not change, depletion decreased the total number of effector CD4 T cells. Data are presented as median and statistical significance calculated using Mann-Whitney U test. *$p<0.05$, **$p<0.01$. Results represent two independent experiments.

Figure S8 – Macrophage depletion caused little change in effector CD4 T lymphocytes in the mesenteric lymph nodes of mice infected with *Nippostrongylus brasiliensis*

CD11b-DTR mice were injected subcutaneously with *N. brasiliensis* (500 L3) at D0. Half of the mice were treated with DTX (25ng/g) when indicated in Figure 9. On D7 or 10 mesenteric lymph node leukocytes isolated from untreated (naïve, n=9), infected (D7 n=8; D10 n=8), and infected DTX treated (D7 n=8; D10 n=9) CD11b-DTR mice were restimulated with PMA plus Ionomycin and stained for flow cytometry to compare cytokine production by CD4+ T lymphocytes. Unstimulated cells from infected mice served as staining controls. A small decrease in the frequency of all cytokine+ CD4+ lymphocytes was observed at D7. Data are presented as median and statistical significance calculated using Mann-Whitney U test. *$p<0.05$. Results represent two independent experiments.

Figure S1

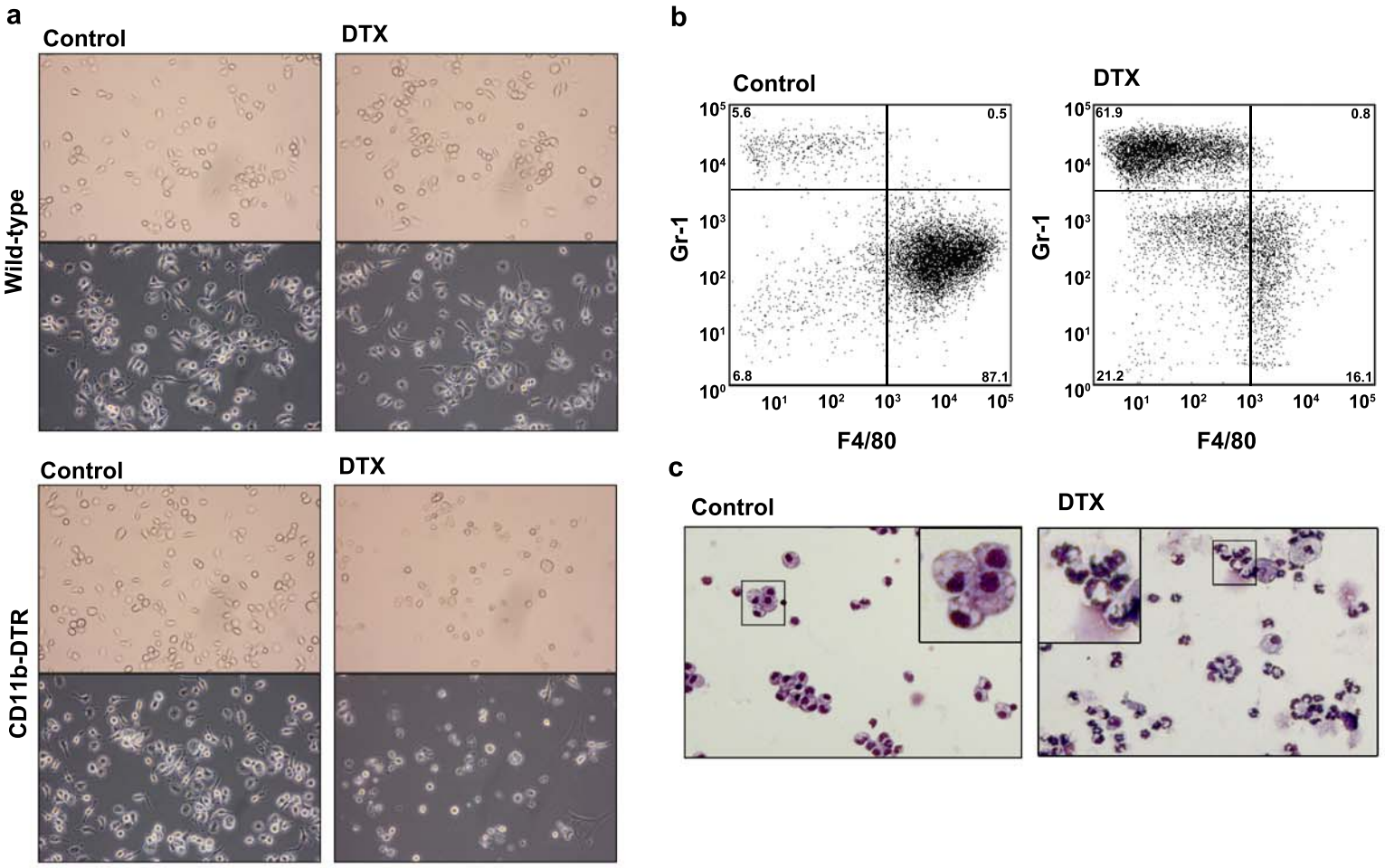

a
Control
DTX
Wild-type
Control
DTX
CD11b-DTR
b
Control
DTX
Gr-1
F4/80
5.6
0.5
6.8
87.1
61.9
0.8
21.2
16.1
c
Control
DTX

Figure S2

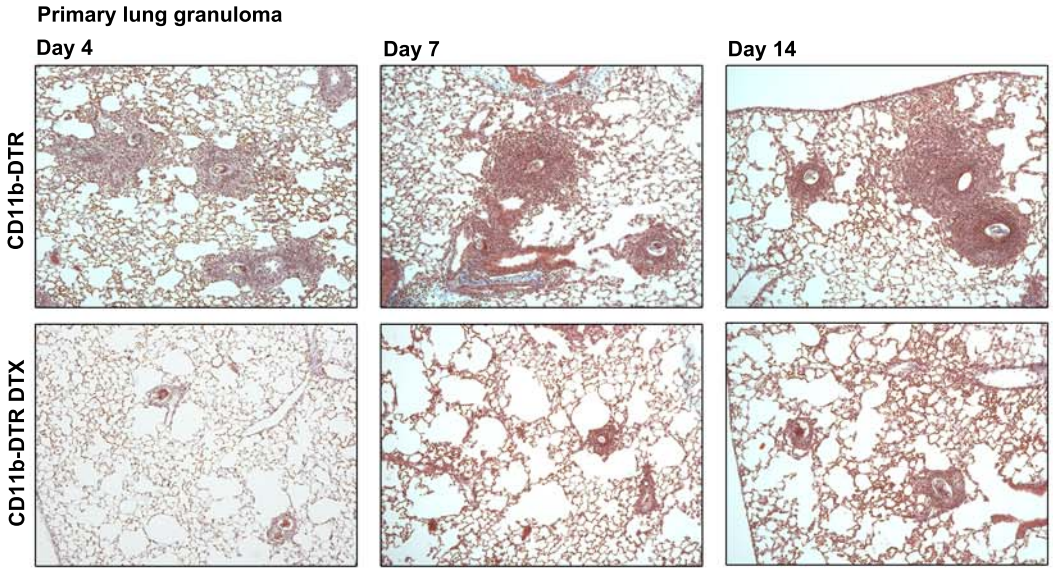

Primary lung granuloma
Day 4
Day 7
Day 14
CD11b-DTR
CD11b-DTR DTX

Figure S3

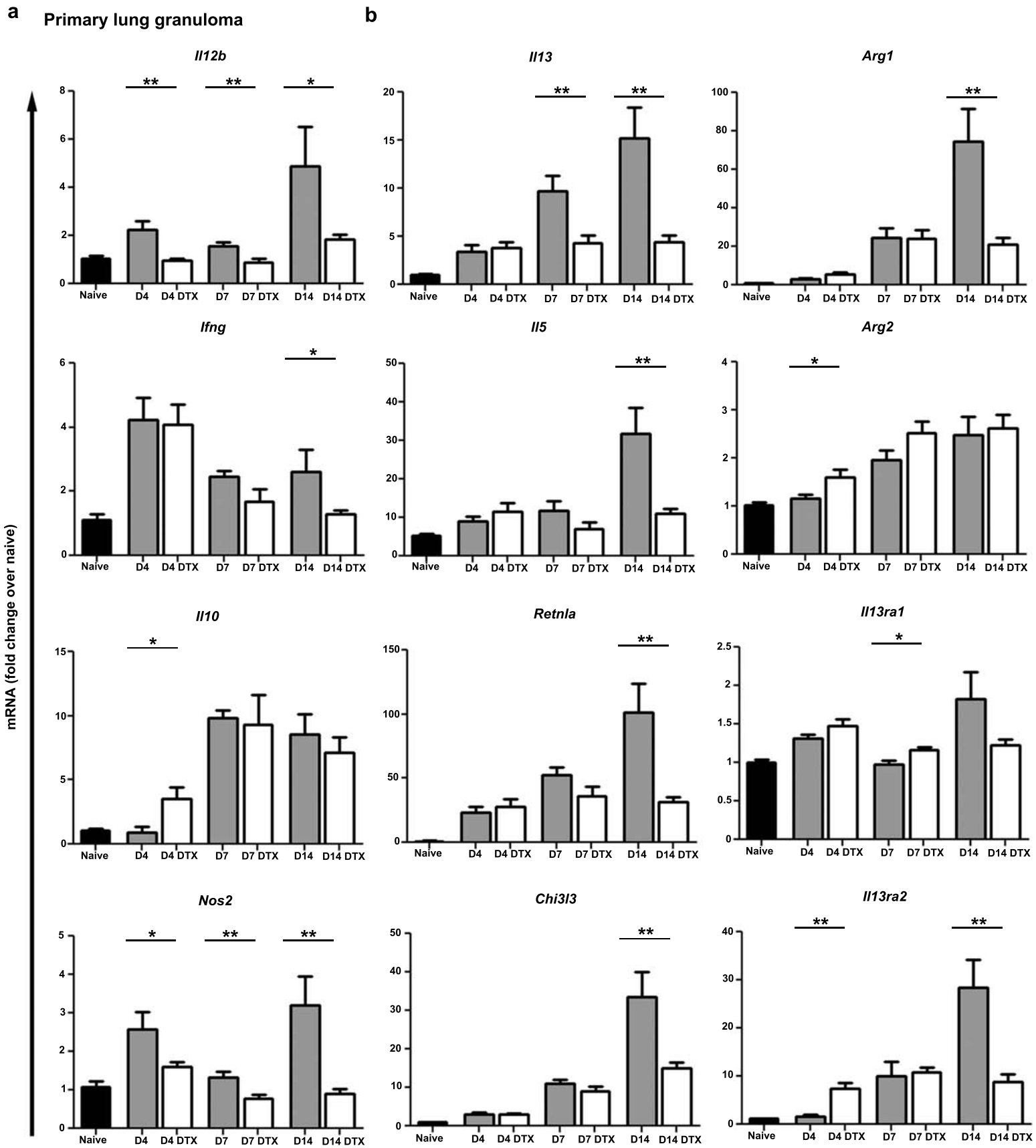

a
Primary lung granuloma
b
mRNA (fold change over naive)
Il12b
Il13
Arg1
Ifng
Il5
Arg2
Il10
Retnla
Il13ra1
Nos2
Chi3l3
Il13ra2
Naive
D4
D4 DTX
D7
D7 DTX
D14
D14 DTX

Figure S4

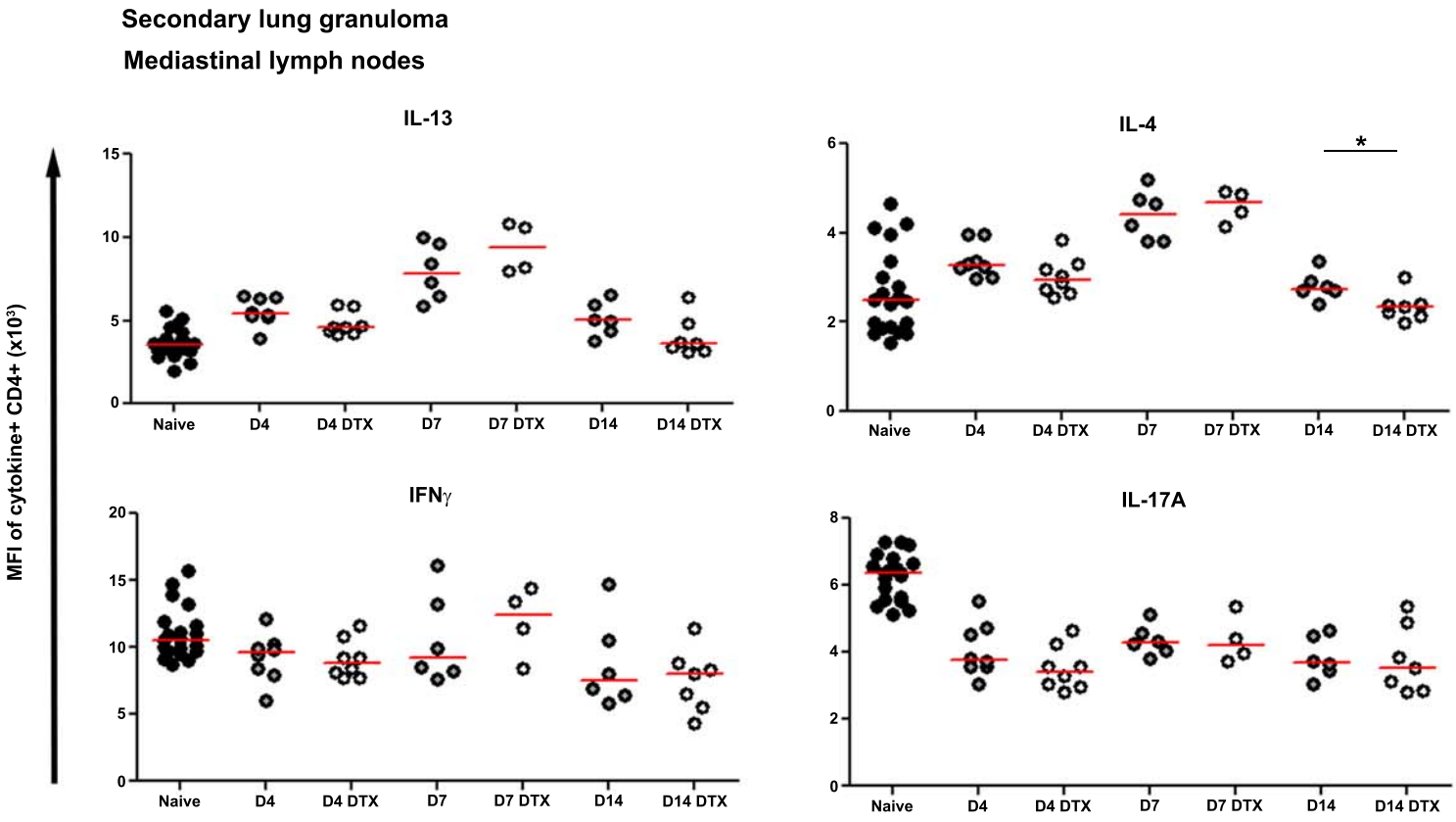

Secondary lung granuloma
Mediastinal lymph nodes
MFI of cytokine+ CD4+ (x10³)
IL-13
IL-4
IFNγ
IL-17A
*
Naive
D4
D4 DTX
D7
D7 DTX
D14
D14 DTX

Figure S5

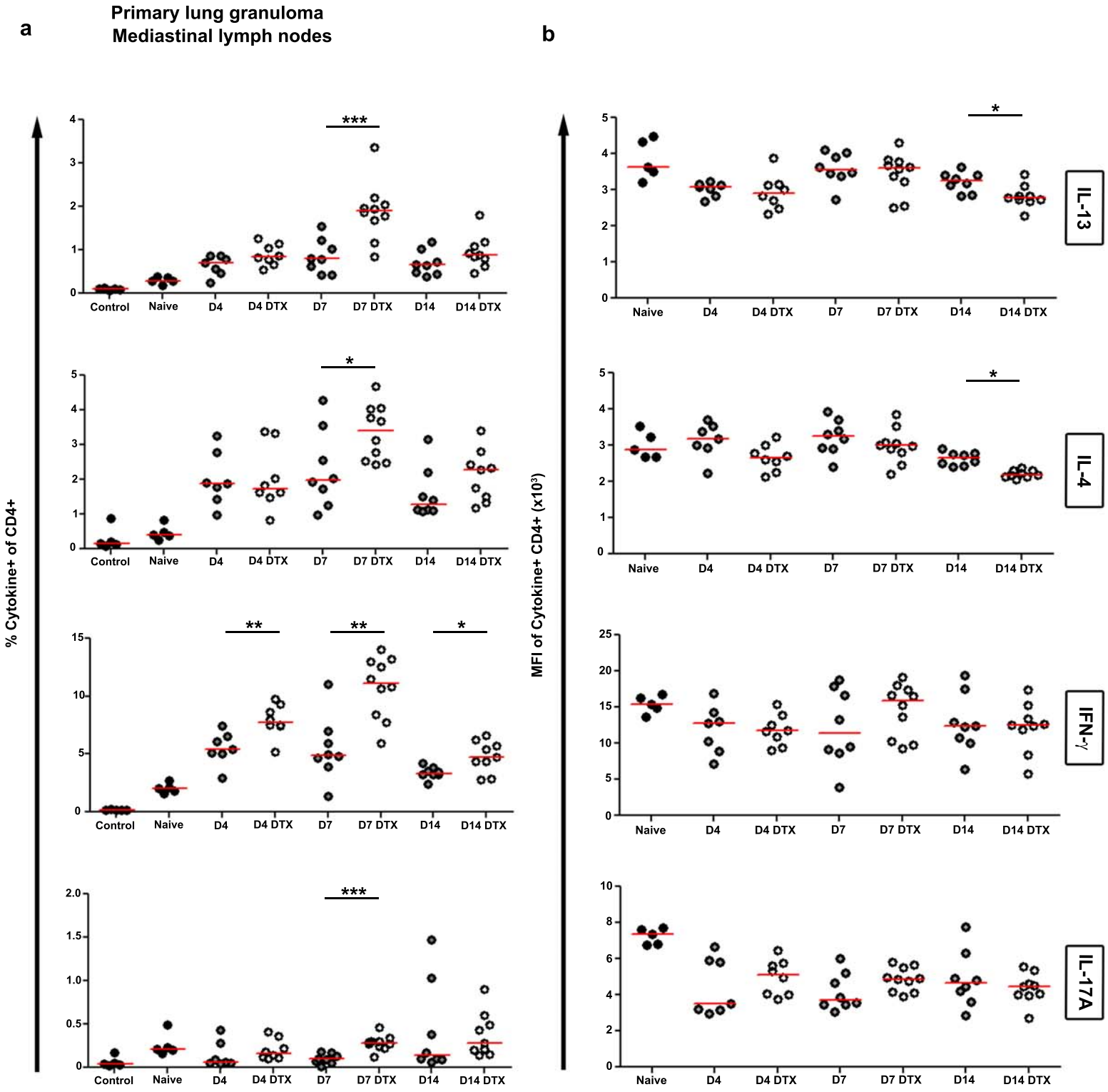

a
Primary lung granuloma
Mediastinal lymph nodes
% Cytokine+ of CD4+
Control
Naive
D4
D4 DTX
D7
D7 DTX
D14
D14 DTX
***
*
**
b
MFI of Cytokine+ CD4+ (x10³)
IL-13
IL-4
IFN-γ
IL-17A

Figure S6

Secondary lung granuloma

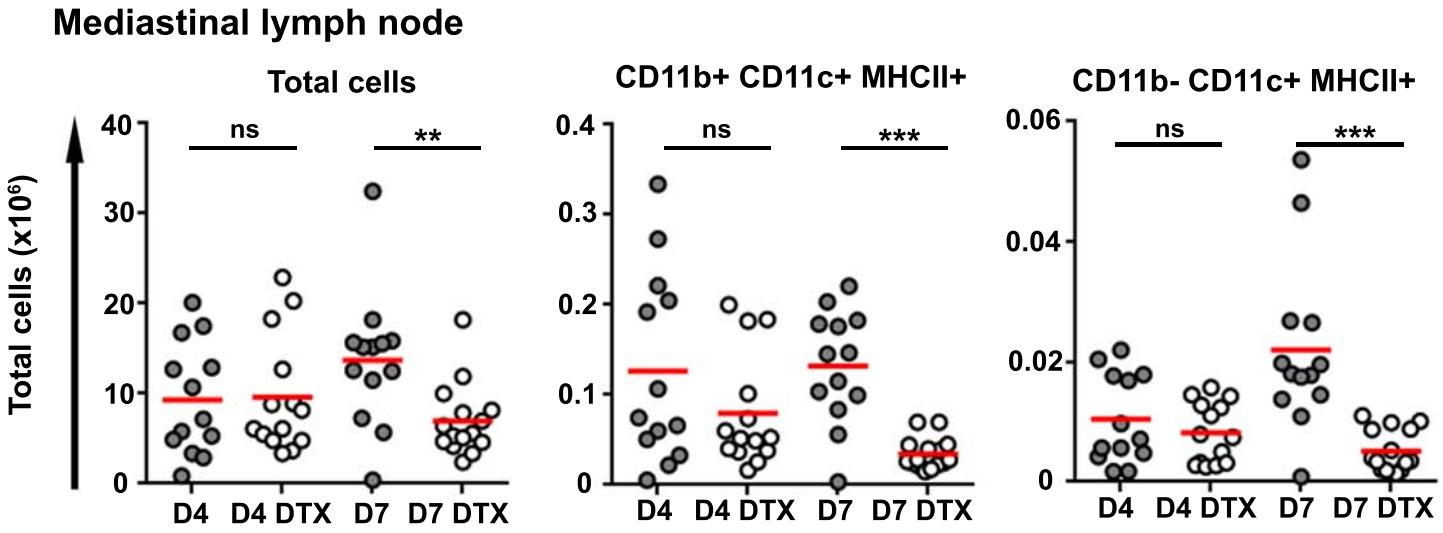


Figure S7

House Dust Mite

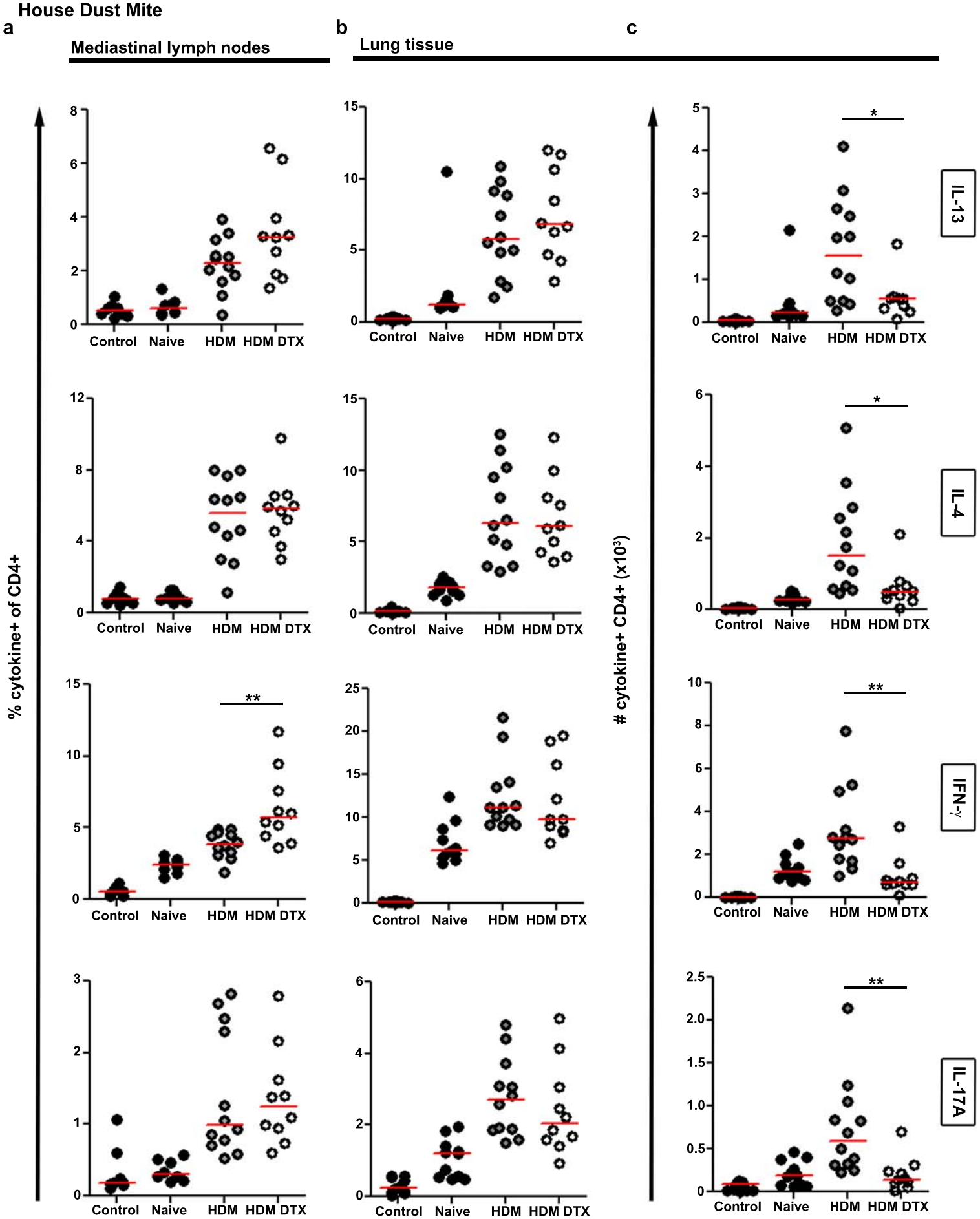
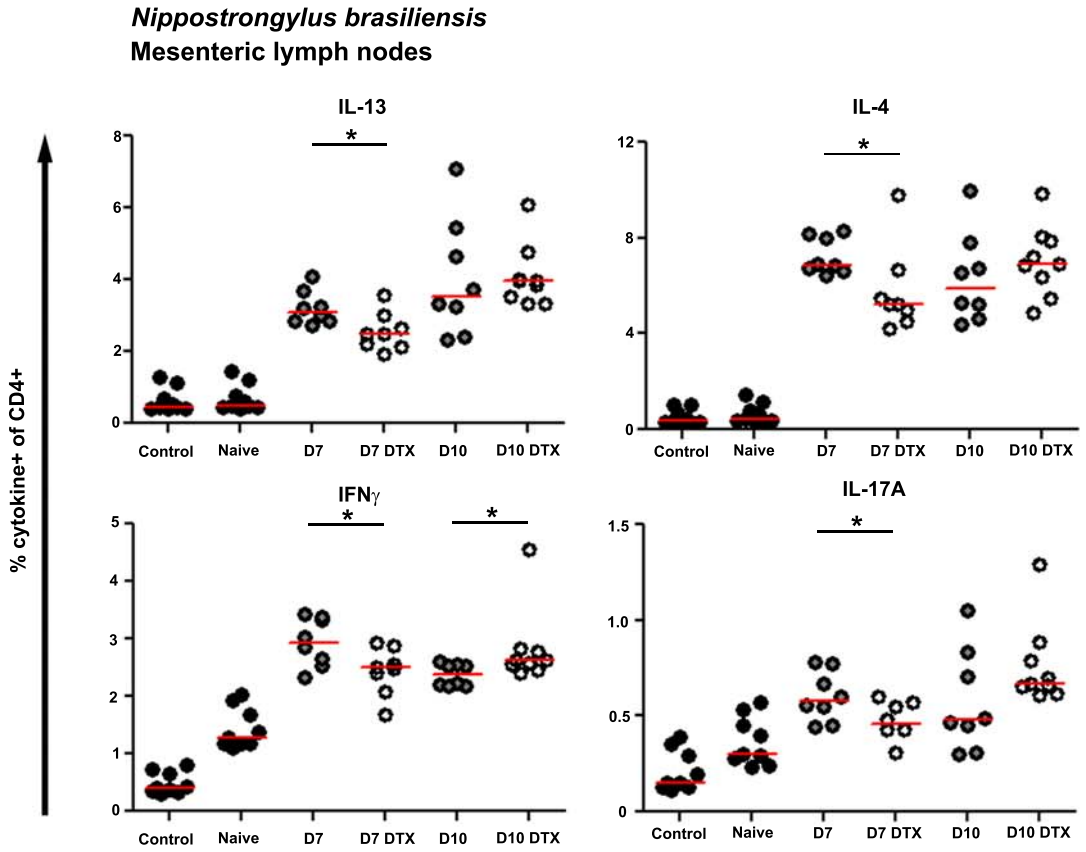


Figure S8


Nippostrongylus brasiliensis
Mesenteric lymph nodes
% cytokine+ of CD4+
IL-13
IL-4
IFNγ
IL-17A
Control
Naive
D7
D7 DTX
D10
D10 DTX
*