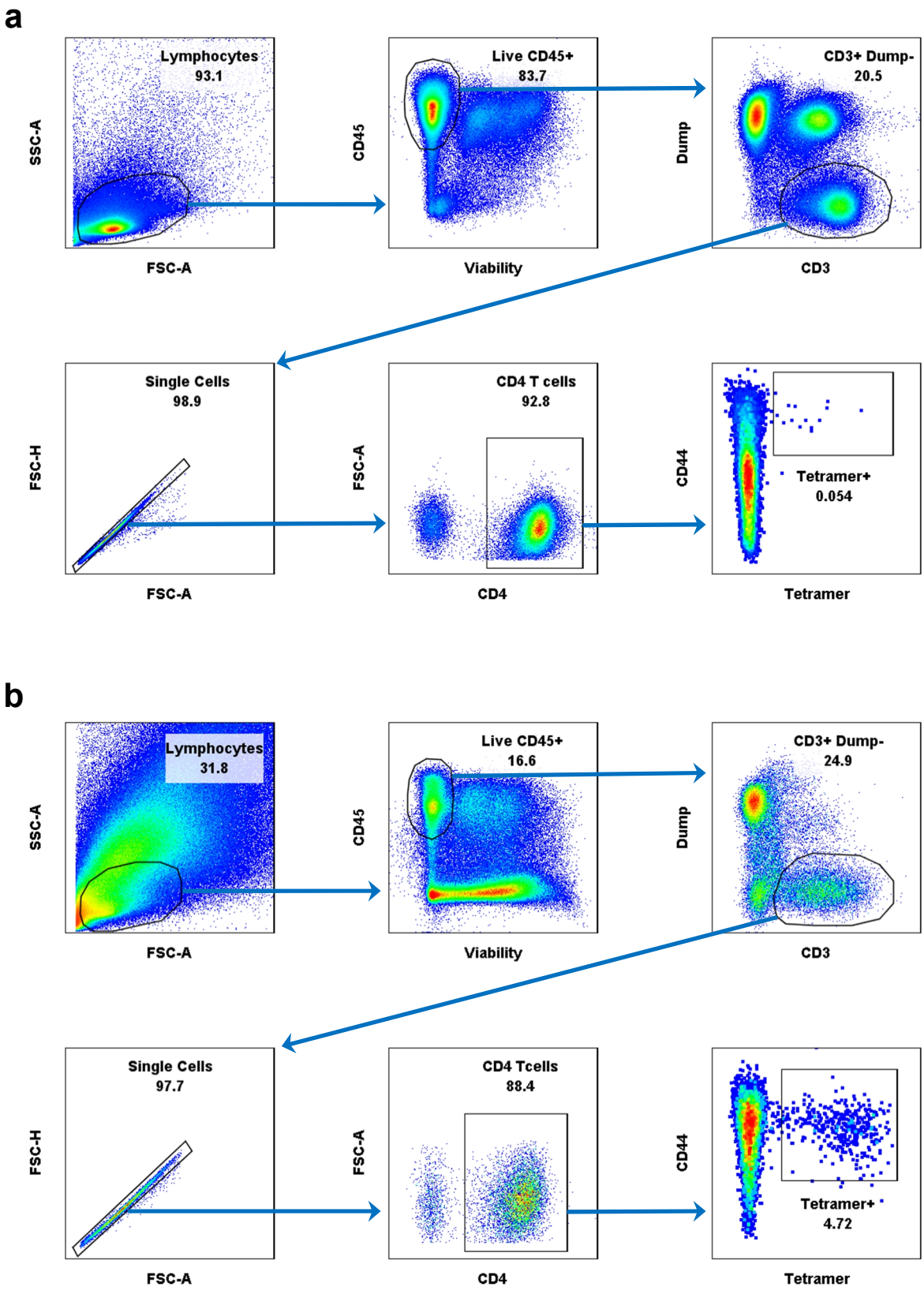

Figure S1 Gating strategy for 2W1S:IA^{b} tetramer^{+} CD4 T cells in the MLNs and colon. Representative plots from BRD509-2W1S-infected mice 6 days p.i. Tetramer^{+} cells are identified from single cell suspension of **a**, MLN and **b**, colonic cells. Cells are identified as live, single CD45^{+}, CD3^{+}, Dump (MHCII, B220, CD8, CD64)$^{-}$, CD4^{+}, CD44^{hi} and tetramer^{+} cells.

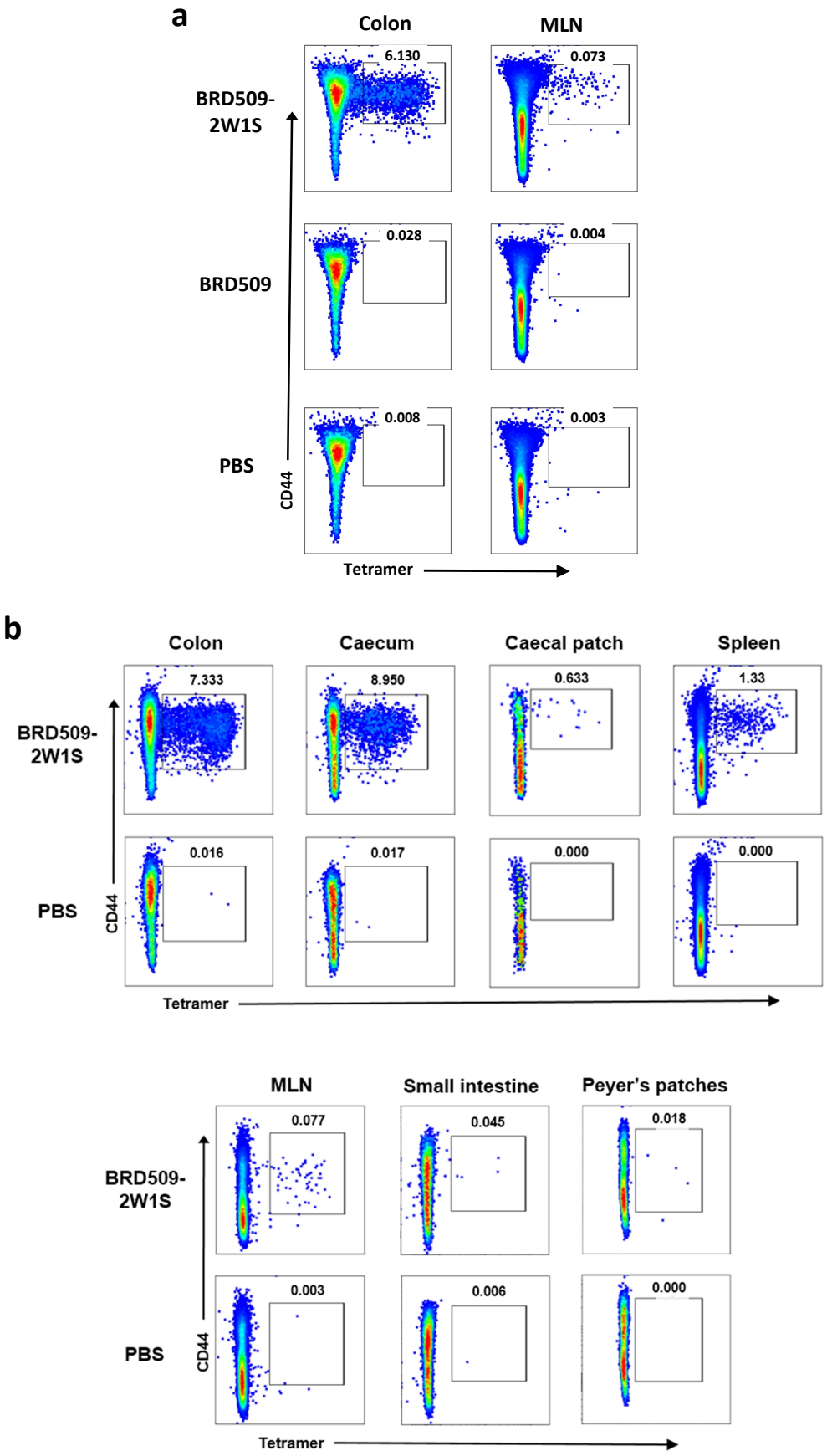

Figure S2 2W1S-specific CD4 T cells are detected in multiple intestinal and lymphoid sites. **a** Representative plots of 2W1S:IA^{b} tetramer^{+} CD4 T cells from the colon and MLN of animals infected with *S.* Tm strain BRD509-2W1S, the non-2W1S-expressing BRD509 parental strain, or mock infected with PBS. **b** Representative plots of tetramer^{+} cells from 7 tissues at day 30 post-infection with BRD509-2W1S (top) or mock-infected with PBS (bottom).

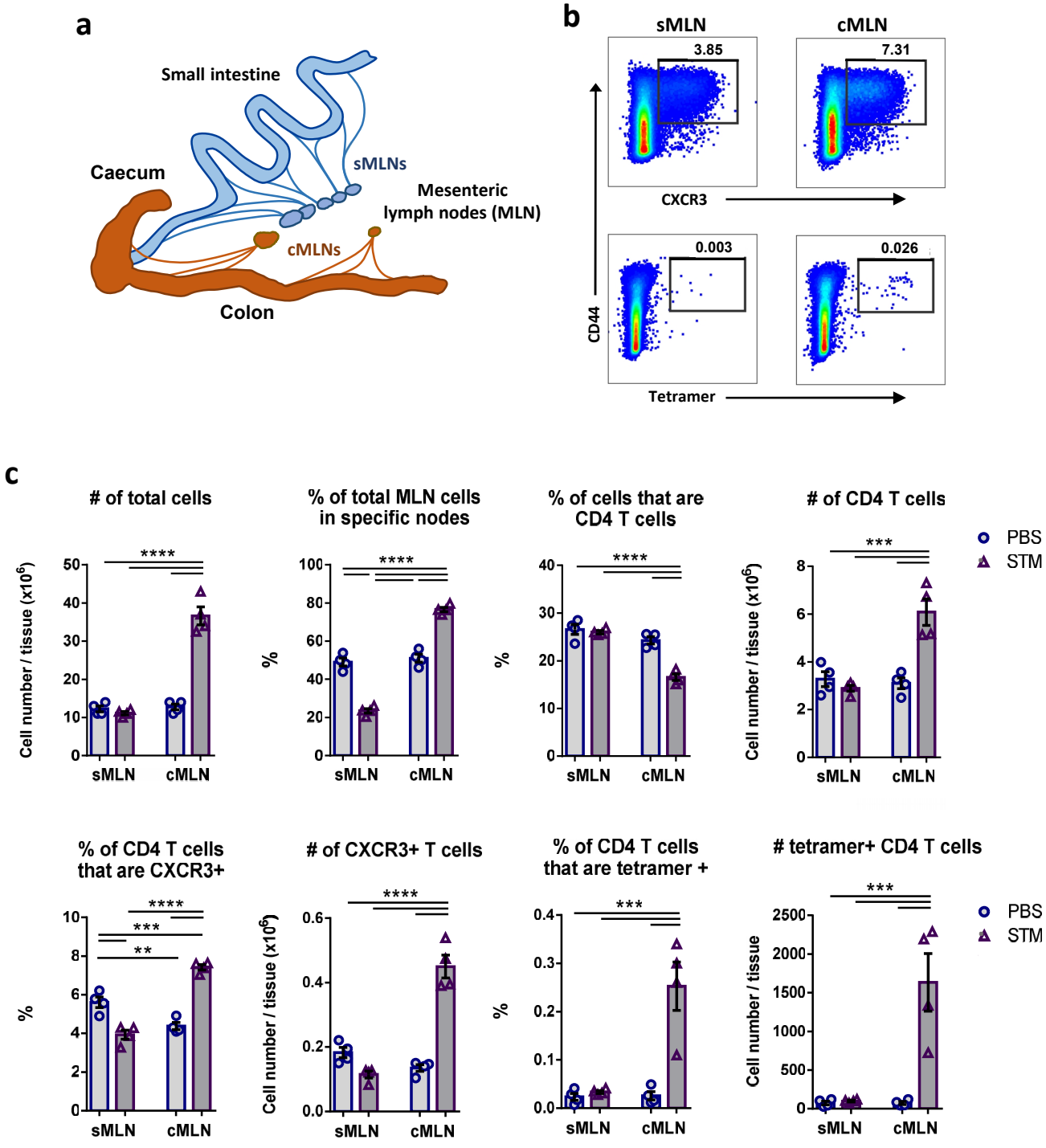

Figure S3 The MLN CD4 T cell response 6 days p.i. is confined to colon and caecum draining nodes. **a** Schematic representation of intestines and MLN, including small intestine draining MLNs (sMLN), and colonic draining MLNs (cMLN). **b** Representative plots of CD4 T cells from sMLN (left) and cMLN (right) that are CXCR3$^+$ (top) and tetramer$^+$ (bottom), 6 days p.i. **c** Graphs showing the number and proportion of total cells, CD4 T cells, and CD4 T cells that are CXCR3$^+$ or tetramer$^+$ at day 6 p.i. Data points represent individual animals (n=4). Mean ± SEM are plotted. Statistical significance calculated by a one-way ANOVA with Tukey's test. *p<.05; **p<.01; ***p<.001; ****p<.0001.

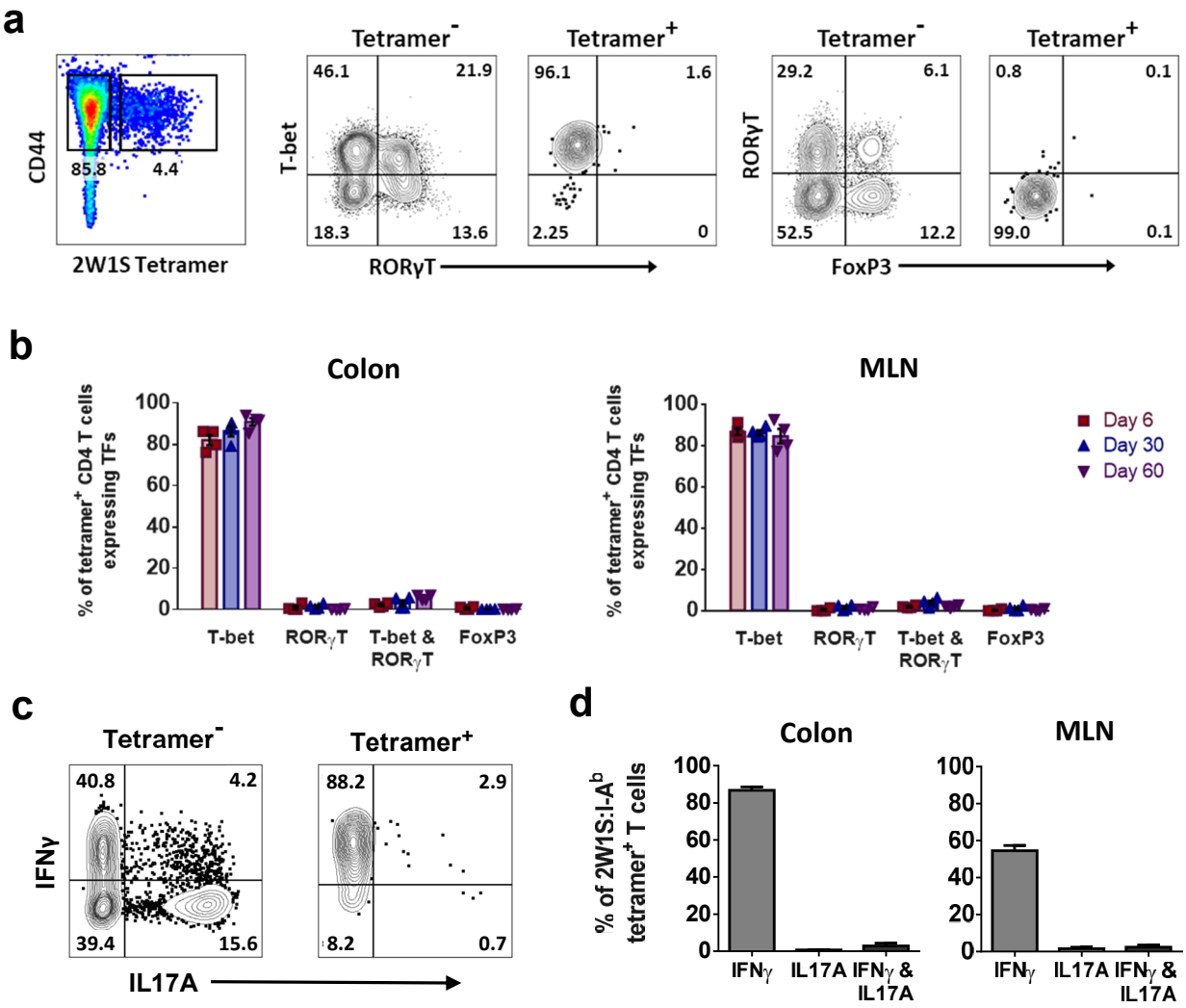

Figure S4 2W1S:I-Ab tetramer$^+$ CD4 T cells in the colon and MLN express T-bet and IFNγ but not RORγT, FoxP3 or IL17A. **a** Representative plots of TF expression by colonic CD4 T cells that are tetramer$^-$ or tetramer$^+$. **b** Tetramer$^+$ cells in the colon (left) and MLN (right) are predominantly T-bet$^+$ and do not express RORγT or FoxP3 at day 6, day 30 and day 60 p.i. **c** Representative plots of IFNγ and IL-17A expression by 2W1S-specific CD4 T cells 11 days p.i. **d** The proportion of 2W1S:I-Ab tetramer$^+$ CD4 T cells expressing IFNγ and/or IL17A in the colon (left) and MLN (right) are shown 11 days p.i. Means ± SEM are plotted.

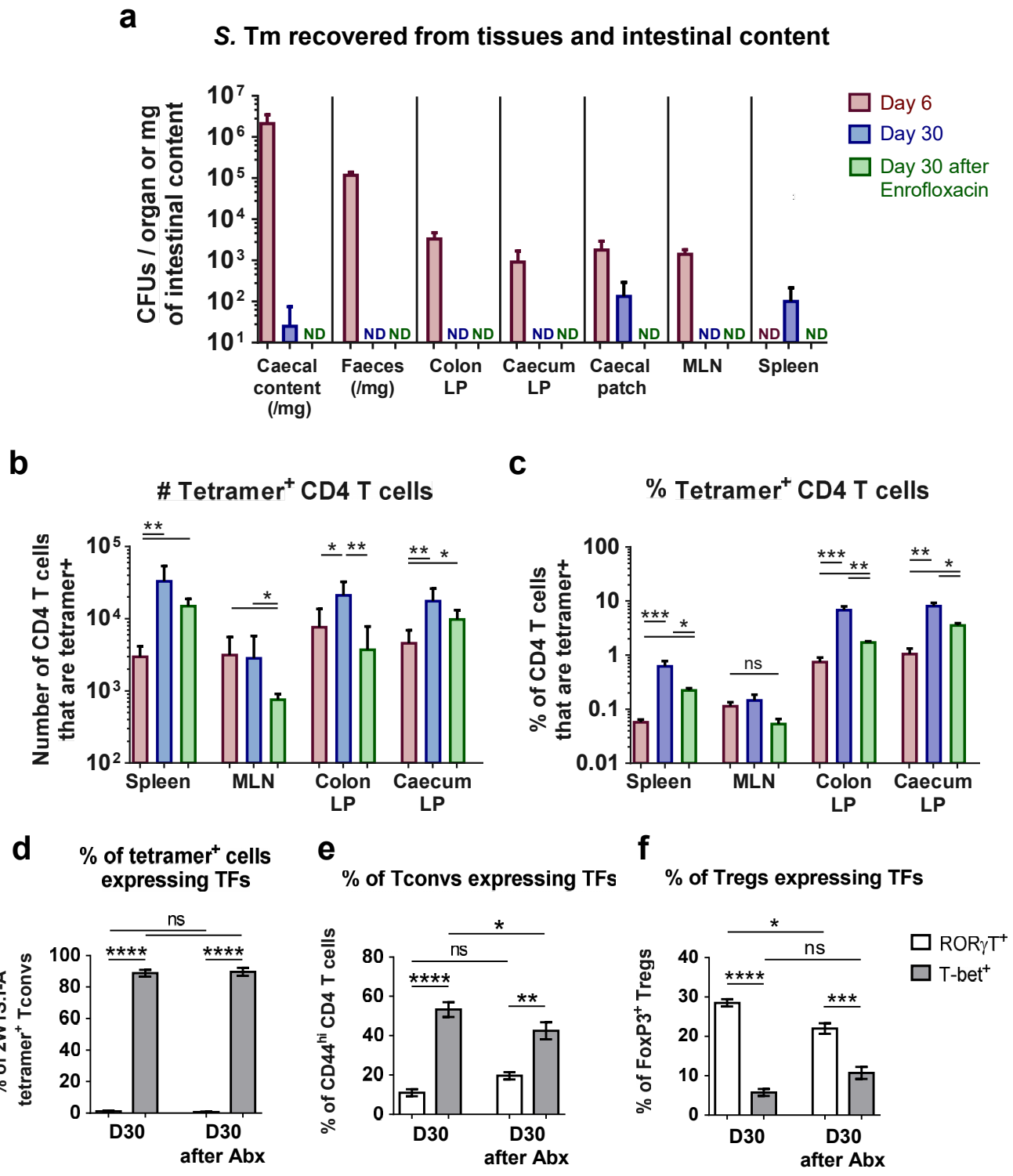

Figure S5. Enrofloxacin treatment for 3 weeks eliminates any detectable *Salmonella* CFUs, reduces the number of tetramer⁺ CD4 T cells, and partially reduces the Th1 bias at day 30 p.i. **a** Samples of caecal content, faeces and tissues were plated following harvest and the number of *S.* Tm CFUs recovered / organ or mg of intestinal content was plotted. **b** The proportion and **c** absolute number of CD4 T cells that are tetramer⁺ at day 6, 30 or 60 p.i. is shown following 24 days of treatment with enrofloxacin in drinking water from 6 days p.i. The proportion of 2W1S-specific T cells (**d**), Tconvs (**e**) and Tregs (**f**) expressing RORγT or T-bet are shown from enrofloxacin-treated or untreated mice at day 30 p.i. Data is from one experiment (n=3-6). Mean ± SEM are plotted. Statistical significance calculated for each tissue by a one-way ANOVA with Tukey's test. ns, not significant; *p<.05; **p<.01; ***p<.001; ****p<.0001; Abx, antibiotics; ND, not detected).

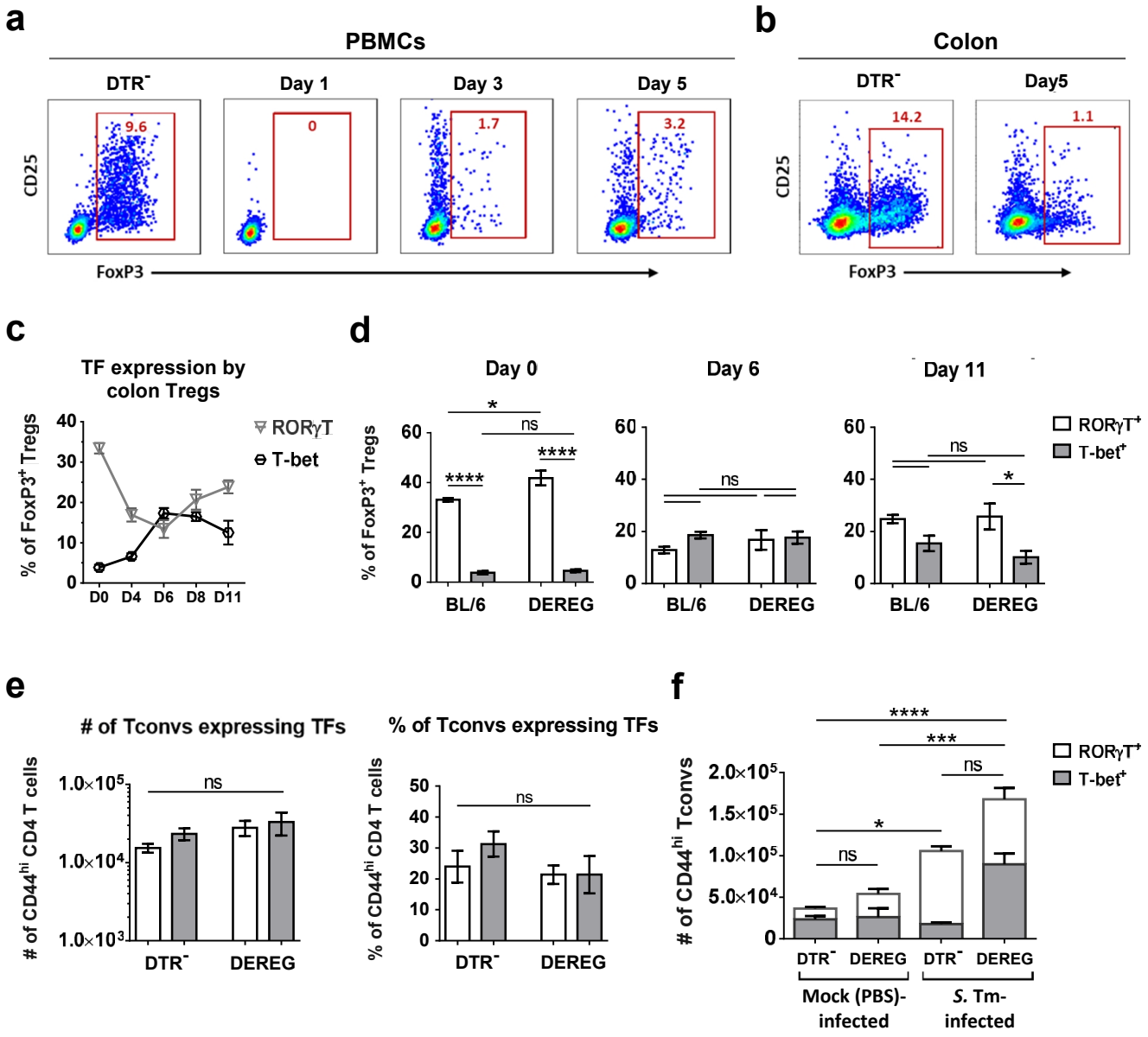

Figure S6 Diphtheria toxin (D.T.) treatment of DEREG mice depletes Tregs in PBMC and colonic lamina propria; Tregs in untreated DEREG mice express similar levels of T-bet and RORγT as C57BL/6 mice; and Treg ablation in mock-infected mice does not significantly shift the Th1-Th17 bias nor increase colonic Tconv numbers, as observed following *S.* Tm infection. **a** Representative plots of CD4 T cells from PBMCs are shown from a WT littermate (left) and DEREG mouse at 1, 3 or 5 days following 2 D.T. treatments, 24 hrs apart. **b** Representative plots show the proportion of colon CD4 T cells that are FoxP3$^+$ 5 days after D.T. treatment. CD4 T cells are identified as described in Fig. 1d. Gate numbers represent the proportion of CD4 T cells that are FoxP3$^+$. **c** The proportion of colon Tregs from C57BL/6 mice that express T-bet or RORγT are plotted following *S.* Tm infection. **d** Graphs compare Treg expression of T-bet or RORγT between C57BL/6 mice and Treg replete (untreated) DEREG mice at day 0, 6 and 11 following *S.* Tm infection. **e** The absolute number (left) and proportion (right) of colonic CD44^{hi} Tconvs that are RORγT$^+$ or T-bet$^+$ are shown 4 days following D.T. treatment in uninfected DEREG mice or wild type (WT) littermates. **f** The number of RORγT$^+$ and T-bet$^+$ Tconvs are stacked to show changes in cell numbers 4 days following Treg depletion in mock- or *S.* Tm-infected mice 6 days p.i. Data represent n=3-8/group. Means ± SEM are plotted. Statistical significance calculated by one-way ANOVA (**d**, **e**) or two-way ANOVA with Holm-Šídák test of compiled columns (**f**) . ns, not significant; *p<.05; **p<.01; ***p<.001; ****p<.0001.

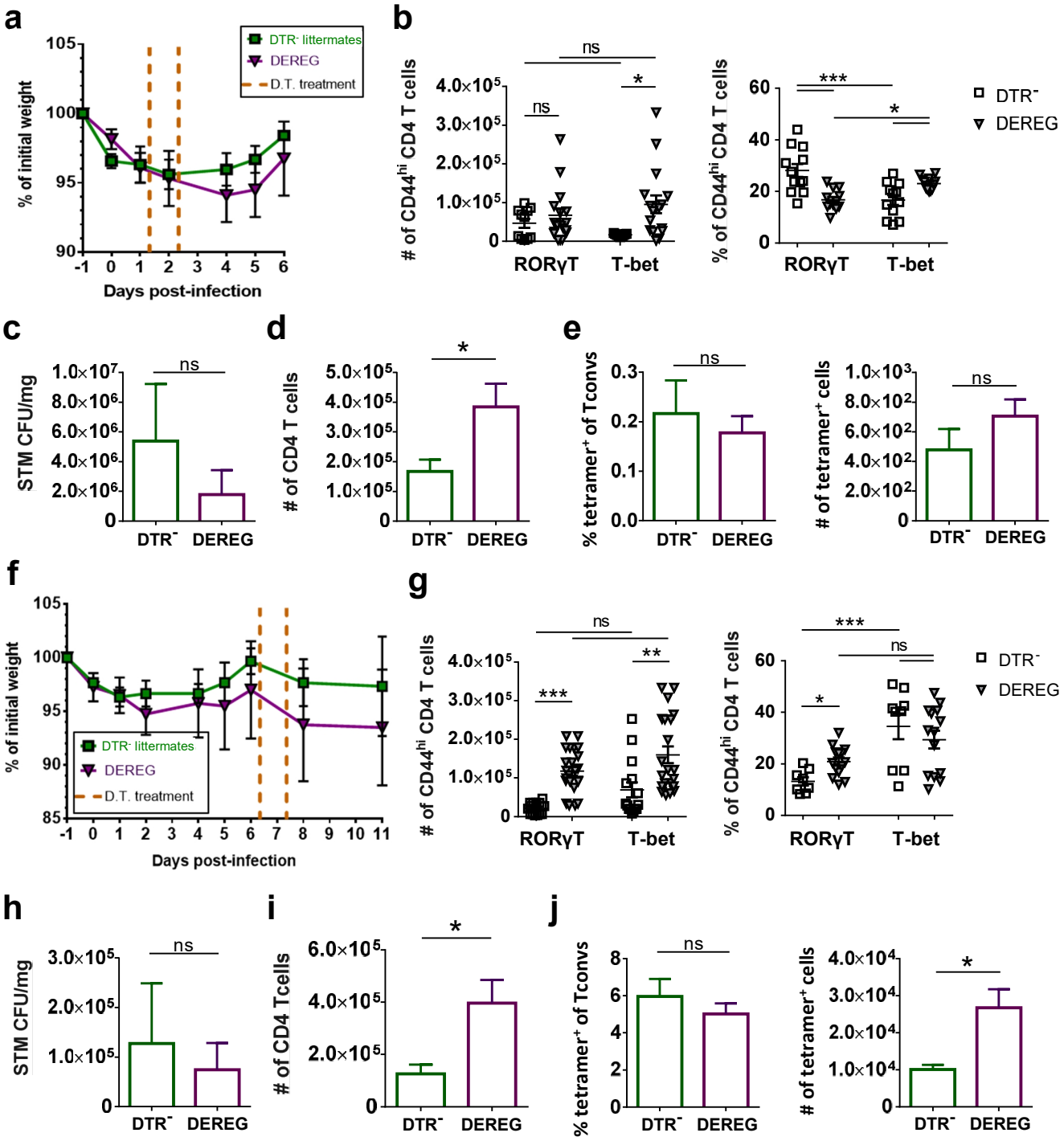

Figure S7 Treg depletion of *S.* Tm-infected mice does not cause significant weight loss, change faecal *S.* Tm burden or tetramer$^+$ cells, but consistently shifts Th bias. **a** Weight loss in DEREG mice and WT littermates following *S.* Tm infection and D.T. treatment (represented by dashed lines) at day 1-2 p.i. **b** Pooled data showing the number (left) and proportion (right) of CD44^{hi} CD4 Tconvs expressing RORγT or T-bet at day 6 p.i. **c** STM CFU recovered from faeces at day 6 p.i. **d** The number of colonic CD4 T cells in DEREG mice and WT littermates are shown. **e** The proportion (left) and number (right) of colonic Tconvs that are tetramer$^+$ are shown. **f** Weight loss shown following STM infection and D.T. treatments at day 6-7 p.i. **g** Pooled data shows the number (left) and proportion (right) of Tconvs expressing RORγT or T-bet at day 11 p.i. **h** *S.* Tm CFU recovered from faeces at day 11 p.i. **i** The number of CD4 T cells, **j** The proportion (left) and number (right) of colonic Tconvs that are tetramer$^+$ are shown. Data are representative examples of three independent experiments (n=3-5) (**c**-**e**, **h**-**j**) or are pooled from 3 independent experiments (**b, g**). Means ± SEM are plotted. Statistical significance calculated by Mann-Whitney test (**c**-**e**, **h**-**j**) or one-way ANOVA with Holm-Šídák test (**b**, **g**). ns, not significant; *p<.05; **p<.01; ***p<.001.

Figure S8 Antibodies used for flow cytometry

Marker	Fluorochrome	Clone	Concentration	Manufacturer
7AAD	N/A	N/A	5µl/sample	Biolegend
B220	BV510	RA3-6B2	1:200	Biolegend
CCR6	BV605	29-2L17	1:100	Biolegend
CD25	PE/Cy7	PC61	1:200	Biolegend
CD3	BV605	17A2	1:100	Biolegend
CD3ε	AF700	eBio500A2	1:100	eBioscience
CD3ε	BUV395	145-2C11	1:100	BD Biosciences
CD4	BUV805	GK1.5	1:200	BD Biosciences
CD4	AF647	GK1.5	1:200	Biolegend
CD4	PE/Cy7	GK1.5	1:200	Biolegend
CD44	BV605	IM7	1:100	Biolegend
CD44	BV785	IM7	1:100	Biolegend
CD45	BV785	30-F11	1:200	Biolegend
CD45	AF700	30-F11	1:200	Biolegend
CD8α	BV510	53-6.7	1:200	Biolegend
CXCR3	BV421	CXCR3-173	1:100	Biolegend
CXCR3	BV605	CXCR3-173	1:100	Biolegend
F4/80	BV510	BM8	1:200	Biolegend
Fixable viability	eFluor 780	N/A	1:1000	Invitrogen
FoxP3	APC	FJK-16s	1:100	eBioscience
FoxP3	PE	FJK-16s	1:100	eBioscience
FoxP3	AF700	FJK-16s	1:100	Invitrogen
GATA3	PerCP/Cy5.5	TWAJ	1:100	eBioscience
Helios	AF488	22F6	1:100	BD Biosciences
Helios	PerCP/Cy5.5	22F6	1:100	Biolegend
I-A/I-E	BV510	M5/114.15.2	1:200	Biolegend
IFNγ	PE	3E4	1:100	eBioscience
IFNγ	PerCP/Cy5.5	XMG1.2	1:100	Biolegend
IL-17A	BV605	TC11-18H10.1	1:100	Biolegend
RORγT	PE	AFKJS-9	1:100	eBioscience
RORγT	APC	B2D	1:100	Invitrogen
T-bet	PE/Cy7	eBio4B10	1:100	eBioscience