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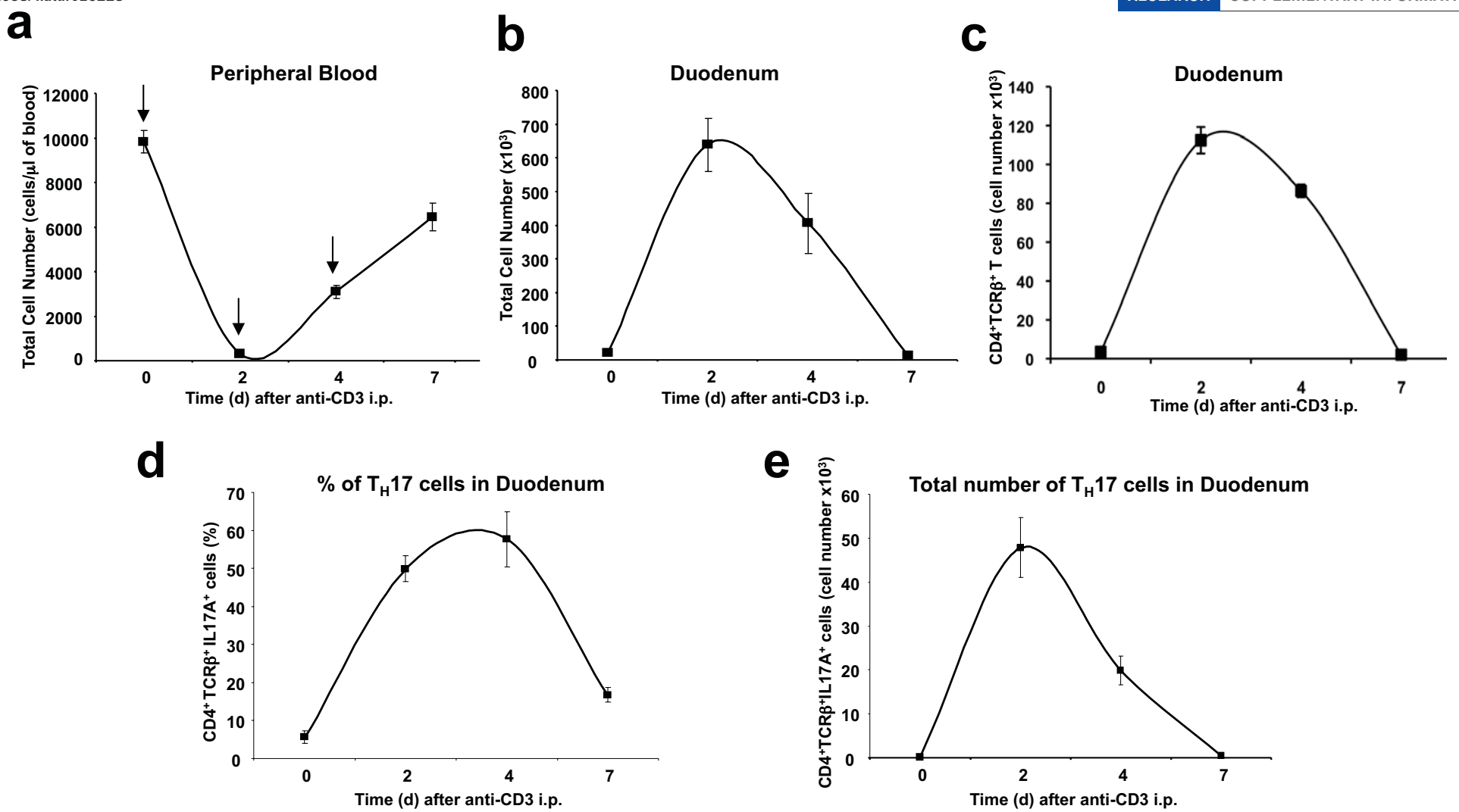
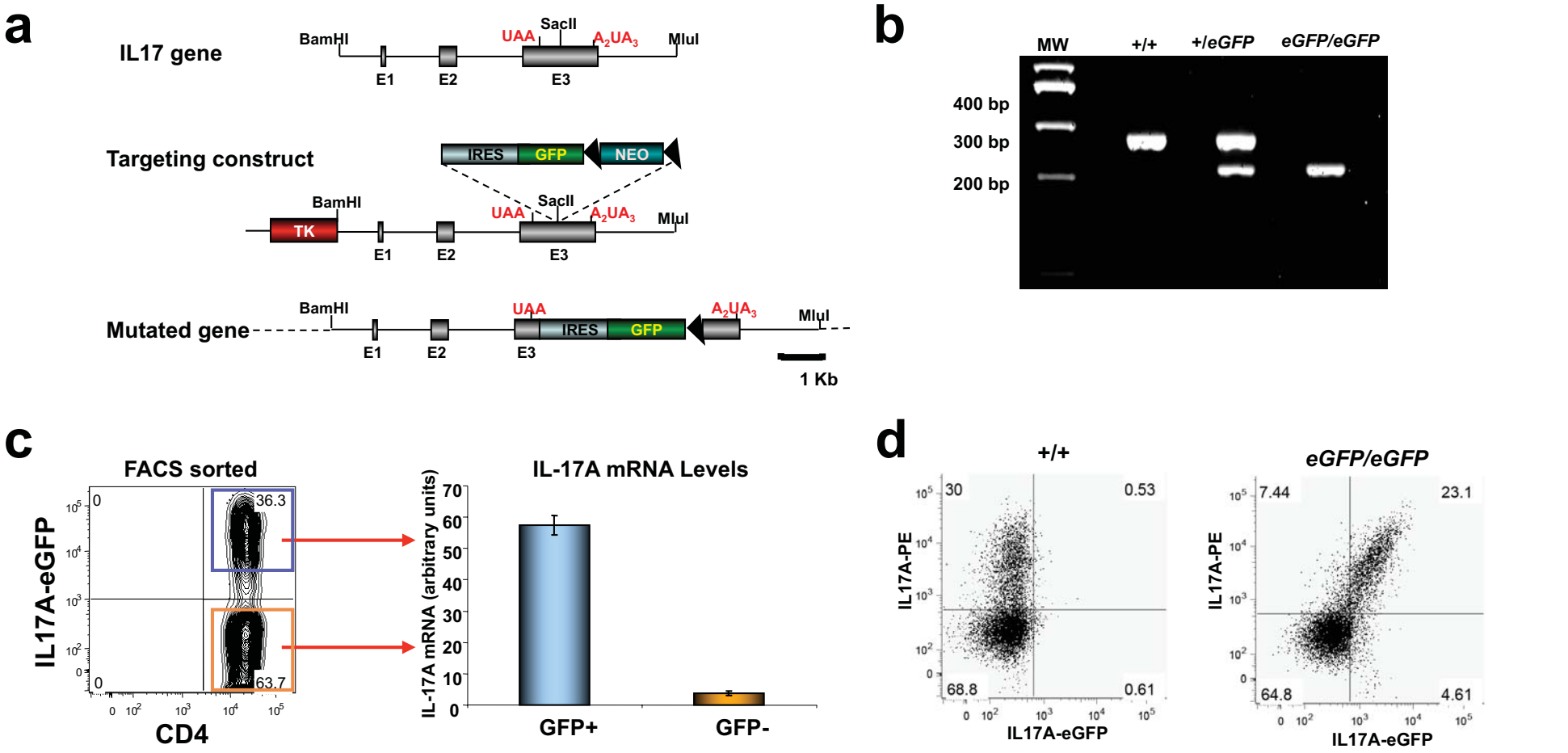
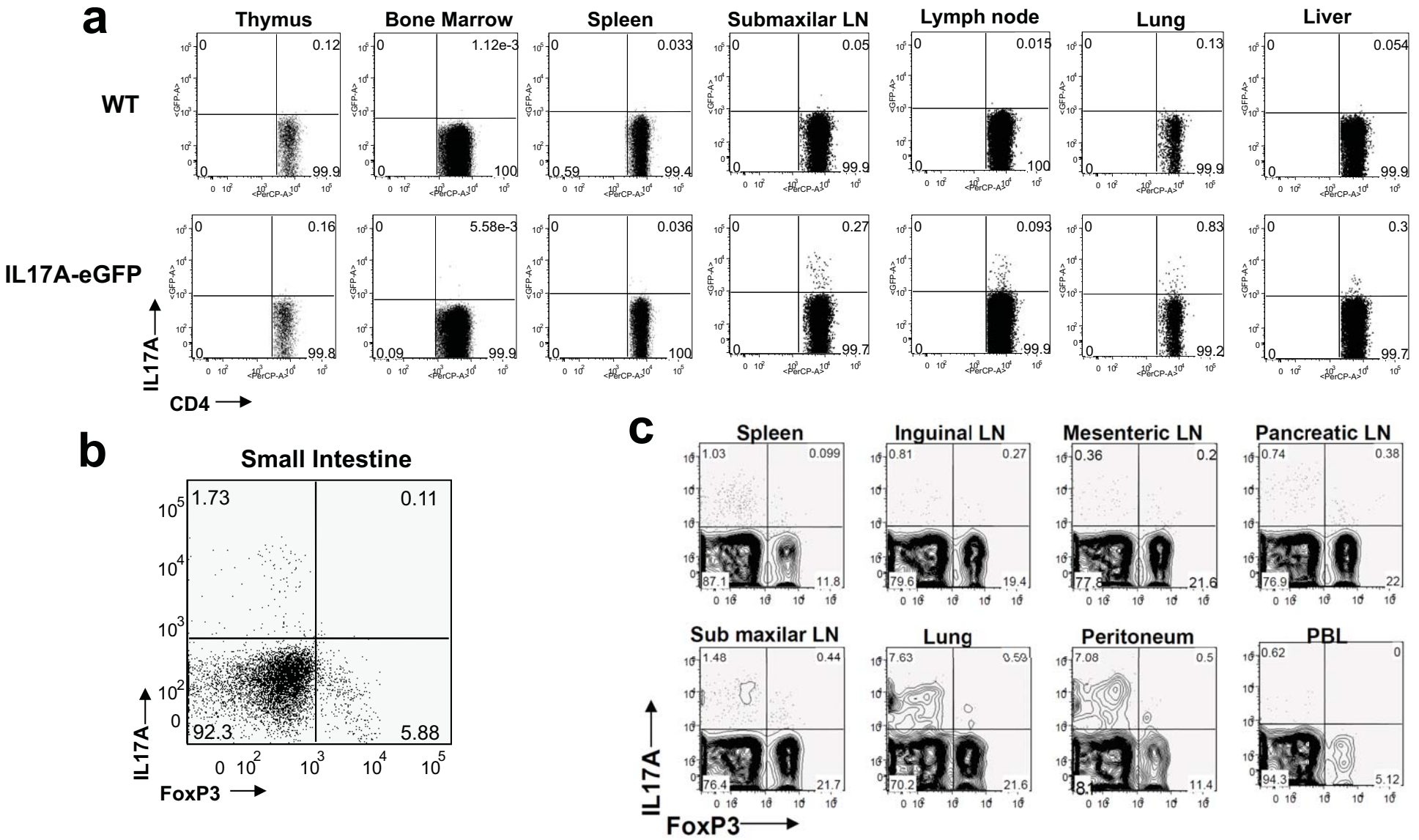


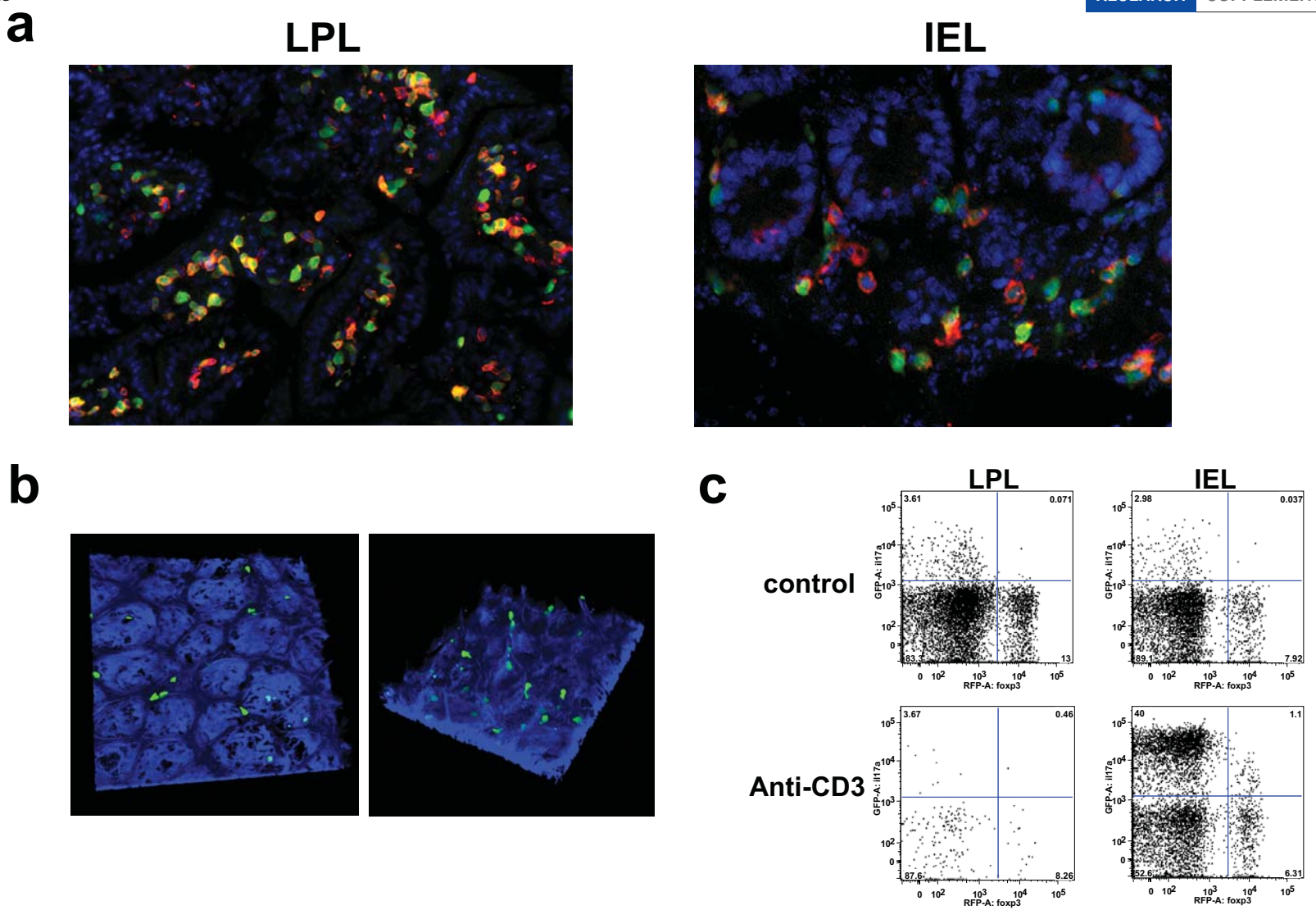
Supplemental Fig. 1. Dynamics of T_H17 cells during CD3-specific treatment.
C57BL/6 mice were treated three times with 20μg of CD3-specific antibody (arrows) and total cell number in the blood (**a**) and in the duodenum (**b**) were counted at different time points during the treatment. The total number of $CD4^+$ $TCR\beta^+$ T cells (**c**), percentage (**d**) and the total number (**e**) of T_H17 in the duodenum was obtained after flow cytometry analysis of IL-$17A^+$ cells gated on $CD4^+TCR\beta^+$ T cells (mean ± s.e.m; n=5). Representative results from three independent experiments.



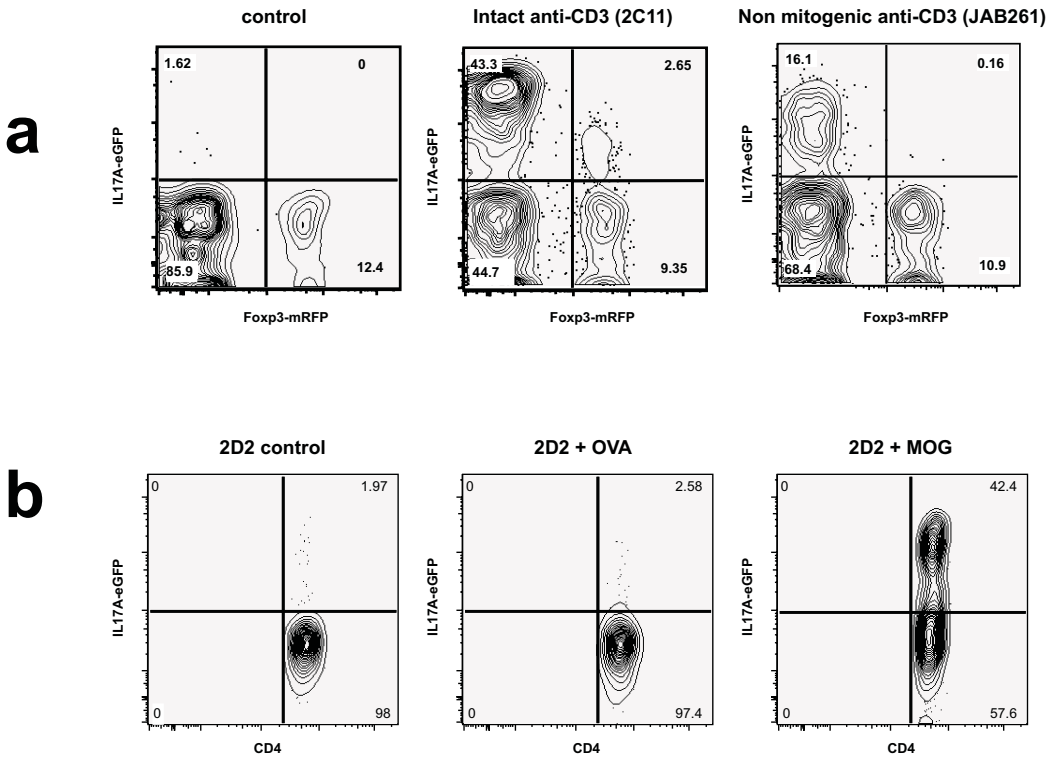
Supplemental Fig. 2. Targeting IRES-eGFP reporter into the mouse IL-17 locus.
(**a**) Maps for mouse IL-17A locus, targeting DNA construct and the targeted IL-17A locus. An 8-kb mouse genomic fragment, including exons 1, 2 and 3 of IL-17A gene, was excised by using BamHI and MluI (*Top*) and cloned into pEasy-Flox vector adjacent to the thymindine kinase (TK) selection marker. A cassette containing IRES-GFP and LoxP-flanked neomycin (Neo) selection marker was inserted into a SacII site between the translation stop codon (UGA) and the polyadenylation signal (A2UA3) of IL-17A gene (*Middle*). A correctly targeted ES cell was used to create chimeras and germ-line-transmitted mice. The Neo gene was removed *in vivo* by using deletor mice transgenic for Cre recombinase to generate mice bearing targeted IL-17A locus (*Lower*). (**b**) PCR genotyping IL-17A-eGFP reporter mice. Three primers were designed to genotype reporter mice. PCR yielded 220-bp product for the wild-type (Wt) IL-17A allele and 290-bp product for targeted IL-17A allele. (**c**) IL-17A mRNA expression in eGFP positive versus eGFP negative cells after sorting. (**d**) Dot plots of PE-anti-IL-17A (Y axis) versus eGFP (X axis) intracellular staining of T_H17 cells from wild-type (left) and homozygous (right) mice.



Supplemental Fig 3. Expression of IL-17A detected *in vivo* by an IL-17A-eGFP reporter mouse. Flow cytometry analysis of IL17A^{+}CD4^{+} T cells in different organs from IL17A-eGFP reporter mice and wild-type littermates in steady state conditions are shown (**a**). Flow cytometry analysis of IL-17A (Y axis) and Foxp3 (X axis) on the small intestine CD4^{+} T cells from IL17A-eGFP x Foxp3-mRFP double reporter mice in steady state conditions (**b**). IL17A-eGFP x Foxp3-mRFP double reporter mice were treated three times with 20μg of anti-CD3 antibody (2C11) (**c**). Four hours after the last injection animals were sacrificed, different organs removed and processed for FACS analysis. Plots are gated on CD4^{+} and numbers in quadrants indicate percent cells in each. Data represents at least 3 different experiments.

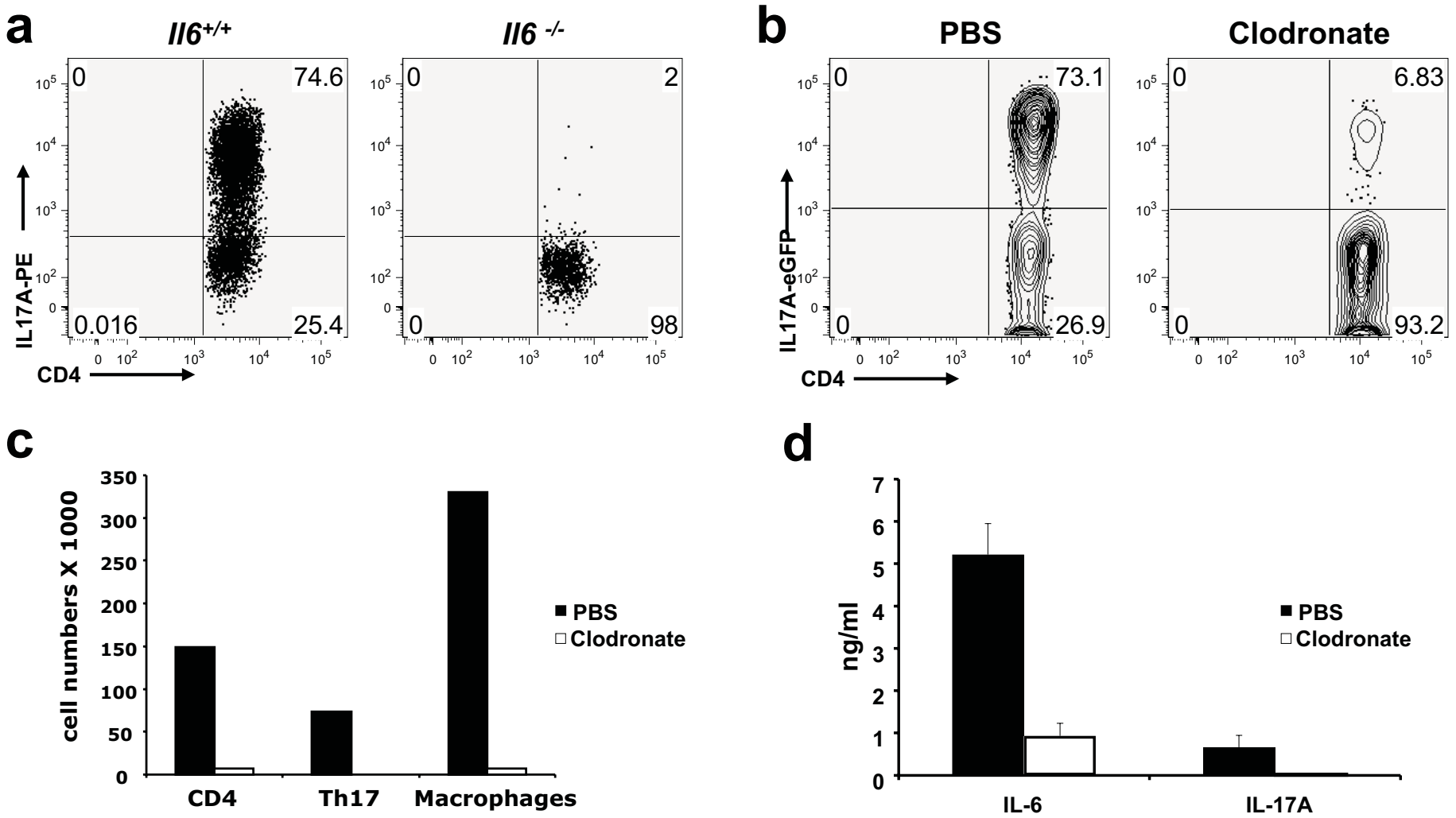


Supplemental Fig. 4. Small Intestine localization of T_H17 cells during CD3-specific antibody treatment.
(**a**) Sections demonstrating that eGFP$^+$CD4$^+$ T cells can be located in the lamina propria compartment (left) and in the intraepithelial compartment (right) of the small intestine. Original magnification 40x. (blue, dapi; red, CD4; green, IL-17A-eGFP). (**b**) 3D reconstruction of small intestine from IL17A-eGFP knock-in mice treated with CD3-specific antibody obtained by multiphoton microscopy analysis. (blue, second harmonics; green, IL-17A-eGFP). (**c**) Flow cytometry analysis of IL-17A (Y axis) and Foxp3 (X axis) on the intestinal LPL (lamina propria lymphocytes) and IEL (intestinal intraepithelial lymphocytes) CD4$^+$ T cells from IL17A-eGFP x Foxp3-mRFP double reporter mice after CD3-specific antibody treatment. All animals were analyzed 100h after the first injection of CD3-specific antibody.

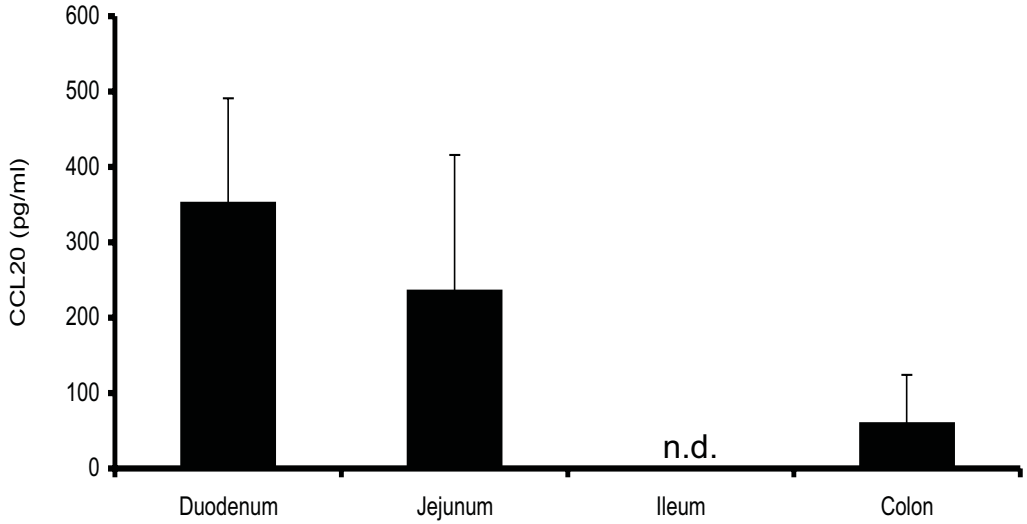


Supplemental Fig. 5. T_H17 cells are increased in the small intestine during non mitogenic anti-CD3 treatment (a) or during antigen specific immunization (b).

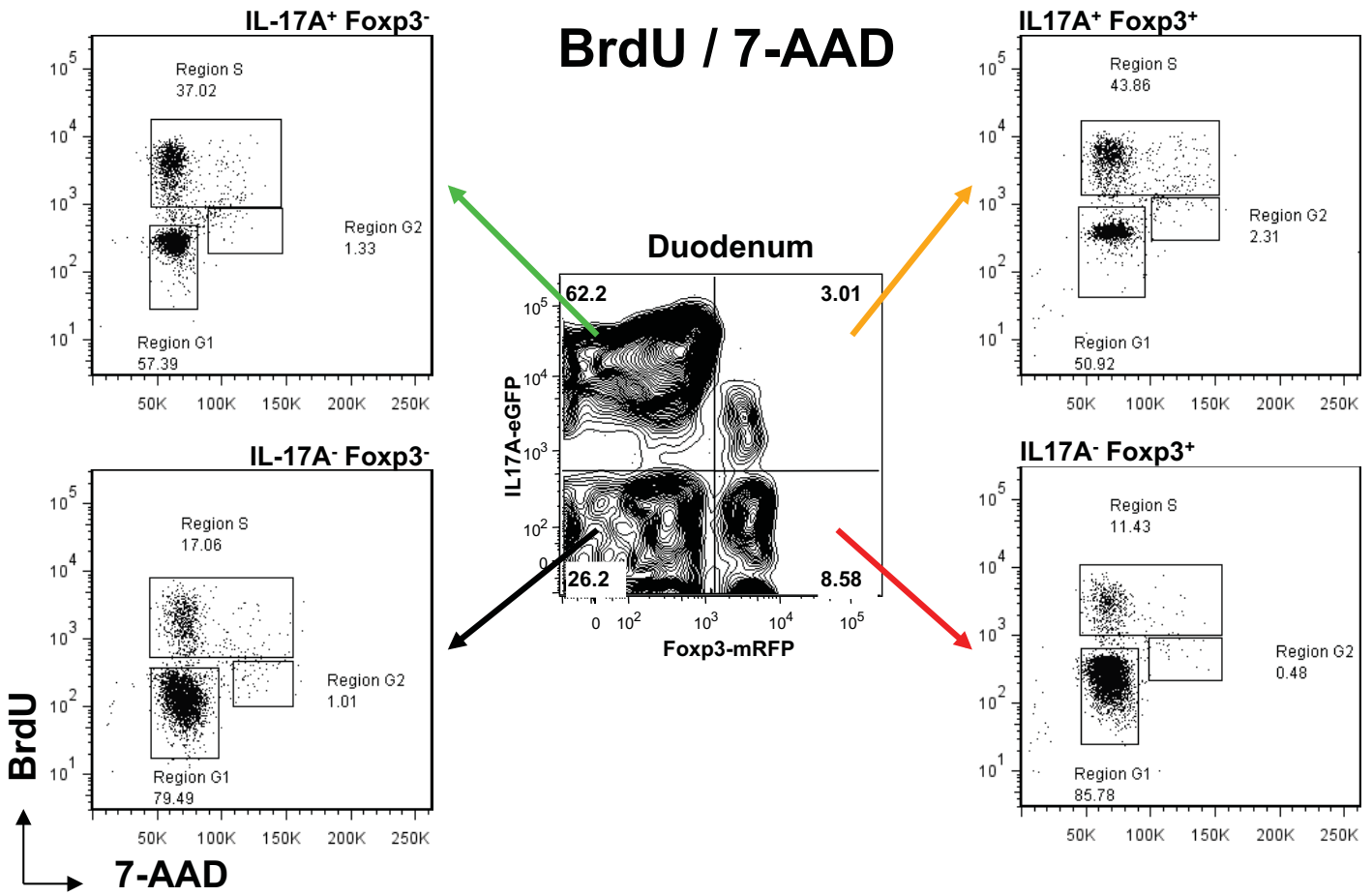
(**a**) Flow cytometry analysis of IL-17A and Foxp3 on intestinal $CD4^+$ T cells from IL17A-eGFP x Foxp3-mRFP double reporter mice 100h after the first injection of CD3-specific antibody or after non-mitogenic anti-CD3 treatment. (**b**) MOG-TCR transgenic mice (2D2 mice) crossed with the IL-17A-eGFP knock-in mice were treated three times with PBS, OVA or MOG peptide (150 mg/i.p. dose; injections on days 0,3 and 6). Four hours after the last injection the presence of T_H17 in the duodenum of the treated mice was evaluated by flow cytometry analysis. (n=4). Experiments were repeated twice with similar results.



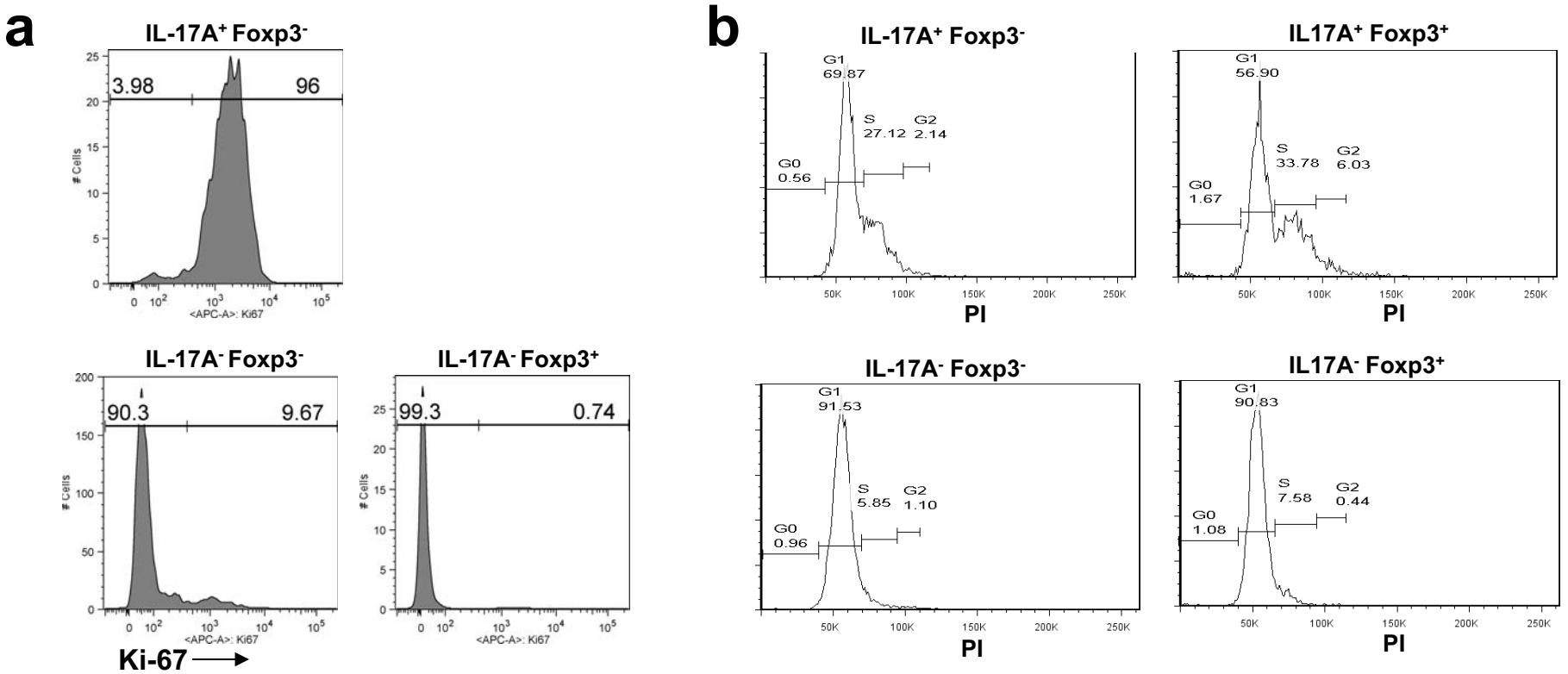
Supplemental Fig. 6. IL-6 secreted by antigen-presenting cells (APCs) is critical for the generation of T_H17 cells during CD3-specific antibody treatment. (a) Flow cytometry analysis of IL-17A intracellular expression in $CD4^+$ T cells from duodenum of wild type and *IL-6*$^{-/-}$ mice treated with CD3-specific antibody.(n=5). Experiment was performed twice with similar results. (b) IL-17A-eGFP reporter mice were treated with clodronate-loaded liposomes or with PBS-loaded liposomes one day before the treatment with CD3-specific antibody. 100h after the first injection T_H17 cells from the duodenum were analyzed by flow cytometry. (n=5). Data represents three independent experiments. (**c**) Total $CD4^+$ T cells, T_H17 cells and Macrophages ($CD11b^+CD11c^-$) from experiment as in (**b**) were quantified. (**d**) Plasma levels of IL-6 and IL-17A in these animals were measured by CBA (mean ± s.e.m; n=5).



Supplemental Fig. 7. CCL20 protein expression in the gastrointestinal tract.
Different parts of the intestine were cultured for two days. CCL20 was measured in the supernatants using ELISA (mean +/- s.e.m.).



Supplemental Fig. 8. T_H17 cells from duodenum actively proliferate during CD3-specific antibody treatment. Proliferation capacity assessed by BrdU/7-AAD analysis on different populations of FACS sorted $CD4^+TCR\beta^+$ T cells from the duodenum of IL-17A-eGFP x Foxp3-mRFP double reporter mice treated with CD3-specific antibody (clone 2C11). Cells were isolated 100h after the first injection. Data represents three independent experiments.



Supplemental Fig. 9. T_H17 cells from duodenum actively proliferate during CD3-specific antibody treatment.
Proliferation capacity assessed by Ki-67 (**a**) or by Propidium Iodide (PI) cell cycle analysis (**b**) on different populations of FACS sorted CD4$^+$TCRβ^+ T cells from the duodenum of IL-17A-eGFP x Foxp3-mRFP double reporter mice treated with CD3-specific antibody (clone 2C11). % of cells that are in different phases of the cell cycle are represented in (**b**). Data represents three independent experiments. (**a**) was analyzed 60h after the first injection and (**b**) 100h after the first injection

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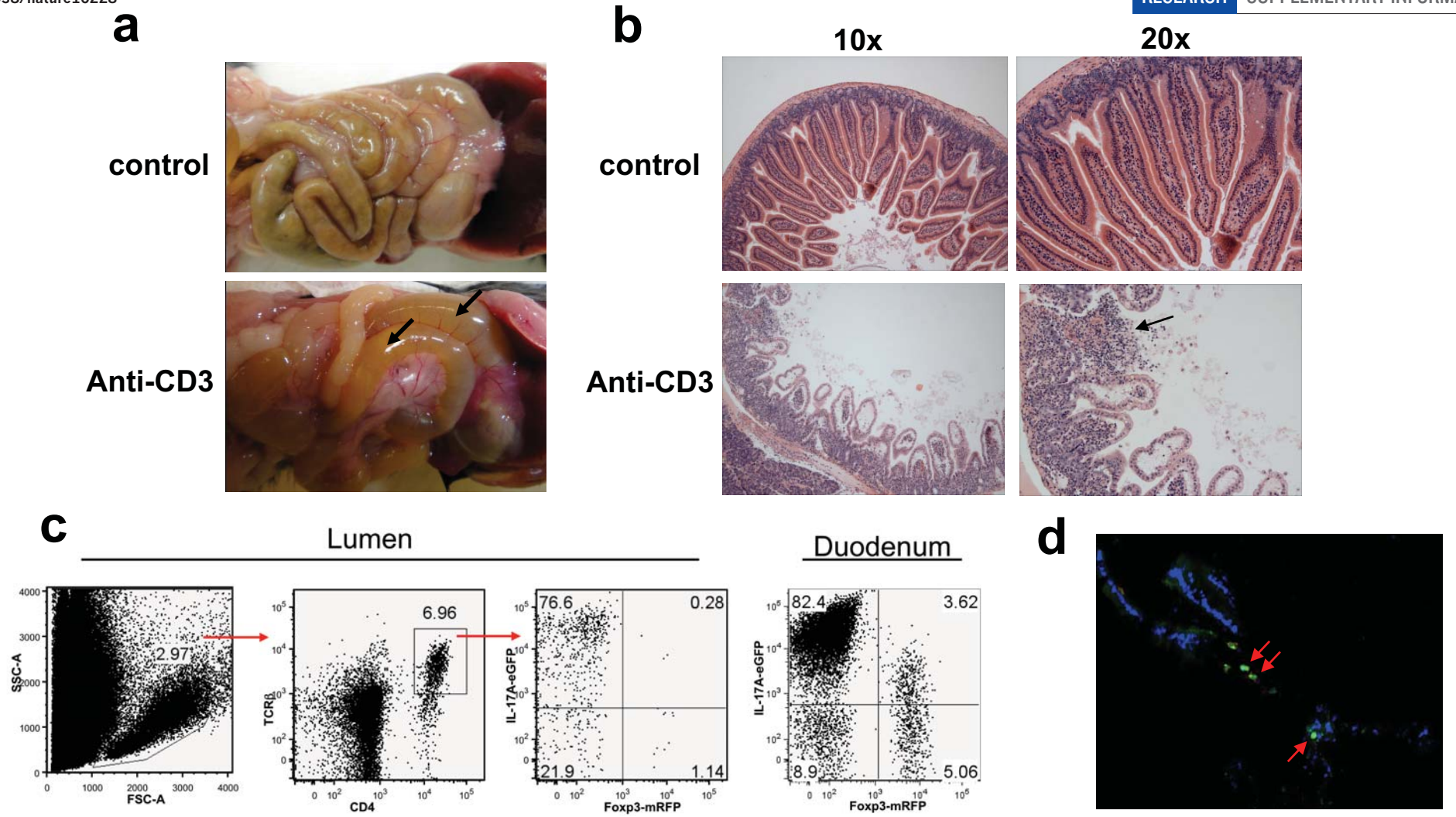
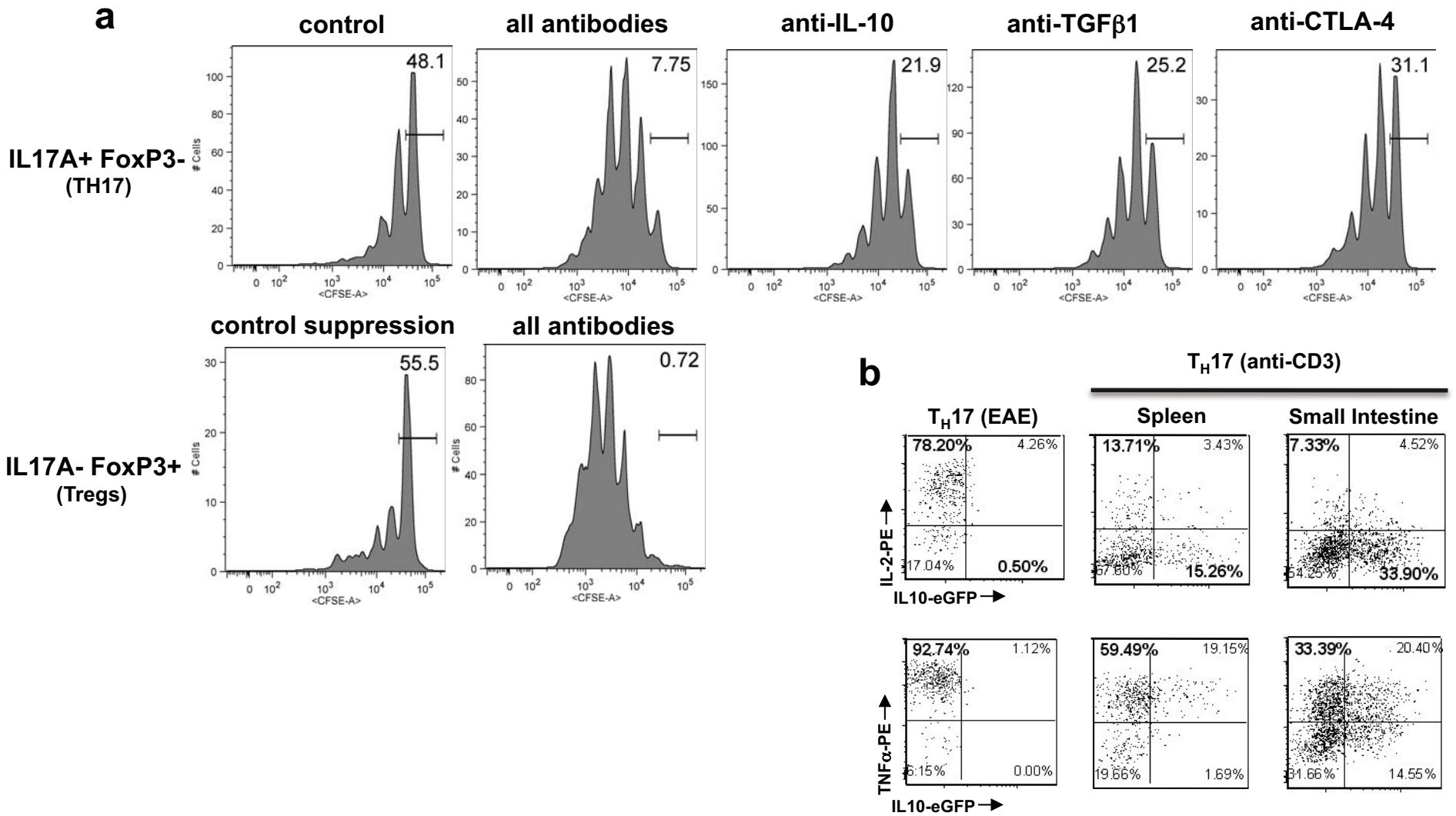
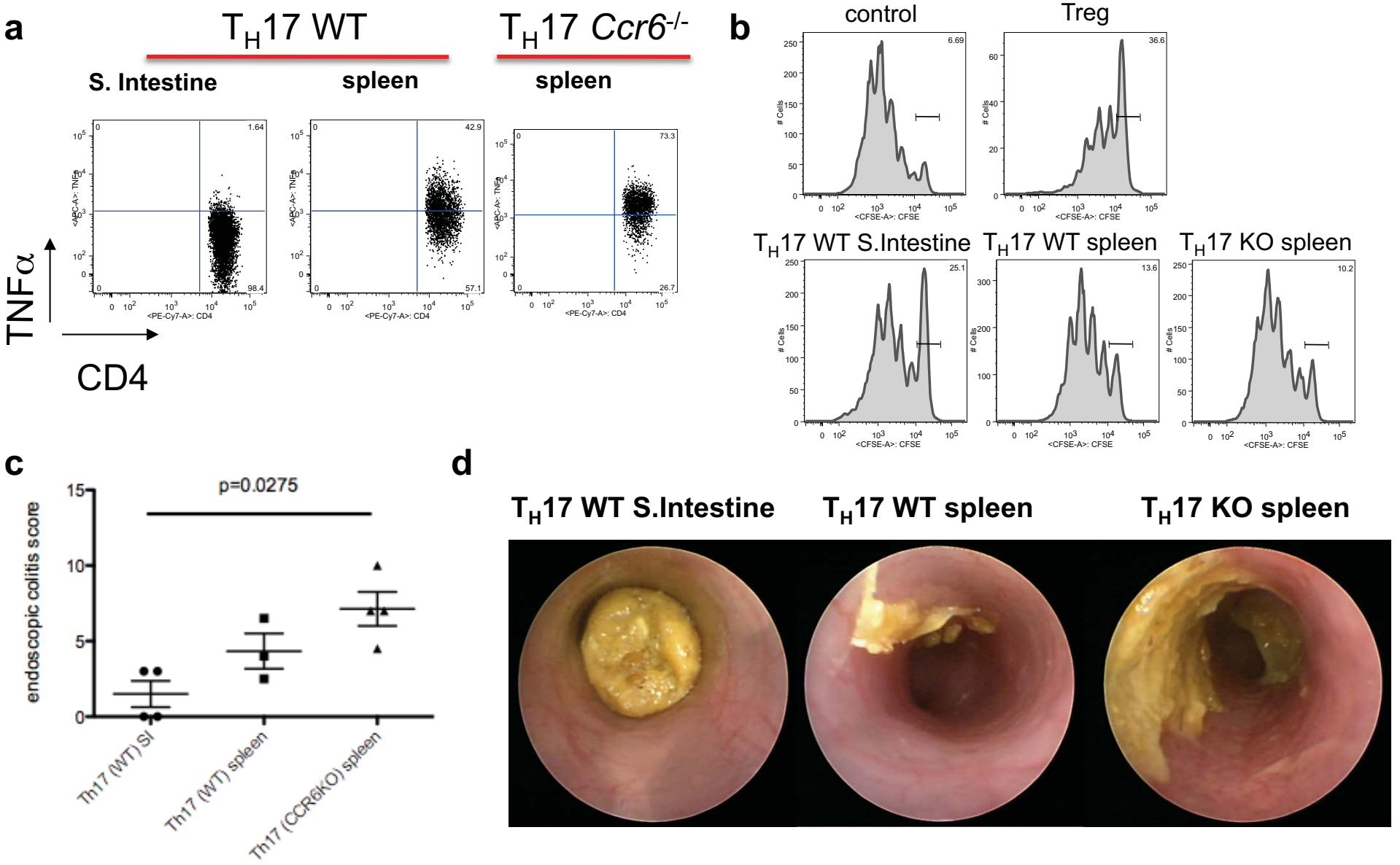


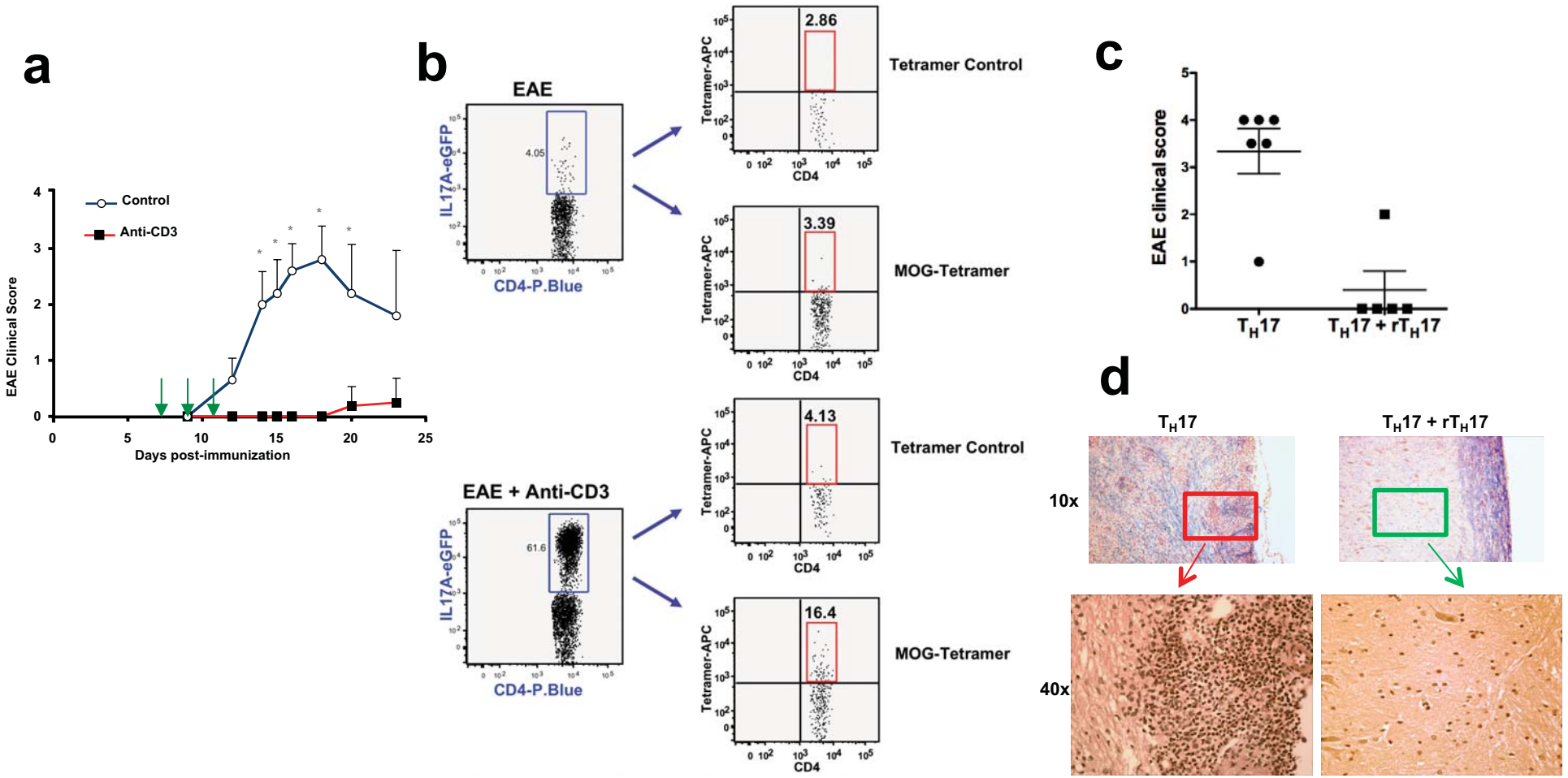
Supplemental Fig. 10. Elimination of T_H17 cells in the intestinal lumen of mice treated with CD3-specific antibody. (**a**) Photographs are of intestine from C57bl/6 mice treated with 3 i.v. injections of CD3-specific antibody (clone 2C11) or control mice. (**b**) Histological sections (H&E staining and magnification was 10x or 20x) from the same treated and control mice shown in (**a**). (**c**) Flow cytometry analysis of the luminal content of IL-17A-eGFP x Foxp3-mRFP treated with CD3-specific antibody. Decreased percentage of Tregs ($Foxp3^+$ T cells) in the lumen (left) was observed when compared to the duodenum (LPL +IEL compartment) (right). Gate is on the $CD4^+TCR\beta^+$ T cells. Numbers in quadrants indicate percent cells in each. (**d**) Immunofluorescence staining of frozen sections of duodenum from IL-17A-eGFP knock-in mice treated with CD3-specific antibody. T_H17 cells were found in the intestinal lumen (arrows). CD4 (red); IL-17A-eGFP (green); cell nuclei (DAPI). Original magnification was 40x. Data are representative of four experiments. Analysis was done 100h after the first injection.



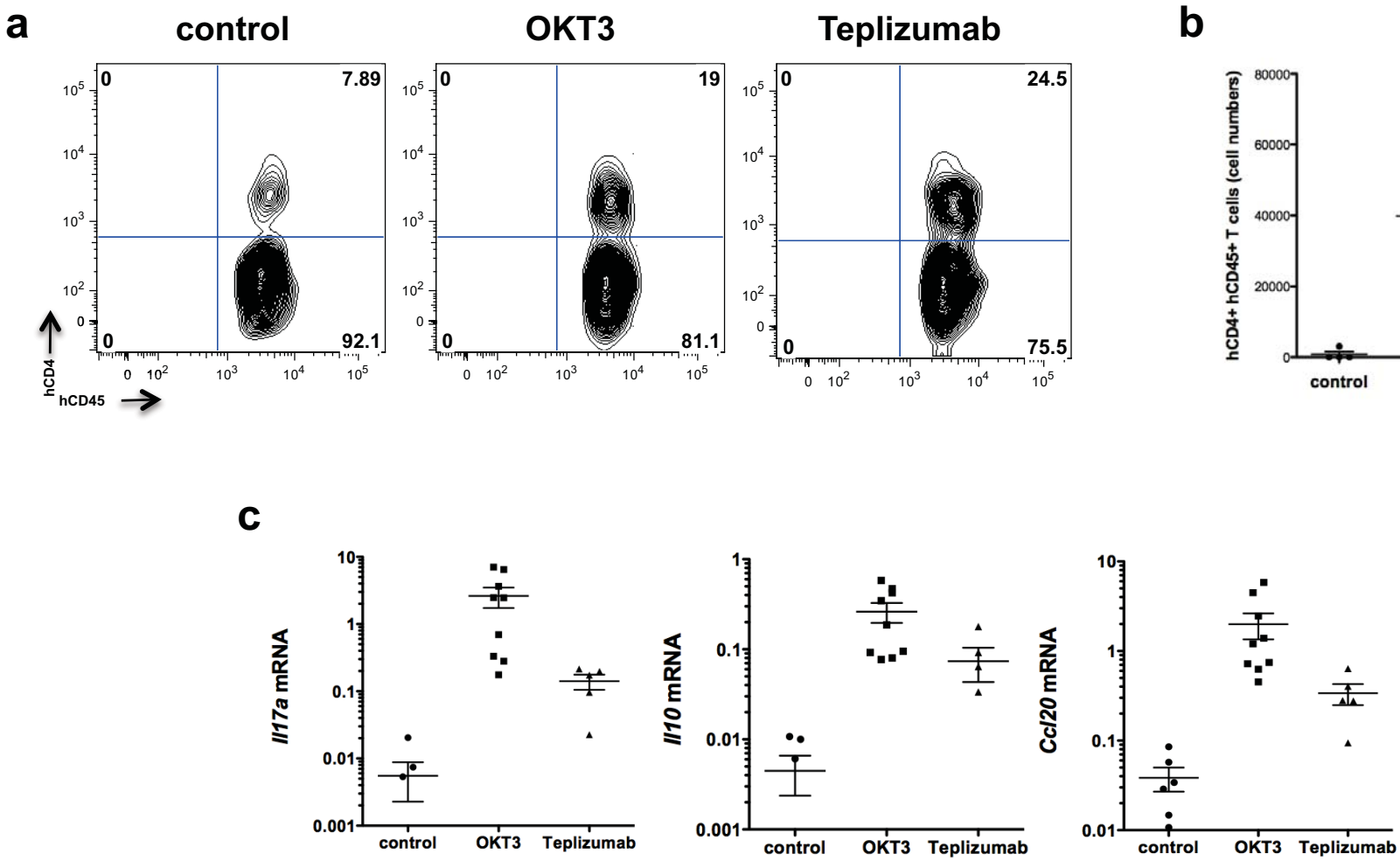
Supplemental Fig. 11. Suppressive capacity of the rT$_H$17 cells is partially dependent on IL-10, CTLA-4 and TGF-β signalling.
(a) Suppression assay performed using sorted eGFP-mRFP+CD4+ (Tregs) or eGFP+mRFP-CD4+ (T$_H$17) cells from IL-17A-eGFP x Foxp3-mRFP double reporter mice treated with CD3-specific antibody. Cells were sorted from duodenum. (Bar represents undivided CFSE labelled CD4$^+$CD25$^-$ responder T cells). Antibodies were added at 10 μg/ml final concentration. (**b**) Expression of IL-2, TNF-α and IL-10 was determined by flow cytometry analysis. Plots are gated on CD4$^+$IL-17A$^+$ T cells from IL-10-eGFP reporter mice with EAE or treated with CD3-specific antibody. Numbers in quadrants indicate percent cells in each. Data are representative of 3 different experiments.



Supplemental Fig. 12. Migration to the small intestine is essential for T_H17 cells to acquire a regulatory phenotype.
IL-17A eGFP+ cells were isolated from the small intestine and spleen of wild type and *Ccr6*$^{-/-}$ mice after CD3-specific antibody treatment. **(a)** Cells were restimulated and intracellular cytokine staining for TNF-α performed. (**b**) Cells were cocultured with CFSE responder cells to assess the *in vitro* suppressive capacity. (**c+d**) Sorted IL-17A eGFP+ cells were transferred into *Rag1*$^{-/-}$ mice, and the colitis development monitored using colonoscopy (mean+/- s.e.m.). Results are representative of two independent experiments.



Supplemental Fig. 13. CD3-specific antibody redirects antigen specific T_H17 cells to the Small Intestine and they acquire a suppressive function *in vivo*. (**a**) EAE was induced in IL-17A-eGFP knock-in mice by immunization with MOG35–55 in complete Freund adjuvant (CFA). A group of mice was treated with 2 μg of CD3-specific antibody (clone 2C11) on days 7, 9 and 11 after immunization (arrows). The course of EAE in these mice is shown as mean clinical score ± s.e.m., n=8, $^*P < 0.05$. (**b**) Flow cytometry analysis using a MOG-specific tetramer on small intestine $CD4^+IL\text{-}17A^+$ T cells purified from EAE-induced animals (IL-17A-eGFP knock-in mice) four hours after the last injection with PBS (upper plot) or with CD3-specific antibody (lower plot). Numbers above outlined areas indicate the frequency of MOG-tetramer$^+$ T_H17 cells. Data are representative of 2 different experiments.
(**c+d**) *In vitro* differentiated IL-17A-eGFP$^+$Foxp3-mRFP$^-$CD4$^+$ T cells from 2D2 mice were either transferred alone (25 x 10^3 cells) or together with IL-17A-eGFP$^+$Foxp3-mRFP$^-$CD4$^+$ T cells isolated from the small intestine of CD3-specific antibody treated 2D2 mice 100 hours after the first injection (25 x 10^3 cells each). Clinical EAE score 9 weeks after the transfer (**c**), and representative section of the spinal cord stained with fast luxol blue and haematoxylin/eosin (**d**) are shown. Each dot represents one mouse, lines indicate mean.



Supplemental Fig. 14. Anti-human-CD3 antibody treatment induces the recruitment of human IL17A$^+$ and IL10$^+$ cells into the duodenum in a humanized mouse system. Flow cytometric analysis (**a**) and cell counts (**b**) showing increased percentage and cell numbers of hCD4$^+$hCD45$^+$ T cells in the duodenum of Balb/c RAG1-/-γc-/- mice two weeks after reconstitution with human PBMCs. Mice were analyzed 5h after treatment with OKT3, Teplizumab or antibody control (10μg/mouse/i.v.). Gate is on human CD45$^+$ T cells. Numbers in quadrants indicate percent cells in each. (**c**) The presence of human *IL17a*, *Il10* and *Ccl20* mRNA in the duodenum of OKT3, Teplizumab and control mice (from the same animals shown in **a**+**b**) was determined by RT-PCR. Data is normalized to human HPRT. Data are cumulative of two experiments performed independently.

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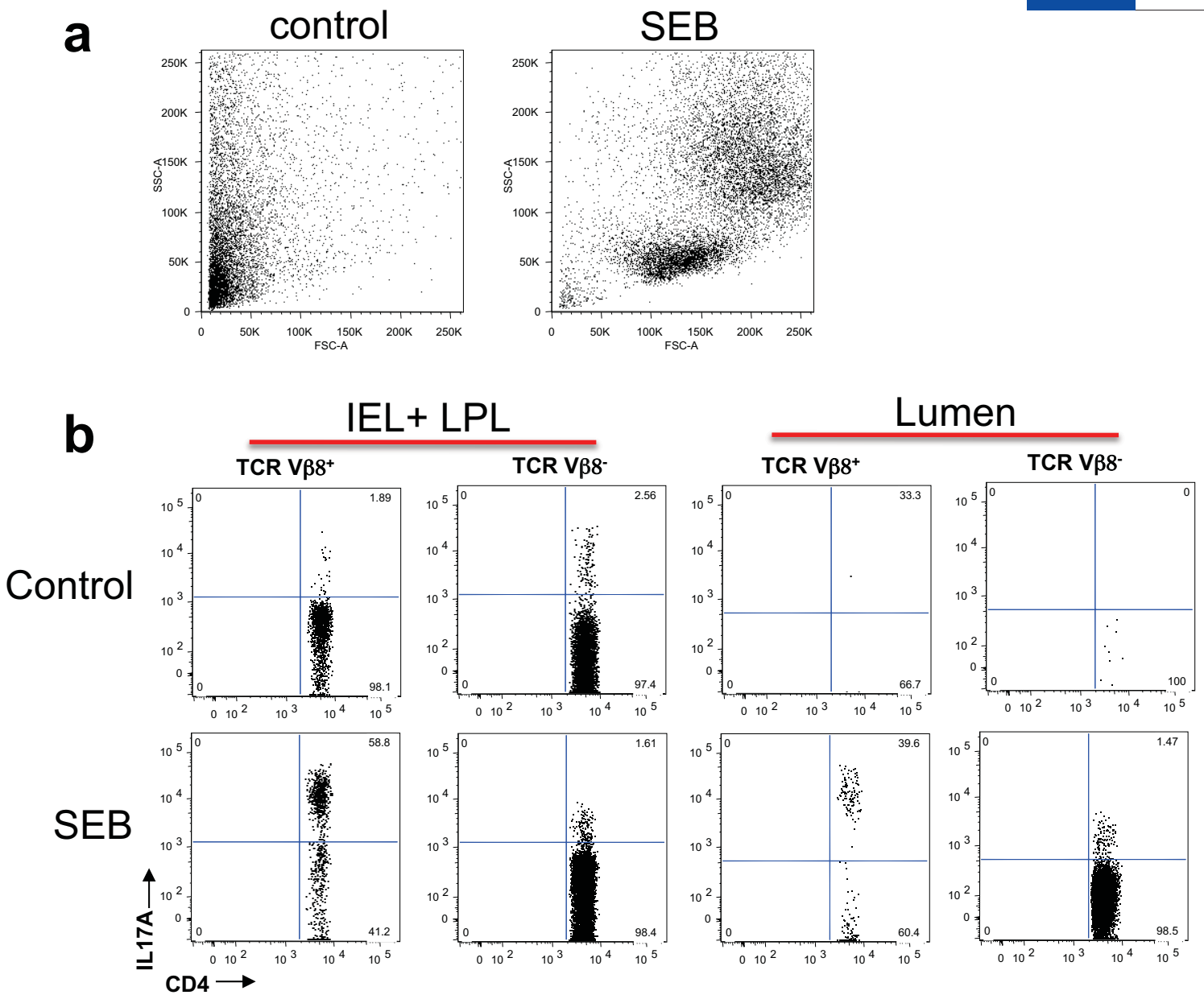
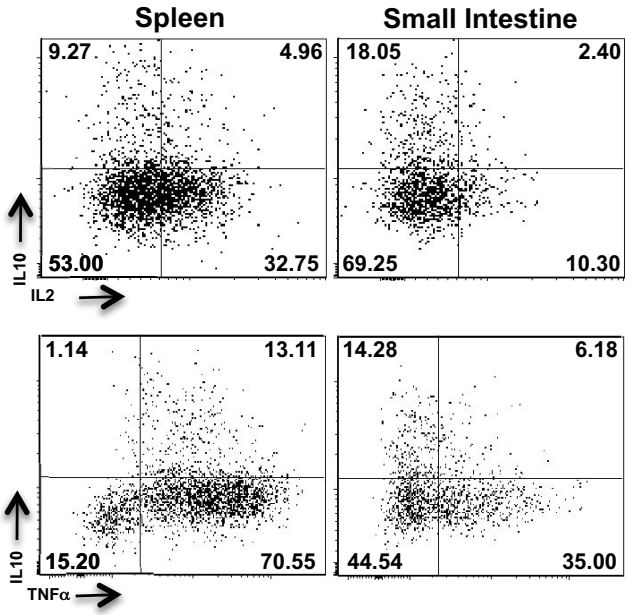
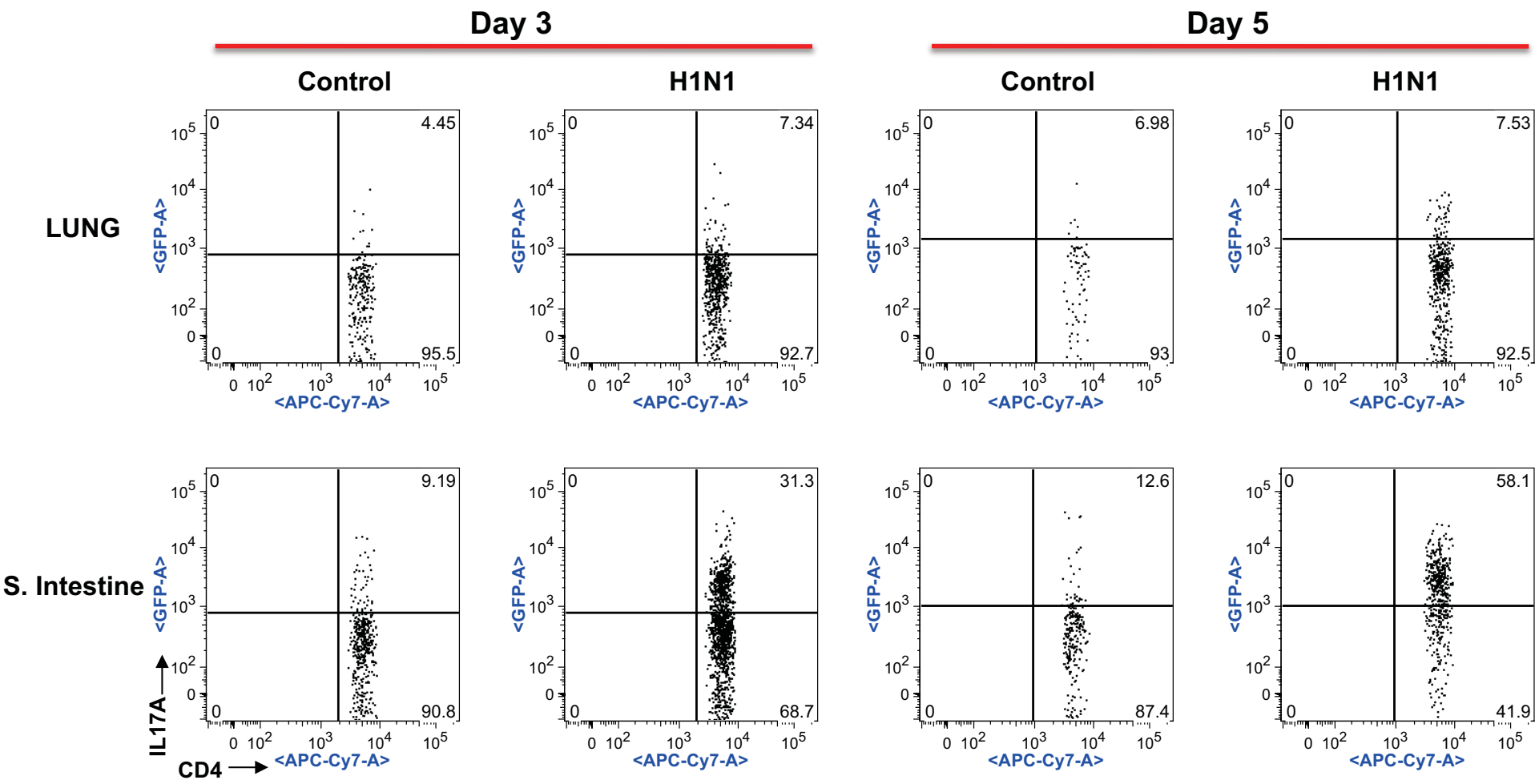


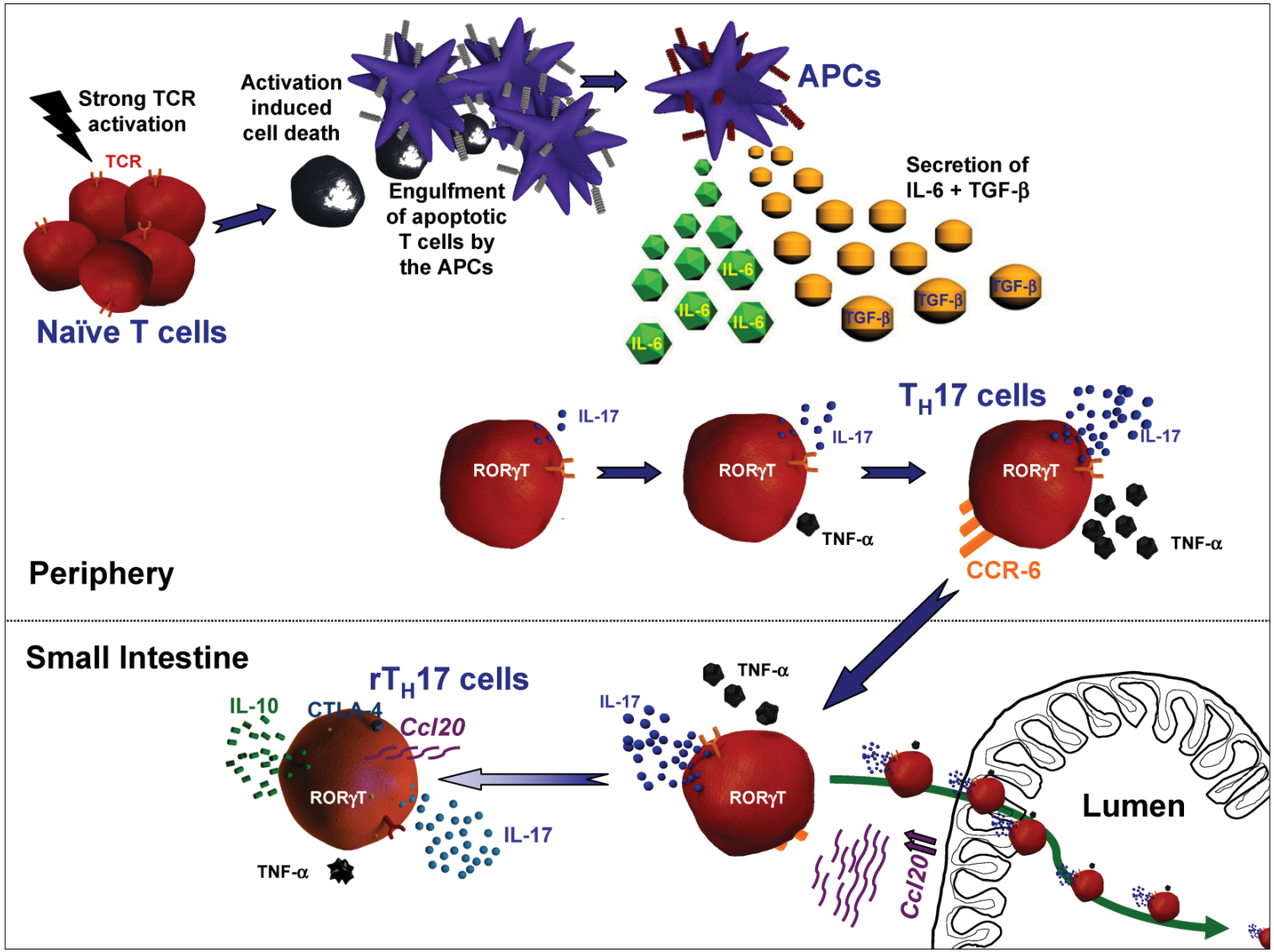
Supplemental Fig. 15. Elimination of T_H17 cells in the intestinal lumen of mice treated with SEB.
(**a** and **b**) Flow cytometry analysis of the luminal content of IL-17A-eGFP mice untreated or after treatment with 3 i.v. injections (0, 48, 96 hours) with 50μg of SEB. Animals were analyzed 4 h after the last injection. (**a**) SSC versus FSC plot of the luminal content of treated and untreated mice analyzed by FACS. (**b**) Flow cytometric analysis showing the presence of IL-17A$^+$ CD4$^+$ T cells in the luminal content of SEB treated animals as well in the LPL+IEL compartments. Gate is on the CD4$^+$TCRβ$^+$ T cells. Numbers in quadrants indicate percent cells in each.



Supplemental Fig. 16. Induction of rT_H17 cells in the small intestine during sepsis. IL-10 eGFP x Foxp3 mRFP double reporter mice were treated with SEB. Cells from the spleen and small intestine were restimulated with PMA and Ionomycin and intracellular cytokine staining for IL-17A, IL-2, TNF-α, and anti-GFP performed. Plots are gated on IL-17A$^+$CD4$^+$ events. Results are representative of at least two independent experiments.



Supplemental Fig. 17. Accumulation of T_H17 cells in the small intestine during Influenza A (H1N1) infection.
Flow cytometric analysis showing the presence of IL-17A$^+$ CD4$^+$ T cells in the lung and small intestine of Influenza A (H1N1) infected mice at indicated time points. Gate is on the CD4$^+$CD44$^+$Vβ8TCR$^+$ cells. Numbers in quadrants indicate percent cells in each. Results are representative of two independent experiments.



Supplemental Fig. 18. Scheme of the fate of T_H17 cells during tolerance induction after a polyclonal T cell activation *in vivo*. After polyclonal T cell activation via TCR, apoptotic T cells will be engulfed by phagocytes. IL-6 and TGF-β are going to be produced by APCs and consequently IL-17A$^+$CCR6$^+$CD4$^+$ T cells (T_H17) are generated in the periphery. T_H17-mediated production of IL17 will induce upregulation of CCL20 in the small intestine attracting and trapping the pro-inflammatory T_H17 cells. In the Small Intestine, part of the T_H17 cells are going to be eliminated in the lumen and others will be reprogrammed in rT_H17 cells with immuno-suppressor functions.