Fate mapping of interleukin 17-producing T cells in inflammatory responses

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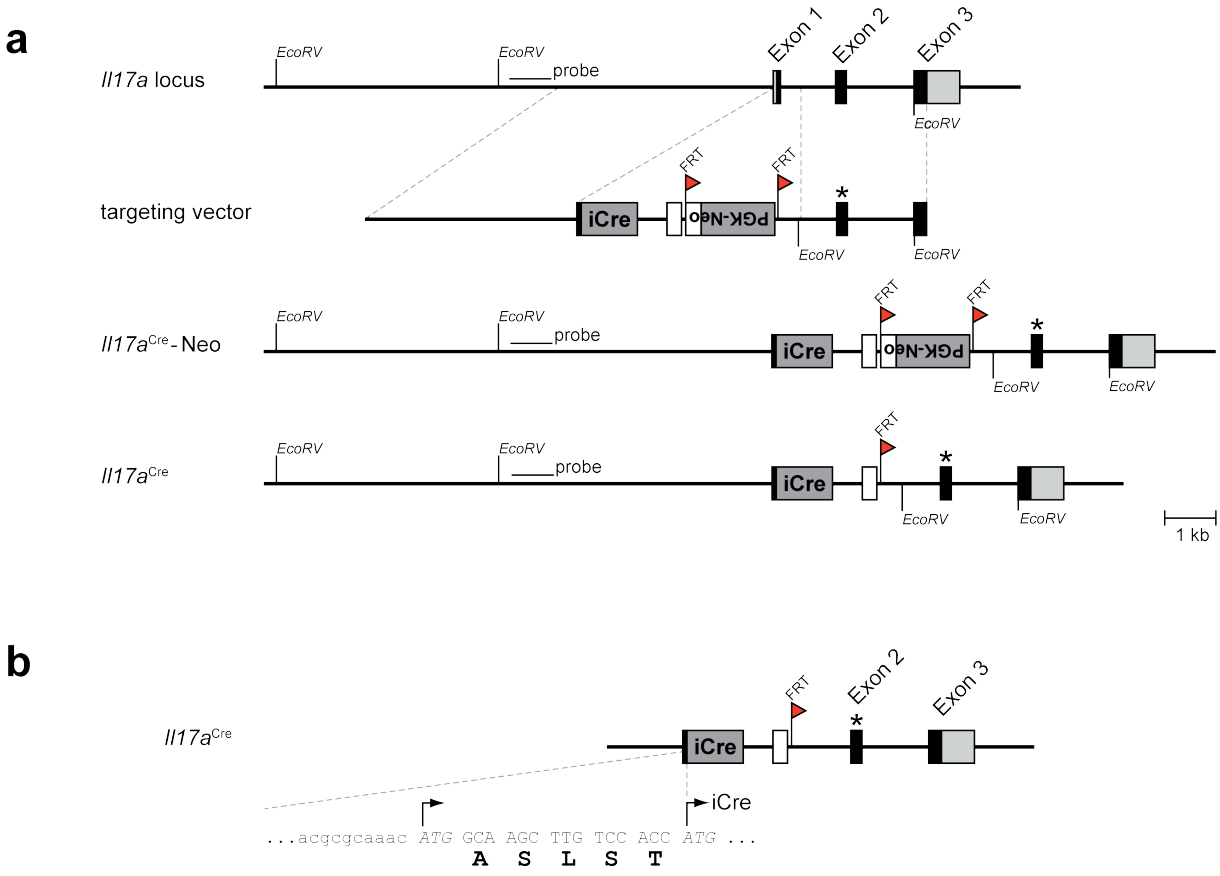
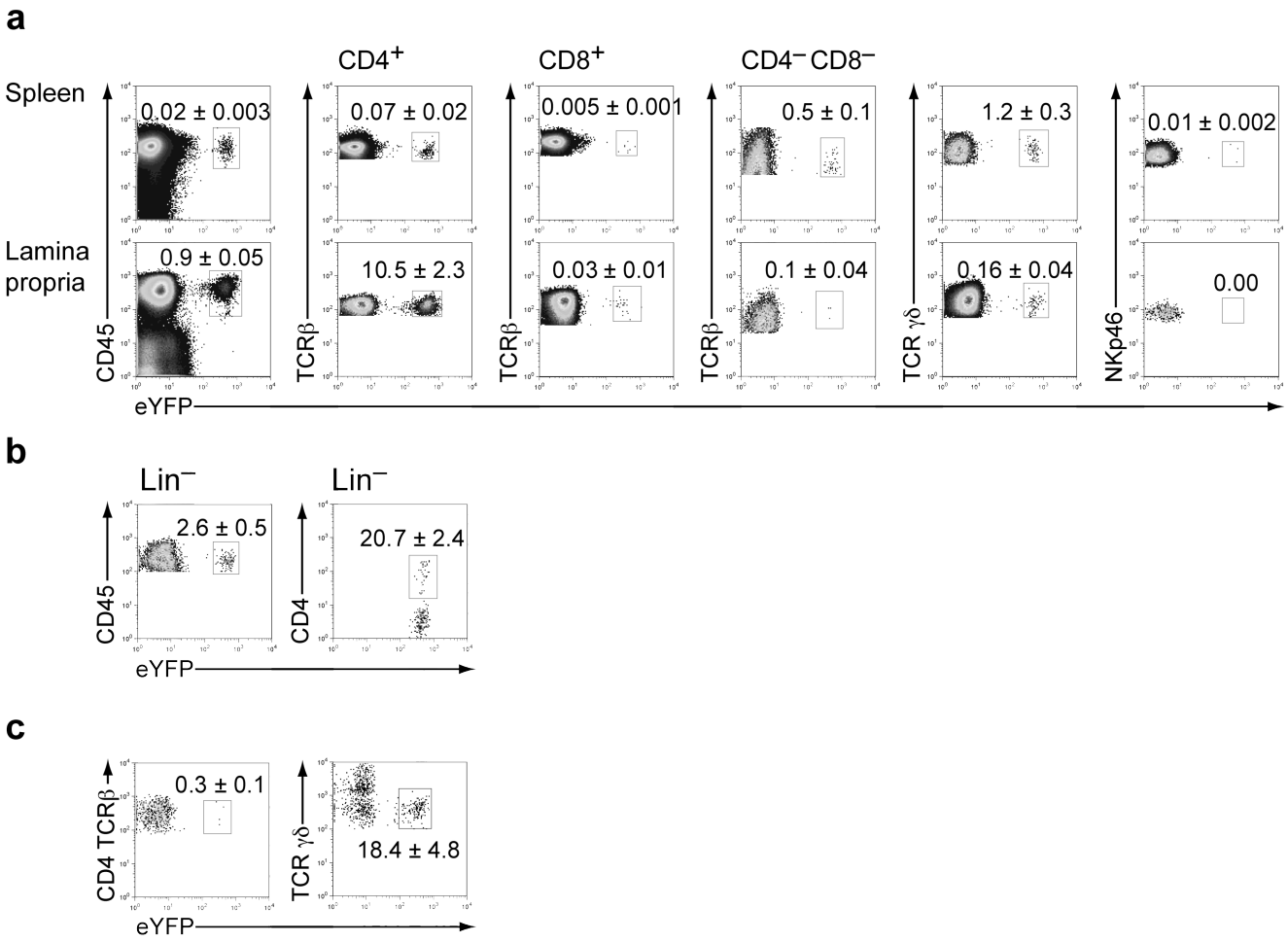


Supplementary Figure 1. Generation of the *Il17a*^{Cre} allele

(a) The endogenous *Il17a* locus, the gene targeting vector, the targeted *Il17a* allele including the Neo resistance gene cassette (*Il17a*^{Cre}-Neo) and the final targeted allele (*Il17a*^{Cre}) after FLPe-mediated recombination are schematically depicted to scale. A minigene composed of a codon improved Cre (iCre) followed by an intron and polyadenylation site derived from SV40 virus. Point mutations introduced into exon 2 of the *Il17a* gene to modify internal ATG-site are symbolized by an asterisk.

(b) Sequence of *Il17a* exon 1. A linker consisting of 5 amino acids was placed between the original *Il17a* ATG-site and the respective start of the iCre minigene.



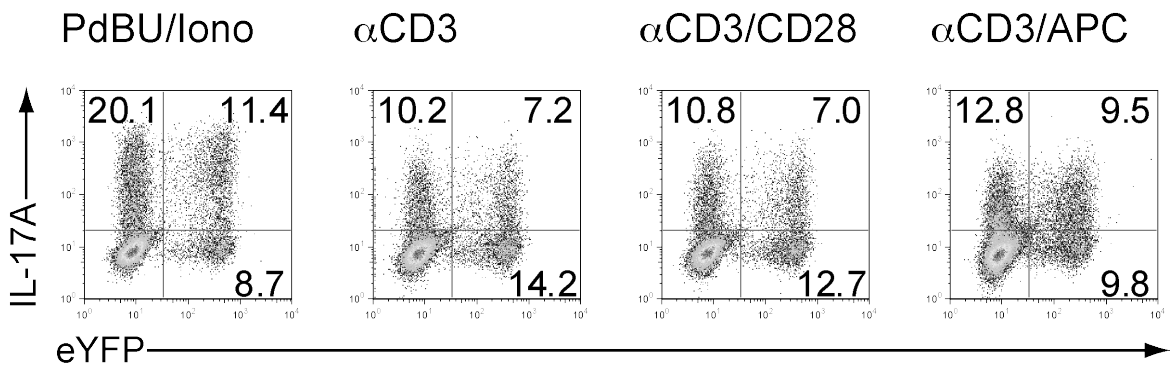
Supplementary Figure 2. Expression of eYFP reporter in the lymphoid tissue of *Il17a*^{Cre} *R26R*^{eYFP} mice

(a) Expression of eYFP in various subsets of splenocytes (top row) or lamina propria cells (bottom row) of non-immune *Il17a*^{Cre} *R26R*^{eYFP} mice. T cell receptor αβ expression was analyzed on CD4$^+$ and CD8$^+$ single positive, or CD4$^-$CD8$^-$ double negative cells.

(b) Expression of eYFP in lineage-marker negative (depleted for: CD3ε, CD8α, CD11b, CD11c, CD19, Gr-1, NK1.1, NKp46), CD45$^+$ lymphoid tissue-inducer-like cells in the lamina propria of reporter mice.

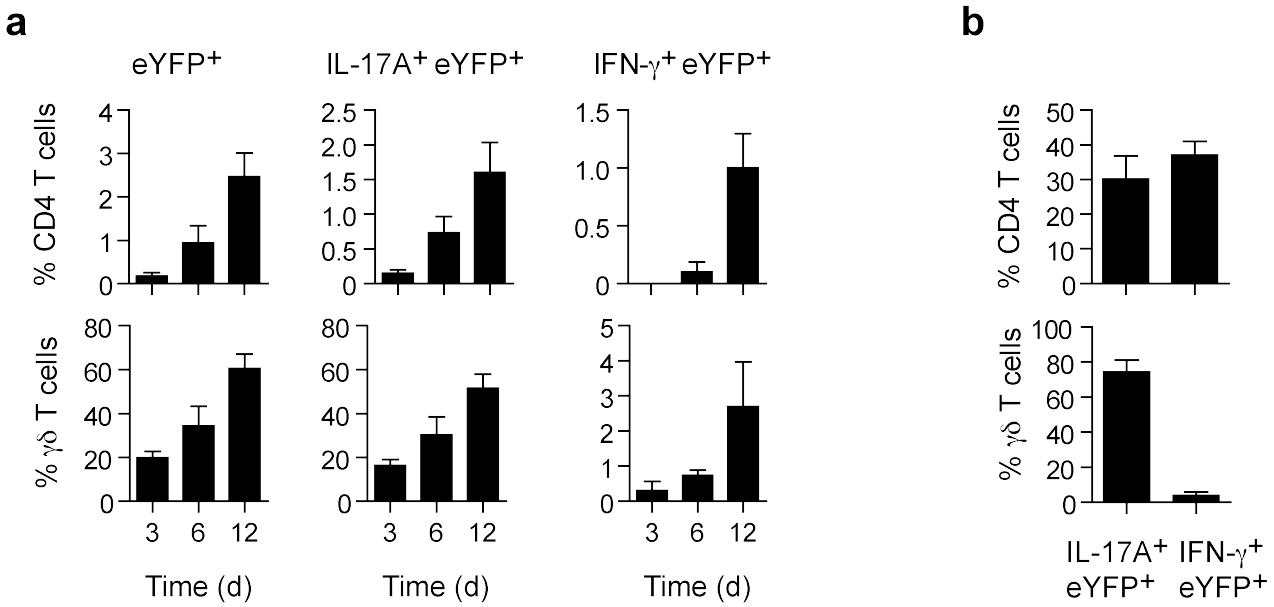
(c) Expression of eYFP on skin CD4 and γδ T cells in reporter mice.

Data are representative of at least three independent experiments. Values in the plots indicate relative frequency ± SD.



Supplementary Figure 3. Induction of eYFP expression in T_H17 cells of *Il17a*^{Cre} *R26R*^{eYFP} mice upon *in vitro* activation

Naïve $CD4^+CD44^{lo}CD25^-$ T cells were cultured under T_H17 conditions for 4 days and restimulated with either PdBU/ionomycin in the presence of brefeldin A for 4 hours; or in overnight cultures with plate-bound anti-CD3 (1 µg/ml), plate-bound anti-CD3/CD28 (1 µg/ml), or anti-CD3 (1 µg/ml) in the presence of T cell-depleted splenocytes. In case of overnight cultures brefeldin A was added for the last 5 hours. Cells were stained for CD4 and intracellular IL-17A. Dot plots show intracellular IL-17A expression versus eYFP in $CD4^+$ T cells. Data are representative for two independent experiments.

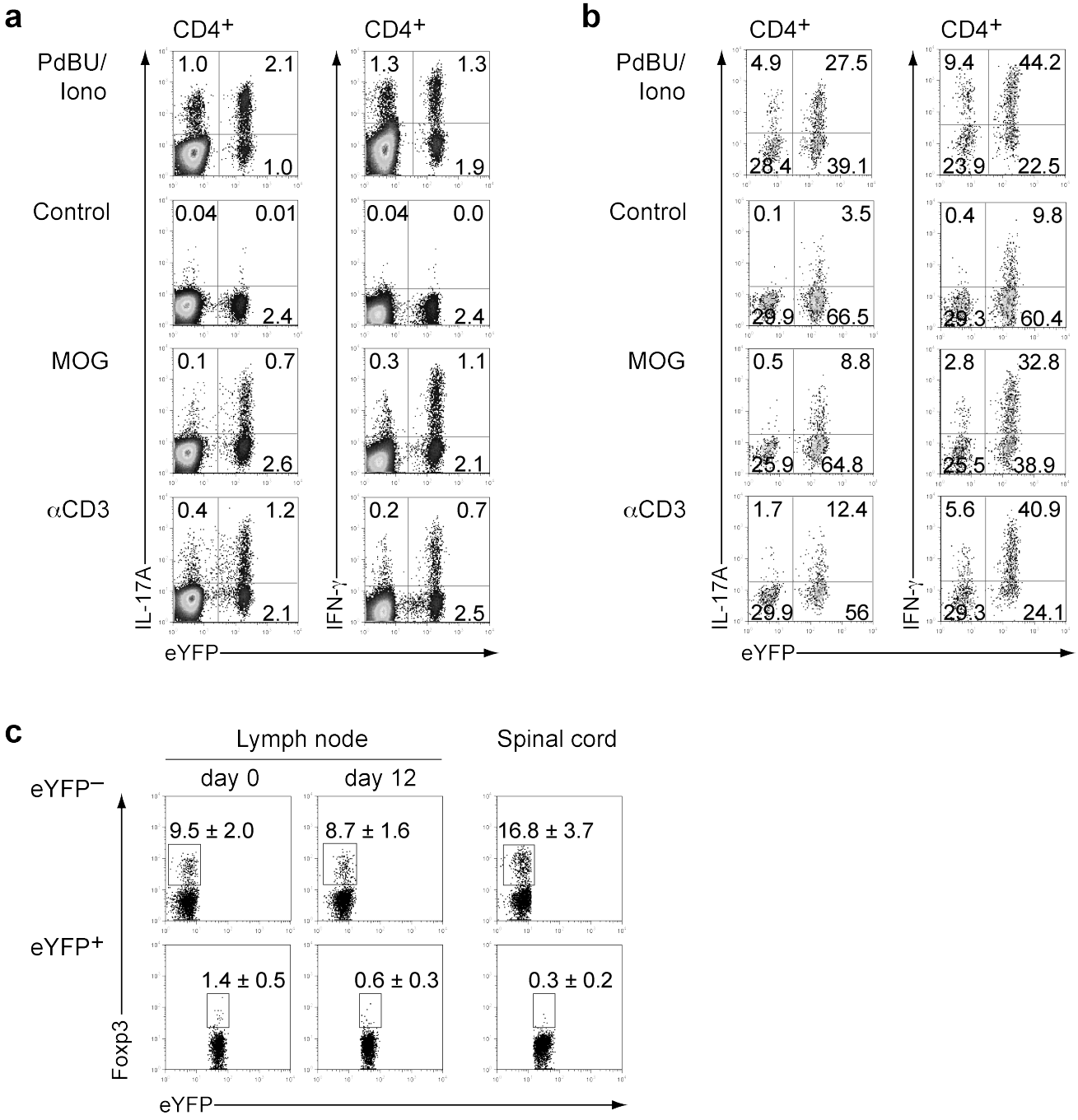


Supplementary Figure 4. Expression of eYFP reporter in T cell subsets in draining lymph node and spinal cord of *Il17a*^{Cre} *R26R*^{eYFP} mice during EAE induction

(a) Bar graphs representing the relative frequency of eYFP$^+$ or cytokine-secreting eYFP$^+$ cells in draining lymph node at indicated days during induction of experimental autoimmune encephalomyelitis (EAE) in *Il17a*^{Cre} *R26R*^{eYFP} mice.

(b) Bar graphs representing the relative frequency of cytokine-secreting eYFP$^+$ cells in the spinal cord at day 15 after EAE induction.

Data represents mean ± SD of three individual mice each.

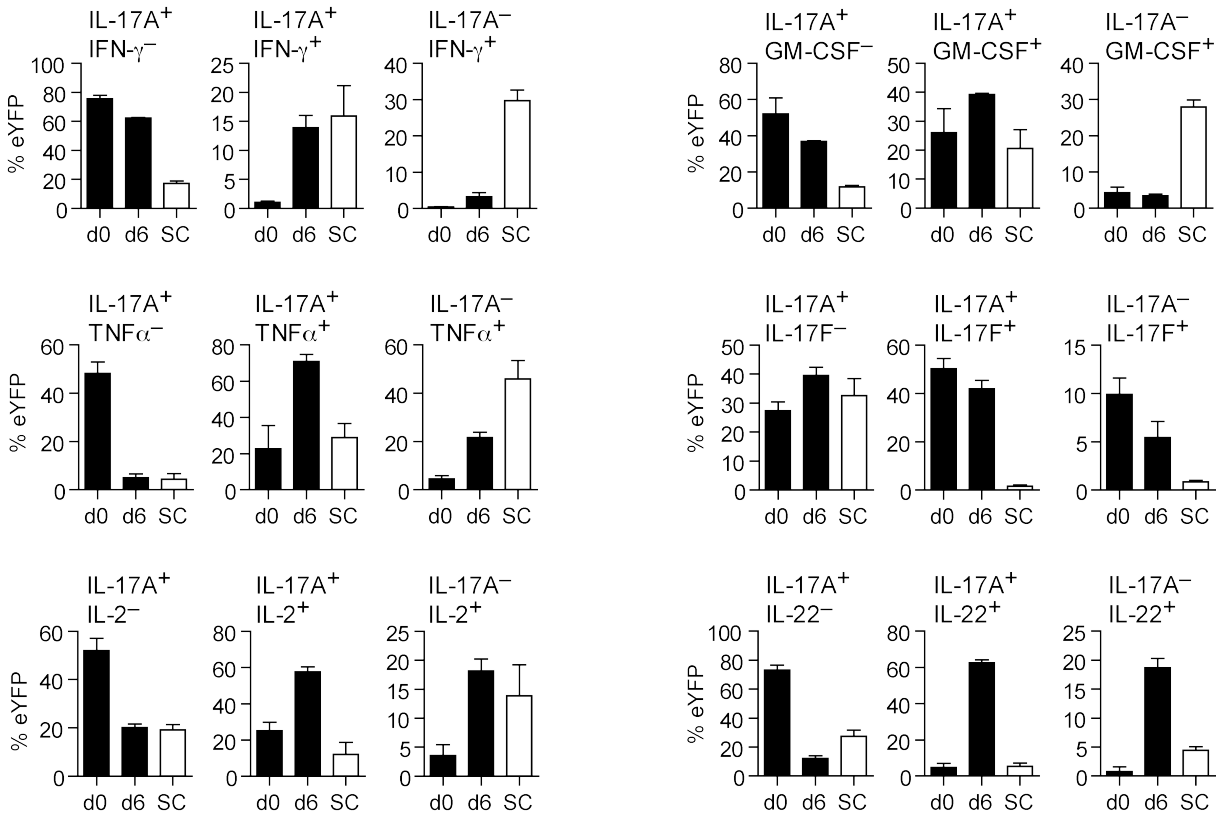


Supplementary Figure 5. Effect of antigen-specific restimulation on IL-17A, eYFP and Foxp3 expression in CD4 T cells in EAE

(a) Cells from draining lymph node at day 12 after EAE induction were restimulated either with PdBU/ionomycin in the presence of brefeldin A for 3 hours; or overnight with MOG peptide (50 µg/ml) and anti-CD3 (1 µg/ml) with brefeldin A added for the last 5 hours. Cells were stained for CD4 and intracellular IL-17A and gated electronically on CD4.

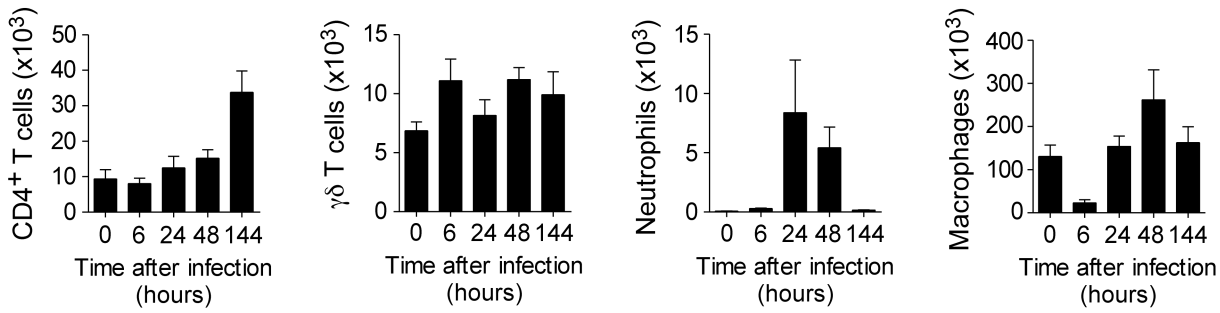
(b) Cells obtained from spinal cord at day 15 after EAE induction were restimulated and stained as in (a).

(c) Representative FACS plots of CD4 T cells form draining lymph node and spinal cord (day 15) during EAE.



Supplementary Figure 6. Correlation of cytokine secretion and eYFP expression in CD4 T cells during EAE

Bar graphs illustrating the relative frequency of cytokine-secreting populations in eYFP^{+} CD4 T cells of lymph nodes at indicated timepoints and spinal cord (SC; day 15) after EAE induction in *Il17a*^{Cre} *R26R*^{eYFP} mice. Data is given as mean ± SD of three individual mice.



Supplementary Figure 7. Cellular composition of skin infiltrates following *Candida albicans* inoculation

Absolute number of CD4 T cells, γδ T cells, neutrophils and macrophages from explants of 1 cm^2 skin infected with *Candida albicans* at the indicated timepoints. Mean ± SD from 3-5 mice per timepoint.

Supplementary Table 1. Specific Expression of *Il17a* and *Ifng* Genes *Ex Vivo* in FACS-purified Lymph Node T Cells after Immunization with MOG/CFA

		Il17a	*Ifng*	*Il17a/Ifng*
$eYFP^+$	$CCR6^+$ (n = 148)	53.4% (79)	4.1% (6)	3.4% (5)
"	$CCR6^-$ (n = 155)	11.0% (17)	43.9% (68)	1.3% (2)

CD4 T cells from peripheral draining lymph nodes were FACS purified at day 4 after immunization with MOG/CFA as described in Experimental Methods. Primers used for multiplex single cell PCR analysis of cytokine loci were each investigated for their expected specificity in these subsets and *in vitro* polarized T_H1 and T_H17 subsets. For all samples *Hprt1* was used as internal control. Percentage of positive cells investigated for co-expression of *Il17* and *Ifng* were normalized to samples positive for *Hprt1*. 172 individual cells were originally isolated from each population; number of individual samples shown in parentheses.

Gene Expression Analysis of Single Cells by Multiplex Reverse Transcriptase-Polymerase Chain Reaction

Multiplex single-cell RT-PCR analysis was performed as previously described (Hu *et al.*, 1997). In brief, single cells were FACS-purified on a MoFlo sorter (Beckman Coulter, UK) and subsequently deposited into 96-well PCR plates using an automated cell deposition unit on a FACSAria II (Becton Dickinson, UK; providing single cells in >99% of the wells, and no wells with more than one cell as assessed by routinely sorting fluorescent beads or cells prior to and after single cell sorting), containing 4 µl lysis buffer and snap frozen at -80ºC. Cell lysates were reverse-transcribed using gene-specific reverse primers and 50 U Superscript II (Invitrogen, UK) in a final volume of 12 µl. First-round PCR was performed by addition of gene specific forward primers in a 30 µl PCR mix containing 1.25 U Taq polymerase (Roche Diagnostics Ltd., UK) and subjected to 35 cycles of PCR. One-microliter aliquots of first-round PCRs were further amplified for 35 cycles using nested gene-specific primers. Second-round PCR products were subjected to gel electrophoresis and visualized by ethidium bromide staining.
Candidate primers for establishing patterns of cytokine transcription by multiplex single cell PCR analysis, were first tested for their lineage specificity and efficiency in cells polarized under T_H1 and T_H17 conditions *in vitro*.

Primers used for Analysis of Single Cells by Reverse Transcriptase-Polymerase Chain Reaction

The external primer sequences used were as follows:
HPRT: GGGGGCTATAAGTTCTTTGC and TCCAACACTTCGAGAGGTCC
IFN-γ: CATGAAAATCCTGCAGAGCC and GGACAATCTCTTCCCCACCC
IL17A: GTGCACCCAGCACCAGCTGA and GGCGGCACTGAGCTTCCCAG

The internal primer sequences used were as follows:
HPRT: GTTCTTTGCTGACCTGCTGG and TGGGGCTGTACTGCTTAACC
IFN-γ: CCTCAGACTCTTTGAAGTCT and CAGCGACTCCTTTTCCGCTT
IL17A: CCAGCTGATCAGGACGCGCAA and TTGCGGTGGAGAGTCCAGGGT

Reference:
Hu, M., Krause, D., Greaves, M., Sharkis, S., Dexter, M., Heyworth, C. & Enver, T. Multilineage gene expression precedes commitment in the hemopoietic system. *Genes Dev.* **11**, 774-785 (1997).