Supporting Information

Supporting Materials and Methods

Realtime PCR primers. GAPD: Forward: 5'CTGGAGAAACCTGCCAAGTA, reverse: 5'TGTTGCTGTAGCCGTATTCA; IL-17A [1]: 5'CTCCAGAAGGCCCTCAGACTAC, 5'AGCTTTCCCTCCGCATTGACACAG; IL-17F [1]: 5'GAGGATAACACTGTGAGAGTTGAC, 5'GAGTTCATGGTGCTGTCTTCC; IL-22: 5'CATGCAGGAGGTGGTACCTT, 5'CAGACGCAAGCATTTCTCAG; IL-23R: 5'TCAGTGCTACAATCTTCAGAGG, 5'GCCAAGAAGACCATTCCCGA; IRF4: 5'GCAATGGGAAACTCCGACAGT, 5'CAGCGTCCTCCTCACGATTGT; RORα [2]: 5'CAATGCCACCTACTCCTGTCC, 5'GCCAGGCATTTCTGCAGC; RORγt [1]: 5'GACAGGGAGCCAAGTTCTCA, 5'CTTGTCCCCACAGATCTTGCA; T-bet: 5'ACCAGAGCGGCAAGTGGG, 5'TGGACATATAAGCGGTTCCCTG.

***In vivo* proliferation assay.** Spleen and peripheral lymph nodes were isolated from Ctrl (*Stim1*$^{fl/fl}$) and *Stim1*$^{fl/fl}$ *Cd4-Cre* mice (C57BL/6 background), red blood cells lysed by hypotonic shock and a single-cell suspension prepared that was labeled with 2.5 μM CFSE (Invitrogen) for 5 min at room temperature. 2x10^7 CFSE-labeled cells were injected i.v. into irradiated (10 Gy) Balb/c mice. Three days later, the recipient mice were sacrificed, splenocytes and peripheral lymph node cells recovered and analyzed by flow cytometry.

***In vitro* proliferation.** T_H1 and T_H17 cells were generated as described in the main text. On day 3 of culture, 20 U/ml IL-2 was added to the medium in addition to the cytokine

supplements used for differentiation of T_H1 and T_H17 cells. T cells were cultured for a total of 8 days in Iscove's modified Dulbecco's medium containing 2 mM L-glutamine, 50 μM β-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% FBS. Cells numbers were obtained on days 0, 4, 6 and 8.

Analysis of cell death. T_H1 and T_H17 cells were pretreated with Fc block (anti-CD16/32, clone 93, eBioscience) and incubated with eFluor 450- conjugated anti-mouse CD4 antibody (L3T4, eBioscience). For analysis of Fas and FasL expression, cells were stained with PE-conjugated anti-mouse CD95 (15A7, eBioscience) and biotin-conjugated anti-mouse CD95L (clone MFL3, eBioscience) antibody followed by APC-conjugated streptavidin (Invitrogen). For analysis of cell death, cells were stained with FITC-conjugated Annexin V and propidium iodide using an apoptosis detection kit (BD Biosciences) according to the manufacturer's instructions. Cells were analyzed using a LSRII cytometer (BD Biosciences) and FlowJo software (Treestar, Ashland, OR).

Supporting Figures

Supporting Information Figure 1. Reduced numbers of STIM2 deficient T cells in the CNS, lymph nodes and spleen of *Rag2*$^{-/-}$ mice after adoptive cotransfer. EAE was induced in *Rag2*$^{-/-}$ mice by adoptive transfer of mononuclear cells from MOG_{35-55} immunized CD45.1^{+} wild-type Ctrl mice, CD45.2^{+} *Stim2*$^{fl/fl}$ *Cd4-Cre* mice or cotransfer of both as described in Figure 5A. Mononuclear cells were harvested on day 12 after immunization with MOG_{35-55} and restimulated *in vitro* with MOG_{35-55} and IL-23 for 3 days; 2 x 10^7 cells were transferred to *Rag2*$^{-/-}$ mice. Mononuclear cells were isolated from the CNS of recipient mice on day 14 after transfer and analyzed for CD45.2 and CD45.1 expression. Shown are the results of one representative experiment (A) and the averaged results from three mice (B). Abbreviation: Stim2^{KO}, *Stim2*$^{fl/fl}$ *Cd4-Cre*.

Supporting Information Figure 2. Normal short-term proliferation of STIM1 deficient T cells *in vivo*. 2x10^7 T cells isolated from lymph nodes and spleen of Ctrl (*Stim1*$^{fl/fl}$) and *Stim1*$^{fl/fl}$ *Cd4-Cre* mice (both C57BL/6) were labeled with CFSE and injected into irradiated (10 Gy) Balb/c mice. 3 days later, T cells were recovered from lymph nodes and spleen of Balb/c mice and analyzed for CFSE dilution by flow cytometry. Histograms show CFSE^{+} CD4^{+} or CFSE^{+} CD8^{+} T cells of donor origin. Numbers represent the percentages of proliferating T cells from one experiment.

Supporting Information Figure 3. STIM1 deficient T_H17 cells fail to expand *in vitro* despite normal viability. CD4^{+} T cells were isolated from spleen and lymph nodes of Ctrl (*Stim1*$^{fl/fl}$) and *Stim1*$^{fl/fl}$ *CD4-Cre* mice. For T_H1 and T_H17 differentiation , 2 x 10^6 and

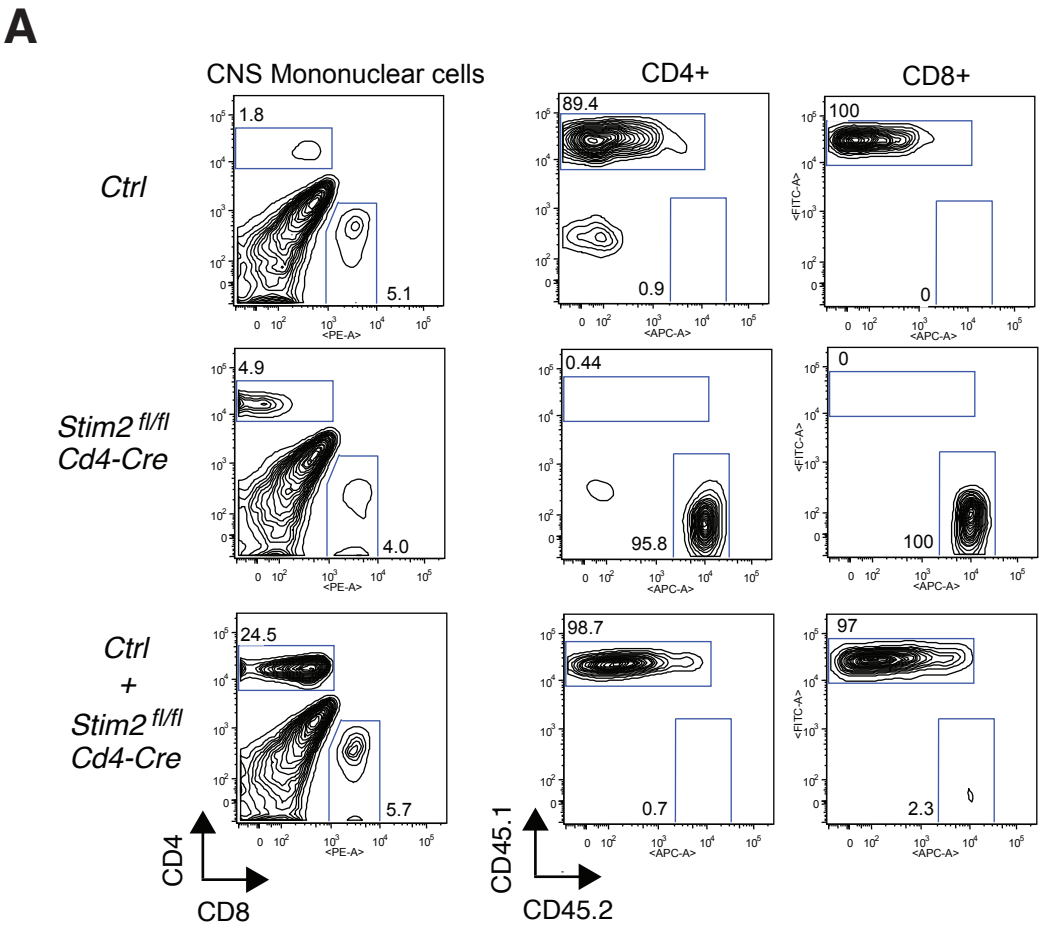
1 x 10^6 $CD4^+$ cells, respectively, were stimulated with anti-CD3 and anti-CD28 under polarizing conditions and cultured for 8 days. **A,** Percent Annexin V^+ (solid lines) and Annexin V^+, PI^+ (dashed lines) of T_H1 or T_H17 cells on days 4, 6 and 8 of culture. **B,** Percent Fas^+ (solid lines) and $FasL^+$ (dashed lines) of T_H1 or T_H17 cells on days 4, 6 and 8 of culture. **C,** Total cell numbers of T_H1 and T_H17 cells on days 0, 4, 6 and 8 of culture. Results are representative of T cells derived from three mice in each group cultured separately. Error bars, SEM. Abbreviations: PI, propidium iodide.

Supporting Information Figure 4. Comparable CXCR3 and CCR7 chemokine receptor expression on STIM1 deficient and Ctrl T_HN cells. $CD4^+$ T cells from Ctrl (*Stim1*$^{fl/fl}$) and *Stim1*$^{fl/fl}$ *Cd4-Cre* mice were cultured *in vitro* for 6 days under non-polarizing (T_HN) conditions. Cells were stained with antibodies to CD4, CXCR3 and CCR7 and analyzed by flow cytometry. Shown are data from one representative experiment (left) and averages of 11 (Ctrl) and 5 (*Stim1*$^{fl/fl}$ *Cd4-Cre*) repeat experiments (right). Error bars, SEM. AU, arbitrary units.

Supporting References

1 **Ivanov, II, McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., Cua, D. J. and Littman, D. R.,** The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 2006. **126**: 1121-1133.

2 **Lau, P., Nixon, S. J., Parton, R. G. and Muscat, G. E.,** RORalpha regulates the expression of genes involved in lipid homeostasis in skeletal muscle cells: caveolin-3 and CPT-1 are direct targets of ROR. *J Biol Chem* 2004. **279**: 36828-36840.


A
CNS Mononuclear cells
CD4+
CD8+
Ctrl
Stim2 fl/fl Cd4-Cre
Ctrl + Stim2 fl/fl Cd4-Cre
1.8
5.1
89.4
0.9
100
0
4.9
4.0
0.44
95.8
0
100
24.5
5.7
98.7
0.7
97
2.3
CD4
CD8
CD45.1
CD45.2
B
CD4+
CD8+
CNS % average
CNS T cell number (x 10^4)
Spleen % average
LN % average
CD45.1+ (Ctrl)
CD45.2+ (Stim2KO)

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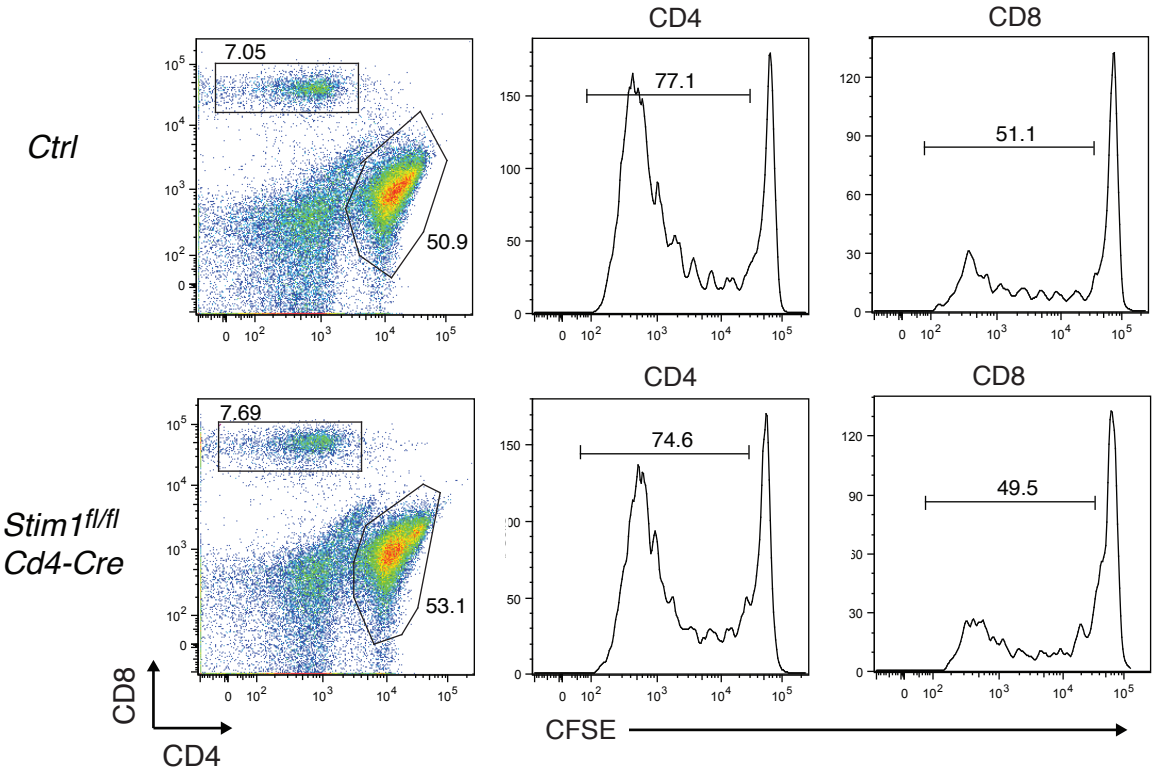
Supporting Information
Fig. 2



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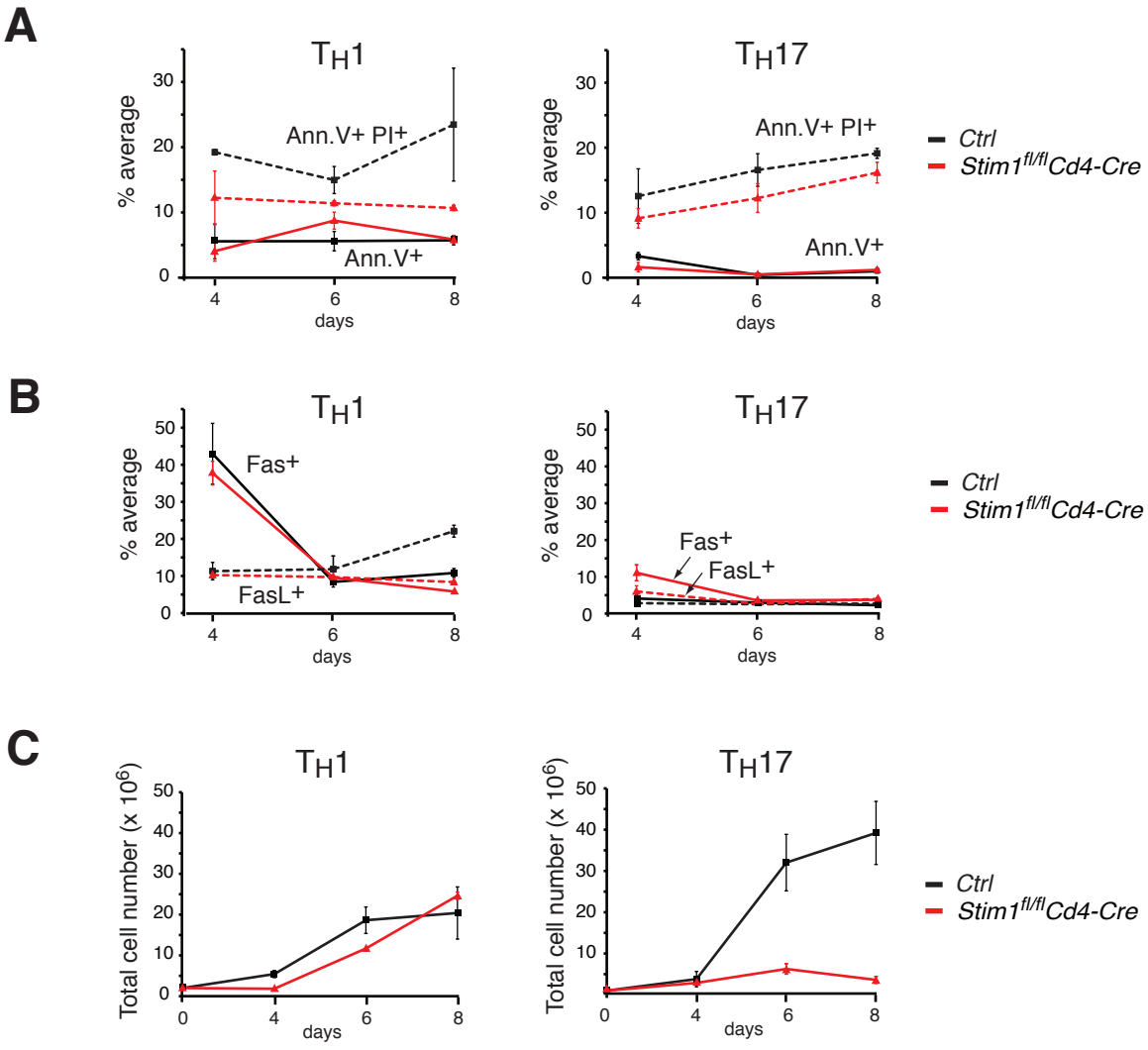
Supporting Information
Fig. 3



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Supporting Information
Fig. 4