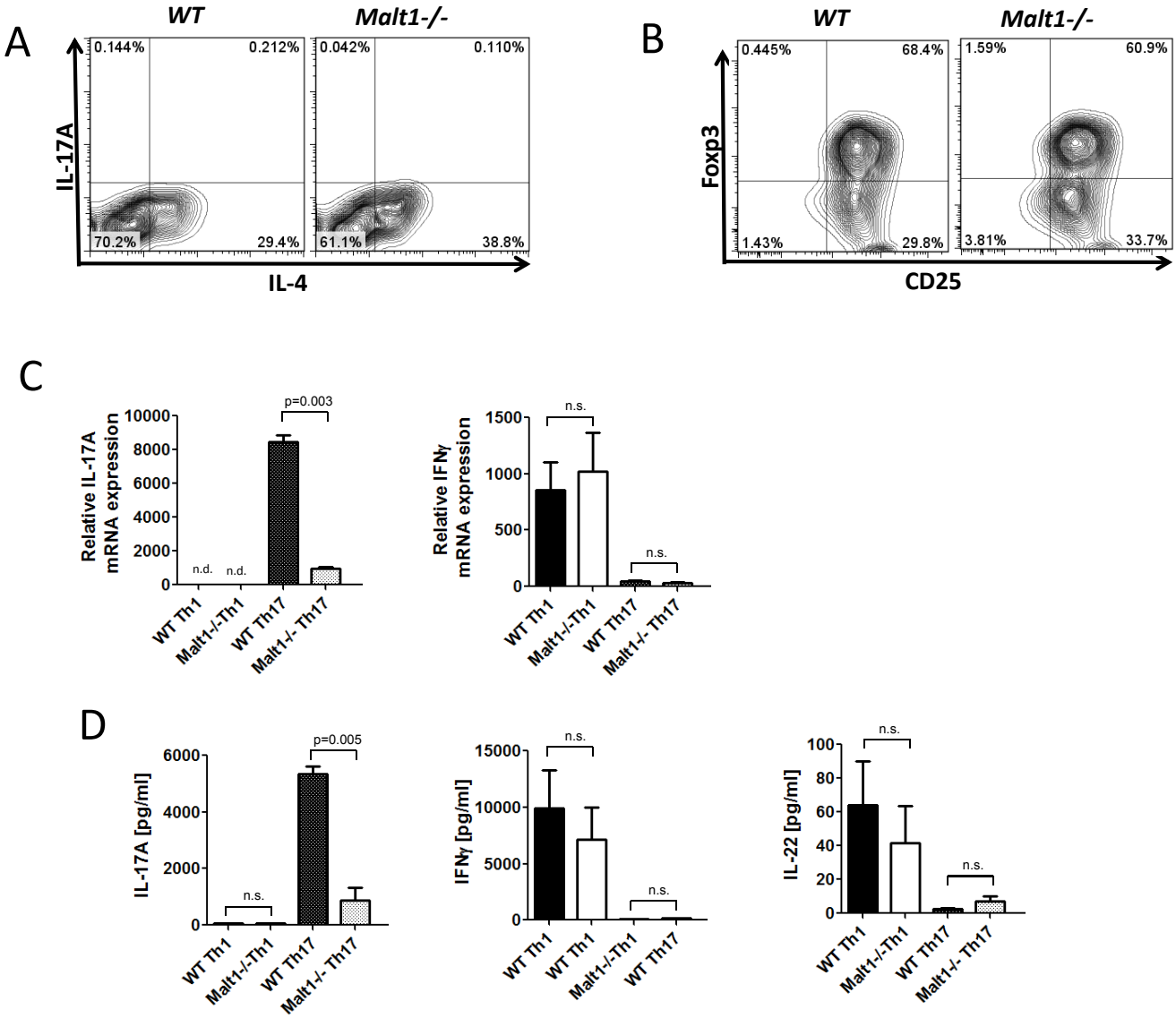
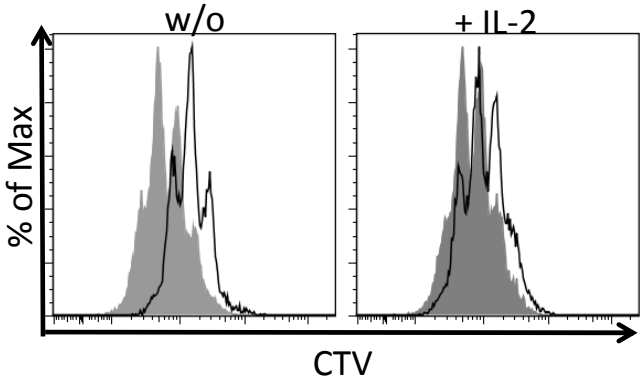

A
WT
Malt1-/-
0.144%
0.212%
0.042%
0.110%
70.2%
29.4%
61.1%
38.8%
IL-17A
IL-4
B
WT
Malt1-/-
0.445%
68.4%
1.59%
60.9%
1.43%
29.8%
3.81%
33.7%
Foxp3
CD25
C
Relative IL-17A mRNA expression
p=0.003
n.d.
n.d.
Relative IFNγ mRNA expression
n.s.
n.s.
WT Th1
Malt1-/-Th1
WT Th17
Malt1-/- Th17
D
IL-17A [pg/ml]
p=0.005
n.s.
IFNγ [pg/ml]
n.s.
n.s.
IL-22 [pg/ml]
n.s.
n.s.

Suppl Fig 1

Supplementary Figure 1: In vitro, MALT1 is essential only for the generation of IL-17-producing Th cells.

Th cell subsets were generated from naïve T cells of *Malt1-/-* and *WT* mice by priming with anti-CD3 antibody (Ab) plus anti-CD28 Ab and treating with subset-specific cytokines for 72h (see below). Primed Th cells were restimulated for 6h with PMA/Iono. Th2 cells were rested for 2 days before restimulation. (A, B) Th2 cells (A) were induced with IL-4, IL-2 plus anti-IFNγ, whereas Treg cells (B) were induced with TGF-β plus IL-2. IL-17A and IL-4 (A), or Foxp3 and CD25 (B), were detected by intracellular staining and flow cytometry. Data are gated on live $CD4^+$ cells and representative of 3 independent experiments. (C, D) Th1 cells were induced with IL-12 and IL-2, whereas Th17 cells were induced with IL-6, TGF-β, IL-2 and anti-IFNγ. (C) Levels of IL-17A mRNA (left) and IFNγ mRNA (right) as quantitated by qRT-PCR. (D) Levels of IL-17A (left), IFNγ (middle) and IL-22 (right) in culture supernatants as quantified by ELISA. Data are the mean ± SEM of 3 independent experiments. n.s., not significant.

A



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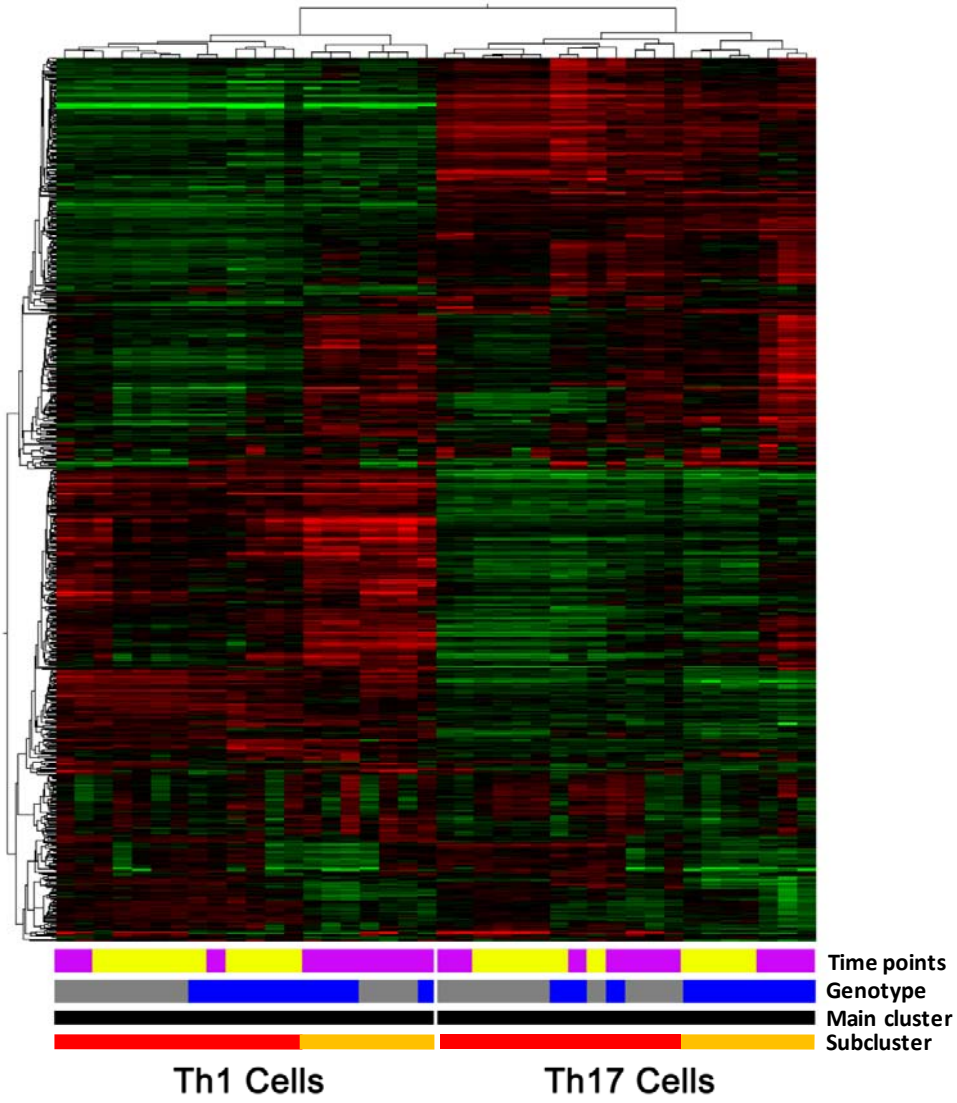
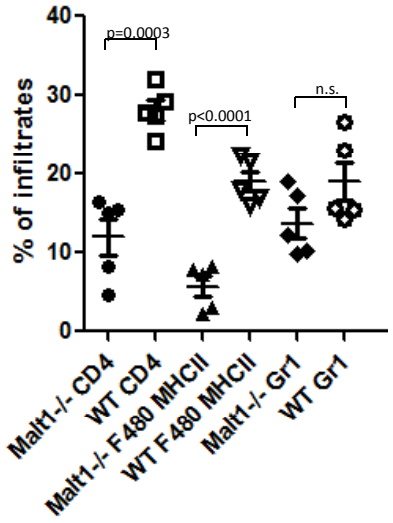


Suppl Fig 2

Supplementary Figure 2: IL-2 restores the proliferation of MALT -deficient Th17 cells.

(A) *Malt1-/-* and *WT* naïve $CD4^+$ T cells were labeled with CellTrace violet (CTV) before Th17 differentiation was induced by priming with anti-CD3/28 including treatment with IL-6, TGF-β and anti-IFNγ in the absence (w/o) or presence (+) of IL-2. After 72h differentiation plus 6h restimulation with PMA/Iono, cell proliferation was analyzed by flow cytometry. Data are gated on live $CD4^+$ T cells and are representative of 3 independent experiments. (B) *Malt1-/-* and *WT* Th1 and Th17 cells were differentiated as described in Suppl. Figure 1. Total mRNA was isolated at 48h and 72h of priming and run on Illumina whole genome microarrays. Unsupervised two-way hierarchical clustering analysis of probes on the array with highest variability in signal overall (Standard Deviation > 0.5) was then performed. Purple = 72h; yellow = 48h; grey = *Malt1-/-*; blue = *WT*. Black = main cluster (Th1 and Th17); Orange and red = Subcluster in Th1 and Th17 clusters.

A



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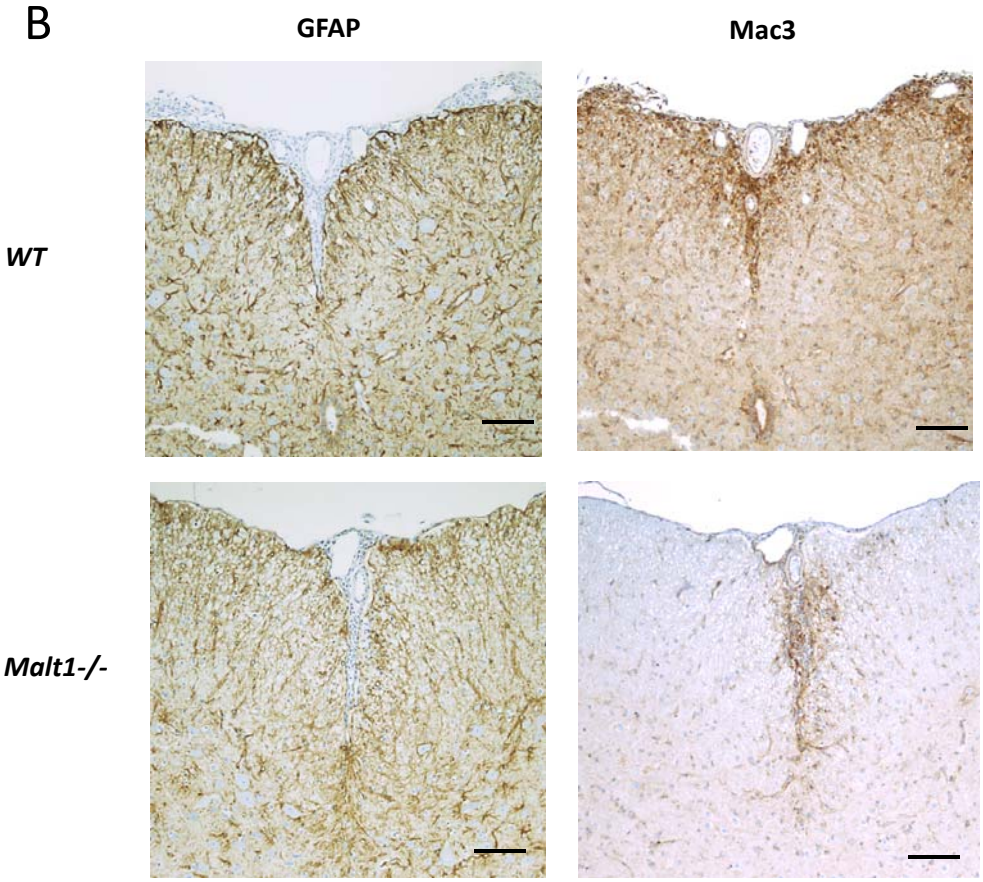
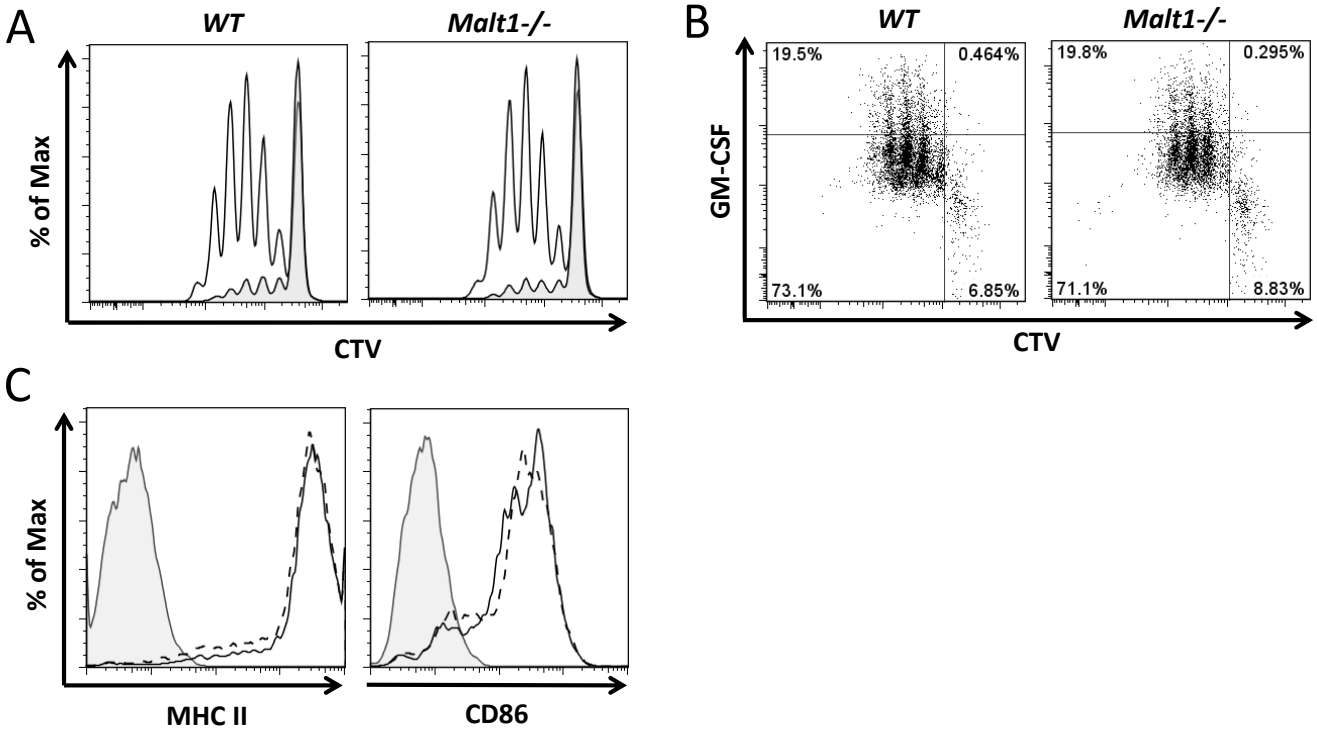


Suppl Fig 3

Supplementary Figure 3: *Malt1-/-* brain shows altered immune cell infiltration patterns after EAE induction.

(A) Immune cells infiltrating the brains of *Malt1-/-* and *WT* mice were isolated by gradient centrifugation on day 14 post-MOG injection. Surface expression of CD4, GR1, F4/80 and MHCII was detected by flow cytometry. Results are expressed as a relative percentage of total infiltrating cells gated on live cell. Data are the mean ± SEM (n=5). (B) Representative histopathological analyses of spinal cord cross-sections from *Malt1-/-* and *WT* mice in Figure 1A 30days post-MOG injection Sections were stained with GFAP to detect reactive astrocytes and Mac3 to detect macrophages. Scale bars, 100µm.

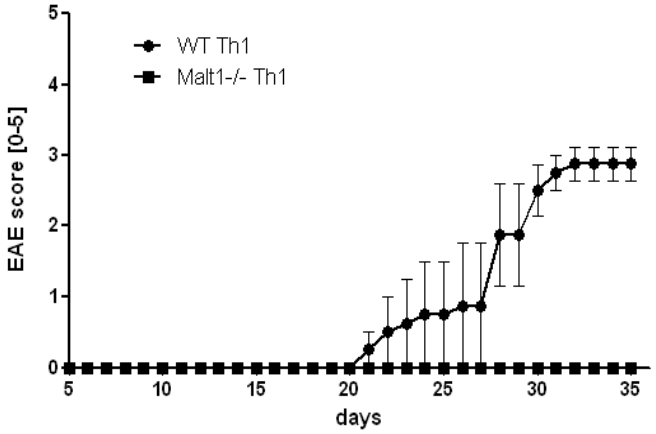

A
WT
Malt1-/-
% of Max
CTV
B
WT
Malt1-/-
GM-CSF
19.5%
0.464%
73.1%
6.85%
19.8%
0.295%
71.1%
8.83%
CTV
C
% of Max
MHC II
CD86

Suppl Fig 4

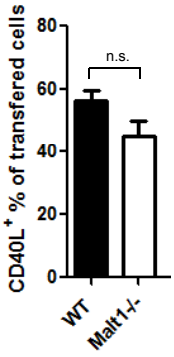
Supplementary Figure 4: MALT1 deficiency does not alter DC function.

(A, B) *Malt1-/-* and *WT* bone marrow-derived dendritic cells (BM-DCs) ($1x10^5$) were activated for 16h with LPS (clear trace) or left untreated (grey), and pulsed with MOG peptide. MOG-loaded *Malt1-/-* and *WT* BM-DCs were incubated with CTV-labeled *WT* 2D2 $CD4^+$ T cells ($2x10^5$), and (A) proliferation and (B) GM-CSF production were measured 72h later by flow cytometry. (C) The LPS-activated WT (solid line) and *Malt1-/-* (dashed line) BM-DCs of (A, B) were assessed for MHCII and CD86 expression by flow cytometry (grey, unstained control). Data are gated on live $CD11c^+$ cells (A) or live CD4=T cells (B,C) and are representative of 3 independent experiments.

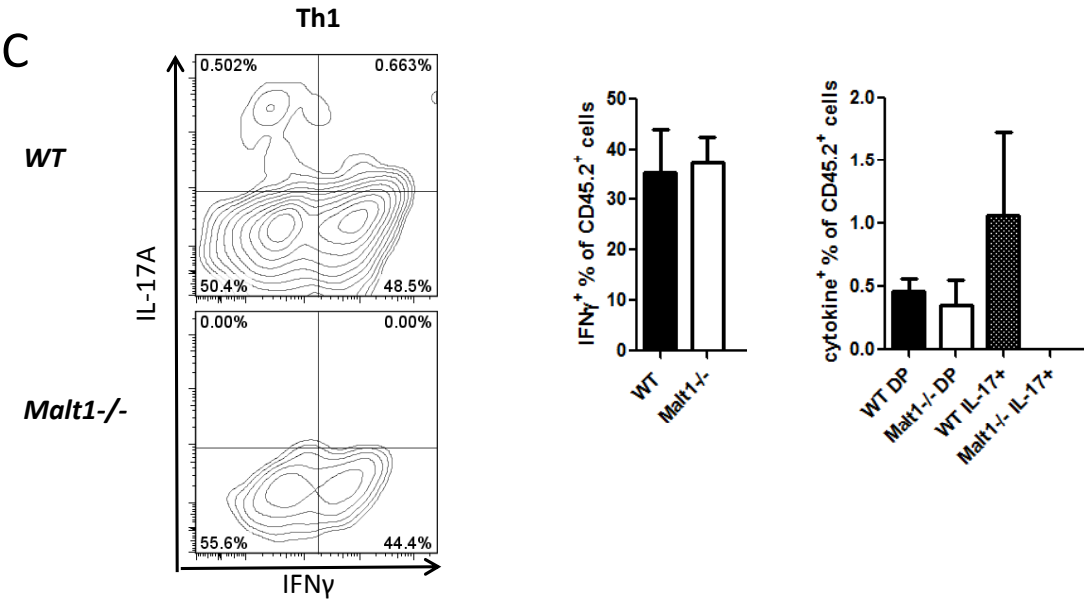
A



B



C



Suppl Fig 5

Supplementary Figure 5: Malt1-/- Th1 cells maintain a characteristic Th1 cytokine profile but fail to induce EAE.

(A) EAE was initiated in *Malt1-/-* and *WT* mice by MOG injection. At 10 days post-injection, mice were sacrificed and *Malt1-/- or WT* $CD4^+$T cells from spleen and LN were cultured for 5 days with MOG peptide, IL-12 and anti-IL-23 before injection into *WT* recipients ($2x10^7$ cells/mouse; n=7 mice /group). Recipients were injected with PT on the day of T cell transfer and again two days later. Clinical signs of EAE were monitored daily as for Figure 4C. (B) $CD45.2^+$ MOG-specific *Malt1-/-* and *WT* naïve T cells were differentiated into Th1 cells as described above and transferred into $CD45.1^+$ transgenic irradiated WT mice. EAE was induced by MOG injection. On day 18, spleen cells were analyzed for surface expression of CD40L by flow cytometry. Results are the percentage of live $CD45.2^+$ $CD4^+$ T cells expressing CD40L. Data are the mean ± SEM (n=5). (C) The cells in (B) were evaluated for expression of IL-17A and IFNγ by intracellular cytokine staining and flow cytometry (left panel). Right panel: Results are expressed as the percentage of live $CD45.2^+$ $CD4^+$ T cells producing IFNγ (left) or IL-17A (right). Data are the mean ± SEM (n=5).

Supplementary table 1
qRT-PCR primer sequences used

Target	Forward 5'-3'	Reverse 5'-3'
HPRT	CTG GTG AAA AGG ACC TCT	TGA AGT ACT CAT TAT AGT CAA GGG CA
IFNγ	ATG AAC GCT ACA CAC TGC ATC	CCA TCC TTT TGC CAG TTC CTC
IL-17A	TTT AAC TCC CTT GGC GCA AAA	CTT TCC CTC CGC ATT GAC AC
IRF4	ACG CTG CCC TCT TCA AGG CTT	TGG CTC CTC TCG ACC AAT TCC
RORα	TCT CCC TGC GCT CTC CGC AC	TCC AGA GAT CTT GCA TGG A
RORγt	TTT GGA ACT GGC TTT CCA TC	AAG ATC TGC AGC TTT TCC ACA
T-bet	GGT GTC TGG GAA GCT GAG AG	GAA GGA CAG GAA TGG GAA CA