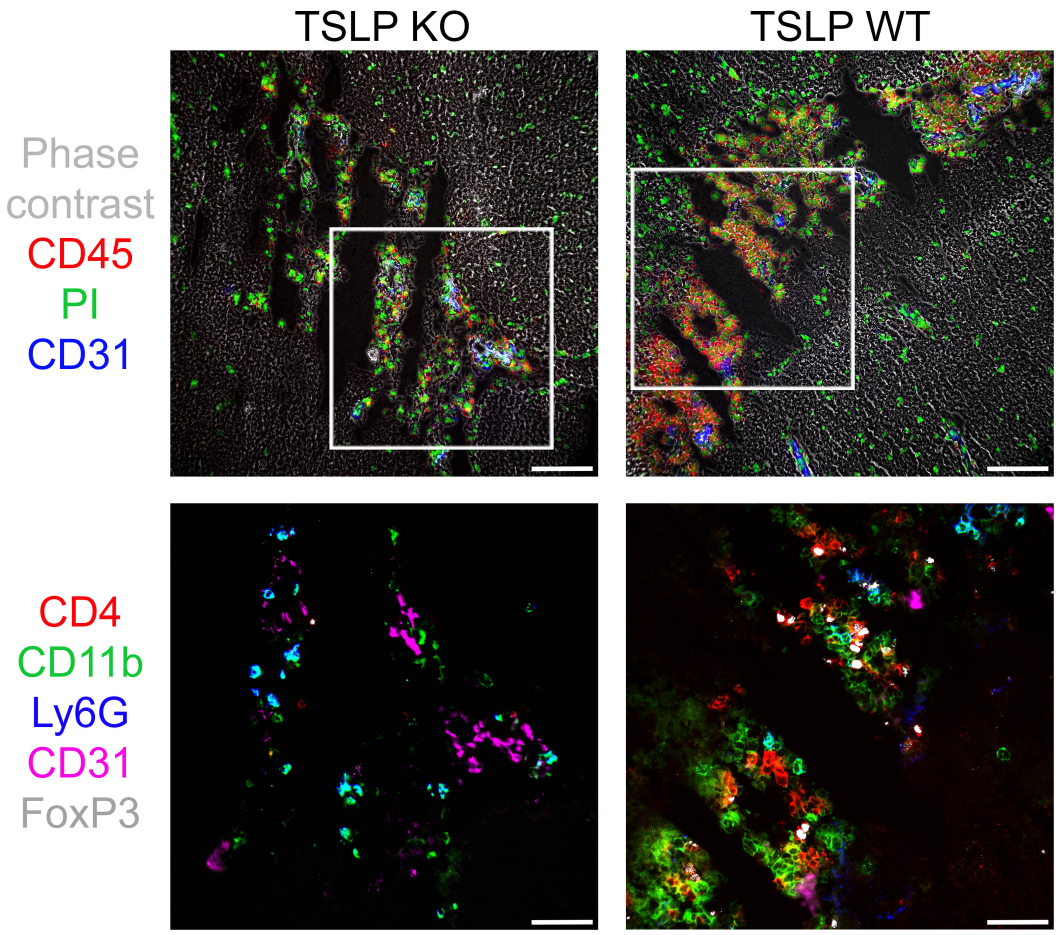
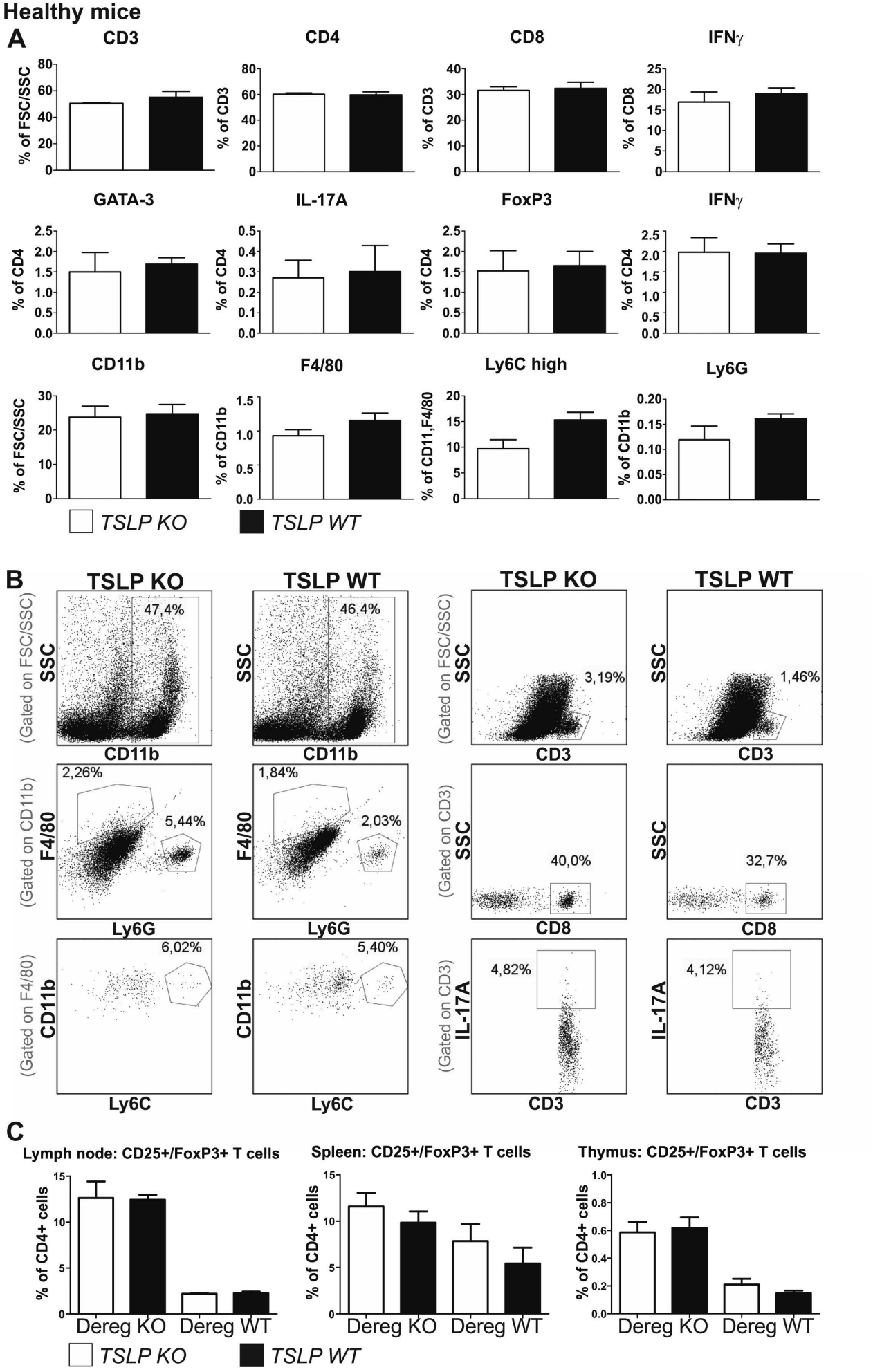
Suppl. Figure 1

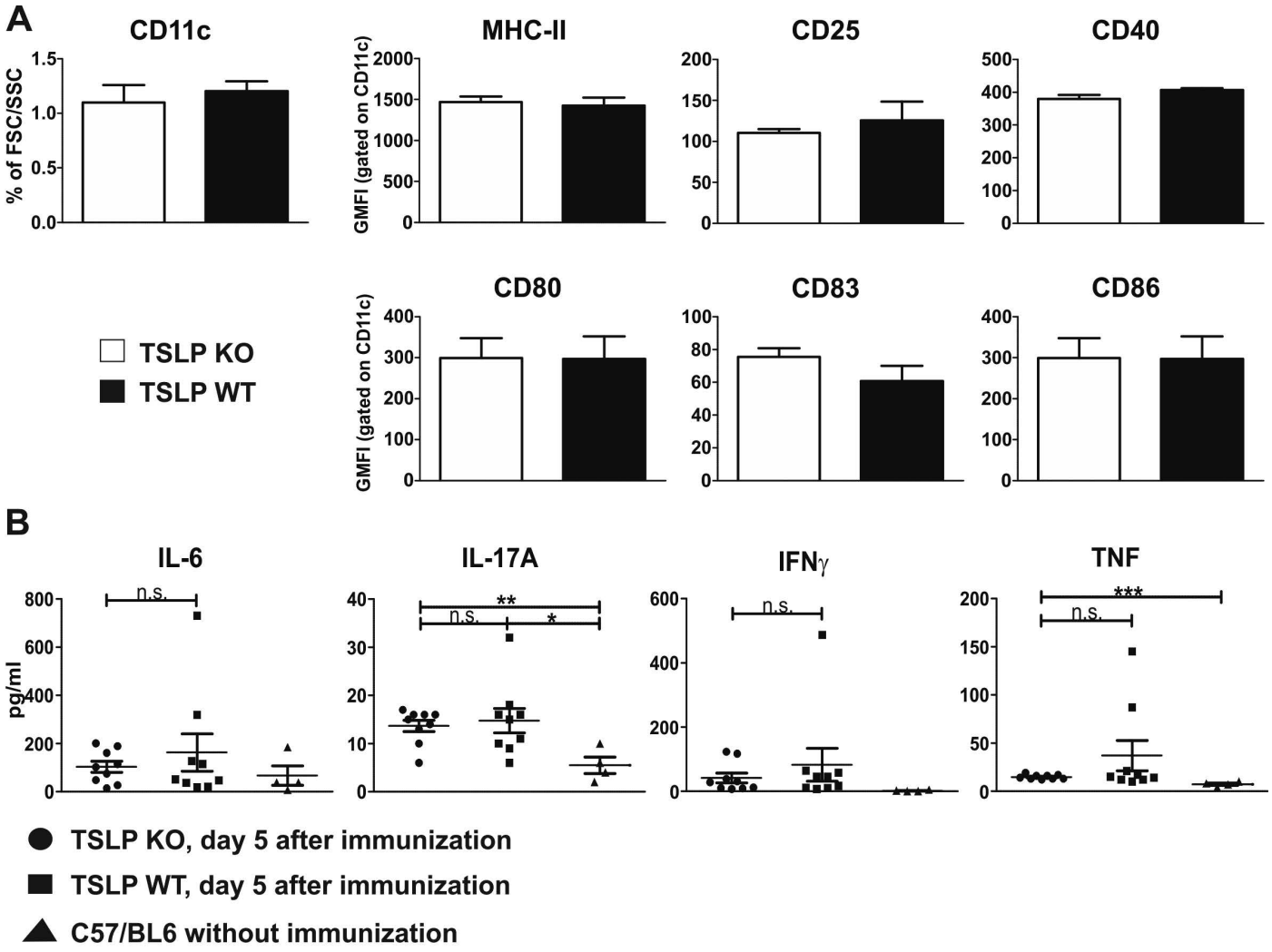


Suppl. Figure 1: The Choroid plexus of TSLP KO mice contained fewer leukocytes in comparison to TSLP WT mice. MELC images of brain harvested from TSLP KO mice and WT mice at day 12 after EAE induction. Upper row: Phase contrast (white), CD45+ leukocytes (red), propidium iodide+ nuclei (green), CD31+ blood vessels (blue). Lower row: CD4+ T cells (red), CD11b+ macrophages / microglial cells (green), Ly6G+ neutrophil granulocytes (blue), CD11b+/Ly6G+ granulocytes (cyan), CD31+ blood vessels (magenta), FoxP3+ regulatory T cells (red with white nuclei). Bar= 50µm.


Healthy mice
A
CD3
CD4
CD8
IFNγ
% of FSC/SSC
% of CD3
% of CD8
GATA-3
IL-17A
FoxP3
IFNγ
% of CD4
CD11b
F4/80
Ly6C high
Ly6G
% of CD11b
% of CD11,F4/80
TSLP KO
TSLP WT
B
TSLP KO
TSLP WT
(Gated on FSC/SSC)
SSC
CD11b
47,4%
46,4%
3,19%
1,46%
CD3
(Gated on CD11b)
F4/80
2,26%
5,44%
1,84%
2,03%
Ly6G
(Gated on CD3)
40,0%
32,7%
CD8
(Gated on F4/80)
Ly6C
6,02%
5,40%
IL-17A
4,82%
4,12%
C
Lymph node: CD25+/FoxP3+ T cells
Spleen: CD25+/FoxP3+ T cells
Thymus: CD25+/FoxP3+ T cells
% of CD4+ cells
Dereg KO
Dereg WT
TSLP KO
TSLP WT

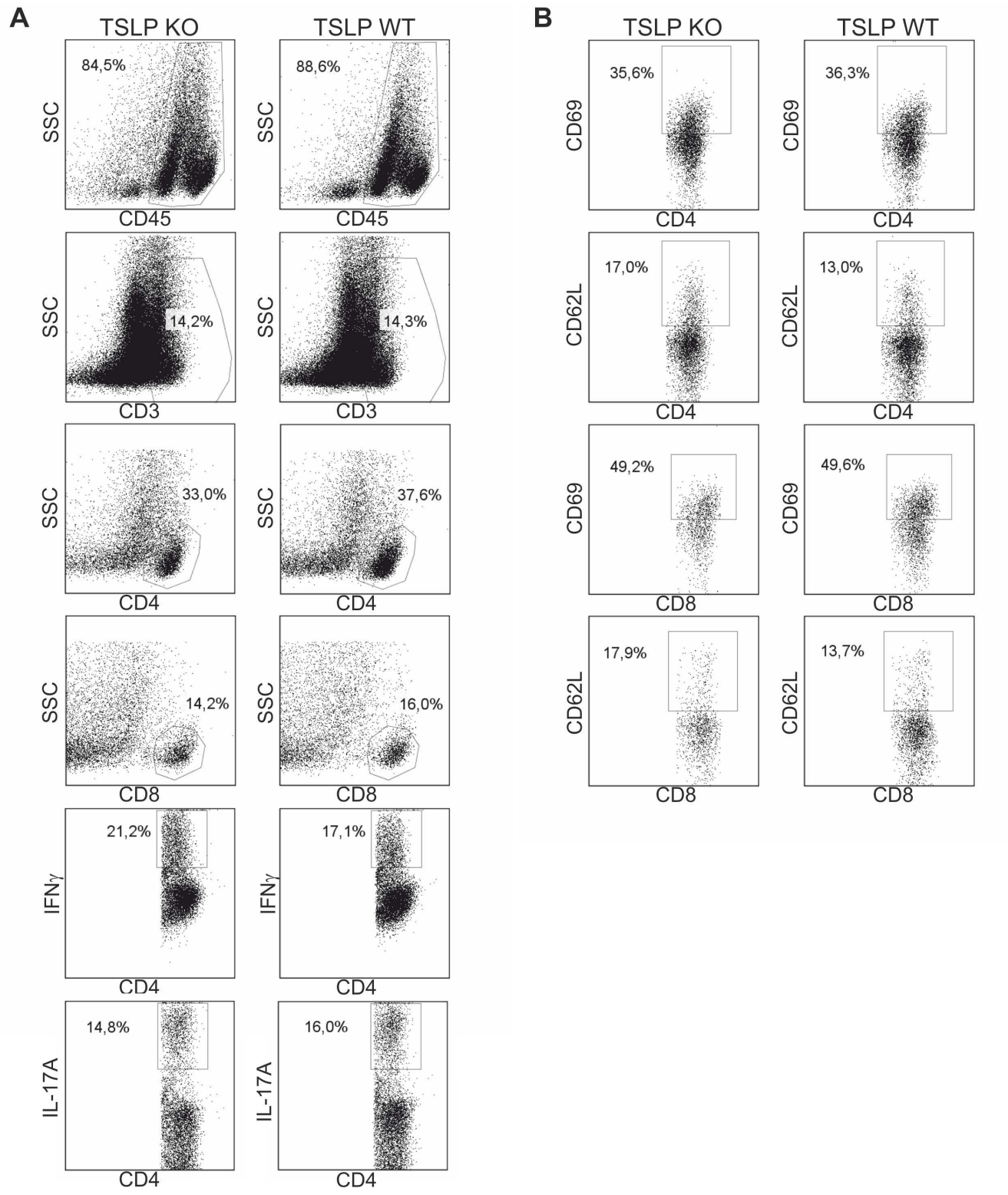
Suppl. Figure 2: In healthy TSLP KO mice no reduction in the numbers of different T cell subtypes, macrophages or neutrophil granulocytes were observed. (A) FACS analyses of inguinal lymph nodes (mean +/- SEM; TSLP KO n=3, TSLP WT n=4). (B) FACS analyses of brain (TSLP KO= pool of 3 mice, TSLP WT= pool of 4 mice). (C) FACS analyses of inguinal lymph nodes, spleen and thymus. Diphteria toxin was injected at day 0 and 1, organs were removed at day 4 (3 mice/group). Data (A, B) (mean +/- SEM) are representative of two and data (C) of three independent experiments.

Suppl. Figure 3



Suppl. Figure 3: No differences in DC maturation or serum cytokine concentrations between TSLP KO and WT mice at day 5 after EAE induction. (A) FACS analysis of spleens. GMFI= Geometric mean fluorescence intensity. The bar charts represent the mean +/- SEM. TSLP KO n=3, TSLP WT n=3. Data are representative of three independent experiments. (B) Cytometric Bead Array (CBA) of serum. TSLP KO n=9; TSLP WT n=9; untreated C57/BL6 n=4; mean +/- SEM; two-tailed unpaired Student t test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$; n.s. = not significant. Data are representative of two independent experiments.

Suppl. Figure 4



Suppl. Figure 4: At day 20 after EAE induction TSLP KO mice and TSLP WT mice show the same extent of CNS inflammation. Flow cytometric analysis of brain. (A) CD45+ cells were gated on FSC/SSC, CD3+ cells were gated on CD45+ cells, CD4+ and CD8+ cells were gated on CD3+ cells. IFNγ+ and IL-17A+ cells were gated on CD4+ cells. (B) CD62L+ and CD69+ cells were gated on CD4+ or CD8+ cells. Results are representative of two independent experiments (pool of three brains each).

Suppl. table S7: qRT-primer sets used in this study

Target	Forward 5´-3´	Reverse 5´-3´
CCL1	CTTACGGTCTCCAATAGCTGC	CCTGAACTCCTGACTACCACAG
CCR8	ACGTCACGATGACCGACTACT	CCCAGCACAAACAAGACGC
CD3e	ATGCGGTGGAACACTTTCTGG	GCACGTCAACTCTACACTGGT
CD4	CAAGCGCCTAAGAGAGATGG	CACCTGTGCAAGAAGCAGAG
CD8a	CCGTTGACCCGCTTTCTGT	TTCGGCGTCCATTTTCTTTGG
CD11b	ATGGACGCTGATGGCAATACC	TCCCCATTCACGTCTCCCA
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
CD19	GGAGGCAATGTTGTGCTGC	ACAATCACTAGCAAGATGCCC
CD45	CAGAAACGCCTAAGCCTAGTTG	AGGCAAGTAGGGACACTTCATAG
FoxP3	CCCAGGAAAGACAGCAACCTT	CCTTGCCTTTCTCATCCAGGA
GATA-3	CTCGGCCATTCGTACATGGAA	GGATACCTCTGCACCGTAGC
HPRT	GTTGGATACAGGCCAGACTTTGTTG	GATTCAACTTGCGCTCATCTTAGGC
IFNγ	AGCGGCTGACTGAACTCAGATTGTAG	GTCACAGTGTTCAGCTGTATAGGG
IL-4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
IL-6	AACCACGGCCTTCCCTACTTC	GCCATTGCACAACTCTTTTCTCAT
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
IL-12p40	TGGTTTGCCATCGTTTTGCTG	ACAGGTGAGGTTCACTGTTTCT
IL-17A	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
IL-17F	CTGGAGGATAACACTGTGAGAGT	TGCTGAATGGCGACGGAGTTC
IL-22	CATGCAGGAGGTGGTACCTT	CAGACGCAAGCATTTCTCAG
RORγt	GACCCACACCTCACAAATTGA	AGTAGGCCACATTACACTGCT
T-bet	GTTCCCATTCCTGTCCTTC	CCTTGTTGTTGGTGAGCTT
TGFβ1	TGGAGCAACATGTGGAACTCTA	AGACAGCCACTCAGGCGTATC
TNFα	ATGAGCACAGAAAGCATGATC	TACAGGCTTGTCACTCGAATT
TSLP	CCCTTCACTCCCCGACAAAAC	CAGTGGTCATTGAGGGCTTCT