Supplementary Figure legends

Supplementary Figure 1. Characterization of polarized human Th17 and Th1 cells. (A) Intracellular cytokine staining was performed for IL-17 by flow cytometry of HD (n = 6) and MS patients (n = 7). **(B)** Intracellular cytokine staining was performed for Th17 and Th1 cells by flow cytometry. **(C)** Quantifiaction of IL17-producing and IL-17/IFNγ producing cells. **(D)** mRNA analysis of the transcription factors *Tbx21, Rorc, Gata3 and Foxp3* was performed in Th17 (n = 6) and Th1 cells (n = 6); normalized to β-actin. Error bars represent mean ± SEM. *, $p < 0.05$; n.s. = not significant, n.d. = not detectable, unpaired Student's t test [A], Mann-Whitney test [C].

Supplementary Figure 2. No impact of glutaminase inhibition or culturing conditions on T cell differentiation and survival. (A) Intracellular cytokine staining was performed for IFNγ, IL-17, FoxP3 and Tbet on Th17 and Th1 cells ± BPTES (each condition n=3) by flow cytometry. **(B)** Live-dead staining was performed by flow cytometry for indicated culture conditions showing no significant differences. Error bars represent mean ± SEM. *, $p < 0.05$; n.s. = not significant, one-way ANOVA with post-hoc Tukey test [A, B].

Supplementary Figure 3. Th17 cells do not express classical neurotoxic molecules. (A) Dot plot representative of FasL and Perforin expression by Th17-differentiated cells after 4 hours TCR stimulation measured by flow cytometry. **(B)** Intracellular cytokine staining was performed for CD4, IFNγ and IL-17 on Th17 and Th1 cells. **(C)** mRNA analysis of the transcription factors *Tbet* and *Rorγt* was performed in Th17 cells (n = 6) in comparison to Th1 cells (n = 6). Bars indicate mean ± SEM. n.d. = not detectable, unpaired Student's t test [B].

Supplementary Figure 4. Expression of IL-17 and VAMP4 on murine Th17 cells. (A) Immunocytochemical staining of CD4 and IL-17 on naïve (upper lane) and Th17-differentiated (lower lane) cells (scale bar = 5 μm). Co-staining was performed with DAPI. (**B)** Polarized Th17 cells from IL-

17-EGFP mice were sorted into IL-17 producing (GFP+) and non-producing (GFP-) Th17 cells and western blotting stainings were performed for VAMP4 and GAPDH (one representative example out of three is shown).

Supplementary Figure 5. Inflammatory cytokine production correlates with glutamate release. (A) Proliferation of differentiated Th17 cells over 72 hours was assessed by [3H]-thymidine labeling and by CFSE-proliferation assay (n = 9). **(B)** Cytokine production (pg/ml) by Th17-skewed cells secreted into the supernatant after 4 hours TCR stimulation measured by cytokine bead array (n = 3). **(C)** Correlation of proinflammatory cytokines IL-17 and TNFα (%) with glutamate secretion (in µM, n = 10-11). Error bars represent mean ± SEM. **(D)** Immunohistochemical staining of $K_V1.3$ in healthy CNS tissue (upper row, scale bar = 50 µm) and in infiltrated EAE lesions (lower row, scale bar = 15 µm). Co-staining with NeuN and CD4 was performed. **(E)** Glutamate secretion levels of primary cortical neuronal cultures after 4-aminopyridine stimulation and MgTX after 24 hours (n = 6 per group). Bars indicate mean ± SEM. *, $p < 0.05$; ns, not significant, one-way ANOVA with post-hoc Tukey test [A, E], unpaired Student's t test [B], Spearman's rank correlation test [C].

Supplementary Figure 6. Transfection efficiency of primary T lymphocytes. (A) Murine Th17 cells were transfected with iGluSnFR. Live dead staining was performed and the transfection efficiency was evaluated via GFP by flow cytometry.

Supplementary Figure 7. Stimulation of CD29 and VCAM1 does not alter T cell functionality. (A) Proliferation of differentiated Th17 cells ± CD29 stimulating-Ab or ± VCAM1 coating over 72 hours was assessed by CFSE-proliferation assay (n = 4). **(B)** Cytokine IL-17 production by Th17-skewed cells after 4 hours TCR stimulation measured by flow cytometry (n = 4). Bars indicate mean ± SEM. ns = not significant, one-way ANOVA with post-hoc Tukey test [A, B].

Supplementary Video legends

Supplementary Video 1. **Effect of MgTX in TN-XXL Rag$^{-/-}$ control conditions.** Intravital two-photon microscopy in the brain stem of living B6.Thy1-TN-XXLxRag2γc$^{-/-}$ anaesthetized mice. Somata and axons are shown (TN-XXL = YFP, CFP; yellow, blue fluorescent proteins). Margatoxin (5 µM) was puffed locally during imaging of control mice.

Supplementary Video 2. **K_V1.3 blockade on Th17 cells reduces Ca^{2+} elevations *in vivo* in EAE.** Passive EAE in B6.Thy1-TN-XXLxRag2γc$^{-/-}$ mice was induced by transfer of 1x10^7 CD4$^+$Th17 cells (B6.RFPx2D2) intravenously. At the peak of disease, intravital two-photon microscopy was performed in the brain stem of living anaesthetized mice. Immune cells (RFP, red fluorescent protein) infiltrate the upper brainstem region and show close proximity with somata and axons (TN-XXL = YFP, CFP; yellow, blue fluorescent proteins). Neuronal free Ca^{2+} levels were assessed by FRET measurements (525 nm/475 nm ratio, false color coded representation). Margatoxin (5 µM) was puffed locally during imaging of EAE mice (for better visualization: red puff at timepoint 35).

Supplementary Tables

Supplementary Table 1. **Clinical and demographic information for patients and controls for CSF samples.**

	NIND	**RRMS**	**p-value**
n	14	16	
Age	49 (34; 55)	37 (27; 45.75)	
Sex	2M 12F	5M 11F	0.9998
Glutamate	12.41 ± 1.581	11.35 ± 1.555	0.6106
Glutamine	612.8 ± 26.72	822.9 ± 83.58	0.0318

Human CSF samples were isolated from RRMS patientes (n = 16) and non-inflammatory neurological disease patients (n= 14). The median (minimum, maximum) of the age and sex is shown. The glutamate and the glutamine levels (mean µM, ±SEM) were assessed for each group.

Supplementary Table 2. **Clinical and demographic information for patients and controls for $CD4^+$ cells.**

	Healthy	**MS**	**p-value**
n	17	32	
Age	29 (26; 33)	36.5 (30.25; 48.50)	
Sex	5M 12F	12M 20F	
EDSS	N.A.	1 (0; 2)	
Disease Duration (yr)	N.A.	2 (1; 7)	
Glutamate	14.57 ± 1.923	21.42 ± 2.713	0.0924
IL-17 %	1.034 ± 0.1541	0.8284 ± 0.2822	0.5836

Human $CD4^+$ cells (>98%) were isolated from PBMCs of healthy donors (n = 17) and MS patients (n= 32). The median (minimum, maximum) of the age, sex, Expanded Disability Status Scale (EDSS) score

and disease duration is shown. The glutamate secretion levels (mean µM, ±SEM) and the IL-17 production (mean %, ±SEM) were assessed for each group.

Supplementary Table 3. **Clinical and demographic information for patients and controls for Th1 and Th17 cells.**

	Healthy	**MS**	**p-value**
n	8	15	
Age	30 (26; 36)	32 (21; 53)	
Sex	4M 4F	6M 9F	0.90
EDSS	N.A.	0 (0; 3.5)	
Disease Duration (yr)	N.A.	3 (1; 7)	

Human $CD4^{+}IL\text{-}17^{+}$ cells were isolated from PBMCs of healthy donors (n = 8) and MS patients (n= 15). The median (minimum, maximum) of the age, sex, Expanded Disability Status Scale (EDSS) score and disease duration is shown. The glutamate secretion levels (mean µM, ±SEM) and the IL-17 production (mean %, ±SEM) were assessed for each group.

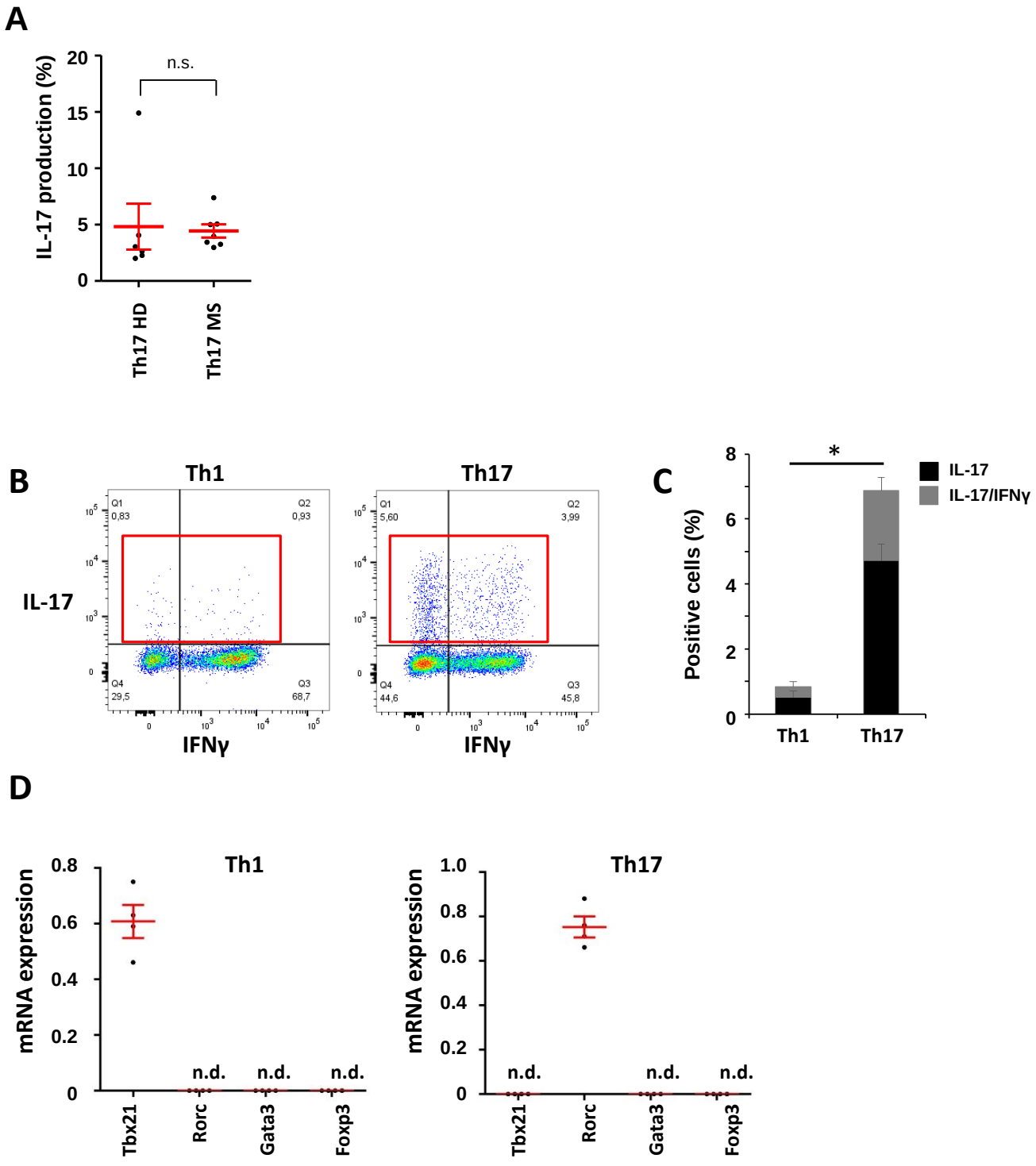
Supplementary Table 4. **qPCR primer information.**

Official gene name	**Primer name**	**Primer sequence**
Homo sapiens actin, beta (AKTB)	**Hs-ßACTIN-F**	**TTAGTTGCGTTACACCCTTTC**
	Hs-ßACTIN-R	**ACCTTCACCGTTCCAGTT**
Homo sapiens glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	**Hs-GAPDH-F**	**TATGACAACAGCCTCAAG**
	Hs-GAPDH-R	**TTCCACGATACCAAAGTT**
Homo sapiens RPS13	**Hs-RPS13-F**	**GAAAGGATAAGGATGCTAAA**
	Hs-RPS13-R	**AGAGGCTGTAGATGATTC**

Homo sapiens Glutaminase	**Hs-GLUTAMINASE-F**	**TGTCTTCAGTCCTGTGTA**
	Hs-GLUTAMINASE-R	**TGTGGTTTATCATCTTCATTC**
Homo sapiens Kv1.3 (KCNA3)	**Hs-KCNA3-F**	**TGAGTAAGTCGGAGTATA**
	Hs-KCNA3-R	**AAGAGTTGGGATTATTGT**
Homo sapiens ATPase D2	**Hs-ATPASED2-F**	**ACTACTGATTATGGTAAC**
	Hs- ATPASED2-R	**ATATAGGTGAGAAATGTG**
Homo sapiens Rorc	**Hs-Rorc-F**	**CTCCATCTTTGACTTCTC**
	Hs-Rorc-R	**GCTGTTCTACTTTCCTTT**
Homo sapiens Gata3	**Hs-Gata3-F**	**AAATACTGAGAGAGGGAGAGA**
	Hs-Gata3-R	**GGTAGCGAAGAGCAGAGA**
Homo sapiens Foxp3	**Hs-Foxp3-F**	**GGCACAATGTCTCCTCCAGAGA**
	Hs-Foxp3-R	**CAGATGAAGCCTTGGTCAGTGC**
Homo sapiens Tbx21	**Hs-Tbx21-F**	**AATGTGACCCAGATGATT**
	Hs-Tbx21-R	**AAAGTAAAGATATGCGTGTT**
Mus musculus actin, beta (Actb)	**Mm-ß-ACTIN-F**	**AATCTTCCGCCTTAATACT**
	Mm-ß-ACTIN-R	**AGCCTTCATACATCAAGT**
Mus musculus glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	**Mm-GAPDN-F**	**CAGCAACTCCCACTCTTC**
	Mm-GAPDN-R	**TGTAGCCGTATTCATTGTCAT**
Mus musculus Rps29-like protein	**Mm-RSP29-F**	**CAAATACGGGCTGAACAT**
	Mm-RSP29-R	**GTCGCTTAGTCCAACTTAA**

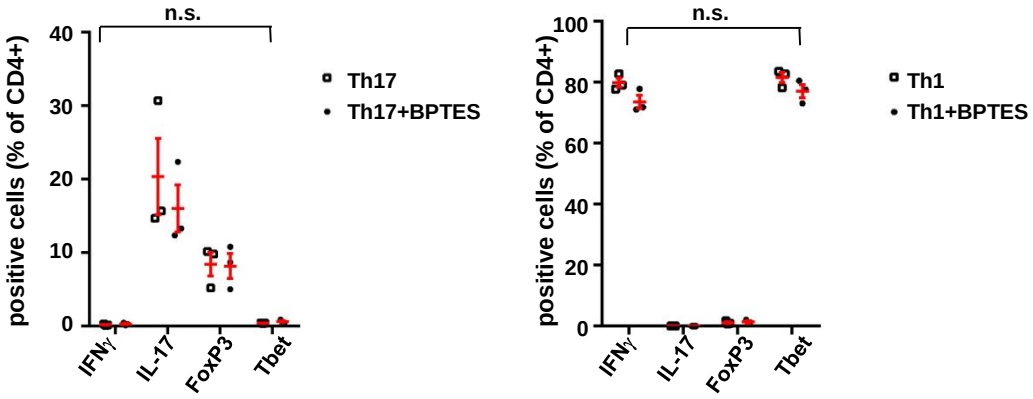
Mus musculus Glutaminase	**Mm-GLUTAMINASE-F**	**TGTGCTCTATTGAAGTGA**
	Mm-GLUTAMINASE-R	**CTCAGTGTATTCCGAACT**
Mus musculus ATPase	**Mm-ATPASE-F**	**CCCATTCTTGAGTTTGAG**
	Mm-ATPASE-R	**CTGTCTTCTTTGCTTAGTT**
Mus musculus Kv1.3 (KCNA3)	**Mm-KCNA3-F**	**GCACCACGAACAATAACC**
	Mm-KCNA3-R	**AATCAAGGGCATACACAGA**
Mus musculus VCAM-1	**Mm-VCAM1-F**	**AGACTACACTGATGAAGAA**
	Mm-VCAM1-R	**GAGGCAAACAAGAGATTT**
Mus musculus VGlut2 (Slc17a6)	**Mm-SLC17A6-F**	**GCTTCTGGTGTCTTATGA**
	Mm-SLC17A6-R	**CAATGCTCTCCTCTATGTA**
Mus musculus VGlut1 (Slc17a7)	**Mm-SLC17A7-F**	**AGTTCTGTCTTCTATGTCTAT**
	Mm-SLC17A7-R	**TGGCATCCTCAATGTATT**
Mus musculus VGlut3 (Slc17a8)	**Mm-SLC17A8-F**	**ATAGAGACAAGCATAGGAGAAG**
	Mm-SLC17A8-R	**GCATAGACAGGCAAGGAT**
Mus musculus Aspartate aminotransferase (GOT2)	**Mm-GOT2-F**	**AGCAGACCACATTACAGAA**
	Mm-GOT2-R	**GGACACGACACTACATCTT**
Mus musculus Vesicle-associated membrane protein 2	**Mm-VAMP2-F**	**CGCAAATACTGGTGGAAA**
	Mm-VAMP2-R	**ATGATGATGATGAGGATGATG**
Mus musculus Vesicle-associated membrane protein 3	**Mm-VAMP3-F**	**AAGTATTGGTGGAAGAAC**
	Mm-VAMP3-R	**CGATGATGATGATGACAA**
Mus musculus Vesicle-associated membrane protein 4	**Mm-VAMP4-F**	**AGGAACTGGACTACTAATC**
	Mm-VAMP4-R	**ATGCTCTTACTATCACTCA**
Mus musculus Synaptosomal-associated protein 23	**Mm-SNAP23-F**	**CGTCTCTTCATCTCTTCATT**
	Mm-SNAP23-R	**AACCAACCAACCAATACC**
Mus musculus RORgt	**Mm-RORgt-F**	**GGATAGAGATAGGATGAC**
	Mm-RORgt-R	**GAAAGGACTGTTATTAGC**
Mus musculus Tbet	**Mm-Tbet-F**	**GTGGAGGTGAATGATGGA**
	Mm-Tbet-R	**ATCTCTGCGTTCTGGTAG**

The primers listed were designed using Beacon Designer Software for designing primers especially for qRT-PCR experiments.

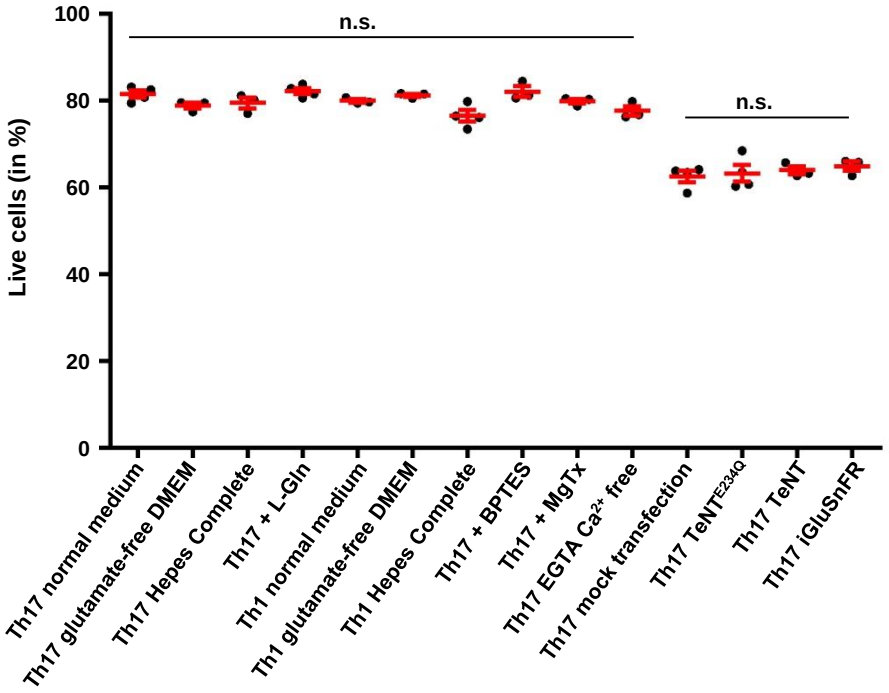

A
IL-17 production (%)
n.s.
Th17 HD
Th17 MS
B
Th1
Th17
IL-17
IFNγ
Q1 0,83
Q2 0,93
Q4 29,5
Q3 68,7
Q1 5,60
Q2 3,99
Q4 44,6
Q3 45,8
C
Positive cells (%)
*
IL-17
IL-17/IFNγ
Th1
Th17
D
Th1
Th17
mRNA expression
n.d.
Tbx21
Rorc
Gata3
Foxp3

Supp 1

A

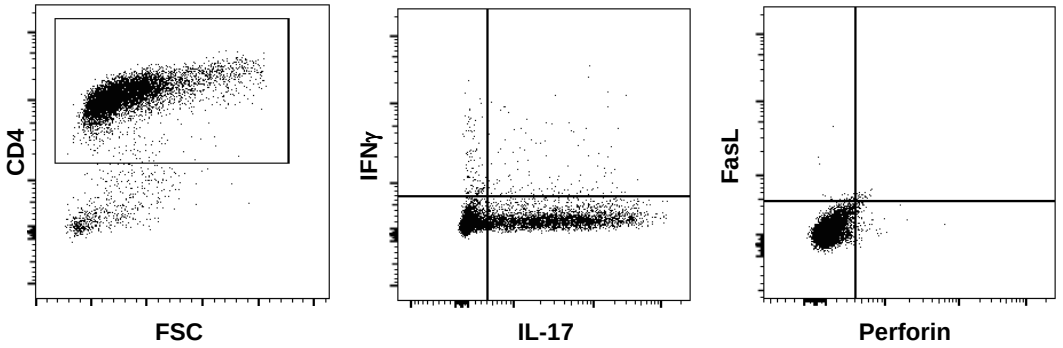

n.s.
positive cells (% of CD4+)
40
30
20
10
0
Th17
Th17+BPTES
IFNγ
IL-17
FoxP3
Tbet
n.s.
positive cells (% of CD4+)
100
80
60
40
20
0
Th1
Th1+BPTES
IFNγ
IL-17
FoxP3
Tbet

B

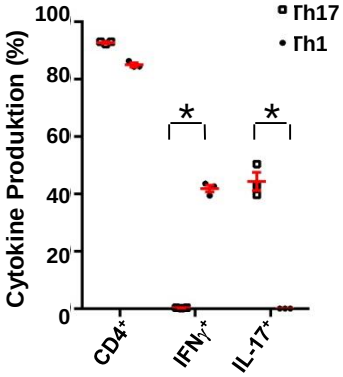

n.s.
n.s.
Live cells (in %)
100
80
60
40
20
0
Th17 normal medium
Th17 glutamate-free DMEM
Th17 Hepes Complete
Th17 + L-Gln
Th1 normal medium
Th1 glutamate-free DMEM
Th1 Hepes Complete
Th17 + BPTES
Th17 + MgTx
Th17 EGTA Ca2+ free
Th17 mock transfection
Th17 TeNTE234Q
Th17 TeNT
Th17 iGluSnFR

Supp 2

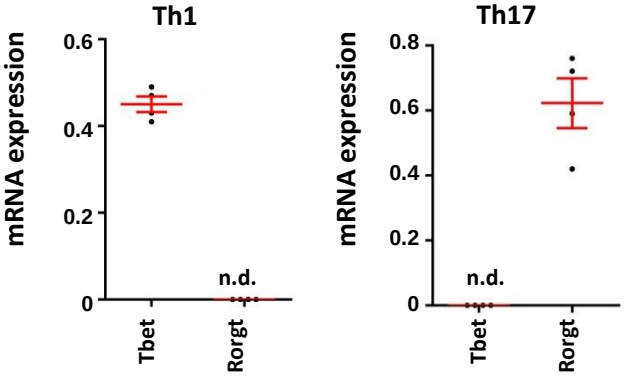
A


CD4
FSC
IFNγ
IL-17
FasL
Perforin

B

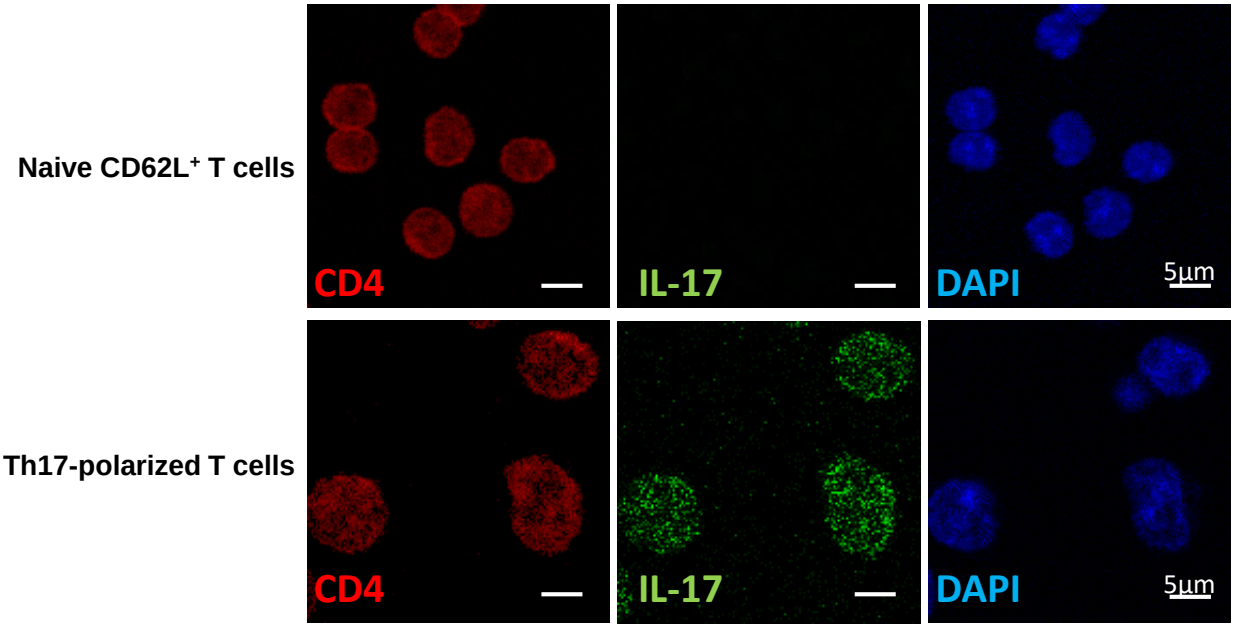

Th17
Th1
Cytokine Produktion (%)
100
80
60
40
20
0
*
*
CD4+
IFNγ+
IL-17+

C

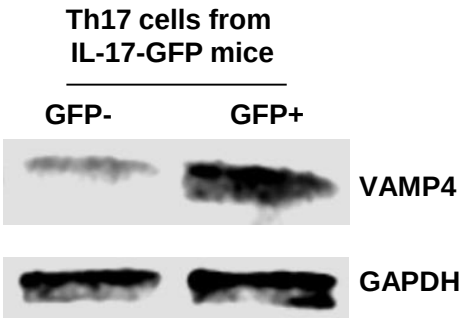

Th1
mRNA expression
0.6
0.4
0.2
0
n.d.
Tbet
Rorgt
Th17
mRNA expression
0.8
0.6
0.4
0.2
0
n.d.
Tbet
Rorgt

Supp 3

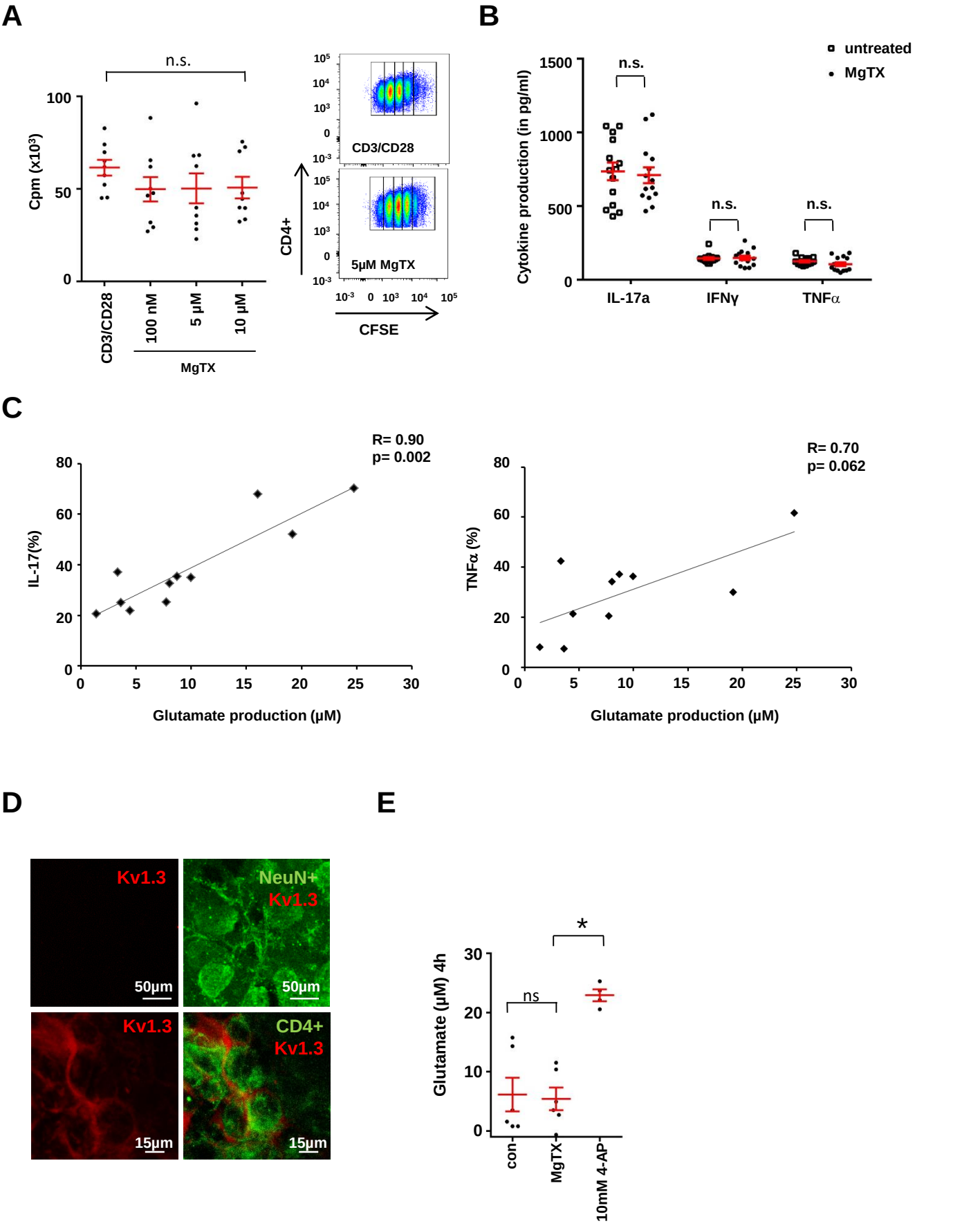
A


Naive CD62L+ T cells
CD4
IL-17
DAPI
5µm
Th17-polarized T cells
CD4
IL-17
DAPI
5µm

B

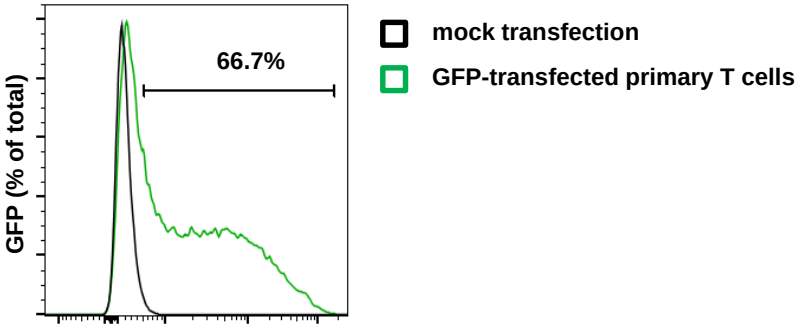

Th17 cells from
IL-17-GFP mice
GFP-
GFP+
VAMP4
GAPDH

Supp 4

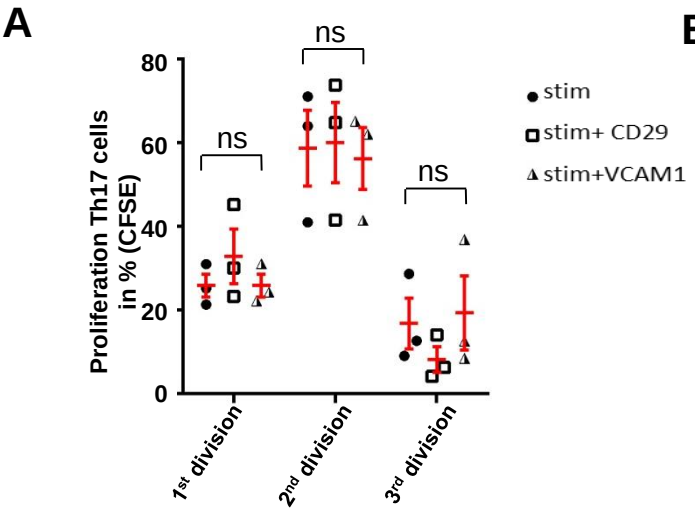

A
n.s.
Cpm (x10³)
100
50
0
CD3/CD28
100 nM
5 µM
10 µM
MgTX
CD3/CD28
5µM MgTX
CD4+
CFSE
B
untreated
MgTX
Cytokine production (in pg/ml)
1500
1000
500
0
n.s.
n.s.
n.s.
IL-17a
IFNγ
TNFα
C
R= 0.90
p= 0.002
IL-17(%)
Glutamate production (µM)
R= 0.70
p= 0.062
TNFα (%)
Glutamate production (µM)
D
Kv1.3
50µm
NeuN+
Kv1.3
50µm
Kv1.3
15µm
CD4+
Kv1.3
15µm
E
Glutamate (µM) 4h
ns
*
con
MgTX
10mM 4-AP

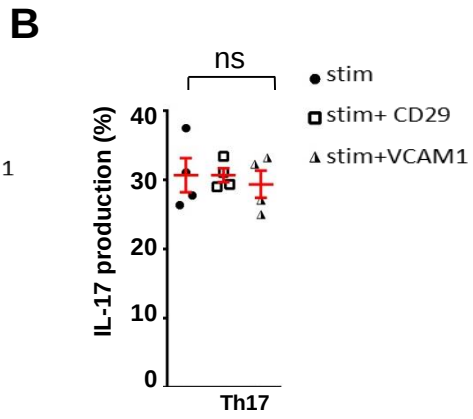
Supp 5

A


66.7%
GFP (% of total)
mock transfection
GFP-transfected primary T cells

Supp 6


A
ns
ns
ns
80
60
40
20
0
Proliferation Th17 cells in % (CFSE)
1st division
2nd division
3rd division
stim
stim+ CD29
stim+VCAM1


B
ns
40
30
20
10
0
IL-17 production (%)
Th17
stim
stim+ CD29
stim+VCAM1

Supp 7