

Supplementary Materials for  
**Tissue-resident memory CD8<sup>+</sup> T cells cooperate with CD4<sup>+</sup> T cells to drive  
compartmentalized immunopathology in the CNS**

Ilena Vincenti *et al.*

Corresponding author: Doron Merkler, [doron.merkler@unige.ch](mailto:doron.merkler@unige.ch)

*Sci. Transl. Med.* **14**, eabl6058 (2022)  
DOI: 10.1126/scitranslmed.abl6058

**The PDF file includes:**

Materials and Methods  
Figs. S1 to S11  
Tables S1, S3 to S5  
References (78–88)

**Other Supplementary Material for this manuscript includes the following:**

Table S2  
Data file S1  
Movies S1 and S2

## Supplementary Materials and Methods

### *Human samples*

To assess a potential role for tissue-resident-memory T cells in humans, we analyzed active/acute and chronic active white matter lesions of patients with MS, lesions from patients with a progressive MS disease course (n=3), lesions of patients with NMOSDs (n=5) and lesions of patients with ADEM (n=4). Autopsies and biopsies were fixed in 4% paraformaldehyde (PFA) and embedded in paraffin according to standardized protocols. Tissue blocks were screened for the presence of demyelination and macrophages. As described earlier (41), hypercellular demyelinated plaques massively infiltrated by myelin-laden macrophages were defined as acute active (n=4) whereas lesions with an inactive core and active edges were categorized as chronic active (n=4). In a separate cohort, we analyzed biopsies of acute active lesions (n=4) and later stage lesions without myelin-laden macrophages (n=3). Their use for scientific purposes was in accordance with institutional ethical guidelines and approved by the ethics committee of the University of Göttingen (Germany) and of the University of Geneva (Switzerland). Informed consent was obtained from all subjects.

A multi-step dye cycling method was performed to analyze the presence of T<sub>RM</sub> (CD8+BCL2+CD69+) and their expression of CD103 and GRANZYME B.

### *Mouse experiments*

We employed intracranial (i.c.) infection of adult GFAP:GP mice with rLCMV-GP33 as an experimental model of transient viral infection of the brain to generate GP33-specific tissue-resident memory CD8<sup>+</sup> T cells (T<sub>RM</sub>). Cross-reactivity of T<sub>RM</sub> with a CNS-restricted self-antigen was modeled by the TAM-inducible expression of LCMV glycoprotein (GP) antigens

in a CNS-restricted cell population (astrocytes). We determined the susceptibility to develop autoimmune disease by monitoring GFAP:GP mice and corresponding GP controls daily. Reactivation of self-reactive T<sub>RM</sub> was associated with development of impaired locomotor function, elimination of cognate-antigen-expressing cells and recruitment of inflammatory cells consisting principally of CD8<sup>+</sup> and CD4<sup>+</sup> T cells, B cells and macrophages. Of note, animals did not reach termination criteria defined by institutional authorities for animal experimentation.

We harnessed the fact that CD4<sup>+</sup> T cells were rapidly recruited within the inflamed CNS following self-reactive T<sub>RM</sub> activation to investigate a potential role of CD4<sup>+</sup> T cell help for T<sub>RM</sub> reactivation. We depleted circulating CD4<sup>+</sup> T cells by antibody-mediated ablation in GFAP:GP mice harboring LCMV-GP33-specific T<sub>RM</sub> and induced cognate-antigen expression in astrocytes. We performed gene expression analysis of helped and unhelped T<sub>RM</sub> from GFAP:GP mice to identify impaired gene signatures. Upregulation of the transcription factor TCF-1 is distinctive of unhelped reactivated T<sub>RM</sub>. The relevance of unhelped T<sub>RM</sub> affected differentiation was assessed by monitoring symptoms and histopathological features of autoimmune disease.

Experiments were reproduced at least once, except scRNA-seq and time lapse microscopy experiments.

### *Mice*

C57Bl/6J GFAP:GP (GFAP-CreERT2<sup>tg/wt</sup>:Stop-GP<sup>flox/wt</sup>) mouse line was generated by crossing mice expressing the TAM-inducible Cre-recombinase under the control of the GFAP promotor (C57Bl/6J GFAP-CreERT2 (29)) with C57Bl/6J Stop-GP mice (30). C57Bl/6J GFAP:GP and C57Bl/6J P14 TCR transgenic mice (78) were bred and lodged under specific-pathogen-free P2 conditions in the animal facilities of the University Medical Center of

Geneva. Male and female sex and age-matched mice between 6 and 8 weeks of age were used for experiments. All animal experiments were authorized by the cantonal veterinary office of Geneva and performed in agreement with the Swiss law for animal protection

#### *Virus infection*

Recombinant LCMV strains were generated and propagated on baby hamster kidney 21 cells BHK21 cells (ATCC CCL-10) (79). Virus stocks were titrated using MC57G cells (ATCC CRL-2295) according to established methods (80). The recombinant LCMV-ΔGP encodes for the glycoprotein (GP) of vesicular stomatitis virus (VSV) instead of its own glycoprotein. The recombinant LCMV-GP33 expresses the signal sequence of LCMV GP harboring the GP<sub>33-41</sub> epitope fused to the VSV-GP instead of the LCMV full-length-GP. For brain virus infection, 10<sup>3</sup> plaque-forming units (PFU) of rLCMV-ΔGP or rLCMV-GP33 were diluted in 30 µl of minimum essential medium (MEM, Gibco) before intracranial injection.

#### *Tamoxifen (TAM) administration*

TAM (Sigma, T5648) was dissolved in sunflower oil/10% ethanol to 10mg/ml. The solution was protected from light and mixed for 20min at 50°C to be dissolved. TAM was administrated intraperitoneally at a dose of 1 mg twice per day for 4 consecutive days.

#### *Purification of naive P14 cells and adoptive P14 cell transfer*

Naive P14 cells from spleen were purified using naive CD8a<sup>+</sup> T Cell Isolation Kit (Miltenyi Biotec, 130-104-075) and subsequently separated by AutoMACS (Miltenyi Biotec). Purity of cells (95%) was confirmed by flow cytometry. 10<sup>5</sup> P14 cells were adoptively transferred intravenously into GFAP:GP and GP mice 1 day prior to viral infection.

#### *Peptide tolerization*

Mice were administered 200 µg of GP33-41 (KAVYNFATM) or GP34-43 (AVYNFATCGI) peptides (GenScript) in incomplete Freund's adjuvant (IFA) i.p. on days 9, 6, and 3 prior to rLCMV-GP33 infection as described previously (31, 81). Control mice received diluent in IFA.

#### *Antibody-mediated depletion*

For depletion of circulating CD8<sup>+</sup> T cells or CD4<sup>+</sup> T cells, mice were treated intraperitoneally twice over a 3 days interval with 200 µg of rat-anti-mouse-CD8 $\alpha$ -depleting antibody (BioXCell, BE0117), 250 µg of rat-anti-mouse-CD4 –depleting antibody (home-made, clone YTS191) or isotype control (BioXCell, BE0090). Anti-CD8 was administered 7 and 4 days before TAM start whereas anti-CD4 was administered 4 and 1 day prior TAM start. For relapse experiments, mice were treated intraperitoneally with 200 µg of mouse-anti-mouse-CD8 $\alpha$  -depleting antibody (Ab00166-2.0, Absolute Antibody) or isotype control (Ab00166-2.3, Absolute Antibody) 7 and 4 days before each TAM administration. Depletion of T cells was confirmed by flow cytometry showing T cell frequencies <0.4% at administration start with TAM.

#### *Rotarod*

Mice were placed on a rotating rod (Rotarod 7650; Ugo Basile Biological Research) constantly accelerating from 10 to 80 rounds per minute for a maximum of 180 s. Endurance time was monitored, and the two best runs out of three at each time point were averaged for analysis. Animals were habituated and trained to the rotarod daily from day -3 to day 0. Displayed values (% latency to fall) are normalized to the running values of mice at day 0.

#### *Immunohistochemistry*

For staining of mouse tissue sections, the following antibodies were used: Alexa Fluor 555-rabbit-anti-GFAP (polyclonal, Dako, Z0334), rabbit-anti-GFP (D5.1, Cell Signaling Technology, 2956S), rat-anti-CD8 (4SM15, eBioscience, 14-0808-82), rabbit-anti-cleaved-caspase3 (polyclonal, Cell Signaling Technology, 9661), rabbit-anti-CD4 (D7D2Z, Cell Signaling Technology, 25229), rat-anti-B220 (RA3-6B2, eBioscience, 14-0452-82), rat-anti-CD107b/Mac3 (M3/84, BioLegend, 108502). For multiplex immunofluorescence stainings, the following antibodies were used: rabbit-anti-TCF-1 (C63D9, Cell Signaling Technology, 6709S), rat-anti-CD8 (4SM16, eBioscience, 14-0195-80), rat-anti-CD3 (CD3-12, Bio-Rad, MCA1477), rabbit-anti-CD4 (D7D2Z, Cell Signaling Technology, 25229), rabbit-anti-von Willebrand Factor (Abcam, ab6994).

Between staining steps, slides were washed with wash buffer (Dako). CNS tissues were collected and fixed in 4% paraformaldehyde (PFA) overnight at 4°C. Dehydrated tissues were embedded in paraffin. Deparaffinized tissue sections (2 µm) were incubated with Dako REAL peroxidase-blocking solution (Dako, K0672) to inactivate endogenous peroxidases and unspecific binding was blocked (PBS/10% FCS). Before staining with rat monoclonal antibodies, tissue sections were blocked with 1% Fab fragment-goat-anti-mouse IgG (Jackson ImmunoResearch, 115-007-003). Tissue sections were incubated with primary antibody in Dako REAL antibody diluent (Dako, S2022). For bright-field microscopic images, bound primary antibodies were visualized using peroxidase-coupled secondary antibody systems (Dako, Vector Laboratories) and polymerized 3,3'-diaminobenzidine (Dako). Nuclei were counterstained with hemalaun. For fluorescence microscopic images, bound primary antibodies were visualized with Alexa Fluor 647-labeled secondary antibodies or with peroxidase-labeled secondary antibodies, followed by Alexa Fluor 488 tyramide signal amplification (TSA, Thermo Fischer Scientific, B40953). Nuclei were stained with DAPI (Invitrogen, D1306).

For immunofluorescent staining of primary astrocyte culture, primary cells were seeded and cultured in a 6-well plate containing a 22mm coverslip that was coated with 0.1 mg/ml Poly-L-Lysin. When cells reached the desired density, culture media was removed, washed with PBS and cells were fixed with 4% PFA. After permeabilization (PBS/0.1% Triton) and unspecific blocking (1% Fab fragment-goat-anti-mouse IgG (Jackson ImmunoResearch, 115-007-003 and PBS/10% FCS)), cells were incubated with mouse-anti-Nogo A (11C7, (82) in Dako REAL antibody diluent (Dako, S2022). Primary bound antibodies were visualized with Alexa Fluor 488–labeled secondary antibodies. Cells were then incubated with Alexa Fluor 647-rabbit-anti-Iba-1 (polyclonal, Wako, 09-19741), Alexa Fluor 555-rabbit-anti-GFAP (polyclonal, Dako Z0334). Nuclei were stained with DAPI (Invitrogen, D1306). Coverslips were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition.

#### *Multiplex immunofluorescence of human tissues*

For staining of human fluorescent microscopy sections, a standardized dye cycling method that allows the staining of all five markers (CD8, CD69, BCL2, CD103, GRANZYME B) or (TCF-1, CD8, CD4, CD31) on the same tissue section was used (48, 83, 84). Coverslipped deparaffinized sections were bleached overnight at 4°C with neutral-white 3-up light-emitting diodes (LED Supply) to minimize autofluorescence. Coverslips were removed at 50°C in PBS. Between staining steps, slides were washed with wash buffer (Dako, S3006). Unspecific binding was blocked (PBS/10% FCS) and sections were incubated overnight at 4°C with rabbit-anti-CD69 (Sigma-Aldrich, HPA050525), msIgG2b-anti-CD8 (4B11, Thermo Fisher Scientific, MA1-80231) and msIgG1-anti-BCL2 (124, Dako, M0887). Bound primary antibodies were visualized with Alexa Fluor 488 goat-anti-rabbit (Jackson ImmunoResearch, 111-545-144), Alexa Fluor 555 goat-anti-msIgG1 (Life Technologies, A21127) and Alexa

Fluor 647 goat-anti-msIgG2b (Life Technologies, A21242). Nuclei were stained with DAPI (Invitrogen, D1306) and slides were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition. For elution of stainings, slides were decoverslipped, incubated for 1h at 50°C with Gendusa stripping buffer (2.5% SDS/1%  $\beta$ -mercaptoethanol in 80 mM tris-HCl (pH 6.8)) and washed in tap water, followed by 50% ethanol and PBS. Elution was verified under the microscope before slides were restained. For restaining, sections were incubated with Dako REAL peroxidase-blocking solution (Dako, K0672), unspecific binding was blocked (PBS/10% FCS) and sections were incubated overnight at 4°C with msIgG2a-anti-GRANZYME B (GrB-7, Monosan, 7029). After incubation with horseradish peroxidase (HRP)-labeled goat-anti-mouse secondary antibody (Dako EnVision), staining was visualized with Alexa Fluor 488 TSA (Thermo Fischer Scientific, B40953). Sections were blocked with PBS/2% NMS, followed by 5% Fab fragment-goat-anti-mouse IgG (Jackson ImmunoResearch, 115-007-003) to block eventual residual antibodies from previous staining steps. Slides were then stained with rabbit-anti-CD103 (EPR4166(2), Abcam, ab129202) and msIgG1-anti-CD8 (C8/144B, Dako, M7103), followed by Alexa Fluor 555 goat-anti-rabbit (Life Technologies, A31572) and Alexa Fluor 647 goat-anti-msIgG1 (Life Technologies, A21240). Nuclei were stained with DAPI (Invitrogen, D1306) and slides were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition.

For the multi-step staining of TCF-1, CD8, CD4 and, CD31 antigen retrieval was performed with Pascal Citrate buffer pH 6.0 and sections were incubated with Dako REAL peroxidase-blocking solution (Dako, K0672) and unspecific binding was blocked (PBS/10% goat serum). Tissue sections were incubated with rabbit-anti-TCF-1 (C63D9, Cell Signaling Technology, 6709S) overnight at 4°C in Dako REAL antibody diluent (Dako, S2022) supplemented with 0.5% Triton X-100.

After 30 minutes incubation with the goat-anti-rabbit HRP (Dako, K4003), bound TCF-1 were visualized using an amplification system, Opal 570 (Akoya, FP1488001KT). CD8 and CD4 detection was achieved by blocking the sections with 10% goat-serum (Vector Laboratories, S-1000) and then applying a mouse IgG2b-anti-CD8 (4B11, Thermo, MA 1-80231) and a mouse IgG1-anti-CD4 (4B12, Invitrogen, MA5-12259) antibody and for 2 hours at RT. Bound CD8 and CD4 were visualized using a goat-anti-mouse IgG1 Alexa Fluor 647 (Life, A21240) and a goat anti -mouse IgG2b Alexa Fluor 488 (Life, A21141) diluted in DAPI. Slides were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition. For the second-round of staining, coverslips were removed, and the sections eluted as describe previously. The tissue was blocked with 10% goat serum prior incubation with the primary antibodies. Section were incubated with a rabbit-anti-CD31 (Abcam, ab134168) for two hours at RT. Bound antibodies were visualized using a goat-anti-rabbit Alexa Fluor 488 (Jackson ImmunoResearch, 111-545-144) and a donkey-anti-rat Alexa Fluor 647 (Life, A21449) diluted in DAPI. Slides were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition. Sections were incubated with an autofluorescence quencher at the end of each round of staining (Autofluorescence Quenching kit, Vector Laboratories; SP-8400).

#### *Multiplex immunofluorescence of mouse tissue sections*

After deparaffination and antigen retrieval (Pascal Citrate pH 6.0), sections were incubated with Dako REAL peroxidase-blocking solution (Dako, K0672) to inactivate endogenous peroxidases and unspecific binding was blocked (PBS/10% FCS). Tissue sections were incubated with rabbit-anti-TCF-1 overnight at 4°C in Dako REAL antibody diluent (Dako, S2022) supplemented with 0.5% Triton X-100.

After 30 minutes incubation with the goat-anti-rabbit HRP, bound TCF-1 were visualized using Opal 570 (Akoya, FP1488001KT). For CD8+ T cell detection, sections were blocked

with 1% Fab fragment-goat-anti-mouse IgG (Jackson ImmunoResearch, 115-007-003) and then incubate with rat-anti-CD8 antibody for 2 hours at RT. Bound anti-CD8 antibody was visualized using a donkey-anti-rat Alexa Fluor 647 (Jackson ImmunoResearch, 712-605-153) diluted in DAPI and slides were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition.

For the second-round of staining, coverslips were removed, and the sections were incubated for 1h at 50°C with Gendusa stripping buffer (2.5% SDS/1%  $\beta$ -mercaptoethanol in 80 mM tris-HCl (pH 6.8)) and washed in tap water, followed by 50% ethanol and PBS. Elution was verified under the microscope. Before applying the new primary antibodies, sections were blocked with 1% Fab fragment-goat-anti-mouse IgG and 10% goat-serum (Vector Laboratories, S-1000). Sections were incubated with rat anti-CD3 and rabbit-anti-CD4 for 2 hours at RT. Bound antibodies were visualized using a goat-anti-rabbit Alexa Fluor 488 (Jackson ImmunoResearch, 111-545-144) and donkey-anti-rat Alexa Fluor 647 (Jackson ImmunoResearch, 712-605-153) diluted in DAPI and slides were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition. The section preparation for the third round was performed as described above. Tissue section was blocked with 10% goat serum prior incubation with the primary antibodies. Section were incubated with rabbit-anti-von Willebrand Factor (Abcam, ab6994) for two hours at RT. Bound antibodies were visualized using a goat-anti-rabbit Alexa Fluor 488 (Jackson ImmunoResearch, 111-545-144) diluted in DAPI and slides were mounted in Fluoromount aqueous mounting medium (Sigma-Aldrich, F4680) for image acquisition.

### *Image analysis*

Immunostained sections were scanned using Panoramic Digital Slide Scanner 250 FLASH II (3DHISTECH) in 200 $\times$  magnification. Most quantifications were performed manually using Panoramic Viewer software (3DHISTECH). Where possible, the examiner was blinded to

the experimental group. For the quantification of immune cell infiltration and astrogliosis, positive cells were automatically quantified using Definiens Developer software using a custom-made script. For representative images, white balance was adjusted, and contrast was enhanced using the tools “levels,” “curves,” “brightness,” and “contrast” in Photoshop CS6 (Adobe). All modifications were acquired uniformly on the entire image.

#### *Time-lapse imaging of brain-derived $T_{RM}$*

Primary astrocyte cultures were prepared from P1-P2 neonatal mice. Briefly, meninges were removed, cortex were triturated with trypsin and incubated for 10 min at 37°C. Tissues were washed and homogenized in DMEM-10 (DMEM with 10% FCS, 50U/ml penicillin/streptomycin). Cell suspensions were cultured in DMEM-10 into 25 cm<sup>2</sup> tissue culture flasks in a humidified 5% CO<sub>2</sub> incubator at 37°C. Medium was changed each 2-3 days to remove death cells. As soon as confluent, primary astrocyte cultures were trypsinized and cultured in Poly-L-lysine coated 8-well  $\mu$ -slides (IBIDI, 80821) at 50'000 cells / well in T cell medium (DMEM, 10% FCS, 2mM L-Glutamine, 100U/ml penicillin/streptomycin, 50  $\mu$ M beta-mercaptoethanol). When attached, astrocytes were pulsed with 1  $\mu$ M KAVYNFATM peptide for 1h at 37°C and washed twice.  $T_{RM}$  were sorted (CD11b-CD4-CD45.1+) from GFAP:GP mice 5 days after TAM administration, labeled with Hoescht-33342 dye (Abcam, ab228551) and 25'000  $T_{RM}$  cells were added on top of astrocytes culture after collection of the first image. Cell cultures were harvested in T cell medium containing 1  $\mu$ M ethidium-1-homodimer (EthD-1; Invitrogen, L3224). XYT sequential imaging was performed in a humidified 5% CO<sub>2</sub> chamber at 37°C at 1 frame per 3 min under a 200x magnification with a Axio Observer Z1 with Definite Focus 2 (Zeiss) with a 385nm LED for Hoescht-33342-labeled TRM, 511nm LED for EthD-1 dye and phase contrast. Background noise was subtracted and dying/living astrocytes were quantified with ImageJ 1.53m software (NIH) by

analyzing the signal intensity in the EthD-1 channel. For minimal distance to  $T_{RM}$  calculation, Imaris 9.8.0 (Bitplane) and a custom Matlab R2021a (The MathWorks) script were used to consider interactions of living or dying astrocytes with a  $T_{RM}$  lasting for at least 3 consecutive frames.

### *Flow cytometry*

The following fluorophore-conjugated antibodies were used: PE anti-BCL2 (BCL/10C4), PE/PECy7/BUV496 anti-CD4 (GK1.5), FITC/Pacific Blue/BV605/BUV805 anti-CD8a (53-6.7), PerCPCy5.5/BUV661 anti-CD11b (M1/70), PECy5.5 anti-CD11c (N418), APCCy7 anti-CD44 (IM7), BUV563 anti-CD45 (30-F11), APC/Pacific Blue/PE anti-CD45.1 (A20), PerCPCy5.5/BV570 anti-CD45R/B220 (RA3-6B2), PECy7 anti-CD49d (R1-2), BV605 anti-CD64 (X54-5/7.1), FITC anti-CD69 (H1.2F3), BUV737 anti-CD80 (16-10A1), AlexaFluor-647/PE anti-CD103 (2E7), FITC anti-CD107a (1D4B), AlexaFluor 700 anti-CD206 (C068C2), biotin anti-CD274 (MIH5), AlexaFluor 647 anti-Cx3cr1 (SA011F11), BV510 anti-F/480 (BM8), PE-eFluor610 anti-FOXP3 (FJK-16s), AlexaFluor 647 anti-GRANZYME B (GB11), BV421 anti-IFN- $\gamma$  (XMG1.2), PECy7 anti-Ki-67 (SolA15), BV480 anti-Ki-67 (B56), FITC anti-Ly6C (AL-21), BV650 anti-Ly6G (1A8), SuperBright 780 anti-MerTK (DS5MMER), BB700 anti-I-A/I-E (M5/114.15.2), PerCPCy5.5/BV711 anti-NK1.1 (PK136), BUV395 streptavidin (BD Biosciences), AlexaFluor 647 anti TCF-1 (C63D9), PECy7/PECy5 anti-TCRb (H57-597), BV421 anti-Siglecf (E50-2440), PECy7 anti-TNF $\alpha$  (MP6-XT22). For detection of virus-specific CD8<sup>+</sup> T cells, Db-GP33-41-tetramer (provided by the National Institutes of Health Tetramer Core Facility) and Kb-GP34-43-tetramer (Beckman Coulter) were used. Dead cells were excluded using Zombie Aqua or NIR Fixable Viability Kits (BioLegend).

Peripheral blood samples were obtained by facial vein puncture in heparin and incubated with directly-labeled antibodies and tetramers in FACS buffer (PBS/2.5%FCS/10mM EDTA/0.01% NaN<sub>3</sub>). Erythrocytes were lysed and cells fixed using BD FACS Lysing Solution (BD Biosciences, 420301). For the preparation of CNS-infiltrating leukocytes, mice were anesthetized and transcardially perfused with PBS. CNS tissues were minced, digested in DMEM with Collagenase A (1 mg/ml, Roche) and DNase I (0.1 mg/ml, Roche) at 37°C for 1h and homogenized using 70-µm cell strainers (BD Biosciences). Leukocytes were separated using a discontinuous Percoll gradient (30/70%). Surface staining was carried out with directly labeled antibodies and tetramers in FACS buffer. Cell numbers were quantified using AccuCheck Counting Beads (Invitrogen, PCB100). For intracellular staining of GRANZYME B and cytokines, cells were fixed and permeabilized using commercial permeabilization buffer set (BioLegend, 421002). Intracellular staining of Ki-67, FoxP3 and TCF-1 was performed using FoxP3/Transcription Factor staining buffer set (eBioscience, 00-5523-00) according to manufacturer's instructions. To assess degranulation and intracellular cytokine production, isolated leukocytes were cultured for 5h in the presence of 5 µg/ml FITC-anti-CD107a antibody, monensin (BioLegend) and Brefeldin (BioLegend). Cells were stimulated with 1 µM KAVYNFATM peptide. For high-dimensional FACS analysis, samples were stained with antibodies listed in Table S1, data were acquired on a FACSymphony (BD Biosciences) and analyzed as previously described (85).

Flow cytometric samples were acquired on a BD LSRFortessa (BD Biosciences) using BD FACSDiva (BD Biosciences, v8.0.2) and Attune NxT Acoustic Focusing Cytometer (Thermo Fisher, V3.1.2) using appropriate filter sets and compensation controls. Gates were assigned according to appropriate control populations, such as fluorescence minus one stainings. In experiments which required high purity, P14 cells were sorted using Aria II flow cytometer

within a laminar flow hood (BD Biosciences). Data were analyzed using FlowJo software (Treestar, V10).

#### *RNA-seq and scRNA-seq samples preparation*

To perform RNA-seq, P14 bT<sub>RM</sub> cells (CD45.1<sup>+</sup> CD44<sup>hi</sup> CD69<sup>+</sup>) were FACS-sorted from GP,  $\alpha$ CD8 $\alpha$  GFAP:GP and  $\alpha$ CD4 $\alpha$ CD8 $\alpha$  GFAP:GP mice 7 days after start of TAM. Each biological replicate ( $n = 3$ ) represents 10'000 cells sorted in RLT buffer with 1%  $\beta$ -mercaptoethanol and processed with RNAeasy Plus Micro Kit (Qiagen) following manufacturer's instructions.

RNA integrity was assessed with a Bioanalyzer (Agilent Technologies) and total RNA was quantified with a Qubit fluorimeter (Life Technologies). All samples exhibited a RIN over 8. Reverse transcription and cDNA amplification were performed using the SMART-Seq v4 kit (Clontech) according to manufacturer's specifications, starting with 0.5 ng of total RNA as input. Library preparation were generated using 200 pg of cDNA with Nextera XT kit (Illumina). Libraries molarity and quality were assessed with Qubit and Tapestation using a DNA High sensitivity chip (Agilent Technologies). Libraries were pooled and sequenced on the Illumina HiSeq 4000 sequencer (TruSeq SBS, SE; 1 $\times$ 50 bp; 40 Mio reads/sample). Basecalls generated by Illumina's Real Time Analysis (RTA) software were demultiplexed to fastq files with bcl2fastq. The quality control was done using FastQC (Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>).

For scRNA-seq of CD4<sup>+</sup> T cells, brain-infiltrating CD4<sup>+</sup> cells (CD11b<sup>-</sup> NK1.1<sup>-</sup> B220<sup>-</sup> TCRb<sup>+</sup> CD8a<sup>-</sup> CD4<sup>+</sup>) were FACS-sorted from GP and  $\alpha$ CD8 $\alpha$  GFAP:GP mice 7 days after

start of TAM. Cells were adjusted to a concentration of  $3 \times 10^5$  cells/ml in PBS, 0.04% BSA before proceeding with droplet generation.

Single cell 10X libraries were constructed from isolated single cells following the Chromium Single Cell 5'V(D)J Reagent Kits User Guide (CG000166). Briefly, single cells were co-encapsulated with gel beads (10X Genomics) in droplets using two lanes of one Chromium Chip A (10X Genomics, 230027). VDJ-enriched and gene expression libraries construction were carried out using the Chromium Single Cell 5' Library Construction Kit (v3, 10X Genomics). Reverse transcription, preparation of single-cell transcriptome and libraries construction were performed following manufacturer's instructions. Final libraries were pooled and sequenced on Illumina NextSeq 500 platform (PE, 100 cycles) with a concentration of 1.8 pM with 5% PhiX. Resulting FASTQ files were demultiplexed and subsequently used as input to cellranger (10x Genomics).

For scRNA-seq of T<sub>RM</sub>, brain-infiltrating P14 cells (CD11b- NK1.1- B220- CD4- TCRb+ CD8a+ CD45.1+) were FACS-sorted from GP,  $\alpha$ CD8 $\alpha$  GFAP:GP and  $\alpha$ CD4+ $\alpha$ CD8 $\alpha$  GFAP:GP mice 7 days after start of TAM. Each biological replicate ( $n = 2$ ) was adjusted to a concentration of  $3 \times 10^5$  cells/ml in PBS, 0.04% BSA before proceeding with droplet generation. Single cell 10X libraries were constructed from isolated single cells following the Chromium Single Cell 3'Reagent Kits User Guide (CG000183 Rev B). Briefly, single cells were co-encapsulated with gel beads (10X Genomics) in droplets using 6 lanes of one Chromium Chip G (10X Genomics). Library construction was carried out using the Chromium Single Cell 3' Library Kit (v3, 10X Genomics). Reverse transcription, preparation of single-cell transcriptome and libraries construction were performed following manufacturer's instructions. Final libraries were pooled and sequenced on Illumina HiSeq 4000 sequencer (PE, 100 cycles) with a concentration of 2 pM with 10% PhiX. Resulting FASTQ files were demultiplexed and subsequently used as input to cellranger (10x Genomics).

### *RNA-seq data processing*

FastQ reads were mapped to the ENSEMBL reference genome (GRCm38.96) using STAR version 2.4.0j (86) with standard settings except that any reads mapping to more than one location in the genome (ambiguous reads) were discarded ( $m = 1$ ). A unique gene model was used to quantify the number of reads per gene. Briefly, the model considers all annotated exons of all annotated protein-coding isoforms of a gene to create a unique gene where the genomic regions of all exons are considered coming from the same RNA molecule and merged together.

All reads overlapping the exons of each unique gene model were reported using featureCounts version 1.4.6-p1 (87). Gene expressions were reported as raw counts and in parallel normalized in RPKM in order to filter out genes with low expression value (1 RPKM) before calling for differentially expressed genes. Library size normalizations and differential gene expression calculations were performed using the package edgeR (v3.28.0, differential expression analysis of digital gene expression data) (37) designed for the R software (v3.6.2). Candidate genes were filtered to a minimum of 1.5-fold change (aCD8a GFAP:GP vs aCD8+aCD4 GFAP:GP) and FDR-corrected  $p$ -value  $< 0.05$ .

Circulating and T<sub>RM</sub> core signatures originated from (36) studying the transcriptional regulation of T<sub>RM</sub> cell formation in tumors and non-lymphoid tissues.

### *scRNA-seq data processing*

For CD4 gene expression analysis, fastq reads were aligned to the reference transcriptome (mm10-3.0.0) using the count function of the 10x genomics software cellranger (v3.0.1). Directories containing the output filtered featured matrices were supplied to the functions Read10x and CreateSeuratObject from the R package Seurat (v3.1.1) (70). Cells containing more than 20% mitochondrial counts were removed from the analysis. Data was log

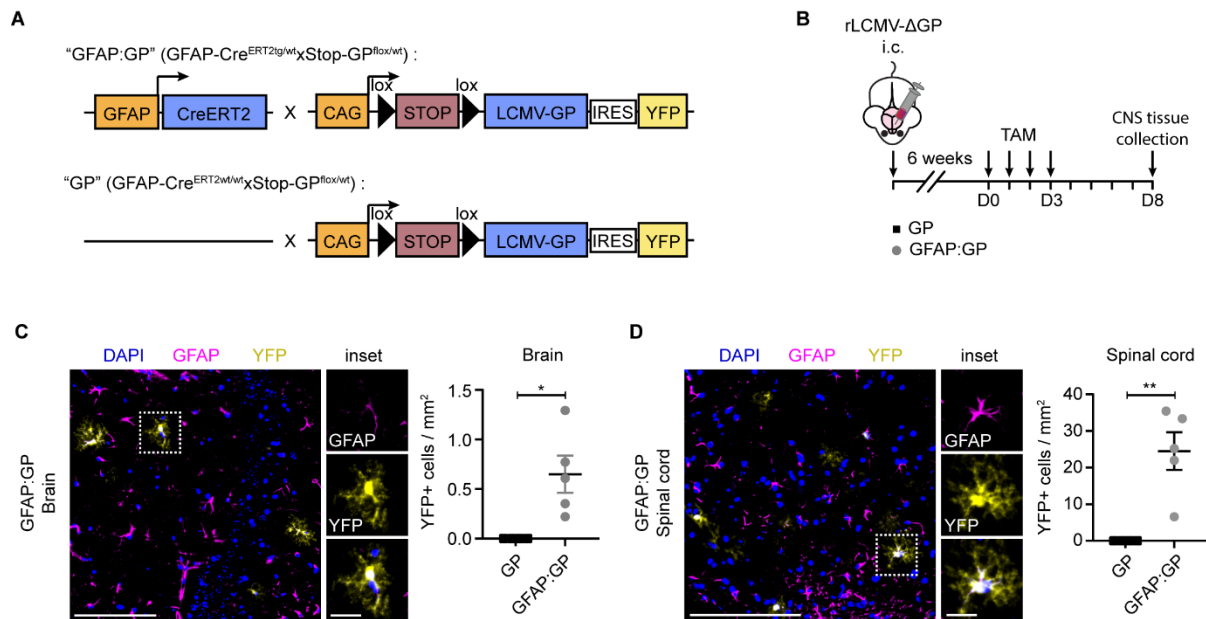
normalized by a scaling factor of 10000 and subsequently scaled using the ScaleData function under default parameters. 2000 variable features were selected using the "vst" selection method and used as input to principal component analysis (PCA). The first ten dimensions supplied to Seurat's FindNeighbors function. Unsupervised clustering was performed using a cluster resolution of 0.5 as suggested by the developers (70). Uniform manifold approximation projection (UMAP) was calculated using the first ten dimensions. Differential gene expression analysis between clusters was performed using the Wilcoxon Rank Sum test in the FindMarkers function with the logfc threshold set to 0.25. Cluster defining genes were restricted to the output of the FindMarkers function and were subsequently ranked according to average log fold-change. Heatmaps, feature plots, and violin plots were produced by supplying genes of interest to the functions DoHeatmap, FeaturePlot, and VlnPlot functions in Seurat, respectively. For repertoire analysis, fastq files were aligned to the murine reference (refdata-cellranger-vdj-GRCm38-alt-ensembl-3.1.0) using the vdj function in Cellranger (v5.0.1). Those cells containing exactly one TCR beta and one TCR alpha chain (806 cells) were merged into clones based on identical amino acid CDR3 sequence appended from both chains. Cells containing matching barcodes in both VDJ and GEX libraries were displayed on the UMAP using the FeaturePlot function in Seurat with the identity set to clonal frequency as calculated from the VDJ libraries.

For  $T_{RM}$  analysis, FastQ reads were aligned to the reference transcriptome (mm10-3.0.0) using 10x genomics software cellranger (v3.0.1) by specifying "Single Cell 3' v3 as input". Directories containing the output filtered featured matrices were supplied to the functions Read10x and CreateSeuratObject from the R package Seurat (v3.1.1) (88). Cells containing more than 20% mitochondrial counts were removed from the analysis. Data was log normalized by a scaling factor of 10000 and subsequently scaled using the ScaleData function under default parameters. 2000 variable features were selected using the "vst" selection

method and used as input to principal component analysis (PCA). The first ten dimensions supplied to Seurat's FindNeighbors function. Unsupervised clustering was performed using a cluster resolution of 0.5 as suggested by the developers (88). Uniform manifold approximation projection (UMAP) was calculated using the first ten dimensions. Differential gene expression analysis between groups and between clusters was performed using the Wilcoxon Rank Sum test in the FindMarkers function with the logfc.threshold set to 0.25. Cluster defining genes were restricted to the output of the FindMarkers function and were subsequently ranked according to average log fold-change. Heatmaps, feature plots, and violin plots were produced by supplying genes of interest to the functions DoHeatmap, FeaturePlot, and VlnPlot functions in Seurat, respectively.

## Supplementary Figures

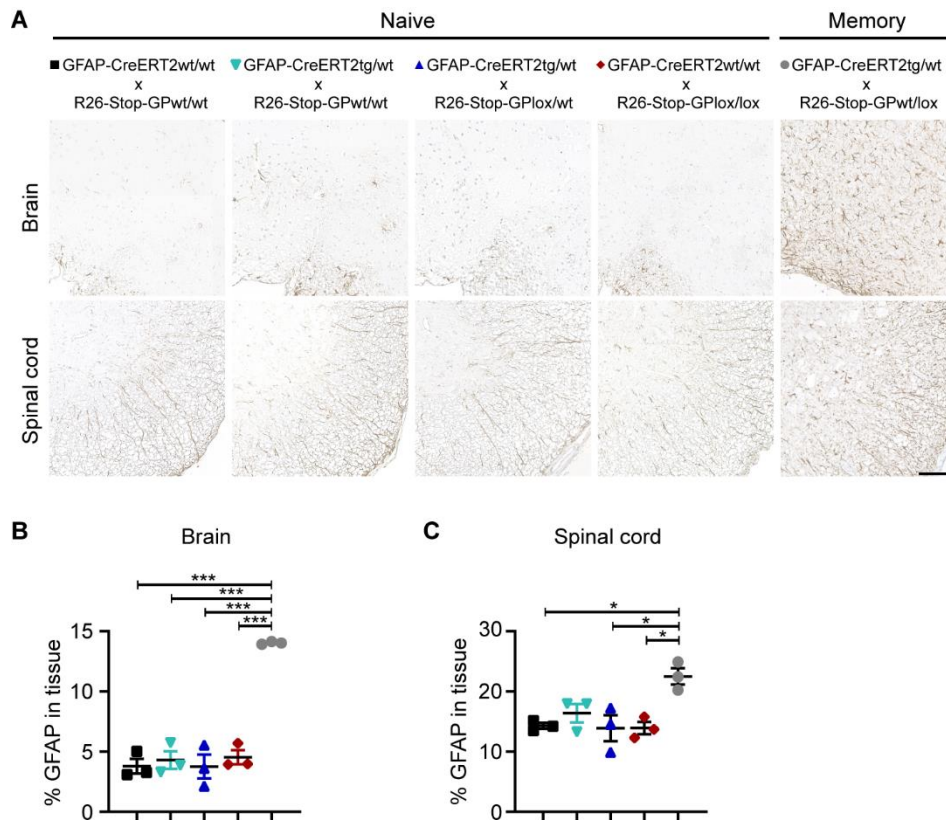
Fig S1.



**Figure S1: Tamoxifen-inducible Cre-recombination drives conditional YFP reporter expression in GFAP:GP mice.**

(A) Schematic representation of the mouse model. GFAP:GP (GFAP-Cre<sup>ERT2</sup> tg/wt;Stop-GP<sup>fllox/wt</sup>) mouse line express tamoxifen-inducible Cre-recombinase (Cre<sup>ERT2</sup>) under the control of the astrocyte-specific promotor (GFAP). Binding of tamoxifen (TAM)-metabolites to ERT2 mediates the translocation of the Cre recombinase to the nucleus and thus the conditional expression of LCMV glycoprotein (GP) and reporter gene YFP in astrocytes. (B) Experimental setup: GP or GFAP:GP adult mice were infected intra-cerebrally (i.c.) with  $1 \times 10^4$  PFU of attenuated recombinant LCMV lacking GP (rLCMV-ΔGP). At least six weeks later, memory mice were treated with TAM intraperitoneally (i.p.) for four consecutive days to induce GP expression in astrocytes of GFAP:GP mice. (C-D) (Left) Representative immunostainings for YFP reporter and astrocytes (GFAP) in (C) brain or (D) spinal cord sections of indicated groups five days after TAM administration termination. Nuclei are visualized by 4',6-diamidino-2-phenylindole (DAPI). Scale 150 μm; inset 20 μm. (Right) Quantification of YFP+ cells/mm<sup>2</sup> of tissue in indicated groups. Symbols represent one individual mouse and bars represent mean ± SEM (C-D: n= 4-5). \*, P < 0.05; \*\*, P < 0.01; unpaired t-test (C-D); (C-D) One representative out of at least two independent experiments is shown.

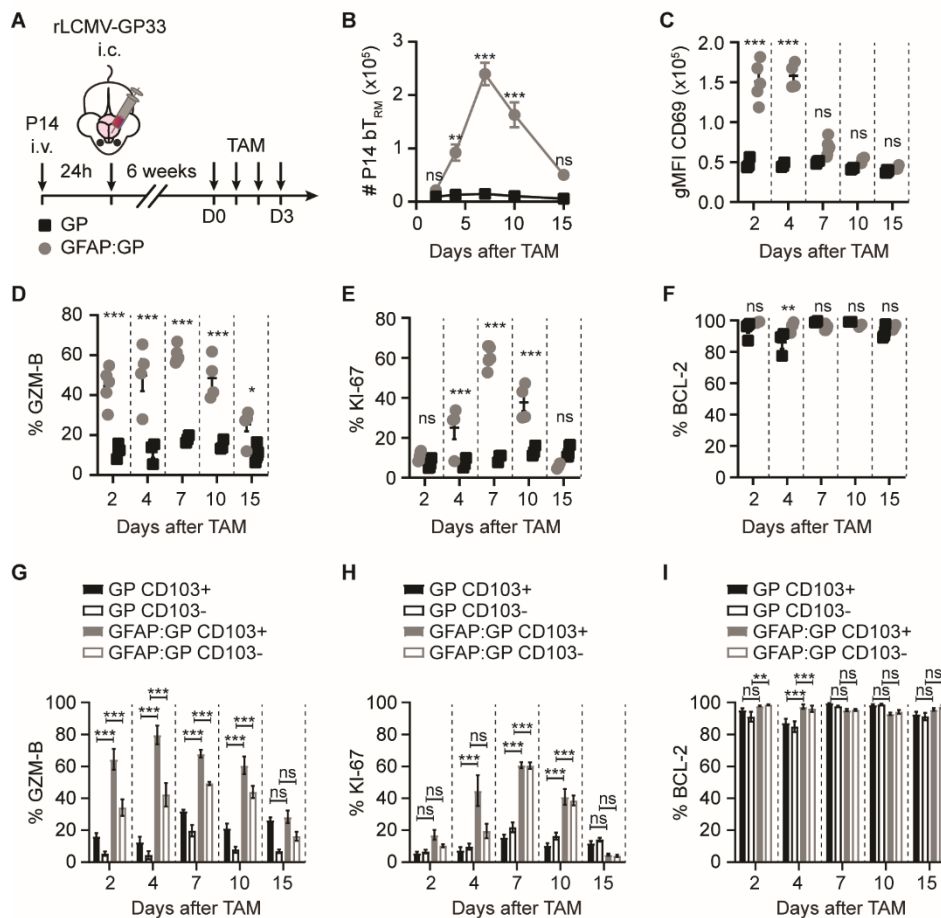
Fig. S2



**Figure S2. TAM administration per se does not alter astrocyte status.**

Naïve mice of indicated genotypes or GFAP:GP memory mice were treated with TAM i.p. for four consecutive days. Tissues were collected five days after tamoxifen administration termination. **(A)** Representative immunostainings for GFAP in brain and spinal cord sections of indicated groups to visualize astrogliosis. **(B-C)** Quantification of GFAP signal coverage among total tissue in **(B)** brain or **(C)** spinal cord sections of indicated groups. Scale 100  $\mu$ m. Symbols represent one individual mouse and bars represent mean  $\pm$  SEM (B-C: n= 3). \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ ; one-way ANOVA followed by Tukey's multiple comparisons test (B-C). Experiment has been performed once.

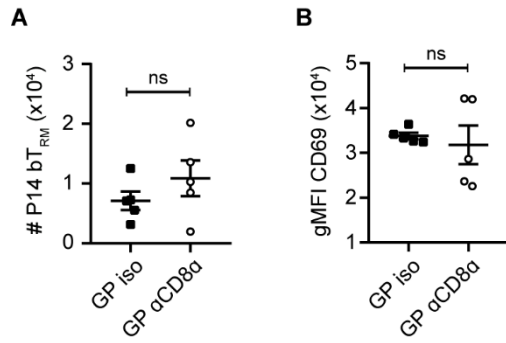
Fig. S3



**Figure S3. Kinetics of reactivation and expansion of P14+ T<sub>RM</sub> subsets following tamoxifen administration in GFAP-GP mice.**

(A) Experimental setup: CD45.1+ TCR transgenic P14 cells were adoptively transferred into adult CD45.2+ GP or GFAP:GP mice 24h before i.c. infection with rLCMV-GP33. At least six weeks later, mice were treated with TAM i.p. for four consecutive days to induce GP expression in astrocytes of GFAP:GP mice. (B) Flow cytometric enumeration of brain infiltrating P14 cells in GFAP:GP and GP mice at indicated days after the first TAM injection. (C) CD69 gMFI and frequencies of (D) GRANZYME B (GzmB)-, (E) Ki-67- and (F) BCL2-expressing P14 cells at indicated days after the first tamoxifen injection. Frequencies of (G) GzmB- (H) Ki-67- and (I) BCL2-expressing P14 CD103+ and CD103- T<sub>RM</sub> subsets at indicated days after the first TAM administration. Symbols represent one individual mouse and bars represent mean  $\pm$  SEM, except for (B), where data represent mean  $\pm$  SEM (B-I: n= 3-7). \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001, ns: not significant; two-way ANOVA followed by Sidak's multiple comparisons test (B-I); (B-I) One representative out of at least two independent experiments is shown.

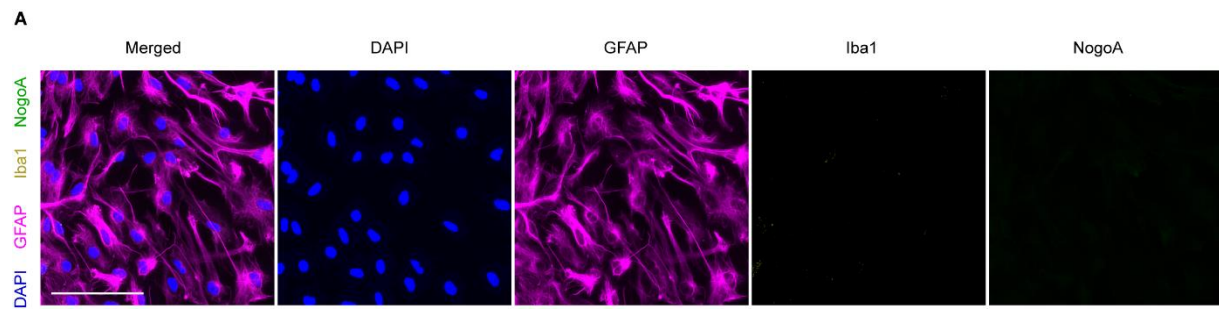
Fig. S4



**Figure S4. T<sub>RM</sub> number and activation in the brain are unaltered by administration of anti-CD8α depleting antibody in the circulation.**

CD45.1+ P14 cells were adoptively transferred into adult CD45.2+ GP mice 24h before i.c. infection with rLCMV-GP33. At least six weeks later, mice received anti-CD8α-depleting antibody (αCD8α) or isotype control (iso) and were treated with TAM i.p. for four consecutive days. **(A)** Quantification of brain infiltrating P14 cells in GP mice 4 days after the first TAM injection. **(B)** CD69 gMFI of P14 cells in GP mice four days after the first TAM injection. Symbols represent one individual mouse and bars represent mean ± SEM (A-B: n= 5). ns: not significant; unpaired t-test (A-B); (A-B) One representative out of at least two independent experiments is shown.

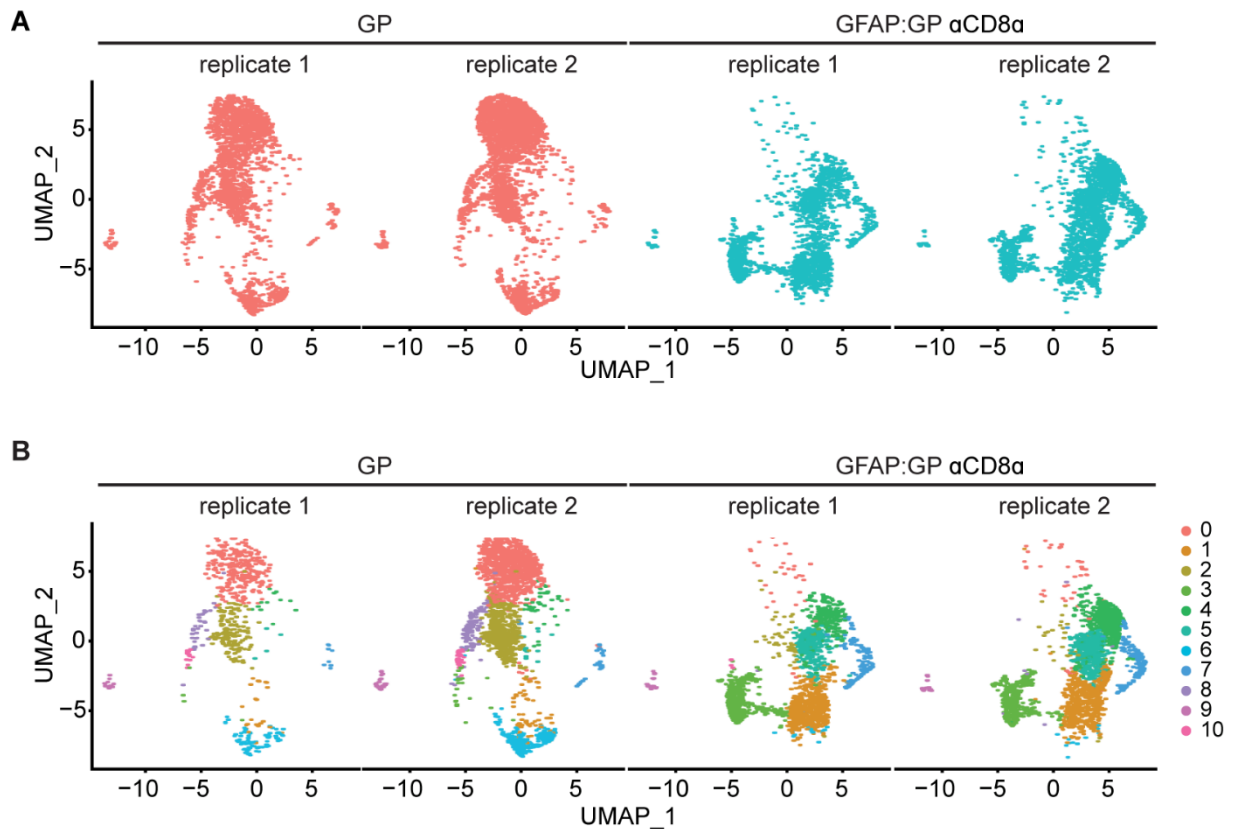
Fig. S5



**Figure S5. Purity of primary astrocyte culture.**

Primary astrocytes cultures were prepared from P1-P2 neonatal mice and cultured for at least 15 days before time-lapse microscopy performed in Figure 2K-M. (A) Representative immunostainings for DAPI (nuclei), GFAP (astrocytes), Iba1 (microglia/macrophages) and NogoA (oligodendrocytes). Scale 100  $\mu$ m.

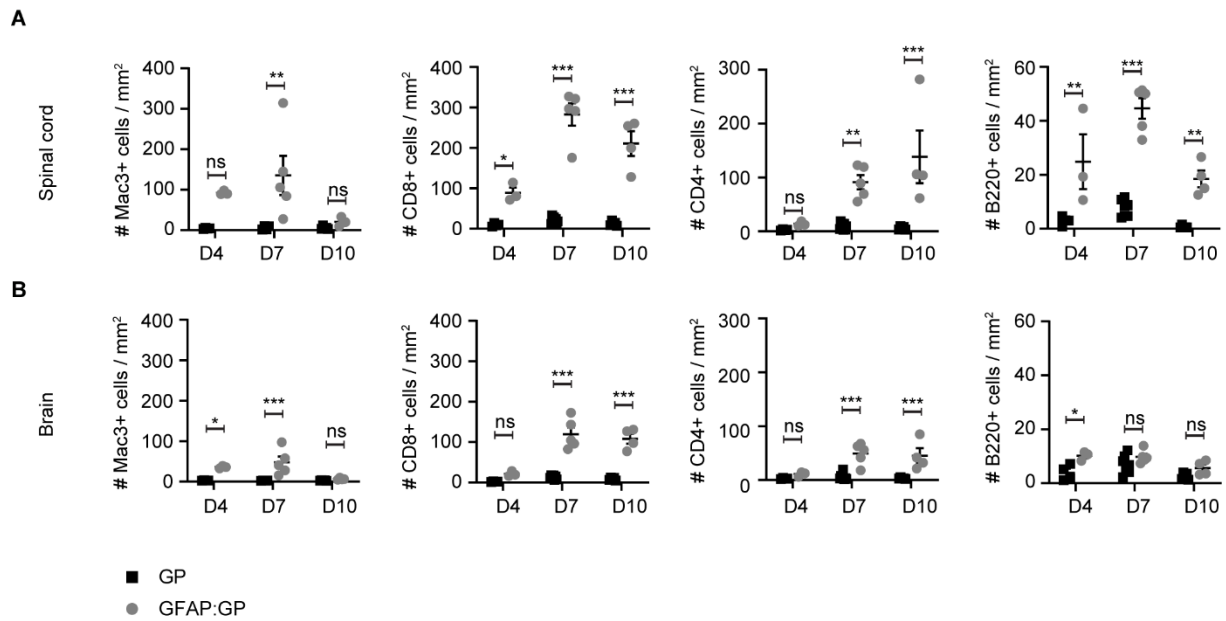
Fig. S6



**Figure S6. scRNAseq replicates.**

CD45.1+ P14 cells were adoptively transferred into adult CD45.2+ GP or GFAP:GP mice 24h before i.c. infection with rLCMV-GP33. At least six weeks later, mice were treated with anti-CD8a depleting antibody or isotype control. One week after depletion, mice were treated with TAM i.p. for four consecutive days to induce GP expression in astrocytes of GFAP:GP mice. P14 cells were isolated from brain of indicated groups 7 days following TAM administration and FACS-sorted for single-cell-RNA sequencing (scRNAseq) analysis. **(A)** Individual replicates of cells in Figure 3F. **(B)** Individual replicates of clustering shown in Figure 3G. Symbols represent one individual cell (A-B: n=2). (A-B) Experiment has been performed once.

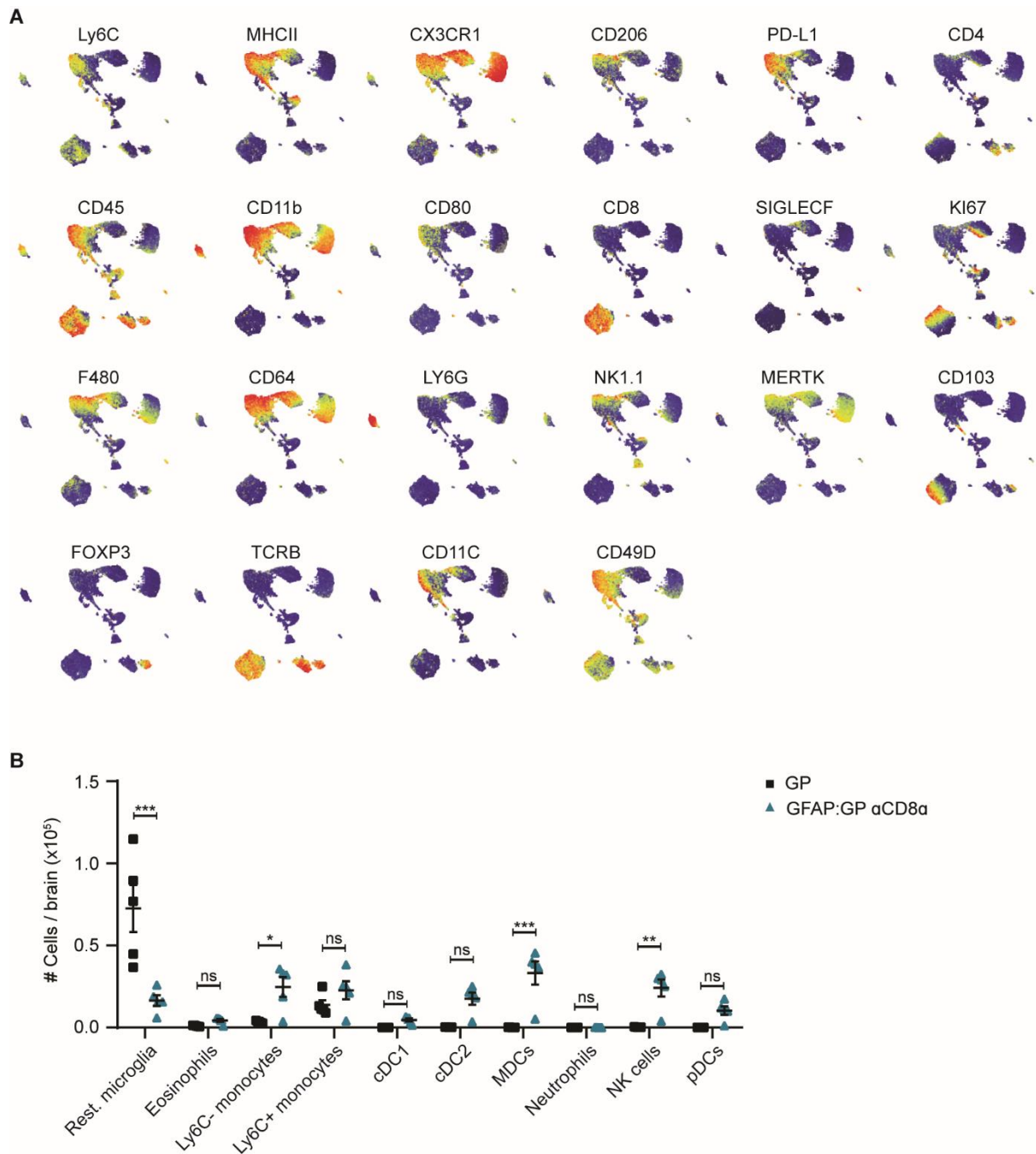
Fig. S7



**Figure S7. Recruitment and activation of immune cell subsets following TAM administration in GP and GFAP:GP memory mice.**

GP and GFAP:GP mice were infected i.c. with rLCMV-GP33. At least six weeks later, mice were treated with TAM i.p. for four consecutive days to induce GP expression in astrocytes of GFAP:GP mice. Tissues were collected at indicated days after TAM administration. Numbers of Mac3+, CD8+, CD4+ and B220+ positive cells/mm<sup>2</sup> of tissue in **(A)** spinal cord or **(B)** brain are shown. Symbols represent one individual mouse and bars represent mean  $\pm$  SEM (A-B: n=3-8). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ , ns: not significant; two-way ANOVA followed by Sidak's multiple comparisons test (A-B). (A-B) Experiment has been performed once.

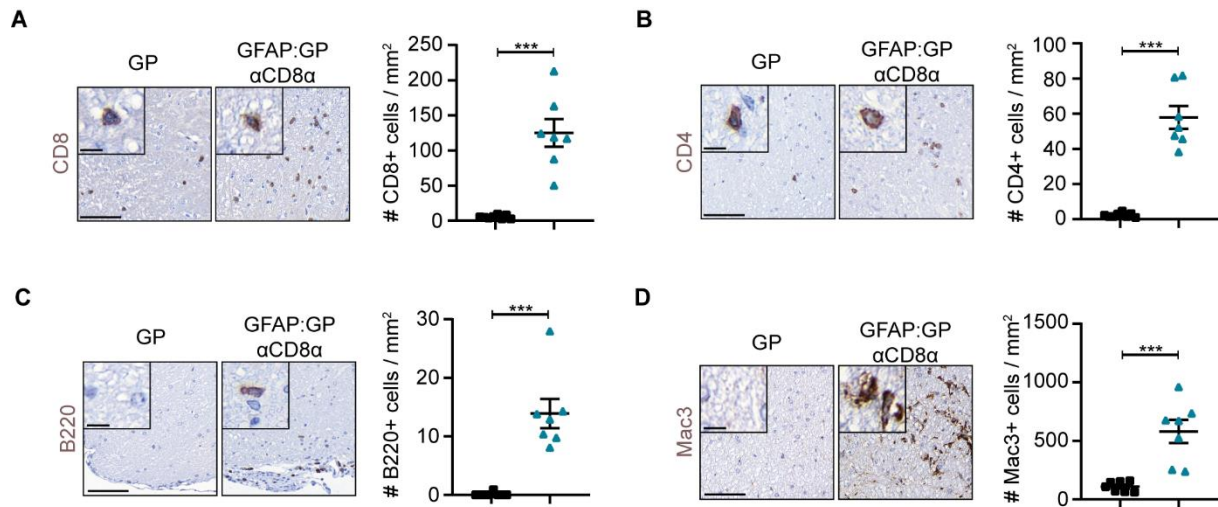
Fig. S8



**Figure S8. Fluorescence cytometry marker expression across brain leukocytes.**

CD45.1+ P14 cells were adoptively transferred into adult CD45.2+ GP or GFAP:GP mice 24h before i.c. infection with rLCMV-GP33. At least six weeks later, mice received anti-CD8 $\alpha$ -depleting antibody ( $\alpha$ CD8 $\alpha$ ) or isotype control (iso) and were treated with tamoxifen (i.p.) for four consecutive days. **(A)** Fluorescence cytometry panel markers overlaid onto the merged UMAP from Figure 4A. **(B)** Cell numbers of less abundant brain infiltrating leukocytes in indicated groups. Symbols represent one individual mouse and bars represent mean  $\pm$  SEM (n=5). \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001, ns: not significant; two-way ANOVA followed by Sidak's multiple comparisons test (B). (A-B) Experiment has been performed once.

Fig. S9



**Figure S9. Activated T<sub>RM</sub> orchestrate local inflammation upon encounter of cognate-self-antigen in the CNS.**

CD45.1+ P14 cells were adoptively transferred into adult CD45.2+ GP or GFAP:GP mice 24h before i.c. infection with rLCMV-GP33. At least six weeks later, mice were treated with anti-CD8a depleting antibody or isotype control. One week after depletion, mice were treated with TAM i.p. for four consecutive days to induce GP expression in astrocytes of GFAP:GP mice. Tissues were collected 7 days after TAM administration. **(A-D)** (Left) Representative immunostainings of (A) CD8+, (B) CD4+, (C) B220+ and (D) Mac3+ cells in spinal cord sections of indicated groups. Scale: 80  $\mu$ m; inset: 10  $\mu$ m. (Right) Numbers of positive cells/mm<sup>2</sup> of tissue. Symbols represent one individual mouse and bars represent mean  $\pm$  SEM (A-C: n=7-8; D: n=7). \*\*\*, P < 0.001; unpaired t-test (A-D); (A-D) One representative out of at least two independent experiments is shown.

**A**

Blood

# CD8 /  $\mu\text{L}$

D0 D7

\*\*\* NS \*\*\* NS

Days after TAM

**B**

Blood

# CD4 /  $\mu\text{L}$

D0 D7

\*\*\* NS \*\*\* NS

Days after TAM

**C**

Day 7 after TAM  
Brain

# CD4 ( $\times 10^5$ )

ns

GP GFAP:GP  $\alpha\text{CD}8\alpha$  GFAP:GP  $\alpha\text{CD}8\alpha + \alpha\text{CD}4$

**D**

UMAP\_2

UMAP\_1

GP GFAP:GP  $\alpha\text{CD}8\alpha$

**E**

UMAP\_2

UMAP\_1

0: Resting  
1: Resting Col5<sup>hi</sup>  
2: Th17-like  
3: CD52<sup>hi</sup>  
4: Treg  
5: Th1-like  
6: Treg Il10<sup>hi</sup> Gzmb<sup>hi</sup>  
7: Proliferating  
8: Naive/CM  
9: Naive/CM  
10: M

**F**

% cells

Cluster

GP GFAP:GP  $\alpha\text{CD}8\alpha$

**G**

0 1 2 3 4 5 6 7 8 9 10

NR4A3  
MPOY  
IFITB  
ABO21B  
CDK11B  
COL4A  
UBASH3  
CD44  
FAU  
RC3H1  
ECC1  
ATP10A  
ZEB2  
TNFSF8  
RPLN2  
CD82  
S100A4  
HSP70A  
WDR7  
KLF2  
S100B1  
ITGB8  
PKNOX1  
ITGAV  
BAZ2  
HSBP1  
HSPA1A  
OMAZ1  
JUN  
CAPO1  
G2M5  
C20orf42  
GATA  
STMN1  
TUSK1  
ANKRD  
PCOLCE  
TUBA1B  
IGFBP4  
EF1  
KLFP  
SELL  
GPM6  
GIM5  
FCMB1  
STRA3  
G274  
CTD8  
CTD8  
CTD8  
CTD8  
CTD8

**H**

*Col5* *Ccr7* *Sell* *Rora* *Cd52*

*Foxp3* *Ilt2ra* *Il1f0* *Gzmb* *Tbx21*

*Cd69* *Mki67* *Gzma* *Gzmk* *Prf1*

Expression

**I**

GzmB - Day7

□ CD8 GFAP:GP  $\alpha\text{CD}8\alpha$   
■ CD4 GP  
● CD4 GFAP:GP  $\alpha\text{CD}8\alpha$

Events

GzmB

% GzmB+

**J**

clone = 1  
clone > 1

Cell fraction

GP GFAP:GP

**K**

clone = 1  
clone > 1

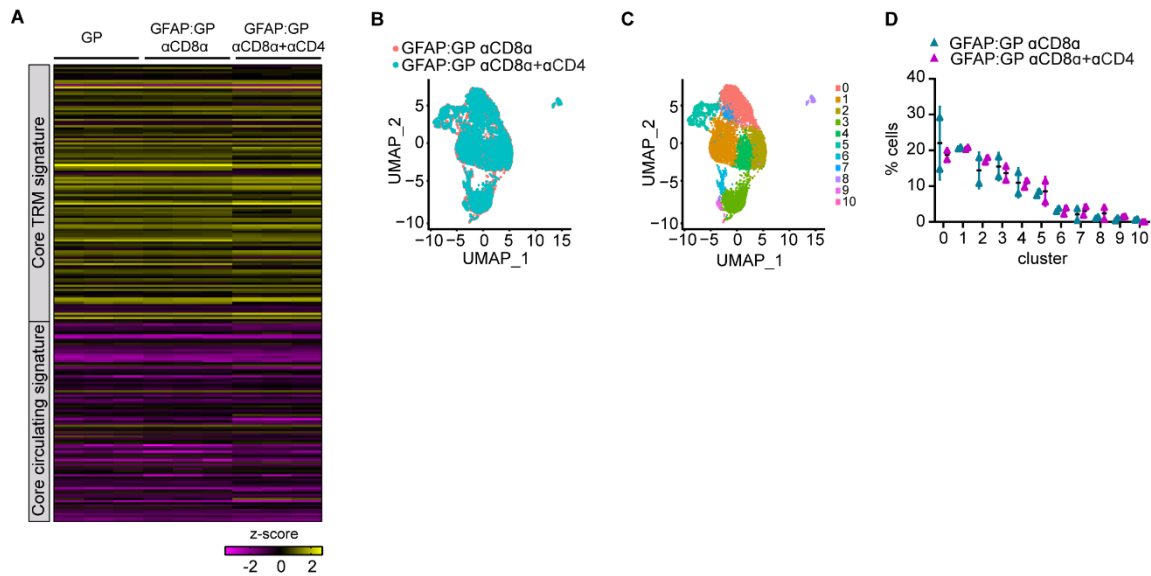
UMAP\_2

UMAP\_1

CD45.1+ P14 cells were adoptively transferred into adult CD45.2+ GP or GFAP:GP mice 24h before i.c. infection with rLCMV-GP33. At least six weeks later, mice were treated with anti-CD8 $\alpha$  depleting antibody ( $\alpha$ CD8 $\alpha$ ), anti-CD8 $\alpha$  and anti-CD4 depleting antibody

( $\alpha$ CD8 $\alpha$ + $\alpha$ CD4) or isotype control. At day 0, mice were treated with TAM i.p. for four consecutive days to induce GP expression in astrocytes of GFAP:GP mice. **(A-B)** Quantification of (A) CD8<sup>+</sup> and (B) CD4<sup>+</sup> T cells in the blood at day 0 (D0) and day 7 (D7) following TAM administration. **(C)** Quantification of CD4<sup>+</sup> T cells in the brain 7 days following TAM administration in indicated groups. **(D)** UMAP visualization of 1681 live and singlet-gated B220<sup>−</sup>CD11b<sup>−</sup>NK1.1<sup>−</sup>CD8<sup>−</sup>TCRb<sup>+</sup>CD4<sup>+</sup> single-cell transcriptomes. Each symbol represents a cell and color corresponds to group identity. **(E)** Seurat clustering of cells in D colored by transcriptional cluster. **(F)** Proportion of cells from indicated groups in clusters identified by unsupervised clustering in E. **(G)** Heatmap of scRNA-seq data for the averaged expression of the top 5 differentially expressed genes ranked according to average log fold-change within each cluster. **(H)** Expression of selected genes in the UMAP visualization shown in D. **(I)** (Left) Representative histograms of GRANZYME B (GzmB) expression and (right) frequencies in brain-infiltrating CD4<sup>+</sup> or CD8<sup>+</sup> T cells 7 days after TAM administration in indicated groups by flow cytometry. **(J)** Fraction of cells having one clone (red) or more than one clone (blue) from paired single-cell TCR sequencing. **(K)** UMAP visualization of clones shown in J colored by the number of cells per clone. Symbols represent one individual mouse and bars represent mean  $\pm$  SEM, except for (D-E, H), where data represents one individual cell and (F) where data represents one individual sample (A-C: n=3-5; D-H: n=1; I: n=3-4). \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001, ns: not significant; two-way ANOVA followed by Sidak's multiple comparisons test (A-B) one-way ANOVA followed by Tukey's multiple comparisons test (C, I). (A-C) One representative out of at least two independent experiments is shown. (D-K) Experiment has been performed once.

Fig. S11



**Figure S11. Administration of a depleting anti-CD4 T cell antibody does not affect the relative cluster representation of reactivated P14 T<sub>RM</sub> after tamoxifen administration in GFAP:GP mice.**

CD45.1+ P14 cells were adoptively transferred into adult CD45.2+ GP or GFAP:GP mice 24h before i.c. infection with rLCMV-GP33. At least six weeks later, mice were treated with anti-CD8a depleting antibody (αCD8α), anti-CD8α and anti-CD4 depleting antibody (αCD8α+αCD4) or isotype control. At day 0, mice were treated with TAM i.p. for four consecutive days to induce GP expression in astrocytes of GFAP:GP mice and brain were collected at day 7. **(A)** Heatmap showing Z scores of RNA-seq data for the average expression of core T<sub>RM</sub> or core circulating signature genes within a particular sample of indicated groups. **(B)** UMAP visualization of ~12'000 live and singlet-gated B220-CD11b-NK1.1-CD4-TCRb+CD8+CD45.1+ single-cell transcriptomes in indicated groups. Each symbol represents a cell. **(C)** Seurat clustering of cells in B, identifying eleven clusters. **(D)** Proportion of cells from indicated groups in clusters identified in C. Symbols represent one individual cell excepted in (D) where data represents two individual sample (A: n=3; B-D:n=2). (A-D) Experiment has been performed once.

**Table S1. Antibodies used for high-dimensional FACS analysis.**

| SURFACE        |             |                 |                        |                          |          |
|----------------|-------------|-----------------|------------------------|--------------------------|----------|
| Antigen        | Clone       | Fluorochrome    | Isotype                | Manufacturer             | Dilution |
| CD45           | 30-F11      | BUV 563         | rat IgG2b              | BD Bioscience            | 1:400    |
| CD8            | 53-6.7      | BUV 805         | rat IgG2a              | BD Bioscience            | 1:100    |
| CD4            | GK1.5       | BUV 496         | rat IgG2b              | BD Bioscience            | 1:200    |
| B220           | RA3-6B2     | BV 570          | rat IgG2a              | BioLegend                | 1:100    |
| CD80           | 16-10A1     | BUV 737         | armenian hamster IgG2a | BD Bioscience            | 1:100    |
| CD11b          | M1/70       | BUV 661         | rat IgG2b              | BD Bioscience            | 1:500    |
| TCR beta chain | H57-597     | PE-Cy5          | armenian hamster IgG1  | BioLegend                | 1:300    |
| CD11c          | N418        | PE-Cy5.5        | armenian hamster IgG   | Thermo Fisher Scientific | 1:500    |
| Siglec-F       | E50-2440    | BV 421          | rat IgG2a              | BD                       | 1:100    |
| NK1.1          | PK136       | BV 711          | rat IgG2a              | BioLegend                | 1:100    |
| Ly-6C          | AL-21       | FITC            | rat IgM                | BD Bioscience            | 1:800    |
| CD274          | MIH5        | biotin          | rat IgG2b              | ThermoFisher Scientific  | 1:200    |
| CD49d          | R1-2        | PE-Cy7          | rat IgG2b              | BioLegend                | 1:200    |
| Ly6G           | 1A8         | BV 650          | rat IgG2a              | BioLegend                | 1:200    |
| F4/80          | BM8         | BV 510          | rat IgG2a              | BioLegend                | 1:100    |
| I-A / I-E      | M5/114.15.2 | BB700           | rat IgG2b              | BD Bioscience            | 1:800    |
| CD103          | 2E7         | PE              | armenian hamster IgG1  | BioLegend                | 1:100    |
| CD64           | X54-5/7.1   | BV 605          | mouse IgG1             | BioLegend                | 1:100    |
| CX3CR1         | SA011F11    | AF647           | rat IgG2a              | Biolegend                | 1:100    |
| CD206          | C068C2      | AlexaFluor 700  | rat IgG2a              | BioLegend                | 1:100    |
| MerTK          | DS5MMER     | SuperBright 780 | rat IgG2a              | Thermo Fisher Scientific | 1:200    |
| Intracellular  |             |                 |                        |                          |          |
| FOXP3          | FJK-16s     | PE-eFluor610    | rat IgG2a              | Thermo Fisher Scientific | 1:200    |
| Ki-67          | B56         | BV 480          | mouse IgG1             | BD Bioscience            | 1:200    |
| Streptavidin   |             | BUV395          |                        | BD Bioscience            | 1:400    |

**Table2: Provided as separate Excel file**

**Table S3. Genome-wide transcriptome analysis of P14 T<sub>RM</sub> isolated 7 days after tamoxifen treatment from helped and unhelped GFAP:GP mice.** List of differentially expressed genes in unhelped versus helped P14 T<sub>RM</sub> (FC > 1.5, p < 0.05).

| Gene name | log2FoldChange | p-adj    |
|-----------|----------------|----------|
| Gkn3      | 9.10           | 2.75E-12 |
| Ighg2b    | 7.36           | 3.37E-06 |
| Ttr       | 6.73           | 5.88E-06 |
| Enpp2     | 5.74           | 3.20E-05 |
| Igkj2     | 4.67           | 1.91E-03 |
| Tpm2      | 4.32           | 5.49E-10 |
| Clu       | 3.58           | 1.29E-04 |
| Igkc      | 3.56           | 2.92E-05 |
| Wtip      | 3.47           | 2.97E-05 |
| Des       | 3.35           | 6.06E-07 |
| Myl9      | 3.33           | 6.12E-04 |
| Igkj5     | 3.17           | 4.00E-02 |
| Acta2     | 3.08           | 2.82E-04 |
| Sncg      | 2.98           | 1.86E-04 |
| Cdkn1c    | 2.70           | 1.48E-03 |
| Sdc2      | 2.45           | 6.27E-04 |
| Thbs1     | 2.40           | 4.79E-03 |
| Gas6      | 2.38           | 2.85E-02 |
| Mcam      | 2.32           | 4.09E-04 |
| Tagln     | 2.17           | 9.83E-06 |
| Gja4      | 2.15           | 2.88E-02 |
| Mgp       | 2.04           | 2.29E-02 |
| Sdc4      | 2.04           | 2.75E-02 |
| Siglech   | 2.02           | 8.73E-05 |
| Mustn1    | 1.99           | 1.38E-03 |
| Tcf7      | 1.95           | 6.47E-10 |
| Oas2      | 1.81           | 7.37E-05 |
| Crispld2  | 1.79           | 4.89E-02 |
| Tnfsf8    | 1.77           | 5.52E-07 |
| Hck       | 1.76           | 4.80E-04 |
| Fads2     | 1.76           | 2.85E-02 |
| Tox       | 1.68           | 2.49E-07 |
| S1pr3     | 1.65           | 3.21E-02 |
| Susd2     | 1.59           | 1.97E-02 |

|                |      |          |
|----------------|------|----------|
| <b>Cpa3</b>    | 1.51 | 4.53E-02 |
| <b>Tinagl1</b> | 1.51 | 4.86E-02 |
| <b>Ms4a4c</b>  | 1.46 | 3.94E-17 |
| <b>Ighm</b>    | 1.45 | 3.37E-06 |
| <b>Ccl2</b>    | 1.45 | 1.31E-02 |
| <b>Ltbp4</b>   | 1.43 | 4.41E-02 |
| <b>Ccl12</b>   | 1.39 | 3.65E-02 |
| <b>Zfp395</b>  | 1.38 | 2.12E-03 |
| <b>Art2b</b>   | 1.37 | 1.87E-04 |
| <b>Oasl2</b>   | 1.35 | 7.20E-05 |
| <b>Zfp691</b>  | 1.33 | 4.31E-02 |
| <b>Asb2</b>    | 1.30 | 6.70E-03 |
| <b>Acvr2a</b>  | 1.29 | 1.38E-03 |
| <b>Cxcl10</b>  | 1.25 | 2.49E-07 |
| <b>Sparc</b>   | 1.24 | 5.82E-06 |
| <b>C4b</b>     | 1.23 | 1.13E-03 |
| <b>Tyro3</b>   | 1.20 | 1.44E-02 |
| <b>Eomes</b>   | 1.19 | 7.91E-05 |
| <b>Tmem119</b> | 1.18 | 1.09E-03 |
| <b>Plxdc2</b>  | 1.17 | 6.23E-03 |
| <b>Gstk1</b>   | 1.17 | 3.58E-02 |
| <b>Ly86</b>    | 1.16 | 1.53E-02 |
| <b>Depdc1b</b> | 1.16 | 3.10E-02 |
| <b>Cd81</b>    | 1.15 | 2.30E-05 |
| <b>Cd40lg</b>  | 1.15 | 2.58E-02 |
| <b>Prr11</b>   | 1.15 | 4.00E-02 |
| <b>Gbp10</b>   | 1.14 | 3.11E-10 |
| <b>Ahr</b>     | 1.12 | 8.96E-03 |
| <b>Samd3</b>   | 1.11 | 1.10E-09 |
| <b>Anln</b>    | 1.09 | 3.09E-03 |
| <b>Cd83</b>    | 1.08 | 1.05E-04 |
| <b>P2ry12</b>  | 1.05 | 4.12E-02 |
| <b>Unc5a</b>   | 1.05 | 6.23E-03 |
| <b>Eme1</b>    | 1.01 | 4.00E-02 |
| <b>Sdc3</b>    | 1.00 | 6.58E-08 |
| <b>Cd200r1</b> | 1.00 | 7.50E-08 |
| <b>Pgm2l1</b>  | 1.00 | 4.05E-02 |
| <b>Itgb1</b>   | 0.98 | 7.90E-22 |
| <b>Ifit3</b>   | 0.96 | 2.90E-07 |
| <b>Csf1r</b>   | 0.96 | 2.76E-03 |
| <b>Perp</b>    | 0.95 | 1.49E-02 |
| <b>Nuf2</b>    | 0.95 | 3.99E-03 |
| <b>Pimreg</b>  | 0.94 | 3.23E-02 |
| <b>Ccr7</b>    | 0.94 | 6.61E-03 |

|                 |      |          |
|-----------------|------|----------|
| <b>Chst2</b>    | 0.92 | 2.95E-05 |
| <b>Plk1</b>     | 0.91 | 5.27E-04 |
| <b>Sh2b3</b>    | 0.90 | 7.78E-05 |
| <b>Nrp1</b>     | 0.89 | 3.08E-06 |
| <b>Lgmn</b>     | 0.89 | 7.60E-03 |
| <b>Rsad2</b>    | 0.88 | 4.52E-06 |
| <b>Gprasp1</b>  | 0.87 | 2.36E-02 |
| <b>Shcbp1</b>   | 0.86 | 4.79E-03 |
| <b>Zfp831</b>   | 0.86 | 3.95E-02 |
| <b>Ckb</b>      | 0.85 | 2.85E-02 |
| <b>Cmpk2</b>    | 0.84 | 2.16E-04 |
| <b>E2f8</b>     | 0.84 | 1.61E-02 |
| <b>Serpini1</b> | 0.84 | 7.67E-03 |
| <b>Ifi44</b>    | 0.83 | 2.01E-03 |
| <b>Ifit1</b>    | 0.81 | 2.79E-05 |
| <b>Usp33</b>    | 0.80 | 6.90E-04 |
| <b>Ifit1bl1</b> | 0.80 | 2.49E-07 |
| <b>Ckap2l</b>   | 0.79 | 1.39E-02 |
| <b>Aurka</b>    | 0.78 | 2.96E-02 |
| <b>BC147527</b> | 0.78 | 7.42E-05 |
| <b>Oplah</b>    | 0.76 | 3.74E-02 |
| <b>Cdca3</b>    | 0.76 | 1.66E-03 |
| <b>Ube2c</b>    | 0.76 | 7.89E-03 |
| <b>Rad54l</b>   | 0.75 | 2.16E-02 |
| <b>Pde6d</b>    | 0.75 | 2.35E-02 |
| <b>C1qc</b>     | 0.75 | 1.68E-03 |
| <b>Ect2</b>     | 0.74 | 9.85E-03 |
| <b>Itprid2</b>  | 0.73 | 2.79E-03 |
| <b>Cenpf</b>    | 0.73 | 3.01E-02 |
| <b>Tdrd7</b>    | 0.73 | 1.20E-02 |
| <b>Ccna2</b>    | 0.72 | 5.07E-06 |
| <b>Kif18b</b>   | 0.72 | 1.45E-02 |
| <b>Foxm1</b>    | 0.72 | 3.52E-04 |
| <b>Oasl1</b>    | 0.72 | 1.48E-02 |
| <b>C1qb</b>     | 0.71 | 1.32E-02 |
| <b>Ptpn4</b>    | 0.71 | 4.80E-04 |
| <b>Tgtp2</b>    | 0.70 | 6.67E-04 |
| <b>Irf7</b>     | 0.69 | 9.71E-06 |
| <b>Hmmr</b>     | 0.69 | 4.05E-02 |
| <b>Gbp8</b>     | 0.69 | 1.50E-05 |
| <b>Dusp7</b>    | 0.69 | 2.24E-02 |
| <b>Fbxo5</b>    | 0.69 | 4.71E-02 |
| <b>H2-Aa</b>    | 0.68 | 2.08E-02 |
| <b>Cdc25b</b>   | 0.67 | 2.97E-05 |

|                 |       |          |
|-----------------|-------|----------|
| <b>Chtf18</b>   | 0.67  | 2.59E-02 |
| <b>Birc5</b>    | 0.67  | 1.18E-03 |
| <b>Kif11</b>    | 0.67  | 2.86E-05 |
| <b>Ccl3</b>     | 0.66  | 2.11E-05 |
| <b>C1qa</b>     | 0.66  | 2.65E-02 |
| <b>Ncapg</b>    | 0.66  | 1.34E-03 |
| <b>Ccrl2</b>    | 0.66  | 8.53E-03 |
| <b>Slfn5</b>    | 0.66  | 3.05E-02 |
| <b>Kif20a</b>   | 0.66  | 3.01E-02 |
| <b>Rgcc</b>     | 0.65  | 1.15E-02 |
| <b>Ube2l6</b>   | 0.65  | 1.73E-03 |
| <b>Cd101</b>    | 0.65  | 5.72E-05 |
| <b>Spc25</b>    | 0.65  | 3.65E-02 |
| <b>Spag5</b>    | 0.64  | 1.53E-02 |
| <b>Dse</b>      | 0.64  | 2.76E-03 |
| <b>Spc24</b>    | 0.64  | 4.00E-02 |
| <b>Zcchc18</b>  | 0.63  | 1.63E-02 |
| <b>Tuba1b</b>   | 0.63  | 1.11E-04 |
| <b>Tnf</b>      | 0.63  | 3.22E-03 |
| <b>Xcl1</b>     | 0.63  | 6.48E-05 |
| <b>Rrm2</b>     | 0.63  | 9.89E-05 |
| <b>Krba1</b>    | 0.62  | 4.95E-02 |
| <b>Tubb5</b>    | 0.62  | 1.20E-04 |
| <b>Nusap1</b>   | 0.61  | 2.38E-04 |
| <b>Ifi214</b>   | 0.61  | 2.27E-04 |
| <b>Vav3</b>     | 0.61  | 5.49E-03 |
| <b>Ifi208</b>   | 0.60  | 2.27E-04 |
| <b>Nfkbid</b>   | 0.59  | 3.47E-04 |
| <b>Ly75</b>     | -0.59 | 3.51E-04 |
| <b>Fbxo32</b>   | -0.59 | 3.52E-04 |
| <b>Il2ra</b>    | -0.60 | 5.88E-03 |
| <b>Crtc1</b>    | -0.60 | 2.85E-02 |
| <b>Vegfa</b>    | -0.60 | 2.81E-02 |
| <b>Prf1</b>     | -0.60 | 1.87E-04 |
| <b>Zyg11b</b>   | -0.60 | 1.82E-04 |
| <b>Gsto1</b>    | -0.61 | 2.35E-02 |
| <b>Il18r1</b>   | -0.61 | 4.97E-02 |
| <b>Tgfbr3</b>   | -0.62 | 1.17E-04 |
| <b>Dennd3</b>   | -0.63 | 3.57E-03 |
| <b>B4galnt4</b> | -0.63 | 1.11E-04 |
| <b>Rora</b>     | -0.64 | 5.70E-05 |
| <b>Adam9</b>    | -0.65 | 2.12E-03 |
| <b>Socs2</b>    | -0.65 | 3.46E-02 |
| <b>Pmepa1</b>   | -0.66 | 2.30E-02 |

|                  |              |          |
|------------------|--------------|----------|
| <b>Hk2</b>       | <b>-0.67</b> | 2.99E-05 |
| <b>Sema4f</b>    | <b>-0.68</b> | 8.13E-03 |
| <b>Irs2</b>      | <b>-0.68</b> | 1.72E-05 |
| <b>Pwwp2b</b>    | <b>-0.68</b> | 6.53E-03 |
| <b>Il18rap</b>   | <b>-0.69</b> | 6.29E-04 |
| <b>Fkbp5</b>     | <b>-0.69</b> | 5.97E-04 |
| <b>Itgax</b>     | <b>-0.70</b> | 4.17E-04 |
| <b>Emid1</b>     | <b>-0.70</b> | 5.27E-04 |
| <b>Cd7</b>       | <b>-0.71</b> | 1.24E-02 |
| <b>Gstt2</b>     | <b>-0.71</b> | 1.61E-02 |
| <b>Cirbp</b>     | <b>-0.74</b> | 1.82E-04 |
| <b>Ern1</b>      | <b>-0.76</b> | 7.98E-07 |
| <b>Arl14ep</b>   | <b>-0.77</b> | 1.00E-04 |
| <b>AA467197</b>  | <b>-0.77</b> | 5.73E-07 |
| <b>Sv2c</b>      | <b>-0.83</b> | 8.63E-03 |
| <b>Tnfrsf13b</b> | <b>-0.83</b> | 3.05E-02 |
| <b>Ifitm2</b>    | <b>-0.84</b> | 1.10E-05 |
| <b>Lzts1</b>     | <b>-0.89</b> | 1.80E-02 |
| <b>Gzma</b>      | <b>-0.92</b> | 7.81E-07 |
| <b>Dusp14</b>    | <b>-0.93</b> | 3.65E-02 |
| <b>Styk1</b>     | <b>-0.95</b> | 2.53E-05 |
| <b>Smpdl3b</b>   | <b>-0.95</b> | 3.01E-02 |
| <b>Ifitm1</b>    | <b>-1.02</b> | 2.75E-12 |
| <b>Klrg1</b>     | <b>-1.04</b> | 2.37E-03 |
| <b>Gpnmb</b>     | <b>-1.07</b> | 3.12E-02 |
| <b>Myo10</b>     | <b>-1.10</b> | 1.05E-09 |
| <b>Fut4</b>      | <b>-1.12</b> | 2.85E-02 |
| <b>Mt1</b>       | <b>-1.13</b> | 3.49E-06 |
| <b>Ier3</b>      | <b>-1.14</b> | 2.88E-02 |
| <b>Pqlc1</b>     | <b>-1.19</b> | 1.13E-05 |
| <b>Lrrc29</b>    | <b>-1.21</b> | 4.89E-02 |
| <b>Dmwd</b>      | <b>-1.23</b> | 3.10E-02 |
| <b>Ankrd13b</b>  | <b>-1.24</b> | 2.39E-02 |
| <b>Vcam1</b>     | <b>-1.36</b> | 4.80E-02 |
| <b>Prr5</b>      | <b>-1.43</b> | 2.35E-02 |
| <b>Gzmc</b>      | <b>-1.44</b> | 5.25E-10 |
| <b>Tff1</b>      | <b>-1.48</b> | 2.24E-02 |
| <b>Klra3</b>     | <b>-1.50</b> | 1.53E-02 |
| <b>Esm1</b>      | <b>-1.55</b> | 5.53E-14 |
| <b>Dgat2</b>     | <b>-1.57</b> | 4.67E-07 |
| <b>Mt2</b>       | <b>-1.63</b> | 1.12E-04 |
| <b>Bcl2l15</b>   | <b>-1.67</b> | 1.46E-03 |
| <b>Emilin2</b>   | <b>-1.71</b> | 4.48E-02 |
| <b>Gpr141</b>    | <b>-1.83</b> | 3.16E-06 |

|                |              |          |
|----------------|--------------|----------|
| <b>Alpk2</b>   | <b>-2.00</b> | 4.95E-10 |
| <b>Ptgs2</b>   | <b>-2.07</b> | 1.21E-03 |
| <b>Il1r2</b>   | <b>-2.08</b> | 2.64E-04 |
| <b>Ltb4r1</b>  | <b>-2.14</b> | 3.26E-08 |
| <b>Ermn</b>    | <b>-2.46</b> | 9.71E-06 |
| <b>Ch25h</b>   | <b>-3.22</b> | 6.67E-04 |
| <b>Slc38a5</b> | <b>-3.32</b> | 6.75E-03 |
| <b>Gzmf</b>    | <b>-3.37</b> | 1.40E-09 |
| <b>Lrg1</b>    | <b>-4.48</b> | 2.67E-04 |
| <b>Ackr1</b>   | <b>-5.42</b> | 6.52E-05 |
| <b>Sele</b>    | <b>-6.27</b> | 4.50E-05 |

**Table S4. Single-cell sequencing statistics of P14 cells isolated 7 days after tamoxifen treatment from GP, helped and unhelped GFAP:GP mice. Sequencing statistics.**

| <b>sample ID</b> | <b>Cells</b> | <b>Mean reads<br/>per cell</b> | <b>Median genes<br/>per cell</b> | <b>Sequencing<br/>saturation (%)</b> |
|------------------|--------------|--------------------------------|----------------------------------|--------------------------------------|
| aCD8+aCD4_1_s1   | 3148         | 99114                          | 700                              | 75.2                                 |
| GP_1_s2          | 2420         | 148284                         | 1567                             | 83.4                                 |
| aCD8_1_s3_       | 2784         | 123843                         | 586                              | 78.5                                 |
| GP_2_s4_         | 3011         | 99755                          | 1491                             | 78.7                                 |
| aCD8+aCD4_2_s5   | 2650         | 112845                         | 678                              | 76.6                                 |
| aCD8_2_s6        | 3212         | 102641                         | 897                              | 74.6                                 |

**Table S5. Human tissue samples used for multiplexed stainings.**

| Case N° | Age (years) | Sex | Tissue type | Disease type                | Disease duration (years) | Brain region                         | Lesion type                    |
|---------|-------------|-----|-------------|-----------------------------|--------------------------|--------------------------------------|--------------------------------|
| 1       | 61          | F   | biopsy      | CIS <sup>1</sup>            | NA                       | Frontal white matter                 | acute active demyelinating     |
| 2       | 42          | F   | biopsy      | RR-MS <sup>1</sup>          | 19                       | frontal white matter                 | acute active demyelinating     |
| 3       | 25          | F   | biopsy      | NA <sup>1</sup>             | NA                       | occipital white matter               | acute active demyelinating     |
| 4       | 52          | F   | biopsy      | CIS <sup>1</sup>            | Approx. 3 weeks          | Periventricular white matter (left)  | acute active demyelinating     |
| 5       | 45          | F   | autopsy     | RR-MS <sup>1</sup>          | 12                       | frontal white matter                 | chronic active demyelinating   |
| 6       | 51          | F   | autopsy     | RR-MS <sup>1</sup>          | 13                       | fronto-parietal white matter         | chronic active demyelinating   |
| 7       | 17          | F   | autopsy     | RR-MS <sup>1</sup>          | NA                       | parietal white matter                | chronic active demyelinating   |
| 8       | 45          | M   | autopsy     | RR-MS <sup>1</sup>          | 14                       | frontal periventricular white matter | chronic active demyelinating   |
| 9       | 75          | M   | autopsy     | SP-MS <sup>1</sup>          | 32                       | cerebral white matter                | chronic active demyelinating   |
| 10      | 45          | F   | biopsy      | Progressive MS <sup>1</sup> | NA                       | cerebral white matter                | acute active demyelinating     |
| 11      | 63          | F   | biopsy      | Progressive MS <sup>1</sup> | 22                       | cerebral white matter                | acute active demyelinating     |
| 12      | 42          | F   | biopsy      | NMOSD <sup>1</sup>          | Approx. 2 weeks          | cerebral white matter                | acute astrocyte depleting      |
| 13      | 44          | F   | autopsy     | NMOSD <sup>1</sup>          | 16 months                | spinal cord                          | acute astrocyte depleting      |
| 14      | 41          | F   | biopsy      | NMOSD <sup>1</sup>          | Few weeks                | cerebral white matter                | acute astrocyte depleting      |
| 15      | 69          | F   | autopsy     | NMOSD <sup>1</sup>          | 5 months                 | cerebral white matter                | acute astrocyte depleting      |
| 16      | 49          | F   | autopsy     | NMOSD <sup>1</sup>          | 4 months                 | spinal cord                          | acute astrocyte depleting      |
| 17      | 41          | F   | biopsy      | ADEM <sup>2</sup>           | Few days                 | cerebral white matter                | acute perivenous demyelinating |
| 18      | 61          | M   | biopsy      | ADEM <sup>2</sup>           | NA                       | cerebral white matter                | acute perivenous demyelinating |
| 19      | 18          | M   | biopsy      | ADEM <sup>2</sup>           | 2 weeks                  | cerebral white matter                | acute perivenous demyelinating |

|    |    |   |        |                    |          |                       |                                |
|----|----|---|--------|--------------------|----------|-----------------------|--------------------------------|
| 20 | 62 | M | biopsy | ADEM <sup>2</sup>  | Few days | cerebral white matter | acute perivenous demyelinating |
| 21 | 41 | M | biopsy | CIS <sup>3</sup>   | < 1 y    | cerebral white matter | acute active demyelinating     |
| 22 | 30 | F | biopsy | CIS <sup>3</sup>   | < 1 y    | cerebral white matter | acute active demyelinating     |
| 23 | 28 | M | biopsy | CIS <sup>3</sup>   | < 1 y    | cerebral white matter | acute active demyelinating     |
| 24 | 55 | M | biopsy | CIS <sup>3</sup>   | < 1 y    | cerebral white matter | acute, active demyelinating    |
| 25 | 51 | M | biopsy | RR-MS <sup>3</sup> | 8        | cerebral white matter | late acute, post-demyelinating |
| 26 | 39 | F | biopsy | CIS <sup>3</sup>   | < 1 y    | cerebral white matter | late acute, post-demyelinating |
| 27 | 49 | F | biopsy | CIS <sup>3</sup>   | < 1 y    | cerebral white matter | late acute, post-demyelinating |

RR-MS: relapsing-remitting MS; SP-MS: secondary progressive MS; CIS: clinical isolated syndrome; NA: not available; NMOSD: neuromyelitis optima spectrum disorders; ADEM: acute disseminated encephalomyelitis

<sup>1</sup>: Used in Fig. 5B-F; <sup>2</sup>: Used in Fig. 5B-D; <sup>3</sup>: Used in Fig. 5G-H