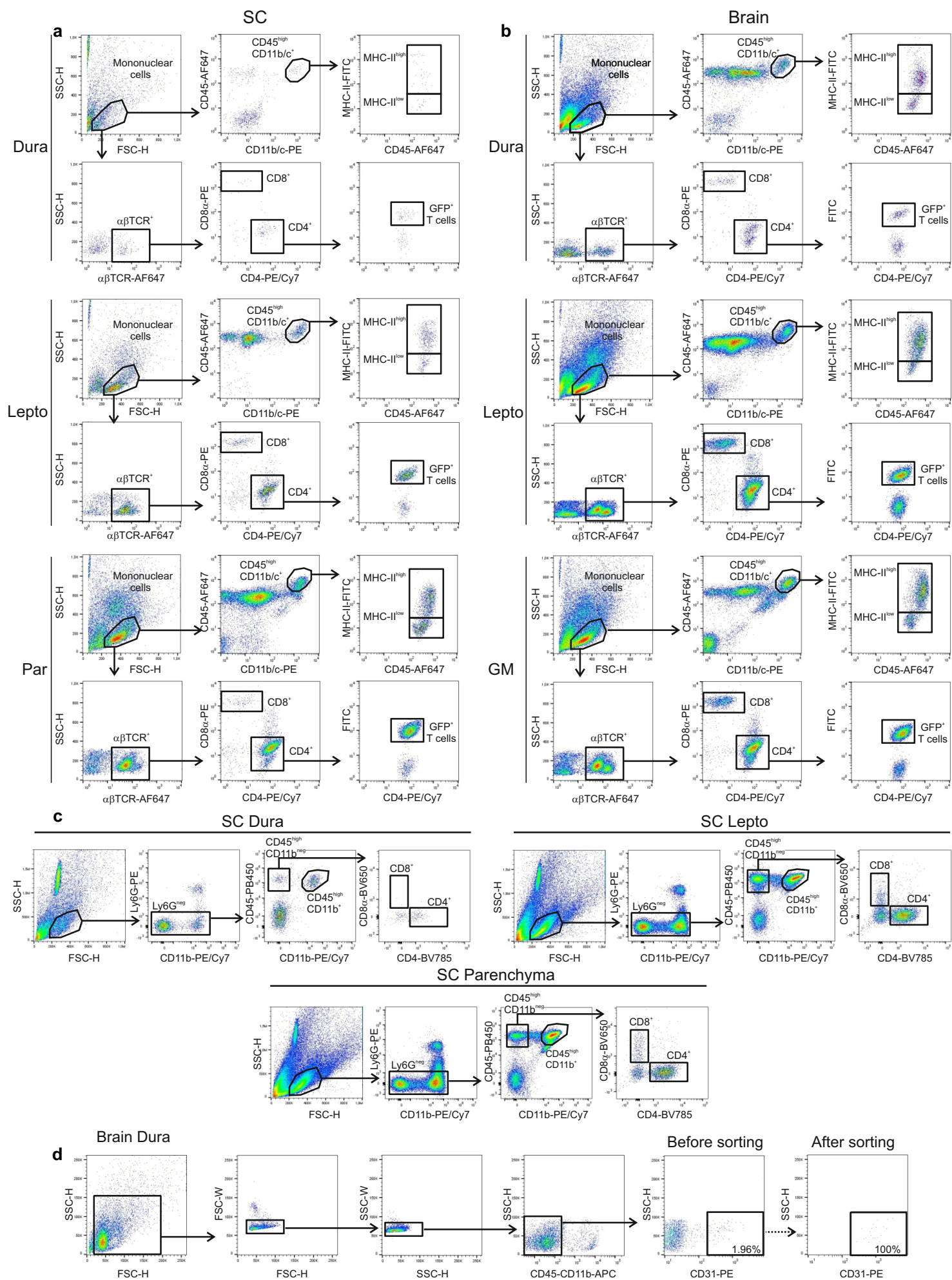

Supplementary information

Distinct roles of the meningeal layers in CNS autoimmunity

In the format provided by the
authors and unedited



Supplementary Figure 1: Flow-cytometry gating strategies for the characterization of immune cells and endothelial cells in the CNS. **a**, Representative plots for analyzing T cell and myeloid cell subsets in the indicated rat SC compartments at the peak of T_{MBP} cell infiltration. Transfer EAE. **b**, Representative plots for analyzing T cell and myeloid cell subsets in the indicated rat brain compartments at the peak of T_{bSYN} cell infiltration. Transfer EAE. **c**, Representative plots for analyzing the indicated T cell and myeloid cell subsets in the indicated mouse SC compartments at the peak of active MOG-EAE. **d**, Representative plots depicting the strategy for isolation of rat endothelial cells. Numbers indicate the purity of the sample before and after the sorting procedure. Brain dura. Naïve animals.

Supplementary Table 1: Clinical data.

Case n. in figure	Case n.	Age (years)	Gender	Diagnosis	Disease duration (years)
1	A64/17	38	f	MS	>10
2	A109/13	41	f	MS	>10
3	A112/12	44	f	SPMS	21
4	A3/11	46	f	PPMS	>10
5	A2/10	47	f	SPMS	24
6	A113/13	49	f	PPMS	31
7	A4/20	56	m	SPMS	>10
8	A40/16	57	f	SPMS	32
9	A36/14	59	m	SPMS	26
10	A38/14	60	m	SPMS	22
11	A65/17	64	f	SPMS	50
12	A140/12	64	f	SPMS	37
13	A120/11	66	m	MS	20
	B351/19	33	m	Lymphoplasmacytic pachymeningitis	
	A20/20	47	m	Aortic dissection with cardiac tamponade	
	A44/20	67	m	Sepsis	

Supplementary Table 2: Primer/probe combinations used for quantitative PCR.

Gene (<i>Abbreviation</i>)	Forward primer (5'-3')	Reverse primer (5'-3')	Probe (FAM-5'-3'-TAMRA)
β -actin (<i>Actb</i>)	GTACAACCTCCTTGCAGCTCC T	TTGTCGACGACGAGCGC	CGCCACCAGTTCGCCATGGT
Interferon gamma (<i>Ifng</i>)	AACAGTAAAGCAAAAAAGGA TGCATT	TTCATTGACAGCTTTGTGCTG G	CGCCAAGTTCGAGGTGAACAA CCC
Interleukin-17A (<i>Il17a</i>)	GAGTCCCGGAGAATTC CAT	GAGTACCGCTGCCTTCACTG T	ATGTGCCTGATGCTGTT
Vascular cell adhesion protein-1 (<i>Vcam1</i>)	ACATGAGGGTGCTCC TGTGA	GGTGGCATTTCGAGAGGA	TGTGCCAGCGAGGGTCTACCA GCTCCT
Fibronectin (Fn1)	TGATCTTTGAGGAACATGGCT TT	GCAGGTATGGTCTTGGCCTA AG	AACCACGCCACCCACTGCGG
Intercellular adhesion molecule-1 (<i>Icam</i>)	GGAGACAGCAGACCACTGTG CTT	CTCGCTCTGGGAACGAATAC A	ACTGTGGCACCACGC
Claudin-5 (<i>Cldn5</i>)	CGGGCGTCCAGAGTTCAGT	TAGACGTAGTTCTTC TTGTCGTAATCG	CCAGTCAAGTACTCAGCACCAA GGCGA
Occludin (<i>Ocln</i>)	CCTAATGTGGAAGAGTGGGT TAAAAA	GTCGACTCTTTCCGC ATAGTCA	CACACAAGACATGCCTCCACCC CC
Chemokine (C-X-C motif) ligand 9 (<i>Cxcl9</i>)	TTG CCC CAA GCC CTA ACT G	ACC CTT GCT GAA TCT GGG TCT AG	CAT CGC TAC ACT GAA GAA CGG AGA TCA
Chemokine (C-X-C motif) ligand 10 (<i>Cxcl10</i>)	CGT GCT GCT GAG TCT GAG T	GTC TCA GCG GCT GTT CAT	CTC AAG GGA TCC CTC TCG CAA GAA C
Chemokine (C-X-C motif) ligand 11 (<i>Cxcl11</i>)	GGT TCC AGG CTT CGT TAT GTT C	AAC TTC CTT GAT TGC TGC CAT T	CTG TCT TTG CAT CGA CCG CGG AGT

Supplementary Legends

Supplementary Videos

Supplementary Video 1: Differential permeability of brain meningeal vessels to solutes. First part of the video: Experimental set-up allowing the simultaneous in vivo visualization of pial and leptomeningeal vessels through a thinned skull window. Second part of the video: Assessment of dural and pial vessel permeability to solutes. Intravital TPLSM recording was performed immediately after i.v. injection of 3kDa Dextran Texas Red (TR, in magenta). The dye leaked out from the dural vessels already 2min after injection and after 40min was taken up by local phagocytes (white open arrows in the video). No leakage was observed from the leptomeningeal vessels during the observation time. Green: Blood vessels labeled by i.v. injection of 70kDA FITC-Dextran.

Supplementary Video 2: Locomotion behavior of intravascular T_{bSYN} , T_{MBP} and T_{OVA} cells in hemispheric dural and leptomeningeal vessels of the brain. Intravital TPLSM recordings performed on day 3 p.t. 30min time-lapse videos and corresponding time-projections. Turquoise: Antigen- specific T cells; Red: Blood vessels.

Supplementary Video 3: Effect of VLA-4 blockade on T cell adhesion to meningeal vessels. Intravital TPLSM recordings depicting the intravascular locomotion behavior of T_{OVA} cells (day 3 p.t.) in leptomeningeal and hemispheric dural vessels before and 60min after i.v. injection of VLA-4 mAbs. 30min time-lapse recordings and corresponding time-projections. Turquoise: T_{OVA} cells; Red: Blood vessels.

Supplementary Video 4: Changes in T cell motility upon induction of dural inflammation. Motility behavior of T_{OVA} cells (day 3p.t.) recorded by intravital TPLSM in the brain hemispheric dura before (steady state), 150min and 210min after transcranial application of IFN γ and TNF α . 30min time-lapse videos and corresponding time-projections. Red: T_{OVA} cells; White: Blood vessels; Blue: Collagen.

Supplementary Video 5: In vivo TPLSM visualization of T cell locomotion in the distinct brain meningeal layers. Single plane, maximal projection and 3D reconstruction of the dural and leptomeningeal layers. Turquoise: T_{OVA} cells (day 3.5 post transfer); Green and orange: Leptomeningeal and dural phagocytes, respectively.

Supplementary Video 6: Locomotion behavior of T_{OVA} cells in the leptomeninges and dura. Intravital TPLSM recordings on day 3.5 after transfer of T_{OVA} cells in the hemispheric leptomeninges and overlying dura. 30min time-lapse videos. Turquoise: Antigen-specific T cells; Green and red: Leptomeningeal and dura macrophages, respectively; Yellow: Tracks of extravascular T_{OVA} cells.

Supplementary Video 7: Interactions between T cells and dural phagocytes. Depicted is a T_{OVA} cell (false color: turquoise) establishing serial contacts (false color: yellow) with local phagocytes (red) during 30min intravital TPLSM recording performed on day 3.5 p.t. Green: Other T_{OVA} cells in dura.

Supplementary Video 8: T cell motility in the dura upon local Ag application. Motility behavior of T_{OVA} cells recorded on day 3.5 p.t. in the brain dura before (steady state) and 120min after transcranial application of OVA peptide. Yellow arrows indicate stationary T cells. 30min TPLSM time-lapse videos. Turquoise: T_{OVA} cells; Red: Dura macrophages; Blue: Collagen.

Supplementary Video 9: Real-time detection of T cell activation in the dura upon local application of the cognate antigen. Intravital TPLSM recording performed on brain dura 3.5 days p.t. of OVA-reactive T cells expressing NFAT biosensor. Before transcranial application of OVA peptide, in the circled T cell. NFAT is located in the cytosol that appears green. 120min upon Ag application, in the circled OVA-reactive T cell, NFAT translocates from the cytosol to the nucleus, indicated by the red nucleus turning yellow. Overview 30min TPLSM time-lapse videos and magnified areas of interest. Green: NFAT-YFP; Red: Histone H2B-mCherry labelling the nucleus; Blue: Collagen.

Supplementary Video 10: T cell trafficking in the dura lymphatic vessels. Intravital TPLSM recording in a Prox1-eGFP rat depicting a T_{bSYN} cell (yellow circle) located inside a lymphatic vessel on day 3.5 p.t. Red: T_{bSYN} cells; Green: lymphatic vessels; Blue: Collagen. 30min time-lapse videos.

Supplementary Video 11: T cell trafficking in the sinus. Motility behavior of T_{OVA} and T_{bSYN} cells in the sagittal sinus on day 3 p.t. 16min TPLSM time-lapse videos and corresponding time-

projections. Attenuation of laser intensity by blood cells limits T cell detection in the central part of the sinus. Turquoise: T_{OVA}/ T_{bSYN} cells; Red: Blood vessels.

Supplementary Video 12: Extravascular locomotion behavior of T_{bSYN} cells in the leptomeninges, hemispheric dura and sagittal sinus. Note the less straight and more confined movement of T_{bSYN} cells in the leptomeninges. 25min time-lapse intravital TPLSM recordings on day 3.5 after transfer. Turquoise: T_{bSYN} cells. Red: Leptomeningeal and dural macrophages; Yellow: Tracks of extravascular T_{bSYN} cells.