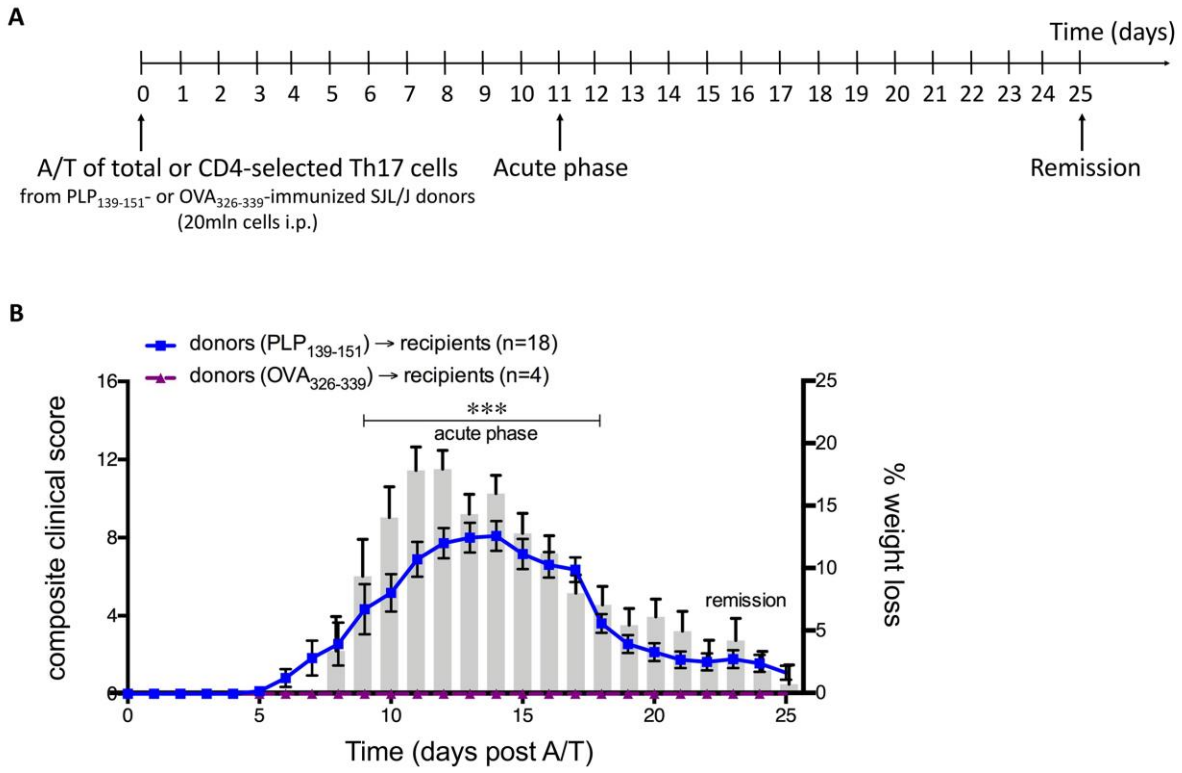
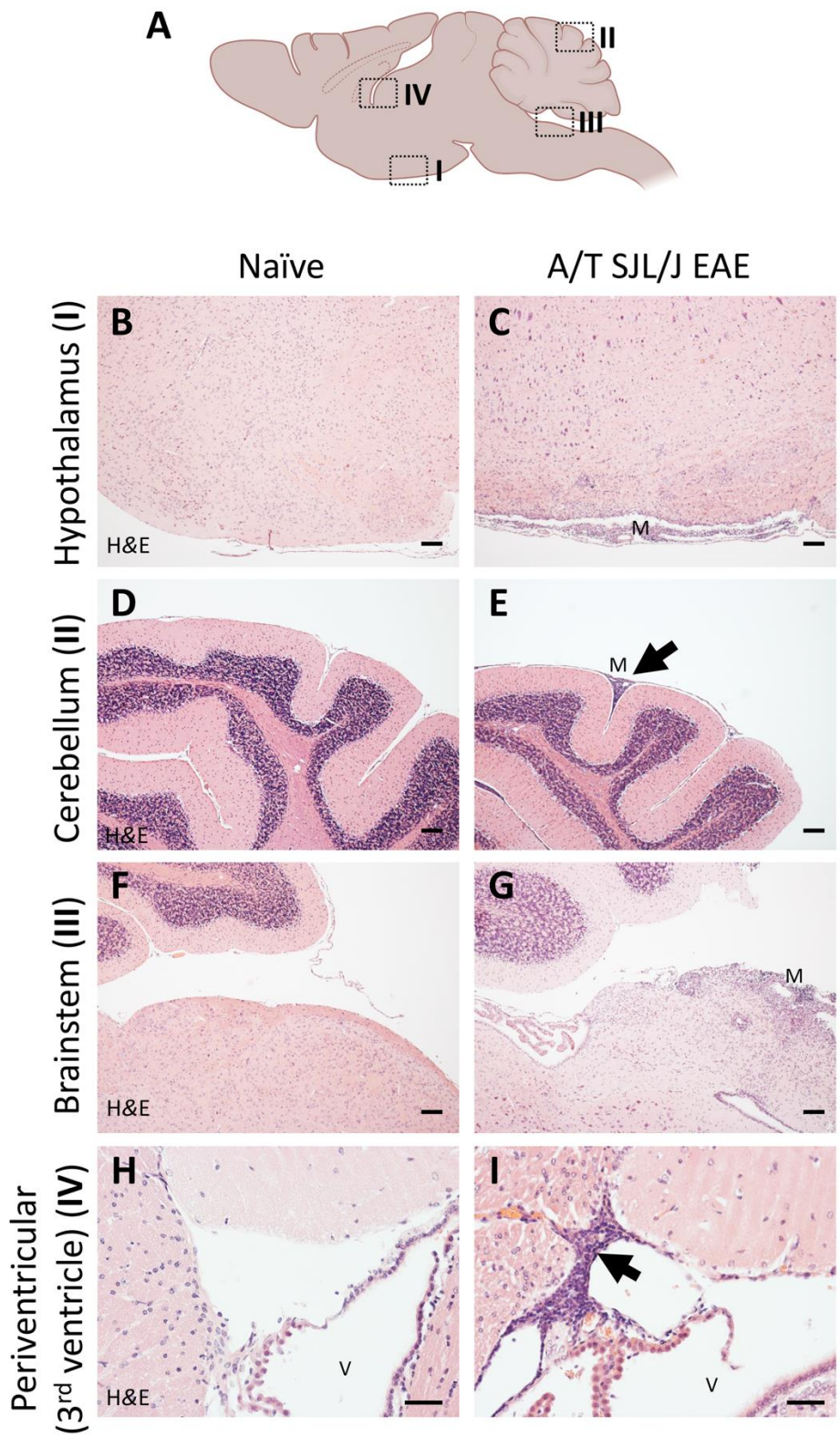
Supplementary Figures

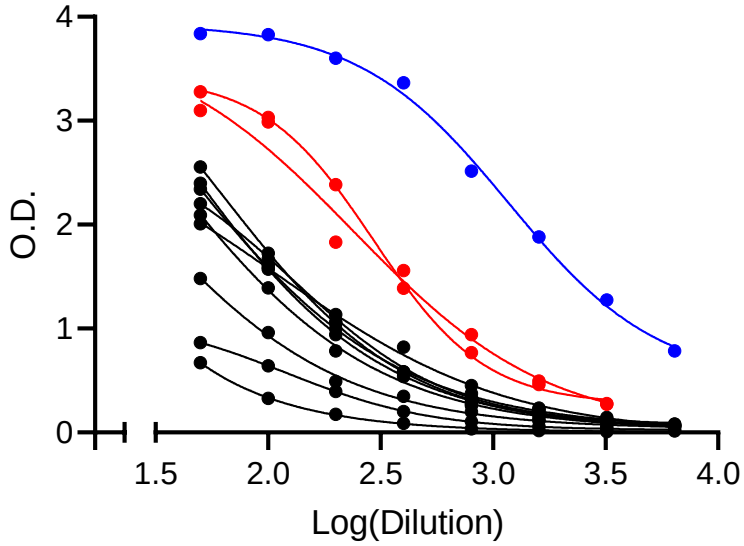


Supplementary Fig. 1. Schematic of A/T SJL/J EAE induction protocol, experimental timeline and clinical course. (**A**) Total or CD4-selected Th17 lymphocytes from 6-10 week old SJL/J donor mice immunized with either $PLP_{139-151}$ (n=9) or $OVA_{326-339}$ (n=2) in CFA, were harvested at day 9 post-immunization and adoptively transferred into 6-10 week old SJL/J recipient mice (n=18 received from $PLP_{139-151}$-immunized donors; n=4 received from $OVA_{326-339}$-immunized donors). Mice were sacrificed either at acute phase (day 11 post-A/T) or remission (25 days post-A/T). (**B**) The graph illustrates the composite clinical score (16-point scale) of recipient mice. Values are shown as mean +/- S.E.M. Statistical analysis by two-way ANOVA with $p<0.0001$. Changes in weight were monitored throughout the experiment

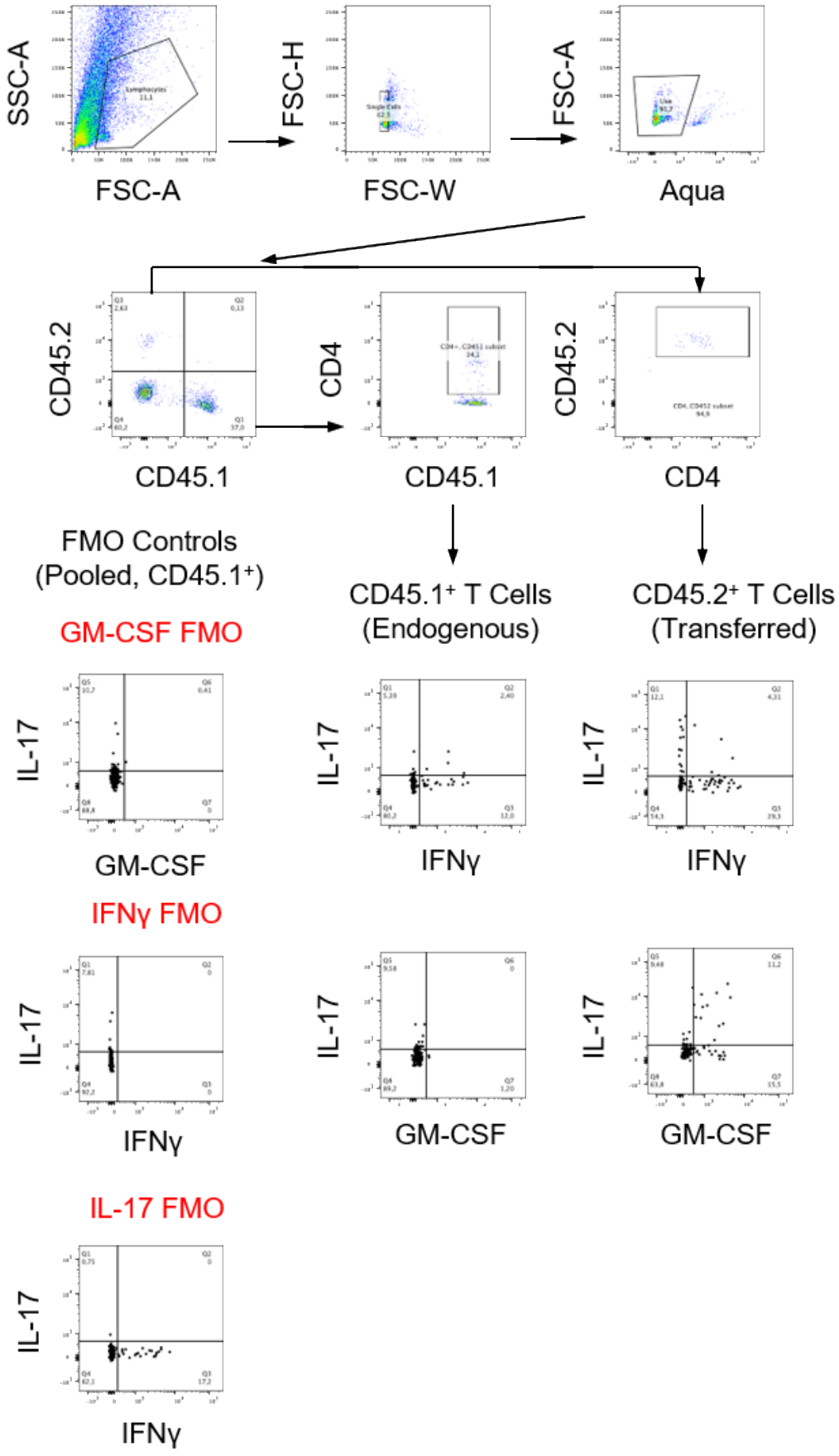


Supplementary Fig. 2. Meningeal and periventricular inflammation in A/T SJL/J EAE mice. (**A**) Diagram of the mouse brain sectioned through the sagittal neuraxis, showing areas of the hypothalamus (**I**), cerebellum (**II**), brainstem (**III**) and periventricular (3rd ventricle)

region (**IV**) imaged in **B-I**. (**B-I**) Inflammation as assessed by Hematoxylin & Eosin (HE) staining at day 11 (acute phase) post-A/T. Aggregates of cells (in blue) are seen in meninges lining the hypothalamus (**C**), the cerebellum (**E**) and the brainstem (**G**) as well as periventricular inflammation at the 3rd ventricle (arrow in **I**) in A/T SJL/J EAE mice at the acute phase. Such aggregates of meningeal cells are not detected in naïve mice (**B**, **D, F, H**), as expected. Scale bar in **B-G** is 200 μm. Scale bar **H-I** is 50 μm.

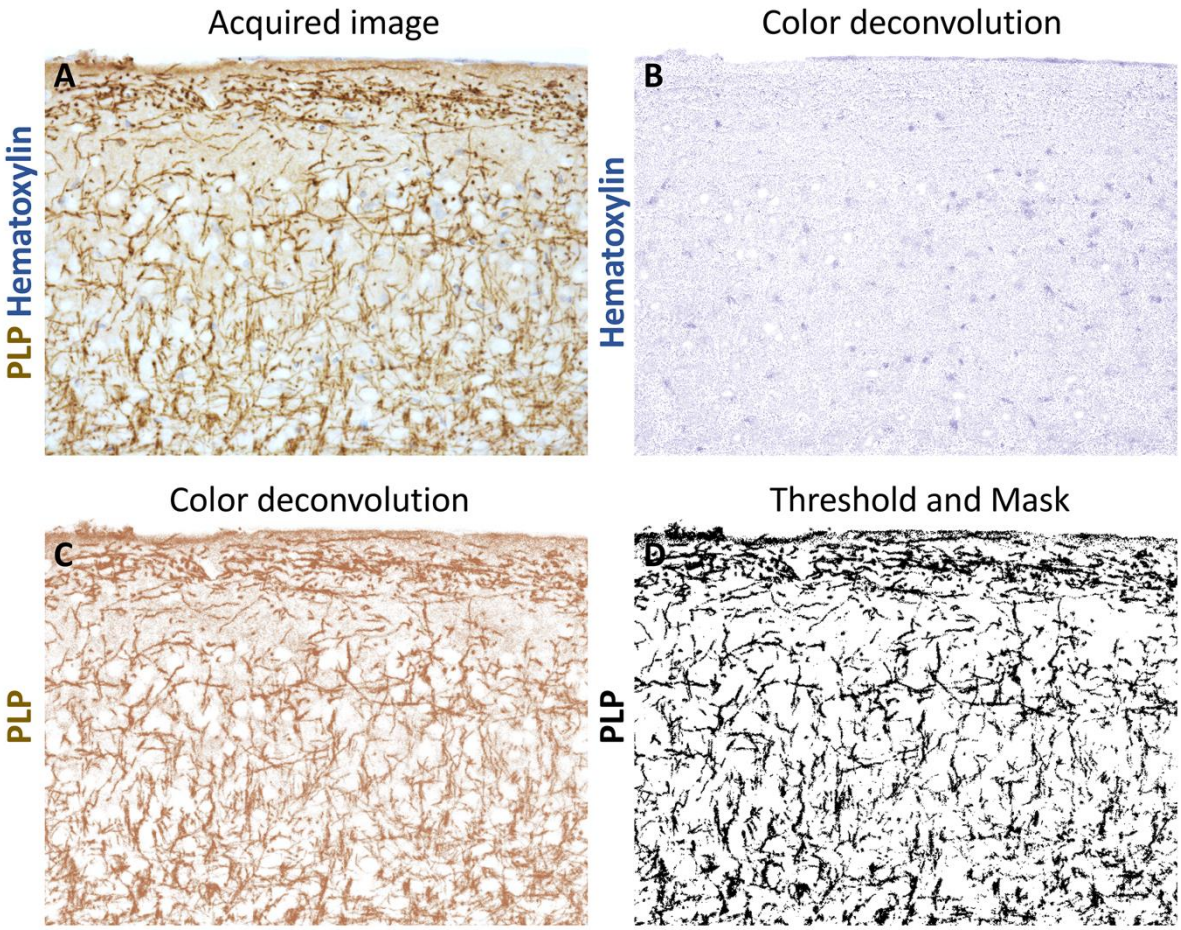


Supplementary Fig. 3. Example of anti-mouse MOG IgM plate results and dilution curves from sera. Anti-MOG IgM was measured by ELISA. Each serum sample was plated in a 1:2 serial dilution and curves were generated using a 4-parameter logistic regression. IC50 was taken from each curve and then normalized to a hMOG-immunized C57Bl/6 pooled control lot that was run on the same plate. Black curves denote hMOG-immunized C57Bl/6 mice (n=9). Red curves denote unimmunized C57Bl/6 mice (n=2). Blue curve denotes hMOG-immunized *Aicda*$^{-/-}$ mouse (n=1).


SSC-A
FSC-A
FSC-H
FSC-W
FSC-A
Aqua
CD45.2
CD45.1
CD4
CD45.1
CD45.2
CD4
FMO Controls
(Pooled, CD45.1+)
CD45.1+ T Cells
(Endogenous)
CD45.2+ T Cells
(Transferred)
GM-CSF FMO
IL-17
GM-CSF
IFNγ
IFNγ
IFNγ FMO
IL-17
IFNγ
IL-17
GM-CSF
IL-17
GM-CSF
IL-17 FMO
IL-17
IFNγ

35

Supplementary Fig. 4. Gating strategy for CD4$^+$ T cells following EAE. Samples were gated on lymphocytes>singlets>live cells as a starting point. Cells were further segmented by CD45.1 versus CD45.2 markers to determine the endogenous or transferred source of hematopoietic cells. Next, CD4$^+$ were gated from CD45$^+$ cells to visualize T cells. Finally, CD45.X$^+$CD4$^+$ were then assayed for the production of GM-CSF, IFNγ, and IL-17. Double-producers of IL-17/IFNγ and IL-17/GM-CSF were also determined from these plots. FMO (Fluorescence minus one) controls are shown from pooled samples.



Supplementary Fig. 5. Example of image processing for quantification analysis. Example of red/green/blue (RGB) image of (**A**) proteolipid protein (PLP)/Hematoxylin staining of the somatosensory cortex from a naïve mouse acquired at 20x magnification using a light microscope (Axioscope, Zeiss). Using the color deconvolution plugin in ImageJ 1.15s (National Institutes of Health), the RGB image was separated into single color channels for (**B**) hematoxylin and (**C**) PLP. (**D**) In ImageJ, the single-color channel for PLP was subjected to thresholding to create a mask that captures the specific staining. The region of interest (ROI) was outlined using the freehand tool. The ROI was then subjected to the "area fraction" measurement to quantify the percentage of thresholded staining per ROI.

Supplementary tables

Supplementary table 1: Incidence of meningeal and periventricular inflammation in A/T SJL/J EAE

	Meningeal Cortex	Meningeal Hypothalamus	Meningeal Cerebellum	Meningeal Brainstem	Periventricular (4rd ventricle)	Periventricular (3th ventricle)
Naïve	0/3	0/3	0/3	0/3	0/3	0/3
A/T SJL/J EAE acute[1]	12/12$^{\Delta}$	11/12 (92%)$^{\Delta}$	9/12 (75%)	4/12 (33%)	1/12 (8%)	5/12 (42%)

[1]Female 6-10 week old SJL/J mice received an adoptive transfer of PLP-primed Th17 $CD4^+$ T cells and were assessed for meningeal and periventricular inflammation by Hematoxylin & Eosin (HE) staining at day 11 (acute) post-adoptive transfer.
Delta ($^{\Delta}$) indicates statistically significant changes between naïve mice and A/T SJL/J EAE mice at acute stage of the disease; $p \leq 0.05$ by Kruskal-Wallis test with Dunn's correction for multiple comparisons.

Supplementary table 2. Antibodies for immunohistochemistry and flow cytometry

Primary antibody	Clone	Target	Dilution	Antigen retrieval	Source
Immunohistochemistry					
PLP	mAb, plpc1	Proteolipid protein (myelin)	1:100	EDTA	Bio-Rad, MCA839G
TPPP/p25	mAb, EPR3316	tubulin polymerization promoting protein	1:500	EDTA	Abcam, ab92305
Iba-1	mAb, ERP16589	ionized calcium binding adaptor molecule 1	1:8000	Citrate	Abcam, ab178847
GFAP	pAb	Glial fibrillary acidic protein (astrocytes)	1:4000	EDTA	Dako, Z0334
NeuN	mAb, A60	Neuronal nuclei (neurons)	1:1000	EDTA	Millipore, MAB377
mtHSP70	mAb, 30A5	Mitochondrial heat shock protein 70 (metabolic stress)	1:50	EDTA	Enzo life science, ADI-SPS-825-F
8OHG	pAb	8-Hydroxy-2′-guanosine (oxidative injury)	1:500	EDTA	Abcam, Ab10802
Fibronectin*	mAb, GW20021F	stroma	1:300	-	Sigma, 53-0452-83
CD45-AF594*	mAb, 30-F11	Leukocytes and macrophages	1:200	-	Biolegend, 103144
CD3-AF594*	mAb, 17A2	T cells	1:100	-	Biolegend, 100240
B220-AF488*	mAb, RA3-6B2	B cells	1:100	-	eBioscience, 53-0452-82
Flow cytometry					
CD4-FITC	mAb, RM4-5	MHC class II restricted T cells	1:200	-	eBioscience, 11-0042
CD4-PE	mAb, RM4-5	MHC class II restricted T cells	1:100	-	eBioscience, 12-0042

CD8-BV711	mAb, 53-6.7	MHC class I restricted T cells	1:200	-	Biolegend, 100748
CD3-APC	mAb, 17A2	T cells	1:200	-	eBioscience, 17-0032
B220-BV605	mAb,RA3-6B2	B cells	1:200	-	Biolegend, 103244
CD19-PE	mAb, eBio1D3	B cells	1:200	-	eBioscience, 12-0193
CD45.1-APCeF780	mAb, A20	Lymphocytes and macrophages	1:200	-	eBioscience, 47-0453
CD45.1-APC	mAb, A20	Lymphocytes and macrophages	1:200	-	eBioscience, 17-0453
CD45.2-eF450	mAb, 104	Lymphocytes and macrophages	1:200	-	eBioscience, 48-0454
GM-CSF-FITC	mAb, MP1-22E9	Granulocyte-macrophage colony-stimulating factor	1:200	-	eBioscience, 11-7331
IL-17a-PerCP-Cy5.5	mAb, eBio17B7	Interleukin 17 alpha	1:100	-	eBioscience, 45-7177
IFNγ-PE-Cy7	mAb, XMG1.2	Interferon gamma	1:100	-	eBioscience, 25-7311
Brefeldin A					
mAb, monoclonal antibody; pAb, polyclonal antibody; EDTA, 10mM Tris 1mM EDTA buffer pH 9.0; Citrate, 10mM citrate buffer pH 6.0; the asterisk indicates antibodies used on frozen sections					