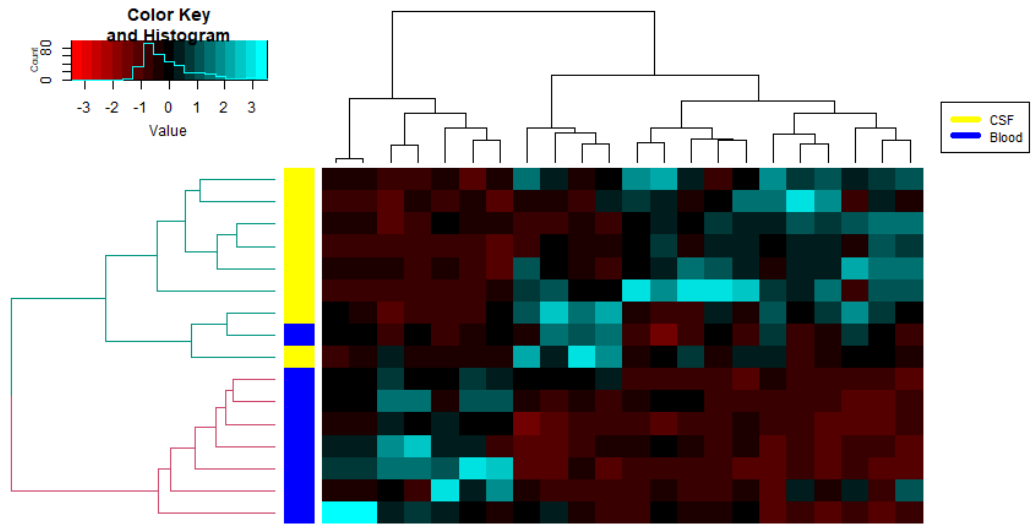
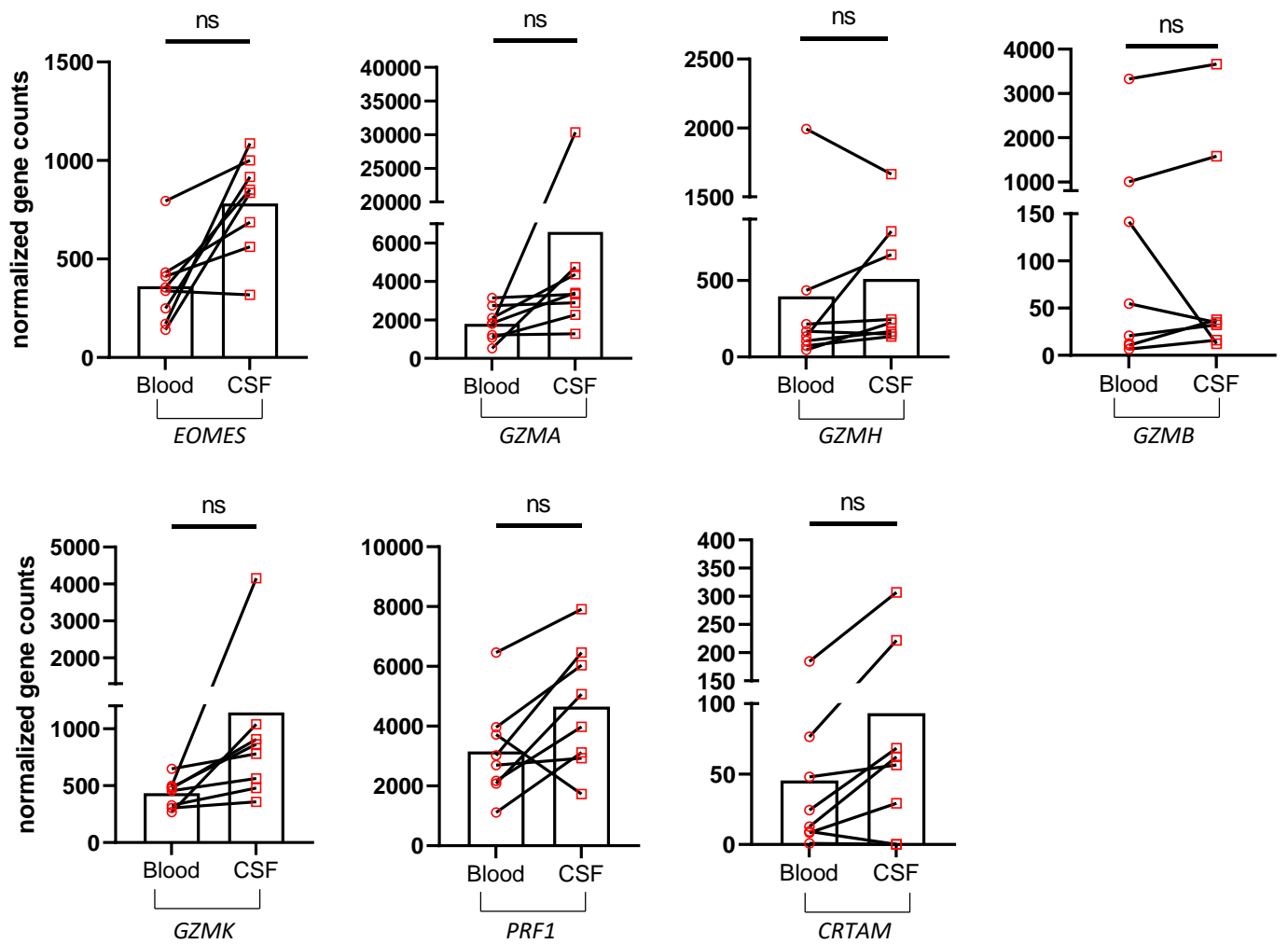


# eFigure 1

A



B



**eFigure 1. RNA sequencing analysis of CSF-T non fh (Tnfh) cells from CIS patients.** (A) Clustering analysis of differentially expressed genes of CSF-Tnfh cells compared to blood Tnfh cells in CIS patients. Red and blue represent low and high expression levels, respectively. (B) Representative plots of normalized gene counts for *EOMES* and genes implicated in

cytotoxicity (*GZMA*, *GZMH*, *GZMK*, *PRF1* and *CRTAM*) in Tnfh-CSF cells compared to paired blood. p-values were calculated using the DESeq2 script. Bars indicate the mean.

eTable 1

Cohort	Subjects, n	Form of disease, n	Gender, female, n (%)	Age in years, median (range) <sup>a</sup>	Disease duration in months, median (range) <sup>b</sup>	EDSS, median (range)	Diagnosis at last follow-up
<b>Peripheral Tfh cell phenotype</b>							
HC	39	NA	28 (70)	36 (21-51)	NA	NA	
RRMS in relapse <sup>c</sup>	18	-	13 (72)	32 (18-54)	1 (0.25-240)	2 (1-7)	
RRMS in remission	23	-	19 (80)	36 (22-53)	24 (1-348)	1 (0-3.5)	
<b>Tfh cell phenotype in CSF and paired blood</b>							
RRMS	10	Relapse, 7	6 (60)	30 (18-46)	0.75 (0.3-144)	1.25 (0-2)	
		Remission, 3					
<b>Transmigration assays of Tfh cells</b>							
HC	8	NA	8 (100)	37 (22-44)	NA	NA	
<b>Luminex analysis of cytokines and chemokines concentration in the CSF</b>							
NIND	8	NA	5 (63)	36 (23-49)	NA	NA	
RRMS	22	Relapse, 11	15 (68)	29.5 (23-46)	0.9 (0-84)	1 (0-4)	
		Remission, 11					
<b>RNAseq analysis of CSF-infiltrating Tfh cells</b>							
CIS	8	Relapse, 7	6 (75)	24.5 (18-44)	0.5 (0.1-2)	2.5 (0-7)	6 MS conversion, 2 high-risk CIS

**eTable 1. Clinical and demographic characteristics of patients and controls**<sup>a</sup>At the time of sampling<sup>b</sup>Time from diagnosis to sampling<sup>c</sup>RRMS according to the McDonald (2017) criteria

EDSS = expanded disease severity score; HC = healthy controls; RRMS = relapsing-remitting multiple sclerosis; PMS = progressive multiple sclerosis; NIND = non-inflammatory neurological disease; CIS : Clinically Isolated Syndrome.

eTable 2

Gene symbol	Adjusted p-value	Fold-change
<b>List of genes implicated in lymphocyte activation pathway</b>		
HLA-DPB1	0.019301	1.937085
HLA-DRB1	0.011878	2.025261
PRF1	0.01881	1.858922
SLAMF1	0.001308	1.818446
EOMES	0.000644	2.649259
TNFSF14	4.29E-05	2.642076
FADD	0.019206	1.962298
TBX21	0.001308	2.201296
EXOSC3	0.022725	1.867988
CRTAM	0.049593	2.004678
SLAMF7	0.015849	2.076454
TNFAIP8L2	0.001308	2.103573
LDLR	0.000179	2.931987
PLEK	0.008907	2.095458
CST7	0.008256	2.084952
<b>List of genes implicated in canonical IL-12 pathway</b>		
FASLG	0.003253	2.184575
GZMA	0.000894	2.537099
IL1R1	0.039516	-1.54976
EOMES	0.000644	2.649259
TBX21	0.001308	2.201296

**Table 2. List of differentially expressed genes in CSF cTfh cells implicated in lymphocyte activation pathway and canonical IL-12 pathway.**