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Supplemental Information

Chronic Peripheral Inflammation Causes a Region-Specific Myeloid Response in the Central Nervous System

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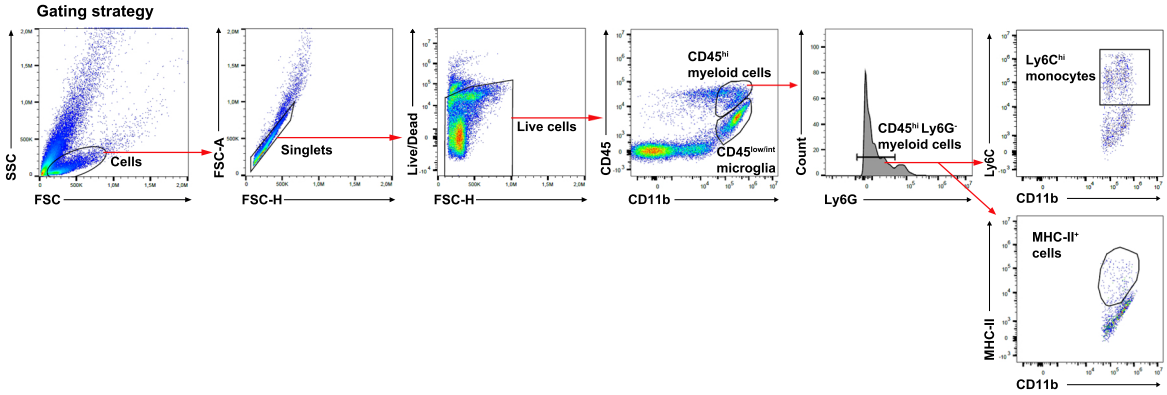
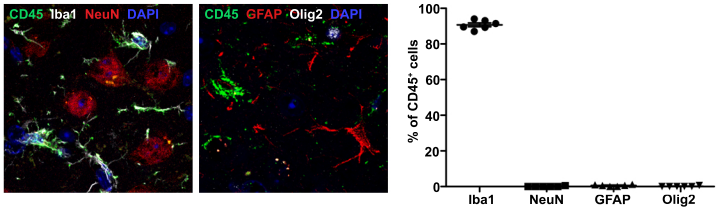

Gating strategy
SSC
FSC
Cells
FSC-A
FSC-H
Singlets
Live/Dead
FSC-H
Live cells
CD45
CD11b
CD45hi
myeloid cells
CD45low/int
microglia
Count
Ly6G
CD45hi Ly6G-
myeloid cells
Ly6C
CD11b
Ly6Chi
monocytes
MHC-II
CD11b
MHC-II+
cells

Figure S1

Figure S1. Gating strategy for flow cytometry experiments. Related to Figures 1,2, and 6. Cells were gated on CD11b and CD45 for the separation of $CD11b^{+}CD45^{low/int}$ homeostatic microglia from $CD11b^{+}CD45^{hi}$ myeloid cells. $CD11b^{+}CD45^{hi}$ myeloid cells were further gated on $Ly6G^{-}$ to exclude granulocytes and characterized regarding Ly6C and MHC-II expression levels.

a


CD45 Iba1 NeuN DAPI
CD45 GFAP Olig2 DAPI
% of CD45+ cells
100
80
60
40
20
0
Iba1
NeuN
GFAP
Olig2

b

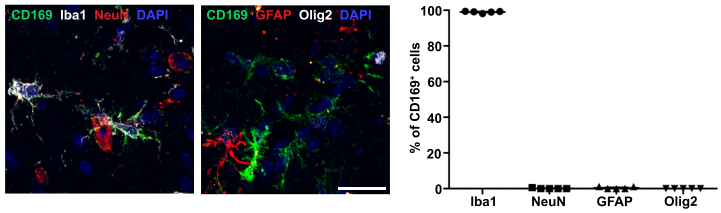
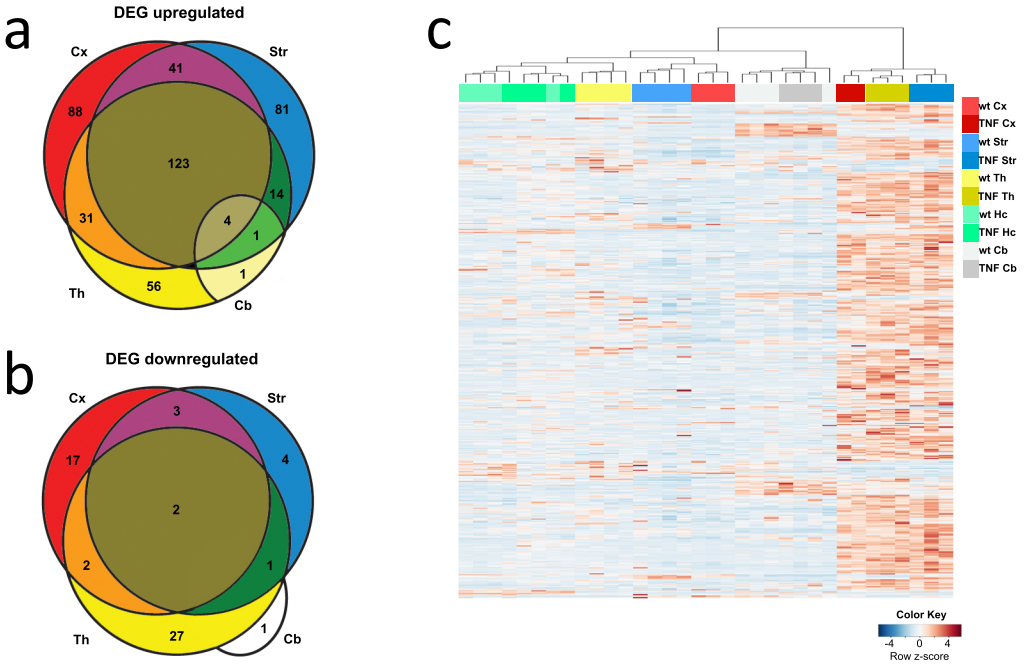
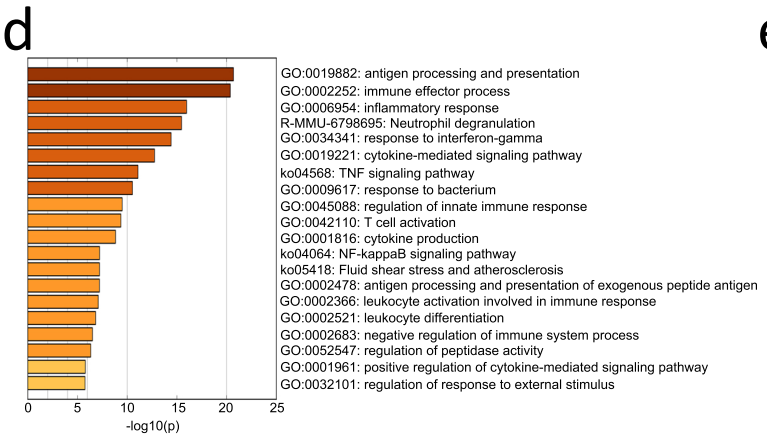
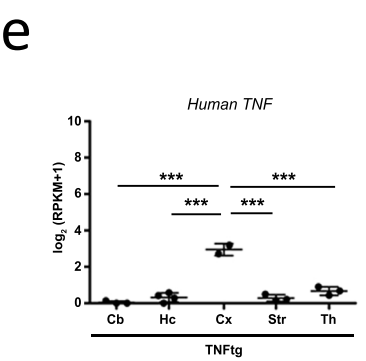

CD169 Iba1 NeuN DAPI
CD169 GFAP Olig2 DAPI
% of CD169+ cells
100
80
60
40
20
0
Iba1
NeuN
GFAP
Olig2

Figure S2

Figure S2. Specific expression of CD45 and CD169 in myeloid cells. Related to Figures 2 and 6. (a) Expression rates of Iba1, NeuN, GFAP, and Olig2 in $CD45^+$ cells. (b) Expression rates of Iba1, NeuN, GFAP, and Olig2 in $CD169^+$ cells. Scale bars: 20 µm. Data are derived from the thalamus of TNFtg mice and representative of n=6 (a) or n=5 (b) animals. Data are shown as mean ± S.E.M.


a
DEG upregulated
Cx
Str
Th
Cb
41
88
81
123
14
31
4
1
56
1
b
DEG downregulated
Cx
Str
Th
Cb
3
17
4
2
2
1
27
1
c
wt Cx
TNF Cx
wt Str
TNF Str
wt Th
TNF Th
wt Hc
TNF Hc
wt Cb
TNF Cb
Color Key
-4
0
4
Row z-score


d
GO:0019882: antigen processing and presentation
GO:0002252: immune effector process
GO:0006954: inflammatory response
R-MMU-6798695: Neutrophil degranulation
GO:0034341: response to interferon-gamma
GO:0019221: cytokine-mediated signaling pathway
ko04568: TNF signaling pathway
GO:0009617: response to bacterium
GO:0045088: regulation of innate immune response
GO:0042110: T cell activation
GO:0001816: cytokine production
ko04064: NF-kappaB signaling pathway
ko05418: Fluid shear stress and atherosclerosis
GO:0002478: antigen processing and presentation of exogenous peptide antigen
GO:0002366: leukocyte activation involved in immune response
GO:0002521: leukocyte differentiation
GO:0002683: negative regulation of immune system process
GO:0052547: regulation of peptidase activity
GO:0001961: positive regulation of cytokine-mediated signaling pathway
GO:0032101: regulation of response to external stimulus
0
5
10
15
20
25
-log10(p)


e
Human TNF
log2 (RPKM+1)
10
8
6
4
2
0
***
***
***
***
Cb
Hc
Cx
Str
Th
TNFtg

FigureS3

Figure S3. Regional inflammatory response in the brain of TNFtg mice revealed by bulk RNA-seq. Related to Figure 3. (a) Venn diagram showing the regional overlap of upregulated genes in TNFtg mice. (b) Venn diagram showing the regional overlap of downregulated genes in TNFtg mice. (c) Heat map showing hierarchical clustering of all individual samples based on n=497 genes differentially expressed in brain regions of TNFtg mice compared to wt mice. (d) Heat map indicating the most significantly enriched GO-terms in n=127 genes commonly upregulated in the cortex (Cx), striatum (Str), and thalamus (Th) of TNFtg mice. (e) Expression levels of human TNF in the Cx, Str, Th, Hc and Cb of TNFtg mice. FASTQ files were mapped onto UCSC genome build hg38. Data are depicted as mean ± S.E.M., ***p<0.001.

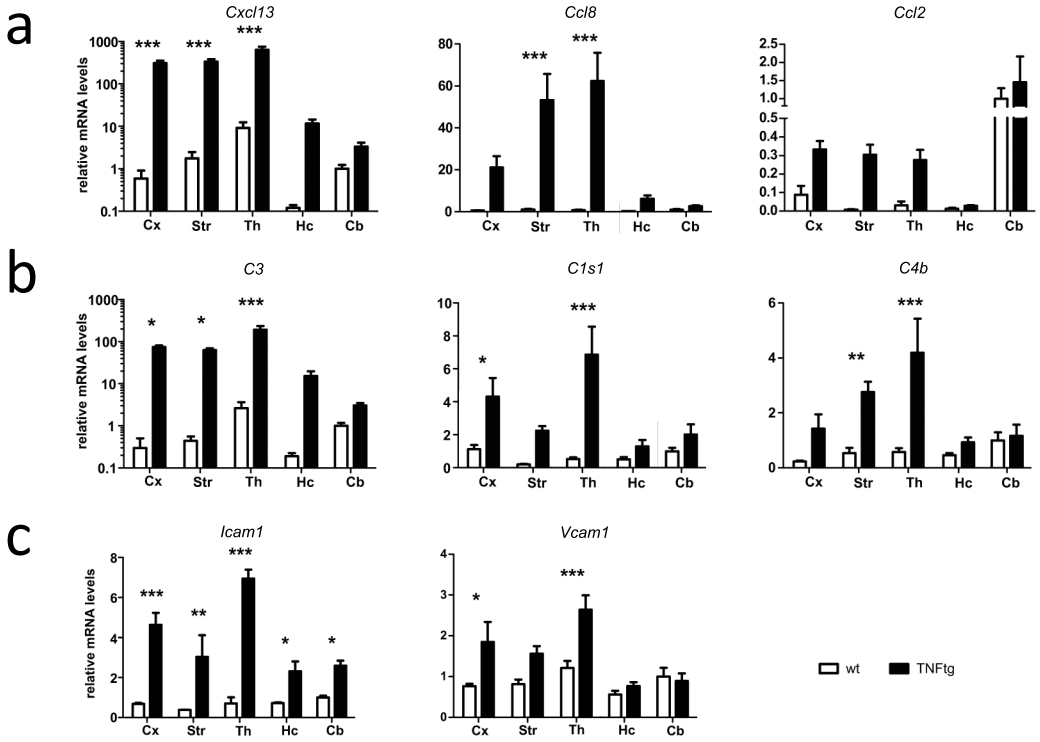

a
Cxcl13
Ccl8
Ccl2
relative mRNA levels
Cx
Str
Th
Hc
Cb
b
C3
C1s1
C4b
c
Icam1
Vcam1
wt
TNFtg

Figure S4

Figure S4. Regional transcriptional inflammatory response in the brain of TNFtg mice confirmed by qPCR. Related to Figure 3. (a) Transcriptional levels of the chemokines *Cxcl13*, *Ccl8*, and *Ccl2* in brain regions of wt and TNFtg mice. (b) Regional transcriptional levels of genes encoding the complement factors *C3*, *C1s1*, and *C4b*. (c) Regional mRNA levels of *Icam1* and *Vcam1* in wt and TNFtg mice. Data are representative of n=4 animals per genotype and region from two independent experiments and are presented as mean ± S.E.M. Analysis was performed using two-way ANOVA followed by Bonferroni's post-hoc test, *p<0.05, **p<0.01, ***p<0.001.

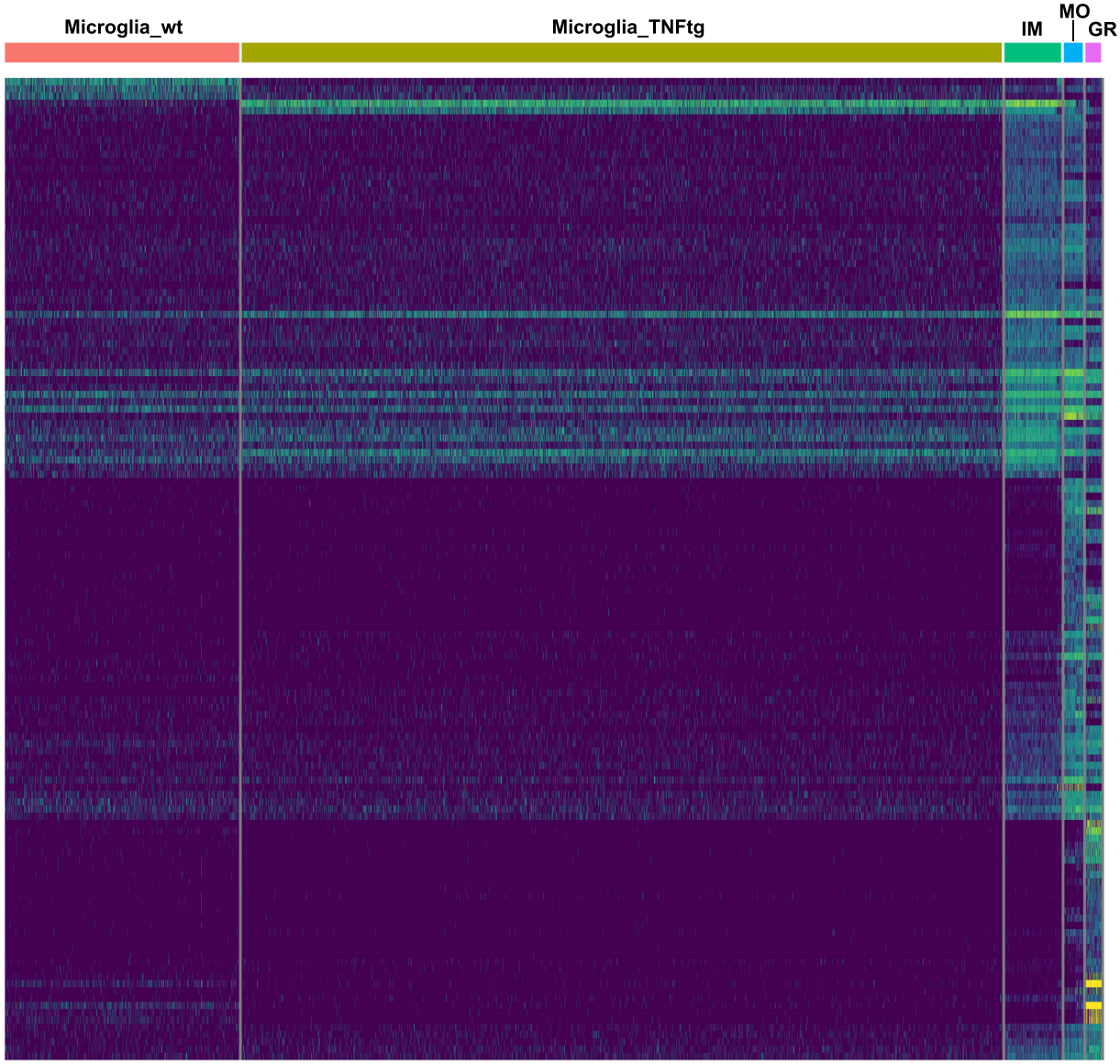
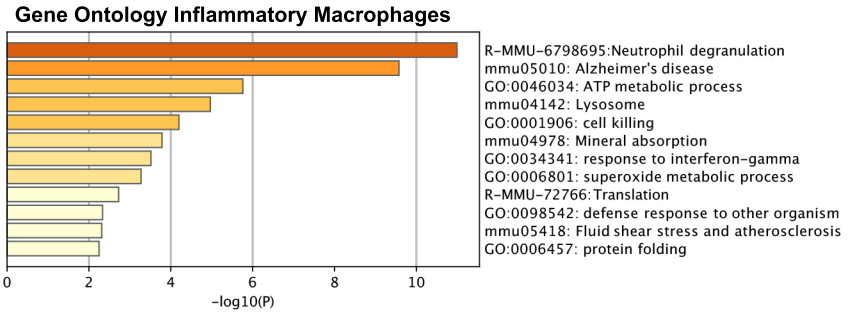


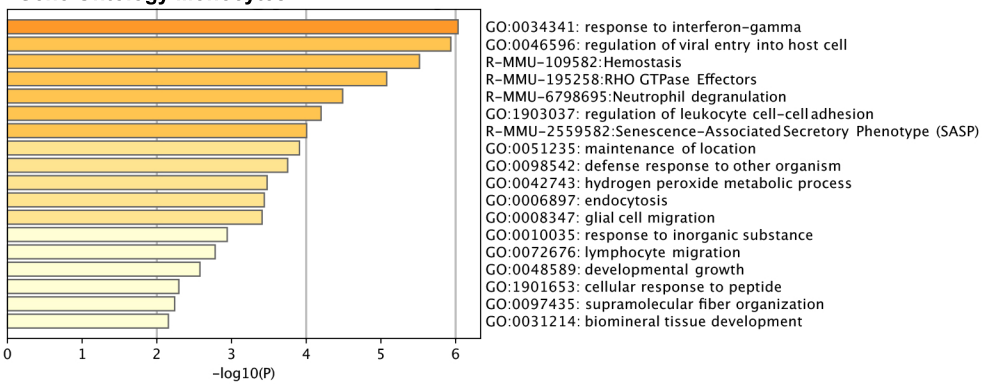
Figure S5

Figure S5. Heat map of differentially expressed genes in myeloid cell clusters. Related to Figure 4. IM: Inflammatory macrophages, MO: Monocytes, GR: Granulocytes.

a



b



c

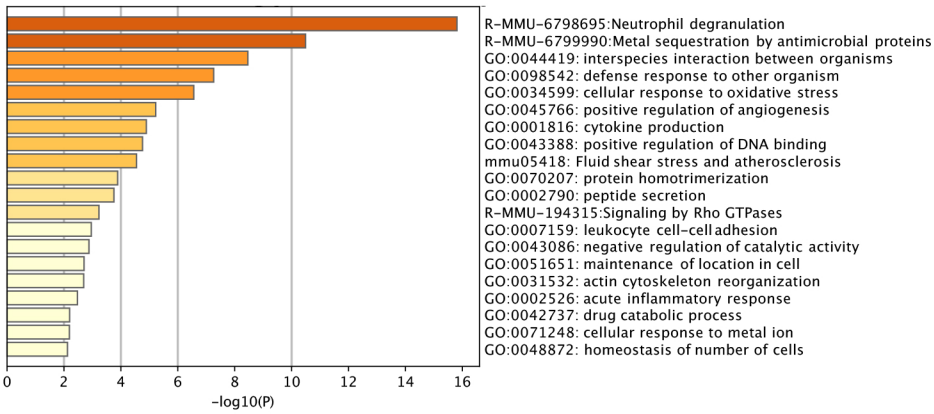


Figure S6

Figure S6. Gene ontology of myeloid clusters identified by scRNA-seq. Related to Figure 4. (a) Enriched Gene Ontology (GO) terms in the inflammatory macrophage cluster (cluster 2). (b) Enriched GO terms in the cluster of monocytes. (c) Enriched GO terms in granulocytes.

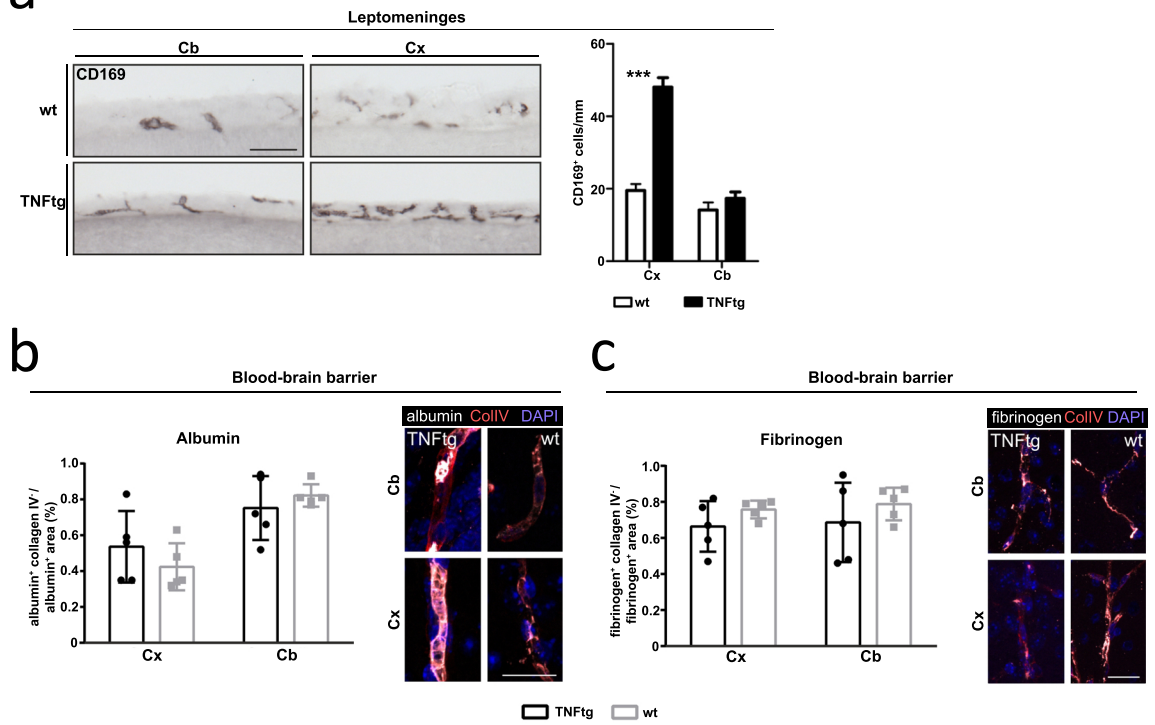

a
Leptomeninges
Cb
Cx
CD169
wt
TNFtg
***
CD169+ cells/mm
60
40
20
0
Cx
Cb
wt
TNFtg
b
Blood-brain barrier
Albumin
albumin+ collagen IV+ / albumin+ area (%)
1.0
0.8
0.6
0.4
0.2
0
Cx
Cb
albumin ColIV DAPI
TNFtg
wt
Cb
Cx
c
Blood-brain barrier
Fibrinogen
fibrinogen+ collagen IV+ / fibrinogen+ area (%)
1.0
0.8
0.6
0.4
0.2
0
Cx
Cb
fibrinogen ColIV DAPI
TNFtg
wt
Cb
Cx
TNFtg
wt
d
wt/TNFtg mice
Evans Blue
(25 mg/kg i.v.)
Analysis
4h
0
1
14
age (weeks)
Lung

Figure S7

Figure S7. Analysis of CNS borders in TNFtg mice. Related to Figure 5. (a) Densities of $CD169^+$ leptomeningeal macrophages in vicinity to the cortex (Cx) and cerebellum (Cb) of wt (n=5) and TNFtg mice (n=6). Scale bar: 50 µm. (b, c) Intensity-adjusted fraction of albumin (b)- and fibrinogen (c)-positive area not overlapping with collagen IV (ColIV) staining in the cortex (Cx) and cerebellum (Cb) of wt and TNFtg mice (n=5 per group). Scale bars: 20 µm. (d) Experimental scheme of Evans Blue injection and in-situ image of the lung as a positive control for Evans Blue uptake. Data are shown as mean ± S.E.M. Analysis was performed using two-way ANOVA followed by Bonferroni's post-hoc test, ***p<0.001.