nature immunology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41590-018-0091-5

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Fatal demyelinating disease is induced by monocyte-derived macrophages in the absence of TGF-β signaling

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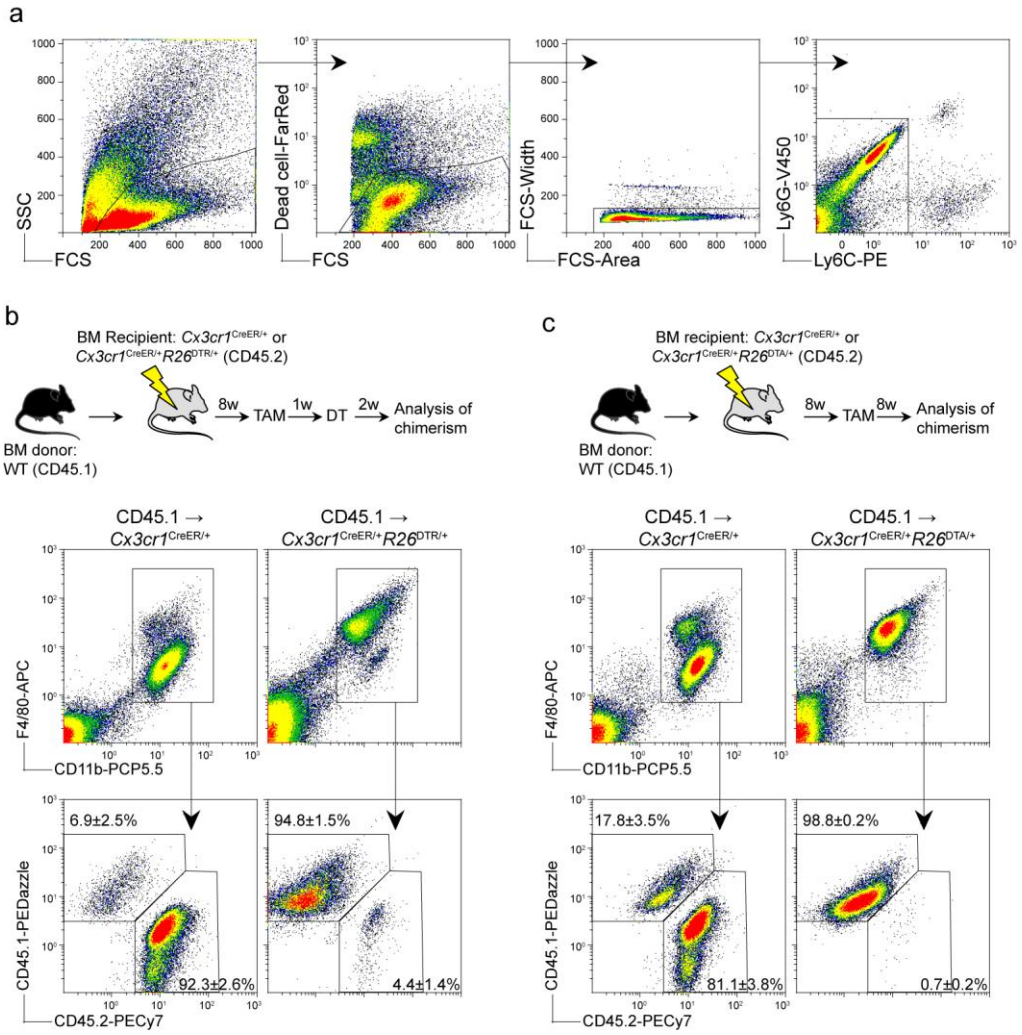
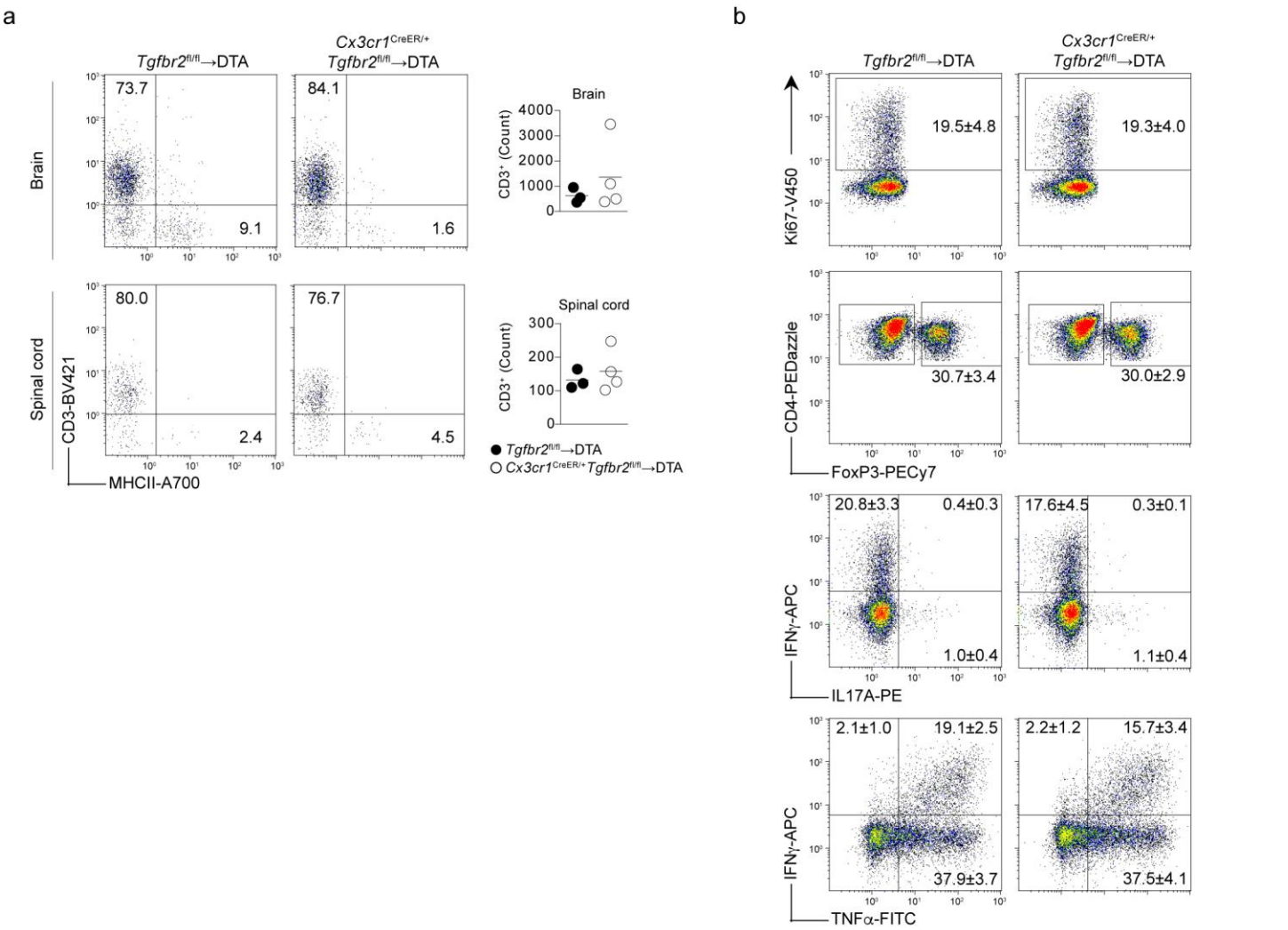


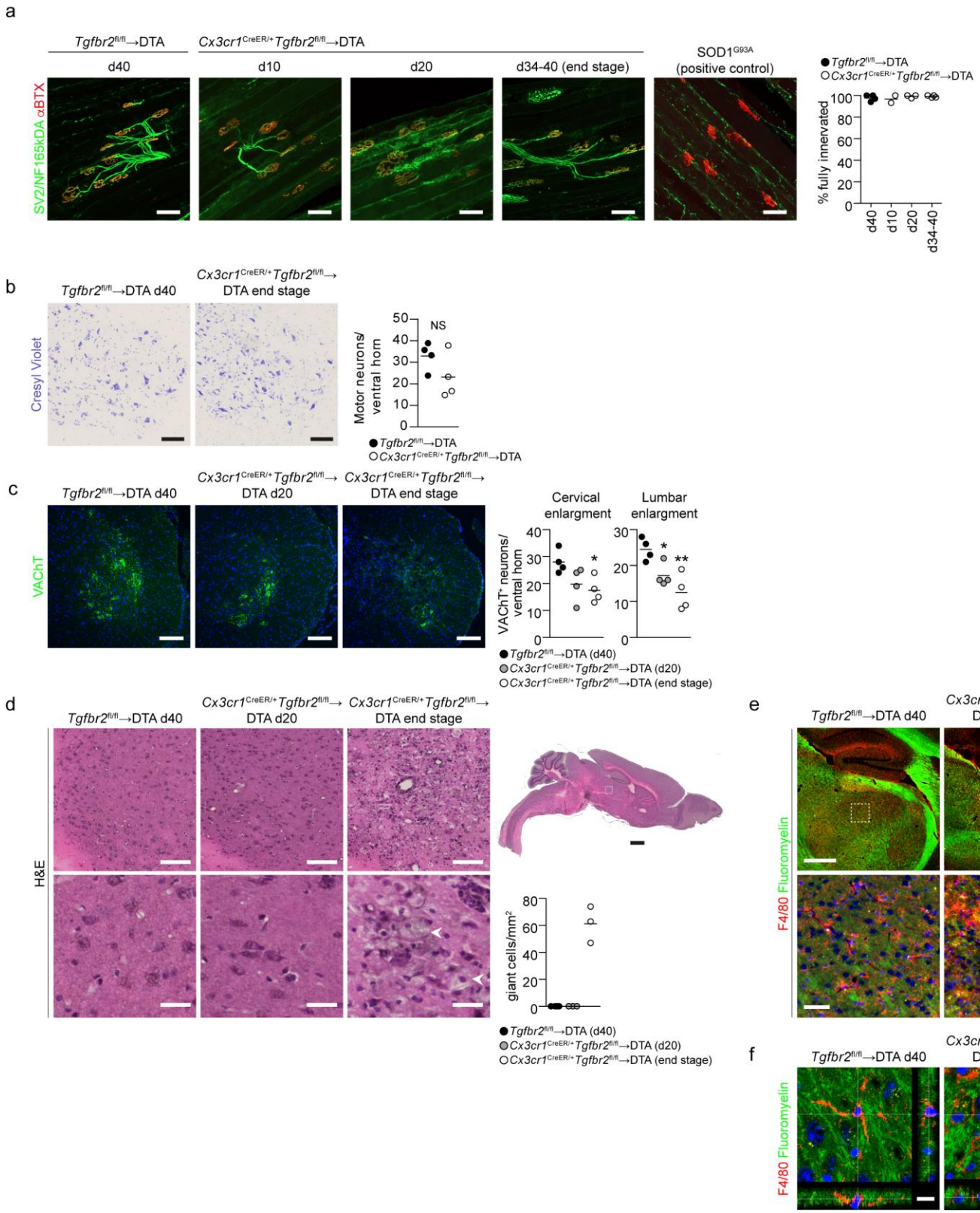
Supplementary Figure 1

Repopulation of microglia by peripheral myeloid cells in chimeric *Cx3cr1*$^{CreER/+}$*R26*$^{DTR/+}$ and *Cx3cr1*$^{CreER/+}$*R26*$^{DTA/+}$ mice (**a**) Gating strategy of CNS cells employed in the study. (**b-c**) Analysis of CNS, top panel gated on live, singlet and Ly6C^{-}Ly6G^{-} cells in (**b**) CD45.1→*Cx3cr1*$^{CreER/+}$*R26*$^{DTR/+}$ and (**c**) CD45.1→*Cx3cr1*$^{CreER/+}$*R26*$^{DTA/+}$ chimeras. Values in dot plots are mean±s.d. of (**b**) n=5 and 6 mice from two pooled experiments and (**c**) n=5 mice/group from one of two independent experiments.



Supplementary Figure 2

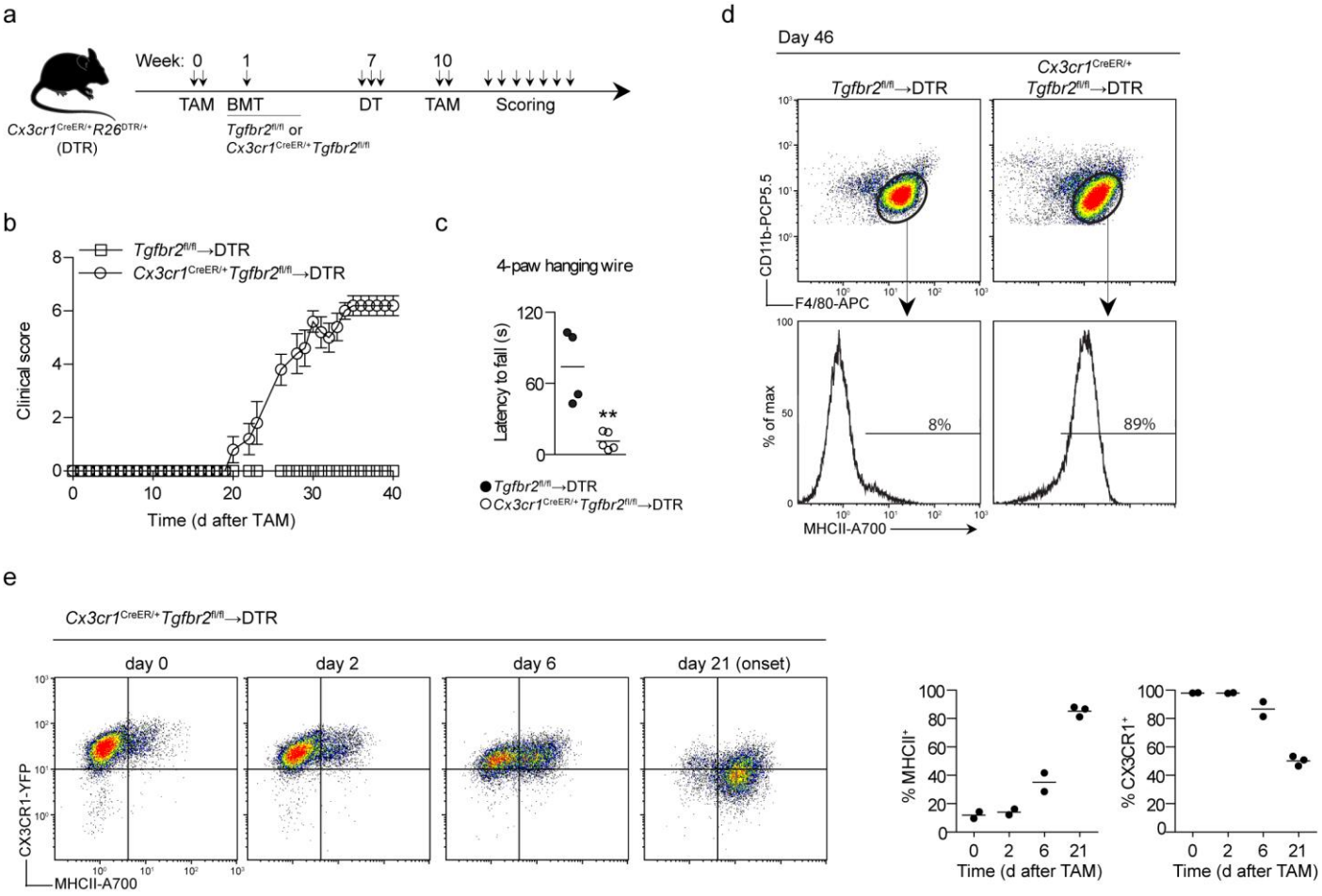
Lack of adaptive immunological changes in *Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$→DTA chimeras
(**a**) Analysis of T cell (CD11b^{-}CD45^{hi}CD3^{+}) and B cell (CD11b^{-}CD45^{hi}MHCII^{+}) numbers in the brain and spinal cord of the indicated chimeras at day 20 after TAM. Lines in graphs represent mean values of n=3 and 4 mice. The experiment was performed once. (**b**) Analysis of proliferating T cells, CD4^{+}FoxP3^{+} regulatory T cells and IFN-γ, IL-17 and TNF-α producing CD4^{+} T cells in the spleens of the indicated chimeras after onset of motor symptoms (day 20 after TAM). Values in plots are mean±s.d. of n=6 and 5 mice. The experiment was performed once.



Supplementary Figure 3

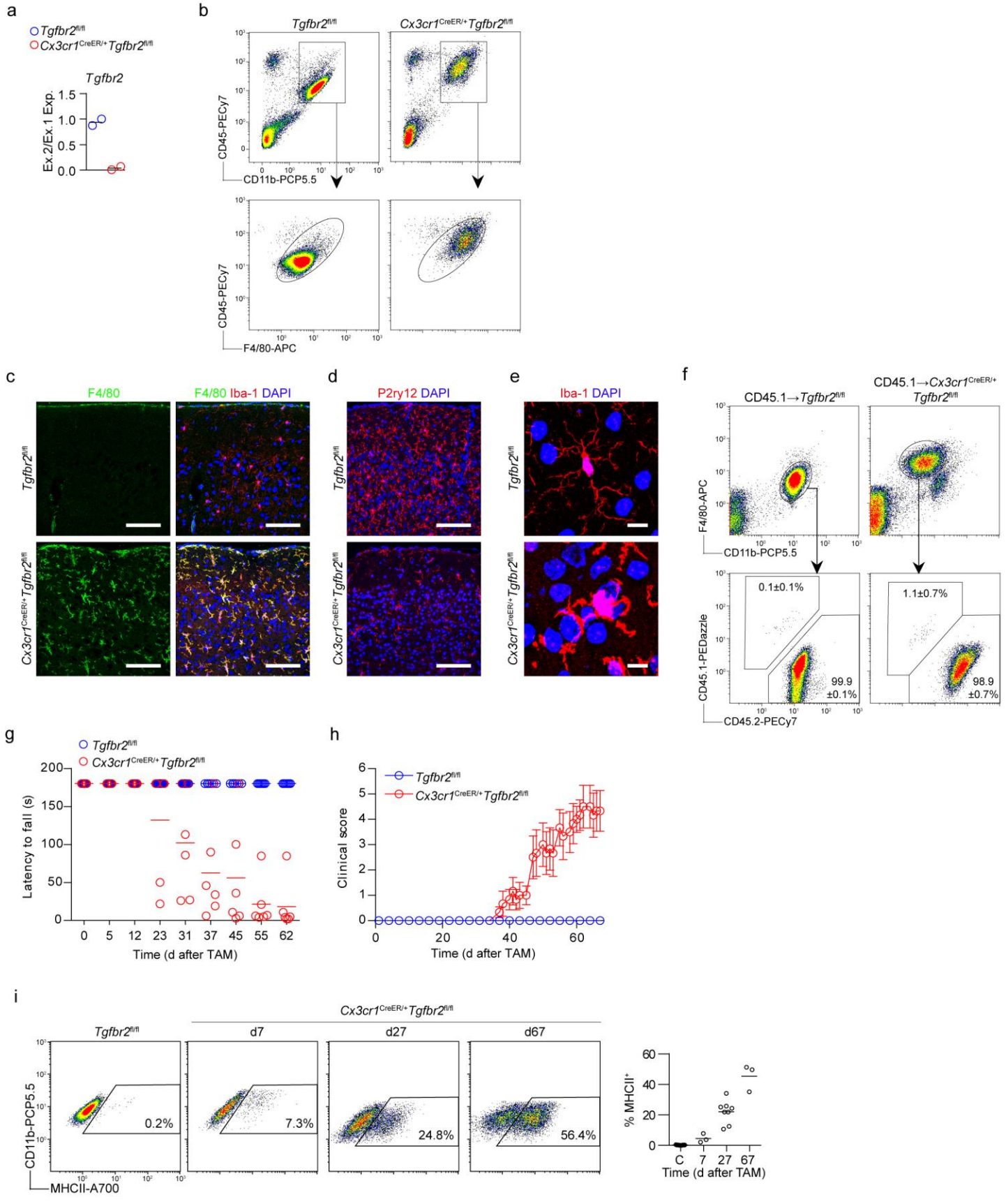
Analysis of lower motor neuron pathology and thalamic lesions in *Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$→DTA chimeras

(**a**) Analysis of innervation of neuromuscular junctions in the tibialis anterior muscle of *Tgfbr2*$^{fl/fl}$→DTA and *Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$→DTA chimeras. Innervation was quantified by assessing overlay of synaptic vesicle protein (SV2) and neurofilament (NF) 165 kDA staining with α-BTX labeled endplates. Positive control is tibials anterior muscle from SOD^{G93A} (day 140) mice illustrating complete denervation. Lines in graph are mean values of n = 4, 2, 3, 4 mice/group and at least 40 motor endplates/mouse. Scale bar 50µm. (**b**) Enumeration of cresyl violet stained motor neurons (>200µm^2) in the dorsal horns of the cervical enlargement. Lines in graph are mean values of n=4 mice/group and at least 5 sections/mouse. NS=not significant P=0.1633 Student's two-tailed unpaired t-test. Scale bar 100µm. (**c**) Enumeration of VAChT$^+$ motor neurons in the ventral horns of the spinal cord. Representative images are taken from the lumbar enlargement. Lines are mean values of n= 4, 3, 4 mice and at least 4 sections/mouse. * P= 0.0354 (cervical) and * P=0.0487 ** P=0.0034 (lumbar) 1-way ANOVA Dunnett's Multiple Comparison test. Scale bar 200µm. (**d**) Hematoxylin and eosin staining visualizing lesions in the thalamus in end stage KO→DTA mice. Lesions were quantified based on the presence of giant cells (white arrow) with an area >150µm^2. Lines are mean values of n=3 mice/group and 2 sections/mouse. Scale bar 100µm (top panel) and 25µm (bottom panel). (**e**) F4/80 and fluoromyelin staining in the thalamus. Images are representative of n=3 mice/group. Scale bar 500µm and 50µm. (**f**) Confocal images of F4/80 and fluoromyelin staining in the thalamus. Images are representative of n=3 mice/group. Scale bar 10µm.



Supplementary Figure 4

Loss of *Tgfbr2* in CX3CR1^{+} macrophages after niche colonization leads to onset of motor phenotype
(**a**) Experimental setup of *Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$→DTR chimeras. DTR mice were given TAM (two doses, 48h apart) to recombine CNS-resident microglia, followed by irradiation and bone marrow transplantation (BMT) from *Tgfbr2*$^{fl/fl}$ or *Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$ donors one week later. After reconstitution 6 weeks later, mice were given DT to deplete microglia. *Tgfbr2* expression was targeted in repopulated CX3CR1^{+} monocyte-derived macrophages 3 weeks later by TAM administration. (**b**) Clinical score assessing motor symptoms on the indicated days after TAM. Values in plot are mean±s.e.m. of n=4 and 5 mice. The experiment was performed once. (**c**) 4-paw hanging wire test to assess grip strength performed at onset of motor symptoms (day 20 after TAM). Lines are mean values of n=4 and 5 mice. ** P=0.0032. Student's two-tailed unpaired t-test. (**d**) Analysis of CNS by flow cytometry at day 46 (end stage). Top panel gated on CD11b^{+}CD45^{+}Ly6C^{-}. The data is representative of n=2 mice/group. (**e**) Kinetic analysis of the transformation of monocyte-derived macrophages after TAM. Gated on CD11b^{+}CD45^{+}Ly6C^{-}Ly6G^{-}F4/80^{hi}. Lines in graphs are mean values of n=2, 2, 2, 3 mice.


a
Tgfbr2fl/fl
Cx3cr1CreER/+Tgfbr2fl/fl
Tgfbr2
Ex.2/Ex.1 Exp.
b
Tgfbr2fl/fl
Cx3cr1CreER/+Tgfbr2fl/fl
CD45-PECy7
CD11b-PCP5.5
F4/80-APC
c
F4/80
F4/80 Iba-1 DAPI
d
P2ry12 DAPI
e
Iba-1 DAPI
f
CD45.1→Tgfbr2fl/fl
CD45.1→Cx3cr1CreER/+ Tgfbr2fl/fl
F4/80-APC
CD11b-PCP5.5
0.1±0.1%
99.9 ±0.1%
1.1±0.7%
98.9 ±0.7%
CD45.1-PEDazzle
CD45.2-PECy7
g
Latency to fall (s)
Time (d after TAM)
h
Clinical score
Time (d after TAM)
i
d7
d27
d67
0.2%
7.3%
24.8%
56.4%
CD11b-PCP5.5
MHCII-A700
% MHCII+
C
7
27
67
Time (d after TAM)

Supplementary Figure 5

Loss of *Tgfbr2* in CNS-resident microglia leads to slowly progressing motor disease
(**a**) qRT-PCR analysis of sorted Ly6C^{-}CD11b^{+}F4/80^{low} (*Tgfbr2*$^{fl/fl}$) and Ly6C^{-}CD11b^{+}F4/80^{hi} (*Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$) microglia day 7 after TAM. Deletion of *Tgfbr2* was assessed by the expression of the floxed exon 2 divided by the unfloxed exon 1. n=2 samples/group sorted from pools of 1-5 mice/sample. (**b**) CNS analysis by flow cytometry 7 days after TAM. Top panel is gated on live, singlet and Ly6C^{-}Ly6G^{-} cells. Dot plots are representative of three independent experiments. (**c-d**) Immunofluorescence for (**c**) F4/80 and Iba-1 or (**d**) P2ry12 in cortex day 7 after TAM. Images are representative of n=3mice/group. Scale bar 100µm. (**e**) Confocal images of one Iba-1^{+} microglial cell in the cortex to visualize morphology. Images are representative of n=3 mice/group. Scale bar 10µm. (**f**) CNS analysis of head-protected CD45.1→*Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$ chimeras analyzed 3 weeks after TAM administration. Top panel is gated on live, singlet and Ly6C^{-}Ly6G^{-} cells. Values in plots are mean±s.d. of n=7 and 6 mice. The experiment was performed once. (**g-h**) Development of motor symptoms in n=7 *Tgfbr2*$^{fl/fl}$ and n=6 *Cx3cr1*$^{CreER/+}$*Tgfbr2*$^{fl/fl}$ mice. (**g**) 4-paw hanging wire test to assess grip strength (max time 180 sec) performed on the indicated days after TAM. Lines represent mean values. (**h**) Clinical score assessing motor symptoms. Values are mean±s.e.m. The experiment was performed once. (**i**) Time course analysis of MHCII surface expression on CD11b^{+}CD45^{+}Ly6C^{-}Ly6G^{-} microglia on the indicated days after TAM. Lines represent mean values of n=15, 3, 8, 3 mice.