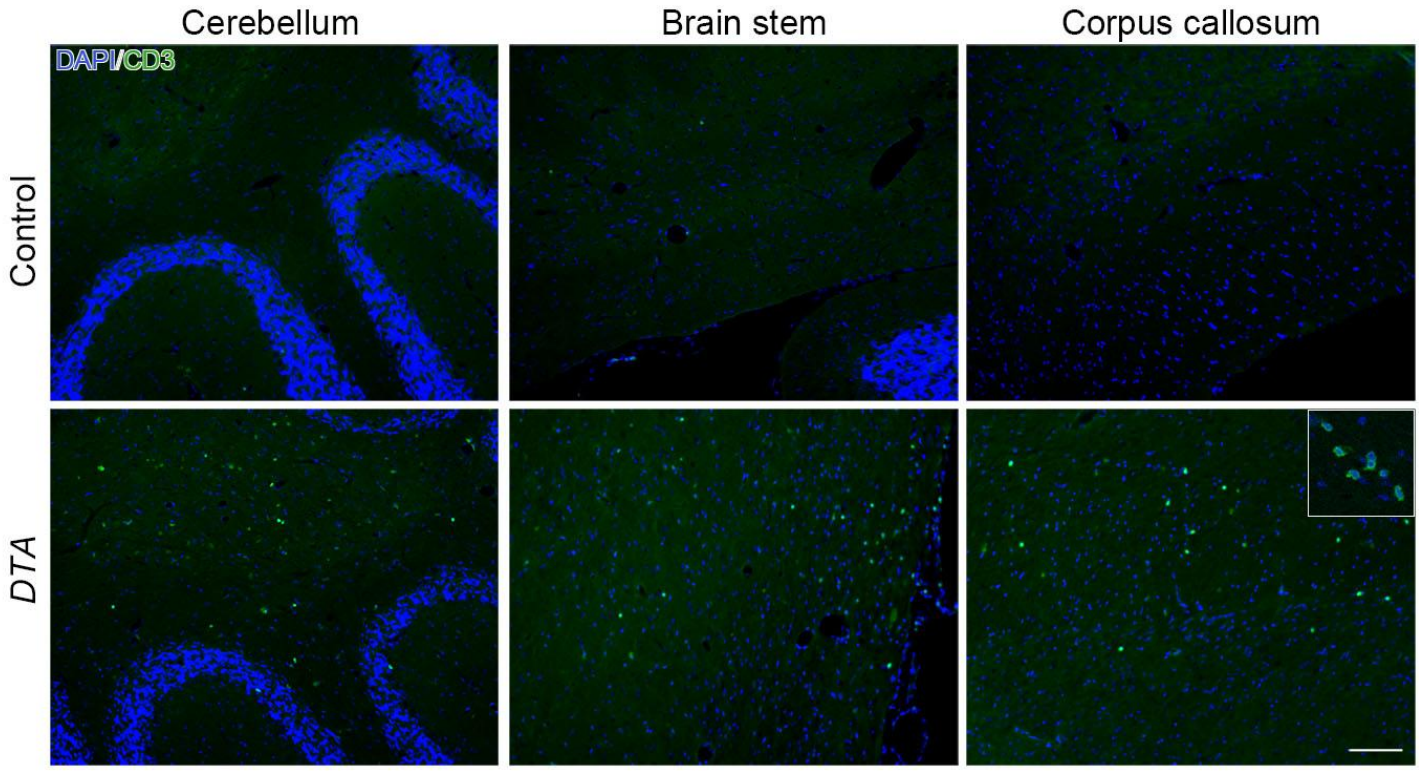
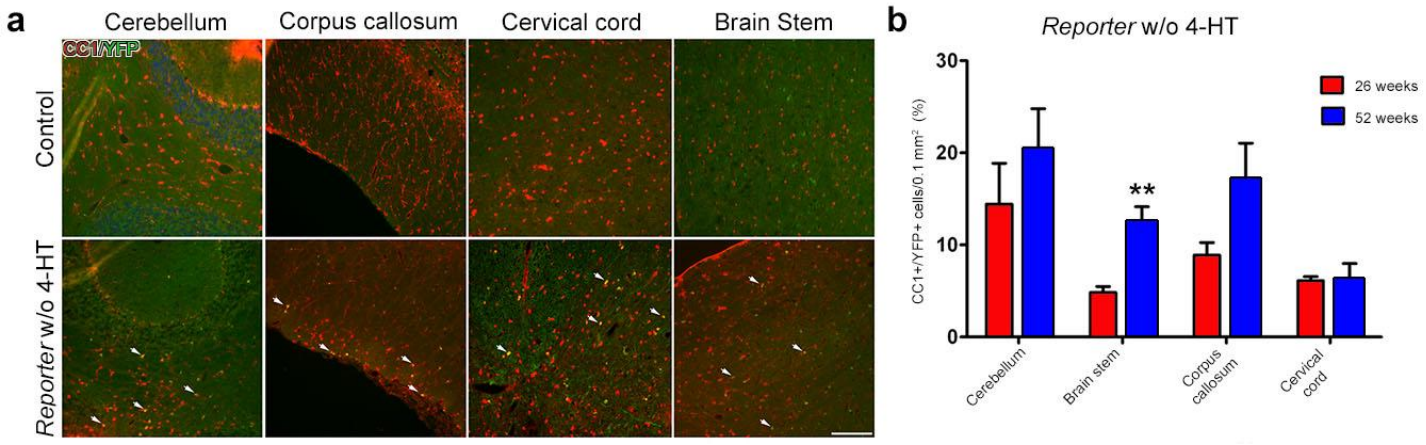



Supplementary Figure 1

**T cell infiltration in the CNS of tamoxifen-treated DTA mice during the late-onset disease.**

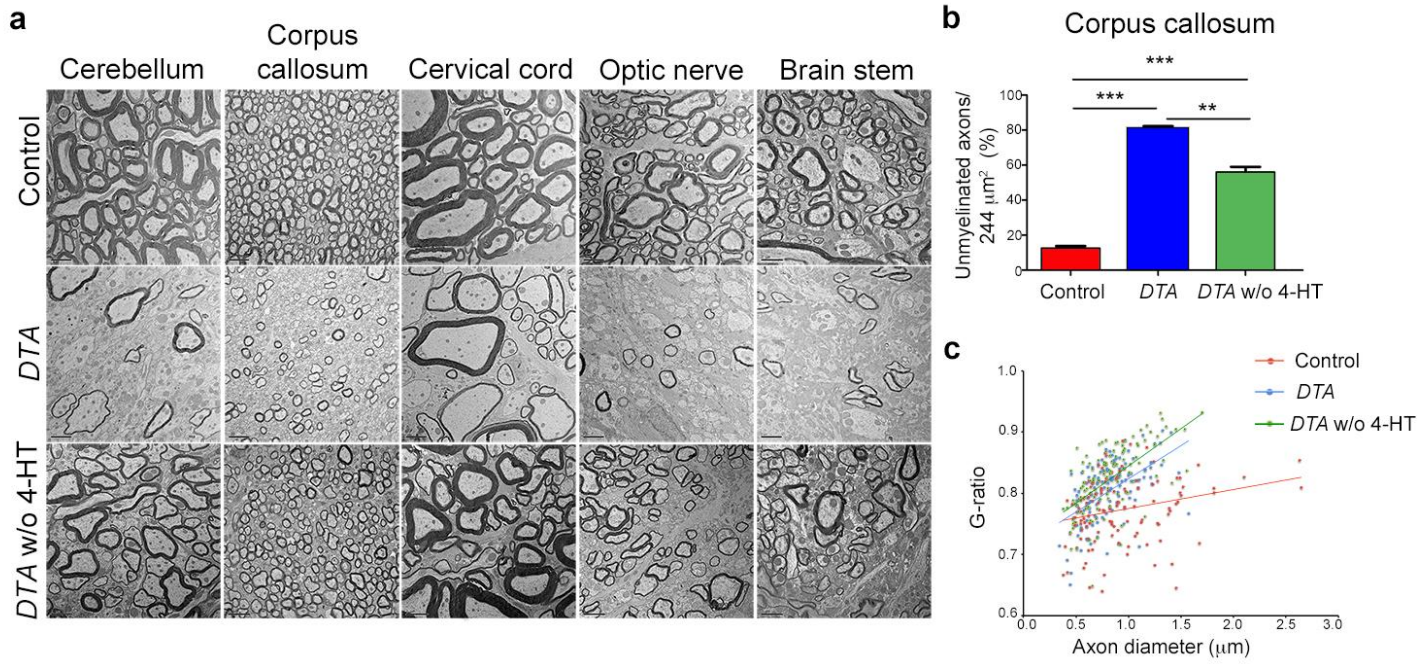
Staining for CD3 (green) revealed increased T cell infiltration in different areas of the CNS in tamoxifen-treated *PLP/CreER*$^{T}$;*ROSA26-eGFP-DTA* (DTA) mice (lower panel) at 53 weeks post-injection (p.i.) compared with that in control (*ROSA26-eGFP-DTA*) mice (upper panel). Higher magnification of the CD3+ cells is shown in the inset (lower panel, corpus callosum). Images are representative of three mice/genotype. Scale bar: 100 μm. Sections were counterstained with DAPI (blue) to show cell nuclei.




**Supplementary Figure 2**

**Tamoxifen-independent recombination in oligodendrocytes of the reporter mice.**

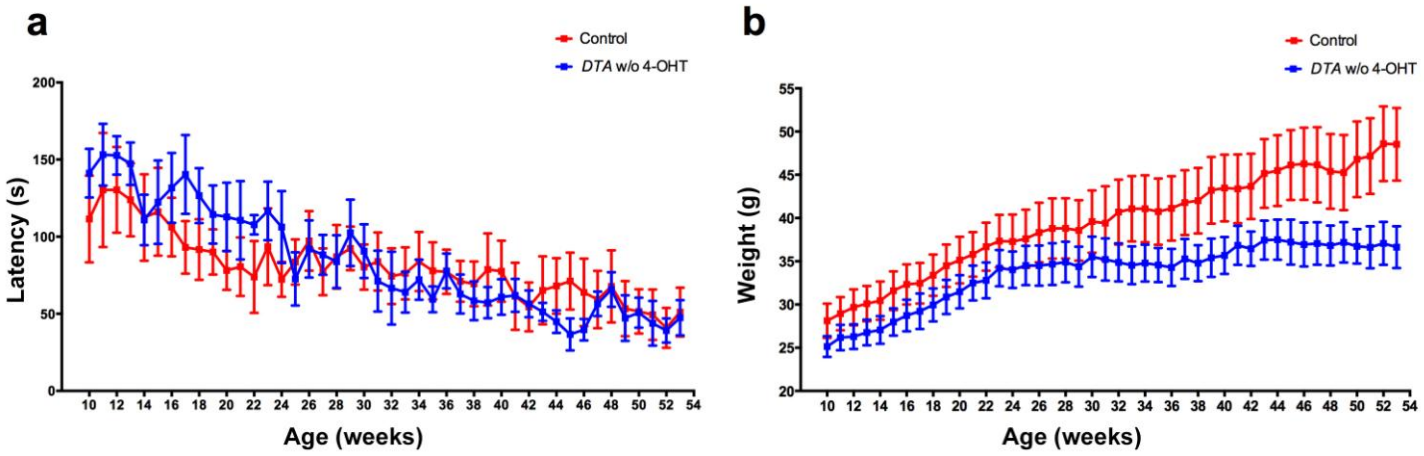
(**a**) Expression of the reporter gene protein YFP (green) was detected in a subpopulation of oligodendrocytes stained for CC-1 (red) in all CNS areas examined of the untreated *PLP/CreER$^{T}$;RosaEYFP* (reporter w/o 4-HT; lower panel) mice at the age of 52 weeks. CC1/YFP stainings in corresponding areas of control littermate mice (*RosaEYFP*) are shown in the upper panel. The CC1/YFP double positive cells (yellow) are marked by arrows. Images are representative of three mice/genotype. Scale bar: 100 μm. (**b**) Counts of the CC1/YFP double positive cells labeled in different CNS areas of the untreated reporter mice showed that tamoxifen-independent recombination occurs in ~ 6%-14% of oligodendrocytes at 26 weeks and it tends to be increased in most areas (6%-20%) at 52 weeks (brain stem $p$ =0.0084). Graph in **b** shows means (+ SEM), n=4 mice per group, two-tailed unpaired Student's $t$ test, **$p$ <0.01.




**Supplementary Figure 3**

**Tamoxifen-independent demyelination in untreated DTA mice.**

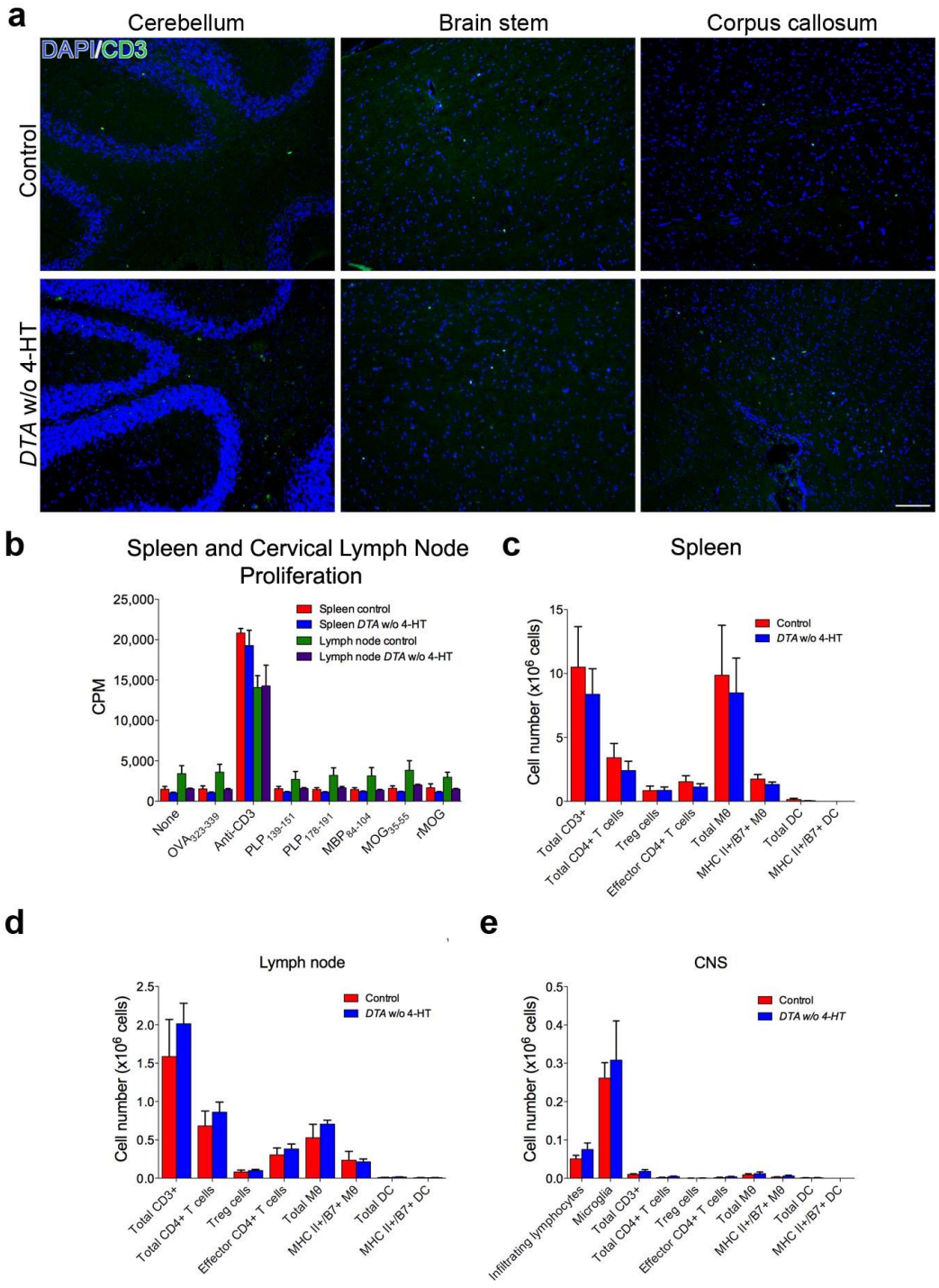
(**a**) Electron microscopy analysis demonstrated normal myelin levels in the cervical cord and milder demyelination in the cerebellum, corpus callosum, optic nerve and brain stem areas of the untreated *PLP/CreER$^T$;ROSA26-eGFP-DTA* (DTA w/o 4-HT) mice as compared to tamoxifen-treated *PLP/CreER$^T$;ROSA26-eGFP-DTA* (DTA) mice, which showed more severe myelin loss in the same areas. Images are representative of three mice/genotype. Scale bars: 2 μm. (**b**) Morphometric analysis of the corpus callosum area showed that numbers of unmyelinated axons were significantly lower (-30%) in the 52-week old untreated DTA mice as compared to tamoxifen-treated DTA mice at 53 weeks post-injection suggesting milder myelin loss in the former animals. Control versus tamoxifen-treated DTA mice: $p<0.0001$, control vs. untreated DTA mice: $p=0.0001$, tamoxifen-treated DTA mice versus untreated DTA mice: $p=0.0013$. Graph in **b** shows means (+ SEM), n=3 for control and tamoxifen-treated DTA mice, n=4 for untreated DTA mice. ***$p$ <0.001, **$p$ <0.01, one way ANOVA with Tukey's post-hoc test. (**c**) Scatter plots of g-ratios versus axon diameters indicate significantly increased g-ratios (thinner myelin) in axons of the corpus callosum in both the tamoxifen-treated DTA (mean g ratio of 0.80 ± 0.004) and the untreated DTA mice (mean g ratio of 0.82 ± 0.005) as compared to controls (*ROSA26-eGFP-DTA,* mean g ratio of 0.77 ± 0.005). Control versus tamoxifen-treated DTA mice: $p<000.1$ and control versus untreated DTA mice: $p<000.1$, one-way Anova followed by Tukey's *post-hoc* analysis test. Forty axons/mouse, n=3 mice per group.




**Supplementary Figure 4**

**The untreated DTA mice do not develop a late-onset clinical phenotype.**

**(a-b)** The untreated *PLP/CreER*$^{T}$;*ROSA26-eGFP-DTA* (DTA w/o 4-HT) mice displayed normal coordination skills on the rotarod (**a**) and they did not show significant weight loss (**b**) as compared to control littermate (*ROSA26-eGFP-DTA*) mice during the time points that the tamoxifen-treated DTA mice were severely affected. Data are presented as the mean (± SEM), n= 5 mice/genotype for each data point shown in the graphs in **a** and **b**, $p > 0.05$; two-way ANOVA with Bonferroni post-hoc analysis.

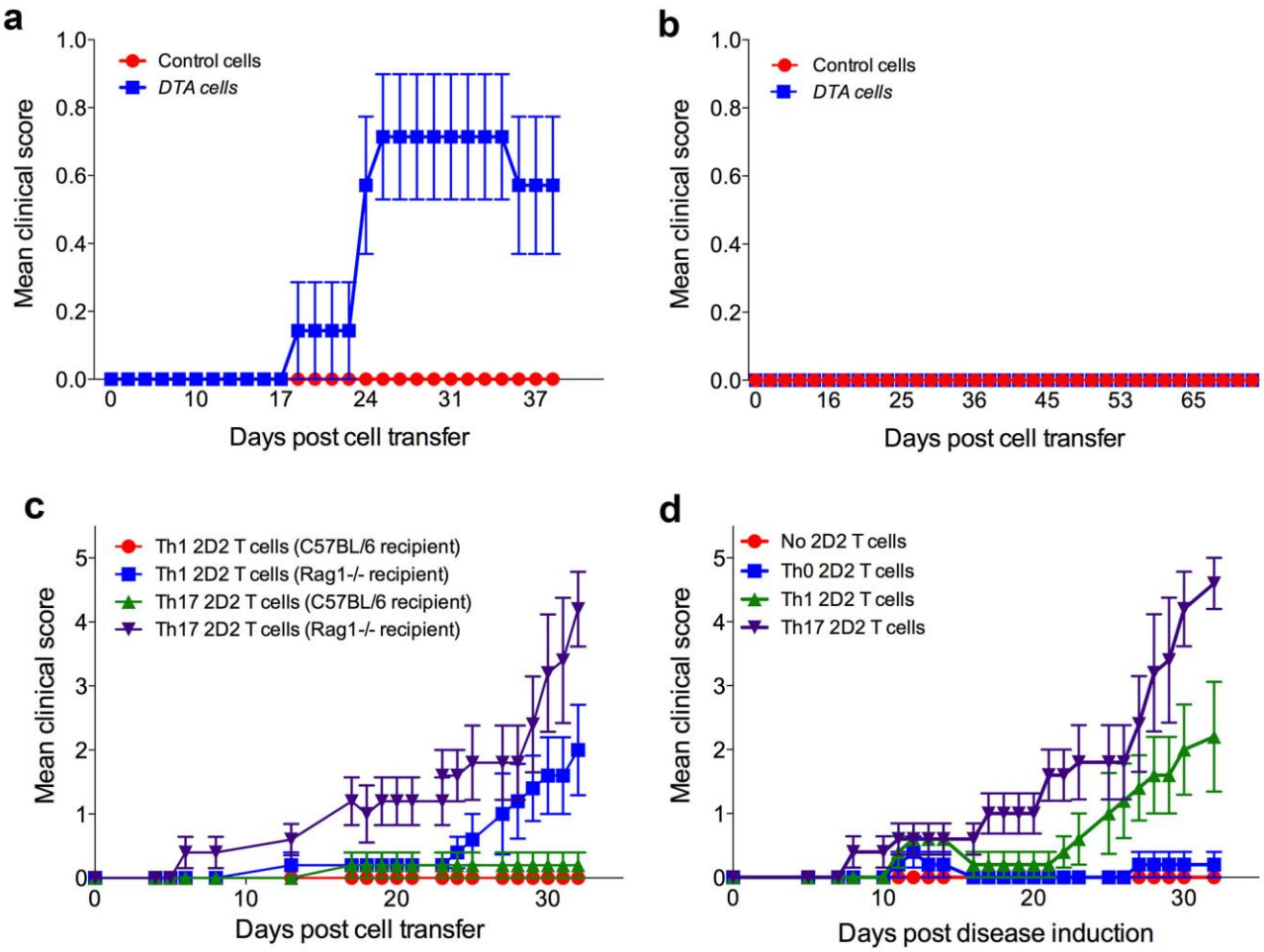



**Supplementary Figure 5**

**Tamoxifen-independent demyelination in untreated DTA mice does not elicit an immune response against myelin.**

(**a**) Staining for the pan T cell marker CD3 showed similar, low numbers of T cells in different CNS areas of the 52-week old untreated *PLP/CreER$^T$;ROSA26-eGFP-DTA* (DTA w/o 4-HT) mice and in corresponding CNS areas of their littermate controls (*ROSA26-eGFP-DTA* mice). Images are representative of three mice/genotype. Scale bar: 100 μm. Spleen, cervical lymph nodes, and CNS samples were collected from age matched control littermate mice and untreated DTA mice. (**b**) Total splenoctyes (1x10$^6$ cells per well) were cultured in the presence of medium alone, anti-CD3 (1μg/ml),

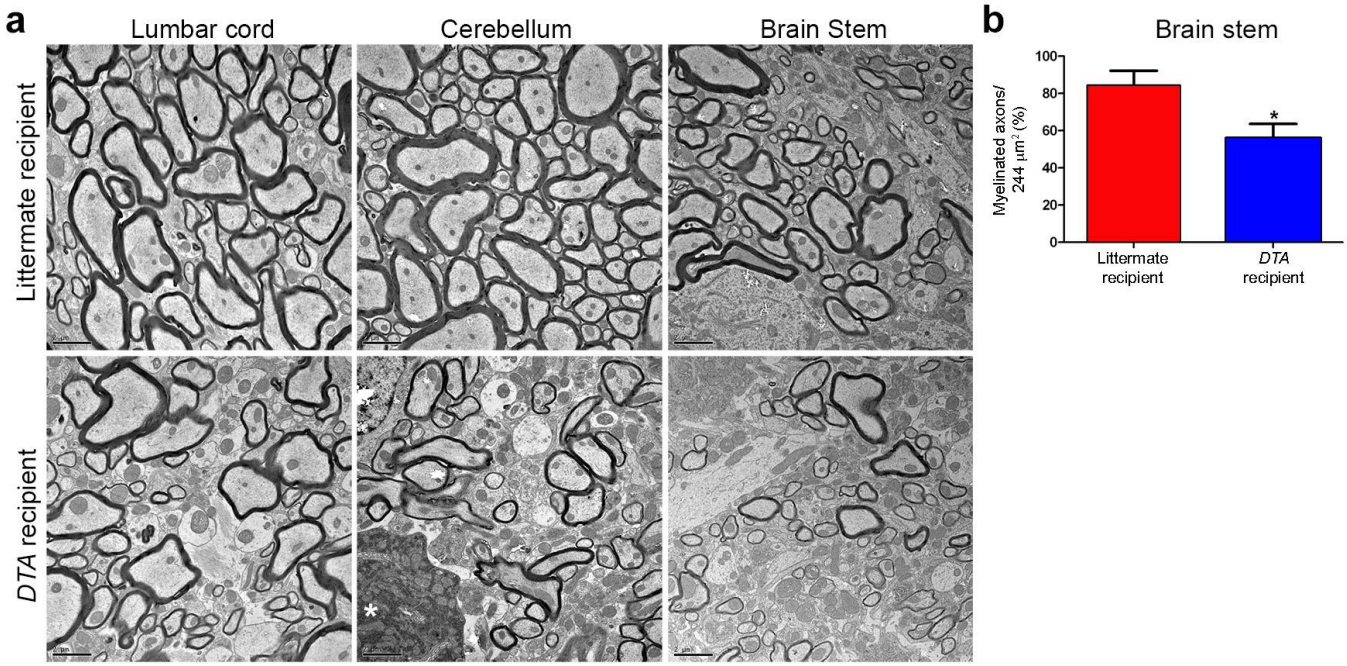
$OVA_{323-339}$, $PLP_{139-151}$, $PLP_{178-191}$, $MBP_{84-104}$, $MOG_{35-55}$, and whole recombinant rat MOG protein (10μg/ml). The cultures were pulsed with tritiated thymidine (1μCi) at 24 hours and cultures were harvested at 72 hours post culture to determine the level of cellular proliferation. Cells from (**c**) spleen, (**d**) cervical lymph nodes, and (**e**) CNS were stained for the presence of total infiltrating lymphocytes (CD45hi), microglia (CD45lo/CD11b+), total CD3+, total CD4+ T cells (CD3+/CD4+), $T_{reg}$ cells (CD3+/CD4+/CD25+/FoxP3+), effector CD4+ T cells (CD3+/CD4+/CD44hi/FoxP3-), total monocytes (Mθ; CD3-/CD11b+/CD11c-), and total dendritic cells (DC; CD3-/CD11b-/CD11c+). No significant difference in the number of the various immune cells populations were found within the spleen, cervical lymph nodes, or the CNS (n=3) via two-way ANOVA with Bonferroni post-hoc analysis for differences between control mice and tamoxifen-treated DTA mice. The data are presented as the means (+ SEM). Sections in **a** were counterstained with DAPI (blue) to visualize cell nuclei. Abbreviations: CPM, counts per minute.




**Supplementary Figure 6**

**Adoptive transfer of MOG-specific T cells derived from tamoxifen-treated DTA mice and 2D2 TCR transgenic mice into naive *Rag1*$^{-/-}$ mice versus wild-type C57BL/6 mice.**

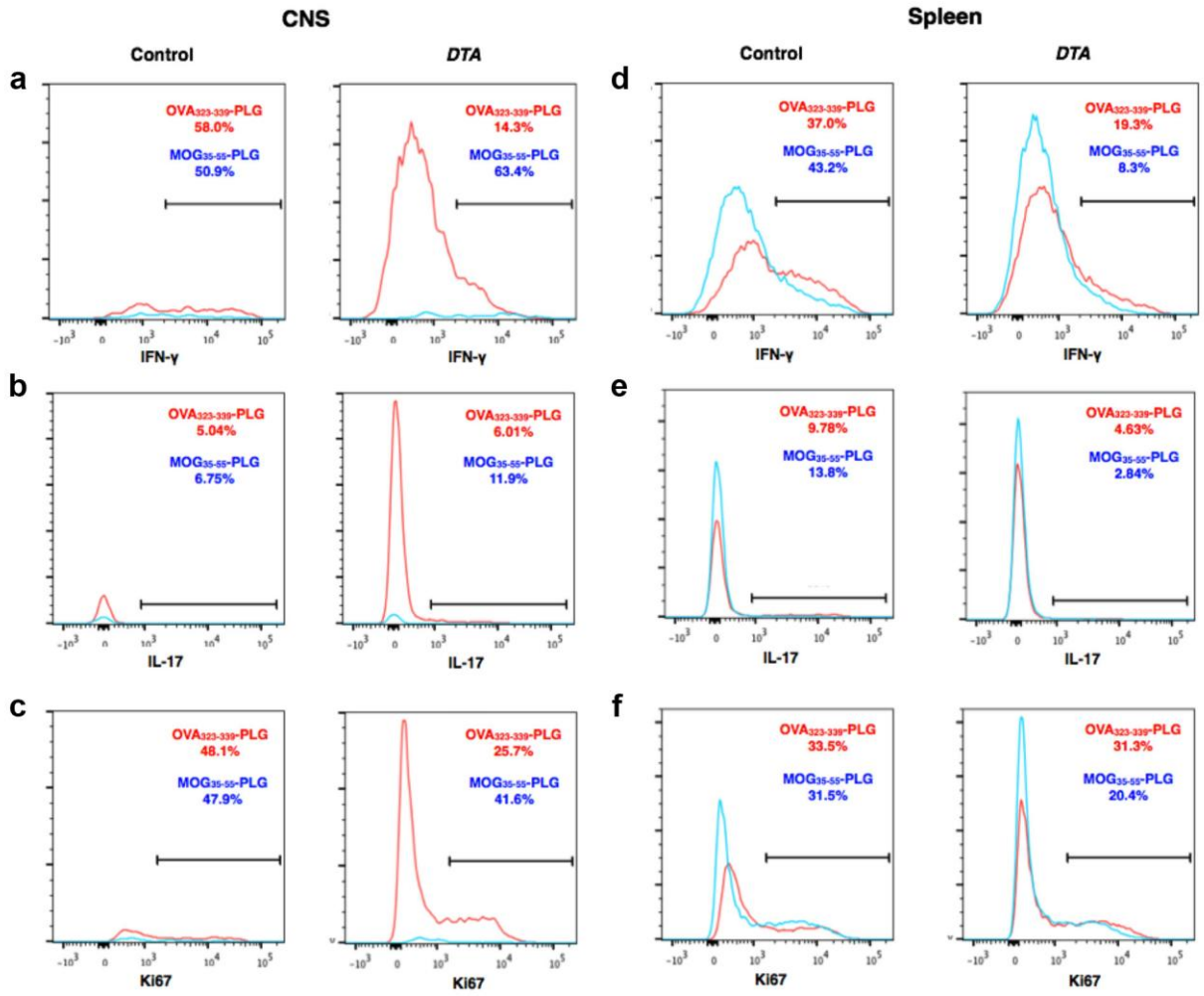
Splenic cells from tamoxifen-treated *PLP/CreER*$^{T}$;*ROSA26-eGFP-DTA* (DTA) mice at 40-52 weeks post-injection or age-matched control (*ROSA26-eGFP-DTA*) mice were cultured for 72 h at in the presence of $MOG_{35–55}$ peptide (20 μg/ml) plus IL-12 (10ng/ml) and IL-2 (100U/ml) . Cultured cells ($10x10^6$ blast cells) from tamoxifen-treated DTA mice were transferred into either naïve *Rag1*$^{-/-}$ recipient mice (n=7) (**a**) or naïve wildtype C57BL/6 recipient mice (n=7) (**b**). Naïve CD4+ T cells were isolated from 2D2 $MOG_{35–55}$-specific TCR transgenic mice, and activated in the presence plate-bound anti-CD3 (1ug/ml) plus various Teff cell lineage promoting conditions as indicated. 2D2 T cells ($3x10^6$ cells per mouse) activated in the presence of Th1 cell- [IL-12 (10ng/ml), IL-2 (100U/ml), and anti-IL-4 (1μg/ml)] or Th17 cell- [TGF-β (10ng/ml), IL-6 (50ng/ml), IL-23 (10ng/ml), anti-IL-4 (1μg/ml), and anti-IFN-γ (1μg/ml)] promoting conditions were transferred into *Rag1*$^{-/-}$ mice versus wildtype C57BL/6 mice (n=5), and the recipient mice were followed for disease (**c**). 2D2 T cells ($3x10^6$ cells per mouse) activated in the presence of Th0- [IL-2 (100U/ml)], Th1 cell- or Th17 cell-promoting conditions were transferred into *Rag1*$^{-/-}$ mice (n=5), and the recipient mice were followed for disease (**d**). One representative experiment of three is presented. The data is presented as the mean Clinical Score (± SEM).




**Supplementary Figure 7**

**Adoptive transfer of MOG-specific T cells derived from tamoxifen-treated DTA mice causes mild demyelination in naive *Rag1*$^{-/-}$ mice.**

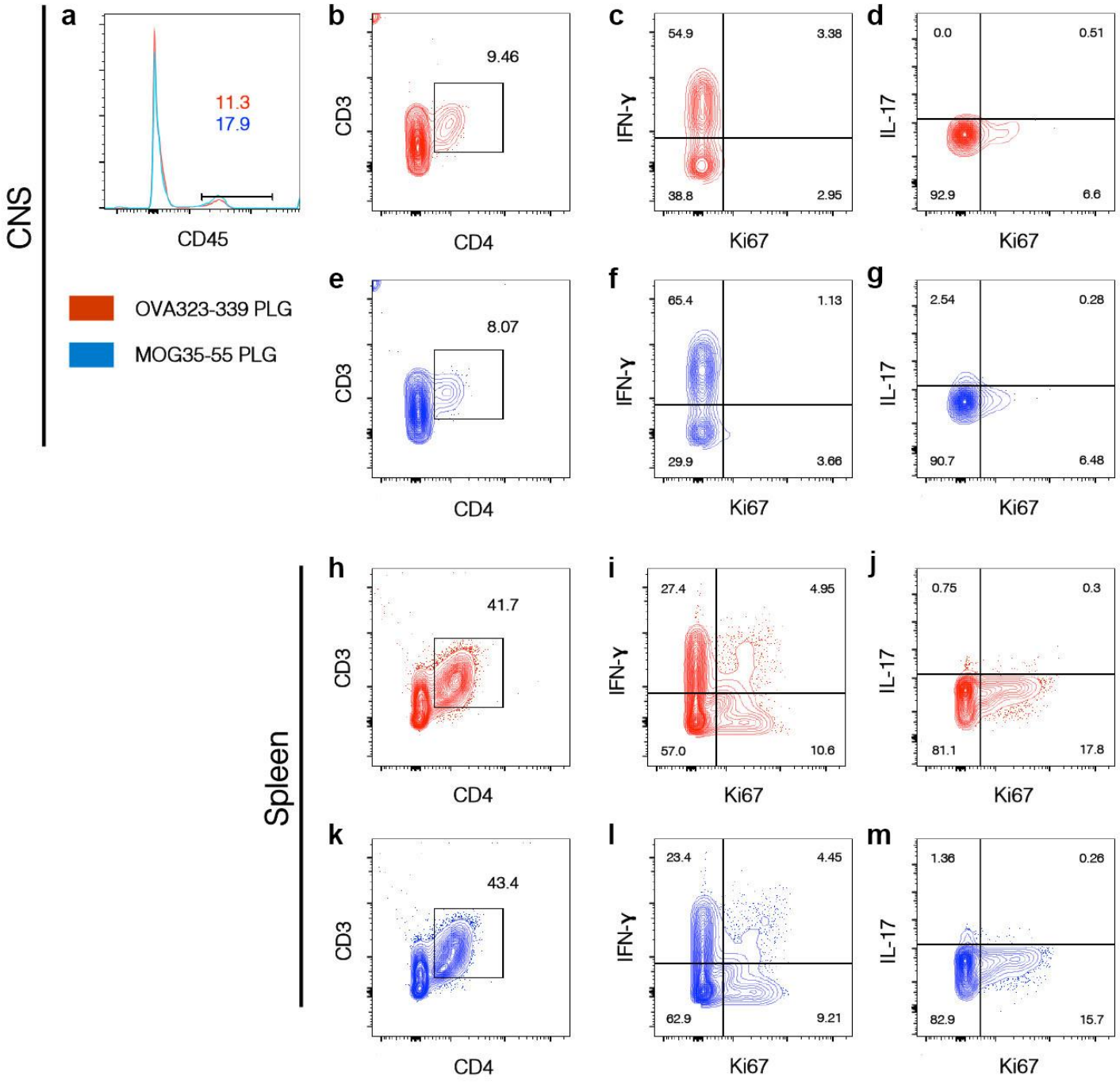
Splenic cells from tamoxifen-treated *(PLP/CreER*$^{T}$*;ROSA26-eGFP-DTA*) DTA mice at 40-52 weeks post-injection or age-matched control (*ROSA26-eGFP-DTA*) mice were cultured for 72 h at in the presence of $MOG_{35–55}$ peptide (20 μg/ml). Cultured cells from tamoxifen-treated DTA mice ($2x10^6$ blast cells) were transferred into naïve *Rag1*$^{-/-}$ recipient mice (**a**) Forty-two days after transfer, Electron microscopy analysis revealed foci of myelin loss in the lower lumbar spinal cord and cerebellar white matter in *Rag1*$^{-/-}$ mice inoculated with T cells from tamoxifen-treated DTA mice (DTA recipient) as compared with those inoculated with cells from control mice (littermate recipient). Inflammatory cells were also frequently detected in the cerebellum (asterisk). More-extensive demyelination was seen in the brain stem gray matter. Images are representative of three mice/genotype. Scale bars: 2 μm. (**b**) Demyelination in the brain stem of *Rag1*$^{-/-}$ mice inoculated with cells from tamoxifen-treated DTA mice was shown by significantly reduced mean (+ SEM) numbers of myelinated axons (~30% fewer, $p$=0.0363). N=3 mice/group; *$p$ <0.05 with two-tailed unpaired Student's $t$ test.




**Supplementary Figure 8**

**$MOG_{35–55}$-PLG-induced tolerance inhibits activated DTA mouse-derived $MOG_{35–55}$-specific T cell entry into the CNS.**

Representative flow plots for the data presented in **Fig. 7c** and **e** are presented. The data is presented as the (**a**) IFN-γ+, (**b**) IL-17+, and (**c**) Ki67+ singlet live CD45hi/CD4+ T cells within the CNS from control (*ROSA26-eGFP-DTA*) or tamoxifen-treated *PLP/CreER$^T$;ROSA26-eGFP-DTA* (DTA)-derived cell recipient mice that received $OVA_{323-339}$-PLG (Red line) versus $MOG_{35-55}$-PLG treatment (Blue line). Similar analyses were completed for splenocyte samples assessing (**d**) IFN-γ+, (**e**) IL-17+, and (**f**) Ki67+ singlet live CD45hi/CD4+ T cells.




**Supplementary Figure 9**

**$MOG_{35–55}$ tolerance inhibits $CD4^+$ T cell infiltration, proliferation and production of the proinflammatory cytokines in the CNS of the tamoxifen-treated DTA mice.**

Representative flow plots of cells isolated from the CNS (**a**-**g**) and the spleens (**h**-**m**) of the tamoxifen-treated *PLP/CreER$^T$;ROSA26-eGFP-DTA* (DTA) mice at 39 weeks post-injection after receiving a single treatment with the $MOG_{35-55}$-PLG or the $OVA_{323-339}$-PLG control peptide at 32 weeks post-injection. Lower numbers of the total infiltrating lymphocytes (CD45hi+ stained cells) were detected in the CNS of the $MOG_{35-55}$-tolerized DTA mice, as compared to the

$OVA_{323-339}$-tolerized (control) DTA mice (n=6). The later mice also showed, lower numbers of the effector T cells (CD3+/CD4+ double positive cells) and proliferating interferon-γ-positive effector T cells (Ki67+/INF-γ+ stained cells) and no changes for the proliferating IL-17-positive effector T cells (Ki67+/IL-17+ stained cells). No differences were detected in the numbers of the different immune cell populations in the spleens between the two groups of mice.

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