

Additional file 1

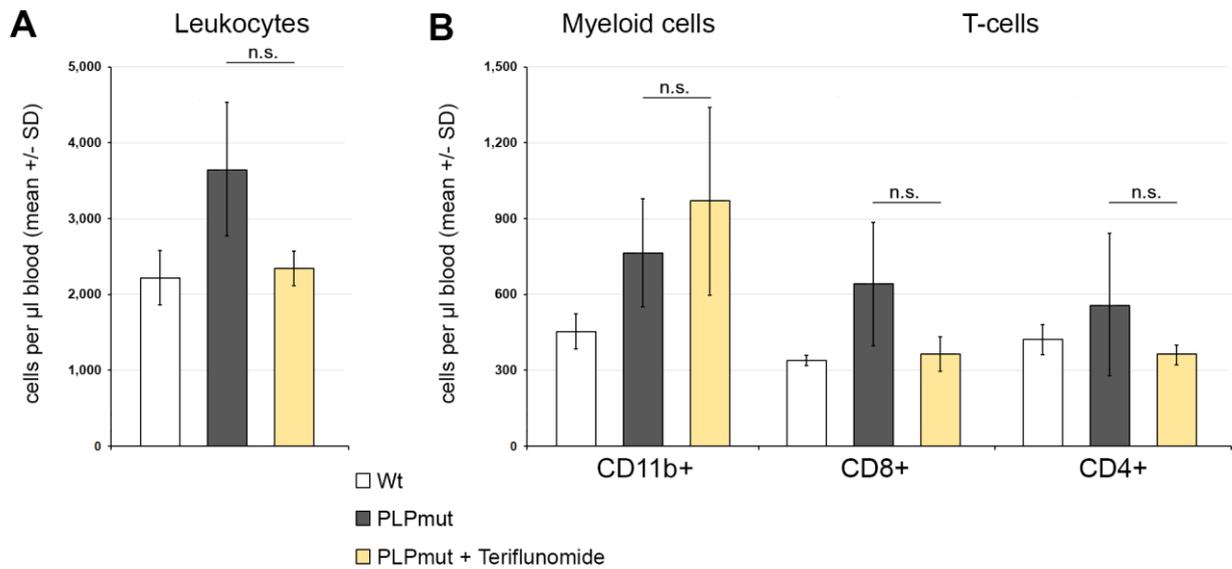


Figure S1

Lack of impact of teriflunomide treatment on blood leukocyte numbers.

(A, B) Effect of preventive treatment on total leukocyte (A) and on myeloid cell and T-lymphocyte numbers (B) in the peripheral blood. Based on flow cytometry, no significant changes of leukocyte numbers were detectable. One-way ANOVA and Tukey's *post hoc* tests. $n = 5$ mice per group.

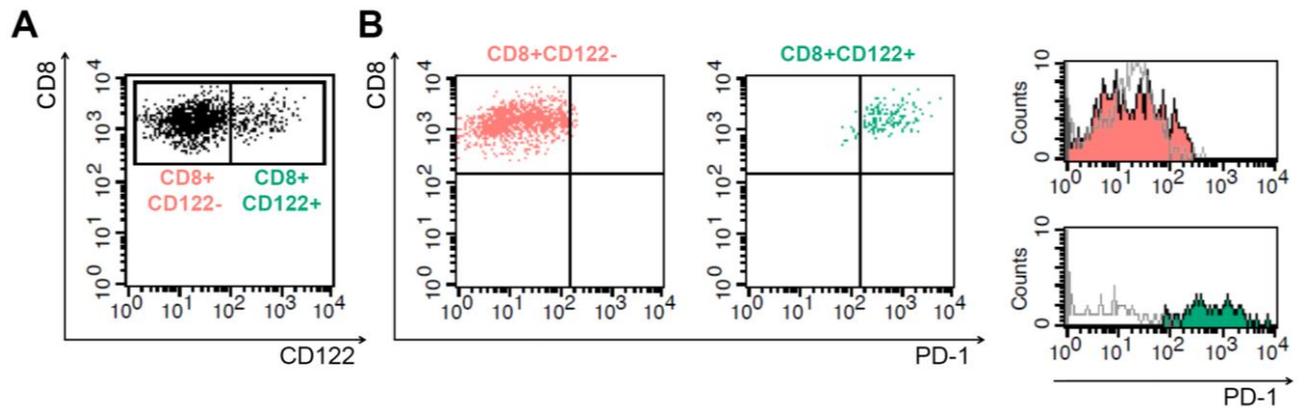


Figure S2

Confirmation of PD-1 expression as a marker of CD8+CD122+ regulatory T-cells in the CNS of *PLPmut* mice.

(A) Representative example of flow cytometric analysis of T-lymphocytes freshly isolated from brains of untreated *PLPmut* mice gated for CD8 and CD122 expression. The majority of the CNS-derived CD8+ T-cells are CD122- effector cells (red); only few cells are double-positive for CD8 and CD122 (green) and thus regulatory T-cells. (B) Representative example of flow cytometric analysis of gated CD8+CD122- (red) and CD8+CD122+ (green) T-lymphocytes from brains of untreated *PLPmut* mice analysed for PD-1-coexpression. Only CD8+CD122+ regulatory cells coexpress PD-1. Right histograms display corresponding counts for CD8+CD122- (red) and CD8+CD122+ cells (green) and their coexpression of PD-1. Appropriate isotype control counts are represented in grey.

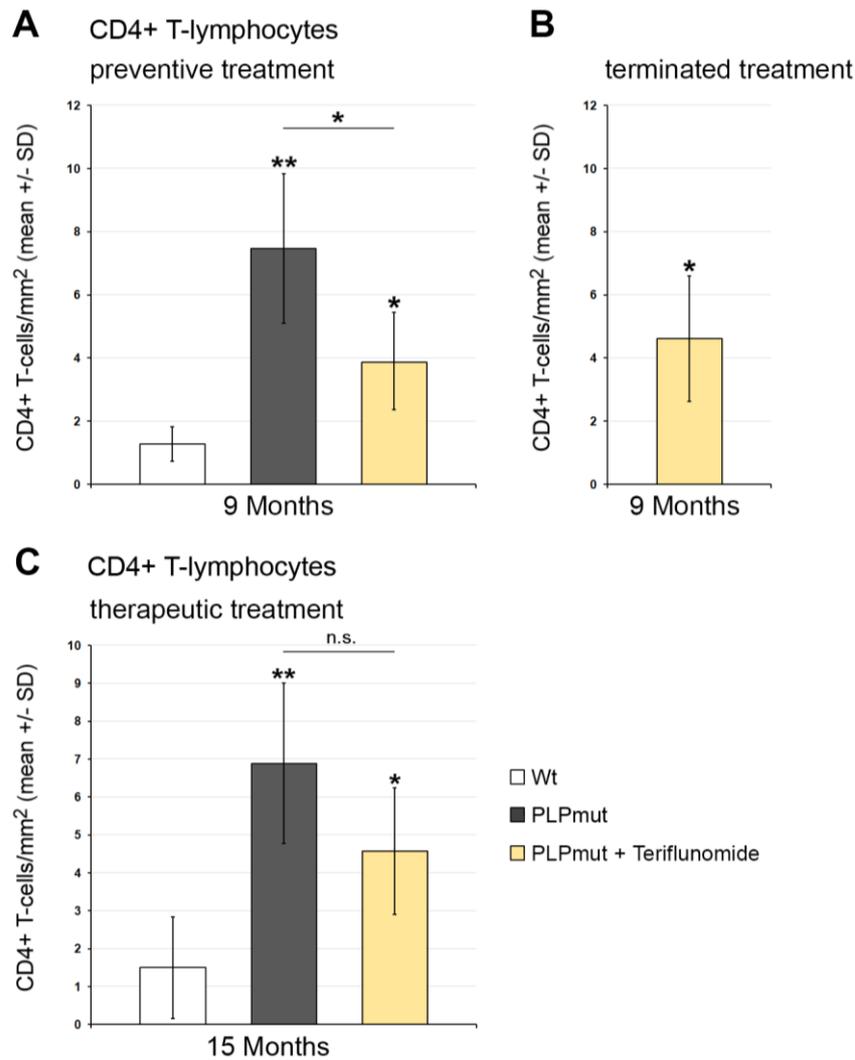


Figure S3

Preventive, but neither terminated nor therapeutic treatment impairs the increase of CD4+ T-lymphocyte numbers in *PLPmut* mice.

(A) Quantification of CD4+ T-lymphocytes in optic nerve sections of *Wt* and *PLPmut* mice and in *PLPmut* mice after 150 days of preventive treatment. Like CD8+ T-lymphocytes (Figure 2), the numbers of CD4+ T-cells were significantly increased in the untreated *PLPmut* mice; this elevation was attenuated by preventive treatment. (B) Quantification of CD4+ T-lymphocytes in optic nerve sections of *PLPmut* mice after terminated treatment. Treatment termination did not lead to an overshoot or rebound of T-cell numbers, but failed to significantly preserve reduction of T-cell numbers. (C) Quantification of CD4+ T-lymphocytes in optic nerve sections of *Wt* and *PLPmut* mice and in *PLPmut* mice after 150 days of therapeutic treatment. Therapeutic treatment did not significantly reduce the increase of CD4+ T-cell numbers in *PLPmut* mice. Note generally lower numbers of CD4+ T-

cells in comparison to CD8+ T-cells (compare to Figure 2). One-way ANOVA and Tukey's *post hoc* tests. * $P < 0.05$, ** $P < 0.01$. $n = 5$ mice per group.

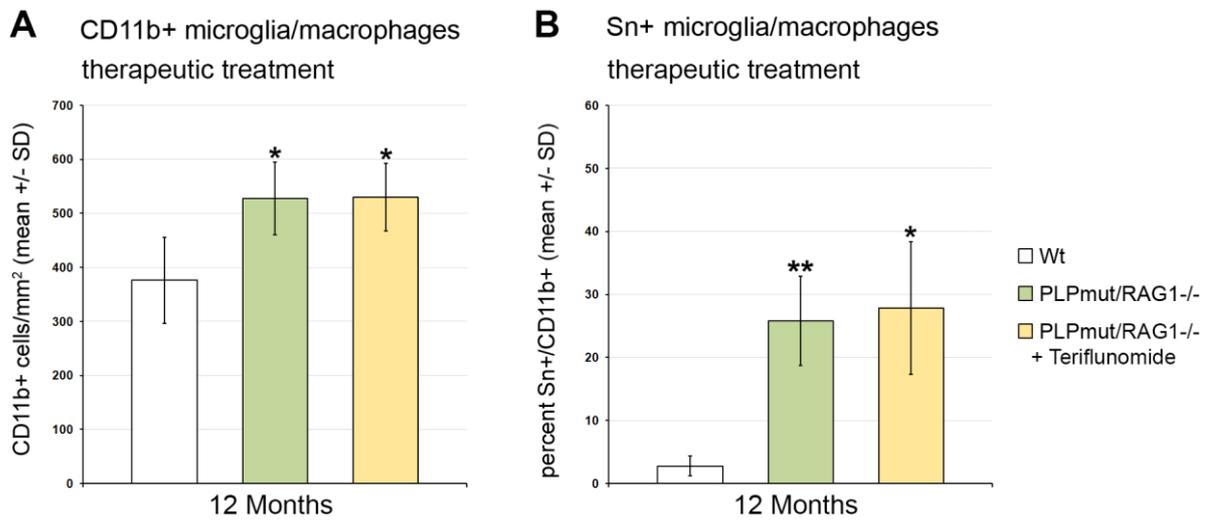


Figure S4

Lack of evidence for an immune-unrelated effect of therapeutic teriflunomide treatment.

(A) Quantification of CD11b+ and (B) Sn+ activated microglia/macrophages in optic nerves of *Wt*, *PLPmut/Rag1^{-/-}* and therapeutically-treated *PLPmut/Rag1^{-/-}* mice. Treatment had no further effect on (in comparison with *PLPmut* reduced) CD11b+ or Sn+ microglia/macrophage numbers in *PLPmut* mice lacking mature adaptive immune cells. One-way ANOVA and Tukey's *post hoc* tests. * $P < 0.05$, ** $P < 0.01$. $n = 5$ mice per group.

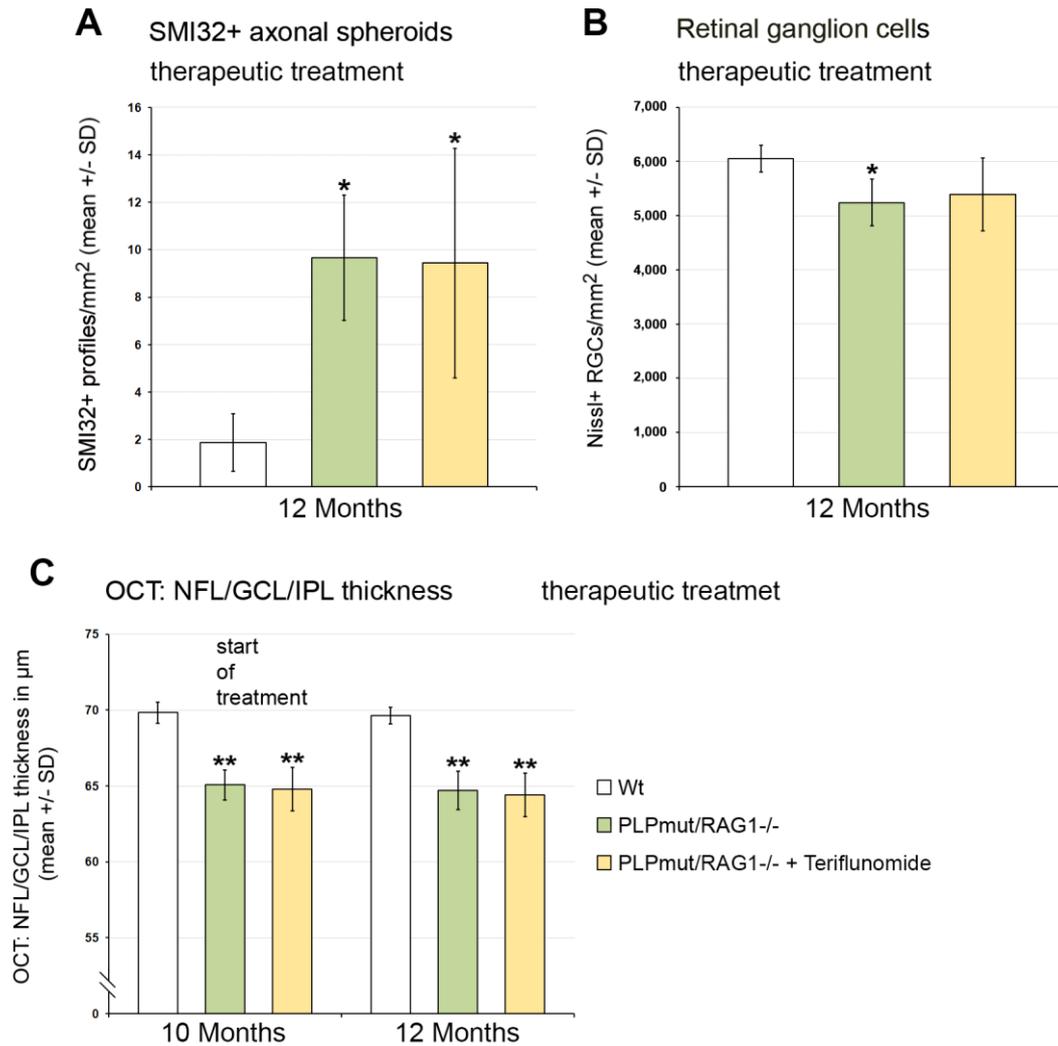


Figure S5

Lack of evidence for an immune-unrelated effect of therapeutic teriflunomide treatment on histopathological features.

(A) Quantification of SMI32+ axonal spheroids in optic nerves, (B) of cresyl violet-stained retinal ganglion cells and of inner retinal composite layer thickness in *Wt*, *PLPmut/Rag1^{-/-}* and therapeutically-treated *PLPmut/Rag1^{-/-}* mice by OCT. Treatment failed to further ameliorate the corresponding histopathological alterations which have been attenuated by RAG1-deficiency. One-way ANOVA and Tukey's *post hoc* tests. * $P < 0.05$, ** $P < 0.01$. $n = 5$ mice per group.

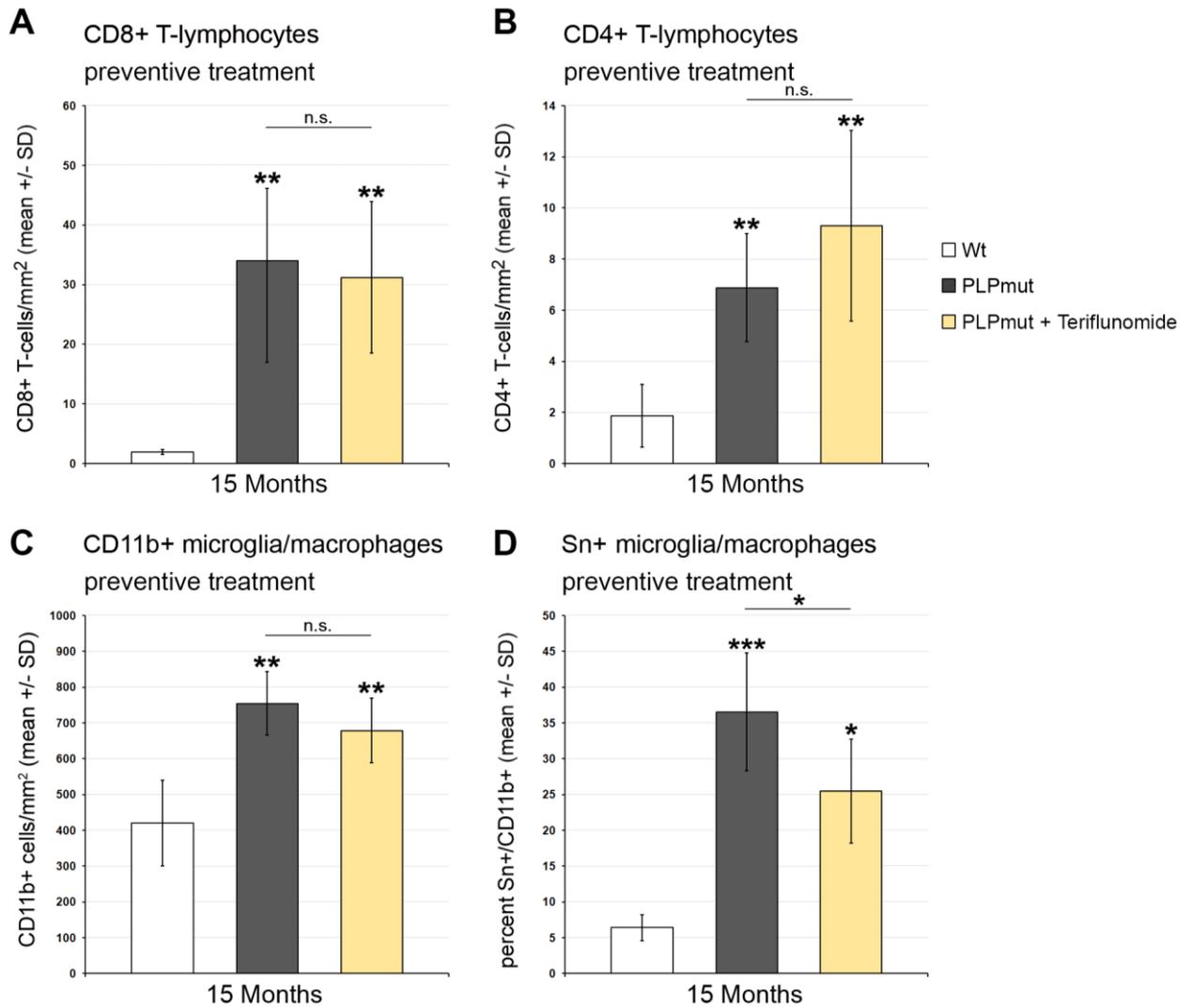


Figure S6

Long-term preventive treatment does not reduce T-lymphocyte and microglia/macrophage numbers but leads to a reduction of activated Sn+ microglial cell numbers.

(A) Quantification of CD8+ T-lymphocytes, (B) CD4+ T-lymphocytes, (C) total numbers of microglia/macrophages and (D) Sn+ microglial cells in longitudinal sections of optic nerves of *Wt*, *PLPmut* and *PLPmut* mice after long-term preventive treatment. Only activated Sn+ microglial cell numbers were still reduced after long-term treatment conditions. One-way ANOVA and Tukey's *post hoc* tests. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. $n = 5$ mice per group.