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Supplemental Information

Fingolimod and Teriflunomide Attenuate Neurodegeneration in Mouse Models of Neuronal Ceroid Lipofuscinosis

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Supplemental figures and legends

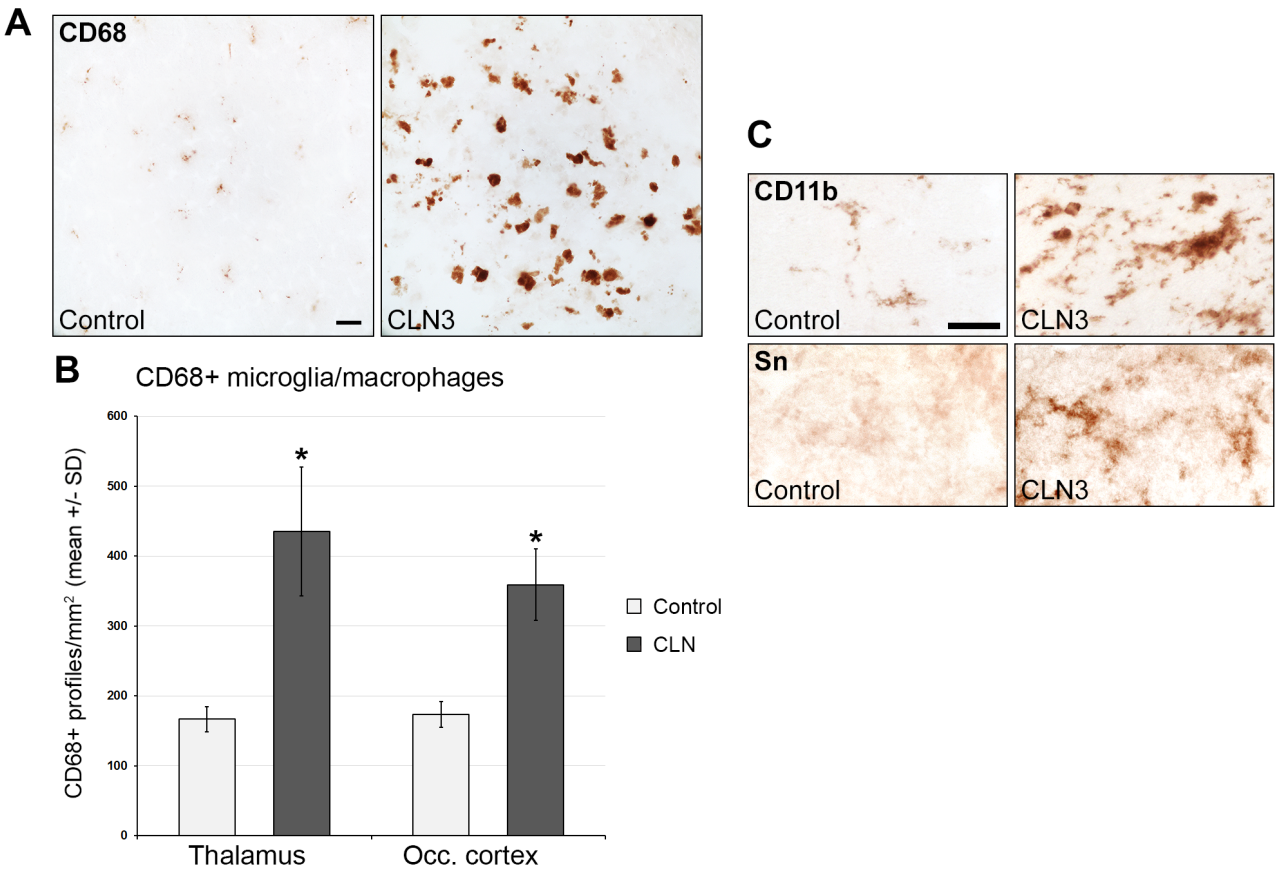

Figure S1
Increased numbers of microglia/macrophage-like cells in the CNS of CLN disease patients. (**A**) Representative light microscopic images of immunohistochemically labeled CD68+ microglia/macrophages in occipital cortex autopsy sections from a CLN3 patient (BBN_16328) and a control patient (BBN_15741). Scale bars: 30 µm. (**B**) Quantification of CD68+ cells: the numbers of CD68+ microglia/macrophages were significantly increased in the thalamus and occipital cortex of CLN disease patients compared with controls (n = 3 patients per group). Student's t test. *P < 0.05. (**C**) CD11b and sialoadhesin (Sn) immunoreactivity were also increased in brain autopsy sections from CLN disease patients compared with controls.

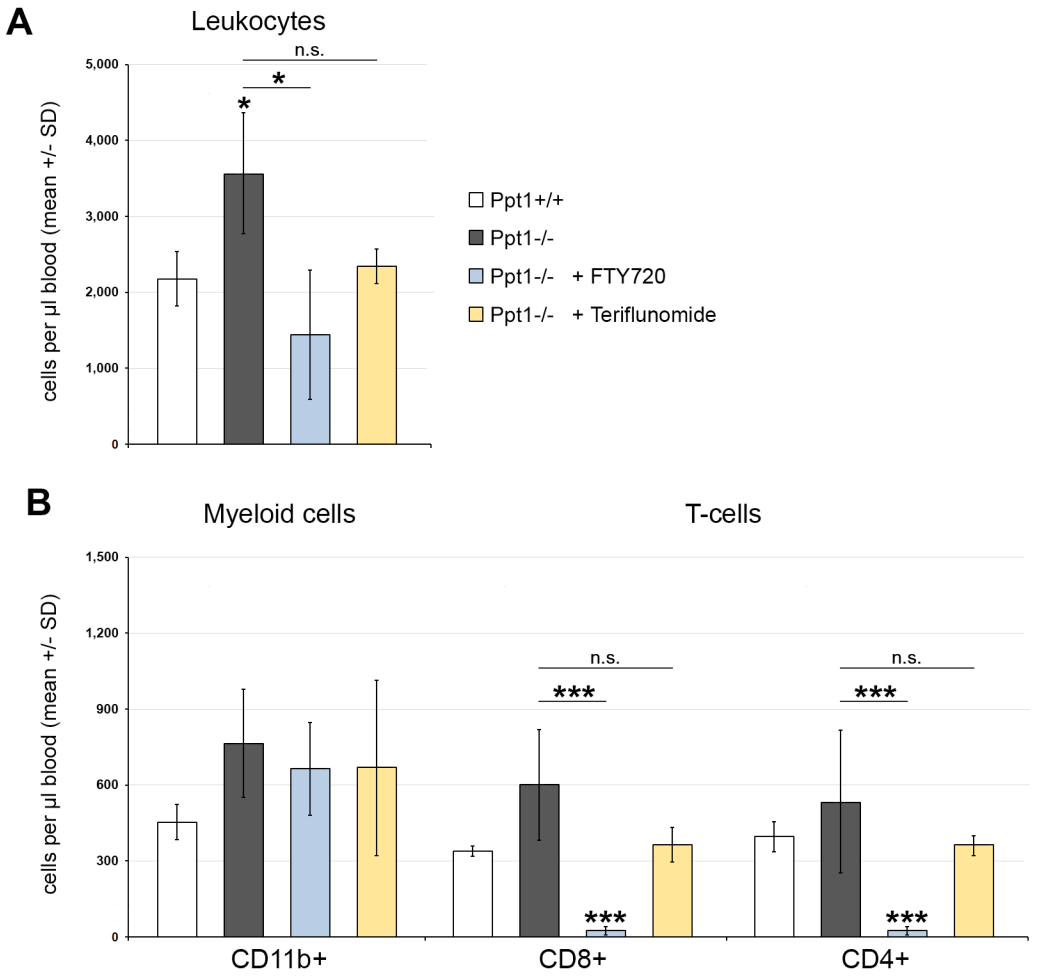

Figure S2

Fingolimod but not Teriflunomide depletes circulating T-lymphocytes. (**A**) Total blood leukocyte counts by flow cytometry from 6-month-old *Ppt1*$^{+/+}$, *Ppt1*$^{-/-}$, Fingolimod-treated *Ppt1*$^{-/-}$ (+ FTY720) and Teriflunomide-treated *Ppt1*$^{-/-}$ (+ Teriflunomide) mice. The numbers of blood leukocytes were significantly increased in the CLN1 disease model compared with Wt mice. Blood leukocyte numbers were reduced by treatment with Fingolimod but not significantly by treatment with Teriflunomide for 150 days (n = 5 mice per group). One-way ANOVA and Tukey's *post hoc* tests. *$P < 0.05$, n.s. = not significant. (**B**) CD11b+ myeloid cell counts, CD8+ T-cell counts and CD4+ T-cell counts by flow cytometry in blood from 6-month-old *Ppt1*$^{+/+}$, *Ppt1*$^{-/-}$, Fingolimod-treated *Ppt1*$^{-/-}$ (+ FTY720) and Teriflunomide-treated *Ppt1*$^{-/-}$ (+ Teriflunomide) mice. The numbers of blood myeloid cells and T-cells showed a non-significant tendency towards increase in the CLN1 disease model compared with Wt mice. Blood T-cell numbers were strongly reduced by treatment with Fingolimod but not significantly by treatment with Teriflunomide for 150 days (n = 5 mice per group). One-way ANOVA and Tukey's *post hoc* tests. ***$P < 0.001$. n.s. = not significant.

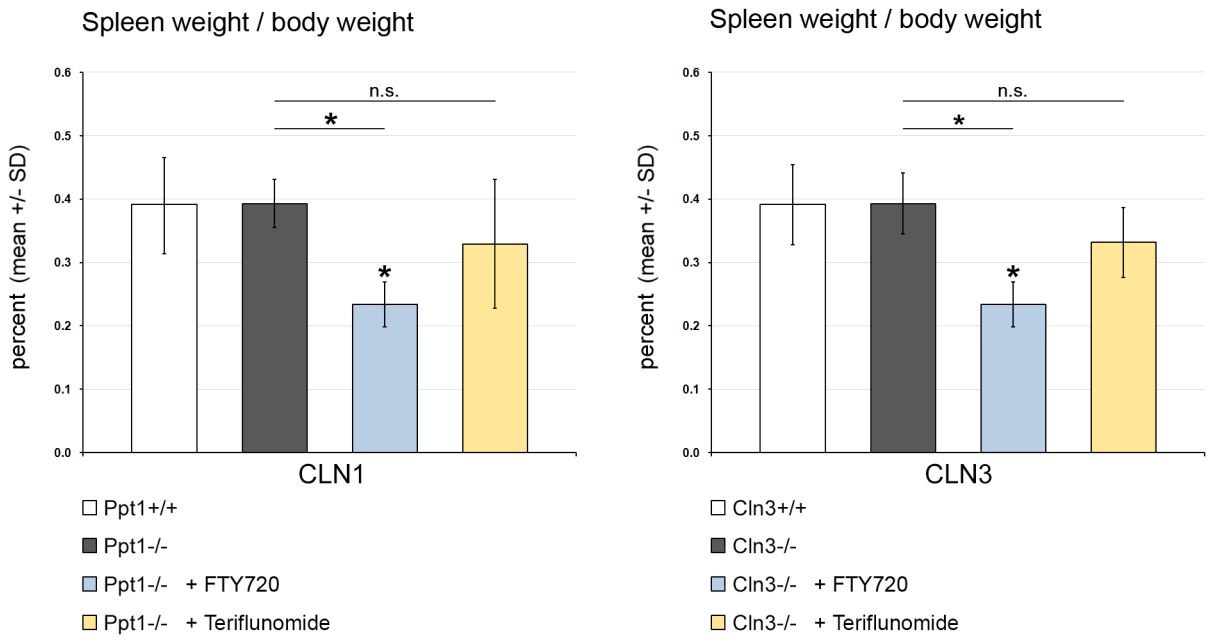

Figure S3

Fingolimod but not Teriflunomide reduces spleen weight in CLN1 and CLN3 disease models. Spleen weight related to body weight from 6-month-old *Ppt1*$^{+/+}$, *Ppt1*$^{-/-}$, Fingolimod-treated *Ppt1*$^{-/-}$ (+ FTY720) and Teriflunomide-treated *Ppt1*$^{-/-}$ (+ Teriflunomide) mice and 17-month-old *Cln3*$^{+/+}$, *Cln3*$^{-/-}$, Fingolimod-treated *Cln3*$^{-/-}$ (+ FTY720) and Teriflunomide-treated *Cln3*$^{-/-}$ (+ Teriflunomide) mice. Spleen weight was significantly reduced by treatment with Fingolimod but not significantly by treatment with Teriflunomide for 150 days (n = 5 mice per group). One-way ANOVA and Tukey's *post hoc* tests. *$P < 0.05$, n.s. = not significant.

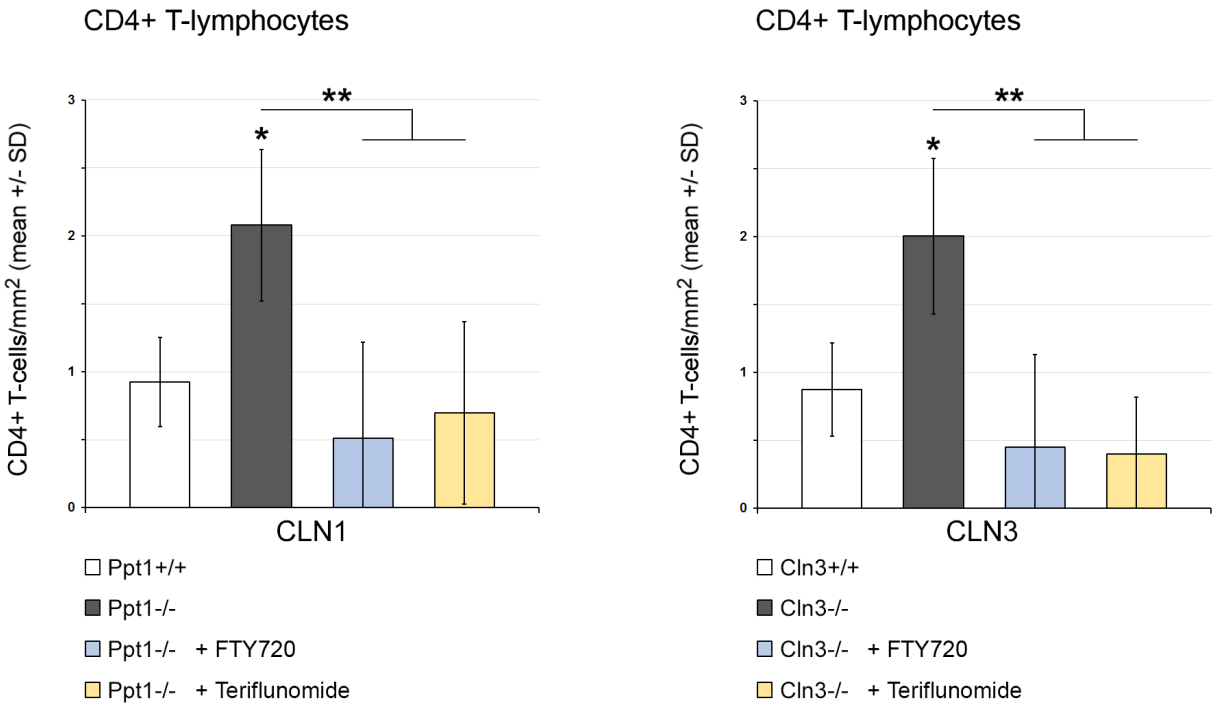

Figure S4

Fingolimod and Teriflunomide prevent the elevation of CD4+ T-lymphocyte numbers in CLN1 and CLN3 disease models. Quantification of CD4+ T-lymphocytes in longitudinal optic nerve sections from 6-month-old *Ppt1*$^{+/+}$, *Ppt1*$^{-/-}$, Fingolimod-treated *Ppt1*$^{-/-}$ (+ FTY720) and Teriflunomide-treated *Ppt1*$^{-/-}$ (+ Teriflunomide) mice and 17-month-old *Cln3*$^{+/+}$, *Cln3*$^{-/-}$, Fingolimod-treated *Cln3*$^{-/-}$ (+ FTY720) and Teriflunomide-treated *Cln3*$^{-/-}$ (+ Teriflunomide) mice. The numbers of CD4+ T-cells were significantly increased in the CLN1 and CLN3 disease models compared with Wt mice. This increase was prevented in both models by treatment with Fingolimod as well as Teriflunomide for 150 days (n = 5 mice per group). One-way ANOVA and Tukey's *post hoc* tests. *$P < 0.05$, **$P < 0.01$.

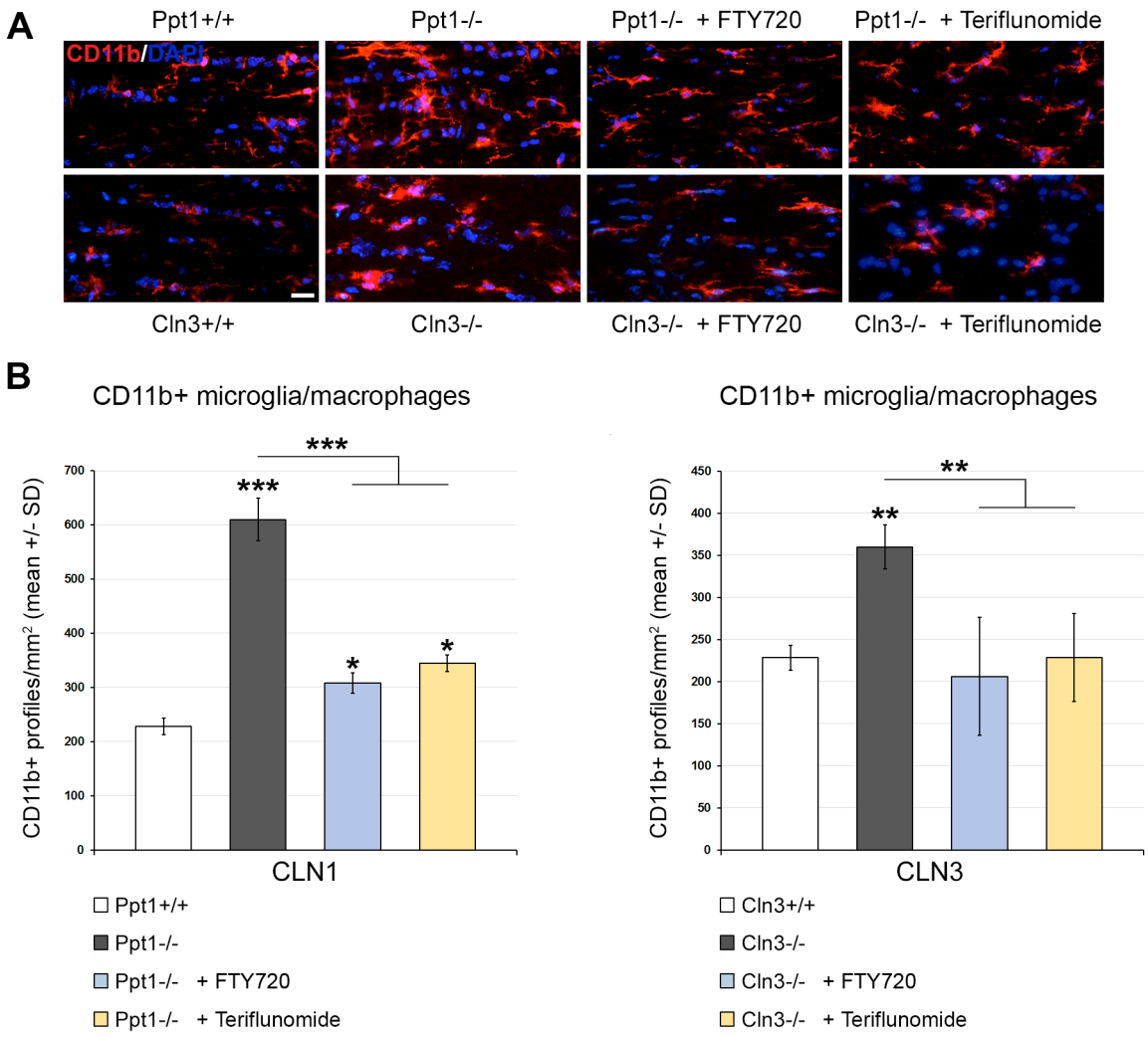

Figure S5

Fingolimod and teriflunomide prevent the increase in CD11b+ microglia/macrophage numbers in CLN1 and CLN3 disease models. (**A**) Representative immune fluorescence microscopy of CD11b+ microglia/macrophages in longitudinal optic nerve sections from 6-month-old *Ppt1*$^{+/+}$, *Ppt1*$^{-/-}$, fingolimod-treated *Ppt1*$^{-/-}$ (+ FTY720) and teriflunomide-treated *Ppt1*$^{-/-}$ (+ Teriflunomide) mice (top) and 17-month-old *Cln3*$^{+/+}$, *Cln3*$^{-/-}$, fingolimod-treated *Cln3*$^{-/-}$ (+ FTY720) and teriflunomide-treated *Cln3*$^{-/-}$ (+ Teriflunomide) mice (bottom). Scale bar: 20 µm. (**B**) Quantification of CD11b+ microglia/macrophages in optic nerve sections: the numbers of CD11b+ microglia/macrophages were significantly increased in the untreated CLN1 and CLN3 disease models compared with Wt mice. This increase was prevented in both models by treatment with fingolimod as well as with teriflunomide for 150 days (n = 5 mice per group). One-way ANOVA and Tukey's *post hoc* tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.