Supplementary files for:

Incomplete remyelination via therapeutically enhanced oligodendrogenesis is sufficient to recover visual cortical function

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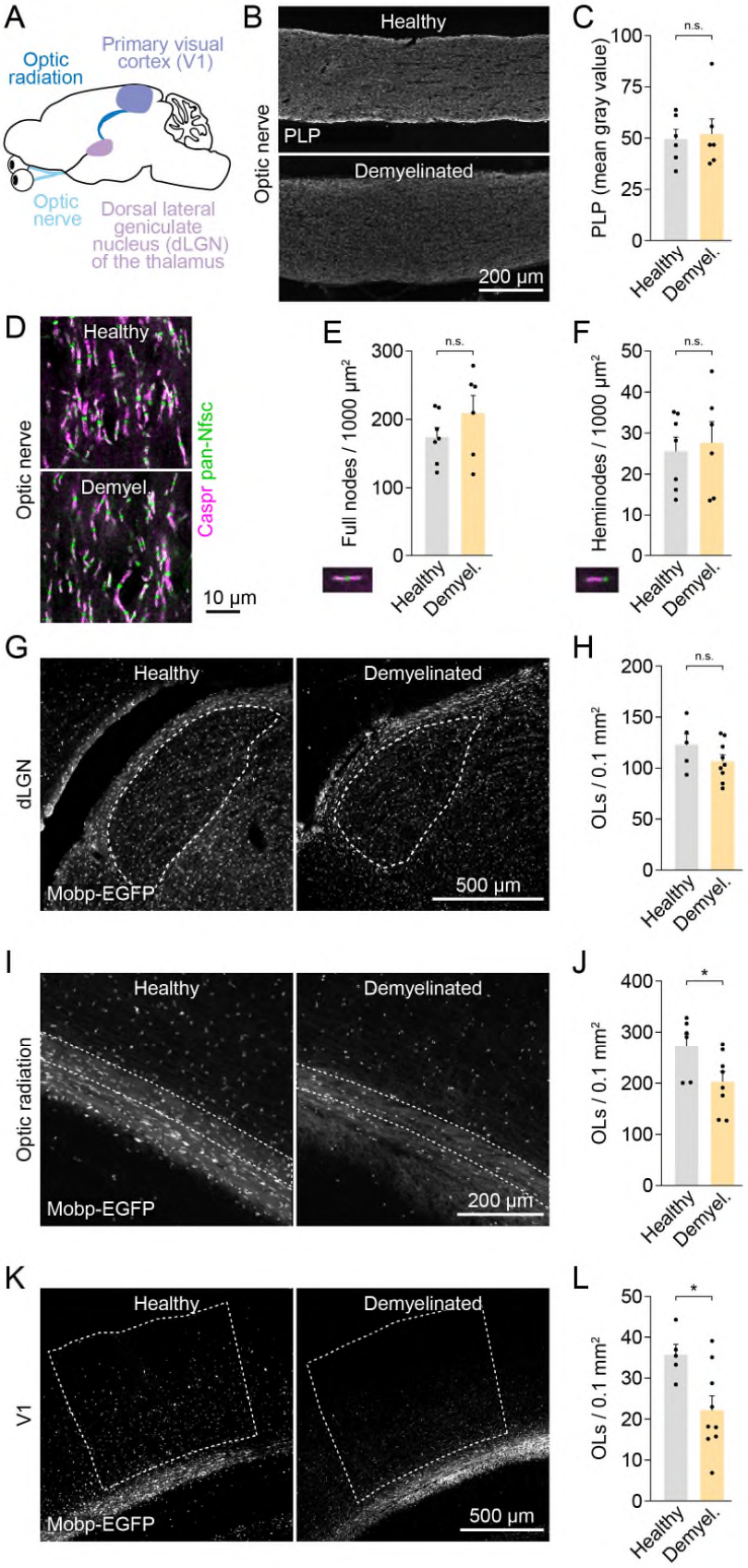


Supplementary Fig. 1: Characterization of visual pathway demyelination in acute cuprizone model.

(A) Schematic of mouse visual pathway.

(B) Representative images of optic nerves immunostained for proteolipid protein (PLP) in healthy and demyelinated mice.

(C) PLP levels in the optic nerve are not different between groups.

(D) Representative images of immunostained nodes of Ranvier (Pan-Nfsc+) and paranodes (Caspr+ Pan-Nfsc+) in healthy and demyelinated mice.

(E-F) Cuprizone does not alter the density of nodes of Ranvier (E; bilaterally expressing Caspr and Pan-Nfsc) or heminodes (F; unilaterally expressing Caspr and Pan-Nfsc) in the optic nerve, suggesting myelin is preserved.

(**G, I, K**) Representative images of Mobp-EGFP+ oligodendrocytes in different visual pathway regions of healthy and demyelinated mice.

(**H**) Oligodendrocyte numbers are similar between groups in the dLGN.

(**J, L**) Cuprizone causes a decrease in oligodendrocyte density in the optic radiation (J) and primary visual cortex (L).

In **C**, t test (t(10)=0.29, p=0.78). Healthy: n=6, demyelinated: n=6.

In **E**, t test (t(11)=1.25, p=0.24). Healthy: n=7, demyelinated: n=6.

In **F**, t test (t(11)=0.35, p=0.35). Healthy: n=7, demyelinated: n=6.

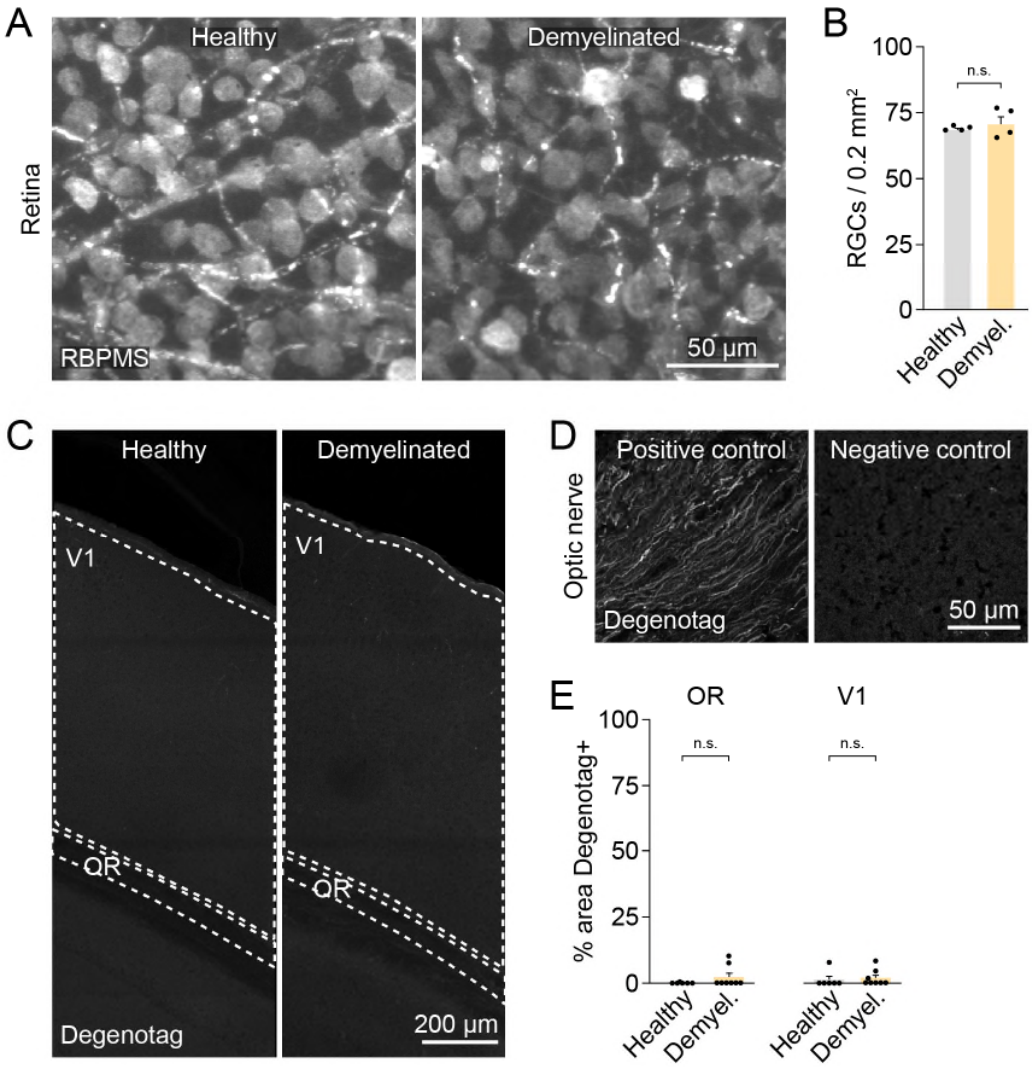
In **H**, t test (t(12)=1.4, p=0.19). Healthy: n=5, demyelinated: n=9.

In **J**, t test (t(12)=2.28, *p=0.042). Healthy: n=6, demyelinated: n=8.

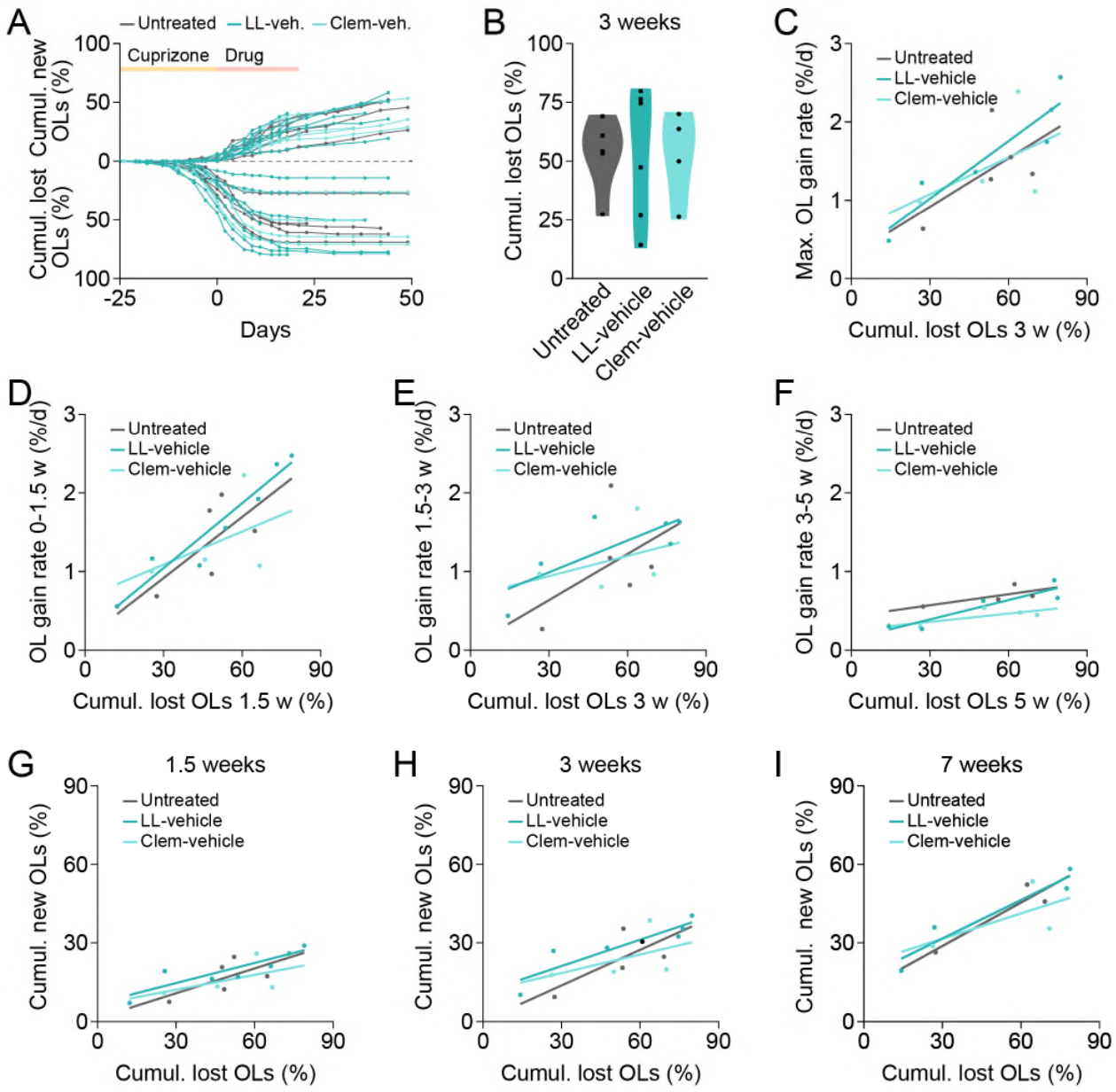
In **L**, t test (t(12)=2.66, *p=0.021). Healthy: n=5, demyelinated: n=9.

n.s. not significant, *p<0.05; mean±SEM; n=mice; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.

dLGN=dorsal lateral geniculate nucleus, V1=primary visual cortex.



Supplementary Fig. 2: No evidence of degeneration of neurons or axons in acute cuprizone model.
(A) Representative images of RGCs immunostained for RBPMS in healthy and demyelinated mice.
(B) Cuprizone does not affect the density of RGCs.
(C) Representative coronal brain sections with immunostaining for Degenotag in optic radiation and V1 of both groups.
(D) Degenotag antibody can sensitively detect axon degeneration induced by maintaining an optic nerve *ex vivo* for 6 h (positive control) and is specific, not detecting degeneration in an acutely fixed optic nerve (negative control).
(E) Cuprizone does not cause axon degeneration as evidenced by lack of Degenotag staining in optic radiation (left) or V1 (right).
In **B**, t test ($t(6)=0.78$, $p=0.47$). Healthy: n=4, demyelinated: n=4.
In **E** OR, t test ($t(12)=1.31$, $p=0.22$). Healthy: n=6, demyelinated: n=8. In **E** V1, t test ($t(12)=0.37$, $p=0.72$). Healthy: n=6, demyelinated: n=8.
n.s. not significant, mean±SEM; n=mice; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
RGC=retinal ganglion cell, V1=primary visual cortex.



Supplementary Fig. 3: Magnitude and dynamics of oligodendrocyte loss and gain are comparable between untreated and vehicle-treated mice.

(A) Cumulative OL loss and gain as a percentage of baseline OLs in individual mice over time (untreated: n=5, LL-341070 vehicle: n=7, clemastine vehicle: n=4).

(B) OL loss by 3 weeks did not differ between groups in mean or variance.

(C) ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on maximum OL gain rate is not significant.

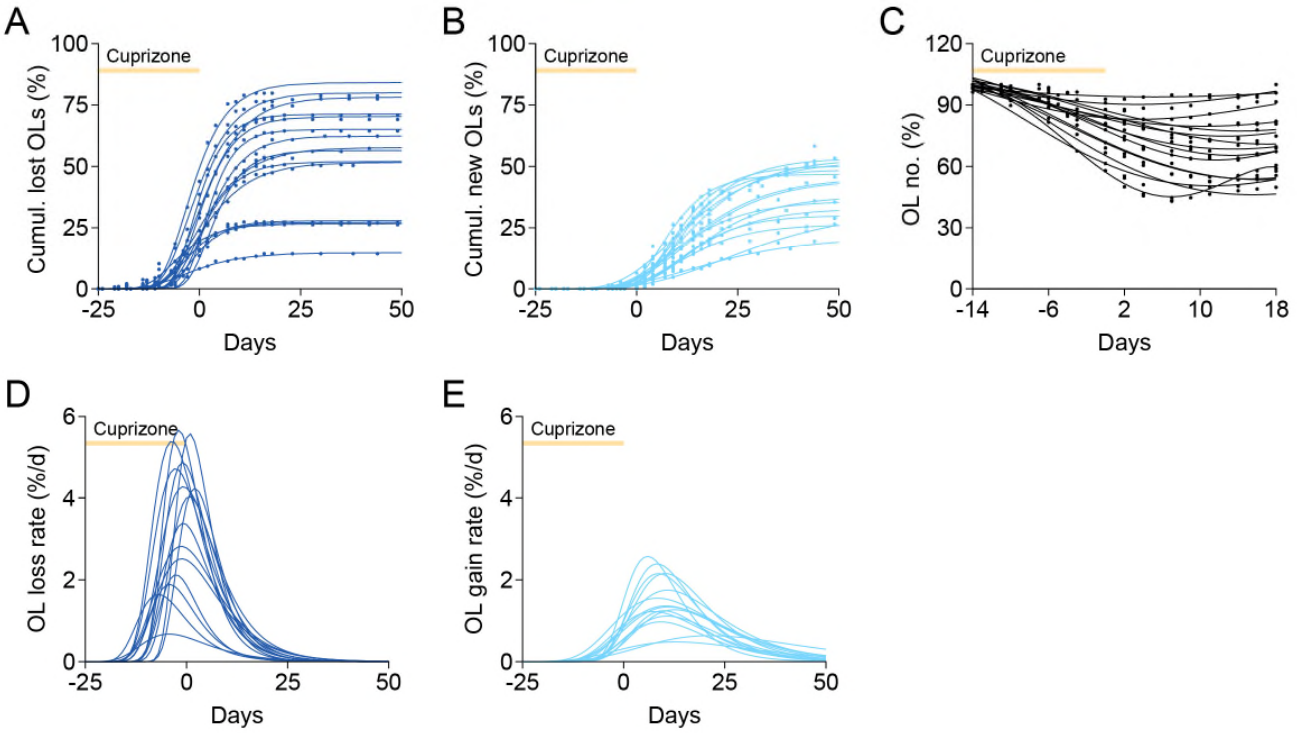
(D) ANCOVA with unequal slopes shows effect of cumulative OL loss but not treatment or the interaction between cumulative OL loss and treatment on OL gain rate from 0-1.5 weeks.

(E) ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on OL gain rate from 1.5-3 weeks is not significant.

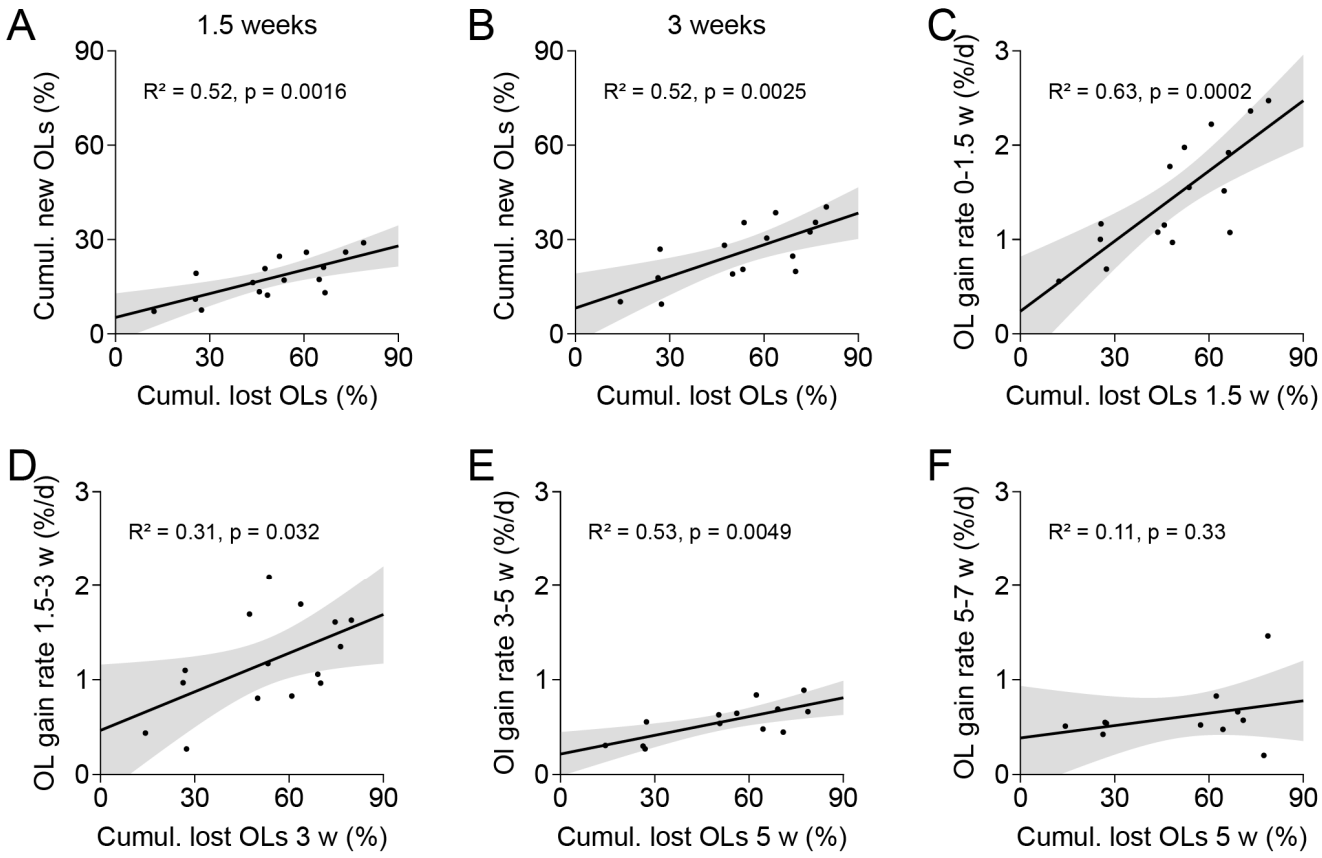
(F) ANCOVA with unequal slopes shows effect of treatment and OL loss but no effect of interaction between OL loss and treatment on OL gain rate from 3-5 weeks. Difference between untreated and clemastine vehicle.

(G-I) ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on cumulative OL gain at 1.5 weeks (G), 3 weeks (H), and 7 weeks (I) is not significant.

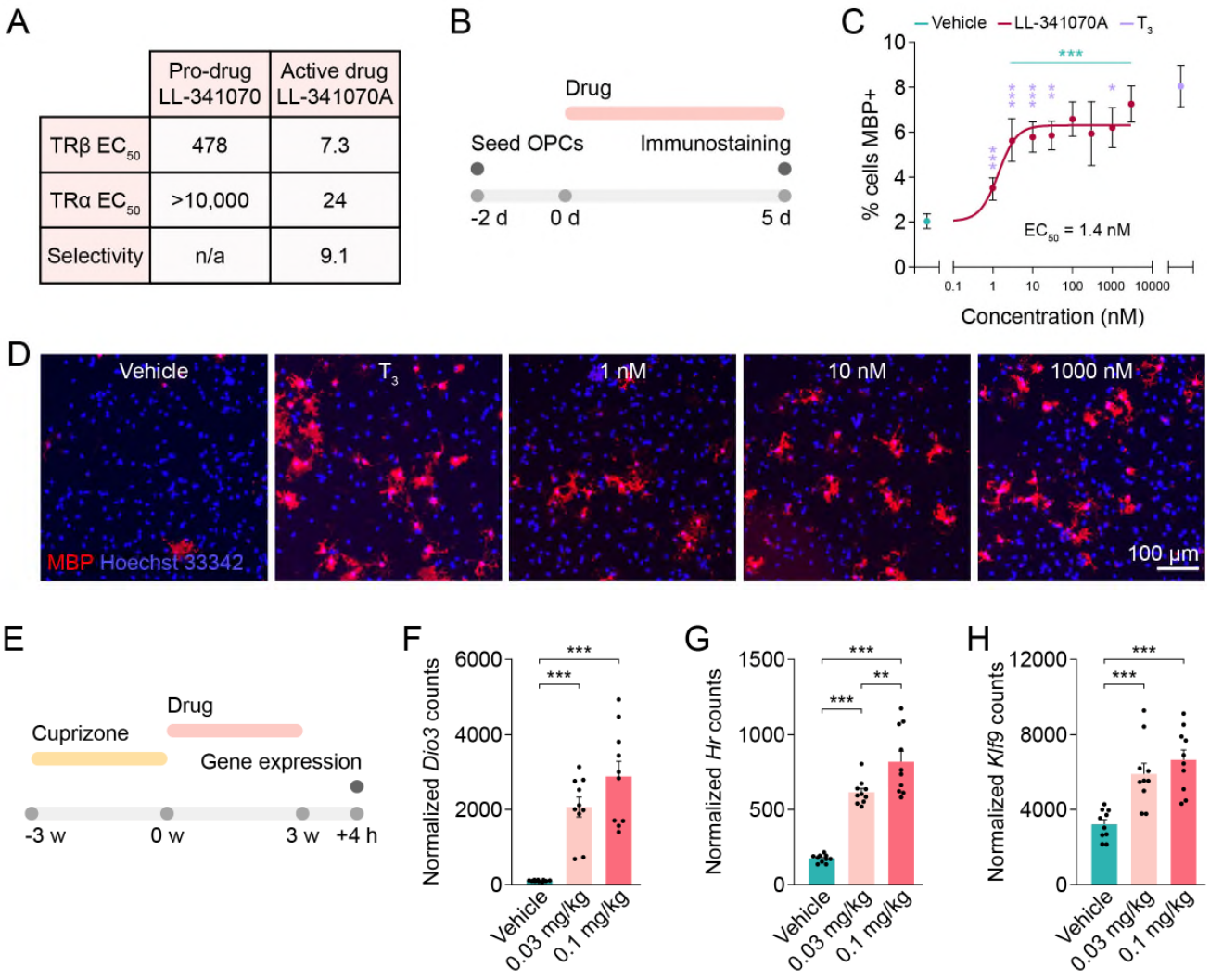
In **B**, ANOVA (F(2, 12)=0.0013, p=1). Brown-Forsythe (F(2, 12)=1.82, p=0.2). Untreated: n=5, LL-341070 vehicle: n=6, clemastine vehicle: n=4.
In **C**, ANCOVA with unequal slopes (F(5, 9)=2.72, p=0.092). Untreated: n=5, LL-341070 vehicle: n=6, clemastine vehicle: n=4.
In **D**, ANCOVA with unequal slopes (F(5, 10)=4.21, *p=0.026). Effects: treatment (F(2)=0.33, p=0.72), cumulative OL loss (F(1)=9.93, *p=0.010), interaction (F(2)=0.41, p=0.67). Untreated: n=5, LL-341070 vehicle: n=7, clemastine vehicle: n=4.
In **E**, ANCOVA with unequal slopes (F(5, 9)=1.02, p=0.46). Untreated: n=5, LL-341070 vehicle: n=6, clemastine vehicle: n=4.
In **F**, ANCOVA with unequal slopes (F(5, 7)=6.57, *p=0.014). Effects: treatment (F(2)=4.84, *p=0.048), OL loss (F(1)=10.83, *p=0.013), interaction (F(2)=1.02, p=0.41). Tukey's HSD (Untreated vs. clemastine vehicle: *p=0.041). Untreated: n=4, LL-341070 vehicle: n=5, clemastine vehicle: n=4.
In **G**, ANCOVA with unequal slopes (F(5, 10)=2.74, p=0.082). Untreated: n=5, LL-341070 vehicle: n=7, clemastine vehicle: n=4.
In **H**, ANCOVA with unequal slopes (F(5, 9)=2.69, p=0.093). Untreated: n=5, LL-341070 vehicle: n=6, clemastine vehicle: n=4.
In **I**, ANCOVA with unequal slopes (F(5, 4)=2.96, p=0.16). Untreated: n=3, LL-341070 vehicle: n=4, clemastine vehicle: n=3.
N=mice; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data. OL=oligodendrocyte, LL=LL-341070, clem=clemastine.



Supplementary Fig. 4: Modeling oligodendrocyte dynamics over time.
(A-B) Three parameter Gompertz growth curves fit to cumulative OL loss (A) and gain (B) over time for individual mice. $R^2 \geq 0.98$ for all mice. Only mice with imaging data out to at least 3 weeks post-cuprizone were used in growth curve modeling (n=15 untreated/vehicle-treated mice).
(C) Quintic curves fit to OL number over time for individual mice. $R^2 \geq 0.86$ for all mice. Only mice with imaging data out to at least 3 weeks post-cuprizone were used in quintic curve modeling (n=15 untreated/vehicle-treated mice). Modeling tracked the data most accurately between -14 days and 18 days so modeled values were used from only this period.
(D-E) First derivatives of three parameter Gompertz growth curves for cumulative OL loss and gain for individual mice (n=15 untreated/vehicle-treated mice) represent OL loss (D) and gain (E) rates over time.
OL=oligodendrocyte.



Supplementary Fig. 5: Oligodendrocyte gain correlates with oligodendrocyte loss over time.
(A) Cumulative OL gain at 1.5 weeks correlates with cumulative OL loss at 1.5 weeks (F(1,14)=15.15, n=16).
(B) Cumulative OL gain at 3 weeks correlates with cumulative OL loss at 3 weeks (F(1,13)=13.99, n=15).
(C) OL gain rate from 0-1.5 weeks correlates with cumulative OL loss at 1.5 weeks (F(1,14)=23.70, n=16).
(D) OL gain rate from 1.5-3 weeks correlates with cumulative OL loss at 3 weeks (F(1,13)=5.74, n=15).
(E) OL gain rate from 3-5 weeks correlates with cumulative OL loss at 5 weeks ((F(1,11)=12.34, n=13).
(F) OL gain rate from 5-7 weeks does not correlate with cumulative OL loss at 5 weeks (F(1,9)=1.06, n=11).
Linear regression; line of best fit ± 95% CI; n=mice. See Supplementary Data 1 for statistical details and Source Data file for source data.
OL=oligodendrocyte.



Supplementary Fig. 6: LL-341070 TRβ selectivity and ability to induce OPC differentiation and thyroid hormone target gene expression.

(**A**) Luciferase reporter assay for TRα or TRβ activity. LL-341070A, the active metabolite of LL-341070, is potent against TRβ (EC_{50} = 7.3 nM) and is 9.1-fold more potent for TRβ than TRα. The prodrug LL-341070 is substantially less potent against both receptors.

(**B**) Timeline of OPC differentiation assay. Drug = vehicle (0.1% DMSO), various concentrations (1, 3, 10, 30, 100, 300, 1000, and 3000 nM) of LL-341070A, or 10 ng/mL (15.4 nM) T_3 as a positive control.

(**C**) The active metabolite LL-341070A increases OPC differentiation *in vitro* in a concentration-dependent manner (EC_{50} = 1.4 nM), with maximum efficacy similar to that of T_3. Data are reported as % of live Hoechst 33342+ cells expressing oligodendrocyte protein MBP.

(**D**) Representative images of OPC differentiation assay.

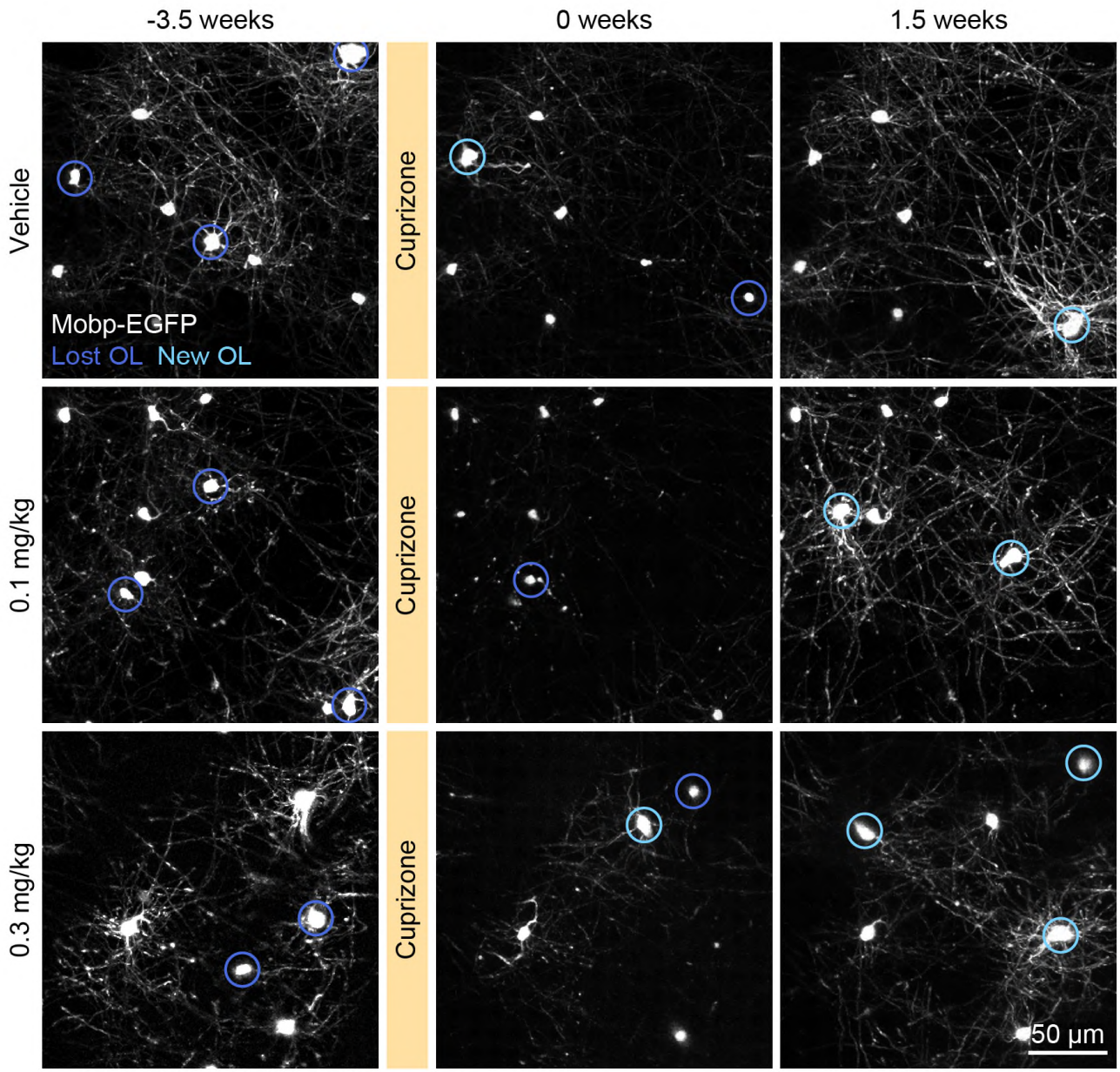
(**E**) Timeline of cuprizone and drug administration to rats for gene expression assay. Drug = vehicle (1% NMP, 1% solutol), 0.03mg/kg and 0.1mg/kg LL-341070.

(**F-H**) LL-341070 increases thyroid hormone target genes *Dio3* (F), *Hr* (G), and *Klf9* (H) in the brain.

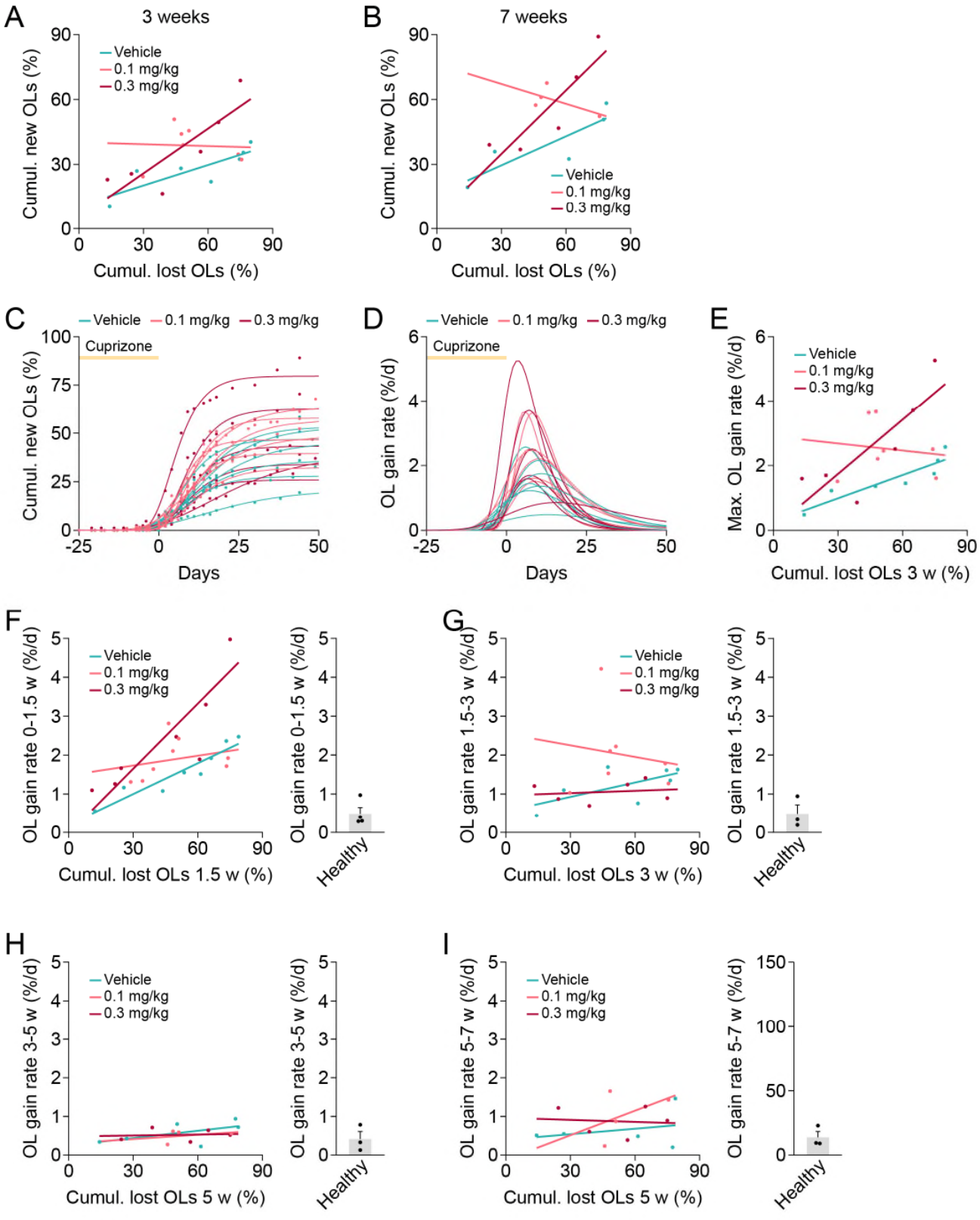
In **C**, ANOVA (F(9, 50)=26, ***p<0.001). Tukey's HSD for all comparisons. For clarity, differences from vehicle and T_3 are shown on graph, while differences from 1nM are listed here: 1nM vs. 3nM: **p=0.0015; 1nM vs. 10nM: ***p=0.0006; 1nM vs. 30nM: ***p<0.0004; 1nM vs. 300nM: ***p=0.0002; 1nM vs. 100nM, 1nM vs. 1000nM, 1nM vs. 3000nM: ***p<0.0001. See Supplementary Data 1 for details of all comparisons. N=6 wells for all treatments.

In **F**, ANOVA (F(2, 27)=26.56, ***p<0.0001). Tukey's HSD (vehicle vs. 0.03mg/kg: ***p<0.0001; vehicle vs. 0.1mg/kg: ***p<0.0001; 0.03mg/kg vs. 0.1mg/kg: p=0.11). N=10 for all treatments.

In **G**, ANOVA (F(2, 27)=58.38, ***p<0.0001). Tukey's HSD (vehicle vs. 0.03 mg/kg: ***p<0.0001; vehicle vs. 0.1mg/kg: ***p<0.0001; 0.03mg/kg vs. 0.1mg/kg: **p=0.0064). N=10 for all treatments.
In **H**, ANOVA (F(2, 27)=15.02, ***p<0.0001). Tukey's HSD (vehicle vs. 0.03mg/kg: ***p=0.0010; vehicle vs. 0.1mg/kg: ***p<0.0001; 0.03mg/kg vs. 0.1mg/kg: p=0.51). N=10 for all treatments.
*p<0.05, ** p<0.01, ***p<0.001; mean±SD in C; mean±SEM in F-H; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
OPC=oligodendrocyte precursor cell.



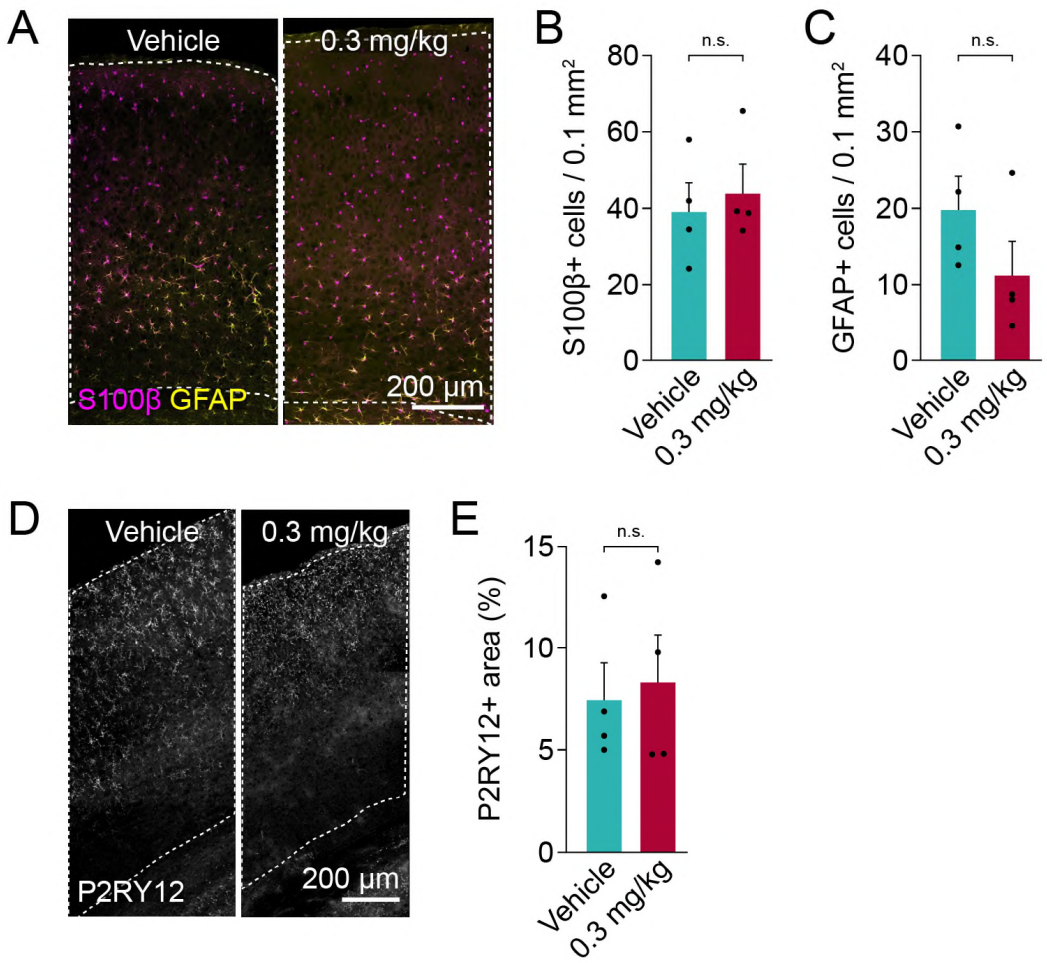
Supplementary Fig. 7: Representative images of V1 oligodendrocytes in thyromimetic- and vehicle-treated mice. Representative images of V1 OLs in mice treated with vehicle, 0.1mg/kg LL-341070, or 0.3mg/kg LL-341070 at baseline (-3.5 weeks), end of cuprizone (0 weeks), and 1.5 weeks post-cuprizone. Lost OLs (dark blue) and new OLs (light blue) are encircled.
OL=oligodendrocyte, V1=primary visual cortex.



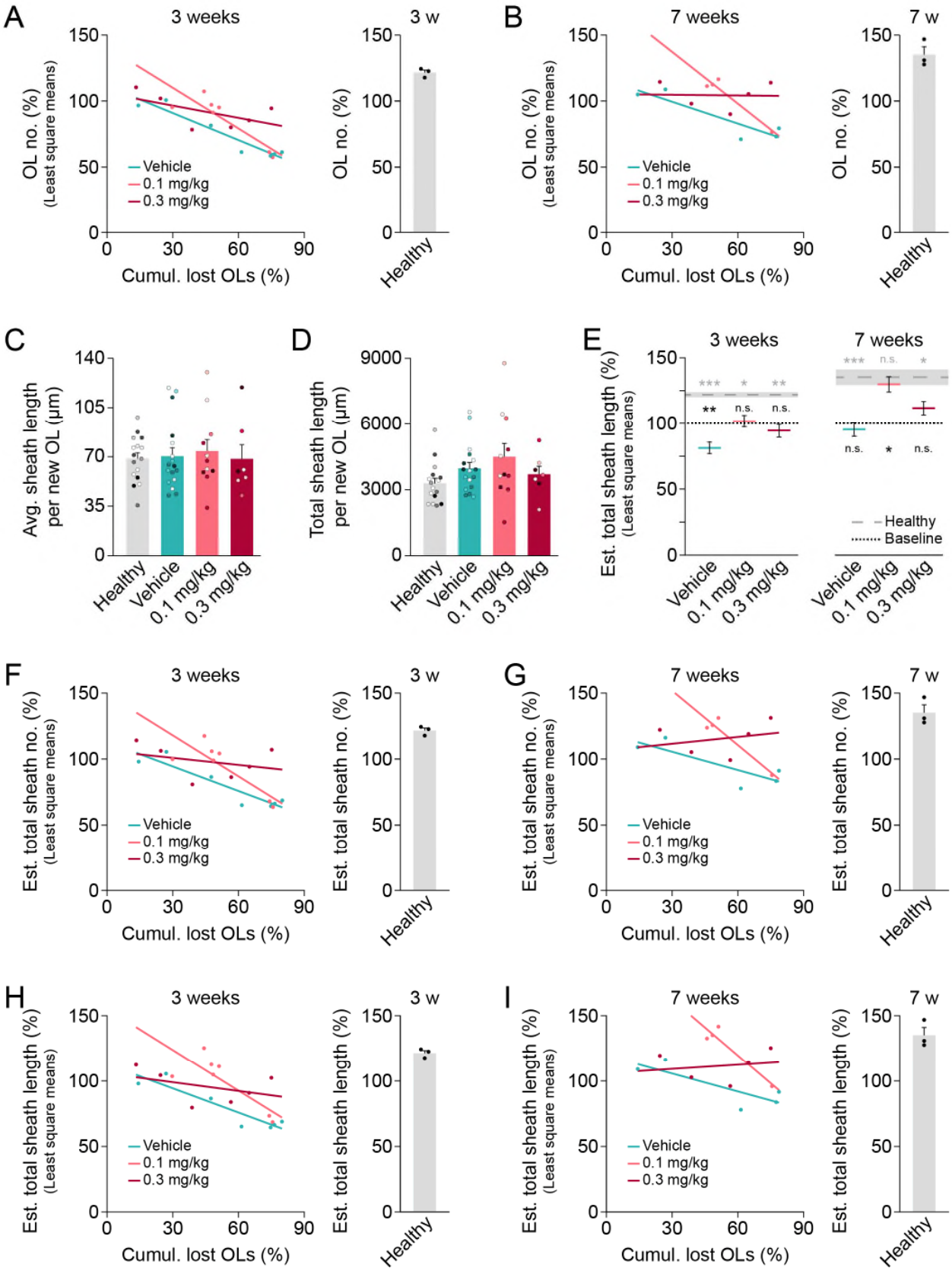
Supplementary Fig. 8: Supporting evidence that thyromimetic treatment enhances oligodendrocyte gain during remyelination.

(A) ANCOVA with unequal slopes shows effect of treatment and OL loss but no effect of interaction between OL loss and treatment on cumulative OL gain at 3 weeks.

(B) ANCOVA with unequal slopes shows effect of treatment but no effect of cumulative OL loss or interaction between OL loss and treatment on cumulative OL gain at 7 weeks.
(C) Three parameter Gompertz growth curves fit to cumulative OL gain over time for individual mice. R^2>0.97 for all mice. Only mice with imaging data out to at least 3 weeks post-cuprizone were used in growth curve modeling (vehicle: n=7, 0.1 mg/kg: n=7, 0.3 mg/kg: n=6).
(D) First derivatives of three parameter Gompertz growth curves for cumulative OL gain for individual mice represent OL gain rates over time (vehicle: n=7, 0.1 mg/kg: n=7, 0.3 mg/kg: n=6) and were used to derive maximum OL gain rate.
(E) ANCOVA with unequal slopes shows effect of treatment and cumulative OL loss but no effect of interaction between OL loss and treatment on maximum OL gain rate.
(F) Left: ANCOVA with unequal slopes shows effect of treatment and cumulative OL loss, and interaction between OL loss and treatment on OL gain rate from 0-1.5 weeks. Right: OL gain rate from 0-1.5 weeks in healthy mice.
(G-I) Left: ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on OL gain rate from 1.5-3 weeks (G), 3-5 weeks (H), and 5-7 weeks (I) is not significant. Right: OL gain rate from 1.5-3 weeks (G), 3-5 weeks (H), and 5-7 weeks (I) in healthy mice.
In **A**, ANCOVA with unequal slopes (F(5, 14)=5.18, **p=0.0067). Effects: treatment (F=4.14, *p=0.039), cumulative OL loss (F=9.18, **p=0.009), interaction (F=3.29, p=0.068). Vehicle: n=7, 0.1mg/kg: n=7, 0.3mg/kg: n=6.
In **B**, ANCOVA with unequal slopes (F(5, 8)=7.38, **p=0.0072). Effects: treatment (F=6.1, *p=0.025), cumulative OL loss (F=5.05, p=0.055), interaction (F=4.02, p=0.062). Vehicle: n=5, 0.1mg/kg: n=4, 0.3mg/kg: n=5.
In **E**, ANCOVA with unequal slopes (F(5, 14)=4.95, *p=0.0081). Effects: treatment (F=5.46, *p=0.018), cumulative OL loss (F=6.77, *p=0.021), interaction (F=3.39, p=0.063). Vehicle: n=7, 0.1mg/kg: n=7, 0.3mg/kg: n=6.
In **F** left, ANCOVA with unequal slopes (F(5, 18)=11.20, ***p<0.0001). Effects: treatment (F=9.34, **p=0.0017), cumulative OL loss (F=29.60, ***p<0.0001), interaction (F=5.93, *p=0.011). Vehicle: n=8, 0.1mg/kg: n=9, 0.3mg/kg: n=7.
In **G** left, ANCOVA with unequal slopes (F(5, 14)=1.59, p=0.23). Vehicle: n=7, 0.1mg/kg: n=7, 0.3mg/kg: n=6.
In **H** left, ANCOVA with unequal slopes (F(5, 9)=1.19, p=0.39). Vehicle: n=6, 0.1mg/kg: n=4, 0.3mg/kg: n=5.
In **I** left, ANCOVA with unequal slopes (F(5, 8)=0.48, p=0.78). Vehicle: n=5, 0.1mg/kg: n=4, 0.3mg/kg: n=5.
Mean±SEM in F-I right; n=mice; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
OL=oligodendrocyte.

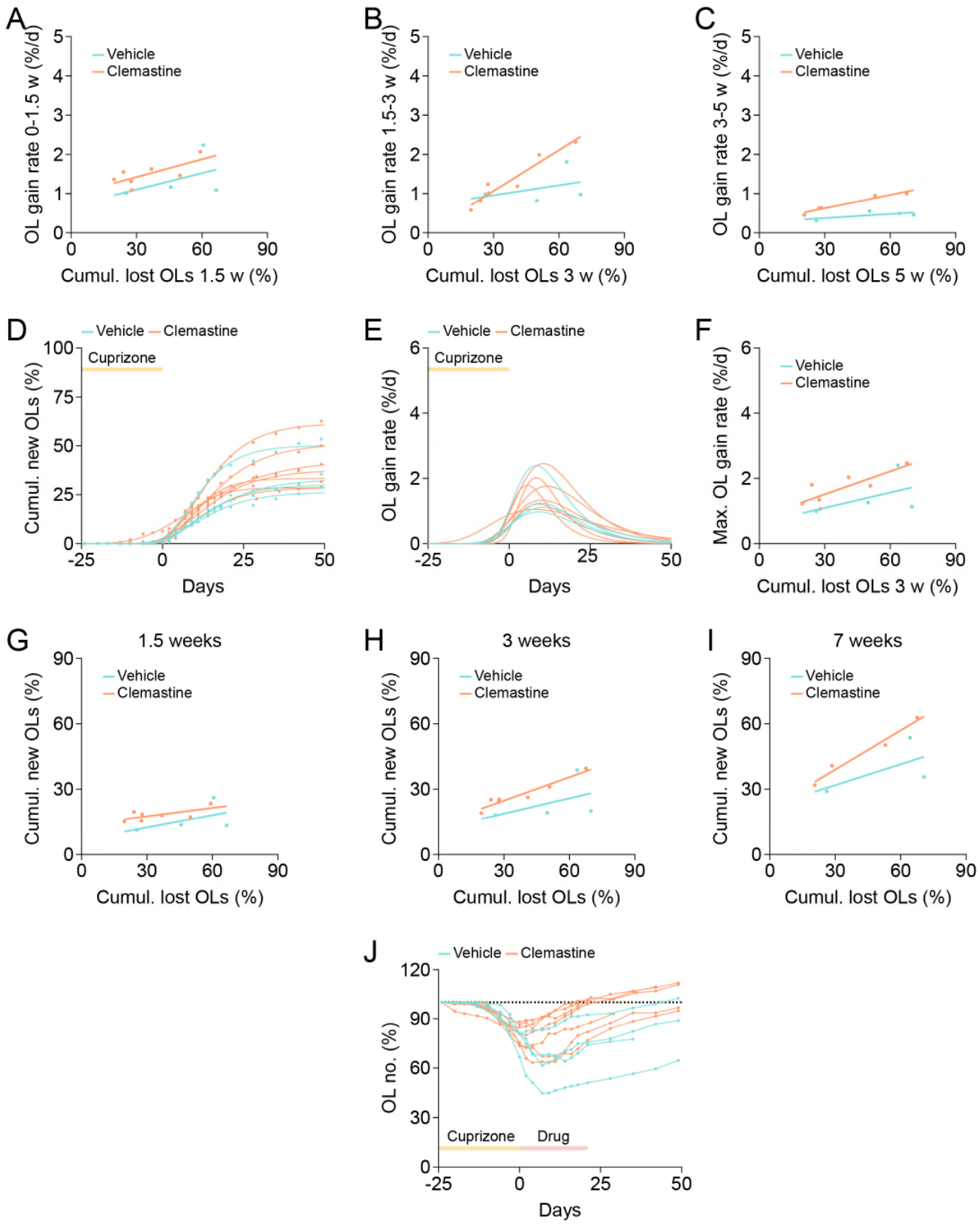


Supplementary Fig. 9: Thyromimetic does not alter astrocytic and microglial responses to cuprizone.
(A) Representative images 3 weeks post-cuprizone of immunostaining for astrocytes with a pan-astrocytic marker (S100β) and with a marker of reactive astrocytes (GFAP) in the V1 of demyelinated mice treated with 0.3mg/kg LL-341070 or vehicle.
(B) Density of overall astrocytes in V1 is unaffected by LL-341070 treatment.
(C) Density of reactive astrocytes in V1 is unaffected by LL-341070 treatment.
(D) Representative images 3 weeks post-cuprizone of immunostaining for homeostatic microglia with P2RY12 in V1 of demyelinated mice treated with 0.3mg/kg LL-341070 or vehicle.
(E) Percentage area with homeostatic microglia is unaffected by LL-341070 treatment.
In **B**, t test ($t(6)=0.48$, $p=0.65$). Vehicle: n=4, LL-341070: n=4.
In **C**, t test ($t(6)=1.41$, $p=0.21$). Vehicle: n=4; LL-341070: n=4.
In **E**, t test ($t(6)=0.3$, $p=0.77$). Vehicle: n=4; LL-341070: n=4.
n.s. not significant; mean±SEM; n=mice; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
V1=primary visual cortex.



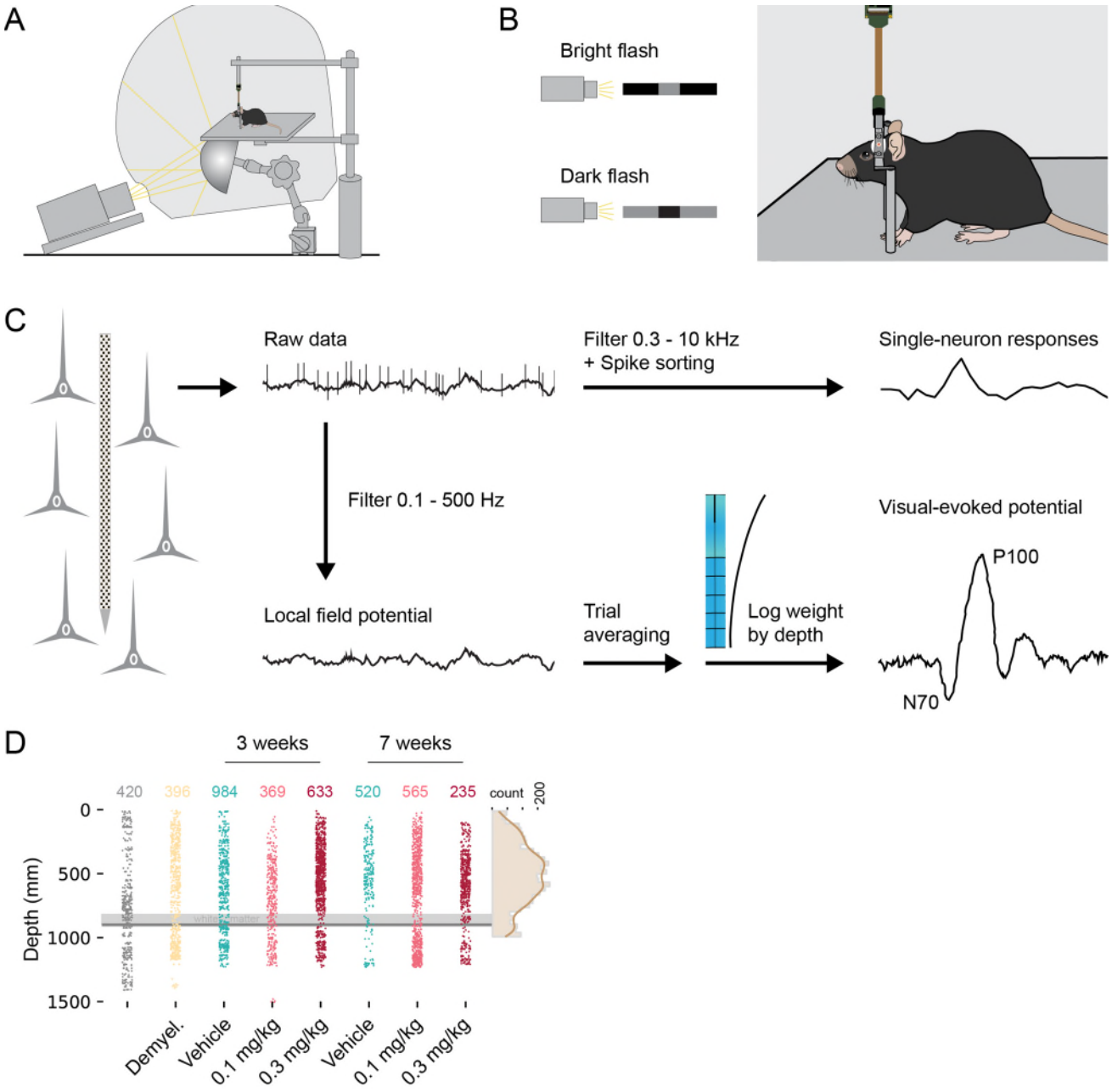
Supplementary Fig. 10: Supporting evidence that thyromimetic restores oligodendrocytes to baseline levels and myelin to healthy levels.

(A-B) Left: ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on OL number at 3 weeks (A) and 7 weeks (B) was used to derive least square mean and SEM for OL number of treatment groups. Right: OL number in healthy mice at 3 weeks (A) and 7 weeks (B).
(C-D) No difference in average sheath length (C) or total sheath length (D) made by individual new OLs generated during remyelination by mice treated with vehicle, low dose, or high dose LL-341070, or in healthy mice.
(E) Low and high dose LL-341070-treated mice restore baseline levels of total myelin length by 3 weeks (left) and low dose treated mice reach healthy levels of total myelin length by 7 weeks (right).
(F-G) Left: ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on estimated total sheath number at 3 weeks (F) and 7 weeks (G) was used to derive least square mean and SEM for estimated total sheath number of treatment groups. Right: Estimated total sheath number in healthy mice at 3 weeks (F) and 7 weeks (G).
(H-I) Left: ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on estimated total sheath length at 3 weeks (H) and 7 weeks (I) was used to derive least square mean and SEM for estimated total sheath length of treatment groups. Right: Estimated total sheath length in healthy mice at 3 weeks (H) and 7 weeks (I).
In **C**, ANOVA ($F(3,47)=0.13$, $p=0.94$). Vehicle: n=17 OLs from 7 mice, 0.1mg/kg: n=11 OLs from 6 mice, 0.3mg/kg: n=7 OLs from 5 mice, healthy: n=16 OLs from 7 mice.
In **D**, ANOVA ($F(3,47)=1.98$, $p=0.13$). Vehicle: n=17 OLs from 7 mice, 0.1mg/kg: n=11 OLs from 6 mice, 0.3mg/kg: n=7 OLs from 5 mice, healthy: n=16 OLs from 7 mice.
In **E** 3w, ANOVA ($F(3, 19)=15.4$, ***$p<0.0001$). Dunnett's (vehicle vs. healthy: ***$p=0.0001$; 0.1mg/kg vs. healthy: *$p=0.013$; 0.3mg/kg vs. healthy: **$p=0.0011$). One-sample t tests (vehicle vs baseline: $t(6)=5.29$, **$p=0.0018$; 0.1mg/kg vs baseline: $t(6)=0.50$, $p=0.64$; 0.3mg/kg vs baseline: $t(5)=1.45$, $p=0.21$). Vehicle: n=7, 0.1mg/kg: n=7, 0.3mg/kg: n=6, healthy: n=3. In **E** 7w, ANOVA ($F(3, 13)=10.61$, ***$p=0.0008$). Dunnett's (vehicle vs. healthy: ***$p=0.0009$; 0.1mg/kg vs. healthy: $p=0.88$; 0.3mg/kg vs. healthy: *$p=0.037$). One-sample t tests (vehicle vs. baseline: $t(4)=0.92$, $p=0.41$; 0.1mg/kg vs. baseline: $t(3)=5.11$, *$p=0.015$; 0.3mg/kg vs. baseline: $t(4)=2.29$, $p=0.084$). Vehicle: n=5, 0.1mg/kg: n=4, 0.3 mg/kg: n=5; healthy: n=3.
n.s. not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$; mean±SEM A-B right, C-D, F-I right; treatments: least square mean±SEM and healthy: mean±SEM in E; n=mice unless otherwise specified; data points color-coded by mouse in C; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
OL=oligodendrocyte.

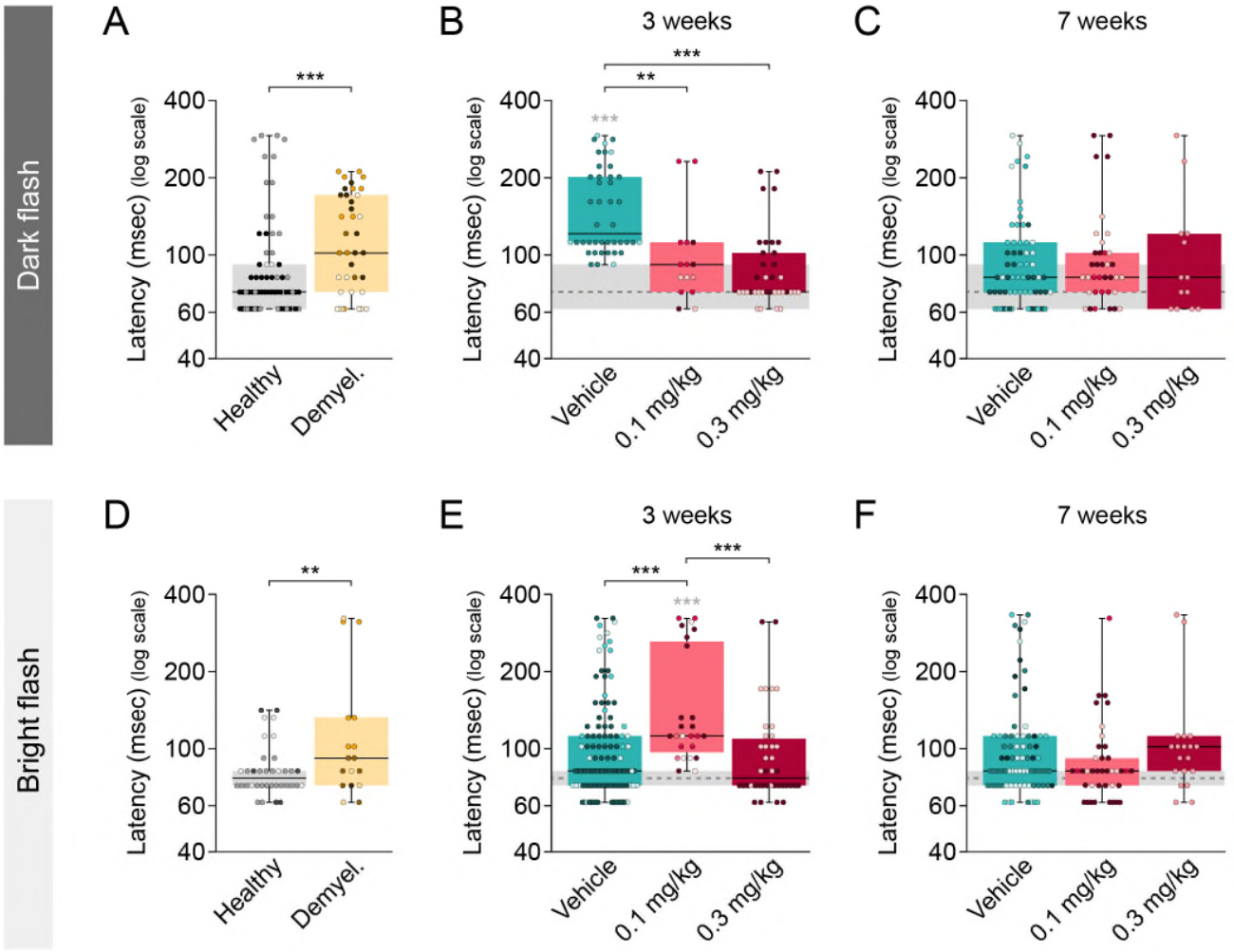


Supplementary Fig. 11: Supporting evidence that clemastine has subtler and more protracted actions on remyelination.

(A) ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on OL gain rate from 0-1.5 weeks is not significant.
(B) ANCOVA with unequal slopes shows effect of cumulative OL loss, but not treatment or interaction between cumulative OL loss and treatment on OL gain rate from 1.5-3 weeks.
(C) ANCOVA with unequal slopes shows effect of cumulative OL loss, treatment, and interaction between cumulative OL loss and treatment on OL gain rate from 3-5 weeks.
(D) Three parameter Gompertz growth curves fit to cumulative OL gain over time for individual mice. R^2>0.98 for all mice. Only mice with imaging data out to at least 3 weeks post-cuprizone were used in growth curve modeling (vehicle: n=4, clemastine: n=7).
(E) First derivatives of three parameter Gompertz growth curves for cumulative OL gain for individual mice represent OL gain rates over time (vehicle: n=4, clemastine: n=7) and were used to derive maximum OL gain rate.
(F) ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on maximum OL gain rate is not significant.
(G-I) ANCOVA with unequal slopes for effect of treatment, cumulative OL loss, and interaction between treatment and cumulative OL loss on cumulative OL gain at 1.5 weeks (G), 3 weeks (H), and 7 weeks (I) is not significant.
(J) OL number in individual mice over time (vehicle: n=7, clemastine: n=4). Dashed line at 100%.
In **A**, ANCOVA with unequal slopes (F(3,7)=1.15, p=0.39). Vehicle: n=4, clemastine: n=7.
In **B**, ANCOVA with unequal slopes (F(3,7)=7.51, *p=0.014). Effects: treatment (F=3.77, p=0.094), cumulative OL loss (F=12.66, **p=0.0093), interaction (F=4.64, p=0.068). Vehicle: n=4, clemastine: n=7.
In **C**, ANCOVA with unequal slopes (F(3, 5)=22.18, **p=0.0026). Effects: treatment (F=45.29, **p=0.0011), cumulative OL loss (F=25.28, **p=0.0040), interaction (F=7.05, *p=0.045). Vehicle: n=4, clemastine: n=5.
In **F**, ANCOVA with unequal slopes (F(3,7)=2.21, p=0.18). Vehicle: n=4, clemastine: n=7.
In **G**, ANCOVA with unequal slopes (F(3,7)=1.24, p=0.37). Vehicle: n=4, clemastine: n=7.
In **H**, ANCOVA with unequal slopes (F(3,7)=2.97, p=0.11). Vehicle: n=4, clemastine: n=7.
In **I**, ANCOVA with unequal slopes (F(3,3)=3.23, p=0.18). Vehicle: n=3, clemastine: n=4.
N=mice; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data. OL=oligodendrocyte.

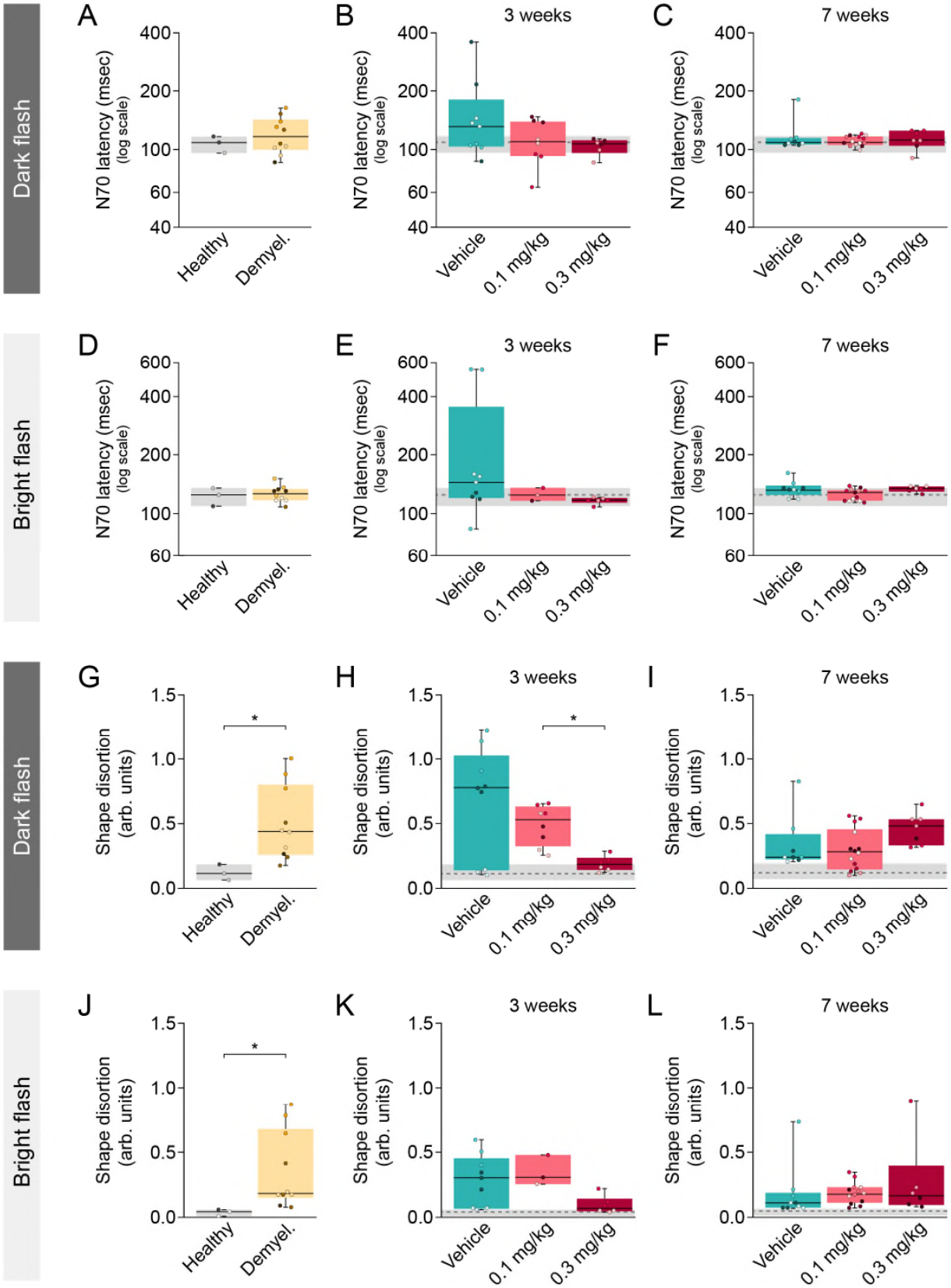


Supplementary Fig. 12: Neuronal recordings using Neuropixel probes.
(A) Schematic representation of the immersive visual stimulation dome.
(B) During the recording, mice are presented with a bright flash stimulus (50 ms increase in luminance) and a dark flash stimulus (50 ms decrement in luminance).
(C) Two signals can be recovered from raw Neuropixels recordings: single-neuron responses are identified through spike sorting (top), while visual evoked responses (VEPs) are obtained from a sum of trial-averaged local field potentials weighted by depth.
(D) Number and depth of single neurons recorded from each group. Marginal distribution of depth of single units from pial surface shown in beige (right), demonstrating a slight bias towards deeper cortical layers.



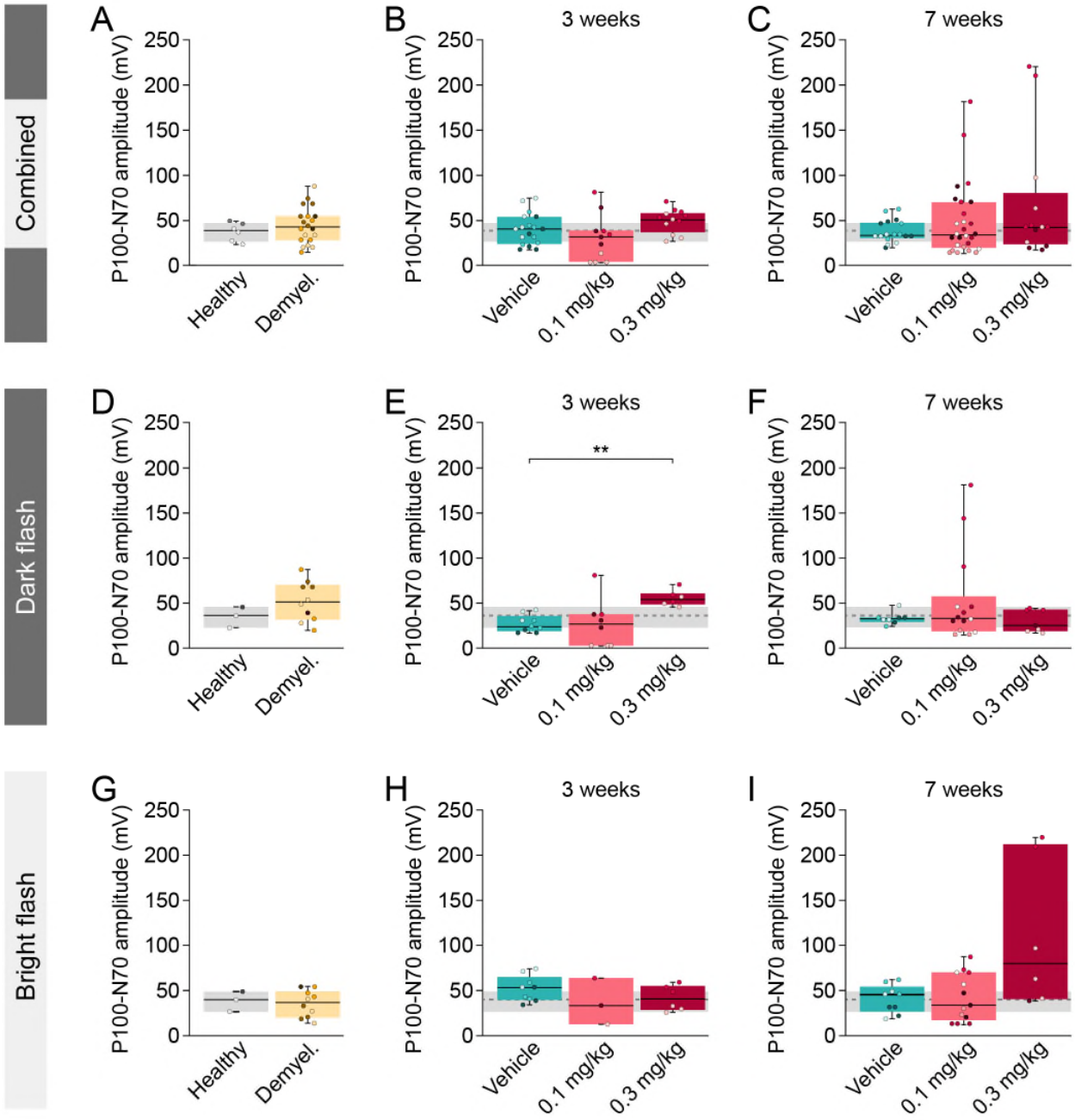
Supplementary Fig. 13: Single-neuron responses to dark and bright flash.
(A) Single-neuron responses to dark flash are delayed after cuprizone-induced demyelination.
(B) Treatment with either low or high dose LL-341070 reestablished healthy neuronal latencies to dark flash at 3 weeks.
(C) Single-neuron responses to dark flash are normalized to healthy values in all groups after 7 weeks of remyelination.
(D) Single-neuron responses to bright flash are delayed after cuprizone-induced demyelination.
(E) Treatment with either high dose of LL-341070 or vehicle reestablished healthy neuronal latencies to bright flash at 3 weeks.
(F) Single-neuron responses to bright flash are normalized to healthy values in all groups after 7 weeks of remyelination.
In **A**, Wilcoxon (Z=11.52, ***p=0.0007). Healthy: n=68 neurons from 5 probes from 3 mice, demyelinated: n=38 neurons from 7 probes from 5 mice.
In **B**, Kruskal-Wallis (F(3)=48.3, ***p<0.0001). Steel-Dwass (vehicle vs. healthy: ***p<0.0001; 0.1mg/kg vs. vehicle: **p=0.002; 0.3mg/kg vs. vehicle: ***p<0.0001). Vehicle: n=42 neurons from 5 probes from 2 mice, 0.1mg/kg: n=15 neurons from 4 probes from 4 mice, 0.3mg/kg: n=36 neurons from 4 probes from 2 mice, healthy: n=68 neurons from 5 probes from 3 mice.
In **C**, Kruskal-Wallis (F(3)=3.1, p=0.38). Vehicle: n=68 neurons from 10 probes from 4 mice, 0.1mg/kg: n=38 neurons from 8 probes from 3 mice, 0.3mg/kg: n=13 neurons from 1 probe from 1 mouse, healthy: n=68 neurons from 5 probes from 3 mice.
In **D**, Wilcoxon (Z=6.7, **p=0.0097). Healthy: n=36 neurons from 6 probes from 3 mice, demyelinated: n=17 neurons from 4 probes from 4 mice.

In **E**, Kruskal-Wallis (F(3)=25.84, ***p<0.0001). Steel-Dwass (0.1mg/kg vs. healthy: ***p<0.0001; vehicle vs. 0.1mg/kg: ***p=0.0005; 0.3mg/kg vs. 0.1mg/kg: ***p=0.0004). Vehicle: n=115 neurons from 8 probes from 3 mice, 0.1mg/kg: n=25 neurons from 6 probes from 4 mice, 0.3mg/kg: n=40 neurons from 4 probes from 2 mice, healthy: n=36 neurons from 6 probes from 3 mice.
In **F**, Kruskal-Wallis (F(3)=9.6, *p=0.022). Steel-Dwass (no significant comparisons). Vehicle: n=76 neurons from 11 probes from 4 mice, 0.1mg/kg: n=39 neurons from 6 probes from 3 mice, 0.3mg/kg: n=19 neurons from 3 probes from 1 mouse.
**p<0.01, ***p<0.001, median, IQR (box), min./max. (whiskers); data points color-coded by mouse; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.



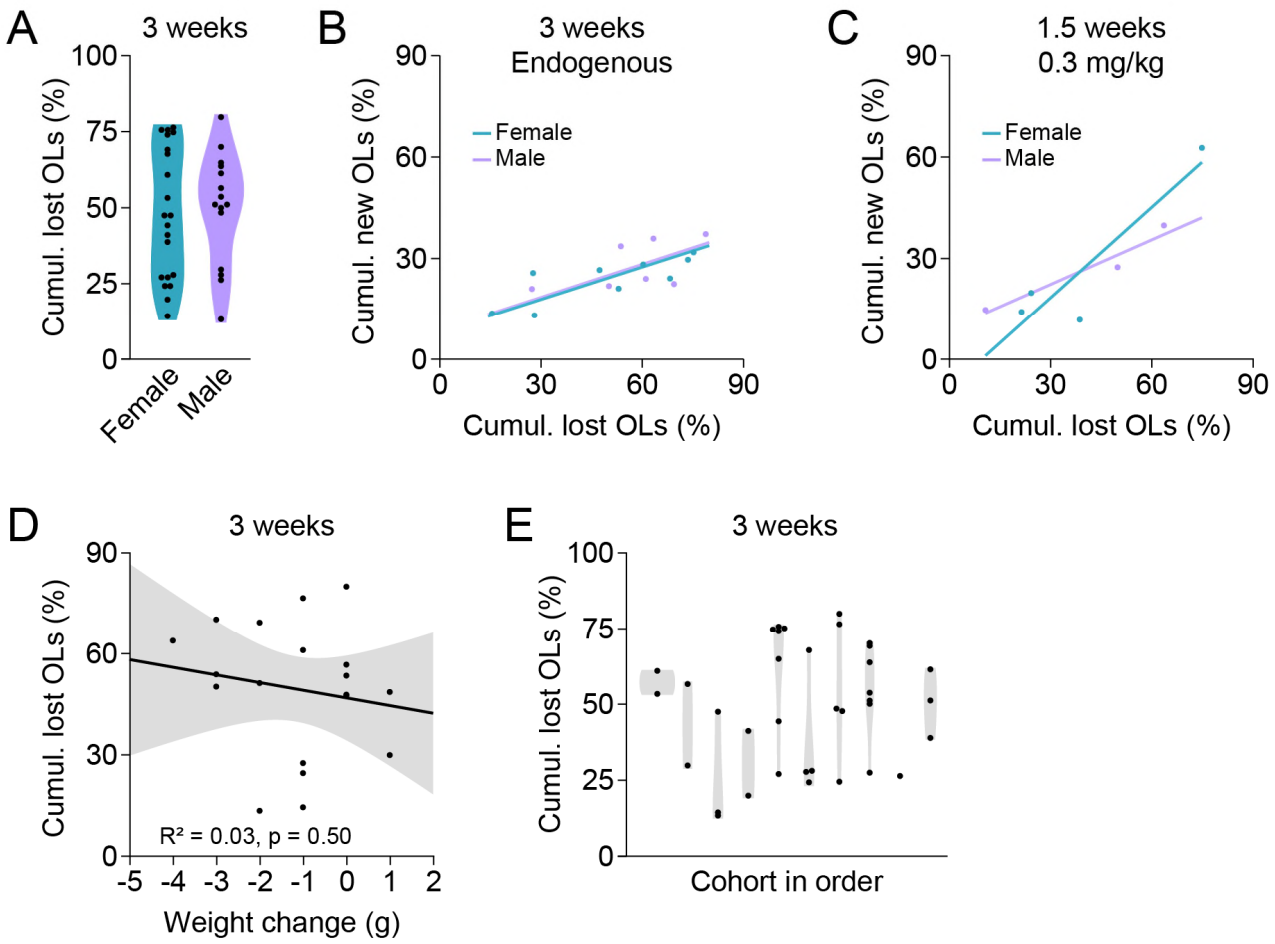
Supplementary Fig. 14: VEP responses to dark and bright flash.

(A) VEP N70 latency to dark flash is not altered by demyelination.
(B-C) All groups show similar VEP N70 latencies to dark flash as healthy after 3 weeks (B) and 7 weeks (C) of remyelination.
(D) VEP N70 latency to bright flash is not altered by demyelination.
(E-F) All groups show similar VEP N70 latencies to bright flash as healthy after 3 weeks (E) and 7 weeks (F) of remyelination.
(G) Demyelination alters VEP shape to dark flash.
(H) High dose treatment with LL-341070 provides a larger benefit to altered VEP shape to dark flash than low dose treatment after 3 weeks.
(I) VEP shape to dark flash is equivalent across groups after 7 weeks.
(J) Demyelination alters VEP shape to bright flash.
(K-L) VEP shape to bright flash is equivalent across groups after 3 weeks (K) and 7 weeks (L).
In **A**, Wilcoxon (Z=-0.42, p=0.61). Healthy: n=3 probes from 2 mice, demyelinated: n=10 probes from 4 mice.
In **B**, Kruskal-Wallis (F(3)=1.66, p=0.64). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=8 probes from 3 mice, 0.3mg/kg: n=6 probes from 2 mice, healthy: n=3 probes from 2 mice.
In **C**, Kruskal-Wallis (F(3)= 0.86, p = 0.83). Vehicle: n=8 probes from 3 mice, 0.1mg/kg: n=14 probes from 5 mice, 0.3mg/kg: n=7 probes from 3 mice, healthy: n=3 probes from 2 mice.
In **D**, Wilcoxon (Z=-0.79, p=0.43). Healthy: n=3 probes from 2 mice, demyelinated: n=10 probes from 4 mice.
In **E**, Kruskal-Wallis (F(3)=6.03, p=0.11). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=3 probes from 3 mice 0.3mg/kg: n=6 probes from 2 mice, healthy: n=3 probes from 2 mice.
In **F**, Kruskal-Wallis (F(3)=6.28, p=0.099). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=13 probes from 5 mice, 0.3mg/kg: n=6 probes from 3 mice, healthy: n=3 probes from 2 mice.
In **G**, Wilcoxon (Z=-2.28, p=0.022). Healthy: n=3 probes from 2 mice, demyelinated: n=10 probes from 4 mice.
In **H**, Kruskal-Wallis (F(3)=9.68, *p=0.022). Steel-Dwass (0.3 mg/kg vs 0.1 mg/kg: *p=0.019). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=8 probes from 3 mice, 0.3mg/kg: n=6 probes from 2 mice, healthy: n=3 probes from 2 mice.
In **I**, Kruskal-Wallis (F(3)=11.46, **p=0.0095). Steel-Dwass (no significant comparisons). Vehicle: n=8 probes from 3 mice, 0.1mg/kg: n=14 probes from 5 mice, 0.3mg/kg: n=7 probes from 3 mice, healthy: n=3 probes from 2 mice.
In **J**, Wilcoxon (Z=-2.45, *p=0.014). Healthy: n=3 probes from 2 mice, demyelinated: n=10 probes from 4 mice.
In **K**, Kruskal-Wallis (F(3)= 11.2, *p=0.011). Steel-Dwass (no significant comparisons). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=3 probes from 3 mice, 0.3mg/kg: n=6 probes from 2 mice, healthy: n=3 probes from 2 mice.
In **L**, Kruskal-Wallis (F(3)= 9.85, *p=0.02). Steel-Dwass (no significant comparisons). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=13 probes from 5 mice, 0.3 mg/kg: n=6 probes from 3 mice, healthy: n=3 probes from 2 mice.
*p<0.05; median, IQR (box), min./max. (whiskers); data points color-coded by mouse; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
VEP=visual evoked potential.



Supplementary Fig. 15: Amplitude of VEP responses is minimally affected by demyelination and remyelination.
(A) The difference in amplitude of P100 and N70 VEP peaks to flash (dark and bright) is not altered by demyelination.
(B-C) All groups show similar P100-N70 amplitudes to flash (dark and bright) as healthy mice after 3 weeks (B) and 7 weeks (C) of remyelination.
(D) P100-N70 VEP amplitude to dark flash is not altered by demyelination.
(E) High dose LL-341070 increases P100-N70 VEP amplitude to dark flash compared to vehicle-treated mice.
(F) All groups show similar P100-N70 amplitudes to dark flash as healthy mice after 7 weeks of remyelination.
(G) P100-N70 VEP amplitude to bright flash is not altered by demyelination.
(H-I) All groups show similar P100-N70 amplitudes to bright flash as healthy mice after 3 weeks (H) and 7 weeks (I) of remyelination.
In **A**, Wilcoxon (Z=-0.7, p=0.48). Healthy: n=6 VEPs from 3 probes from 2 mice, demyelinated: n=20 VEPs from 10 probes from 4 mice.

In **B**, Kruskal-Wallis (F(3)=6.22, p=0.10). Vehicle: n=18 VEPs from 9 probes from 3 mice, 0.1mg/kg: n=11 VEPs from 3 probes from 3 mice, 0.3mg/kg: n=12 VEPs from 6 probes from 2 mice, healthy: n=6 VEPs from 3 probes from 2 mice.
In **C**, Kruskal-Wallis (F(3)=0.78, p=0.85). Vehicle: n=17 VEPs from 9 probes from 3 mice, 0.1mg/kg: n=27 VEPs from 13 probes from 5 mice, 0.3mg/kg: n=13 VEPs from 6 probes from 3 mice, healthy: n=6 VEPs from 3 probes from 2 mice.
In **D**, Wilcoxon (Z=-1.1, p=0.27). Healthy: n=3 probes from 2 mice, demyelinated: n=10 probes from 4 mice.
In **E**, Kruskal-Wallis (F(3)=11.03, *p=0.012). Steel-Dwass (0.3mg/kg vs. vehicle: **p=0.0097). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=8 probes from 3 mice, 0.3mg/kg: n=6 probes from 2 mice, healthy: n=3 probes from 2 mice.
In **F**, Kruskal-Wallis (F(3)=0.79, p=0.85). Vehicle: n=8 probes from 3 mice, 0.1mg/kg: n=14 probes from 5 mice, 0.3mg/kg: n=7 probes from 3 mice.
In **G**, Wilcoxon (Z=0.084, p=0.93). Healthy: n=3 probes from 2 mice, demyelinated: n=10 probes from 4 mice.
In **H**, Kruskal-Wallis (F(3)=3.2, p=0.36). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=3 probes from 3 mice, 0.3 mg/kg: n=6 probes from 2 mice, healthy: n=3 probes from 2 mice.
In **I**, Kruskal-Wallis (F(3)= 5.3, p=0.15). Vehicle: n=9 probes from 3 mice, 0.1mg/kg: n=13 probes from 5 mice, 0.3mg/kg: n=6 probes from 3 mice, healthy: n=3 probes from 2 mice.
** $p < 0.01$; median, IQR (box), min./max. (whiskers); data points color-coded by mouse; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
VEP=visual evoked potential.



Supplementary Fig. 16: No influence of sex, weight change, or cohort.
(A) No difference in mean or variance of cumulative oligodendrocyte loss at 3 weeks between female and male mice. All mice from all demyelinating groups are included.
(B) ANCOVA with unequal slopes shows effect of cumulative OL loss but not sex or the interaction between cumulative OL loss and sex on cumulative OL gain at 3 weeks (when the remyelination response has mostly concluded) in untreated/vehicle-treated mice.
(C) ANCOVA with unequal slopes for effect of cumulative OL loss, sex, and the interaction between cumulative OL loss and sex on cumulative OL gain at 1.5 weeks (the period of effect of 0.3mg/kg LL-341070) is not significant in mice treated with 0.3mg/kg LL-341070.
(D) No relationship between the amount of weight change in mice during the cuprizone diet (a proxy for amount of cuprizone chow consumed) and cumulative OL loss at 3 weeks. Demyelinating mice from any group with weight data are included.
(E) No effect of cohort on mean or variance of cumulative OL loss at 3 weeks. All mice from all demyelinating groups are included.
In **A**, t test (t(34)=0.25, p=0.80). Brown-Forsythe (F(1, 34)=1.68, p=0.20). Female: n=21, male: n=15.
In **B**, ANCOVA with unequal slopes (F(3, 12)=3.89, *p=0.037). Effects: sex (F=0.04, p=0.84), cumulative OL loss (F=9.21, *p=0.010), interaction (F=0.0008, p=0.98). Female: n=9, male: n=7.
In **C**, ANCOVA with unequal slopes (F(3, 3)=5.83, p=0.091). Female: n=4, male: n=3.
In **D**, linear regression (F(1,17)=0.48, n=19).
In **E**, ANOVA (F(9, 26)=1.83, p=0.11). Brown-Forsythe (F(8, 26)=0.20, p=0.99).
Line of best fit ± 95% CI in D; n=mice; two-sided statistical tests. See Supplementary Data 1 for statistical details and Source Data file for source data.
OL=oligodendrocyte.

Supplementary Tables

Week	Day	Low loss (25%)	High loss (75%)
1	7	106.1283633	79.5632633
2	14	109.5408416	84.4152916
3	21	112.9533199	89.2673199
4	28	116.3657982	94.1193482
5	35	119.7782765	98.9713765
6	42	123.1907548	103.8234048
7	49	126.6032331	108.6754331
8	56	130.0157114	113.5274614
9	63	133.4281897	118.3794897
10	70	136.840668	123.231518
11	77	140.2531463	128.0835463
12	84	143.6656246	132.9355746
13	91	147.0781029	137.7876029
14	98	150.4905812	142.6396312
15	105	153.9030595	147.4916595
16	112	157.3155378	152.3436878
17	119	160.7280161	157.1957161
18	126	164.1404944	162.0477444
19	133	167.5529727	166.8997727
19.6	137	169.5029603	169.6723603

Supplementary Table 1: Modeled future oligodendrocyte numbers in low and high loss untreated/vehicle-treated mice.

Untreated/vehicle-treated mice with low loss (25%) and high loss (75%) are estimated to have equal numbers of oligodendrocytes in an additional 19.6 weeks following the end of imaging. See Supplementary Data 1 for modeling details and Source Data file for source data.

Week	Day	Healthy	Vehicle	0.1 mg/kg	0.3 mg/kg
0	0	135	87	107	104
1	7	138.01	90.71	111.41	108.41
2	14	141.02	94.42	115.82	112.82
3	21	144.03	98.13	120.23	117.23
4	28	147.04	101.84	124.64	121.64
5	35	150.05	105.55	129.05	126.05
6	42	153.06	109.26	133.46	130.46
7	49	156.07	112.97	137.87	134.87
8	56	159.08	116.68	142.28	139.28
9	63	162.09	120.39	146.69	143.69
10	70	165.1	124.1	151.1	148.1
11	77	168.11	127.81	155.51	152.51
12	84	171.12	131.52	159.92	156.92
13	91	174.13	135.23	164.33	161.33
14	98	177.14	138.94	168.74	165.74
15	105	180.15	142.65	173.15	170.15
16	112	183.16	146.36	177.56	174.56
17	119	186.17	150.07	181.97	178.97
18	126	189.18	153.78	186.38	183.38
19	133	192.19	157.49	190.79	187.79
20	140	**195.2**	161.2	**195.2**	192.2
21	147	198.21	164.91	199.61	196.61
22	154	201.22	168.62	204.02	201.02
22.14	204	**201.65**	169.15	204.65	**201.65**
68	476	339.68	339.28	406.88	403.88
68.57	529	**341.4**	**341.4**	409.4	409.4

Supplementary Table 2: Modeled future oligodendrocyte numbers in treatment groups and healthy mice.
Mice treated with low dose LL-341070, high dose LL-341070, and vehicle are estimated to achieve healthy levels of oligodendrocyte numbers in an additional 20, 22.14 and 68.57 weeks following the end of imaging, respectively. See Supplementary Data 1 for modeling details and Source Data file for source data.