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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Two-photon imaging data was collected using Zen 2012 SP5 (Black, Zeiss), Immunofluorescence data was acquired using software LAS X {Leica Microsystems}, Neuropixels data was recorded using Open Ephys GUI {https://open-ephys.org/}
Data analysis	Two-photon images were registered using ImageJ (v1.46j and v1.54f). Oligodendrocytes were tracked across time using custom-made macros (ImageJ v1.51p) and myelin was traced in Simple Neurite Tracer {(ImageJ v1.54f). Quantification of nodes of Ranvier was carried out using ImageJ (v1.54f). Action potential isolation and sorting from raw electrophysiology data was done using Kilosort (2.5). Analysis of action potentials and evoked field potentials was done using custom Python-based analysis scripts. Satistical analysis was performed in JMP Pro (v17, SAS) or Prism 7 (GraphPad).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Source data are provided in the Source Data file. Data analysis notebooks and scripts for electrophysiology data are available on github (denmanlab/remyelination) 104. All data that support the findings will be shared on an unrestricted basis; requests should be directed to the corresponding authors. Inquiries concerning LL-341070 should be directed to www.autobahtx.com.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample sizes, but sample sizes used in this study are comparable to those reported in previous publications (see References 21, 41, 77).
Data exclusions	No animals were excluded from our datasets. For the VEP data, the VEP signal was manually inspected by an investigator blinded to the experimental conditions, and signals deemed to have low quality were excluded from further analyses.
Replication	Experimental groups were replicated in multiple cohorts with multiple experimental groups per cohort. For longitudinal imaging experiments, treatment groups were repeated in the following number of cohorts: untreated = 2; LL-341070 vehicle, 0.1 mg/kg, and 0.3 mg/kg = 5; clemastine = 3; clemastine vehicle = 2; healthy = 2. For electrophysiology, treatment groups were repeated in the following number of cohorts: healthy = 1; demyelinated = 3; LL-341070 vehicle = 3; LL-341070 0.1 mg/ml = 4; and LL-341070 0.3 mg/ml = 2.
Randomization	Allocation of mice to experimental groups was random, except for the following considerations. Sex was distributed between treatment

groups when possible. Mice were randomly allocated to blinded groups either prior to or during cuprizone treatment. In some instances, the level of oligodendrocyte loss during cuprizone was assessed prior to mouse allocation to attempt to ensure an even distribution of oligodendrocyte loss levels between blinded groups.

Both male and female mice were used for experiments, with sex being distributed between treatment groups. No evidence of sexual dimorphism was observed (Supplementary Fig. 16A-C).

Blinding

Experiments were blinded to treatment group during data collection and image or electrophysiological analysis. Autobahn Therapeutics prepared LL-341070 at two different concentrations, as well as its vehicle, and blinded the identity of each solution before shipment to our laboratory. Clemastine and its vehicle were blinded in house by experimenters unaffiliated with the project prior to administration to mice.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies:
- Rabbit anti-Caspr (Abcam ab34151; RRID:AB_869934) 1:500
- Chicken anti-pan-Nfsc (R&D Biosystems AF3235; RRID:AB_10890736) 1:500
- Rat anti-PLP (gift from Dr. Wendy Macklin) 1:500
- Mouse anti-Degenotag (Encor Biotechnology MCA-1D44; RRID:AB_2923483) 1:500
- Mouse anti-GFAP (Sigma G3893; RRID:AB_477010) 1:1000
- Rabbit anti-S100beta (Abcam AB52642; RRID:AB_882426) 1:500
- Rabbit anti-P2RY12 (Anaspec AS-55043A) 1:1000
- Guinea pig anti-RPBMS (PhosphoSolutions 1832-RBPMS; RRID:AB_2492226) 1:100

Secondary antibodies:
- Alexa Fluor® 488 Donkey anti-chicken IgY (Jackson Immunoresearch Laboratories 703-545-155) 1:500
- Alexa Fluor™ 568 Donkey anti-rabbit IgG (Thermo Fischer A10042) 1:500
- Alexa Fluor 568 Goat anti-rat IgG (Thermo Fischer A11077) 1:500
- Alexa Fluor 568 Donkey anti-mouse IgG (Thermo Fischer A11004) 1:500
- DyLight 405 Donkey anti-mouse IgG (Jackson Immunoresearch Laboratories 715-475-150) 1:500
- FITC Donkey anti-guinea pig 1:200

Validation

Validation was performed by the vendors. Mouse anti-DegenoTag was first tested on degenerated and control optic nerves to validate specificity (Supplementary Fig. 2). All other antibodies had been previously validated in house.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

All animals used in this study were aged 6-8 weeks at the start of the experimental timelines. C57BL/6 MOBP-EGFP (MGI:4847238) mice were used for two-photon imaging.

Wild animals

N/A

Reporting on sex

Sex was distributed between groups. No effects of sex were detected on oligodendrocyte loss or gain magnitude or in response to remyelination therapy (Supplementary. Fig. 16).

Field-collected samples

N/A

Ethics oversight	All animal experiments were conducted in accordance with protocol 00056 approved by the Animal Care and Use Committee at the University of Colorado Anschutz Medical Campus.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*