Inhibition of Gli1 mobilizes endogenous neural stem cells for remyelination

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Enhancing repair of myelin is an important but still elusive therapeutic goal in many neurological disorders1. In multiple sclerosis, an inflammatory demyelinating disease, endogenous remyelination does occur but is frequently insufficient to restore function. Both parenchymal oligodendrocyte progenitor cells and endogenous adult neural stem cells resident within the subventricular zone are known sources of remyelinating cells2. Here we characterize the contribution to remyelination of a subset of adult neural stem cells, identified by their expression of Gli1, a transcriptional effector of the sonic hedgehog pathway. We show that these cells are recruited from the subventricular zone to populate demyelinated lesions in the forebrain but never enter healthy, white matter tracts. Unexpectedly, recruitment of this pool of neural stem cells, and their differentiation into oligodendrocytes, is significantly enhanced by genetic or pharmacological inhibition of Gli1. Importantly, complete inhibition of canonical hedgehog signalling was ineffective, indicating that the role of Gli1 both in augmenting hedgehog signalling and in retarding myelination is specialized. Indeed, inhibition of Gli1 improves the functional outcome in a relapsing/remitting model of experimental autoimmune encephalomyelitis and is neuroprotective. Thus, endogenous neural stem cells can be mobilized for the repair of demyelinated lesions by inhibiting Gli1, identifying a new therapeutic avenue for the treatment of demyelinating disorders.

Remyelination in the adult human and mouse brains is performed by two cell types: oligodendrocyte progenitor cells (OPCs) and neural stem cells (NSCs). OPCs, which are present in the parenchyma of healthy brain as well as in, and around, multiple sclerosis lesions2, can be identified by their expression of the NG2 proteoglycan and platelet-derived growth factor receptor alpha (PDGFR-α). They respond locally to demyelination by generating oligodendrocytes although do not migrate long distances during remyelination3. NSCs present in the subventricular zone (SVZ), express gial fibrillary acidic protein (GFAP) and Nestin, and are normally quiescent. In response to demyelination, cells in the adult SVZ can generate oligodendrocytes4, including, presumptively, in patients with multiple sclerosis2.

The signals that activate and recruit NSCs to lesion sites and promote their local differentiation into oligodendrocytes remain poorly understood. A candidate to regulate NSCs is sonic hedgehog (Shh), an important morphogen during CNS development that is required for the generation of most oligodendrocytes during development5 and for the maintenance of stem cells in the adult SVZ6. Shh is therefore an attractive candidate to expand the pool of remyelinating cells available for repair. Indeed, Shh levels have been reported to increase in remyelinating lesions6. Canonical Shh signalling is mediated by interactions of the hedgehog receptor patched (Ptc) with the G-protein-coupled transmembrane co-receptor smoothened (Smo). Binding of Shh to Ptc relieves inhibition of Smo and thereby activates the Gli family of zinc-finger transcription factors7. Of the three Gli proteins, Gli1 is the only one whose transcription is driven by Shh signalling and its expression is therefore considered a sensitive readout of sustained, high-level activation of this pathway8,9.

In this study, we have examined remyelination by the Shh-responsive (that is, Gli1−/−) pool of NSCs, which is concentrated in the ventral SVZ and comprises ~25% of NSCs10. To genetically map Gli1−/− NSCs, we crossed Gli1CreERT2/− mice with the Rosa-CAG-EGFP (RCE) reporter11 to generate Gli1CreERT2/− mice in which tamoxifen treatment results in permanent expression of cytoplasmic green fluorescent protein (GFP) in all Gli1-expressing cells and their progeny12,13; see Supplementary Table 1 for a summary of these and other mouse lines used in this study. GFP-labelled cells correspond to NSCs in the SVZ and a subset of astrocytes, but not OPCs or oligodendrocytes. We then followed the fate of these GFP+ cells after inducing demyelination in the mouse corpus callosum (CC) either by (1) dietary cuprizone14 or (2) direct, stereotactic injection of the detergent lysophosphatidyl-choline (LPC)15.

At 6 weeks of dietary cuprizone, corresponding to peak demyelination, GFP-expressing cells were recruited to areas of demyelination. In contrast, no labelled cells were observed in the CC of controls (Fig. 1a). At 2 weeks of remyelination, after removal of cuprizone from the diet, GFP-expressing cells in the CC (17.3 cells ± 2.6 per section) differentiated exclusively into glia, primarily oligodendroglia, namely PDGFR−/− OPCs (9.8 ± 8.7%) and CC1−/− oligodendrocytes (40.2 ± 15.1%), as well as GFAP-expressing astrocytes (15.5 ± 4.1%, Fig. 1b, c); other markers are shown in Extended Data Fig. 1a. Approximately 30% of the GFP+ cells in the CC remained unspecified at this time. None of the GFP-labelled cells expressed neuronal (NeuN) or microglial (Iba1 and CD11b) markers (data not shown). Ten weeks after recovery from cuprizone diet, the numbers of GFP+ cells in the demyelinated CC increased from about 17 to 48 cells per section and consisted of PDGFR−/− OPCs (18.4 ± 1.8%), CC1−/− oligodendrocytes (58.3 ± 5.3%) and GFAP+ astrocytes (28.7 ± 11.6%) (Fig. 1c), accounting for all the GFP+ cells in the CC. These results suggest that Gli1−/− NSCs continue to generate glial cells in the CC for a prolonged period after demyelination. In addition to the cells within the CC, GFP-labelled cells located outside the CC frequently increased with demyelination; these correspond to a subset of protoplasmic astrocytes that are responsive to Shh16.

The newly generated, NSC-derived oligodendrocytes remyelinated axons, as evidenced by GFP-labelled processes that flanked nodes of Ranvier (Fig. 1e), overlapped with the paranodal marker Caspr (Extended Data Fig. 1b), and co-expressed myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP), but not the Schwann cell myelin protein P0 (Extended Data Fig. 1b). Immunoelectron microscopy of the CC in Gli1CreERT2/− mice crossed to a membrane GFP reporter (Rosa-TdTomato–mGFP) demonstrated GFP labelling in compact myelin sheaths surrounding axons (Fig. 1e), corroborating that these NSCs form remyelinating oligodendrocytes. For comparison, we also fate-mapped the entire pool of NSCs, in healthy brains and after demyelination using a NestinCreERT2/− driver line15.
that preferentially labels the SVZ (Fig. 1b). We also observed GFP+ cells outside the SVZ, including some cells within the healthy CC (Fig. 1b). The cells present in the healthy CC were largely astrocytes (93.81 ± 3.34%); a small proportion were OPCs (1.81 ± 1.57%) and oligodendrocytes (4.42 ± 0.29%) (Fig. 1b, d). Two weeks after recovery from cuprizone, there was a striking increase in the numbers of labelled cells in the CC, consistent with a recent report2, associated with an increase in the percentages of OPCs (28.49 ± 18.52%) and oligodendrocytes (9.35 ± 3.25%) and a commensurate reduction in the proportion of astrocytes (59.72 ± 9.54%) (Fig. 1b, d). These results do not distinguish whether the oligodendrocytes present in the CC during remyelination were generated primarily from NSCs, from pre-existing precursors within the CC or both. They emphasize that, in contrast to the broader Nestin+ pool of NSCs, Gli1 demarcates a distinct set of NSCs in the SVZ that are recruited only upon demyelination and preferentially fated to oligodendroglia.

To assess whether cells that enter demyelinated lesions in the CC were actively responding to Shh, we analysed Gli1 expression using Gli1CreLacZ mice, which express nuclear LacZ from the Gli1 locus15. In healthy brains, Gli1 was expressed by cells in the cortex, basal forebrain and ventral SVZ (Extended Data Fig. 2a, b). NSCs that co-expressed GFAP and Gli1 were also present in the human SVZ (Extended Data Fig. 2c). Outside the SVZ, labelled cells co-expressed GFAP (data not shown) but not PDGFR-α (Extended Data Fig. 2b) and thus correspond to a subset of mature astrocytes but not OPCs, as previously reported17. No LacZ+ cells were present in the CC during or after recovery from cuprizone- or LPC-mediated demyelination (data not shown), further indicating that Shh-responsive cells do not arise from within the callosum. Thus, although NSCs in the SVZ actively respond to Shh, their progeny, upon entry into the callosum, do not. These results are consistent with the minimal expression of Gli proteins by OPCs or oligodendrocytes during development (http://web.stanford.edu/group/barres_lab/brain_rnaseq.html) and indicate Shh signalling is decreased in OPCs during both normal development and active remyelination.

A time-course analysis of fate-mapped cells in Gli1Cre+ mice strongly suggests NSCs emigrate from the SVZ into the CC after demyelination (Extended Data Fig. 2d–f) and argues against transdifferentiation of Gli1-expressing astrocytes in the cortex as a source of labelled cells. Together, these data indicate that a Shh-responsive pool of NSCs in the SVZ are recruited specifically to the demyelinated CC where they downregulate Gli1 and differentiate into mature, myelinating oligodendrocytes.

Downregulation of Gli1 expression by remyelinating SVZ-derived OPCs raises the possibility that inhibiting Shh signalling might further augment remyelination— a notion that contrasts with previous studies showing that Shh signalling can promote repair after CNS injury19 and is required for remyelination20. We first analysed the effects of a partial loss of Shh signalling by fate mapping NSCs in Gli1 null (Gli1CreRCE) versus heterozygous (Gli1Cre+;RCE) mice. Consistent with our hypothesis, there were many more GFP-labelled cells in the remyelinating CC of Gli1 nulls (63 ± 6.5% per section) than in Gli1 heterozygotes (17.3 ± 2.6% per section, Fig. 2a, b), and a much higher percentage of these were mature oligodendrocytes in the nulls (81.3 ± 4.4%) than in the heterozygotes (40.2 ± 15.1%, Fig. 2c). Overall, there were ~7.5-fold more GFP-labelled, mature oligodendrocytes in the Gli1 null versus heterozygous mice. The proportion of labelled OPCs in the nulls versus heterozygotes was similar (5.22 ± 2.5% versus 9.8 ± 8.7%, Fig. 2c) whereas that of GFAP-expressing astrocytes was significantly reduced (2.6 ± 1.3% versus 15.5 ± 4.1%, Fig. 2c). Finally, overall myelin levels in the CC were increased in the Gli1 nulls compared with heterozygotes 3 weeks after cessation of cuprizone (Fig. 2d), strongly suggesting this enhanced NSC remyelination is physiologically significant.
While cuprizone primarily demyelinates the CC, it also has modest effects on other white matter tracts\textsuperscript{44}. Accordingly, labelled oligodendrocytes in the Gli1 nulls were also present in white matter tracts at sites distant from the SVZ including the lateral striatum, anterior commissure (Extended Data Fig. 3a, b) and the optic nerve (data not shown). No GFP-labelled cells were seen in the CC or other white matter tracts of heterozygotes or nulls on a control diet (Fig. 2a). Thus, in the adult, this effect of Gli1 is specific to remyelination.

Meylination also started significantly earlier in the Gli1 nulls than in the heterozygotes (Extended Data Fig. 4a, b). In addition, the CC were slightly larger on average in the adult Gli1 nulls versus heterozygotes (Extended Data Fig. 6a, b). There was no difference in the proliferation of NSCs of Gli1 nulls versus heterozygotes on a normal diet, on a cuprizone-fed diet (Extended Data Fig. 6a, b). There was no difference in the proliferation of NSCs of Gli1 nulls versus heterozygotes on a normal diet, on a cuprizone-fed diet (Extended Data Fig. 6a, b).

As Gli1 is expressed only upon sustained, high-level Shh signalling, we asked whether complete abrogation of this pathway also enhanced myelination. In contrast to loss of Gli1, loss of canonical Shh signalling by conditional ablation of smoothened in Gli1-expressing NSCs (Gli1\textsuperscript{CE/+};Smoo\textsuperscript{M2}RCE mice) did not increase the numbers of labelled cells in the CC or alter their cell fates (Fig. 2a–c). Thus, loss of Gli1 is distinct from loss of Shh signalling, emphasizing the specificity of the effects of Gli1.

We also examined the effects of activating canonical Shh signalling in the presence or absence of Gli1. To this end, we expressed an activated (M2) form of Smo\textsuperscript{M2} in NSCs upon tamoxifen treatment in Gli1 heterozygous (Gli1\textsuperscript{CE/+};Smoo\textsuperscript{M2}RCE) and null (Gli1\textsuperscript{CE/+};Smoo\textsuperscript{M2}) mice; the activated SmoM2 allele is itself fused to yellow fluorescent protein, allowing fate mapping. Again, labelled cells were only detected in the CC in mice that had undergone demyelination (Extended Data Fig. 5a). In the Gli1\textsuperscript{CE/+};Smoo\textsuperscript{M2} mice, many of the GFP\textsuperscript{+} cells (5.6 ± 2.9 per section) in the CC (Extended Data Fig. 5b) were oligodendrocyte progenitors (57.5 ± 23.2%, Extended Data Fig. 5c), a much higher proportion than in Gli1\textsuperscript{CE/+} mice (9.8 ± 8.7%). These results agree with previous studies that showed that increasing the levels of Shh in the brain enhances the generation of OPCs but blocks their maturation\textsuperscript{23}. The Gli1\textsuperscript{null};Smo\textsuperscript{M2} mice had many more GFP\textsuperscript{+} labelled cells in the CC than the Gli1\textsuperscript{null};Smo\textsuperscript{M2} mice (47.2 ± 25.7 versus 5.6 ± 2.9 per section, Extended Data Fig. 5b) and a significantly greater proportion of these were mature oligodendrocytes (52.5 ± 10.5% versus 28.8 ± 3.8%). On average, the Gli1\textsuperscript{null};Smo\textsuperscript{M2} mice had ~16 times as many oligodendrocytes derived from the Shh-responsive NSC pool than the Gli1\textsuperscript{null};Smo\textsuperscript{M2} mice (Extended Data Fig. 5b, c). These results indicate loss of Gli1 has an even greater effect in the context of active Shh signalling, promoting robust recruitment and relieving an arrest of NSCs differentiation into oligodendrocytes in the remyelinating CC.

Other analyses revealed that loss of Gli1 results in a significant increase in proliferation in Shh-responsive SVZ NSCs but only at the onset of demyelination, namely 3 weeks of cuprizone treatment (Extended Data Fig. 6a, b). There was no difference in the proliferation of NSCs of Gli1 nulls versus heterozygotes on a normal diet, on a cuprizone diet at 4, 5 or 6 weeks or 2 weeks after removal of cuprizone (data not shown). The higher proliferation at 3 weeks may contribute to preservation of the stem-cell pool, which was unchanged in the SVZ of Gli1 nulls (see Extended Data Fig. 6c). We did not detect any increase in Shh levels with demyelination or a significant difference in Shh levels between Gli1 heterozygotes versus nulls (Extended Data Fig. 6d–f). Thus, the major effects of loss of Gli1 in NSCs appear to be enhanced differentiation and recruitment to lesion sites and an increase in proliferation during demyelination.

These findings suggested that Gli1 might be a useful therapeutic target to promote remyelination. To test this possibility, we infused GANT61, a small molecule inhibitor of Gli1\textsuperscript{23} into the lateral ventricle of Gli1\textsuperscript{CE/+} mice via a mini-osmotic pump; we corroborated inhibition of Gli1 by the reduction of its messenger RNA (mRNA) levels by quantitative PCR (qPCR) (Extended Data Fig. 7a). A similar inhibition of Gli1 mRNA levels was observed when the drug was administered by intraperitoneal injection and oral gavage, indicating GANT61 can cross the blood–brain barrier efficiently. We infused GANT61 in Gli1\textsuperscript{CE/+} mice during the last 2 weeks of the cuprizone diet and continued it for an additional 2 weeks off cuprizone for a total of 4 weeks. Mice that received GANT61 versus vehicle had significantly greater numbers of labelled cells (43.8 ± 22.1 versus 6.4 ± 5 per section,
Fig. 3a, b) and a significantly greater proportion of these were oligodendrocytes (65.4 ± 7.5% versus 21.4 ± 18.7%, Fig. 3c). Administration of GANT61 was well tolerated and did not deplete the NSCs in the SVZ (Extended Data Fig. 7b, c). Importantly, mice receiving extended treatment with GANT61, namely during the last 3 weeks of the cuprizone diet and 6 weeks thereafter for a total of 9 weeks, had more myelin in the CC than mice similarly treated with vehicle (Fig. 3d). Thus, the Gli1 inhibitor enhanced the recruitment and differentiation of Shh-responsive NSCs into oligodendrocytes at sites of demyelination, promoting remyelination.

These effects of GANT61 are specific to NSCs as there were no effects on remyelination by OPCs on the basis of their fate mapping in NG2CreERT2 transgenic mice crossed to the RCE reporter (Extended Data Fig. 8). The lack of an effect of GANT61 on OPC remyelination is consistent with the absence of Gli1 expression by these cells. These results also indicate that enhanced repair by NSCs resulting from GANT61 treatment does not come at the expense of OPC remyelination: rather, it is additive.

To address the therapeutic potential of inhibiting Gli1, we examined the effects of GANT61 in a relapsing-remitting model of experimental autoimmune encephalitis (RR-EAE), a physiologically relevant model of inflammatory demyelination and remyelination. In this model, RR-EAE is induced by injecting proteolipid protein (PLP) peptide into wild-type SJL mice; the severity of the clinical phenotype of the initial attack correlates with the extent of spinal cord inflammation, whereas the late-stage neurological disability in later relapses correlates with axonal loss probably resulting from cumulative injury including chronic demyelination. GANT61 was administered by daily oral gavage either prophylactically (that is, at the onset of PLP immunization) or therapeutically (that is, at the onset of symptoms). Neither treatment protocol altered the induction of EAE or the severity of the acute attack (about day 12), suggesting GANT61 did not affect the immune response (Fig. 4a). In agreement, Gli1 was not expressed by cells in the lymphocytic or monocytic lineages isolated from the spleen, thymus or liver of healthy (Extended Data Fig. 9) or cuprizone-treated mice (data not shown).

Of note, GANT61 reduced the severity of the first relapse (around day 27) and significantly enhanced functional recovery during and after the second relapse (around day 46) compared with vehicle treatment (Fig. 4a). We therefore examined myelin levels, axon pathology and motor neuron numbers in the (prophylactic, therapeutic) GANT61- and vehicle-treated lumbar spinal cords at the end of the second relapse phase (that is, day 53). In electron microscopy images, all three groups had significant spinal cord pathology, with disruption of fascicles of myelinated axons and pathology of individual myelinated axons most evident in vehicle-treated compared with the GANT61-treated groups (Fig. 4b, c). In all EAE groups, pathology was most pronounced in small-diameter axons (<0.5 μm, Extended Data Fig. 10a–c) which were increased in numbers and had significantly lower G ratios (ratio of axon diameter to myelinated axon diameter); the reduction in axon diameter and corresponding increase in G ratios are probably due to axonal atrophy and suggestive of active demyelination. We did not detect significant numbers of unmyelinated or thinly myelinated axons in any group (Extended Data Fig. 10a–c). While thin myelin sheaths have long been considered the hallmark of remyelination, recent studies suggest remyelination performed by neural stem cells or in the spinal cord can be of normal thickness. Analysis of MBP levels supported significantly higher levels of myelin in the drug- versus vehicle-treated groups (Fig. 4f).

The numbers of lower motor neurons, identified by co-staining for NeuN and choline acetyltransferase (ChAT), were significantly reduced in the spinal cords of vehicle-treated (16.93 ± 1.92 per section) versus healthy control mice (27.59 ± 5.9 per section, Fig. 4d, e). Numbers of lower motor neurons in the GANT61-treated groups were intermediate and closer to controls (prophylactic: 23.4 ± 4.3 per section; therapeutic: 26.93 ± 8.4 per section), providing strong presumptive evidence of neural protection, potentially because of reduced axon pathology and/or remyelination. The relative preservation of lower motor neurons is consistent with the reduction in axonal pathology and probably accounts for improved functional outcomes. Taken together, these results suggest inhibition of Gli1 enhances remyelination and thereby protects neurons from degeneration in this EAE model.

Figure 4 | GANT61 improves functional outcomes and is neuroprotective in a RR-EAE model. a, EAE clinical scores after prophylactic or therapeutic treatment with GANT61 compared with vehicle administration. N = 9 mice per group. Data are mean ± s.e.m., *P < 0.05, two-way analysis of variance with Tukey’s multiple comparison test. b, c, Electron micrographs from the ventral lumbar spinal cords of control, GANT61- and vehicle-treated EAE mice shows axonal pathology (b) including axolysis (arrow) and dense axoplasm (arrowhead). Quantification of 500 axons per group from three mice per group (c) shows a higher proportion of pathological axons in vehicle-treated group compared with GANT61-treated EAE mice. Scale bar, 2 nm. N = 3 mice per group. d, e, Alpha motor neurons labelled for NeuN and ChAT in the lumbar spinal cords of control, vehicle- and GANT61-treated EAE mice (d). Quantification shows more motor neurons in the GANT61- versus vehicle-treated group (e). Scale bar, 50 μm. N = 3 mice per group. f, Quantification for the area of MBP expression shows lowest levels of myelin in the vehicle-treated group. N = 3 mice per group. Data are mean ± s.e.m. Student’s t-test.
model without altering the immune response. Gli1 is primarily expressed in the spinal cord by a subset of astrocytes within the par enchymal grey matter and by cells located around the central canal, a site of spinal cord NSCs but not by NG2 OPCs (Extended Data Fig. 10d). These findings suggest the effects of inhibiting Gli1 on myelination and neuroprotection are mediated by direct effects on neural stem cells or potentially by indirect effects mediated by par enchymal astrocytes.

These studies demonstrate that inhibiting Gli1 appears to be a well tolerated and effective strategy for mobilizing and enhancing the differentiat ion of a resident population of Shh-responsive neural stem cells. The findings of elevated levels of myelin, reduced axon pathology and preservation of lower motor neurons in a RR-EAE model further support its therapeutic potential. This approach may therefore be useful in aiding repair in multiple sclerosis and other demyelinating neurological disorders. The findings also highlight that different pools of remyelinating cells (that is, NSCs and OPCs) might require different strategies to promote their remyelination of axons, raising the possibility of using combinatorial strategies to enhance repair.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

No statistical methods were used to predetermine sample size. 

Fate mapping and demyelination. Ten-week-old mice were administered 5 mg tamoxifen (Sigma) in corn oil on alternate days for a total of four intraperitoneal injections. NestinCE/lacZ+, Gli1CE/lacZ, Gli1Ce/Znb/aZ, Gli1plaeZ+/+ and Gli1Ce/+;SmoM2/+ mice, maintained on Swiss-Webster background, were fed 0.4% cuprizone31. Gli1CE/M2, Gli1CE/Znb/aZ, SmoM2+, NG2CE and ShhCE/M2 mice were maintained in C57Bl/6 background and fed 0.2% cuprizone diet to obtain comparable demyelination in the CC31 (Supplementary Table 1). No labelling was seen in the absence of tamoxifen administration. For the LPC model, 2 μl of 1% LPC (Calbiochem) were stereotaxically injected into the CC at 1.5 mm anterior, 1.2 mm lateral and 2.2 mm ventral to the bregma. For intraperitoneal and oral routes, 50 mg kg⁻¹ GANT61 dissolved in ethanol:corn oil (1:4) was administered daily for 4 weeks.

Immunostaining. For Black-Gold (Millipore) myelin staining, mice were perfused with 4% PFA, and 20 μm coronal cryosections were stained according to the manufacturer’s protocol. For all other analyses, mice were perfused with Prepar (Anatech) and 20 μm coronal cryosections were processed for immunofluorescence with rabbit or chicken anti-GFP (1:1,000, Invitrogen) and one of the following antibodies: rat anti-PDGFα-5 (1:200, BD Biosciences); rabbit anti-NG2 (1:200, Millipore); anti-S100β (1:600, Dako) and β4-spectrin (1:4,000, from M. Rasband); mouse anti-CC1 (1:400, Calbiochem), anti-GFAP (1:400, Sigma), anti-NeuN (1:200, Millipore), anti-Shh (1:500, DSHB), anti-MOG (1:50, Sigma) and anti-MBP (1:500, Millipore); goat anti-LacZ (1:2,000, Biolegend) and anti-ChAT (1:200, Millipore); chicken anti-P0 (1:200, Millipore); guinea pig anti-Caspr (1:3,000, from M. Bhat) and anti-Sox10 (1:1,000, from M. Wegner). Cryosections (30 μm) of an autopsy specimen of a healthy human brain (provided by New York University Brain Bank of Columbia University) were stained with rabbit anti-Gli1 (1:2,000, Abcam) and mouse anti-GFAP (1:400, Sigma). Secondary antibodies were goat or donkey anti-species conjugated with Alexafluors (1:1,000, Molecular Probes). Nuclei were counterstained with Hoechst 33258 (1:5,000, Invitrogen). Fluorescent images were obtained as Z-stacks of 1 μm optical sections using a confocal laser-scanning microscope (LSM 510, Zeiss) and processed using Adobe Photoshop. At least ten sections per mouse were analysed and data from three to five mice were combined to determine the average and standard deviation. The investigators were blinded to allocation during experiments and outcome assessment. Student’s t-test was performed to calculate P values.

Flow cytometry. Cells from the thymus, spleen and liver were stained with the following conjugated antibodies: CD4 (RM4-5), CD8 (53-6-7), CD19 (6D5), Ly6c (HK1.4), CD11b (M1/70), CD11c (N418), CD45 (30-F11), PDCA-1 (927), B220 (RA3-6B2) and CD3 (145-2C11). Antibodies were purchased from eBioscience or Biolegend and the staining was performed according to the manufacturer’s instructions. DAPI (Invitrogen) was always used to exclude dead cells from the analysis. Stained cells were analysed on a LSRII flow cytometer (BD) and data processed using FlowJo (Tree Star). The investigators were blinded to the genotype of the cells being sorted.

EdU labelling. EdU (Invitrogen; 200 mg kg⁻¹) dissolved in saline was administered by intraperitoneal injection 1 day before harvesting the brains. EdU was visualized using the AlexaFluor-594 Click-iT EdU Cell Proliferation Assay Kit (Invitrogen) according to the manufacturer’s instructions. 

EAE model. The EAE experiments were randomized. The PLP-induced EAE model was performed and scored using the EAE induction kit (Hooke Labs) on 8-week-old female SJL mice according to the manufacturer’s instructions. We used a random number generator to assign each mouse to a group and used an inclusion criteria of clinical score of 2 or above for final analysis (N = 9 per group). Mice received either vehicle (ethanocorn oil (1:4)) or 50 mg kg⁻¹ GANT61 via oral gavage until the end of the experiment, namely day 53 after induction. Separate investigators performed the clinical scoring and the drug treatments and were blinded to each other; the histological analysis was also done blinded.

Electron microscopy. Immunoelectron microscopy was performed on brain sections to detect mGFP in the myelin wraps around axons in the CC according to a previous study32. The investigators were blinded to allocation during experiments and outcome assessment.

qPCR. mRNA was extracted from the forebrains of three mice in each group using the RNeasy kit (Qiagen) and reverse-transcribed to complementary DNA using the qScript cDNA Synthesis Kit (Quanta Biosystems). qPCR was performed on a Stratagene MX3000P thermal cycler. Primers used were Gli1 (forward, 5’-GCC ATG TGC CTC AAA C-3’; reverse, 5’-GGA GCT GCT GCT TCA TTA-3’); Gli2 (forward, 5’-AGA GAC AGC AGA AGC TAT GCCCAA-3’; reverse, 5’-TGG GCA GCC TCC ATT CGT TTC ATA-3’); GAPDH (forward, 5’-GTT GAT AAC GGA TTT GGC-3’; reverse, 5’-CGG TGC TGT CGG TGC-3’). The data were analysed using the relative gene expression and Student’s t-test was used to calculate P values.

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Extended Data Figure 1 | Gli1-expressing cells generate oligodendrocytes after demyelination. Additional markers used to analyse fate-mapped Shh-responsive NSCs are shown. a, Two weeks after removal from cuprizone diet, GFP-labelled cells in the CC of Gli1\textsuperscript{CE/1} mice co-expressed the oligodendrocyte progenitor markers PDGFR-\(\alpha\), NG2 and Sox10, the mature oligodendrocyte marker CC1, and the astrocytic markers GFAP and S100\(\beta\). Scale bar, 10 \(\mu\)m. b, Four weeks after removal from cuprizone diet, GFP-labelled processes co-localized with myelin proteins MBP and MOG but not with peripheral myelin protein P0. GFP-labelled processes also overlaid the axonal, paranodal marker Caspr. \(N = 5\) mice per group. Scale bar, 10 \(\mu\)m.
Extended Data Figure 2 | Gli1-expressing neural stem cells in the SVZ egress and generate labelled cells in the CC. a, Expression of Gli1 in the forebrain was confirmed in Gli1\textsuperscript{nLacZ/1} mice by immunofluorescence for LacZ. Labelled cells were observed in the SVZ, cortex and basal forebrain but not in the CC of mice. The right panel shows the magnified images for the corresponding boxes in the left panel. N = 5 mice. Scale bar, 50 μm. b, Double staining for PDGFR-α and LacZ in the Gli1\textsuperscript{nLacZ/1} forebrain does not show any co-labelled cells, indicating that Gli1 is not expressed by OPCs. N = 5 mice. Scale bar, 50 μm. c, The ventral SVZ lining the body of the lateral ventricle from a human brain specimen shows co-localization of Gli1 with GFAP\textsuperscript{+} cells (yellow arrow) as well as a Gli1\textsuperscript{+} cell not expressing GFAP (arrowhead). N = 1 brain. Scale bar, 50 μm. d-f, Time-course analysis of the SVZ and CC of Gli1\textsuperscript{CE/1} mice stereotactically injected with saline (control, left panels) or LPC (right panels) to induce demyelination. N = 3 mice per group. No labelled cells were seen within the CC after saline injections; areas of ingress into the LPC-injected CC are boxed. At 1 day post-lesion (d.p.l.) (d), GFP-labelled cells diverted towards the CC; at 2 d.p.l. (e), a few labelled cells were seen within the CC; at 6 d.p.l. (f), many GFP\textsuperscript{+} cells had accumulated at the site of LPC injection (arrowhead). Scale bar, 50 μm.
Extended Data Figure 3 | Neural stem cells generate oligodendrocytes in various white matter tracts in Gli1-null brains upon demyelination.

a, b, Fate-mapped Gli1-null cells migrated into the anterior commissure (AC) (a) and striatum (b) after cuprizone-mediated demyelination (inset shows the area of the forebrain) in Gli1Null (Gli1CE/nLacZ) mice. Scale bar, 50 μm.

c, Two weeks after removal from cuprizone diet, GFP-labelled cells are present throughout the CC of Gli1Null (Gli1CE/nLacZ) mice. N = 5 mice per group. Scale bar, 100 μm.
Extended Data Figure 4 | Myelination starts earlier in developing Gli1 null mice. a, Gli1<sub>null</sub> (Gli1<sup>Null</sup>) mice (right panel) show increased MBP levels in the forebrain at postnatal day 9 (P9) compared with Gli1<sub>heterozygote</sub> (Gli1<sup>Het</sup>) mice. b, Quantification of the extent of MBP expression at P9 in the CC of Gli1 nulls (47.12 ± 10.44%) versus heterozygotes (29.71 ± 1.77%) corroborates that myelination is accelerated. N = 5 mice per genotype. c, Analysis of healthy adult forebrain shows the intensity of Black-Gold myelin stain in Gli1 heterozygotes and nulls was comparable. d, Quantification of the sizes of the CC shows the CC in Gli1<sup>Null</sup> (Gli1<sup>nLacZ/nLacZ</sup>) was slightly larger on average than that in Gli1<sup>Het</sup> (Gli1<sup>nLacZ/+</sup>) mice, although the difference was not statistically significant. N = 5 mice per genotype. e, Quantification of G ratios from electron micrographs of healthy Gli1<sup>Het</sup> (Gli1<sup>nLacZ/+</sup>) and Gli1<sup>Null</sup> (Gli1<sup>nLacZ/nLacZ</sup>) mice revealed no difference in the thickness of myelin sheaths in the CC. N = 3 mice per genotype. Scale bar, 50 μm. Data are mean ± s.e.m., Student's t-test.
Extended Data Figure 5 | Effects of gain of smoothened function in Gli1-het versus Gli1-null cells during remyelination. Gli1Het (Gli1CE/+) Gli1Het;SmoM2 (Gli1CE/+;SmoM2) and Gli1Null;SmoM2 (Gli1CE/nLacZ;SmoM2) mice were injected with tamoxifen, fed either a regular or a cuprizone-supplemented diet for 6 weeks and analysed by immunofluorescence 2 weeks after removal of cuprizone. a, GFP+ cells are only seen in the CC of mice on cuprizone diet (right) and not in the control mice (left). b, Quantification of the GFP+ cells in the CC shows significantly higher numbers of cells in Gli1Null;SmoM2 mice compared with Gli1Het and Gli1Het;SmoM2 mice. c, Quantification of the proportion of GFP-labelled co-expressing glial markers in the CC of cuprizone-treated Gli1Het, Gli1Het;SmoM2 and Gli1Null;SmoM2 mice shows an increase in percentage of GFP-labelled OPCs (PDGFR-a+) in Gli1Het;SmoM2 mice and mature oligodendrocytes (CC1+) in Gli1Null;SmoM2 mice. N = 3 mice per group for each genotype. Data are mean ± s.e.m., Student’s t-test.
Extended Data Figure 6 | Proliferation of NSCs and expression of Shh in Gli1-null mice. 

a, b, At the start of demyelination (3 weeks of cuprizone diet), Gli1\(^{-}\)Null (Gli1\(^{-}\)null\(\text{nLacZ/nLacZ}\)) brains have a higher proportion of proliferating nLacZ\(^{+}\) neural stem cells indicated by the percentage of EdU-incorporating cells co-expressing nLacZ in the SVZ compared with Gli1\(^{+}\)Het (Gli1\(^{+}\)null\(\text{nLacZ/nLacZ}\)) brains, namely 31.68.9\% versus 8.2\%5.3\%, respectively. \(N = 3\) mice per group for each genotype. Scale bar, 50 \(\mu\)m.

c, The numbers of fate-mapped Gli1 neural stem cells in the SVZ were quantified as the proportion of GFP\(^{+}\) cells co-expressing GFAP in Gli1\(^{+}\)Het (Gli1\(^{-}\)null\(\text{CE/CE}\)) and Gli1\(^{-}\)Null (Gli1\(^{-}\)null\(\text{CE/nLacZ}\)) mice at 2 weeks of recovery from cuprizone diet. The percentage of GFAP\(^{+}\)GFP\(^{+}\) cells in the SVZ of mice receiving cuprizone diet was comparable to those on a control diet, suggesting that the stem cell pool is not depleted during remyelination. \(N = 3\) mice per group for each genotype. Data are mean \(\pm\) s.e.m., Student’s \(t\)-test.

d, Fate-mapping of Shh expressing cells using an mGFP reporter labels neurons in the basal forebrain (left) with their neurites reaching the ventral SVZ (right) in Gli1\(^{+}\)Het and Gli1\(^{-}\)Null brains.

e, Immunostaining of Gli1\(^{-}\)Null mice shows Shh in the SVZ and CC after demyelination is mostly co-localized to GFAP-expressing cells. Thus, Shh is produced by neurons of the basal forebrain and binds to a responsive set of astrocytes and neural stem cells.

f, Quantification of the proportional area of the CC expressing Shh does not show any significant difference between Gli1\(^{+}\)Het and Gli1\(^{-}\)Null mice either on control or cuprizone diet. \(N = 3\) mice per group for each genotype. Data are mean \(\pm\) s.e.m., Student’s \(t\)-test. Scale bar, 50 \(\mu\)m.
Extended Data Figure 7 | GANT61 reduces Gli1 levels but does not deplete neural stem cells in the SVZ. a, Relative expression of Gli1 and Gli2 mRNA in the forebrain of Gli1<sup>Cre/+</sup> mice was examined by qPCR after administration of GANT61 (50 mg/kg/day) for 4 weeks. GANT61 decreases the mRNA levels of Gli1 significantly without changing Gli2 levels. N = 3 mice per group. Data are mean ± s.e.m., Student’s t-test. b, c, The numbers of fate-mapped Gli1<sup>+</sup> neural stem cells in the SVZ were analysed by immunofluorescence as the proportion of GFP<sup>+</sup> cells co-expressing GFAP in Gli1<sup>Cre/+</sup> mice treated with vehicle or GANT61 at 2 weeks of recovery from cuprizone diet (b). The percentage of GFAP<sup>+</sup> GFP<sup>+</sup> cells in the SVZ of mice treated with GANT61 was comparable to those treated with vehicle, suggesting that the stem cell pool is not depleted by GANT61 (c). N = 3 mice per group. Data are mean ± s.e.m., Student’s t-test.
Extended Data Figure 8 | Pharmacological inhibition of Gli1 does not affect OPC recruitment or differentiation during remyelination. a, NG2CE/1 mice were treated with two doses of intraperitoneal tamoxifen to sparsely label OPCs and analysed at 2 weeks of recovery from cuprizone. Scale bar, 50 μm. b, Numbers (~40 GFP cells per field) and c, proportions of GFP-labelled OPCs (PDGFR-α) and mature oligodendrocytes (CC1) were similar in the GANT61- versus the vehicle-treated mice in the CC, indicating GANT61 does not alter OPC remyelination. Scale bar, 50 μm. N = 3 mice per group. Data are mean ± s.e.m., Student’s t-test.
Extended Data Figure 9 | Gli1 is not expressed by immune cells of spleen, thymus and liver of healthy mice. a–d, Cells from the spleen, liver and thymus of tamoxifen-treated Gli1Het (Gli1CE/1) and Gli1Null (Gli1CE/nLacZ) mice were analysed by flow cytometry for GFP expression. Wild-type mice did not express GFP and were used as controls. Representative flow cytometry scatter plots showing absence of GFP expression in CD45⁻/CD3⁻/CD4⁻/CD19⁻ T and B cells (a), CD45⁻/CD3⁻/CD19⁻/B220⁻/MPDCA⁺ plasmacytoid dendritic cells (b), CD45⁻/CD3⁻/CD19⁻/B220⁻/CD11b⁻ macrophages, monocytes, dendritic and natural killer (NK) cells (c) and CD45⁻/CD3⁻/CD19⁻/B220⁻/CD11b⁻ cells (d) in Gli1CE/1 and Gli1CE/nLacZ mice. N = 3 mice per genotype.
Extended Data Figure 10 | Effects of GANT61 on spinal cord axons in the PLP-induced EAE model. a, Scatter plot of $G$ ratios with respect to axonal diameters ($n = 500$ axons in three mice per group, exponential trend line). b, Analysis of electron microscopy images showing the relative proportion of axons binned by their diameters in the four groups. c, Analysis of electron microscopy images, indicating the $G$ ratios of axons relative to their diameters in the four groups ($N = 500$ axons in three mice per group; data are mean ± s.e.m., Student’s $t$-test). d, Immunofluorescence image of a spinal cord section from $Gli1^{\text{nLacZ}+}$ mice shows that LacZ is not expressed by NG2$^+$ OPCs. The inset shows expression of LacZ in the germinal zone around the central canal. $N = 3$ mice. Scale bar, 50 μm.