Transected axons fail to regrow across anatomically complete spinal cord injuries (SCI) in adults. Diverse molecules can partially facilitate or attenuate axon growth during development or after injury1–3, but efficient reversal of this regrowth failure remains elusive4. Here we show that three factors that are essential for axon growth during development but are attenuated or lacking in adults—(i) neuron intrinsic growth capacity5–7, (ii) growth-supportive substrate10,11, and (iii) chemoattraction12,13—are all individually required and, in combination, are sufficient to stimulate robust axon regrowth across anatomically complete SCI lesions in adult rodents. We reactivated the growth capacity of mature descending propriospinal neurons with osteopontin, insulin-like growth factor 1 and ciliary-derived neurotrophic factor before SCI14,15; induced growth-supportive substrates with fibroblast growth factor 2 and epidermal growth factor; and chemoattracted propriospinal axons with glial-derived neurotrophic factor6,17 delivered via spatially and temporally controlled release from biomaterial depots18,19, placed sequentially after SCI. We show in both mice and rats that providing these three mechanisms in combination, but not individually, stimulated robust propriospinal axon regrowth through astrocyte scar borders and across lesion cores of non-neural tissue that was over 100-fold greater than controls. Stimulated, supported and chemoattracted propriospinal axons regrew a full spinal segment beyond lesion centres, passed well into spared neural tissue, formed terminal-like contacts exhibiting synaptic markers and conveyed a significant return of electrophysiological conduction capacity across lesions. Thus, overcoming the failure of axon regrowth across anatomically complete SCI lesions after maturity required the combined sequential reinstatement of several developmentally essential mechanisms that facilitate axon growth. These findings identify a mechanism-based biological repair strategy for complete SCI lesions that could be suitable to use with rehabilitation models designed to augment the functional recovery of remodelling circuits.

We tested the hypothesis that the failure of adult central nervous system (CNS) axons to regrow across complete SCI lesions is due to a combined lack of several mechanisms that are required for developmental axon growth. We targeted descending propriospinal neurons because after incomplete SCI they spontaneously form new intraspinal circuits that relay functionally meaningful information past lesions20–22. Thus, short-distance regrowth of transected propriospinal axons across complete SCI lesions has the potential to find new neuronal targets and form new relay circuits. To reactivate intrinsic propriospinal neuronal growth capacity, which is attenuated in adult CNS neurons23–25, we tested two approaches that have previously been successful with retinal and corticospinal neurons: using adeno-associated viral vectors (AAV) to deliver either phosphatase and tensin homologue (PTEN) knockdown (AAV-shPT), or to express osteopontin, insulin-like growth factor 1 (IGF1) and ciliary-derived neurotrophic factor (CNTF) (AAV-OIC)14,15. To increase axon growth-supportive substrates such as laminin10,11,19,24, we delivered fibroblast growth factor 2 (FGF2)23 and epidermal growth factor (EGF)26. To chemoattract12,13 propriospinal axons, we delivered glial-derived growth factor (GDNF) because propriospinal neurons express GDNF receptors (GDNFR), increase GDNFR expression after SCI14 and regrow axons into GDNF-secreting grafts17, and because SCI lesions lack GDNF19. To provide temporally controlled and spatially targeted delivery of growth factors or function-blocking antibodies, we used biomaterial depots of synthetic hydrogels18,19 placed sequentially into SCI lesion centres and into caudal spared neural tissue (Fig. 1a, Extended Data Fig. 1). AAV injected one segment rostral to SCI lesions efficiently targeted propriospinal neurons, including neurons expressing GDNFR (Extended Data Fig. 2a, b). These manipulations were tested alone and in combinations, first in adult mice and then in adult rats with severe crush SCI causing anatomically complete lesions, across which there is no spontaneous regrowth of descending or ascending axons19. Propriospinal axon regeneration was quantified as tract-tracer-labelled axons that regrew to lesion centres or beyond (Fig. 1b–d, Extended Data Figs. 2c–e, 3, 4). Mice with SCI only or SCI plus empty hydrogel depots exhibited few or no axons at lesion centres. Individual interventions, AAV-shPT or AAV-OIC alone, or depots with FGF and EGF alone or GDNF alone, did not significantly increase this number. Combined delivery of all three growth factors, FGF, EGF and GDNF in one or two depots, but without AAVs, led to modest but significantly increased axon regrowth. Combined delivery of AAV-shPT plus FGE and GDNF did not significantly increase axon numbers compared with FGE, EGF and GDNF alone or with control AAV delivering a nonsense sequence plus FGE, EGF and GDNF. By contrast, combined delivery of AAV-OIC plus FGE, EGF and GDNF synergistically facilitated robust propriospinal axon regrowth. In mice with two sequentially placed depots, this regrowth passed through non-neural lesion cores and their astrocyte scar borders, and penetrated well into spared grey matter (Figs. 1–3, Extended Data Figs. 3, 4). In these mice, total axon regrowth past lesion centres was over 100-fold greater than SCI only or SCI plus empty depots (Fig. 1c). Regrowing propriospinal axons expressed detectable GDNFR (Fig. 1e) and followed irregular paths (Fig. 1d) consistent with regrowing, as opposed to spared, axons27. Biotinylated dextran amine (BDA) tract-tracer did not label axons of passage, such as serotonin axons (Extended Data Fig. 2d, e). In all cases, no BDA-labelled axons were present 3 mm past lesion centres, confirming that lesions were complete (Fig. 1b–d, Extended Data Figs. 3, 4).

To dissect potential cellular and molecular mechanisms underlying this robust axon regrowth, we first examined axon–substrate interactions (Fig. 2, Extended Data Figs. 5, 6). FGF with EGF significantly
increased known axon-supportive substrate molecules \(^1\), laminin, fibronectin and collagen in SCI lesions (Fig. 2a, Extended Data Fig. 5a), whereas potentially inhibitory chondroitin sulfate proteoglycans (CSPG) \(^2\) were not significantly altered (Fig. 2g). FGF with EGF significantly increased astrocyte proliferation and density (Fig. 2b), yet despite this increase, stimulated and chemoattracted propriospinal axons regrew robustly through and beyond proximal (Fig. 2c) and distal (Fig. 3a) astrocyte scar borders, consistent with observations for sensory axons \(^3\). FGF with EGF also increased stromal cell density in the lesion core (Fig. 2a, Extended Data Fig. 6b). Axons transitioned readily from regrowing among astrocytes in proximal scar borders to regrowing among stromal cells in lesion core (Fig. 2c, Extended Data Fig. 6a), often oriented along stromal cells or blood vessels and circumventing inflammatory cell clusters (Fig. 2c, Extended Data Fig. 6a–c). Some regrowing axons partially contacted cells expressing Schwann cell markers (Extended Data Fig. 6d). Thus, appropriately stimulated and
To probe more broadly the effects of prolonged FGF and EGF treatment on astrocytes and other cells in SCI lesions, we conducted genome-wide sequencing of astrocyte-specific ribosome-associated RNA and RNA from non-astrocyte cells at two weeks after SCI. At this time point, FGF and EGF treatment continued to significantly regulate many genes. The most significantly regulated gene networks were associated with astrocyte proliferation and development, and with non-astrocyte inflammatory responses (Extended Data Fig. 7a–c).

We next identified mechanisms required to achieve propriospinal axon regrowth beyond lesion cores and distal borders into spared grey matter. In early experiments with one depot of AAV-OIC plus FGF, EGF and GDNF in lesion cores, we noted that propriospinal axons regrew robustly to surround and encircle depots, but did not pass beyond (Extended Data Fig. 4). We therefore injected a second depot of GDNF into spared grey matter caudal to injuries at one week after the first depot (Extended Data Fig. 1b). In mice with two such spatially and temporally separated depots, axons regrew robustly across lesion cores and distal astrocyte borders and routinely reached a full spinal segment past lesion centres (Fig. 1b–d, Extended Data Fig. 3a–c), demonstrating that chemoattraction is required to draw robust regrowth of mature endogenous axons into spared neural tissue beyond injuries. Notably, the second depot was placed at nine days after SCI, indicating that GDNF efficiently chemoattracted regrowing axons across already formed distal astrocyte scar borders without altering CSPG levels (Fig. 3a; Extended Data Fig. 3b). Starting just beyond lesion borders (Fig. 3b, c), propriospinal axons regrowing in spared grey matter intermingled with NeuN neurons, and some formed terminal-like swellings that contacted neurons, colocalized with the pre-synaptic marker synaptophysin and were in apposition with the post-synaptic marker homer (Fig. 3b–d). Such contacts were found wherever regrowing axons were present in grey matter, up to a full spinal segment (1,500 μm) beyond lesion centres. As expected and discussed below, over-ground locomotion did not improve in these experiments focused on dissecting the mechanisms required to achieve axon regrowth across lesions (Extended Data Fig. 2f).

We next tested whether our findings could be extended to rats, in which lesion core pathophysiology has been proposed to be more similar to humans. As expected, rats exhibited little or no propriospinal axon regrowth after SCI with empty hydrogel. Axon regrowth was not increased by AAV-OIC alone, and only minimally by FGF, EGF and GDNF alone. By contrast, and consistent with our observations in mice, rats given combined AAV-OIC plus two depots of FGF, EGF and GDNF exhibited robust propriospinal axon regrowth that routinely reached a full spinal segment or more past lesion centres and penetrated well into spared grey matter around the second depot but not further (Fig. 4a, b, Extended Data Figs. 8, 9). Total axon regrowth past lesion centres was over 140-fold greater in rats with AAV-OIC plus FGF, EGF and GDNF versus SCI with empty hydrogel (Fig. 4b). AAV-derived tract-tracer, red fluorescent protein (RFP), was not expressed by axons of passage such as serotonin axons. Moreover, in contrast to the robust regrowth of RFP-labelled propriospinal axons, serotonin axons exhibited no regrowth (Extended Data Fig. 9c), indicating that our growth factor depots did not simply alter the lesion core environment to broadly enable regrowth of all axon types. Regrowing propriospinal axons that reached spared grey matter intermingled with NeuN neurons and some axons formed terminal-like swellings that contacted neurons, colocalized with synaptophysin and were in apposition with homer (Fig. 4c). As in mice, over-ground locomotion of rats did not improve in these experiments that probed the mechanisms required for axon regrowth but did not provide rehabilitation to elicit use-dependent plasticity (Extended Data Fig. 9d). Nevertheless, to look for potential basic functionality of regrown propriospinal axons, we measured electrophysiological signals across lesions. Rats with SCI only exhibited essentially no conduction above background levels across lesions, whereas rats with AAV-OIC plus FGF, EGF and GDNF exhibited conduction at about 25% of control levels at 2 mm past lesions, which disappeared by 5 mm past lesions (Fig. 4d), indicating that propriospinal

Fig. 3 | Stimulated and chemoattracted mouse propriospinal axons regrow past astrocyte scar distal borders into grey matter and form synapse-like contacts with neurons. a, b, Surveys and details (boxed areas) of BDA-labelled axon regrowth across distal borders and into grey matter. c, Detail of b (outlined region) and 3D view of synapse-like contact of BDA-labelled terminal with the post-synaptic marker, homer, on NeuN neuron. d, Synapse-like BDA-labelled terminals with overlapping pre- and post-synaptic markers, synaptophysin (Syn) and homer.

attracted axons regrew in contact with multiple cell types. Notably, over 98% of regrowing propriospinal axons in SCI lesions had at least one surface continually in contact with laminin, whereas axon surfaces in mature uninjured tissue rarely contacted laminin (Fig. 2d, e). Many regrowing axons also contacted fibronectin or collagen (Extended Data Fig. 5a). Simultaneous in vivo delivery of anti–CD29, an integrin-function-blocking antibody, significantly prevented most axon regrowth (Fig. 2f, Extended Data Figs. 3d, 4), demonstrating that regrowth required integrin-dependent axon–substrate interactions with laminin, fibronectin or collagen.

Nevertheless, upregulation of permissive substrate alone was not sufficient to attract activated axon regrowth. AAV-OIC plus depots of only FGF with EGF exhibited no significant regrowth, whereas AAV-OIC plus FGF, EGF and GDNF did (Fig. 1c, Extended Data Figs. 3d, 4), demonstrating that axon regrowth also required chemoattraction.

Notably, AAV-OIC plus FGF, EGF and GDNF stimulated axons regrew robustly through dense areas of CSPGs, including in direct contact with brevican or CSPG4 (also known as NG2) in both astrocyte scars and non-neural lesion cores. This regrowth occurred along surfaces with high laminin expression (Fig. 2h, i, Extended Data Fig. 5b, c), consistent with in vitro observations that CSPG inhibition is relative rather than absolute, such that increasing laminin overrides CSPG presence.

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axon regrowth was associated with a significant return of conduction capacity that correlated with the distance of regrowth past lesions. Biological repair of anatomically complete SCI will require axon regrowth across lesions with non-neural tissue cores and astrocyte limitans borders to reach spared grey matter and form new circuits. A mechanistic understanding of why spontaneous axon regrowth fails in adults is fundamental to creating beneficial interventions. Our findings, in both mice and rats, strongly support the hypothesis that adult axon regrowth across such lesions fails primarily because of the simultaneous absence or inadequate presence of three types of mechanisms essential for facilitating developmental axon growth: (i) neuron intrinsic growth capacity, (ii) supportive substrate, and (iii) chemoattraction. We show that each of these mechanisms is required, and that in combination—not but individually—they are sufficient to achieve robust axon regrowth across lesions in spite of the presence of putative growth inhibitors. We extend previous observations that providing individual growth-facilitators is not sufficient to achieve meaningful axon regrowth across complete lesions and combinations can improve regrowth. Importantly, our findings identify chemoattraction as critically required for robust axon regrowth, and point towards the need to identify chemoattractants effective for other axon populations desirable to target after SCI. The different response of propriospinal axon regrowth was associated with a significant return of electrophysiological conduction across lesions, there was, as expected, no detectable improvement of locomotor function, consistent with accumulating evidence that new circuits formed after complete SCI cannot be expected to acquire function spontaneously, but will require rehabilitation that fosters their integration into functional networks through use-dependent plasticity. Our findings provide proof-of-concept evidence that robust and physiologically active descending propriospinal axon regrowth can be achieved across anatomically complete SCI lesions, and identify a mechanism-based biological repair strategy for such lesions that can be tested in conjunction with targeted rehabilitation paradigms designed to augment synapse remodelling and functional recovery of remodelling circuits.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0467-6.

Received: 28 February 2018; Accepted: 13 July 2018; Published online 29 August 2018.

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Surgical procedures for mice. All surgeries on mice were performed at UCLA under general anaesthesia with isoflurane in oxygen-enriched air using an operating microscope (Zeiss), and rodent stereotaxic apparatus (David Kopf). AAV injections were made two weeks before SCI to allow for the implantation and were targeted at propriospinal neurons between one and two segments rostral to the planned locations of SCI lesions after laminectomy of a single vertebra. AAV (see below) were injected into two sites (one on each side of the cord, 0.25 μl (AAV2/9 OPN: 1 × 1013, IGF: 5 × 1012, CNFT: 5 × 1012 genome copies per ml in sterile saline)) 0.6 mm below the surface at 0.15 μl per minute using glass micropipettes (ground to 50- to 100-μm tips) connected via high-pressure tubing (Kopf) to 10-μl syringes under the control of microinfusion pumps. Severe crush SCIs were made at the level of T10 after laminectomy of a single vertebra by using No. 2 Dumont forceps (Fine Science Tools). The second syringe was used to inject a low-viscosity (storage modulus measured at 10 rad/s and 10% strain) 2.5%, Bayer Health Care AG, 5–10 mg per kg, subcutaneously) were provided for injections as described above. Timelines of all injections are provided in Extended Data Fig. 1. All mice received analgesic cargoes as described36–38. Cargo molecules were as follows. Human recombinant FGF2, EGF and GDNF were purchased from PeproTech: (i) human FGF2 (FGF-basic) (154 amino acids) Cat#100-188-100U, Lot#091608 C0617; (ii) human EGF Cat#AF-100-15–100UG, Lot#0816AFC05 B2317; (iii) human GDNF Cat#405-10-100UG, Lot#0606BE4A2517. Integrin-function blocking hamster anti-rat CD29 monoclonal antibody was purchased from BD Bioscience as a custom order at 10.3 mg/ml (product #624084; lot#7165896). All surgeries on rats were performed under general anaesthesia with isoflurane in oxygen-enriched air using an operating microscope (Zeiss). Histology and immunohistochemistry. After terminal anaesthesia by barbiturate overdose mice or rats were perfused transcardially with 4% paraformaldehyde and spinal cords processed for immunofluorescence as described39–35. Primary antibodies were: rabbit anti-GFAP (1:2,000; Dako); rat anti-GFAP (1:1,000, ThermoFisher); chicken anti-GFAP (1:1,000, Novus Biologicals); rabbit anti NeuN (1:2,000, Millipore); goat anti-NG2 (CSPG4) (E. G. Hughes and D. W. Bergles41); rat anti-PECAM-1 (1:200, BD Biosciences); guinea pig anti-homer1 (1:600, Synaptic Systems GmbH); rabbit anti-NG2 (CSPG4) (E. G. Hughes and D. W. Bergles41); rabbit anti-CD29 (5 μg/ml, ThermoFisher); sheep anti-BrdU (1:300, Maine Biotechnology Services); rabbit anti-HSV-TK (1:1,000,15,38); goat anti-CD13 (1:1,000, R&D systems); rabbit anti-amin (1:100, Sigma); rabbit anti-fibronectin (1:500, Millipore); rabbit anti-catenin (1:500, Novus Biologicals); mouse anti-NeuN (1:2,000, Millipore); mouse anti-CSPG4 (1:100, Sigma); rabbit anti-brevican (BCAN) (1:300, Novus Biologicals); guinea pig anti-NG2 (CSPG4) (E. G. Hughes and D. W. Bergles41); rat anti-PECAM-1 (1:200, BD Biosciences); guinea pig anti-homer1 (1:600, Synaptic Systems GmbH); rabbit anti-synaptophysin (1:600, Dako); rabbit anti-TRPV1 (1:1,000, Rockland); chicken anti-RFP (1:500, Novus Biologicals); goat anti-GFP (1:1,000, Novus Biologicals). Fluorescence secondary antibodies were conjugated to: Alexa 488 (green) or Alexa 594 (red). Sections were coverslipped using ProLong Gold anti-fade reagent (Invitrogen). Sections were examined and photographed using deconvolution fluorescence microscopy and scanning confocal laser microscopy (Zeiss). Tiled scans of individual whole sections were prepared using a ×20 objective and the scanning function of a Leica Aperio Versa 200 Microscope (Leica) available in the UCLA Translational Pathology Core Laboratory. Composite surveys were prepared from tiled scans of multiple sections from the same animals oriented and overlaid using Imaris software (9.1.2.64 Bit, Bitplane, Oxford Instruments).

Axon quantification. Axons labelled by tract tracing using BDA or RFP were quantified using image analysis software (NeuroLucida, 9.14.35 12 Bit, MicroBrightField) using the computer-driven microscope regulated in the x, y and z axes (Zeiss) by using the same experimental conditions. Using NeuroLucida, lines were drawn across horizontal spinal cord sections at SCI lesion centres and at regular distances beyond (Fig. 1c, Extended Data Fig. 3a, b) and the number of axons crossing these lines was counted by observers blind to experimental conditions. Multiple sections were drawn along the spinal cord to count the number of axons that crossed each line. The number of axons crossing each line was counted and then averaged across all sections to obtain the number of axons per section.
through the middle of the cord, in which propiosiolipin axons were densest, were counted per mouse or rat and expressed as total intercepts per location per animal. To determine the efficacy of axon transection after SCI, we examined labelling 3 mm distal to SCI lesion centres in mice and 5 mm distal to lesion centres in rats, with the intention of eliminating animals that had labelled axons at this location on the grounds that these mice may have had incomplete lesions. However, essentially all mice or rats that had met the strict behavioural inclusion criterion of no hindlimb movements 2 days after severe crush SCI exhibited no detectable axons 3 mm or 5 mm, respectively, distal to SCI lesions, regardless of treatment group.

Quantification of immunohistochemically stained areas. Sections stained for laminin 1, fibronectin, collagen 1a1 or CSPG were scanned using constant exposure settings. Single channel immunofluorescence images were converted to black and white and thresholded (Fig. 2a) and the amount of stained area was measured in different tissue compartments using NIH Image (v.1.51) software.

Quantification of astrocyte proliferation and density. To quantify astrocyte proliferation and the number of astrocytes in the immediate scar border previously defined as zone 1, we used a well-characterized transgenic mouse line that expresses thymidine kinase in astrocyte cell bodies, thereby facilitating quantification of cell number and co-localization with other markers.

To quantify the proportion of newly proliferated astroglia in the scar boarder, we injected daily single doses of the cell division marker, bromodeoxyuridine (BrDU, Sigma), 100 mg/kg/day dissolved in saline plus 0.007% NaOH on days 2–7 after SCI. Newly proliferated astrocytes were quantified by determining the percentage of astrocytes stained for both GFAP, thymidine kinase and BrDU in zone 1. Total astrocyte numbers in the immediate scar border (zone 1) were determined by counting the number of cell per unit area of tissue. Cell counts were performed using stereological image analysis software (StereoInvestigator, 9.14.5.32 Bit, and NeuroLucida, 9.14.5 32 Bit, MicroBrightField) operating a computer-driven microscope regulated in the x, y and z axes (Zeiss).

Dot blot. For the dot blot immunosassay of laminin 1, fibronectin, collagen 1 or CSPG, spinal cord tissue blocks were lysed and homogenized in standard RIPA (radio-immuno-precipitation-assay) buffer. LDS (lithium dodecyl sulfate) buffer (Life Technologies) was added to the post-mitochondrial supernatant and 2 μl containing 2 μg/μl protein was spotted onto a nitrocellulose membrane (Life Technologies), set to dry and incubated overnight with primary antibodies: rabbit anti-laminin 1 (1:4,000, Sigma); rabbit anti-fibronectin (1:7,000, Millipore); rabbit anti-collagen 1a1 (1:7,000, Novus Biologicals); mouse anti-chondroitin sulfate antibody (CS5–6, 1,300, Sigma Aldrich), an IgM monoclonal antibody that detects glyco-mieties of all CSPGs. Immunoreactivity was detected on X-ray film with HRP-conjugated secondary antibody (1:5,000) and chemiluminescent substrate (Thermo Fisher). Densitometry measurements of immunoreactivity were obtained using ImageJ software (NIH) and normalized to total protein (Ponceau S) density.

Raw images of dot blots are provided as Supplementary Fig. 1.

Isolation and sequencing of RNA from astrocyte cells. Using mice expressing an mGFAP-RiboTag transgene, RNA was evaluated as previously described from uninjured mice, and mice two weeks after SCI after treatment with hydrogel depots that either contained no cargo (empty depots) or delivered FGF + EGF (Extended Data Fig. 1a). In brief, spinal cords were rapidly dissected out of the spinal canal and the central 3 mm of the lower thoracic lesion including the lesion core and 1 mm rostral and caudal were rapidly removed and snap-frozen in liquid nitrogen. Haemagglutinin immunoprecipitation of astrocyte ribosomes and ribosome-associated mRNA was carried out as described.

The non-precipitated flow through from each sample was collected for analysis of non-astrocyte total RNA. Haemagglutinin and flow-through samples underwent on-column DNA digestion using the RNase-Free DNase Set (Qiagen) and RNA purified with the RNeasy Plus Micro kit (Qiagen). Integrity of the eluted RNA was analysed using a 2100 Bioanalyzer (Agilent) with the RNA Pico chip, average RNA integrity number (RIN) = 7.9 ± 1.4. RNA concentration determined using the RiboGreen RNA Assay kit (Life Technologies). cDNA was generated from 5 ng of immunoprecipitate or flow-through RNA using the Nugen Ovation 2 RNA-Seq System V2 kit (Nugen). One microgram of cDNA was fragmented using the Nugen Ovation 2 RNA-Seq System V2 kit (Nugen). One microgram of cDNA was fragmented using the Covaris M220. Paired-end libraries for multiplex sequencing were generated from 300 ng of fragmented cDNA using the Apollo 324 automated library preparation system (Wafergen Biosystems), enriched over ten cycles of PCR and purified with Agencourt AMPure XP beads (Beckman Coulter). All samples were analysed by an Illumina NextSeq 500 Sequencer (Illumina) using 75-bp pair-end sequencing. The number of reads obtained are between 38.0 to 71.0 million (average 52.1 million). Sequences were aligned to mouse mm10 genome using STAR aligner (v.2.4.0j).

Uniquely aligned reads were between 57.1 to 87.0% (average 73.9%). Read counts were determined using HT-seq (v.0.6.0). Differential expression analysis was conducted using the edgeR package (v.3.20.1) after removal of low count genes (five counts for at least two samples). Three samples from astrocyte samples were excluded owing to low RIN values (<5.7), no samples were excluded for non-astrocyte samples.

Rat electrophysiology. Terminal electrophysiological assessments were carried out as previously described four weeks after SCI. In brief, animals were anaesthetized with urethane (1.5 g/kg; intraperitoneally) and core body temperature was maintained at 37 °C using a self-regulating heated pad connected to a rectal probe. Depth of anaesthesia was continually monitored by assessing withdrawal reflexes and respiratory rate. After laminectomy to expose the injury site and 7 mm rostral and caudal to the lesion site by removal of one vertebra on either side of the injury, the dura was removed, and the exposed spinal cord was covered with warm mineral oil to prevent drying. For stimulation, a tungsten bipolar concentric electrode was positioned intraspinally at the rostral-most point of the laminectomy. A silver ball electrode was used to record any evoked activity from the surface of the exposed spinal cord at various locations (above, 2 mm below, and 5 mm below the lesion).

Stimulation was delivered in 200 μs square wave pulses at the maximum amplitude possible before large motor responses were evoked (typically between 600 μA and 800 μA) and at a frequency of 0.75 Hz using a STG 4004 stimulus generator (Multi-Channel Systems). Evoked activity was amplified and recorded using an A-M Systems differential amplifier, PowerLab and LabChart Pro acquisition and analysis system (AD Instruments). For analysis, 30 traces from each recording site were averaged and the peak to peak amplitude of the evoked potential was quantified.

Statistics, power calculations, group sizes and reproducibility. Statistical evaluations of repeated measures were conducted by one-way ANOVA with post hoc independent pair-wise analysis as per Bonferroni, or by Student’s t-test (Prism, 7.0c, GraphPad). For one-way ANOVA statistical evaluations, F-values are also reported in the online Source Data files in the format F(degree of freedom 1, degree of freedom 2) = X. The degrees of freedom are computed as degree of freedom 1 = k – 1, in which k is the number of compared treatments, and degree of freedom 2 = n – k in which n is the total number of samples across the treatment groups. For Student’s two-tailed t-test (Prism, 7.0c, GraphPad), t value and degrees of freedom are reported in the format t(degree of freedom) = X. Power calculations were performed using G*Power Software v.3.1.9.2. For quantification of histologically derived neuroanatomical outcomes such as numbers of axons or percentage of area stained, group sizes were used that were calculated to provide at least 80% power when using the following parameters: probability of type I error (α) = 0.05, a conservative effect size of 0.25, 3–10 treatment groups with multiple measurements obtained per replicate. All graphs show mean ± s.e.m. as well as individual values as dot plots. All bar graphs are overlaid with dot plots in which each dot represents the value for one animal to show the distribution of data and the number (n) of animals per group. Files of all individual values are provided as Source Data. The main experiments testing propriospinal axon regrowth across SCI lesions in animals treated with SCI + AAV-OIC + 2D + FGF + EGF + GDNF and the main control groups (SCI + 1D empty, SCI + AAV-OIC + 1D empty, SCI + 2D + FGF + EGF + GDNF) were repeated independently three times in different groups of mice, and in three different groups of rats with similar results. Other experiments testing propriospinal axon regrowth across SCI lesions in animals in all other groups were repeated independently at least twice in different groups of mice with similar results. For all photomicrographs of histological tissue, staining experiments were repeated independently with tissue from at least four, and in most cases six, different animals with similar results.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. Files of Source Data of individual values for all quantitative figures are provided with the paper. Raw images of dot blots are provided as Supplementary Fig. 1. RNA-seq data are available at the NCBI Gene Expression Omnibus under accession number GSE111529. Other data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Extended Data Fig. 1 | Experimental models and timelines. Mice or rats received different combinations of procedures including adeno-associated virus (AAV) injections, complete crush SCI, injections of one or two depots of hydrogel containing different molecular cargos and injections of biotinylated dextran amine (BDA) for axonal tract-tracing. AAV injections were made two weeks before SCI to allow time for molecular expression and were targeted at propriospinal neurons (PrSp) between one and two segments rostral to planned locations of SCI lesions. AAVs were used to deliver either potential axon-growth reactivating molecules, GFP to identify targeted neurons or RFP as an axonal tract-tracer. Complete crush SCI lesions were placed at the level of spinal segment T10. Two days after SCI, all animals were behaviourally evaluated for completeness of SCI and only animals with functionally complete SCI were included in subsequent experimental steps. Additional animals with complete SCI were evaluated without hydrogel injections (SCI only). a, Schematic and timeline of one-depot experiments. Two days after complete crush SCI, animals received hydrogel injections targeted to the centre of the non-neural lesion core. These depots (D₁) contained different molecular cargos as listed in the schematic. Depots without cargo were referred to as ‘empty’. b, Schematic and timeline of two-depot experiments. Two days after complete crush SCI, animals received a D₁ hydrogel injection into the centre of the non-neural lesion core to deliver the growth factors FGF + EGF + GDNF. Nine days after SCI, the animals received a second hydrogel injection (D₂) targeted to spared neural tissue 1 to 2 mm caudal to the lesion centre to deliver GDNF to sequentially chemoattract propriospinal axons that had regrown into the lesion core. BDA injections for axonal tract-tracing were targeted at propriospinal neurons between one and two segments rostral to SCI lesions and were placed at the time of injecting either D₁ (a) or D₂ (b). Tissue was collected for evaluation at either two or four weeks after SCI. Electrophysiological evaluations were conducted at four weeks after SCI. For abbreviations, see Extended Data Table 1.
Extended Data Fig. 2 | AAV targeting, axon tracing and axon quantification. 

a, AAV targeting of green fluorescent protein (GFP) to propriospinal neurons. Multi-fluorescent, survey (left) and detail (right, boxed area) confocal images of horizontal section through mouse grey (gm) and white (wm) matter. Essentially all NeuN+ propriospinal neurons targeted with AAV express GFP.

b, GDNFR expression by AAV-targeted propriospinal neurons. Multi-fluorescent, orthogonal 3D confocal images show that AAV-targeted propriospinal neurons express GDNFR.

c, BDA tract-tracing of propriospinal axons in uninjured mouse. Multi-fluorescent, survey images show tract-tracing of propriospinal axons using biotinylated dextran amine (BDA) in tiled confocal scans of horizontal section from uninjured mouse. Hatched area indicates densely labelled location of BDA injections.

d, BDA tract-tracing compared with 5HT staining. Survey and orthogonal 3D confocal detail from an area proximal to the SCI lesion shows a complete lack of overlap of BDA-labelling and 5HT immunohistochemistry, indicating that BDA-tracing did not label 5HT axons of passage.

e, Survey images of the same field examined with different filters show BDA-labelled propriospinal axons (bottom image) regrowing robustly past the astrocyte scar proximal border and through the non-neural lesion core; by contrast, 5HT axons (top and bottom image) did not regrow into or through the lesion core.

f, Open field hindlimb locomotion. Data are mean ± s.e.m., n = 6 mice per group.

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Extended Data Fig. 3 | Procedures for quantification of BDA-labelled propriospinal axons after SCI. a, Schematics show demarcation of SCI lesion centre (Cn) and evenly spaced lines beyond the lesion centre placed by image analysis software (Neurolucida, MicroBrightField) for quantification of axon intercepts in horizontal tissue sections of mice with SCI and one (D₁) or two (D₁ + D₂) hydrogel depots. b, Multi-fluorescent, survey images show BDA-labelled axons and GFAP-labelled astrocytes that demarcate astrocyte scar proximal borders and distal borders around the non-neural lesion core after SCI. The hydrogel of the empty depot (left) was tagged with a blue fluorescent label for visualization. Note the essential absence of axons passing the astrocyte scar (AS) proximal border to reach the lesion centre (Cn) or beyond in the mouse with SCI plus empty depot (left), in contrast to the large number of axons that regrew through the lesion core and passed beyond the distal astrocyte scar border into spared grey matter in the mouse with full treatment of stimulatory AAV plus growth factors (right). GFAP staining shows that the SCI lesions are anatomically complete across the entire width of the spinal cord in both cases. Note that the second depot was placed at nine days after SCI, by which time the distal astrocyte scar border was essentially formed35. Note also that astrocytes do not migrate into the depots, potentially giving the mistaken impression of cavity formation when looking only at the GFAP channel alone. Nevertheless, examination of other fluorescence channels shows that depot sites clearly contain DAPI-stained stromal cells and BDA-positive axons. c, Large area survey images of BDA-labelled axons in composite mosaic scans of horizontal sections. In a control mouse (top) that received SCI plus empty depot, few axons reach the lesion centre, almost none pass beyond and no axons are present at 3 mm. In the treated mouse (middle) that received stimulatory AAV plus growth factors, many axons regrow through the lesion core and reach or pass 1.5 mm beyond the lesion centre, which is the equivalent length of a full thoracic spinal segment in mice46. Note also that there are no axons present at 3 mm, demonstrating that the SCI lesion was complete and that axons that are found past the lesion centre represent axon regrowth after SCI in response to the experimental manipulations. In an uninjured mouse (bottom), there are many labelled axons at the distance equivalent to 3 mm beyond the location of SCI in injured mice. d, Numbers of axon intercepts at lesion centres for all experimental groups. Data are mean ± s.e.m., dots in graphs show numbers and distribution of individual mice per group. NS, not significant versus SCI only; #P < 0.01, versus SCI only and not significant versus each other; **P < 0.01; ***P < 0.001, versus all other groups; one-way ANOVA with Bonferroni, F(12, 57) = 22.3.
Extended Data Fig. 4 | BDA tract-tracing of propriospinal axons after SCI and different treatment conditions. Survey images show tiled mosaic scans of horizontal sections from representative mice of all experimental conditions. Experimental treatment conditions are listed in the upper left of each scan. Mouse identification numbers are given in the upper right. Axon regrowth was quantified by counting axon intercepts with lines drawn through lesion centres and at regular intervals beyond by using image analysis software.
Extended Data Fig. 5 | Stimulated, supported and chemottracted mouse propriospinal axons regrow through the lesion core in contact with various substrate molecules, including putatively inhibitory CSPGs. a, Left and left middle, multi-fluorescent, detail images show BDA-labelled axons regrowing along and among surfaces decorated with fibronectin or collagen. Right middle, orthogonal 3D confocal images of the outlined areas show direct contact between BDA-labelled axons and fibronectin or collagen. Right, quantification of fibronectin or collagen levels and dot blots. Data are mean ± s.e.m. of density, n = 4 mice per group. *P < 0.01, one-way ANOVA with Bonferroni, $F(3, 12) = 13.0$ for fibronectin dot blot and $F(3, 12) = 10.2$ for collagen dot blot. b, Left and middle, multi-fluorescent, detail images show BDA-labelled axons regrowing along and among surfaces decorated with brevican (BCAN). Right, orthogonal 3D confocal image of the outlined area shows direct contact between BDA-labelled axons and BCAN. c, Multi-fluorescent, orthogonal 3D confocal images show BDA-labelled axons regrowing along and in direct contact with surfaces decorated with both CSPG4 and laminin (arrows).
Extended Data Fig. 6 | BDA tract-tracing of propriospinal axon regrowth after SCI along and among different cell types. a, Multiple channel fluorescent images show the same BDA-labelled axon transitioning from contact with GFAP-positive astrocytes in proximal scar border to contact with CD13+ stromal cells in the lesion core. Numbers and arrows indicate the same locations in images of different combinations of fluorescent markers: 1, axons in contact with astrocyte processes; 2, axons in contact with both astrocyte process and stromal cell; 3, axons in contact with stromal cell. b, Multiple channel fluorescent images show axons regrowing along, and following the trajectory of, stromal cells (S) while circumventing clusters of inflammatory cells (Inf) in the lesion core. c, Multiple channel fluorescent images show axons (A) regrowing along the trajectory of blood vessels (bv) in contact with stromal cells that are present on endothelia (E) positive for platelet endothelial cell adhesion molecule (PECAM). d, Multiple channel fluorescent images show BDA-labelled propriospinal axons and cells expressing the combinatorial Schwann cell markers, p75 and SOX10, in the lesion core. Some stromal cells expressing only SOX10 but not p75 are also visible. Numbers and arrows indicate the same locations in images of different combinations of fluorescent markers: 1, axons in partial contact with cells expressing Schwann cell markers; 2, axons not in detectable contact with Schwann cells. Note that some axons are partially in contact with, and partially not in contact with, Schwann cells in lesion core.
Extended Data Fig. 7 | Comparison of genomic data from astrocytes and non-astrocyte cells from mice with or without FGF + EGF after SCI. a, Heat maps showing significantly differentially expressed genes (DEGs) derived by RNA-seq of mRNAs from spinal cord tissue of mice treated with SCI + 1D + FGF + EGF (D+GF), and the expression of these genes in mice treated with SCI + 1D empty (D-Em), at two weeks after SCI. Data are shown for mRNAs derived selectively from astrocytes or from all other cell types (non-astrocytes), isolated as previously described. Red, upregulated; green, downregulated; relative to SCI only. n = 3 mice per group; FDR < 0.1 for differential expression. b, Total numbers of significant DEGs in astrocytes and non-astrocytes from mice as shown in the heat map in a. Red and green numerical values indicate significantly upregulated and downregulated genes, respectively. Relative to SCI only, over 900 astrocyte genes and over 300 non-astrocyte genes were significantly up- or downregulated in mice after 12 days of growth factor treatment, which were not significantly altered by treatment with empty depots. c, Top five networks of genes significantly altered by SCI + 1D + FGF + EGF that were not altered by SCI + 1D empty after SCI relative to SCI only, as identified by unbiased analysis (Ingenuity). Full RNA-seq data are available at NCBI Gene Expression Omnibus under accession GSE111529.

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Extended Data Fig. 8 | RFP tract-tracing of propriospinal axons after SCI and different treatment conditions in rats. a, Large area survey images of RFP-labelled axons in composite mosaic scans of horizontal sections. Tracer-injection sites are denoted by RFP-PrSp. a, Multiple channel fluorescent images showing BDA-labelled axons and GFAP-labelled astrocytes that demarcate astrocyte scar proximal borders and distal borders around the non-neural lesion core after SCI. GFAP-staining shows that SCI lesions were anatomically complete across the entire width of the spinal cord, with large lesion cores in a control rat (left), and in a rat treated with stimulatory AAV plus growth factors (right). In the control rat, few axons reach the lesion centre or beyond. In the treated rat, many axons regrow through the lesion core and reach or pass 3 mm beyond the lesion centre, which is the equivalent length of a full thoracic spinal segment in rats. b, Completeness of SCI lesions was confirmed in all rats used in qualitative and quantitative evaluations by confirming that no axons were present at 5 mm or more past lesion centres, as shown here for control rats (top) and treated rats (middle), whereas in uninjured rats, abundant labelled axons are present at an equivalent distance past the RFP-injection site.
Extended Data Fig. 9 | Growth factor induction of laminin, comparison of propriospinal and serotonin axons, and locomotor evaluations of rats after SCI without and with treatments. a, b, Survey images show laminin IHC in tiled mosaic scans of horizontal sections from representative rats. b, Top, mean ± s.e.m. quantification of laminin IHC in rats as per cent area per linear μm², (n = 4 rats per group, dark coloured lines = means, lighter coloured shaded areas = s.e.m., colours indicate experimental groups as shown in graph below). Bottom, total laminin in rats summarized as mean ± s.e.m. area under the curve as calculated from graph above. (ns non-significant, **P < 0.005, one-way ANOVA/Bonferroni, F(2, 9) = 15.04). c, Multiple channel fluorescent images show RFP-labelled propriospinal axons and immunohistochemically stained serotonin (5HT) axons in rats after SCI + AAV-OIC + 2D + FGF + EGF + GDNF. The two survey images on the left show the same field with different filters. Note in the survey images on the left, and in the higher magnification image in centre, that RFP-labelled propriospinal axons regrow robustly past the astrocyte scar proximal border and through the non-neural lesion core. By contrast, 5HT axons did not regrow into or through the lesion core. The image on the right shows an orthogonal 3D confocal detail from an area proximal to the SCI lesion, demonstrating a complete lack of overlap of RFP labelling and 5HT immunohistochemistry, indicating that RFP tracing did not label 5HT axons of passage. d, Open field hindlimb locomotor score at various times after SCI in rats assessed using a 6-point scale in which 5 is normal walking and 0 is no movement of any kind. Data are mean ± s.e.m., n = 6 per rats group.
Extended Data Table 1 | Abbreviations used in the text and figures

| Abbreviation | Description |
|--------------|-------------|
| 1D           | animals receiving 1 hydrogel depot |
| 2D           | animals receiving 2 hydrogel depots |
| αCD29        | anti-CD29 function-blocking antibody |
| AAV          | adeno-associated virus |
| BCAN         | brevican |
| BDA          | biotinylated dextran amine (axon tract tracer) |
| CD29         | integrin beta-1 |
| Cn           | lesion center |
| CNTF         | ciliary-derived neurotrophic factor |
| CSPG         | chondroitin sulfate proteoglycan |
| D₁           | hydrogel depot in lesion center |
| D₂           | hydrogel depot in spared neural tissue |
| EGF          | epidermal growth factor |
| FGF2         | fibroblast growth factor 2 (basic) |
| GDNF         | glial derived neurotrophic factor |
| GFP          | green fluorescent protein |
| IGF          | insulin-like growth factor |
| Inf          | Inflammatory cells |
| OIC          | osteopontin, IGF plus CNTF |
| PECAM        | platelet endothelial cell adhesion molecule |
| PrSp         | propriospinal neurons |
| PTEN         | phosphatase and tensin homolog |
| RFP          | red fluorescent protein (axon tract tracer) |
| shPTEN       | short hairpin RNA against PTEN |
| S            | stromal cells |
| Syn          | synaptophysin |
| TP           | total protein |