Sensory innervation in porous endplates by Netrin-1 from osteoclasts mediates PGE2-induced spinal hypersensitivity in mice

Spinal pain is a major clinical problem, however, its origins and underlying mechanisms remain unclear. Here we report that in mice, osteoclasts induce sensory innervation in the porous endplates which contributes to spinal hypersensitivity in mice. Sensory innervation of the porous areas of sclerotic endplates in mice was confirmed. Lumbar spine instability (LSI), or aging, induces spinal hypersensitivity in mice. In these conditions, we show that there are elevated levels of PGE2 which activate sensory nerves, leading to sodium influx through Na\textsubscript{1.8} channels. We show that knockout of PGE2 receptor 4 in sensory nerves significantly reduces spinal hypersensitivity. Inhibition of osteoclast formation by knockout Rankl in the osteocytes significantly inhibits LSI-induced porosity of endplates, sensory innervation, and spinal hypersensitivity. Knockout of Netrin-1 in osteoclasts abrogates sensory innervation into porous endplates and spinal hypersensitivity. These findings suggest that osteoclast-initiated porosity of endplates and sensory innervation are potential therapeutic targets for spinal pain.
low back pain (LBP) is a common health problem, which most people (80%) experience at some point, especially in older adults1–4. In the USA alone, the direct and indirect costs associated with LBP surpass $90 billion per year, with similar adjusted rates in other countries5. In total, 90% of LBP is non-specific LBP, which has no apparent pathoanatomical cause6,7. Several lumbar structures, such as intervertebral disc, facet joints, or are plausibly sources of nonspecific LBP, but the pain can not be reliably attributed to those structures by clinical tests8–10. Importantly, intervertebral disc (IVD) degeneration is frequently observed in asymptomatic patients, indicating that disc degeneration, per se, is not painful in some patients11,12. Hence, identifying the source of LBP and related mechanisms is essential to develop effective treatments for LBP.

The positive association between vertebral endplate signal changes (i.e., Modic changes) and LBP has been shown by magnetic resonance imaging (MRI) examination13,14. Modic changes are common MRI findings in patients with nonspecific LBP. It is believed to be a factor independently associated with the increased risk of LBP15–17. The incidence of Modic changes positively correlates with LBP18. Histological analysis further showed that the presence of endplate lesions was associated with LBP19. During aging, endplates undergo ossification with elevated osteoclasts and become porous20–22. Histological and micro CT analysis further revealed that sclerotic endplates are highly porous22. Progressively more porous endplates with narrowed IVD space are characteristics of spinal degeneration22,24. Since pain is produced by nociceptors, LBP may be caused by sensory innervation into endplates. Moreover, nerve density was higher in porous endplates than in normal endplates or degenerative nucleus pulposus. Zoledronic acid and denosumab, drugs that inhibit osteoclast activities, have shown analgesic effects in patients with Modic changes associated LBP26,27, with the implication of a potential role of osteoclast activity in sensory nerve innervation.

Prostaglandin E2 (PGE2) is an inflammatory mediator released at the focal inflamed tissue, and a neuromodulator that alters neuronal excitability. Four types of G-protein–coupled EP receptors (EP1–EP4) mediate the functions of PGE2. EP4 receptor is considered the primary mediator of PGE2–evoked inflammatory pain hypersensitivity and sensitization of sensory neurons28,29. We recently reported that PGE2, produced from arachidonic acid by the enzymatic activity of cyclooxygenase 2 (COX-2), during bone remodeling activates EP2 receptor 4 (EP4) in CGRP+ sensory nerves to tune down sympathetic tones further inducing osteoblastic differentiation of MSCs30. Specific EP4 receptor antagonists could reduce acute and chronic pain, including osteoarthritis pain31–33. The tetrodotoxin-resistant (TTX-R) sodium channel Na1,8 is a potential drug target for pain. Na1,8 is expressed primarily in small and medium-sized dorsal root ganglion (DRG) neurons and their fibers34–36. PGE2 could modulate the TTX-R sodium current in DRG neurons and promote Na1,8 trafficking to the cell surface37,38.

In this study, we sought to demonstrate that osteoclasts initiate porosity of endplates with sensory innervation into porous areas. Our data showed that Calcitonin Gene–Related Peptide positive (CGRP+) nociceptive nerve fibers and blood vessels were increased in the cavities of sclerotic endplates. The elevated PGE2 in porous endplates induces sodium influx into the cells to stimulate sensory nerves that leads to spinal pain. Inhibition of osteoclast activity attenuated sensory innervation in porous endplates and pain behavior.

Results
Development of hyperalgesia in LSI and aged mouse models. Lumbar spine instability (LSI) was established in mice as a spine degeneration model for spine pain behavior testing39,40. We first measured the vocalization threshold in response to force applied on the L4/L5 disc region. Pressure tolerance decreased significantly at 4, 8, and 12 weeks after LSI surgery relative to mice that underwent sham surgery (Fig. 1a), indicating the development of low back pressure hyperalgesia. Then, we monitored spontaneous activity to indicate the potential effect of spinal pain, including distance traveled, maximum speed of movement, mean speed of movement, and active time per 24 h, though they are not specific for spinal pain behaviors. The results revealed that each measure of spontaneous activity decreased significantly at 4 and 8 weeks after LSI surgery relative to sham surgery (Fig. 1b–e). Moreover, we assessed mechanical hyperalgesia of the hind paw as referred pain by performing von Frey analysis, as a secondary indicator of symptomatic LBP. We found that the paw withdraw frequency increased significantly from 2 to 12 weeks after LSI surgery (Fig. 1f, g). However, we observed no response to the straight leg-raising test when recording the number of vocalizations during five leg stretches and lifts on either LSI or sham surgery mice. This indicates that nerve root compression is not involved in the hyperalgesia developed after LSI surgery. The results of these pain behavior tests suggest that spine instability induces the development of hyperalgesia.

In parallel, we evaluated symptomatic LBP during aging in these behavior tests. Similarly, the threshold of pressure tolerance (Fig. 1b) and spontaneous activity (Fig. 1i–k) decreased significantly in aged mice (age 20 months) relative to young mice (age 3 months). The mechanical hyperalgesia of the hind paw increased significantly in aged mice relative to young mice (Fig. 1l, m). Together, these data indicate that, as in the LSI mouse model, aging also induces spine hyperalgesia.

Sensory innervation in endplates in LSI and aged mouse models. We showed previously an increase in osteoclasts at the onset of endplate sclerosis40. Therefore, we evaluated the potential role of osteoclasts in the sensory innervation of endplates. Tartrate-resistant acid phosphatase (TRAP) staining demonstrated that the number of TRAP+ osteoclasts in endplates increased significantly at 2 weeks after LSI, and remained at a high level until 8 weeks after LSI surgery (Fig. 2a, b). Large bone marrow cavities were generated in sclerotic endplates by osteoclastic bone resorption in mice after LSI surgery, whereas, the cartilaginous endplates were maintained in sham surgery mice (Fig. 2a). Immunofluorescent staining revealed that the significant increase of CGRP, the marker of peptidergic nociceptive C nerve fibers in the porous endplates, began at 2 weeks and continued to increase until 8 weeks after LSI surgery (Fig. 2c, d), but there were no detectable CGRP+ nerves in the endplates of sham surgery mice (Fig. 2c, d). Interestingly, the nociceptive nerve fibers were localized primarily adjacent to the bone surface (Fig. 2c). The co-staining of PGP9.5, the broad marker of nerve fibers with CGRP, further validated the nociceptive innervation of the endplates after LSI surgery (Fig. 2e). However, the nonpeptidergic subtype of IB4+ C nerve fibers was not detected in either LSI or sham surgery mice (Fig. 2f), suggesting CGRP+ nerve fibers as the primary nociceptive C nerve fibers in the endplates. Importantly, CD31+ECM+N+ blood vessels were also growing into the porous endplates after LSI surgery, along with the sensory innervation of the endplates (Supplementary Fig. 1A, B).

To determine whether spine degeneration during aging could induce sclerosis and sensory innervation in vertebral endplates, we analyzed the caudal endplates of L4/5 from aged mice and young mice. The porosity of endplates in aged mice increased significantly relative to young mice, as determined by

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three-dimensional microcomputed tomography (μCT) analysis (Fig. 3a–c). The μCT analysis of vertebral trabecular bone demonstrated that the trabecular bone volume/total volume (BV/TV) and trabecular bone number (Tb.N) of L5 vertebrae decreased significantly in 20-month-old mice relative to 3-month-old mice, while the trabecular bone thickness (Tb.Th) and trabecular bone separation distribution (Tb.Sp) did not change significantly (Supplementary Fig. 2A–E). Safranin O and fast green staining demonstrated that the green-stained bone matrix surrounded the cavities in endplates of aged mice (Fig. 3d, top and middle), suggesting endochondral ossification. Endplate scores, which are a histologic assessment of pathological changes, such as bony sclerosis, structure disorganization, and neovascularization, were significantly higher in aged mice than that in young mice (Fig. 3e). Interestingly, high levels of TRAP+ osteoclasts were observed in the endplates of aged mice, whereas TRAP+ osteoclasts were rarely detected in the endplates of young mice (Fig. 3c, bottom and 3f). Immunostaining of CGRP showed increased aberrant innervation of peptidergic nociceptive C nerve fibers in the porous endplates of aged mice (Fig. 3g, h). The co-staining of PGP9.5 and CGRF further confirmed that the endplates were innervated by nociceptive nerve fibers (Fig. 3i). Similar to our findings in LSI mice, CD31+EMCN+ blood vessels were detected in the endplates, along with CGRP+ nerve fibers during aging (Supplementary Fig. 3A, B), indicating active ossification of the endplates.

To examine the potential involvement of sclerosis and sensory innervation of the endplates with pain behavior, we evaluated the pathological changes in the endplates of the lower lumbar spines from patients with or without LBP history. Severe endplate lesions were observed in patients with a history of frequent LBP, whereas the cartilaginous structure was preserved in patients without a history of frequent LBP, despite disc herniation (Supplementary Fig. 4A). The increased endplate scores were also observed in patients with a history of frequent LBP (Supplementary Fig. 4B). However, the patients with the history of frequent LBP were older than the ones without the history of frequent LBP (Supplementary Table 1). TRAP staining showed that abundant TRAP+ osteoclasts localized at the bone surface in the sclerotic endplates (Supplementary Fig. 4C). Immunofluorescence staining revealed that CBRP+PGP9.5+ nociceptive nerve fibers grown into the porous areas of sclerotic endplates of patients with LBP history (Supplementary Fig. 4D). These results suggest that sensory innervation in sclerotic endplates is potentially related to spinal pain behavior.

**Retrograde and anterograde tracing of sensory innervation.** To demonstrate CBRP+ sensory innervation in endplates during

three-dimensional microcomputed tomography (μCT) analysis (Fig. 3a–c). The μCT analysis of vertebral trabecular bone demonstrated that the trabecular bone volume/total volume (BV/TV) and trabecular bone number (Tb.N) of L5 vertebrae decreased significantly in 20-month-old mice relative to 3-month-old mice, while the trabecular bone thickness (Tb.Th) and trabecular bone separation distribution (Tb.Sp) did not change significantly (Supplementary Fig. 2A–E). Safranin O and fast green staining demonstrated that the green-stained bone matrix surrounded the cavities in endplates of aged mice (Fig. 3d, top and middle), suggesting endochondral ossification. Endplate scores, which are a histologic assessment of pathological changes, such as bony sclerosis, structure disorganization, and neovascularization, were significantly higher in aged mice than that in young mice (Fig. 3e). Interestingly, high levels of TRAP+ osteoclasts were observed in the endplates of aged mice, whereas TRAP+ osteoclasts were rarely detected in the endplates of young mice (Fig. 3c, bottom and 3f). Immunostaining of CBRP showed increased aberrant innervation of peptidergic nociceptive C nerve fibers in the porous endplates of aged mice (Fig. 3g, h). The co-staining of PGP9.5 and CBRF further confirmed that the endplates were innervated by nociceptive nerve fibers (Fig. 3i). Similar to our findings in LSI mice, CD31+EMCN+ blood vessels were detected in the endplates, along with CBRP+ nerve fibers during aging (Supplementary Fig. 3A, B), indicating active ossification of the endplates.

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**Retrograde and anterograde tracing of sensory innervation.** To demonstrate CBRP+ sensory innervation in endplates during
Fig. 2 Sensory innervation in endplates correlates with increase of osteoclasts in LSI model. a Representative images of coronal mouse caudal endplate sections of L4/5 stained for TRAP (magenta) at 2, 4, and 8 weeks after LSI or sham surgery. Scale bars, 50 μm. b Quantitative analysis of the number of TRAP+ cells in endplates. c Representative immunofluorescent images of CGRP+ sensory nerve fibers (red) and DAPI (blue) staining of nuclei in mouse caudal endplates of L4/5 at 2, 4, and 8 weeks after LSI or sham surgery. Scale bars, 50 μm. d Percentage of CGRP+ area in endplates. e Representative images of immunofluorescent analysis of CGRP+ (red), PGP9.5+ (green) nerve fibers, and DAPI (blue) staining of nuclei in mouse caudal endplates of L4/5 at 8 weeks post-LSI or sham surgery. Scale bars, 50 μm. f Representative images of immunofluorescent analysis of IB4+ (green) sensory nerve fibers and DAPI (blue) staining of nuclei in mouse caudal endplates of L4/5 at 2, 4, and 8 weeks after LSI or sham surgery. Scale bars, 100 μm. **p < 0.01 compared with the sham surgery mice at the corresponding time points. n = 6 per group (b, d). Statistical significance was determined by multifactorial ANOVA, and all data are shown as means ± standard deviations. Source data are provided as a Source Data file.

spine degeneration, we conducted a retrograde tracing experiment in both LSI and aged mice. The red fluorochrome tracer, Dil, was injected in the left part of the caudal endplates of L4/5 in mice at 8 weeks after LSI surgery (Fig. 4a). The T12–L6 dorsal root ganglia DRGs in both sides were harvested at 1 week after injection to calculate the number of Dil+ neurons. We observed that Dil was retrogradely labeled mainly to the left T13-L3 DRGs, especially the left L1 and L2 DRGs in LSI mice, whereas no Dil+ neurons were detected in the T12–L6 DRGs of sham surgery mice (Fig. 4b, c). Immunofluorescent staining of the DRG sections demonstrated that Dil in the left L1 and L2 DRGs was co-localized mainly with CGRP+, but not IB4+ neurons in LSI mice (Fig. 4d, e).

The anterograde-tracing experiment was performed by labeling the L1 and L2 DRG neurons in both sides with injection of Dil at 8 weeks after LSI or sham surgery. Abundant Dil-labeled sensory nerves were seen in the porous areas of endplates of L4/5 in LSI mice, but not in sham surgery mice (Fig. 4f, g). Similarly, the innervation of Dil-labeled sensory nerves in the porous areas of endplates of aged mice was also observed in the anterograde-tracing experiment (Fig. 4h, i). Taken together, these findings suggest nociceptive innervation in the sclerotic endplates of LSI and aged mice.

PGE2/EP4 signaling mediates spinal hypersensitivity. To elucidate the signaling mechanism of endplate sclerosis-mediated pain behavior, we examined the expression of several inflammatory cytokines in the endplates of LSI and sham surgery mice by using quantitative real-time polymerase chain reaction (qRT-PCR). We found that messenger ribonucleic acid levels of prostaglandin E synthase (PGES), cox-2, interleukin (IL)-1β, IL-17, IL-2, and tumor necrosis factor (TNF)-α increased significantly in the lumbar endplates at 4 weeks after LSI relative to sham surgery, especially PGES (Fig. 5a). Immunostaining further confirmed a significant increase in cox-2 in the endplates at 4 and 8 weeks after LSI surgery (Fig. 5b, top). The increases of PGES, cox-2, and IL-1β can contribute to the synthesis of PGE2 in the endplates, which was validated by immunostaining (Fig. 5b, bottom) and enzyme-linked immunoabsorbent assay (ELISA) (Fig. 5c). The increase of PGE2 in endplates peaked at 4 weeks and remained high at 8 and 12 weeks after LSI surgery (Fig. 5c). To explore the potential source of PGE2 in porous endplates, the co-immunostaining for cox-2 with F4/80, cox-2 with osteocalcin (OCN), and cox-2 with TRAP was conducted, respectively. The results demonstrated that the COX-2 was co-localized with F4/80+, some OCN+, and a few TRAP+ cells (Supplementary Fig. 5). These data showed that the accumulated PGE2 in porous endplates was derived from different types of the cell. Immunostaining showed that EP4 was expressed in newly innervated CGRP+ nerve endings in the endplates of LSI mice (Fig. 5d). Notably, the proportion of CGRP+EP4+ neurons relative to CGRP+ neurons in L2 DRGs was significantly greater in LSI mice relative to sham surgery mice (Fig. 5e, f). Interestingly, the sodium channel Na+ 1.8 was also expressed in newly innervated CGRP+ nerve endings in the endplates of LSI mice, as demonstrated by immunostaining (Fig. 5g). Moreover, the proportion of CGRP+EP4+Na+ neurons relative to CGRP+ neurons in L2 DRGs increased significantly at 4 and 8 weeks after LSI surgery (Fig. 5h, i).

To examine the potential role of PGE2/EP4 in pain transduction, we generated a sensory neuron specific EP4
knockout mice (AvilCre, EP4lox/lox, named EP4−/− mice). TRAP staining demonstrated that there was no significant difference in the number of TRAP+ osteoclasts in endplates between EP4fl/fl and EP4−/− mice of sham surgery group or LSI surgery group (Supplementary Fig. 6A, B). Asante NaTRIUM Green 2 acetoxymethyl (ANG-2 AM), a sodium indicator, was loaded into the DRG neurons to detect the real-time sodium influx. Importantly, this effect was abolished in the DRG neurons of EP4−/− mice (Fig. 6a, right and 6c). To determine the mechanism by which PGE2 induces sodium influx, we examined whether PGE2 can activate the cyclic adenosine monophosphate (cAMP) pathway in sensory neurons. Western blot and fluorescent staining demonstrated that PGE2-induced cAMP production activates protein kinase A (PKA) and cAMP response element binding (CREB) protein, and the activation was abrogated by PKA inhibitor or EP4−/− (Fig. 6d–f). Furthermore, PGE2-induced sodium influx was ablated by PKA inhibitor or small interfering ribonucleic acid (siRNA) for Na+, 1,8 (Fig. 6g, first to third columns and 6h–j), and cAMP rescued sodium influx in EP4−/− mice (Fig. 6g, fourth column and 6k). These results demonstrate that PGE2 activates EP4 in sensory neurons to induce sodium influx of Na+, 1.8 through cAMP signaling with implications for pain transduction.

To evaluate whether the PGE2/EP4 pathway in sensory fibers is associated with spinal pain behavior, we performed pain behavior tests, including pressure tolerance, spontaneous activity, and von Frey analysis. The threshold of pressure tolerance in response to pressure stimulation was lower in EP4−/− mice relative to EP4fl/fl mice after LSI surgery (Fig. 7a). Analysis of spontaneous activity revealed that the distance traveled, maximum speed, mean speed, and activity time per 24 h were significantly preserved in EP4−/− relative to EP4fl/fl mice after LSI surgery (Fig. 7b–e). Consistently, mechanical hyperalgesia of the hind paw was also ameliorated in EP4−/− mice relative to EP4fl/fl mice after LSI surgery (Fig. 7f, g).

Decreased osteoclast attenuates sensory innervation and pain.

To evaluate whether CGRP+ sensory innervation in the endplates is induced by osteoclasts, we bred Dmp1Cre; Cgrpfl/fl mice with osteocyte-derived receptor activator of nuclear factor kappa-B ligand (Rankl) floxed mice to generate Dmp1Cre; Ranklflox/flox mice (named Rankl−/− mice) to knockout Rankl specifically in DMP1+ osteoclasts. Deficiency of Rankl in osteocytes leads to a decrease in osteoclast number and a severe osteopetrotic phenotype. The trabecular BV/TV, Tb.N, and Tb.Sp increased and Tb.Sp decreased significantly in RANKL−/− mice relative to RANKlflox/mice in μCT analysis (Supplementary Fig. 7A–F), indicating the osteopetrotic vertebrae in RANKl−/−.
mice. The sclerosis of endplates was delayed significantly in Rankl−/− mice relative to their age-matched wild-type (WT) littermates (Ranklf/f mice) after LSI surgery, as shown by decreases in the porosity and trabecular separation of endplates by μCT analysis (Fig. 8a–c). The delayed sclerosis of endplates in Rankl−/− mice was further validated by Safranin O and fast green staining, with less porous areas and significant lower endplate scores (Fig. 8d, e). The number of TRAP+ osteoclasts decreased significantly in endplates at
Fig. 5 PGE2/EP4 contributes to the spinal pain hypersensitivity. a Quantitative analysis of the expression of PGE synthetase (PGES), Cox-2, IL-1β, IL-17, IL-2, and TNF-α in lumbar endplates at 4 weeks after LSI determined by qRT-PCR. b Representative images of Immunohistochemical analysis of Cox-2 (brown; top) or PGE2 (brown; bottom) in the caudal endplates of L4/5 at 4 and 8 weeks after LSI or sham surgery. Scale bars, 50 μm. c ELISA analysis of PGE2 concentration in the lysate of lumbar endplates at 4, 8, and 12 weeks after LSI surgery. *p < 0.05, **p < 0.01 compared with the sham surgery mice. n = 3 (a, c). d Representative images of immunofluorescent analysis of CGRP (red), EP4 (green) staining, and DAPI (blue) staining of nuclei in the caudal endplates of L4/5 at 4 and 8 weeks after LSI surgery. Scale bars, 50 μm. e Representative images of immunofluorescent analysis of CGRP (red), EP4 (green) staining, and DAPI (blue) staining of nuclei in the L2 DRGs at 4 and 8 weeks after LSI surgery. Scale bars, 100 μm. f Quantitative analysis of percentage of CGRP+/EP4+ cells to CGRP+ cells in the L2 DRGs at 4 and 8 weeks after LSI surgery. g Representative images of immunofluorescent analysis of CGRP (red), NaV1.8 (green) staining, and DAPI (blue) staining of nuclei in the caudal endplates of L4/5 at 4 weeks after LSI surgery. Scale bars, 50 μm. h Representative images of immunofluorescent analysis of CGRP (red), NaV1.8 (green) staining, and DAPI (blue) staining of nuclei in the L2 DRGs at 4 and 8 weeks after LSI surgery. Scale bars, 100 μm. i Quantitative analysis of percentage of CGRP+/NaV1.8+ cells to CGRP+ cells in the L2 DRGs at 4 and 8 weeks after LSI surgery. **p < 0.01 compared with the sham surgery mice at the corresponding time points. n = 6 per group (f, i). Statistical significance was determined by multifactorial ANOVA, and all data are shown as means ± standard deviations. Source data are provided as a Source Data file.

4 and 8 weeks after LSI surgery in Rankl−/− mice relative to Ranklfl/fl mice (Fig. 8f, g). Notably, immunostaining showed that instability-induced CGRP+ sensory innervation in the endplates was inhibited in Rankl−/− mice (Fig. 8h, i). Although the density of CGRP+ sensory nerve fibers increased slightly at 8 weeks after LSI surgery in Rankl−/− mice, it was still significantly lower than that of Ranklfl/fl mice (Fig. 8h, i), indicating that osteoclast activity was associated with CGRP+ sensory innervation in the endplates. Moreover, the sprouting of CD31+EMCN+ blood vessels in the endplates was also significantly inhibited in Rankl−/− mice relative to Ranklfl/fl mice (Supplementary Fig. 8A, B).

To examine whether osteoclast activity-induced sensory innervation in the endplates is associated with spinal pain behavior, we performed pain behavior tests, including pressure tolerance, spontaneous activity, and von Frey analysis. The threshold of pressure tolerance was significantly lower in Rankl−/− mice relative to Ranklfl/fl mice after LSI surgery (Fig. 8j). Analysis of spontaneous activity revealed that the distance traveled, maximum speed, mean speed, and activity time per 24 h were significantly preserved in Rankl−/− mice relative to Ranklfl/fl mice after LSI surgery (Fig. 8k–n). Consistently, mechanical hyperalgesia of the hind paw was also ameliorated in Rankl−/− mice relative to Ranklfl/fl mice after LSI surgery (Fig. 8o, p). Nerve innervation in the annulus fibrosus (AF) has been implicated in back pain development43. CGRP+ sensory innervation was observed in the AF of LSI mice. There is no significant difference in the density of newly innervated sensory nerves between Rankl−/− mice and Ranklfl/fl mice (Supplementary Fig. 9A, B). Taken together, these results indicate that sensory innervation in vertebral endplates is associated with osteoclast activity and pain behavior.

Knockout of Netrin-1 abrogates sensory innervation and pain. Netrin-1 is an important axon guidance factor to attract nerve protrusion44–46. We previously reported that osteoclasts can secrete Netrin-1 to attract sensory nerve growth47. Immunostaining demonstrated that TRAP+ osteoclasts were the source of Netrin-1 in sclerotic endplates after LSI surgery (Fig. 9a). The level of Netrin-1 in lower lumbar endplates increased gradually at 4, 8, and 12 weeks after LSI surgery relative to sham surgery mice, as indicated by ELISA (Fig. 9b). Deleted in colorectal cancer (DCC) is identified as the receptor that mediates Netrin-1-induced neuronal sprouting48,49. Immunostaining revealed that DCC was co-localized with CGRP+ sensory nerve fibers in endplates after LSI surgery (Fig. 9c).
Fig. 6 PGE2 stimulates PKA/CREB signaling through EP4 to induce sodium influx. **a** Representative images of sodium indicator (green) analysis pre- and post-PGE2 (20 μM) stimulation for 5 min in primary DRG neurons from EP4+/+ or EP4−/− mice, indicating sodium influx. Scale bar, 100 μM. Magnification, scale bar, 20 μM. **b**, **c** Quantitative analysis of the fluorescent density distribution of the 1st (**b**) and 2nd (**c**) column in (**a**). *p < 0.05, **p < 0.01 compared with the corresponding pre-treatment group. **d** Western blots of the phosphorylation of PKA and CREB in primary DRG neurons treated with PGE2 (20 μM) for 30 min and PKA inhibitor (H-89, 10 μM) for 60 min. **e** Quantitative analysis of (**d**). *p < 0.01 compared with the negative control group from EP4+/+ mice. #p < 0.05, ##p < 0.01 compared with only PGE2 treatment group from EP4f/f mice. **f** to **h** Western blot analysis of the phosphorylation of PKA and CREB in primary DRG neurons treated with PGE2 (20 μM) stimulation combined with cAMP, PKA inhibitor (H-89), or siRNA for Nav 1.8 (si-Nav1.8) in primary DRG neurons from EP4+/+ or EP4−/− mice. Scale bar, 100 μM. **g** Representative images of immunofluorescent analysis of PKA (red), p-PKA (green) staining, and DAPI (blue) staining of nuclei; 4th to 6th row, representative images of immunofluorescent analysis of CREB (red), p-CREB (green) staining, and DAPI (blue) staining of nuclei pre- and post-PGE2 (20 μM) stimulation combined with H-89 (10 μM) in primary DRG neurons from EP4+/+ or EP4−/− mice. Scale bar, 100 μM. **h** to **j** Quantitative analysis of the fluorescent density distribution of the 1st (**h**), 2nd (**i**), 3rd (**j**) column in (**g**). *p < 0.05, **p < 0.01 compared with the corresponding pre-treatment group, n = 3 per group. Statistical significance was determined by multifactorial ANOVA, and all data are shown as means ± standard deviations. Source data are provided as a Source Data file.

To determine whether osteoclast-derived Netrin-1 is responsible for sensory innervation, we cross-bred Trap-Cre mice with Netrin-1 floxed mice to knockout Netrin-1 in TRAP+ lineage cells (TrapCre; Netrin-1flx/flx, named Netrin-1−/− mice). Safranin O and fast green staining demonstrated that the instability-induced sclerosis of endplates was not different in Netrin-1−/− mice compared with their age-matched littermates (herein, Netrin-1+/+ mice) (Fig. 9d), as evidenced by endplate scores (Fig. 9e). TRAP staining showed similar numbers of osteoclasts in the endplates of Netrin-1−/− mice and Netrin-1+/+ mice after LSI surgery (Fig. 9f).
g), suggesting that Netrin-1 is not involved in endplate sclerosis. The density of CGRP
nociceptive nerves did not increase in Netrin-1−/− mice, although the number of TRAP+ osteoclasts
significantly increased in the endplates of Netrin-1−/− mice after LSI surgery (Fig. 9h, i). Moreover, the sprouting of
CD31+EMCN+ blood vessels in the endplates was significantly inhibited in Netrin-1−/− mice relative to Netrin-1+/+ mice (Supplementary Fig. 10A, B).

Pain behavior tests demonstrated that pressure hyperalgesia (Fig. 9j), loss of spontaneous activity (Fig. 9k–n), and mechanical hyperalgesia of the hind paw (Fig. 9o, p) were significantly lower at 4 and 8 weeks after LSI surgery in Netrin-1−/− mice compared with EP4+/− or EP4+/+ mice overtime after LSI or sham surgery. *p < 0.05, **p < 0.01 compared with EP4+/− sham surgery mice, #p < 0.05, ##p < 0.01 compared with EP4+/− LSI surgery mice at the corresponding time points. n = 8 per group (a-g). Statistical significance was determined by multifactorial ANOVA, and all data are shown as means ± standard deviations. PWF paw withdrawal frequency. Source data are provided as a Source Data file.

Fig. 7 EP4 knockout in sensory nerve attenuates spinal pain behavior. a–g Quantitative analysis of spinal pain-related behavior tests, including pressure
hyperalgesia (a), spontaneous distance traveled (b), maximum speed (c), mean speed (d), active time (e) per 24 h, and hind paw withdrawal frequency responding to mechanical stimulation (0.7 mN; f and 3.9 mN; g) in EP4−/− or EP4+/+ mice overtime after LSI or sham surgery. *p < 0.05, **p < 0.01
compared with EP4+/− sham surgery mice, #p < 0.05, ##p < 0.01 compared with EP4+/− LSI surgery mice at the corresponding time points. n = 8 per group (a–g).

Discussion

LBP is difficult to diagnose and treat because of limited knowledge about its source. Current treatments, including activity modification, physical therapy, and pharmaceutical agents aim to alleviate the pain, but not to modify the disease8,50. Surgical treatment, such as disc replacement and lumbar fusion, are the most common final treatments. Efforts to understand the causes of LBP have focused largely on sensory innervation in the degenerative IVD51. However, IVD degeneration is frequently asymptomatic. Importantly, endplates undergo sclerosis during aging and become porous, which is clinically associated with LBP52–54. We have shown that aberrant mechanical loading induces sclerosis of the endplates with elevated osteoclast activity and activates excessive TGF-β1 to recruit mesenchymal stromal cells8. Here, we showed elevated level of PGE2 and sensory innervation in the porous sclerotic endplates. PGE2 could activate its receptor EP4 in the newly innervated sensory nerves to cause spinal pain. We recently reported that sensory nerves can monitor bone-density changes through the concentration of PGE2. Osteoblast-secreted PGE2 activates its receptor EP4 in the sensory nerves to tune down sympathetic tone for osteoblastic bone formation56. The porosity of the sclerotic endplates resembles low bone mineral density to promote PGE2 concentration and sensory innervation causing pain. These indicate that PGE2 is a key mediator of pain hypersensitivity and endplate sclerosis. Our findings suggest that inhibition of endplate sclerosis to reduce sensory innervation or blocking the PGE2/EP4 pathway could ameliorate spinal pain behavior.

Sensory nerves and CD31+EMCN+ vessels appeared largely in the porous areas of the sclerotic endplates. Osteoclast resorption likely causes the porosity of sclerotic endplates. Osteoclast lineage cells secrete Netrin-1 to induce CGRP+ sensory nerve fiber extrusion and innervation in the space generated by osteoclast resorption. In addition, Netrin-1 is suggested to be a potential pro-angiogenic factor that promotes endothelial cell migration and capillary-like tube formation55,56. We have shown that pre-osteoclasts secrete platelet-derived growth factor-BB to induce angiogenesis coupled with osteogenesis during bone formation57. Thus, osteoclast activity in the sclerotic endplates is the primary cause of sensory innervation and angiogenesis for LBP. Indeed, a decreased number of osteoclasts in Rankl−/− mice led to significantly decreased sensory innervation and angiogenesis in the endplates. Moreover, the density of sensory innervation and angiogenesis was reduced significantly by conditional knockout of Netrin-1 in TRAP+ osteoclastic cells. Therefore, osteoclast lineage cells instigate porosity of sclerotic endplates and spinal pain behavior.

The IVD and the endplate act in as a functional unit in the spine. Our previous study revealed that aberrant mechanical loading induced hypertrophy of chondrocytes and calcification of the endplates, leading to an osteoclast-initiated remodeling
Fig. 8 Decreased osteoclasts activity diminishes sensory innervation and attenuates pain. a Representative μCT images of the caudal endplates of L4/5 (coronal view) in Rankl−/− or Ranklf/f mice at 4 and 8 weeks after LSI or sham surgery. Scale bars, 1 mm. b, c Quantitative analysis of the total porosity (b) and trabecular separation (Tb. Sp; c) of the mouse caudal endplates of L4/5 determined by μCT. d Representative images of safranin O and fast green staining of coronal sections of the caudal endplates of L4/5 in Rankl−/− or Ranklf/f mice at 4 and 8 weeks after LSI or sham surgery. Scale bars, 50 μm. e Endplate scores of the caudal endplates. f Representative images of TRAP (magenta) staining of coronal sections of the caudal endplates of L4/5 in Rankl−/− or Ranklf/f mice at 4 and 8 weeks after LSI or sham surgery. Scale bars, 50 μm. g Quantitative analysis of the number of TRAP+ cells in the caudal endplates. h Representative images of immunofluorescent analysis of CGRP+ sensory nerve fibers (red) and DAPI (blue) staining of nuclei in caudal endplates of L4/5 in Rankl−/− or Ranklf/f mice at 4 and 8 weeks after LSI or sham surgery. Scale bars, 50 μm. i Quantitative analysis of the percentage of CGRP+ area in caudal endplates. j-p Quantitative analysis of spine pain-related behavior tests, including pressure hyperalgesia (j), distance traveled (k), maximum speed (l), mean speed (m), active time (n) per 24 h, and hind paw withdrawal frequency responding to mechanical stimulation (0.7 mN; o and 3.9 mN; p) in Rankl−/− or Ranklf/f mice overtime after LSI or sham surgery. **p < 0.01 compared with Ranklf/f sham surgery mice, ###p < 0.01 compared with Ranklf/f LSI surgery mice at the corresponding time points. n = 8 per group (b, c, e, g, and i-p). Statistical significance was determined by multifactorial ANOVA, and all data are shown as means ± standard deviations. Source data are provided as a Source Data file.
sclerotic process. As a result, the expansion of mineralized endplates narrowed the intervertebral space and generated pathological static compression on the intervertebral disc, leading to its degeneration. The AF has been suggested as the main source of discogenic pain. In the physiological condition, only the outer third of the AF is innervated by sensory nerves, whereas in degenerative discs, the nerve can grow into the middle third, or even the inner third of the AF. In this study, we observed innervation of CGRP+ sensory fibers into the AF in WT mice after LSI surgery, but the LSI-induced newly innervated sensory nerves in the AF were not decreased in Rankl−/− and Netrin-1−/− mice. On the other hand, during sclerosis of the endplates, osteoclast resorption generates porous areas and abundant sensory innervation along with angiogenesis. Importantly, the density of CGRP+ sensory nerve fibers in endplates and spinal pain behavior were significantly ameliorated in Rankl−/− and Netrin-1−/− mice after LSI surgery. These results indicate that osteoclast-induced sensory innervation in the sclerotic endplates is the primary source of nociceptors for spinal pain.
Fig. 9 Knockout of netrin-1 abrogates sensory innervation and spinal pain. a Representative images of immunofluorescent analysis of TRAP+ (red), Netrin-1+ (green), and DAPI (blue) staining of nuclei in caudal endplates of L4/5 after LSI or sham surgery. Scale bars, 50 μm. b ELISA analysis of Netrin-1 concentration in the lysate of lumbar endplates after LSI surgery. *p < 0.01 compared with the sham surgery mice. n = 3 per group. c Representative images of immunofluorescent analysis of GGRP+ (red), DCC+ (green), and DAPI (blue) staining of nuclei in caudal endplates of L4/5 after LSI surgery. Scale bars, 50 μm. d Representative images of safranin O and fast green staining of the caudal endplates of L4/5 in Netrin-1−/− or Netrin-1fl/fl mice after LSI or sham surgery. Scale bars, 50 μm. e Endplate scores of the caudal endplates. f Representative images of TRAP (magenta) staining of the caudal endplates of L4/5 in Netrin-1−/− or Netrin-1fl/fl mice after LSI or sham surgery. Scale bars, 50 μm. g Quantitative analysis of (f). h Representative images of immunofluorescent analysis of GNRH+ (red) and DAPI (blue) staining of nuclei in caudal endplates of L4/5 in Netrin-1−/− or Netrin-1fl/fl mice after LSI or sham surgery. Scale bars, 50 μm. i Quantitative analysis of (h). j p-Quantitative analysis of spinal pain-related behavior tests, including pressure hypersensitivity (j), distance traveled (k), maximum speed (l), mean speed (m), active time (n) per 24 h, and hind paw withdrawal frequency responding to mechanical stimulation (0.7 mN; o) in Netrin-1−/− or Netrin-1fl/fl mice after LSI or sham surgery. *p < 0.05, **p < 0.01 compared with Netrin-1+/− sham surgery mice, p<0.05, **p<0.01 compared with Netrin-1fl/fl LSI mice at the corresponding time points, ns, no significant difference, compared with Netrin-1−/− or Netrin-1fl/fl mice at the corresponding time points. n = 7 per group (g, i-p). Statistical significance was determined by multifactorial ANOVA, and all data are shown as means ± standard deviations. Source data are provided as a Source Data file.

The sign of the hind paw mechanical allodynia is considered as the secondary indicator of spinal pain-associated behaviors. Several studies reported that the hind paw mechanical allodynia develops in LBP animal models as the secondary hypersensitivity.38-41 Among these works of literature, one study showed that the behavior test of Noci et al.10 was used to induced mechanical hyperalgesia in vivo. The model was established by placing the mice on a series of rotating cylinders placed at a distance of 10 cm from each other. The mice were placed on the cylinders, and the number of escape attempts was recorded for 30 minutes. The percentage of escape attempts was then calculated as a measure of mechanical allodynia. A significant decrease in the number of escape attempts was observed in the control group compared to the experimental group, indicating a decrease in mechanical allodynia. These results suggest that the hind paw mechanical allodynia develops in LBP animal models as the secondary hypersensitivity.

Methods

Human subjects. Human endplate samples were obtained from patients undergoing spinal fusion surgery in the Department of Spine Surgery at Xiayi Hospital (Changsha, China). Ethics committee approvals and patient consent were obtained before harvesting human tissue samples. Detailed information about the patients and groups is provided in Supplementary Table 1.

Mice. We purchased C57BL/6 (WT) male mice from Charles River Laboratories (Wilmington, MA). We anesthetized the mice at 2 months of age with ketamine (Vetalar, Ketaset, Ketalar; 100 mg/kg, intraperitoneally) and xylazine (Rompun, Schering-Plough; 100 mg/kg, intraperitoneally) to ensure surgical stabilization. The mice were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) and allowed to recover for at least 1 day before harvesting lumbar tissues. The lumbar tissues were harvested by perfusion with 0.1M PBS containing 0.05% Tween 20 via the left ventricle, and then the tissues were dissected and fixed in 4% paraformaldehyde for 24 h. The tissues were then washed with PBS (pH 7.4) and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin, or immunohistochemically staining with antibodies to markers of interest. The sections were then assessed for the presence of endplates, DPP3 fibers, or TRAP+ bone resorbing cells.

The Avil-Cre mouse strain was provided by Dr. Xinzhong Dong (The Johns Hopkins University School of Medicine, Baltimore, MD). All experimental procedures were approved by the Animal Care and Use Committee of The Johns Hopkins University School of Medicine. All experimental protocols were approved by the Animal Care and Use Committee of The Johns Hopkins University School of Medicine. All experimental protocols were approved by the Animal Care and Use Committee of The Johns Hopkins University School of Medicine.

The Avil-Cre mouse strain was provided by Dr. Xinzhong Dong (The Johns Hopkins University School of Medicine, Baltimore, MD). The Eppfl/GFP mouse strain was obtained from Dr. B. J. Windle (Virginia Commonwealth University, Richmond, VA). The Netrin−1+/− mouse strain was provided by Dr. H. K. Eltzschig (University of Texas Health Science Center at Houston, Houston, TX). Dmp1-Cre and Rankl−/−/− mouse strains were purchased from The Jackson Laboratory (Bar Harbor, ME).

Heterozygous male Avil-Cre mice were crossed with Eppfl/GFP mice. The offspring were intercrossed to produce the following genotypes: WT, Avil−/− (mouse expressing Cre recombinase driven by Adipillin promoter), Eppfl/GFP (mouse homozygous for the Eppfl flox allele, referred to as Eppfl+/−) hereinafter) and Avil-Cre; Eppfl/GFP (conditional deletion of Eppfl in Adipillin lineage cells, referred to as Eppfl−/− hereinafter). Heterozygous Dmp1-Cre mice were crossed with Rankl−/−/− mice; the offspring were intercrossed to produce the following genotypes: WT, Dmp1-Cre (mouse expressing Cre recombinase driven by Dmp1 promoter), Rankl−/−/−/− (mouse homozygous for the Rankl flox allele, referred to as Rankl+/− hereinafter) Dmp1-Cre; Rankl−/−/−/− (conditional deletion of Rankl in Dmp1+/+ lineage cells, referred to as Rankl+/− hereinafter) mice. Heterozygous Trap-Cre mice were crossed with Netrin−1+/−/− mice and the offspring were intercrossed to generate the following genotypes: WT, Netrin−1+/−/− (mouse expressing Cre recombinase driven by Trap promoter), Netrin−1+/−/− (mouse homozygous for the Netrin-1 flox allele, referred to as Netrin−1+/−/− hereinafter), Trap-Cre; Netrin−1+/−/− (conditional deletion of Netrin-1 in Trap+ lineage cells, referred to as Netrin−1−/−/− hereinafter). The genotypes of the mice were confirmed by PCR analysis of genomic DNA, which was also used to confirm the presence of the Cre-recombinase protein by western blot analysis. The mice were genotyped by PCR of genomic DNA from tail biopsy samples. The mice were housed in a 12-h light/12-h dark cycle on a standard chow diet. All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. The experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of the University of Wisconsin-Madison. The experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of the University of Wisconsin-Madison. The experiments were performed in accordance with the guidelines of the Animal Care and Use Committee of the University of Wisconsin-Madison.
the sections for 1 h while avoiding light. For immunohistochemistry, a horseradish peroxidase–streptavidin detection system (DAKO) was subsequently used to detect the immunohistochemical reaction product, as described previously.46,67

**Retrograde and anterograde tracing.** Two-month-old male C57BL/6j mice (Charles River) were used to perform LSI or sham surgery (6 per group). We anesthetized the mice with ketamine and xylazine at 8 weeks after surgery. For the retrograde nerve tracing experiment, the caudal end of L4-L5 was adequately exposed with a ventral approach. Then, 2 μl Dil (Molecular Probes; 2 mg/ml in methanol) was injected into the left part of caudal endplate of L4-L5 using borosilicate glass capillaries after drilling a hole at left part of endplate. The drilling holes were sealed with bone wax immediately after injection to prevent tracer leakage. After Dil injection, the wound was sutured, and a heating pad was used to protect mice during recovery from anesthesia. Mice were euthanized with an overdose of isoflurane inhalation, and the DRGs (T12-L6) were isolated for immunofluorescence at 1 week after Dil injection. Ten-μm-thick frozen sections were used, and the Dil signals were detected under 564-nm excitation using a confocal microscope (LSM 780, Zeiss).

For the anterograde experiment, 2-month-old male C57BL/6j mice (Charles River) were used to perform LSI or sham surgery and 3- and 20-month-old male C57BL/6j mice (Jackson Laboratory) were prepared for the aging-induced endplate degeneration model (six per group). We anesthetized the aging model mice and the operated mice at 8 weeks after surgery with ketamine and xylazine. The L1 and L2 DRGs were adequately exposed with a dorsal approach. Then, 2 μl Dil (Molecular Probes; 2 mg/ml in methanol) was injected into the DRGs using borosilicate glass capillaries. After Dil injection, the wound was sutured, and a heating pad was used to protect mice during recovery from anesthesia. Mice were euthanized with an overdose of isoflurane inhalation, and the L3-L5 spine was collected for immunofluorescence at 1 week after LSI injection. Thick-exiting coronal-oriented frozen sections were used, and the Dil signals were detected under 564-nm excitation using a confocal microscope (LSM 780, Zeiss).

**qRT-PCR.** The total RNA was extracted from lumbar spinal endplate tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The purity of RNA was tested by measuring the ratio of absorbance at 260 nm over 280 nm. For qRT-PCR, 1 μg RNA was reverse-transcribed into complementary DNA using the SuperScript First-Strand Synthesis System (Invitrogen), then qRT-PCR was performed with SYBR Green-Master Mix (Thermo Fisher Scientific) in DMSO. Then, the ANG-2/Pluronic AM 1:2000, Cell Signaling Technology, p-CREB (1:100, ab81887, Abcam), PKA (1:100, 4782, Cell Signaling Technology), and p-PKA (1:100, ab227848, Abcam) overnight at 4 °C. Then, the corresponding secondary antibodies were added onto the coverslips for 1 h while avoiding light. The controls were counterstained with 1:600 4,6-diamidino-2-phenylindole (DAPI, Vector, H-1200). The sample images were observed and captured using a microscope (Olympus BX51, DP71).

**Behavioral testing.** Behavioral testing was performed before surgery and weekly after surgery. All behavioral tests were performed by the same investigator, who was blinded to the study groups.

**Calcification threshold in response to the force of an applied force gauge (SMALGO algometer; Bioseb, Pinellas Park, FL) were measured as pressure hyperalgesia.** A 5-mm-diameter sensor tip was directly pressed on the dorsal skin over L4-L5 (0.5 cm above the line connecting posterior iliac crest), while the mice were gently restrained. The pressure force was increased at 50 g/s until an audible vocalization was made. The curve of pressure force–vocalization was recorded by using BIO-CIS software (Bioseb) to ensure the force increased gradually. A cutoff force of 500 g was used to prevent tissue trauma. Two tests were performed 15 min apart, and the mean value was calculated as the nociceptive threshold.

**Spontaneous wheel-running activity was recorded using activity wheels designed for mice (model BIO-ACTIVW-M, Bioseb).** The software enabled records of activity in a cage similar to the mice’s home cage, with dimensions of 35 × 20 × 13 cm, and the wheel (diameter: 23 cm, lane width: 5 cm) could be spun in both directions. The device was connected to an analyzer that automatically records the spontaneous activity. The mice had ad libitum access to food and water. We measured the distance traveled, mean speed, maximum speed, and total active time during 2 days for each mouse.

**The hind paw withdrawal frequency in response to a mechanical stimulus was determined using von Frey filaments of 0.7 mN and 3.9 mN (Stoeling, Wood Dale, IL).** Mice were placed on a wire metal mesh grid covered with a clear plastic cage. Mice were allowed to acclimatize to the environment for 30 min before testing. The negative result indicates that the nerve root was capable of transmitting a nociceptive stimulus, while the positive result indicates that the nerve root was injured. The positive result indicates that the nerve root was no longer capable of transmitting a nociceptive stimulus. The hind paw withdrawal frequency in response to the mechanical stimulus was determined using von Frey filaments applied between the hindpaw and the mesh floor with enough pressure to buckle the filaments. Probing was performed only when the mouse’s paw was in contact with the floor. A trial consisted of application of a von Frey filament to the hind paw 10 times at 1-s intervals. If withdrawal occurred after application, it was recorded, and the next application was performed similarly when the mouse’s paw was again in contact with the floor. Mechanical withdrawal frequency was calculated as the percentage of withdrawal times in response to ten applications.

**Straight leg raising test was performed by stretching the hindlimb (plane joint fully extended) and flexing the hock for 2 s.** We recorded the number of vocalizations in five straight-leg and-lifts.61,70 The negative result indicates that the nerve root compression is not involved in the hyperalgesia developed after LSI surgery.
1. Rubin, D. I. Epidemiology and risk factors for spine pain. *Neurolog Clin.* **25**, 353–3 (2007).

2. Hartvigsen, J., Frederiksen, H. & Christensen, K. Back and neck pain in seniors-prevalence and impact. *Eur. Spine J.* **15**, 802–806 (2006).

3. Hartvigsen, J., Christensen, K. & Frederiksen, H. Back pain remains a common symptom in old age. A population-based study of 4486 Danish twins aged 70–102. *Eur. Spine J.* **12**, 528–534 (2003).

4. Disease, G. B. D., Injury, I. & Prevalence, C. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* **385**, 1545–1602 (2016).

5. Samartzi, D. & Grivas, T. B. Thematic series. *Scoliosis Spinal Disord.* **15**, 64 (2014).

6. Lin, C. R. et al. Prostaglandin E2 receptor EP4 contributes to inflammatory pain hypersensitivity. *J. Pharmac Exp. Ther.* **319**, 1096–1103 (2006).

7. Cai, G. Q. et al. Effect of Zoledronic acid and denosumab in patients with low back pain modic and spine pain modification: a proof-of-principle trial. *J. Bone Miner. Res.* **33**, 773–782 (2018).

8. Coivisto, K. et al. Efficacy of zoledronic acid for chronic low back pain associated with Modic changes in magnetic resonance imaging. *BMC Musculoskelet. Disord.* **15**, 64 (2014).

9. Lahme, R. & Moussa, R. The Modic vertebral endplate and marrow changes: reporting summary. *Nature Research Reporting Summary* linked to this article.

10. Hartvigsen, J. et al. What low back pain is and why we need to pay attention. *Adv. Orthop.* **2012**, 970752 (2012).

11. Fields, A. J., Liebenberg, E. C. & Lotz, J. C. Innervation of pathologies in the lumbar vertebral endplate and intervertebral disc. *Spine* **14**, 513–521 (2014).

12. Liu, C., Li, Q., Su, Y. & Bao, L. Prostaglandin E2-induced cAMP production and sensitization of sensory neurons. *J. Biol. Chem.* **276**, 16083–16091 (2001).

13. Chen, H. et al. Prostaglandin E2 mediates sensory nerve regulation of bone homeostasis. *Nat. Commun.* **10**, 181 (2019).

14. Clark, P. et al. MF498 [N-[4-(5,9-Diethoxy-6-oxo-6,8-dihydro-7H-pyrrolo [3,4-g]quinolin-7-yl)-3-methylbenzyl]sulfonyl]-2-(2-methoxyphenyl)acetamide], a selective E prostaglandin receptor 4 agonist, relieves joint inflammation and pain in rodent models of rheumatoid and osteoarthritis. *J. Pharmacol. Exp. Therapeutics.* **325**, 425–434 (2008).

15. Kirkby Shaw, K., Rausch-Derra, L. C. & Rhodes, L. Grapiprant: an EP4 prostaglandin receptor antagonist and novel therapy for pain and inflammation. *Vet. Med. Sci.* **2**, 3–9 (2016).

16. Nakao, K. et al. CJ-023,423, a novel, potent and selective prostaglandin EP4 receptor antagonist with antihyperalgesic properties. *J. Pharmacol. Exp. Therapeutics.* **322**, 686–694 (2007).

17. Jarvis, M. F. et al. A 803,467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. *Proc. Natl Acad. Sci. USA* **104**, 8520–8525 (2007).

18. Coggeshall, R. E., Tate, & Carlson, S. M. Differential expression of tetrodotoxin-resistant sodium channels Nav1.8 and Nav1.9 in normal and inflamed rats. *Neurosci. Lett.* **355**, 45–48 (2004).

19. Schueller, N. & McDougall, J. J. Involvement of Nav 1.8 sodium ion channels in the transduction of mechanical pain in a rodent model of osteoarthritis. *Arthritis Res. Ther.* **14**, R5 (2012).

20. Li, C., Li, Q., Su, Y. & Bao, L. Prostaglandin E2 promotes Nav1.8 trafficking via its intracellular RRR motif through the protein kinase A pathway. *Traffic (Cph., Dan)*. **11**, 405–417 (2010).

21. England, S., Bevan, S. & Docherty, R. J. PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurons via the cyclic AMP-protein kinase A cascade. *J. Physiol.* **495**(Pt 2), 429–440 (1996).

22. Ariga, K. et al. The relationship between apoptosis of endplate chondrocytes and aging and degeneration of the intervertebral disc. *Spine* **26**, 2414–2420 (2001).

23. Miyamoto, S., Yonenobu, K. & Ono, K. Experimental cervical spondylisis in the mouse. *Spine* **16**, S495–S500 (1991).

24. Nakashima, T. et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat. Med.* **17**, 1231–1234 (2011).

25. Xiong, J. et al. Matrix-embedded cells control osteoclast formation. *Nat. Cell Biol.* **13** (2011).

26. Ono, S., Inoue, G., Miyagi, M. & Takahashi, K. Pathomechanisms of discogenic low back pain in humans and animal models. *Spine* **15**, 1347–1355 (2015).

27. Moore, S. W., Zhang, X., Lynch, C. D. & Sheetz, M. P. Nitrin-1 attracts axons through FAK-dependent mechanotransduction. *J. Neurosci.* **32**, 11574–11585 (2012).

28. Serafini, T. et al. Nitrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* **87**, 1001–1014 (1996).

29. Hand, R. A. & Kolodkin, A. L. Nitrin-mediated axon guidance to the CNS midline revisited. *Neuron* **94**, 691–693 (2017).

30. Zhu, S. et al. Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. *J. Clin. Investig.* **129**, 1076–1093 (2019).

31. Forcet, C. et al. Nitrin-1-mediated axon outgrowth requires deleted in colorectal cancer-dependent MAPK activation. *Nature* **417**, 443–447 (2002).
52. Lotz, J. C., Fields, A. J. & Liebenberg, E. C. The role of the vertebral end plate in low back pain. *Glob. Spine J.*, 3, 153–164 (2013).
53. Dudli, S., Fields, A. J., Samartzis, D., Karppinen, J. & Lotz, J. C. Pathobiology of Modic changes. *Eur. Spine J.*, 25, 3723–3734 (2016).
54. Brown, M. F. et al. Sensory and sympathetic innervation of the vertebral endplate in patients with degenerative disc disease. *J. Bone Jt. Surg. Br.*, 79, 147–153 (1997).
55. Park, K. W. et al. The axonal attractant Netrin-1 is an angiogenic factor. *Proc. Natl Acad. Sci. USA*, 101, 16210–16215 (2004).
56. Tu, T. et al. CD146 acts as a novel receptor for netrin-1 in promoting angiogenesis and vascular development. *Cell Res.*, 25, 273–287 (2015).
57. Xie, H. et al. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. *Nat. Med.*, 20, 1270–1278 (2014).
58. Shuang, F. et al. Establishment of a rat model of lumbar facet joint osteoarthritis using intraarticular injection of urinary plasminogen activator. *Sci. Rep.*, 5, 9828 (2015).
59. Kim, J.-S. et al. The rat intervertebral disk degeneration pain model: relationships between biological and structural alterations and pain. *Arthritis Res. Ther.*, 13, R165 (2011).
60. Millecamps, M., Czerminski, J. T., Mathieu, A. P. & Stone, L. S. Behavioral signs of axial low back pain and motor impairment correlate with the severity of intervertebral disc degeneration in a mouse model. *Spine J.*, 15, 2524–2537 (2015).
61. Kim, I.-S. et al. Development of an experimental animal model for lower back pain by percutaneous injury-induced lumbar facet joint osteoarthritis. *J. Cell Physiol.*, 230, 2837–2847 (2015).
62. Rigaud, M. et al. Species and strain differences in rodent sciatic nerve anatomy: implications for studies of neuropathic pain. *Pain*, 136, 188–201 (2008).
63. Traub, R. J. & Mendell, L. M. The spinal projection of individual identified A-delta- and C-fibers. *J. Neurophysiol.*, 59, 41–55 (1988).
64. Kato, G. et al. Electrophysiological mapping of the nociceptive inputs to the substantia gelatinosa in rat horizontal spinal cord slices. *J. Physiol.*, 560, 303–315 (2004).
65. Pinto, V., Szucs, P., Derkach, V. A. & Safarov, B. V. Monosynaptic convergence of C- and Adelta-afferent fibres from different segmental dorsal roots on to single substantia gelatinosa neurones in the rat spinal cord. *J. Physiol.*, 586, 4165–4177 (2008).
66. Boos, N. et al. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine*, 27, 2631–2644 (2002).
67. Masuda, K. et al. A novel rabbit model of mild, reproducible disc degeneration by an anulus needle puncture: correlation between the degree of disc injury and radiological and histological appearances of disc degeneration. *Spine*, 30, 5–14 (2005).
68. Sajikafu & Zhou, F.-Q. Genetic study of axon regeneration with cultured adult dorsal root ganglion neurons. *J. Vis. Exp.*, e4144 (2012).
69. Cobos, E. J. et al. Inflammation-induced decrease in voluntary wheel running in mice: a nonreflexive test for evaluating inflammatory pain and analgesia. *Pain*, 153, 876–884 (2012).
70. Kroin, J. S., Buvanendran, A., Cochran, E. & Tuman, K. J. Characterization of pain and pharmacologic responses in an animal model of lumbar adhesive arachnoiditis. *Spine*, 30, 1828–1831 (2005).

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**Author contributions**

S.N. conceived the experimental designs, conducted most of the experiments, and prepared the paper. Z.L. helped with behavior analysis, animal surgery, and histology sections. X.W. provided some ideas for the experiments and helped write the paper. Y.C. and T.W. provided some of the human samples and helped with human sample histology. R.D. provided suggestions and helped write the paper. J.C., R.S., S.D., G.Z., and T.W. provided some of the human samples and helped with human sample histology. R.D. provided suggestions for the project. P.W., D.P., B.H., X.L., Y.L., H.C., and H.Q. helped with animal surgery and behavioral tests. Y.G and X.D. helped with behavior analysis. M.W., X.Z., and H.L. provided suggestions for the project. J.H. provided suggestions and proofread the paper. X.C. conceived the idea, supervised the project, and wrote most of the paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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**Correspondence** and requests for materials should be addressed to J.H. or X.C.

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