Supplementary Information

IGFBPL1 in DRG Nociceptors Drives Neuropathic Pain and Neuroimmune Crosstalk via IGF1R–ERK Signaling

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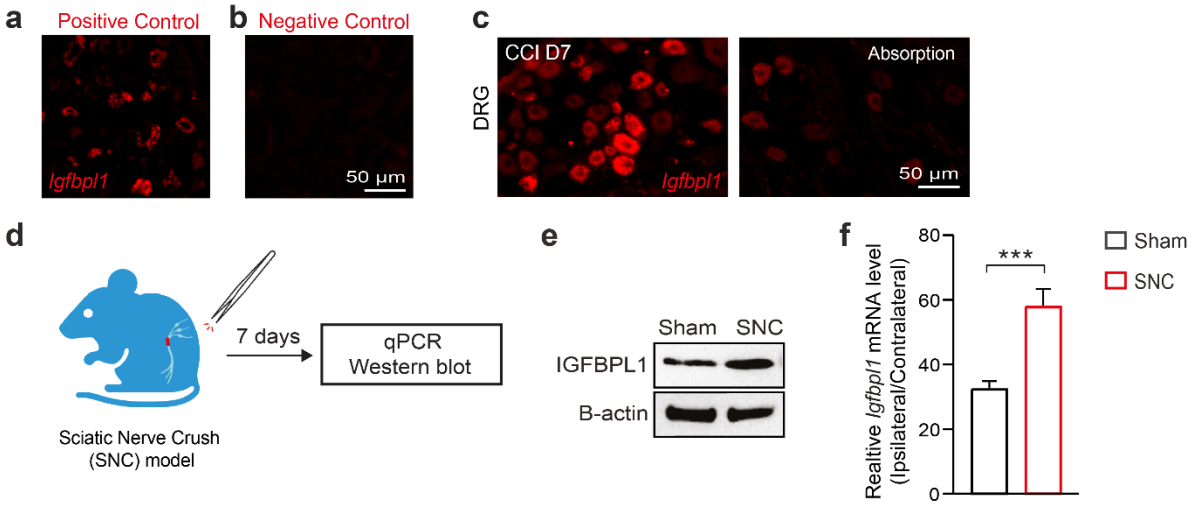
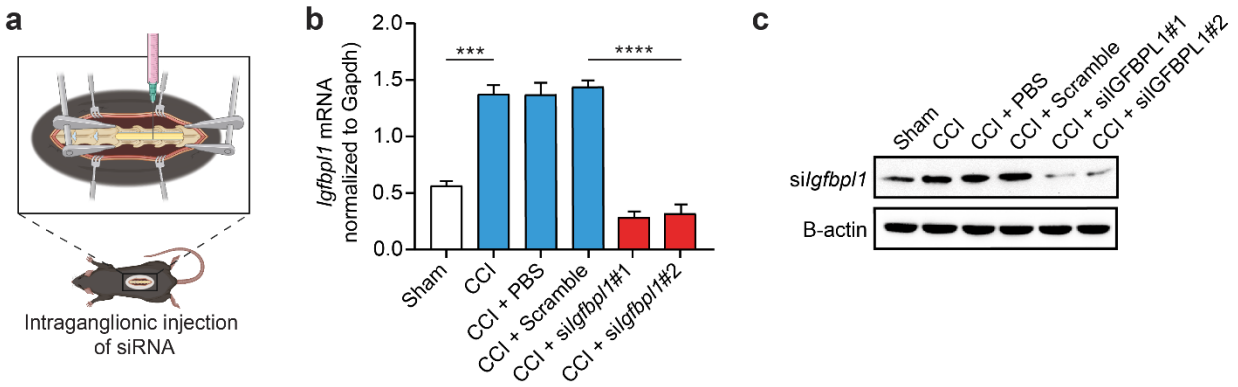
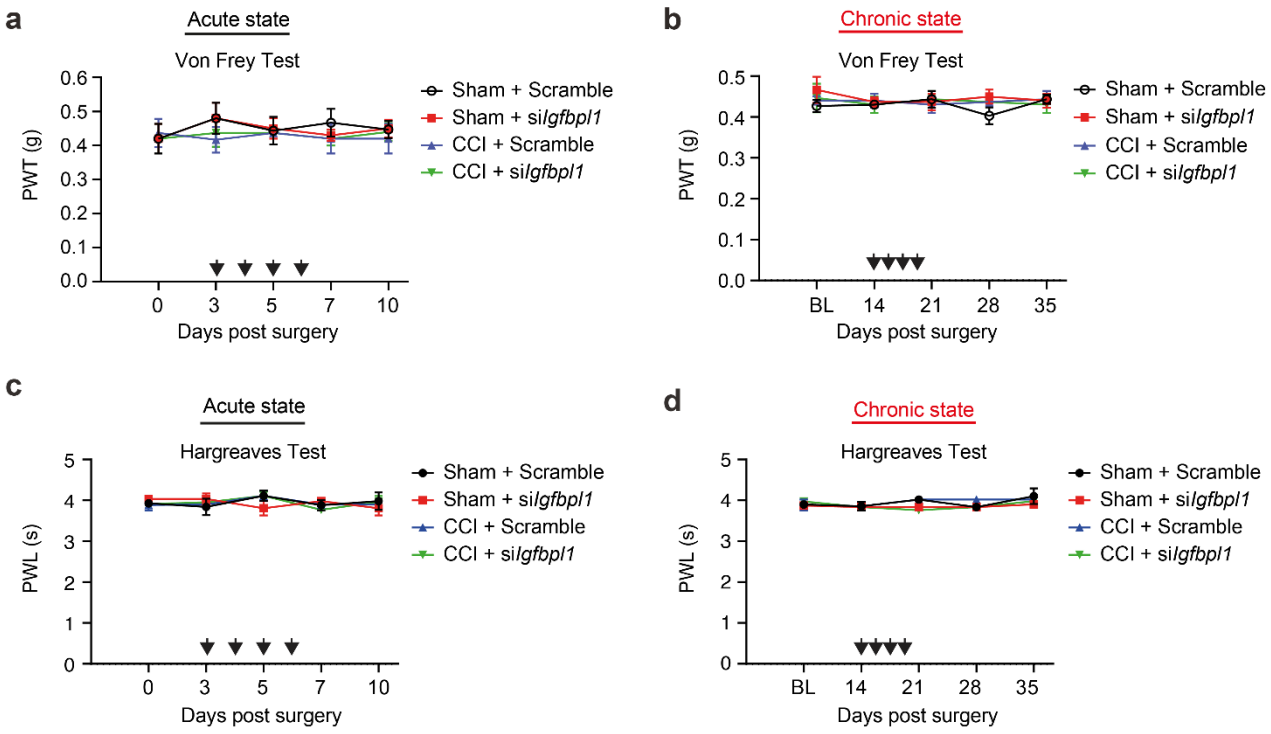


Supplementary Fig. 1: The specificity of *Igfbpl1* probe and antibody, and the upregulation of *Igfbpl1* mRNA and IGFBPL1 protein in Sciatic nerve crush (SNC). (**a**,**b**) RNAscope ISH of DRG sections of adult mice with the positive (**a**) and negative control (**b**). **c** The staining of DRG with *Igfbpl1* antibody without (left) or with (right) absorption with *Igfbpl1* peptide in Sham (**c**) or CCI mice (**d**) 7-day post-CCI surgery. $n = 3$ mice per group. Scale bar, 50 μm. **d**–**f**) Schematic representation of Sciatic Nerve Crush (SNC) model, where the sciatic nerve of the animal was subjected to crush injury. Tissue samples were collected on day 7 post-injury and analyzed by Western blot (**d**) and qPCR (**e**). A significant increase in *Igfbpl1* mRNA and IGFBPL1 protein expressions were observed in the SNC compared to the Sham. β-actin used as a loading control. Data are presented as mean ± SEM. ***$P < 0.001$. Student t test (F). $n = 3$ mice per group.



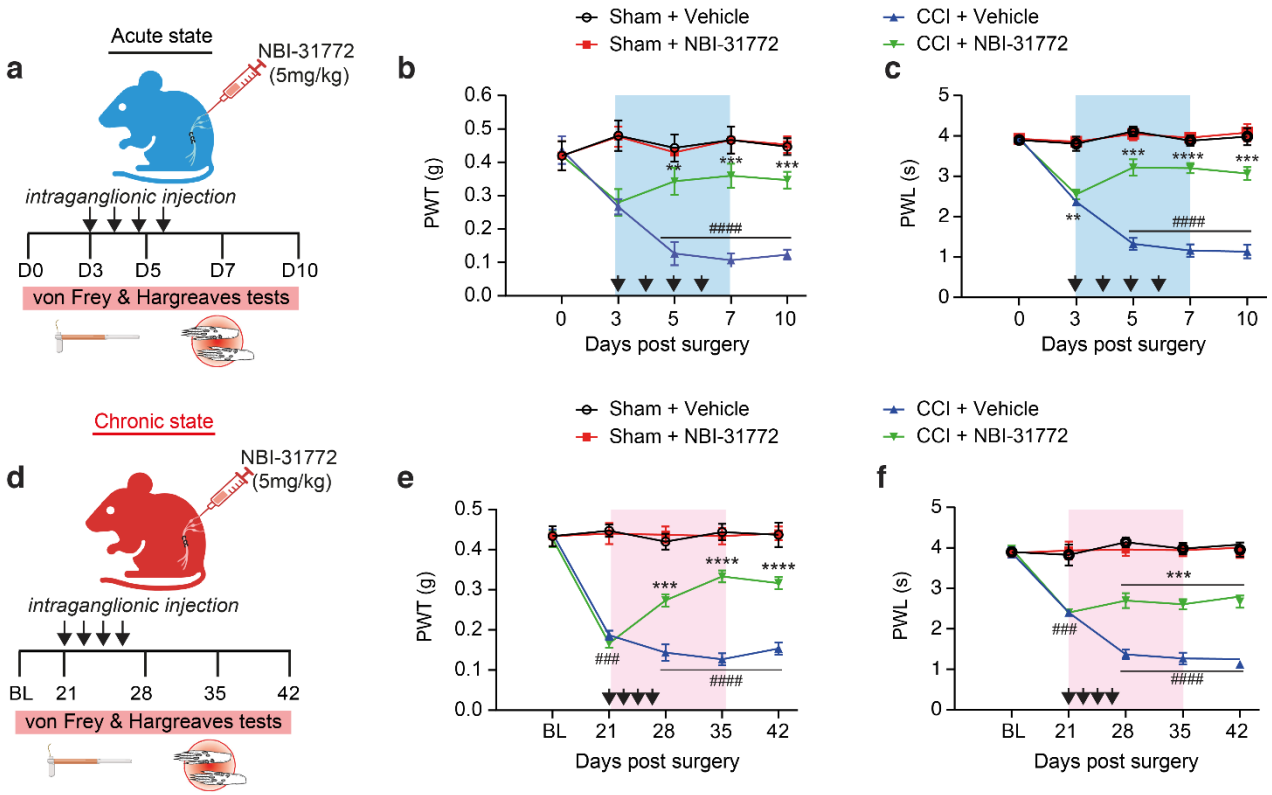
Supplementary Fig. 2: *In vivo* knockdown of IGFBPL1 and validation.

(**a**–**c**) Experimental scheme (**a**) and validation of si*Igfbpl1* knockdown efficiency assessed by qRT-PCR (**b**) and western blot (**c**) after DRG-targeted injection. Student's t-test, $n = 4$ mice per group; ***$P < 0.001$, and ****$P < 0.0001$. All data are presented as mean ± SEM.



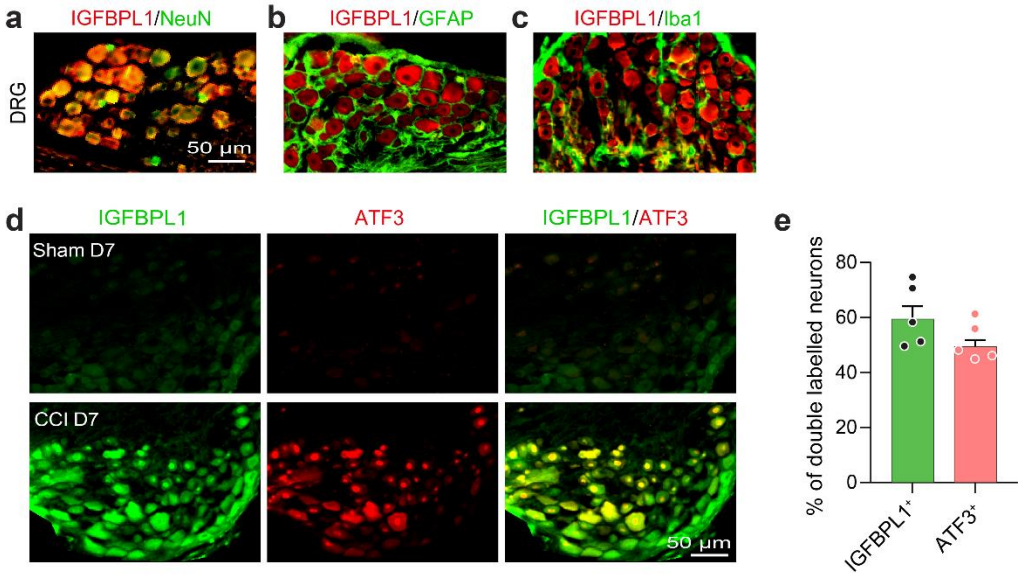
Supplementary Fig. 3: Behavioral evaluation of mechanical and thermal pain sensitivity in acute and chronic states following peripheral nerve Injury.

(**a**–**d**) Behavioral tests evaluating paw withdrawal responses in acute and chronic states using the Von Frey and Hargreaves tests across experimental groups: Sham + Scramble, Sham + si*Igfbpl1*, CCI + Scramble, and CCI + siIGFBPL1. Acute state (Von Frey Test): Intraganglionic injection of si*Igfbpl1* in the contralateral DRG on post-CCI days 3 to 6 (acute phase) or days 14 to 17 (chronic phase) failed to attenuate CCI-induced mechanical allodynia (**a**,**c**) and thermal hyperalgesia (**b**,**d**). Two-way ANOVA followed by post hoc Bonferroni's test, *n* = 12 mice per group. All data are presented as mean ± SEM.



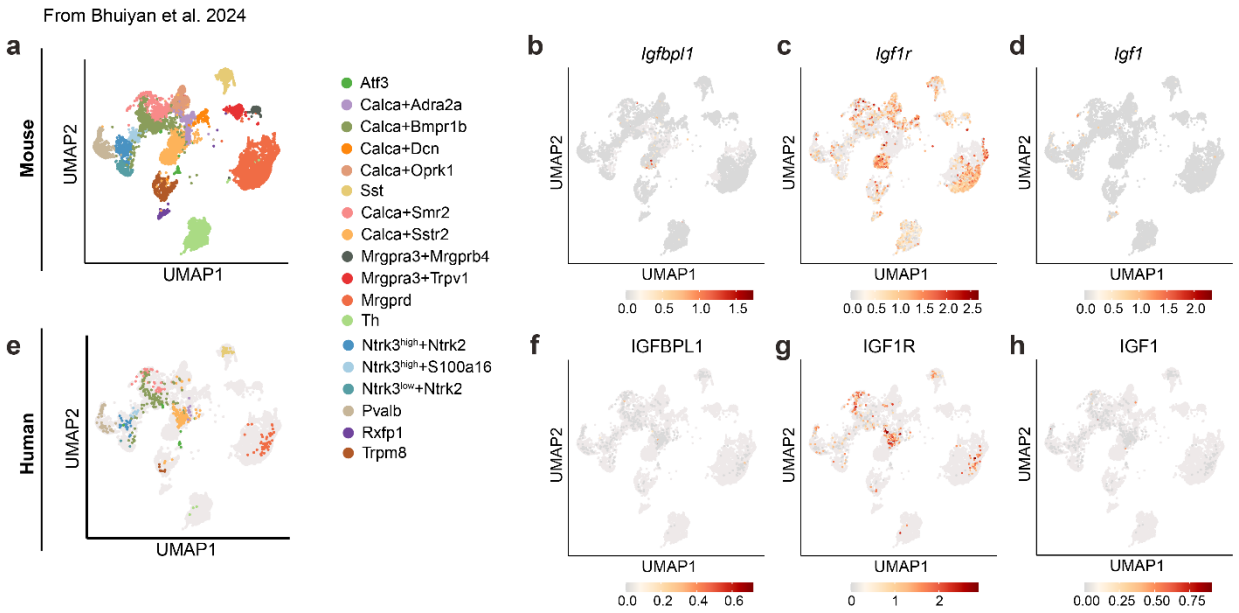
Supplementary Fig. 4: Pharmacological inhibition of IGFBP attenuated CCI-induced neuropathic pain.

(**a**–**d**) Experimental scheme displaying timeline behavioral assessment after intraganglionic injection of NBI-31772 or vehicle in the ipsilateral DRG in Sham or CCI mice. (**b**,**c**) Mechanical (**b**) and thermal (**c**) hyperalgesia in mice after NBI-31772 injection during the acute phase. (**e**,**f**) Mechanical (**e**) and thermal (**f**) hyperalgesia in mice after NBI-31772 injection during the chronic phase. Two-way ANOVA followed by post hoc Bonferroni's test, n = 12 mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$; $^{\#\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$. All data are presented as mean ± SEM.



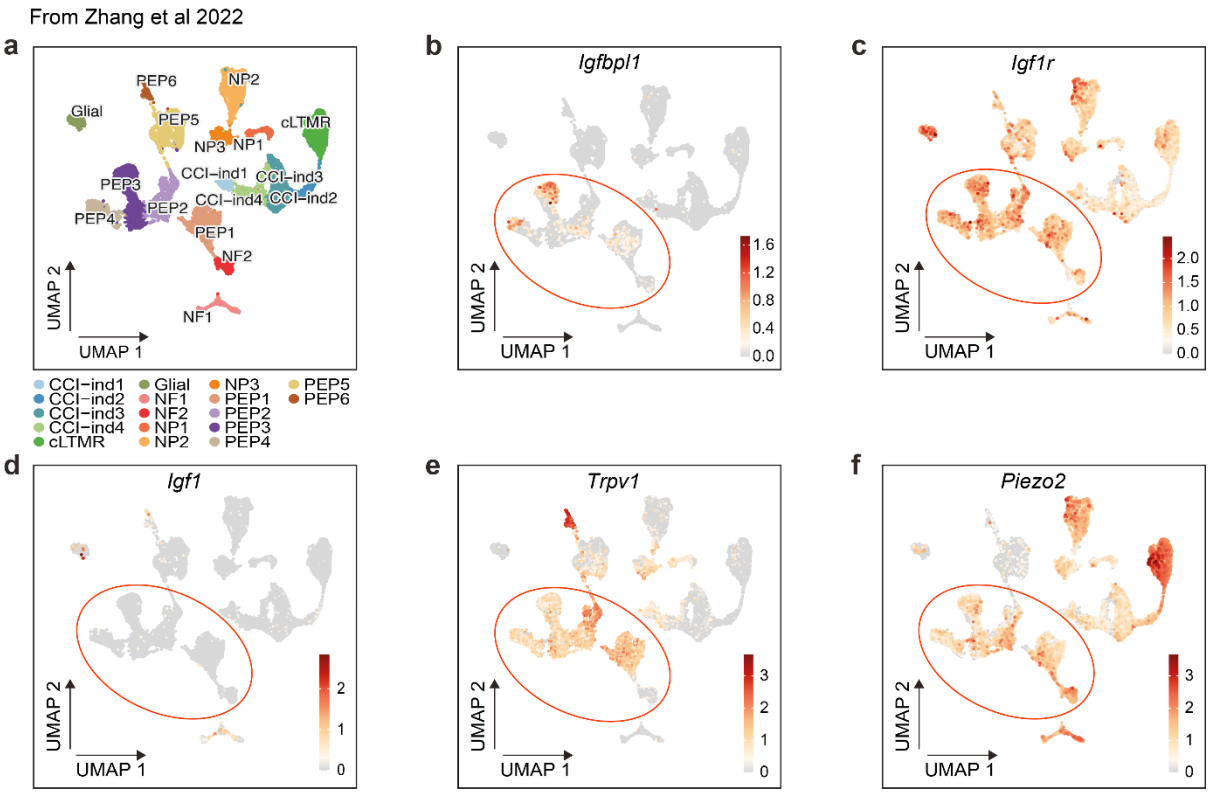
Supplementary Fig. 5: The cell type distribution of IGFBPL1 and co-localization with ATF3 in the DRG.

(**a**–**d**) Representative images of co-localization of IGFBPL1 with NeuN (**a**), GFAP (**b**) and Iba-1 (**c**) in mouse DRG. Scale bar, 50 μm. **f** Double staining of IGFBPL1 and ATF3 in the DRG. Scale bar, 50 μm. **e** Percentage of double-labeled neurons in IGFBPL1^{+} and ATF3^{+} double-labeled neurons.

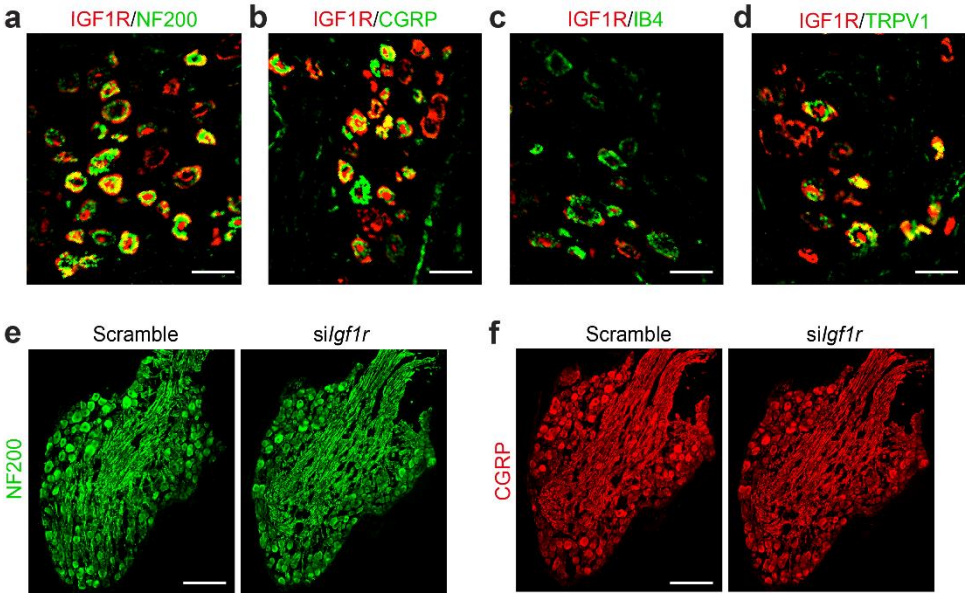


Supplementary Fig. 6: *Igfbpl1* expression in the mouse and human DRGs.

(**a**–**h**) UMAPs displaying clusters of DRG neuronal subtypes (**a**,**e**), injury-induced gene *Atf3* and the expression of IGF-related genes in mouse (*Igfbpl1*, *Igf1r* and *Igf1*) (**b**–**d**) and human (*IGFBPL1*, *IGF1R* and *IGF1*) (**f**–**h**) from scRNA-seq dataset[43].

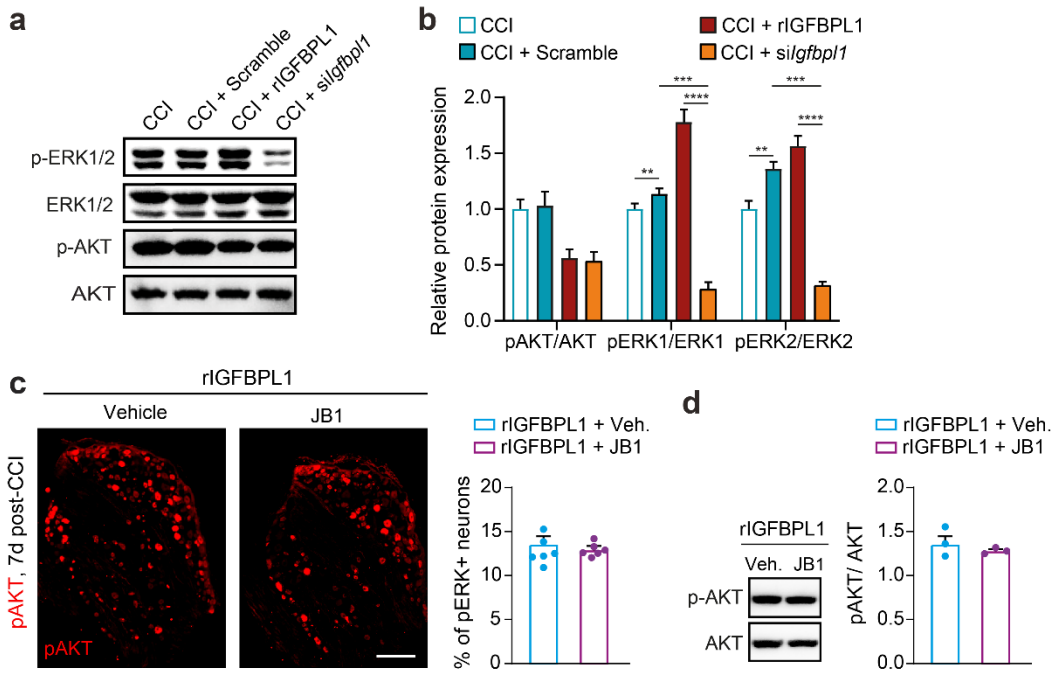


Supplementary Fig. 7: *Igfbpl1* expression in the mouse DRG neurons after CCI. (**a**–**f**) UMAP displaying 17 clusters of DRG cell populations (neurons and glia cells), and the expression patterns of *Igfbpl1*, *Igf1r*, *Igf1*, *Trpv1* and *Piezo2*. High *Igfbpl1* expression is localized predominantly within specific neuronal subpopulations (indicated by red circle). From scRNA-seq dataset[44].



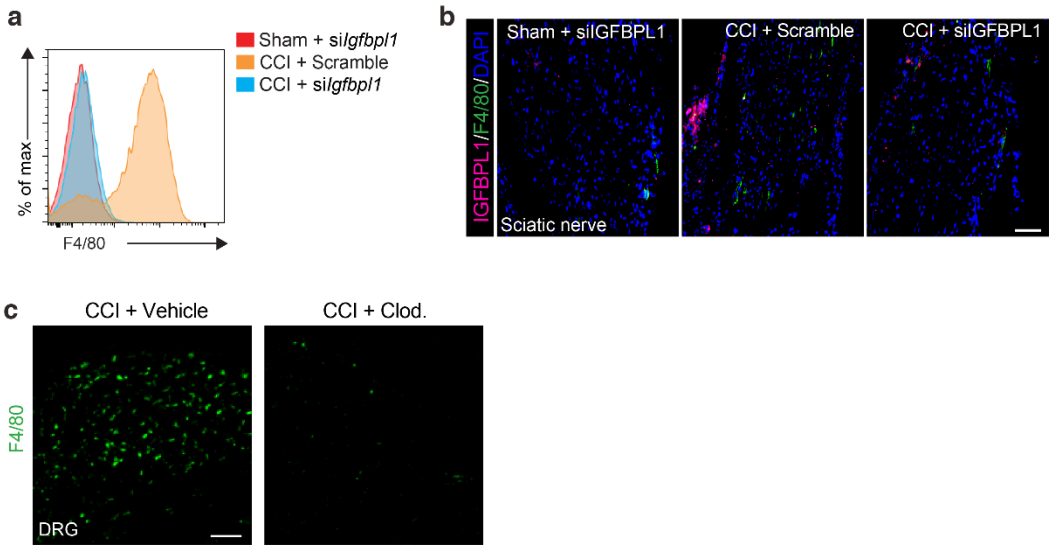
Supplementary Fig. 8: Distribution of *Igf1r* mRNA in the DRG and the effects of IGF1R knockdown.

(**a**–**g**) Representative image of RNAscope showing the co-localization of *Igf1r* with NF200 (**a**), CGRP (**b**), IB4 (**c**) and TRPV1 (**d**) in the DRG. (**e**) Percentage of double-labeled neurons in NF200$^+$, CGRP$^+$, IB4$^+$ and TRPV1$^+$ neurons. *Igf1r* knockdown mice show normal distribution patterns of NF200 (**e**), and CGRP (**f**) in the DRG. Percentage of NF200$^+$ and CGRP$^+$ neurons. *n* = 3 mice/group. Scale bar, 50 μm and 100 μm.



Supplementary Fig. 9: The effect of pAKT pathway in IGFBPL1 and IGF1R-mediated pain behaviors.

(**a** ,**b**) Western blot analysis and quantifications showing pERK (**a**) and pAKT (**b**) expression in DRG neurons from mice with *Igfbpl1* knockdown or rIGFBPL1 overexpressed conditions. (**c**,**d**) Representative IHC staining and quantification of pAKT after intraganglionic JB-1 and rIGFBPL1 injection. $n = 6$ mice. Scale bar, 100 μm. (**d**) Immunoblotting analysis and quantification of pAKT protein expression in DRG neurons after JB-1 and rIGFBPL1 injection in CCI mice. $n = 4$ mice per group. Two-way ANOVA followed by post hoc Bonferroni's test, $n = 8$ mice per group. *$P < 0.05$, and **$P < 0.01$. All data are presented as mean ± SEM.



Supplementary Fig. 10: The effects of IGFBPL1 inhibition and macrophage depletion on DRG macrophages.

a Flow cytometry analysis of F4/80$^+$ cells isolated from DRGs following intraganglionic injection of si*Igfbpl1* (*n* = 3 independent experiments). **b** Representative immunostaining images showing IGFBPL1$^+$ cells (magenta), F4/80$^+$ macrophages (green), and nuclei (DAPI, blue), with reduced infiltrating macrophages after *Igfbpl1* knockdown (*n* = 5 mice per group; scale bar, 50 μm). **c** Representative immunostaining images of F4/80$^+$ cells in DRGs collected from vehicle or clodronate-treated mice after rIGFBPL1 injection. Chlodronate treatment reduced the number of macrophages (scale bar, 50 μm).

Table S1. List of antibodies used in this study

Antibody	Species	Company	Cat. No.	RRID
anti-IGFBPL1	Goat	R&D	Cat# AF4130	AB_2279980
anti-IGF-1	Mouse	R&D	Cat# AF791	AB_2248752
anti-IGF1R	Mouse	R&D	Cat# MAB391	AB_2122409
anti-pIGF1R	Rabbit	SCB	Cat# sc-81499	AB_2797366
IgG	Rabbit	CST	Cat# 2729P	AB_1031062
anti-pERK1/2	Rabbit	CST	Cat# 4370	AB_2315112
anti-ERK1/2	Rabbit	CST	Cat# 4695	AB_390779
anti-pAKT	Rabbit	CST	Cat# 9271	AB_329828
anti-AKT	Rabbit	CST	Cat# 9272	AB_329827
NF200	Mouse	Millipore	Cat# MAB5266	AB_11212783
IB4-FITC	Mouse	Sigma	Cat#L2895	AB_477295
CGRP	Rabbit	CST	Cat#14959S	AB_2650536
TRPV1	Mouse	Abcam	Cat#ab203103	AB_443297
TRPV1	Rabbit	Alomone labs	Cat#ACC-030	AB_10008453
GS	Rabbit	ABclonal	Cat#A21822	AB_2735178
NeuN	Rabbit	Abcam	Cat#ab177487	AB_444290
NeuN	Mouse	CST	Cat#94403S	AB_2830192
GFAP	Mouse	CST	Cat#3670S	AB_2223032
IBA-1	Rabbit	ProteinTech	Cat#10904-1-AP	AB_2315043
TUBB3	Rabbit	Abcam	Cat#ab52623	AB_880896
ATF3	Rabbit	CST	Cat#18665S	AB_2723386
CD11b	Rat	BioLegend	101212 (APC)	AB_312784
F4/80	Rat	BioLegend	123110 (PE)	AB_893653
CD68	Rat	BioLegend	137012 (APC)	AB_893654

Table S2. The specific primers used for qRT-PCR in this study

Gene	**Forward Sequence**	**Reverse Sequence**
Igfbpl1_1	ATGCCCCCTCGAGATATTCA	CTCGCACCTGGACAGCTATA
Igfbpl1_2	GTGAGGGCTGTGCCTACCC	CATCACATGCGGTCATCGGG
Igf1r	GTACCGGCACAACTACTGCT	AGCCTGCTTCTCAGCTTCAG
Tnf-α	TGATCGGTCCCCAAAGGGAT	TGTCTTTGAGATCCATGCCGT
Il-6	CAACGATGATGCACTTGCAGA	GTGACTCCAGCTTATCTCTTGGT
Il-1β	TGCCACCTTTTGACAGTGATG	AAGGTCCACGGGAAAGACAC
Ccr2	ATCCACGGCATACTATCAACA	CAAGGCTCACCATCATCGTAG
Gapdh	ATCACTGCCACCCAGAAGACT	ATGCCAGTGAGCTTCCCGTT

Table S3. The siRNA sequences used in this study

Target gene	**Sequence sense (5’ to 3’)**	**Sequence antisense (5’ to 3’)**
IGFBPL1_847	GCUCCCGAUGACCGCAUGUTT	ACAUGCGGUCAUCGGGAGCTT
IGFBPL1_482	GCGAGUUCGCUCCUGUGGUTT	ACCACAGGAGCGAACUCGCTT
Neg. Control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUCGGAGAATT