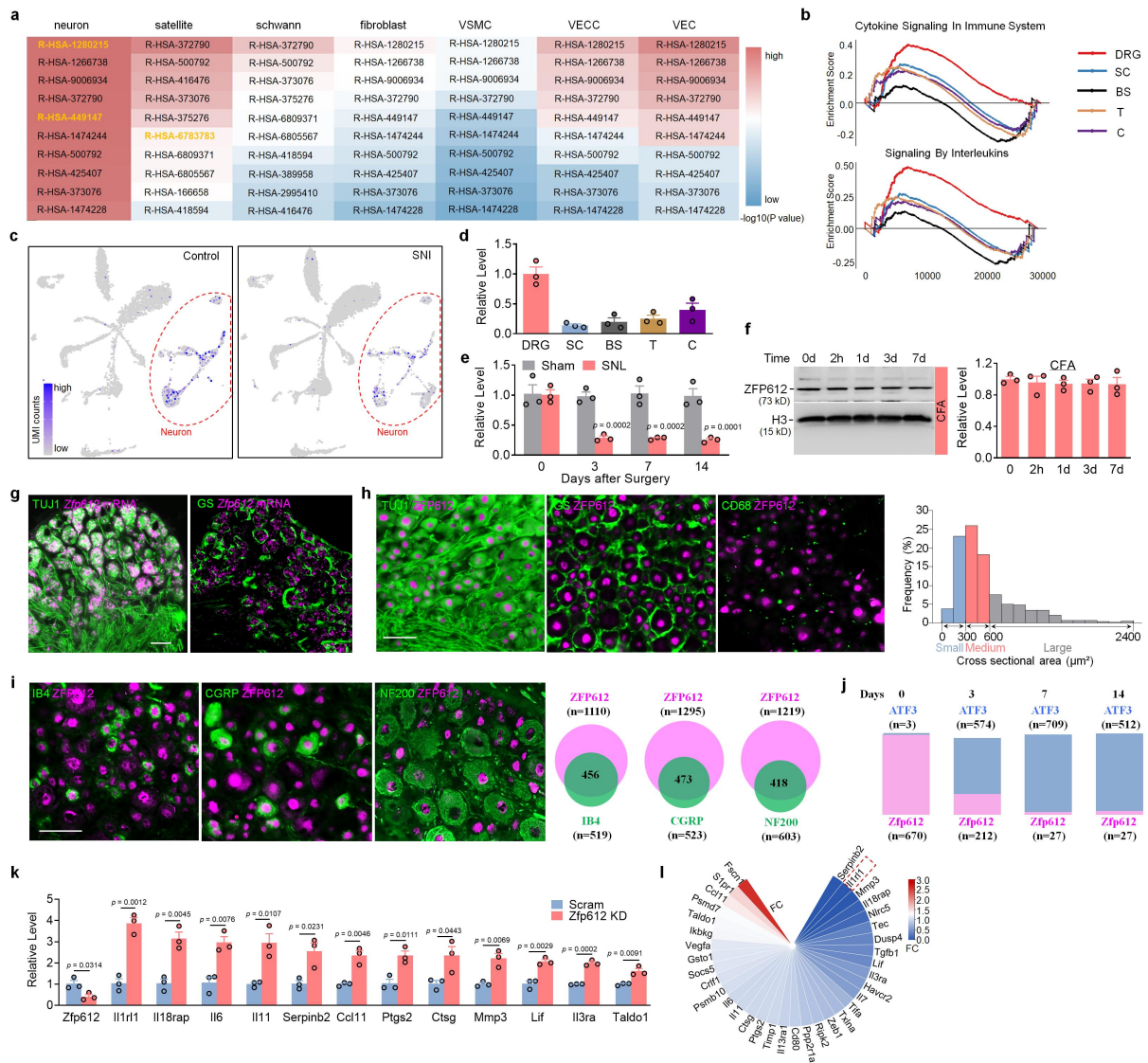
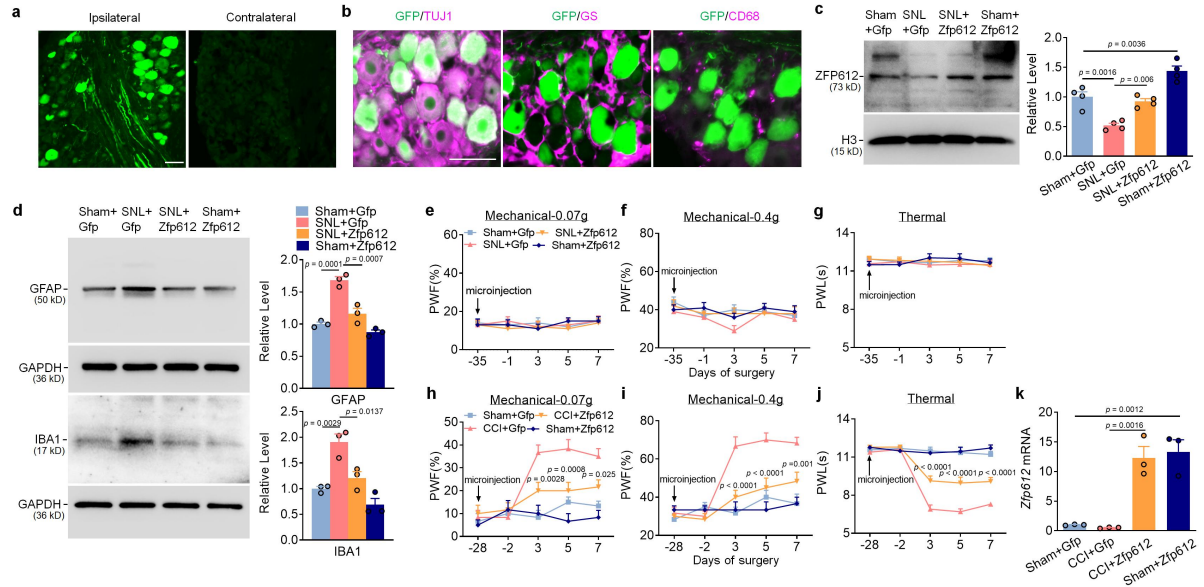


Supplementary Figures and Figure legends

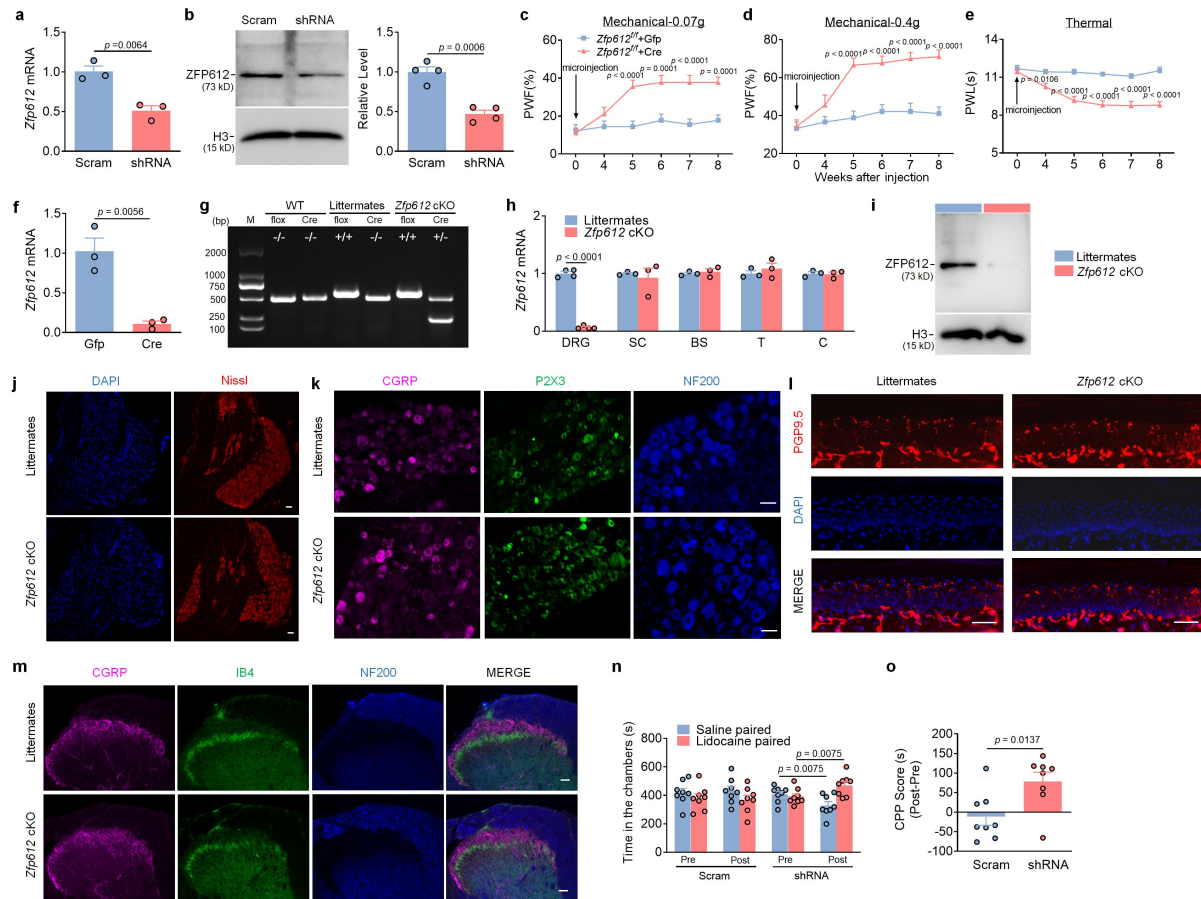


Supplementary Fig. 1 Immune responses in DRG distinct cell types and different pain transduction loci after nerve injury, and the distribution characteristics of ZFP612 in DRG. a, Reactome pathway analyses for the differentially expressed genes in DRG neuron, satellite glial cell, Schwann cell, fibroblast, VSMC, VECC, and VEC 7 days after nerve injury using GSEA. The top 10 enriched pathways in each cell type were displayed, and the immune-related pathways were highlighted yellow. **b**, GSEA curves of indicated pathways in DRG, SC, BS, T, and C 7 days after SNL. **c**, UMAP visualization of single-cell transcriptomic profiles from control or SNI DRG on day 7 post-surgery. Cells were colored according to the expression level of *Zfp612*, and the red dashed circles highlighted the neuron-enriched clusters. **d**, The quantitative analysis for the enrichment of ZFP612 in DRG, SC, BS, T, and C under normal condition. *n* = 3 repeats/tissue. **e**, qRT-PCR of *Zfp612* mRNA in L4 DRG after

surgery. n = 3 repeats/group/time point. **f**, Immunoblot of ZFP612 in L3/4 DRGs after plantar CFA injection. n = 3 repeats/time point. **g**, Representative images of ISH for *Zfp612* mRNA with TUJ1 or GS in the DRG. Scale bar: 50 μ m. n = 3 mice. **h,i**, Representative images of IF for ZFP612 with TUJ1, GS, CD68 (**h**) or different neuronal markers (**i**) in the DRG. Histogram showed the distribution of ZFP612-positive somata: Large, 29%; Medium, 44.1%; Small, 26.9% (**h**). Venn diagram showed the number of neurons double-stained by ZFP612 and NF200, CGRP or IB4 (**i**). n = 5 mice. Scale bar: 50 μ m. **j**, Histogram showed the number of ATF3-positive or *Zfp612* mRNA-positive neurons on different days after SNL. n = 3 mice/time point. **k**, qRT-PCR of the top 12 upregulated genes in interleukin signaling pathway after ZFP612 knockdown. n = 3 repeats/group. **l**, Heatmap displaying the fold change (FC) of the 35 interleukin signaling genes in SNL DRG pre-microinjection with AAV5-*Zfp612* compared to AAV5-*Gfp* by RNA-seq. *P* values determined by two-tailed unpaired Student's *t*-test (**k**), one-way ANOVA (**f**)/two-way ANOVA (**e**) followed by *post hoc* Tukey test. Mean $\pm$ SEM.

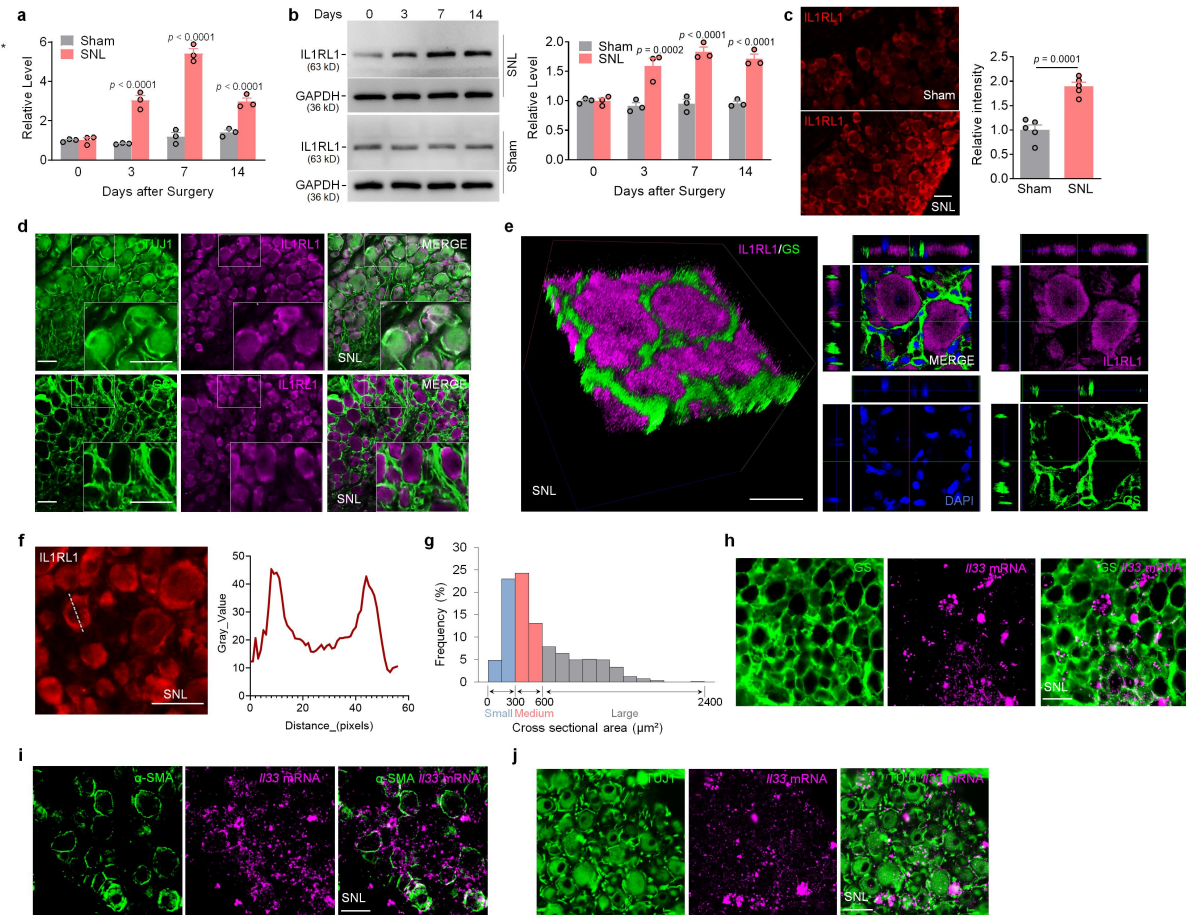


Supplementary Fig. 2 Validation of DRG neuronal ZFP612 delivery *in vivo* and its effect on nerve injury-induced spinal glial activities and pain hypersensitivities. **a**, Representative images of GFP expression in the ipsilateral and contralateral L4 DRG on week 6 after microinjection with AAV5-*Zfp612* (Zfp612) or AAV5-*Gfp* (Gfp) into ipsilateral L4 DRG. $n = 3$ mice. Scale bar: 50 μm . **b**, Representative images of co-localization of GFP with TUJ1, GS, or CD68 in the AAV5-injected DRG. $n = 3$ mice. Scale bar: 50 μm . **c**, Immunoblot of ZFP612 on day 7 after SNL or Sham surgery in the L4 DRG pre-microinjected with AAV5-*Zfp612* (Zfp612) or AAV5-*Gfp* (Gfp) 5 weeks before surgery. $n = 4$ repeats/group. **d**, Effect of microinjection with AAV5-*Zfp612* (Zfp612) or AAV5-*Gfp* (Gfp) into the ipsilateral L4 DRG on the levels of GFAP and IBA1 in the lumbar enlargement segment 7 days after SNL or Sham surgery. $n = 3$ repeats/group. **e-g**, Effect of microinjection with AAV5-*Zfp612* (Zfp612) or AAV5-*Gfp* (Gfp) into the ipsilateral L4 DRG on paw withdrawal responses to mechanical (e,f) and heat (g) stimuli on the contralateral side. $n = 10$ mice/group. **h-j**, Effect of AAV5-*Zfp612* (Zfp612) into the ipsilateral L3/4 DRGs on CCI-induced mechanical allodynia (h,i) and heat hyperalgesia (j) on the ipsilateral side compared to AAV5-*Gfp* (Gfp). $n = 6$ mice/group. **k**, qRT-PCR of *Zfp612* mRNA on day 7 after CCI or Sham surgery in the L3/4 DRGs pre-microinjected with AAV5-*Zfp612* (Zfp612) or AAV5-*Gfp* (Gfp). $n = 3$ repeats/group. P values determined by one-way ANOVA followed by *post hoc* Tukey test (c,d,k) and two-way RM ANOVA followed by *post hoc* Tukey test (e-j). Mean $\pm$ SEM.

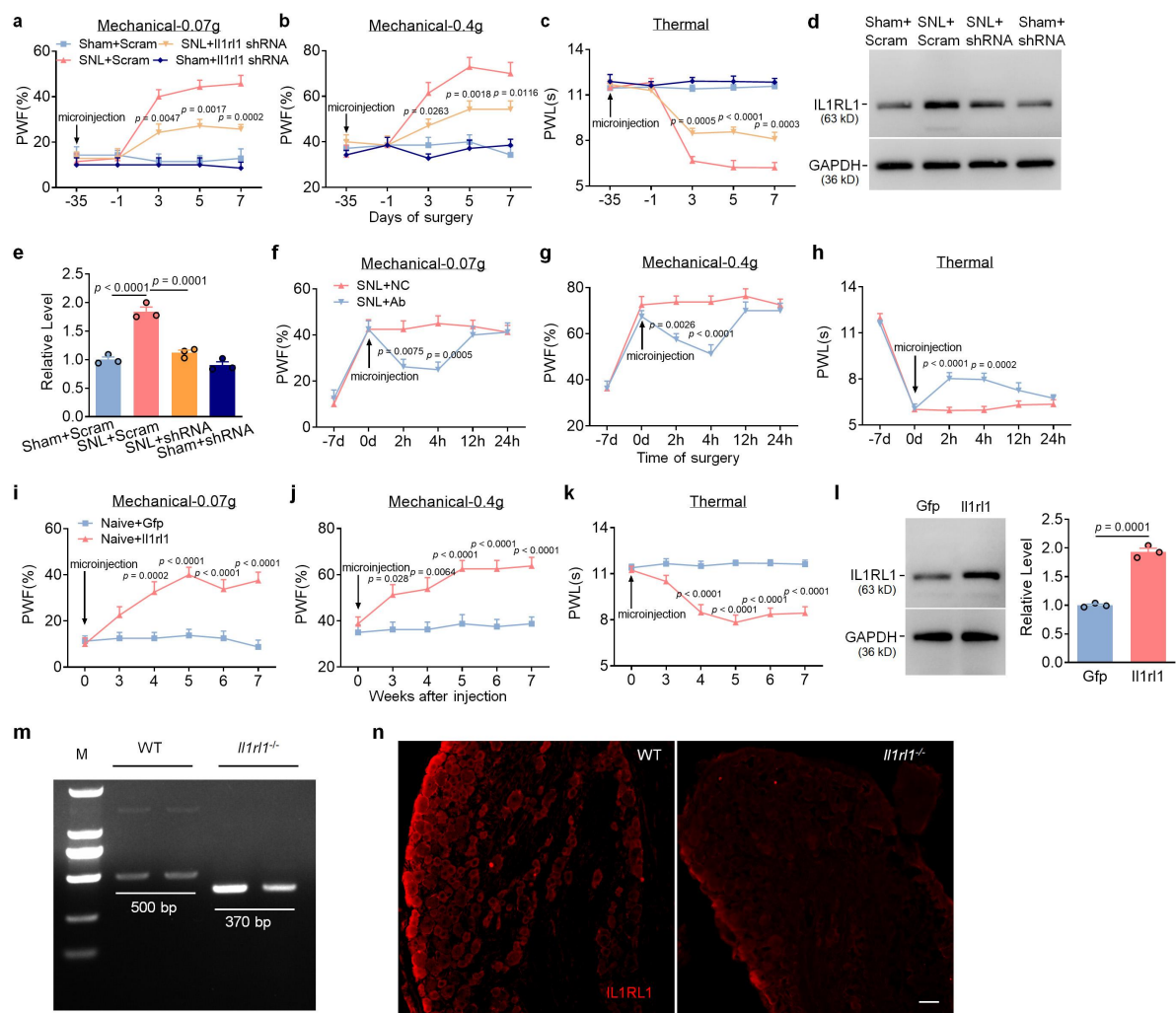


Supplementary Fig. 3 DRG loss of *Zfp612* induces increased spinal glial activities and pain hypersensitivity, and displays no deficits in sensory neurons and peripheral or central innervation. **a,b**, qRT-PCR of *Zfp612* mRNA (**a**) ($n = 3$ repeats/group) and immunoblot of ZFP612 protein (**b**) ($n = 4$ repeats/group) in L3/4 DRGs 8 weeks after microinjection with AAV5-*Zfp612* shRNA (shRNA) or AAV5-*Zfp612* scrambled shRNA (Scram). **c-e**, Effect of microinjection with AAV5-*Cre* (Cre) or AAV5-*Gfp* (Gfp) into the ipsilateral L3/4 DRGs of *Zfp612^{fl/fl}* mice on paw withdrawal responses to mechanical (**c,d**) and heat (**e**) stimulation. $n = 9$ mice/group. **f**, qRT-PCR of *Zfp612* mRNA in L3/4 DRGs 8 weeks after microinjection with AAV5-*Cre* (Cre) or AAV5-*Gfp* (Gfp). $n = 3$ repeats/group. **g**, PCR-based genotyping of WT, Littermates and *Zfp612* cKO mice. **h**, qRT-PCR of *Zfp612* mRNA in the DRG ($n = 4$ repeats/group), SC, BS, T, and C ($n = 3$ repeats/group) from Littermates and *Zfp612* cKO mice. **i**, Representative immunoblot image of the ZFP612 expression in the DRG from Littermates and *Zfp612* cKO mice. **j**, Effect of DRG *Zfp612* deletion on the architecture of DRG. $n = 3$ mice/group. Scale bar: 50 μ m. **k**, Representative images of the CGRP-, P2X3-, and NF200-positive neurons in L4 DRG from the Littermates

and *Zfp612* cKO mice. n = 3 mice/group. Scale bar: 50 μ m. **l,m**, Effect of DRG *Zfp612* deletion on the intraepidermal nerve fibers in glabrous skin of hind paw (l) and on the central terminals of CGRP-, IB4-, and NF200-positive neurons in the spinal dorsal horn (m). n = 3 mice/group. Scale bar: 50 μ m. **n**, Effect of microinjection with AAV5-*Zfp612* shRNA (shRNA) or AAV5-scrambled shRNA (Scram) into L3/4 DRGs of naive mice on the time spent in saline- or lidocaine-paired chambers 8 weeks after injection. Pre: pre-conditioning. Post: post-conditioning. n = 8 mice/group. **o**, DRG microinjection with AAV5-*Zfp612* shRNA (shRNA) produced spontaneous pain in naive mice compared to AAV5-scrambled shRNA (Scram). n = 8 mice/group. *P* values determined by two-tailed unpaired Student's *t*-test (a,b,f,h,o) and two-way RM ANOVA followed by *post hoc* Bonferroni's test (c-e,n). Mean $\pm$ SEM.

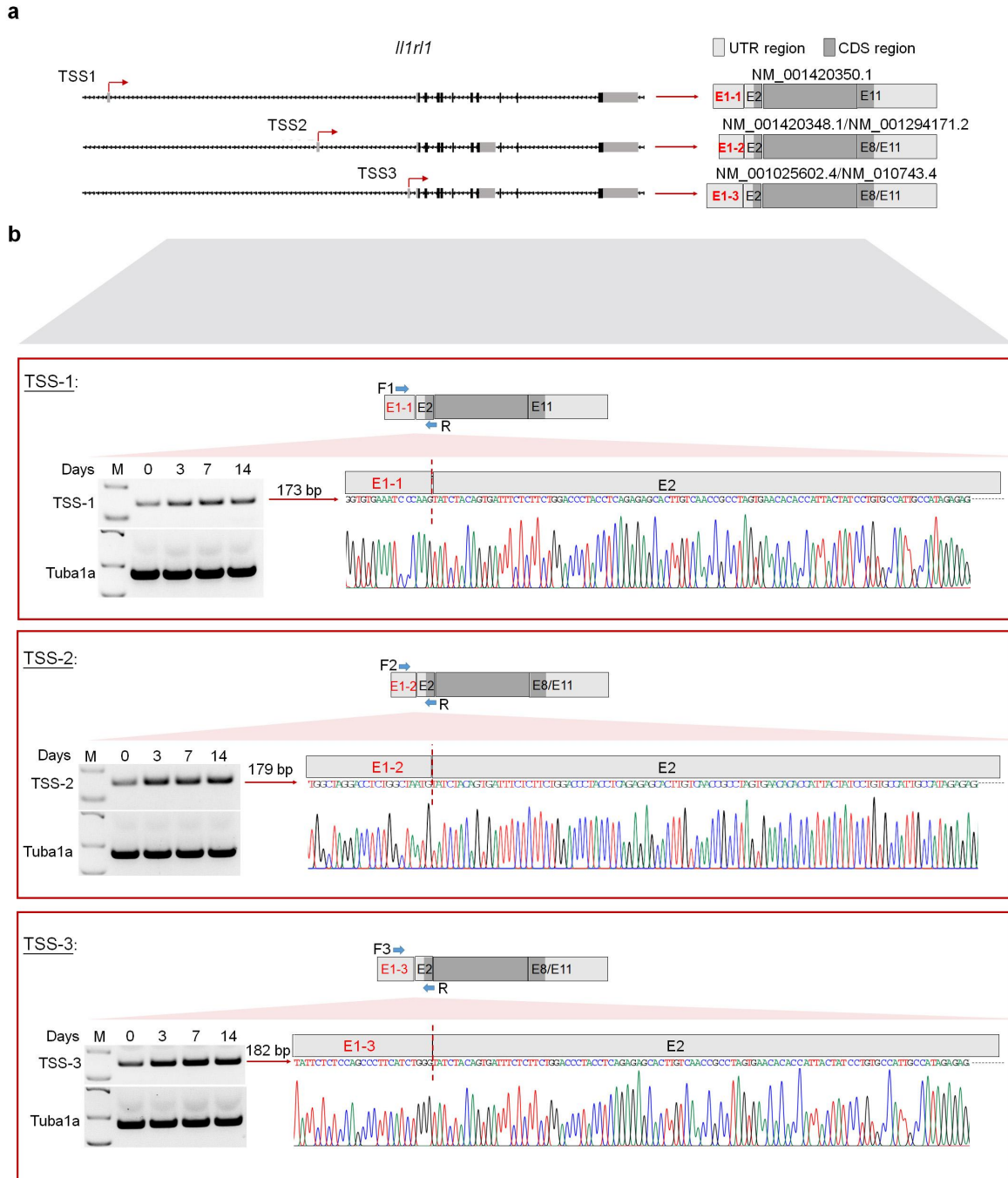


Supplementary Fig. 4 The expression and distribution characteristics of IL1RL1 and IL-33 in DRG. **a,b**, qRT-PCR of *Il1rl1* mRNA (a) and immunoblot of IL1RL1 protein (b) in the ipsilateral L4 DRG after SNL or Sham surgery. $n = 3$ repeats/group/time point. **c**, Representative IF images of IL1RL1 and analysis of IL1RL1 intensity in L4 DRG after SNL or Sham surgery. $n = 5$ mice/group. **d**, Representative IF images of IL1RL1 and TUJ1 or GS in the injured DRG 7 days after SNL. $n = 3$ mice/group. Scale bar: 50 μ m. **e**, Representative IF images of the three-dimensional distribution between IL1RL1 and GS in injured DRG 7 days after SNL. $n = 3$ mice. Scale bar: 25 μ m. **f**, Representative IF image of the distribution intensity of IL1RL1 over each neuron in SNL DRG. $n = 3$ mice. Scale bar: 50 μ m. **g**, Histogram shows the distribution of IL1RL1-positive somata in L4 DRG: Large, 34.8%; Medium, 37.4%; Small, 27.9%. $n = 3$ mice. **h-j**, ISH combined with IF for *Il33* mRNA with GS (h), α -smooth muscle actin (α -SMA) (i), or TUJ1 (j) in the SNL DRG. $n = 3$ mice. Scale bar: 50 μ m. P values determined by two-tailed unpaired Student's t -test (c) and two-way ANOVA followed by *post hoc* Tukey test (a,b). Mean $\pm$ SEM.

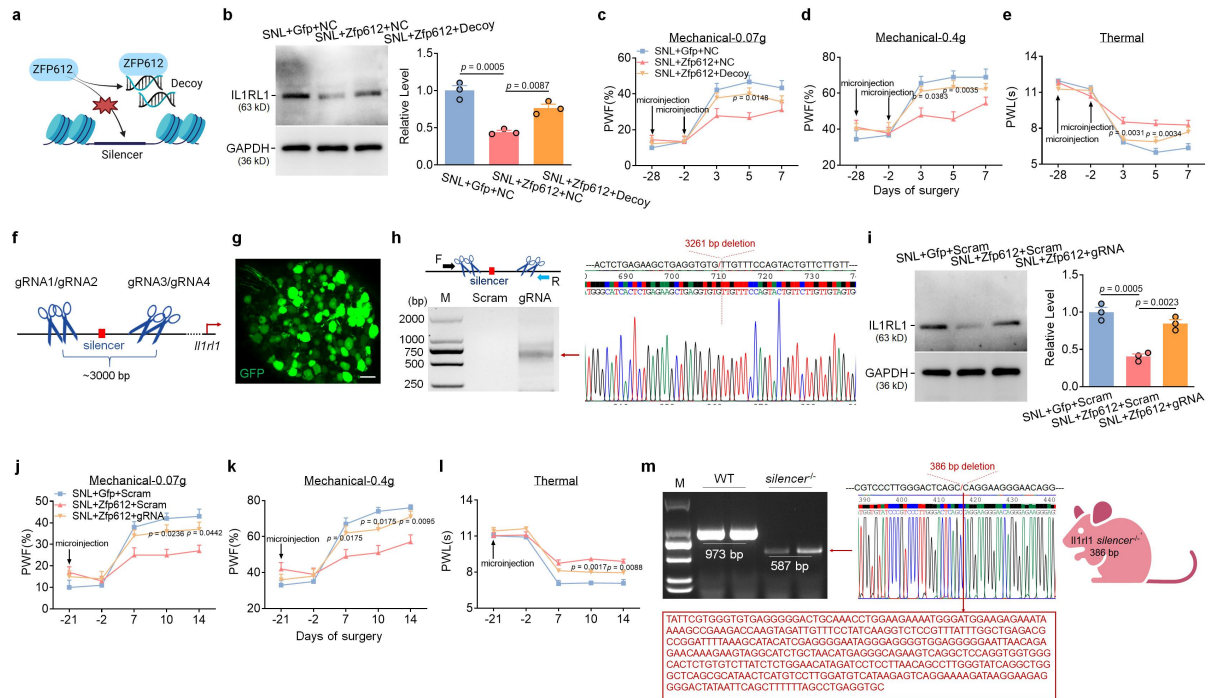


Supplementary Fig. 5 The algescic effect of IL1RL1 in DRG neurons. **a-c**, Effect of AAV5-*Il1rl1* shRNA (*Il1rl1* shRNA) into the ipsilateral L4 DRG on SNL-induced mechanical allodynia (**a**,**b**) and heat hyperalgesia (**c**) compared to AAV5-scrambled shRNA (Scram). $n = 7$ mice/group. **d,e**, Immunoblot of IL1RL1 on day 7 after SNL or Sham surgery in the L4 DRG pre-microinjected with AAV5-*Il1rl1* shRNA (shRNA) or AAV5-scrambled shRNA (Scram). $n = 3$ repeats/group. **f-h**, Effect of post-microinjection with IL1RL1 neutralization antibody (Ab) or negative control IgG (NC) into the SNL DRG on paw withdrawal responses to mechanical (**f**,**g**) and heat (**h**) stimuli. $n = 8$ mice/group. **i-k**, Effect of microinjection with AAV5-*Il1rl1* (*Il1rl1*) or AAV5-*Gfp* (*Gfp*) into the L3/4 DRGs of naive mice on paw withdrawal responses to mechanical (**i**,**j**) and heat (**k**) stimulation. $n = 8$ mice/group. **l**, Immunoblot of IL1RL1 in the ipsilateral L3/4 DRGs 7 weeks after microinjection with AAV5-*Il1rl1* (*Il1rl1*) or AAV5-*Gfp* (*Gfp*). $n = 3$ repeats/group. **m**, PCR-based genotyping of WT and *Il1rl1*^{-/-} mice. **n**,

Representative IF images of IL1RL1 expression in L4 DRG of WT and *Il1rl1*^{-/-} mice. n = 3 mice/group. Scale bar: 50 μ m. *P* values determined by two-tailed unpaired Student's *t*-test (l), one-way ANOVA followed by *post hoc* Tukey test (e), two-way RM ANOVA followed by *post hoc* Tukey test (a-c) and two-way RM ANOVA followed by *post hoc* Bonferroni's test (f-k). Mean $\pm$ SEM.

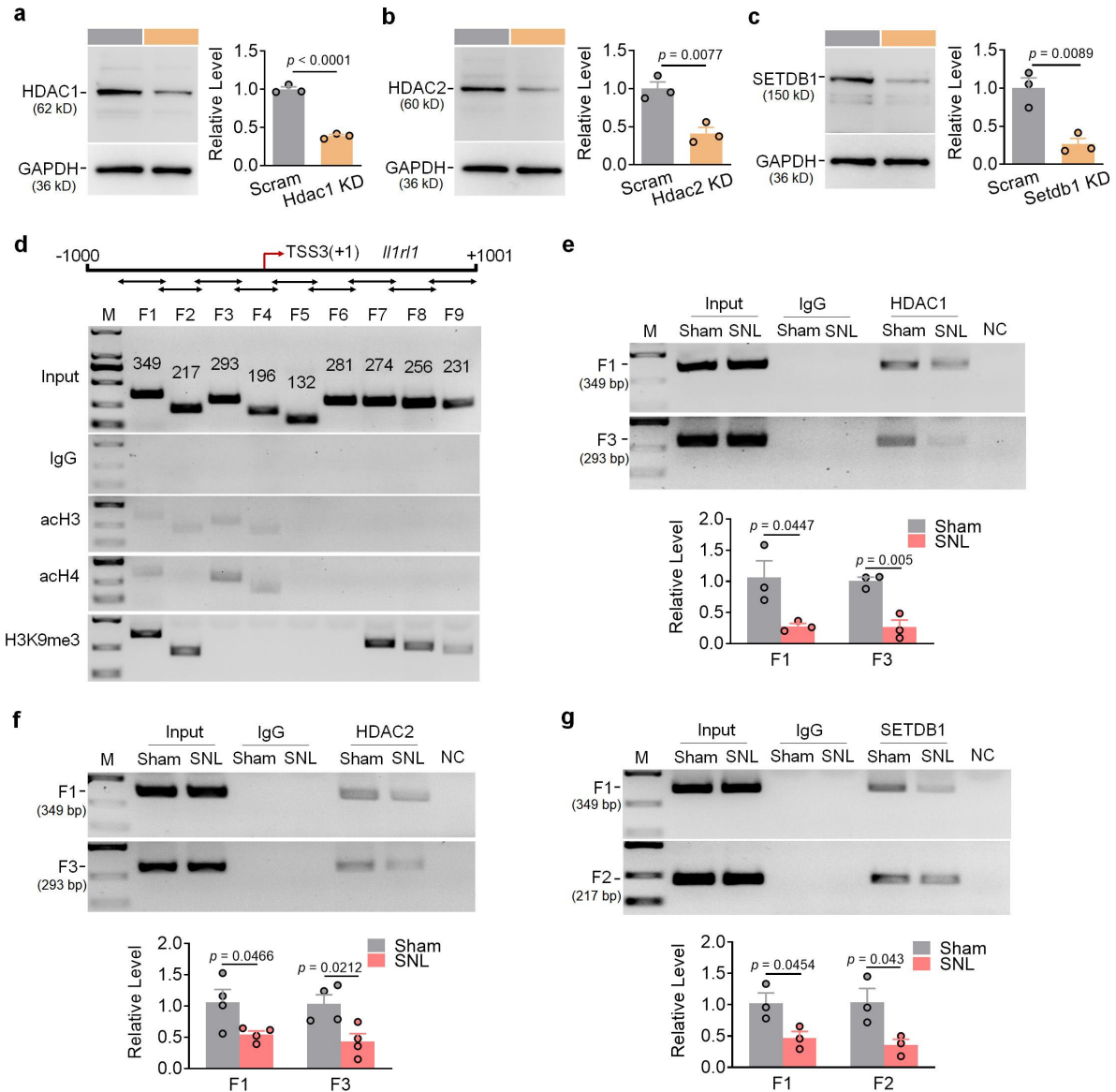


Supplementary Fig. 6 The validation of *Il1rl1* transcript isoforms transcribed from distinct TSSs of *Il1rl1* gene in DRG. **a**, Schematic representation of the *Il1rl1* genome and the corresponding transcripts deriving from TSS1, TSS2, and TSS3 of *Il1rl1* gene. UTR: untranslated region, CDS: coding sequence, E: exon. **b**, PCR-based representative images of the transcripts TSS-1, TSS-2, and TSS-3 originating from TSS1, TSS2, and TSS3 of *Il1rl1* gene, respectively, in DRG on days 0, 3, 7 and 14 after SNL and the sequence chromatogram of excised band indicated. Forward primers 1-3 (F1, F2 and F3) targeting exon1-1 (E1-1), exon1-2 (E1-2), and exon1-3 (E1-3), respectively. R: reverse primer.



Supplementary Fig. 7 Effect of blocking ZFP612 binding to silencer or excising the silencer of *Il1rl1* on ZFP612-mediated repression of IL1RL1 and relief of nociceptive hypersensitivity after peripheral nerve injury. **a**, Schematic graph showed the blockade of ZFP612 binding to silencer of *Il1rl1* by decoy DNA. **b**, Effect of decoy DNA (Decoy) or control DNA (NC) on the repressive role of AAV5-*Zfp612* (Zfp612) in IL1RL1 expression on day 5 after SNL. n = 3 repeats/group. **c-e**, Effect of decoy DNA (Decoy) or control DNA (NC) on the analgesic role of AAV5-*Zfp612* (Zfp612) in SNL-induced mechanical allodynia (c,d) and heat hyperalgesia (e). n = 9 mice/group. **f**, Schematic graph showed the excision of *Il1rl1* silencer by CRISPR/Cas9 with 4 guiding RNAs (gRNA1-4) around silencer. **g**, Representative GFP expression in L4 DRG on day 14 after SNL from the mice pre-microinjected with AAV5-*Zfp612* plus LV-gRNA 3 weeks before SNL surgery. n = 3 mice. **h**, PCR-based detection of silencer-included fragment excision of *Il1rl1* on day 14 after SNL from the mice pre-microinjected with LV-gRNA (gRNA) or LV-scrambled gRNA (Scram) 3 weeks before SNL surgery. Sequence chromatogram of excised band with regions of interest denoted. n = 2 repeats. M: ladder marker. **i**, Effect of LV-gRNA (gRNA) or LV-scrambled gRNA (Scram) on the repressive role of AAV5-*Zfp612* (Zfp612) in IL1RL1 expression on day 14 after SNL. n = 3 repeats/group. **j-l**, Effect of LV-gRNA (gRNA) or LV-scrambled gRNA (Scram) on the analgesic role of AAV5-*Zfp612* (Zfp612) in SNL-induced mechanical

allodynia (j,k) and heat hyperalgesia (l). n = 10 mice/group. **m**, Representative image of PCR-based genotyping of WT and *Il1rl1 silencer*^{-/-} mice, and the validation of the target sequence deletion by sequence chromatogram. M: ladder marker. *P* values determined by one-way ANOVA followed by *post hoc* Tukey test (b,i) and two-way RM ANOVA followed by *post hoc* Tukey test (c-e,j-l). Mean ± SEM. **a** and **m** were created in BioRender. Jiang, B. (2025) <https://BioRender.com/yvqn90b>.



Supplementary Fig. 8 Validation of HDAC1, HDAC2 and SETDB1 knockdown, and enrichments of acH3/acH4/H3K9me3 and HDAC1/HDAC2/SETDB1 in P3 region of *Il1rl1* in DRG. **a-c**, Immunoblot of HDAC1, HDAC2, or SETDB1 in L3/4 DRGs of naive mice 3 days after intrathecal injection with *Hdac1* (a), *Hdac2* (b), or *Setdb1* (c) siRNA (KD) compared to the scrambled siRNA (Scram). $n = 3$ repeats/group. **d**, Four regions (F1, F2, F3, F4), three regions (F1, F3, F4), and five regions (F1, F2, F7, F8, F9) from the *Il1rl1* promoter were successfully immunoprecipitated by acH3, acH4, and H3K9me3 antibody, respectively, in naive L4 DRG. $n = 2$ repeats. Input: total purified fragments. M: ladder marker. TSS3 (+1): transcription start site 3 as +1. **e**, ChIP-qPCR of HDAC1 occupancy in F1 and F3 of *Il1rl1* in injured DRG 7 days after Sham or SNL surgery. $n = 3$ repeats (18 mice)/group. **f**, ChIP-qPCR

of HDAC2 occupancy in F1 and F3 of *Il1rl1* in injured DRG 7 days after Sham or SNL surgery. n = 4 repeats (24 mice)/group. **g**, ChIP-qPCR of SETDB1 occupancy in F1 and F2 of *Il1rl1* in injured DRG 7 days after Sham or SNL surgery. n = 3 repeats (18 mice)/group. *P* values determined by two-tailed unpaired Student's *t*-test (a-c,e-g). Mean $\pm$ SEM.