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Peer Review File

ZFP612 controls mouse neuropathic pain through epigenetic repression of Il1rl1 within the silencer–promoter loop in primary sensory neurons

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The study investigates the role of ZFP612 in mediating neuropathic pain via IL1RL1 regulation in DRG neurons following peripheral nerve injury. Using cross-species DRG neurons (monkey, mouse, human) and multiple injury paradigms (SNL, CCI, SNI, CFA), the authors demonstrate that ZFP612 downregulation unlocks the expression of IL1RL1, driving neuronal interleukin signaling and in vivo hypersensitivity. While the findings are novel and mechanistically detailed, there are some issues that deserve to be clarified.
1. The title emphasizes 3D epigenomics while findings focus on local transcriptional repression (eg intronic silencer deletion, ZFP612-IL1RL1 axis) rather than genome-wide 3D chromatin reorganization. I suggest reframing at it may sound misleading
2. The first part of the introduction makes overgeneralization. The opening conflates heterogeneous neurological disorders (eg diabetic neuropathy, GBS, neurodegenerative disorders) that do not share the same mechanisms underlying the occurrence of pain. Moreover, in these conditions pain can occur early (eg GBS) or be chronic (DN). References 1-11 and 13 seem misaligned. All this weakens the rationale of the study. I suggest narrowing the context to conditions aligned to the experimental models used
3. SNL, SNI and CCI models recapitulate pain related to traumatic nerve injury but do not mimic several other clinically relevant disorders causing neuropathic pain (eg DN). This should be mentioned and discussed as a limitation.
4. ZFP612 expression is reported across any neuron subtypes (small, medium, large size), then specifically in CGRP, IB4, NF200-positive ones. The authors may like to comment on how ZFP612 knockdown enhance excitability in nociceptive neurons, given the in vivo effect.
5. The effect of ZFP612 delivery and silencing on astrocytes and microglia is nicely demonstrated. The authors may like to comment on the relevance of these data on the proposed DRG neuron-autonomous mechanism of neuropathic pain induction and hypersensitivity rescue. This would be in keeping with the bulk of literature indicating that neuropathic pain mechanisms rely on changes in both neurons and glial cells, and that microglia are quickly activated by DRG neuron-derived pro-inflammatory factor after peripheral nerve injury. Moreover, these data are confined in the extended figures and are quite difficult to be found out. I suggest reshaping the order of the paragraph and of the correspondent images to give emphasis to these findings.
6. Data are limited to 7 days post-injury except for one CCI experiment (ie 14 days), thus it is difficult to infer on chronic pain conditions. This should be mentioned as a limitation.
7. Does ZFP612 remain suppressed in chronic pain models? Is ZFP612 downregulation transient or irreversible? These points have implications for translational relevance and should be discussed.
8. Within the text, Pathogenic genes and Pathogenic immune genes are mentioned. Genes themselves are not inherently pathogenic; rather, it is their mutations, dysregulation, or aberrant expression that can lead to pathogenic effects. I suggest using the term “immunogenic genes” or “pro-inflammatory genes” if referring to inflammation rather than the immune response.
9. The discussion lacks mention of clinical translatability. Demonstrating the role of ZFP612 as an epigenetic regulator of IL1RL1 and its impact on neuropathic pain, along with evidence that restoring ZFP612 or modulating IL1RL1 alleviates pain in preclinical models, suggests the ZFP612/IL1RL1 axis as a potential therapeutic target. Additionally, this study may provide a mechanistic framework for novel biomarkers, such as ZFP612 downregulation in injured DRG neurons, which could aid in early diagnosis or patient stratification.

10. Figures contain an excessive number of images, making them compressed and leaving little space for headings. This makes it difficult for the reader to easily grasp the main information.
11. Check the text length according to NatComm guidelines: Methods are typically less than 3000 words. Figure legends are limited to 350 words each. As a guide, references should not exceed 70.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
The Authors identified zinc finger protein 612 (ZFP612) as a repressor of Interleukin 1 receptor-like 1 (Il1rl1) transcription in dorsal root ganglion (DRG) sensory neurons. Following nerve injury, the expression of ZFP612 is downregulated. This downregulation contributes to the activation of interleukin signaling and the development of neuropathic pain. Mechanistically, ZFP612 binds to a silencer region located between transcription start sites TSS1 and TSS2/3 in the Il1rl1 promoter. It recruits repressive epigenetic modifiers, such as HDAC1, HDAC2, and SETDB1, to the silencer-P3 loop, maintaining Il1rl1 in a silent state. The injury-induced downregulation of ZFP612 removes these repressive modifications, allowing for the transcription of the three Il1rl1 transcripts.

I would like to compliment the Authors on their well-designed and thorough experimental approach, which includes various positive and negative controls, a substantial amount of data derived from large input material, and the clarity of the manuscript. This work is original and emphasizes the role of chromatin architecture and epigenetic mechanisms in the neuronal response to injury. The conclusions drawn are well-supported by the data, with each claim backed by two independent loss- and gain-of-function approaches.

I do have a few comments:
- In Extended Fig. 1a, it would be helpful to visualize the neuron-specific expression of ZFP612 by plotting its expression in the UMAP plots under both sham and SNI conditions.

- Regarding Fig. 5b, it is unclear why the Authors chose a semiquantitative PCR to analyze the 3C interactions. Utilizing a qPCR approach (https://www.nature.com/articles/nprot.2007.243) would be a more effective strategy for detecting differences in interactions between conditions, as they may have overlooked changes in interactions induced by injury.

- In Fig. 3, the Authors used a reciprocal Co-IP approach to identify the formation of a repressive complex. While this method, along with the observation that the factors occupy the same genomic region, suggests the presence of a complex, it cannot be confirmed with certainty. A potential solution would be to conduct a re-ChIP experiment (https://pubmed.ncbi.nlm.nih.gov/22113276/). If this approach proves challenging due to the required starting input material, I suggest rephrasing this aspect or discussing it further in the discussion section.

- In Figs. 3h-j, the Authors employed siRNAs against HDAC1/2 and SETDB1. It may be beneficial to demonstrate the reduction of these proteins through Western blot analysis.

Minor points:
-On some occasions, the charts and heatmaps lack a legend, making it unclear whether we are viewing expression data as FPKM, relative expression, or z-scores. Examples include Fig. 1c, 1e, 1m, and Fig. 2b.
-In many charts, particularly those related to the manipulation of ZFP612 expression through gain or loss of function, the legends are spread beneath the different charts, making them difficult to locate.
- In line 290, the phrase should read “these repressive effectS.”

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed all the issue raised

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature

Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
This work is original and emphasizes the role of chromatin architecture and epigenetic mechanisms in the neuronal response to injury, the activation of immune-related genes, and neuropathic pain. The conclusions are supported by the data. It represents an important advancement to the field.

No revision required.

Response to the Reviewer #1 comments:

We appreciate the reviewer for the positive comments.

1) The title emphasizes 3D epigenomics while findings focus on local transcriptional repression (eg intronic silencer deletion, ZFP612-IL1RL1 axis) rather than genome-wide 3D chromatin reorganization. I suggest reframing at it may sound misleading.

We agree with the reviewer. Indeed, our findings focus on the local epigenetic repression of *Il1rl1* on the basis of the innate silencer-promoter loop rather than the genome-wide 3D chromatin reorganization. To avoid this misleading, we have reframed the title as follow: "ZFP612 controls neuropathic pain through epigenetic repression of *Il1rl1* within the silencer-promoter loop in primary sensory neurons" (page 1, lines 2-3).

2) The first part of the introduction makes overgeneralization. The opening conflates heterogeneous neurological disorders (eg diabetic neuropathy, GBS, neurodegenerative disorders) that do not share the same mechanisms underlying the occurrence of pain. Moreover, in these conditions pain can occur early (eg GBS) or be chronic (DN). References 1-11 and 13 seem misaligned. All this weakens the rationale of the study. I suggest narrowing the context to conditions aligned to the experimental models used.

We appreciate the reviewer's helpful suggestions. To avoid overgeneralization in the first part of the introduction, we have narrowed the context to peripheral nerve injury-induced neuropathic pain aligned with our experimental models (SNL, SNI, and CCI) used in this study, and carefully updated references to accurately support our focused narrative (page 2, lines 34-35; page 3, lines 53-54; page 4, lines 98-100).

3) SNL, SNI and CCI models recapitulate pain related to traumatic nerve injury but do not mimic several other clinically relevant disorders causing neuropathic pain (eg DN). This should be mentioned and discussed as a limitation.

We agree with the reviewer's suggestion. As reported in the previous literatures (PMID: 30887021; PMID: 35867896; PMID: 35304484), neuropathic pain induced by traumatic nerve injury, chemotherapy, diabetes mellitus, or other neurological diseases exhibits differential transcriptome profiles in DRG. This suggests that each type of neuropathic pain may have a unique and specific molecular basis for neuropathic genesis. ZFP612 is downregulated in response to traumatic nerve injury, however, the expression of ZFP612 in other pain models is not characterized in this study. This point has been acknowledged as a limitation and mentioned in the Discussion section (page 16, lines 455-458).

4) ZFP612 expression is reported across any neuron subtypes (small, medium, large

size), then specifically in CGRP, IB4, NF200-positive ones. The authors may like to comment on how ZFP612 knockdown enhance excitability in nociceptive neurons, given the in vivo effect.

We appreciate the reviewer's thoughtful suggestions. As shown in our results, both ZFP612 and its target IL1RL1 are broadly distributed across small-, medium-, and large-sized DRG neurons. IL1RL1 is well-documented for its effects on the activation of NF-κB, MAPKs, JAK2/STAT3 and subsequent the production of pro-inflammatory mediators like TNF-α, IL-1β and IL-6, which directly enhances the excitability of DRG neurons including nociceptive neurons (PMID: 34225736; PMID: 31087583; PMID: 26310268; PMID: 18427980), and/or on the inhibition of A-type potassium channels primarily in small-sized nociceptive neurons to facilitate the excitability (PMID: 35265208). Thus, the hyperexcitability of nociceptive neurons induced by ZFP612 downregulation likely results from the upregulation of IL1RL1 and its mediated neuro-inflammatory cascades and ion channels dysfunctions in DRG neurons.

In addition, it is proved that medium/large DRG neurons also undergo functional changes following nerve injury and contribute to neuropathic pain. The increased spontaneous ectopic activity was found in injured myelinated afferents and corresponding medium/large DRG neurons (PMID: 10899197; PMID: 10781925; PMID: 10196451). Silencing pain-associated genes (e.g., Nav 1.6, Kcna2 antisense RNA, NIS-lncRNA) mainly expressed in medium/large DRG neurons mitigated neuropathic pain and prevented abnormal spontaneous activity in injured medium/large DRG neurons (PMID: 23792947; PMID: 3577548; PMID: 25686526). CGRP and SP in injured myelinated fibers and medium/large DRG neurons were markedly increased as early as 2 days after nerve injury (PMID: 19242687; PMID: 16680762). Clearly, peripheral nerve injury also increases the excitability of medium/large DRG neurons, which drives the production of pro-inflammatory mediators and the release of neurotransmitters from primary afferents and leads to spinal cord dorsal horn central sensitization.

In summary, the nociceptive effect induced by ZFP612 downregulation may be attributed to the IL1RL1-mediated increased hyperexcitability of small/medium/large DRG neurons and the release of primary afferent transmitters, consequently resulting in central sensitization in the spinal dorsal horn. According to the reviewer's suggestion, we have expanded the discussion on these points in the revised manuscript (page 19, lines 543-550).

5) The effect of ZFP612 delivery and silencing on astrocytes and microglia is nicely demonstrated. The authors may like to comment on the relevance of these data on the proposed DRG neuron-autonomous mechanism of neuropathic pain induction and hypersensitivity rescue. This would be in keeping with the bulk of literature indicating that neuropathic pain mechanisms rely on changes in both neurons and glial cells, and that microglia are quickly activated by DRG neuron-derived pro-inflammatory factor after peripheral nerve injury. Moreover, these data are confined in the extended

figures and are quite difficult to be found out. I suggest reshaping the order of the paragraph and of the correspondent images to give emphasis to these findings.

We acknowledge the reviewer's helpful suggestions. Under neuropathic pain conditions, DRG neuron-derived pro-inflammatory mediators such as CSF1, CCL2 and ATP sequentially activate the spinal microglia and astrocytes, synergistically promoting central sensitization (PMID: 26642091; PMID: 27811267). The anti-nociceptive effect produced by ZFP612 overexpression in neuropathic pain likely results from the silencing of IL1RL1 and subsequent inactivation of neuro-inflammatory cascades in injured DRG neurons. These inactive signaling pathways in injured sensory neurons cause the reduction in the production of pro-inflammatory mediators and the decrease in the release of primary afferent transmitters, further resulting in the attenuation of central sensitization in spinal dorsal horn. In support of this conclusion, we found that rescuing ZFP612 downregulation in injured DRG relieved SNL-induced hyperactivation in spinal dorsal horn microglia and astrocytes. This point has been comprehensively discussed in the Discussion section (page 19-20, lines 552-562). Moreover, to give emphasis to these findings, we have placed the results regarding ZFP612-mediated spinal glial activities from Extended Data to the current Fig. 2g,k, and further reshaped the order of the sentences in the Results section correspondingly (page 7, lines 183-185; page 7, lines 197-198).

6) Data are limited to 7 days post-injury except for one CCI experiment (ie 14 days), thus it is difficult to infer on chronic pain conditions. This should be mentioned as a limitation.

We acknowledge the reviewer's useful feedback. Peripheral nerve injury-induced nociceptive hypersensitivity occurs at 2-3 days post-surgery, reach a peak around 7 days post-surgery and persists for at least 3 months in preclinical animal models of neuropathic pain. We chose 7 days post-surgery for mechanistic studies. At this time point, nociceptive hypersensitivity reaches a peak, which represents typical molecular/biological changes in injured DRG. We expect similar mechanisms to occur in later chronic phase beyond 7 days post-surgery, as ZFP612 is downregulated in injured DRG time-dependently from day 3 to day 14 post-surgery in this study and maintains downregulated over 28 days evidenced by the sequencing data (PMID: 30335683). Although the 7-day post-injury time point captures main features of the development and maintenance of neuropathic pain (PMID: 23792947; PMID: 29682596), its utility in inferring on long-term chronic pain conditions remains limited. We have acknowledged 7-day limitation and discussed this point in the Discussion section (page 19, lines 539-542).

7) *Does ZFP612 remain suppressed in chronic pain models? Is ZFP612 downregulation transient or irreversible? These points have implications for translational relevance and should be discussed.*

Indeed, ZFP612 remains suppressed during the development and maintenance of neuropathic pain, including 3, 7, and 14 days after traumatic nerve injury, and such suppression even shows no signs of recovery as evidenced by the sequencing data that Zfp612 maintains downregulated over 28 days after nerve injury (PMID: 30335683). This sustained downregulation suggests that nerve injury may induce irreversible suppression of ZFP612. However, we acknowledge that ZFP612 expression in later chronic phase beyond 14 days post-injury warrants further confirmation and whether ZFP612 is also suppressed in other chronic pain models remains unclear. This limitation has been explicitly discussed in the Discussion section in our revised manuscript (page 16, lines 452-455).

8) *Within the text, Pathogenic genes and Pathogenic immune genes are mentioned. Genes themselves are not inherently pathogenic; rather, it is their mutations, dysregulation, or aberrant expression that can lead to pathogenic effects. I suggest using the term "immunogenic genes" or "pro-inflammatory genes" if referring to inflammation rather than the immune response.*

We sincerely appreciate the reviewer's insightful comment regarding the precise use of genetic terminology. We agree that genes themselves are not intrinsically pathogenic, but rather their dysregulation can lead to disease phenotypes. We have replaced the terms "Pathogenic genes" and "Pathogenic immune genes" throughout the manuscript with the more accurate descriptor "immunogenic genes", which better reflects their role in inflammation-related pathogenesis (page 3, lines 75-77).

9) *The discussion lacks mention of clinical translatability. Demonstrating the role of ZFP612 as an epigenetic regulator of IL1RL1 and its impact on neuropathic pain, along with evidence that restoring ZFP612 or modulating IL1RL1 alleviates pain in preclinical models, suggests the ZFP612/IL1RL1 axis as a potential therapeutic target. Additionally, this study may provide a mechanistic framework for novel biomarkers, such as ZFP612 downregulation in injured DRG neurons, which could aid in early diagnosis or patient stratification.*

We sincerely thank the reviewer for highlighting the clinical implications of our findings. As suggested, we have now expanded the discussion on the translational potential of the ZFP612/IL1RL1 axis in neuropathic pain management. Specifically, we discuss:

Therapeutic Targeting: Given the epigenetic regulation of IL1RL1 by ZFP612, pharmacological or genetic approaches to restore ZFP612 (e.g., gene therapy, small-molecule activators) or inhibit IL1RL1 (e.g., neutralizing antibodies, receptor antagonists) could represent novel therapeutic strategies for neuropathic pain (page 20, lines 562-563; page 20, lines 572-575).

Biomarker Potential: We further emphasize that ZFP612 downregulation in injured DRG neurons may serve as a diagnostic biomarker for early nerve injury detection or a predictive biomarker for patient stratification (page 16, lines 449-450).

This could facilitate personalized treatment strategies in clinical settings.

10) *Figures contain an excessive number of images, making them compressed and leaving little space for headings. This makes it difficult for the reader to easily grasp the main information.*

We appreciate the reviewer's valuable feedback regarding figure presentation. We acknowledge that some figures appear overly compressed due to the inclusion of multiple panels. To improve clarity and readability, we have implemented the following modifications in the revised manuscript:

1. Splitting Fig. 1 (including both expression, distribution, and function of ZFP612) into separate figures (now designated as Fig. 1 (primarily containing the expression and distribution data for ZFP612) and Fig. 2 (primarily containing the phenotype data)).
2. Splitting Fig. 1k (containing both the monkey and human data) into separate panels (now designated as Fig. 1j (for monkey) and Fig. 1k (for human being).
3. Consolidating repetitive experimental results, like consolidating Extended Data Fig. 1j,k into current Extended Data Fig. 1j, Extended data Fig. 4a,b into current Extended Data Fig. 4a.
4. Moving less critical data like the validation of *Zfp612* mRNA timecourse from Fig. 1f to supplementary materials (current Extended Data Fig. 1e).
5. Increasing white space between panels and enlarging key labeling and headings throughout the Figures and Extended Data Figures.

These adjustments have significantly improved figure legibility while maintaining all essential information. We believe the revised versions will greatly facilitate readers' understanding of our key findings.

11) *Check the text length according to NatComm guidelines: Methods are typically less than 3000 words. Figure legends are limited to 350 words each. As a guide, references should not exceed 70.*

We appreciate the reviewer's careful remind of the journal's formatting requirements. For the Methods section, it has been thoroughly edited to maintain conciseness while preserving all critical technical details and make the word count of the Methods close to 3000 words. For the Figure legends, we have systematically revised all legends to enhance clarity while strictly adhering to the 350-word limit. For the References, we carefully curated the reference list to include only the most essential citations and controlled it well within the 70-reference limit.

Response to the Reviewer #2 comments:

We thank the co-reviewer for the time and suggestions for our paper.

Response to the Reviewer #3 comments:

We thank the reviewer for the positive comments.

In Extended Fig. 1a, it would be helpful to visualize the neuron-specific expression of ZFP612 by plotting its expression in the UMAP plots under both sham and SNI conditions.

We appreciate the reviewer's helpful suggestion. We have plotted the neuron-specific expression of Zfp612 in the UMAP plots under both sham and SNI conditions (now presented in Extended Data Fig. 1c) and added it in the Results section (page 6, line 142).

Regarding Fig. 5b, it is unclear why the Authors chose a semiquantitative PCR to analyze the 3C interactions. Utilizing a qPCR approach (https://www.nature.com/articles/nprot.2007.243) would be a more effective strategy for detecting differences in interactions between conditions, as they may have overlooked changes in interactions induced by injury.

We agree with the reviewer's insightful suggestion. 3-C technology identifies chromatin interaction sites by quantifying ligation frequencies between an anchor fragment and multiple candidate genomic fragments, with the highest interaction frequency among the neighbored fragments indicating the most significant spatial proximity. Compared to the traditional semi-quantitative PCR widely used in 3-C (PMID: 36697821; PMID: 29648668; PMID: 20360044; PMID: 31848476), the real-time TaqMan PCR technology offers superior sensitivity for detecting interaction differences with available locus-specific primers and probes (PMID: 17641637). Particularly, the requirement for the specificity of 3C-qPCR primers targeting each fragment is extremely strict, ensuring no non-specific amplification products from the genomic template (PMID: 17971780; PMID: 17641637). However, due to the complexity of the reconstructed 3-C library after DRG genome digestion and re-ligation, it is an uncertainty that all the primers are capable of amplifying its re-ligated fragments specifically. Under this context, it is suggested that semi-quantitative PCR be used to analyze the expected PCR product for accurate comparison between different loci after normalization (PMID: 22903059; PMID: 17641637). Indeed, during our designing primers for the fragments from I to IX of *Il1rl1* genome, some 3-C primer such as primer I inevitably generated weak non-specific products. To ensure the expected PCR products from fragment I to IX between conditions comparable, semi-quantitative PCR was eventually adopted for 3-C analyses based on the established protocols (PMID: 11847345; PMID: 36697821; PMID: 22903059). We have acknowledged semi-quantitative PCR limitation and discussed this point in the Discussion section (page 19, lines 529-532).

In Fig. 3, the Authors used a reciprocal Co-IP approach to identify the formation of a

repressive complex. While this method, along with the observation that the factors occupy the same genomic region, suggests the presence of a complex, it cannot be confirmed with certainty. A potential solution would be to conduct a re-ChIP experiment (https://pubmed.ncbi.nlm.nih.gov/22113276/). If this approach proves challenging due to the required starting input material, I suggest rephrasing this aspect or discussing it further in the discussion section.

We acknowledge the reviewer's useful feedback. Although our reciprocal Co-IP and ChIP assays strongly suggest the presence of the ZFP612 repressive complex in the same genomic region, we agree that re-ChIP assay would be a better solution to validate this conclusion. However, due to the small size of mouse DRG, the starting input material for each ChIP experiment requires at least 6 DRGs per group/repeat. Accordingly, the starting material for the re-ChIP experiment would be approximately 60 DRGs per group/repeat (PMID: 22113276), and this is a great challenge with the sacrifice of several hundreds of mice. Following the reviewer's suggestion, this point has been mentioned and discussed in the Discussion section (page 18, lines 508-510).

In Figs. 3h-j, the Authors employed siRNAs against HDAC1/2 and SETDB1. It may be beneficial to demonstrate the reduction of these proteins through Western blot analysis.

We appreciate the reviewer's valuable suggestion regarding the validation of siRNA knockdown efficiency. We have now included Western blot analyses demonstrating the significantly reduced levels of HDAC1, HDAC2, and SETDB1 proteins following siRNA treatment (see Extended Data Fig. 8a-c). The corresponding description has been added to the Results section (Page 11, lines 314-315).

Minor points:

On some occasions, the charts and heatmaps lack a legend, making it unclear whether we are viewing expression data as FPKM, relative expression, or z-scores. Examples include Fig. 1c, 1e, 1m, and Fig. 2b.

We agree with the reviewer's helpful suggestions. We have systematically reviewed all Figures and ensured that proper legends are now included for all charts and heatmaps in the Panel or its Figure Legend, including:

Included clear labeling of **Relative expression** in Fig. 1c;

Included clear labeling of **Log-normalized expression** in Fig. 1e;

Included clear labeling of **Z-score** scales in Fig. 1m;

Included clear labeling of **Normalized read coverages** in Fig. 3a;

Added **relative expression** in the Figure Legend of Fig. 3b (Page 42, line 1182-1183);

Included clear labeling of **unique molecular identifier (UMI) counts** in Extended Data Fig. 1c.

In many charts, particularly those related to the manipulation of ZFP612 expression through gain or loss of function, the legends are spread beneath the different charts, making them difficult to locate.

According to the reviewer's suggestion, we have consolidated the scattered legends beneath different charts (3 charts) into one chart throughout the Figures and Extended Data Figures to make them easy to locate, including:

Fig. 2a-c (ZFP612 gain-of-function experiments);
Fig. 2d-f (ZFP612 gain-of-function experiments);
Fig. 2h-j (ZFP612 loss-of-function experiments);
Fig.2p-r (ZFP612 and IL1RL1 loss-of-function experiments);
Fig.3l-n (Silencer loss-of-function experiments);
Extended Data Fig.2e-g (ZFP612 gain-of-function experiments);
Extended Data Fig. 2h-j (ZFP612 gain-of-function experiments);
Extended Data Fig. 3c-e (ZFP612 loss-of-function experiments);
Extended Data Fig. 5a-c (IL1RL1 loss-of-function experiments);
Extended Data Fig. 5f-h (IL1RL1 loss-of-function experiments);
Extended Data Fig. 5i-k (IL1RL1 gain-of-function experiments);
Extended Data Fig. 7c-e (Silencer loss-of-function experiments);
Extended Data Fig. 7j-l (Silencer loss-of-function experiments).

In line 290, the phrase should read "these repressive effectS.".

We appreciate the reviewer's careful check. We have corrected the specific phrase to "these repressive effects" in page 10, line 285 and thoroughly examined the verb tense, person, and number agreement to ensure grammatical consistency throughout the manuscript.