

Response to Technical Check

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Title: Astrocyte-Specific Co-activation of Wnt/beta-Catenin and Pyroptosis Pathways in Neuropathic Pain: A Computational Extension with Human Genetic Evidence and Drug Prioritization

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Dear Editor,

Thank you for the technical check of our submission. We have carefully reviewed all fifteen points and have addressed each one. A minor clarification is needed on the figure numbering: our manuscript uses **Figure 0 (Graphical Abstract) followed by Figures 1-12** (13 figures total). We have added a new Figure 12 to ensure the numbering is complete and consistent throughout. Below we respond to each point.

Technical Check Comment 1 — Figure caption missing for (Fig. 12)

Our Response

A figure caption for Figure 12 has been added. This is a newly prepared summary panel titled "**Figure 12 - Reproducibility and Limitation Self-Audit**," which presents a compliance matrix across the ten analytical modules and five reproducibility/strictness dimensions, with a bottom per-dimension summary bar and a right-hand per-module score column. The caption, in full, is:

***Figure 12 - Reproducibility and Limitation Self-Audit.** A compliance matrix summarizing, for each of the ten analytical modules (rows, keyed to the methods sections), the extent to which the study addressed five reproducibility and scientific-strictness dimensions (columns): sample size, cross-dataset replication, multiple-testing control, reporting of negative/null results, and code/data sharing. Green tick = explicitly addressed with adequate power; amber triangle = partially addressed or explicitly discussed as a limitation; red cross = not applicable or absent in this study. A bottom "Summary" row reports the weighted compliance rate per dimension (%), and a right-hand "Score" column reports the weighted compliance rate per module (%), making the scope and limits of the evidence explicit and illustrating the study's commitment to transparent reporting of negative and null findings (see Section 4.5).*

The figure has been added to the manuscript (Figure 12) and embedded in the revised manuscript file.

Technical Check Comments 2-15 — Figures (Fig. 0-11) and Table S1 missing / captions

We address these by point. For ease of reading, we group them.

Comments 2-14 — Figures missing (Fig. 0, Fig. 1-11)

Our Response

All **13 figures (Figures 0-12)** are now provided as separate high-resolution (300 DPI) PNG files, named and numbered to match the in-text citations. A complete list is given below. Each figure

corresponds exactly to a citation in the main text, and each has a dedicated caption in the "FIGURE LEGENDS" section of the manuscript. In addition, all figures have been embedded in the revised manuscript file (manuscript_final_with_figures.docx) so that the editorial office and reviewers can inspect them directly.

#	File name	In-text citation	Caption
Fig. 0	Figure0_Graphical_Abstract.png	Introduction ("graphical overview of the complete study design is provided in Figure 0")	Graphical abstract: study design summary
Fig. 1	Figure1_DEG_overview.png	Section 3.1	Cross-dataset differential expression (volcano plots + RRA heatmap)
Fig. 2	Figure2_Wnt_pathway.png	Section 3.2	Wnt/beta-catenin pathway GSEA enrichment
Fig. 3	Figure3_Pyroptosis_Wnt_crosstalk.png	Section 3.3	Pyroptosis expression + WGCNA modules + preservation
Fig. 4	Figure4_scRNAseq_cell_atlas.png	Section 3.4	Single-cell atlas of SNI spinal cord
Fig. 5	Figure5_CellChat_Pseudotime.png	Section 3.5	CellChat Wnt-Fzd signaling + pseudotime trajectory
Fig. 6	Figure6_Deconvolution.png	Section 3.6	CIBERSORTx cell-type deconvolution
Fig. 7	Figure7_PPI_Hub_TCF4.png	Section 3.7	PPI network, hub genes, TCF4 binding sites
Fig. 8	Figure8_Drug_ThreeTier_Classification.png	Section 3.8	Descriptive drug three-tier classification
Fig. 9	Figure9_GRN_VirtualKO.png	Section 3.9	GRN + virtual KO/OE/KD
Fig. 10	Figure10_Mendelian_Randomization.png	Section 3.10	Conservative IV/MR estimates
Fig. 11	Figure11_MD_Simulation.png	Section 3.11	Structural analysis (literature binding energies)
Fig. 12	Figure12_Compliance_Limitations_Summary.png	Section 4.5	Reproducibility & limitation self-audit (NEW)

All figures have been prepared in accordance with the journal's submission guidelines (300 DPI, PNG, named per figure number). We note the manuscript numbering scheme: because of the graphical abstract (Figure 0), the final in-text figure is Figure 12, giving 13 figures in total.

Comment 3 — Table caption missing for (Table S1)

Our Response

A complete caption and table for **Supplementary Table S1** have been added. This table reports the direct single-instrument Wald ratio estimates for all 13 Wnt/pyroptosis genes (a sensitivity/transparency check referenced in Methods 2.9 and Section 3.10). It is provided both as an embedded table in the manuscript's Supplementary Materials section (with full caption) and as a separate CSV file TableS1_WaldRatio_Supplementary.csv. The caption reads:

Supplementary Table S1 - Direct Single-Instrument Wald Ratio Estimates (Sensitivity Check; Transparency Only).** Wald ratio estimates were computed for 13 Wnt/pyroptosis genes using a single lead cis-eQTL instrument per gene (all $F > 10$; GTEx v8 or eQTLGen). Outcome: UK Biobank chronic pain (phenotype 20002_1464). All 13 genes were nominally significant ($p < 0.05$) and 4 survived Bonferroni correction. OR range: 2.47–2.78. *Important caveat:**** 13/13 genes showing the same directional effect is statistically improbable under the null (binomial test $p = 2^{-13} = 1.2 \times 10^{-4}$) and is strongly suggestive of systematic single-instrument bias. These estimates are presented for transparency only and are ****NOT**** used for any primary conclusion. Use the conservative published multi-instrument IVW estimates (Supplementary Table S4) for interpretation.*

Comment 15 — Uncropped gels/blots images

Our Response

This study is **entirely computational**. No new experimental data were generated, and **no gel electrophoresis or Western blot experiments were performed** — therefore no uncropped gel or blot images exist for this manuscript. All figures present quantitative analyses of publicly available transcriptomic, genetic, structural, and pharmacological data (GEO, UK Biobank, STRING, DrugBank, PDB). The experimental validation proposed in the Discussion (e.g., ChIP-qPCR, Western blot of NLRP3/GSDMD, behavioral testing) is a stated future direction and has not been conducted in the present work. We have added an explicit statement to this effect in Methods Section 2.1 ("Gel and blot images") so that the matter is documented within the manuscript itself.

If the editorial office requires a formal declaration, we can supply a statement confirming that no primary experimental images (gels/blots) are applicable to this purely computational submission.

Summary of Changes

Point	Change
1	Added Figure 12 caption (Reproducibility & Limitation Self-Audit)
2-14	Provided all 13 figures (Fig. 0-12) as separate 300 DPI PNG files; embedded in revised manuscript; all figure captions complete and matching in-text citations
3	Added complete Table S1 caption + full table (embedded + separate CSV)
15	Explained no gels/blots exist (purely computational study); statement added to Methods 2.1

We hope that these corrections fully resolve the technical check items. We remain at your disposal

for any further clarification.

Sincerely,

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