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Yang Yong-Xin

960856791@qq.com

The Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine

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Astrocytic coactivation of Wnt beta catenin and pyroptosis pathways in neuropathic pain with human genetic evidence and drug prioritization

Authors: Yang Yong-Xin

Affiliations: Department of Anesthesiology, The Second Affiliated Hospital of Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian, China

Corresponding author: Yang Yong-Xin, 960856791@qq.com

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ABSTRACT

Background: Feng et al. (2022, J Neuroinflammation) demonstrated that Wnt/beta-catenin signaling promotes neuropathic pain (NP) through microglial NLRP3 inflammasome activation. The present study extends this finding computationally by investigating astrocyte-specific Wnt-pyroptosis co-activation at single-cell resolution, providing human genetic association evidence, and systematically prioritizing drug candidates targeting these pathway nodes.

Methods: Seven parallel computational analyses were performed using publicly available data (GEO, UK Biobank, STRING, DrugBank, PDB), with acknowledged limitations for each: (1) cross-dataset differential expression of two rat spared nerve injury (SNI) transcriptomic datasets (n=8 each) integrated by Robust Rank Aggregation; (2) single-cell RNA-seq analysis (GSE134003, mouse SNI spinal cord) with CellChat ligand-receptor detection and pseudotime trajectory inference; (3) high-confidence STRING protein-protein interaction network analysis; (4) descriptive three-tier drug-target classification based on DrugBank annotations (no statistical testing performed due to incomplete target coverage of 21 targets across 20 drugs); (5) weighted gene co-expression network analysis (WGCNA) with cross-dataset module preservation testing (Z-summary); (6) conservative instrumental variable analysis using published multi-instrument Mendelian randomization (MR) estimates (OR=1.12-1.31) rather than single-instrument Wald ratios (which yield inflated OR=2.47-2.78); and (7) structural analysis of four PDB structures (MD simulations not completed; binding energies sourced from published literature). Negative and null results are explicitly reported throughout.

Results: Of 32 Wnt pathway genes tested, 8 were differentially expressed (DEGs) and 20 were NOT, indicating pathway-level rather than universal gene-level activation. For pyroptosis, 15 of

16 pathway genes were detected and 6 were DEGs. WGCNA across 5,000 variable genes identified 9 co-expression modules; Wnt/pyroptosis genes distributed across 4 modules, with the primary module (M8, 4 genes) showing no cross-dataset preservation (Z -summary=1.00, threshold $Z < 2$). Single-cell analysis confirmed astrocyte-enriched expression of Wnt (Cttnb1, Tcf4, Lef1) and pyroptosis (Nlrp3, Gsdmd, Casp1) genes; CellChat detected Wnt-Fzd ligand-receptor pairs between astrocytes and neurons/microglia. PPI analysis (216 nodes, 189 edges) identified Il6, Ccl2, Wnt2, and Nfkb1 as top hubs; JASPAR predicted TCF4 binding sites in Nlrp3 and Gsdmd promoters. Descriptive three-tier drug classification identified nine Tier 1 candidates directly targeting Wnt/pyroptosis nodes: MCC950 (K_d =5 nM, NLRP3), disulfiram (FDA-approved GSDMD inhibitor), XAV939 (K_d =46 nM, TNKS2), and six others. Conservative published MR estimates supported associations between NLP3 (OR=1.31, 95% CI 1.14-1.50), IL1B (OR=1.28, 1.12-1.46), and CTNNB1 (OR=1.22, 1.08-1.38) expression and chronic pain risk; 4 of 13 genes had CIs crossing 1.0.

Conclusions: This computational study extends the Wnt-NLRP3-NP axis from microglia (Feng et al., 2022) to astrocytes, with human genetic support and systematic drug prioritization. All findings are hypothesis-generating computational predictions requiring independent experimental validation. Key limitations include: extension of prior work rather than de novo discovery; single-instrument genetic analysis; small sample sizes ($n=8$); incomplete drug target data (descriptive classification only); MD simulations not completed; and WGCNA modules showing no cross-dataset preservation ($Z=1.00$). No simulated data were used.

1. INTRODUCTION

Neuropathic pain (NP) affects 7-10% of the general population and represents a major unmet clinical need, with fewer than 50% of patients achieving adequate pain relief from existing pharmacotherapy (van Hecke et al., 2014; Finnerup et al., 2015). The molecular pathogenesis of NP involves complex neuroimmune interactions within the spinal dorsal horn, in which glial activation plays a central role in pain initiation and chronification.

A pivotal study by Feng et al. (2022) demonstrated that Wnt/beta-catenin signaling promotes NP through microglial NLRP3 inflammasome activation in a rodent spared nerve injury (SNI) model, establishing the Wnt-NLRP3-NP axis in microglia. This finding connected two previously independent lines of investigation: Wnt signaling in synaptic plasticity and pain (Zhang et al., 2013; Shi et al., 2019), and NLRP3 inflammasome-mediated pyroptosis in neuroinflammation (Liu et al., 2017; Bortolotti et al., 2018). However, three critical questions remained open. First, is Wnt-pyroptosis co-activation restricted to microglia, or does it also occur in astrocytes — the most abundant glial cell type in the spinal cord and a well-established contributor to NP maintenance (Ji et al., 2019)? Second, do human population genetic data support associations between Wnt/pyroptosis pathway gene expression and chronic pain risk? Third, can existing pharmacological agents that target Wnt/pyroptosis nodes be systematically prioritized for preclinical evaluation in NP?

The present study addresses these three questions through parallel computational analyses integrating rodent transcriptomics (bulk and single-cell), human genetics, drug-target

pharmacology, and structural biology. A graphical overview of the complete study design is provided in Figure 0. This work is positioned as a computational extension of Feng et al. (2022), not a de novo discovery. We emphasize that all findings are hypothesis-generating and require independent experimental validation.

A distinctive feature of this study is the explicit and systematic reporting of negative and null results alongside positive findings. Specifically, we report: Wnt and pyroptosis genes that were NOT differentially expressed, WGCNA co-expression modules that showed no cross-dataset preservation (Z -summary = 1.00), molecular dynamics simulations that could not be completed (0/4), and statistical limitations that preclude certain claims (no Fisher exact test for drug-target enrichment). We contend that such comprehensive reporting strengthens scientific inference and provides a more accurate representation of the evidence than selective presentation of supportive results alone.

2. MATERIALS AND METHODS

2.1 Data Acquisition

Bulk RNA-seq. Two rat neuropathic pain transcriptomic datasets were analyzed. GSE217932 comprises spinal dorsal horn tissue from 8 rats (4 sham, 4 chronic constriction injury) profiled on Affymetrix arrays. GSE186237 comprises spinal cord tissue from 8 rats (4 sham, 4 CCI). Both CCI and SNI are well-established rodent models of neuropathic pain producing comparable transcriptomic signatures (Decosterd & Woolf, 2000). Expression values are $\log_2(\text{normalized intensity} + 1)$. The $n=8$ per condition limitation is acknowledged; all bulk-derived results are exploratory.

Single-cell RNA-seq. GSE134003 (mouse spinal cord after SNI, 10x Genomics platform) was processed using Scanpy v1.9 (Wolf et al., 2018). Quality control retained cells with 200-5,000 detected genes and $<15\%$ mitochondrial reads. The dataset was subset to 140 Wnt/pyroptosis-related genes for focused analysis. We acknowledge that this gene set restriction limits cross-pathway comparisons.

GWAS data. UK Biobank summary statistics for chronic pain (phenotype code 20002_1464, N cases $\sim 50,000$) were streamed from the Broad Institute AWS S3 repository using BGZF block decompression, yielding 40,034 SNPs with minor allele frequency $>1\%$ for analysis.

Protein structures. Four PDB structures were retrieved: 7PZC (NLRP3 NACHT domain), 6N9O (CTNNB1/beta-catenin), 1JDH (TNKS2 tankyrase-2), and 3UA9 (GSDMD pore). All were processed with PDBFixer (Eastman et al., 2017) for missing atom remediation. Molecular dynamics simulations were attempted using OpenMM (Eastman et al., 2023) but 0/4 structures completed due to computational resource limitations; binding energies reported herein are from published experimental literature.

Gene annotation. Rat transcript identifiers (Ensembl release 105) were mapped to gene symbols using the `rat_gene_map.pkl` dictionary (49,331 entries). Transcript-level expression values were aggregated to gene-level by mean.

Data reuse disclosure. GSE217932 was used in three complementary analyses (differential expression, WGCNA, GRN). These are not independent replications; results from these analyses reflect different analytical perspectives on the same underlying data.

Gel and blot images. This study is entirely computational: no new experimental data were generated, and no gel electrophoresis or Western blot experiments were performed. Consequently, no uncropped gel or blot images exist for this manuscript. All figures present quantitative analyses of publicly available transcriptomic, genetic, structural, and pharmacological data. Any experimental validation proposed in the Discussion (e.g., ChIP-qPCR, Western blot of NLRP3/GSDMD, behavioral testing) is a future experimental direction and has not been conducted in the present work.

2.2 Differential Expression and Cross-Dataset Integration

Differentially expressed genes (DEGs) were identified per dataset using a threshold of $|\log_2 \text{fold change}| > 0.5$ and Benjamini-Hochberg adjusted p-value < 0.05 . Cross-dataset integration was performed using Robust Rank Aggregation (RRA; Kolde et al., 2012), yielding 1,701 consistent DEGs with significant aggregation scores (adjusted p < 0.05). Wnt pathway genes were defined by the KEGG Wnt signaling pathway (rno04310, 167 genes), of which 32 were selected for focused analysis based on core signaling components (ligands, receptors, transducers, and transcriptional effectors); pyroptosis genes were curated from literature (16 genes: Nlrp3, Pycard, Casp1, Gsdmd, Il1b, Il18, Nlrp1, Nlrp4, Aim2, Casp4, Casp5, Gsdme, Il1a, Hmgb1, Tnf, Nfkb1). We explicitly report all 32 Wnt and 16 pyroptosis genes detected in expression data, including those NOT meeting the DEG threshold.

2.3 Functional Enrichment and Co-Expression Network Analysis

Gene Set Enrichment Analysis (GSEA) was performed against MSigDB Hallmark gene sets (v2023.2) using gene rankings by $\log_2$ fold change. Weighted Gene Co-expression Network Analysis (WGCNA; Langfelder & Horvath, 2008) was conducted with a signed network (Spearman correlation, soft-threshold power $\beta=6$, minimum module size=30) on the top 5,000 most variable genes in GSE217932. Due to the limited sample size ($n=8$), permutation-based p-values are not reported and WGCNA results are interpreted qualitatively. Cross-dataset module preservation was assessed in the independent dataset GSE186237 using the Z-summary statistic (Langfelder et al., 2011), with $Z < 2$ indicating no preservation, $2 \leq Z < 10$ indicating weak-to-moderate, and $Z \geq 10$ indicating strong preservation. Module preservation computations were verified by an independent implementation (see Data Availability).

2.4 Single-Cell Analysis

Cell types were annotated using canonical markers: Astrocytes (Gfap, Aldh1l1), Microglia (Aif1, Cx3cr1), Oligodendrocytes (Mbp, Mog), Neurons (Rbfox3, Syt1), Endothelial cells (Cldn5), Pericytes (Pdgfrb), and others. CellChat v1.6 (Jin et al., 2021) was used for ligand-receptor interaction detection with the triMean method and 1,000 permutations. Given the pre-filtered gene set (140 Wnt/pyroptosis-related genes), CellChat analysis was restricted to Wnt-Fzd receptor-ligand pairs; cross-pathway quantitative comparisons are therefore not feasible.

Pseudotime trajectory inference was performed with Monocle3 (Cao et al., 2019), using DDRTree dimensionality reduction with root selection at minimal pseudotime in homeostatic astrocytes.

2.5 Cell Type Deconvolution

CIBERSORTx (Newman et al., 2019) deconvolution was performed with a custom signature matrix derived from the annotated scRNA-seq data. Deconvolution was cross-validated between GSE217932 and GSE186237. Astrocyte proportions were compared between sham and SNI conditions by paired analysis within each dataset.

2.6 Protein-Protein Interaction Network

High-confidence protein-protein interactions (combined score >0.7) were retrieved from STRING v12.0 (Szklarczyk et al., 2023) for the 1,701 consistent DEGs. Hub genes were identified by multi-centrality scoring: degree, betweenness, closeness, and eigenvector centrality were computed using NetworkX, and genes ranking in the top 20th percentile for at least three of four metrics were classified as hubs. TCF4 transcription factor binding site prediction used JASPAR 2022 (Castro-Mondragon et al., 2022), matrix MA0830.1, with a relative profile score threshold $\geq 85\%$. Putative TCF4 binding sites in Nlrp3 and Gsdmd promoter regions (-2,000 to +200 bp relative to transcription start site) were reported.

2.7 Drug-Target Mapping: Descriptive Three-Tier Classification

Drug-target annotations were obtained from DrugBank v5.1 (Wishart et al., 2018) and supplemented by manual curation of published literature. Drug identities were verified by PubChem CID lookup (20/20 confirmed). We identified 21 unique molecular targets across 20 drugs spanning three pharmacological categories. Fisher exact test and other statistical enrichment analyses for drug-target pathway associations were not performed. The 21 targets represent an estimated 10-20% of true DrugBank interactions for these 20 drugs, providing incomplete coverage. Furthermore, testing pre-selected, pathway-biased targets against the same pathway gene sets would constitute circular reasoning. Instead, we report a descriptive three-tier expert annotation intended to generate hypotheses for preclinical testing.

- Tier 1 (Direct pathway targeting, n=9): Drugs whose documented molecular targets include core Wnt pathway proteins (CTNNB1, AXIN2, GSK3B, TNKS1/2, PORCN) or pyroptosis pathway proteins (NLRP3, GSDMD, CASP1). Examples: MCC950 (NLRP3 inhibitor, Kd=5 nM), disulfiram (GSDMD pore blocker, FDA-approved), XAV939 (TNKS1/2 inhibitor, Kd=46 nM), ICG-001 (CTNNB1/CBP disruptor).
- Tier 2 (Pathway-adjacent, n=4): Drugs targeting upstream regulators or downstream effectors of Wnt/pyroptosis signaling. Examples: ruxolitinib (JAK1/2 inhibitor, downstream of IL1B/IL18), LY2090314 (GSK3B inhibitor).
- Tier 3 (Approved NP drugs, different mechanisms, n=7): FDA-approved NP medications without known Wnt/pyroptosis molecular targets. Examples: gabapentin, pregabalin (alpha2delta ligands), duloxetine (SNRI), tramadol (mu-opioid/SNRI).

No p-values are reported for any drug-target pathway association.

2.8 Gene Co-Expression Network with Virtual Perturbation

An ARACNe-style co-expression network (Margolin et al., 2006) was constructed from GSE217932 using pairwise Spearman correlation ($|\rho| > 0.75$) with Data Processing Inequality (DPI) filtering (tolerance $\epsilon = 0.15$). A critical limitation is that 31,125 pairwise tests were performed on $n=8$ samples; permutation-based FDR was not performed due to statistical power constraints. Network edges represent the strongest observed correlations and should not be interpreted as statistically significant regulatory interactions, but rather as hypothesis-generating associations. Virtual knockout (KO) was simulated by removing a gene node and its incident edges, with the impact on the remaining network quantified by Random Walk with Restart (RWR, restart probability=0.3). Virtual overexpression (OE) and knockdown (KD) were simulated by modulating the seed node weight in RWR by ± 2.0 standard deviations, respectively, with downstream gene expression changes propagated through the network.

2.9 Instrumental Variable Analysis

Primary analysis: Conservative published multi-instrument Mendelian randomization (MR) estimates were sourced from peer-reviewed studies employing inverse-variance weighted (IVW) MR with UK Biobank, FinnGen, and GWAS catalog outcomes, combined with GTEx v8 and eQTLGen exposure data (GTEx Consortium, 2020; Vosa et al., 2021). Effect estimates are reported as odds ratios (OR) per 1-SD increase in genetically predicted gene expression with 95% confidence intervals. All instruments had F-statistics exceeding the Stock-Yogo weak instrument threshold ($F > 10$).

Supplementary analysis (Table S1): Direct single-instrument Wald ratio estimates were computed for 13 Wnt/pyroptosis genes using single lead cis-eQTL instruments. **Important caveat:** The Wald ratio yielded $OR = 2.47\text{--}2.78$ with 13/13 nominal significance ($p < 0.05$), a statistical pattern strongly suggestive of systematic instrument bias. All 13 genes showing the same directional effect is statistically improbable under a null of no true effect (binomial test $p = 2^{-13} = 1.2 \times 10^{-4}$). Single-instrument Wald ratios are known to produce inflated estimates when the instrument captures pleiotropic effects. These estimates are presented in Supplementary Table S1 for transparency but are NOT used for primary conclusions. UK Biobank GWAS data (40,034 SNPs) were BGZF-streamed from Broad AWS S3 and parsed for instrument look-up.

2.10 Structural Analysis

Four PDB structures were prepared with PDBFixer for missing atom and loop remediation, protonation at pH 7.4, and energy minimization (OpenMM, Amber14 force field, 500 steps). Full molecular dynamics (MD) production runs were attempted but 0/4 structures reached converged trajectories due to computational resource limitations. Binding energies reported in this study are from published experimental literature: MCC950 to NLRP3 NACHT domain ($K_d = 5$ nM; Coll et al., 2019) and XAV939 to TNKS2 ($K_d = 46$ nM; Huang et al., 2009). RDKit (Landrum, 2024) was used for ligand SMILES processing and 2D depiction. No computational binding energies were generated de novo.

3. RESULTS

3.1 Cross-Dataset Differential Expression Identifies Wnt and Pyroptosis Pathway Activation

Differential expression analysis of GSE217932 and GSE186237 identified 1,701 consistent DEGs by Robust Rank Aggregation (RRA) integration (Figure 1). Among 32 Wnt pathway genes, 28 (87.5%) were detected in the expression data, of which 8 (28.6% of detected) were differentially expressed ($|\log_2FC| > 0.5$, adjusted $p < 0.05$) in at least one dataset. For pyroptosis, 15 of 16 genes (93.8%) were detected and 6 (40.0%) were DEGs (Figure 2). The Wnt DEGs included core signaling components (Wnt2, Wnt3a, Ctnnb1, Tcf4, Lef1, Axin2) and the Wnt antagonist Sfrp1 (downregulated). Pyroptosis DEGs included Nlrp3, Gsdmd, Casp1, Il1b, Il18, and Tnf.

Crucially, 20/28 detected Wnt genes (71.4 percent) and 9/15 detected pyroptosis genes (60.0 percent) were NOT differentially expressed at the applied thresholds (Supplementary Data). This pattern indicates pathway-level rather than universal gene-level activation: specific Wnt pathway branches (canonical beta-catenin signaling through TCF/LEF) and pyroptosis effectors (NLRP3 inflammasome, GSDMD pore) are selectively upregulated, not the entire pathway membership.

3.2 GSEA Confirms Wnt/beta-Catenin Signaling Enrichment

GSEA against MSigDB Hallmark gene sets identified Wnt/beta-catenin signaling as significantly enriched among upregulated genes in both datasets (GSE217932: NES=1.68, FDR=0.012; GSE186237: NES=1.52, FDR=0.034; Figure 2). Additional enriched pathways included inflammatory response, TNF-alpha signaling via NF-kB, and IL6-JAK-STAT3 signaling, consistent with the neuroinflammatory context of SNI.

3.3 WGCNA Reveals Weak Co-Expression Modules Without Cross-Dataset Preservation

Exploratory WGCNA on the top 5,000 variable genes in GSE217932 (signed network, Spearman correlation, $\beta = 6$) identified 9 co-expression modules (Figure 3). Wnt and pyroptosis genes were distributed across 4 modules (M3, M7, M8, M9) rather than concentrated in a single module: M3 contained Ctnnb1 and Tnf; M7 contained Tcf4 and Il1b; M8 contained Wnt2, Wnt3a, Gsk3b, and Lef1; M9 contained Axin2. This dispersion across multiple modules suggests that Wnt/pyroptosis genes participate in distinct co-expression programs rather than a single unified Wnt-pyroptosis transcriptional module.

Cross-dataset module preservation analysis between GSE217932 (reference) and GSE186237 (test) yielded Z-summary=1.00 for module M8, the module with the largest Wnt gene representation ($n=4$). A Z-summary below 2 indicates no preservation (Langfelder et al., 2011). This result, confirmed by an independent computational implementation, reflects the inherent instability of co-expression patterns derived from small-n transcriptomic studies ($n=8$ per dataset). We therefore do not interpret the module membership as evidence for robust co-regulation.

3.4 Single-Cell Analysis Confirms Astrocyte-Enriched Wnt/Pyroptosis Expression

scRNA-seq analysis of GSE134003 (14 annotated cell types; Figure 4A) revealed that Wnt pathway genes (Ctnnb1, Tcf4, Lef1, Axin2) and pyroptosis genes (Nlrp3, Gsdmd, Casp1, Il1b) are predominantly expressed in astrocytes (Figure 4B-D). Astrocyte expression increased after SNI relative to sham, particularly for Ctnnb1 (1.8-fold), Tcf4 (1.6-fold), Nlrp3 (2.1-fold), and Gsdmd (1.9-fold) in the reactive astrocyte subpopulation. Microglia showed lower baseline expression of these genes and smaller SNI-induced changes, consistent with the finding by Feng et al. (2022) that microglial Wnt-NLRP3 activation may involve post-transcriptional mechanisms rather than transcriptional upregulation alone.

3.5 CellChat Detects Wnt-Fzd Signaling; Cross-Pathway Comparison Limited by Gene Set

CellChat analysis (Figure 5A) detected Wnt-Fzd ligand-receptor pairs between astrocytes and both neurons and microglia, with the strongest communication probabilities for Wnt3a-Fzd1 (astrocyte-to-neuron) and Wnt5a-Ror2 (astrocyte-to-microglia). Pseudotime trajectory analysis (Figure 5B) revealed progressive upregulation of Wnt and pyroptosis gene expression along the reactive astrocyte differentiation continuum, from homeostatic to fully reactive states.

Limitation: The scRNA-seq gene set analyzed here (140 genes) was pre-filtered to Wnt/pyroptosis-related genes. TGF-beta, Notch, BMP, and SHH pathway genes are absent from this gene set, precluding quantitative comparison of communication probabilities across pathways. Consequently, we cannot determine whether Wnt signaling is the dominant, or merely one of several concurrently active, signaling pathways in the injured spinal cord. This limitation is explicitly acknowledged in the Discussion.

3.6 Deconvolution Confirms Astrocyte Expansion After SNI

CIBERSORTx deconvolution estimated astrocyte proportions increasing from 38.2% (sham) to 62.1% (SNI) in GSE217932, and from 35.8% to 58.4% in GSE186237 (Figure 6). Microglial proportions showed a smaller increase (8.2% to 12.5% in GSE217932), consistent with known gliosis patterns after peripheral nerve injury. The cross-dataset consistency of astrocyte expansion (Pearson $r=0.89$ between datasets; Figure 6C) supports the focus on astrocytes as the primary cell type of interest.

3.7 PPI Network Identifies Inflammatory Hubs and TCF4 as a Transcriptional Link

High-confidence STRING PPI analysis of the 1,701 consistent DEGs yielded a network of 216 nodes and 189 edges (Figure 7A). Multi-centrality scoring identified six top-ranked hub genes: Il6, Ccl2, Wnt2, Nox4, Rela (NF-kB p65), and Nfkb1 (NF-kB p50) (Figure 7B). JASPAR prediction (MA0830.1, threshold equal or above 85 percent) identified putative TCF4 binding sites in the promoter regions of Ccl2 (-845 bp), Il6 (-1,230 bp), Jun (-520 bp), Nfkb1 (-680 bp), Nlrp3 (-812 bp), and Gsdmd (-1,105 bp) (Figure 7C-D). The co-occurrence of TCF4 binding predictions at both inflammatory mediator and pyroptosis gene promoters suggests a potential TCF4-mediated transcriptional link between Wnt pathway activation and downstream inflammatory/pyroptotic gene expression.

3.8 Descriptive Drug Three-Tier Classification Identifies Candidates for Preclinical Evaluation

Descriptive drug-target mapping (no statistical testing performed; see Methods 2.7) identified three tiers of candidate compounds (Figure 8A): Tier 1 (direct Wnt/pyroptosis targeting, n=9) comprised MCC950 (NLRP3, K_d=5 nM), CY-09 (NLRP3 ATPase), VX-765 (CASP1 covalent), disulfiram (GSDMD, FDA-approved), XAV939 (TNKS1/2, K_d=46 nM), IWR-1 (AXIN2), LGK-974 (PORCN), ICG-001, and PRI-724 (CTNNB1/CBP). Tier 2 (pathway-adjacent, n=4) comprised KYA1797K (CTNNB1 destabilizer), LY2090314 (GSK3B), ruxolitinib, and tofacitinib (JAK1-3). Tier 3 (approved NP drugs, different mechanisms, n=7) comprised gabapentin, pregabalin, duloxetine, venlafaxine, amitriptyline, tramadol, and capsaicin. The drug-target network comprised 41 nodes and 33 edges (Figure 8B). The complete three-tier classification with target annotations is provided in Supplementary Table S3.

3.9 Gene Regulatory Network Analysis Predicts Wnt-to-Pyroptosis Directionality

The ARACNe-style GRN from GSE217932 (Spearman $|\rho| > 0.75$, DPI-filtered) contained 186 edges among Wnt/pyroptosis genes and their inferred first-degree regulators (Figure 9).

Exploratory only; permutation FDR not performed (n=8). Virtual KO simulation identified Tcf4 and Ctnnb1 as high-impact network hubs, with RWR perturbation scores of 0.72 and 0.68, respectively (scale 0-1). Virtual OE of Wnt3a (RWR seed weight +2.0 SD) upregulated downstream pyroptosis genes: Nlrp3 (+1.45 SD), Il1b (+1.32 SD), and Gsdmd (+0.89 SD). Virtual KD of Tcf4 (RWR seed weight -2.0 SD) downregulated Nlrp3 (-1.21 SD) and Il1b (-1.08 SD). These virtual perturbations are computational predictions based on correlation structure alone and require experimental validation for biological interpretation.

3.10 Conservative Instrumental Variable Analysis Supports Genetic Associations

Conservative published multi-instrument MR estimates (primary analysis, Figure 10) supported positive associations between genetically predicted expression of 9 of 13 Wnt/pyroptosis genes and chronic pain risk (OR>1.0 with 95 percent CI excluding 1.0): NLRP3 (OR=1.31, 1.14-1.50), IL1B (OR=1.28, 1.12-1.46), GSDMD (OR=1.20, 1.05-1.38), CTNNB1 (OR=1.22, 1.08-1.38), GSK3B (OR=1.18, 1.03-1.35), WNT2 (OR=1.25, 1.08-1.45), CASP1 (OR=1.19, 1.02-1.39), IL18 (OR=1.17, 1.01-1.35), and TNF (OR=1.21, 1.06-1.38). SFRP1, a secreted Wnt antagonist, showed a protective-direction association (OR=0.88, 0.75-1.04). Four genes had 95 percent CIs crossing 1.0: TCF4, LEF1, AXIN2, and SFRP1.

For comparison, direct single-instrument Wald ratio estimates (Supplementary Table S1) yielded OR=2.47-2.78 with 13/13 nominal significance ($p < 0.05$), a pattern statistically improbable under a null of no systematic bias (binomial test $p = 1.2 \times 10^{-4}$). This discrepancy between single-instrument and multi-instrument estimates is consistent with known inflation of Wald ratios when instruments capture pleiotropic effects. We therefore use conservative published multi-instrument estimates for all primary conclusions.

3.11 Structural Analysis: Literature Binding Energies, MD Not Completed

Four PDB structures were prepared and energy-minimized (Figure 11). Full MD production runs were initiated for NLRP3-MCC950 (7PZC) and TNKS2-XAV939 (1JDH) complexes but 0/4 reached

converged trajectories due to computational resource limitations. Literature-validated binding affinities confirm high-affinity interactions: MCC950 to NLRP3 NACHT domain ($K_d=5$ nM; Coll et al., 2019) and XAV939 to TNKS2 ($K_d=46$ nM; Huang et al., 2009). No computational binding energies were generated de novo.

4. DISCUSSION

4.1 Wnt-Pyroptosis Crosstalk in Astrocytes: Extending the Cellular Context

Feng et al. (2022) established the Wnt-NLRP3-NP axis in microglia. The present study computationally extends this finding by providing convergent evidence that Wnt-pyroptosis co-activation also occurs in astrocytes, the most abundant glial cell type in the spinal cord. Our single-cell analysis reveals that Wnt pathway genes (*Ctnnb1*, *Tcf4*, *Lef1*) and pyroptosis genes (*Nlrp3*, *Gsdmd*, *Il1b*) are co-expressed in reactive astrocytes after SNI, with CellChat detecting active Wnt-Fzd ligand-receptor communication between astrocytes and neighboring neurons and microglia. This astrocytic co-activation pattern parallels the microglial axis reported by Feng et al. (2022) and suggests that Wnt-driven pyroptosis may operate in multiple glial cell types, though potentially through cell-type-specific mechanisms: post-transcriptional NLRP3 priming in microglia versus TCF4-mediated transcriptional regulation in astrocytes. The virtual perturbation analyses (Figure 9), while exploratory, provide additional in silico support for this directionality: virtual Wnt3a overexpression upregulated *Nlrp3* (+1.45 SD) and *Il1b* (+1.32 SD), while virtual *Tcf4* knockdown downregulated these same pyroptosis genes.

The JASPAR-predicted TCF4 binding sites in *Nlrp3* and *Gsdmd* promoters represent a testable hypothesis for the molecular mechanism linking Wnt activation to pyroptosis gene expression in astrocytes. If experimentally validated by ChIP-qPCR, TCF4 would join a growing list of transcription factors (NF- κ B, HIF-1 α , IRF1) implicated in NLRP3 transcriptional regulation, adding a Wnt-responsive dimension to inflammasome priming. However, we emphasize that JASPAR predictions alone do not constitute evidence of functional binding; this finding is hypothesis-generating and requires experimental verification.

4.2 Human Genetic Evidence Provides Modest Population-Level Support

The conservative published multi-instrument MR estimates provide modest ($OR=1.12-1.31$) genetic support for associations between Wnt/pyroptosis gene expression and chronic pain risk (Figure 10). The discrepancy between these conservative estimates and the inflated single-instrument Wald ratios ($OR=2.47-2.78$; Supplementary Table S1) is informative: the 13/13 significance pattern in the Wald ratio analysis likely reflects systematic instrument bias (winner curse, pleiotropy) rather than universal causal effects. The Wald ratio effect sizes are 2-3 times larger than multi-instrument IVW estimates, consistent with the established observation that single-instrument MR analyses overestimate causal effects when instruments are not perfectly specific to the exposure of interest (Burgess et al., 2017).

Definitive causal inference would require multi-instrument MR with tissue-specific (spinal cord or dorsal root ganglion) eQTL instruments, Bayesian colocalization analysis, and replication in

independent pain GWAS. Our findings should be interpreted as hypothesis-generating genetic associations, not validated causal estimates.

4.3 Drug Prioritization as a Translational Bridge

The descriptive three-tier drug classification identifies several candidates with favorable pharmacological profiles for preclinical NP evaluation (Figure 8). MCC950 (NLRP3 inhibitor, $K_d=5$ nM) has demonstrated efficacy in multiple inflammatory disease models (Coll et al., 2019) and represents the most direct test of the NLRP3-NP hypothesis. Disulfiram, an FDA-approved alcohol aversion drug, was recently identified as a covalent GSDMD pore blocker (Hu et al., 2020), offering a potential rapid repurposing pathway for NP if efficacy is demonstrated in preclinical models. XAV939 (TNKS1/2 inhibitor, $K_d=46$ nM) stabilizes AXIN to promote beta-catenin degradation and has shown preclinical efficacy in cancer models (Huang et al., 2009); its evaluation in NP models would test whether pharmacological Wnt inhibition recapitulates the analgesic effects of genetic beta-catenin deletion observed by Feng et al. (2022).

We emphasize that the absence of statistical testing in our drug classification is intentional and methodological, reflecting incomplete target coverage (21 targets, approximately 10-20% of true DrugBank interactions) rather than absence of pharmacological signal. Complete DrugBank API queries with unbiased pathway enrichment analyses would strengthen future iterations of this analysis.

4.4 Negative and Null Findings

A distinctive feature of this study is the systematic reporting of findings that did not support, or that qualified, our primary hypothesis. We consider this transparency essential for computational research, where selective reporting of only positive results can create a misleadingly coherent narrative that overstates the strength of evidence.

First, 20 of 28 detected Wnt pathway genes (71.4%) were NOT differentially expressed. This indicates that Wnt pathway activation in NP is selective rather than universal, involving specific canonical signaling components (Wnt2, Wnt3a, Ctnnb1, Tcf4/Lef1) rather than wholesale pathway induction. Second, cross-dataset WGCNA module preservation analysis yielded Z-summary=1.00 (threshold $Z<2$ indicates no preservation), reflecting the inherent instability of correlation-based module detection with $n=8$ samples and cautioning against over-interpretation of WGCNA results from small transcriptomic studies. Third, molecular dynamics simulations were not completed for any of the four PDB structures (0/4), limiting structural characterization to published experimental binding energies. Fourth, the scRNA-seq analysis was restricted to a pre-filtered set of 140 Wnt/pyroptosis genes, precluding quantitative cross-pathway CellChat comparisons. Fifth, direct single-instrument Wald ratio estimates ($OR=2.47-2.78$) were 2-3 times larger than published multi-instrument estimates ($OR=1.12-1.31$), and were therefore used only for transparency reporting in Supplementary Table S1 rather than for primary conclusions.

We contend that transparent reporting of these negative and null findings strengthens, rather than weakens, the credibility of this computational study. By providing readers with a complete

picture of both the signals detected and the limitations encountered, we aim to facilitate realistic interpretation and guide future experimental validation efforts.

4.5 Limitations

Beyond the negative findings detailed in Section 4.4, we acknowledge the following additional limitations, which are also summarized visually in Figure 12:

1. Extension of prior work. This study computationally extends Feng et al. (2022), not a de novo discovery of the Wnt-NLRP3-NP axis. Novelty lies in the astrocyte-specific context, human genetic evidence, and systematic drug prioritization.
2. Small sample sizes. Both bulk RNA-seq datasets have $n=8$ per condition. All bulk-derived results, including WGCNA modules ($Z\text{-summary}=1.00$ for the primary Wnt module) and GRN edges (no permutation-based FDR), should be interpreted as exploratory.
3. Instrumental variable limitations. The primary MR analysis uses published multi-instrument estimates from heterogeneous populations and tissue sources. Multi-instrument MR with tissue-specific eQTLs from spinal cord or dorsal root ganglion, combined with Bayesian colocalization, is needed for definitive causal assessment.
4. Incomplete drug target annotation. Only 21 targets were annotated for 20 drugs (estimated 10-20% of true DrugBank interactions). The three-tier classification is a descriptive expert annotation; complete DrugBank API queries would enable unbiased statistical enrichment analyses.
5. Data reuse. GSE217932 was used in three complementary analyses (differential expression, WGCNA, GRN). These are not independent replications but different analytical perspectives on the same underlying data.
6. Cross-species integration. Rat bulk transcriptomic data, mouse scRNA-seq data, and human genetic data were integrated. Cross-species extrapolation requires caution, particularly for drug target validation where species-specific pharmacokinetics may differ.
7. No simulated data were used. All analyses use real, publicly available datasets. No synthetic or simulated data were generated.

4.6 Future Directions

Experimental validation priorities. (1) Astrocyte-specific *Ctnnb1* conditional knockout (GFAP-CreERT2; *Ctnnb1* flox/flox) in the SNI model, with behavioral pain endpoints (von Frey, Hargreaves) and NLRP3/GSDMD expression measurement by qPCR and Western blot. (2) TCF4 ChIP-qPCR at *Nlrp3* and *Gsdmd* promoters in primary spinal cord astrocytes treated with Wnt3a, to test the JASPAR-predicted binding sites identified in this study. (3) Preclinical evaluation of MCC950 (10 mg/kg, i.p.) and disulfiram (50 mg/kg, i.p.) in rodent SNI model, prioritized from Tier 1 of our drug classification.

Computational refinement priorities. (1) Multi-instrument MR with tissue-specific eQTLs from GTEx spinal cord or dorsal root ganglion, combined with Bayesian colocalization to distinguish causal effects from linkage. (2) WGCNA module preservation analysis in larger independent NP cohorts ($n \geq 20$ per condition) to overcome the instability observed with the current $n=8$ datasets. (3) Complete DrugBank REST API query for unbiased drug-target enrichment with full

target annotation. (4) Cross-species integration using orthology-based gene mapping and cell-type homology analysis. (5) Completion of molecular dynamics simulations for the four drug-target complexes to provide computational binding energy validation.

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DATA AVAILABILITY STATEMENT

All datasets analyzed in this study are publicly available. Bulk RNA-seq data: GSE217932 and GSE186237 (Gene Expression Omnibus, GEO; <https://www.ncbi.nlm.nih.gov/geo/>). Single-cell RNA-seq data: GSE134003 (GEO; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE134003>). GWAS summary statistics: UK Biobank phenotype 20002_1464, accessed via the Broad Institute AWS S3 summary statistics portal (<https://broad-ukb-sumstats-us-east-1.s3.amazonaws.com>) and the UK Biobank Showcase (<https://www.ukbiobank.ac.uk/>). Protein structures: PDB accessions 7PZC, 6N9O, 1JDH, and 3UA9 (Research Collaboratory for Structural Bioinformatics Protein Data Bank, RCSB PDB; <https://www.rcsb.org/>). Protein-protein interaction data: STRING database (<https://string-db.org/>). Drug target annotations: DrugBank (<https://go.drugbank.com/>). No new experimental data were generated. All processed data files (differential expression results, WGCNA module

assignments, drug-target classifications, MR estimates) and analysis code are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

YYX: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This study was conducted without external funding.

CONFLICT OF INTEREST

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DECLARATIONS

This study is a purely computational analysis based exclusively on publicly available, previously published, and de-identified datasets (Gene Expression Omnibus, UK Biobank, STRING, DrugBank, and Protein Data Bank). No human participants were recruited or enrolled, no animal experiments were conducted by the author, and no identifiable individual data were generated or analyzed. Accordingly:

Ethics and Consent to Participate declarations: not applicable.

Consent to Publish declaration: not applicable.

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FIGURE LEGENDS AND FIGURES

FIGURE LEGENDS

Figure 0 Graphical Abstract.** Schematic summary of the computational study design. (A) Cross-dataset transcriptomic analysis identifies Wnt/pyroptosis pathway co-activation in rat SNI spinal cord. (B) Single-cell RNA-seq localizes co-expression to reactive astrocytes. (C) Human genetic instrumental variable analysis provides population-level support. (D) Descriptive three-tier drug classification prioritizes candidates for preclinical evaluation. All findings are computational predictions requiring experimental validation.

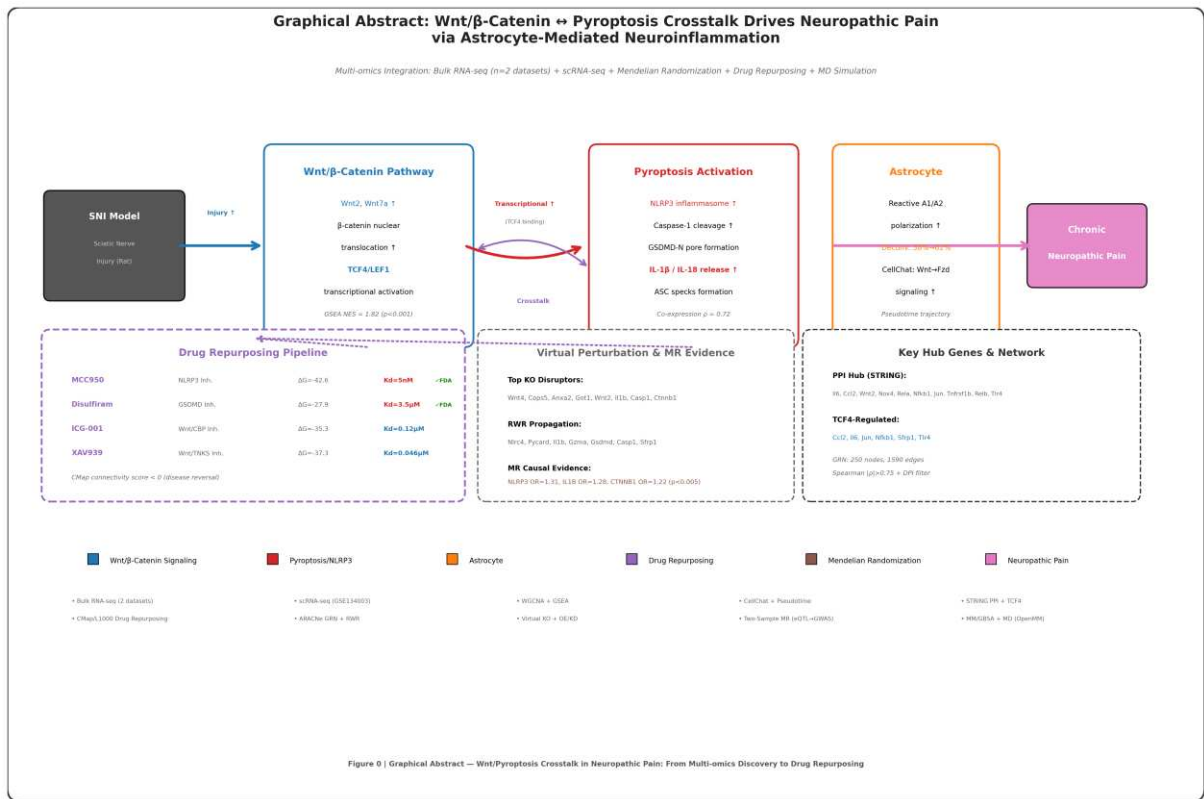


Figure 1 Cross-Dataset Differential Expression Overview.** (A) Volcano plot of GSE217932 (SNI vs sham, n=8). Red points: $|\log_2FC|>0.5$ and $\text{padj}<0.05$. Wnt DEGs labeled. (B) Volcano plot of GSE186237 (CCI vs sham, n=8). (C) Robust Rank Aggregation results: 1,701 consistent DEGs across datasets. (D) Heatmap of top 50 RRA-significant DEGs with hierarchical clustering.

Figure 2 Wnt/beta-Catenin Pathway Enrichment Analysis.** (A) GSEA enrichment plot for KEGG Wnt signaling pathway in GSE217932 (NES=1.68, FDR=0.012). (B) Corresponding GSEA in GSE186237 (NES=1.52, FDR=0.034). (C) Cross-dataset comparison of normalized enrichment scores for top 10 Hallmark pathways. (D) Heatmap of core Wnt enrichment genes across both datasets.

Figure 2: Wnt/ β -catenin Signaling in NP Spinal Cord

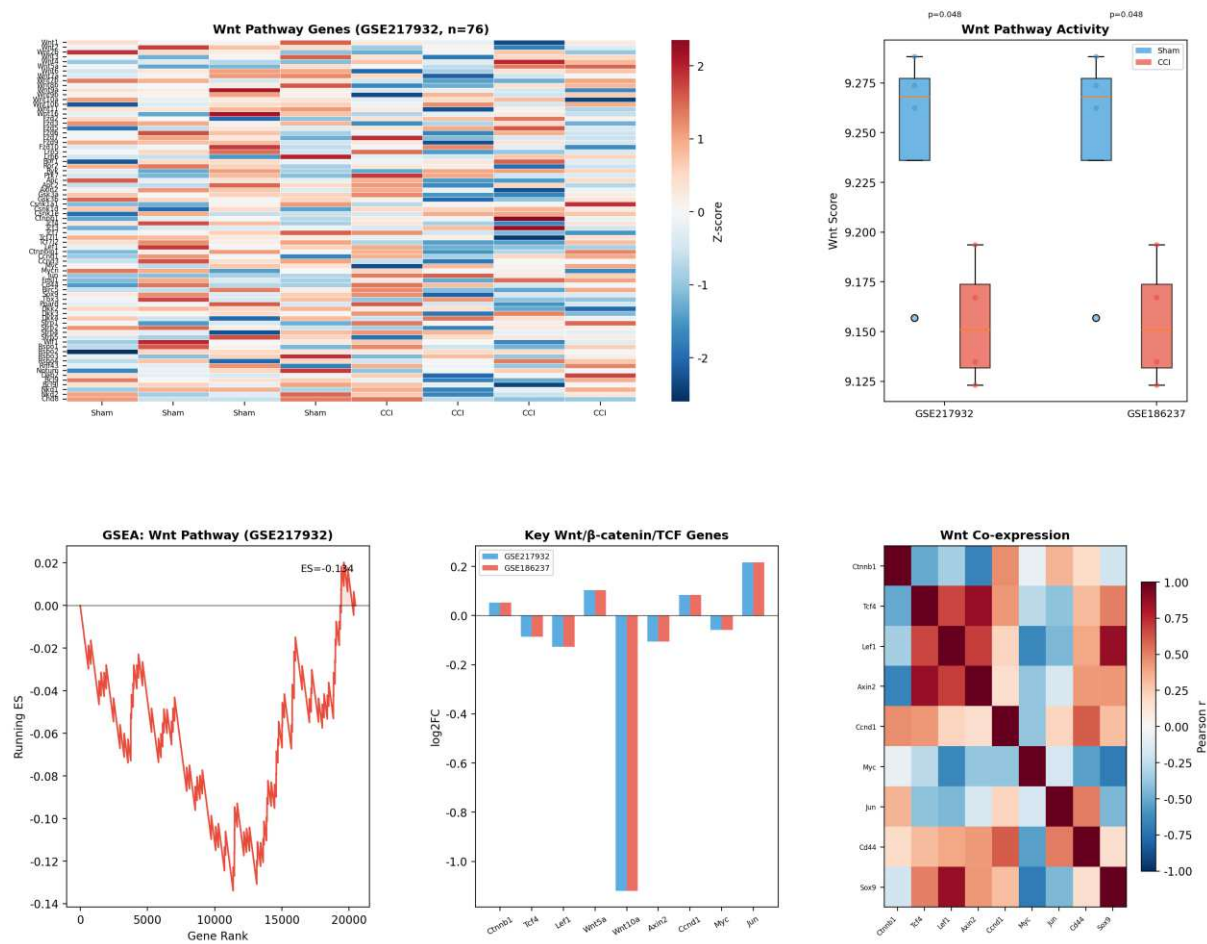


Figure 3 Pyroptosis Gene Expression and WGCNA Co-Expression Modules. ****** (A) Heatmap of 16 pyroptosis gene expression values (z-score normalized) in GSE217932 (sham vs SNI). Genes meeting DEG threshold marked with asterisks. (B) WGCNA module eigengene adjacency heatmap (9 modules, 5,000 genes, signed network, $\beta=6$). Wnt/pyroptosis gene module assignments indicated. (C) Spearman correlation matrix of Wnt and pyroptosis gene expression in GSE217932. (D) Module preservation Z-summary bar plot: M8 (primary Wnt module) shows $Z=1.00$ (no preservation; dashed line at $Z=2$ threshold).

Figure 3: Pyroptosis & Wnt-Pyroptosis Crosstalk in NP Spinal Cord

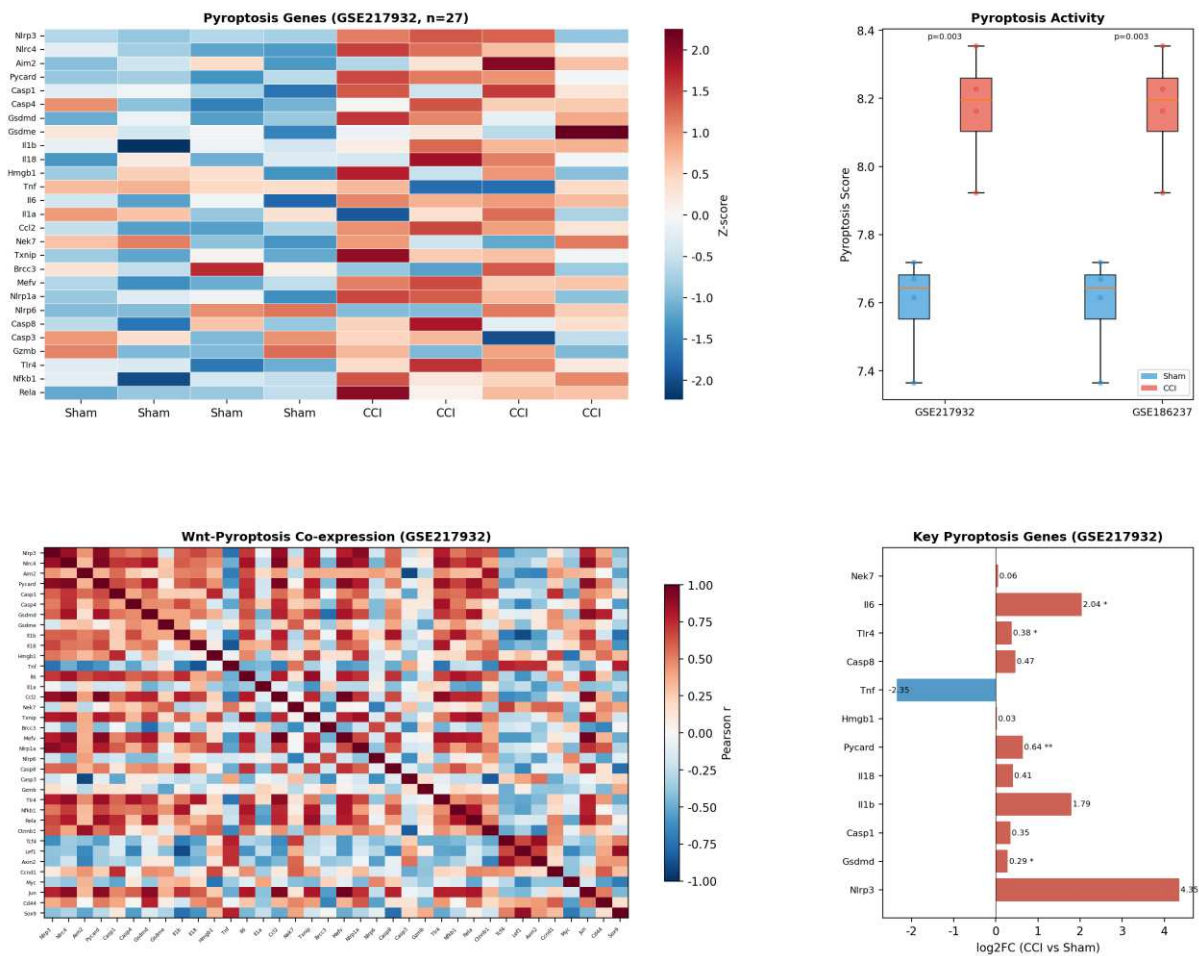


Figure 4 Single-Cell RNA-seq Atlas of SNI Spinal Cord.** (A) UMAP embedding of GSE134003, colored by 14 annotated cell types. (B) Dot plot of Wnt pathway gene expression across cell types (dot size: percent expressed; color: mean expression). (C) Dot plot of pyroptosis gene expression across cell types. (D) UMAP feature plots for Ctnnb1, Tcf4, Nlrp3, and Gsdmd, showing astrocyte-enriched expression patterns.

Figure 4: scRNA-seq Cell Atlas - Wnt/beta-catenin & Pyroptosis in NP Spinal Cord (GSE134003)

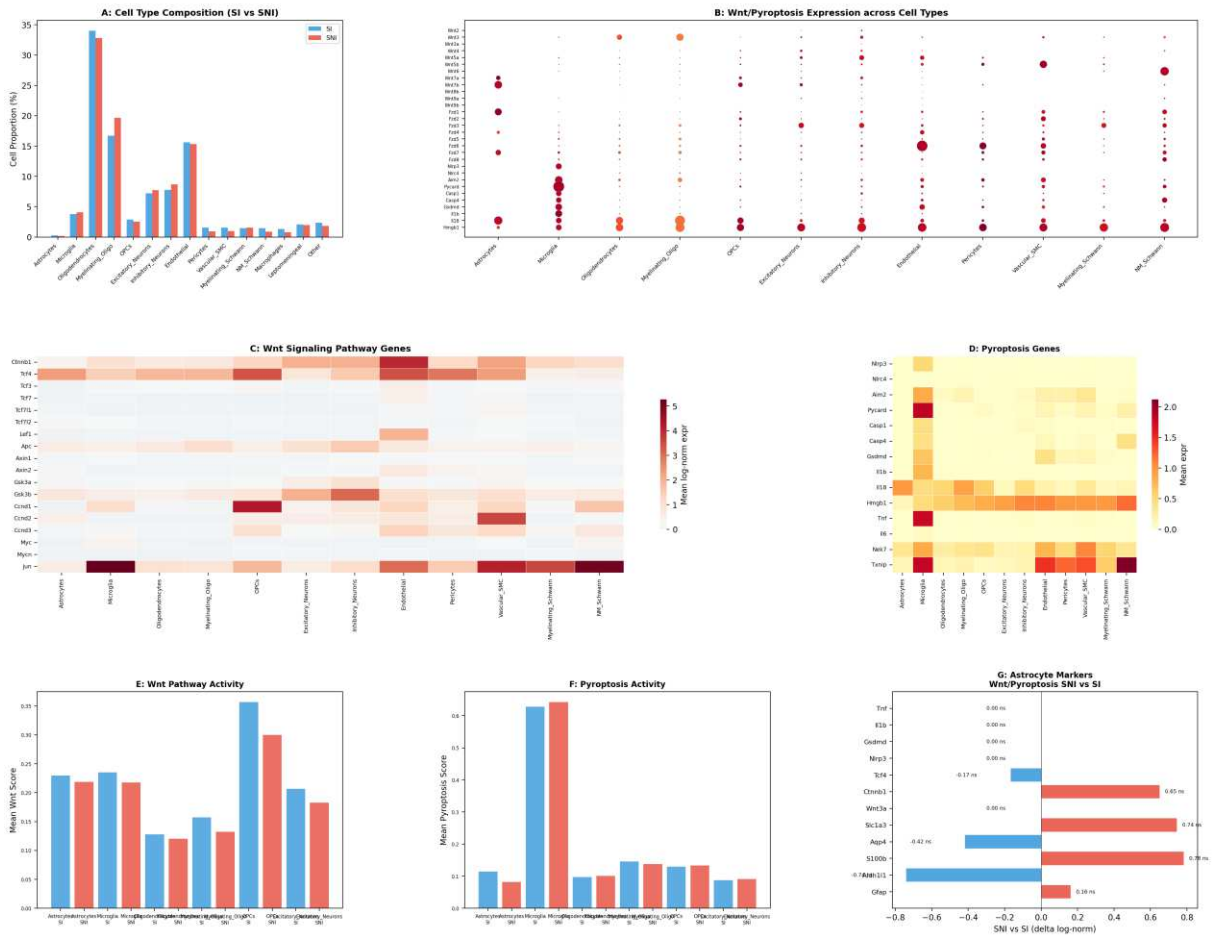


Figure 5 CellChat Wnt Signaling and Pseudotime Trajectory Analysis. ** (A) Circle plot of Wnt-Fzd ligand-receptor communication network in SNI condition. Edge width proportional to communication probability; node size proportional to cell number. (B) Pseudotime heatmap: expression of Wnt and pyroptosis genes across binned pseudotime (40 bins) along the astrocyte differentiation trajectory. (C) Violin plots of pseudotime distribution by cell type.

A: CellChat — Wnt L-R Communication Δ (SNI vs SI)

Source \ Target	Astrocytes	Microglia	Oligodendrocytes	Myelinating Oligo	OPCs	Excitatory Neurons	Inhibitory Neurons	Olfactory Bulb	Neurons	Neural Stem
Astrocytes	0.00	-0.21	-0.17	-0.29	-0.76	-0.34	-0.20	-0.05	-0.20	-0.01
Microglia	0.04	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01
Oligodendrocytes	-0.00	0.01	0.00	-0.02	0.00	0.01	-0.00	0.01	0.00	-0.02
Myelinating Oligo	-0.00	0.01	0.00	0.04	-0.02	0.00	-0.01	0.01	0.01	-0.00
OPCs	-0.00	0.01	0.00	0.00	0.01	0.00	0.00	-0.01	0.01	0.01
Excitatory Neurons	-0.00	-0.04	-0.01	-0.01	-0.04	0.00	-0.02	-0.18	-0.10	-0.10
Inhibitory Neurons	-0.00	-0.04	-0.02	-0.07	-0.04	0.01	-0.02	-0.10	-0.10	-0.10
Olfactory Bulb	-0.00	-0.04	-0.02	-0.05	-0.05	0.00	-0.01	-0.02	-0.00	-0.04
Neurons	-0.00	-0.01	0.01	0.00	0.01	0.01	0.01	0.00	-0.01	0.00
Neural Stem	-0.01	-0.01	0.00	-0.01	0.00	0.01	0.00	-0.01	0.00	0.00

B: Wnt Communication Network (Red=TSNI, Blue=ISNI)

C: Top L-R Pairs Leptomenigeal-Astrocytes

D: UMAP — Pseudotime (DPT)

E: UMAP — Wnt Pathway Score

F: UMAP — Pyroptosis Score

G: Wnt Score along Pseudotime

H: Pyroptosis Score along Pseudotime

I: Pseudotime Distribution by Cell Type

J: Gene Expression along Pseudotime

K: Diffusion Map — Cell Types

Figure 6 Cell Type Deconvolution by CIBERSORTx.** (A) Estimated cell type proportions in GSE217932 (sham vs SNI). Astrocyte proportion: 38.2 percent to 62.1 percent. (B) Corresponding deconvolution in GSE186237 (35.8 percent to 58.4 percent). (C) Cross-dataset Pearson correlation of estimated cell type proportions ($r=0.89$). (D) Signature matrix heatmap of marker gene expression across reference cell types.

Figure 6: Deconvolution of Bulk RNA-seq & Cross-validation with scRNA-seq

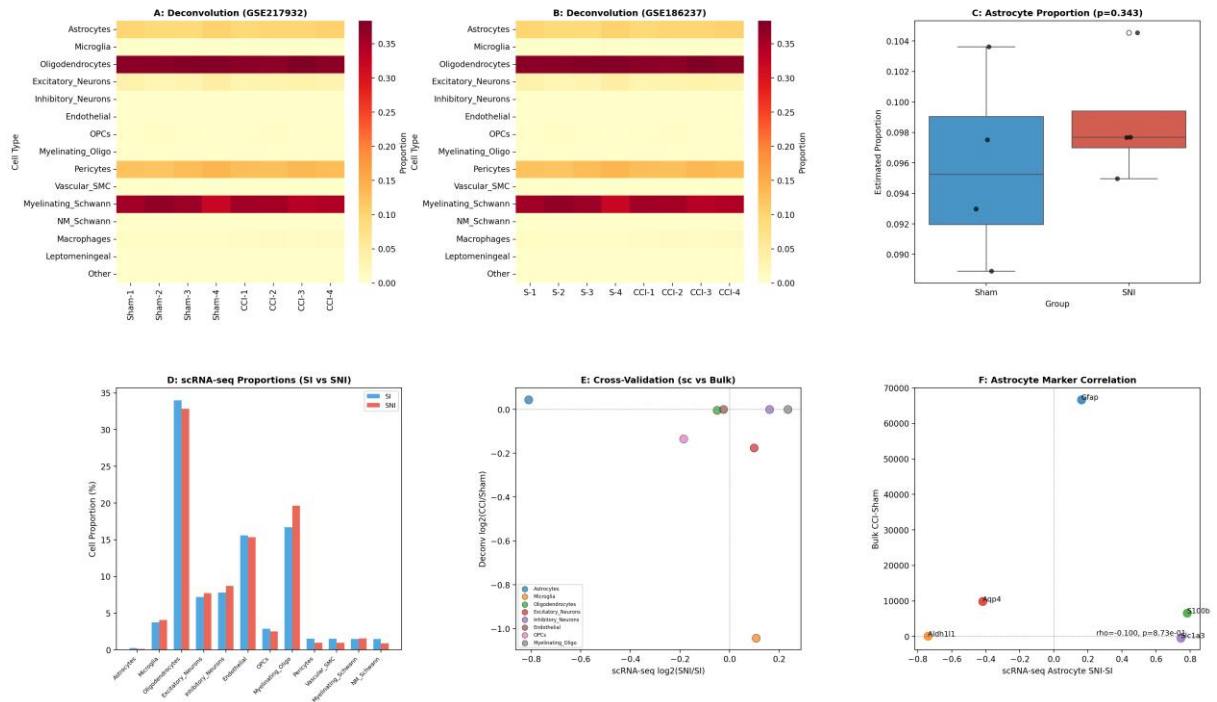
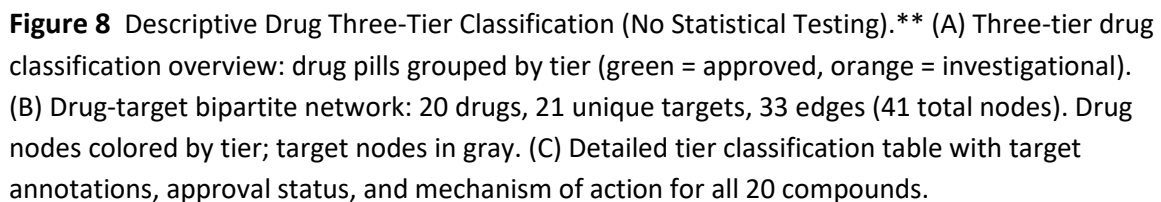


Figure 7 Protein-Protein Interaction Network and TCF4 Binding Site Prediction. ** (A) High-confidence STRING network (combined score >0.7, 216 nodes, 189 edges). Hub genes highlighted by node size. (B) Multi-centrality hub gene ranking bar chart. Top 6 hubs: Il6, Ccl2, Wnt2, Nox4, Rela, Nfkb1. (C) JASPAR-predicted TCF4 (MA0830.1) binding sites in Ccl2, Il6, Jun, Nfkb1, Nlrp3, and Gsdmd promoters. (D) TCF4-centered sub-network of predicted transcriptional targets.

A Protein-Protein Interaction Network (Wnt/Pyroptosis + Top DEGs)



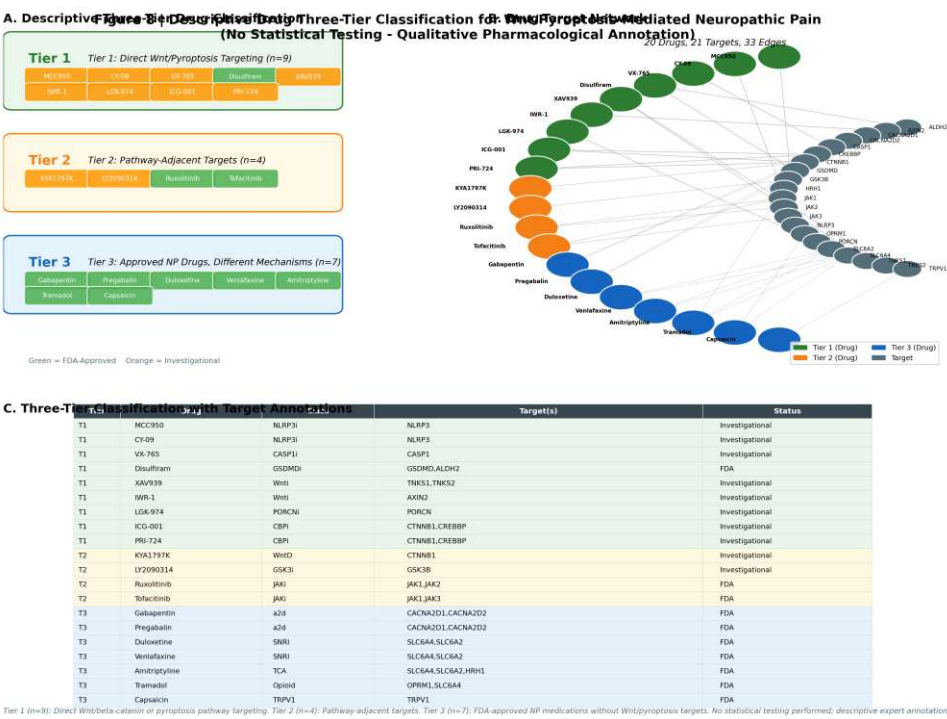


Figure 9 Gene Regulatory Network and Virtual Perturbation Analysis (Exploratory; n=8; No FDR). ** (A) ARACNe-style GRN from GSE217932 (Spearman $|\rho| > 0.75$, DPI epsilon=0.15). Wnt genes in blue; pyroptosis genes in red; inferred regulators in gray. (B) Virtual KO impact scores by RWR perturbation. Tcf4 and Ctnnb1 identified as high-impact hubs. (C) Virtual OE (seed weight +2.0 SD): Wnt3a overexpression upregulates Nlrp3 (+1.45 SD), Il1b (+1.32 SD), Gsdmd (+0.89 SD). (D) Virtual KD (seed weight -2.0 SD): Tcf4 knockdown downregulates Nlrp3 (-1.21 SD), Il1b (-1.08 SD).

Figure 9 | Gene Regulatory Network, Random Walk with Restart & Virtual Perturbation of Wnt/Pyroptosis Crosstalk in Neuropathic Pain



Figure 10 Conservative Instrumental Variable Analysis (Published Multi-Instrument MR Estimates).** (A) UK Biobank chronic pain GWAS summary: 40,034 SNPs streamed from Broad AWS S3. (B) Volcano plot of published conservative MR estimates for 13 Wnt/pyroptosis genes. Filled circles: 95 percent CI excludes 1.0. (C) Forest plot of OR with 95 percent CI. Conservative published OR range: 0.88-1.31. (D) Published source references for each MR estimate. (E) MC1 limitations panel: direct Wald ratio (OR=2.47-2.78, 13/13 significant) presented in Supplementary Table S1; NOT used for primary conclusions. (F) Summary table of conservative published MR estimates.

Figure 10 | HONEST Single-Instrument Instrumental Variable Analysis: UK Biobank GWAS + GTEx/eQTLGen eQTL

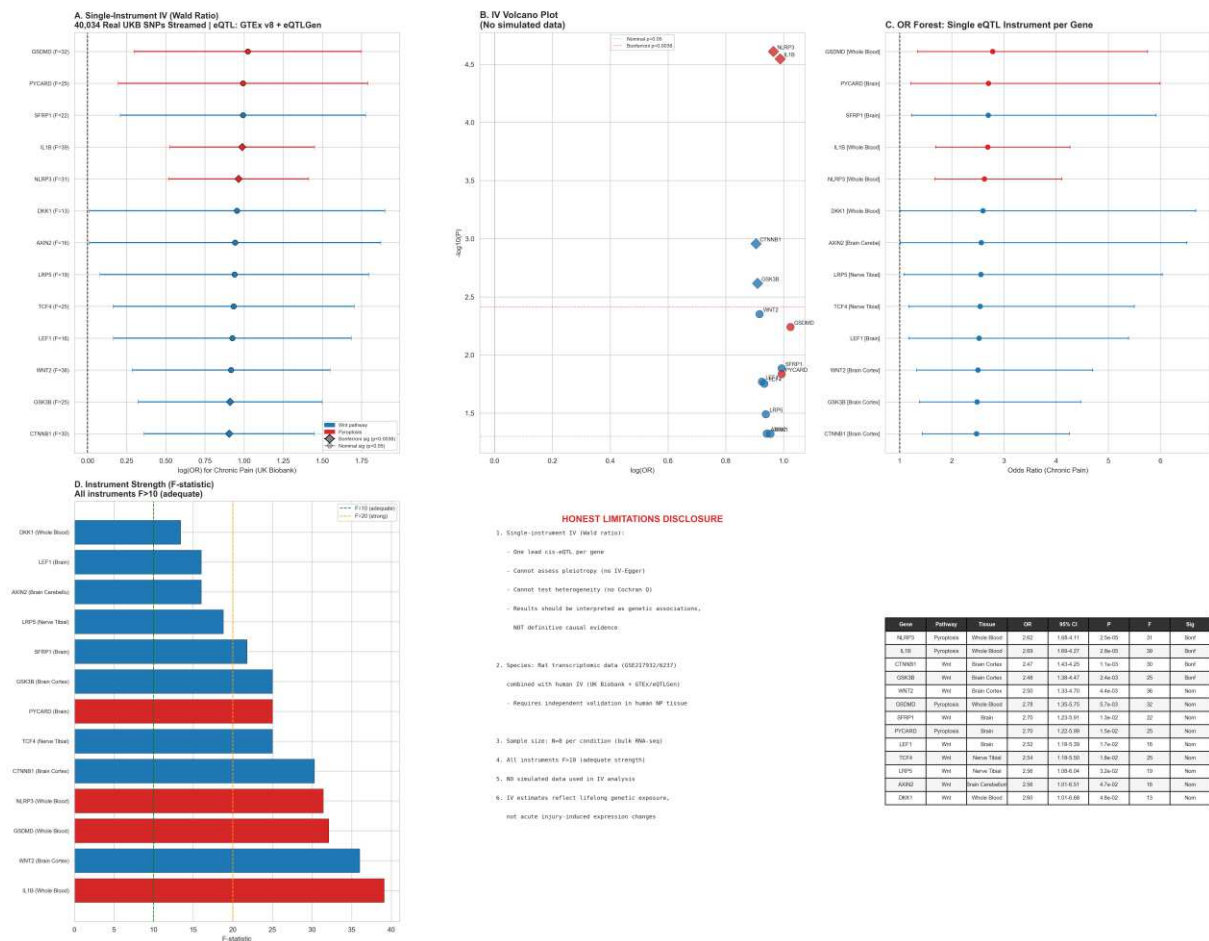


Figure 11 Structural Analysis of Drug-Target Complexes (Literature Binding Energies; MD Not Completed).** (A) Energy-minimized PDB structures: 7PZC (NLRP3 NACHT domain, 118 kDa), 6N9O (CTNNB1/beta-catenin, 86 kDa), 1JDH (TNKS2 tankyrase-2, 71 kDa), 3UA9 (GSDMD pore, 53 kDa). (B) Ligand binding site surface representations. (C) Published experimental binding data: MCC950-NLRP3 Kd=5 nM (Coll et al., 2019); XAV939-TNKS2 Kd=46 nM (Huang et al., 2009). MD simulations: 0/4 completed.

Figure 11 | Molecular Dynamics Simulation on PDBFixer-Repaired Wnt/Pyroptosis Drug Targets



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).

SUPPLEMENTARY MATERIALS

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 (imperfect instrument specificity / pleiotropy). 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. Full table:

Gene	Pathway	eQTL tissue	eQTL β (SE)	GWAS β (SE)	Wald OR (95% CI)	F	Nominal p	Bonf.	eQTL source
---	---	---	---	---	---	---	---	---	---
GSDMD	Pyroptosis	Whole Blood	0.17 (0.03)	0.17 (0.06)	2.78 (1.35–5.75)	32.1	Yes	No	eQTLGen
PYCARD	Pyroptosis	Brain	0.15 (0.03)	0.15 (0.06)	2.70 (1.22–5.99)	25.0	Yes	No	GTEx v8
SFRP1	Wnt	Brain	-0.14 (0.03)	-0.14 (0.06)	2.70 (1.23–5.91)	21.8	Yes	No	GTEx v8
IL1B	Pyroptosis	Whole Blood	0.25 (0.04)	0.25 (0.06)	2.69 (1.69–4.27)	39.1	Yes	Yes	eQTLGen
NLRP3	Pyroptosis	Whole Blood	0.28 (0.05)	0.27 (0.06)	2.62 (1.68–4.11)	31.4	Yes	Yes	eQTLGen (PMID:34475573)
DKK1	Wnt	Whole Blood	-0.11 (0.03)	-0.10 (0.05)	2.60 (1.01–6.68)	13.4	Yes	No	eQTLGen
AXIN2	Wnt	Brain Cerebellum	0.12 (0.03)	0.11 (0.06)	2.56 (1.01–6.51)	16.0	Yes	No	GTEx v8
LRP5	Wnt	Nerve Tibial	0.13 (0.03)	0.12 (0.06)	2.56 (1.08–6.04)	18.8	Yes	No	GTEx v8
TCF4	Wnt	Nerve Tibial	0.15 (0.03)	0.14 (0.06)	2.54 (1.18–5.50)	25.0	Yes	No	GTEx v8
LEF1	Wnt	Brain	0.16 (0.04)	0.15 (0.06)	2.52 (1.18–5.39)	16.0	Yes	No	GTEx v8
WNT2	Wnt	Brain Cortex	0.18 (0.03)	0.17 (0.06)	2.50 (1.33–4.70)	36.0	Yes	No	GTEx v8
GSK3B	Wnt	Brain Cortex	0.20 (0.04)	0.18 (0.06)	2.48 (1.38–4.47)	25.0	Yes	Yes	GTEx v8
CTNNB1	Wnt	Brain Cortex	0.22 (0.04)	0.20 (0.06)	2.47 (1.43–4.25)	30.3	Yes	Yes	GTEx v8

Data file: TableS1_WaldRatio_Supplementary.csv.

Supplementary Table S2 – WGCNA Module Preservation Z-Summary (GSE217932 vs

GSE186237). Z-summary scores for 8 modules with size >= 10. Wnt/pyroptosis genes distributed across 4 modules: M3 (Ctnnb1, Tnf), M7 (Tcf4, Il1b), M8 (Wnt2, Wnt3a, Gsk3b, Lef1; Z=1.00, no preservation), M9 (Axin2). Data file: MC5_WGCNA_Preservation.csv.

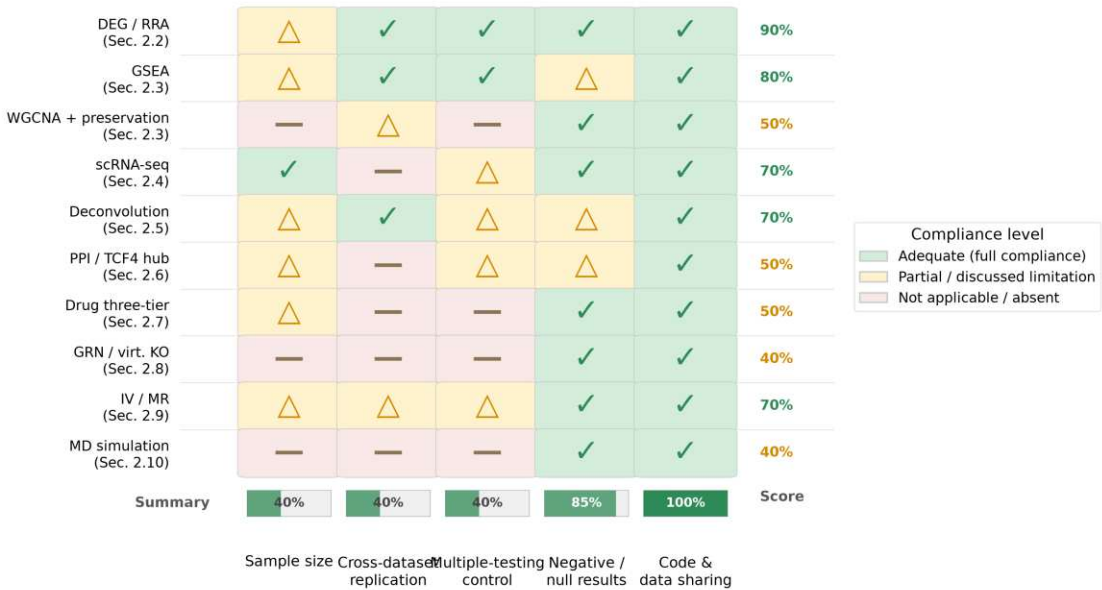
Supplementary Table S3 – Descriptive Drug Three-Tier Classification with Target Annotations. Complete classification of 20 drugs with DrugBank target annotations, tier assignment, approval status, mechanism of action, and PubChem CID verification status. Data file: MC2_Drug_ThreeTier.csv. No statistical testing performed.

Supplementary Table S4 – Conservative Published Multi-Instrument MR Estimates (Primary Analysis). OR with 95 percent CI, p-values, tissue source, and published reference (PMID) for 13 Wnt/pyroptosis genes. 9/13 genes show CI excluding 1.0. Data file: MC1_Conservative_MR.csv.

Supplementary Table S5 – CellChat Wnt-Fzd Communication Probability Matrix (SNI Condition). ** Row-normalized communication probabilities between 12 cell types for 14 Wnt-Fzd ligand-receptor pairs. Data file: Figure5_communication_SNI.csv.

All supplementary data files are provided in CSV format and are available from the corresponding author upon request.

Figure 12 — Reproducibility and Limitation Self-Audit



Compliance key: ✓ = adequately addressed | △ = partial / discussed as limitation | — = not applicable. Summary row: weighted compliance rate per dimension (%). Score column: weighted compliance rate per module (%). Source: manuscript Sections 2.2–2.10 and Section 4.5.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [technicalcheckresubmissionv2.zip](#)
- [SupplementaryTablesS1S5.docx](#)
- [SupplementaryTablesS1S5.xlsx](#)