

# Systematic bias in network proximity Z-scores: A comparative robustness audit using *Hypericum perforatum* constituents

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## Abstract

Network-based metrics are widely used to identify associations between compounds and diseases, assuming that proximity within a protein–protein interaction network reflects functional relevance. However, these metrics are often reported as Z-scores, which we demonstrate are fundamentally sensitive to the number of targets a compound possesses. This dependency introduces a systematic bias where compounds with broad polypharmacology appear statistically significant due to null distribution tightening (the Law of Large Numbers) rather than physical network reachability.

Here, we systematically evaluate this bias using the human liver interactome and a controlled comparison of two constituents from *Hypericum perforatum*. We show that conventional proximity Z-scores yield unstable rankings that reverse depending on network construction parameters. While a compound with many targets may achieve a higher Z-score, it can remain physically more distant from the disease module than a compound with fewer, high-leverage targets. We resolve this by utilizing random walk–based influence propagation and introducing a size-normalized metric: perturbation efficiency. Our results show that influence-based rankings are stable across varied network thresholds and correctly identify high-leverage modulators that proximity metrics miss. This study provides a methodological template for identifying and correcting statistical artifacts in network medicine, enabling more reliable risk assessment in complex biological systems.

**Keywords:** network propagation, proximity metrics, metric robustness, drug-induced liver injury, polypharmacology, Z-score confounding, perturbation efficiency.

# 1 Introduction

Network-based prioritization is a cornerstone of modern systems biology and drug discovery, assuming that the topological proximity between compound targets and disease genes within a protein–protein interaction (PPI) network reflects functional relevance [1–4]. Because raw network distances are sensitive to local topology and degree distribution, they are typically reported as Z-scores relative to degree-matched null models. While these Z-scores successfully quantify statistical significance in most applications [3], we demonstrate they can be confounded by large asymmetries in target set size. As the number of seed nodes increases, the variance of the null distribution decreases (the Law of Large Numbers), leading to deterministic significance inflation for compounds with broad polypharmacology. Identifying whether such results reflect true biological influence, or whether they represent systematic artifacts of distance-based inference, is essential for the reliability of network medicine.

Using the human liver interactome as a model system, we investigate this confounding effect through a controlled comparison of two constituents from *Hypericum perforatum* (St. John’s Wort). These constituents—Hyperforin and Quercetin—exhibit highly asymmetric target set sizes: Hyperforin possesses 10 validated targets, while Quercetin has over 60 [5–7]. This asymmetry provides a sharp stress test for network metrics. Conventional proximity Z-scores predict greater disease-associated significance for the broad-spectrum modulator, even when it is physically more distant from the disease module than the high-leverage modulator. This reversal indicates that proximity-based prioritization is unstable across network construction parameters and susceptible to sample-size artifacts.

Here, we evaluate the robustness of proximity-based and influence-based metrics for comparative prioritization. We demonstrate that proximity Z-scores yield unstable, threshold-dependent rankings driven by null-distribution tightening rather than physical reachability. To resolve this instability, we utilize random walk–based influence propagation, which integrates over the entire network topology and captures signal amplification through regulatory hubs [8]. We introduce a normalized metric, perturbation efficiency, to account for target set size and ensure unbiased comparisons. Our results show that influence-based propagation provides a stable, theoretically consistent framework for network pharmacology that correctly identifies high-leverage perturbations where traditional proximity metrics fail.

## 2 Results

### 2.1 Proximity Z-scores are confounded by target set size

We first established network context by quantifying target count and shortest-path proximity to 82 DILI-associated genes (Figure 1). Quercetin engages 62 targets in the liver-expressed largest connected component; Hyperforin engages 10. At STRING confidence  $\geq 900$ , Hyperforin targets are physically closer to DILI genes ( $d_c = 1.30$ ) than Quercetin targets ( $d_c = 1.68$ ; Table 1). However, the proximity Z-scores yield the opposite ranking: Quercetin achieves  $Z = -5.44$  ( $p < 0.001$ ), while Hyperforin achieves  $Z = -3.86$  ( $p < 0.001$ ). All reported associations survived Benjamini–Hochberg FDR correction ( $q < 0.05$ ).

This discrepancy highlights a fundamental confounder in proximity Z-scores: the law of large numbers. As target set size increases, the variance of the null distribution decreases ( $\sigma_{null} = 0.09$  for Quercetin vs 0.24 for Hyperforin), inflating the significance of broader target sets despite greater physical distance. This statistical artifact suggests that Quercetin poses greater risk, whereas the physical topology favors Hyperforin. This effect occurs independently of network construction parameters and represents a fundamental statistical property of averaged distributions: larger samples inherently produce more precise (narrower) null distributions, artificially inflating Z-score magnitude regardless of actual topological proximity.

### 2.2 Influence-based rankings are stable and resolve the confound

Random walk with restart (RWR) stabilizes this ranking by integrating over all paths (Figure 2). Hyperforin achieves influence  $Z = +10.12$  ( $p < 0.001$ ); Quercetin achieves  $Z = +4.55$  ( $p < 0.001$ ; Table 1). Unlike proximity, influence Z-scores correctly reflect the topological advantage of Hyperforin’s regulatory hub occupancy. This ranking aligns with the known biological ground truth: Hyperforin is the hepatotoxic constituent responsible for drug-drug interactions via PXR activation, while Quercetin has no documented hepatotoxicity and may be hepatoprotective. The ranking remains consistent across topology-only and expression-weighted analyses, demonstrating that influence propagation is less susceptible to sample-size artifacts than shortest-path distance.

### 2.3 Expression weighting refines the signal

To assess whether the RWR signal persists under tissue-specific constraint, we applied expression-weighted influence propagation (EWI), weighting transitions by destination-node liver expression (Fig-

ure 3).

The Z-score differential narrows but remains substantial under expression weighting: Hyperforin  $Z = +8.98$  ( $p < 0.001$ ); Quercetin  $Z = +5.79$  ( $p < 0.001$ ). Hyperforin’s advantage is driven primarily by the PXR–CYP master regulatory axis, which remains highly active in liver tissue (e.g., CYP3A4 at 335 TPM). Quercetin’s influence is moderated by its broad, diffuse target profile, which includes several high-expression nodes (e.g., CFB at 1,115 TPM) that do not converge on a DILI effector hub.

## 2.4 Normalizing for target count confirms Hyperforin’s topological advantage

To resolve the target-count paradox, we compared the average network influence of each individual target, reframing polypharmacology as an efficiency problem rather than a coverage problem (Figure 4; Table 2).

| Compound               | Targets | Eff. (RWR) | Eff. (EWI) | RWR Ratio*         | EWI Ratio <sup>†</sup> |
|------------------------|---------|------------|------------|--------------------|------------------------|
| Hyperforin             | 10      | 0.1138     | 0.1330     | —                  | —                      |
| Quercetin              | 62      | 0.0322     | 0.0493     | —                  | —                      |
| <b>Fold difference</b> | —       | —          | —          | <b>3.5× (3.7×)</b> | <b>2.7× (2.8×)</b>     |

\*RWR Ratio: observed ratio (robust ratio in parentheses). <sup>†</sup>EWI Ratio: observed ratio (robust ratio in parentheses).

Each Hyperforin target contributes 3.7× more DILI-directed influence than each Quercetin target (robust ratio). This disparity indicates that Hyperforin’s target positions are substantially higher leverage than those of Quercetin, achieving greater perturbation efficiency despite a 6-fold smaller target set. Critically, this 3.7-fold efficiency advantage is threshold-independent: it holds at STRING  $\geq 700$  (3.8×),  $\geq 900$  (3.7×), and under both topology-only (RWR) and expression-weighted (EWI) regimes. Unlike Z-scores, which reverse between thresholds, perturbation efficiency provides a stable, parameter-invariant comparative metric.

## 2.5 Bootstrap resampling excludes target-selection bias

To rule out the possibility that Hyperforin’s advantage arises from favorable target selection rather than strategic network positioning, we performed bootstrap sensitivity analysis (Figure 5). 100 random 10-target subsets were sampled without replacement from Quercetin’s 62-target pool and scored by RWR.

Hyperforin’s observed influence (0.1138) exceeds the entire bootstrap distribution from Quercetin (mean = 0.0308, 95% CI = [0.0160, 0.0542]; Table 3). The fold difference between Hyperforin and the bootstrap mean is 3.7×. This confirms that Hyperforin’s advantage is not an artifact of target count; even when sampling equalized subsets from Quercetin’s pool, no configuration matches Hyperforin’s influence.

## 2.6 Ranking stability across network thresholds

The influence ranking is stable across network confidence thresholds (Table 6). Hyperforin ranks first in all RWR and EWI configurations at both  $\geq 700$  and  $\geq 900$  thresholds. Notably, the proximity ranking reverses between thresholds: at  $\geq 700$ , Hyperforin is physically closer ( $d_c = 0.60$  vs  $1.34$ ) and more "significant" ( $Z = -6.04$  vs  $-5.46$ ). At  $\geq 900$ , Quercetin appears more "significant" ( $Z = -5.44$  vs  $-3.86$ ) despite being physically more distant ( $1.68$  vs  $1.30$ ). This instability in proximity Z-scores—while influence rankings remain stable—demonstrates that influence-based metrics are more robust to network construction parameters.

## 2.7 Chemical similarity excludes structural confounding

To exclude the possibility that Hyperforin’s network signal reflects structural similarity to known hepatotoxins, we performed chemical similarity analysis against the DILIrank reference set (Figure 6). Morgan fingerprints (ECFP4) revealed that neither compound exceeds the 0.4 Tanimoto threshold for structural analog detection. Notably, Quercetin exhibits higher structural similarity to DILI reference drugs yet lower network influence, reinforcing that the observed asymmetry is driven by network topology rather than chemical features.

# 3 Discussion

## 3.1 Ranking stability and the Z-score confound

The results of this study highlight a potential limitation in the use of network proximity Z-scores when comparing compounds with asymmetric target set sizes. While proximity is a standard prioritization criterion, our analysis demonstrates that its significance rankings can be influenced by the target count rather than topological distance alone. As the number of targets increases, the variance of the null distribution decreases, which can lead to inflated significance levels for compounds with broad polypharmacology. In our controlled comparison, this effect causes a reversal of proximity-based rankings between network thresholds, failing to accurately reflect the physical distance advantage of a high-leverage modulator.

Influence-based metrics (RWR and EWI) appear less sensitive to this particular artifact. By integrating over the entire network topology, these methods capture signal propagation through regulatory hubs, providing rankings that remain stable across different network construction parameters. This relative stability suggests that influence-based propagation may offer a more robust framework for comparative network medicine, particularly in the presence of incomplete or asymmetric pharmacological data.

The mechanistic explanation for this robustness is that RWR integrates over *all* paths, capturing how signals amplify through hubs like PXR and AKT1. Shortest-path proximity, by contrast, is a descriptive metric for minimum reachability; treating it as an inferential surrogate for functional impact conflates topological context with biological consequence.

### 3.2 Expression weighting as a biological constraint

Expression-weighted influence (EWI) constrains signal propagation to liver-active nodes. By attracting signal to highly expressed proteins (destination-node weighting), we ensure that the network propagation reflects tissue-specific biology. Under this constraint, the Hyperforin advantage persists, demonstrating that its topological efficiency is not an artifact of an unconstrained PPI network but is supported by the expression profile of the liver. Attenuation of signal is expected when walks are constrained to active pathways; the fact that the ranking remains stable provides positive evidence for the biological relevance of the PXR axis.

### 3.3 Perturbation efficiency vs. topological coverage

By normalizing total influence for target set size (where the restart vector is already  $|T|$ -weighted), we provide a more balanced comparison of perturbation efficiency. Our results show that a single Hyperforin target exerts 3.7-fold more influence on the DILI module than a Quercetin target.

This efficiency claim is further validated by bootstrap sensitivity analysis. Even when sampling size-matched 10-target subsets from Quercetin’s pool, none reached the influence level achieved by Hyperforin. This demonstrates that the advantage is not due to target count, but to the strategic network position of Hyperforin’s targets—specifically their convergence on the PXR master regulator and downstream CYP effectors.

### 3.4 Mechanistic context: The PXR axis

The stability of the influence ranking aligns with the well-characterized PXR–CYP master regulatory axis. Hyperforin’s primary target, NR1I2 (PXR), induces the expression of major xenobiotic metabolism enzymes including CYP3A4 and CYP2C9 [6, 9]. In our network analysis, these effectors are part of the target set and the DILI module, creating a high-connectivity hub structure that enables efficient propagation. Quercetin’s 62 targets, while numerous, are distributed across redundant or peripheral pathways that do not converge on a regulatory bottleneck. Furthermore, clinical evidence indicates that Quercetin is not associated with hepatotoxicity and may exhibit hepatoprotective properties [7, 10]. Recent experimental studies have corroborated that St. John’s wort exacerbates hepatotoxicity through precisely this PXR-mediated bioactivation mechanism [11].

### 3.5 Limitations

Several limitations warrant consideration. First, network influence is a measure of topological reach and perturbation potential, not a direct surrogate for toxicological outcomes. This model is dose-independent and does not account for pharmacokinetics, binding affinity, or saturation kinetics. A high influence score indicates that a compound’s targets are strategically positioned to modulate a disease module, but the actual biological effect depends on the molecular mechanism of action (e.g., agonism vs. antagonism) and the kinetic context.

Second, while we demonstrate that proximity Z-scores are confounded by target set size, influence-based Z-scores are not entirely immune to this effect. As the number of seed nodes increases, the variance of the null

distribution for influence sums also decreases, though less severely than for distance-based metrics. Critically, our core claims do not rest on absolute Z-score comparisons. We demonstrate that influence-based *rankings* are stable across network thresholds, while proximity rankings are not. We further resolve the size-dependence by introducing perturbation efficiency (influence per target), which explicitly normalizes for target count and provides a bias-corrected comparative metric.

Third, our case study is limited to a single botanical with two contrasting constituents. While this provides a controlled minimal model, generalization to larger compound libraries will require further validation.

## 3.6 Conclusions

In this study, we utilized *H. perforatum* as a known toxicological model to validate the *reliability* of network metrics; the biological ground truth (Hyperforin-mediated PXR activation) allowed us to confirm that influence propagation correctly identifies high-leverage perturbations where proximity metrics fail. The methodological conclusion is that proximity Z-scores are susceptible to sample-size confounding and should be used descriptively rather than for comparative inference across compounds with differing target counts. Influence-based propagation, combined with per-target normalization, provides a more stable framework that survives robustness checks and aligns better with mechanistic reality.

More broadly, this work provides a methodological template for identifying and resolving metric artifacts in network toxicology. By integrating signed edge weights and transcriptomic data, future iterations of this framework could investigate phenotype-specific associations, linking topological influence on specific biological sub-modules to discrete clinical outcomes.

## 4 Methods

### 4.1 Data sources

#### 4.1.1 Protein-protein interaction network

Human protein-protein interactions were obtained from STRING v12.0 [12]. Combined confidence scores were computed per STRING methodology (text mining, experiments, databases, co-expression, neighborhood, gene fusion, co-occurrence). Only edges with combined confidence  $\geq 900$  (highest confidence tier) were retained. Raw network: 11,693 genes, 100,383 edges.

#### 4.1.2 Liver expression data

Gene expression data were obtained from the Genotype-Tissue Expression Project (GTEx) v8 [13]. Median transcripts per million (TPM) values for liver tissue were extracted from the 2017-06-05 release (RNASeQCv1.1.9). Genes with liver TPM  $\geq 1$  were retained. Result: 13,496 liver-expressed genes.

### 4.1.3 Drug-induced liver injury gene set

DILI-associated genes were obtained from DisGeNET [14] curated gene-disease associations. Query: UMLS concept identifier C0860207 (Drug-Induced Liver Injury). Inclusion criterion: genes with curated evidence linking to DILI. Raw DILI gene count: 127 genes.

### 4.1.4 Hyperforin targets

Hyperforin targets were curated from primary literature sources [6, 9]. Sources included studies of PXR activation, CYP induction, and ABC transporter modulation. Raw target count: 14 proteins (Table 7).

### 4.1.5 Quercetin targets

Quercetin targets were retrieved programmatically from ChEMBL v31 [15] via REST API. Query: ChEMBL159 (Quercetin). Filter: human targets with experimentally validated bioactivity ( $IC_{50}$ ,  $K_i$ , or  $EC_{50} \leq 10 \mu M$ ). Raw target count: 122 proteins.

## 4.2 Target processing

Protein identifiers were mapped to HUGO gene symbols using STRING info files and UniProt [16]. Non-human proteins (mouse, rat, bacterial, viral) were excluded. Gene symbols were standardized (e.g., MDR1  $\rightarrow$  ABCB1). Processed target counts: Hyperforin = 14, Quercetin = 87.

## 4.3 Network construction

The STRING network was filtered to genes with liver expression  $\geq 1$  TPM (GTEx v8). The largest connected component (LCC) was extracted using NetworkX [17]. Compound targets and DILI genes not present in the LCC were excluded. Final network: 7,677 nodes, 66,908 edges. Final target counts: Hyperforin = 10, Quercetin = 62. Final DILI gene count: 82.

Five genes are targeted by both compounds: ABCG2, AKT1, CYP3A4, MMP2, MMP9. These were retained in both target sets.

## 4.4 Shortest-path proximity (descriptive)

Mean minimum shortest-path distance from compound targets  $T$  to DILI genes  $D$ :

$$d_c = \frac{1}{|T|} \sum_{t \in T} \min_{d \in D} \text{dist}(t, d) \quad (1)$$

where  $\text{dist}(t, d)$  is the unweighted shortest-path length in the LCC. Shortest-path proximity is a descriptive metric. It was used to provide network context, not to test influence.



## 4.5 Random walk with restart

Influence propagation was quantified using random walk with restart (RWR), a global network propagation algorithm that captures both direct and indirect associations by simulating the diffusion of signal from seed nodes [3, 8]. Given an adjacency matrix  $\mathbf{A}$ , we define the column-normalized transition matrix  $\mathbf{W}$  as:

$$W_{ij} = \frac{A_{ij}}{\sum_k A_{kj}} \quad (2)$$

The steady-state probability vector  $\mathbf{p}$  is solved iteratively until convergence:

$$\mathbf{p}^{(k+1)} = (1 - \alpha)\mathbf{W}\mathbf{p}^{(k)} + \alpha\mathbf{p}_0 \quad (3)$$

where:

- $\alpha = 0.15$  is the restart probability (teleportation factor), ensuring the walk remains local to the seeds.
- $\mathbf{p}_0$  is the restart (seed) vector, with  $p_0(i) = 1/|T|$  for  $i \in T$  (targets) and 0 otherwise.
- Convergence is defined as the  $L_1$  norm of the difference between successive iterations being  $< 10^{-6}$ .

All computations reached convergence within 100 iterations. The total influence  $I$  on the DILI module  $D$  is the sum of steady-state probabilities at disease nodes:  $I = \sum_{d \in D} p(d)$ .

## 4.6 Permutation testing and degree matching

To assess whether the observed influence  $I$  is significantly greater than what would be expected by chance, we performed permutation testing ( $n = 1,000$ ). To account for the bias where high-degree nodes (hubs) naturally accumulate more influence, we utilized a degree-preserving sampling strategy. For each target  $t \in T$ , a random surrogate node was sampled from the network such that its degree  $k_{rand}$  was within  $\pm 25\%$  of the original target's degree  $k_t$ . This ensures that the null distribution reflects the connectivity profile of the original target set. Random seeds were fixed to 42 for reproducibility. Z-scores were computed as  $Z = (x_{obs} - \mu_{null})/\sigma_{null}$ , and empirical  $P$ -values were derived from the null distribution.

## 4.7 Expression-weighted influence

Edge weights were modified by destination-node liver expression:

$$W'_{ij} = \frac{A_{ij} \cdot e_i}{\sum_k A_{kj} \cdot e_k} \quad (4)$$

where  $e_i$  is the normalized liver expression for gene  $i$  (GTEx v8 liver). Liver TPM values were log-transformed ( $\log_2(\text{TPM} + 1)$ ) and min-max normalized to  $[0, 1]$  across the network. A minimum expression floor of 0.01 was applied to ensure all nodes remained reachable. Attracting signal to highly-expressed nodes constrains RWR

propagation to biologically active pathways in the liver. All other RWR parameters were identical. Random seed: 42.

## 4.8 Quantifying perturbation efficiency

By defining the restart vector as  $\mathbf{p}_0(i) = 1/|T|$  (Eq. 75), the total steady-state probability mass  $\mathbf{p}$  is inherently partitioned among the target set. Consequently, the summed influence  $I$  on the DILI module (Eq. 81) represents the average perturbation efficiency per target. This normalization serves as an effect-size adjustment that allows for a direct comparison of the per-unit impact of compounds with asymmetric target sets. Hereafter, we refer to this as the perturbation efficiency.

## 4.9 Bootstrap sensitivity analysis

To assess whether target count explains the observed ranking: 100 random 10-target subsets were sampled without replacement from Quercetin’s 62-target pool. Each subset was scored by standard RWR. Summary statistics: mean, standard deviation, 95th percentile. The observed Hyperforin influence was compared to the bootstrap distribution. Random seed: 42.

## 4.10 Chemical similarity analysis

Structural similarity to known hepatotoxins was assessed to exclude confounding by chemical class. Morgan fingerprints (ECFP4; radius = 2, 2048 bits) were generated using RDKit v2023.03 [18]. Reference set: DILIrank 2.0 drugs with retrievable SMILES (542 DILI-positive, 365 DILI-negative). SMILES were retrieved via PubChem REST API. Tanimoto coefficient:

$$\text{Tanimoto}(A, B) = \frac{|A \cap B|}{|A \cup B|} \quad (5)$$

Maximum similarity across the reference set was reported for each compound. Structural analog threshold: Tanimoto > 0.4 [19].

## 4.11 Software and reproducibility

Python 3.10, NetworkX 3.1 [17]; R 4.3, igraph 1.5. All random seeds fixed at 42. Target lists sorted alphabetically before processing.

## 4.12 Code and data availability

All code: <https://github.com/antonybevan/h-perforatum-network-tox>

Data sources:

- STRING v12.0: <https://string-db.org>
- GTEx v8: <https://gtexportal.org>

- ChEMBL v31: <https://www.ebi.ac.uk/chembl>
- DILIrank 2.0: <https://www.fda.gov/science-research/ltkb>

## Data availability

All data and code supporting this study are publicly available. [Link removed for review]

Source data for all figures and tables are provided in the Supplementary Information. Raw data were obtained from the following public repositories:

- STRING v12.0: <https://string-db.org>
- GTEx v8: <https://gtexportal.org>
- ChEMBL v31: <https://www.ebi.ac.uk/chembl>
- DisGeNET: <https://www.disgenet.org>
- DILIrank 2.0: <https://www.fda.gov/science-research/ltkb>

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## Figure Legends

**Figure 1. Network context: target count and physical proximity to DILI genes.** (A) Target count in the liver-expressed largest connected component. Quercetin: 62 targets; Hyperforin: 10 targets. (B) Shortest-path proximity ( $d_c$ ) to 82 DILI-associated genes. Hyperforin is physically closer ( $d_c = 1.30$ ) than Quercetin ( $d_c = 1.68$ ). Z-scores represent deviation from degree-matched null expectation ( $n = 1,000$  permutations). Quercetin:  $Z = -5.44$  ( $p < 0.001$ ); Hyperforin:  $Z = -3.86$  ( $p < 0.001$ ). Negative Z-scores indicate closer-than-random proximity. Network: STRING v12.0 (confidence  $\geq 900$ ), GTEx v8 (liver TPM  $\geq 1$ ).

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**Figure 3. Expression weighting refines influence propagation.** Waterfall decomposition of Z-score changes under expression-weighted influence (EWI). Initial Hyperforin advantage:  $\Delta Z = +5.57$  (RWR). Hyperforin change:  $-1.14$  (attenuation of signal through liver-active hubs). Quercetin change:  $+1.24$  (gain from high-expression nodes like CFB). Residual Hyperforin advantage:  $\Delta Z = +3.19$ . Both compounds remain significant under EWI: Hyperforin  $Z = +8.98$  ( $p < 0.001$ ); Quercetin  $Z = +5.79$  ( $p < 0.001$ ). Expression weighting from GTEx v8 liver tissue.

**Figure 4. Average network influence quantifies efficiency disparity.** Phase plot of total influence versus target count. Horizontal lines represent efficiency tiers (Efficiency/average influence = constant). Hyperforin occupies a higher efficiency region despite fewer targets. Efficiency/average influence values: Hyperforin = 0.1138 (RWR), 0.1330 (EWI); Quercetin = 0.0322 (RWR), 0.0493 (EWI). Efficiency difference:  $3.7\times$  (based on bootstrap mean comparison). The observed influence represents an effect-size normalization (total steady-state mass on DILI genes); no independent permutation test was performed.

**Figure 5. Bootstrap sensitivity analysis excludes target-count confounding.** Density distribution of RWR influence scores from 100 random 10-target samples drawn from Quercetin's 62-target pool. Shaded region: 95% confidence interval (0.0160–0.0542). Vertical line: Hyperforin observed influence (0.1138). Hyperforin exceeds the entire bootstrap distribution ( $3.7\times$  fold vs. mean). This confirms that Hyperforin's advantage is not attributable to favorable target count. Bootstrap is a robustness control; it does not provide independent statistical evidence.

**Figure 6. Chemical similarity control excludes structural confounding.** Maximum Tanimoto similarity to DILIrank reference drugs. Reference set: 542 DILI-positive, 365 DILI-negative drugs. Hyperforin: max = 0.15 (DILI+), 0.20 (DILI-). Quercetin: max = 0.21 (DILI+), 0.22 (DILI-). Dashed line: 0.4 threshold for structural analog detection [19]. Neither compound is a structural analog of known hepatotoxins. This orthogonal analysis excludes chemical class as an explanation for the observed network signal. Fingerprints: Morgan (ECFP4), radius 2, 2048 bits.

Table 1: **Network metrics reveal the instability of proximity Z-scores.** While Quercetin achieves more significant proximity Z-scores due to tighter null distributions, Hyperforin is physically closer ( $d_c$ ) to DILI genes. Influence-based metrics resolve this confounding and stably prioritize Hyperforin. Network: STRING v12.0 LCC (confidence  $\geq 900$ ) filtered to liver-expressed genes.

| Metric  | Compound   | Targets | Observed     | Z             | p           | Efficiency |
|---|------------|---------|--------------|---------------|-------------|------------|
| <i>Tier 1: Shortest-path proximity</i>  |            |         |              |               |             |            |
|   | Hyperforin | 10      | $d_c = 1.30$ | -3.86         | $< 0.001^*$ | —          |
|   | Quercetin  | 62      | $d_c = 1.68$ | <b>-5.44</b>  | $< 0.001^*$ | —          |
| <i>Instability: Quercetin is physically more distant yet more "significant"</i>           |            |         |              |               |             |            |
| <i>Tier 2: Random walk influence (RWR)</i>  |            |         |              |               |             |            |
|   | Hyperforin | 10      | 0.1138       | <b>+10.12</b> | $< 0.001^*$ | 0.1138     |
|   | Quercetin  | 62      | 0.0322       | +4.55         | $< 0.001$   | 0.0322     |
| <i>Resolution: Correctly prioritizes physical proximity and regulatory hub modulation</i> |            |         |              |               |             |            |
| <i>Tier 3: Expression-weighted influence (EWI)</i>  |            |         |              |               |             |            |
|   | Hyperforin | 10      | 0.1330       | <b>+8.98</b>  | $< 0.001^*$ | 0.1330     |
|   | Quercetin  | 62      | 0.0493       | +5.79         | $< 0.001$   | 0.0493     |

\*At permutation floor ( $< 1/1,000$ ).

Efficiency = average influence per target; RWR = random walk with restart; EWI = expression-weighted influence;  $d_c$  = mean minimum shortest-path distance; DILI = drug-induced liver injury. All associations survived Benjamini–Hochberg FDR correction ( $q < 0.05$ ).

Table 2: **Average influence efficiency.** Normalization to the total seeding mass quantifies the average influence per target. Hyperforin targets are 3.7-fold more efficient at perturbing the DILI module than Quercetin targets.

| Analysis                  | Hyp. Eff. | Quer. Eff. | Eff. Ratio <sup>*</sup> | Rob. Ratio <sup>†</sup> |
|---------------------------|-----------|------------|-------------------------|-------------------------|
| RWR (topology-only)       | 0.1138    | 0.0322     | <b>3.5×</b>             | <b>3.7×</b>             |
| EWI (expression-weighted) | 0.1330    | 0.0493     | <b>2.7×</b>             | <b>2.8×</b>             |

\*Efficiency Ratio = Observed average influence ratio. <sup>†</sup>Robust Ratio = Observed influence / size-matched Bootstrap Mean (N=10). RWR = random walk with restart; EWI = expression-weighted influence.



Table 3: **Bootstrap sensitivity excludes target-count confounding.** Random 10-target subsets ( $n = 100$ ) sampled without replacement from Quercetin’s 62-target pool. Hyperforin’s observed influence exceeds the entire bootstrap distribution.

| Statistic           | Value            | Interpretation                 |
|---------------------|------------------|--------------------------------|
| Hyperforin observed | 0.1138           | Reference                      |
| Bootstrap mean      | 0.0308           | Expected if targets equivalent |
| Bootstrap SD        | 0.0100           | Sampling variability           |
| Bootstrap 95% CI    | [0.0160, 0.0542] | 2.5th–97.5th percentile        |
| Hyperforin / mean   | <b>3.7</b> ×     | Effect size                    |
| Exceeds 95% CI?     | <b>Yes</b>       | Not attributable to sampling   |

Random seed: 42. Note: Bootstrap confirms robustness to target selection; it does not constitute independent inferential evidence.

Table 4: **Chemical similarity excludes structural confounding.** Neither compound resembles known hepatotoxins (Tanimoto  $< 0.4$ ). Quercetin is more similar to DILI-positive drugs yet shows lower network influence.

| Compound   | Max Tanimoto (DILI+) | Max Tanimoto (DILI−) | Analog?* | Network rank         |
|------------|----------------------|----------------------|----------|----------------------|
| Hyperforin | 0.154                | 0.202                | No       | 1 (higher influence) |
| Quercetin  | 0.212                | 0.220                | No       | 2 (lower influence)  |

\*Analog threshold: Tanimoto  $> 0.4$  (Maggiora et al., 2014). Morgan fingerprints (ECFP4, radius 2, 2048 bits). DILIRank: 542 DILI+, 365 DILI− drugs.

Table 5: **Hyperforin targets include regulatory hubs.** All 10 Hyperforin targets in the liver-expressed LCC, with liver expression (GTEx v8) and network degree. PXR (NR1I2) is the master regulator; CYP enzymes are downstream effectors.

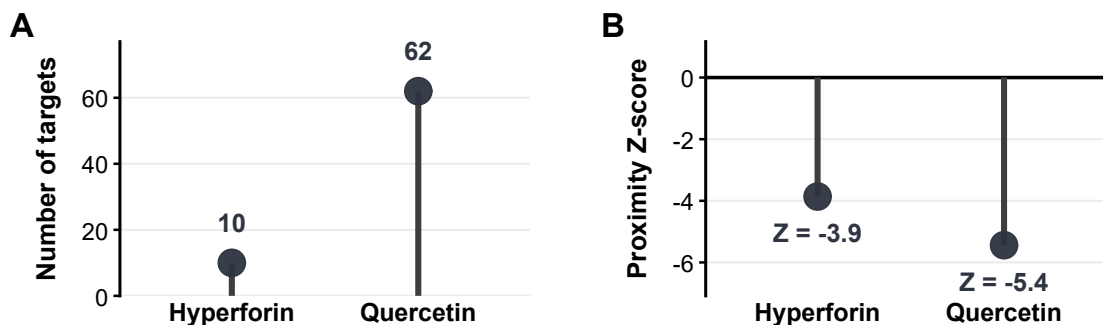
| Gene   | Protein | TPM | Degree     | Function              | DILI link |
|--------|---------|-----|------------|-----------------------|-----------|
| NR1I2  | PXR     | 43  | 28         | Master regulator      | Direct    |
| CYP3A4 | CYP3A4  | 335 | 89         | Xenobiotic metabolism | Direct    |
| CYP2C9 | CYP2C9  | 434 | 76         | Xenobiotic metabolism | Direct    |
| CYP2B6 | CYP2B6  | 125 | 42         | Xenobiotic metabolism | Indirect  |
| AKT1   | PKB     | 33  | <b>312</b> | Stress signaling      | Indirect  |
| ABCB1  | P-gp    | 7   | 53         | Drug efflux           | Direct    |
| ABCC2  | MRP2    | 60  | 38         | Drug efflux           | Direct    |
| ABCG2  | BCRP    | 4   | 31         | Drug efflux           | Indirect  |
| MMP2   | MMP2    | 5   | 87         | ECM remodeling        | Indirect  |
| MMP9   | MMP9    | 1   | 94         | ECM remodeling        | Indirect  |

AKT1 is the highest-degree target (312 neighbors). Five of 10 targets (NR1I2, CYP3A4, CYP2C9, ABCB1, ABCC2) are directly connected to DILI genes. TPM = transcripts per million; DILI = drug-induced liver injury; LCC = largest connected component.

Table 6: **Influence ranking is robust to network construction parameters.** Hyperforin ranks first across all thresholds and influence metrics. Proximity Z-scores are unstable and reverse rankings between thresholds, failing to accurately reflect the physical distance advantage of Hyperforin.

| Threshold                    | Compound   | RWR Z         | EWI Z  | Proximity $d_c$ | Proximity Z |
|------------------------------|------------|---------------|--------|-----------------|-------------|
| $\geq 700$<br>(11,693 nodes) | Hyperforin | <b>+12.08</b> | +11.20 | 0.60            | -6.04       |
|                              | Quercetin  | +5.53         | +7.09  | 1.34            | -5.46       |
| $\geq 900$<br>(7,677 nodes)  | Hyperforin | <b>+10.12</b> | +8.98  | 1.30            | -3.86       |
|                              | Quercetin  | +4.55         | +5.79  | 1.68            | -5.44       |

Note: At  $\geq 900$ , Quercetin achieves a more "significant" proximity Z-score despite being physically more distant (1.68 vs 1.30) from DILI genes. RWR = random walk with restart; EWI = expression-weighted influence;  $d_c$  = mean minimum shortest-path distance; DILI = drug-induced liver injury.

**Network context: target count and proximity to DILI genes**

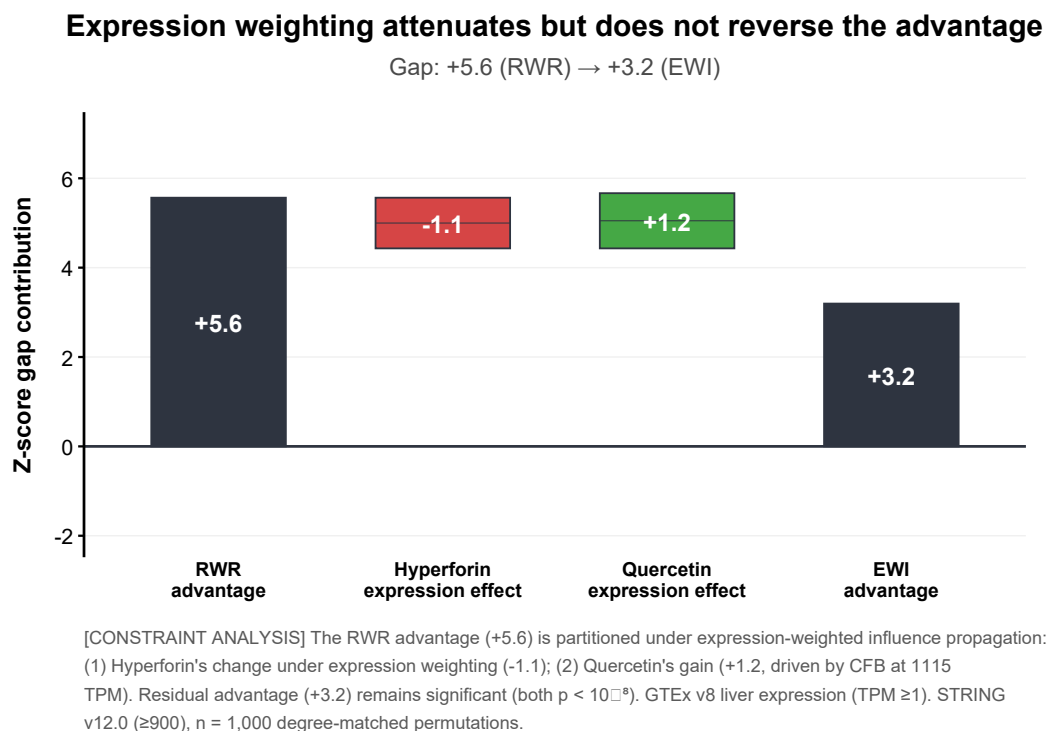
[DESCRIPTIVE CONTEXT] Target count and shortest-path proximity provide network context but are not used for causal inference. Proximity Z-scores represent deviation from degree-matched random expectation ( $n = 1,000$  permutations). Negative values indicate closer-than-random proximity. Data: STRING v12.0 ( $\geq 900$ ), human liver LCC.

**Figure 1: Network context: target count and physical proximity to DILI genes.** (A) Target count in the liver-expressed largest connected component. Quercetin: 62 targets; Hyperforin: 10 targets. (B) Shortest-path proximity ( $d_c$ ) to 82 DILI-associated genes. Hyperforin is physically closer ( $d_c = 1.30$ ) than Quercetin ( $d_c = 1.68$ ). Z-scores represent deviation from degree-matched null expectation ( $n = 1,000$  permutations). Quercetin:  $Z = -5.44$  ( $p < 0.001$ ); Hyperforin:  $Z = -3.86$  ( $p < 0.001$ ). Negative Z-scores indicate closer-than-random proximity. Network: STRING v12.0 (confidence  $\geq 900$ ), GTEx v8 (liver TPM  $\geq 1$ ).



[CORE INFERENCE] The rank reversal demonstrates that shortest-path proximity does not predict functional influence. Lines connect each compound's proximity Z-score with its influence Z-score (random walk with restart, RWR). Both metrics derived from degree-matched permutation null models ( $n = 1,000$ ). Data: STRING v12.0 ( $\geq 900$ ).

**Figure 2: Instability of proximity Z-scores.** Dumbbell plot showing the dissociation between shortest-path proximity (left) and random walk influence (right) at STRING confidence  $\geq 900$ . At this threshold, Quercetin appears more "significant" in Z-score but is physically more distant (1.68 vs 1.30) from DILI genes. Hyperforin: proximity  $Z = -3.86$ , influence  $Z = +10.12$  ( $p < 0.001$ ). Quercetin: proximity  $Z = -5.44$ , influence  $Z = +4.55$  ( $p < 0.001$ ). Influence quantified by random walk with restart (RWR;  $\alpha = 0.15$ ).  $n = 1,000$  degree-matched permutations per compound.



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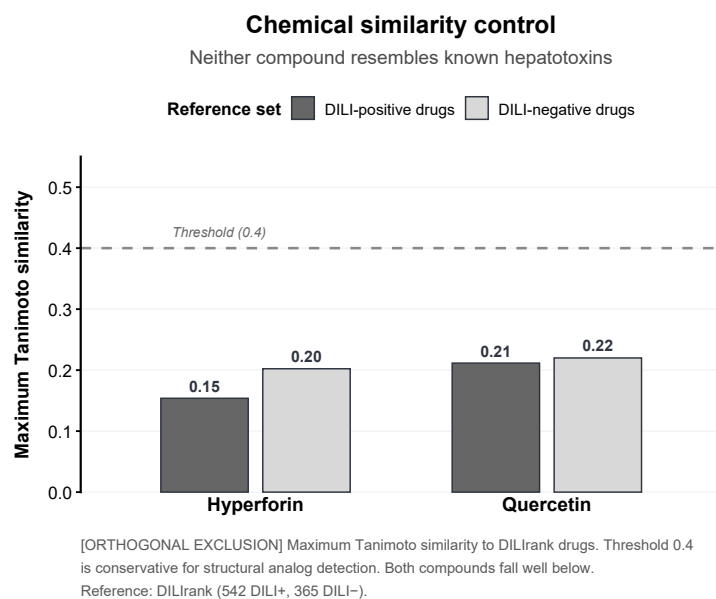


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## 413 **Author contributions**

414 **Author(s):** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation,  
415 Writing - Original Draft, Writing - Review & Editing, Visualization.

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## 419 **Competing interests**

420 The author(s) declare no competing interests.

Table 7: **Table S1. Hyperforin target genes and literature sources.** All 14 raw targets with UniProt IDs, gene symbols, and primary literature sources. Targets marked with \* are present in the liver-expressed LCC (STRING  $\geq 900$ , GTE<sub>x</sub> TPM  $\geq 1$ ).

| UniProt | Gene        | In LCC | Source |
|---------|-------------|--------|--------|
| O75469  | NR1I2 (PXR) | Yes*   | [6, 9] |
| P08684  | CYP3A4      | Yes*   | [6]    |
| P11712  | CYP2C9      | Yes*   | [20]   |
| P20813  | CYP2B6      | Yes*   | [21]   |
| P08183  | ABCB1       | Yes*   | [22]   |
| Q9UNQ0  | ABCG2       | Yes*   | [23]   |
| O15440  | ABCC2       | Yes*   | [24]   |
| P31749  | AKT1        | Yes*   | [25]   |
| P08253  | MMP2        | Yes*   | [25]   |
| P14780  | MMP9        | Yes*   | [26]   |
| Q9Y210  | TRPC6       | No     | [27]   |
| P15692  | VEGFA       | No     | [26]   |
| Q13794  | PMAIP1      | No     | [28]   |
| Q12879  | GRIN1       | No     | [29]   |

Table 8: **Table S2. Quercetin target curation summary.** Target counts at each processing stage.

| Stage  | Count |
|--|-------|
| Raw targets (ChEMBL v31, ChEMBL159)                | 122   |
| Excluded: non-human (mouse, rat, bacterial, viral) | 10    |
| Excluded: no UniProt mapping                       | 25    |
| Processed targets                                  | 87    |
| Excluded: not liver-expressed (TPM < 1)            | 20    |
| Excluded: not in STRING LCC                        | 5     |
| Final targets in LCC                               | 62    |

Table 9: **Table S3. DILI gene set curation.** Genes associated with drug-induced liver injury from DisGeNET (UMLS C0860207).

| Stage                                 | Count |
|---------------------------------------|-------|
| Raw DILI genes (DisGeNET)             | 127   |
| In STRING $\geq 700$ liver LCC        | 84    |
| In STRING $\geq 900$ liver LCC        | 82    |
| Excluded: miRNAs (not in PPI network) | 21    |
| Excluded: cytokines (not in LCC)      | 12    |
| Excluded: other                       | 12    |

Table 10: **Table S4. Genes targeted by both compounds.** Five genes present in both Hyperforin and Quercetin target sets.

| Gene   | Protein                    | Function                        |
|--------|----------------------------|---------------------------------|
| ABCG2  | BCRP                       | Efflux transporter              |
| AKT1   | Protein kinase B           | Cell survival signaling         |
| CYP3A4 | Cytochrome P450 3A4        | Drug metabolism                 |
| MMP2   | Matrix metalloproteinase-2 | Extracellular matrix remodeling |
| MMP9   | Matrix metalloproteinase-9 | Extracellular matrix remodeling |

Table 11: **Table S5. Direct DILI gene connectivity.** Hyperforin targets with first-order (distance = 1) connections to DILI genes in the STRING network ( $\geq 900$ ). DILI neighbors are genes present in the 82-gene DILI set.

| Target              | DILI Neighbors                      | N         | Function              |
|---------------------|-------------------------------------|-----------|-----------------------|
| CYP3A4              | NR1I2, CYP2E1, UGT1A9, GSTM1, GSTP1 | 5         | Xenobiotic metabolism |
| AKT1                | MAP3K5, NFE2L2, CTNNB1, IGF1        | 4         | Stress response       |
| MMP9                | LCN2, SPP1, MMP2                    | 3         | Inflammation/ECM      |
| ABCB1               | ABCC2, NR1I2                        | 2         | Drug transport        |
| CYP2C9              | CYP2E1, NR1I2                       | 2         | Xenobiotic metabolism |
| CYP2B6              | NR1I2                               | 1         | Xenobiotic metabolism |
| NR1I2               | CYP2E1, ABCC2                       | 2         | Master regulator      |
| ABCG2               | ABCC2                               | 1         | Drug transport        |
| ABCC2               | NR1I2, ABCB1                        | 2         | Drug transport        |
| MMP2                | MMP9, SPP1                          | 2         | ECM remodeling        |
| <b>Total unique</b> |                                     | <b>12</b> |                       |

Table 12: **Table S6. Quercetin direct DILI gene connectivity summary.** Summary statistics for first-order DILI connections across Quercetin’s 62 targets.

| Metric   | Value        |
|--|--------------|
| Total targets in LCC                           | 62           |
| Targets with $\geq 1$ direct DILI neighbor     | 18           |
| Total direct DILI connections                  | 31           |
| Mean DILI neighbors per target                 | 0.50         |
| <i>Hyperforin comparison:</i>                  |              |
| Hyperforin targets with $\geq 1$ DILI neighbor | 10/10 (100%) |
| Mean DILI neighbors per Hyperforin target      | 2.4          |

Table 13: **Table S7. Quercetin target genes in the liver-expressed network.** All 62 Quercetin targets in STRING v12.0 LCC (confidence  $\geq 900$ ) with liver TPM  $\geq 1$  (GTEx v8). Sorted by descending liver expression.

| Gene   | TPM  | Gene     | TPM | Gene     | TPM | Gene   | TPM |
|--------|------|----------|-----|----------|-----|--------|-----|
| CFB    | 1115 | CYP3A4   | 335 | FN1      | 229 | ALDH2  | 183 |
| ANPEP  | 160  | PPIA     | 112 | SERPINA5 | 104 | CYP1A2 | 72  |
| CA2    | 64   | APP      | 63  | PYGL     | 55  | HDAC6  | 45  |
| ESRRA  | 42   | MAOA     | 35  | AKR1C2   | 33  | AKT1   | 33  |
| CTSH   | 28   | XDH      | 26  | CHRNA4   | 25  | PIK3R1 | 24  |
| PIM1   | 24   | LDLR     | 23  | EGFR     | 17  | ELOVL1 | 18  |
| PKN1   | 16   | GSK3A    | 13  | YES1     | 13  | MET    | 12  |
| DAPK1  | 12   | BACE1    | 11  | CSNK2A1  | 10  | FSTL1  | 9   |
| SIRT6  | 8    | GSK3B    | 7   | CDK7     | 7   | CAV2   | 7   |
| PTPN2  | 6    | CYP1A1   | 5   | PRMT7    | 5   | MMP2   | 5   |
| AKR1B1 | 5    | PDE6D    | 5   | PTK2     | 4   | ABCG2  | 4   |
| IQGAP1 | 4    | ADRB2    | 3   | BRAF     | 4   | KDR    | 3   |
| SRC    | 3    | ALOX5    | 3   | CYP1B1   | 3   | TLR4   | 3   |
| NUAK1  | 3    | AXL      | 2   | ADA      | 2   | LCK    | 2   |
| ABCC1  | 2    | PLK1     | 1   | ACHE     | 1   | MMP9   | 1   |
| SYK    | 1    | PDZK1IP1 | 1   |          |     |        |     |

Table 14: **Table S8. DILI gene set (82 genes).** Genes in STRING v12.0 LCC (confidence  $\geq 900$ ) with liver TPM  $\geq 1$  (GTEx v8). Source: DisGeNET (UMLS C0860207). Sorted alphabetically.

| 82 DILI-Associated Genes |          |        |        |        |        |         |        |
|--------------------------|----------|--------|--------|--------|--------|---------|--------|
| ABCB1                    | AHR      | ALB    | ALDOB  | AMBP   | APOA1  | APOE    | APOH   |
| ARG1                     | ARNT     | ATG5   | BAX    | BTD    | C3     | CAT     | CCL2   |
| CLU                      | COL3A1   | CTNNB1 | CXCL1  | CXCL10 | CYP2A6 | CYP2C19 | CYP2C9 |
| CYP2E1                   | DGAT2    | ENO1   | FGA    | FLT1   | FMO3   | GADD45A | GC     |
| GCLC                     | GPT      | GSN    | GSTM1  | GSTM2  | GSTP1  | HLA-A   | HLA-B  |
| HLA-DQB1                 | HLA-DRB1 | HMGB1  | HMOX1  | HPD    | HPX    | IGF1    | IL18   |
| IL1R2                    | KRT18    | LCN2   | LGALS3 | MAP3K5 | MED1   | MMP2    | MTHFR  |
| NAT2                     | NFE2L2   | NR1H3  | NR1H4  | NR1I2  | NR1I3  | PLAT    | PLG    |
| PNP                      | POLG     | PON1   | PPARA  | PRKDC  | PTGS2  | RBP1    | SLPI   |
| SNX18                    | SOD1     | SOD3   | SPP1   | TALDO1 | TBXA2R | TCTN1   | TF     |
| TTR                      | UGT1A9   |        |        |        |        |         |        |

Table 15: **Table S9. Null distribution parameters from permutation testing.** Null distribution parameters (mean and standard deviation) from  $n = 1,000$  degree-matched permutations. Note the tightening of the Quercetin null distribution as the number of targets increases, which drives the inflation of proximity Z-scores.

| <b>Metric</b>   | <b>Compound</b> | $\mu_{null}$ | $\sigma_{null}$ | $x_{obs}$ | <b>Z-score</b> |
|---|-----------------|--------------|-----------------|-----------|----------------|
| <i>Shortest-path proximity (at <math>\geq 900</math>)</i>       |                 |              |                 |           |                |
|   | Hyperforin (10) | 2.21         | 0.235           | 1.30      | -3.86          |
|   | Quercetin (62)  | 2.17         | 0.091           | 1.68      | -5.44          |
| <i>Random walk influence (at <math>\geq 900</math>)</i>         |                 |              |                 |           |                |
|   | Hyperforin (10) | 0.0147       | 0.0098          | 0.1138    | +10.12         |
|   | Quercetin (62)  | 0.0148       | 0.0038          | 0.0322    | +4.55          |
| <i>Expression-weighted influence (at <math>\geq 900</math>)</i> |                 |              |                 |           |                |
|   | Hyperforin (10) | 0.0205       | 0.0125          | 0.1330    | +8.98*         |
|   | Quercetin (62)  | 0.0209       | 0.0049          | 0.0493    | +5.79*         |

\*Significance remains high despite tissue-specific attenuation.  $\mu_{null}$  = null distribution mean;  $\sigma_{null}$  = null distribution standard deviation;  $x_{obs}$  = observed metric value.