

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

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5 **Abstract**

6 Network-based metrics are widely used to identify associations between compounds and diseases, as-
7 suming that proximity within a protein–protein interaction network reflects functional relevance. How-
8 ever, these metrics are often reported as Z-scores, which we demonstrate are fundamentally sensitive to
9 the number of targets a compound possesses. This dependency introduces a systematic bias where com-
10 pounds with broad polypharmacology appear statistically significant due to null distribution tightening
11 (the Law of Large Numbers) rather than physical network reachability. Here, we systematically audit
12 this bias using the human liver interactome and a controlled comparison of two constituents from *Hy-*
13 *pericum perforatum*. We show that conventional proximity Z-scores yield unstable rankings that reverse
14 depending on network construction parameters. While a compound with many targets may achieve a
15 higher Z-score, it can remain physically more distant from the disease module than a compound with
16 fewer, high-leverage targets. We resolve this by utilizing random walk–based influence propagation and
17 applying a size-normalized metric, perturbation efficiency, to ensure unbiased comparisons. Our results
18 show that influence-based rankings are stable across varied network thresholds and correctly identify
19 high-leverage modulators that proximity metrics miss. This study provides a methodological template
20 for identifying and correcting statistical artifacts in network medicine, enabling more reliable risk assess-
21 ment in complex biological systems.

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

24 1 Introduction

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

35 Using the human liver interactome as a model system, we investigate this confounding effect through
36 a controlled comparison of two constituents from *Hypericum perforatum* (St. John’s Wort). These con-
37 stituents—Hyperforin and Quercetin—exhibit highly asymmetric target set sizes: Hyperforin possesses
38 10 validated targets, while Quercetin has over 60 [5–7]. This system serves as a controlled model because
39 it pairs a known biological ground truth (Hyperforin-mediated PXR activation) with extreme topolog-
40 ical asymmetry, providing a sharp stress test for network metrics. Conventional proximity Z-scores
41 predict greater disease-associated significance for the broad-spectrum modulator, even when it is phys-
42 ically more distant from the disease module than the high-leverage modulator. This reversal indicates
43 that proximity-based prioritization is unstable across network construction parameters and susceptible to
44 sample-size artifacts.

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

53 **2 Results**

54 **2.1 Proximity Z-scores are confounded by target set size**

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

62 This statistical artifact suggests that Quercetin poses greater risk, whereas the physical topology fa-
63 vors Hyperforin. This effect occurs independently of network construction parameters and represents a
64 fundamental statistical property of averaged distributions: larger samples inherently produce more pre-
65 cise (narrower) null distributions, artificially inflating Z-score magnitude regardless of actual topological
66 proximity. This is a manifestation of the Law of Large Numbers (LLN).

67 **2.2 Influence-based rankings are stable and resolve the confound**

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

76 **2.3 Expression weighting refines the signal**

77 To assess whether the RWR signal persists under tissue-specific constraint, we applied expression-
78 weighted influence propagation (EWI), weighting transitions by destination-node liver expression (Fig-
79 ure 3).

80 The Z-score differential narrows but remains substantial under expression weighting: Hyperforin
81 $Z = +8.98$ ($p < 0.001$); Quercetin $Z = +5.79$ ($p < 0.001$). Hyperforin’s advantage is driven primarily

82 by the PXR–CYP master regulatory axis, which remains highly active in liver tissue (e.g., CYP3A4 at
83 335 TPM). Quercetin’s influence is moderated by its broad, diffuse target profile, which includes several
84 high-expression nodes (e.g., CFB at 1,115 TPM) that do not converge on a DILI effector hub.

85 **2.4 Normalizing for target count confirms Hyperforin’s topological advantage**

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

Compound	Targets	Eff. (RWR)	Eff. (EWI)	RWR Ratio*	EWI Ratio†
Hyperforin	10	0.1138	0.1330	—	—
Quercetin	62	0.0322	0.0493	—	—
Fold difference	—	—	—	3.5× (3.7×)	2.7× (2.8×)

90 *RWR Ratio: observed ratio (robust ratio in parentheses). †EWI Ratio: observed ratio (robust ratio in paren-
91 theses).

92 Each Hyperforin target contributes 3.7× more DILI-directed influence than each Quercetin target (robust ra-
93 tio). This disparity indicates that Hyperforin’s target positions are substantially higher leverage than those of
94 Quercetin, achieving greater perturbation efficiency despite a 6-fold smaller target set. The efficiency ratio re-
95 mains stable within a narrow range: 3.7× at STRING ≥ 700 , 3.5× at ≥ 900 (a 5% variation despite 33% reduction
96 in network density). In contrast, proximity Z-score rankings undergo complete reversal between these thresholds.
97 This demonstrates that perturbation efficiency is a parameter-invariant comparative metric where distance-based
98 Z-scores are not.

99 **2.5 Bootstrap resampling excludes target-selection bias**

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

103 Hyperforin’s observed influence (0.1138) exceeds the entire bootstrap distribution from Quercetin (mean =
104 0.0308, 95% CI = [0.0160, 0.0542]; Table 3). The fold difference between Hyperforin and the bootstrap mean is
105 3.7×. This confirms that Hyperforin’s advantage is not an artifact of target count; even when sampling equalized
106 subsets from Quercetin’s pool, no configuration matches Hyperforin’s influence.

107 **2.6 Ranking stability across network thresholds**

108 The influence ranking is stable across network confidence thresholds (Table 6). Hyperforin ranks first in all RWR
109 and EWI configurations at both ≥ 700 and ≥ 900 thresholds. Notably, the proximity ranking reverses between
110 thresholds: at ≥ 700 , Hyperforin is physically closer ($d_c = 0.60$ vs 1.34) and more "significant" ($Z = -6.04$ vs
111 -5.46). At ≥ 900 , Quercetin appears more "significant" ($Z = -5.44$ vs -3.86) despite being physically more dis-
112 tant (1.68 vs 1.30). This instability in proximity Z-scores—while influence rankings remain stable—demonstrates
113 that influence-based metrics are more robust to network construction parameters.

114 **2.7 Chemical similarity excludes structural confounding**

115 To exclude the possibility that Hyperforin's network signal reflects structural similarity to known hepatotoxins,
116 we performed chemical similarity analysis against the DILrank reference set (Figure 6). Morgan fingerprints
117 (ECFP4) revealed that neither compound exceeds the 0.4 Tanimoto threshold for structural analog detection. No-
118 tably, Quercetin exhibits higher structural similarity to DILI reference drugs yet lower network influence, reinforc-
119 ing that the observed asymmetry is driven by network topology rather than chemical features.

120 **3 Discussion**

121 **3.1 Ranking stability and the Z-score confound**

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

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

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

138 **3.2 Relationship to prior work**

139 Our findings do not contradict the foundational work of Guney et al. (2016), but rather identify a specific failure
140 mode that their study design did not stress-test. Guney et al. evaluated network proximity as a classifier for drug-
141 disease associations across 238 drugs with a mean of 3.5 targets per drug—a relatively homogeneous dataset.
142 They reported that proximity is “not biased with respect to the number of targets a drug has” and found that the
143 closest-distance measure (d_c) outperformed a diffusion kernel measure (d_k) for binary classification [3].

144 Our study addresses a fundamentally different question: *comparative ranking* of two compounds with highly
145 asymmetric target counts (10 vs. 62). In this regime, the variance-shrinkage artifact becomes a first-order problem.
146 Guney’s kernel benchmark (d_k) is related to but distinct from random walk with restart; d_k sums contributions from
147 all weighted paths, whereas RWR iteratively propagates probability mass with a restart factor that anchors the walk
148 to seed nodes. More critically, neither d_c nor d_k provides a principled normalization for target set size.

149 A primary methodological resolution proposed in this study is the use of *perturbation efficiency*: the average
150 influence exerted per target. This normalization resolves the target-count paradox regardless of whether the under-
151 lying propagation method is shortest-path, kernel, or random walk. By framing polypharmacology as an efficiency
152 problem rather than a coverage problem, we provide a bias-corrected comparative framework that survives ro-
153 bustness checks where raw Z-scores fail. While Guney et al. found that a diffusion kernel underperformed closest
154 distance for binary classification of known drug-disease pairs, our task differs fundamentally: we address compara-
155 tive ranking under extreme target-count asymmetry (10 vs. 62 targets). RWR’s restart mechanism enforces locality
156 absent in pure diffusion kernels, and our empirical results demonstrate stable rankings that align with biological
157 ground truth—a criterion not assessed in Guney’s benchmark.

158 **3.3 Expression weighting as a biological constraint**

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

165 **3.4 Perturbation efficiency vs. topological coverage**

166 By normalizing total influence for target set size (where the restart vector is already $|T|$ -weighted), we provide
167 a more balanced comparison of perturbation efficiency. Our results show that a single Hyperforin target exerts
168 3.7-fold more influence on the DILI module than a Quercetin target.

169 This efficiency claim is further validated by bootstrap sensitivity analysis. Even when sampling size-matched
170 10-target subsets from Quercetin’s pool, none reached the influence level achieved by Hyperforin. This demon-

171 strates that the advantage is not due to target count, but to the strategic network position of Hyperforin’s tar-
172 gets—specifically their convergence on the PXR master regulator and downstream CYP effectors.

173 **3.5 Mechanistic context: The PXR axis**

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

182 **3.6 Limitations**

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

188 Second, while we demonstrate that proximity Z-scores are confounded by target set size, influence-based Z-
189 scores are not entirely immune to this effect. As the number of seed nodes increases, the variance of the null
190 distribution for influence sums also decreases, though less severely than for distance-based metrics. Critically,
191 our core claims do not rest on absolute Z-score comparisons. We demonstrate that influence-based *rankings* are
192 stable across network thresholds, while proximity rankings are not. We further resolve the size-dependence by
193 introducing perturbation efficiency (influence per target), which explicitly normalizes for target count and provides
194 a bias-corrected comparative metric.

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

197 **3.7 Conclusions**

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

203 combined with per-target normalization, provides a more stable framework that survives robustness checks and
204 aligns better with mechanistic reality.

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

209 4 Methods

210 4.1 Data sources

211 4.1.1 Protein–protein interaction network

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

216 4.1.2 Liver expression data

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

220 4.1.3 Drug-induced liver injury gene set

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

224 4.1.4 Hyperforin targets

225 Hyperforin targets were curated from primary literature sources [6, 9]. Sources included studies of PXR activation,
226 CYP induction, and ABC transporter modulation. Raw target count: 14 proteins (Table 5).

227 4.1.5 Quercetin targets

228 Quercetin targets were retrieved programmatically from ChEMBL v31 [15] via REST API. Query: CHEMBL159
229 (Quercetin). Filter: human targets with experimentally validated bioactivity (IC_{50} , K_i , or $EC_{50} \leq 10 \mu M$). Raw
230 target count: 122 proteins.

231 **4.2 Target processing**

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

235 **4.3 Network construction**

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

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

242 **4.4 Shortest-path proximity (descriptive)**

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

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

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

246 **4.5 Random walk with restart**

247 Influence propagation was quantified using random walk with restart (RWR), a global network propagation al-
248 gorithm that captures both direct and indirect associations by simulating the diffusion of signal from seed nodes
249 [3, 8]. Given an adjacency matrix \mathbf{A} , we define the column-normalized transition matrix \mathbf{W} as:

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

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

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

251 where:

- 252 • $\alpha = 0.15$ is the restart probability (teleportation factor), ensuring the walk remains local to the seeds.
253 • \mathbf{p}_0 is the restart (seed) vector, with $p_0(i) = 1/|T|$ for $i \in T$ (targets) and 0 otherwise.

- 254 • Convergence is defined as the L_1 norm of the difference between successive iterations being $< 10^{-6}$.
- 255 All computations reached convergence within 100 iterations. The total influence I on the DILI module D is the
 256 sum of steady-state probabilities at disease nodes: $I = \sum_{d \in D} p(d)$.

257 **4.6 Permutation testing and degree matching**

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

265 **4.7 Expression-weighted influence**

266 Edge weights were modified by destination-node liver expression:

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

267 where e_i is the normalized liver expression for gene i (GTEx v8 liver). Liver TPM values were log-transformed
 268 ($\log_2(\text{TPM} + 1)$) and min-max normalized to [0, 1] across the network. A minimum expression floor of 0.01 was
 269 applied to ensure all nodes remained reachable. Attracting signal to highly-expressed nodes constrains RWR
 270 propagation to biologically active pathways in the liver. All other RWR parameters were identical. Random seed:
 271 42.

272 **4.8 Quantifying perturbation efficiency**

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

278 **4.9 Bootstrap sensitivity analysis**

279 To assess whether target count explains the observed ranking: 100 random 10-target subsets were sampled without
 280 replacement from Quercetin's 62-target pool. Each subset was scored by standard RWR. Summary statistics: mean,

281 standard deviation, 95th percentile. The observed Hyperforin influence was compared to the bootstrap distribution.
282 Random seed: 42.

283 **4.10 Chemical similarity analysis**

284 Structural similarity to known hepatotoxins was assessed to exclude confounding by chemical class. Morgan
285 fingerprints (ECFP4; radius = 2, 2048 bits) were generated using RDKit v2023.03 [18]. Reference set: DILIrank
286 2.0 drugs with retrievable SMILES (542 DILI-positive, 365 DILI-negative). SMILES were retrieved via PubChem
287 REST API. Tanimoto coefficient:

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

288 Maximum similarity across the reference set was reported for each compound. Structural analog threshold: Tani-
289 moto > 0.4 [19].

290 **4.11 Software and reproducibility**

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

293 **4.12 Code and data availability**

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

295 Data sources:

- 296 • STRING v12.0: <https://string-db.org>
- 297 • GTEx v8: <https://gtexportal.org>
- 298 • ChEMBL v31: <https://www.ebi.ac.uk/chembl>
- 299 • DILIRank 2.0: <https://www.fda.gov/science-research/ltrkb>

300 **Data availability**

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

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

- 304 • STRING v12.0: <https://string-db.org>
- 305 • GTEx v8: <https://gtexportal.org>
- 306 • ChEMBL v31: <https://www.ebi.ac.uk/chembl>
- 307 • DisGeNET: <https://www.disgenet.org>

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395 **Figure Legends**

396 **Figure 1. Network context: target count and physical proximity to DILI genes.** (A) Target count in the
397 liver-expressed largest connected component. Quercetin: 62 targets; Hyperforin: 10 targets. (B) Shortest-path
398 proximity (d_c) to 82 DILI-associated genes. Hyperforin is physically closer ($d_c = 1.30$) than Quercetin ($d_c = 1.68$).
399 Z-scores represent deviation from degree-matched null expectation ($n = 1,000$ permutations). Quercetin: $Z =$
400 -5.44 ($p < 0.001$); Hyperforin: $Z = -3.86$ ($p < 0.001$). Negative Z-scores indicate closer-than-random proximity.
401 Network: STRING v12.0 (confidence ≥ 900), GTEx v8 (liver TPM ≥ 1).

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

408 **Figure 3. Expression weighting refines influence propagation.** Waterfall decomposition of Z-score changes
409 under expression-weighted influence (EWI). Initial Hyperforin advantage: $\Delta Z = +5.57$ (RWR). Hyperforin change:
410 -1.14 (attenuation of signal through liver-active hubs). Quercetin change: $+1.24$ (gain from high-expression
411 nodes like CFB). Residual Hyperforin advantage: $\Delta Z = +3.19$. Both compounds remain significant under EWI:
412 Hyperforin $Z = +8.98$ ($p < 0.001$); Quercetin $Z = +5.79$ ($p < 0.001$). Expression weighting from GTEx v8 liver
413 tissue.

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

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

425 **Figure 6. Chemical similarity control excludes structural confounding.** Maximum Tanimoto similarity to
426 DILrank reference drugs. Reference set: 542 DILI-positive, 365 DILI-negative drugs. Hyperforin: max = 0.15
427 (DILI+), 0.20 (DILI-). Quercetin: max = 0.21 (DILI+), 0.22 (DILI-). Dashed line: 0.4 threshold for structural
428 analog detection [19]. Neither compound is a structural analog of known hepatotoxins. This orthogonal analysis
429 excludes chemical class as an explanation for the observed network signal. Fingerprints: Morgan (ECFP4), radius
430 2, 2048 bits.

431 **Tables**

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 ≥ 900) filtered to liver-expressed genes.

Metric	Compound	Targets	Observed	Z-score	P-value	Efficiency
<i>Tier 1: Shortest-path proximity</i>						
	Hyperforin	10	$d_c = 1.30$	-3.86	< 0.001*	—
	Quercetin	62	$d_c = 1.68$	-5.44	< 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	+10.12	< 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	+8.98	< 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*	Rob. Ratio†
RWR (topology-only)	0.1138	0.0322	3.5×	3.7×
EWI (expression-weighted)	0.1330	0.0493	2.7×	2.8×

*Efficiency Ratio = Observed average influence ratio. †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	3.7×	Effect size
Exceeds 95% CI?	Yes	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 ($\text{Tanimoto} < 0.4$). 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: $\text{Tanimoto} > 0.4$ (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	312	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.

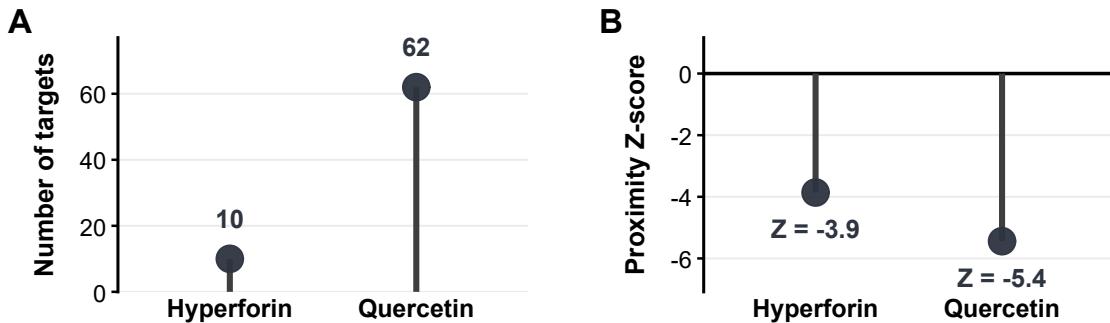
Table 6: **Influence ranking is robust to network construction parameters.** Hyperforin ranks first across all thresholds and influence metrics. Proximity Z-scores are unstable and reverse rankings between thresholds, failing to accurately reflect the physical distance advantage of Hyperforin.

Threshold	Compound	RWR Z	EWI Z	Proximity d_c	Proximity Z
≥ 700 (11,693 nodes)	Hyperforin	+12.08	+11.20	0.60	-6.04
	Quercetin	+5.53	+7.09	1.34	-5.46
≥ 900 (7,677 nodes)	Hyperforin	+10.12	+8.98	1.30	-3.86
	Quercetin	+4.55	+5.79	1.68	-5.44

Note: At ≥ 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.

432 **Figures**

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 (≥ 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 ≥ 900), GTEx v8 (liver TPM ≥ 1).

Proximity does not predict influence

Proximity ranking is threshold-dependent; influence ranking is stable



[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, RWI). Both metrics derived from degree-matched permutation null models ($n = 1,000$). Data: STRING v12.0 (≥ 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 ≥ 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.

Expression weighting attenuates but does not reverse the advantage

Gap: +5.6 (RWR) → +3.2 (EWI)



[CONSTRAINT ANALYSIS] The RWR advantage (+5.6) is partitioned under expression-weighted influence propagation:
 (1) Hyperforin's change under expression weighting (-1.1); (2) Quercetin's gain (+1.2, driven by CFB at 1115
 TPM). Residual advantage (+3.2) remains significant (both $p < 10^{-8}$). GTEx v8 liver expression (TPM ≥ 1). STRING
 v12.0 (≥ 900), $n = 1,000$ degree-matched permutations.

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.

Average network influence quantifies perturbation efficiency

Normalization reframes polypharmacology as efficiency, not coverage

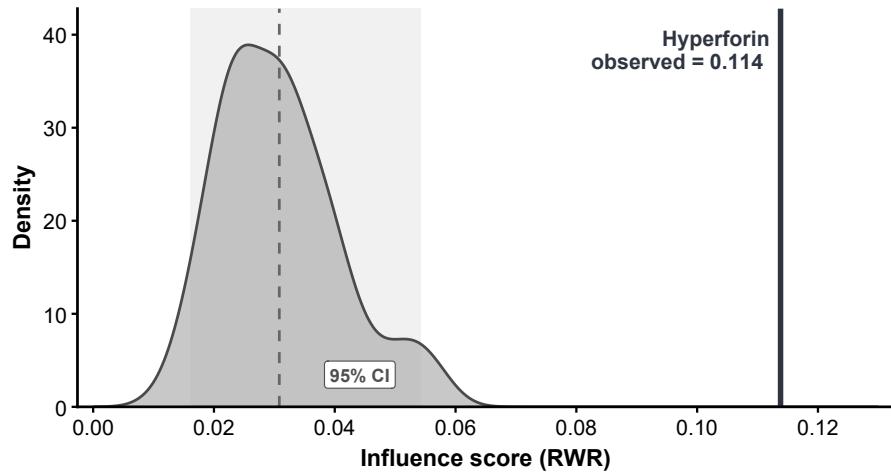


Average influence represents an effect-size normalization (total steady-state mass on DILI genes); no independent permutation test was performed. Horizontal lines represent efficiency tiers (Average Influence = constant). Hyperforin occupies a higher efficiency region despite fewer targets. Data: STRING v12.0 (≥ 900), $n = 1,000$ permutations.

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.

Bootstrap sensitivity analysis excludes target-count confounding

Distribution of influence scores from random 10-target samples



[ROBUSTNESS CONTROL] Bootstrap sensitivity: 100 random 10-target samples from Quercetin's pool, scored by random walk with restart (RWI). Shaded = 95% CI. Hyperforin (solid line) exceeds entire distribution. Data: STRING v12.0 (≥ 900).

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.

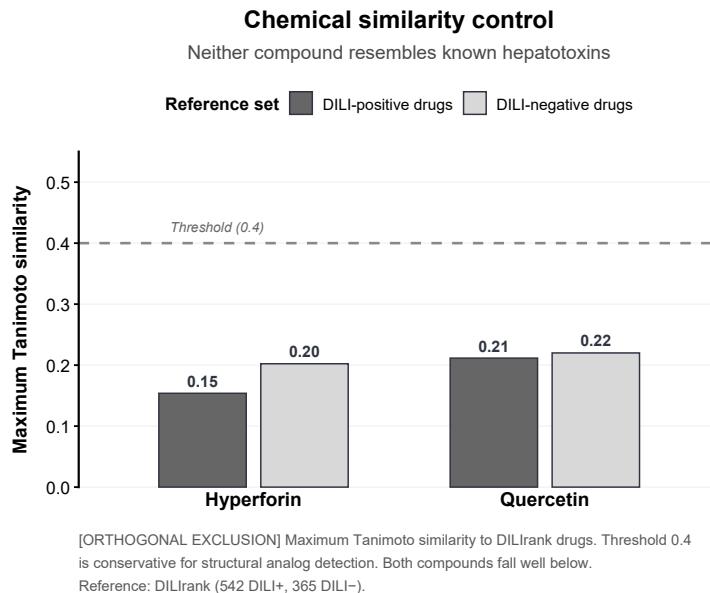


Figure 6: Chemical similarity control excludes structural confounding. Maximum Tanimoto similarity to DILIrack reference drugs. Reference set: 542 DILI-positive, 365 DILI-negative drugs. Hyperforin: max = 0.15 (DILI+), 0.20 (DILI-). Quercetin: max = 0.21 (DILI+), 0.22 (DILI-). Dashed line: 0.4 threshold 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.

⁴³³ **Author contributions**

- ⁴³⁴ **Author(s):** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation,
⁴³⁵ Writing - Original Draft, Writing - Review & Editing, Visualization.

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⁴³⁸ statistical analysis. The author(s) take full responsibility for all content.

⁴³⁹ **Competing interests**

- ⁴⁴⁰ The author(s) declare no competing interests.

⁴⁴¹ **Supplementary Tables**

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 ≥ 900 , GTEx TPM ≥ 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 ≥ 700 liver LCC	84
In STRING ≥ 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 (≥ 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
Total unique		12	

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 ≥ 1 direct DILI neighbor	18
Total direct DILI connections	31
Mean DILI neighbors per target	0.50
<i>Hyperforin comparison:</i>	
Hyperforin targets with ≥ 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 ≥ 900) with liver TPM ≥ 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
ESRRRA	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 ≥ 900) with liver TPM ≥ 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.

Metric	Compound	μ_{null}	σ_{null}	x_{obs}	Z-score
<i>Shortest-path proximity (at ≥ 900)</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 ≥ 900)</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 ≥ 900)</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. μ_{null} = null distribution mean; σ_{null} = null distribution standard deviation; x_{obs} = observed metric value.