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Desmoplasia-Aware h-Foci Expose Repurposing Limits to Prioritize Targeted Degradation and Cell-Therapy Anchors in Pancreatic Cancer

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Highlights

- Continuous h selection resolves four cell-localized PDAC network foci
- Seven repeated reversers collapse to zero after independent hard gates
- Sixty-one hubs are routed by compartment and disease direction
- Surface, extracellular and intracellular anchors receive distinct modalities

Abstract

Expression-signature reversal and molecular docking can generate apparently positive repurposing claims without testing therapeutic validity. We analysed 353 pancreatic tissues, a continuous-h differential network, 48,570 tumour-atlas cells and KRAS-mutant LINCS perturbations. Of 14,954 genes, 4,048 were concordant; a 295-node backbone contained four cell-localized h-foci and 61 second-order hubs. Seven of 541 active-FDA perturbagens passed repeated reversal, but all failed mechanism or clinical gates; metformin reversed nine of 12 signatures despite two negative randomized trials. We therefore routed the 61 hubs by compartment and disease direction: 9 were cell-surface, 9 extracellular/secreted, 16 internal-membrane/organelle and 27 soluble/nuclear. CDH3 emerged as a clinically calibrated surface anchor, while GPRC5A and LAMC2 were high-contrast non-hub backbone nodes. LAMC2 requires extracellular lysosomal targeting rather than canonical PROTAC; SAMD9L and XAF1 received degradation vetoes. This falsification-to-modality framework yields testable cell-therapy, antibody and degradation hypotheses without converting computational proximity into efficacy claims.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is sustained by oncogenic epithelial programs and a dense, immunologically active stroma that constrains perfusion, drug delivery and therapeutic response [1,2]. Bulk tumour profiles therefore mix malignant, fibroblast, myeloid, endothelial and residual pancreatic lineages; a drug-repurposing design that ignores this organization can reverse a composition signal rather than a disease mechanism. Single-cell studies have resolved diverse cancer-associated fibroblast and immune states, but donor dependence and tumour-only sampling remain frequent inferential traps [3-5].

Expression-signature reversal is attractive because it can connect a human disease state to perturbational profiles without selecting a target in advance [6,7]. Molecular docking is then commonly added as an apparently orthogonal validation. Both layers are permissive: reversal varies with lineage, genotype, concentration and time, while docking can

produce plausible poses for compounds that never reach the tumour or benefit patients. Independent assessments have reported limited reproducibility and poor clinical calibration of signature-reversion predictions; docking scores likewise do not constitute binding or efficacy measurements [8-10].

A negative repurposing result need not end target discovery. Surface antigens can route engineered cells, antibody–drug conjugates (ADCs) and bispecific engagers; extracellular proteins can be neutralized or directed to lysosomes; intracellular proteins may be eligible for proteolysis-targeting chimaeras (PROTACs) or molecular glues. These routes are not interchangeable. Canonical PROTACs require an intracellular binder and productive E3-ligase ternary complex, whereas lysosome-targeting chimaeras (LYTACs) and cell-surface E3-ligase strategies operate on extracellular or transmembrane proteins [11,12]. Target abundance alone is also insufficient: disease direction, causal dependency, epitope accessibility, internalization and normal-tissue expression must be independent gates.

We therefore designed a falsification-calibrated PDAC study in which null drug selection was allowed, then asked whether the same multiscale network could yield a modality-qualified target atlas. Three independent bulk cohorts defined a directionally concordant signature; a continuous h-cutoff extracted data-adaptive network focus without specifying an edge count; a donor-aware tumour atlas localized those foci; and LINCS scoring was restricted to an auditable KRAS-mutant pancreatic context. Active Drugs@FDA status, directional mechanism and randomized clinical evidence were independent gates. The 61 second-order hubs were subsequently routed by subcellular compartment and direction, with high-contrast non-hub nodes retained only as explicitly labelled extensions. The goal was to replace “reversal plus docking equals drug” with testable, modality-specific hypotheses and equally explicit vetoes.

Results

Audited Cohorts Prevent Pseudo-Replication

The Blueprint proposed TCGA, ICGC and several GEO collections as discovery cohorts. TCGA and ICGC contain predominantly tumours and could not estimate a primary tumour–normal effect without an unmatched external reference. GSE62452 was also not independent: its first 90 arrays correspond to the same 45 tumour–adjacent pairs distributed as GSE28735. We therefore excluded it instead of double-counting donors (Figure. 1). The frozen evidence base comprised GSE28735 (45 pairs), GSE15471 (36 pairs after averaging technical replicates) and GSE71729 (145 primary tumours and 46 normal pancreata), totalling 226 tumours and 127 normal tissues [13-15].

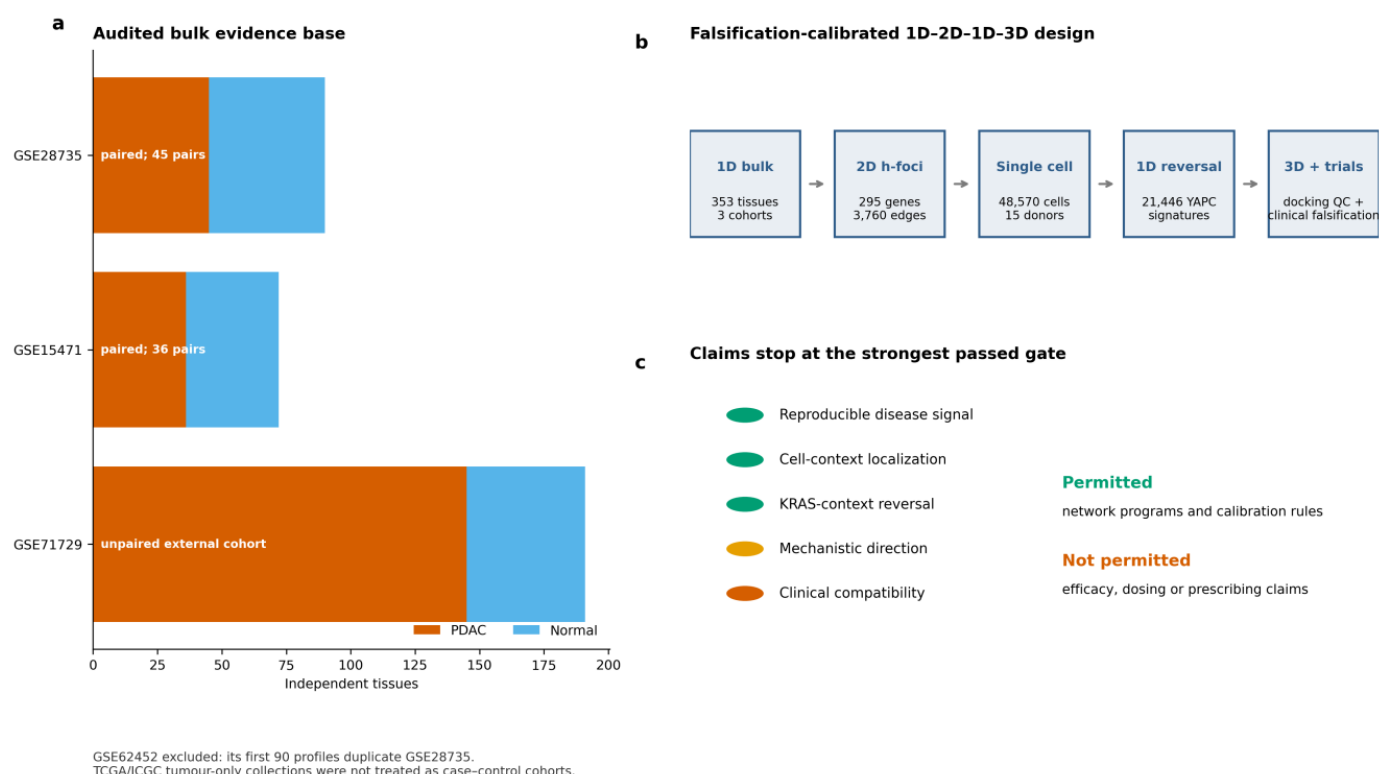


Figure 1: Study Design and Data Audit. a) Independent PDAC and Normal Tissues Retained after Sample-Identity Review; 81 Paired Donors were Analysed in GSE28735 and GSE15471, and GSE71729 Supplied 145 Independent Primary Tumours and 46 Normal Pancreata. b) The 1D–2D–1D–3D Workflow. c) Interpretation Boundary. GSE62452 was Excluded Because its First 90 Profiles Duplicate GSE28735; TCGA/ICGC Tumour-only Collections were not Treated as Case–Control Cohorts

Alt Text: Three audited bulk PDAC cohorts and a five-stage falsification workflow. GSE62452 is excluded as a duplicate; claims stop before efficacy or prescribing.

Across 14,954 common genes, random-effects synthesis identified 4,305 at $FDR < 0.01$. Requiring identical direction in all three cohorts and $I^2 < 75\%$ retained 4,048 genes: 2,186 upregulated and 1,862 downregulated (Figure. 2a). Cohort-effect Spearman correlations ranged from 0.45 to 0.64. Upregulated programs included interferon responses, epithelial–mesenchymal transition, apical junction, mitotic spindle and KRAS signalling; prominent concordant genes included LAMC2, GJB2, CDH3, SLC6A14 and GPRC5A (Figure. 2b–d). These are tissue-level effects and are not interpreted as malignant-cell-specific until the single-cell localization step.

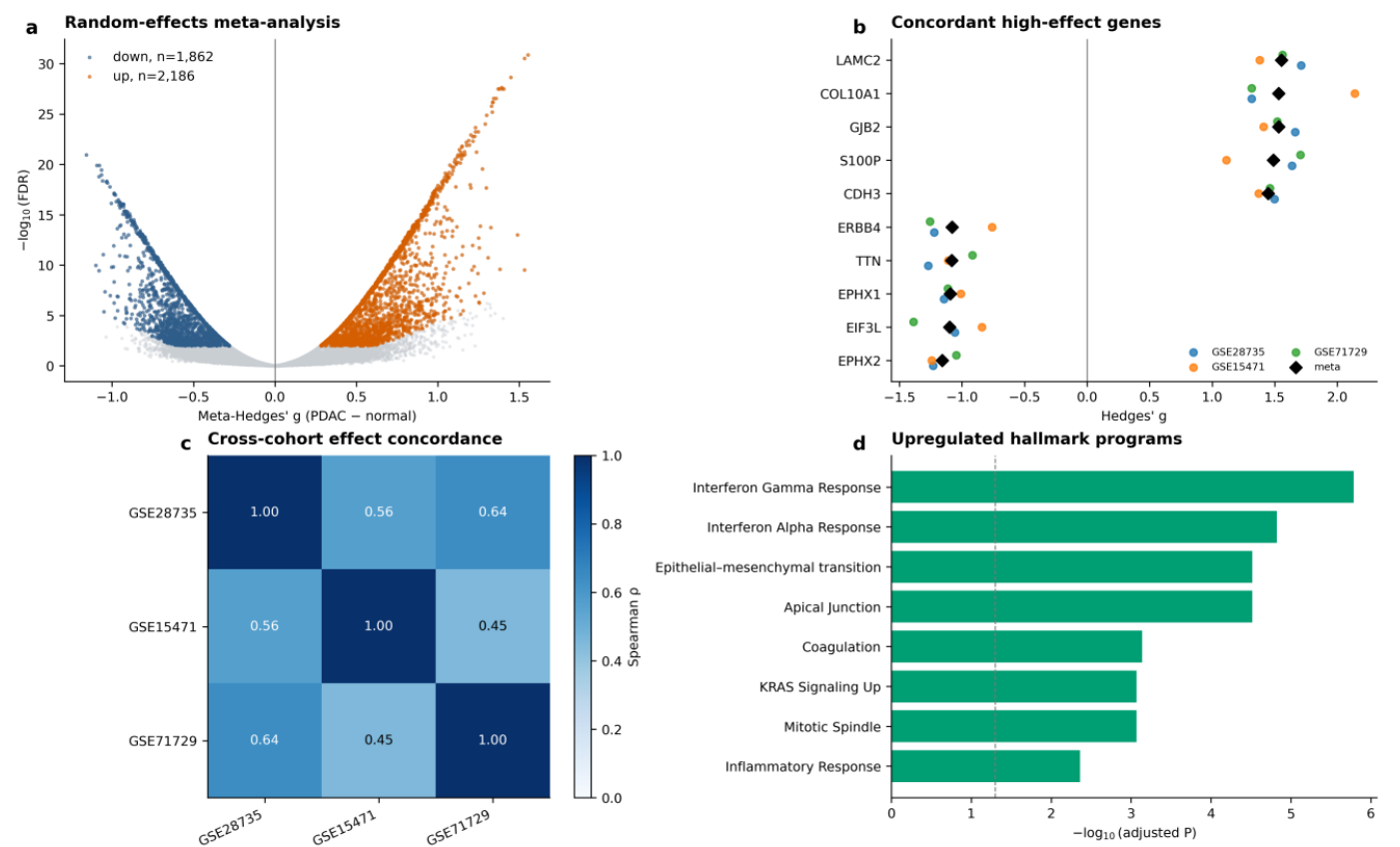


Figure 2: Cross-Cohort Bulk PDAC Signature. a) Random-Effects Meta-Hedges' g Versus Benjamini–Hochberg FDR for 14,954 Shared Genes; Coloured Points Meet $FDR < 0.01$, Identical Direction and $I^2 < 75\%$. b) Cohort and Meta Effects for Representative Genes. c) Spearman Correlations across Cohort-Level Effects. d) Enrichr/MSigDB Hallmark Results for Upregulated Genes; the Dashed Line Denotes Adjusted $P=0.05$. All Tests Were Two-Sided

Alt Text: Random-effects meta-analysis of 14,954 shared genes showing 4,048 robust genes, concordant cohort effects and interferon, EMT and KRAS-associated enrichment.

Continuous h Selection Resolves Four Microenvironmental Foci

The 150 strongest robust upregulated and 150 strongest robust downregulated genes generated 44,850 differential-correlation candidates. For each edge, the weight combined absolute tumour–normal correlation changes with the larger absolute condition-specific correlation. The continuous h intersection selected 3,727 edges at weight 0.0831 without specifying K; 33 high-betweenness inter-community bridges produced a connected 3,760-edge, 295-node backbone. Louvain community detection yielded four h-foci and the second continuous h pass identified 61 hubs [16,17].

Focus 1 contained 109 genes and was enriched for epithelial–mesenchymal transition; focus 3 contained 85 genes and was enriched for interferon- γ response. Focus 2 (90 genes) and focus 4 (11 genes) were interpreted jointly with cell localization rather than assigned from enrichment alone. LIPH, RAB3D, SAMD9L, XAF1, DDX60, MX1, DPEP1, EPSTI1 and CDH3 ranked among the highest hub scores (Figure. 3a,b). Across 40 stratified bootstraps, median Jaccard similarity was 0.4446 for continuous h, 0.4419 for an oracle top-K comparator whose K was learned from the full-data h solution, and 0.2745 for a fixed $FDR < 0.05$ plus $|\Delta r| \geq 0.20$ rule (Figure. 3c). The empirical advantage over oracle top-K was small; the methodological advantage is that h does not require a user-chosen K.

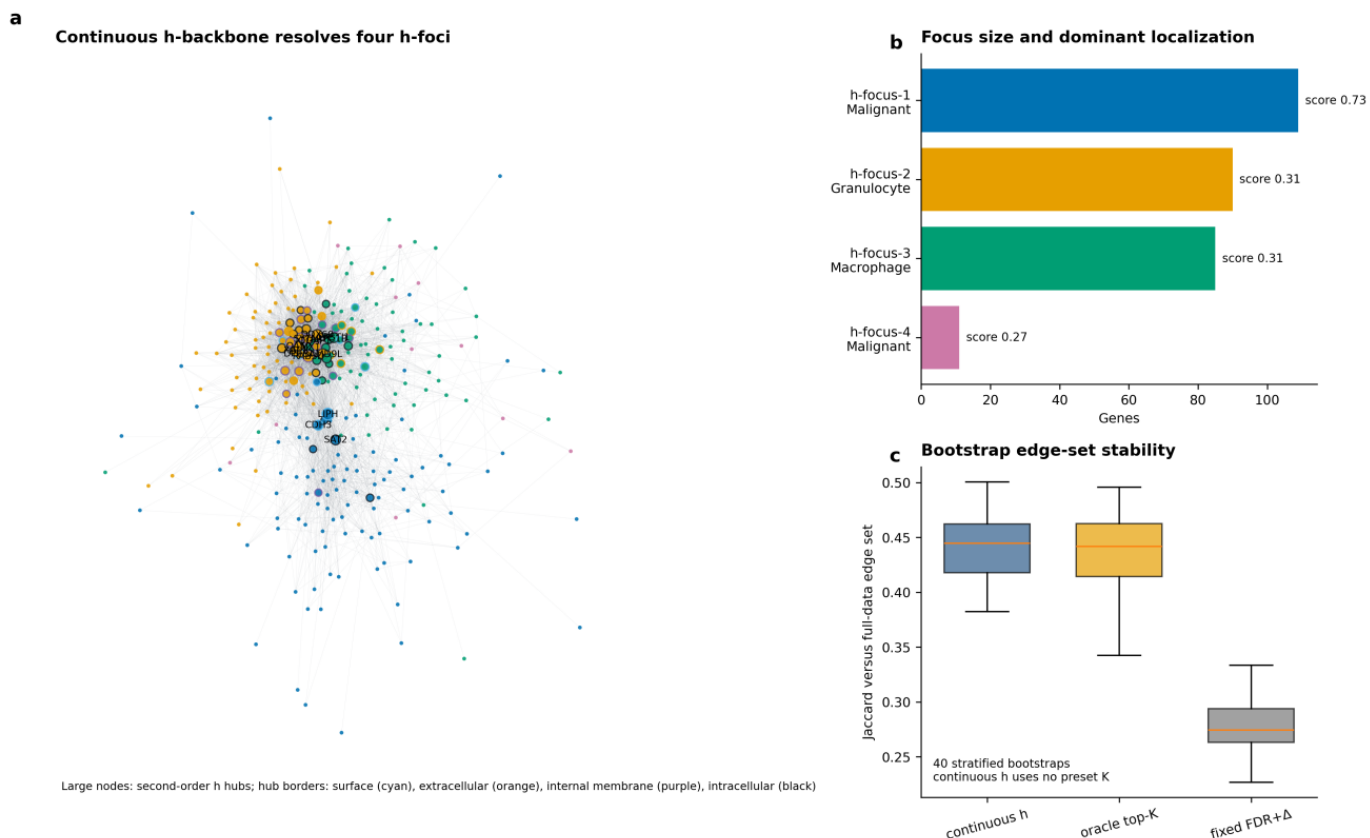


Figure 3: Continuous h-Backbone and h-foci. a) The Final 295-Node, 3,760-Edge Graph; Large Nodes are 61 Second-Order h Hubs. b) Focus Size and Strongest Donor-Level Cell Localization. c) Jaccard Similarity to the Full-Data Edge Set Across 40 Stratified Bootstraps. Boxes Show Median and Interquartile Range; Whiskers Extend to $1.5 \times \text{IQR}$. The oracle top-K Comparator uses $K=3,727$ Learned from the Full-Data h Solution, whereas Continuous h Specifies no K

Alt Text: A 295-gene differential co-expression backbone with four coloured h-foci, second-order hubs and bootstrap stability compared with oracle top-K and fixed thresholds.

Tumour Single-Cell Data Localize, but Do Not Re-Test, the Disease Contrast

The Steele atlas contributed 48,570 cells from 16 PDAC samples; 37,985 cells had cell-type annotations and were conservatively collapsed to 15 donor identifiers [3]. Of 295 network genes, 294 were measured, including 60 of 61 second-order hubs. We pseudobulked donor–cell-type groups with at least 30 cells, centred each gene within donor and computed disease-direction-weighted focus scores. Twenty of 32 focus–cell-type tests passed global $\text{FDR} < 0.05$.

Focus 1 localized most strongly to malignant cells (mean score 0.730, $\text{FDR} = 2.17 \times 10^{-7}$); focus 2 to granulocytes (0.308, $\text{FDR} = 9.77 \times 10^{-4}$); focus 3 to macrophages (0.311, $\text{FDR} = 2.36 \times 10^{-6}$); and focus 4 to malignant cells (0.273, $\text{FDR} = 1.88 \times 10^{-3}$) (Figure. 4). These associations partition the bulk network across tumour compartments. Because the atlas is tumour-only, they neither establish tumour–normal single-cell differential expression nor demonstrate causal epithelial–stromal signalling.

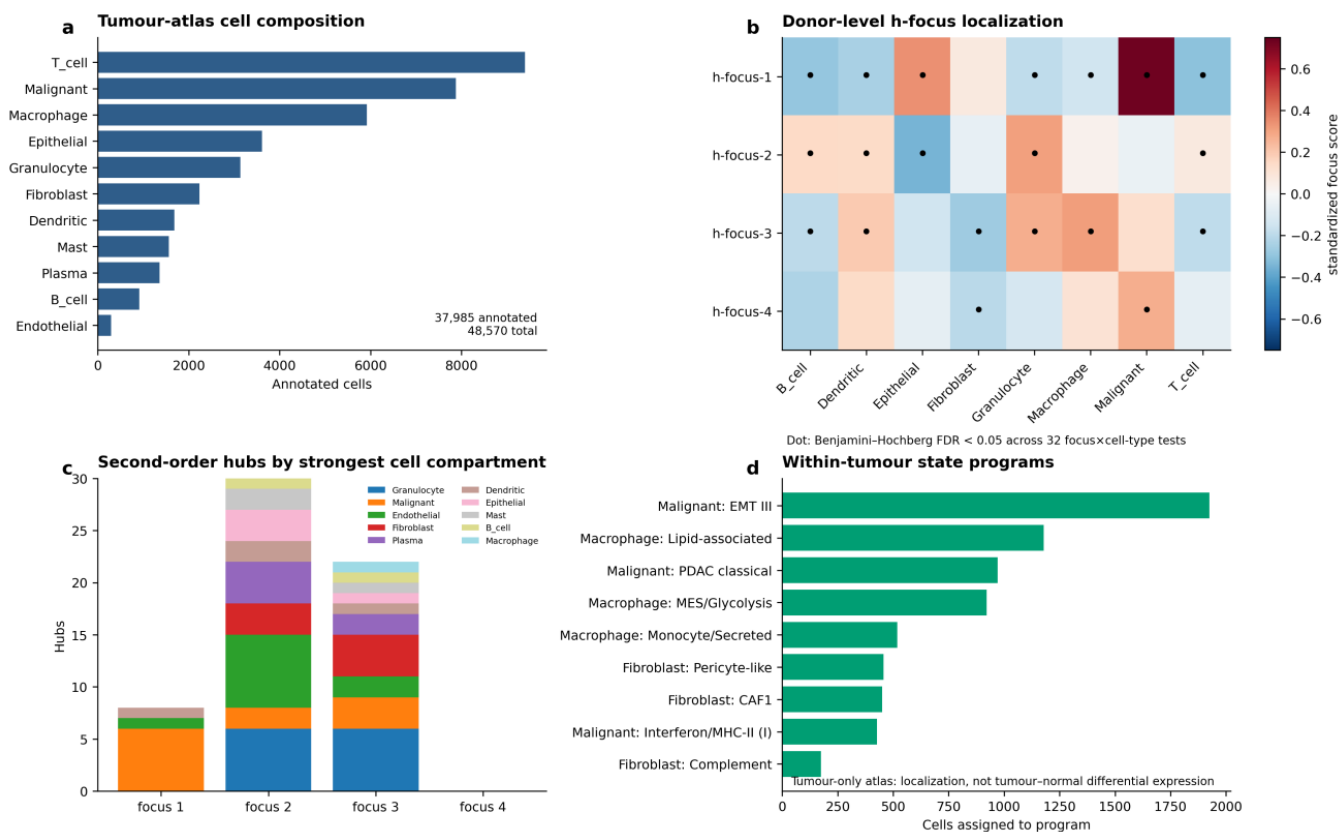


Figure 4: Donor-Aware Single-Cell Localization in the Steele Tumour Atlas. a) Annotated Cell Counts. b) Direction-Weighted Focus Scores after Centring Each Gene within Donor; Dots Mark Benjamini–Hochberg FDR<0.05 across 32 One-Sample, Two-Sided t-Tests. c) Cell Compartment with the Largest Specificity z Score for each Second-Order Hub. d) Curated within-Tumour Programs. The Atlas Contains Tumour Samples Only; these Panels do not Test Tumour–Normal Differential Expression

Alt Text: Tumour-only single-cell atlas cell counts, donor-level focus-by-cell-type scores, hub localization and within-tumour state programs.

A Coverage Audit Changes the KRAS Gate

The prespecified LINCS gate named PANC-1, MIA PaCa-2, AsPC-1 and HPAC. The current SigCom LINCS chemical collection exposed none of those cell lines; using non-pancreatic cells or silently dropping the genotype requirement would have violated the protocol. YAPC was the sole represented pancreatic model with documented KRAS V12/G12V mutation and KRAS amplification [18,19]. We therefore enumerated all 21,446 YAPC chemical signatures deterministically, intersected perturbation names with active Drugs@FDA products before scoring, and retained 7,221 signatures representing 541 drugs [20].

The frozen transcriptional rule required at least five returned signatures, a reversal fraction of at least 0.60 and a median reversal z of at least 1.64. Only triclabendazole, metformin, ivabradine, pimavanserin, propranolol, bupropion and riluzole passed (Figure. 5a,b). Six lacked a directionally compatible, multiscale human-PDAC target bridge. For example, HCN4 and CHRNA4 were robustly downregulated, making additional channel inhibition discordant; HTR2A and ADRB2 were non-robust; and triclabendazole lacked an established human PDAC target at approved exposure. Metformin alone advanced to clinical calibration.

Metformin Defines a Computational-Positive–Clinical-Negative Standard

Metformin reversed nine of 12 YAPC signatures (fraction 0.75; median reversal z 1.85) and had a plausible energy-stress rationale. It nevertheless failed two randomized phase-II PDAC trials. In the double-blind Kordes trial (n=121), six-month overall survival was 56.7% with metformin and 63.9% with placebo (P=0.41); median overall survival yielded HR 1.056 (95% CI 0.72–1.55) [21]. In the Reni trial (n=60), six-month progression-free survival was 42% with metformin and 52% in controls (P=0.61), and the trial stopped for futility [22]. Consequently, the final candidate count was zero. Metformin is not a disappointing hit; it is the negative standard that reveals the false-positive boundary of the computational workflow.

Standard therapies further showed that reversal is not necessary for efficacy. Gemcitabine and paclitaxel reversed only two of 12 and one of 12 YAPC signatures, respectively, while fluorouracil reversed seven of 12 (Figure. 5c). These values do not rank clinical regimens; they demonstrate that L1000 reversal interrogates one mechanism and cannot be used as a universal efficacy surrogate.

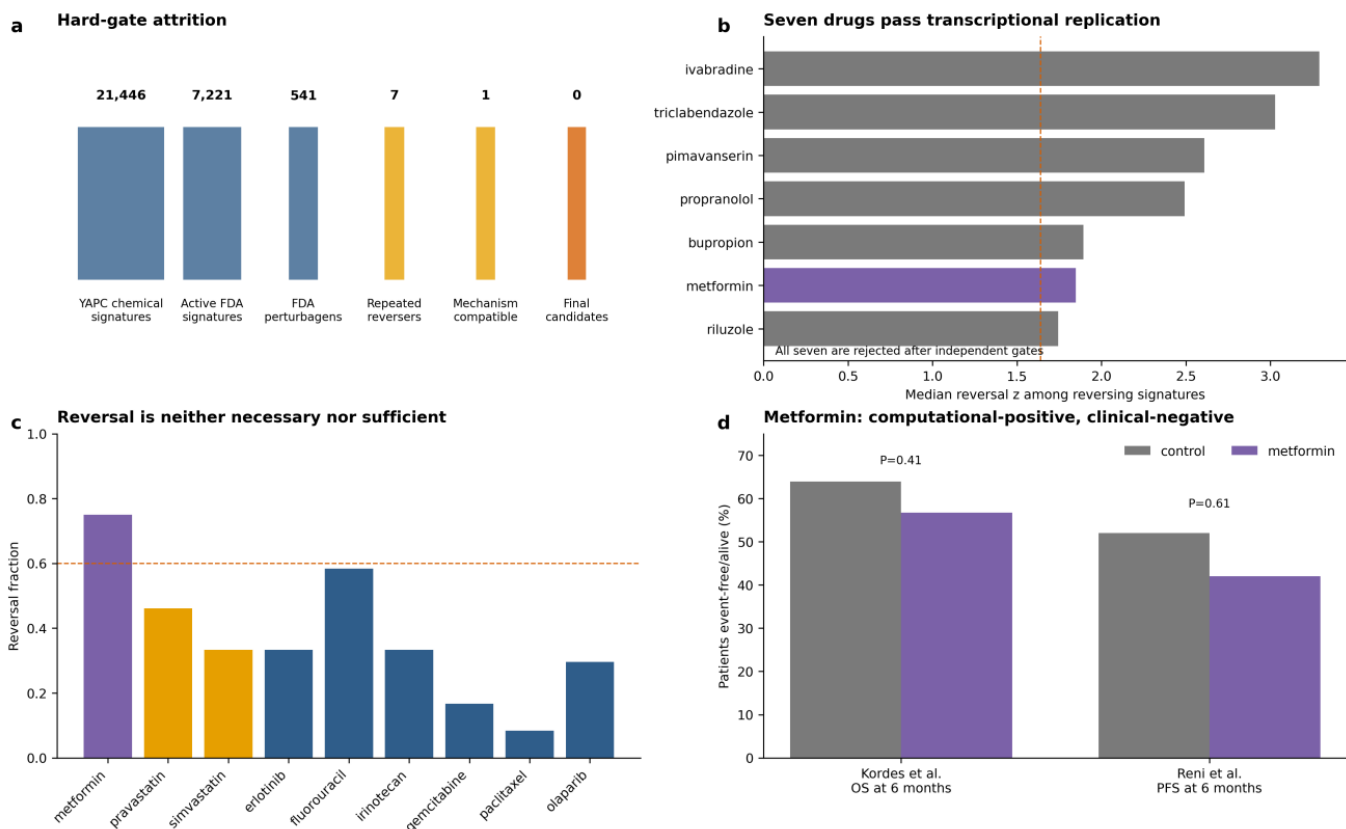


Figure 5: KRAS-Context Reversal and Clinical Falsification. a) Attrition from all YAPC Chemical Signatures to Zero Final Candidates. b) Seven Drugs met the Frozen Transcriptional Rule (at least Five Signatures, Reversal Fraction ≥ 0.60 and Median Reversal $z \geq 1.64$); none Survived Mechanism and Clinical Gates. c) Reversal Fractions for the Clinical-Negative Control, Structural Near-Misses and Standard Therapies. d) Six-Month Outcomes in Two Randomized Phase-II Metformin Trials: overall Survival in Kordes et al. and Progression-Free Survival in Reni et al. P Values are as Reported by the Trials

Alt Text: Drug attrition from 21,446 YAPC signatures and 541 active-FDA perturbagens to seven repeated reversers and zero final candidates; metformin is positive computationally but negative in two randomized phase-II trials.

Structural Compatibility Cannot Rescue a Transcriptional Near-Miss

HMGR was consistently upregulated across the three cohorts (meta-Hedges' $g=0.508$, $FDR=1.9 \times 10^{-6}$, $I^2=0\%$), providing a biologically coherent mevalonate anchor [23]. Pravastatin nevertheless failed the frozen transcriptional gate: six of 13 signatures reversed (fraction 0.462; median reversal $z=1.50$). Simvastatin was weaker still (five of 15; fraction 0.333) and a randomized PDAC phase-II trial had found no benefit from adding low-dose simvastatin to gemcitabine [24].

We used the 2.22-Å human HMGR-atorvastatin structure (PDB 1HWK) as an exact structural control [25,26]. Three-seed native atorvastatin redocking recovered the crystallographic pose with median heavy-atom RMSD 0.808 Å (maximum 0.830 Å) and median Vina score -8.848 kcal mol $^{-1}$. Pravastatin cross-docking returned a median score of -7.573 kcal mol $^{-1}$ and occupied the established pocket (Figure. 6a,b). This result confirms protocol pose recoverability and pocket compatibility. It does not estimate affinity, pancreatic exposure or efficacy and therefore cannot override failed reversal replication or class-level clinical caution [10,27].

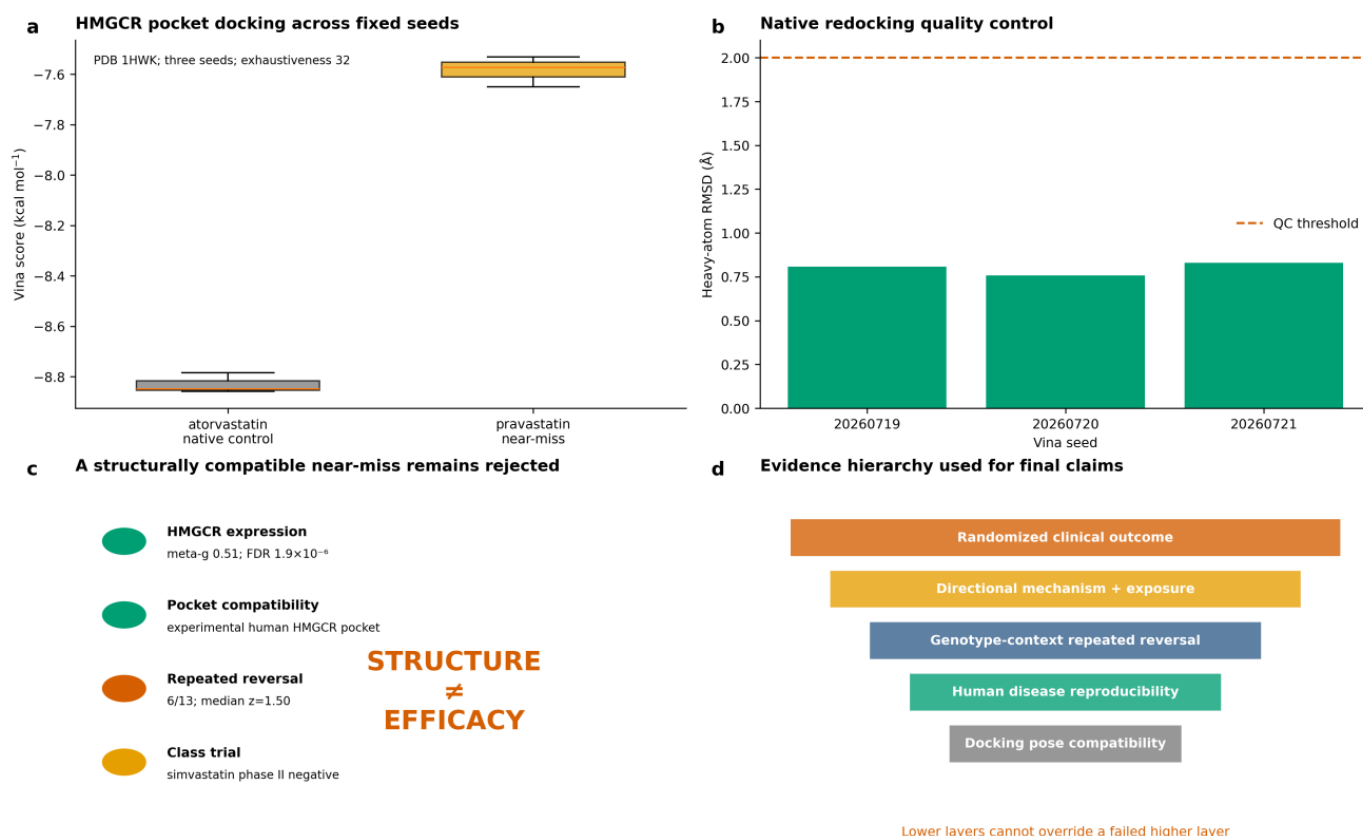


Figure 6: Structural Compatibility Cannot Rescue a Failed Higher Gate. a) Vina Scores Across Three Fixed Seeds for Native Atorvastatin Redocking and Pravastatin Cross-Docking in Human HMGCR (PDB 1HWK, 2.22 Å); Boxes Show Median and Range. b) Native Heavy-Atom Redocking RMSD; the Dashed Line is the Prespecified 2 Å QC Limit. c) Pravastatin Passes HMGCR Expression and Pocket-Compatibility Checks but Fails the Frozen Reversal Rule; A Randomized Simvastatin PDAC Trial Supplies Class-Level Caution. d) Evidence Hierarchy. Docking Scores do not Estimate Affinity, Tumour Exposure or Efficacy

Alt Text: Three-seed human HMGCR docking controls and an evidence hierarchy showing that pravastatin pocket compatibility cannot rescue failed transcriptomic replication or negative class-level clinical evidence.

Spatial Routing Converts h-foci into Modality-Qualified Anchors

The 61 second-order hubs were divided into four coarse compartments: 9 cell-surface, 9 extracellular/secreted, 16 internal-membrane/organelle and 27 intracellular soluble/nuclear proteins (Figure. 7b). Compartment alone did not determine action. Twenty-eight hubs with positive tissue-level effects remained eligible for target-specific validation, 6 interferon-context hubs were held for functional perturbation, and 27 hubs were vetoed from destructive targeting because they were downregulated or carried tumour-suppressive directionality. The matrix thereby turns a two-bin “membrane versus intracellular” split into a direction-aware routing rule.

CDH3 was the strongest surface-accessible second-order hub (meta-Hedges’ $g=1.448$, $FDR=2.31\times10^{-29}$; malignant-cell specificity $z=2.23$). However, it is a calibrated engineering anchor, not an unqualified recommendation: the P-cadherin ADC PCA062 produced one partial response among 47 patients and was discontinued for limited activity, while the CDH3×CD3 bispecific PF-06671008 was terminated after limited activity and frequent cytokine-release syndrome [28,29]. Any new CDH3 format must therefore outperform these historical efficacy and safety constraints.

GPRC5A and LAMC2 were among the strongest PDAC effects in the full backbone but were not members of the 61-hub set; we retain them as explicitly labelled high-contrast extensions. GPRC5A ($g=1.370$, $FDR=3.10\times10^{-28}$) localized to malignant cells and has independent PDAC protein and functional evidence, supporting antibody-binding, internalization, ADC and CAR feasibility tests rather than a clinical claim [30]. LAMC2 ($g=1.552$, $FDR=1.31\times10^{-31}$) is a malignant-localized laminin-332 extracellular-matrix component with PDAC dependency evidence [31]. Its location excludes canonical cytosolic PROTAC; neutralizing antibodies or a prototype LYAC provide the anatomically coherent route [11].

Two actual hubs supply small-molecule test hypotheses. LGALS3 ($g=1.086$) localized across malignant and macrophage compartments and has genetic PDAC evidence; selvigaltin (GB1211), an oral galectin-3 inhibitor with first-in-human pharmacokinetic data, is proposed only for organoid–CAF–myeloid co-culture with knockout–drug concordance [32,33]. MGLL ($g=1.030$) is a membrane-associated serine hydrolase with emerging PDAC functional evidence; JZL184 or another selective MGLL inhibitor is a mechanistic tool that must agree with CRISPR or inducible-degron phenotypes

[34]. Atovaquone is retained as a lower-priority complex-III/hypoxia comparator, not a metformin replacement or proven stromal-penetrating PDAC drug [35]. Finally, SAMD9L and XAF1 were designated degradation-veto controls because interferon-induced growth restriction and pro-apoptotic tumour-suppressor evidence makes their destruction directionally unsafe [36,37].

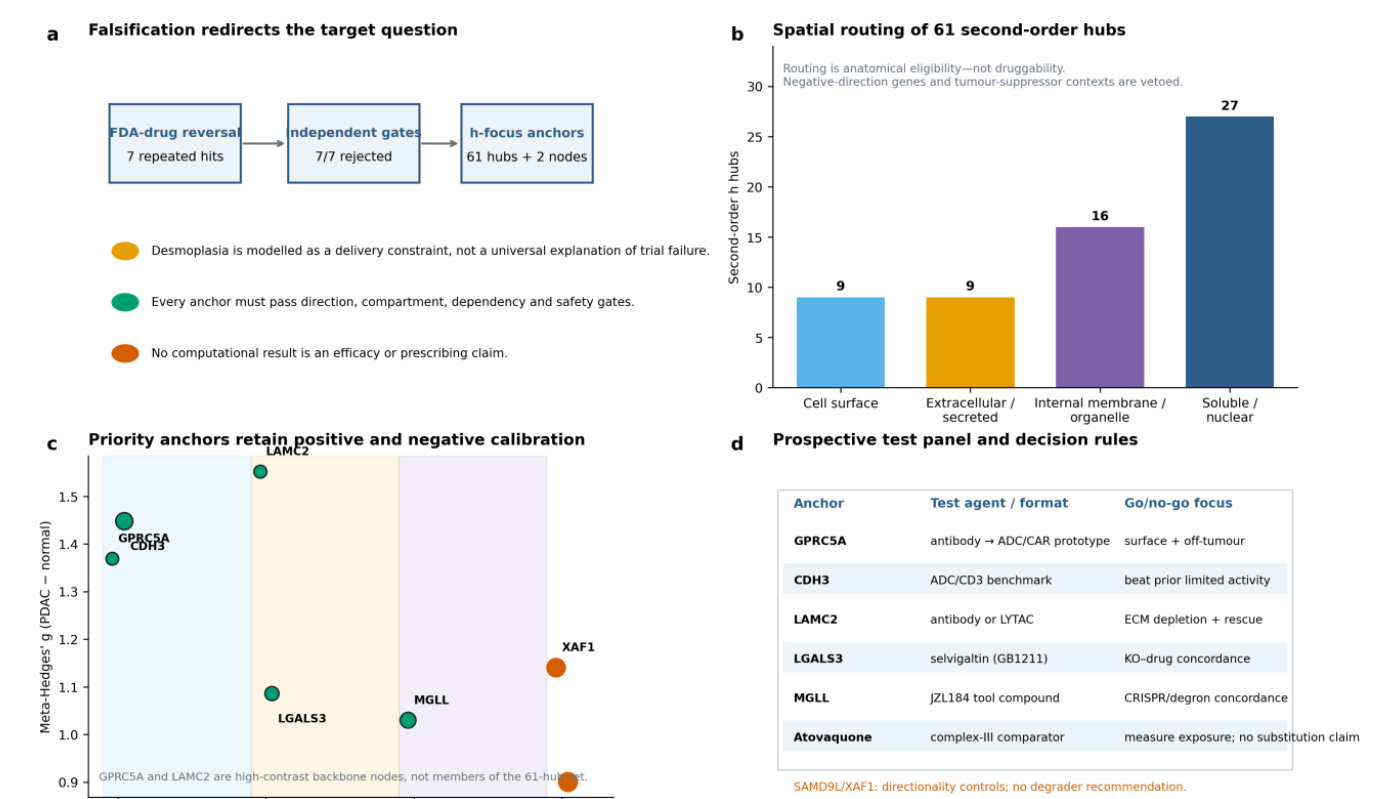


Figure 7: Whole-Cell Modality Map. a) Failure of all Seven Repeated Reversers Redirects the Question from Drug Nomination to Target Qualification. b) Coarse Spatial Routing of the 61 Second-Order Hubs: 9 Cell-Surface, 9 Extracellular/Secreted, 16 Internal-Membrane/Organelle and 27 Soluble/Nuclear Proteins. c) Priority Anchors Plotted by Compartment and Tissue-Level Meta-Effect. GPRC5A and LAMC2 are Explicitly Labelled as High-Contrast Non-Hub Backbone Nodes; SAMD9L and XAF1 are Degradation-Veto Controls. d) Prospective Formats and Predeclared go/no-go Tests. Routing Denotes Anatomical Eligibility, not Druggability or Efficacy

Alt Text: A falsification-to-modality map showing seven of seven repeated drug reversers rejected, the spatial routing of 61 second-order hubs, calibrated priority anchors and a prospective organoid and co-culture test panel.

Discussion

This study was designed to permit the answer “none.” Three bulk cohorts, a stable data-adaptive network and donor-level cell localization produced a coherent PDAC representation, yet the drug arm ended with zero final candidates. That null set is the central methodological result. A pipeline that must output a drug will convert context dependence, incomplete perturbational coverage and structurally permissive poses into false confidence; a pipeline that includes independent clinical-negative calibration can stop.

Metformin provides a particularly informative boundary. Its repeated YAPC reversal would have made it a strong conventional repurposing hit, and docking against a selected metabolic target could easily have added another positive panel. Two randomized trials already show why that evidence stack is insufficient. Conversely, standard cytotoxics can be clinically useful without strongly reversing a 24-hour disease signature. Reversal is therefore neither sufficient nor necessary for clinical efficacy. The appropriate role of LINCS is hypothesis triage within a clearly defined lineage, genotype, dose and time, followed by mechanism, exposure, safety and clinical falsification.

The h-foci results add biological value independently of drug promotion. A malignant EMT focus, a granulocyte-associated focus, a macrophage interferon focus and a smaller malignant ion-transport focus capture the fact that PDAC is not a single homogeneous expression vector. Continuous h achieved essentially the same stability as an oracle top-K edge set while avoiding a manually specified K. The method should not be claimed to dominate top-K numerically in every dataset; its strength is objective cardinality selection and the ability to reveal multiple local cores. Future applications could formalize several local solutions as h-foci before any perturbational query.

Limitations of the Study

The spatial map converts that biological structure into an experimental routing system. Surface proteins are candidates for binding and internalization assays before ADC, CAR-T or bispecific construction; extracellular proteins require neutralization or lysosomal routing; internal targets require causal dependency, a ligand and compatible E3 biology before a degrader is even designed. This is a whole-cell coverage framework, not a claim of complete therapeutic access. Dense stroma, heterogeneous antigen density, T-cell exclusion, endosomal routing and normal-tissue expression remain independent obstacles that computational localization cannot solve.

The positive and negative anchor pairs are deliberately inseparable. GPRC5A provides a discovery-stage surface hypothesis, whereas CDH3 carries clinical evidence that antigen expression alone did not deliver broad activity. LAMC2 supports an extracellular-degradation hypothesis but simultaneously demonstrates why a canonical PROTAC label would be anatomically wrong. LGALS3 and MGLL supply pharmacologic test points with genetic or functional comparators. SAMD9L and XAF1 prevent the common directional error of equating upregulation with a degradation mandate. This calibration is the target-level analogue of the metformin negative standard.

Important limitations remain. The bulk cohorts are microarray datasets with incomplete stage, grade and purity metadata, so we used matched contrasts and cross-cohort concordance rather than fabricate unavailable covariate adjustment. Adjacent pancreas is not a pristine healthy control. The single-cell atlas is tumour-only and supports localization, not replication of the disease contrast. Current LINCS coverage reduced the genotype gate to one KRAS-amplified YAPC line, preventing lineage replication and excluding co-culture responses from fibroblasts or macrophages. FDA name matching establishes product status, not intratumoural exposure or safe combination dosing. Finally, docking used a rigid experimental pocket and cannot capture pharmacokinetics, protein dynamics or tumour efficacy.

The next decisive experiment is not another docking run. A staged panel should quantify GPRC5A and CDH3 surface protein and internalization against normal lung and pancreas counter-screens; compare new CDH3 formats with PCA062/PF-06671008 liabilities; test LAMC2 neutralization or lysosomal depletion with recombinant-protein rescue; and evaluate selvigaltin, an MGLL tool inhibitor and atovaquone in KRAS-genotyped organoid-CAF-myeloid systems under measured oxygen and exposure conditions. Every pharmacologic phenotype should agree with orthogonal genetic perturbation. No result in this study supports prescribing or testing a candidate outside an approved protocol.

STAR★METHODS

Key Resources Table

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Human PDAC bulk microarray	NCBI GEO	GSE28735; GSE15471; GSE71729
Human PDAC single-cell atlas	Steele et al., 2020 / 3CA	doi:10.1038/s43018-020-00121-4
Perturbational signatures	SigCom LINCS	l1000_cp; YAPC
Active US products	US FDA	Drugs@FDA release 2026-07-21
Human HMGCR structure	RCSB PDB	1HWK
Pravastatin coordinates	PubChem	CID 54687
Spatial-modality annotations	This study	PDAC_61_hub_spatial_modality_map.csv
Prospective experimental panel	This study	PDAC_preclinical_test_panel.csv
Analysis code	This study	Repository DOI pending

Resource Availability

Lead Contact

Further information and requests should be directed to Fred Y. Ye.

Materials Availability

This study generated no new biological materials.

Data Availability

All primary data are public at the accession and structure identifiers listed in the Sources worksheet. Derived tables and exact source hashes accompany this manuscript. A public repository DOI is 10.5281/zenodo.21626401.

Code Availability

Executable analysis and figure-generation scripts, frozen query outputs, docking inputs and pose files accompany this manuscript. A public repository URL and software license saved at <https://zenodo.org/records/21626401>.

Experimental Model and Study Participant Details

All analyses used de-identified public human tissue or tumour-atlas data. Original studies obtained consent and ethics approval as reported in their source publications. No new participants were recruited.

Method Details

Study Design and Data Provenance

The prospective Blueprint was treated as a launch protocol, but infeasible or dependence-violating elements were changed transparently. Public records were accessed on 22 July 2026. GSE28735, GSE15471 and GSE71729 were downloaded from GEO. GSE62452 was excluded after accession and expression-level identity review established overlap with GSE28735. Large public raw matrices are not redistributed; accession URLs and SHA-256 values are supplied.

Bulk Preprocessing and Effect Synthesis

Series-matrix values were log₂ transformed when required. Platform probes were mapped to HGNC symbols and collapsed by median. Technical replicate arrays in GSE15471 were averaged within donor and condition. GSE28735 and GSE15471 used Hedges-corrected paired standardized mean change; GSE71729 used Hedges' g with Welch tests. Gene effects and variances were combined by DerSimonian–Laird random-effects meta-analysis [38]. Benjamini–Hochberg adjustment controlled FDR [39]. Robust genes required meta-FDR<0.01, identical direction in all cohorts and I²<75%.

Differential Co-Expression and Continuous h

The top 150 robust upregulated and 150 robust downregulated genes were standardized within cohort and pooled by condition. For each pair, Fisher-transformed tumour and normal correlations were compared. Edge weight was $(|r_{\text{tumour}} - r_{\text{normal}}|/2) \times \max(|r_{\text{tumour}}|, |r_{\text{normal}}|)$. Ordered weights $w(k)$ were retained while $w(k) \geq k/M$, where M is the number of candidate edges; the last intersection defines continuous h and its data-adaptive cardinality [16]. A second continuous h selection on approximate edge betweenness recovered sparse inter-community bridges. Louvain communities defined h -foci; a continuous h pass over a 0.50 strength, 0.25 degree and 0.25 betweenness composite defined second-order hubs [17]. Forty stratified donor/sample bootstraps compared continuous h , oracle top- K and fixed FDR/ Δr selection.

Pathway and Single-Cell Localization

Enrichr queried MSigDB Hallmark 2020, Reactome 2022, GO Biological Process 2023 and KEGG 2021 Human [40]. Steele/3CA counts and metadata were analysed without cell-level replication claims [3]. Samples 11A/11B were conservatively collapsed to one donor identifier. Donor–cell-type pseudobulks required at least 30 cells. Within each donor, gene logCPM was centred and scaled across available cell types; focus scores averaged disease-direction-signed z values. One-sample two-sided t -tests across donors were globally FDR adjusted over 32 tests.

LINCS Enumeration and Reversal Scoring

The disease query comprised the 150 strongest robust upregulated and 150 strongest robust downregulated genes. SigCom LINCS metadata were audited before scoring [19]. Because the prespecified PANC-1, MIA PaCa-2, AsPC-1 and HPAC chemical signatures were absent, YAPC was used as the sole available pancreatic KRAS V12/G12V-amplified context [18]. All YAPC chemical signatures were retrieved in stable identifier order. Perturbagen names were normalized, salts were collapsed, and signatures were intersected with prescription or OTC products in active Drugs@FDA marketing categories dated 21 July 2026 [20]. LDP3 rank-two-sided characteristic-direction scores defined reversal as negative z -up and negative z -down; reversal z was $-(z_{\text{up}} + z_{\text{down}})/\sqrt{2}$.

Frozen Candidate and Clinical Gates

The candidate ranking was written to disk and hashed before final clinical adjudication. The transcriptomic gate required at least five returned signatures, reversal fraction ≥ 0.60 and median reversal $z \geq 1.64$. Passing drugs were checked against the robust bulk signature and h -backbone for a directional human-PDAC mechanism. Randomized clinical evidence was then treated as a higher-order falsification layer. A null final set was prespecified as admissible. The exact frozen table SHA-256 is a1ea234f8ed1713f9bffd132278045de8b1999291ce09036f379ba5986af6b96.

Structural Analysis

Human HMCCR PDB 1HWK (2.22 Å) and its native atorvastatin ligand supplied the experimental pocket [25,26]. Receptor and ligands were prepared with Meeko; Vina 1.2.7 used a 24×24×24 Å box centred on the native ligand, exhaustiveness 32, nine poses and fixed seeds 20260719, 20260720 and 20260721 [27]. Native redocking required median heavy-atom RMSD<2 Å. PubChem pravastatin was cross-docked only after being labelled a transcriptional near-miss. Scores are reported as docking outputs, not free energies.

Spatial Annotation, Directional Gating and Experimental Routing

All 61 second-order hubs were manually assigned to cell-surface, extracellular/secreted, internal-membrane/organelle or intracellular soluble/nuclear classes using canonical UniProt and Human Protein Atlas gene-level localization, with row-level source links retained in the derived matrix. Donor–cell-type mean logCPM and specificity z scores were calculated from the same Steele pseudobulk matrix used for focus localization. A negative PDAC meta-effect vetoed destructive targeting. SAMD9, SAMD9L and XAF1 were separately vetoed because published tumour-suppressive or pro-apoptotic function contradicted degradation [36,37]. Surface routing required a positive effect and triggered antibody/ADC/CAR/bispecific eligibility—not prioritization—pending extracellular-epitope, internalization and normal-tissue safety experiments. Extracellular proteins were routed to neutralizing antibodies or LYTACs, not canonical PROTACs [11]. Intracellular routes required target-specific dependency, ligandability and productive E3 engagement. GPRC5A and

LAMC2 were included in the priority table as high-contrast non-hub backbone extensions and were never counted among the 61 hubs.

Quantification and Statistical Analysis

Analyses used Python 3.12, NumPy 2.3.5/2.5.1, pandas 2.2.3, SciPy 1.17.0, NetworkX 3.6.1, RDKit 2025.3.3, Meeko 0.7.1, Vina 1.2.7, openpyxl 3.1.5 and Matplotlib 3.10.8. All inferential tests were two-sided unless stated. The package contains scripts, derived tables, figures in SVG and 300/600-dpi PNG, structure inputs, six docking pose files, source hashes and a formula-audited workbook. Public raw matrices are referenced rather than duplicated.

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Author Contributions

F.Y.Y. conceived the study and is responsible for data interpretation and manuscript approval. Detailed CRediT roles should be confirmed before submission.

Declaration of Interests

The author declares no competing interests.

Declaration of Generative AI and AI-Assisted Technologies

ChatGPT 5.6 Sol and Gemini 3.6 Flash assisted code generation, English-language drafting, figure layout and consistency checking. The named author is responsible for idea creation, research design, data analyses, result interpretations and final text.

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