

Supplementary Information

Bias-Controlled Computational Prioritisation of Therapeutic Hypotheses From Heterogeneous Molecular Data Identifies PLK1 as the Leading MTX-Combination Candidate in Choriocarcinoma

This supplementary file provides the expanded methods, sensitivity analyses and detailed tables that support the main manuscript. It is not intended as a separate study.

1. Supplementary Materials and Methods

1.1 Network Propagation and Robustness Analyses

Protein and phosphoprotein effect sizes from the MTX-sensitive JEG-3 versus MTX-resistant JEG-3R comparison were used as weighted seeds for random walk with restart propagation across STRING, OmniPath, BioGRID and ReactomeFI networks. Because the originating SILAC comparison lacked independent biological replicates, seed weights were treated as effect-size evidence rather than inferential statistics.

Ranking stability was evaluated by leave-one-network-out analysis, omics-layer dropout and random seed dropout. Two global STRING null models were used:

1. In the edge-degree-preserving null, STRING edges were randomised by double-edge swaps before propagation scores were recalculated.
2. In the seed-degree-matched null, the STRING graph was held fixed while mapped seeds were replaced with genes from matched degree bins and positive seed weights were reassigned.

Each null model used 1,000 permutations. Benjamini-Hochberg correction was applied across the 10,746-gene evaluated universe.

1.2 Public Pharmacogenomic Analyses

Pan-cancer DepMap CRISPR gene effects and PRISM MTX area-under-the-curve values were analysed using lineage- and proliferation-adjusted regression. Negative MTX-AUC coefficients indicate that MTX-resistant models tend to show stronger dependency on the evaluated target.

These analyses were used as public-data stress tests because trophoblastic MTX-response measurements were sparse.

For each evaluated target, the pan-cancer model was:

CRISPR gene effect $\sim$ standardised MTX AUC + cancer lineage + standardised proliferation signature.

The proliferation signature was calculated from available expression values for the predefined proliferation-gene set in the archived analysis code. Models used HC3 heteroskedasticity-robust standard errors. Higher MTX AUC indicates lower MTX sensitivity; therefore, a negative MTX-AUC coefficient is the supportive direction.

Three DepMap choriocarcinoma models were identified from model metadata: JAR (ACH-001529), JEG-3 (ACH-001530) and T3M-3 (ACH-002021). Their basal CRISPR dependencies were summarised descriptively and compared with pan-cancer distributions. None had public PRISM MTX-AUC coverage.

1.3 External Transcriptomic Context and Pathway Enrichment

Five non-trophoblastic MTX-resistance GEO datasets were summarised by candidate-level random-effects meta-analysis. GSE88873 JEG-3R versus JEG-3 effects were reported separately because the sensitive arm contained one sample. Preranked enrichment analysis was performed over Hallmark and Reactome gene-set libraries without mechanism-keyword prefiltering. Nominal permutation p values were adjusted within each dataset-library combination.

1.4 Normal-Placenta Single-Cell Context Projection

The top 100 upregulated and top 100 downregulated genes from the descriptive GSE88873 comparison were projected onto the GSE89497 normal-placenta single-cell TPM matrix. Of these genes, 98 upregulated and 96 downregulated genes mapped to the reference. AddModuleScore-like expression-bin-matched control correction was applied to $\log_{10}(\text{TPM})$ values. The MTX-resistance score was calculated as the upregulated-module score minus the downregulated-module score.

The reference contained two source prefixes (HE8W and HE24W). Because cytotrophoblast, syncytiotrophoblast and stromal cells occurred only within HE8W, subtype contrasts were

restricted to the shared HE8W source prefix. Cell-level tests are descriptive and do not represent independent patient-level replication.

As a predefined-panel sensitivity analysis, PLK1, PRKDC, PARP1, WEE1, ATR, CHEK1, CLIP1 and TP53 were projected onto GSE89497 and the Vento-Tormo E-MTAB-6701 normal first-trimester atlas. The fixed panel score was the mean per-gene z score after log1p expression transformation. A non-overlapping proliferation module used MKI67, TOP2A, PCNA, MCM2, MCM5, CCNB1, CDK1 and AURKB. E-MTAB-6701 trophoblast cells were mapped as VCT to CTB, SCT to STB and EVT to EVT, then pseudobulked by individual and subtype. Paired Wilcoxon tests used individuals rather than cells as replicates. A small-sample sensitivity model estimated subtype coefficients after proliferation-score adjustment using individual fixed effects and individual-clustered standard errors.

1.5 Drug-Combination Evidence

DrugComb records were collapsed to independent antifolate x target class x drug pair x cell line units. MTX combinations were evaluated separately from pralatrexate and other antifolates. Sparse classes were reported descriptively.

1.6 Virtual RNA Expression-Window Proxy

DepMap log2(TPM+1) values were back-transformed for the three choriocarcinoma models. Median tumour-model TPM values were compared descriptively with Human Protein Atlas consensus nTPM values in selected critical normal tissues. Platform differences, drug exposure, dose-response and adverse events are not modelled. The reported ratios are RNA expression-window proxies, not pharmacologic therapeutic indices or patient-level toxicity estimates.

1.7 Candidate Ranking and Cross-Modal Integration

Transparent MCDA integrated network support, robustness, pharmacogenomic direction, external context, actionability, direct combination evidence, pan-essentiality risk and normal-expression risk. Weight uncertainty was tested in 3,000 perturbation simulations and by leave-one-component-out analysis.

Each component was min-max normalised across the predefined eight-target panel. Supportive components increased the score and liability components reduced it:

MCDA score = 0.17(network consensus) + 0.14(degree-aware support) + 0.12(seed-dropout stability) + 0.10(pharmacogenomic direction) + 0.10(external context) + 0.17(actionability) + 0.10(direct MTX-combination evidence) - 0.06(pan-essentiality penalty) - 0.04(normal-expression penalty).

These were fixed analysis weights selected for transparent sensitivity analysis, not preregistered parameters and not learned model coefficients. The component definitions, normalised values, fixed weights, 3,000 Dirichlet weight perturbations and leave-one-component-out results are supplied as machine-readable tables.

Because the available evidence sources concentrate signal within overlapping cell-cycle and DNA-damage-repair programs, they do not satisfy the independence assumption required for combined-probability tests. Cross-modal support was therefore summarised without combining p values: candidates were ranked within each evidence source separately, and the number of sources ranking a candidate among the top three was reported as a descriptive concordance measure. Sources with missing coverage were recorded as unavailable and were neither imputed nor treated as negative evidence. Source-specific statistics remained primary.

1.8 GNN Label-Design Audit

A supervised GCN or GraphSAGE model was not added as a validation layer. Known anticancer drug targets encode actionability, whereas housekeeping genes are not validated negatives for MTX-combination vulnerability. A small local audit compared available DNA-damage-response positive proxies with housekeeping negative proxies. Mean DepMap expression alone separated these proxy groups with a maximum univariate AUC of 0.975 after allowing orientation. A GNN trained on these labels could therefore learn housekeeping expression status rather than disease-specific MTX-combination biology.

Any future GNN benchmark should use experimentally anchored MTX-combination sensitiser and non-sensitiser labels, degree- and expression-matched negatives, leakage-resistant network splits, calibration analysis and simple baselines including logistic regression, degree-only models, label propagation and random walk with restart.

1.9 Exploratory In-Silico Synergy Prioritisation

The public TDC DrugComb DrugSyn file contained 297,098 records across 59 NCI-60 cell lines and 128 unique training SMILES. A fixed-seed 65,000-row subset was used to fit ExtraTrees regression models for ZIP and Bliss scores. Symmetric Morgan-fingerprint pair features were evaluated as a chemistry-only prior. Morgan fingerprints used radius 2 and 128 bits. A second model appended a 16-component RNA embedding learned from TDC RNA-seq features. JEG-3 DepMap log2(TPM+1) values were matched to the TDC RNA feature map and projected into that embedding.

Each ExtraTrees model used 72 trees and random seed 20260601. Random-row, cold drug-pair and cold cell-line splits used a 20% test fraction. Candidate-level predictions included tree-level standard deviation as an internal model-spread indicator. JEG-3 gene matching covered 74.4% of the TDC RNA feature map. Its projected PCA norm was 274.7, slightly above the NCI-60 maximum of 267.2. None of the proposed inhibitors was an exact TDC training compound. These estimates should therefore be read as domain-shift exploratory assay-ordering priors, not as calibrated JEG-3 synergy predictions.

1.10 Normal-Placenta Trophoblast-State Map Overlay

The E-MTAB-6701 expression API payload included atlas embedding coordinates for individual cells. After intersection with the predefined-panel score table, 14,034 trophoblast cells were visualized: 9,327 CTB, 1,235 STB and 3,472 EVT cells. The embedding was not recalculated from the restricted gene panel.

The visualisation shows trophoblast-state annotations, the predefined eight-gene score, the separate proliferation score and the standardised residual of the eight-gene score after linear adjustment for proliferation score and donor indicators. These cell-level overlays are descriptive only. Donor-level pseudobulk contrasts and the donor-clustered proliferation-adjusted sensitivity model remain the primary analyses.

1.11 Reproducibility Metadata and Public-Input Provenance

The reproducibility archive records the public-resource accession, release or version identifier, local cache location and source URL in public_input_manifest.csv. DepMap files were obtained from DepMap Public 26Q1, released on 2026-04-01. DrugComb screening records used the

versioned DrugComb_summary_table_v1.4.csv export. GEO and ArrayExpress accessions are reported in the main manuscript and in the manifest. Exact local source files and processed outputs are enumerated in supplementary_file_index.csv.

The proliferation-adjusted E-MTAB-6701 sensitivity models were fit on donor-subtype pseudobulk rows using:

outcome ~ cell_type + proliferation_score + donor.

CTB was the reference subtype. Standard errors were clustered by donor with small-sample correction. These models are sensitivity analyses because only seven donors were represented.

2. Supplementary Tables

Supplementary Table 1. Candidate Ranking under Fixed, Entropy and AHP Weighting

The fixed weights are the analyst-specified values used in the primary analysis. Entropy weights are derived from the dispersion of the normalised component matrix; AHP weights are the principal eigenvector of a Saaty pairwise comparison matrix (consistency ratio 0.023). PLK1 ranks first under all three strategies.

Gene	Fixed weight score	Fixed rank	Entropy weight score	Entropy rank	AHP weight score	AHP rank	Monte Carlo rank 95% interval	Monte Carlo P(top 3)
PLK1	0.811	1	0.643	1	0.884	1	1-1	1.0
PARP1	0.587	2	0.181	4	0.738	2	2-3	0.977
PRKDC	0.557	3	0.216	3	0.688	3	3-4	0.906
TP53	0.522	4	0.243	2	0.619	4	2-4	0.117
CLIP1	0.287	5	0.037	5	0.361	5	5-6	0.0
CHEK1	0.229	6	-0.011	6	0.346	6	5-6	0.0
ATR	0.179	7	-0.027	7	0.308	7	7-8	0.0
WEE1	0.148	8	-0.051	8	0.214	8	7-8	0.0

Weight vectors under each strategy.

Criterion	Fixed	Entropy	AHP
network_consensus	0.17	0.0284	0.289
degree_aware	0.14	0.0575	0.1553
seed_dropout	0.12	0.0917	0.1116
pharmacogenomic_direction	0.1	0.0931	0.0797

external_context	0.1	0.061	0.0569
actionability	0.17	0.05	0.2138
direct_mtx_combination	0.1	0.3977	0.0408
toxicity_penalty	0.06	0.1134	0.0299
normal_expression_penalty	0.04	0.1072	0.0228

Rank agreement between weighting strategies.

Comparison	Spearman rho	Kendall tau
Fixed vs entropy	0.905	0.786
Fixed vs AHP	1.0	1.0
Entropy vs AHP	0.905	0.786

Supplementary Table 2. Fixed Transparent-MCDA Analysis Weights

Component	Weight	Direction
Network consensus	0.17	Supportive
Degree-aware support	0.14	Supportive
Seed-dropout stability	0.12	Supportive
Pharmacogenomic direction	0.10	Supportive
External context	0.10	Supportive
Actionability	0.17	Supportive
Direct MTX-combination evidence	0.10	Supportive
Pan-essentiality penalty	0.06	Liability
Normal-expression penalty	0.04	Liability

Weights are fixed analysis parameters reported for auditability. They were not preregistered and were not learned from outcome labels.

Supplementary Table 3. Global Network-Null Sensitivity across Final Candidates

Gene	Edge-swap empirical p	Edge-swap BH-FDR	Seed-placement empirical p	Seed-placement BH-FDR
PLK1	0.017	0.479	0.048	0.896
PRKDC	0.033	0.524	0.052	0.902
PARP1	0.044	0.552	0.089	0.982

CLIP1	0.050	0.564	0.153	0.987
TP53	0.065	0.581	0.123	0.982
WEE1	0.074	0.591	0.145	0.985
ATR	0.197	0.800	0.291	1.000
CHEK1	0.199	0.803	0.296	1.000

Supplementary Table 4. External MTX-Resistance Random-Effects Meta-Analysis

Gene	Pooled log2FC	95% CI	BH-FDR	I2 (%)	JEG-3 descriptive log2FC
PLK1	0.557	-0.084 to 1.197	0.364	88.9	-0.178
PRKDC	-0.255	-0.550 to 0.041	0.364	67.4	-0.320
PARP1	0.262	-0.376 to 0.900	0.562	95.3	-0.035
WEE1	-0.067	-0.323 to 0.189	0.695	80.8	-0.057
ATR	-0.033	-0.336 to 0.270	0.831	79.1	-0.082
CHEK1	-0.151	-0.511 to 0.209	0.562	82.7	-0.342
TP53	-0.285	-0.696 to 0.125	0.411	74.5	-0.401
CLIP1	-0.307	-0.782 to 0.168	0.411	54.4	0.099

Supplementary Table 5. Recurrent Hallmark Programs from Preranked Enrichment Analysis

Pathway	Dataset N	Median NES	Minimum FDR
Epithelial Mesenchymal Transition	4	1.468	0.021
E2F Targets	3	2.168	0.021
G2-M Checkpoint	3	1.882	0.021
Apoptosis	3	-1.299	0.021
TNF-alpha Signalling via NF-kB	3	-1.507	0.021
Unfolded Protein Response	3	-1.744	0.055

Supplementary Table 6. Disease-Context DepMap CRISPR Audit with Pan-Cancer Comparison

Gene	Choriocarcinoma	Choriocarcinoma	Pan-cancer	Fraction	BH-FDR vs non-	Interpretation
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	models	median gene effect	median gene effect	with effect < -1	choriocarcinoma	
PLK1	3	-2.735	-2.778	1.000	0.795	Strong basal dependency; not disease-selective
WEE1	3	-2.305	-2.633	1.000	0.404	Strong basal dependency; pan-essentiality concern
CHEK1	3	-1.794	-1.745	1.000	0.833	Strong basal dependency; pan-essentiality concern
ATR	3	-1.215	-1.101	1.000	0.795	Strong basal dependency; pan-essentiality concern
PARP1	3	-0.277	-0.197	0.000	0.624	Modest basal dependency
PRKDC	0	NA	NA	NA	NA	Missing CRISPR coverage
TP53	3	0.678	0.238	0.000	0.334	No basal dependency
CLIP1	3	-0.018	0.019	0.000	0.795	Minimal basal dependency

Supplementary Table 7. HE8W-Only Single-Cell Context Contrasts

Score	Contrast	EVT n	Reference n	EVT median	Reference median	Median difference	Interpretation
MTX-resistance score	EVT vs CTB	440	248	-0.228	-0.042	-0.186	Resistance score lower in EVT

MTX-resistance score	EVT vs STB	440	64	-0.228	0.342	-0.570	Resistance score lower in EVT
MTX-resistance score	EVT vs STR	440	615	-0.228	0.086	-0.314	Resistance score lower in EVT
EVT-invasion control score	EVT vs CTB	440	248	0.692	-1.834	2.526	Positive-control behaviour
EVT-invasion control score	EVT vs STB	440	64	0.692	-1.135	1.827	Positive-control behaviour
EVT-invasion control score	EVT vs STR	440	615	0.692	-0.119	0.811	Positive-control behaviour

Cell-level p values are available in the machine-readable table but are not interpreted as patient-level inferential statistics.

Supplementary Table 8. Trophoblast-State Map Descriptive Summary

Cell type	Cells	Donors	Median 8-gene score	Median proliferation score	Median adjusted residual z
CTB	9,327	7	0.045	-0.162	0.086
STB	1,235	6	-0.311	-0.613	-0.250
EVT	3,472	7	-0.051	0.278	-0.150

Cell-level medians are descriptive and must not be treated as independent biological replicates.

Supplementary Table 9. Vento-Tormo Donor-Level Predefined-Panel Context Projection

Analysis	Contrast or term	Sources	Estimate	P value	BH-FDR	Interpretation
Paired Wilcoxon	EVT vs CTB	7	Median paired difference - 0.181	0.0156	0.0893	Eight-gene score lower in EVT; nominal paired support only

Paired Wilcoxon	EVT vs STB	6	Median paired difference 0.168	0.3125	0.4808	Not supported
Proliferation-adjusted sensitivity model	EVT vs CTB	7	Coefficient - 0.196	7.74e-7	1.04e-5	Negative EVT coefficient retained after proliferation adjustment
Proliferation-adjusted sensitivity model	STB vs CTB	7	Coefficient - 0.116	1.22e-4	8.26e-4	Negative STB coefficient retained after proliferation adjustment

Cells were pseudobulked by individual and trophoblast subtype before analysis. The adjusted model is a small-sample sensitivity analysis. These normal-placenta findings do not validate the panel in tumour tissue, establish MTX-specific sensitisation or justify a patient digital-twin claim.

Supplementary Table 10. Collapsed MTX-Combination Evidence from DrugComb

DrugComb records were collapsed to independent antifolate x target class x drug pair x cell line units, with methotrexate separated from other antifolates.

Antifolate	Target class	Independent units	Cell lines	Drug pairs	Median ZIP	Evidence status
Methotrexate	PLK1	1	1	1	11.916	Descriptive only: sparse
Methotrexate	PARP1	79	40	2	-0.466	Multi-unit aggregate not supportive
Methotrexate	PRKDC	0	0	0	NA	No direct public evidence

Supplementary Table 11. Exploratory MTX-Combination In-Silico Prioritisation

Rank	Target class	Candidate drug	JEG-3 projected	Tree SD	Chemistry-only ZIP	JEG-3 projected	Nearest TDC-drug	Exact TDC
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			ZIP			Bliss	Tanimoto	compound
1	PRKDC	peposertib	0.721	3.990	0.197	-0.378	0.462	No
2	PARP1	talazoparib	0.322	3.439	-0.237	0.254	0.403	No
3	PLK1	onvansertib	-0.230	3.390	-0.435	-1.482	0.487	No
4	PARP1	olaparib	-0.279	2.885	-0.675	-0.180	0.447	No
5	PRKDC	AZD7648	-0.410	4.814	0.288	-1.351	0.500	No
6	PLK1	volasertib	-1.624	3.269	-1.563	-2.335	0.506	No

The ranking is suitable for ordering dose-matrix assays only. The JEG-3 projection is outside the NCI-60 training domain, and tree-level standard deviation is not a calibrated confidence interval. These values do not replace the orthogonal evidence-based recommendation to test PLK1 inhibition first.

Supplementary Table 12. Cross-Platform Virtual RNA Expression-Window Proxy

Gene	Models	Median tumour TPM	Bone marrow nTPM	Highest selected critical normal tissue	Highest critical-normal nTPM	Tumour / bone marrow	Tumour / highest critical normal	Tox-burden proxy
ATR	3	14.30	1.2	testis	4.9	11.91	2.92	0.34
PRKDC	3	60.81	17.7	thymus	28.6	3.44	2.13	0.47
WEE1	3	72.73	21.7	liver	54.0	3.35	1.35	0.74
CLIP1	3	55.66	17.0	liver	43.2	3.27	1.29	0.78
PLK1	3	51.09	30.2	thymus	40.9	1.69	1.25	0.80
TP53	3	39.75	18.4	thymus	44.0	2.16	0.90	1.11
CHEK1	3	22.04	24.9	bone marrow	24.9	0.89	0.89	1.13
PARP1	3	91.08	27.7	lymph node	117.2	3.29	0.78	1.29

Supplementary Table 13. Lineage- and Proliferation-Adjusted DepMap/PRISM Stress Test

Gene	N	MTX-AUC coefficient	P value	BH-FDR	Model status
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PLK1	301	-0.055	0.081	0.565	Model fitted
PRKDC	12	NA	NA	NA	Not estimable due to insufficient data
PARP1	301	0.005	0.541	0.758	Model fitted
WEE1	301	-0.001	0.978	0.978	Model fitted
ATR	301	-0.025	0.403	0.706	Model fitted
CHEK1	301	-0.027	0.290	0.677	Model fitted
TP53	301	-0.034	0.170	0.594	Model fitted
CLIP1	301	0.002	0.779	0.909	Model fitted

Supplementary Table 14. Cross-modal rank concordance across evidence sources.

Gene	Network rank (edge-swap)	Network rank (seed-matched)	DepMap rank (adjusted)	GEO meta rank	Sources available	Ranked top 3	Concordance
PLK1	1	1	1	1	4	4	Concordant (all sources)
PRKDC	2	2	n/a	2	3	3	Incomplete coverage
TP53	5	4	2	3	4	2	Partial
PARP1	3	3	5	6	4	2	Partial
CLIP1	4	6	6	4	4	0	Non-concordant
CHEK1	8	8	3	5	4	1	Non-concordant
WEE1	6	5	7	7	4	0	Non-concordant
ATR	7	7	4	8	4	0	Non-concordant

Ranks are computed separately within each evidence source from the source-specific p values already reported; no p values are combined. PRKDC lacks DepMap CRISPR gene-effect

coverage and is flagged as not rank-comparable; missing coverage is never imputed or treated as negative evidence.

Supplementary Table 15. Preclinical Validation Roadmap

This roadmap translates the public-data prioritisation into testable preclinical hypotheses. It is not a clinical treatment recommendation.

Target	Candidate drugs	Biomarker stratum	Schedule hypothesis	Pharmacodynamic readouts	Main risk	Evidence position
PLK1	onvansertib; volasertib	proliferation/G2M-high	MTX pretreatment then intermittent PLK1 inhibition	p-H3; Cyclin B1; sub-G1	myelosuppression; proliferating normal tissue	Lead hypothesis; direct MTX DrugComb evidence is one cell line only
PARP1	olaparib; talazoparib	HRD-like; BRCA/RAD51-low	MTX pretreatment then PARP inhibition	PAR; gammaH2AX; RAD51	hematologic toxicity; broad normal expression	Secondary stratified hypothesis; aggregate DrugComb signal is not favourable
PRKDC	peposertib; AZD7648	NHEJ-high; replication-stress-high	MTX pretreatment then DNA-PK inhibition	DNA-PKcs pS2056; gammaH2AX; 53BP1	DNA-repair toxicity; sparse public coverage	Secondary mechanistic hypothesis; no direct public MTX-combination evidence

Supplementary Table 16. Comparison with Recent Multi-Criteria Target-Prioritisation Approaches (2023-2026)

Evidence layers are counted as distinct data modalities integrated into the ranking model. Entries were verified against the primary publications; PMIDs and DOIs are given in the final column.

Study	Disease context	Heterogeneous evidence layers	Permutation-based bias control	Normal-tissue toxicity	Ranking uncertainty quantified	Integration model	Citation	PMID / DOI

				constraint				
Present study (2026)	Choriocarcinoma, MTX combination	6	Yes - two degree-preserving permutation nulls, all-gene FDR over 10,746 genes	Yes - pan-essentiality penalty and normal-tissue RNA counterscreen	Yes - 3,000-draw weight perturbation, leave-one-component-out, entropy and AHP reweighting	Yes - rank-based cross-modal concordance without an independence assumption	-	-
Buniello et al., Open Targets Platform	Pan-disease resource	Many (association evidence aggregated across sources)	No permutation-based null model	Yes - safety widget, pan-cancer dependency flags, mouse knock-out phenotypes	No formal uncertainty quantification of the prioritisation score	Weighted aggregation; user-adjustable weights	Nucleic Acids Res 2025;53:D1467-D1475	39657122 / 10.1093/nar/gkae1128
Ghosh & Fortino	Pan-cancer drug combinations	3-4 (drug targets, disease genes, adverse-effect genes, networks)	Network proximity with randomisation-based significance	Yes - explicitly models adverse-reaction potential alongside efficacy	Not reported as ranking uncertainty	Network propagation plus gene-set enrichment; no MCDA weighting	Comput Struct Biotechnol J 2025;27:65-77	41230525 / 10.1016/j.csbj.2024.12.003
Lee et al., co-essentiality network	17 TCGA cancer types	4-5 (CRISPR co-essentiality, PPI, co-expression, co-methylation, pathways)	Yes - degree-controlled modularity Z-score normalised by permutation	No normal-tissue toxicity consideration	P values reported; no ranking confidence intervals	Network propagation from driver genes; no explicit weighting model	Genomics Proteomics Bioinformatics 2025;23:qzaf070	41002174 / 10.1093/gpbnl/qzaf070
Sannigrahi et al., DepMap pipeline	Head and neck squamous cell carcinoma	6 (DepMap, DGIdb, DAVID, Open Targets, GDSC/PRISM, CCLE/TCGA)	Filtering and effect-size thresholds; no permutation null	Partly - removes common-essential and core-fitness genes shared with normal tissue	No formal uncertainty quantification	Sequential filtering rather than weighted scoring	Mol Oncol 2024;18:336-349	37997254 / 10.1002/1878-0261.13558
Gu & Chen, GETgene-AI	Pancreatic ductal adenocarcinoma	5 (mutation, expression, known targets, PPI centrality, literature via LLM)	Threshold sensitivity only; no permutation null	Yes - negative weights for expression in brain, liver, GI and kidney	No confidence intervals or perturbation analysis	Weighted scoring calibrated against clinical-trial frequency	Front Syst Biol 2025;5:1649758	- / 10.3389/fsysb.2025.1649758

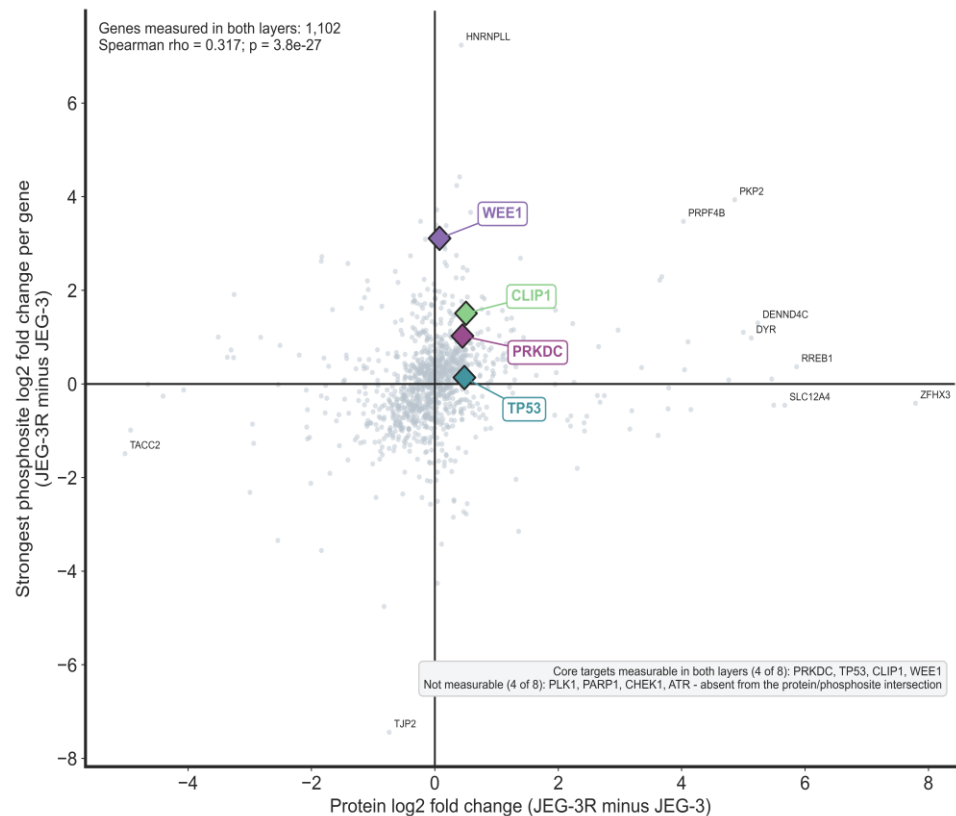
Lin, Dai & Pusztai	Breast cancer	3-4 (CRISPR functional screen plus multi-omics)	Not reported	Not reported	Not reported	Screen-driven candidate selection	Breast Cancer Res Treat 2025;214:319-327	40888991 / 10.1007/s10549-025-07817-0
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Supplementary Table 17. Framework Portability Checklist across Gynaecologic Malignancies

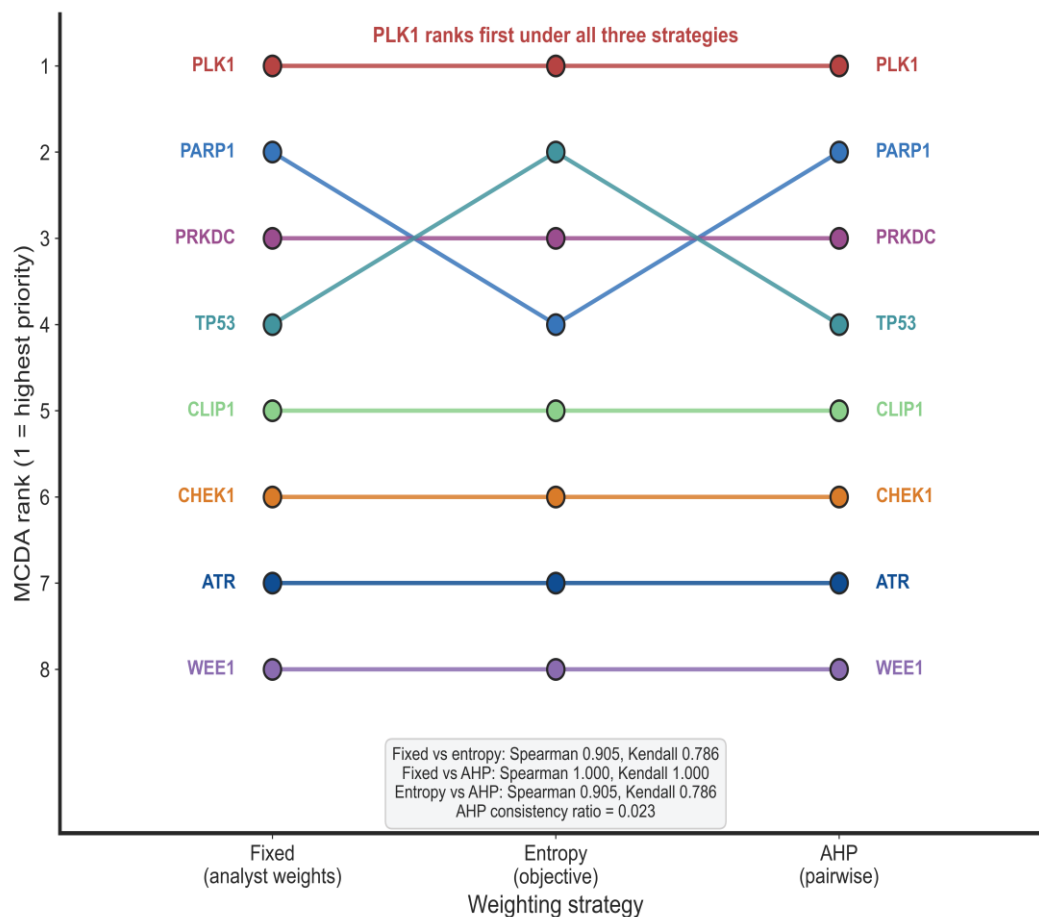
Availability refers to public resources for ovarian, endometrial and cervical cancer. Only the disease-context seed is unavailable, because methotrexate is not a standard agent in those diseases and matched resistant models have not been generated.

Workflow layer	Data requirement	Resource used in this study	Availability for other gynaecologic malignancies	Comment
1. Disease-context seed	Matched sensitive vs resistant proteomic and phosphoproteomic comparison	SILAC JEG-3 / JEG-3R (Georgiou et al.)	Not available	MTX is not a standard agent in ovarian, cervical or endometrial cancer, so matched MTX-resistant models with paired proteomic and phosphoproteomic profiling have not been generated. This is the binding constraint.
2. Network propagation	Any curated protein interaction resource	STRING, OmniPath, BioGRID, ReactomeFI	Fully available	Species-level resources; no disease-specific input required.
3. Bias controls	The interaction graph only	Degree-preserving edge swap and seed-degree-matched null on STRING	Fully available	Both null models are computed from the graph and the seed set; they transfer unchanged.
4. Pharmacogenomic stress test	CRISPR gene effect and drug sensitivity for disease-matched cell lines	DepMap Public 26Q1, PRISM Repurposing	Available	DepMap covers ovarian (n>40), endometrial and cervical lines, better than the three choriocarcinoma models available here.
5. External transcriptomic context	Independent resistance datasets for the same agent	5 non-trophoblastic MTX-resistance GEO series	Partly available	Platinum- and taxane-resistance series are plentiful; MTX-resistance series are not.
6. Drug-combination evidence	Curated synergy records containing the index agent	DrugComb, TDC DrugSyn	Available	Coverage of MTX combinations is sparse in every disease, as documented here.
7. Single-cell lineage context	A normal-tissue single-cell reference for the tissue of origin	GSE89497, E-MTAB-6701 placenta atlases	Available	Normal ovary and endometrium atlases exist (Human Cell Atlas).
8. Normal-tissue counterscreen	Normal-tissue expression reference	Human Protein Atlas consensus nTPM	Fully available	Tissue-agnostic resource.
9. MCDA and uncertainty	The component matrix from steps 1-8	Fixed, entropy and AHP weights; 3,000-draw perturbation	Fully available	Purely computational; no additional data required.

3. Supplementary Figures

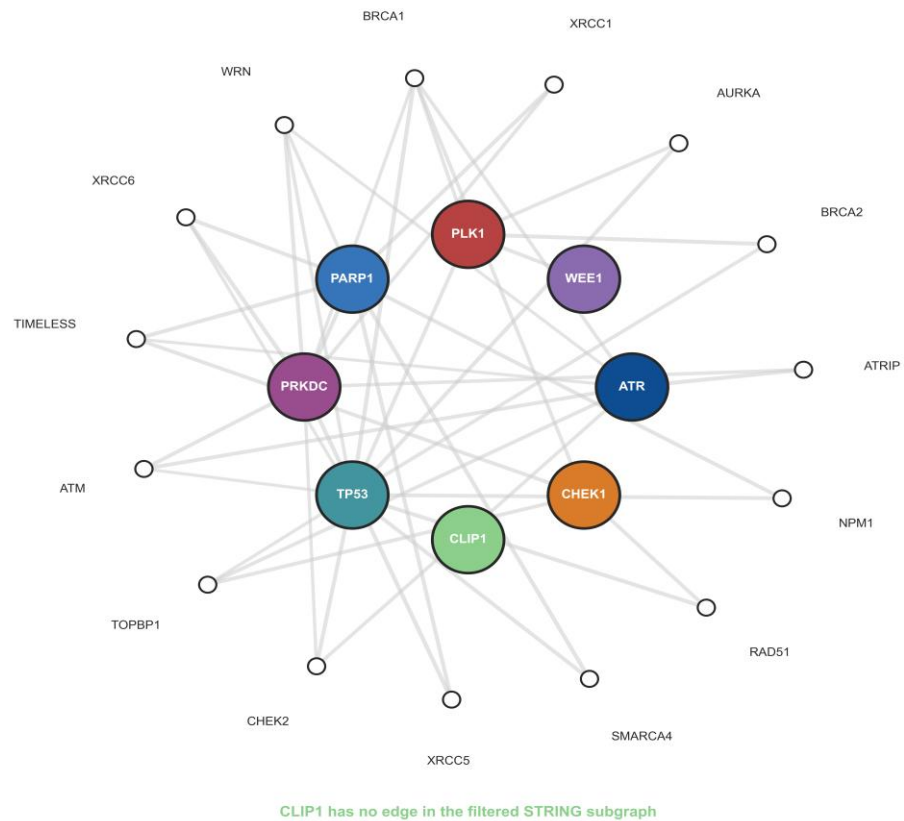


Supplementary Fig. 1. Descriptive multi-omics effect-size concordance. Protein log₂ fold changes are plotted against the strongest absolute phosphosite log₂ fold change per gene while retaining the phosphosite direction. Among 1,102 genes measured in both layers, Spearman rho was 0.317. Red diamonds show measured core targets. The originating SILAC comparison has no independent biological replicates, so the plot is an effect-size visualisation rather than an inferential analysis. All eight candidate targets are labelled where measurable. Four (CLIP1, PRKDC, TP53, WEE1) were quantified in both the protein and phosphoprotein layers; PLK1, PARP1, CHEK1 and ATR are absent from the protein/phosphosite intersection and therefore cannot be displayed.

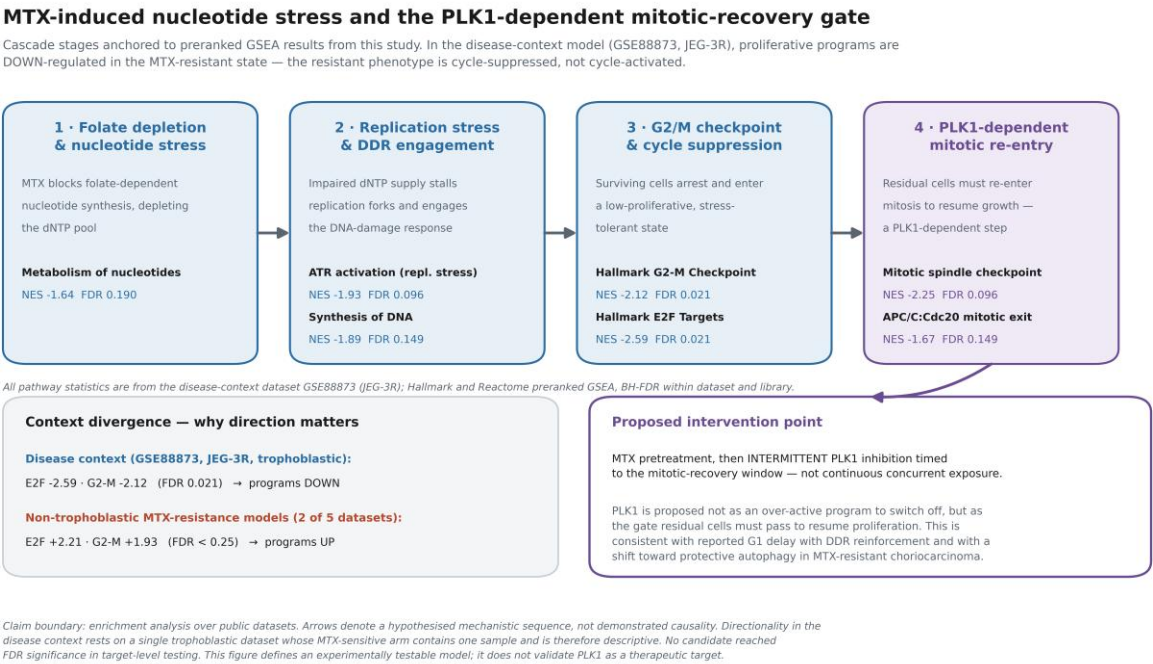


Supplementary Fig. 2. Ranking stability across weighting strategies. The predefined eight-target panel was rescored using the fixed analysis weights, entropy weights derived objectively from the dispersion of the normalised component matrix, and analytic hierarchy process weights obtained from the principal eigenvector of a Saaty pairwise comparison matrix. PLK1 ranks first

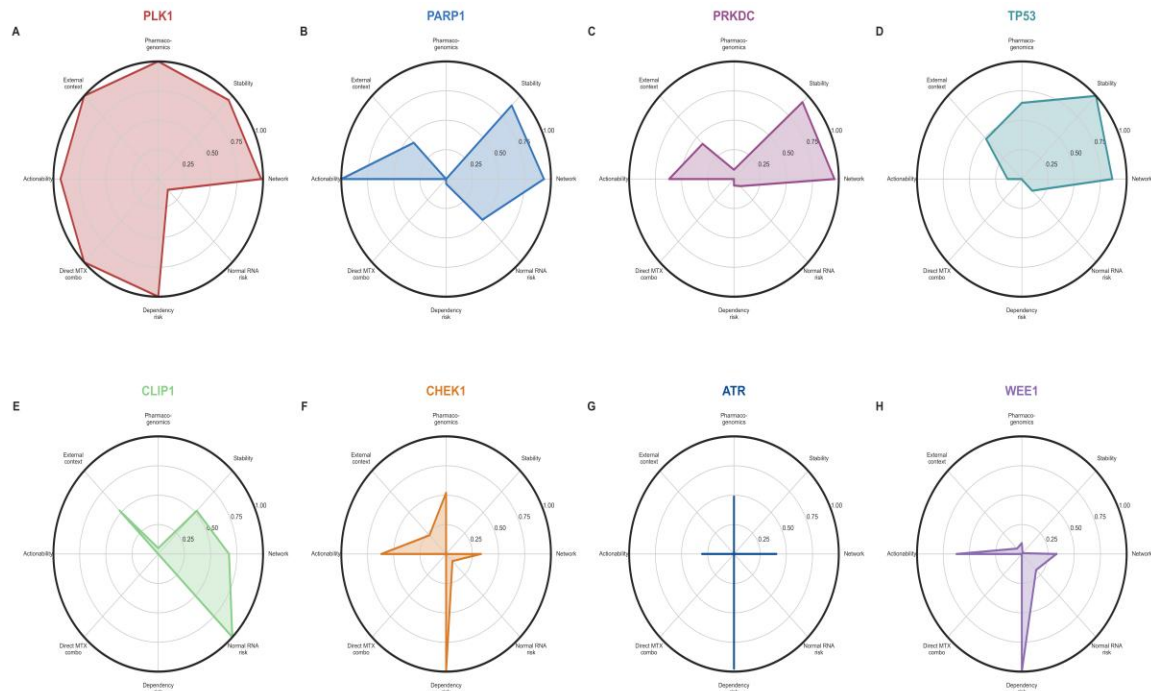
under all three strategies. Rank agreement with the fixed weights is complete for the analytic hierarchy process and high for entropy weighting. Monte Carlo weight-perturbation results are reported in Supplementary Table 1.



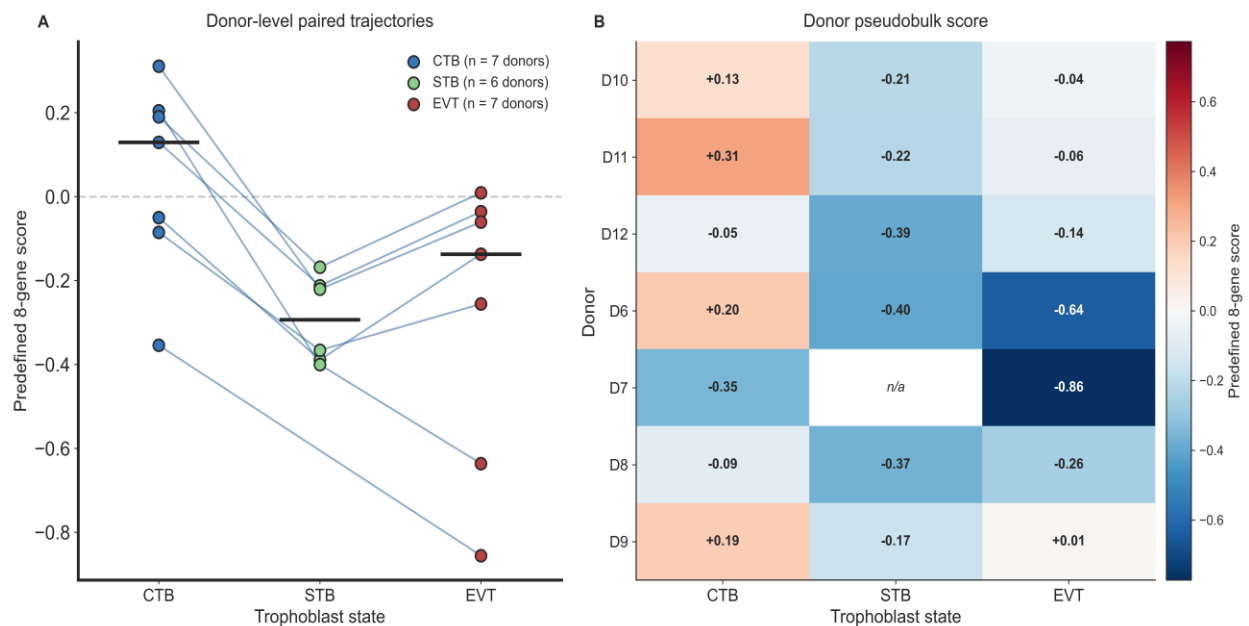
Supplementary Fig. 3. Mechanistic STRING PPI context of the predefined eight-target panel. Coloured nodes show the eight core targets. Pale nodes show the top 15 direct bridge nodes ranked by the number of core-target links and mean STRING weight. CLIP1 is retained as an isolated panel member because it has no edge in the filtered STRING subgraph. The network is a mechanistic context visualisation, not proof of target-mediated MTX resistance.



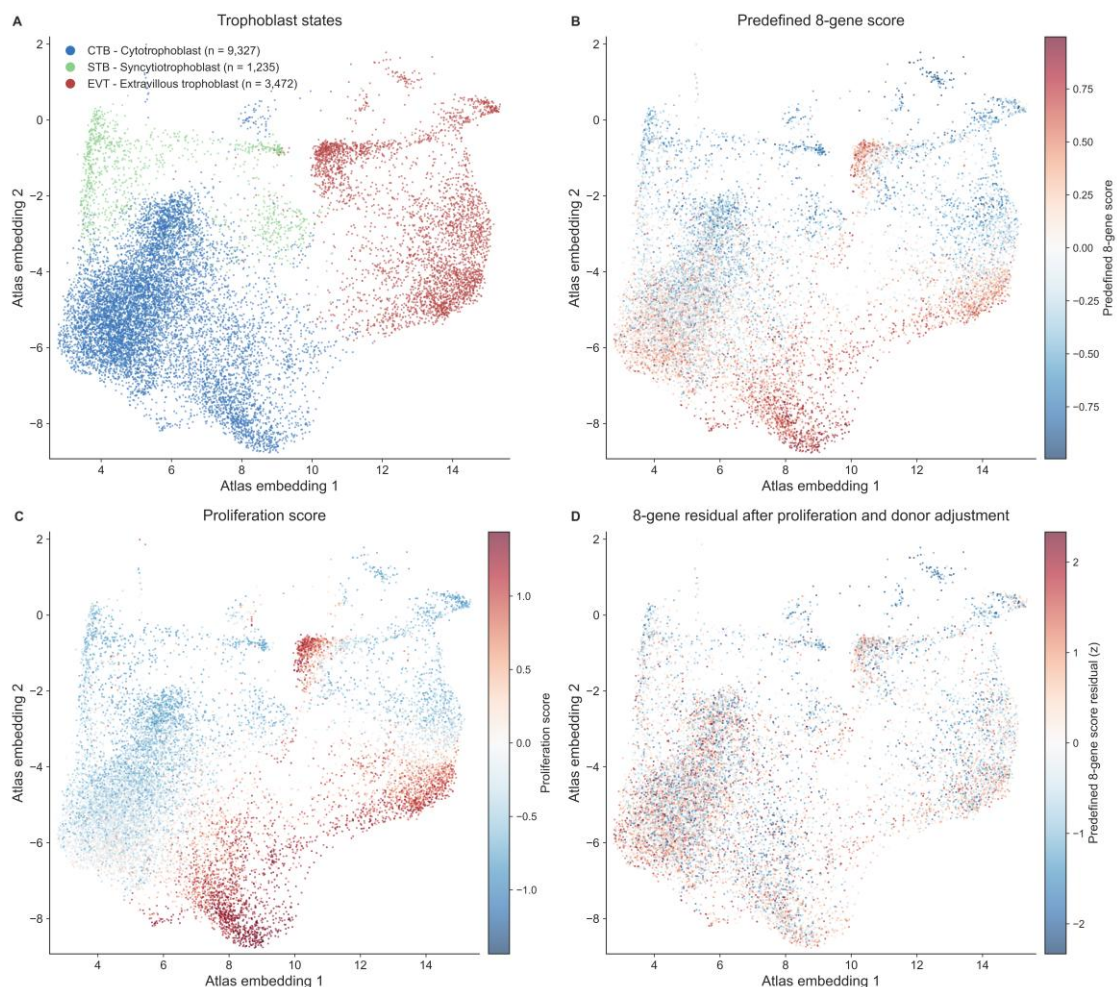
Supplementary Fig. 4. MTX-induced nucleotide stress and the PLK1-dependent mitotic-recovery gate. Cascade stages are anchored to preranked GSEA results from this study. In the disease-context dataset GSE88873 (JEG-3R), proliferative programs are downregulated in the MTX-resistant state (Hallmark E2F Targets NES −2.59; Hallmark G2-M Checkpoint NES −2.12; BH-FDR 0.021), whereas two of five non-trophoblastic MTX-resistance datasets show the same programs enriched in the opposite direction. PLK1 is therefore proposed not as an over-active program to be switched off, but as the gate through which residual, cycle-suppressed cells must pass in order to resume proliferation. Arrows denote a hypothesised mechanistic sequence, not demonstrated causality; directionality in the disease context rests on a single trophoblastic dataset whose MTX-sensitive arm contains one sample and is therefore descriptive.



Supplementary Fig. 5. Core eight-target MCDA component profiles. Each radar chart shows normalised network, stability, pharmacogenomic, external-context, actionability, direct-MTX-combination and risk components. Dependency risk and normal-RNA risk are liabilities; the remaining axes are supportive components. Small multiples avoid overplotting between targets.

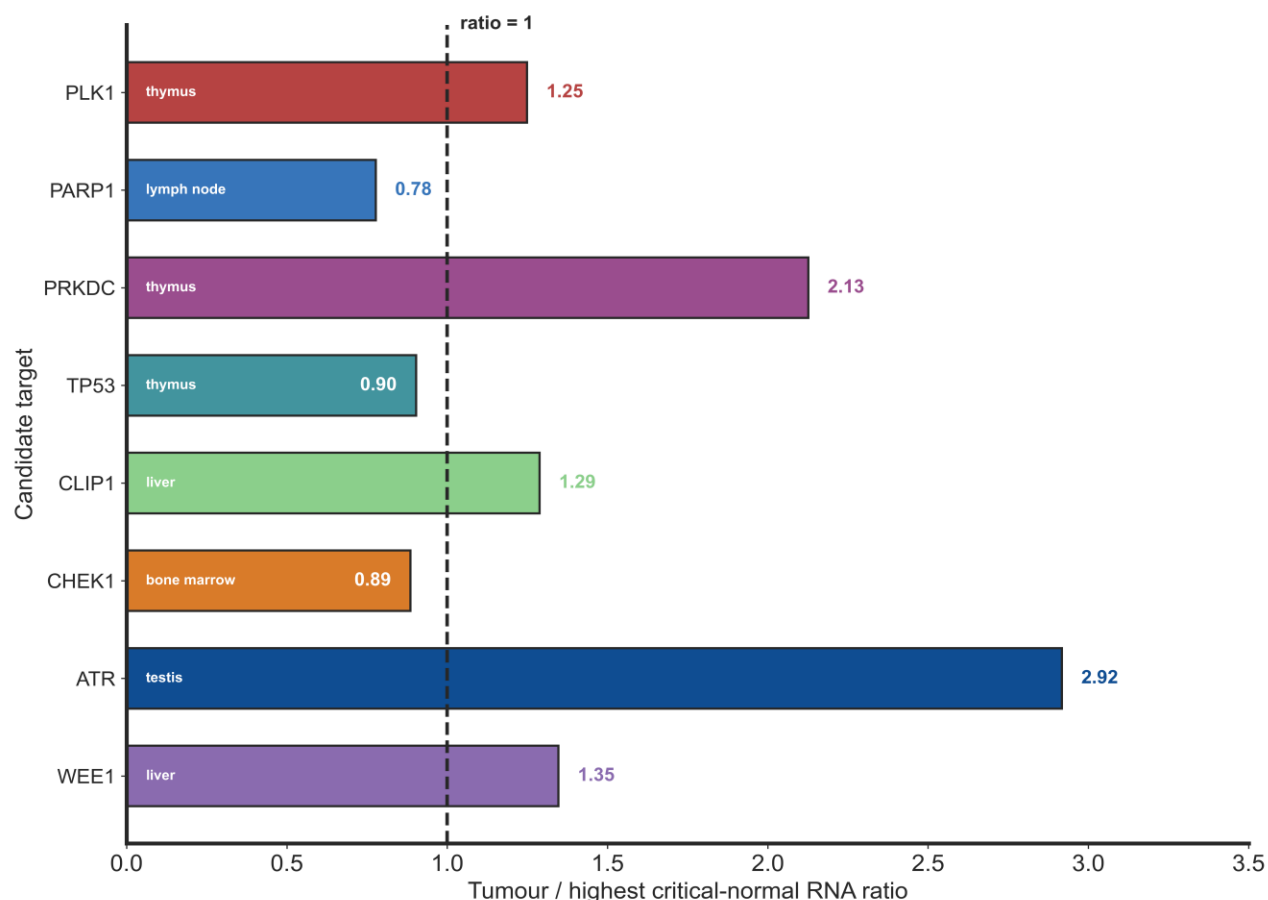


Supplementary Fig. 6. Vento-Tormo E-MTAB-6701 donor-level normal-placenta context projection. The predefined eight-gene score was calculated after $\log_{1p}(\text{CPM})$ transformation and per-gene standardisation, then pseudobulked by individual and trophoblast subtype. (A) Lines connect available subtype summaries within individuals; horizontal bars mark the per-state median. (B) Donor pseudobulk score per individual and state; n/a marks an individual with no annotated cells of that state. This is a normal-placenta context projection, not validation in tumour tissue or a patient digital twin.



Supplementary Fig. 7. Normal-placenta trophoblast-state context projection using E-MTAB-6701 atlas embedding coordinates. Panels show CTB, STB and EVT annotations; the predefined eight-gene score; the separate proliferation score; and the eight-gene-score residual after adjustment for proliferation and donor indicators. The overlays are descriptive visualisations.

Donor-level pseudobulk contrasts remain primary. The map does not validate tumour-tissue biology or MTX-specific sensitisation.



Supplementary Fig. 8. Exploratory cross-platform RNA expression-window proxy. Bars show the ratio of median RNA expression in three DepMap choriocarcinoma models to the highest selected critical-normal-tissue RNA expression from the Human Protein Atlas. Values above one indicate higher median tumour-model RNA expression than the highest selected critical normal tissue. This cross-platform ratio is a toxicity-awareness proxy, not a pharmacologic therapeutic index.