

Online Resource 1

Supplementary Figures S1–S10

Single-cell and spatial transcriptomics define a C3-centred multicellular ecology in colorectal liver metastasis

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Feifei Ma¹ and Yige Yu^{2*}

¹ Department of Anesthesia, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325047, China

² Department of Pharmacy, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

* Correspondence: Yige Yu, yuyige321@163.com

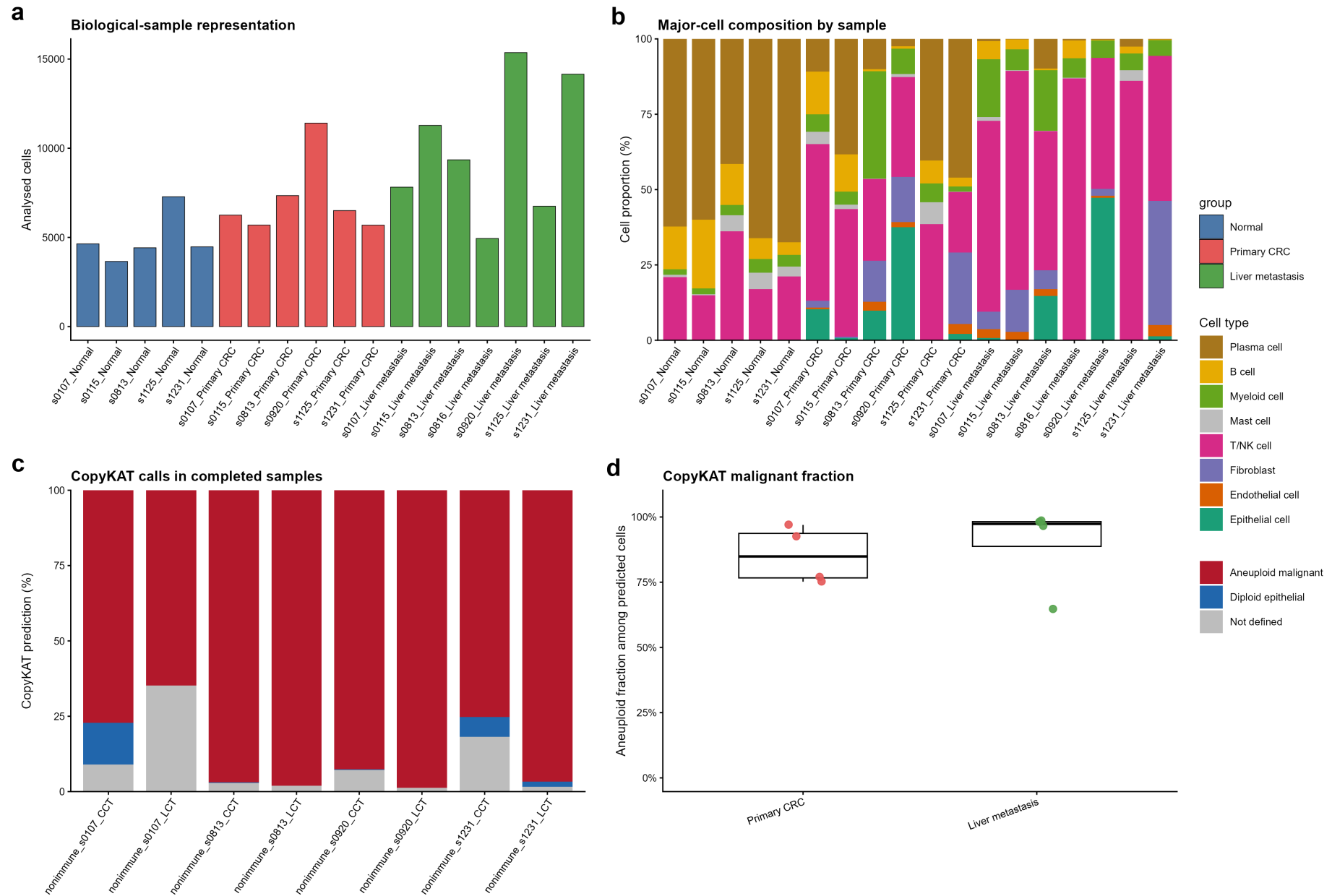


Fig. S1 Sample representation and CopyKAT quality audit. (a) Cells contributed by each biological-sample unit after quality control. (b) Major-cell-type composition within each sample. (c) Relative frequencies of CopyKAT aneuploid, diploid and not-defined calls among epithelial cells in samples with completed inference. (d) Fraction of epithelial cells classified as aneuploid. The merged atlas contains sorting-enriched datasets and does not estimate unbiased whole-tissue abundance

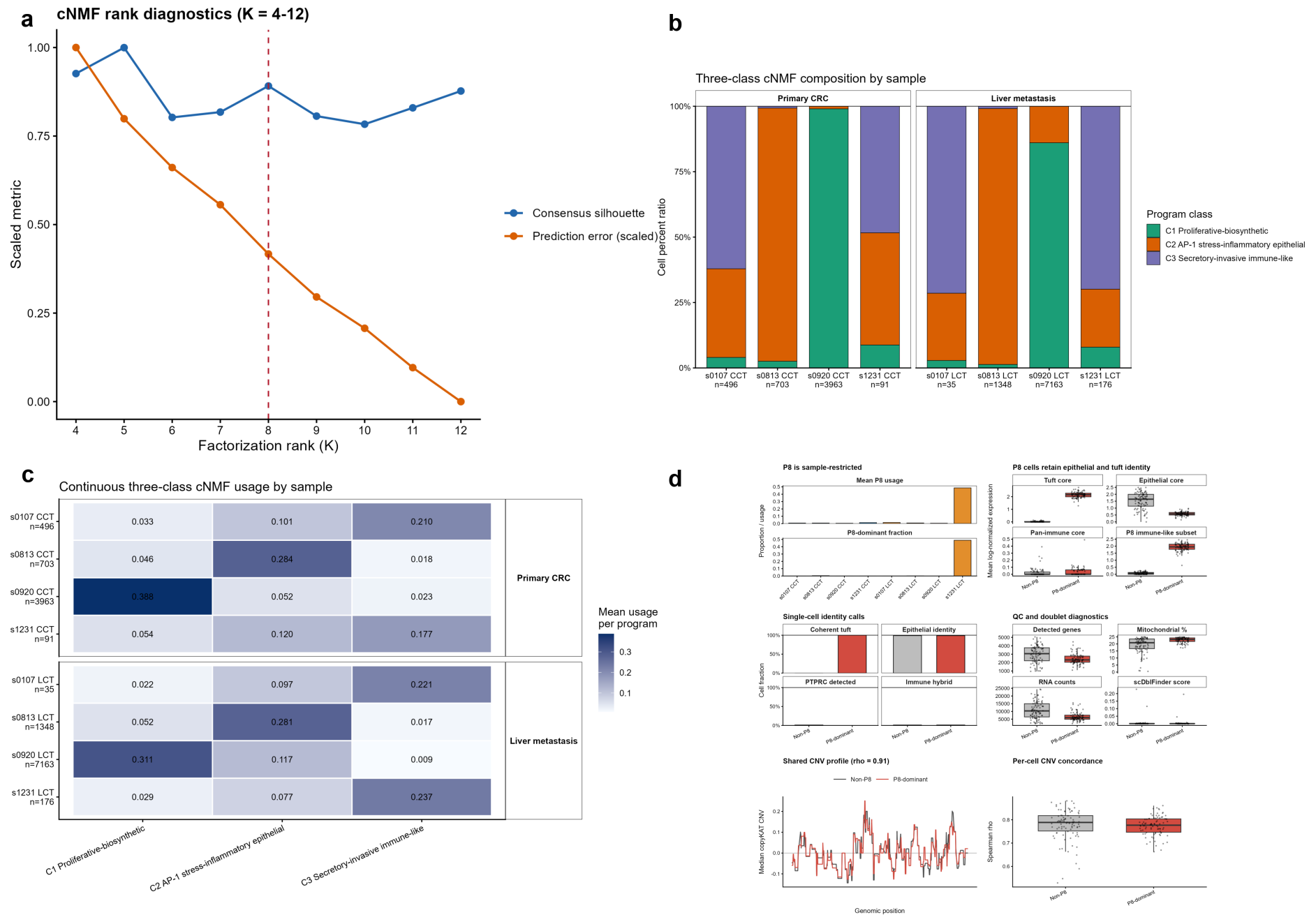


Fig. S2 cNMF rank selection, sample support and program-8 sensitivity. (a) Consensus silhouette and scaled reconstruction error across K=4–12; the dashed line marks K=8. (b) Meta-program proportions by sample. (c) Mean meta-program usage by sample. (d) Program-8 sensitivity analysis showing its sample-restricted tuft/chemosensory component

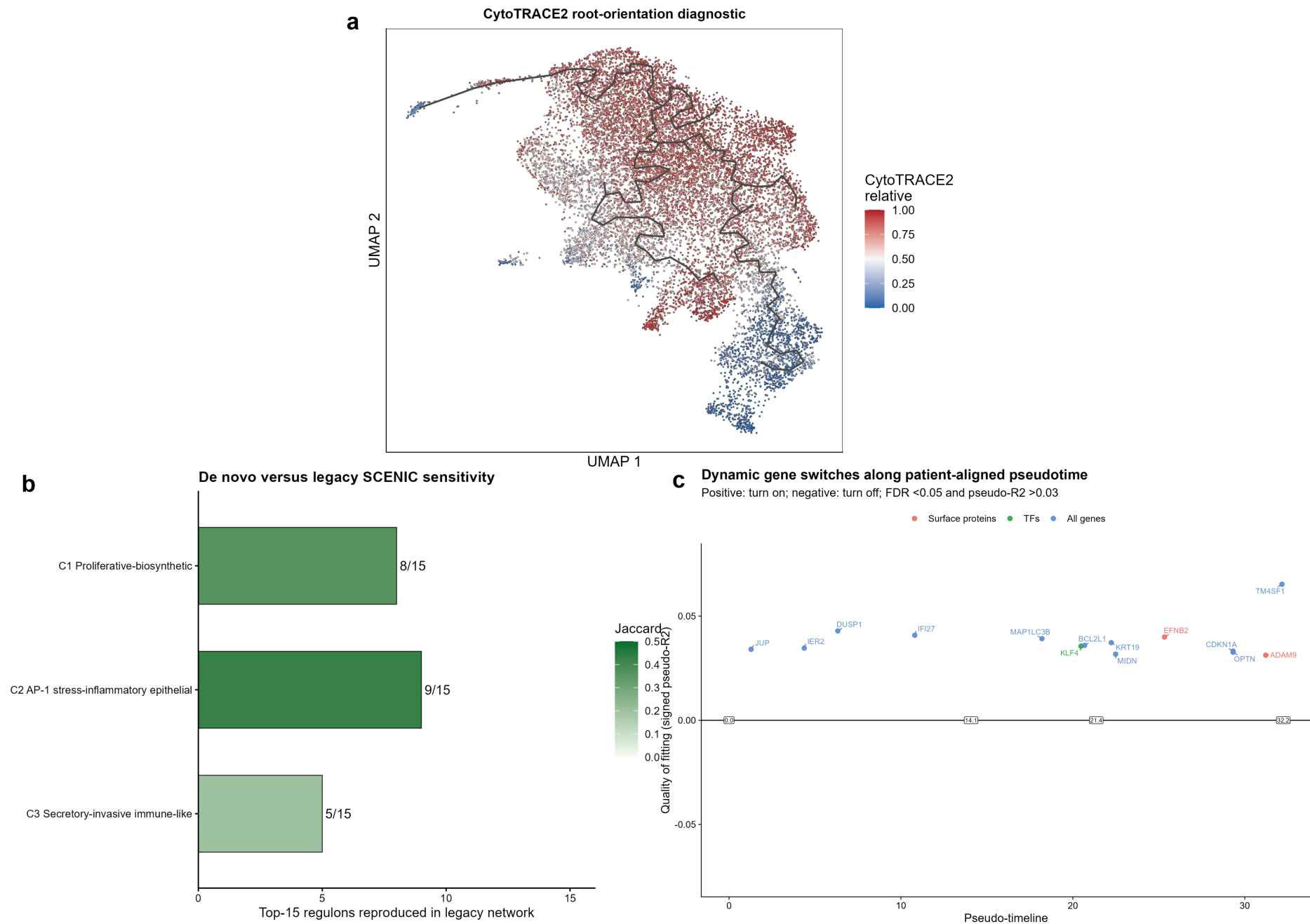


Fig. S3 CytoTRACE2 orientation and regulatory sensitivity. (a) CytoTRACE2 potency used to orient the trajectory root. (b) Reproduction of the top 15 class-associated regulons between de novo and legacy SCENIC networks. (c) High-confidence GeneSwitches events along patient-aligned pseudotime

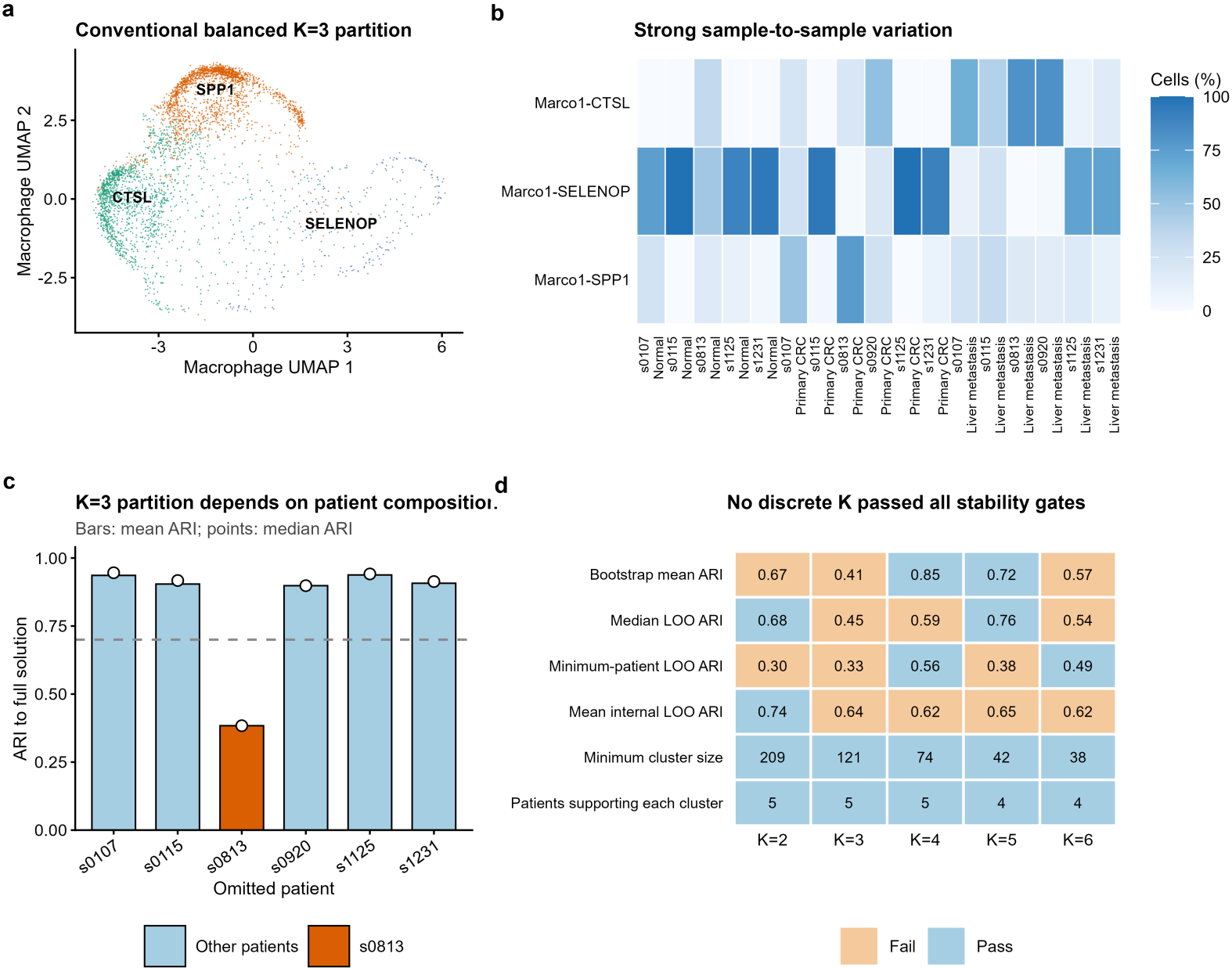


Fig. S4 Instability of discrete macrophage subclustering. (a) Representative balanced K=3 macrophage partition. (b) Sample-to-sample variation in cluster composition. (c) Leave-one-patient-out adjusted Rand indices showing sensitivity to the dominant s0813 profile. (d) Prespecified stability criteria across K=2–6. Failure to identify a stable partition motivated continuous program analysis

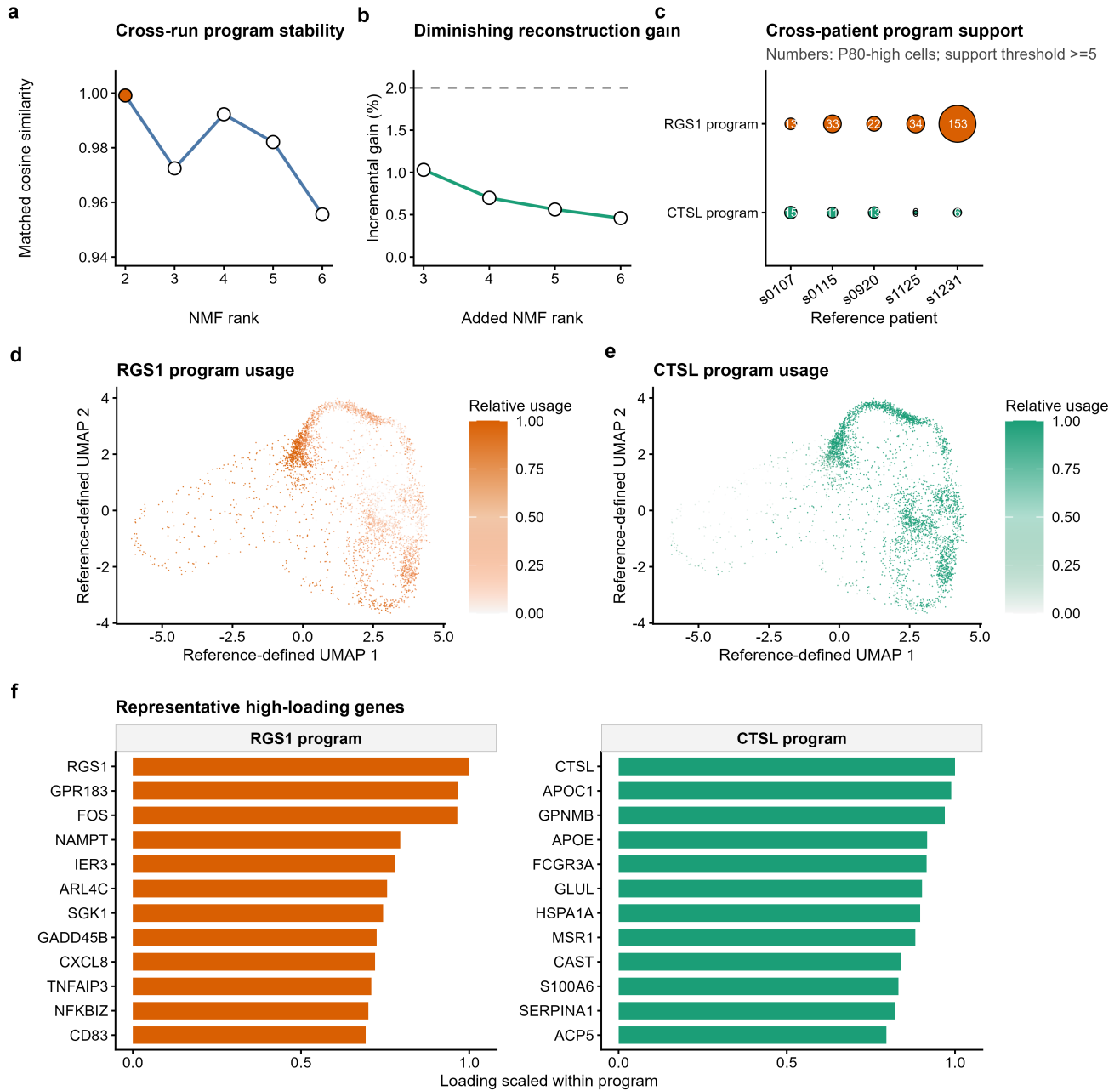


Fig. S5 Robust rank-2 RcppML NMF programs in macrophages. (a) Cross-run program stability. (b) Incremental reconstruction gain across ranks. (c) Cross-patient program support. (d,e) Continuous RGS1- (d) and CTSL-program (e) usage. (f) Representative high-loading genes. Program usage is continuous and is not a categorical macrophage annotation

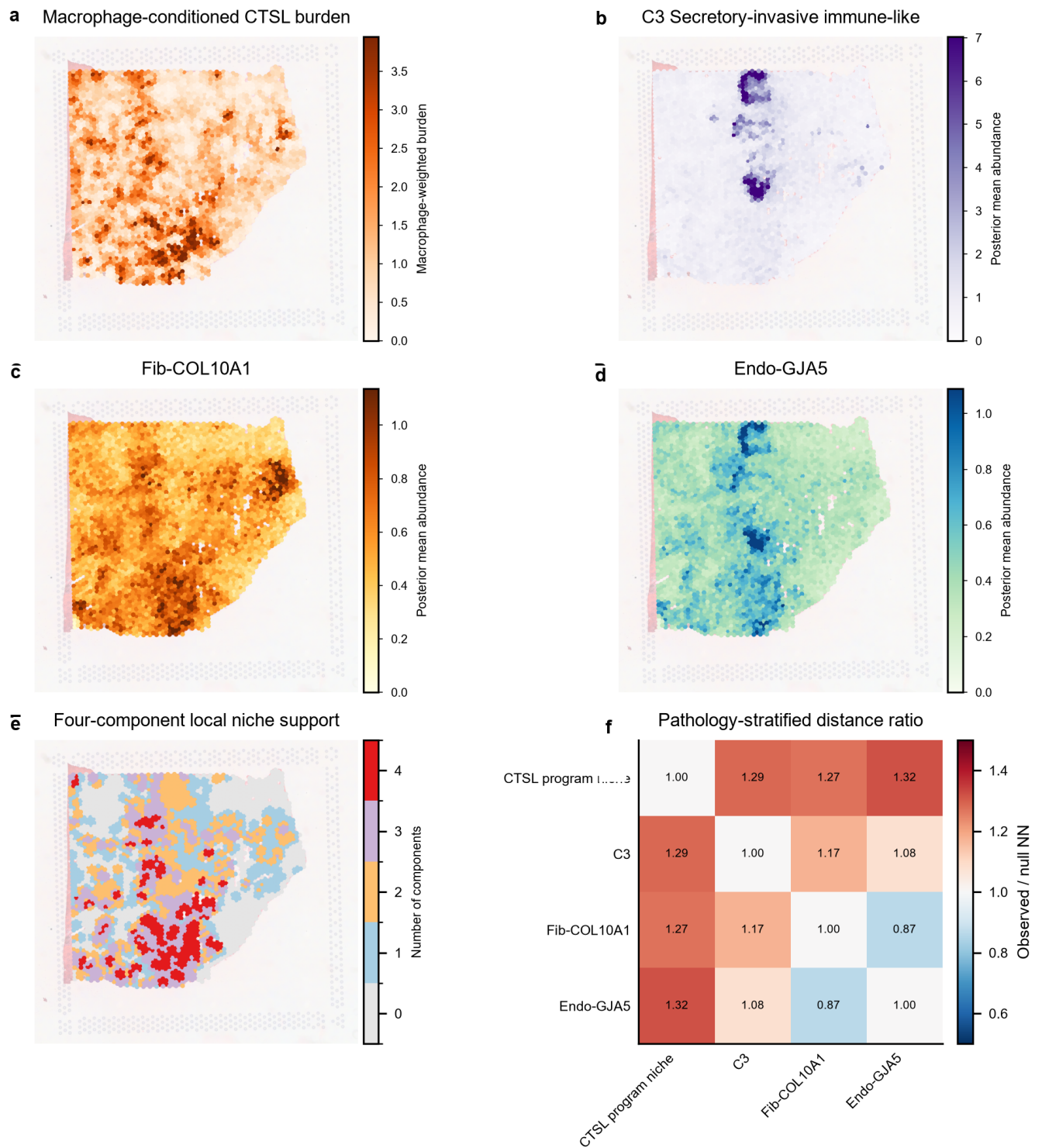


Fig. S6 Spatial validation in GSE217414 section GSM6716964. (a) Macrophage-conditioned CTSL-program burden. (b) C3 abundance. (c) Fib-COL10A1 abundance. (d) Endo-GJA5 abundance. (e) Four-component local-niche support. (f) Pathology-stratified distance ratios. Hotspots were defined independently for each feature

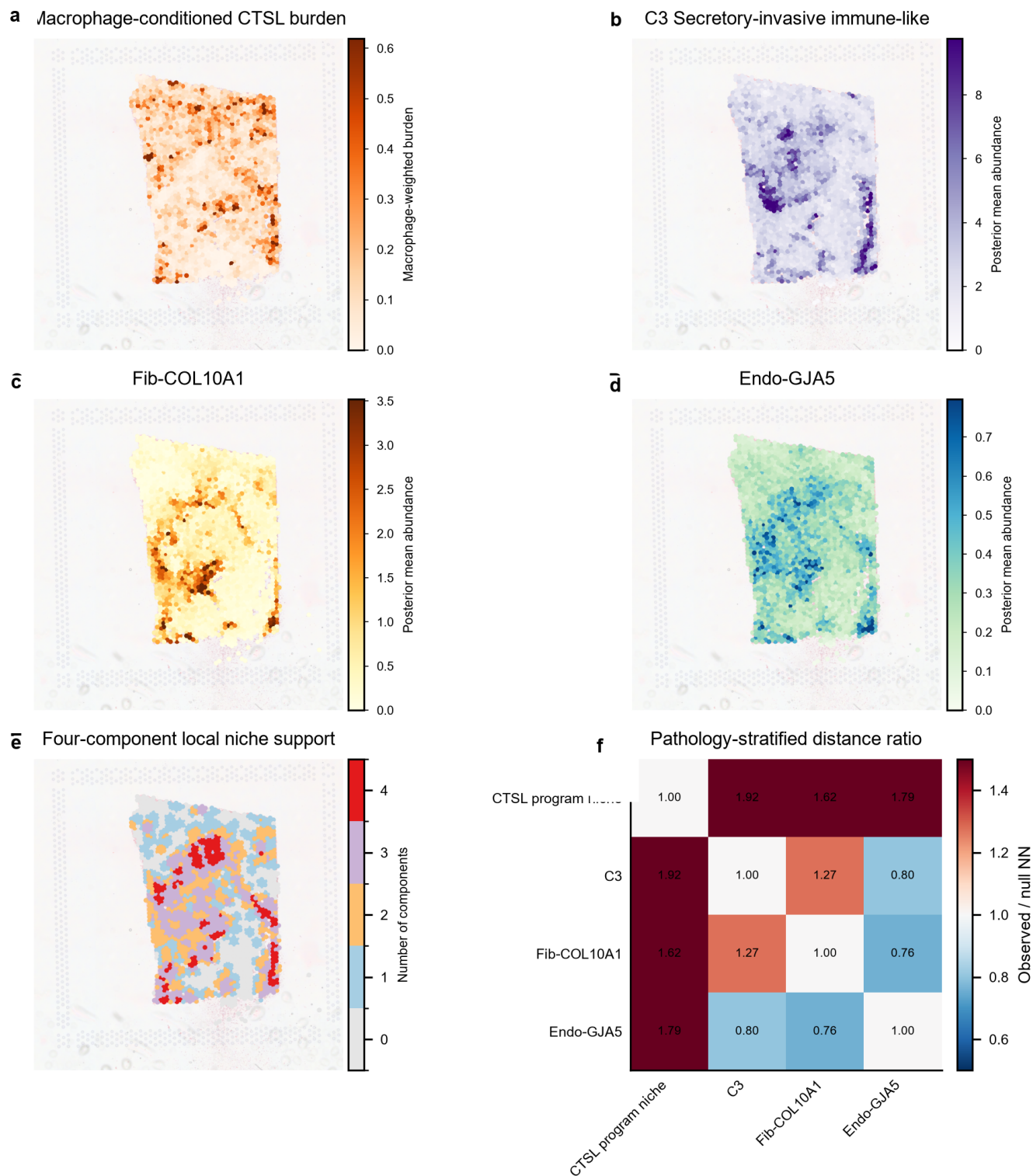


Fig. S7 Spatial validation in GSE217414 section GSM6716965. (a) Macrophage-conditioned CTSL-program burden. (b) C3 abundance. (c) Fib-COL10A1 abundance. (d) Endo-GJA5 abundance. (e) Four-component local-niche support. (f) Pathology-stratified distance ratios. The section illustrates inter-section spatial variation

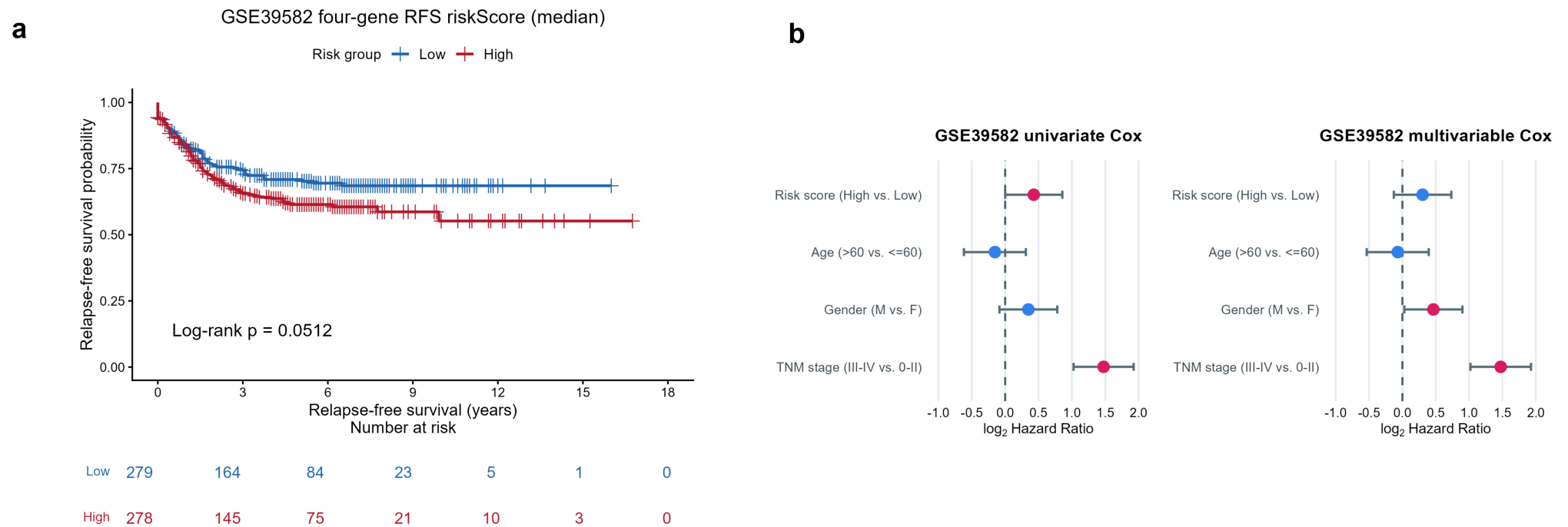


Fig. S8 Locked external validation of the four-gene recurrence model. (a) Kaplan–Meier recurrence-free survival in GSE39582 using locked TCGA coefficients and a cohort-specific median risk threshold. (b) Univariable and clinically adjusted Cox models

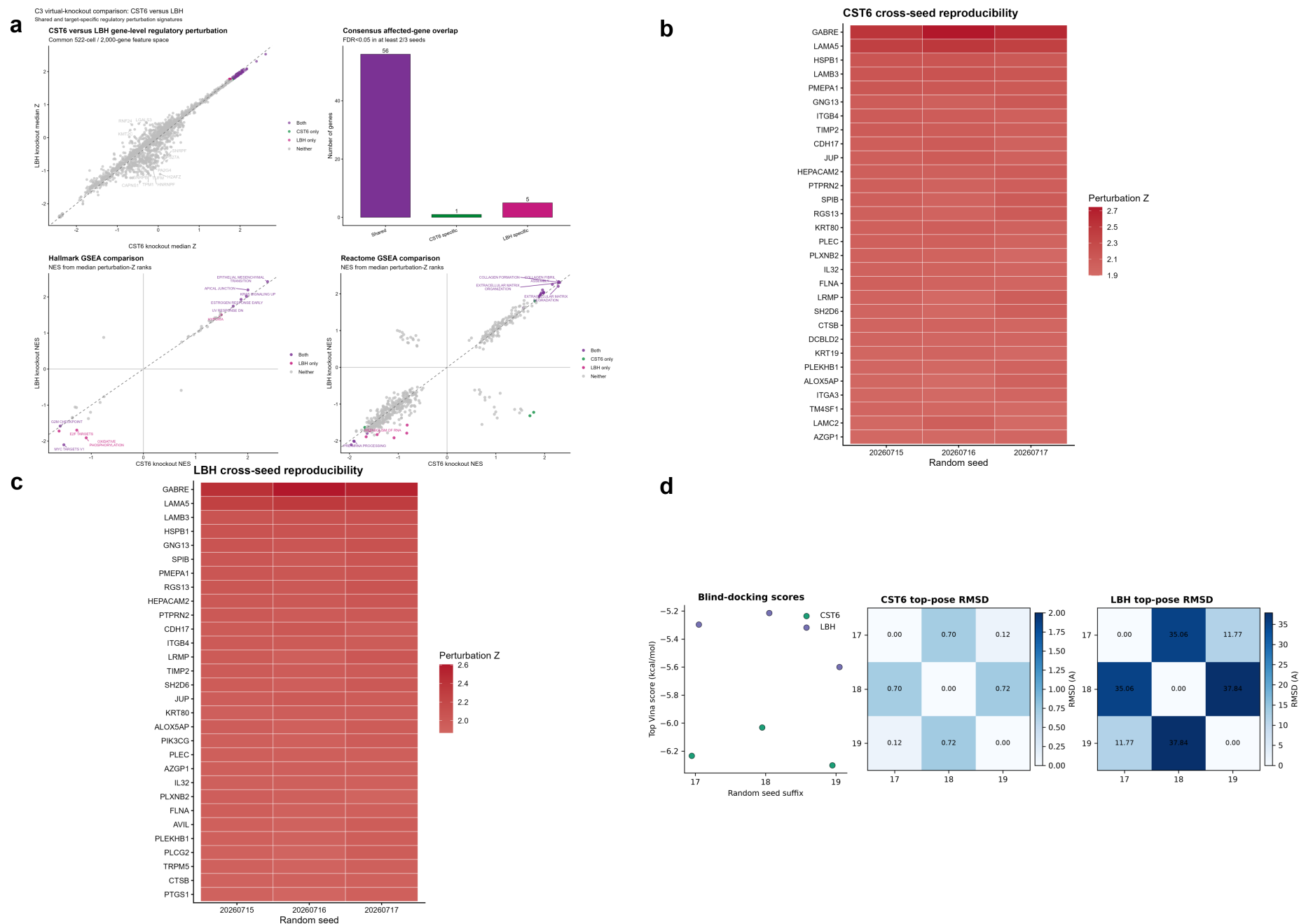


Fig. S9 Virtual-knockout reproducibility and docking quality. (a) CST6- and LBH-knockout score concordance, consensus affected-gene overlap and pathway enrichment. Consensus genes had FDR<0.05 in at least two seeds. (b,c) Cross-seed reproducibility after CST6 (b) or LBH (c) knockout. (d) AutoDock Vina scores and pairwise RMSDs among top-ranked PD-0325901 poses. Docking does not demonstrate target engagement

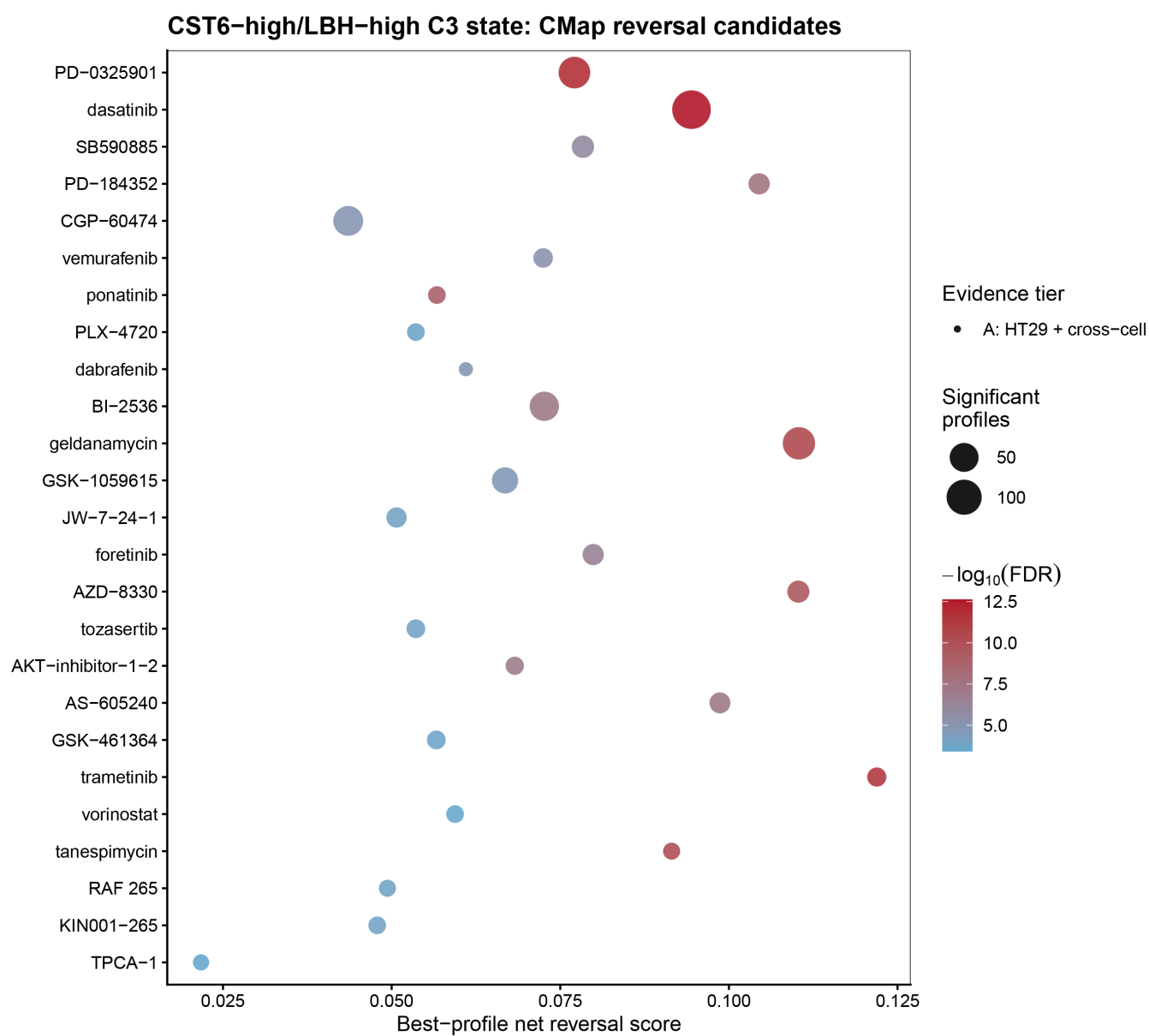


Fig. S10 Connectivity Map identifies candidate compounds predicted to reverse the CST6-high/LBH-high C3 state. Bubble plot of the top 25 Tier A LINCS L1000 compounds predicted to reverse the CST6-high/LBH-high C3 transcriptional signature