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SPACE: Spatially resolved multiomic analysis for high-throughput CRISPR screening in 3D models

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Introduction

High-content single-cell perturbation screens are pivotal for elucidating gene functions and uncovering novel biology, yet conventional methods necessitate cell dissociation, forfeiting critical spatial information essential for dissecting cell-cell interactions and tissue architecture in complex microenvironments. While spatial CRISPR screening mitigates this partially, existing technologies are constrained by hypothesis-driven phenotyping panels limited to sparse RNA or protein coverage, curtailing comprehensive gene function assessment and discovery breadth.

To overcome these barriers, we developed **SPACE** (**SPA**tial **C**ell **E**xploration) assay, a pioneering platform that fuses whole-transcriptome profiling, CRISPR perturbations (sgRNAs and associated Unique Guide Identifiers UGIs), and multiplexed protein detection at single-cell resolution within intact 3D tissue contexts. SPACE delivers unbiased, transcriptome-wide readouts alongside 68 protein markers (64 CosMx® IO protein panel targets plus 4 morphological markers), vastly expanding phenotypic landscapes in spatial screens. As the highest-plex coverage multimodal CRISPR assay to date, SPACE achieves this at unprecedented scale and affordability – profiling hundreds of spheroids encompassing tens of thousands of cells per slide – largely outperforming sequencing-based alternatives in efficiency.

SPACE Assay Design

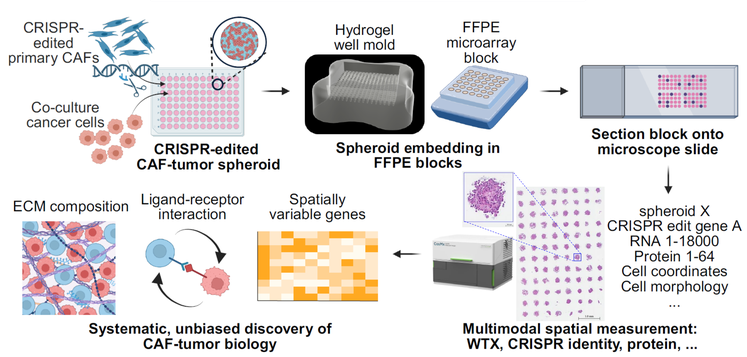

Fig. 1 Diagram of SPACE experimental workflow. Primary cancer associated fibroblasts (CAF) are CRISPR edited, co-cultured with BxPC3 cancer cells in ultra-low attachment plates to allow spheroid formation, and fixed. The spheroids are then embedded in FFPE blocks while maintaining 96-well arrayed format for CRISPR identity confirmation. The sectioned slides undergo SPACE profiling of CRISPR molecules, transcriptome and protein on the same slide

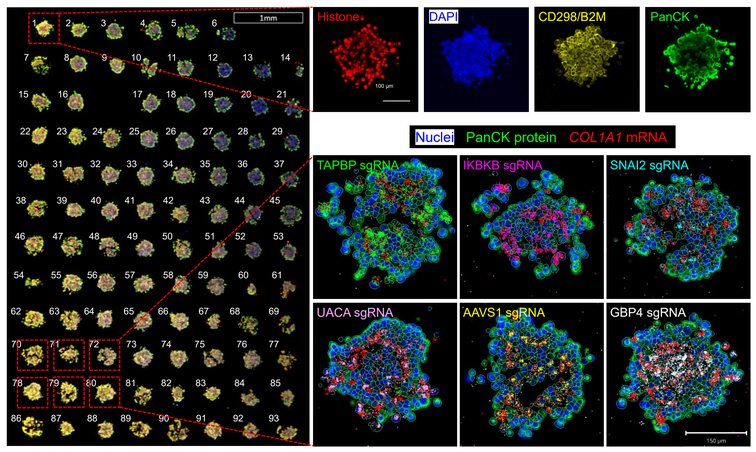

Fig. 2 Example images from SPACE workflow. Left: morphological staining of whole slide containing a spheroid array. Top right: Morphological staining markers. Bottom right: composite images of representative spheroids, showing different synthetic sgRNAs, COL1A1 mRNA, PanCK protein and nuclear staining.

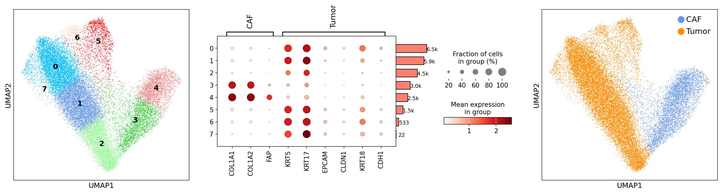

Fig. 3 Primary cell identification. Left: cell clustering using RNA data. Middle: Differentially expressed genes across main clusters. Right: Cell annotation with key marker genes.

SPACE Assay Characterization

RNA detection performance

Sample Location	Section-13	Section-18	Section-14
Panel Coverage	WTX + CRISPR	WTX	6K + CRSIPR
Organoid Size (mm^2)	2.55	2.5	2.23
Total Cells Segmented	24,548	23,294	22,234
Mean Cell Size (um^2)	103.7	107.2	100.5
Mean Transcripts / Cell	2,188	2,405	1,492
Median Transcripts / Cell	1,887	2,033	1,297
Mean Unique Genes / Cell	1,518	1,516	874
Median Unique Genes / Cell	1,362	1,386	819
Mean Transcripts / um^2	21.1	22.4	14.8

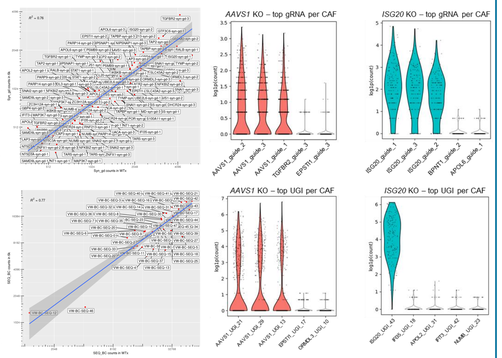

Fig. 4 SPACE is compatible with different panel sizes, showing consistent detection of CRISPR targets with high specificity. Left: Summary table for SPACE performance in RNA detection. Middle: Correlation of detected CRISPR molecules (upper: CRISPR sgRNAs; lower: UGIs) count between WTX+CRISPR (x axis) and 6K+CRISPR (y axis). Right: Counts per CAF cell of the top 5 most abundant sgRNAs and UGIs in AAVS1-KO and ISG20-KO spheroids.

SPACE Revealed Novel Biomarkers in CAF

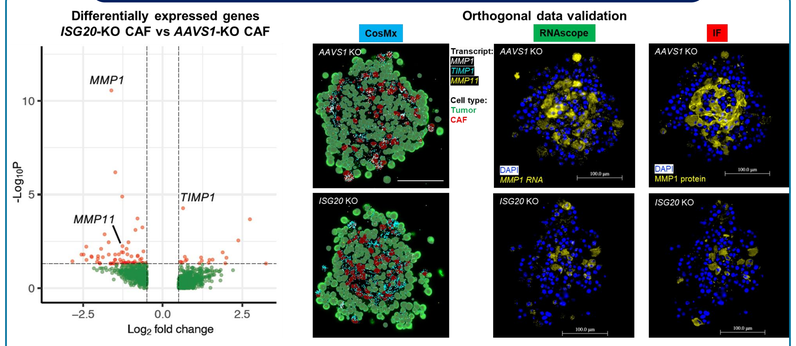

Fig. 5 In ISG20-KO CAF, SPACE identified several MMPs significantly downregulated, which were later validated with orthogonal assays at both RNA and protein levels.

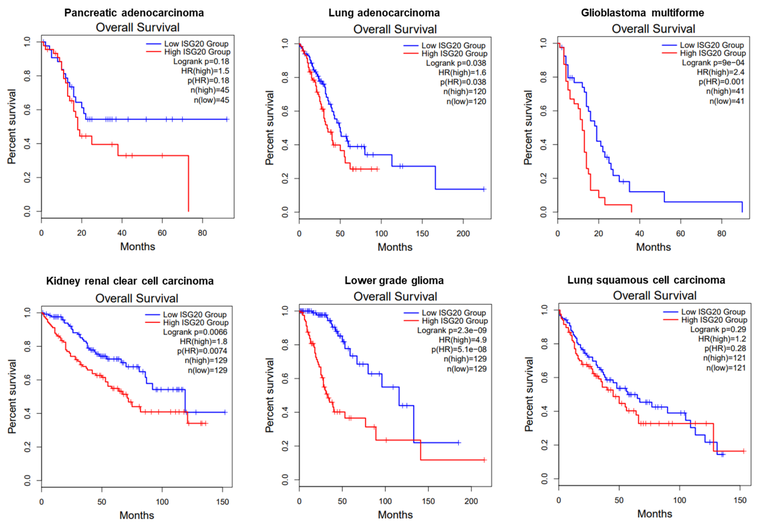

Fig. 6 Kaplan-Meier plots of patient overall survival in different tumor types categorized as high-ISG20 or low-ISG20 subgroups. (Data source: TCGA/GTEx)

SPACE Enabled Spatially Informed LR Analysis

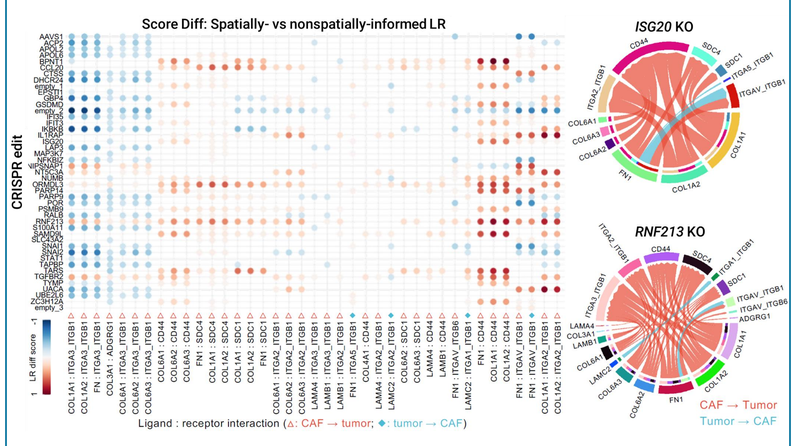

Fig. 7 Left: Score differences between the spatially informed and nonspatially informed LR analysis for each CRISPR edits (blue – high LR interaction score in nonspatially informed analysis; red – high LR interaction score in spatially informed analysis). Only LR pairs identified in more than 5 KO conditions are presented. Right: Chord diagrams showing the spatially informed LR pairs specific to ISG20-KO and RNF213-KO spheroids compared to nonspatially informed analysis (LR pairs with score difference > 0).

Same-Sample Multiomic SPACE

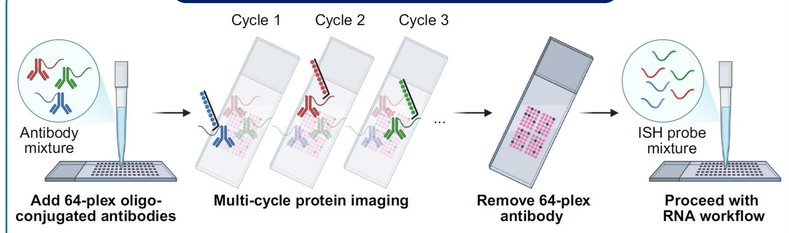

Fig. 8 Diagram of multiomic SPACE workflow. Samples were first stained with a CosMx 64-plex protein panel, followed by the SPACE WTX workflow for RNA profiling.

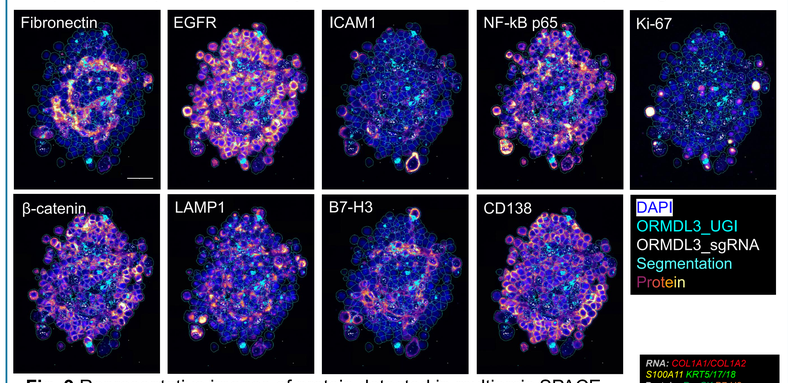

Fig. 9 Representative images of protein detected in multiomic SPACE.

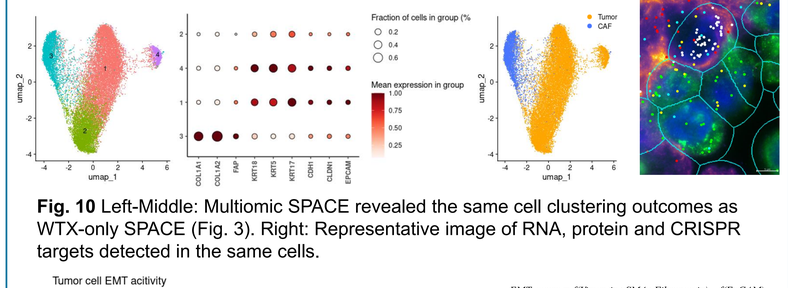

Fig. 10 Left-Middle: Multiomic SPACE revealed the same cell clustering outcomes as WTX-only SPACE (Fig. 3). Right: Representative image of RNA, protein and CRISPR targets detected in the same cells.

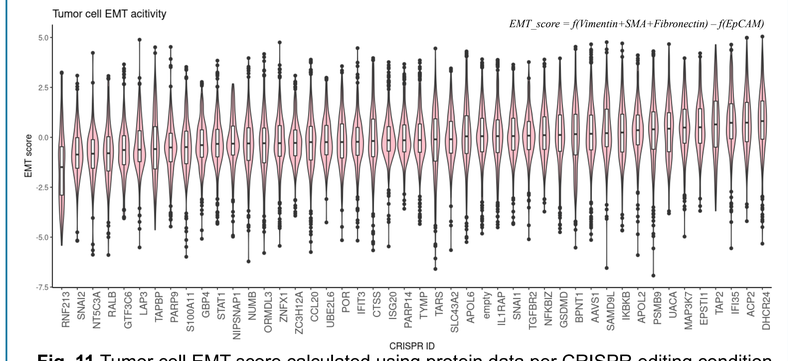

Fig. 11 Tumor cell EMT score calculated using protein data per CRISPR editing condition

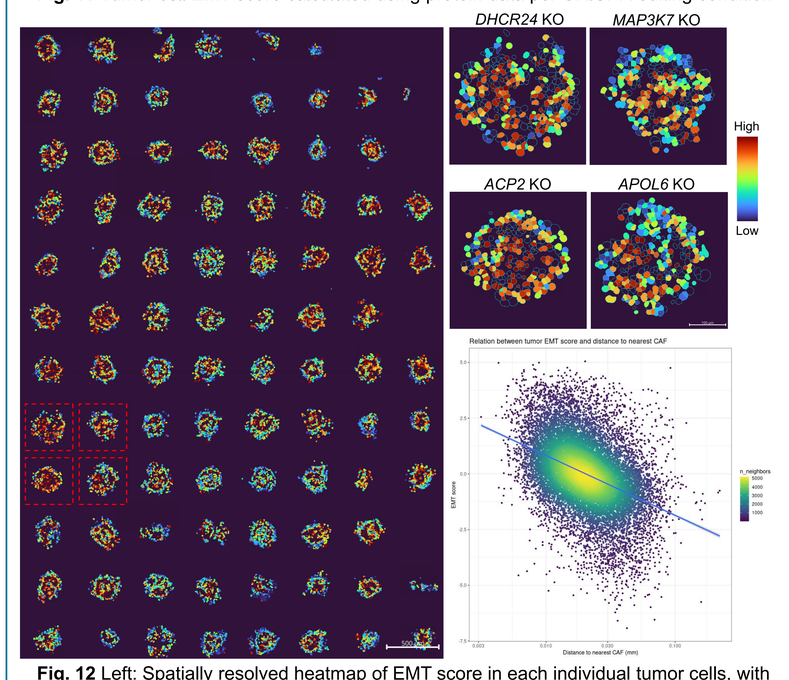

Fig. 12 Left: Spatially resolved heatmap of EMT score in each individual tumor cells, with 4 spheroids zoomed in (Upper right). Lower right: Tumor cell EMT score negatively correlates with their distance to the closest CAF cell.

Summary

- SPACE integrates whole transcriptome, multiplexed proteins, and CRISPR sgRNA ID at subcellular resolution in 3D models, providing unparallel phenotypic depth.
- SPACE scales to hundreds of spheroids per slide for cost-effective screening.
- SPACE is FFPE-compatible and supports multimodal, orthogonal cross-validation.
- SPACE preserves spatial context to reveal new regulators and mechanisms.
- SPACE accelerates drug discovery with disease-relevant functional genomics datasets.