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 Human 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

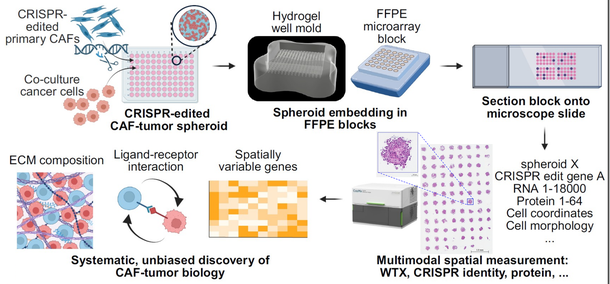

Fig. 1 Diagram of SPACE experimental workflow. Primary cancer associated fibroblasts (CAF) are CRISPR edited, co-cultured with BxPC3 cancer cells in ultra-low attachment plates to allow spheroid formation, and fixed. The spheroids are then embedded in FFPE blocks while maintaining 96-well arrayed format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

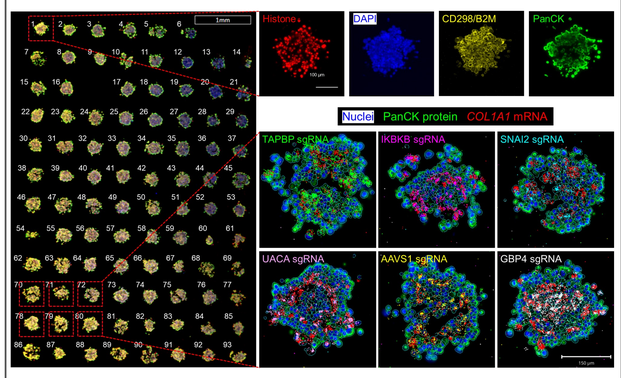

Fig. 2 Example images from SPACE workflow. Left: morphological staining of whole slide containing a spheroid array. Top right: Morphological staining markers. Bottom right: composite images of representative spheroids, showing different synthetic sgRNAs, COL1A1 mRNA, PanCK protein and nuclear staining.

SPACE Assay Characterization

RNA detection performance

Sample Location	Section-13	Section-18	Section-14
Panel Coverage	WTX + CRISPR	WTX	6K + CRISPR
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

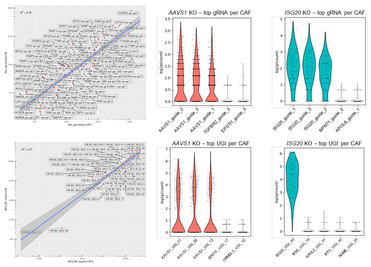

Fig. 3 SPACE is compatible with different panel sizes, showing consistent detection of CRISPR targets with high specificity. Left: Summary table for SPACE performance in RNA detection. Middle: Correlation of detected CRISPR molecules (upper: CRISPR sgRNAs; lower: UGIs) count between WTX+CRISPR (x axis) 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.

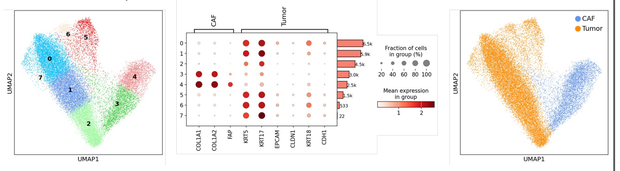

Fig. 4 Primary cell identification. Left: cell clustering using RNA data. Middle: Differentially expressed genes across main clusters. Right: Cell annotation with key marker genes.

SPACE Revealed Novel Biomarkers in CAF

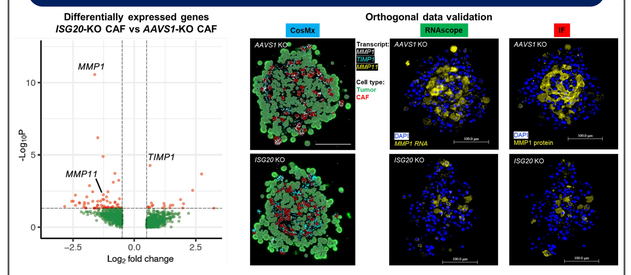

Fig. 5 In ISG20-KO CAF, SPACE identified several MMPs significantly downregulated, which were later validated with orthogonal assays at both RNA and protein levels.

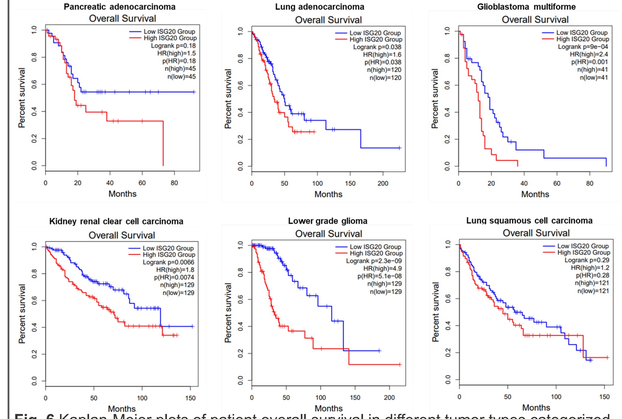

Fig. 6 Kaplan-Meier plots of patient overall survival in different tumor types categorized as high-ISG20 or low-ISG20 subgroups. (Data source: TCGA/GTEx)

SPACE Enabled Spatially Informed LR Analysis

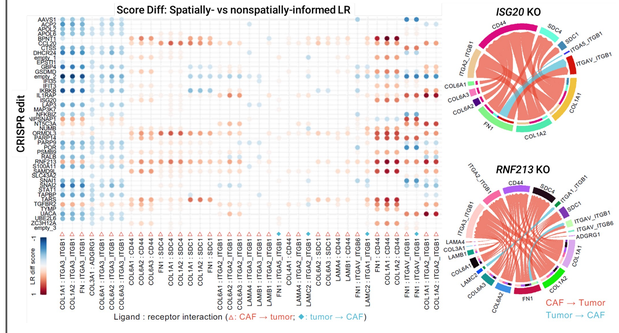

Fig. 7 Left: Score differences between the spatially informed and nonspatially informed LR analysis for each CRISPR edits (blue – high LR interaction score in nonspatially informed analysis; red – high LR interaction score in spatially informed analysis). Only LR pairs identified in more than 5 KO conditions 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

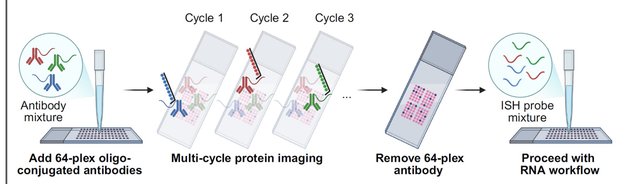

Fig. 8 Diagram of multiomic SPACE workflow. Samples were first stained with CosMx 64-plex protein panel, followed by the SPACE WTX workflow for RNA profiling.

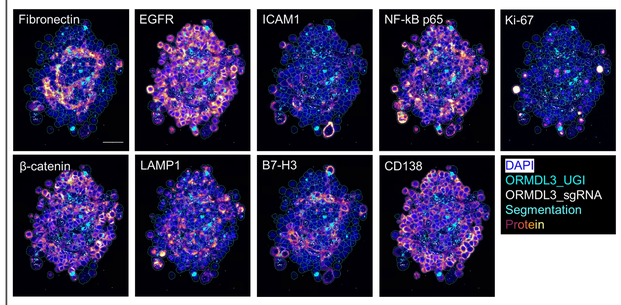

Fig. 9 Representative images of protein detected in multiomic SPACE.

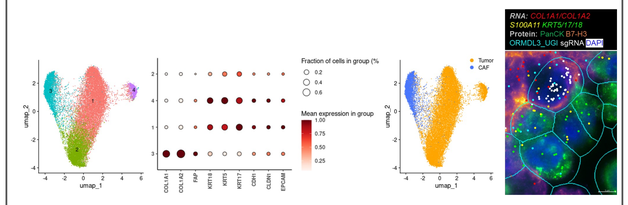

Fig. 10 Left-Middle: Multiomic SPACE revealed the same cell clustering outcomes as WTX-only SPACE (Fig. 3). Right: Representative image of RNA, protein and CRISPR targets detected in the same cells.

SPACE for Spatial Phenotyping

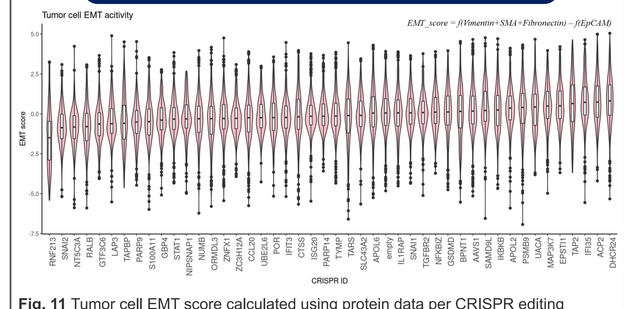

Fig. 11 Tumor cell EMT score calculated using protein data per CRISPR editing condition

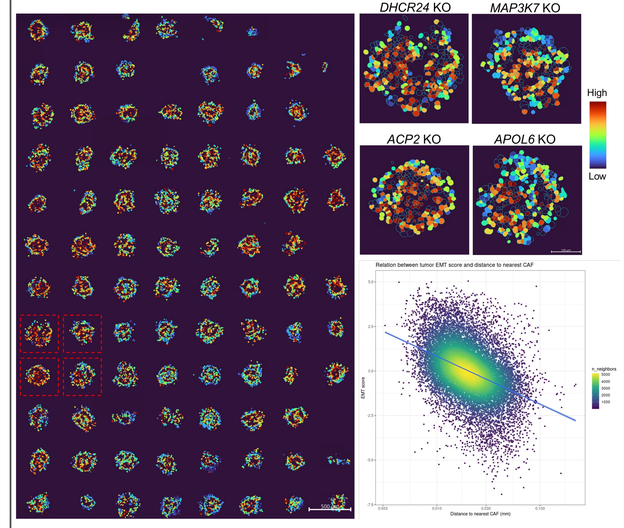

Fig. 12 Left: Spatially resolved heatmap of EMT score in each individual tumor cells, with 4 spheroids zoomed in (Upper right). Lower right: Tumor cell EMT score negatively correlates with their distance to the closest CAF cell.

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.

Reference:
bioRxiv 2025.09.14.675819;
doi: https://doi.org/10.1101/2025.09.14.675819

bioRxiv 2024.11.27.625536;
doi: https://doi.org/10.1101/2024.11.27.625536

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