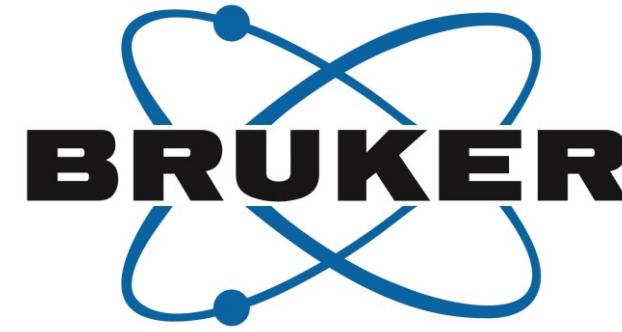


# Whole transcriptome spatial CRISPR screening of tumor cells and spheroids with nanometer resolution



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## Introduction

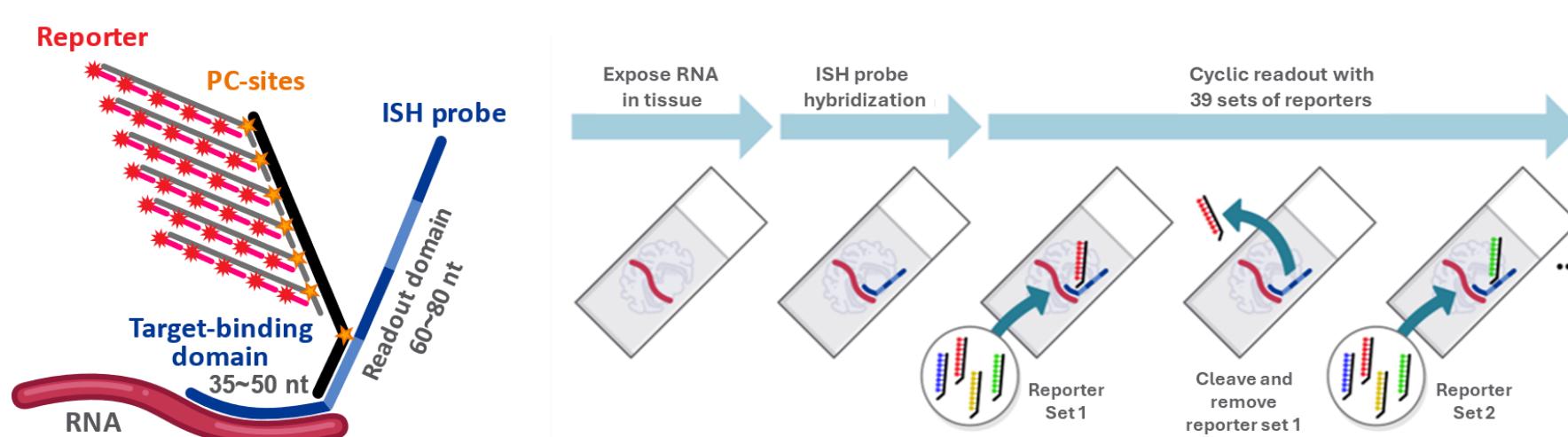
The CosMx® Spatial Molecular Imager (SMI) Whole Transcriptome (WTX) assay is a transformative platform for spatially resolved single-cell transcriptomics, offering unprecedented insights into cancer biology. This study presents an optimized, end-to-end protocol for in vitro cell-based assays, enabling high-resolution spatial mapping of RNA molecules. This approach is pivotal for dissecting tumor microenvironments, cell-cell interactions, and the molecular underpinnings of cancer.

In cultured cancer cell models, the CosMx WTX assay consistently quantified tens of thousands of transcripts across diverse cell lines, achieving high reproducibility and strong concordance with bulk RNA-seq data. This robust performance supports precise transcriptomic profiling, enabling effective clustering, accurate annotation, and deep functional insights into cancer cell heterogeneity.

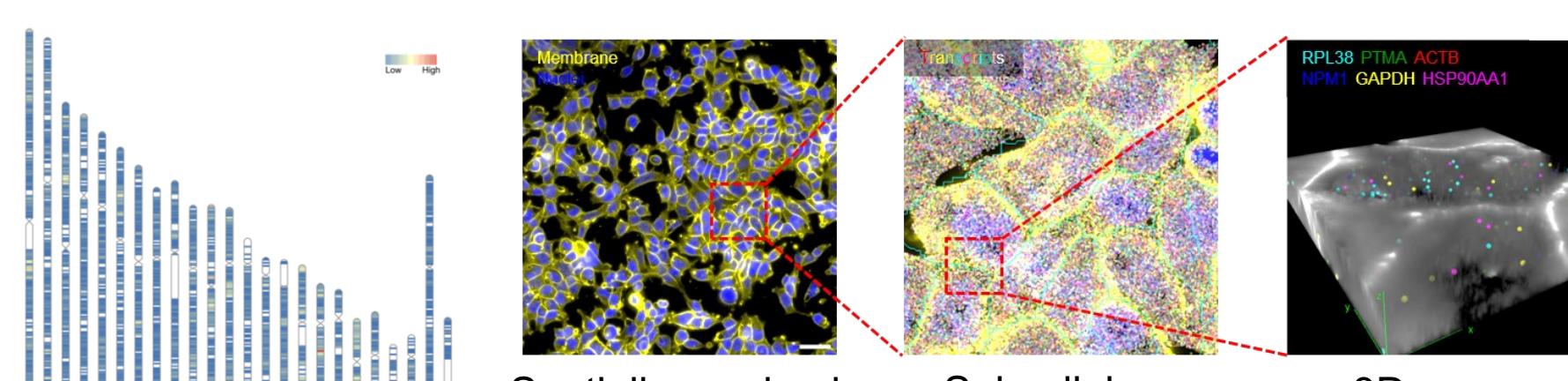
The CosMx imager's versatility and cost-effectiveness make it a cornerstone for single-cell analysis. We demonstrate its utility in high-throughput CRISPR screening, where it accurately identifies introduced CRISPR modules and correlates genetic edits with spatially resolved, transcriptome-wide responses. This paradigm-shifting strategy was validated in both cancer cell lines and spheroid arrays, highlighting its broad applicability.

In summary, the CosMx WTX assay provides a powerful, multi-dimensional platform that bridges fundamental cancer research with translational applications. By revolutionizing our understanding of cancer biology, this technology holds immense potential to accelerate the development of novel diagnostics and therapies, ultimately improving patient outcomes.

## CosMx WTX Assay Design

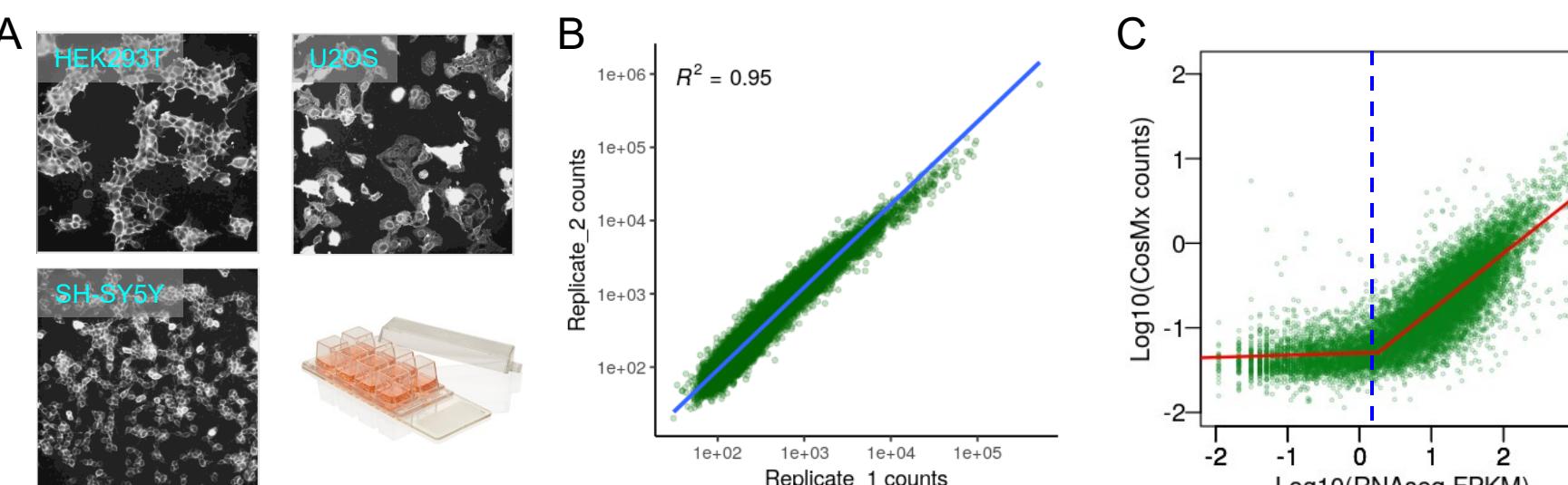


**Fig. 1** Schematic of the CosMx probe design and cyclic hybridization. Target RNAs are first bound to a set of primary ISH probes, followed by hybridization with photocleavable, fluorescent secondary "reporters". The "hybridization-imaging-cleavage-rehybridization" is programmed to barcode ~19,000 gene targets in human transcriptome.

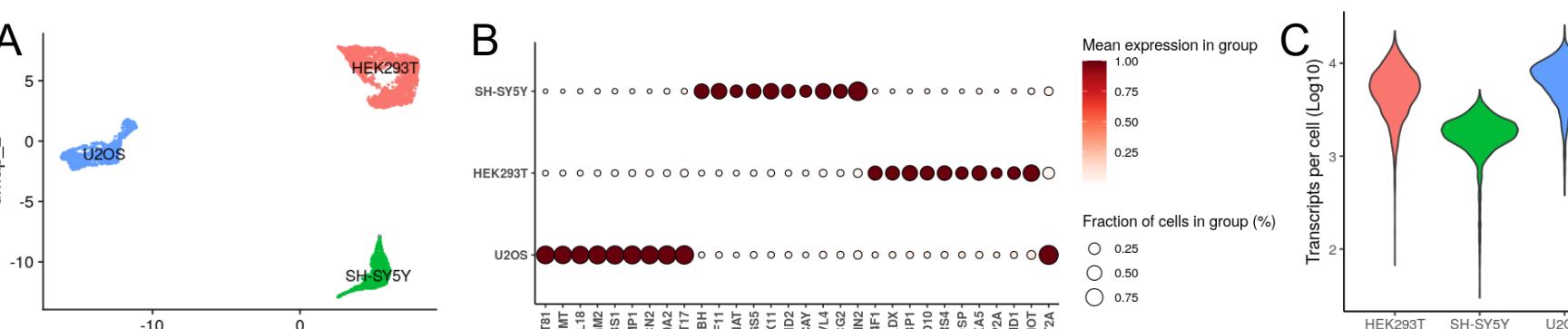


**Fig. 2** The CosMx WTX assay enables full coverage of human protein-coding genes (genomic loci as shown in the T2T karyotype density diagram), and visualization in spatial context with subcellular resolution and 3D localization capability.

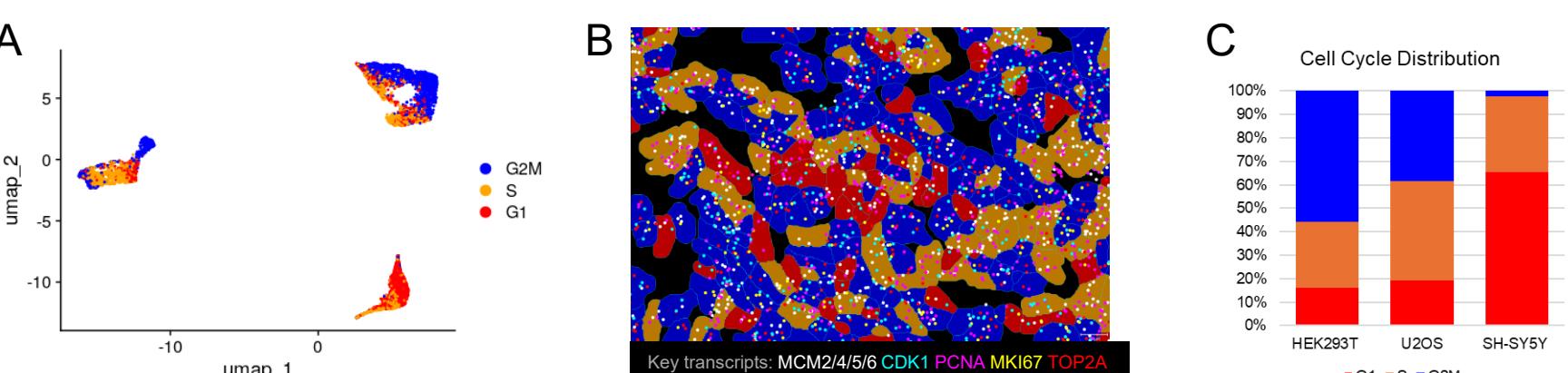
## CosMx WTX Assay Characterization in Cultured Cells



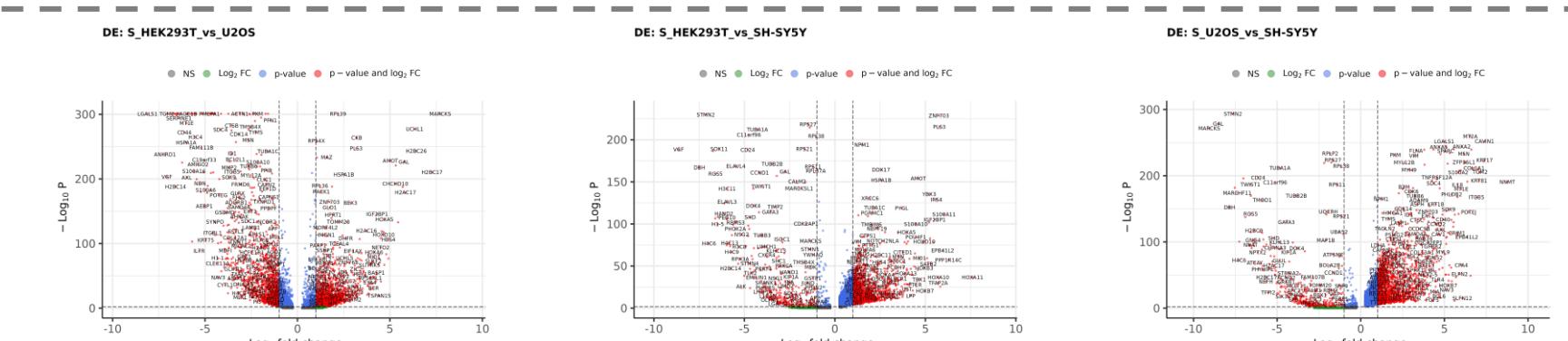
**Fig. 3** (A) CosMx WTX assay can be adapted to diverse culture formats, (B) highly reproducible, and (C) concordant with standard RNA-seq results.



**Fig. 4** The whole-transcriptome coverage enables accurate cell annotation.

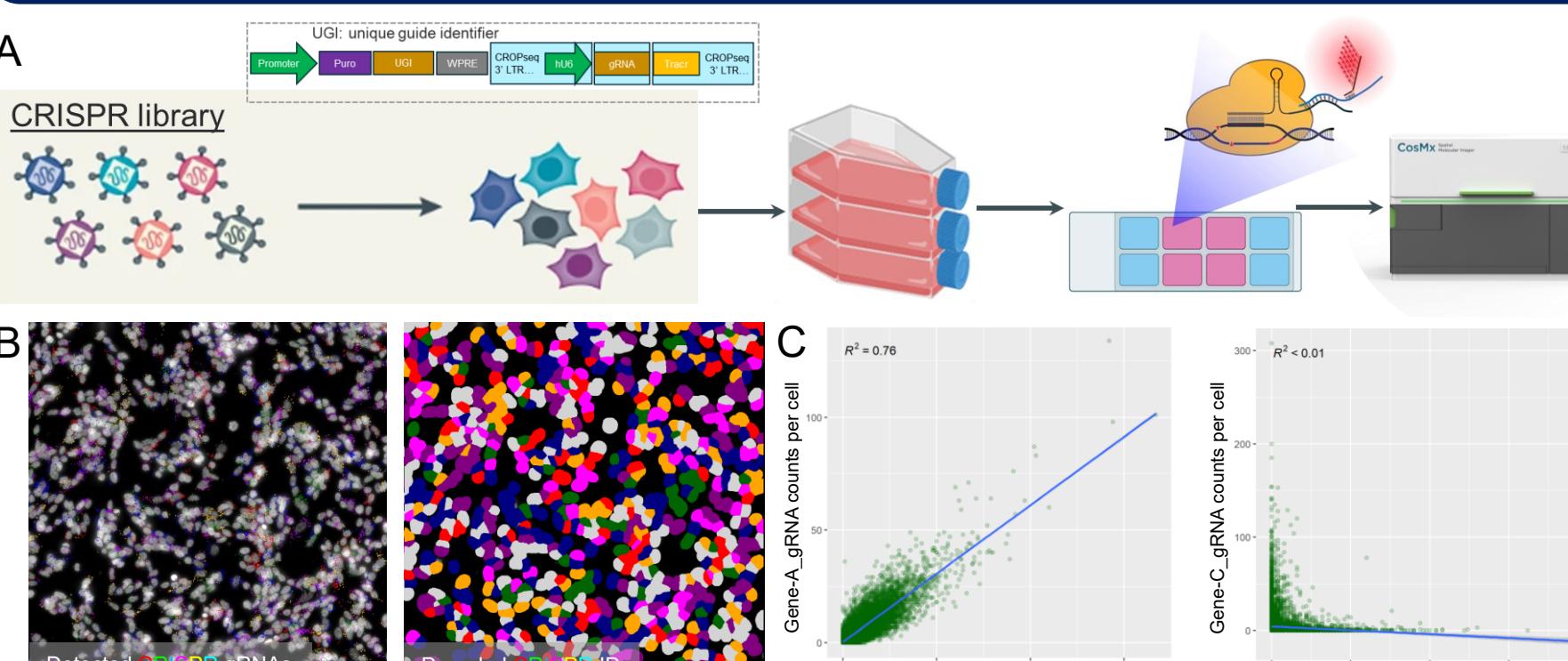


**Fig. 5** Spatial evaluation of cell cycle provides new insights of cancer cell growth.

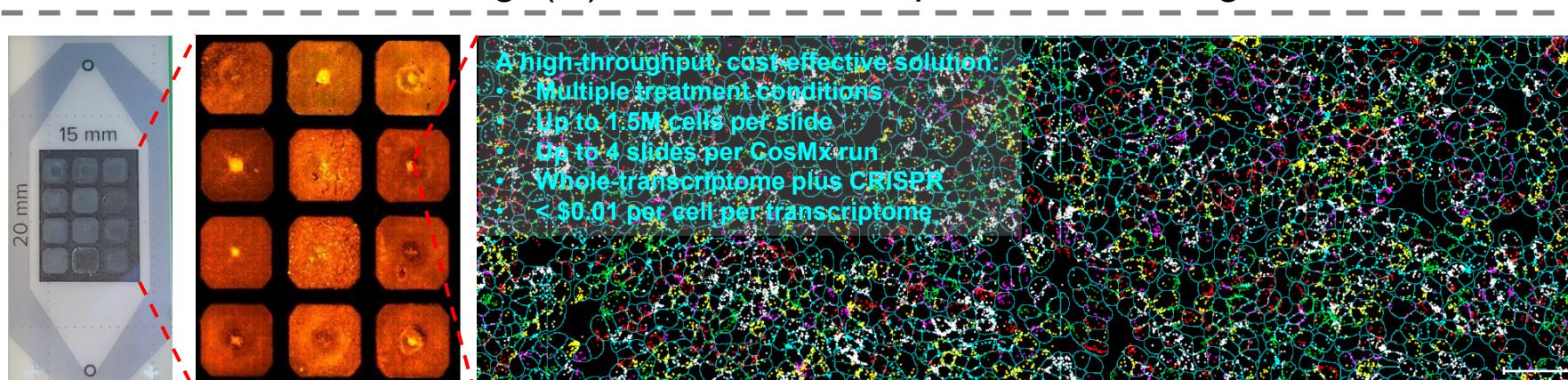


**Fig. 6** DE analysis reveals distinct expression profile for each cell type in S-phase.

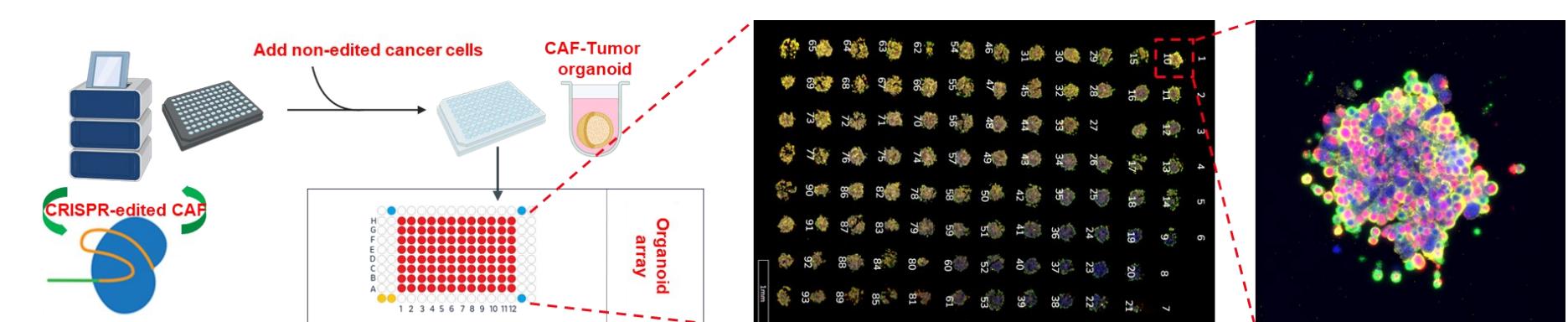
## High-Throughput CRISPR Screening of Cancer Cells



**Fig. 7** (A) The schematic of assay workflow; (B) High sensitivity for gRNA detection and CRISPR ID decoding; (C) Co-detection of paired CRISPR gRNAs and UGIs.



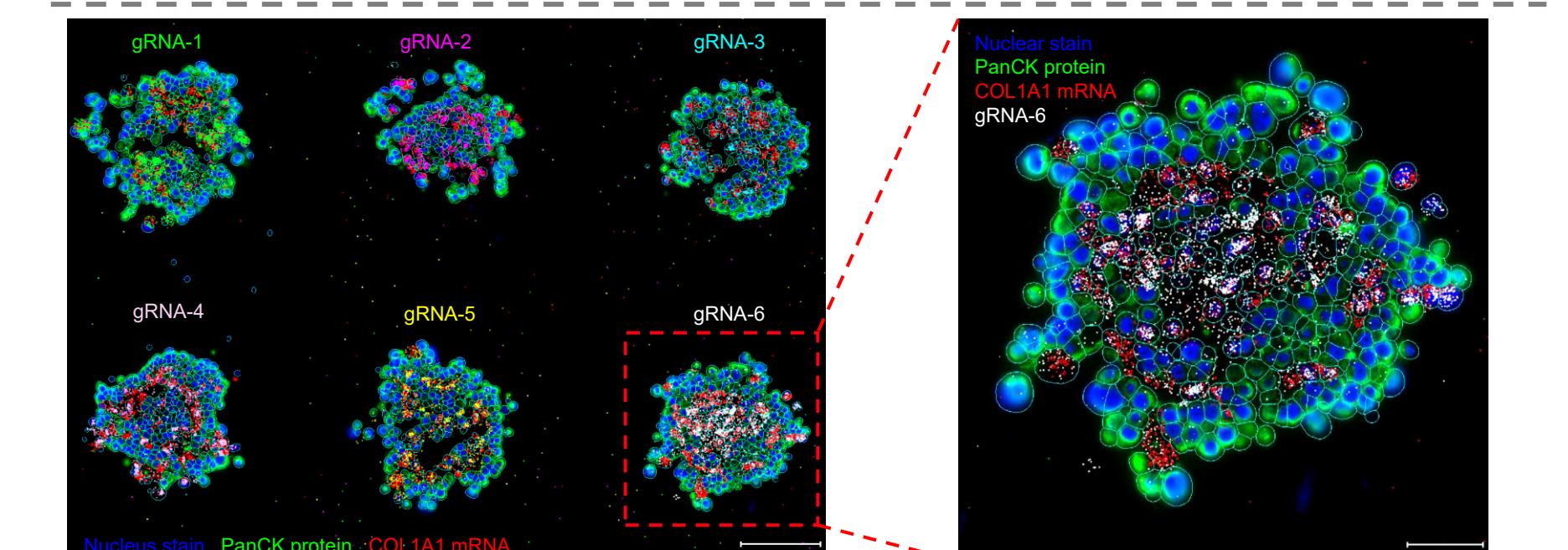
## CosMx WTX for CRISPR Screening of Tumor Spheroids



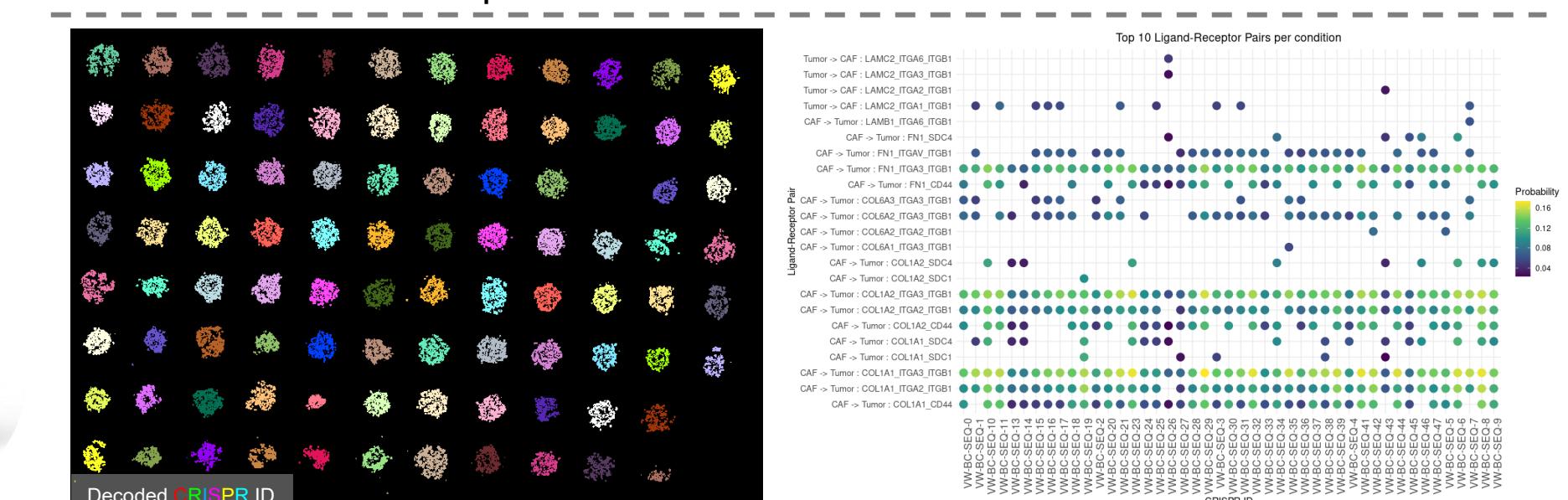
**Fig. 8** The schematic of spatial transcriptome assay with cancer spheroid array

Sample	Serial section-13	Serial section-14
Assay Coverage	WTX + CRISPR	6k-plex + CRISPR
Total Scanning Area (mm <sup>2</sup> )	24.09	24.09
Number of Cells	24,604	23,184
Mean Cell Size (μm <sup>2</sup> )	138.5	139.3
Mean Transcripts Per Cell	2,702	1,779
Median Transcripts Per Cell	2,458	1,567
90-Percentile Transcripts Per Cell	4,845	3,331
Mean Unique Genes Per Cell	1,789	967
Median Unique Genes Per Cell	1,677	926
Mean Transcripts Per um <sup>2</sup>	19.5	12.9
Negprobes Per Plex Per Cell	0.027	0.048
Falsecodons Per Plex Per Cell	0.009	0.016

**Fig. 9** (A) Compared to the 6k-plex panel, the WTX assay generates more data per cell, while maintaining the same detection efficiency for synthetic CRISPR gRNAs.



**Fig. 10** Spatial localization of mRNAs and gRNAs to CRISPR-edited CAF cells in individual tumor-CAF spheroid.



**Fig. 11** Parallel evaluation of multiple spheroids on the same slide enables spatially informed, unbiased cell-cell interactions analysis, such as LR analysis.

## Conclusion

The CosMx WTX assay revolutionizes spatial single-cell analysis with unmatched transcriptomic coverage and performances, enabling high-throughput spatial CRISPR screening in various cancer models. With tens of times lower cost per cell than traditional methods, this imager empowers the biopharma industry to accelerate drug discovery and development with unprecedented efficiency. Its multimomics potential positions CosMx WTX assay as a game-changer, unlocking integrated genomic, transcriptomic, proteomic and spatial insights to drive the next generation of precision therapeutics and improve patient outcomes.

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