A novel cell biology platform maps dynamic cell-cell interactions to CRISPR perturbations at high throughput

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INTRODUCTION

Unbiased, systems biology approaches to studying the immune system have been critical for unveiling immunological insights on health and disease. Large datasets on cell types, their function, secretion products, and associated genes can be aggregated and insights around their relationships extrapolated. However, a few challenges remain. First, how cells interact with other cells remains difficult to study given the absence of technologies that measure interactions between single-cells at high-throughput. Second, high-throughput technologies largely capture static snapshots. Given the immune system is a dynamic entity, it is possible that critical nuances in cell-cell interactions, including the strength or kinetics of T-cell priming by dendritic cells, remain underexplored.

To overcome these limitations, we leverage a novel cell biology platform that links genetic perturbations to cell function at the resolution of single cell-cell interactions with longitudinal context. Tens of thousands of individual edited cells are paired with cells of interest using light-guided polymerization of bio-compatible hydrogel compartments, enabling cell imaging over days to weeks. Single cell sequencing data can be mapped to functions detected by imaging (e.g., morphology, secreted and surface proteins), because cells can be lysed to generate barcoded libraries within compartments for mRNA and sgRNA sequencing. We utilized this platform for cancer immunotherapy by developing a single-cell T cell priming assay to study antigen presentation by CRISPR-edited DCs. Individual DCs from a pool of edited cells are compartmentalized with one or more T cells, and T cell responses measured by imaging activation markers and proliferation. We aim to map genomic edits with enhanced T cell priming to specific DC genes, which would pave the way for therapies aiming to enhance DC function in tumor microenvironments and improve polyclonal tumor-specific T cell priming and T cell-mediated tumor killing.

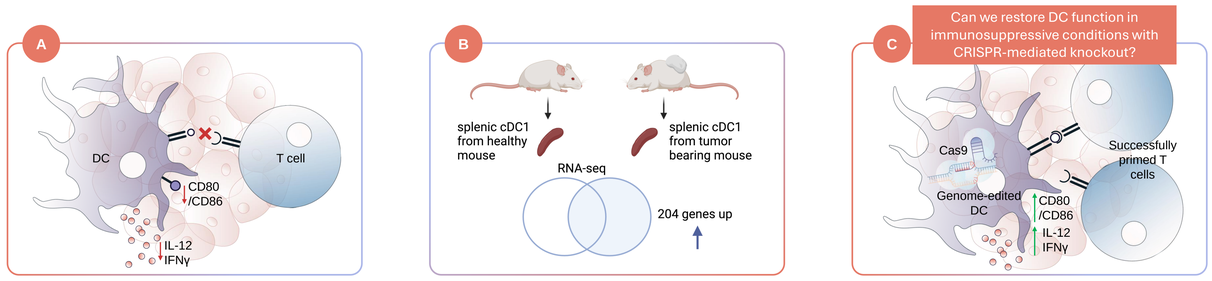


FIGURE 1: Dendritic cell functions are impaired during tumorigenesis

(A) Dendritic cells (DCs) are subjected to immunosuppressive factors released by tumor and tumor-associated cells which result in decreased ability of DCs to prime T cells and contribute to tumor escape. **(B)** *Ex vivo* RNA-seq revealed 204 genes upregulated in splenic cDC1 from tumor-bearing mice on d30 after LLC cells injection compared to healthy controls. **(C)** Our goal for this project is to conduct a CRISPR screen perturbing these differentially expressed genes to identify candidates that can rescue the DC function and potentially enhance T cell priming under immunosuppressive conditions.

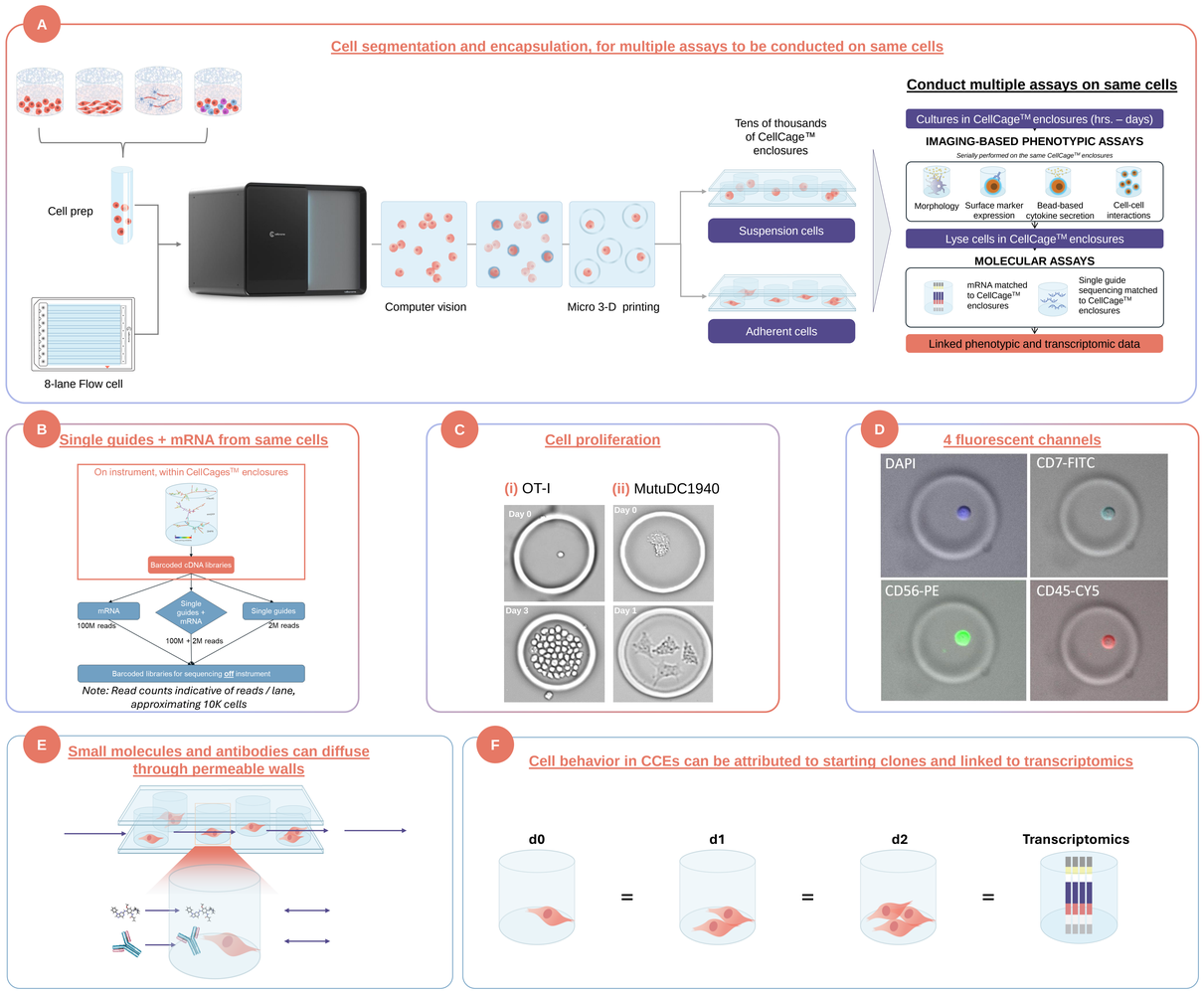


FIGURE 2: Novel micro-3D printing technology enables high-throughput evaluation of cell-extrinsic phenotypes

(A) Schematic of workflow for novel Cellanome technology enabling the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures. Tens of thousands of suspension or adherent cells are mixed with hydrogel precursor and loaded on an 8-lane flow cell. Positions of cells are identified and CellCage™ enclosures are generated around cells with light-guided polymerization in an automated fashion. Bio-compatible CellCage™ enclosures can be formed around single cells, multiple cells, or cells with objects (e.g., cytokine beads). CellCage™ enclosures are permeable to reagents enabling long-term culturing and a variety of imaging-based, longitudinal phenotypic and functional assays to be performed on the same cells (e.g., small molecules, immunofluorescent antibodies). Cells can be lysed within CellCage™ enclosures to generate cDNA for downstream library prep and sequencing off the instrument. **(B)** Cellanome's molecular assay workflow enables flexible generation of robust sequencing data from mRNA, sgRNAs, or both by conducting separate preps in parallel. cDNA is processed on the flow cell while mRNA and / or sgRNA library prep are performed off the platform. **(C) i)** Brightfield imaging of activated primary mouse OT-I T cells (suspension) or **ii)** MutuDC1940 cells (adherent) on d3 or d1, respectively, of culture in CellCage™ enclosures. **(D)** Fluorescent imaging of NK92 cells in CellCage™ enclosures following staining with α-CD7-FITC, α-CD56-PE, α-CD45-CY5 and DAPI. Same individual cells were imaged across 4 channels with Cellanome's technology. **(E)** Reagents can be diffused through semi-permeable CCE walls. This feature can be used to deliver nutrients, small molecules, and antibodies to all CCEs at once, at any point of the experiment. **(F)** Cells within CCEs are serially imaged for longitudinal, multi-functional analysis, before being processed within the CCEs for transcriptomic analysis. This enables the generation of highly parallel, integrated single-CCE datasets.

FIGURE 3: Establishing T-cell priming assay with single dendritic cells using Cellanome technology

(A) Schematic overview of single-DC:OT-I T-cell priming assay with Cellanome technology. MutuDC1940 cells are loaded onto the flow cell and pulsed with OVA (SIINFEKL) peptide. Primary mouse OT-I cells mixed with hydrogel precursor are loaded onto the flow cell, followed by encapsulation of single DCs with OT-I cells in CellCage™ enclosures. Time-lapse brightfield and fluorescent images are collected to evaluate T-cell proliferation (cell counts with brightfield) and activation levels measured via fluorescent antibody detection of CD69 / CD71 expression. **(B)** Imaging of single MutuDC1940 cells with OT-I cells in CellCage™ enclosures at different timepoints. **(C) i), ii)** Quantification of CD69 expression in >7000 CellCage™ enclosures across a flow cell. Each dot represents a CellCage™ enclosure and the average CD69 signal by all cells in that CellCage™ enclosure. The intensity of CD69 and percentage of CellCage™ enclosures positive for CD69 increases from 0 to 24 hours, indicating successful OT-I priming by individual DCs across the flow cell. **(D)** Distribution of number of CellCage™ enclosures by number of OT-I cells compartmentalized, enabling analysis of T-cell priming by DCs to be normalized to the starting number of OT-I cells at d0.

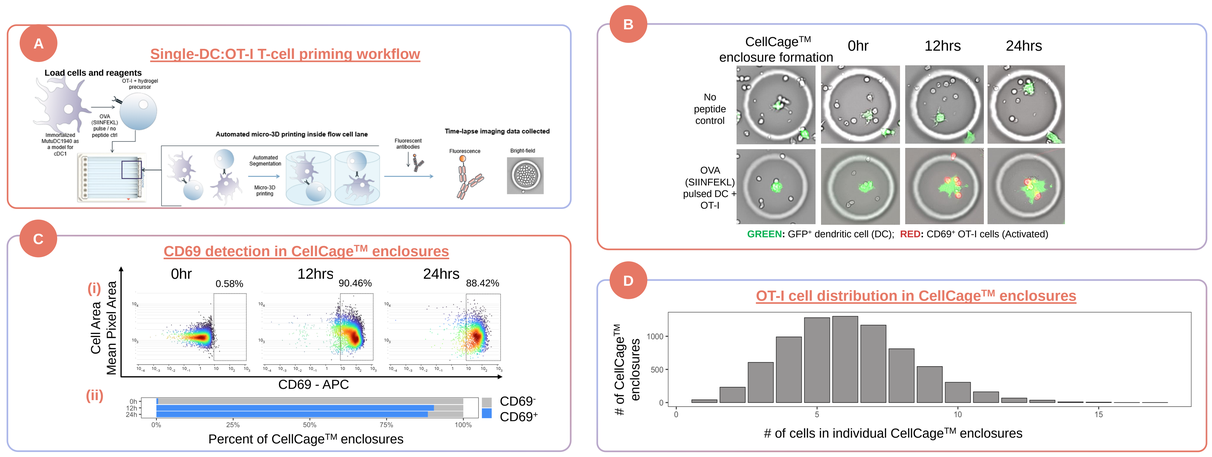
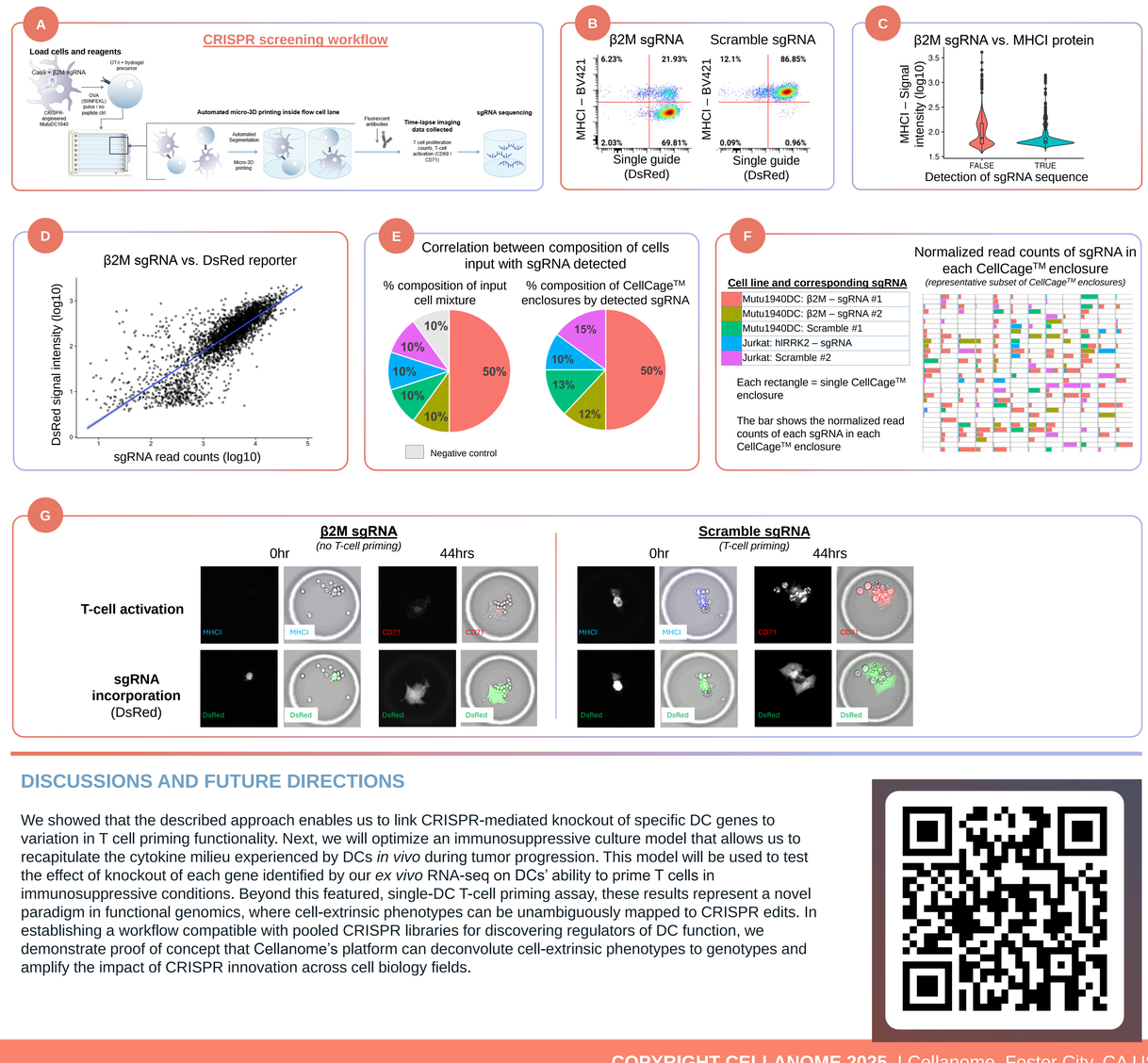


FIGURE 4: CRISPR screening workflow detects sgRNA from single-DC:T cell priming assay

(A) Schematic showing how a single-DC:OT-I culturing workflow can be modified for compatibility with sgRNA detection. Cas9-MutuDC1940 cells edited with β2M targeting sgRNA are loaded onto the flow cell as described in Figure 3. DC and OT-I cells are both lysed in CellCage™ enclosures and barcoded cDNA generated to prepare libraries and sequence sgRNA off the instrument. **(B)** Knockout efficiency for β2M sgRNA in Cas9-Mutu1940DC cells was measured via MHCI expression by flow cytometry. **(C)** Testing β2M sgRNA knockout efficiency in single cells on Cellanome platform by comparing MHCI surface protein levels via fluorescent imaging with sgRNA detection. **(D)** sgRNA expression was correlated with intensity of dsRed reporter included in lentiviral construct. **(E)** 5 cell lines edited with different sgRNAs were mixed at defined percentages and loaded onto the same lane in a flow cell, their sgRNA sequenced, and the distribution of read counts in each cell type analyzed across CellCage™ enclosures. Composition of detected sgRNA as a % of ~4K CellCage™ enclosures was compared to composition of input cell mixture. **(F)** Normalized read counts of sgRNA were plotted for individual CellCage™ enclosures, with each rectangle representing a single CellCage™ enclosure. Bar length indicates the read count for respective sgRNA in the specific CellCage™ enclosure. **(G)** CRISPR-edited Cas9-MutuDC1940 cells were pulsed with OVA peptide, stained with α-MHCI, and encapsulated with OT-I cells in individual CellCage™ enclosures, which were then labeled with α-CD71 to monitor OT-I cells' activation. β2M KO Cas9-Mutu1940DC cells were negative for MHCI staining, and did not exhibit ability to activate OT-I cells, whereas Cas9-MutuDC1940 cells edited with scramble sgRNA were positive for MHCI protein expression and successfully activated OT-I cells.



DISCUSSIONS AND FUTURE DIRECTIONS

We showed that the described approach enables us to link CRISPR-mediated knockout of specific DC genes to variation in T cell priming functionality. Next, we will optimize an immunosuppressive culture model that allows us to recapitulate the cytokine milieu experienced by DCs *in vivo* during tumor progression. This model will be used to test the effect of knockout of each gene identified by our *ex vivo* RNA-seq on DCs' ability to prime T cells in immunosuppressive conditions. Beyond this featured, single-DC T-cell priming assay, these results represent a novel paradigm in functional genomics, where cell-extrinsic phenotypes can be unambiguously mapped to CRISPR edits. In establishing a workflow compatible with pooled CRISPR libraries for discovering regulators of DC function, we demonstrate proof of concept that Cellanome's platform can deconvolute cell-extrinsic phenotypes to genotypes and amplify the impact of CRISPR innovation across cell biology fields.