Linking CRISPR Perturbations to Transcriptomic and Live-Cell Phenotypes at Single-Cell Resolution in Primary Immune Cells

Ana Margarida Meireles[1], Eloise Pariset[2], Raúl A. Reyes Hueros[1], Yiqi Zhou[2], Aparna Natarajan[2], Niranjan Srinivas[2], Shan Sabri[2], Pier Federico Gherardini[2], Gary P. Schroth[2], Katie Geiger-Schuller[1]

[1]Genentech, South San Francisco, CA, USA; [2]Cellanome, Foster City, CA, USA

ABSTRACT

Current CRISPR screens typically require tradeoffs between scale and complexity of cellular phenotypes. High-scale approaches are optimized for narrow phenotypic readouts, making it challenging to **integrate functional, morphological, and genomic data at the resolution of individual cells**, whereas plate-based approaches that integrate multiple readouts are low-scale. These limitations hamper comprehensive insights into the effects of genetic perturbations - especially in complex, heterogeneous cell systems.

We address this gap with a novel high-throughput platform that enables **longitudinal, multi-modal profiling of single cells by linking CRISPR perturbations to cellular function, morphology, and transcriptomics in a single, integrated assay**. Using this system, we screened perturbations in immune signaling pathways in murine **bone marrow-derived macrophages (BMDMs)** under inflammatory stimulation. Specifically, BMDMs were CRISPR-edited to target **153 genes including 13 in the NF-κB signaling pathway** using four sgRNAs per gene, alongside 40 non-expressed gene controls and 60 non-targeting controls, enabling robust statistical analysis of perturbation effects.

Central to this approach is the use of **CellCage™ Enclosures (CCEs)**—light-guided, biocompatible hydrogel compartments that dynamically enclose tens of thousands of live single cells or co-cultures per run. CCEs support concurrent execution of multiple phenotypic assays—including longitudinal, high-content imaging of morphology and surface markers, followed by transcriptomic and single guide RNA capture—on the same individual cells. This unified workflow generates rich, multi-dimensional datasets comprising matched imaging and sequencing data at scale.

Following 12 hours of lipopolysaccharide (LPS) stimulation, BMDMs within CCEs were stained and imaged to quantify expression of **inflammatory surface marker CD40 and PD-L1**, along with **cellular morphology**. The same cells were then lysed to simultaneously recover polyadenylated **sgRNAs and single-cell mRNA**. After separate sequencing, the guide and cDNA libraries were integrated with matched phenotypic data at the CCE level, enabling precise mapping of each perturbation to its corresponding transcriptomic and functional signatures.

To interpret these **large, high-dimensional datasets**, the platform provides an integrated computational pipeline that leverages advanced tools from deep learning, computer vision, data compression, and genomics. This enables robust association of CRISPR edits with diverse and complex phenotypes, including cell-extrinsic effects, across tens of thousands of single cells.

This platform enables us to extend the phenotypic scope of our BMDM CRISPR screen, allowing for high-resolution, multiplexed analysis of primary immune cells. It accelerates both discovery and validation of genotype-to-phenotype relationships in immunogenomics by enabling comprehensive **linkage of perturbations to specific cells and their associated mRNA profiles, morphology, and surface marker expression**.

Introduction to Cellanome Technology

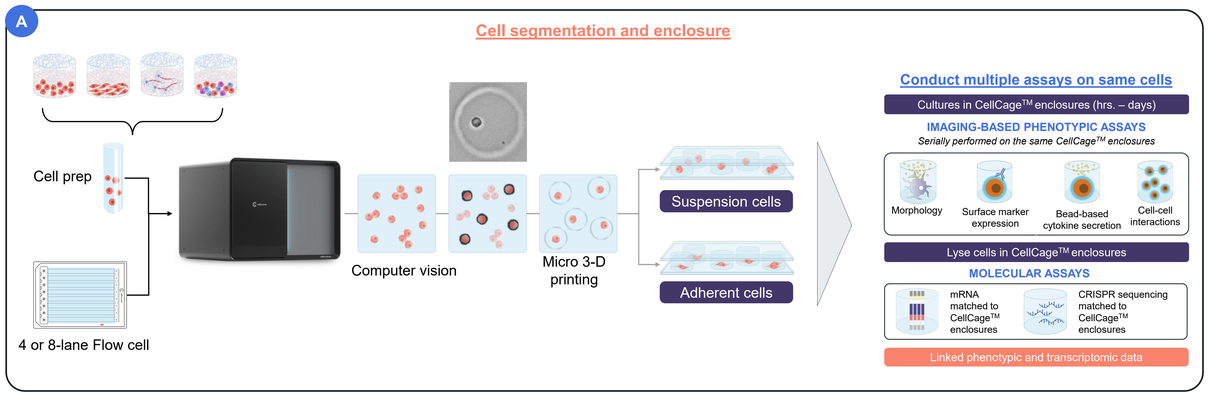
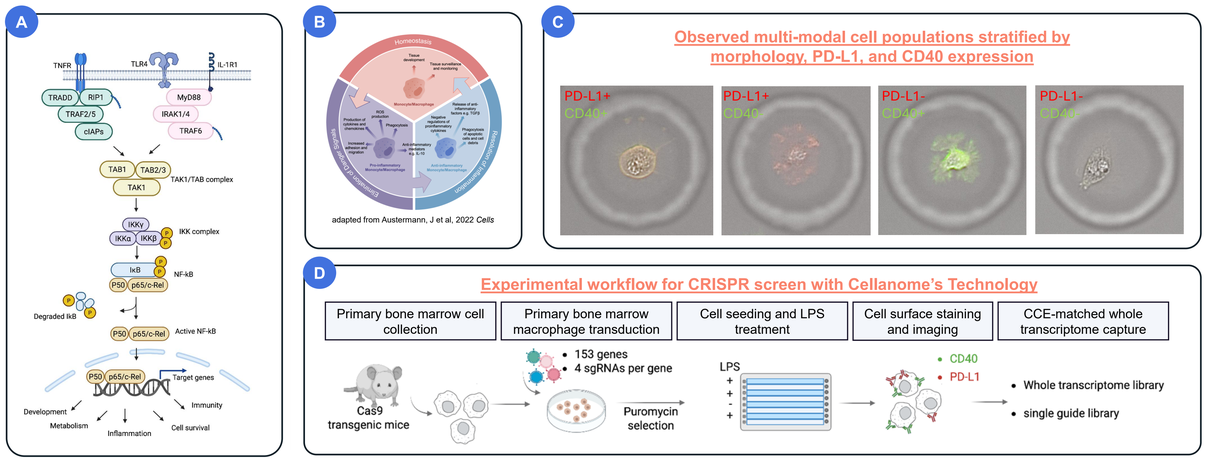
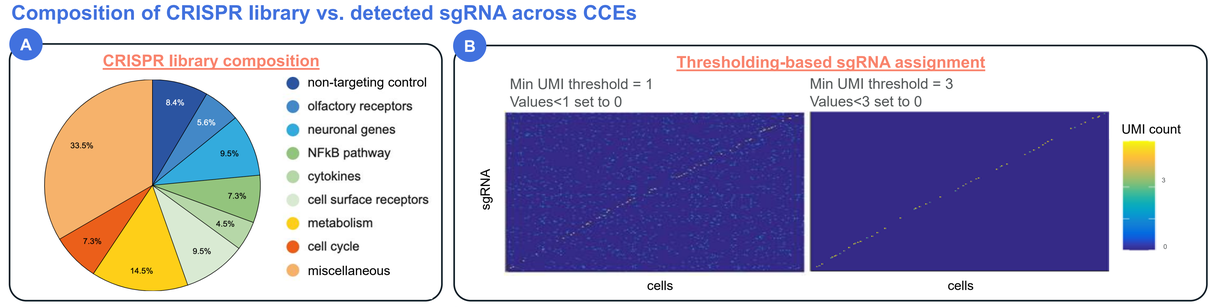


Fig 1. Novel micro-3D printing technology enables high-throughput evaluation of cell-extrinsic phenotypes
(A) Schematic of workflow for novel Cellanome technology enabling the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures (CCEs). Tens of thousands of suspension or adherent cells are mixed with hydrogel precursor and loaded on a 4 or 8-lane flow cell. Positions of cells are identified and CCEs are generated around cells with light-guided polymerization in an automated fashion. Bio-compatible CCEs can be formed around single cells, multiple cells, or cells with objects (e.g., cytokine capture beads). CCEs 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 CCEs 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)** Representative images cropped to CCE dimensions of primary bone marrow-derived macrophages (BMDM, adherent) on D0 and D1, respectively. **(D)** Fluorescent imaging of BMDM cells in CCEs following staining with CD40-PE, Annexin-FITC, PD-L1-APC, and NK92 cells stained with DAPI. **(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, single-CCE datasets that integrate morphology, surface markers, and live cell data to both transcriptomics and specific CRISPR perturbations.

Establishing a novel CRISPR screen assaying morphology, protein, and transcriptomics



Composition of CRISPR library vs. detected sgRNA across CCEs



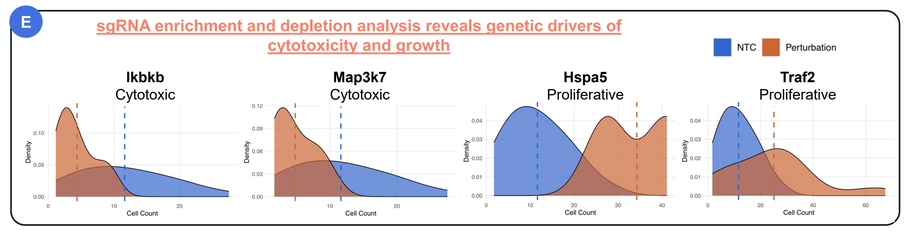


Fig 3. Composition of sgRNA detected with Cellanome platform closely aligns with library input and expected cellular phenotypes (A) We used a CRISPR sgRNA library, targeting 163 genes with 4 sgRNAs per gene, including 10 nonessential control genes (olfactory receptor genes) along with 60 non-targeting control (NTC) sgRNAs. The library was composed of components of the canonical NF-κB pathway (13 genes), along with various other metabolic and signal transduction genes. **(B)** sgRNAs were assigned to cells based on UMI thresholding. Whereas a UMI threshold of 1 shows many cells as being multiple infected, a UMI threshold 3 assigns only one guide per cell. **(C)** All sgRNAs presented in viral library (top) were detected in BMDMs at end point (bottom). **(D)** sgRNAs detected with the Cellanome technology were highly correlated with input library. **(E)** sgRNAs distributions were normalized and compared to non-targeting control helping uncover perturbations that increase or decrease sgRNA representation.

Effect of CRISPR perturbation on gene expression

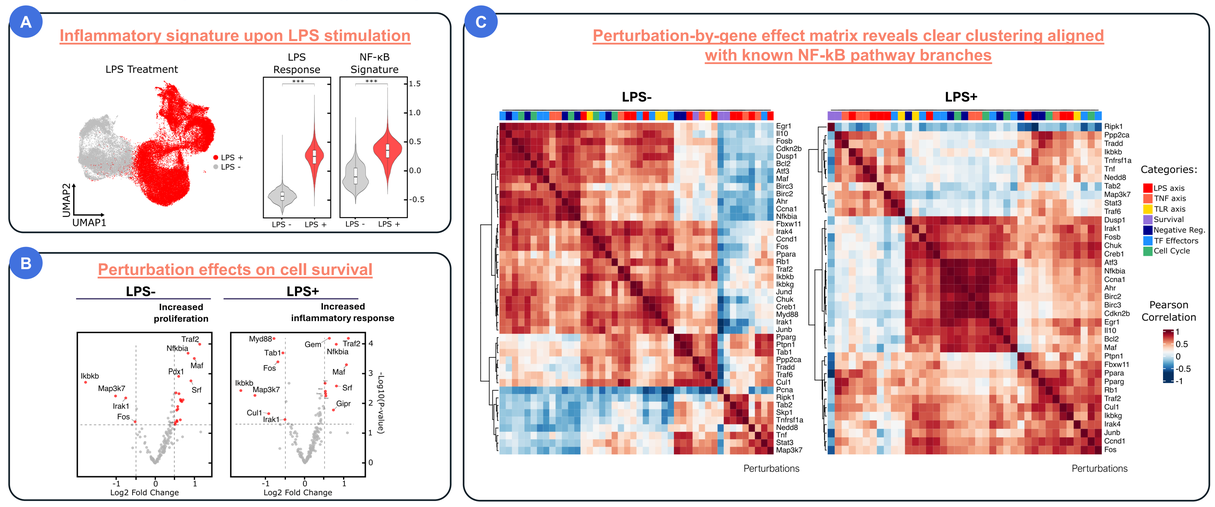


Fig 4. Multi-modal CRISPR screen in BMDMs reveals branch-aligned transcriptomic clustering (A) UMAP embedding corrected by Harmony integration, highlighting mock-treated (LPS-, grey) and stimulated (LPS+, red) conditions. Violin plots illustrating the distributions of LPS response and NF-κB signature scores derived from non-targeting controls (NTC). Highly significant differences between LPS– and LPS+ conditions are indicated by significance (FDR < 0.05, log2FC > 0.5). **(B)** Volcano plots showing results from the viability assays comparing gene perturbations to NTC, normalized by baseline sgRNA distributions in lentivirus; left and right plots represent LPS- and LPS+ conditions, respectively. Statistical significance was determined using a two-sided Wilcoxon rank sum test, with p-values adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) correction method (FDR < 0.05, log2FC > 0.5). **(C)** Heatmap illustrating Pearson correlations between gene perturbations in LPS– and LPS+ conditions, based on PCA of the collapsed beta-matrix. Hierarchical clustering annotations of sgRNA categories are indicated at the top of the heatmap.

DISCUSSION

Cellanome's cutting-edge platform, featuring CellCage™ enclosures, enables robust detection of matched sgRNA, whole transcriptomes, cell surface protein expression, and cellular morphology from the same cells. This multi-modal integration provides a powerful approach to dissect complex signaling networks.

Key Findings:

LPS-Induced Protein Expression: LPS stimulation in bone marrow-derived macrophages (BMDMs) increased the expression of PD-L1 and CD40 compared to unstimulated cells. Ongoing studies aim to define specific stimulation effects on PD-L1 and CD40 levels.

sgRNA Enrichment Analysis: We identified genetic perturbations that negatively affect cell viability:

- In both LPS-stimulated and unstimulated cells: Ikbkb, Map3k7, Irak1 (core NF-κB pathway components).
- In LPS-stimulated cells only: MyD88, Tab1 (upstream signaling adaptors in the NF-κB pathway).
- Identified that perturbations promoting cellular proliferation were more frequently observed in unstimulated cells, suggesting that inflammatory stress suppresses growth-promoting genetic disruptions.

Pathway Mapping: A perturbation-by-gene effect matrix revealed clustering consistent with known NF-κB pathway branches. Core NF-κB components, negative regulators, and downstream effectors formed distinct functional clusters.

Our CRISPR screen in BMDMs demonstrates the ability of the Cellanome platform to reconstruct NF-κB pathway topology and identify inflammation-specific essential genes. This technology's integration of transcriptomic, proteomic, and morphological data offers a comprehensive framework to unravel complex cellular signaling networks, advancing our understanding of inflammation and immune responses.

Interested in collaborating? Please reach out!