Linking CRISPR Perturbations to Live-Cell Behavior, Morphology, and Paired Transcriptomics in Single Cells

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ABSTRACT

Pooled CRISPR screens typically force a tradeoff between scale and phenotypic depth. High-throughput approaches rely on a single proxy readout, usually the transcriptome, while plate-based assays that combine multiple readouts are low-scale. When the proxy and the biology of interest diverge, important perturbations get deprioritized, and some hits move forward without functional evidence. Many perturbations change morphology, protein regulation, or cell-cell behavior without producing a proportional transcriptomic signature.

Cellanome PERTURB-LINK (described in Orcutt-Jahns, Reyes Hueros, Meireles et al., bioRxiv 2026) technology **links guide identity to live-cell function, morphology, surface protein expression, and the single-cell transcriptome of the same cell**. At its core are permeable **CellCage Enclosures (CCEs)** that encapsulate single cells or co-cultures, maintain them through longitudinal imaging over days to weeks, and end with in-situ lysis that **co-captures single guide RNA and mRNA**. Single guide and cDNA libraries are sequenced separately and reintegrated with the matched phenotypic data at the CCE (same-cell) level.

PERTURB-LINK was applied to murine bone marrow-derived macrophages (BMDMs) under LPS stimulation, targeting **153 NF-κB regulators with 4 sgRNAs per gene**. After 12 h of LPS, cells in CCEs were imaged for **CD40 and PD-L1 staining and brightfield morphology**, then lysed for paired transcriptome and guide capture. Of ~100k starting cells, PERTURB-LINK yielded ~60 cells per guide and ~250 cells per targeted gene. Guides targeting expressed genes significantly downregulated their intended target compared to non-targeting controls, with 56 of 153 targeted genes (~36%) showing significant on-target knockdown, consistent with prior Perturb-seq datasets.

The screen recovered canonical NF-κB biology and resolved perturbations into distinct layers of a multimodal hit list. Some perturbations changed all measured modalities, such as Myd88, a core NF-κB regulator. Others acted primarily on cell morphology, such as Arc, or primarily on surface protein expression, such as Ppp1r15a, with little matched transcriptomic signature. A transcriptome-only screen would have captured only part of this biology. By **linking guide identity to function, morphology, surface protein, and transcriptome in the same single cell**, the Cellanome platform refines transcriptional hit lists by adding functional support and expands them by detecting RNA-silent phenotypic hits. It is well-suited for screens in primary immune cells, co-culture systems, and adherent cells where guide, behavior, morphology, and transcriptome must stay linked at the single-cell level.

Introduction to Cellanome Technology

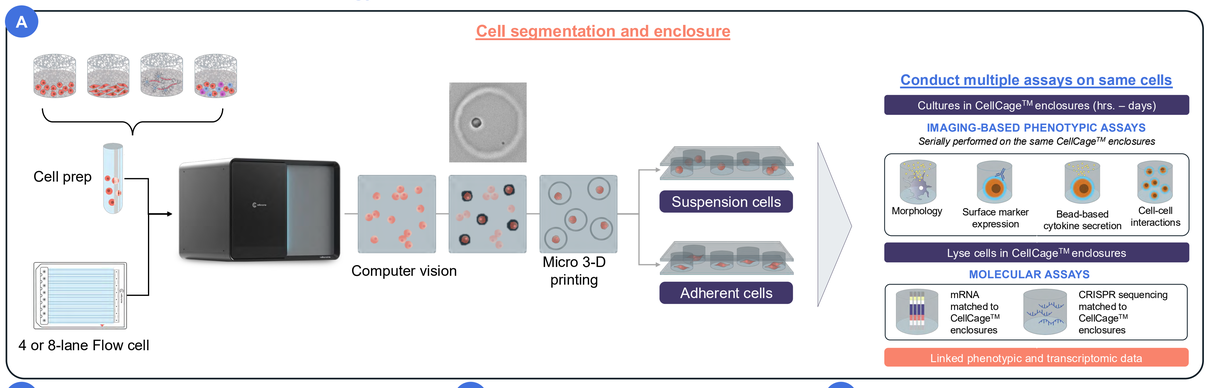


Fig 1. Novel micro-3D printing technology enables high-throughput evaluation of cell-extrinsic phenotypes
(A) Schematic of workflow for Cellanome technology enabling the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures (CCEs). Tens of thousands of suspension or adherent cells are mixed with hydrogel precursor and loaded on an 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 Jurkat cells on D0, D1, D2, and D3, 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.

Validating single-cell guide capture and assignment for pooled CRISPR screens on the Cellanome platform

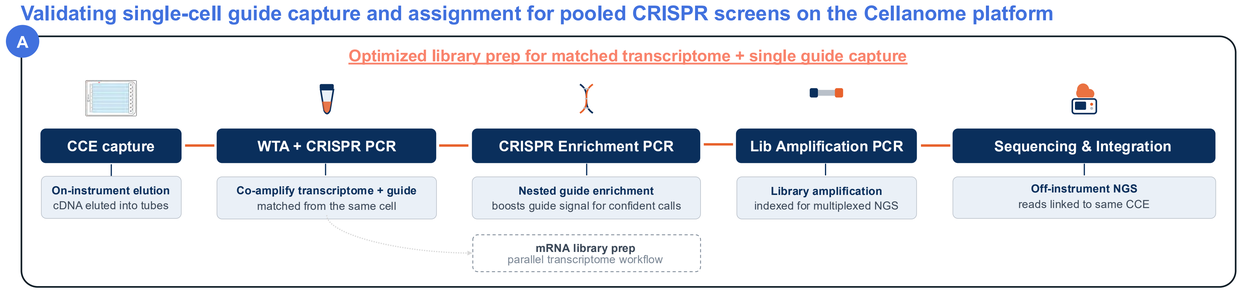
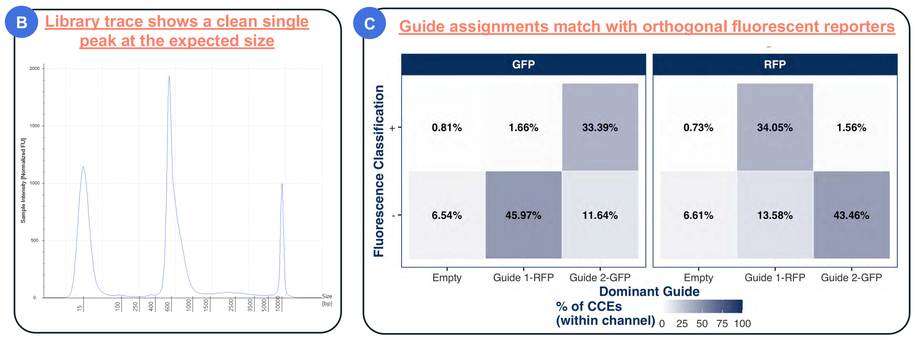
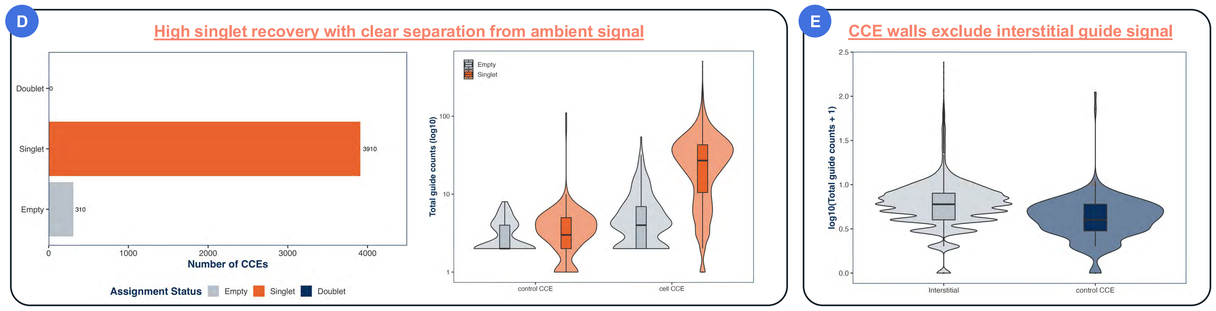


Fig 2. Single-cell guide capture and assignment are accurate and enclosure-specific on the Cellanome platform

(A) Matched transcriptome + guide library-prep workflow. cDNA is eluted on-instrument from CCEs, then split into parallel preps: single guides are co-amplified with the whole transcriptome from the same cell, selectively enriched to enable sensitive guide detection, and indexed for multiplexed sequencing, while matched mRNA libraries are built in parallel. Both are sequenced off-instrument by Next Generation Sequencing (NGS), and reads are linked back to CCEs.



(B) Guide-library size distribution. Agilent D5000 TapeStation electropherogram showing a single library peak at the expected size. **(C)** Guide calls validated against orthogonal fluorescence. Per-CCE fluorescence classification (+/−) versus dominant guide for the GFP and RFP channels (thresholds: GFP log10 > 1, RFP log10 > 0.5; values are % of all CCEs within each channel). RFP-marked Guide 1 is predominantly RFP+ (34.1%) and GFP-marked Guide 2 predominantly GFP+ (33.4%), while mismatched fluorescence is rare (≤1.7%).



(D) Guide assignment across CCEs. Left: assignment status - 3,910 CCEs (93%) received a single guide (Singlet), 310 (7%) were Empty, and 0 were Doublet. Right: single guide counts (log10) for Empty vs Singlet CCEs, split by control CCE and cell CCE. **(E)** Guide counts (log10(x+1)) in interstitial space (n = 18,080) vs control CCEs (n = 255); control CCEs carry lower ambient signal, indicating enclosure walls exclude interstitial guide.

Linking morphology, surface protein expression, transcriptomics, and guide identity

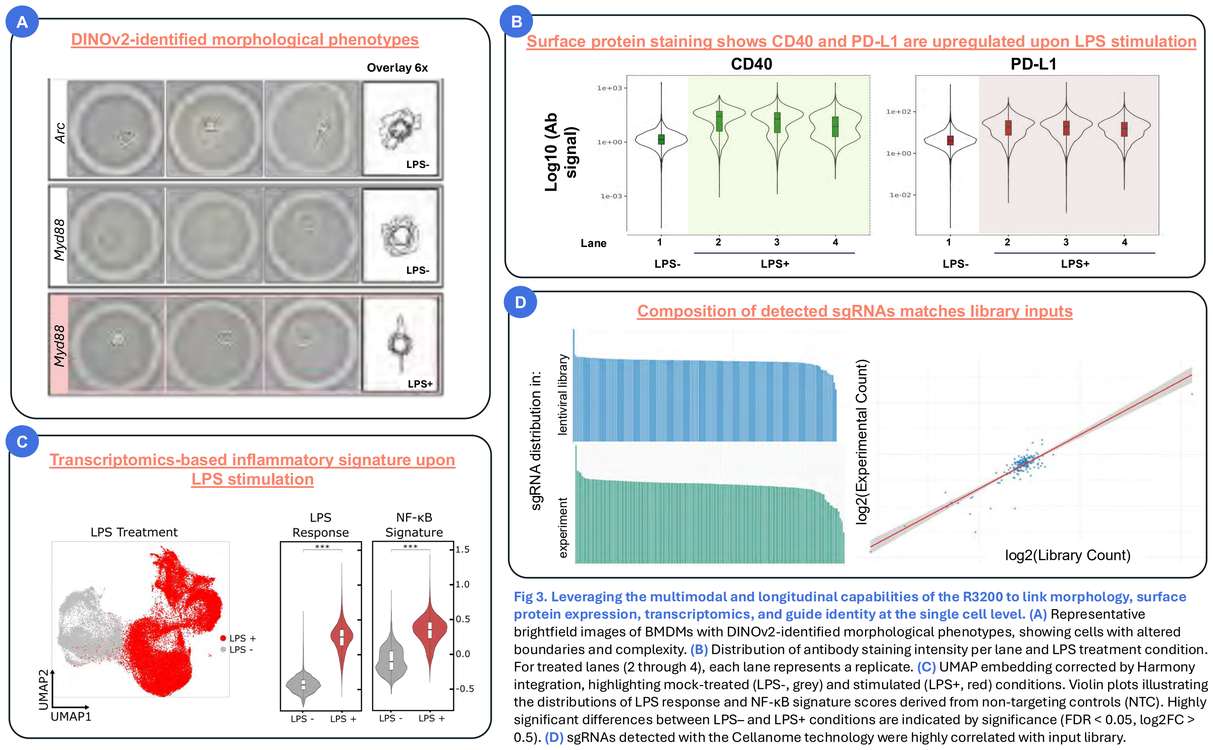


Fig 3. Leveraging the multimodal and longitudinal capabilities of the R3200 to link morphology, surface protein expression, transcriptomics, and guide identity at the single cell level. **(A)** Representative brightfield images of BMDMs with DINOv2-identified morphological phenotypes, showing cells with altered boundaries and complexity. **(B)** Distribution of antibody staining intensity per lane and LPS treatment condition. For treated lanes (2 through 4), each lane represents a replicate. **(C)** 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). **(D)** sgRNAs detected with the Cellanome technology were highly correlated with input library.

Multimodal profiling reveals NF-kB regulators invisible to transcriptomics alone

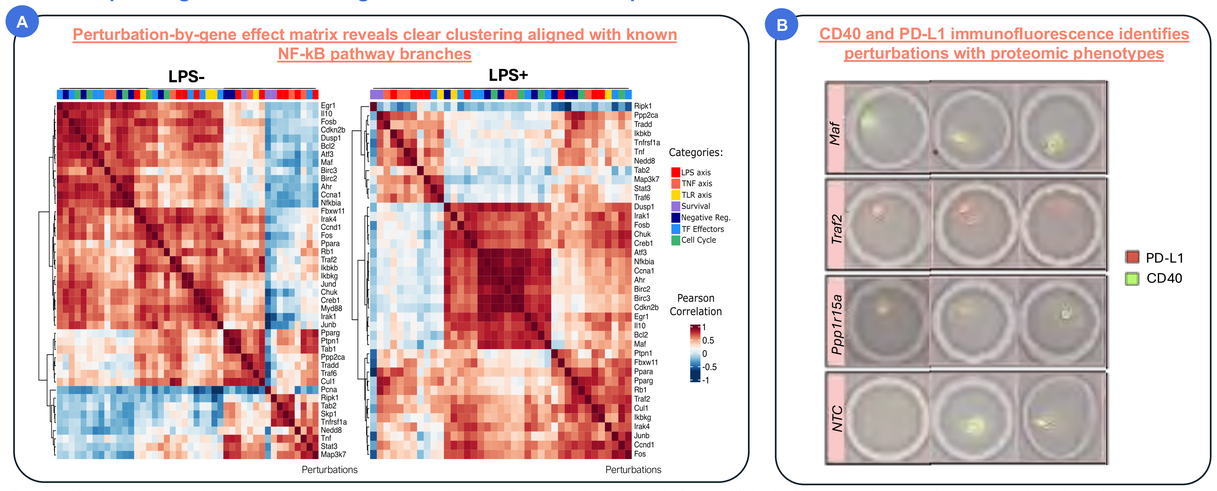


Figure 4. Multimodal integration resolves complementary layers of the NF-κB perturbation hit list. Having established guide, morphology, protein, and transcriptomic readouts individually, we next combined them at the single-cell level to ask which perturbations act through which modality. **(A)** Correlation heatmap of perturbation-by-gene transcriptomic effects (glmGamPoi beta matrix, PCA-based cosine similarity) resolves core NF-κB pathway components, negative regulators, and survival-linked perturbations into distinct functional clusters, recovering known pathway topology directly from guide-linked transcriptomes. **(B)** CD40 (green) and PD-L1 (red) immunofluorescence in the same CCEs identifies perturbations - including *Maf*, *Traf2*, and *Ppp1r15a* - with significant surface protein phenotypes, several of which showed minimal corresponding transcriptomic signature in (A). Together, these panels show that no single modality captures the full functional impact of a perturbation: transcriptomic profiling reconstructs pathway-level structure, while paired protein and morphological readouts surface RNA-silent hits that would otherwise be missed or deprioritized. This same-cell linkage across all modalities is what allows PERTURB-LINK to move from a single ranked hit list to a multi-layered functional map of perturbation effects.

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, removing the scale-vs-depth tradeoff that limits current pooled screening approaches.

Traditional pooled CRISPR screens must choose between throughput and phenotypic richness: transcriptome-only approaches scale well but can miss perturbations that act on function, morphology, or protein expression without a proportional transcriptional signature, while multi-readout plate-based assays capture that richness only at low scale and without single-guide resolution. PERTURB-LINK, built on Cellanome's CellCage Enclosure (CCE) platform, removes this tradeoff by deterministically linking guide identity to live-cell morphology, surface protein expression, and the full single-cell transcriptome - all from the same cell, at pooled-screen scale.

Underpinning this approach is accurate single-cell guide capture and assignment, which we validated directly before applying the platform biologically. Using a defined HeLa reporter pool carrying two guides with orthogonal fluorescent markers as ground truth, the optimized library preparation workflow produced clean, correctly sized guide libraries and assigned a single guide to 92.6% of enclosures with no detectable doublets. Detected guide identity showed high concordance with the independent fluorescent reporter (mismatched calls ≤1.7%). The CCE walls excluded interstitial guide signal, keeping empty control CCEs cleaner than the surrounding interstitial space. Together these results confirm that guide identity is captured and assigned accurately and enclosure-specifically at single-cell resolution, the prerequisite for the deterministic multi-modal linking on which PERTURB-LINK depends.

We demonstrated this in a 153-gene NF-κB regulator screen in LPS-stimulated BMDMs. The screen recovered canonical pathway biology, confirming data quality, and resolved perturbations into distinct hit classes that a transcriptome-only readout would not have distinguished: perturbations with concordant effects across all modalities (Myd88), perturbations acting predominantly through morphology (Arc), and perturbations acting predominantly through surface protein expression (Ppp1r15a) with little transcriptomic signature. This layered hit list illustrates the core value proposition of the platform: refining transcriptional hit lists with functional support and expanding them by surfacing RNA-silent but biologically meaningful perturbations.

More broadly, this application is one instance of a general capability. Because CCEs support longitudinal live-cell imaging, in-situ lysis, and compatibility with both suspension and adherent cells, PERTURB-LINK extends naturally to other pooled perturbation contexts, including primary immune cells, co-culture and cell-cell interaction assays, and adherent cell systems, where guide identity, dynamic behavior, morphology, and transcriptome must remain linked at single-cell resolution to correctly prioritize hits. As pooled screening moves toward richer functional readouts, platforms like this one offer a path to screens that are both high-throughput and phenotypically deep.

Questions?