Unveiling genes in dendritic cells that enhance T cell priming through functional multiplexed single cell-cell interaction analysis

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1 Can we reprogram DCs to resist cancer-driven suppression?

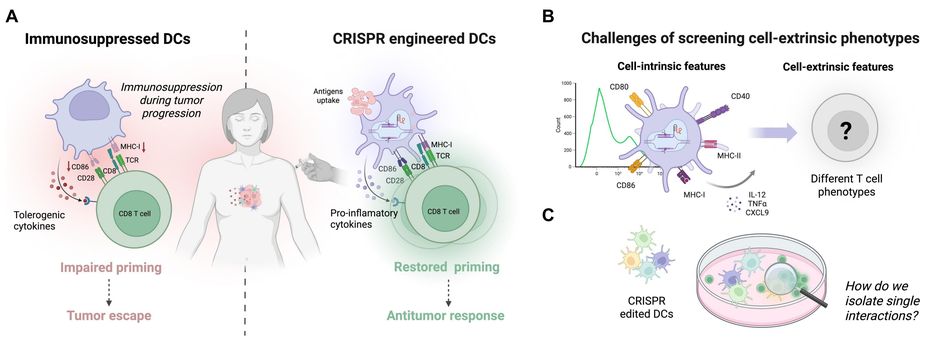


Figure 1. Genomic engineering to restore function in tumor-impaired DC. (A) Tumor-induced local and systemic immunosuppression impairs DC activation and T cell priming. Genomic engineering may counteract this suppression and enhance antitumor immunity. **(B)** Conventional Perturb-seq assays capture cell-intrinsic phenotypes, which may not reflect functional outcomes of cell-cell interactions such as T cell priming. **(C)** Conventional co-culture assays cannot trace DC–T cell contacts or the outcomes of single priming events, restricting cell-extrinsic perturbations to bulk co-cultures and limiting throughput.

2 High-throughput screening with Cellanome's CellCage™ enclosure technology

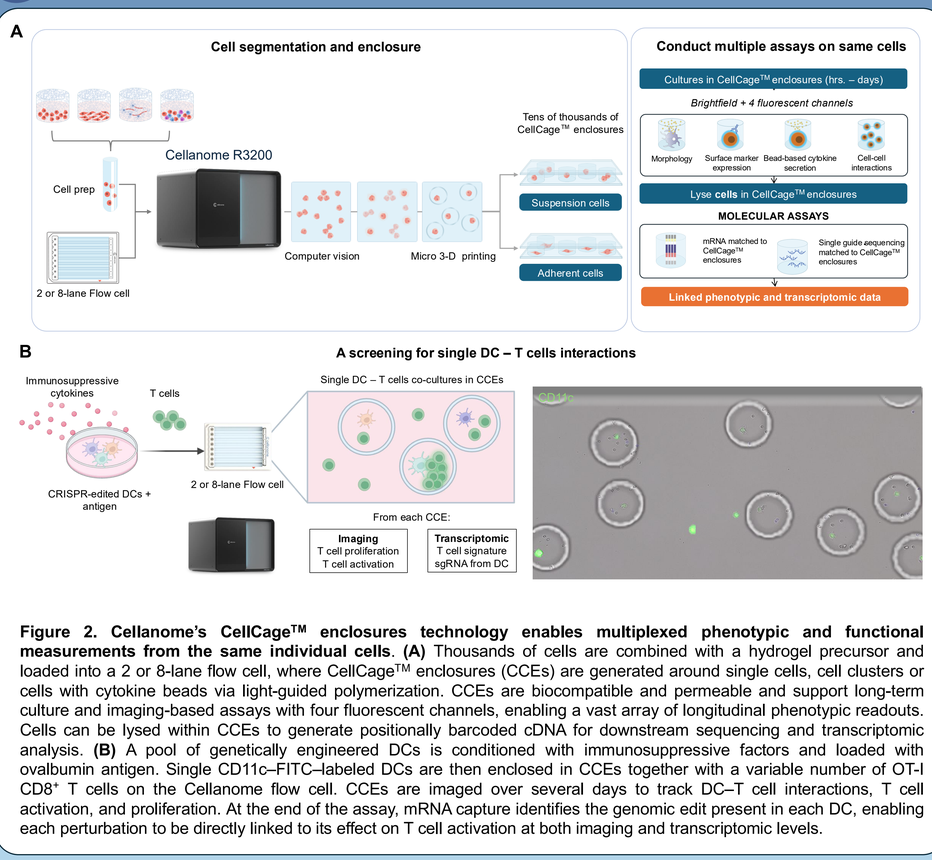


Figure 2. Cellanome's CellCage™ enclosures technology enables multiplexed phenotypic and functional measurements from the same individual cells. **(A)** Thousands of cells are combined with a hydrogel precursor and loaded into a 2 or 8-lane flow cell, where CellCage™ enclosures (CCEs) are generated around single cells, cell clusters or cells with cytokine beads via light-guided polymerization. CCEs are biocompatible and permeable and support long-term culture and imaging-based assays with four fluorescent channels, enabling a vast array of longitudinal phenotypic readouts. Cells can be lysed within CCEs to generate positionally barcoded cDNA for downstream sequencing and transcriptomic analysis. **(B)** A pool of genetically engineered DCs is conditioned with immunosuppressive factors and loaded with ovalbumin antigen. Single CD11c–FITC–labeled DCs are then enclosed in CCEs together with a variable number of OT-I CD8⁺ T cells on the Cellanome flow cell. CCEs are imaged over several days to track DC–T cell interactions, T cell activation, and proliferation. At the end of the assay, mRNA capture identifies the genomic edit present in each DC, enabling each perturbation to be directly linked to its effect on T cell activation at both imaging and transcriptomic levels.

3 Modeling priming under immunosuppression

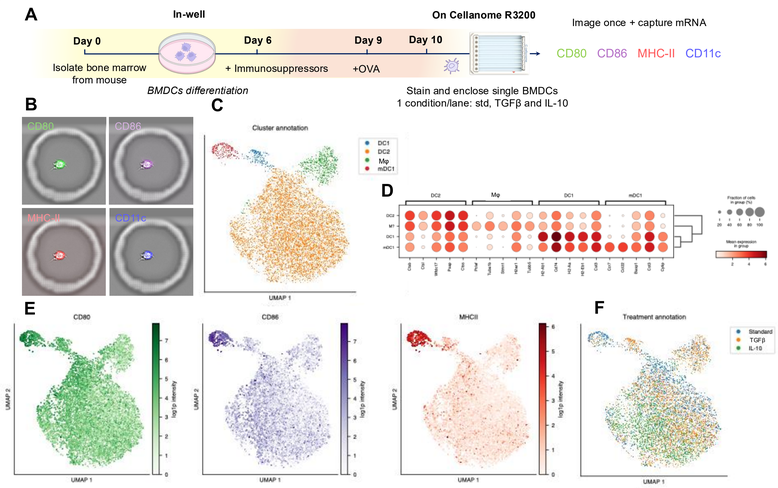


Figure 4. Imaging and transcriptomic profiling of immunosuppressed BMDCs reveal changes in population composition. **(A)** BMDCs are conditioned with IL-10 and TGF-β prior to single-cell enclosing for imaging and mRNA capture on the Cellanome flow cell. **(B)** Imaging of CD11c, CD80, CD86 and MHC-II on the flow cell. **(C)** UMAP of integrated data from three lanes. **(D)** Marker genes defining BMDC subsets. **(E)** Protein-level expression of CD80, CD86, and MHC-II overlaid on the UMAP. **(F)** UMAP stratified by treatment.

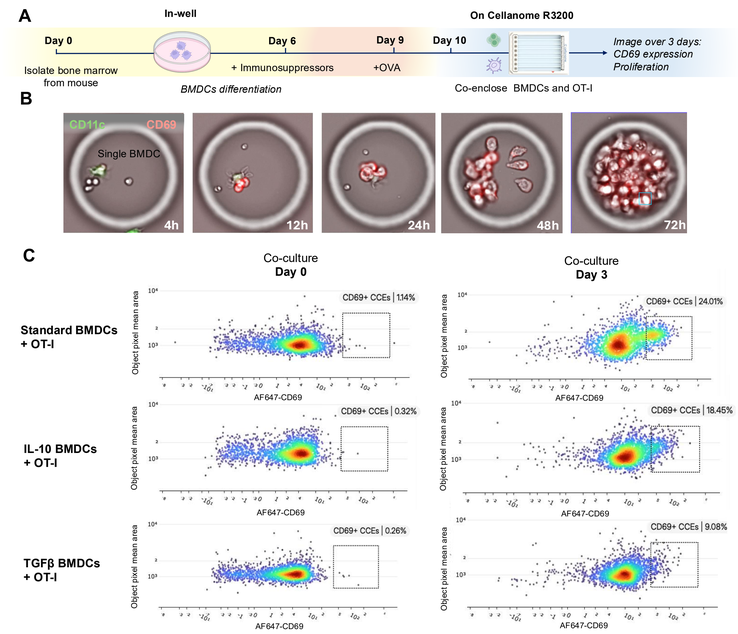


Figure 3. Optimization of a T cell priming assay under immunosuppressive conditions using Cellanome's CellCage™ enclosure technology. (A) Bone-marrow derived dendritic cells (BMDCs) are conditioned with IL-10 or TGF-β and co-enclosed with OT-I T cells on the Cellanome flow cell for the priming assay. **(B)** CCEs are imaged for 3 days to assess proliferation and CD69 expression. **(C)** The percentage of CD69⁺ CCEs is shown at t_0 and day 3.

4 Linking genomic edits to functional outcomes

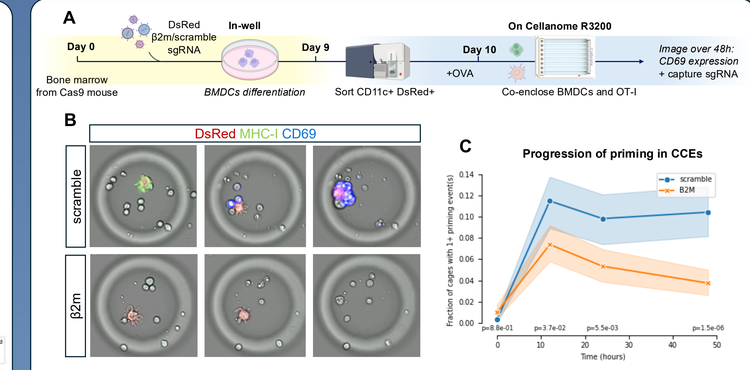
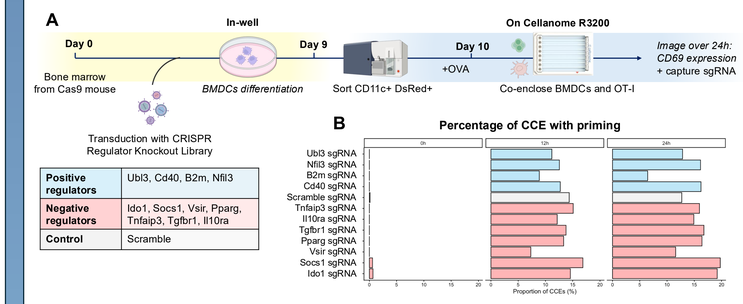


Figure 5. Functional validation of B2m knockout in BMDCs. (**A**) H11-Cas9 bone marrow is transduced with sgRNAs targeting β2m or a scramble control. After differentiation, DsRed⁺ CD11c+ BMDCs are tested in the priming assay. (**B**) MHC-I surface levels correlate with BMDCs priming ability. (**C**) Quantification of priming efficiency over time, shown as the percentage of CCEs with successful T priming events.



Positive regulators	Ubl3, Cd40, B2m, Nfil3
Negative regulators	Ido1, Socs1, Vsir, Pparg, Tnfaip3, Tgfbr1, Il10ra
Control	Scramble

Figure 6. Functional CRISPR perturbations of DC genes involved in T cell activation . **(A)** A focused library of sgRNAs targeting known regulators of DC-mediated T cell priming is delivered into H11-Cas9 bone marrow, and DsRed⁺ CD11c+ BMDCs are tested in the priming assay. **(B)** Percentage of CCEs showing successful priming stratified by identity of expressed sgRNA.

5 Conclusions and future directions

Platform Overview
Cellanome CellCage™ enclosure technology enables longitudinal imaging, transcriptomics, and CRISPR perturbations in individual DC–T cell co-cultures at high throughput.

Key Findings
- Immunosuppressive factors reduce the ability of BMDCs to prime T cells.
- Combined imaging and RNA-seq reveal altered BMDC subset composition under immunosuppressive conditions.
- CRISPR perturbations in Cas9-expressing BMDCs map specific gene knockouts to differences in T cell priming.

Get in

Future Directions
- Expand perturbation screens to identify novel DC regulators under immunosuppressive conditions

Genomic edit → **DC phenotype** → **T cell phenotype**

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