Longitudinal Multi-Modal Single-Cell Profiling Reveals Functional Heterogeneity In Human Preadipocytes

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Introduction

Adipogenesis requires coordinated morphological and molecular transitions as mesenchymal precursors differentiate into lipid-laden adipocytes. While single-cell technologies have unveiled cellular heterogeneity in adipogenesis, they cannot link transcriptomic states to functional outcomes to the same single cells: mature adipocytes are lost during enzymatic dissociation, and transcriptomics cannot fully predict functional phenotypes. These constraints prevent comprehensive understanding of adipocyte specification and metabolic disease mechanisms. To address this, we developed the Cellanome R3200, a platform that encloses and cultures thousands of single live human preadipocytes within semi-permeable hydrogel CellCage™ Enclosures (CCEs), enabling longitudinal multi-modal profiling. We combined time-resolved live-cell imaging to link morphological changes, lipid droplet accumulation, and viability assessment, to single-cell transcriptomics from the same individual cells. Longitudinal tracking revealed substantial asynchrony in differentiation kinetics, with marked cell-to-cell variation in timing and quantity of lipid droplet formation. Using regularized regression with bootstrap stability selection, we identified 227 genes whose expression predicts lipid accumulation. Top positive predictors included *CCND1*, a known regulator of adipogenesis, as well as *AKR1B1* and *THY1* with less characterized roles in adipogenic lineages. Negative predictors included *IGFBP6* and *ADIRF*, whose role in adipogenesis is still not fully understood. Critically, even within transcriptomic similar clusters, we observed additional functional heterogeneity in lipid accumulation and droplet morphology - discordance that would be missed without integrated imaging data. By linking molecular and functional data at single-cell resolution, this approach enables resolution of molecular drivers of functional phenotypes, with broad applicability to other dynamic stem cell differentiation processes.

Cellanome R3200 Links Longitudinal Live Cell Imaging With Transcriptomics

A On-Instrument CellCage™ Formation Unlocks Paired Multimodal Profiling

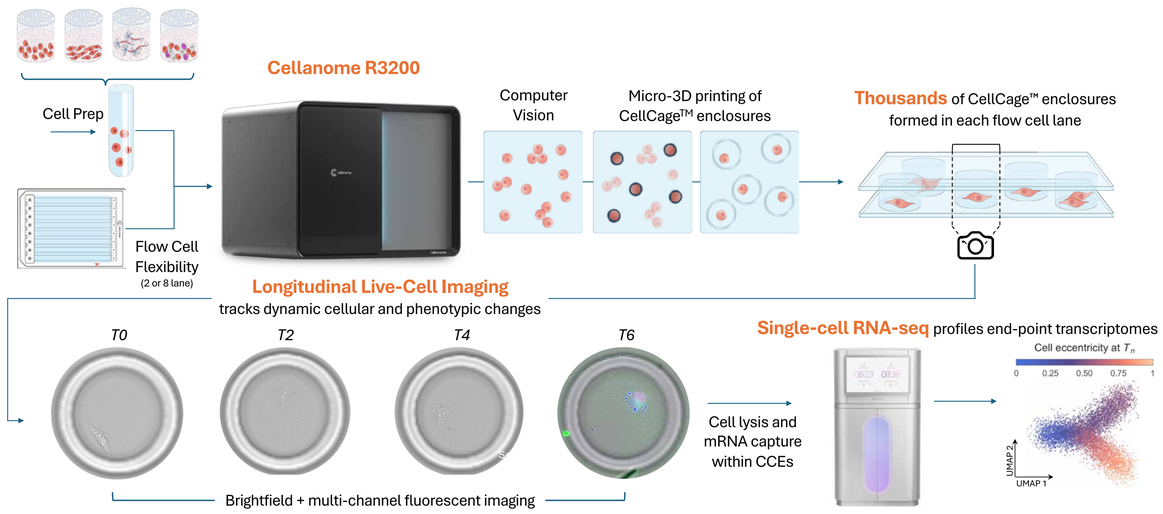
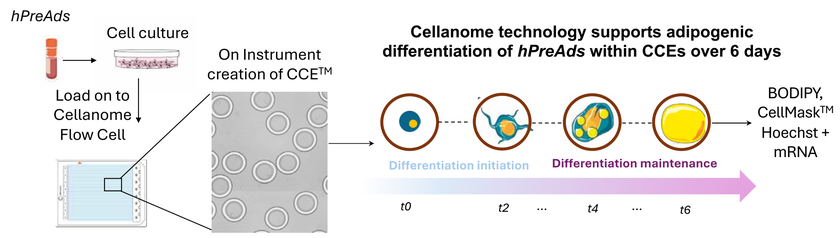


Fig 1. Cellanome's CellCage™ Technology enables paired longitudinal imaging and transcriptomics in the same cells. (A) Tens of thousands of cells are mixed with hydrogel precursor and loaded on a 2- or 8-lane flow cell. Cell positions are identified and CellCage™ Enclosures (CCEs) automatically generated around cells with light-guided hydrogel polymerization. Bio-compatible CCEs can be formed around single cells, multiple cells, or cells with objects (e.g., cytokine 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 are lysed within CCEs, and then cDNA is generated for downstream library prep and sequencing off the instrument.

Longitudinal Live-Imaging of Differentiating Preadipocytes

A Preadipocytes Differentiation on Cellanome's Flow Cells



B Longitudinal Live-cell Imaging

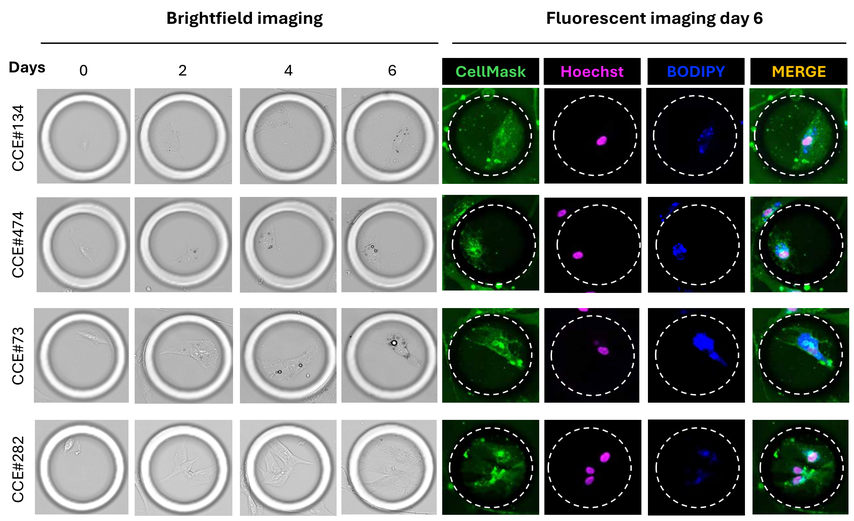
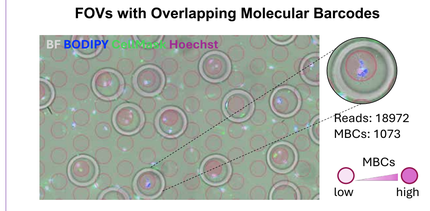


Fig 2. Establishing a workflow to induce adipogenic differentiation on Cellanome's flow cells.

(A) Schematic of human primary preadipocyte (*hPreAds*) differentiation induction on flow cell. Human preadipocytes were thawed and expanded in tissue culture flasks under appropriate culture conditions. Early passages *hPreAds* (p2-p4) were detached, loaded with hydrogel precursor on flow cells and CCEs were generated around single cells. Cells were cultured in differentiation initiation media for 2 days, then switched to differentiation maintenance media for up to 6 days. Medium was refreshed every 24 hours. Brightfield images were captured at 0, 2 , 4 and 6 days. At 6 days, cells were stained for BODIPY (lipid droplet), CellMask™ (cytoplasm) and Hoechst 33342 (nuclei), imaged and processed for same-cell mRNA.
(B) Representative brightfield and fluorescence images (10x) of live differentiating *hPreAds* within CCEs at 0, 2, 4 and 6 days. Fluorescence staining for CellMask™ (cytoplasm, green), Hoechst 33342 (nuclei, purple), BODIPY (lipid droplet, blue) shown individually and as merged images. Heterogeneous differentiation kinetics were observed at the single-cell level in *hPreAds* within CCEs, with cells accumulating lipid droplets as early as 2 days, cells showing increased lipid droplet content at 6 days, and cells evading differentiation and continuing to cycle, with no BODIPY signal.

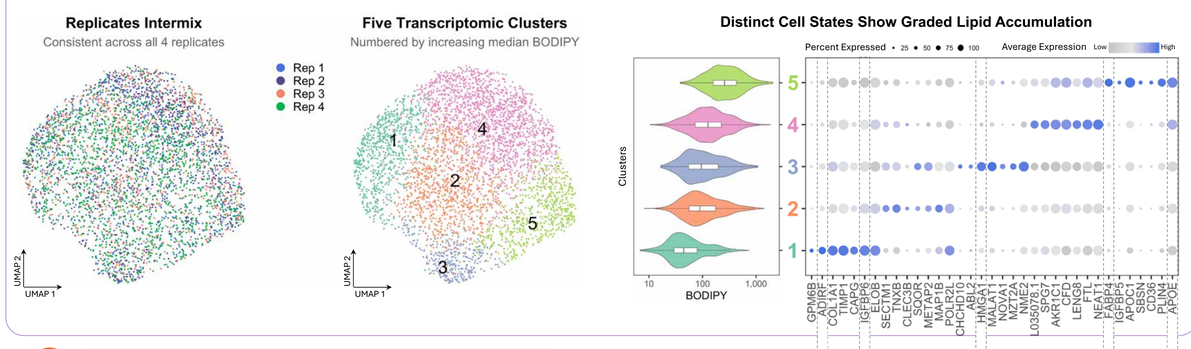
Multi-Modal Characterization of Adipogenesis at Single-Cell Resolution

A Paired Morphology and Transcriptomics Resolve Reproducible, Marker-Defined Cell States



Quality Control Summary Metrics

		Sequencing-based			Morphology-based		
Replicate	Total CCEs	Median MBCs/CCE	Median Reads/CCE	Median Genes/CCE	Median BODIPY MFI (A.U.)	Median Cell Area (PX)	Median Eccentricity
Rep 1	983	1,530	18,774	728	113.5	366,388	0.22
Rep 2	809	1,165	21,297	606	84.0	369,400	0.23
Rep 3	1,051	1,174	13,395	615	96.1	364,310	0.22
Rep 4	1,467	1,775	13,841	764	136.6	361,837	0.21
All replicates	**4,310**	**1,386**	**16,318**	**673**	**108.4**	**364,760**	**0.22**



B Lipid Accumulation Across a Differentiation Continuum

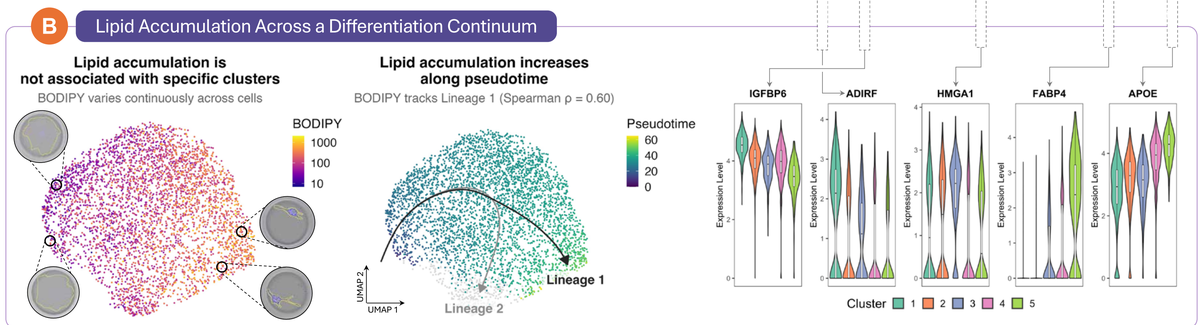


Fig 3. Linking transcriptome to lipid accumulation in differentiating preadipocytes. (A) Single-cell profiling of 4,310 CCEs across four replicates links transcriptome to lipid content in differentiating preadipocytes. Replicates intermix in the integrated UMAP and resolve into five clusters ordered by increasing median BODIPY, each with distinct markers. **(B)** Lipid (BODIPY) varies continuously rather than partitioning by cluster, yet rises along a differentiation pseudotime (Slingshot Lineage 1), revealing lipid accumulation as an asynchronous continuum.

Predicting the Drivers of Lipid Accumulation and Successful Differentiation

A A Regression on Continuous BODIPY Recovers Lipid Genes that Discrete Clusters Miss

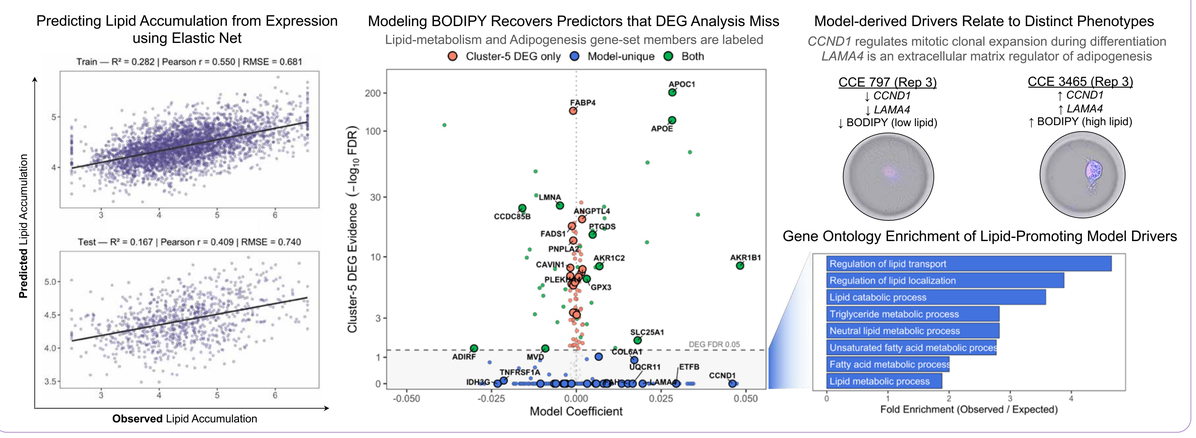


Fig 4. Predicting lipid accumulation nominates the genes driving successful differentiation. (A) An elastic-net model explains continuous per-CCE lipid signal (Pearson r = 0.41). Its "model-unique" drivers – selected by the model yet falling below the cluster DEG significance line ($-\log_{10}$ FDR) – are missed by DEG analysis, are enriched for lipid transport and metabolism, and separate low- from high-lipid enclosures.

Conclusion / Next Steps

Cellanome R3200 enables longitudinal, multi-modal single-cell profiling of human preadipocyte differentiation, revealing heterogeneous adipogenic trajectories and identifying gene drivers of lipid accumulation by paired imaging and transcriptomic analysis. We are now extending this work through deeper temporal profiling at 12 days, automated segmentation-based quantification of lipid droplet dynamics, and pooled CRISPR screens to functionally interrogate candidate regulators. We believe that leveraging this framework will help advance our understanding of stem cell fate decisions across mesenchymal lineages, opening new avenues for targeting cellular drivers of obesity and metabolic disease.

Questions?