Longitudinal Imaging of the Same Cell Anchors Transcriptional Trajectories of Adipogenic Commitment

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INTRODUCTION Biological processes unfold over days but are reconstructed from snapshots of different cells, so a cell's dynamics and its molecular endpoint are linked by inference, not observation. The Cellanome R3200 forms CellCage™ Enclosures (CCEs) around single cells, coupling longitudinal imaging to endpoint single-cell RNA-seq of the same cell. We applied it to human adipogenesis, a slow, asynchronous differentiation. Preadipocytes at baseline and 7 and 12 d after induction were imaged daily for lipid and sequenced with short and long reads across 9,985 CCEs. Short-read clusters span proliferative (*HMGA1*) to lipid-laden (*APOE*, *FABP4*) states, and pseudotime tracks imaged lipid in the same cells. Long reads resolve the isoform carrying this signal: differential transcript usage from 0 to 12 days reveals coordinated isoform switching in metabolic, cytoskeletal and matrix genes, invisible to short reads. An autoencoder over longitudinal DINOv2 embeddings clusters cells by morphodynamic trajectory; one, shrinking and rounding as stress fibers disassemble, reaches a high-lipid state, an observed ordering that validates pseudotime. Imaging and sequencing the same cell resolves commitment dynamics that snapshots can only infer.

CellCage™ Enclosures Unite Morphology and Transcriptomics

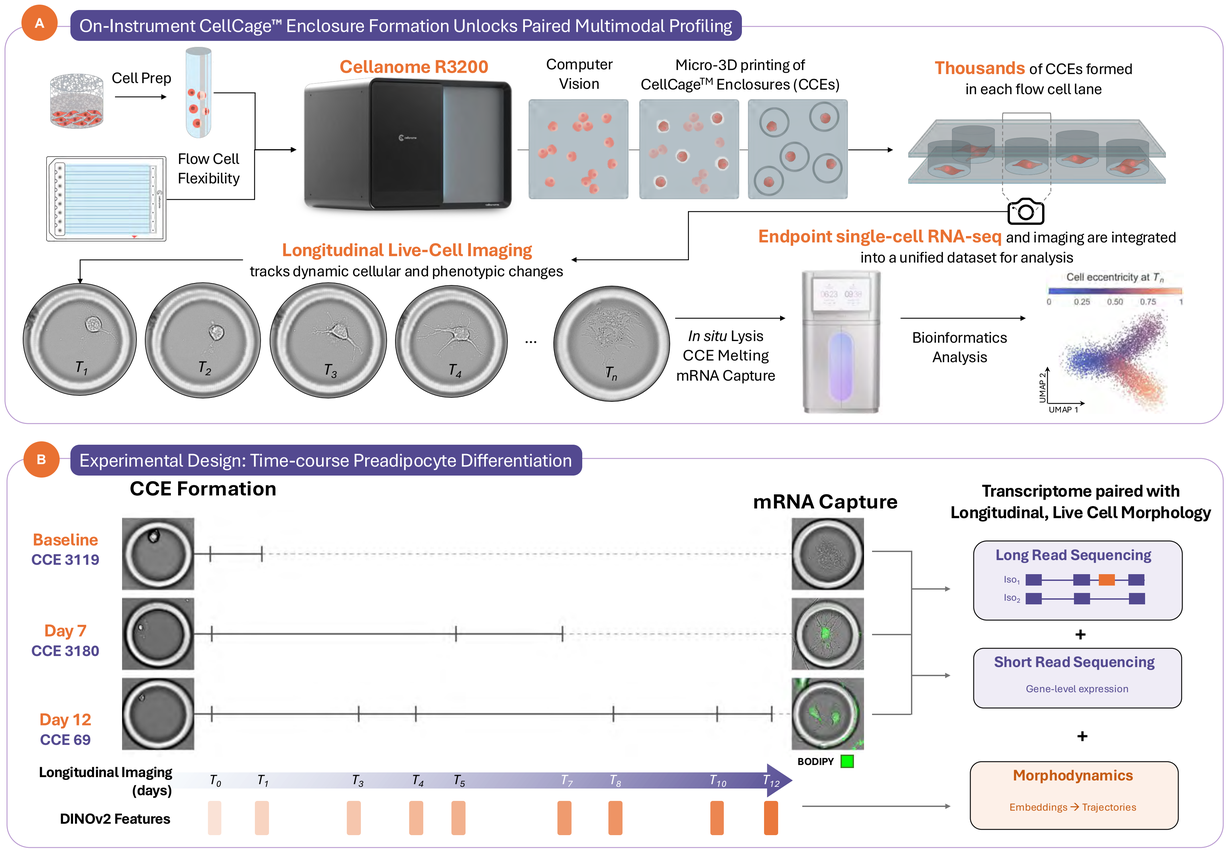


Fig. 1. CellCage™ Enclosures pair longitudinal imaging with endpoint sequencing of the same cell. (A) On the Cellanome R3200, cells are located by computer vision and enclosed in micro-3D-printed CellCage™ Enclosures (CCEs), thousands per lane. Each CCE is imaged repeatedly (T_1 to T_n), then lysed *in situ* for mRNA capture, linking imaging features to the transcriptome of the same cell. **(B)** Preadipocytes enclosed in CCE at baseline, 7 d and 12 d after induction were imaged daily (DINOv2 features per frame), stained with BODIPY for lipid, and captured at endpoint, pairing long-read isoform, short-read gene and morphodynamic trajectory layers per CCE.

Long Reads Resolve Isoform Usage Across Differentiation

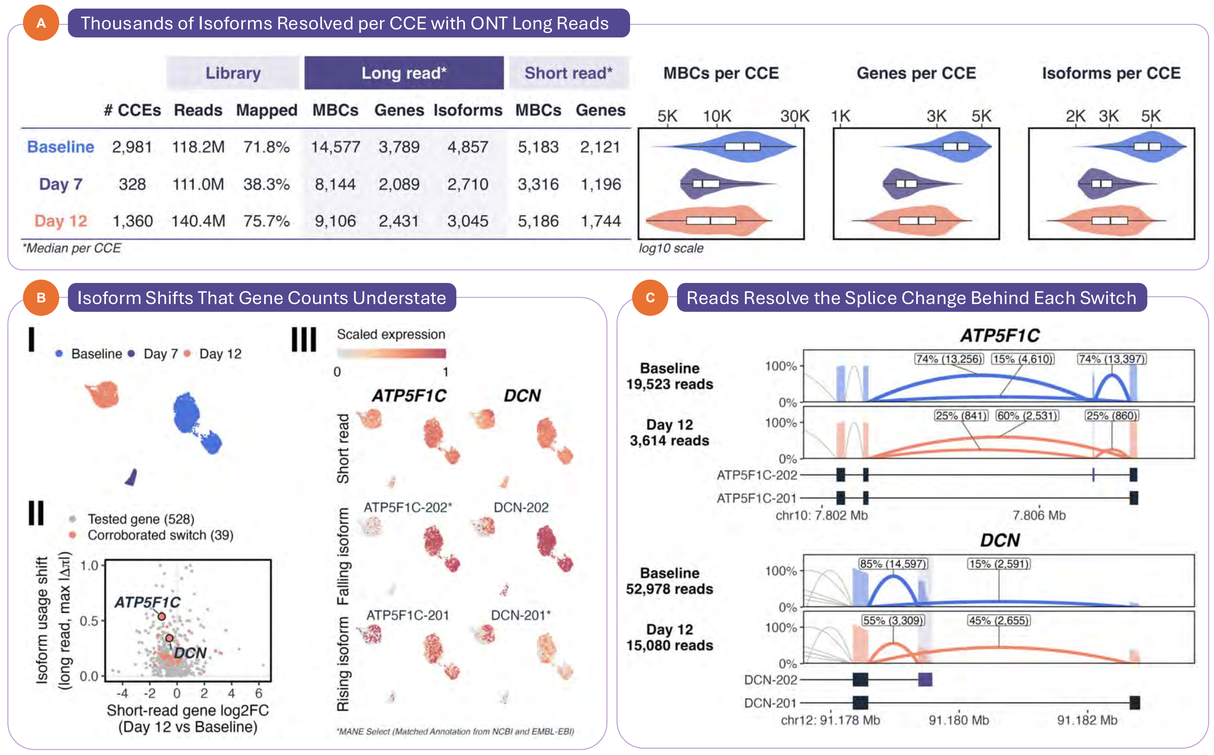
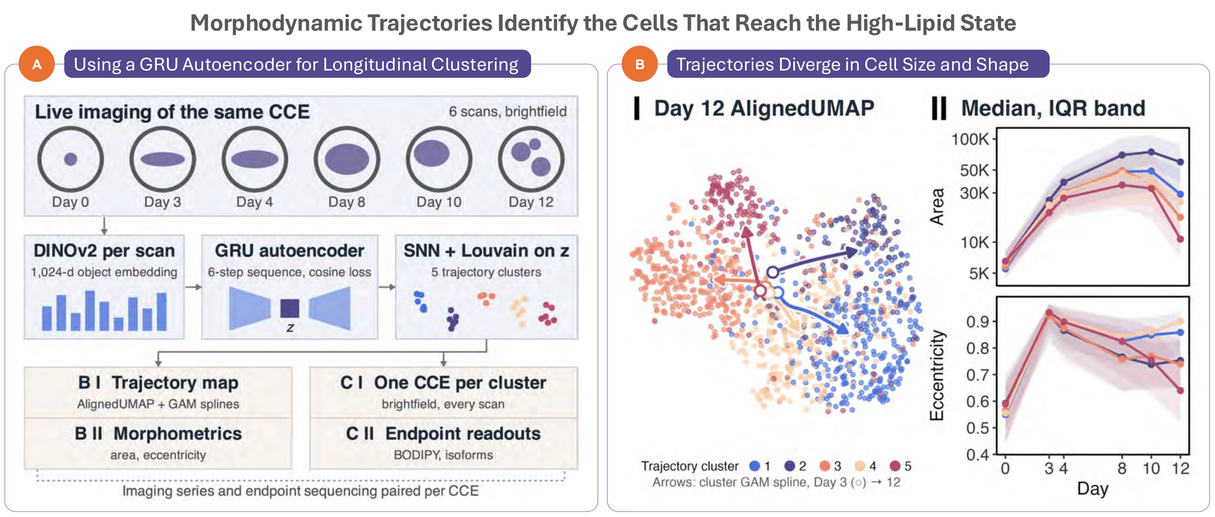


Fig. 3. Long reads resolve isoform switches that gene-level counts understate. (A) Long- and short-read metrics per CCE for 4,669 CCEs at baseline, 7 d and 12 d. **(B)** Isoform UMAP by timepoint (I). Of 528 genes tested between baseline and 12 d, 39 switch isoforms. The picked two (*ATP5F1C*, *DCN*) change only modestly at gene level in short reads (II). Short-read expression and isoform share per CCE (III). (C) Junction usage: *ATP5F1C* skips exon 9 (74% → 25% inclusion), and *DCN* shifts toward a distal first exon (85% → 55% proximal).

Morphodynamic Trajectories Identify the Cells That Reach the High-Lipid State



Lipid Accumulation Links Transcriptomic State to Function

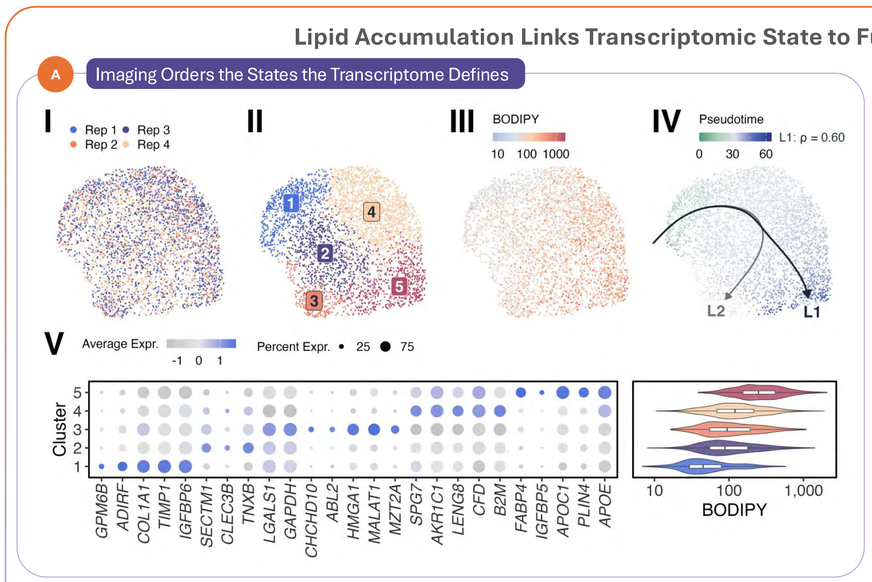
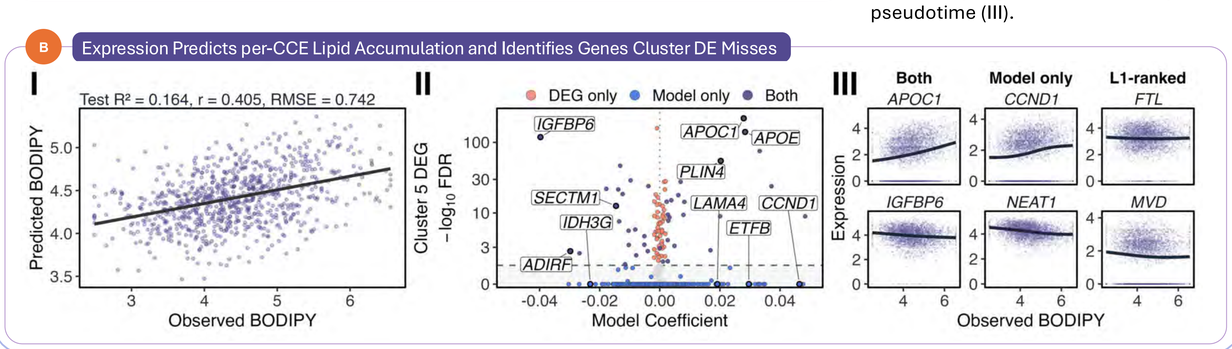


Fig. 2. Lipid accumulation orders transcriptomic state and is predictable from expression in single CCEs. (A) 4,310 CCEs at 7 d, integrated across four replicates (I), form five clusters numbered by increasing median BODIPY (II). BODIPY varies continuously across the embedding (III) and rises along Slingshot Lineage 1 (IV). Markers run from *COL1A1* to *FABP4* and *APOE* (V). **(B)** An elastic net on 829 genes predicts Hoechst-normalized BODIPY in held-out CCEs (I). Against cluster-5 differential expression (dashed, FDR 0.05), 177 genes are selected only by the model, including *CCND1* and *LAMA4* (II). Expression against BODIPY for the genes rising and falling most with lipid among shared markers and model-only genes, and for model-only genes ranked by L1 pseudotime (III).



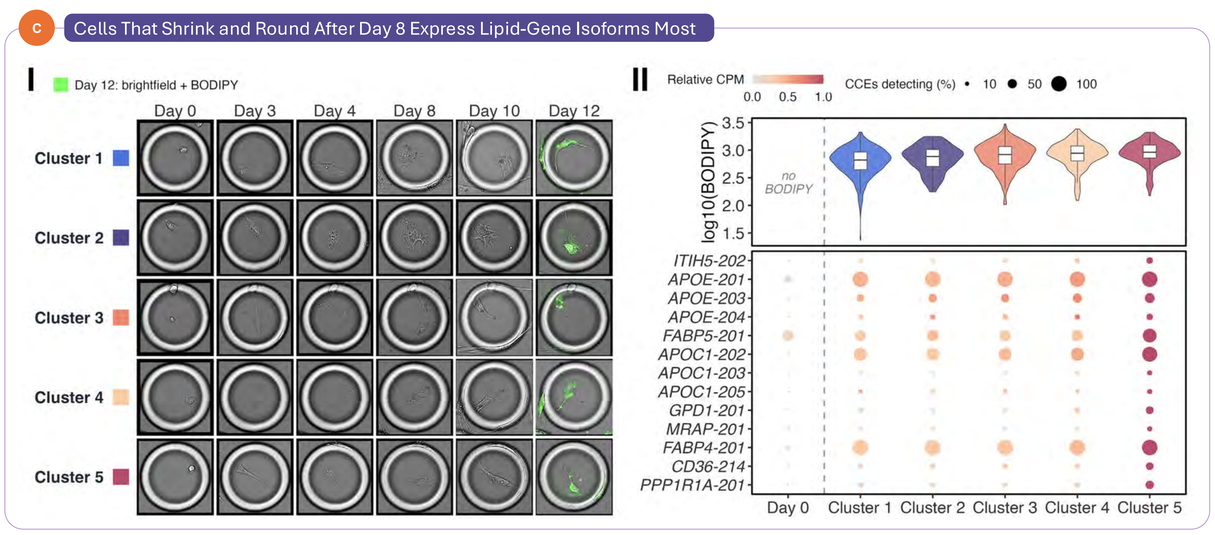


Fig. 4. Morphodynamic trajectories give an observed temporal order to adipogenic commitment. (A) A GRU autoencoder encodes six DINOv2 scans (0 to 12 d) per CCE; Louvain clustering gives five trajectories, numbered by median BODIPY as in Fig. 2. **(B)** 12 d AlignedUMAP with GAM splines (I); per-object area and eccentricity (II). **(C)** One CCE per cluster (I); BODIPY and long-read CPM of 13 cluster 5 vs 1 marker isoforms (II). Cluster 5 shrinks and rounds after 8 d (eccentricity 0.83 → 0.64) and expresses every isoform most.

CONCLUSIONS

- **Same-cell readouts:** imaging plus short- and long-read RNA-seq of the same cell across 9,985 CCEs.
- **Lipid orders state:** pseudotime tracks BODIPY (ρ = 0.60); expression predicts it in held-out CCEs.
- **New lipid genes:** 177 genes, incl. CCND1 and LAMA4, are found by the model but missed by cluster DE.
- **Isoform switching:** ATP5F1C-202 exon inclusion falls from 74% to 25% by day 12, unseen by short reads.
- **Observed order:** one morphodynamic trajectory shrinks and rounds, a benchmark for pseudotime.

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