

cellanome

Multi-Modal Single-Cell Phenotyping Resolves *bona fide* Senescent Cells in Human Preadipocytes

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Introduction

Cellular senescence drives age-related tissue dysfunction, but reliably identifying bona fide senescent cells remains a fundamental challenge: no single biomarker is sensitive and specific, and existing single-cell technologies measure complementary features in separate samples, leaving per-cell co-occurrence unobserved. We developed the Cellanome platform, which integrates live-cell culture in CellCage™ enclosures (CCEs), tens of thousands of semi-permeable hydrogel compartments each encircling a single cell or ensemble, with programmable reagent delivery, longitudinal time-lapse imaging, and multimodal phenotyping linking fluorescence-based measures to end-point RNA. In etoposide-treated primary human preadipocytes, a model of age-related metabolic disease, we reproducibly induced senescence and measured, per-cell, the whole transcriptome and five readouts: SA-βgal activity, cell morphology, image-based proliferation history, p21/CDKN1A mRNA, and the SenMayo transcript signature. Per-cell integration of imaging and transcriptomic hallmarks reveals a multimodally senescent population (p21+, large, non-dividing, SA-βgal+, SenMayo+; ~8% of etoposide-treated cells), providing a foundation for high-content senolytic and senomorphic screening.

Cellanome R3200 Links Longitudinal Live Cell Imaging With Transcriptomics

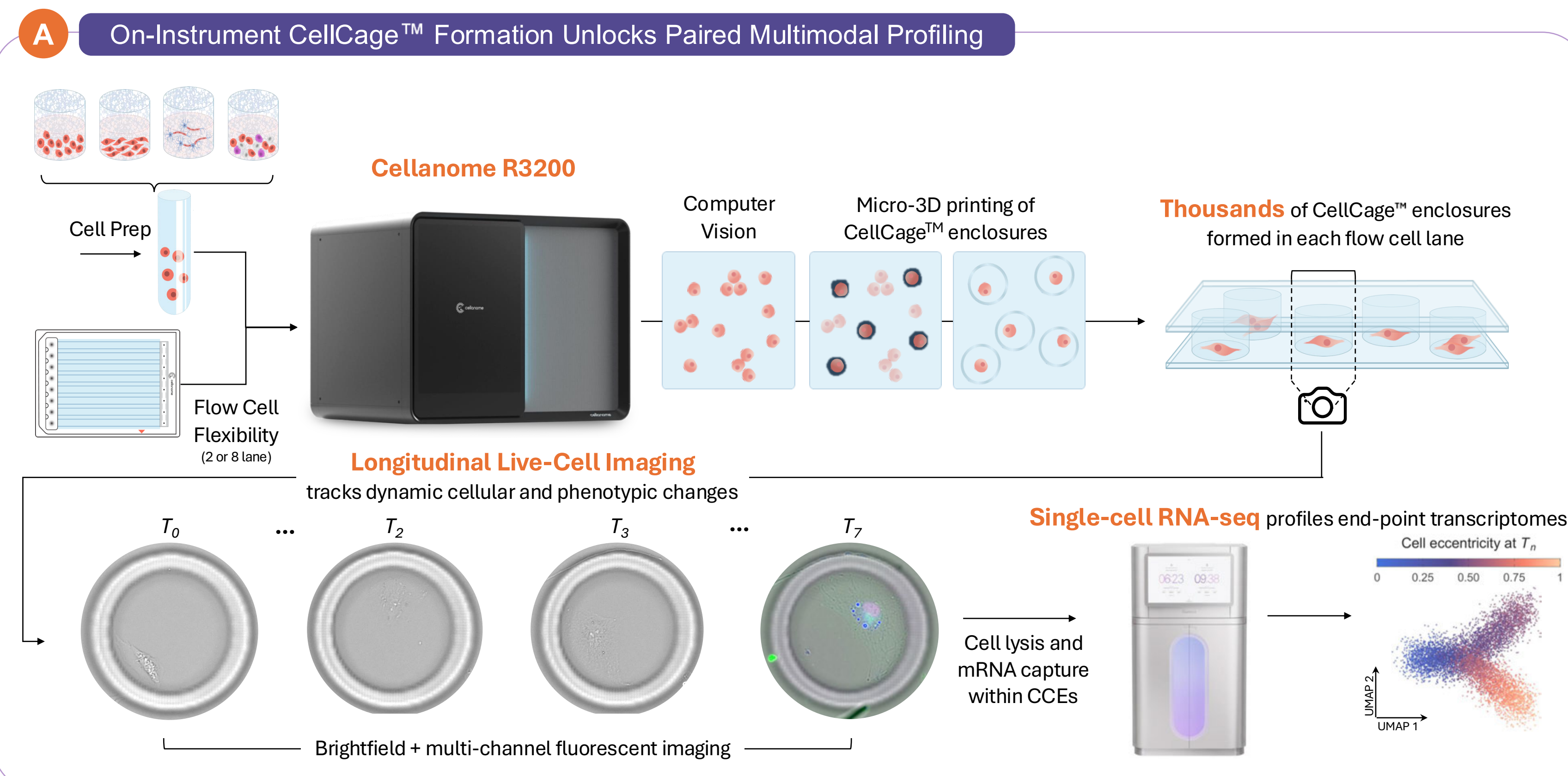


Fig 1. Cellanome's CellCage™ Technology enables paired longitudinal imaging and transcriptomics in the same cells. (A) Tens of thousands of cells are mixed with hydrogel precursor and loaded on a 2- or 8-lane flow cell. Cell positions are identified and Enclosures (CCEs) automatically generated around 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 Senescence-Induced Human Preadipocytes

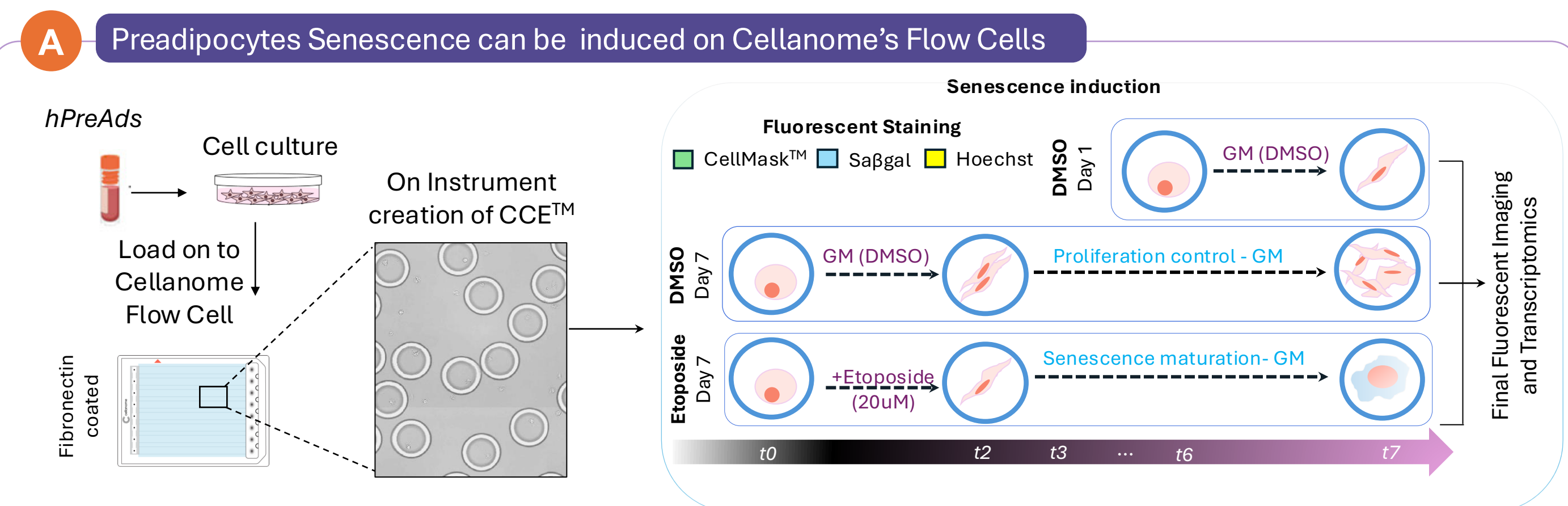
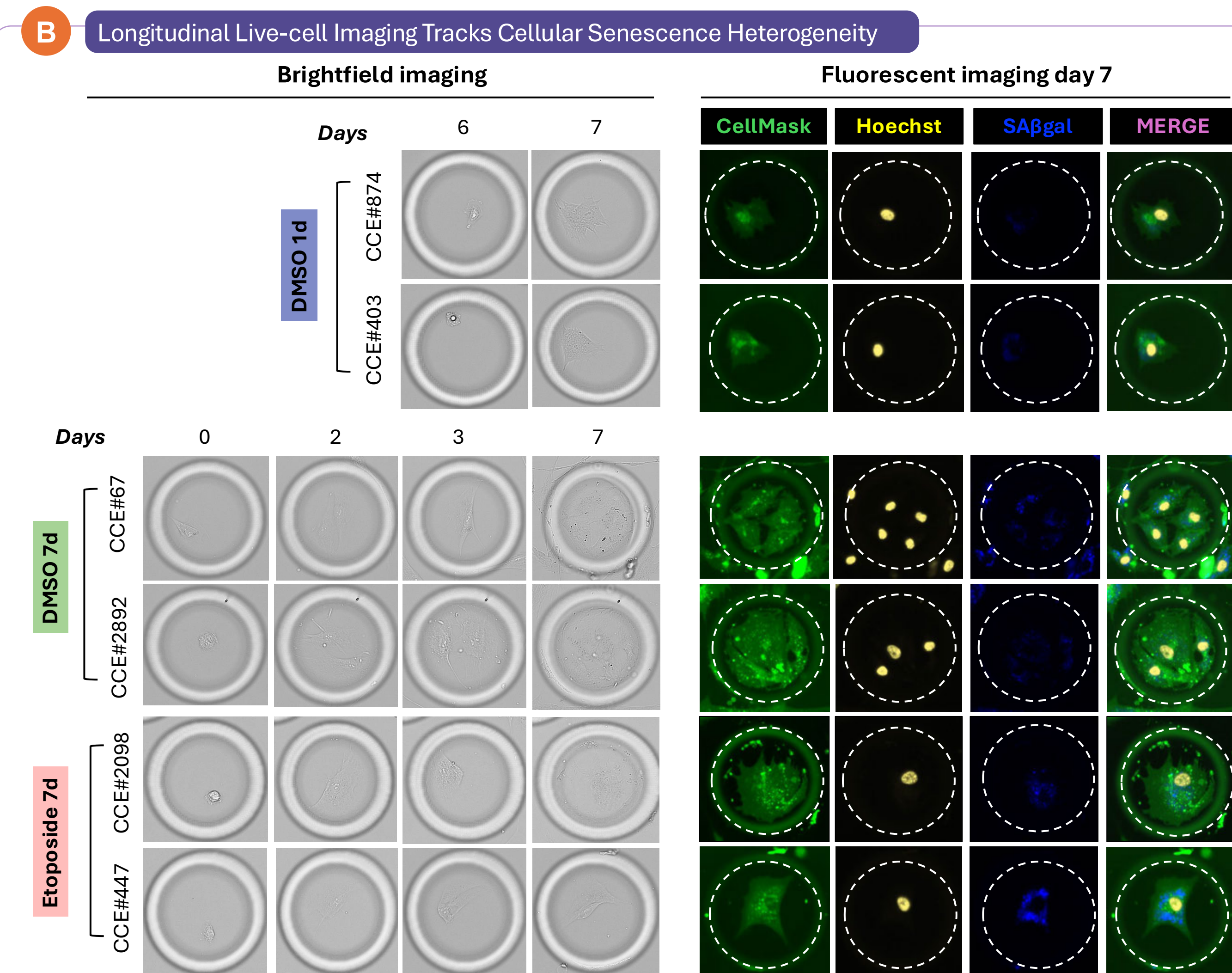


Fig 2. Establishing a workflow to induce cellular senescence on Cellanome's flow cells.

(A) Schematic of hPreAdS senescence induction on flow cells. hPreAdS were expanded, then early-passage hPreAdS (p2–p3) were loaded onto fibronectin-coated flow cells and encapsulated in single-cell CCEs. DMSO and Etoposide Day 7 conditions were treated ± Etoposide (20 μM) in GM for 2 days, washed out, then maintained in GM for 5 more days (7 days total). The DMSO Day 1 condition was loaded on day 6 and cultured for 1 day in GM (DMSO), serving as a single day control distinct from the 7-day proliferation control (DMSO Day 7). On day 7, all lanes were stained with CellMask™, SA-βgal, and Hoechst, imaged, and processed for mRNA. BF images: days 0, 2, 3, 6, 7; fluorescence: day 7. (B) Representative brightfield and fluorescence images (10x) of live hPreAdS within CCEs at 0, 2, 3, 6 and 7 days. Fluorescence staining for CellMask™ (cytoplasm, green), Hoechst (nuclei, purple), SA-βgal (blue) shown individually and as merged images.



Etoposide-Induced Senescence Is Visible in Cell Shape and Transcriptomics

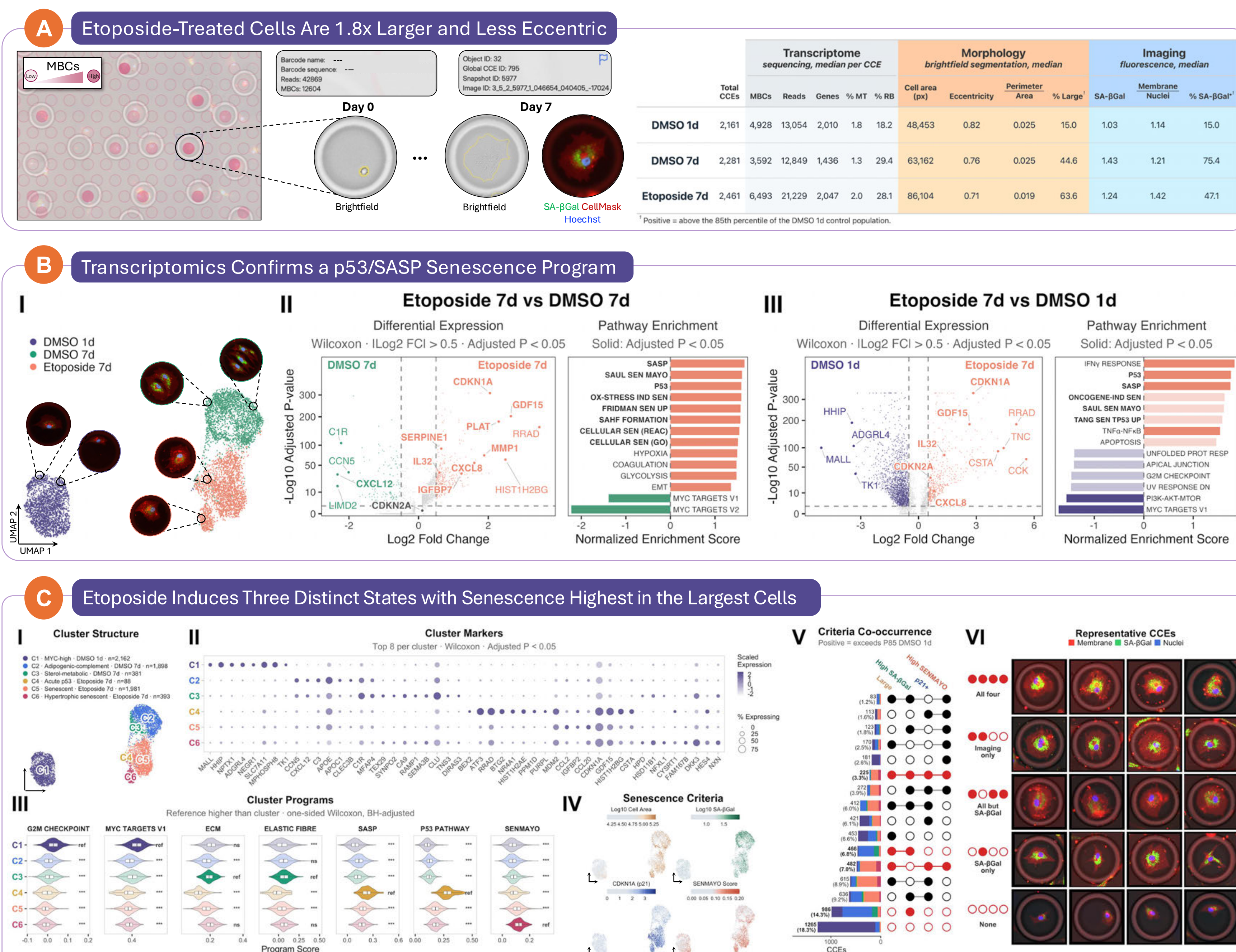


Fig 3. A morphological and transcriptomic definition of etoposide-induced senescence. Primary human preadipocytes in CellCage™ Enclosures (CCEs), profiled by scRNA-seq plus morphology and fluorescence imaging from the same CCE (6,903 after QC). (A) Etoposide cells are 1.4-1.8x larger than the vehicles and less eccentric than either. (B) CDKN1A/p21 is the most significantly induced gene, CDKN2A/p16 unchanged against the 7-day vehicle; p53 and curated senescence sets enriched, MYC targets depleted; SENMAYO genes bold. (C) Etoposide-treatment resolved CDKN1A-positive cells into three clusters; C6 has the largest cells and highest SenMayo score, p53/SASP peaks in the small C4 cluster. 8.1% of etoposide-treated CCEs meet all four criteria, against 1.1% and 0.05% in the DMSO vehicles.

Senolytics Deplete Multimodally Defined Senescent Preadipocytes

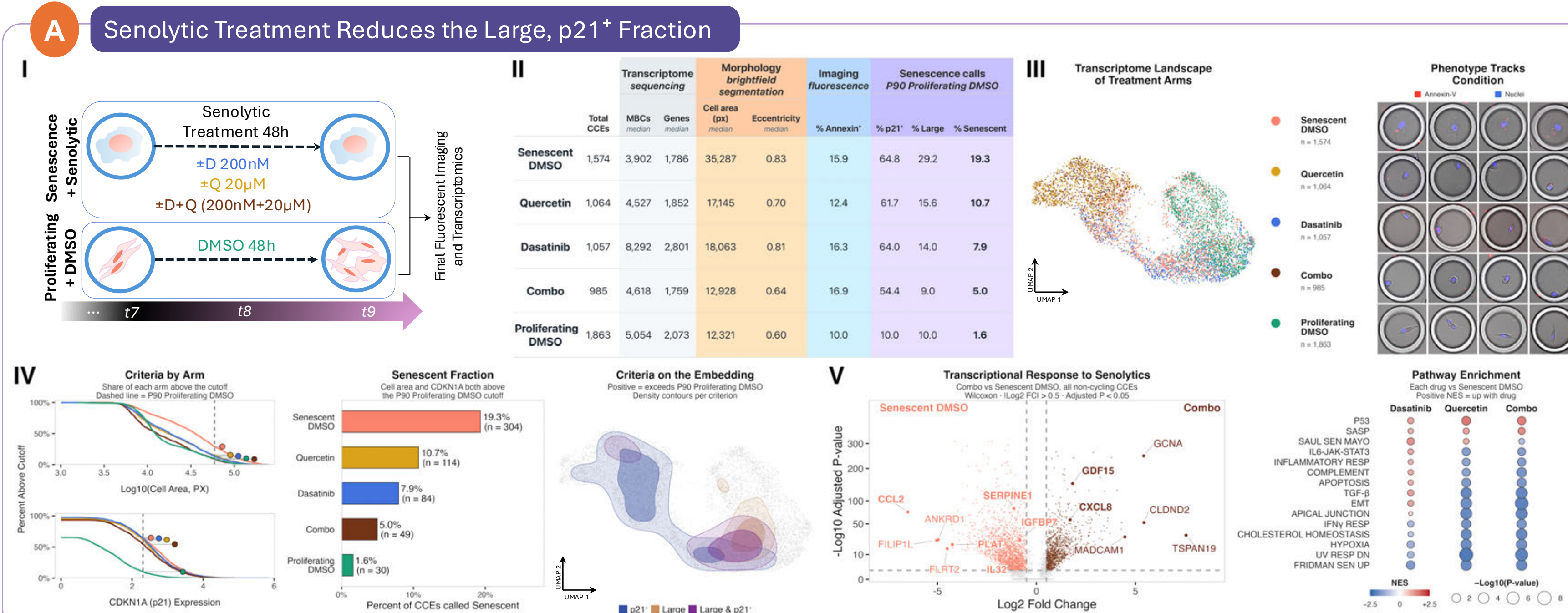


Fig 4. Senolytics deplete senescent preadipocytes. (A) Senolytic treatment reduces cell size back toward the proliferating baseline. The senescent (Large & p21+) fraction falls from 19% (Senescent DMSO) to 10% (Quercetin) and 8% (Dasatinib), down to 5% under the combination of the two (Combo). Relative to Senescent DMSO, Quercetin and Combo deplete known senescence signatures, TGF-β, EMT, and IFNγ response, but leave p53/SASP intact.

Conclusion / Next Steps

We demonstrate that Cellanome's technology can decipher cellular senescence by generating multi-modal datasets within the same individual cells, converging multiple senescence measurements, validating SENMAYO score gene sets, and integrating morphological features with gene expression in a single experiment. This system enabled senolytic drug screening, where we observed a significant reduction in the senescent cell fraction upon dasatinib and quercetin combination treatment. We believe Cellanome's technology will increase confidence in senescence studies by identifying bona fide senescent cells, accelerate tissue-specific senescence atlas initiatives, and clarify senolytic drug specificity, opening the door to future work on tracking senescence kinetics or diffusion of senescence phenotype through cell-cell interactions in co-culture systems.

Questions?

