High-plexy intracellular and surface CITE-Seq offers multiomic insights into cell function

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Abstract

CITE-Seq allows researchers to combine high-plex protein information with whole transcriptome sequencing to gain insights about individual cell states. Due to the harsh fixation and permeabilization required for intracellular (IC) antibody staining, only a few studies have recovered mRNA expression profiles alongside IC protein detection.

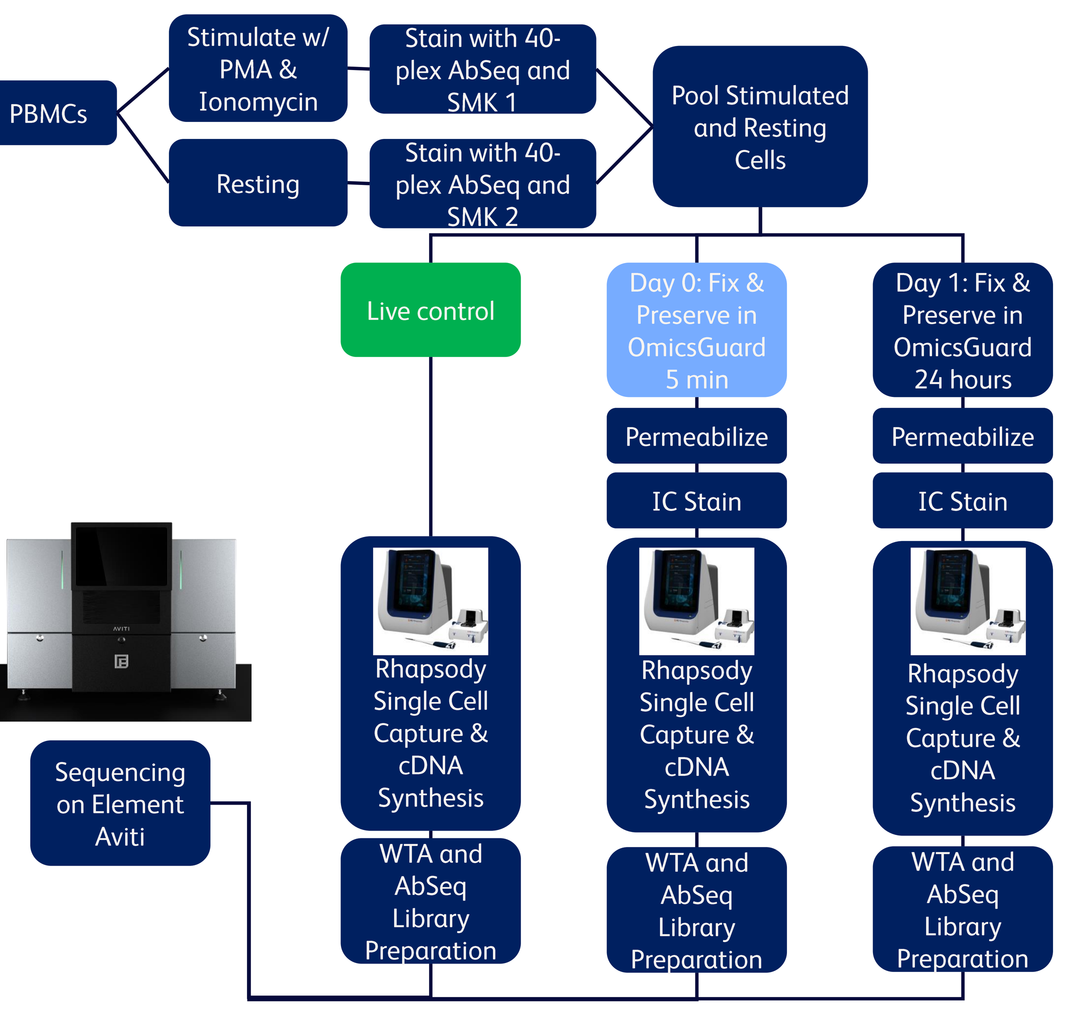
This experiment utilized a novel IC CITE-Seq assay that enables robust profiling of multiple IC protein targets in combination with mRNA and high-plex surface proteins. In this study, we stimulated human peripheral blood mononuclear cells (PBMCs) alongside a resting control. After stimulation, we stained the surface proteins with a 40-plex BD® AbSeq Panel, including the Immune Discovery Panel and human Single-Cell Multiplexing Kit (SMK). Resting and stimulated cells were pooled, and a portion of the sample pool immediately underwent cell capture as a live control using a BD Rhapsody™ single-cell microwell system. The other portion of the pool was stored in BD® OMICS-Guard Sample Preservation Buffer for either 5 minutes or 24 hours followed by permeabilization, IC staining with a 10-plex IC AbSeq Panel (50-plex total, surface and IC), and cells captured on the the same system.

We show IC protein detection for the expected positive targets, including pH2AX, actCaspase-3, and cPARP in the stimulated population, as well as T-Bet, Granzyme B and Helios in the resting lymphocytes. Analysis shows excellent correspondence between IC CITE-Seq protein and transcriptome for T-Bet, Granzyme B and Helios. The gene expression correlation for surface antigen and mRNA between the IC and live control each had R2 values > 0.9. The mRNA sensitivity for the IC sample compared to live control was within whole transcriptome analysis (WTA) assay variation at 86–89% and 95–97% median molecules and genes per cell.

Our data showed that the new IC CITE-Seq assay can provide rich single-cell multiomics information including mRNA and surface and intracellular proteins.

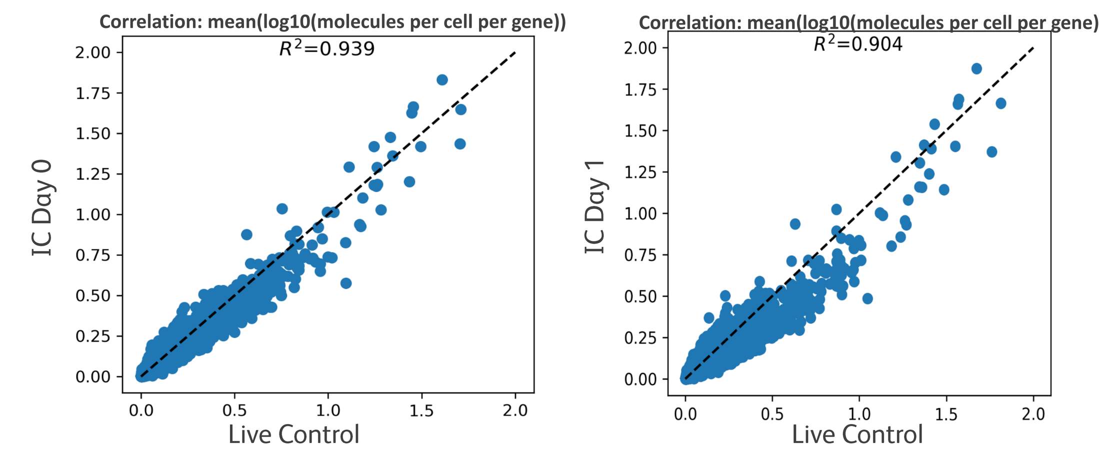
Methods

Thawed peripheral blood mononuclear cells (PBMCs) were either stimulated for 48-hours with phorbol 12-myristate 13-acetate (PMA) and Ionomycin or incubated at rest. After stimulation, the surface proteins were stained with a 40-plex BD® AbSeq Panel, including the Immune Discovery Panel and human Single-Cell Multiplexing Kit (SMK). Resting and stimulated cells were pooled, and a portion of the sample pool immediately underwent cell capture as a live control using a BD Rhapsody™ single-cell microwell system. The other portion of the pool was fixed and preserved in BD® OMICS-Guard Sample Preservation Buffer for either 5 minutes or 24 hours followed by permeabilization, IC staining with a 10-plex IC AbSeq Panel (50-plex total, surface and IC), and cell capture on the the same system. Cell capture beads were subsampled targeting 5000 cells. Libraries were prepared and sequenced on the Element Aviti system.



Results (1)

1A mRNA gene expression correlation R^2>0.9 for IC samples vs Live Control



1B WTA sensitivity for IC day 0 and day 1 samples > 86% of live control

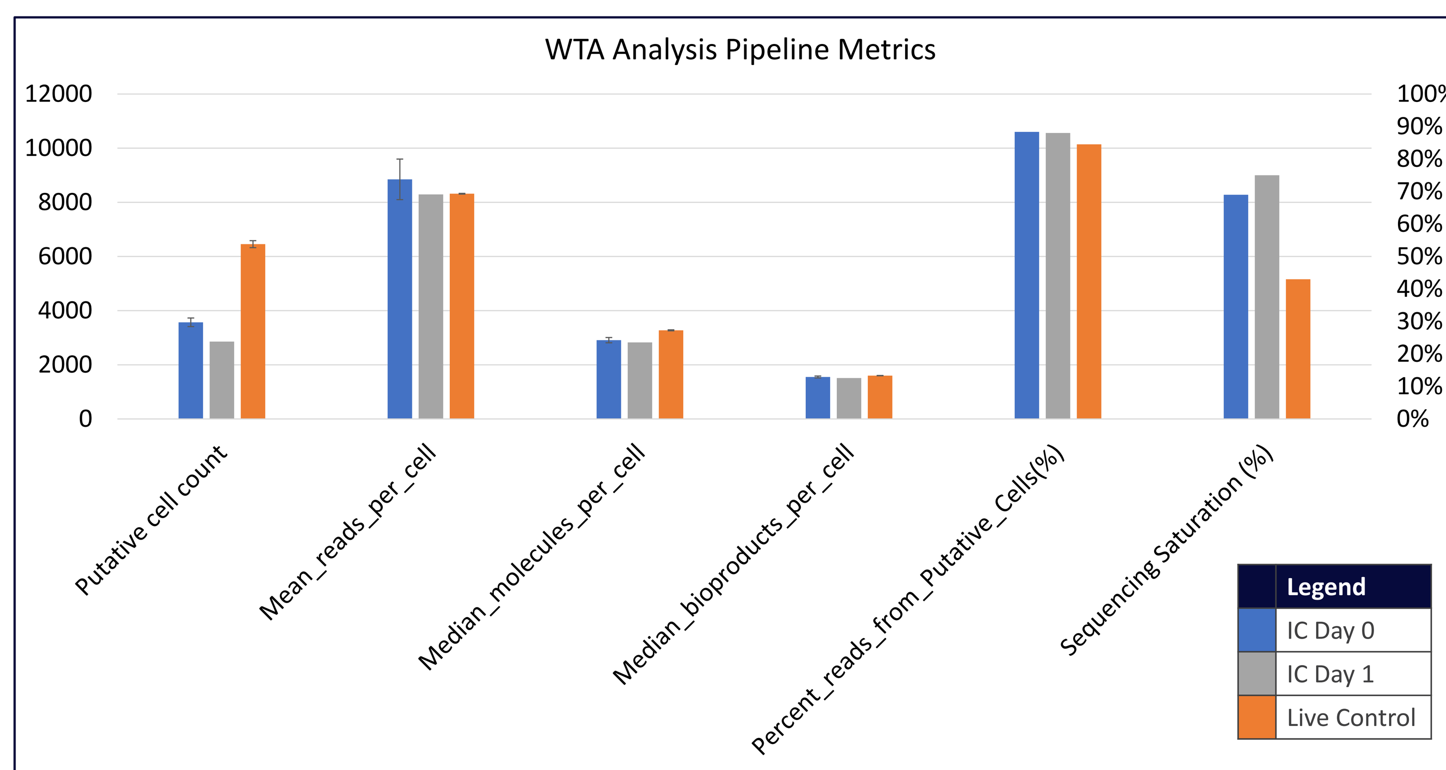
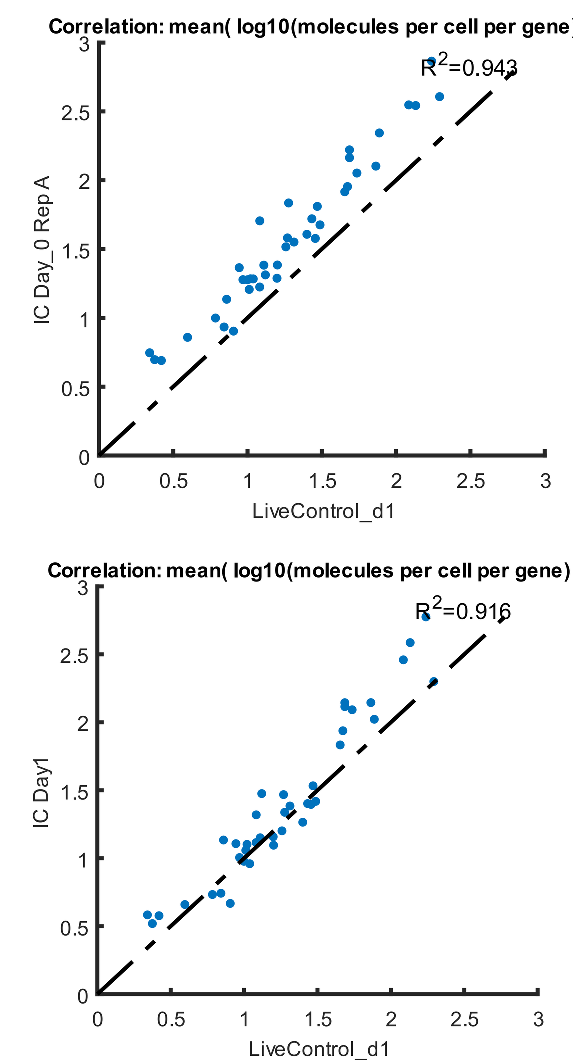


Figure 1A (top) shows the mRNA correlation for the IC Day 0 sample vs Live control (left) and the IC Day 1 sample vs Live control(right) both have R^2 > 0.9.
Figure 1B (bottom) shows the analysis metrics for the whole transcriptome analysis. Samples were normalized to the same number of reads per cell. Day 0 and Day 1 had 86% and 89% median molecules per cell compared to the live control and 95% and 97% of the mean bioproducts per cell compared to the live control.

1C High correlation (R^2 > 0.9) between surface AbSeq detection from IC samples versus live control for both IC day 0 (top) and IC day 1 (bottom)



1D Good surface and IC AbSeq specificity for all samples

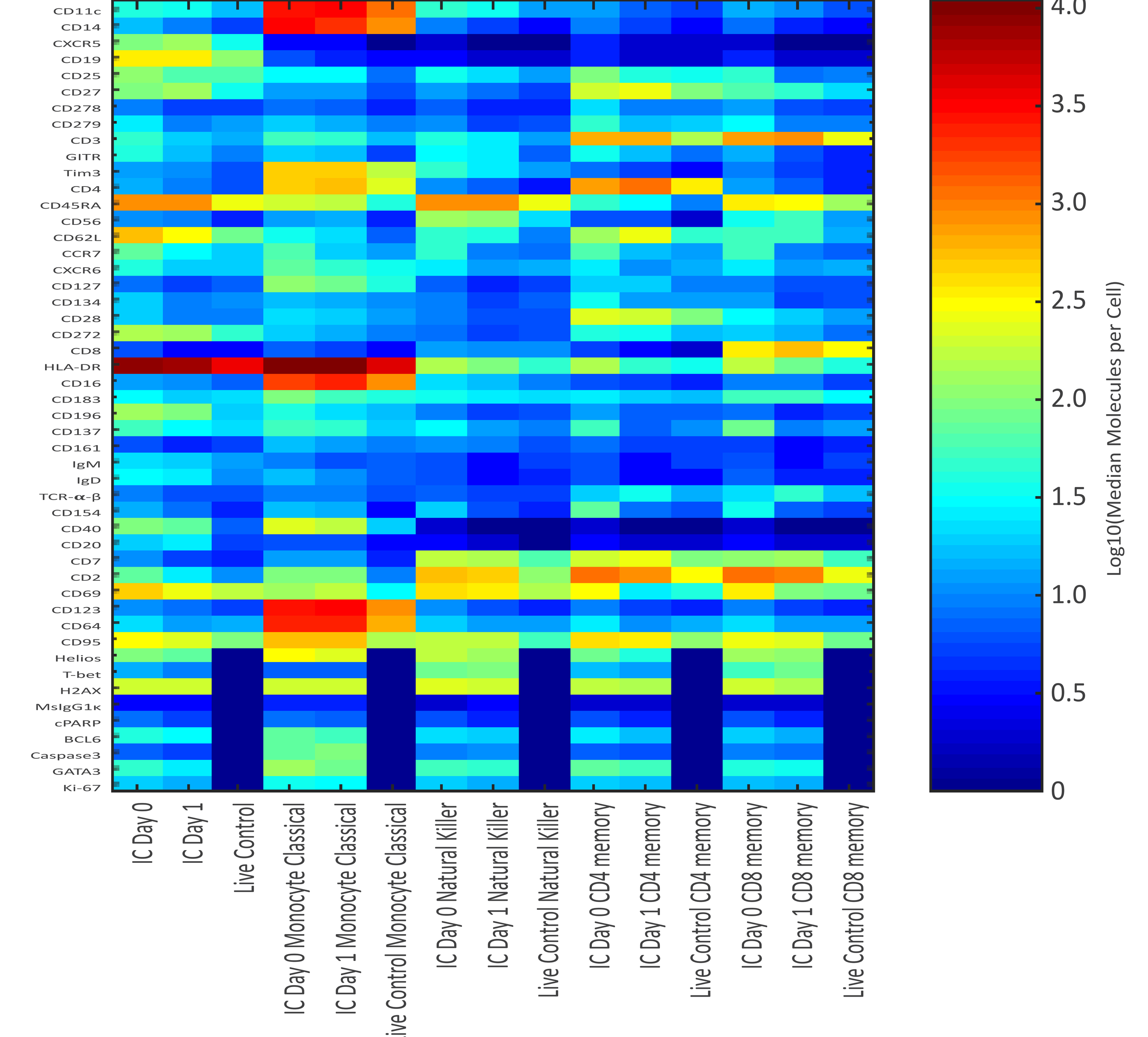
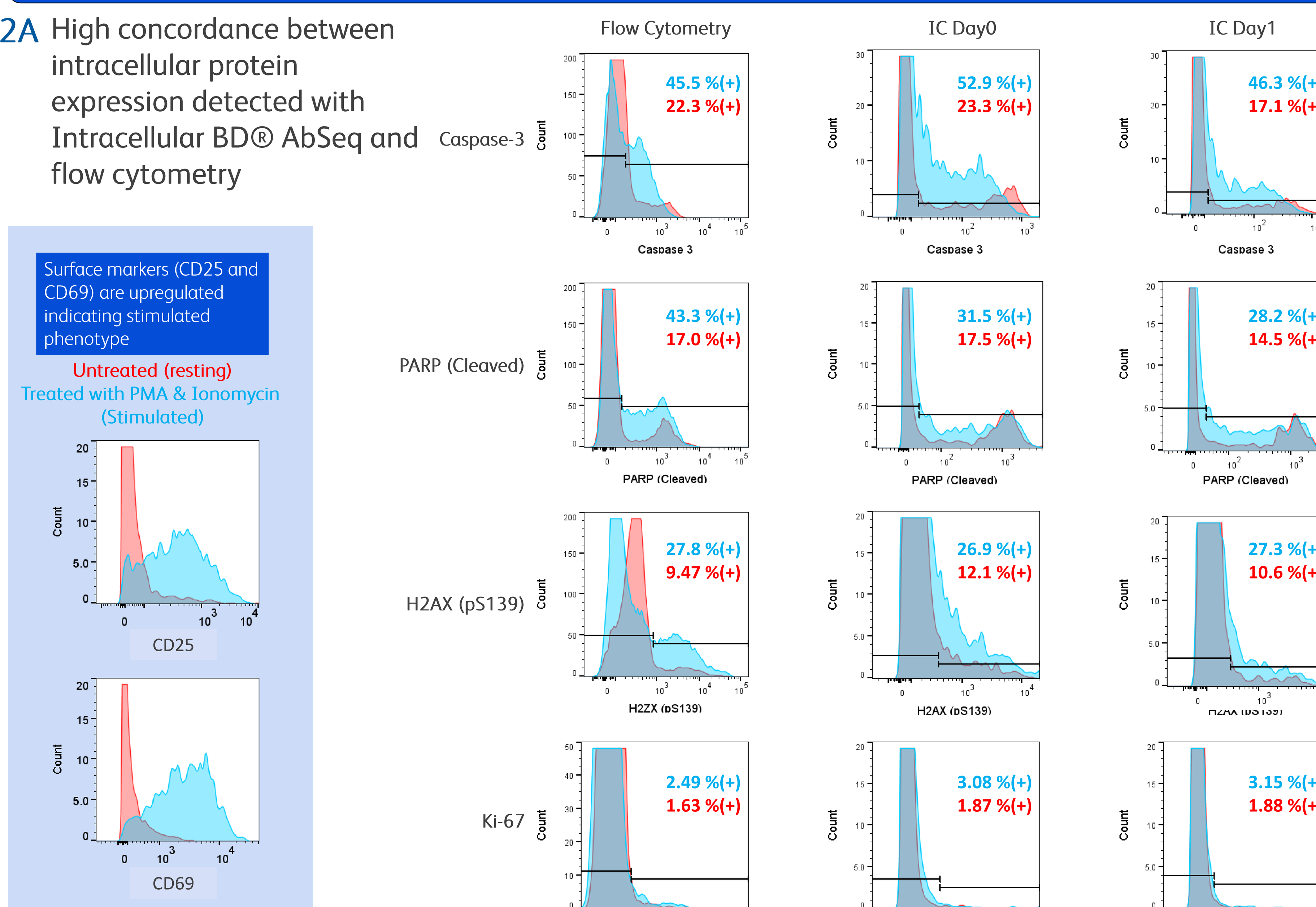


Figure 1D shows a heatmap displaying AbSeq expression for IC Day 0, IC Day 1, and Live control as log10(median molecules per cell) for a select subset of cell types. The IC samples show a similar expression pattern for surface markers compared to live control. This is further demonstrated with the correlation plots in figure 1C which have R2 values > 0.9.

Results (2)

2A High concordance between intracellular protein expression detected with Intracellular BD® AbSeq and flow cytometry



2B T-bet, Helios, and Granzyme B are expressed in expected subset of PBMC lymphocytes

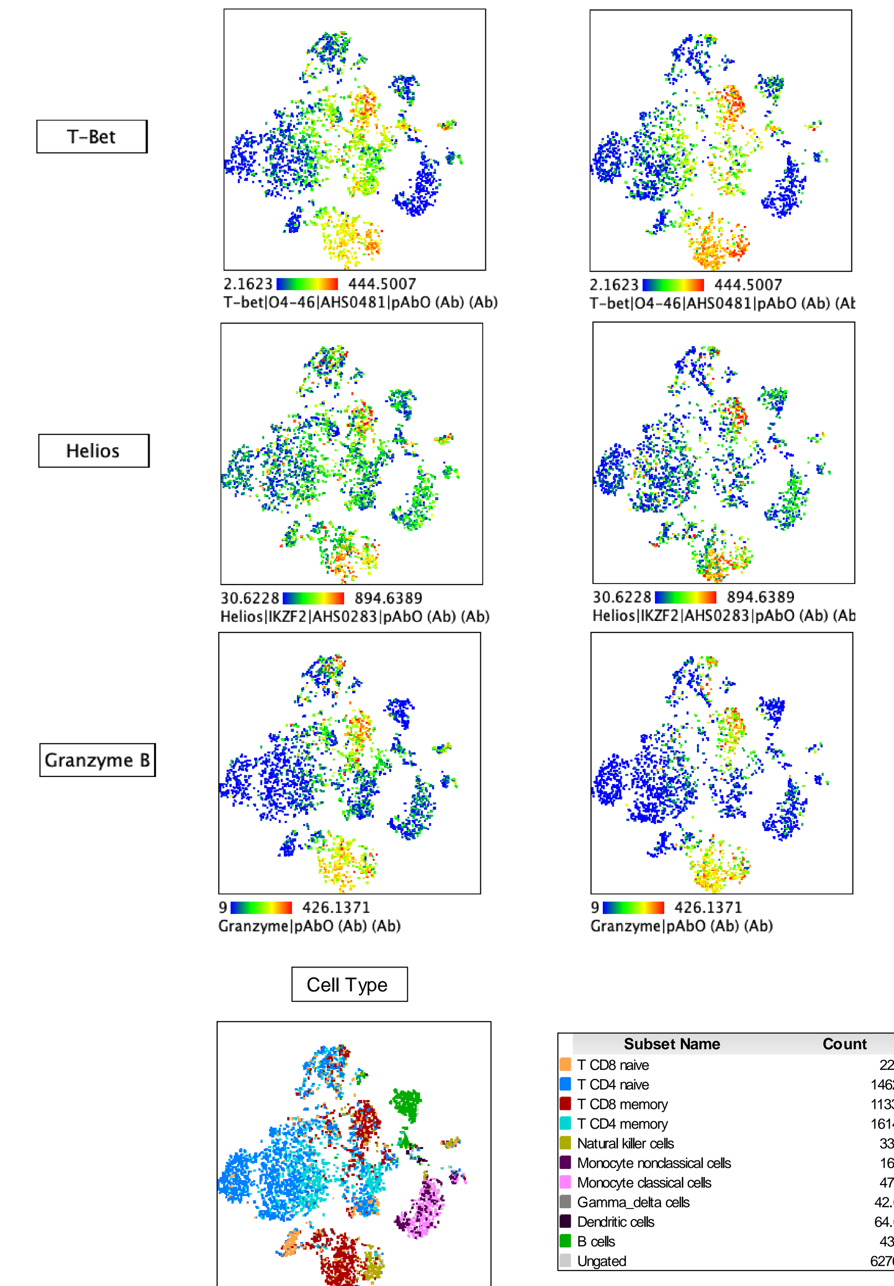


Figure 2A (above) shows histograms for intracellular AbSeq from resting and stimulated samples. Day 0 (middle column) and Day 1 (right column) show similar profiles and percent positive compared to flow cytometry controls (left column). Caspase-3, cPARP, and H2AX are apoptosis markers and show good signal in the stimulated samples. Ki-67 is a proliferation marker and wasn't expected to show strong signal with this stimulation system.
Figure 2B (right) shows tSNE plots overlaid with expression (in molecules per cell) for T-bet, Helios, and Granzyme B. Day 0 (left) and Day 1 (right) show similar expression and signal to noise. The bottom left panel shows the cell type as determined by the WTA analysis pipeline and demonstrates that the expression of the markers is on the expected populations of lymphocytes.

Conclusions

- The IC samples from Day 0 and Day 1 had excellent mRNA gene expression correlation versus the live control (R^2 > 0.9).
- WTA mRNA data from IC samples showed similar sensitivity compared to live control, with Day 0 and Day 1 having within 85% median molecules per cell and within 95% median bioproducts per cell compared to live control.
- Surface AbSeq detection correlation between the IC samples and the live control had and R^2 > 0.9
- The heatmap shows high specificity for AbSeq for IC samples and live control.
- The IC apoptotic markers accurately reflected stimulation status and corresponded to flow controls.
- T-bet, Helios, and Granzyme B were expressed in the expected lymphocyte populations and showed good agreement between Day 0 and Day 1.

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