MOLECULAR PIXELATION
DISSECTING THE SPATIAL ORGANIZATION OF 80 SURFACE PROTEINS IN SINGLE IMMUNE CELLS

Hanna van Ooijen[1], Max Karlsson[1], Vincent van Hoef[1], AnnaLotta Schiller[1], Divya Thiagarajan[1], Louise Leijonancker[1], Maud Schweitzer[1], Prajakta Sathe[1], Steve Glavas[1] Annika Branting[1], Alvaro Martinez Barrio[1], Filip Karlsson[1], Simon Fredriksson[1,2]

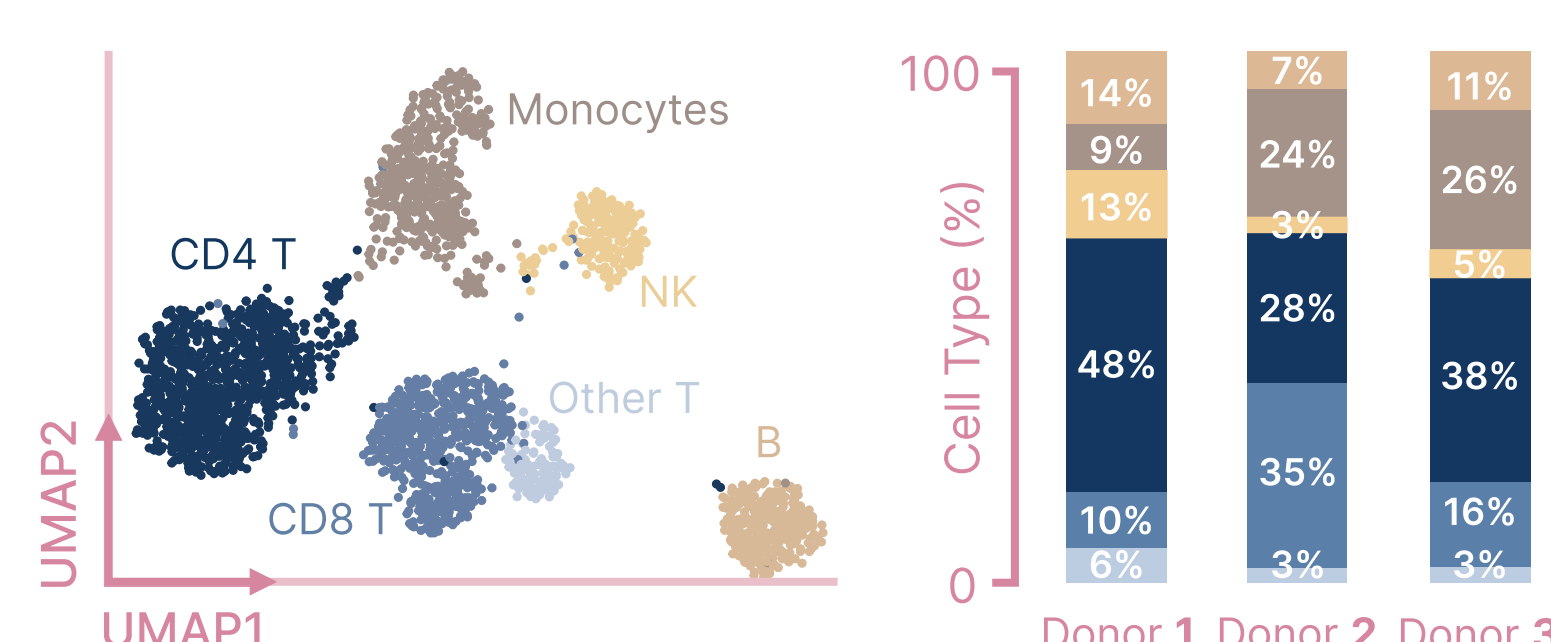[1] Pixelgen Technologies AB, Stockholm, Sweden
[2] Royal Institute of Technology, Department of Protein Science, Stockholm, Sweden
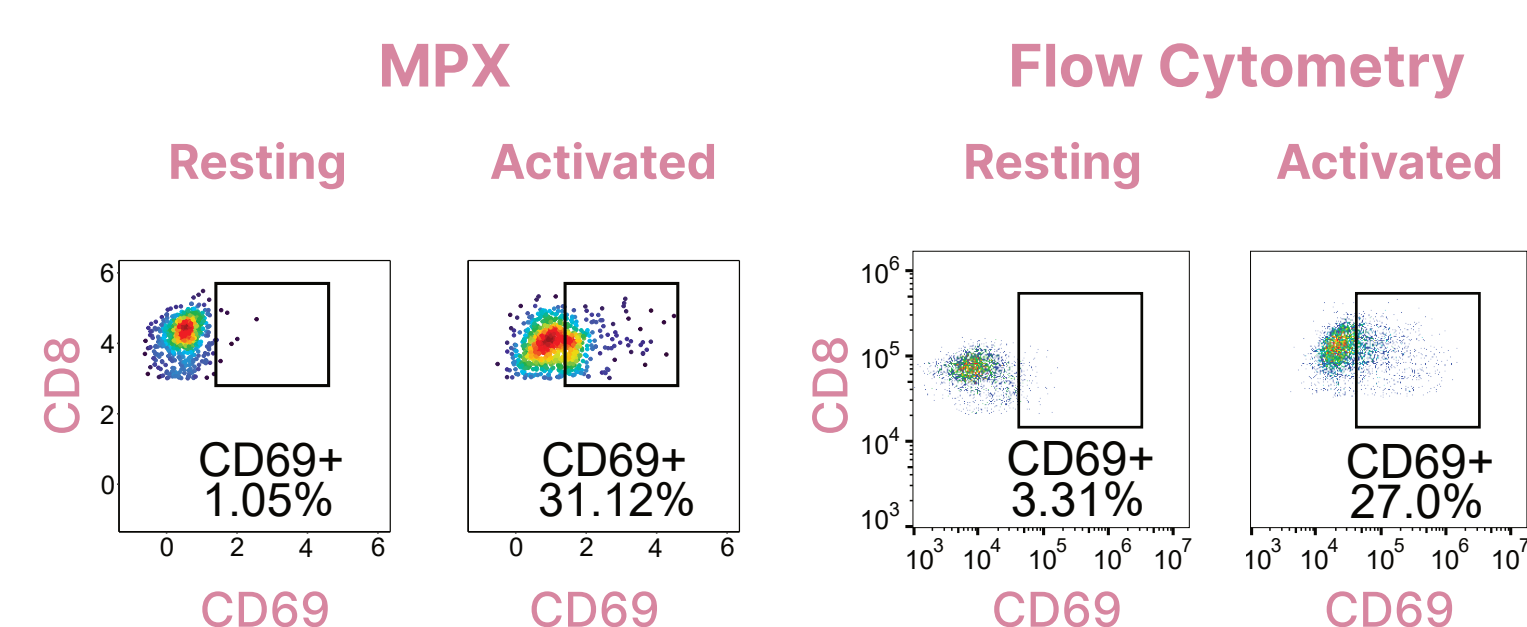
PIXELGEN TECHNOLOGIES

MOLECULAR PIXELATION (MPX) UNCOVERS LARGE-SCALE CHANGES IN THE MEMBRANE PROTEIN ARCHITECTURE OF ACTIVATED T CELLS

T cell activation is traditionally measured through the detection of cytokine production or the expression of activation markers including CD69 and CD25. In this study, we harness the high multiplexing capability and the spatial resolving power of the new technology, Molecular Pixelation (MPX), to show that T cell activation is associated with not only large-scale alterations in protein abundance levels, but also with profound re-organization of membrane proteins. Importantly, this protein re-organization is seen only in a fraction of cells and is not correlated with the expression level, demonstrating how spatial protein analysis can be used for deeper phenotyping and improved characterization of cellular states.
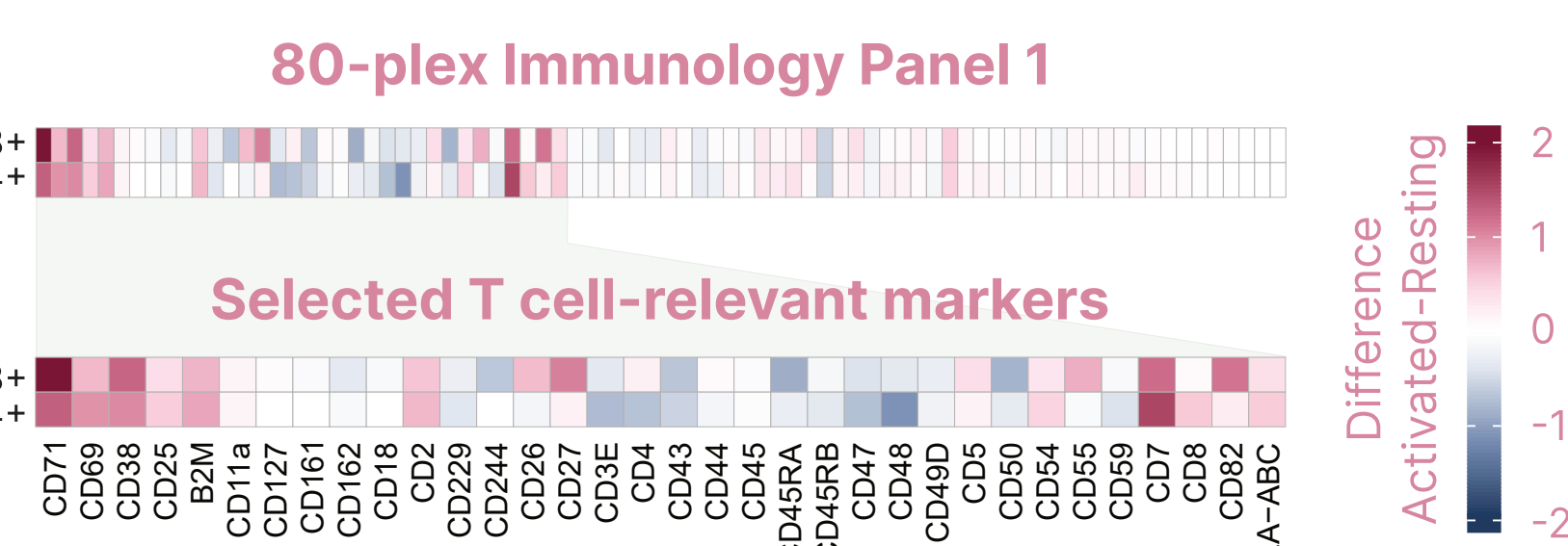
MOLECULAR PIXELATION (MPX) WORKFLOW

Binding of Antibody-Oligo Conjugates	Pixelation A	Pixelation B	Library Prep	Sequencing	Data Analysis
Introducing unique protein barcodes - 80-plex antibody staining of fixed cells in solution	Creating local neighborhoods of proteins in spatial proximity	Connecting the local neighborhoods	Next-generation sequencing library preparation	Next-generation sequencing	Data analysis using Pixelator - Reconstruction of 3D cell surface maps

A new multifaceted technology that provides highly multiplexed, single-cell information about membrane protein abundance, clustering and colocalization.

WANT TO LEARN **MORE?**

I. MPX IDENTIFIES ACTIVATION-INDUCED CHANGES IN **SURFACE PROTEIN ABUNDANCE**

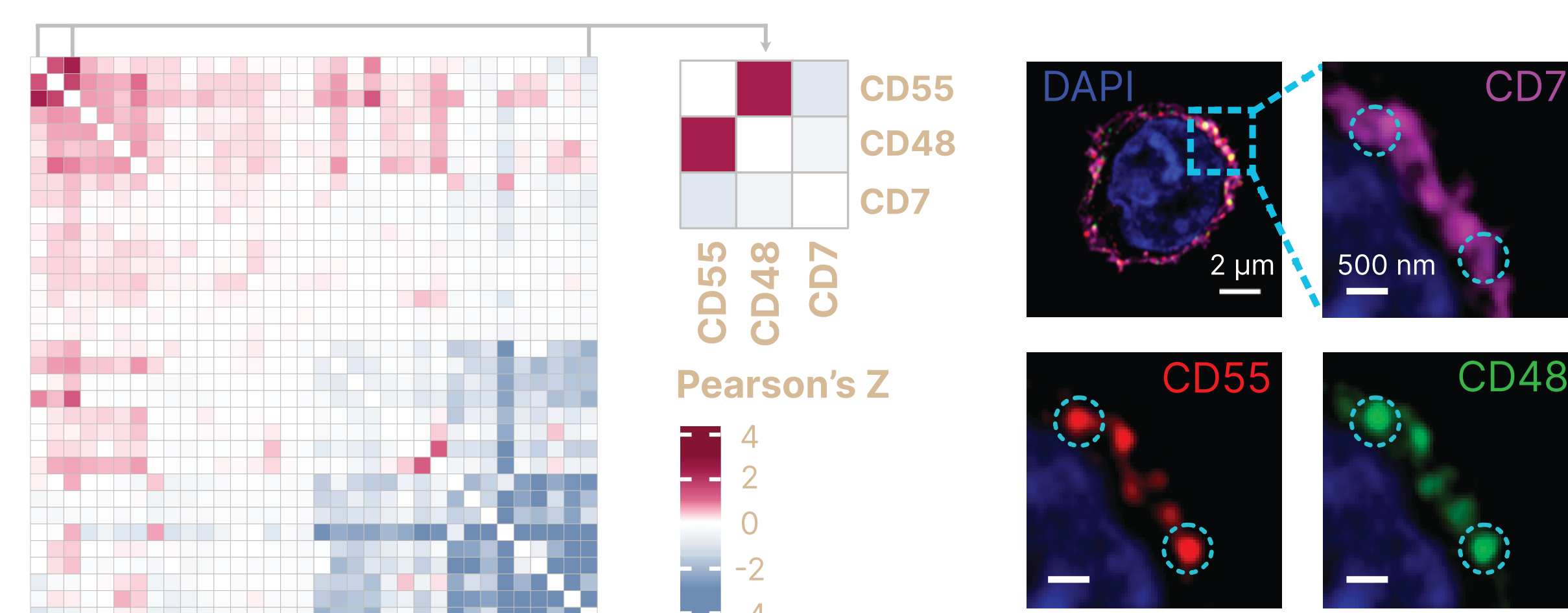The 80-plex Human Immunology Panel I enables the identification of major cell types found in human PBMCs.

80-plex Immunology Panel 1

Selected T cell-relevant markers

More than 30 of the panel markers are expressed in T cells. PHA-activation induces changes in the abundance levels of a large number of these proteins, including increased expression of activation markers CD69, CD71, CD38 and CD25.
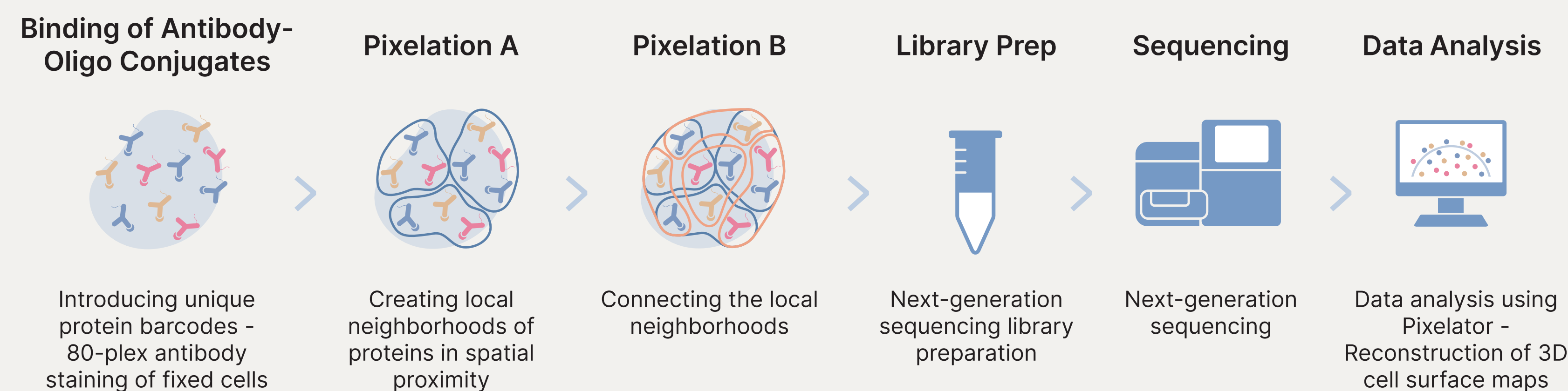
MPX / **Flow Cytometry**

Resting / Activated / Resting / Activated

CD69+ 1.05% / CD69+ 31.12% / CD69+ 3.31% / CD69+ 27.0%

CD69-positive CD8 T cells are detected equally well using MPX as compared to flow cytometry.

II. THE **MPX POLARITY SCORE** UNFOLDS NEW FEATURES OF T CELL ACTIVATION

CD82 Abundance / **CD82 Polarization**

Resting / Activated

Using the MPX polarity measurement, we discovered that a fraction of T cells polarize CD82 into cell surface clusters following activation. Remarkably, this phenotype is not correlated to changes in CD82 abundance, which suggests the existence of a distinct cell state among activated T cells.

Resting T Cell / **Activated T Cell**

CD82 G*i
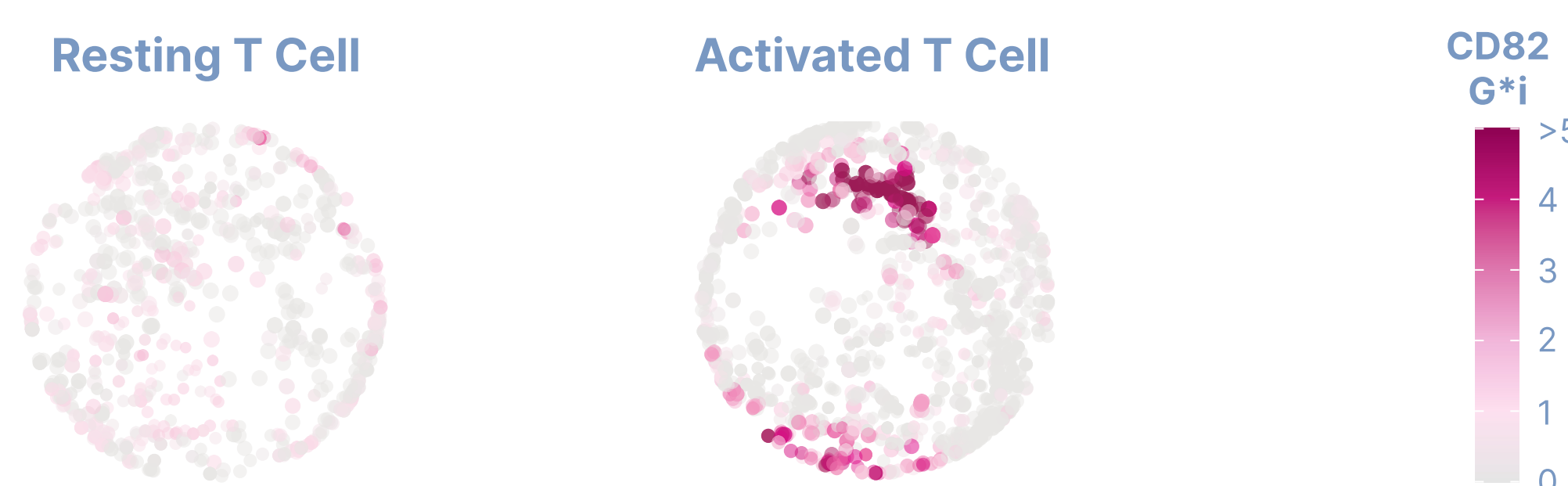
MPX 3D cell visualization highlights how activated T cells present large CD82 clusters.

Resting T Cell / **Activated T Cell**

CD8 DAPI CD82
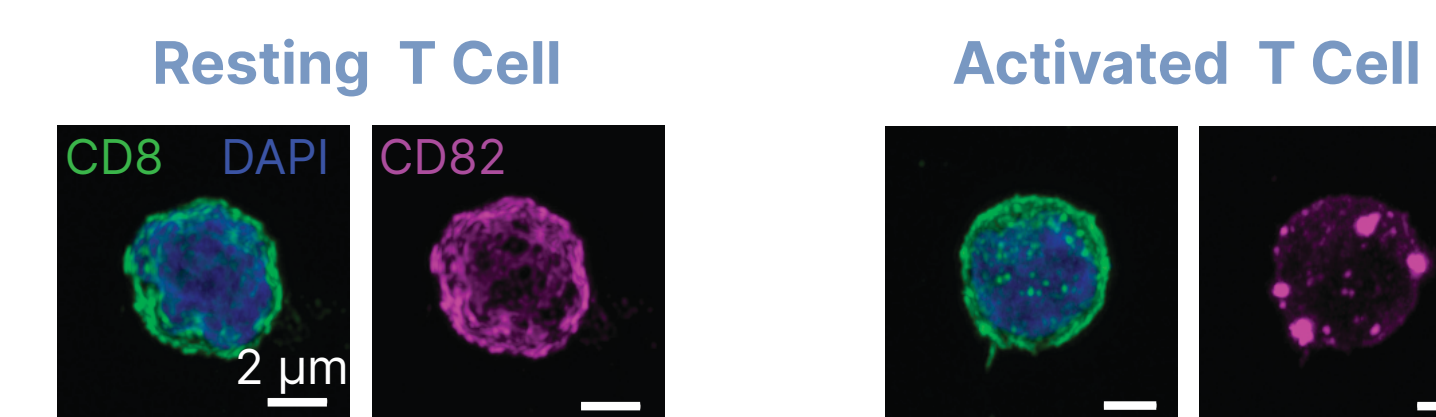
3D confocal microscopy confirmed the observed activation-induced clustering of CD82.

III. THE **MPX COLOCALIZATION SCORE** REVEALS MULTIPROTEIN COLOCALIZATION NETWORKS

CD55 CD48 CD7

Pearson's Z

Protein-protein colocalization can have drastic effects on cellular function. Using the MPX colocalization score, we investigated which proteins colocalize in activated T cells. As an example, we observed a positive colocalization between two GPI-coupled proteins, CD55 and CD48, both previously shown to associate with lipid rafts.

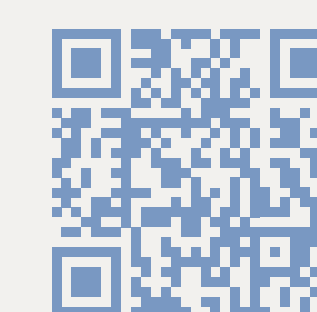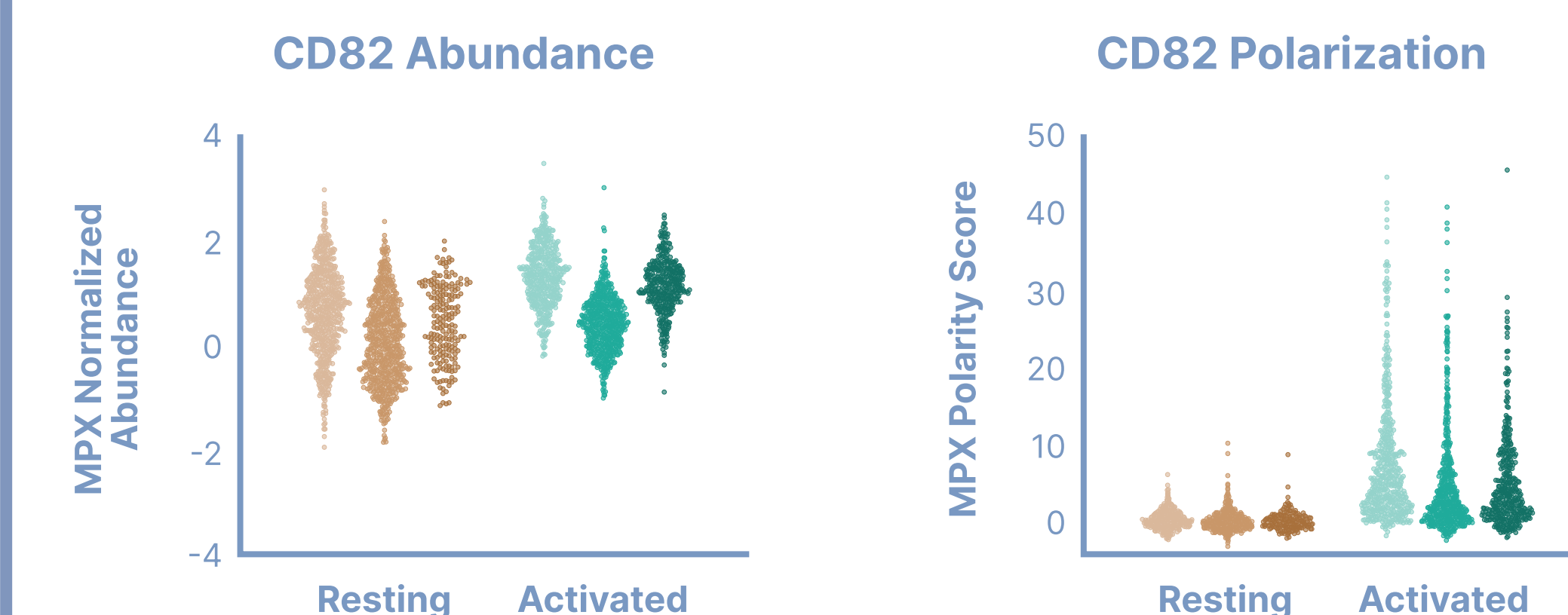DAPI / CD7 / CD55 / CD48

2 µm / 500 nm

Using Airyscanning super-resolution microscopy, we found that CD55 and CD48 colocalized in nanoclusters. Meanwhile, the control marker CD7 was uniformly distributed on the T cell surface.

Activated T Cell

SUMMARY

- MPX can be efficiently applied to examine **immune cell states**, including T cell activation

- The 80-plex panel allows **cell type characterization**, and more than 30 markers display **differential abundance levels** in activated compared to resting T cells

- MPX demonstrates the **existence of a distinct T cell state** presenting clustered CD82

- MPX unravels **multi-protein colocalization networks**, including the enrichment of CD55 and CD48 in nanoclusters at the surface of activated T cells

- By simultaneously mapping the abundance and spatial organization of membrane proteins in single cells, **MPX offers a unique angle to study the complex phenotypes of immune cells**