

# Discovery to targeted assays for fingerprinting flow-sorted immune from healthy human donors

Ariana E. Shannon<sup>1</sup>, Robert Davenport<sup>2</sup>, Yoshi Koguichi<sup>2</sup>, Zihai Li<sup>2</sup>, Mark Rubinstein<sup>2</sup>, Amanda Berg<sup>3</sup>, Gary Valaskovic<sup>3</sup>, Helena Svobodova<sup>3</sup>, Brian C. Searle<sup>1</sup>

<sup>1</sup>Division of Quantitative Health Sciences, Mayo Clinic, Rochester, Mn, <sup>2</sup>New Objective, Inc., Boston, MA, <sup>3</sup>Pelotonia Institute for Immuno-Oncology, Ohio State University, Columbus, OH

## OBJECTIVE

Achieving true precision medicine using mass spectrometry requires the ability to rapidly, confidently, and reproducibly develop fit-for-purpose targeted assays for proteins capable of distinguishing between healthy and disease states. Prior to committing to large-scale studies on precious samples, we need to establish which panels of proteins are important to specific biological processes for healthy tissues in certain cell types.

To support the development of fit-for-purpose targeted assays, we aim to establish baseline proteomic profiles of flow-sorted immune cells from healthy donors and design protein "fingerprint" panels for comprehensive cell-type identification.

## METHODS

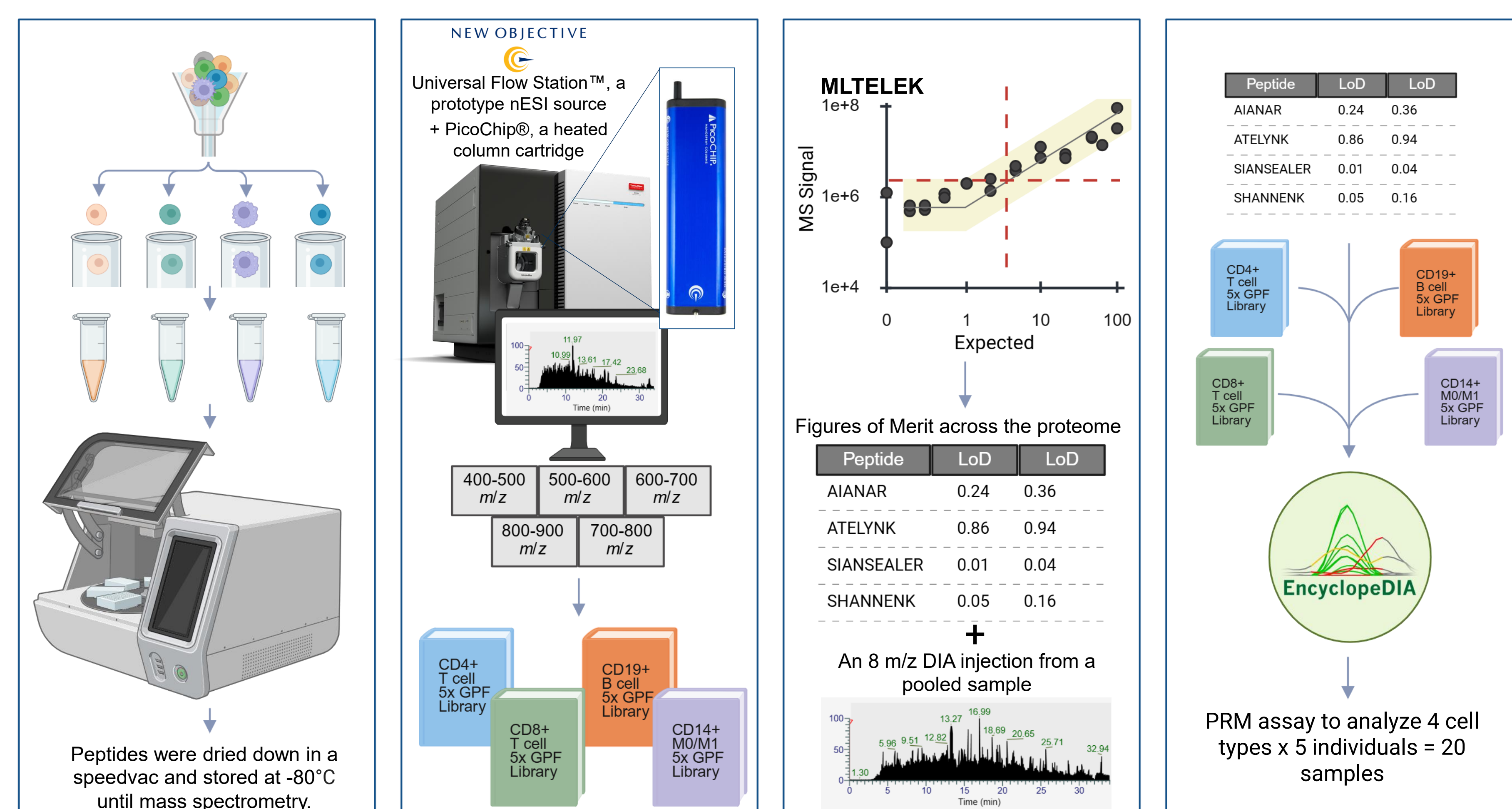


FIGURE 1: Discover-to-targeted workflow

## PROFILING IMMUNE CELLS

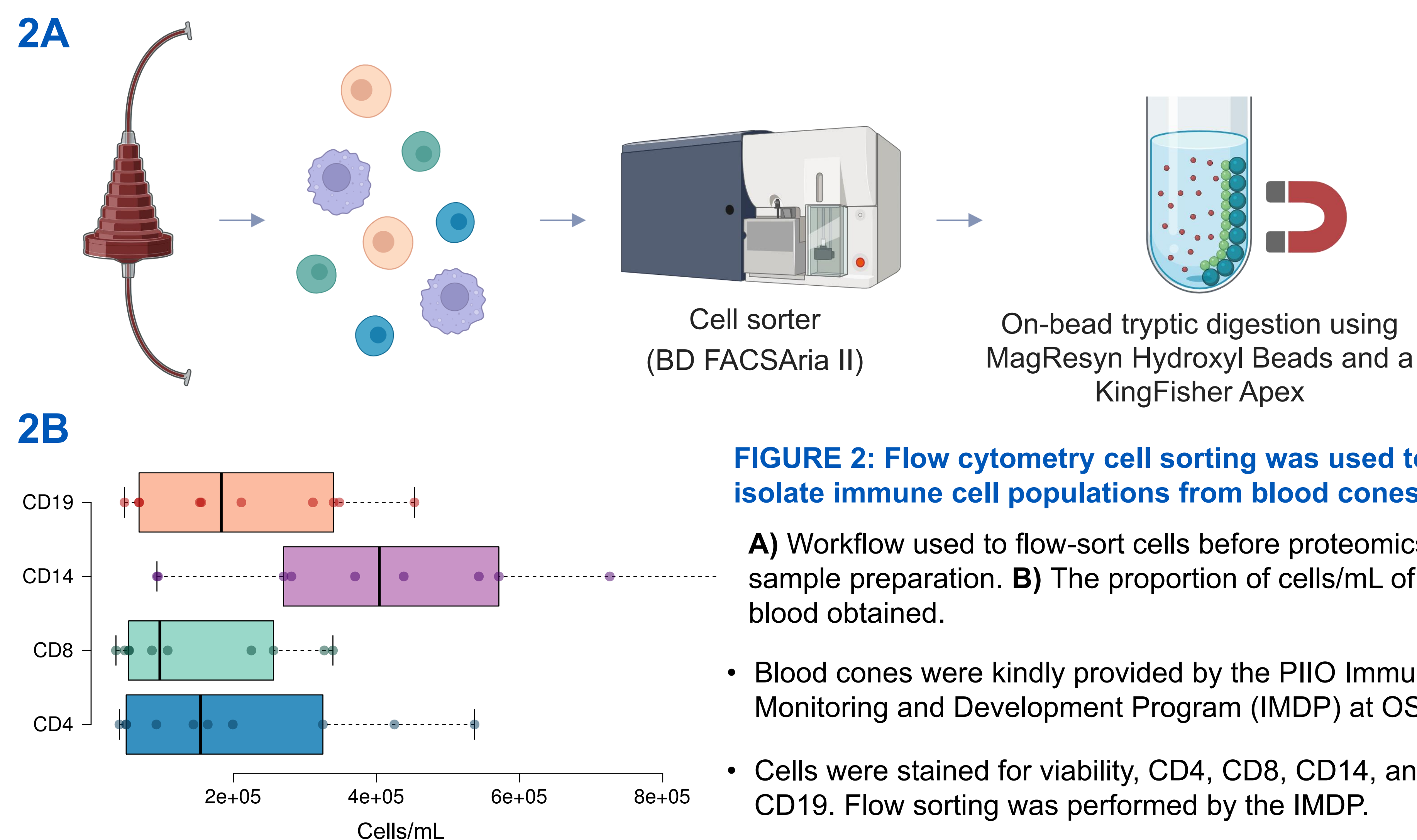


FIGURE 2: Flow cytometry cell sorting was used to isolate immune cell populations from blood cones

A) Workflow used to flow-sort cells before proteomics sample preparation. B) The proportion of cells/mL of blood obtained.

- Blood cones were kindly provided by the PIIO Immune Monitoring and Development Program (IMDP) at OSU.
- Cells were stained for viability, CD4, CD8, CD14, and CD19. Flow sorting was performed by the IMDP.

## REFERENCES

- Lagasse, E. and R G Clerc. "Cloning and expression of two human genes encoding calcium-binding proteins that are regulated during myeloid differentiation." *Molecular and cellular biology* vol. 8,6 (1988): 2402-10. doi:10.1128/mcb.8.6.2402-2410.1988
- Carr SA, Abbatiello SE, Ackermann BL, et al. Targeted peptide measurements in biology and medicine: best practices for mass spectrometry-based assay development using a fit-for-purpose approach. *Mol Cell Proteomics*. 2014;13(3):907-917. doi:10.1074/mcp.M113.036095

Funding provided by the OSU's PIIO Priority Research Funding Program and NIH NIGMS Grant R35 GM150723-04.

## BUILDING TARGETED ASSAYS

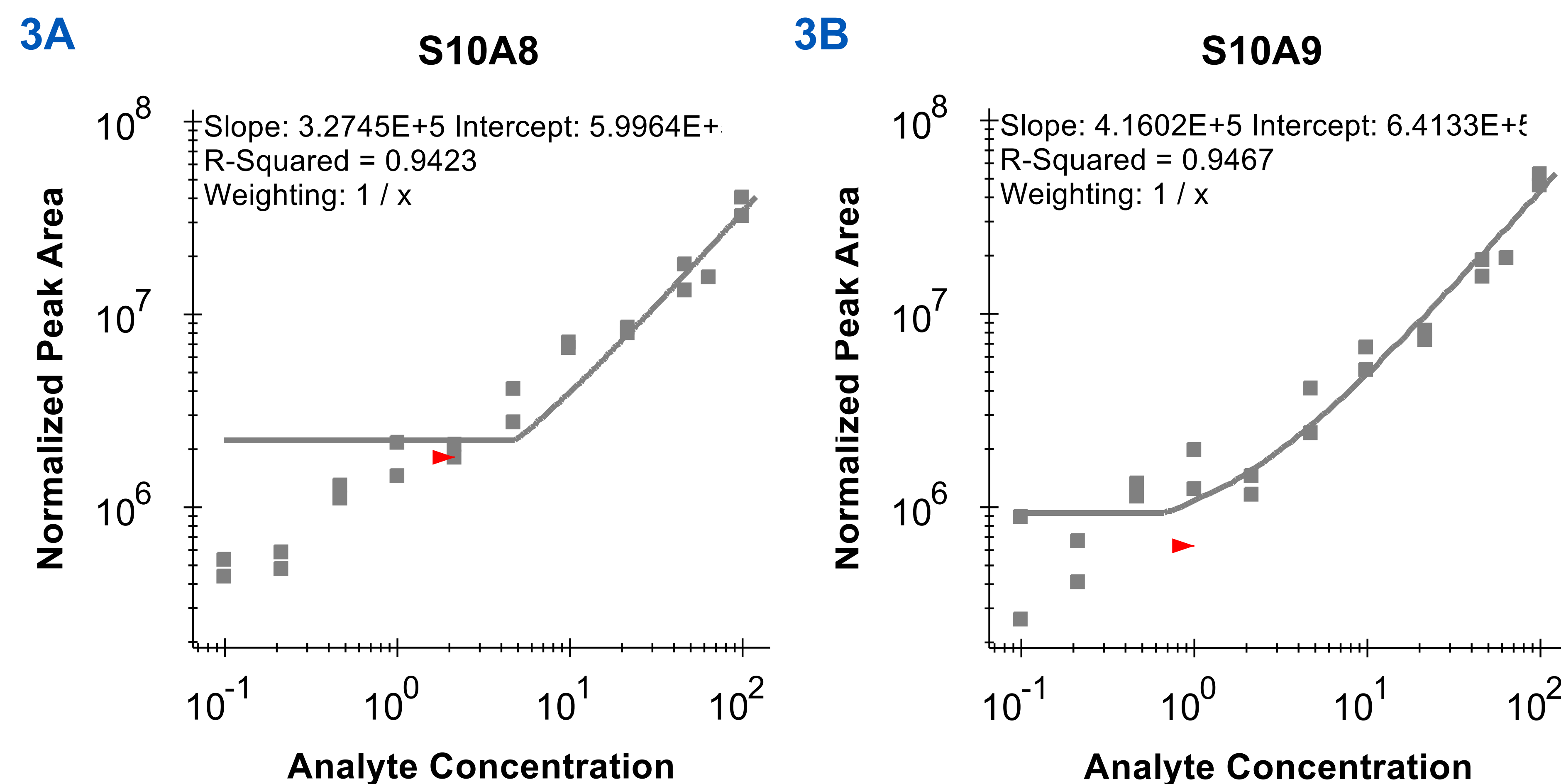


FIGURE 3: Matrix-matched calibration curves to obtain figures-of-merit on sentinel proteins

We performed a matrix-matched calibration curve. An aliquot of pooled sample was dimethyl labelled, and all samples underwent a C18 cleanup. The calibration curves were processed in Skyline to calculate figures-of-merits. The proteins shown are A) S10A8 and B) S10A9 proteins (from the S100A8 and S100A9 genes respectively). These proteins form a heterodimer which mediates an immune response and inflammation in human cells derived from a myeloid lineage.<sup>1</sup>

## CELL TYPE DIFFERENTIATION

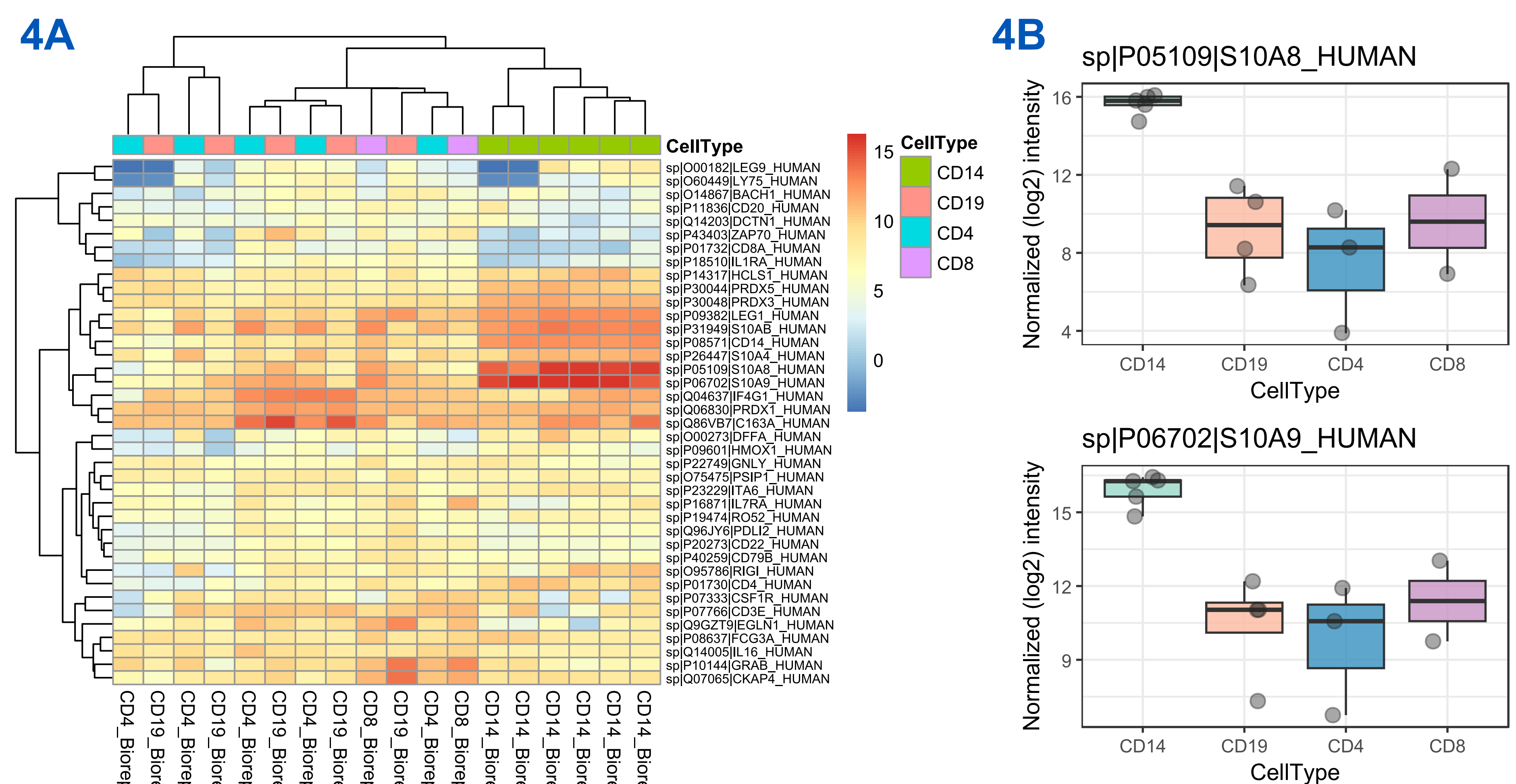


FIGURE 4: TARGETED EXPRESSION OF SENTINEL PROTEINS ACROSS HEALTHY DONORS

A) Heatmap from PRM across a subset of samples analyzed for PRM. The myeloid lineage cells cluster apart from lymphoid lineage cells. B) S100A9 and S100A8 protein expression across individual replicates measured with a PRM assay. The assay targeted 265 peptides, or 135 proteins.

## CONCLUSIONS AND FUTURE WORK

We created a DIA library of peptides detected and quantified across human CD19+ B cells, CD14+ macrophages/monocytes, CD4+ and CD8+ T cells from blood cones available in-house. We can use this library and corresponding sensitivity data (figures-of-merits from calibration curves) to select peptides for "on-the-fly" targeted assays.

Additional assay refinement will be performed to maximize the number of sentinel proteins included in the assay. Further improvement will be done to automatically process calibration curves and identify figures of merits without manual refinement.