

Long-Term Whole Blood Stabilization Preserves Immune Phenotypes for Multiparameter Cytometry



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Introduction

Multiparameter cytometry enables comprehensive characterization of immune populations and phenotypes in clinical samples. However, conventional whole blood workflows typically require processing within 24–72 hours of collection, creating logistical challenges for multicenter clinical trials and potentially introducing variability associated with sample shipment and processing delays.

TokuKit is a three-step, centrifuge-free whole blood preservation method designed to support sample storage at -80°C for up to 24 months, enabling samples to be stabilized at the collection site and analyzed later at a centralized laboratory.

To evaluate long-term preservation of immune phenotypes, whole blood from three healthy donors was analyzed fresh or following TokuKit stabilization and storage for 1, 3, 9, 15, 18, and 24 months using a 41-marker mass cytometry panel supporting analysis of up to 735 immune cell subsets and states. At 24 months, frequencies across 36 immune cell populations remained highly concordant with same-week stabilized samples ($R > 0.99$), while functional marker-defined subsets demonstrated similarly strong concordance ($R = 0.96$). These data support evaluation of long-term whole blood stabilization as an approach for centralized and longitudinal immune profiling.

Methods

Whole blood from three healthy donors was collected into sodium heparin or EDTA vacutainers. For the longitudinal stability assessment, EDTA vacutainers were used. For the fresh versus fixed comparison, both collection types were evaluated.

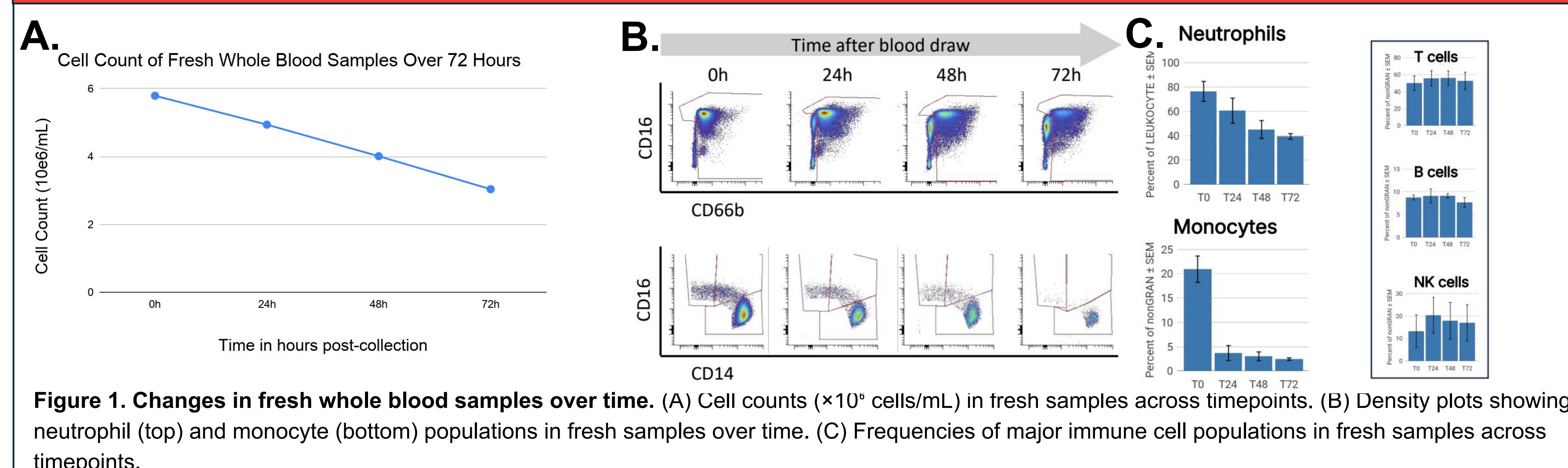
Live samples: Whole blood was diluted in ACK lysis buffer to lyse red blood cells and stained with a 33-marker antibody cocktail.

Fixed samples: Whole blood was fixed within 2 hours of collection using TokuKit and frozen at -80°C in 2 mL aliquots. Samples were evaluated at 1, 3, 9, 15, 18, and 24 months post-fixation. Upon thawing, cells were labeled with a 20-plex palladium barcode, pooled, and stained with the 41-marker antibody cocktail. Cells were labeled with iridium intercalator and acquired on a Helios® mass cytometer (Standard BioTools).

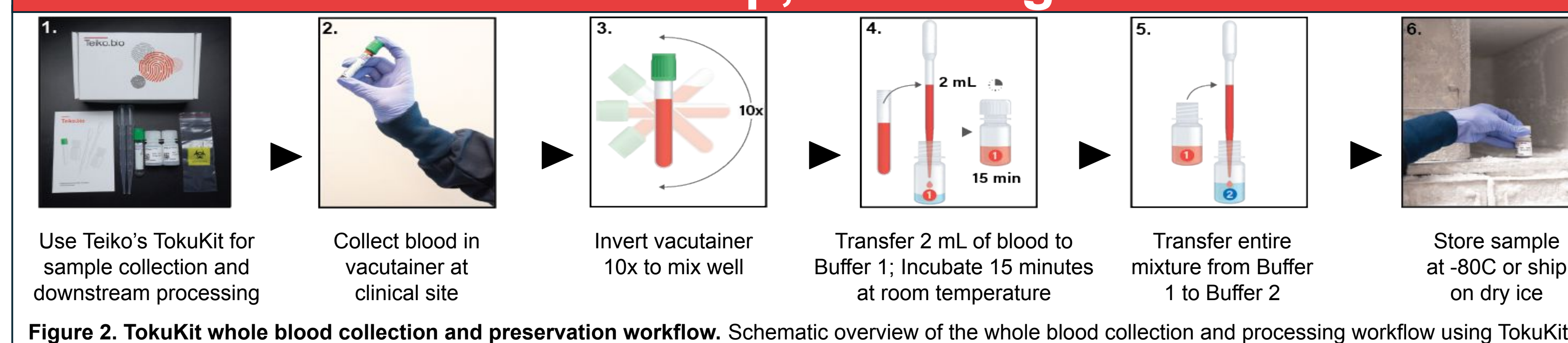
Panel: The 41-marker panel profiles major immune cell lineages and functional states across up to 735 subsets. Populations and subsets with event counts below 100 at any donor-timepoint combination were excluded. No viability dye was included.

Analysis: FCS files were normalized using EQ bead-based batch normalization and debarcoded prior to analysis in CellEngine (CellCarta). Cell populations were identified by manual gating. Phenotypic population frequencies were calculated as a percentage of non-granulocyte populations. Functional marker frequencies were calculated as a percentage of the parent population. Approximately 500,000 to 700,000 total lymphocytes were analyzed per donor per timepoint. Pearson correlation coefficients were calculated between T=0 and T=24 month frequencies across all donor-marker combinations meeting the 100-event threshold.

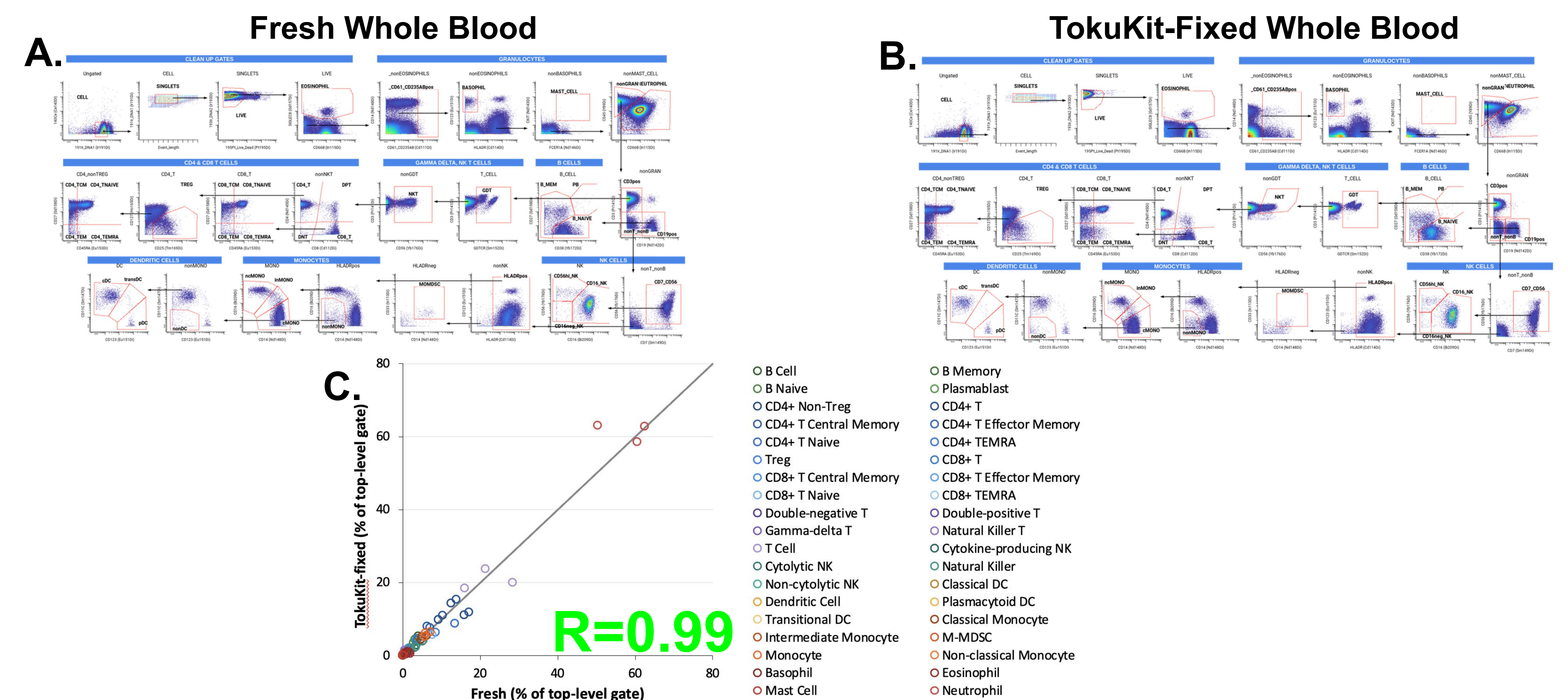
Fresh Samples Show Significant and Non-Uniform Degradation



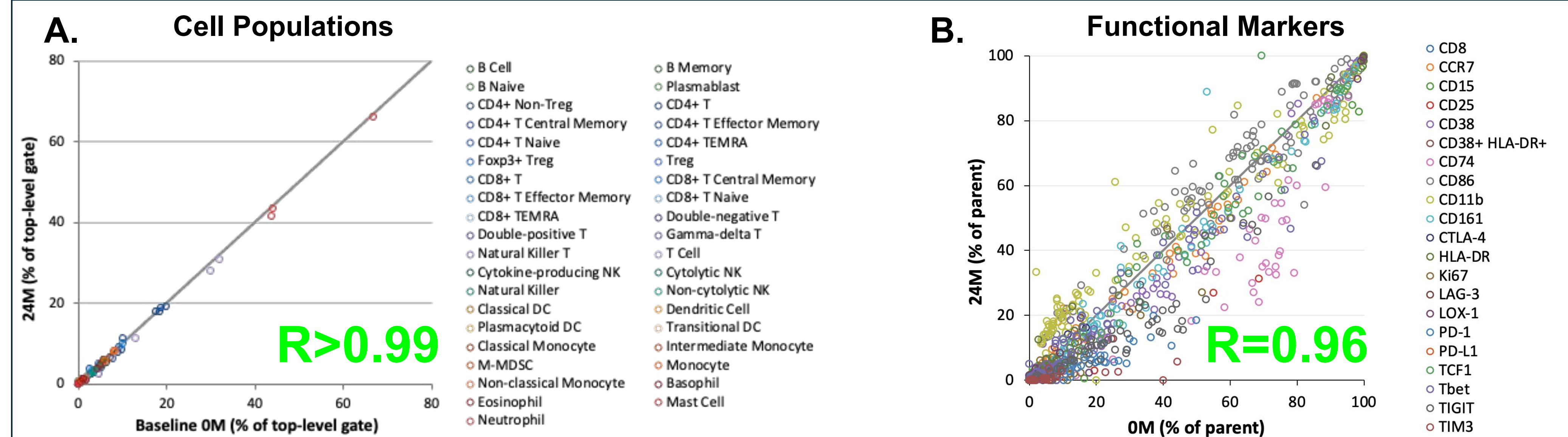
TokuKit is a three-step, centrifuge-free workflow



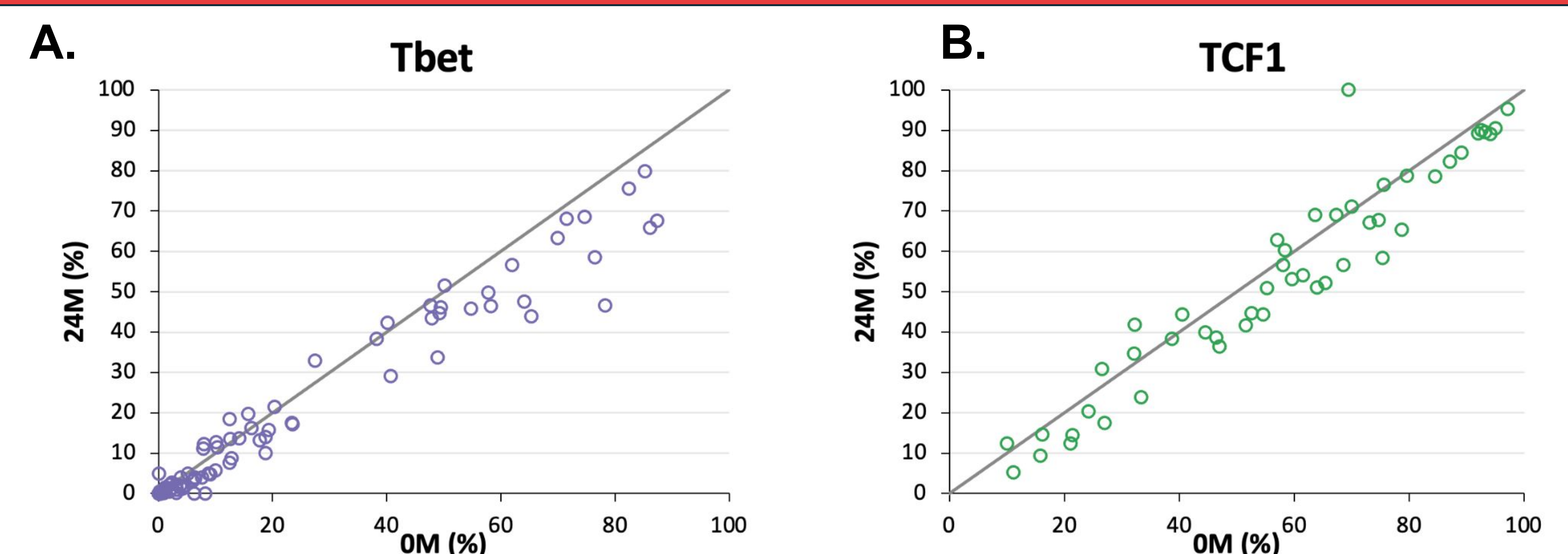
Fresh vs. Fixed Comparison



TokuKit Preserves Cell Populations and Functional Markers up to 24 months



TokuKit Preserves Intracellular markers



Conclusions and Future Directions

- TokuKit enables a scalable workflow for longitudinal and multicenter immune monitoring
 - Eliminates Bias in Live processing and reduced site to site variability
 - Enables consistent longitudinal studies

Explore TokuKit: teiko-labs.com/tokukit | teiko-labs.com | carly@teiko-labs.com