

Cell Mimics Provide Robust Controls for Memory T Cell Phenotyping Panels

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BACKGROUND AND AIMS

Accurate identification of memory T-cell subsets in flow cytometry requires robust and reproducible controls. Traditional use of peripheral blood mononuclear cells (PBMCs) as controls presents challenges, including lot-to-lot variability, limited stability, and batch-dependent marker expression. To address these limitations, we developed synthetic cell mimics for CD4+ and CD8+ naive, stem central memory, central memory, and effector memory subsets using biomarkers and viability components. We also added a surrogate CAR extracellular domain (ECD) to mimic memory phenotypes on CAR-T products. These synthetic mimics offer a reliable, stable alternative for fluorescence minus one (FMO) analysis and immunological assay standardization.

INTRODUCTION

T Cell Memory Mimic™ is engineered to mimic lymphocytes' forward and side scatter and is loaded with biomarkers commonly used to define memory phenotypes. These markers include CD45RA, CD45RO, CCR7, and FAS, in addition to CD3, CD4, and CD8 for identification of helper and cytotoxic T cells. Memory populations were also loaded with the scFv of FMC63, the clone from which many CD19-specific chimeric antigen receptors, or CARs, are derived (Fig 1). The product is intended for identification of different memory subset populations via flow cytometry and the representative gating scheme for population identification is depicted (Fig 2).

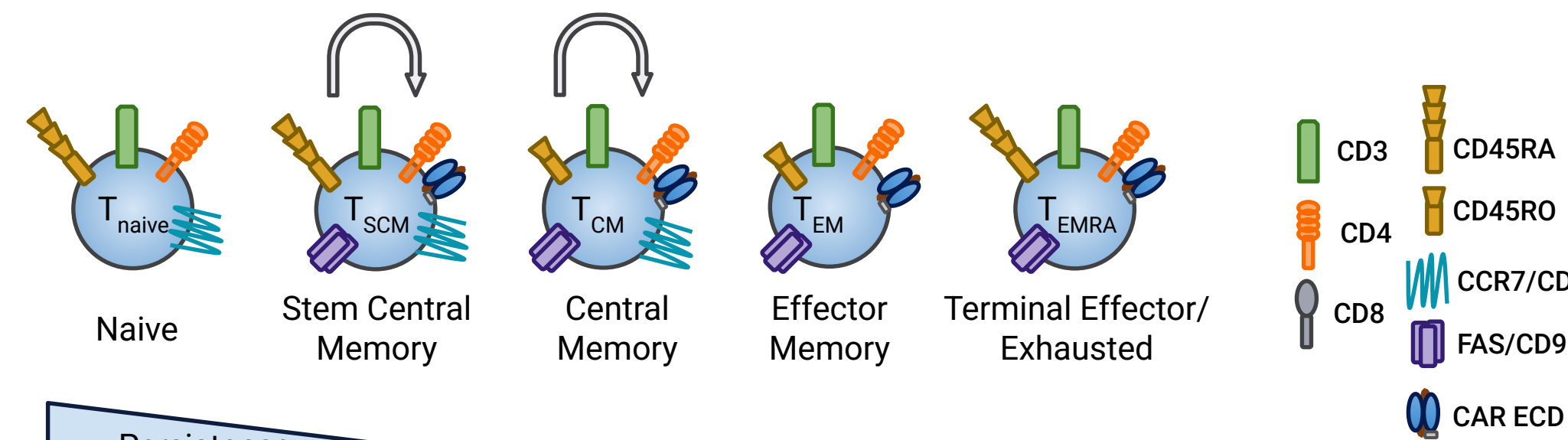


Figure 1. Schematic representation of Slingshot Biosciences T Cell Memory Mimic™

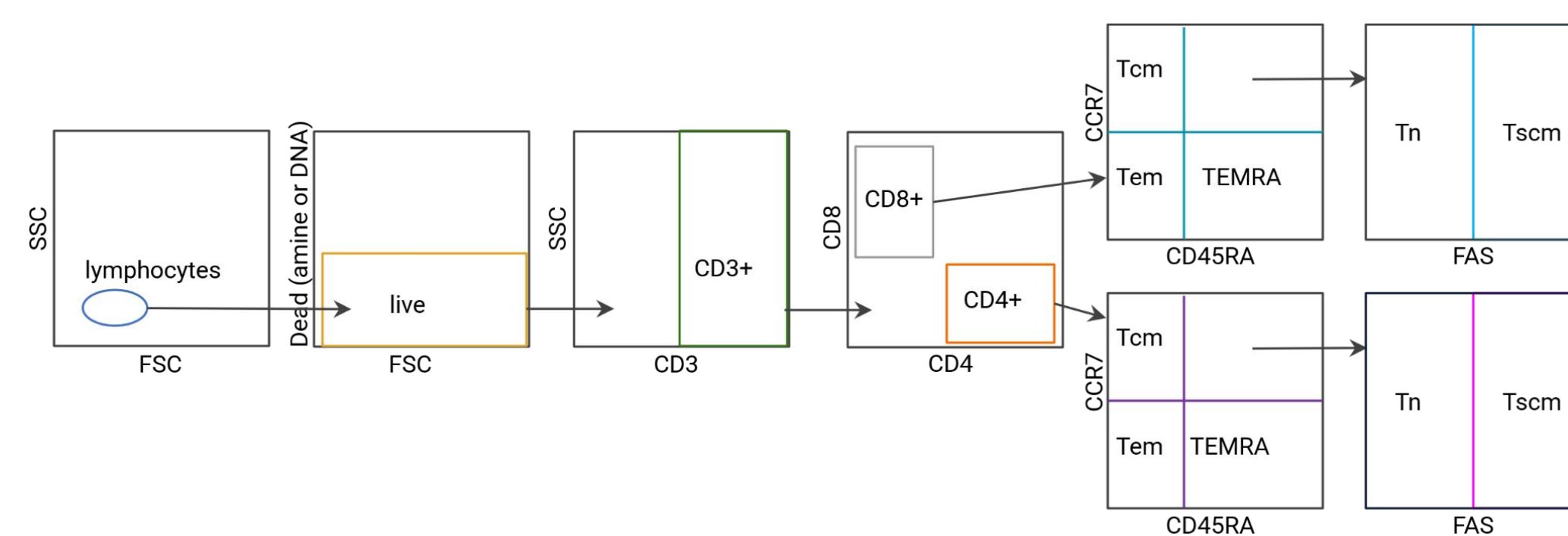


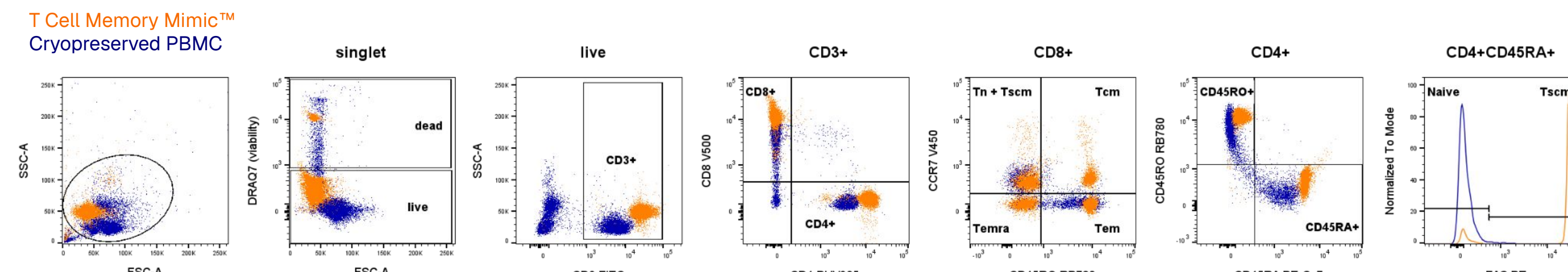
Figure 2. Representative flow cytometry-based gating scheme for T Cell Memory Mimic™

METHODOLOGY

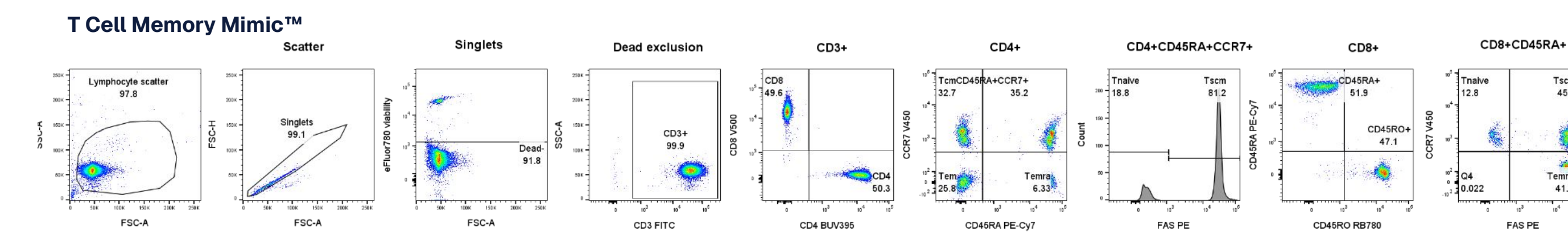
- Synthetic mimics loaded with memory subset biomarkers were stained and analyzed on Cytex Aurora with SpectroFlo and BD FlowJo software.
- PBMC or cultured T cells were stained with same antibodies.
- Accelerated stability was evaluated in a closed vial, lyophilized format.
- Post-reconstitution stability was evaluated by maintaining reconstituted and washed mimics at 4°C for indicated time period prior to staining.
- Mimics loaded with memory T cell markers were also loaded with FMC63 scFv to mimic CD19-specific CAR-T.
- Mimics were stained with several clones for markers commonly used to discriminate memory populations: CCR7, CD45RA, CD45RO, and FAS, as well as the surrogate CAR, DNA-based and amine-reactive fixable viability dyes.

RESULTS: T Cell Memory Mimic™ has markers to identify CD4 and CD8 memory T cell subsets.

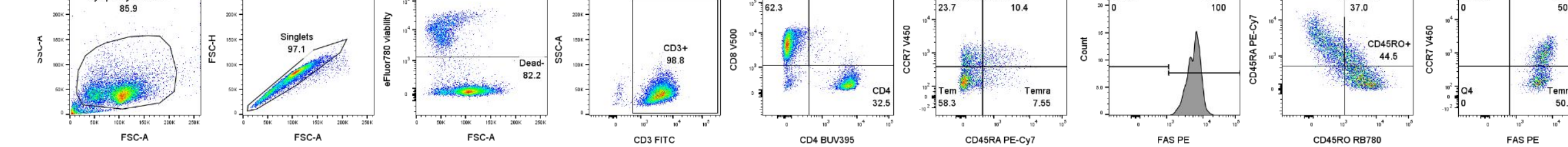
A. T Cell Memory Mimic™ provides distinct CD45RA+ and CD45RO+ populations and provides positive control T_{SCM} population not present in PBMC sample.



B. T Cell Memory Mimic™ contains CD45RA+ populations frequently lost after activation or extended culture.



Activated and rested T cells



Repeat activated T cells

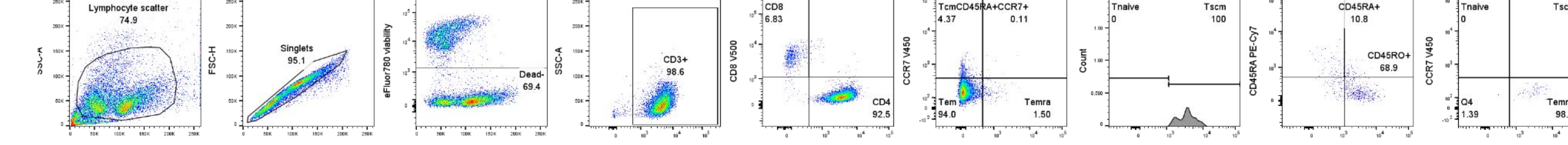
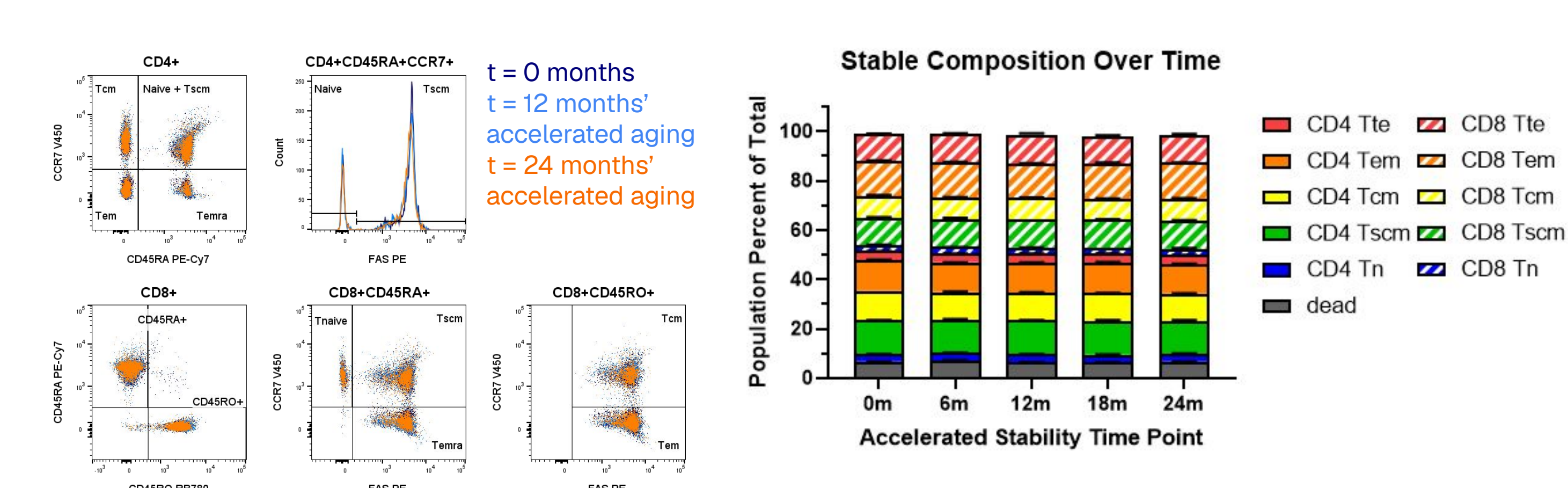


Figure 3. T Cell Memory Mimic™ compared to biological samples.
A. T Cell Memory Mimic™ and cryopreserved PBMCs were reconstituted or thawed and stained for DNA-based viability with lineage identification and memory markers.
B. PBMC were activated with Dynabeads in complete media and cultured with human IL-2 for a further 3 weeks, or re-activated every 7 days and cultured with human IL-2 to mimic T cell exhaustion and cryopreserved in CS10. T Cell Memory Mimic™ and cryopreserved T cells were reconstituted or thawed and stained for fixation-compatible viability, washed and stained for lineage and memory markers as indicated.

RESULTS: Memory markers on T Cell Memory Mimic™ are stable

A. Population frequencies are maintained after accelerated aging to mimic extended storage at -20°C.



B. Positive staining for all biomarkers is retained after reconstitution in staining buffer for 3 days at 4°C.

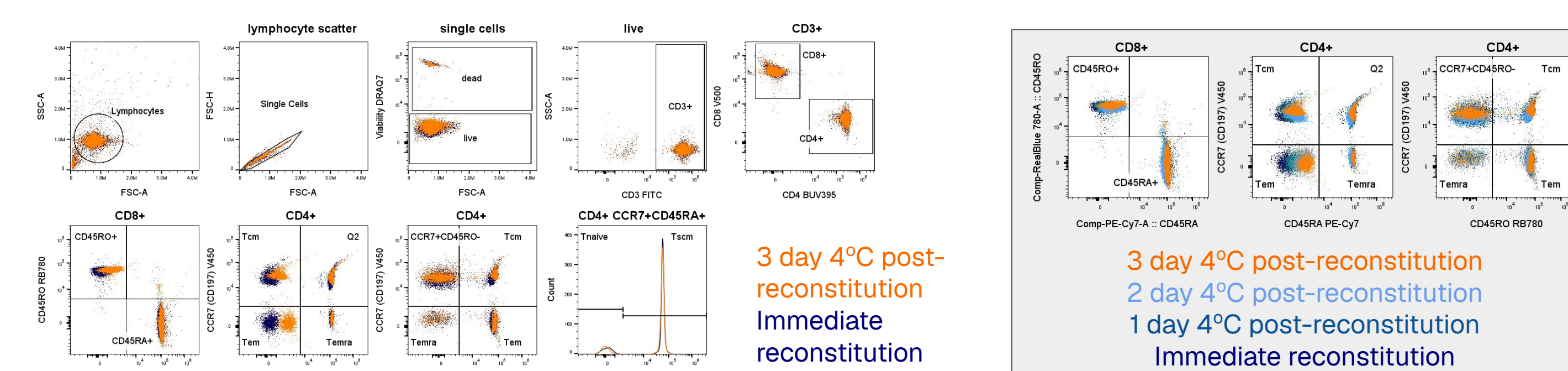
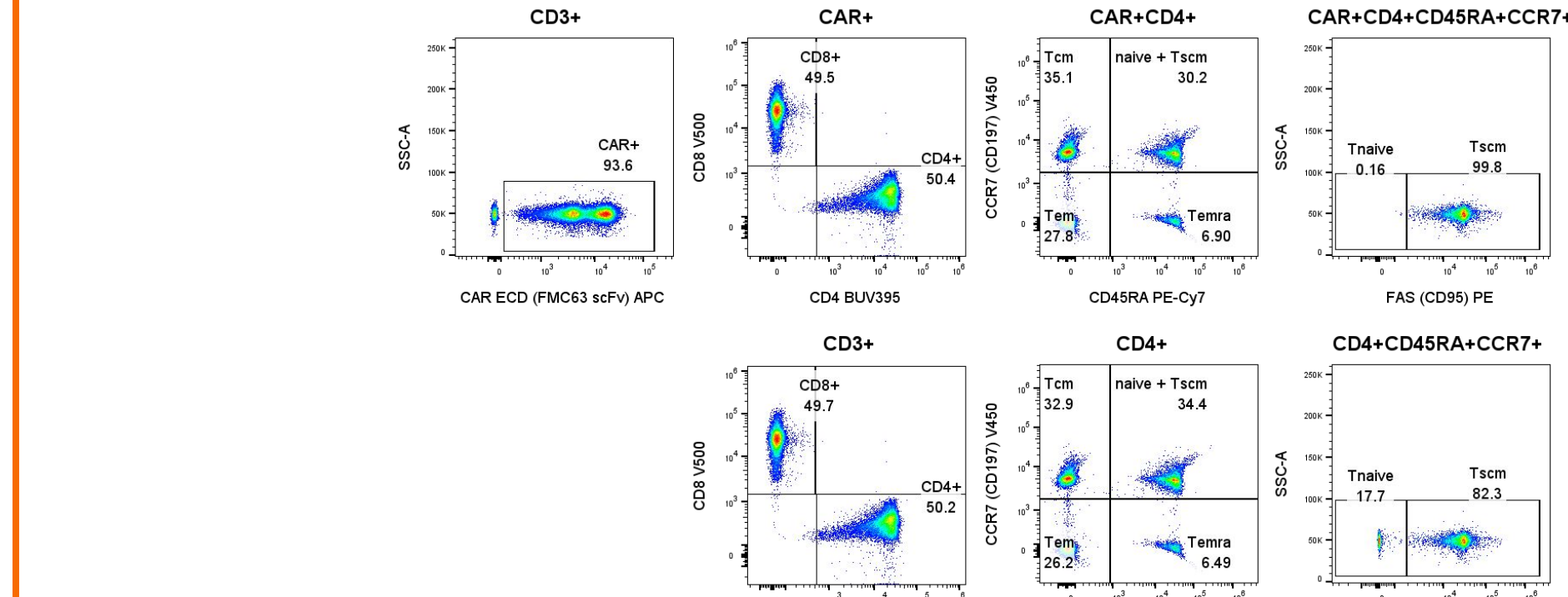


Figure 4. T Cell Memory Mimic™ stability.
A. Accelerated aging in closed vial, lyophilized format was performed and compared to material stored under recommended conditions. Example overlays of 12 months' and 24 months' accelerated aging for memory markers compared to material stored at -20°C are shown, with population frequency of total are graphed as average frequency + SD of 2-3 samples per time point.
B. Samples were reconstituted and washed with staining buffer, then maintained for indicated time at 4°C prior to staining and comparison with freshly reconstituted and stained sample. Positive populations are maintained, but biomarker negative populations do not overlay, indicating product should only be used for positive staining control after extended storage of post-reconstitution.

RESULTS: CAR ECD addition provides stable reference control for CAR-T memory phenotyping

A. FMC63 scFv attached to all memory T cell populations in T Cell Memory Mimic™ can be gated for same memory populations but is not present on naive T cell populations.



B. CAR ECD staining maintained after accelerated aging to mimic extended storage at -20°C.

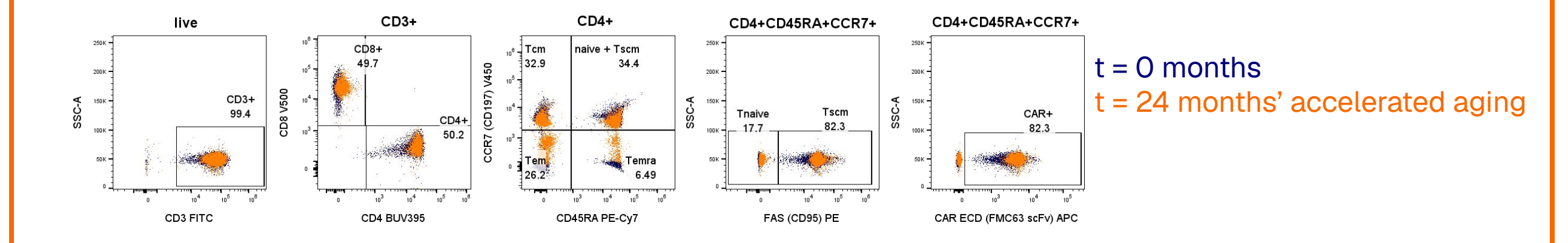


Figure 5. T Cell Memory Mimic™ can be loaded with CAR ECD for in process monitoring controls.
A. Alternate gating of T cell Memory Mimic™ loaded with FMC63 scFv (surrogate for the CAR ECD of Kymrah, Yescarta, Tecartus, Breyanzi).
B. Accelerated aging in closed vial, lyophilized format was performed and compared to material stored under recommended conditions. Overlays of 24 months' accelerated aging are shown.

RESULTS: Biomarker compatibility with common clones

Biomarker	Clone 1	Clone 2	Clone 3
CD3	SK7	UCHT1	OKT3
CD4	SK3	REA623	RPA-T4*
CD8	SK1	REA734	RPA-T8
CD45RA	L48	HI100	5H9
CD45RO	UCHL1	S19021B	REA611
FAS (CD95)	DX2	REA738	n/a
CCR7 (CD197)	150503	GO43H7	REA108
FMC63 scFv	1G10	REA1297	Y45
DNA	DRAG-7	Hoechst	7-AAD
Amine	eFluor™ 780	Zombie UV	Zombie Red

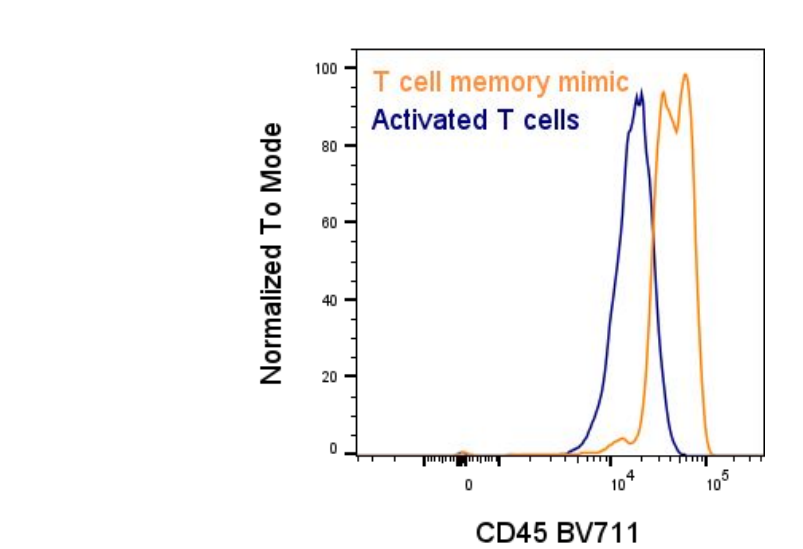


Table 1. T Cell Memory Mimic was stained with multiple clones for included biomarkers. Tested clones are listed in table. Incompatible clones are highlighted in yellow.
Figure 6. T Cell Memory Mimic non-dead populations are reactive with CD45 staining and as bright as activated T cells.

CONCLUSIONS

T Cell Memory Mimic™ provides a stable, non-biohazardous reference control for memory T cell phenotype panels. The multi-clone compatibility and incorporation of a population reactive to DNA- and amine-based viability dyes make them ideal for FMO analysis and template development. Reproducible measurement of population frequencies across users and over time enable standardization of longitudinal monitoring and better gating accuracy, enhancing data confidence and statistical power in immune monitoring and CAR-T workflows vital to gene and cell therapy clinical trials.

REFERENCES

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- Xia, Ying et al. "Reference range of naive T and T memory lymphocyte subsets in peripheral blood of healthy adult." *Clinical and Experimental Immunology* vol. 207,2 (2022): 208-217. doi:10.1093/cei/uxab038