KACTUS

Transforming Tumor Research: Engineering MHCs as Versatile Reagents

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Introduction

When dealing with solid tumors lacking specific biomarkers, the effectiveness of adoptive T cell therapy using TCR-T treatments is limited. We believe that the absence of dependable, multifaceted MHC reagents has been a hindrance in the research and development of treatments for solid tumors. To address this unmet need, we have engineered MHC molecules in several formats with diverse applications and high stability. These improved, highly active MHC molecules have the potential to facilitate solid tumor research and development.

Form 1: Mammalian-Expressed Single-Chain Trimer MHC for High Sensitivity

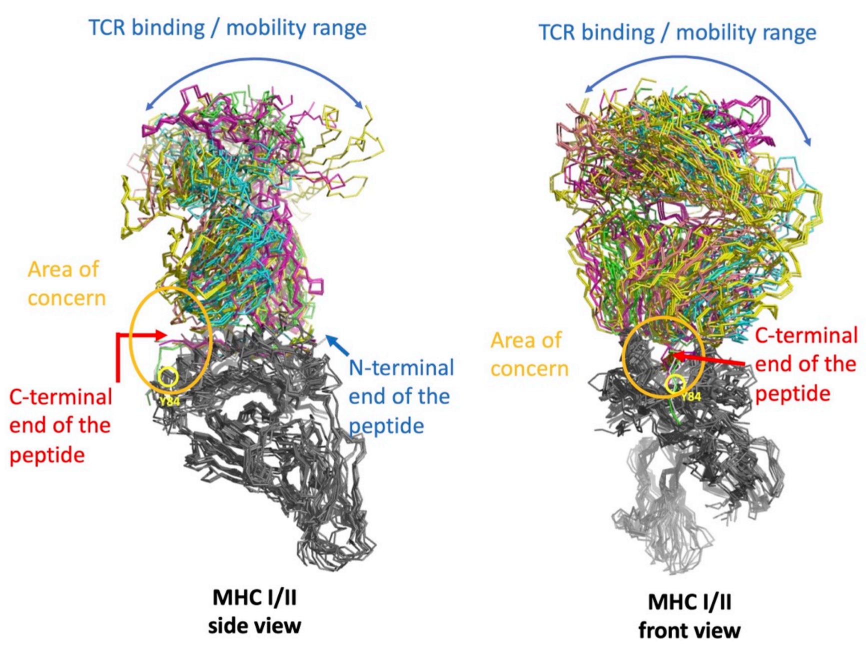


Figure 1. Structural analyses of >200 TCR-peptide MHC I/II complexes

The single-chain trimer (SCT) MHCs incorporate linkers strategically, maintaining MHC integrity and antigen presentation. The SCT design, on the other hand, has been facing scrutiny over worries that its linker might cause interference with TCR. Our structural investigations of over 200 TCR-peptide MHC complexes, however, reveal that SCT MHC's linkers are positioned apart from crucial TCR, peptide, and MHC interfaces (Figure 1). Furthermore, we use mammalian cells to express our SCT MHC rather than the common way of producing it from E. coli expression and then refolding it from the inclusion body. Thus, our SCT MHCs offer improved stability and sensitivity compared to conventional MHC complexes with separate chains, making the SCT design a promising advancement for immune research.

Form 2: Chimeric MHCs for Reduced Off-Targets

Humoral immune responses to MHC-I seldom induce T-cell receptor mimic (TCRm) antibodies. Furthermore, many antibodies are thought to detect the $\alpha 3$ domain of MHC-I and the β_2 microglobulin (β_2m), which are not directly involved in delivering the target peptide. Substituting the MHC-I $\alpha 3$ and β_2m domains with their murine counterparts preserved the MHC's antigen specificity for T-cells and supplied an acceptable antigen for TCRm antibody recognition. Here, we engineered our own chimeric human-murine MHCs designed to be expressed in mammalian systems to retain their innate conformation and activity (Figure 2). This should increase the likelihood of generating peptide-specific antibodies while decreasing the frequency of non-specific antibodies and making screening less labor-intensive.

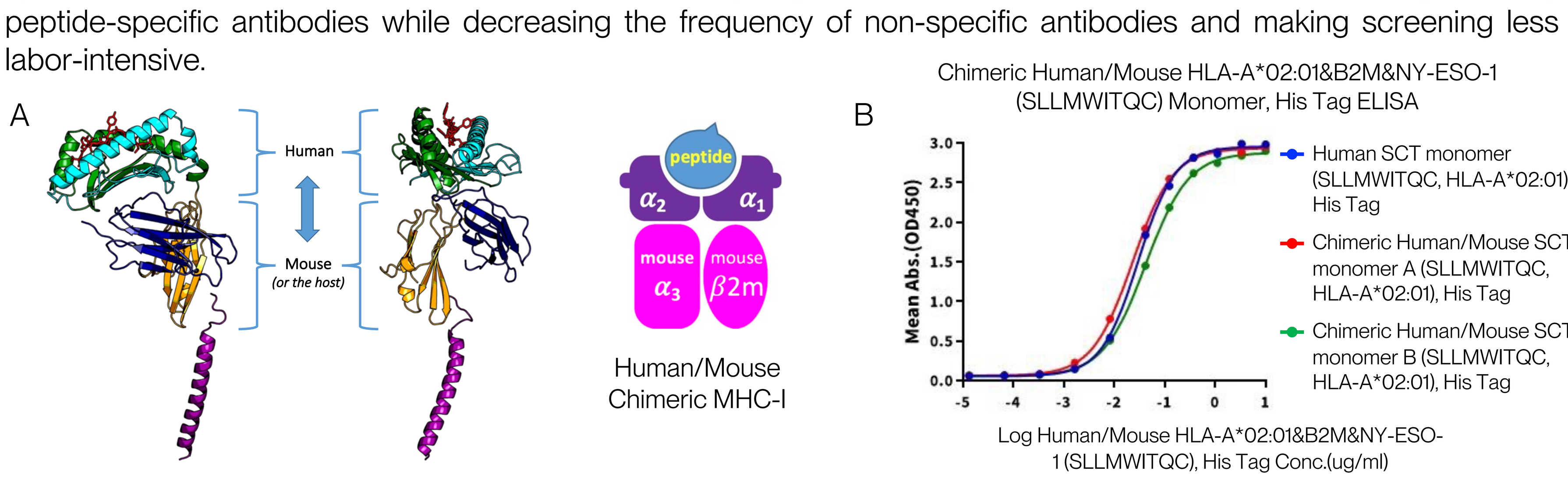


Figure 2. (A) Structure of Chimeric MHC-I molecule. (B) Immobilized chimeric HLA-A*02:01(mα3)&mB2M&WT-1 (SLLMWITQC) monomers and Human SCT monomer (SLLMWITQC, HLA-A*02:01), at 2μg/mL (100μL/well) on the plate. Dose-response curves for anti-NY-ESO-1 antibody, hFc Tag with an EC50 of 29.6/23.0/41.9ng/mL.

Form 3: prMHC (Peptide-Ready MHC) for Rapid Neoantigen Loading

The Peptide-ready MHCs (prMHC) offer unparalleled ease and convenience for scientists. By providing MHC molecules without pre-loaded peptides, scientists gain the flexibility to select and load any peptide of their choice, tailored precisely to their experimental needs.

This ready-to-be-load peptide feature not only saves valuable time spent on peptide-MHC complex preparation (Figure 3), but also offers the highest level of flexibility and experimental control. Our prMHC simplifies the process and enhances the scope of research, making it a versatile and invaluable tool in the field of immunology and beyond.

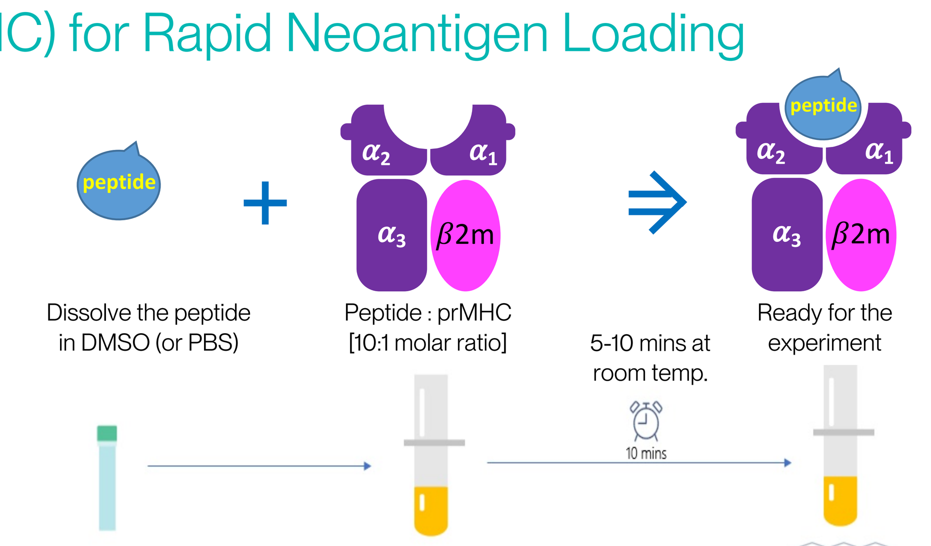


Figure 3. Schematic of protocol for neoantigen loading onto prMHCs.

Form 4: Biotinylated MHC monomers & Fluorescent Peptide-MHC Tetramers

MHC tetramers are complexes of four peptide-MHC biotinylated monomers bound to streptavidin molecules. The enhanced avidity of MHC tetramers and TCR interactions can have a significant impact on detecting antigen-specific T cells. They allow for direct detection, phenotyping, and enumeration of antigen-specific T cells within a polyclonal T cell population. By labeling T cells with fluorescent MHC tetramers, the frequency and distribution of antigen-specific T cells can be determined and sorted by FACS in a cell population. Our MHC tetramers can be produced both in vivo or in vitro in pMHC (peptide-MHC) form or prMHC (peptide-ready MHC) form (Figure 4).

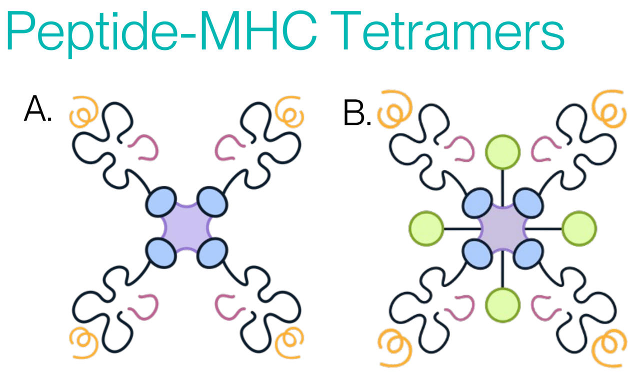


Figure 4. Structural representation of MHC-I Tetramer (A) and Fluorescent MHC-I Tetramer (B).

Case Study 1: The W6/32 mAb validates the mammalian cell-expressed prMHCs and SCT MHCs

The W6/32 mAb is commonly used to study human MHC I structure and function, recognizing a shared epitope on HLA-ABC. Research indeed has demonstrated that W6/32 reactivity is completely dependent on the amino terminus of human β_2-microglobulin (hβ_2m). In other words, the W6/32 cannot recognize bacterially expressed recombinant hβ_2m that has an additional methionine at the amino terminus (Figure 5). Similarly, the W6/32 cannot detect the amino terminus of SCT pMHC hβ_2m that is fused with a glycine linker. In contrast, our prMHC molecule, which has the native amino terminus without methionine, can be recognized by the W6/32. In fact, the W6/32 antibody is more effective in identifying our prMHC compared to the competitor's mammalian-expressed pMHC. Collectively, these data support the validity of our prMHC and SCT MHC that are expressed in mammalian cells.

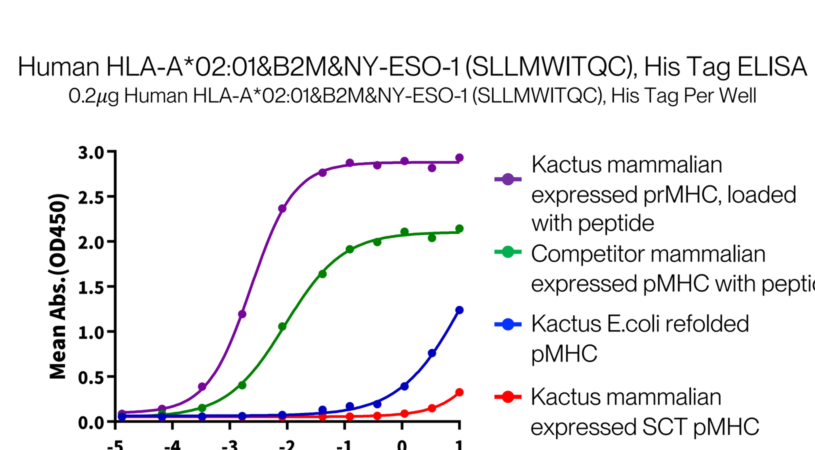
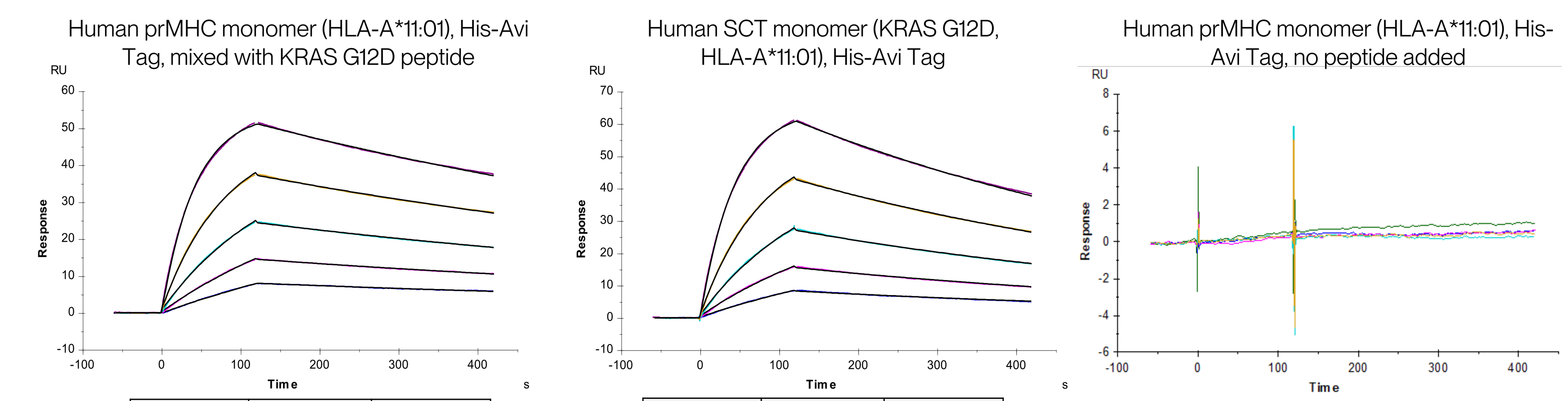


Figure 5. W6/32 mAb binds KACTUS mammalian-expressed loaded prMHC with native N-terminus of β_2m light chain, comparable to a leading competitor. It does not bind SCT pMHC which contains the linker at the N-terminus of β_2m light chain.

Case Study 2: Using prMHCs is simple and matches SCT MHC activity



ka (1/Ms)	ks (1/s)	KD (M)
1.07E+5	1.60E-3	1.49E-8

ka (1/Ms)	ks (1/s)	KD (M)
1.43E+5	1.29E-3	9.00E-9

Figure 6. SPR characterization of KRAS-G12D specific TCR (JDI a41b1) binding with peptide (VVVGADGVGK) loaded prMHC HLA-A*11:01 and SCT MHC (KRAS-G12D, HLA-A*11:01)

Case Study 3: In vivo-formed MHC tetramer is equivalent to standard MHC tetramer

HLA-G is known to bind to natural killer (NK) cells through its receptors. We produced HLA-G using our proprietary in vivo technology in a fluorescent tetrameric form. We then tested its ability to bind to the LILRB2 receptor against a tetrameric HLA-G generated in vitro.

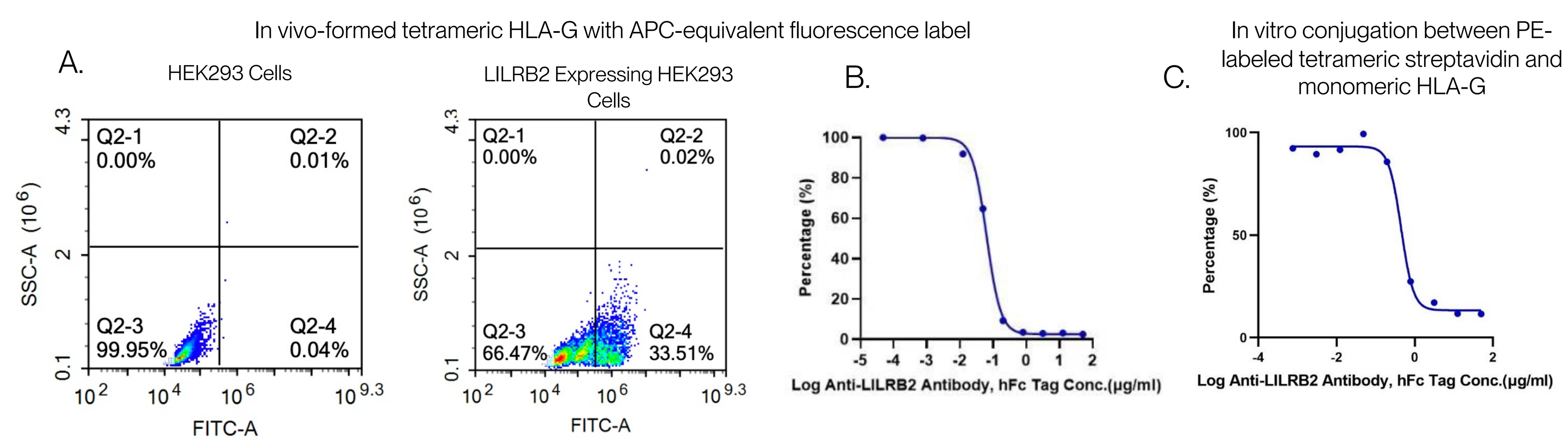


Figure 7. (A) FACS analysis of engineered, in vivo fluorescently charged tetrameric HLA-G, against LILRB2-expressing cells. (B) Serial dilutions of Anti-LILRB2 antibody were added into in vivo formed Red Fluorescent HLA-G Tetramer(His Tag), : Human LILRB2(mFc Tag) binding reactions. The half maximal inhibitory concentration (IC50) is 62.7ng/mL. (C) Serial dilutions of Anti-LILRB2 antibody were added into in vitro assembled PE-Labeled Human HLA-G Tetramer(His Tag): LILRB2(mFc Tag) binding reactions. The half-maximal inhibitory concentration (IC50) is 0.45μg/mL.

Case Study 4: In vivo-formed prMHC tetramers: robust for sorting cells

We examine our fluorescent prMHC tetramer's performance, rapidly loaded with NY-ESO-1 prior to cell sorting Jurkat cells expressing the NY-ESO-1-specific 1G4 TCR. Unlike competitors' MHC tetramers, ours allows peptide loading customization.

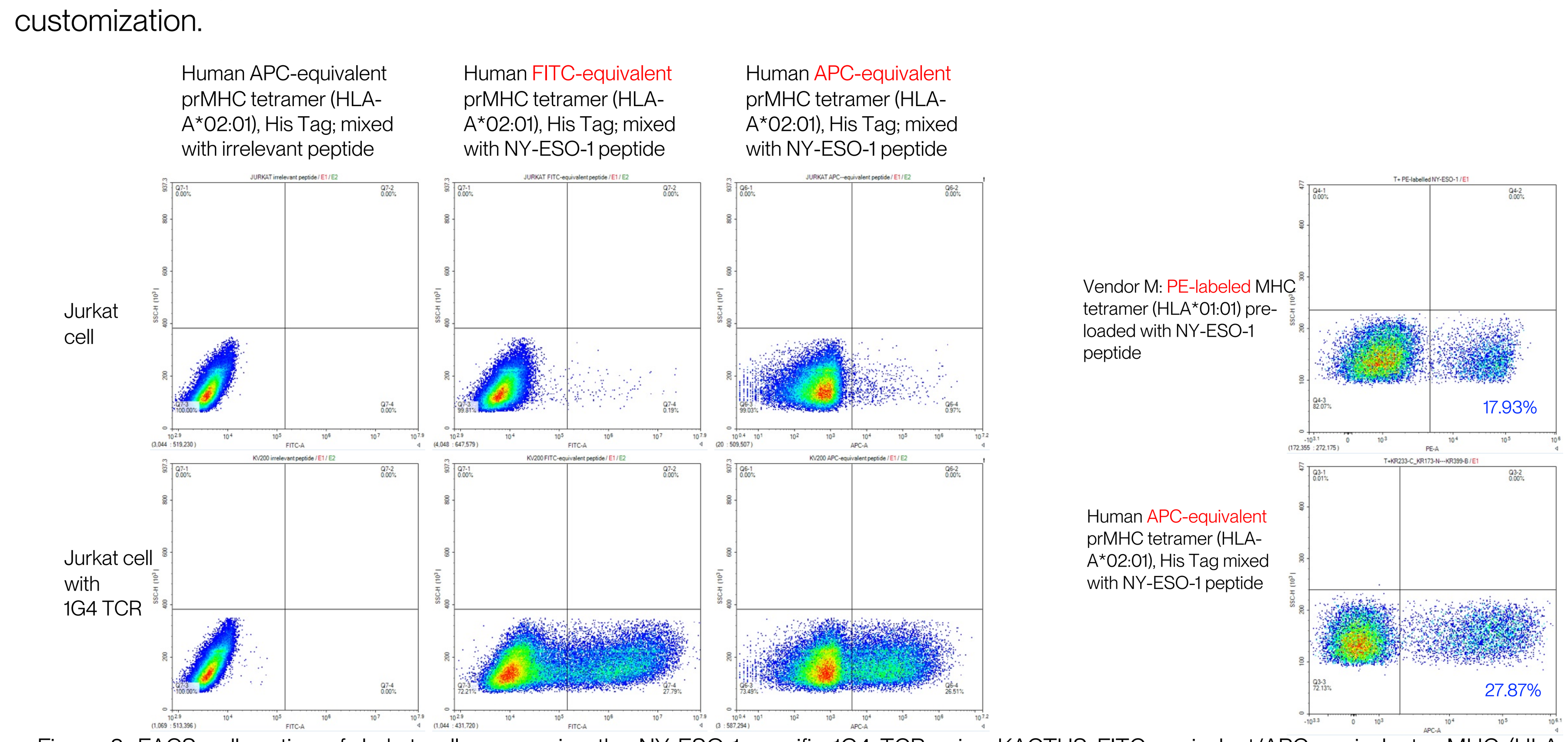


Figure 8. FACS cell sorting of Jurkat cells expressing the NY-ESO-1-specific 1G4 TCR using KACTUS FITC-equivalent/APC-equivalent-prMHC (HLA-A*02:01) tetramers, quickly loaded with NY-ESO-1 peptide. A competitor's PE-labeled NY-ESO-1 MHC (HLA-A*02:01) tetramer is compared.

Case Study 5: Our pMHCs and prMHCs exhibit long-lasting stability

Protein stability is a major concern for biopharmaceuticals. Because of this, we subject our MHCs to rigorous protein stability testing under various storage conditions, including freeze-thaw cycles and freeze-dried solids (Figure 9).

Stability Testing Procedures -> (1) Biotinylated human HLA-A*11:01 -> (2) lyophilization -> (3) reconstitution -> (4) add KRAS G12D peptide

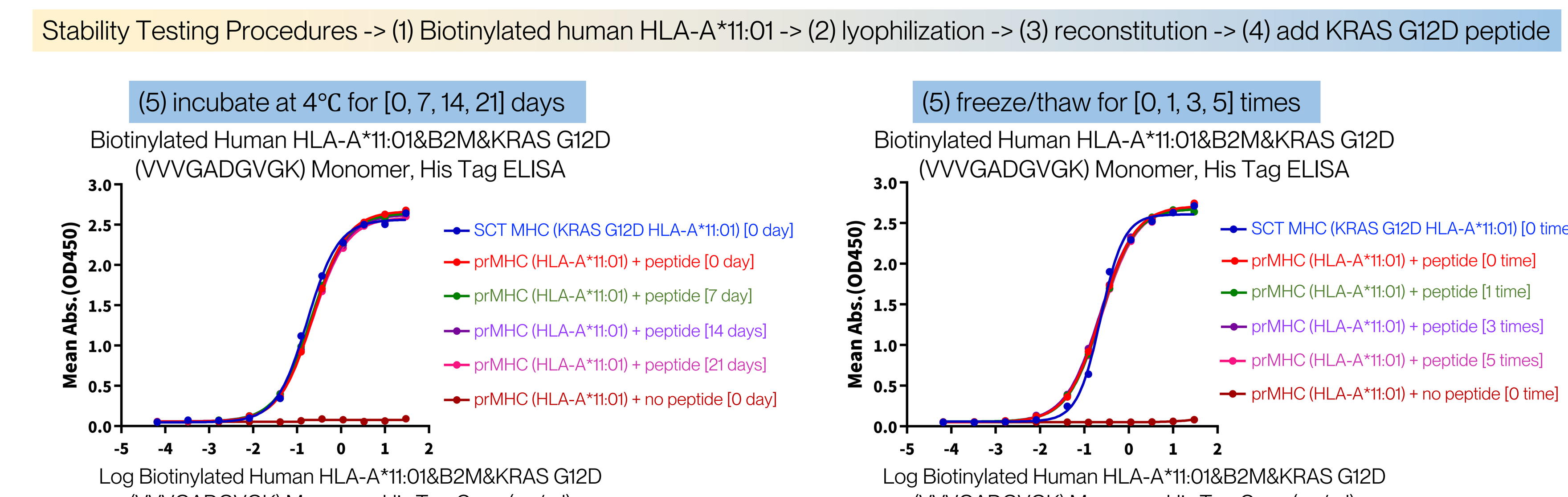


Figure 8. (A) Stability testing of SCT MHC and prMHC (Biotinylated Human HLA-A*11:01&B2M&KRAS G12D (VVVGADGVGK) Monomer) at 4℃ for 21 days. Activity is analyzed using ELISA. (B) Freeze/thaw testing of of SCT MHC and prMHC (Biotinylated Human HLA-A*11:01&B2M&KRAS G12D (VVVGADGVGK) Monomer) at 4℃ for 21 days. Activity is analyzed using ELISA.

Conclusion

- KACTUS proprietary prMHCs cover common HLA alleles such as HLA-A 11:01, HLA-A 02:01, HLA-A 02:03, HLA-A 03:01, etc., which can be utilized for highly efficient neoantigen loading.
- Loading peptides onto prMHC is convenient. This empowers researchers with the customization of experiments, optimizing conditions for specific assays or investigations.
- Peptide-loaded prMHC monomers can be utilized to characterize TCR binding through SPR and BLI and in some situations, ELISA.
- In T-cell sorting, peptide-loaded APC equivalent-prMHC tetramer outperforms competing products.

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