

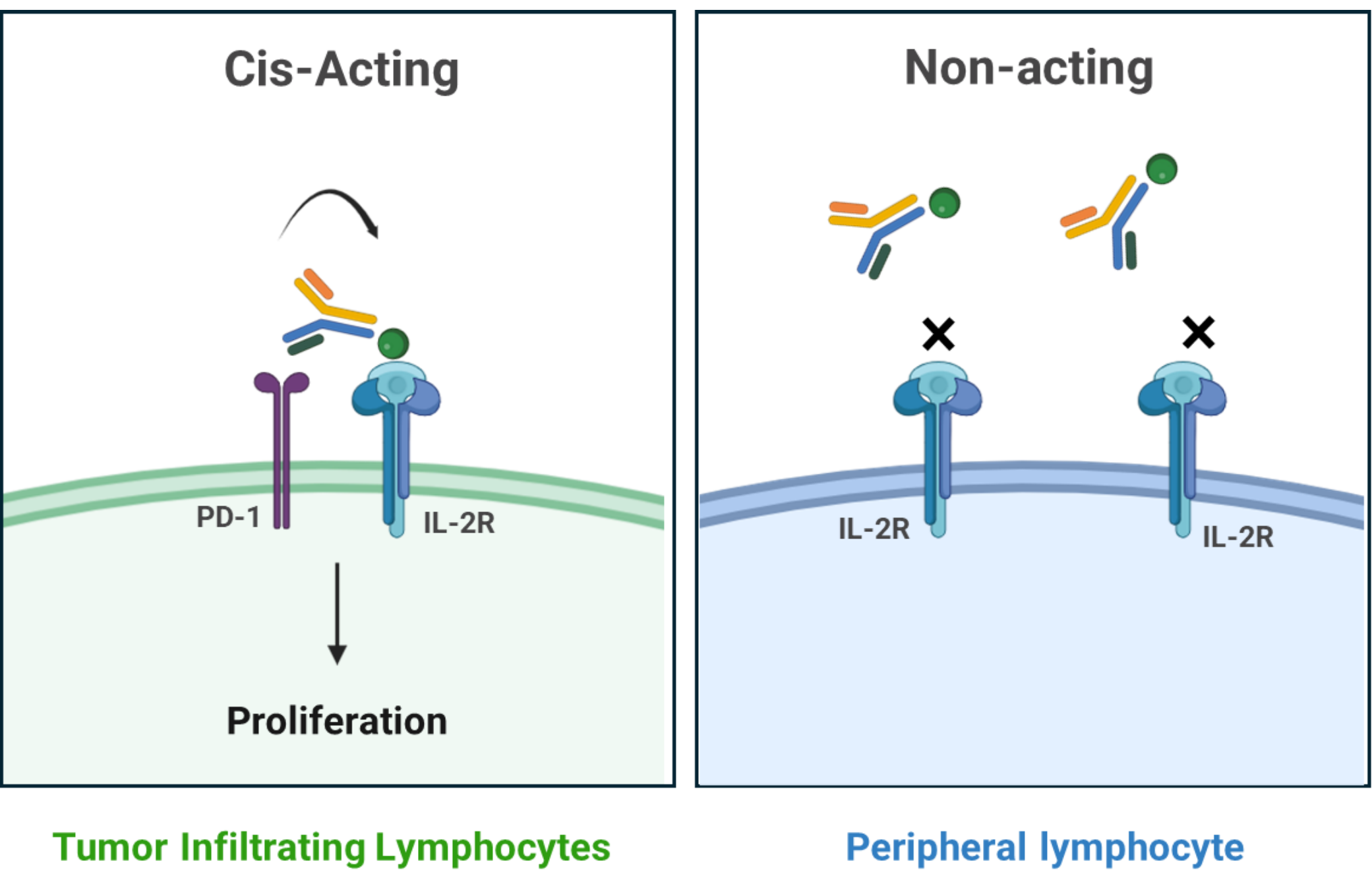


BACKGROUND

Targeted delivery of an interleukin-2 variant (IL-2v) via fusion to an anti-PD-1 antibody has emerged as a promising strategy to overcome resistance to anti-PD-(L)1 therapy. However, unattenuated signaling through the IL-2 receptor β/γ chain (IL-2R β/γ), even in the context of cis-delivery to PD-1⁺ cells, can still lead to systemic toxicities.

Fusion of IL-2v to bispecific antibodies (BsAbs) introduces further challenges associated with dual-antigen targeting and increased risk of adverse events. To address these challenges, we screened IL-2 variants with varying degrees of IL-2R β/γ binding that either have IL-2R α interaction preserved or reduced, for fusion with anti-PD-1-based BsAb to generate novel **tri-specific immuno-cytokines (“Multi-AbKines”)**.

RATIONALE



Rationale for IL-2v screening

Not all IL-2 variants are equally suited for cis-targeted delivery. Optimal IL-2v design requires careful balancing of:

- The degree of IL-2R β/γ attenuation and IL-2R α binding status
- Signaling potency and safety window

Study objective

We functionally screened IL-2 variants to identify lead candidates that optimally support bispecific antibody-mediated cis-activation of TILs via PD-1 targeting, improving anti-tumor efficacy while maintaining a favorable safety profile.

SCREENING STRATEGY



RESULTS

Primary screening based on manufacturability

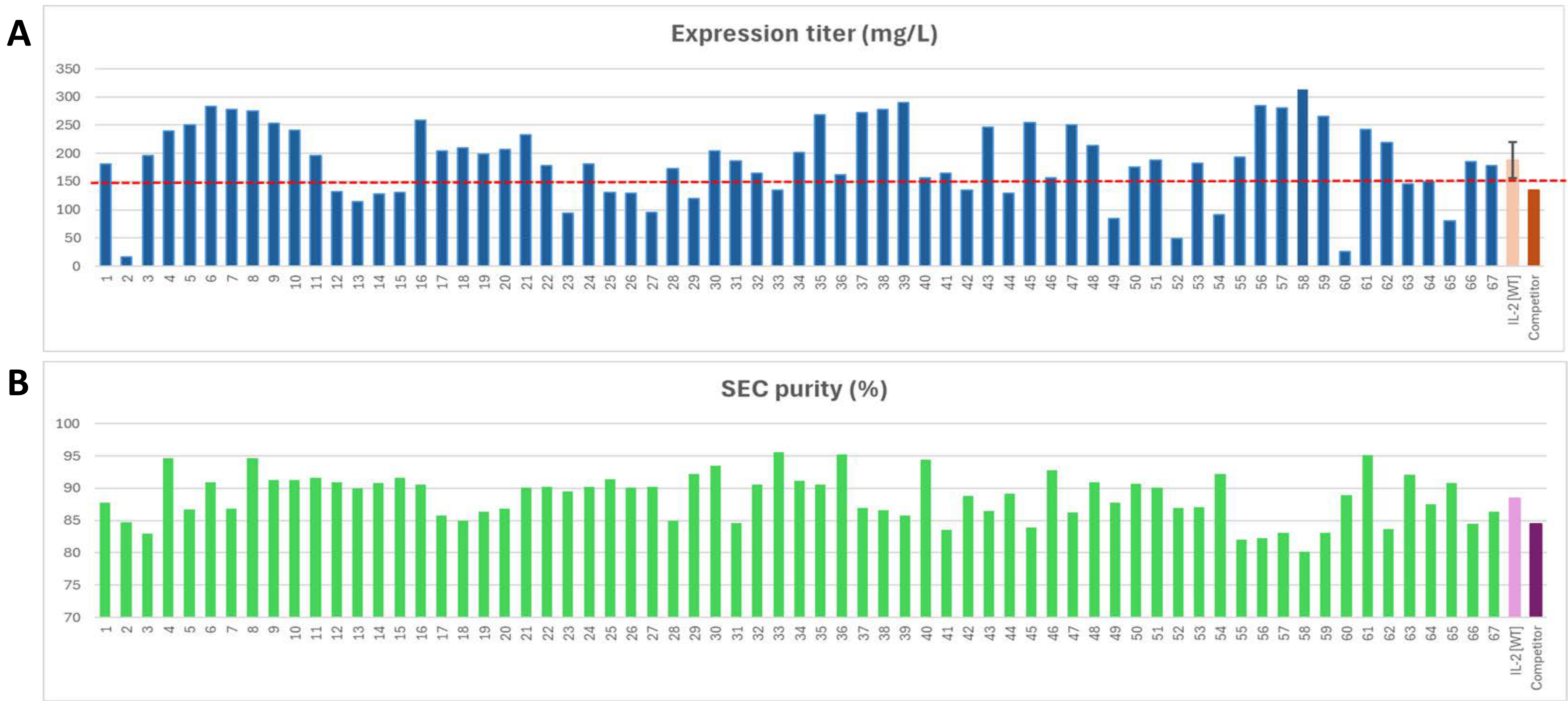


Figure 1. Hit clones were prioritized based on manufacturability, including expression titer and SEC purity of BsAb-containing IL-2v constructs. Clones with equivalent or superior productivity (A) and purity (B) relative to the reference IL-2v format were prioritized.

Distribution of clones according to pSTAT5 signaling levels.

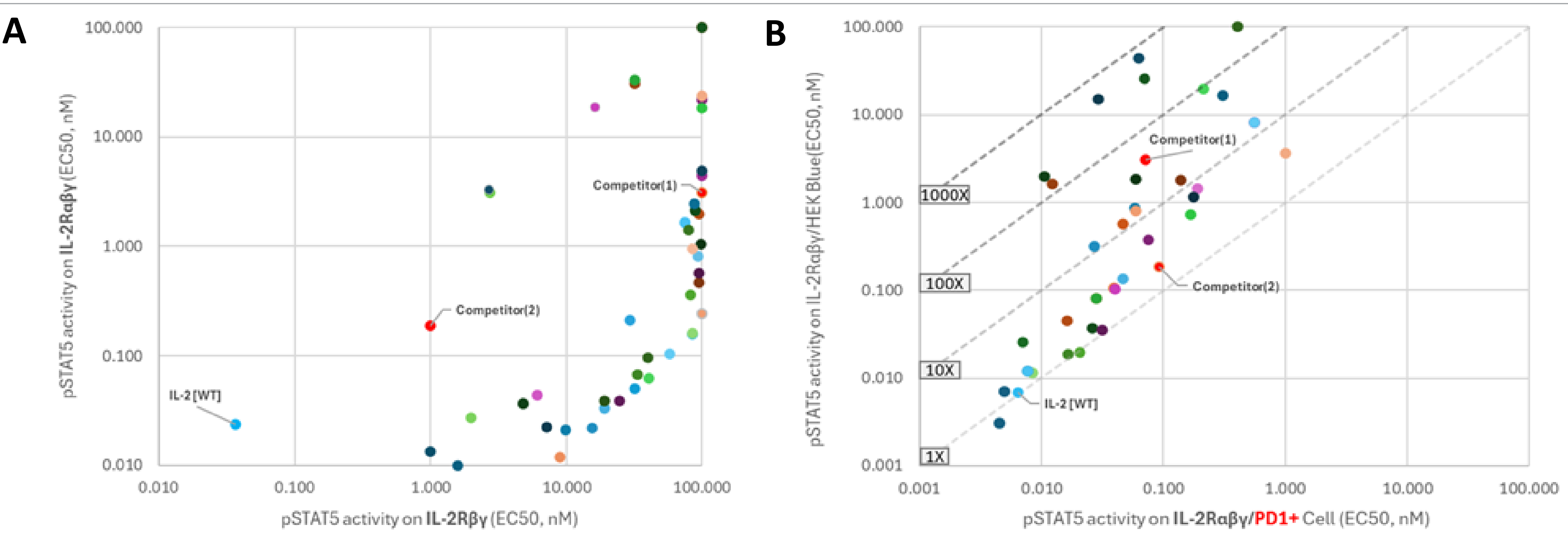


Figure 2. Broad pSTAT5 profiling enabled efficient identification of promising IL-2 variants and expansion of the candidate pool.

In Vitro Binding affinity

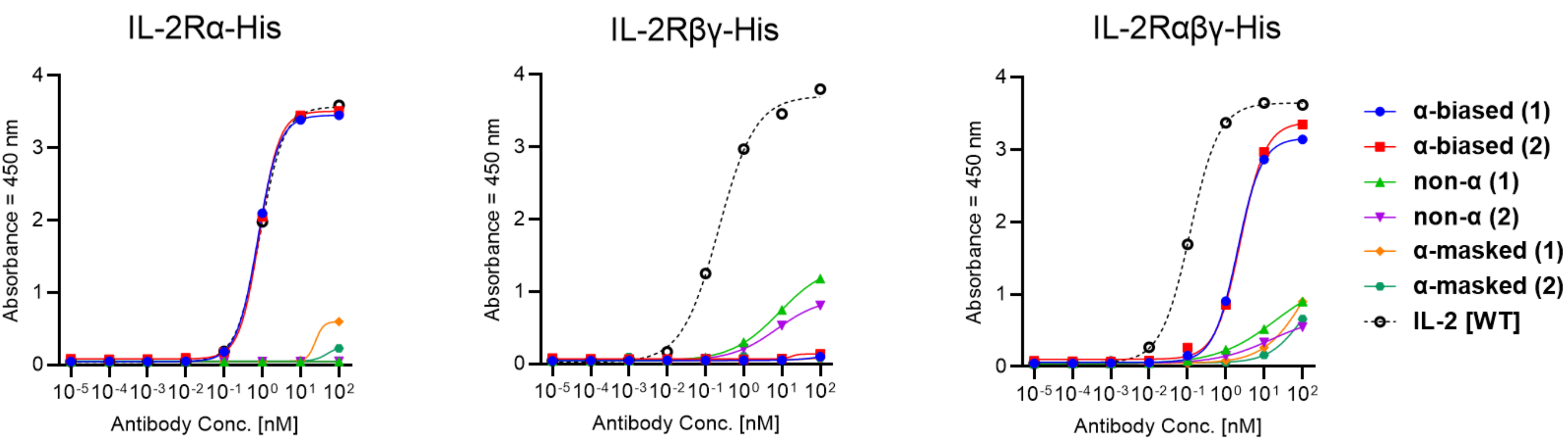


Figure 3. Binding affinity analysis of lead candidate IL-2v-fused BsAb constructs. IL-2 variants were categorized based on IL-2R α binding properties, and their affinities to IL-2R β/γ , and the IL-2R $\alpha\beta/\gamma$ complex were further assessed by ELISA.

In Vitro pSTAT5 signaling

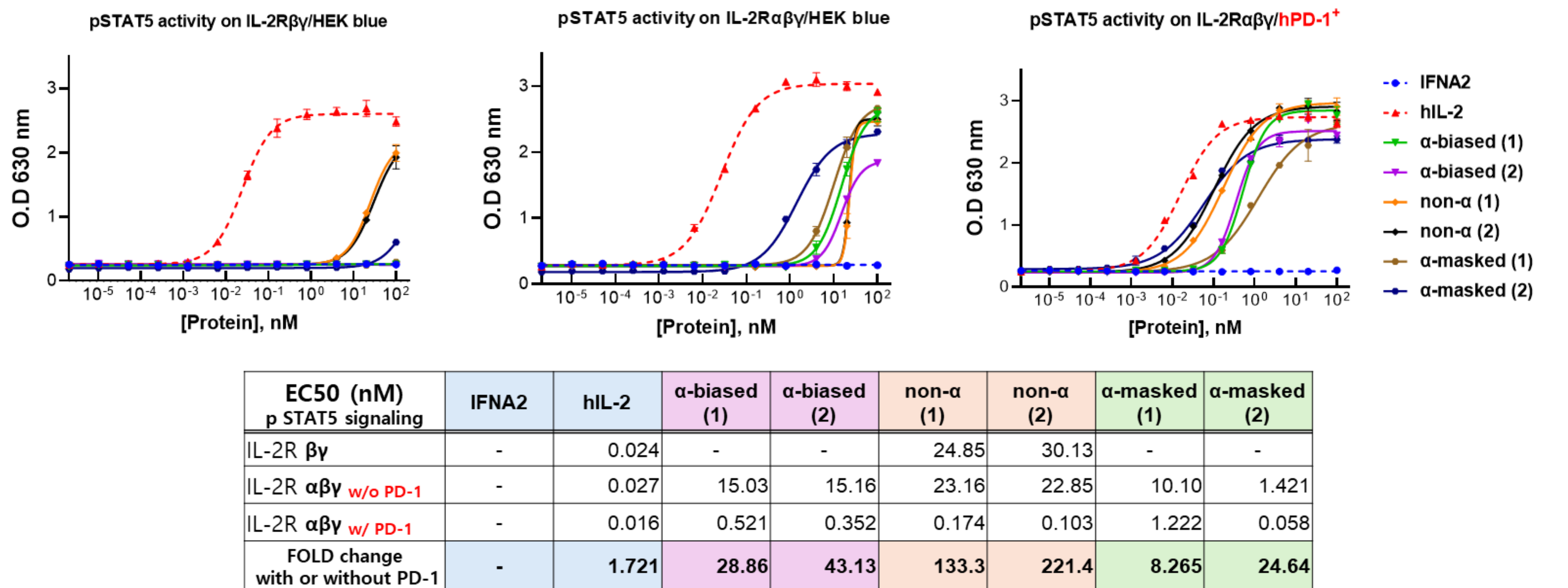


Figure 4. IL-2R α binding affinity critically determines the magnitude and selectivity of downstream signaling. PD-1 anchoring increased signaling potency for all IL-2 muteins, with differential fold enhancement observed across IL-2v formats.

In Vivo anti-tumor efficacy

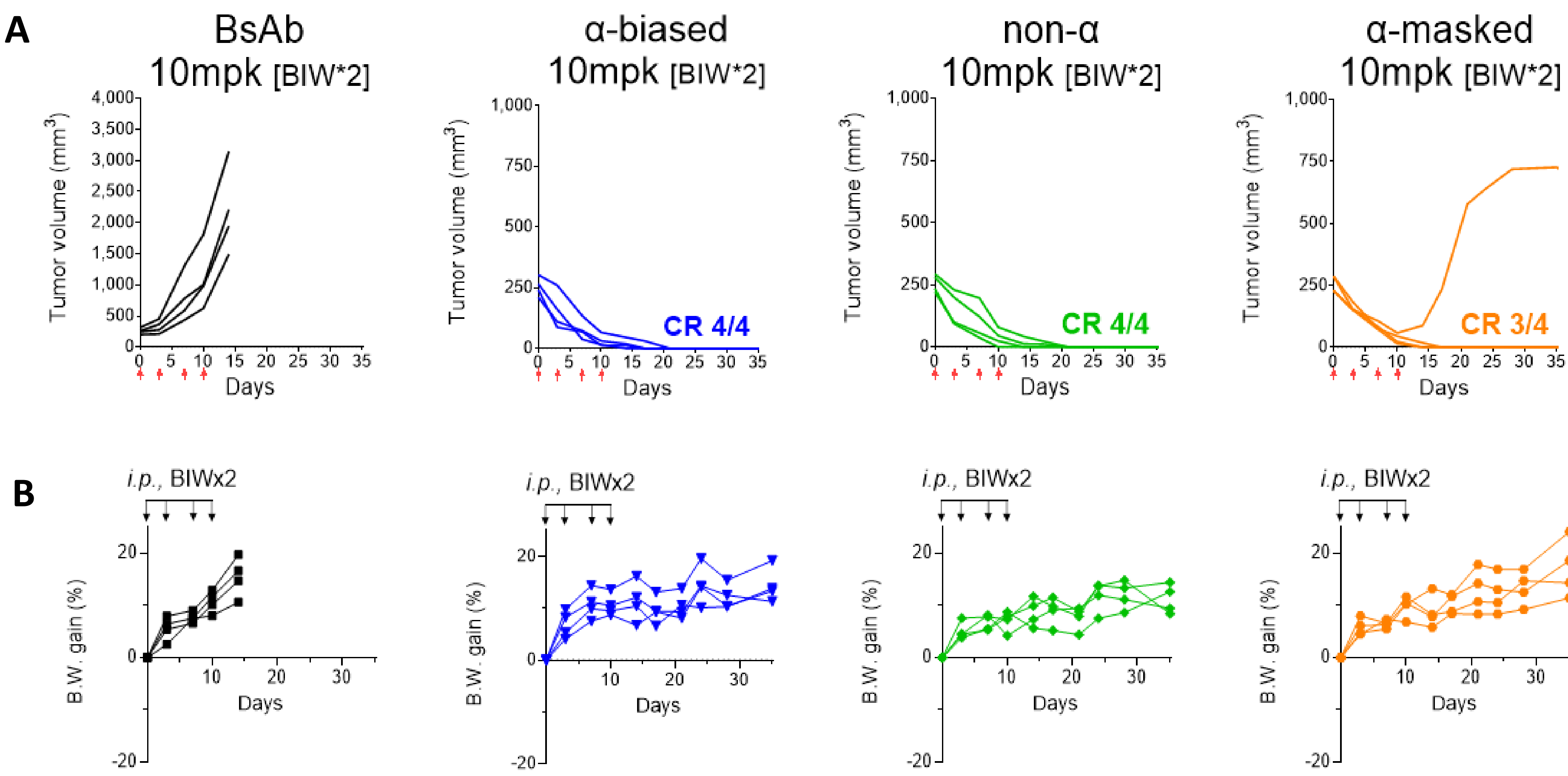


Figure 5. Anti-tumor efficacy of PD-1xTAAxIL-2v Multi-AbKines with distinct IL-2R α binding properties in an MC-38 syngeneic hPD-1 knock-in mouse model. All treatment groups demonstrated an immediate reduction in tumor volume following the first administration (A). No significant body weight loss was observed despite BIW dosing (B).

CONCLUSION

- Our screening approach enabled identification of attenuated IL-2 variants with **reduced IL-2R β/γ binding across distinct IL-2R α -binding categories.**
- The attenuation-based engineering principle is not cytokine-specific. By targeting receptor-binding interfaces, the same strategy can be extended to **other cytokines, enabling tunable immune modulation** across multiple therapeutic contexts.