

Targeted antibody/cytokine fusion protein antagonists to overcome T_{reg}-mediated immunosuppression in tumors



JOHNS HOPKINS
MEDICINE

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INTRODUCTION

- Immune checkpoint inhibitors (ICIs) have transformed cancer therapy by overcoming tumor-driven immune evasion, but efficacy of ICIs is limited by immunosuppressive regulatory T cells (T_{reg}) in the tumor microenvironment
- Single-cell analyses found that a T_{reg} subset with high OX40 and GITR expression was associated with resistance to ICI therapy
- This project aims to develop Treg antagonist immunocytokines (ICs): fusion proteins comprised of affinity-tuned IL-2 antagonists and OX40/GITR-targeting antibody domains
- By selectively inhibit OX40^{high}/GITR^{high} T_{reg} in the TME, this approach could overcome T_{reg}-mediated immunosuppression and synergize with ICIs to enhance anti-tumor immunity

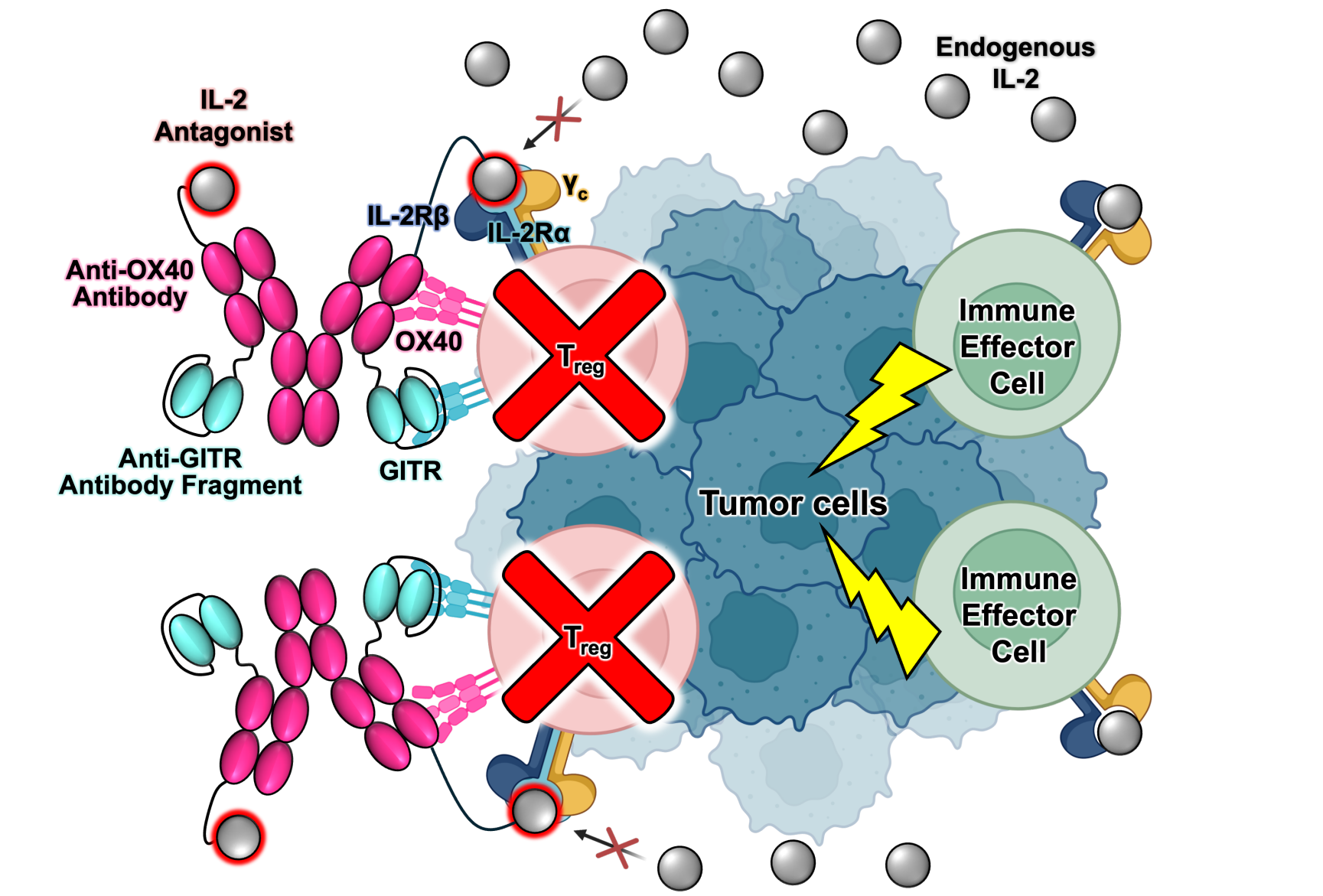


Figure 1. Proposed mechanism of OX40^{high}/GITR^{high} T_{reg} antagonism by antagonist ICs to promote anti-cancer immunity

RESULTS

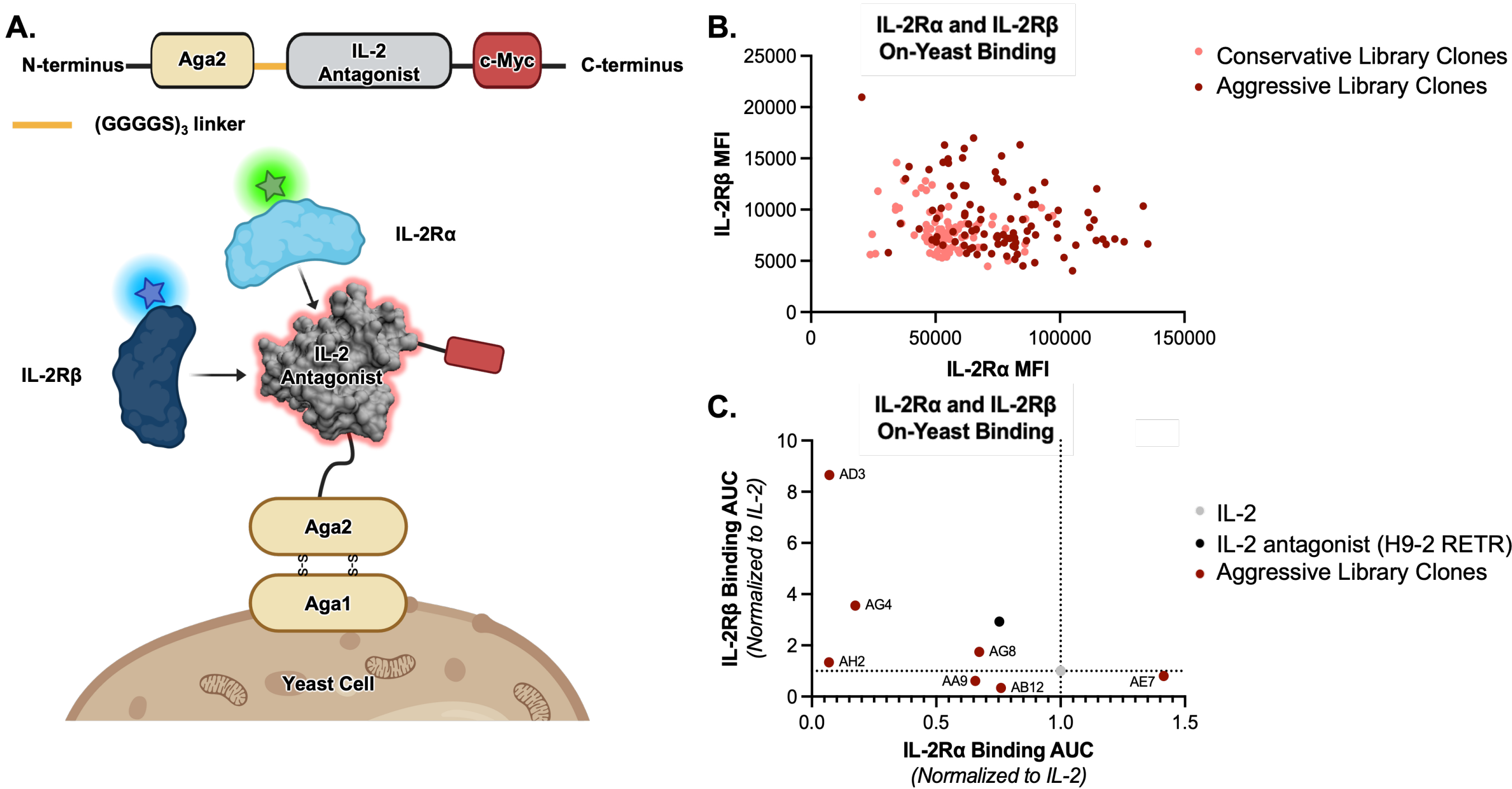


Figure 2. Yeast surface display can be leveraged to evolve IL-2 antagonists with distinct receptor binding properties. (A) Schematic of a yeast surface-displayed IL-2 antagonist binding to fluorescently labeled IL-2Rα and IL-2Rβ; **(B)** Flow cytometry-based binding of 192 clones from the 2 evolved libraries to IL-2Rα and IL-2Rβ; **(C)** IL-2Rα and IL-2Rβ binding profiles of select IL-2 antagonist clones, with binding area under the curve (AUC) values normalized to those of wild-type IL-2.

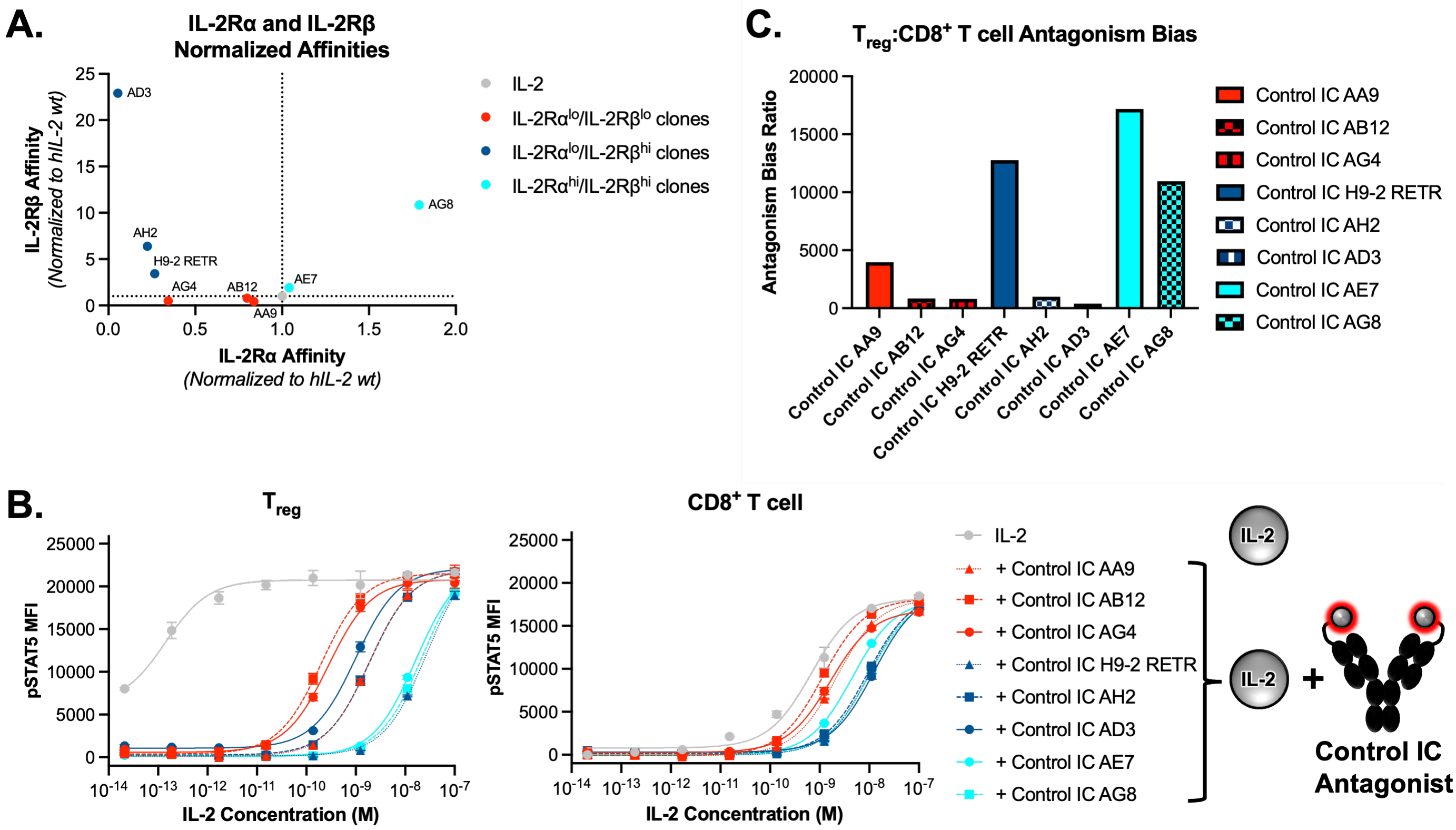


Figure 3. Yeast-derived IC variants show biased antagonism towards T_{reg}. (A) IL-2Rα and IL-2Rβ affinities of dominant negative antagonist clones, normalized to those of wild-type IL-2; **(B)** pSTAT5 mean fluorescence intensity (MFI) in T_{reg} and CD8⁺ T cells after treatment with IL-2 alone (gray) versus IL-2 plus Control IC antagonists (colored); **(C)** Treg:CD8⁺ T cell pSTAT5 antagonism biases of Control IC antagonists.

CONCLUSIONS

- To explore the effects of IL-2Rα and IL-2Rβ affinities on T_{reg} antagonism bias, a panel of IL-2 antagonists were discovered by yeast surface display and sequential selection against IL-2Rα and IL-2Rβ (**Fig. 2A**)
- Error-prone mutagenesis of a dominant negative IL-2 mutein yielded IL-2 antagonists with distinct receptor binding properties relative to wild-type IL-2 (**Fig. 2B-C, Fig. 3A**)
- Upon bivalent conjugation of the IL-2 antagonists to a human IgG1 antibody of irrelevant specificity, Control IC AE7 exhibited 17,000-fold higher pSTAT5 antagonism in T_{reg} over CD8⁺ T cells (**Fig. 3B-C**)
- Preliminary analyses suggest that there are optimal IL-2Rα and IL-2Rβ affinities that maximize T_{reg} antagonism while minimizing CD8⁺ T cell antagonism
- Our lead IC antagonist significantly inhibits IL-2-mediated T_{reg} expansion with minimal effects on CD4⁺ T_{conv} and CD8⁺ T cell counts (**Fig. 4**)
- The lead IL-2 antagonist (clone AE7) will be cloned into different OX40- and GITR-targeted scaffolds (**Fig. 5**)
- OX40- and GITR-targeted antagonists will be evaluated in mouse models of colorectal adenocarcinoma (MC38)
- The development of these T_{reg} antagonist ICs has the potential to overcome T_{reg}-mediated immunosuppression and improve the efficacy of ICIs in cancer therapy

METHODS

- 2 libraries containing >10⁸ IL-2 mutants were created via error-prone mutagenesis, displayed on EBY100 yeast, and screened against IL-2Rα and IL-2Rβ via sequential rounds of MACS/FACS
- IL-2Rα and IL-2Rβ binding affinities of select IL-2 antagonists were determined by biolayer interferometry (Octet)
- IL-2 antagonists with most divergent binding properties were cloned into bivalent IgG1 frameworks, creating IC antagonists
- Proteins were expressed in Expi293 HEK cells and purified by Protein G affinity chromatography followed by size-exclusion chromatography
- Functional antagonism was measured by competition of IL-2-dependent STAT5 phosphorylation (pSTAT5)
- In vivo* immune cell expansion and phenotyping studies were performed in C57BL/6 mice. Spleens were harvested after 4 days of treatment.

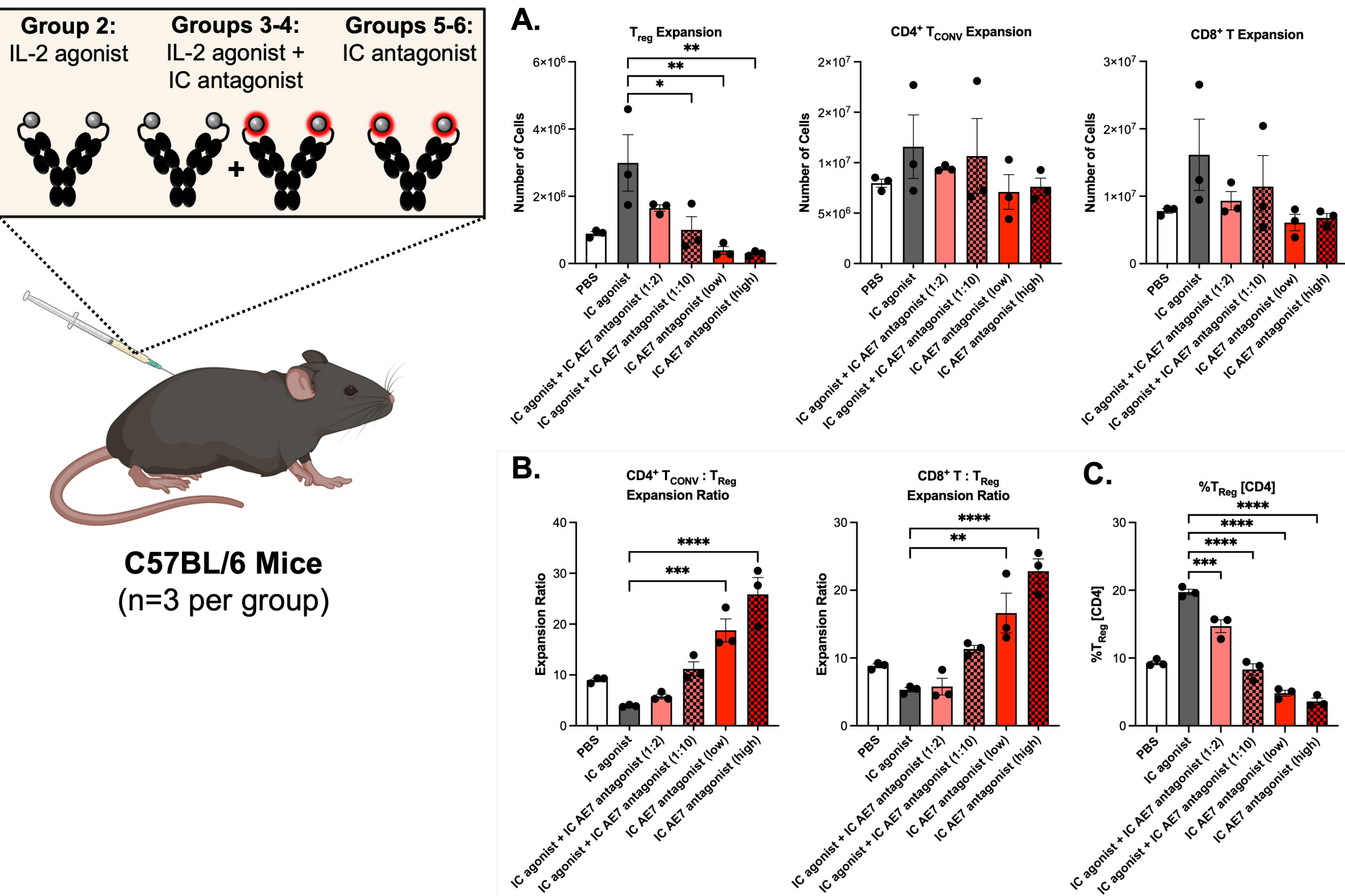


Figure 4. IC antagonists selectively deplete T_{reg} over immune effector cells and inhibit-IL-2-mediated T_{reg} expansion *in vivo*. C57BL/6 mice (n=3) were treated with PBS, bivalent IL-2 agonist (with or without IC antagonist), or IC antagonist alone. Immunophenotyping of splenocytes was performed to evaluate **(A)** T_{reg}, CD4⁺ T_{conv}, and CD8⁺ T cell counts; **(B)** CD4⁺ T_{conv}:T_{reg} and CD8⁺ T cell:T_{reg} ratios. **(C)** %T_{reg} in CD4⁺ T cell compartment

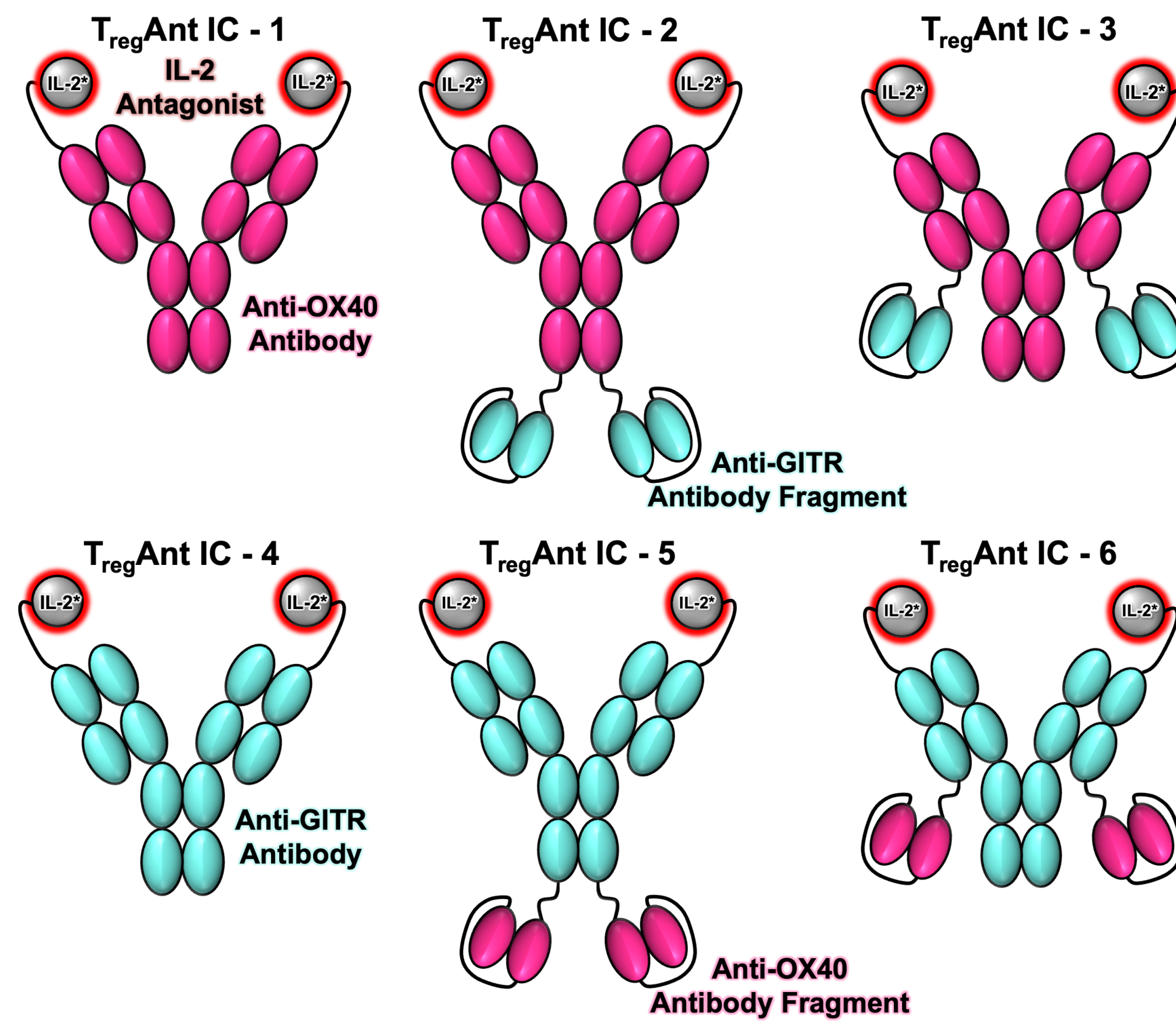


Figure 5. Designs of OX40- and GITR-targeted antagonist ICs

ACKNOWLEDGEMENTS

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