

HER2-targeting TLR7/8 immune-stimulating antibody conjugates elicit robust myeloid activation and anti-tumor immune responses in a TLR- and FcR- dependent manner.



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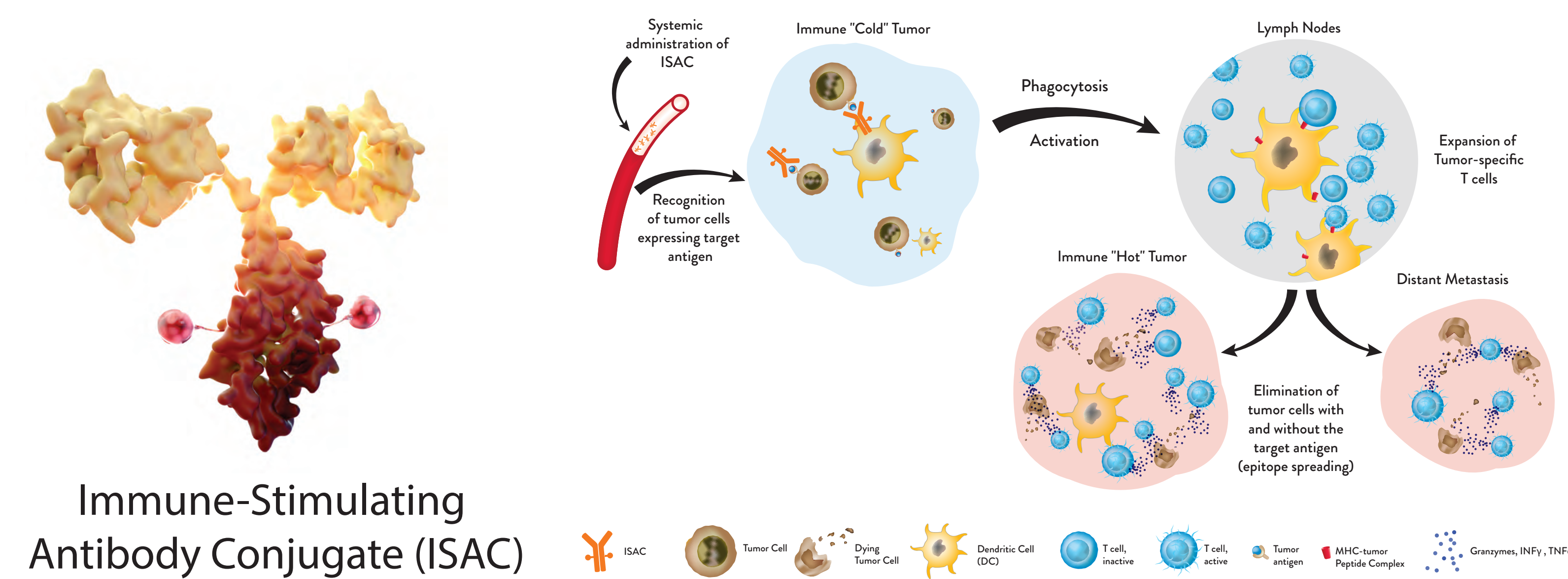
ABSTRACT

Background: Antigen presenting cells (APCs) process tumor antigens and facilitate recruitment of tumor-specific T cells to generate a robust anti-tumor immune response. APCs are present in almost all tumor types, including those that are refractory to checkpoint inhibitors; however, the highly immunosuppressive tumor microenvironment (TME) often renders APCs unable to effectively process and present tumor antigens. To overcome this immunosuppressive TME and rescue the function of tumor-infiltrating APCs, we developed an immune-stimulating antibody conjugate (ISAC) through covalent attachment of a TLR7/8 agonist to a HER2-targeting monoclonal antibody.

Results: In vitro co-cultures with human primary leukocytes and cancer cell lines revealed that ISACs potently activates APCs, leading to increased co-stimulatory molecule expression and secretion of pro-inflammatory cytokines. In myeloid-focused in vivo models utilizing HER2-positive human tumor cell line xenografts, which are resistant to trastuzumab, anti-HER2 ISAC treatment led to tumor regression and clearance. Further mechanistic studies revealed that anti-HER2 ISAC efficacy was TLR- and FcR- dependent. In syngeneic tumor models in which anti-HER2 ISAC treatment led to tumor clearance, animals rechallenged with the parental tumor cell line lacking HER2 antigen expression were protected from tumor growth. In addition, depletion of CD4 and CD8 T cells prior to rechallenge led to loss of immunological memory.

Conclusions: These data provide a strong rationale for pursuing an anti-HER2 TLR7/8 ISAC into the clinic for the potential treatment of HER2-positive cancers.

PROPOSED MECHANISM OF ACTION



HUMAN IN VITRO EXPERIMENTAL DESIGN

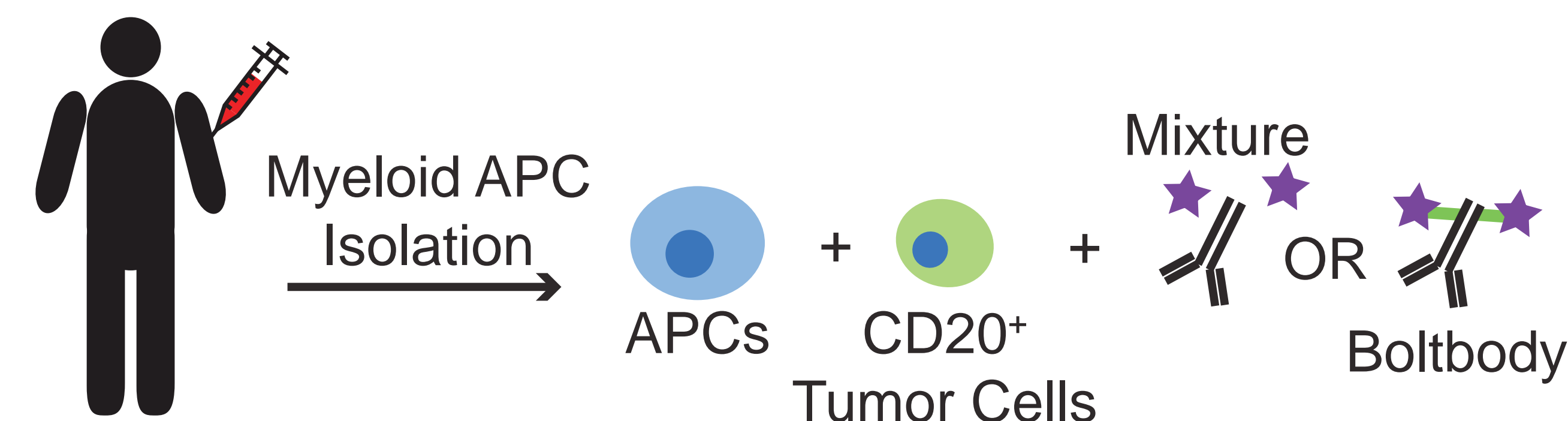


Figure 1: Assessment of ISAC Activity. Myeloid APCs were isolated from healthy donor via negative selection and co-cultured with CFSE-labeled CD20+ tumor cells. After 18 hours, cells were assessed for cellular activation/differentiation via microscopy and flow cytometry (Figures 2-5).

HUMAN IN VITRO RESULTS

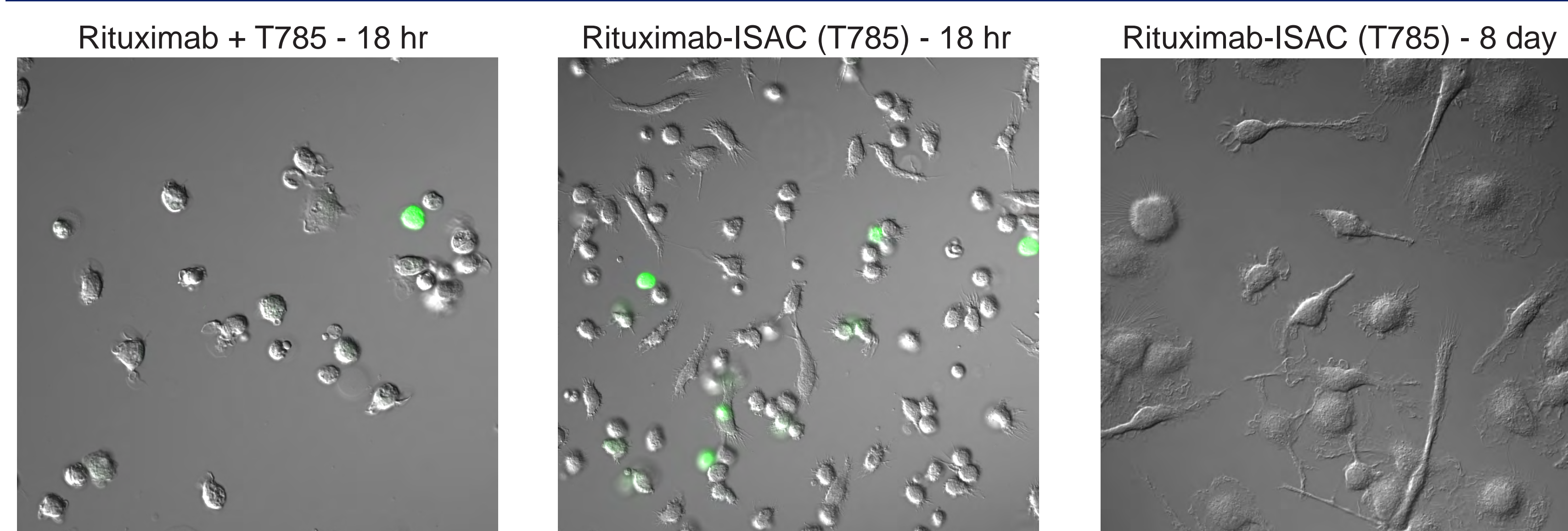


Figure 2: ISACs elicit distinct changes in cellular morphology.

HUMAN IN VITRO RESULTS

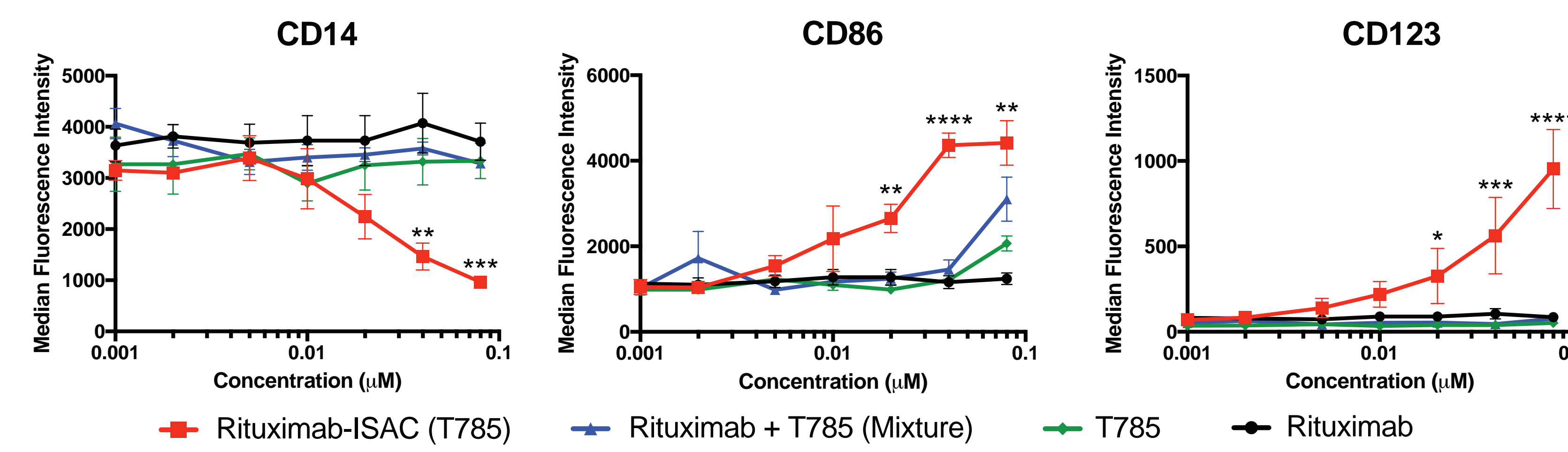


Figure 3: ISACs elicit myeloid activation and DC differentiation.

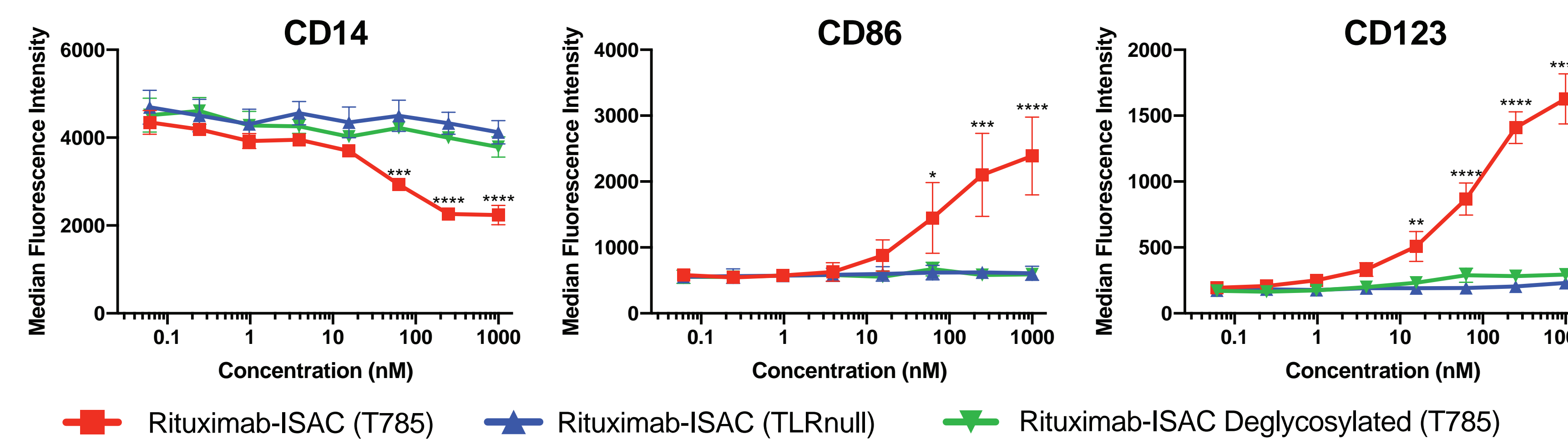


Figure 4: ISACs require intact Fc and TLR engagement for in vitro potency.

IN VIVO EXPERIMENTAL DESIGN

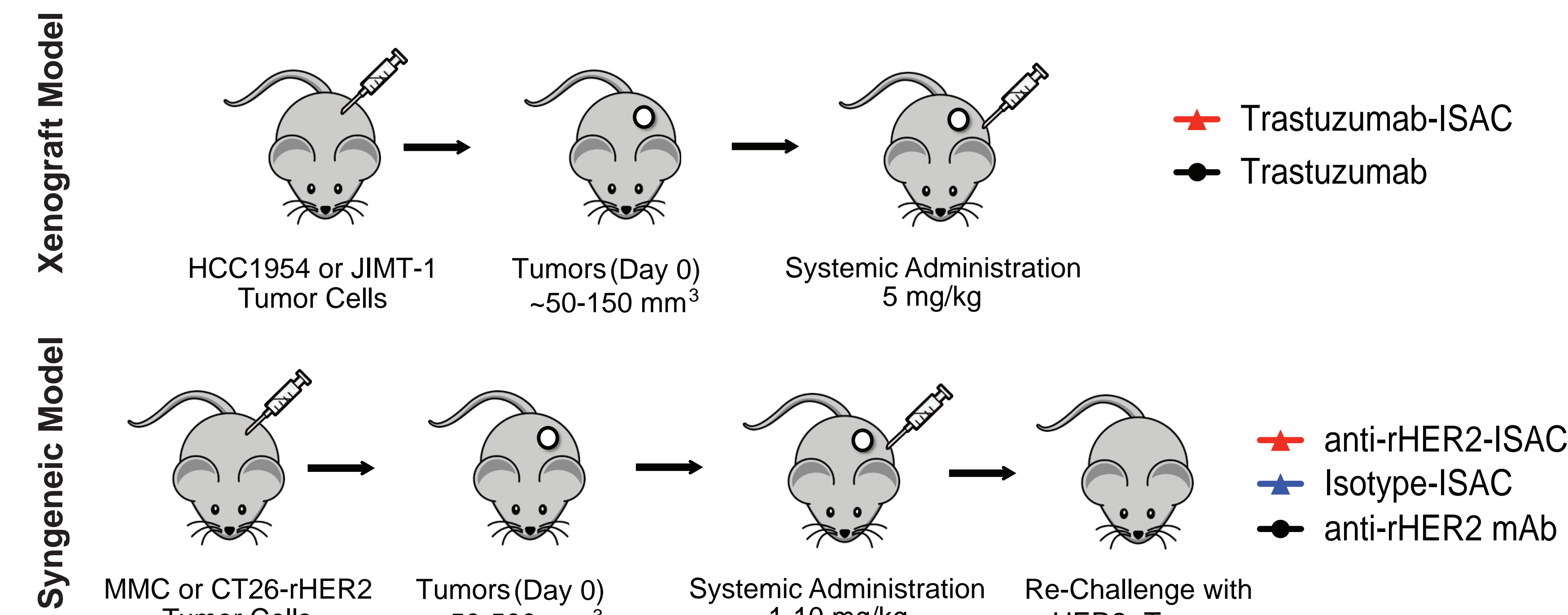


Figure 5: Assessment of ISAC Anti-Tumor Activity. Myeloid-mediated anti-tumor efficacy was assessed in mice that lacked B/T/NK cells with huHER2+ tumor cell lines (Q5Dx6, 5 mg/kg - Figure 6-8), or in immunocompetent syngeneic rHER2+ tumor cell lines (Figure 9-10).

IN VIVO RESULTS

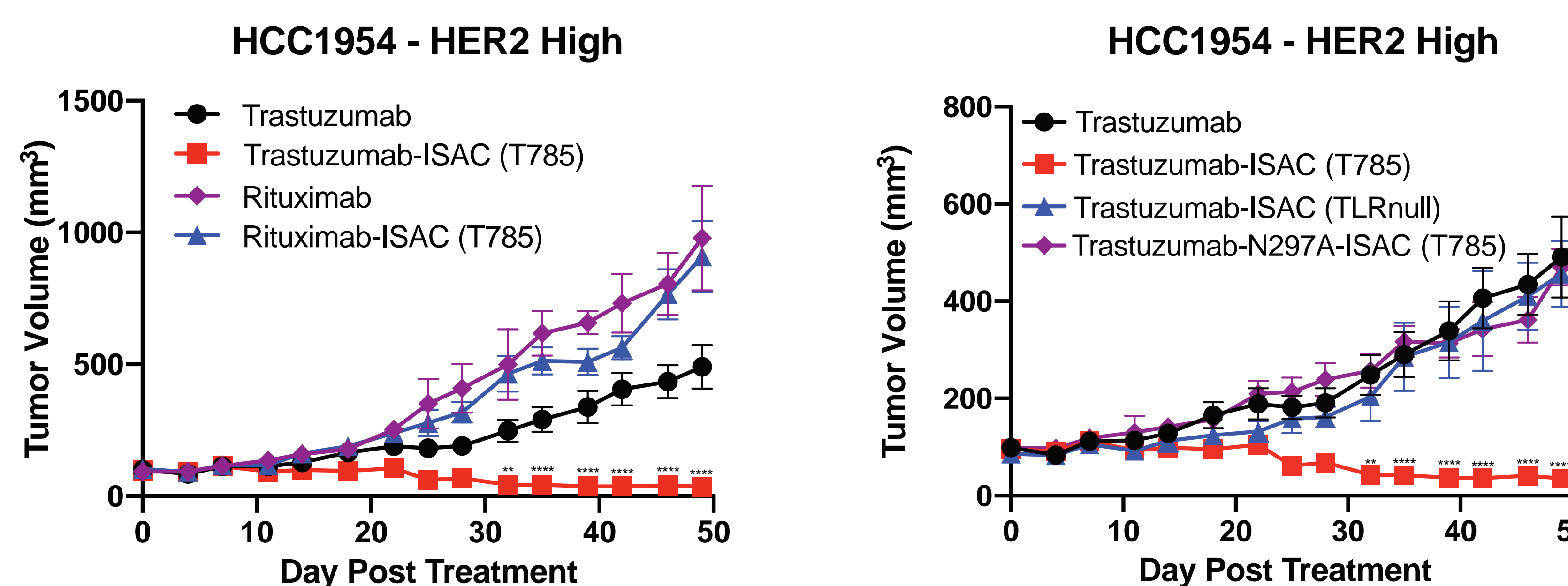


Figure 6: T785-containing ISACs require TLR- and FcR- engagement for anti-tumor efficacy.

IN VIVO RESULTS

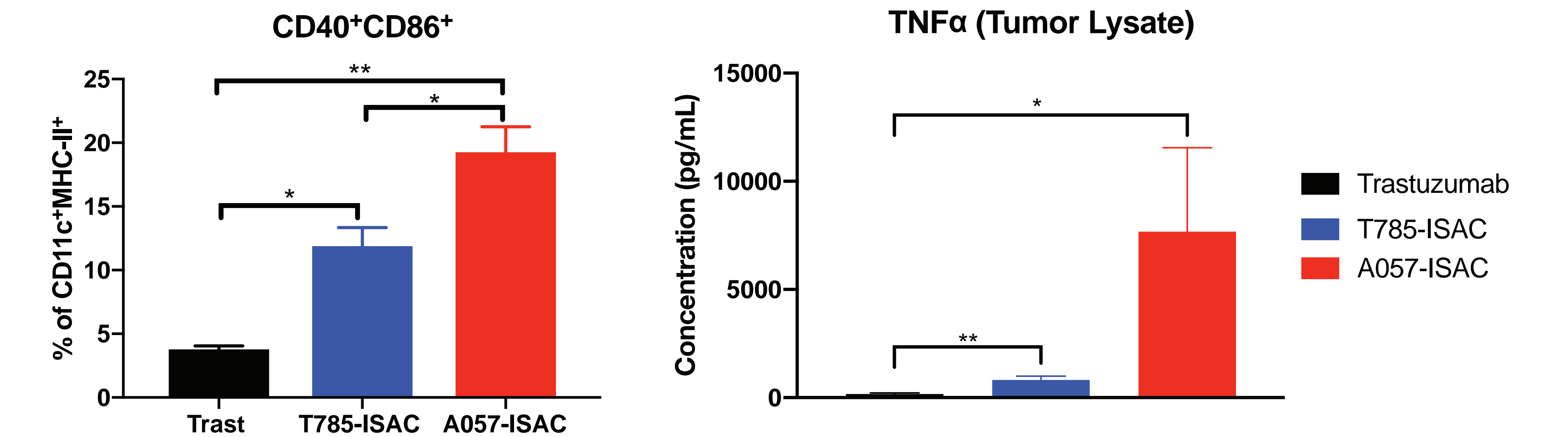


Figure 7: A057-ISACs exhibit greater potency than T785-ISACs.

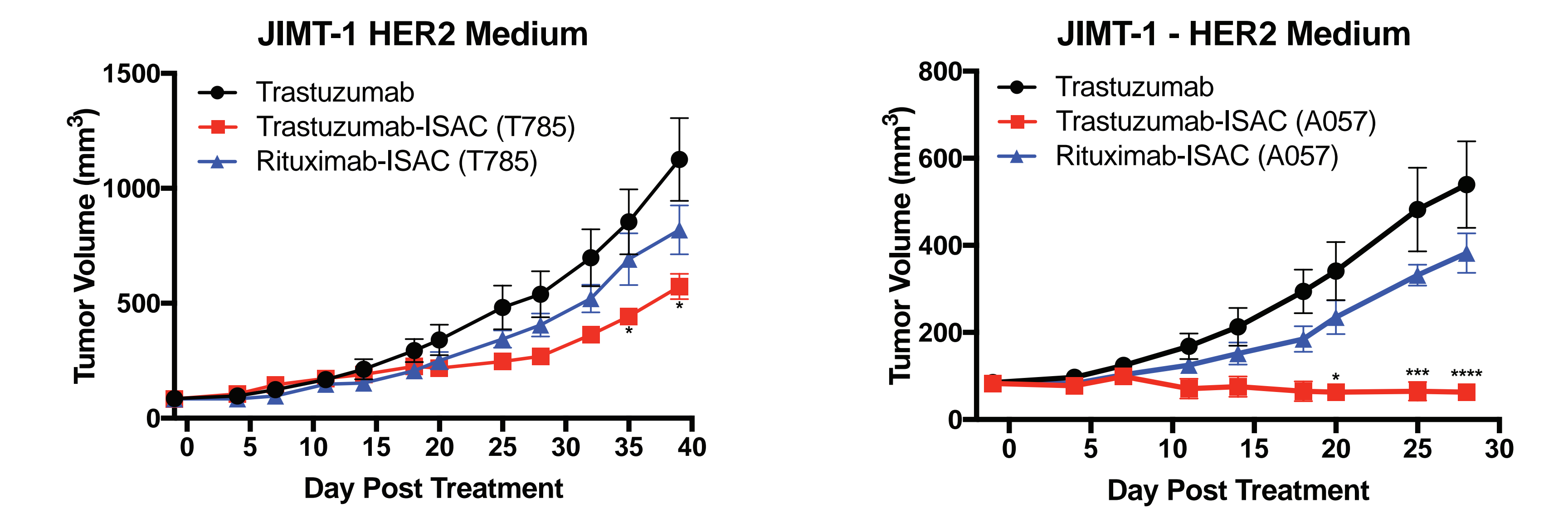


Figure 8: A057-ISACs mediate enhanced efficacy in trastuzumab-resistant model JIMT-1 as compared to T785-ISACs.

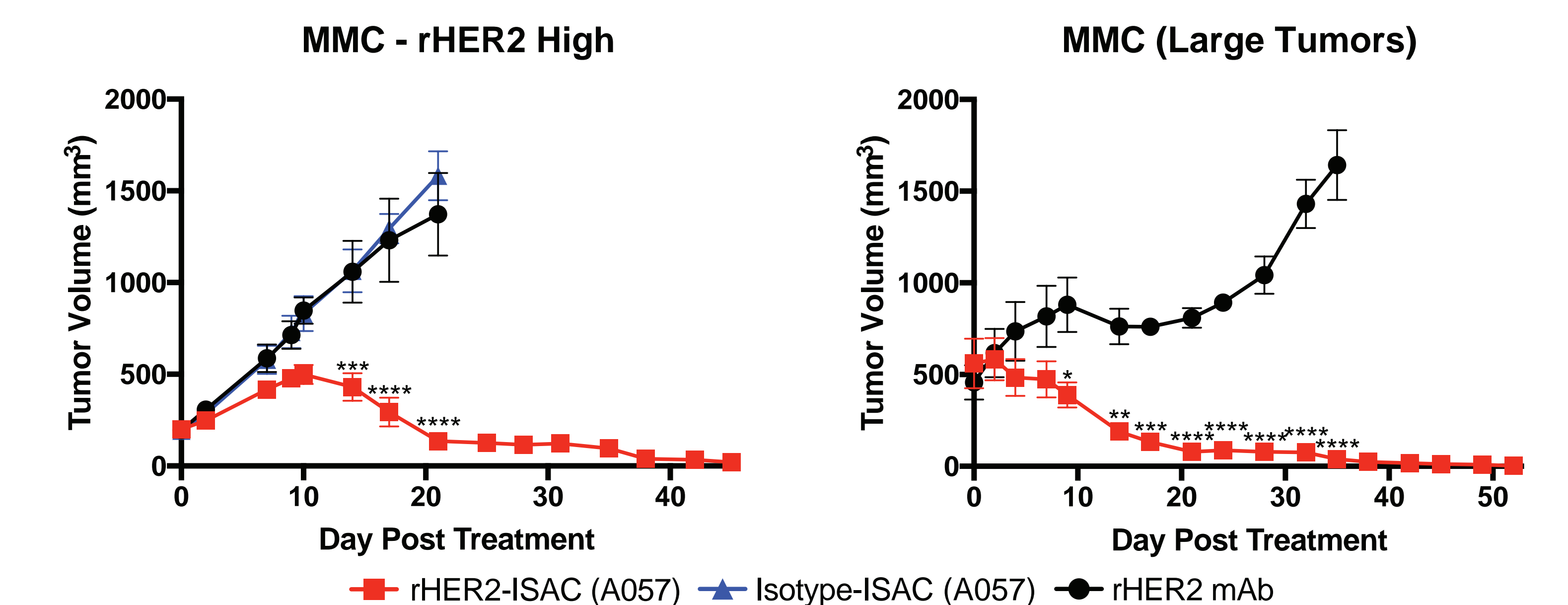


Figure 9: ISACs lead to tumor clearance of tumors >500 mm³ in syngeneic tumor model.

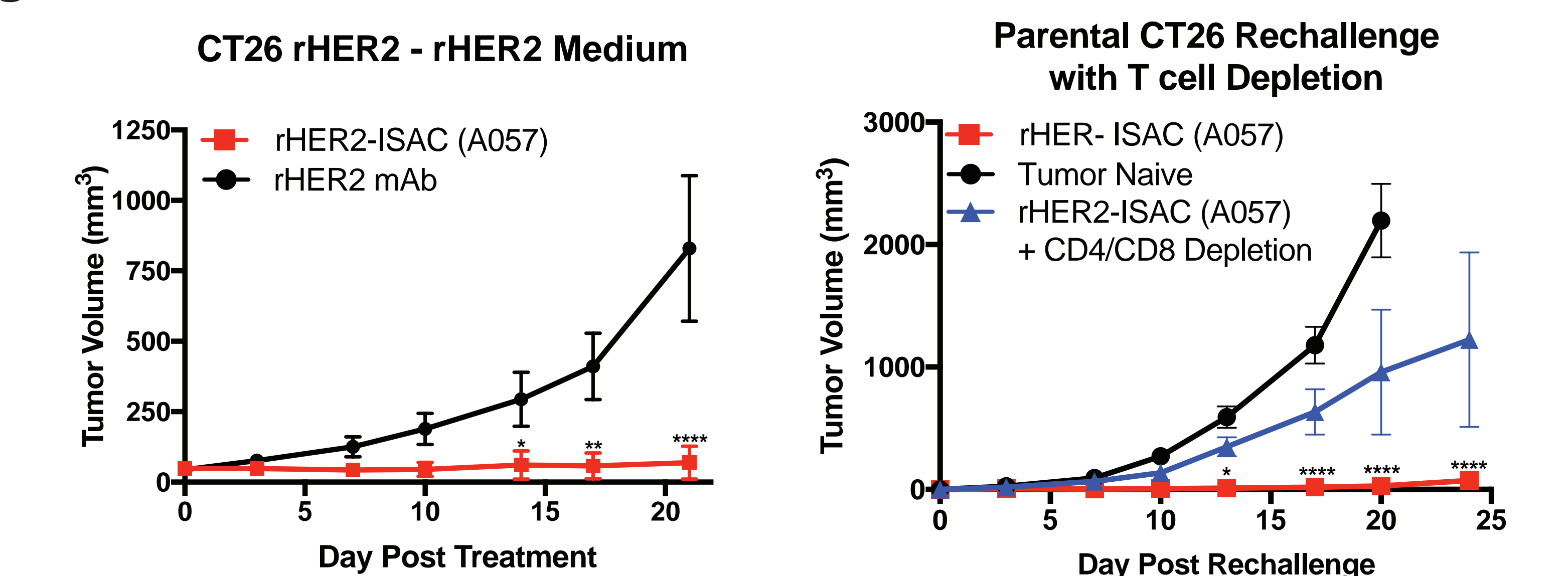


Figure 10: ISACs lead to tumor clearance, epitope spreading and govern T cell-dependent immunologic memory.

KEY FINDINGS

1. ISACs elicit robust myeloid activation and DC differentiation in human cells
2. ISAC treatment leads to anti-tumor efficacy in trastuzumab resistant models in TLR- and FcR- dependent manner
3. ISACs demonstrate efficacy tumors as large as >500 mm³ in volume
4. Tumor clearance following ISAC treatment leads to T cell-dependent immunologic memory