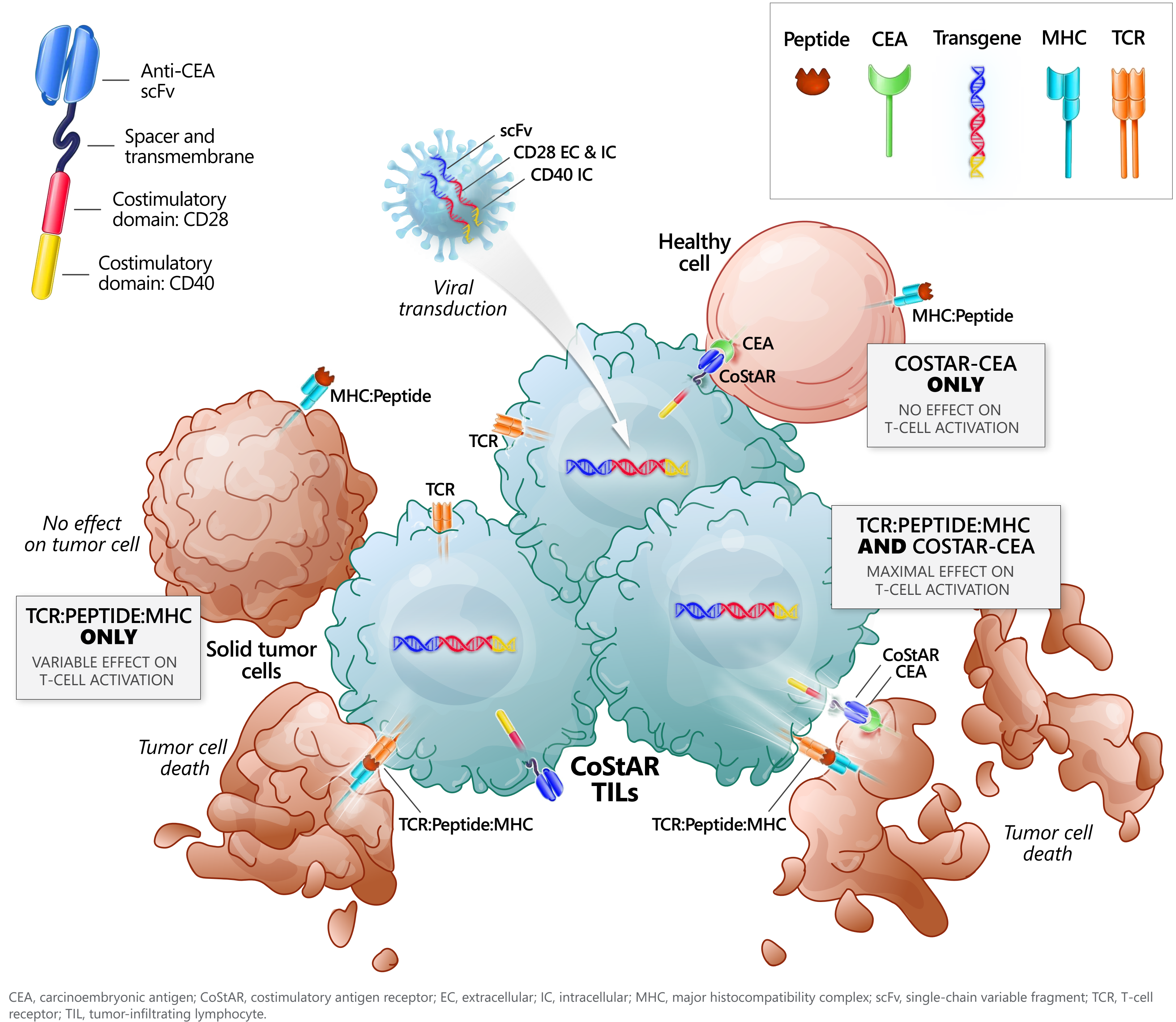


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- Costimulatory signals are a critical component in mounting an effective antitumor response¹
- Prolonged T-cell receptor (TCR) stimulation in the absence of costimulatory signals can lead to T-cell anergy and dysfunction²⁻⁴
- The suppressive tumor microenvironment is characterized by high expression of coinhibitory receptors and poor costimulation^{5,6}
- The novel costimulatory antigen receptor (CoStAR) molecule, delivered to T cells by lentiviral vector transduction, has an extracellular antigen-specific binding domain fused to 2 intracellular costimulatory receptor-derived signaling domains to improve T-cell effector function (**Figure 1**)

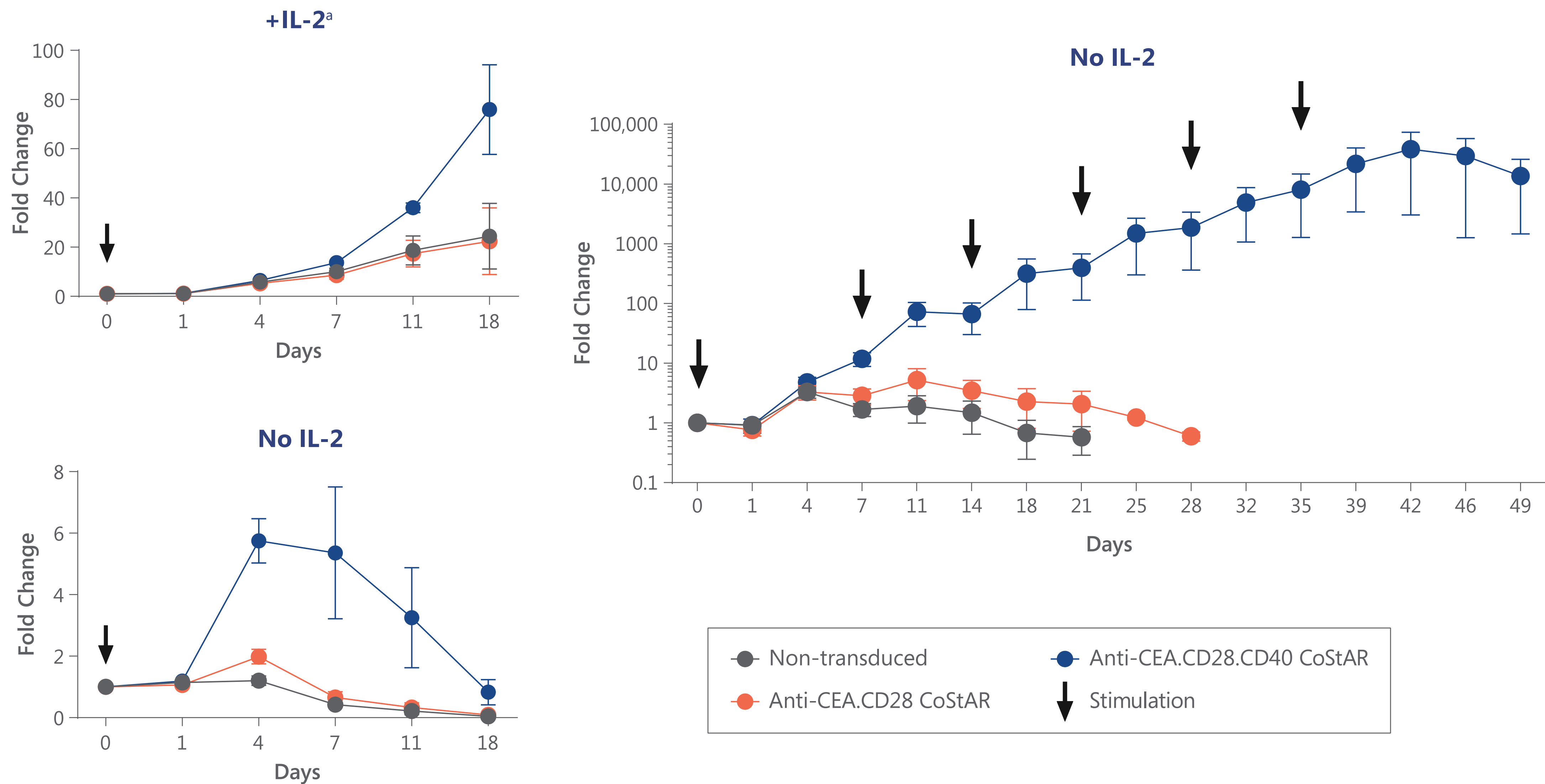
Figure 1. CoStAR Platform Overview



- Expression of CoSTAR by T cells enhances their functional activity, including cytotoxicity, proliferation, and cytokine expression
- CoSTAR-expressing T cells retain their native patient-specific anti-tumor TCR repertoire, which may limit antigen escape and off-tumor toxicity⁷
 - For additional information on the CoSTAR platform, specifically a clinical candidate, please see poster 198 by Sukumanan et al, titled “Costimulatory Antigen Receptor: A Novel Platform That Enhances the Activity of Tumor-Infiltrating Lymphocytes”
- Here, we describe a CoSTAR molecule that contains an extracellular single chain variable fragment (scFv) that binds carcinoembryonic antigen (CEA) and dual intracellular CD28 and CD40 signaling domains

- Primary human T cells from 3 healthy donors were transduced with CoStARs targeting the tumor-associated antigen CEA
 - Data shown herein depict a summary of donors
- Anti-CEA CoStAR T cells were cocultured with CEA+ tumor cells expressing:
 - Membrane-anchored anti-CD3 antibody (OKT3) to provide Signal 1 through TCR/CD3 complex crosslinking
 - CEA protein to provide potent costimulatory Signal 2 through CoStAR
- Anti-CEA CoStAR T cell results were confirmed and extended with an alternative experimental model using a CEA peptide-reactive transgenic TCR^g
- CoStAR signaling domains consisted of CD28 alone (anti-CEA.CD28 CoStAR) or a fusion of CD28 and CD40 (anti-CEA.CD28.CD40 CoStAR)
- Activity was measured by quantifying expression of activation markers, cytokine secretion, proliferation, and analysis of gene expression profiles

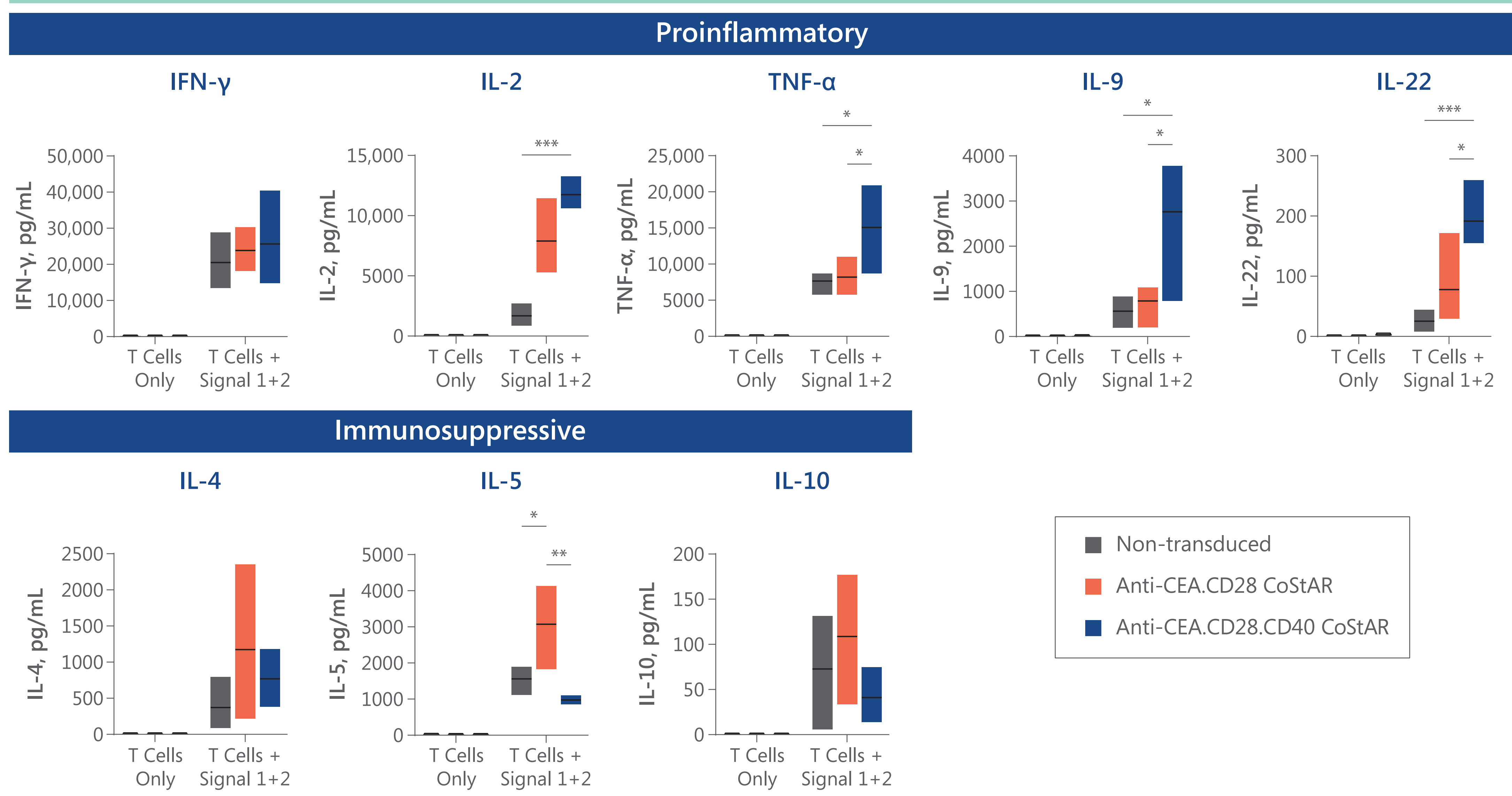
Figure 2. Exogenous IL-2-Independent Proliferation of CoStAR-Expressing T Cells



T cells were stimulated via a cell line containing anti-CD3 antibody and CEA antigen. CEA, carcinoembryonic antigen; CoStAR, costimulatory antigen receptor; IL, interleukin.

- Anti-CEA.CD28.CD40 CoStAR T cells exhibited exogenous interleukin (IL)-2-independent proliferation
- Anti-CEA.CD28.CD40 CoStAR T-cell proliferation was improved compared with anti-CEA.CD28 CoStAR and non-transduced T cells in the presence of exogenously added IL-2
- Sustained, exogenous IL-2-independent cell proliferation by anti-CEA.CD28.CD40 CoStAR T cells was observed upon serial stimulation

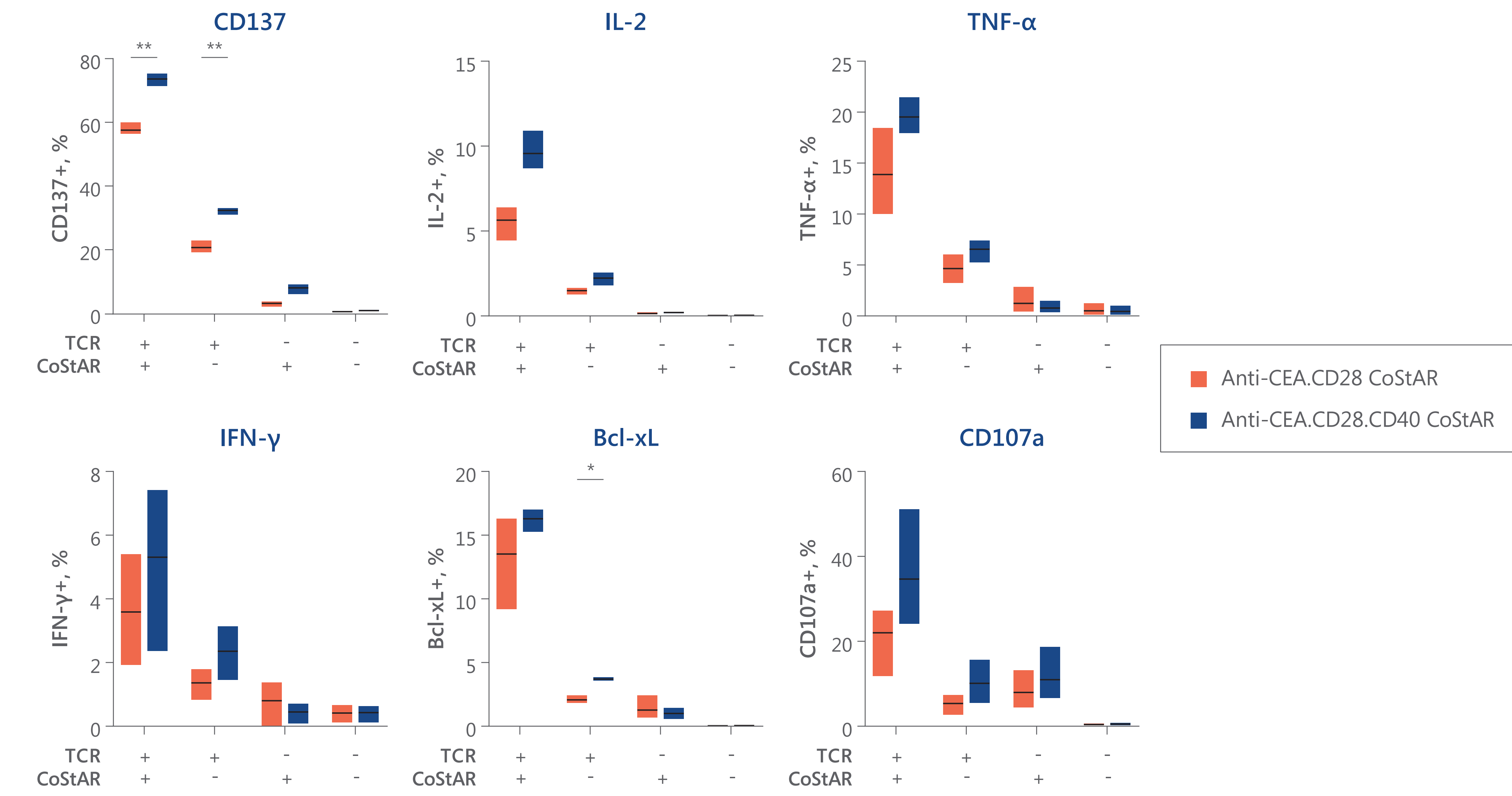
Figure 3. Cytokine Production by CoStAR-Expressing T Cells Cocultured With Targets Expressing OKT3 (Signal 1) and CEA (Signal 2)



Statistical significance was assessed by 2-way analysis of variance with Tukey's multiple comparison test; * $P<.05$, ** $P<.005$, and *** $P<.001$. CEA, carcinoembryonic antigen; CoStAR, costimulatory antigen receptor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

- Coculture of CoSTAR+ T cells with target cells expressing surface-bound OKT3 and CEA revealed enhanced proinflammatory cytokine expression by anti-CEA.CD28.CD40 CoSTAR as compared with anti-CEA.CD28 CoSTAR
- Anti-CEA.CD28.CD40 CoSTAR enhanced the production of multiple proinflammatory cytokines, including IL-2, tumor necrosis factor (TNF)- α , IL-9, and IL-22
- Protein levels of immunosuppressive cytokines (IL-4, IL-5, and IL-10) were unchanged compared with non-transduced controls

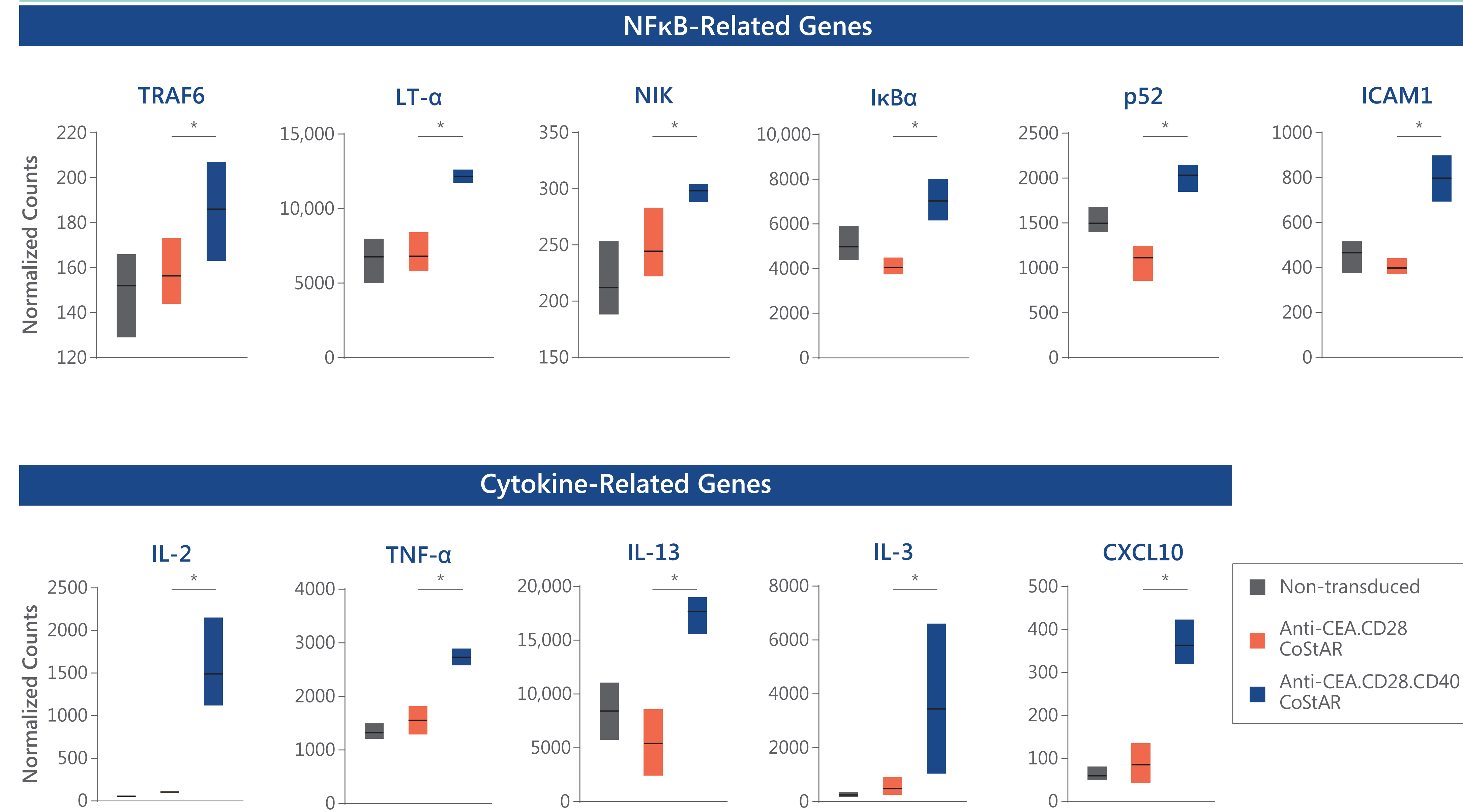
Figure 4. Enhanced T-Cell Effector Function Following Peptide:MHC Binding by Transgenic TCR



TCR represents a CEA-reactive TCR⁴
Statistical significance was assessed by 2-way analysis of variance with Tukey's multiple comparison test; **P* < .05 and ***P* < .005.
CEA, carcinoembryonic antigen; B0-XL, B-cell lymphoma-extra large; CoStAR, costimulatory antigen receptor; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; TCR, T-cell receptor; TNF, tumor necrosis factor.

- T cells expressing an anti-CEA TCR and either anti-CEA.CD28 or anti-CEA.CD28.CD40 CoStAR were cocultured with targets expressing CEA
- Effector activity was assessed by CD137, IL-2, TNF- α , interferon- γ , B-cell lymphoma-extra large (Bcl-xL), and CD107 in vitro model of TCR transfer

Figure 5. Increased NF- κ B Signaling and Cytokine-Related Gene Expression by Anti-CEA.CD28.CD40 CoStAR-Expressing T Cells



Negative binomial test was used on the normalized count data to evaluate statistical significance. Differences among presented genes are statistically significant (**P* < .05). CEA, carcinoembryonic antigen; CoStAR, costimulatory antigen receptor; CXCL10, C-X-C motif chemokine ligand 10; ICAM1, intercellular adhesion molecule 1; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; IL, interleukin; LT-α, lymphotxin alpha; NIK, nuclear factor kappa B-inducing kinase; TNF, tumor necrosis factor; TRAF6, tumor necrosis factor receptor-associated factor 6.

- CoSTAR-expressing T cells were cocultured with targets expressing membrane bound OKT3 and CEA
- Anti-CEA.CD28.CD40 CoSTAR enhanced nuclear factor kappa B (NF- κ B)-related gene transcription and downstream genes, including IL-2, TNF- α , and IL-13
- These findings are consistent with previous work demonstrating that CD40-mediated signaling occurs via the NF- κ B pathway⁸¹⁰

- CoStAR is a lentiviral vector-delivered molecule designed to enhance activation of T cells

- The inclusion of costimulatory domains from CD28 and CD40 in the CoStAR molecule gives rise to markedly increased T-cell activity, including improvement in secretion of proinflammatory cytokines and long-term, exogenous IL-2-independent proliferation

- The novel design of the CoStAR molecule, including CD28 and CD40 signaling motifs, may further improve the performance of T-cell-based therapies, including tumor-infiltrating lymphocytes (TILs)

- Similar observations with an analogous anti-folate receptor alpha (FOLR1) CoStAR have been observed,⁷ indicating broad applicability of the CoStAR platform across target molecules and tumor indications

- A first-in-human clinical study with ITIL-306, an investigational anti-FOLR1 CoStAR TIL product, is planned (sponsored by Instil Bio, Inc.)

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ACKNOWLEDGMENTS

* Medical writing support was provided by Christopher Waldapfel, PharmD, of Instil Bio, Inc., and Ashley Skorusa, PhD, and Jennifer Yang, PhD, of Nexus Global Group Science, with funding from Instil Bio, Inc.

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