



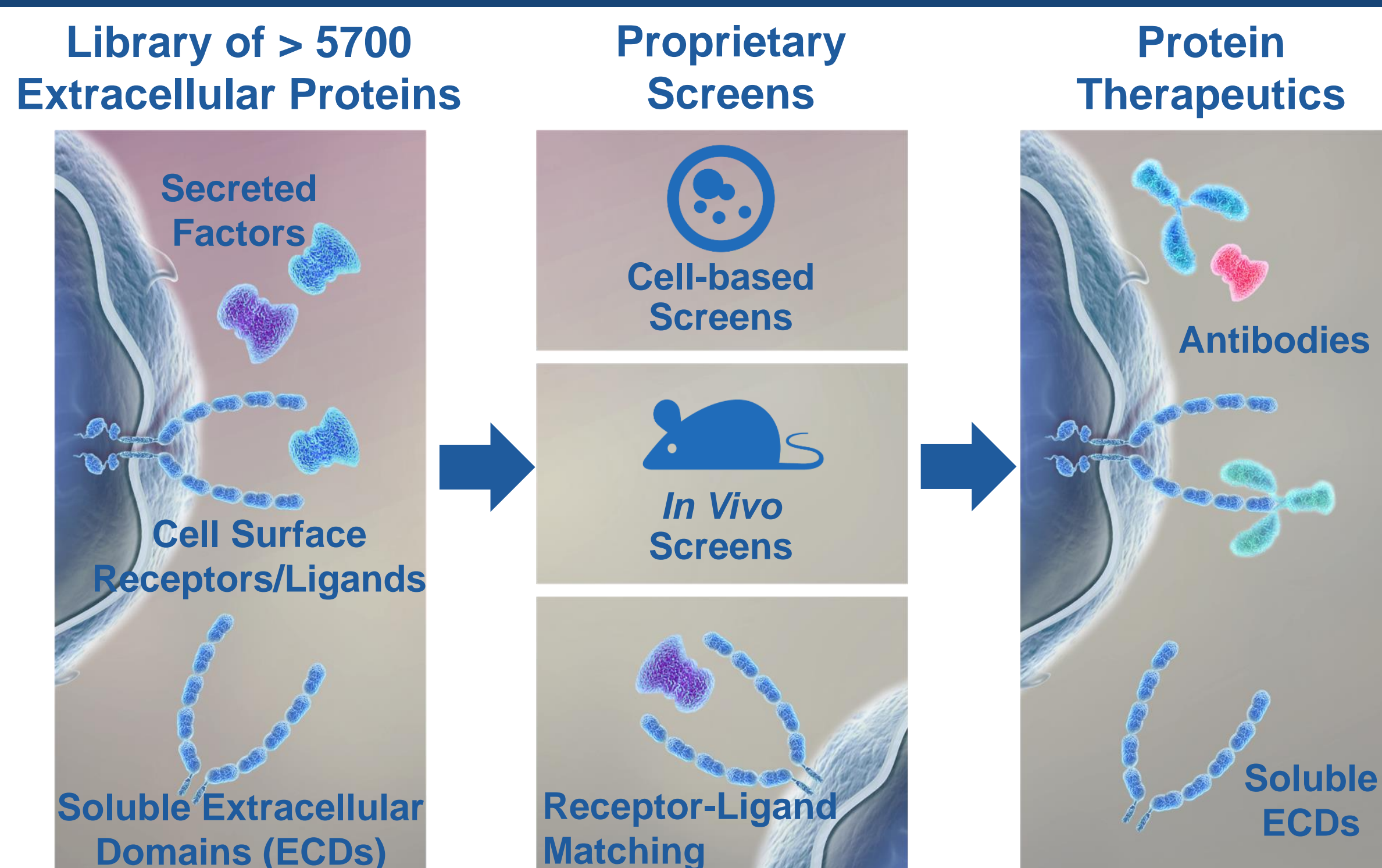
Identification Of Novel T Cell Co-inhibitory And Co-stimulatory Receptors From Screening A Comprehensive Library Of Extracellular Proteins

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Introduction

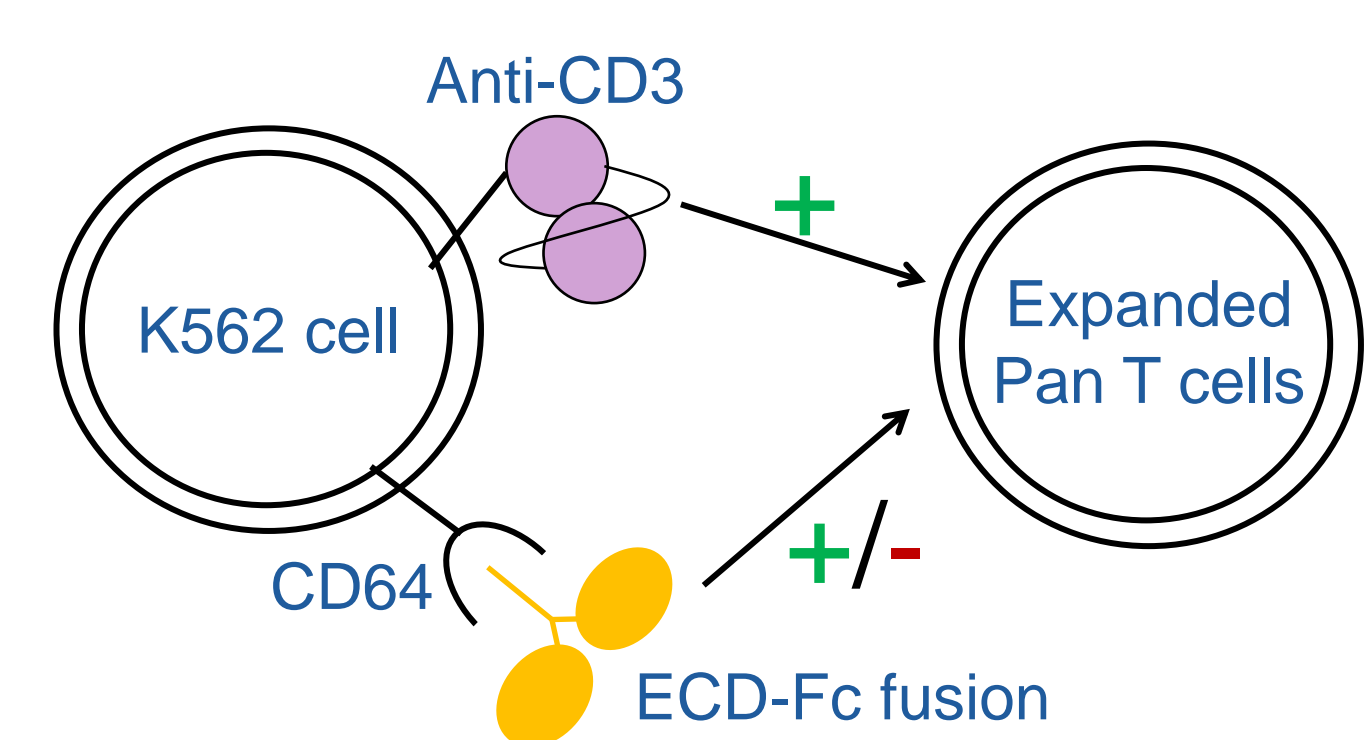
We have developed multiple sophisticated *in vitro*, *in vivo*, and biophysical assay systems for screening our comprehensive library of human extracellular proteins in order to identify novel therapeutics that can reprogram immune cells in the tumor microenvironment. *In vitro* screens of primary human effector and regulatory T cells identified novel proteins that can modulate T cell activation and suppression. We also screened subsets of our library in four distinct syngeneic mouse tumor models (either as single agents, or in combination with checkpoint blockade or vaccine) and identified targets that can modulate an anti-tumor immune response *in vivo*. In addition, we performed a combinatorial biophysical screen for protein-protein interactions within a set of ~700 predicted immune-related transmembrane proteins and identified novel receptor-ligand interaction networks. Using this multi-pronged screening approach, we have confirmed activities of well-known immunomodulatory proteins including GITRL and CD80. Moreover, we have identified novel immune modulators that have activities across multiple *in vitro* and *in vivo* assays, as well as novel interaction partners for these proteins that help us elucidate their mechanisms of action. One such example is OLR1, which we found to be a novel co-stimulator of effector T cells, potentially through a novel interaction with TM9SF1. Additionally, we identified previously-unappreciated mechanisms for proteins with known immunomodulatory properties. One such example is soluble DLL4, which we found to specifically stimulate regulatory T cells and to modulate adaptive immune responses in a tumor model *in vivo*. Taken together, we have developed robust *in vitro* and *in vivo* platforms that allow us to discover new immuno-therapeutic targets that we believe will help address the needs of cancer patients that fail to respond to current immunomodulatory therapies.

FivePrime's Unique Platform: Screening A Library Of The Extracellular Proteome To Identify Novel Targets And Therapeutics

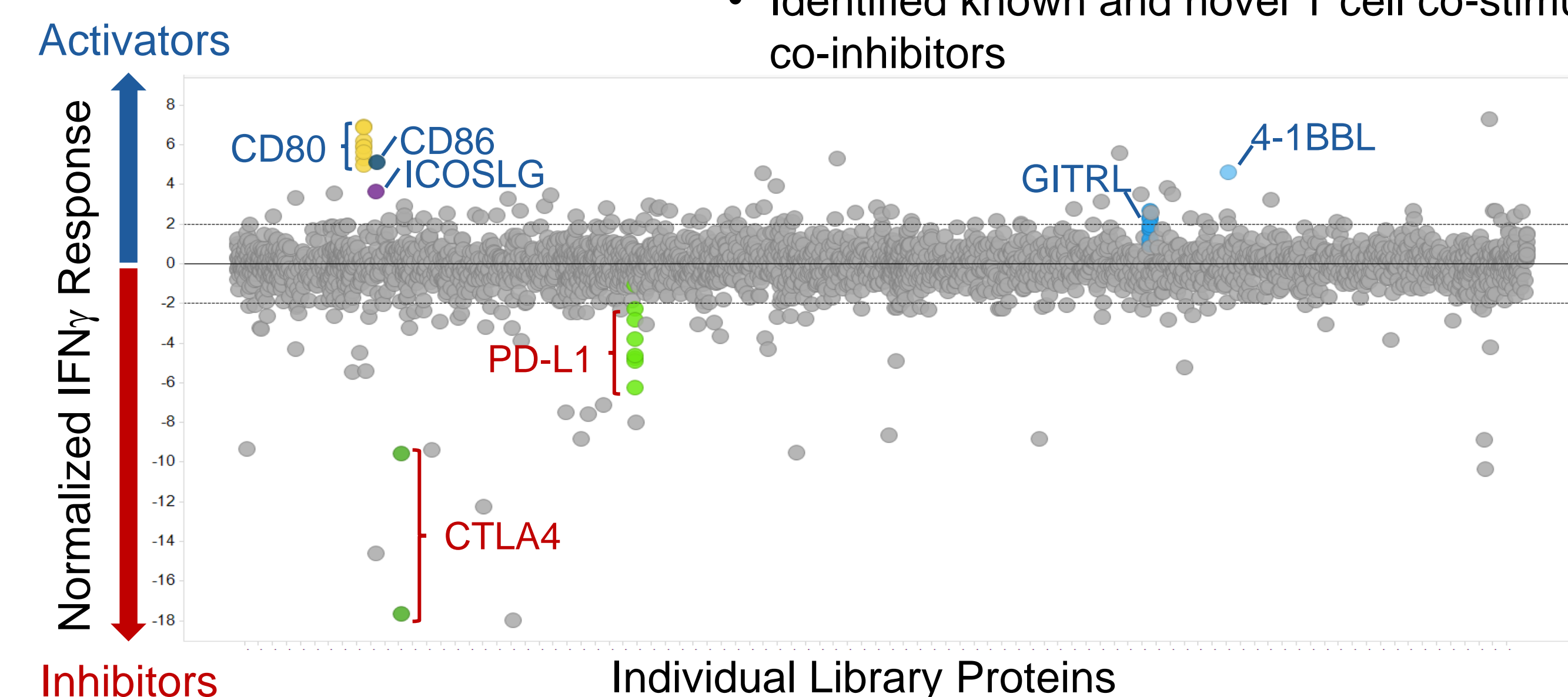


- Secreted proteins and extracellular domains of membrane bound proteins are expressed in a human cell system
- The proteins are screened for functional activity in cell based or biophysical binding studies
- DNA encoding mouse secreted proteins is delivered directly into mouse disease models

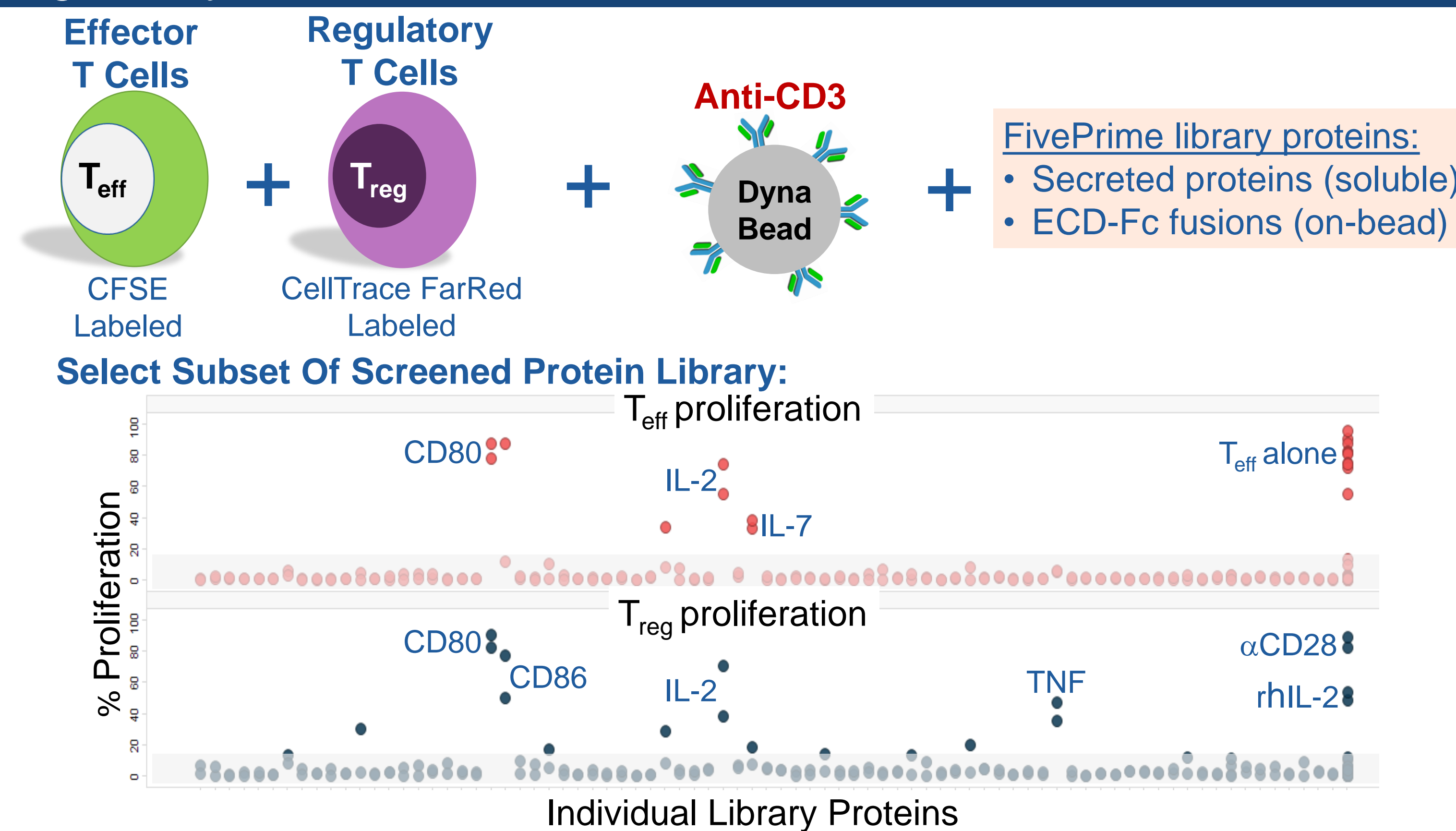
Effector T Cell Screen



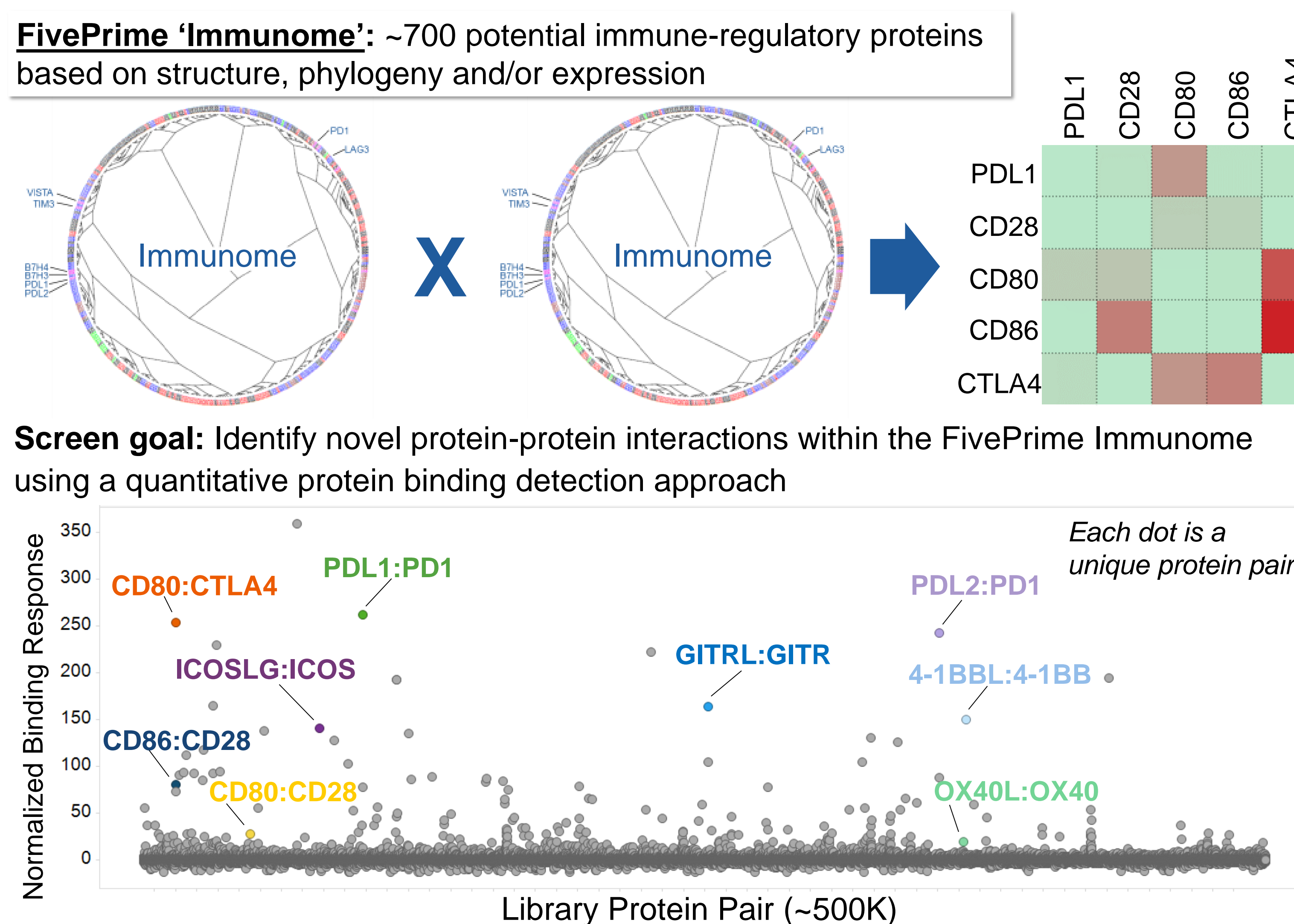
- Engineered K562 artificial antigen presenting cell:
 - Cell-surface anti-CD3 fragment to stimulate T cells
 - Non-signaling CD64 Fc receptor to capture library proteins
- Human pan T cells pre-activated, then rested
- Incubated with FivePrime library of extracellular domain (ECD) – Fc fusion proteins
- Identified known and novel T cell co-stimulators and co-inhibitors



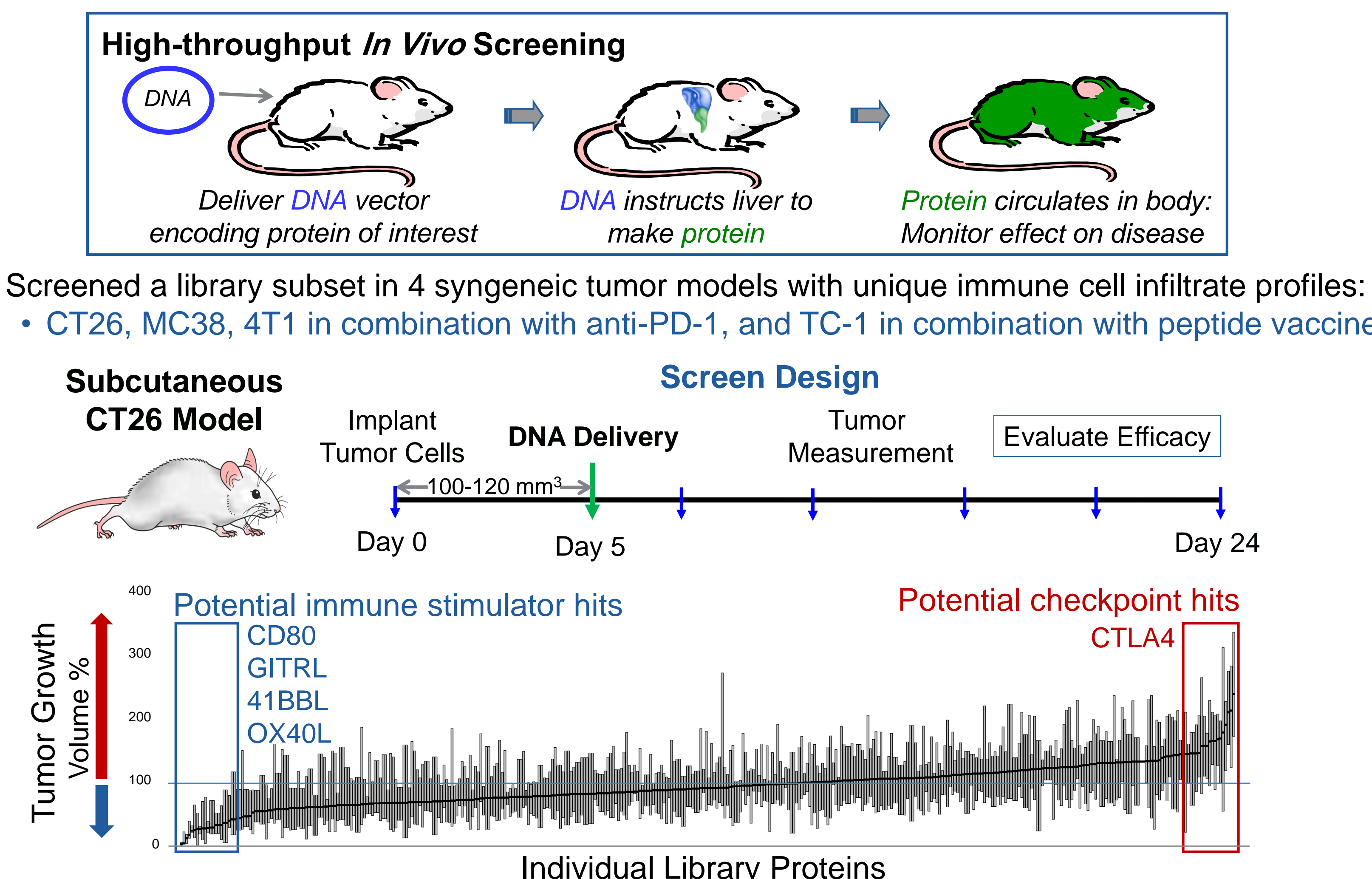
Regulatory T Cell Screen



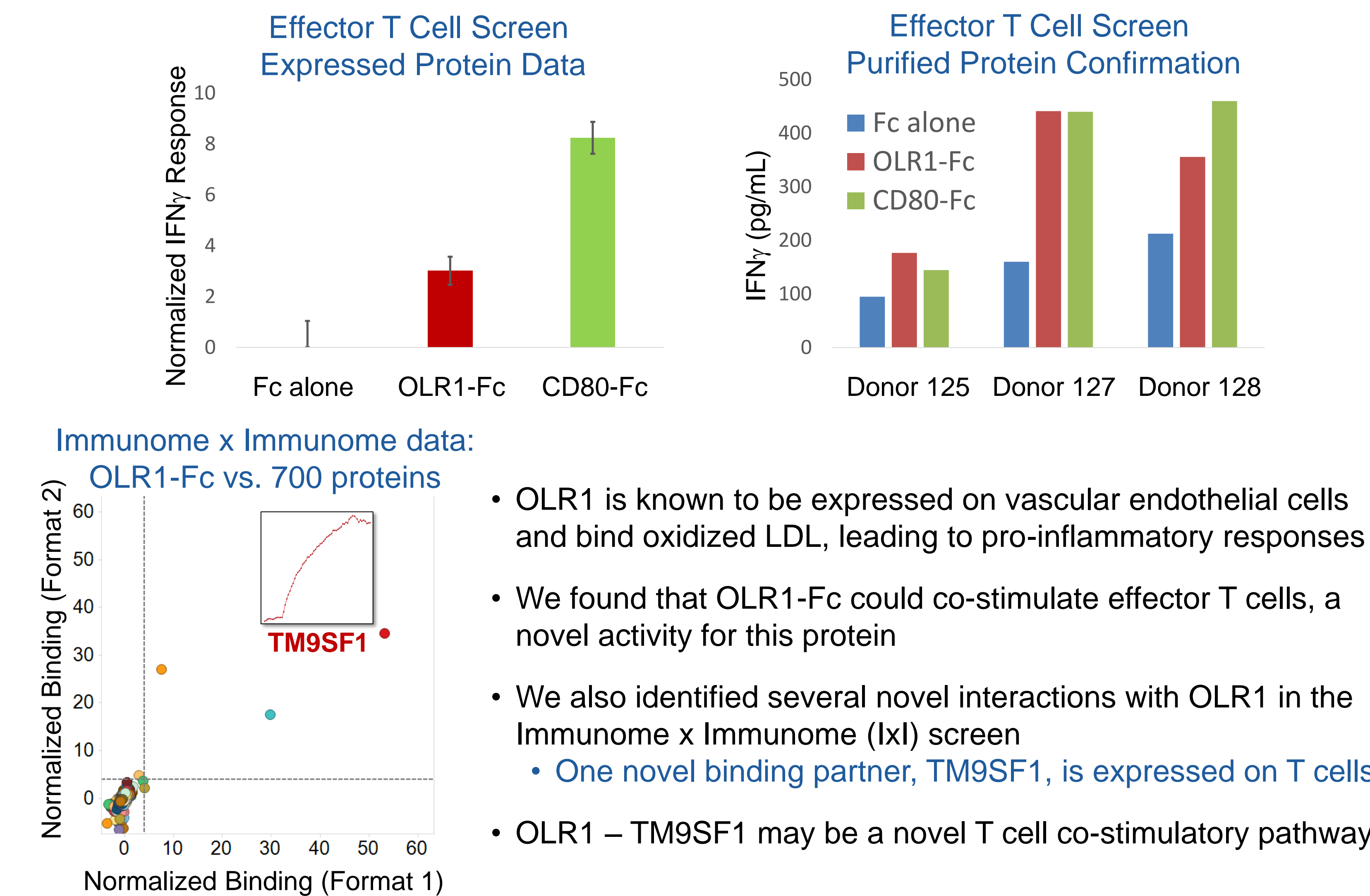
Combinatorial Receptor-Ligand Matching Screen



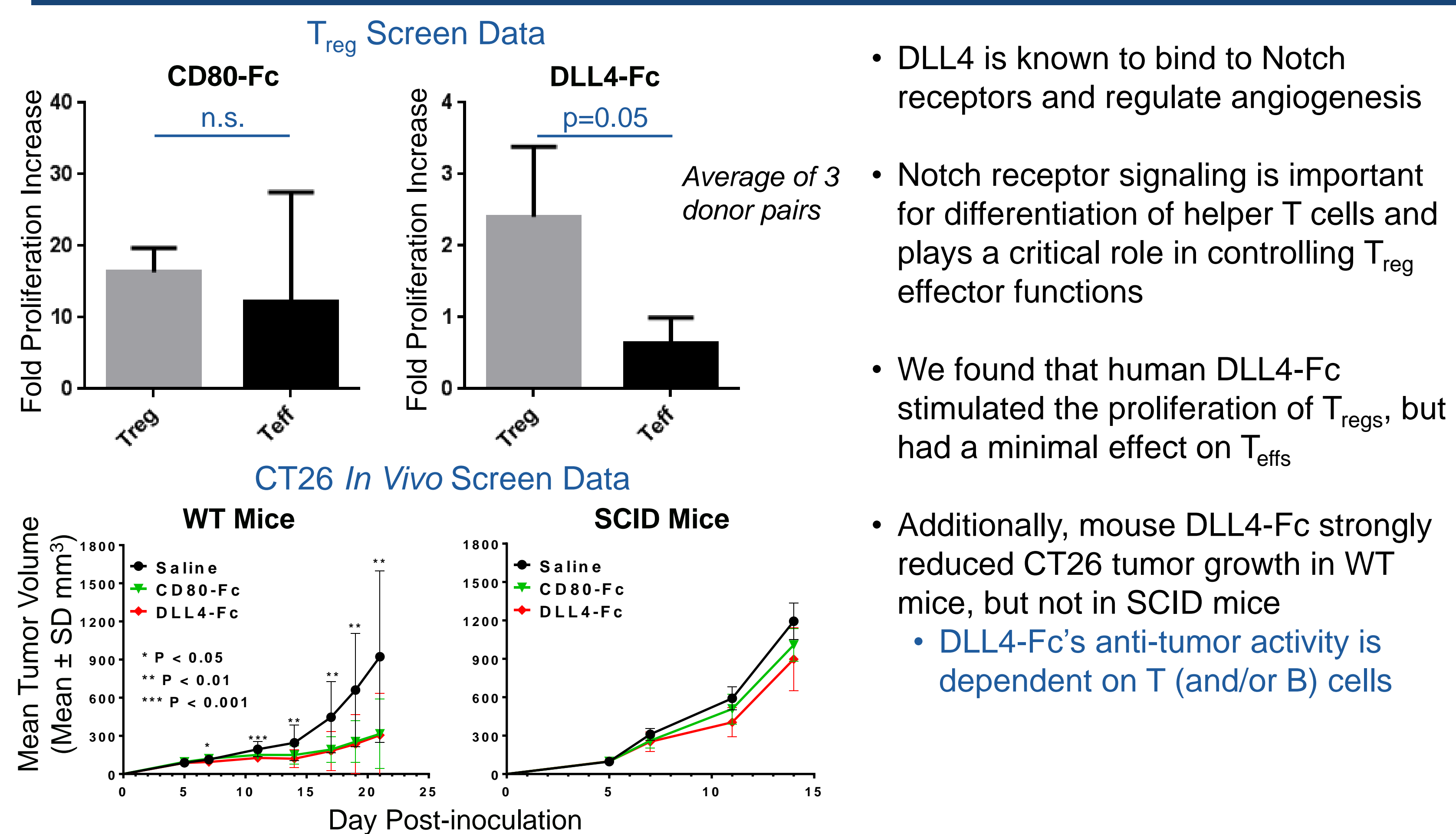
In Vivo Screens In Multiple Syngeneic Tumor Models



Oxidized Low-Density Lipoprotein Receptor 1 (OLR1) Is A Novel T Cell Co-Stimulator For Which We Identified Novel Binding Partners



Soluble Delta-Like Protein 4 (DLL4) Specifically Stimulates Regulatory T Cells & Controls Anti-Tumor Immune Response In Vivo



Summary And Next Steps

