In silico and multi-dimensional functional interrogation of tissue Tregs reveals novel therapeutics for autoimmunity and inflammation

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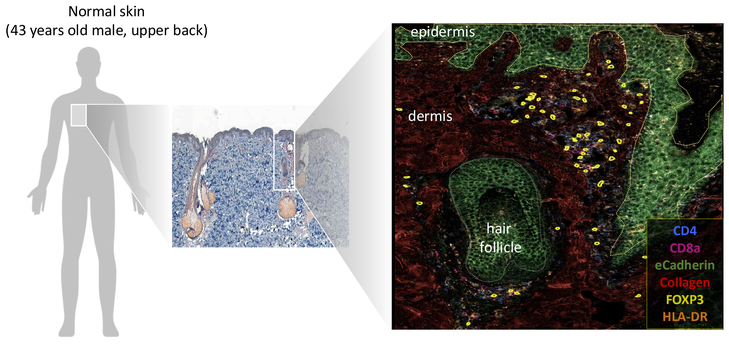
Abstract

As we gain a higher resolution understanding of the immune system's role in homeostasis and individual disease pathologies it has become increasingly clear that different tissue environments can result in disparate roles and functions for a given immune cell type. Understanding the *in situ* tissue-specific action of immune cellular networks may offer important insights into novel therapeutic strategies for inflammatory diseases. As Tregs are master regulators of the immune system, modulation of tissue resident Tregs presents a particularly promising strategy. We have developed a platform to comprehensively dissect their signaling and function with the aim to therapeutically reprogram or enhance Tregs in disease states. We used bulk and single-cell RNA sequencing along with a diverse set of computational analyses to build gene networks characterizing our exclusive collection of healthy and diseased tissues. These analyses led us to identify *in vitro* culturing conditions that recapitulate the expression of many of the same pathways found in primary tissue resident Tregs. In parallel, we developed a suite of functional and phenotypic assays that allow for a comprehensive assessment of modulators of our tissue-like Tregs. With these tools in hand, we can perturb these cells using genetic deletion, gene overexpression, along with molecular treatments and assess the function of human tissue-derived targets. In summary, our platform has the ability to reveal novel tissue Treg focused therapeutic targets for autoimmune and inflammatory diseases.

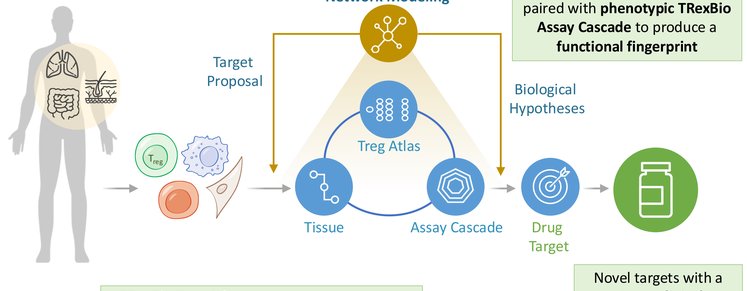
Tregs are localized in certain tissues, but their specific phenotypes and functions have yet to be explored



Cells were defined by nuclei segmentation and unbiased clustering based on different markers. Clusters were then identified as specific cell types with an internally-developed algorithm that uses critical lineage markers. Tregs (shown in yellow) are located in the dermis and interact with different cell types.

Deep Biology Platform combines –omics data, computational modeling and functional screening to ID tissue Treg pathways

High resolution profiling of **human tissue** in health and disease by bulk and single-cell RNA sequencing

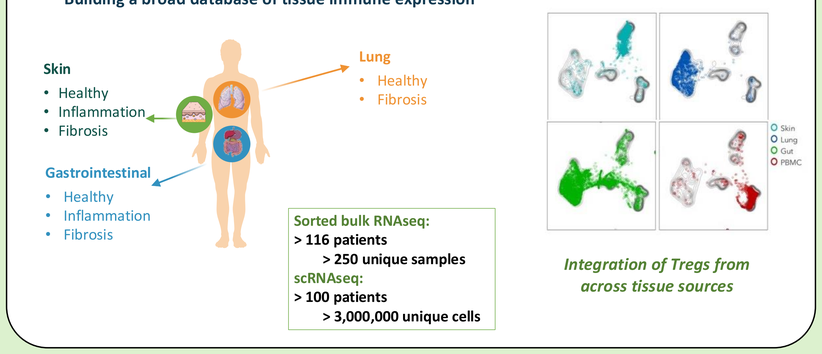


Cutting-edge computational tools integrating human transcriptomics and genetics data paired with **phenotypic TRexBio Assay Cascade** to produce a **functional fingerprint**

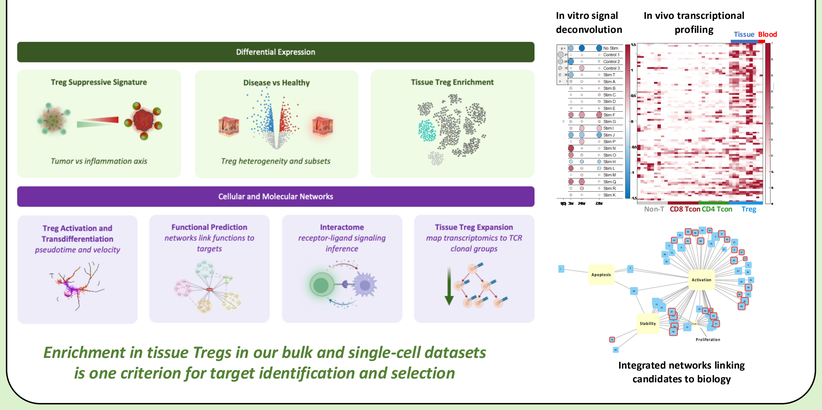
TRexBio Treg Atlas recreates tissue states in blood-derived Tregs generating **tissue-like Tregs**

Novel targets with a strong **clinical hypothesis**

Tissue Database: Proprietary collection of single-cell and bulk RNA-sequencing data across healthy and diseased barrier tissues

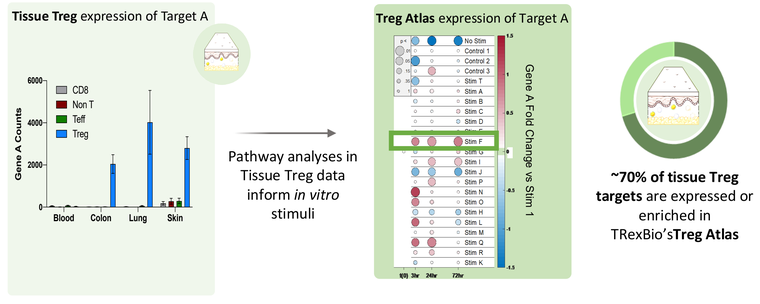


Target ID: Purpose-built computational methods to identify key tissue Treg pathways and reveal novel therapeutic targets



Enrichment in tissue Tregs in our bulk and single-cell datasets is one criterion for target identification and selection

Treg Atlas: System to induce tissue-pathway expression in blood derived Treg *in vitro* using tissue-specific perturbations

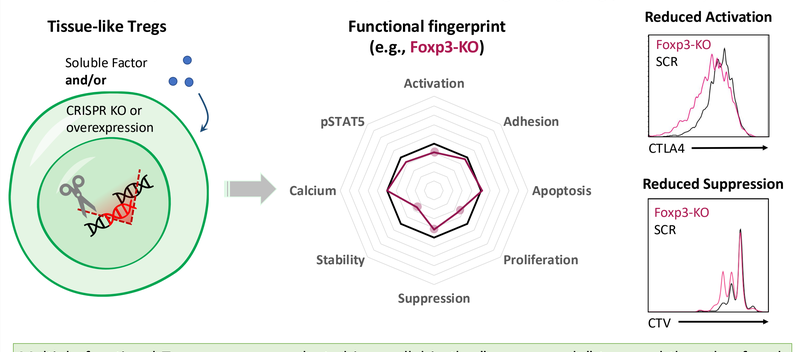


Tissue Tregsare different than Blood Tregs

TRexBio rationally selected stimuli generate tissue-like Tregs

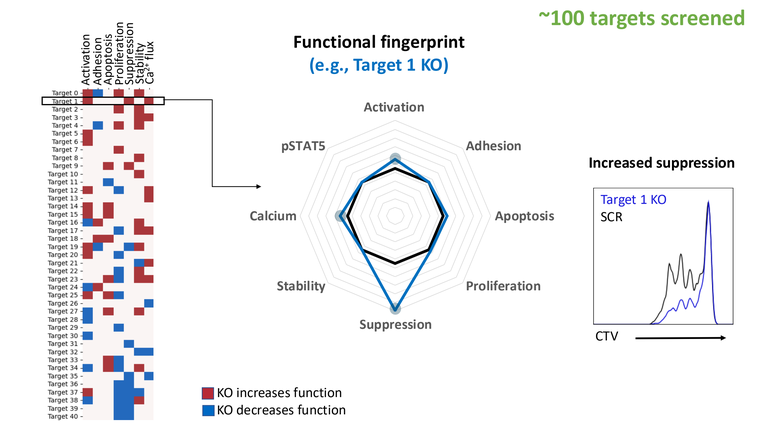
Assay Cascade: Suite of functional assays in primary human Treg designed to reveal clinically relevant pathway biology

Assay cascade was validated by key drivers of Treg biology (e.g., FOXP3-KO)



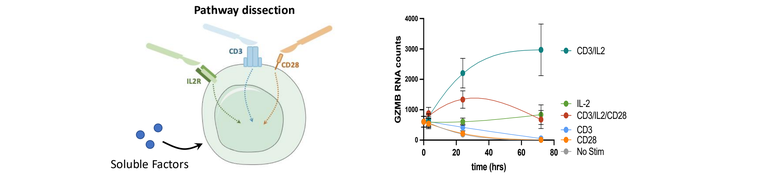
Multiple functional Treg assays are evaluated in parallel in the "assay cascade" to reveal the role of each target in the regulation of Treg functions. Cascade screening was validated with multiple controls: FOXP3 KO led to reduced Treg function as measured by Activation, Proliferation, Stability and Suppression

CRISPR Cascade :Functional screening reveals essential tissue Treg pathways highlighting potential therapeutic targets

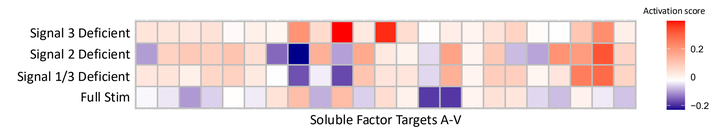


Interrogation of candidates using our Assay Cascade robustly reveals new targets for Treg modulation. The heatmap shows effect on Treg functions for 40 out of the ~100 screened targets with a functional hit using CRISPR KO. Target 1 example shows that KO of this target enhances Treg suppression capacity.

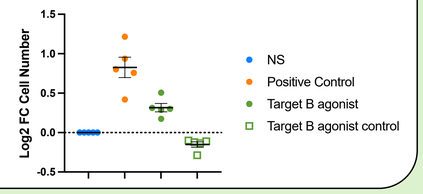
Soluble Factor Screening:Focused activation and proliferation screening reveals targets and pathways regulating Treg activity under diverse stimulatory backgrounds



Gene expression and function are dependent on signaling context



Soluble factor screening identified Target B as a candidate that promotes activation and proliferation of Tregs under stimulation conditions similar to those found in the periphery.



Pipeline: Advancing multiple large molecule therapeutic candidates with the potential to be first-in-class therapeutics

Atopic Dermatitis **TRB-061**	Preclinical	IND Enabling	Phase 1	Phase 2	Phase 3
IMMUNOLOGY **TRB-071**	Preclinical	IND Enabling	Phase 1	Phase 2	Phase 3
IMMUNOLOGY **TRB-051** Partner: Lilly	Preclinical	IND Enabling	Phase 1	Phase 2	Phase 3