

A Novel Anti-EphrinB2 Neutralizing Antibody Suppresses Fibrosis and Modulates Systemic Sclerosis Gene Signatures in Preclinical Models



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Background

Fibrosis is complex pathology characterized by persistent inflammation, aberrant synthesis and accumulation of extracellular matrix (ECM), and ultimately loss of organ function as healthy tissue is replaced by scar tissue. EphrinB2 is a protein hypothesized to play a role in fibroblast activation and has been found to be increased in fibrosing diseases, such as systemic sclerosis (SSc). Although the exact mechanism is not well understood, dysregulation of the EphrinB2-EPHB signaling axis is thought to play a role in fibrotic disease progression. We generated neutralizing antibodies to EphrinB2 and investigated their anti-fibrotic efficacy in preclinical *in vitro* and *in vivo* studies including bleomycin models of skin fibrosis. We further sought to identify genes and pathways relevant in SSc with evidence of preclinical modulation by anti-EphrinB2 antibody treatment.

Methods

Antibodies to EphrinB2 were generated by several approaches with leads undergoing affinity maturation engineering using phage display methods. Bacteria phage expressing these libraries were panned on EphrinB2 extra cellular domain (ECD) protein for multiple rounds of increasing binding stringency. Selected clones with desired binding affinities were sequenced, reformatted, and expressed as human IgG1 antibodies.

Inhibition of EphB4 Receptor phosphorylation was evaluated by treating Human Umbilical Vein Endothelial Cells (HUVECs) with EphrinB2-Fc +/- anti-EphrinB2 antibody or isotype control for 1h and quantifying pEphB4 via ELISA (RayBiotech).

Bleomycin mouse models of skin fibrosis were conducted at Aragen under IACUC approved protocols. Study designs are shown in the Results. Histological analysis was conducted at Dallas Tissue and evaluated by a board-certified pathologist.

Publicly available and proprietary human and mouse skin, lung, and heart gene expression datasets were analyzed, including RNA-seq and microarray data from preclinical fibrosis models and systemic sclerosis clinical trials. Differential expression analysis was performed using DESeq2 (for RNA-seq) and limma (for microarrays), followed by pathway and meta-analyses to identify consistent biological signals across datasets and to understand the transcriptional impact of the neutralizing anti-EphrinB2 antibody.

Results

A novel high affinity anti-EphrinB2 antibody has been generated. This antibody demonstrates EphB4 phosphorylation inhibition in a HUVEC assay and significantly reduced fibrosis in preclinical mouse models of skin fibrosis.

The cross-species genetic meta-analysis identified key transcriptional similarities between human SSc and preclinical rodent models, highlighting conserved dysregulated pathways in disease, including TGF β , JAK-STAT, NF κ B, TNF α , and EGFR signaling. Anti-EphrinB2 administration reversed significant subsets of these dysregulated genes, with robust evidence supporting its impact on fibrosis- and inflammation-related pathways.

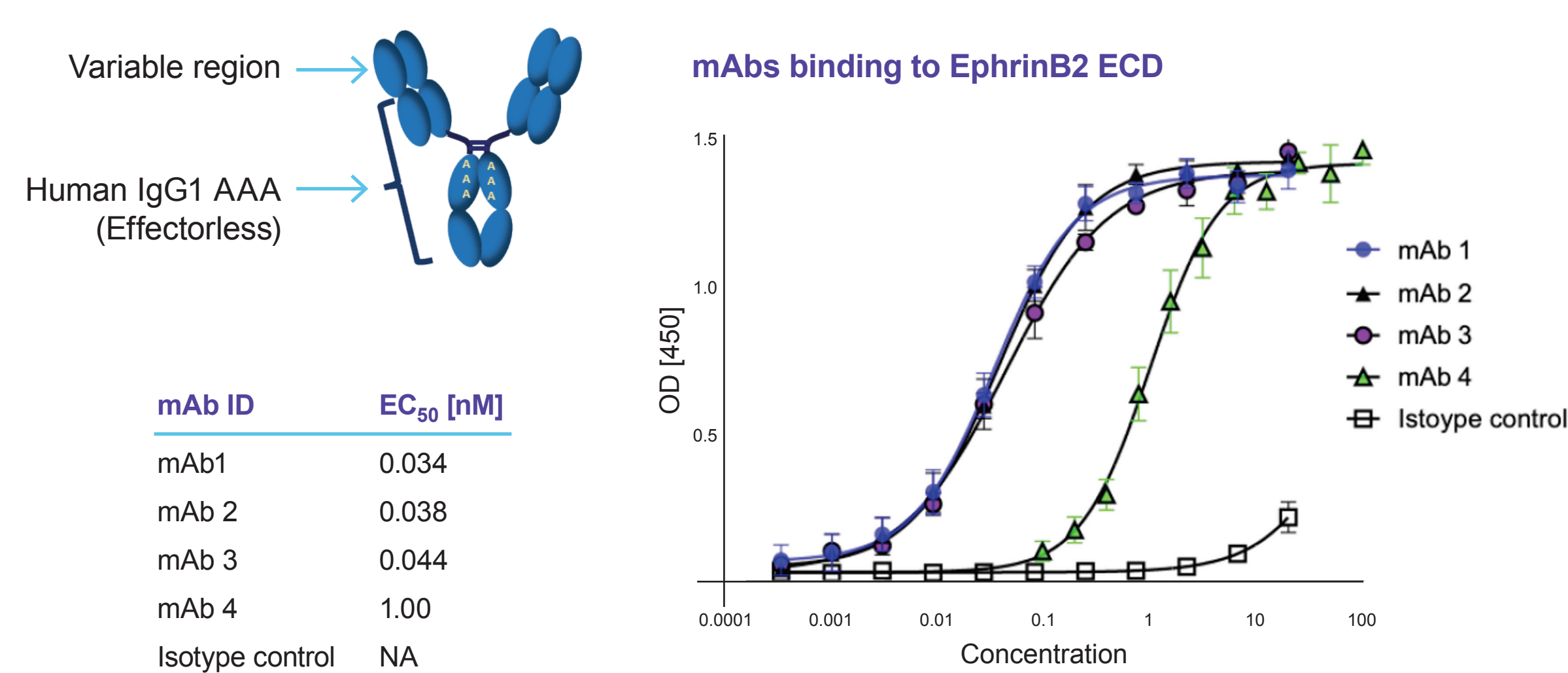
Implications

These findings align with preclinical evidence and further support EphrinB2 neutralization as a promising strategy for SSc treatment. Notably, CCN2, THBS1, and CD14 emerged as top disease-relevant genes suppressed by anti-EphrinB2 treatment, reinforcing their potential use as skin biomarkers. A change in this gene signature could be evaluated in clinical studies of anti-EphrinB2 treatment in SSc patients to identify a potential early signal of efficacy.

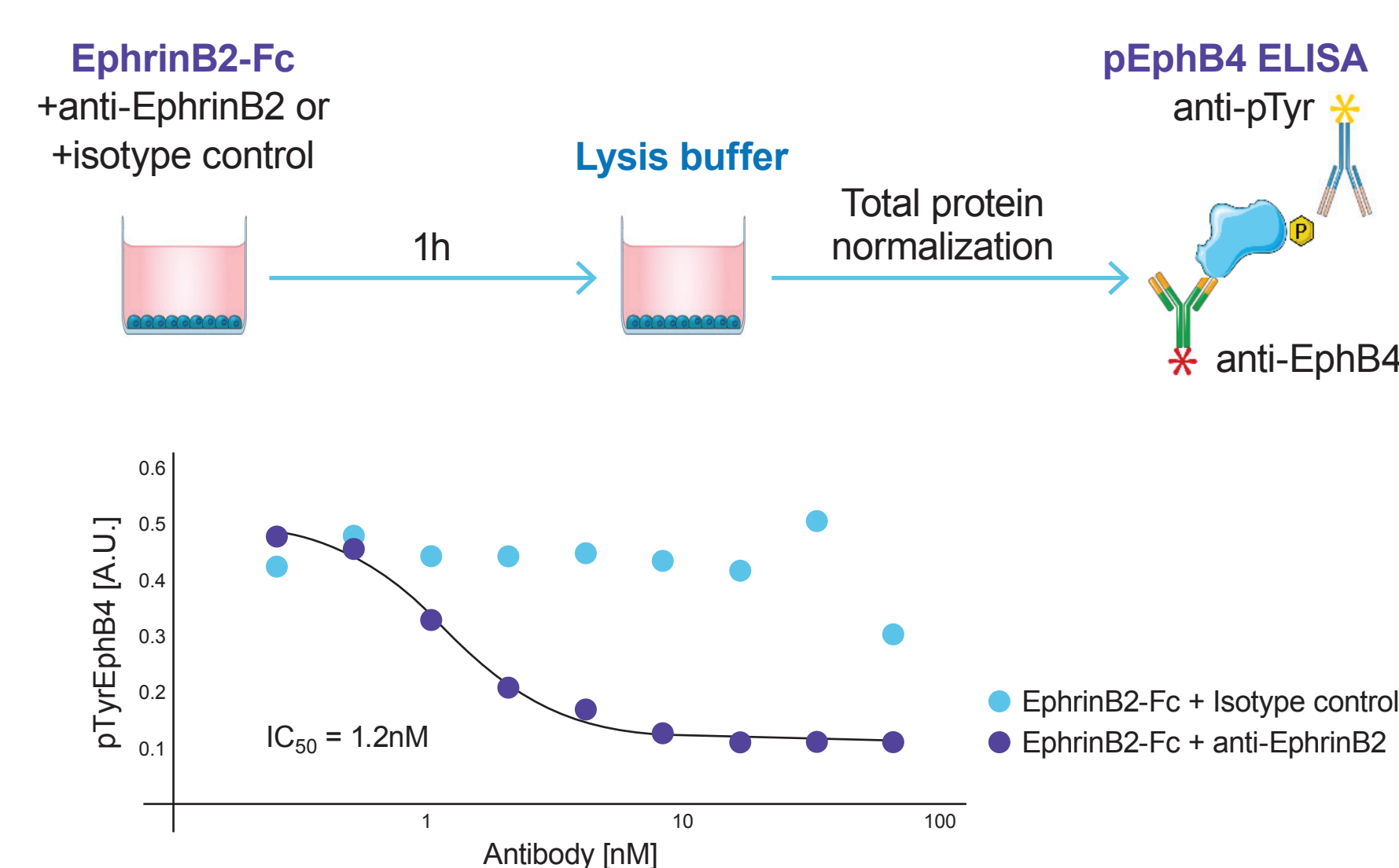
Funding

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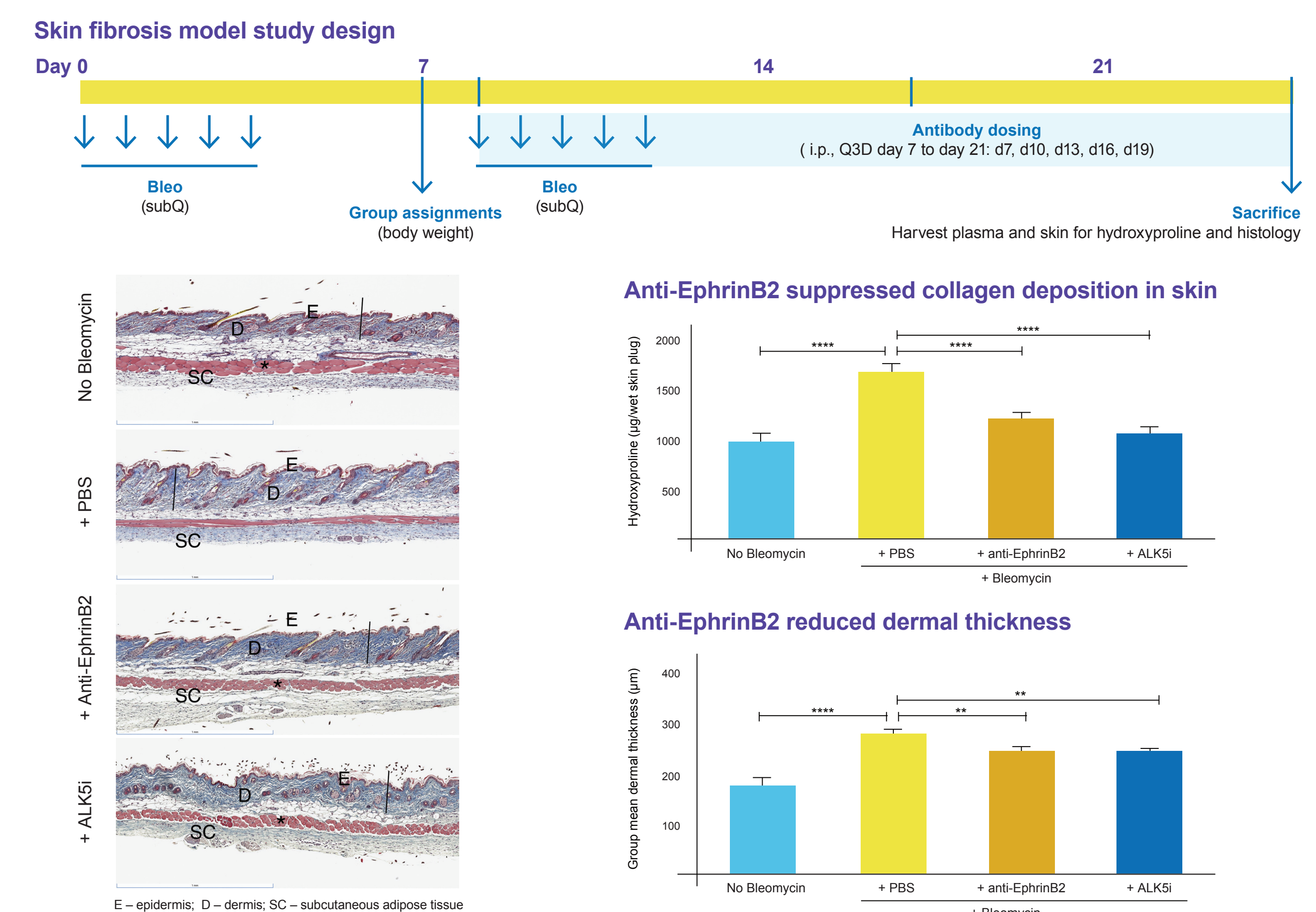
Anti-EphrinB2 human IgG1 monoclonal antibody binds EphrinB2 with high specificity and high affinity



Anti-EphrinB2 inhibits EphB4 phosphorylation in HUVECs

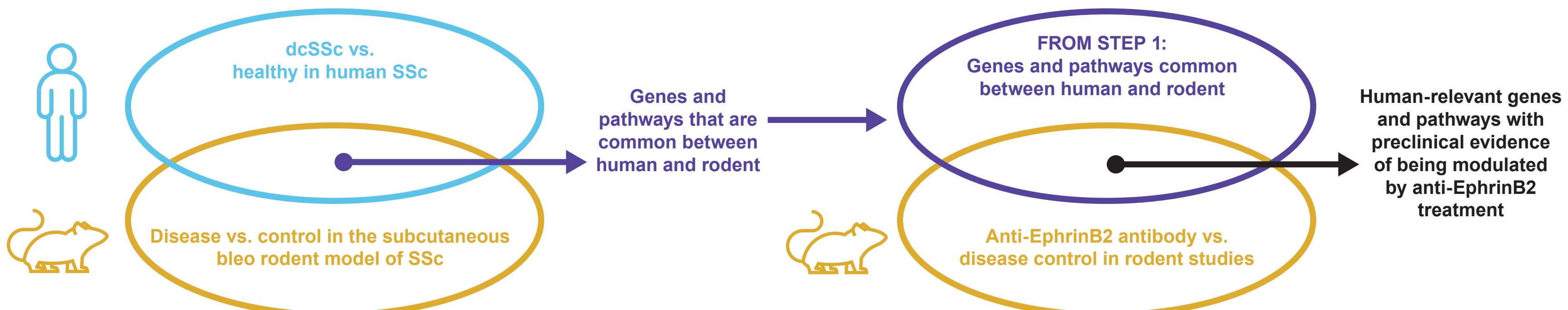


Anti-EphrinB2 neutralizing antibody suppresses skin fibrosis in bleomycin mouse model

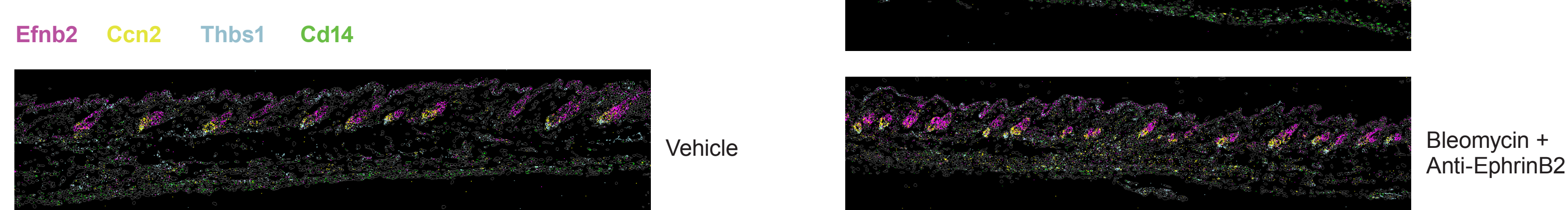


STEP 1: Find overlap between human and rodent SSc data

STEP 2: Identify relevant signature to interrogate clinically

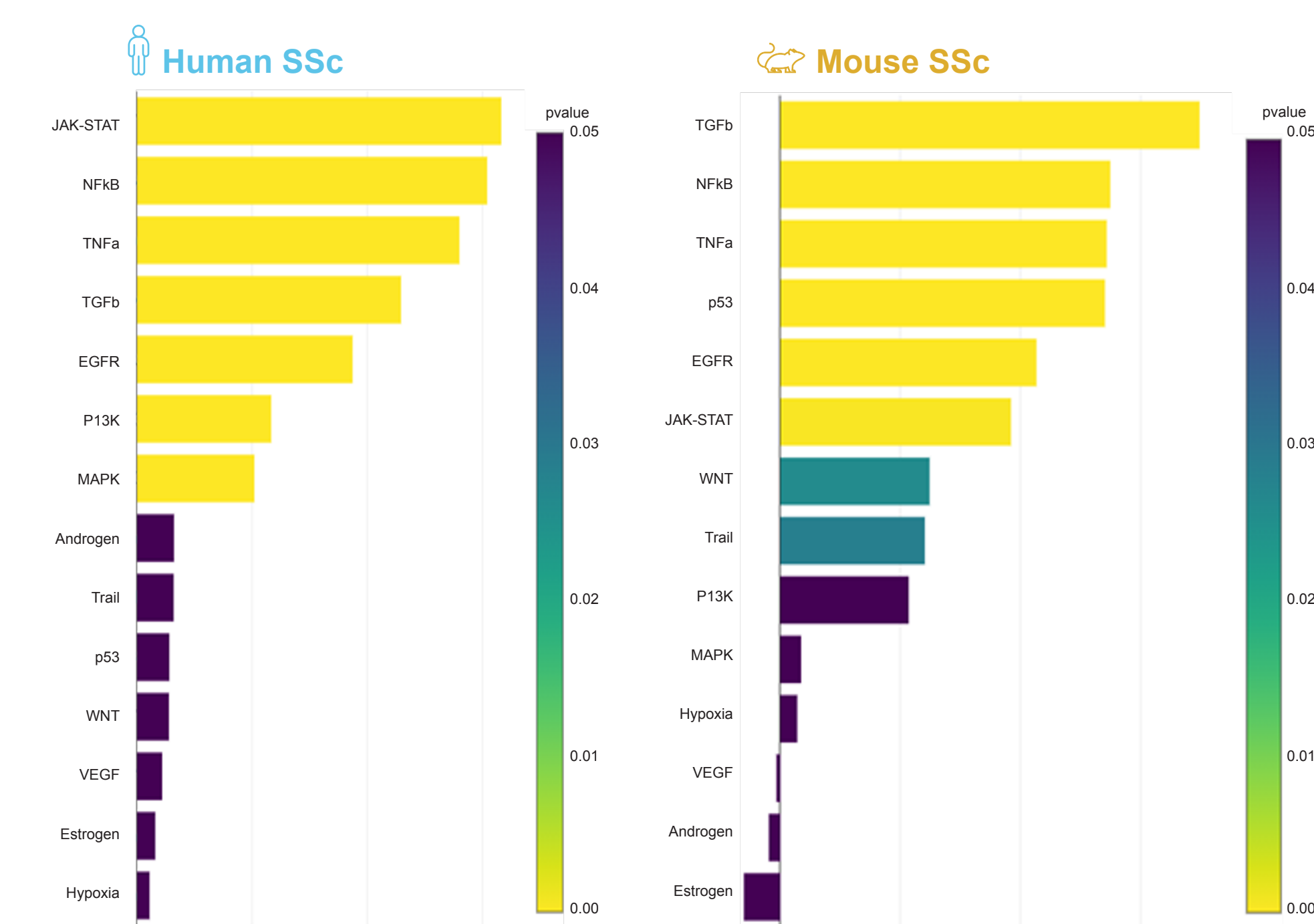


Vizgen spatial transcriptomics of skin bleomycin mouse model enables cell-type understanding of changes in gene expression in a spatially-resolved context



For additional *in vitro* and *in vivo* preclinical data on EphrinB2, see Wilks et al. Mediar Therapeutics, May 2024: "Anti-EphrinB2 neutralizing antibody suppresses fibrosis in preclinical *in vitro* and *in vivo* models" mediartx.com/2024_EphrinCongress_AntiEphrinB2AntibodySuppressesFibrosis

Pathway Analysis of Differentially Expressed Genes in Human SSc and Mouse Models of SSc



Over-enrichment Analysis of Genes Upregulated in Human SSc and Downregulated in Mouse SSc Following Anti-EphrinB2 Treatment

