

9-cis Retinoic Acid Promotes Lymphangiogenesis via IGFBP5/IGF2 Signaling in Lymphatic Endothelial Cells

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Background

- Secondary lymphedema is a chronic, debilitating condition that is a complication after cancer surgery involving lymph node management
- 9-cis retinoic acid (9-cisRA) promotes therapeutic lymphangiogenesis and prevents lymphedema through RXR-alpha dependent signaling in lymphatic endothelial cells (LECs)
- Spatial transcriptomics has revealed several 9-cis RA LEC responsive genes including IGFBP-5
- Objective:** Investigate the role of the IGFBP5/IGF signaling axis as a mediator of 9-cisRA induced lymphangiogenesis

Materials & Methods

- A well-established murine tail lymphedema was used as a model to study secondary lymphedema
- Female mice underwent circumferential tail excision and deep lymphatic vessel ligation to induce lymphedema in the distal tail
- Mice were randomized to daily 9-cis RA or vehicle control via intraperitoneal injections for 6 weeks

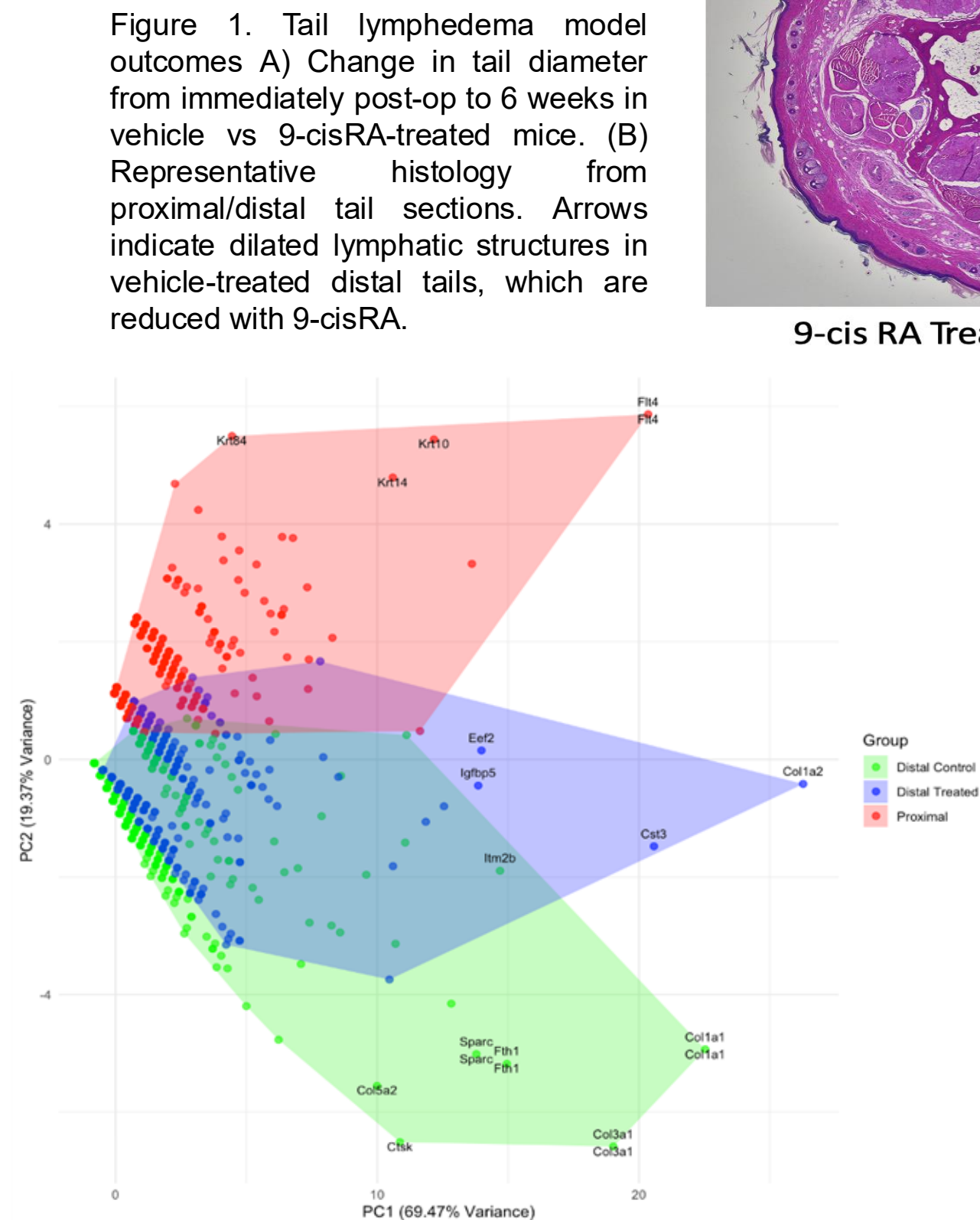
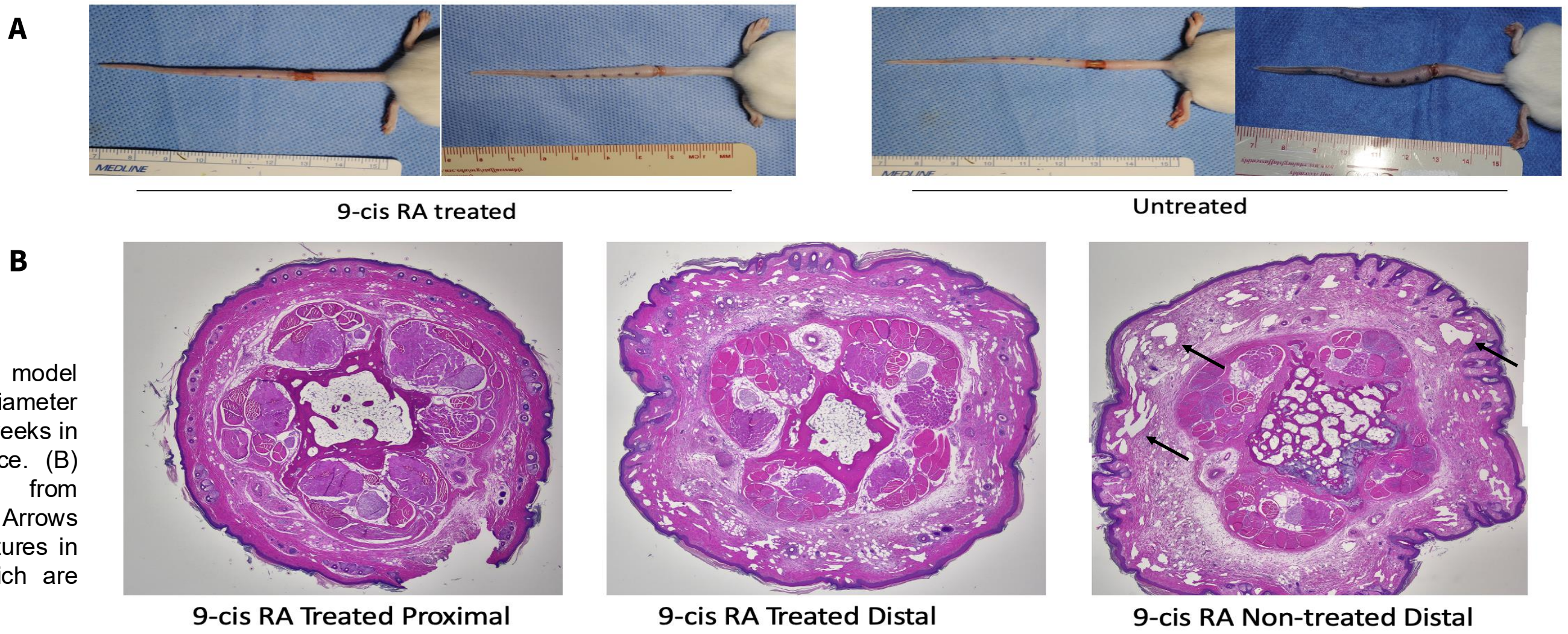


Figure 2. Principal component analysis (PCA) shows sample group separation, highlighting the top genes contributing to variability in each section.

- Spatial transcriptomics analysis was conducted using the 10x Visium platform on mouse tail samples categorized as proximal control, distal control, proximal treated, and distal treated
- Principal component analysis was applied to data set
- Top DEGs in distal 9-cisRA treated section: IGFBP5, eEF2, Cst3, Col1a2

Results

- RT-qPCR confirmed a ~10-fold upregulation of IGFBP5 in LECs (p=0.015; 2 μ M 9-cisRA at 48 hours), with no significant change in HUVECs and fibroblasts
- IGF2 expression was also significantly upregulated (p=0.008) in LECs after 9-cisRA treatment, whereas IGF1 levels remained unchanged. Conversely, HUVECs demonstrated decreased IGF2 expression under the same conditions (p=0.010)

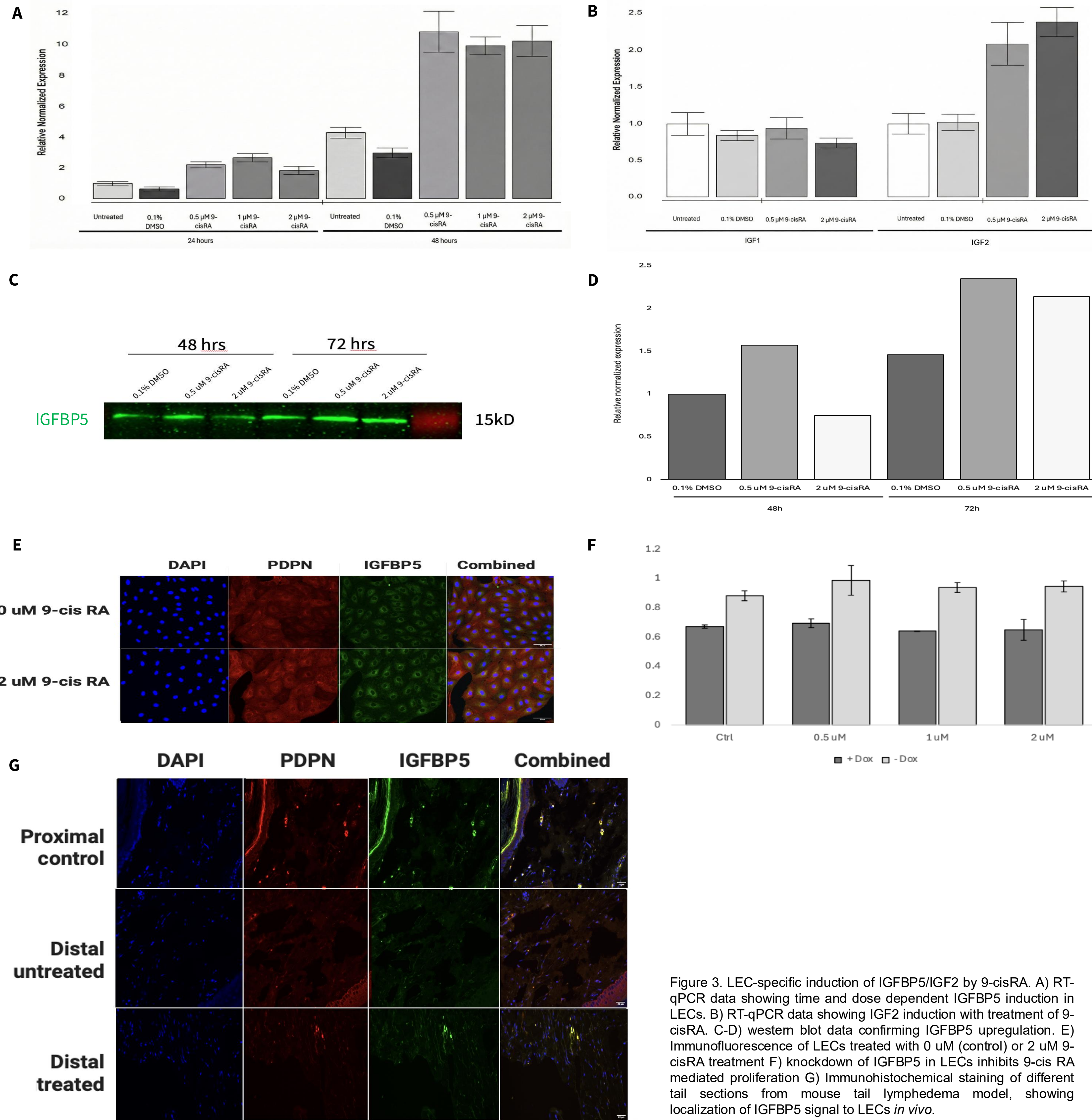


Figure 3. LEC-specific induction of IGFBP5/IGF2 by 9-cisRA. A) RT-qPCR data showing time and dose dependent IGFBP5 induction in LECs. B) RT-qPCR data showing IGF2 induction with treatment of 9-cisRA. C-D) Western blot data confirming IGFBP5 upregulation. E) Immunofluorescence of LECs treated with 0 uM (control) or 2 uM 9-cisRA treatment F) knockdown of IGFBP5 in LECs inhibits 9-cis RA mediated proliferation G) Immunohistochemical staining of different tail sections from mouse tail lymphedema model, showing localization of IGFBP5 signal to LECs *in vivo*.

Results (continued)

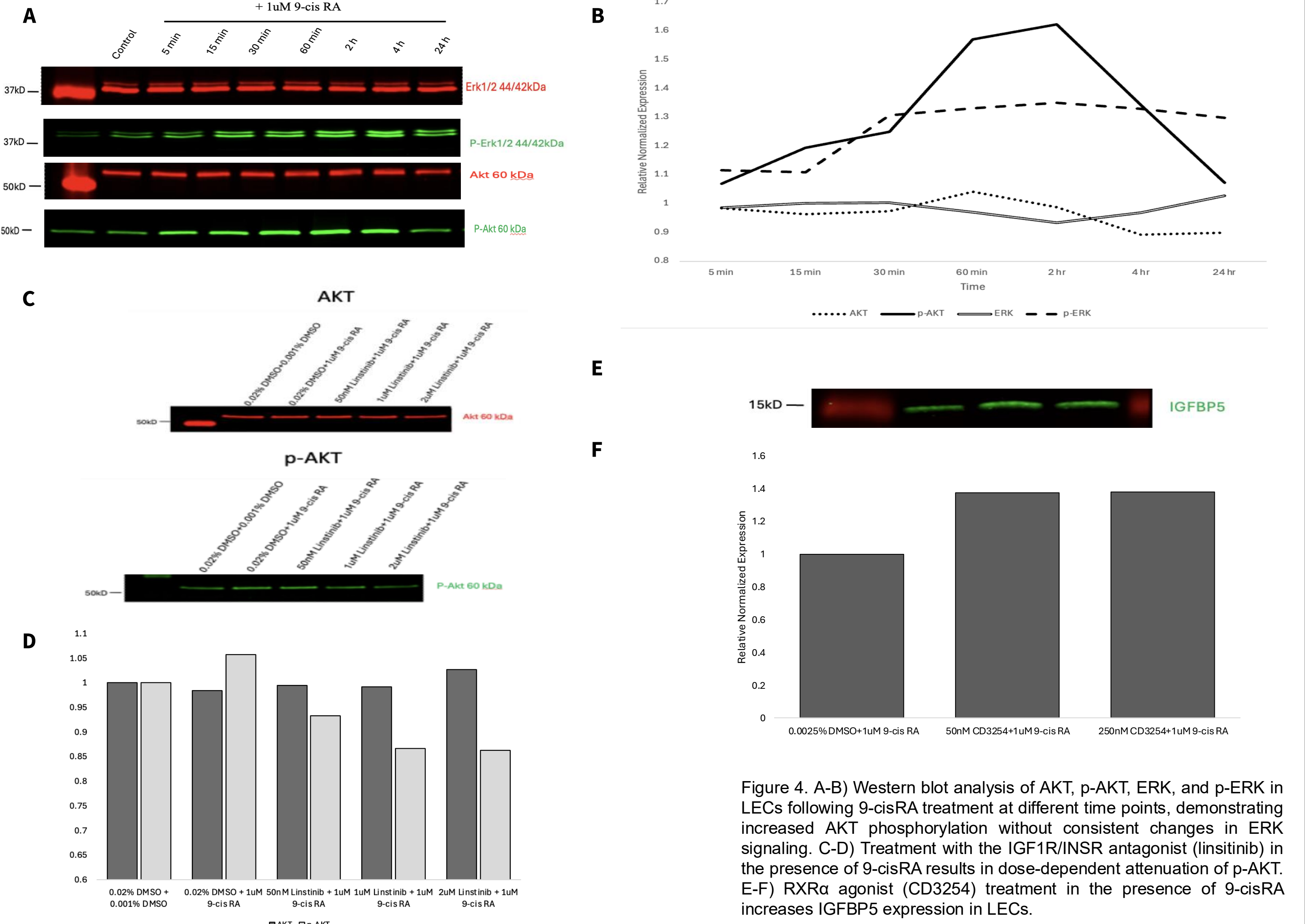


Figure 4. A-B) Western blot analysis of AKT, p-AKT, ERK, and p-ERK in LECs following 9-cisRA treatment at different time points, demonstrating increased AKT phosphorylation without consistent changes in ERK signaling. C-D) Treatment with the IGF1R/INSR antagonist (linsitinib) in the presence of 9-cisRA results in dose-dependent attenuation of p-AKT. E-F) RXRα agonist (CD3254) treatment in the presence of 9-cisRA increases IGFBP5 expression in LECs.

Conclusions & Discussion

- This study reveals that 9-cisRA promotes lymphangiogenesis through a mechanism that is specific to LECs, driven by selective induction of the IGFBP5/IGF signaling axis. Spatial transcriptomics and *in vitro* data showed that 9-cisRA upregulates IGFBP5 and IGF2 exclusively in LECs, which is known to be independent of the classical VEGF-C/D-VEGFR3 signaling pathways.
- This study provides the first mechanistic link between 9-cisRA and IGFBP5 in the context of lymphangiogenesis, highlighting this pathway as a therapeutic target for pharmacologic management of lymphedema.
- Future directions: Establish LEC-conditional IGFBP5 knockout in *in vivo* and *in vitro* models to further interrogate the effect IGFBP5 and binding partners on LEC behavior and 9-cis RA induced lymphangiogenesis

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