

Background

- Lymphatics maintain interstitial fluid homeostasis while injury leads to lymphatic insufficiency and eventually lymphedema.
- Derivatives of vitamin A, such as 13-cis retinoic acid (13-cis RA), all-trans retinoic acid (all-trans RA), and Bexarotene bind RAR and RXR with varying affinities.
- While we hypothesize that RXR is the critical receptor in lymphatic endothelial cells (LECs) responsible for pro-lymphangiogenic responses, this has yet to be proven.
- To address the current gap in treatment options, we aim to determine whether 13-cis RA, all-trans RA, and Bexarotene are capable of positively affecting LECs *in vitro*.

Methods

WST-1 Proliferation Assay

Human LECs were cultured and pre-treated in low serum medium for 24 hours, followed by treatment of either 9-cis RA (2μM), 13-cis RA (2μM), all-trans RA (2μM), Bexarotene (2μM) for 48 hours. Absorbance was measured in a plate reader and the final cell number was extrapolated from a standard curve.

Scratch Assay

Grids were marked under a 6-well tissue culture plate for positional reference and visualization under phase contrast microscopy. The confluent monolayer was scratched. Gap closure was captured via phase contrast microscopy and analyzed.

Endothelial Tube Formation Assay

Maximum endothelial tube structure formation was observed under phase contrast microscopy at 18 hours. Tube formation images were analyzed with the Angiogenesis Analyzer Plugin.

Results

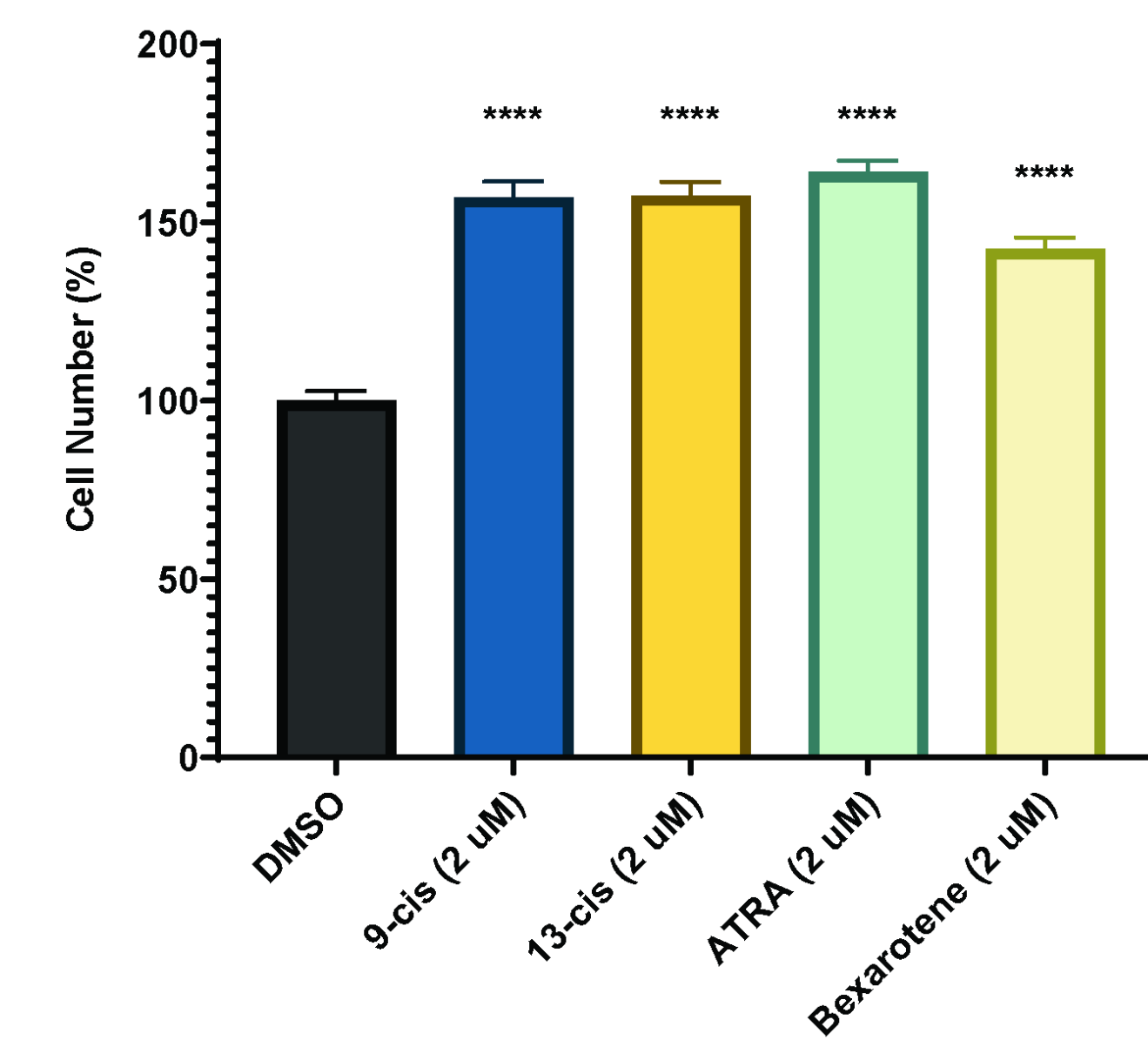


Figure 1. WST-1 Proliferation Assay. Compared to DMSO treated controls, LECs treated with 2μM RAs demonstrated an increase in cell number of at least 145% (p<0.0001).

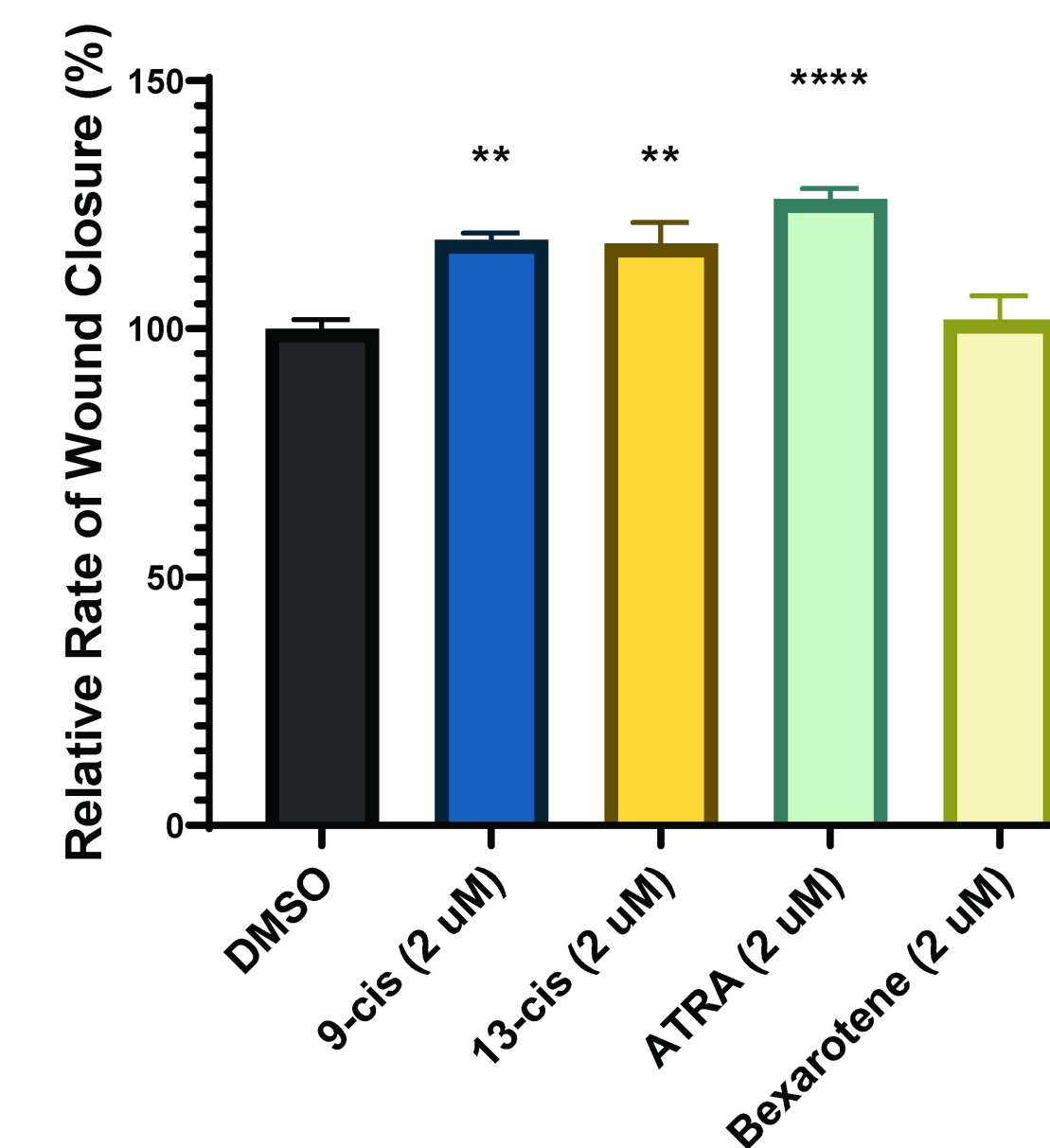
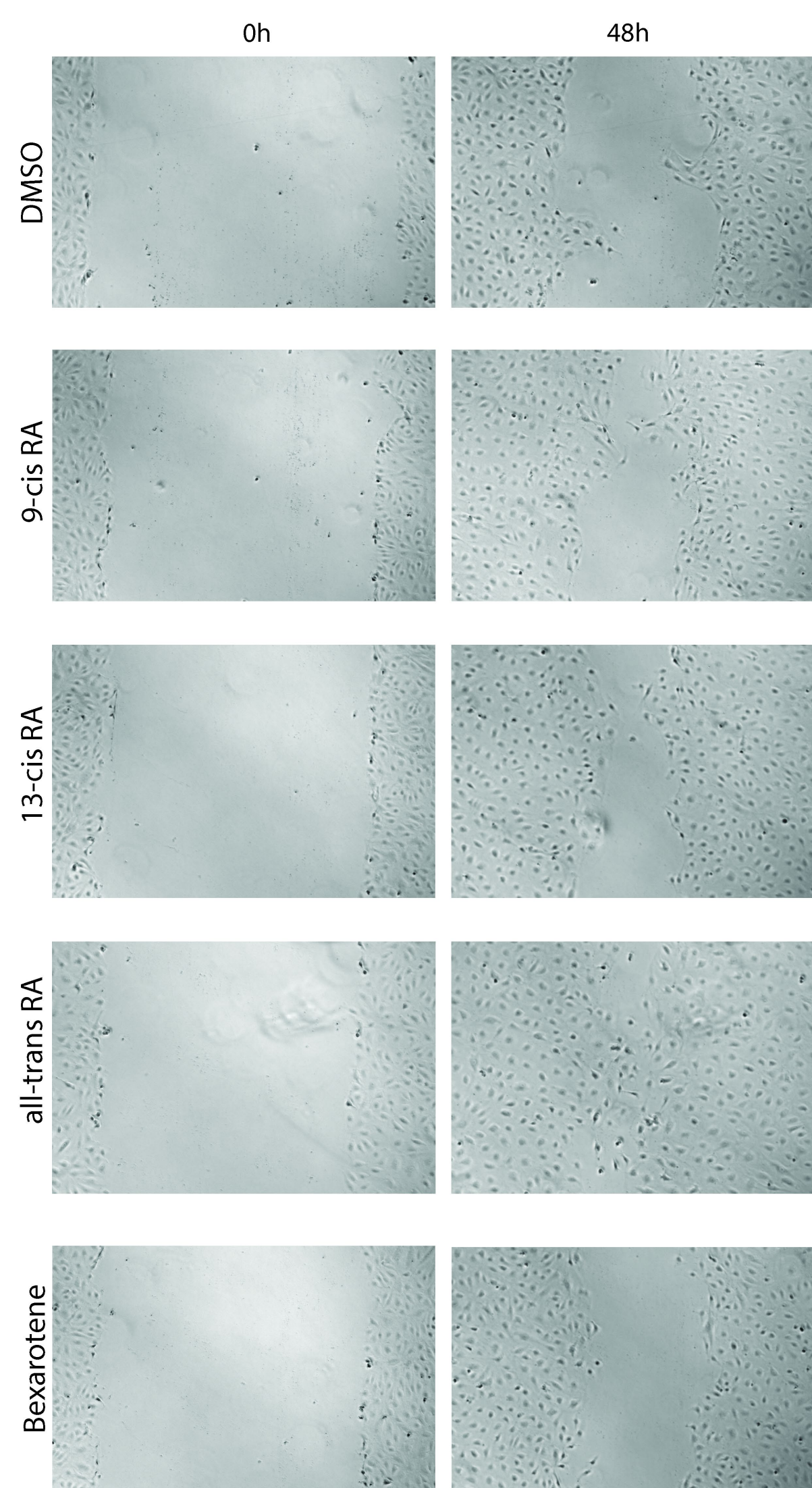


Figure 2. Scratch Assay. Compared to other RAs and controls, Bexarotene treated LECs did not significantly improve in migration.

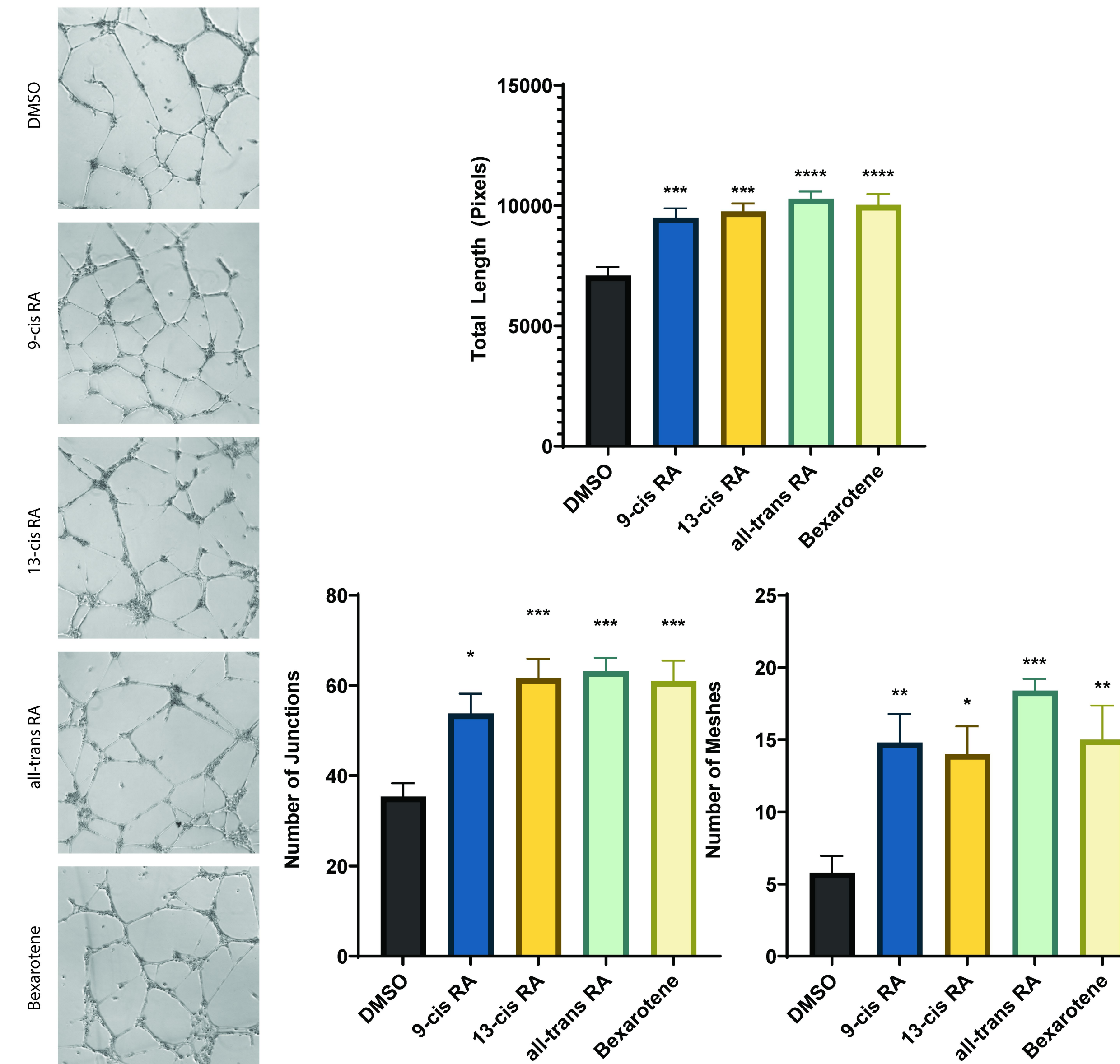


Figure 3. Endothelial Tube Formation Assay. RA treatment resulted in increased cord length (p<0.0001), junctions (p<0.001), and meshes (p<0.01) in tube formation assays.

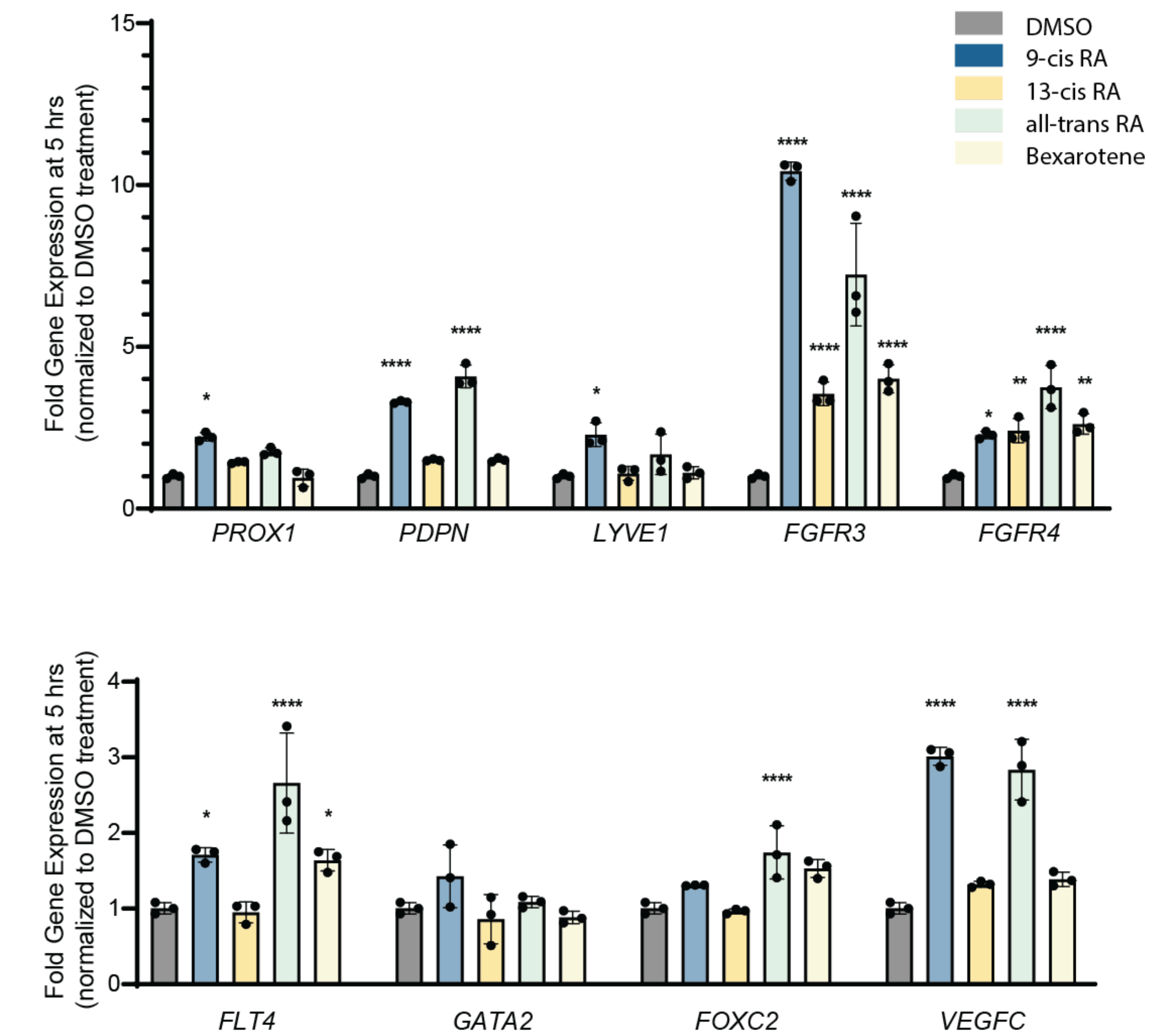


Figure 4. Quantitative PCR of lymphatic endothelial cell markers confirm growth and regenerative behavior after treatment with retinoic acid isoforms.

Conclusions

- Future *in vivo* studies will investigate the effect of additional retinoids on lymphangiogenesis and vessel regeneration using an experimental mouse tail lymphedema model.
- These isoforms could serve as a potential therapeutic agent to treat lymphedema patients.