

Background

- A hallmark of cancer is increased formation of new, abnormal vasculature to feed the growing tumor.
- Blood vessels are lined with endothelial cells, which form tight junctions (TJs) to regulate the transport of molecules.
- Leaky vessels cause metastasis and interfere with drug delivery.
- Polaxamer 188 (**P188**) is an FDA-approved drug shown to have membrane sealing capabilities and postulated to stabilize leaky vessels.

Objectives

- Develop an *in vitro* migration model of endothelial tight junctions.
- Quantify the effects of cancer cells on endothelial tight junctions.
- Apply P188 to restore endothelium permeability and reduce cancer cell migration.

Tumor-Vasculature Model Validation

Cell Types: Mouse Brain Primary Endothelial Cell (MBEC) & Mouse Triple Negative Breast Cancer Cell (4T1)

10,000 MBEC/well forms a monolayer

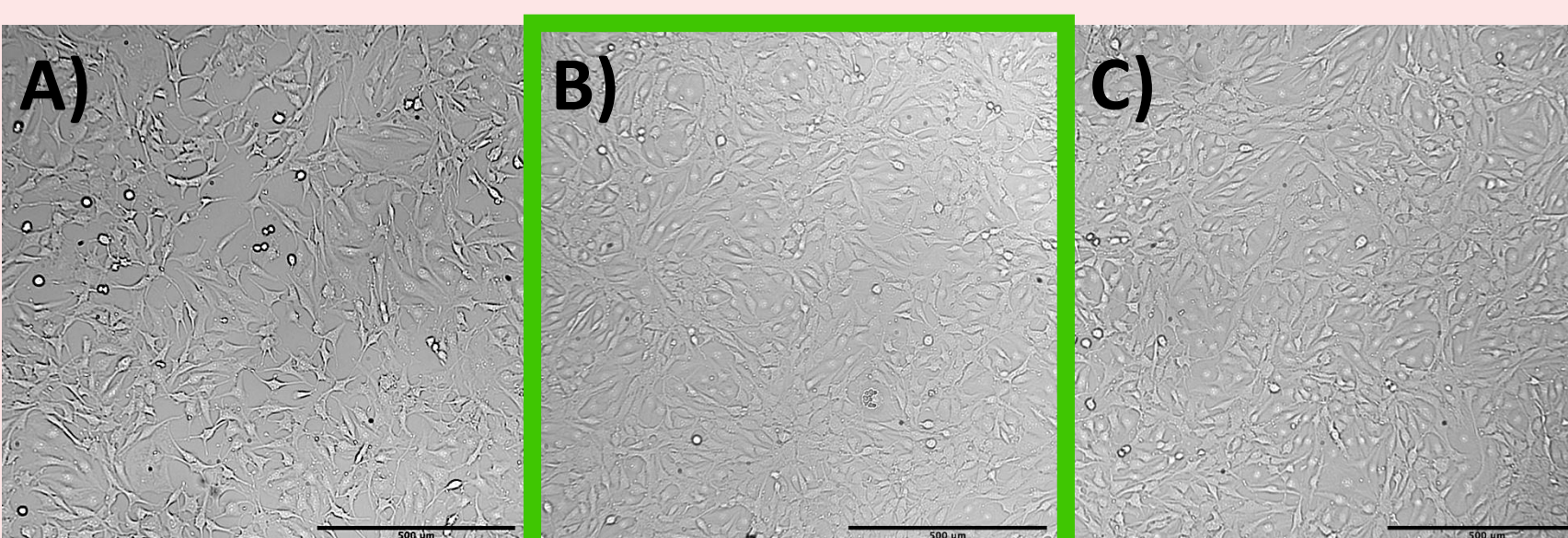


Figure 1. MBEC density experiment 24 hours after seeding A) 5k, B) 10k, C) 15k cells. Scale bar = 500µm.

Media w/ FBS induces 4T1 cell migration

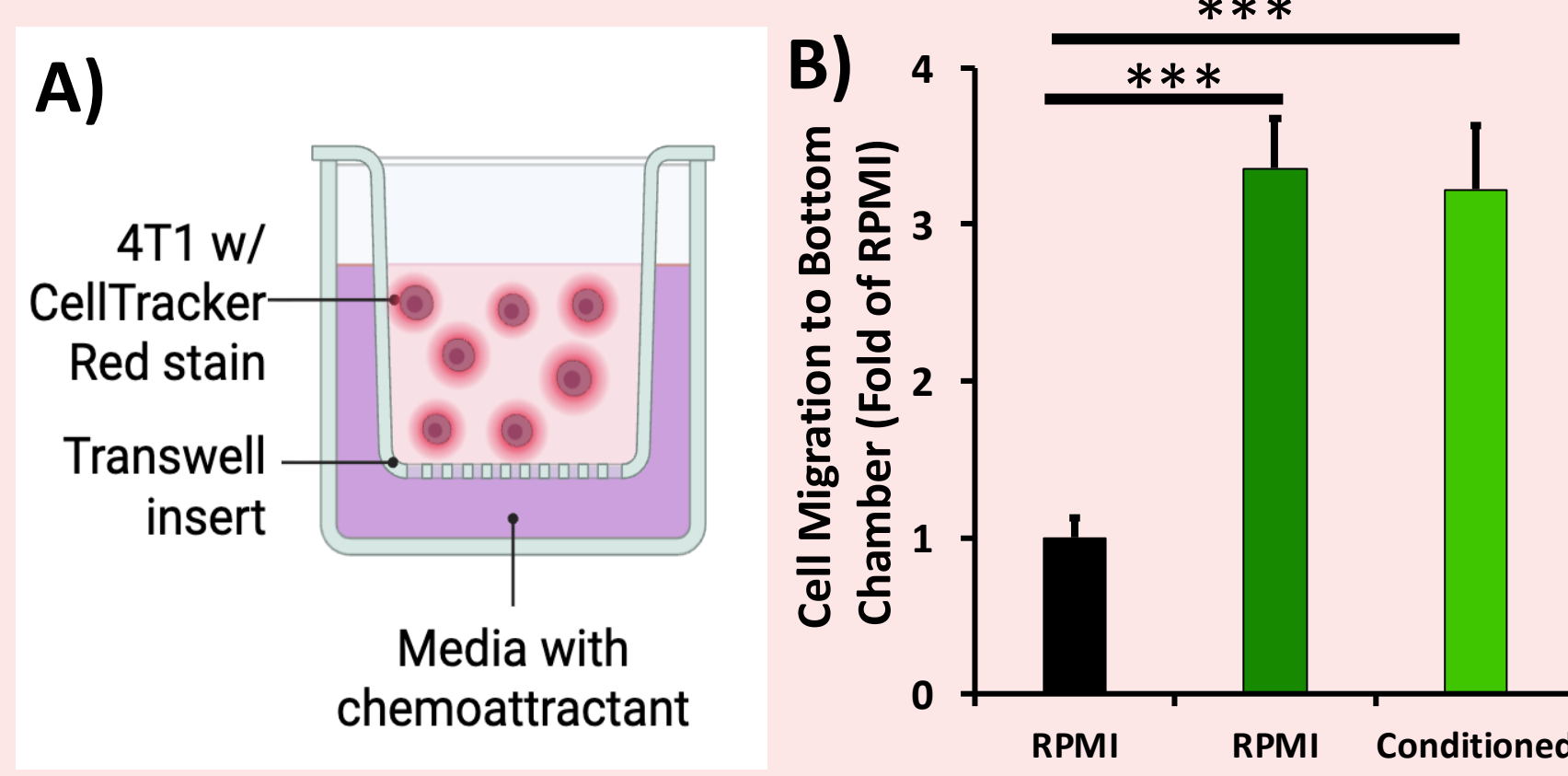


Figure 2. 4T1 cell migration assay A) methods and B) results for chemoattractant selection with 8µm pore Transwells (n=4). *** p<.001.

Breast Cancer Transendothelial Migration

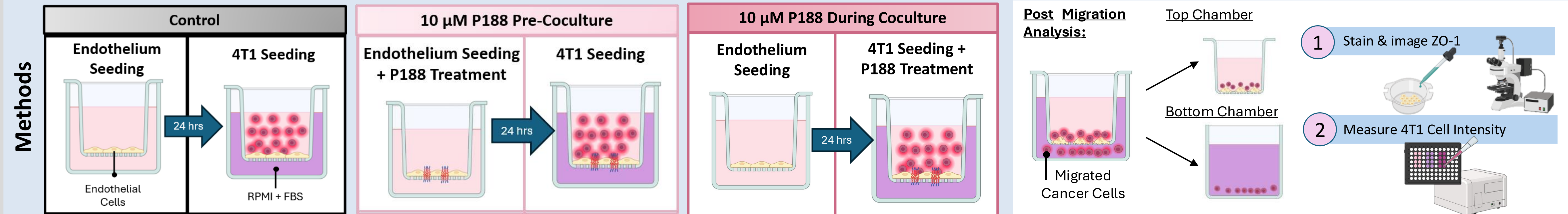


Figure 3. Schematic of transendothelial migration assay methodology.

Breast Cancer Cells Disrupt Tight Junctions

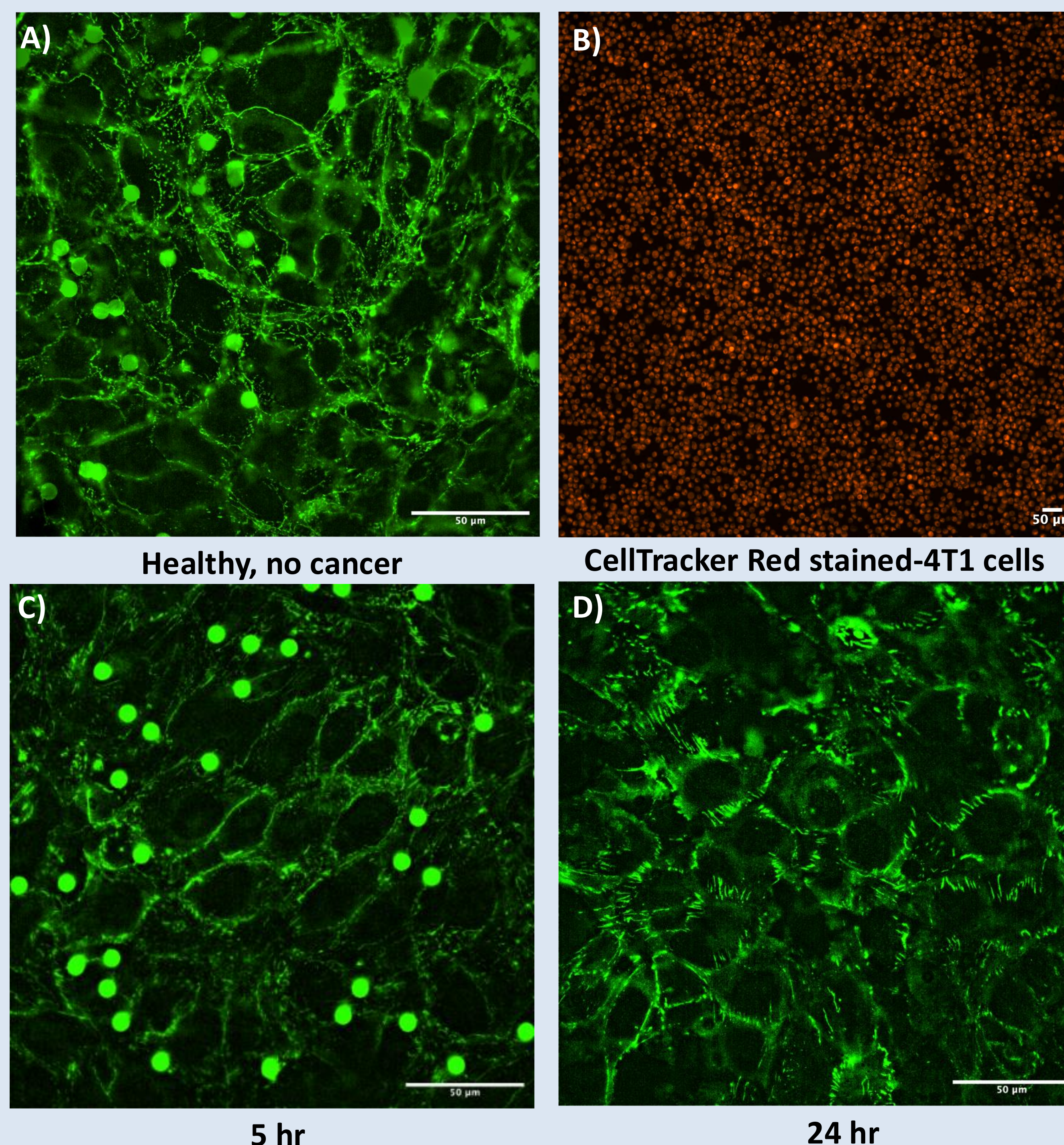


Figure 4. ZO-1 antibody staining for TJs on the transwell of A) cancer-free, C) 5 hrs, and D) 24 hrs of 4T1 transmigration. B) CellTracker Red-stained 4T1 representative. Scale bar = 50µm.

P188 Reduces Transendothelial Migration of Cancer Cells

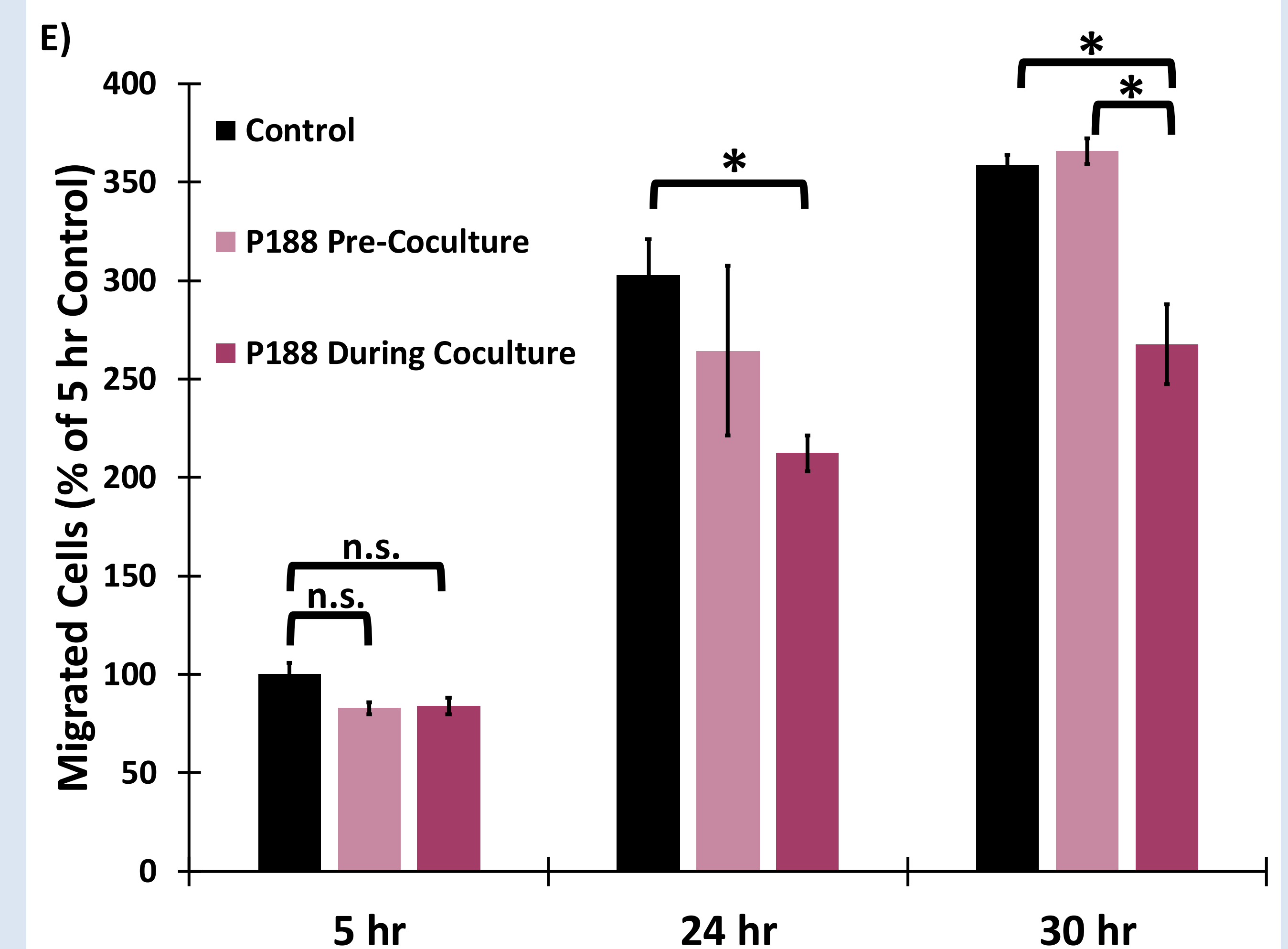


Figure 5. Measured fluorescent intensity of migrated CellTracker Red stained-4T1 cells in the bottom well across 5 hr, 24 hr, and 30 hr timepoints. Mean ± standard error (n = 3) *p < 0.05.

Discussion

- Development of the vascular endothelium model is successfully employed in a transendothelial migration assay.
- Structure of endothelial TJs is impacted by exposure to 4T1 cells over time, producing more jagged and discontinuous ZO-1 compared to the baseline, allowing for the increased extravasation of 4T1 cells into the bottom compartment via transmigration.
- Treatment of P188 during 4T1 exposure decreased the transmigration of 4T1 cells, supporting evidence of its role as a membrane or TJ resealant.
- Pre-treatment of P188 did not reinforce the *in vitro* endothelium against 4T1 transmigration.

Future work: ZO-1 imaging of the P188 treatments at each time point to gain evidence of TJ repair, quantify number of migrated cells, and conduct an additional timepoint to compute a cell migration rate.

Acknowledgements

4T1 cells were generously donated from the Azab Lab at UTSW. This project was supported by the UTA College of Engineering REU and by the Potvin Endowment. This work would not be possible without the mentorship of Dr. Anne Alsup.