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Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2026 Project Summary

1. Student name & home university: Sofia Hutchens, University of Notre Dame

2. ND faculty name & department: Professor Donny Hanjaya-Putra, Department of Aerospace and Mechanical Engineering

3. Summer project title: Rab11a-Dependent GLUT3 Trafficking Influences Lymphatic Endothelial Cell Contractility

4. Briefly describe new skills you acquired during your summer research:
I gained hands-on experience in cell culture, including maintaining LECs and performing siRNA knockdowns. I also gained experience in performing western blot and immunofluorescence (IF) imaging experiments. I developed an understanding of processing and analyzing western blot and IF images using FIJI software, and I learned how to use GraphPad Prism for figure generation and statistical analysis.

5. Briefly share a practical application/end use of your research:
The goal of this project is to normalize dysfunctional lymphatic vasculature by inhibiting glucose transporter GLUT3. The applications of this research would be to help improve drug delivery during cancer treatments as well as restore lymphatic flow and limb function in secondary lymphedema.

6. 50- to 75-word abstract of your project:
Dysfunctional lymphatic vessel growth (lymphangiogenesis) is a hallmark of diseases like lymphedema and cancer. We propose that increased LEC contractility propagates dysfunctional lymphangiogenesis, and GLUT3 is an important part of supporting that process. Additionally, our results indicate that Rab11a helps transport GLUT3 to the cell membrane. Therefore, targeting Rab11a/GLUT3 may help control dysfunctional lymphangiogenesis.

7. References for papers, posters, or presentations of your research:

[1] Johandes, E., Hall, E., Harbut, T., Priebe, K., and Hanjaya-Putra, D., "Blocking Glutamine Transport Normalizes Lymphatic Vessels in Hypoxic Environments by Attenuating Glycolysis."
[2] Kamba, T., and McDonald, D. M., 2007, "Mechanisms of Adverse Effects of Anti-VEGF Therapy for Cancer," Br J Cancer, 96(12), pp. 1788–1795. https://doi.org/10.1038/sj.bjc.6603813.
[3] "Principle, Mechanism, and Application of siRNA Knockdown in Gene Silencing - BOC Sciences." Available: https://www.bocsci.com/resources/principle-mechanism-and-application-of-sirna-knockdown-in-gene-silencing.html.

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One-page project summary that describes problem, project goal, and your research activities / results:

Dysfunctional lymphatic vessel growth (lymphangiogenesis) is a hallmark of diseases like lymphedema and cancer. These leaky vessels contribute to inflammation, poor drug delivery, and provide convenient routes for cancer metastasis. Finding ways to control this abnormal vascularization could help address these challenges [1]. Current treatments work by inhibiting the vascular endothelial growth factors (VEGFs) that drive lymphangiogenesis. However, these therapies harm healthy cells [2]. This research seeks to identify targets that specifically inhibit dysfunctional lymphangiogenesis. As lymphatic endothelial cells (LECs) uniquely use large amounts of glucose to power growth, we evaluated if blocking glucose transport protein GLUT3 could be used to control lymphangiogenesis.

First, we examined whether GLUT3 is necessary for cell contractility and cell-cell junction loss, both of which are critical during vessel formation. To test this, siRNA was used to knock down GLUT1 and GLUT3 in cultured LECs, and the cells were treated with thrombin to force LEC contraction. A non-targeted scramble siRNA was used as a control group. Immunofluorescence imaging revealed that GLUT3 knockdown (KD) was successful in preventing cell-cell junction loss and contraction (**Fig 1a-c**).

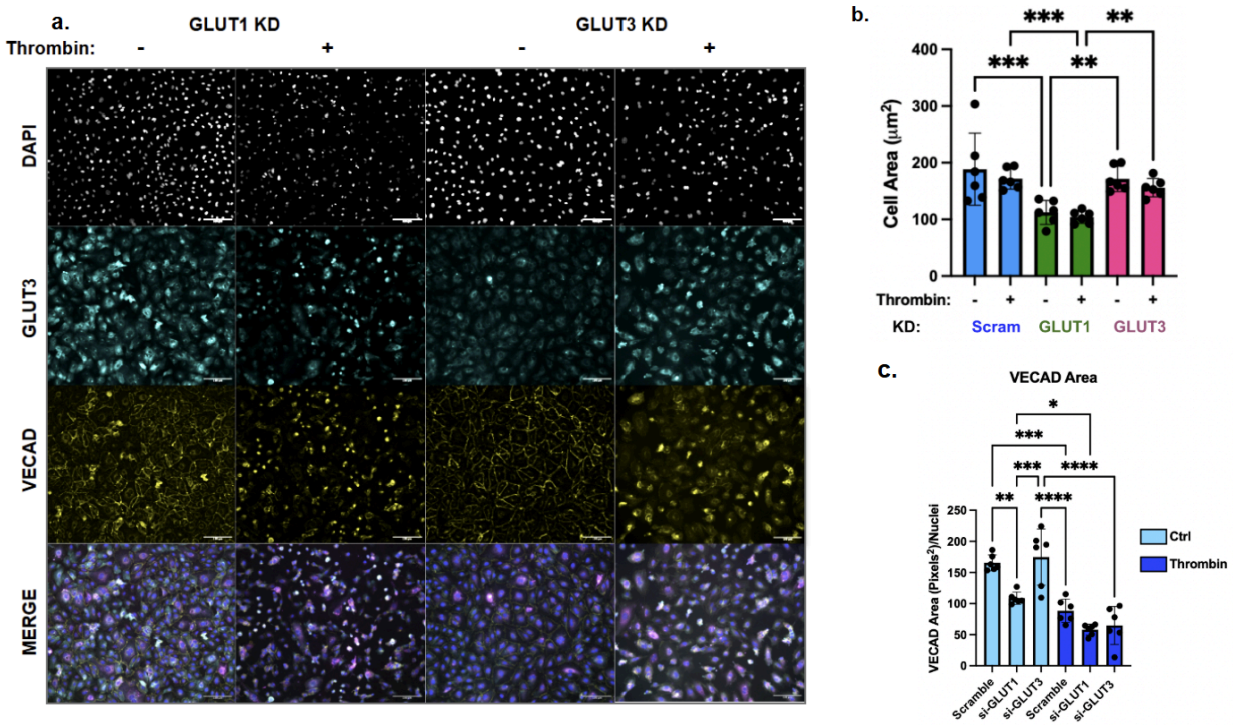

Figure 1. GLUT3 KD attenuates thrombin-induced contraction and influences cell permeability. a) Immunofluorescence images of LECs transfected with 25 nM non-targeting scramble siRNA, si-GLUT1 or si-GLUT3 in the presence (+) or absence (-) of 10 U/mL thrombin (scramble control not pictured). Cells were stained for DAPI (white), GLUT3 (cyan), VECAD (yellow), and CellTracker (magenta, individual channel not pictured). Scale bar (white) = 130 µm. **b)** Average cell size quantified by the area. **c)** Bar graph representation of VECAD Area. Statistical significance determined via one-way ANOVA with Tukey's test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

We also examined the effects of knocking down Rab11a, a protein we hypothesized controls GLUT3 transport to the cell membrane. Rab11a KD LECs were challenged with thrombin, and the changes to the GLUT3 concentration were analyzed using Western blot. A nonsignificant decrease in GLUT3 expression on the membrane was observed with the Rab11a knockdown (**Fig 2)**.

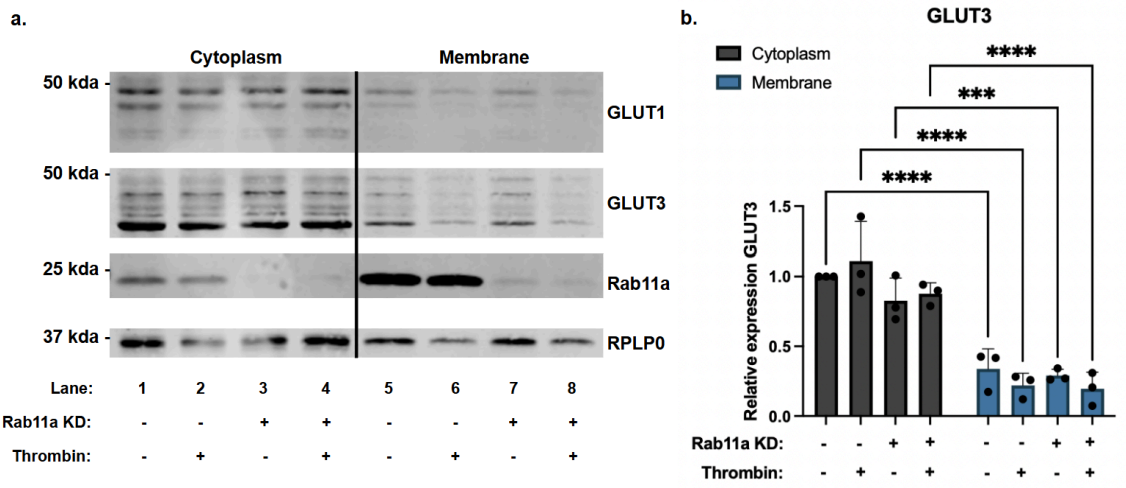

Figure 2. Rab11a KD decreases GLUT3 expression in LECs. a) Western blot of GLUT1, GLUT3, Rab11a, and RPLP0 in the cytoplasm and membrane fractions of LECs following transfection with 15 nM si-Scramble (-) or si-Rab11a (+) along with stimulation with either 0 U/mL (-) or 10 U/mL (+) of thrombin. **b)** Protein expression for GLUT3 was quantified using ImageJ and normalized to RPLP0 and renormalized to the cytoplasm scramble condition. Statistical significance determined via two-way ANOVA with Tukey's test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

In addition, a proximity ligation assay confirmed that Rab11a and GLUT3 colocalize (**Fig 3**). In this assay, a fluorescent signal is produced only when two proteins are in close physical proximity to each other.

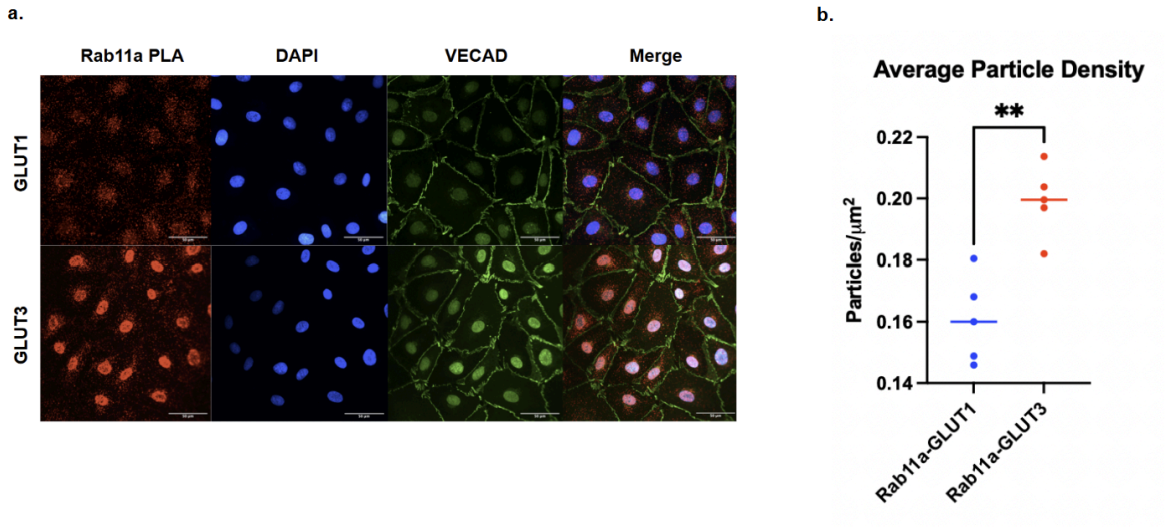

Figure 3. Rab11a and GLUT3 colocalize in LECs. a) Images of Proximity Ligation Assay (PLA) assay detecting respective interactions of Rab11a with GLUT1 and GLUT3. Cells were stained for PLA fluorescent signals (red), DAPI (blue), and VECAD (green). Scale bar (white) = 50 µm. **b)** Quantification of average PLA particle density normalized to cell area. Statistical significance determined via Student's T-test. **P<0.01.

In conclusion, our results indicate that Rab11a transports GLUT3 to the cell membrane, and that GLUT3 is important for cell contraction. Consequently, we identified GLUT3 and Rab11a as novel metabolic targets to control dysfunctional lymphangiogenesis. The potential applications of this research in medicine would be to use inhibitors of Rab11a and GLUT3 to improve drug delivery during cancer treatments and restore lymphatic flow and limb function in secondary lymphedema.