Probing The Relationship Between Partial Epithelial-to-Mesenchymal Transition and Cell Shape in Epithelial Cells

Fareeda Elinam Abu-Juam, Erzsébet Ravasz Regan
Department of Biochemistry and Molecular Biology, College of Wooster

THE COLLEGE OF **WOOSTER**

Background and Significance

Metastatic disease is the cause of 90% of cancer-related deaths. Metastatic potential is increased by the epithelial-to-mesenchymal transition (EMT). This potential is further increased by commitment to a partial EMT (pEMT) process.
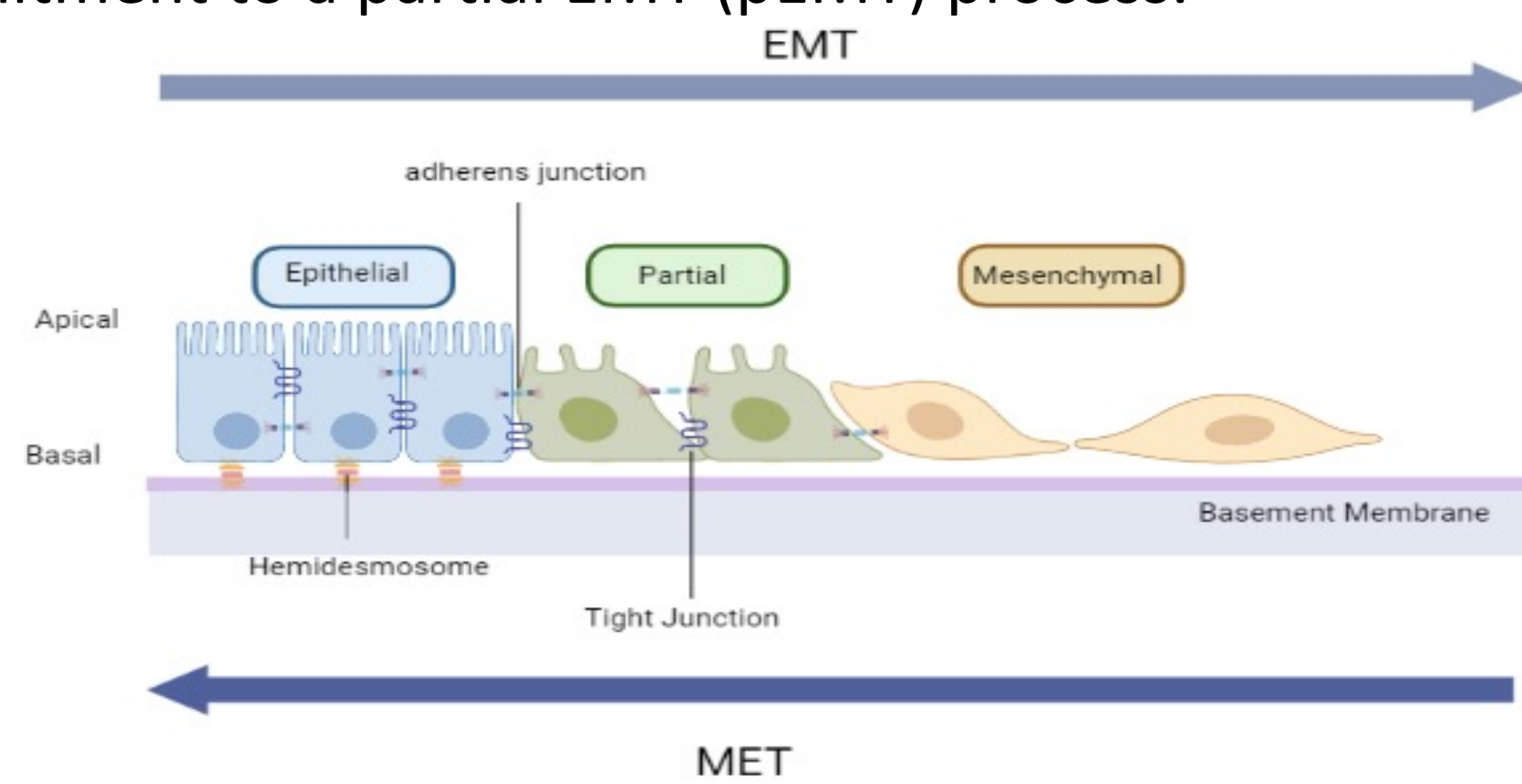
pEMT is stabilized by the biophysical environment of a cell[1]. Expression data, morphology data, bioimage staining and more are used to study this[2-4]. However, they are limited because pEMT results from combinations of molecular identifiers. To combat this, studies have combined these methods. Popularly, morphology data in combination bioimaging gives powerful insight in determining whether cells are in the hybrid states that promote metastasis.
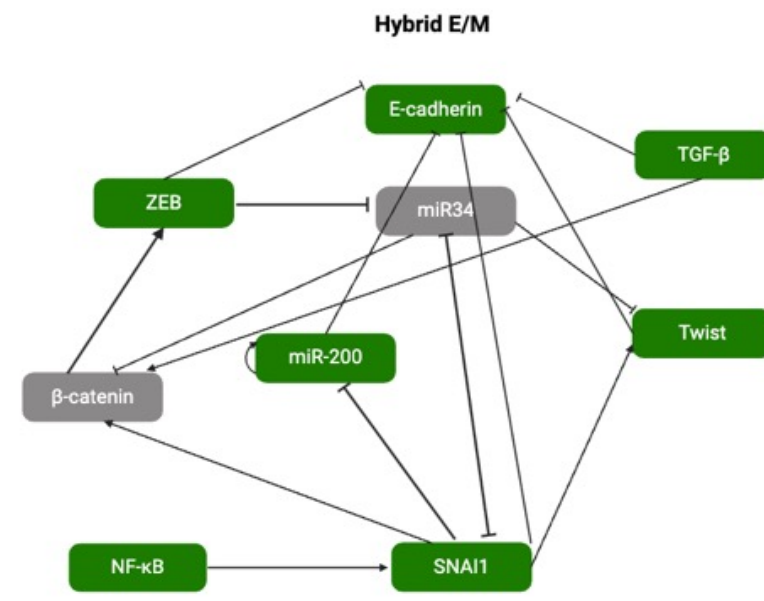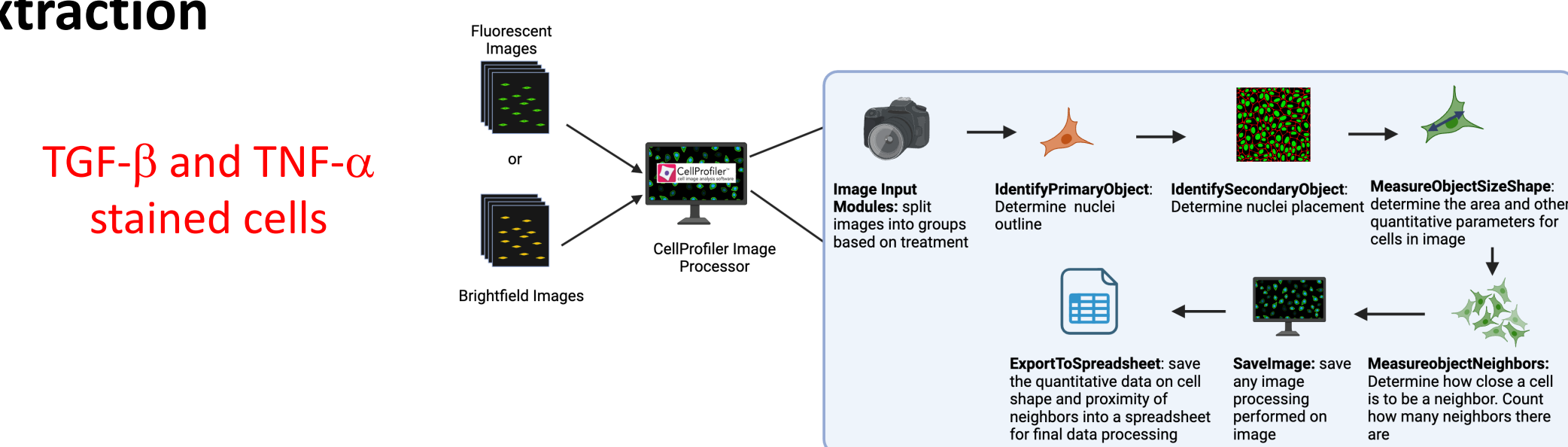
Research Question and Goals

Develop a method that tracks changes in cell shape from images stained with various biomarkers in different environmental conditions, to understand what drives the hybrid E/M phenotype by:
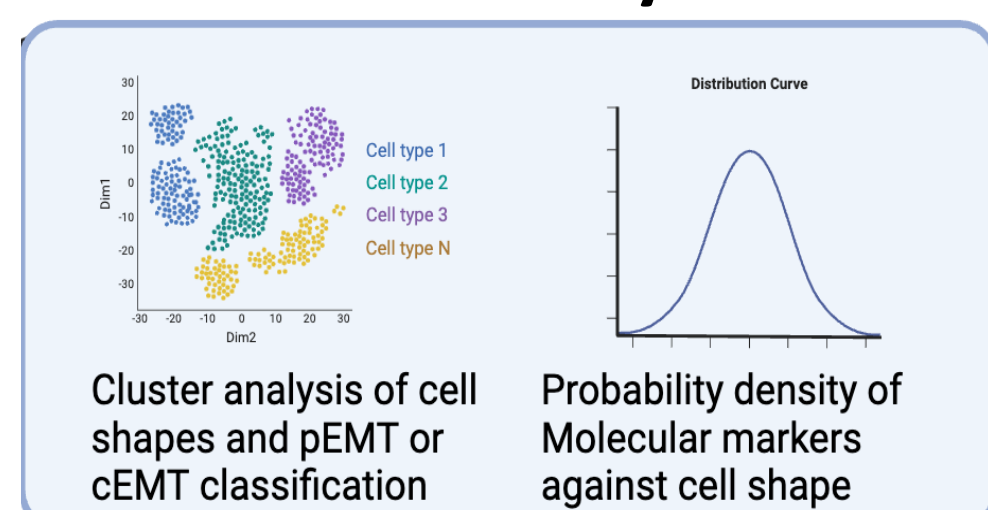
- Identifying environments that stabilize pEMT
- Defining a metric to measure cell shape
- Identifying stable clusters within the data
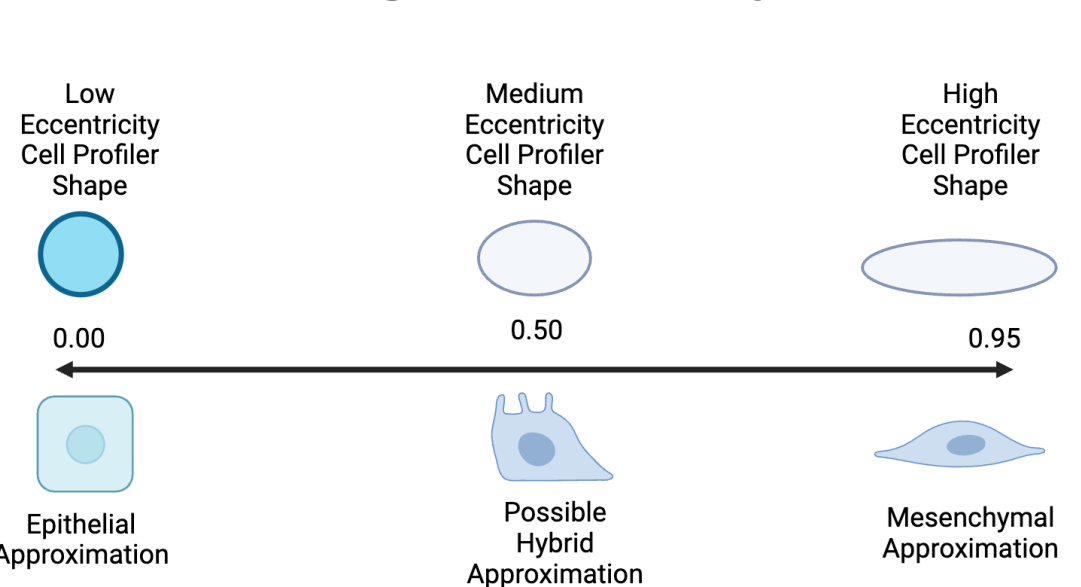
Methods

1. Developing a Pipeline and Assigning Parameters For Feature Extraction

TGF-β and TNF-α stained cells

2. Data Clustering Using K-Means and Distribution Analysis

Cluster analysis of cell shapes and pEMT or cEMT classification

Probability density of Molecular markers against cell shape

3. Defining a Cell Shape Metric

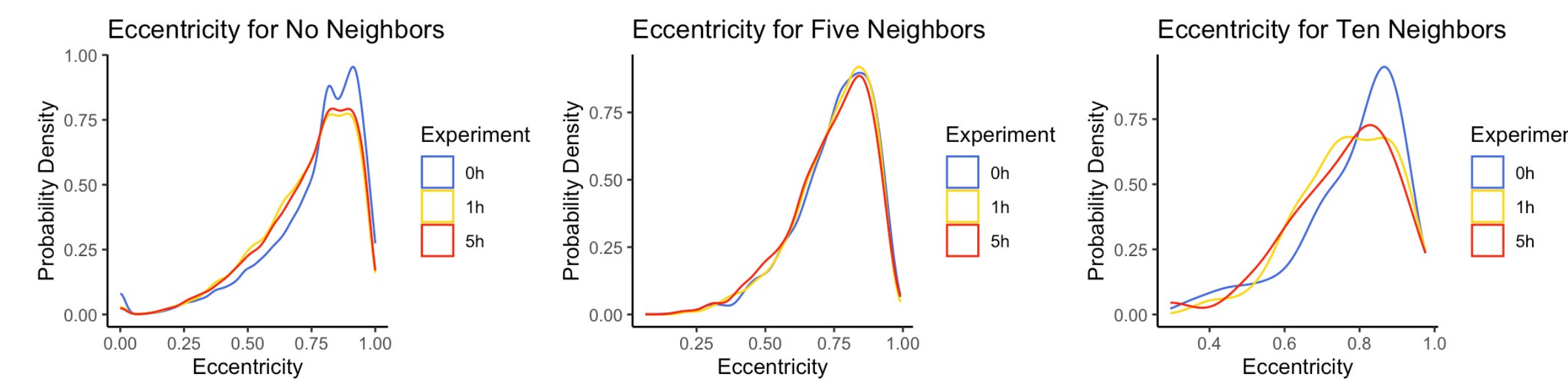| Low Eccentricity Cell Profiler Shape	Medium Eccentricity Cell Profiler Shape	High Eccentricity Cell Profiler Shape
0.00	0.50	0.95
Epithelial Approximation	Possible Hybrid Approximation	Mesenchymal Approximation

Results I: Consideration of The Biophysical Environment Stabilizes pEMT

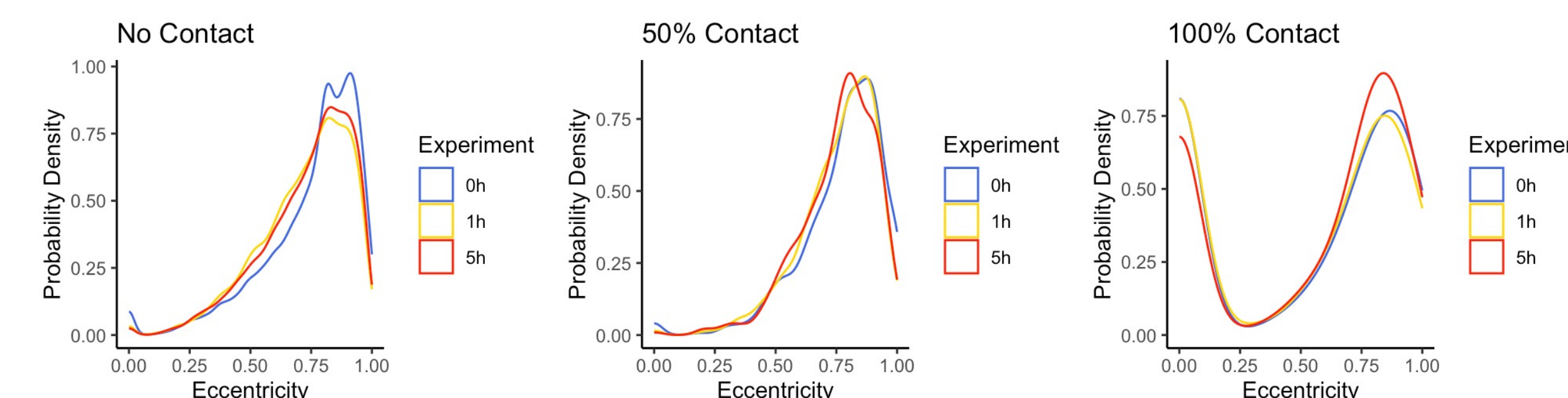**Cells Are Highly Mesenchymal Regardless Of EMT Driver Treatment**

TGF-β Effect Weakened With Increased Neighbor Count

Increased Neighbor Count Favors Epithelial And Hybrid Approximations In TNF-α Treated Cells
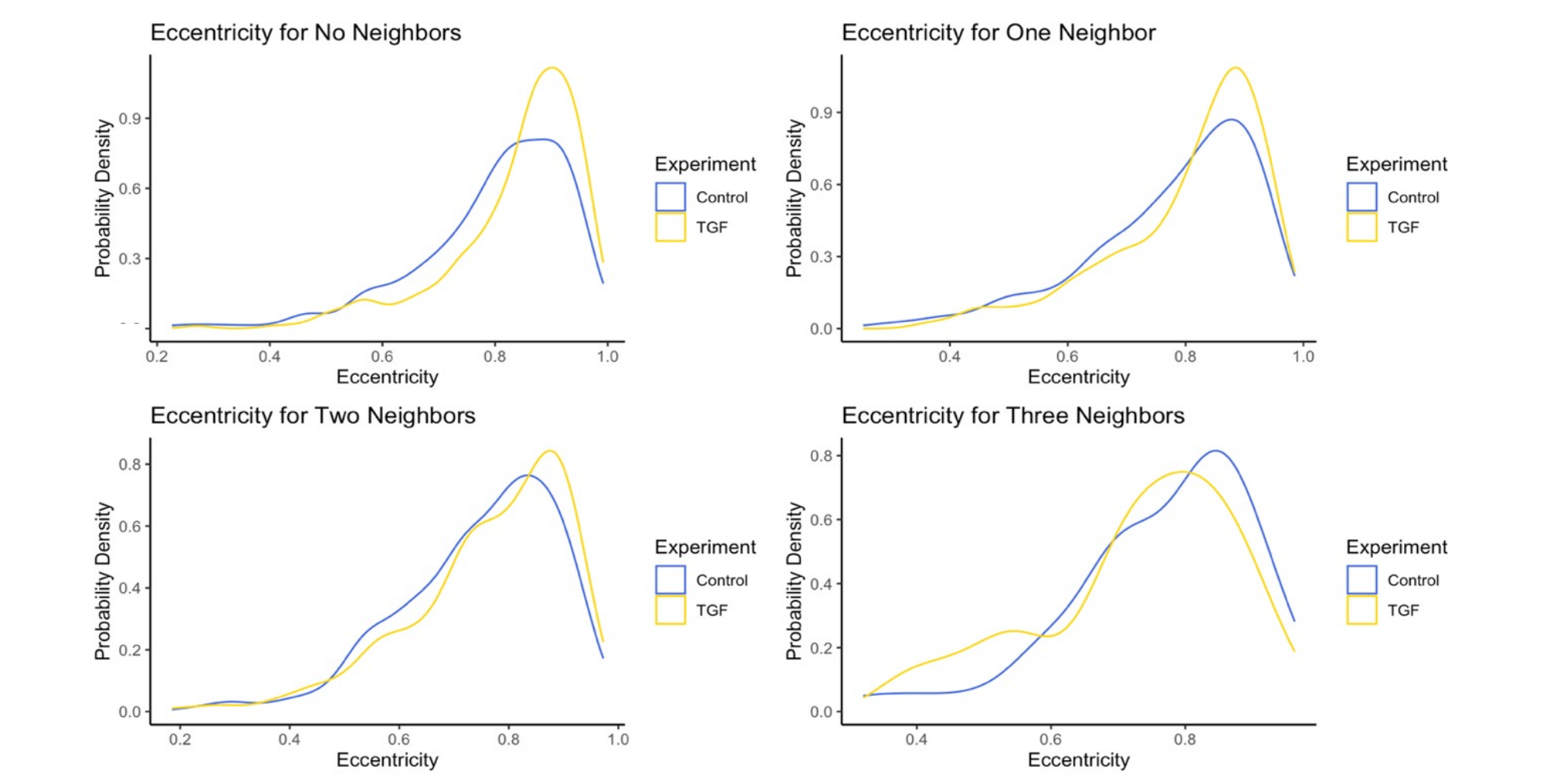
Distinct Cell Shape Populations At 100% Cell-Cell Contact

Results II: Hybrid E/M Cells Are Stabilized in Higher Density, Low Contact Environments

Clustering Results For Cells Treated With TNF-α To Induce NF-κB
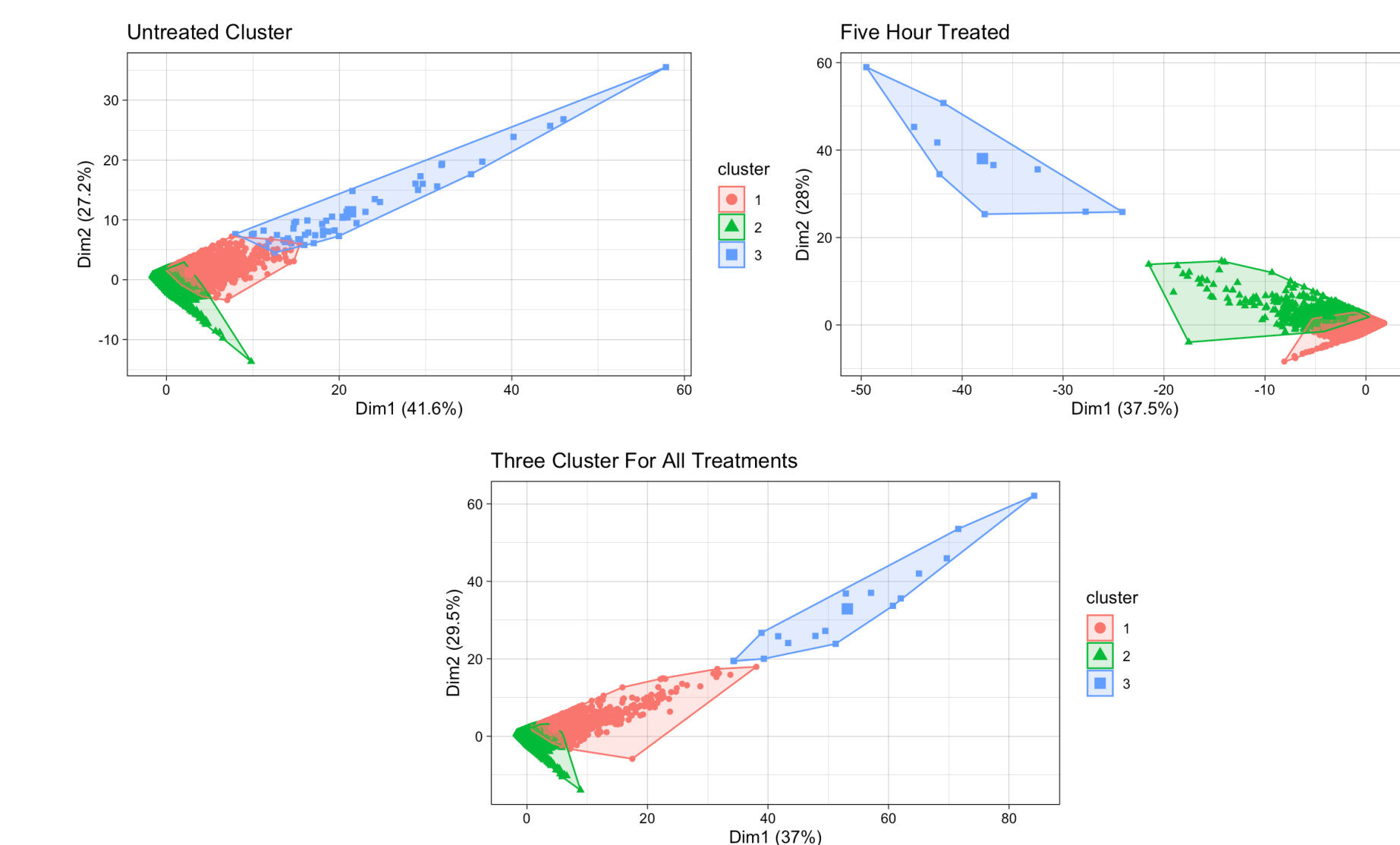
Compactness Scores Support 3 Clusters As The Optimum Cluster Number

Treatment	Two-Cluster Compactness (%)	Three-Cluster Compactness (%)	Four-Cluster Compactness (%)	Five-Cluster Compactness (%)
Untreated	39.7	64.3	45.3	53.5
One-hour	25.2	74.2	54.5	54.5
Five-hour	56.3	72.3	45.8	53.5

The Biophysical Environment Is Key For Distinguishing Between Clusters

Hybrid E/M Cells Identified Via Clustering Averages

Mesenchymal
Hybrid

Conclusions and Limitations

This project resulted in:

1. **A morphology classification metric.** Eccentricity is an easily measurable metric of cell shape however it does not have well defined boundaries.
2. **An adaptable pipeline that focuses on single-cell interactions.** Additional modules that cater to extracting information like image intensity could be used to answer further questions.
3. **Indications of cell microenvironments which stabilize the hybrid E/M phenotype.** Our data showed importance of percent contact and cell density for hybrid commitment.
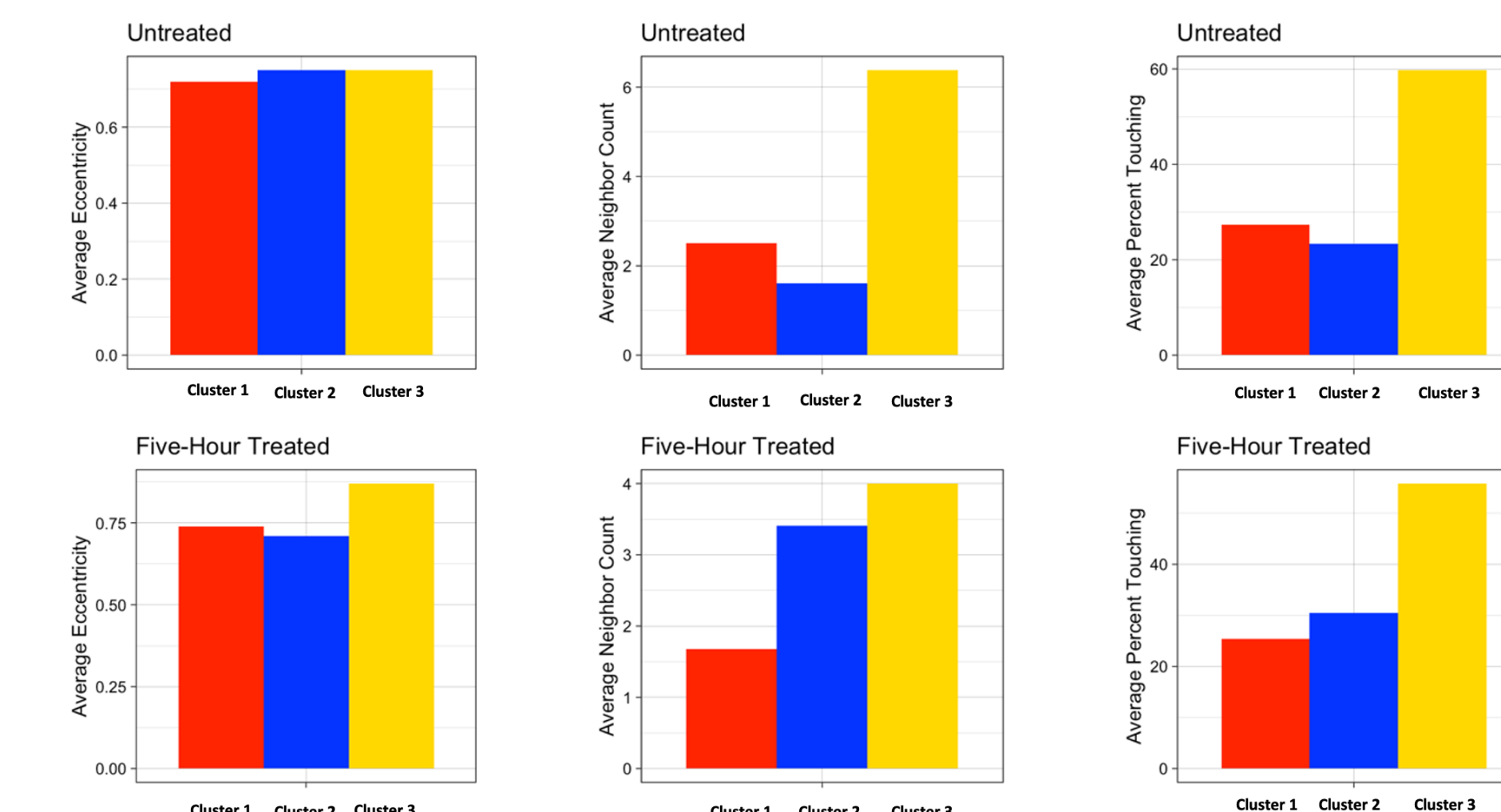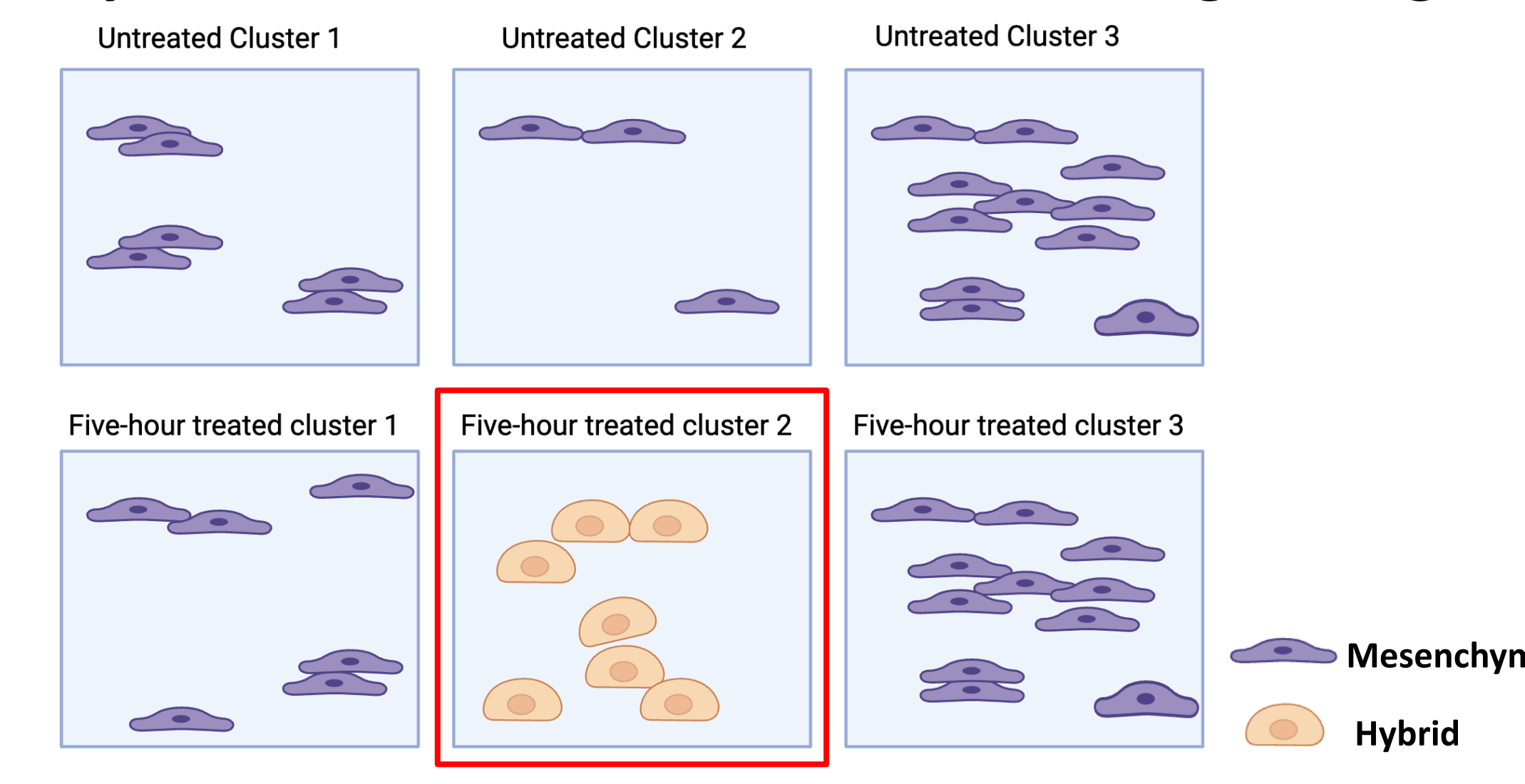
Future Works

To improve upon this project, further work should incorporate:

- Utilizing data using core regulators such as E-cadherin, ZEB and Snai1/2
- Perform analyses on matrix stiffness bioimaging data
- Incorporating a module that measures treatment levels to correlate with shape changes
- Using live cell imaging to track specific changes in cell objects

Acknowledgements

Works Cited

1. Sullivan, E., Harris, M., Bhatnagar, A., Guberman, E., Zonfa, I., and Regan, E. R. (2023) Boolean modeling of mechanosensitive epithelial to mesenchymal transition and its reversal. iScience. 10.1016/j.isci.2023.106321
2. Pastushenko, I., Brisebarre, A., Sifrim, A., Fioramonti, M., Revenco, T., Boumahdi, S., Van Keymeulen, A., Brown, D., Moers, V., Lemaire, S., De Clercq, S., Minguijón, E., Balsat, C., Sokolow, Y., Dubois, C., De Cock, F., Scozzaro, S., Sopena, F., Lanas, A., D'Haene, N., Salmon, I., Marine, J.-C., Voet, T., Sotiropoulou, P. A., and Blanpain, C. (2018) Identification of the tumour transition states occurring during EMT. Nature. 556, 463–468
3. Ren, Z.-X., Yu, H.-B., Li, J.-S., Shen, J.-L., and Du, W.-S. (2015) Suitable parameter choice on quantitative morphology of A549 cell in epithelial–mesenchymal transition. Biosci Rep. 35, e00202
4. Sero, J. E., Sailem, H. Z., Ardy, R. C., Almuttaqi, H., Zhang, T., and Bakal, C. (2015) Cell shape and the microenvironment regulate nuclear translocation of NF-κB in breast epithelial and tumor cells. Mol Syst Biol. 11, 0790