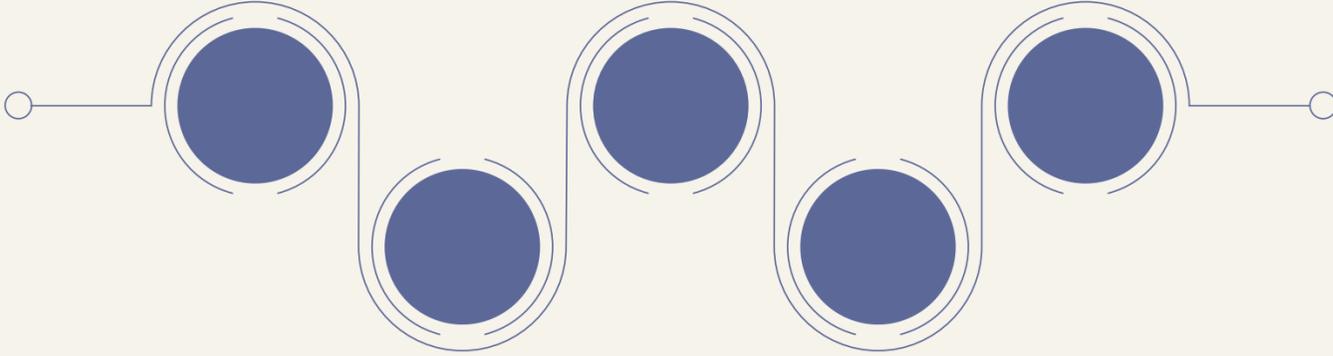




# **EXPLORING THE PIK3CA MUTATION AND PI3K PATHWAY IN HER2+ CANCER**

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# **HER2+ cancer subtypes**

**HER2 is a protein on the surface of breast cells that tell them when to grow**

## **In HER2+ breast cancer:**

- **There are too many HER2+ receptors**
- **Cells get told to “grow” constantly**
- **Leads to aggressive and fast tumor growth**

## **Why it is important:**

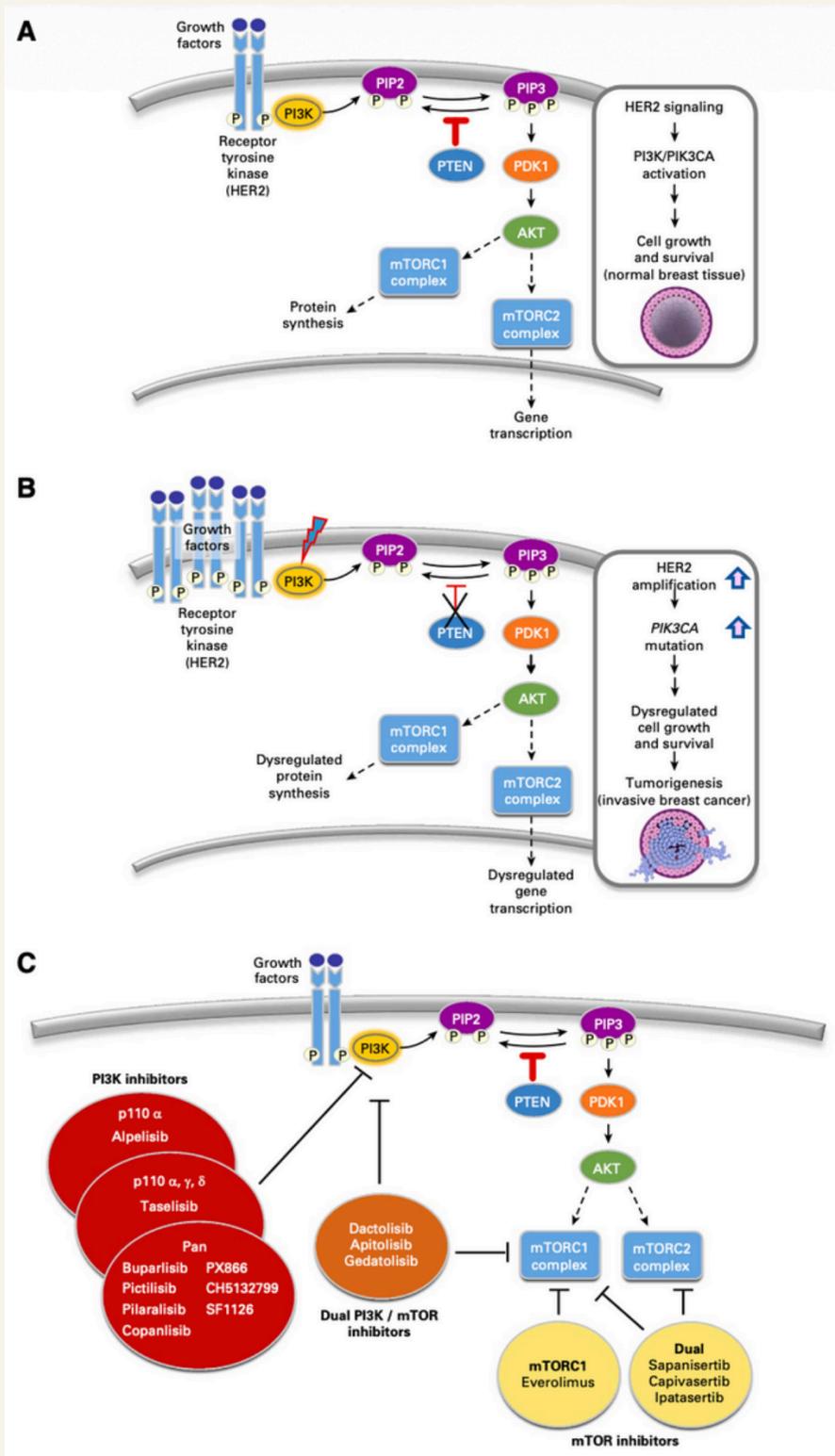
- **They make up 15-20% of all breast cancers**
- **They tend to grow and spread faster than other breast cancers**

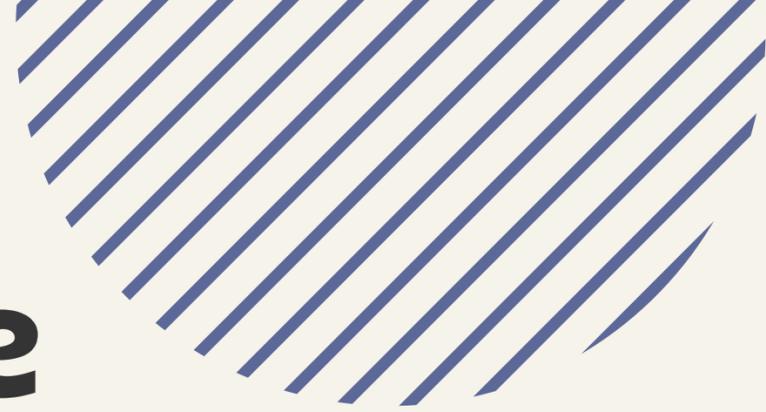
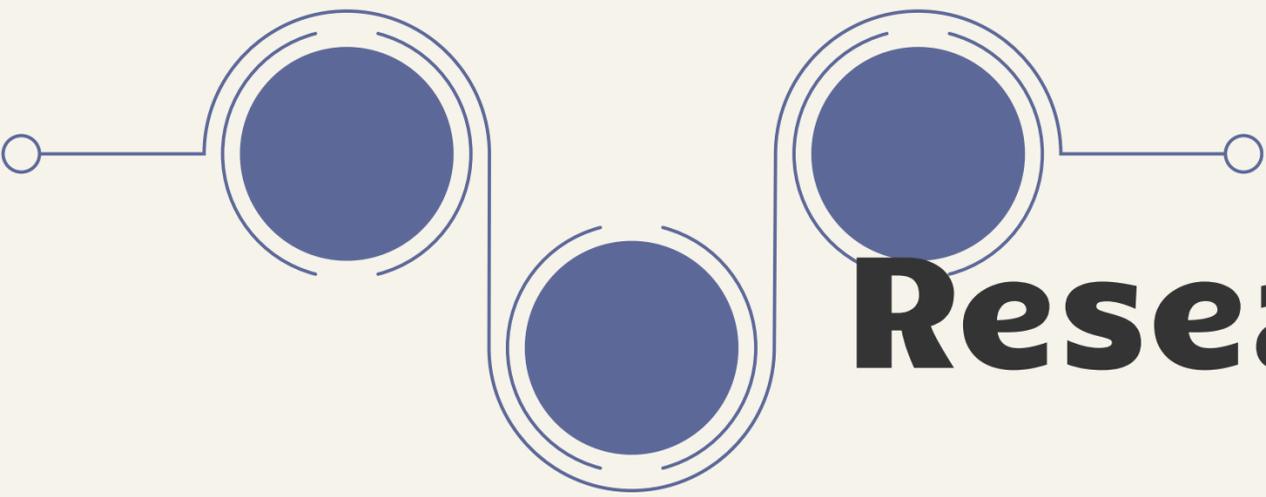


# PI3K Pathway

The PI3K pathway is essential in regulating cell survival, metabolism, and proliferation

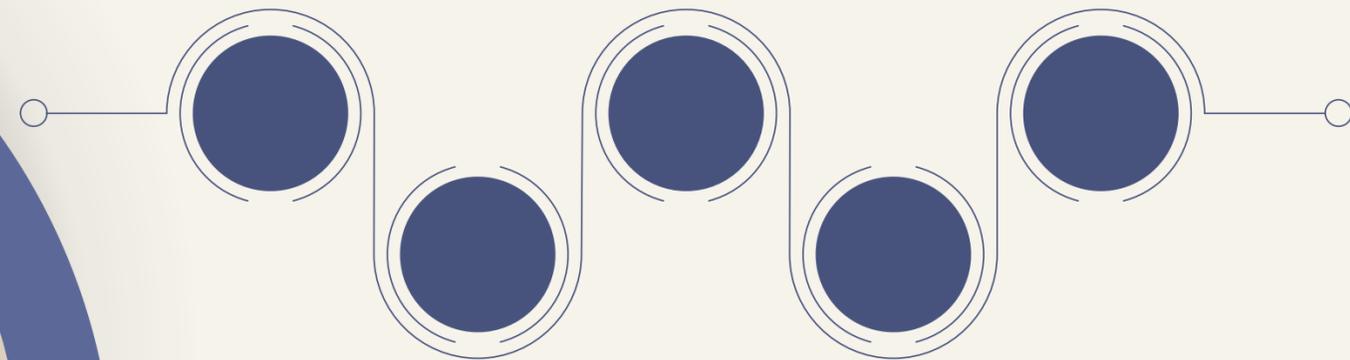
Mutations in the gene that cause the pathway to disrupt (PIK3CA mutation) are common in HER2+ cancers and play a role in tumor progression





# Research Objective

**The goal of the research project is to determine how introducing a common PIK3CA mutation into HER2 positive breast cancer cells changes their gene expression and signaling. By comparing mutant and wild-type cells using RNA-sequencing, we are trying to understand how this mutation influences tumor behavior, including growth, migration, and invasion.**



# The Project

**Two breast cancer cell lines were used**

- **SUM225**
- **SKBR3**

**These lines were genetically modified to have the PIK3CA mutation using CRISPR. A multitude of experiments were/are running to compare these mutated cells to the unmutated cells.**

**The experiments include**

- ***In vitro* and *in vivo* experiments with CRISPR cells - behavior**
- **RNA Sequencing Analysis**

# CRISPR

**CRISPR-Cas9 is a gene-editing system that uses a guide RNA to direct the Cas9 enzyme to a specific DNA sequence. Cas9 makes a cut, and during repair, a precise mutation can be inserted.**

- **In this specific project, CRISPR was used to introduce the PIK3CA mutation into HER2+ breast cancer cells**



# Cell Cultures

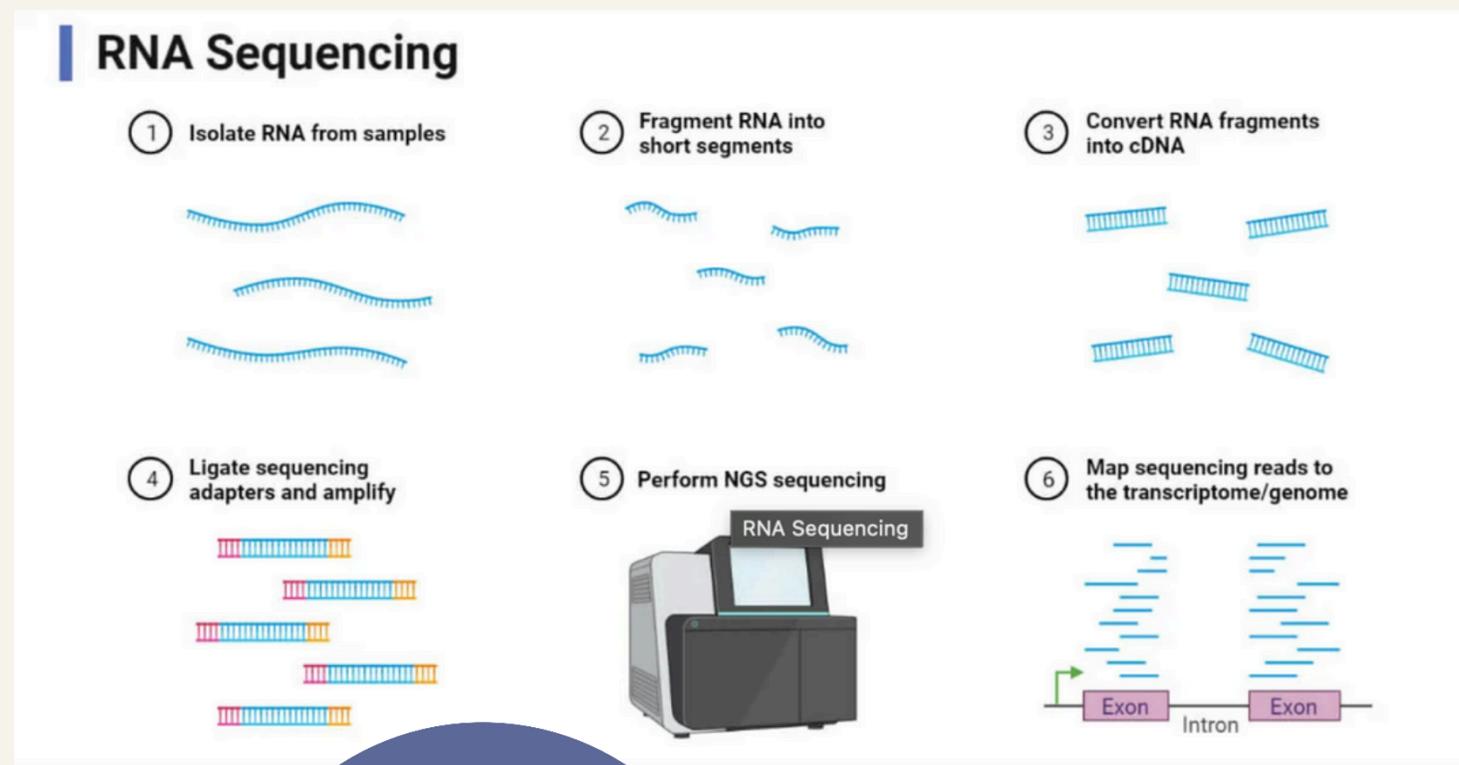
- **Technique used to grow and maintain cells outside the body**
- **Kept in nutrient-rich media under controlled temperature, CO<sub>2</sub>, and sterile conditions**
- **This allows for the study of cell growth patterns in controlled environment.**

**The cell cultures were used to maintain the mutated and wild type cancer cell lines. By culturing the cells it allowed for cells to be used in the RNA sequencing.**

# RNA Sequencing

RNA sequencing is used to measure RNA levels across a transcriptome

- Provides us with a snapshot of what genes are active, upregulated, and downregulated
- Allows for comparison of wild type cells and the mutated cells on the genetic levels



## RNA Seq pipeline

- Rna Extraction
- Library Preparation
- Sequencing
- Quality Control
- Alignment
- Quantification
- Differential Expression Analysis
- Pathway Analysis



# **RNA Seq data generation and analysis**

**The RNA sequencing is done by an outside lab and they send back a spreadsheet of differentially expressed genes.**

**From these files is when the analysis started using the Anschutz RNA seq software.**

**I did multiple Analysis including ORA, direct comparison, and FGSEA.**

**From these analyses, I found a list of upregulated genes and enriched pathways that could be important in the future of the experiment.**



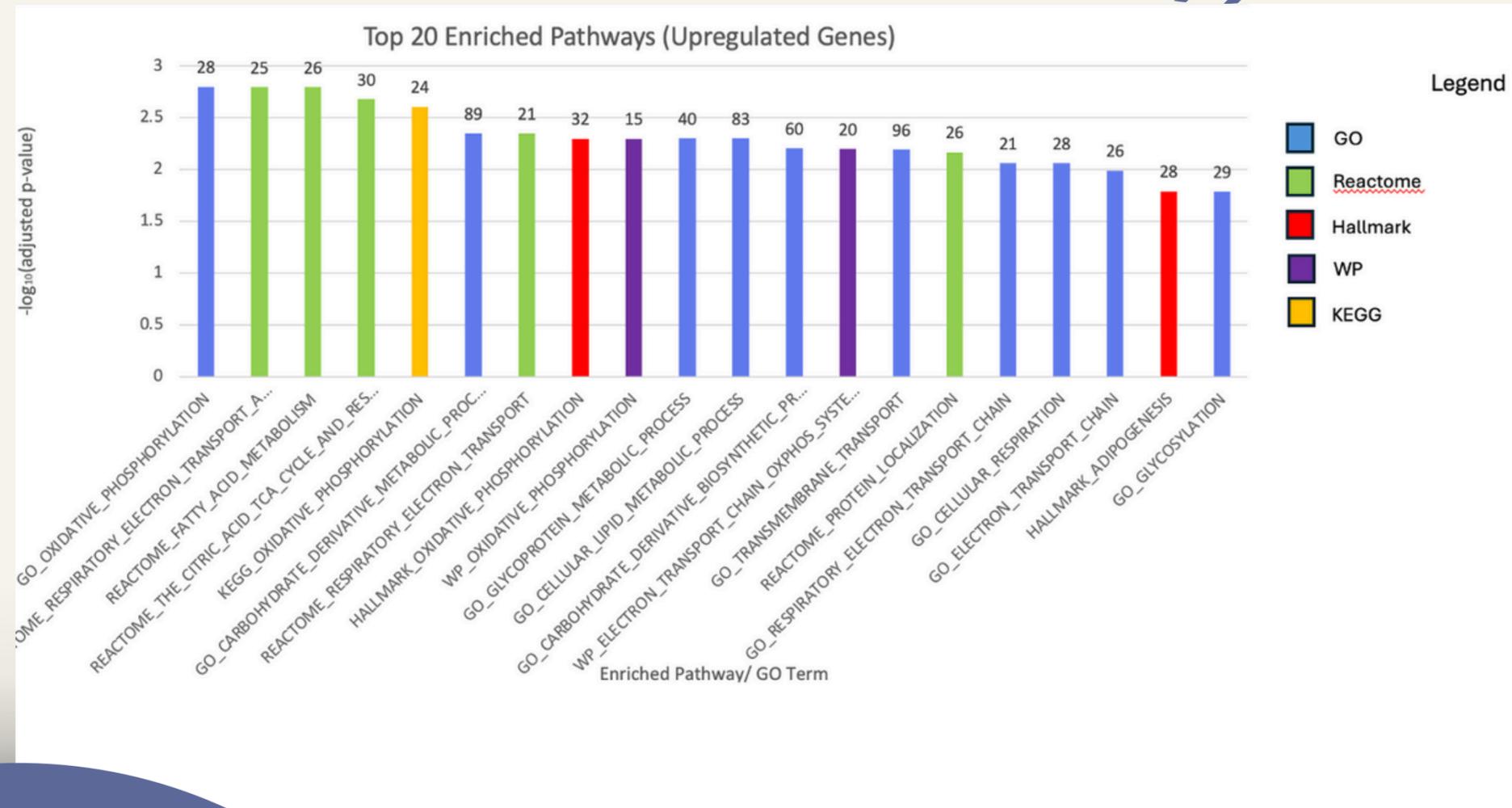
# Results

The analysis shows strong enrichment of

- **Oxidative Phosphorylation**
- **Respiratory Electron Transport Chain**
- **TCA cycle and Fatty Acid Metabolism**

**Multiple Pathways converge on the same themes**

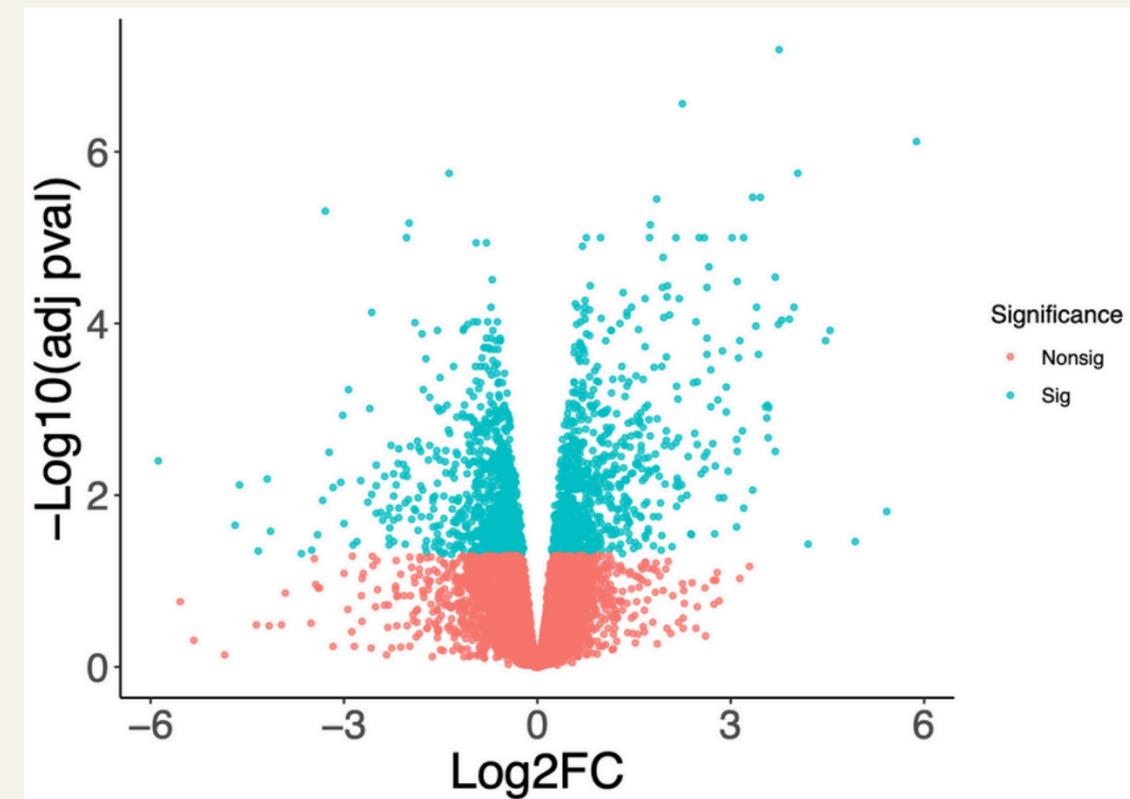
**We can gather that: The PIK3CA mutation drives coordinated upregulation of energy-producing mitochondrial pathways and extracellular matrix remodeling processes.**



# Results

| Gene   | Count |
|--------|-------|
| COL4A1 | 31    |
| FN1    | 24    |
| H2AC18 | 22    |
| HIPK2  | 18    |
| GATA3  | 13    |

**Gene counts of which popped up the most in enriched pathways.**

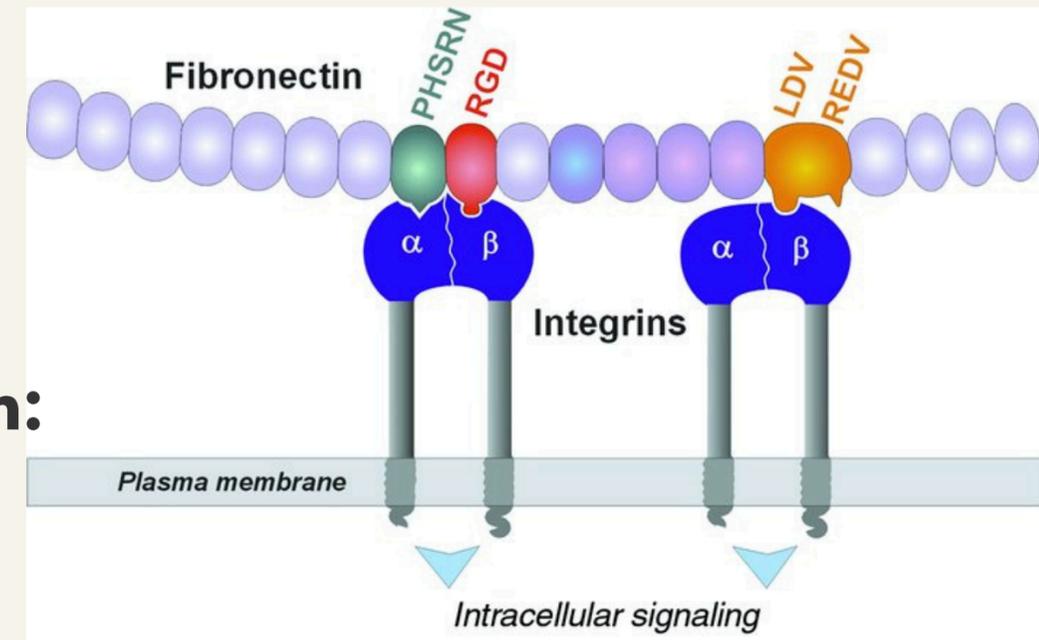


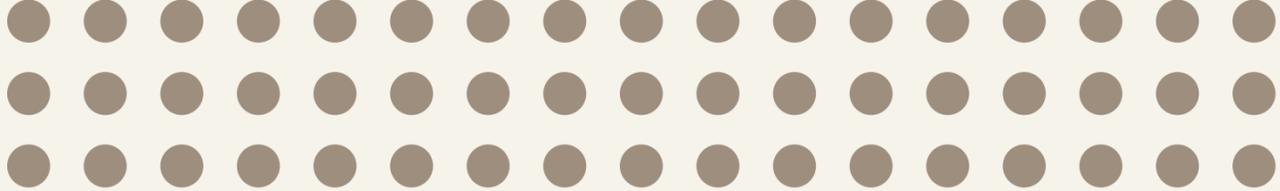
Left is down regulated, right is upregulated. Blue is significant. Red is not significant

**COL4A1, FN1, and GATA3 are the focus**

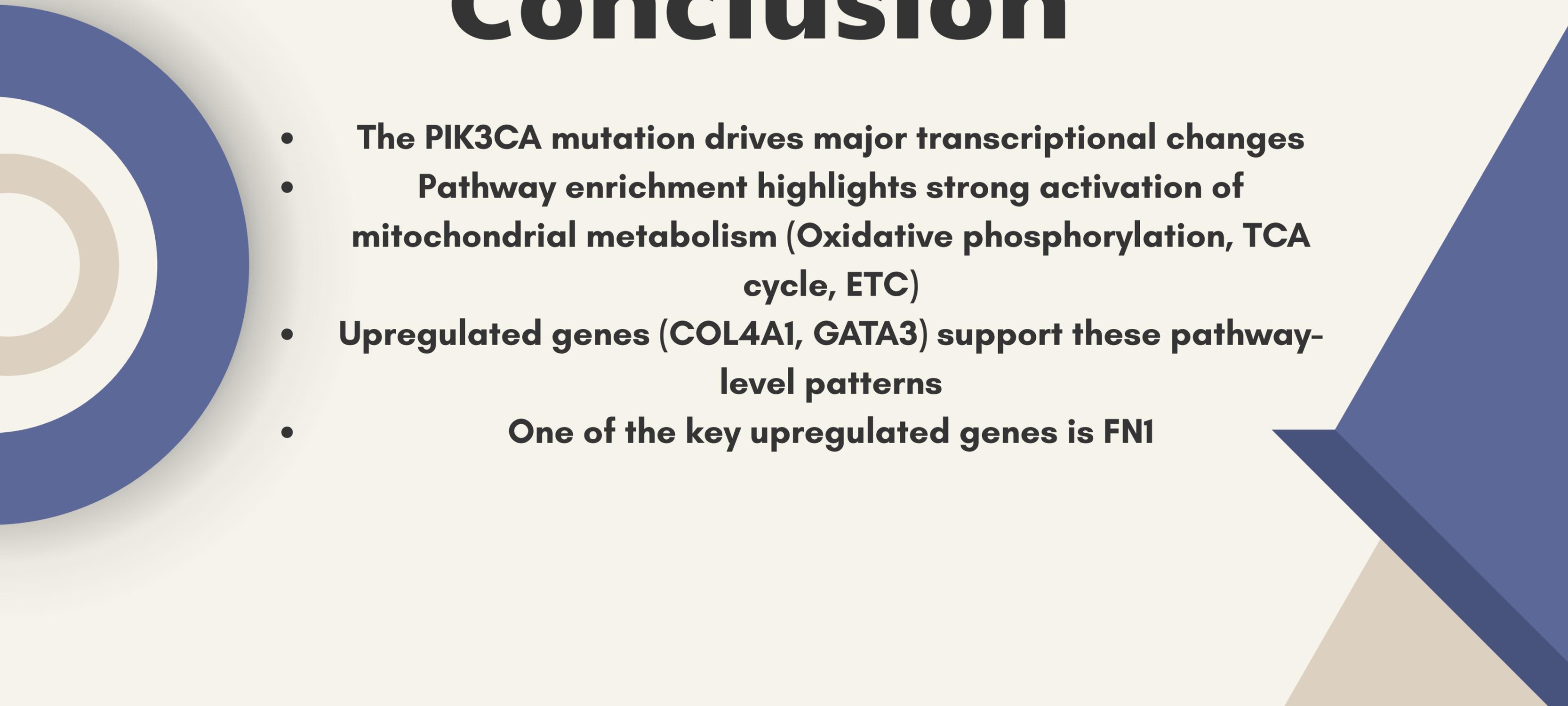
# FN1

- **FN1 is strongly upregulated in PIK3CA mutated cells**
- **Encodes fibronectin, a major ECM protein involved in:**
- **Cell adhesion, Migration, Invasion**
- **FN1 upregulation is well-established in the literature as a marker of aggressive or metastatic breast cancer (Zhang et al., 2022)**
- **Connects directly to enriched pathways: ECM remodeling, focal adhesion, and cell movement**
- **May explain functional behaviors examined in the broader project**



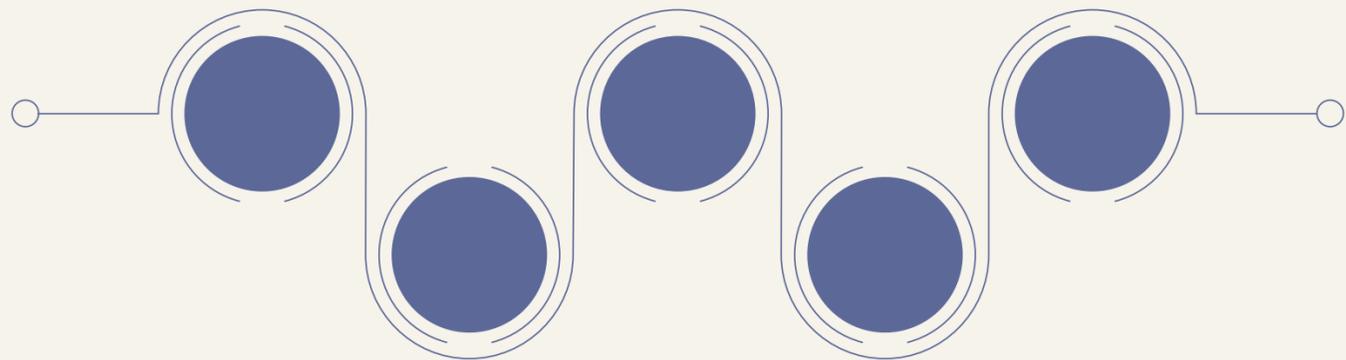


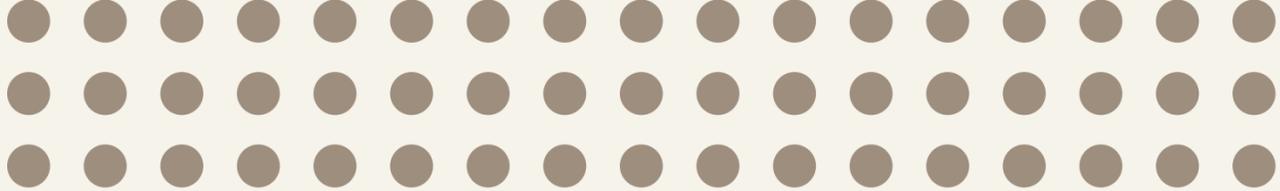
# Conclusion

- **The PIK3CA mutation drives major transcriptional changes**
  - **Pathway enrichment highlights strong activation of mitochondrial metabolism (Oxidative phosphorylation, TCA cycle, ETC)**
  - **Upregulated genes (COL4A1, GATA3) support these pathway-level patterns**
  - **One of the key upregulated genes is FN1**
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# CULTURE EXCHANGE

- **Worked Closely with PB who shared stories and traditions from Indian culture**
- **We talked about Holidays such as Holi and Diwali and the meaning behind them and where they come from, as well as differences In celebrating Thanksgiving.**
- **We shared perspectives on life within the United States coming from different backgrounds**





# Acknowledgment

**Thank you to PB for being such a great mentor**

**Thank you to the Department of Medical Oncology & Elena Shagisultanova, MD, PhD for allowing me to spend time in the lab**

**A special thank you to Liz and Dr.Cenciarelli for giving me this opportunity**

