# KASHATUS LAB: CANCER BIOLOGY

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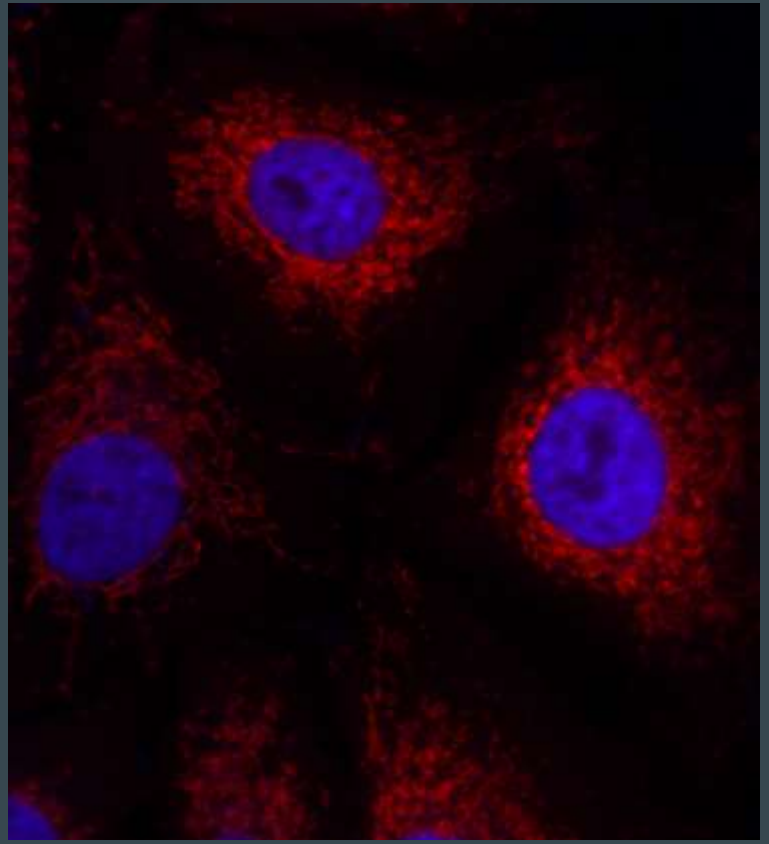
# CANCER CELLS

- Unregulated cell growth - invasive
  - DNA replication functions are broken
  - Divide constantly
- Mutate quickly and frequently
- Metastasis: mutated cancer cells traveling to other parts of the body
- Apoptosis: programmed cell death
  - Damaged/non-functional cells should eliminate themselves
  - Cancer cells avoid apoptosis
- Neoplasm: body of cells originating from mutated cell
  - Neoplasm grows and becomes a lump = tumor
  - Benign: harmless tumor
  - Malignant: super growth, invasive, takes over resources



# ONCOGENES

- Proto-oncogenes: code for proteins that direct normal cell growth
- Oncogene: mutated version of proto-oncogene that can cause cancer
  - Either normal protein that is overexpressed or hyperactive (mutated) with normal expression
- Proto-oncogene → oncogene
  - Deletion/point mutation
  - Gene amplification
  - Chromosomal rearrangement
- Ras Oncogene
  - Codes for small GTPases - hydrolyzes GTP into GDP and P
  - On/off switch in growth signaling pathways
    - GTP is needed for RNA synthesis during transcription

# TUMOR SUPPRESSOR GENES

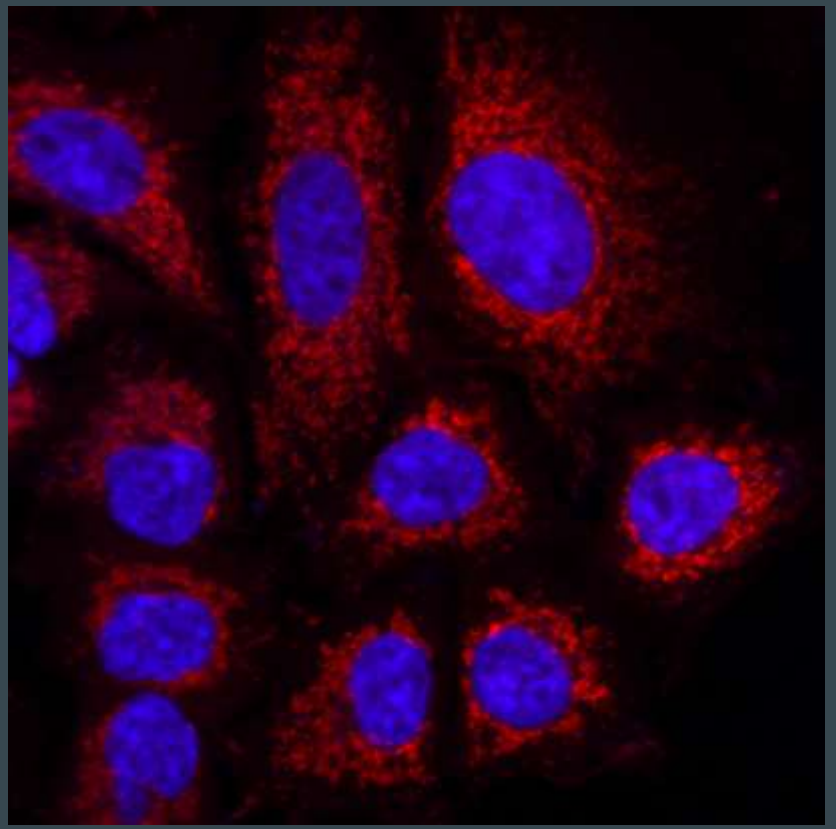
- Safety checks to stop mistakes in cell division that lead to cancer
- Tumor Suppressor genes make proteins that either
  - Have halting effect on cell cycle regulation
  - Promote apoptosis
- Types of tumor suppressors
  - DNA repair proteins
    - Recognize DNA damage
    - Repair damage or initiate cell death
  - Cell cycle repressors
    - Represses genes that are essential for continuation of cell cycle → stops cell cycle
- Two-hit Hypothesis
  - Both alleles must be mutated before protein is altered
    - “Back-up copy” if only one is damaged
  - Mutated tumor suppressor allele = “recessive”
    - Both alleles must be mutated to cause cancerous phenotype
  - Does not apply in mutated oncogene - only need one mutated allele

# MITOCHONDRIA IN CANCER

- Main functions of mitochondria
  - Performing of cellular metabolism
    - Sugars/fats/proteins → ATP and other important molecules
  - Regulation of cell suicide (apoptosis)
- Mitochondrial dynamics: fission and fusion
  - Fission: fragmentation of larger mitochondria into smaller mitochondria
  - Fusion: union of smaller mitochondria into larger mitochondria
  - Influence mitochondrial function
- Mitochondrial dynamics are mediated by many proteins
  - Drp1: fragments mitochondria (“Drp1 divides”)
  - Mfn1/2: and Opa1: fuse/elongate mitochondria (“Mfn1/2 merges”)
  - Cancer hyperactivates proteins that control mitochondrial proteins
- Mitochondrial functions are dysregulated in cancer cells
  - Mutant genes activate specific pathways → How do the pathways change mitochondria?

# THE LAB'S FOCUS: MITOCHONDRIAL DYNAMICS

- Understand the differences in mitochondrial dynamics in different cell lines
- Identify which proteins and pathways ultimately affect mitochondrial dynamics
  - Force "normal cells" to express one hyperactive protein found in cancer
- Compare mitochondria in the normal cell to those in the cell with the cancer protein expressed
- Fused or fragmented phenotype of mitochondria in cells



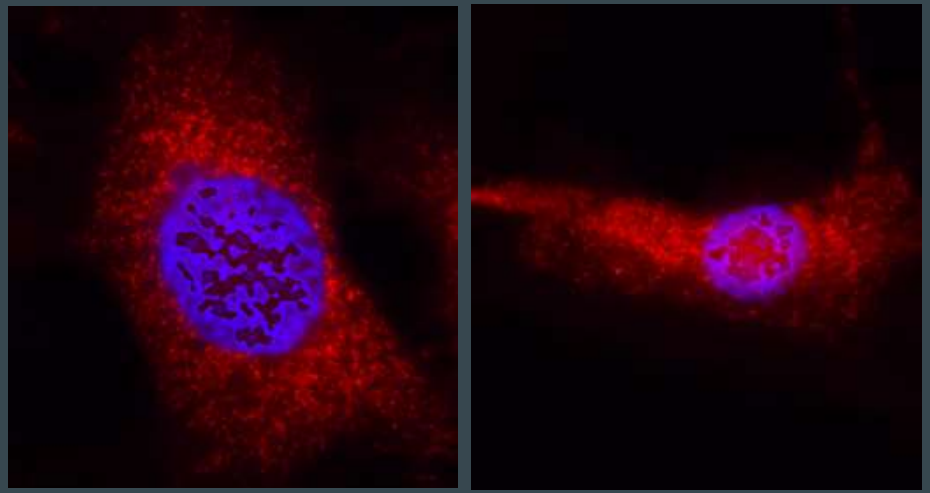
# OVERALL RESEARCH QUESTIONS

1. What groups/pathways of proteins in cancer affect mitochondrial dynamics?
2. How do these pathways affect mitochondrial dynamics?
3. How might different proteins in the same pathways affect mitochondrial dynamics differently?

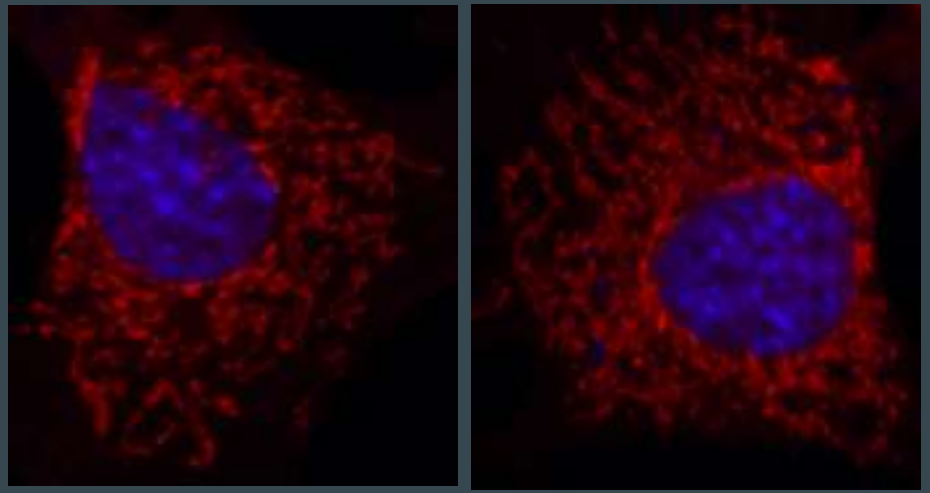
# HOW TO COLLECT CELL IMAGES

- Fluorescently label mitochondria in these cells
- Take images on the microscope
- Measure various features about those mitochondria (length, surface are, etc...)
- After we measure mitochondrial features in different cell lines, we can compare to see if certain proteins lead to more or less mitochondrial fragmentation or fusion

# UNDERSTANDING CELL IMAGES



Fragmented

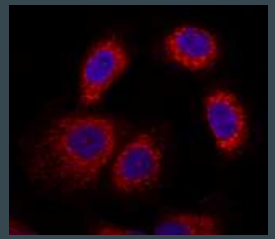


Fused

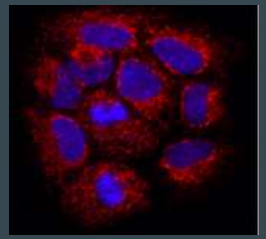
- Mitochondria stained red
- Nucleus stained blue

# MY FOCUS

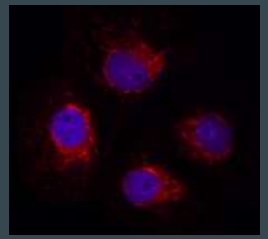
- Comparing the mitochondrial dynamics in 7 different cell lines of lung cancer



A549



H460



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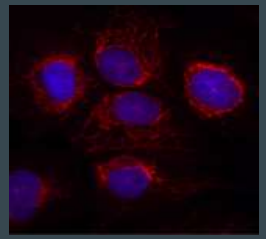

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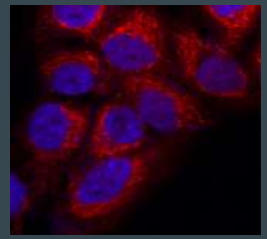

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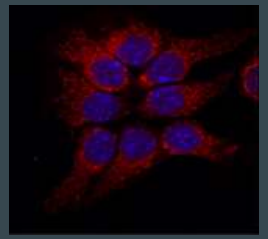
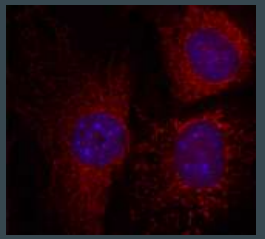
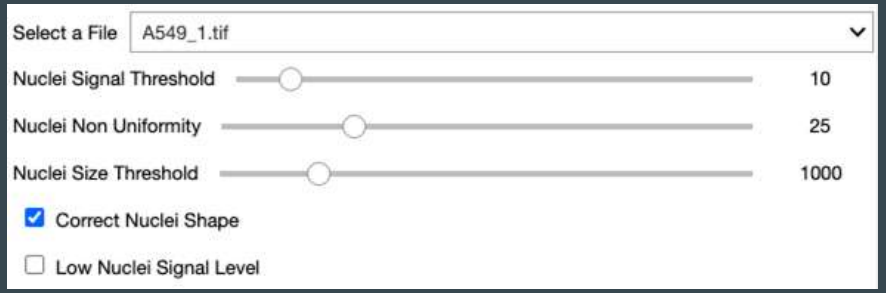


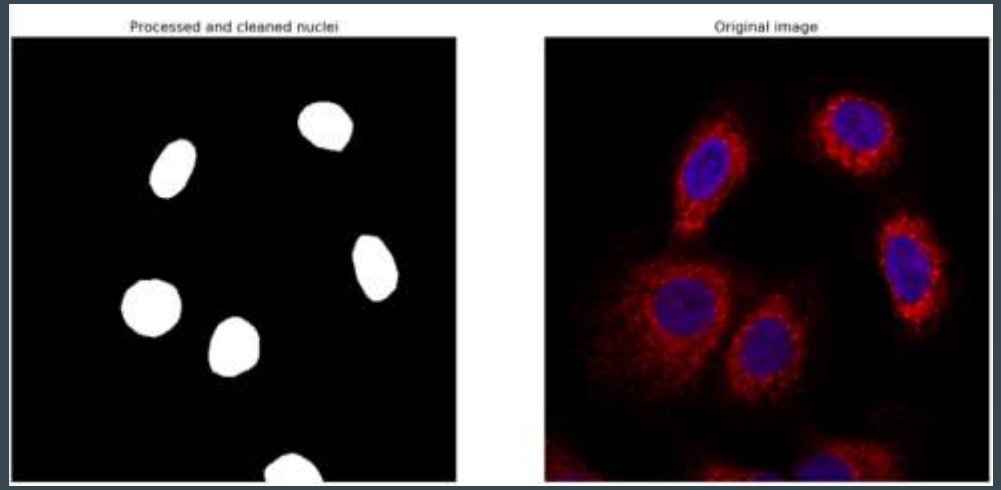
KPY40

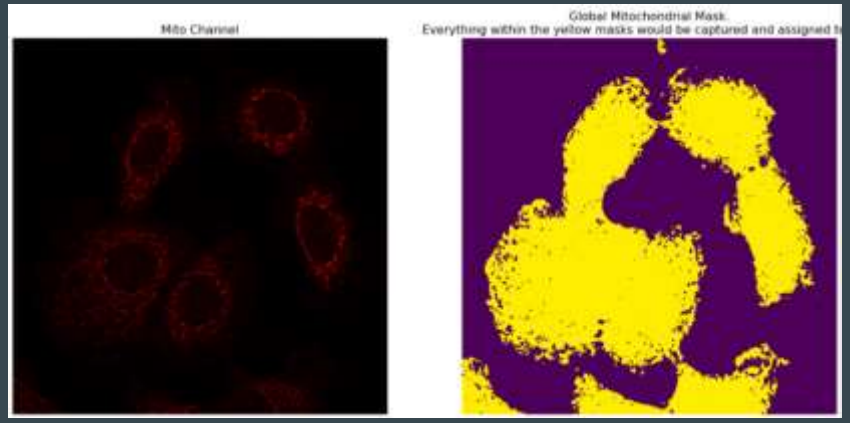


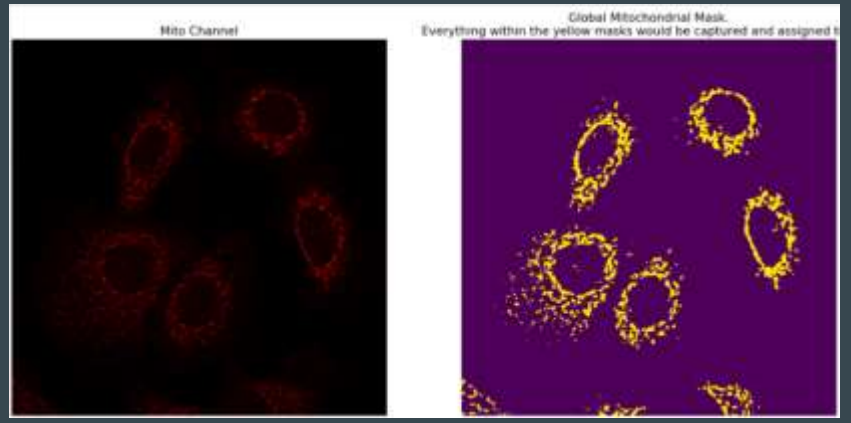
P MEF non-cancerous (control line)

# SOFTWARE STEP ONE: CELL CATCHER

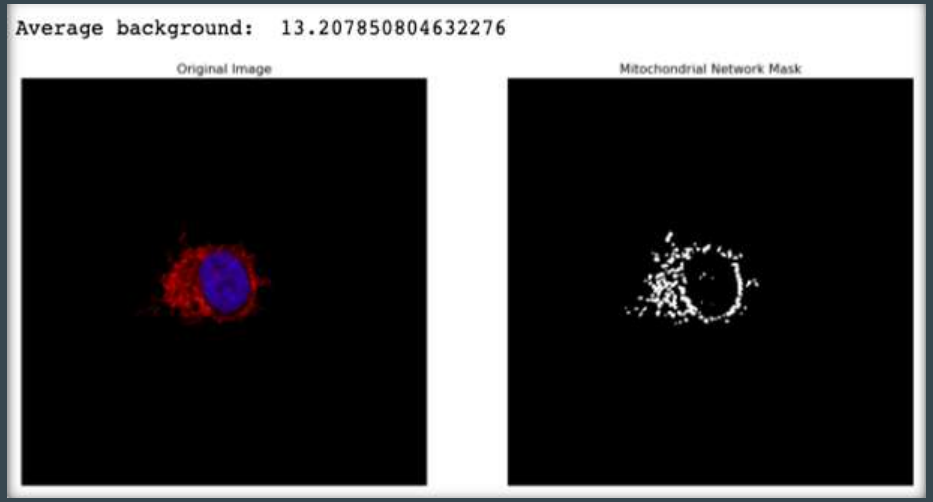


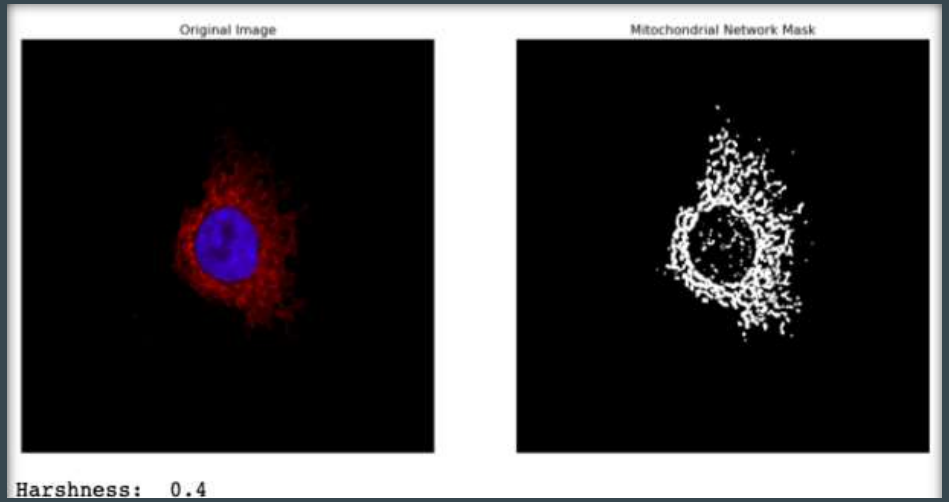



# SOFTWARE STEP TWO: MITO MINER



# SOFTWARE STEP THREE: MIA

**MiA** 0.8.8

**CeMiA Toolkit** 0.6.0

**Kashatus Lab @ UVA** ¶

## Welcome to MiA (Mitochondrial Analyzer)

**MiA is part of CeMiA toolkit that enables you to quantify the segmented miotchondrial netwrk generated by Mito Miner**

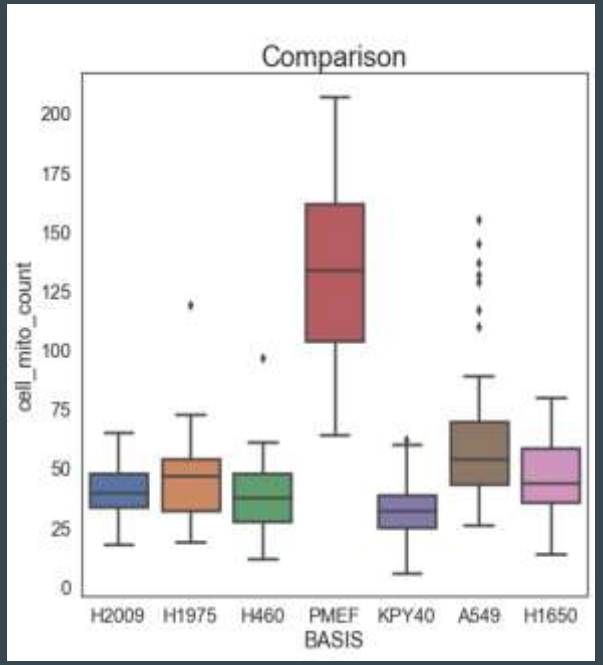
This Jupyter notebook provides you with step-by-step directions to quantify your mitochondria.

## 5) Quantifying all the files

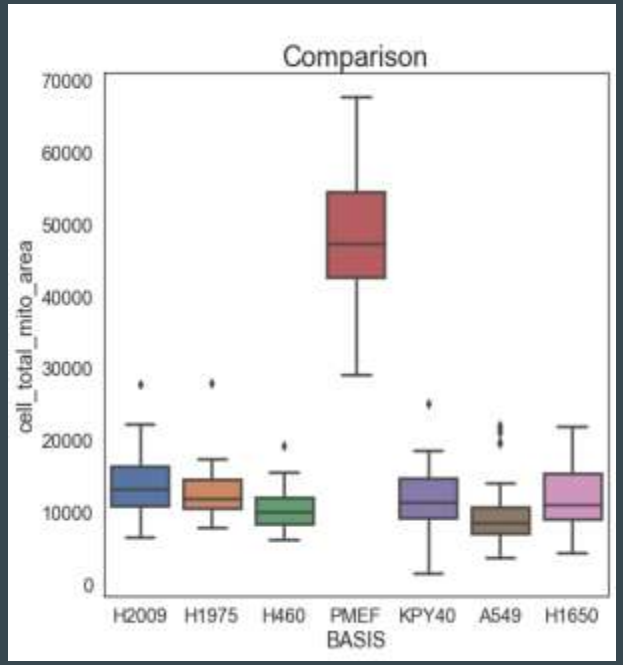
**Just run this block of code, and then go and enjoy your time. Running this block of code may take few hours depending on the number of images you have.**

```
In [*]: cemia.measurement(address,cell_list,output_filename)
        [2.04%] Now quantifying >>> A549_6_cell8_1024_mask.tif
```

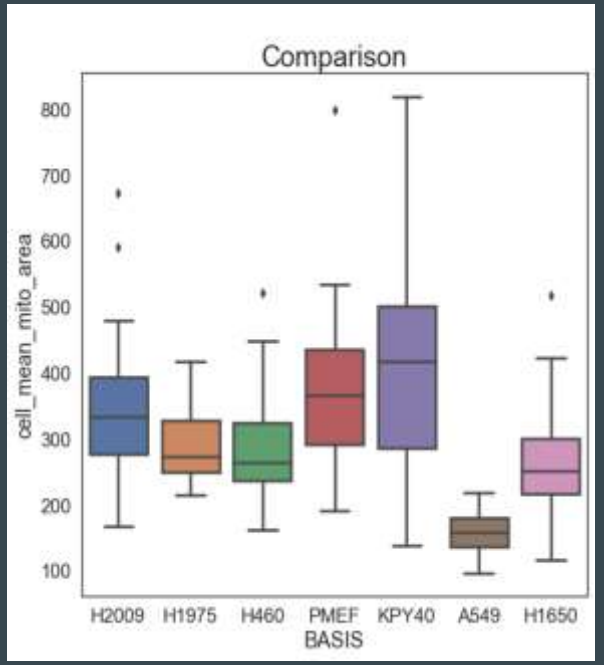
# ANALYZE DATA




MITOCHONDRIA COUNT




TOTAL MITOCHONDRIA AREA




MEAN MITOCHONDRIA AREA

# QUESTIONS?

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