

# Evaluating Ergosterol Peroxide's Impact on TNBC Metastasis

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## ABSTRACT

**INTRODUCTION:** Triple-negative breast cancer (TNBC), constituting 15% of breast cancers, exhibits lower survival rates due to rapid growth and spread. TNBC lacks estrogen receptors (ER), progesterone receptors (PR), and HER2 receptor expression. TNBC is more common in Hispanic and African American women. Racial disparities exist in metastatic TNBC, where ~25% of TNBC patients face metastases, affecting lungs, liver, and brain. Ergosterol peroxide (EP), is a selective anti-TNBC therapeutic, however its anti-metastasis properties have not been investigated until now. In this study, the effects of EP on cancer cell metastasis to the lungs and liver in mice injected with the MDA-MB-231 TNBC cell line were analyzed. **METHODS:** Female athymic nu/nu mice (n=18/group) received GFP-MDA-MB-231 cell injections and were divided into vehicle and EP groups. EP was administered at 100mg/kg BW via oral gavage. Organs were collected, imaged, and analyzed for metastatic foci using ImageJ software. Data analysis utilized Microsoft Excel, GraphPad Prism, and R software. **RESULTS:** Results evidence a highly significant ( $P<0.001$ ) decrease in the metastasized tumor area in EP-treated mice compared to the vehicle group. These significant EP-induced anti-metastasis results were seen for the metastatic foci area in the lung and liver. However, although there were a smaller number of metastatic foci between in the EP-treated group vs. the vehicle mice, this difference was not statistically significant. **CONCLUSION:** This study suggests the treatment with EP has potential anti-metastasis effects which increases the potential of EP as a potent anti-cancer agent.

## INTRODUCTION

### • Triple-Negative Breast Cancer (TNBC) subtype [1-4]

- Highly aggressive subtype of breast cancer;
- Absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor protein (HER2) expression;
- Accounts for about 20% of all breast cancers;
- Approximately 27% of TNBC patients are expected to relapse with distant metastases in lungs, liver, and brain.

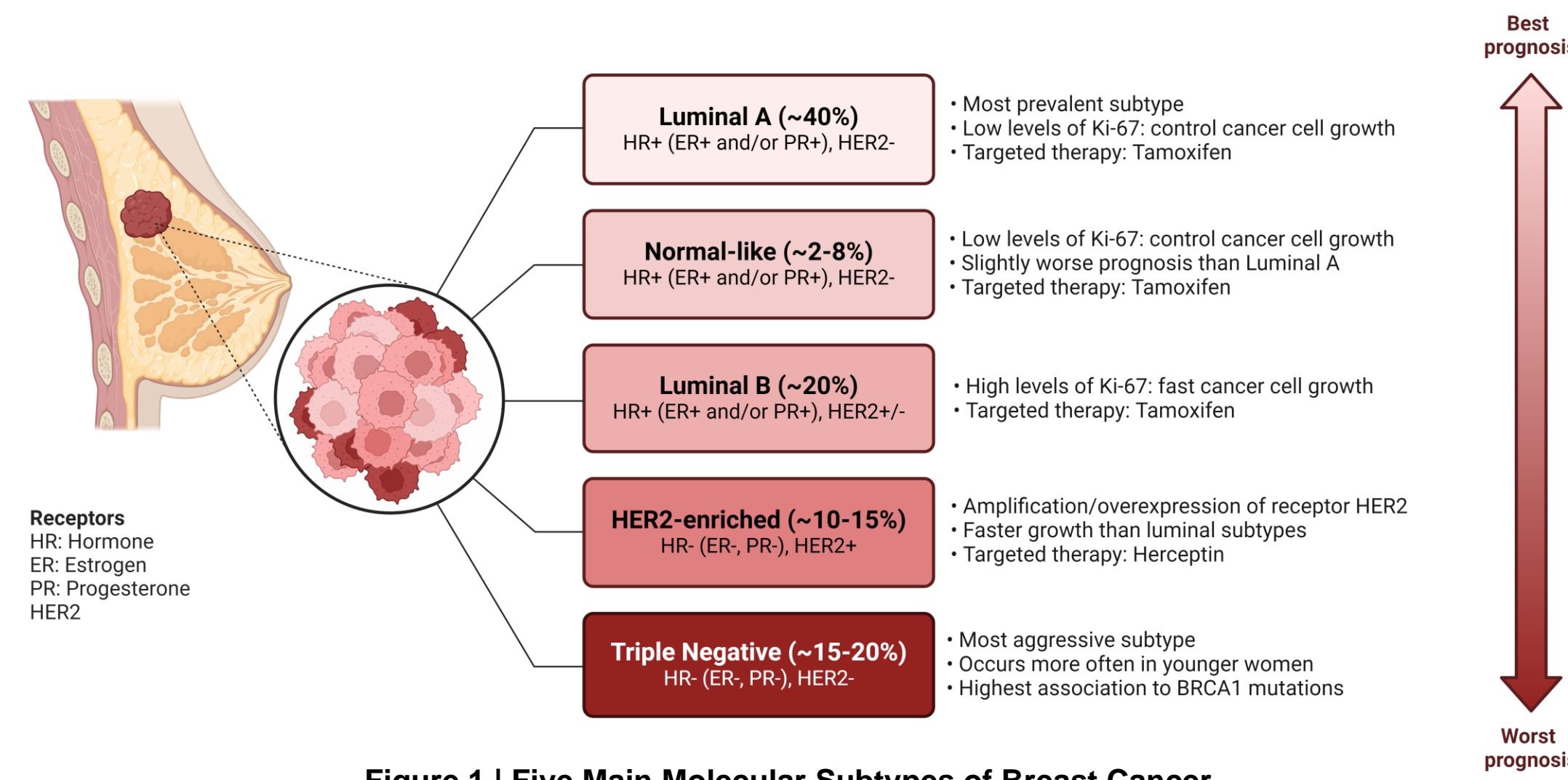


Figure 1 | Five Main Molecular Subtypes of Breast Cancer

### • Ergosterol Peroxide (EP) [5-8]

- Compound isolated from the medicinal mushroom *Ganoderma lucidum*;
- Anti-tumor and anti-proliferative activity;
- Selective effects against cancer cells.

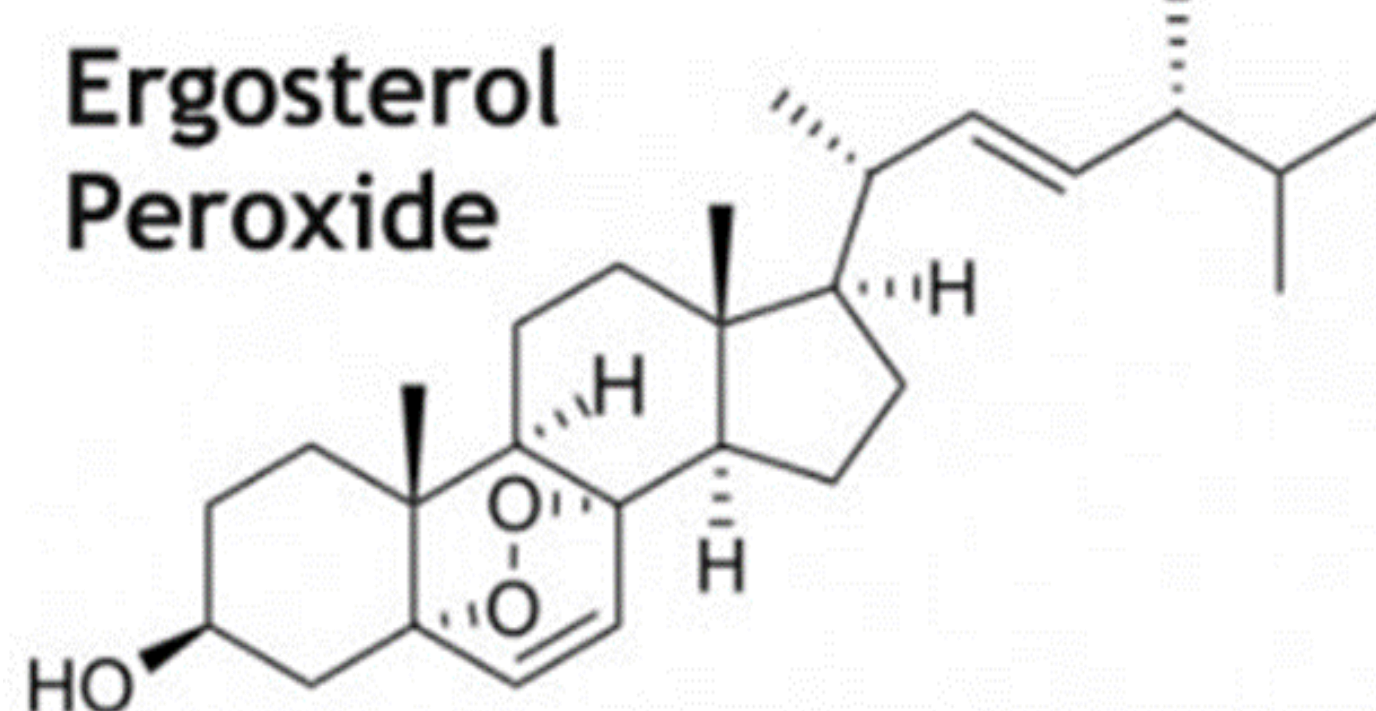


Figure 2 | Molecular Structure of EP



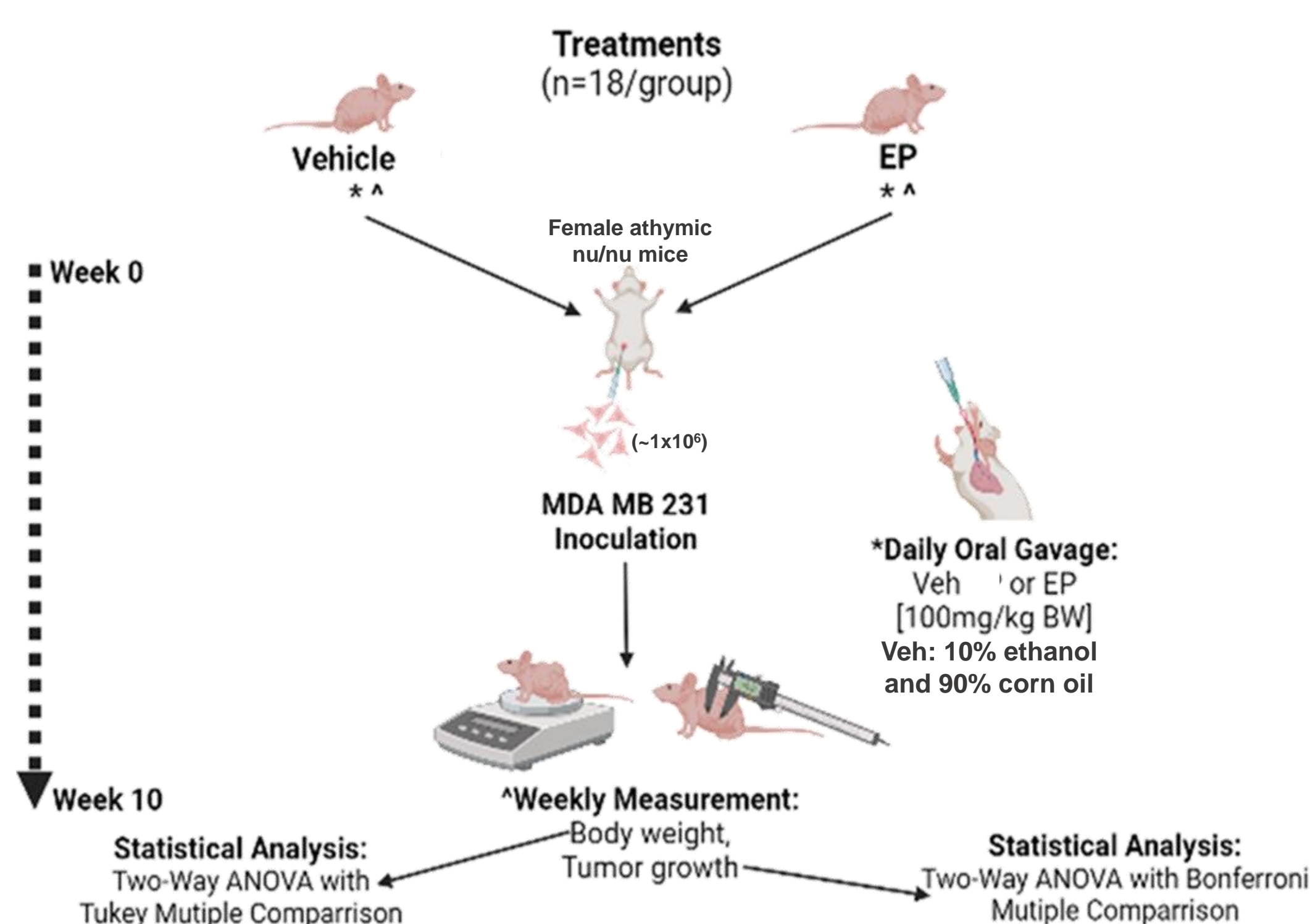
Figure 3 | *Ganoderma lucidum*

## OBJECTIVE

- In this study, the effects of EP on cancer cell metastasis to the lungs and liver in mice injected with the MDA-MB-231 TNBC cell line were analyzed.

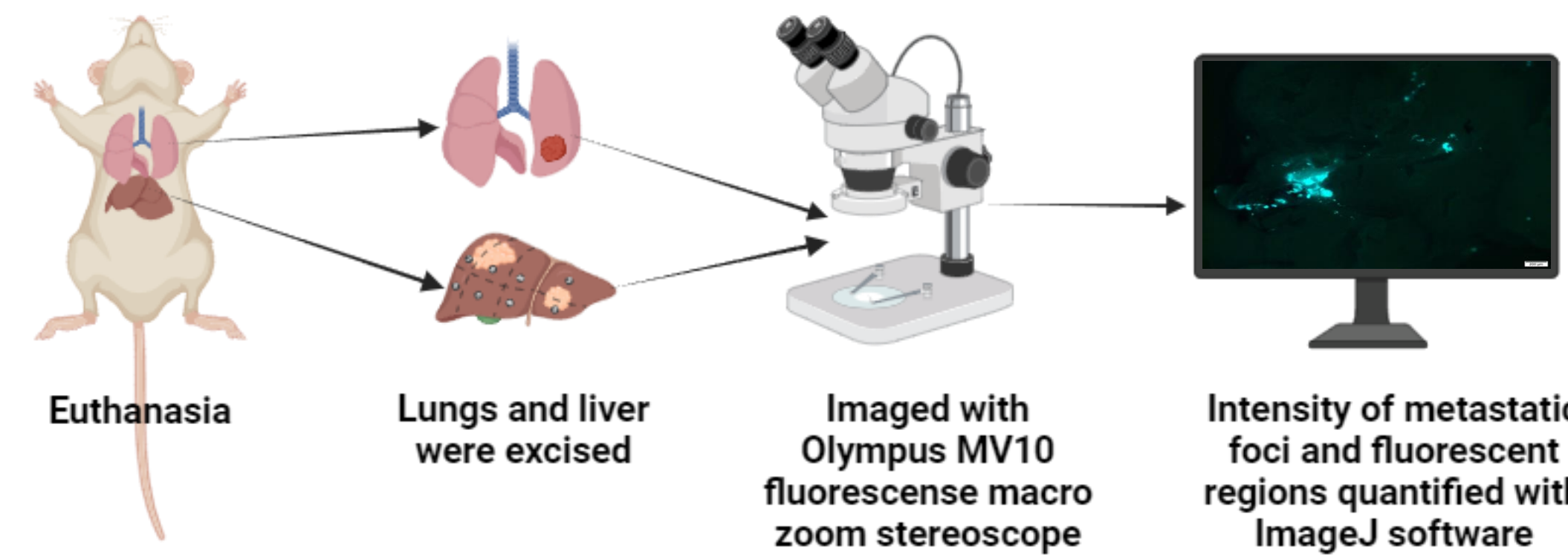
## METHODS

### In Vivo TNBC Study



## METHODS

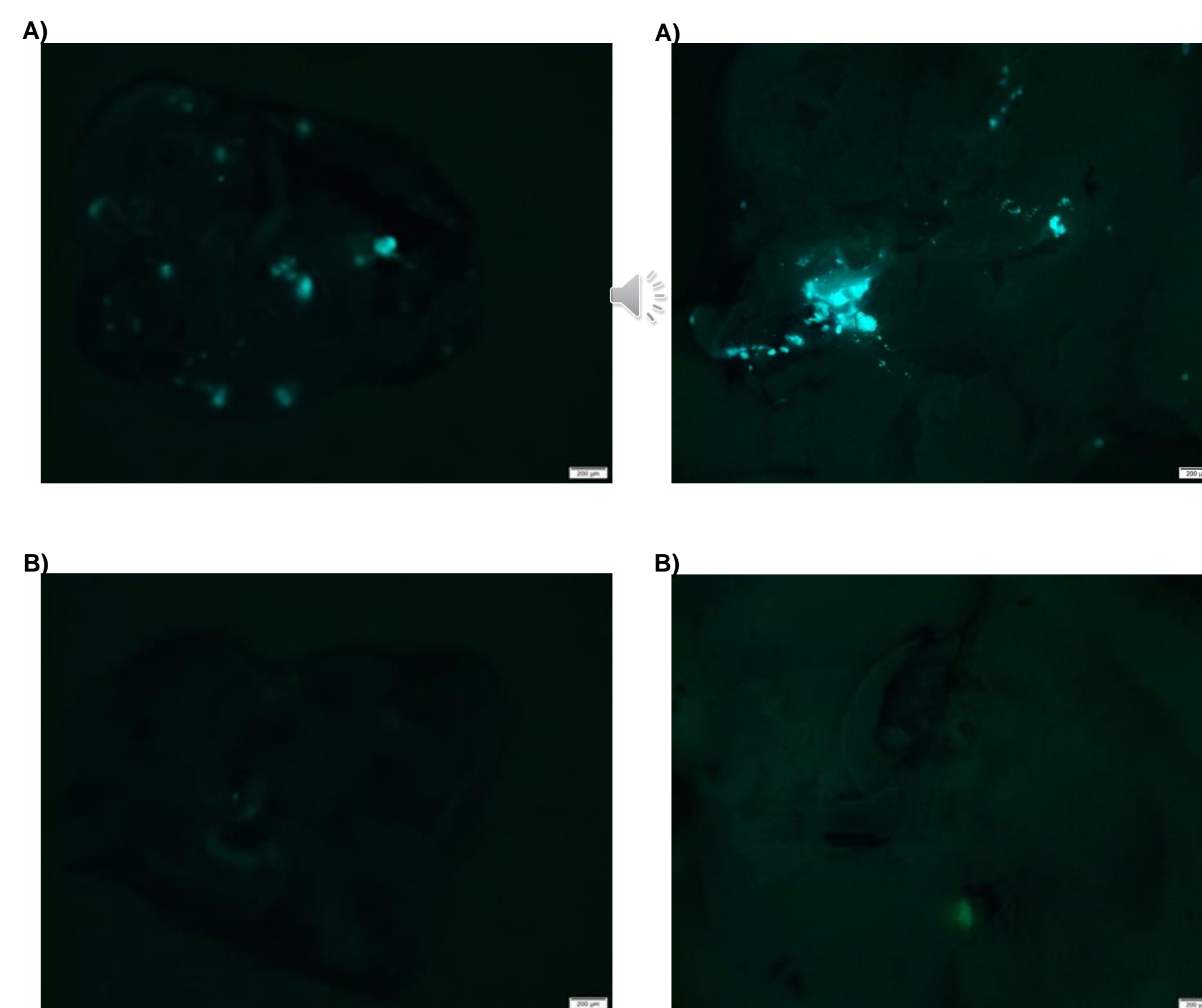
### Metastasis Analysis



## RESULTS

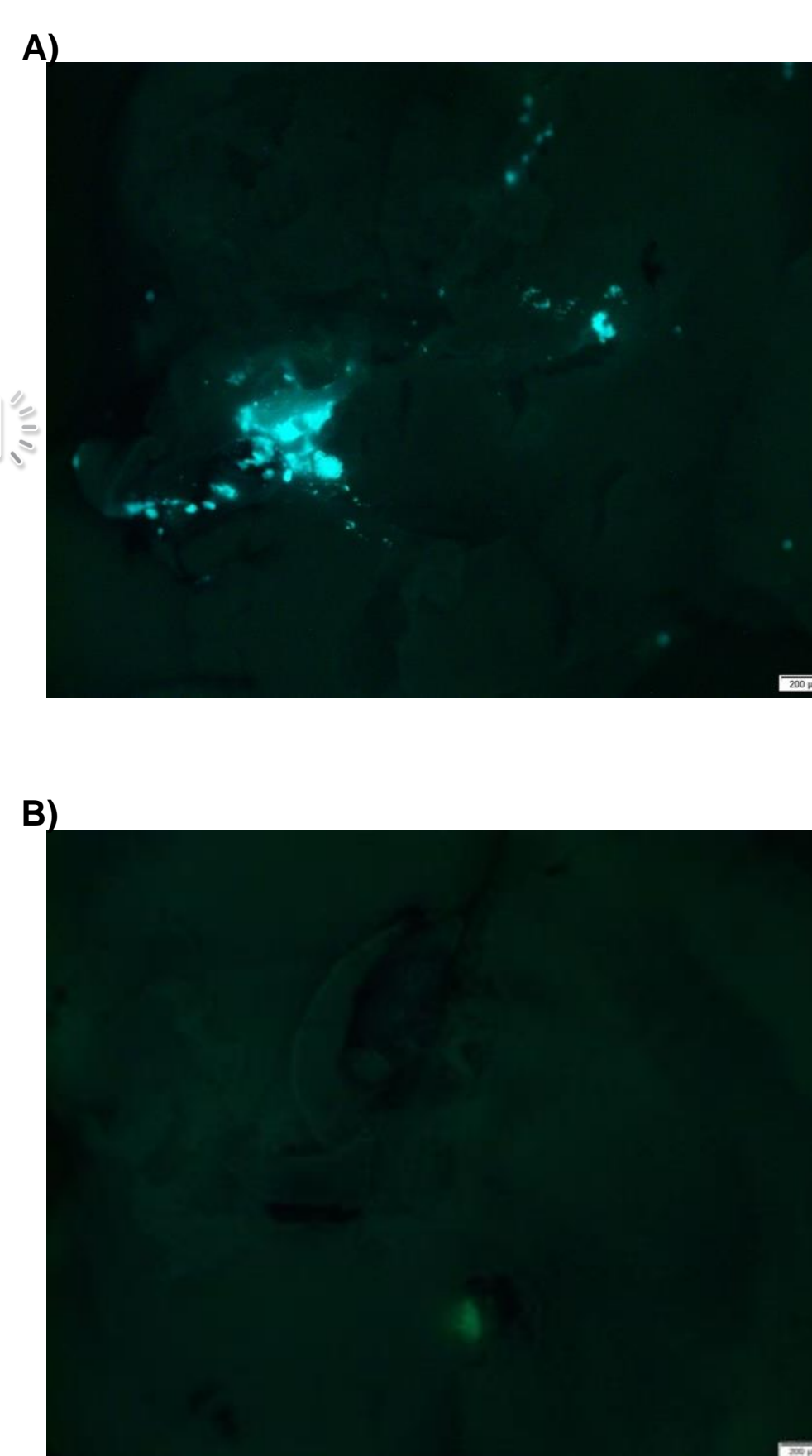
### Fluorescence Stereoscopy Images of Female SCID mice

### Lung Metastases



**Figure 4 | Lung images of EP-treated group revealed a significant decrease in the area and integrated density of metastatic foci. A)** Vehicle group with fluorescent regions in the lung showed the presence of GFP-MDA-MB-231 cells, indicative of metastases. **B)** Image of the experimental group lung display reduced fluorescent areas.

### Liver Metastases

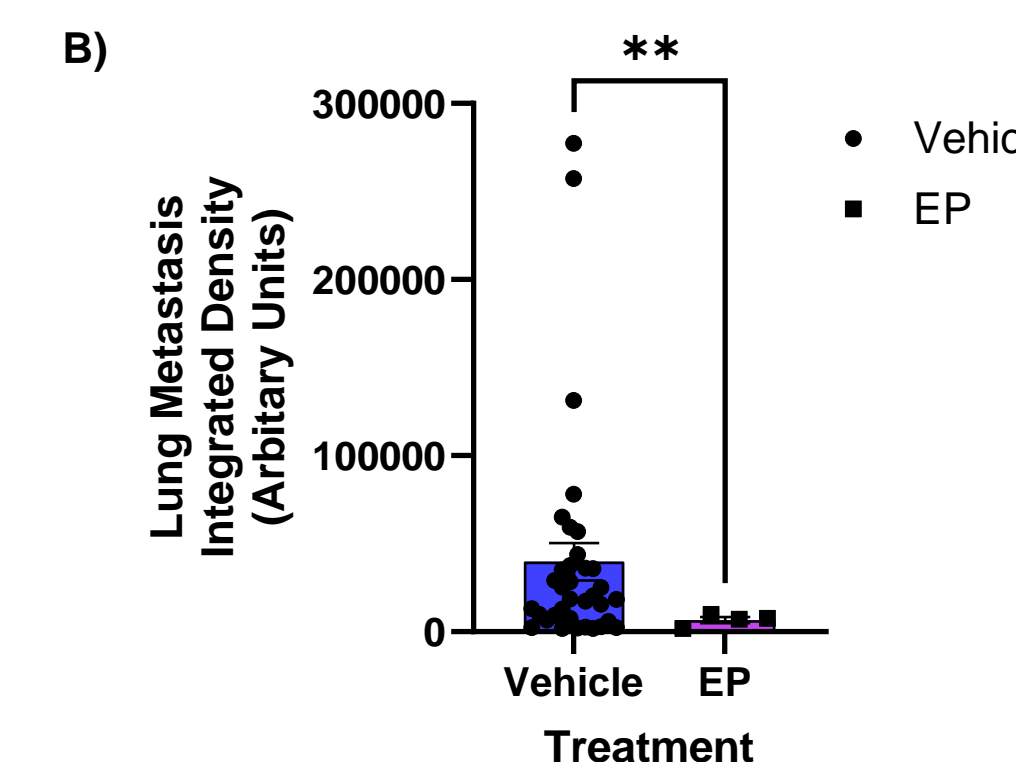
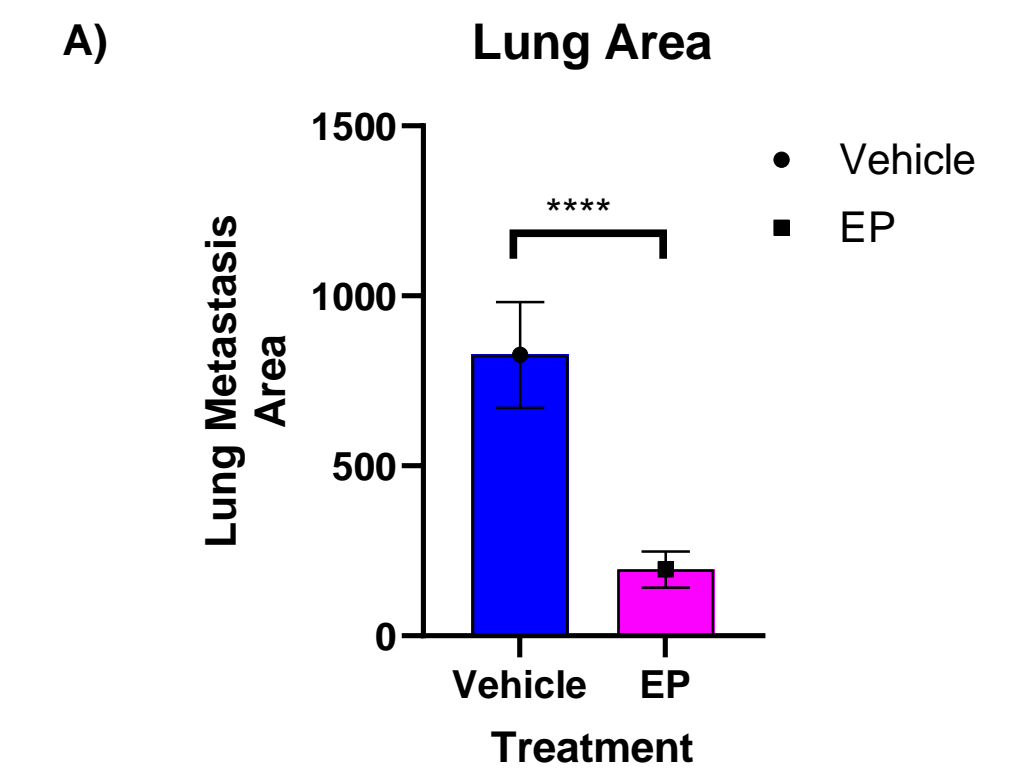


**Figure 5 | Liver images of EP-treated group demonstrate a significant decrease in both the area and integrated density of metastatic foci. A)** Vehicle group with fluorescent regions in the liver image denote the presence of GFP-MDA-MB-231 cells, indicating metastases. **B)** Image of the experimental group displays reduced fluorescent areas.

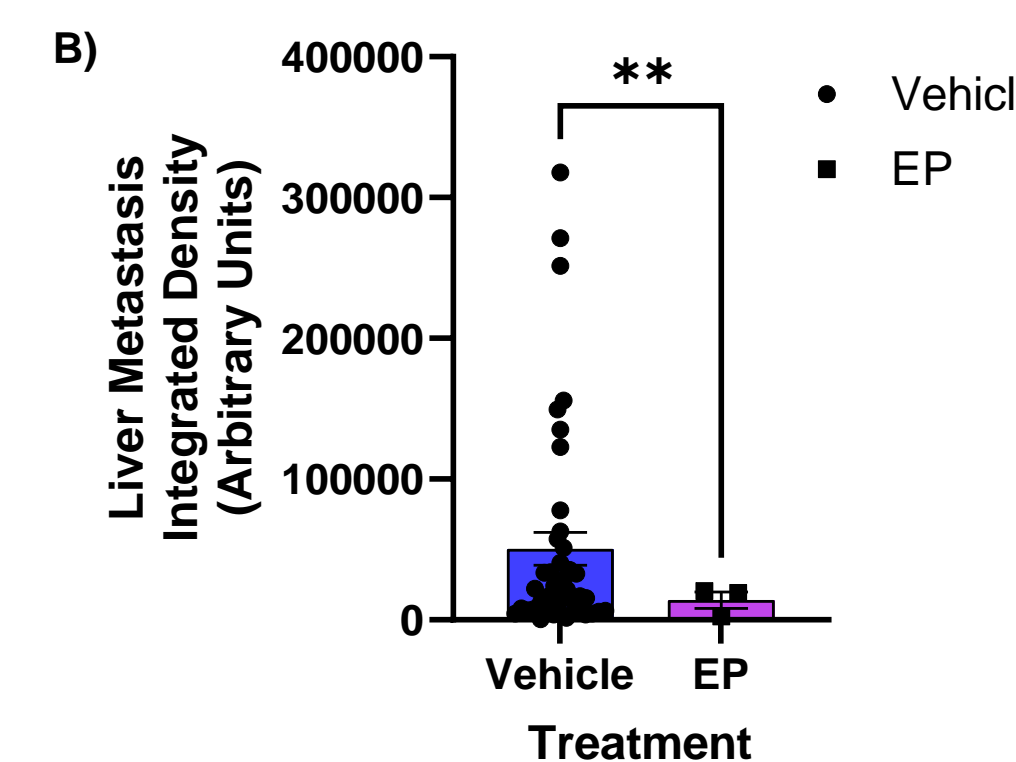
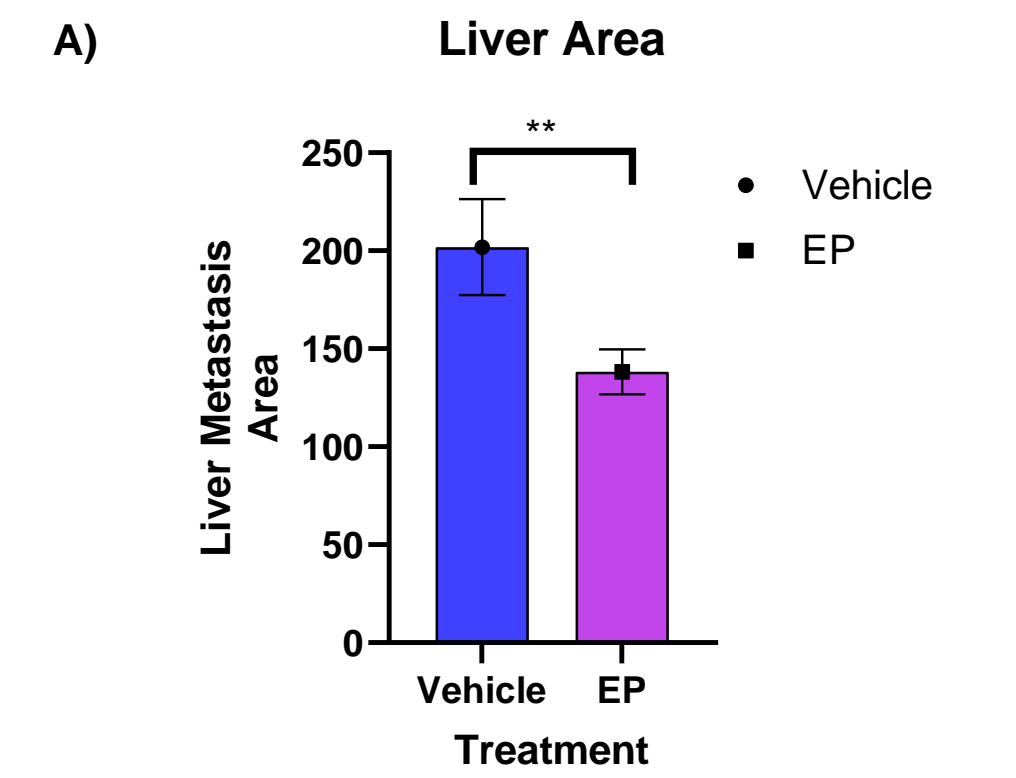
## RESULTS

### Statistical Analysis: Poisson Regression Model

### Lung Metastases



### Liver Metastases



**Figure 6 | Lung images of EP-treated group reveal a significant decrease in the area and integrated density of metastatic foci. A)** Data analysis reveals a highly significant reduction of the lung metastatic foci area (mm<sup>2</sup>) in the EP-treated group. **B)** Biostatistical analysis of the lung metastatic foci integrated density (AU) demonstrates a significant reduction in the EP-treated group compared to the vehicle group.

**Figure 7 | Liver images of EP-treated group demonstrate a significant decrease in both the area and integrated density of metastatic foci. A-B)** Data analysis reveals a significant reduction of the liver metastatic foci area (nm<sup>2</sup>) and integrated density (AU) in the EP-treated group.

## CONCLUSIONS

- Results evidence a highly significant ( $P<0.001$ ) decrease in the metastasized tumor area in EP treated mice compared to the vehicle group.
- These significant EP induced anti-metastasis results were seen for the metastatic foci area in the lung and liver.
- Also, results of metastatic foci integrated density demonstrate a significant reduction in the EP-treated group compared to the vehicle group in both organs.
- In general, the study suggests the treatment with EP has potential anti-metastasis effects which increases the potential of EP as a potent anti-cancer agent.

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