

# Vimentin –A Novel Antimetastatic Target For Breast Cancer Metastasis

# Rationale

- Conventional cancer treatment
  - Targets the primary tumor site
  - Prevents replication, metabolism, signaling etc
- However, metastases are the cause of nearly all cancer deaths in nearly all types of cancers
- So why not target the metastases?

# Chemoprevention

## An anti-metastatic chemopreventative

- Prevent metastasis from ever occurring in high-risk metastatic patients

## What would be the target?

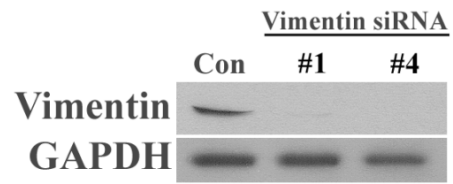
- All metastases have one attribute in common– the ability to move
- Can we precisely target the “movement machinery” but not target anything else

# Vimentin- an antimetastatic target

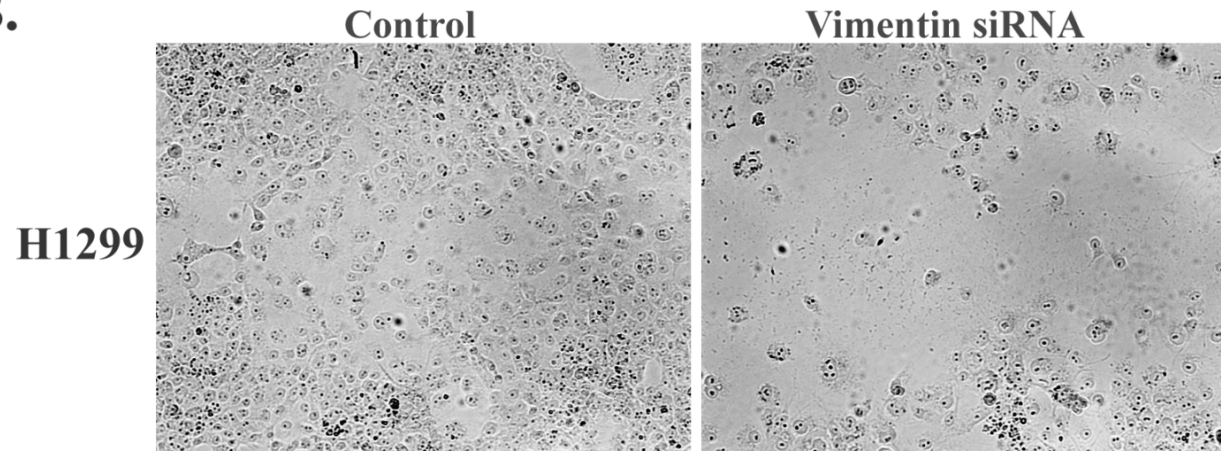
- Vimentin is a classical EMT marker and it is overexpressed in invasive cancers (1000+ publications) and is nearly undetectable in primary, non-aggressive tumors
- In almost all solid tumors increased vimentin correlates with poor prognosis, decreased survival, increased metastasis
- Observed in basic and pre-clinical models, and in the clinic
- It is essential for migration but does not appear to be essential for other cellular processes

# Vimentin is essential for migration

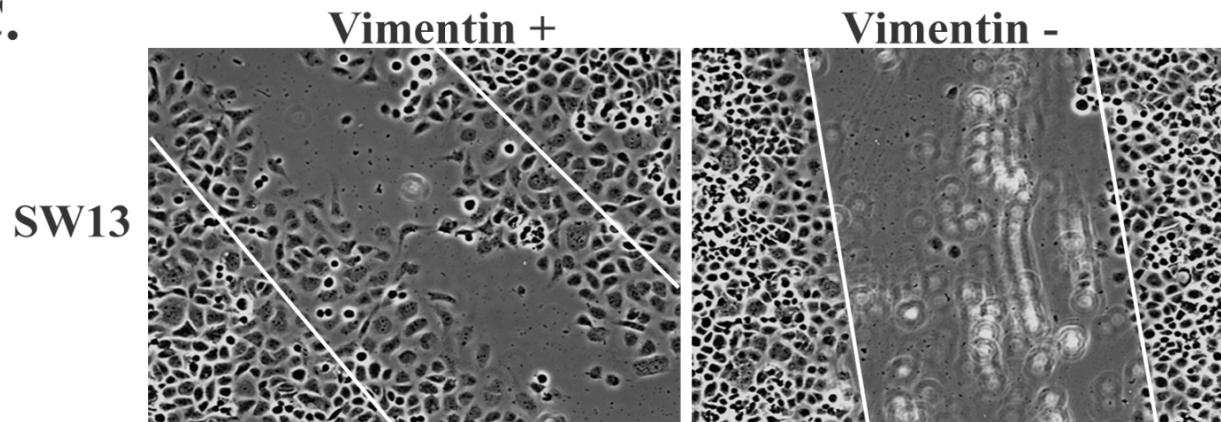
**A.**



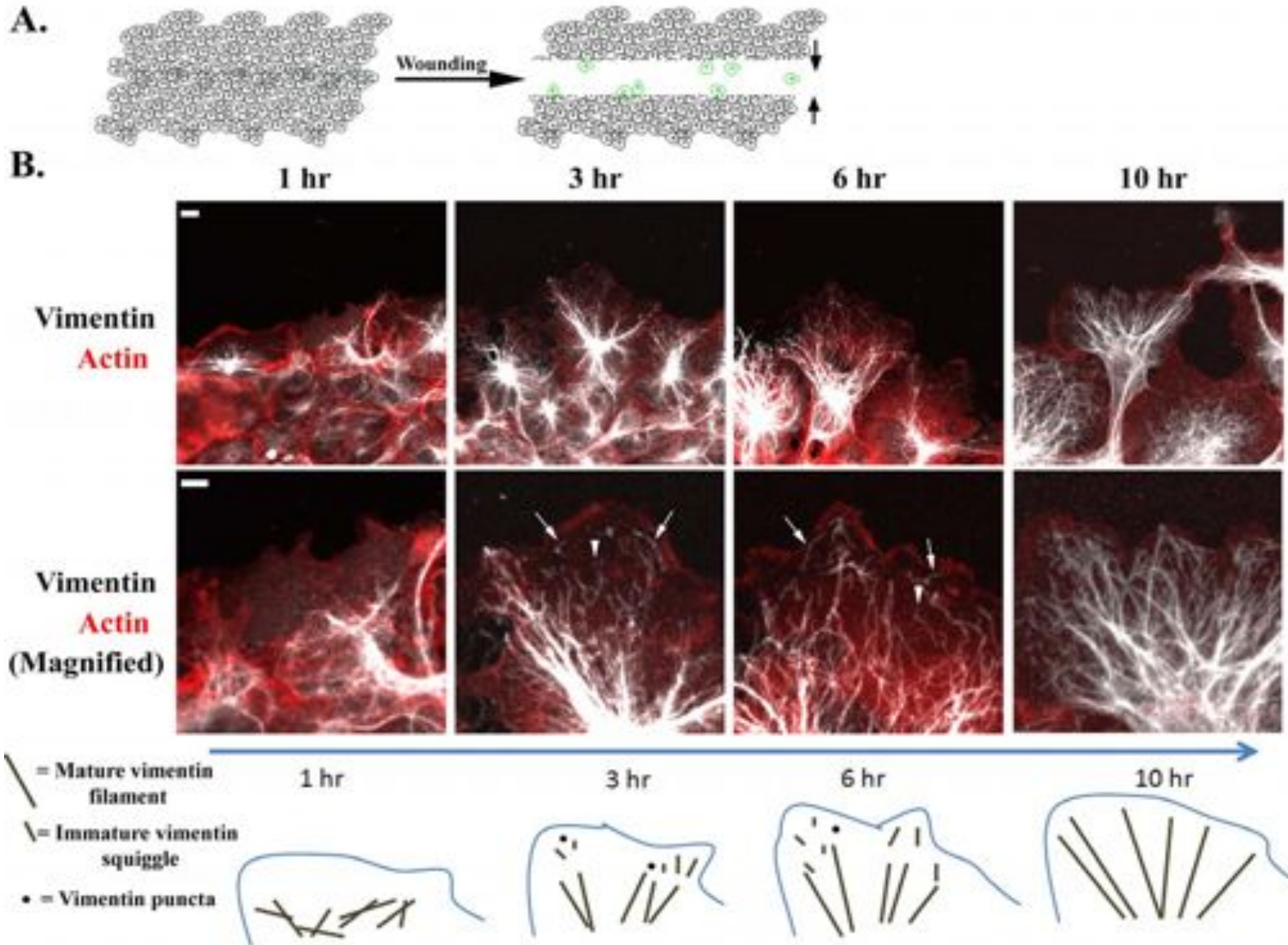
**B.**



**C.**

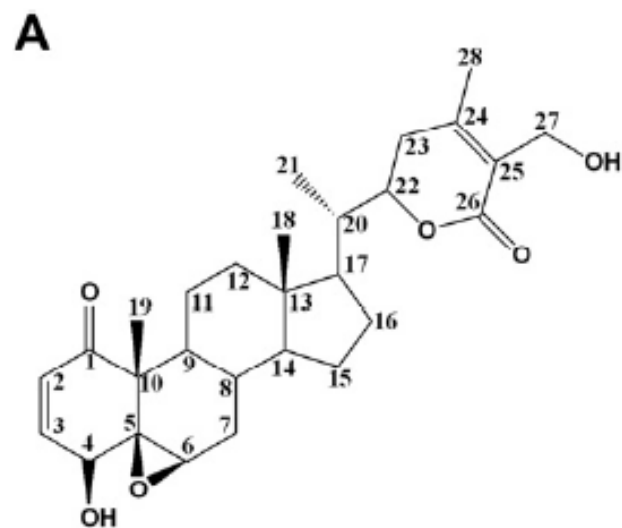


# Vimentin on the Move



# Withaferin A

- Derived from the root extract of *Withania somnifera*
- Directly binds to and targets vimentin (Bargagna-Mohan et al., Chemistry and Biology, 2007)

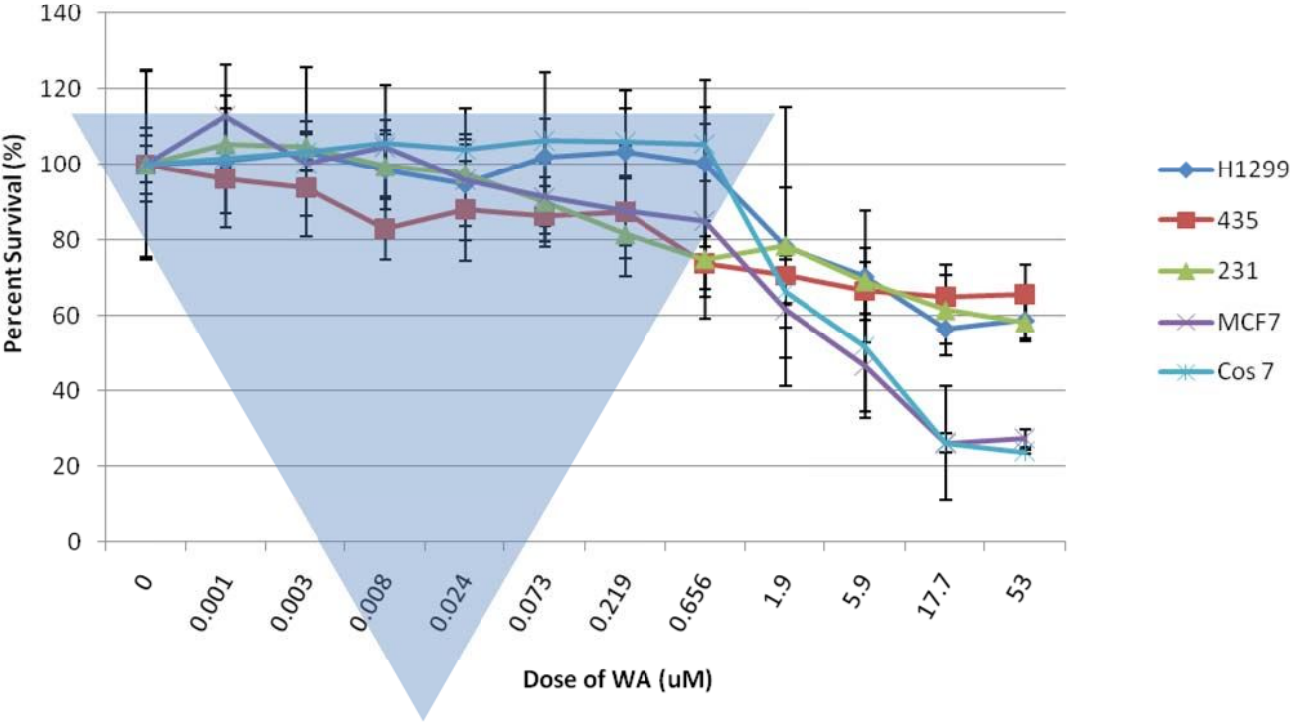


**Withaferin A (WEA)**

1

# Cell Proliferation Assay

## Withaferin A

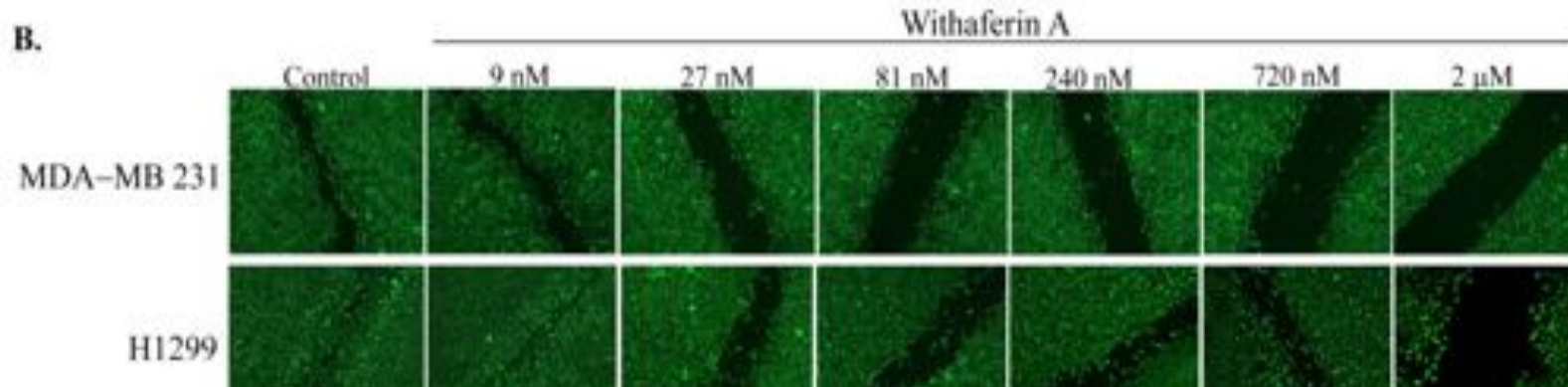


Does not kill cancer cells or normal cells

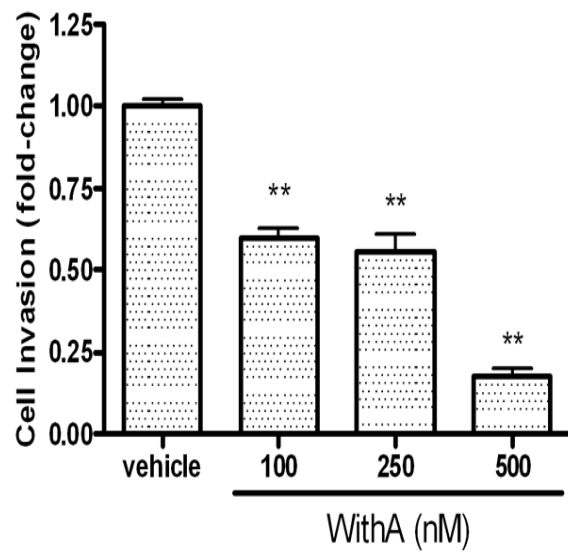
# Cancer cell migration model



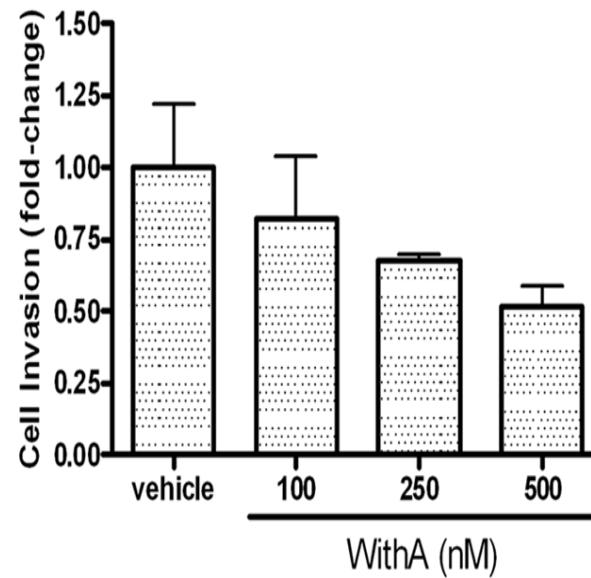
# Cancer Cell Lines



**MDA-MB 231**



**H1299**



# Live cell imaging of Withaferin treated cells

Control



Withaferin A 500 nM

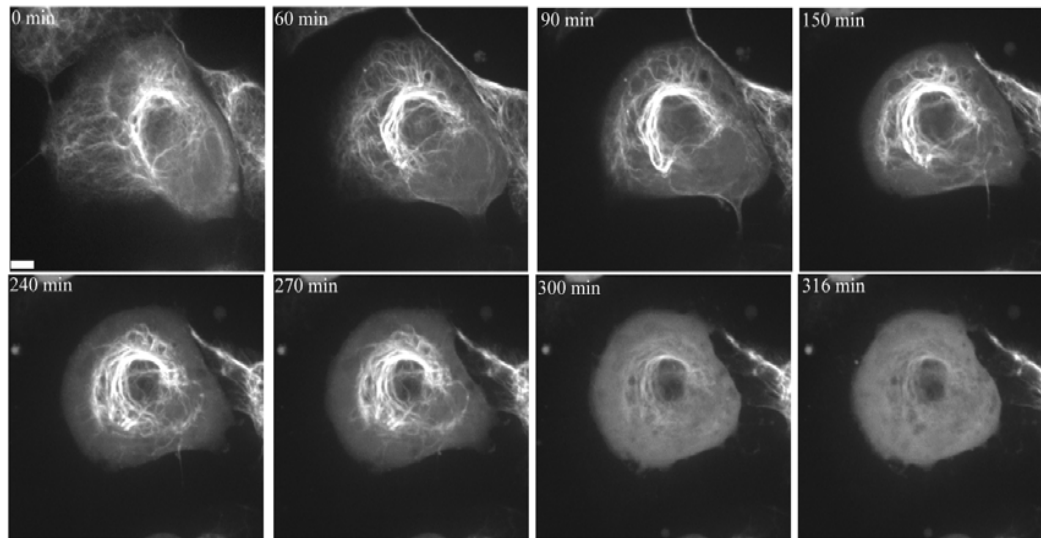


How does Withaferin A perturb  
vimentin function?

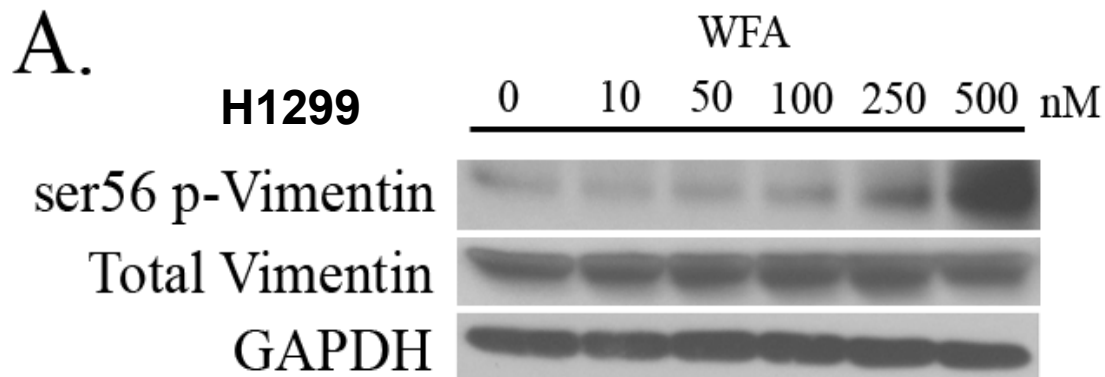
# Live imaging of Withaferin A targeting vimentin



# Live cell imaging of GFP vimentin



## ser56 hyper-phosphorylation

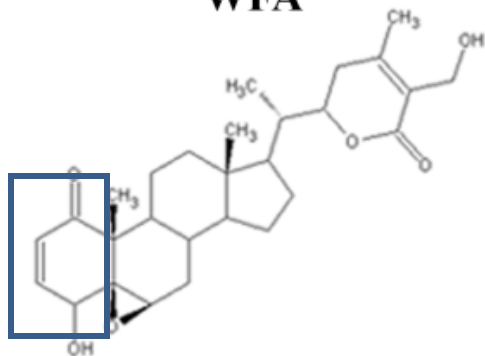


# Structure/Function studies

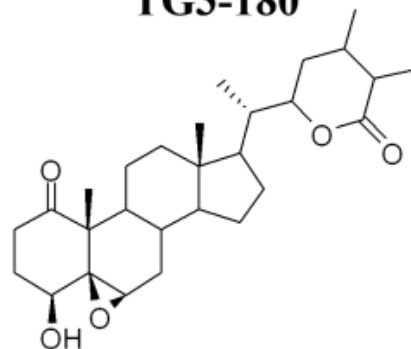
# 'A' ring modified compounds

A.

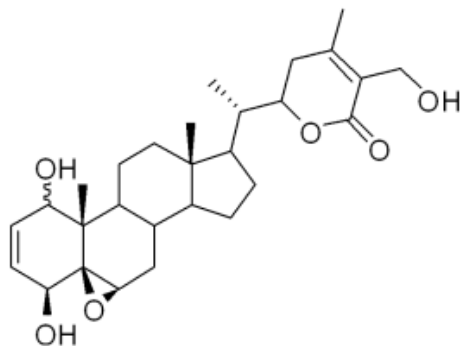
**WFA**



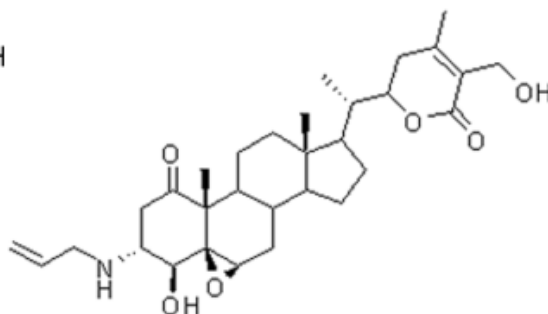
**TG3-180**



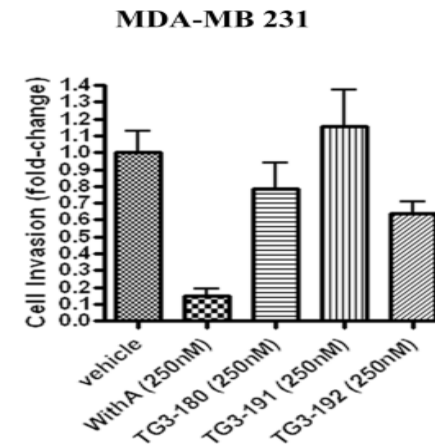
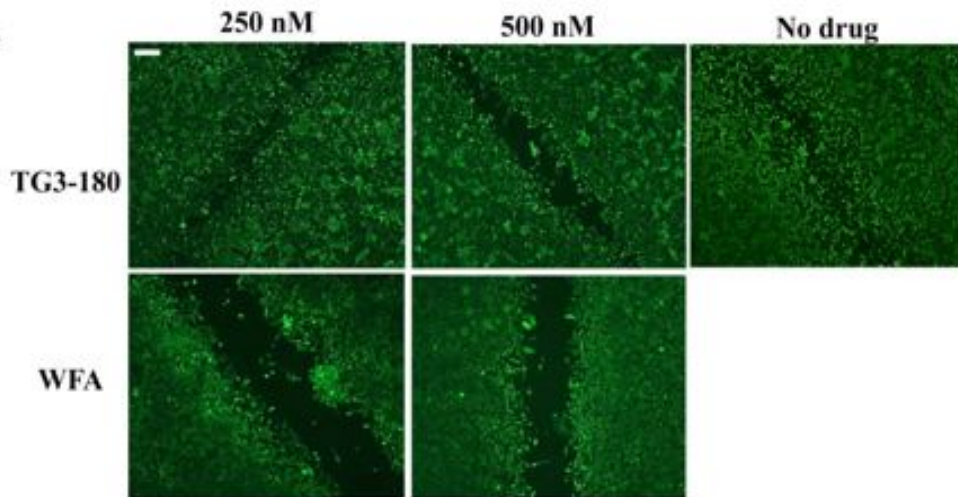
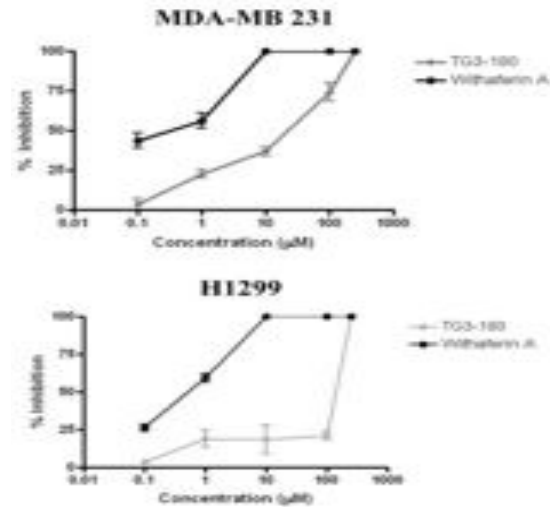
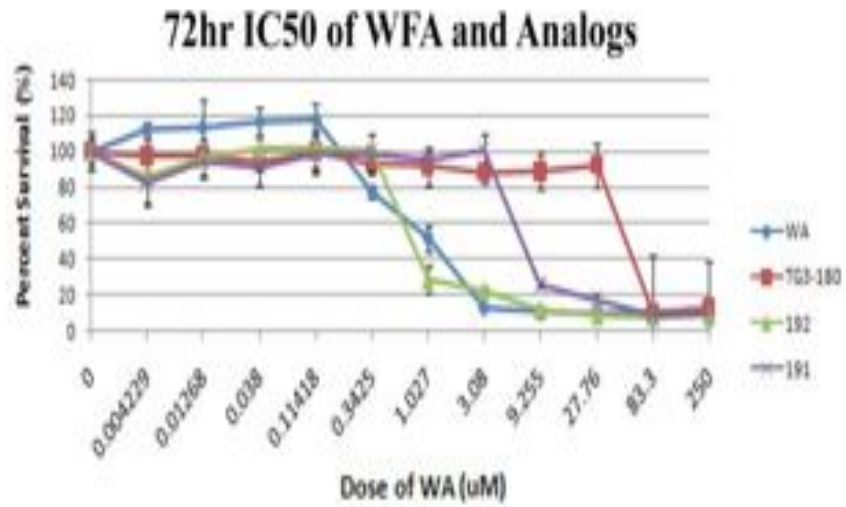
**TG3-191**



**TG3-192**



# Cancer cell migration and Invasion studies



# Anti-Metastatic in vivo Efficacy of Withaferin A

# 4T1 Breast Cancer Model

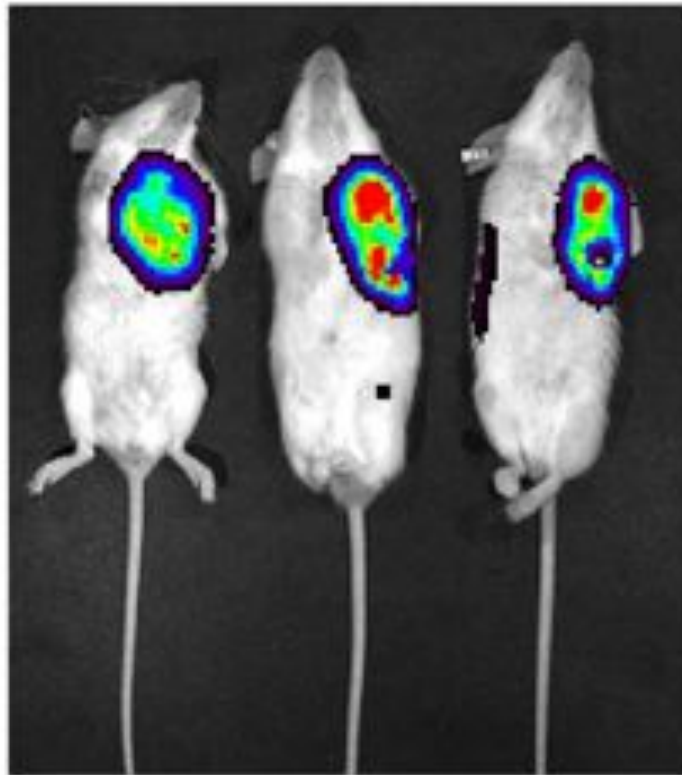
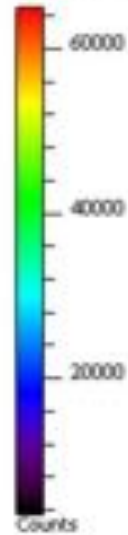
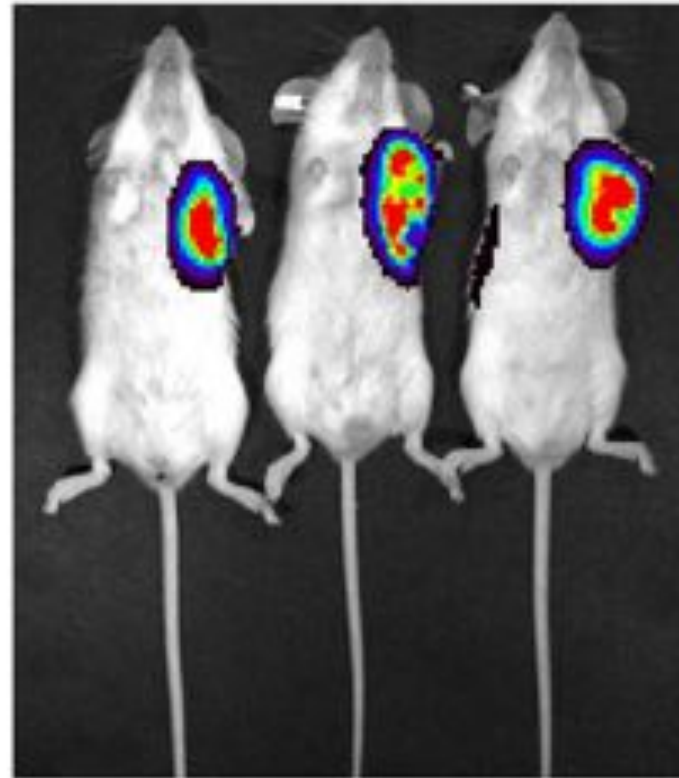


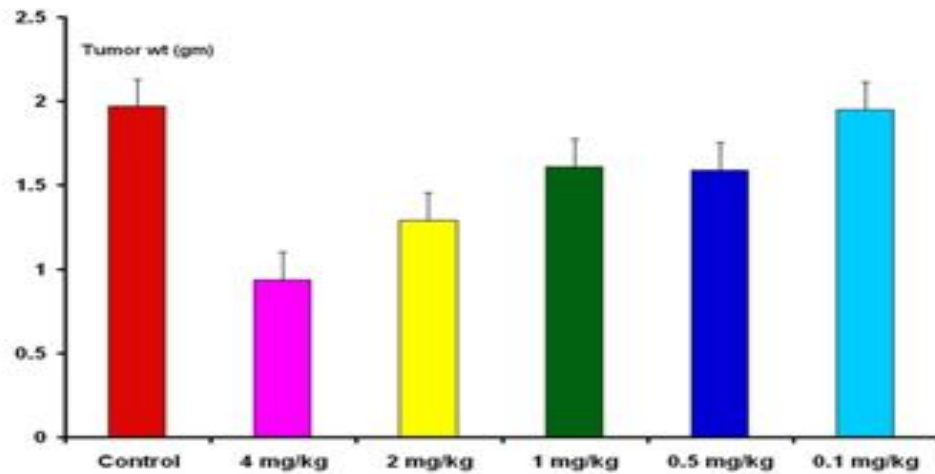
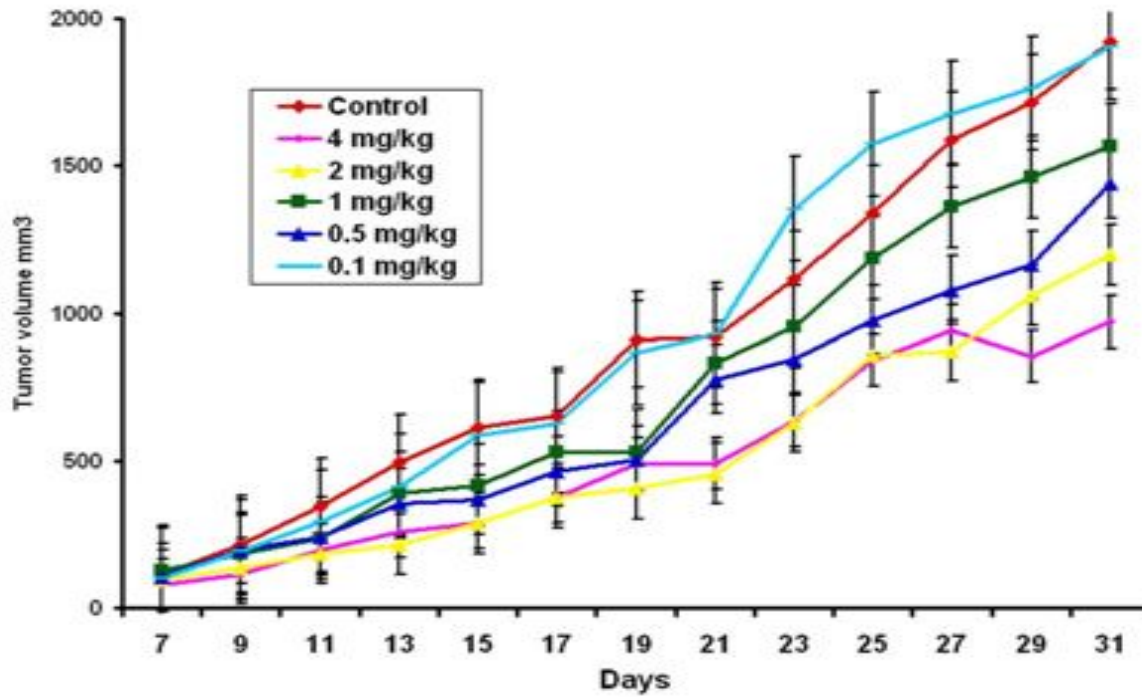
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Max = 69706



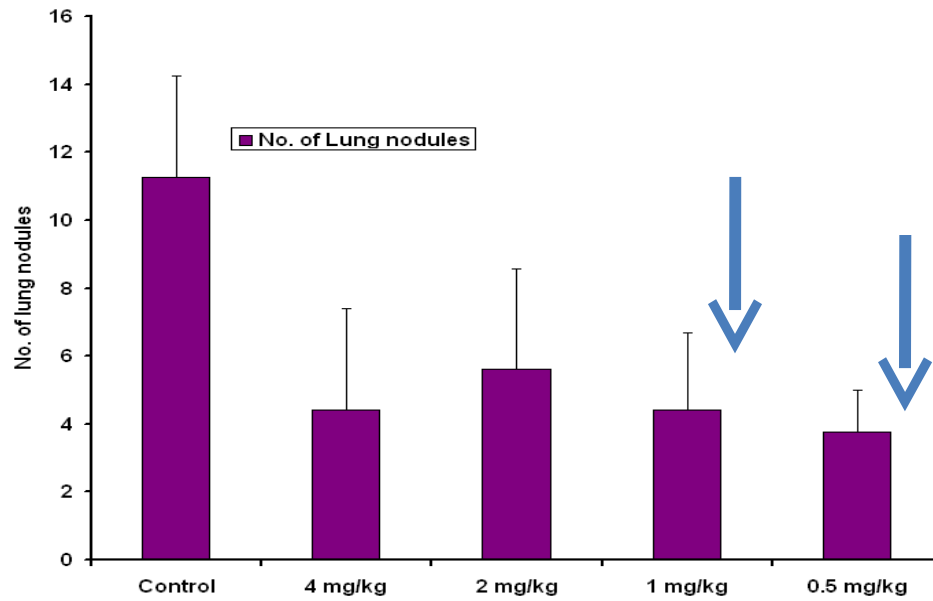
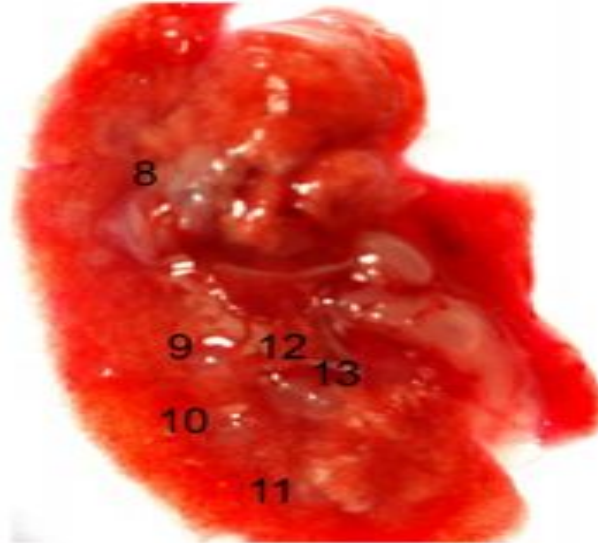
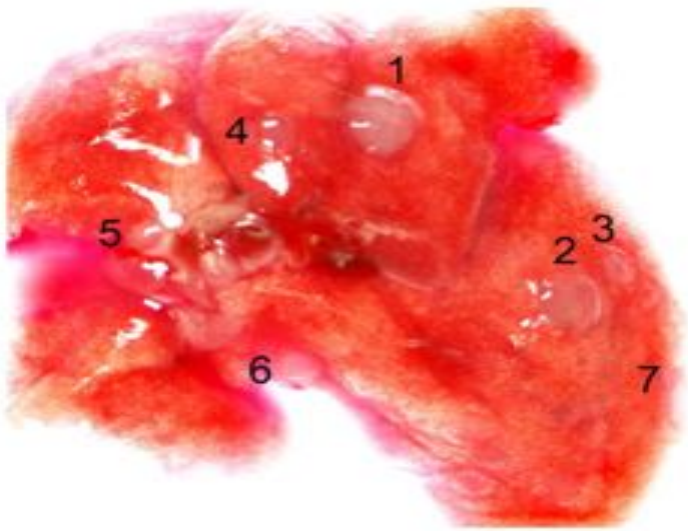
Color Bar  
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Max = 64979



# Animal Therapeutic Data



# Lung nodules



# Conclusions

- Withaferin A can inhibit cancer cell migration and invasion, and has minimal effect on proliferation
- Withaferin A disrupts vimentin leading to hyperphosphorylation of ser56
- Analogs show that the predicted vimentin binding region is essential for activity
- Withaferin A is an *in vivo* anti-metastatic with minimal effect on primary tumor growth at the current dosage schedule

# Acknowledgements

- **Adam Marcus-PhD**      **Department of Chemistry**
- Laura Bender-PhD
- Erik Kline- PhD
- Carrie Eggers
- Katherine Hales
- **Dennis Liotta-PhD**
- James Snyder-PhD
- Thota Ganesh-PhD

## Funding

Godfrey Foundation

American Cancer Society

Georgia Cancer Coalition