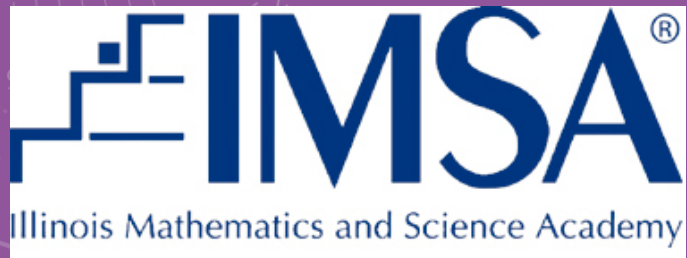




NORTHWESTERN  
UNIVERSITY



# THE DEVELOPMENT OF A HUMANIZED ANTIBODY-TARGETED ABO-SPECIFIC PET PROBE FOR EARLY DIAGNOSTIC IMAGING OF ALZHEIMER'S DISEASE

Adrian Bebenek

Mentors: Ms. Kirsten Viola and Dr. William Klein

Kirsten L. Viola<sup>1</sup>, Adrian Bebenek<sup>1</sup>, Jake Vitrofsky<sup>1</sup>, Maira Bicca<sup>2</sup>, Erika N. Cline<sup>1</sup>, Alex L. Qin<sup>1</sup>, Chad Haney<sup>1</sup>, Ting-Tung Chang<sup>3</sup>, and William Klein<sup>1, 4</sup>

<sup>1</sup> Northwestern University

<sup>2</sup> Universidade Federal de Santa Catarina

<sup>3</sup> Rethink Imaging

<sup>4</sup> Acumen Pharmaceuticals

# TOPICS

- Topic 1 – General Background
- Topic 2 – Molecular Basis
- Topic 3 – Design of PET Probe
- Topic 4 – Preservation of Immunoreactivity
- Topic 5 – NU4PET Preliminary Injections
- Topic 6 – NU4 Histology and Morphology
- Topic 7 – ACU193 Histology and Morphology
- Topic 8 – ACU193PET Preliminary Injections

# BACKGROUND



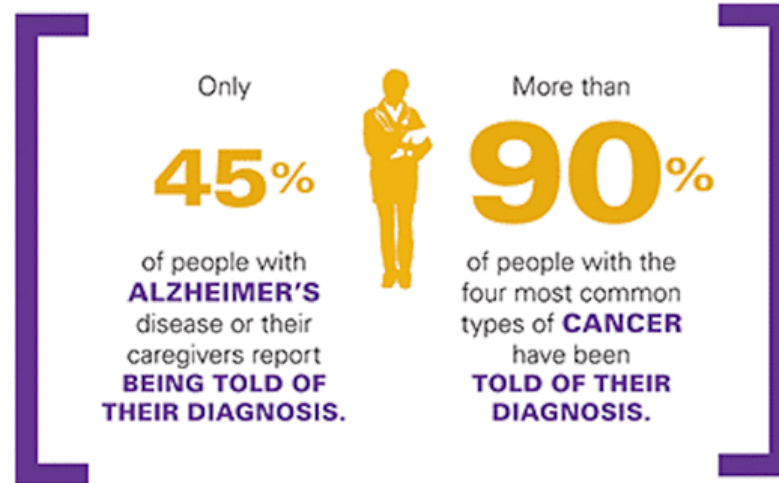
It's the only cause of death in the top 10 in America that **CANNOT BE PREVENTED, CURED OR SLOWED.**



**ALMOST TWO THIRDS** of Americans with Alzheimer's disease are women.



**SENIORS** dies with Alzheimer's or another dementia.



By 2050, these costs could rise as high as **\$1.1 TRILLION.**

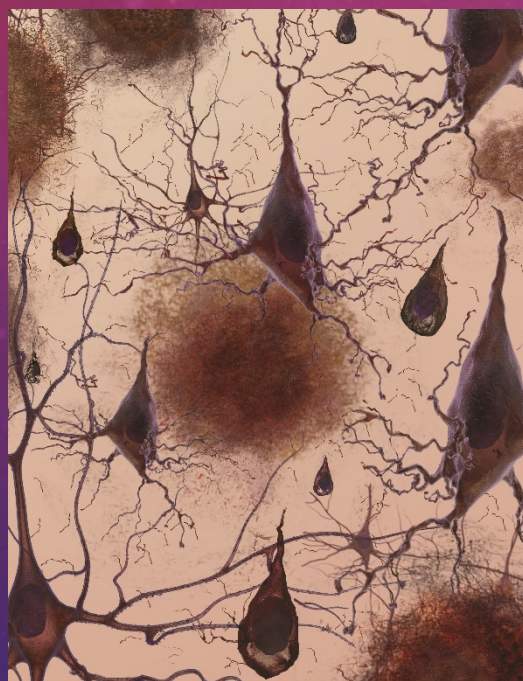
↑

In 2015, Alzheimer's and other dementias will cost the nation **\$226 BILLION.**

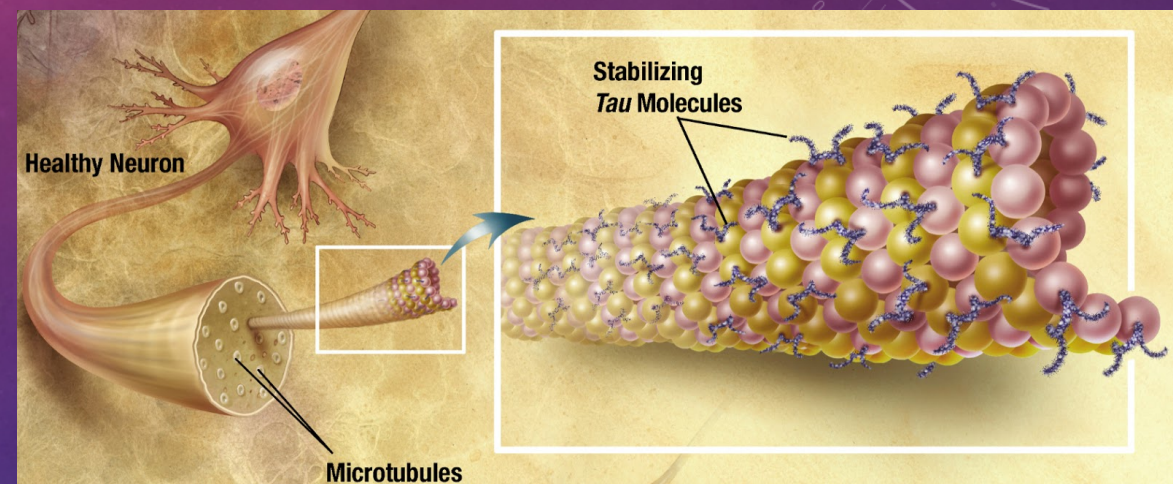
Alzheimers Association 2015

# MOLECULAR BASIS

- Amyloid- $\beta$  plaques
- Neurofibrillary tangles



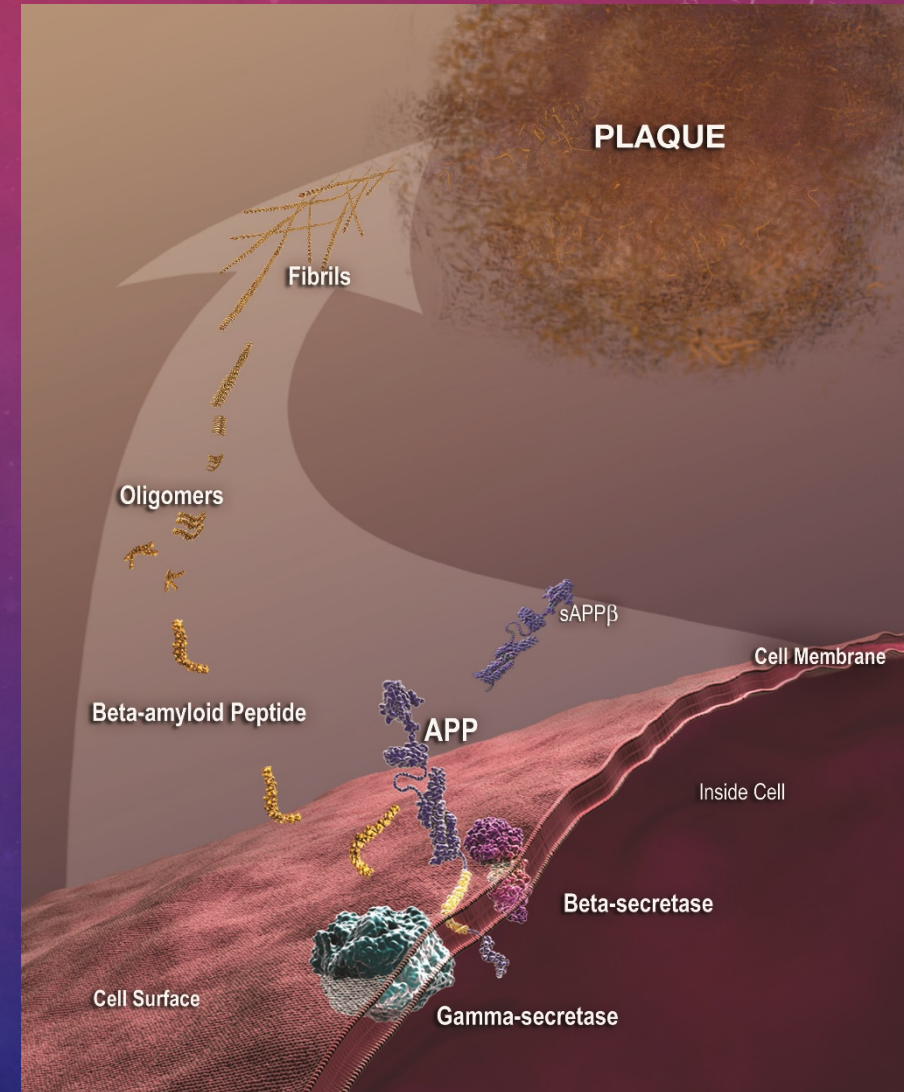
PBS 2016



Lieff 2015

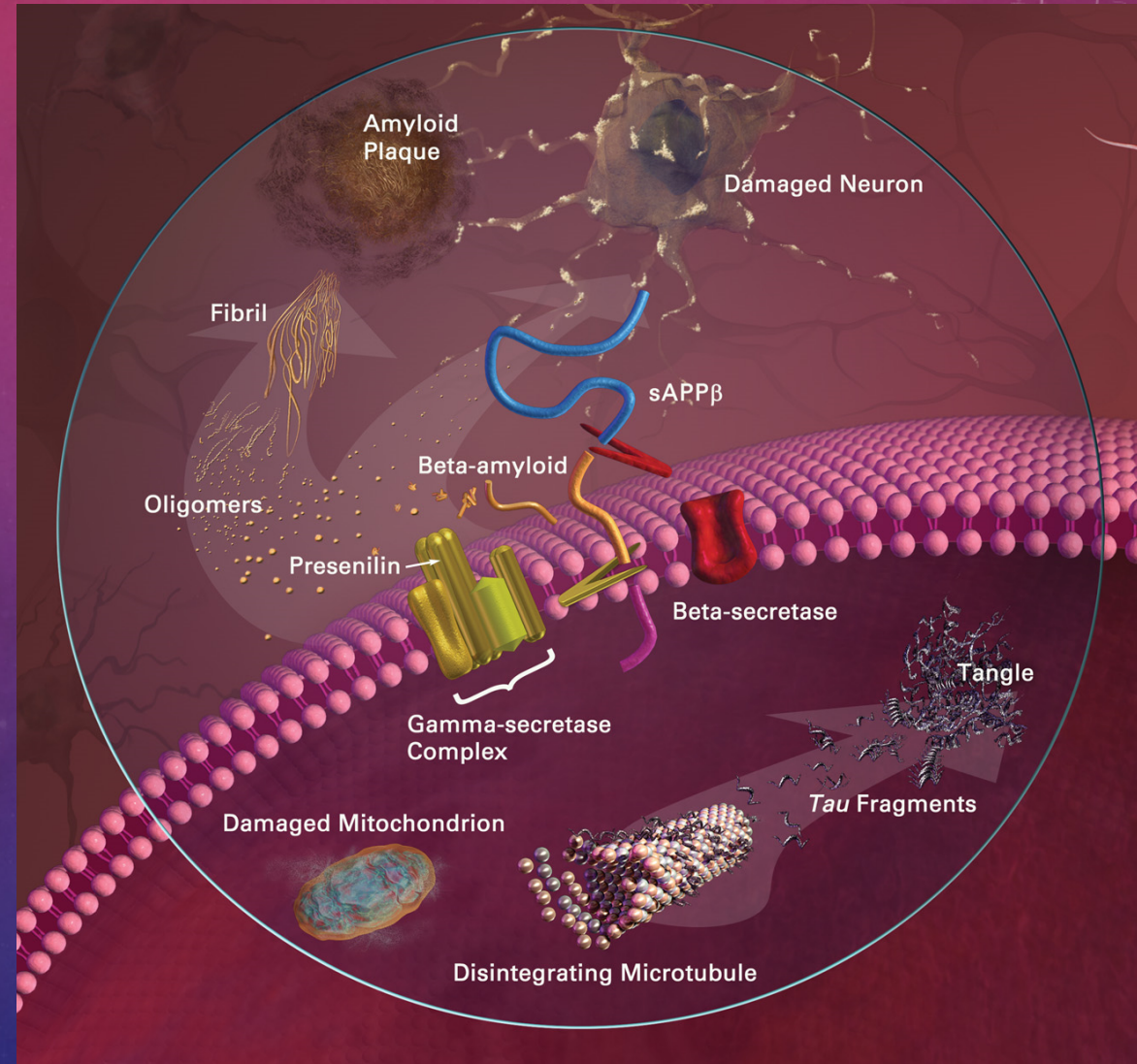
# AMYLOID BETA PLAQUES

- Amyloid Precursor Protein (APP)
- $A\beta_{42}$  vs  $A\beta_{40}$
- Peptide  $\rightarrow$  oligomer  $\rightarrow$  fibril  $\rightarrow$  plaque



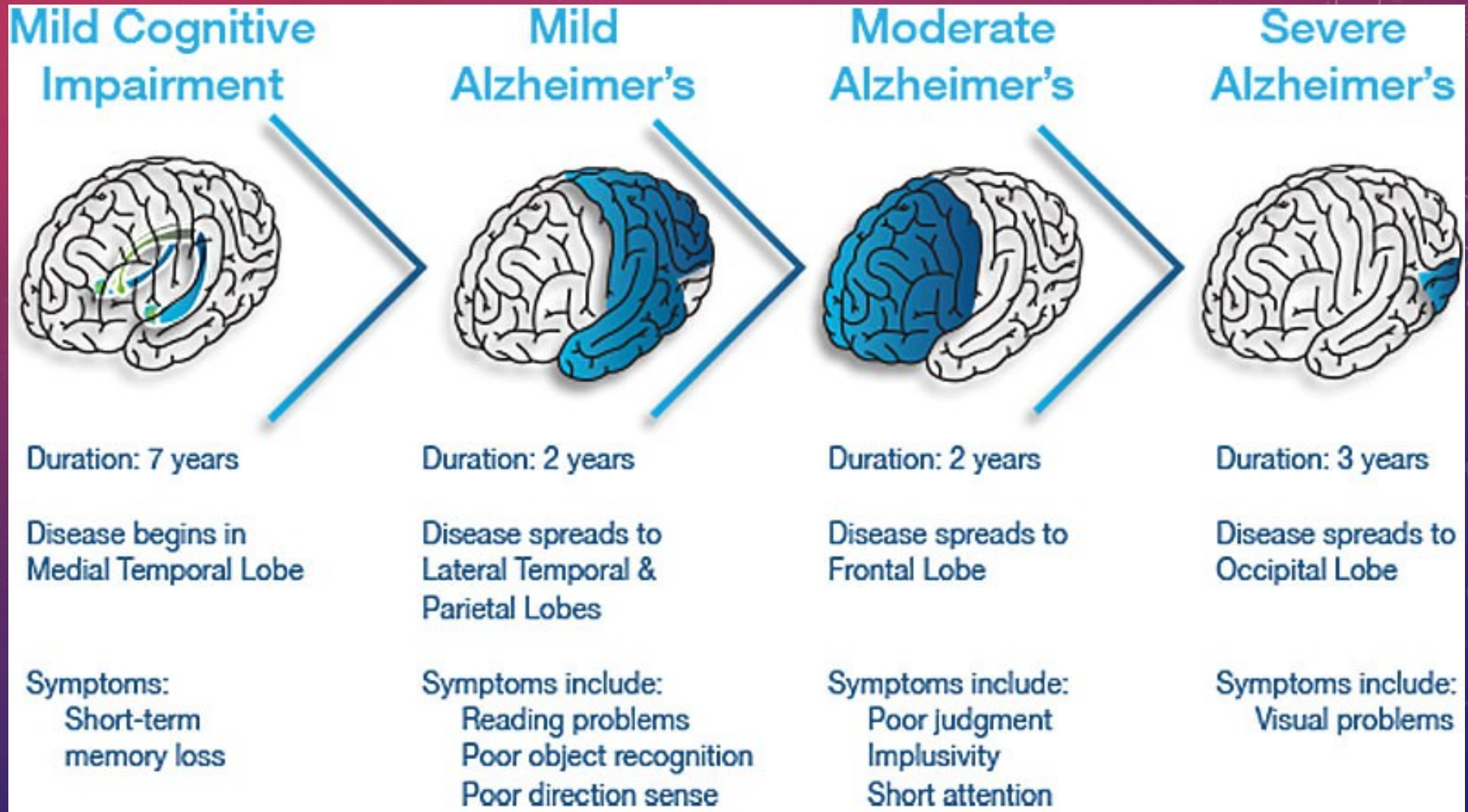
# AMYLOID CASCADE HYPOTHESIS

- Weak correlation between AD and plaques
- Amyloid- $\beta$  oligomers ( $A\beta$ O) initiate neurodegeneration



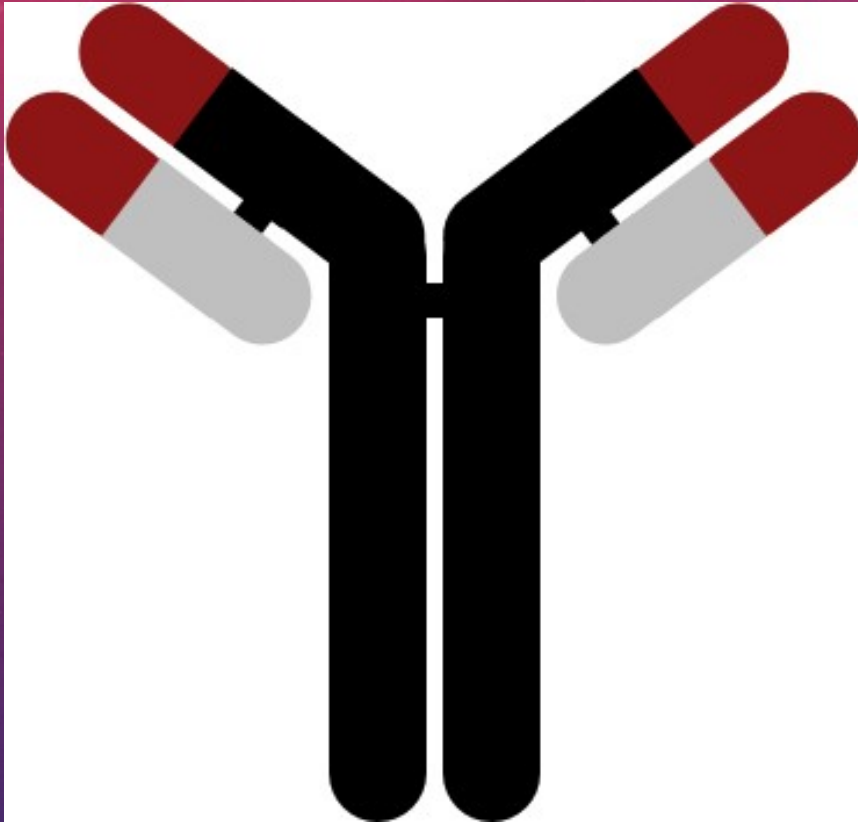
NIA 2016

# BACKGROUND



Medical Care Corporation  
2015

# ANTIBODIES



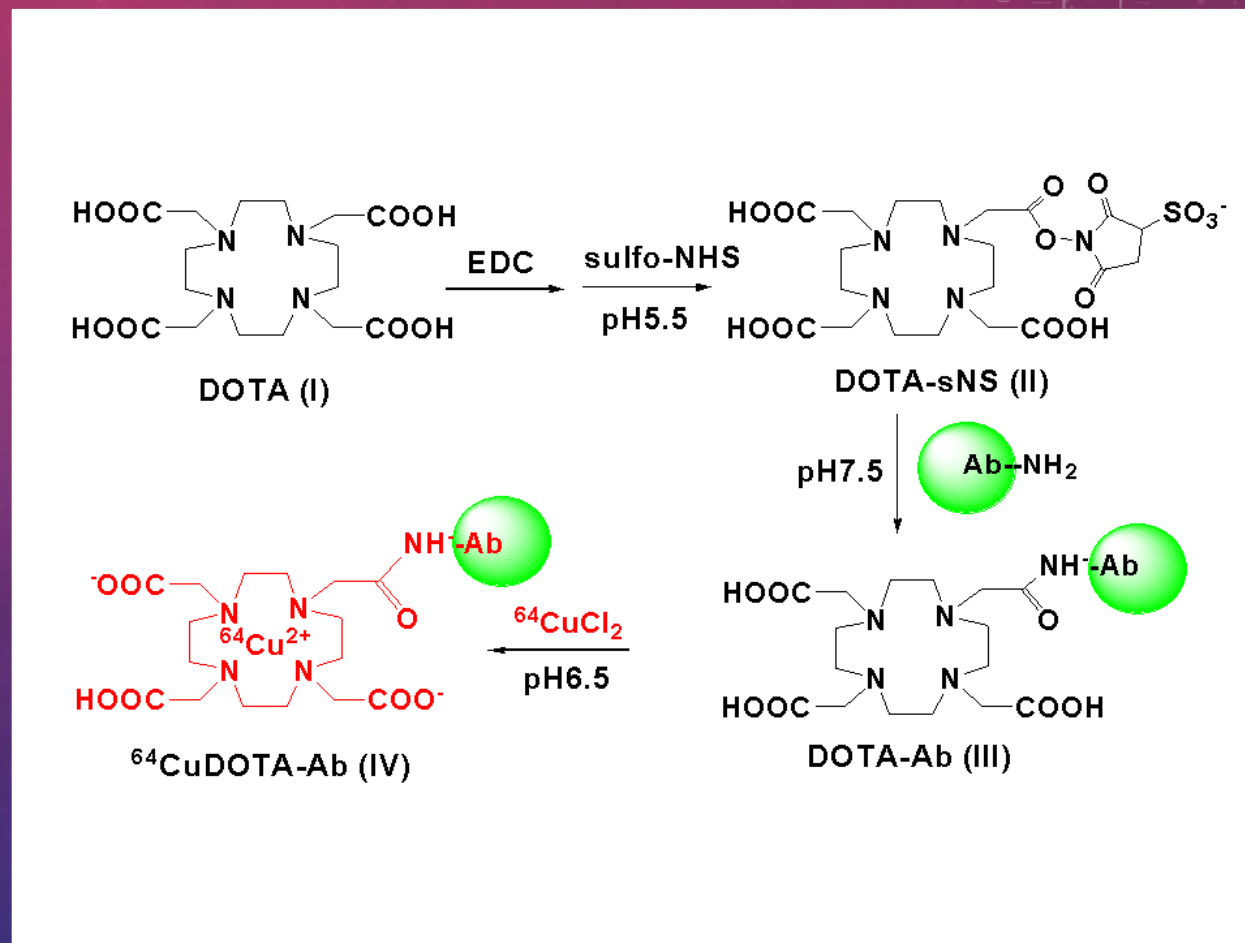
Cell Applications 2015

- Monoclonal
- NU4 - mouse
- ACU193 - human



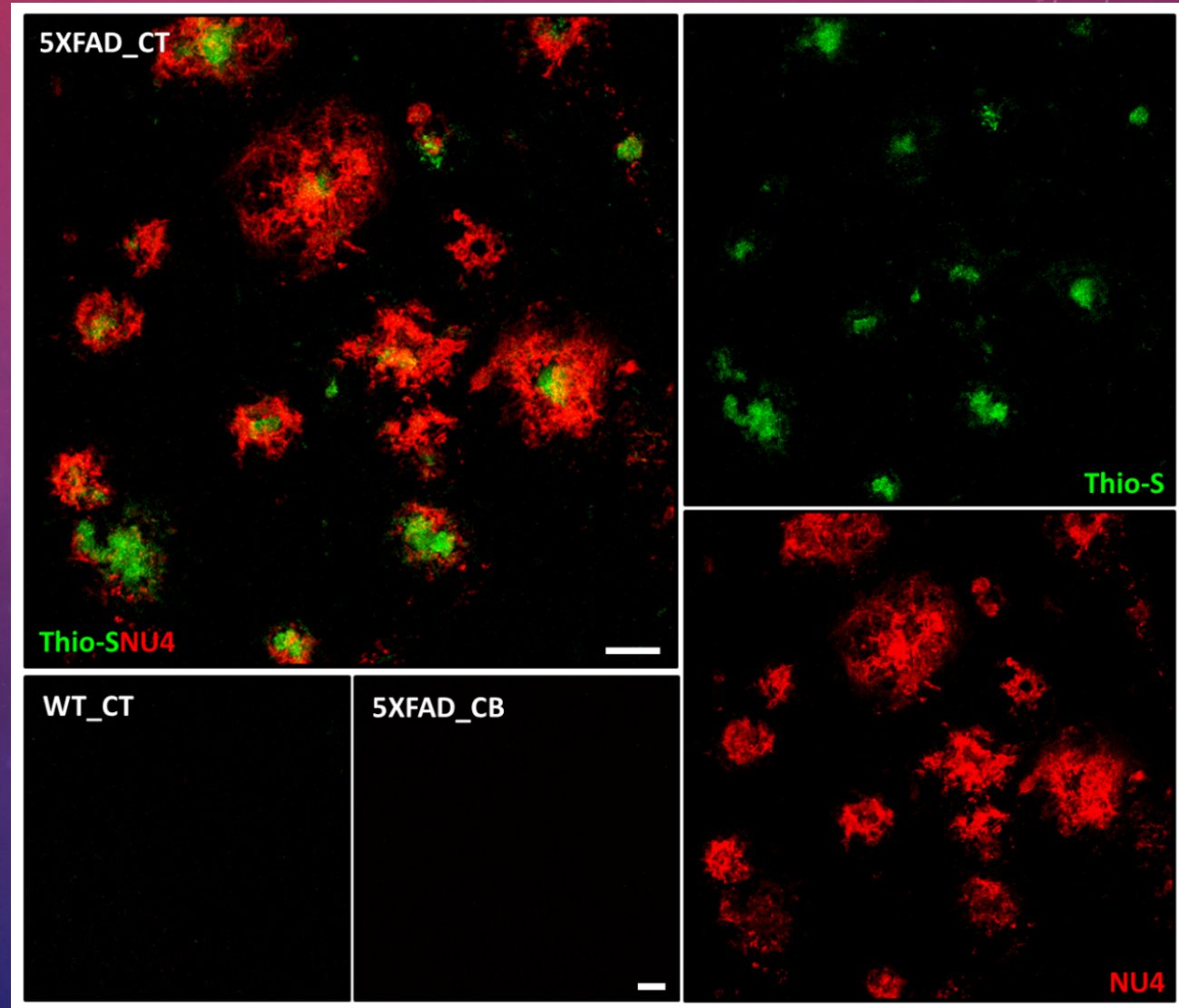
# PET SYNTHESIS

- PET uses radioactive ligand
- Conjugation of DOTA cage to antibody
- Chelation with radioactive compound/element ( $^{64}\text{Cu}$ )



# ABO ARE SEPARATE FROM PLAQUES

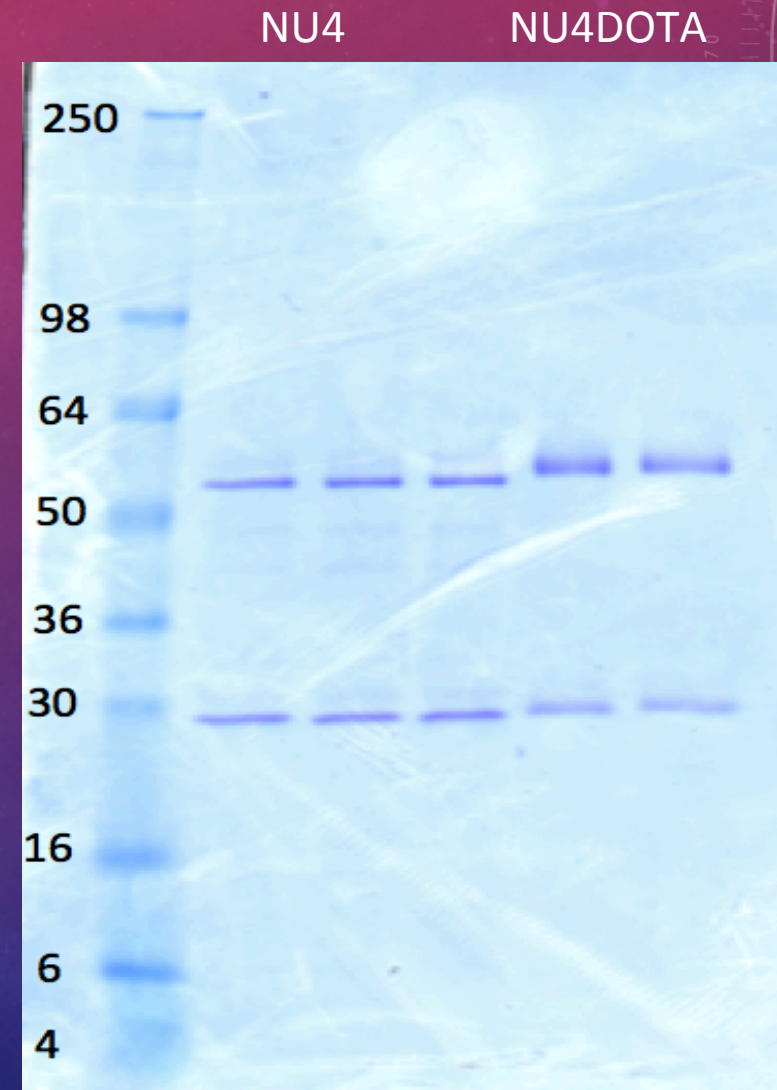
- Demonstrate that A $\beta$ O are separate from plaques
- Immunostained tg and wt mice with 568-NU4 (A $\beta$ O)
- Counterstained with ThioFlavin S (amyloid plaques)



Maira Bicca

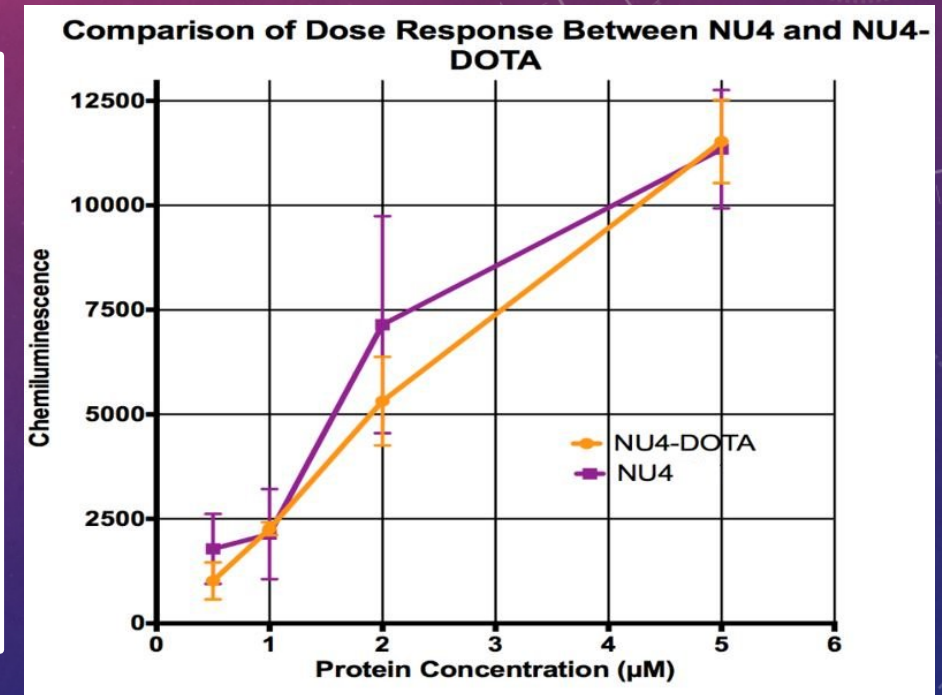
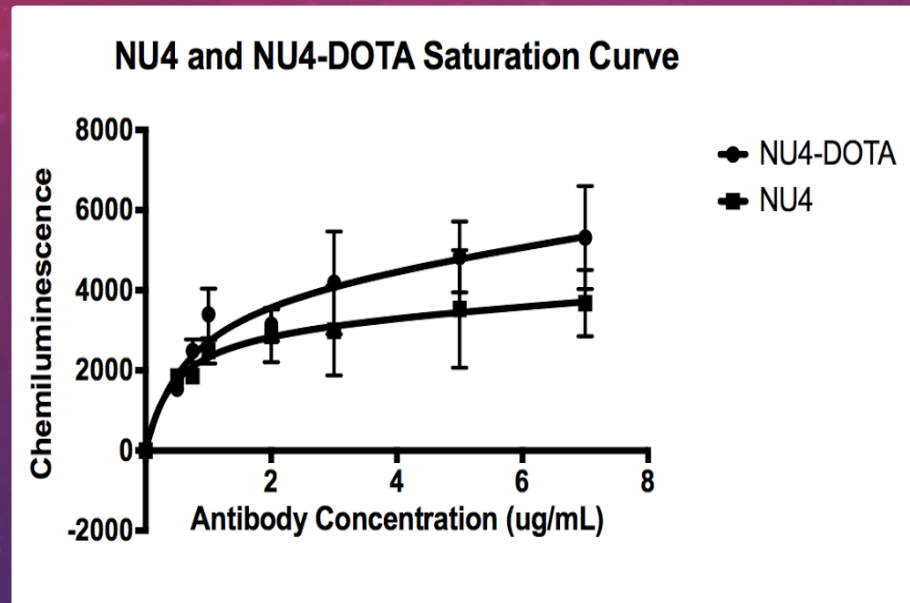
# DOTA BINDS TO THE FC REGION OF NU4

- Electrophoresis
- Confirm DOTA binds to constant region
- NU4: 54.091 kDa
- NU4-DOTA: 56.849 kDa
- 6-7 DOTA bound to heavy chain



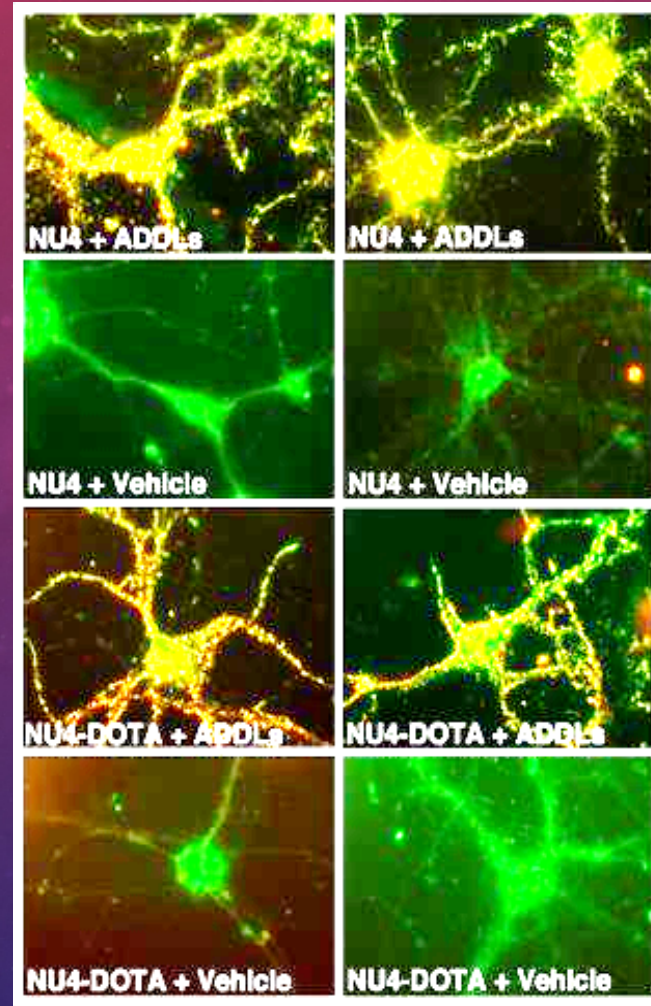
# NU4DOTA RETAINS IMMUNOREACTIVITY TO ABOS

- Dot blots
- $EC_{50}$
- No decrease in immunoreactivity



# NU4-DOTA COLOCALIZES WITH ABOS *IN VITRO*

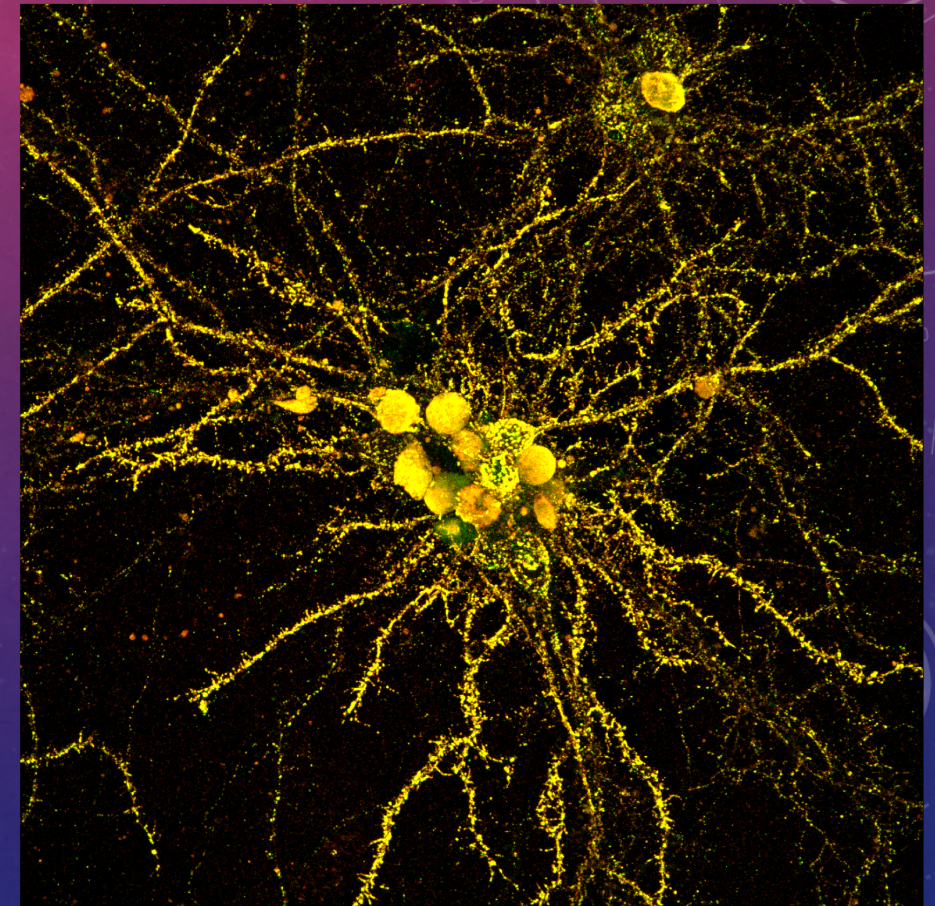
- NU4DOTA and FAM-A $\beta$ Os in primary hippocampal neuron culture
- High levels of colocalization
- Retained immunoreactivity *in vitro*



Maira Bicca

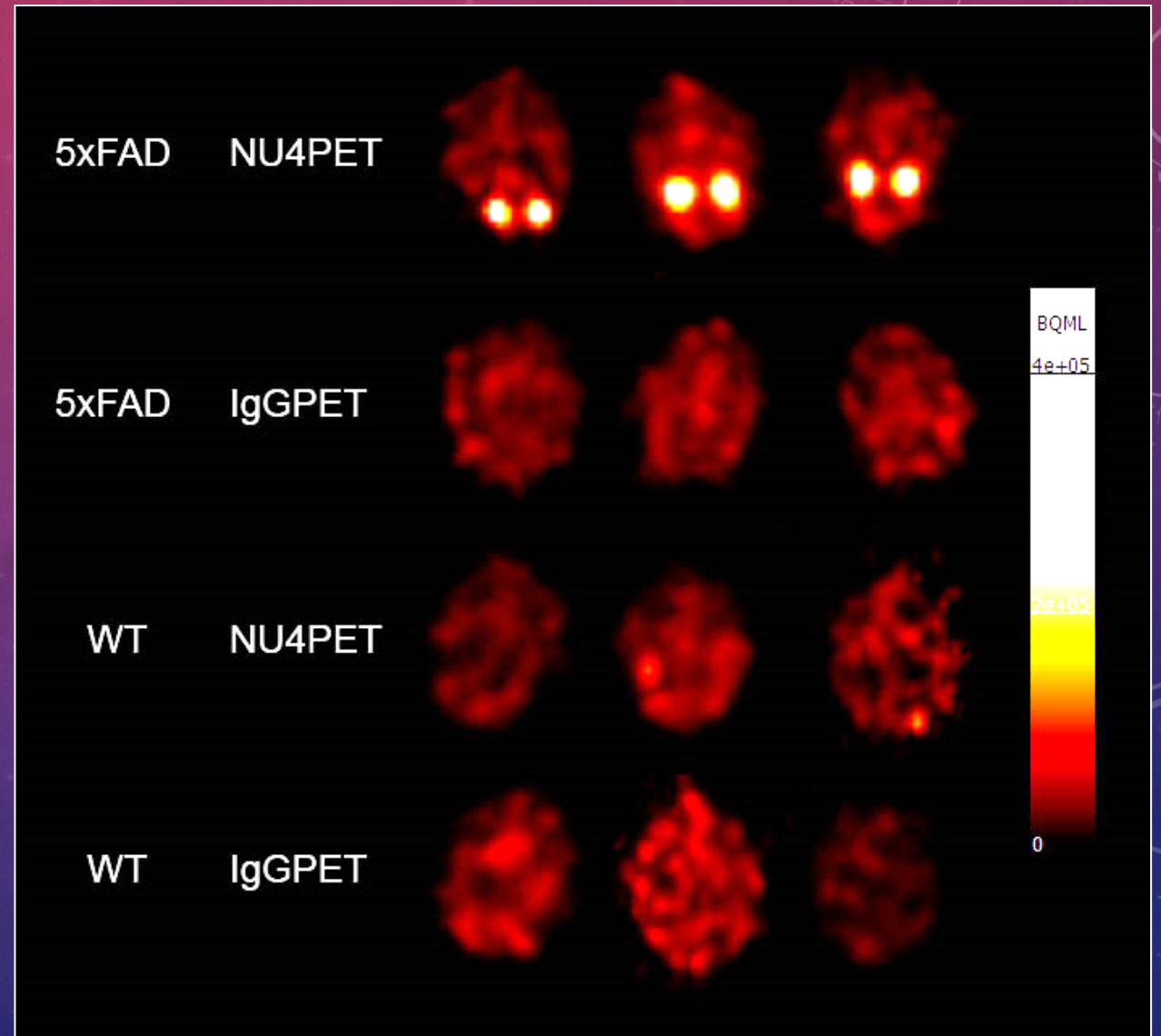
FAM ADDLs/NU4

60x



# NU4PET DEMONSTRATE A STRONG AD DEPENDENT SIGNAL IN MICE

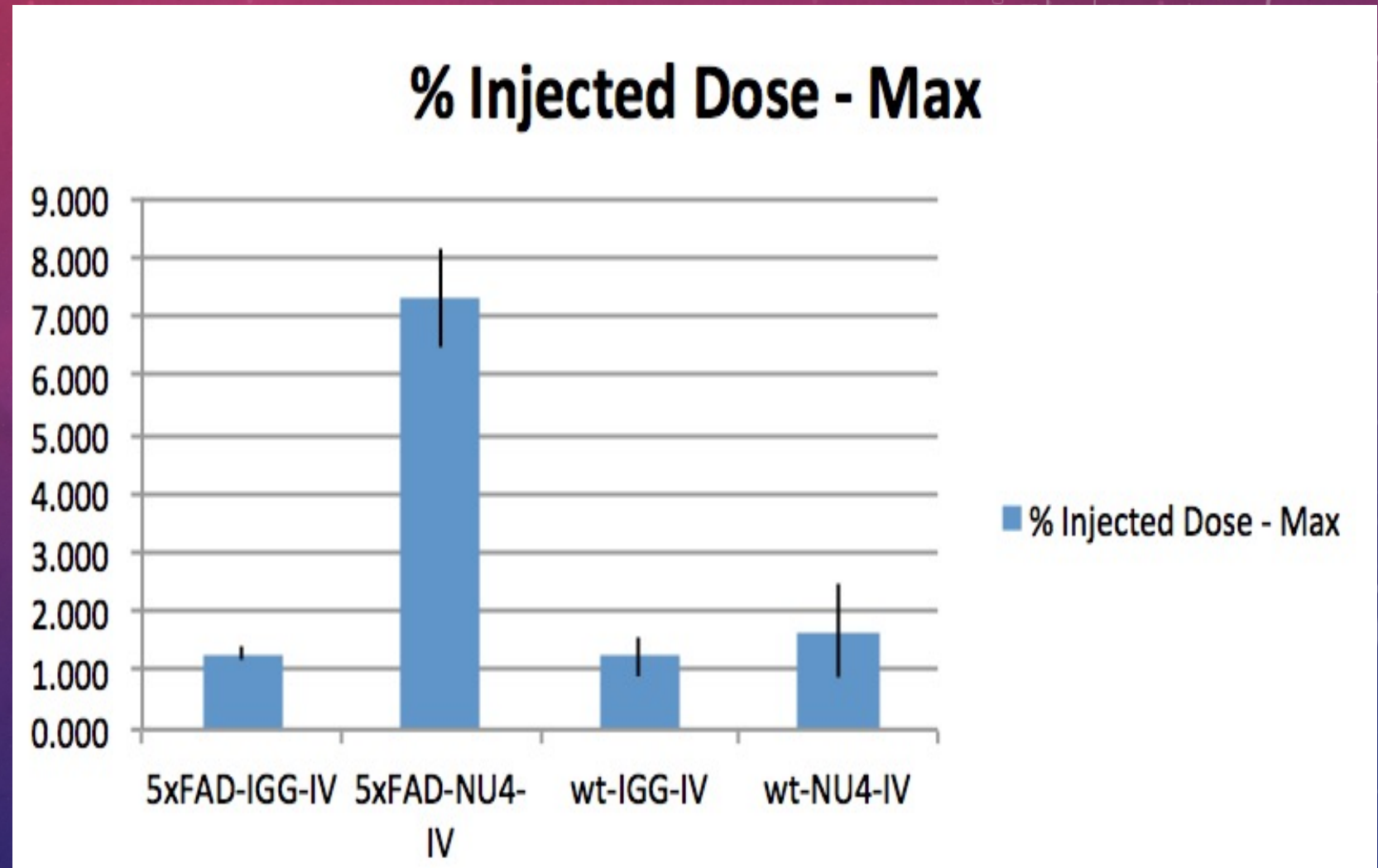
- Tg and Wt mice injected
- NU4PET
- IgGPET
- NU4PET demonstrates a strong AD dependent signal



Ting-Tung Chang

# NU4PET DEMONSTRATES SUBSTANTIAL BRAIN UPTAKE

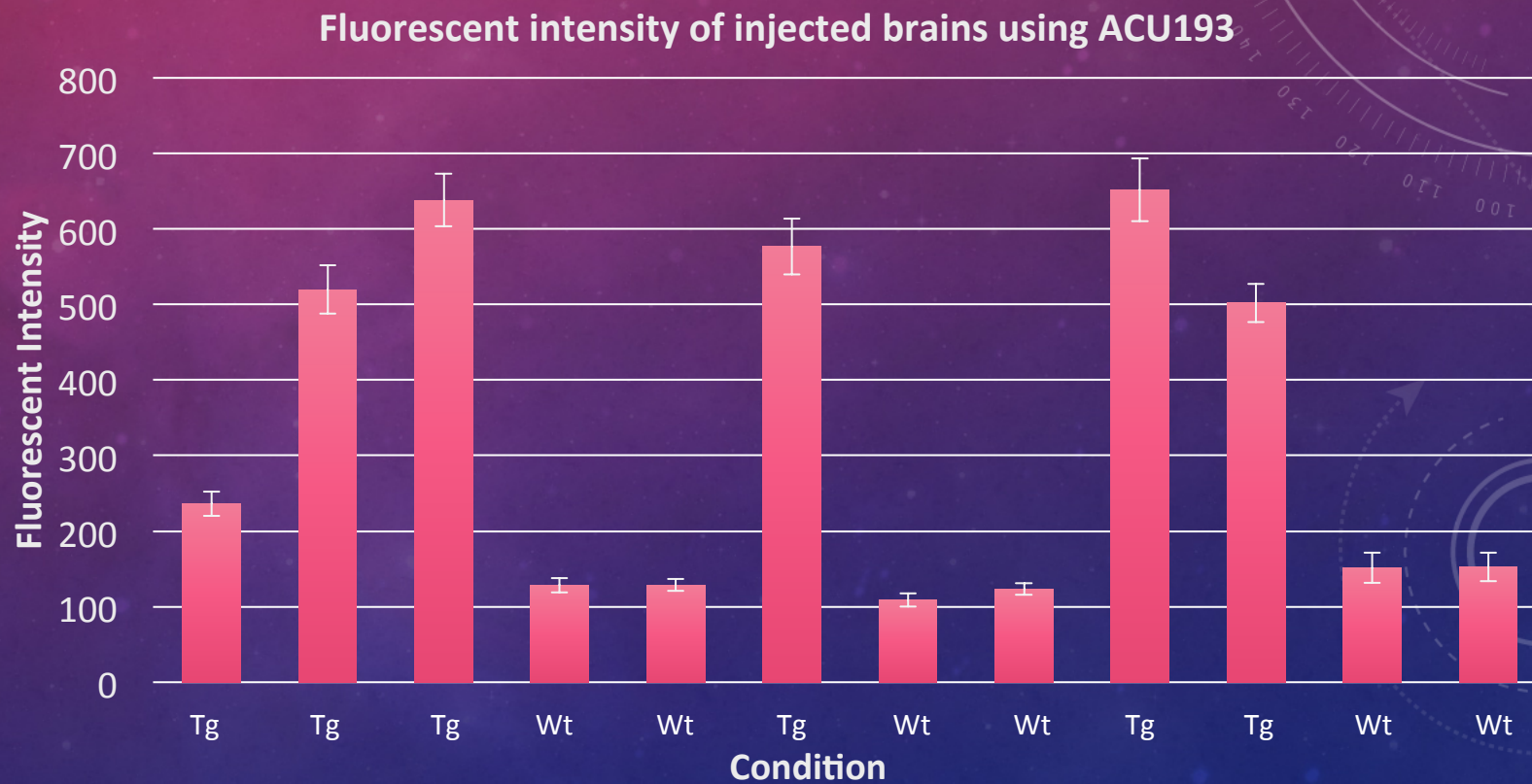
- Blood brain barrier is extremely difficult to cross
- Percent of injected dose retained similar to PiB and Florbetapir



Ting-Tung Chang

# PET SIGNAL CORRELATES TO ACU193 IMMUNOFLUORESCENT INTENSITY

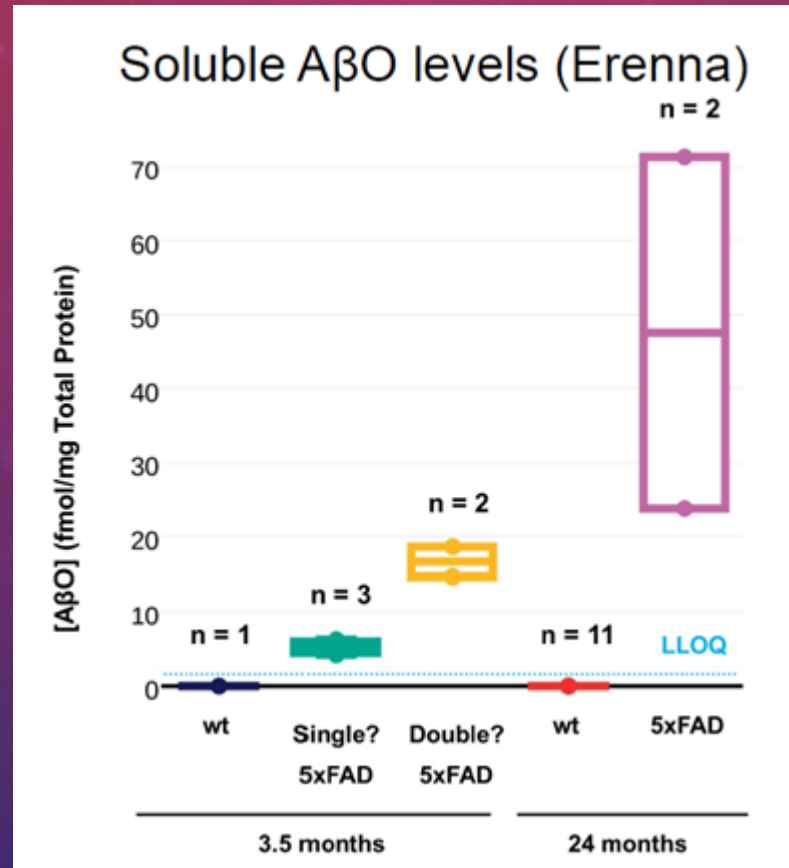
- Immunofluorescently labeled brains with ACU193
- Fluorescent intensity correlates to PET signal



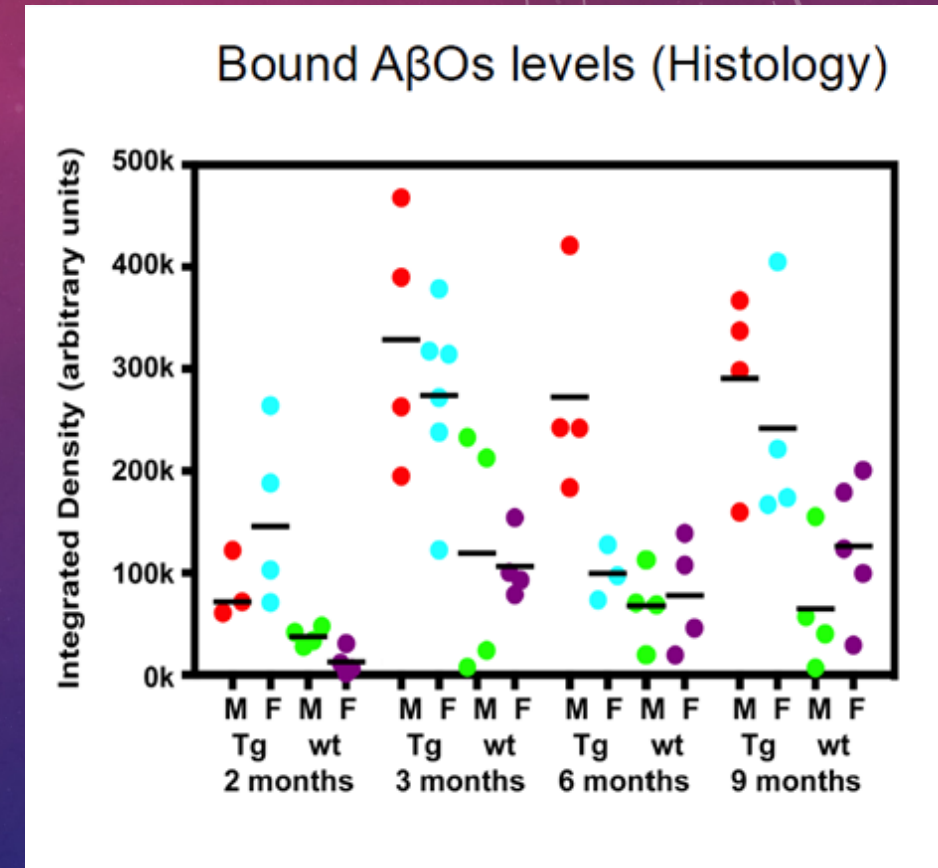


# ABOS ARE DETECTABLE AT 2 MONTHS IN 5XFAD MICE

- Target for diagnosis must appear before onset of symptoms
- ERENNA and histology
- A $\beta$ O<sub>s</sub> are detectable at 2 months

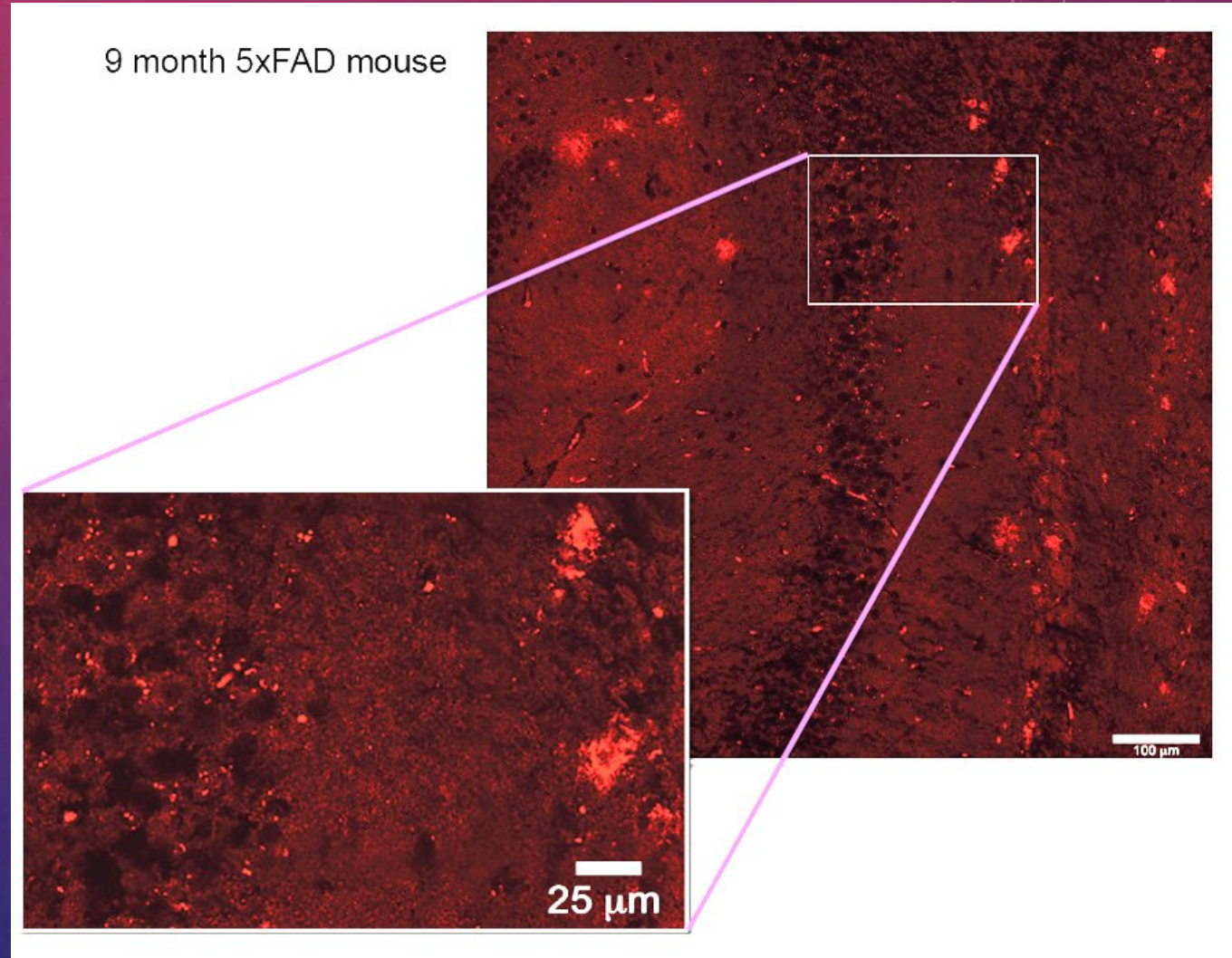


Erika Cline

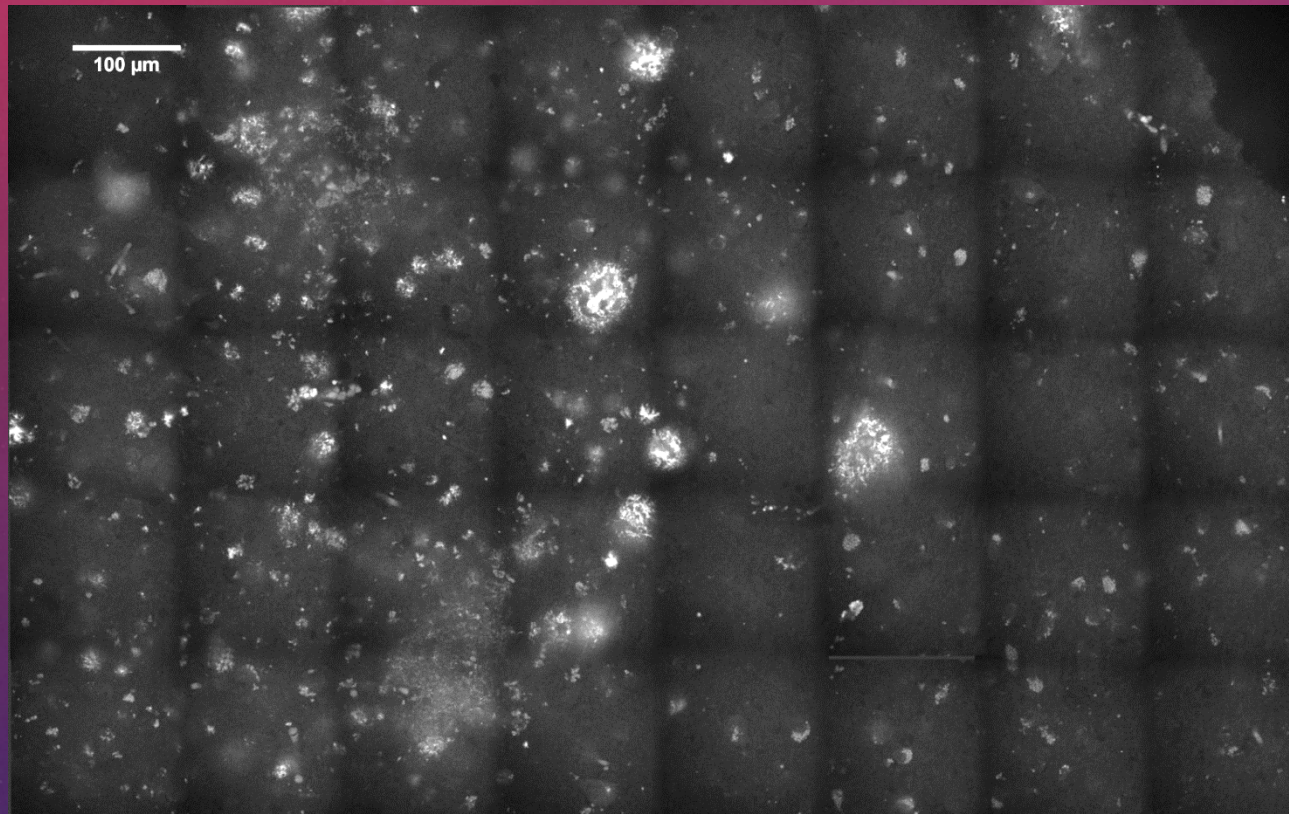


# NU4 DEMONSTRATES PLAQUE-LIKE AND PUNCTATE LABELING OF PYRAMIDAL LAYER IN 5XFAD MOUSE MODEL

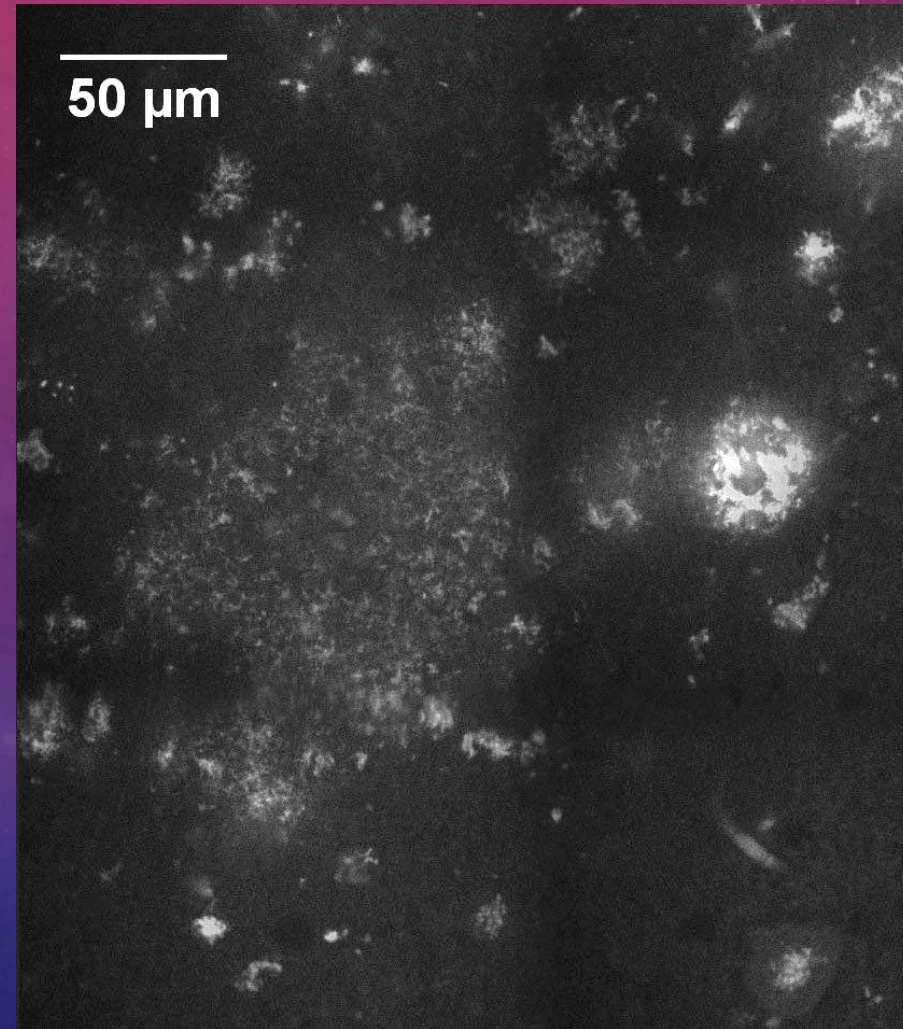
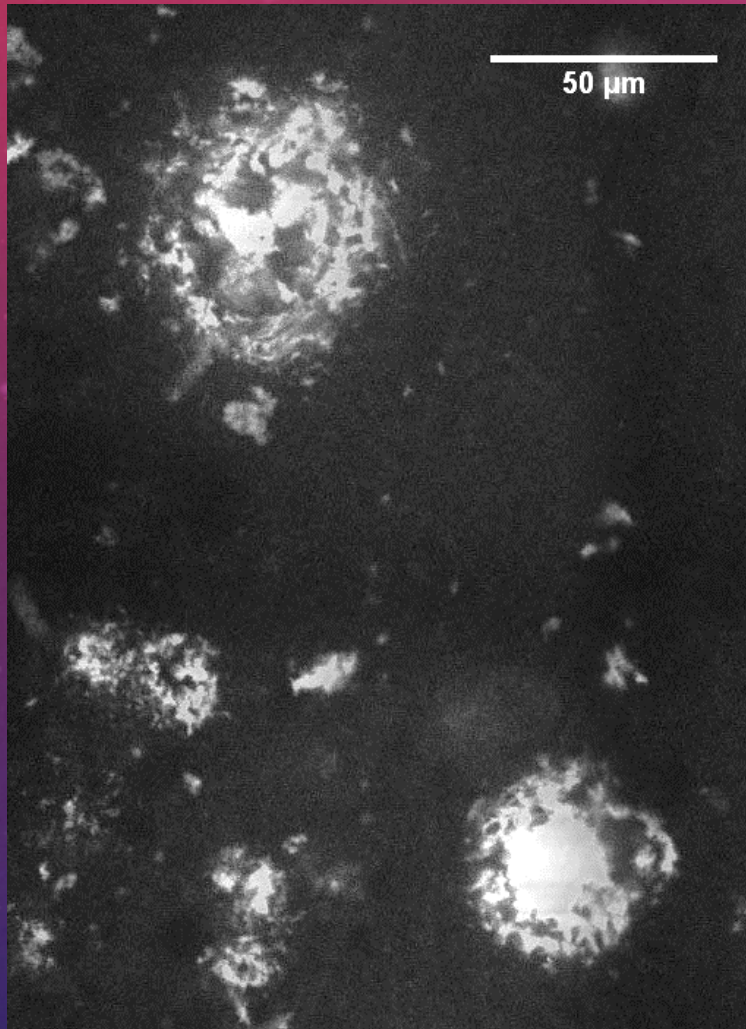
- Identify pathology seen in our 5xFAD mouse model
- Punctate and plaque-like labeling of the dendritic arbors
- A $\beta$ O<sub>4</sub> found primarily in hippocampus, dentate gyrus and along the pyramidal layer



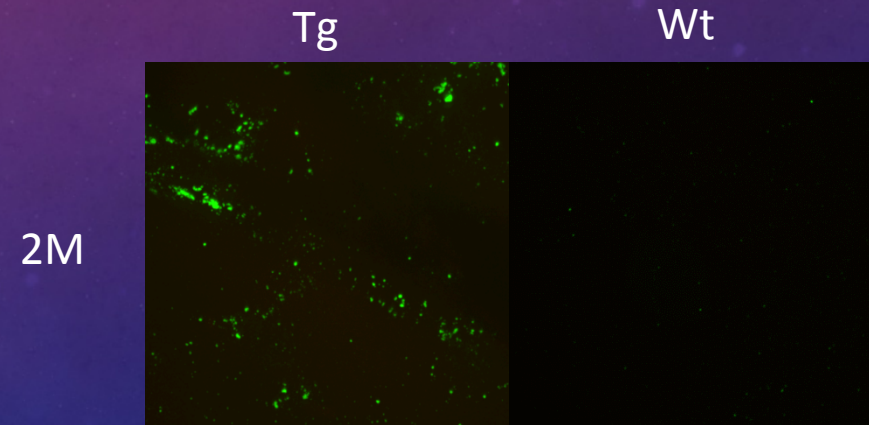
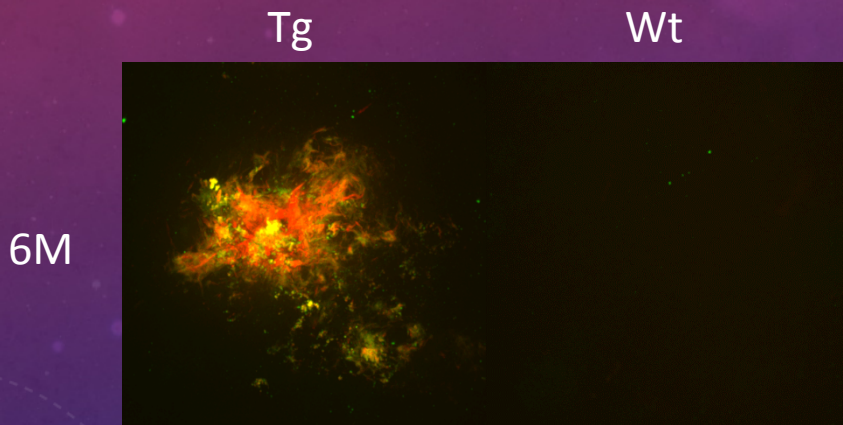
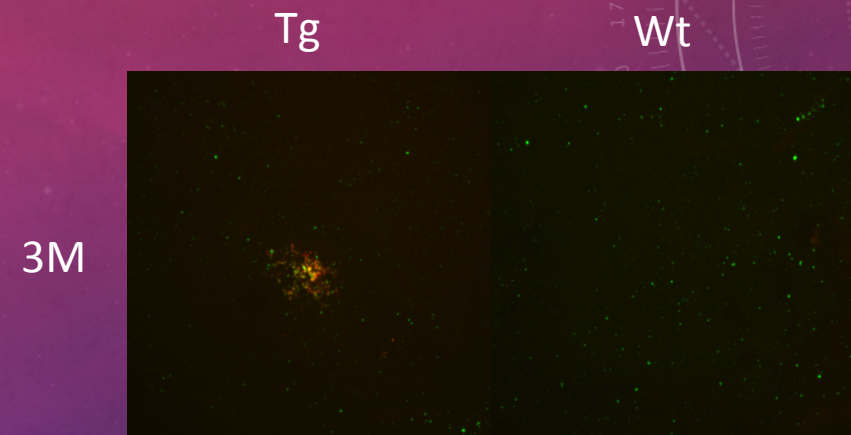
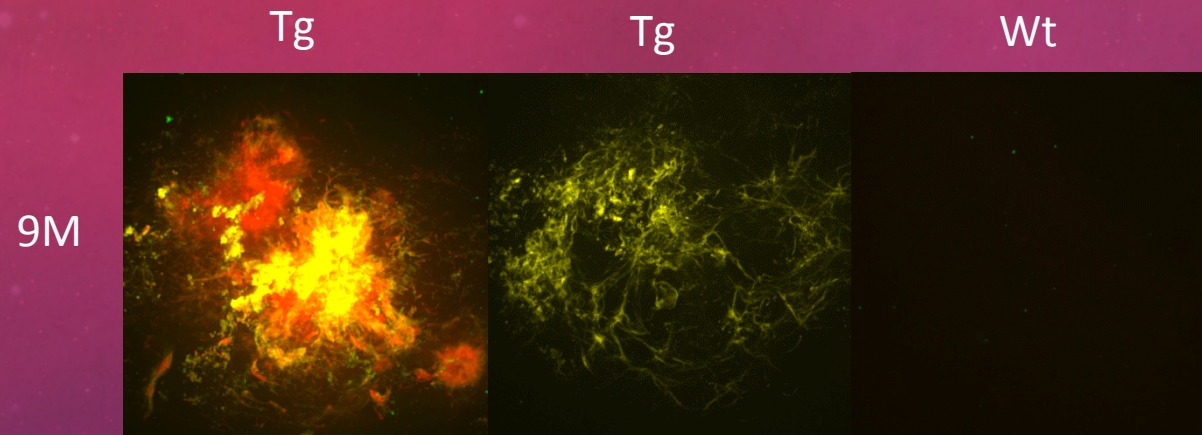
# ACU193 DISTINGUISHES BETWEEN DISEASED AND NONDISEASED HUMAN BRAINS



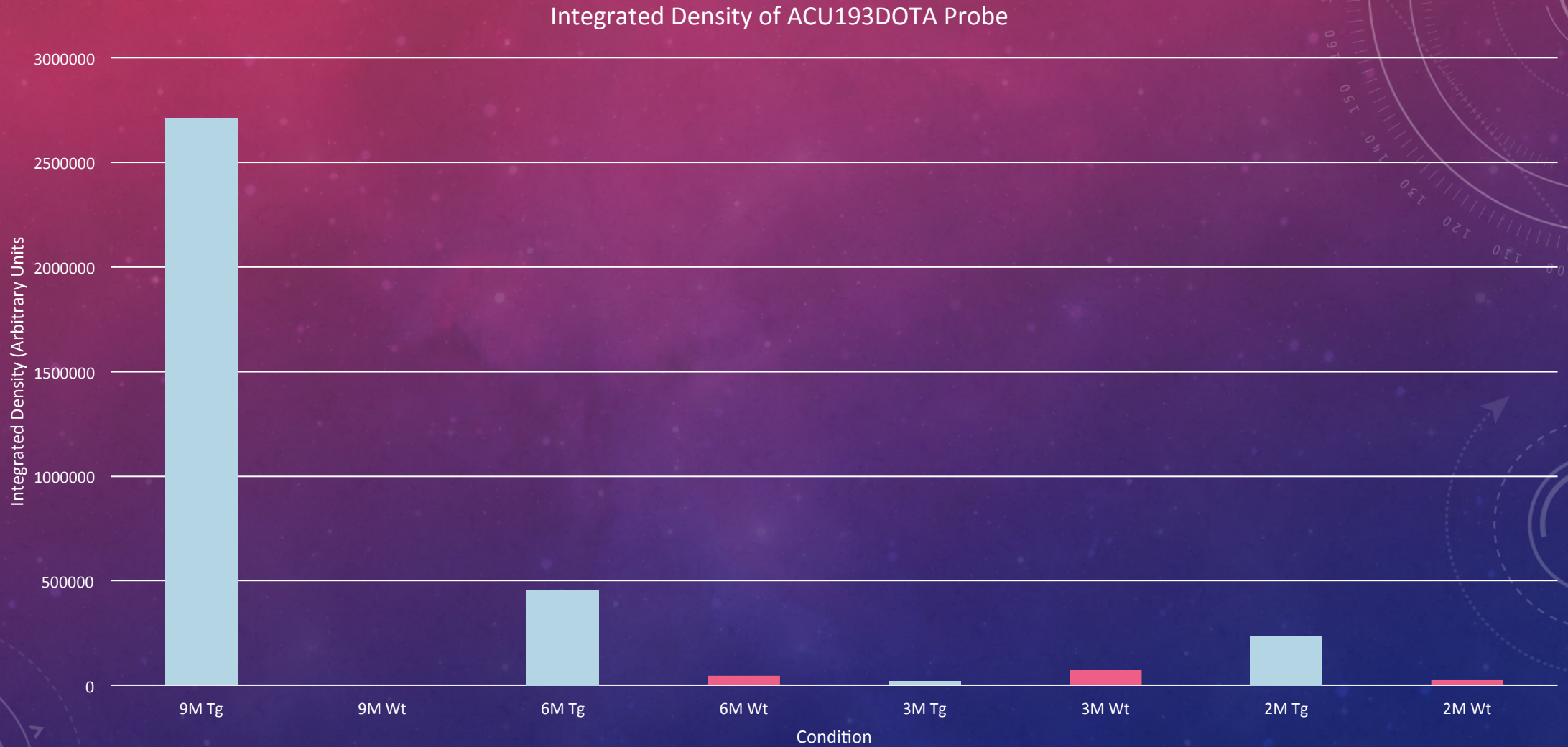
# ACU193 SHOWS PLAQUE-LIKE, PUNCTATE, AND DIFFUSE PATHOLOGY IN HUMAN BRAIN TISSUE



# ACU193DOTA DISTINGUISHES BETWEEN 5XFAD AND WT TISSUES DOWN TO 2 MONTHS (NU4 AND ACU193DOTA)



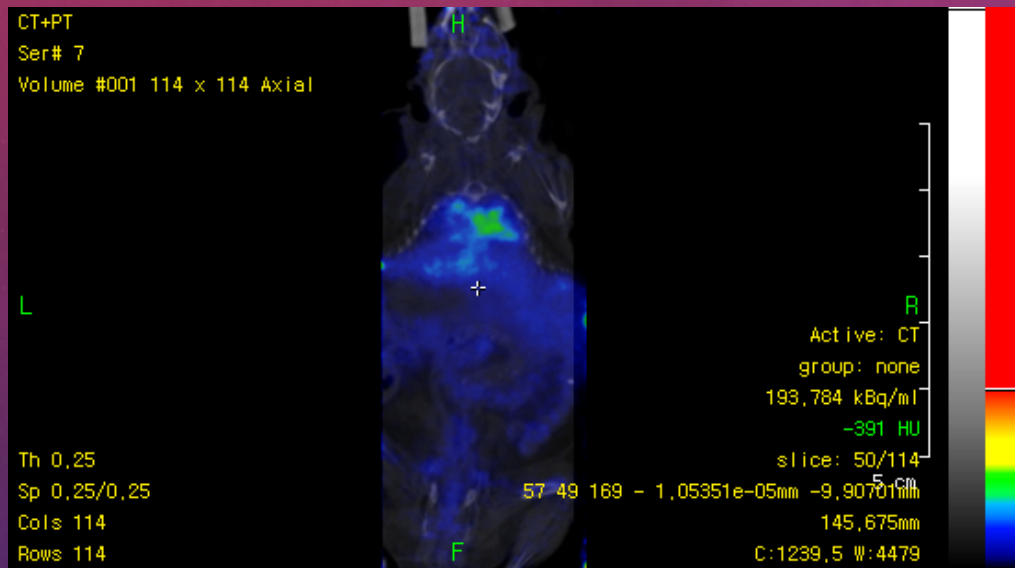
# ACU193DOTA BINDS IN AN AGE DEPENDENT MANNER AND DISTINGUISHES BETWEEN TG AND WT MICE



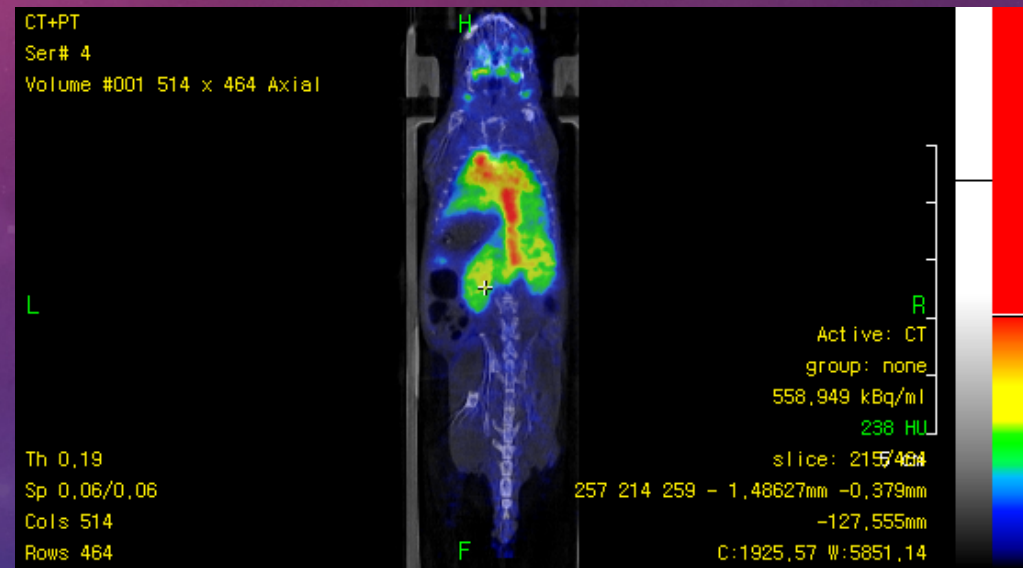
# ACU193PET SHOWS PROMISE IN DISTINGUISHING BETWEEN DISEASED AND NONDISEASED MICE

# DAY 1 RESULTS

## Wild Type



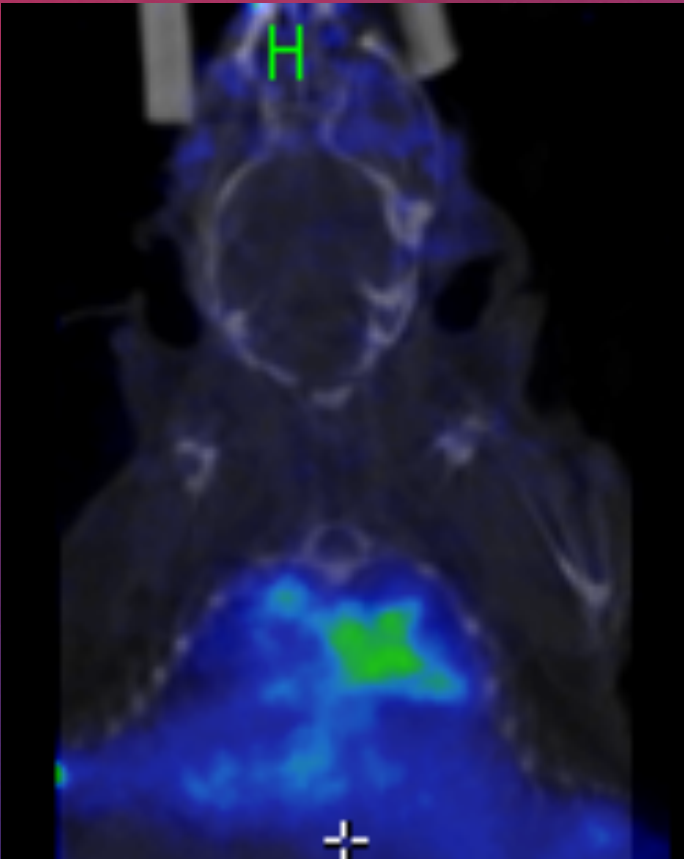
## 5xFAD



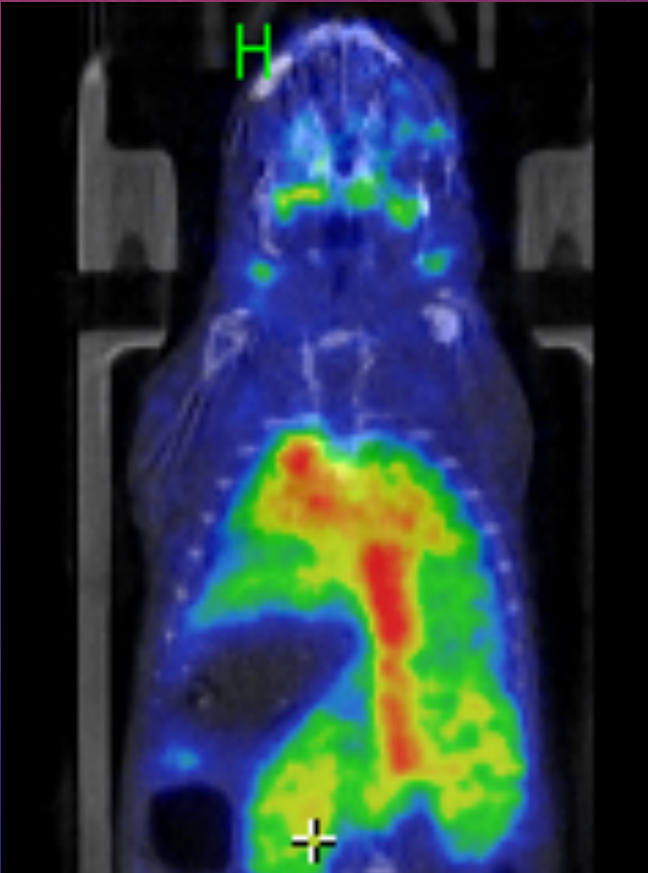


# DAY 1 RESULTS

Wild Type



5xFAD



# CONCLUSION

- ACU193PET demonstrates tremendous potential as an early diagnostic imaging tool for AD
- Future Work:
  - Substantiating AD specific probe signal by autoradiography using mouse and human brain sections
  - Dose curve PET to determine optimal dosing range in mid-stage AD (5xFAD model)
  - Evaluation of gross uptake and clearance in mice in vivo
  - Longitudinal analysis to determine earliest stages at which PET probe detects A $\beta$ O $_s$
  - Quantitative relationship between PET signals, A $\beta$ O $_s$  detected histologically, A $\beta$ O $_s$  detected biochemically, and memory loss
  - Human trial in 18 months
  - Dual MRI/PET

# ACKNOWLEDGEMENTS

First and foremost I would like to thank Ms. Kirsten Viola for allowing me to work on this project with her and for being my mentor. I would like to thank Dr. William Klein for the opportunity to work in his lab and for all of the mentorship that he has also provided. I would also like to thank everyone who I have worked with at Klein Lab and all collaborators outside of Northwestern University for making this possible. Finally, would like to thank the SIR staff at IMSA and my parents for providing me with this opportunity and being a tremendous source of support.

- NIH NIA Award Number AG045637
- NCI CCSG P30 CA060553 and NIH 1S10OD010398-01 for Northwestern University's Center for Advanced Microscopy
- NCI CCSG P30 CA060553 for Northwestern University's Center for Advanced Molecular Imaging

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