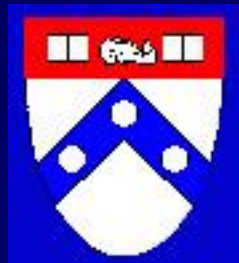


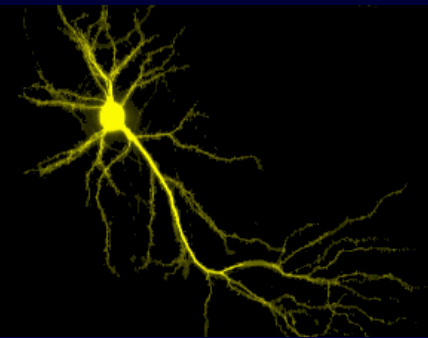
# Targeting Soluble A $\beta$ Oligomers by Passive Immunization for AD Therapy

Virginia M.-Y. Lee, Ph.D.

Center for Neurodegenerative Disease Research  
University of Pennsylvania



# Development of Amyloid Pathology

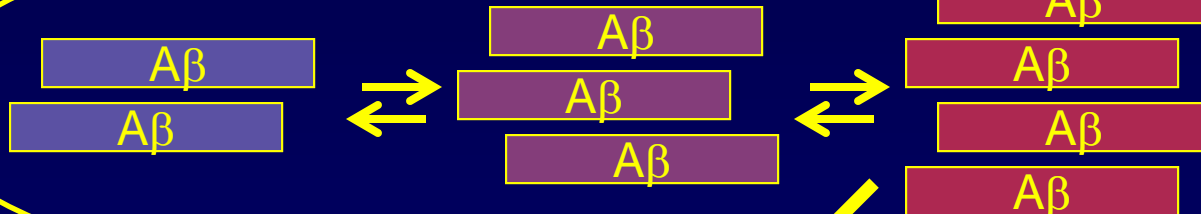


Cellular Metabolism:  
APP, BACE,  $\gamma$ -secretase

→  $A\beta$

**TOXICITY???**

Soluble  $A\beta$  Oligomers

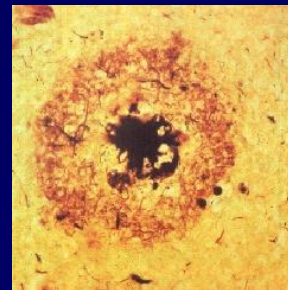


$A\beta$  Fibrillization  
vs.  
 $A\beta$  Degradation

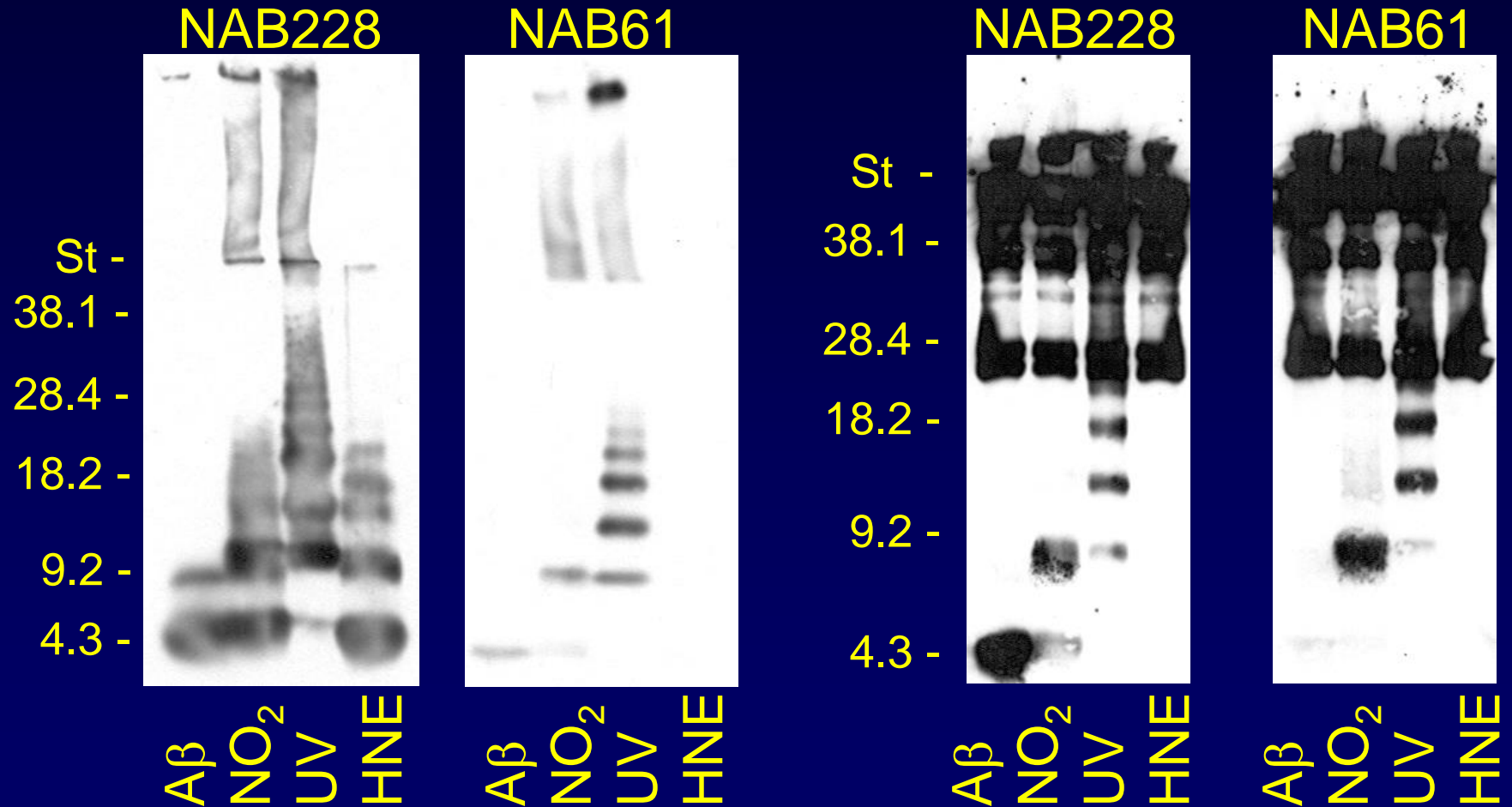
Protofibril

Fibril

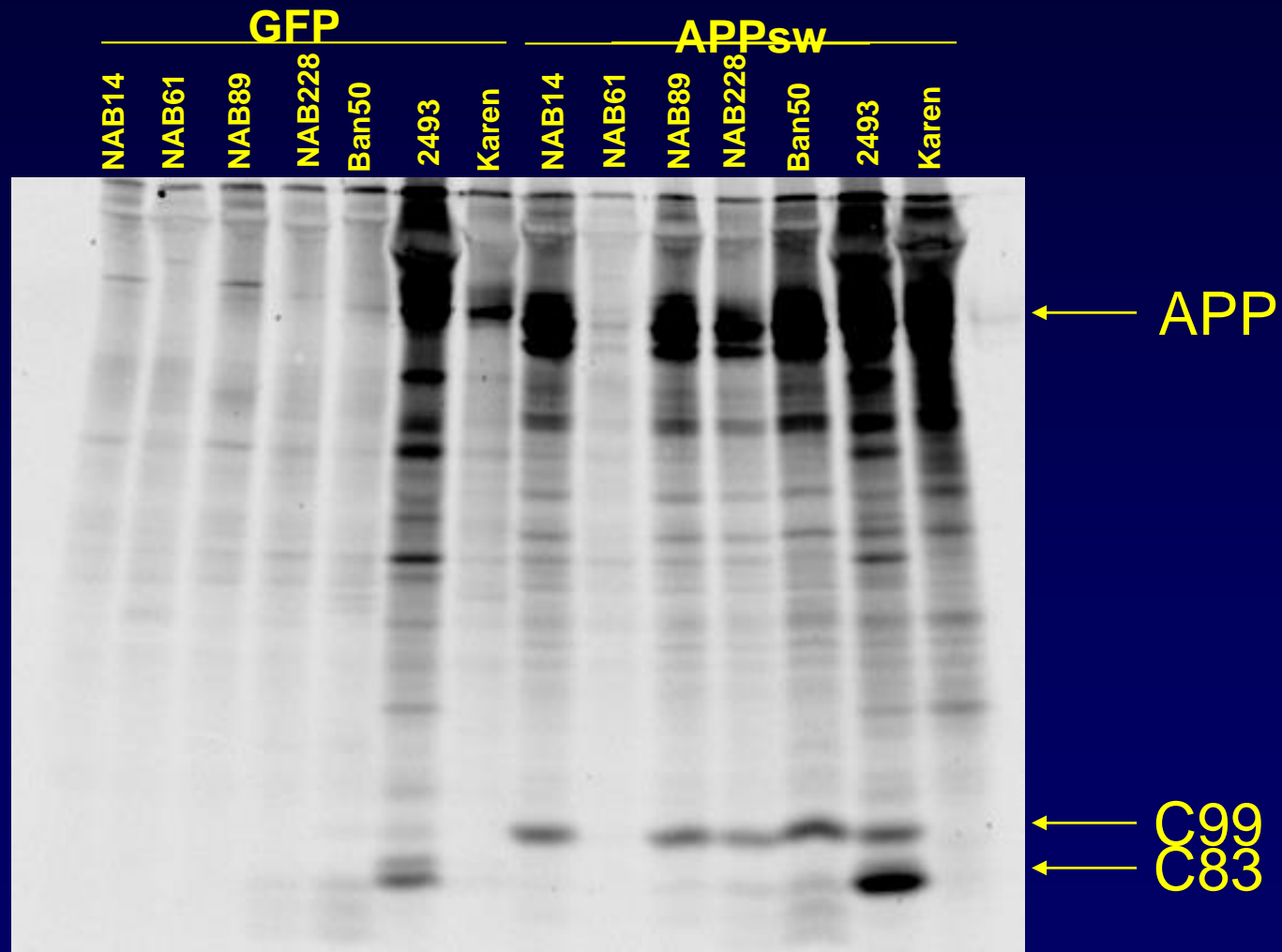
Fibril



# Oligomer-Selective Monoclonal Antibody

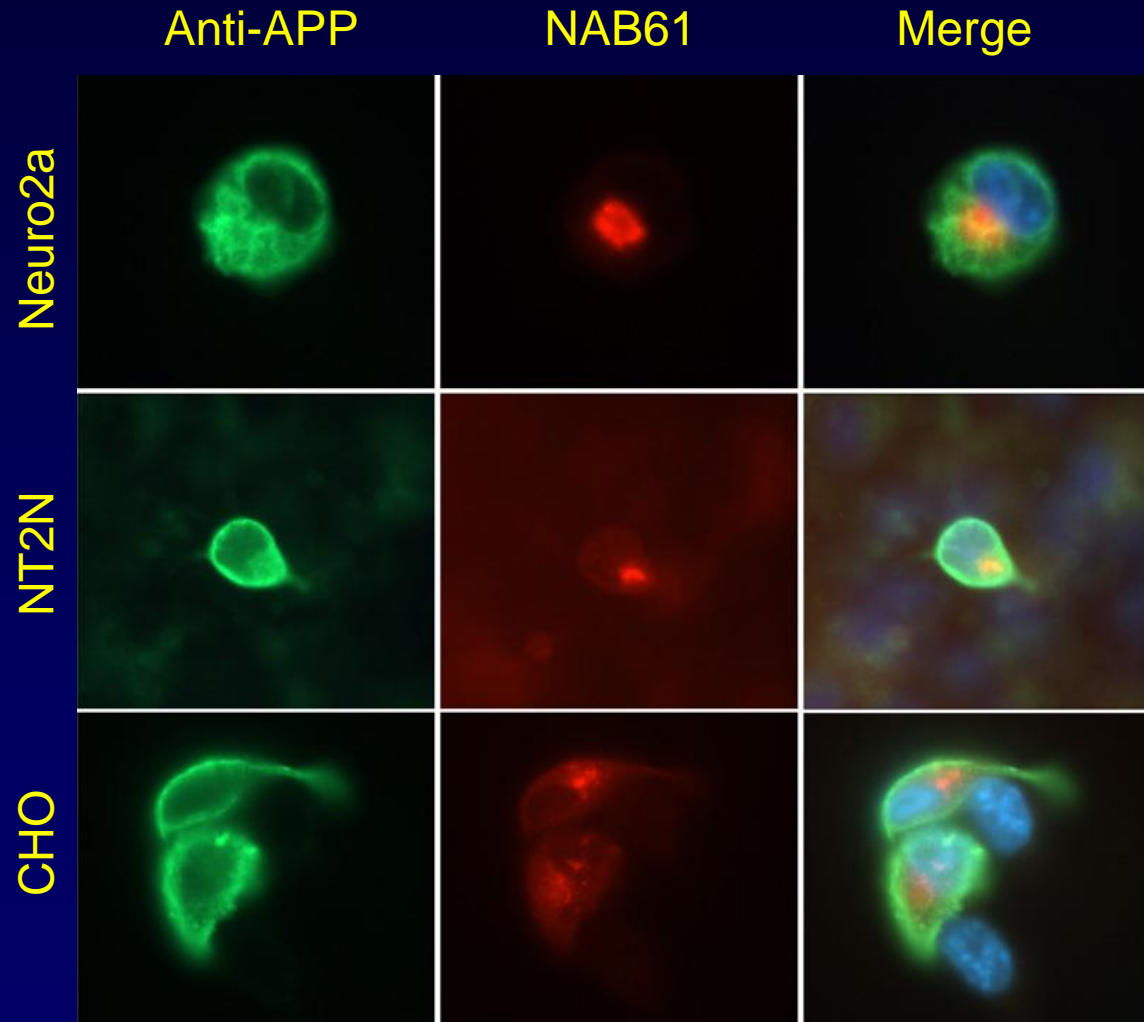


# NAB61 Does Not Recognize APP or C-terminal APP Fragments



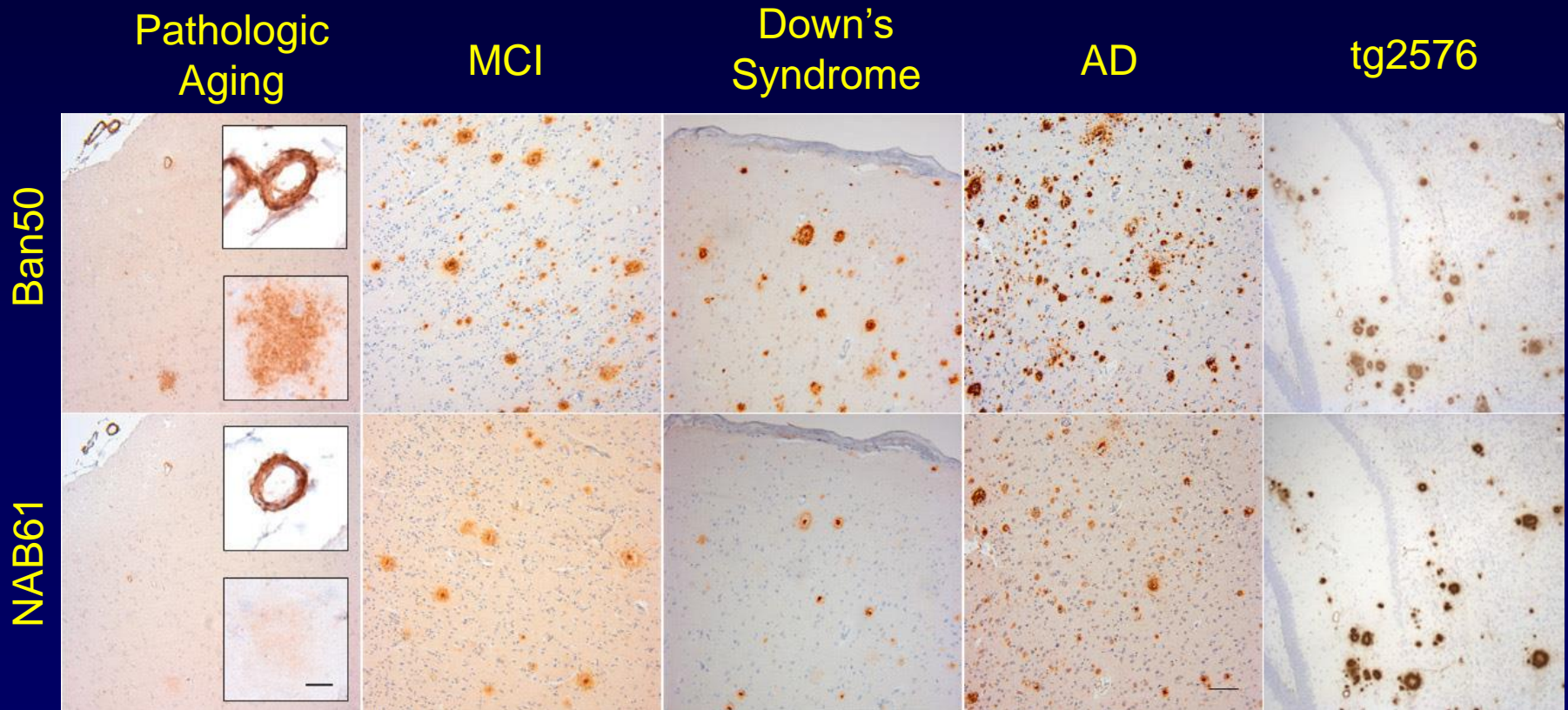
# NAB61 Immunoreactivity Does Not Co-localize with APP

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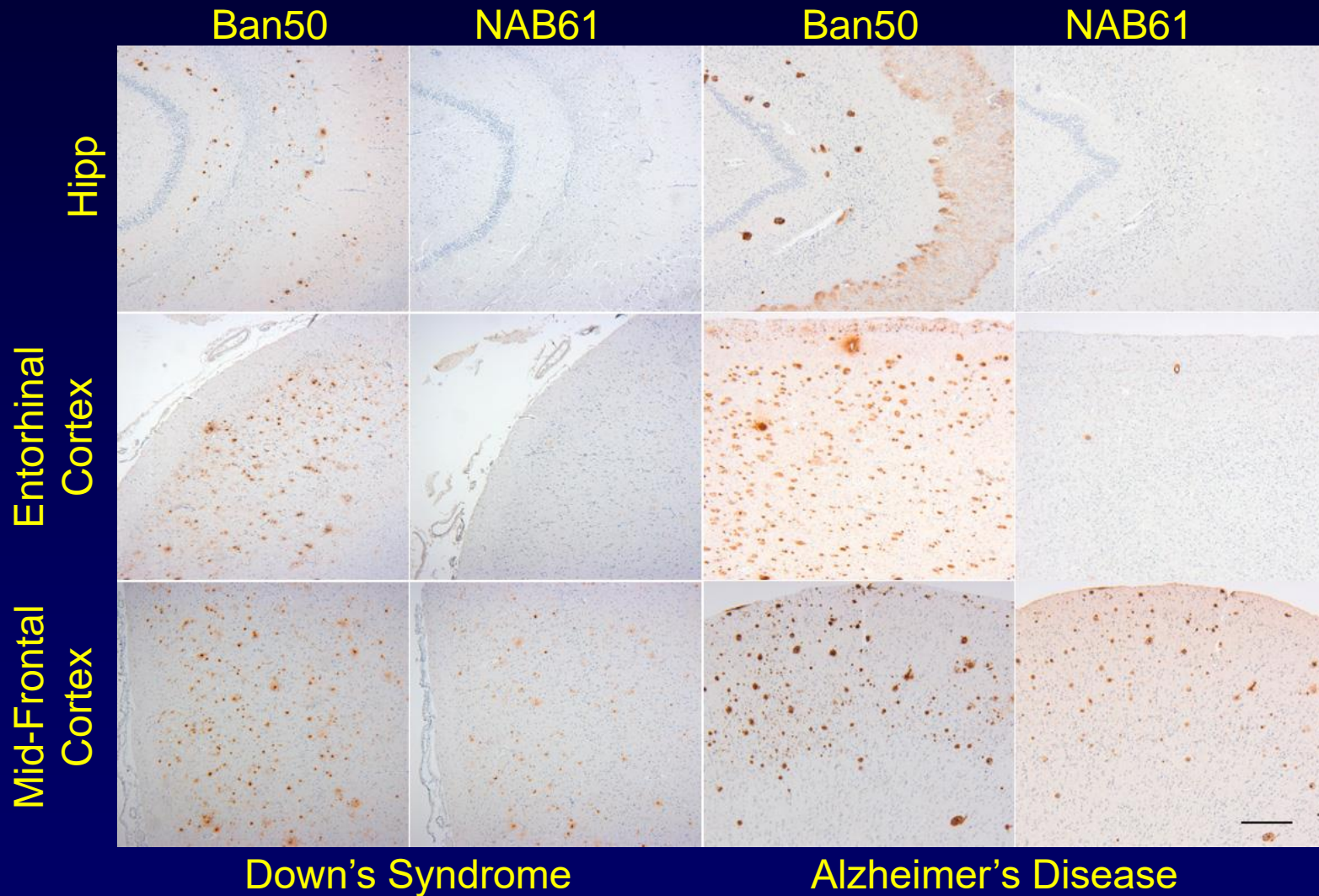
# NAB61 Recognizes Amyloid Deposits

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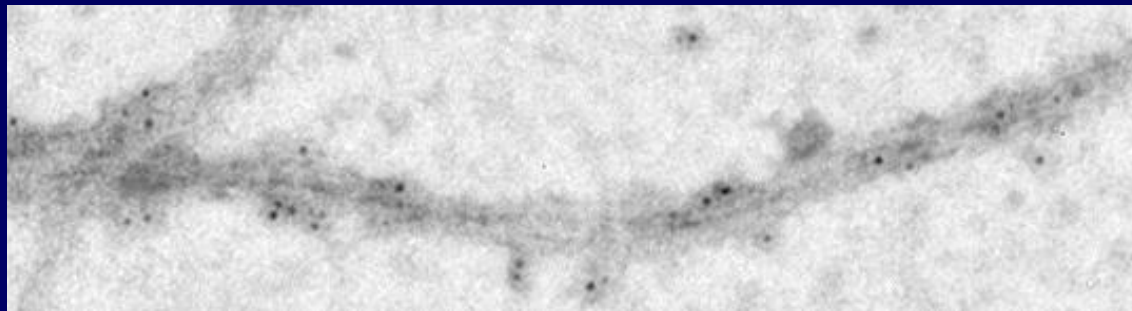
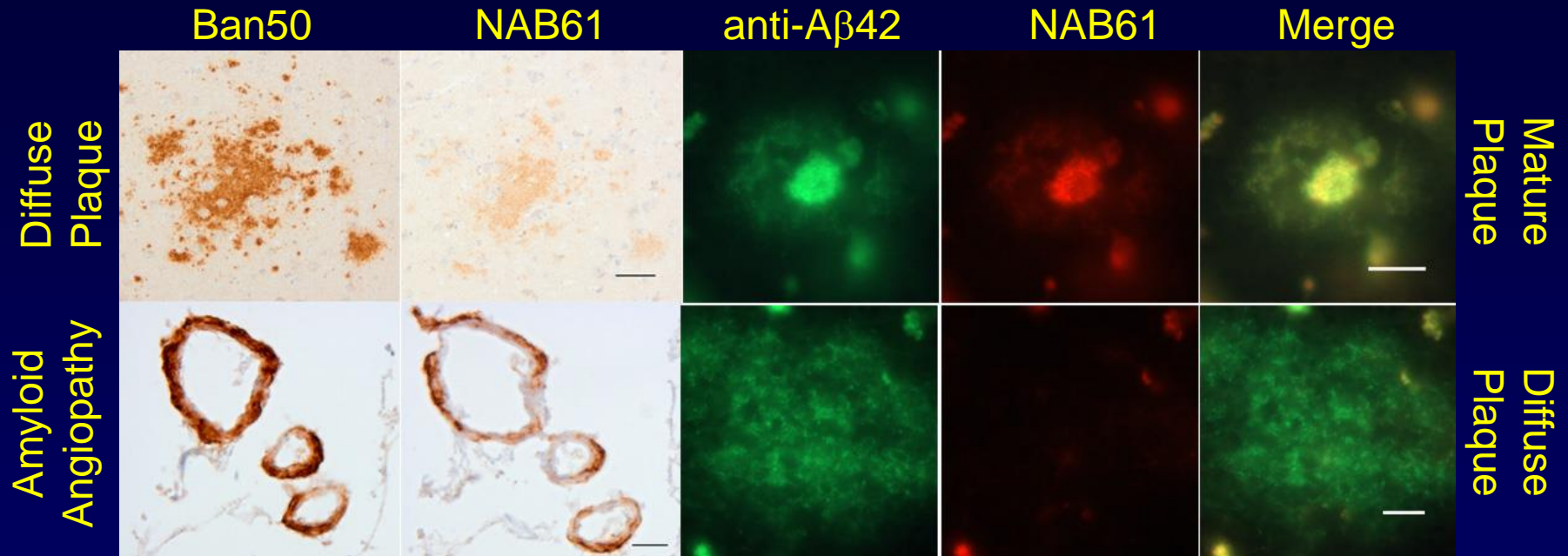




# Regional Specificity of NAB61



# NAB61 Recognizes Fibrillar A $\beta$ , Angiopathy and Mature Senile Plaques





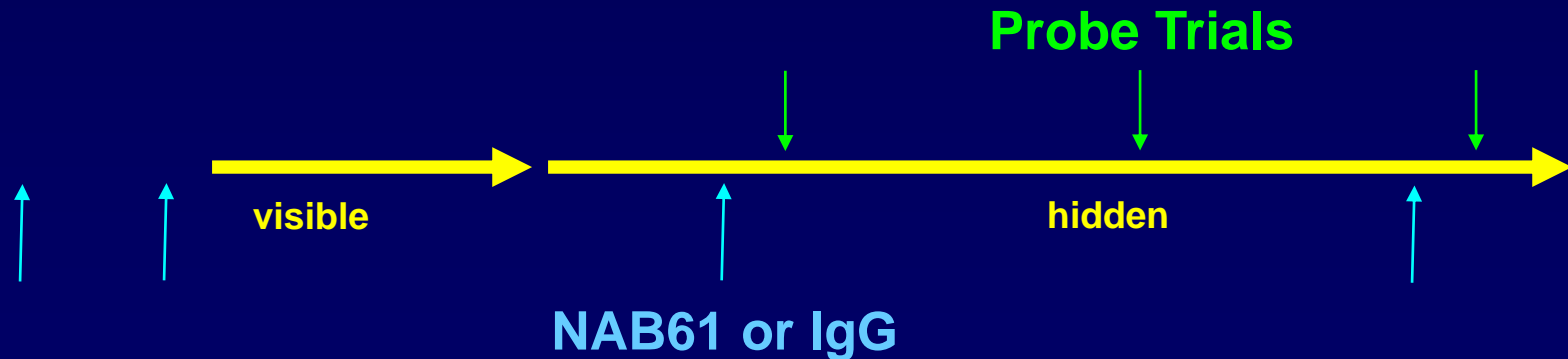
# Mechanisms of A $\beta$ Immunotherapy

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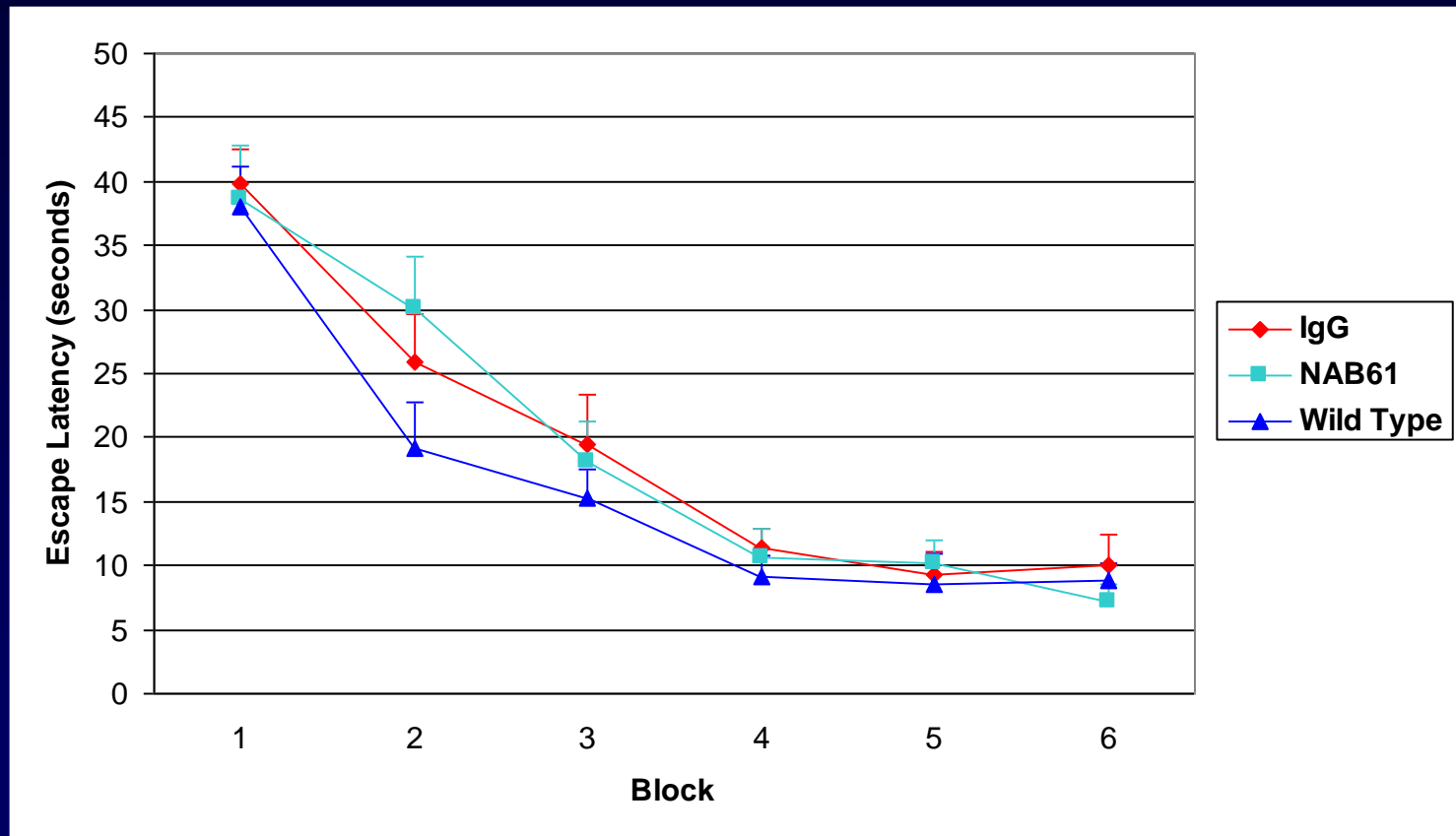
- Active or passive immunization improves cognitive function and inhibits amyloid pathology *in vivo*
- Acute passive immunization improves cognitive function without affecting pathology
  - Synaptotoxic soluble A $\beta$  oligomer???
- Peripheral sink hypothesis
  - Antibodies bind A $\beta$  in plasma and sequester A $\beta$  from the central nervous system
- Central action
  - Antibodies enter the CNS and neutralize A $\beta$

# Targeting Soluble Oligomers by Passive Immunization of Aged tg2576 Mice with NAB61

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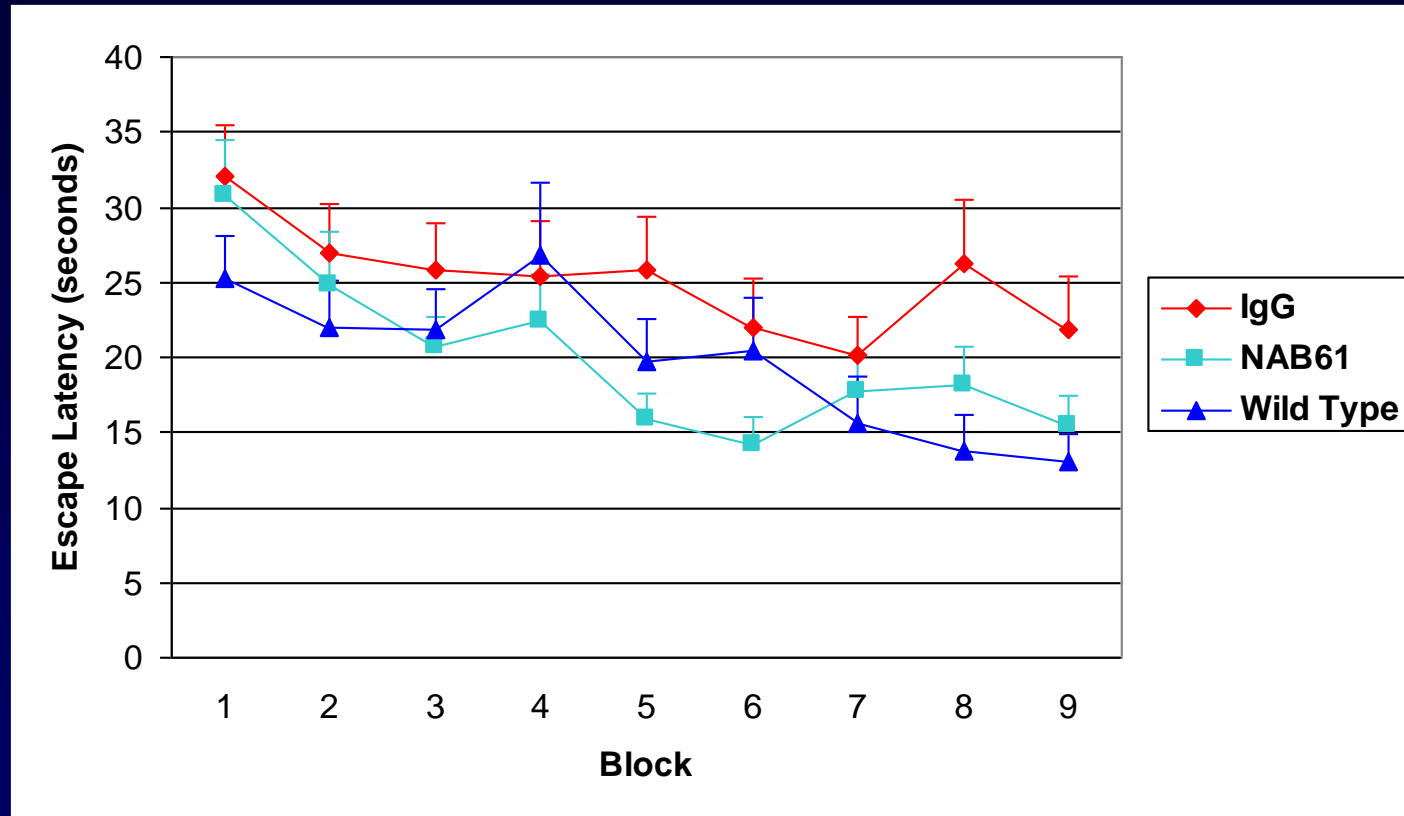


# Passive Immunization Does Not Affect Performance on the Visible Water Maze



Repeated measures ANOVA  $p=0.0952$

# Passive Immunization Reverses Learning and Memory Deficits in Aged tg2576 Mice



Repeated measures ANOVA  $p < 0.0001$

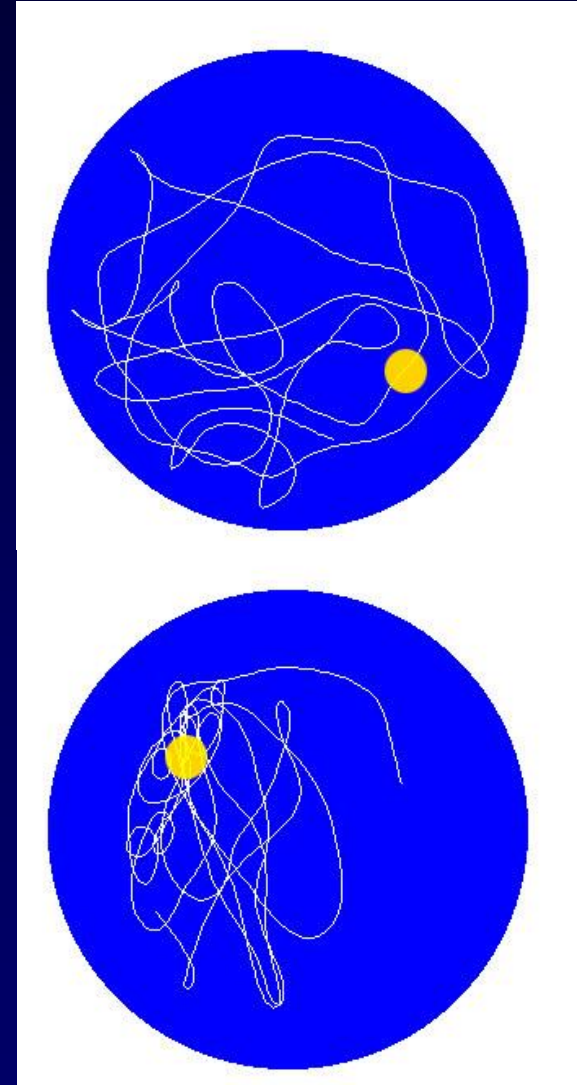
IgG vs. wt or NAB61  $p < 0.001$

wt vs NAB61  $p > 0.05$

# Probe Trial Outcomes

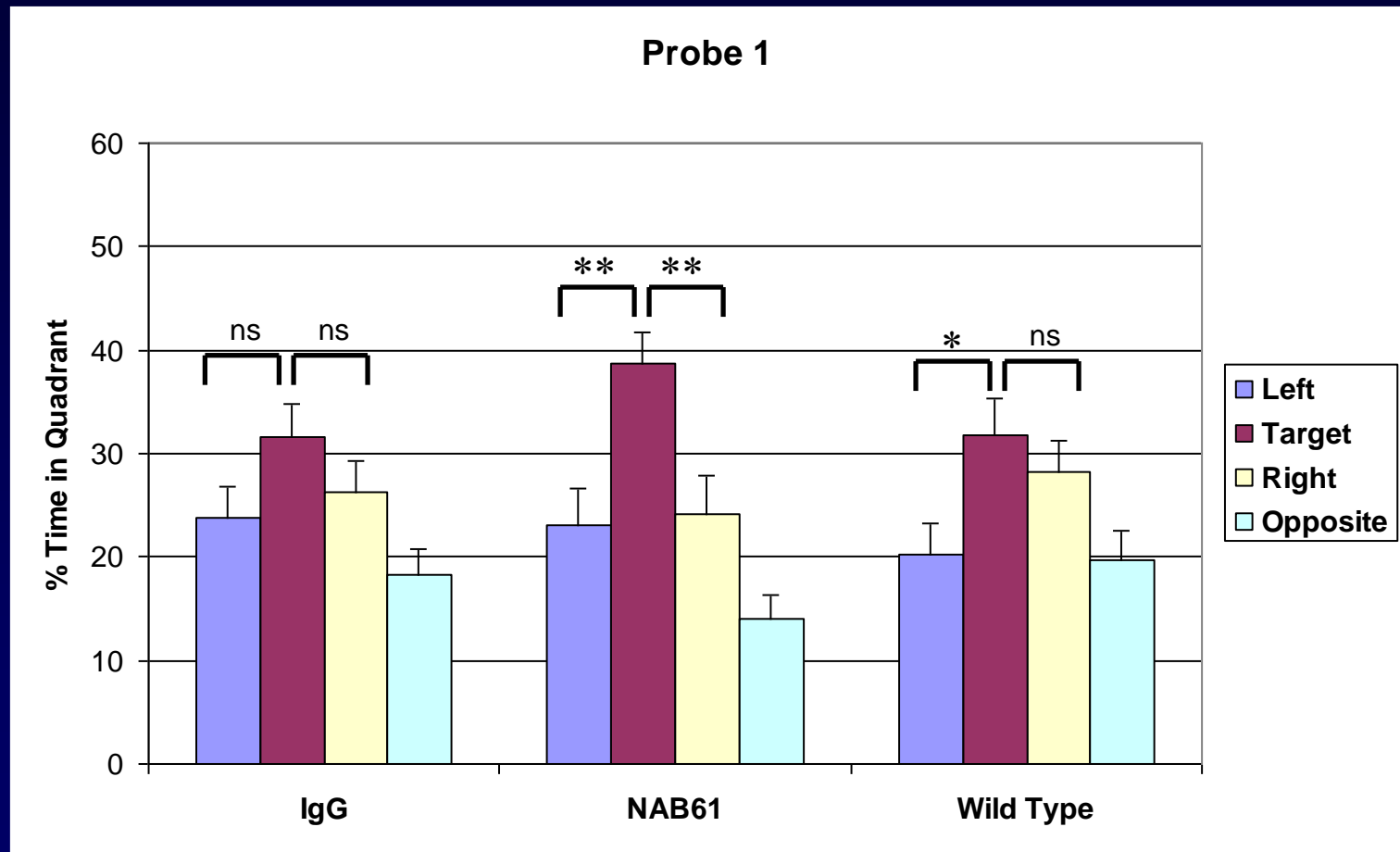
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- Three probe trials (early, middle, late)
- Platform removed from pool for trial of 60 seconds



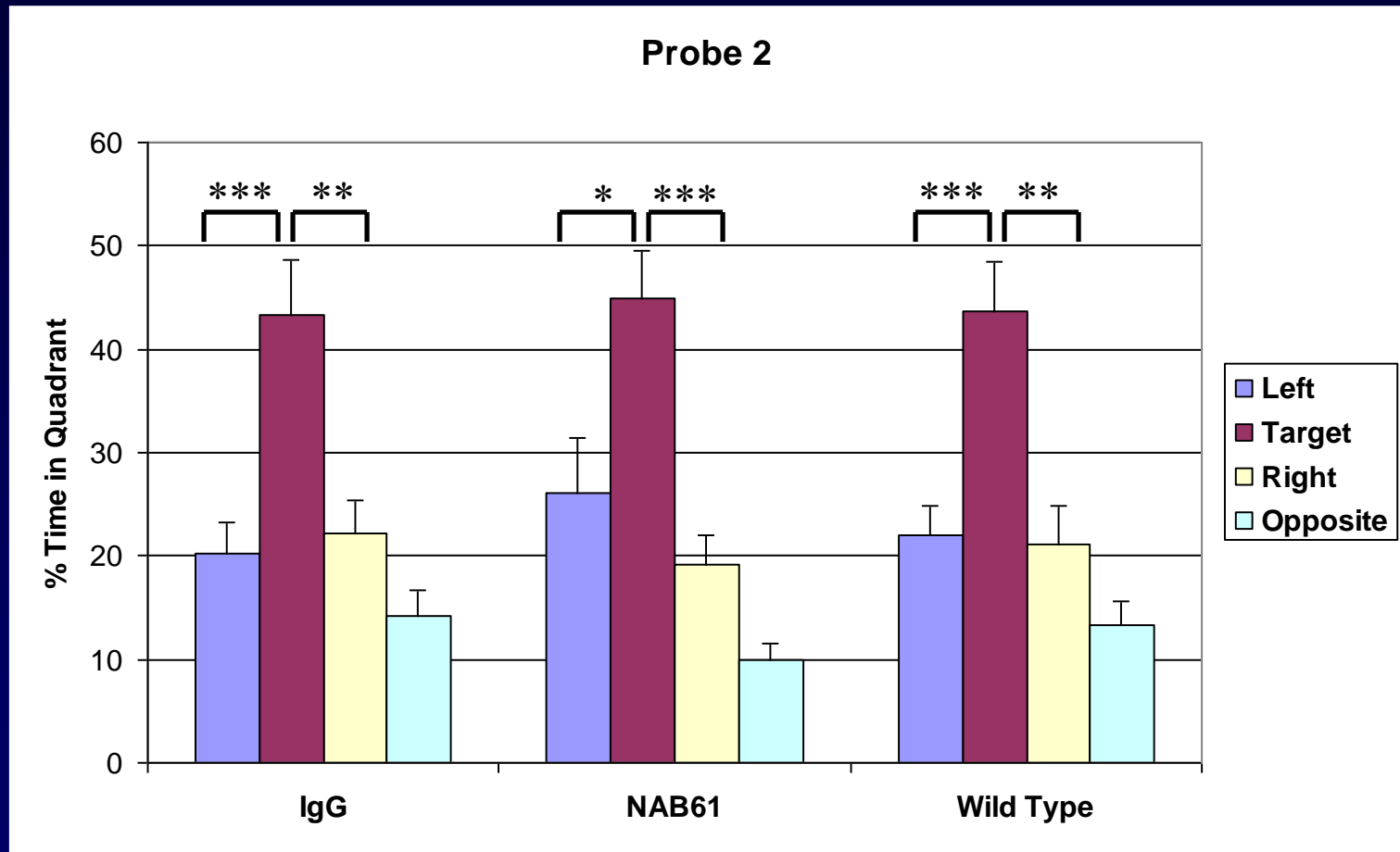


# Spatially Oriented Swimming Behavior: Early Probe

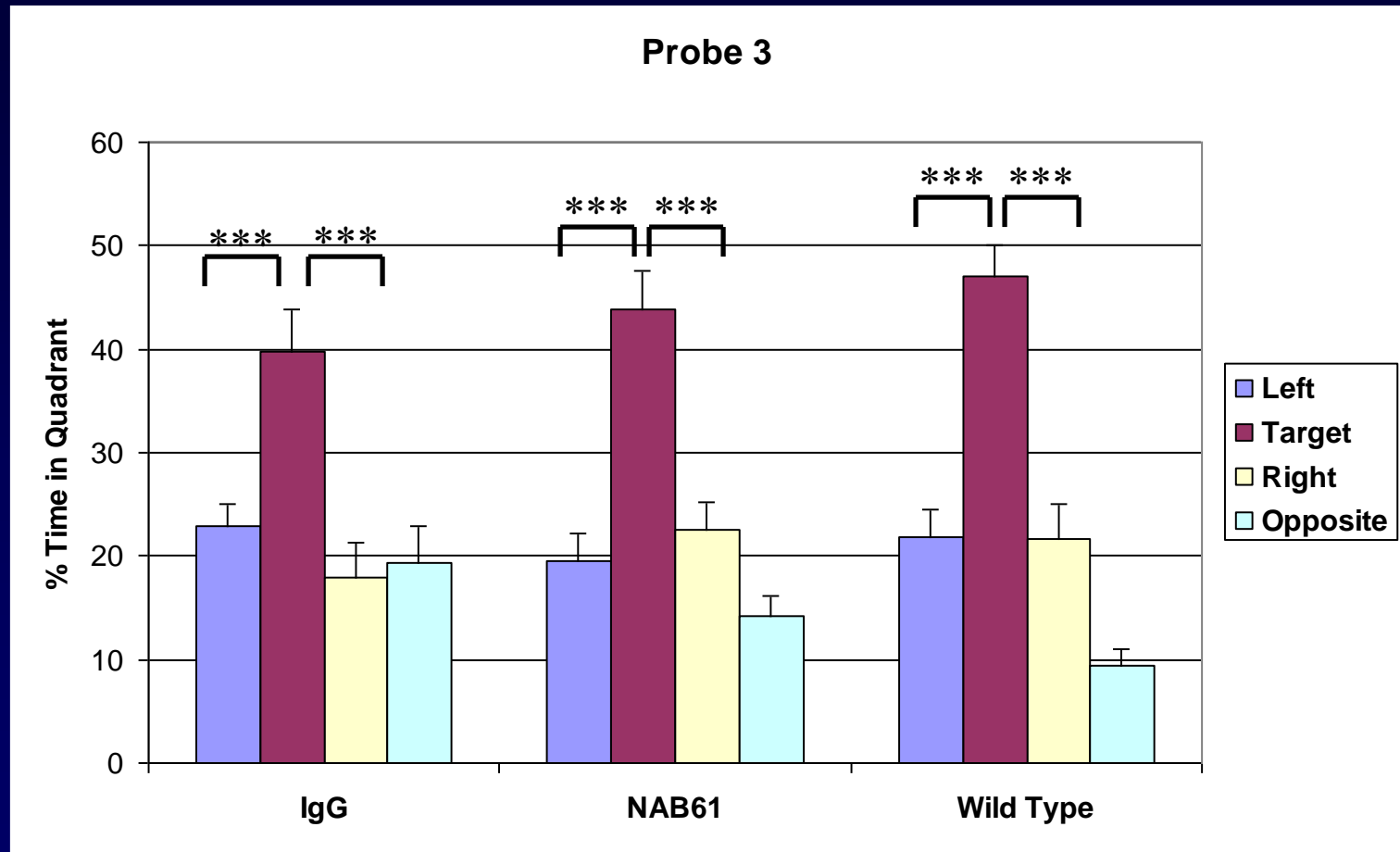


One-way ANOVA  
Fisher's post-hoc, T vs. L, T vs R

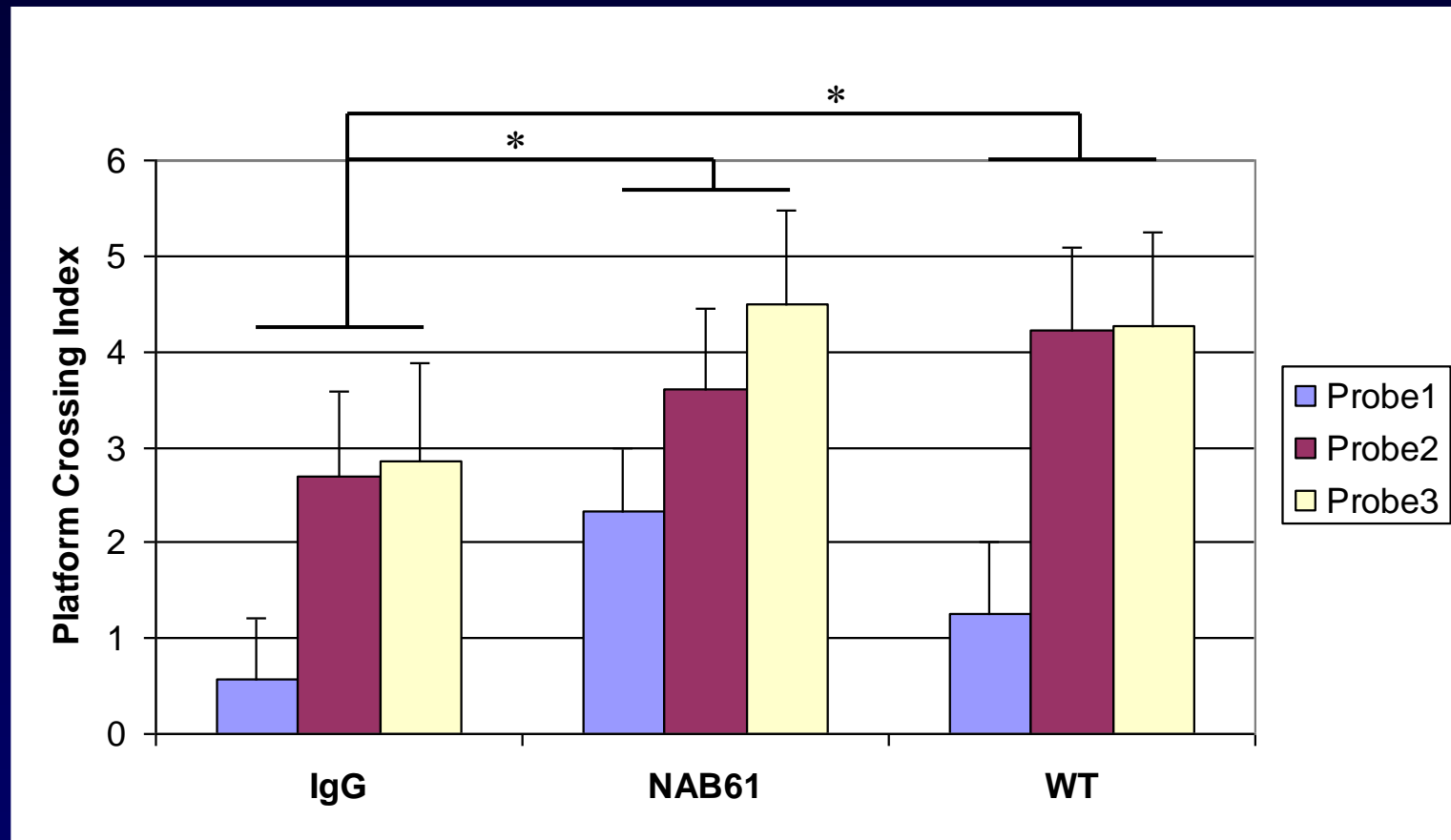
# Spatially Oriented Swimming Behavior: Middle Probe



# Spatially Oriented Swimming Behavior: Late Probe



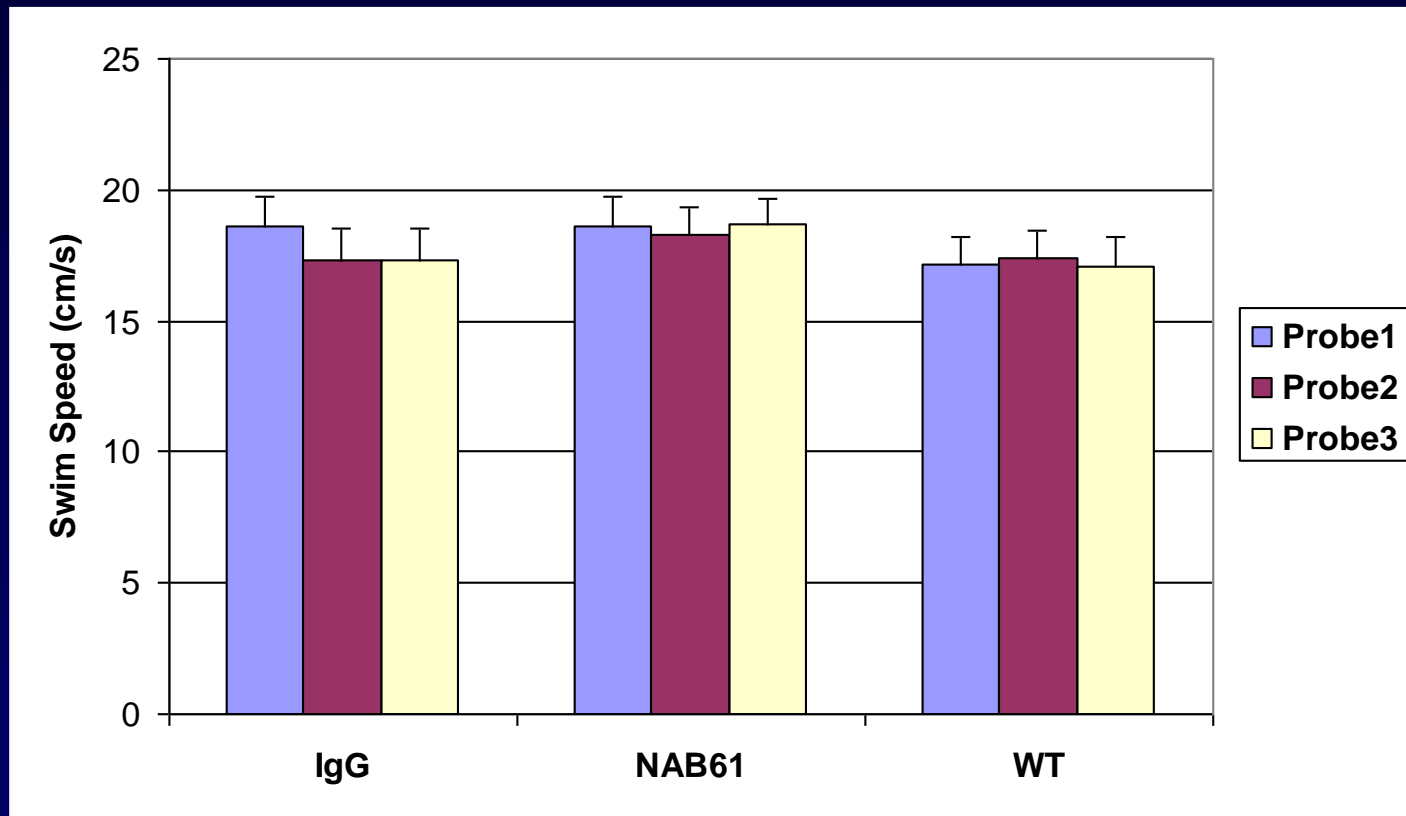
# Spatially Oriented Searching Behavior in NAB61 Immunized tg2576



Platform Crossing Index:  
# Crosses over target platform/  
Average # crosses over other platforms

Repeated measures ANOVA  $p=0.0301$   
(Bonferroni) IgG vs. NAB61  $p<0.05$   
(Neuman-Keul, Fisher) IgG vs. NAB61/wt  $p<0.05$

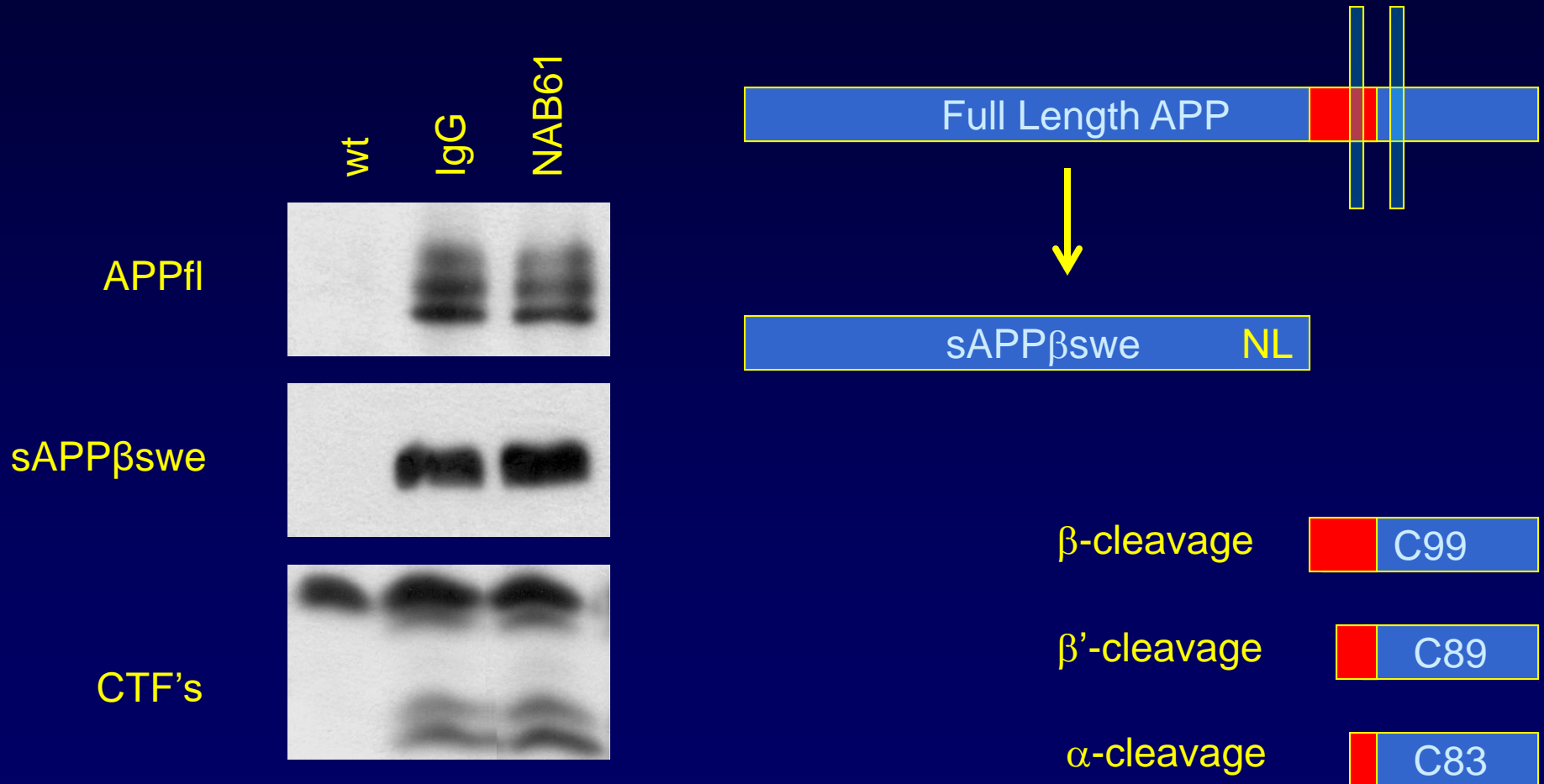
# Improved Behavior is Not Due to Differences in Swimming Speed



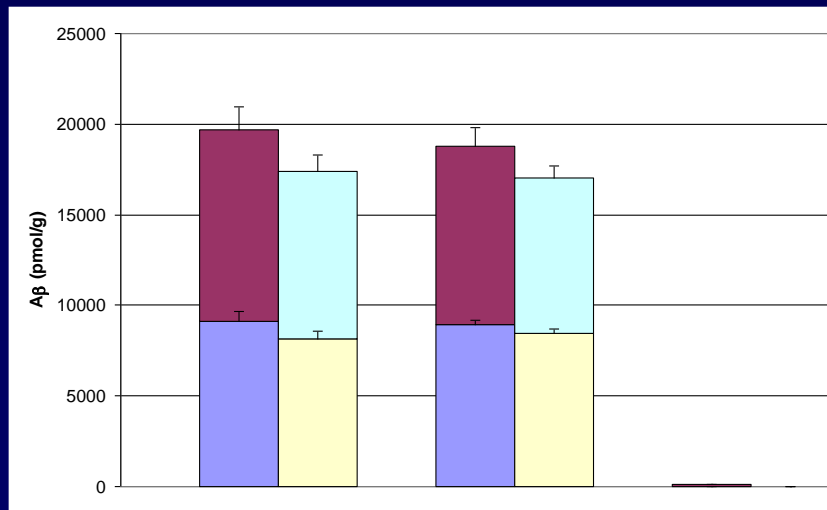
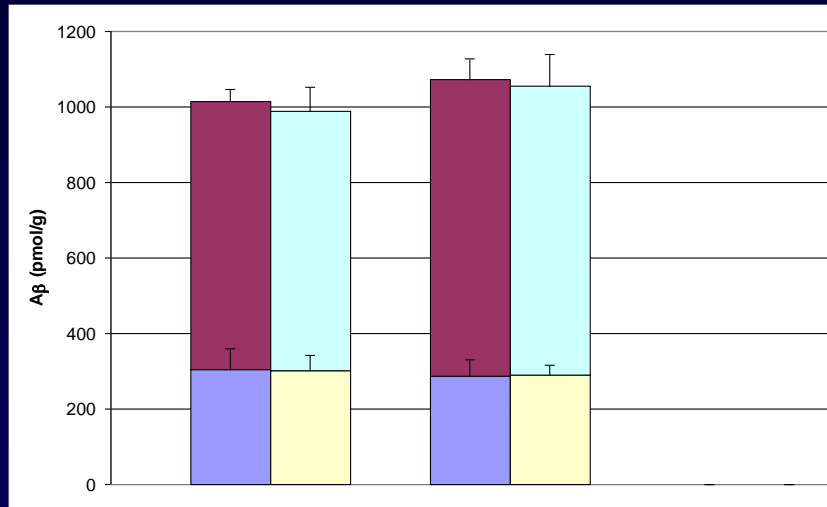
Two-way ANOVA (Group x Probe)  
Treatment  $p=0.3540$   
Probe  $p=0.8284$



# NAB61 Immunization Does Not Affect APP Processing



# Quantification of Brain A $\beta$ After Short Term NAB61 Immunization



**RIPA**

A $\beta$ 42  
Cortex

A $\beta$ 42  
Hipp

A $\beta$ 40  
Cortex

A $\beta$ 40  
Hipp

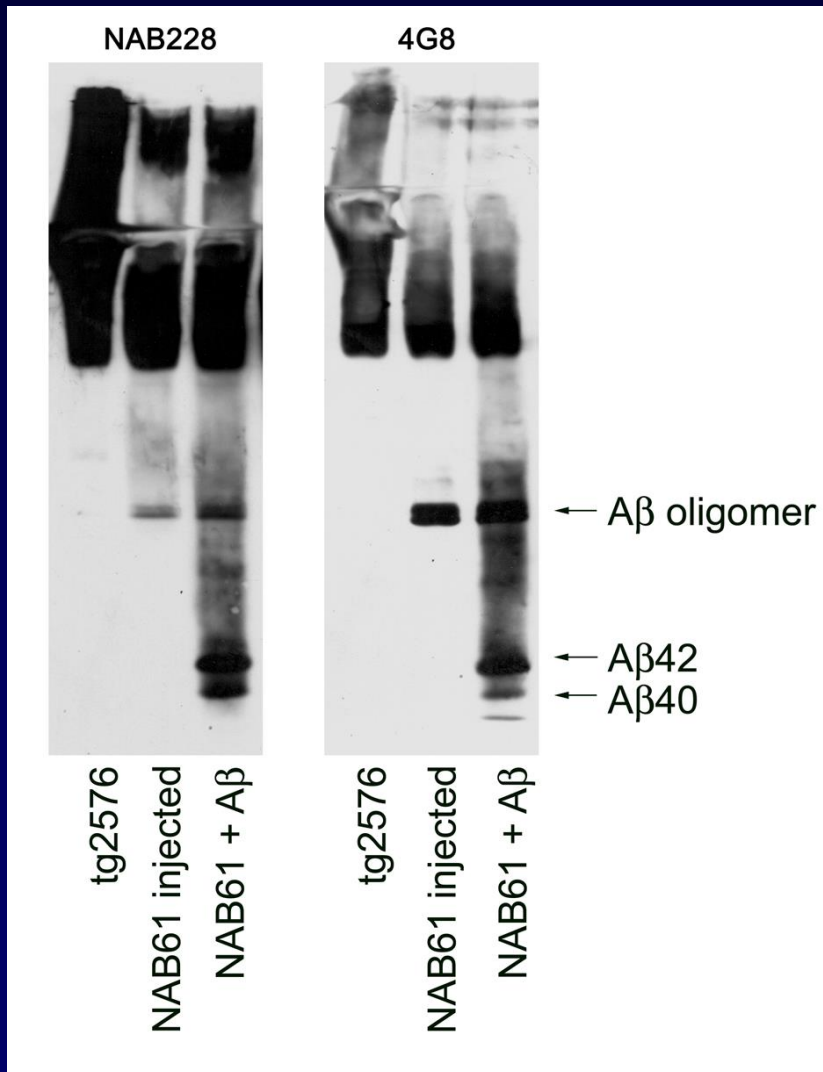
**Formic  
Acid**

**IgG**

**NAB61**

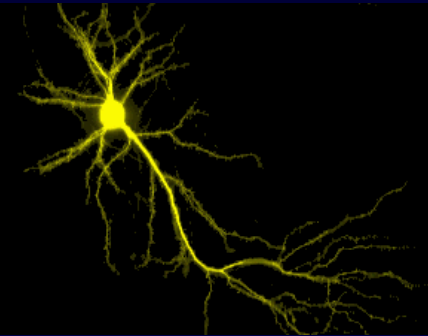
**wt**

# Peripheral Accumulation of Oligomeric A $\beta$ Species???



- tg2576 mice passively immunized with 500  $\mu$ g of NAB61 i.p. or i.v.
- 24 hours post-injection, mice were exsanguinated
- Accumulation of peripheral A $\beta$  was analyzed by electrophoresis of formic acid denatured serum on step gradient acetic acid/urea gels
- Immunoblotting with two different monoclonal antibodies that recognize different epitopes within A $\beta$  demonstrates the accumulation of a higher molecular weight A $\beta$  species

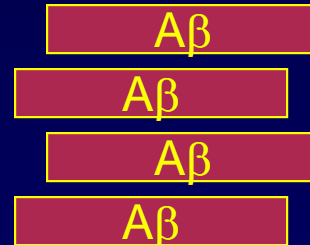
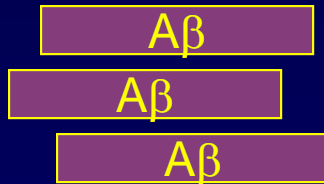
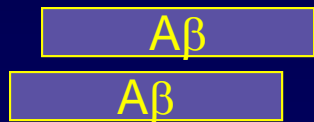
# Production Paradox and Intermediate Impairment



Cellular Metabolism:  
APP, BACE,  $\gamma$ -secretase

→  $A\beta$

Soluble  $A\beta$  Oligomers



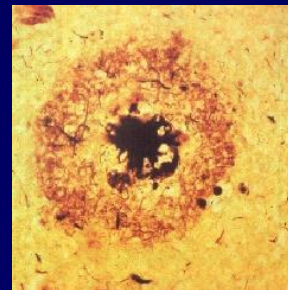
$A\beta$  Fibrillization  
vs.  
 $A\beta$  Degradation

Protofibril



Fibril

Fibril



# Acknowledgements

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- Eddie Lee
- CNDR members
- John Trojanowski
- Ted Abel
- Harry Ischiropoulos
- David Teplow
- Tom Montine

**Supported by grants from the NIA, The Alzheimer's Association  
and the Families of our Patients**





# Some Points For Discussion On Protein Misfolding And Neurodegenerative Diseases

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- Why do only selected brain proteins misfold, fibrillize and deposit with advancing age in neurodegenerative disease brains?
- What is it about the aging that drives this process?
- If misfolding is a core neurodegenerative disease mechanisms, are there interventions to counter misfolding in these disorders?
- Can this be done regardless of the the disease protein?
- When should such therapies be given ( birth, prodrome, onset, etc.)?
- Why do aggregates of misfolded proteins cause neurodegeneration?
- Do they kill by occupying space, by disrupting cellular communications and transport, by loss of function due to sequestration in the aggregates), by toxic gains of functions, by sequestering other key proteins thereby taking them out of action, etc.

# THE SEQUEL TO ADC MEETING

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Society for Neuroscience, Neurobiology of Disease Workshop

**“Protein Misfolding in Neurodegenerative Diseases”**

Friday, 22 Oct., 2004; 8:30 AM – 5:00 PM

## Speakers/Agenda

Dennis Selkoe: Protein Misfolding in Alzheimer's, Parkinson's and Other Neurodegenerative Diseases (with a live or video presentation of an AD or PD patient)

Virginia M.-Y. Lee: Convergence of Tau and Alpha-synuclein Amyloids in Neurodegenerative Diseases

Rick I. Morimoto: Genome-wide Screen for Genes that Regulate Protein Quality Control

Stuart Lipton: Nitrosative/oxidative Stress E3 Ligases and the Ubiquitin Proteasome System in Neurodegeneration

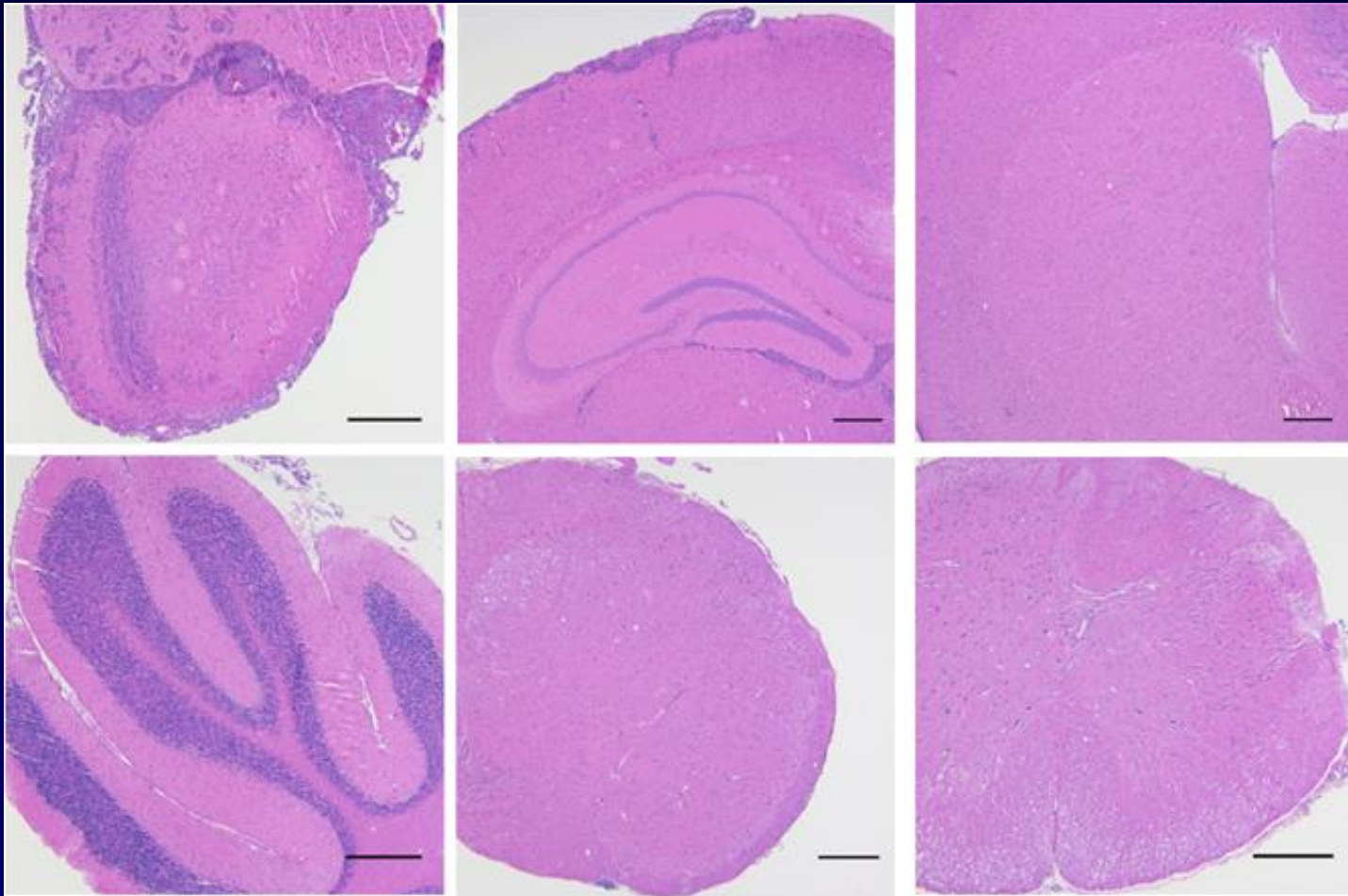
12:00-1:30 Lunch

Breakout Groups: Translational Research in Diseases of Protein Misfolding Each breakout lasts 1-1/2 hour. Students attend 2.



# NAB61 Induced Meningoencephalitis

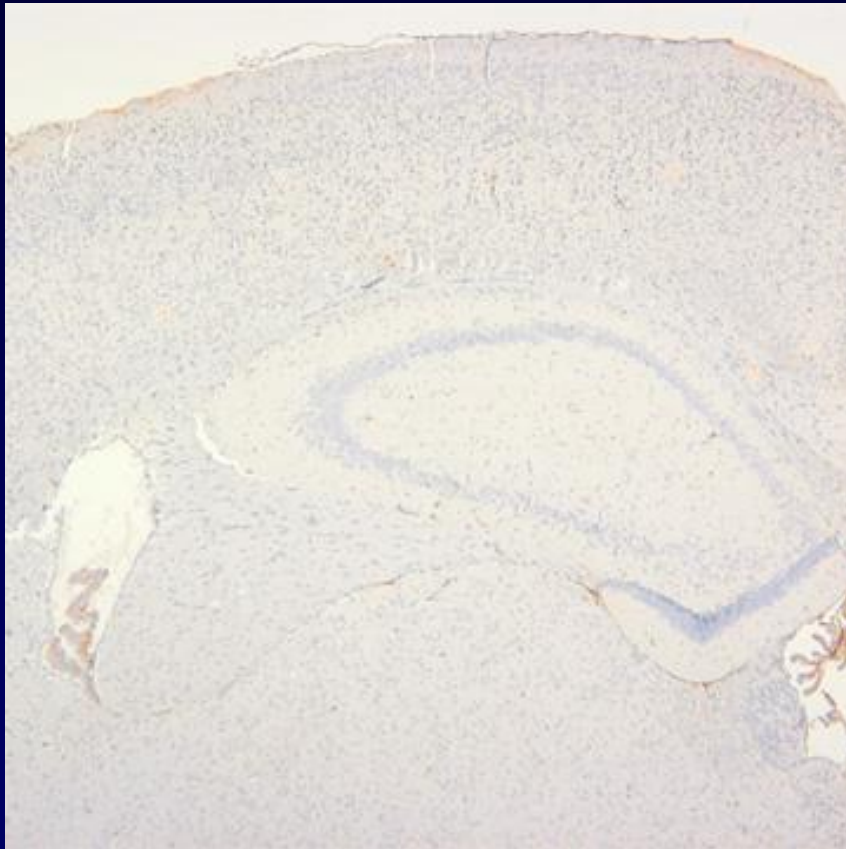
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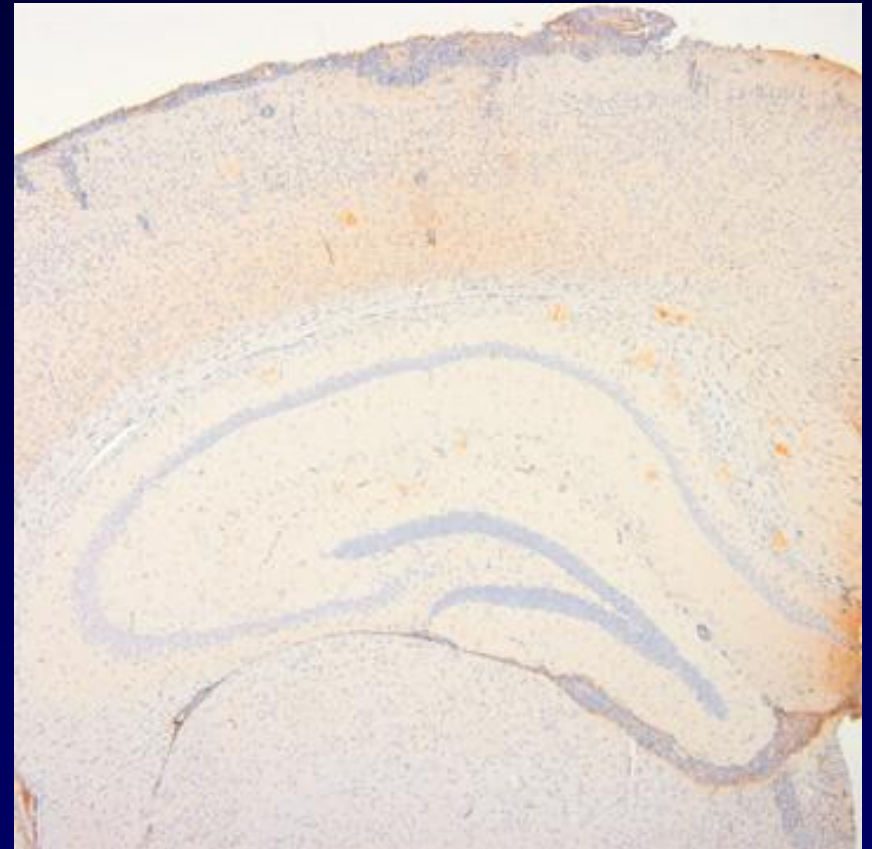


# Disruption of the BBB

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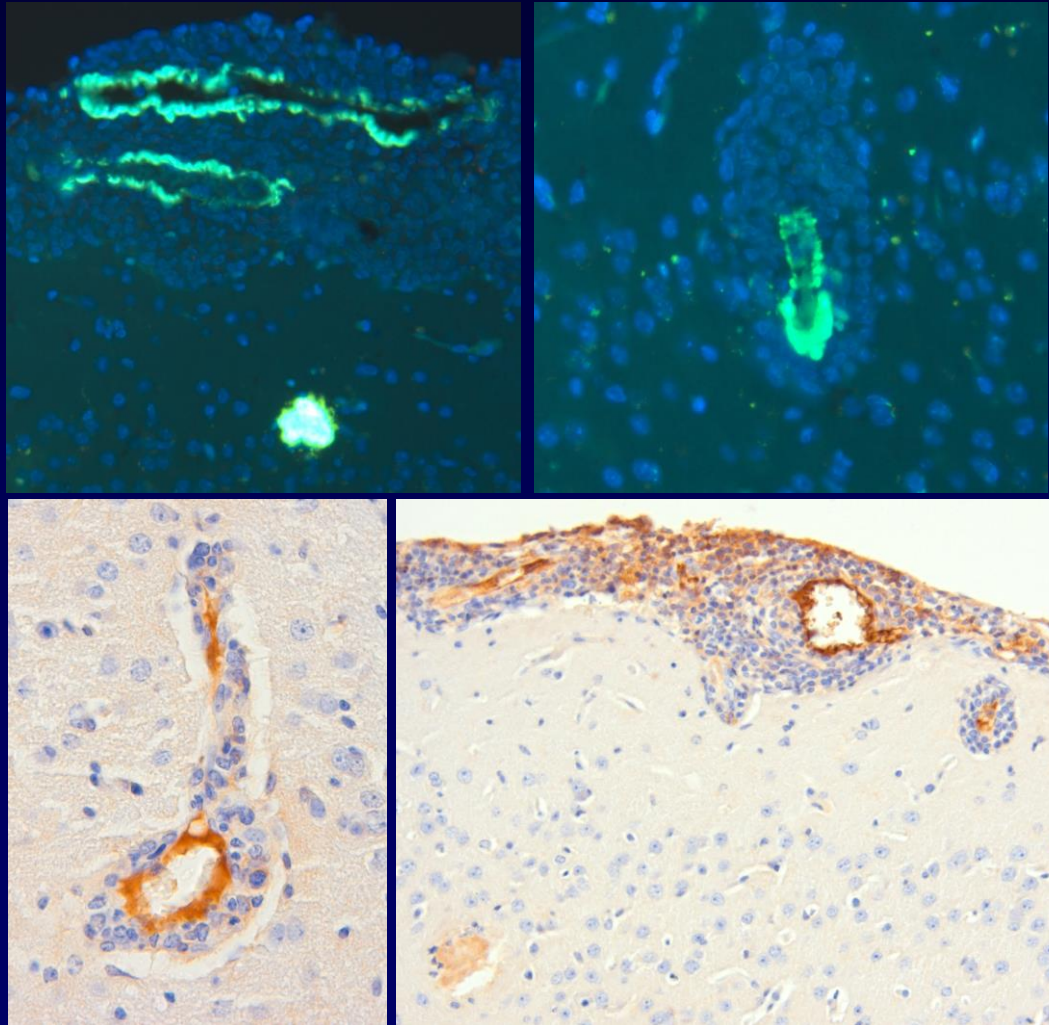
Non-encephalitic  
NAB61 immunized  
tg2576



Encephalitic  
NAB61 immunized  
tg2576

# Vasocentric Mononuclear Infiltrates

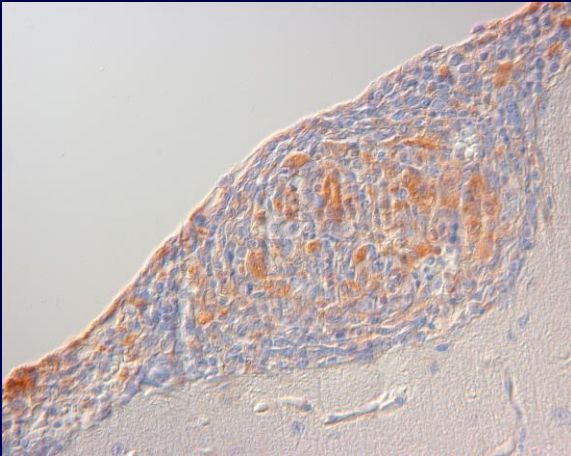
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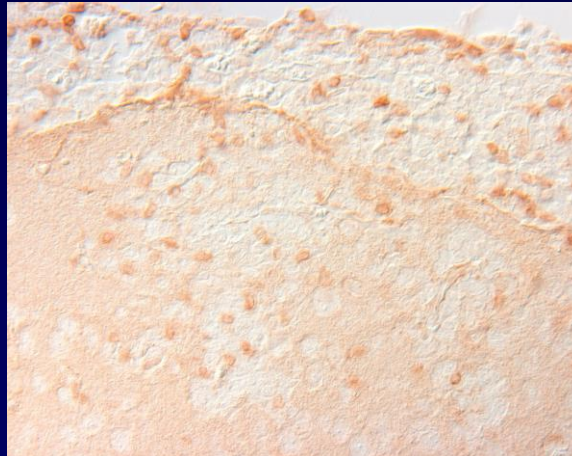
# Lymphocytic Infiltrates

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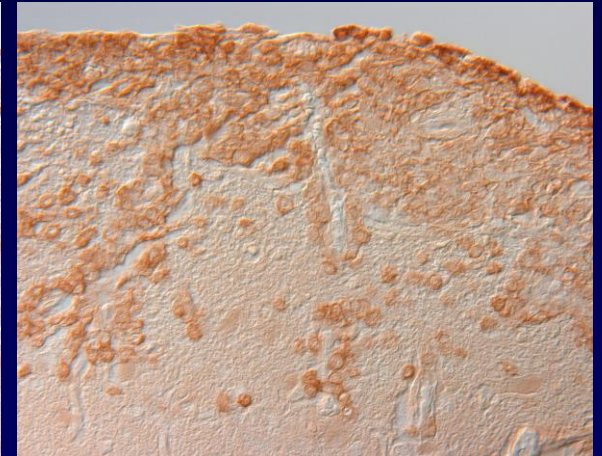
Macrophages (Mac-3)



T Lymphocytes (CD3)

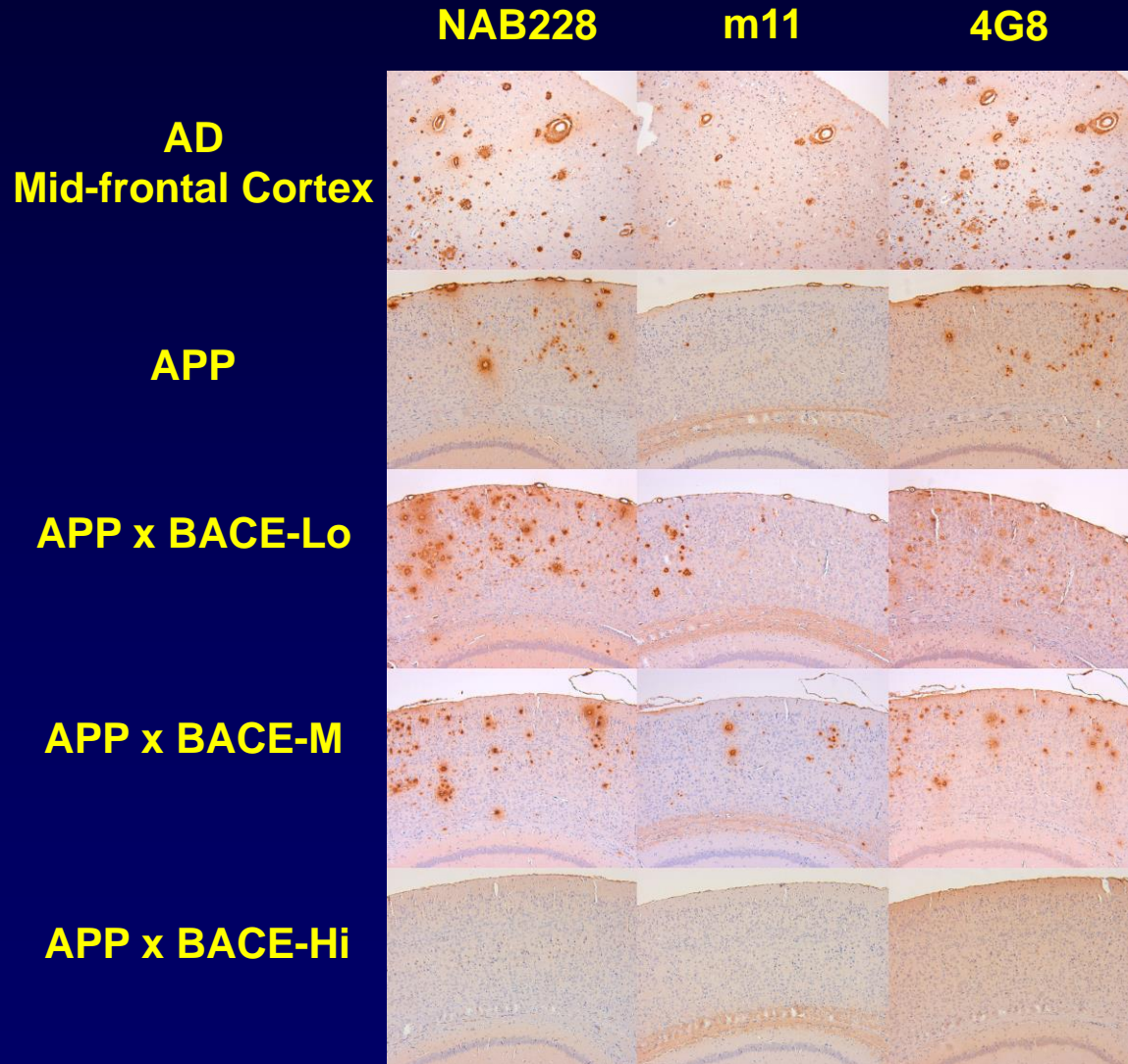


B Lymphocytes (CD45R)

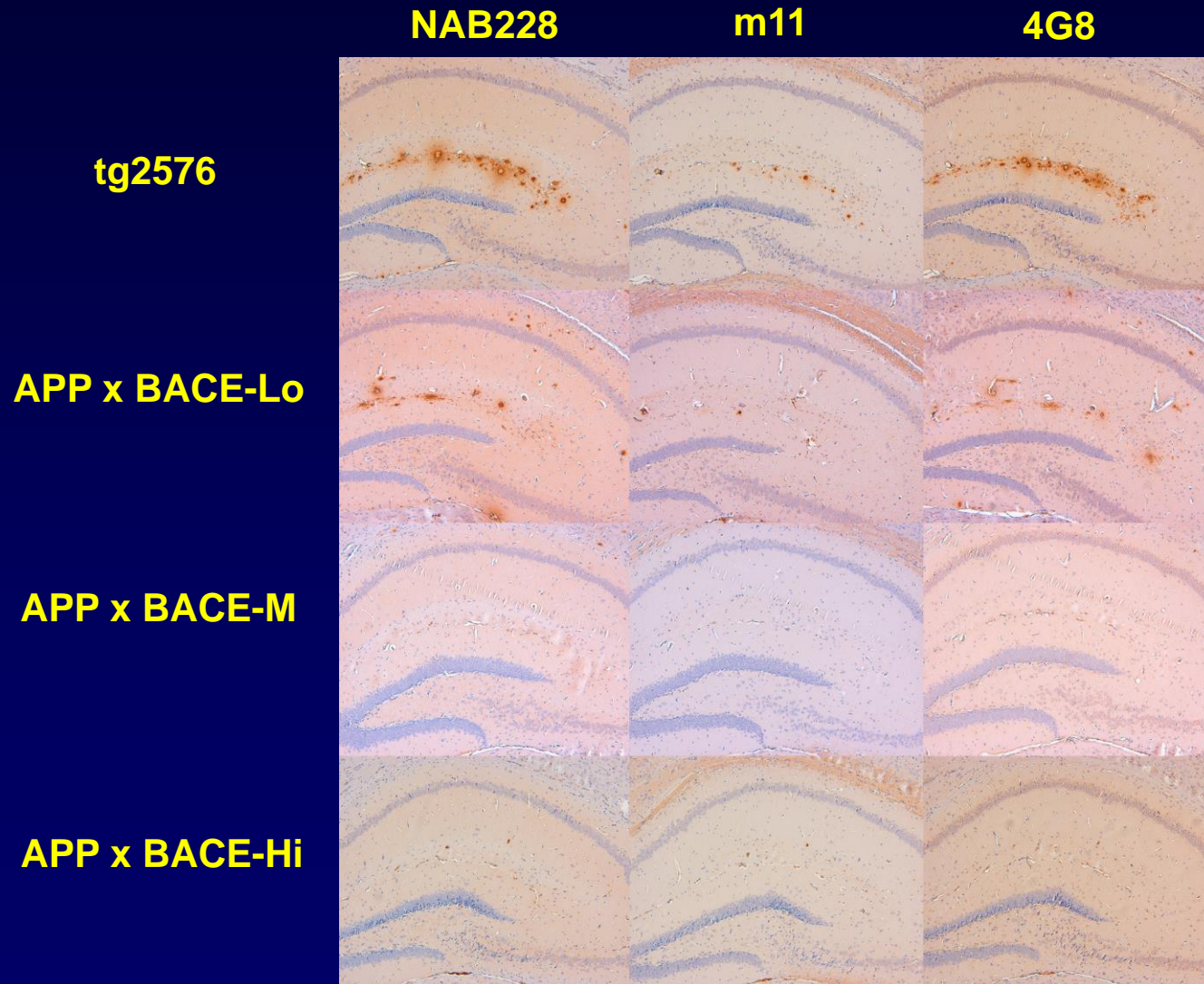




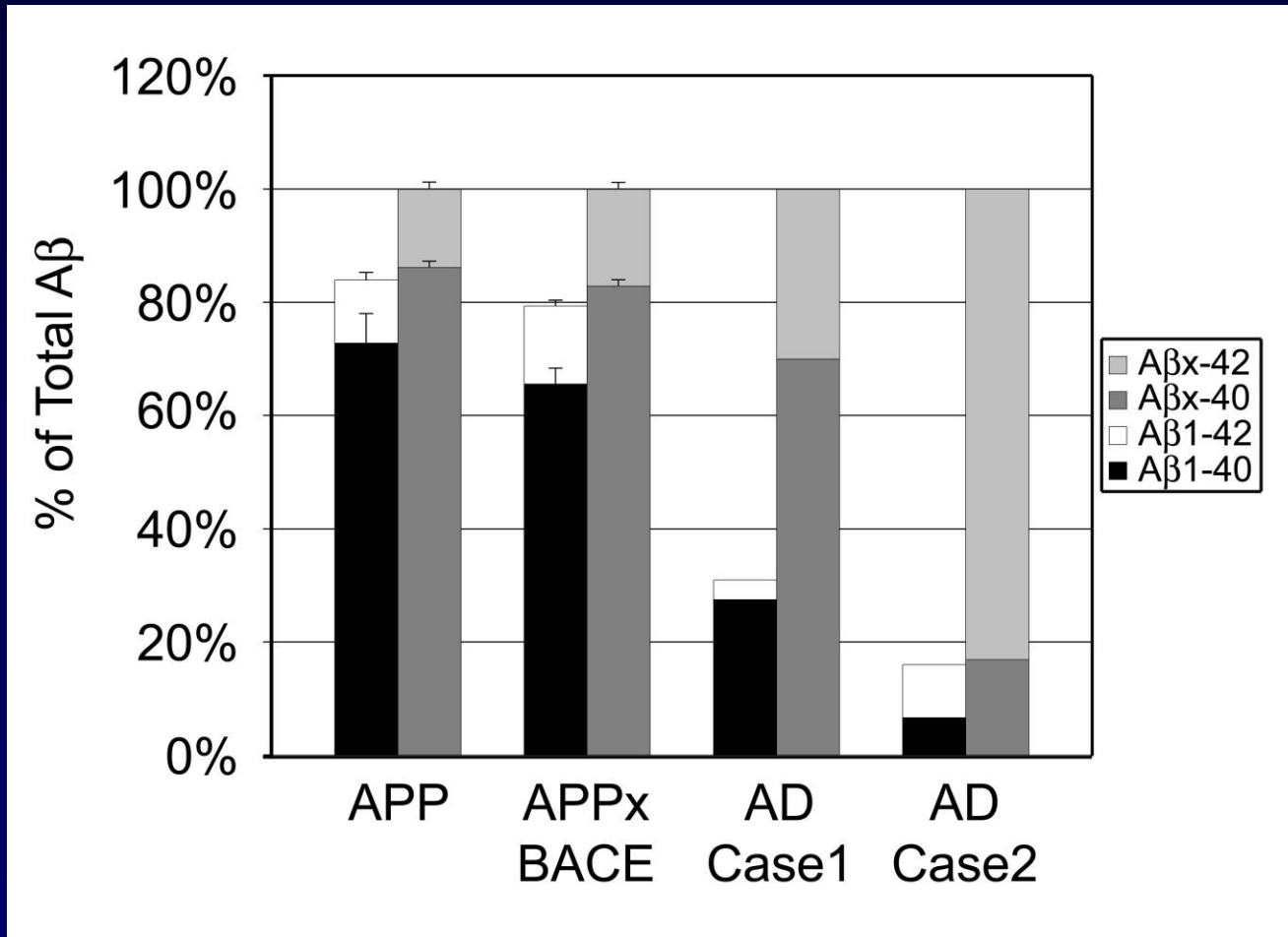
# Limited Deposition of Truncated A $\beta$



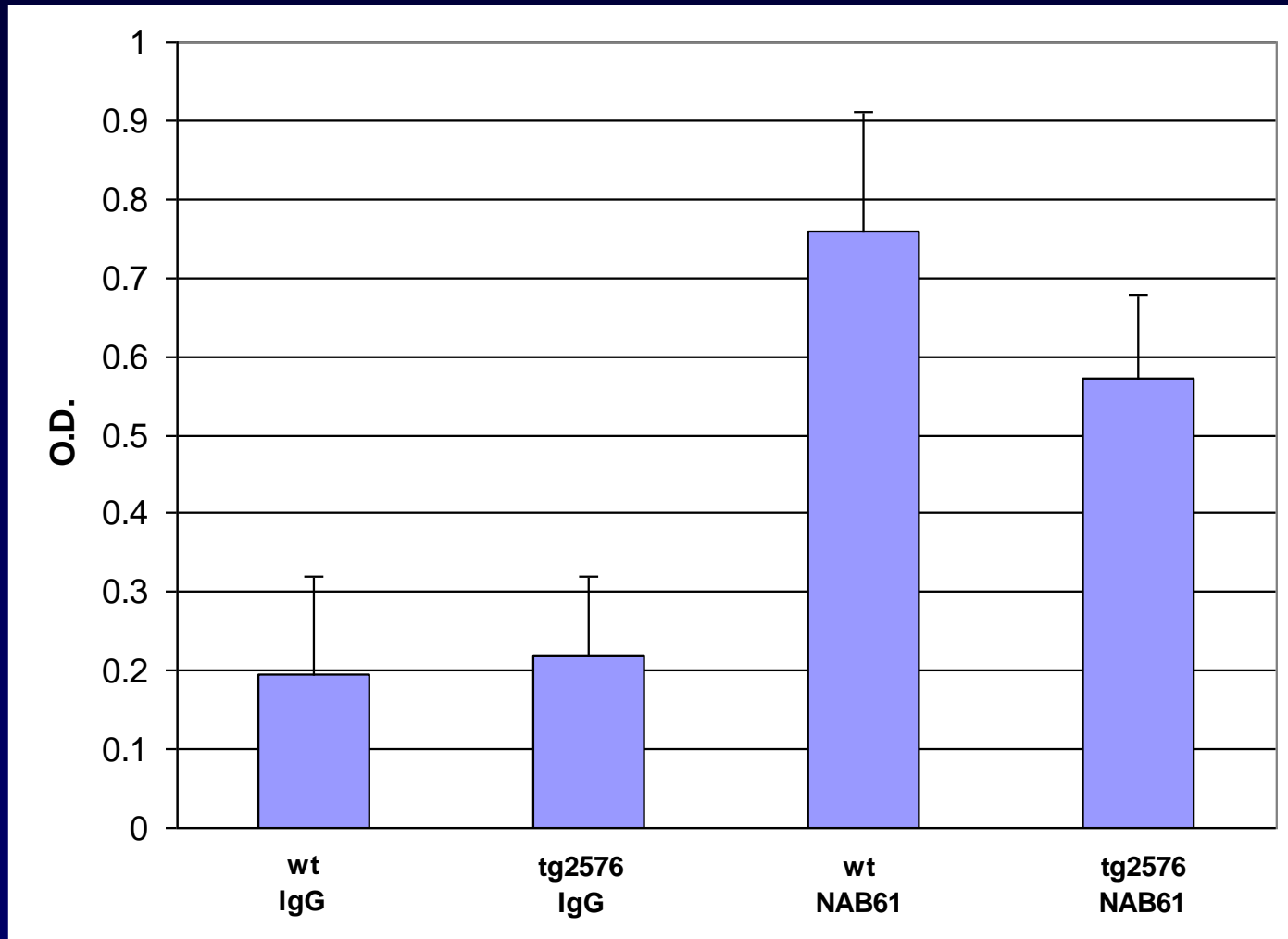
# Limited Deposition of Truncated A $\beta$



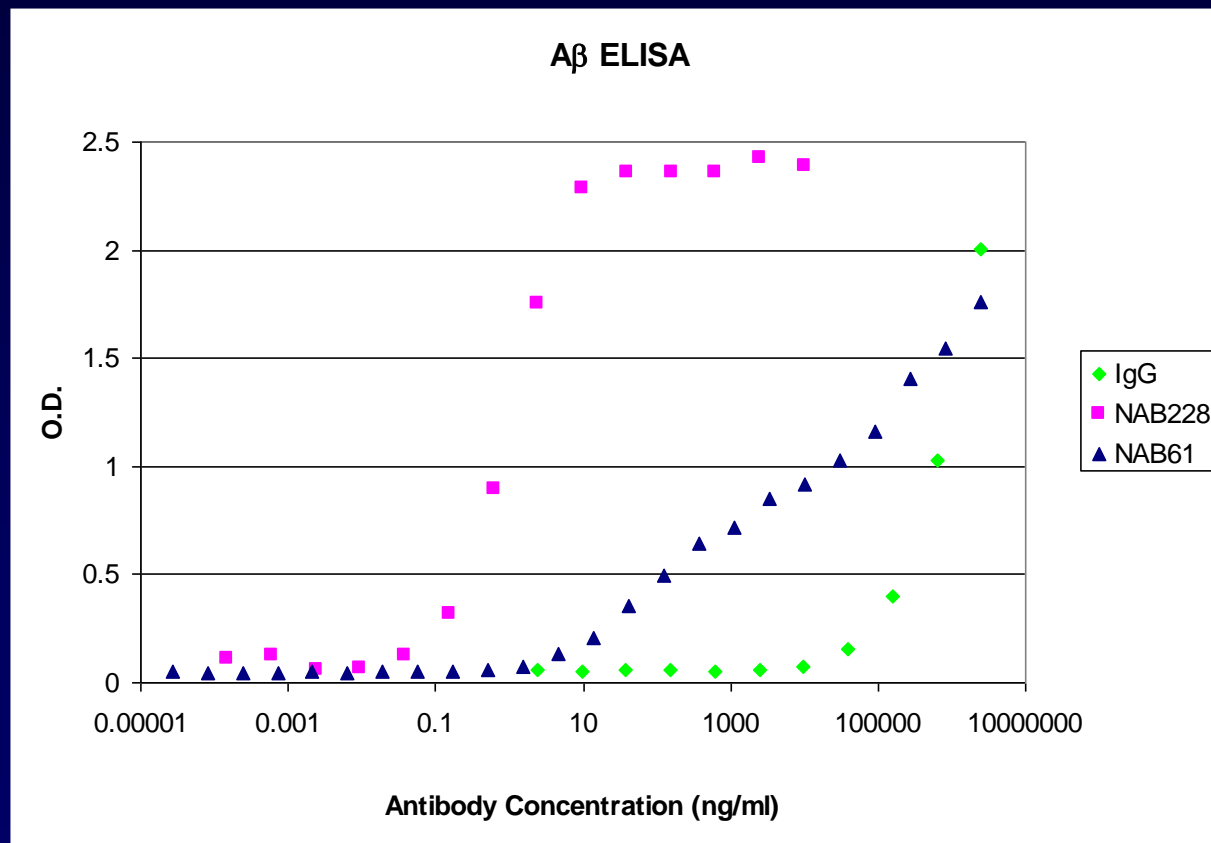
# Limited Deposition of Truncated A $\beta$



# Anti-amyloid Serum Titers



# NAB61 Titer by ELISA





# Serum A $\beta$ After Passive Immunization

