ADC Directors' Meeting
Chicago, April 12, 2008

Amyloid β Degradation & Inflammation: New Therapeutic Strategies in AD

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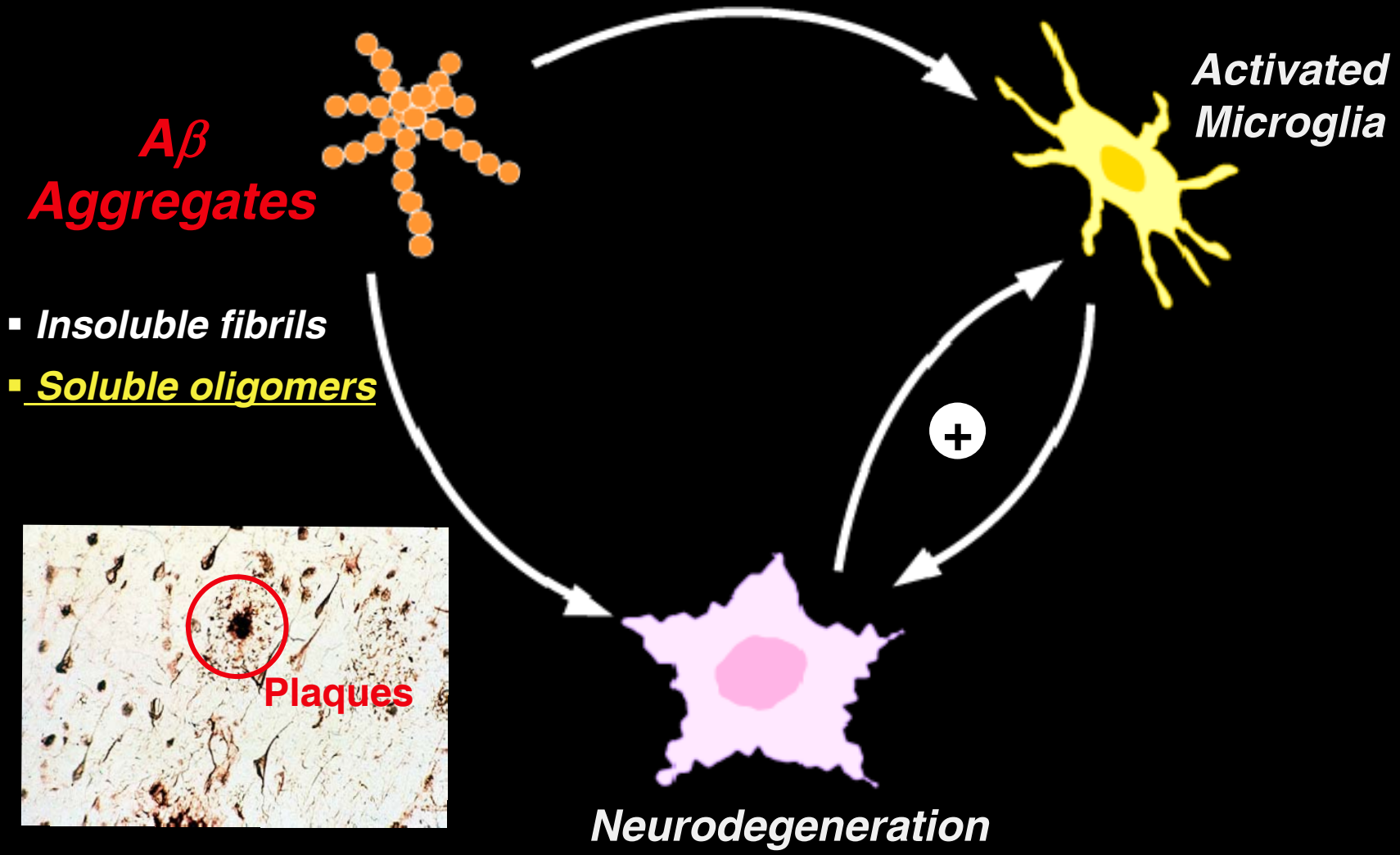
Amyloid β Hypothesis in Alzheimer's Disease



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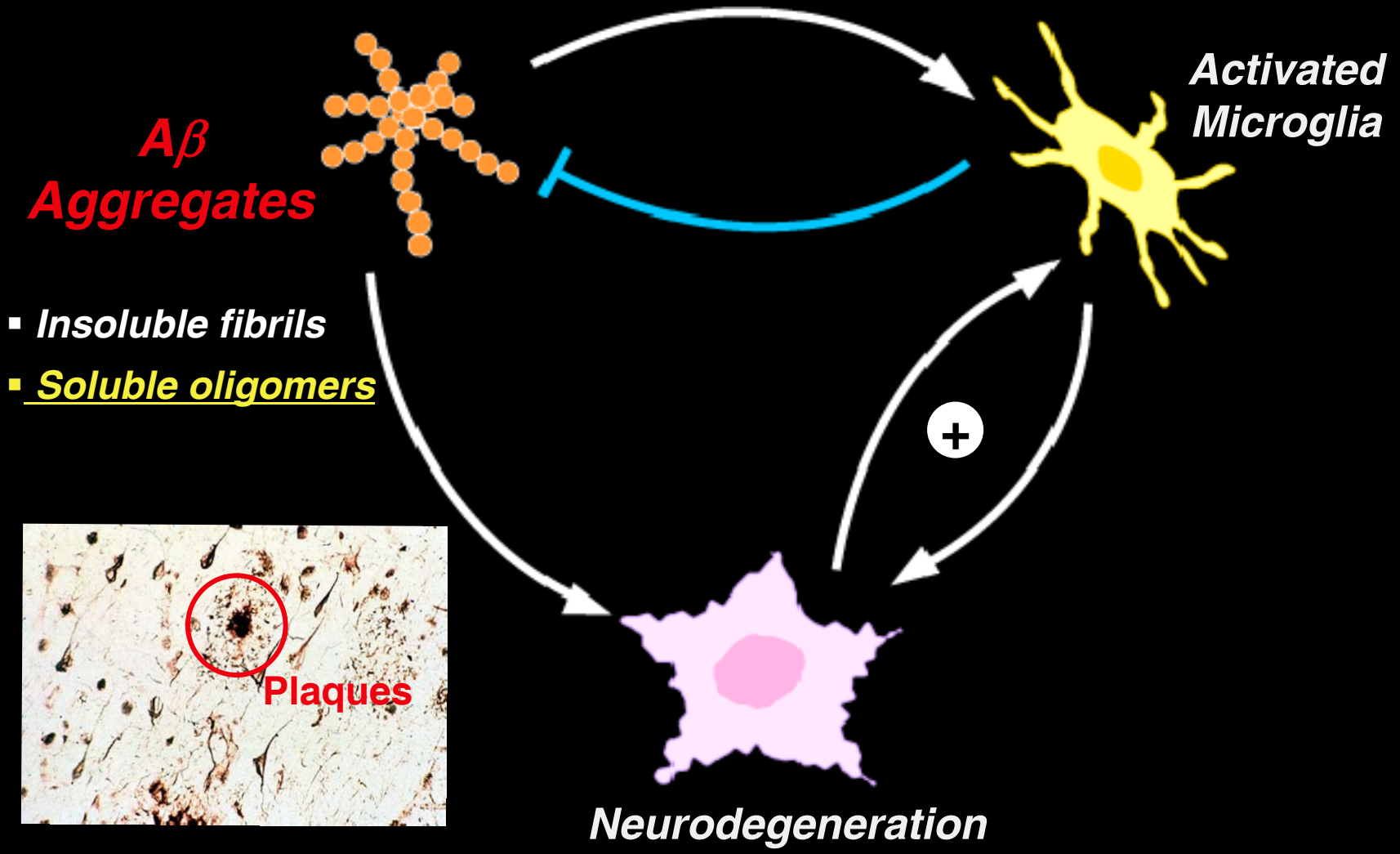
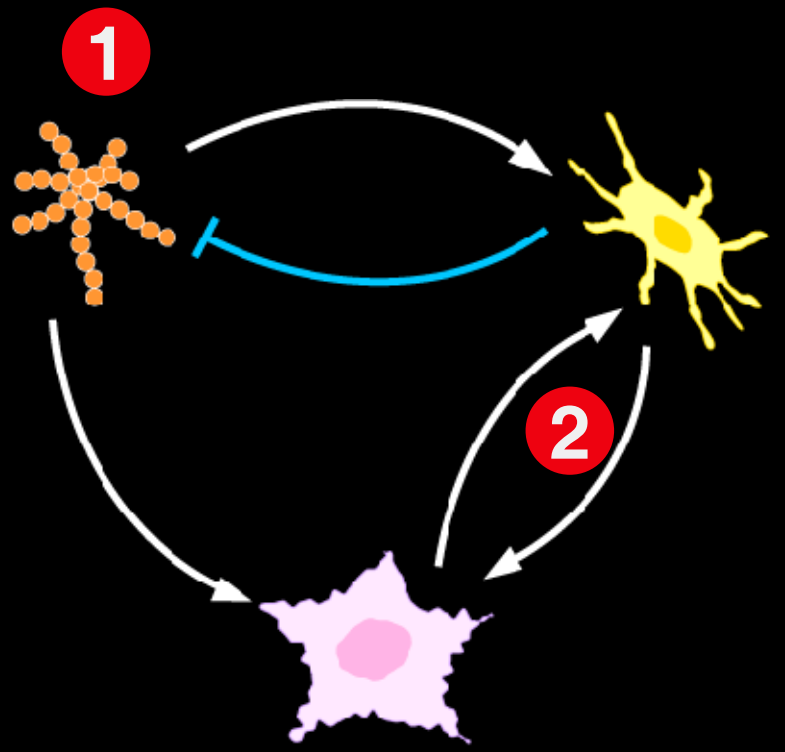


- *Insoluble fibrils*
- *Soluble oligomers*

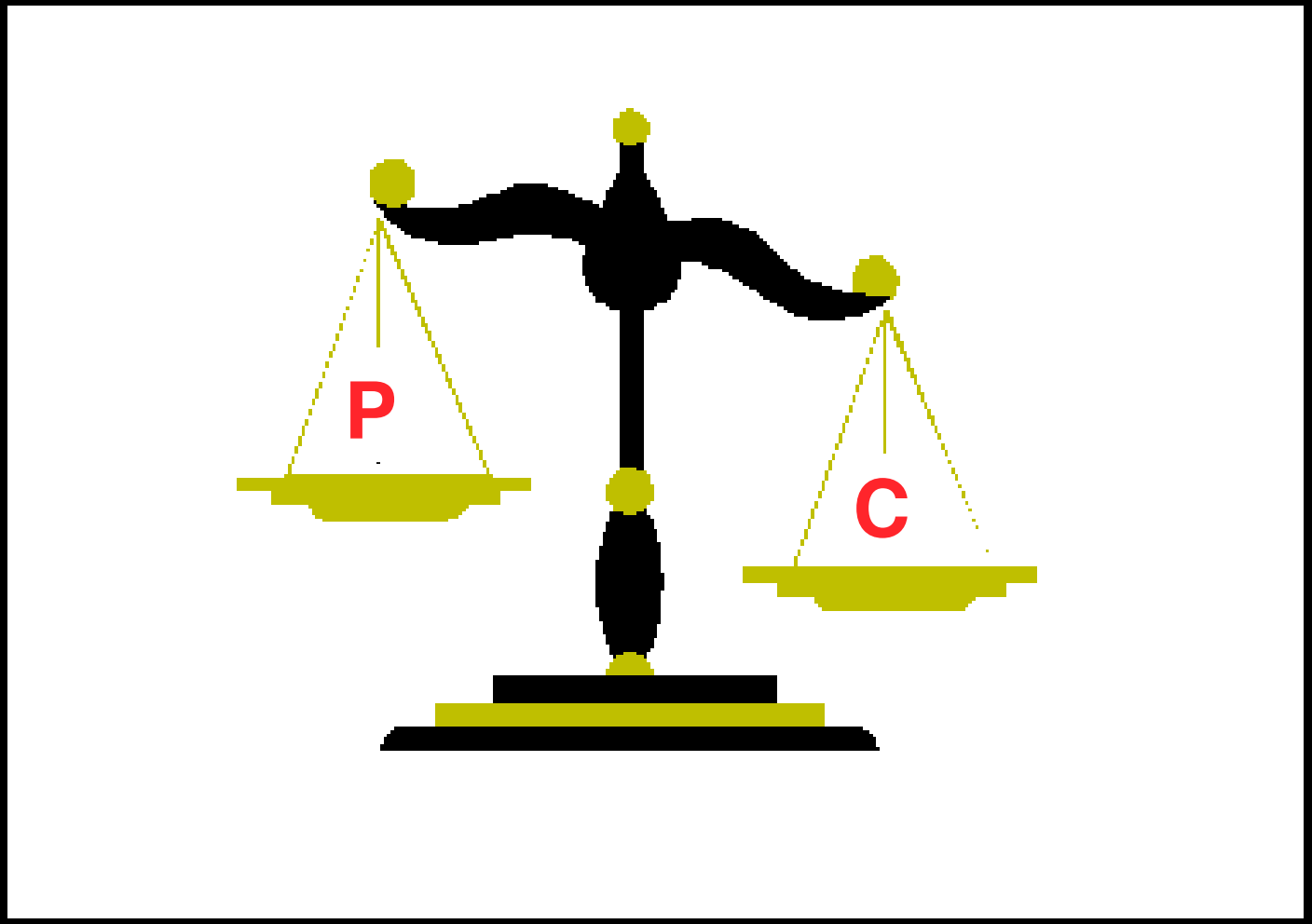
Outline

1. Reduce abnormal accumulation of Aβ by promoting degradation

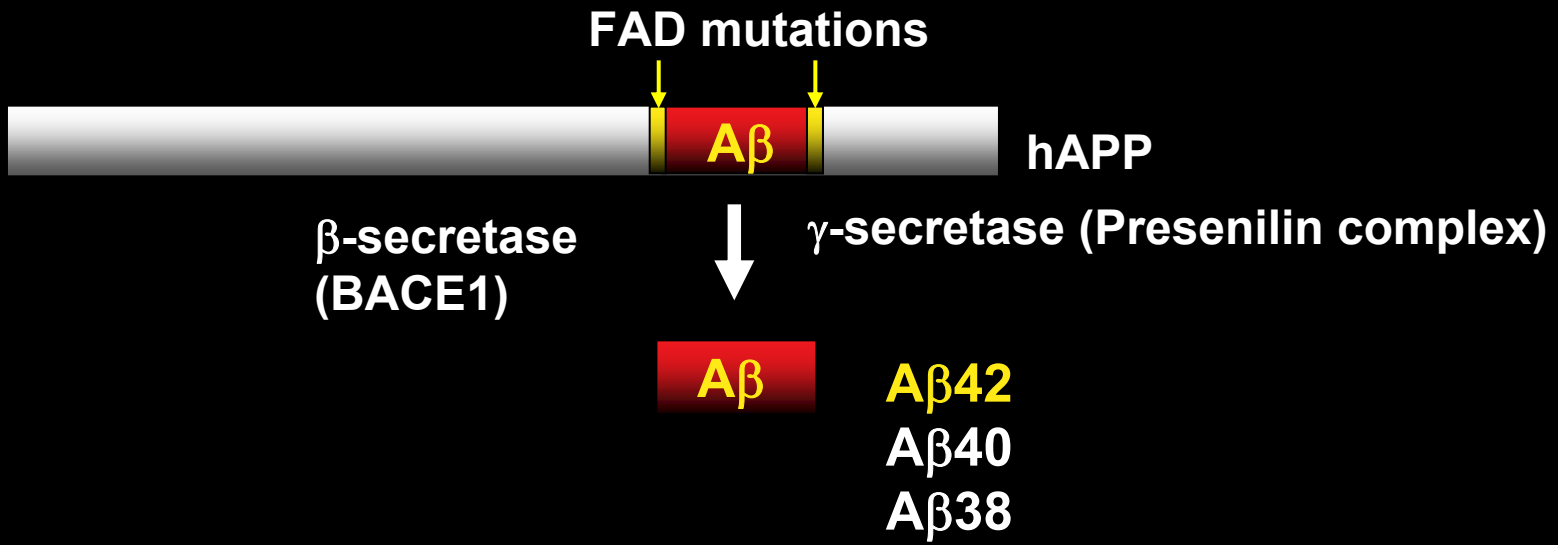
2. Block the toxic pathways in microglia activation



Aβ Accumulation Results From an Imbalance Between Production and Clearance



Generation of Aβ peptides from Amyloid Precursor Protein (APP)



DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA **Aβ1-42**

- More toxic
- More prone to aggregation

The Genetics of Alzheimer's Disease

*Genetic factors are involved in **25 to 40**% of AD cases*

- Early-onset Familial AD

  APP (chromosome 21)
  PSEN1 (chromosome 14)
  PSEN2 (chromosome 1)

↑ **Amyloid β (Aβ42)**

Modified from Tanzi and Bertram, Cell, 2005

The Genetics of Alzheimer's Disease

*Genetic factors are involved in **25 to 40**% of AD cases*

- Early-onset Familial AD

  APP (chromosome 21)
  PSEN1 (chromosome 14)
  PSEN2 (chromosome 1)

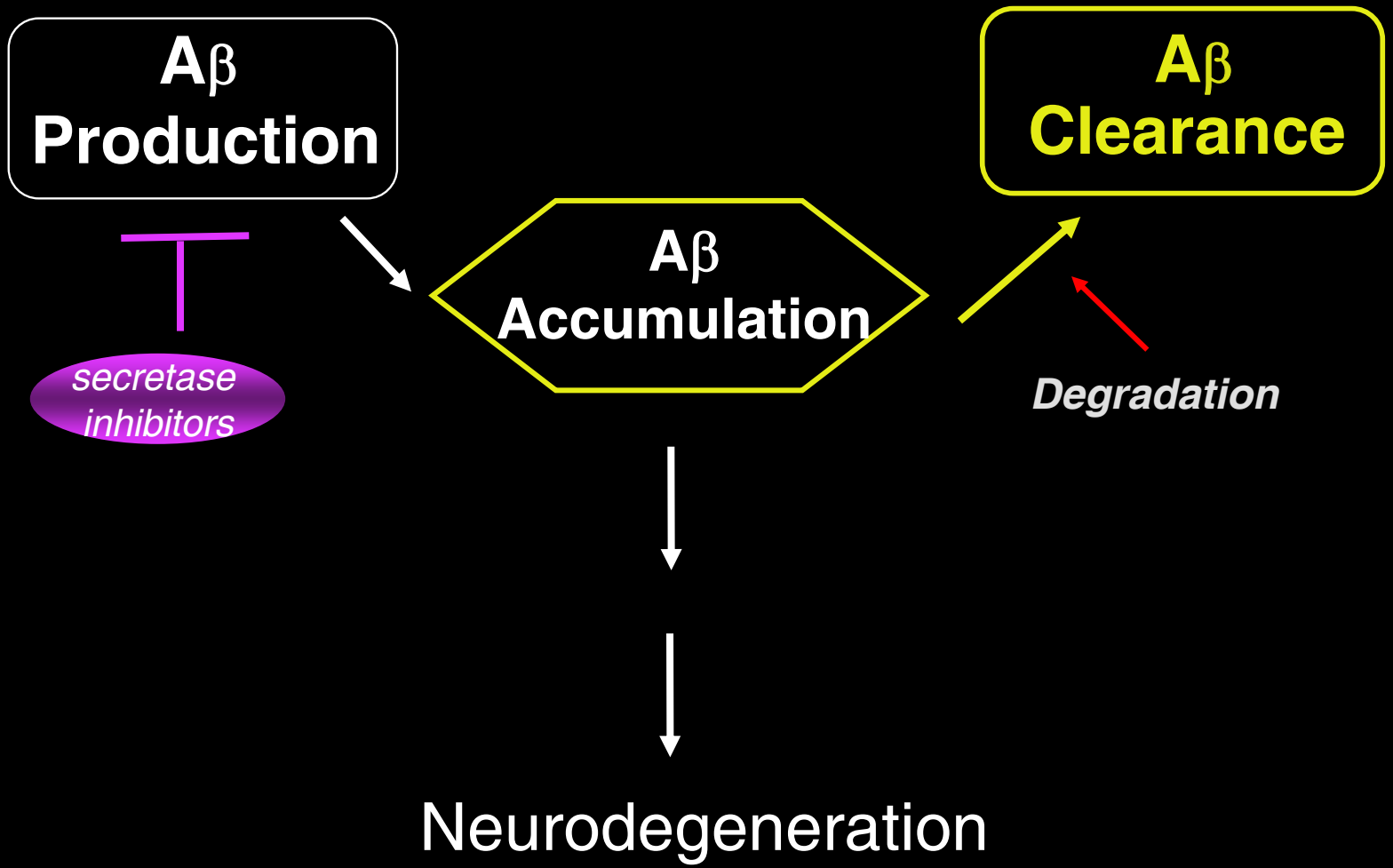
  } *~ 5% AD cases*

- Late-onset Sporadic AD

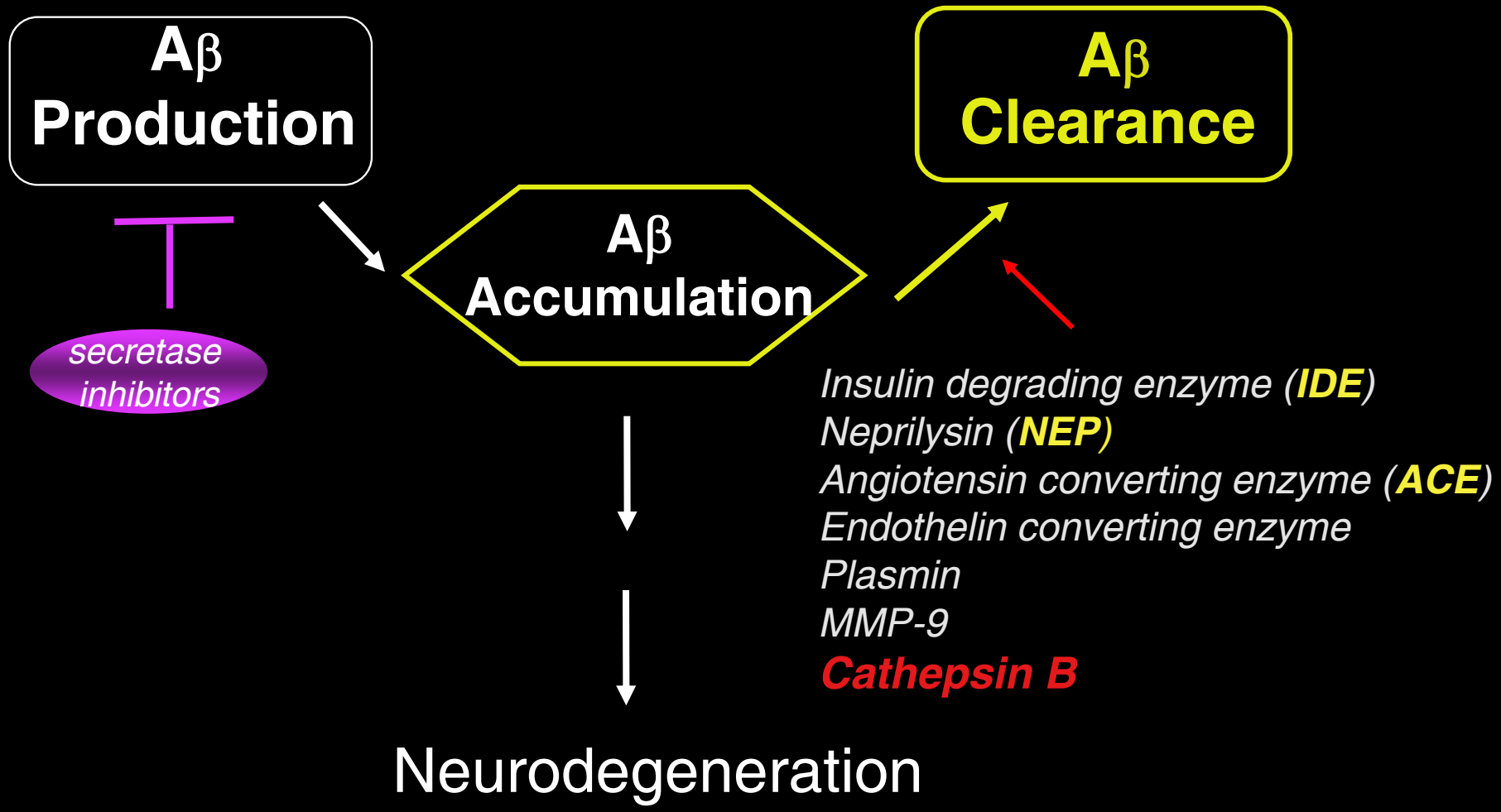
  APOE (chrosome 19) (ε4–allele confers risk)
  > 200 Genes (http://www.alzgene.org)

Modified from Tanzi and Bertram, Cell, 2005

Targeting Aβ Accumulation

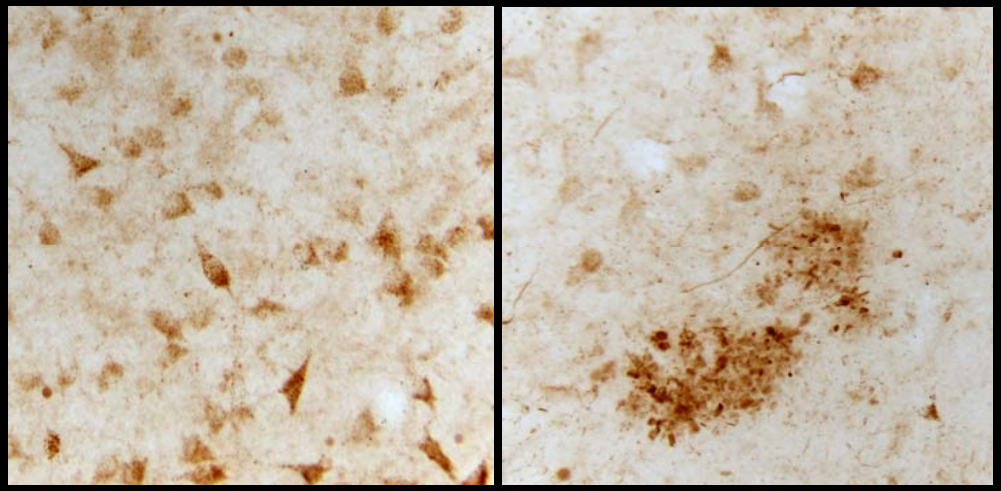


Targeting Aβ Accumulation



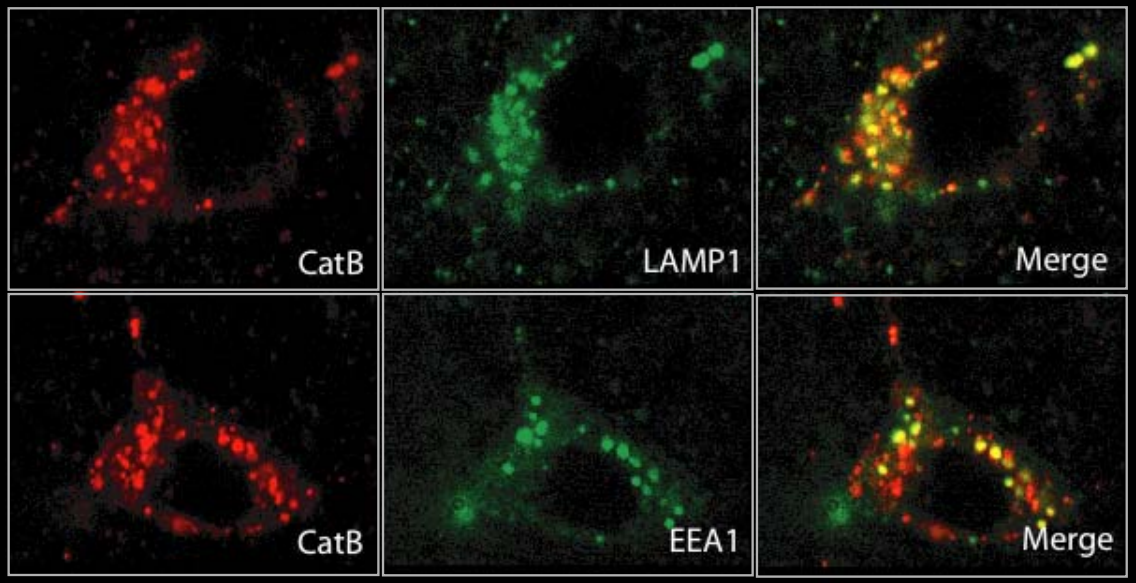
Cathepsin B Is an Cysteine Protease Localized at Amyloid Plaques and Neuronal Endosomes

Control



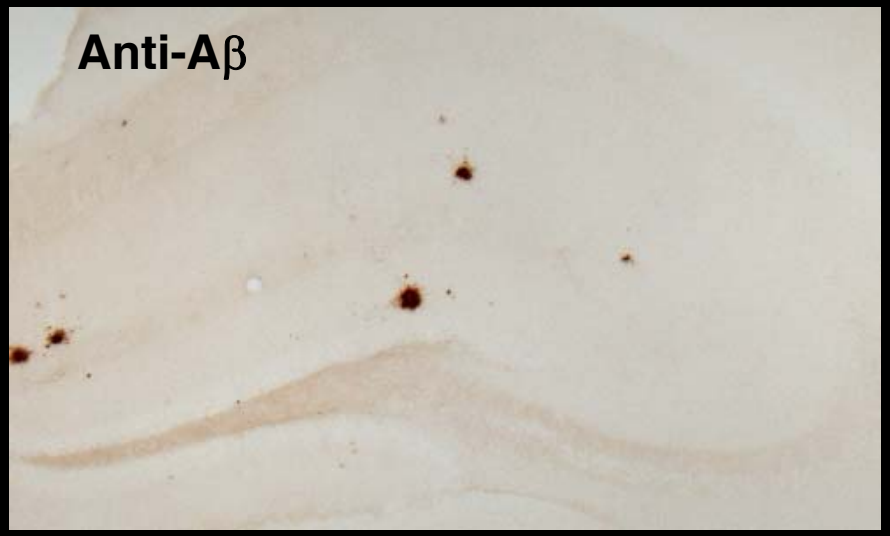
AD

hAPP mice

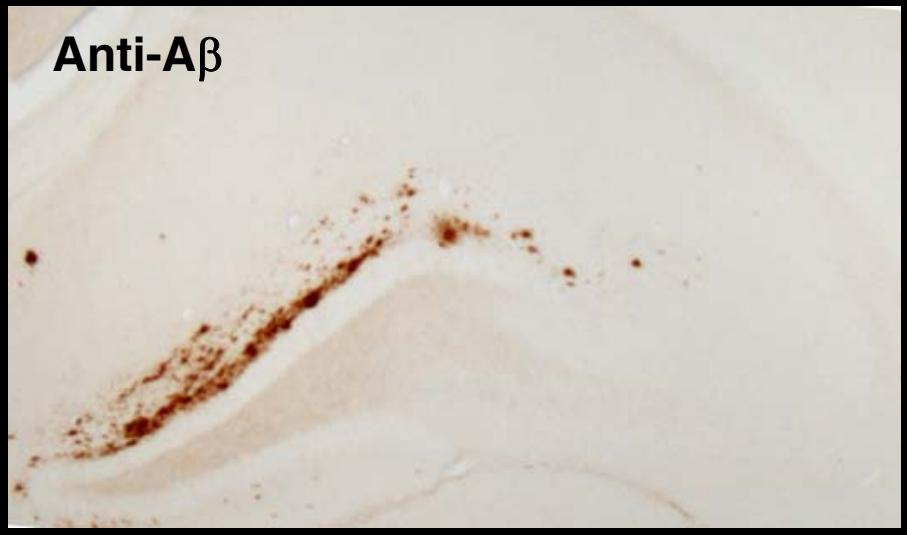


Genetic Ablation of Cathepsin B Increases Aβ Deposition in hAPP Mice

hAPP/CatB$^{+/+}$(n=10)

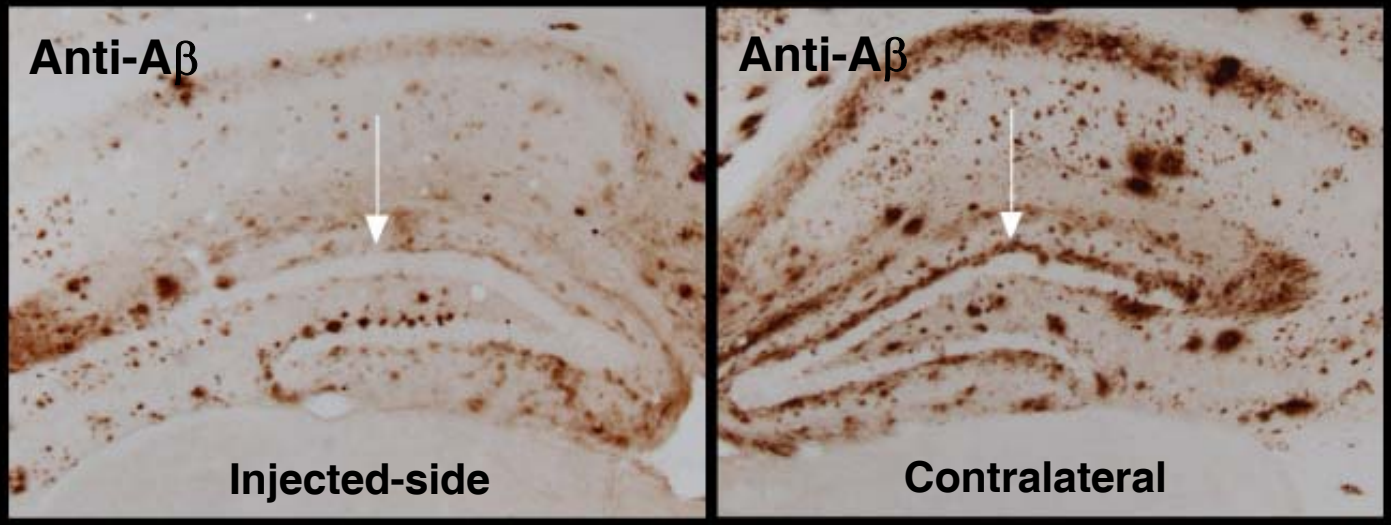


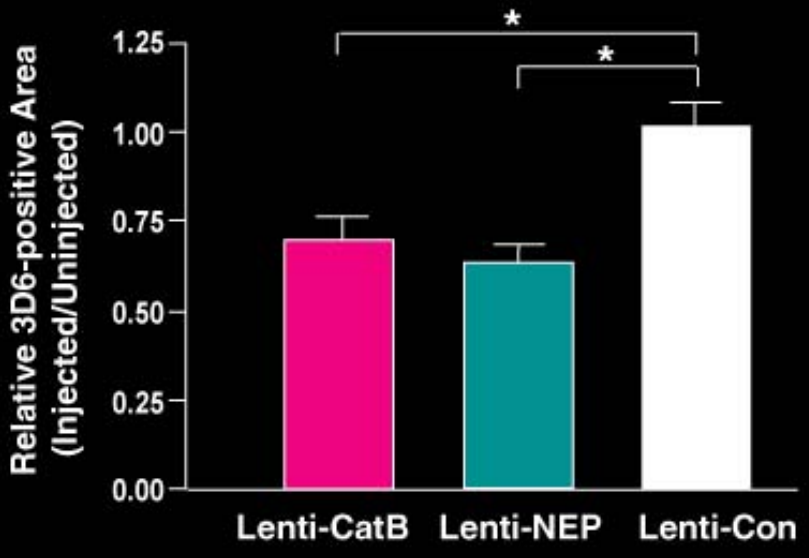
hAPP/CatB$^{-/-}$ (n=12)



Mueller-Steiner et al., Neuron, 51:703-714. (2006)

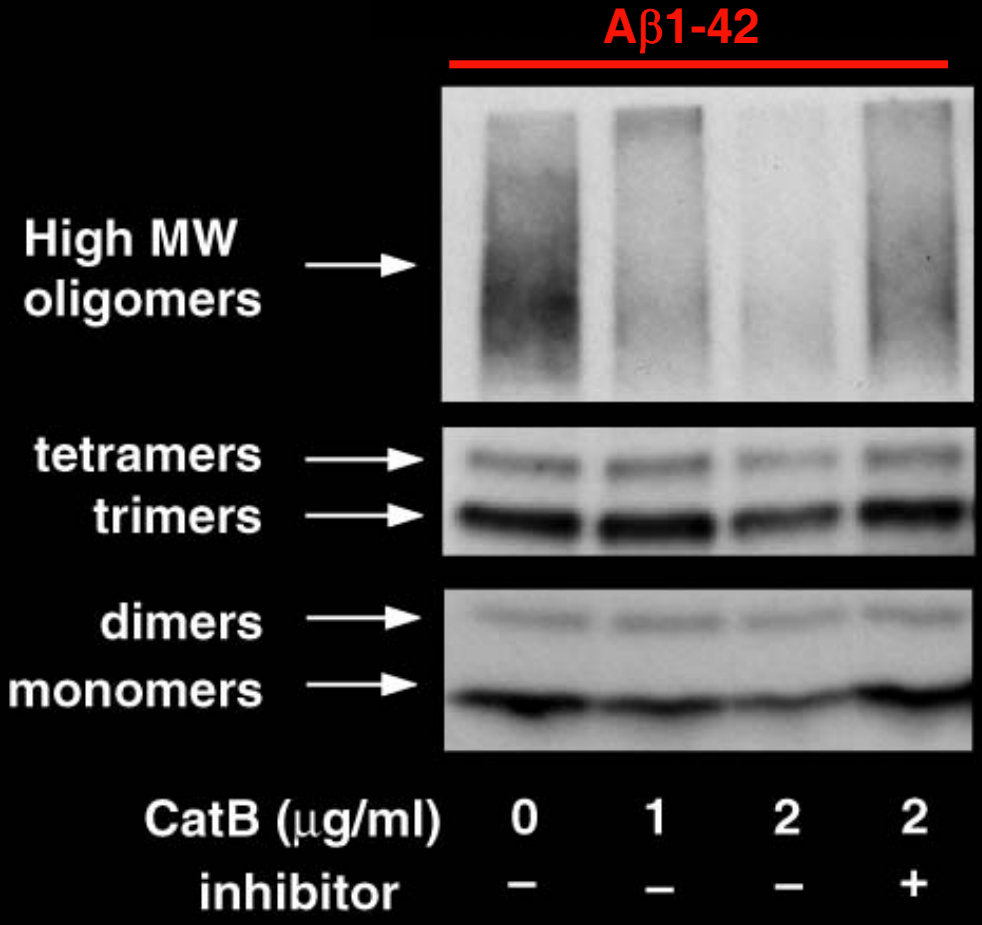
Cathepsin B and Neprilysin Gene Transfer Reduces Aβ Deposits in the Dentate Gyrus of hAPP Mice





Mueller-Steiner et al., Neuron, 51:703-714. (2006)

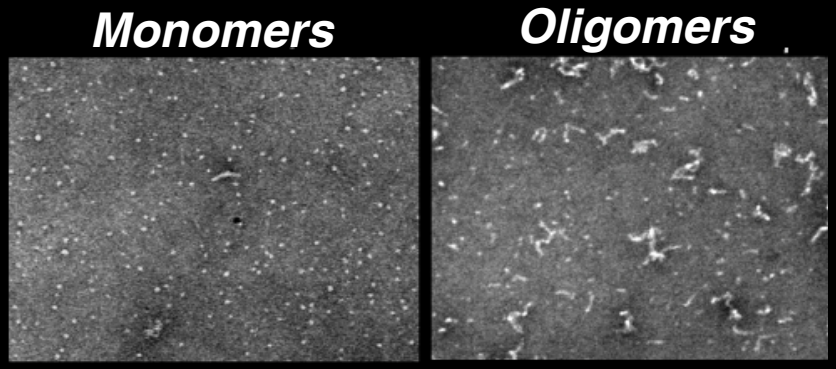
Cathepsin B Degrades Synthetic Aβ1-42 Oligomers Under Cell-free Conditions



Mueller-Steiner et al., Neuron, 51:703-714. (2006)

Analysis of CatB-induced Cleavage of Aβ1-42

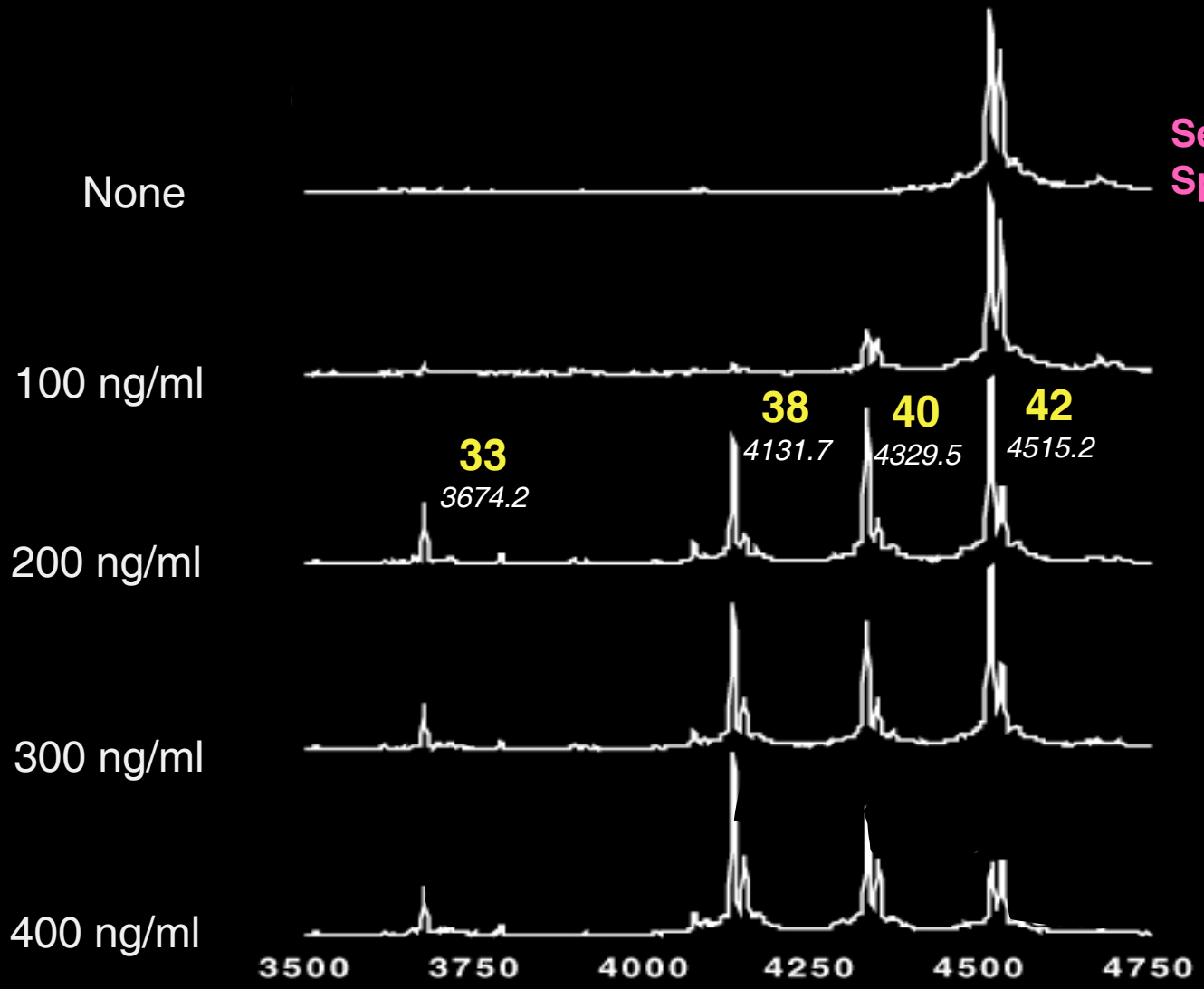
Negative Staining EM



Incubate with purified Cathepsin B

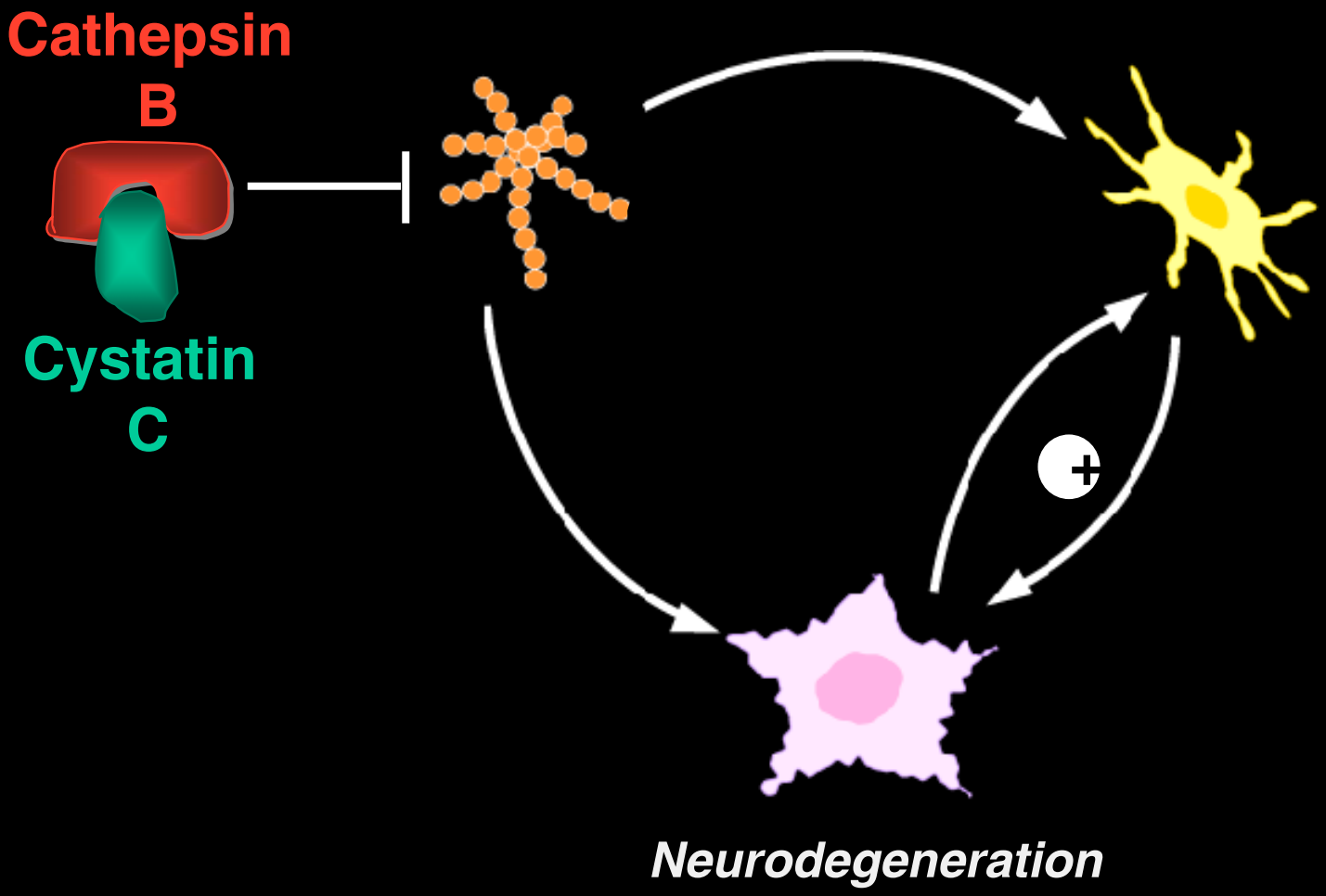
Seldi-TOF Mass Spectrometry (Ciphergen Biosystems)

Cathepsin B Truncates Aβ1-42 in a Dose-Dependent Manner



Seldi-TOF Mass Spectrometry

Cystatin C Is an Endogenous Inhibitor of Cathepsin B



The Genetics of Alzheimer's Disease

- Early-onset Familial AD

  APP (21q21)

  PSEN1 (14q24)

  PSEN2 (1q42)

- Late-onset Sporadic AD

  APOE (19q13) (ε4–allele confers risk)

  > 200 Genes (http://www.alzgene.org)

  (Meta-analysis confirmed: *ACE, CHRNB2,* ***CST3****, ESR1, GAPDHS, IDE, MTHFR, NCSTN, PRNP, PSEN1, TF, TFAM and TNF*..)

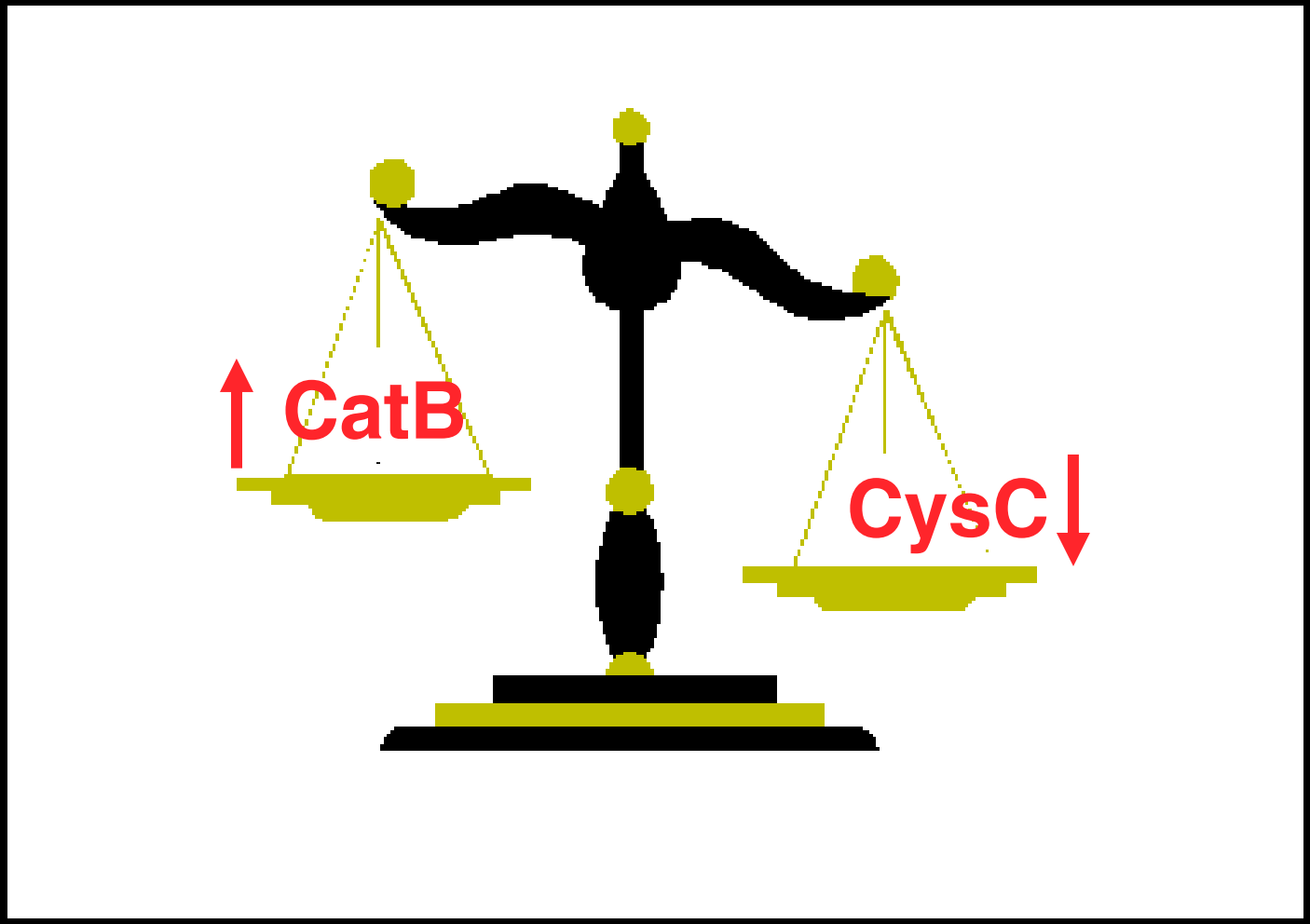
Modified from Tanzi and Bertram, Cell, 2005

Cystatin C in AD

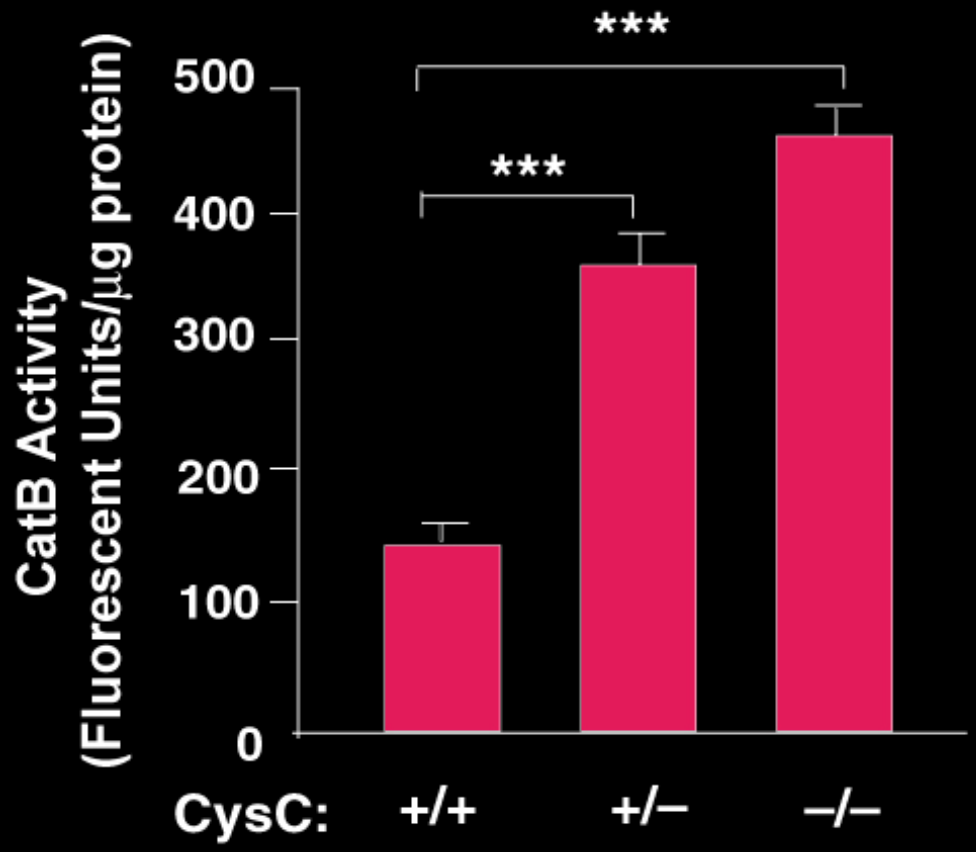
- Polymorphism associated with higher risk for late-onset AD
- Increased in the CSF of AD patients and a subset of neurons in AD-related animal models
- Inhibit Aβ fibrillization through direct binding to Aβ

Kaeser SA et al., & Mi W et al.,
Nature Genetics, 2007

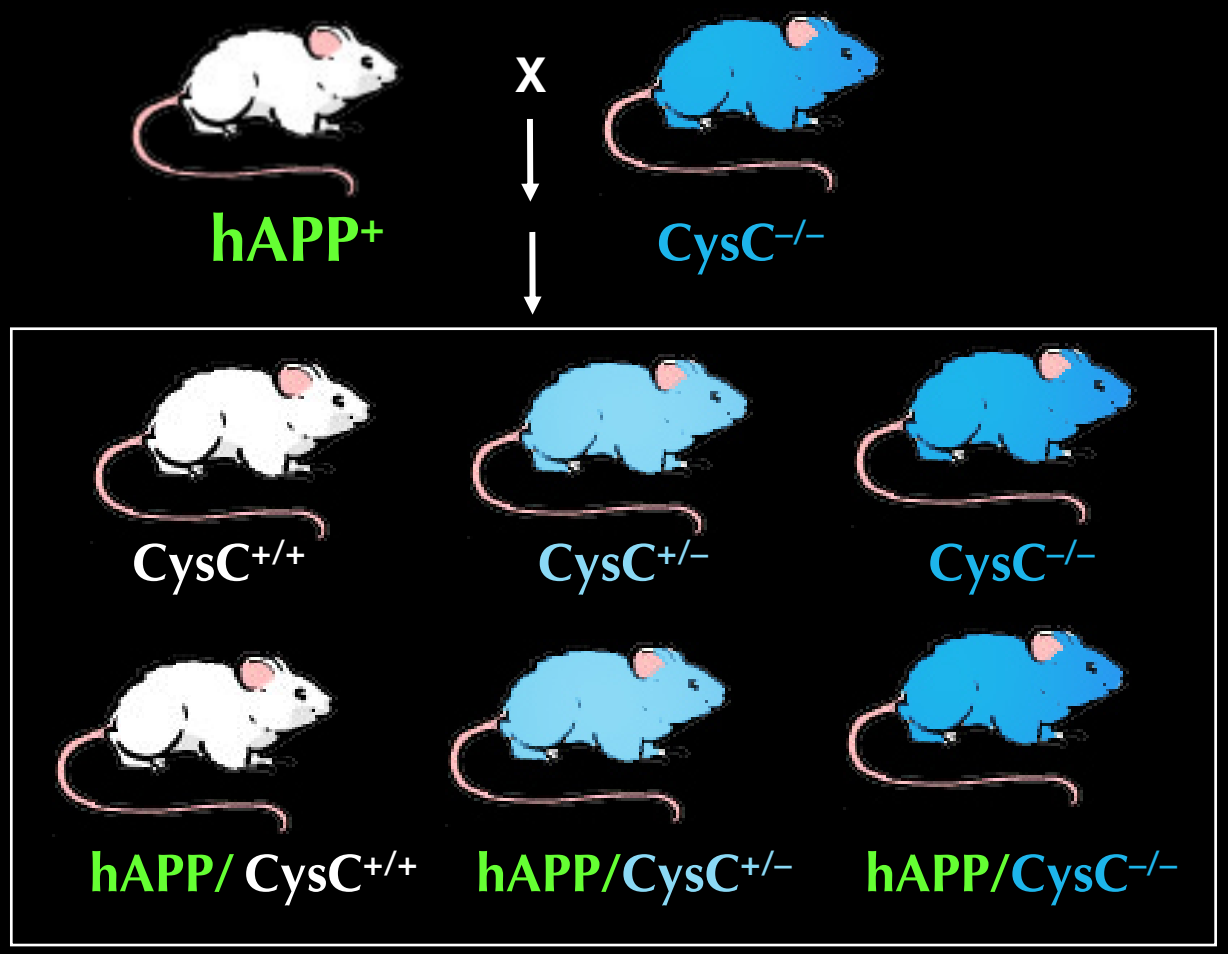
Reducing Cystatin C Will Lower Soluble Aβ by Enhancing CatB Activity



Reduction of Cystatin C Elevates the Activity of CatB



Genetic Inactivation of Cystatin C in hAPP Mice

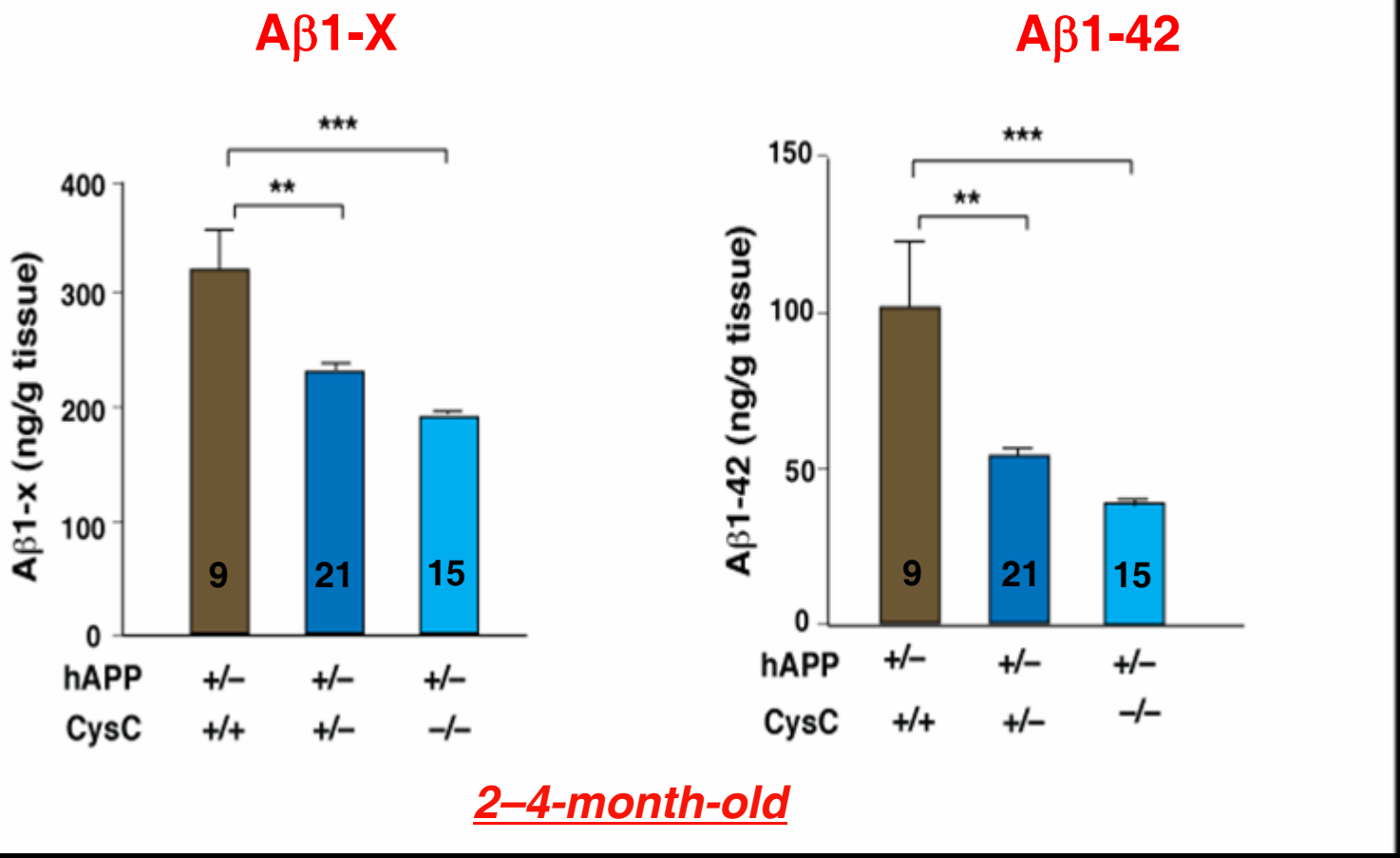


2–4-month-old

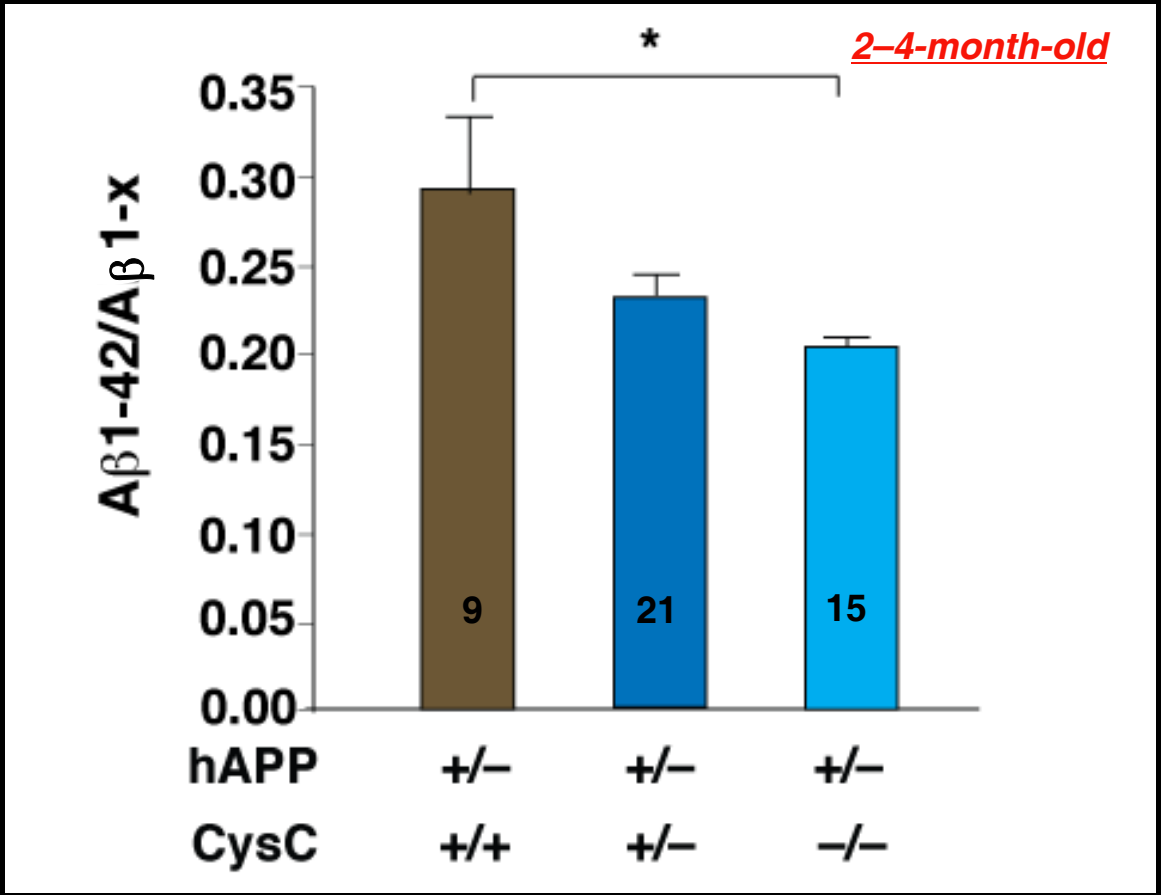
5–8-month-old

8–10-month-old

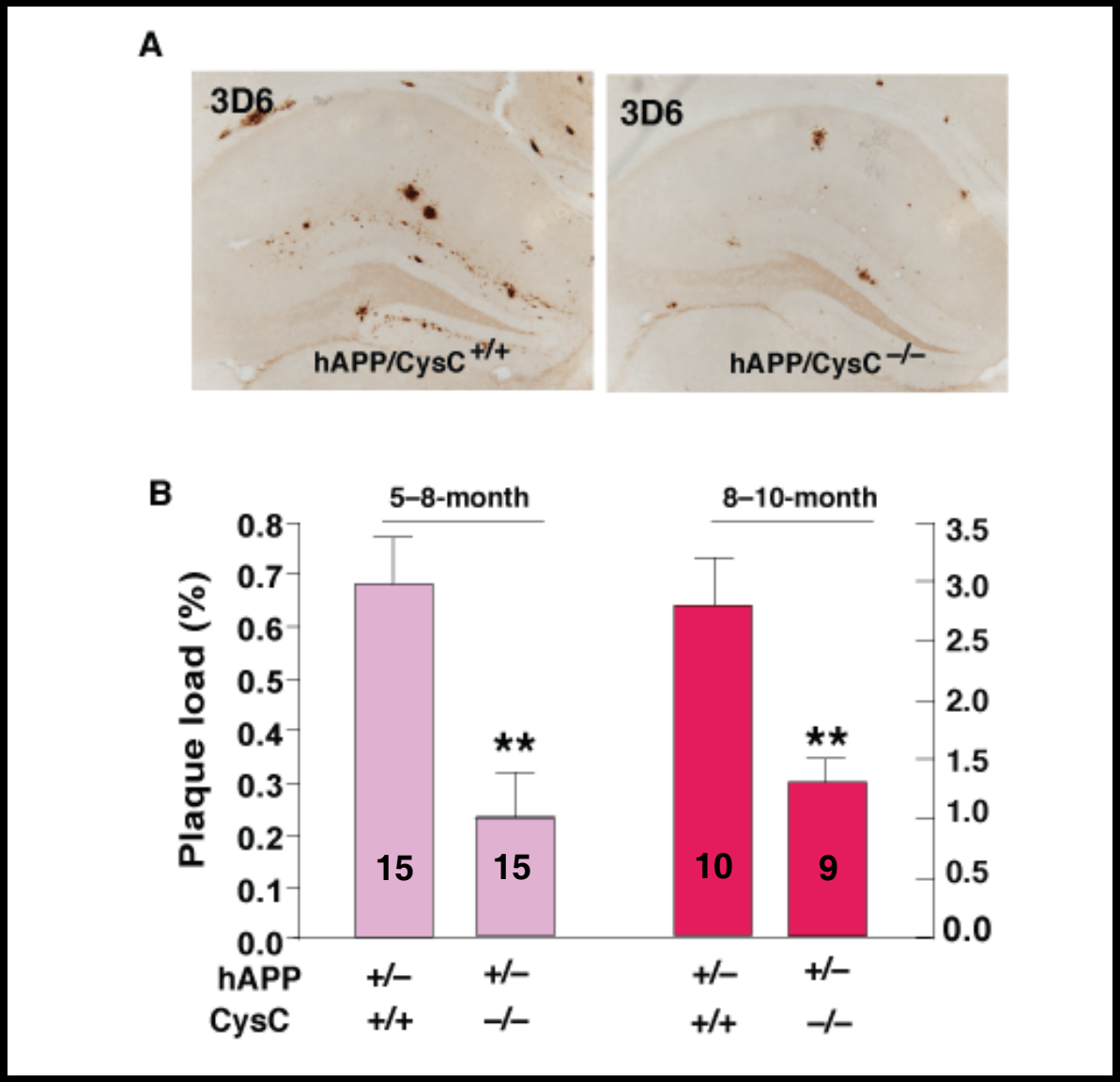
Cystatin C Reduction Lowers Soluble Aβ1-x and Aβ1-42 in Young hAPP Mice



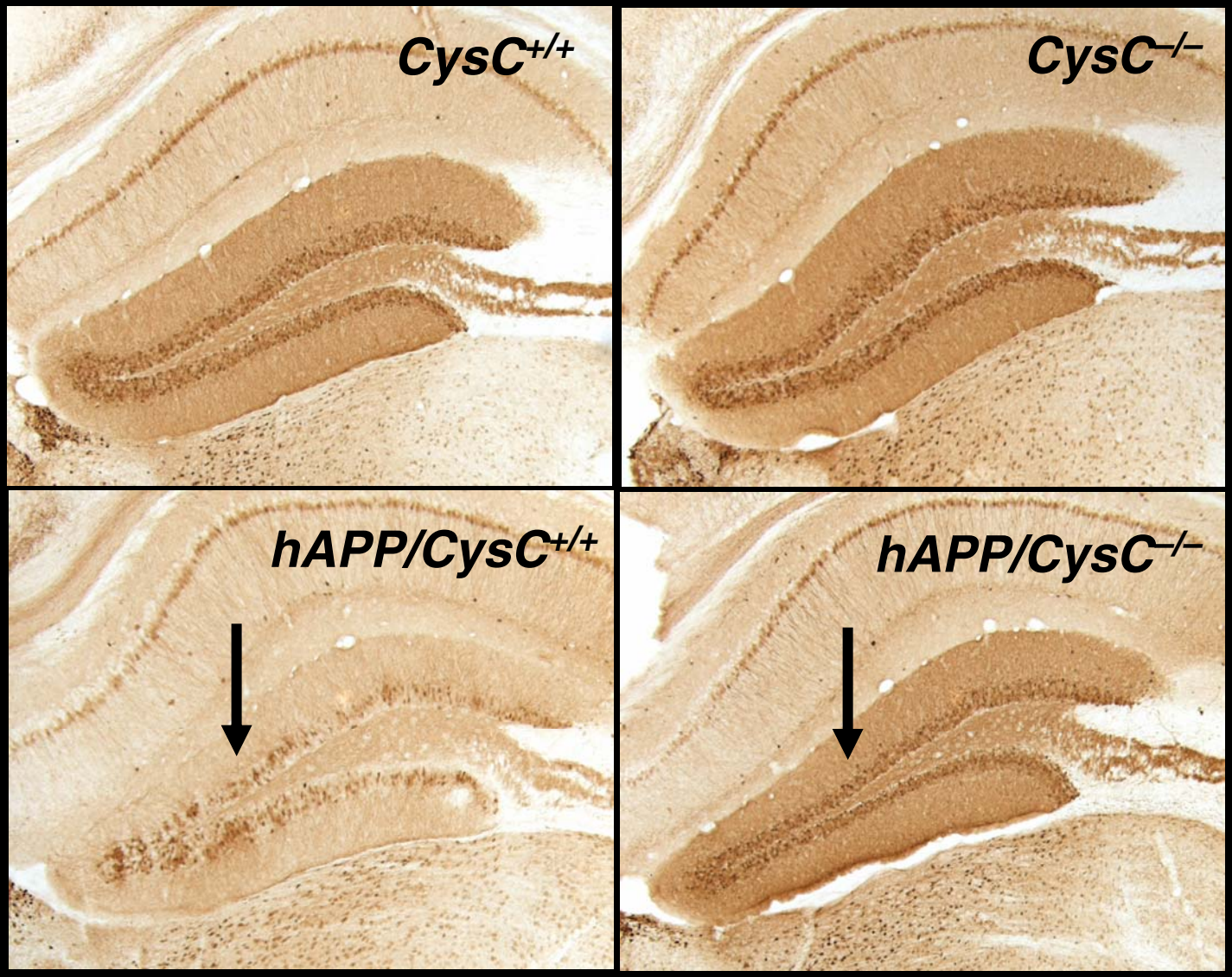
Cystatin C Ablation Lowers the Relative Abundance of Aβ1-42 in Young hAPP Mice



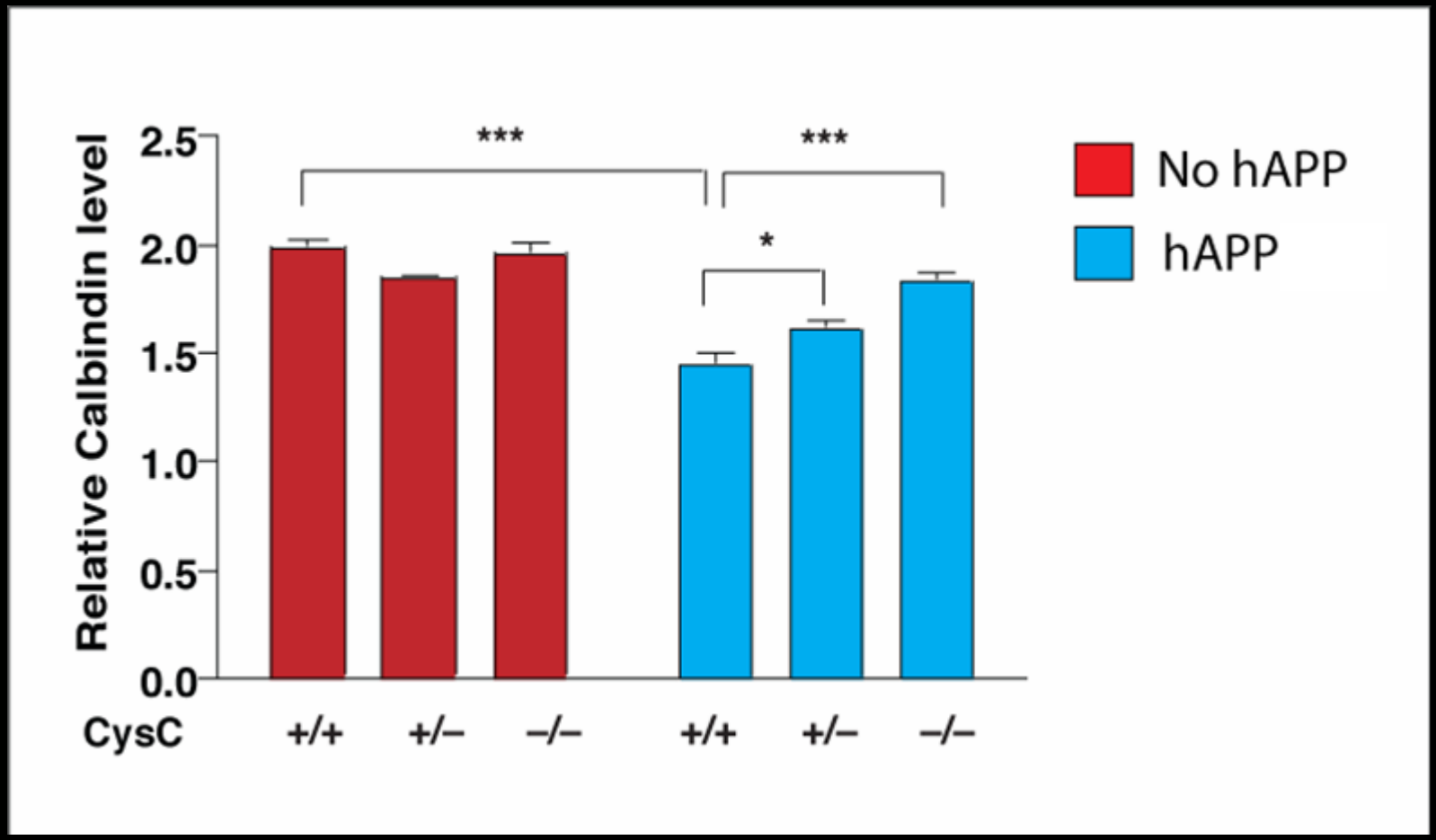
Cystatin C Ablation Reduces Plaque Load in hAPP Mice



Ablation of CysC Prevents Calbindin Depletion in the DG of hAPP mice

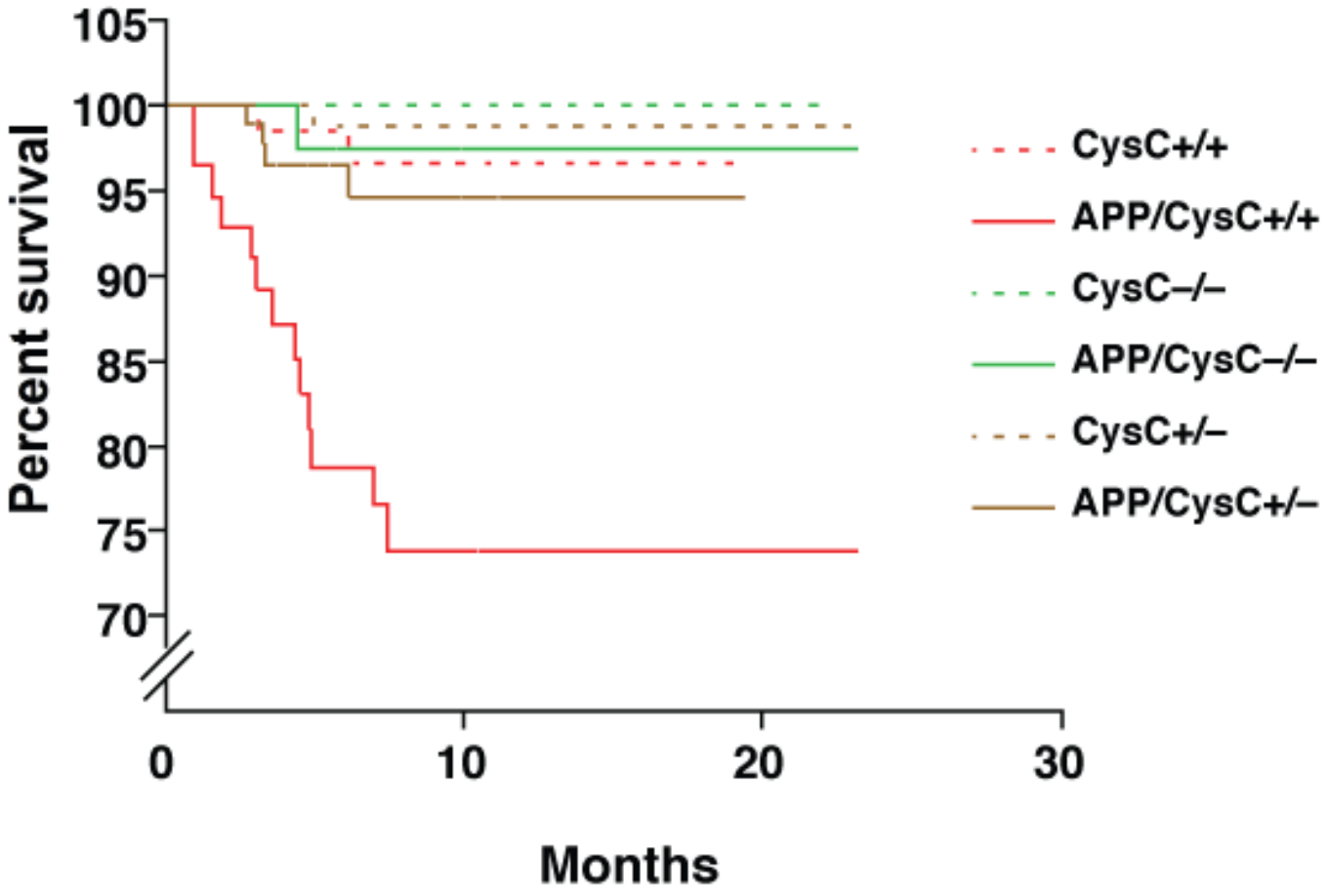


Reduction of CysC Prevents Calbindin Depletion in the DG of hAPP mice

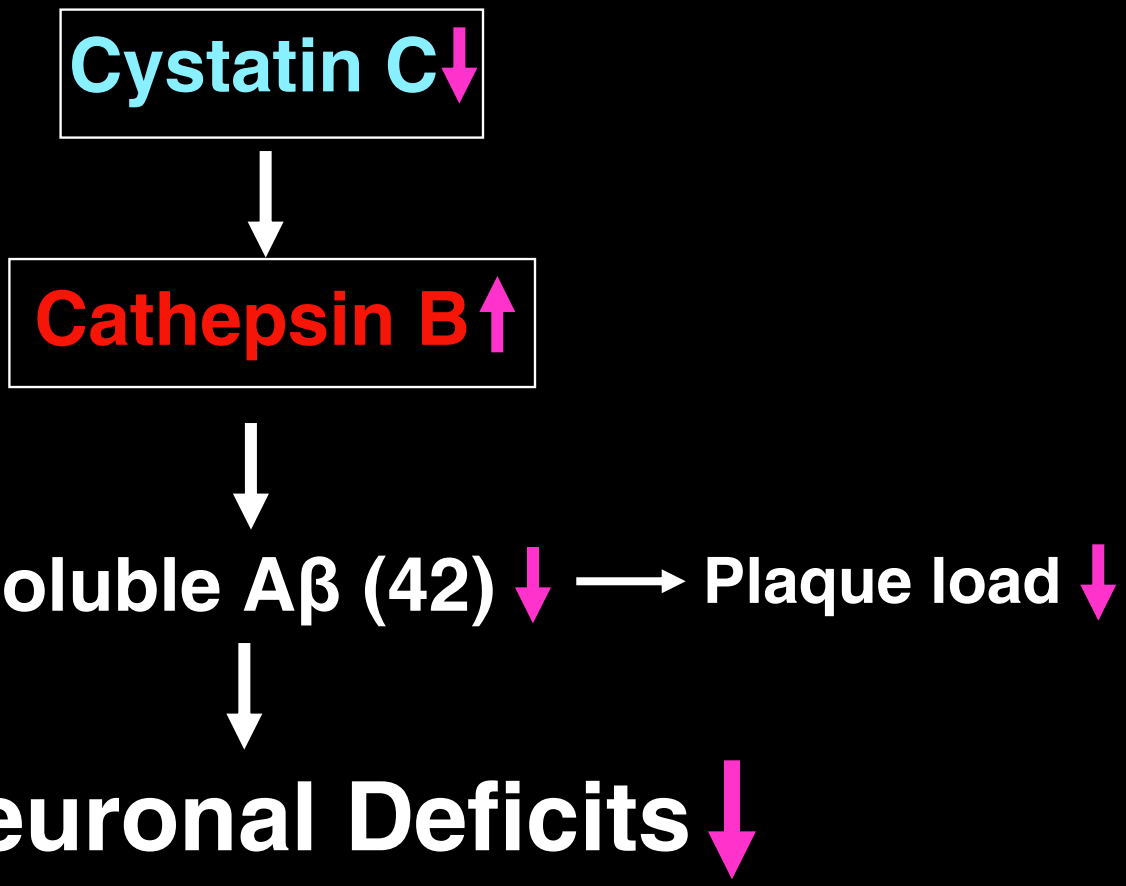


Genotypes	Soluble Aβ	Aβ1–42/Aβ	Plaque	Calbindin	Fos
CST3+/+	*No human Aβ*			+++	+++
CST3+/–				+++	+++
CST3–/–				+++	++
hAPP/CST3+/+	+++	+++	+++	+	+
hAPP/CST3+/–	++	++*	++*	++	++
hAPP/CST3–/–	+	+	+	+++	++

Cystatin C Reduction Abolishes Premature Mortality



Are the Beneficial Effects of Cystatin C Reduction Mediated Through Enhancing CatB Activity?



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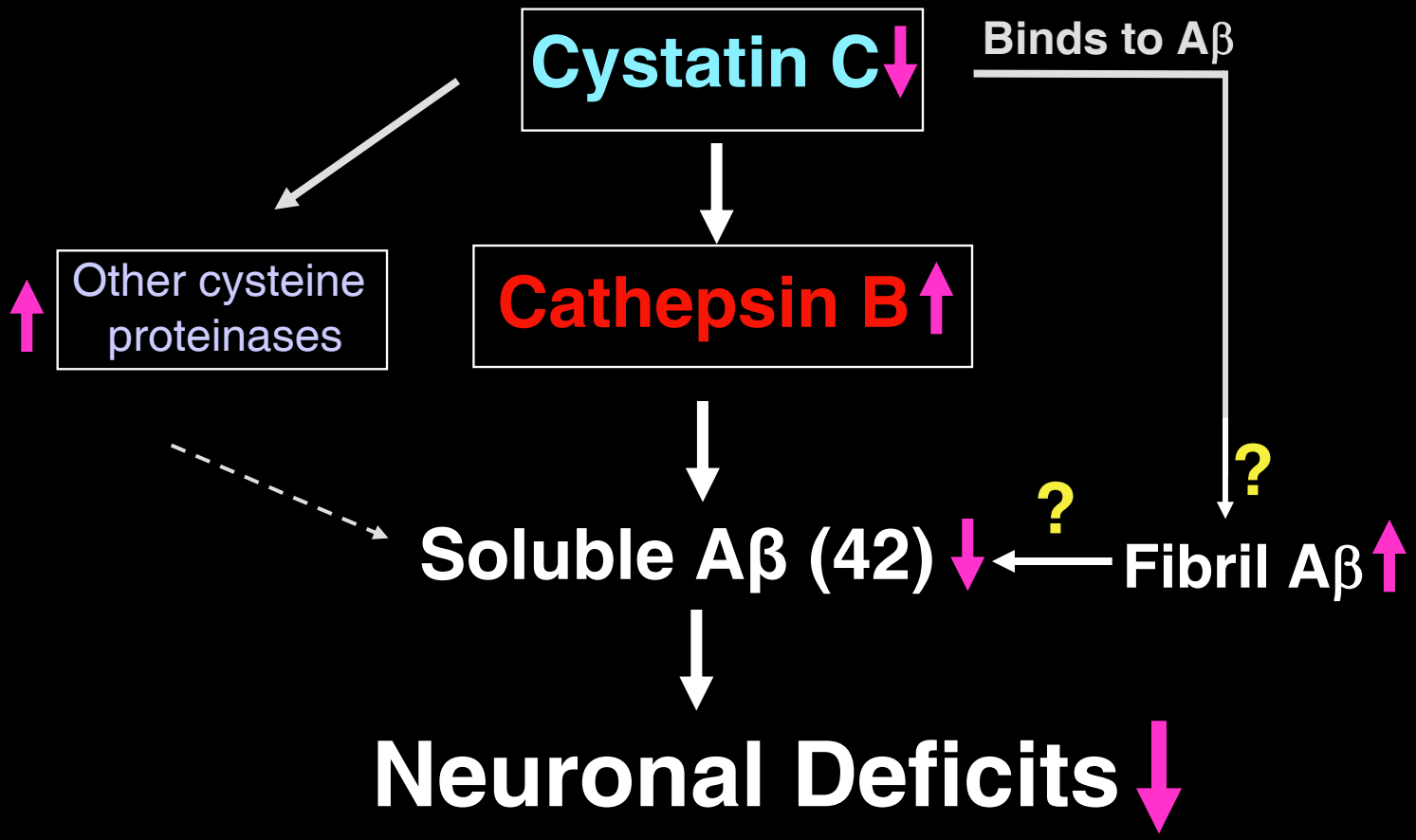

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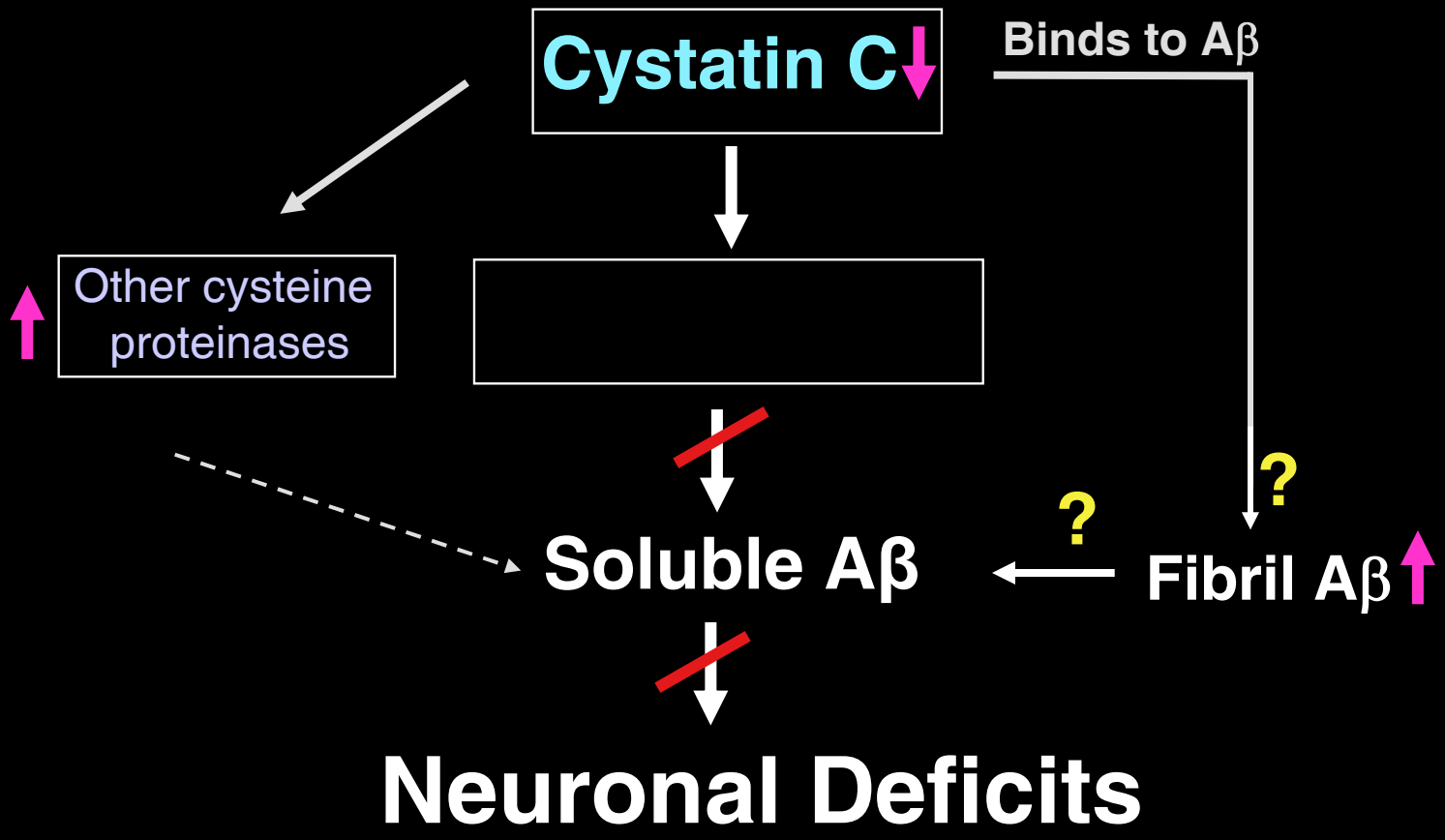


Effects of Cystatin C Reduction in hAPP Mice Lacking CatB

X

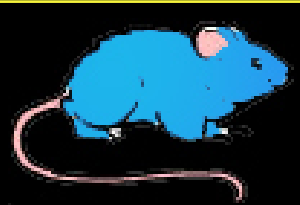
hAPP^{+}/CatB$^{-/-}$ | CysC$^{-/-}$/CatB$^{-/-}$

2–3-month-old

4–6-month-old

CysC$^{+/+}$/CatB$^{-/-}$

CysC$^{+/-}$/CatB$^{-/-}$



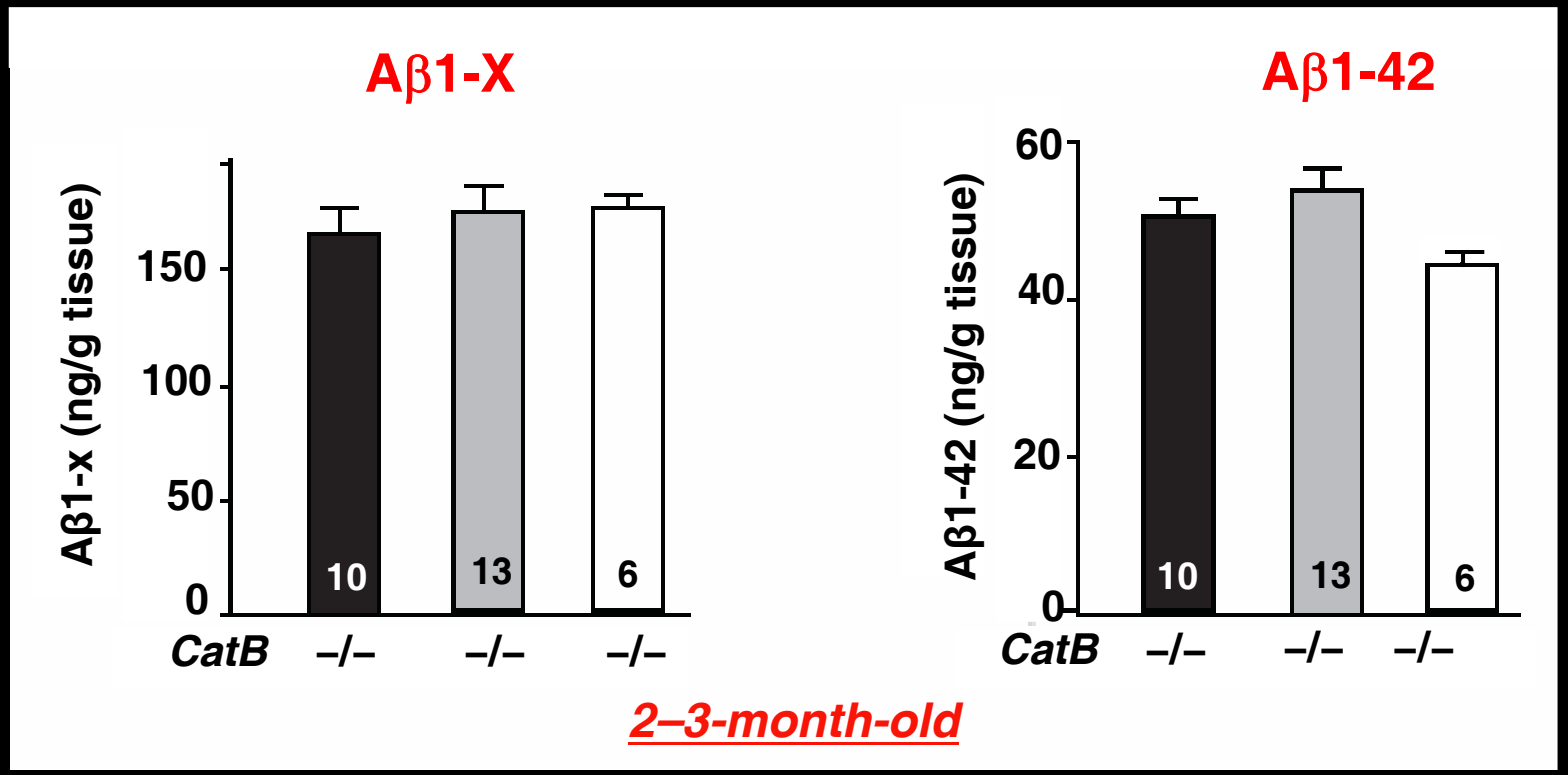
CysC$^{-/-}$/CatB$^{-/-}$

hAPP/CysC$^{+/+}$/CatB$^{-/-}$

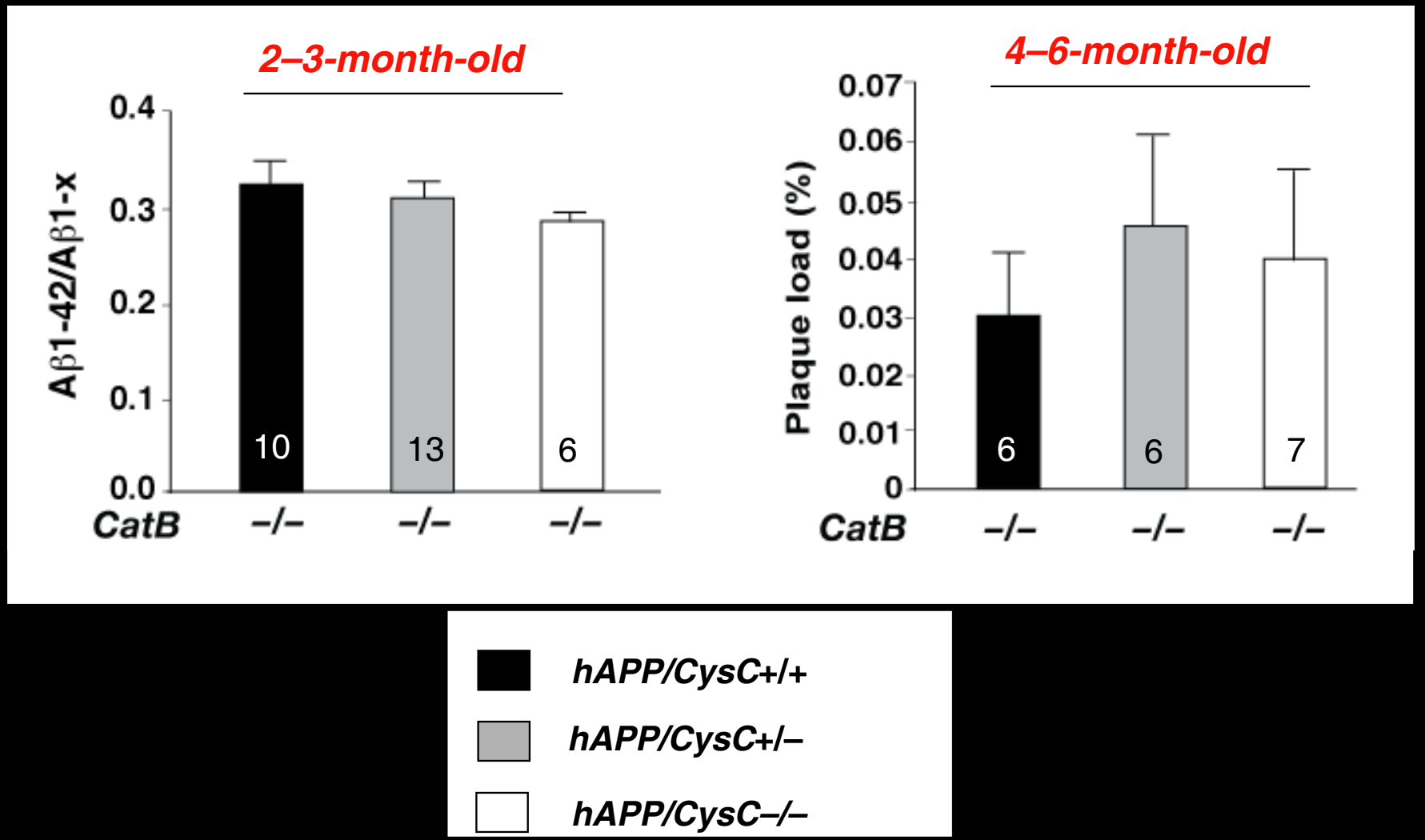
hAPP/CysC$^{+/-}$/CatB$^{-/-}$

hAPP/CysC$^{-/-}$/CatB$^{-/-}$

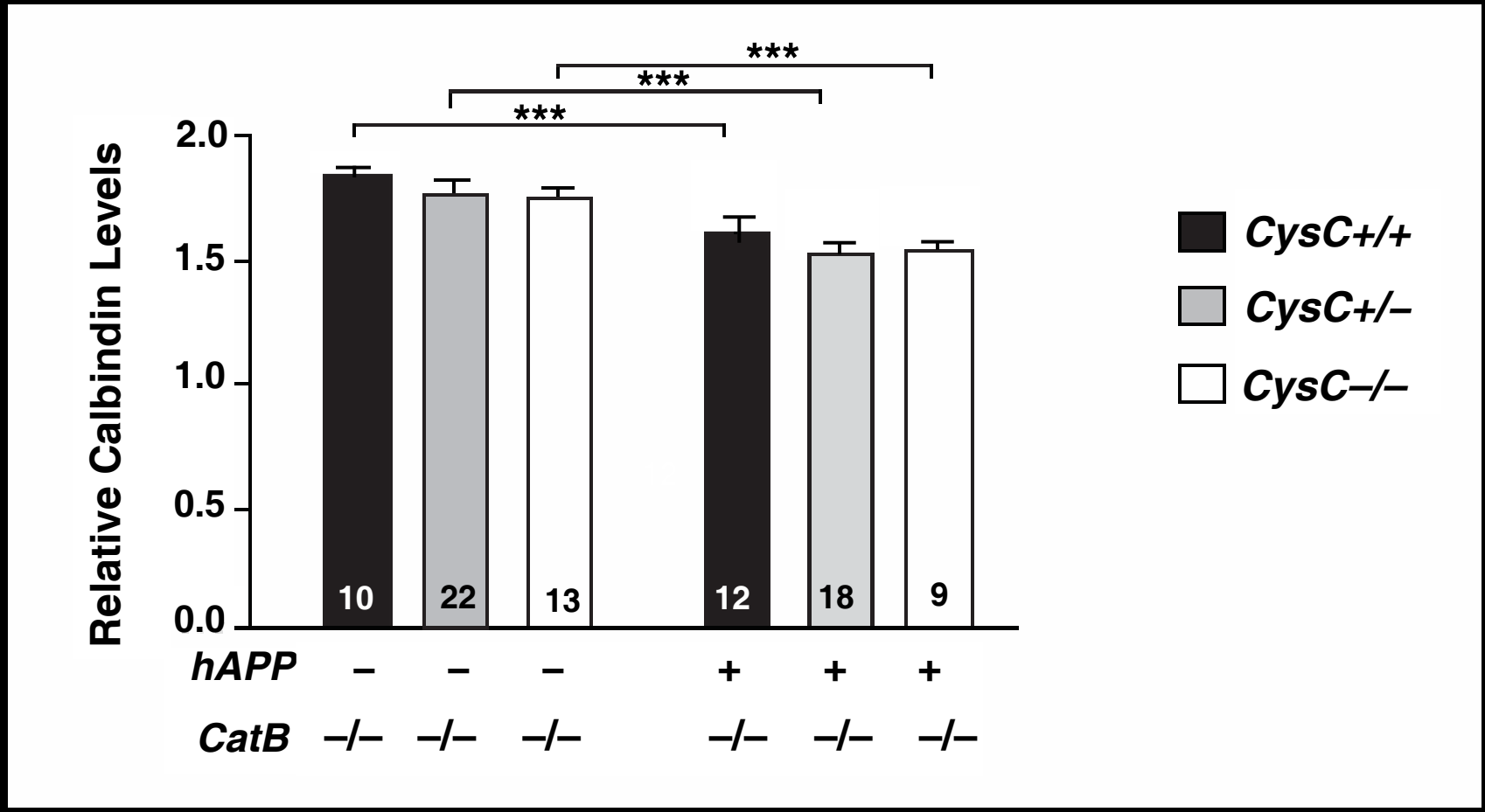
CysC Reduction Failed to Lower Soluble Aβ Levels in the Absence of CatB



Reducing CysC Failed to Lower Relative Abundance of Aβ1-42 and Plaque Load in the Absence of CatB

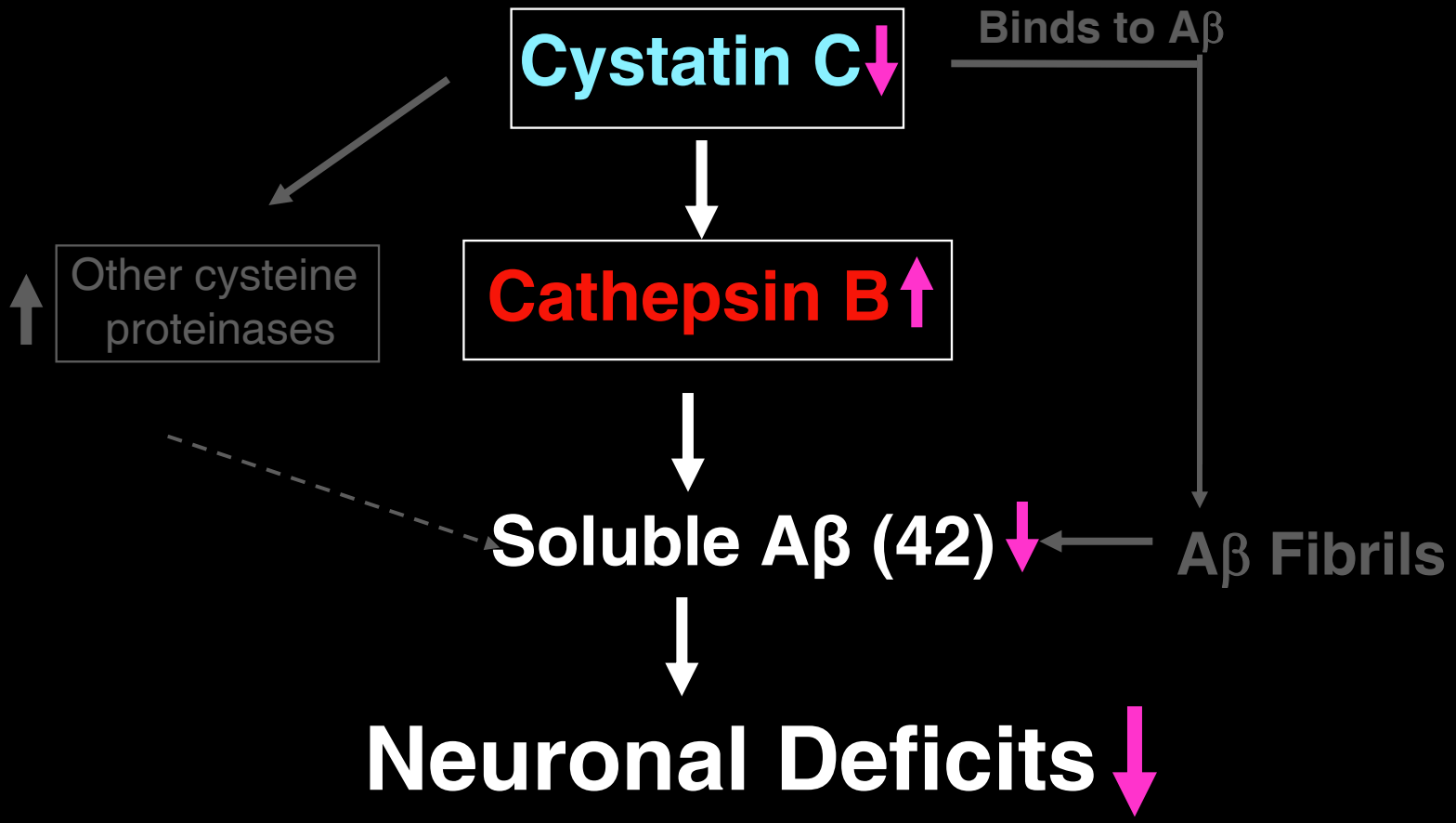


CysC Reduction Did Not Increase Calbindin Levels in the Absence of CatB

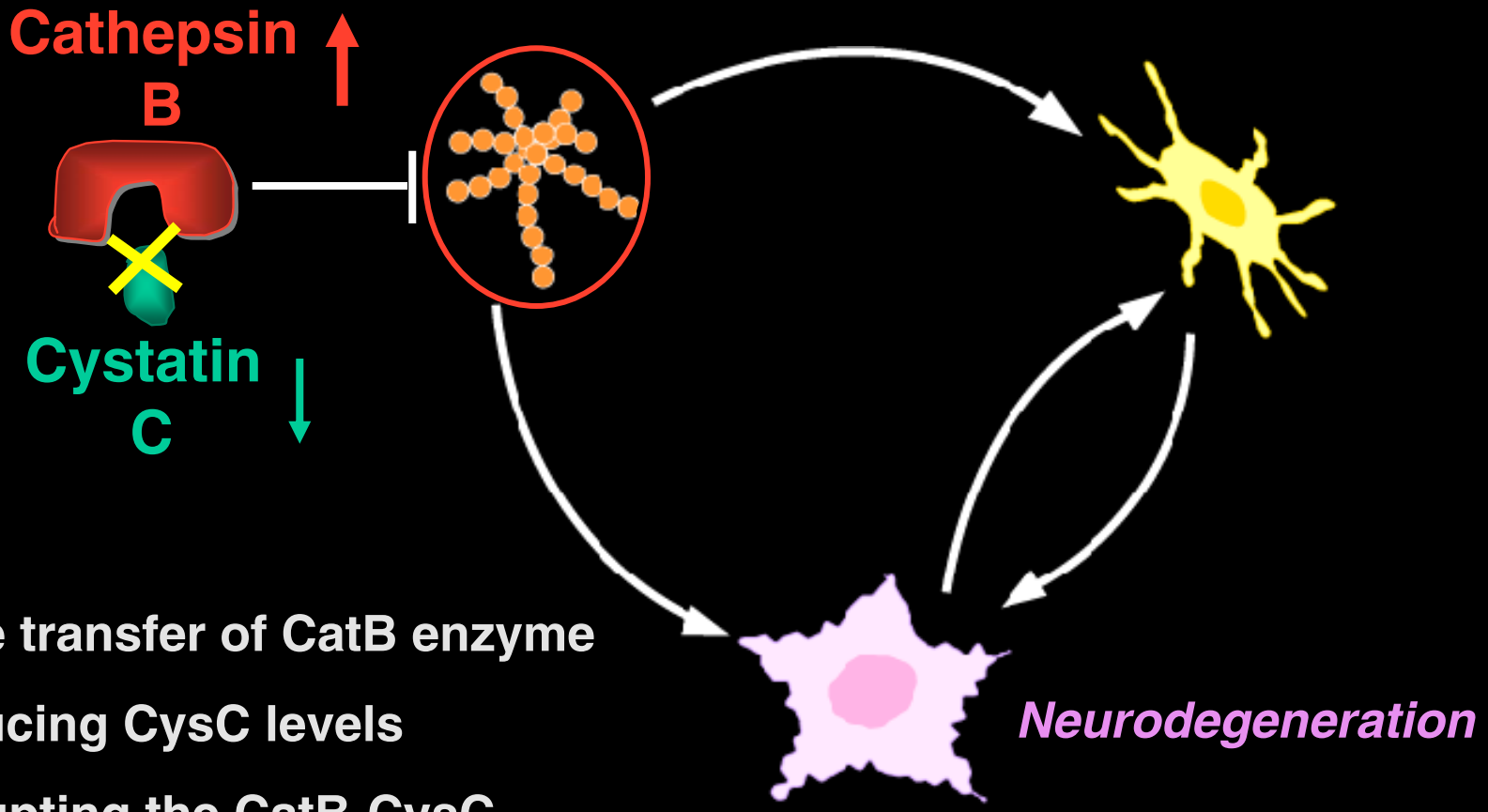


Genotypes	Soluble Aβ	Aβ1–42/Aβ	Plaque	Calbindin	c-Fos
CST3+/+	*No human Aβ*			+++	+++
CST3+/–				+++	+++
CST3–/–				+++	++
hAPP/CST3+/+	+++	+++	+++	+	+
hAPP/CST3+/–	++	++	++	++	++
hAPP/CST3–/–	+	+	+	+++	++
CST3+/+/CatB–/–	*No human Aβ*			+++	+++
CST3+/–/CatB–/–				+++	+++
CST3–/–/CatB–/–				+++	+++
hAPP/CST3+/+/CatB–/–	+++	+++	+++	+	+
hAPP/CST3+/–/CatB–/–	+++	+++	+++	+	+
hAPP/CST3–/–/CatB–/–	+++	+++	+++	+	+

Cystatin C Regulates Soluble Aβ and Neuronal Deficits in a CatB-dependent Manner



New Strategies to Reduce Aβ Accumulation

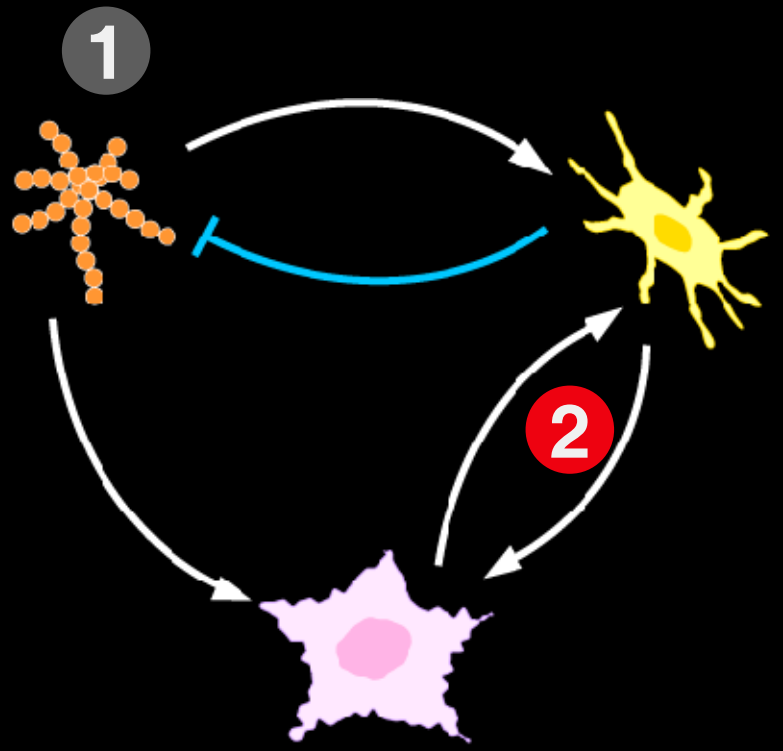


- ✓ Gene transfer of CatB enzyme
- ✓ Reducing CysC levels
- ✓ Disrupting the CatB-CysC interaction

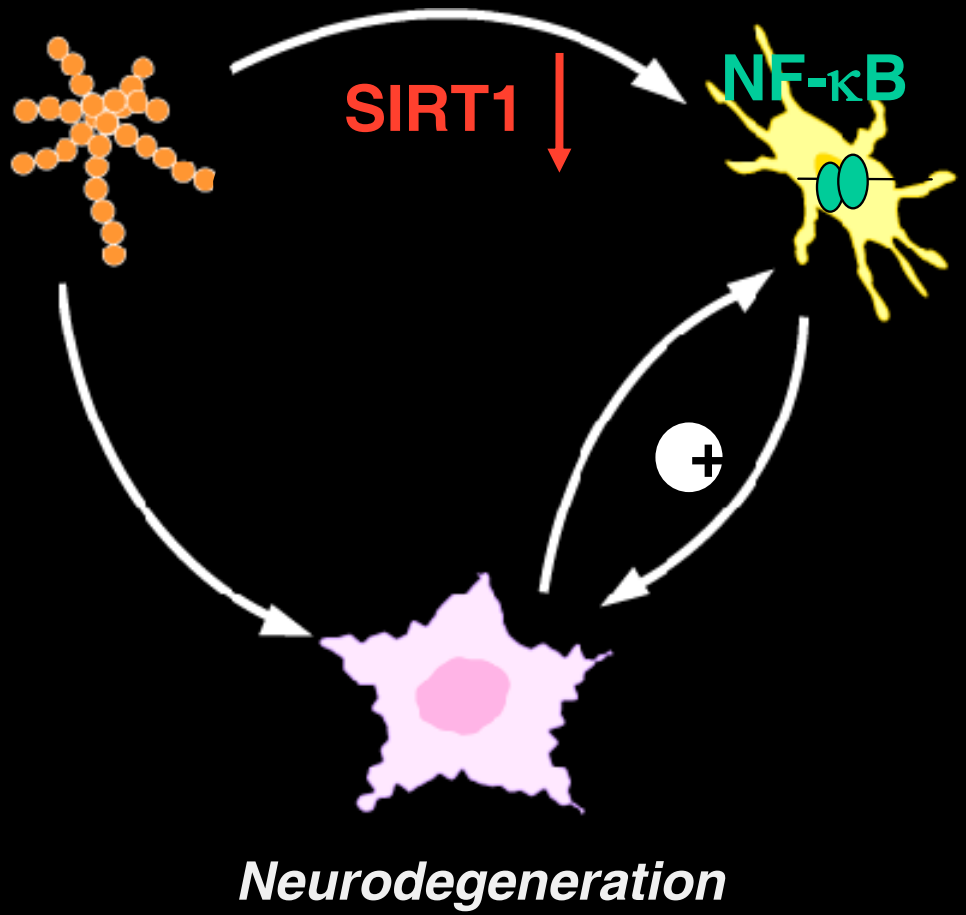
Our Research Focuses

1. Reduce abnormal accumulation of Aβ by promoting degradation

2. Block the toxic pathways in microglia activation



Aβ Downregulates SIRT1 and Activates NF-κB Signaling in Microglia

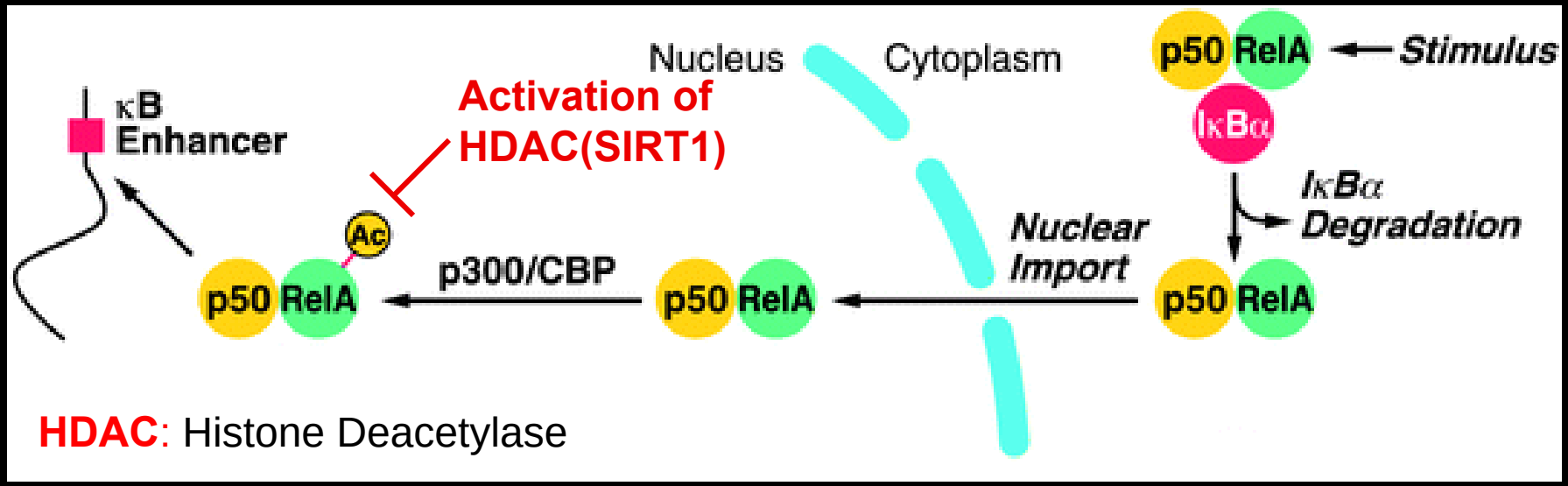


SIRT1

- Class III histone deacetylase
- Deactylate histones and non-histone transcription factors
- A conserved pathway for lifespan extension from protozoa to metazoa
- Modulate multiple processes in neurodegeneration

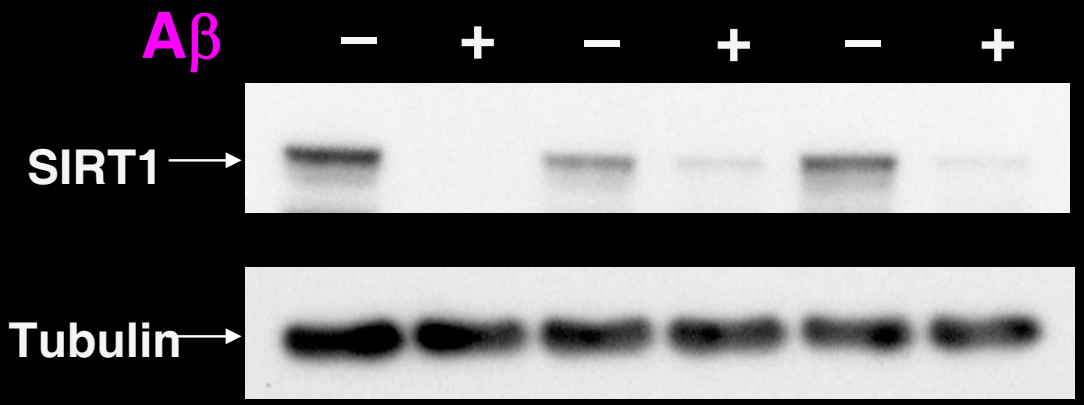
Gan and Mucke, Neuron, 58:10–14. (2008)

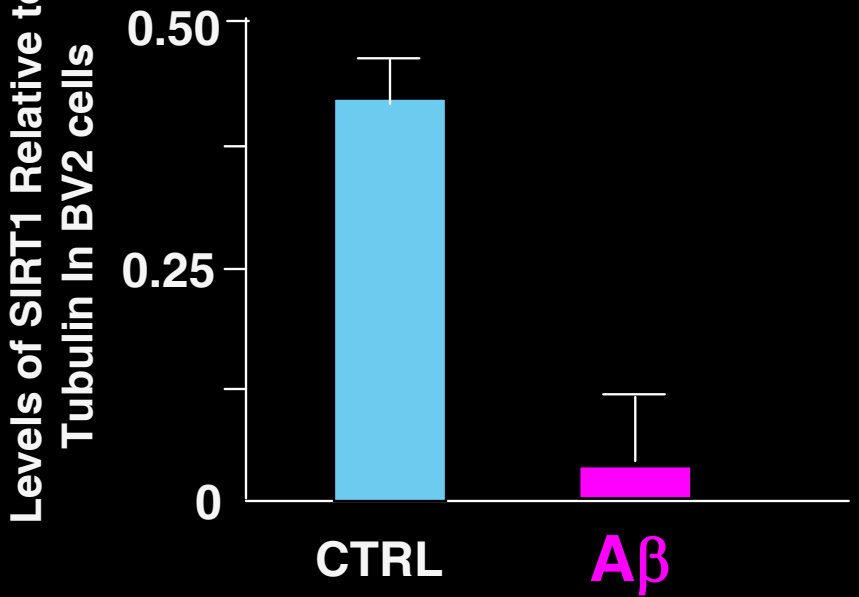
Inhibition of NF-κB Signaling Through Deacetylation by SIRT1

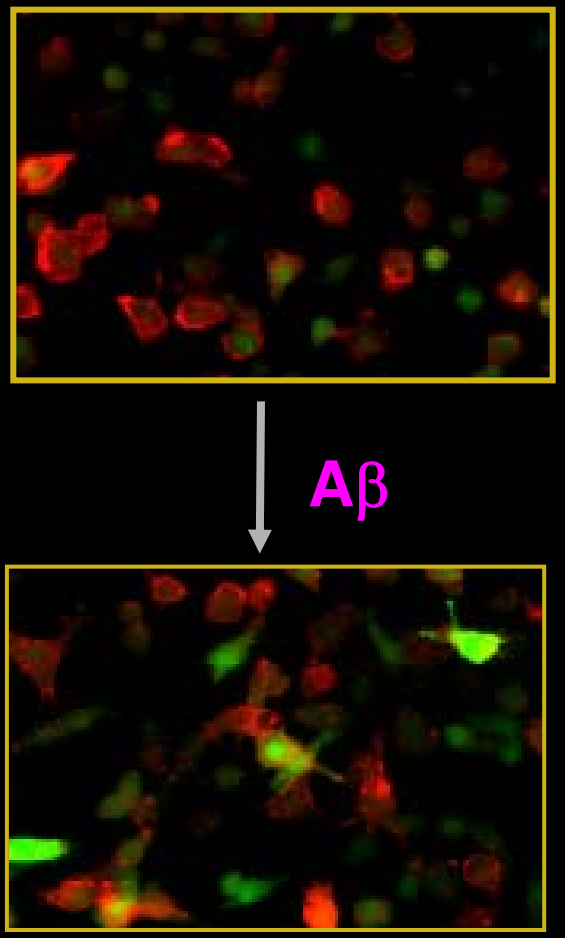


Yeung et al., 2004

Aβ Oligomers Downregulate SIRT1 and Activate NF-κB Activation in Microglial Cells

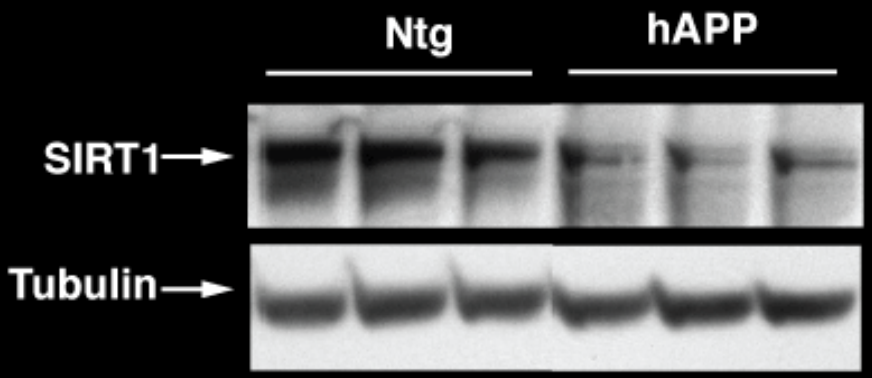


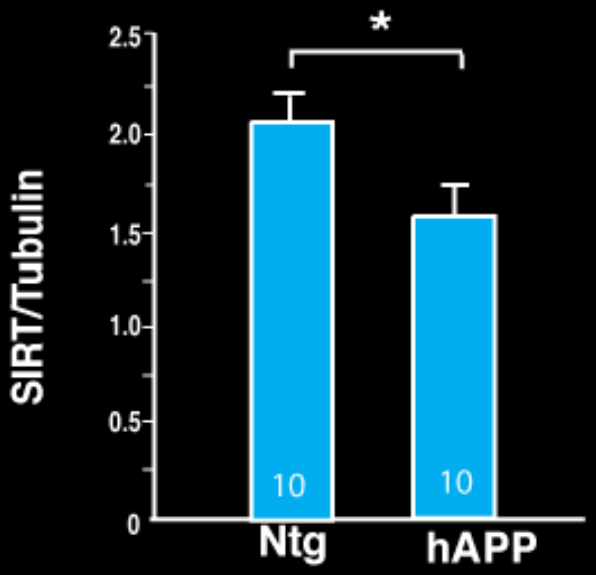




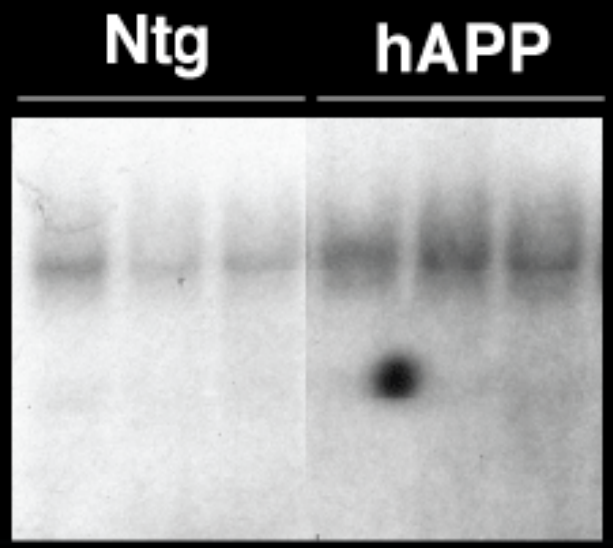
Green: NF-κB activation

SIRT1 Levels Are Downregulated in hAPP Mouse Brains



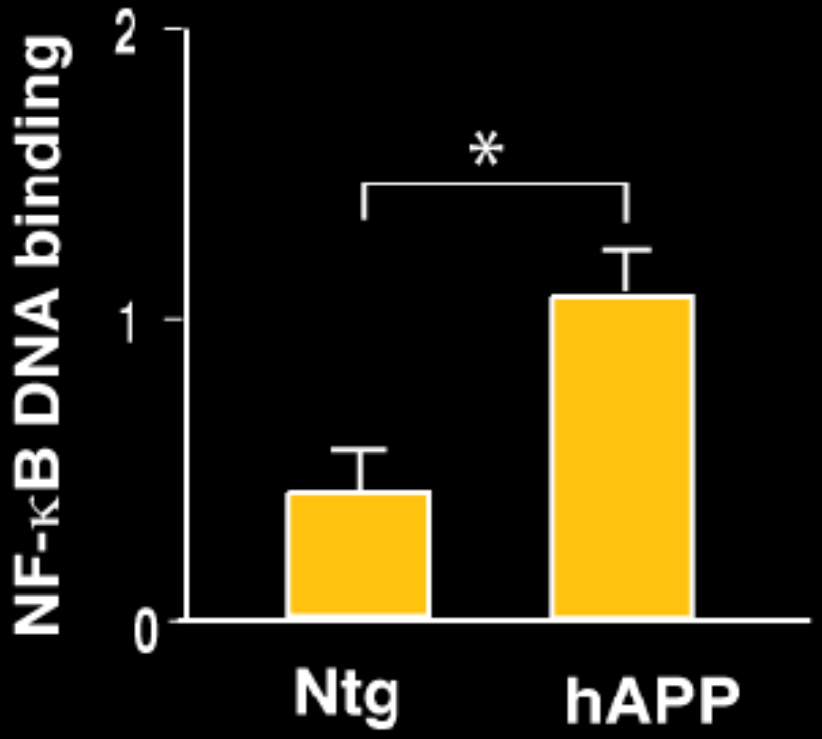


Activation of NF-κB Signaling in Hippocampus of hAPP Mice



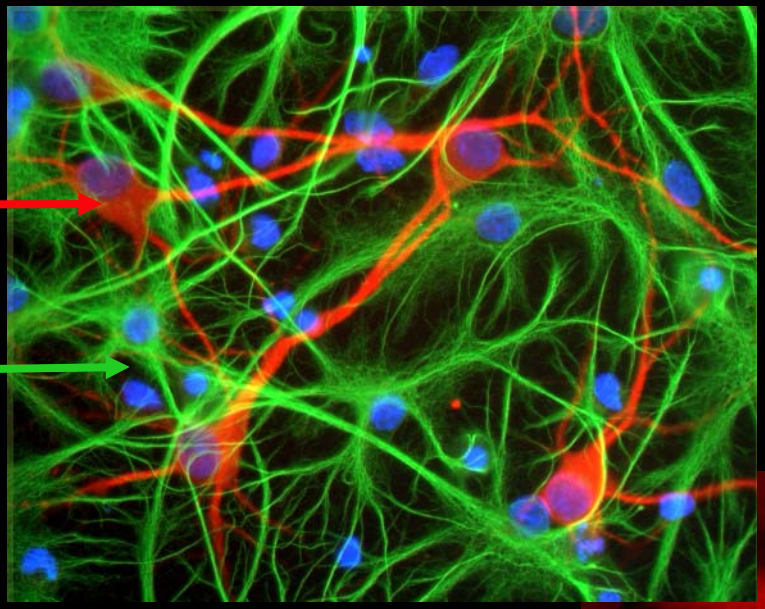
NF-κB DNA/ NE complex

Electrophoretic Mobility Shift Assay (EMSA)



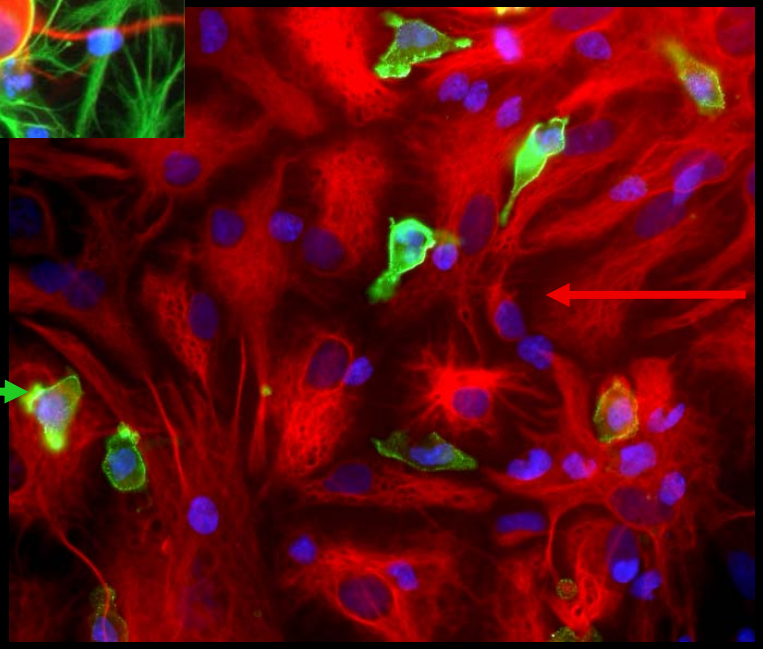
Esposito et al. (Mucke), J. Neuroscience

Cortical Neuron-Glia Mixed Cultures



MAP2
(neurons)

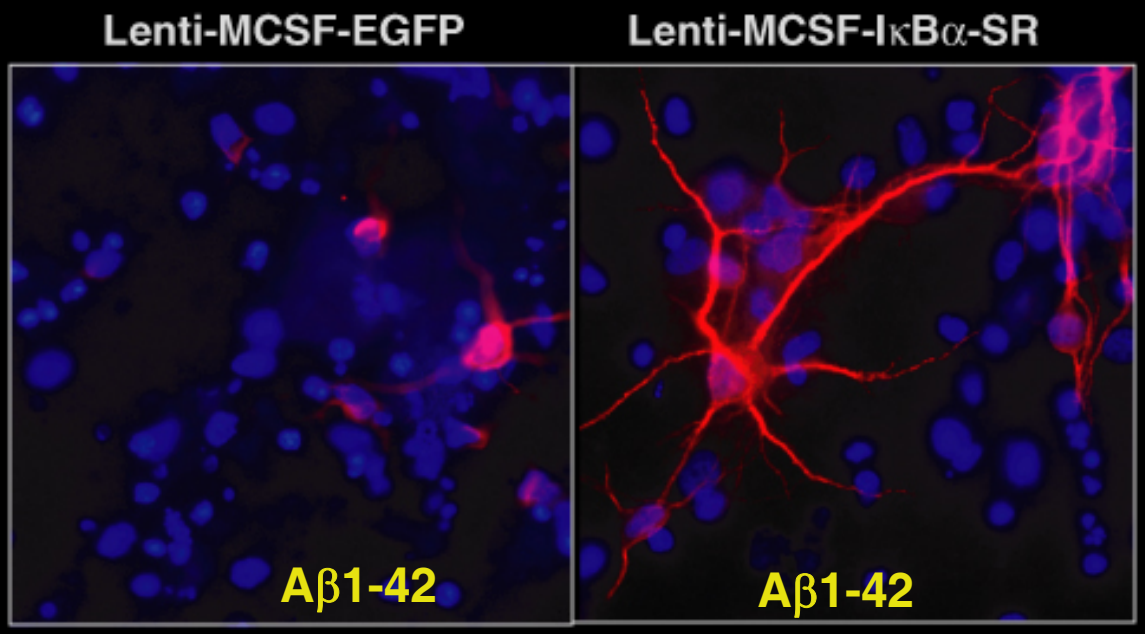
GFAP
(astrocytes)

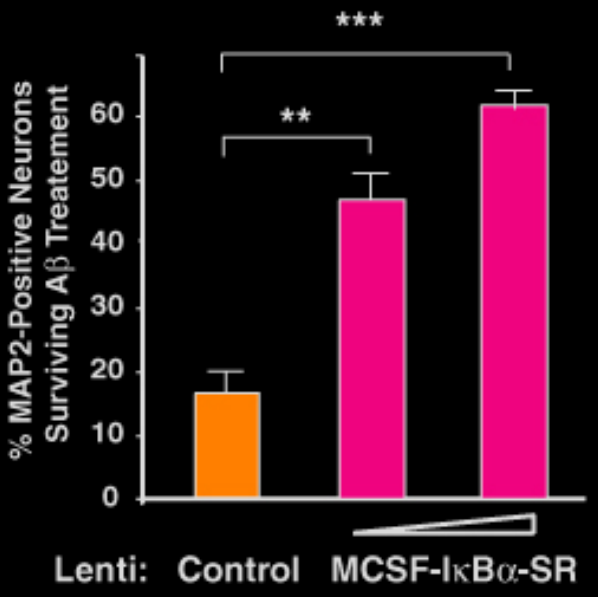


CD11b
(microglia)

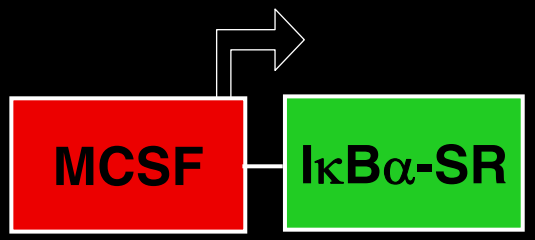
GFAP
(astrocytes)

Targeted Inhibition of NF-κB Signaling in Microglia Protects Against Aβ Toxicity



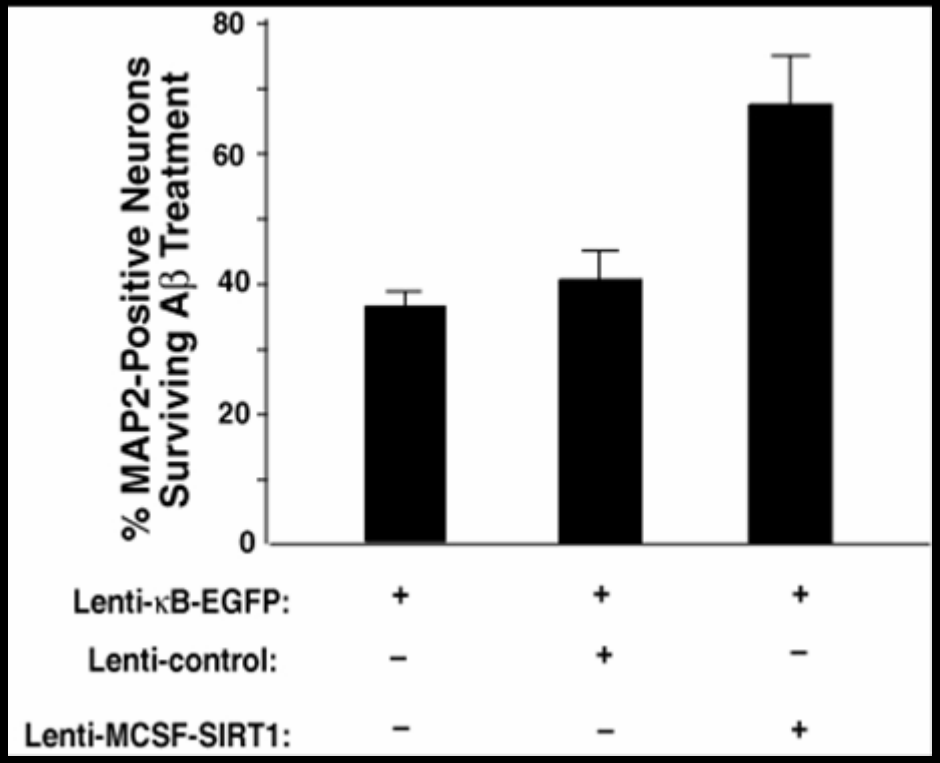


Lenti-MCSF-IκBα-SR

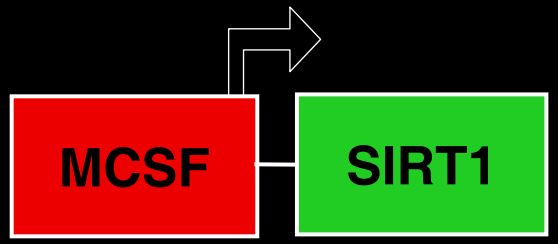


Chen et al., J. Biol. Chem., 280. (2005)

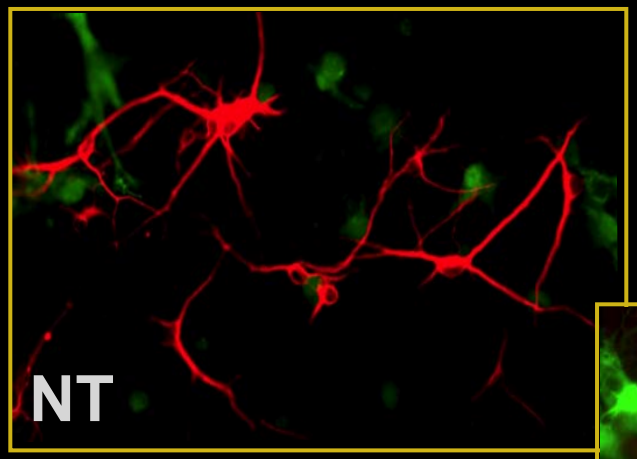
Expression of SIRT1 in Microglia Protects Against Aβ Toxicity

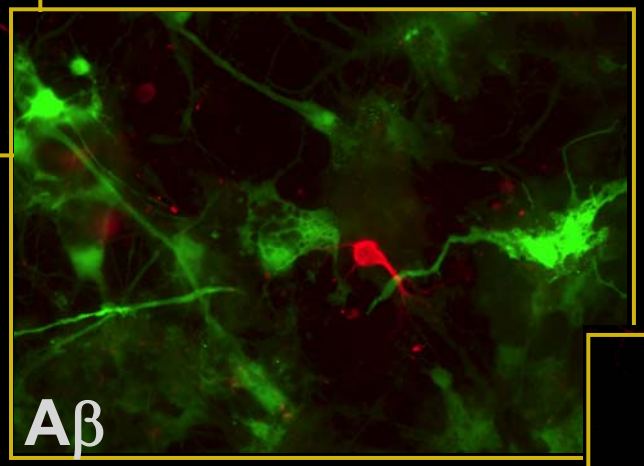


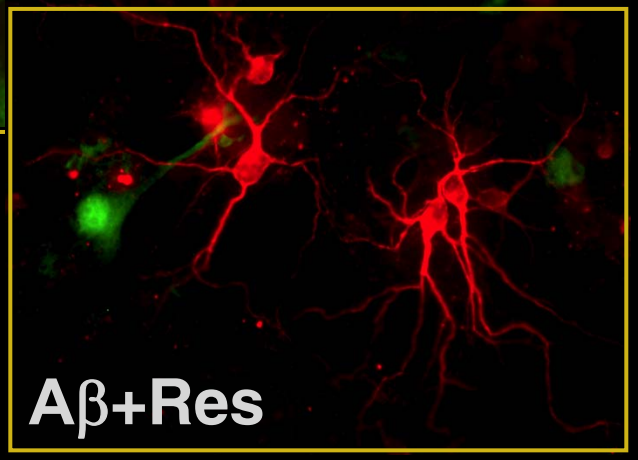
Lenti-MCSF-SIRT1



SIRT1 Agonist Resveratrol Inhibits NF-κB and Protects Against Toxic Effects of Microglia Activation



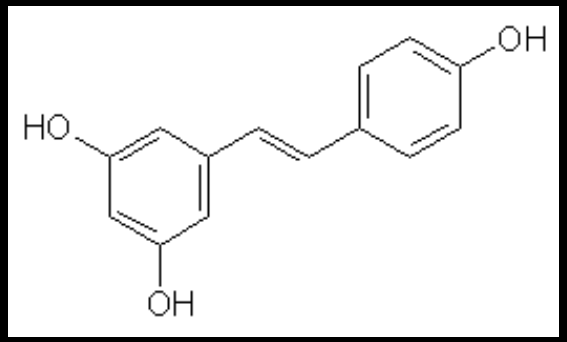




Red: MAP2 (neurons)
Green: NF-κB activation

Chen et al., J. Biol. Chem., 280. (2005)

Resveratrol Exerts Beneficial Effects as a SIRT1 Agonist in Vivo



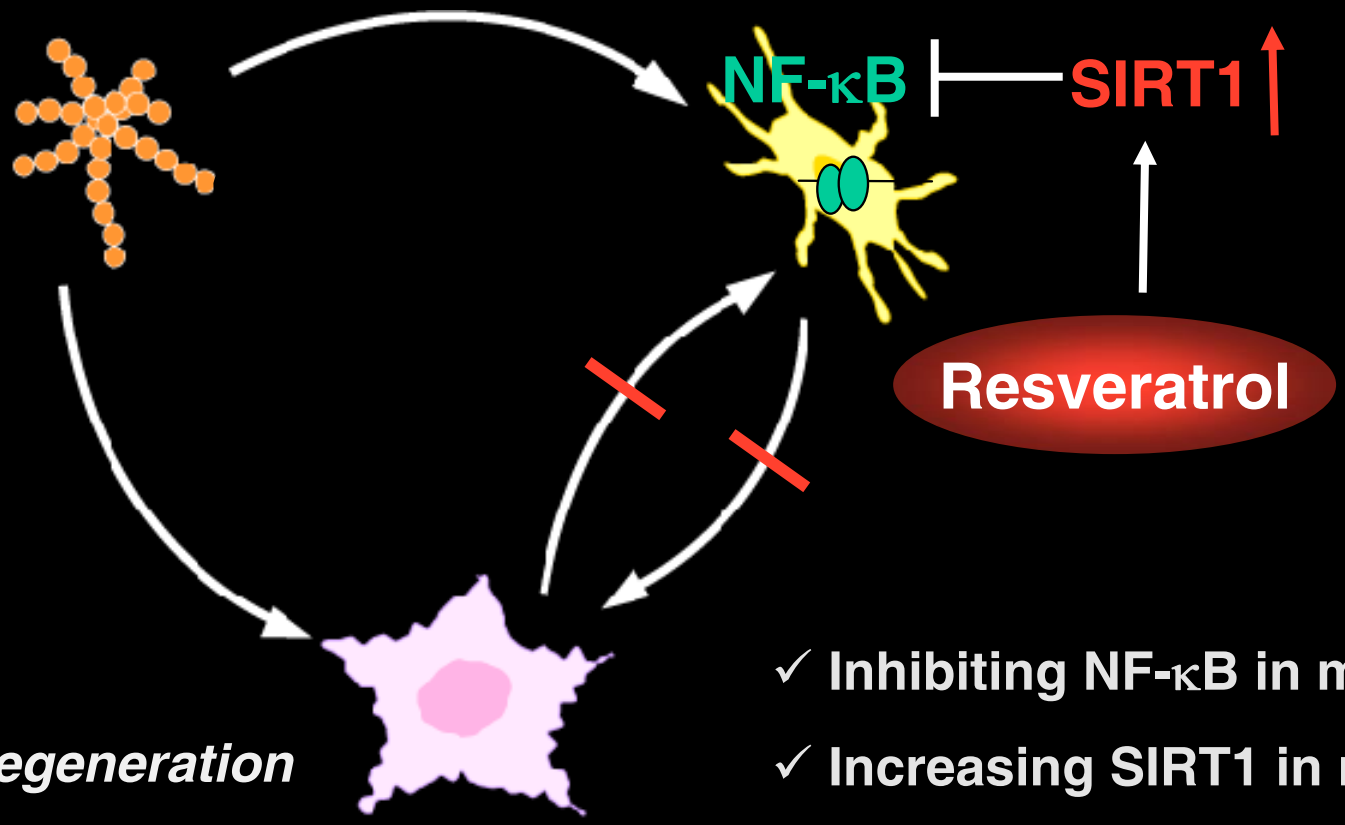
Grapes

Red Wine

Reduces insulin resistance,
Increases mitochondrial function,
Prolongs survival
in mice fed a high-fat diet

Baur et al., Nature, (2006); Lagouge et al., Cell, (2006)

New Strategies to Block the Toxic Effects of Microglia Activation



- ✓ Inhibiting NF-κB in microglia
- ✓ Increasing SIRT1 in microglia
- ✓ Addition of SIRT1 agonists

Acknowledgments

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Brian Halabisky
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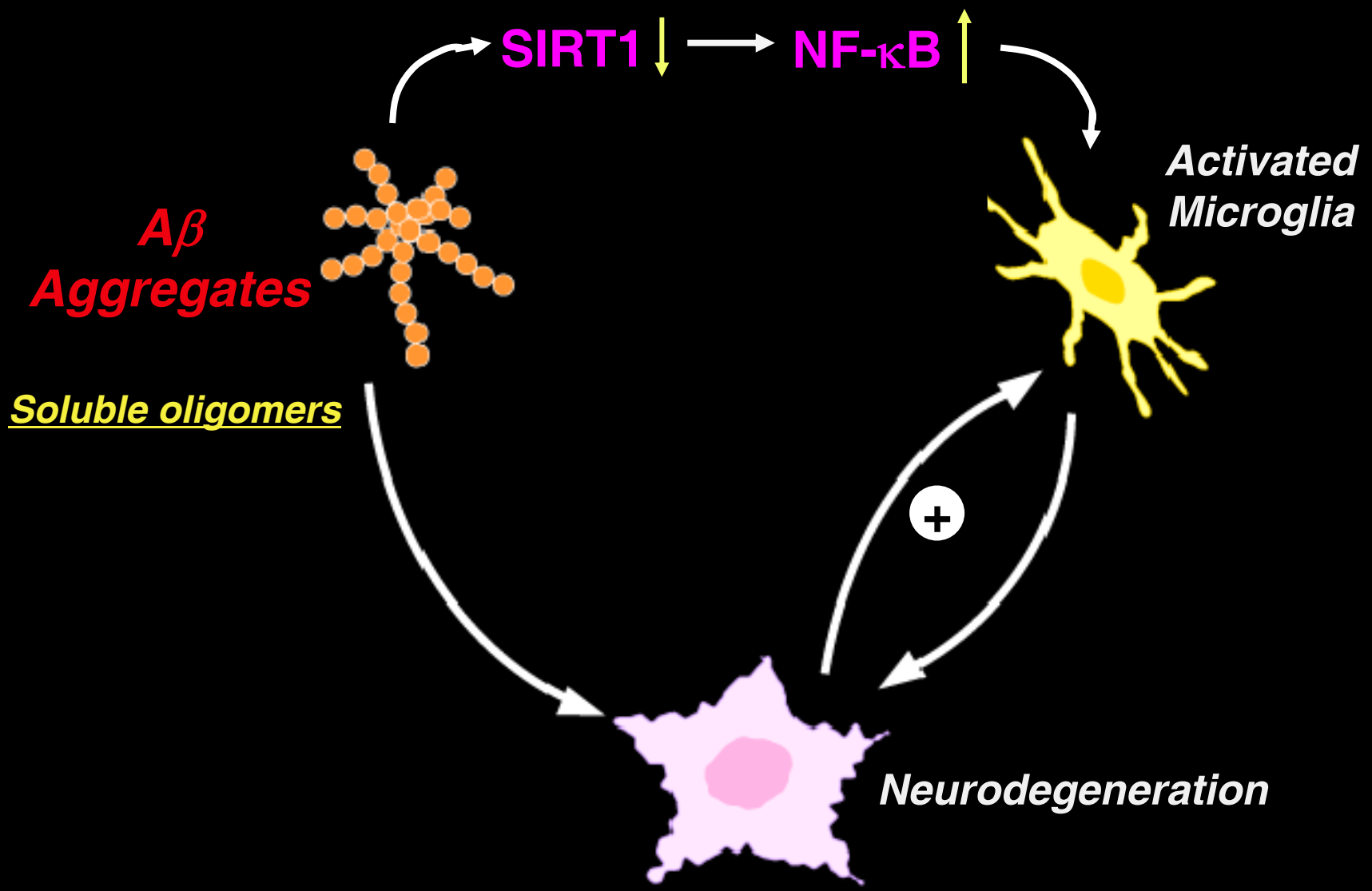
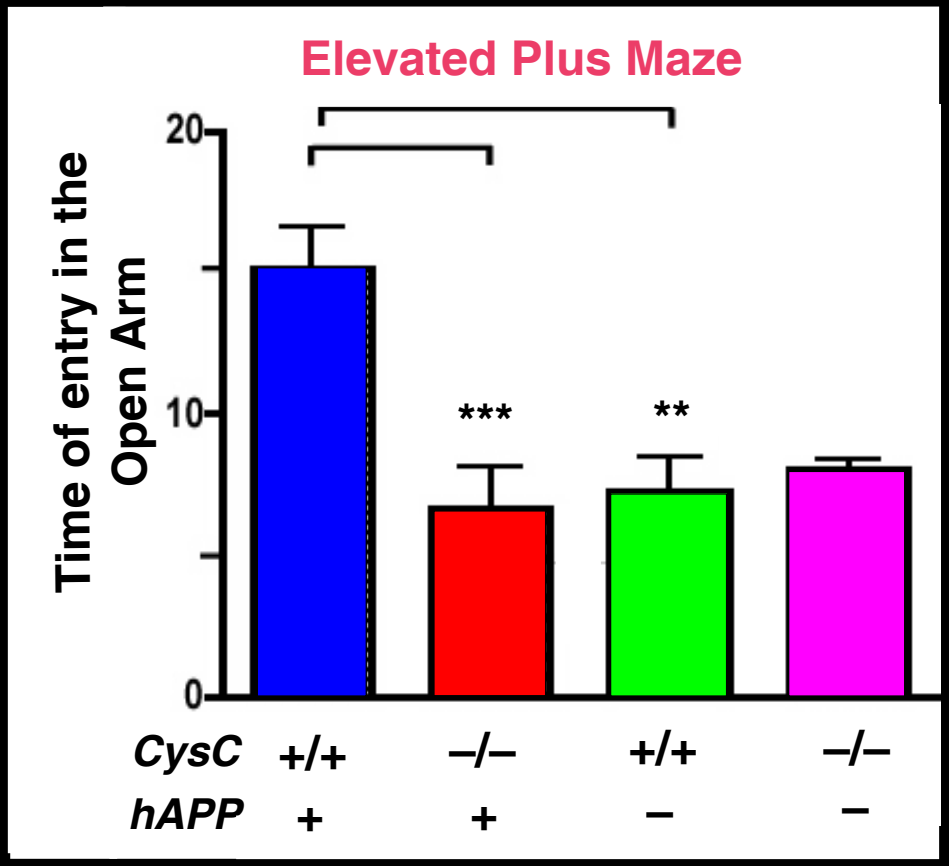
Erik Roberson
Xin Wang
Lennart Mucke
Hidde Plough
Christoph Peters
Eliezer Masliah


SIRT1
NF-κB
Activated Microglia
Aβ Aggregates
Soluble oligomers
+
Neurodegeneration

Ongoing Investigation

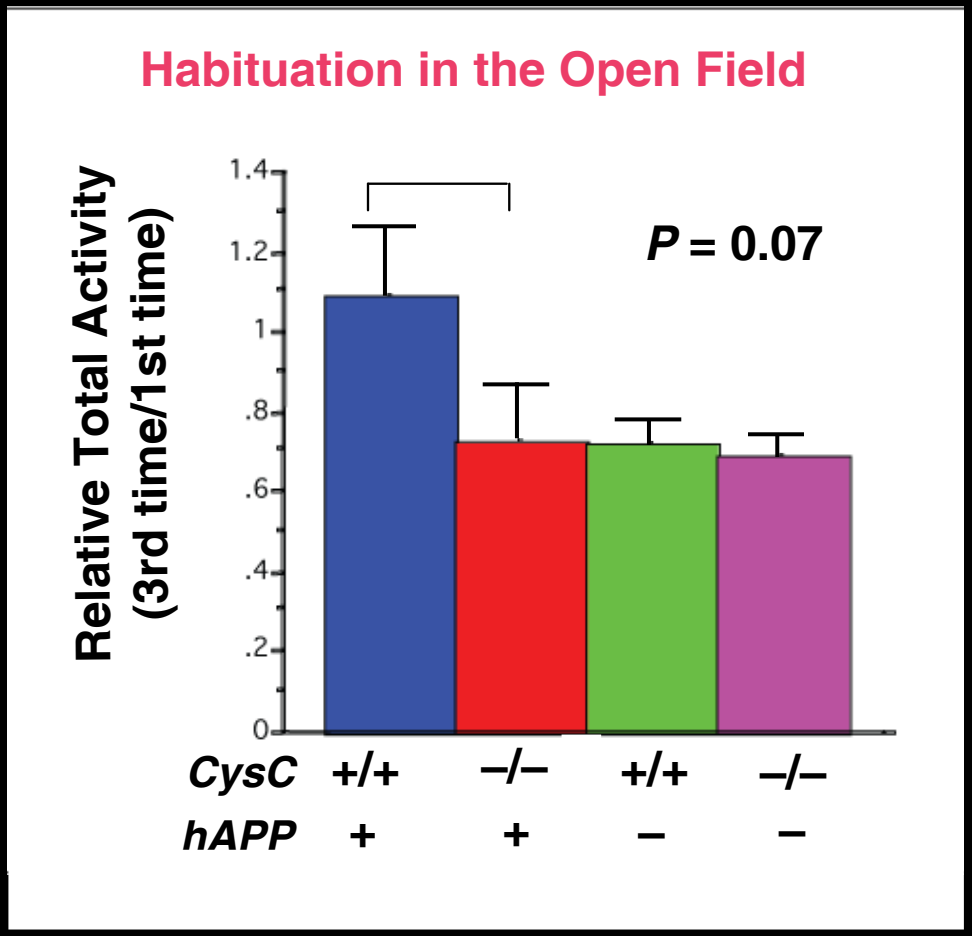
1) Does CysC reduction prevent behavioral deficits in hAPP mice?



- ***Elevated plus maze***
- *Open-field Activity*
- *Morris water maze*
- *Contextual fear conditioning*

Ongoing Investigation

1) Does CysC reduction prevent behavioral deficits in hAPP mice?



- *Elevated plus maze*
- ***Habituation in the open-field***
- *Morris water maze*
- *Contextual fear conditioning*

Ongoing Investigation & Future Plans

2) Where does CysC and CatB interact?

- *Extracellular or intracellular*
- *Neuron or glia (microglia)*
- *Autophagic degradation?*

3) How is the CysC-CatB axis regulated?

4) How to disrupt the CysC-CatB interaction ?

Ongoing Investigation & Future Plans

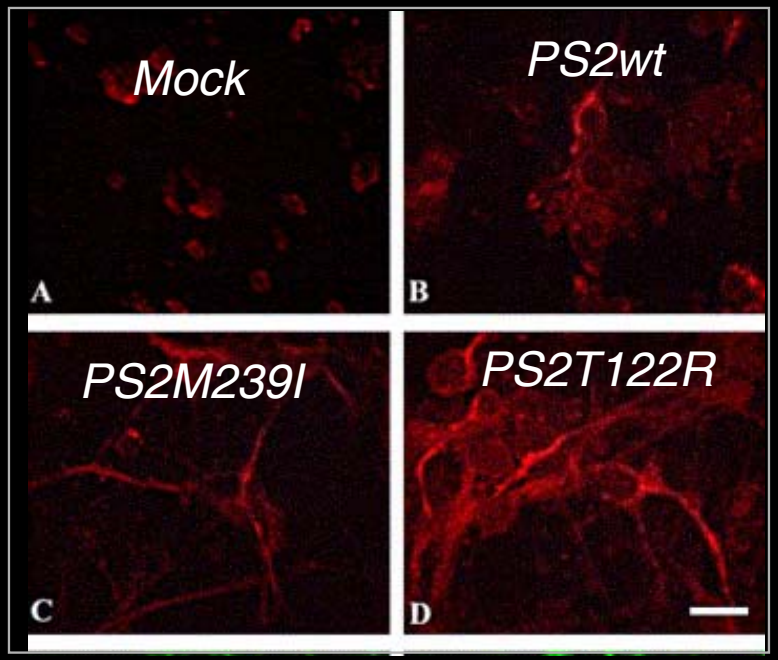
2) Where does CysC and CatB interact?

3) How is the CysC-CatB axis regulated?

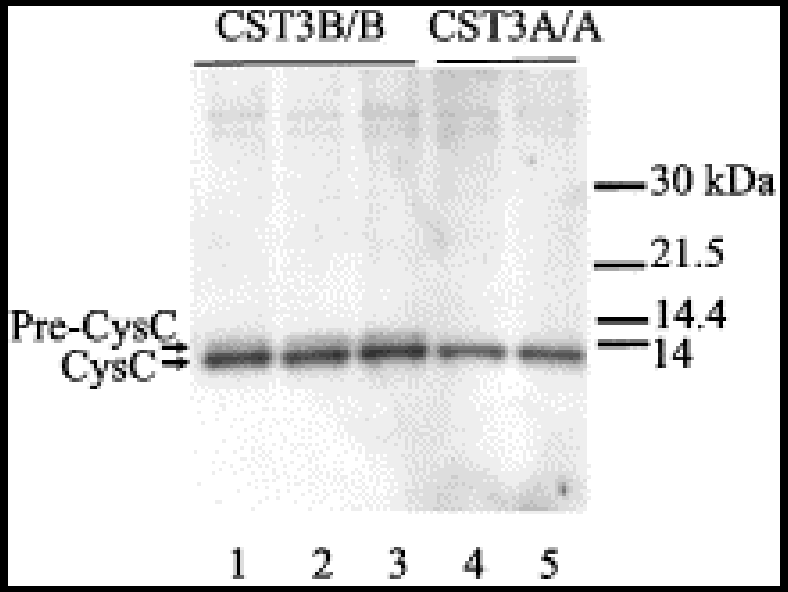
- *Misfolded protein*
- *Aging/environmental factors (stress & inflammation)*
- *Genetic mutations/polymorphism*

4) How to disrupt the CysC-CatB interaction to promote Aβ clearance?

Intracellular Accumulation of CysC Is Associated With CST3 Polymorphism & PS2 Mutations



Ghidoni et al.,
Neurobiology of Aging, 2007



Benussi et al.,
Neurobiology of Disease, 2003

Ongoing Investigation & Future Plans

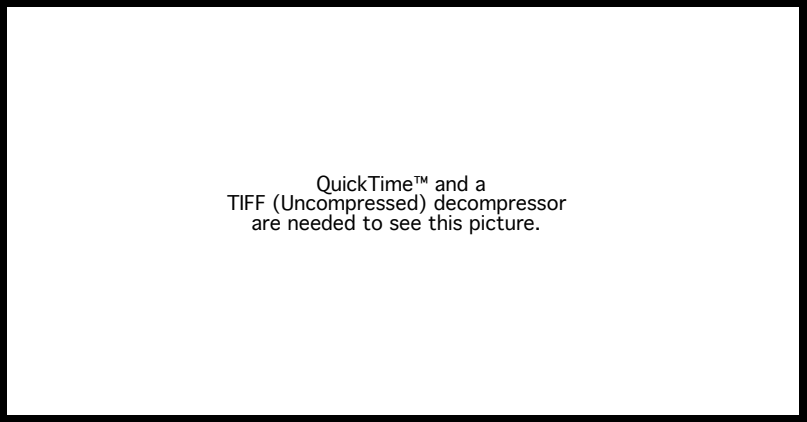
2) Where does CysC and CatB interact?

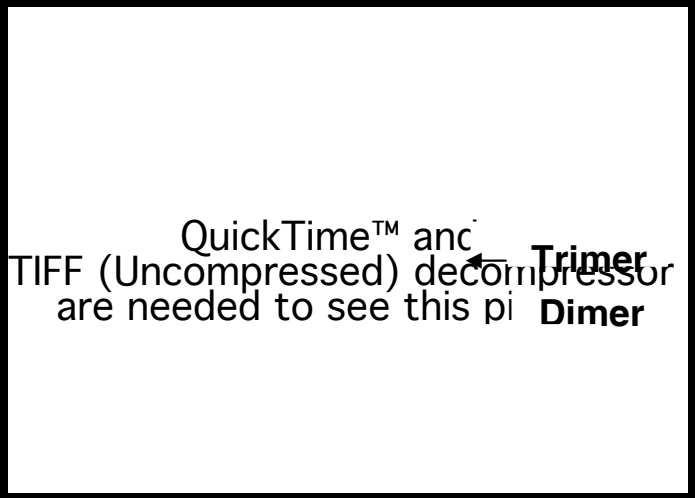
3) How is the CysC-CatB axis regulated?

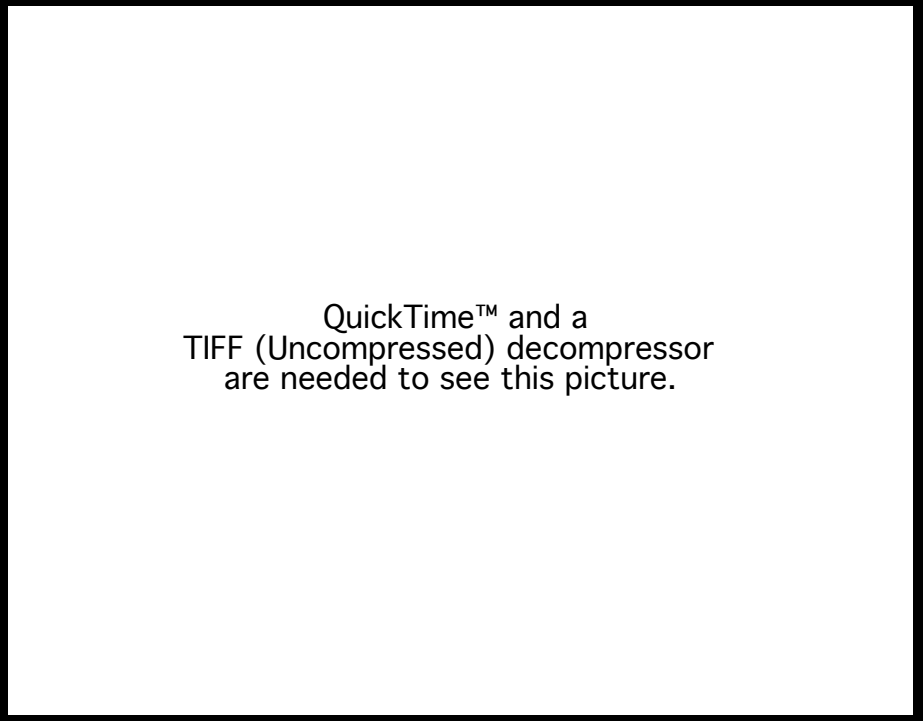
4) How to disrupt the CysC-CatB interaction?

- *Structural basis*
- *Robust cell-based or cell-free assay*
- *Monoclonal antibodies (extracellular)*
- *Small molecule compounds (Intracellular)*

Functional Deficits Induced by Naturally Secreted Aβ Oligomers From CHO Cells Expressing hAPP (7PA2)



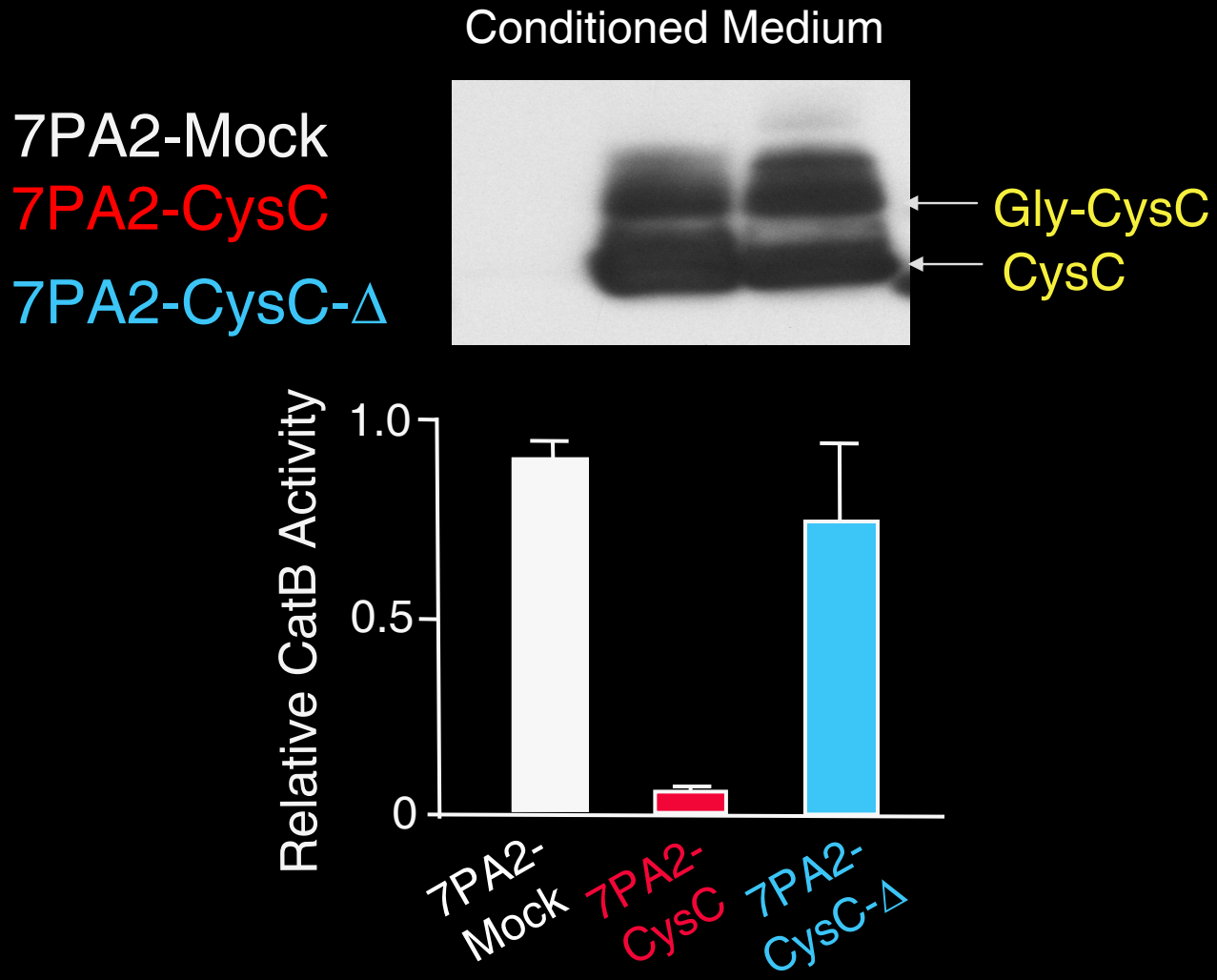




conditioned medium from 7PA2 Cells

Walsh et al., Nature, 2002

A Cell-based Assay to Screen for Inhibitors of CatB-CysC Interaction

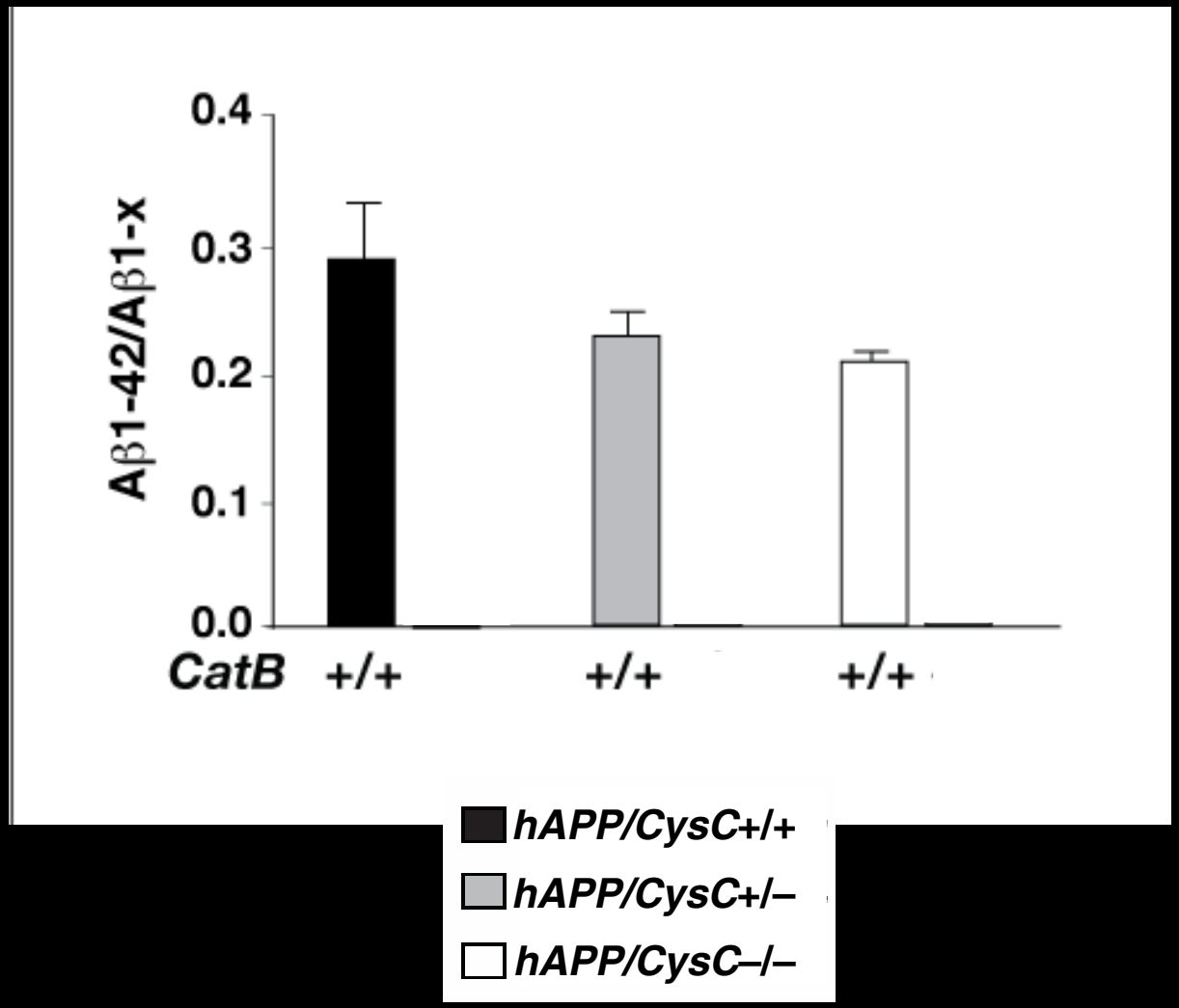


A Cell-based Assay to Screen for Inhibitors of CatB-CysC Interaction

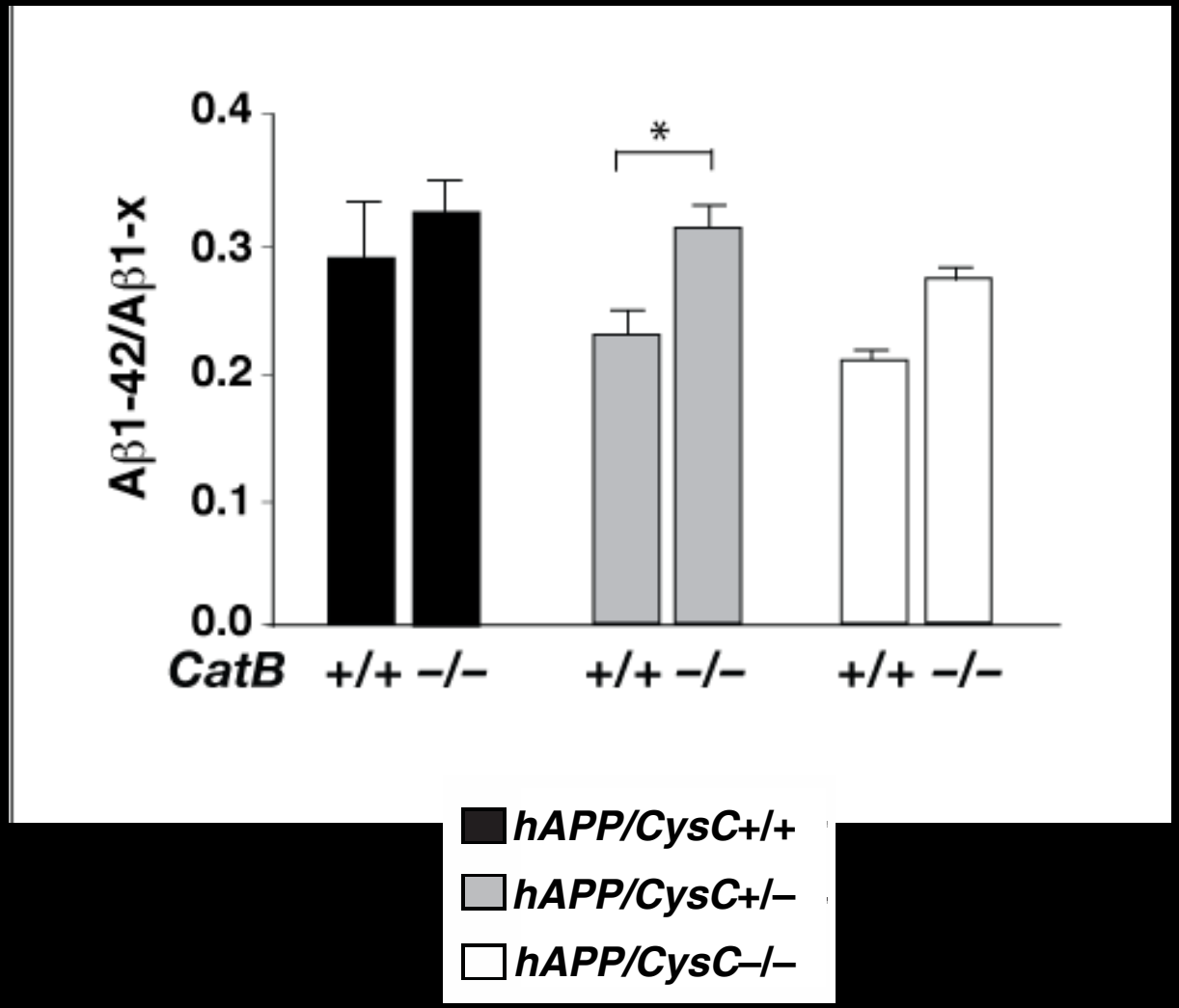
Multiple readouts:

- ✓ CatB activities (*enzymatic assay*)
- ✓ Total Aβ levels (*ELISA*)
- ✓ Levels of Aβ oligomers (*IP-WB*)
- ✓ Toxicity of the conditioned medium (*cell death assay/electrophysiology*)

Reducing CysC Lowers Relative Abundance of Aβ1-42 in the Presence of CatB



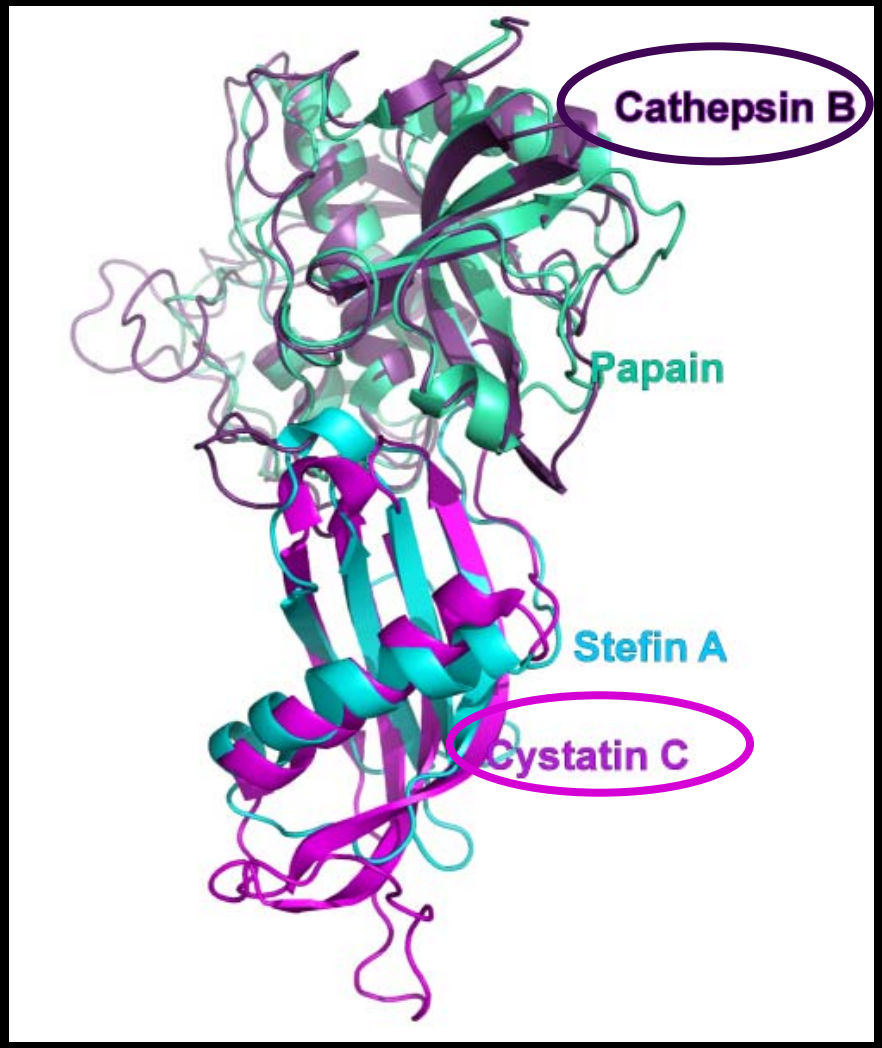
Relative Abundance of Aβ1-42 Remains Similarly High in the Absence of CatB



Structural Basis for the CatB-CysC Interaction

Regions on CysC that confer selectivity to CatB:
- Arg-8 and Leu-9
- Trp106 at the second loop

Regions on CatB that interact with CysC:
-1–16
-53–61
-104–108



Janowski et al. 2005; Jia et al, 1995; Clare Peters-Libeu

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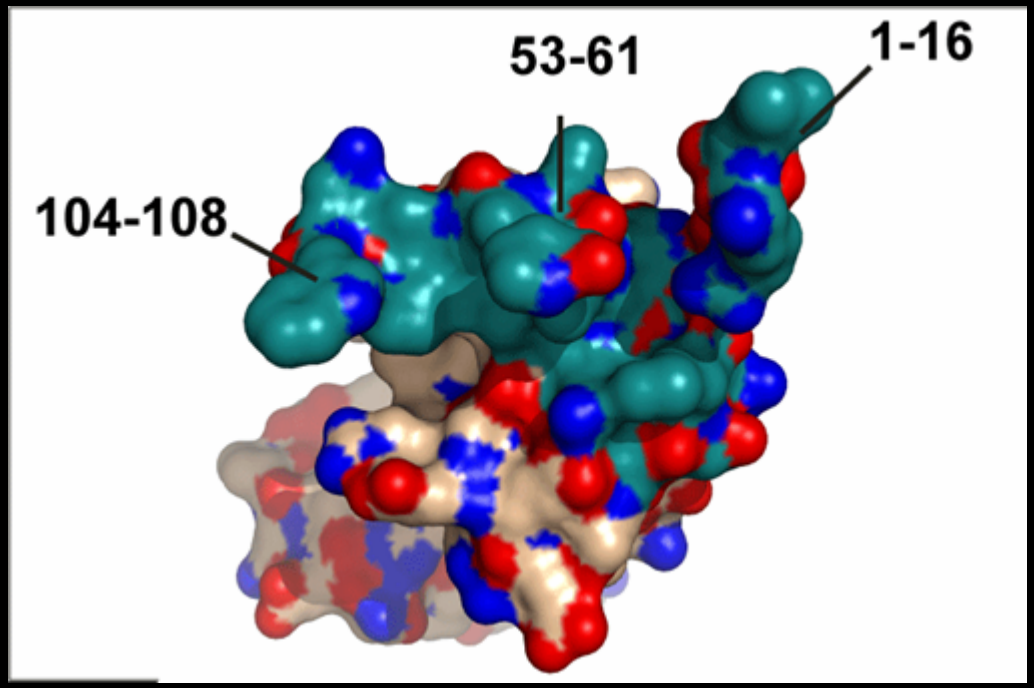
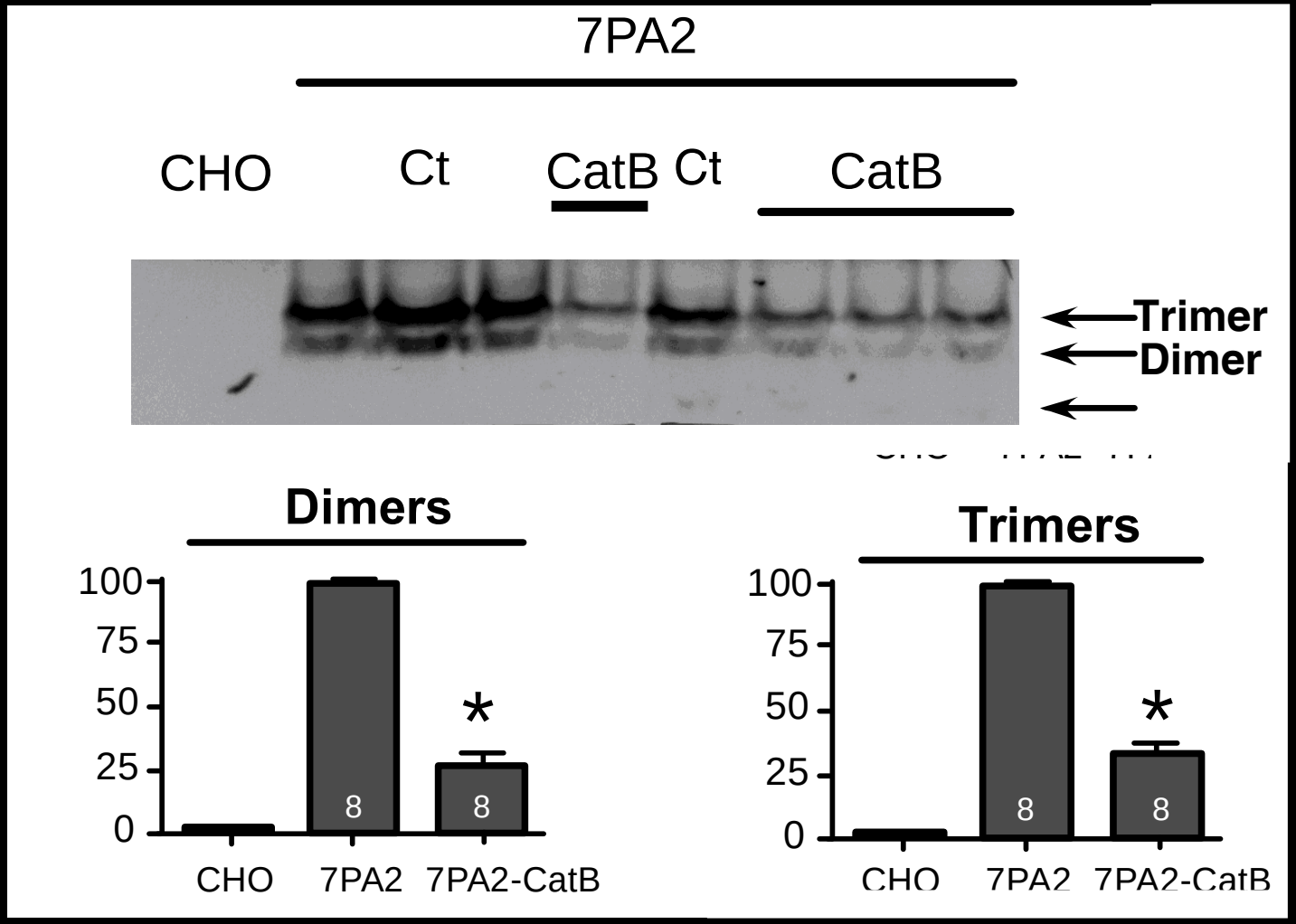


Janowski et al. 2005; Jia et al, 1995; Clare Peters-Libeu

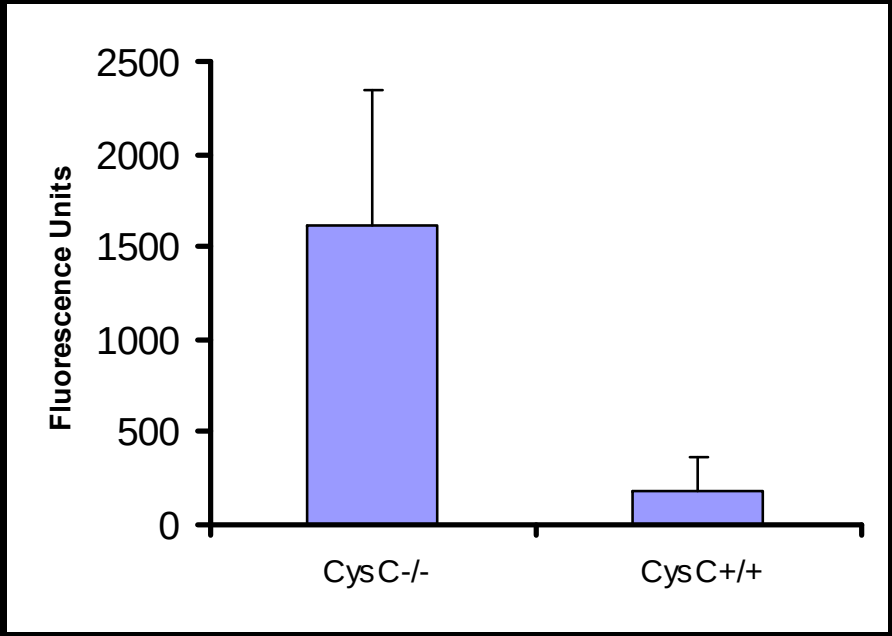
Cathepsin B Reduces Naturally Secreted Aβ Oligomers in CHO Cells Expressing hAPP



M. Cisse, Y. Zhou

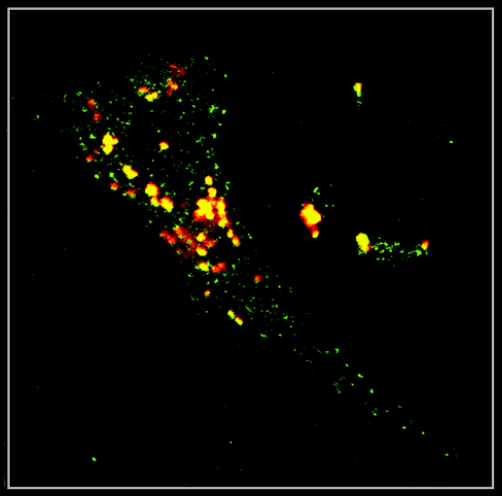
Cystatin C May Interact with Cathepsin B Extracellularly

Cathepsin B activity in CSF



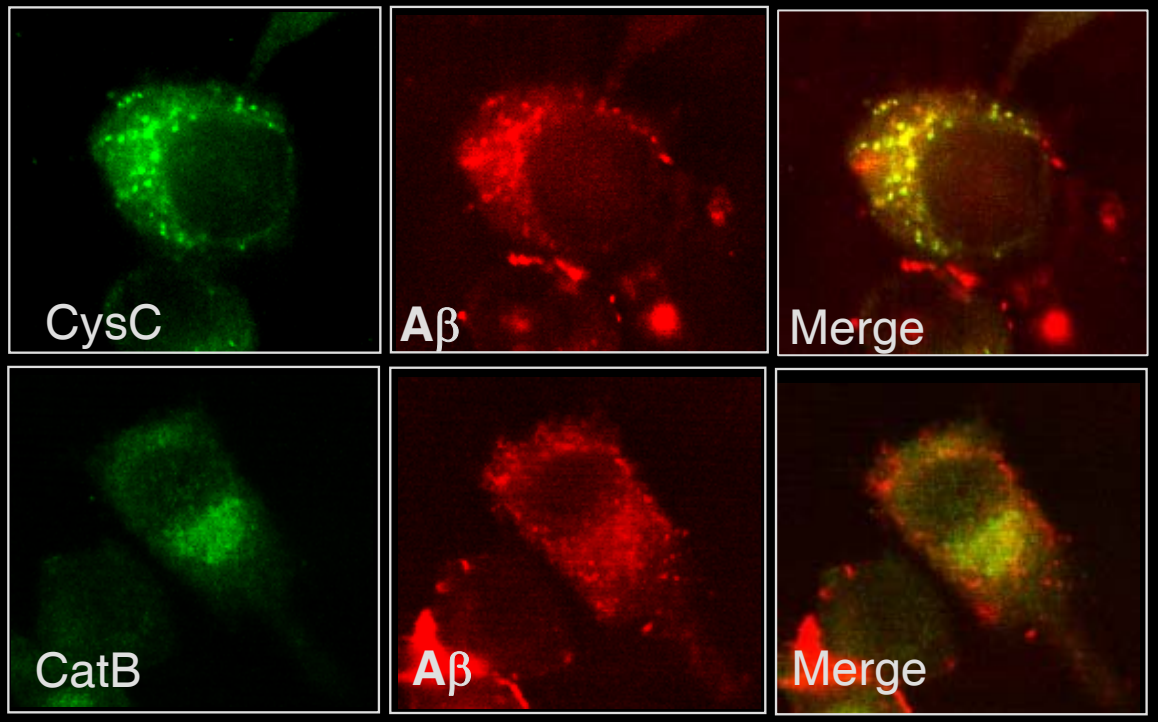
Binggui Sun

CysC-CatB Axis in Aβ Degradation



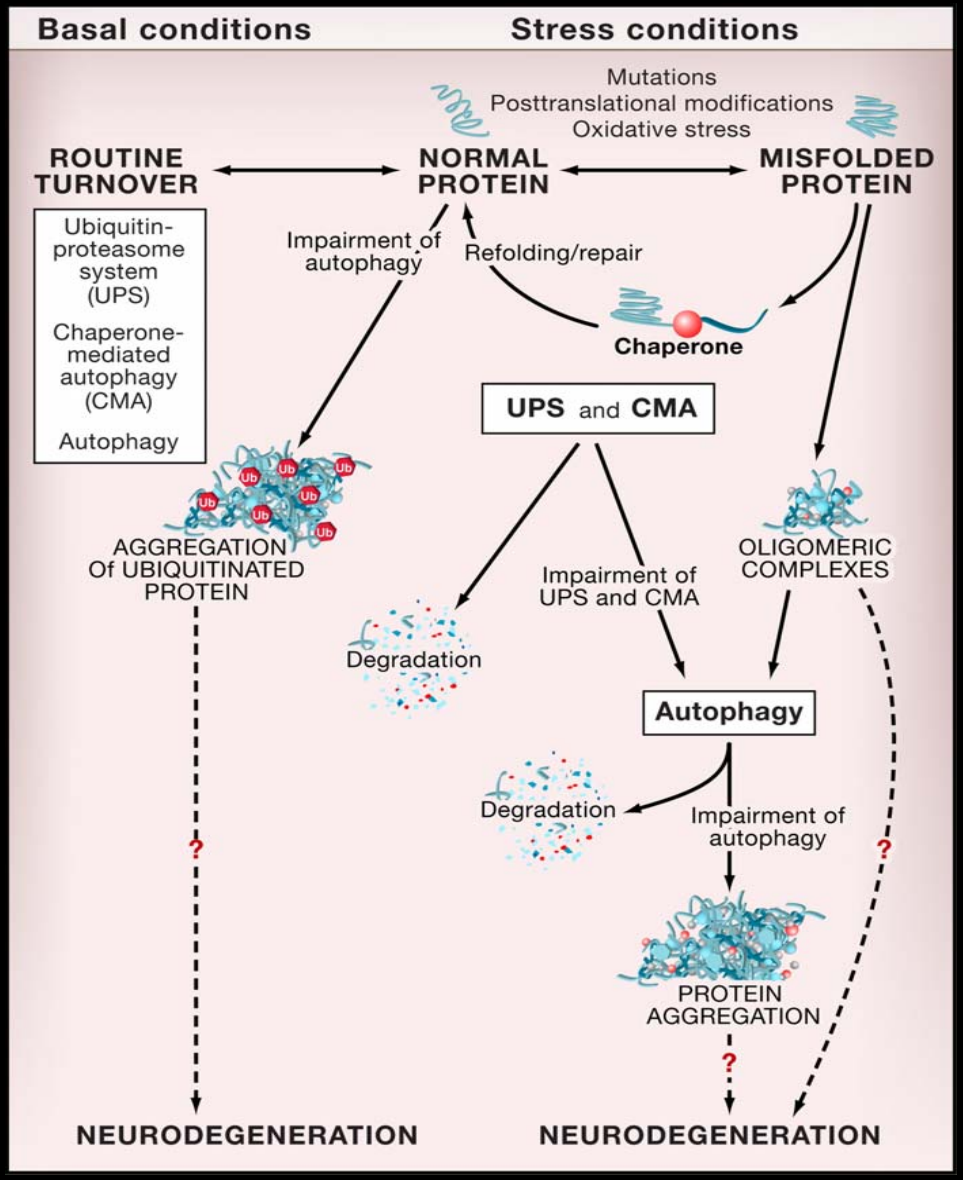
Co-localization of cystatin C with cathepsin B in neurons of AD brains

Deng, et al, Am J Pathol 2001, 59:1061-8



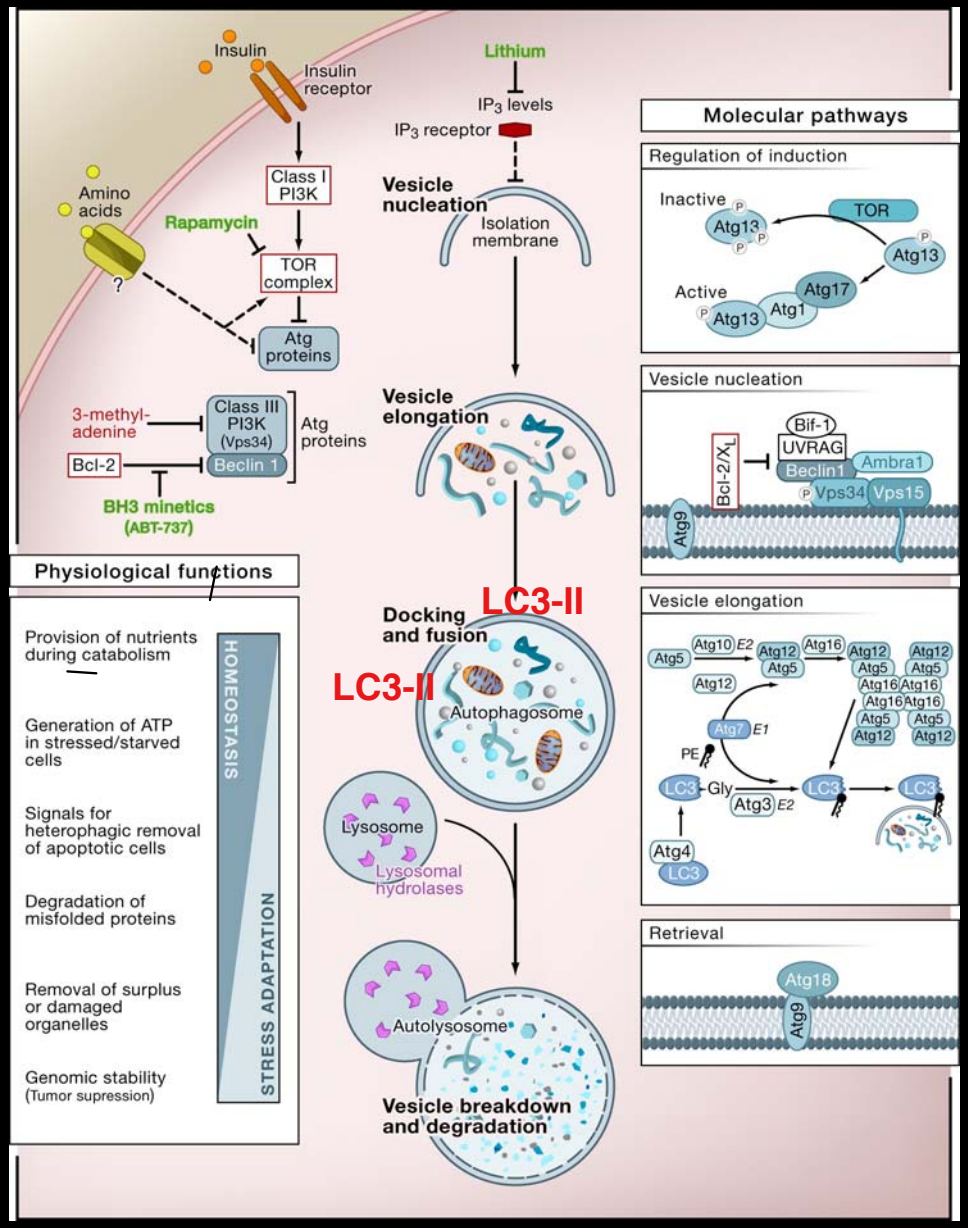
Microglial BV2 Cells

Constitutive Autophagy Plays an Central Role in Degradation of Misfolded Proteins



Levine & Kroemer, Cell, 2008

Macroautophagy Involves Fusion of Lysosomes With Autophagosomes

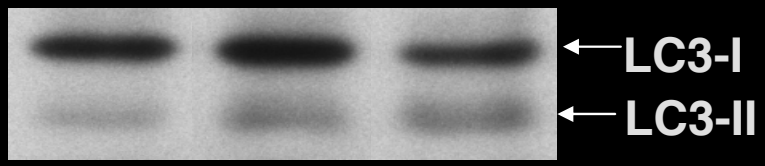


Levine & Kroemer, Cell, 2008

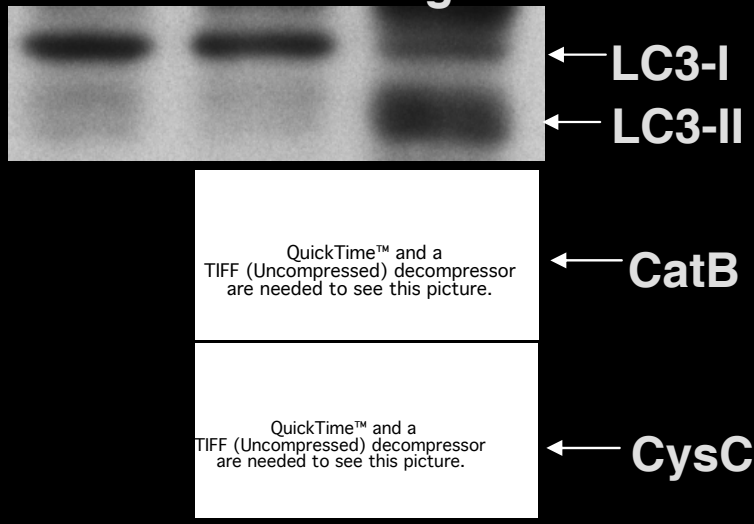
Aβ Activates Autophagy in Microglia: Accompanied by CatB Induction and CysC Reduction

Activation of Autophagy

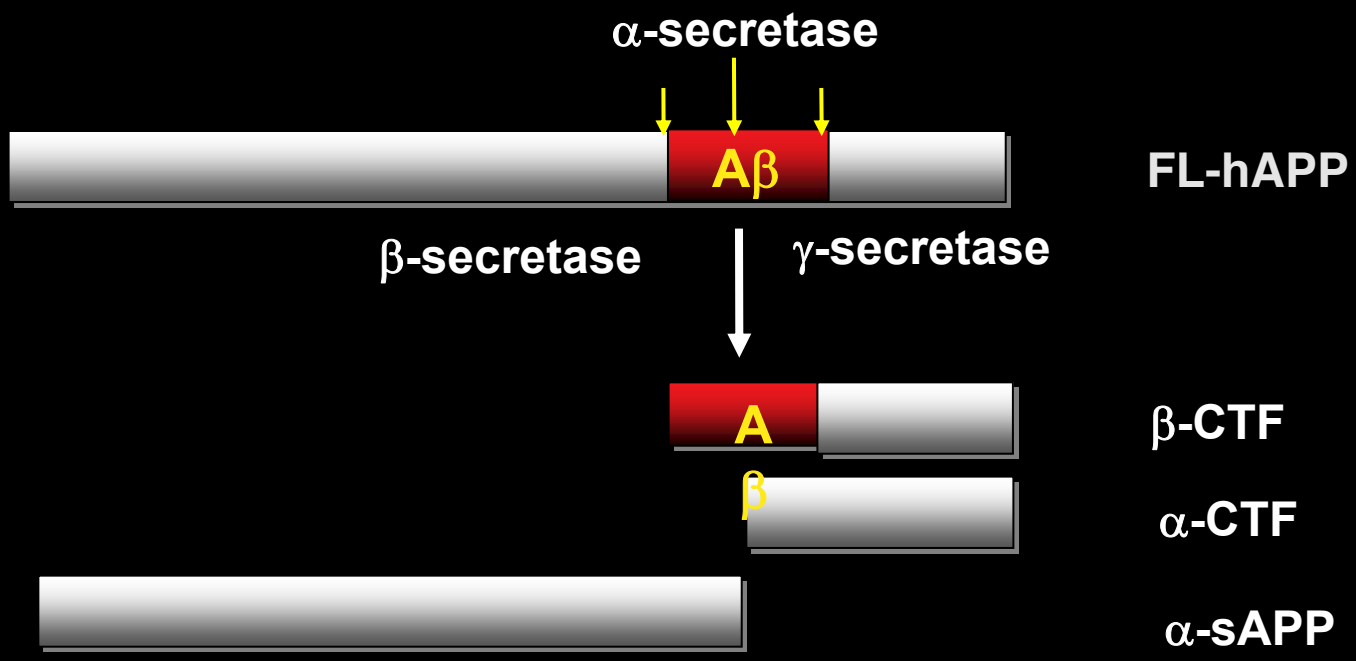
LC3-I → LC3-II



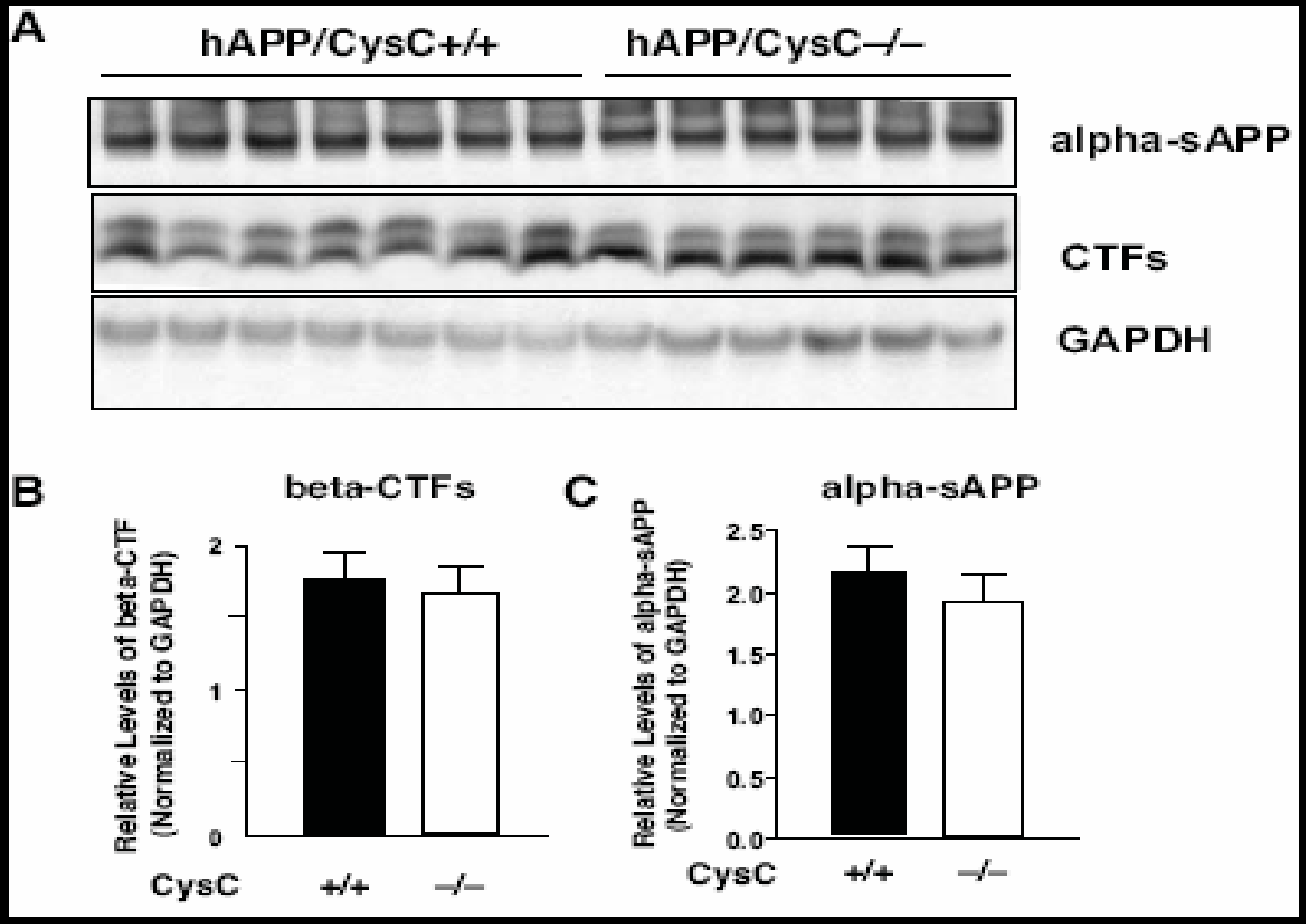
Microglial BV2 Cells



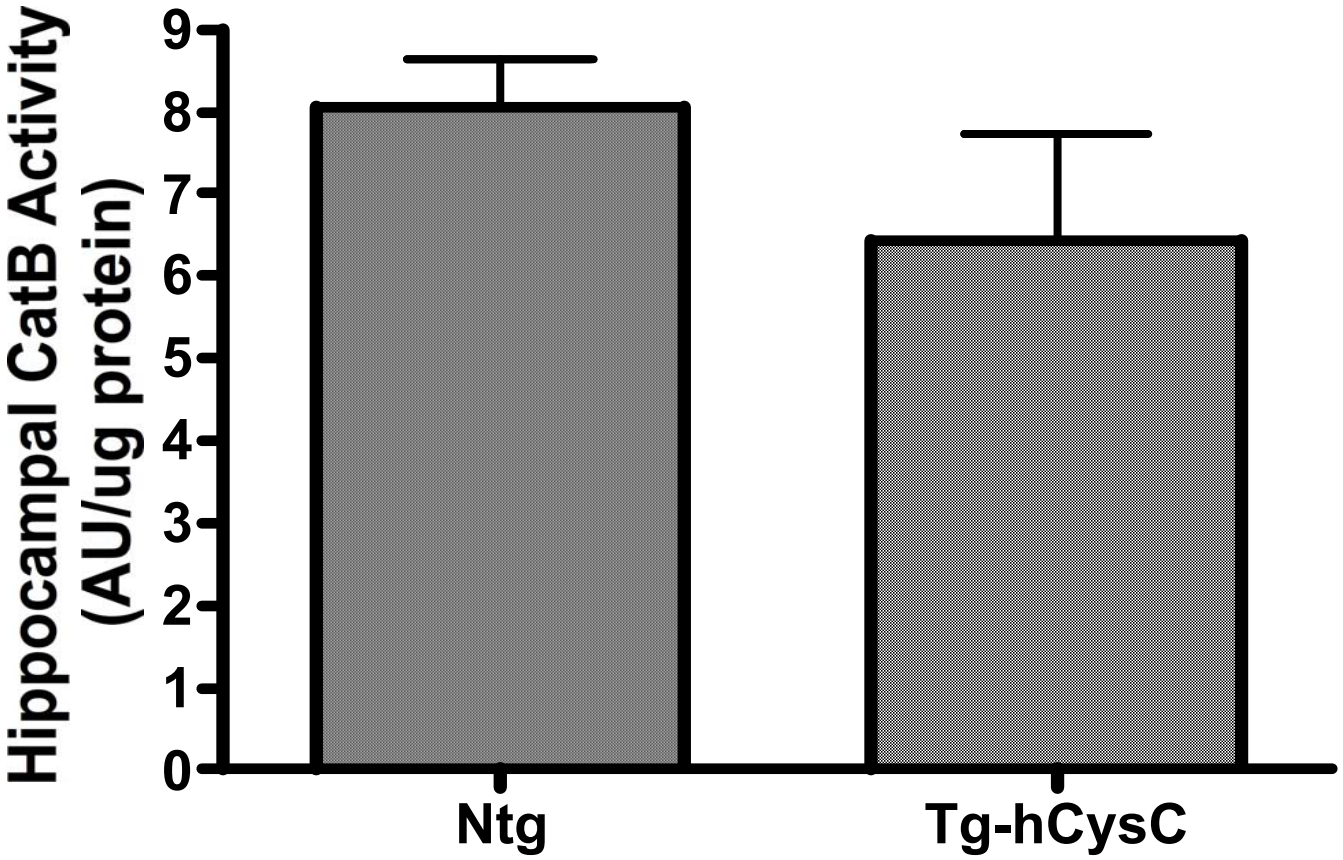
APP Processing and Aβ Generation



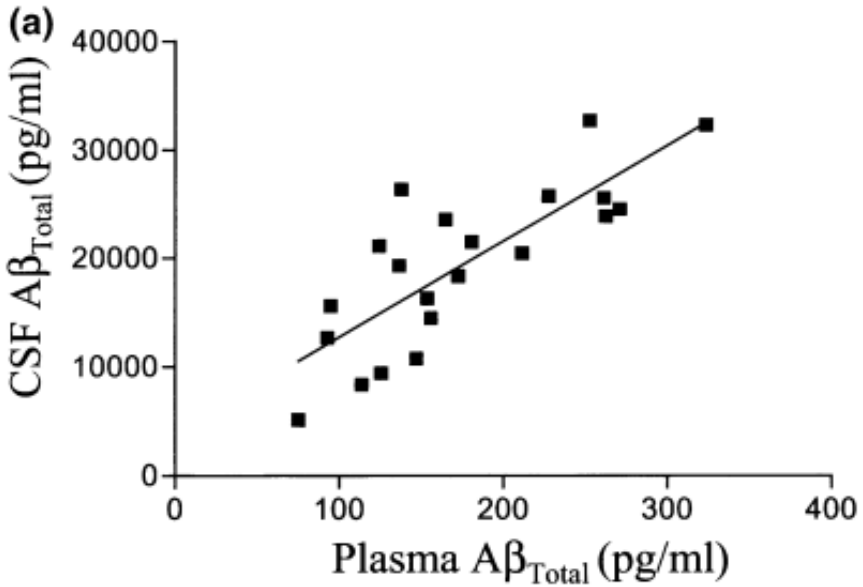
Cystatin C Ablation Does Not Affect hAPP Processing in Vivo



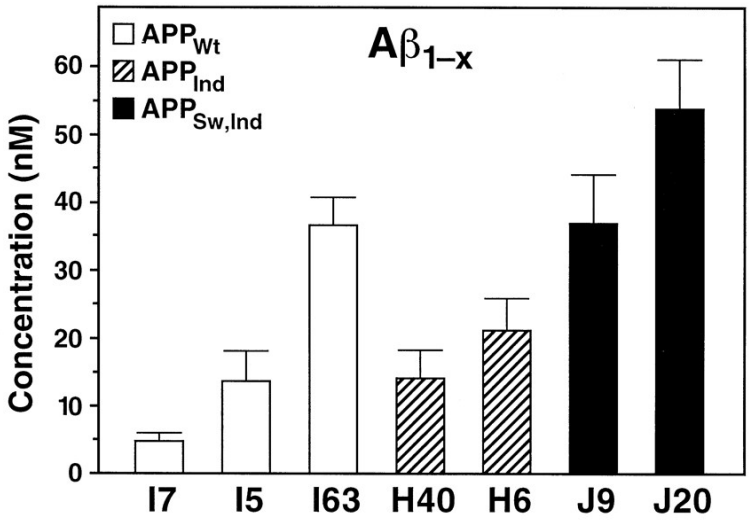
Overexpression of Cystatin C Resulted in Slight Inhibition of CatB



Yungui Zhou and Stephan Kaeser



DeMattos et al., 2002



Mucke et al., 2000

In the CSF
[Ab] ~ 20 ng/ml (4.4 nM)
[CysC] ~ 0.1uM =100 nM >> CatB

In the hippocampus:
[Ab] ~ 50 nM
[CysC] ~ 0.1uM =100 nM >> CatB