

# Discovery of GPCR ligands for GPR3, a GPCR modulating amyloid secretion in Alzheimer's Disease

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Director, Biology

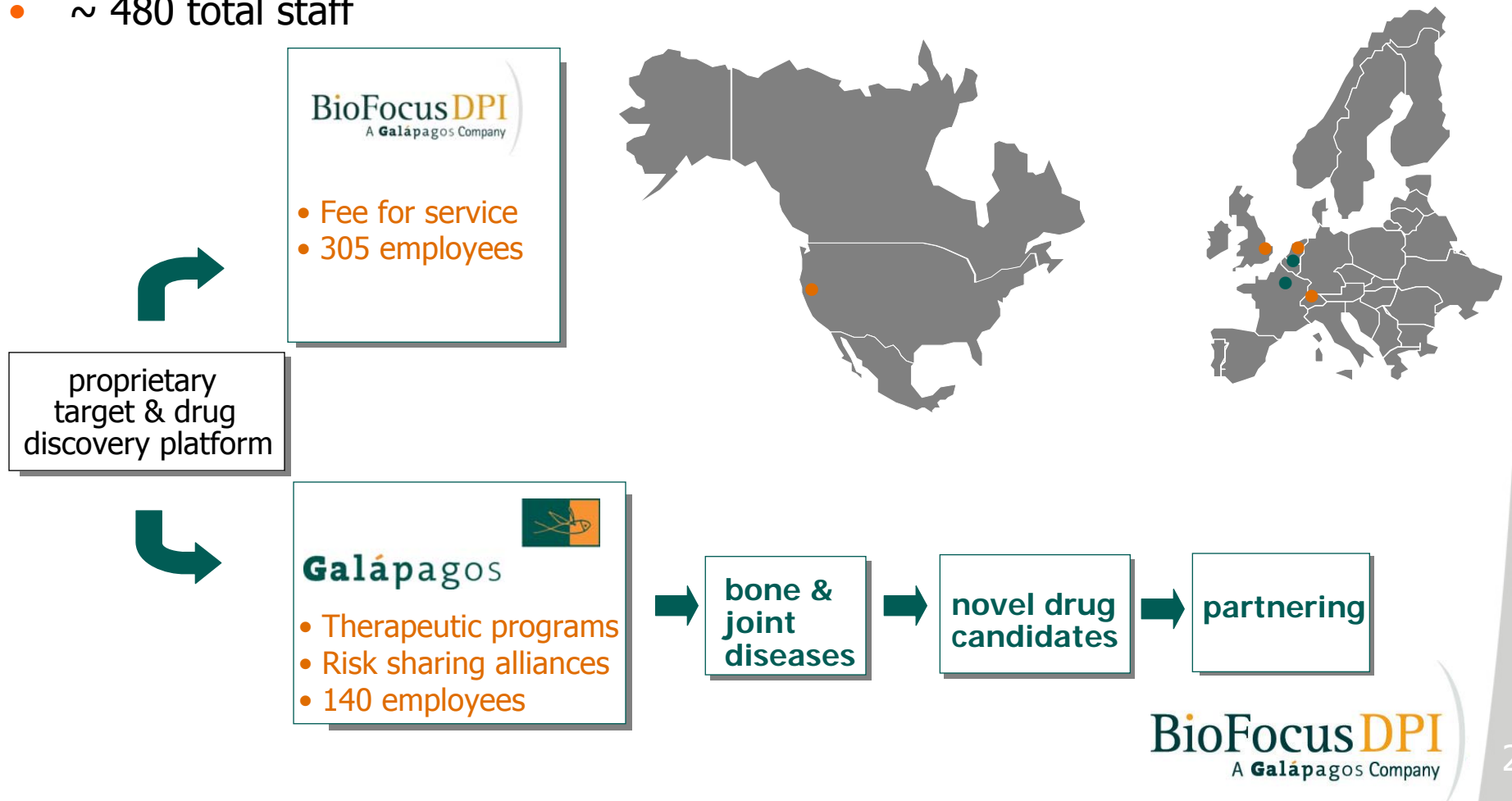
GPCR meeting  
22 September 2009

**BioFocus**DPI  
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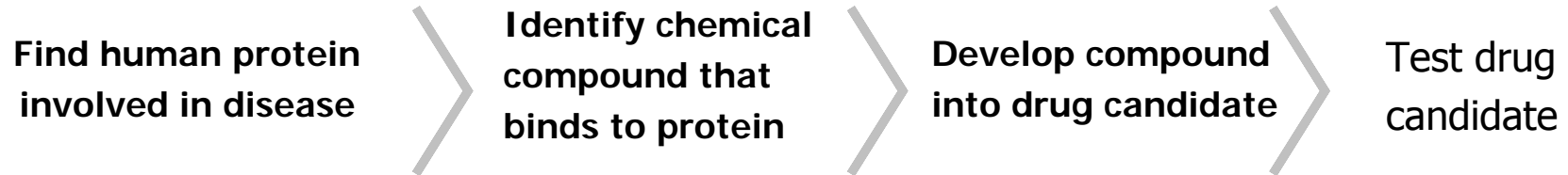
# The Galapagos Group

- Internal therapeutic programs in Belgium and France
- 4 service sites in US and Europe, commercial offices in Boston and Tokyo
- ~ 480 total staff





# BioFocus DPI technology



Target Discovery	Discovery Products	Biology	Medicinal Chemistry	Compound Management
Adenoviral libraries	Chemo-informatics	Assay development	Medicinal chemistry	Selection
Primary cell culture	Library chemistry	HTS	Biological profiling	Procurement
Assay development	Natural products chemistry	Functional screening	PK screening	Storage
Screening		Hit-expansion		Distribution
Bio-informatics		Structural Biology		



# Target Discovery

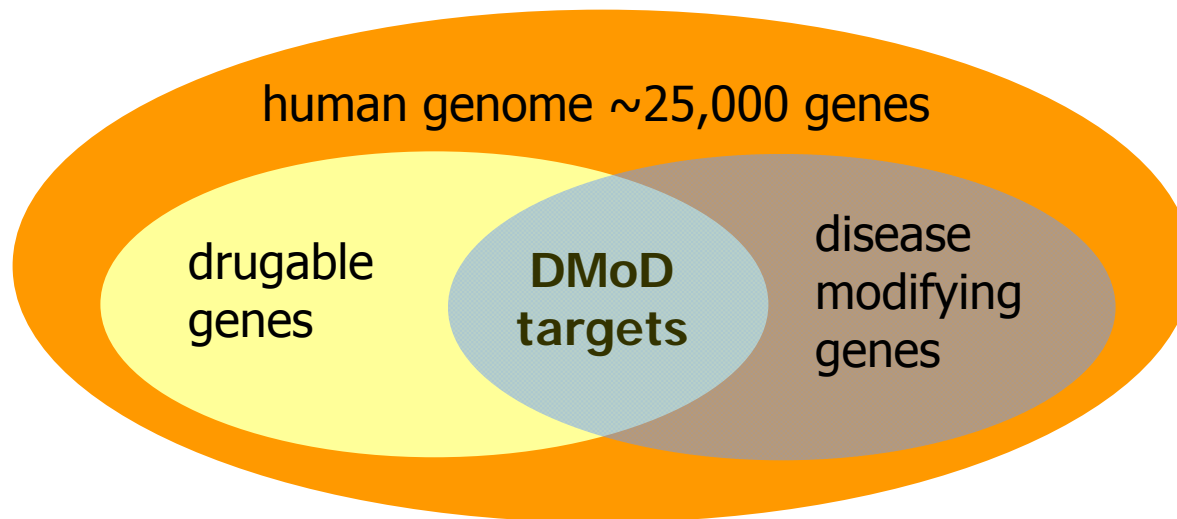
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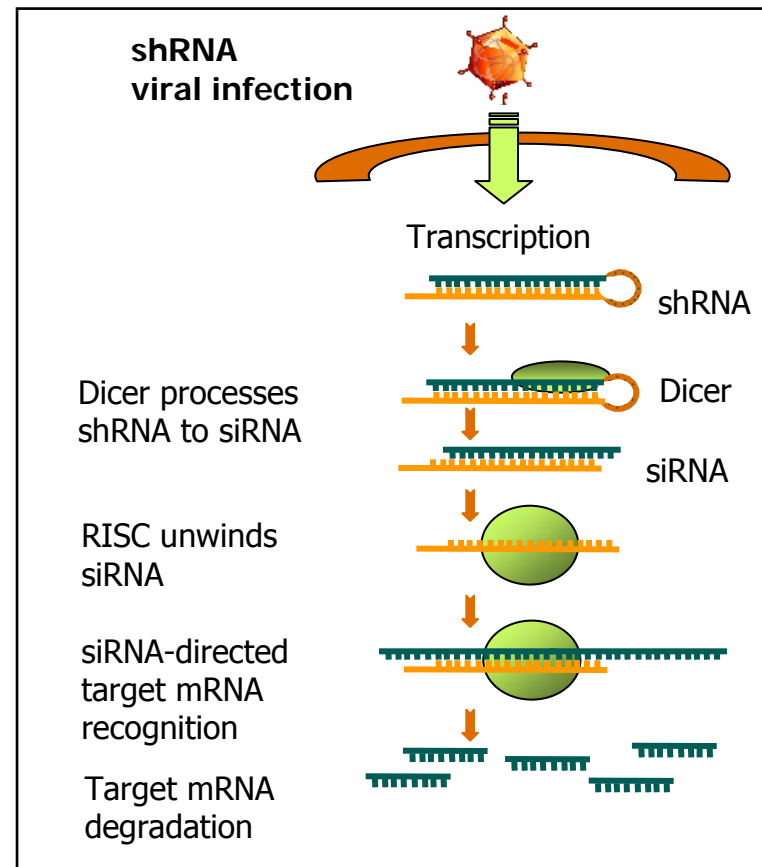
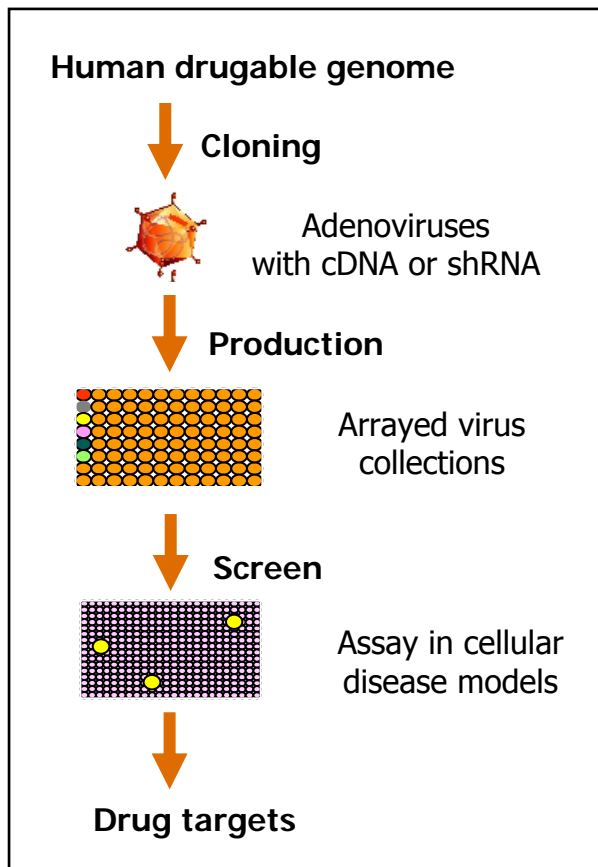
# Target discovery and validation

## Galapagos' approach



- Adenoviral libraries target human disease-modifying genes – functional approach
- *In vitro* disease model established with primary human cells
- Efficient identification of targets that can be modulated by
  - Small molecule compounds
  - Biologics (antibodies, proteins)

# Adenoviral cDNA and shRNA libraries



Nature Biotech. 2002, 20: 1154  
Genome Res. 2003, 13: 2325  
DDT 2005, 10: 1385  
Science 2009, 323: 946

US pat 6,340,595  
US pat 6,413,776  
US pat 7,029,848  
US pat 7,332,337 (fiber variants)

# Libraries: the drugable genome

- Knock-down libraries (SilenceSelect)
  - > 5,300 transcripts, tractable and biologics targets
- Knock-in libraries (FLeXSelect®)
  - > 2,000 human full-length cDNAs
- Arrayed per class, fully annotated
  - Expanding on demand
- Clear IP position

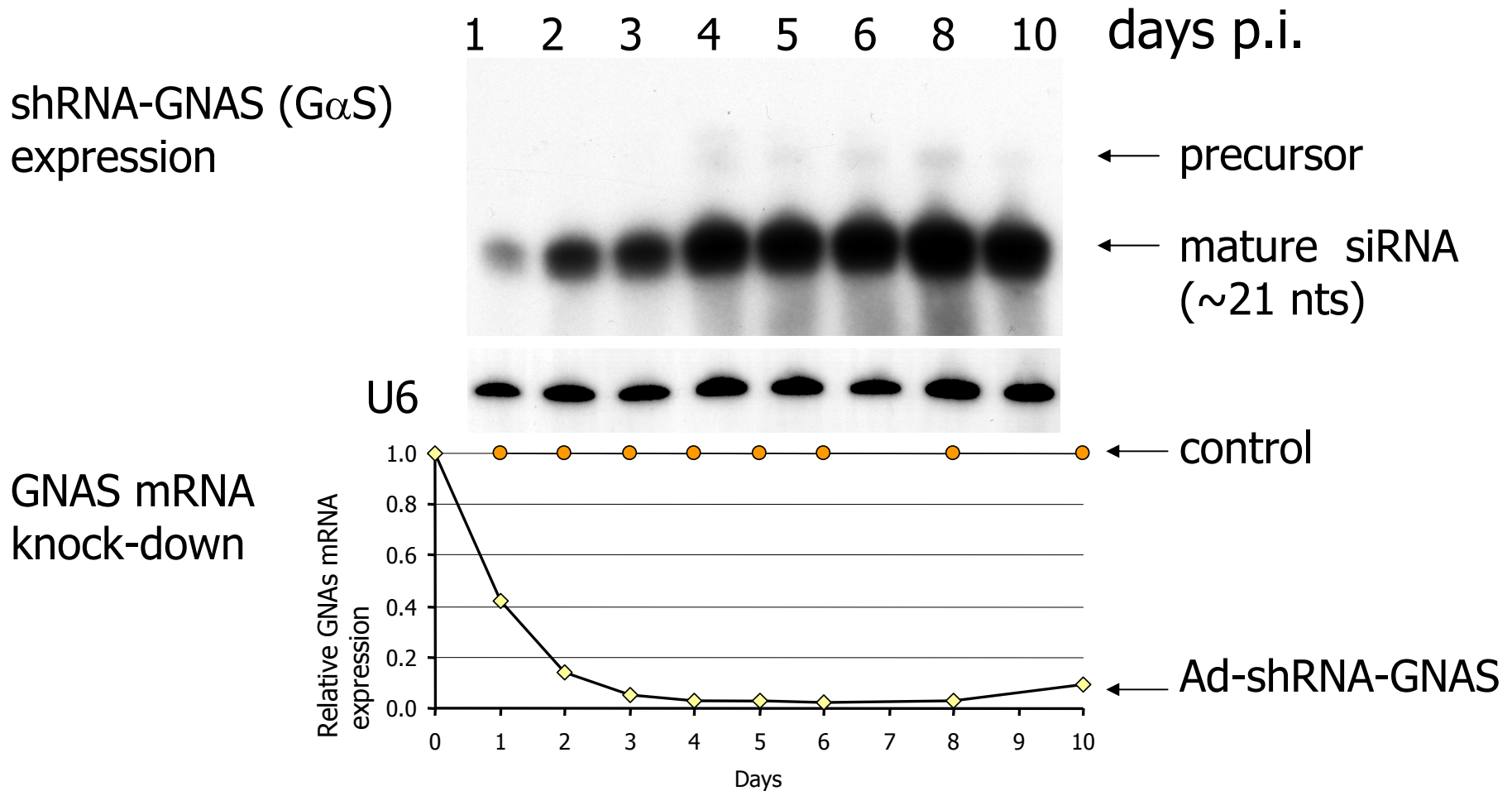
Extracellular	
Adhesion molecule	Hormone (receptor)
Axon guidance	Membrane enzyme
Carrier	Secreted enzyme
Chemokine (receptor)	TNF receptor family
Cytokine (receptor)	Toll like receptor
Extracellular matrix	WNT family member
Growth factor (receptor)	

SilenceSelect	
Class	# transcripts
GPCR	458
Ion channel	367
Kinase	1,128
NHR	68
PDE	41
Protease	723
Phosphatase	312
Other enzymes	723
Receptor	149
Transporter	227
Cytochrome P450	75
<b>Extracellular</b>	<b>1,098</b>
<i>total</i>	<i>5,369</i>



# Adenoviral delivery of shRNA

## Long term expression and RNAi





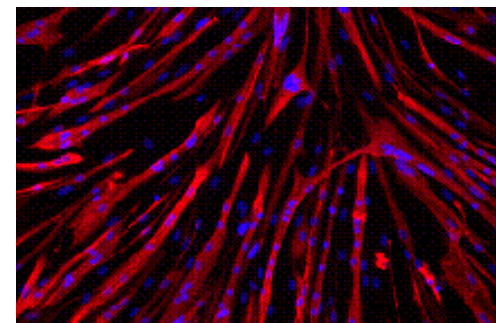
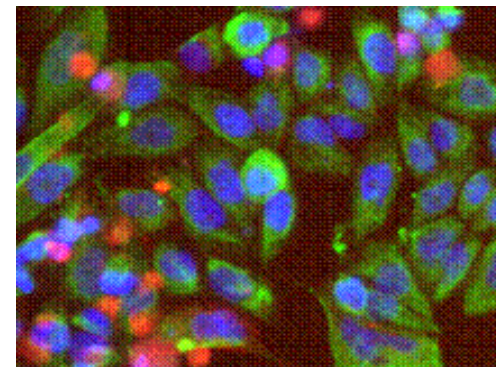
# Tropism of adenovirus

Human primary cells with over 70% transduction (C01 or C20)

- (Pre) adipocytes
- Beta cells
- Bronchial epithelial cells (CF)
- Chondrocytes
- Dendritic cells
- Endothelial cells
- Fibroblasts
- Hepatocytes
- Keratinocytes
- Macrophages
- Mast cells
- Osteoblasts
- Skeletal myoblasts and myotubes
- Synoviocytes
- Mesenchymal stem cells
- Embryonic stem cells
- Astrocytes derived from stem cells
- Cardiomyocytes derived from stem cells
- Motor neurons derived from stem cells
- Striatal neurons derived from stem cells
- Human cell lines (>30)

# Cellular assays

- Adipocyte differentiation and activity
- Amyloid beta regulation
- Bronchial epithelial chloride channel activity
- Chondrocyte differentiation and activity
- Dendritic Cell maturation
- Keratinocyte differentiation/proliferation
- LDL/HDL regulation
- Macrophage activity
- Mast cell degranulation
- Mesenchymal epithelial transition
- Myoblast differentiation
- Neuronal cell death
- Osteoblast differentiation and mineralization
- Synovial fibroblast activity
- Tumor motility
- Tumor invasion



High content screening



# Track record with novel targets

disease area	# Galapagos novel, validated targets	Status of program
RA	19	enters clinical phase 1
OA	20	preclinical candidate
OP	13	lead optimization
Respiratory	28	screening
Alzheimer	17	validated targets
Cardiovascular	16	validated targets
Cystic fibrosis	19	enter screening
Huntington	25	validated targets

Targets: novel starting points for discovery of new drugs



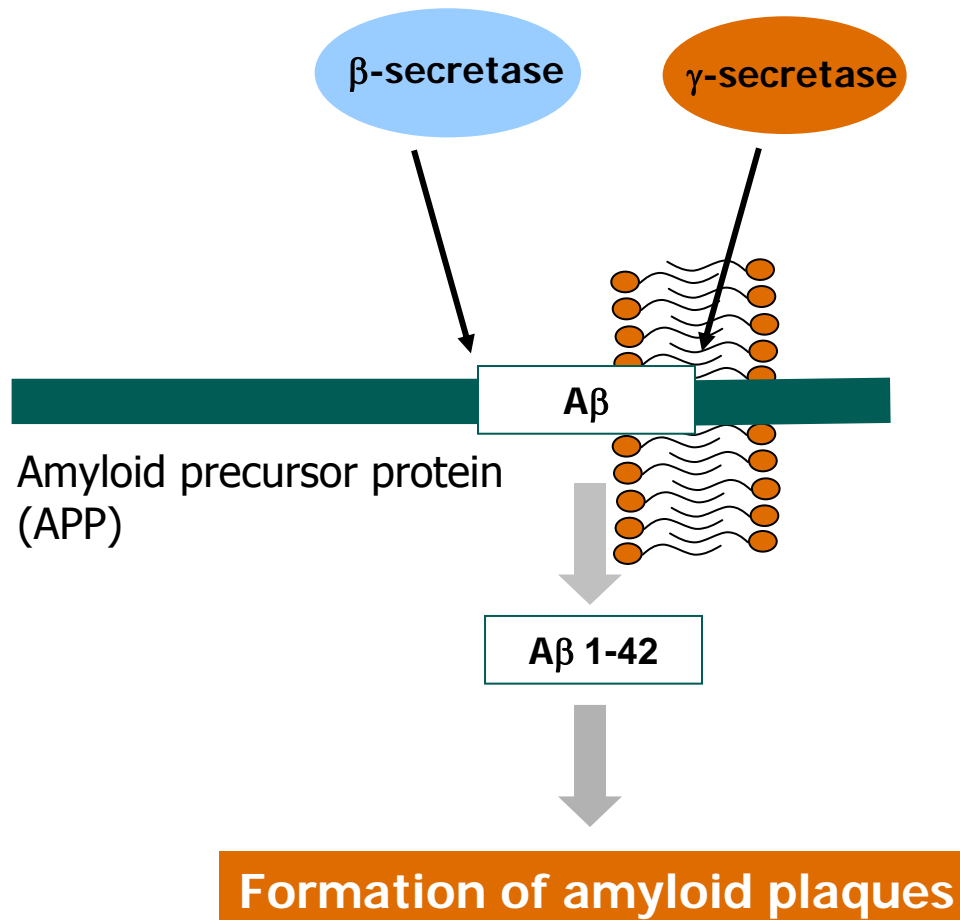
# Alzheimer's Disease

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# APP processing

Leads to  $\beta$ -amyloid ( $A\beta$ ) and plaque formation



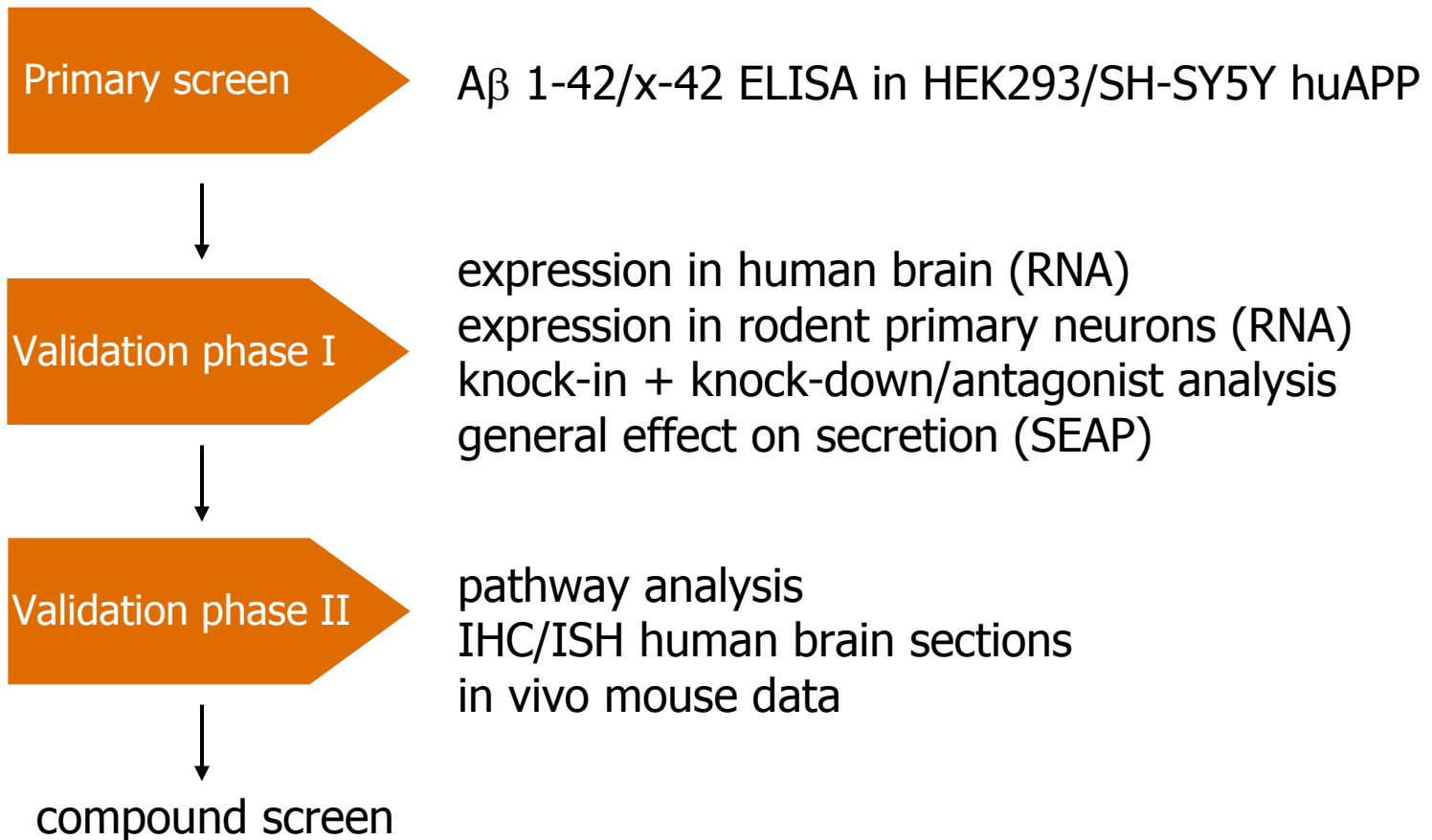


# Alzheimer's Disease

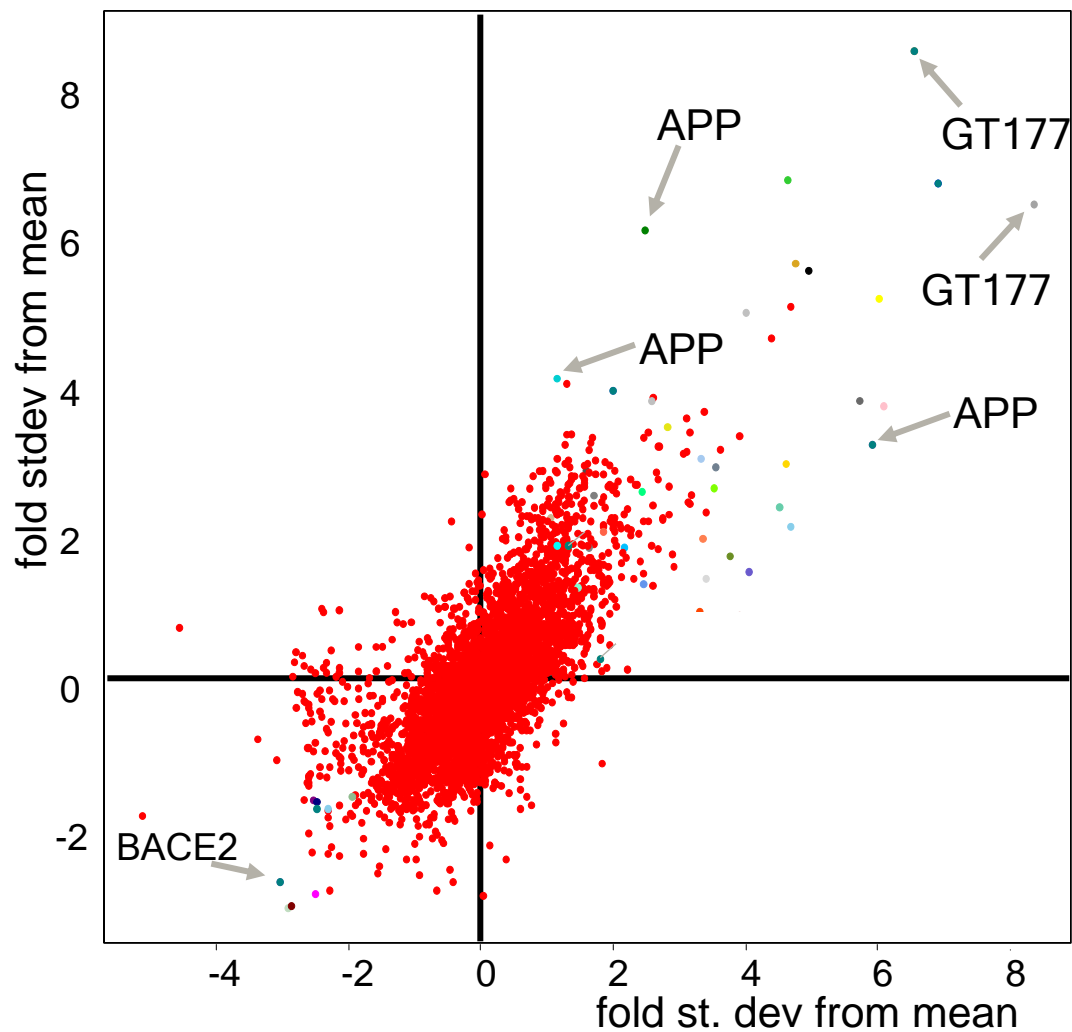
## Critical path

# Galápagos

Drugable  
genome



# Primary screening data (293T, cDNA)





# Primary screen

## Hits already published in literature

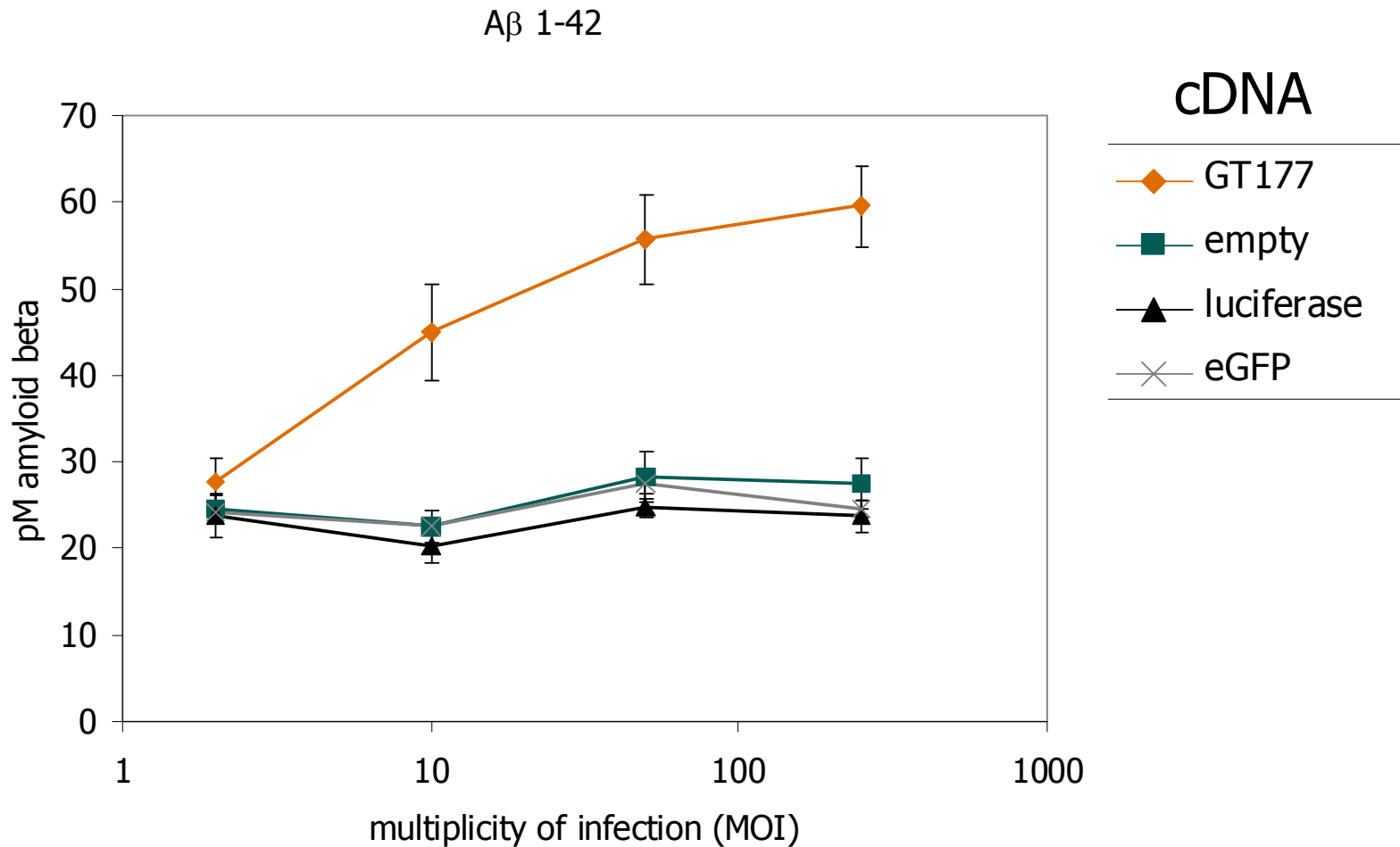
### cDNA knock-in

hit	activity
APP	A $\beta$ 1-42 $\uparrow$
BACE1	A $\beta$ 1-42 $\uparrow$
BACE2	A $\beta$ 1-42 $\downarrow$
CHRM3	A $\beta$ 1-42 $\uparrow$ / $\downarrow$ (sAPP)
HTR2C	A $\beta$ 1-42 $\uparrow$ (sAPP)
PTGER2	A $\beta$ 1-42 $\uparrow$ (APP)

### shRNA knock-down

hit	activity
BACE1	A $\beta$ 1-42 $\downarrow$
ROCK1	A $\beta$ 1-42 $\downarrow$
HTR4	A $\beta$ 1-42 $\downarrow$ (sAPP)
CRHR1	A $\beta$ 1-42 $\downarrow$ (sAPP)
PTGER2	A $\beta$ 1-42 $\downarrow$ (APP)

# GT177: G-protein coupled receptor GPR3

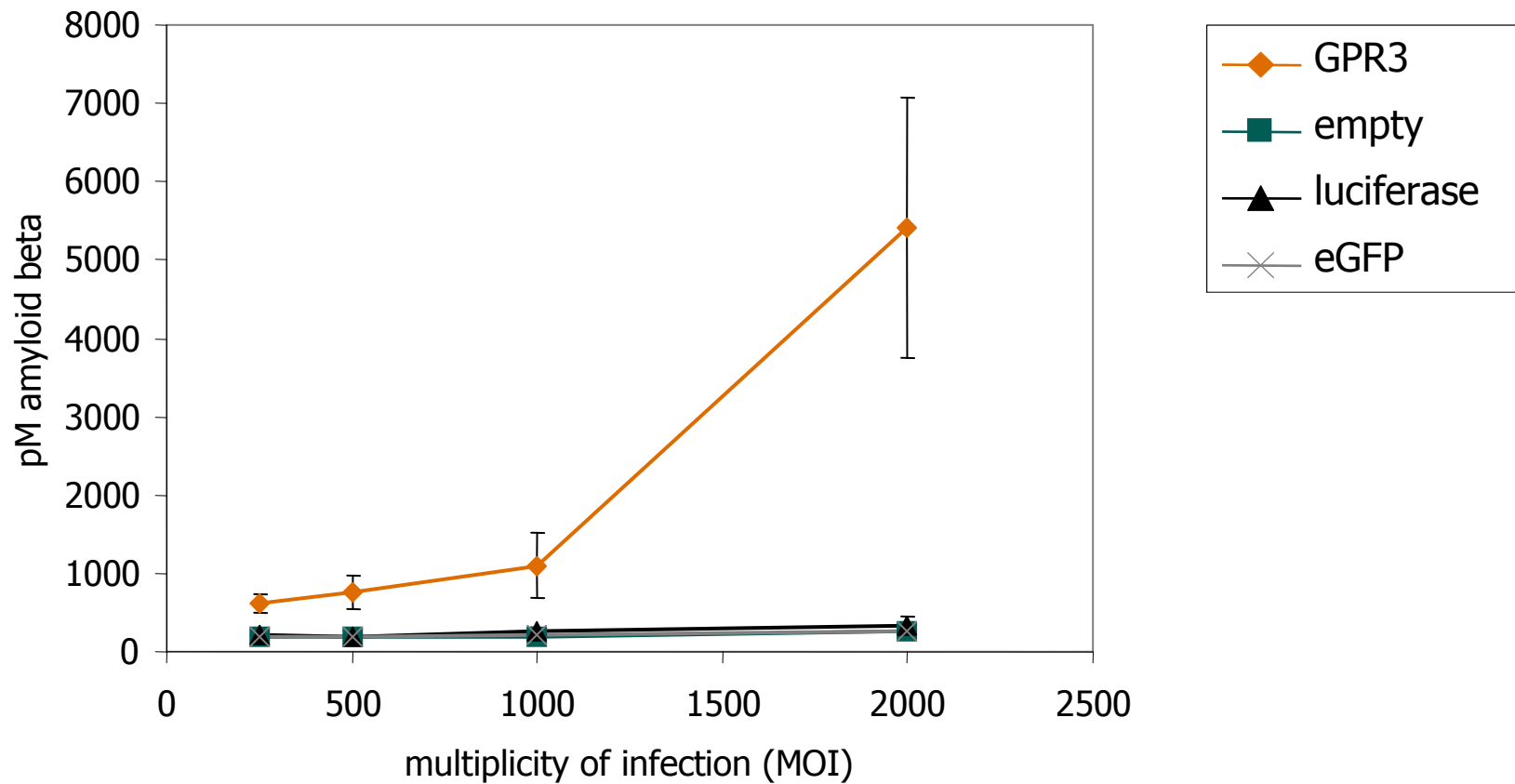




# GPR3 over-expression

## Primary hippocampal neurons

A $\beta$  1-42

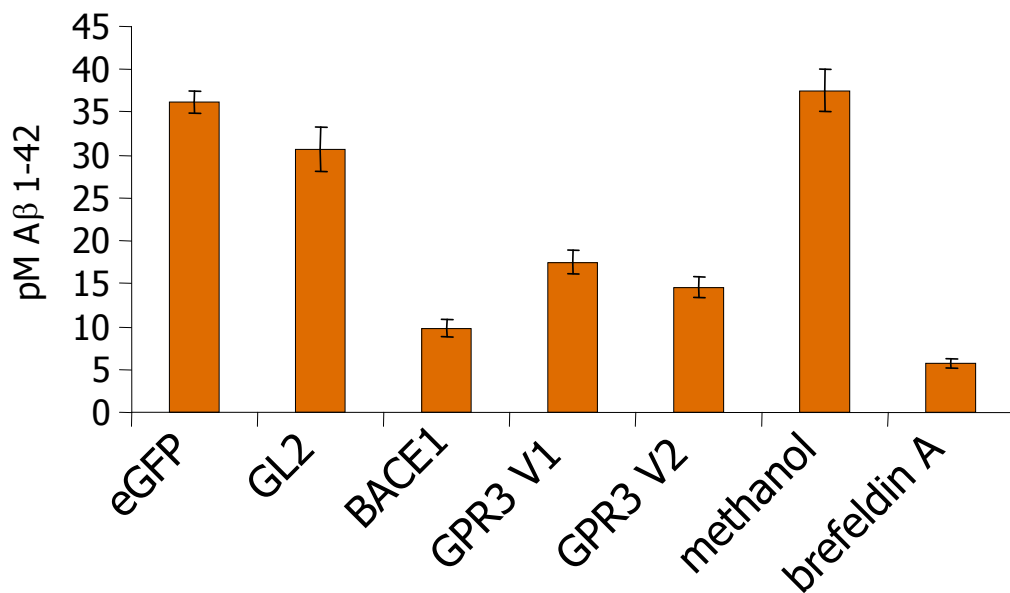




# GPR3 knock-down

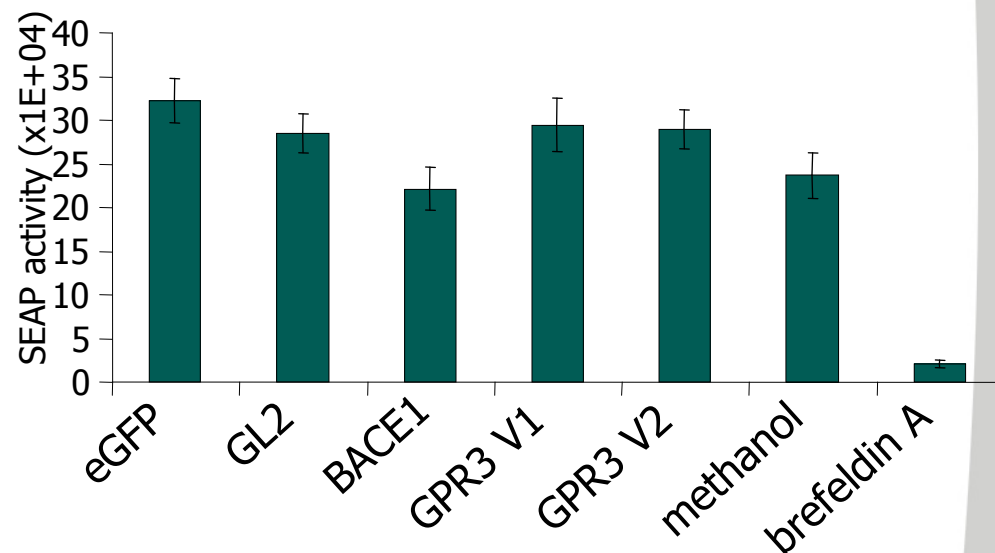
No effect on general secretion, reduction of A $\beta$

A $\beta$  1-42 levels



knock-down Hek293 APPwt

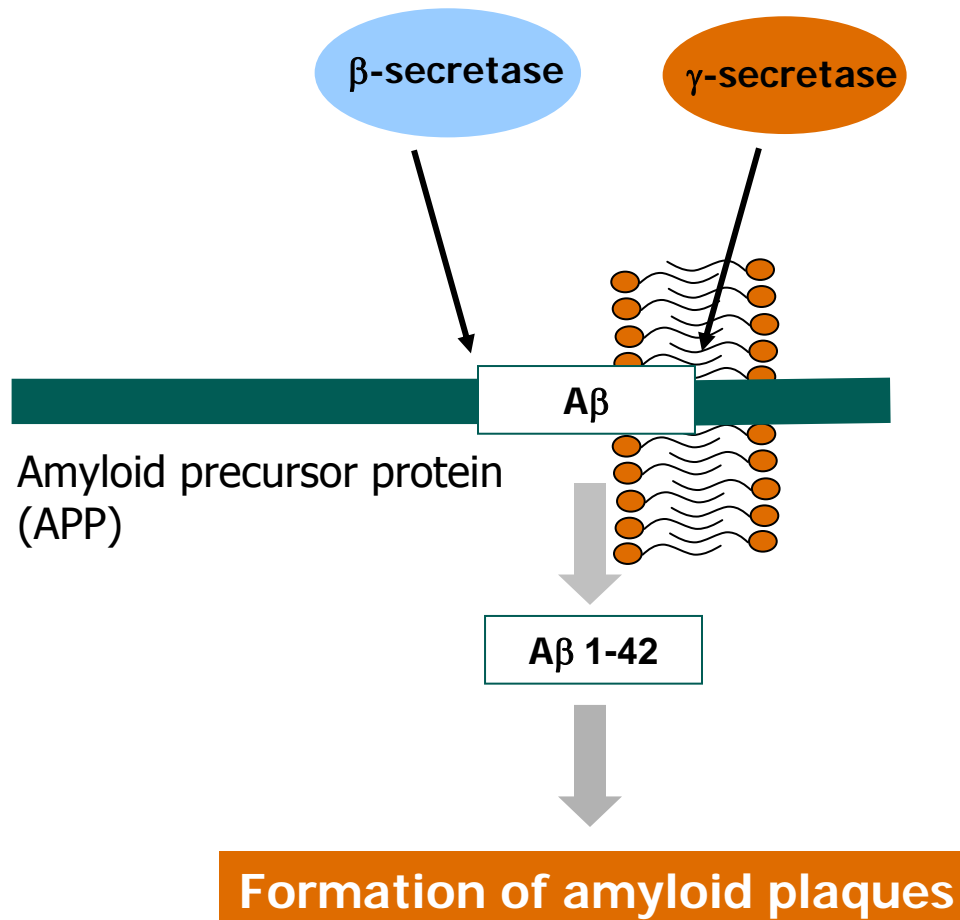
SEAP activity



knock-down Hek293 APPwt

# APP processing

Leads to  $\beta$ -amyloid ( $A\beta$ ) and plaque formation





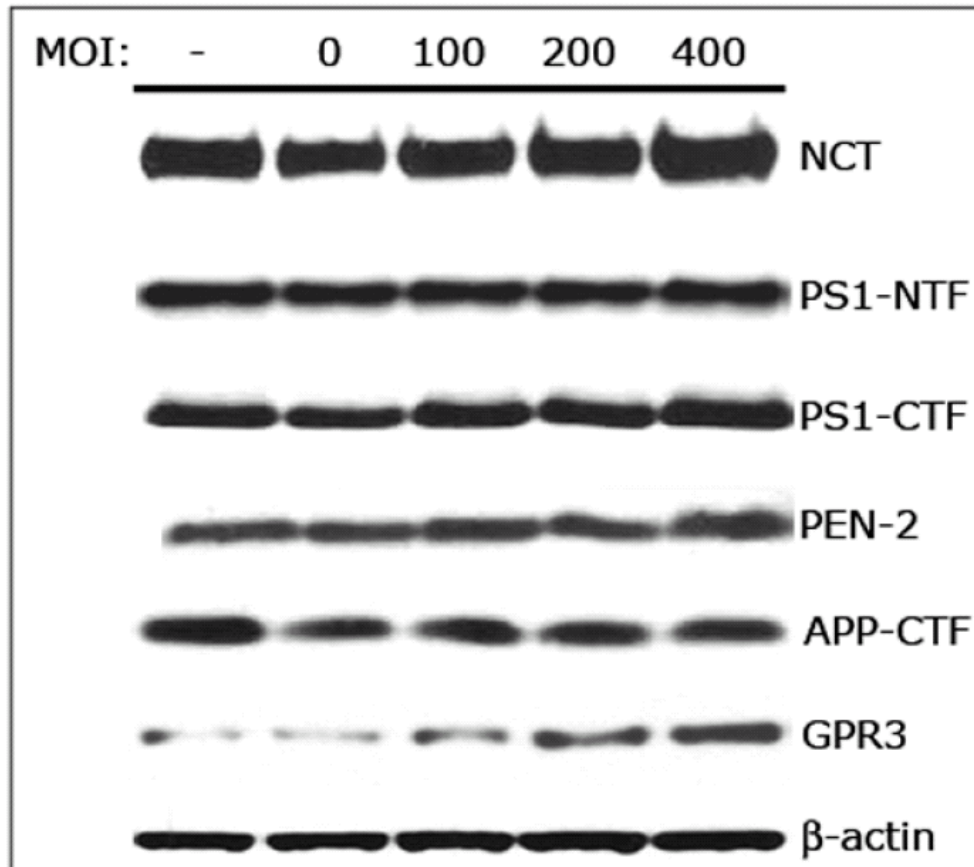
# GPR3

## Overview pathway analysis

<b>pathway</b>	<b>analysis</b>	<b>result of GPR3 expression</b>
$\gamma$ -secretase	C99	increased A $\beta$ 1-42
BACE1	CTF- $\beta$ analysis / IP-MS	no effect
APP	mature and immature APP levels	no effect

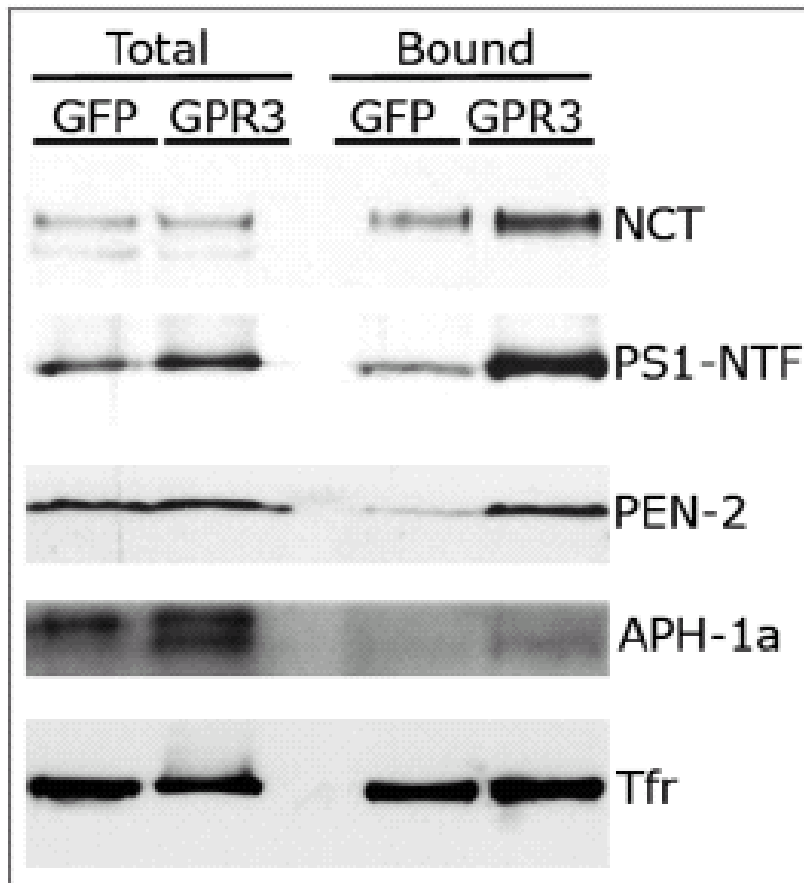
# $\gamma$ -secretase

Individual proteins not altered



# $\gamma$ -secretase

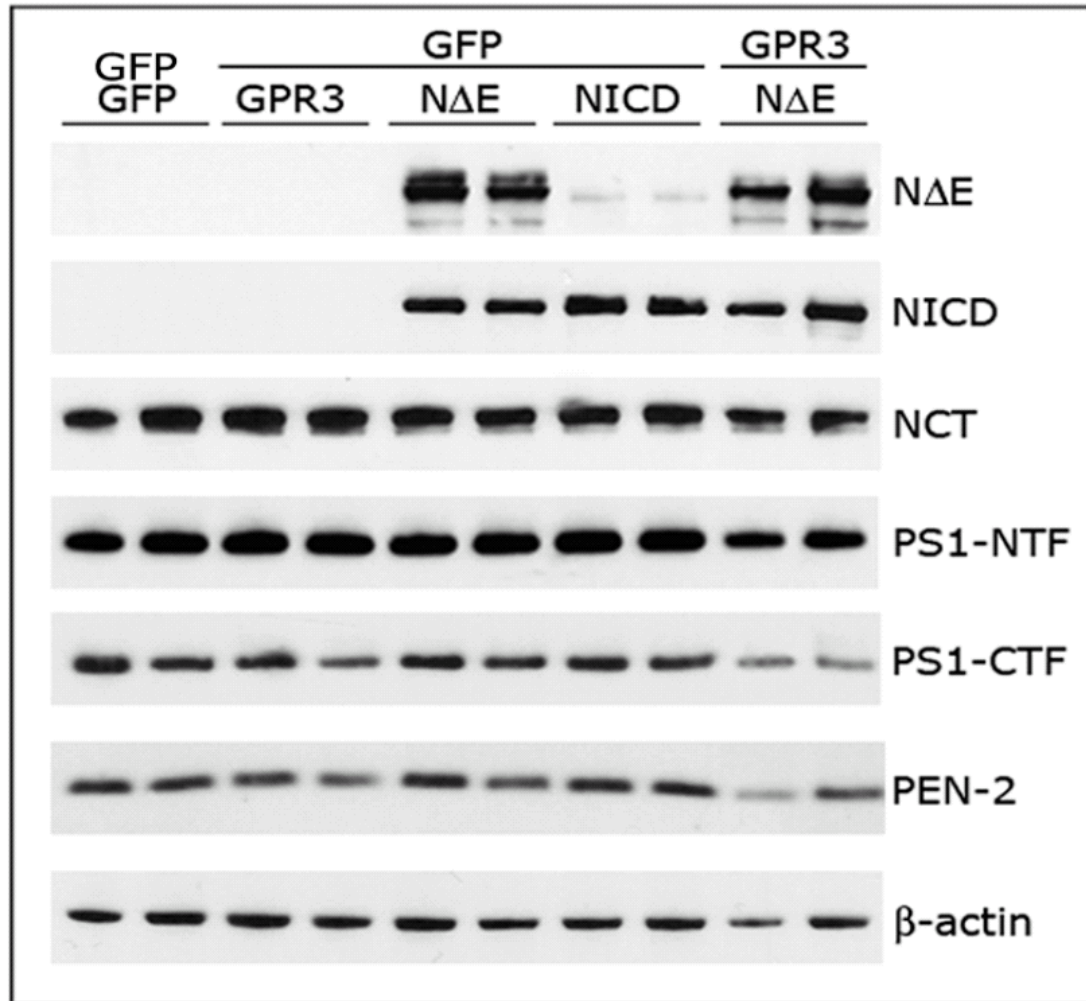
## Cell-surface expression up-regulated by GPR3



Cell-surface biotinylation

# $\gamma$ -secretase

## Notch processing not affected by GPR3 overexpression



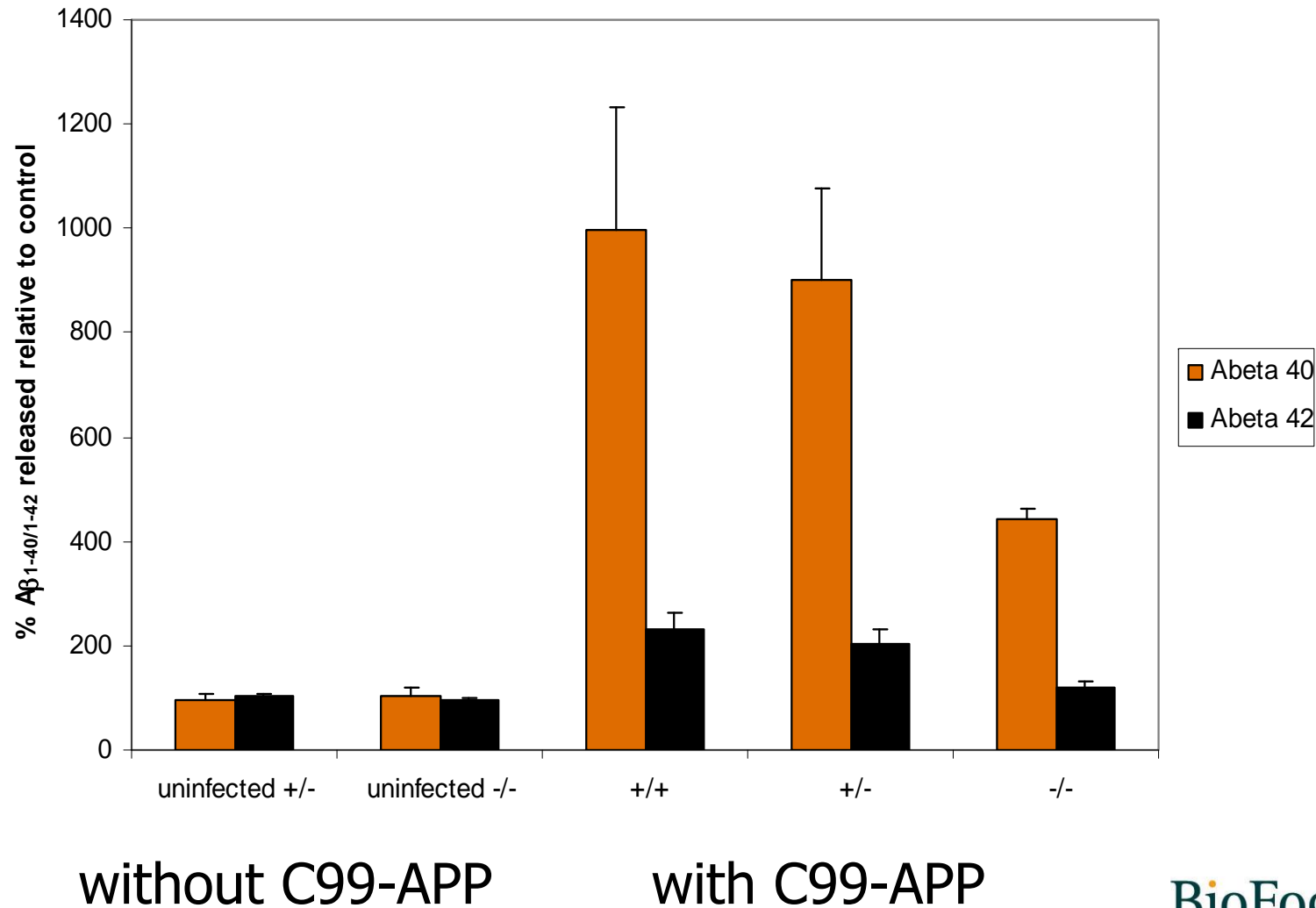
# GPR3 targets $\gamma$ -secretase

- GPR3 regulates cell-surface expression of  $\gamma$ -secretase
- GPR3 does not affect Notch processing through  $\gamma$ -secretase

# GPR3 knock-out mouse

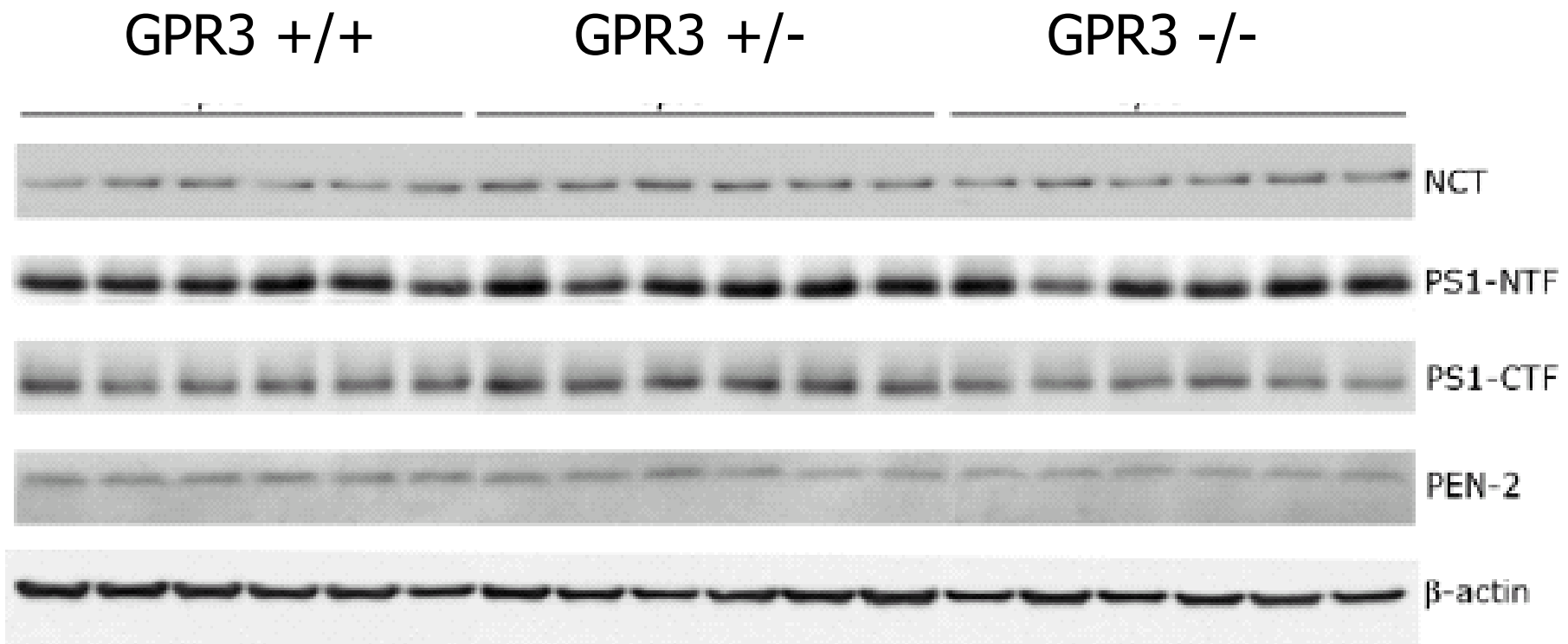
- GPR3 knock-out mouse is available
- No gross phenotype, only lower fertility reported
- Used to validate GPR3 as an AD target
  - Cultured primary neurons
  - $\gamma$ -secretase expression
  - Cross with AD mouse model

# GPR3 $-/-$ primary neurons *in vitro*



# $\gamma$ -secretase in GPR3 knock-outs

Not affected

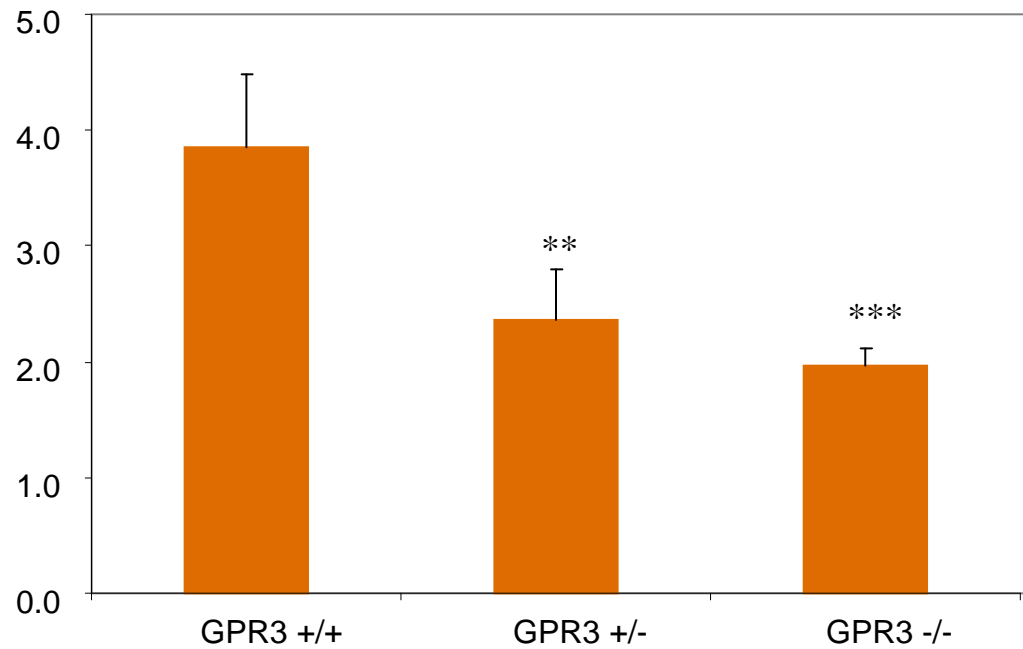




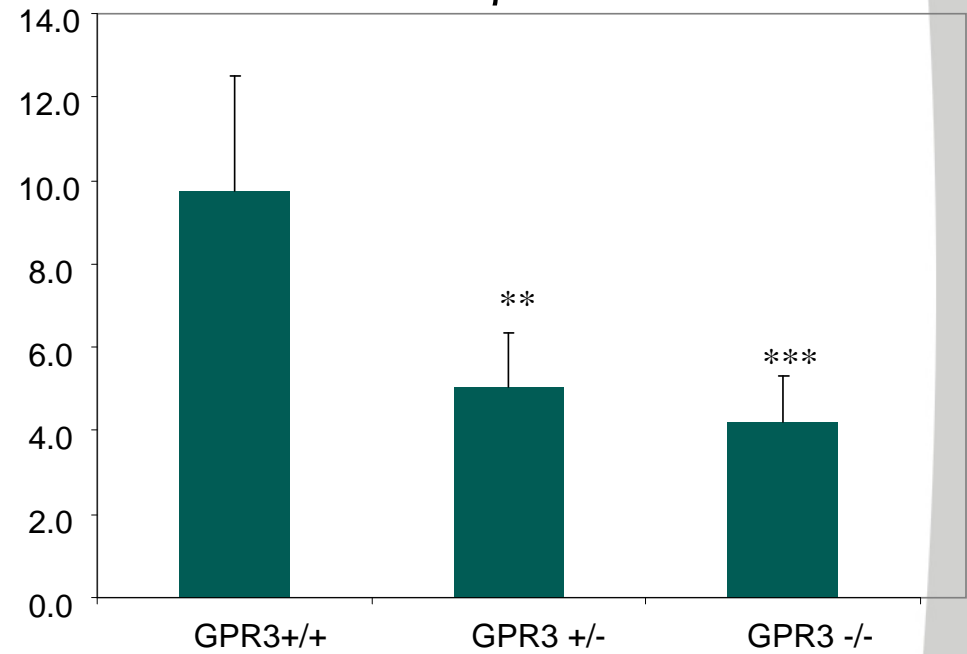
# A $\beta$ release *in vivo*

## APP x PS1 x GPR3 -/- mice

### A $\beta$ 1-40



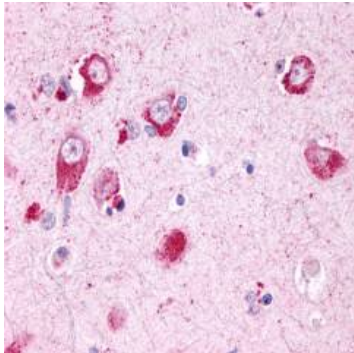
### A $\beta$ 1-42



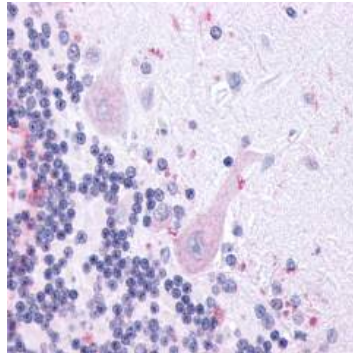
# GPR3

## Expression in human brain (IHC)

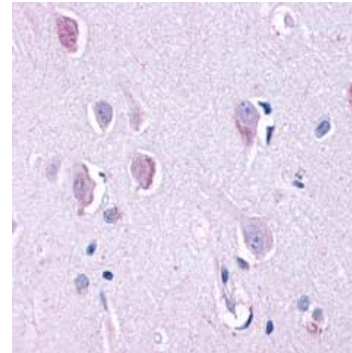
amygdala



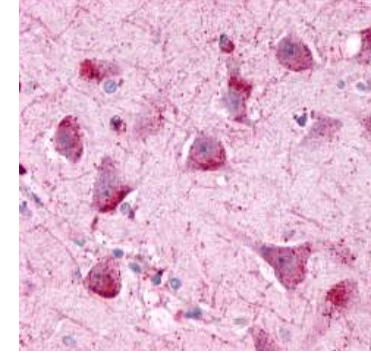
cerebellum, Purkinje neurons



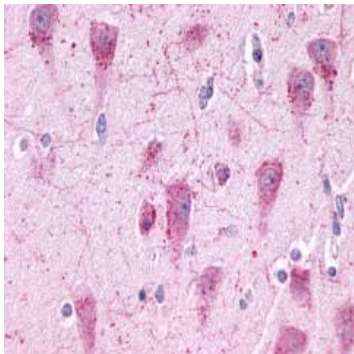
cortex



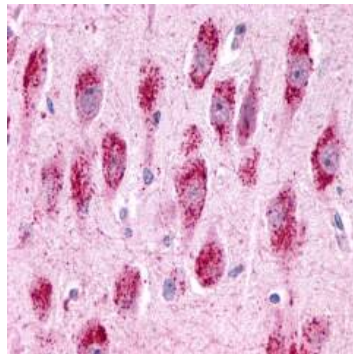
hippocampus – CA4



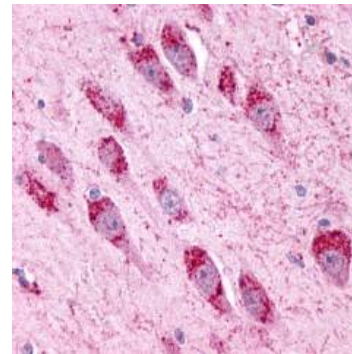
hippocampus - CA1



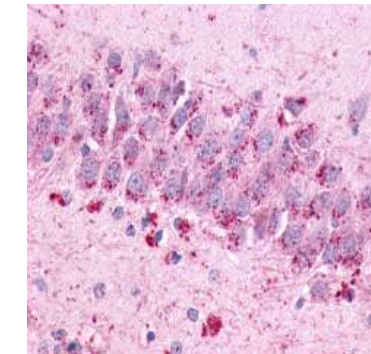
hippocampus - CA2



hippocampus - CA3

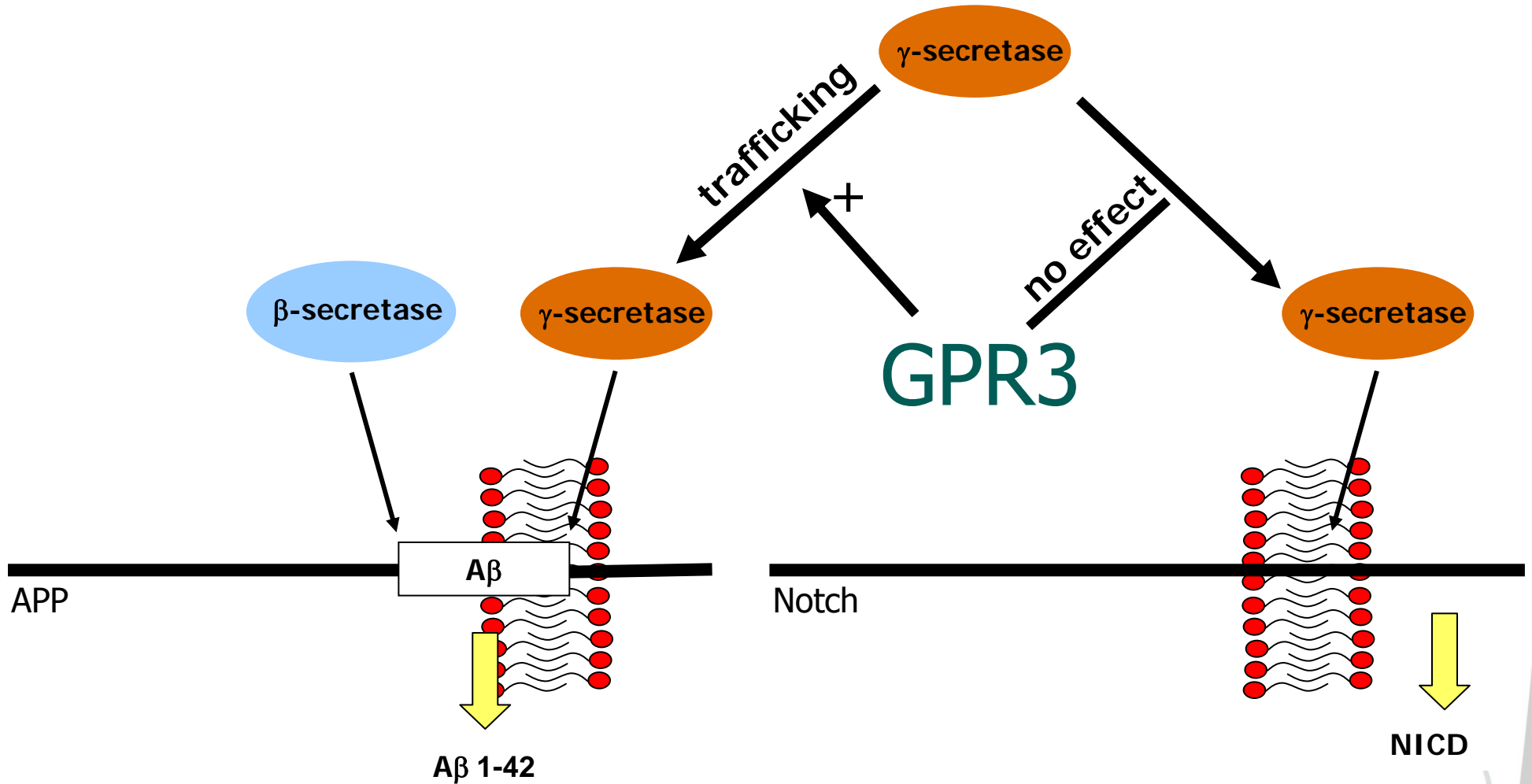


hippocampus – dentate gyrus





# Detailed mechanism of action





# Summary GT177-GPR3

- A $\beta$  release is modulated by GT177
  - knock-in induces A $\beta$  1-40 & 1-42 levels
  - knock-down reduces A $\beta$  1-40 & 1-42 levels
- No effect on general secretion
- Expressed in human brain and rodent neurons
- Limited expression in other tissues
- Indicative for  $\gamma$ -secretase modulator, but no effect on Notch
- Knock-out mice have a clean phenotype
- Knock-out of GT177 reduces A $\beta$  release in gold standard AD mouse model
- Constitutively active class A GPCR



# Acknowledgements

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