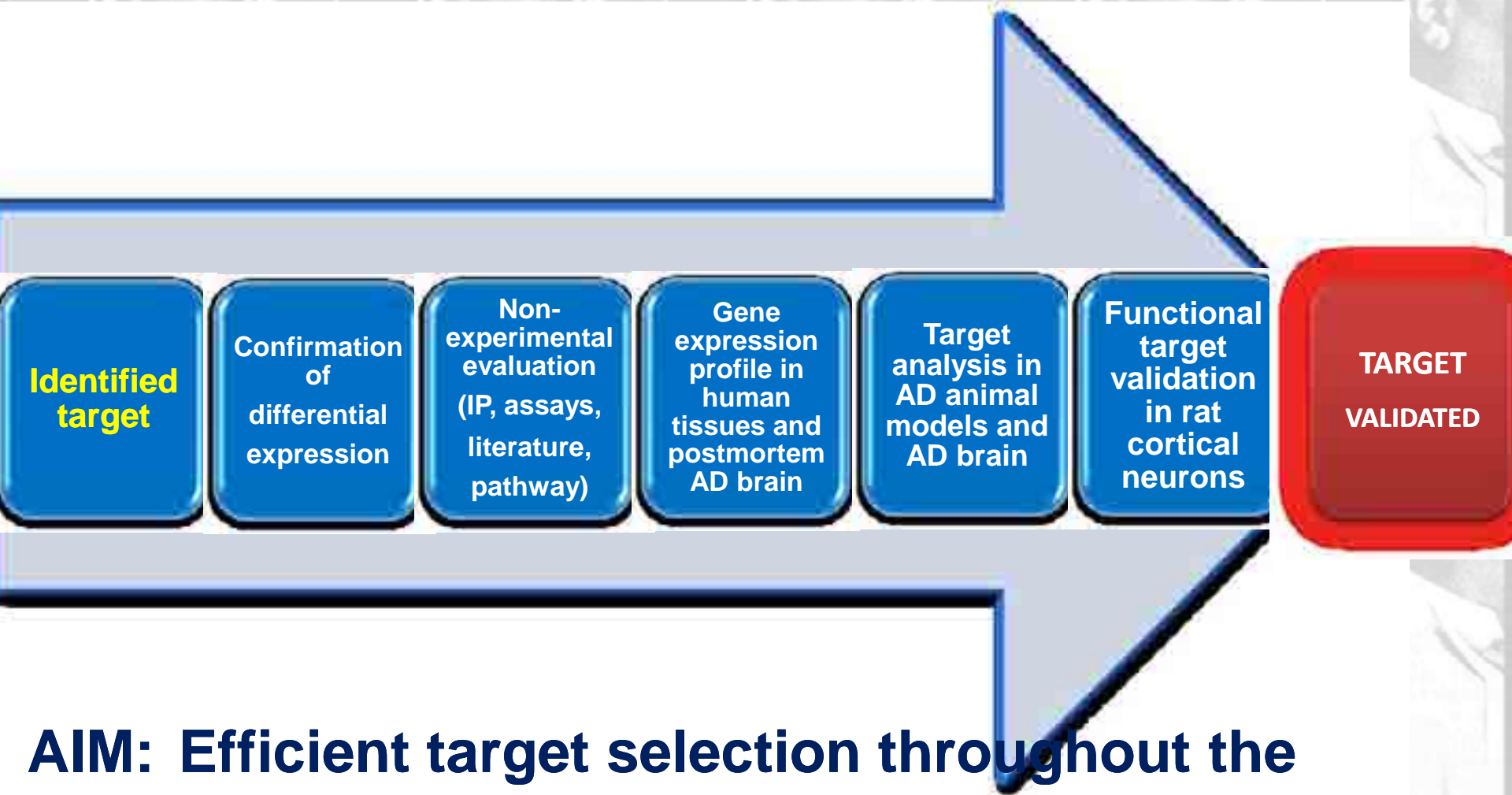


Efficient validation of novel AD targets in a drug discovery context

Giuseppe Pollio

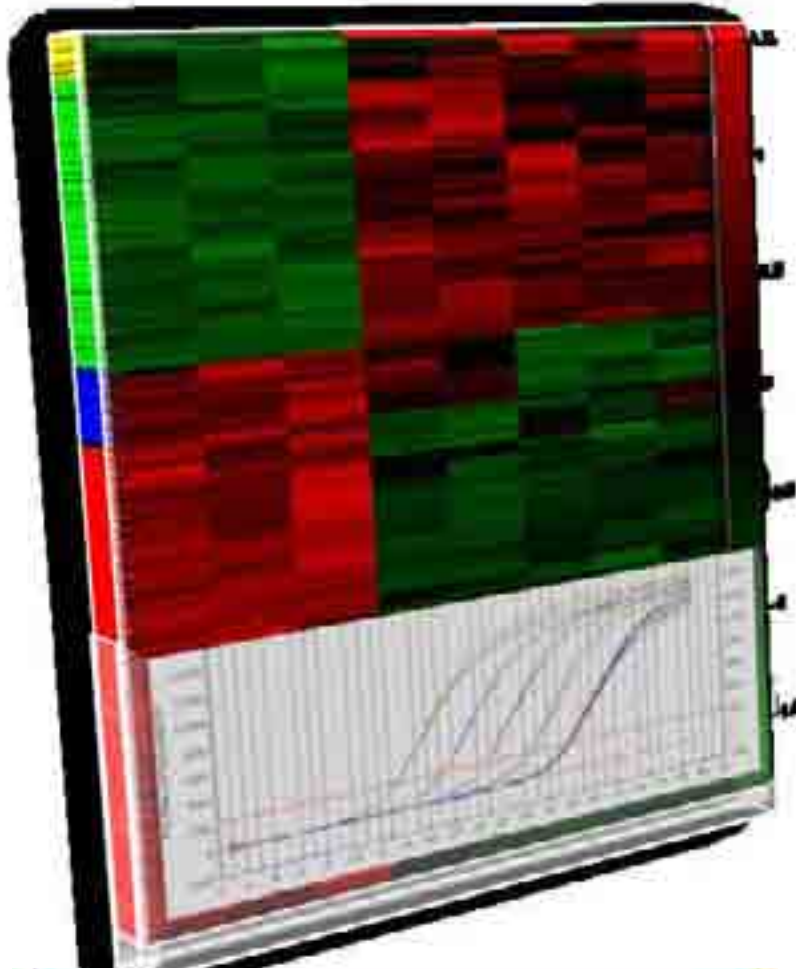
gpollio@sienabiotech.it

Target validation workflow

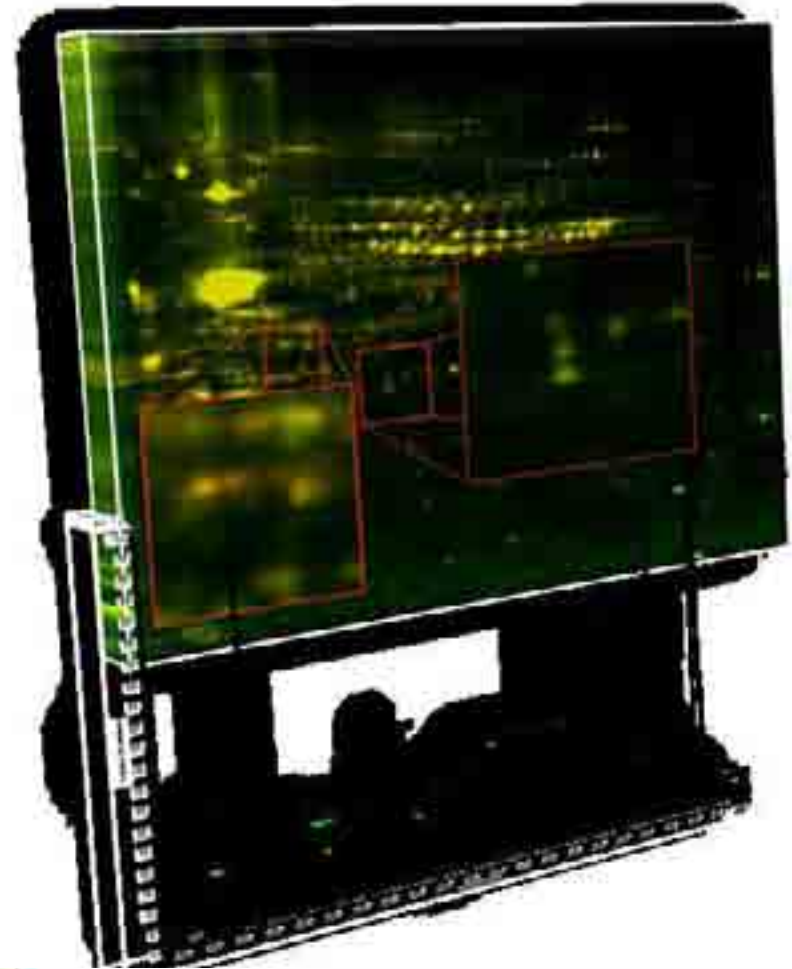


AIM: Efficient target selection throughout the drug discovery process.

Target identification



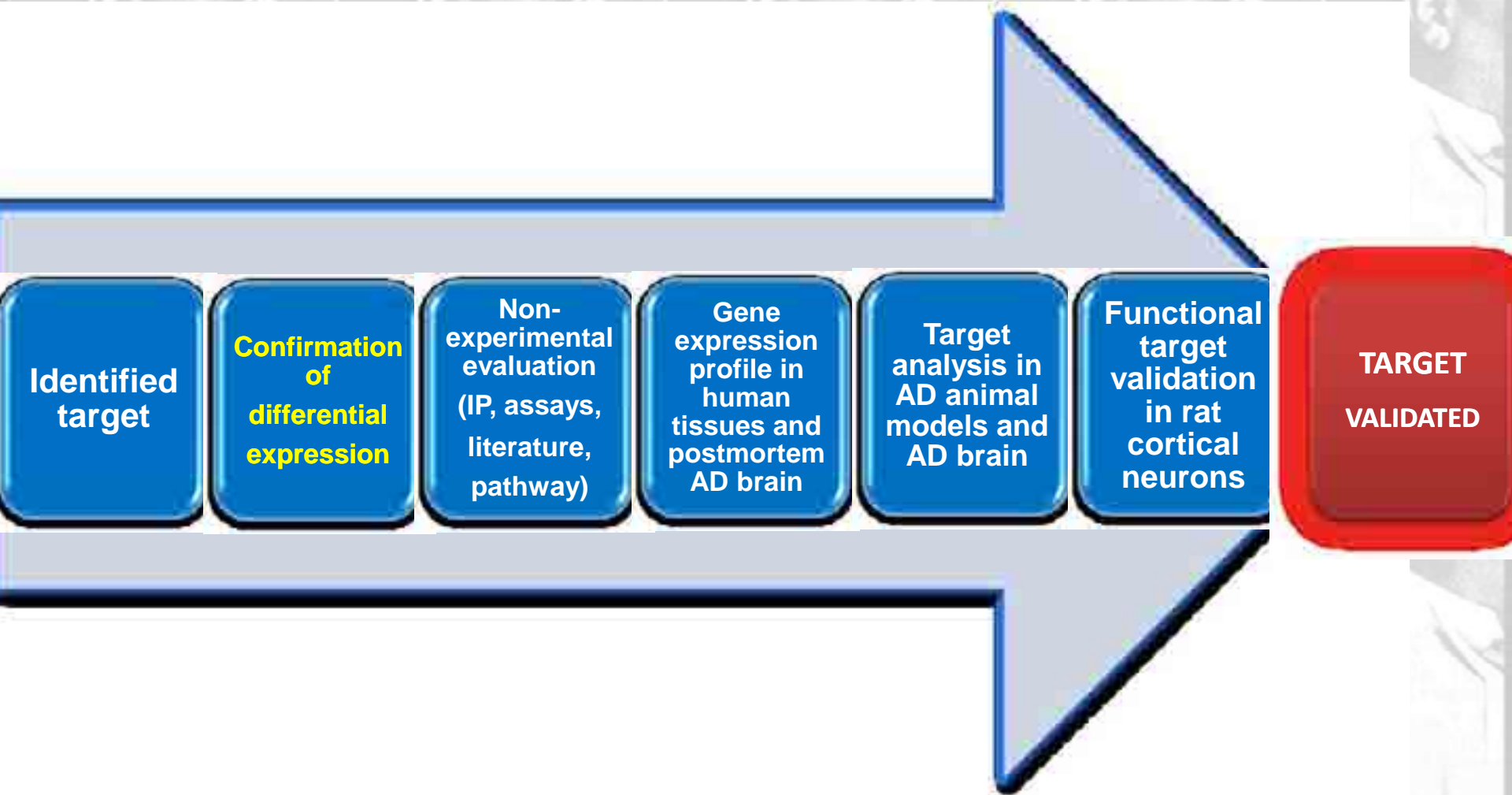
- 96 μ -arrays
- 7 experiments
- 800-1200 diff. expr. genes



- 160 2D-gels, 120 MS-runs
- 69 experiments
- 500-800 diff. mod. prot.

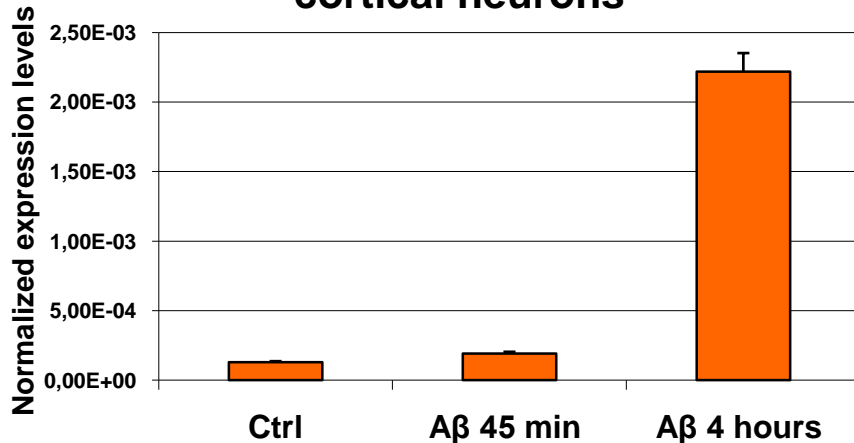
Proteomics

Target validation workflow

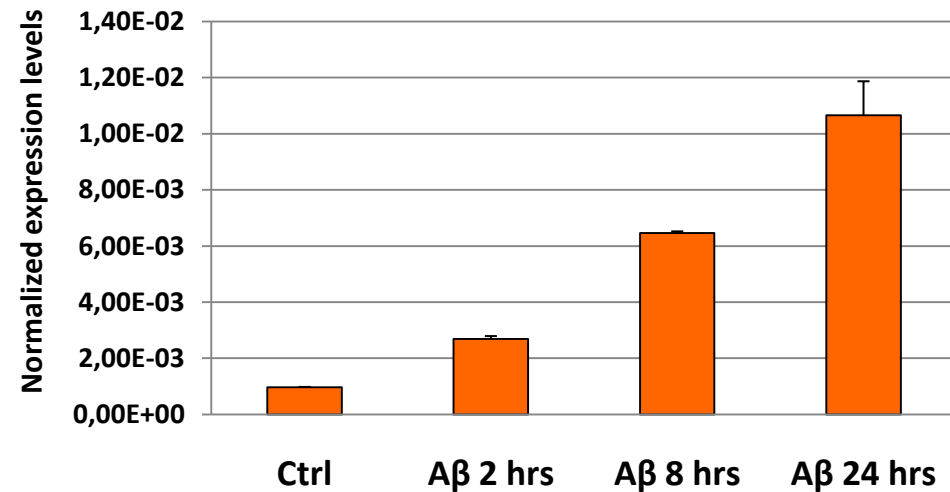


Confirmation of differential expression by QPCR in rat cortical neurons

RnTNFR1a expression in rat cortical neurons



RnTNFR1a expression in rat cortical neurons - Time course -

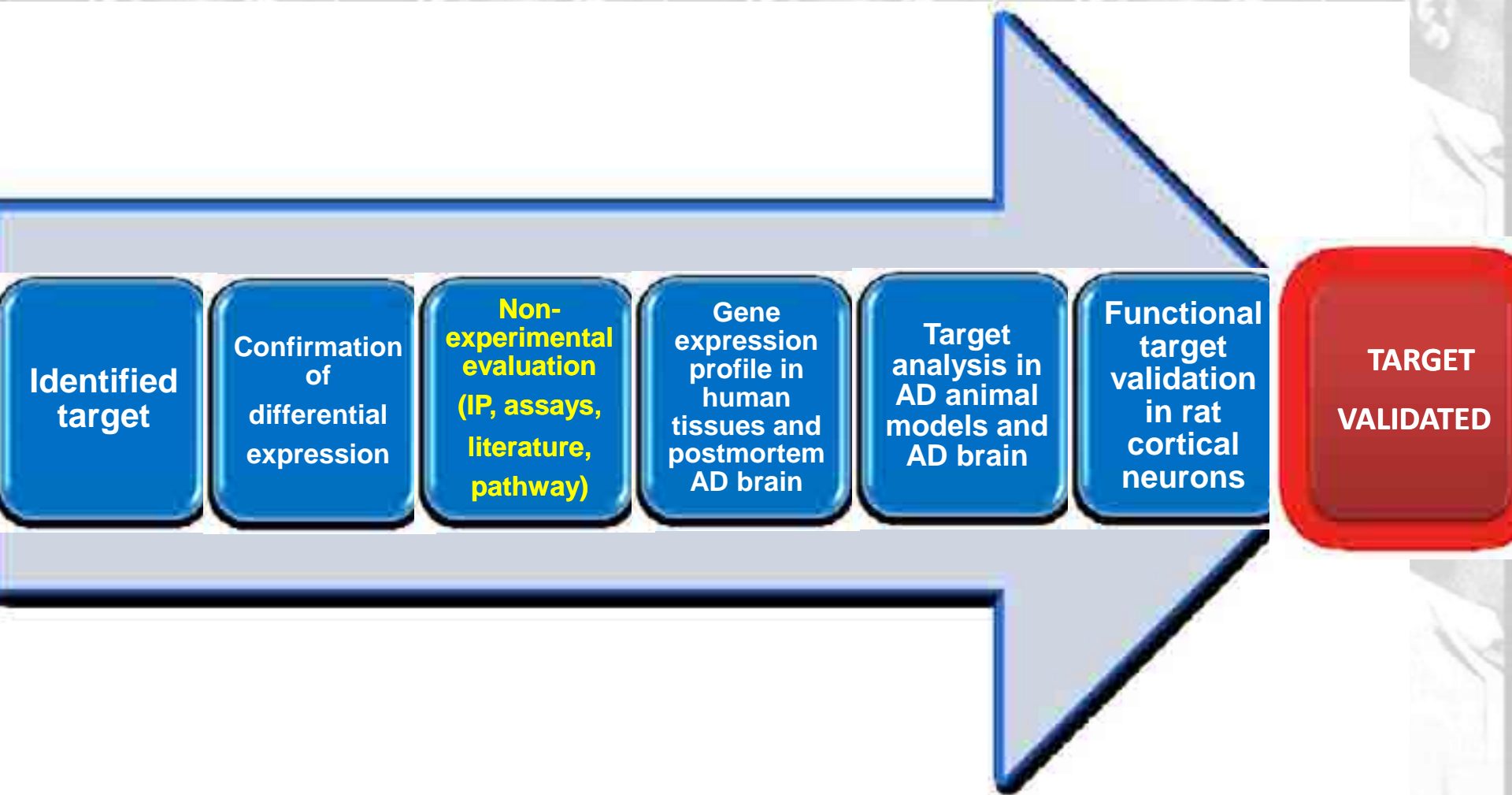


QPCR on the same RNA used for microarray

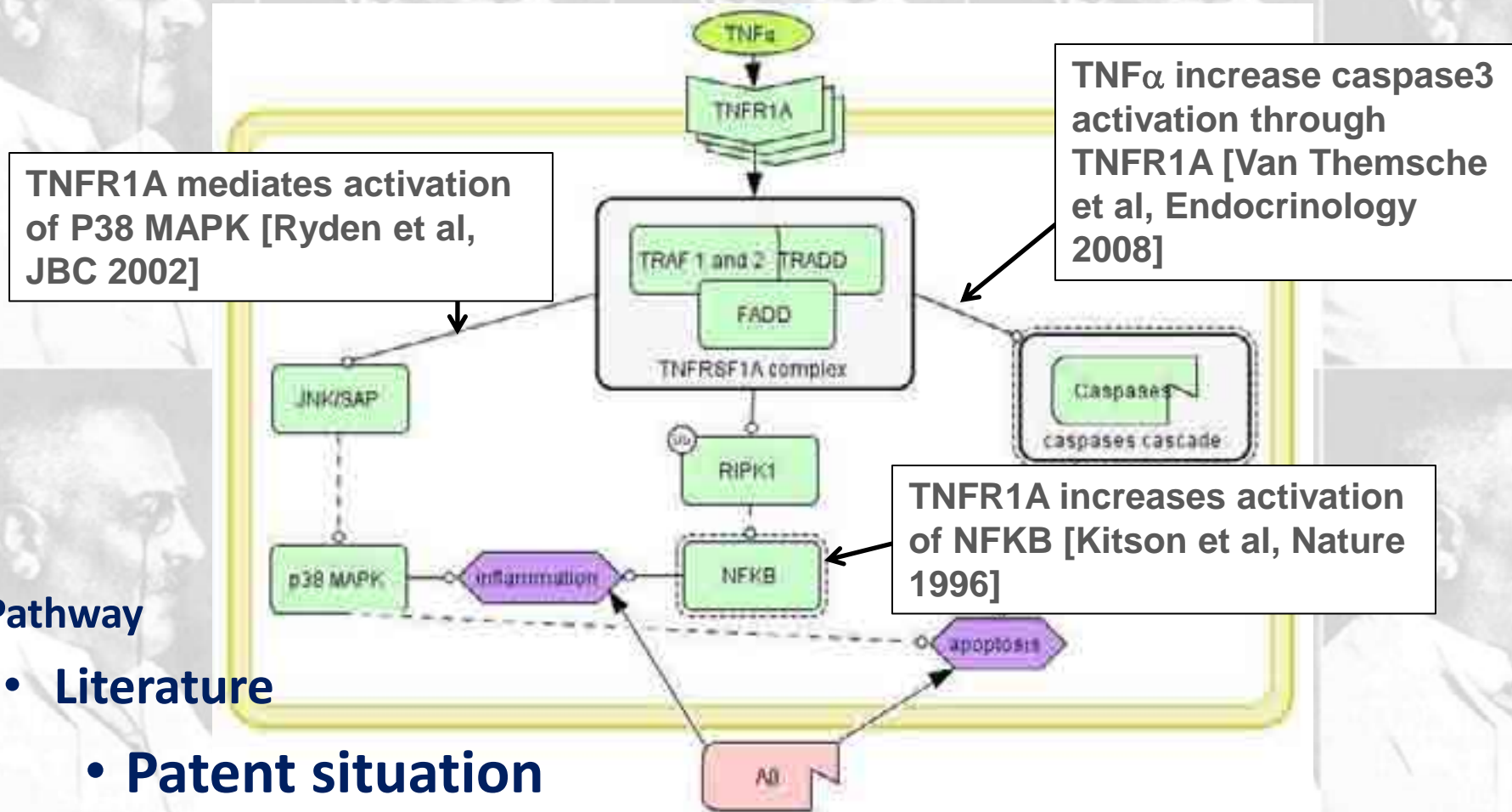
QPCR on a different RNA preparation

QPCRs performed on the same biological preparation used for microarray analysis or in different Aβ-treated RNAs confirmed transcriptomics data

Target validation workflow

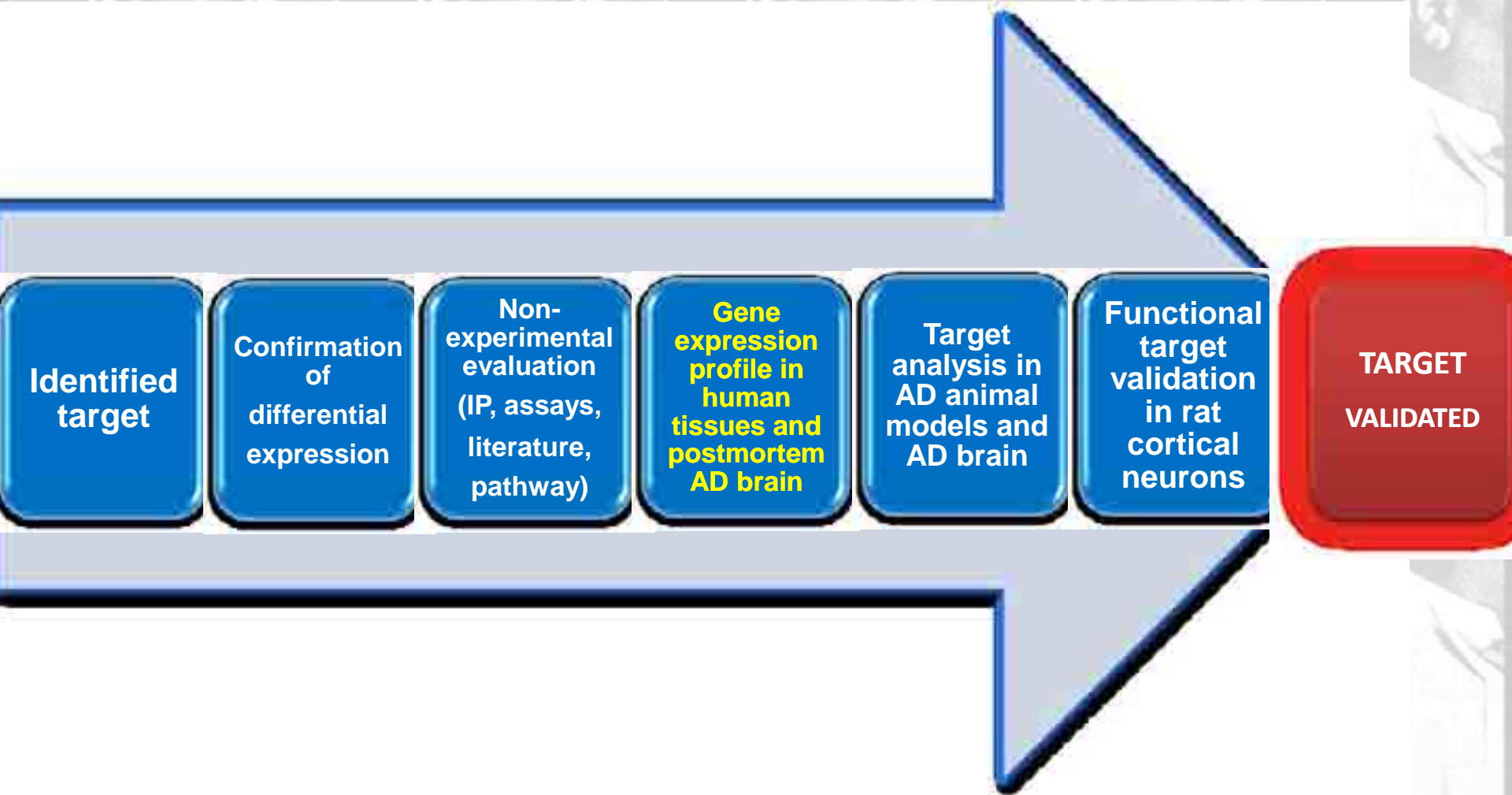


Non-experimental target evaluation

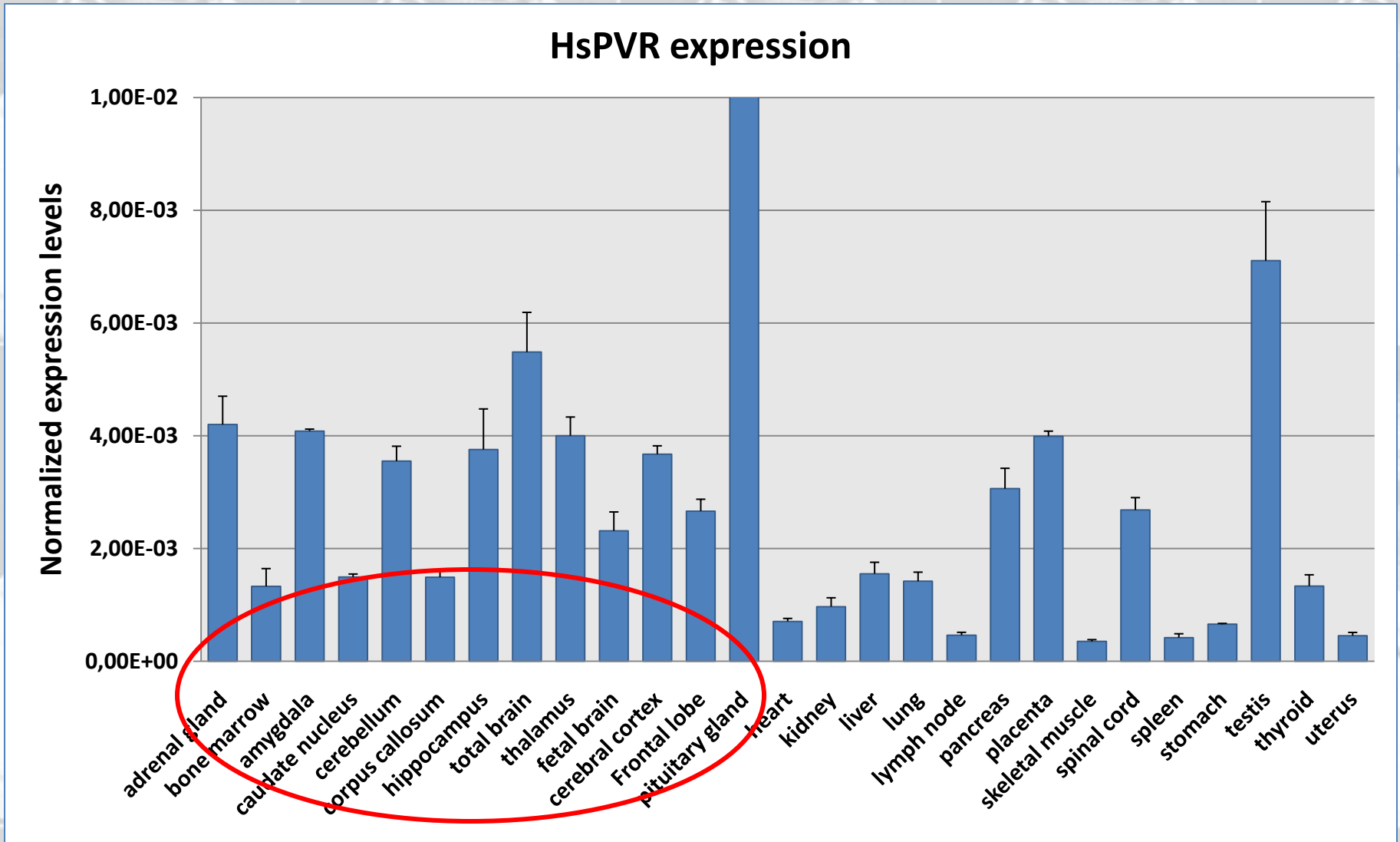


- Pathway
- Literature
- Patent situation
- “Feasibility of screening assays”

Target validation workflow

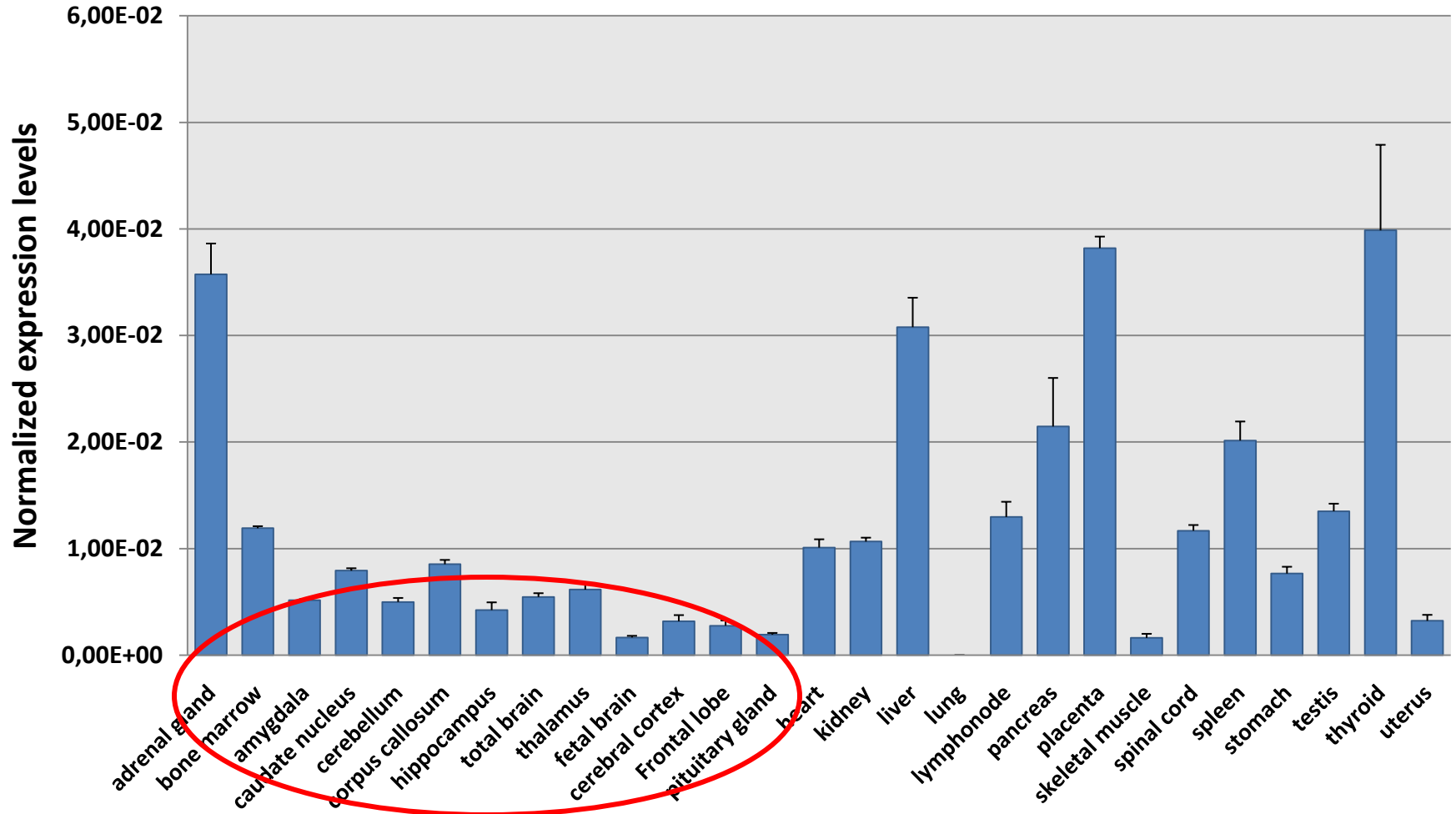


PVR expression in human tissues panel

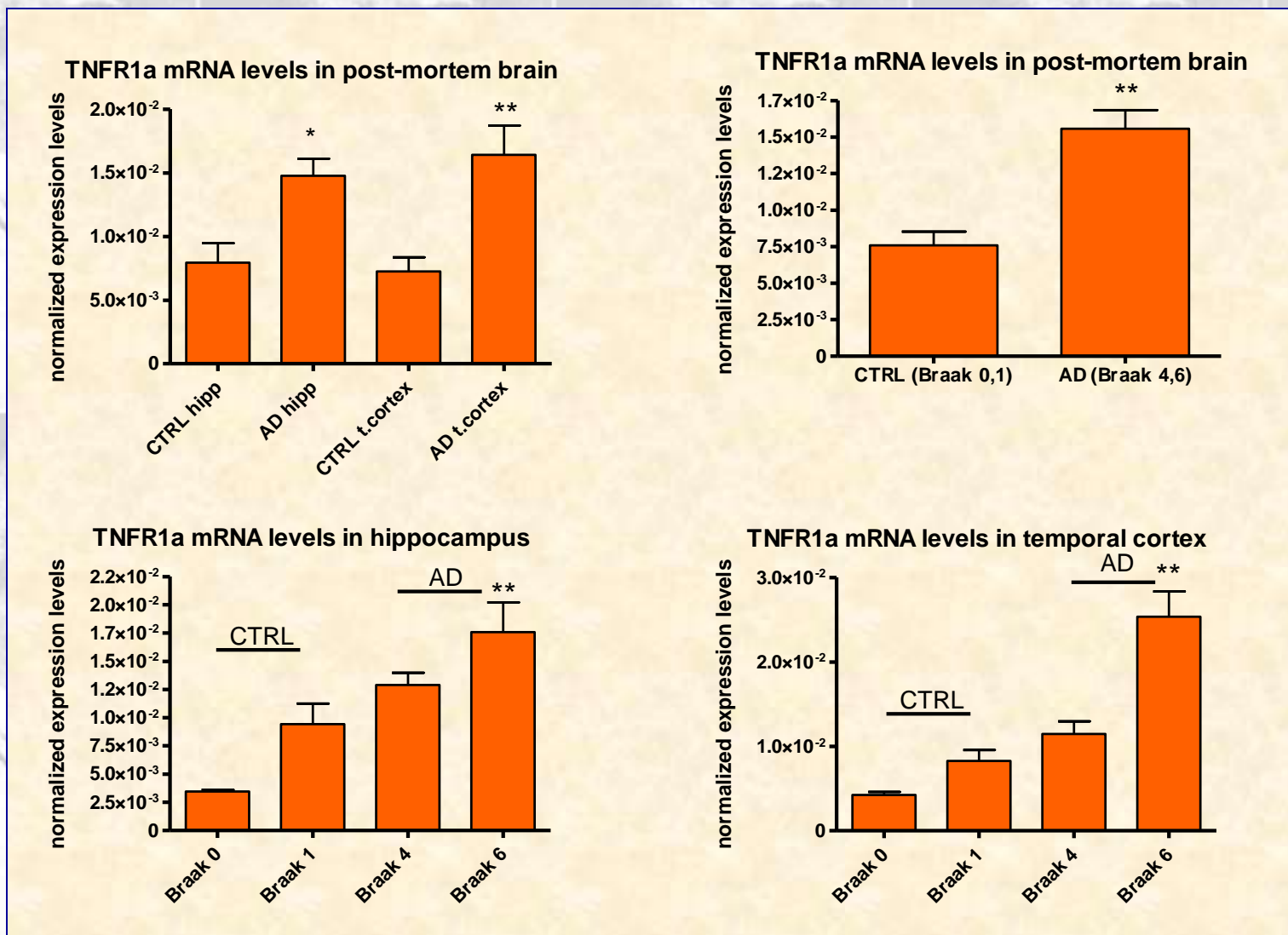


TNFR1a expression in human tissues panel

HsTNFR1a expression



TNFR1a expression in AD brain

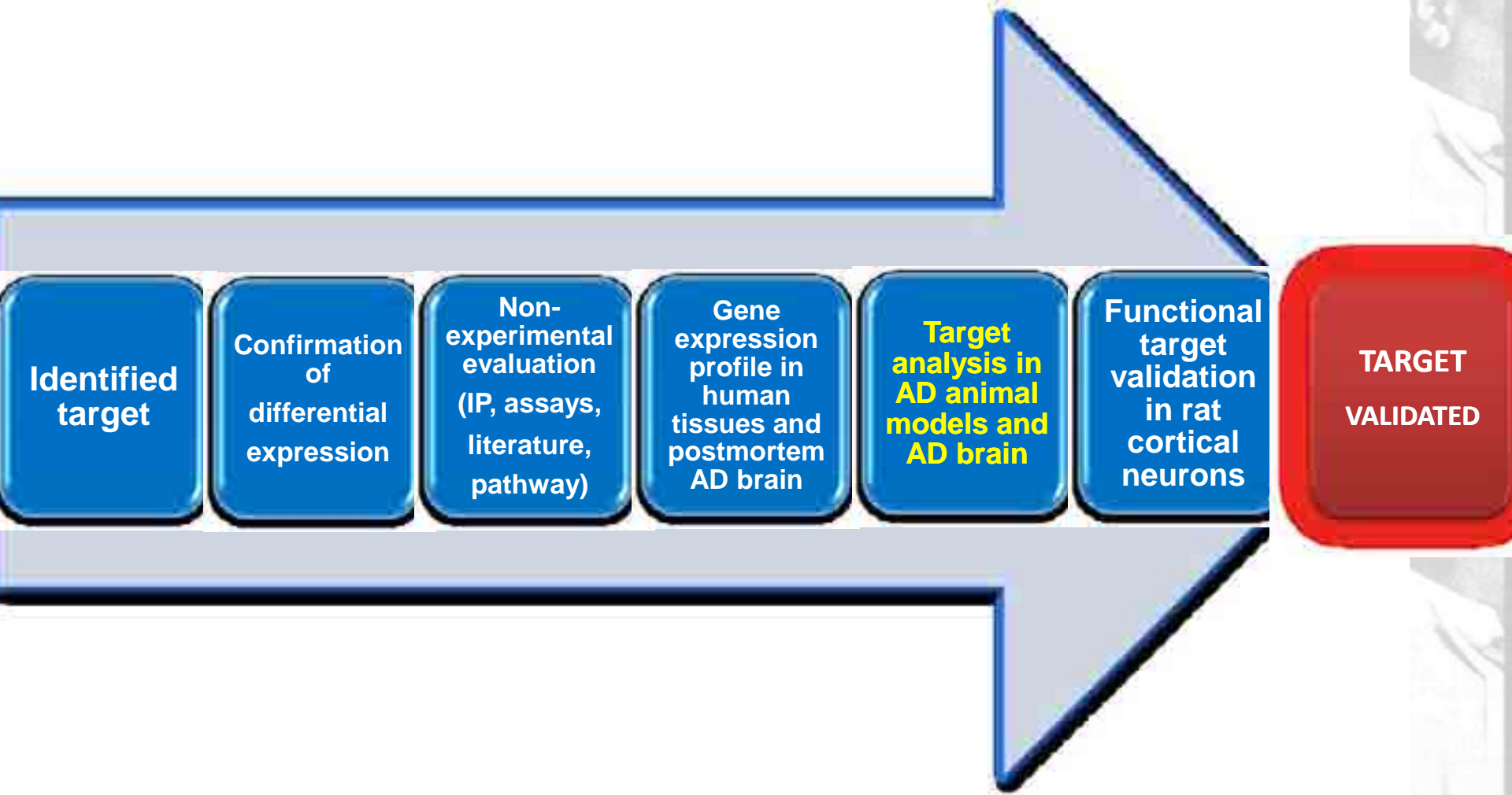


Graph shows Mean \pm SEM of 4 controls and 5 AD tissues analyzed.

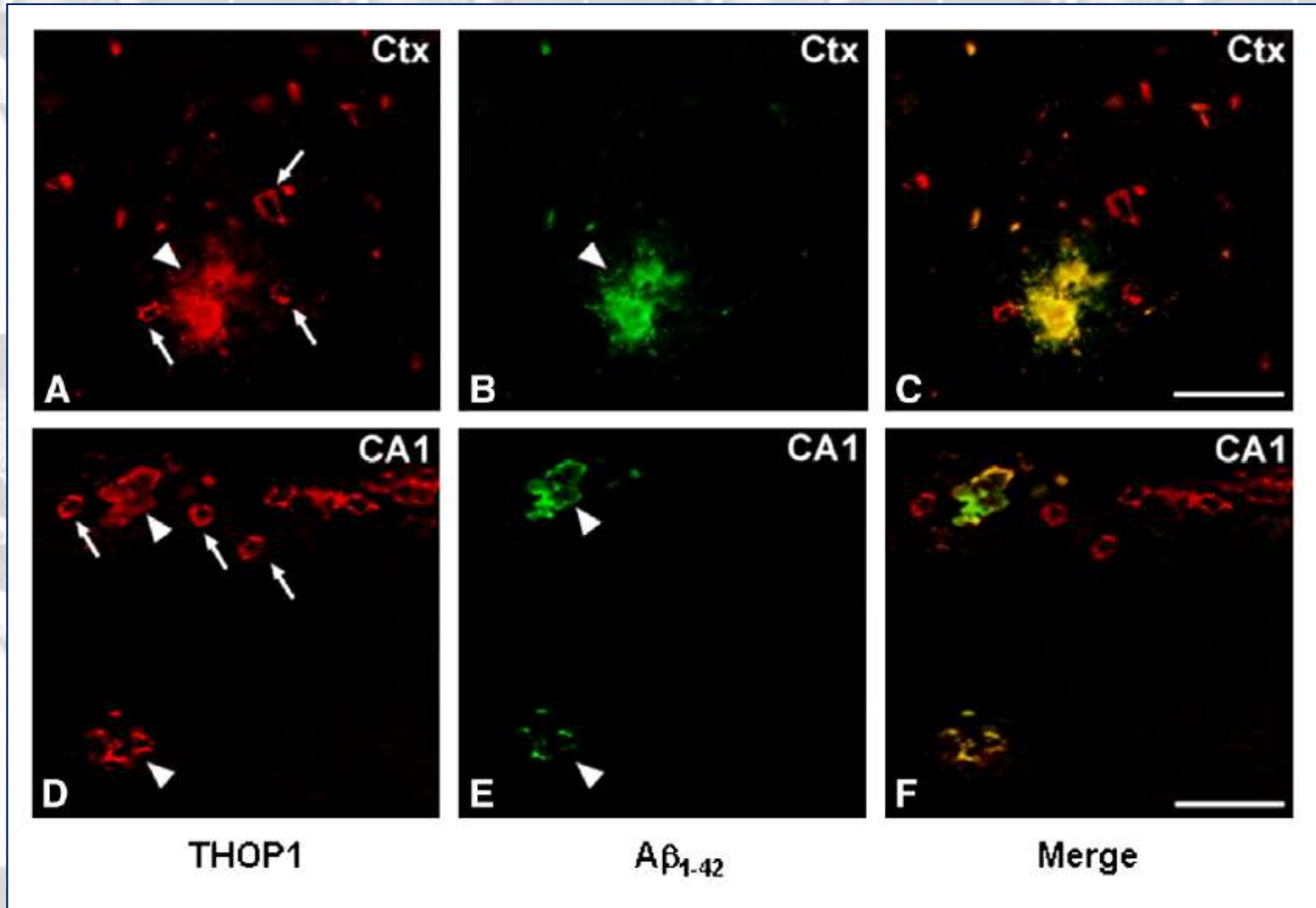
Statistical analysis performed by one way or two-way Anova, followed by Bonferroni multiple comparison test

* $P < 0.05$ ** $P < 0.01$

Target validation workflow



Target analysis in AD animal model: **THOP1** (TgCRND8 APP transgenic mouse)

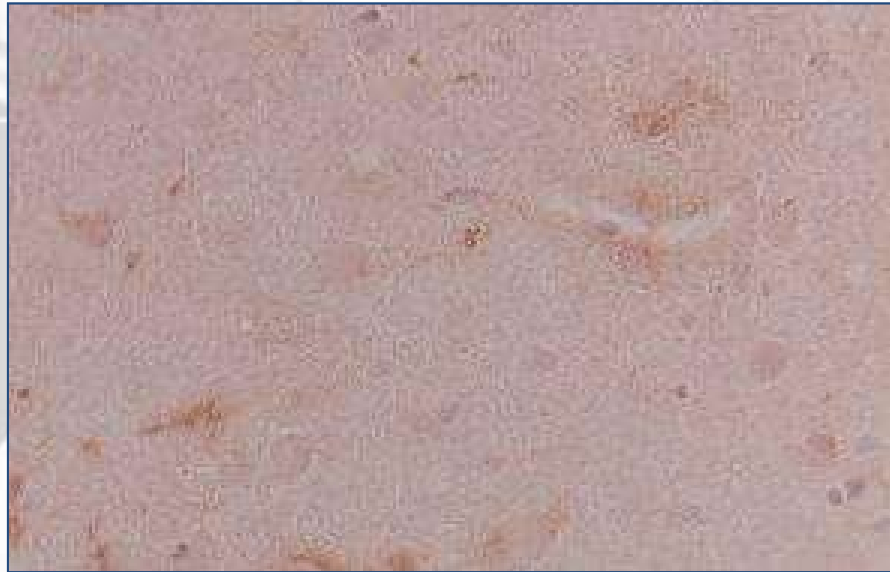


Target analysis in post mortem AD brain: **PVR**

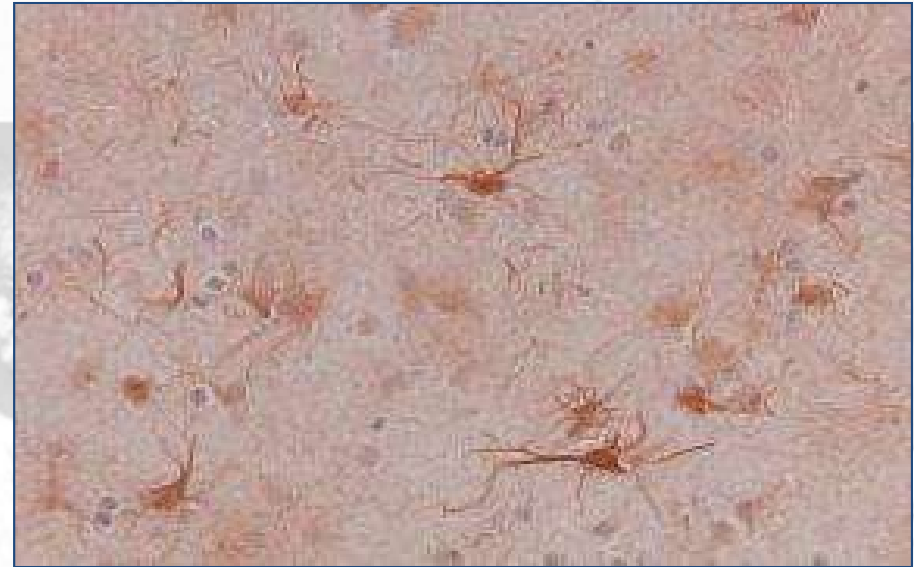
IHC analysis on control and AD temporal cortex

PVR antibody (DAB, brown)

Nuclei stained with hematoxylin (blue)

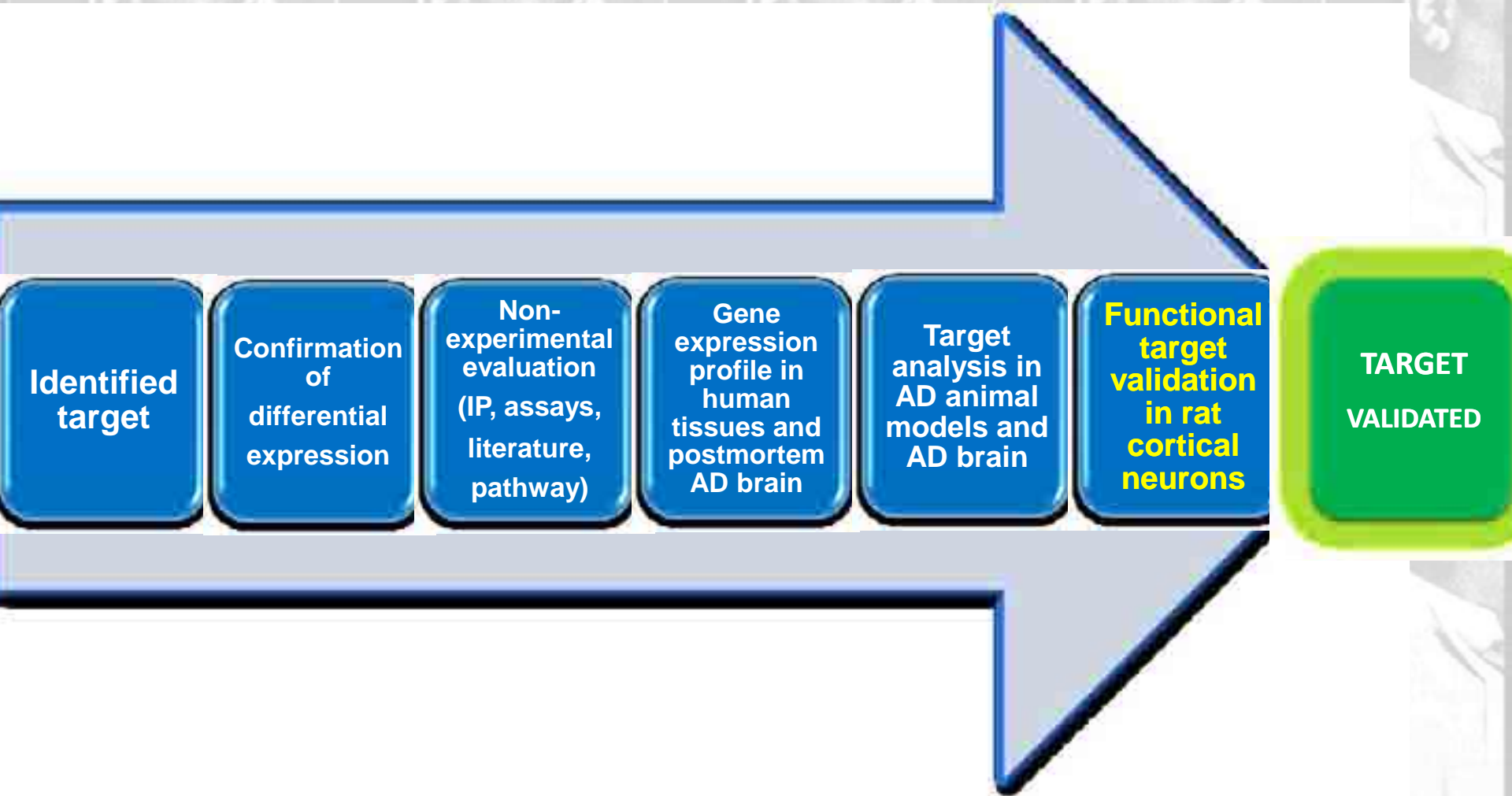


control case without brain pathology



AD case

Target validation workflow



Functional target validation in rat cortical neurons



Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/jneumeth



Short communication

A reporter assay for target validation in primary neuronal cultures[☆]

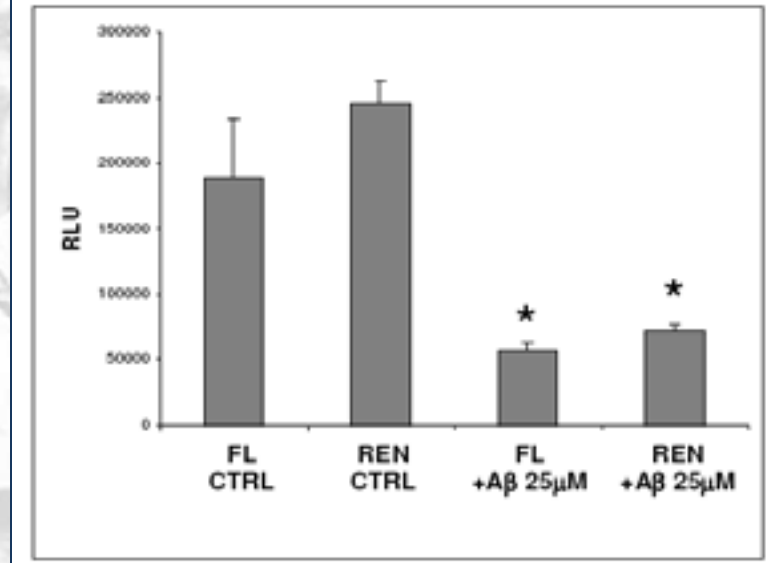
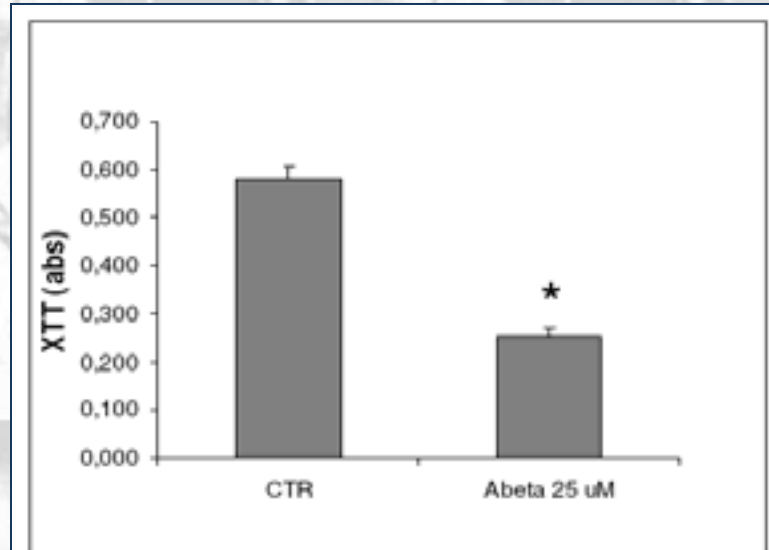
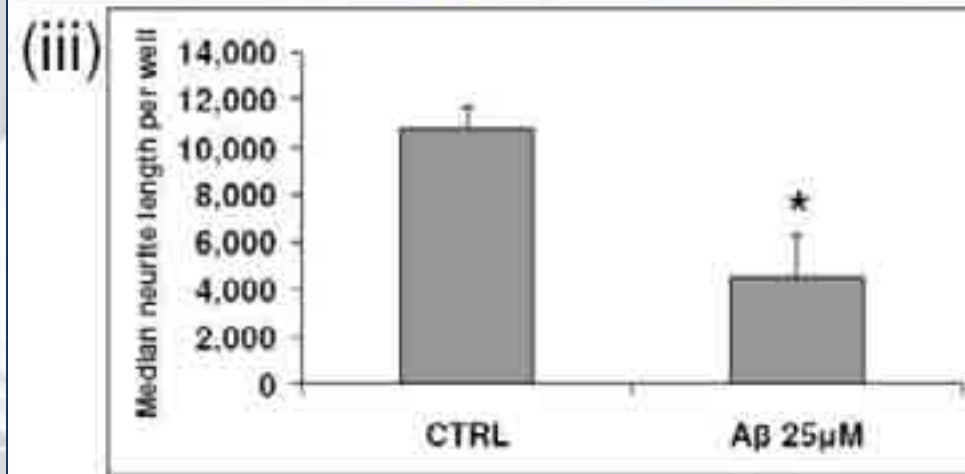
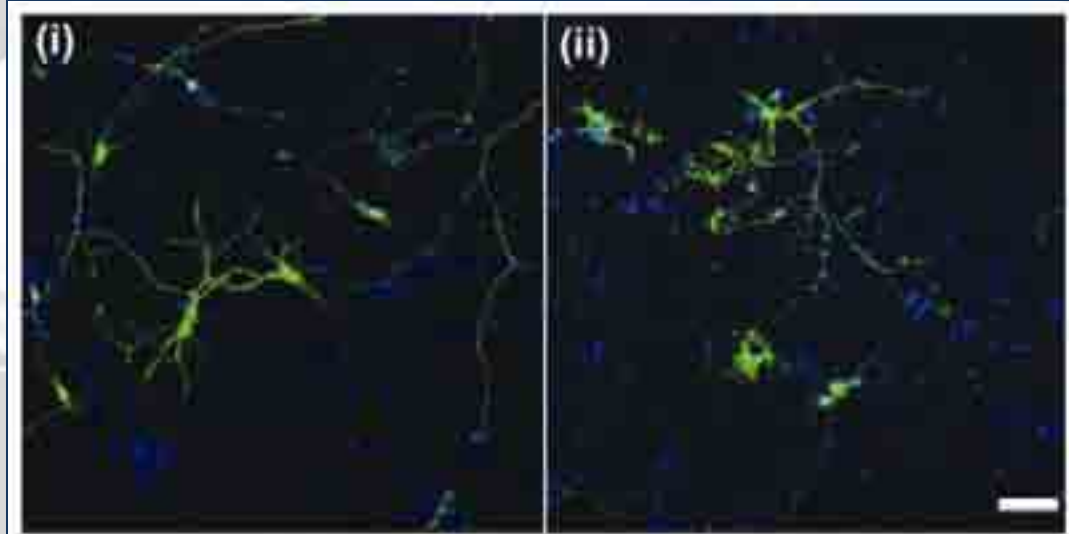
G. Pollio^{*}, R. Roncarati, T. Seredenina, G.C. Terstappen, A. Caricasole

SiRNA Biotech SpA, Via Fiorentina 1, 53100 Siena, Italy

Key advantages of the method described:

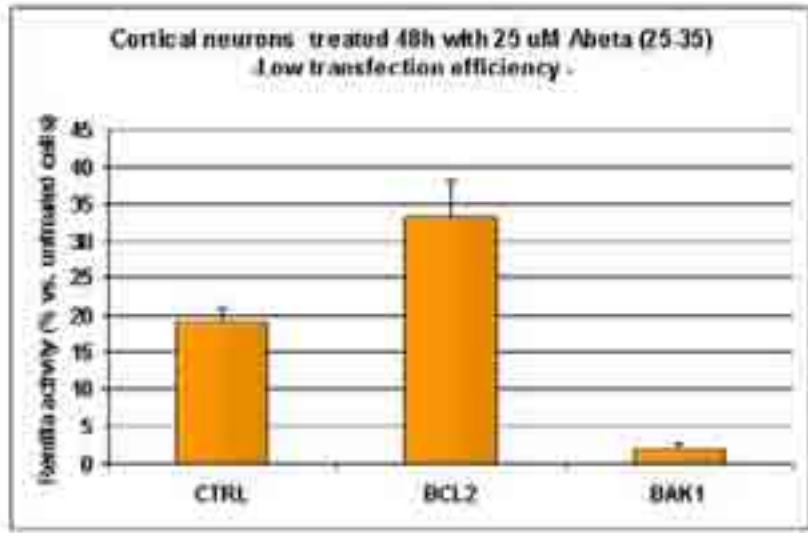
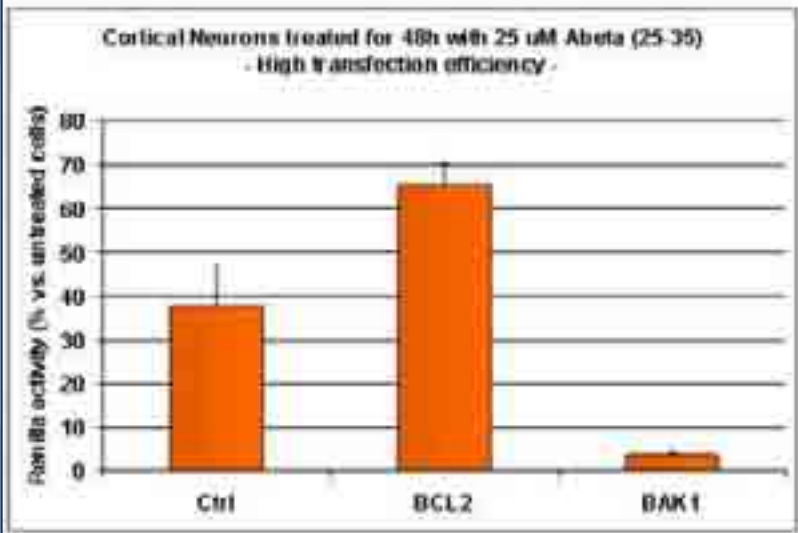
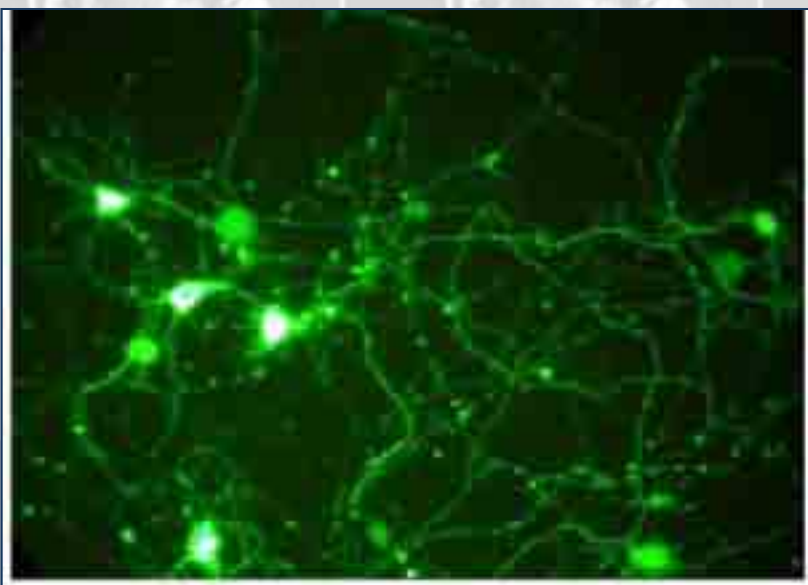
- **Reproducibility:** viability measured in transfected cells recapitulate the whole cell culture situation.
- **Sensitivity:** the use of luciferase reporters allows to use primary neuronal culture by maintaining mild transfection conditions.
- **Flexibility:** the method can be coupled to High Content imaging analysis.

A β_{25-35} treatment in rat cortical neurons

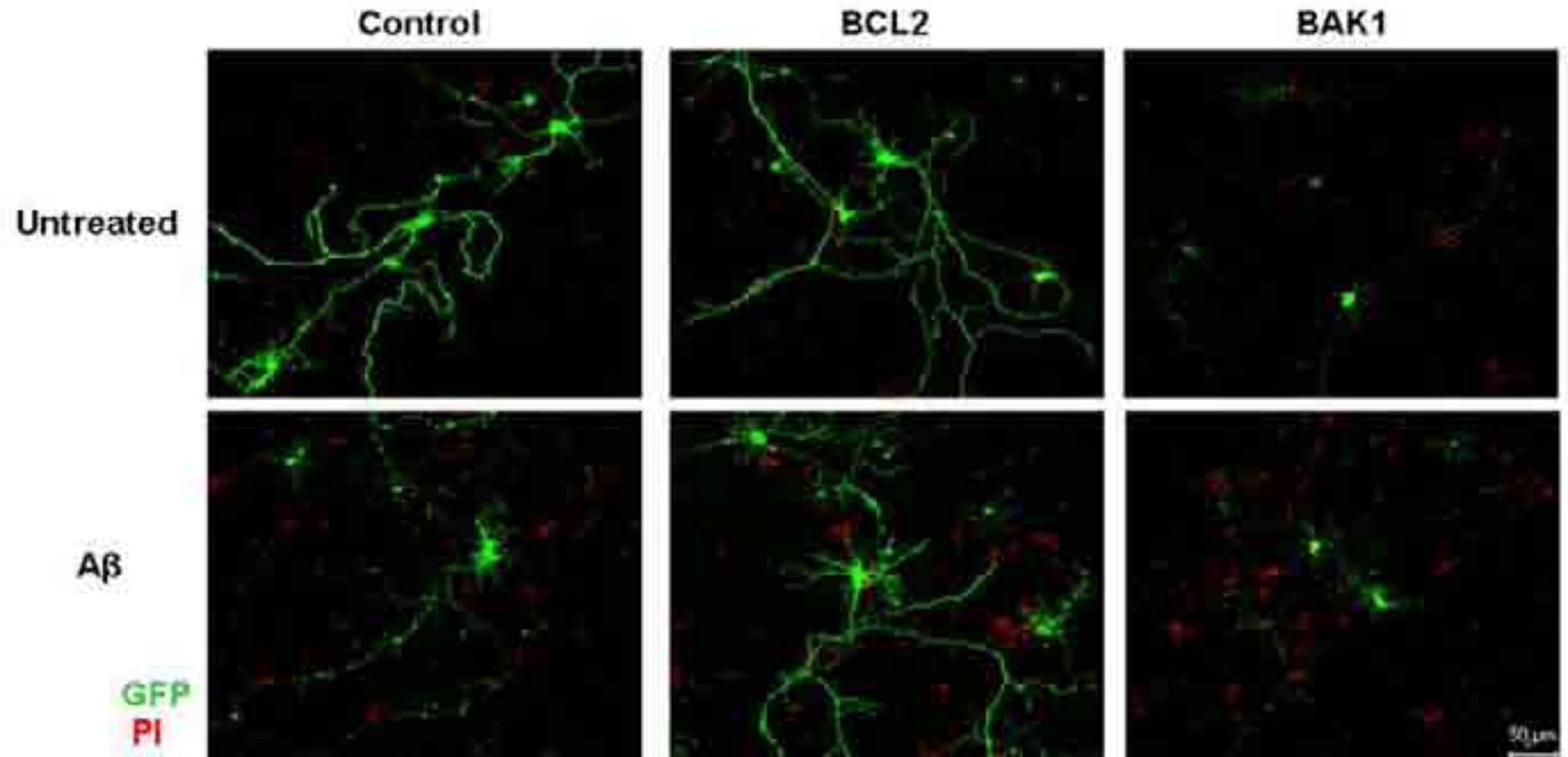


* P < 0.05

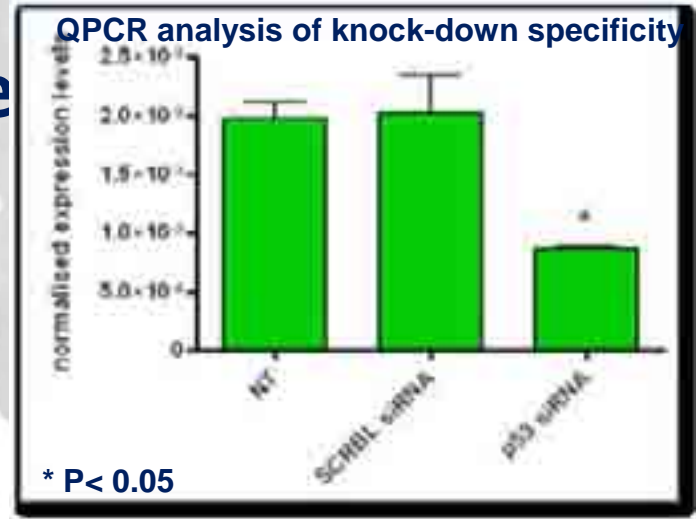
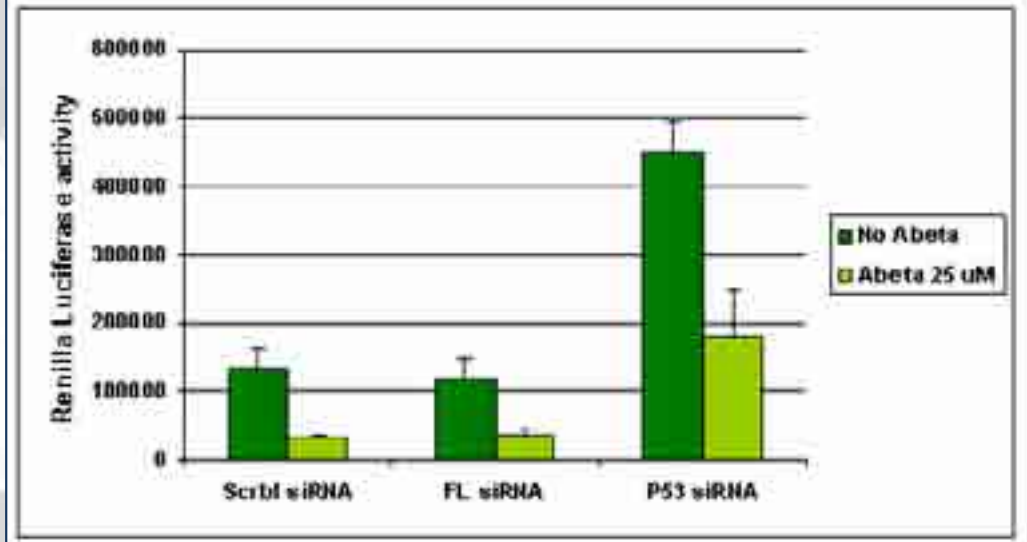
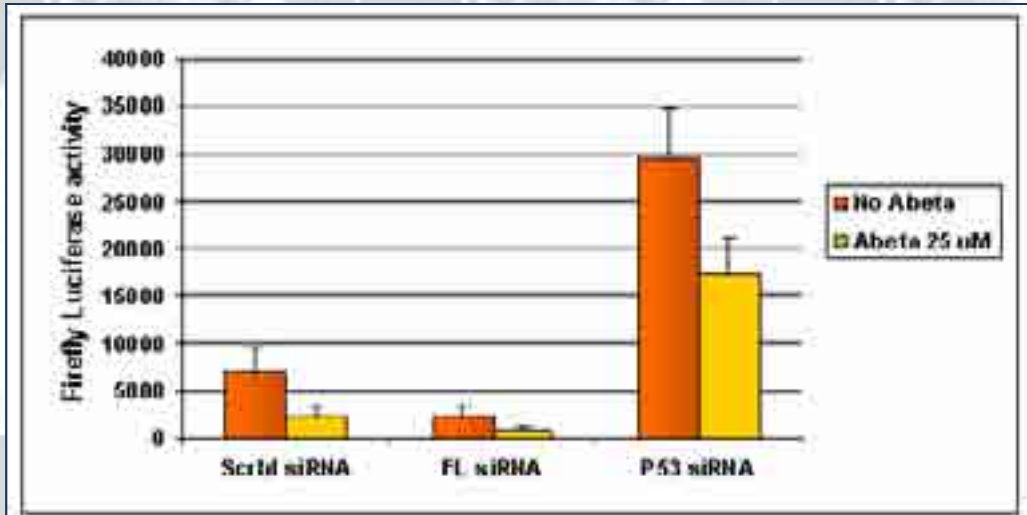
Reporter assay in cortical neurons – no effects from different transfection efficiency



Imaging assay on transfected rat cortical neurons



Knock down of A β -responsive genes



- siRNA knock-down of endogenous toxic genes results in increased cell viability.
- siRNA treatment mimic a pharmacological antagonist intervention.
- False negative results given by gene overexpression are theoretically absent for siRNA knock-down.

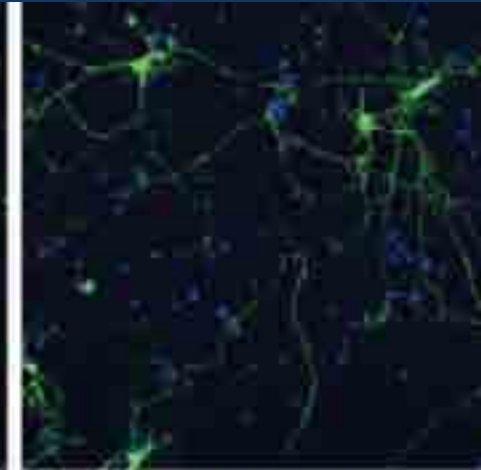
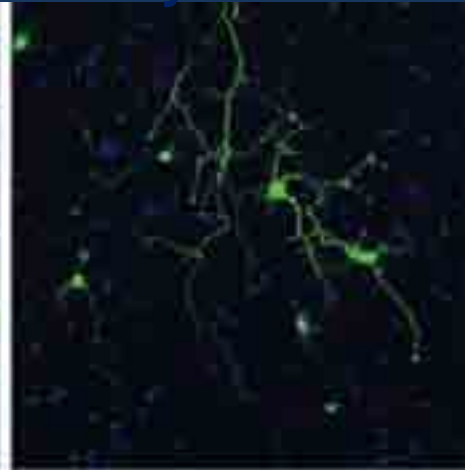
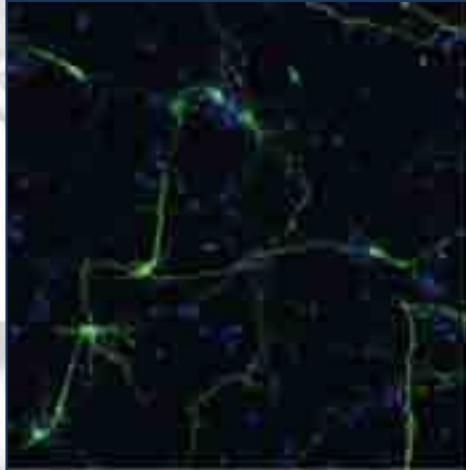
Imaging analysis of siRNA-transfected neurons

Scrb1 siRNA

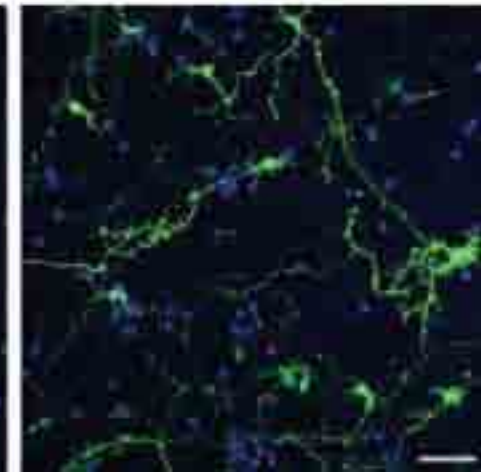
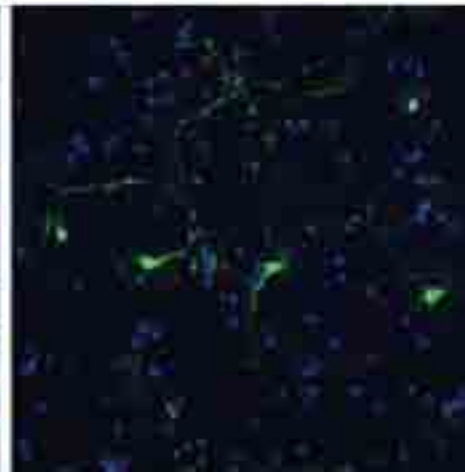
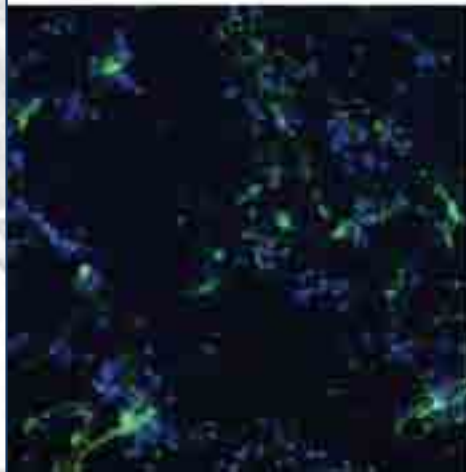
Firefly luc siRNA

P53 siRNA

No A β

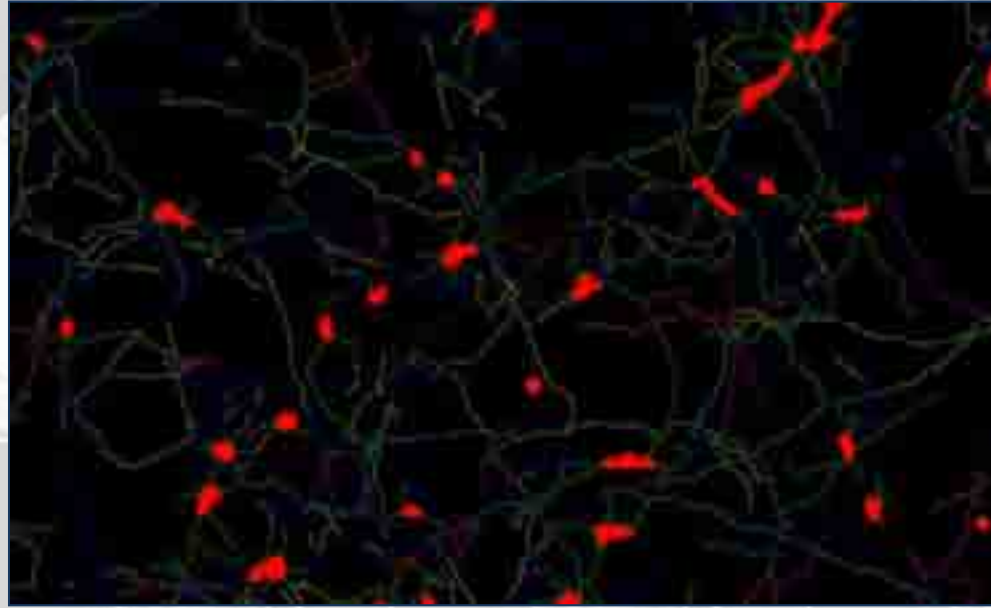


A β 25-35

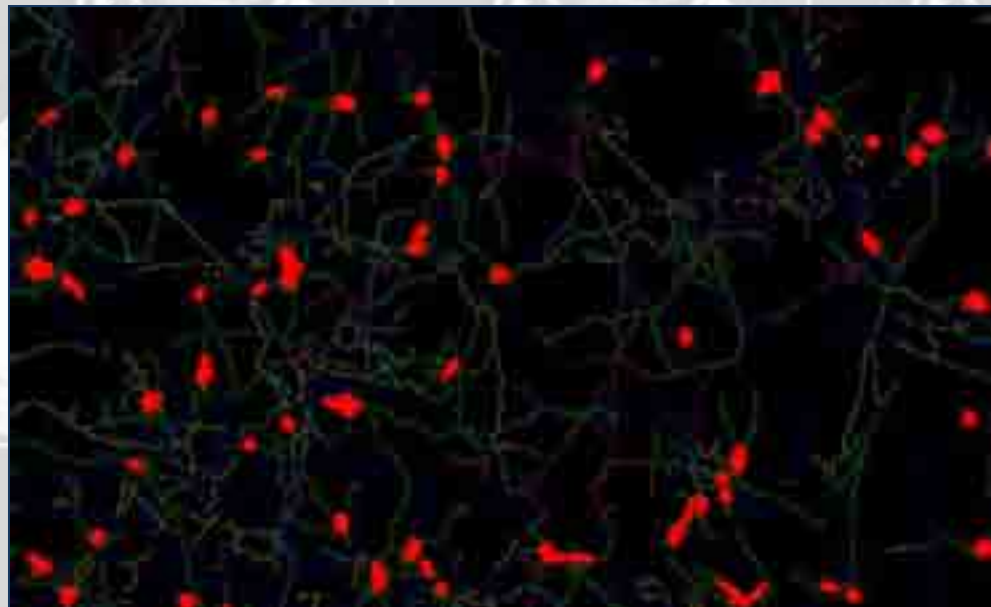


Implementation of automated quantitative neurite outgrowth image analysis on High Content Screening system (BD Pathway 435)

**scrambled
siRNA**



p53 siRNA



TNFR1a functional validation

JCB: ARTICLE

Deletion of tumor necrosis factor death receptor inhibits amyloid β generation and prevents learning and memory deficits in Alzheimer's mice

Ping He,¹ Zhenyu Zhong,¹ Kristina Lindholm,¹ Lilian Berning,¹ Wendy Lee,¹ Cynthia Lemere,² Matthias Staufenbiel,⁴ Rena Li,² and Yong Shen¹

¹Yaskeman Laboratory of Molecular and Cellular Neurobiology and Roberts Center for Alzheimer's Research, Sun Health Research Institute, Scottsdale, AZ 85254
²Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115
³Novartis Pharma Ltd., Nervous System Research, CH-4002 Basel, Switzerland

Firefly luciferase activity



β
5uM

* P<0.05 ** P<0.01 versus respective scrambled controls

The literature finding, while hampering the novelty of our target and preventing further development in a drug discovery context, was extremely useful to validate our target discovery and validation approach with a completely different disease model.

Conclusions on target validation process

- QPCR analysis of identified targets is an important entry point for the validation process, providing the first correlation with cell models and AD patients.
- A series of “Wet” and “Dry” enablers provide robustness to the target validation workflow.
- The functional target validation through RNAi allows us to have a constant pipeline of confirmed genes to be progressed through the drug discovery process.
- Reliability of the target validation approach presented is confirmed by high correspondence of data between our “in vitro” system and “in vivo” models of pathology or “ex vivo” Alzheimer’s tissues.