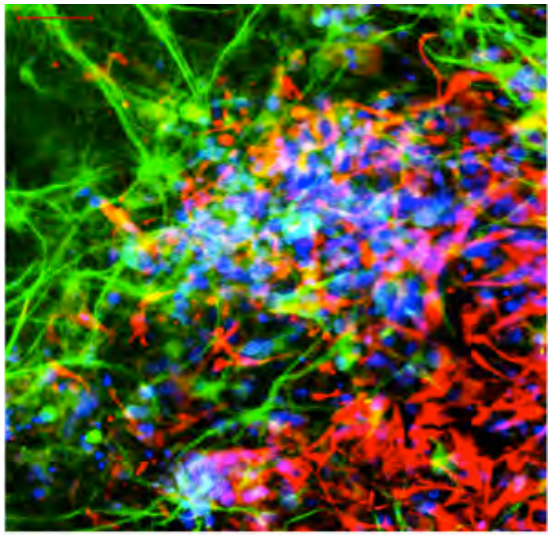
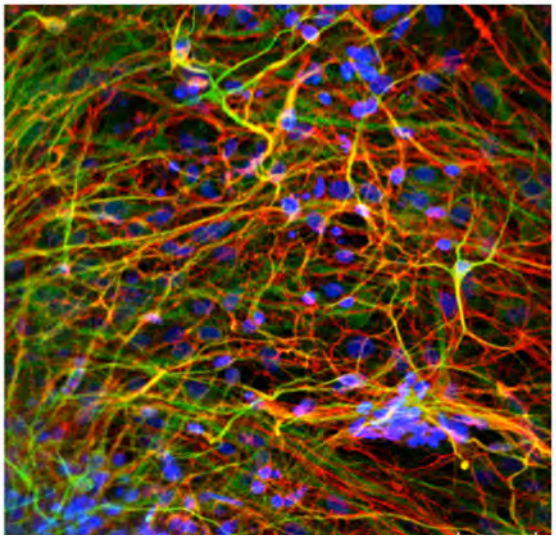
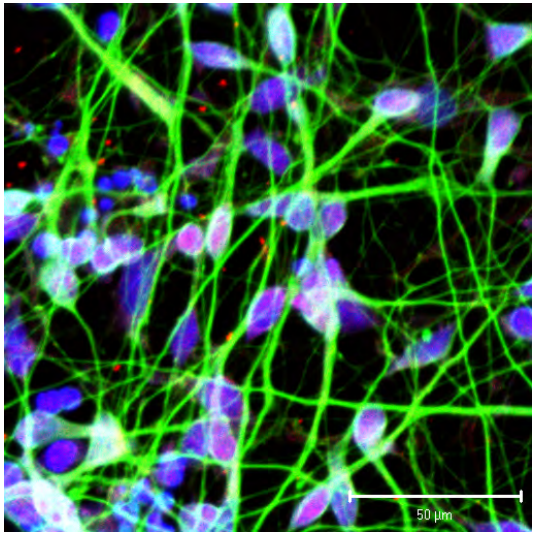
Stem Cells and the Study of Neurodegeneration





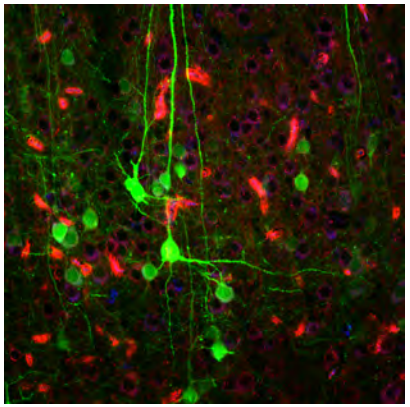


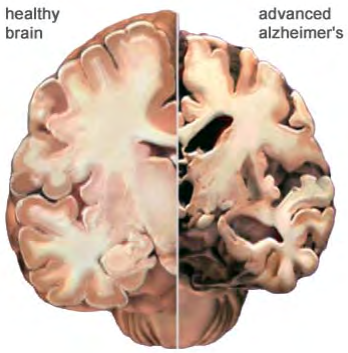
Tracy Young-Pearse, PhD

September 12, 2014

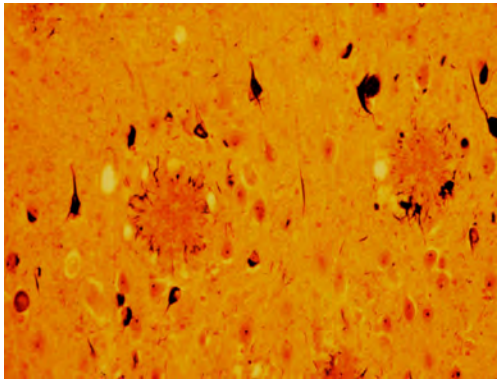
Techniques for studying mechanisms of neurological disease

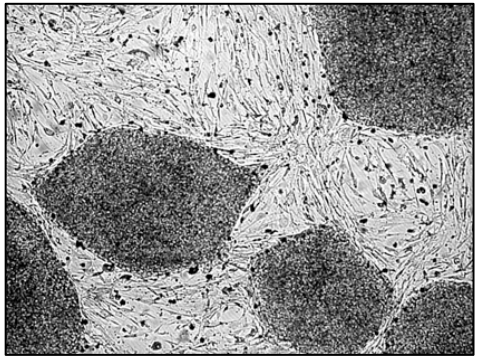
Animal models



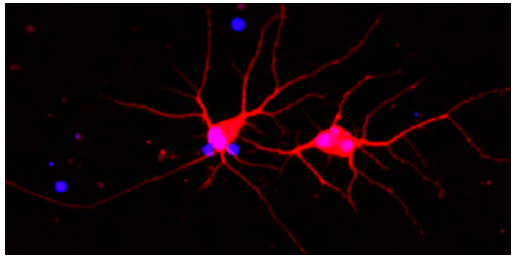


Human subjects
Postmortem analyses, imaging

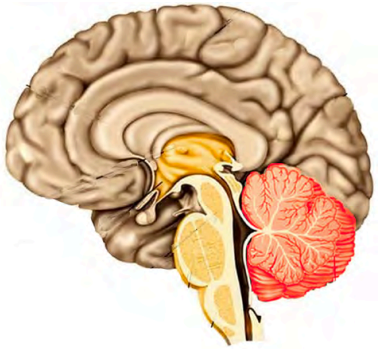




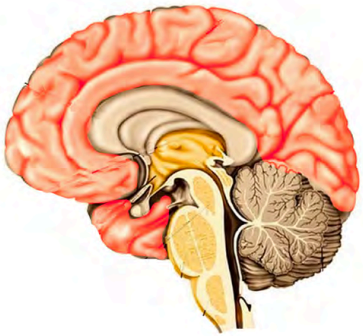
Induced pluripotent stem cells



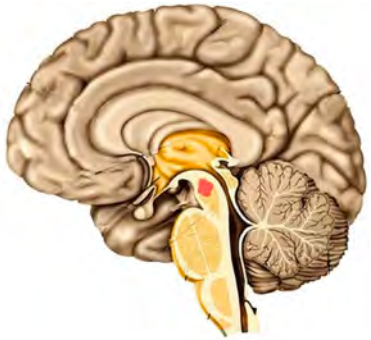
Differential vulnerability to neurodegenerative diseases



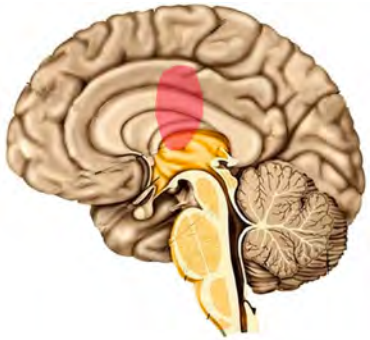
Spinocerebellar ataxia



Alzheimer's disease

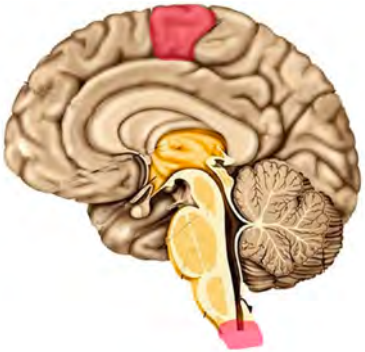


Parkinson's disease

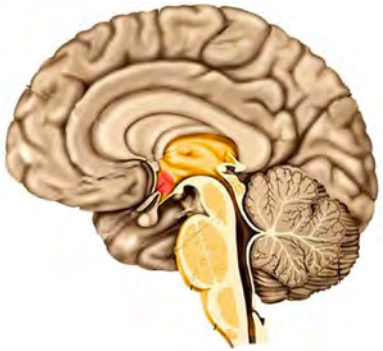


Huntington's disease

Frontotemporal dementia

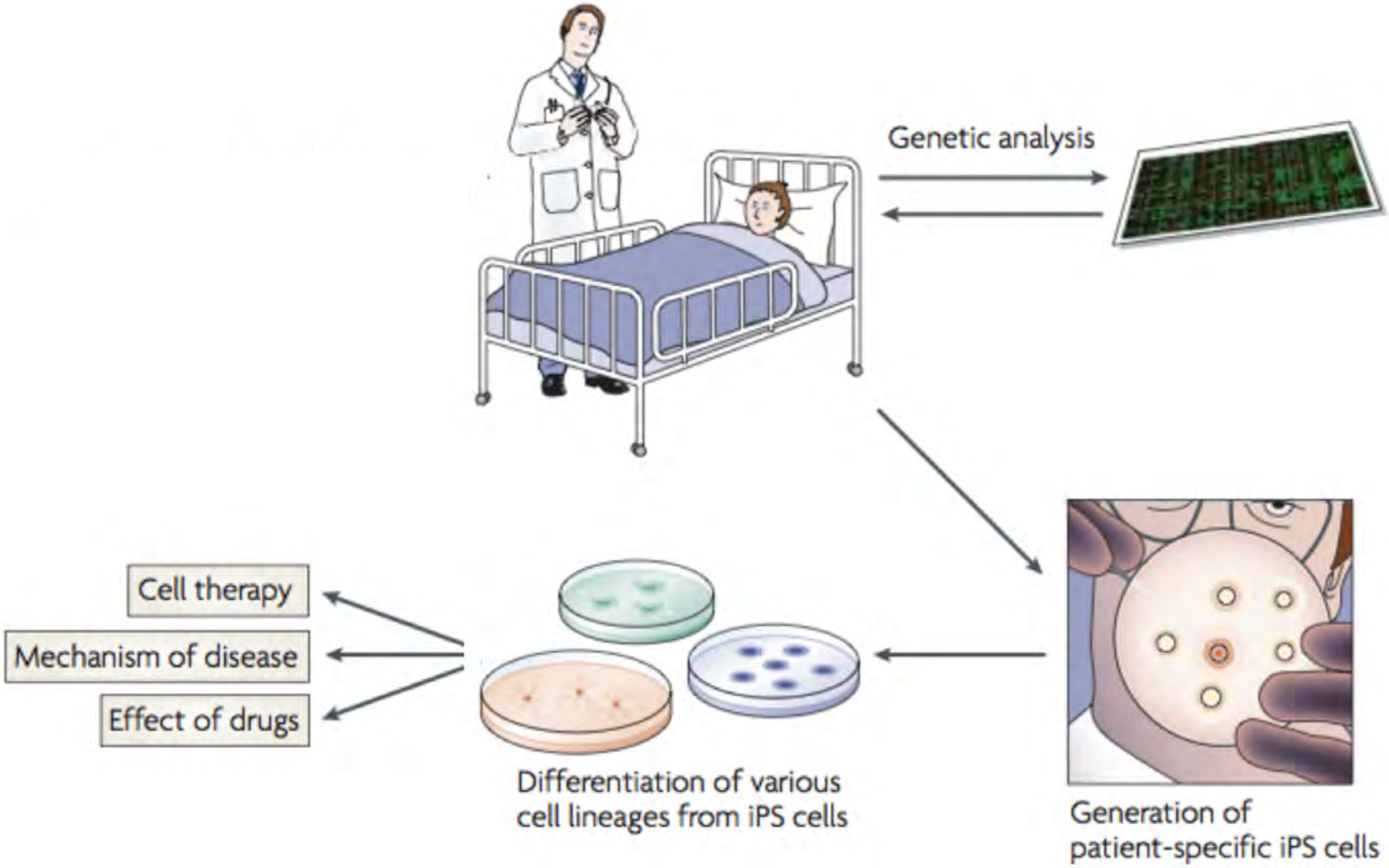


ALS (Lou Gehrig's disease)



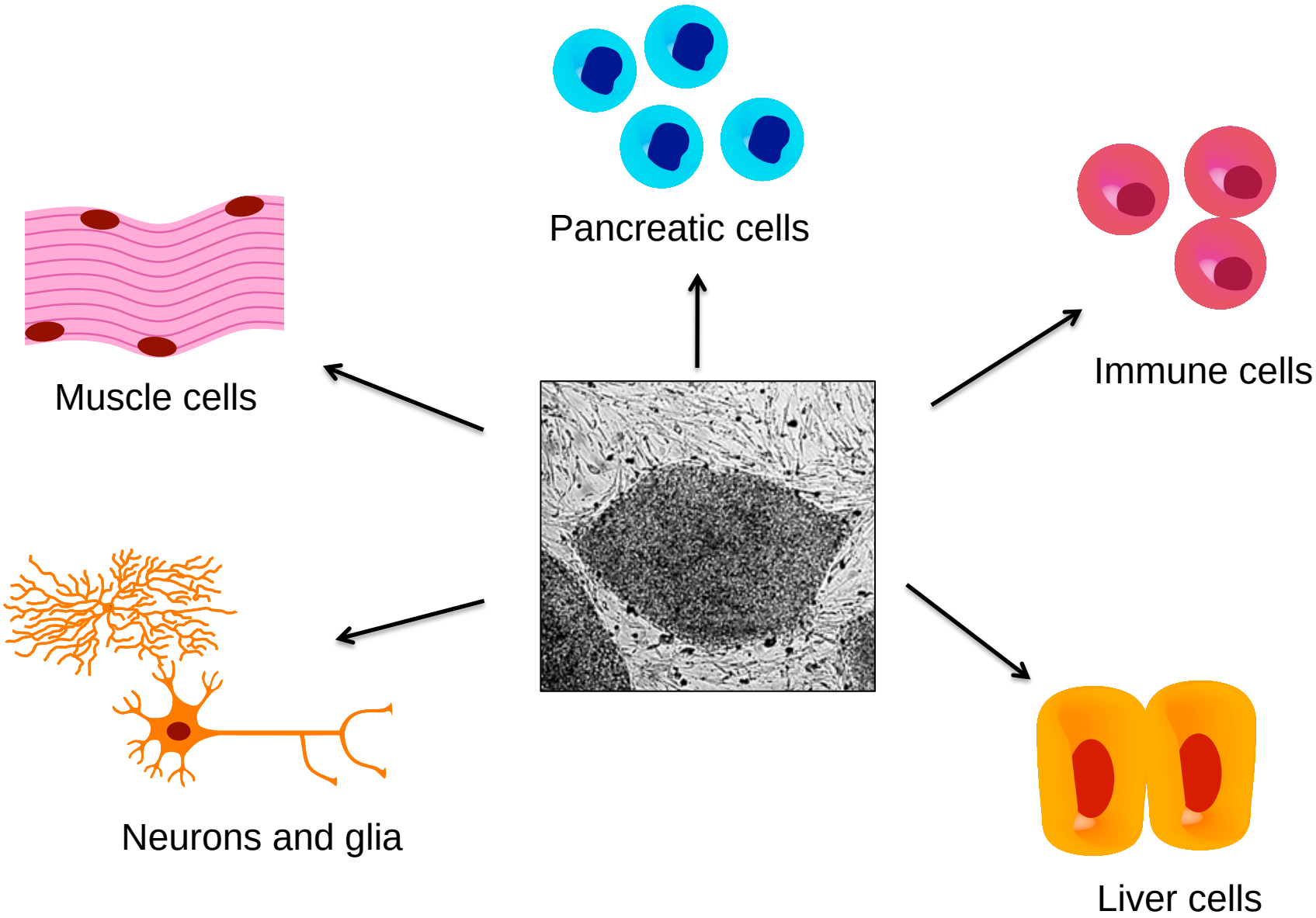
Narcolepsy

Development of stem cells from patients with Alzheimer's disease

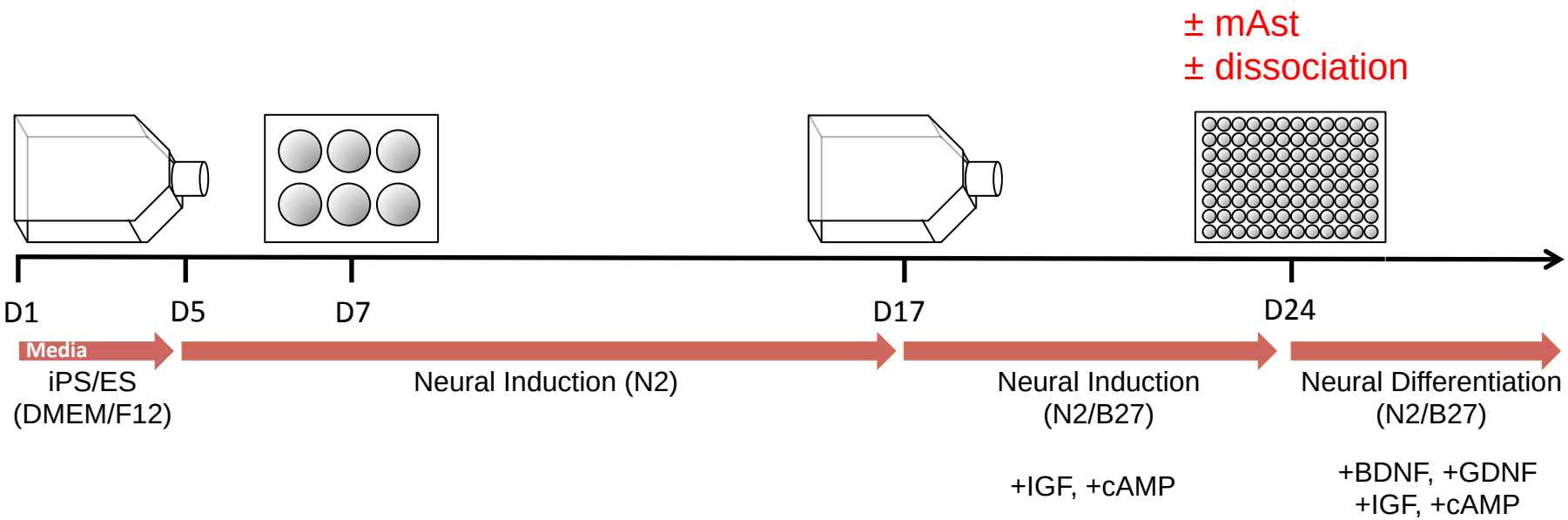


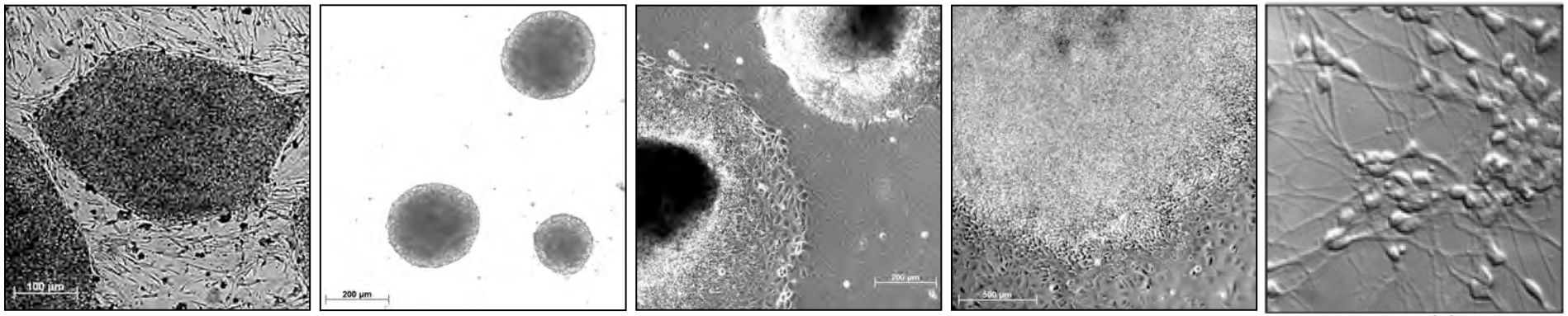
Schematic modified from Nishikawa et al, 2008

Overview of induced pluripotent stem cells (iPSCs)



Human iPSC neural differentiation

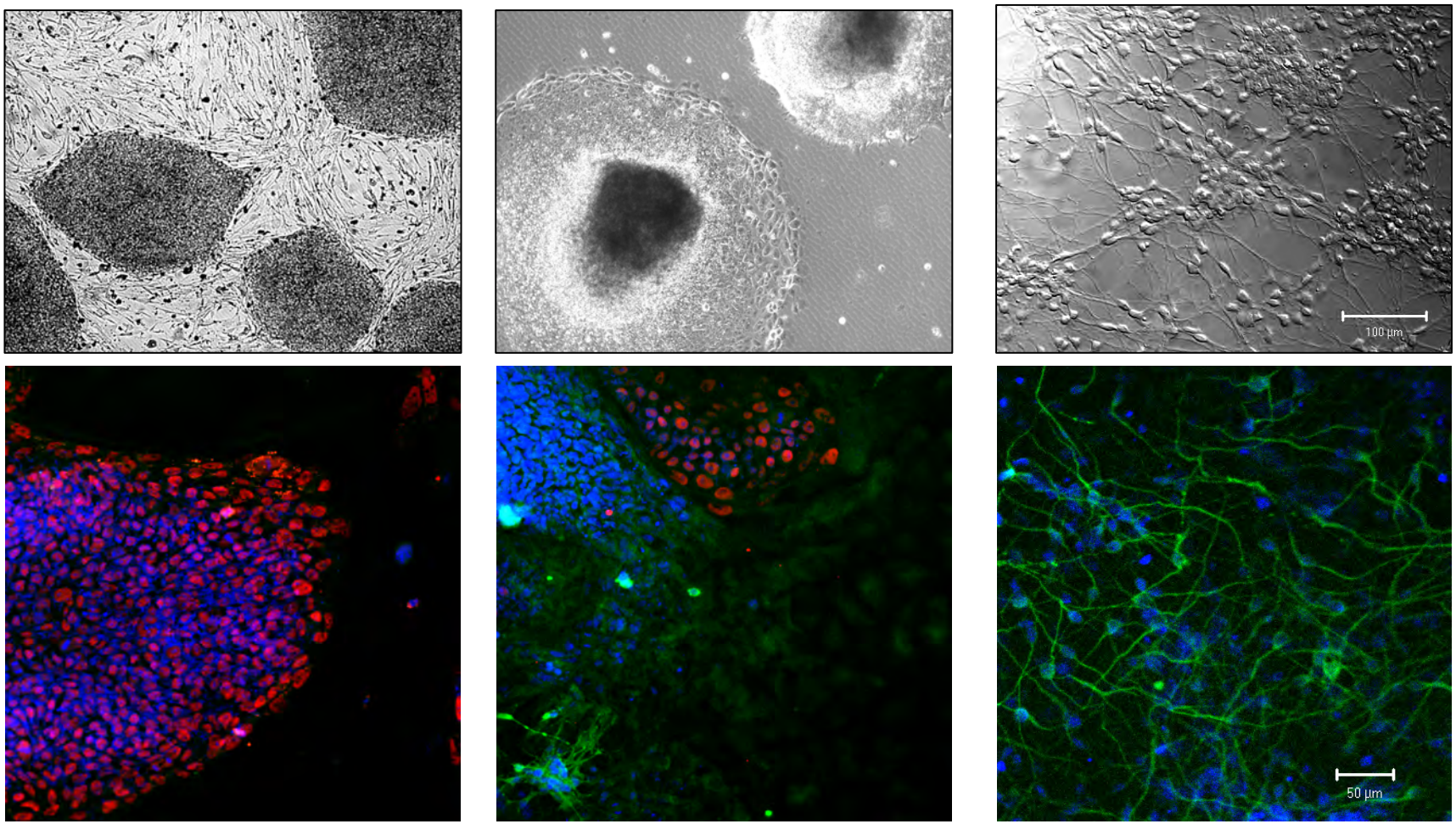




iPS Cells | Day 4 | Day 10 | Day 16 | Day 60

Differentiation of human iPS cells to neuronal fates

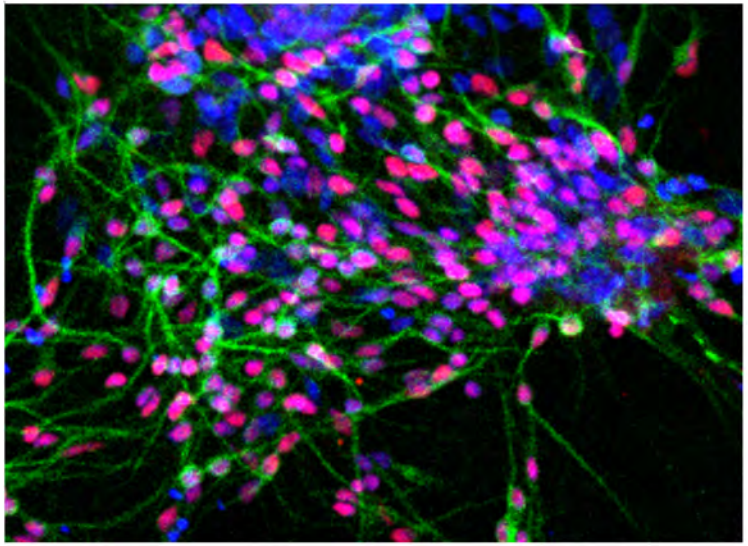
iPS Cells | Day 17 | Day 60

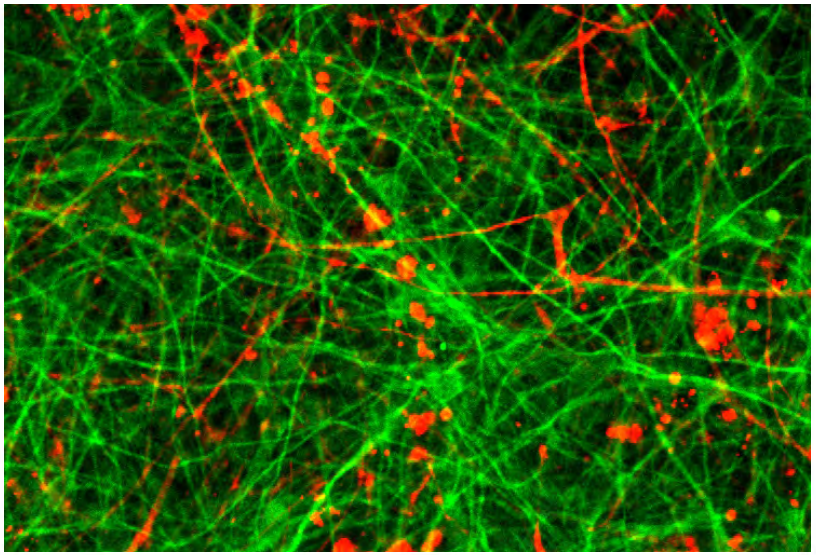


Red = Oct4 = pluripotent cells
Green = MAP2 = neuronal cells
Blue = TOPRO3 = all cells

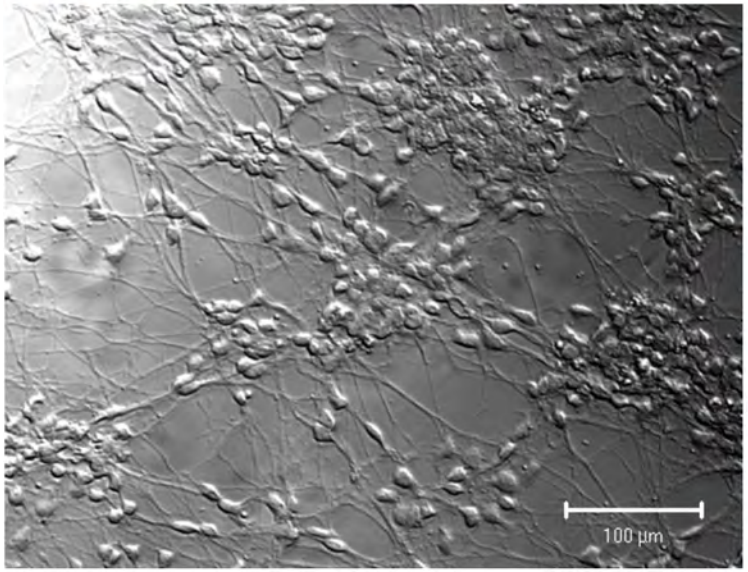
Living neurons from our patients with neurodegenerative diseases

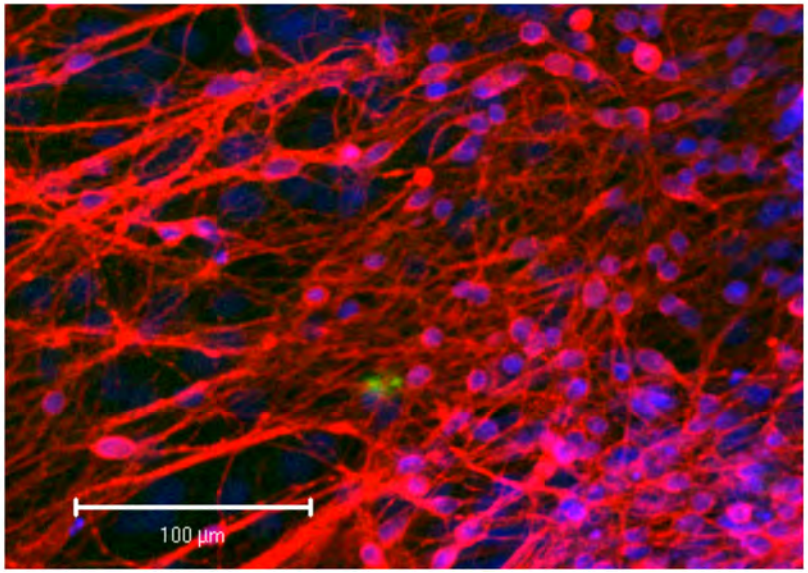
TBR1/MAP2/TOPRO3





MAP2/GFAP

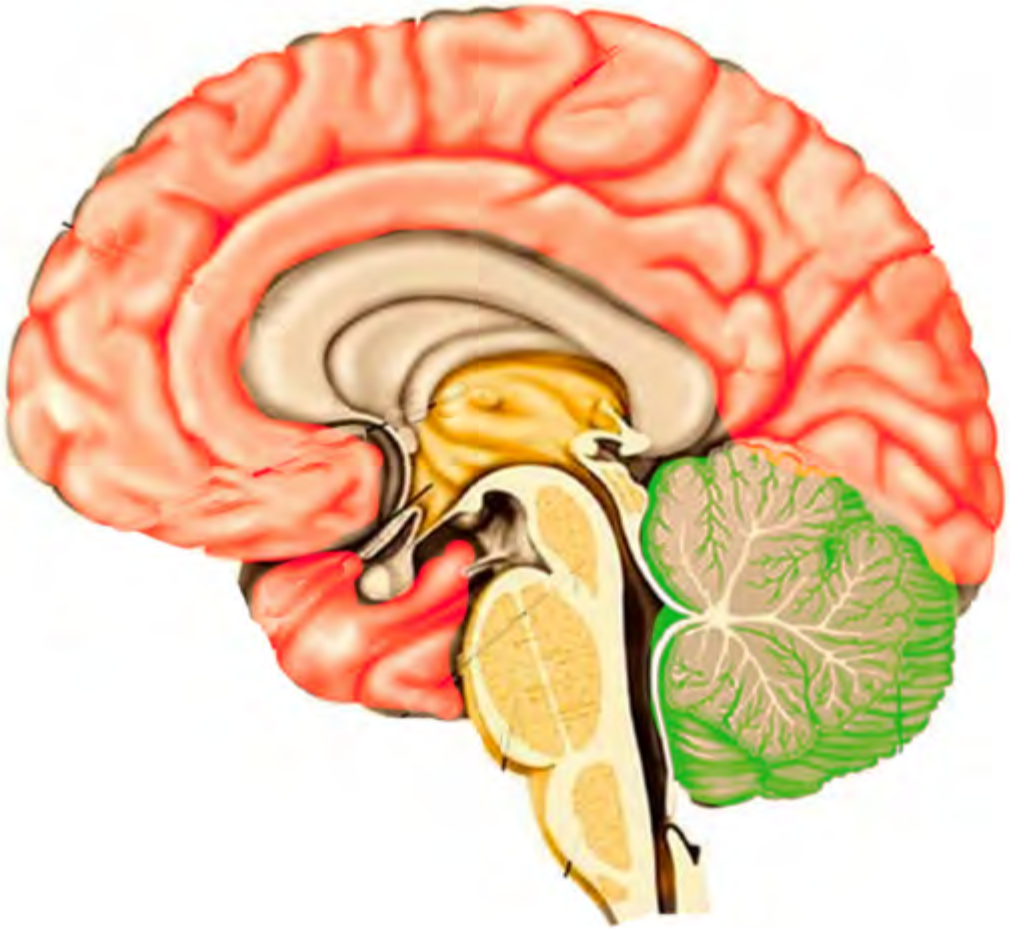




TAU/TOPRO3

Vulnerability to degeneration in Alzheimer's disease differs among brain regions

Cerebral cortex and hippocampus
Memory
Thought
Awareness
Attention
Consciousness



Cerebellum
Movement
Balance
Coordination
Posture

Aβ and Tau accumulate in the cerebral cortex prior to degeneration

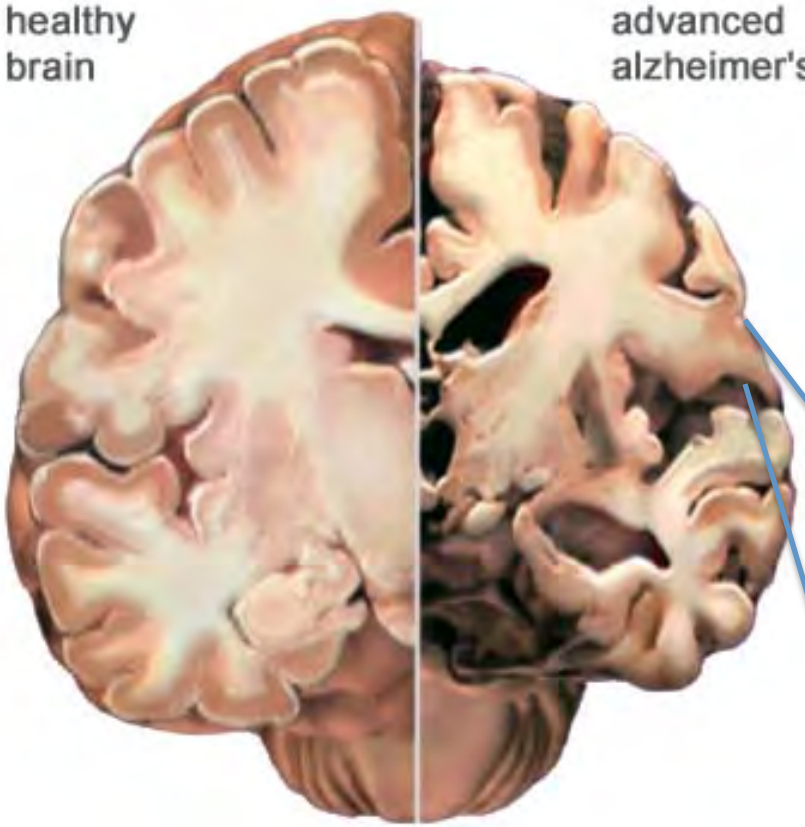
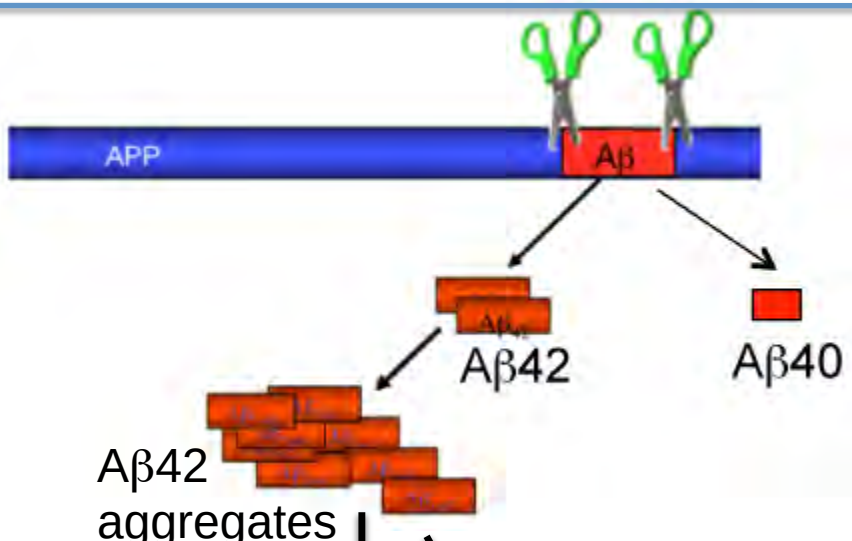
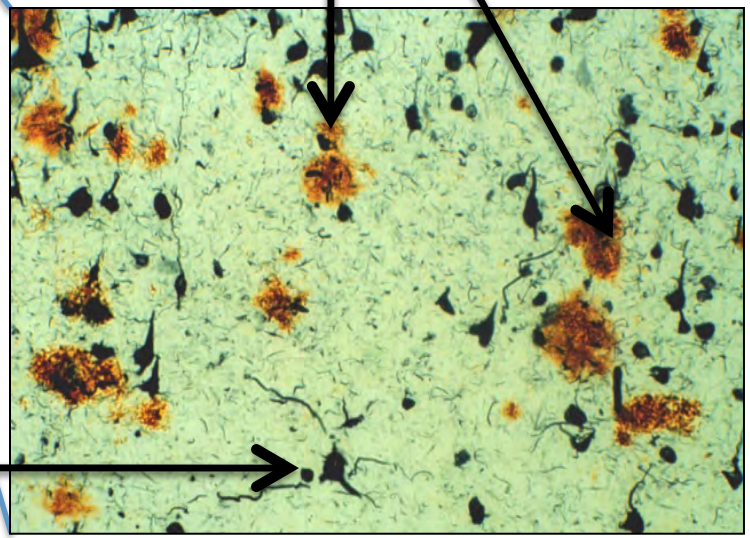


Image courtesy of the Alzheimer's Association

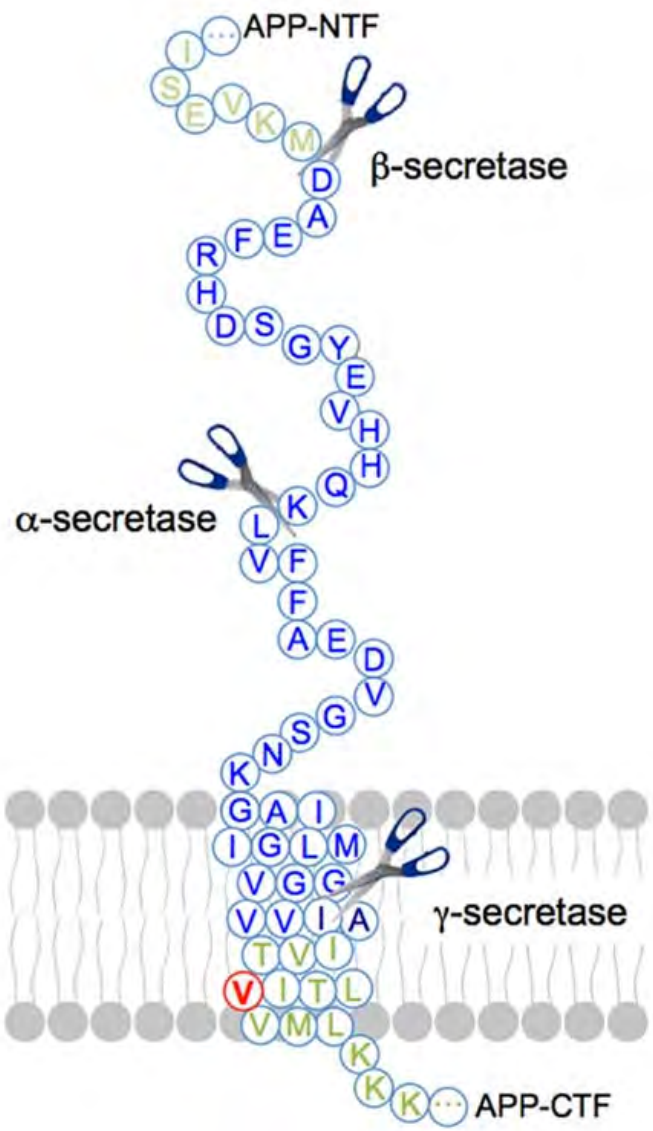


Aβ42 aggregates

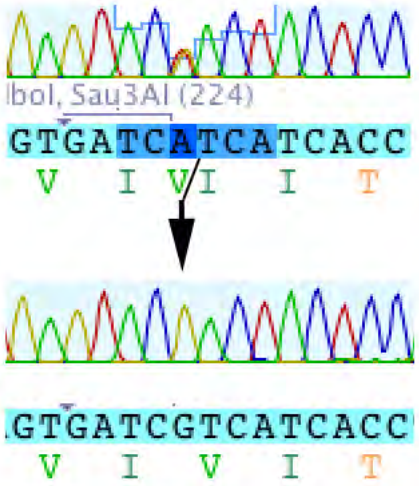


Hyperphosphorylated tau tangles

Mutation of APP causes early-onset Alzheimer's disease



Genome editing with CRISPR/Cas to generate isogenic lines:



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↓

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Gradually rising Aβ42 levels in brain

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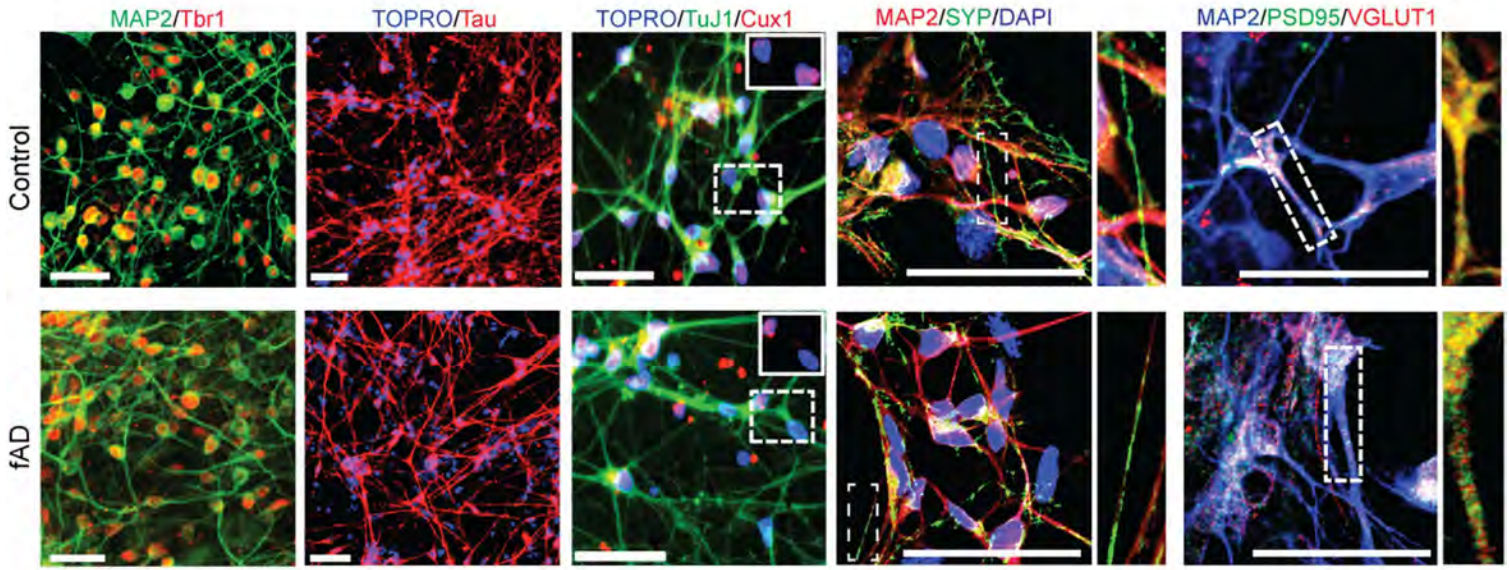
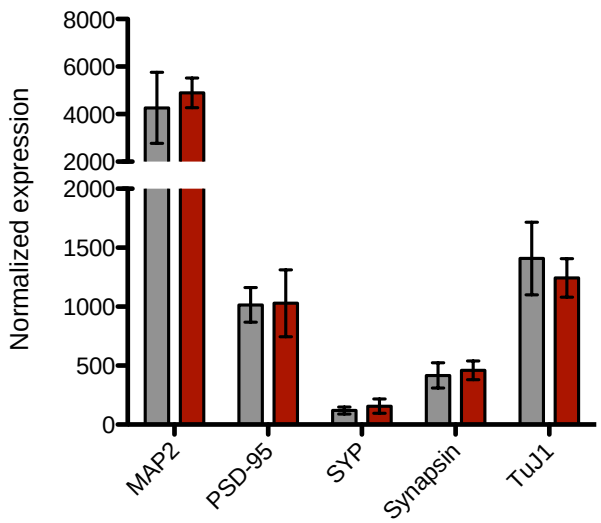
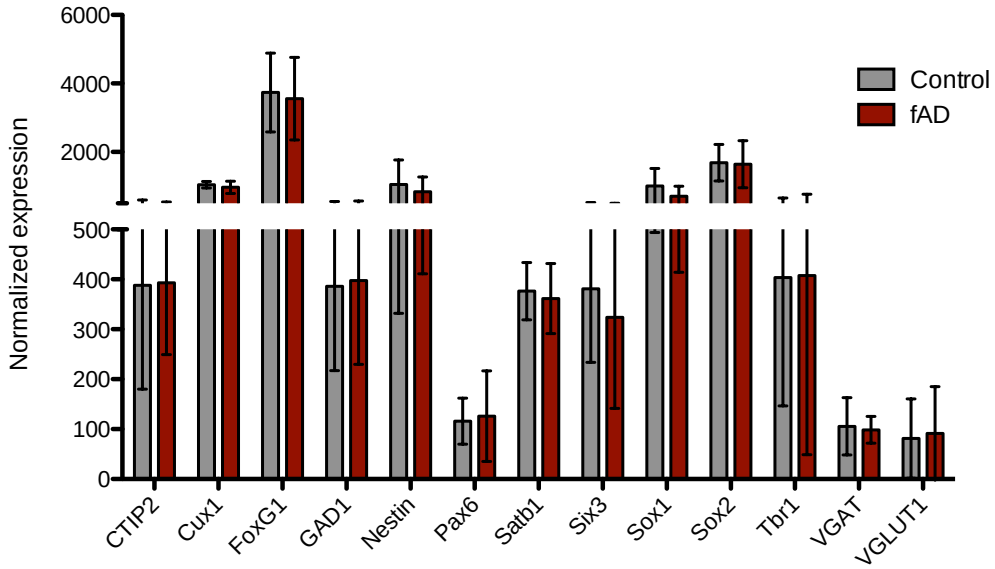
↓

DEMENTIA

APP mutation does not affect neuronal differentiation

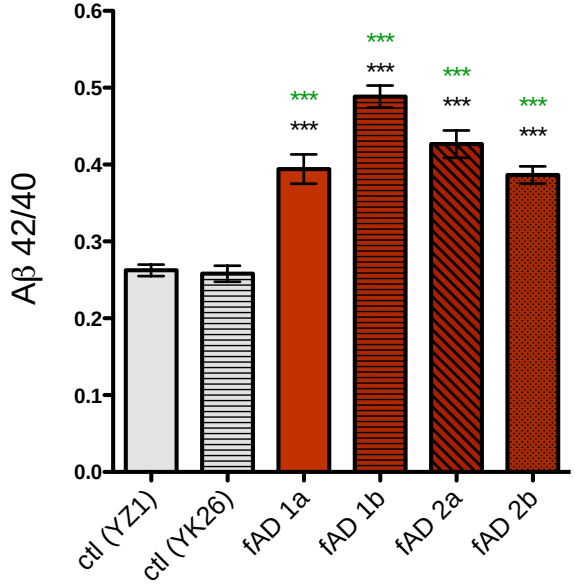


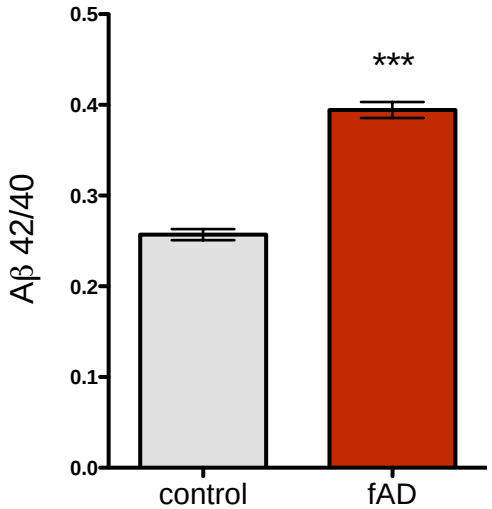


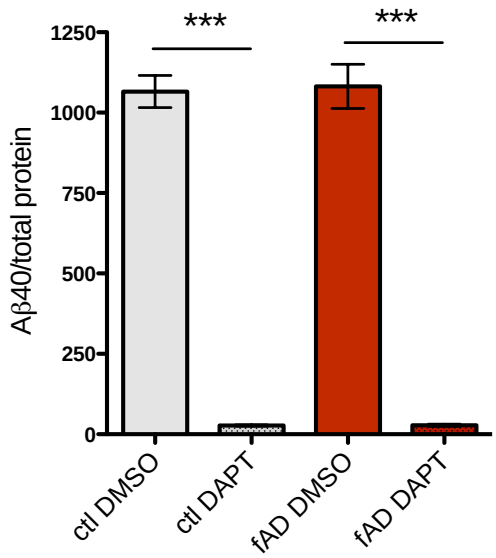


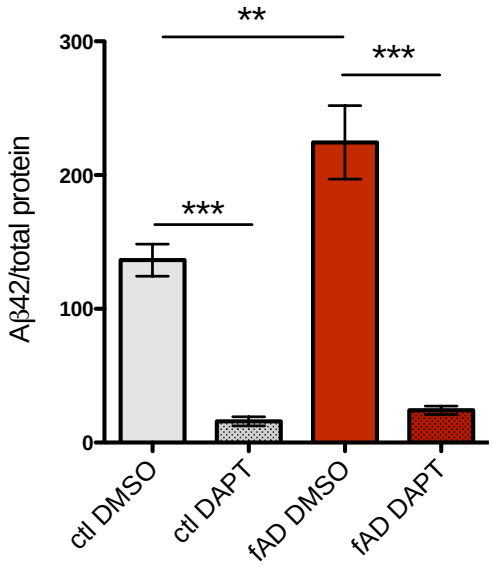
Muratore et al, HMG, 2014

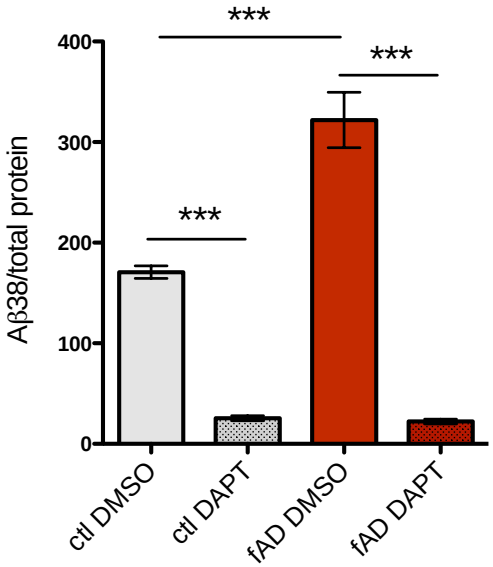
APP mutation leads to increased Aβ42 and Aβ38 production





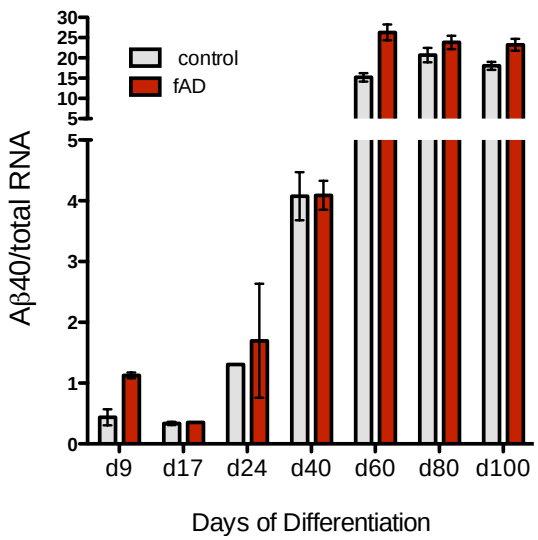


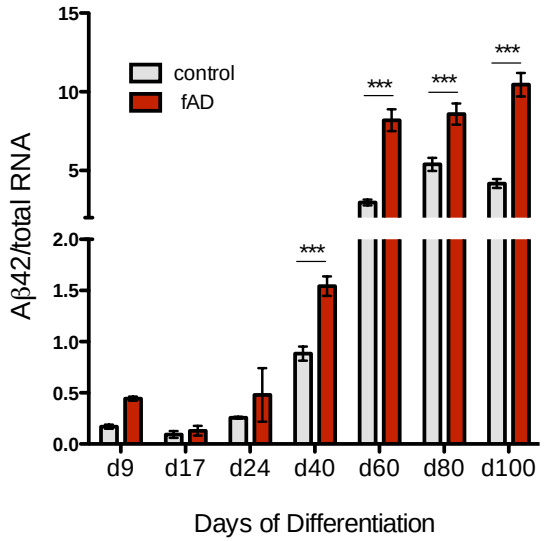


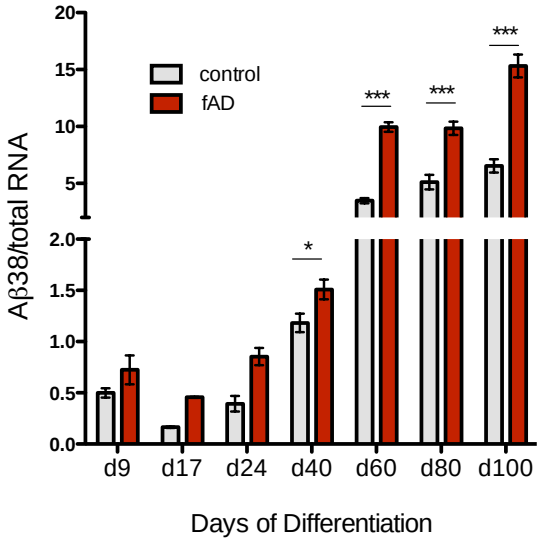


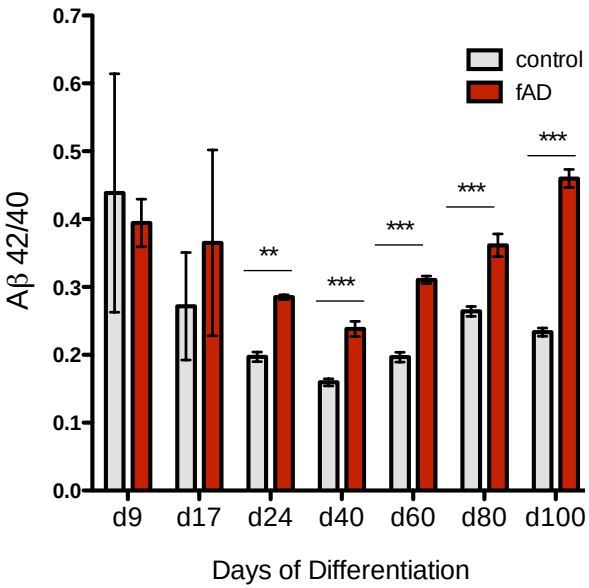
Muratore et al, HMG, 2014

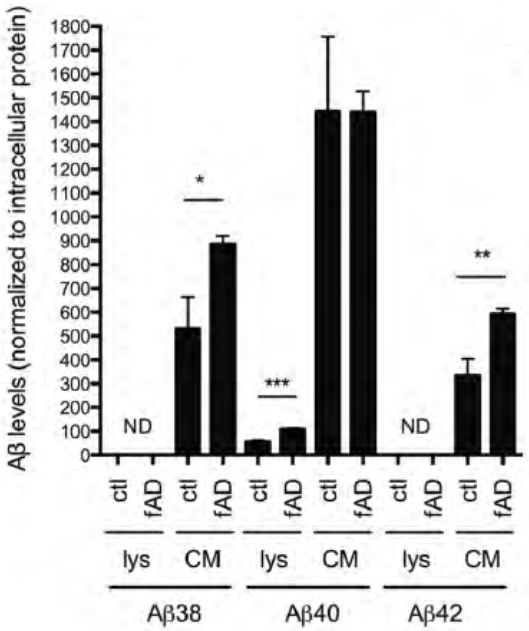
APP cleavage products increase over differentiation from immature to mature neuronal fates





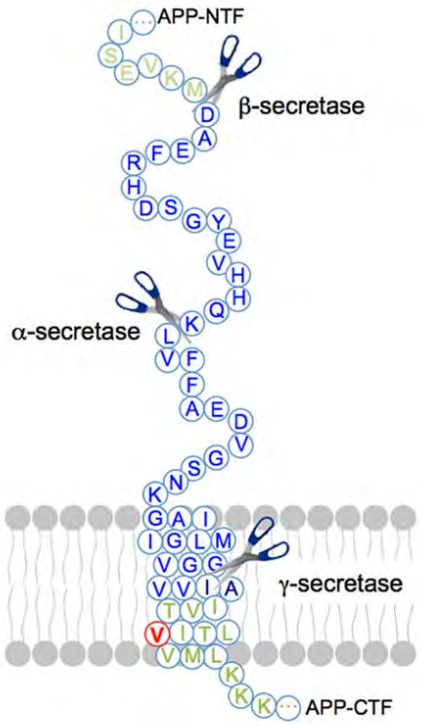


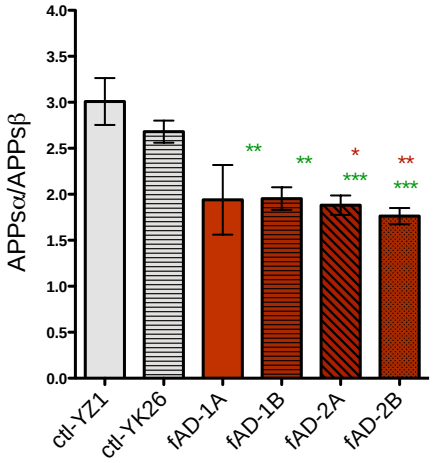


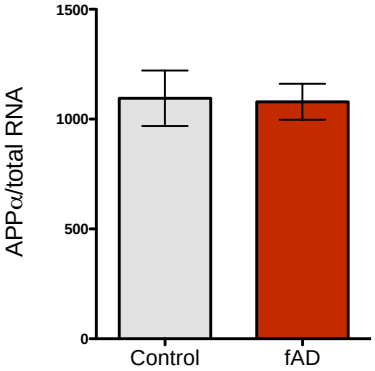


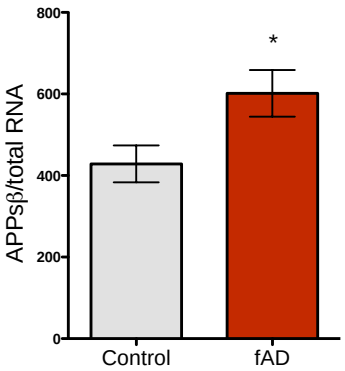
Muratore et al, HMG, 2014

APP mutation leads to increased β-secretase cleavage of APP









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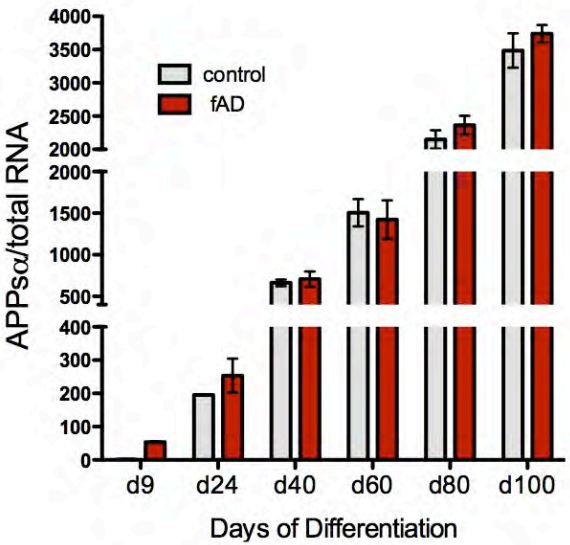
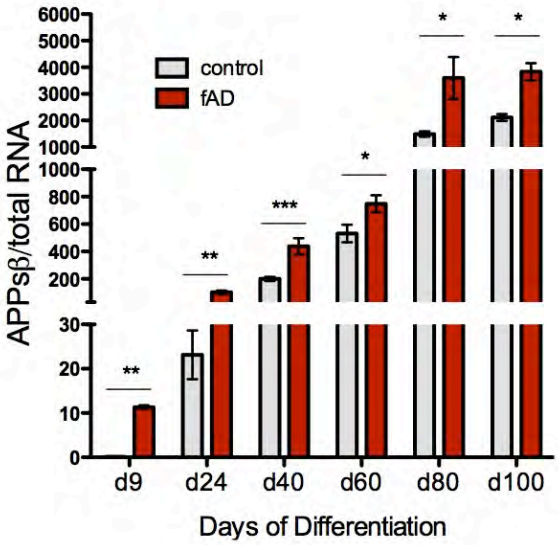
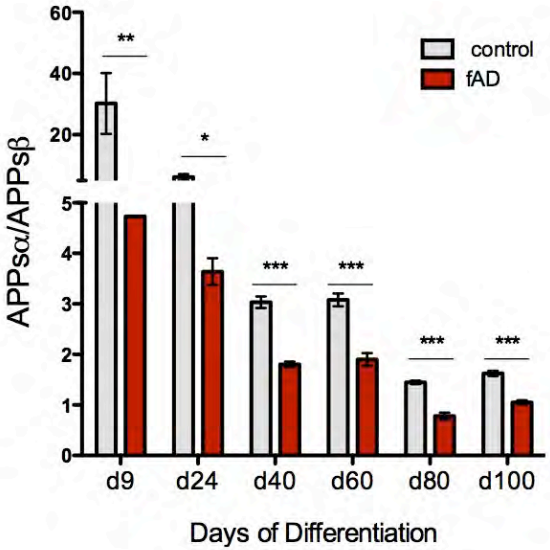
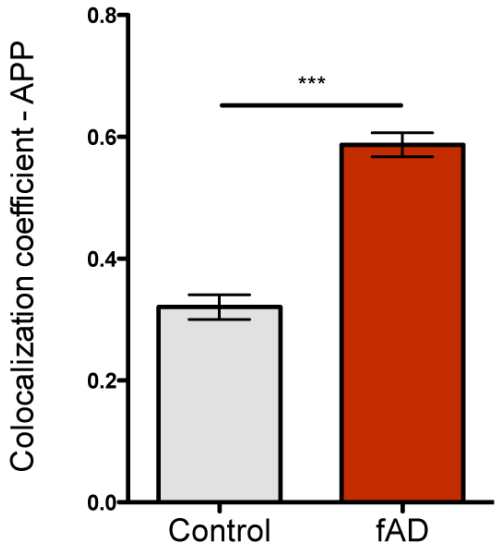


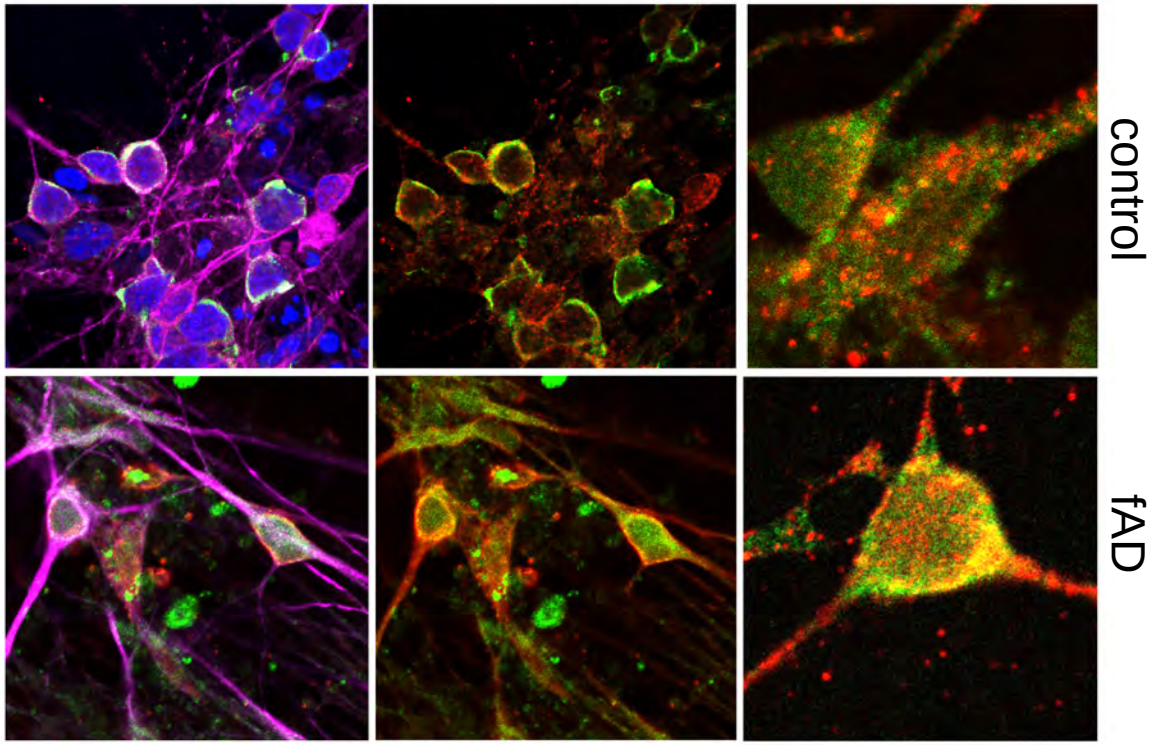




Muratore et al, HMG, 2014

APP subcellular localization is altered in fAD iPSC-derived neurons





EEA-1/APP/MAP2/DAPI

Muratore et al, HMG, 2014

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↓

Altered kinase/phosphatase activities lead to tau-containing tangles

↓

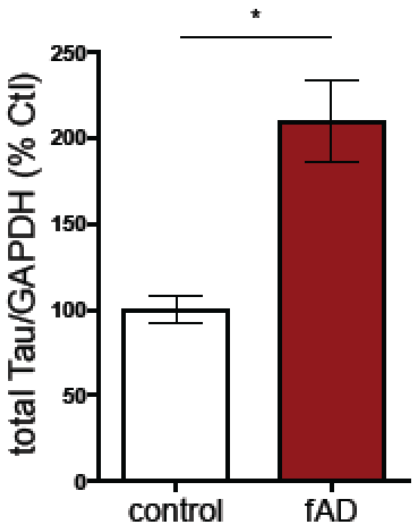
Widespread neuronal/synaptic dysfunction and selective neuronal loss, with attendant neurotransmitter deficits

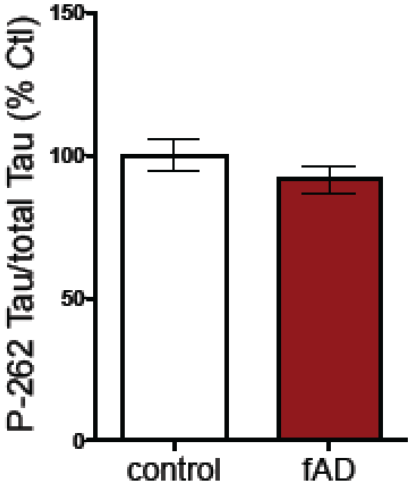
↓

DEMENTIA

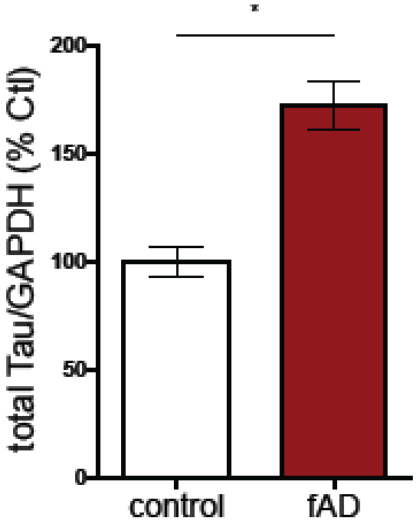
Higher Tau protein levels are observed in fAD-derived neurons

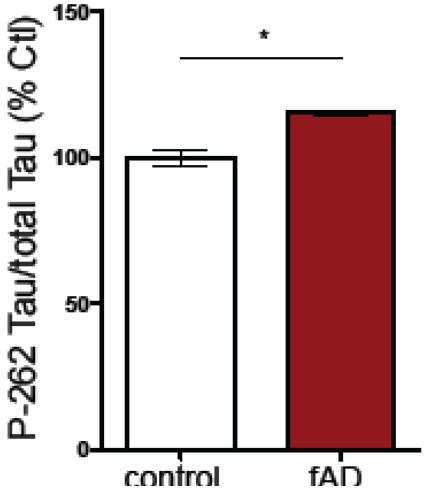
d35





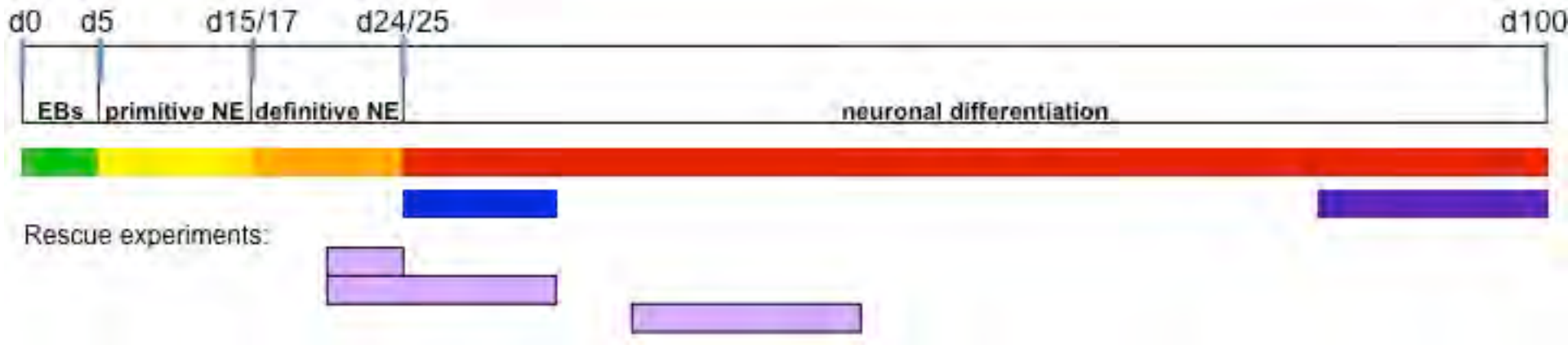
d100



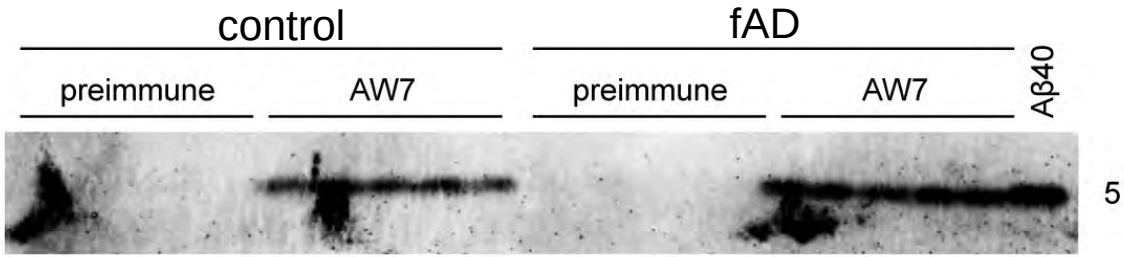


Muratore et al, HMG, 2014

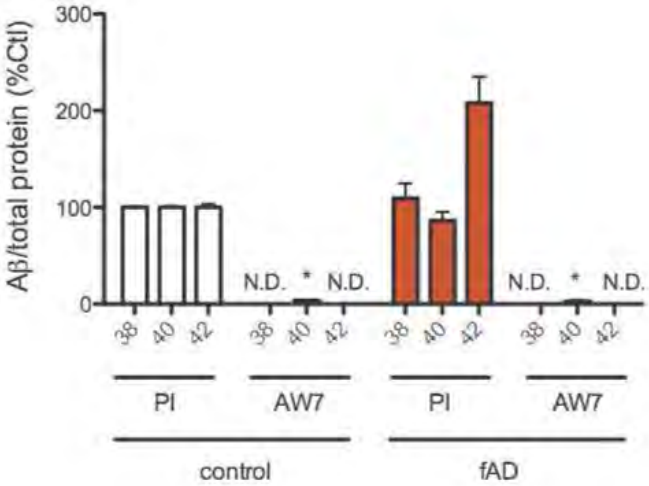
Aβ−specific antibodies (3D6 and AW7) bind secreted Aβ



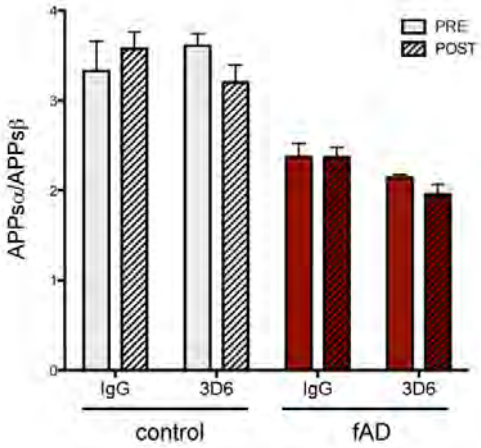
Aβ bound to antibody:



Little unbound Aβ following antibody treatment:

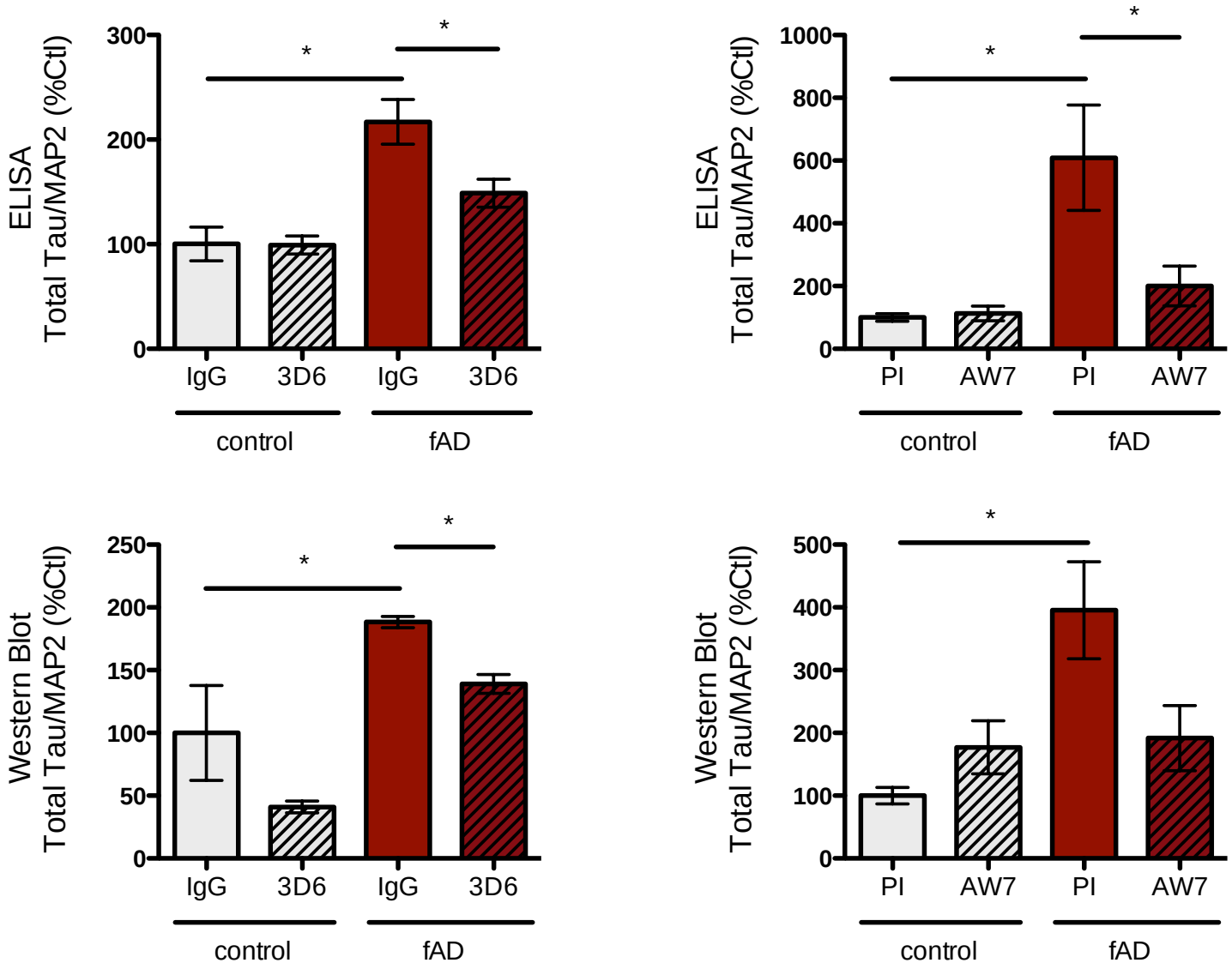


APPs-α and β do not bind to antibody:



Muratore et al, HMG, 2014

Aβ−specific antibodies (3D6 and AW7) rescue the Tau phenotype



Muratore et al, HMG, 2014

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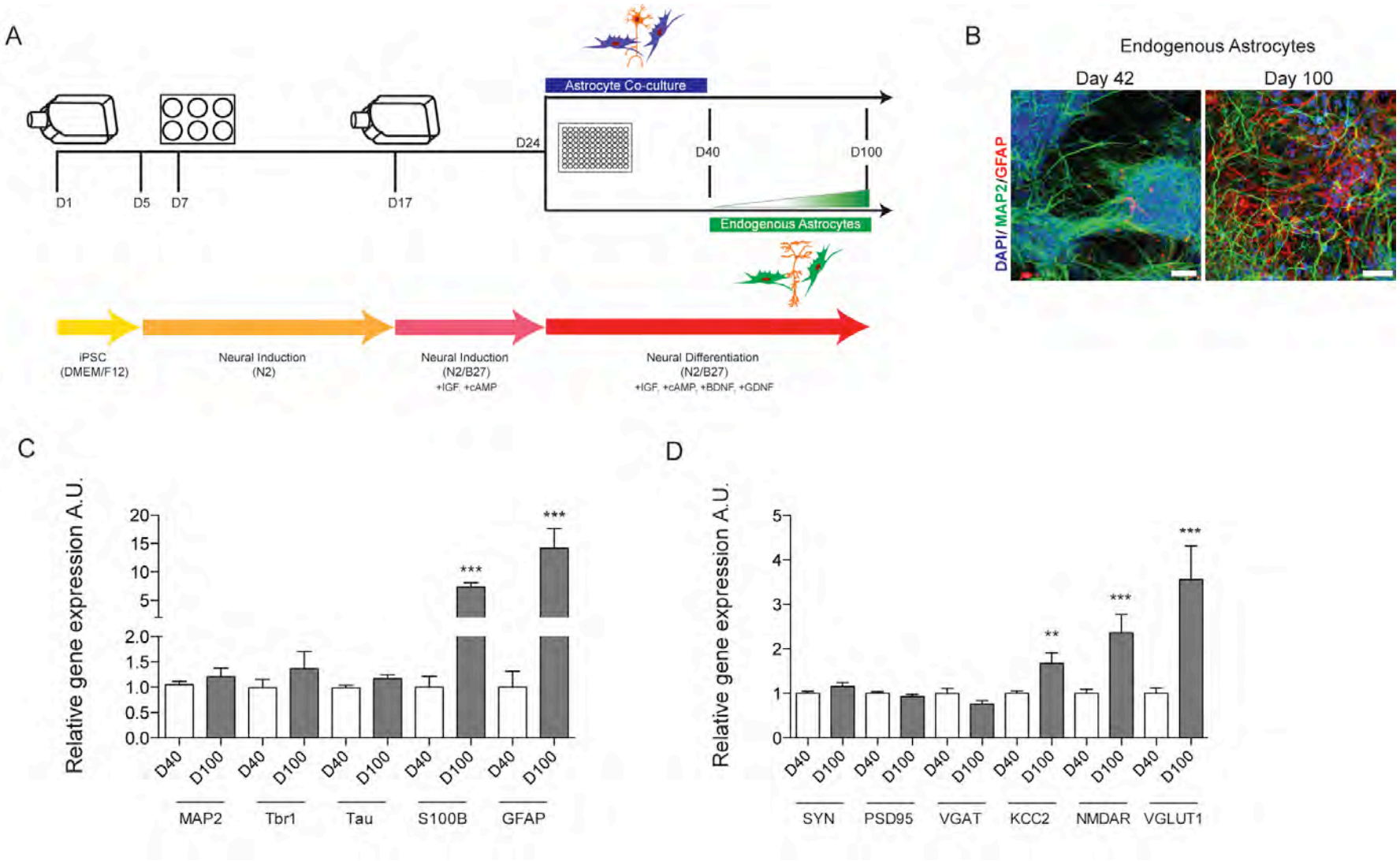
DEMENTIA

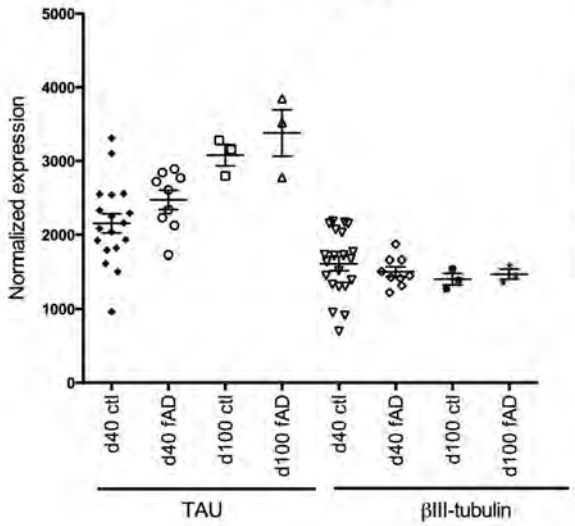
Examining Glial Activation in Alzheimer's Disease



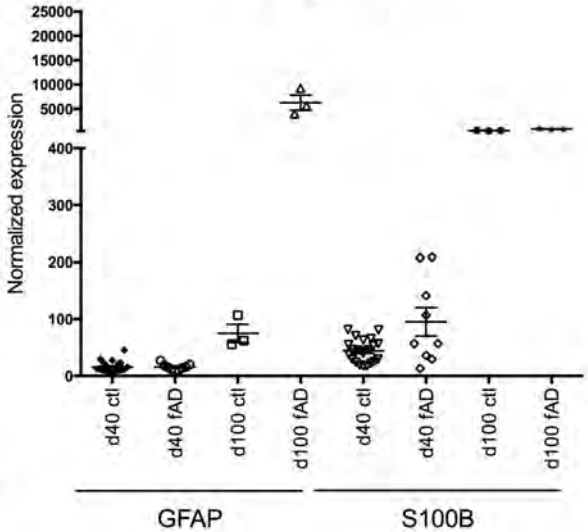
Muratore et al, PLOS ONE, in press

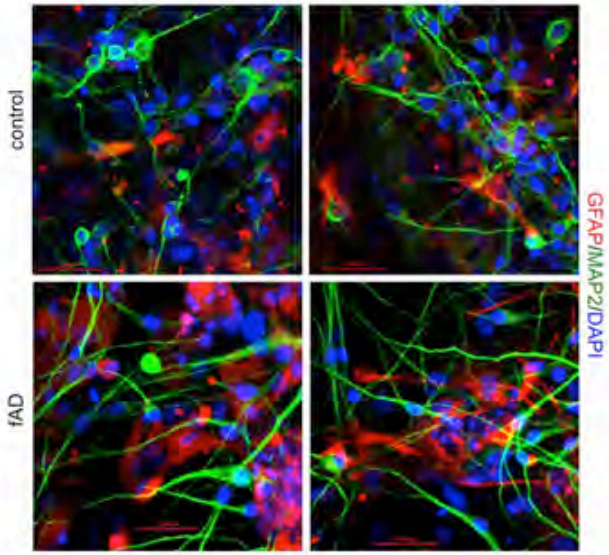
APPV717I induces GFAP expression in astrocytes-derived from iPSCs

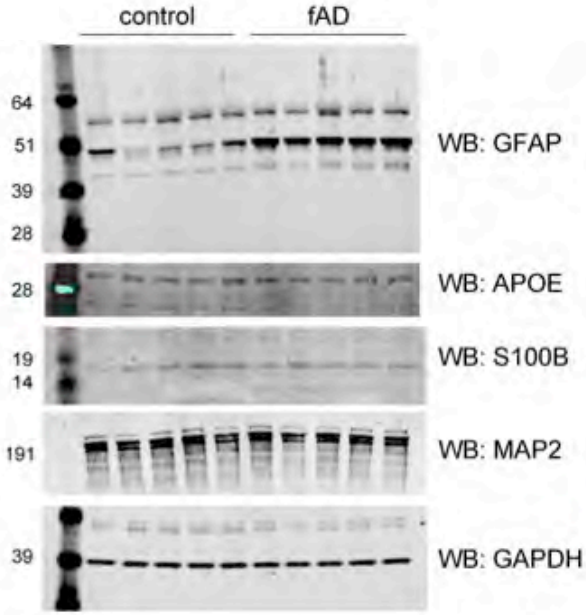
neuronal markers

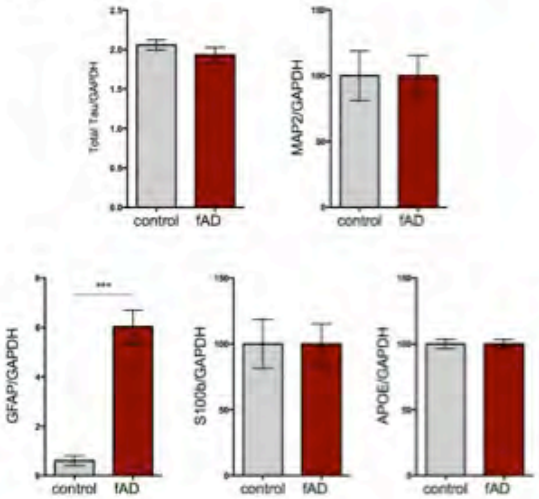


astrocyte markers

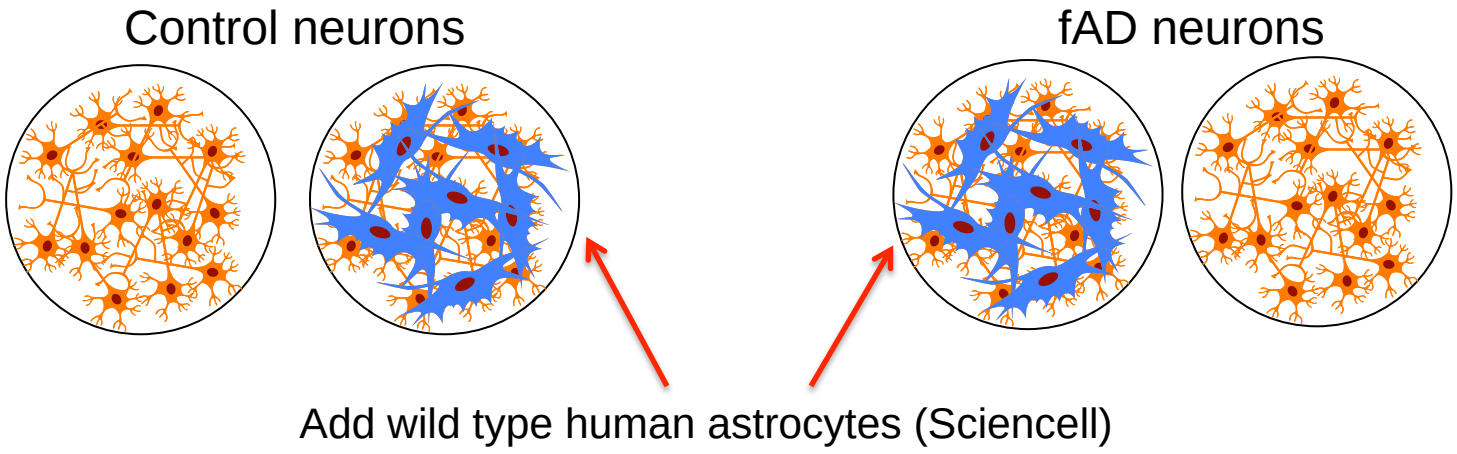


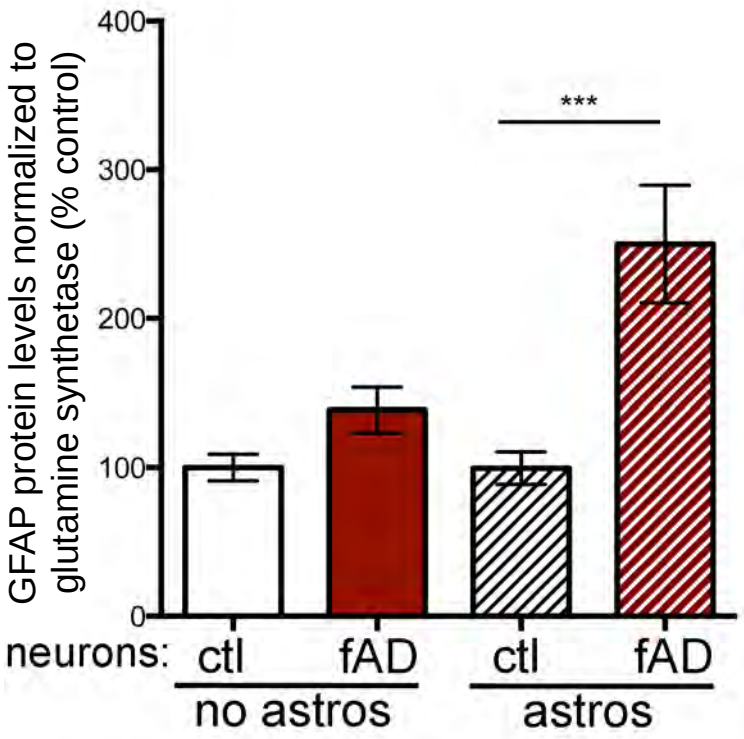




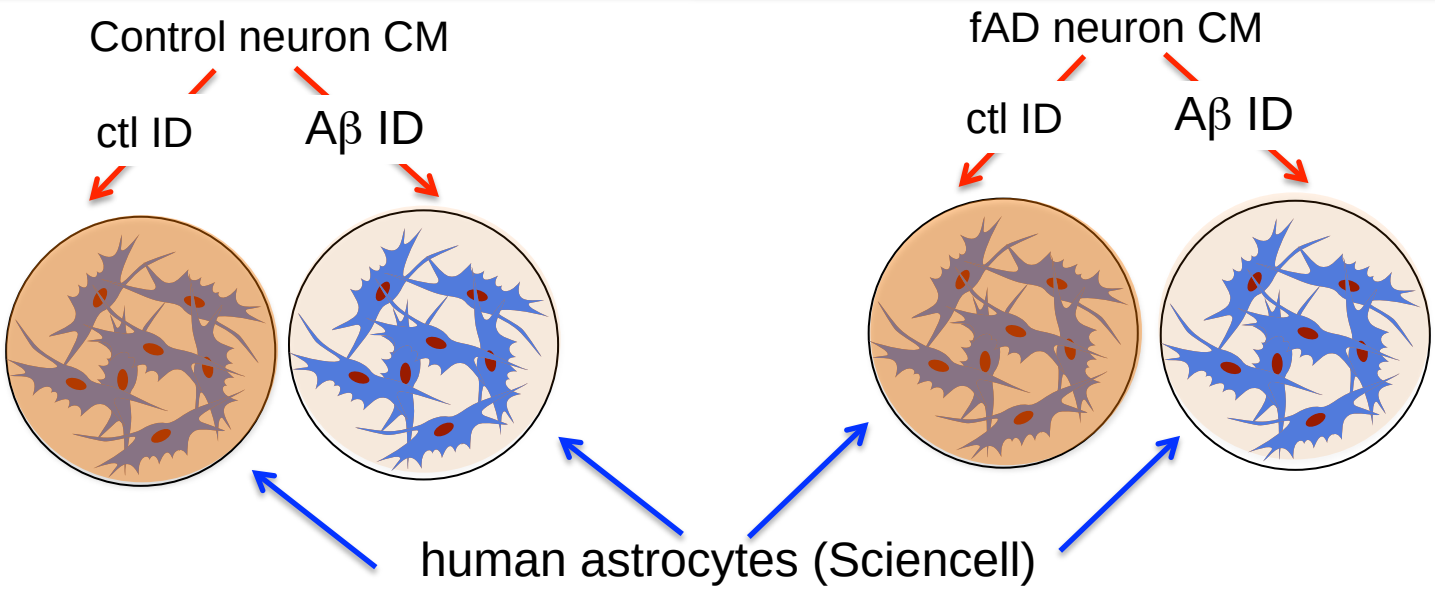


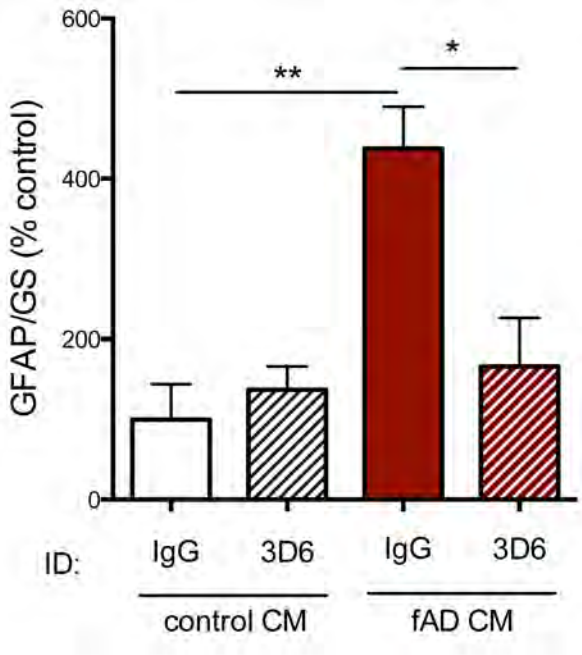
APPV717I induces GFAP expression in human astrocytes





Aβ secreted from fAD neurons induces GFAP expression in human astrocytes





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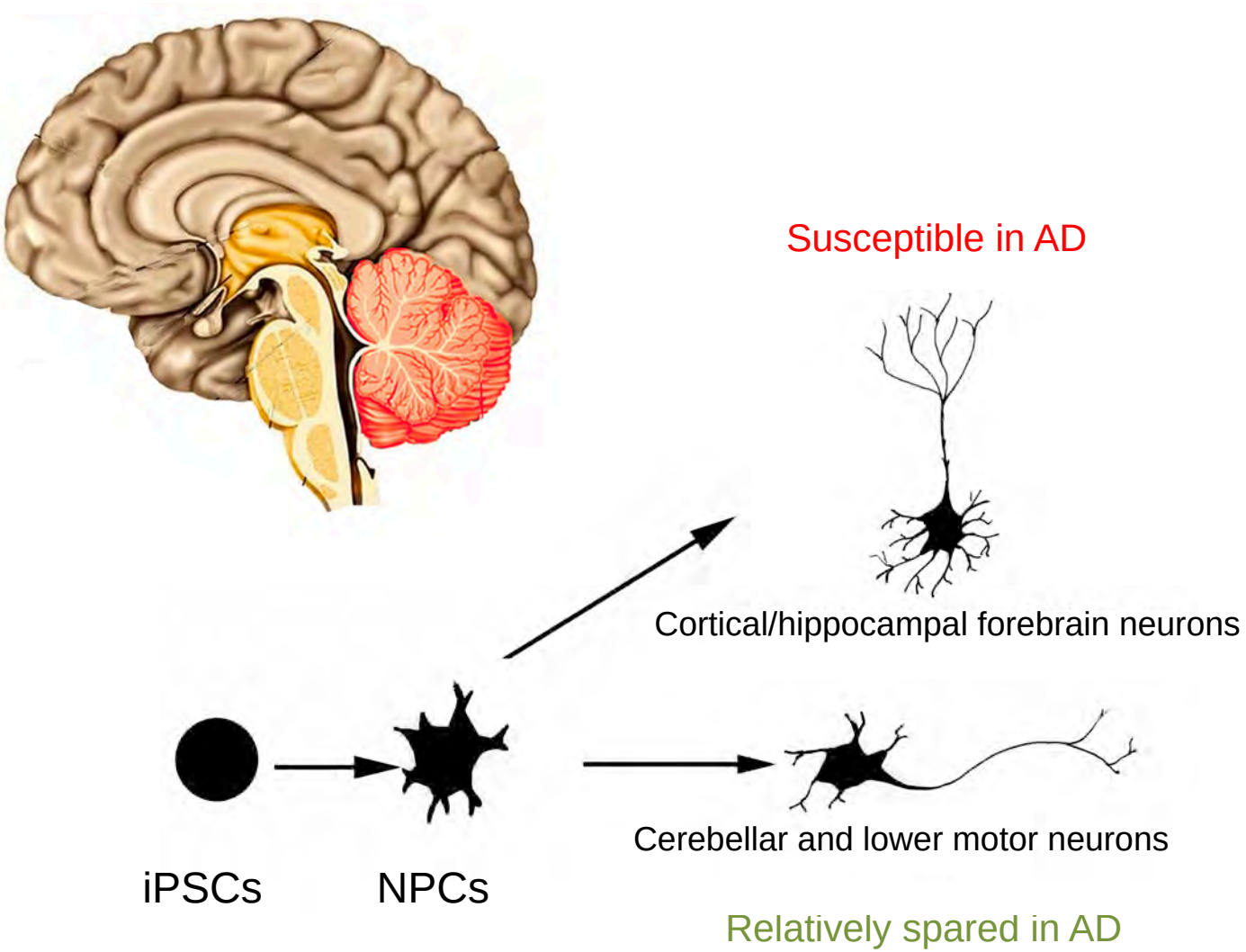
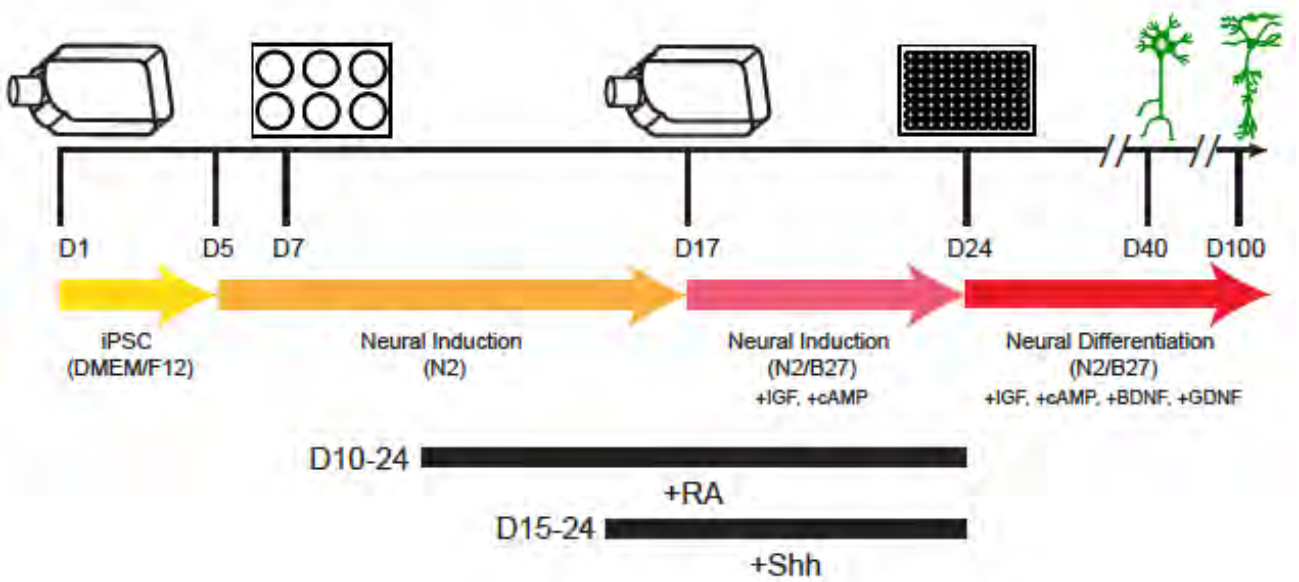
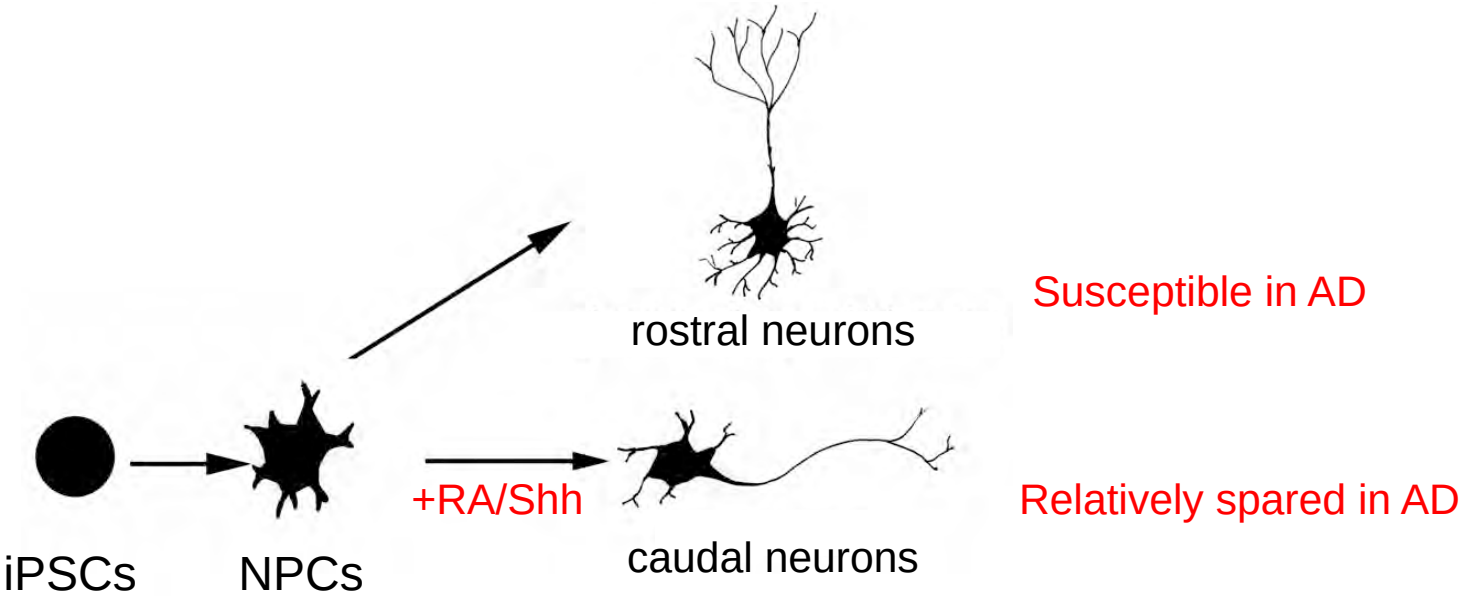
DEMENTIA

Differential vulnerability of neuronal subtypes in Alzheimer's disease

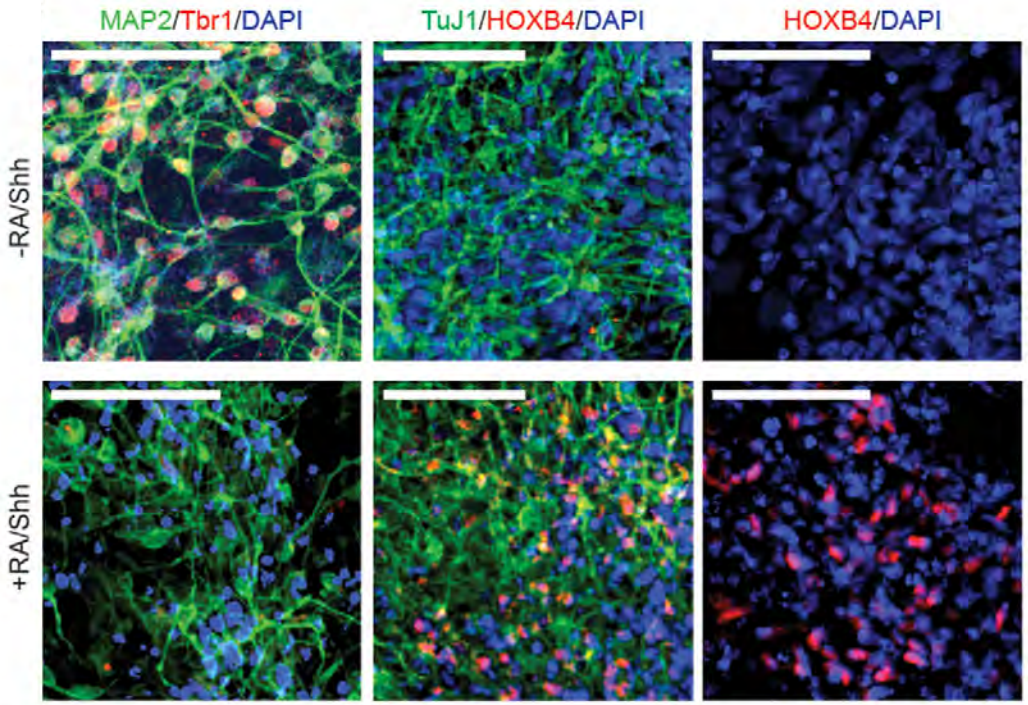


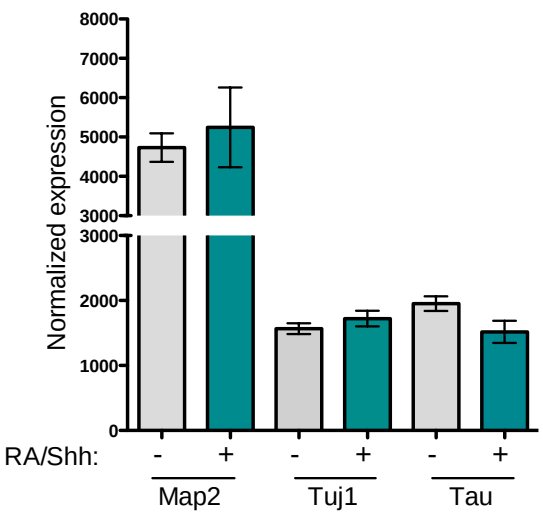
Directed differentiation of iPSCs to alternate neuronal fates

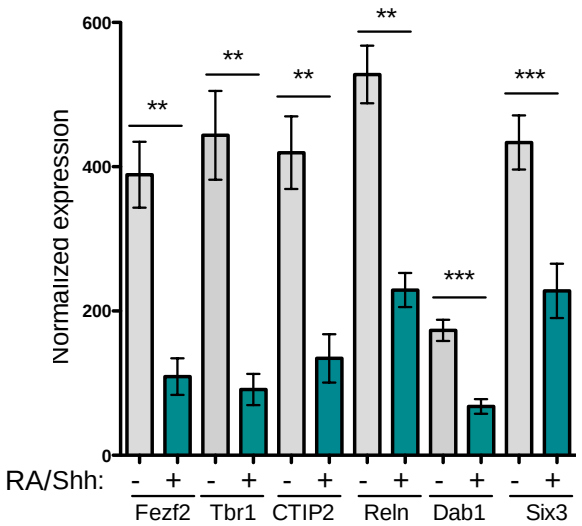


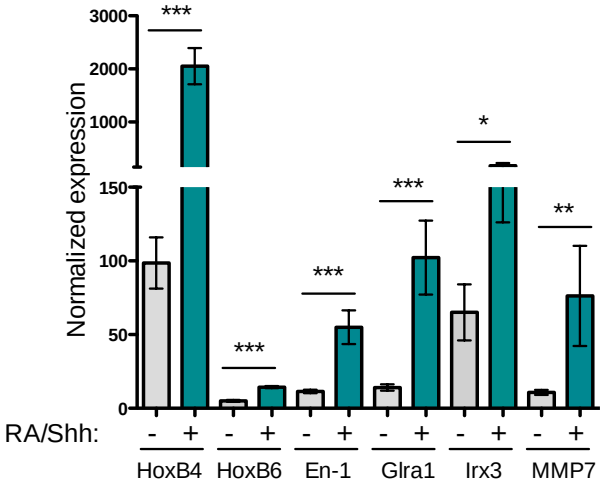


iPSCs directed to caudal neuronal fates show altered expression profiles

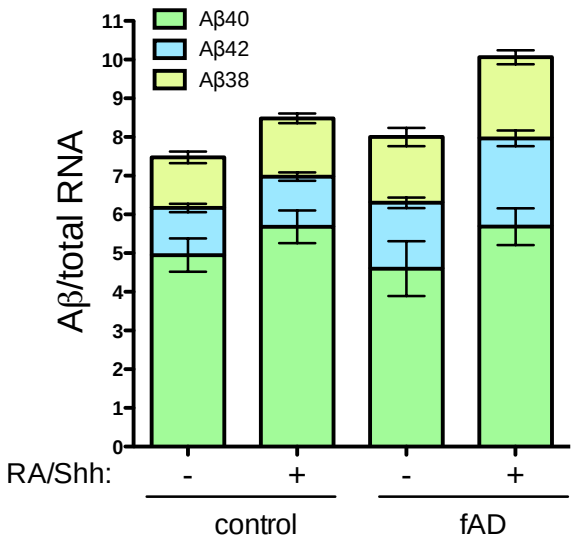


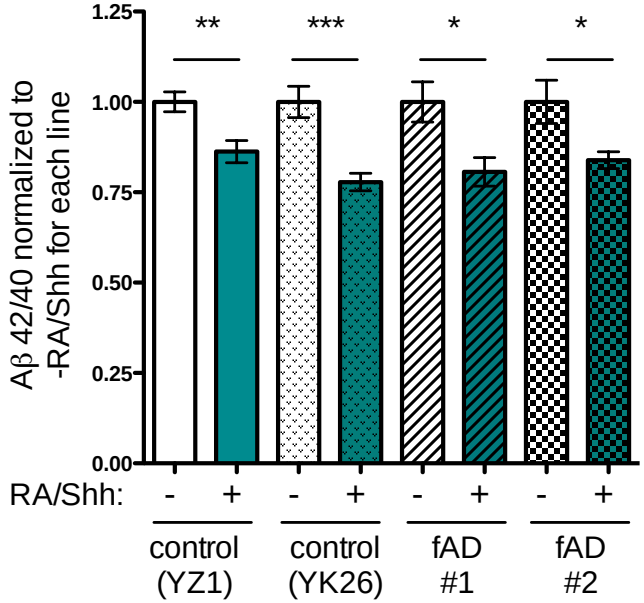


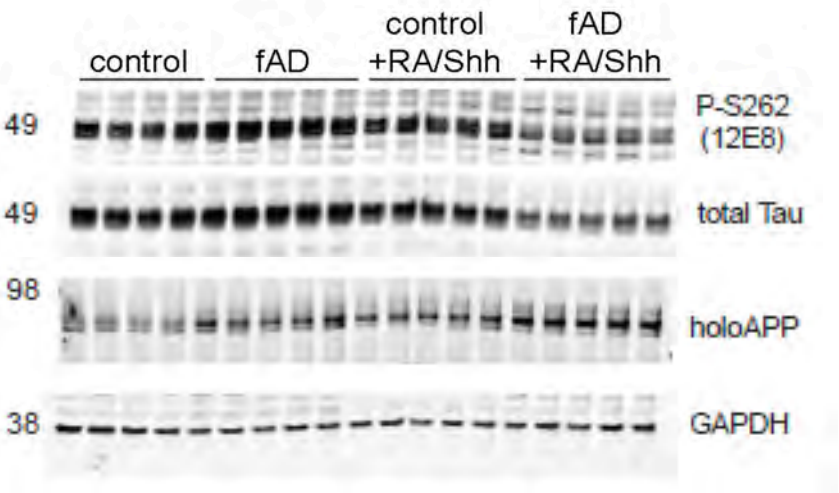


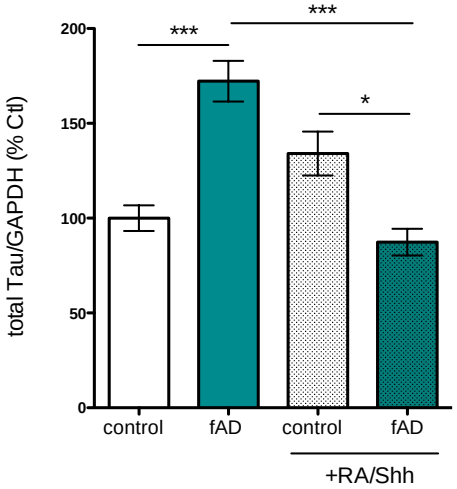


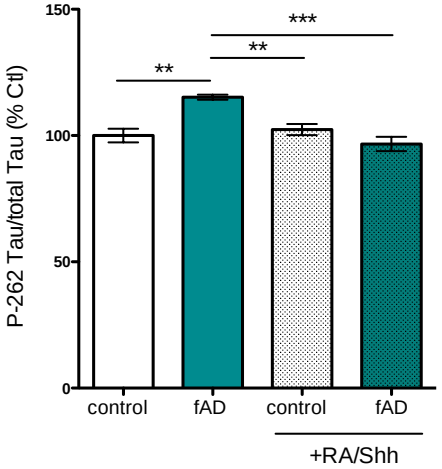
iPSCs directed to a caudal neuronal fate secrete a less toxic ratio of Aβ42/40



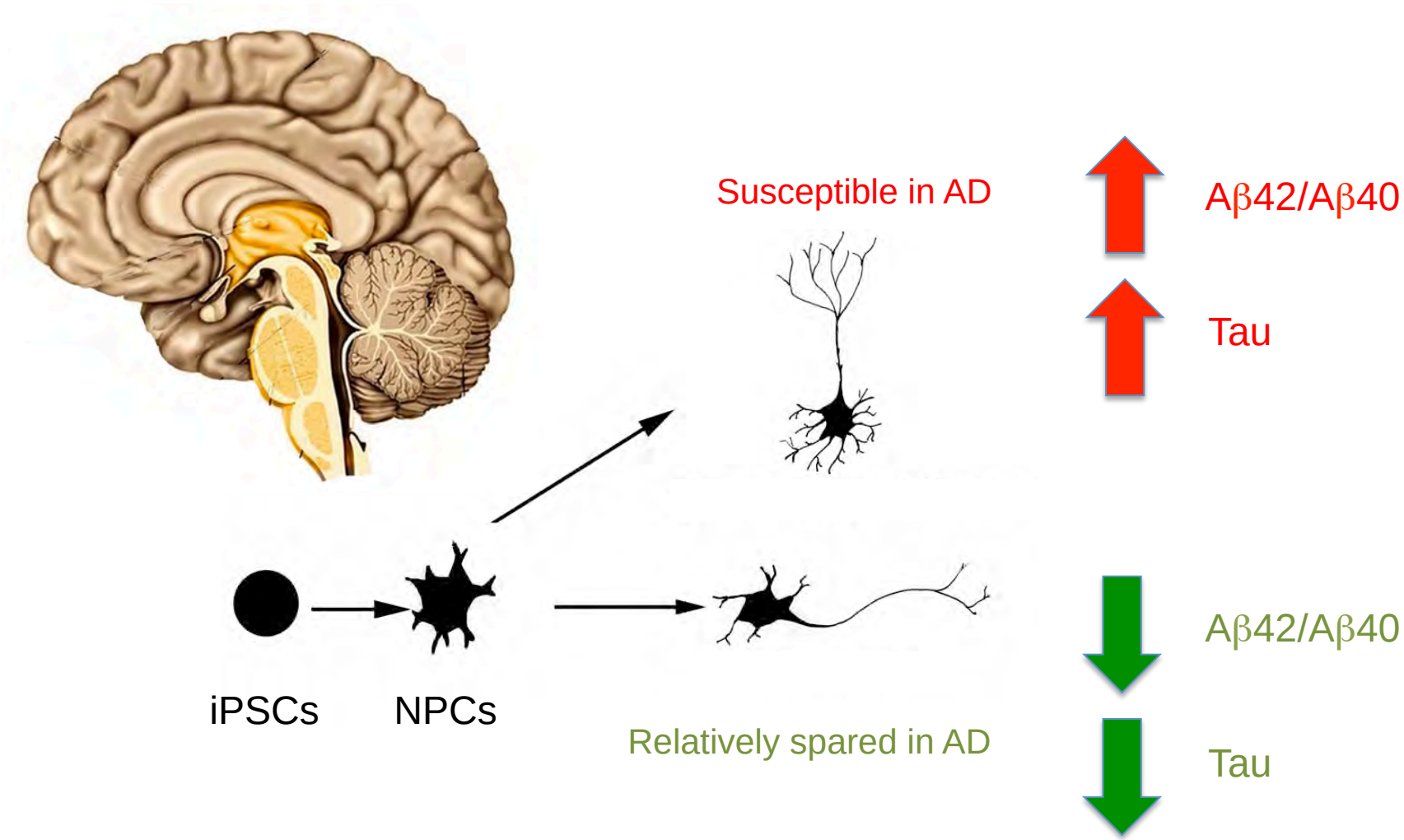




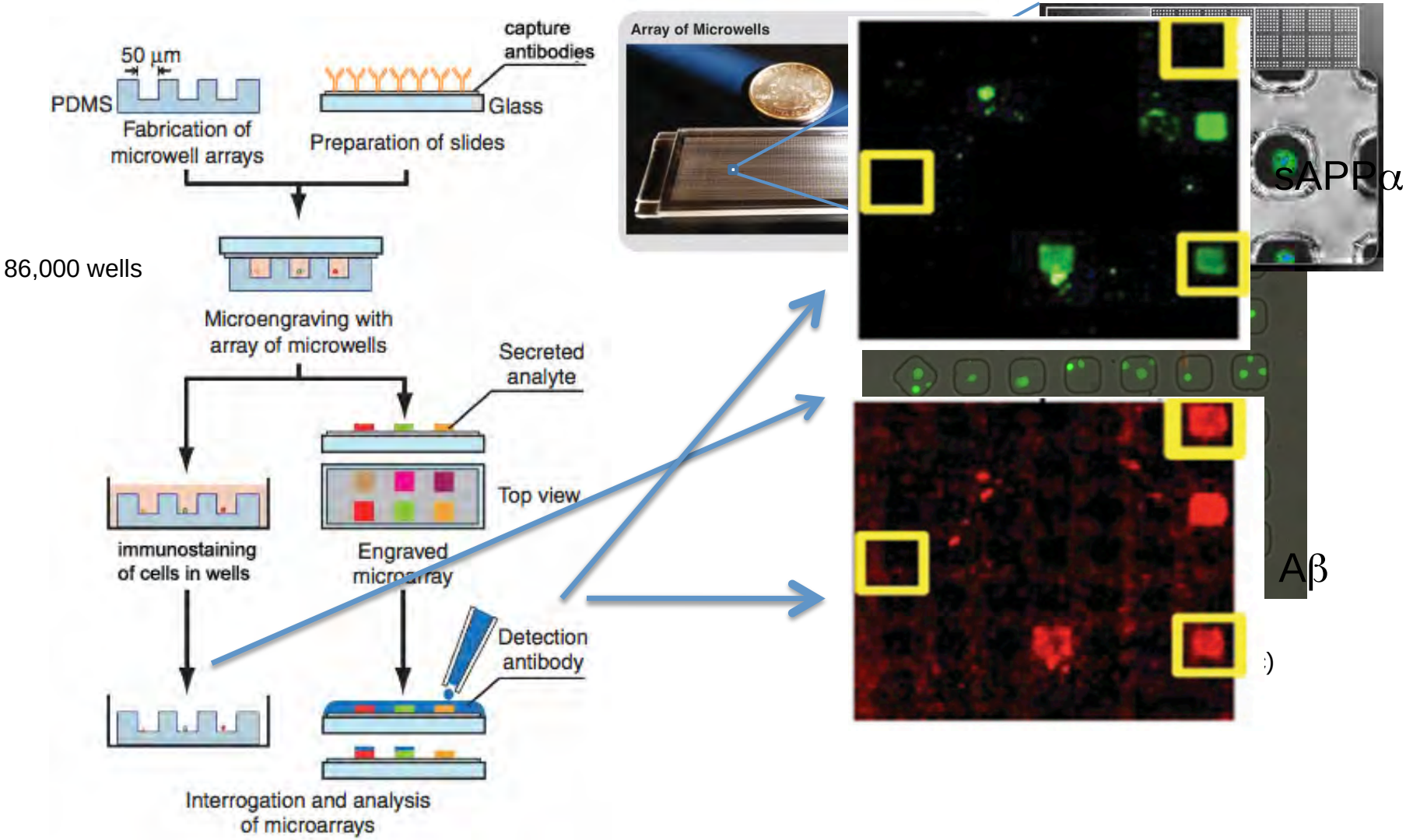




Neurons susceptible to degeneration in Alzheimer's secrete more toxic Aβ

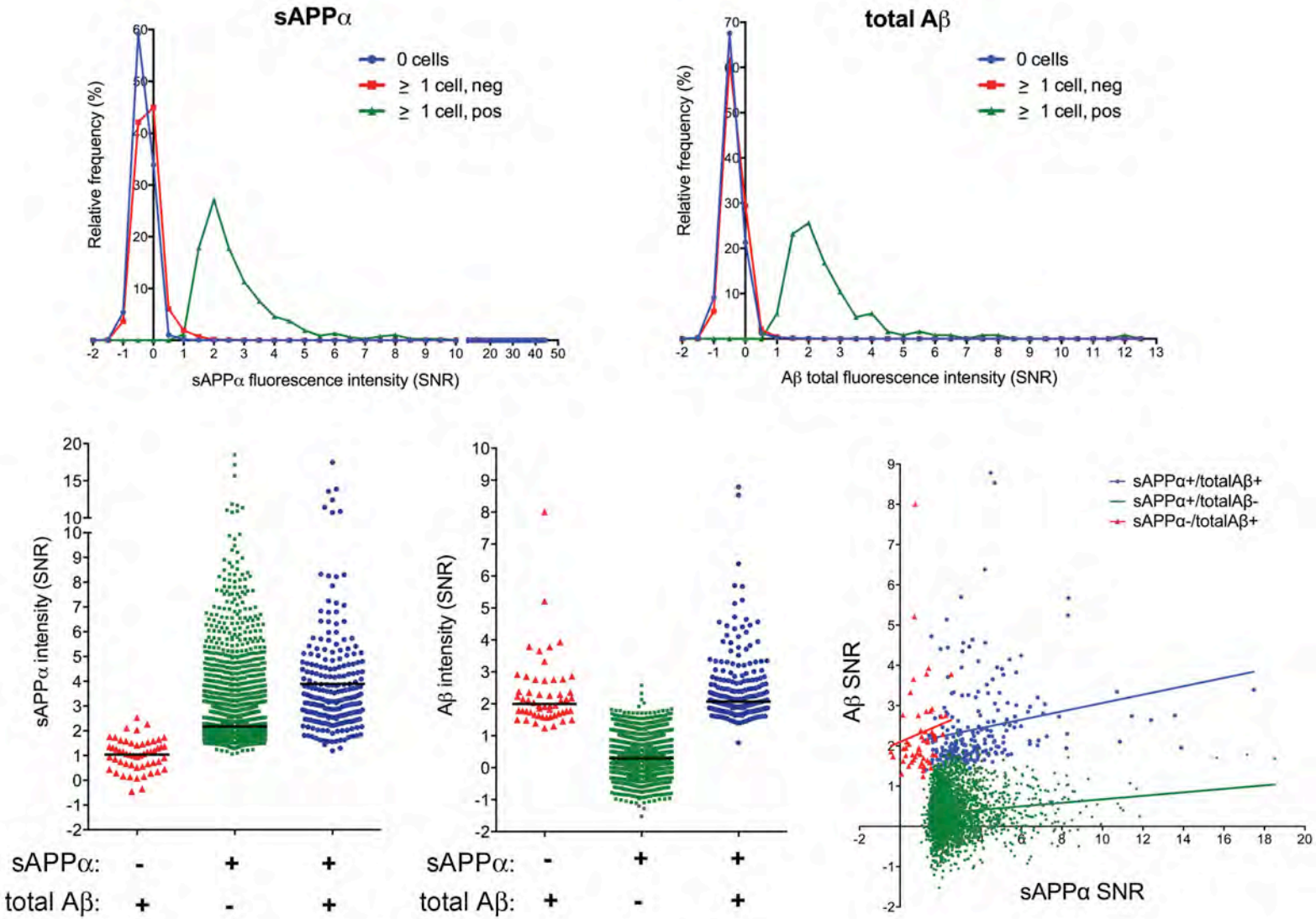


Measuring Aβ and sAPPα levels at the single cell level



Technology developed in JC Love lab (MIT) to study cytokine secretion

Current/Future directions: Measuring Aβ and sAPPα levels at the single cell level



Liao et al, unpublished

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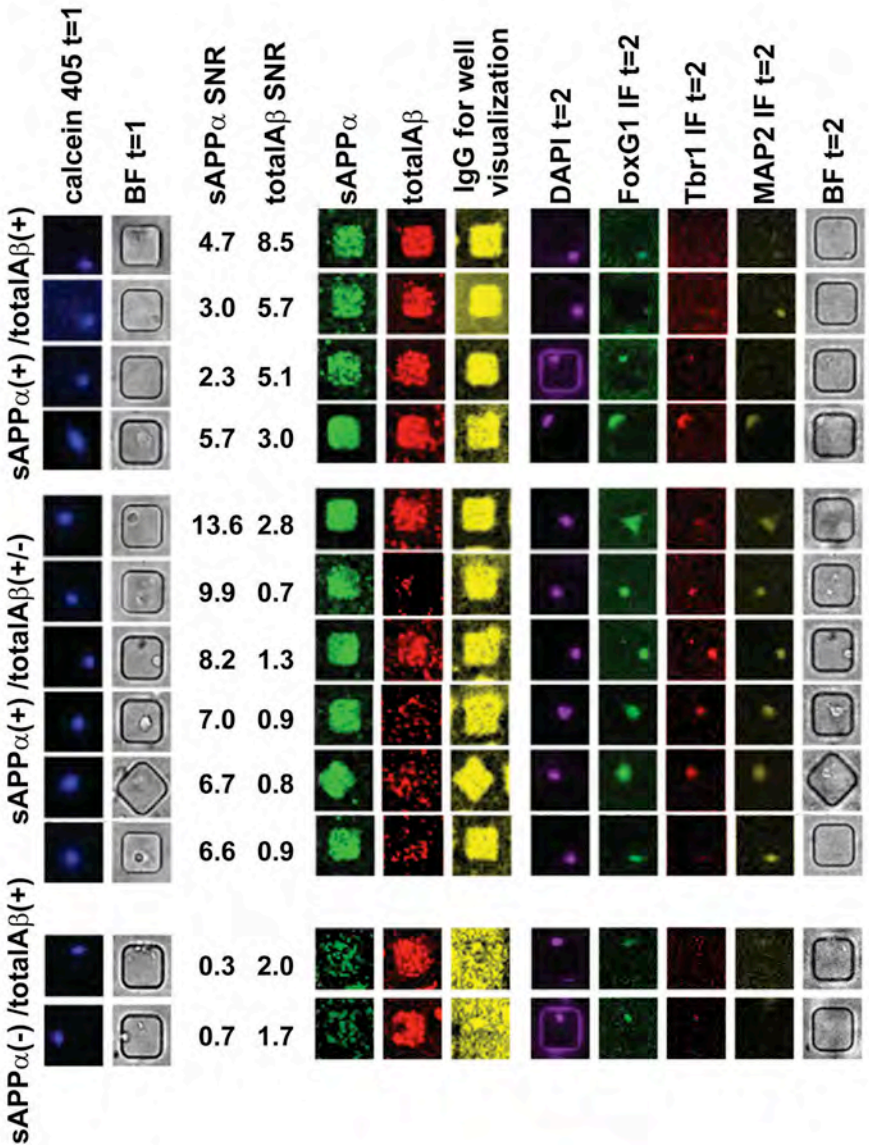

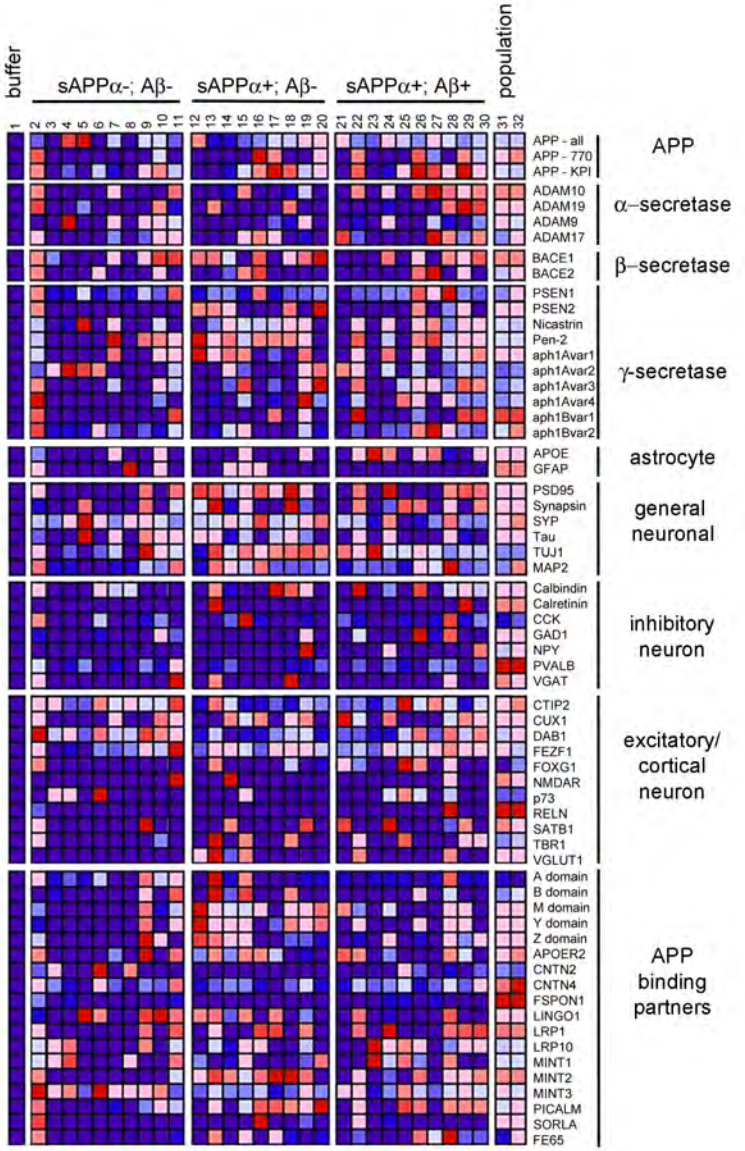

Liao et al, unpublished

Summary of studies of Alzheimer's disease using iPSCs

1. Generation of new stem cell (iPS) lines from patients with early-onset, familial Alzheimer's disease (fAD)

2. In all fates tested, the fAD APP V717I mutation leads to increased total Aβ, Aβ38 and Aβ42 generation

3. Aβ generation increases as stem cells differentiate to neuronal fates

4. Tau protein levels are increased in fAD neurons directed to a rostral but not caudal neuronal fate and Aβ−specific antibodies are able to rescue this phenotype

5. Aβ from fAD neurons stimulates GFAP expression in astrocytes

6. Directing differentiation to caudal fates versus rostral fates alters APP cleavage

7. Microengraving can be used to determine which cell fates secrete the highest levels of Aβ, and compare drug responsiveness in different cell types

Acknowledgments

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Taehwan Shin

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Dominic Walsh, PhD
Chris Love, PhD
Phil De Jager, MD
David Bennett, MD

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Harvard NeuroDiscovery Center