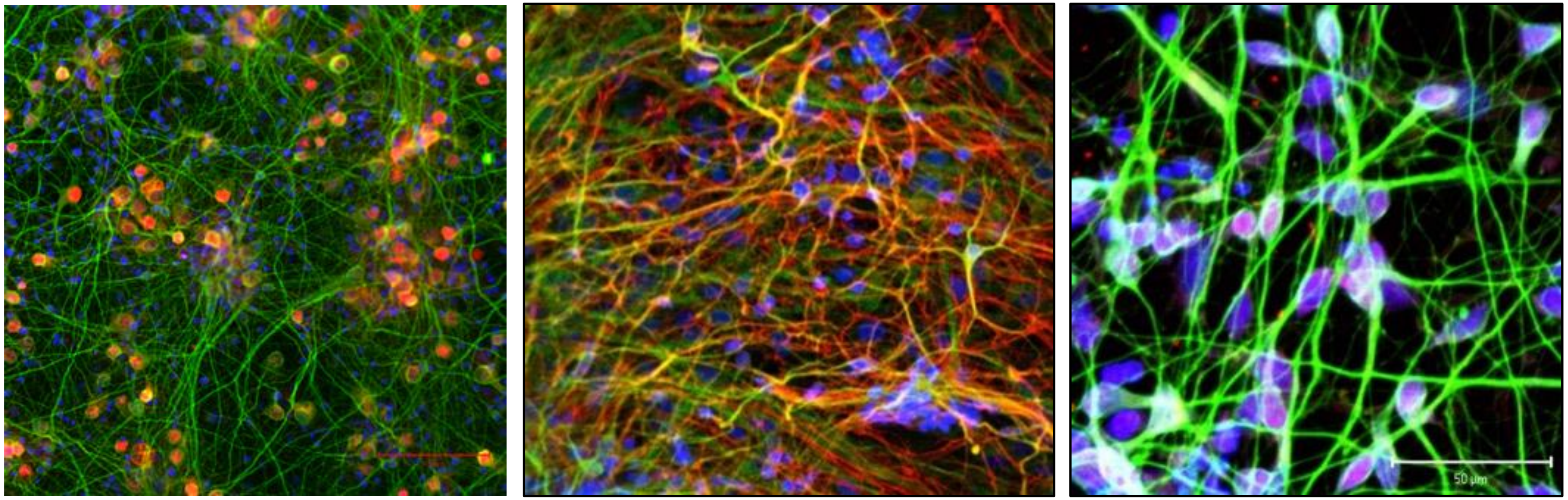
Using iPSCs to Interrogate Heterogeneity in AD and to Develop Assays for Experimental Validation of Novel Targets



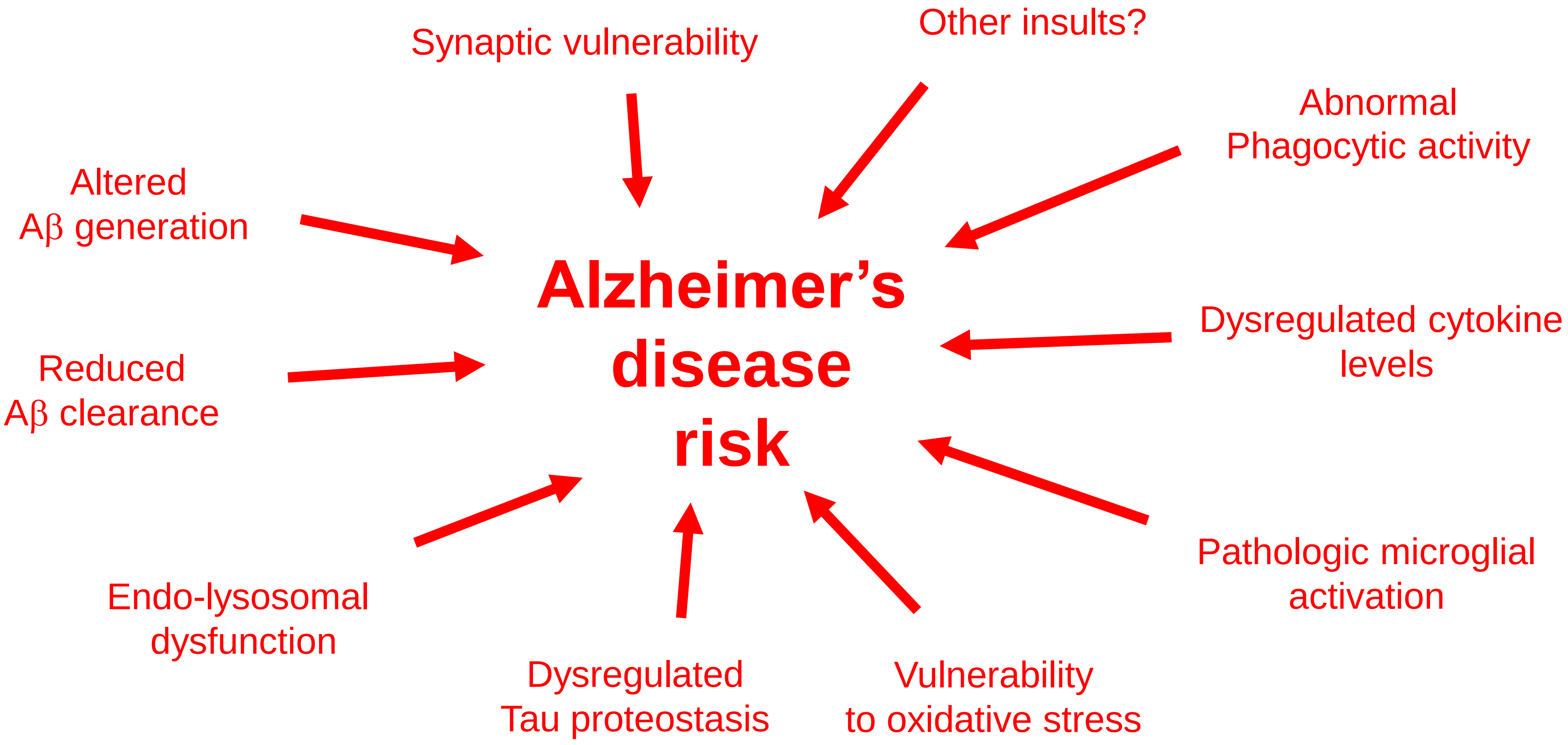
Tracy Young-Pearse, PhD

Associate Professor of Neurology

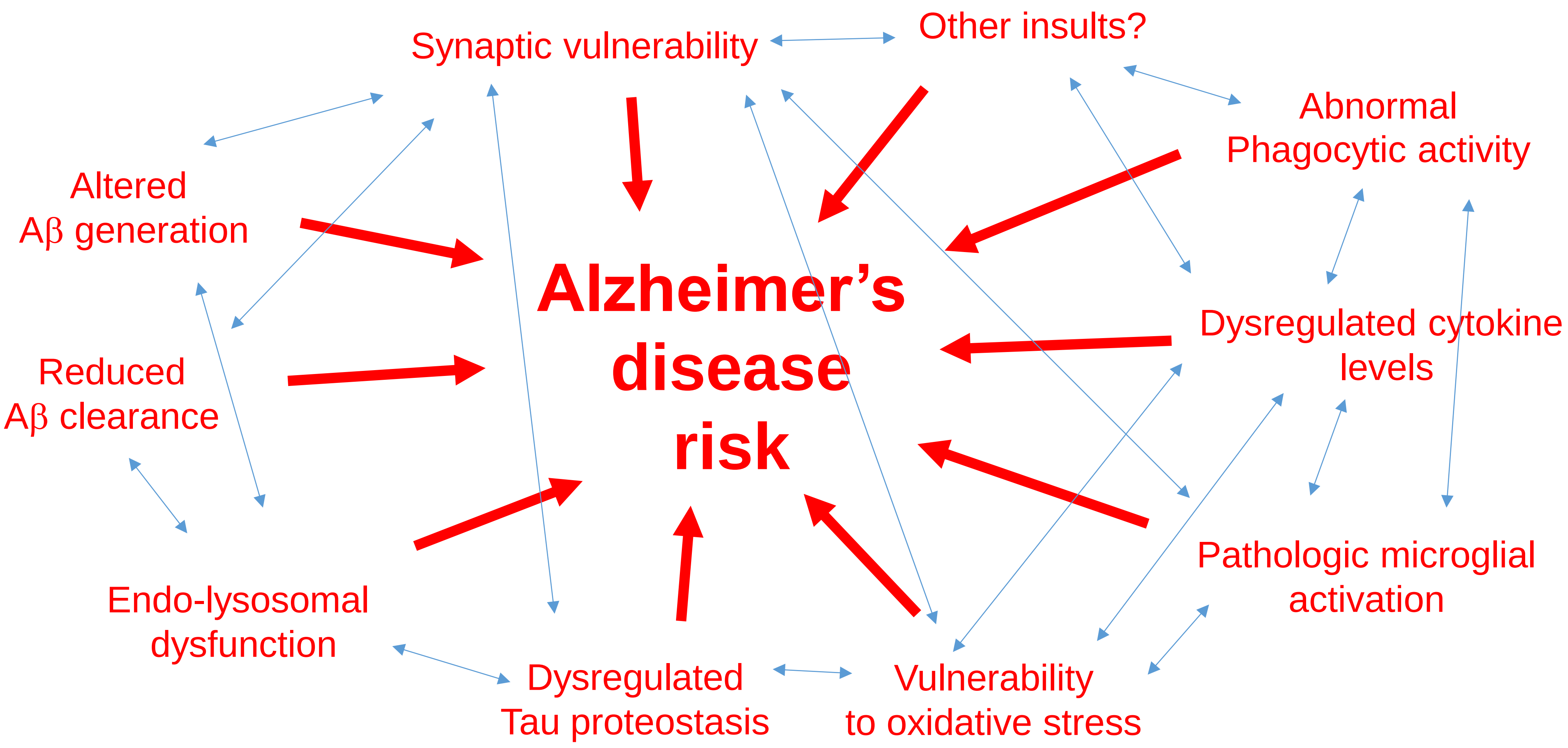
Ann Romney Center for Neurologic Diseases

Harvard Medical School and Brigham and Women’s Hospital

Hypothesis: *Alzheimer's disease has multifactorial etiologies that are in part genetically encoded*

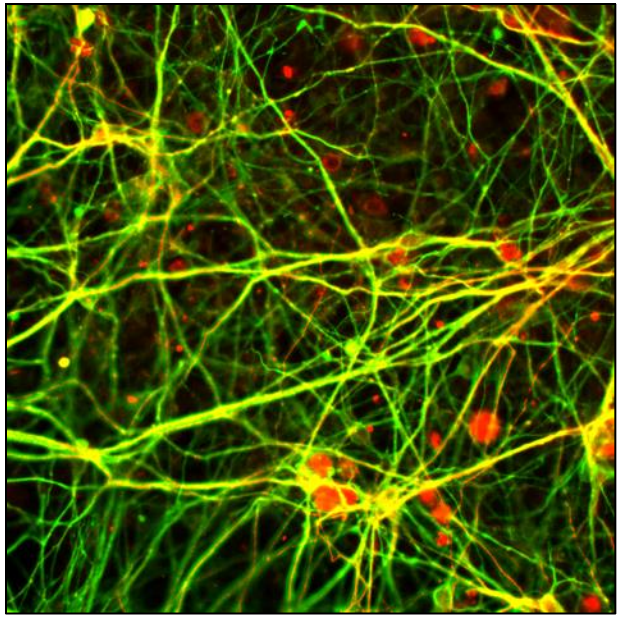


Hypothesis: *Alzheimer's disease has multifactorial etiologies that are in part genetically encoded*

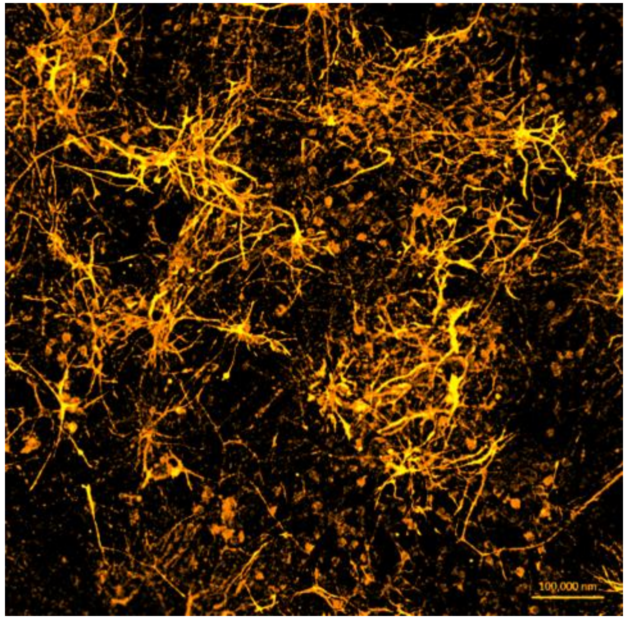


For each new pathway/target identified, which causal process are we trying to rescue?
In which experimental system/cell type(s) should we look?

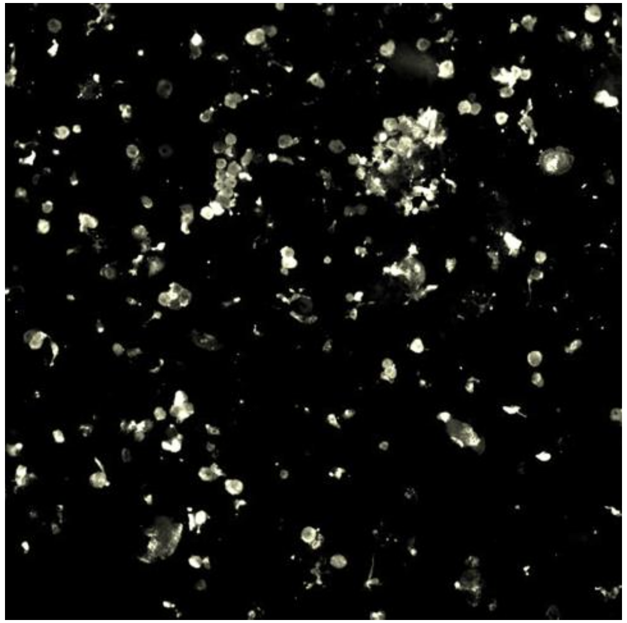
Neurons



Astrocytes



Microglia



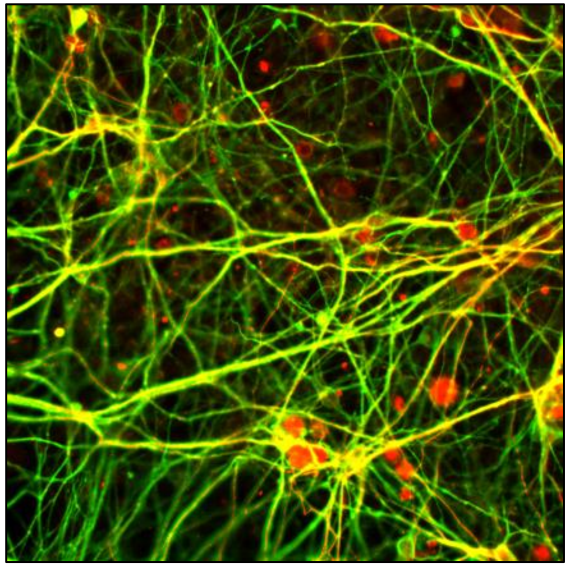
Oligodendrocytes?
Pericytes?
Monocytes/macrophages?
T-cells?

Resources for validating new targets and pathways

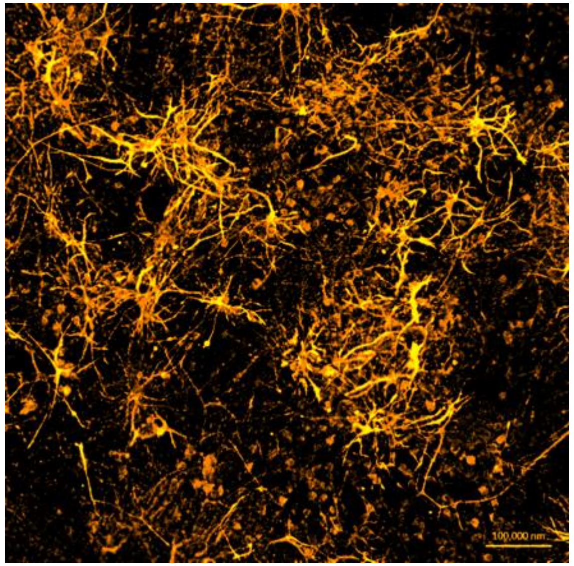
1. A collection of human iPSC lines from ROS and MAP cohorts

2. Cell-based assays

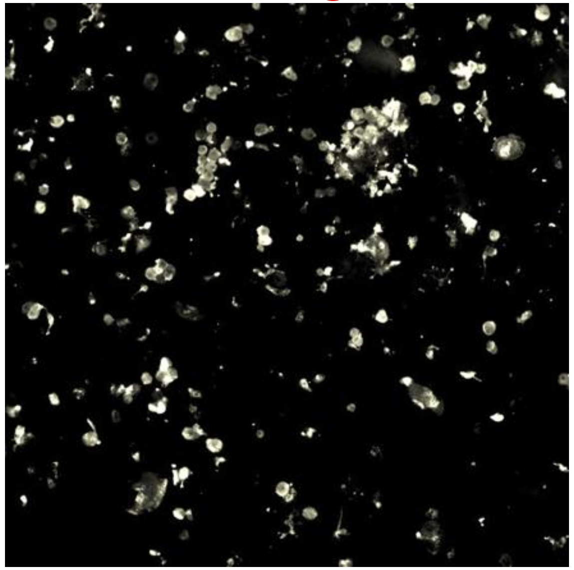
Neurons



Astrocytes



Microglia



Two large cohorts of deeply phenotyped aging Americans

Religious Orders Study (ROS)

- Catholic priests, nuns and brothers
- Started in 1994

Memory and Aging Project (MAP)

- Older men and women in assisted living facilities in the Chicagoland area
- Started in 1997

RUSH UNIVERSITY MEDICAL CENTER

- Free of dementia at enrollment
- Annual clinical evaluations
- Anatomical Gift Act, donate brains (spinal cords, select nerves and muscles at death)
- 3,087 persons enrolled (July 2018)
- 1,481 autopsies (July 2018)
- Follow-up rate among survivors >90%
- Autopsy rate among deceased >90%


PI: David Bennett

Available data from ROS/MAP cohorts

Longitudinal Clinical Data

Cognitive function
19 tests annually

Clinical and pathological diagnoses for AD and other neurological diseases

Functional evaluation
UPDRS
Neurological exam

Neuroimaging
MRI
fMRI
Post-mortem MRI

Biometric data
Circadian rhythm

Other Medical information

Diabetes
Cardiovascular disease
Hypertension
BMI
Diet
Smoking
Drinking
Cancer
Thyroid disease

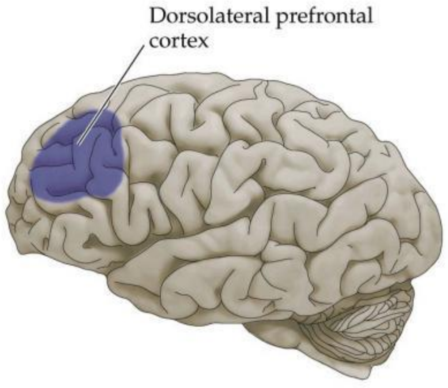
Genotyping

Peripheral blood

Affy 6.0 1000G imputation
APOE status
Genome sequencing

Cell and molecular phenotyping postmortem

Quantitative neuropathology
Amyloid plaques, tangles, Lewy bodies, TDP43 inclusions, vascular pathology



DNA Methylation
Illumina 450K

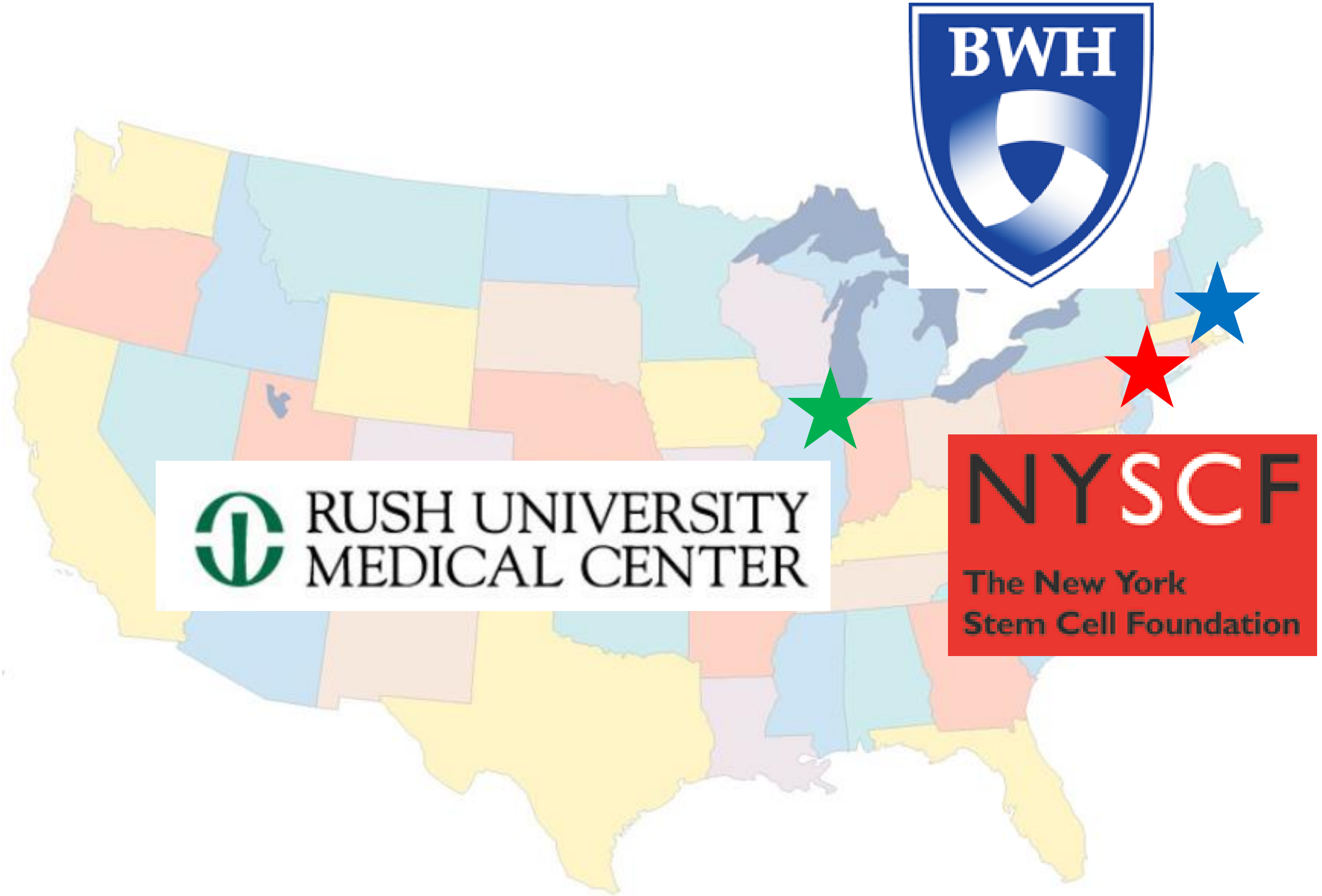
Histone Acetylation
H3K9Ac ChIP Seq

miRNA profile
Nanostring codeset

RNA profile
miRNA & RNAseq

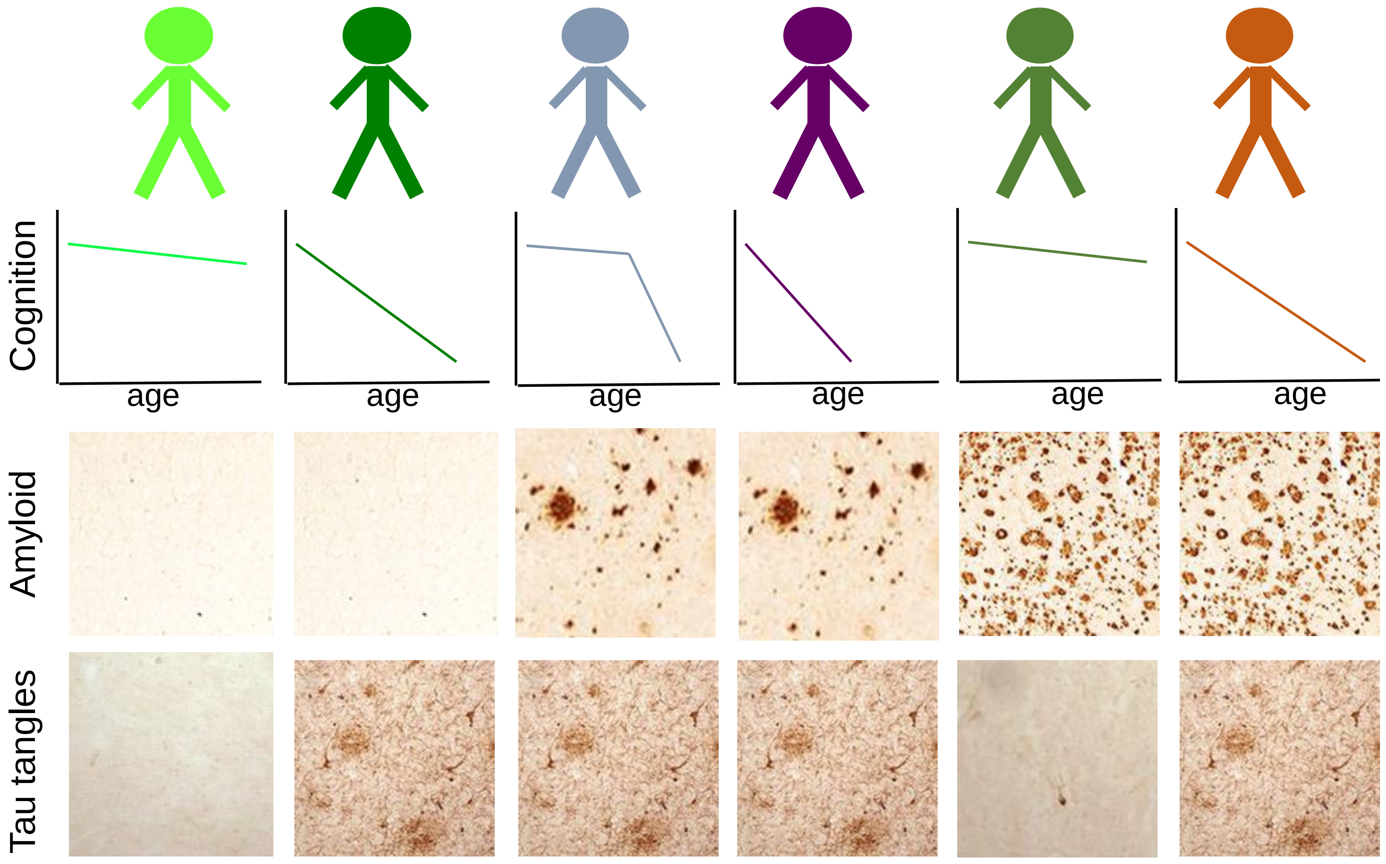
Mass spec profile
Lipids, proteins

Generating iPSC lines from ROS and MAP



- Polyclonal iPSC lines generated from 50 participants using Sendai on PBMCs
- 50 lines generated

A spectrum of cognitive ability and pathology in aging humans



pathology images from Kullmann, Brain, 2013

Inclusion/Exclusion criteria (round 1: 50 participants)

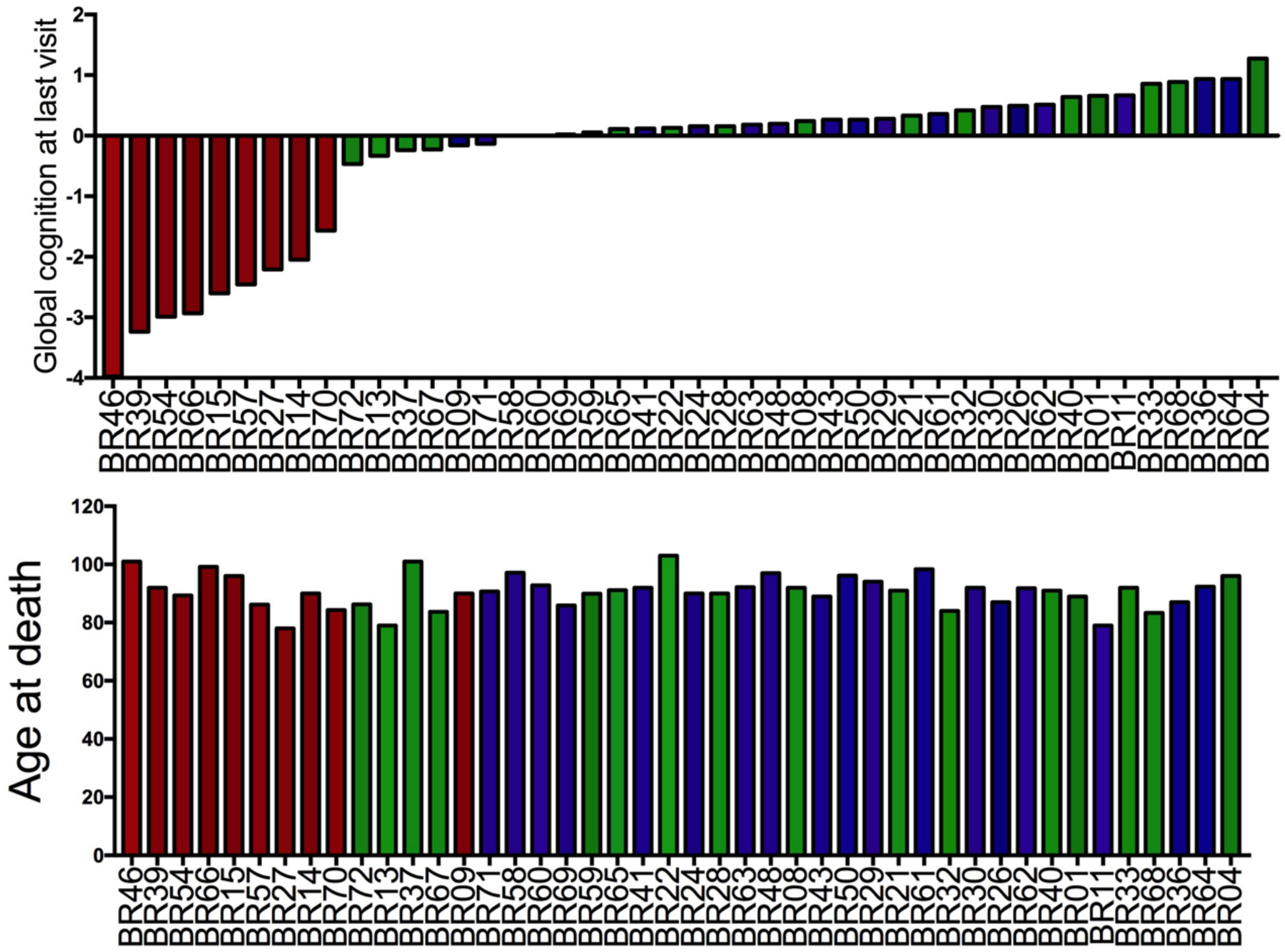
- Older age of death
- No hippocampal sclerosis
- No/minimal TDP43 inclusions
- No/minimal Lewy body pathology
- Low/minimal signs of macro and micro infarcts

Capturing heterogeneity in brain aging with ROS and MAP cohorts

Low AD path, not cognitively impaired

High AD path, not cognitively impaired

High AD path, Alzheimer's disease

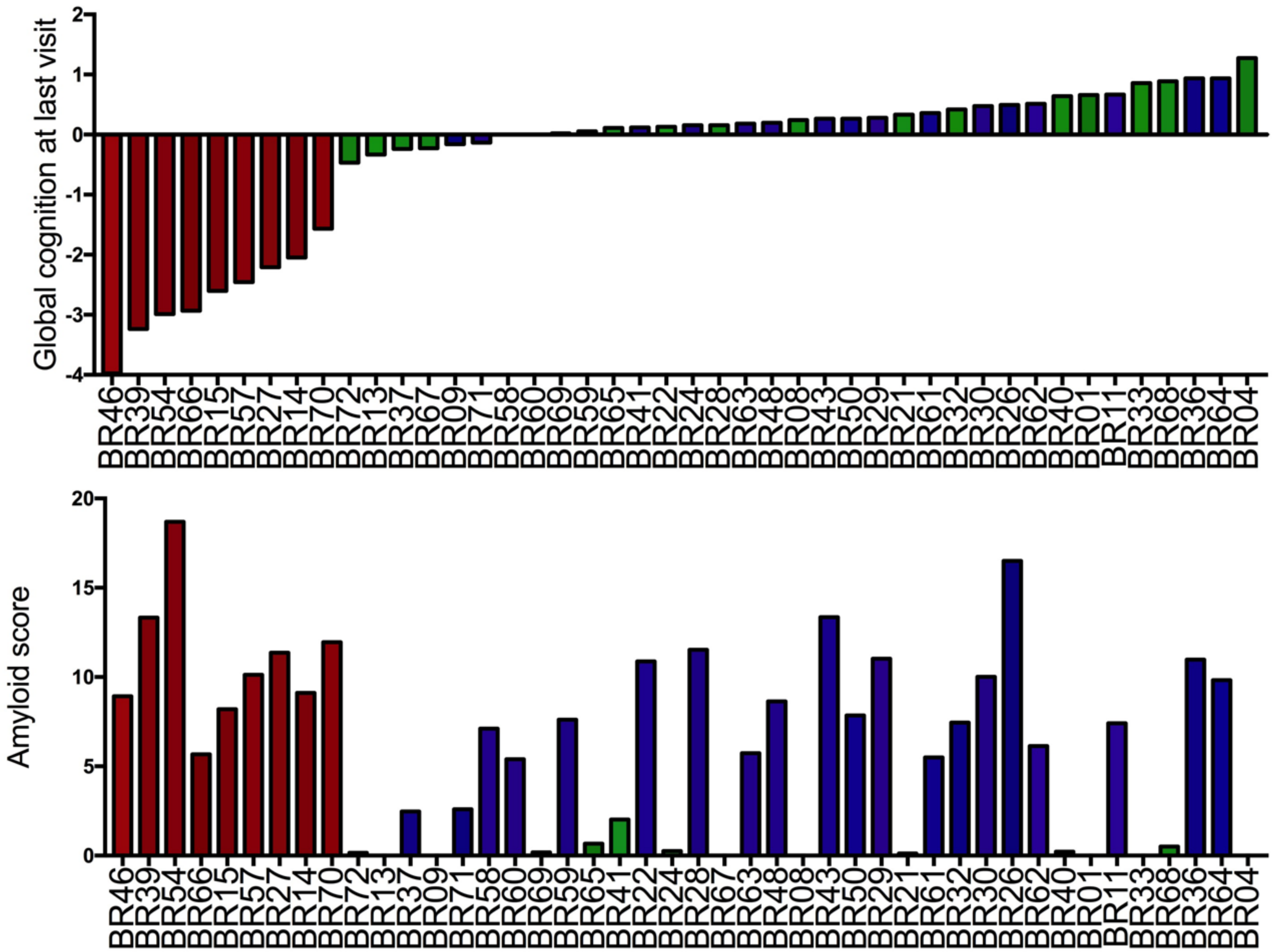


Capturing heterogeneity in brain aging with ROS and MAP cohorts

Low AD path, not cognitively impaired

High AD path, not cognitively impaired

High AD path, Alzheimer's disease

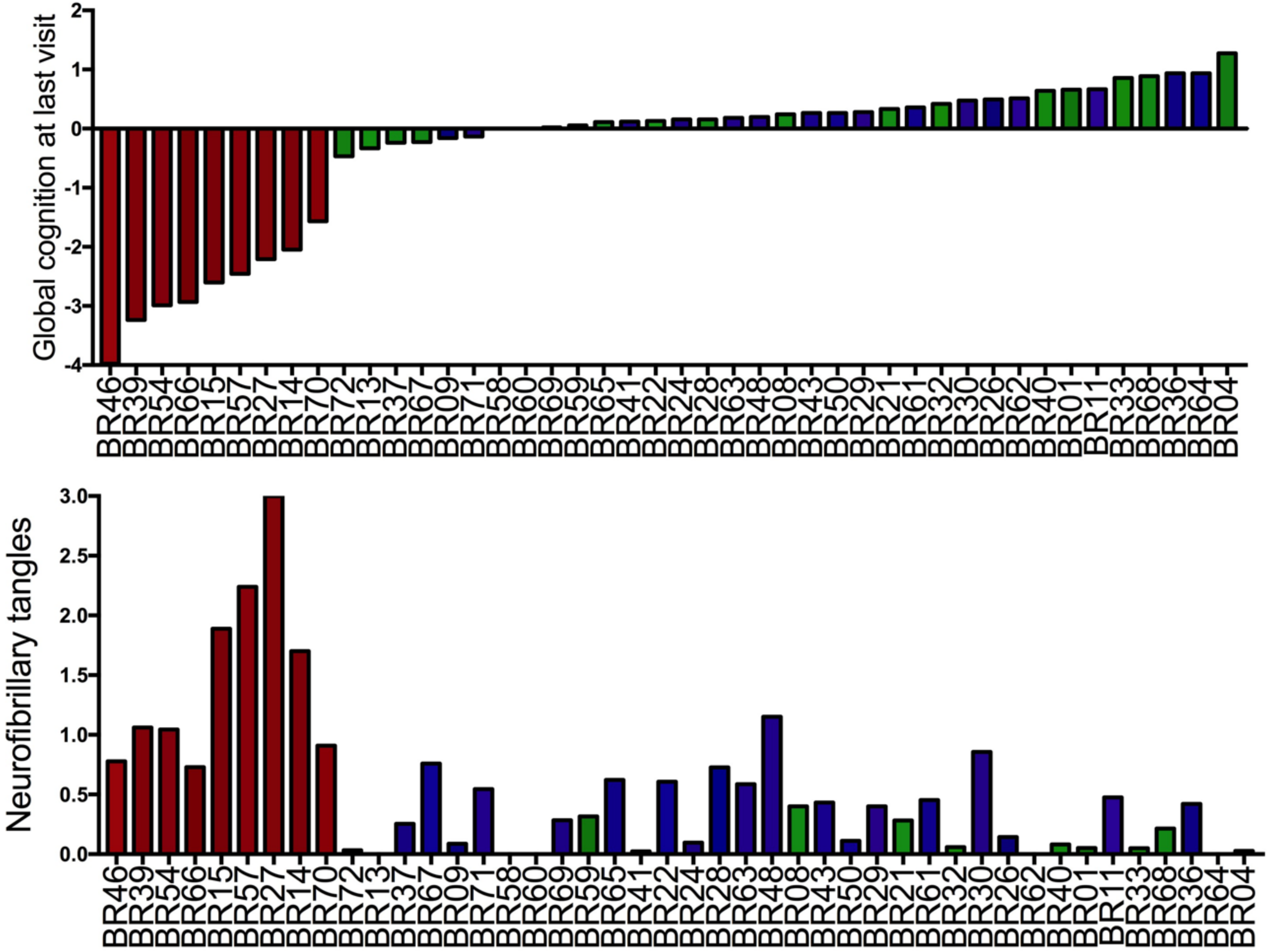


Capturing heterogeneity in brain aging with ROS and MAP cohorts

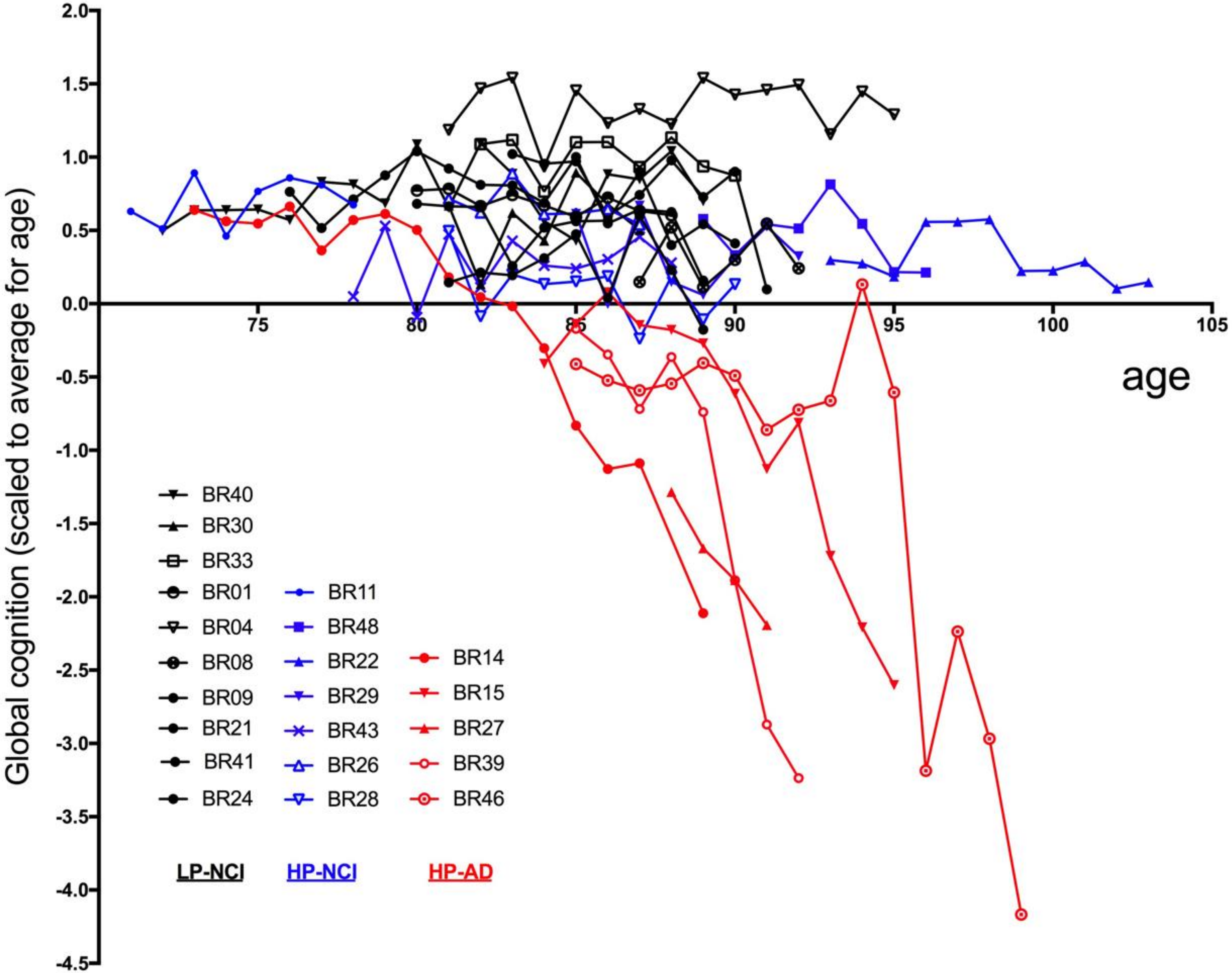
Low AD path, not cognitively impaired

High AD path, not cognitively impaired

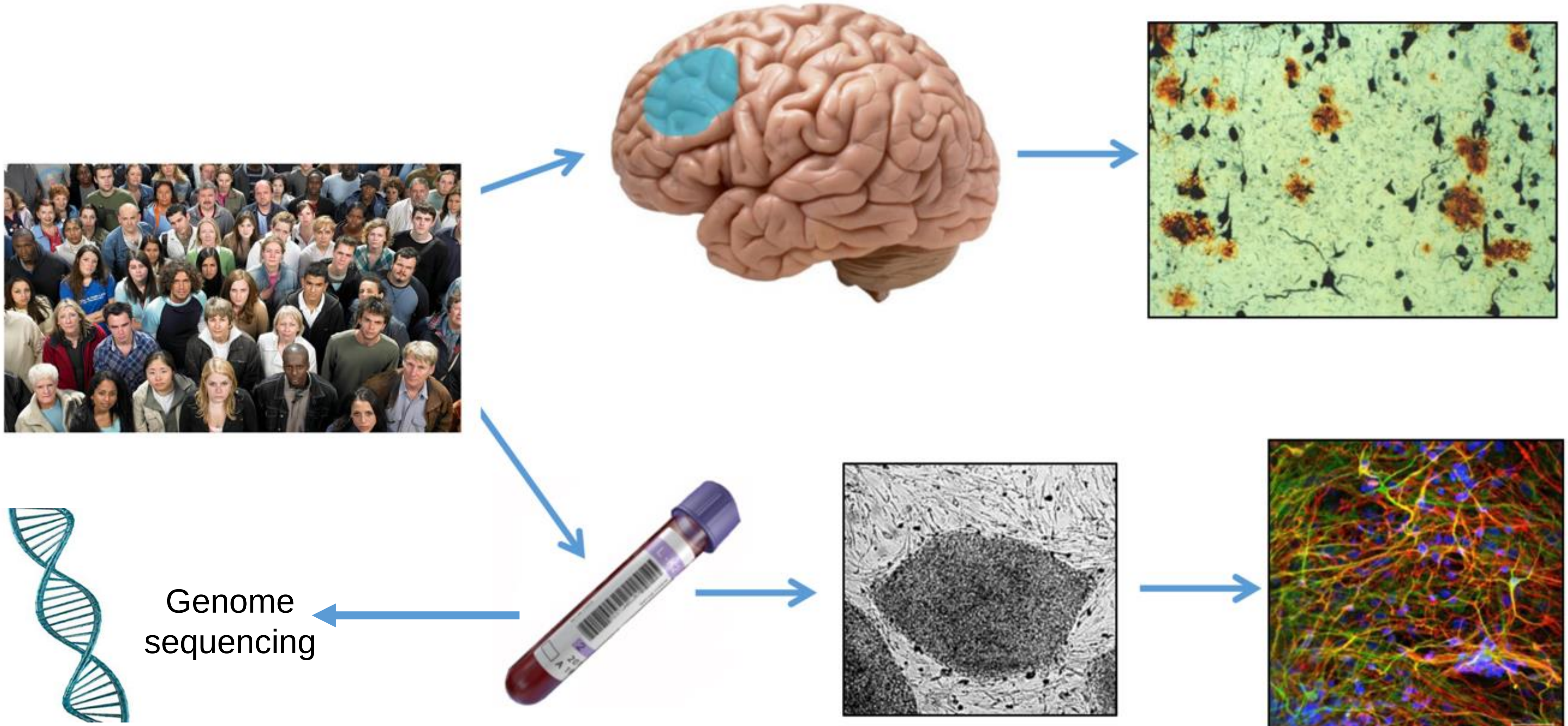
High AD path, Alzheimer's disease

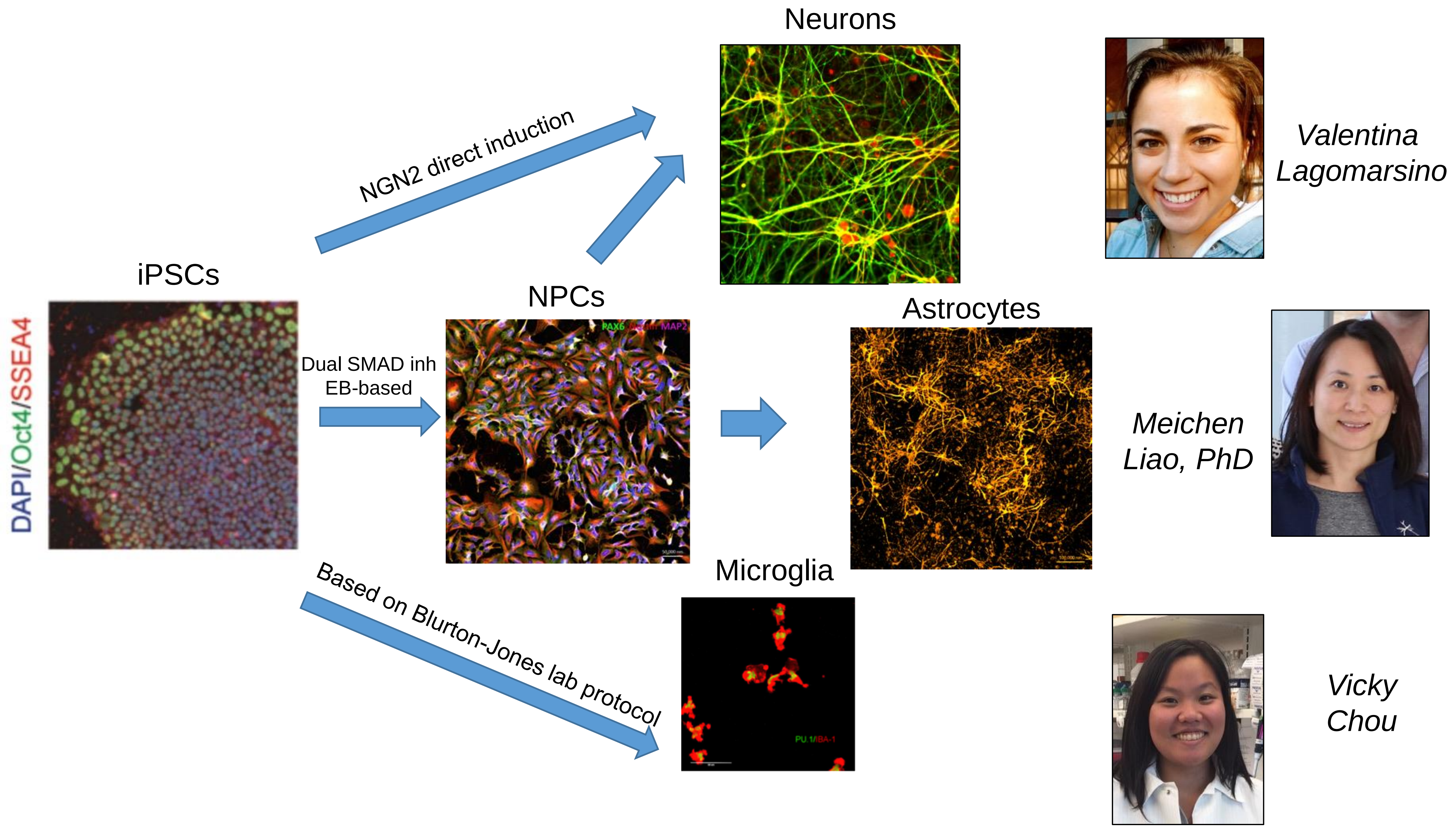


Global cognitive status over time in the participants represented in the first 21 lines



Can iPSC-derived cells predict late-onset "sporadic" Alzheimer's disease: onset, progression, subtyping and treatment responsiveness?

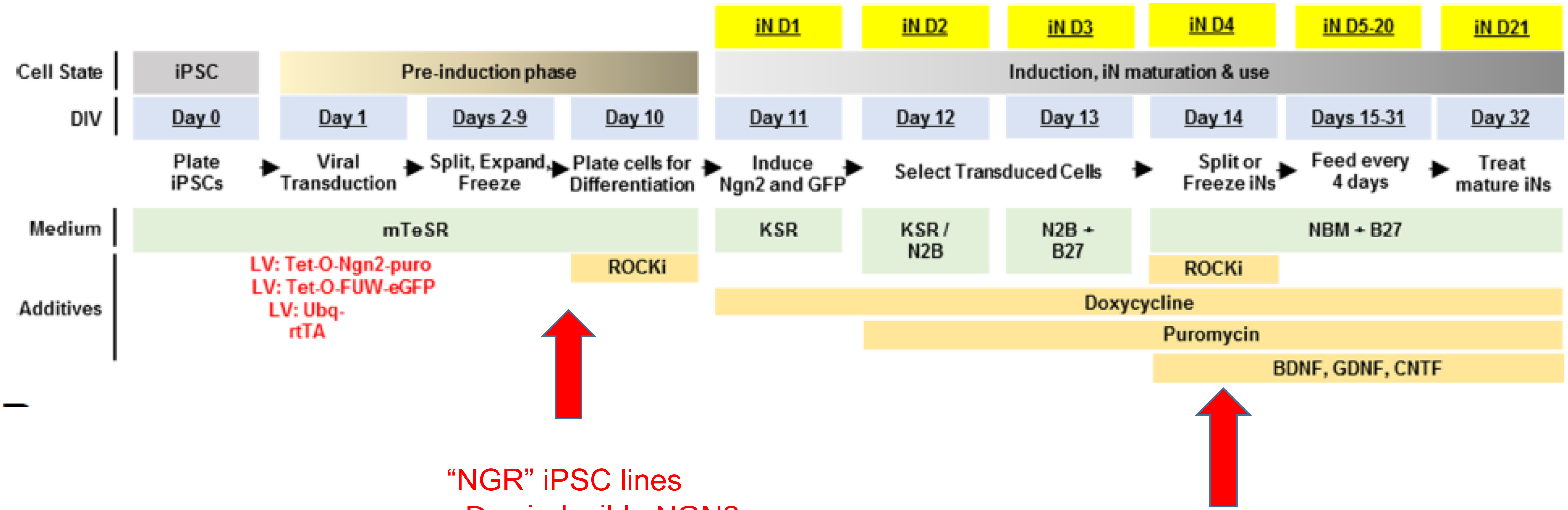



Neurons
NGN2 direct induction
iPSCs
DAPI/Oct4/SSEA4
NPCs
Dual SMAD inh
EB-based
Astrocytes
Microglia
Based on Blurton-Jones lab protocol
PU.1/IBA-1
Valentina Lagomarsino
Meichen Liao, PhD
Vicky Chou

Rapid Single-Step Induction of Functional Neurons from Human Pluripotent Stem Cells

Yingsha Zhang,[1] ChangHui Pak,[1,6] Yan Han,[1,6] Henrik Ahlenius,[3,4] Zhenjie Zhang,[5] Soham Chanda,[1,3,4] Samuele Marro,[3,4] Christopher Patzke,[1] Claudio Acuna,[1] Jason Covy,[1] Wei Xu,[1,2] Nan Yang,[3,4] Tamas Danko,[1,3] Lu Chen,[5] Marius Wernig,[3,4] and Thomas C. Südhof[1,2,5,*]

** Modified by Eggan lab, with minor modifications from TYP lab*



"NGR" iPSC lines
- Dox inducible NGN2

D4 iNs

Generating and banking stem cells, neurons, and glia from ROS and MAP iPSCs

iPSC
Lines
50

NGR iPSCs:
NGN2 inducible with Dox addition
43

DAY 4 INs:
Four days post dox
43

NPCs:
Neural progenitor cells
33

Astrocytes
33

Microglia
3

Resources for validating new targets and pathways

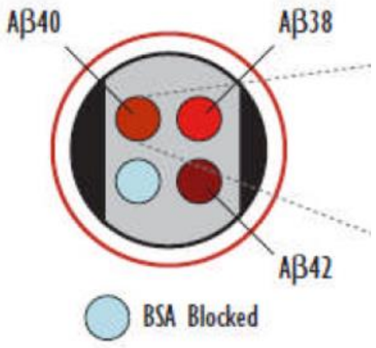
1. A collection of human iPSC lines from ROS and MAP cohorts

2. **Cell-based assays**

Assays developed for human induced neurons from ROS/MAP subjects

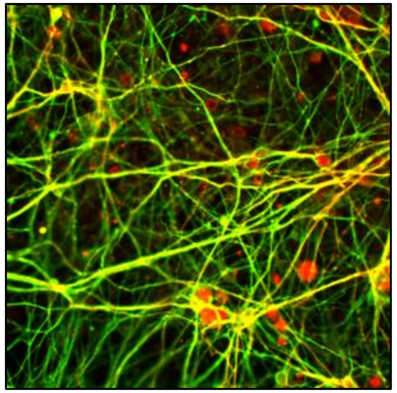
APP processing

Aβ42
Aβ40
Aβ37
Aβ38
Aβ43
Oligomeric Aβ
sAPPβ
sAPPa
APP holoprotein
CTFs



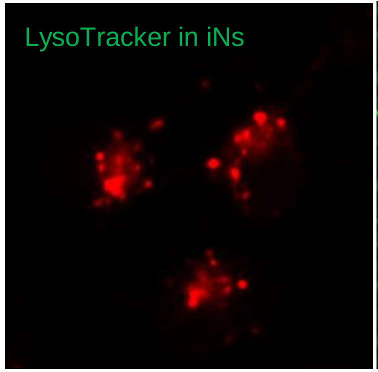
Tau levels

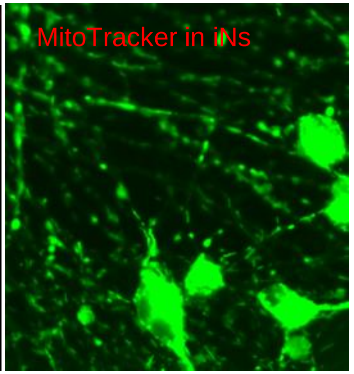
pS202/T205 Tau
p231 Tau
P181 Tau
Tau full length
Tau N-term
Tau C-term



Imaging-based assays

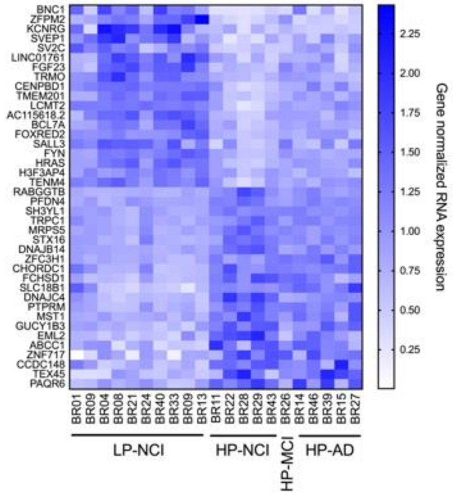
Lysosome size, number, localization
Mitochondria size, number, localization
Endosome size, number, localization
Synaptic puncta number
Oxidative stress





-Omics

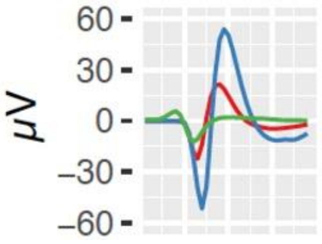
RNAseq
Proteomics



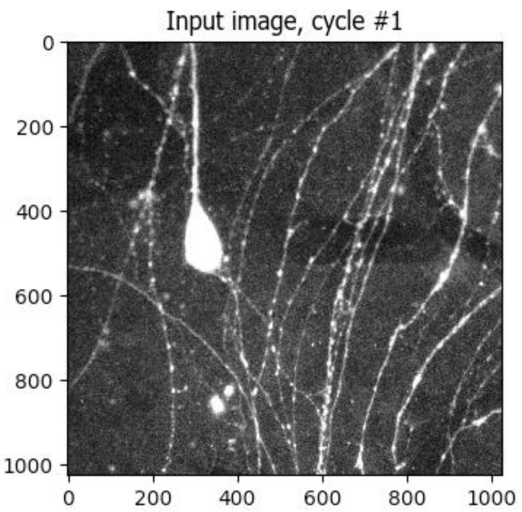
Longitudinal

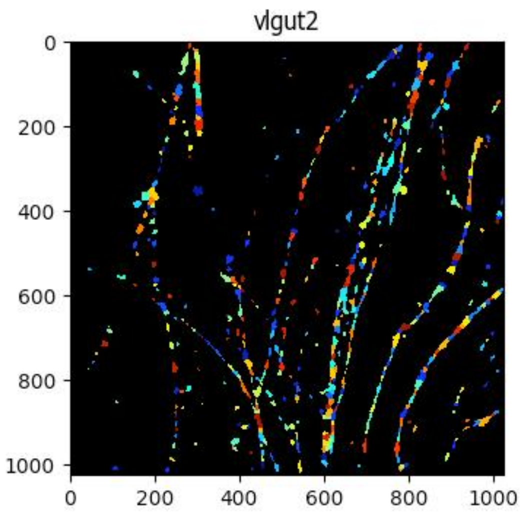
Incucyte

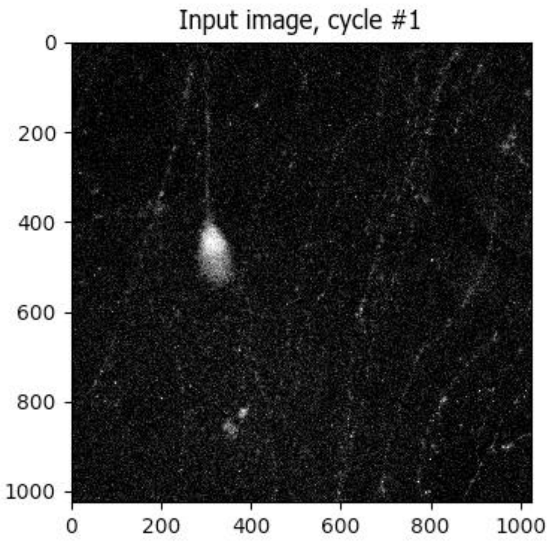
MEA

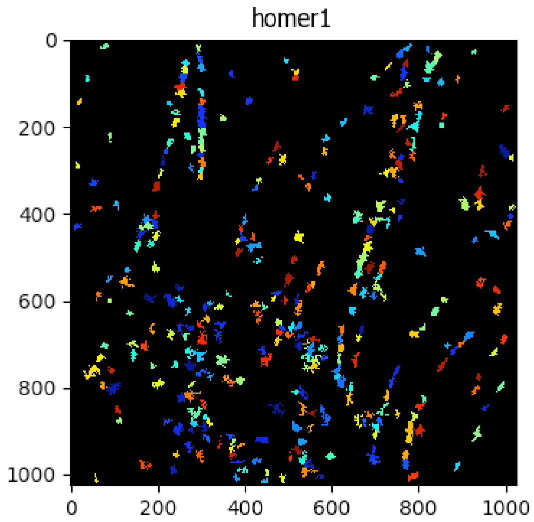


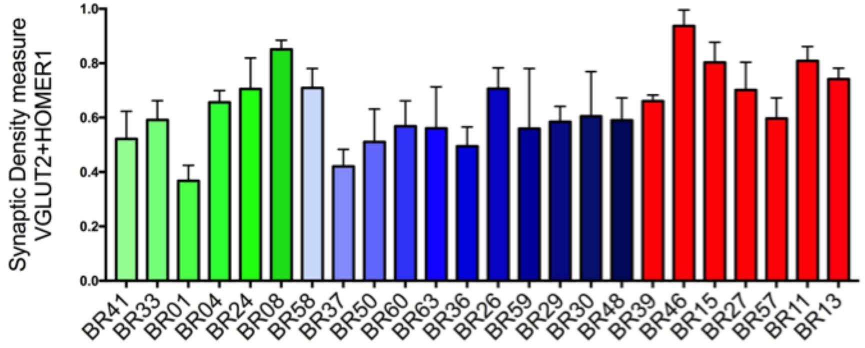
Measuring synaptic puncta number in human iNs



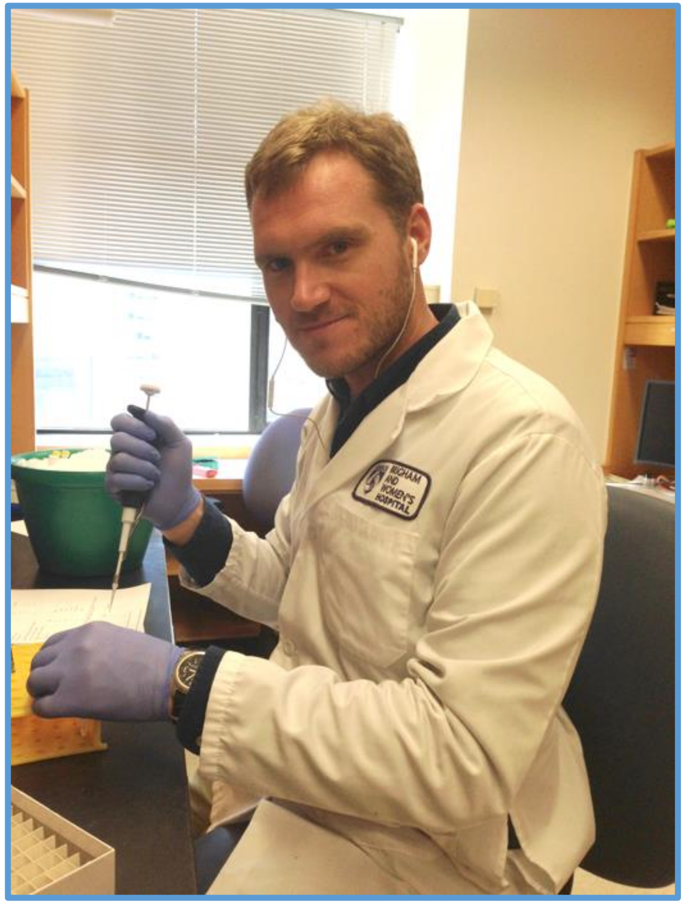




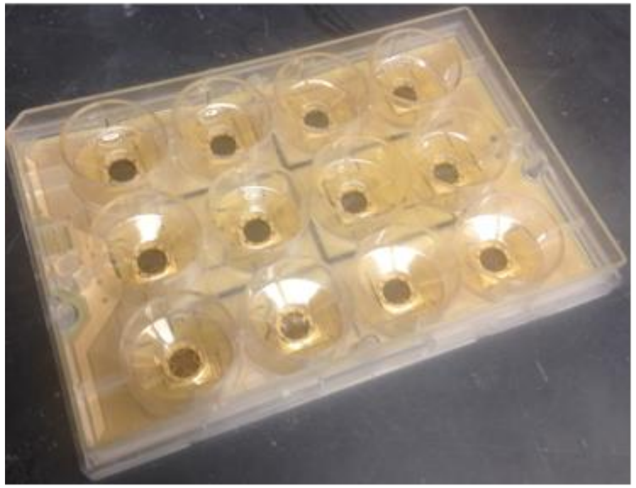


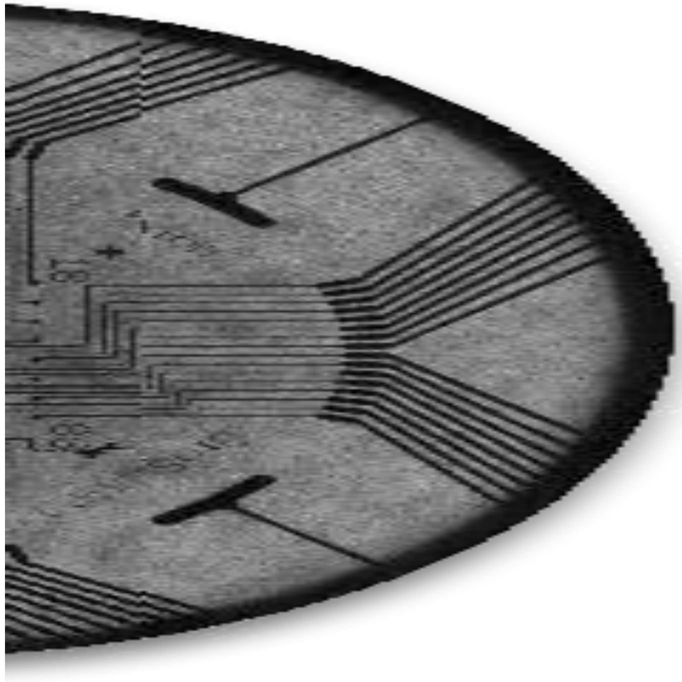


Developing an assay for measuring neuronal activity



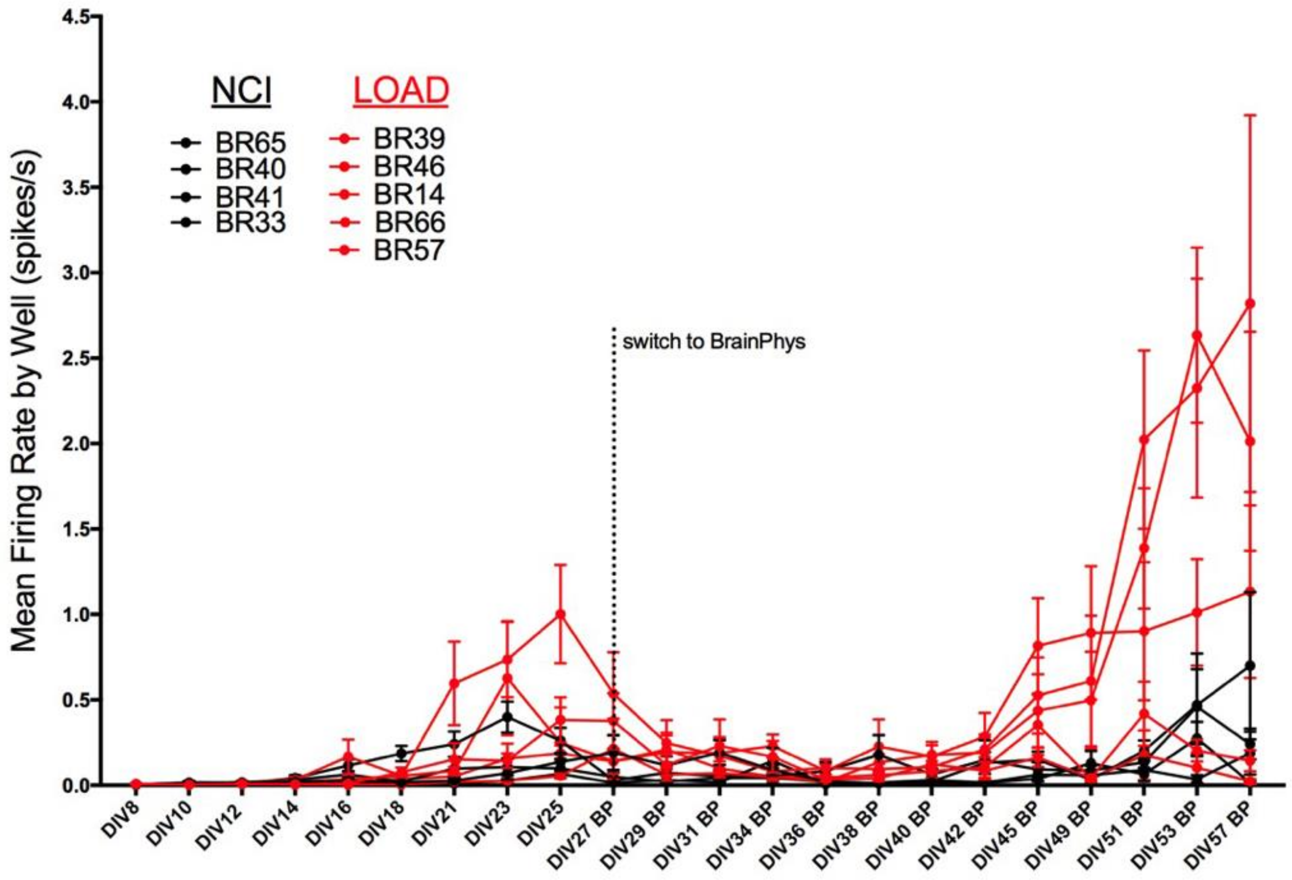
Joe Negri, PhD candidate



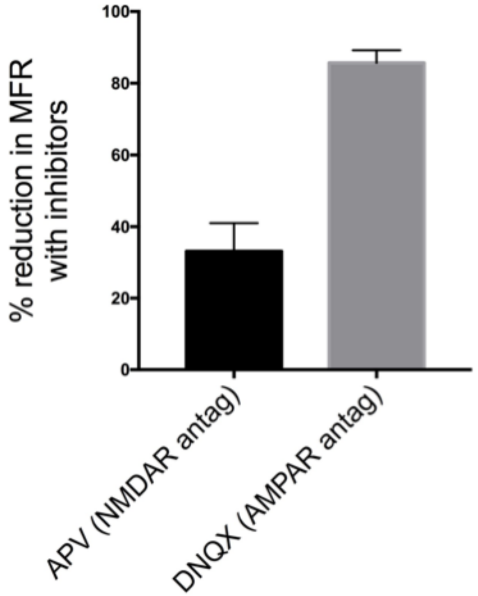


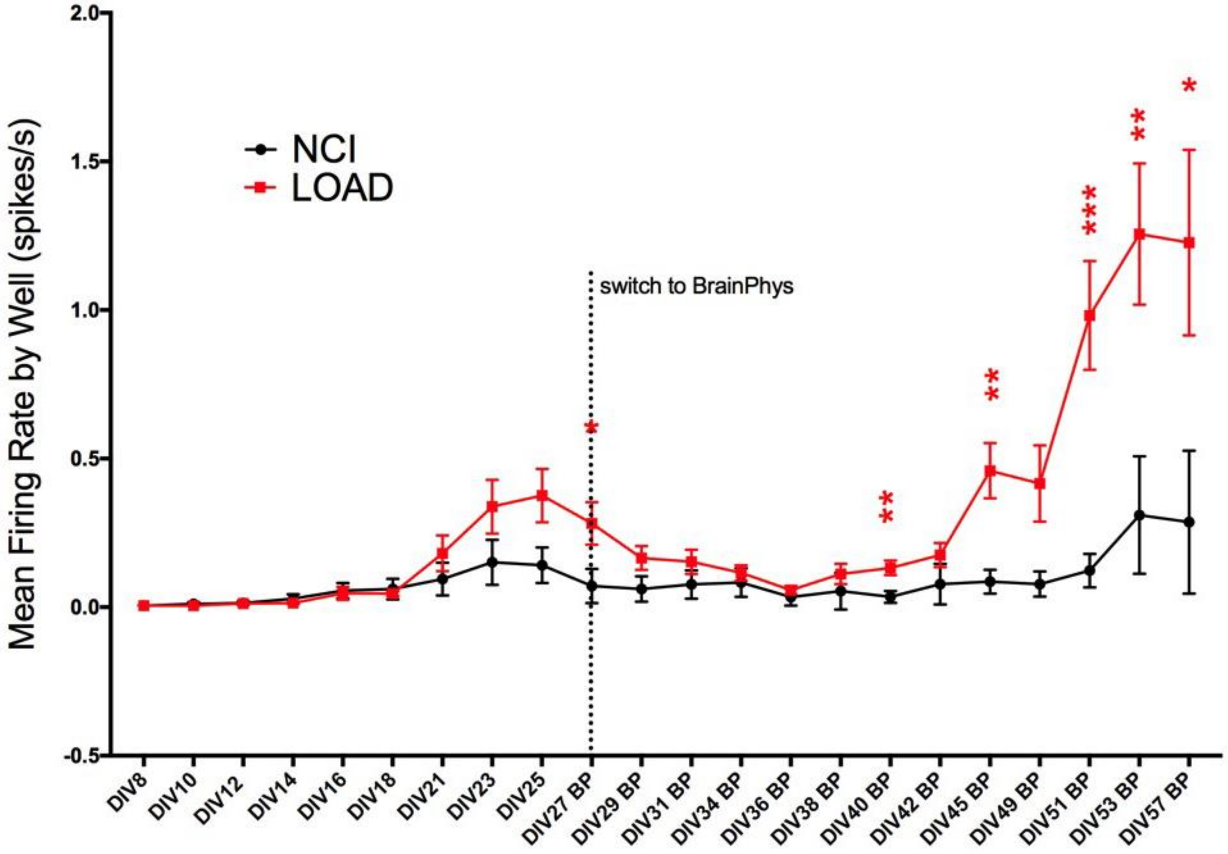
Axion Maestro MEA reader

Firing Frequency of “control” (not cognitively impaired, no pathology) versus LOAD iNs over time Using the Axion Maestro MEA reader



Firing Frequency of control (not cognitively impaired, no pathology) versus LOAD iNs over time
Using the Axion Maestro MEA reader

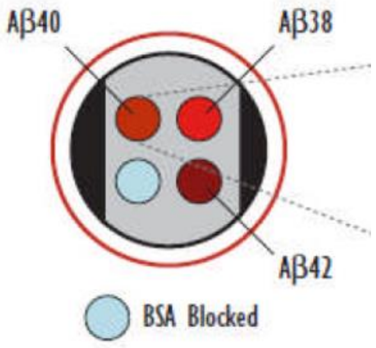




Assays developed for human induced neurons from ROS/MAP subjects

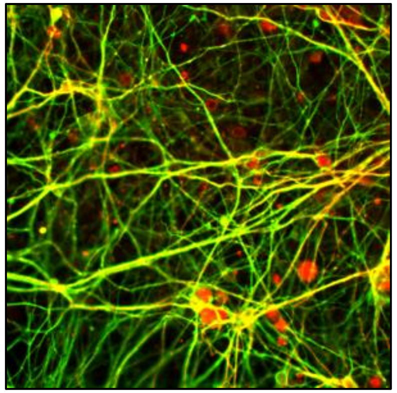
APP processing

Aβ42
Aβ40
Aβ37
Aβ38
Aβ43
Oligomeric Aβ
sAPPβ
sAPPa
APP holoprotein
CTFs



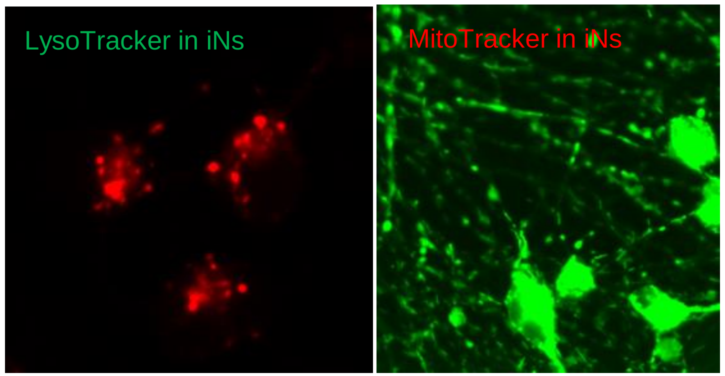
Tau levels

pS202/T205 Tau
p231 Tau
Tau full length
Tau N-term
Tau C-term



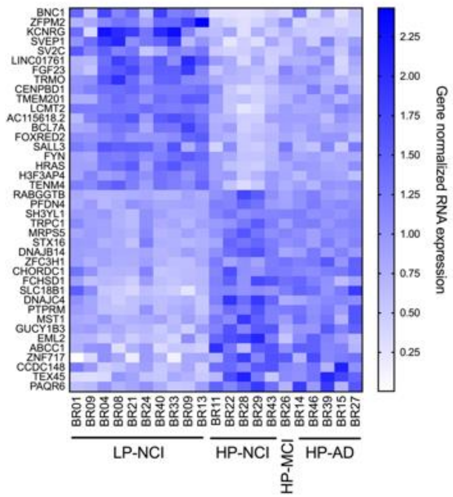
Imaging-based assays

Lysosome size, number, localization
Mitochondria size, number, localization
Endosome size, number, localization
Synaptic puncta number
Oxidative stress



-Omics

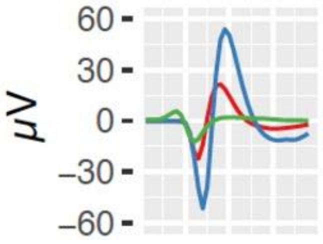
RNAseq
Proteomics



Longitudinal

Incucyte

MEA



APP processing

Tau levels

Imaging-based assays

-Omics

Longitudinal

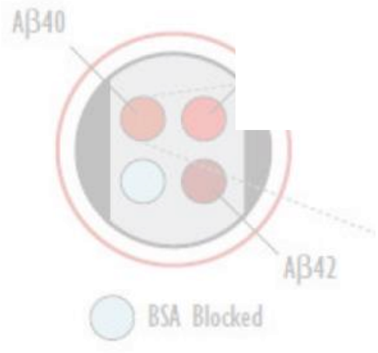
Aβ42

Changes in these metrics in response to a stimulus:

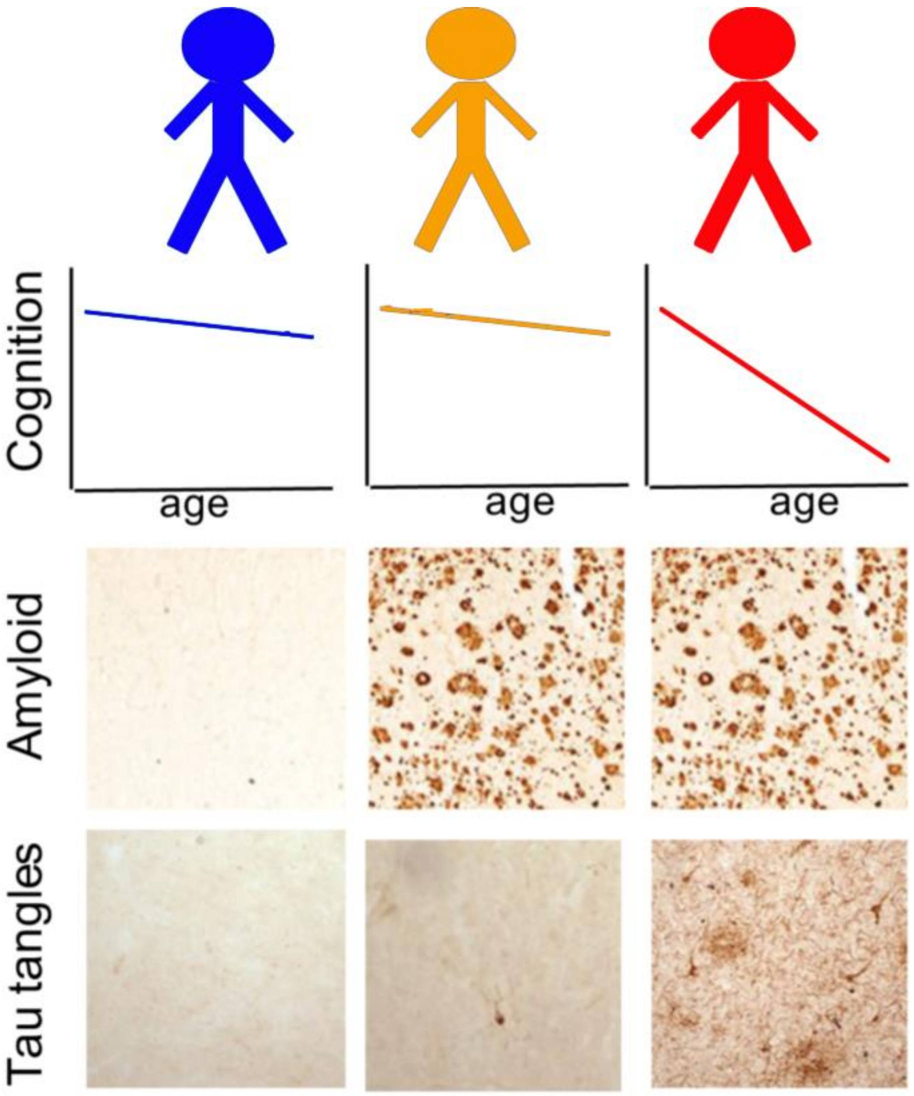
1) genetic perturbation (shRNA, CRISPR-based modulation)

2) chemical treatment (drugs, small molecules)

3) Brain-derived neurotoxic species

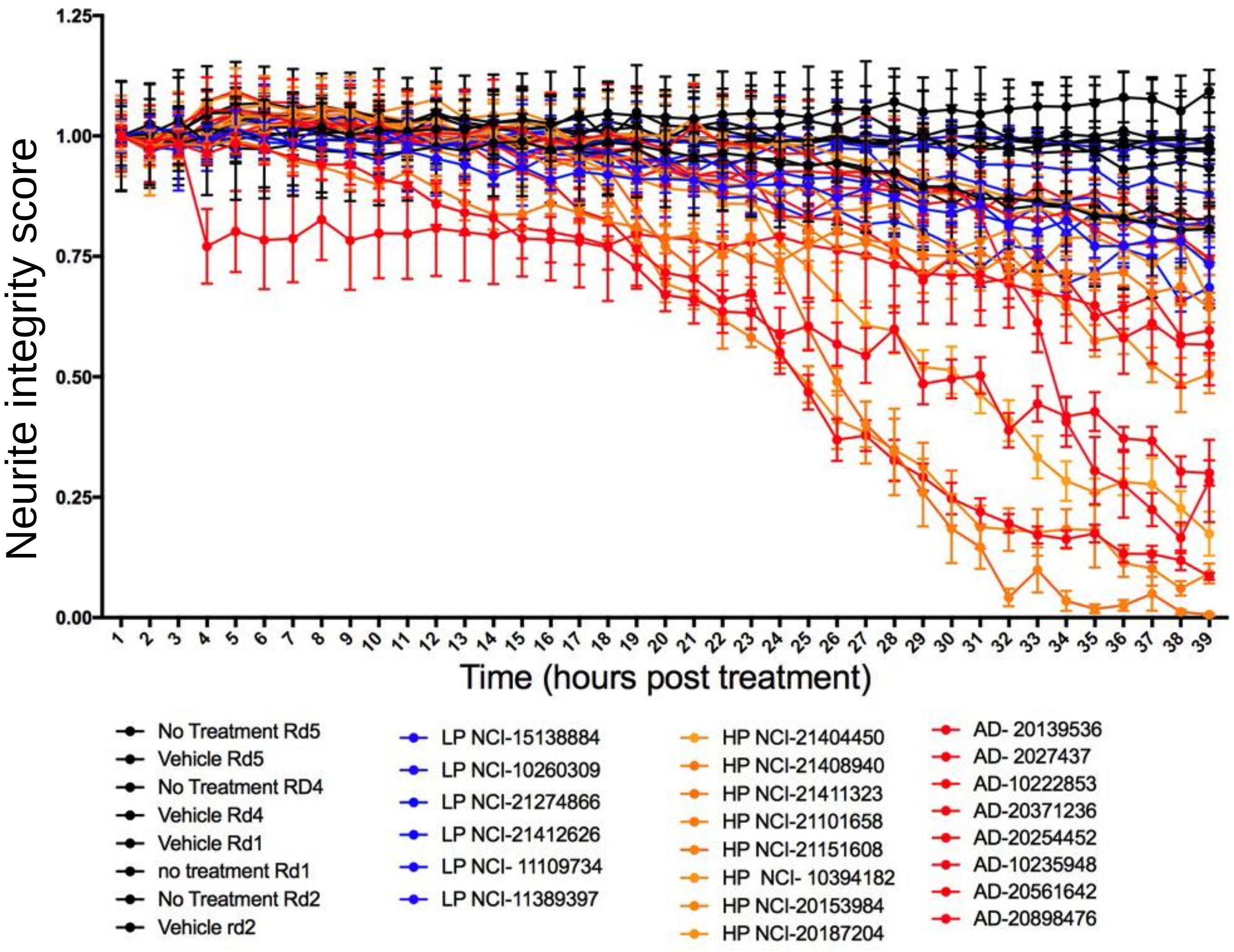


Brain-derived neurotoxic species as a perturbation



Brain extracts from 27 individuals
in ROS/MAP examined

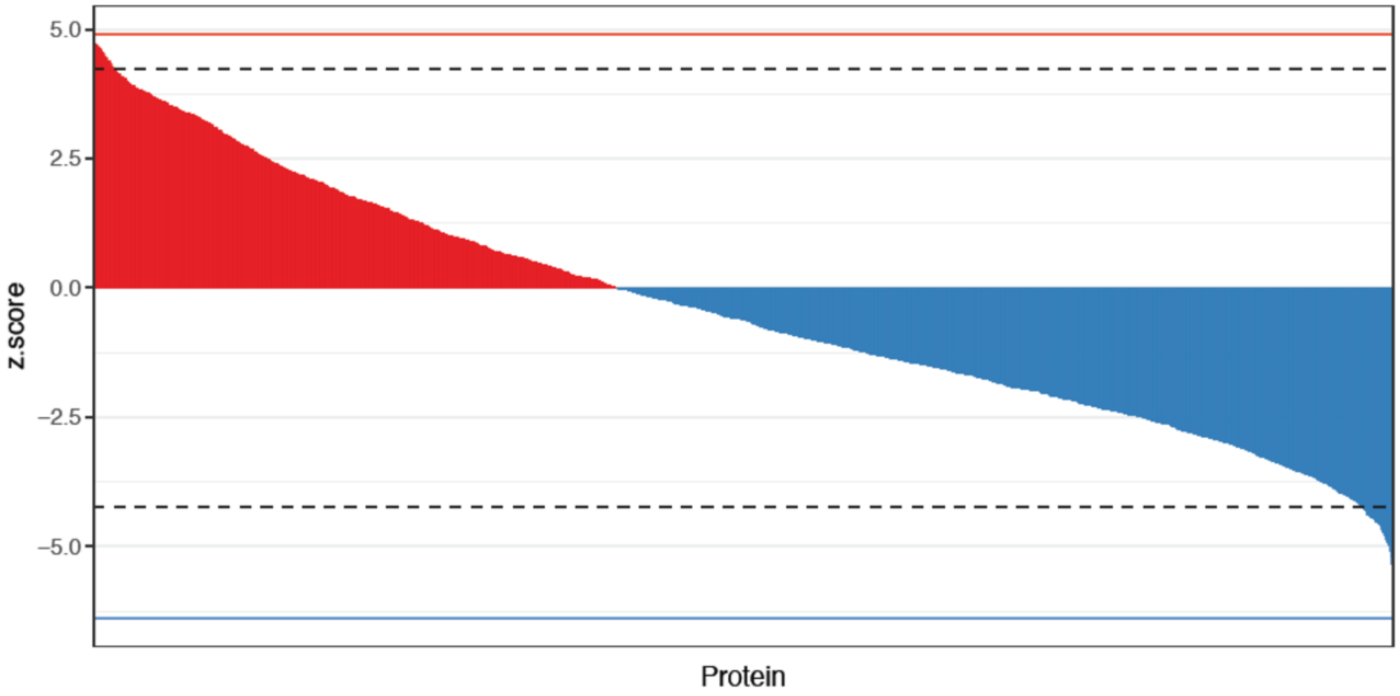
Responsiveness of human iNs to human brain extracts across the clinical and pathological spectrum



Proteomic profiling of human brain extracts and correlations with effects on neurite integrity

<u>EMORY proteomics</u>

3500 proteins identified,
Spearman correlation,
correcting for multiple comparisons
86 proteins significantly correlated

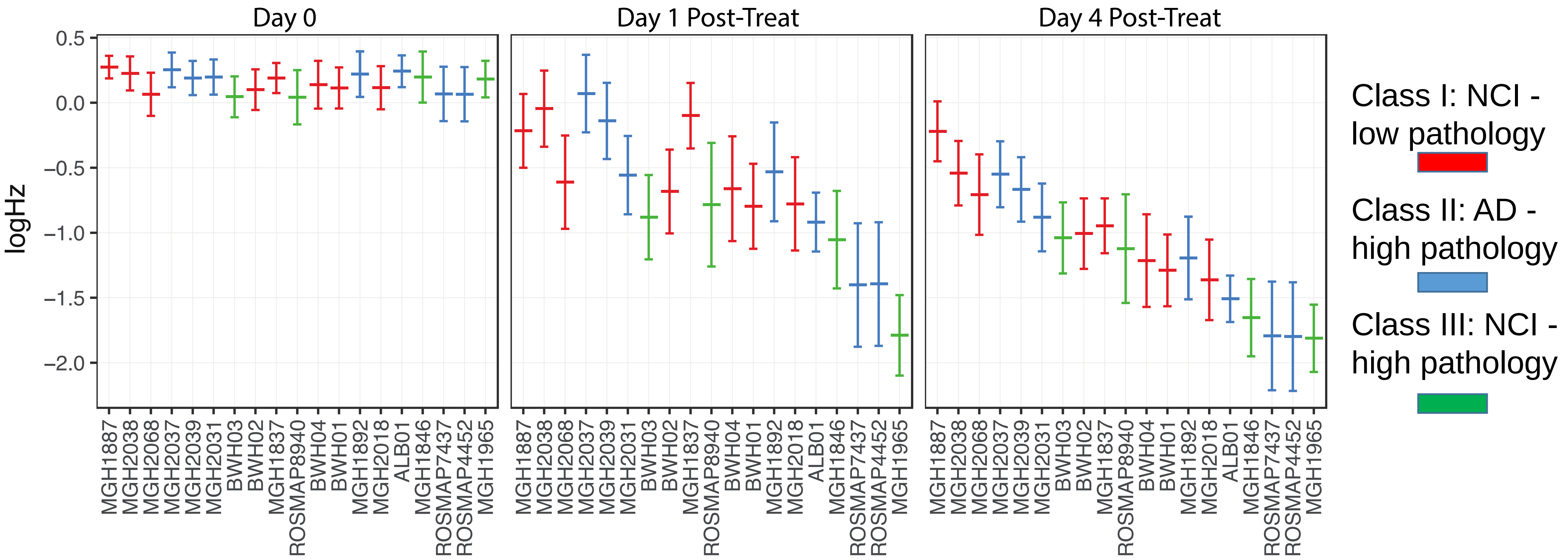


Effects of human brain extracts by diagnosis class; individual, on mean firing rate

Incorporating data from 19 individuals across 22 experiments:

Effect of individual on spontaneous firing

*lme: logHz ~ Individual * Time, random (experiment, well)*

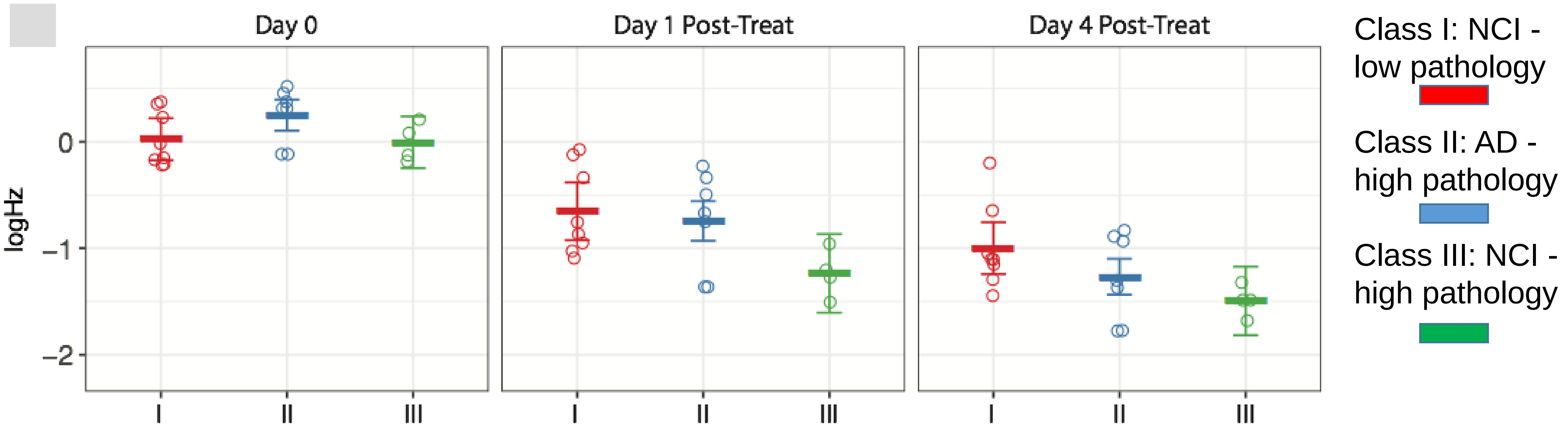


Effects of human brain extracts by diagnosis class; individual, on mean firing rate

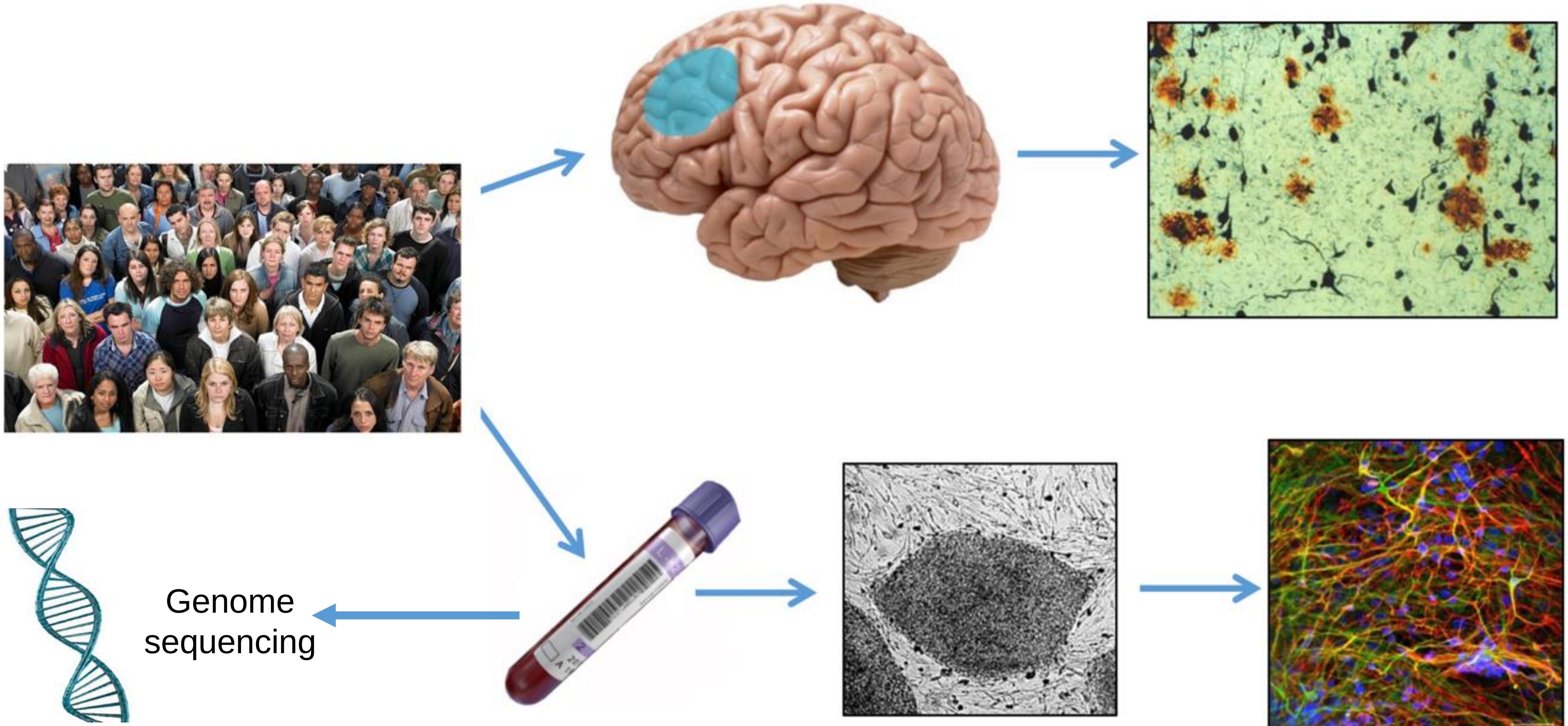
Incorporating data from 19 individuals across 22 experiments:

Effect of individual on spontaneous firing

*lme: logHz ~ Individual * Time, random (experiment, well)*



Probing heterogeneity in Alzheimer’s disease and the aging human brain using iPSCs



Acknowledgments

Young-Pearse Lab
Christina Muratore, PhD
Meichen Liao, PhD
Sarah Sullivan, PhD
Chun-I Wu, PhD
Marty Fernandez, PhD
Joe Negri
Walter Taylor
Valentina Lagomarsino
Dana Callahan
Deepika Kurup
Connie Zhou
Amy He
Robert Smith
Julie Merchant
Hyo Lee
Vicky Chou
Richard Pearse, PhD

AD collaborators
Dennis Selkoe, MD – BWH/HMS
Dominic Walsh, PhD – BWH/HMS
Chris Love, PhD – MIT
Kevin Eggan, PhD - Harvard
Lori Chibnik, PhD - Harvard
Chris Gaiteri, PhD - RUSH
Sara Mostafavi, PhD – UBC
Betsy Bradshaw, PhD - Columbia
Michael Wolfe, PhD – KU
Nick Seyfried, PhD – Emory
Allan Levey, PhD - Emory
Scott Noggle, PhD - NYSCF
Phil De Jager, MD PhD - Columbia
David Bennett, MD - RUSH

NATIONAL INSTITUTE ON AGING
National Institutes of Health

HSCI
HARVARD STEM CELL INSTITUTE

BrightFocus™ Foundation

NIMH
National Institute of Mental Health

Biggest thank you to the participants of ROS and MAP and our generous donors who funded the generation of the iPSC lines