Journal Report

---- Identification of evolutionarily conserved gene networks mediating neurodegenerative dementia

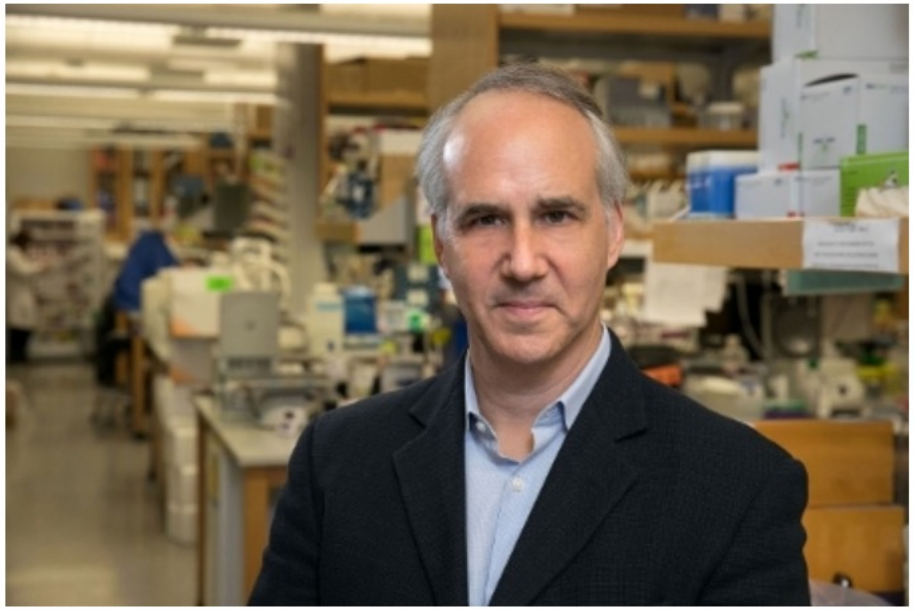
Xiangjie Zhao

December 27, 2018

中国科学院遗传与发育生物学研究所
INSTITUTE OF GENETICS AND DEVELOPMENTAL BIOLOGY
CHINESE ACADEMY OF SCIENCES

中国科学院
CHINESE ACADEMY OF SCIENCES

About PI



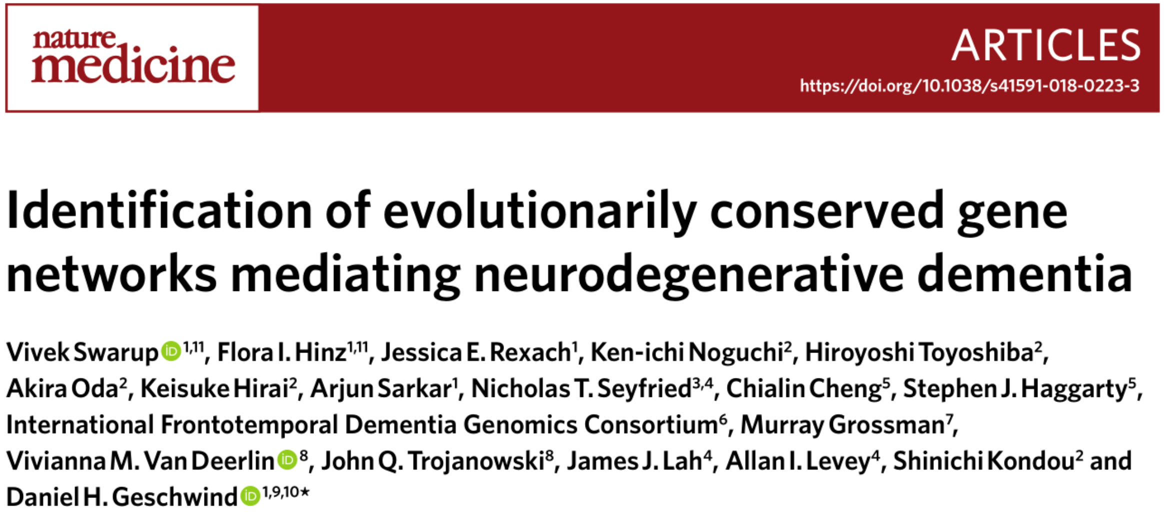
Daniel H. Geschwind

Principal Investigator
Gordon and Virginia Macdonald Distinguished Professor

Senior Associate Dean and Associate Vice Chancellor of Precision Health in the UCLA Health System and David Geffen School of Medicine
Director, UCLA Center for Autism Research and Treatment (CART)
2506 Gonda 695 Charles E. Young Dr. South Los Angeles CA 90095-1761
p: 310 794-6570
f: 310 267-2401
dhg@mednet.ucla.edu ✉

Research

1. Neurodevelopmental and neurodegenerative disease Focusing on **autism spectrum disorders** (自闭症), **dementia** (痴呆), and repair of the damaged nervous system.
2. Genetics, genomics, neurobiology and system biology



nature medicine

ARTICLES

https://doi.org/10.1038/s41591-018-0223-3

Identification of evolutionarily conserved gene networks mediating neurodegenerative dementia

Vivek Swarup[1,11], Flora I. Hinz[1,11], Jessica E. Rexach[1], Ken-ichi Noguchi[2], Hiroyoshi Toyoshiba[2], Akira Oda[2], Keisuke Hirai[2], Arjun Sarkar[1], Nicholas T. Seyfried[3,4], Chialin Cheng[5], Stephen J. Haggarty[5], International Frontotemporal Dementia Genomics Consortium[6], Murray Grossman[7], Vivianna M. Van Deerlin[8], John Q. Trojanowski[8], James J. Lah[4], Allan I. Levey[4], Shinichi Kondou[2] and Daniel H. Geschwind[1,9,10*]

Background

Section 1. Experimental design

Section 2. Identification of disease-relevant mRNA modules

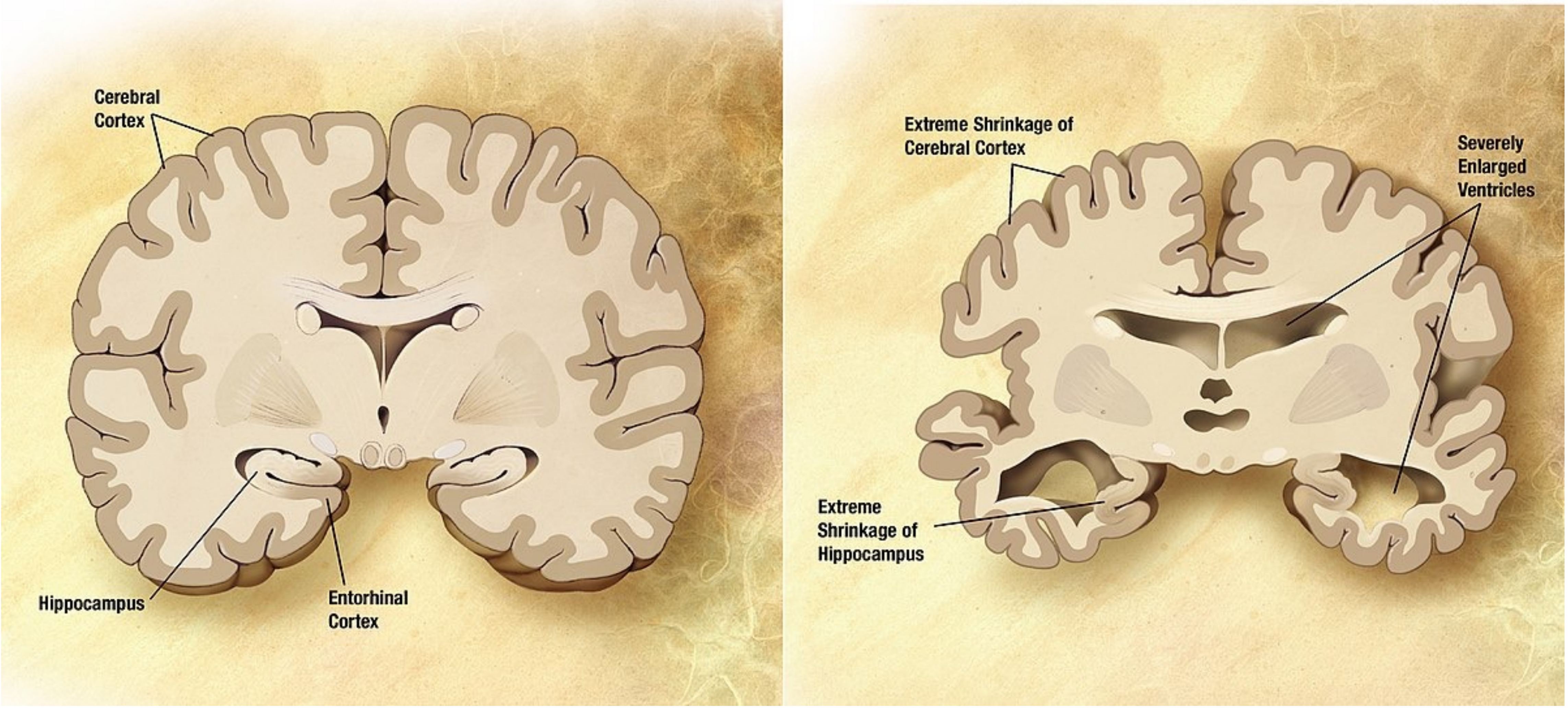
Section 3. Reproducibility of modules across mouse and human

Section 4. Identification of potential miRNA drivers

Section 5. Identification of small molecules normalizing the modules

Summary

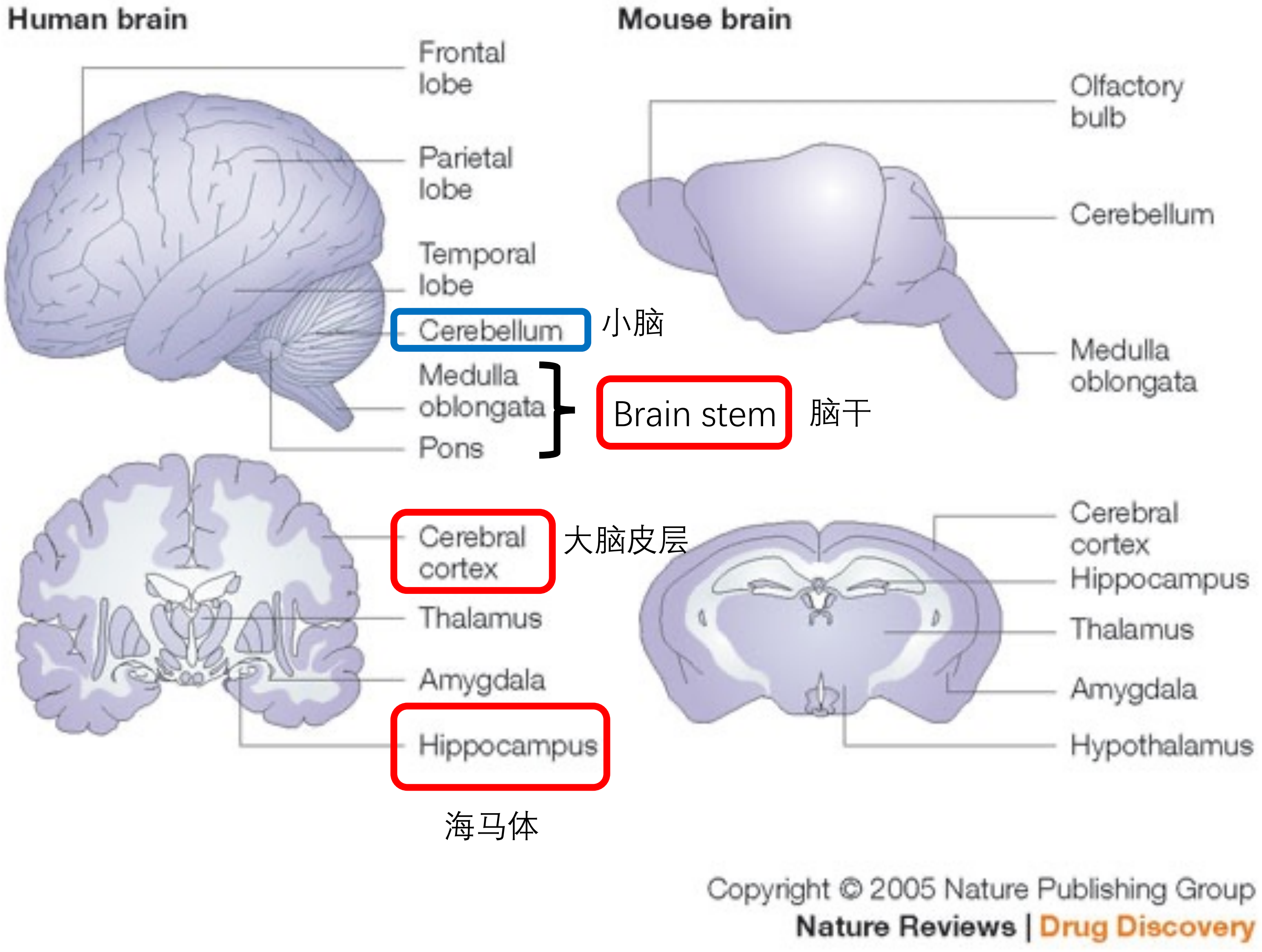
Neurodegeneration Dementia



Comparison of a normal aged brain (left) and the brain of a person with Alzheimer's disease (right)

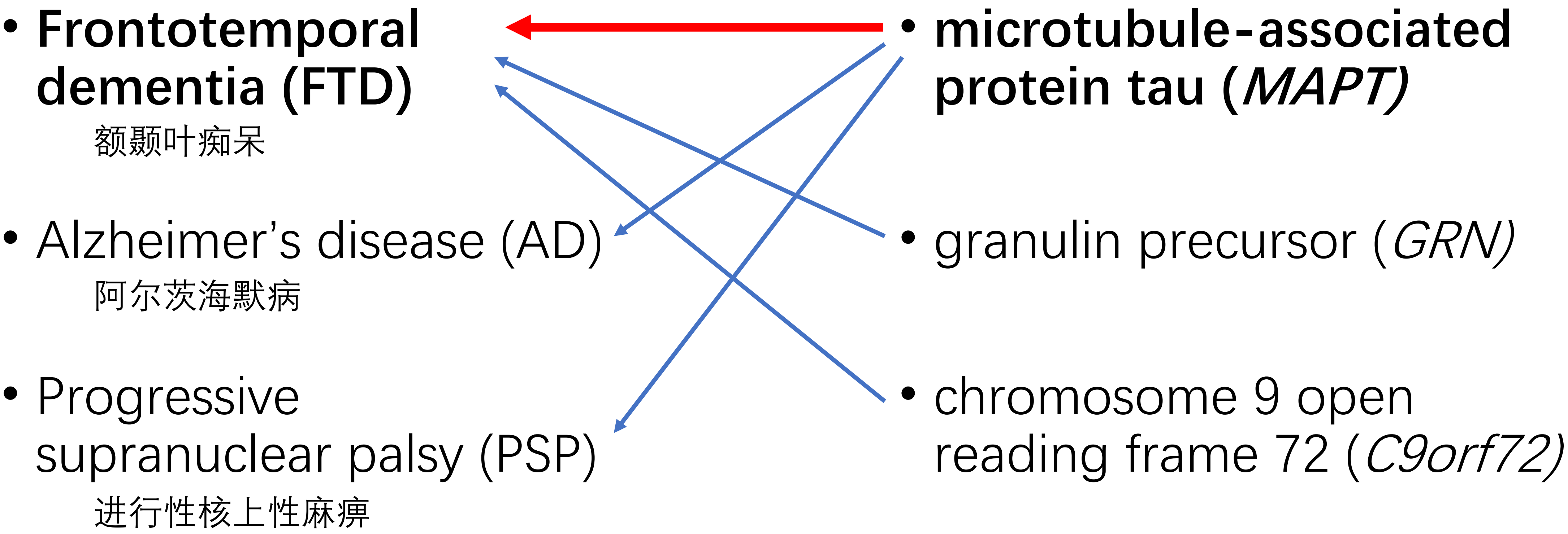
---- Wikipedia

Vulnerable variation of brain areas on tau-induced neurodegeneration



Nature Reviews | Drug Discovery

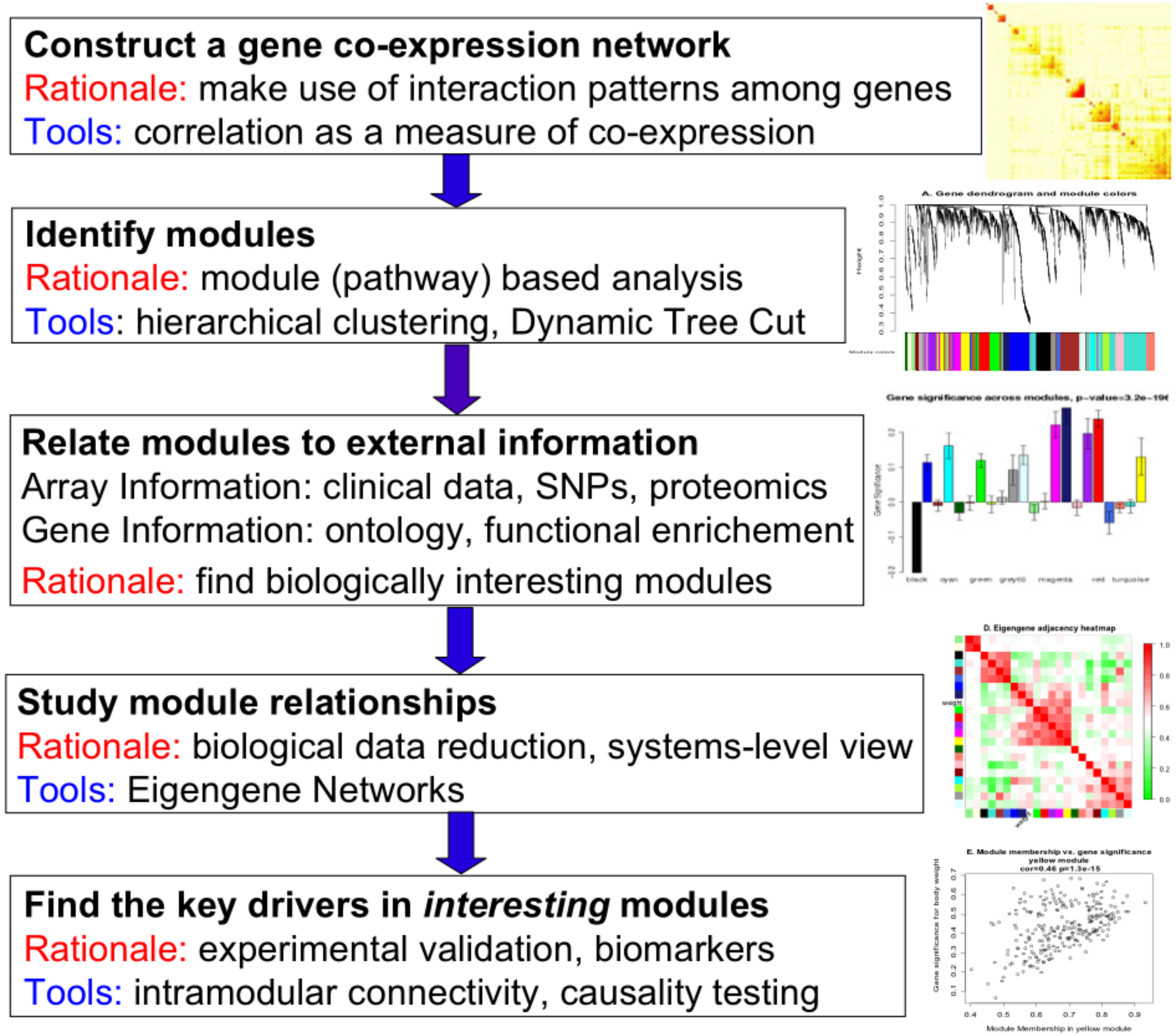
Neurodegenerative dementias and their related genes



- **Frontotemporal dementia (FTD)**
  额颞叶痴呆
- Alzheimer’s disease (AD)
  阿尔茨海默病
- Progressive supranuclear palsy (PSP)
  进行性核上性麻痹

- **microtubule-associated protein tau (*MAPT*)**
- granulin precursor (*GRN*)
- chromosome 9 open reading frame 72 (*C9orf72*)

Weighted gene co-expression network analysis (WGCNA)



Construct a gene co-expression network
Rationale: make use of interaction patterns among genes
Tools: correlation as a measure of co-expression

Identify modules
Rationale: module (pathway) based analysis
Tools: hierarchical clustering, Dynamic Tree Cut

Relate modules to external information
Array Information: clinical data, SNPs, proteomics
Gene Information: ontology, functional enrichement
Rationale: find biologically interesting modules

Study module relationships
Rationale: biological data reduction, systems-level view
Tools: Eigengene Networks

Find the key drivers in *interesting* modules
Rationale: experimental validation, biomarkers
Tools: intramodular connectivity, causality testing

Peter Langfelder et al., BMC Bioinformatics (2008)

Weighted gene co-expression network analysis (WGCNA)

Term	Definition
Co-expression network	undirected, weighted Node: gene expression profile Edge: determined by the pairwise correlation between gene expressions.
Module	cluster of highly interconnected genes
Module eigengene	the first principal component of a given module; can be regarded as a representation of the module
Hub gene	Genes inside a co-expression module tending to have high connectivity

Peter Langfelder et al., BMC Bioinformatics (2008)

Background

Section 1. Experimental design

Section 2. Identification of disease-relevant mRNA modules

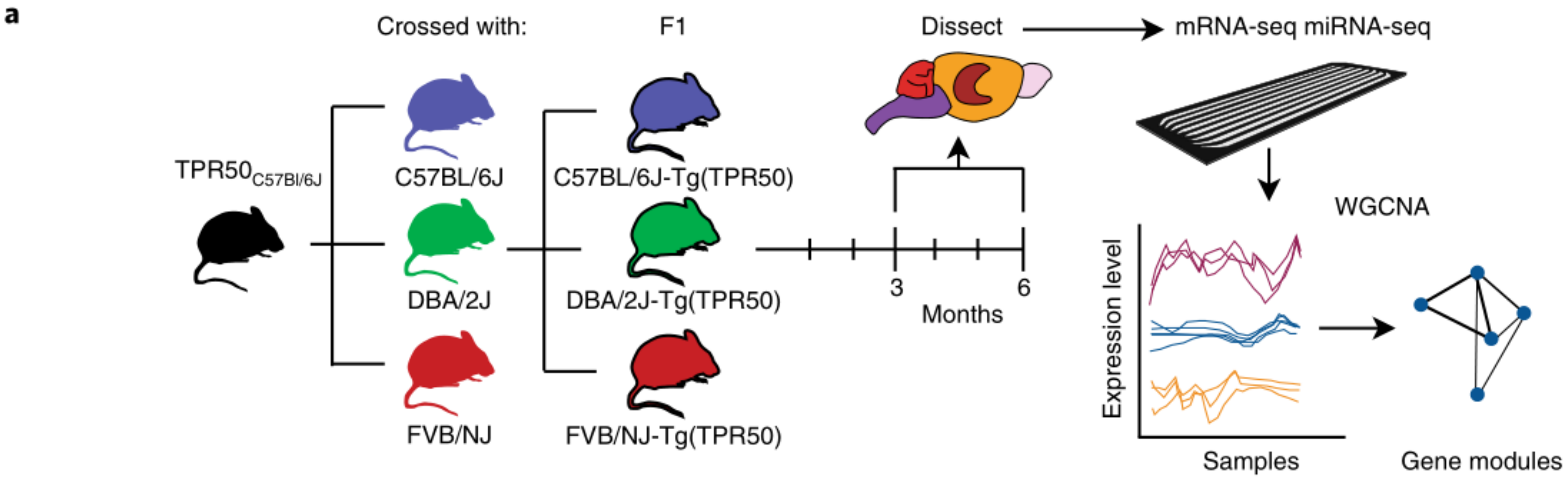
Section 3. Reproducibility of modules across mouse and human

Section 4. Identification of potential miRNA drivers

Section 5. Identification of small molecules normalizing the modules

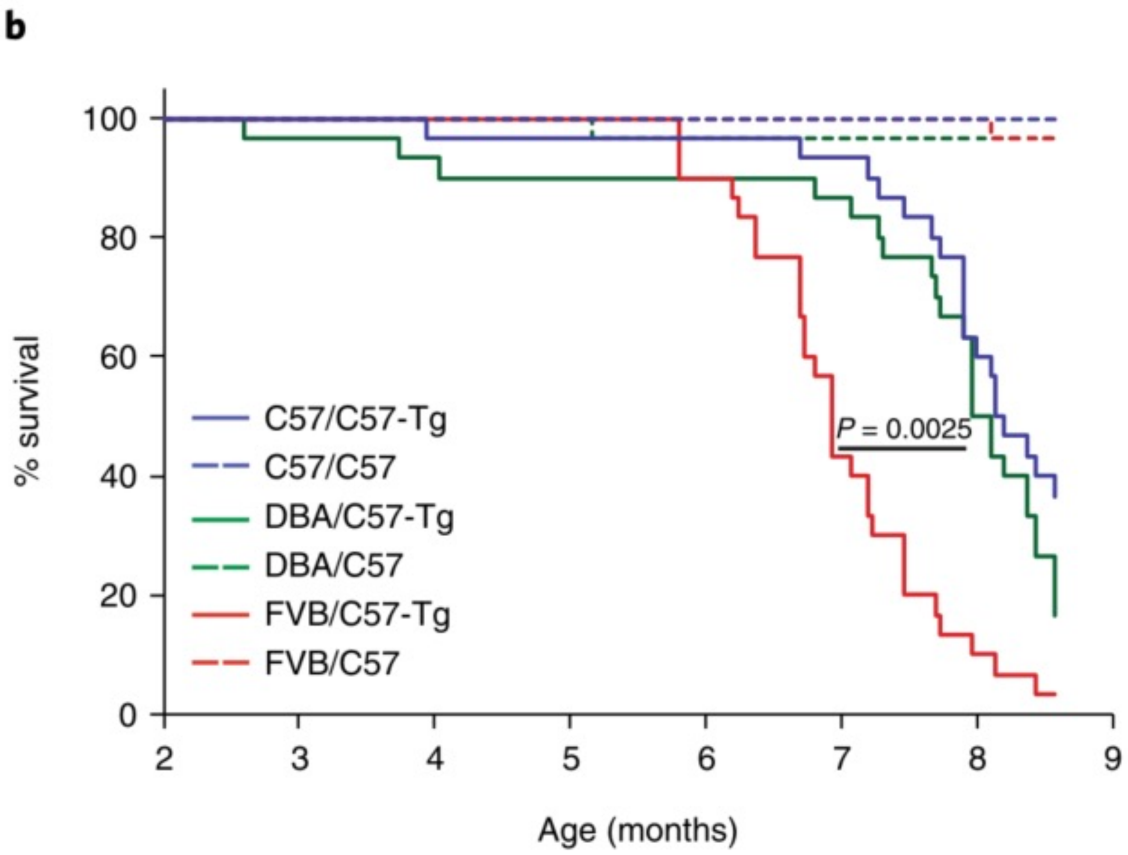
Summary

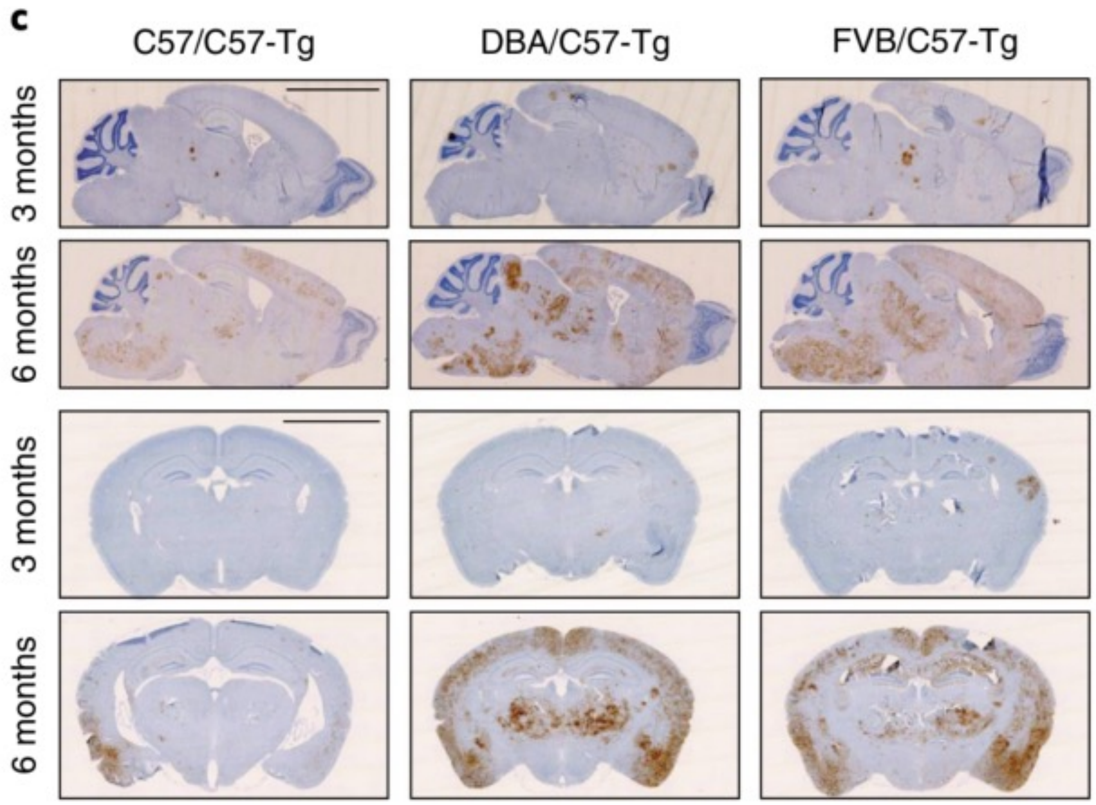
Schematic of the experimental design



TPR50 mice: overexpressing tau^{P301S} mutation in the human *MAPT* gene (4R2N isoform)

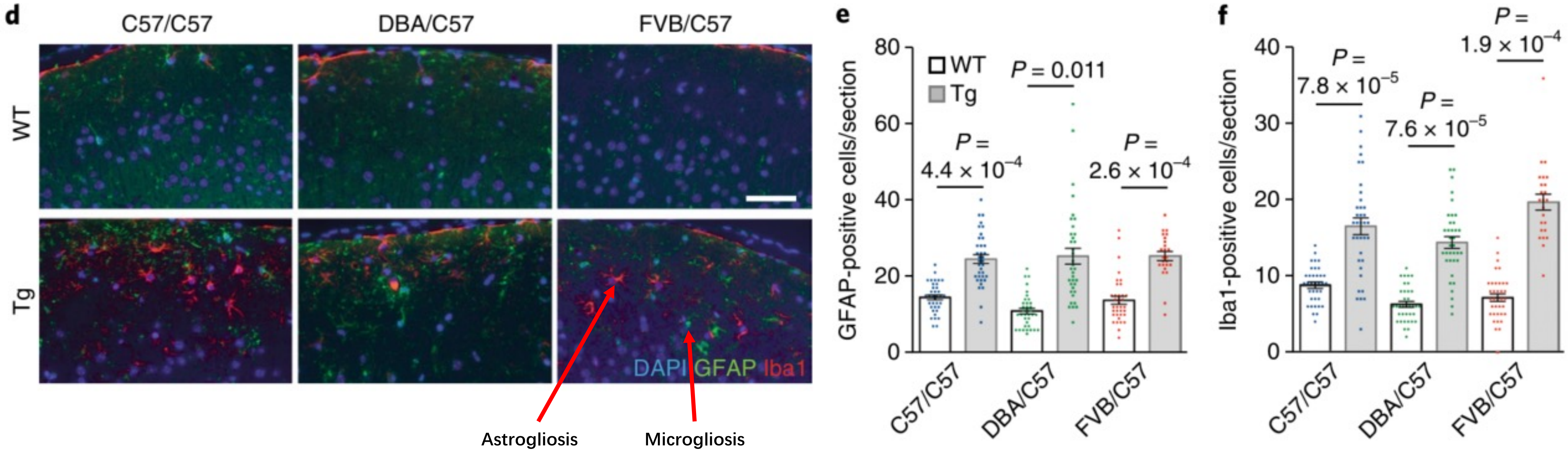
All of three F1 crosses share key features of the disease





Accumulation of hyper-phosphorylated tau in the cortex and hippocampus

All of three F1 crosses share key features of the disease



Astrogliosis
(星形胶质细胞增生)

Microgliosis
(小胶质细胞增生)

Background

Section 1. Experimental design

Section 2. Identification of disease-relevant mRNA modules

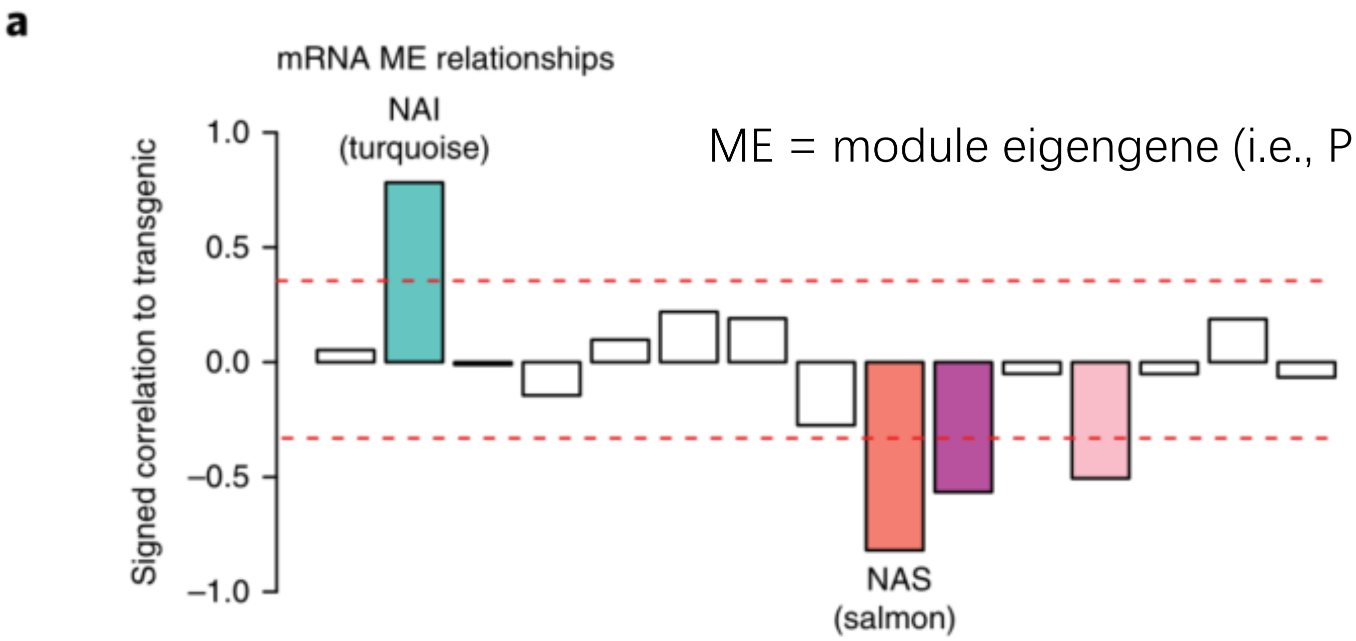
Section 3. Reproducibility of modules across mouse and human

Section 4. Identification of potential miRNA drivers

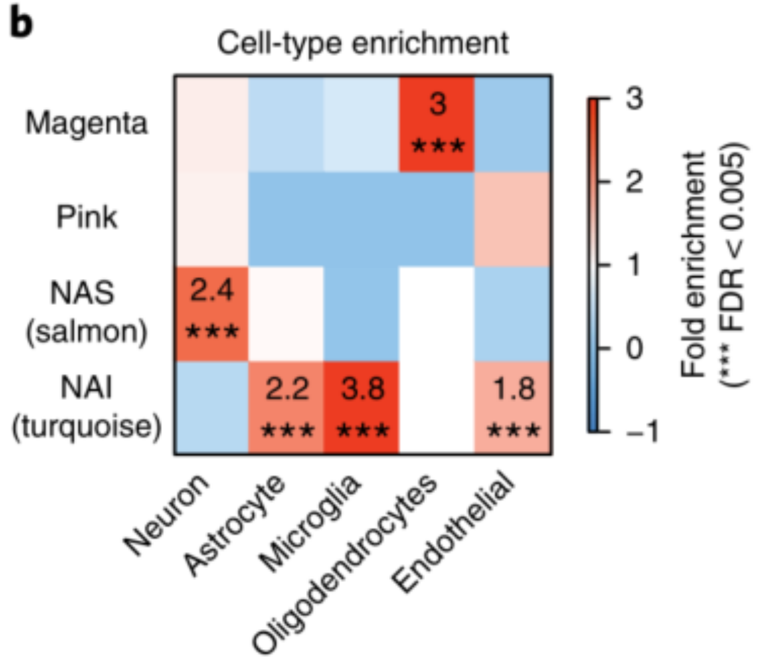
Section 5. Identification of small molecules normalizing the modules

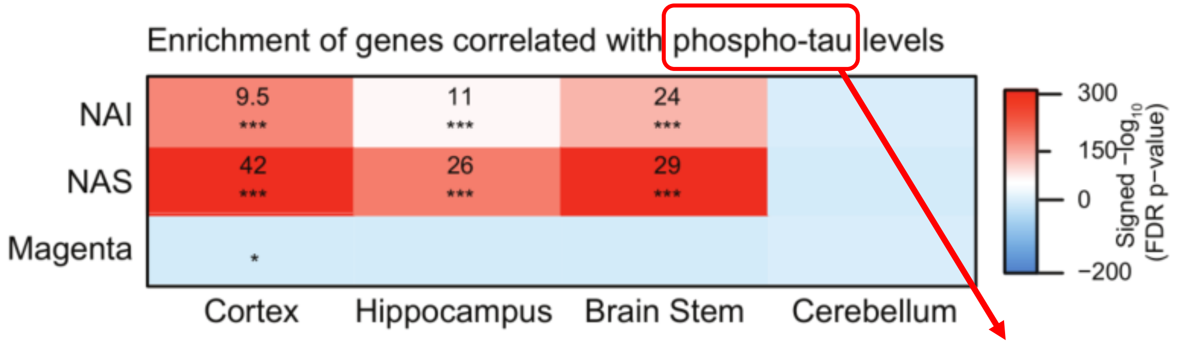
Summary

Identification of 4 mRNA modules in cortex



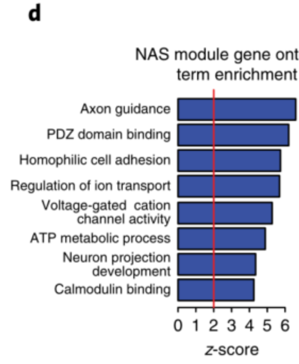
ME = module eigengene (i.e., PCA1)

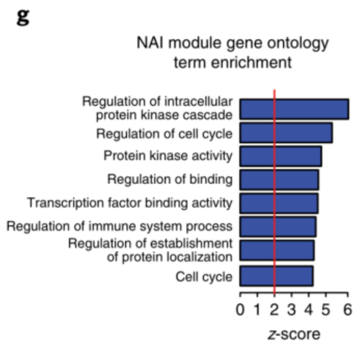




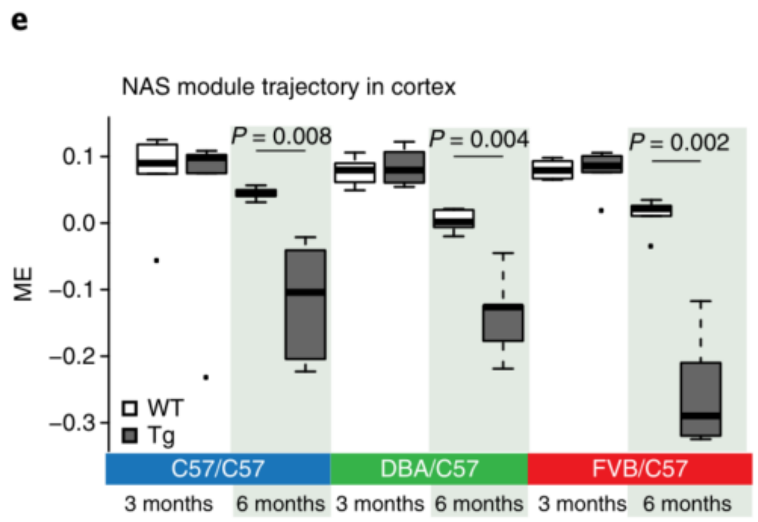
Hyper-phosphorylated tau: a disease marker of FTD and AD

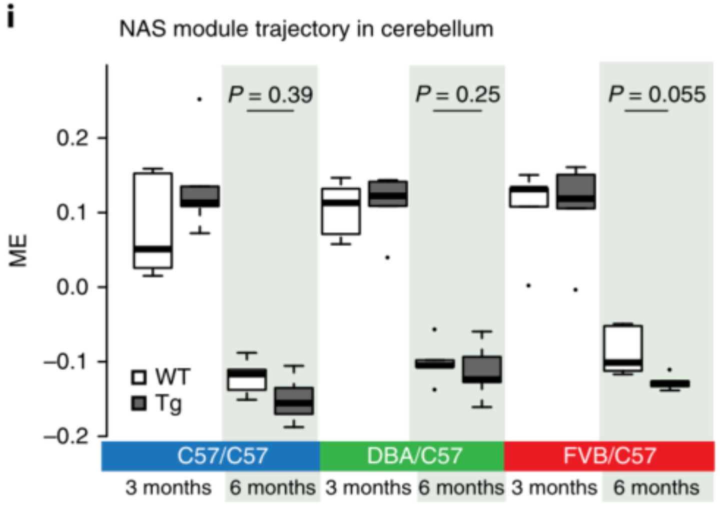
neurodegeneration-associated **synaptic** (NAS)
neurodegeneration-associated **inflammatory** (NAI)

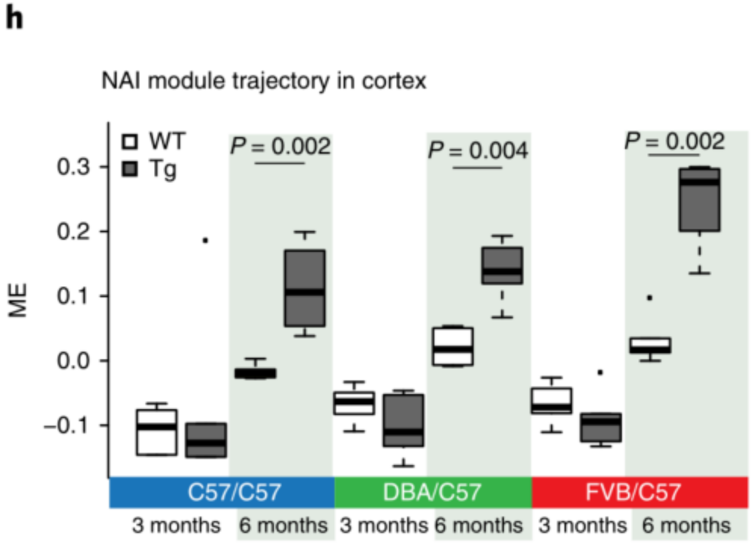


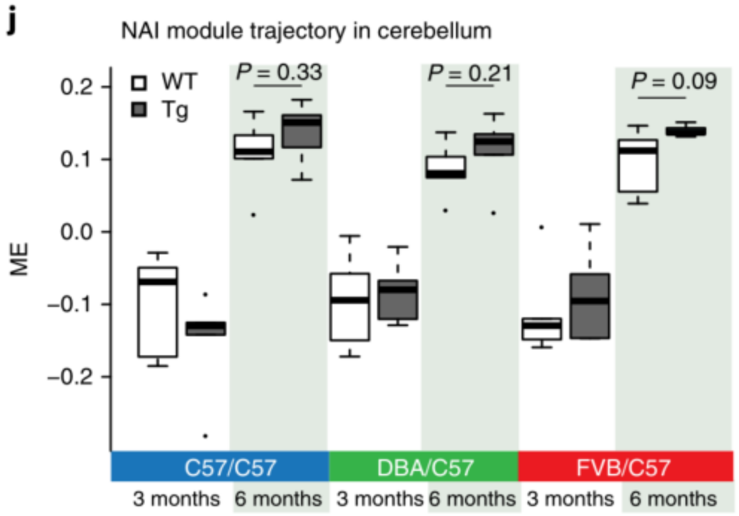


Trajectory of the module eigengene





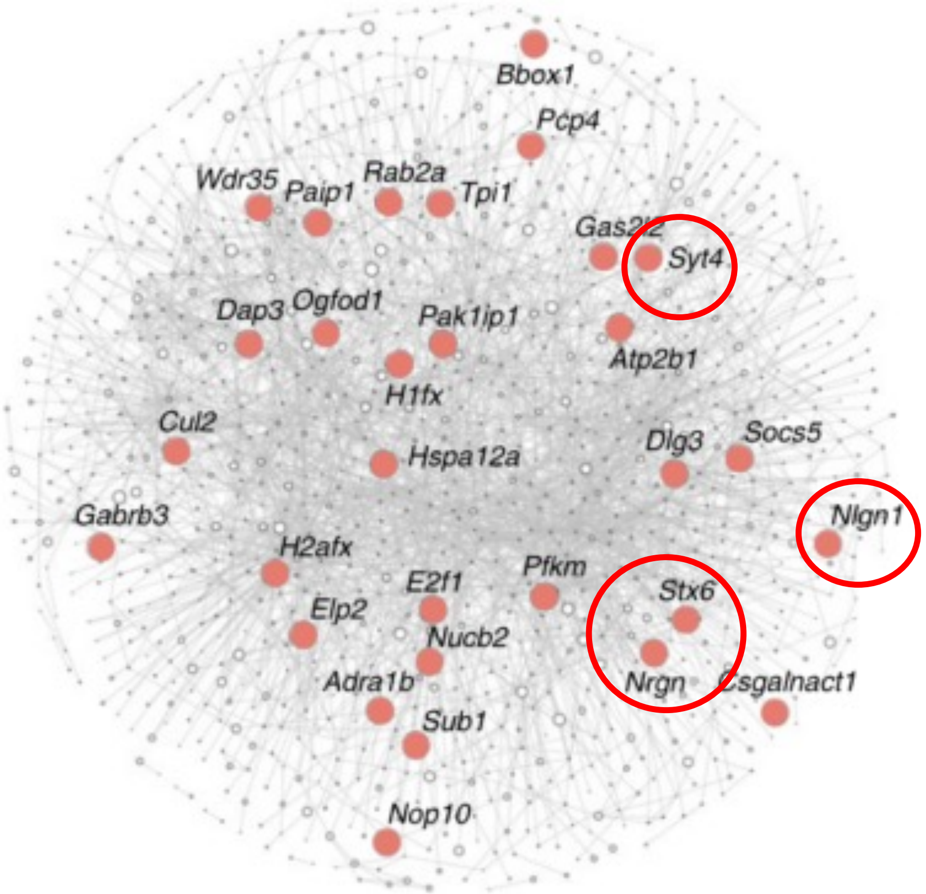




Coexpression-PPI network

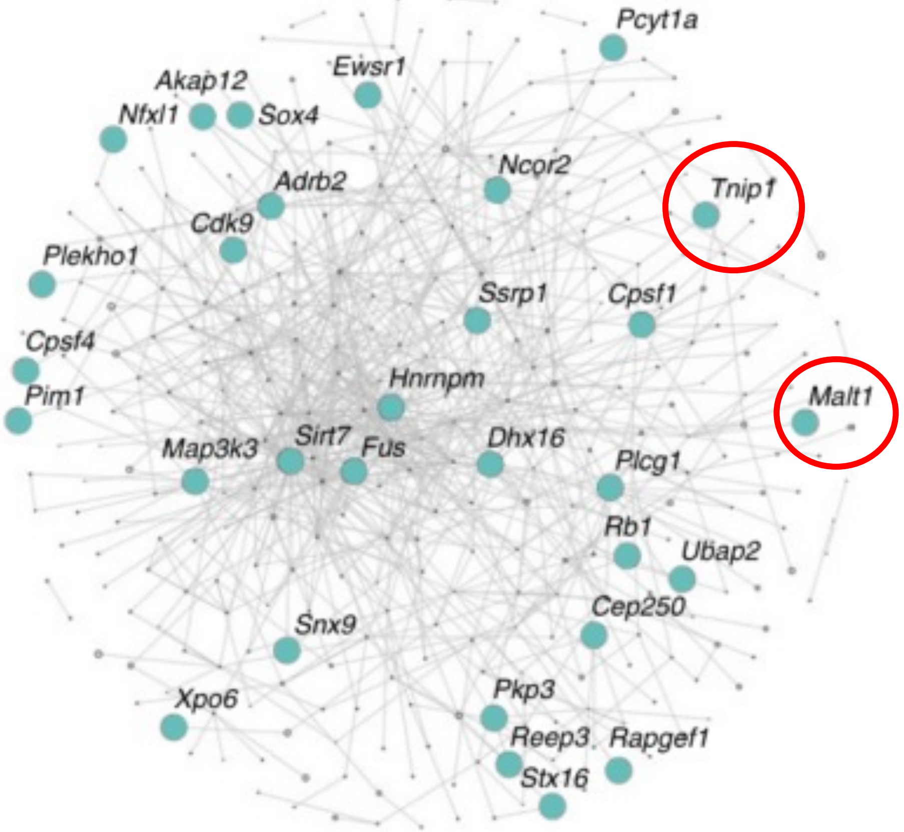
c

NAS module



f

NAI module



Background

Section 1. Experimental design

Section 2. Identification of disease-relevant mRNA modules

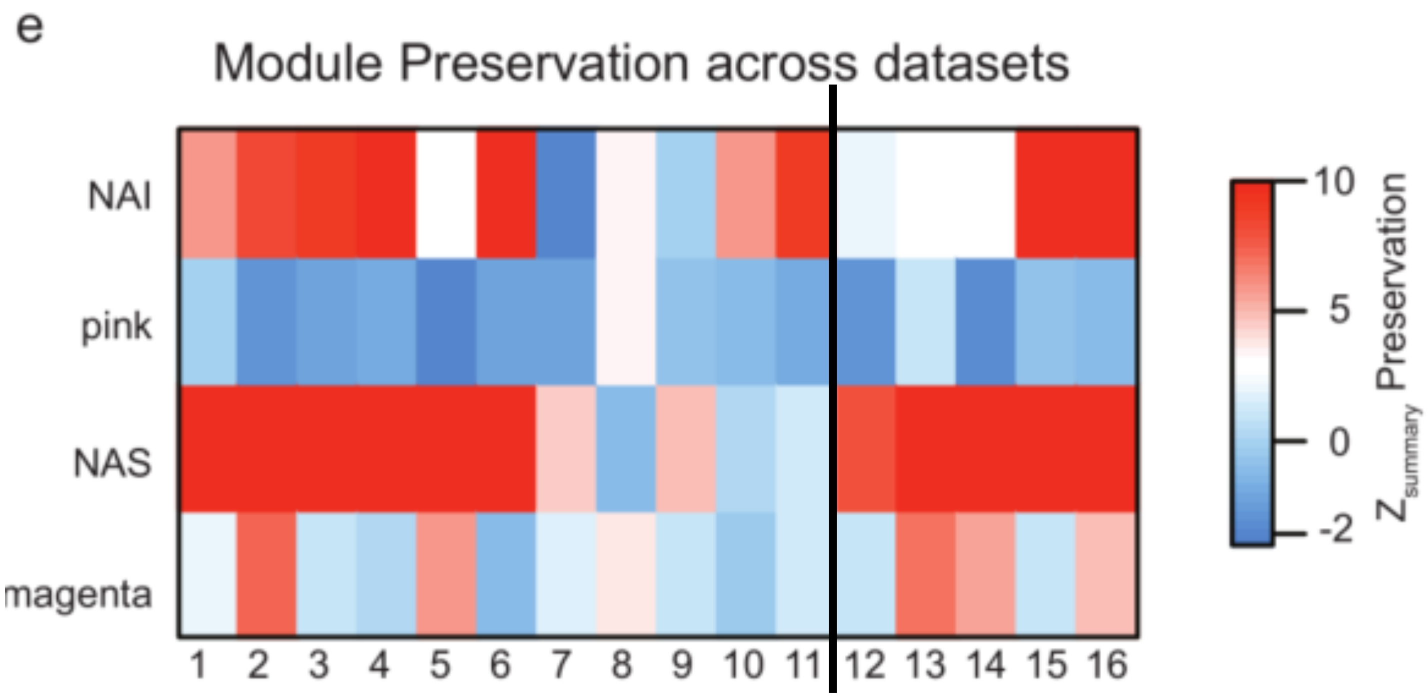
Section 3. Reproducibility of modules across mouse and human

Section 4. Identification of potential miRNA drivers

Section 5. Identification of small molecules normalizing the modules

Summary

NAS and NAI are preserved across different public datasets



1 - Tg4510 Mouse Cortex (*Wes et. al., 2014*)
2 - Tg4510 Mouse Cortex
3 - Tau_{P301L} Mouse Cortex (*Matarin et. al., 2015*)
4 - APP/PS1 Mouse Cortex (*Matarin et. al., 2015*)
5 - CRND8 Mouse Cortex
6 - Progranulin Knockout Mouse Cortex (*Lui et. al., 2016*)
7 - PS19 Mouse Hippocampus
8 - Tg4510 Mouse Microglia
9 - PS2APP Mouse Sorted Neurons (Srinivasan et al., 2016)
10 - PS2APP Mouse Sorted Microglia (Srinivasan et al., 2016)
11 - PS2APP Mouse Sorted Astrocytes (Srinivasan et al., 2016)

---

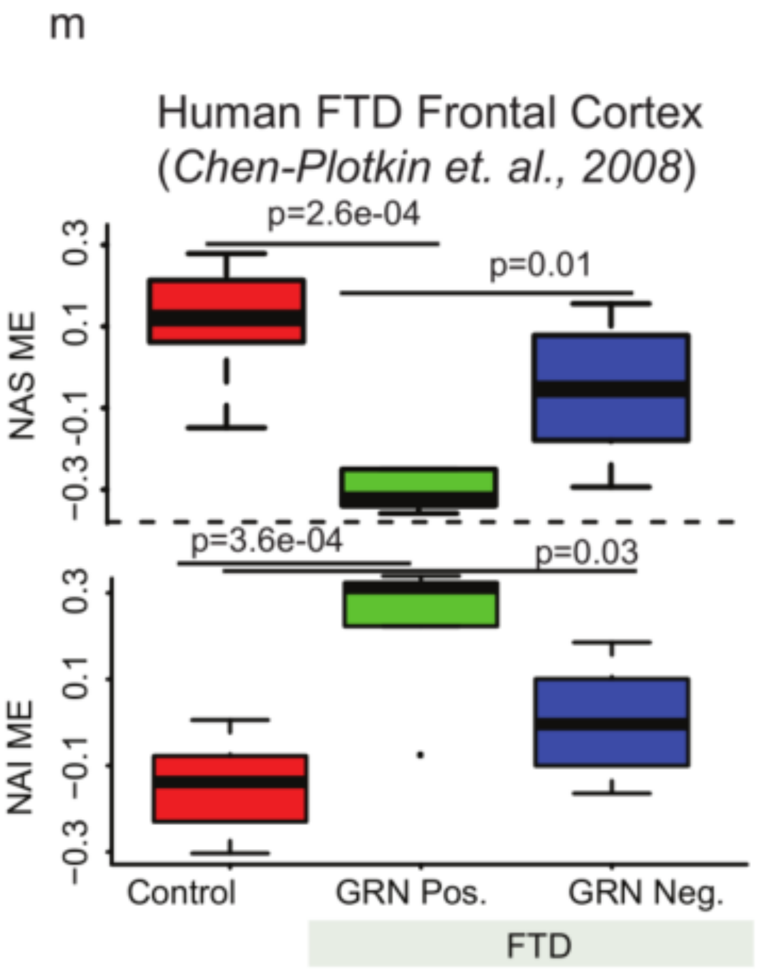
12 - Human FTD iPSC-derived neurons (*Almeida et. al., 2012*)
13 - Human FTD Frontal Cortex (*Chen-Plotkin et. al., 2008*)
14 - Human AD Frontal Cortex *(Zhang et al., 2013)*
15 - Human AD Temporal Cortex *(Allen et al., 2016)*
16 - Human PSP Temporal Cortex *(Allen et al., 2016)*

$$Z_{summary} = \frac{Z_{density} + Z_{connectivity}}{2}.$$

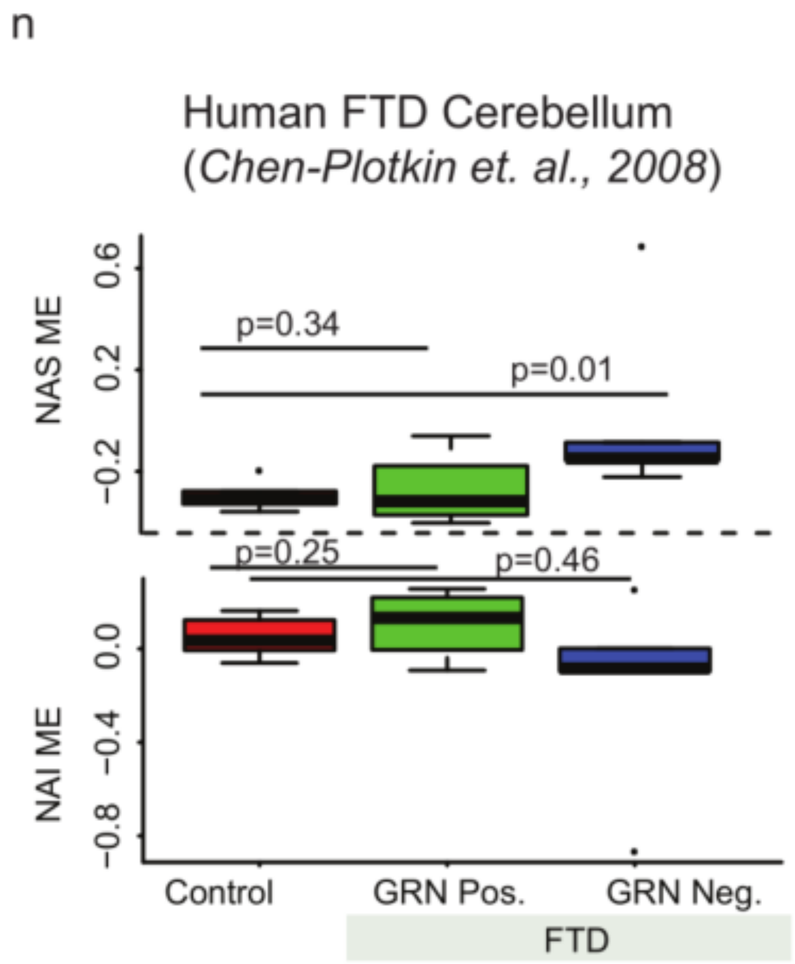
Measures module's network preservation (network structure similarity)

Peter Langfelder et al., PLoS Computational Biology (2011)

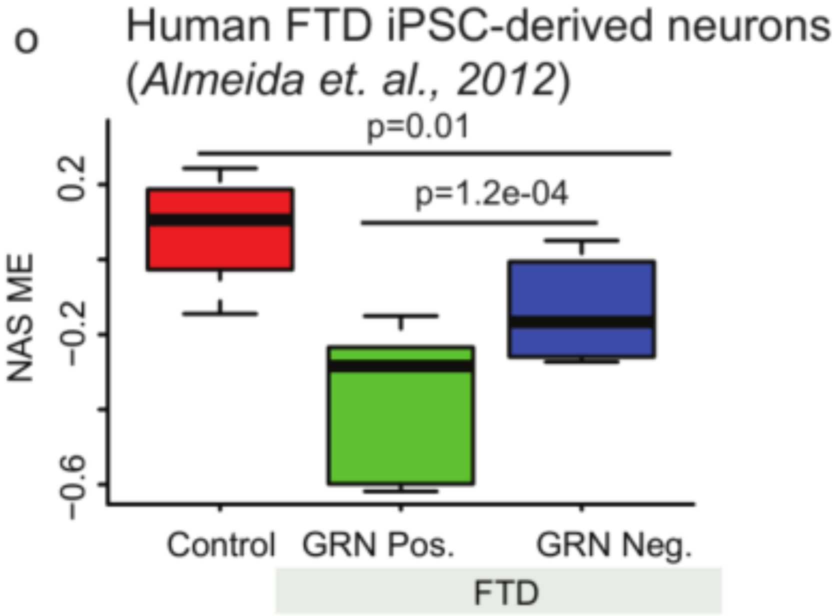
NAS and NAI are preserved across different public datasets



Human FTD Frontal Cortex (*Chen-Plotkin et. al., 2008*)

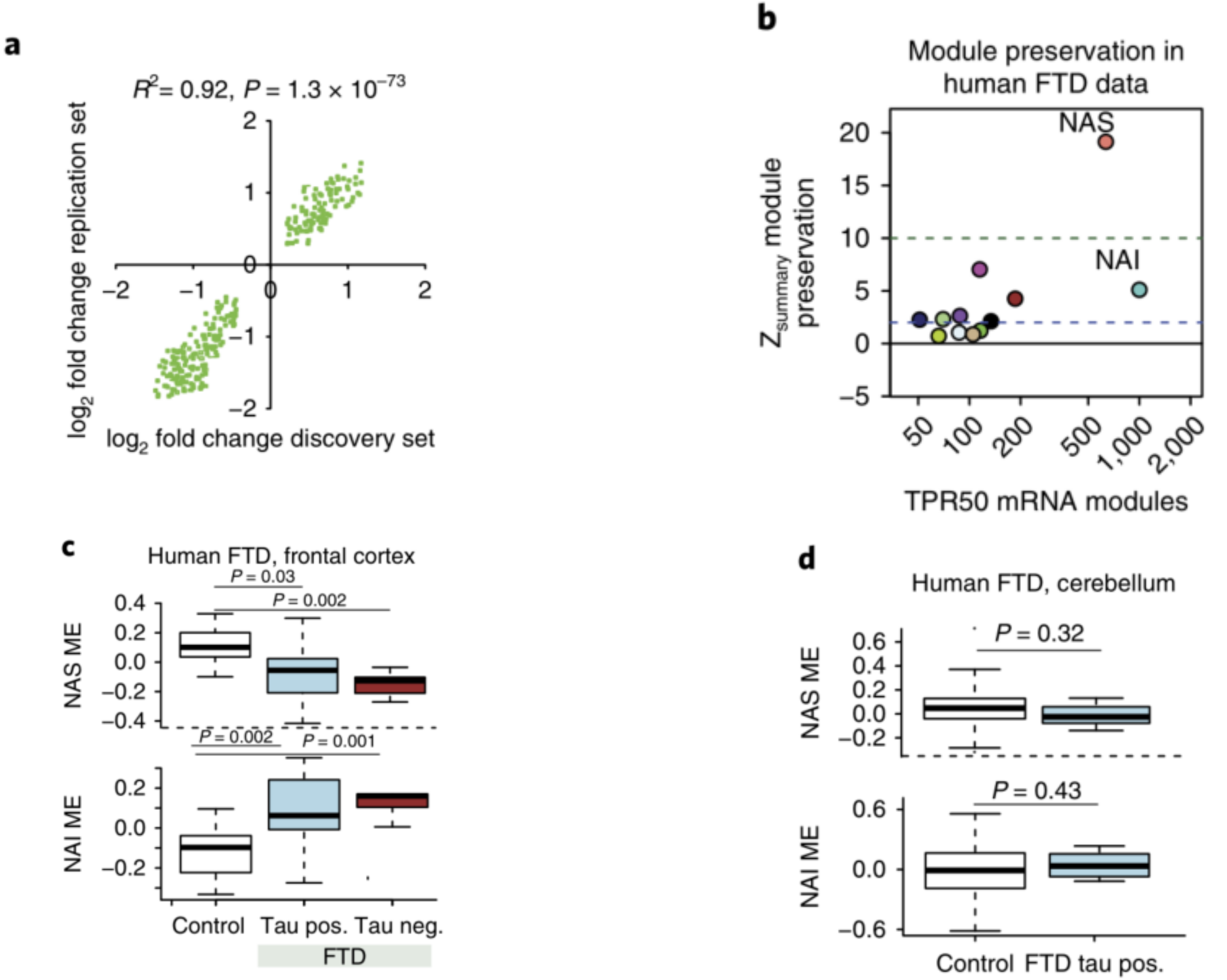


Human FTD Cerebellum (*Chen-Plotkin et. al., 2008*)

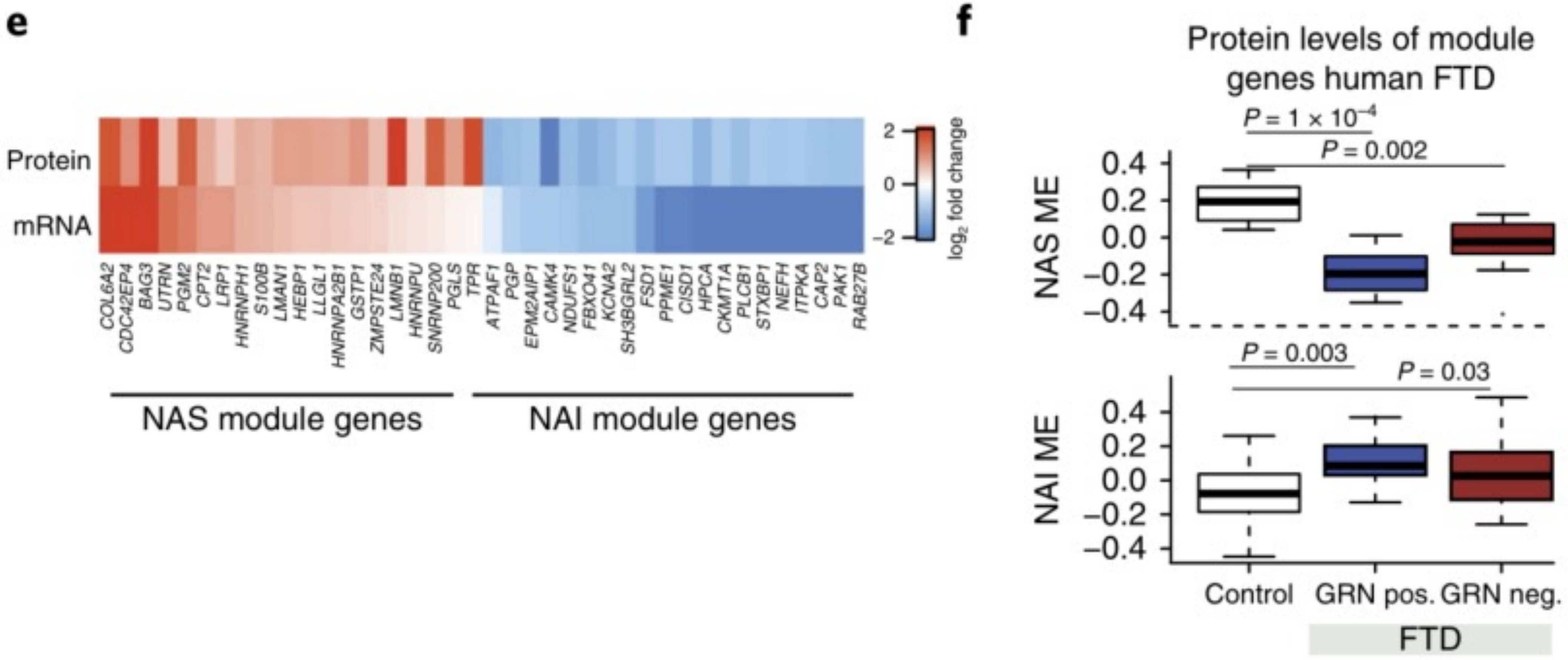


Human FTD iPSC-derived neurons (*Almeida et. al., 2012*)

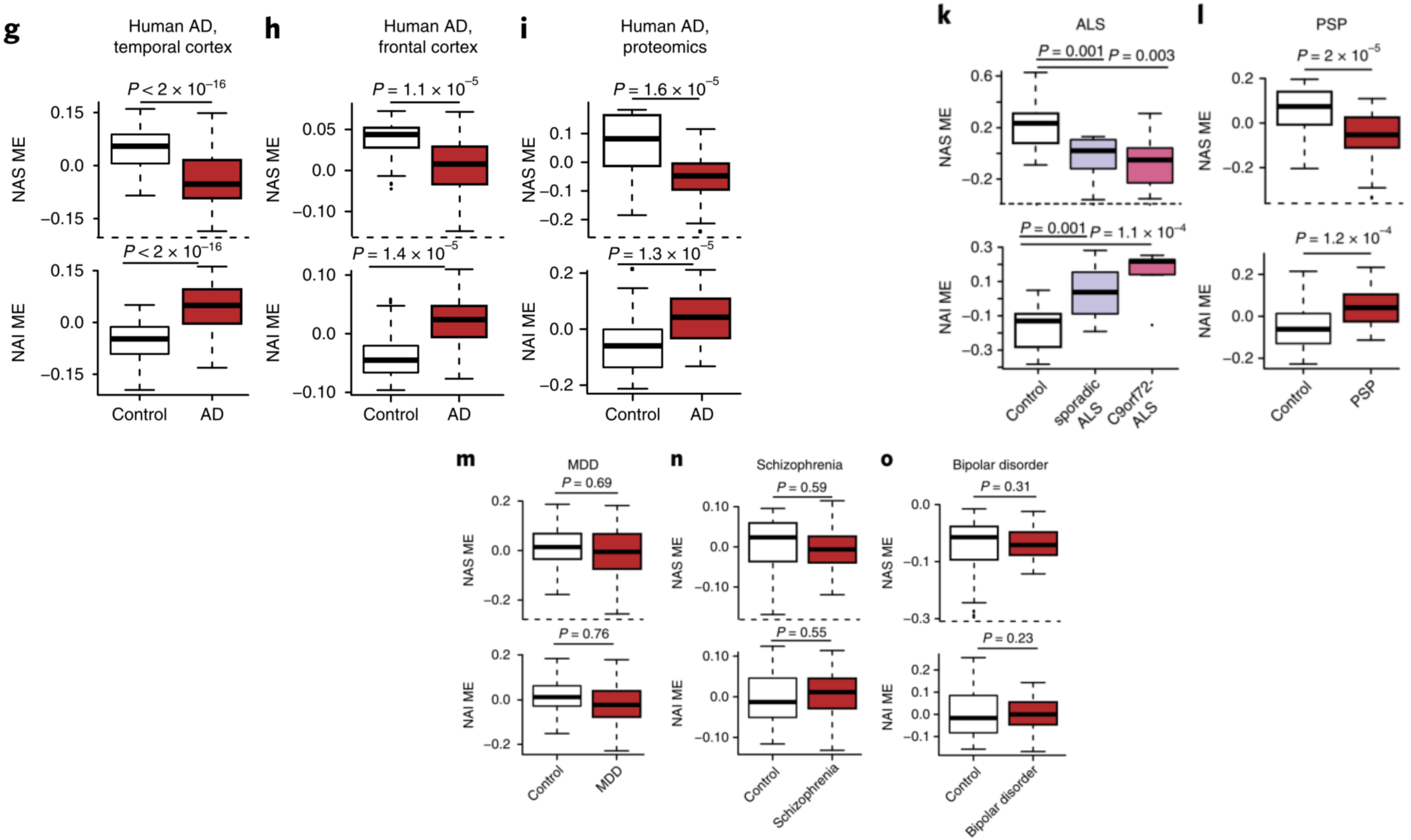
Preservation in newly generated human mRNA-seq data



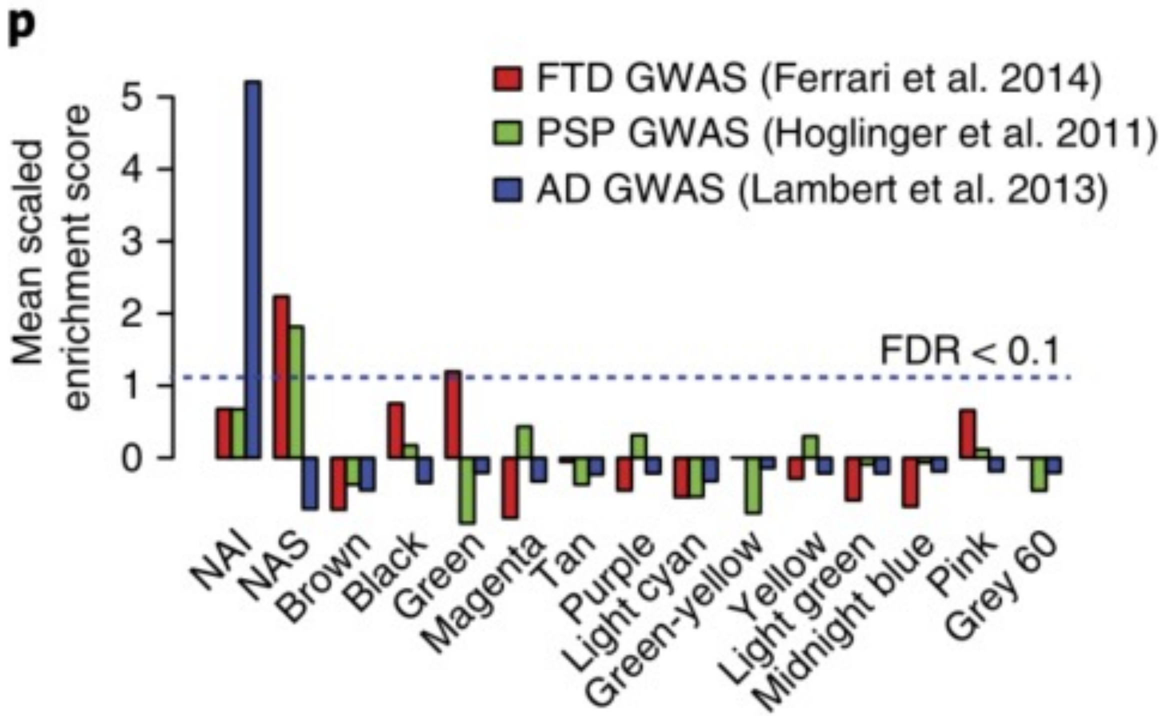
Similar expression patterns at the mRNA and protein level

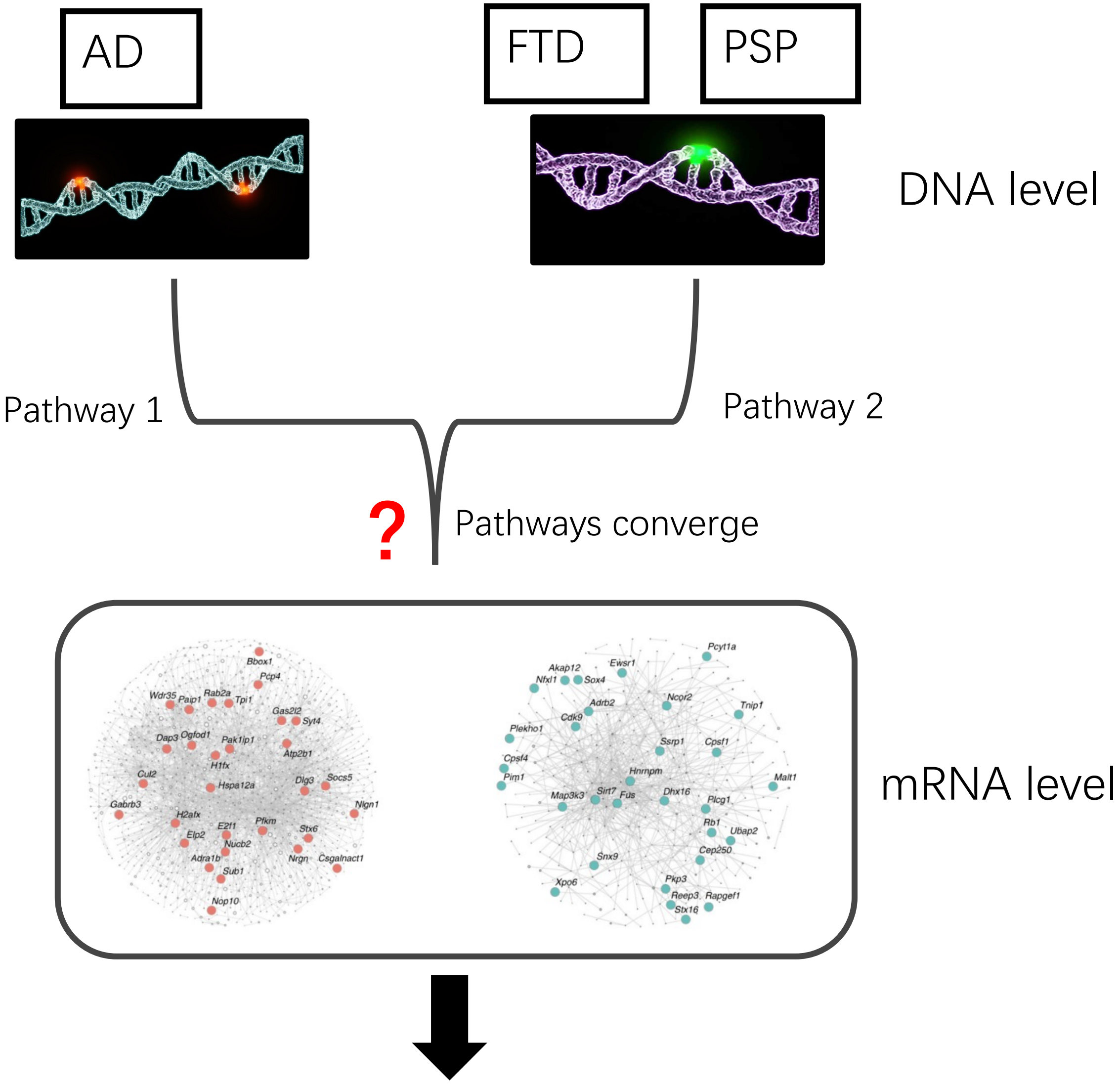


NAS and NAI are specific to neurodegenerative syndromes



Assessment of genetic risk within modules





AD

FTD

PSP

DNA level

Pathway 1

Pathway 2

?

Pathways converge

mRNA level

Background

Section 1. Experimental design

Section 2. Identification of disease-relevant mRNA modules

Section 3. Reproducibility of modules across mouse and human

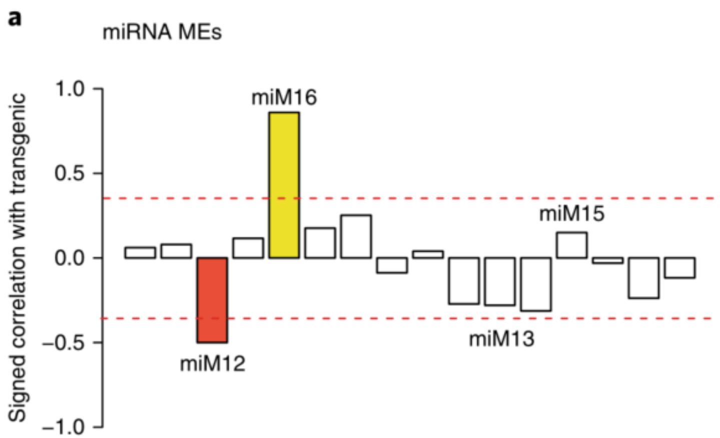
Section 4. Identification of potential miRNA drivers

Section 5. Identification of small molecules normalizing the modules

Summary

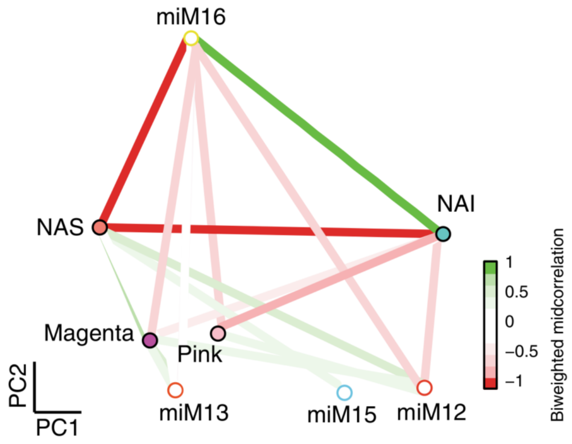
Identification of two miRNA modules

a

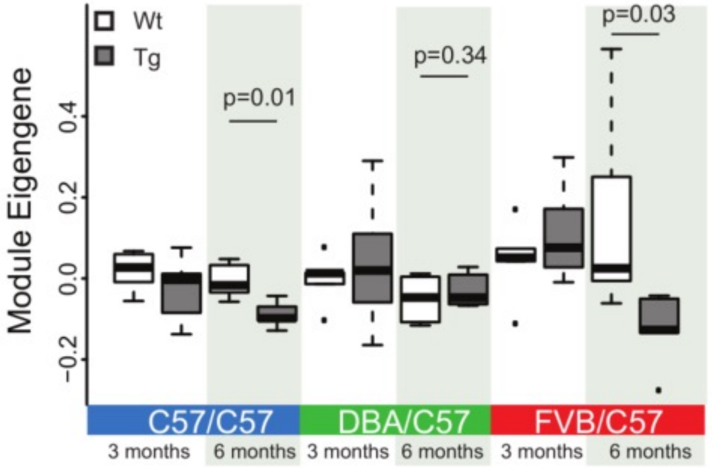


miRNA MEs

d

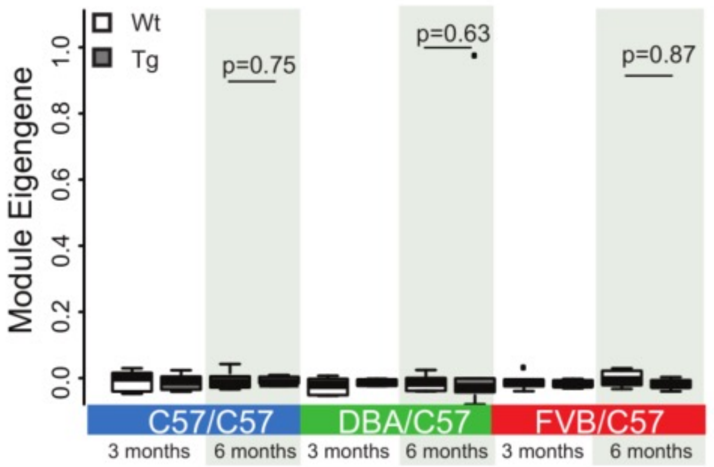


a



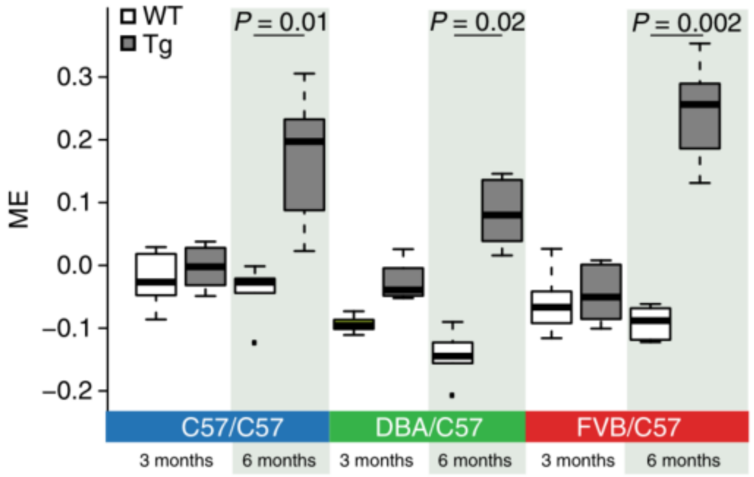
Expression of miM12 in Cortex

b



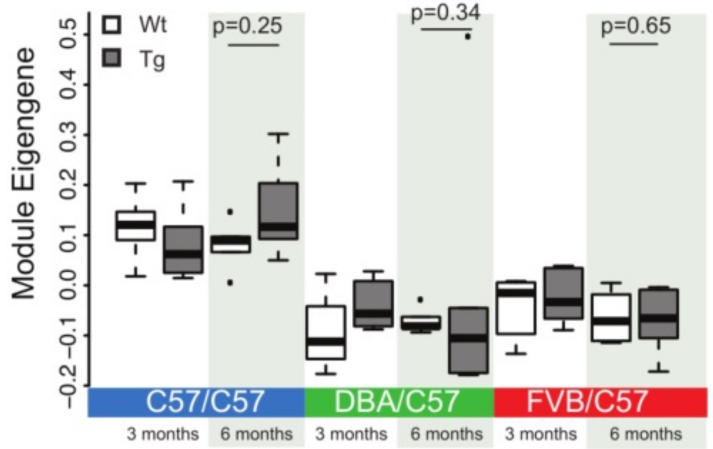
Expression of miM12 in Cerebellum

c



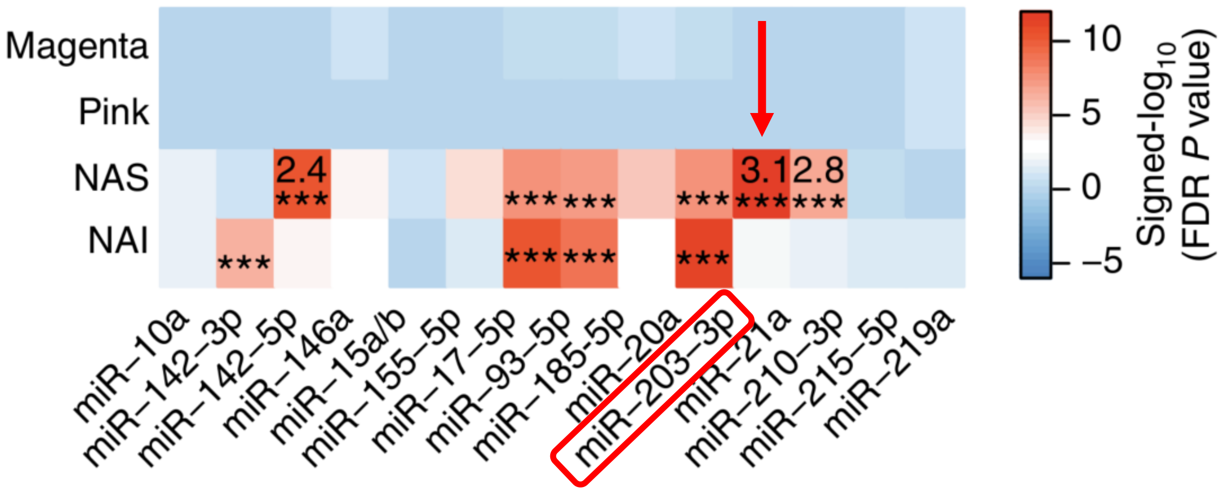
miM16 module trajectory in cortex

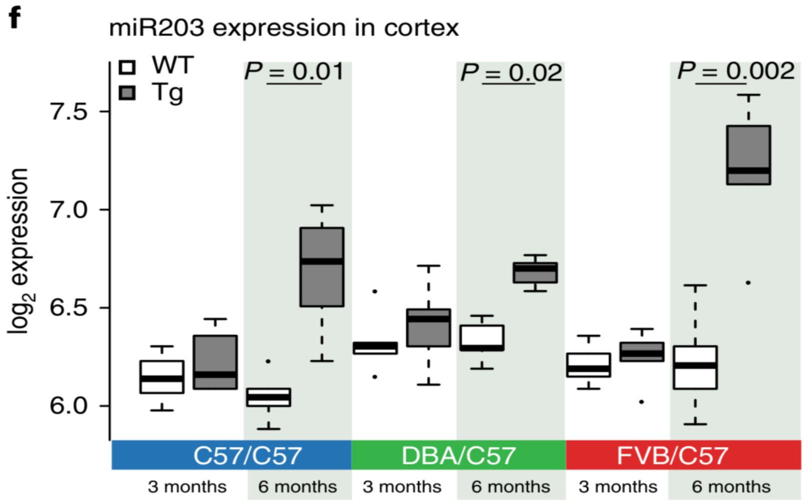
e

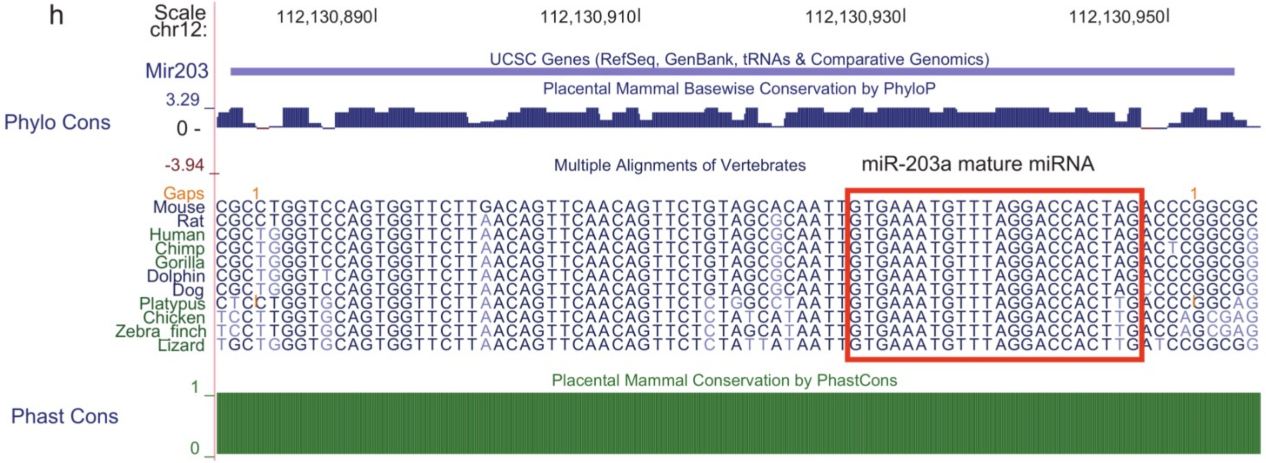


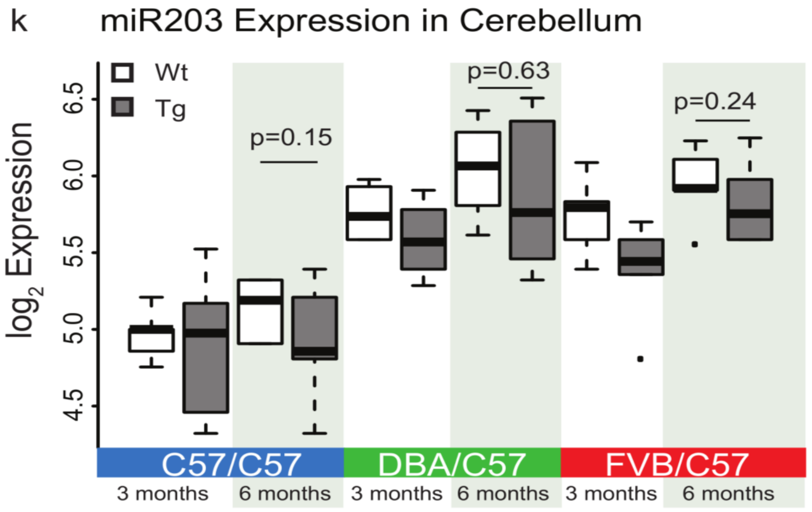
miM16 Module Trajectory in Cerebellum

Enrichment of miM16 module miRNA predicted targets in the mRNA modules

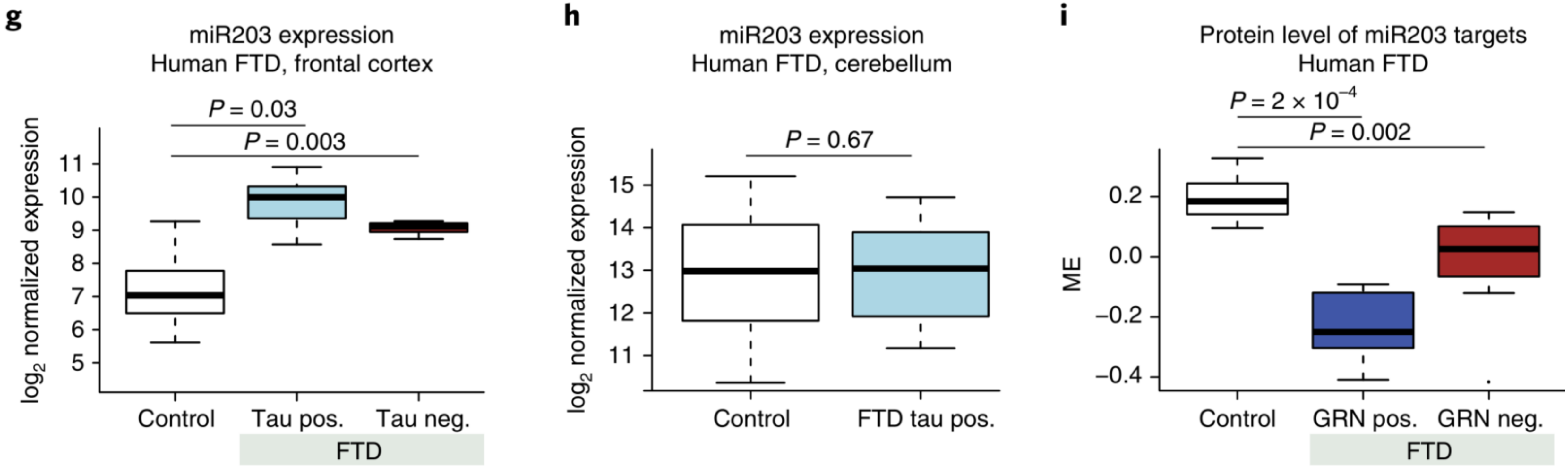






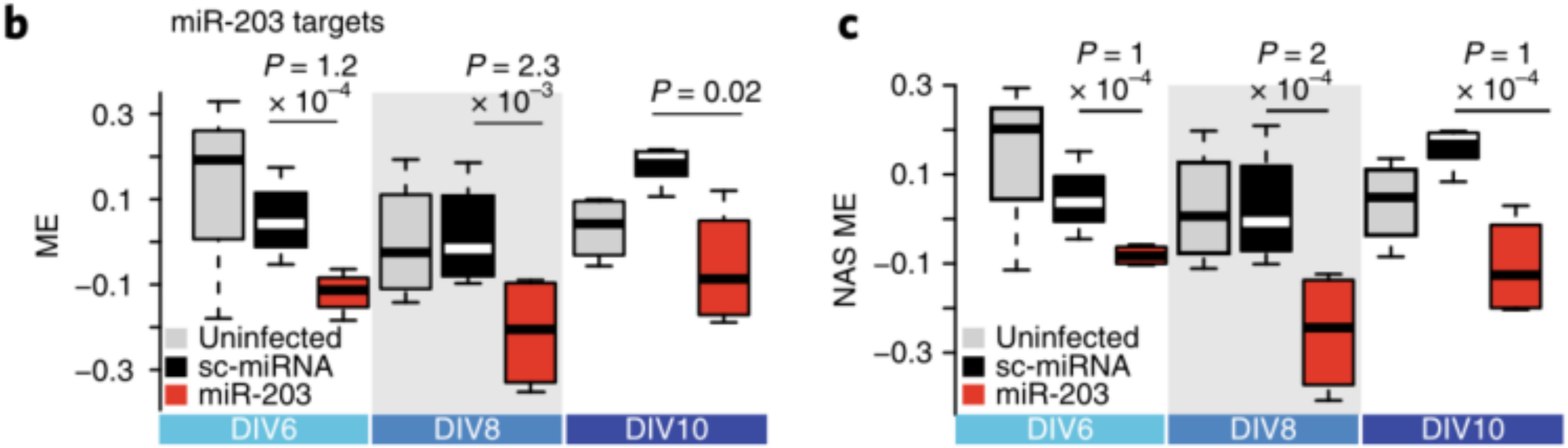


miR-203 expression and protein level of its predicted targets in human



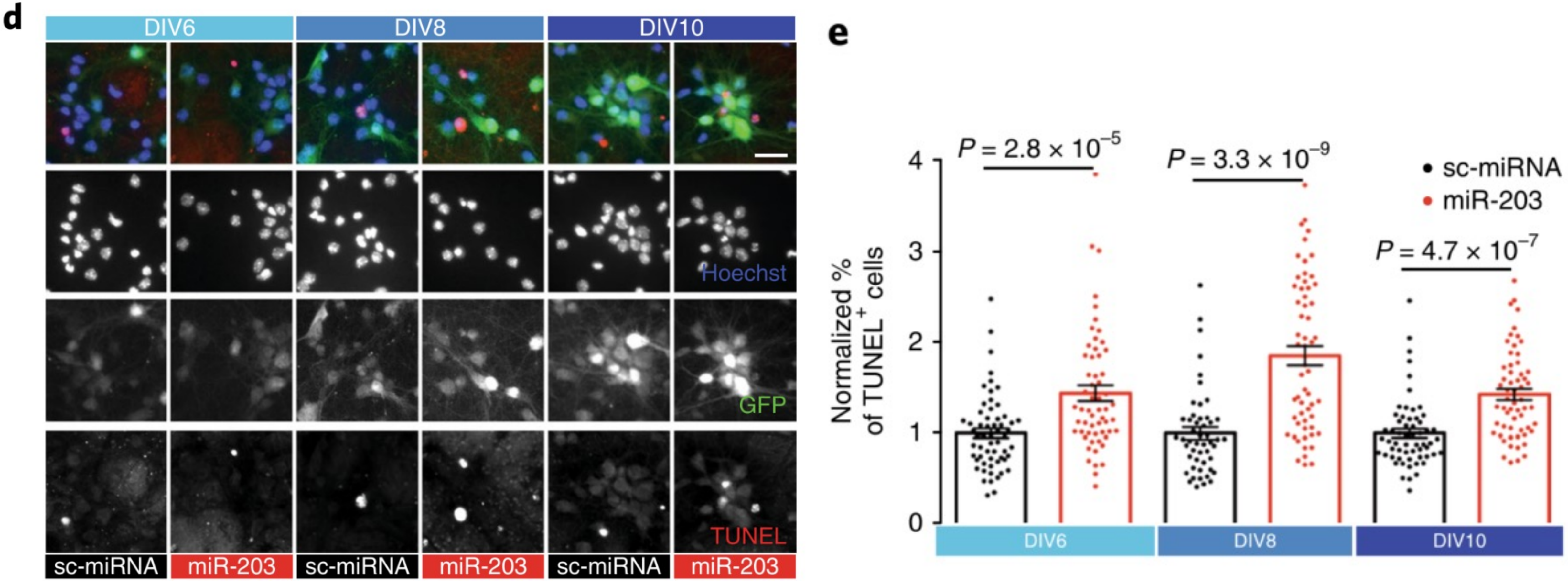
Overexpression of miR-203 in vitro (cortical mouse neuronal cultures)

control
sc = scrambled



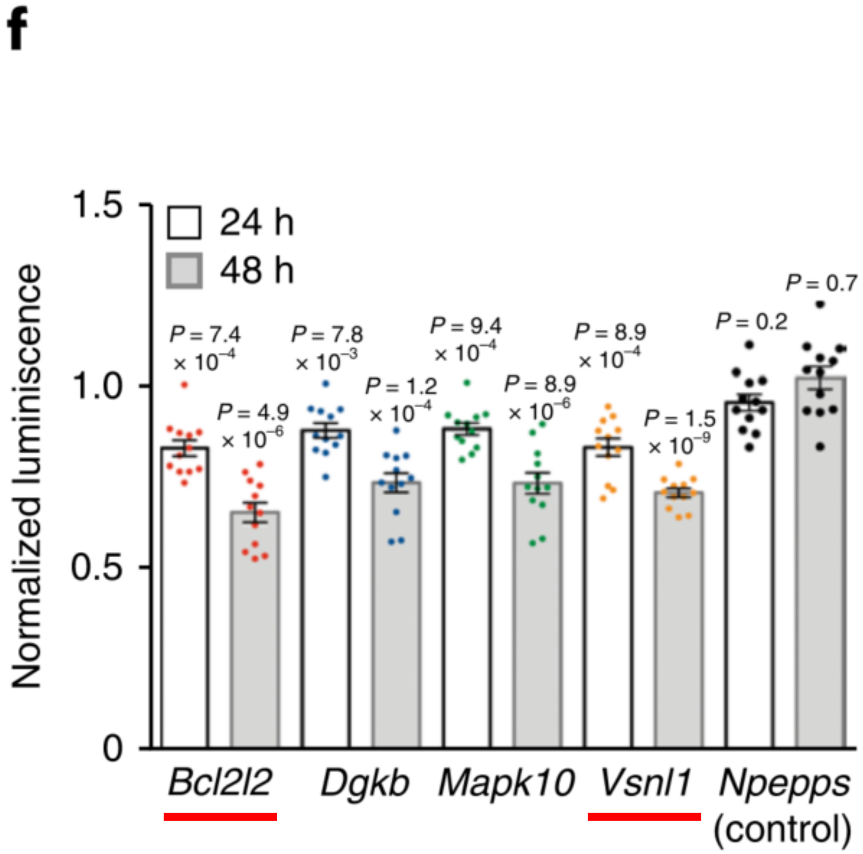
DIV = days in vitro

Overexpression of miR-203 in vitro (cortical mouse neuronal cultures)

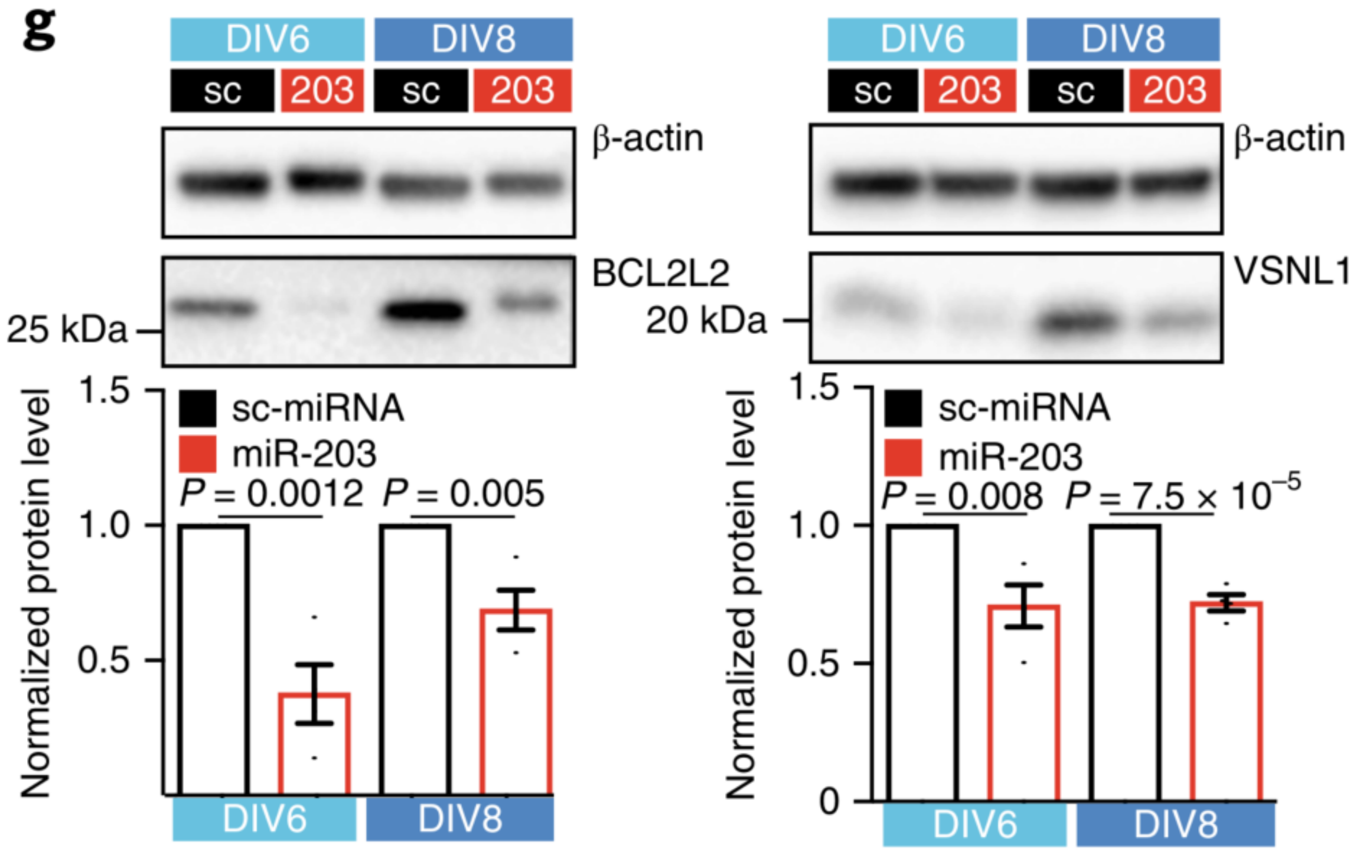


Increased number of apopstosis cells

Overexpression of miR-203 in vitro (cortical mouse neuronal cultures)



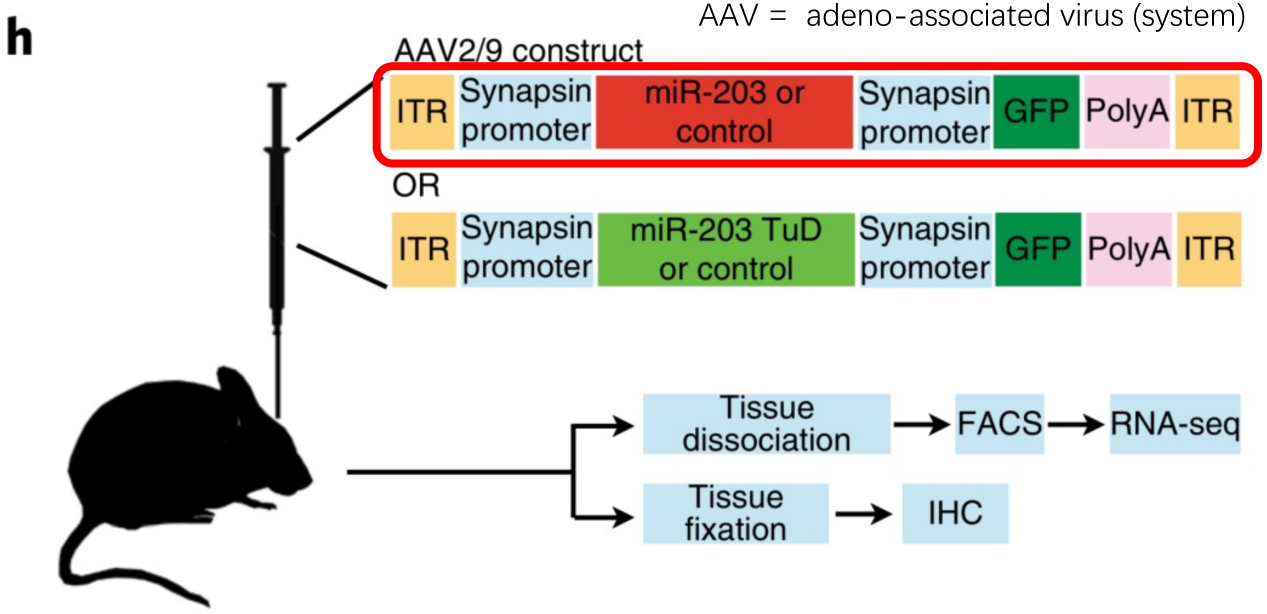
Luciferase reporter assay

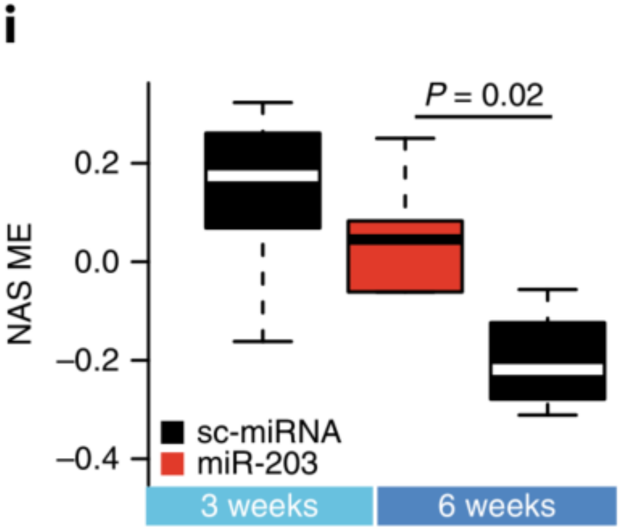


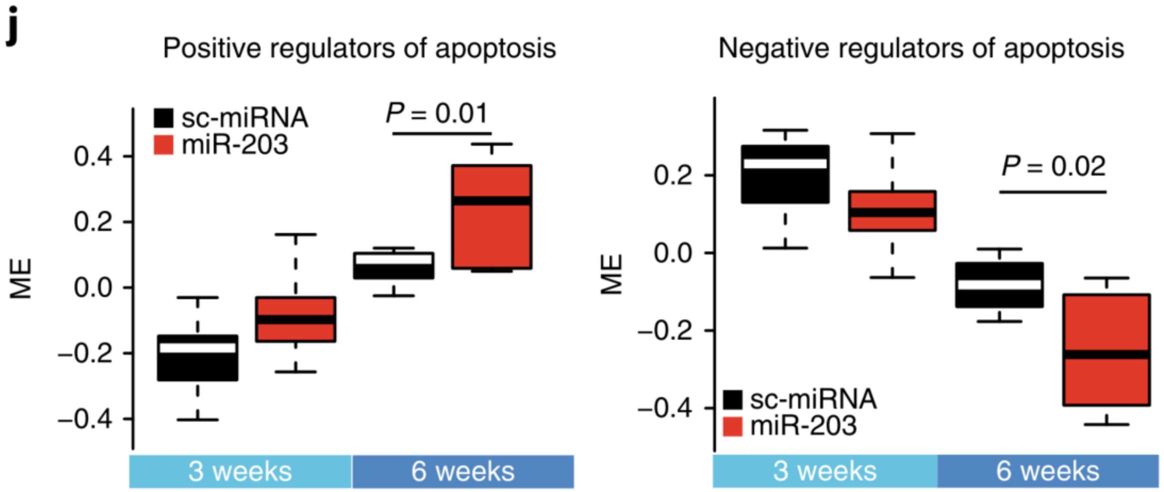
Western blot

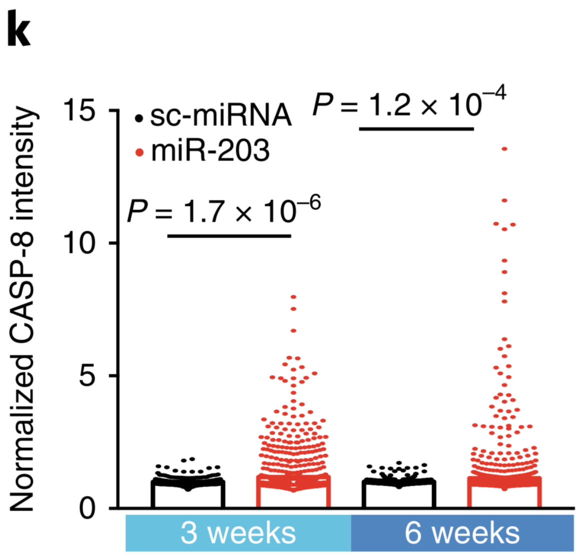
Overexpression of miR-203 in vivo (WT mouse)

AAV = adeno-associated virus (system)



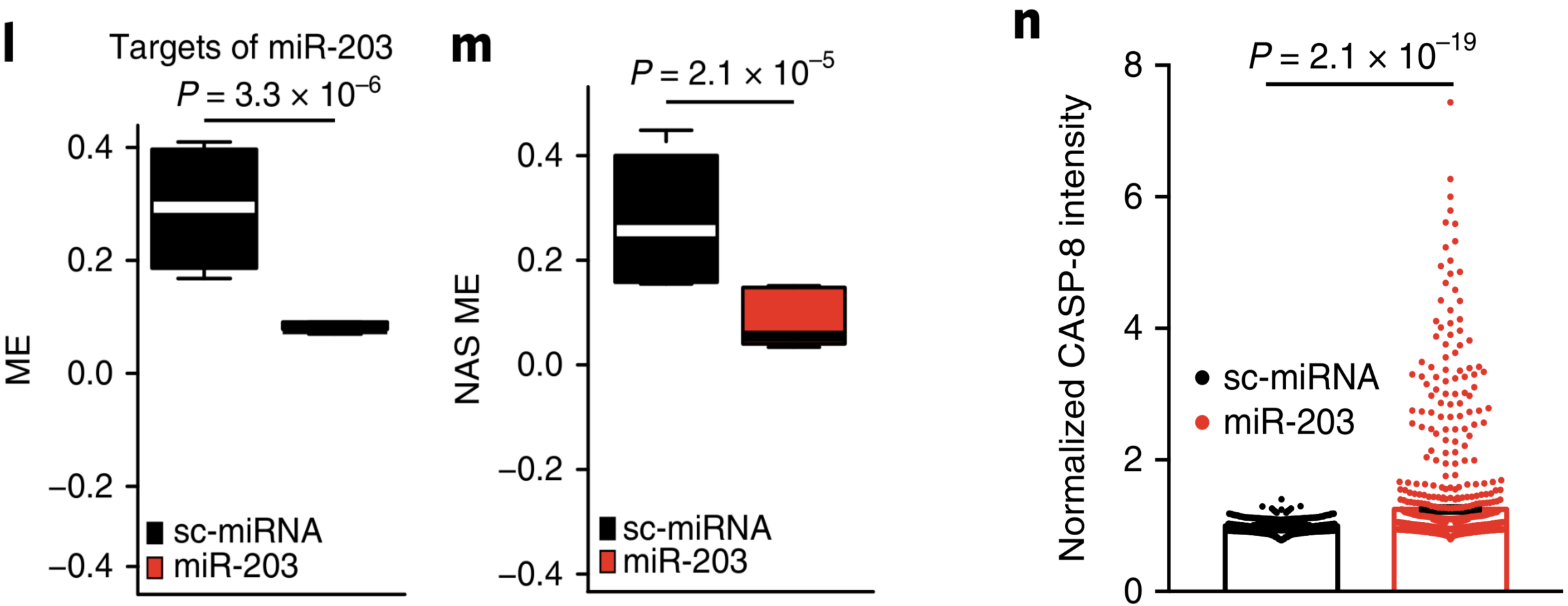




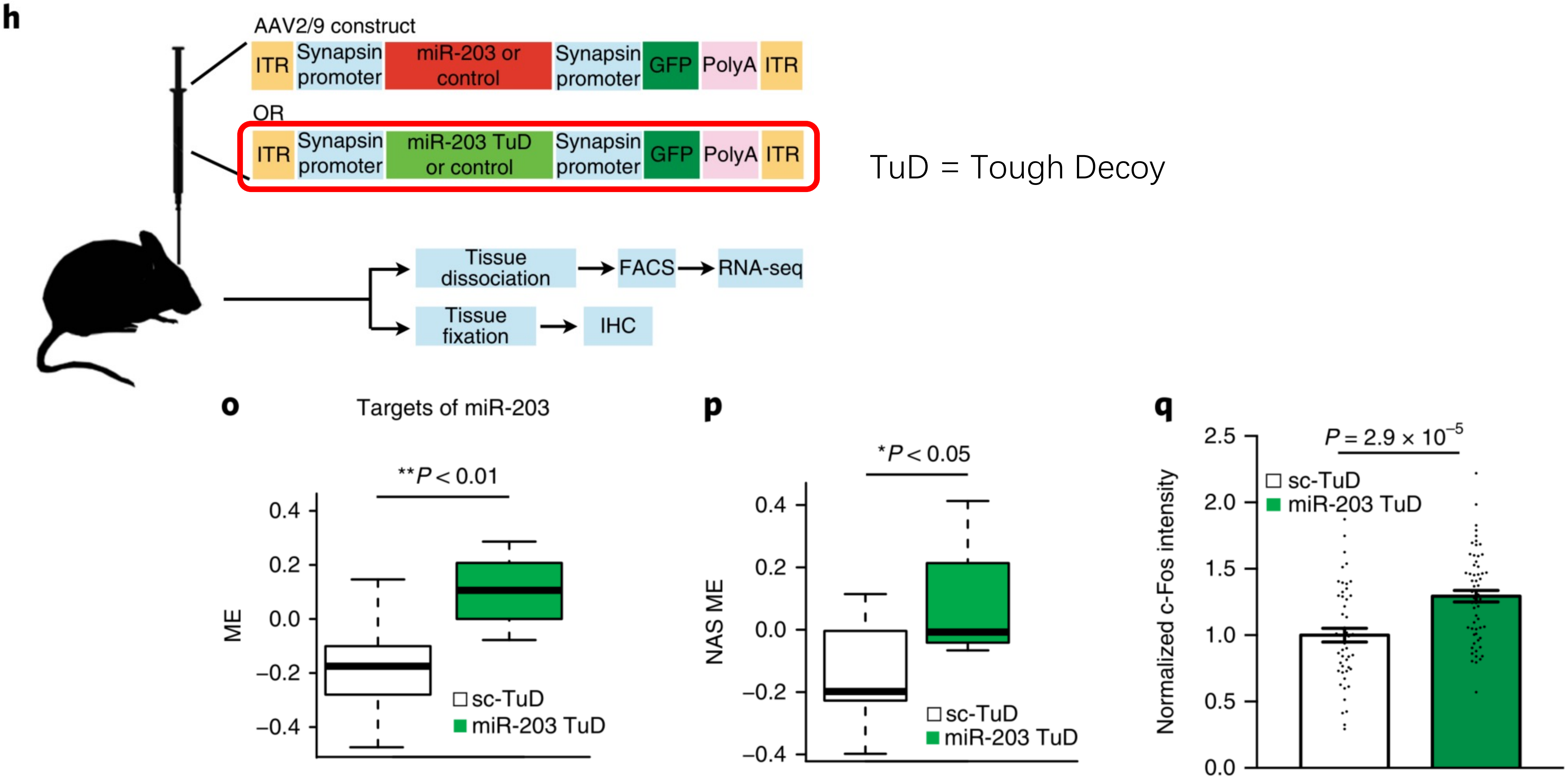


Overexpression of miR-203 in vivo (Tg4510 mouse)

overexpressing a mutant form of human tau



Inhibition of miR-203 in vivo (Tg4510 mouse)



TuD = Tough Decoy

Background

Section 1. Experimental design

Section 2. Identification of disease-relevant mRNA modules

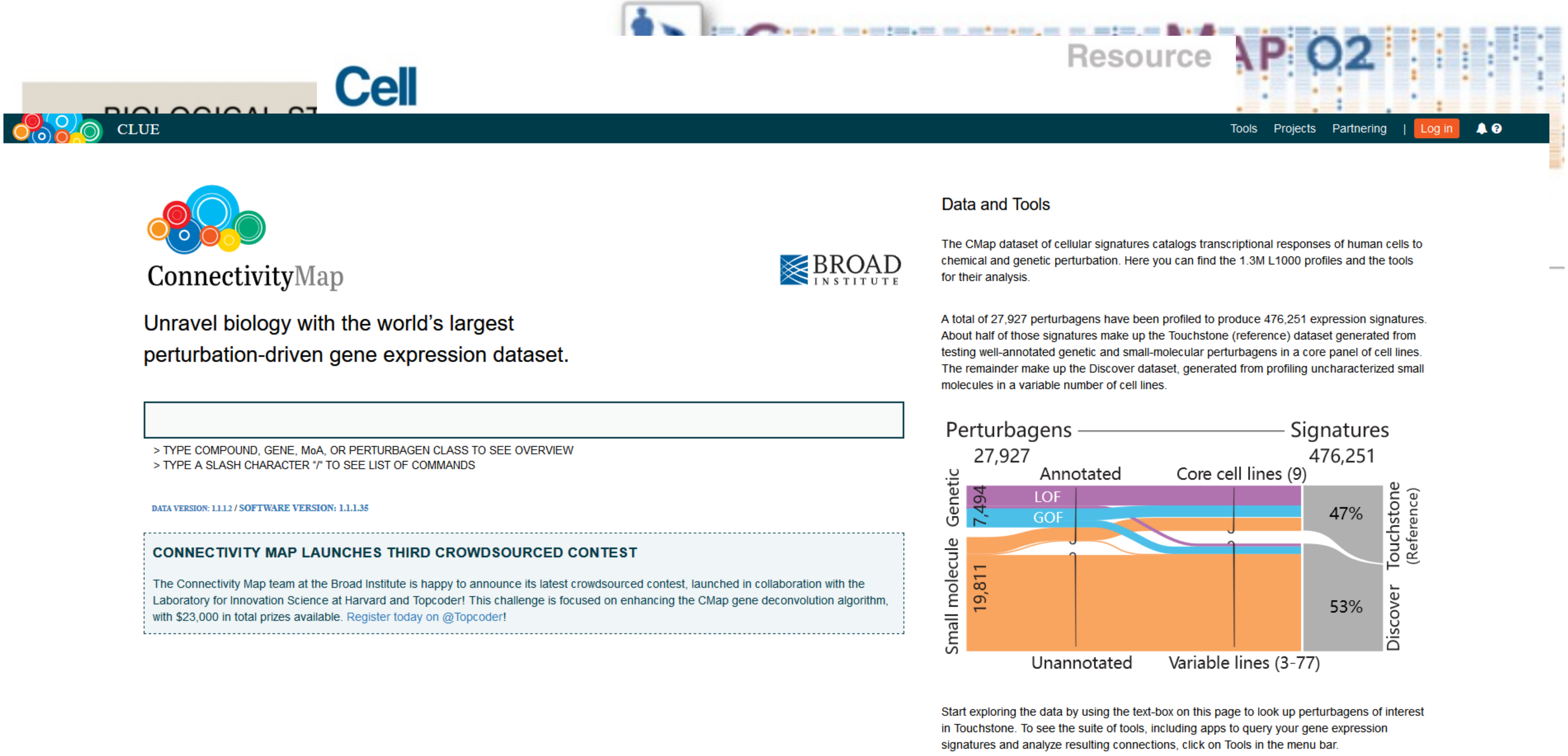
Section 3. Reproducibility of modules across mouse and human

Section 4. Identification of potential miRNA drivers

Section 5. Identification of small molecules normalizing the modules

Summary

Brief introduction of CMap database



Unravel biology with the world's largest perturbation-driven gene expression dataset.

> TYPE COMPOUND, GENE, MoA, OR PERTURBAGEN CLASS TO SEE OVERVIEW
> TYPE A SLASH CHARACTER "/" TO SEE LIST OF COMMANDS

DATA VERSION: 1.1.1.2 / SOFTWARE VERSION: 1.1.1.35

CONNECTIVITY MAP LAUNCHES THIRD CROWDSOURCED CONTEST

The Connectivity Map team at the Broad Institute is happy to announce its latest crowdsourced contest, launched in collaboration with the Laboratory for Innovation Science at Harvard and Topcoder! This challenge is focused on enhancing the CMap gene deconvolution algorithm, with $23,000 in total prizes available. Register today on @Topcoder!

Data and Tools

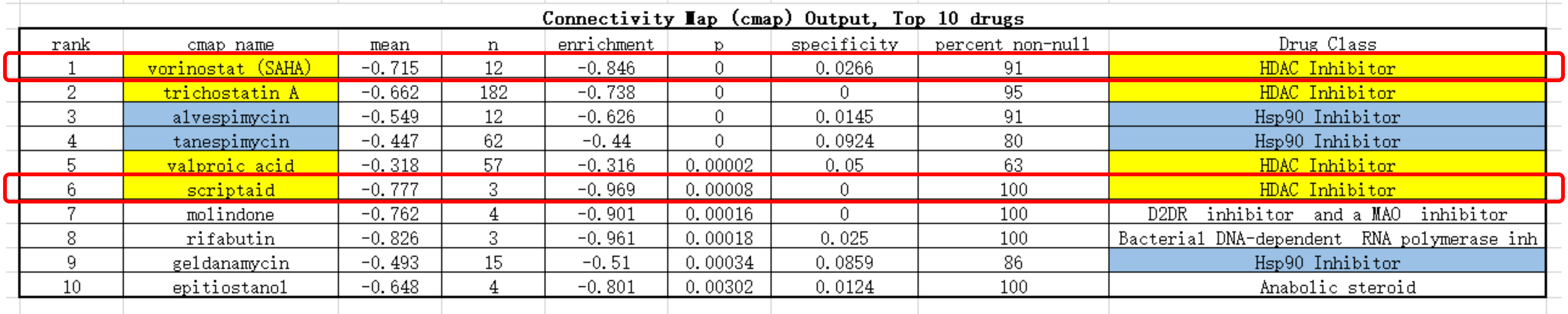
The CMap dataset of cellular signatures catalogs transcriptional responses of human cells to chemical and genetic perturbation. Here you can find the 1.3M L1000 profiles and the tools for their analysis.

A total of 27,927 perturbagens have been profiled to produce 476,251 expression signatures. About half of those signatures make up the Touchstone (reference) dataset generated from testing well-annotated genetic and small-molecular perturbagens in a core panel of cell lines. The remainder make up the Discover dataset, generated from profiling uncharacterized small molecules in a variable number of cell lines.

Start exploring the data by using the text-box on this page to look up perturbagens of interest in Touchstone. To see the suite of tools, including apps to query your gene expression signatures and analyze resulting connections, click on Tools in the menu bar.

Cmap_old: https://portals.broadinstitute.org/cmap/
Cmap_new: https://clue.io

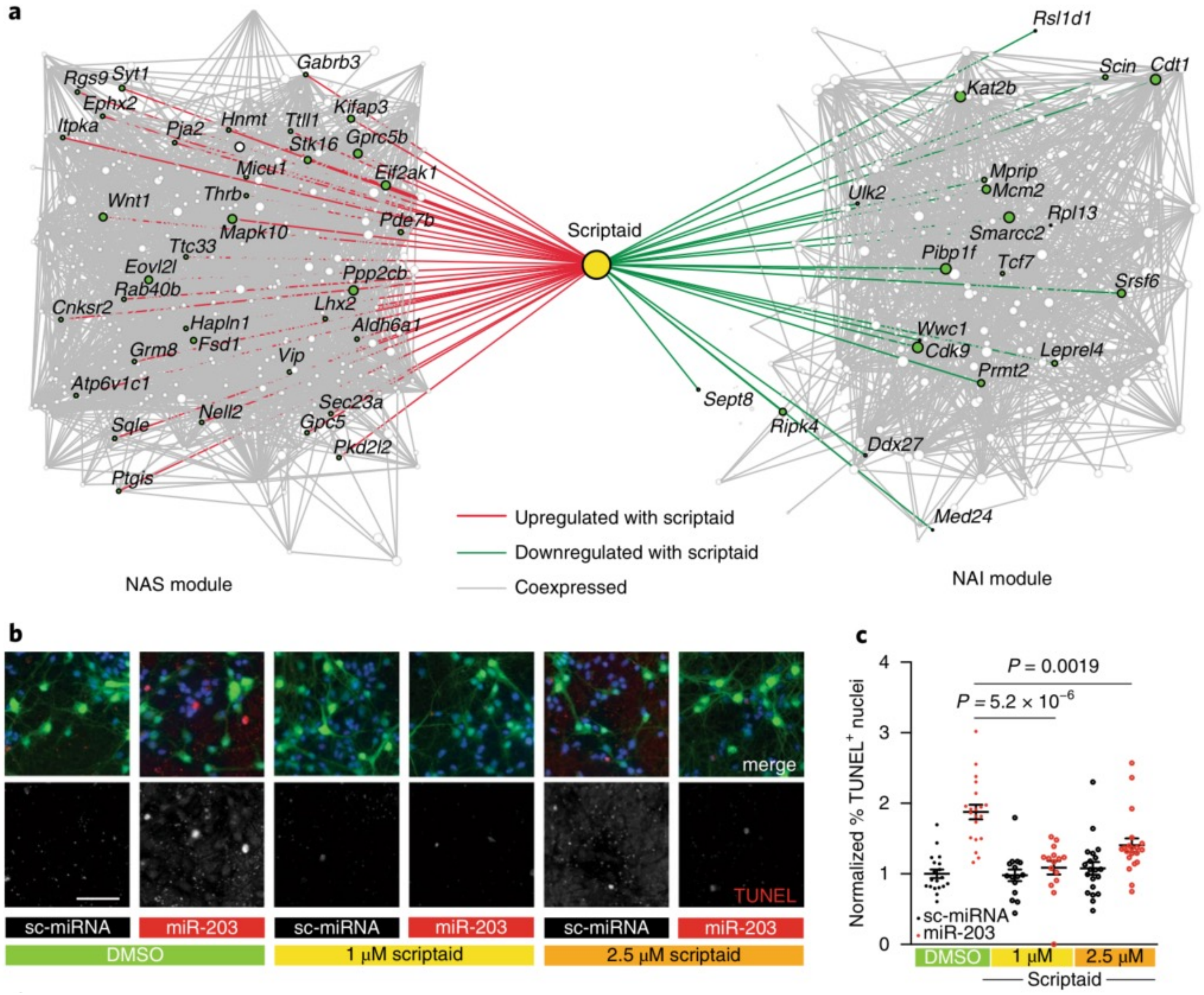
Identification of small molecules predicted to reverse the NAS or NAI changes



Connectivity Map (cmap) Output, Top 10 drugs

rank	cmap name	mean	n	enrichment	p	specificity	percent non-null	Drug Class
1	vorinostat (SAHA)	-0.715	12	-0.846	0	0.0266	91	HDAC Inhibitor
2	trichostatin A	-0.662	182	-0.738	0	0	95	HDAC Inhibitor
3	alvespimycin	-0.549	12	-0.626	0	0.0145	91	Hsp90 Inhibitor
4	tanespimycin	-0.447	62	-0.44	0	0.0924	80	Hsp90 Inhibitor
5	valproic acid	-0.318	57	-0.316	0.00002	0.05	63	HDAC Inhibitor
6	scriptaid	-0.777	3	-0.969	0.00008	0	100	HDAC Inhibitor
7	molindone	-0.762	4	-0.901	0.00016	0	100	D2DR inhibitor and a MAO inhibitor
8	rifabutin	-0.826	3	-0.961	0.00018	0.025	100	Bacterial DNA-dependent RNA polymerase inh
9	geldanamycin	-0.493	15	-0.51	0.00034	0.0859	86	Hsp90 Inhibitor
10	epitiostanol	-0.648	4	-0.801	0.00302	0.0124	100	Anabolic steroid

Scriptaid inhibition of miR-203-induced cell death in vitro

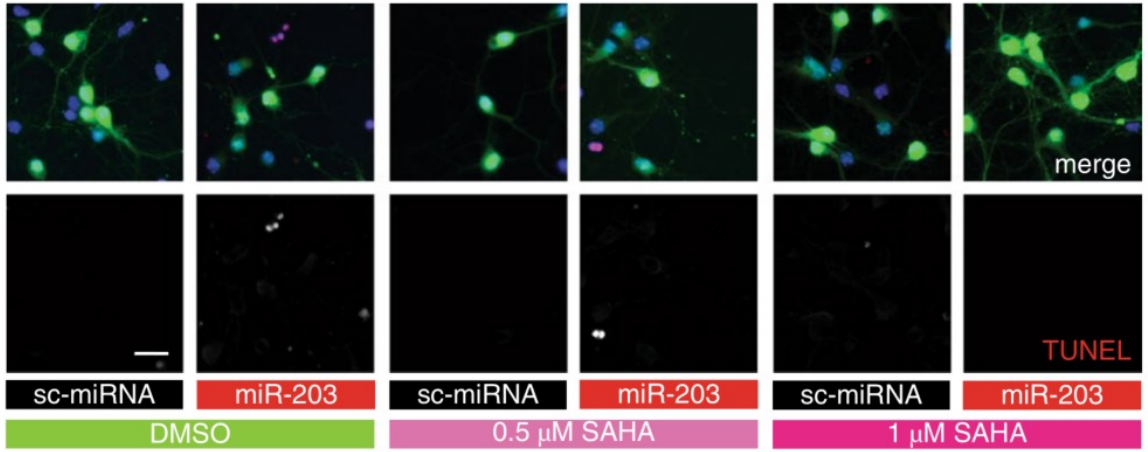


DMSO = dimethylsulfoxide
(control)

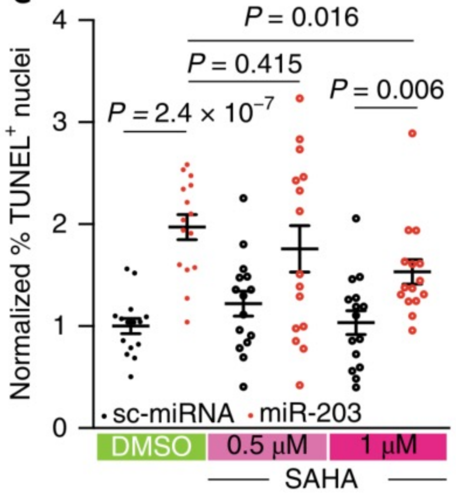
SAHA inhibition of miR-203-induced cell death in vitro

SAHA = suberanilohydroxamic acid (i.e., vorinostat, 伏立诺他)

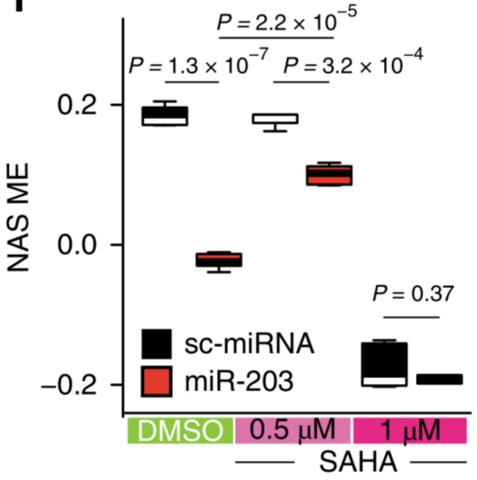
d



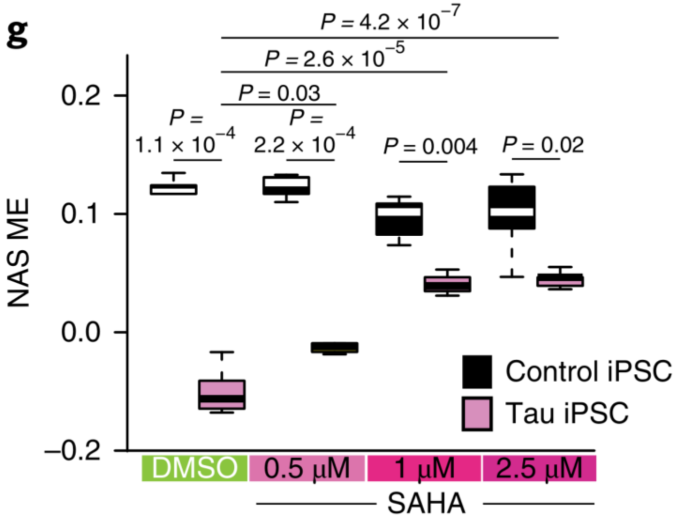
e



f



g



Background

Section 1. Experimental design

Section 2. Identification of disease-relevant mRNA modules

Section 3. Reproducibility of modules across mouse and human

Section 4. Identification of potential miRNA drivers

Section 5. Identification of small molecules normalizing the modules

Summary

- Identifying two neurodegenerative dementia-relevant gene coexpression modules that are preserved in mice and human
- Overexpression of miR-203, a hub of a putative regulatory miRNA module, recapitulates mRNA coexpression patterns, establishing this miRNA as a regulator of neurodegeneration
- Identifying small molecules that can normalize the disease-associated modules and validating this experimentally

Thanks for
attention!