Integrative Proteomics for Novel Target and Biomarker Discovery

Allan Levey and the Emory Proteomics AMP-AD Team

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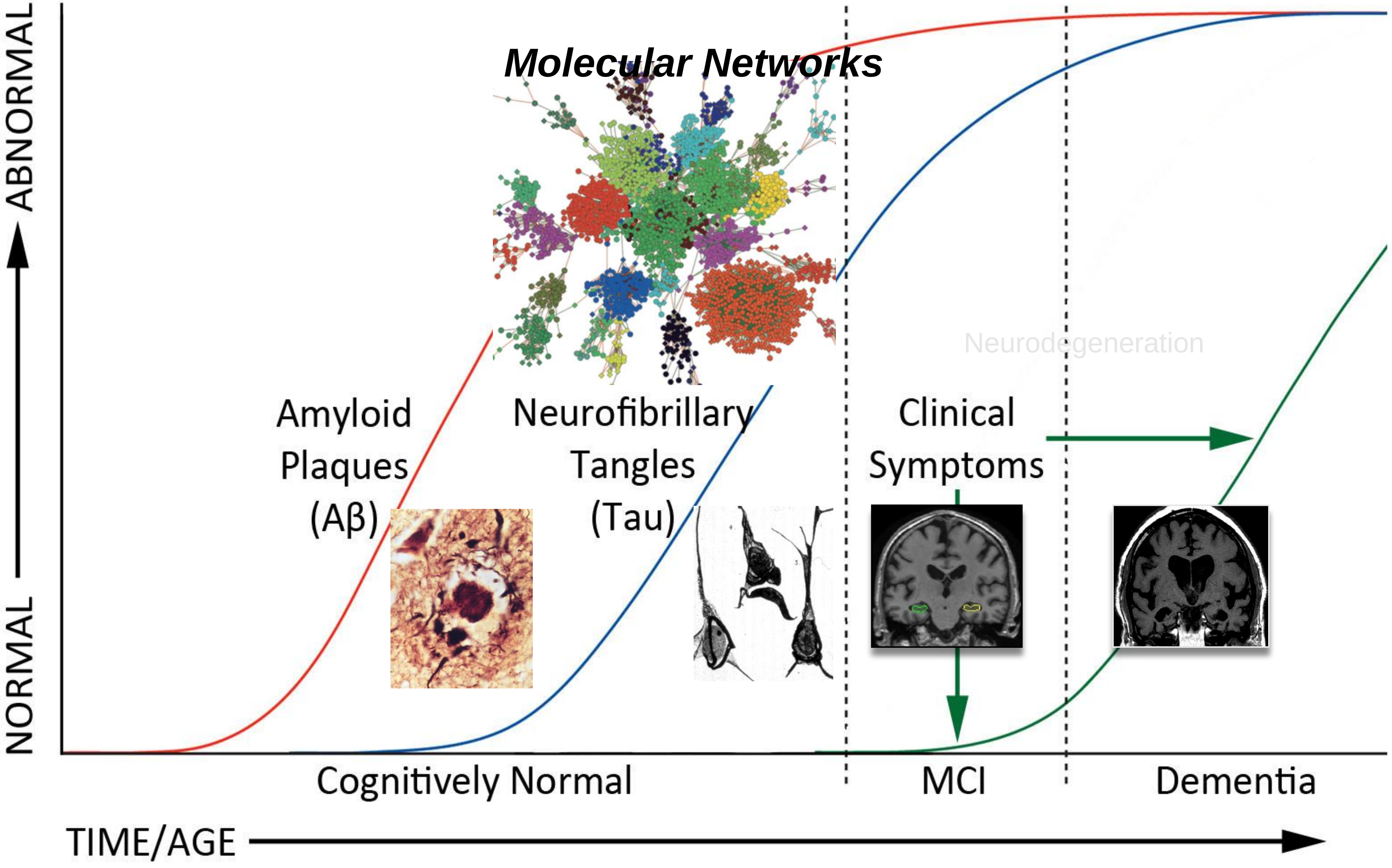
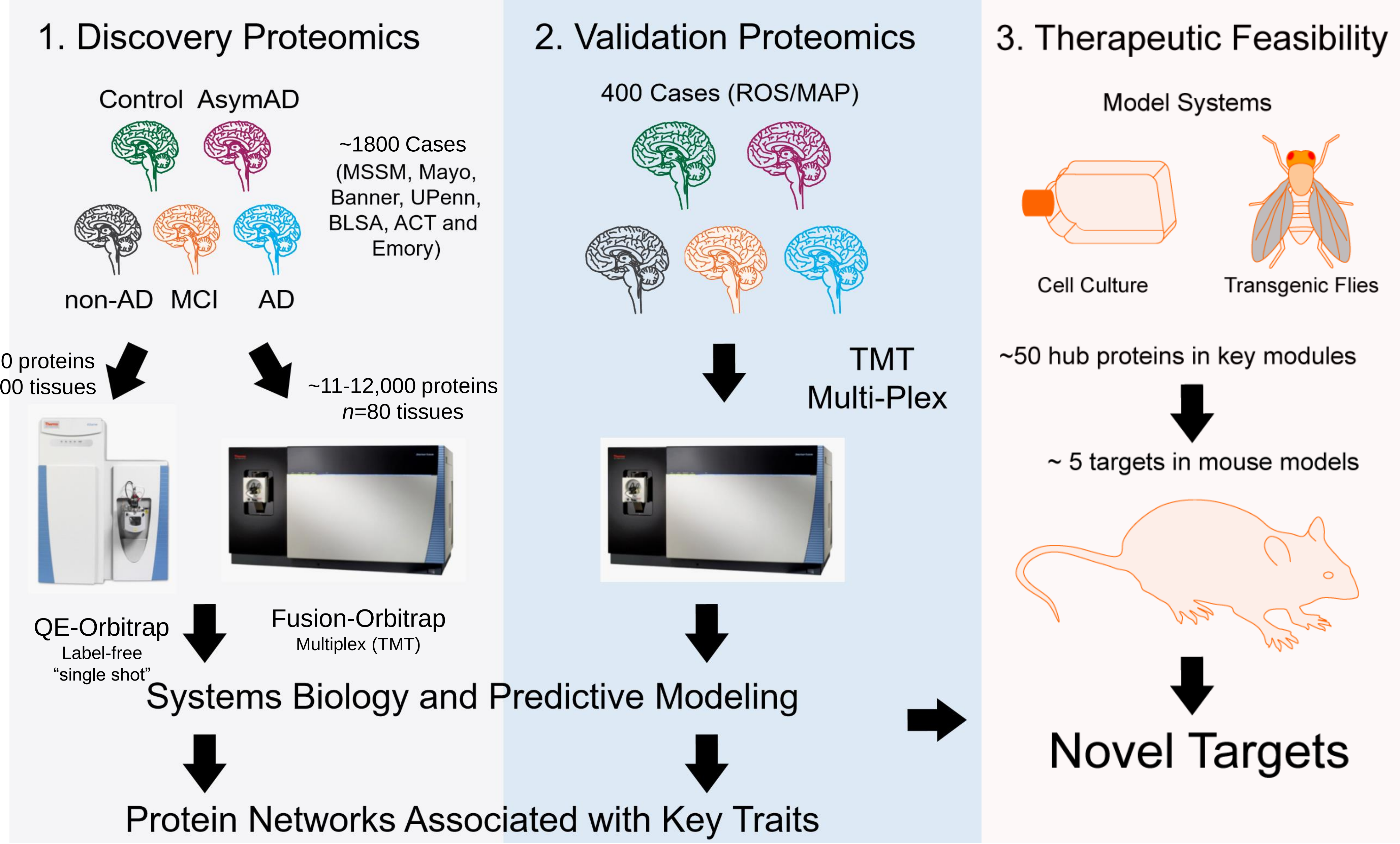
Emory School of Medicine
Atlanta, GA

Goal: Identify Pathophysiological Processes Linked to Asymptomatic AD, Dementia Symptoms, and Progressive Neurodegeneration



AMP-AD: Integrative Proteomics for Novel Target and Biomarker Discovery



1. Discovery Proteomics

Control AsymAD

non-AD MCI AD

~1800 Cases (MSSM, Mayo, Banner, UPenn, BLSA, ACT and Emory)

~3,000 proteins
n=1,000 tissues

~11-12,000 proteins
n=80 tissues

QE-Orbitrap
Label-free
“single shot”

Fusion-Orbitrap
Multiplex (TMT)

2. Validation Proteomics

400 Cases (ROS/MAP)

TMT
Multi-Plex

Systems Biology and Predictive Modeling

Protein Networks Associated with Key Traits

3. Therapeutic Feasibility

Model Systems

Cell Culture

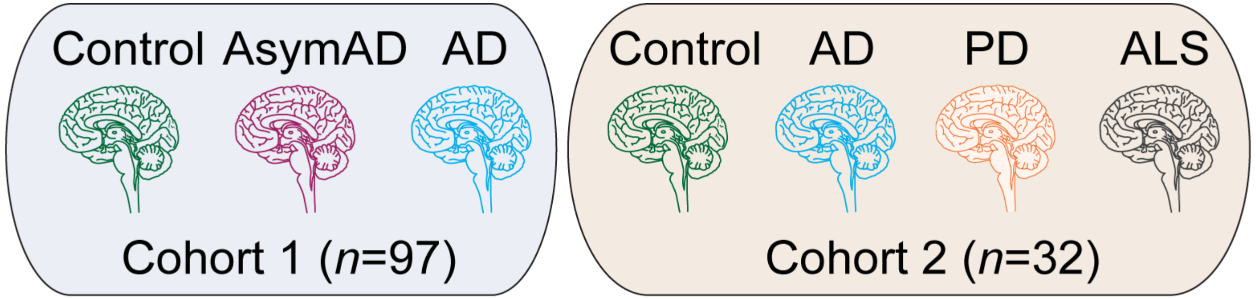
Transgenic Flies

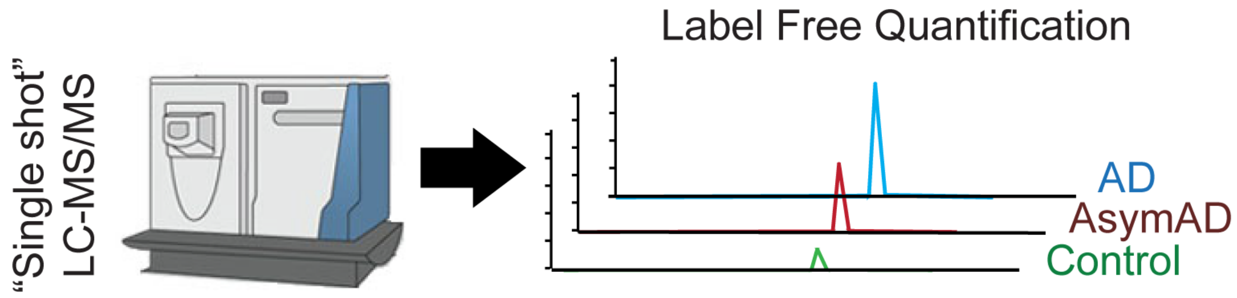
~50 hub proteins in key modules

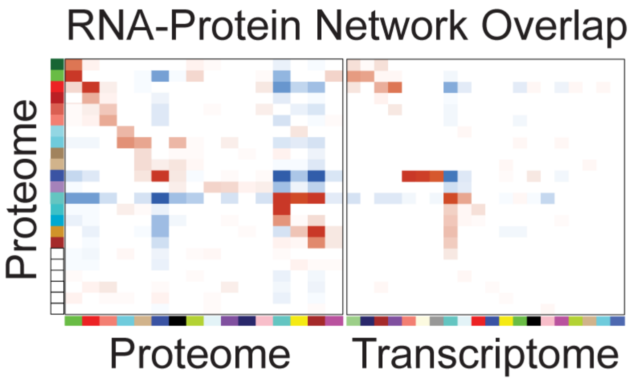
~ 5 targets in mouse models

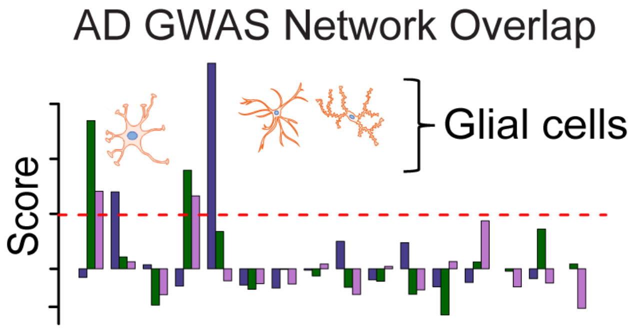
Novel Targets

A Multi-network Approach Identifies Protein-Specific Co-expression in AD









- 129 human cortical tissues
- 64,938 peptides mapped to 5,130 proteins
- Network analysis (~2500 proteins identified in >90% of cases) showed 16 modules of co-expressed proteins
- Modules associated with brain cell types overlapped in protein and RNA networks
- 10 protein modules correlated with cognition and Alzheimer's disease (AD) pathology
- Many protein-based modules were distinct from those in RNA-directed networks
- AD risk loci converged in glial-related modules in the proteome and transcriptome

Seyfried NT, et al (2017) *Cell Systems* **4**:60-72

Cell Systems

AMP-AD Proteomics of Human Postmortem Brain

Table 1. AMP-AD Brains Processed for Mass Spectrometry

AMP-AD Team/Cohort	Cortical Region	# Samples Analysed				
		Con	Asym AD	AD	Other*	Total
Emory						
Emory ADRC	DLPFC	48	8	63	68	187
BLSA	DLPFC / Precuneus	28	29	40	-	97
Adult Changes Thought	DLPFC	12	15	39	-	66
Banner ADRC	DLPFC	61	40	100	-	201
Penn ADRC	DLPFC	42	19	50	258	369
Mayo/UF ADRC	Temporal	31	-	84	84	199
Mt. Sinai (MSSM) ADRC	DLPFC	51	-	154	61	266
ROS/MAP** Rush ADRC	DLPFC	149	-	292	-	441
Total		422	111	822	471	**1826**

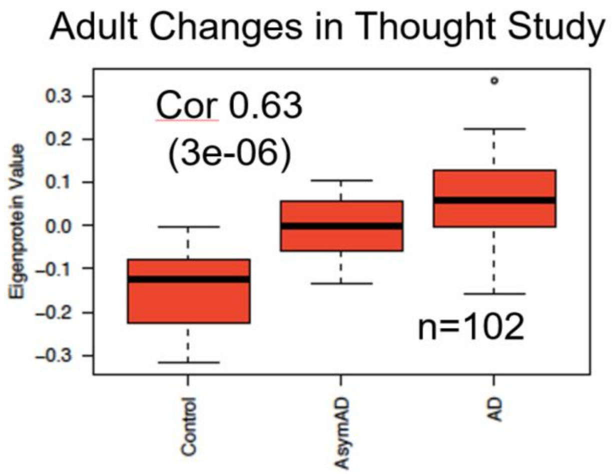
*Other includes non-AD diseases - FTLD, ALS, DLB, PD, MSA, Picks, PSP
**ROS/MAP in progress; to be completed summer 2018

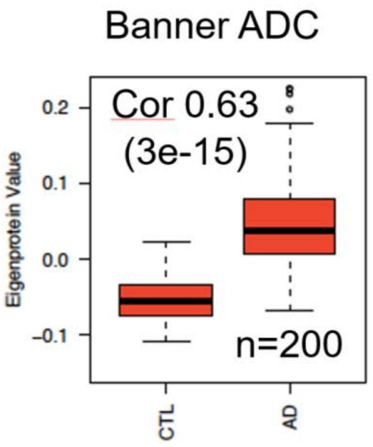
Synapse

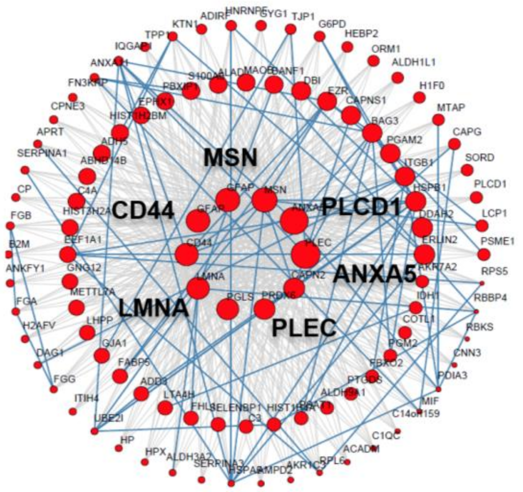
AMP-AD Knowledge Portal

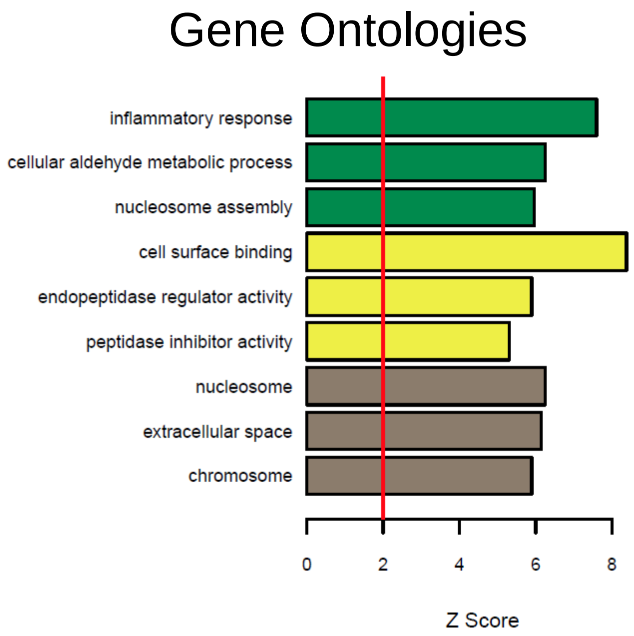
Data is publically available:
www.synapse.org/ampad

Consensus Networks Reveal Modules & Key Drivers Linked to AD Phenotypes

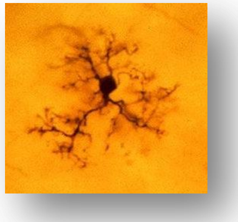








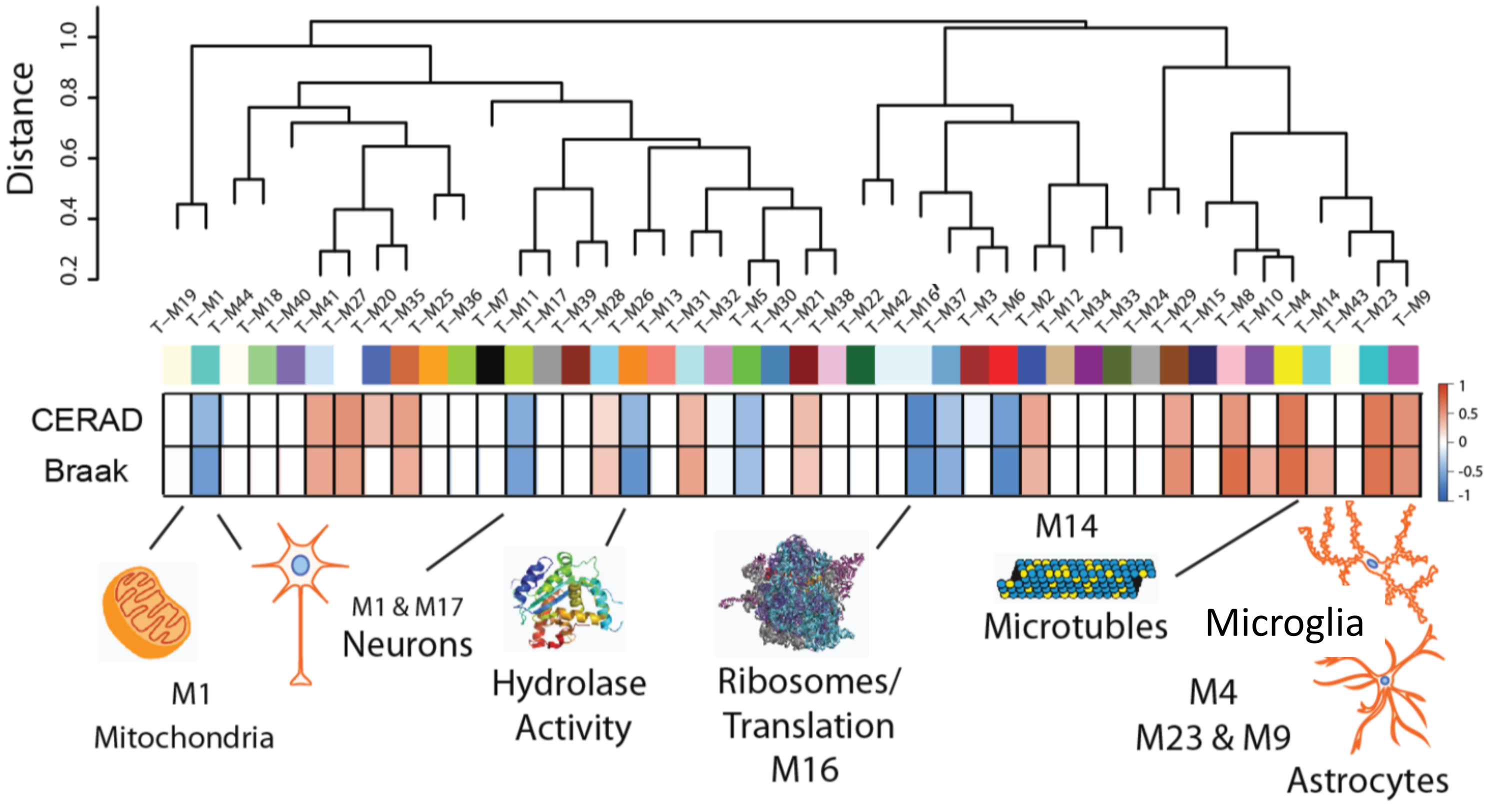

Astrocytes


Microglia

- **Highly significant across all cohorts**
- **AD GWAS enriched (APOE, MAPT, CLU, C4A, +more)**
- **Validation of hub proteins in Drosophila models and human brain pathology**

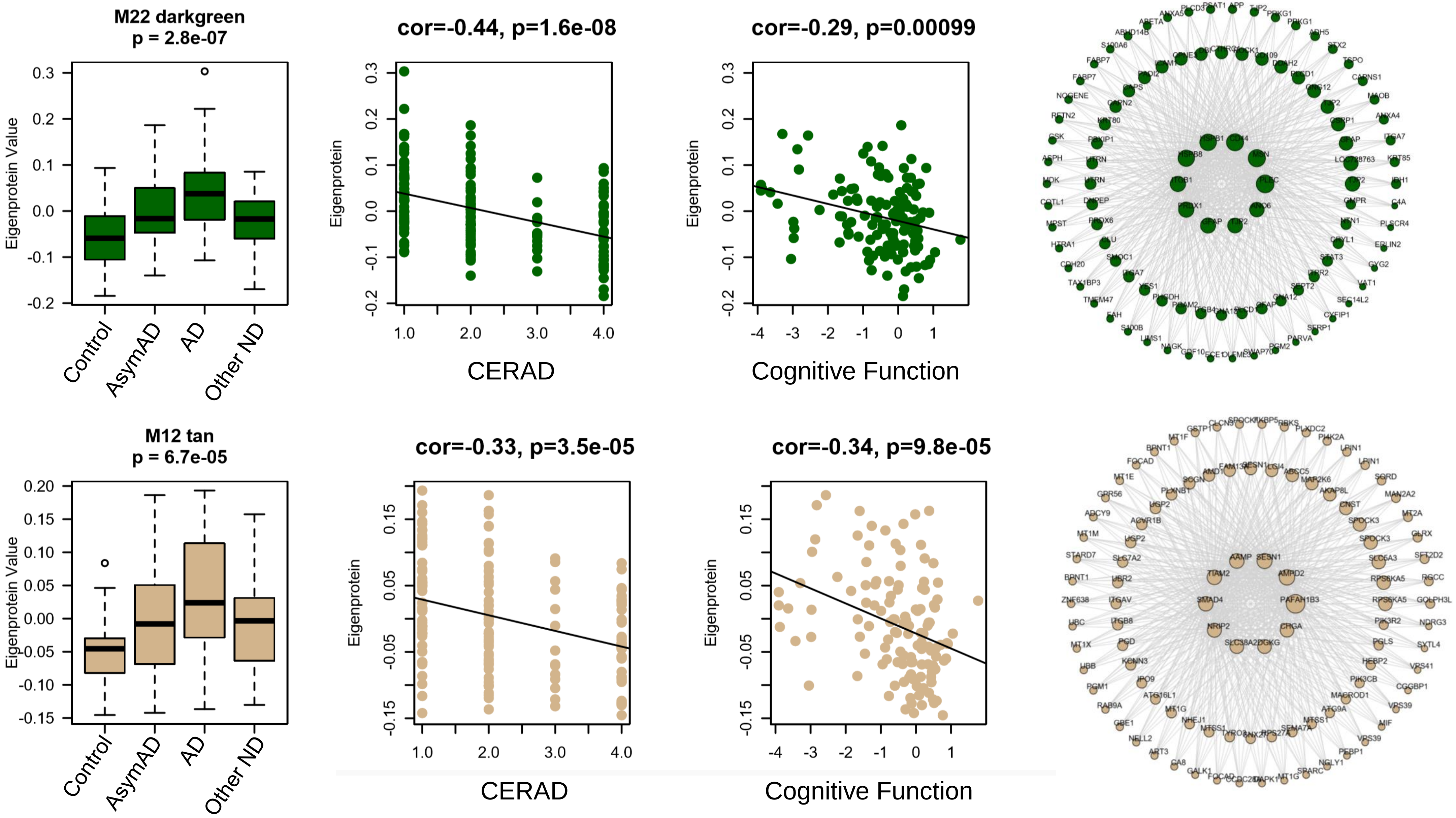
Enhancing the depth and throughput of the AD proteome with multiplex tandem mass tags (TMT)

Protein Co-expression Network Analysis

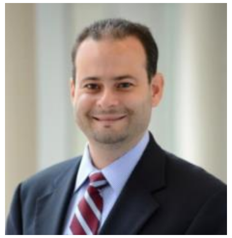


- TMT-MS quantified **~4-fold more proteins** (11-12,000 proteins equivalent to **~65% of expressed brain transcripts**) with no missing values across cases.
- Modules associated with **brain cell types and pathophysiologies**
- The TMT-MS network identified ~**3-fold more modules**, including new modules associated with AD phenotypes while detecting all modules identified in LFQ-MS networks
- Only ~**40-50% of modules overlap** with the **brain transcriptome**

TMT-MS of the ROS/MAP cohort validates AD specific immune-related modules strongly associated with cognitive dysfunction

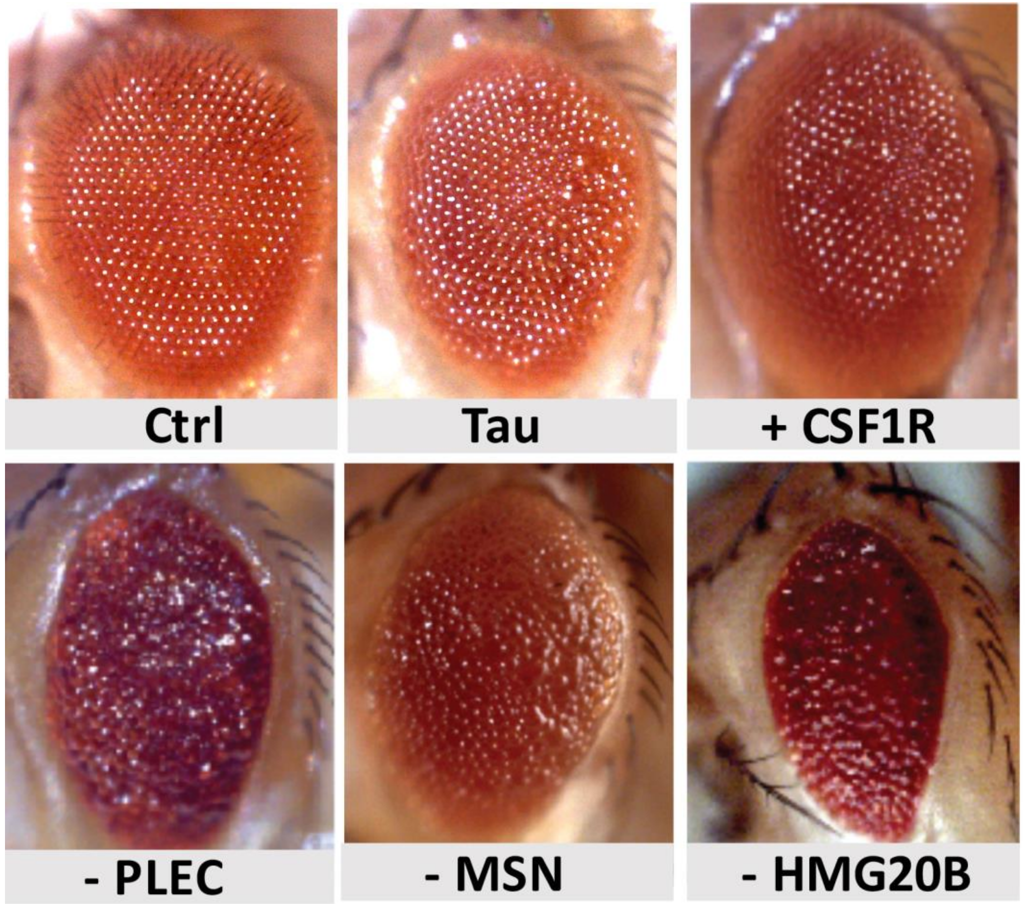


Cross-validation of AMP-AD key drivers in *Drosophila* and human AD brain



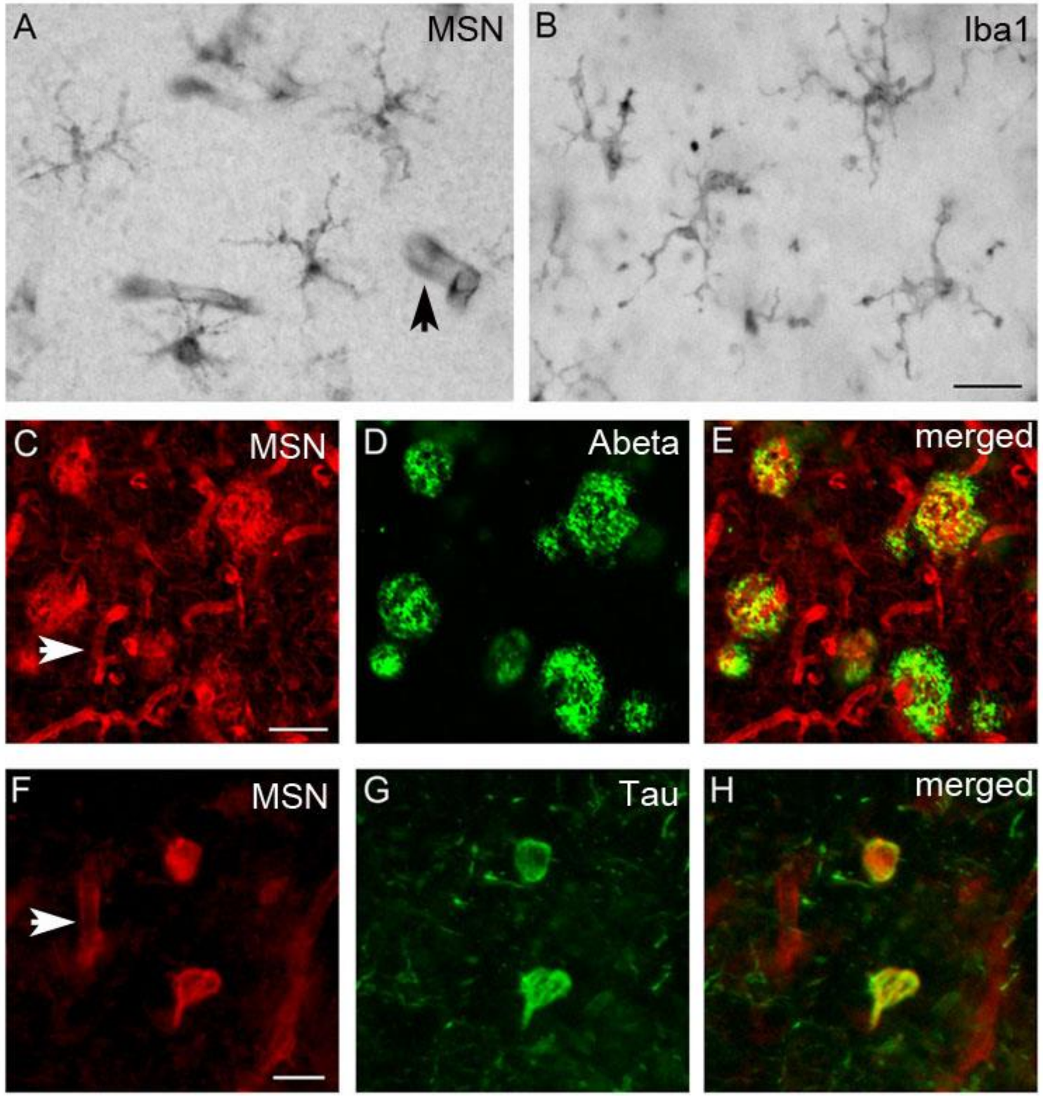
Josh Shulman
Baylor

Validation of AMP-AD key drivers in *Drosophila*



Drivers genetically modify Tau toxicity

Moesin (MSN) associates with AD pathology

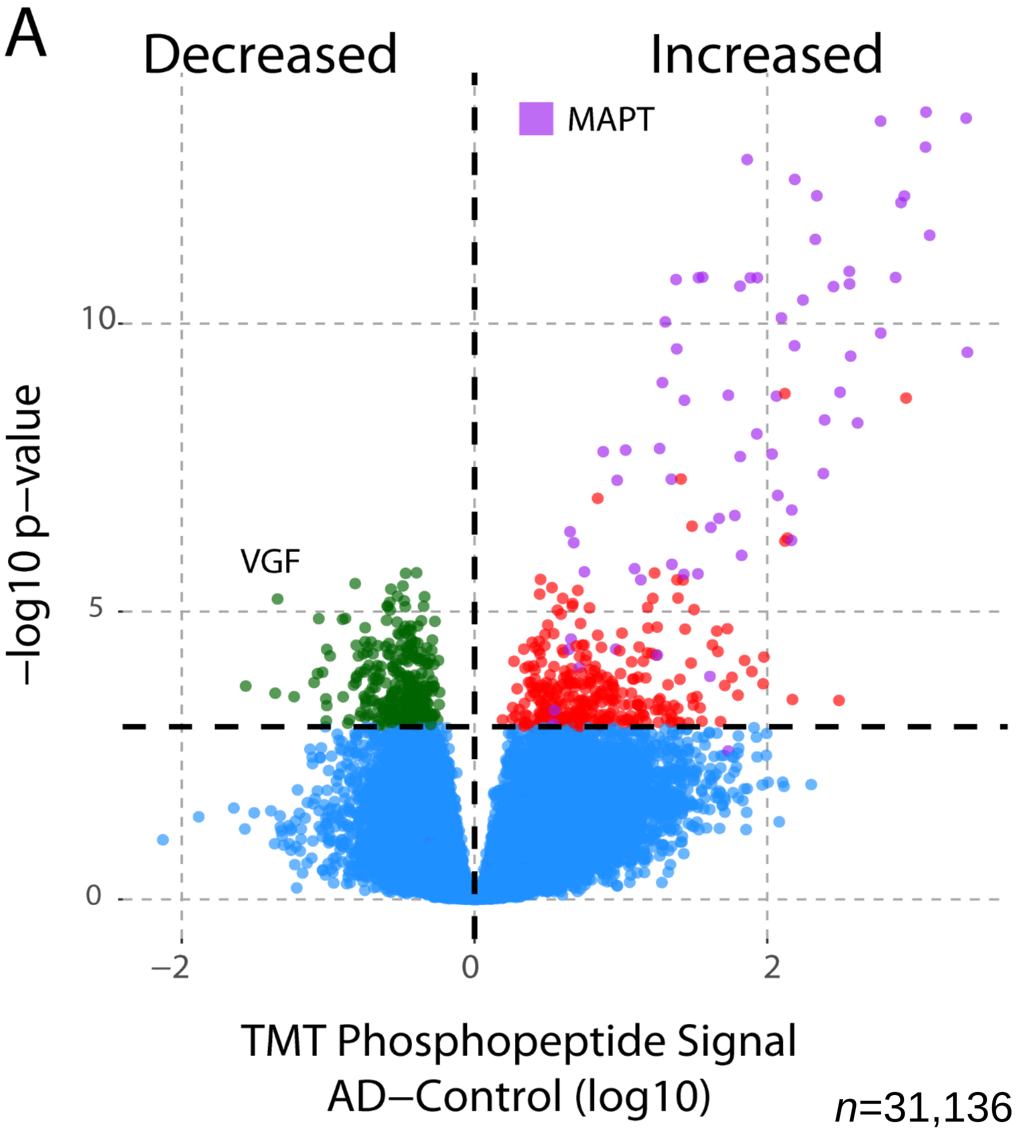


Consortium Targets

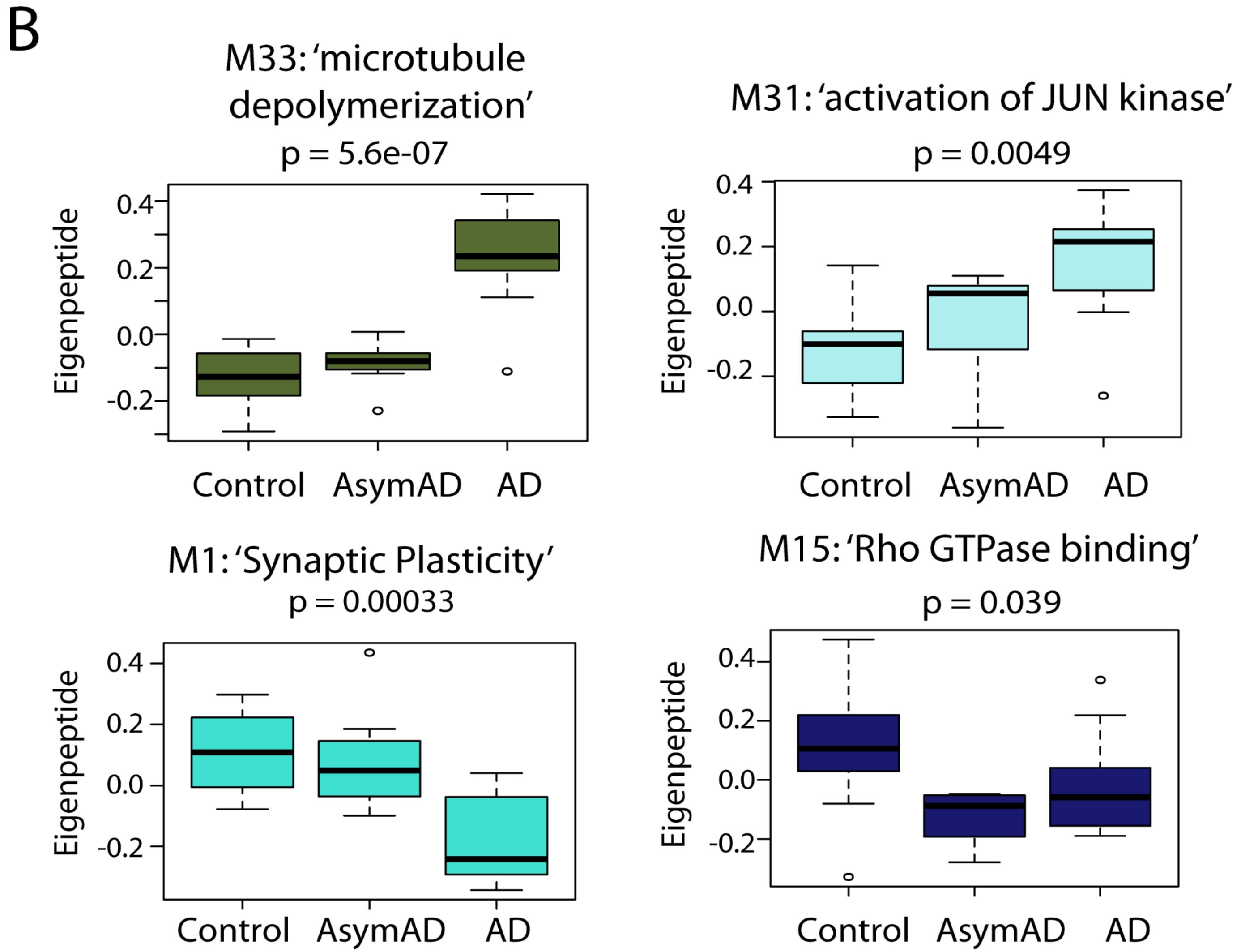
Mayo/UF
Rush/Broad
Emory

Phosphoproteomics reveals disease associated signaling networks in AD

AD brain phosphoproteome



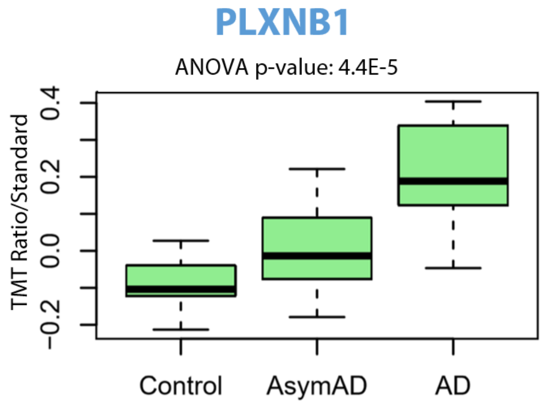
Phosphoproteome Network Analysis



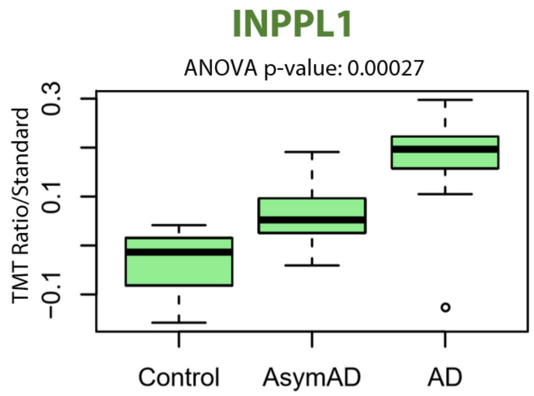
Validating AMP-AD nominated targets by TMT-MS

		Proteome			Phosphoproteome		Isoforms
	AMP-AD Targets (n=29)	LFQ	TMT	Significant (AD/CT)	Total Unique Phosphopeptides	Significant (AD/CT)	Splice variants
Rush-Broad	AK4	x	x	0	4	0	1
	FBXO2	x	x	x	4	3	1
	FERMT2	x	x	x	2	1	1
MSSM	INPP5D	0	x	x	2	1	1
	PLXNB1	0	x	x	3	0	1
	ATP6V1A	x	x	0	12	4	1
	GABRB2	0	x	x	4	0	1
	VGF	x	x	x	5	3	1
Duke	LPIN1	0	x	x	3	0	1
	SGPP1	0	x	0	2	0	1
	CDYL	0	x	0	2	0	1
	NR1H4	0	0	0	0	0	0
	SPTLC3	0	0	0	0	0	0
Emory	CD44	x	x	x	5	4	1
	EPB41L3	x	x	x	63	24	3
	MSN	x	x	x	3	2	1
	PLCD1	x	x	x	12	3	1
	PLEC	0	x	x	97	51	3
	PTRHD1	x	x	x	0	0	1
Harvard	HDAC1	0	x	x	0	0	1
	REST	0	0	0	0	0	0
Mayo/UF	CSF1	0	0	0	0	0	0
	CSF1R	0	0	0	0	0	0
	PLCG2	0	x	0	1	0	1
	CFH	0	x	0	0	0	1
	FCER1G	0	x	0	0	0	1
	INPPL1	0	x	x	5	4	1
	Il10	0	0	0	0	0	0
	IL10RA	0	0	0	1	0	0
	Total	10	22	15	230	100	26

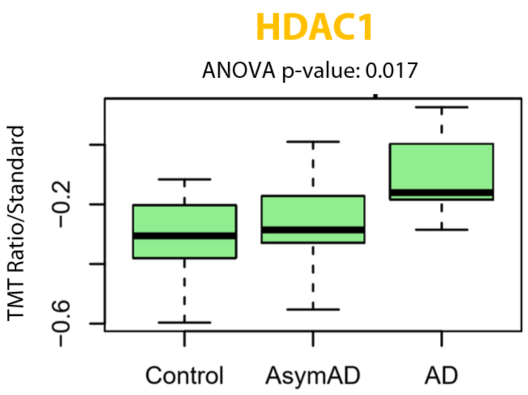
>2 fold more targets with TMT-MS


PLXNB1

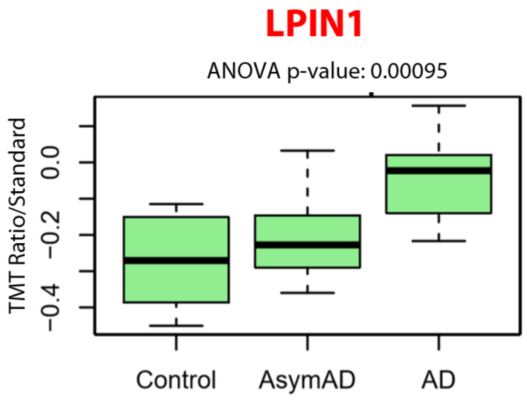
ANOVA p-value: 4.4E-5


INPPL1

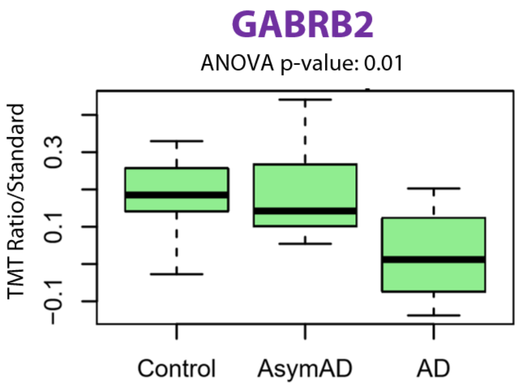
ANOVA p-value: 0.00027


HDAC1

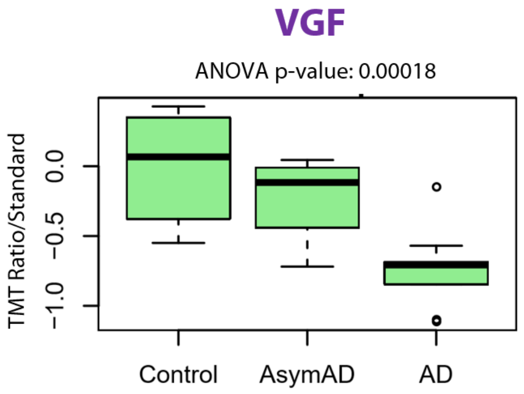
ANOVA p-value: 0.017


LPIN1

ANOVA p-value: 0.00095


GABRB2

ANOVA p-value: 0.01


VGF

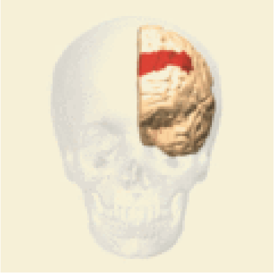
ANOVA p-value: 0.00018

Assessing Disease Specificity Across the UPenn Cohort

Control	**AD**	**CBD**	**PSP**	**ALS**	**FTLD-TDP**	**PD**	**PDD**	**MSA**	**Other**
n=14	n=16	n=7	n=11	n=16	n=9	n=13	n=17	n=5	n=6

PRM Validation
UPenn Cohort

Prefrontal Cortex (DLPC)



Maotian Zhou

n=114 cases and 9 pooled standards across 3 separate batched randomized by diagnosis

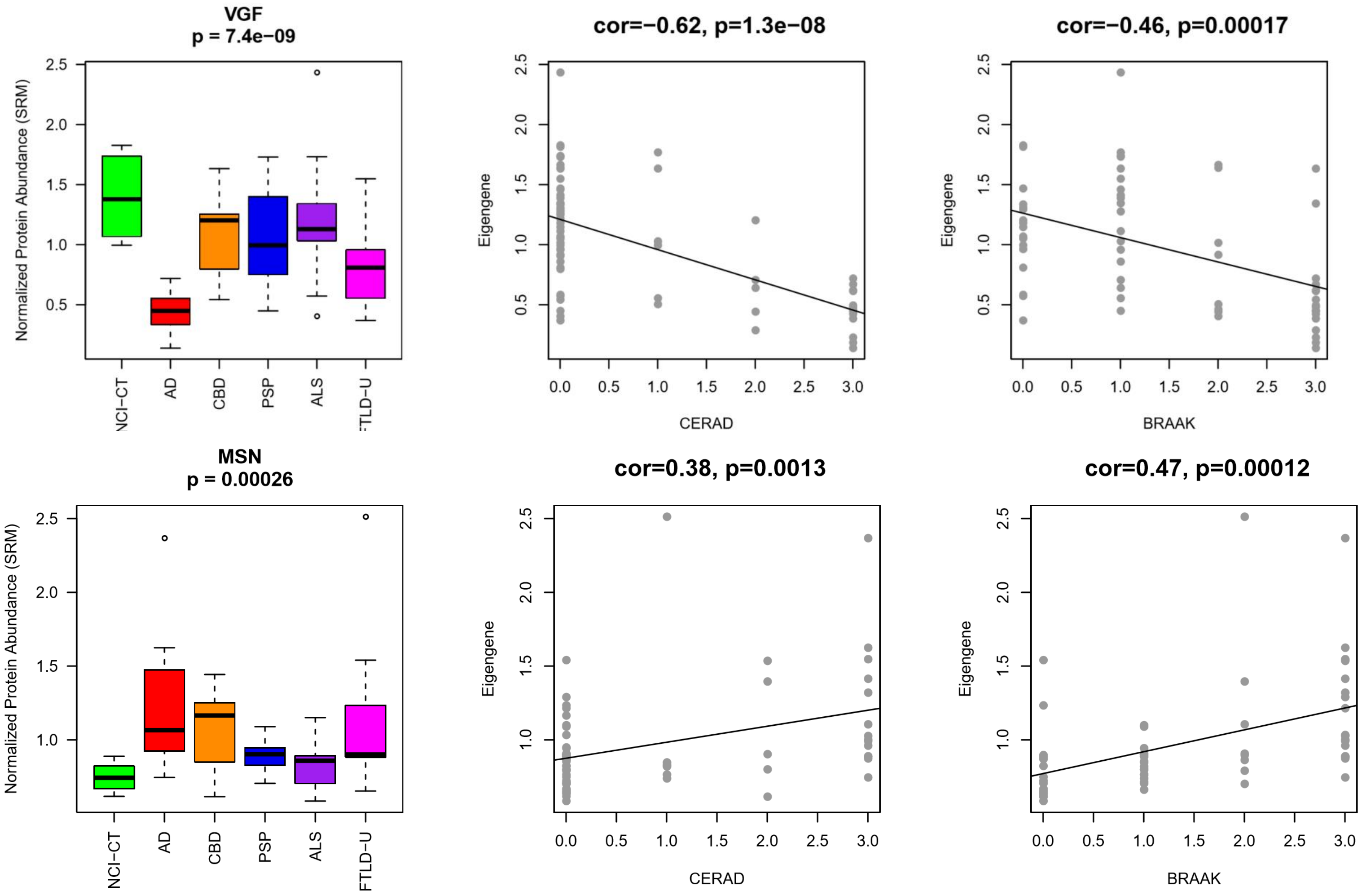
300 Top Targets
+Aβ and (phospho)Tau
1-3 peptides/target
= 720 peptides

Skyline

Wingo T et. al., *J Proteome Res*. 2017 **16**:3336-3347

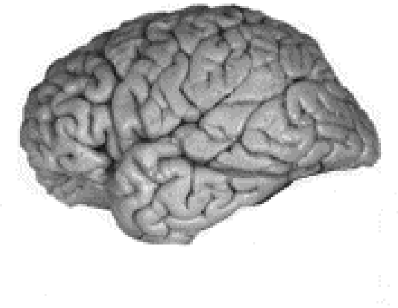
Top AD Protein Targets in UPENN Brain Quantified by PRM Across Diseases

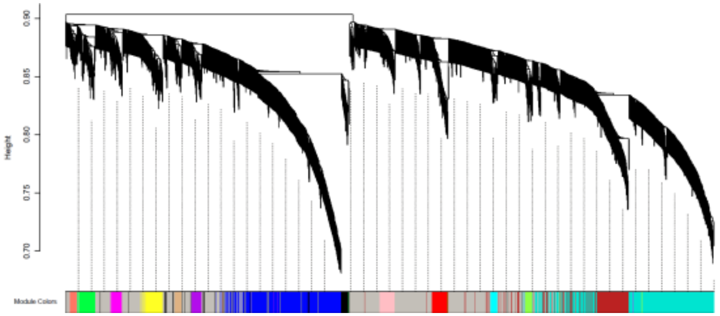
PRM Validation
UPenn Cohort

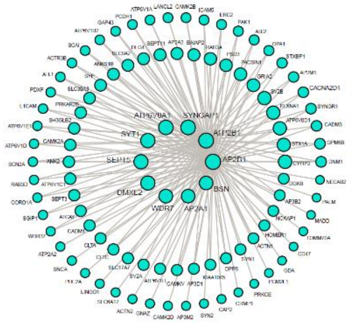


Comprehensive and Integrated Analysis of the Human Brain and CSF Proteome in AD

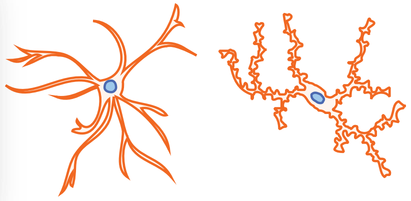
From Brain



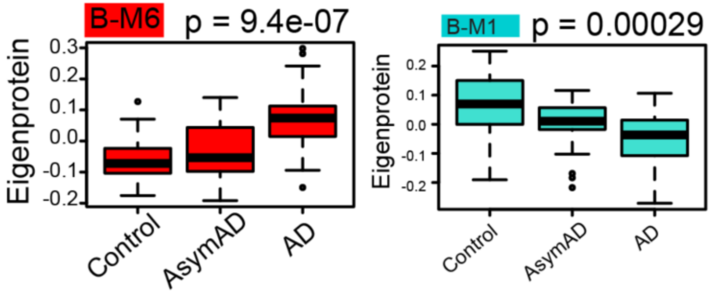




Communities
of Proteins



Relate to biological function
and processes



Hub Proteins

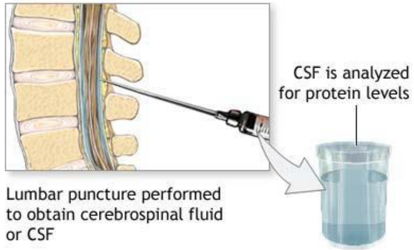
PLEC ANXA5 MSN
GFAP CD44 LMNA

Hub Proteins

AP2B1 ATP6V0A1 SYT1
BSN ATP6V0D1 ATP2B1

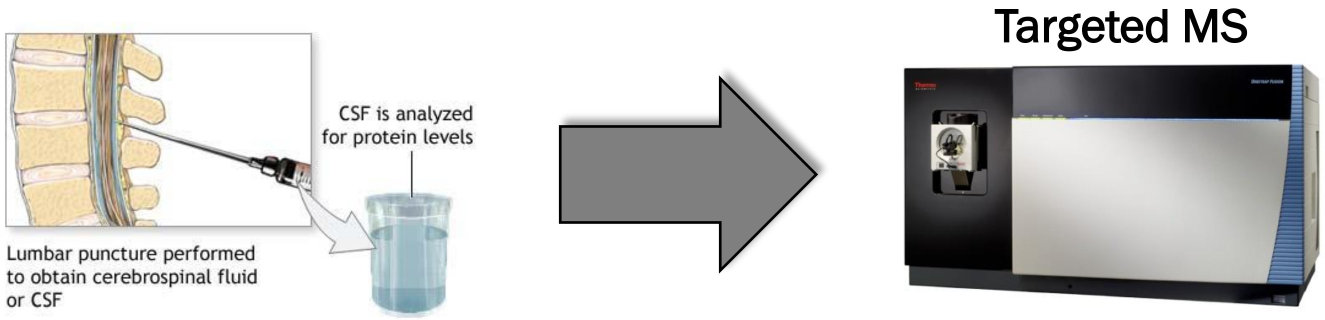
Assess Module-phenotype
Relationships

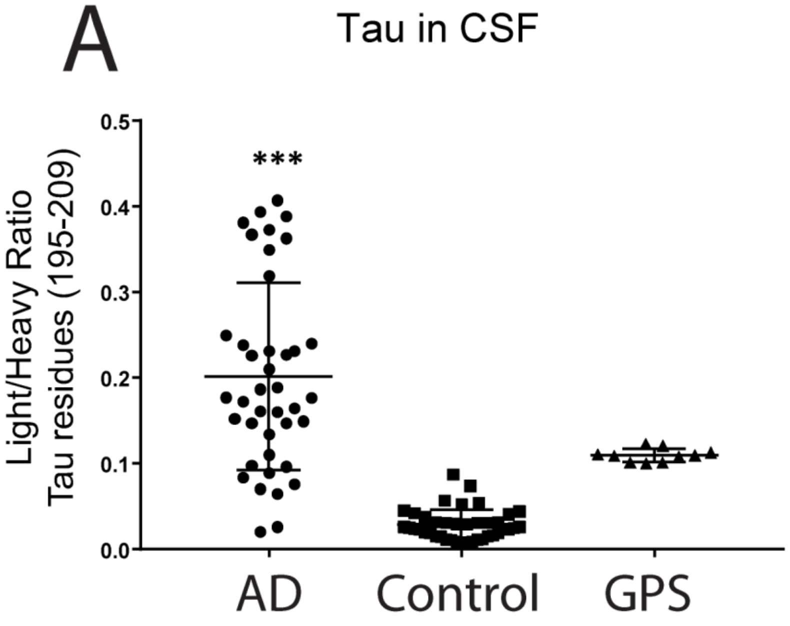
To CSF

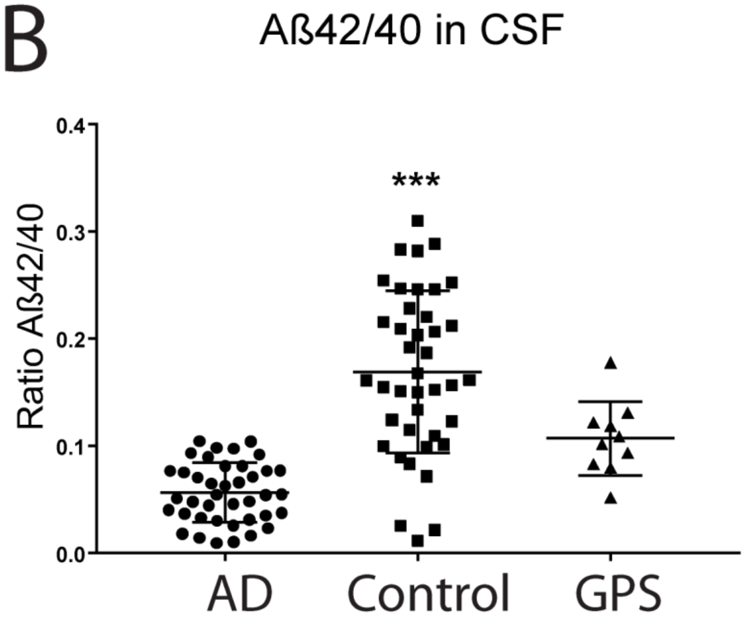


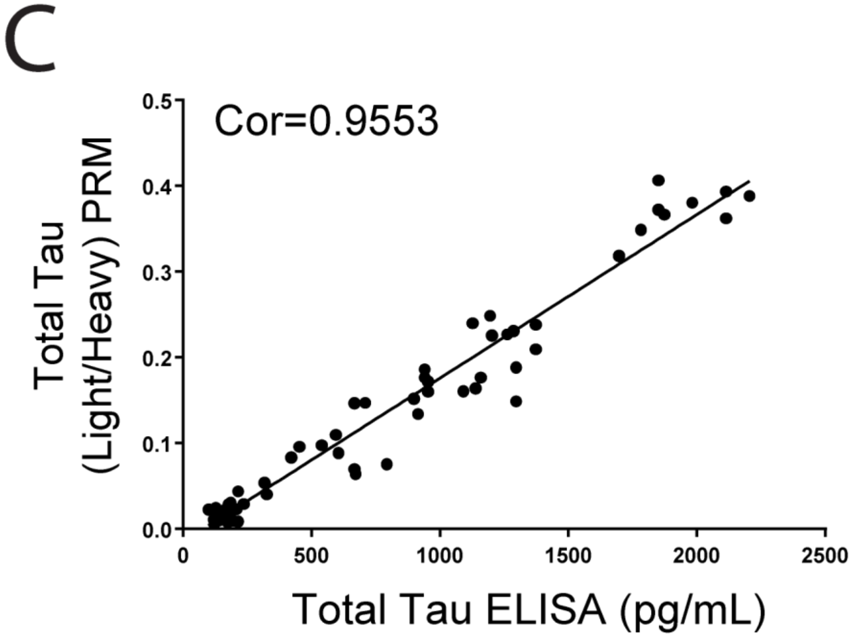
Module-Based
Biomarkers

Quantification of AD biomarkers (Aβ and Tau) in CSF by Mass Spectrometry (MS)



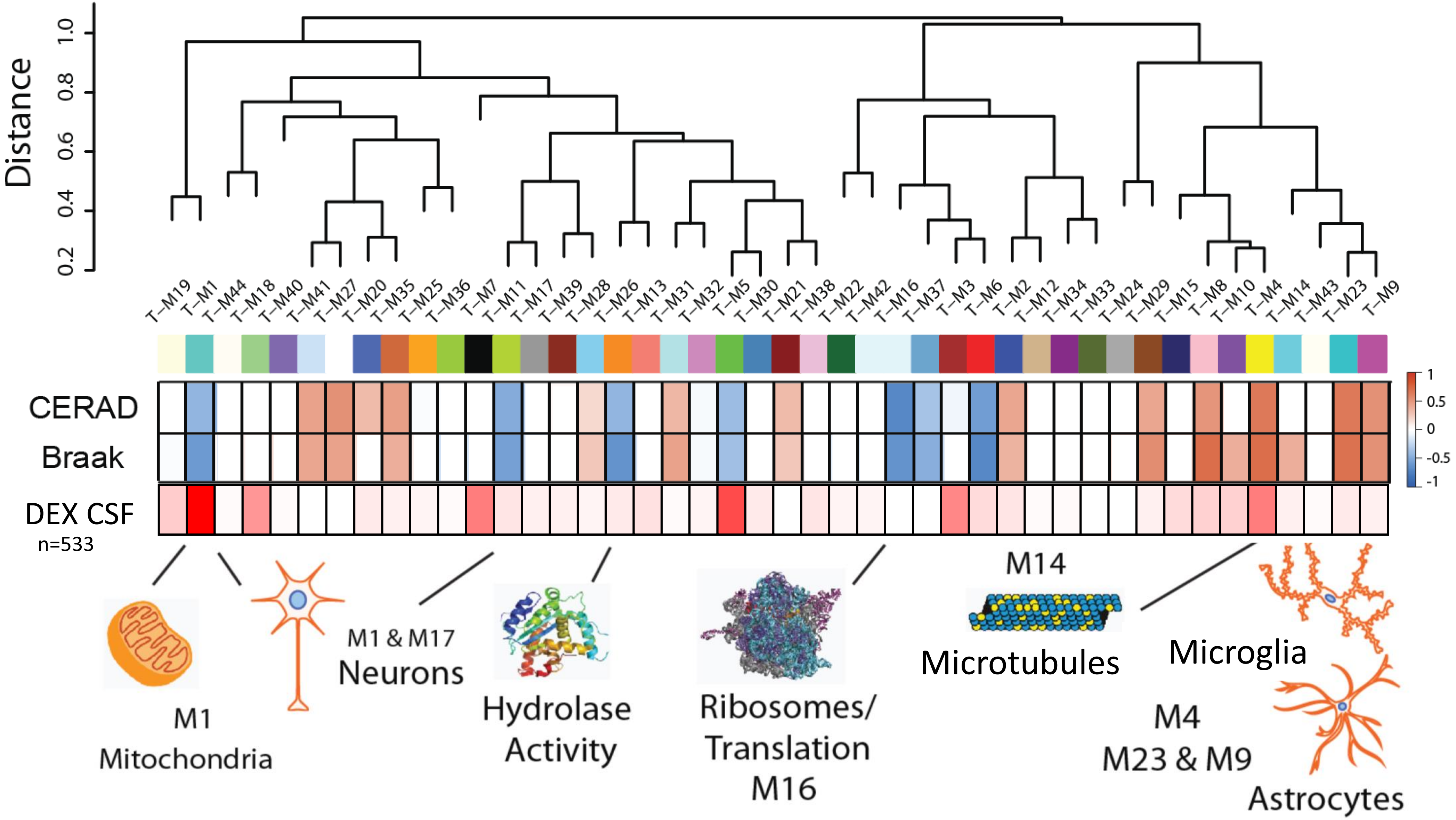






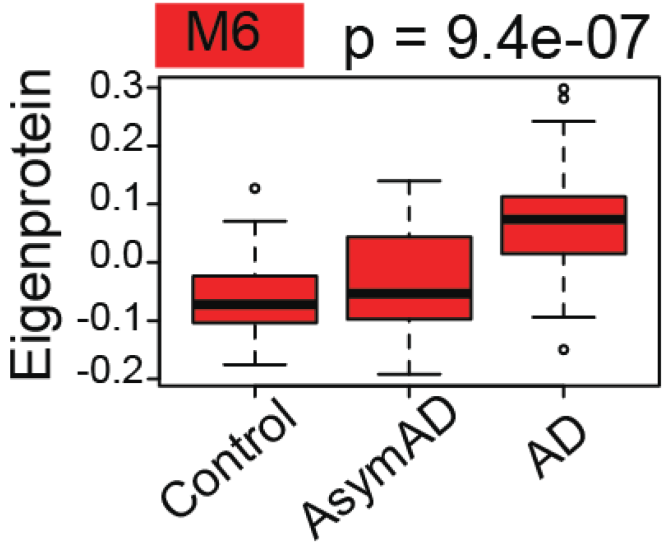
*** t-test p-value <0.001

Discovery of AD Biomarkers in CSF (Individual) by TMT-MS



1171/1737 Peptides and 329/533 Gene Products

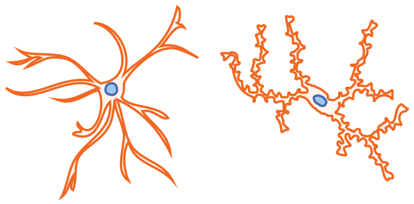
Quantification of AD Brain Networks in Human CSF by MS



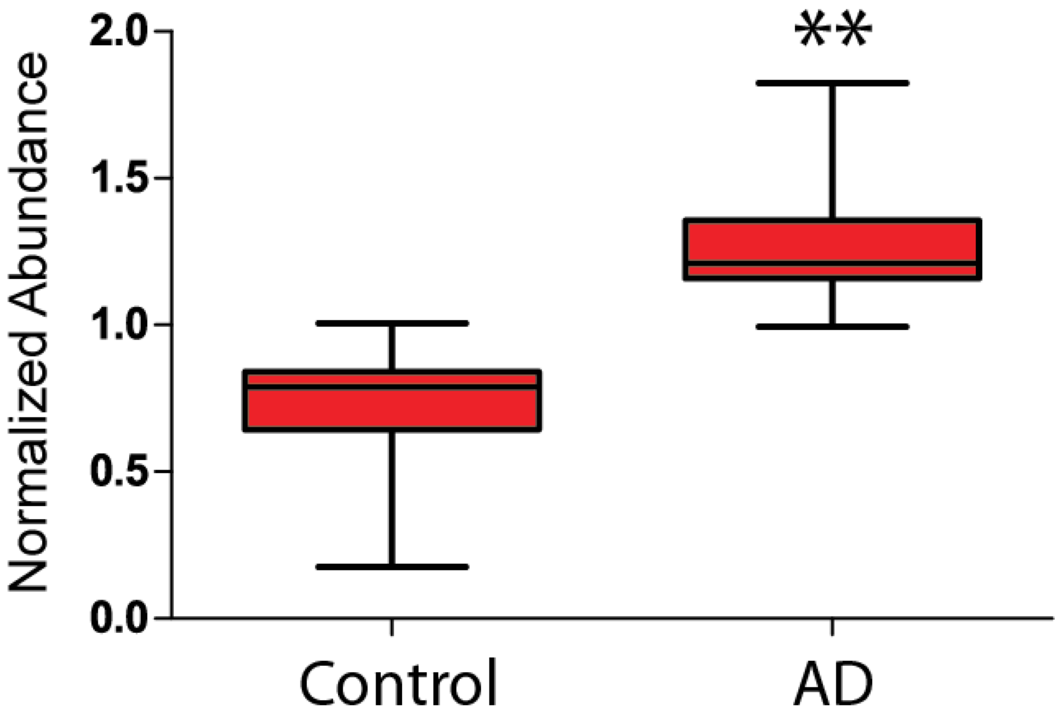
Hub Proteins

PLEC ANXA5 MSN

GFAP CD44 LMNA



25 proteins



*** t-test $p<0.001$

From Brain to Plasma: Proteomic Biomarker Discovery and Validation in the US and UK

M²OVE-AD
Molecular Mechanisms of the Vascular Etiology of Alzheimer's Disease

EMORY UNIVERSITY

Allan Levey, Ihab Hajjar
& Nick Seyfried

UNIVERSITY OF OXFORD

Simon Lovestone

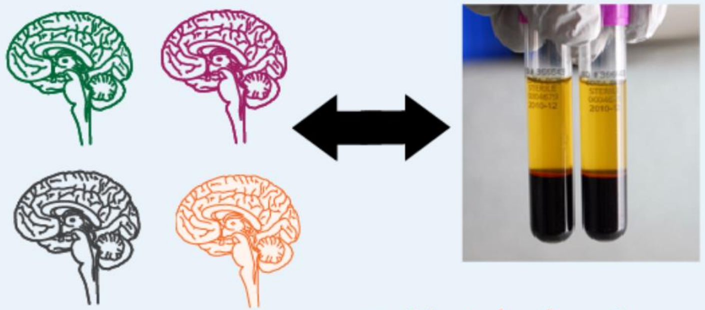
Duke UNIVERSITY

Rima Kaddurah-Daouk

1. Discovery Phase

ROS/MAP

400 brains and antemortem plasma



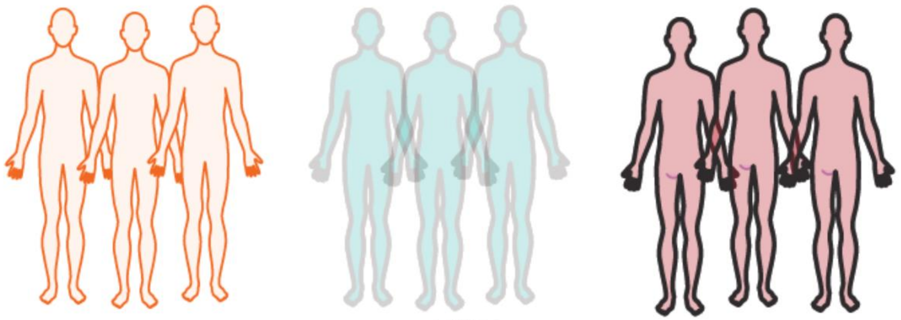
Lipidomics

Metabolomics
Proteomics

Systems Biology and Predictive Modeling

Interrated Networks Associated with Key Traits

2. Validation Phase



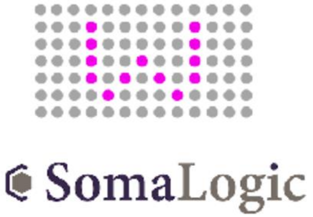
Emory

EMIF
(European Medical
Information Framework)

DPUK
(Dementia Platform UK)

n=1-2,000

Proteomics



100 driver
proteins

Brain-Blood Derived
Plasma Biomarkers Panels

Acknowledgements

EMORY
UNIVERSITY

Nick Seyfried
Eric Dammer
Maotian Zhou
Lingyan Ping
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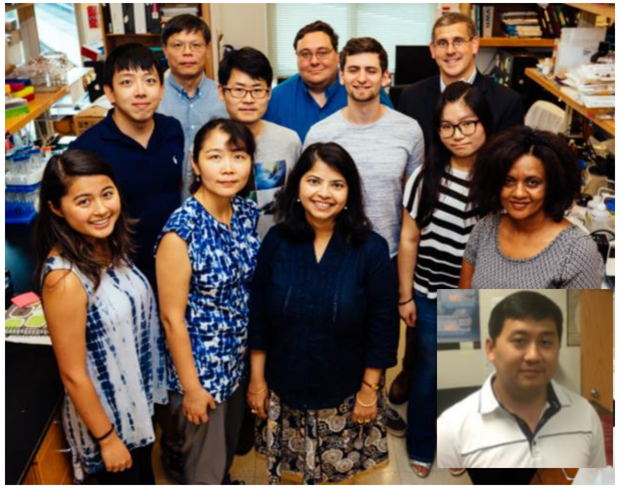
Marla Gearing
Chad Hales
Thomas Wingo
Divya Nandakumar


Emory Proteomics Core


Goizueta Emory ADRC

BCM®
Baylor College of Medicine

Josh Shulman

AMP-AD Collaborations

Columbia University
Medical Center

UCLA
David Geffen
School of Medicine

Rush University
Medical Center

Harvard
Medical School

Penn
University of Pennsylvania

Johns Hopkins
School of Medicine

Icahn
School of
Medicine at
Mount
Sinai

Institute for
Systems Biology

Mayo
Clinic

UF
University of
Florida

The Jackson
Laboratory
*Leading the search
for tomorrow's cures*

Duke
University

Banner
Alzheimer's
Institute

University of
Oxford

Biogen

abbvie

Lilly

gsk

Funding:

NIH
National Institute
on Aging

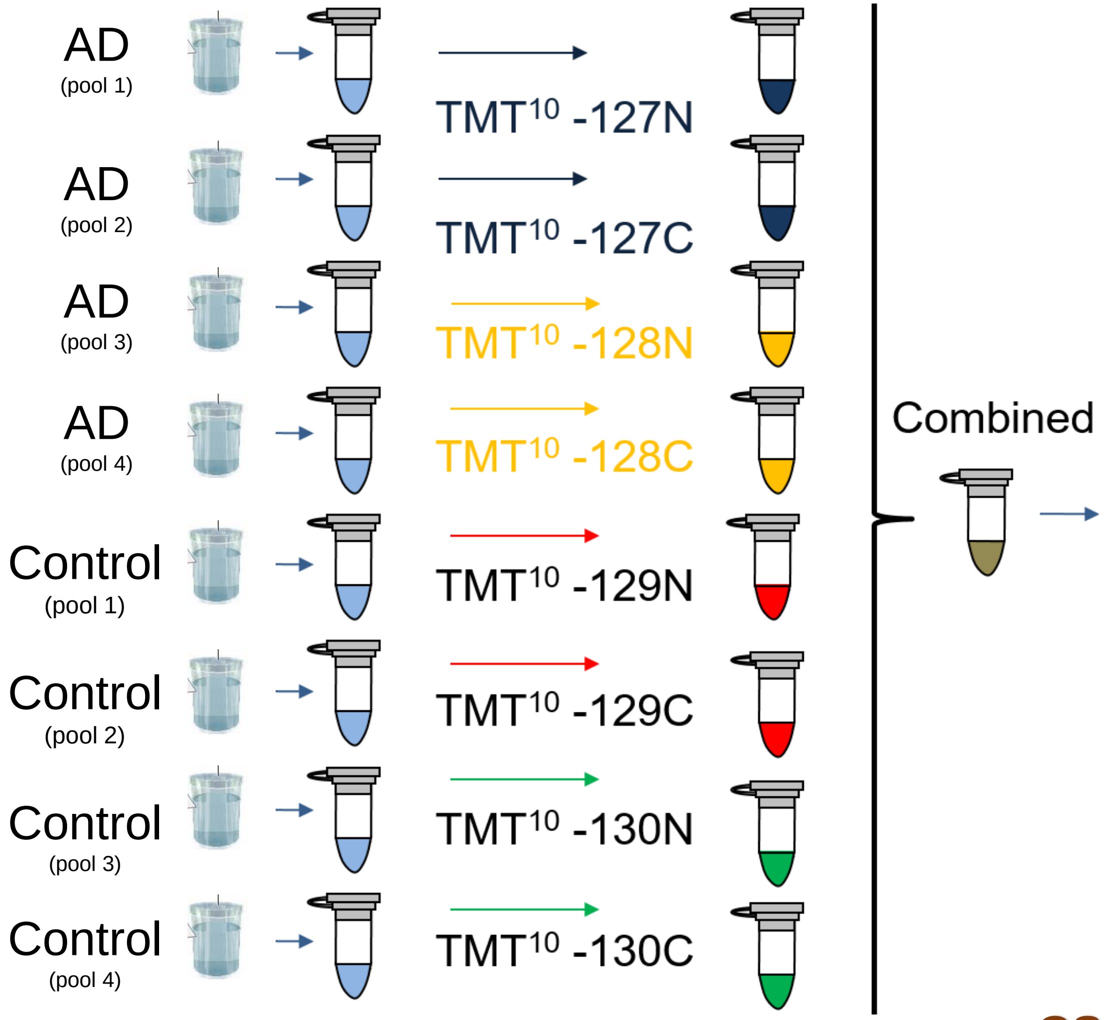
FNIH
Foundation for the
National Institutes of Health

U01AG046161
RF1AG057471
P50AG025688

R01AG057330
RF1AG057470
R01AG053960

Comprehensive Quantification of the CSF Proteome by TMT

CSF (130 uL)



AD (pool 1) TMT^{10} -127N

AD (pool 2) TMT^{10} -127C

AD (pool 3) TMT^{10} -128N

AD (pool 4) TMT^{10} -128C

Control (pool 1) TMT^{10} -129N

Control (pool 2) TMT^{10} -129C

Control (pool 3) TMT^{10} -130N

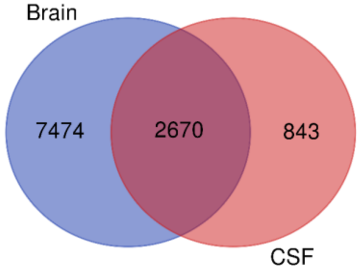
Control (pool 4) TMT^{10} -130C

Combined

High pH Fractionation

30 fractions

Orbitrap Fusion



Approximately ***~72% of proteins in CSF*** were also found in brain

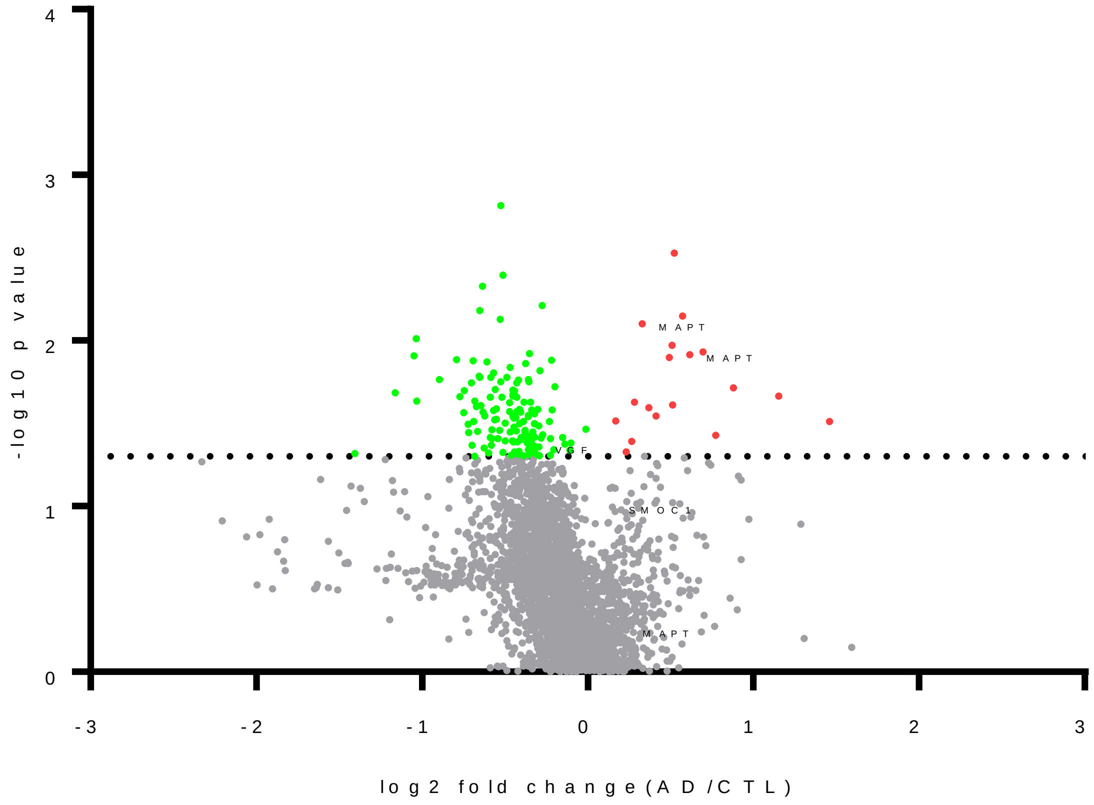
30 nanoLC-MS/MS runs (2 hr gradient)

Uniprot database search

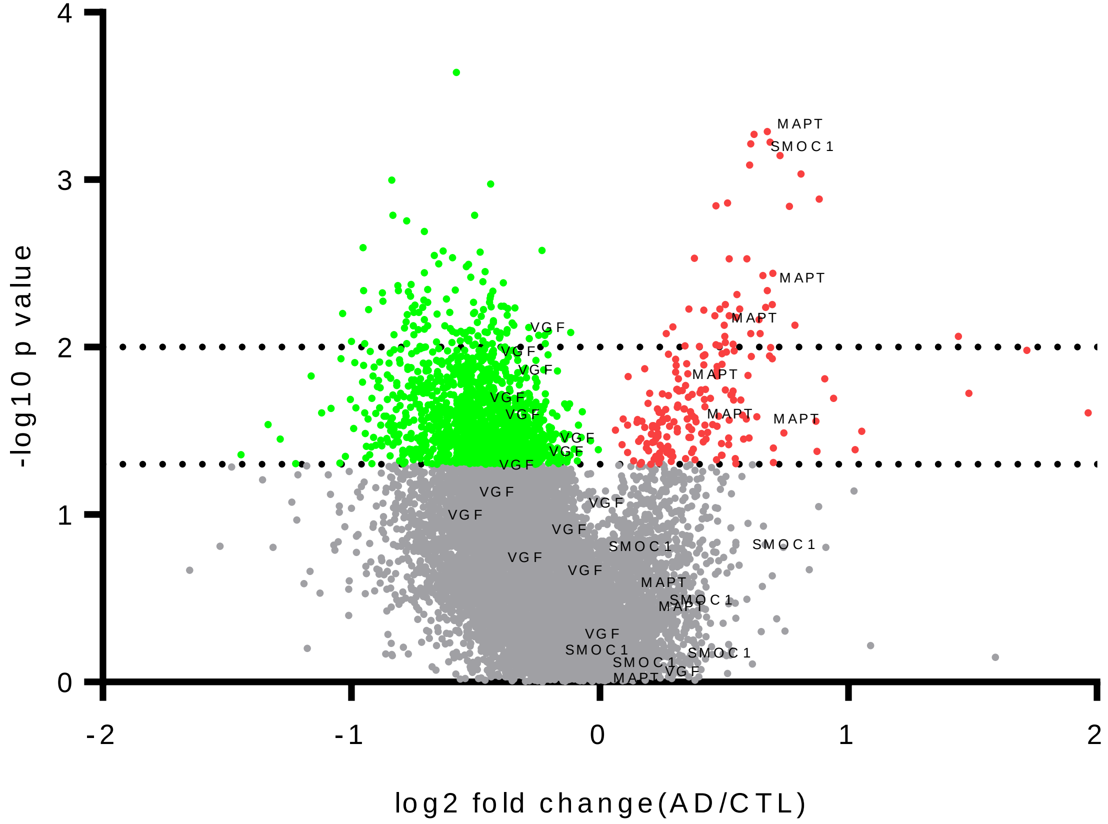
CSF Pools: 30796 peptides mapping to **3,678** protein groups

Discovery of AD Biomarkers in CSF (Individual) by TMT-MS

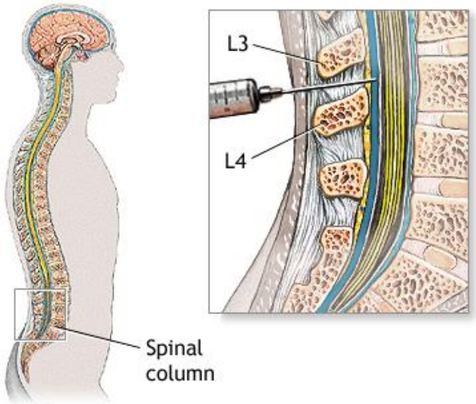
Total Proteins (*n*= 4,209)

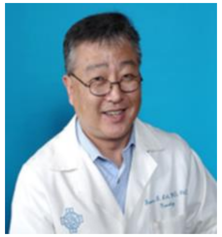


Total Peptides (*n*= 26,252)



Validation of AD Biomarkers in CSF: Parallel Reaction Monitoring (PRM)

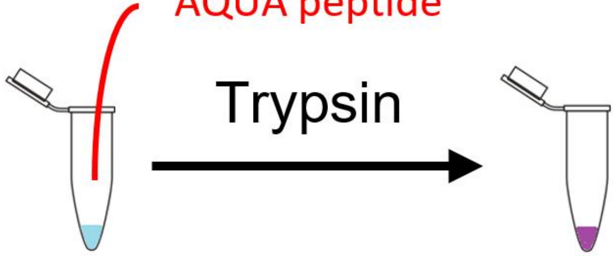




Parallel Reaction Monitoring (PRM)

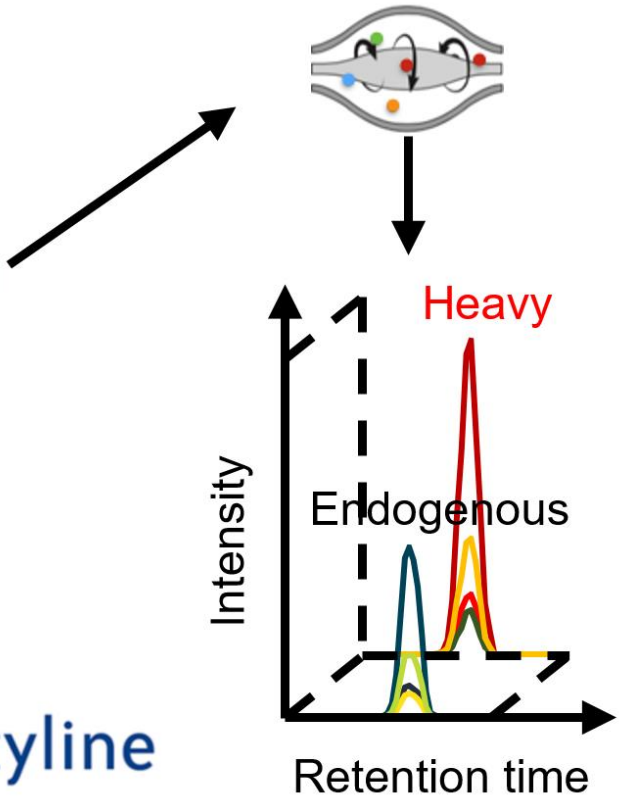
20 µl CSF

"Heavy" isotope labeled AQUA peptide



Trypsin

C_{18} Desalt

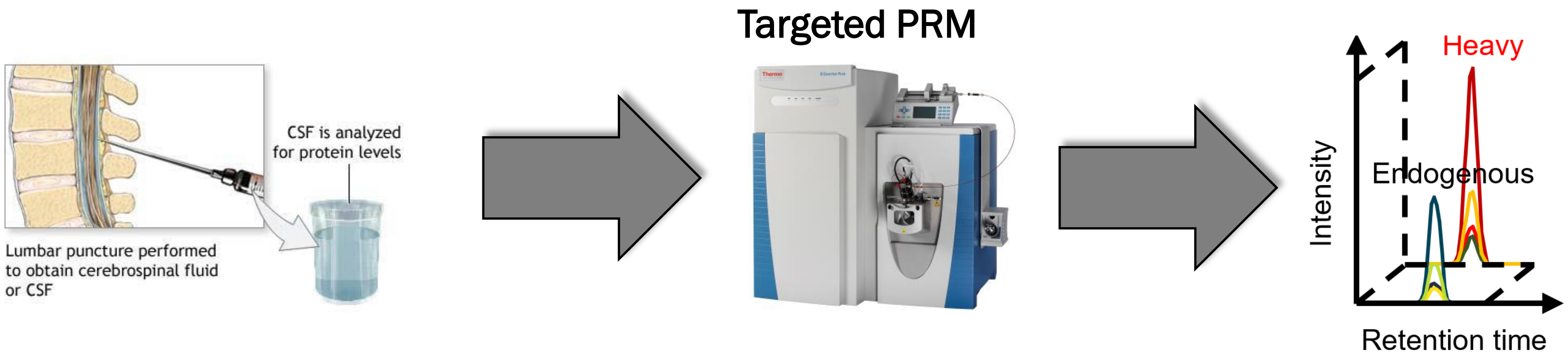


n=40 AD
n=40 non-AD controls

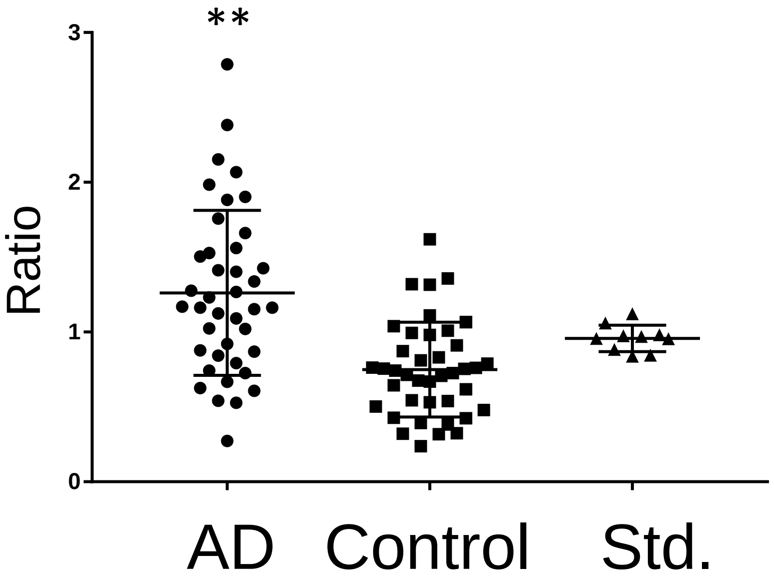
Skyline

Wingo T et. al., *J Proteome Res*. 2017 **16**:3336-3347

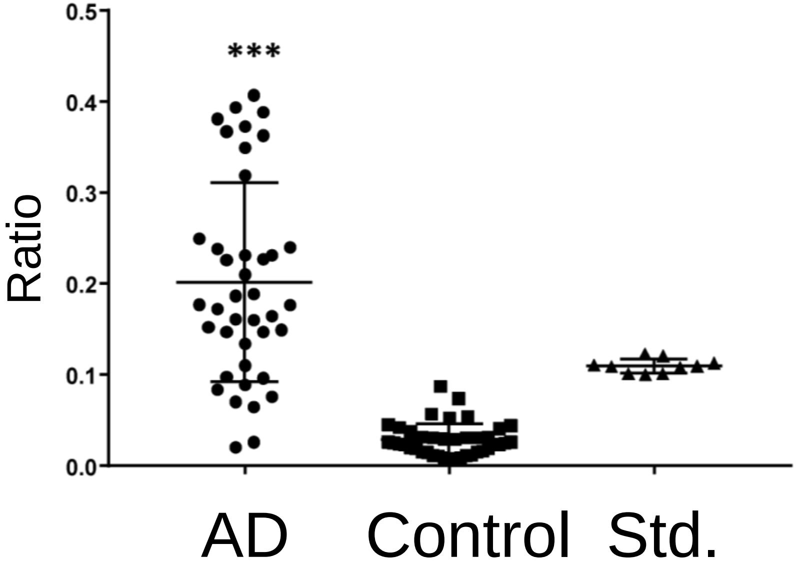
Validation of AD Biomarkers in CSF by PRM



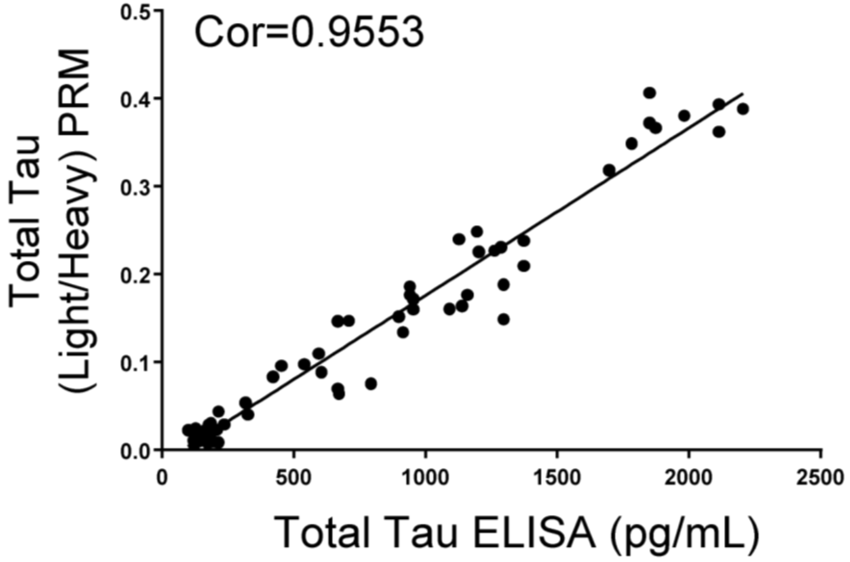
Osteopontin (SPP1)



Tau (MAPT)

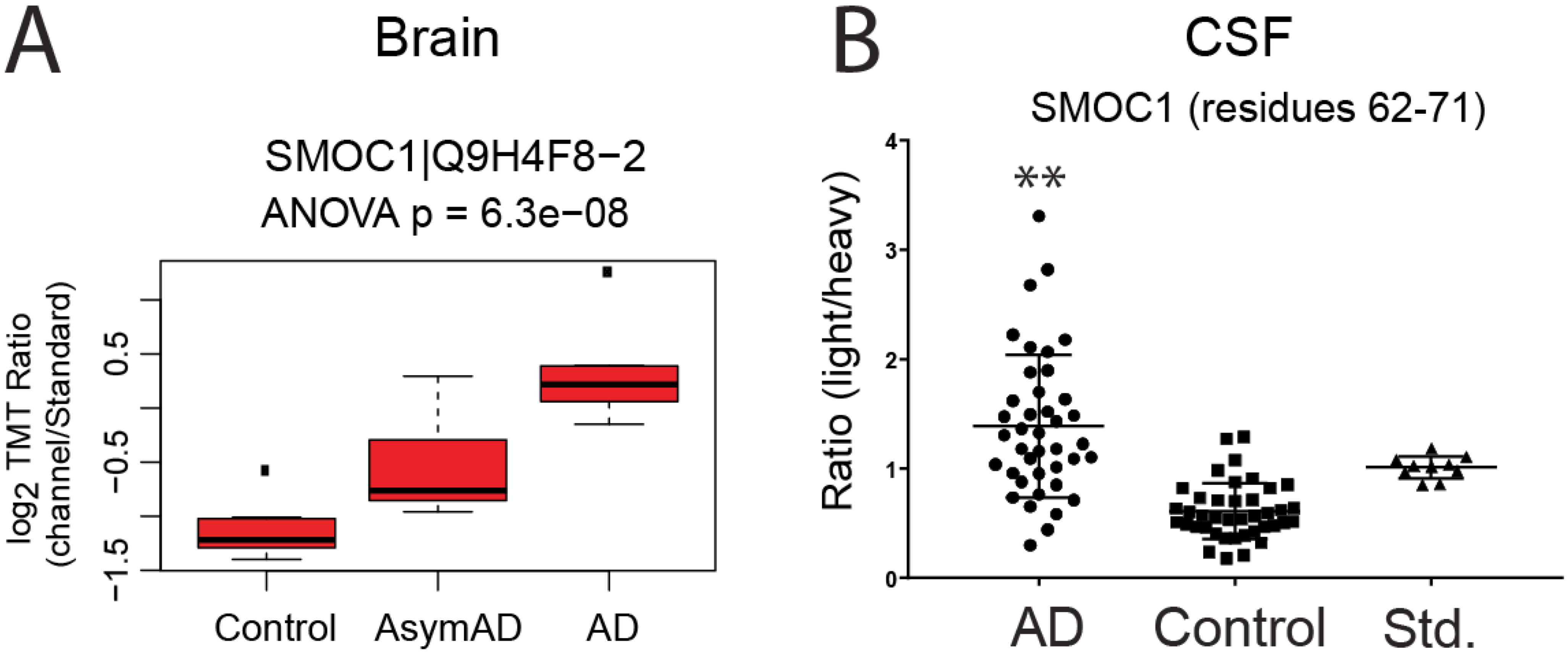


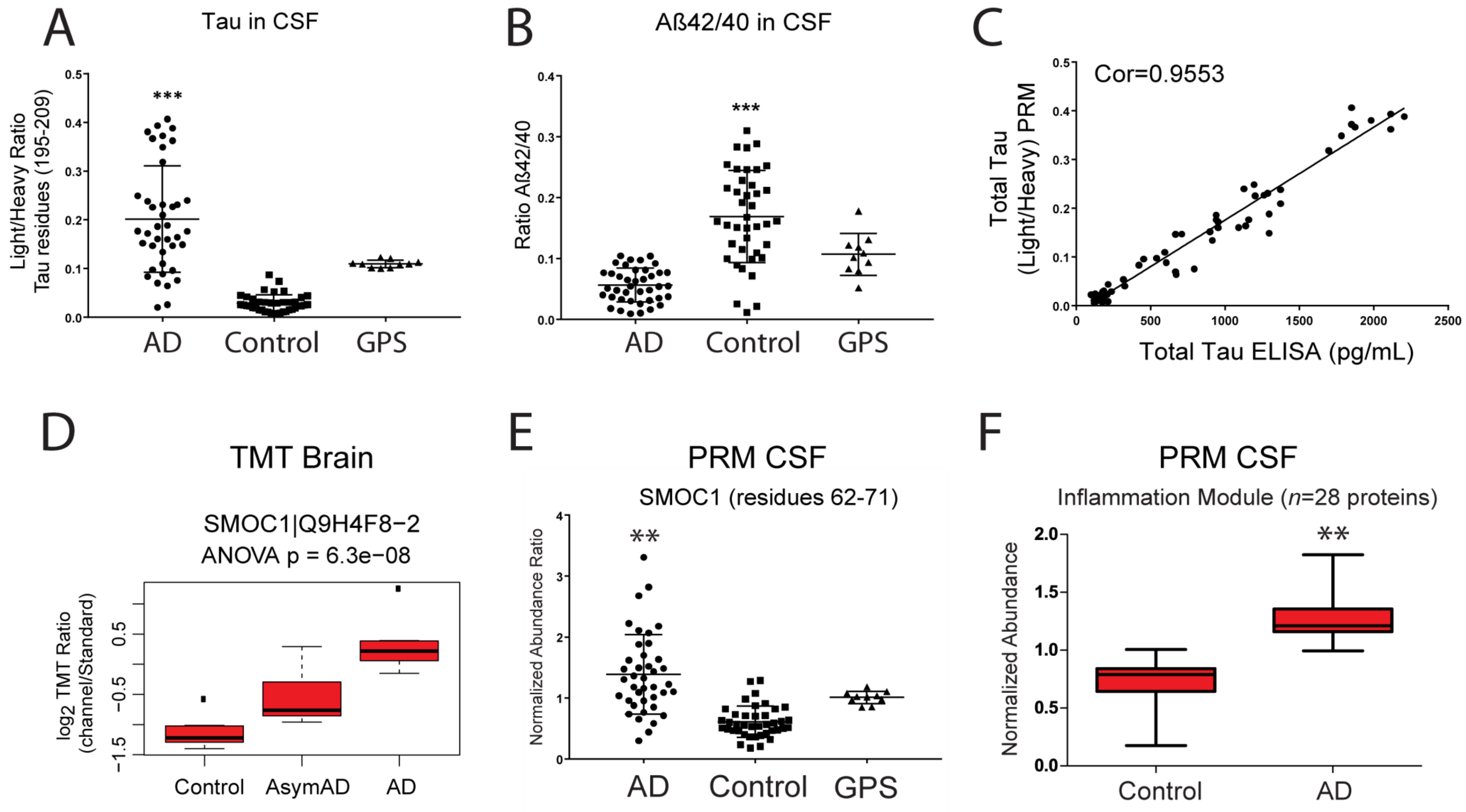
Tau (MAPT)



** t-test p-value <0.05, *** t-test p-value <0.001

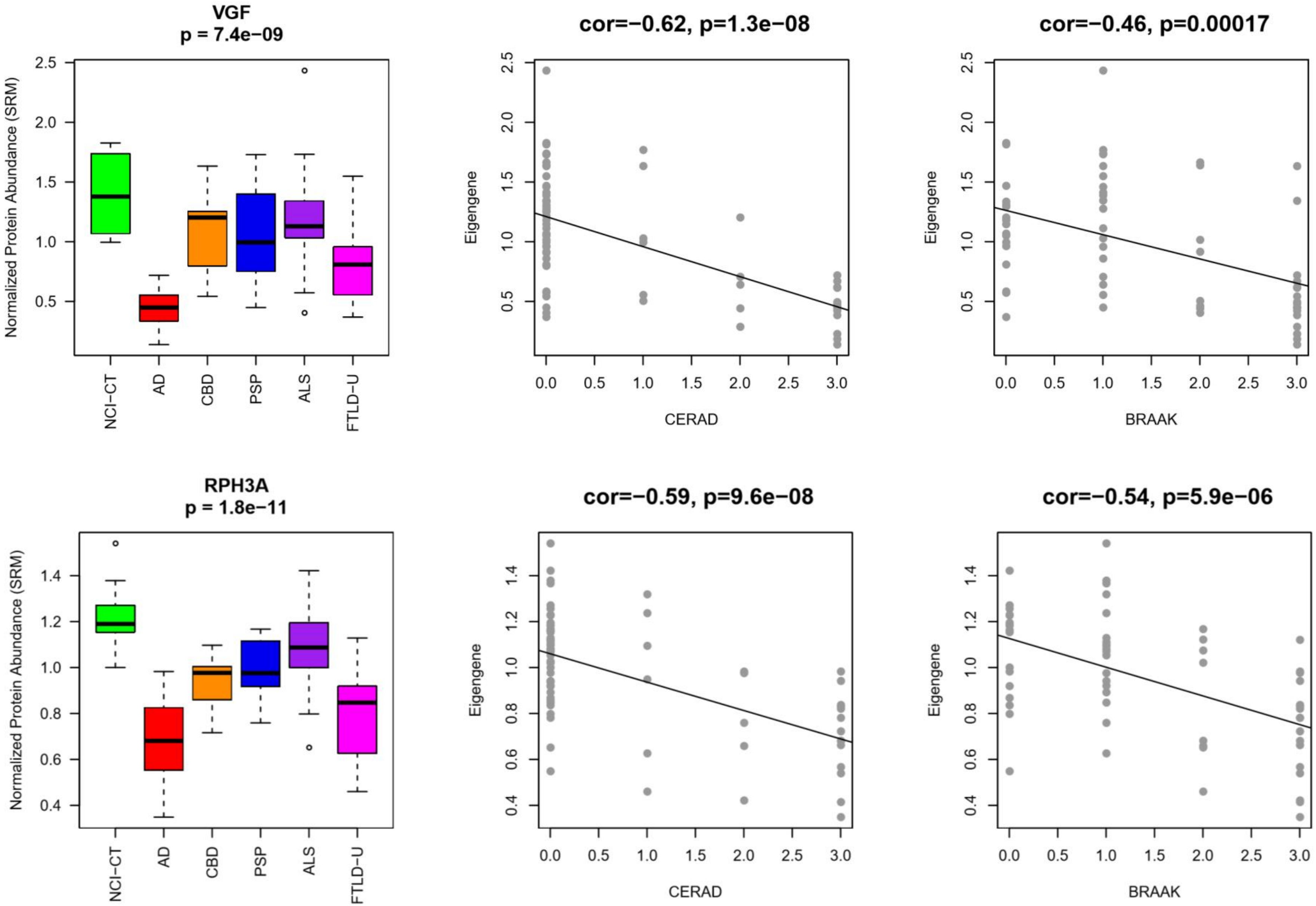
SMOC1 Validation: Brain and CSF




A
Tau in CSF
Light/Heavy Ratio
Tau residues (195-209)
0.5
0.4
0.3
0.2
0.1
0.0
***
AD
Control
GPS
B
Aß42/40 in CSF
Ratio Aß42/40
0.4
0.3
0.2
0.1
0.0
***
AD
Control
GPS
C
Cor=0.9553
Total Tau
(Light/Heavy) PRM
0.5
0.4
0.3
0.2
0.1
0.0
0
500
1000
1500
2000
2500
Total Tau ELISA (pg/mL)
D
TMT Brain
SMOC1|Q9H4F8−2
ANOVA p = 6.3e−08
log2 TMT Ratio
(channel/Standard)
0.5
−0.5
−1.5
Control
AsymAD
AD
E
PRM CSF
SMOC1 (residues 62-71)
Normalized Abundance Ratio
4
3
2
1
0
**
AD
Control
GPS
F
PRM CSF
Inflammation Module (n=28 proteins)
Normalized Abundance
2.0
1.5
1.0
0.5
0.0
**
Control
AD

Top AD Protein Targets in UPENN Brain Quantified by PRM Across Diseases

PRM Validation
UPenn Cohort



Top AD Protein Targets in UPENN Brain Quantified by PRM Across Diseases

PRM Validation
UPenn Cohort