A Systems Approach to AD

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Gene Expression Profiling

- Our initial efforts used serial analysis of gene expression – SAGE
- Identification of over 200 transcripts modulated by Abeta
- ATF4, Uch-L1 among most interesting
- Information overload

Array Based Methods

- Quick and easy
- Relatively low cost
- Information overload
- Key transcription factors may not change in expression levels
- Huge number of cases needed for accuracy

Problem: Can't Distinguish Between the Driver and the Passengers

A Systems Approach

Reverse Engineering Signaling from Expression

- Develop an Human Neuron Interactome
  - A map of all possible molecular interactions. Serves as a road map. Basically you can look up a street in the index and then go to map coordinates to find out where you are.
  - This requires gene expression data from neurons that are subject to different conditions – location, age, disease, sex etc. to uncover variations.

CAN WE DIRECTLY ANALYSE AD CHANGES IN THE HUMAN BRAIN?

Use human postmortem brain tissue

Use multiple brain regions

control brains

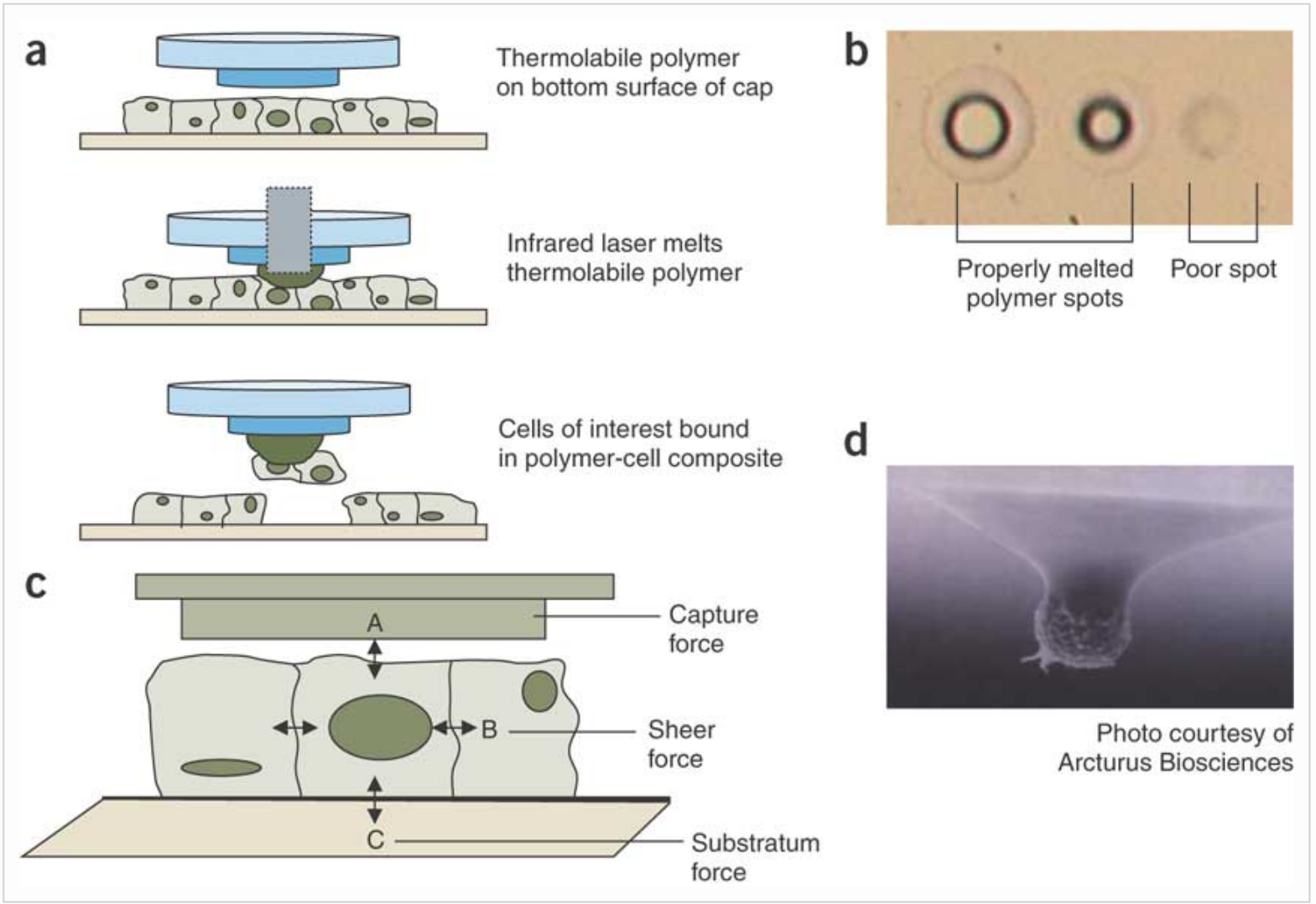
brains with AD pathology but no clinical dementia

brains from patients with AD pathology and clinical dementia

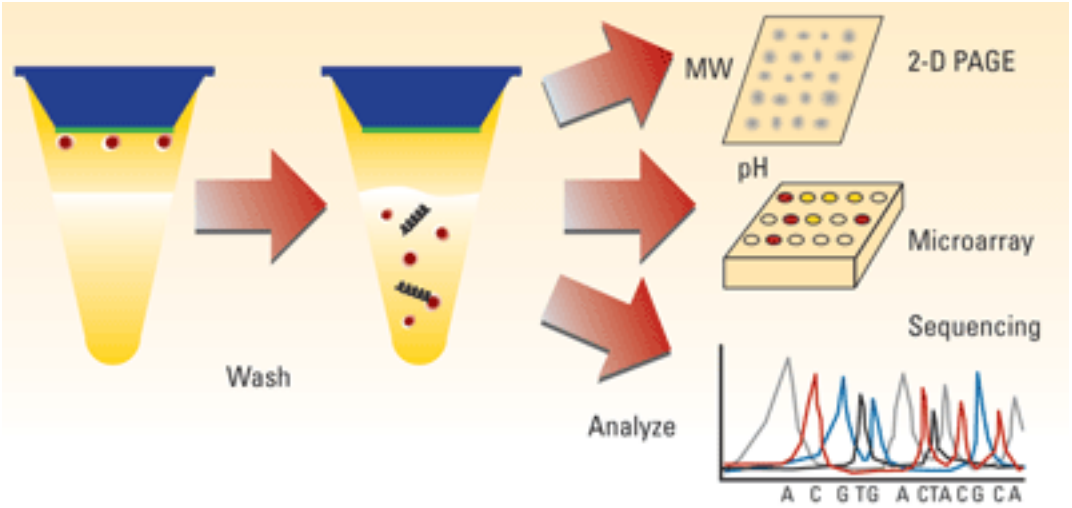
Key Questions –

- Do we use all the cell types?
- Do we sample diseased areas or non-diseased areas?

Decision to use laser captured neurons



(**a**) A thermolabile polymer is placed on a tissue section on a glass slide. An infrared laser melts the polymer in the vicinity of the laser pulse. The resulting polymer-cell composite is removed from the tissue. (**b**) Properly melted polymer spots have a dark outer ring and a clear center, indicating that the polymer has melted and is in direct contact with the slide. Only cells lying within the diameter of the black melted polymer will be targeted for microdissection with each laser pulse. Poor spots have a fuzzy appearance, lacking a distinct black ring. (**c**) Physical forces involved in LCM include an upward adhesive force between the substratum and the tissue, lateral forces between the cells, and a downward adhesive force between the polymer and the cells. (**d**) A single cell is bound to the thermolabile polymer following microdissection with the infrared laser-capture technique. Photo courtesy of Arcturus Biosciences.



Labor Intensive for Large Number of Cases

OUR SECRET LABOR SAVING WEAPON

Liang, W.S.*, et al.* Altered neuronal gene expression in brain regions differentially affected by Alzheimer's disease: a reference data set. *Physiol Genomics* **33**, 240-256 (2008).

Liang, W.S.*, et al.* Neuronal gene expression in non-demented individuals with intermediate Alzheimer's Disease neuropathology. *Neurobiol Aging* **31**, 549-566 (2010).

Liang, W.S.*, et al.* Gene expression profiles in anatomically and functionally distinct regions of the normal aged human brain. *Physiol Genomics* **28**, 311-322 (2007).

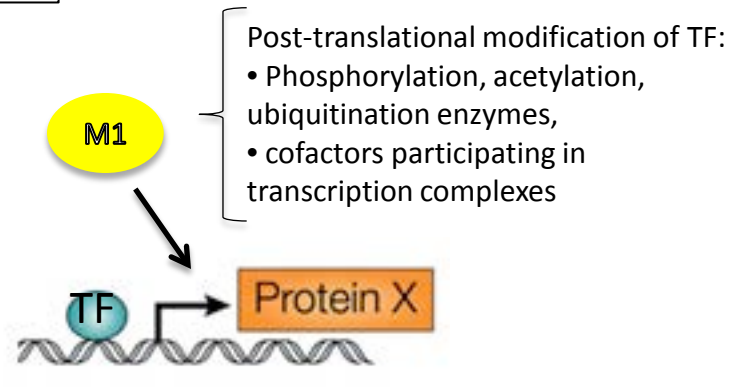
Liang, W.S.*, et al.* Alzheimer's disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. *Proc Natl Acad Sci U S A* **105**, 4441-4446 (2008).

Interactome:
"cell-specific genome-wide maps of *transcriptional* and *post-translational* interactions"

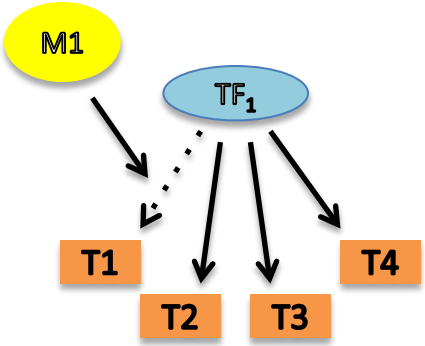
First step: build the interactome

Two main algorithms

ARACNe: "Algorithm for the reconstruction of Accurate cellular Networks"
-> dissection of transcriptional interaction networks



MINDy: "Modulator Inference by Network Dynamics"
-> algorithm for the inference of post-translational modulators of transcription factor activity



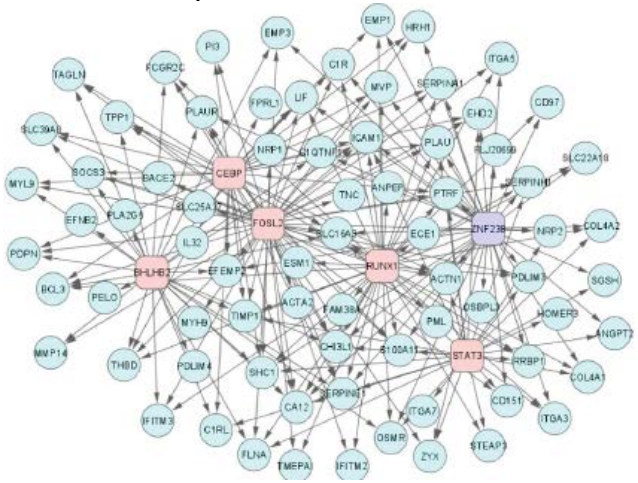
Interactome:
"cell-specific genome-wide maps of *transcriptional* and *post-translational* interactions"

Second step: interrogate the interactome

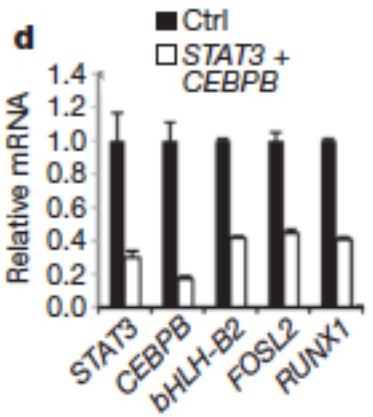
Comparing two phenotypes: gene expression signature.
We try to identify the candidate master regulators of this cell phenotype.
We validate the master regulator using biochemical tools.

And finally place these master regulators within an appropriate signaling and protein interaction context, determine the modulators which can be new targets to regulate these driver genes.

Master regulators of the molecular signature relative to a specific treatment



Biochemical validation

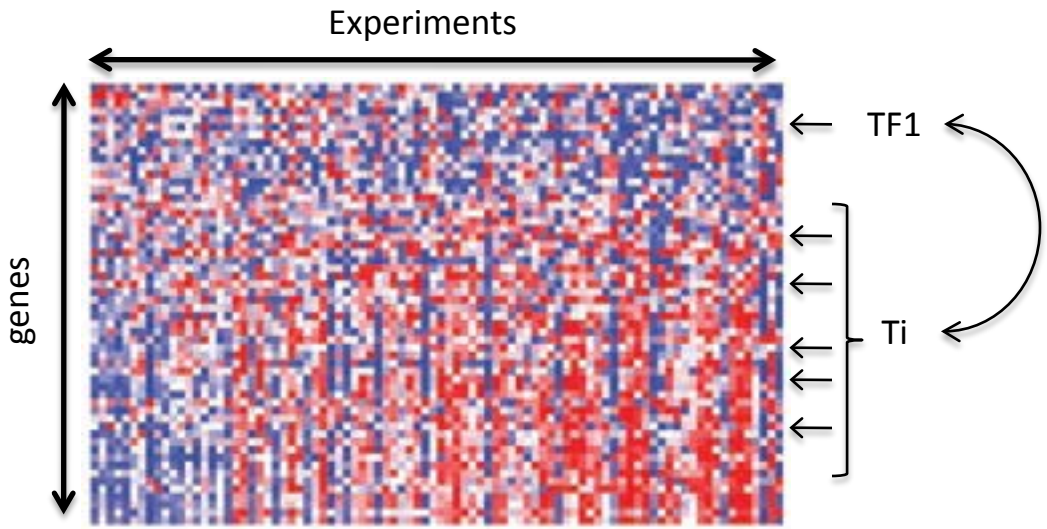


ARACNe : direct transcriptional interactions

First step:
list all the proteins interacting with each TF

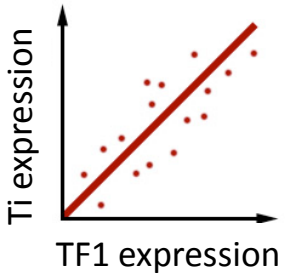
3 inputs: GEP database
TF of interest
List of potential TF targets

Microarray expression profile data

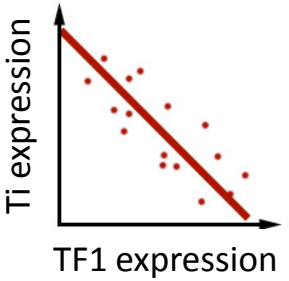


"mutual information (mi)": measure of correlation

Positive correlation?

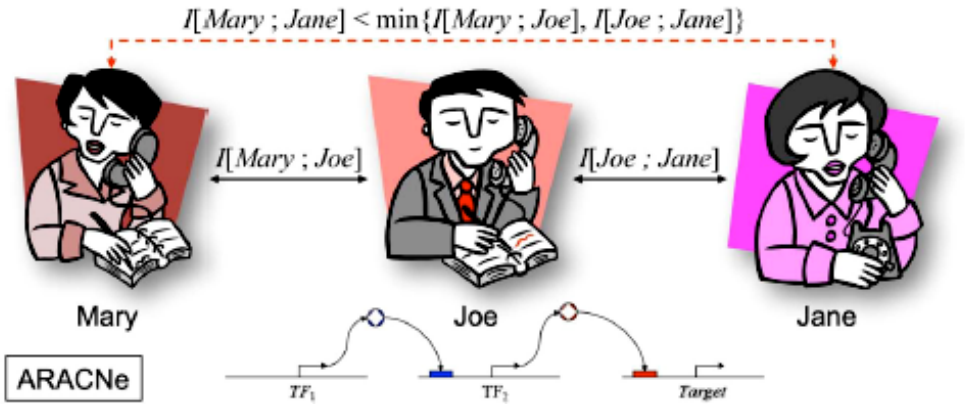


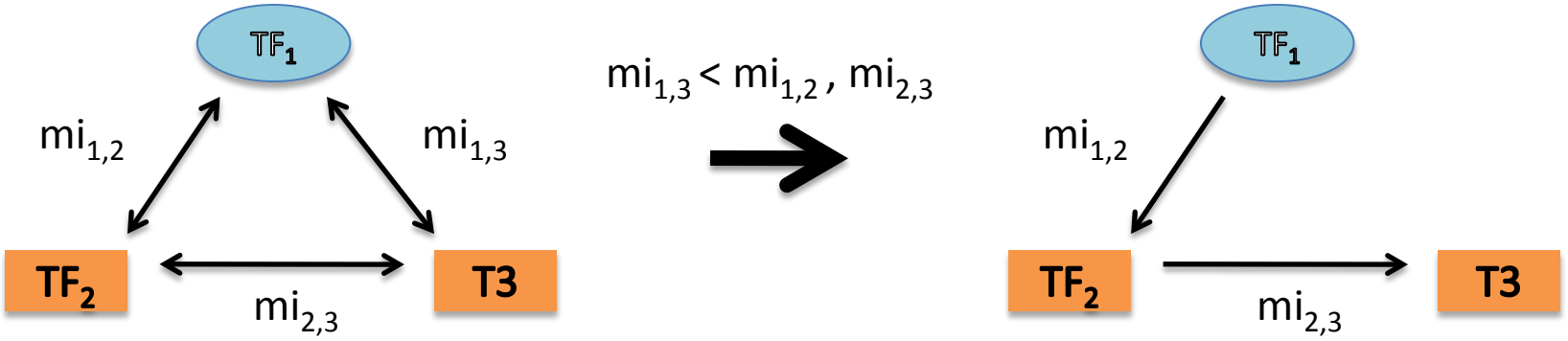
Negative correlation?



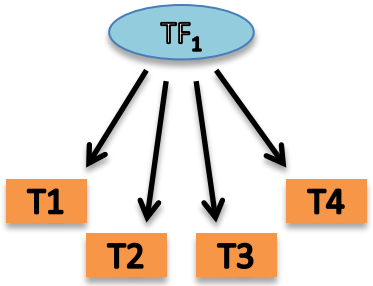
ARACNe : direct transcriptional interactions

Second step:
Remove the proteins that are *indirectly* interacting with each TF





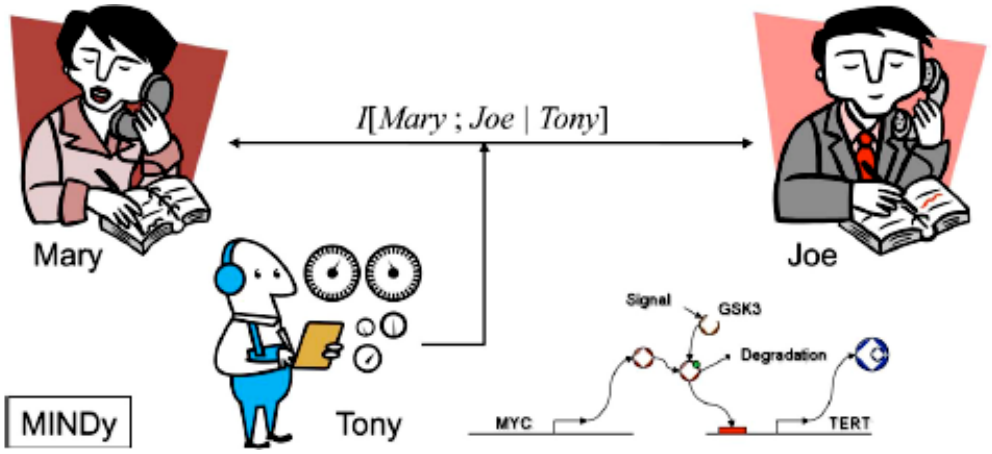
ARACNe : direct transcriptional interactions

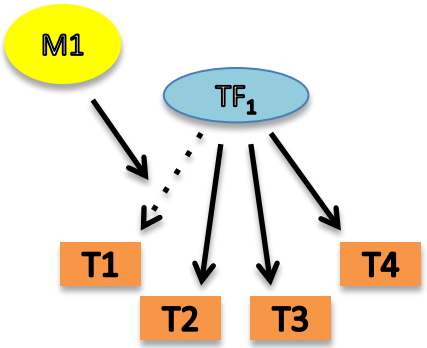


First interactome: ARACNe

Need: 100 – 150 samples (cell treatments)

MINDy : post-translational modulations

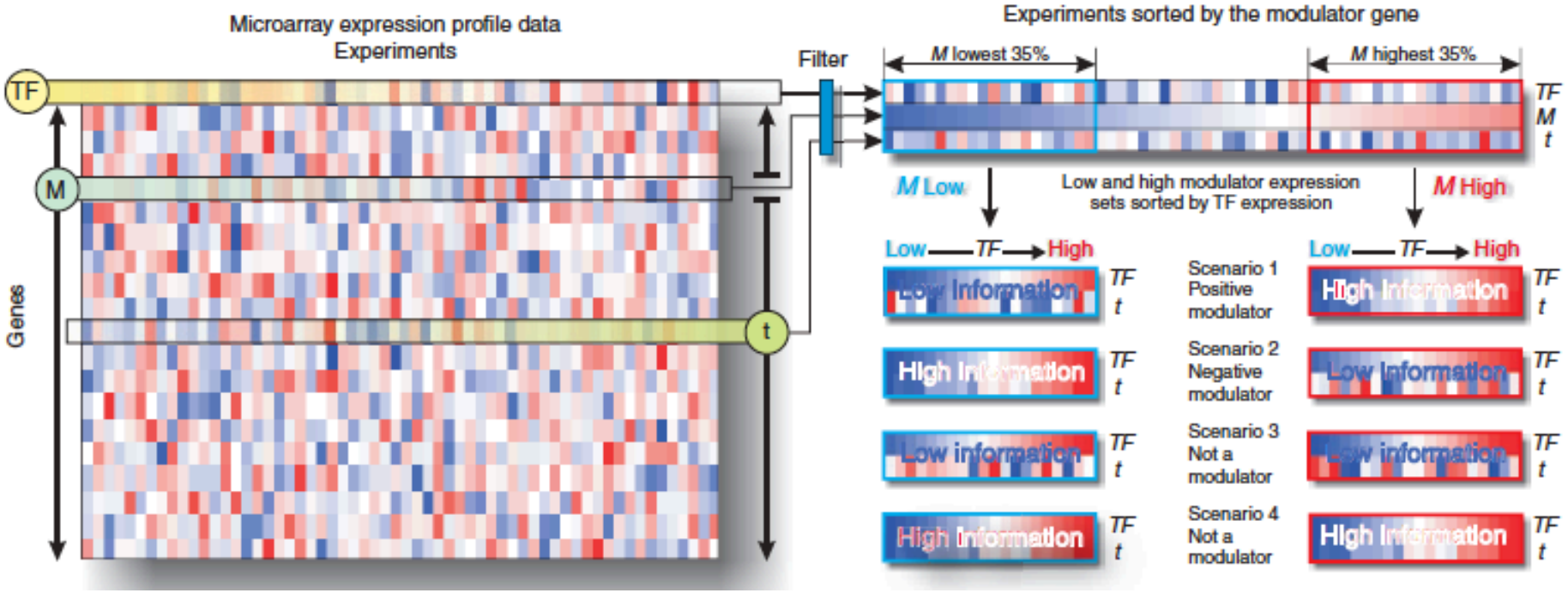




MINDy : post-translational modulations

4 inputs: GEP database
TF of interest
List of potential modulator genes
List of potential TF targets

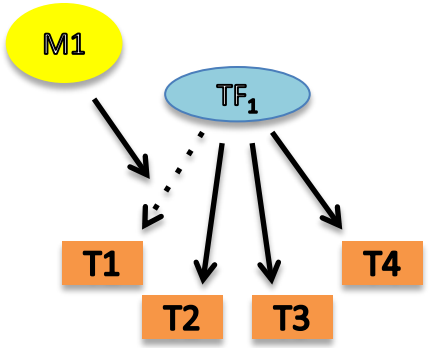
Is M a modulator of the transcriptional activity: TF -> t ?



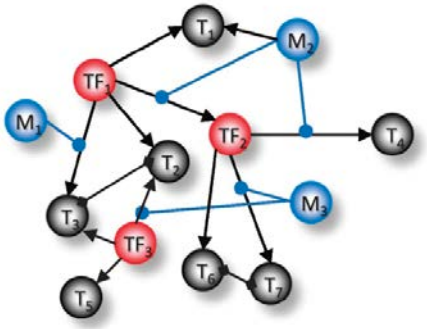
MINDy : post-translational modulations

Second interactome: MINDy

Need: 250 cell treatments



In combination, ARACNe and MINDy can produce high-accuracy, genome-wide maps of molecular interactions, including transcriptional and post-translational pathways, that are cell-context specific



Interrogate the interactome to find the master regulators of a specific phenotype

Gene expression profile data set

Specific phenotype
(Aβ treatment for ex)

Molecular signature: list of genes whose expression differs from the control

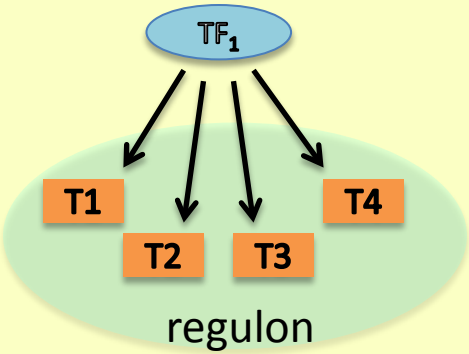
Interrogate ARACNe and MINDy

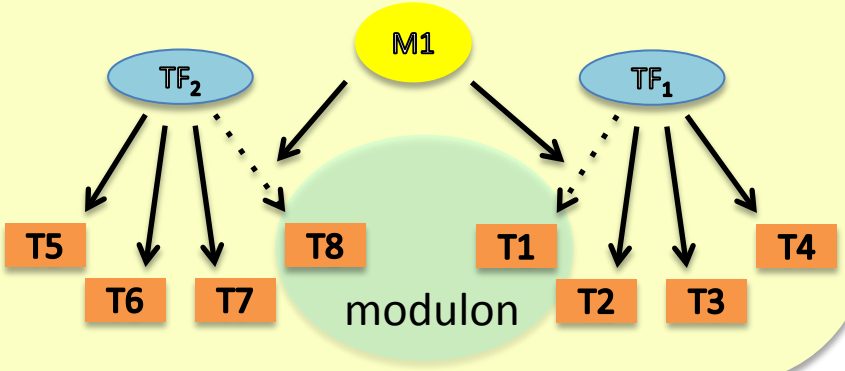
Master regulators

"Gene sets"

TFi ↔ Gene list of targets: "regulon"

Mi ↔ Gene list of all regulons affected by Mi: "modulon"





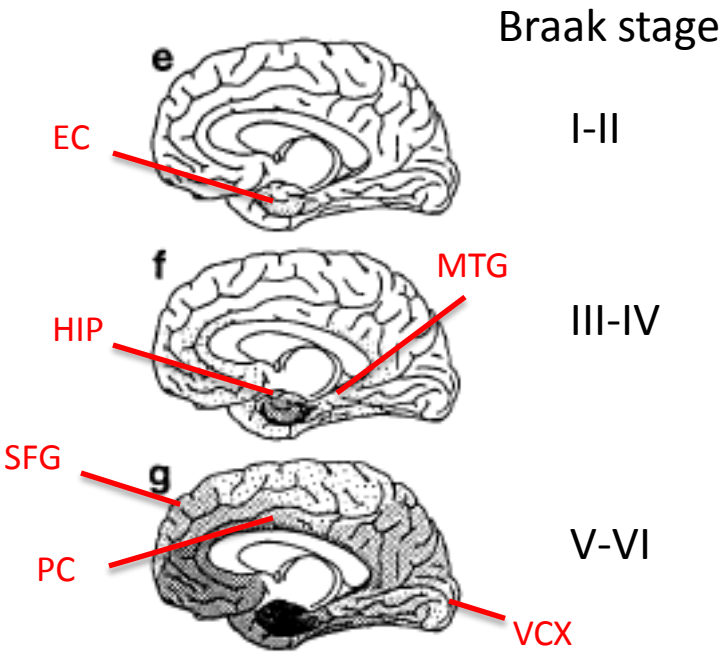
Master regulators

Determine the MRs driving AD phenotype

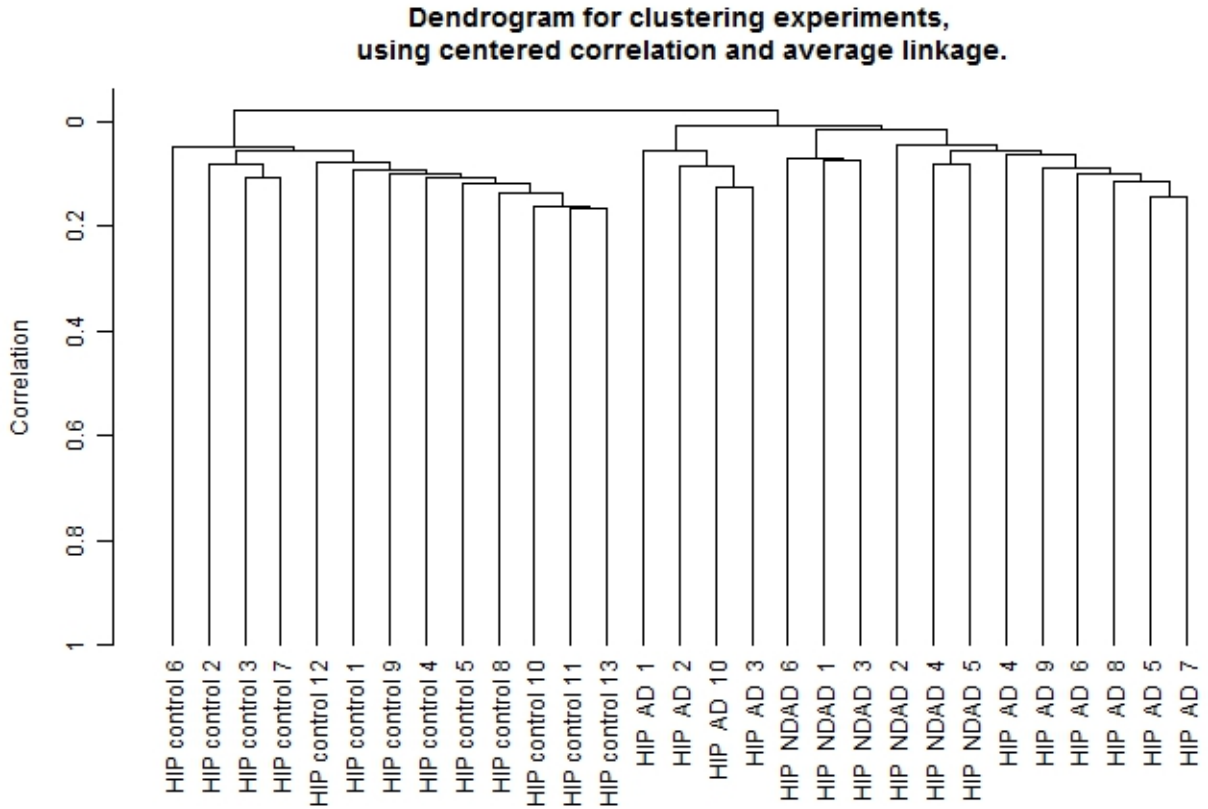
Online: Gene Expression Omnibus website
"Alzheimer's disease and the normal aged brain"
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi

- 150 individual brains sampled
- Laser Capture Microssection of non tangles bearing pyramidal neurons from 6 brain regions differently affected by the disease
- Expression profile on Affymetrix U133 Plus 2.0 Array

Region	Control	NDAD	AD
Age (y)	79.8 ± 9.1	86.6 ± 5.3	79.9 ± 6.9
Braak Stage	I - II	II - IV	V - VI
CERAD (neuritic plaque density)	Infrequent	Moderate / frequent	Moderate / frequent
Clinical diagnostic	Non demented	Non demented	Demented
1_EC	11	6	9
2_HIP	13	6	10
3_MTG	11	6	16
4_PC	13	5	9
5_SFG	10	6	23
6_VCX	12	5	19

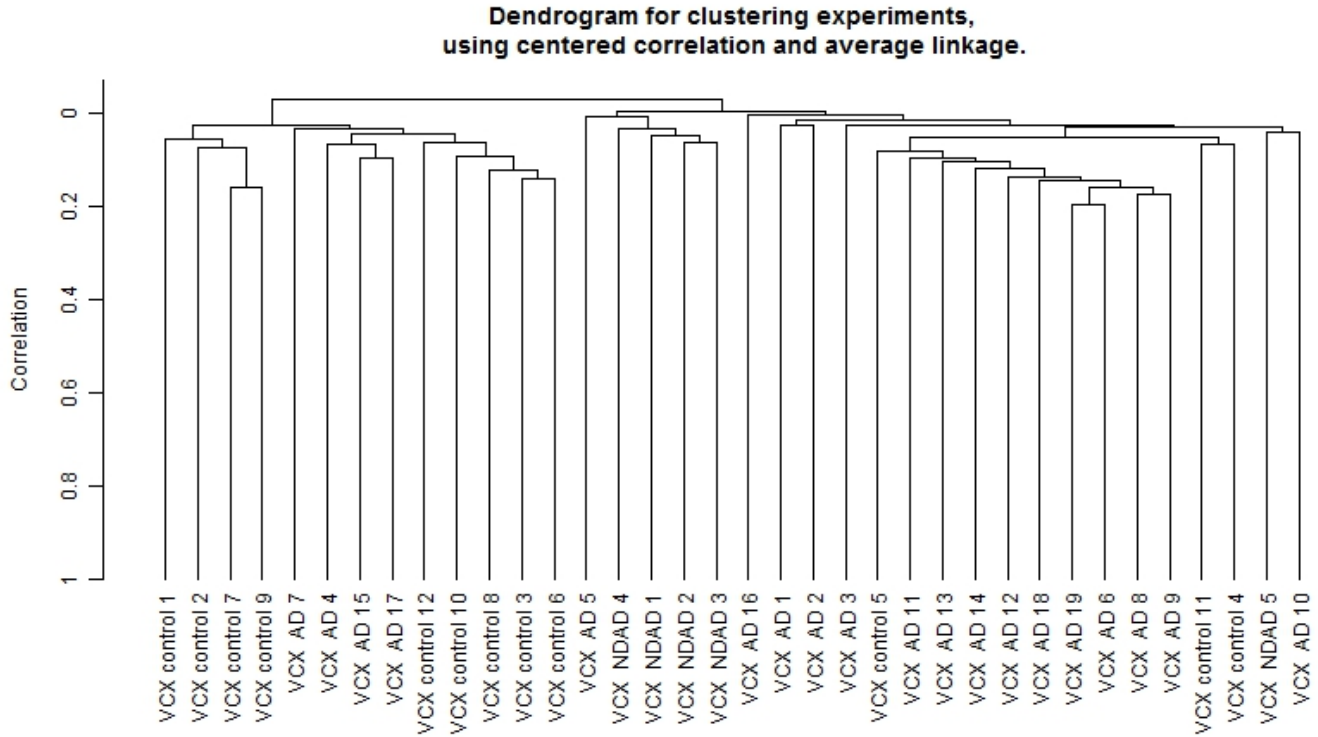


Clustering analysis of the database



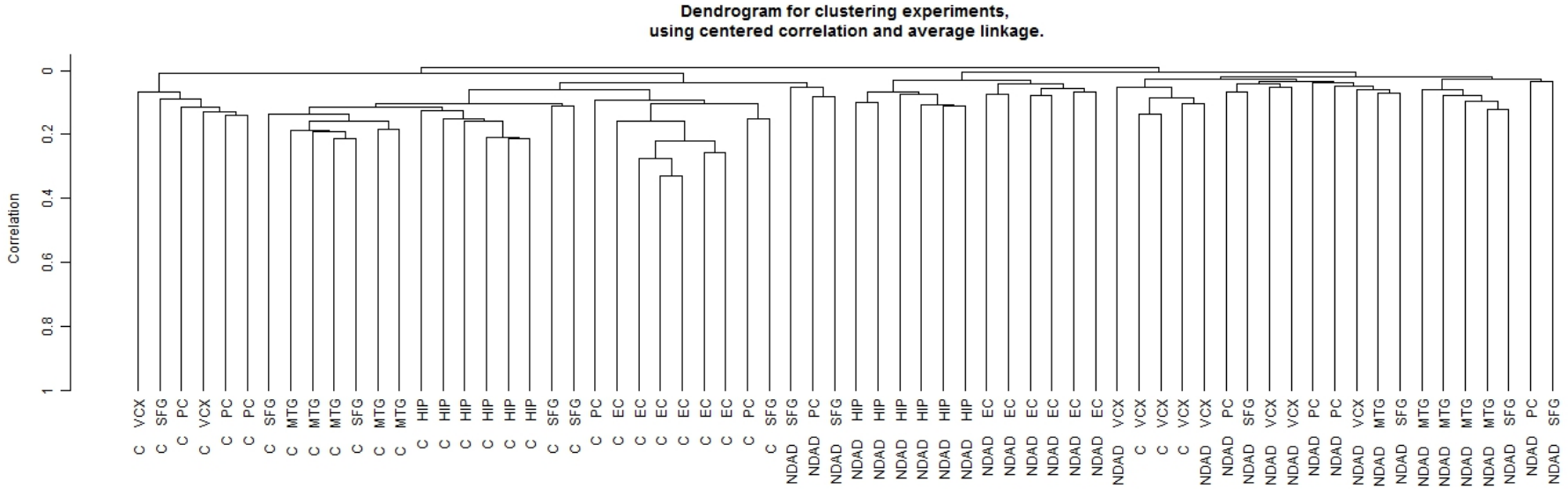
Clustering of the samples by diagnosis

Clustering analysis of the database



As expected, VCX not very specific to the disease

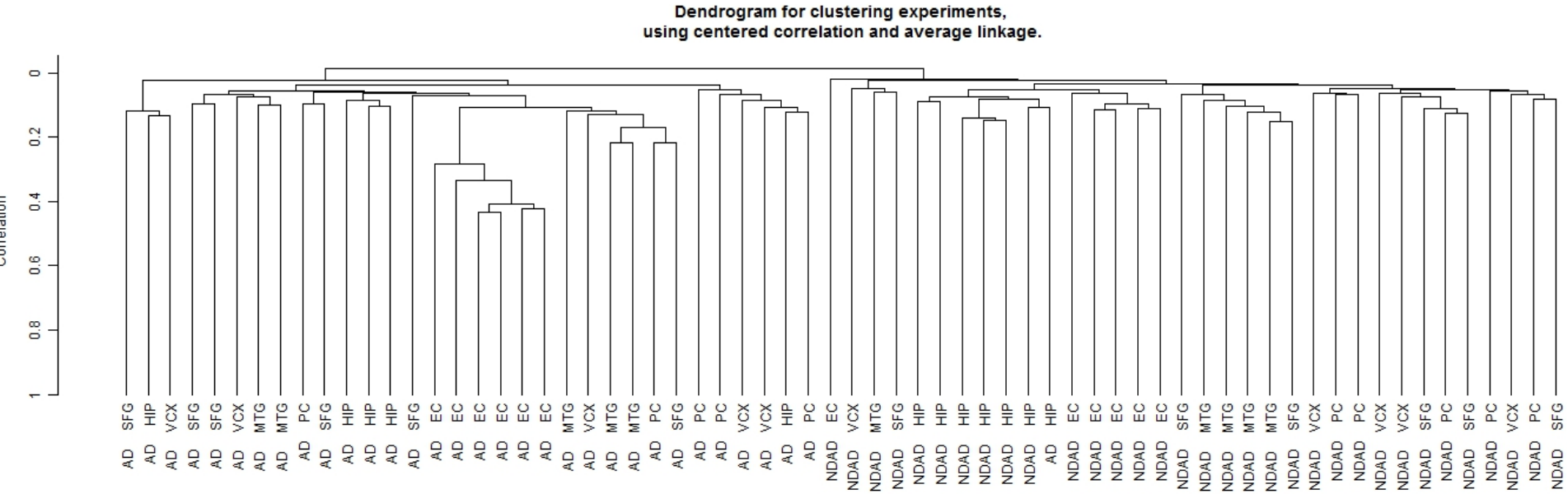
Clustering analysis of the database



Very encouraging clustering of the samples

- First clustering by disease state
- Second clustering by region

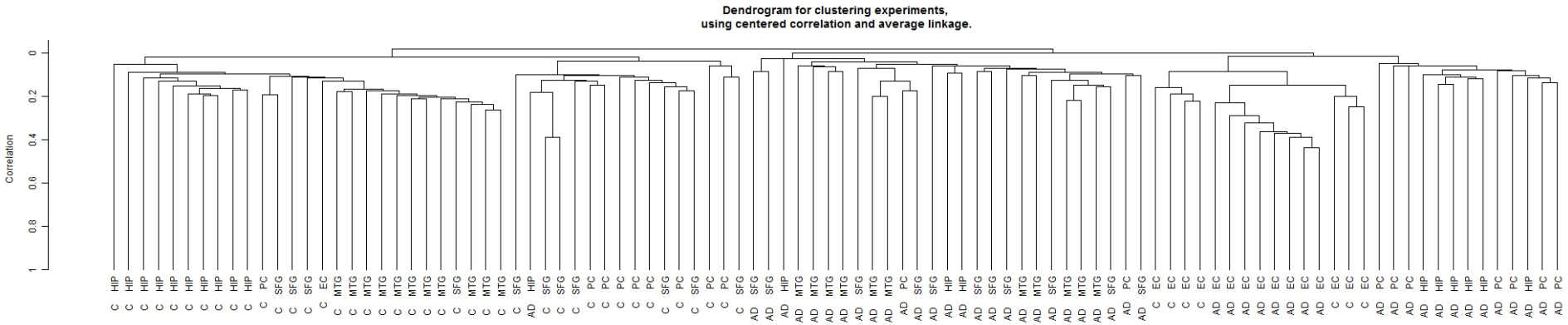
Clustering analysis of the database



Data Quality reflected in Clustering

- First clustering by disease state
- Second clustering by region

Clustering analysis of the database

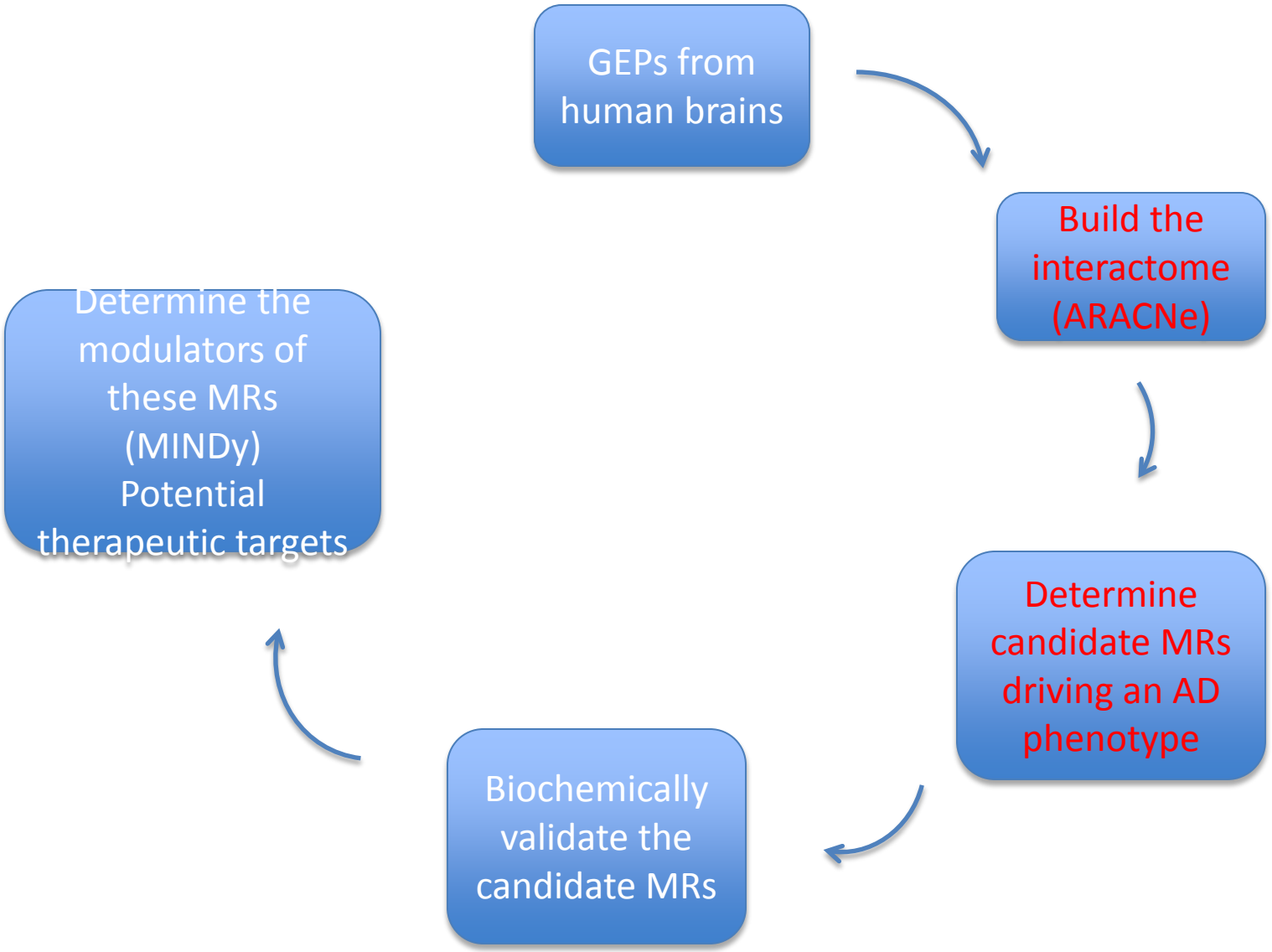


Very encouraging clustering of the samples

- First clustering by disease state
- Second clustering by region

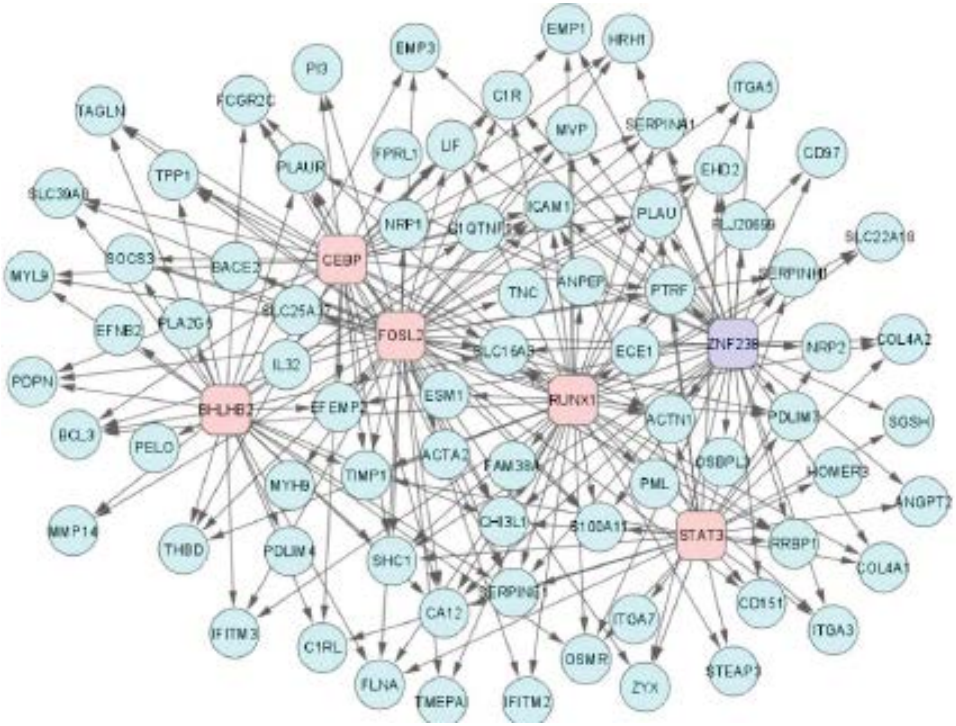
For EC:
C=AD ≠ ND

Determine the MRs driving AD phenotype

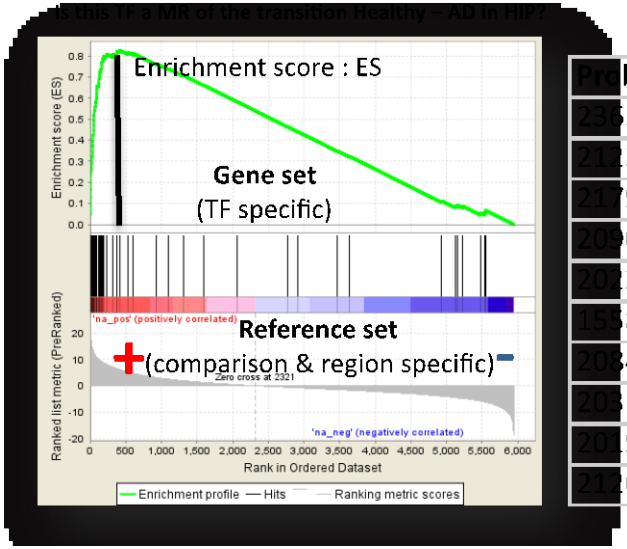


ARACNe

ARACNe run with 193 GEPs:
Network with 500,000 interactions



Enrichment plot



Master Regulators driving the progression Healthy-AD in hippocampus

Probe	Gene	GeneName	Average
236557_at	ZBTB38	zinc finger and BTB domain containing 38	11.5
212382_at	TCF4	transcription factor 4	12.875
217951_s_at	PHF3	PHD finger protein 3	17.333
209088_s_at	UBN1	ubinuclein 1	20
202221_s_at	EP300	E1A binding protein p300	21.571
1558733_at	ZBTB38	zinc finger and BTB domain containing 38	21.778
208445_s_at	BAZ1B	bromodomain adjacent to zinc finger domain, 1B	21.857
203176_s_at	TFAM	transcription factor A, mitochondrial	22.75
201901_s_at	YY1	YY1 transcription factor	23.875
212079_s_at	MLL	myeloid/lymphoid or mixed-lineage leukemia (trithorax hor	24.1

MARINa analysis: Master Regulators driving AD phenotype in HIP

C-AD, HIP: 117 MRs found

MARINA:
Rank genes according to enrichment scores

Good overlap

REGRESSION:
Rank genes according to the percentage of genes they regulate in the molecular signature

MARINA

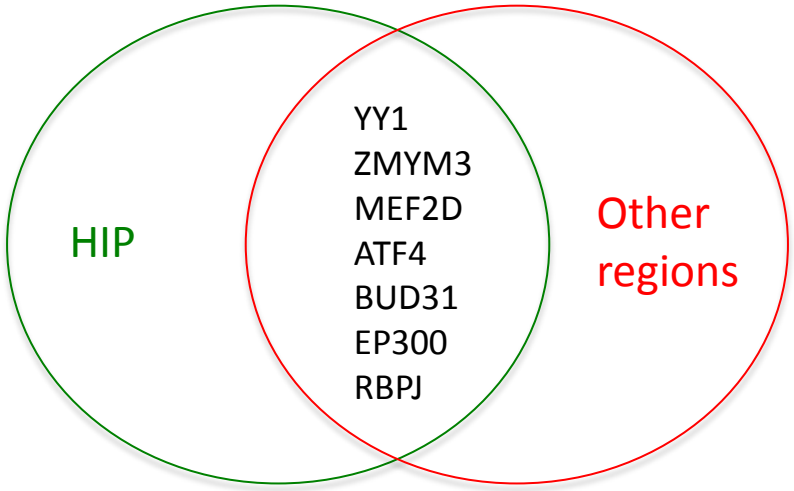
GeneName	GeneDesc	
ZBTB38	zinc finger and BTB domain containing 38	
KDM5A	lysine (K)-specific demethylase 5A	
PHF3	PHD finger protein 3	
LMO7	LIM domain 7	
C21orf66	chromosome 21 open reading frame 66	
PPP1R10	protein phosphatase 1, regulatory (inhibitor	* In Regression
ZBTB38	zinc finger and BTB domain containing 38	
UBN1	ubinuclein 1	
ZNF800	zinc finger protein 800	
ZNF710	zinc finger protein 710	
KDM5A	lysine (K)-specific demethylase 5A	
KLF9	Kruppel-like factor 9	
ZC3H11A	zinc finger CCCH-type containing 11A	
BAZ1B	bromodomain adjacent to zinc finger doma	* In Regression
YY1	YY1 transcription factor	
ZFR	zinc finger RNA binding protein	* In Regression
NFAT5	nuclear factor of activated T-cells 5, tonicity	* In Regression
ZNF532	zinc finger protein 532	* Top in Regression
MLL	myeloid/lymphoid or mixed-lineage leukem	* In Regression
THRA	thyroid hormone receptor, alpha (erythrobl	* In Regression (Different Probe)

REGRESSION

GeneName	GeneDesc	
ZNF532	zinc finger protein 532	* In Marina
MLL	myeloid/lymphoid or mixed-lineage leukem	* In Marina
ZFR	zinc finger RNA binding protein	* In Marina (Different Probe)
NFAT5	nuclear factor of activated T-cells 5, tonicity	* In Marina
PPP1R10	protein phosphatase 1, regulatory (inhibitor	* In Marina
BAZ1B	bromodomain adjacent to zinc finger doma	* In Marina
ZNF419	zinc finger protein 419	
THRA	thyroid hormone receptor, alpha (erythrobl	* In Marina (Different Probe)
CTBP1	C-terminal binding protein 1	
ZFP91	zinc finger protein 91 homolog (mouse)	

Selection of targets to test

- MRs that came up in HIP list
- MRs that overlapped with other regions

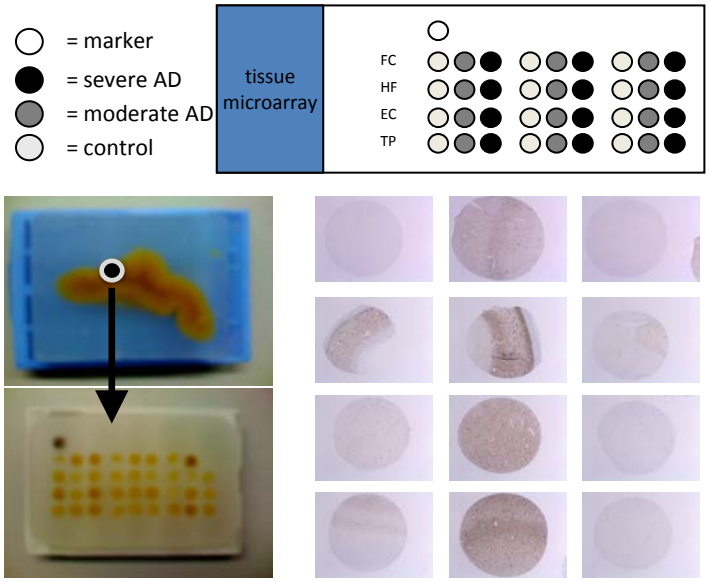


Validation of the targets

1. Cases selection

Groups	Control	Moderate AD	Severe AD
CERAD score	0-A	B	C
Braak stage	Up to IV	V-VI	VI
NFT frequency (frontal/parietal cortex)	None	Low (3-6 NT/)	High (> 7 NT/)

2. IHC on tissue microarray

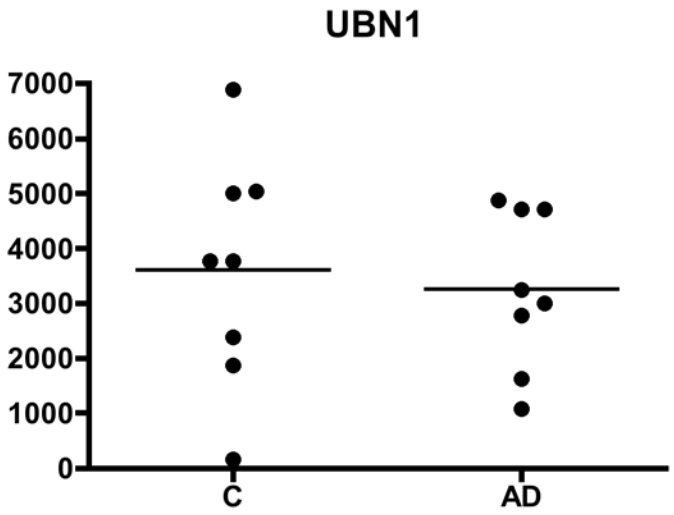


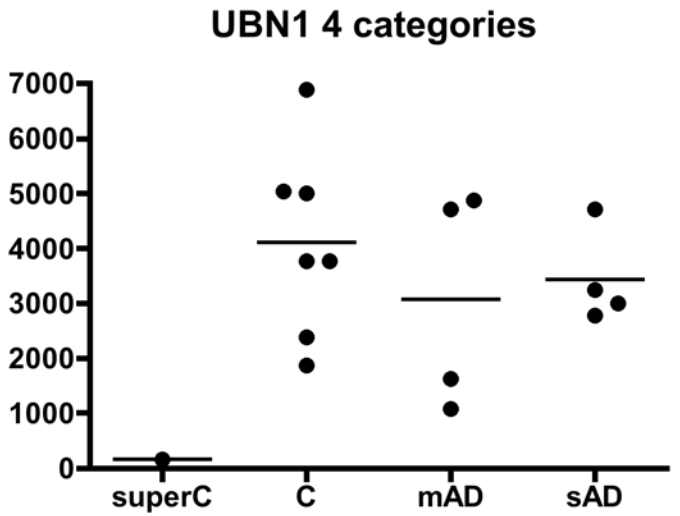
3. Western blots

Careful, Quantitative Neuropathology Essential

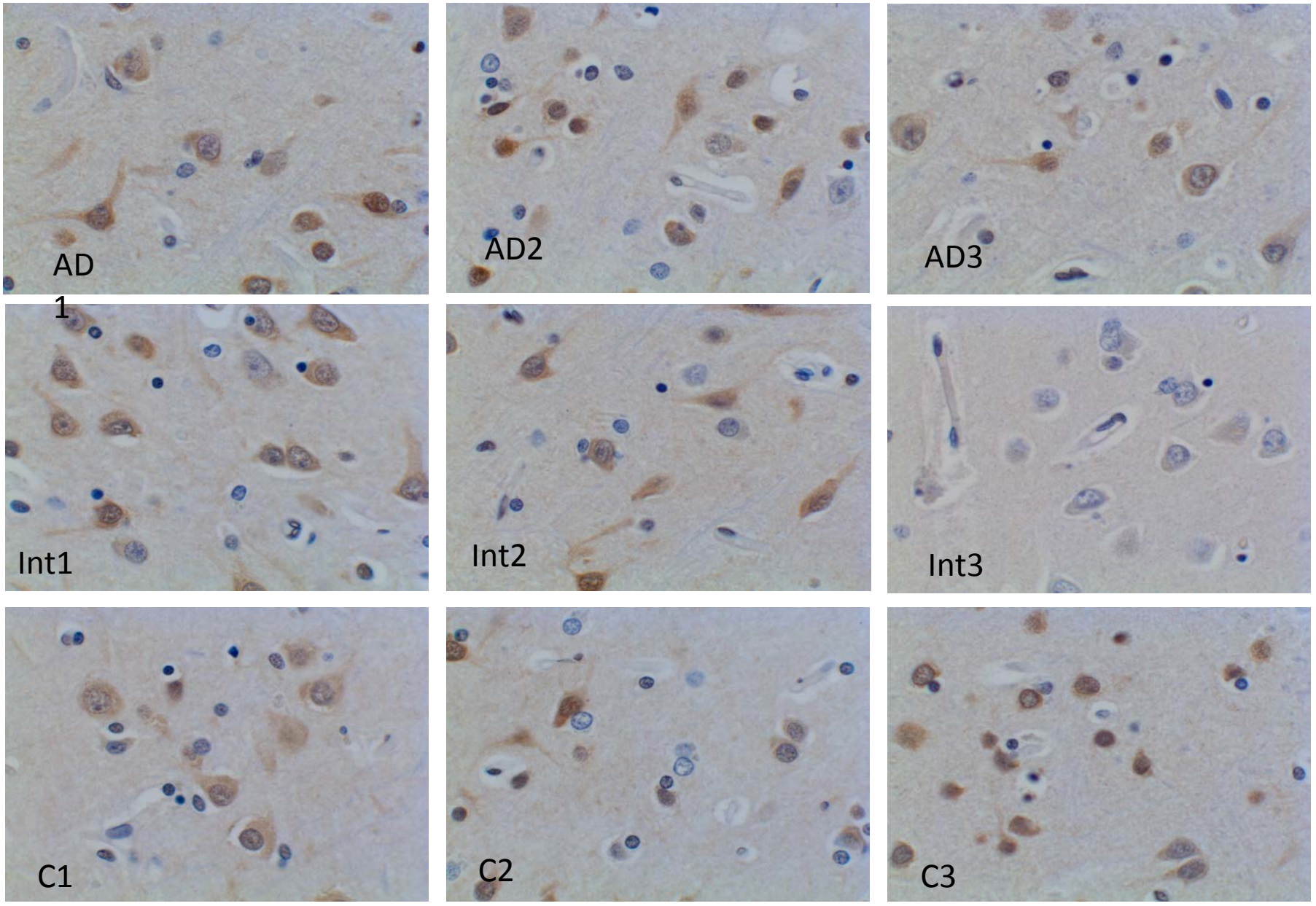
- What is a “Control”?
  - Age matched without dementia?
  - Age matched with other neurological disease?
  - Young brain from accident victim?
  - Low Braak and CERAD scores
  - Supercontrols (very old with minimal AD sigmata)
- No “right” answer but can really influence results.

Controls versus supercontrols - WB for ubinuclein





MEF2D FC

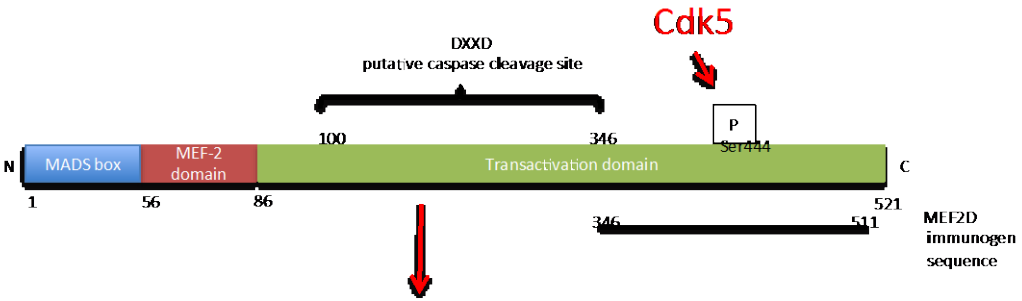


MEF2D - WB

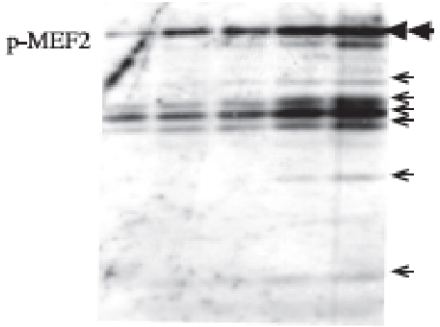
Cyclin-Dependent Kinase 5 Mediates Neurotoxin-Induced Degradation of the Transcription Factor Myocyte Enhancer Factor 2

Xiaoli Tang,[1]* Xuemin Wang,[1]* Xiaoming Gong,[1] Ming Tong,[1] David Park,[2] Zhengui Xia,[3] and Zixu Mao[1]

[1]Department of Medicine, Rhode Island Hospital and Brown Medical School, Providence, Rhode Island 02903, [2]Neuroscience Research Institute, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5, and [3]Departments of Environmental and Occupational Health Sciences and Pharmacology, University of Washington, Seattle, Washington 98195



Caspase cleavage

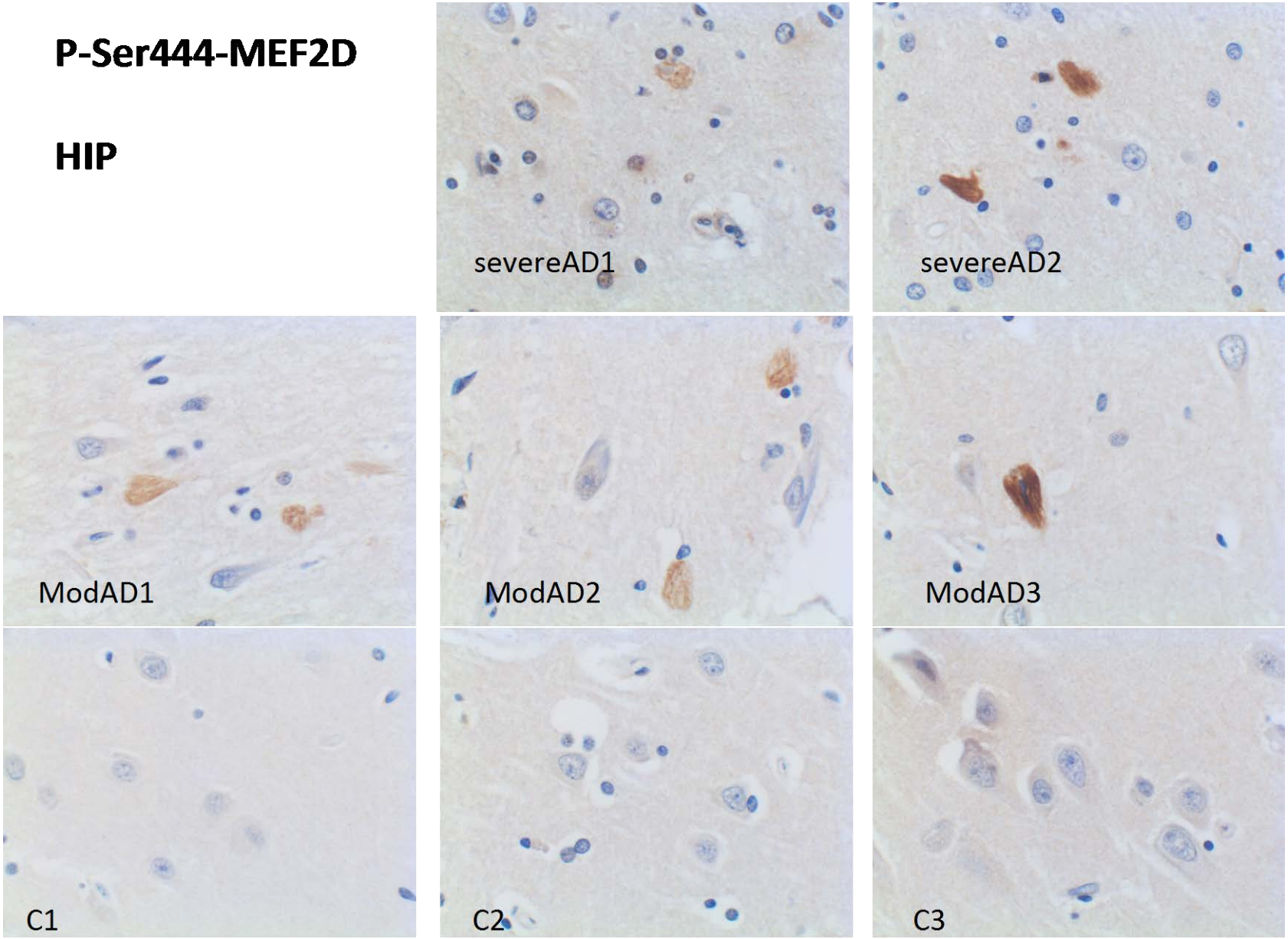


Phosphorylation of MEF2A and MEF2D at a site recognized by Cdk5 accompanies or parallels the caspase-mediated cleavage of MEF2 and neuronal death after neurotoxic insults.

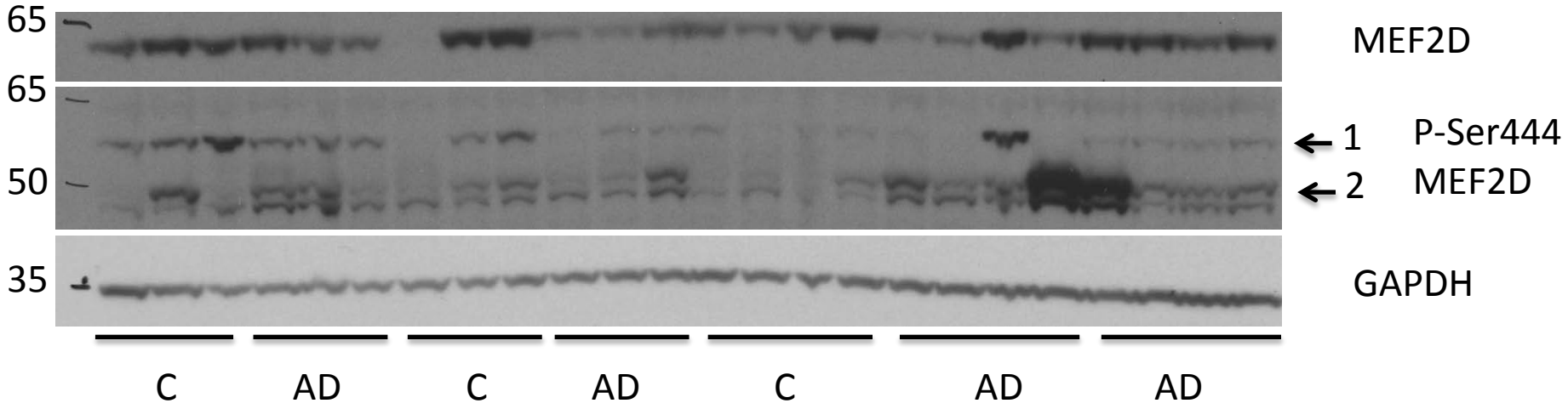
MEF2D - HIC

P-Ser444-MEF2D

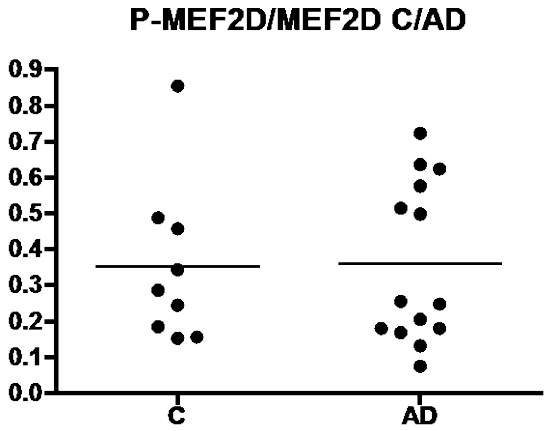
HIP



MEF2D - WB



Band #1 Densitometry analysis



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Cdk5-Mediated Inhibition of the Protective Effects of Transcription Factor MEF2 in Neurotoxicity-Induced Apoptosis

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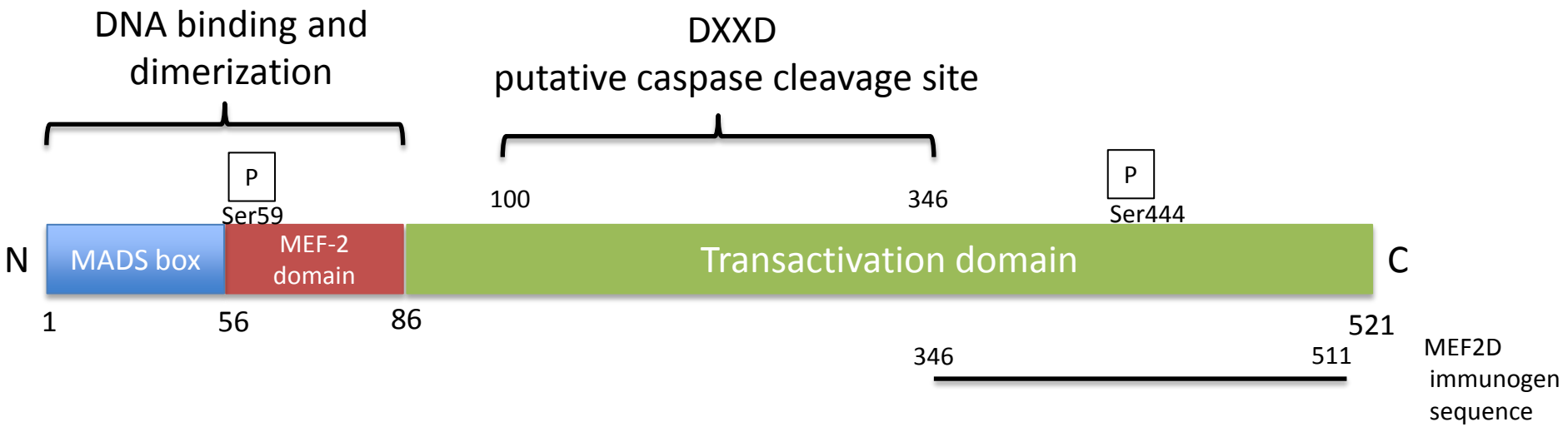
Summary

Neurotoxic insults deregulate Cdk5 activity, which leads to neuronal apoptosis and may contribute to

prehensive molecular mechanisms that couple toxic insults to apoptosis remain to be elucidated.

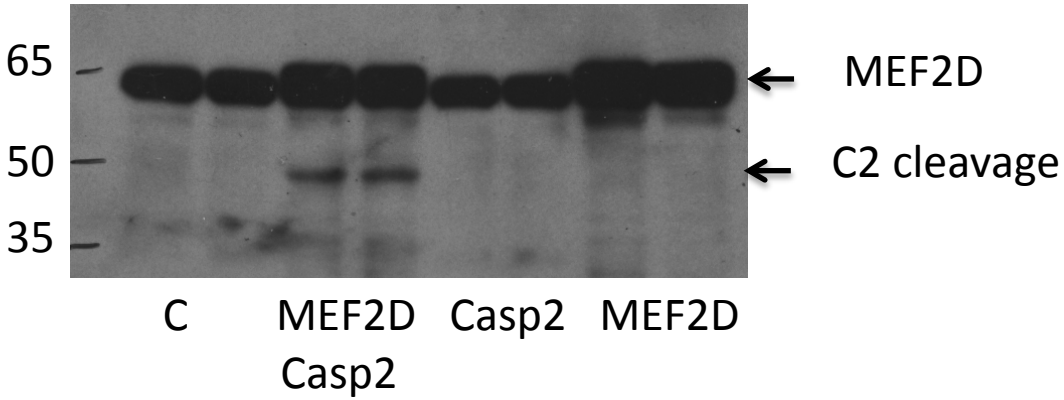
Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase whose activity is highest in postmitotic neurons. In contrast to classic Cdks whose activity is regulated during cell cycle by cyclins, Cdk5 is inactive in cell cycle (Grant et al., 2001). Instead, its activation requires association of Cdk5 with its neuron-enriched regulators p35 or p39 (Dhavan and Tsai, 2001). In vivo Cdk5 activity is tightly regulated. Complete lack of Cdk5 is clearly destructive to CNS, with inactivation of either p35 or Cdk5 resulting in profound neurological defects (Ko et al., 2001; Li et al., 2002; Tanaka et al., 2001). Yet, too much Cdk5 activity is also toxic to cells, particularly neurons, leading to neuronal apoptosis under either physiological or pathological conditions (Ahuja et al., 1997; Gao et al., 2001; Patrick et al., 1999; Zhang and Johnson, 2000; Zhang et al., 1997). Neurotoxicity could deregulate Cdk5 activity either through generating a more stable proteolytic product of p35, p25, or by stabilizing Cdk5/p35 complex. The ensuing inappropriate gain-of-function of Cdk5 has been proposed to play a key role in the molecular events linking neurotoxic insults to the degeneration of neurons found in

MEF2D

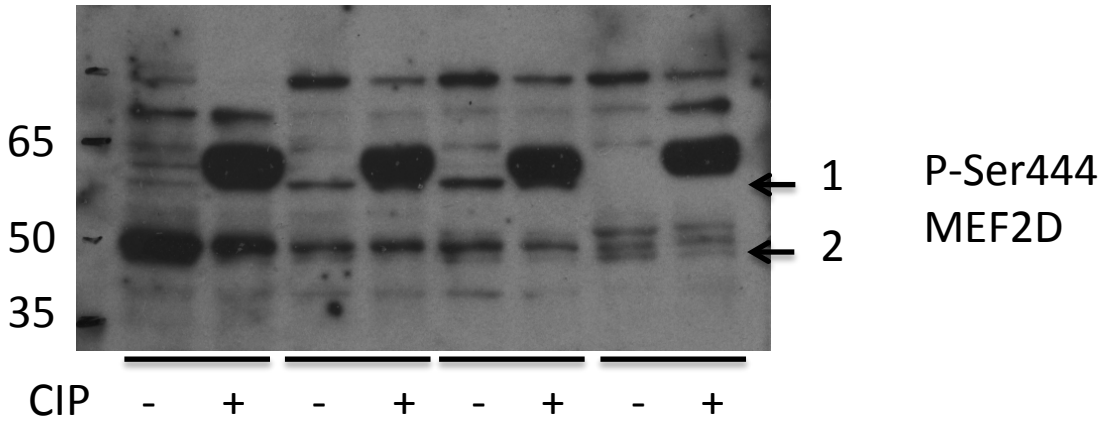

DNA binding and dimerization
DXXD
putative caspase cleavage site
P
Ser59
100
346
P
Ser444
N
MADS box
MEF-2 domain
Transactivation domain
C
1
56
86
521
346
511
MEF2D immunogen sequence

MEF2D

Caspase 2 cleavage *in vitro*

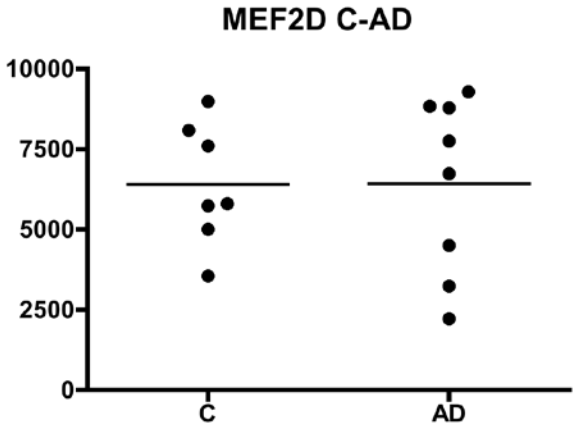


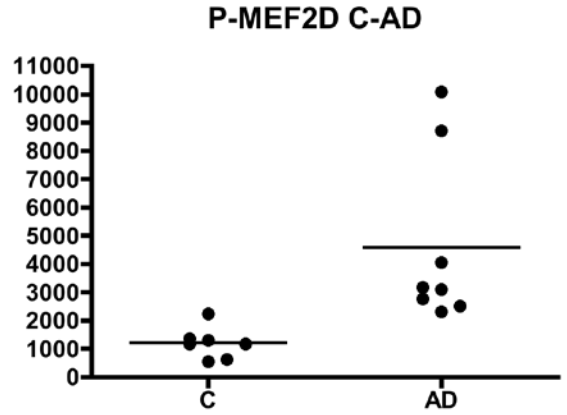
Phosphatase treatment

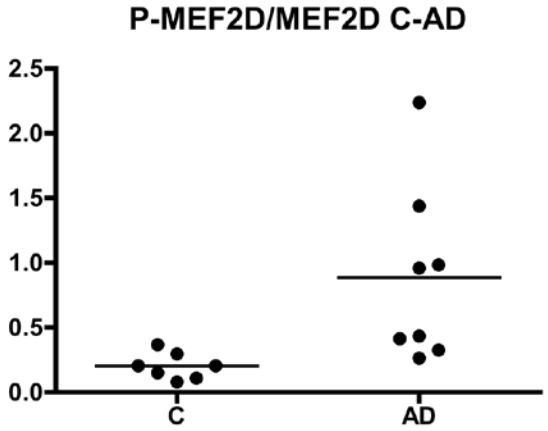


MEF2D

Band #2 : cleaved P-Ser444-MEF2D densitometry analysis







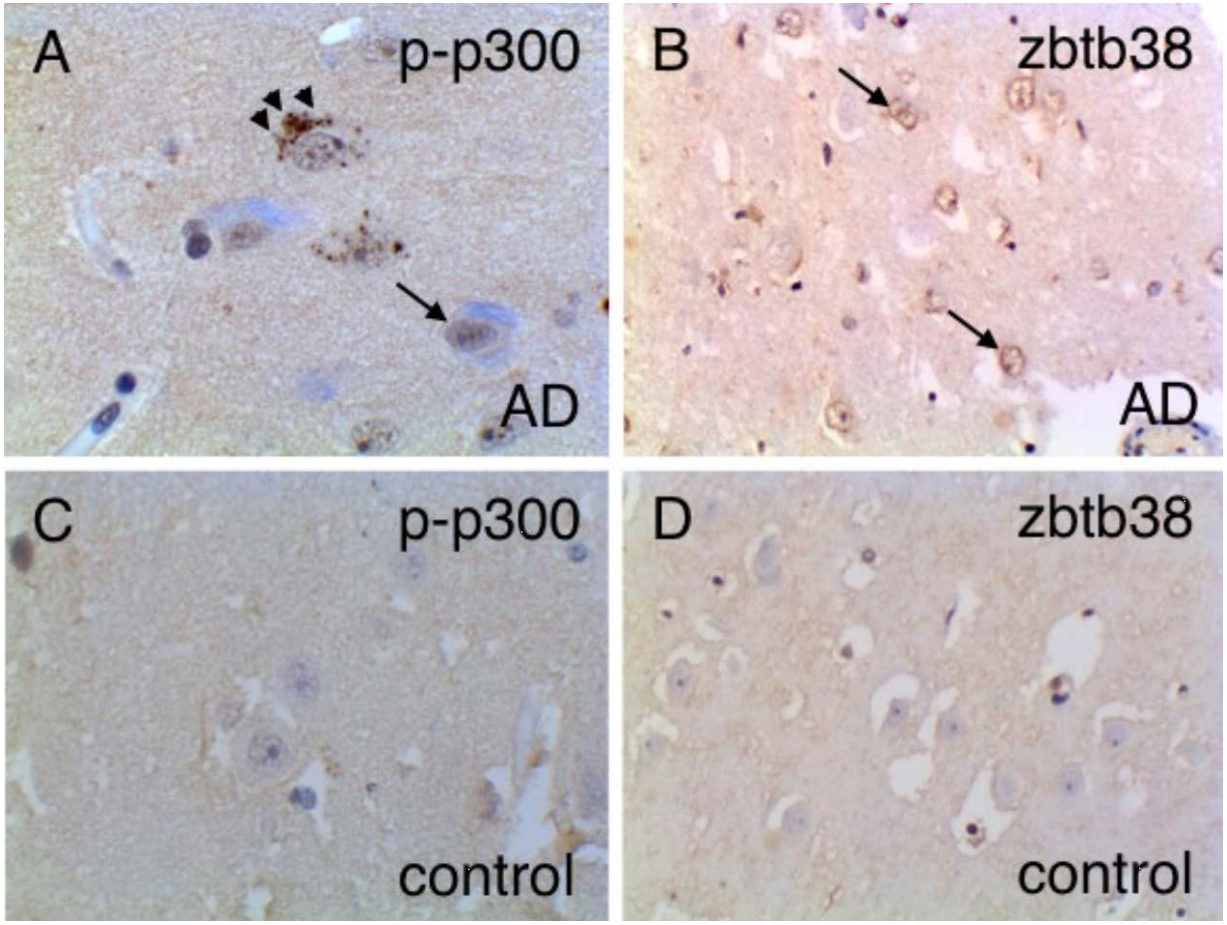
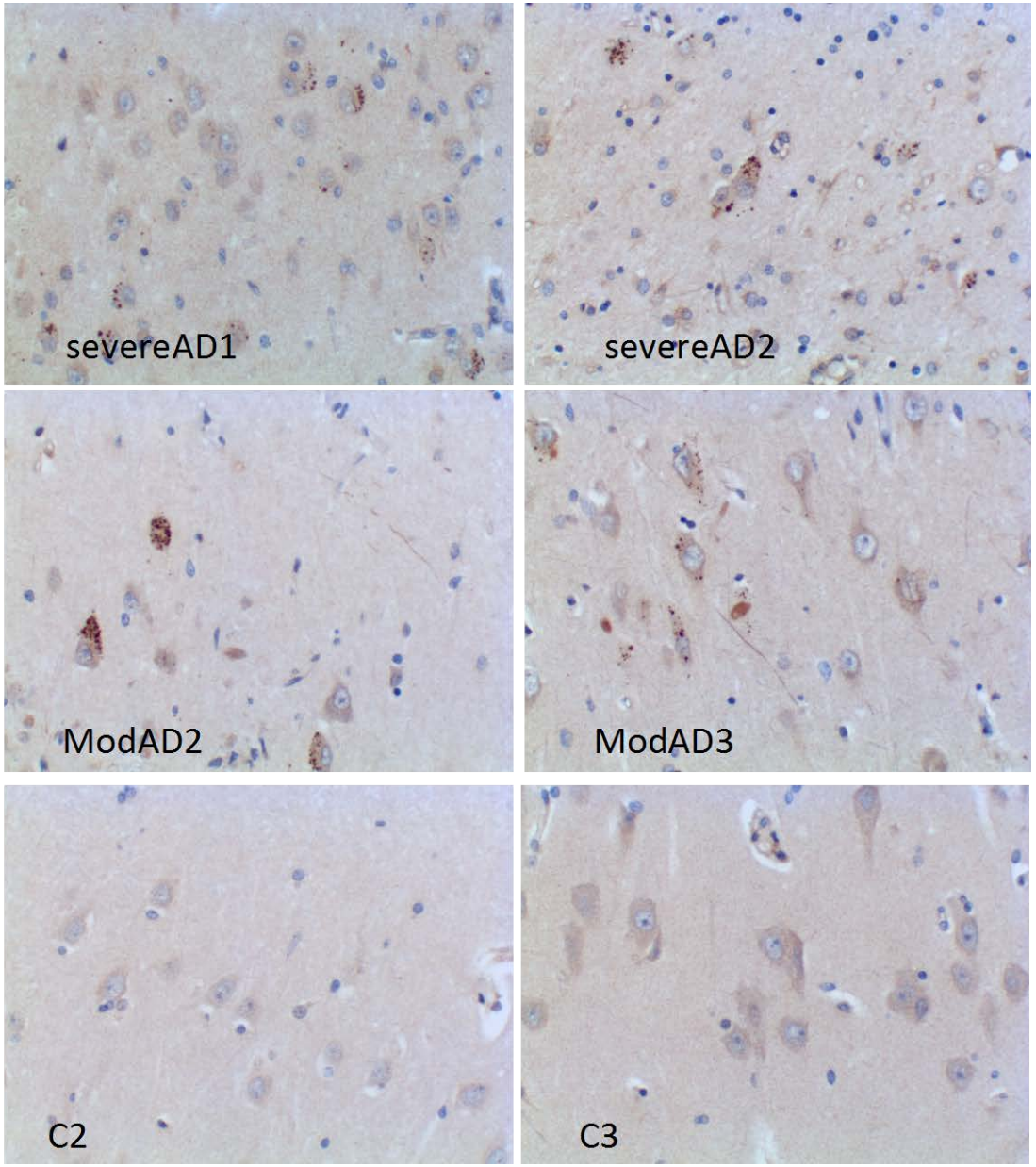


Fig 5. Immunohistochemistry on paraffin sections from the hippocampus from AD patients (*n*=3) demonstrates nuclear label (arrows) with phospho-p300 (A and C) and zbtb38 (B and D) antisera in CA1 pyramidal neurons. Cytoplasmic granular label with phospho-p300 (arrowheads) is also seen.
Controls (*n*=3) are negative (C and D). A, C = 400x; B, D = 200x.

EP300

P-Ser1834-p300

HIP



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Akt Phosphorylation of p300 at Ser-1834 Is Essential for Its Histone Acetyltransferase and Transcriptional Activity†

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The PI3K/Akt pathway plays a critical role in the regulation of gene expression induced by numerous stimuli. p300, a transcriptional coactivator, acts in concert with transcription factors to facilitate gene expression. Here, we show that Akt is activated and translocated to the nucleus in response to tumor necrosis factor alpha. Nuclear Akt associates with p300 and phosphorylates its Ser-1834 both in vivo and in vitro. The phosphorylation induces recruitment of p300 to the *ICAM-1* promoter, leading to the acetylation of histones in chromatin and association with the basal transcriptional machinery RNA polymerase II. These two events facilitate ICAM-1 gene expression and are abolished by the p300 S1834A mutant, inhibitors of PI3K/Akt, or small interfering RNA of Akt. Histone acetylation is attributed to the Akt-enhanced intrinsic histone acetyltransferase (HAT) activity of p300 and its association with another HAT, p/CAF. Our study provides a new insight into the molecular mechanism by which Akt promotes the transcriptional potential of p300.

MINDy analysis: Master Modulators driving AD phenotype

MINDy was run for HIP only

5 Master Modulators of the 117 Master Regulators previously determined show up across all comparisons (C-AD, C-NDAD, NDAD-AD):

Master Modulators of C-AD comparison in HIP:

Mod	Mod Description	MRtarget
TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box)	66
MACF1	microtubule-actin crosslinking factor 1	61
TANK	TRAF family member-associated NFKB activator	57
RICS	Rho GTPase-activating protein	54
GNAS	GNAS complex locus	47
HIPK2	homeodomain interacting protein kinase 2	47
INSR	insulin receptor	47
APLP2	amyloid beta (A4) precursor-like protein 2	46
PPM1A	protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform	46
RORA	RAR-related orphan receptor A	46

Conclusions

- Systems approaches can yield data and identify targets that are unobtainable by expression analysis alone and once the interactome is made it can be queried with a relatively small number of samples at low cost
- It can be applied to small regulatory RNA molecules as well as mRNA
- The algorithms are available online without cost

- The approach can be extended to integrate GWAS data with expression data

- Validation is required and this is more if difficult the tissue of interest cannot be cultured. For example, relatively easy to validate in cancer where the tumor in question can be cultured. Can readily validate interactome with RNAi. What is the “model” for a laser dissected post-mortem neuron?

    - IPS neurons?
    - Human neuronal cell lines (Sy5y)?
    - Human non-neuronal cells?

- For the moment we are stuck with stain and grind. Perhaps Path-RNAseq.

- The approach works best with public data sharing – NACC funded research critical to our results.

The Workers

- Shelanski Lab
  - Soline Aubry
  - Nike Beaubier
  - Warren McGee
- NYSCF
  - Scott Noggle
  - Andrew Sproul
- Califano Lab
  - Celine Lefebvre
  - Will Shin
- John Crary