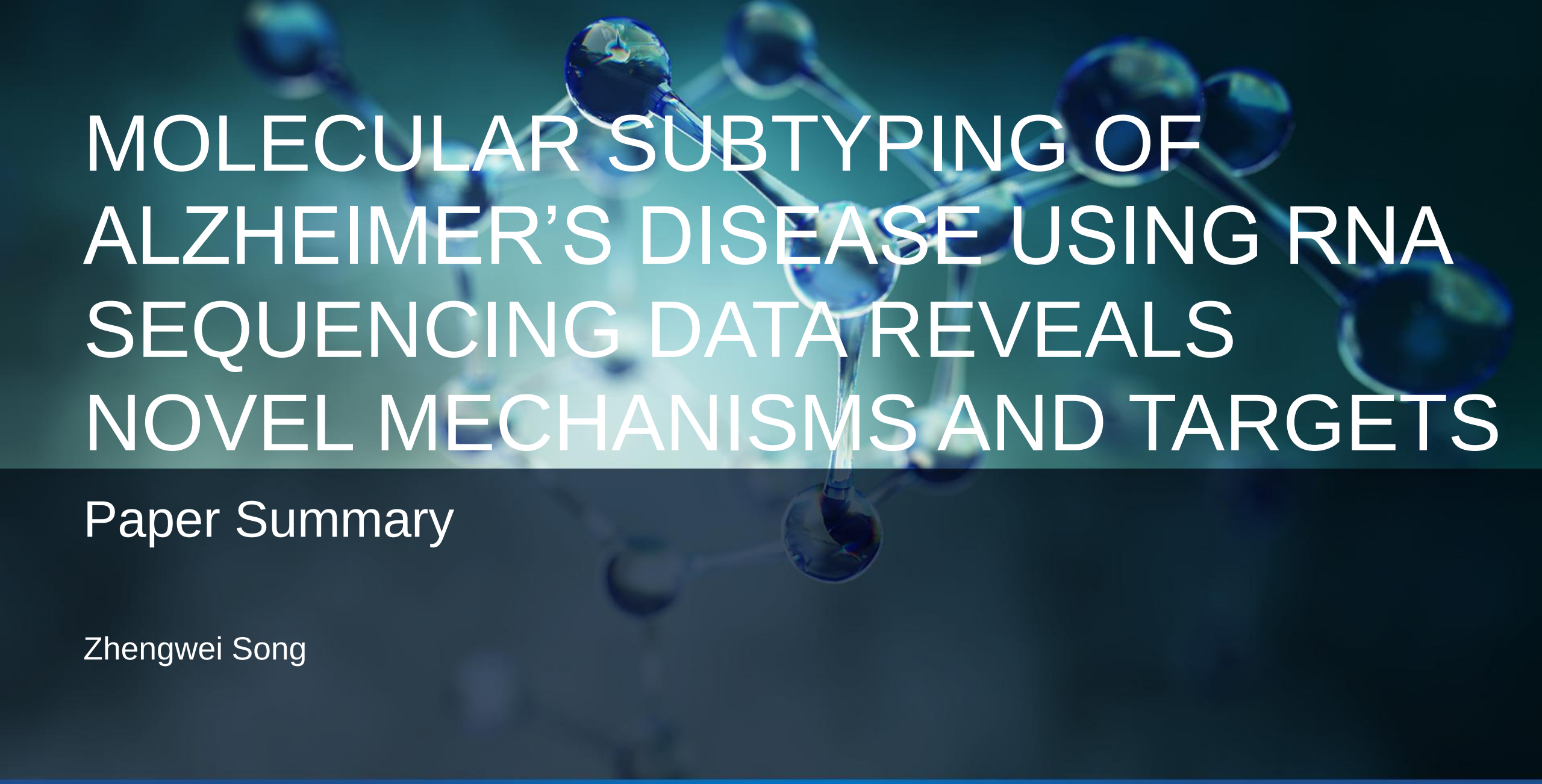

MOLECULAR SUBTYPING OF ALZHEIMER'S DISEASE USING RNA SEQUENCING DATA REVEALS NOVEL MECHANISMS AND TARGETS

Paper Summary

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Outline

Data Processing → Clustering → Associations → Key drivers of each subtype → Cell-type of each subtype →

Normalization

WSCNA

Subtype ~ Clinical & pathological phenotypes, APOE variants

Up- or Down-regulated

Mean change in cell-type proportion

Known AD genetic risk markers → Validation in ROSMAP → Validation in AD mouse models → Discussion

↑ or ↓

WSCNA

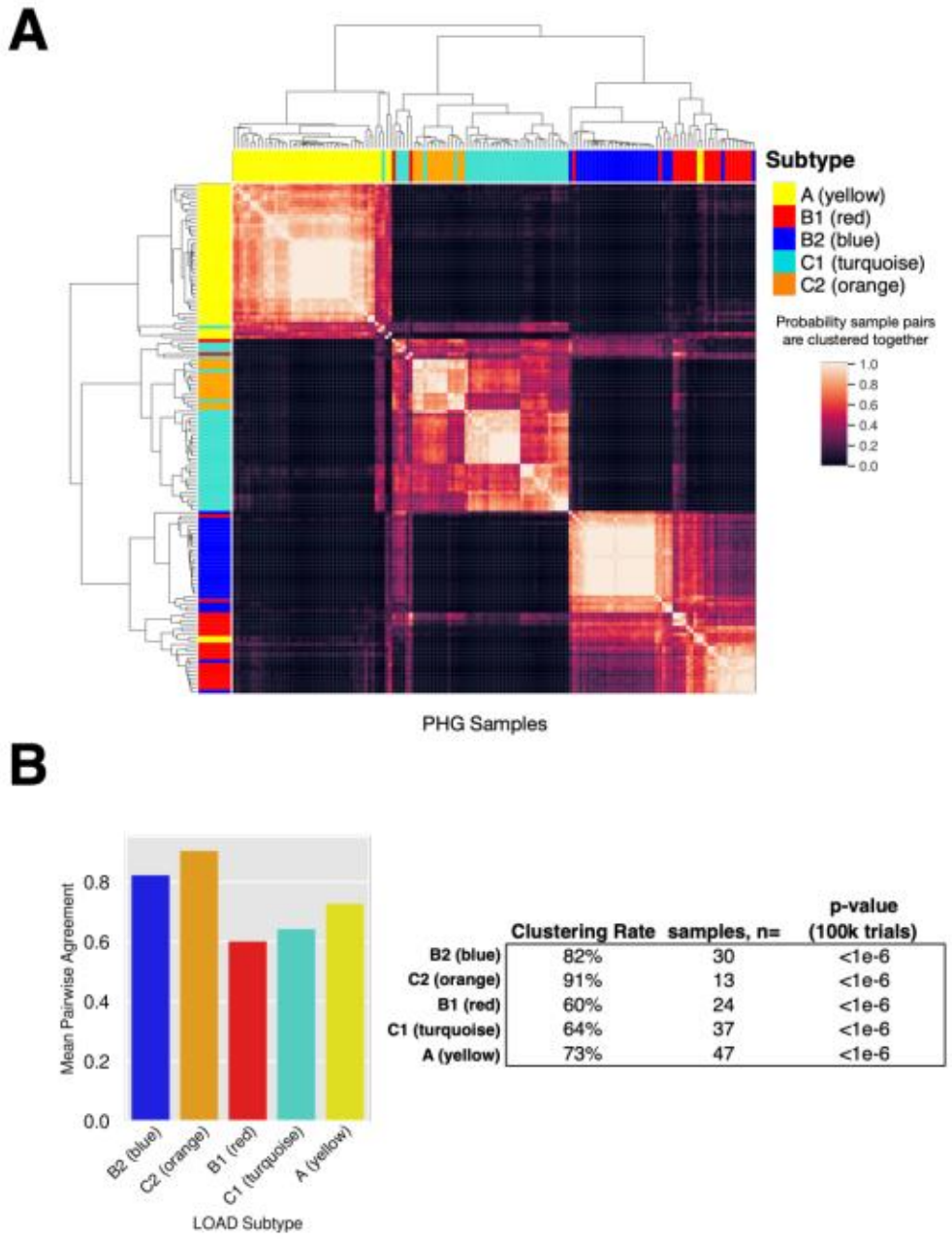
GSEA enrichment

Indicators / Data processing

- Data: MSBB-AD PHG transcriptomic data (RNA-seq data)
- Either Normalization by neuronal cell-type proportion SPVs and dementia severity CDR
  - CDR measures neurocognitive decline / AD dementia / AD stages
  - SPVs measure Neuronal loss (Confounder)
  - Causal : SPVs→CDR； SPVs→Neuronal cell-type

Clustering

- Methods
  - Hierarchical, k-means, WSCNA and MEGENA
- Cluster stability
  - The rate at which sample pairs group together into the same subtypes upon repeated re-clustering on a random subset of the input data



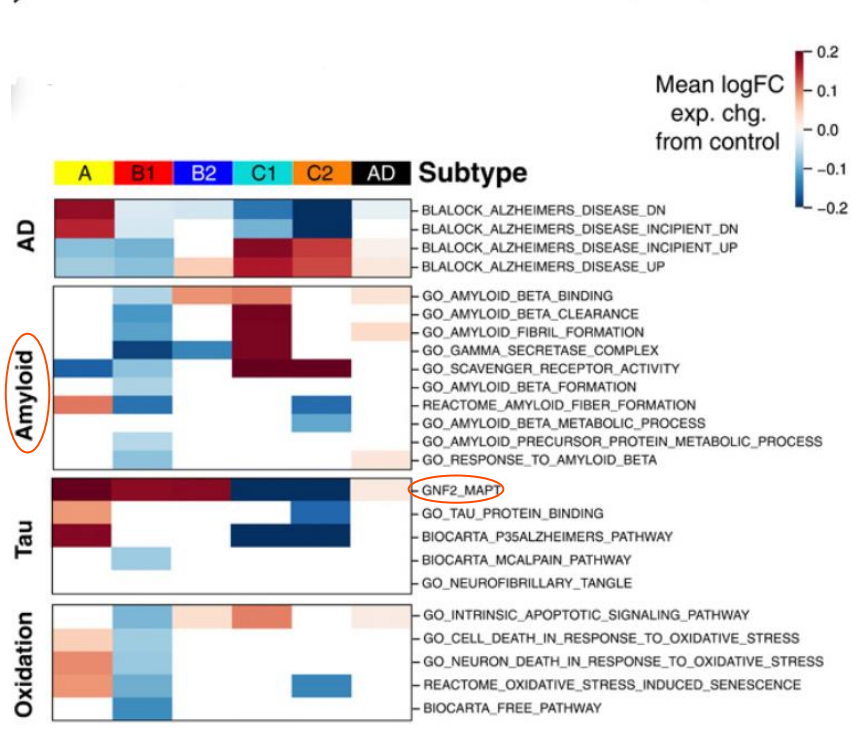
	Clustering Rate	samples, n=	p-value (100k trials)
B2 (blue)	82%	30	<1e-6
C2 (orange)	91%	13	<1e-6
B1 (red)	60%	24	<1e-6
C1 (turquoise)	64%	37	<1e-6
A (yellow)	73%	47	<1e-6

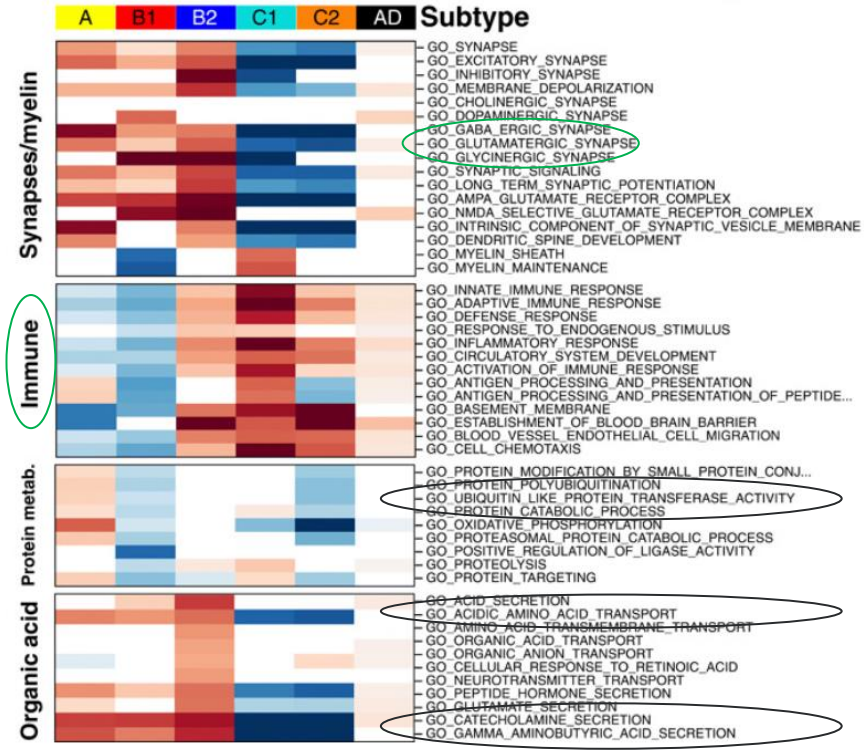
Clustering

- Molecular signatures
  - Aβ-, tau-related pathways

Weak enrichment across all AD participants, while strong enrichment in the subtypes

  - Synaptic depression vs. excitation
  - Increased immune

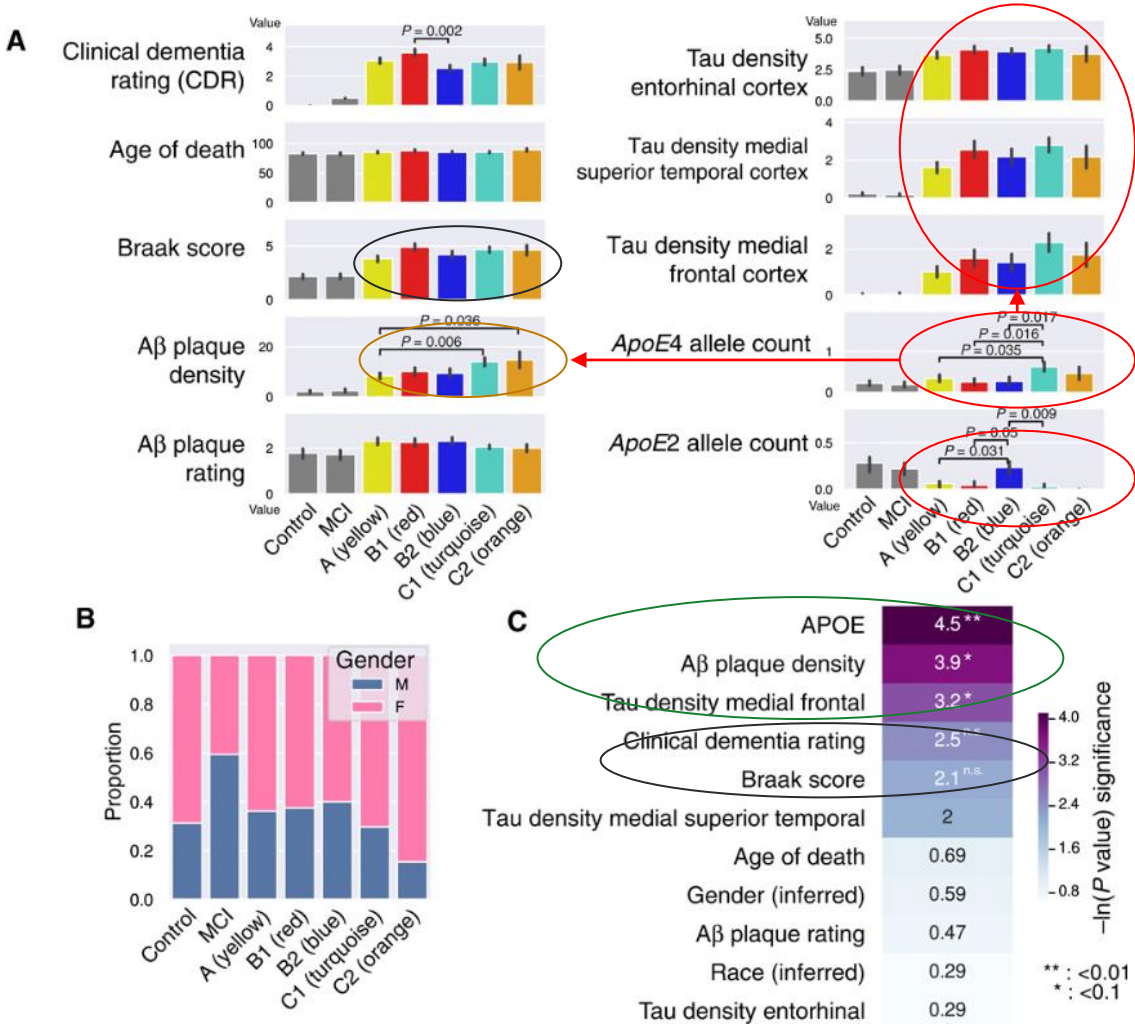




  - Protein degradation-related genes: ubiquitination, polyubiquitination are up-regulated in class A
  - Organic acid-related genes: acid secretion, acidic amino acid transport are up-regulated in class B
  - It's likely that AD subtypes may be characterized by either Aβ activity predominant (class C) or microtubule associated protein tau (MAPT)–activity predominant (class A + B)

Association with subtypes

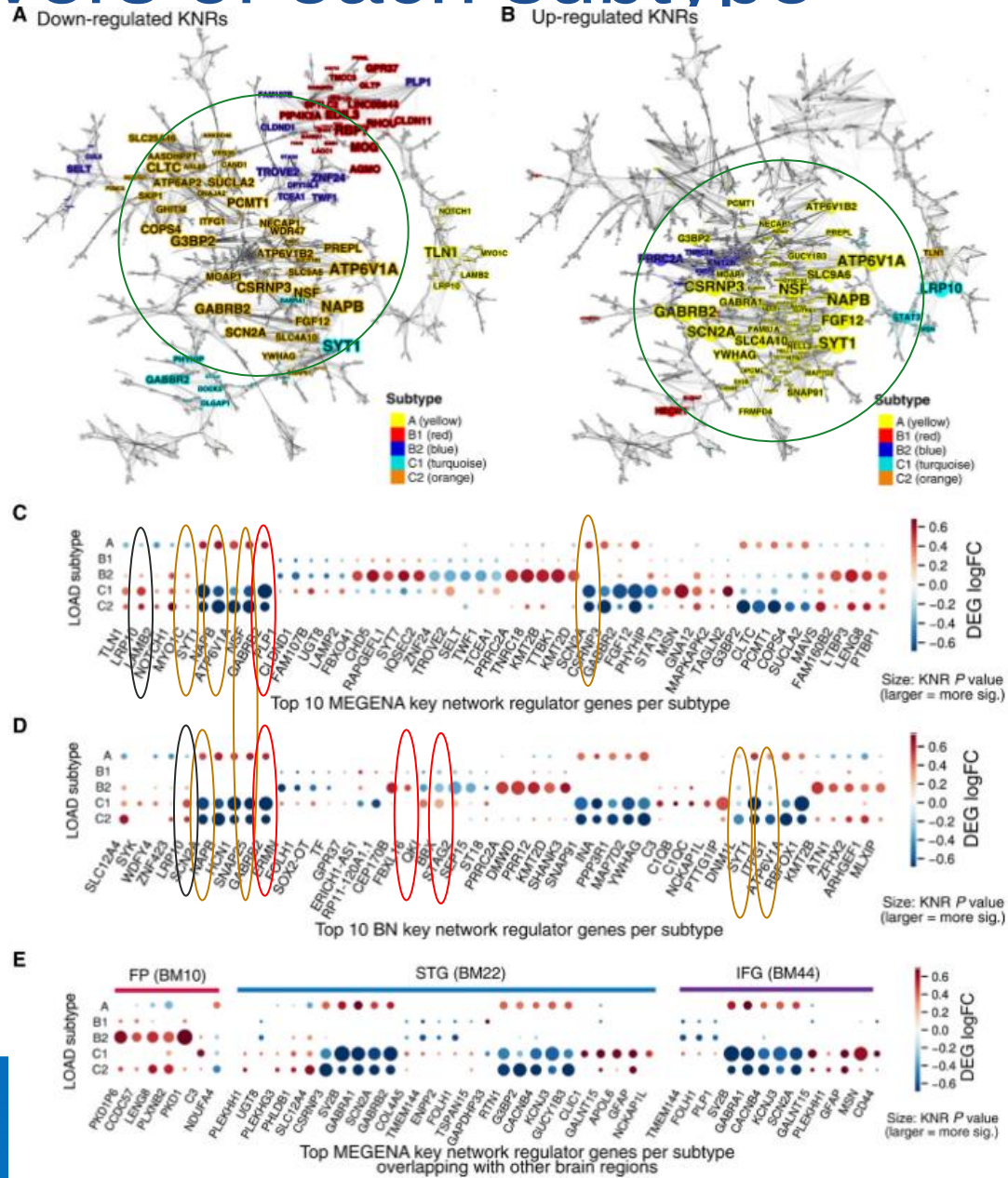
- Clinical & pathological phenotypes, APOE variants (Kruskal-Wallis one-way ANOVA)
  - tau NFT levels in the medial frontal cortex
  - Aβ mean plaque density,
  - APOE e4 and APOE e2 allele counts
  - Larger Aβ plaque burden in class C, while no sig difference in cognitive decline (CDR)
  - APOE may modulate AD pathogenesis and contribute to some molecular signatures in a portion of subtypes
  - Both CDR and Braak score are not associated with the AD subtypes



Up- and Down-regulated key drivers of each subtype

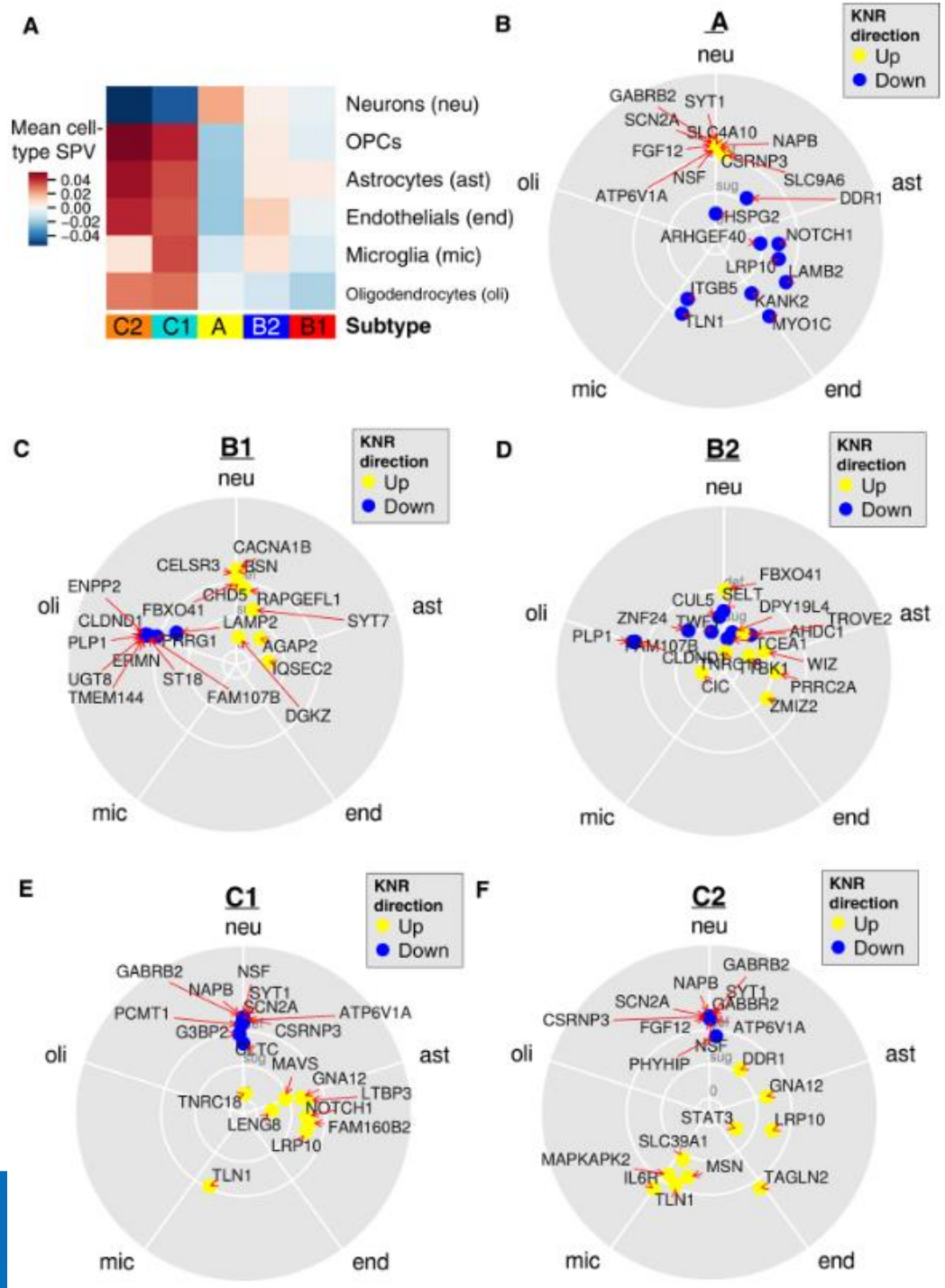
- Methods: Multiscale embedded gene coexpression network analysis (MEGENA); Bayesian causal network (BN) inference
  - Specific gene modules are subtype specific
  - Class C down-regulated pathogenic genes are predicted to be regulated by GABRB2, SYT1, ATP6V1A, and SCN2A in both models
  - Class B down-regulated oligodendrocytic genes are predicted to be regulated by PLP1, ERMN, QKI, and STAG2 in both models
  - Class A up-regulated genes GABRB2, LRP10, SYT1, and PREPL however are down-regulated in class C

It's likely that both the class A and class C subtypes result from either inhibitory or excitatory dysregulation along a single axis in specific neuronal processes.



Cell-type specificity

- Methods: Cell-type proportion analysis
- Mean change in cell-type proportion
  - Class A: Neurons ↑; OPCs, ast, end ↓
  - Class B: oli ↓
  - Class C: Neurons ↓; oli, ast, OPCs, end ↑
- Expression levels of AD subtype key regulators across different cell types
  - Class A: Neurons (GABRB2, SYT1, and SCN2A) ↑; astrocytes, endothelial cells, and microglia (LRP10, NOTCH1, MYO1C, and TLN1) ↓; neuron remodeling and activity
  - Class B: oligodendrocytes (PLP1, UGT8, CLDND1, ERMN, and ENPP2) ↓; demyelinating process
  - Class C: Neurons (ATP6V1A, SCN2A, GABRB2, and NAPB)↓; microglia (TLN1, MSN, and IL6R), endothelial cells (TAGLN2), astrocytes (LRP10, GNA12, and LTBP3) ↑; neuroinflammatory destruction

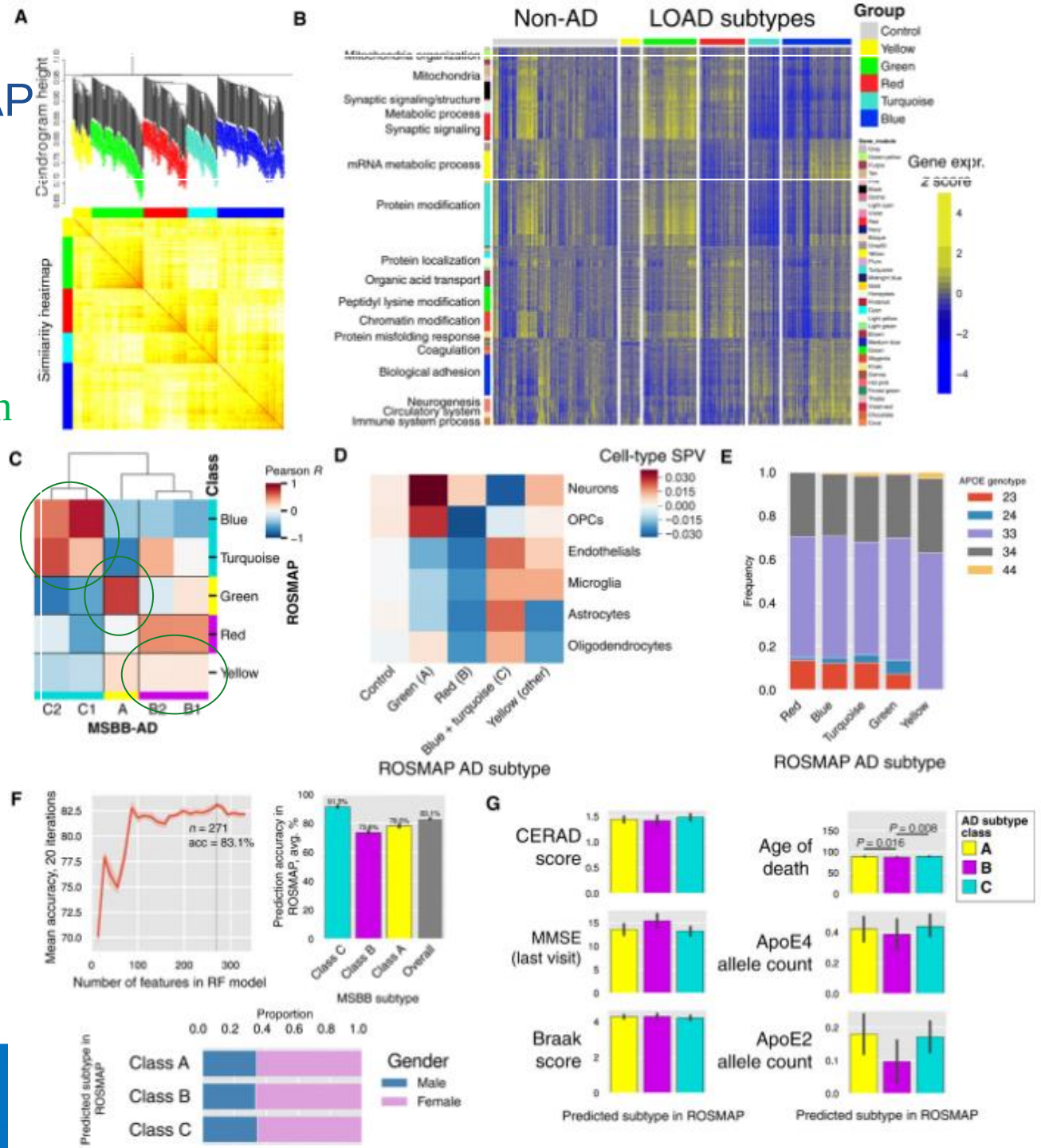


Known AD genetic risk markers

- Methods: Intersect the AD risk genes compiled by the International Genomics of Alzheimer's Project (IGAP) Consortium (6) and the predicted subtype-specific key regulators
- 49 key regulators have genetic loci associated with AD
  - Class A: AMPH, MEF2C, and EPDR1 ↑
  - Class B: PICALM (B2), PSMC6 ↓
  - Class C: AMPH, MEF2C, and EPDR1, PSMC6 ↓

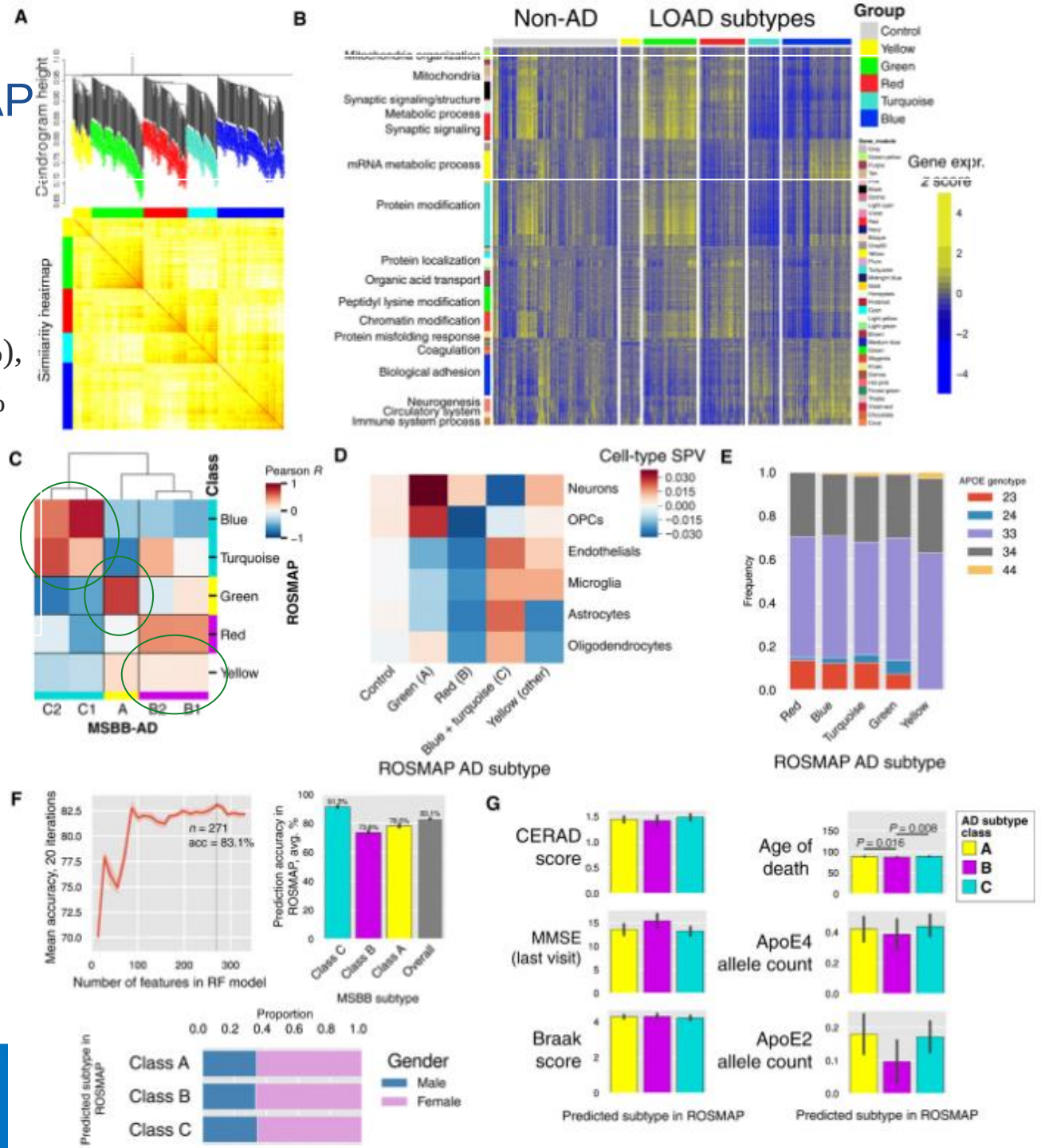
Validation of MSBB-AD subtypes in ROSMAP

- Methods: WSCNA
- C: Most subtypes in the ROSMAP cohort well match certain subtypes from the PHG in the MSBB-AD cohort, except the yellow subtype in the ROSMAP (Pearson cor coef b/w 0.6 & 0.8)
- D:
  - Class A: neuronal and OPC ↑; Others ↓
  - Class B: OPC ↓
  - Class C: neuronal ↓; microglia and endothelial ↑
- E: Class other: *APOE* e2 ↓; APOE e4 allele dosage is not significantly associated
- Not associated with clinical and pathological traits
  - sex, cognitive scores, age, Consortium to Establish a Registry for Alzheimer's Disease (CERAD) pathologic scores, MMSE, and Braak staging



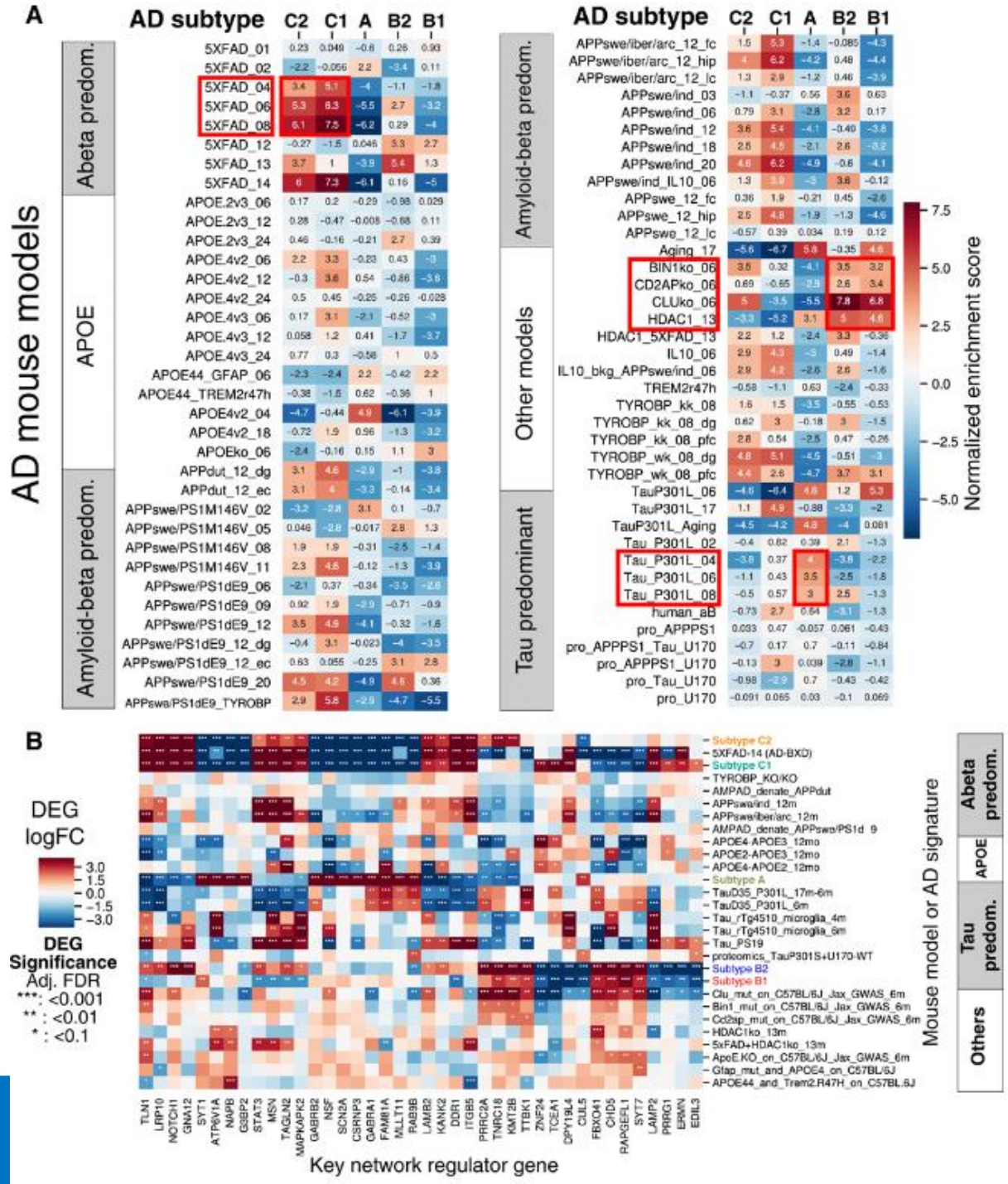
Validation of MSBB-AD subtypes in ROSMAP

- Across different cohorts
- Method: RF model with maximum accuracy
- F: Classifier model reaches an accuracy of 83.1% (SD, 1%), with class C (Aβ predominant) prediction peaking at 91.3% (SD, 1%)
- G: Similar to the ROSMAP subtypes, the subtypes do not show a sig. difference in cognition, CERAD score, MMSE, Braak score, APOE, or sex



Validation of MSBB-AD subtypes in AD mouse models

- Data: 19 mouse model studies of AD
- Method: GSEA enrichment
- A:
  - Class A (tau-predominant): TauP301L, opposite to 5XFAD and APP
  - Class B: CLU (apoJ-), CD2AP and BIN1 mutant models
  - Class C (Aβ predominant): 5XFAD (familial), APP Dutch (inflammatory), and APP Swedish (amyloid) mice
- B:
  - Many KNRs of the Aβ- and tau-predominant subtypes have consistent expression changes in both human and mouse models



Discussion

- Identified five molecular subtypes of AD in three major classes
  - The hippocampal area demonstrates the greatest subtyping signal
  - Independent of age and disease severity
  - Well conserved across different independent cohorts
  - Each subtype has a unique set of key regulator genes, many of them are AD genetic risk genes
  - Each existing mouse model of AD may match to a particular subset of human AD subtypes but not all subtypes simultaneously
- The molecular subtypes cannot be fully explained by diff. in pathologic variables (Aβ, tau accumulation) or by diff. in APOE risk allele genotype b/w participants
- Only about one-third of the AD cases carry consistent hallmarks (e.g., increased immune response and decreased synaptic signaling) of a “typical” AD presentation (class C), while the rest show opposite molecular gene regulation and other complex changes across multiple pathways and cell types (classes A and B)

Discussion

- The bidirectional nature of certain key regulator genes (e.g., GABRB2 and ATP6V1A) across the identified AD subtypes is notable
- APOE genotype is highly associated with some of the AD subtypes

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Unused slides / Appendix