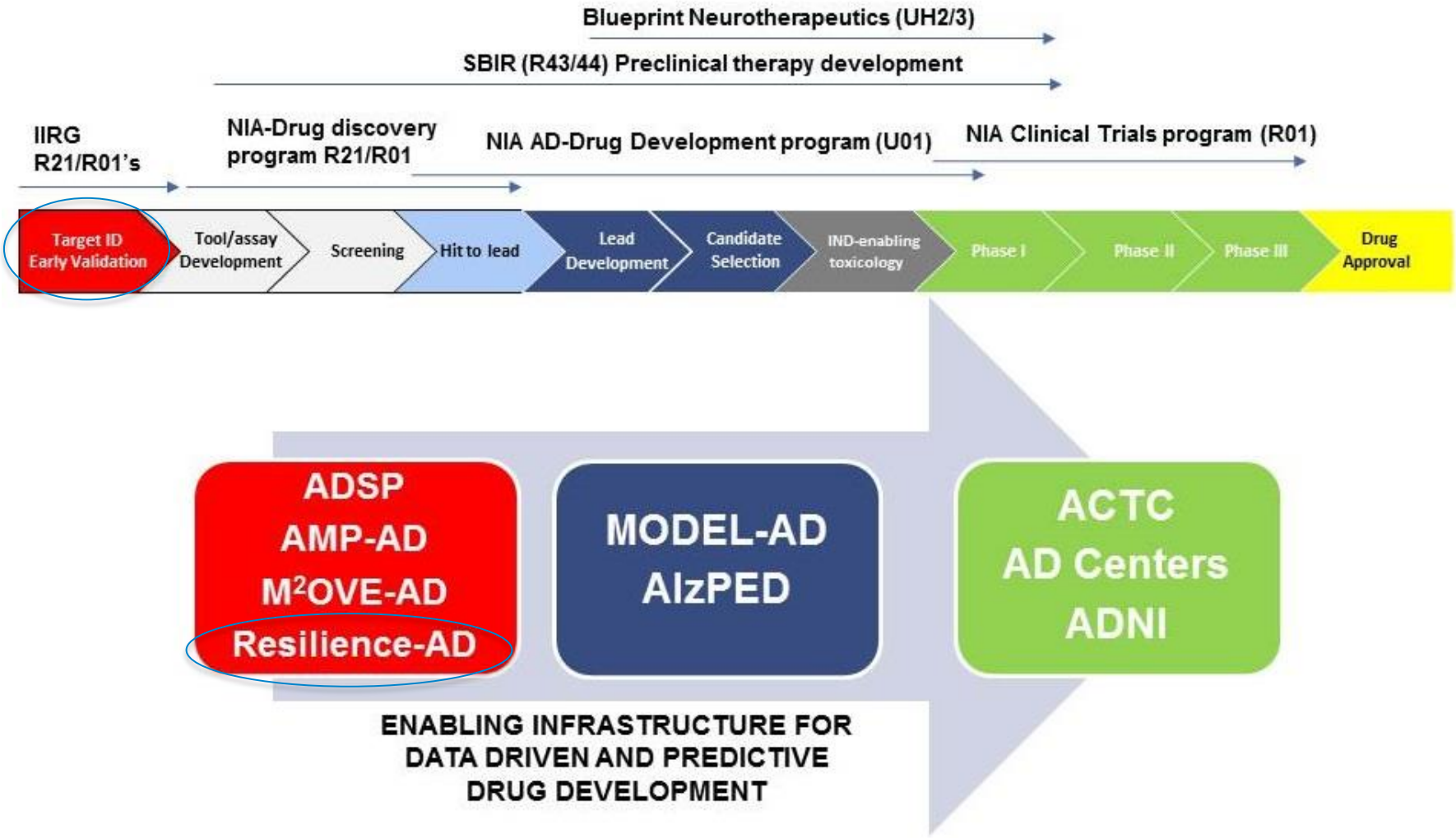
NIA and Trans-NIH translational pipeline for AD and ADRD



Understanding the Complex Biology of Resilience to AD RFA-AG-17-061

• Morgan Levine, *Yale:* Identify underlying biological interactions and networks that explain why some e4 carriers do not develop Alzheimer's disease.

• Sean Bendall, *Stanford: Multiplexed imaging to define resilience to AD.*

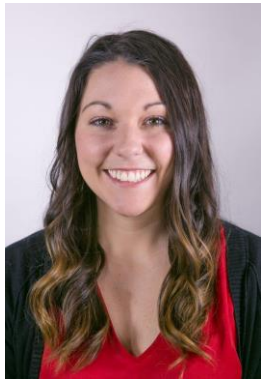
• Catherine Kaczorowski, *The Jackson Laboratory:* Discovery of genetic modifiers to high-risk AD mutations using mouse populations that model humans

• Nir Barzilai, *Einstein School of Medicine:* Discovery of longevity and resilience genes through population studies of Ashkenazi Jews.

• Chris Gaiteri, *Rush:* The goal of the proposed study is to identify the molecular networks underlying resilience to AD, other age-associated neuropathologies and risk factors associated with resilience.

• Bin Zhang, *Icahn Institute at Mount Sinai:* Develop and validate molecular network models underlying cognitive resilience to AD risk.

Systems genetics identifies modifiers of AD risk and resilience



Sarah Neuner

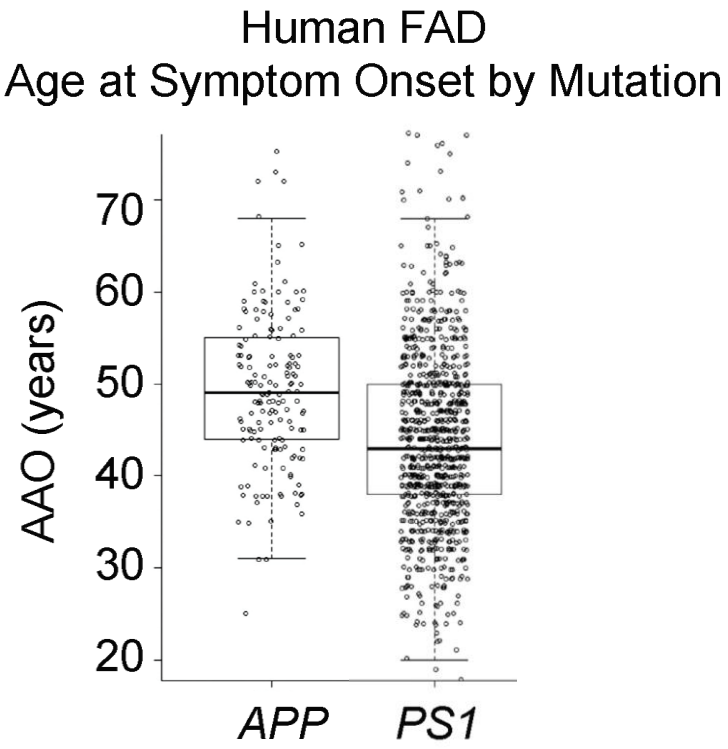
Catherine C. Kaczorowski

Associate Professor and Evnin Family Endowed Chair in Alzheimer's Research

The Jackson Laboratory

Leading the search for tomorrow's cures

Individual differences in the age at first symptom onset (AAO) implicates modifiers

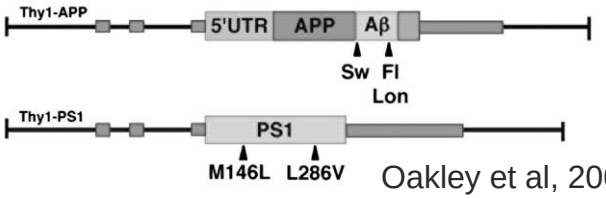


- Variation not explained by sex
- *APOE* genotype explains ~2-3 yrs
- Protective factors exist in humans that delay onset of FAD
- Asymptomatic AD/resilience difficult to study in human populations

Ryman et. al., *Neurology*, 2014

Development of AD-BXD panel to model individual differences in disease susceptibility

5XFAD transgene



Oakley et al, 2006

AD-BXD Schematic

AD model

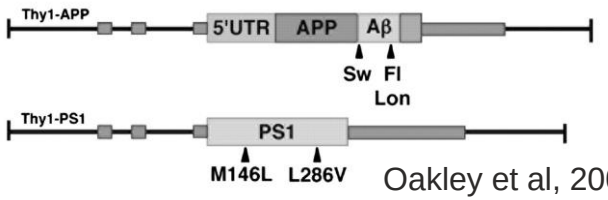
B6-5XFAD

- Combine two well-established mouse resources:
  - 5XFAD mouse model of AD

Neuner et al, unpublished

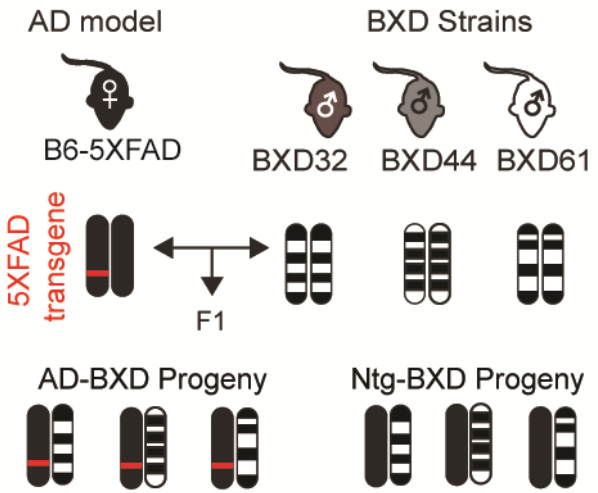
Development of AD-BXD panel to model individual differences in disease susceptibility

5XFAD transgene



Oakley et al, 2006

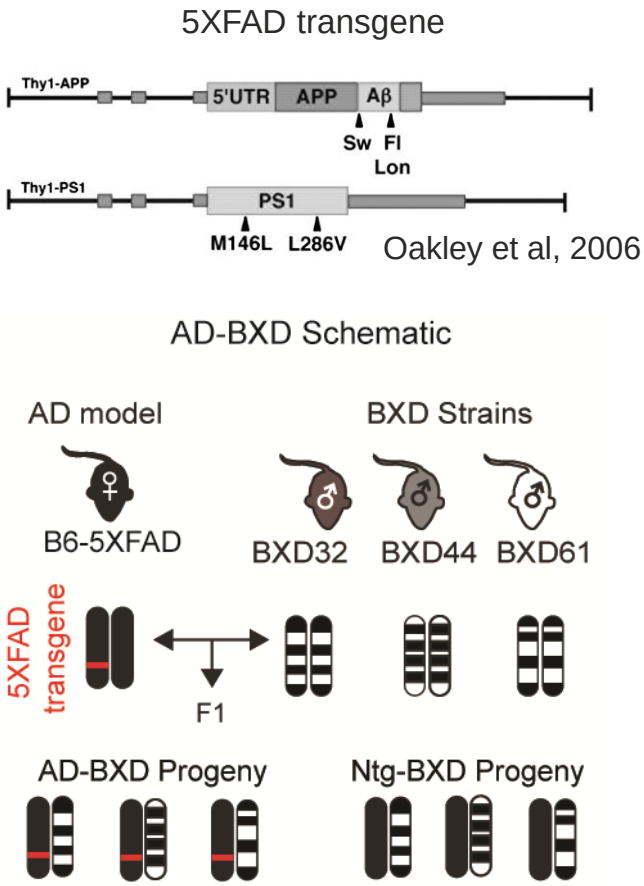
AD-BXD Schematic

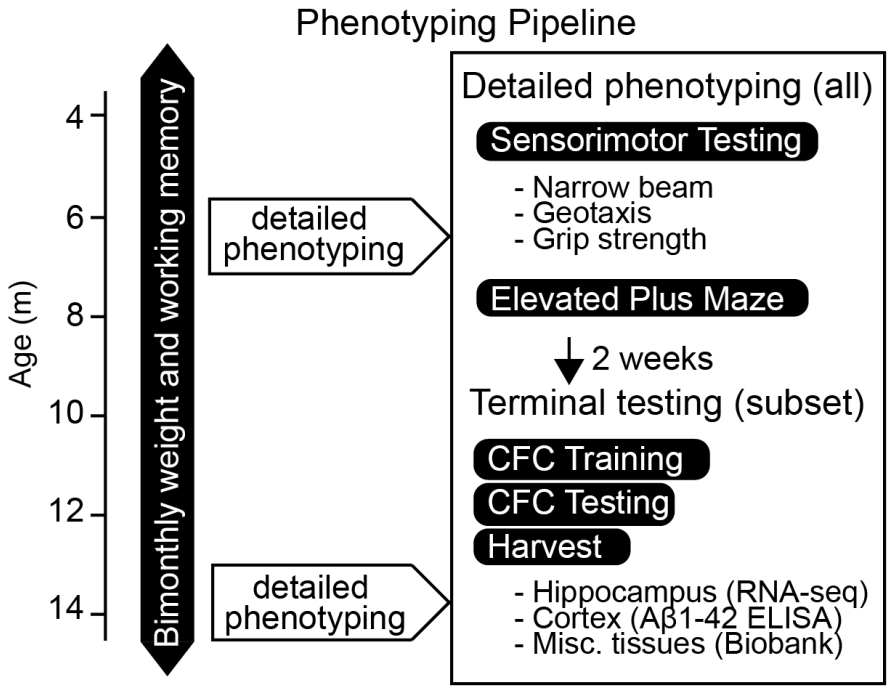


- Combine two well-established mouse resources:
  - 5XFAD mouse model of AD
  - BXD genetic reference panel
- Defined ‘high-risk’ genotypes
- Replicable

Neuner et al, unpublished

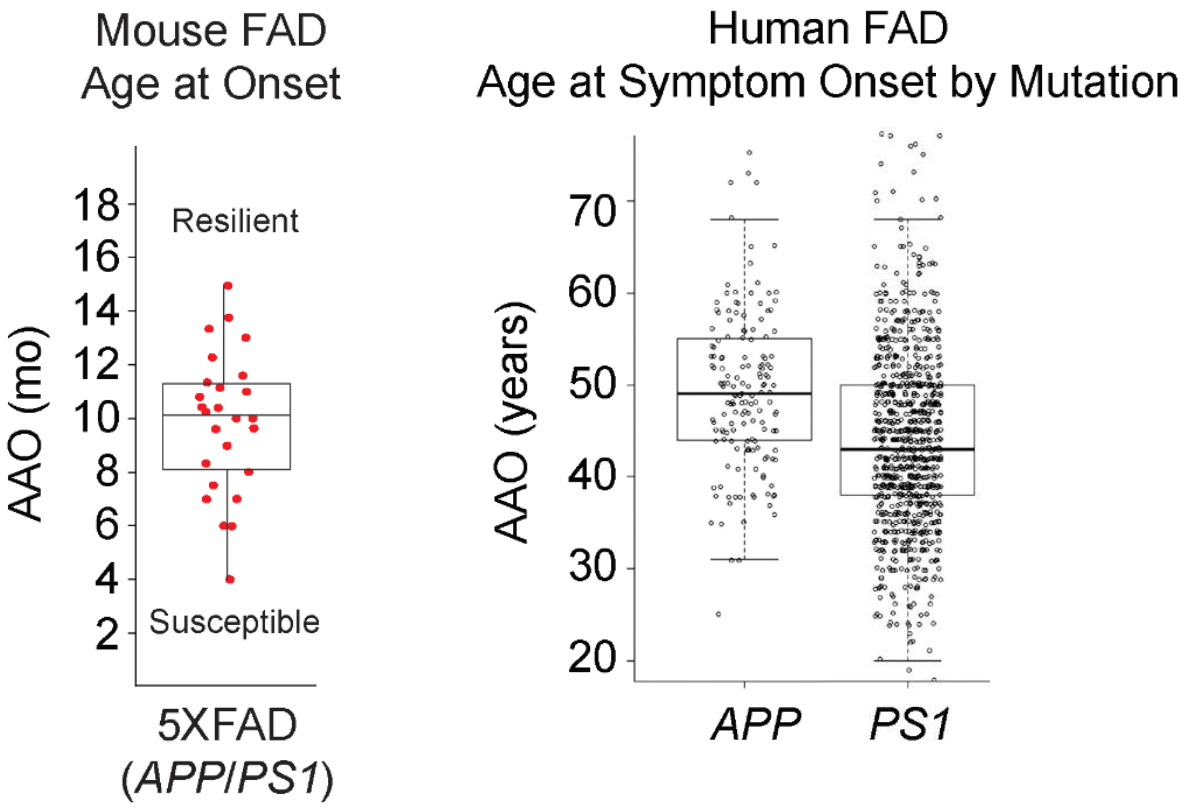
Development of AD-BXD panel to model individual differences in disease susceptibility





Neuner et al, unpublished

Variation in age at onset (AAO) in AD-BXDs parallels that observed in human FAD population



5XFAD on **C57BL/6J** is highly resilient (AAO = +15 mo)

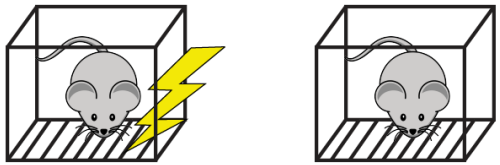
Human data from Ryman et. al., *Neurology*, 2014

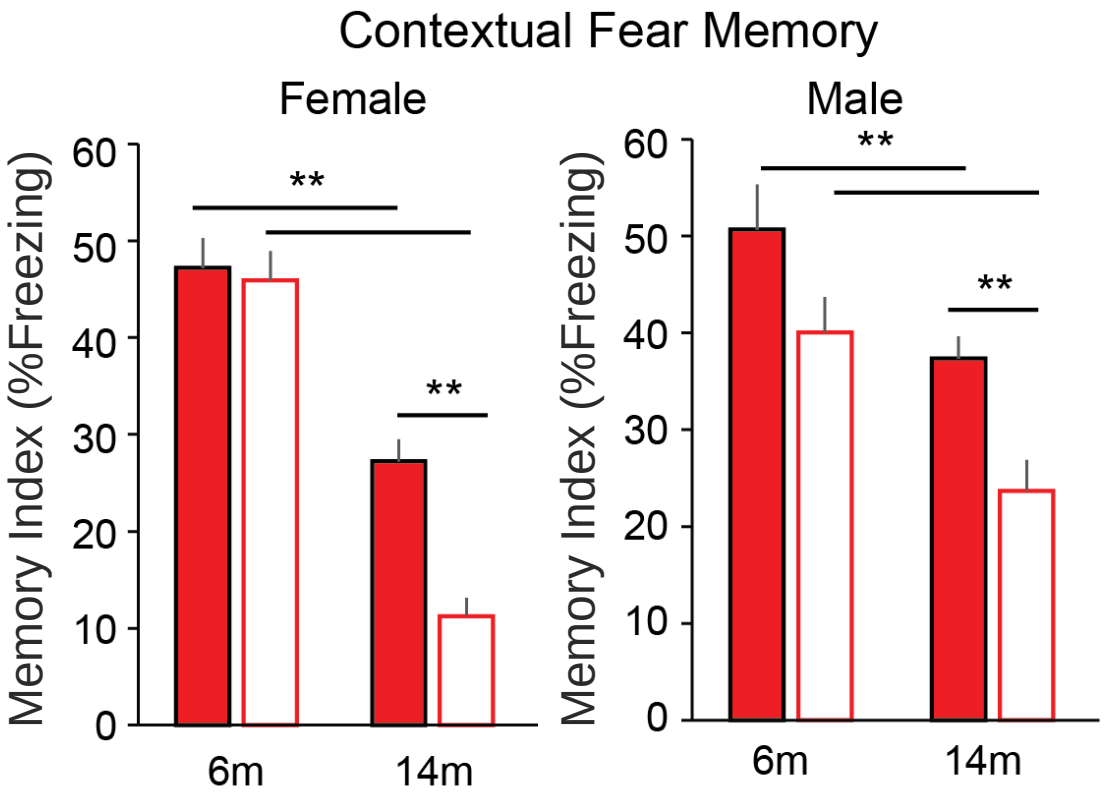
Is the AD-BXD panel sensitive to genetic variants in *Apoe?*

- Receptor-binding region amino acids critical for function
- Single missense polymorphism segregates *B* and *D Apoe* alleles
- Missense SNP causes D allele to most closely match human APOEε4 (RDR)

human position	130																							171	176
Apoe ε2	R	L	G	A	D	M	E	D	V	C	G	R	L	V	Q	Y	R	G	E	V	Q	A	M	L	G	Q	S	T	E	E	L	R	V	R	L	A	S	H	L	R	K	L	R	K	R	L	L	R	D	A	D	D	L	Q	K	C	L	A	V	Y
Apoe ε3	R	L	G	A	D	M	E	D	V	C	G	R	L	V	Q	Y	R	G	E	V	Q	A	M	L	G	Q	S	T	E	E	L	R	V	R	L	A	S	H	L	R	K	L	R	K	R	L	L	R	D	A	D	D	L	Q	K	R	L	A	V	Y
Apoe ε4	R	L	G	A	D	M	E	D	V	R	G	R	L	V	Q	Y	R	G	E	V	Q	A	M	L	G	Q	S	T	E	E	L	R	V	R	L	A	S	H	L	R	K	L	R	K	R	L	L	R	D	A	D	D	L	Q	K	R	L	A	V	Y
Apoe B6	R	L	G	A	D	M	E	D	L	R	N	R	L	G	Q	Y	R	N	E	V	H	T	M	L	G	Q	S	T	E	E	I	R	A	R	L	S	T	H	L	R	K	M	R	K	R	L	M	R	D	A	E	D	L	Q	K	R	L	A	V	Y
Apoe D2	R	L	G	A	D	M	E	D	L	R	N	R	L	G	Q	Y	R	N	E	V	H	T	M	L	G	Q	S	T	E	E	I	R	A	R	L	S	T	H	L	R	K	M	R	K	R	L	M	R	D	A	D	D	L	Q	K	R	L	A	V	Y
mouse position	122																							163	168

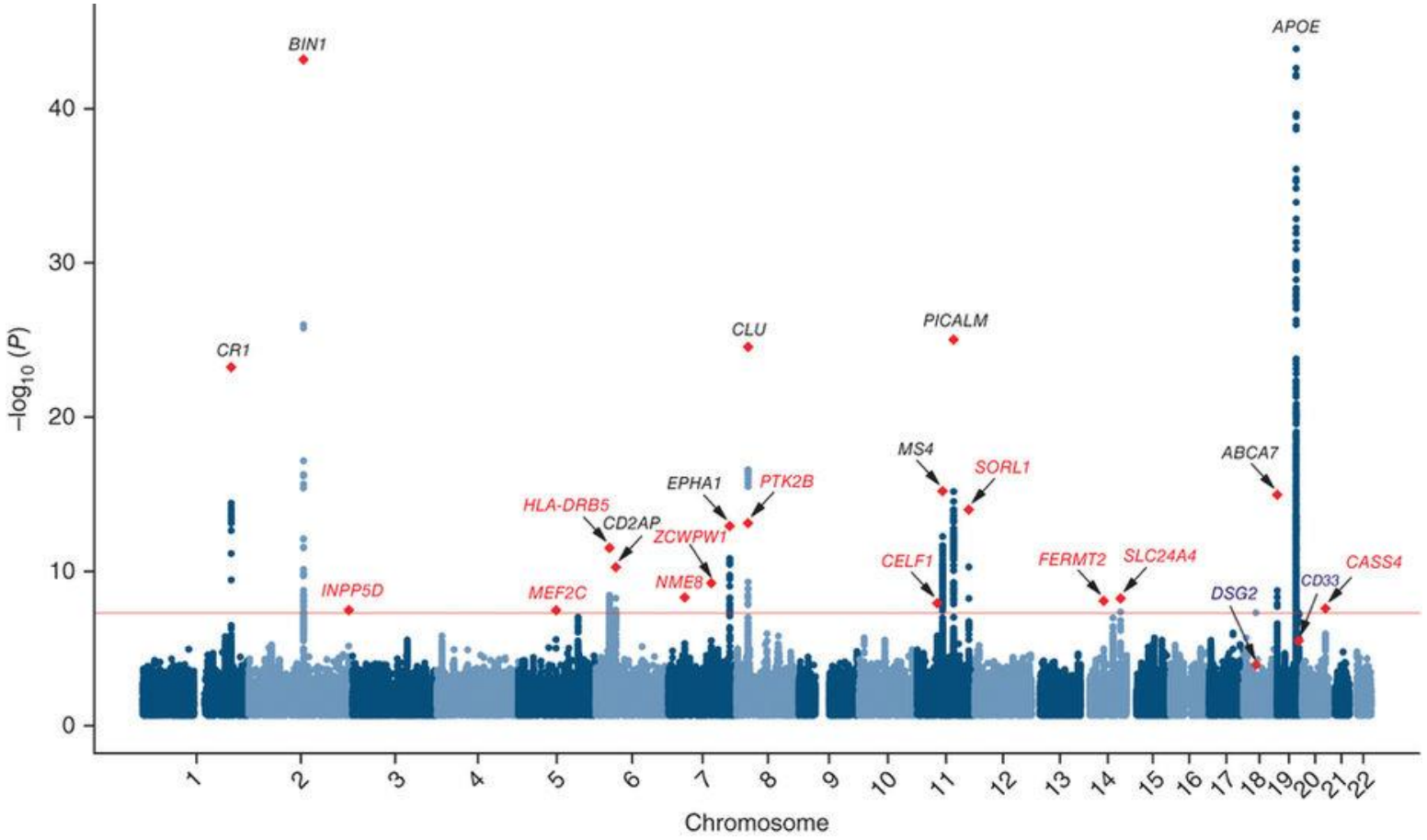
D allele of Apoe represents ε4-equivalent 'susceptibility' allele in AD-BXDs





Neuner et al, unpublished

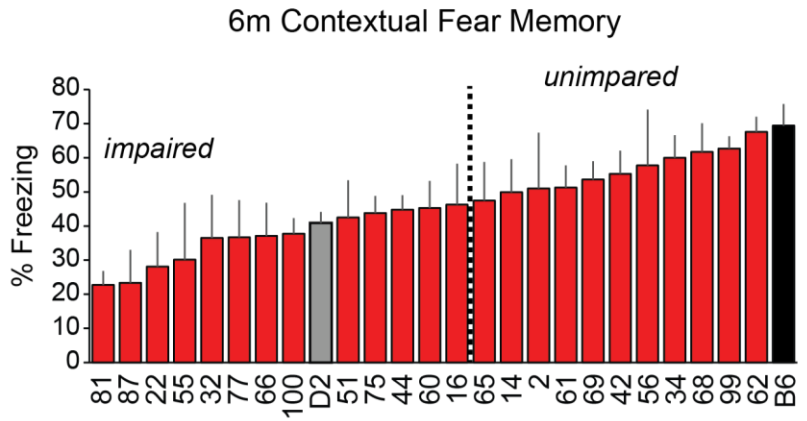
Is the AD-BXD panel is sensitive to variation in multiple AD risk loci?

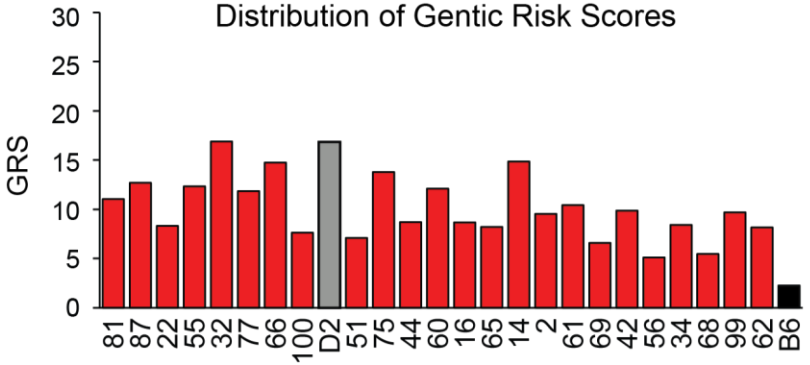


Lambert et al., 2014

Is the AD-BXD panel is sensitive to variation in other AD risk loci?

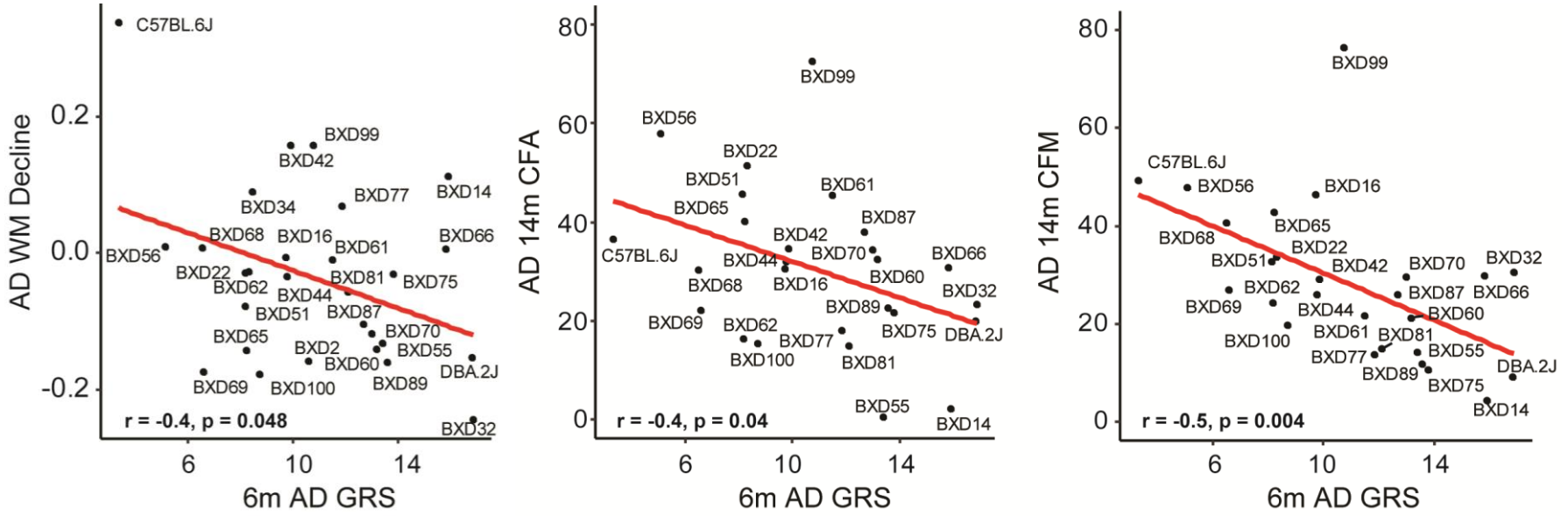
Gene	Mouse Chr.	6m CFM AD				
		Risk allele	Odds ratio	95 % CI	Z stat	Pval
Inpp5d	1	*B*	1.30	0.28 - 6.3	0.36	0.72
Cr1l	1	*D*	2.00	0.41 - 9.8	0.85	0.39
Celf1	2	*D*	1.50	0.30 - 7.4	0.50	0.62
Cass4	2	*D*	1.50	0.30 - 7.4	0.50	0.62
Zcwpw1	5	*D*	1.63	0.34 - 8.0	0.61	0.54
Epha1	6	*D*	1.60	0.33 - 7.8	0.58	0.56
Cd33	7	*D*	1.67	0.30 - 9.2	0.59	0.56
Picalm	7	*D*	3.60	0.71 - 18.3	1.55	0.12
Sorl1	9	*D*	2.50	0.50 - 12.6	1.11	0.27
Abca7	10	*D*	1.17	0.24 - 5.6	0.19	0.85
Slc24a4	12	*D*	3.60	0.71 - 18.3	1.55	0.12
Rin3	12	Located within same region as *Slc24a4*				
Mef2c	13	*B*	0.86	0.18 - 4.1	0.19	0.85
Nme8	13	*D*	1.40	0.30 - 6.6	0.42	0.67
Clu	14	*D*	5.50	0.84 - 36.2	1.77	0.08
Ptk2b	14	Located within same region as *Clu*				
Fermt2	14	*D*	1.83	0.32 - 10.6	0.68	0.50
Cd2ap	17	-	1.00	0.21 - 4.7	0.00	1.00
H2-Eb1	17	*D*	1.05	0.22 - 5.0	0.06	0.95
Trem2	17	*B*	1.20	0.25 - 5.8	0.23	0.82
Bin1	18	*D*	1.33	0.28 - 6.3	0.36	0.72





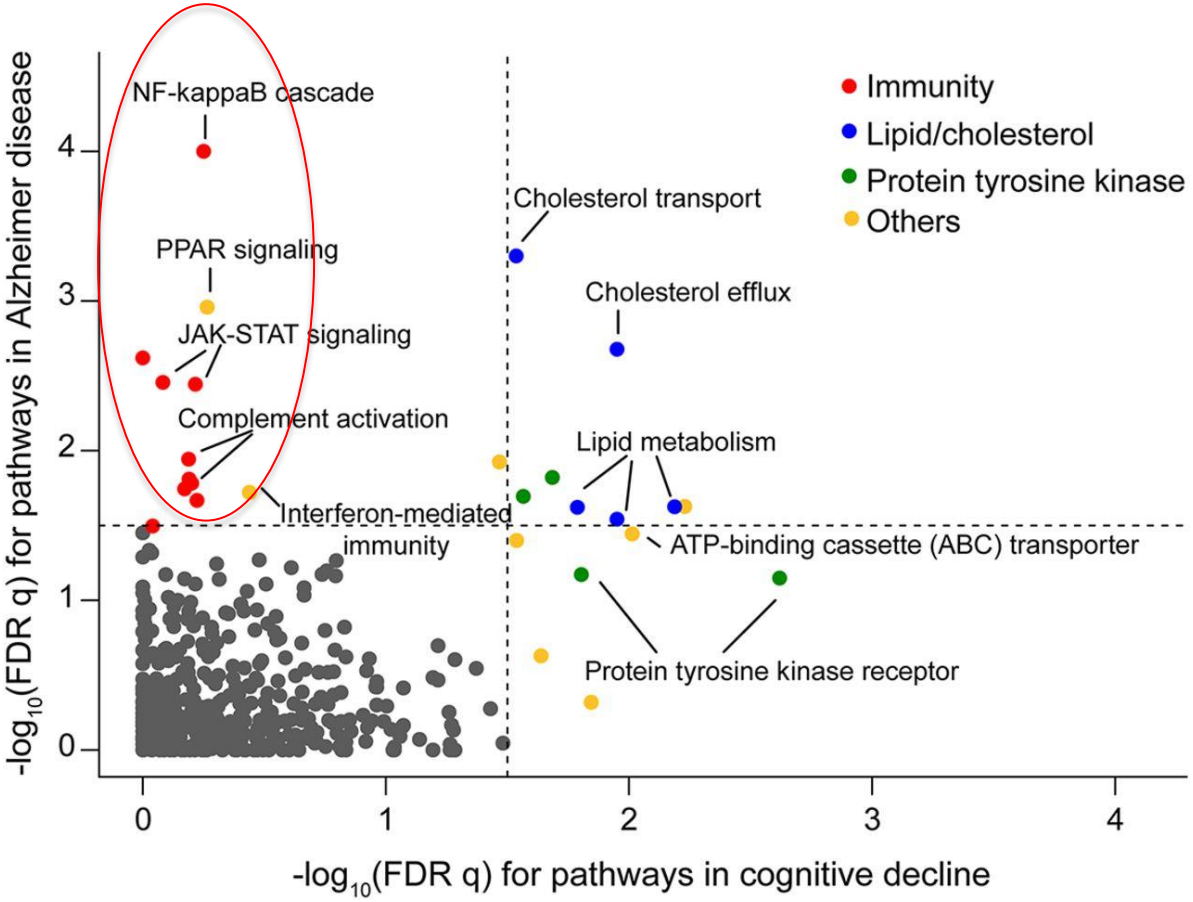
Neuner et al, unpublished

AD-BXD panel is sensitive to variation in known AD risk loci



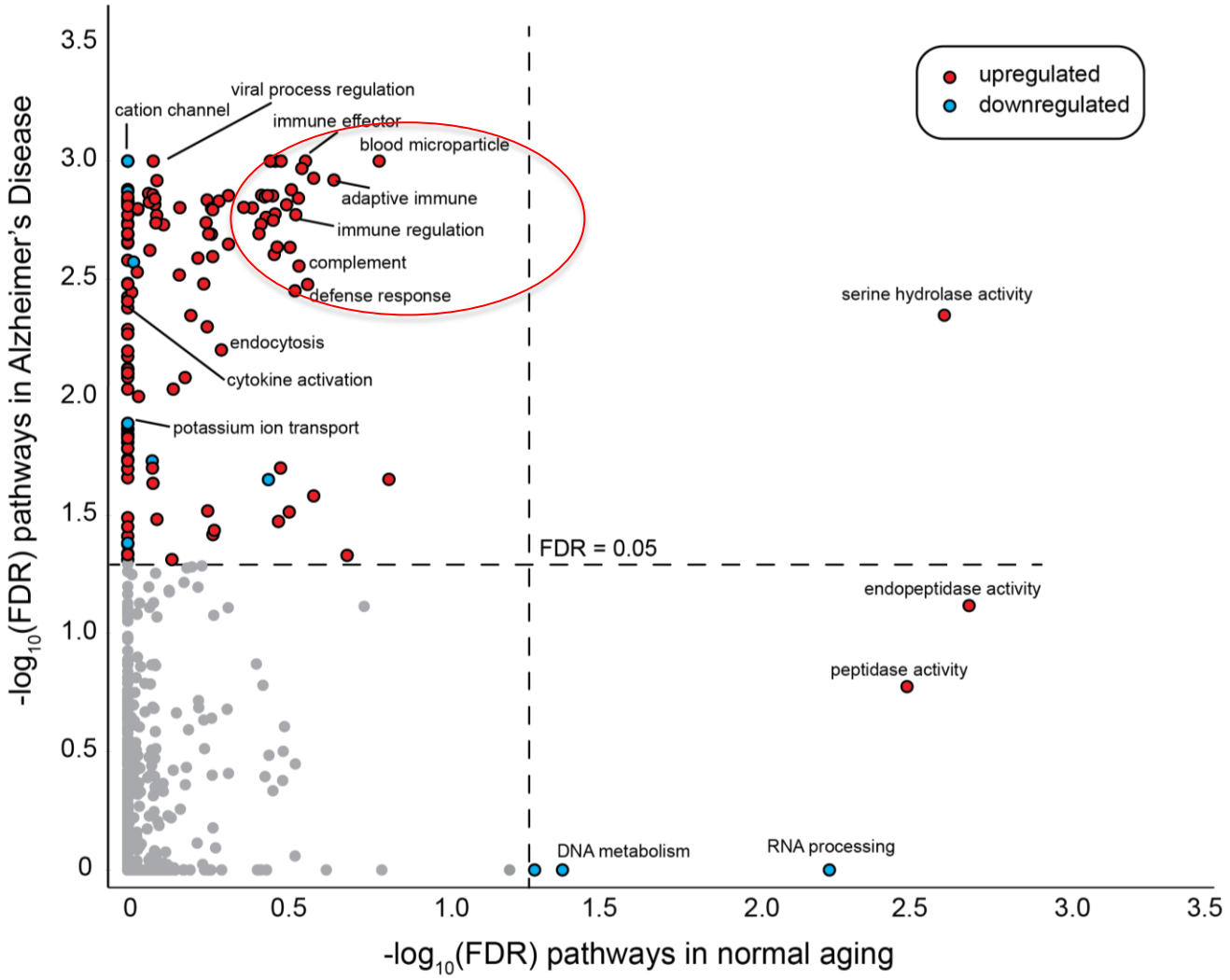
Neuner et al, unpublished

Do pathways enriched in AD genome-wide studies overlap with pathways enriched in AD-BXDs?



Raj et al., 2017

Pathway enrichment analysis of normal cognitive aging and AD highlights immune mechanisms in AD-BXDs



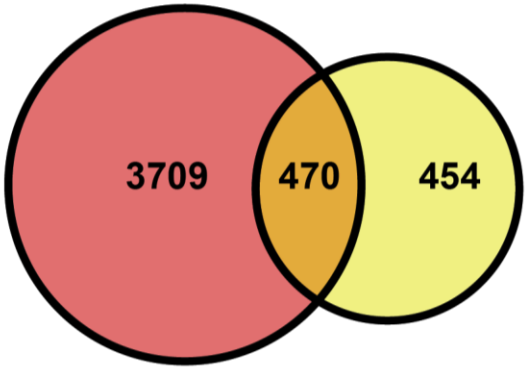
Heuer and Neuner, unpublished

Overlap of genes involved immune enrichment in AD-BXD mice and humans with AD

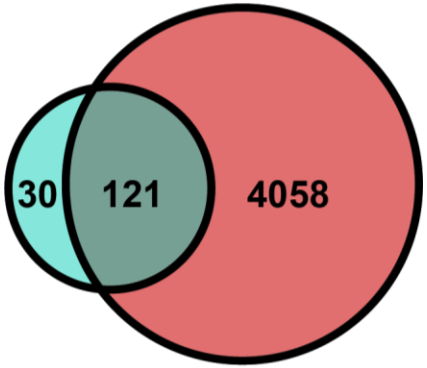
Genes upregulated in AD-BXDs

Zhang et. al. immune *yellow* module

Jones et. al. AD GWAS genes in ≥2 modules



J = 0.10
p = 0.002



J = 0.03
p = 0.002

Heuer and Neuner, unpublished

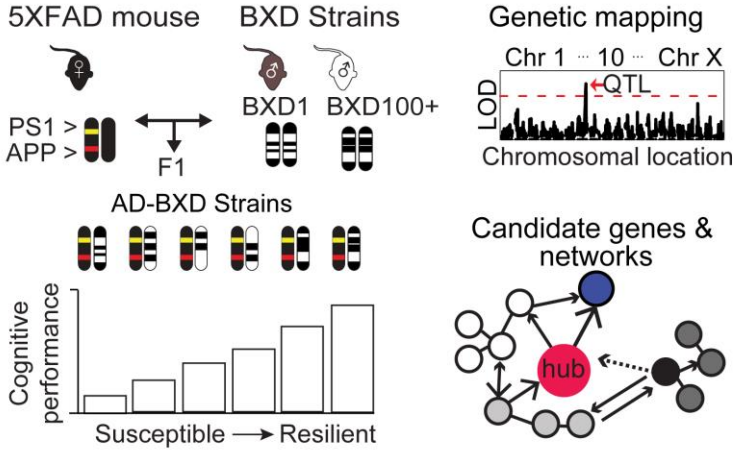
- **AD-BXD panel represents new model for human AD**
  - Variation in AAO mirrors that in human populations
  - Our panel displays sensitivity to known AD risk loci
  - High concordance in expression of AMP-AD Targets between mouse AD-BXD panel and human cohorts (not shown)

- **Approach identified two critical reasons why mouse models may have historically failed for AD:**
  - Lack of genetic diversity
  - Poorly aligned preclinical assays

- **Goal: Identify gene networks and novel drivers of resilience to AD (i.e. potential new drug targets)**

New Resilience-based Targets for Disease Prevention: A Mouse-Human-Mouse Discovery Pipeline

Aim 1: Define resilient genetic and molecular signatures



Aim 2: Prioritize based on human relevance

Resilience to AD (RAD) database

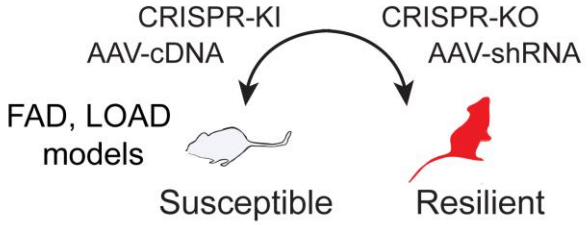
AMP-AD

DIAN
Dominantly Inherited Alzheimer Network

ROS/MAP
Rush Alzheimer's Disease Center

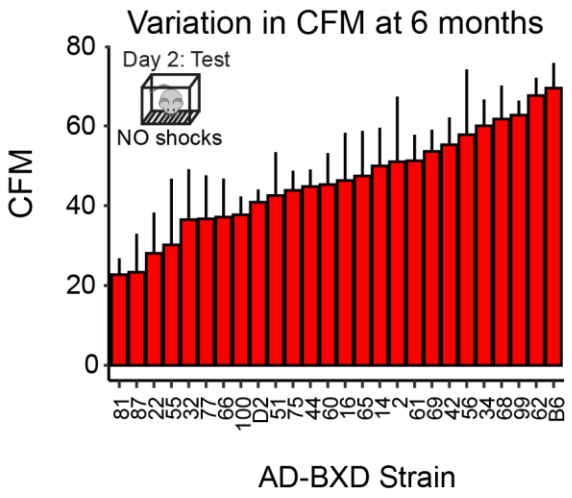
ADNI
Alzheimer's Disease Neuroimaging Initiative

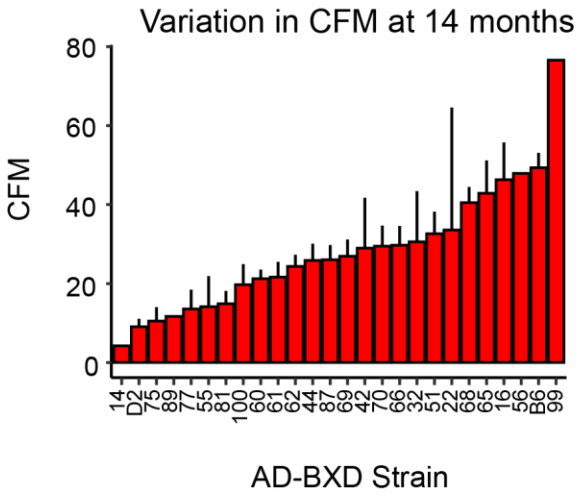
Aim 3: Validate new and *a priori* candidates in AD models

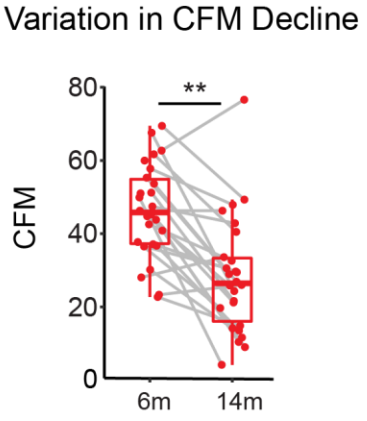


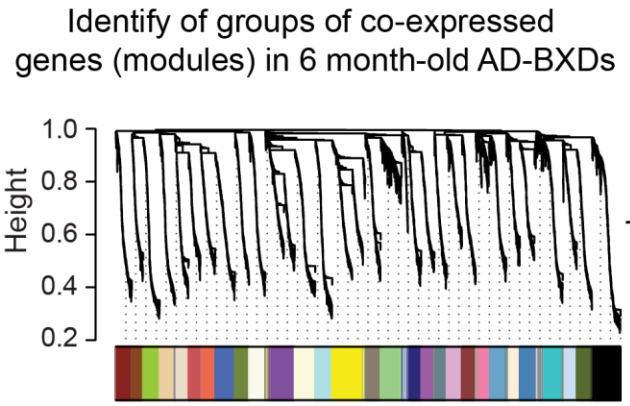
NIA Resilience AD Consortium R01AG057914

Networks of co-expressed genes at pre-symptomatic time points can predict late-disease cognitive resilience

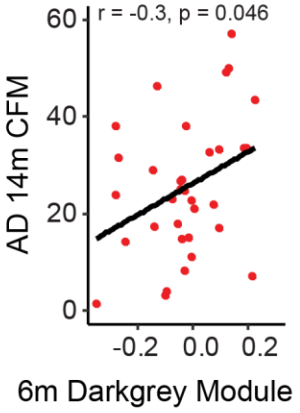








Relate 6 month-old (presymptomatic) modules to 14 month-old (symptomatic) cognitive function

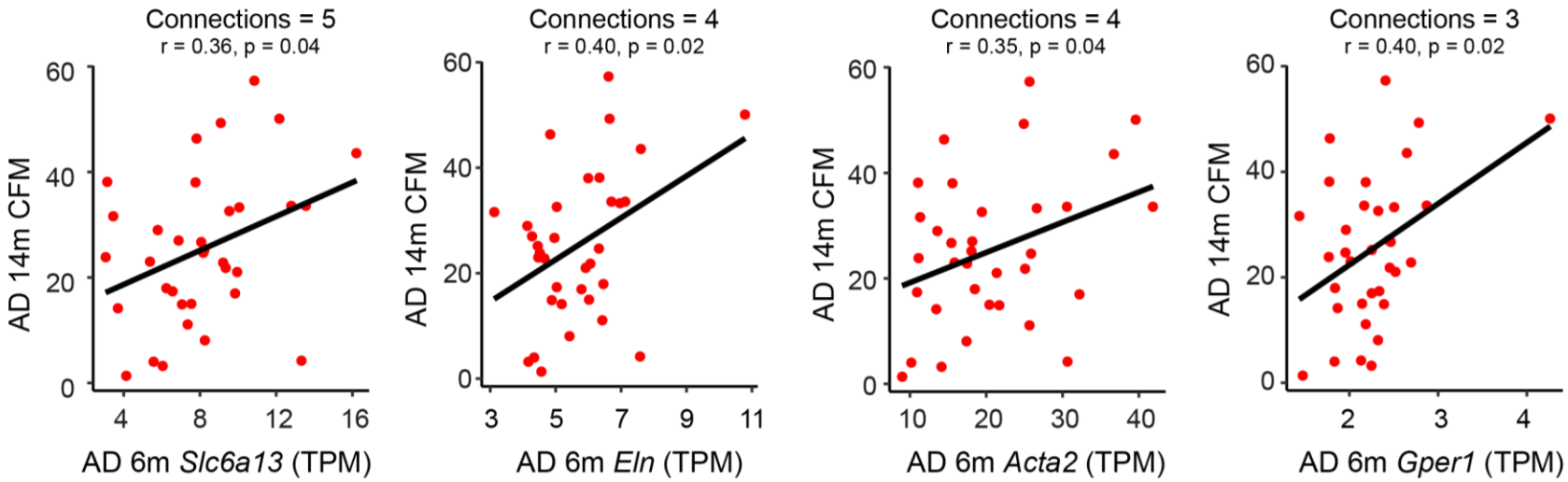


Neuner et al, unpublished

Candidate gene prioritization highlights putative resilience factors

Prioritize candidate genes by:

1. Correlation with late-disease cognitive status
2. Hub gene status as measured by number of direct connections after partial correlation

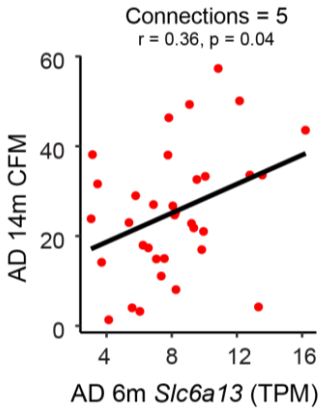


Neuner et al, unpublished

Slc6a13 as putative resilience factor and blood biomarker

Prioritize candidate genes by:

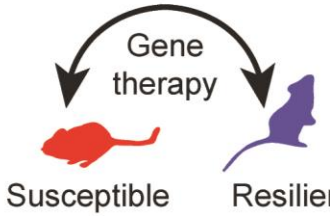
1. Correlation with late-disease cognitive status
2. Hub gene status as measured by number of direct connections after partial correlation



- GABA transporter 2
- Expressed at blood-brain barrier
- AD blood biomarker (Long et. al 2016)
- Expression modified by estrogen levels

Back into the mouse to test causality

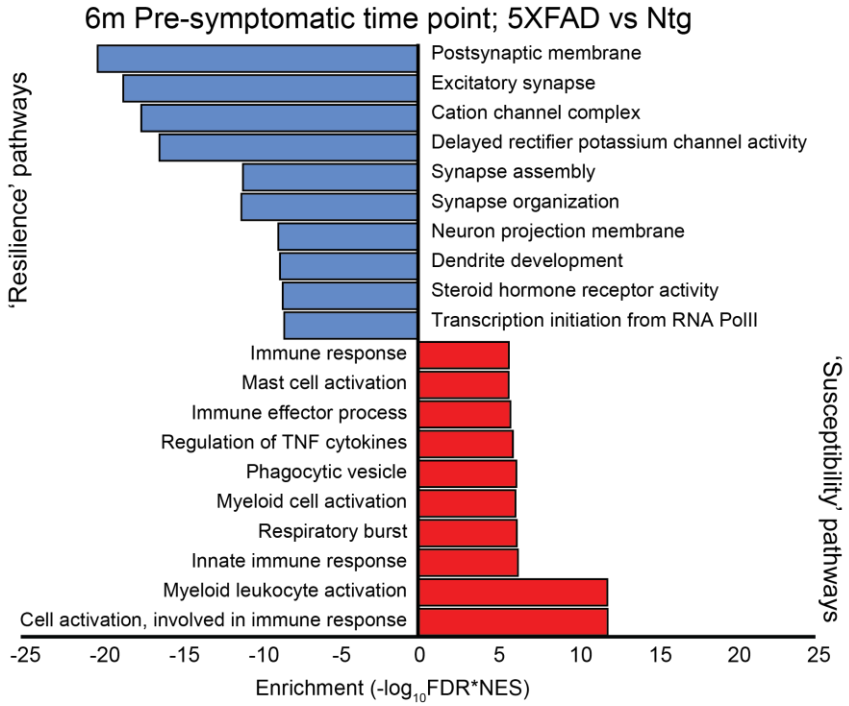
Validate in new AD models

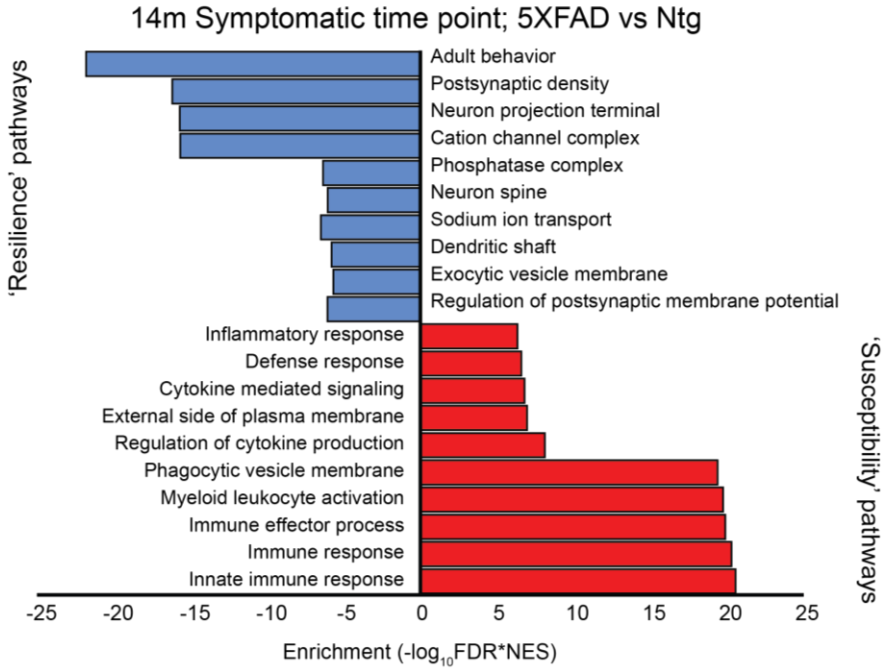


http://amp.pharm.mssm.edu/Harmonizome/gene/SLC6A13

Neuner et al, unpublished

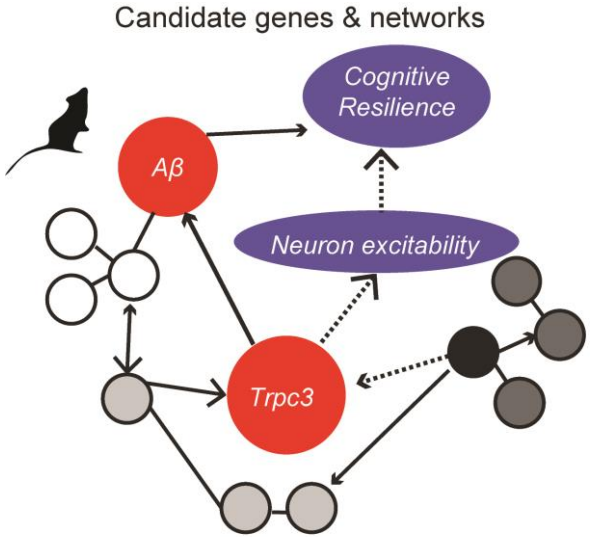
‘Resilience’ pathways in AD are related to neuron function terms: focus on ion channels and receptors

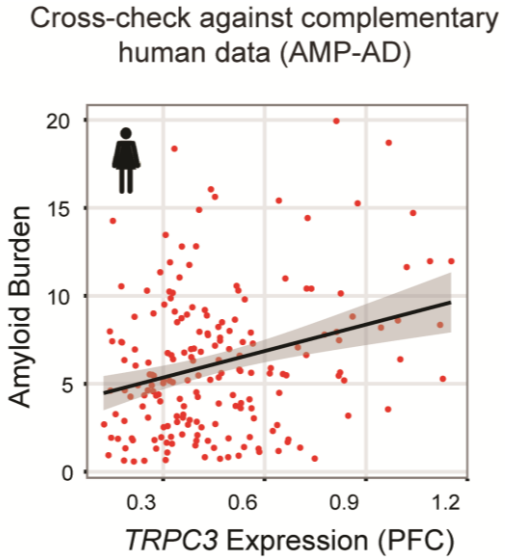


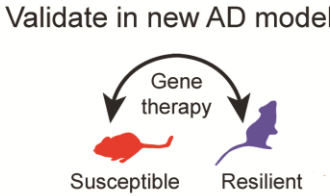


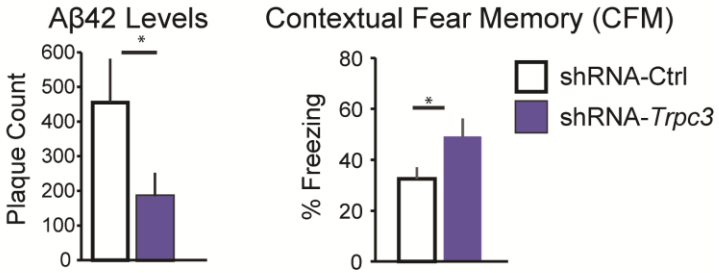
Neuner et al, unpublished

A Mouse-Human-Mouse Discovery Pipeline
Proof of concept – *Trpc3*









Neuner, Hohman, Bennett, De Jager, Schneider et al., unpublished

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AFAR (PI: Kaczorowski, completed)
AFAR (PI: Wilmott, role: mentor, completed)
NIA K99/R00AG039511 (PI: Kaczorowski, completed)
NIA NRSA F31AG050357 (PI: Neuner, role: mentor)
AA Fellowship (PI: Dunn, role: mentor)
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BrightFocus Foundation (2016-2019)
Award from Glenn Foundation for Medical Research
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NIA R01 AG057914 (PI: Kaczorowski, 2017-2022)

Tony and Judith Evnin (Evnin Family Chair)

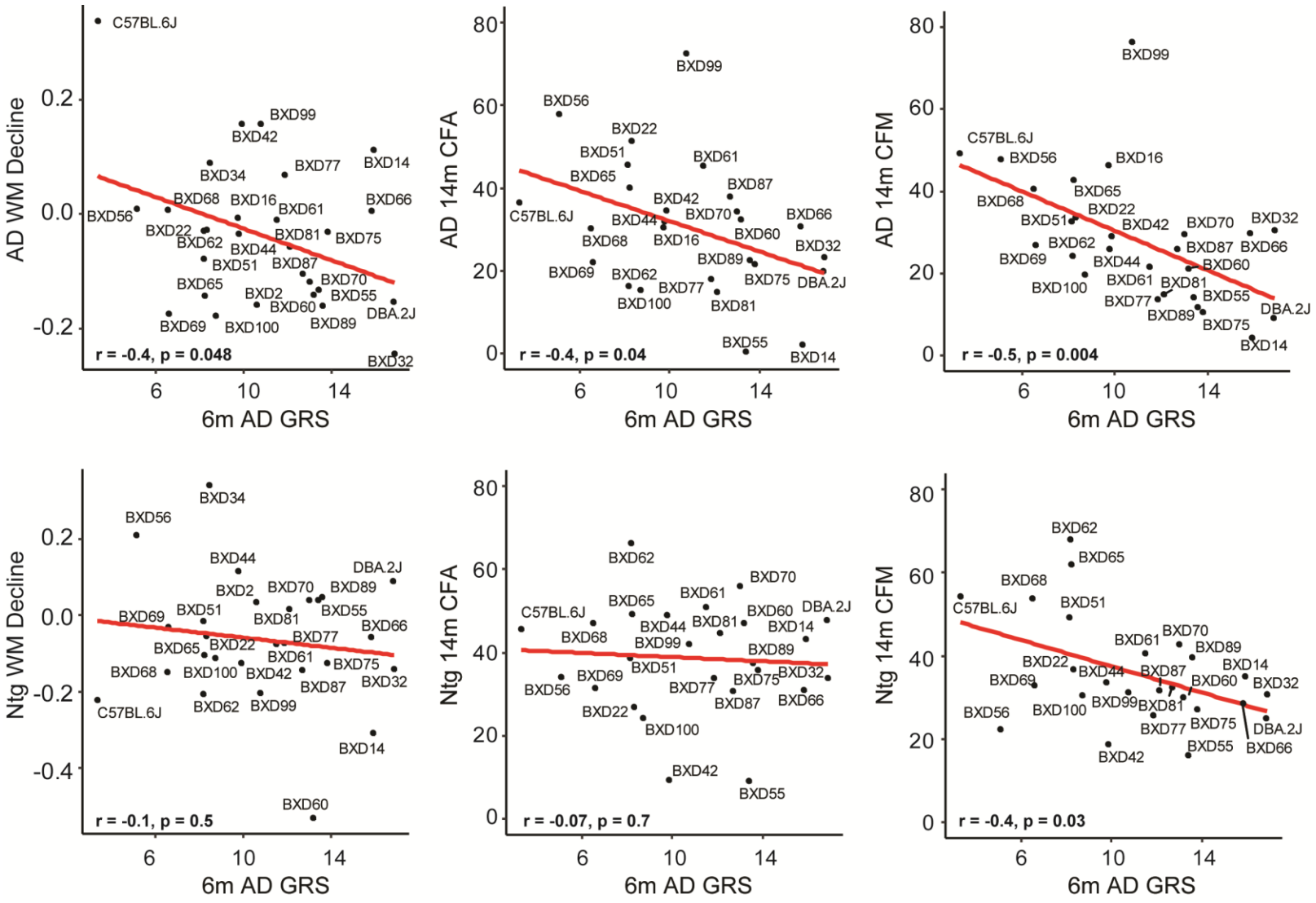
NIH National Institute on Aging

Alzheimer's Disease Research

afar Dorothy Dillon Eweson Lecture Series on the Advances in Aging Research

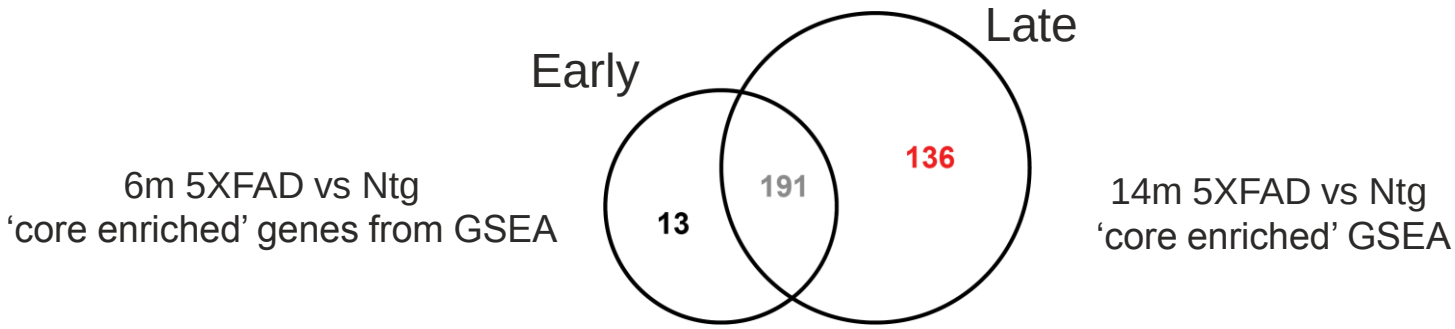
GLENN FOUNDATION FOR MEDICAL RESEARCH

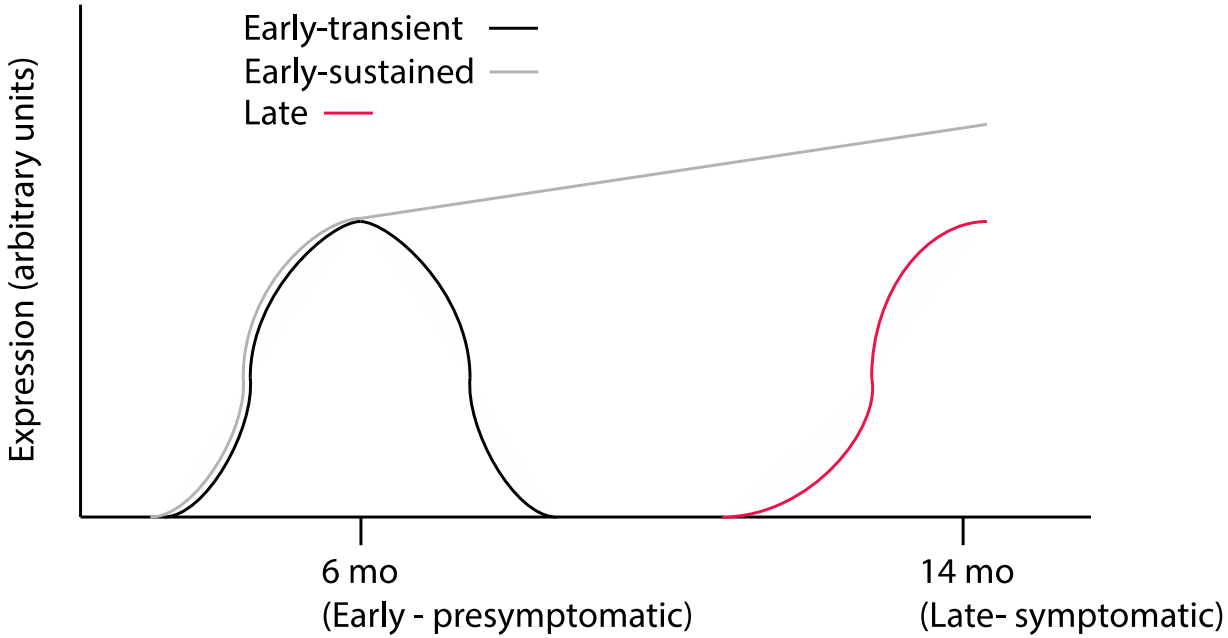
AD-BXD panel is sensitive to variation in known AD risk loci



Neuner et al., unpublished

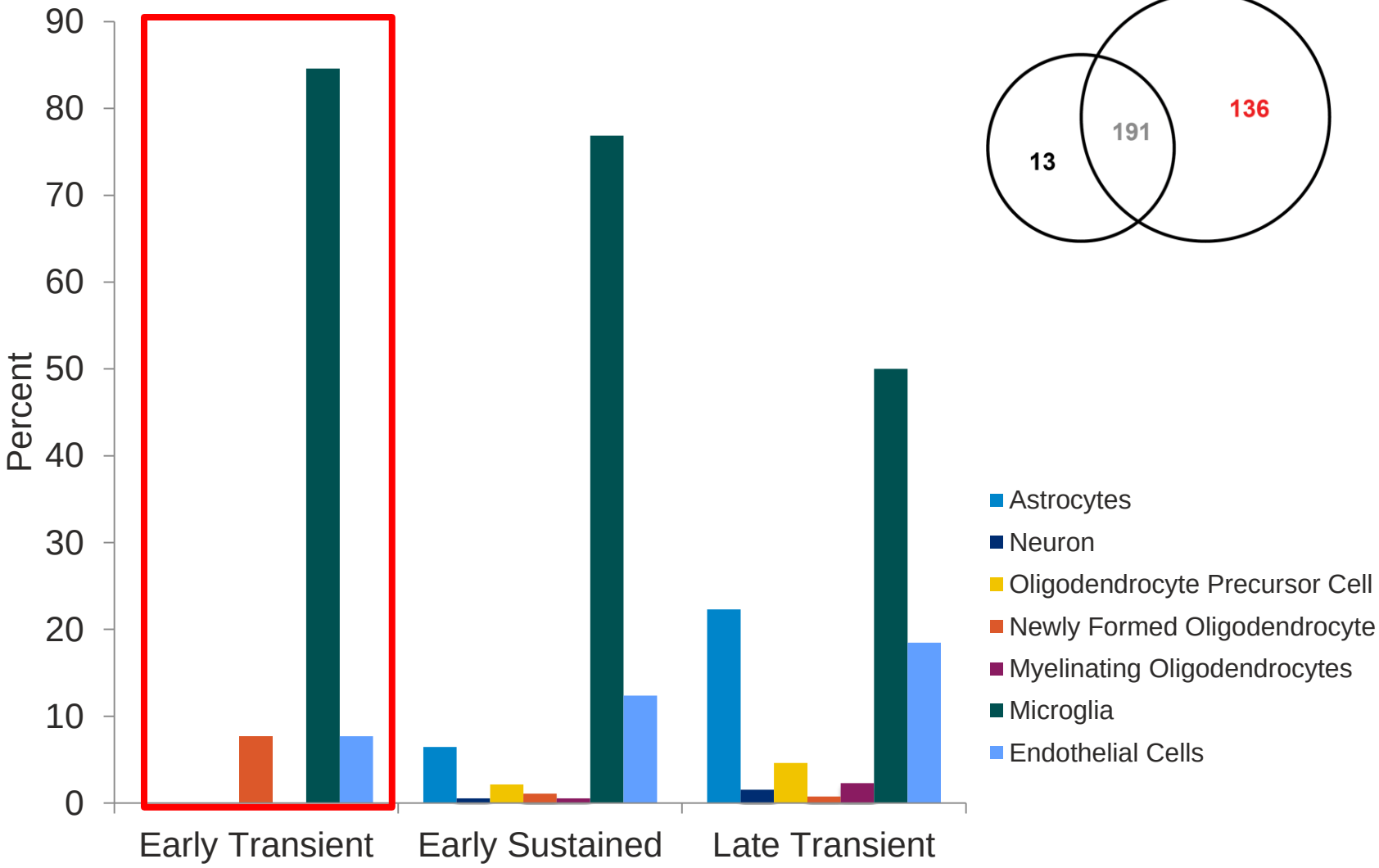
GSEA of DE Genes from AD-BXDs compared to Ntg-BXDs
Top Positively Enriched GO Biological Processes





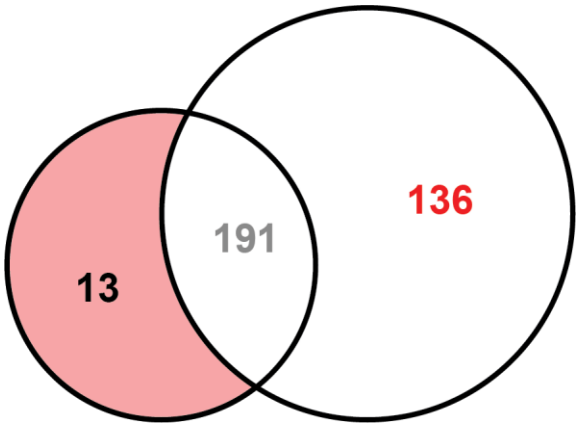
Heuer et al., unpublished

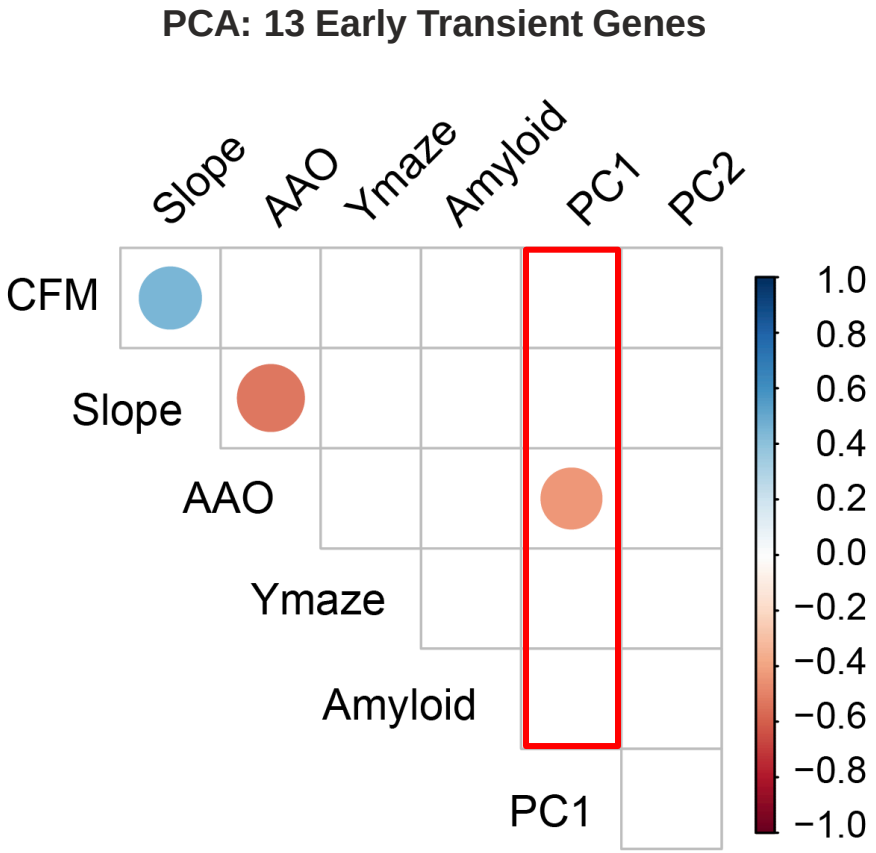
Cell-Type assignment of GSEA “Immune” genes implicate microglia



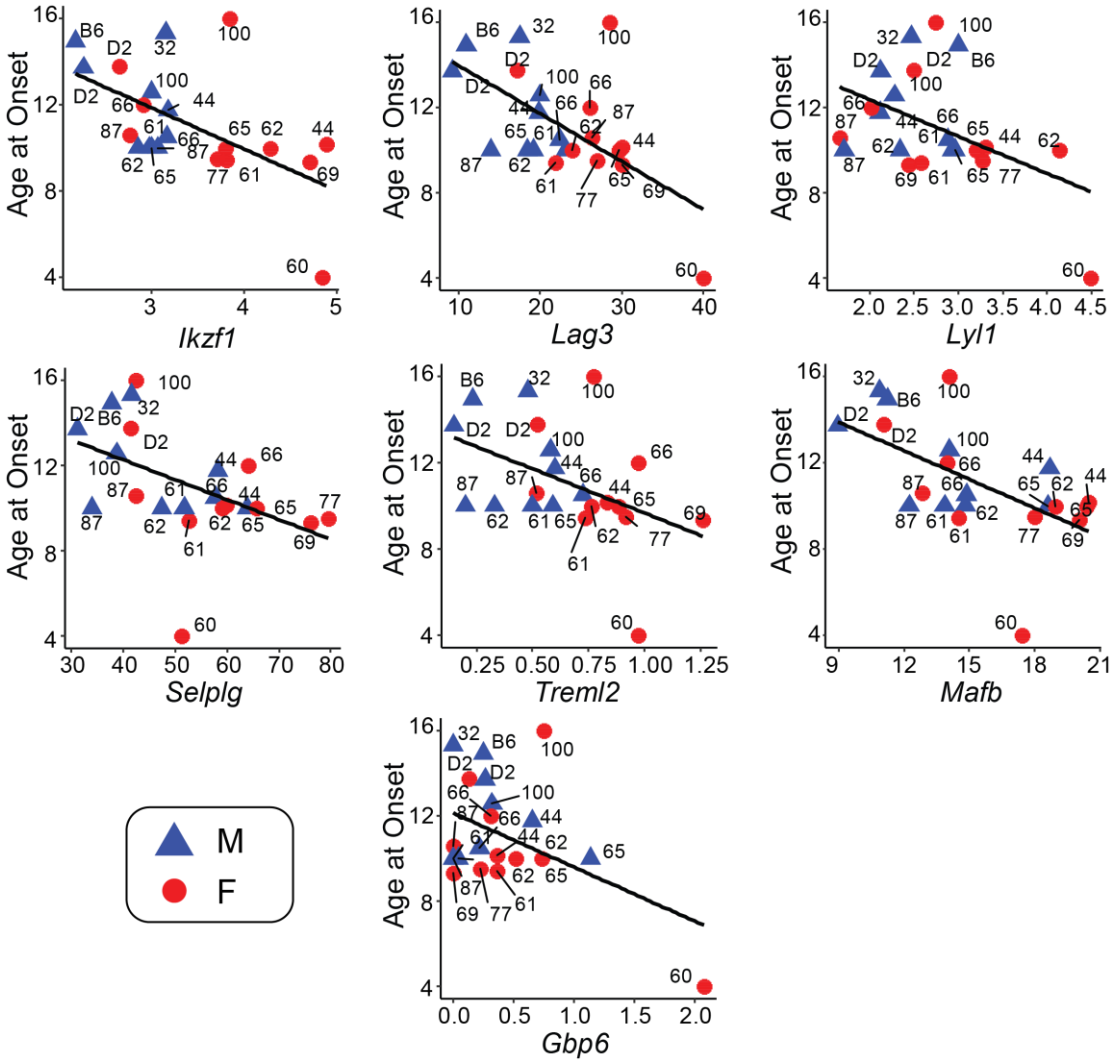
Zhang, Y., et al, J Neuro, 2014

The expression of early-transient genes is correlated with age at onset (AAO), and not amyloid

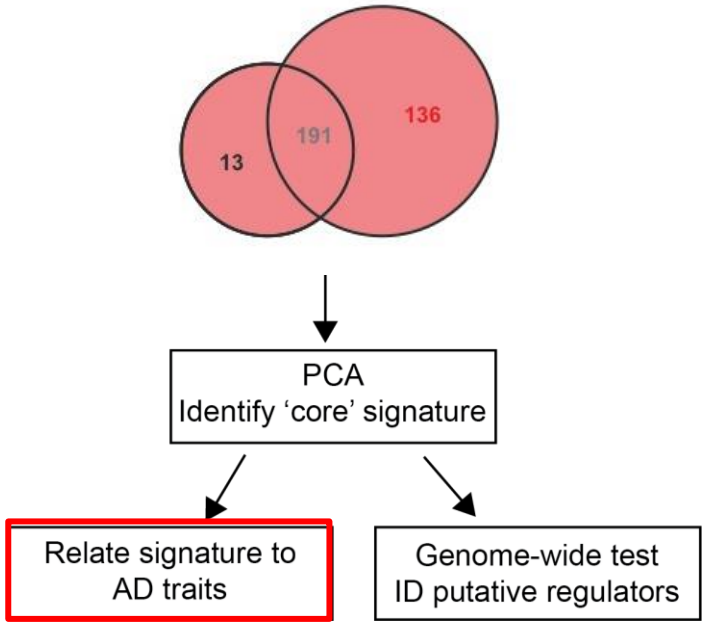


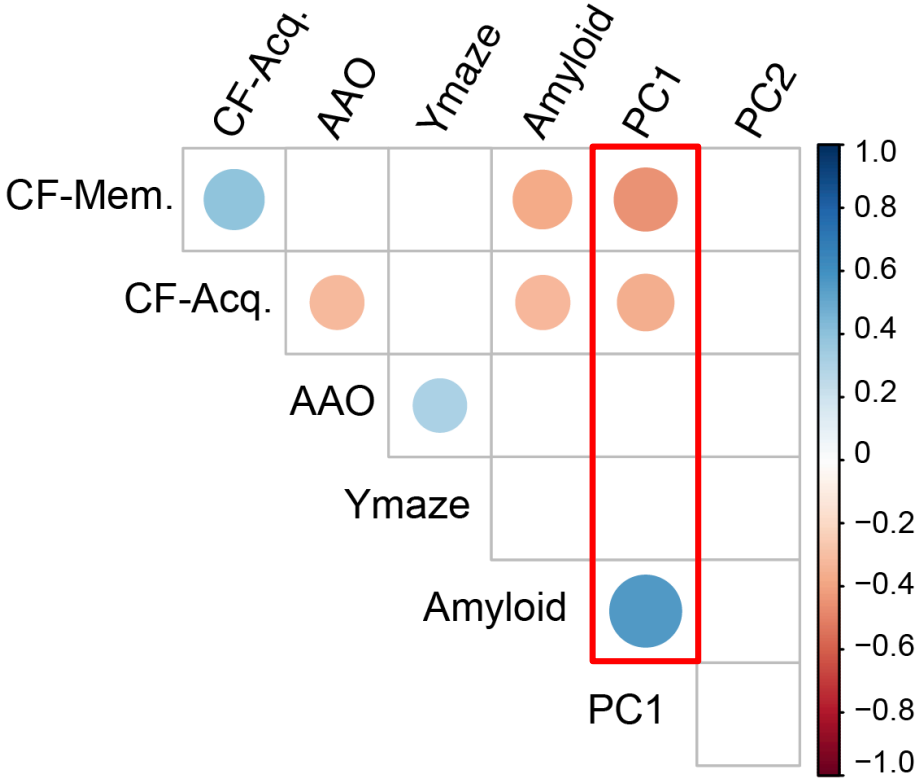


Higher expression of early-transient genes is related to earlier age at onset (AAO) of cognitive impairment



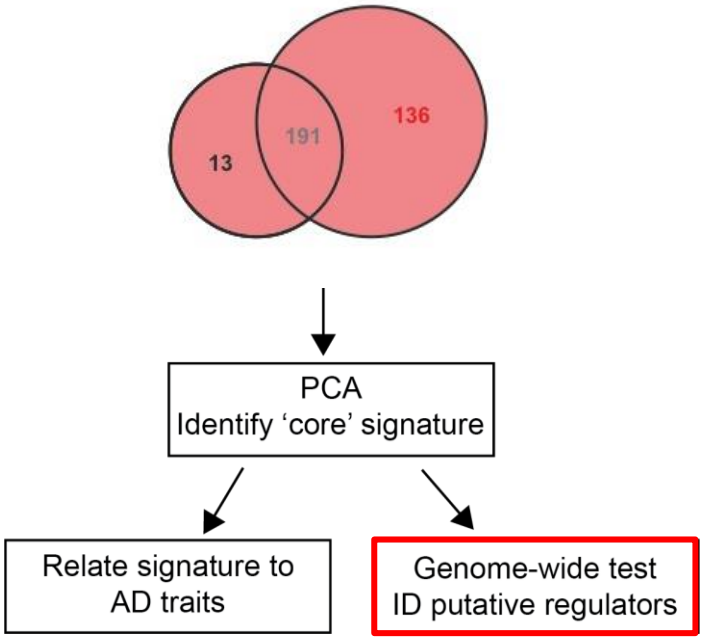
Microglia “core” gene expression (PC1) correlate with performance on short- and long-term memory

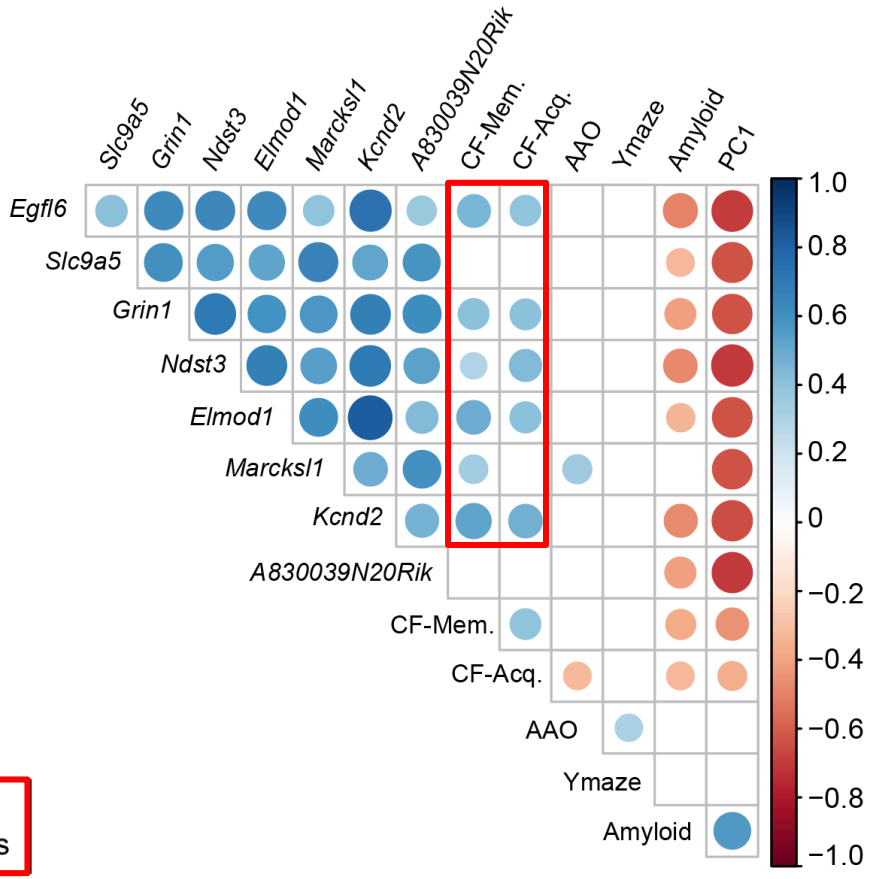




- Higher expression of “core” microglia genes corresponds to poorer performance on CF acquisition and memory
- Higher expression of “core” microglia genes corresponds to higher amyloid levels detected by ELISA assay

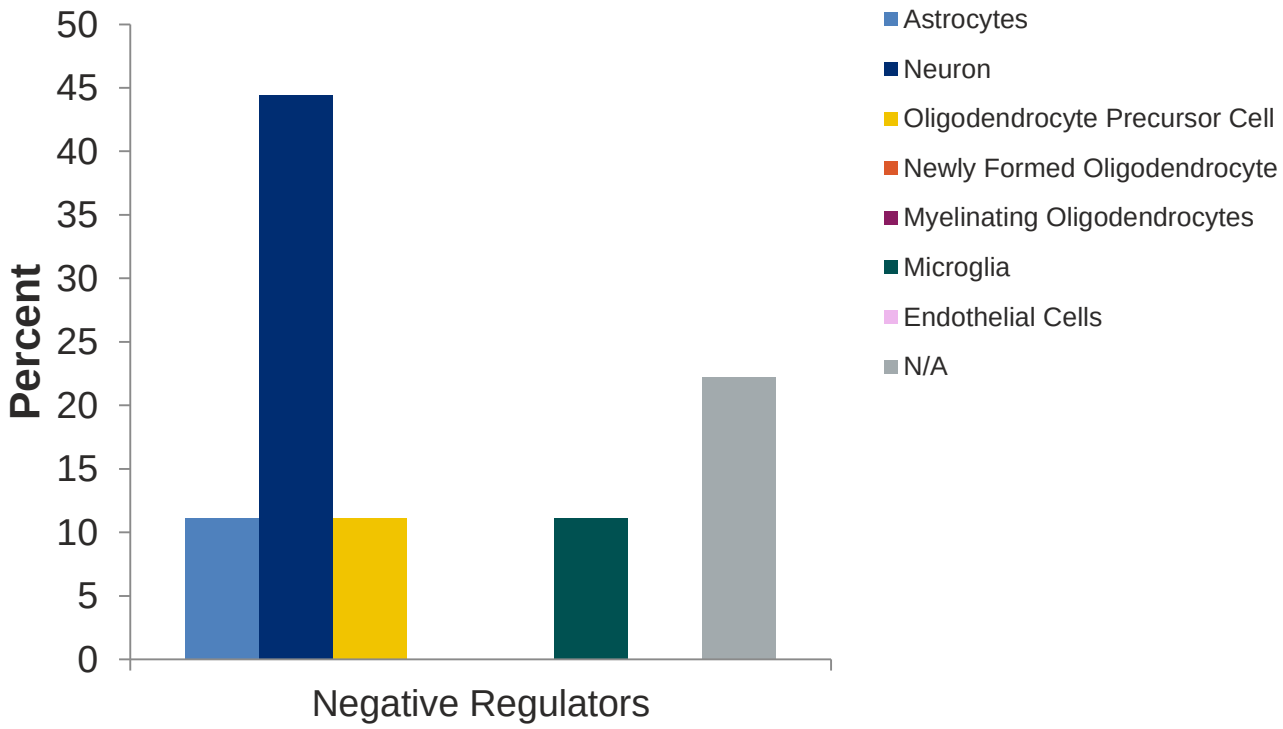
Genome-wide search reveals negative regulators of microglia “core” genes (PC1) as drivers of cognitive resilience





- Higher expression of negative regulators of microglia (PC1) corresponds to better CF acquisition and memory
- Higher expression of negative regulators of microglia (PC1) also corresponds to lower amyloid levels by ELISA

Cell-type specific enrichment of negative regulators suggests neuron involvement

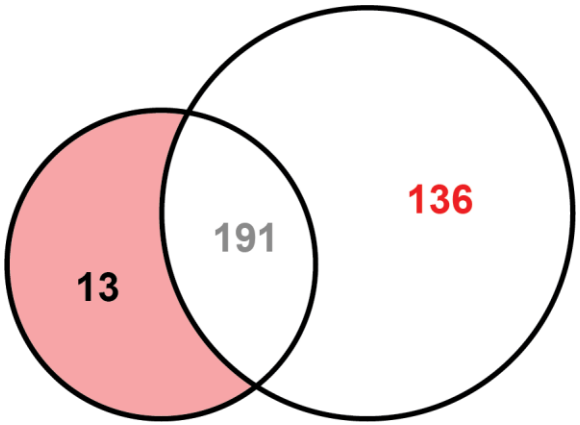


Zhang, Y., et al, J Neuro, 2014

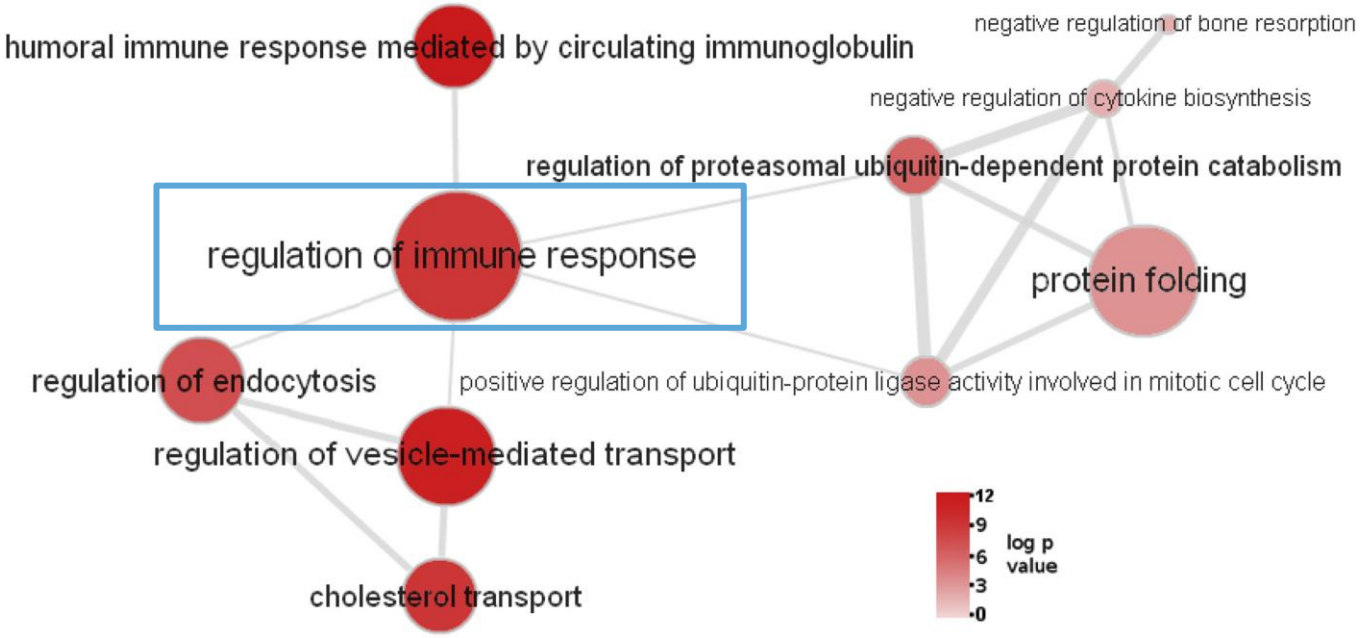
Overall Summary

- Synergy of mouse-to-human discovery will maximize the capabilities of each approach and minimizes the limitations.
  - Complementary to ongoing efforts by MODEL-AD (IU/JAX)
- Because clinical and pathological hallmarks of FAD parallel those of LOAD cases, we expect results from our mouse studies will generalize to LOAD.
- We expect our new AD models will improve predictive validity of preclinical studies and **accelerate discovery** of therapeutics to promote resilience and delay, treat or even cure AD.

Higher expression of early-transient genes is related to earlier age at onset (AAO) of cognitive impairment

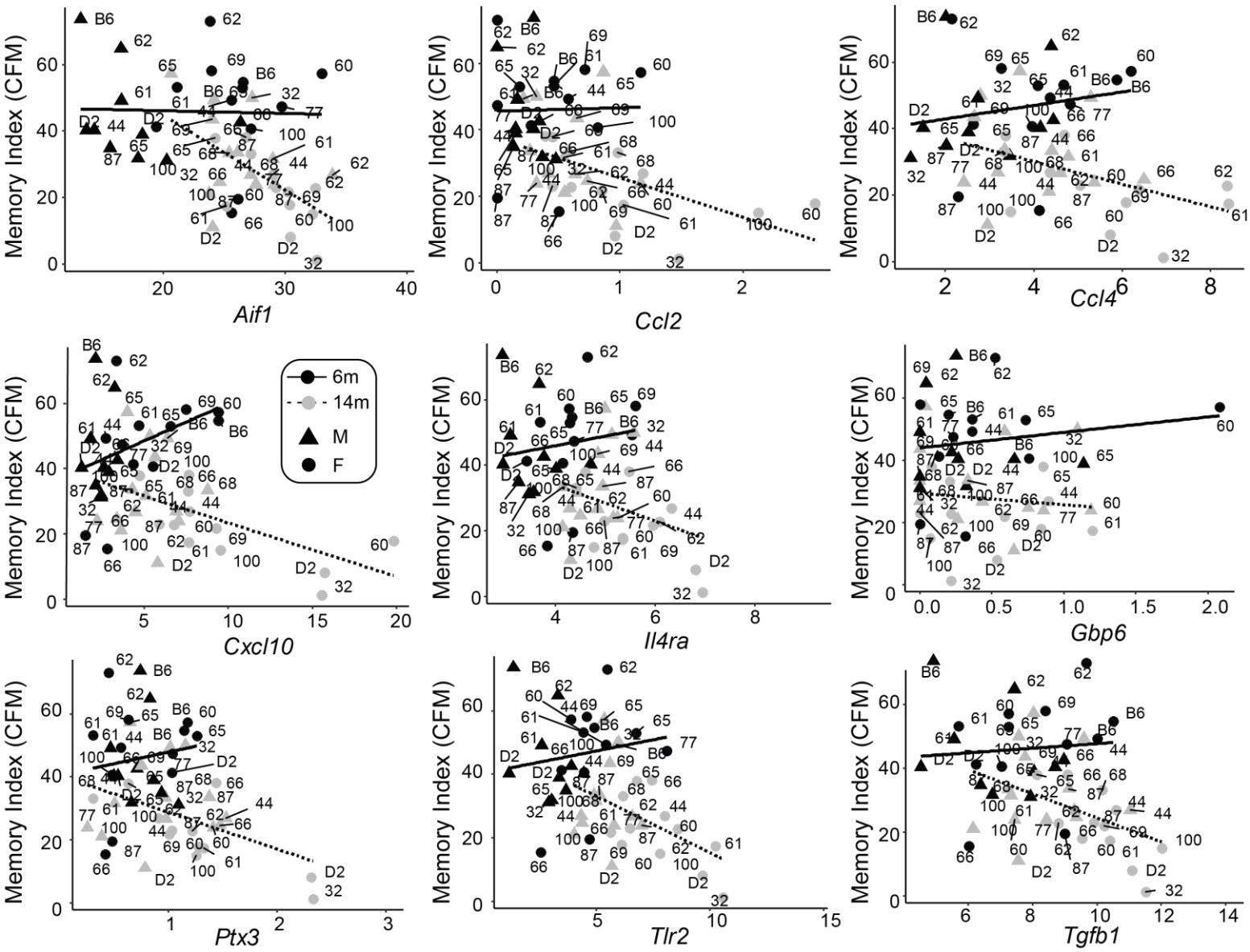


Immune response pathways enriched for association with AD SNPs from IGAP

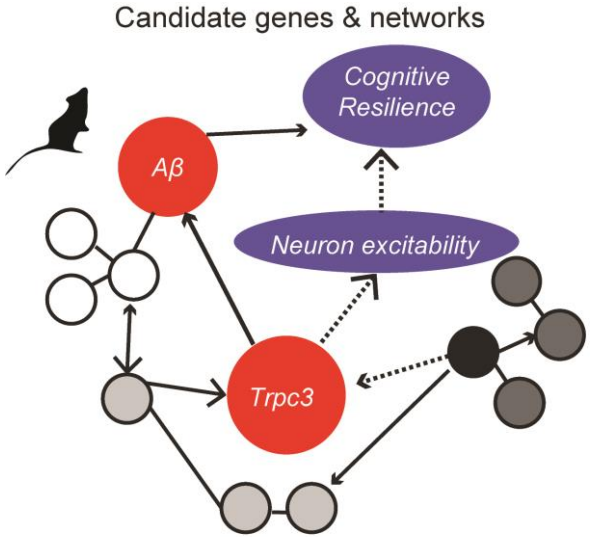


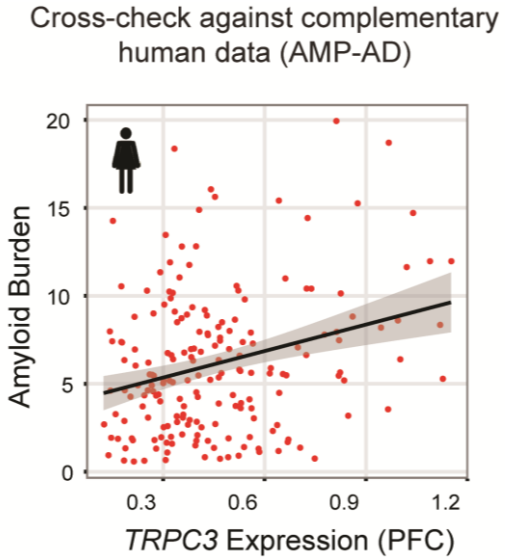
Jones et al., 2015

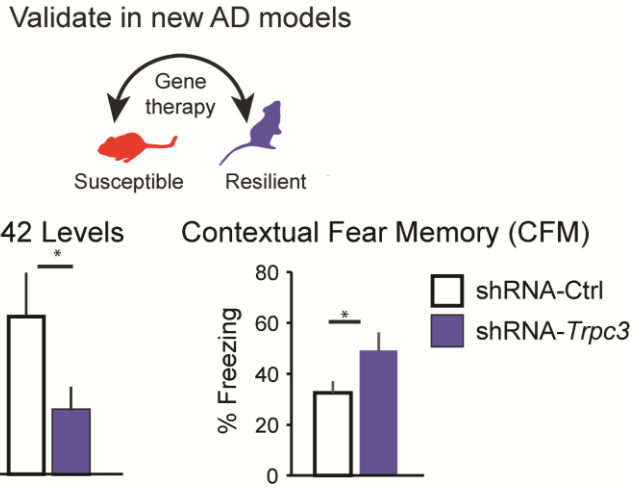
Microglial “core” genes negatively correlate with cognitive deficits in late-stages of the disease (14 mo)



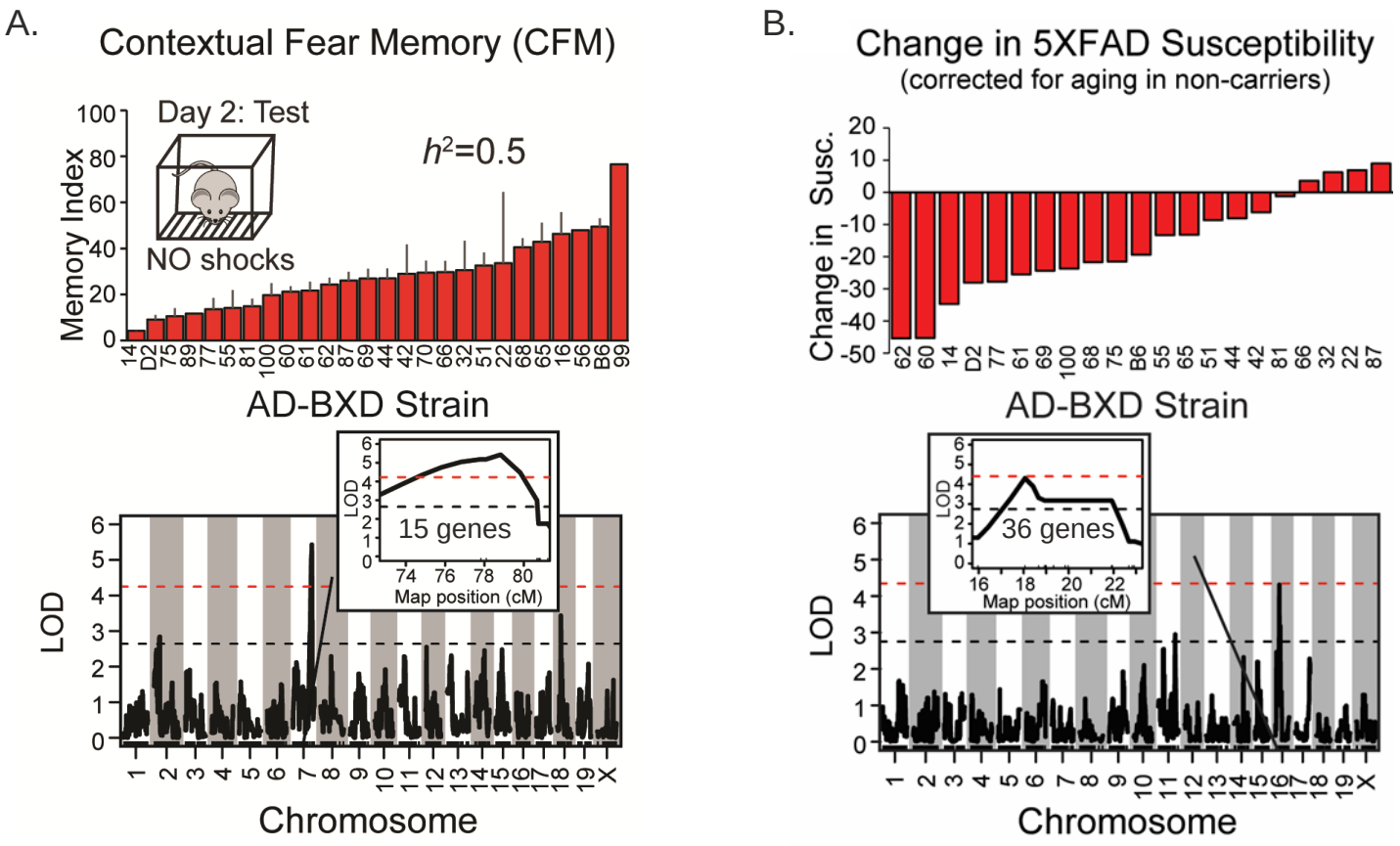
A Mouse-Human-Mouse Discovery Pipeline
Proof of concept – *Trpc3*



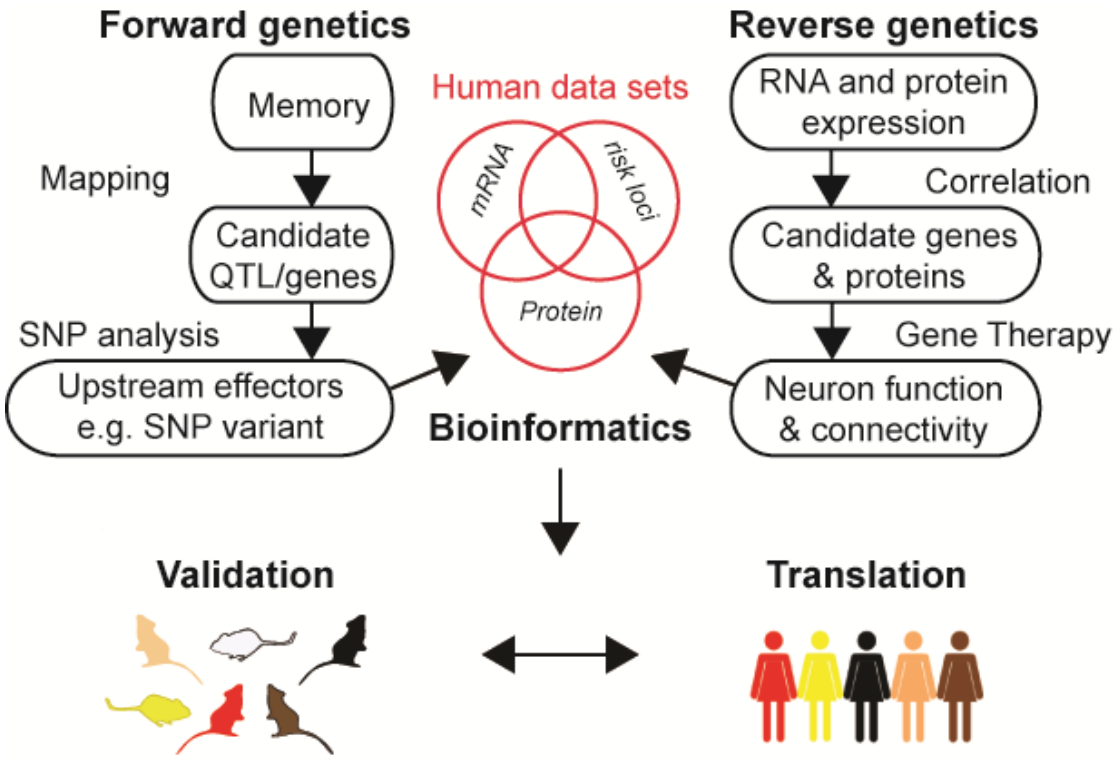




Genetic mapping identifies two *novel* QTLs containing modifiers of cognitive symptoms in AD

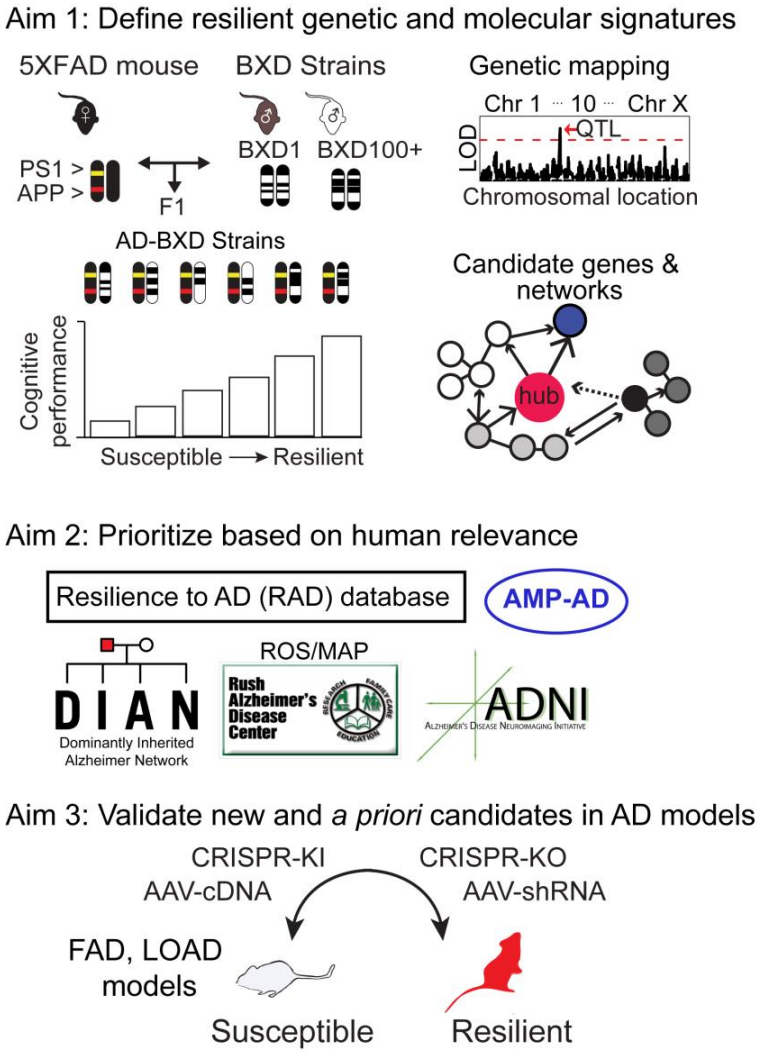


Bridging the gap: “Bench to bedside and back to bench”



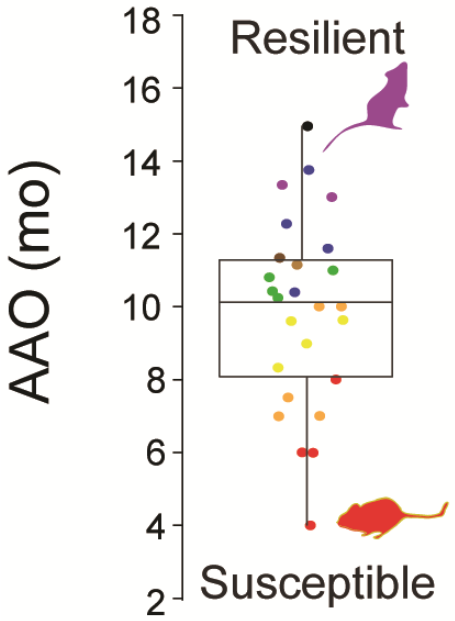
- Understanding the complex biology of resilience to Alzheimer’s disease

Systems genetics analysis of resilience to AD to identify novel drug targets (R01s, 2017-2022)



NIA Resilience AD Consortium R01AG057914

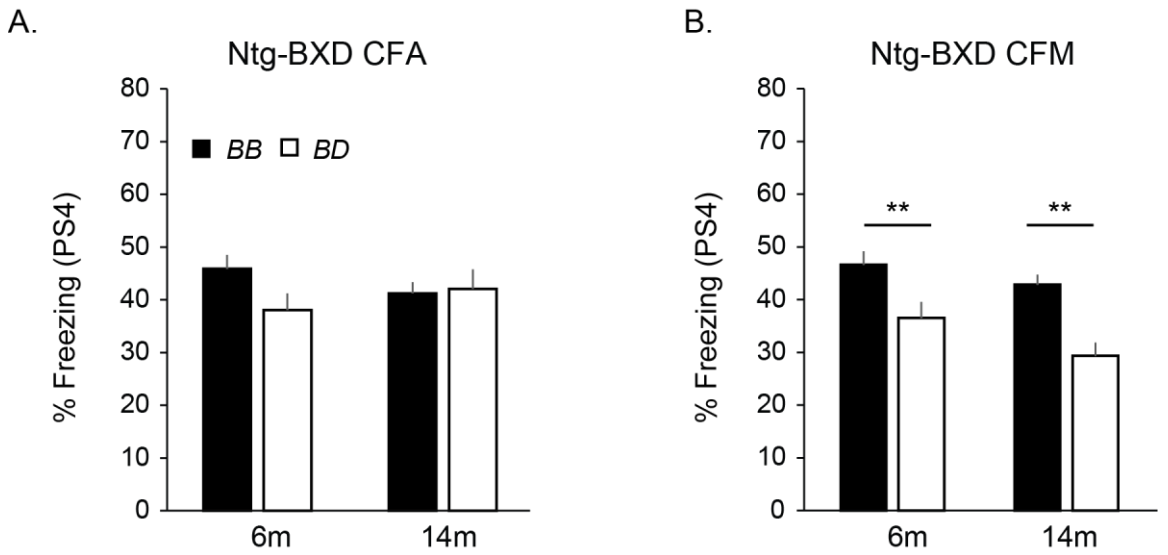
Identification of genetic correlates of age at first symptom onset in the AD-BXD panel



Gene	Corr	pval
Snx25	-0.24	0.015
Prnp	-0.23	0.023
Inpp5d	-0.30	0.003
Clu	-0.25	0.013
Picalm	-0.34	4E-04
Rab10	-0.29	0.004
Hist1h2ad	0.45	3E-06
Gucy2e	0.45	3E-06
Atp1a4	0.44	5E-06
Nudt19	-0.51	8E-08
Ctla2b	-0.50	1E-07
Ccl24	-0.50	2E-07

Neuner et al., unpublished

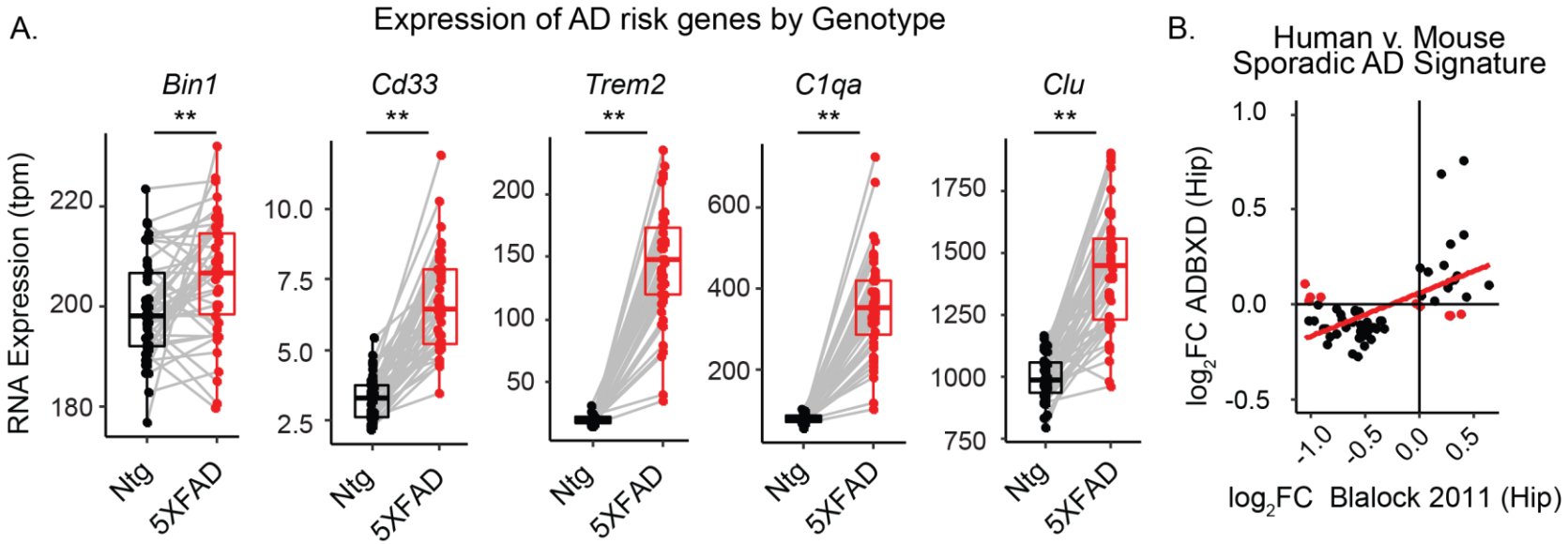
Apoe genotype effects CFM, but not CFA, in Ntg-BXD mice.



- Introduce D2 variant into B6 background to test hypothesis Apoe allele swap is sufficient to recapitulate cognitive deficits in normal aging and AD

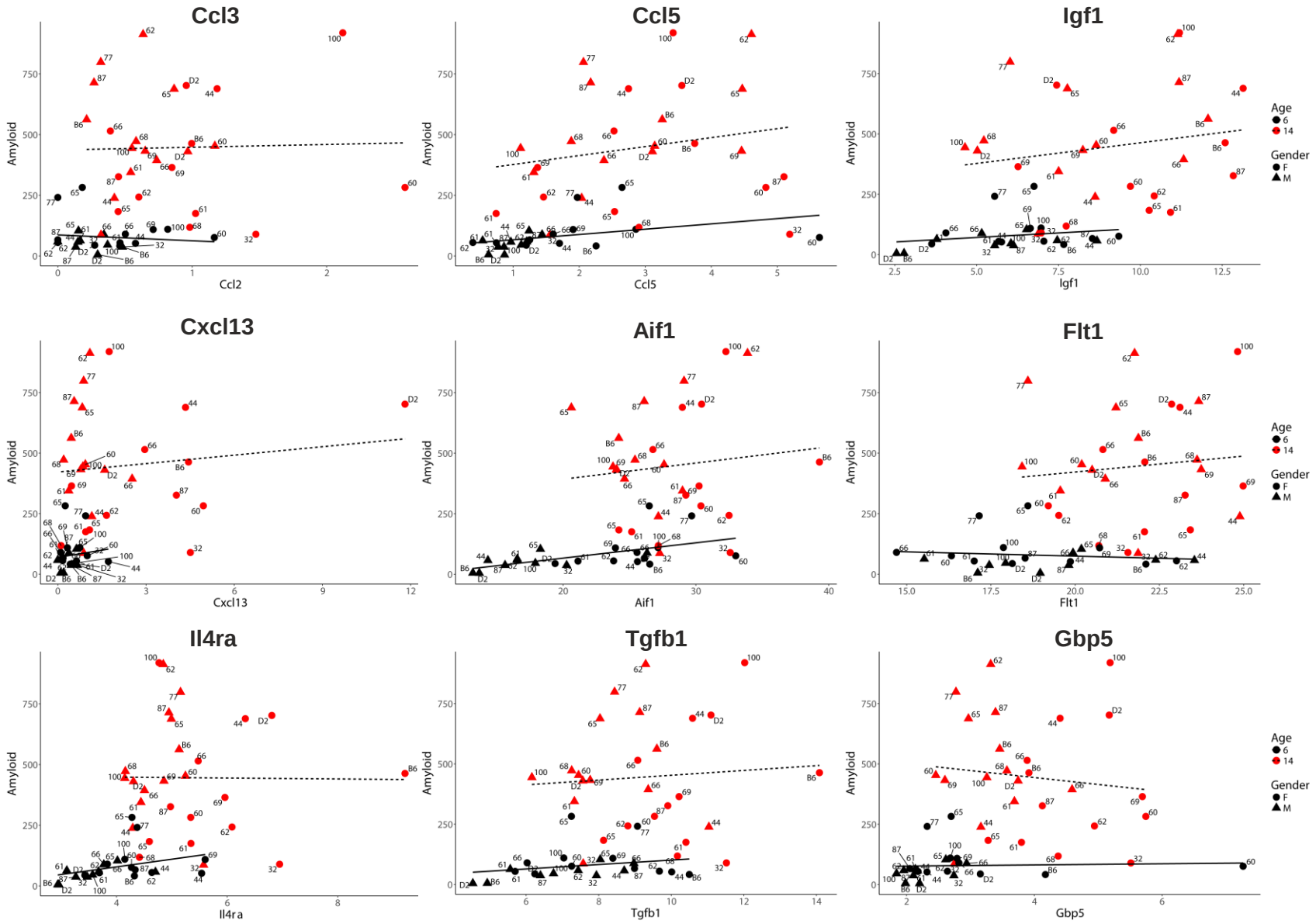
Neuner et al., unpublished

Genetic background modifies AD-associated transcriptome and enhances concordance with human AD signature.



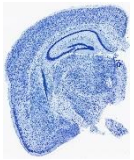
Neuner et al., unpublished

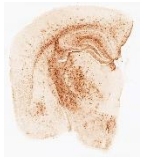
Microglial “core” genes only weakly correlate with amyloid (6 and 14mo)

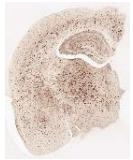


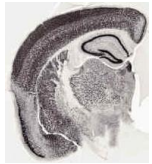
Whole brain region- and cell-type specific quantification

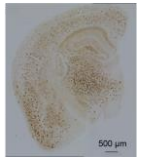
Input: Raw images

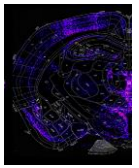

Nissl


GFAP

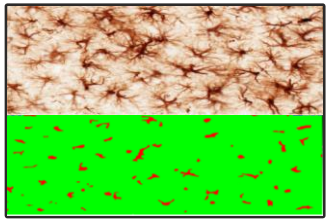

IBA1


NEUN


Aβ

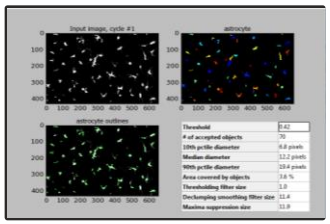

Arc-GFP

ilastik: interactive learning and segmentation toolkit
Sommer et. al 2011



Pixel classification

- Train on diverse images
- Refine “uncertainty” in classification



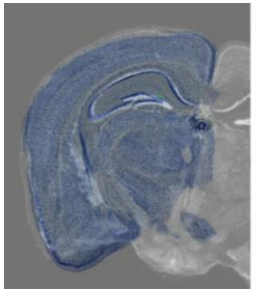
Object classification

- Train on diverse images
- Identify objects based on relevant parameters

QuickNII: image registration onto Allen Brain Atlas

Section alignment

- Nearest Nissl approach



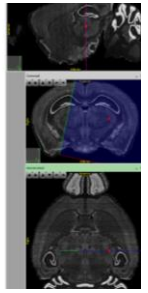
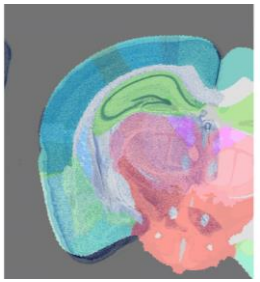
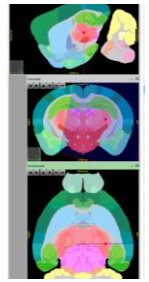


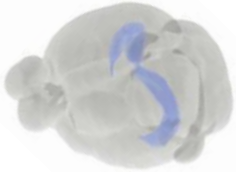
Image registration

- Define regional boundaries





Output: Region-specific quantification
3D reconstruction/visualization



Bjaalie, Bachelder and Neuner, unpublished