

Utility of the B6.APP^{SAA} knock-in mouse model for Alzheimer's disease pre-clinical testing.

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INTRODUCTION

Alzheimer's disease (AD) is a complex and progressive neurodegenerative disease characterized by the development of amyloid plaques, tau neurofibrillary tangles, and neurodegeneration with associated cognitive decline. There is currently no cure, with limited treatments showing varying levels of efficacy.

Preclinical AD research primarily utilizes first-generation transgenic models like 5xFAD, APP/PS1, or 3xTg. However, there are numerous confounding variables related to transgenic mouse models of AD. **Improving preclinical models of AD is necessary to bring novel diagnostic tools and therapeutic agents to the market.** In addressing the translational gap between transgenic animal models and human clinical trials, the Model Organism Development and Evaluation for Late-Onset Alzheimer's Disease (MODEL-AD) consortium has developed novel second-generation knock-in models of FAD and LOAD.

AIMS

To aid in preclinical development, we conducted a follow-up in-depth phenotyping evaluation of the second-generation APP^{SAA} knock-in mouse model (Jax #034711; Xia et al. 2022).

B6.APP^{SAA} MODEL OF AD

The B6.APP^{SAA} knock-in mouse model was engineered on the C57BL/6 background with insertion of three mutations to the mouse APP genomic locus to humanize the mouse A β 1-42 region: G676R, F681Y and R684H. Additionally, three FAD mutations were also inserted: KM670/671NL (Swedish), E693G (Arctic) and T714I (Austrian) (Xia et al. 2022).

B6.APP^{SAA} MICE ACCUMULATE A β ₁₋₄₂ IN AN AGE-DEPENDENT MANNER

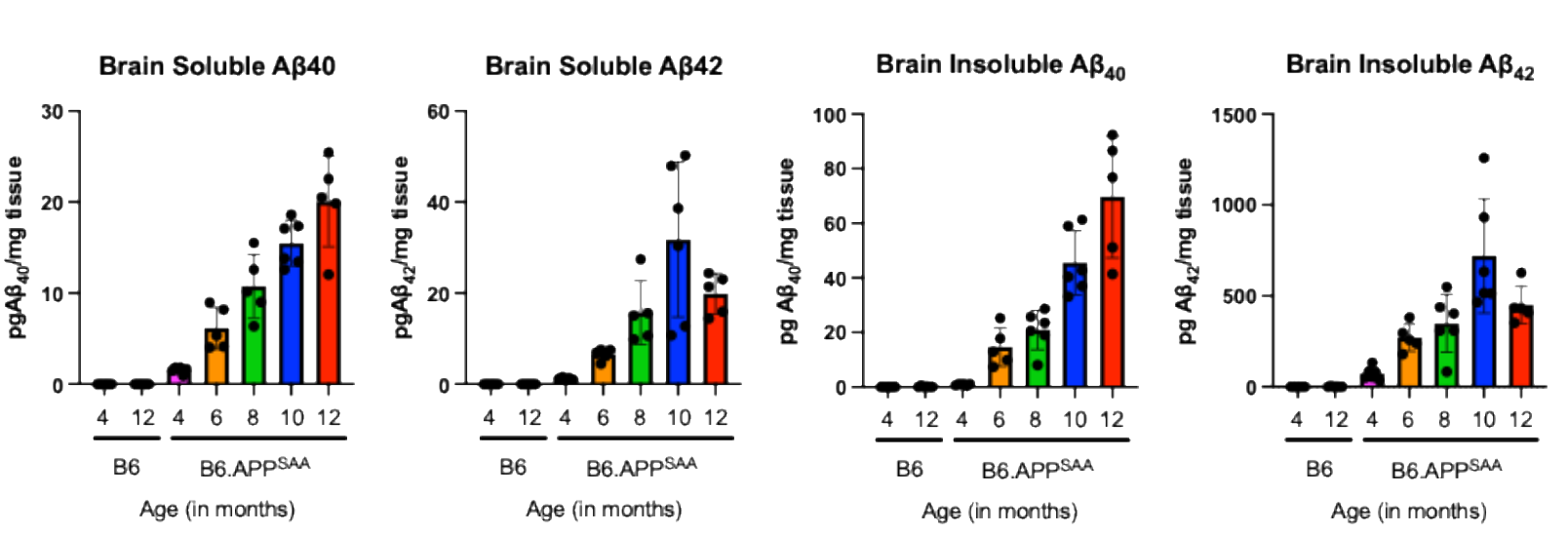


FIGURE 1. Soluble and insoluble proteins were extracted from PBS perfused brains with 0.4% DEA and formic acid, respectively, followed high-speed centrifugation and analyzed using the 6E10 V-PLEX A β MSD kit (#K15200E).

METHODS

Brain preparation for TissueVision: Brains were double labeled by IP injection of methoxy-X04 for amyloid plaques and 24 hours later DyLight594 was injected into the tail vein to label vasculature. Mice were perfused with 1x PBS and then 4% PFA. Intact brains were dissected and post-fixed for 12-24 hours at 4°C. **TissueVision Platform:** Embedded brains are placed into TissueVision's Marinus Imaging platform to undergo processing via serial imaging of the block face, tissue sectioning, and automated slide mounting to create a series of physical mounted slides. The TIFF images were aligned to the Allen Institute Common Coordinate Framework (CCF) and then segmented to reveal methoxy-X04 plaque density and other metrics (e.g. vasculature as imaged by DyLight) by brain region according to the Allen Brain Atlas. All data is made available through a high-performance terabyte visualization portal. **IBEX:** Brains went through a sucrose gradient (24hrs at 15% and 24hrs at 30%) before multiplex immunohistochemistry was performed using iterative bleaching extends multiplexity (IBEX) technique. Sections were mounted on chrome alum-gelatin adhesive before continuing with immunohistochemical labeling with antibodies against IBA1, GFAP, LAMP1, A β (6E10), NeuN. Fluorophores were bleached between antibody labeling with diluted LiBr4 (95% lithium borohydride, 1mg/ml). Slides were imaged on the Leica THUNDER imaging system and visualized with iMARIS software (Oxford Instruments). **Context-dependent fear memory (Figure 4A):** First, mice were trained to associate an auditory tone with an electrical shock in Context A (environment 1 + tone + shock). Next, in Context B (environment 2 + tone), the fear-context memory is extinguished by only using the auditory tone for two days. Retrieval (Context B) and renewal (Context A) are probe trials to determine whether mice recall the context-specific memories. Time immobile is used to quantify associative-memory activity. **Synaptic loss (Figure 4C):** Dendrites and spines were quantified with NeuronStudio after sparse labeling using a dual viral approach to drive GFP expression in CA1-to-mPFC neurons and X34 staining of amyloid plaques (blue) in 12-month-old mice. **Plasma biomarkers:** The mouse NULISA™ (Alamar Biosciences) panel was used to obtain relative quantification of 120 protein analytes in plasma from mice aged 4- to 16-months-of-age. **Data Analysis:** Data analysis was performed using GraphPad Prism and analyzed with normality tests, and according to statistical assumptions, either a one-way ANOVA with Tukey's post-hoc test or a two-way ANOVA with Holm-Sidak's post-hoc test. p values ≤ 0.05 were considered statistically significant.

AGE-DEPENDENT PLAQUE DEPOSITION

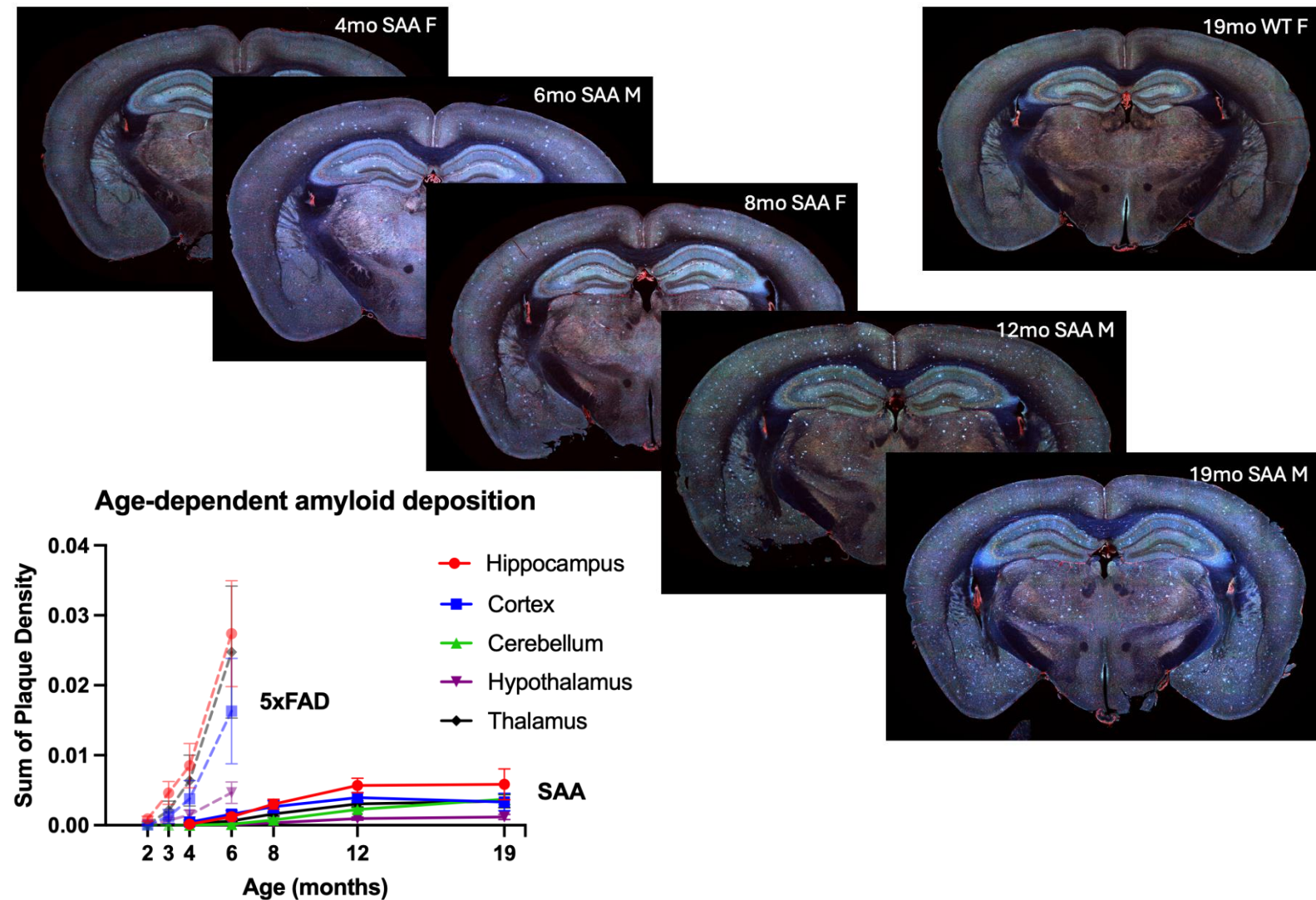


FIGURE 2. A. Representative images showing age-dependent plaque accumulation in B6.APP^{SAA} mice from 4- to 19-months-of-age. No methoxy-X04 signal is detected in 19-month-old WT controls. **B.** Volumetric quantification of plaque density revealed the rate of plaque deposition peaks between 4- and 12-months-of-age before plateauing. In comparison, 5x^{FAD} mice show rapid development of pathology from 4- to 6-months of age. Importantly, there were no sex-specific differences in amyloid pathology in B6.APP^{SAA} mice.

AGE-DEPENDENT PLAQUE-ASSOCIATED AD PATHOLOGY

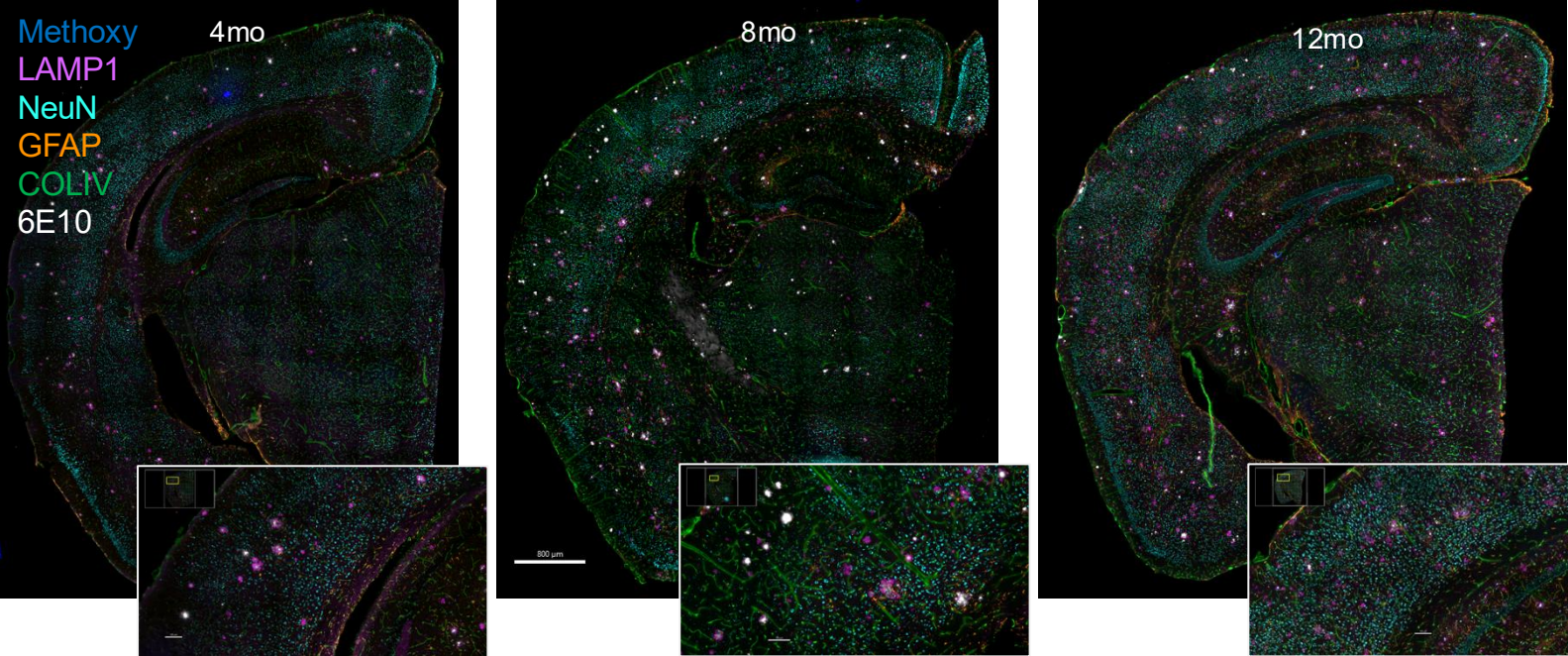


FIGURE 3. Representative IBEX images of A β -associated proteins in the brain of a 4-, 8-, and 12-month-old B6.APP^{SAA} mice. At 4-months, dystrophic neurons stained with LAMP1 (magenta) are apparent surrounding nascent A β plaques stained with methoxy-X04 (blue) and 6E10 (white) in the cortex. GFAP (orange) is apparent surrounding plaques by 8-months-of-age. All pathology increases with age.

MEMORY DEFICITS AND SYNAPSE LOSS IN B6.APP^{SAA} MICE BY 12-MONTHS

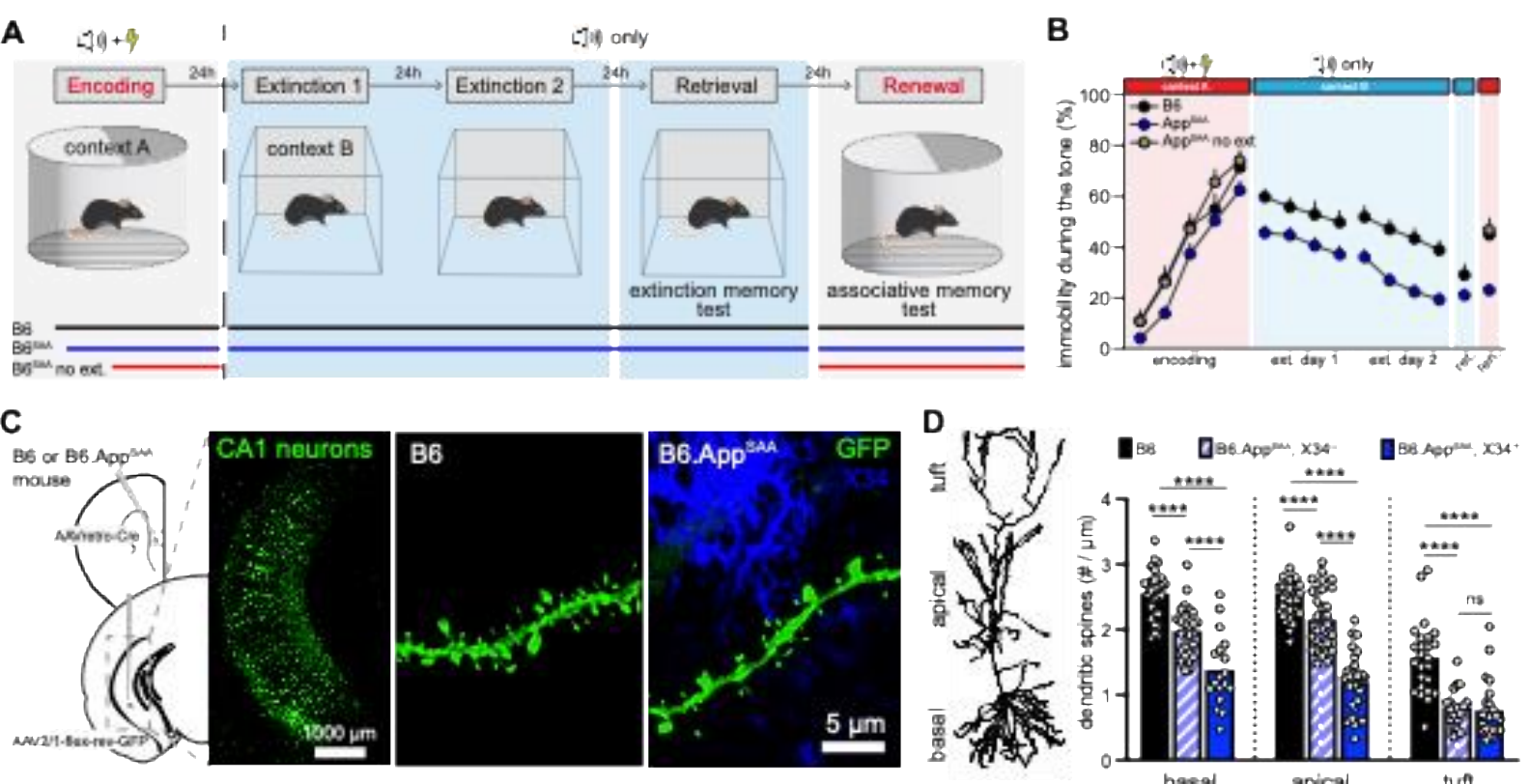
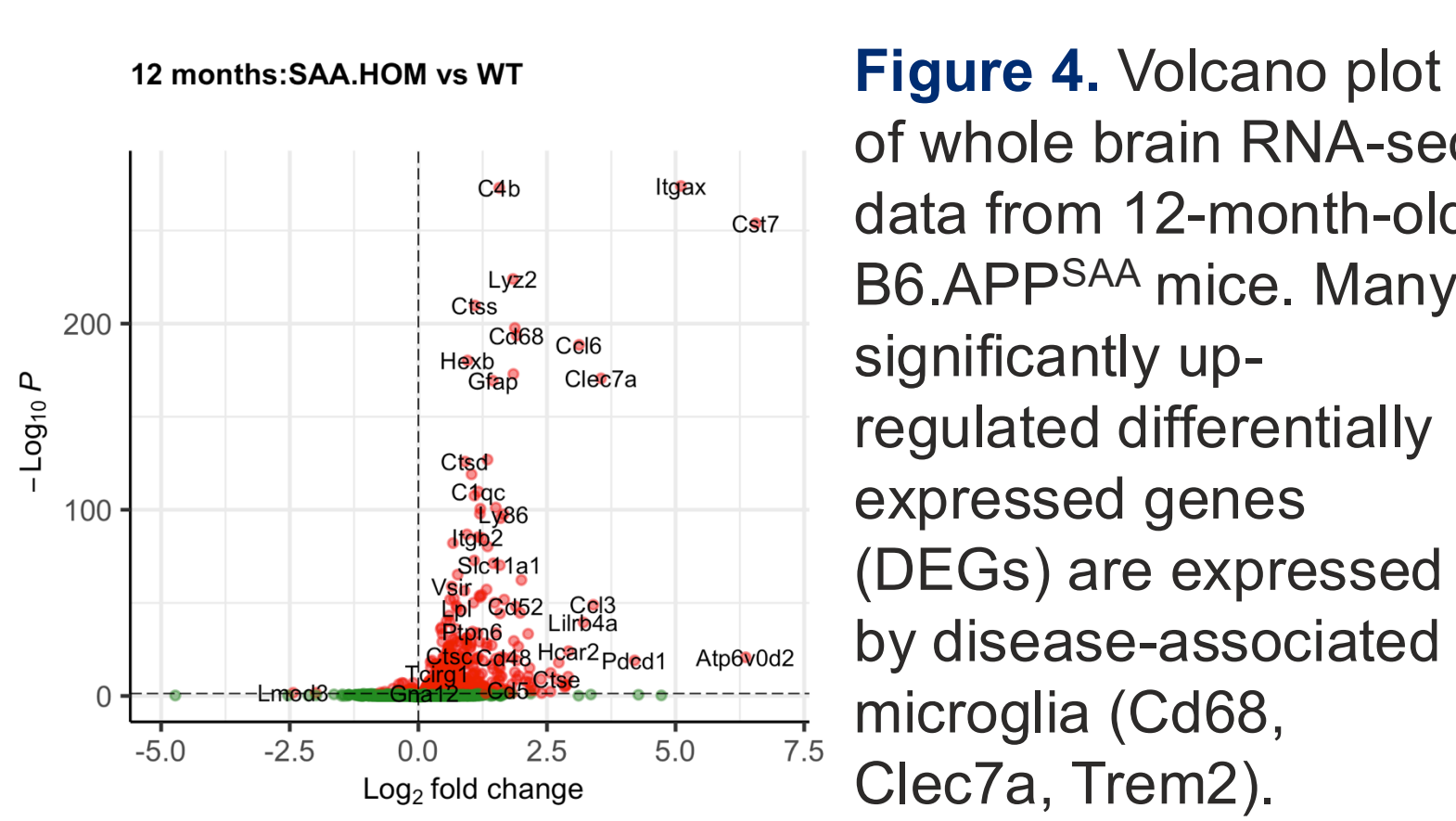


Figure 4. A. 12-month-old B6.APP^{SAA} mice were tested in a context-dependent extinction paradigm. **B.** B6.APP^{SAA} mice show deficits in memory extinction. Omitting extinction (gray dot) shows the original memory remains intact. **C.** GFP labeling of CA1-to-PFC pyramidal neurons using an AAVretro system. **D.** Dendritic branches from B6.APP^{SAA} mice had reduced spine densities. In proximity to plaques, spine densities were further reduced by ~50% on apical oblique and basal branches. ****p ≤ 0.001 .

INCREASED EXPRESSION OF DISEASE-ASSOCIATED MICROGLIAL GENES IN 12-MONTH B6.APP^{SAA} MICE



BRAIN TRANSCRIPTOMICS ALIGN WITH AMP-AD MODULES AT 12 MONTHS

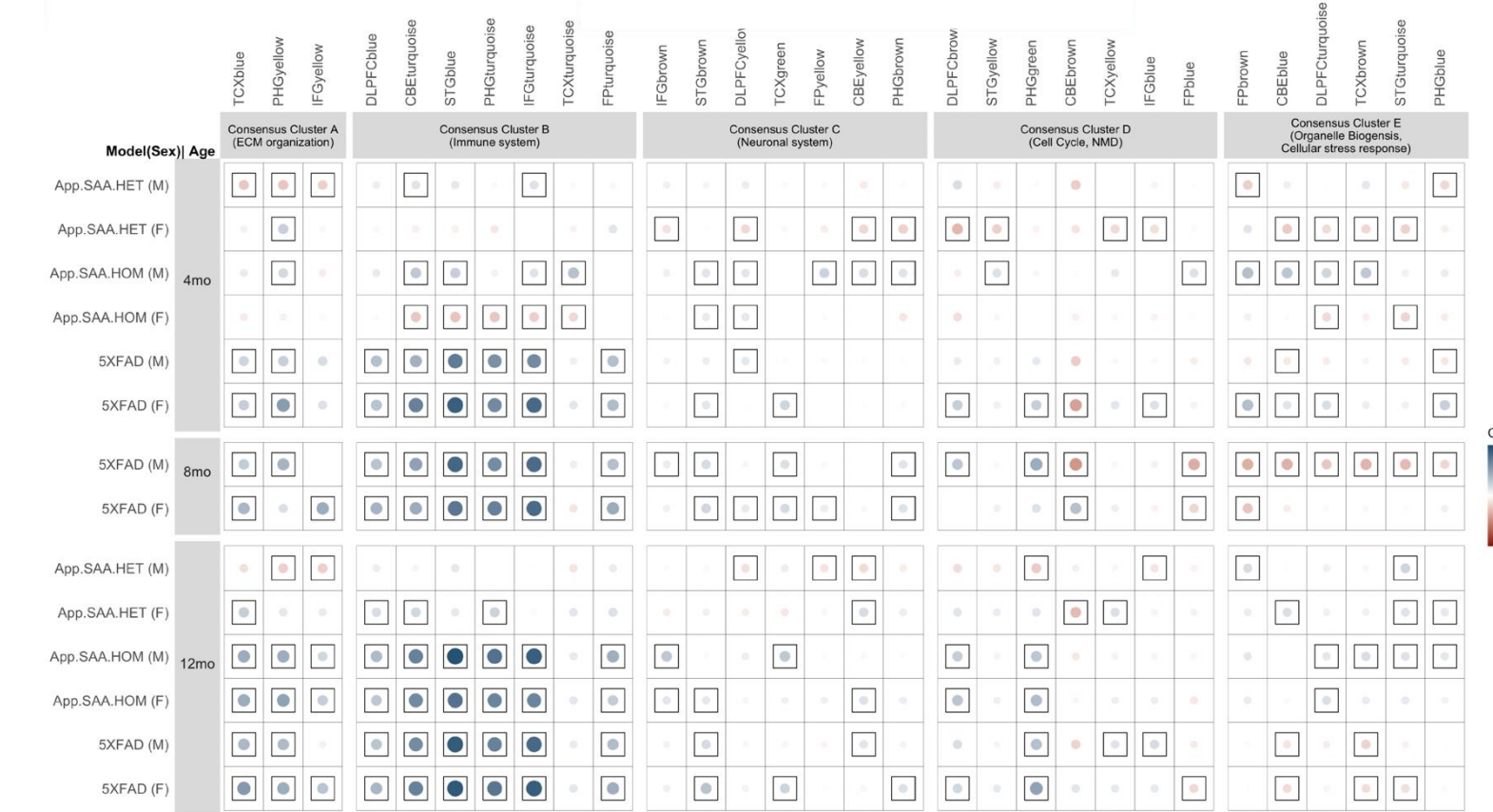


Figure 5. Whole brain transcriptomics shows an age-dependent increase in alignment to AMP-AD consensus modules (Preuss et al., 2020). By 4-months, the 5x^{FAD} mouse model has strong correlations that are sustained until 12 months while B6.APP^{SAA} mice show minimal correlation at 4-months, but by 12-months they show similar alignment to the 5x^{FAD} model at 4-months.

Positive correlation is denoted by blue dots and negative correlation by red dots. Size and intensity of indicate relative strengths of correlations. Boxes indicates statistical significance at p ≤ 0.05 .

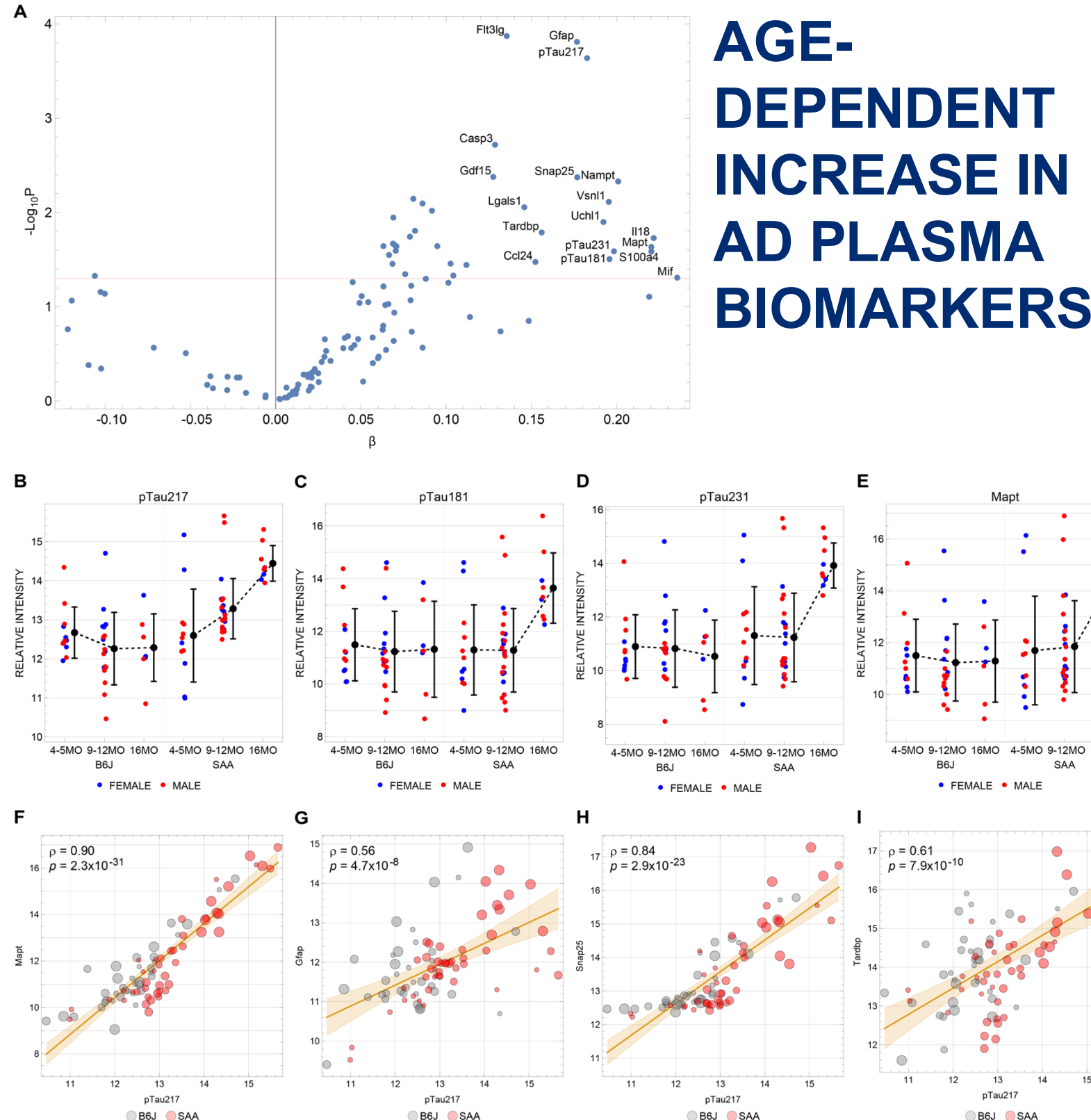


Figure 6. A. Age-by-genotype interaction effects for Alamar NULISA biomarker panel. All tau forms are affected. **B-E.** p-tau-217 is increased earliest, but all tau species are elevated by 16 months. **F-I.** Correlations of key biomarkers with p-tau-217. Dot size denotes animal age.

B6.APP^{SAA} CONCLUSIONS

B6.APP^{SAA} mice are a second-generation knock-in APP AD model. Pre-clinical characterization shows:

- Age-dependent accumulation of amyloid:**
 - Plaques appear by 4-months, accumulate through 12-months, and plateau until 19-months.
 - Soluble and insoluble A β ₄₀ and A β ₄₂ increase in a stepwise manner from 4- to 12-months.
 - There are no sex-specific effects in plaque accumulation in the B6.APP^{SAA} model.
- A β -associated pathology:** Age-dependent accumulation of A β plaques is accompanied by neuroinflammation and LAMP1.
- Neurodegeneration:** Significant loss of dendritic spines in CA1-to-mPFC neurons by 12-months that was exacerbated in proximity to plaques.
- Cognitive deficits:** Spine loss was accompanied by deficits in context-dependent memory.
- Translatability:** Transcriptomic alignment at 12-months-of-age with AMP-AD consensus modules from clinical results.
- Biomarkers:** Clinically relevant plasma biomarkers like Snap25, Gfap and Tardbp are age-associated.

FUTURE DIRECTIONS: B6.APP^{SAA} x MAPT GENE REPLACEMENT MODEL

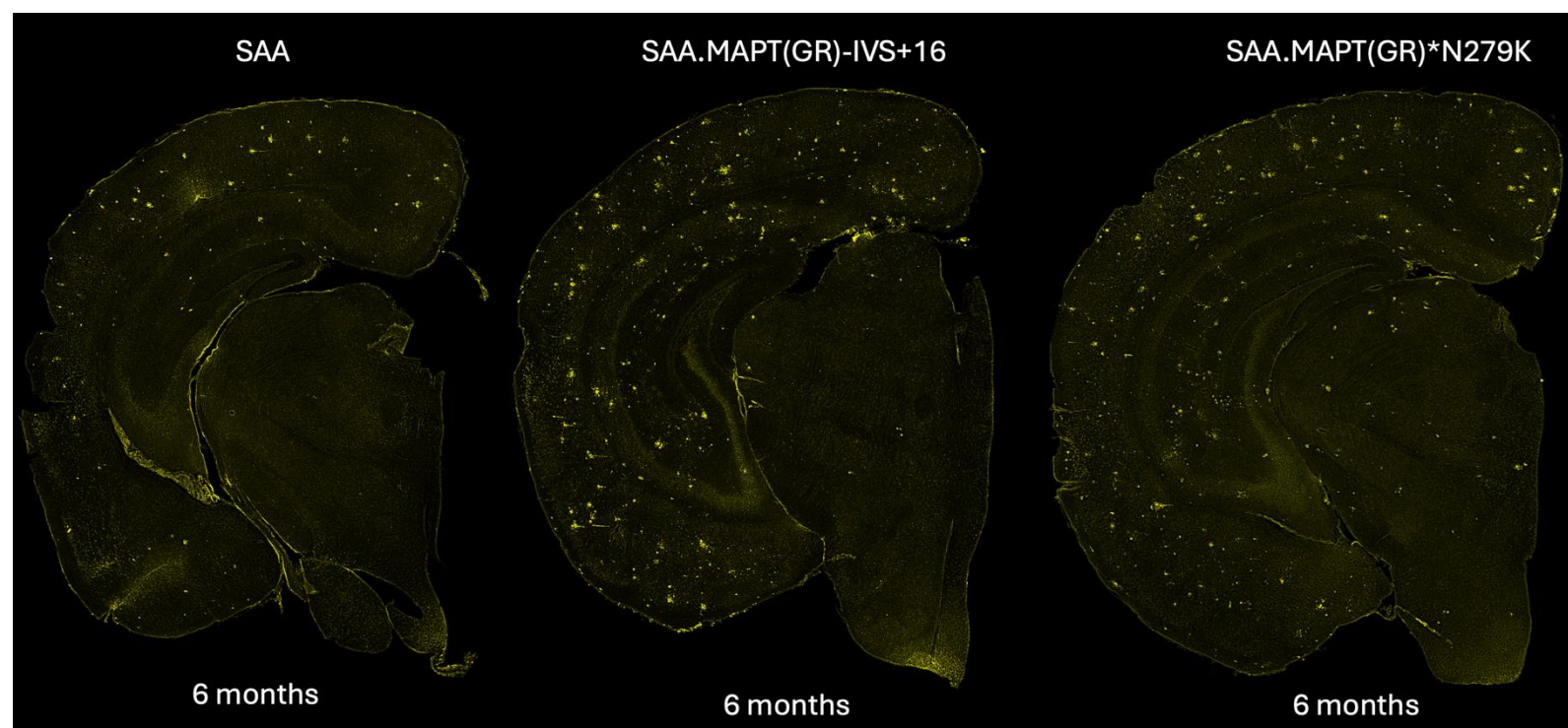


Figure 8. Representative images of 6E10 staining of B6.APP^{SAA} brains with either a SNP that alters splicing of exon 10 or the N279K risk variant have significantly more 6E10 staining than B6.APP^{SAA} control mice. We are currently analyzing age-associated proteomic, metabolomic, and lipidomic data sets as well as analysis of plasma biomarkers.

Model	Use:	JAX #
B6.APP ^{SAA}	Amyloid KI	034711
B6.hAPP	Amyloid KI control	033013
MAPT 10IVS+16 C>T	FTD	36664
MAPT(H1.0*)N279K-GR	FTD	35794
MAPT(H1.0)-GR	FTD control	035398

Since distinct genetic backgrounds can influence neuroinflammatory responses and vascular amyloid (CAA), we are moving the APP^{SAA} allele to various non-B6 backgrounds. We are also in the process of combining B6.APP^{SAA} mice with other AD risk alleles, including risk variants of *APOE*, *MAPT*, and *Klotho*.

ACKNOWLEDGEMENTS

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