



National Institute
on Aging

Sex-Specific Behavioral and Transcriptional Effects of Immune Checkpoint Signaling Blockade by PD1/PDL1 in APP/PS1 Mice

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Introduction

Alzheimer's disease (AD): Progressive cognitive decline, Amyloid- β accumulation, Neuroinflammation, Gliosis and synaptic dysfunction

Immune checkpoint signaling: PD-1/PD-L1 regulates peripheral immune responses, Emerging therapeutic target for AD

Sex differences: Sex influences AD progression and neuroimmune responses, yet sex-specific effects of PD-1/PD-L1 blockade remain poorly understood.

Knowledge gap: The impact of chronic PD-1/PD-L1 blockade in aged APP/PS1 mice and the role of sex-specific responses have not been well characterized.

Objective: Determine whether chronic PD-1/PD-L1 blockade improves behavior and alters neuroimmune gene expression in aged APP/PS1 mice.

Hypothesis: Chronic PD-1/PD-L1 blockade would attenuate AD-associated cognitive deficits and modulate neuroinflammatory, glial, metabolic, and sex-dependent molecular pathways in aged APP/PS1 mice.

Methods

Animals

9-month-old mice
WT | APP/PS1

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Monthly injections

4 months (*i.p.*)

IgG (0.017 mg/g) = control (CNT)
anti-PD-1 (0.017 mg/g)
anti-PDL1 (0.05 mg/g) } Treated (TRT)

Behavior assessment

Morris water maze
Elevated plus maze
Cross Maze

Gene Expression Analysis

Pre-frontal cortex collection

microglial activation
neuroinflammation
astrocyte reactivity
metabolism
vascular function
senescence
sex hormone signaling.

Statistical Analysis

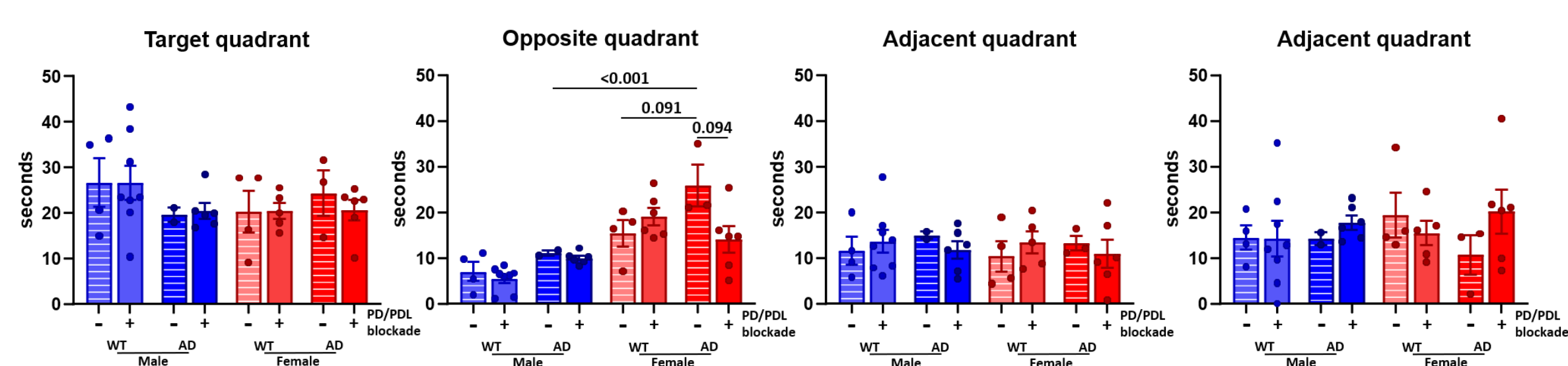
Behavioral data were analyzed with 3-way ANOVA (sex, treatment and strains as factors) and molecular data were analyzed using 2-way ANOVA to evaluate the effects of strain and treatment, followed by Sidak post hoc comparisons.

Results

Sex-Dependent Modulation of Neuroimmune Gene Expression Following Chronic PD-1/PD-L1 Blockade in APP/PS1 Mice

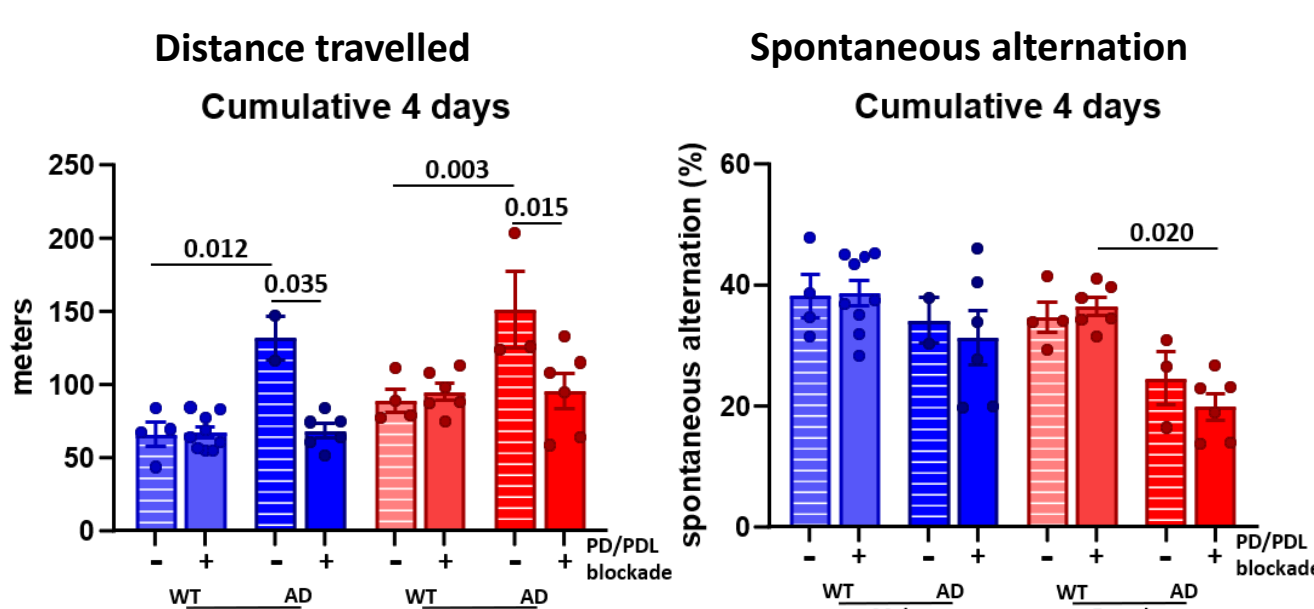
PD-1/PD-L1 treatment partially restored spatial search strategy in female APP/PS1 mice.

Spatial Memory - Morris Water Maze – Probe test



PD-1/PD-L1 Blockade Restores Exploratory Activity Without Altering Alternation

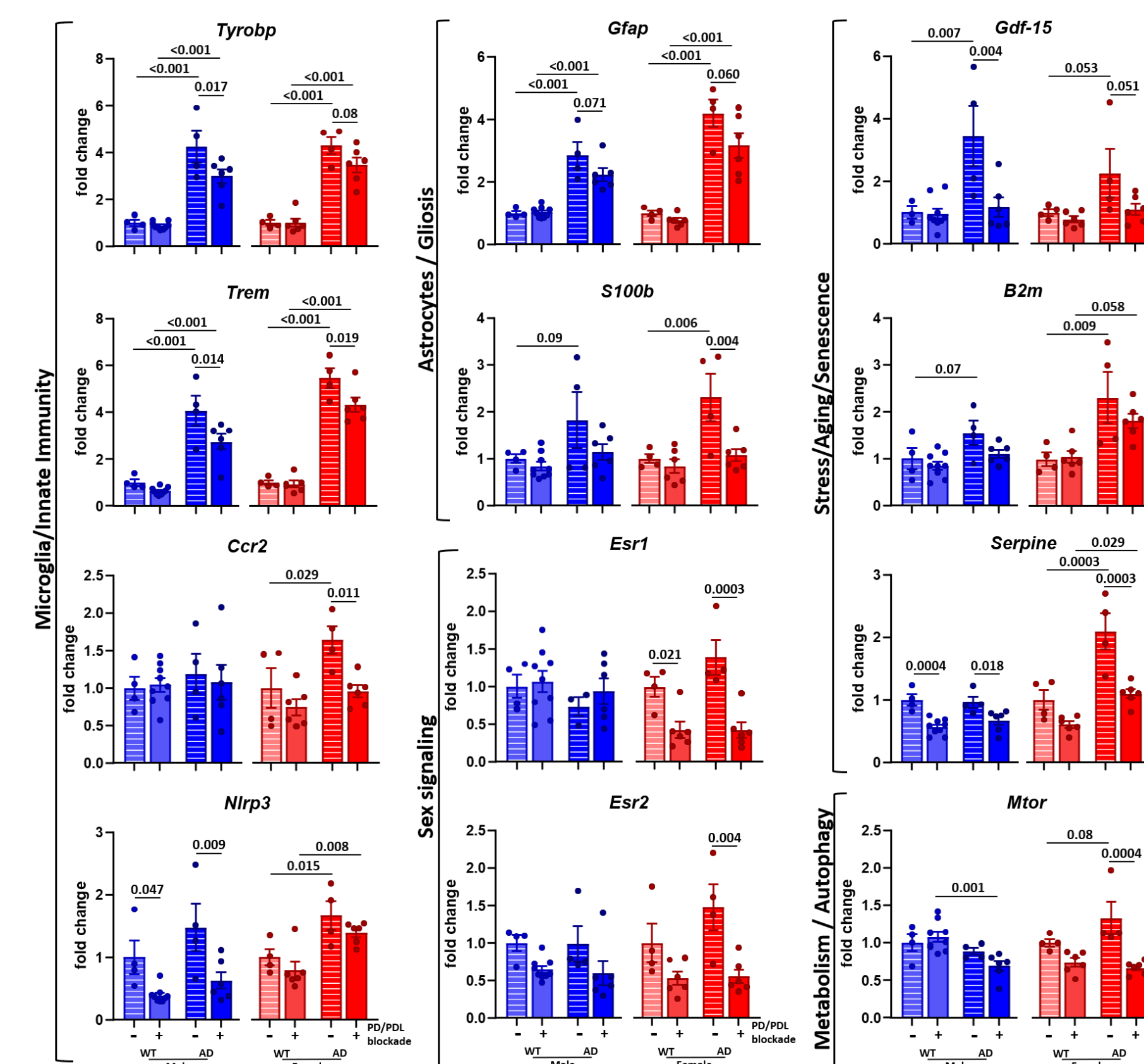
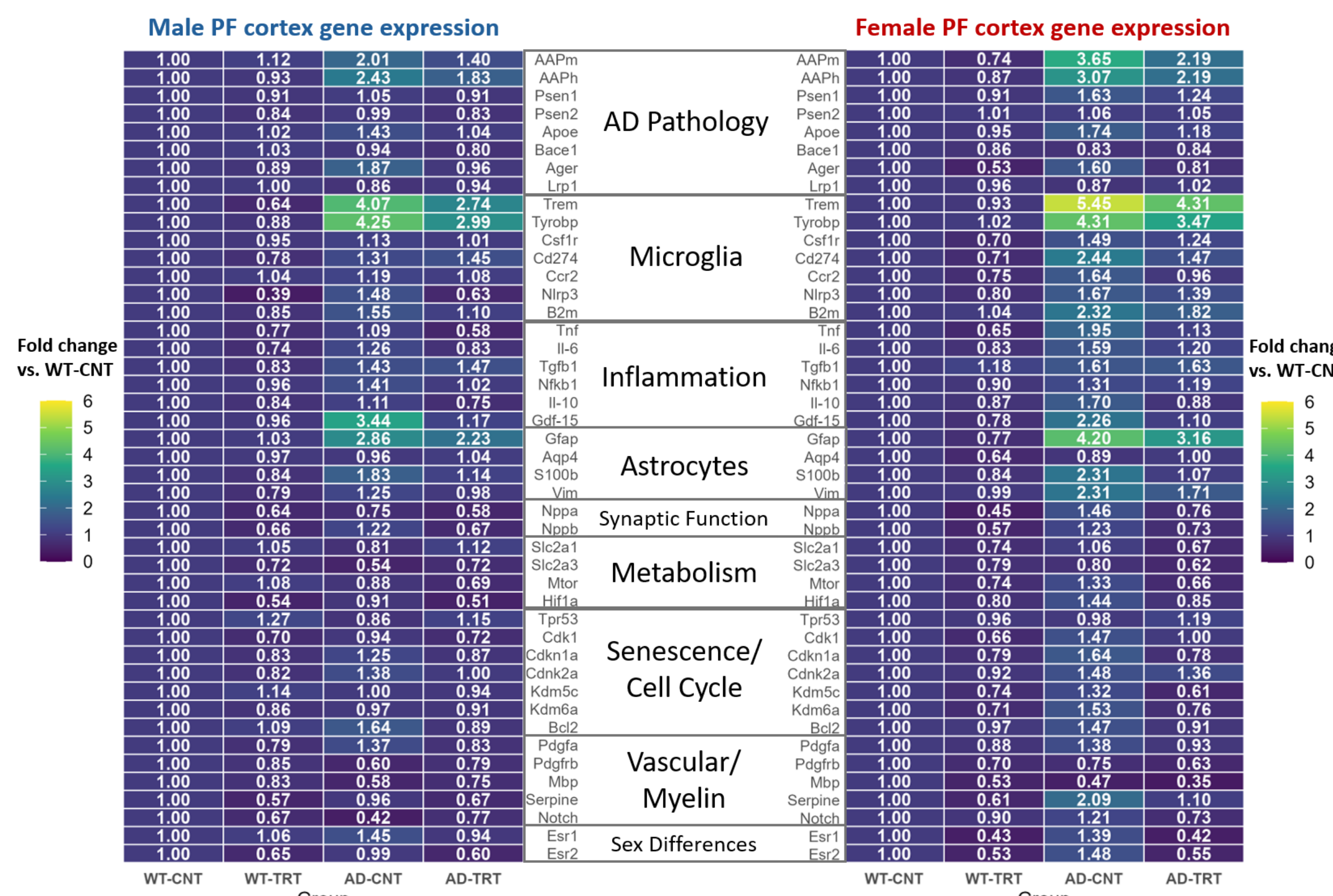
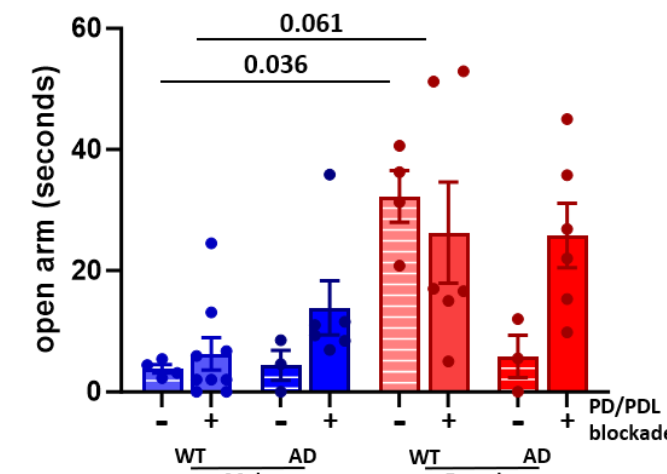
Spatial Memory - Cross Maze



Chronic PD-1/PD-L1 Blockade Reduces Anxiety-Like Behavior

Anxiety-like Behavior - Elevated cross Maze

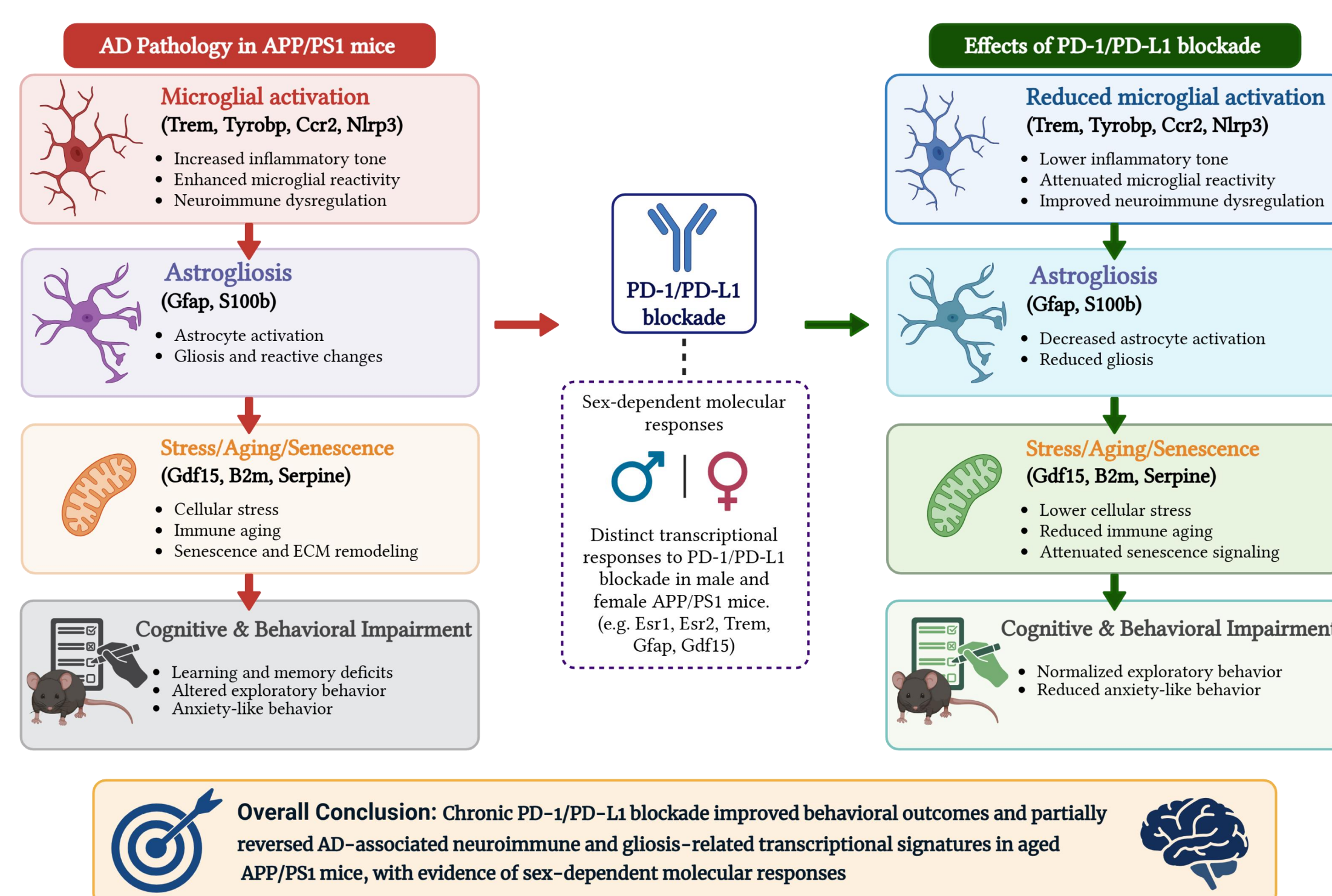
Time spent in open arm



Conclusions

- Chronic PD-1/PD-L1 blockade improved behavioral outcomes in aged APP/PS1 mice.
- Treatment attenuated AD-associated neuroimmune and gliosis-related transcriptional signatures in the prefrontal cortex.
- Microglial (*Trem*, *Tyrobp*, *Ccr2*), astrocytic (*Gfap*), and stress-related (*Gdf15*) pathways were particularly responsive to treatment.
- Male and female mice exhibited distinct molecular responses, suggesting sex-dependent mechanisms.
- These findings support immune checkpoint modulation as a potential co-therapeutic strategy for Alzheimer's disease.

PD-1/PD-L1 Blockade Attenuates Neuroinflammation and Improves Behavior in APP/PS1 mice



Future Directions

- Quantify amyloid burden and glial morphology to determine whether transcriptional changes are accompanied by neuropathological improvement.
- Investigate sex-specific mechanisms underlying treatment responsiveness.

References

- Rosenzweig N, Dvir-Szternfeld R, Tsitsou-Kampeli A, Keren-Shaul H, Ben-Yehuda H, Weill-Raynal P, et al. PD-1/PD-L1 checkpoint blockade harnesses monocyte-derived macrophages to combat cognitive impairment in a tauopathy mouse model. *Nat Commun.* 2019;10(1):465.
- Marsh SE, Abud EM, Lakatos A, Karimzadeh A, Yeung ST, Davtyan H, et al. The adaptive immune system restrains Alzheimer's disease pathogenesis by modulating microglial function. *Proc Natl Acad Sci U S A.* 2016;113(9):E1316-25.
- Matsubara Y, Gonzalez L, Kiwan G, Liu J, Langford J, Gao M, et al. PD-L1 (Programmed Death Ligand 1) Regulates T-Cell Differentiation to Control Adaptive Venous Remodeling. *Arterioscler Thromb Vasc Biol.* 2021;41(12):2909-22.

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AAIC 26

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