

Neuron Death Increases with Age, AD, MCI, Down Syndrome and is Halted By GM-CSF, with Implications for Other Disorders

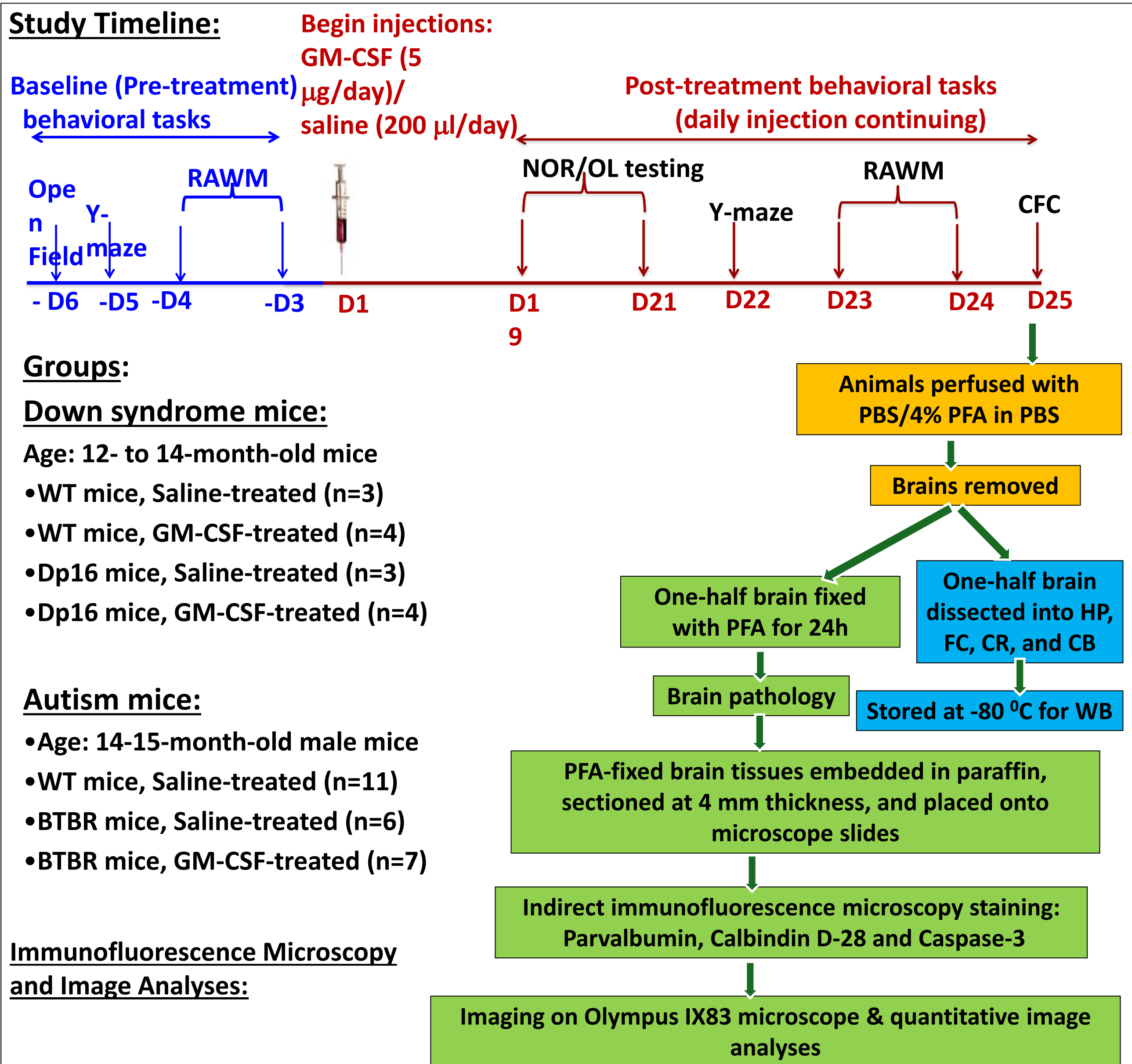
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Introduction

Treatment with granulocyte-macrophage colony-stimulating factor (GM-CSF), an FDA approved immune system modulator used for other conditions, shows benefits in animal models of Alzheimer's disease (AD), Parkinson's disease (PD), Down syndrome (DS), stroke, West Nile Virus, and normal aging, and in clinical trials of participants with mild-to-moderate AD or PD. We previously reported that GM-CSF treatment of the Dp16 mouse model of DS rescues cognitive deficits, reduces astrogliosis, and increases the number of hippocampal interneurons (PMID: 35307513). Our recently published data show that the elevated plasma concentrations of UCH-L1 in participants with mild-to-moderate AD in our phase II clinical trial were significantly reduced after treatment with recombinant human GM-CSF (sargamostim) to levels of young children, and treatment with recombinant rat GM-CSF significantly reduced hippocampal neuronal death in AD rats (PMID: 41421353). Aging leads to an increased risk of developing AD and age-associated cognitive decline. People with DS are especially susceptible and experience cognitive challenges throughout their lives, and every person with DS has AD brain neuropathology by age 40 and the majority develop AD dementia by age 50-60. Approximately 20% of children with DS also have autism and show characteristic behavioral/neuropathological symptoms. Our recent cross-sectional study of typical, community dwelling, karyotypically normal, healthy control participants showed that plasma concentrations of UCH-L1, a marker of neuronal cell loss/death, and NfL, a marker of axon damage, show exponential increases with age starting in early childhood (PMID: 39072024). To understand the age-associated neurodegeneration and inflammation in DS, we measured plasma levels of UCH-L1, NfL, and GFAP in a cohort of 316 participants with DS and compared them to a cohort of 317 karyotypically normal (disomy 21) healthy control participants across the lifespan. We also determined the effects of GM-CSF treatment on neuronal loss by assessing parvalbumin and calbindin-28 staining, and on neuronal cell death by Caspase-3 staining in the brains of Dp16 mice. Finally, we examined behavioral phenotypes and brain pathology in a mouse model of autism spectrum disorder (ASD) after GM-CSF or saline treatments.

Methods



Plasma samples from participants with DS:

Plasma concentrations of UCH-L1, NfL, and GFAP were assessed in 316 participants with DS age 2-60 using the Quanterix SIMOA platform and compared to previous findings from karyotypically normal, disomic, healthy, age-matched controls.

Results 1: DS mice Treated With GM-CSF or Saline

1. GM-CSF Treatment Significantly Increases the Number of Parvalbumin (PV) Positive Purkinje Cells in the Cerebellum of Dp16 Mice.

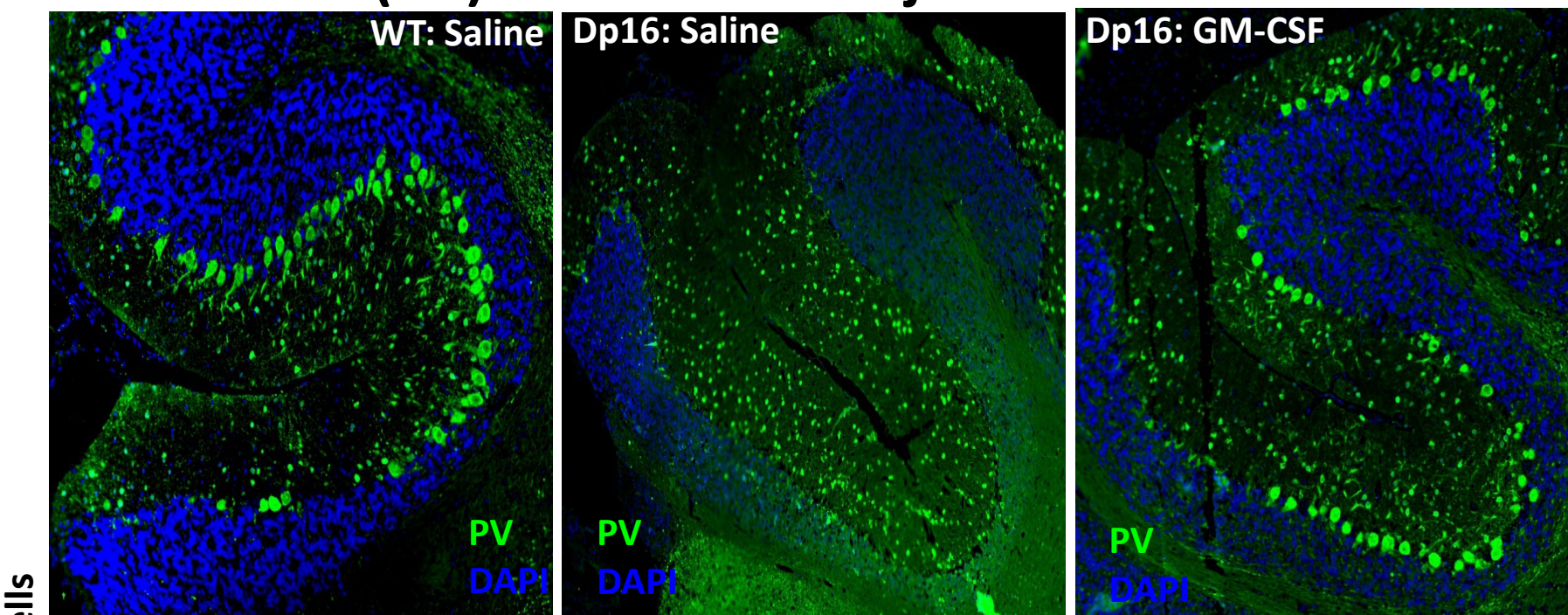


Figure 1. Indirect immunofluorescence microscopy images show parvalbumin (PV)-positive Purkinje cells in the cerebellum of Dp16 mice treated with saline or GM-CSF. Saline-treated Dp16 mice showed a reduced number of PV-positive Purkinje cells in the cerebellum compared to wild-type (WT) mice. In contrast, treatment of Dp16 mice with GM-CSF led to a significant increase in the number of PV-positive Purkinje cells in the cerebellum compared to saline-treated Dp16 mice (n=3-4 mice/group).

3. GM-CSF Treatment Significantly Reduces Neuronal Death in the CA1 and Dentate Gyrus in the Hippocampi of Dp16 Mice

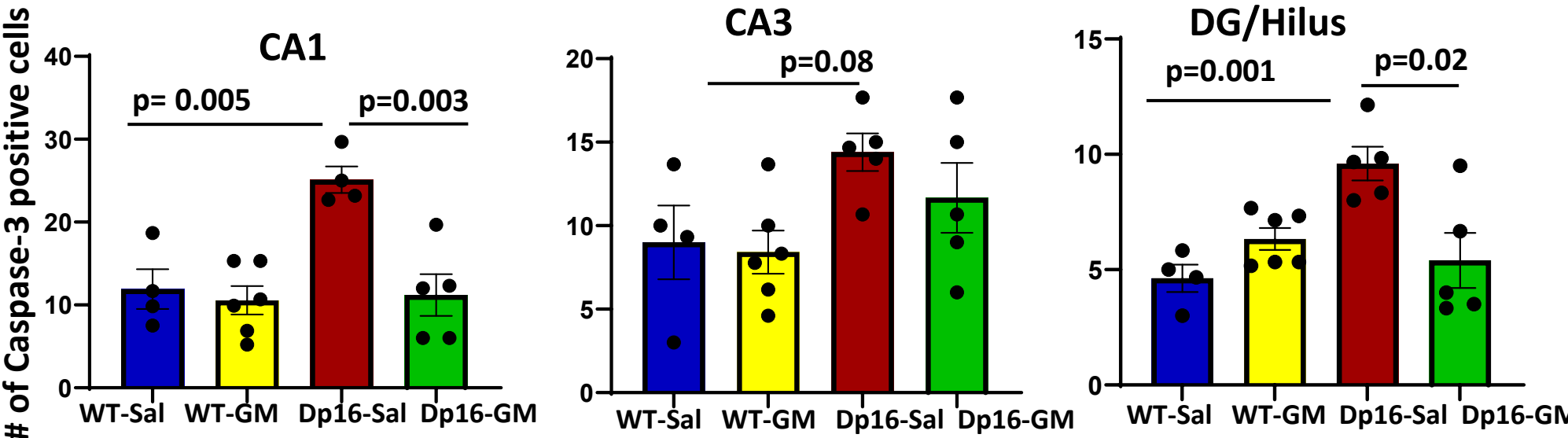


Figure 3. The numbers of apoptotic cells (Caspase-3 positive) were counted in the CA1, CA3, and dentate gyrus (DG/hilus) regions of the hippocampus in WT and Dp16 mice treated with saline or GM-CSF. Saline-injected Dp16 mice show significantly higher numbers of Caspase-3-positive cells in the CA1 and DG/hilus regions compared to WT mice. In contrast, treatment of Dp16 mice with GM-CSF significantly reduced the number of Caspase-3-positive cells in these hippocampal subregions compared to saline-injected Dp16 mice (n=4-6 mice/group).

2. GM-CSF Treatment Significantly Increases the Number of Parvalbumin-Positive Interneurons in the Frontal Cortex of Dp16 Mice.

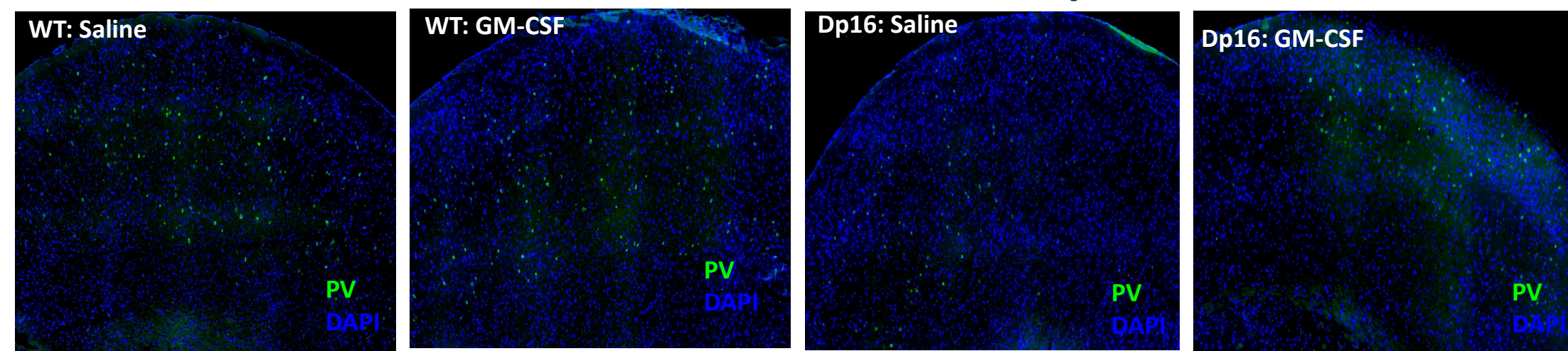


Figure 2. Immunofluorescence microscopy images show parvalbumin (PV)-positive interneurons in the frontal cortex (FC) of Dp16 mice treated with saline or GM-CSF. Saline-treated Dp16 mice showed a significantly reduced number of PV interneurons in the FC compared to WT mice. In contrast, treatment of Dp16 mice with GM-CSF led to a significant increase in the number of PV-positive interneurons in the FC compared to saline-treated Dp16 mice (n=3-4 mice/group).

4. Increased Levels and Rates of Age-Associated Neurodegeneration/Inflammation in the Plasma of Individuals with DS

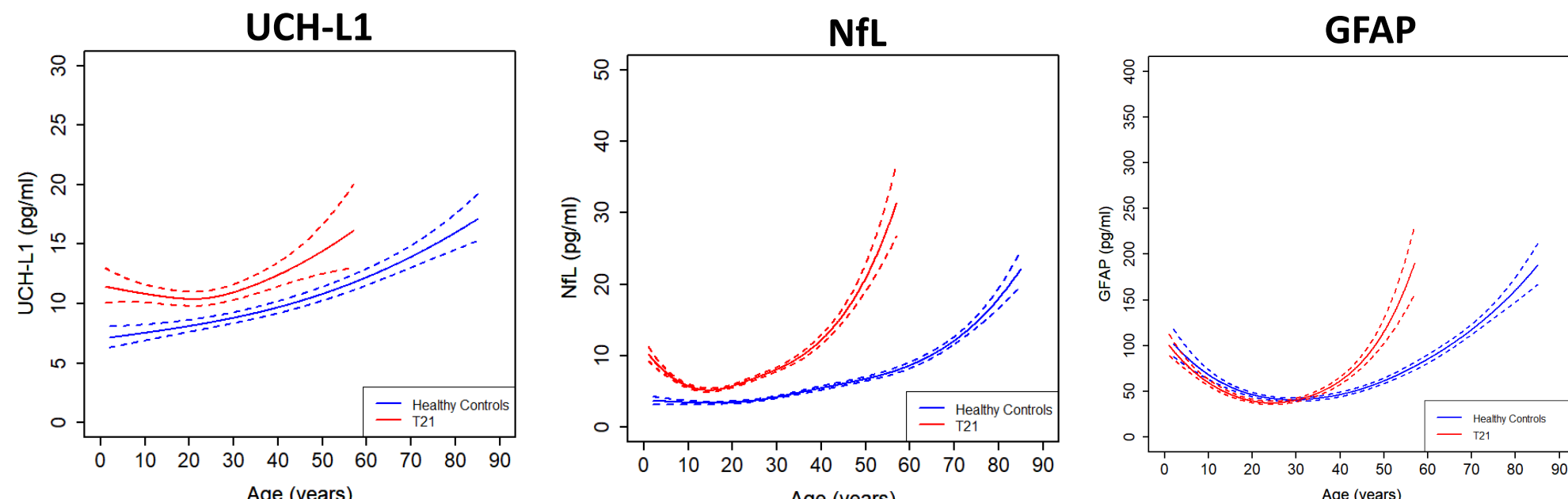


Figure 4. Plasma concentrations of UCH-L1, a marker for neuronal cell death, NfL, a marker of neuron axonal damage, and GFAP, a marker of neuroinflammation, were assayed using the Quanterix SIMOA® platform in plasma samples from a cohort of 317 karyotypically normal (disomy 21) healthy control participants across the lifespan and from a cohort of 316 participants with DS (T21). The levels of plasma UCH-L1 (neuronal loss) and NfL (axonal damage) rise exponentially throughout life from early childhood, and plasma levels of GFAP (inflammation/astrogliosis) rise exponentially from age 40 in karyotypically normal, healthy control participants. The average concentrations of plasma biomarkers of neuronal death/loss (UCH-L1), axonal damage (NfL), and inflammation (GFAP) are higher in participants with DS/T21 and increase exponentially and at faster rates with increasing age compared to karyotypically normal, healthy control participants.

Results 2: Autism mice Treated With GM-CSF or Saline

5. GM-CSF Treatment Improves Cognition in a Mouse Model of Autism.

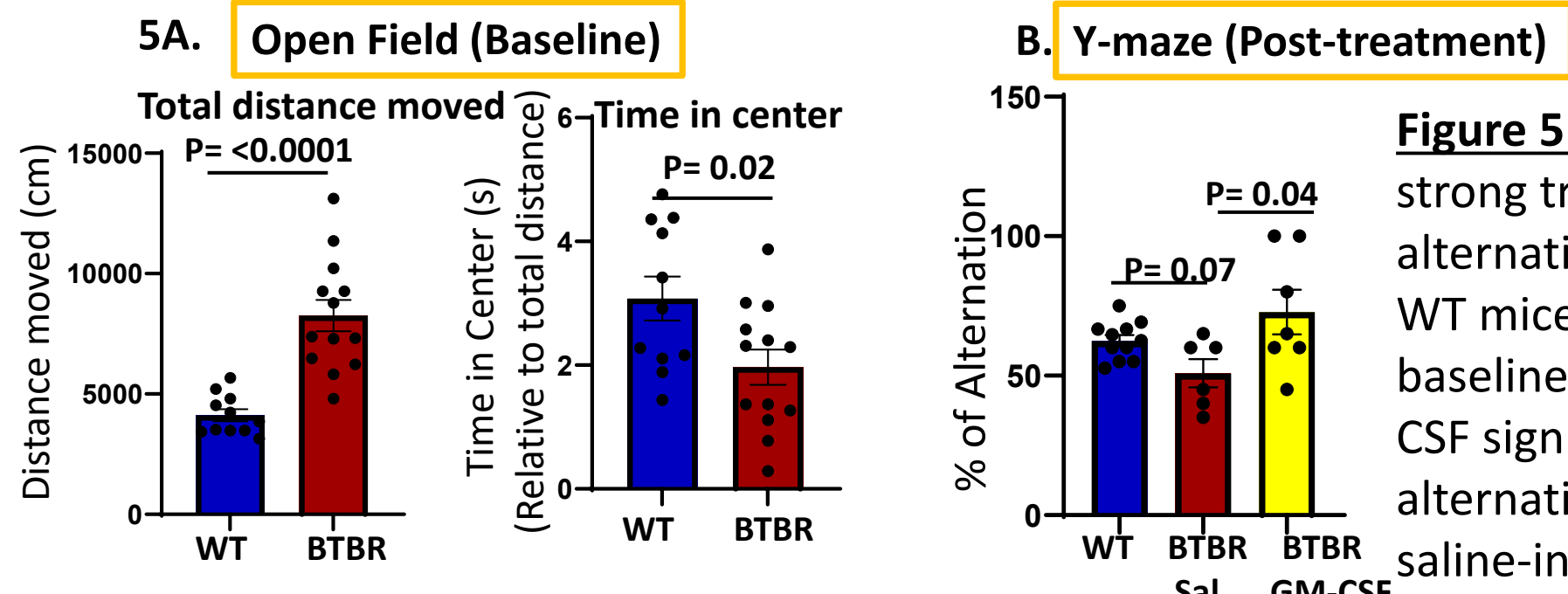


Figure 5A. 14-16-month-old male BTBR mice showed a significant increase in total distance traveled relative to wild-type control mice (WT) in the open field test at baseline. BTBR mice also spend significantly less time in the center compared to WT mice (n=11-13).

Figure 5B. BTBR mice showed a strong trend to having a lower alternation rate compared to WT mice in the Y-maze at baseline. Treatment with GM-CSF significantly increased the alternation rate compared to saline-injected BTBR mice (n=6-11).

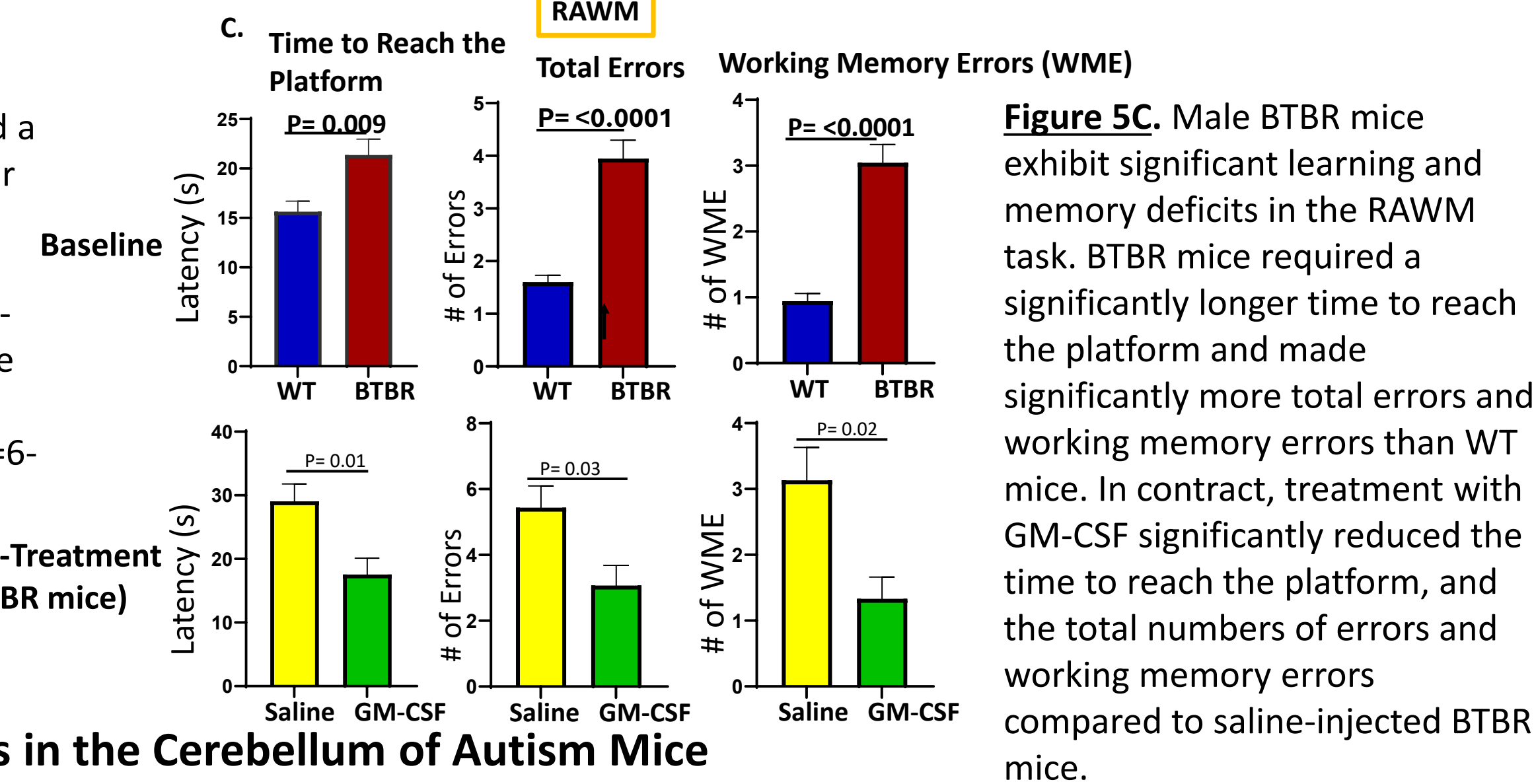


Figure 5C. Male BTBR mice exhibit significant learning and memory deficits in the RAWM task. BTBR mice required a significantly longer time to reach the platform and made significantly more total errors and working memory errors than WT mice. In contrast, treatment with GM-CSF significantly reduced the time to reach the platform, and the total numbers of errors and working memory errors compared to saline-injected BTBR mice.

6. GM-CSF Treatment Improves Purkinje Cell Morphological Abnormalities in the Cerebellum of Autism Mice

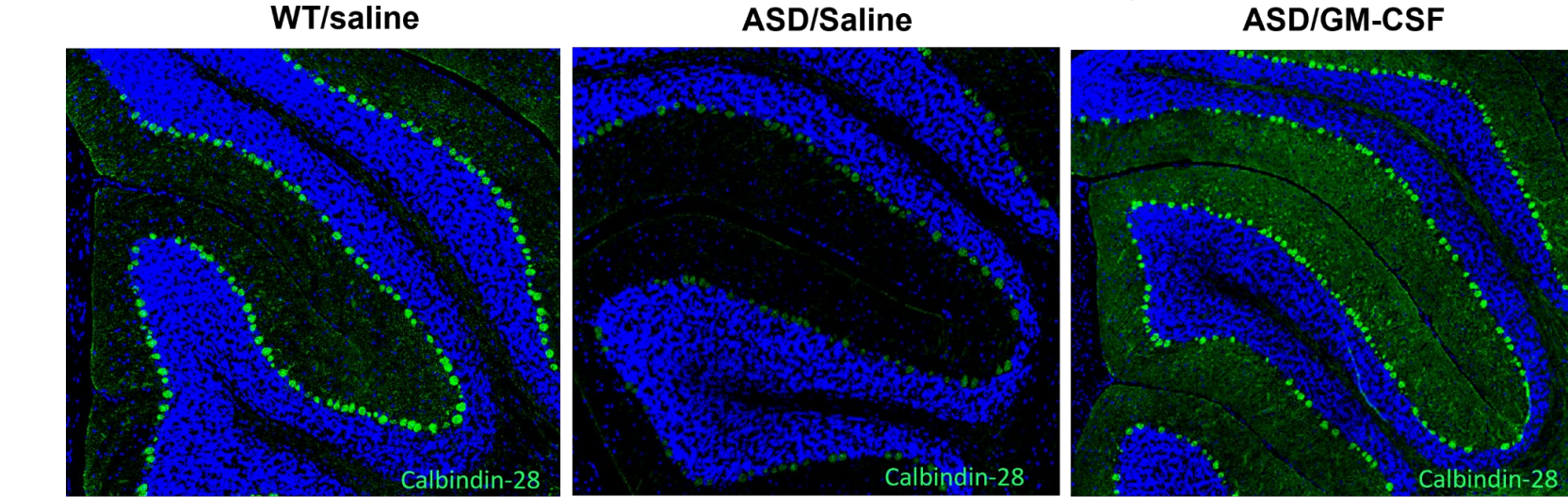


Figure 6. Immunofluorescence microscopy images show Calbindin-positive Purkinje cells in the cerebellum of WT (saline-injected) and autism (BTBR mice injected with saline or GM-CSF) mice. Saline-injected autism mice showed a reduced number of Calbindin-positive Purkinje cells in the cerebellum compared to saline injected WT mice. In contrast, autism mice treated with GM-CSF showed increased number of Calbindin-positive Purkinje cells compared to saline-injected autism mice.

Conclusions

1. GM-CSF treatment rescues the abnormal Purkinje cell morphology and interneurons in the brains of DS mice.
2. GM-CSF treatment rescues neuronal cell death/apoptosis in the brains of DS mice.
3. The concentrations of plasma UCH-L1 (neuronal loss) and NfL (axonal damage) rise exponentially throughout life from early childhood, and plasma levels of GFAP (inflammation/astrogliosis) rises exponentially from age 40 in karyotypically normal, healthy control participants.
4. Levels of plasma biomarkers of neuronal loss (UCH-L1), axonal damage (NfL), and inflammation/astrogliosis (GFAP) are higher in participants with DS and increase exponentially and faster with age compared to karyotypically normal, healthy control participants.
5. GM-CSF treatment improves cognition and restores morphological abnormalities of Purkinje cells in autism mice.