YMTHE, Volume *29*

Supplemental Information

Waning efficacy in a long-term AAV-mediated gene therapy study in the murine model of Krabbe disease

Gregory J. Heller, Michael S. Marshall, Yazan Issa, Jeffrey N. Marshall, Duc Nguyen, Emily Rue, Koralege C. Pathmasiri, Miriam S. Domowicz, Richard B. van Breemen, Leon M. Tai, Stephanie M. Cologna, Stephen J. Crocker, Maria I. Givogri, Mark S. Sands, and Ernesto R. Bongarzone

Supplementary Material

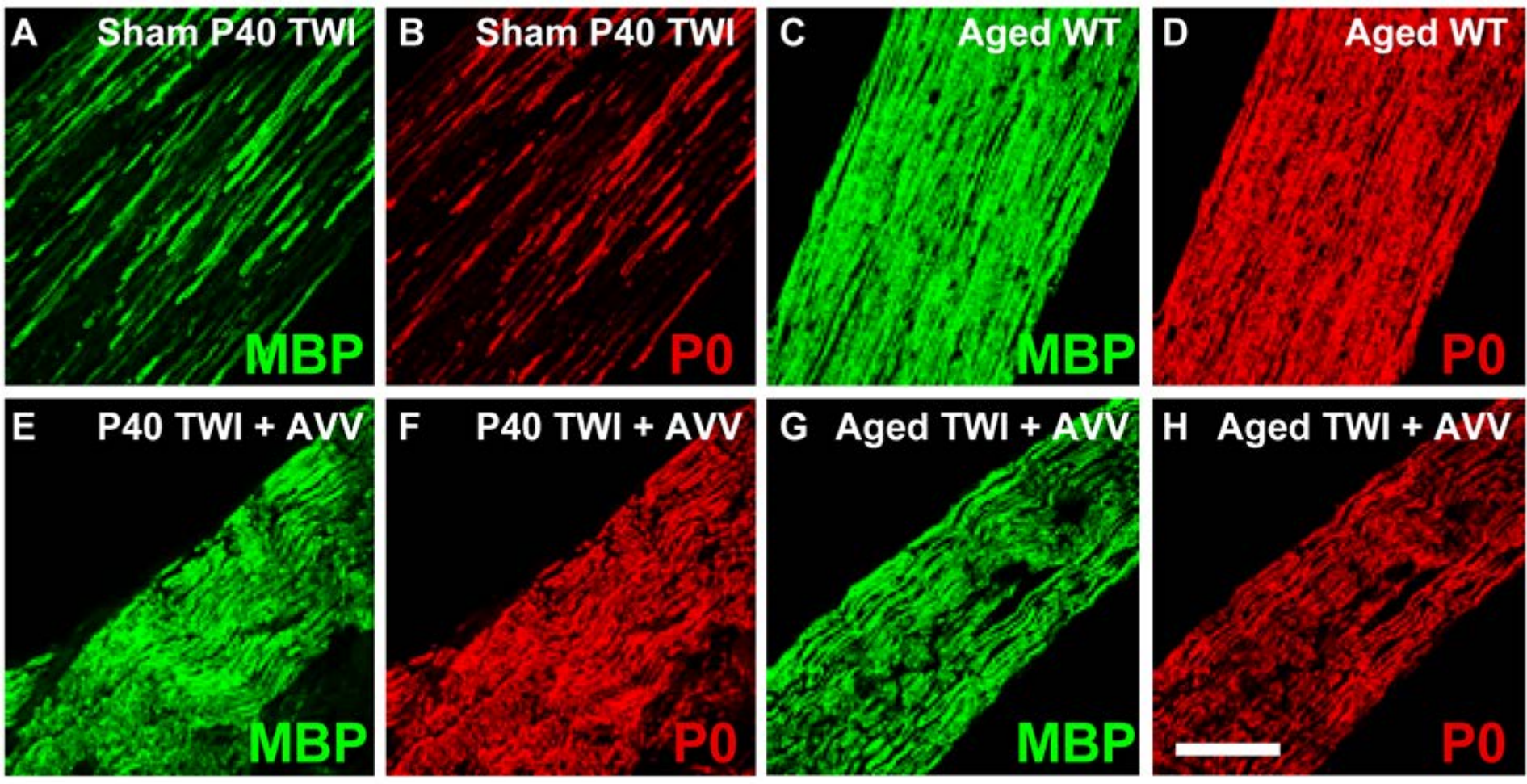
S1. AAV therapy protects PNS myelination. Sciatic nerve tissue was stained with MBP (green) and P0 (red) to examine myelination within the PNS. Myelination in sciatic nerves at P40 and in aged TWI+AVV was largely preserved in TWI+AAV (**E-H**) as compared to WT (**C,D**). Sham P40 TWI nerves (**A-B**) show extensive myelin loss. Scale bar A-H: 1 mm.

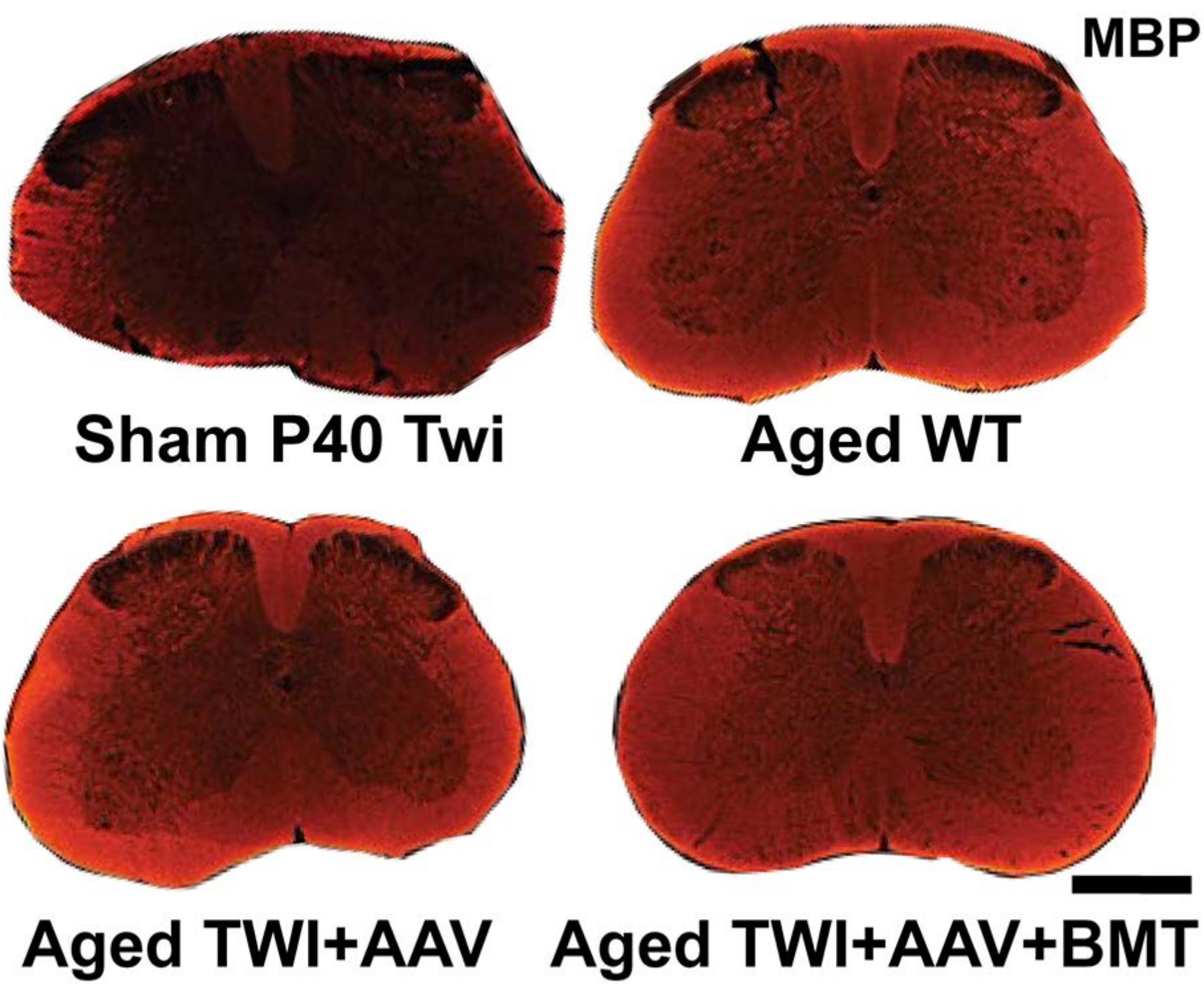
S2. Spinal cords of aged treated TWI are devoid of demyelinating plaques. Serial sections of spinal cord were stained with MBP (red). Pathological analysis of focal lesions or significant pathological changes were not detected in cords of aged TWI+AAV and TWI-AAV+BMT. Scale bar 0.5 mm.

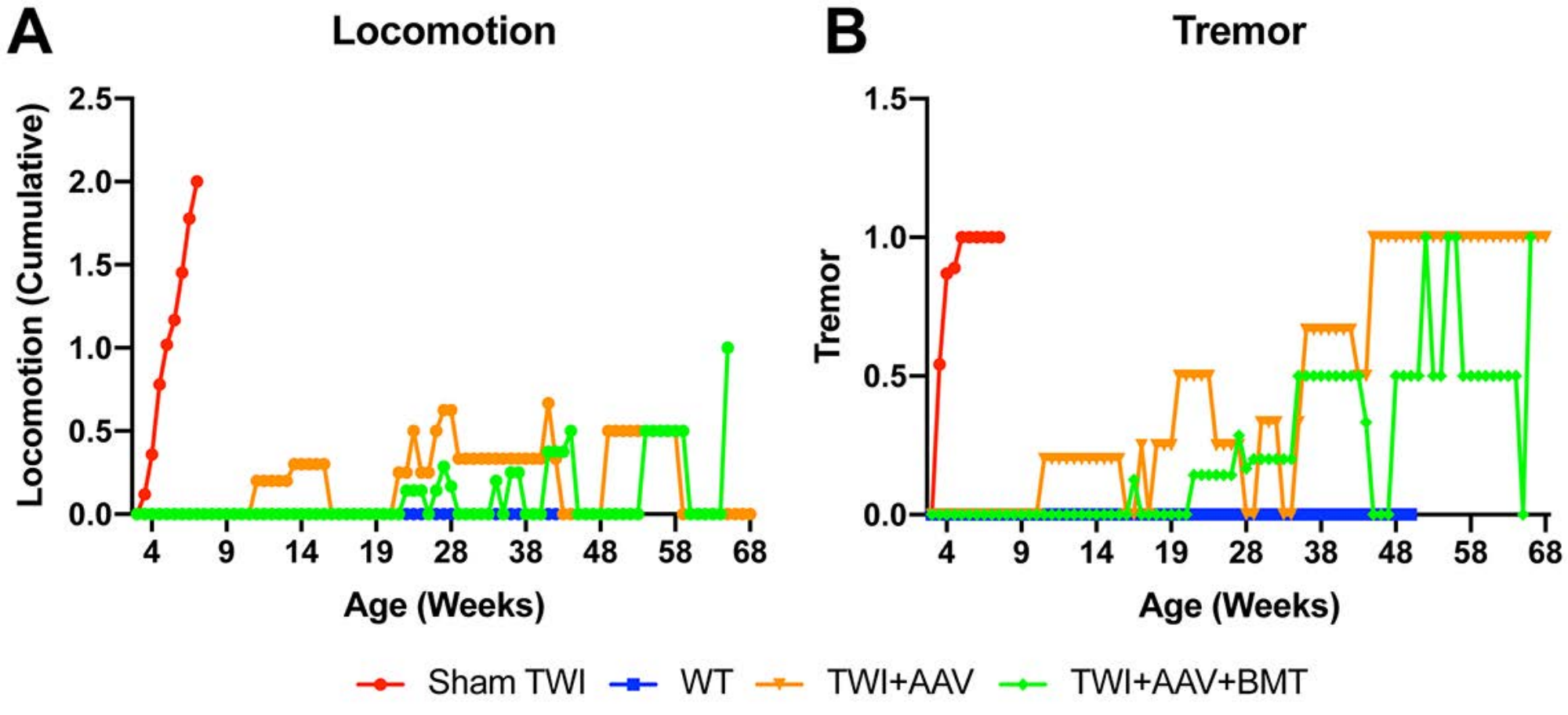
S3. Locomotor ability and Tremor in aged treated TWI. Locomotor function (**A**) was measured, and presence of tremor (**B**) was determined twice a week until 20 weeks and then once per week thereafter. Locomotor ability was scored as follows: normal-0, waddling-1, partial paralysis-1.5, and full paralysis-2. Tremor was scored as no tremor-0 or tremor present-1. This revealed that tremor develops in most treated animals at 16 weeks and locomotor ability shows signs of decline at 20 weeks. (sham TWI n=25, WT n=7, TWI-AAV n=5, TWI-AAV+BMT n = 8)

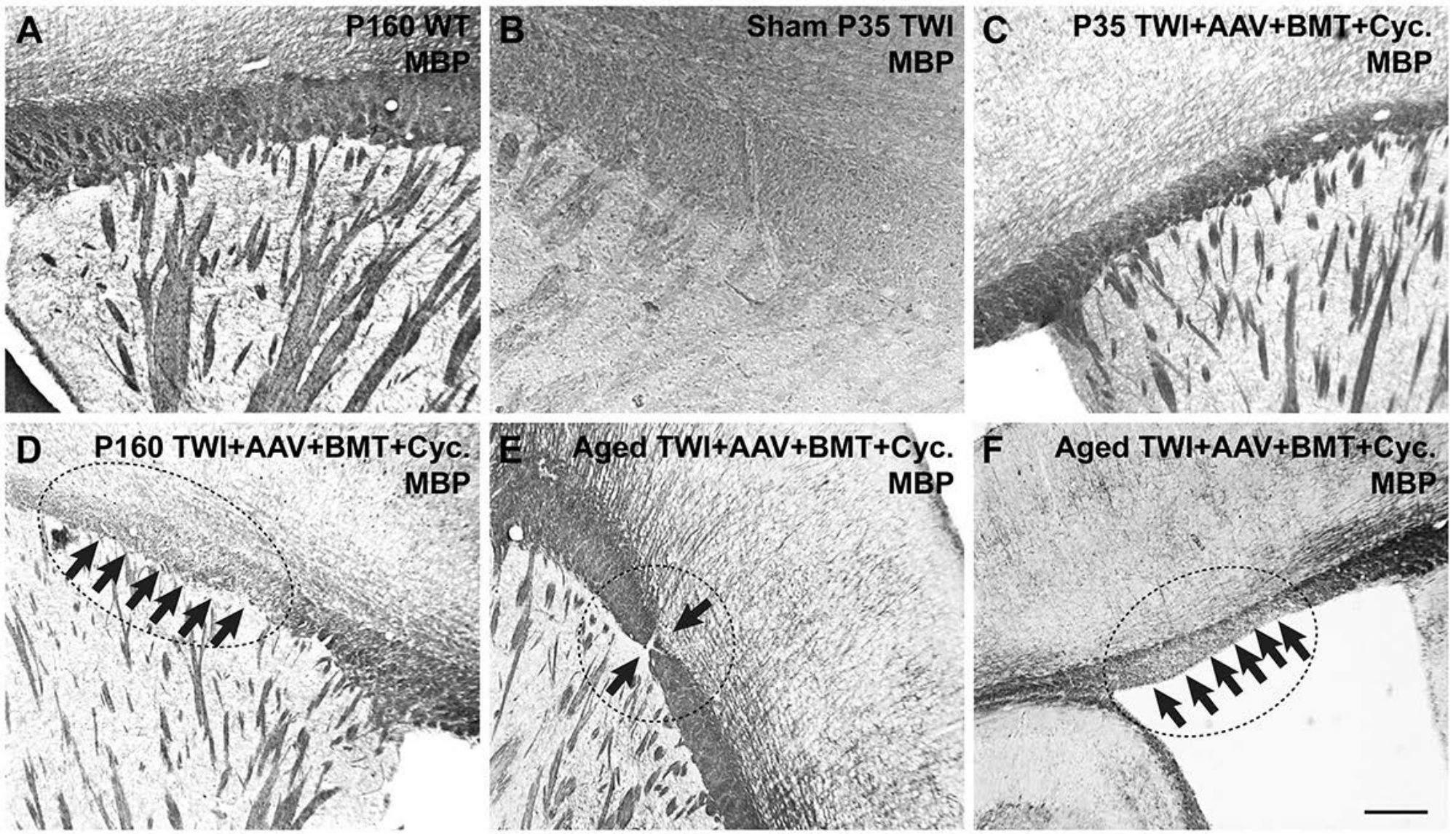
S4. Interlaboratory cross-examination of late-onset multi-focal demyelination in aged treated TWI. Tissue from P160 WT (**A**), sham P35 TWI (**B**), and TWI treated with a combination of AAV9-GALC, BMT, and L-Cycloserine (Cyc) (**C-F**) were immunostained for MBP. At P35 (**C**) treated animals exhibit healthy myelination comparable to the WT. At P160 (**D**) and later aged (>400 days of age) timepoints (**E,F**) the animals show multifocal demyelination (dotted circle and arrows). Images provided by Dr. Marks Sands. Scale Bar A-F: 50 μm.

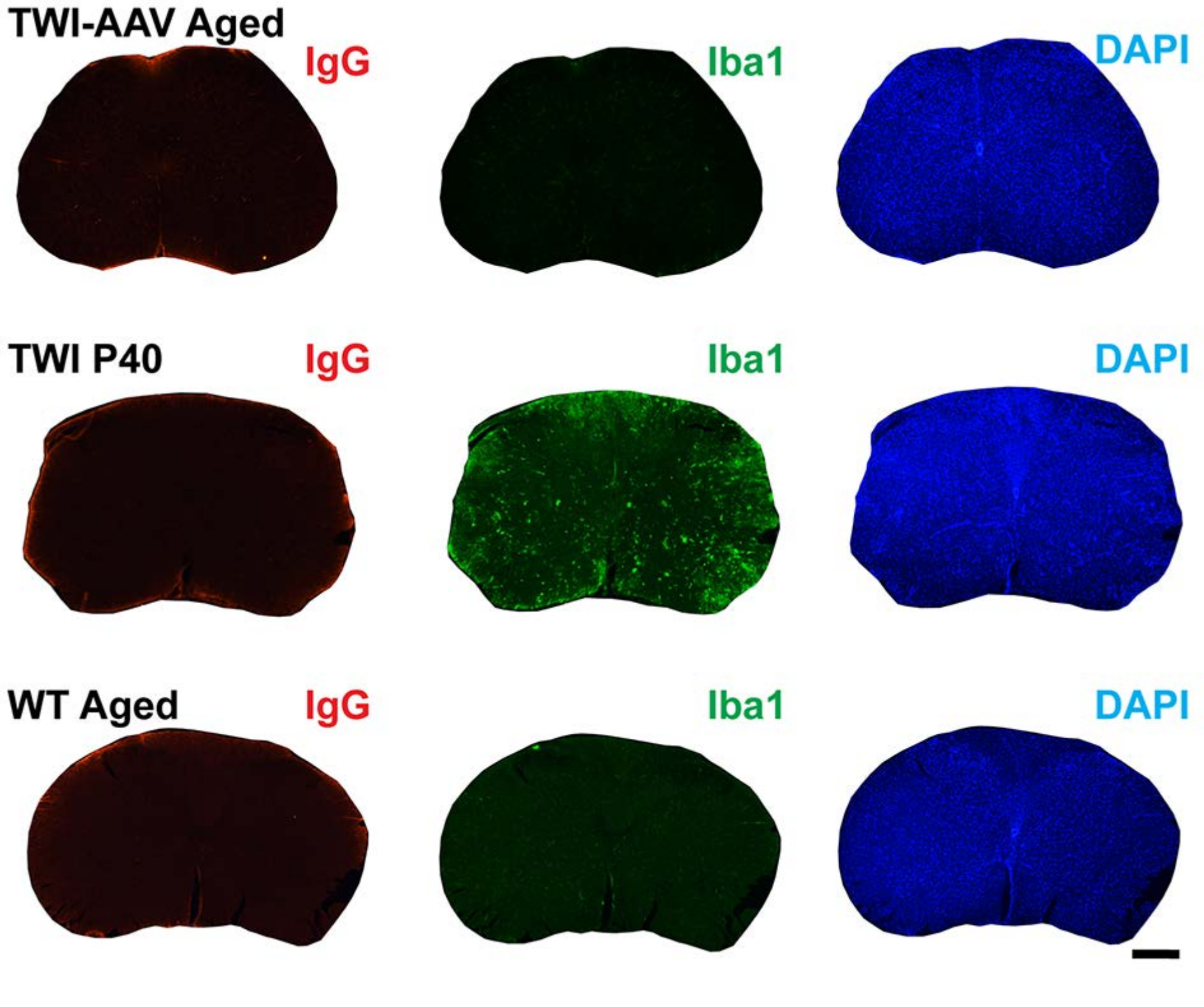
S5. Spinal cords from treated TWI lack leakage of plasma proteins. Serial spinal cord sections were stained for IgG (red) (a general read out protein of plasma extravasation) and IBA1 (green). IgG and microglia were undetectable within spinal cords of aged TWI+AAV. Sham TWI and aged WT also exhibited no IgG but, in contrast, sham TWI contained extensive microgliosis. Scale bar 0.5 mm.


A Sham P40 TWI
MBP
B Sham P40 TWI
P0
C Aged WT
MBP
D Aged WT
P0
E P40 TWI + AVV
MBP
F P40 TWI + AVV
P0
G Aged TWI + AVV
MBP
H Aged TWI + AVV
P0


MBP
Sham P40 Twi
Aged WT
Aged TWI+AAV
Aged TWI+AAV+BMT


A
Locomotion
Locomotion (Cumulative)
2.5
2.0
1.5
1.0
0.5
0.0
4
9
14
19
28
38
48
58
68
Age (Weeks)
B
Tremor
Tremor
1.5
1.0
0.5
0.0
4
9
14
19
28
38
48
58
68
Age (Weeks)
Sham TWI
WT
TWI+AAV
TWI+AAV+BMT


A
P160 WT
MBP
B
Sham P35 TWI
MBP
C
P35 TWI+AAV+BMT+Cyc.
MBP
D
P160 TWI+AAV+BMT+Cyc.
MBP
E
Aged TWI+AAV+BMT+Cyc.
MBP
F
Aged TWI+AAV+BMT+Cyc.
MBP


TWI-AAV Aged
IgG
Iba1
DAPI
TWI P40
IgG
Iba1
DAPI
WT Aged
IgG
Iba1
DAPI