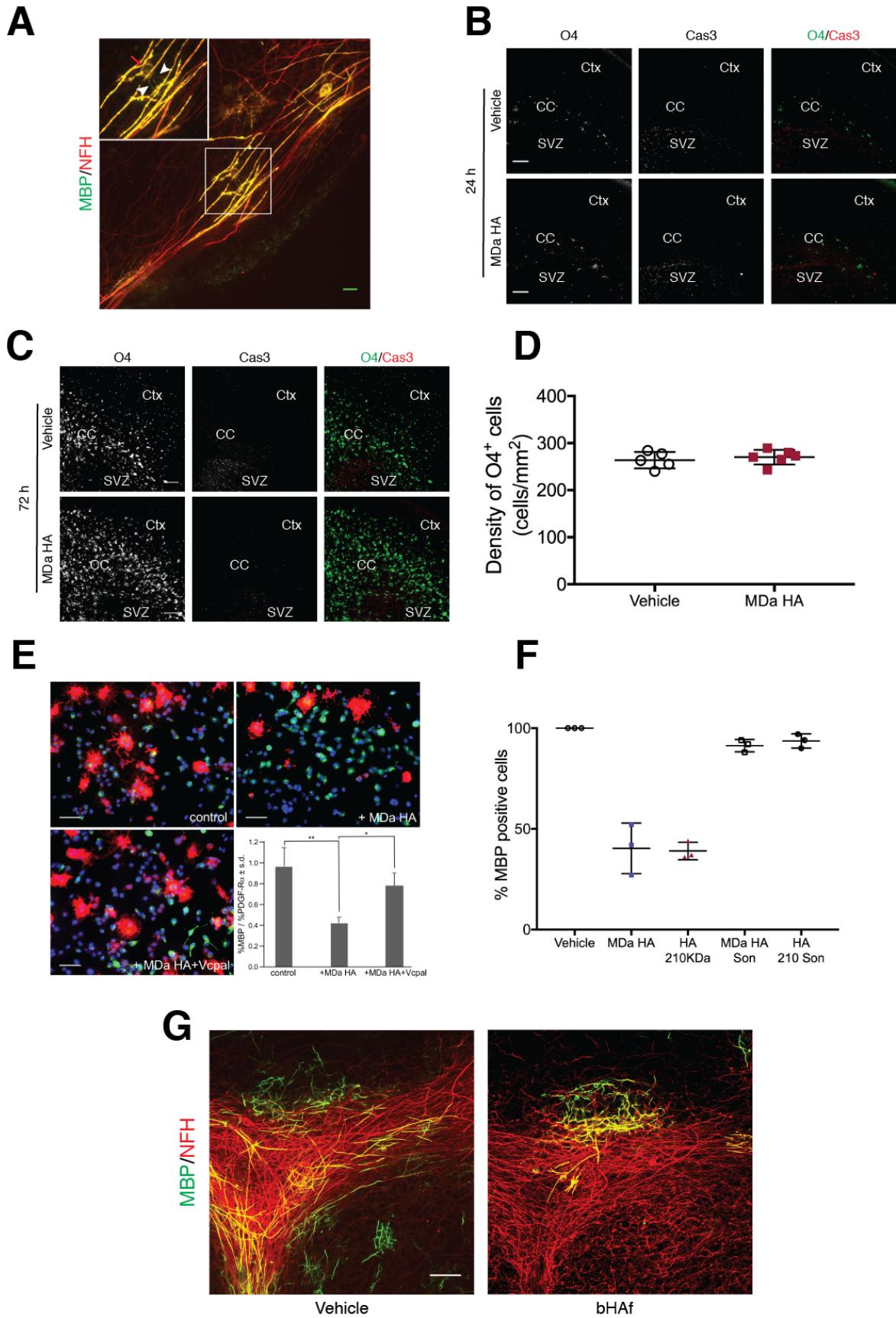


Supplemental Figure 1.

A) Staining controls for HABP visualized in rat forebrain slice cultures. Representative images of the corpus callosum (CC) and the overlying cerebral cortex (Ctx) stained with either biotinylated HABP alone (upper panel) or with rhodamine conjugated streptavidin alone to detect endogenous biotin (lower panel). No specific staining was noted for either control. Occasional auto-fluorescent blood vessels were noted.

B) Real time PCR analysis of expression of HA synthases and hyaluronidases from P4 H-I (red bars) vs. control (gray bars) animals.

A: n=2 animals per condition at P4. B: control n=2 and H-I n=4 animals at P4. Scale Bar: 150 μ m.



Supplemental Figure 2.

A) Chronic culture of rat forebrain slices until DIV 21 is accompanied by axonal myelination. Representative fluorescent confocal image of early axonal myelination in callosal white matter visualized with MBP and 200 kDa neurofilament H (NFH). Inset depicts a MBP⁺ OL (red; arrow) initiating apparent contacts with axons (white arrowheads).

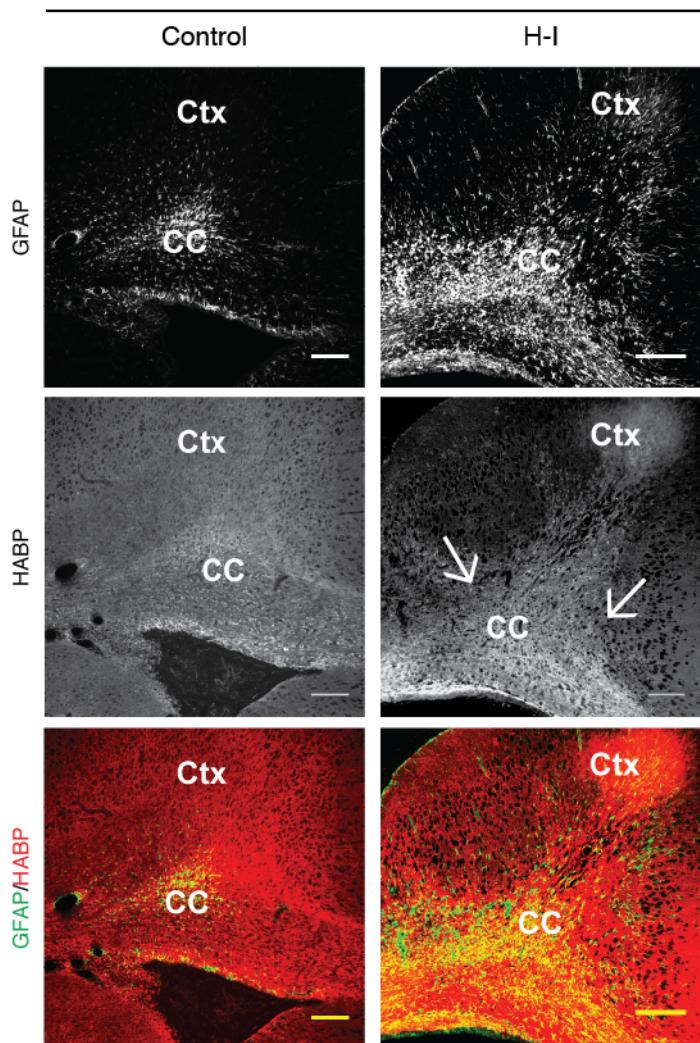
(B, C and D) MDa HA treatment does not promote OPC death. Representative images of the supracallosal radiation white matter of the corpus callosum (CC), Sub-Ventricular Zone (SVZ) and overlying cerebral cortex (Ctx) from rat forebrain slice cultures stained with O4 antibody and Caspase3 (Cas3) after treatment with or without MDa HA (2000 kDa; 50nM) for 24 h (D) or 72 h (E). O4 staining visualized OPCs in the corpus callosum. Cas3 labeled cells were observed in the SVZ with little or no staining in other regions. Cas3⁺O4⁺ co-labeled cells were rarely found. F) Quantification of the density of O4⁺ OPCs in the forebrain slice cultures after 8-10 days of MDa HA (2000 kDa; 50nM) treatment. No change in the density of O4⁺ OPCs was noted with or without treatment.

E) MDa HA de-polymerization is mediated by hyaluronidase activity. Primary OPC cultures were incubated with differentiation media (T3 and CNTF) for 4 days after which they were fixed and stained with, PDGFR- α (green; to label OPCs), MBP (red; to label mature OLs) and DAPI (blue; to label nuclei). Cultures were either treated with DMSO (Control) or MDa HA (50nM). The cultures were co-treated with Vcpal (25 μ M) where indicated. Quantification of % MBP⁺ mature OLs over % PDGFR- α ⁺ OPCs (right lower panel). Treatment with MDa HA attenuated OPC maturation but was rescued by Vcpal co-treatment.

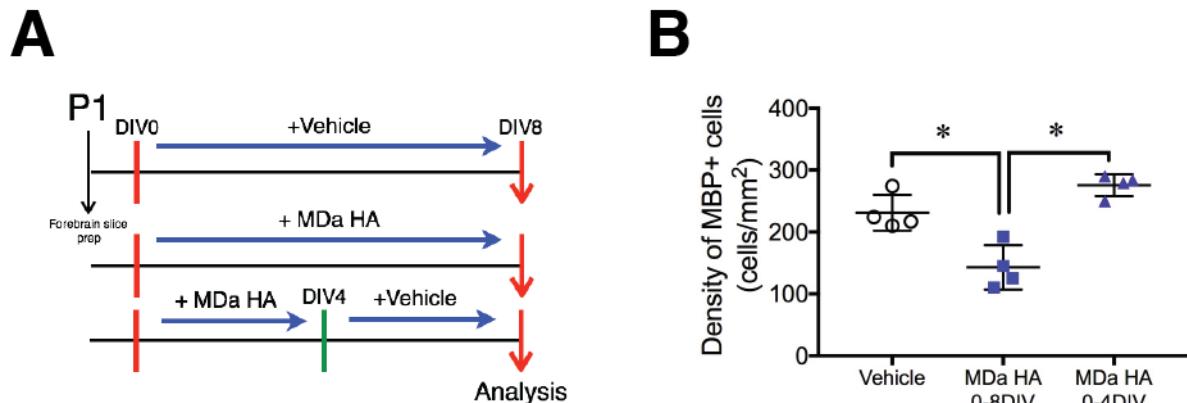
F) Sonication of MDa HA or bHAf leads to loss of bioactivity. Forebrain slice cultures exposed to either sonicated MDa HA (2000 kDa; 50nM) or bHAf (210 kDa; 100nM) preparations did not display a block in OPC maturation and had no decrease in density of MBP⁺ mature OLs. In contrast, un-sonicated MDa HA (50nM) and bHAf (100nM) preparations retain bioactivity to block OPC maturation.

G) Neonatal rat slices cultured for 21 days. Representative images of myelinated axons in callosal white matter visualized with MBP and 200 kDa neurofilament H (NFH) after treatment with PBS (vehicle) or bHAf (210 kDa; 100nM).

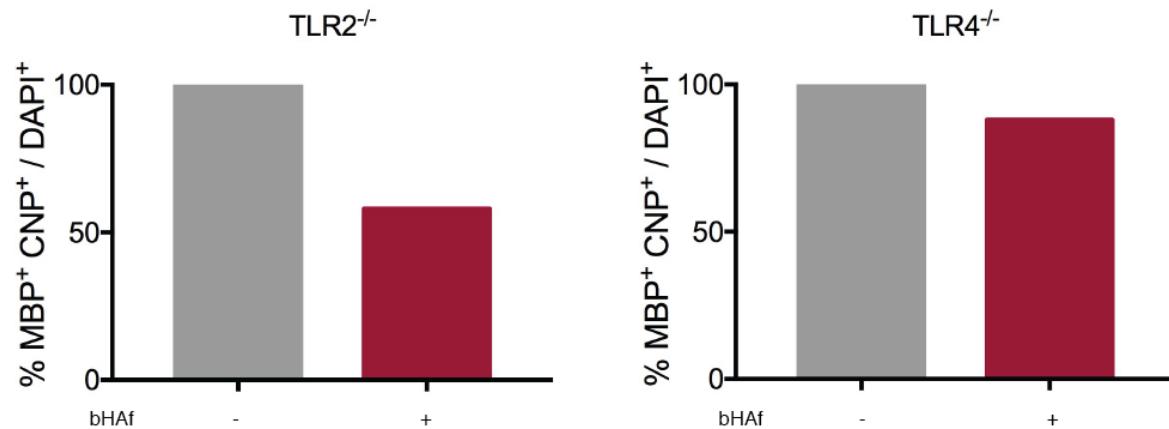
A-D: n=1-2 animals per condition from separate litters; 3 slices analyzed per treatment condition per animal; 3-6 slices total. E and F n=2 separate culture preparations. G: n=3 animals per condition from separate litters; 3 slices analyzed per treatment condition per animal, 9 slices total. **p<0.001 and *p<0.05 by Student's t test (E). Mean \pm SEM (D, E and F). Scale Bars: 75 μ m A and E; 150 μ m B, C and G.



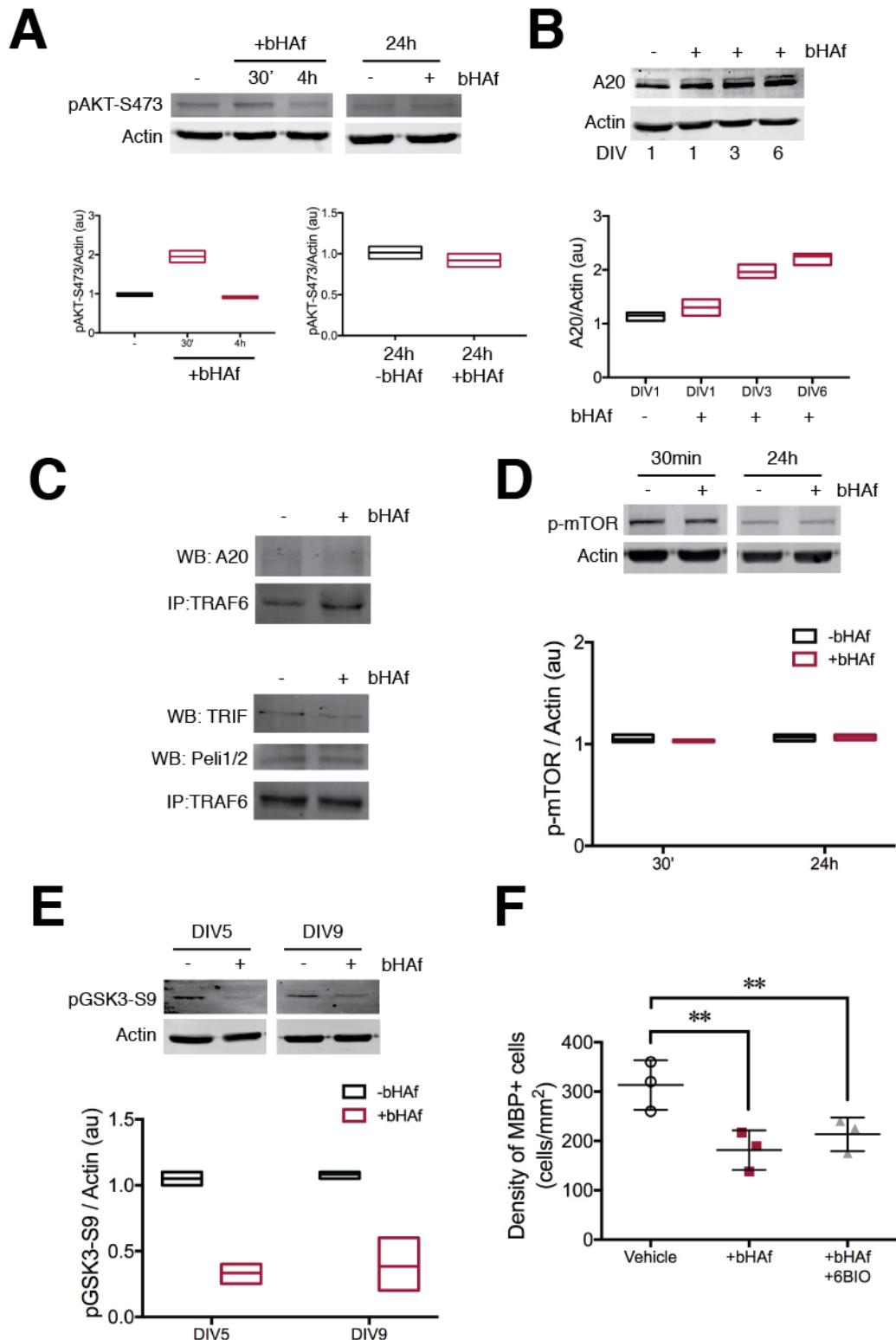
Supplemental Figure 3: MDa HA levels recover at P21. Representative images of the corpus callosum (CC) and overlying cortex (Ctx) obtained from P21 animals stained with glial fibrillary acidic protein (GFAP) and hyaluronan binding protein (HABP) that compare un-injured controls with animals that sustained H-I at P3. Note that in the H-I group there is increased HABP staining in the corpus callosum (arrows) relative to control. Increased deposition of MDa HA (right panel) coincides with regions of GFAP⁺ astrogliosis that demarcate areas of resolving WMI relative to un-injured controls (left panel). n=4 animals per group. Scale Bar: 300 μ m.



Supplemental Figure 4: Persistent MDa HA treatment is required to attenuate OPC maturation in-vitro in rat slice cultures. A) Schematic of the in-vitro chronic WMI slice culture assay to test the hypothesis that persistent generation of bHAF by MDa HA de-polymerization attenuates OPC maturation. B) Transient treatment (DIV0-4) with MDa HA followed by treatment with vehicle (DIV5-8) is unable to attenuate the density of MBP⁺ mature OLs in contrast with persistent treatment (DIV0-8). n=2 animals per condition from separate litters; 2 slices analyzed per treatment condition per animal, 4 slices total. *p<0.05 by ANOVA. Mean \pm SD.



Supplemental Figure 5: bHAf attenuates maturation of TLR2^{-/-} but not TLR4^{-/-} OPCs in a cell autonomous fashion. Primary OPC cultures generated by immunopanning from either P6-P7 TLR2^{-/-} or TLR4^{-/-} mouse pups were induced to differentiate with or without bHAf (210 kDa, 500nM) for 4 days and then fixed and stained with cyclic nucleotide phosphodiesterase (CNP), myelin basic protein (MBP) and DAPI. Quantification of total number of cells treated with or without bHAf shows that the generation of CNP⁺MBP⁺ OLs is attenuated by at least 40% in the TLR2^{-/-} OPCs (left panel) but not in TLR4^{-/-} OPCs (right panel). CNP⁺MBP⁺ OLs and DAPI⁺ cells were counted from 10 random sites from 4 coverslips per condition. n=1 culture experiment.



Supplemental Figure 6.

A) bHAf (210 kDa; 100nM) induces transient AKT activation at 30 minutes in murine slice cultures that attenuates at 4 and 24 hours. Representative blots (upper panel) and quantification (lower panel). Blots were probed with phospho-AKT (pAKT-S473) and Actin (loading control).

B) bHAf treatment caused an increase in total A20 protein levels in rat slices cultured between DIV1 and DIV6. Representative western blot (upper panel) and quantification (lower panel). Blots were probed with A20 and Actin (loading control). Protein lysates were prepared from slices cultured with or without bHAf (HA210 kDa; 100nM) treatment.

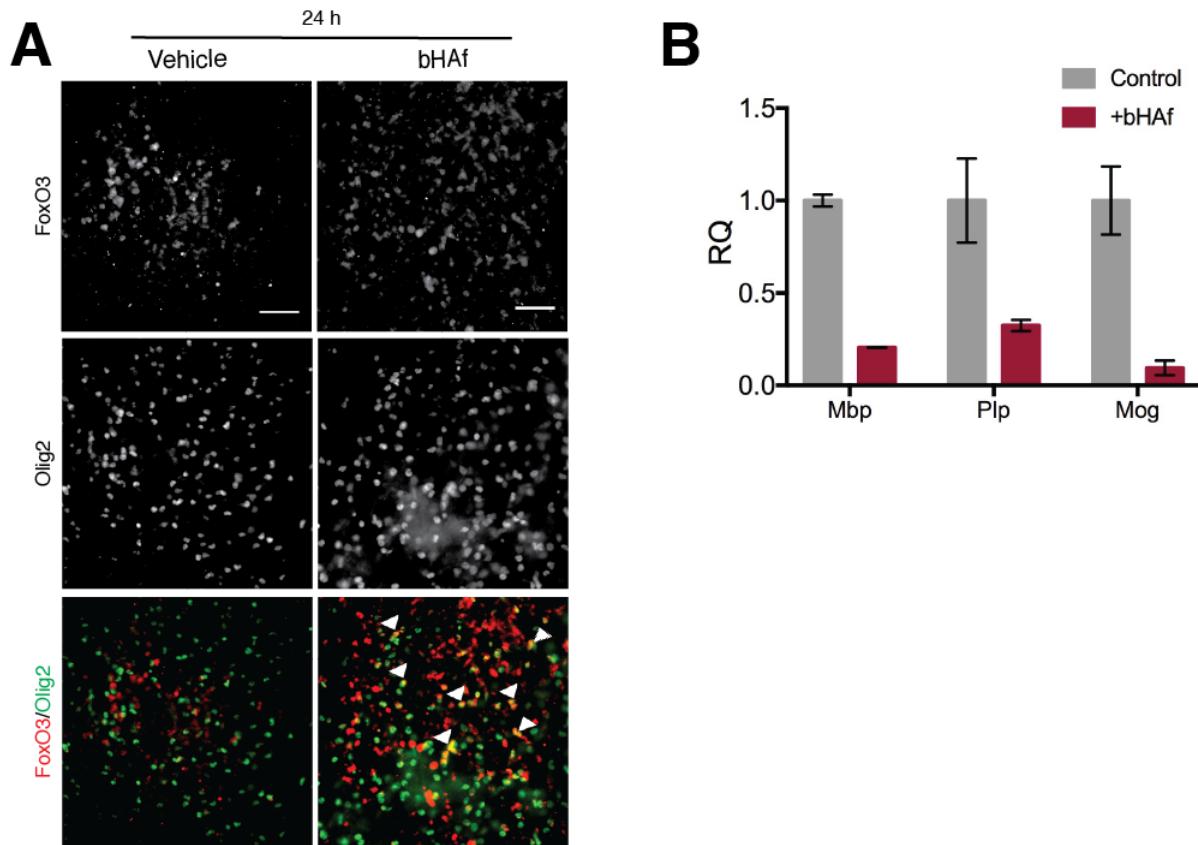
C) Neither A20 nor Pellino1/2 is recruited to the TRIF-TRAF6 complex in response to bHAf. Lysates prepared from forebrain slices with or without bHAf treatment (HA210 kDa; 100nM) for 24 h were subjected to immunoprecipitation (IP) using the TRAF6 antibody. The corresponding blots were probed with TRIF, TRAF6, A20 and Pellino1/2 antibodies.

C) mTOR is not regulated by bHAf (210 kDa; 100nM). Representative western blots (upper panel) and quantification (lower panel). Blots were probed with phospho-mTOR (p-mTOR) and Actin (loading control).

D) GSK3 β Ser9 is de-phosphorylated in bHAf (210 kDa; 100nM) treated slices. Representative western blots (upper panel) and quantification (lower panel) are shown. Blots were probed with phospho-GSK3 β (pGKS3-S9) and Actin (loading control).

E) GSK3 β Ser9 inhibition by 6BIO does not rescue the negative effects of bHAf on OPC maturation. Quantification of MBP $^+$ cells in the entire corpus callosum in forebrain slices after 7 days in culture. Slices were either treated with DMSO (Vehicle) or bHAf (HA210 kDa; 100nM) or both bHAf and 6BIO (20 μ M).

A: n=2 animals (P3/P4 mouse pups) per condition from 2 separate litters; 2 slices analyzed per treatment condition. B-D: n=2 animals per condition from separate litters; 2 slices analyzed per treatment condition. E: n= 2 animals per condition from separate litters; 6 slices total, 3 slices analyzed per animal. **p<0.001 by ANOVA. Mean \pm SEM (A, B, D and E); Mean \pm SD (F).

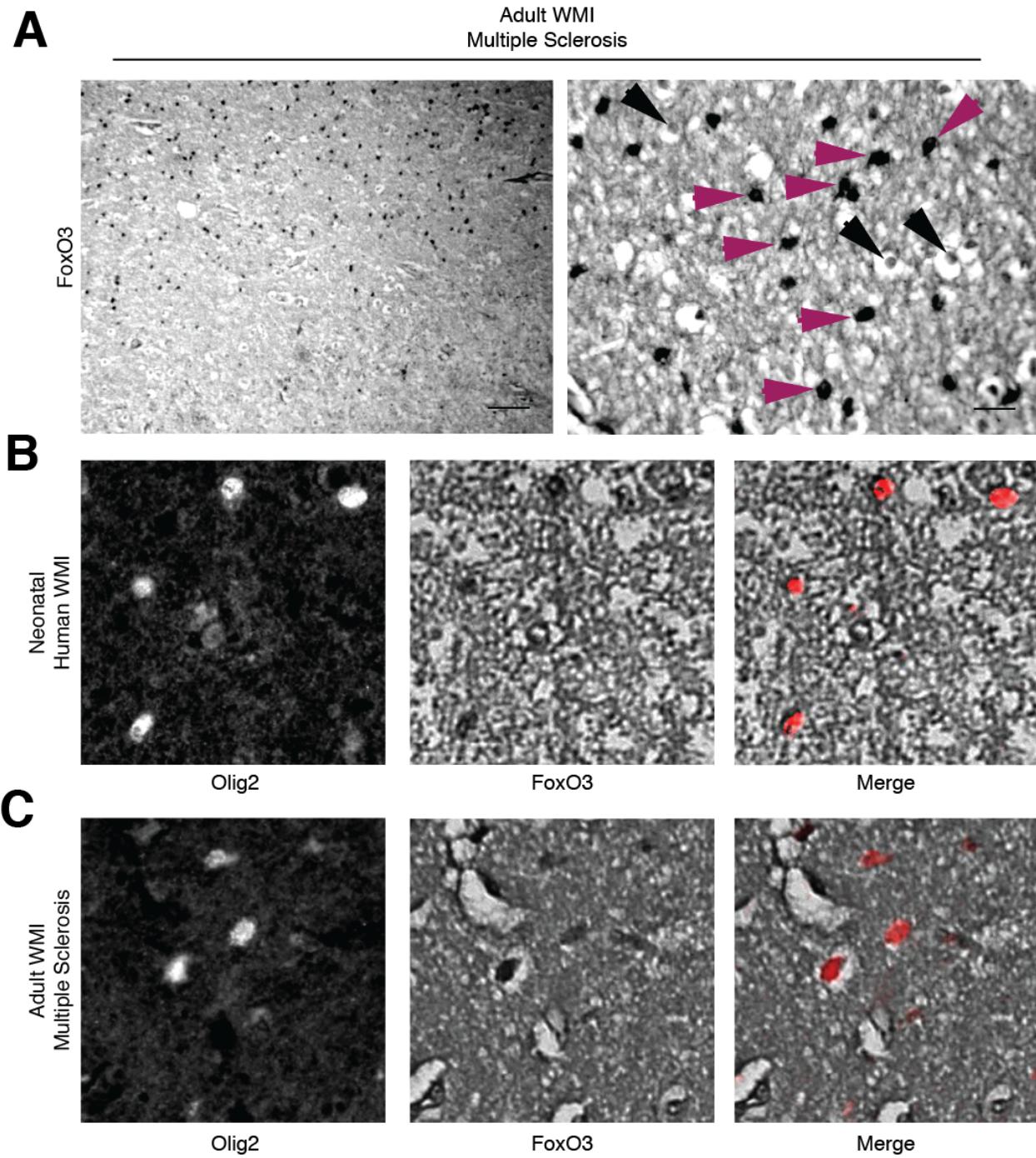


Supplemental Figure 7:

A) FoxO3 nuclear labeling in rat slice cultures after 24 h bHAf treatment is increased in Olig2-labeled OL lineage cells. Representative corpus callosum images from rat forebrain slices treated with or without bHAf (210 kDa; 100nM; 24 h) and stained with FoxO3 and Olig2. bHAf markedly increased double-labeled Olig2⁺FoxO3⁺ cells (white arrowheads) vs. PBS-treated controls.

B) Real time PCR analysis of expression of myelin associated genes in differentiating OPC cultures with (red bars) or without (grey bars) bHAf (210 kDa; 500nM; 4 days) treatment.

A: n=3 animals per condition from 3 separate litters; 9 slices total, 3 slices analyzed per condition per animal. B: n=1 culture experiment. Mean \pm SEM. Scale Bar: 150 μ m.



Supplemental Fig. 8: FoxO3 robustly labels reactive astrocytes and co-localizes with the OL marker, Olig2, in human neonatal and adult WMI. (A, left panel; scale bar 100 μ m) FoxO3 expression demarcated the boundaries of MS plaques. Shown here is a chronic active lesion from case 1 (Supplemental Table 1B; scale bar 25 μ m) and a detail of the numerous reactive astrocytes (red arrows) in this lesion (A, right panel). Note the many FoxO3-labeled nuclei interspersed among these astrocytes (black arrows). Panels in B, C provide higher power

representative images of the data shown in Fig. 8C. B) Images from case 1 (Supplemental Table 1A) with preterm human diffuse WMI depicts the co-localization of cells fluorescently labeled for Olig2 (left panel) and colorometrically labeled for FoxO3 (middle panel). The merge shows the extent of co-localization indicated in red. C) Images from core of a chronic active MS plaque from case 3 (Supplemental Table 1B) shows the co-localization of cells stained for Olig2 and FoxO3. The merge indicates the extent of co-localization in red.

Supplemental Table 1**Table S1A. Summary of Clinical and Neuropathological Data for four cases of preterm human diffuse WMI analyzed from Paraffin-Embedded Archival Tissue**

Case Number	PCA at birth (weeks)	Survival time (weeks)	Gender	Primary Neuropathological Diagnosis	Secondary Neuropathological Diagnosis	Studies
1 (7)*	32	2	M	Chronic Diffuse WMI	Neuronal loss and gliosis in Thalamus; Kernicterus in subthalamic body, floor 4 th ventricle and pontomedullary junction	FoxO3/Olig2
2 (12)*	25	9	M	Chronic Diffuse WMI	Subcortical heterotopias; hydromyelia in spinal cord	FoxO3
3 (13)*	25	13	F	Chronic Diffuse WMI	Partial agenesis of CC	FoxO3
4 (17)*	26	22	F	Chronic Diffuse WMI	Myelination delay; astrocytosis in centrum ovale	FoxO3

* Case number as originally reported in Buser et. al., 2012 (17).

PCA= Post Conceptual Age

Table S1B. Summary of Clinical and Neuropathological Data for four cases of chronic active multiple sclerosis (MS) analyzed from Paraffin-Embedded Archival Tissue

Case Number	Age at Death (years)	Duration of MS (years)	Gender	Primary Neuropathological Diagnosis	Secondary Neuropathological Diagnosis	Studies
1	53	10	M	Chronic Active and Active Remodeling	Caudate lacune	FoxO3/MBP; FoxO3
2	49	25	F	Chronic Active	Diffuse cerebellar and cortical atrophy	FoxO3
3	88	N/A	F	Chronic Active	Braak I Cerad 0 Thal A β 0	FoxO3/Olig2
4	60	4	F	Chronic Active	N/A	FoxO3/GFAP

