Supplementary Materials

Supplementary Methods

Generation of mutant strains. Eif2b4$^{Arg484Trp/Arg484Trp}$: The RP23-119J7 BAC clone was used to generate the 5' homology arm (~3.5 kb), 3' homology arm (~5.3 kb), and conditional region (~0.5 kb). The c.1450C>T (NM_010122.2) point mutation, located in 3' homology arm, was introduced by site-directed mutagenesis. The fragments were cloned in the LoxFtNwCD vector sequentially and confirmed by restriction digestion and end-sequencing. The final vector also contained loxP sequences flanking the conditional KO region (~0.5 kb), the Neo expression cassette (for positive selection of the ES cells) flanked by FRT sequences (for the subsequent removal of the Neo cassette), and a diphtheria toxin (DTA) expression cassette (for negative selection of the potentially targeted ES cells). Not-linearized vector DNA was electroporated into C57BL/6 ES cells and selected with G418. One hundred and ninety-two ES clones were selected for PCR based screening and six potential targeted clones were selected for expansion and further analysis. Based on additional Southern and PCR/sequencing confirmation analysis, two clones were confirmed to be correctly targeted. Southern blot confirmation of targeting was performed using a 300bp BamHI/AvrII 5' fragment and a 230bp BamHI/AvrII 3' fragment as probes. 5' probe detected a WT band of 12.9 kb and a mutant targeted band of 5.0 kb. 3' probe detected a WT band of 12.9 kb and a mutant targeted band of 8.7 kb. The following male chimeras had been generated: 98% (4), 90% (6), 85% (2), 80% (2), 75% (2), 70% (3), 60% (2), 50% (1), and 20% (1).

Eif2b5$^{Arg191His/Arg191His}$: The RP23-5E22 BAC clone was used to generate the 5' homology arm (~3.5 kb) and the 3' homology arm (~3.6 kb). The c.572G>A (NM_172265) point mutation, located in exon 4 of 5' homology arm, was introduced

by site-directed mutagenesis. The fragments were cloned in the LoxNwCD vector sequentially, and were confirmed by restriction digestion and end-sequencing. The final vector also contains loxP sequences flanking the Neo expression cassette (for positive selection of the ES cells), and a DTA expression cassette (for negative selection of the potentially targeted ES cells). Not-linearized vector DNA was electroporated into C57BL/6 ES cells and selected with G418. One hundred and ninety-two ES clones were selected for PCR based screening and two potential targeted clones were selected for expansion and further analysis. Based on additional Southern and PCR/sequencing confirmation analysis, only one clone was confirmed to be correctly targeted. Southern blot confirmation of targeting was performed using a 280bp HindIII 5' fragment and a 410bp SpeI 3' fragment as probes. 5' probe detected a WT band of 10.6 kb and a mutant targeted band of 4.6 kb. 3' probe detected a WT band of 12.3 kb and a mutant targeted band of 8.3 kb. The following male chimeras had been generated: 85% (2), 80%, 75%, 60%, 55%, 50% (2), 45% (2), 40% (2).

Targeting and ES cell work was performed by Caliper Discovery Alliances and Services (Hanover, MD, USA). The neo cassette was removed by crossing the heterozygous *Eif2b4*$^{R484W/WT}$ or *Eif2b5*$^{R191H/WT}$ mice with Cre recombinase expressing mice. Genotyping for routine maintenance was performed by PCR using for the *2b4*^{ho} mice the forward 5'-AAC AAA CAG GTT TCT AAG GTG CTA TTG G-3' and reverse primer 5'-TGG GAG TGC CAC TCT GCC TGG-3'. The primers produce a 738bp product from the WT and a ~838bp product from the mutant allele. For the *2b5*^{ho} genotyping for routine maintenance was performed by PCR using forward 5'-GGT TCA TAG GAC TCT TTG AAA CCA G-3' and reverse primer 5'-GAC AAA ACC CTA GAT TTG GTT CC-3'. The primers produce a 936bp product from the WT and a ~800bp product from the mutant allele.

Behavioral testing. The top unit of each cage contained an array of infrared LEDs and an infrared-sensitive video camera used for video-tracking. The behavior of mice was video-tracked (Noldus Information Technology, Wageningen, The Netherlands) and parsed into 20 behavioral parameters (Synaptologics BV, Amsterdam, The Netherlands), as previously described (61) (Supplementary Table 2).

Neuromuscular function was assessed by sensing the peak amount of force (N) mice applied in grasping a pull bar connected to a force meter (Columbus instruments, Columbus, OH, USA). Mice were allowed to grasp the pull bar 5 times with front paws only, followed by grasping 5 times with front and hind paws. The mean of each 5 repetitions was taken as grip strength (62).

Motor function was tested in a balance beam test (63) and the paw-print test (64). To obtain footprints, the hind- and forefeet of the mice were coated with blue and red nontoxic paints, respectively. The animals were then allowed to walk along a 100-cm-long, 10-cm-wide runway into an enclosed box. A fresh sheet of white paper was placed on the floor of the runway for each run. The footprint patterns were analyzed for four step parameters (all measured in centimeters): (1) stride length was measured as the average distance of forward movement between each stride; (2) hind-base width and (3) front-base width were measured as the average distance between left and right hind footprints and left and right front footprints, respectively. These values were determined by measuring the perpendicular distance of a given step to a line connecting its opposite preceding and proceeding steps. (4) Distance from left or right front footprint/hind footprint overlap was used to measure uniformity of step alternation. When the center of the hind footprint fell on top of the center of the preceding front footprint, a value of zero was recorded. When the footprints did not overlap, the distance between the center of the footprints was recorded. For each

step parameter, three values were measured from each run, excluding footprints made at the beginning and end of the run where the animal was initiating and terminating movement, respectively. The mean value of each set of three values was used in subsequent analysis.

The balance beam test scores the ability of mice to traverse a stationary horizontal rod and measures sensorimotor coordination as assessed by the latency to cross the beam and number of foot slips. Mice were placed at a platform at the start of a wide training beam (100 cm long, 5 cm wide) and allowed to walk along a into an enclosed box. All mice had three training runs on a wide beam, and on the subsequent day were given 3 runs on a narrow beam (1 cm wide). An observer scored the latency to traverse the beam, as well as the number of times a paw slipped off the beam.

References

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Supplementary Figures

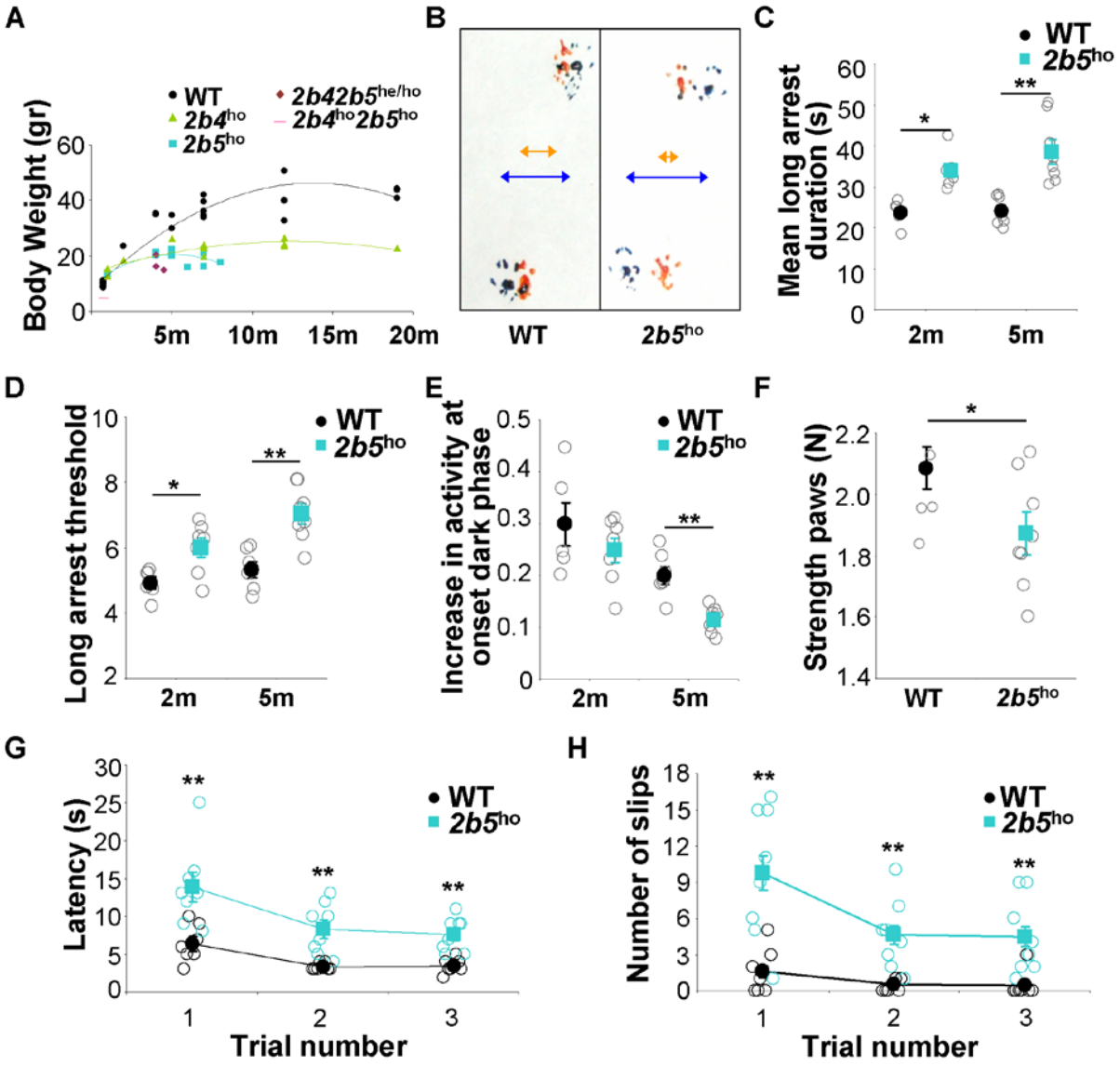

Figure S1. Behavioral phenotyping of VWM mouse models. (**A**) All VWM mutant mice reach a significantly lower body weight than WT animal. Shown are data for male mice; each data point represents one mouse with a trend-line representing the average weight for each genotype (WT *n*=18; *2b4*^{ho} *n*=24; *2b5*^{ho} *n*=13; *2b42b5*$^{he/ho}$ *n*=3; *2b4*^{ho}*2b5*^{ho} *n*=3). (**B**) In the paw-print test the *2b5*^{ho} mice show gait ataxia with a wider base of the hind limbs (blue prints). (**C-E**) Analysis of spontaneous behavioral phenotypes of WT and *2b5*^{ho} mice in the home-cage show significant differences in mean long arrest duration (**C**), long arrest threshold (**D**) and increase in activity at the onset of the dark phase (**E**). (**F-H**) Compared to WT littermates, the *2b5*^{ho} mice show impaired grip strength (**F**) and performance on balance beam (**G-H**) tests.
Data points represent individual mice with solid data points indicating the mean ± SEM (**C-H**). * = *p*<.05; ** = *p*<.01. (**A**) Mann-Whitney U test, (**C-H**) ANOVA. (**C-H**) *n*=10.

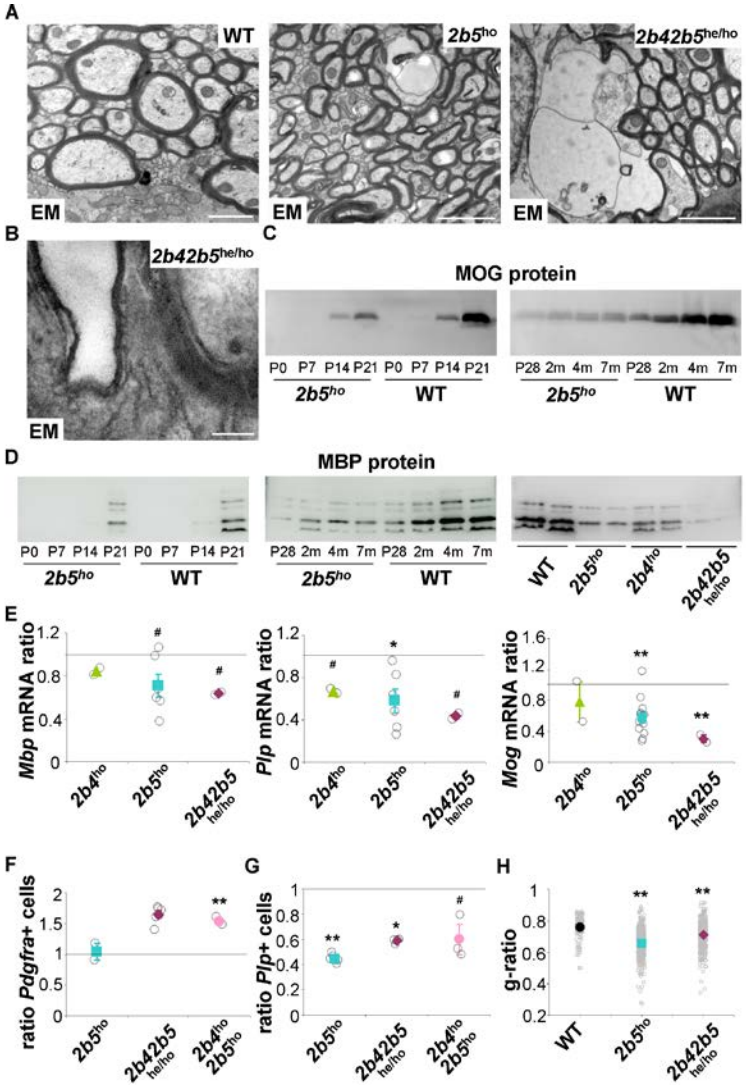

Figure S2. Myelin and oligodendrocyte maturation in VWM mutant mice. (**A-B**) Electron microscopy of 7-month-old *2b5*^{ho} and 4-month-old *2b42b5*$^{he/ho}$ mice shows vacuoles, which are absent in WT mice. Pictures are representative of at least 2 experiments. Higher magnification shows that vacuoles are surrounded by myelin strands, indicating that vacuoles are intramyelinic (**B**). (**C**-**D**) Protein levels of MOG (**C**) and MBP (**D**) are decreased in *2b5*^{ho} mice at all ages on western blot. The amount of MBP protein is decreased in the 19-month-old *2b4*^{ho} and 4-month-old *2b42b5*$^{he/ho}$ mice as well (**D**). The different MBP isoforms have molecular weights of (from top to bottom) 21.5, 18.5, 17 and 14kDa. (**E**) mRNA levels of the mature myelin proteins *Mbp*, *Plp* and *Mog* are significantly decreased in 7-month-old *2b5*^{ho} and 4-month-old *2b42b5*$^{he/ho}$ mice. In 7-month-old *2b4*^{ho} mice, only *Plp* mRNA is significantly decreased (WT *n*=6; *2b4*^{ho} *n*=2; *2b5*^{ho} *n*=6; *2b42b5*$^{he/ho}$ *n*=2). In situ hybridization shows significantly increased numbers of *Pdgfrα*-expressing cells (**F**) in P21 *2b4*^{ho}*2b5*^{ho} mice compared to age-matched controls (WT *n*=14; 7-month-old *2b5*^{ho} *n*=13; 4-month-old *2b42b5*$^{he/ho}$ *n*=6; *2b4*^{ho}*2b5*^{ho} *n*=3) and significantly decreased number of *Plp*-expressing cells (**G**) in P21 *2b4*^{ho}*2b5*^{ho}, 4-month-old *2b42b5*$^{he/ho}$ and 7-month-old *2b5*^{ho} mice compared to age-matched controls (WT *n*=8; *2b5*^{ho} *n*=6; *2b42b5*$^{he/ho}$ *n*=4; *2b4*^{ho}*2b5*^{ho} *n*=3). (**H**) In 7-month-old *2b5*^{ho} and 4-month-old *2b42b5*$^{he/ho}$ mice, the g-ratio is significantly lower than in 7-month-old WT mice (WT *n*=126; *2b5*^{ho} *n*=446; *2b42b5*$^{he/ho}$ *n*=404).
(**E**-**G**) Data points represent ratios of mutant over WT, the latter represented by the line. Solid data points indicate mean ratio of mutant over WT ± SEM. (**H**) Data points represent individual fibers with solid data point indicating the mean ± SEM. (**E**-**G**) Student's *t*-test, (**H**) Mann-Whitney U test. # = significant at $p<.05$ without Bonferroni correction, * = $p<.05$; ** = $p<.01$. (**A**) scalebars = 1µm (**B**) scalebar = 100nm

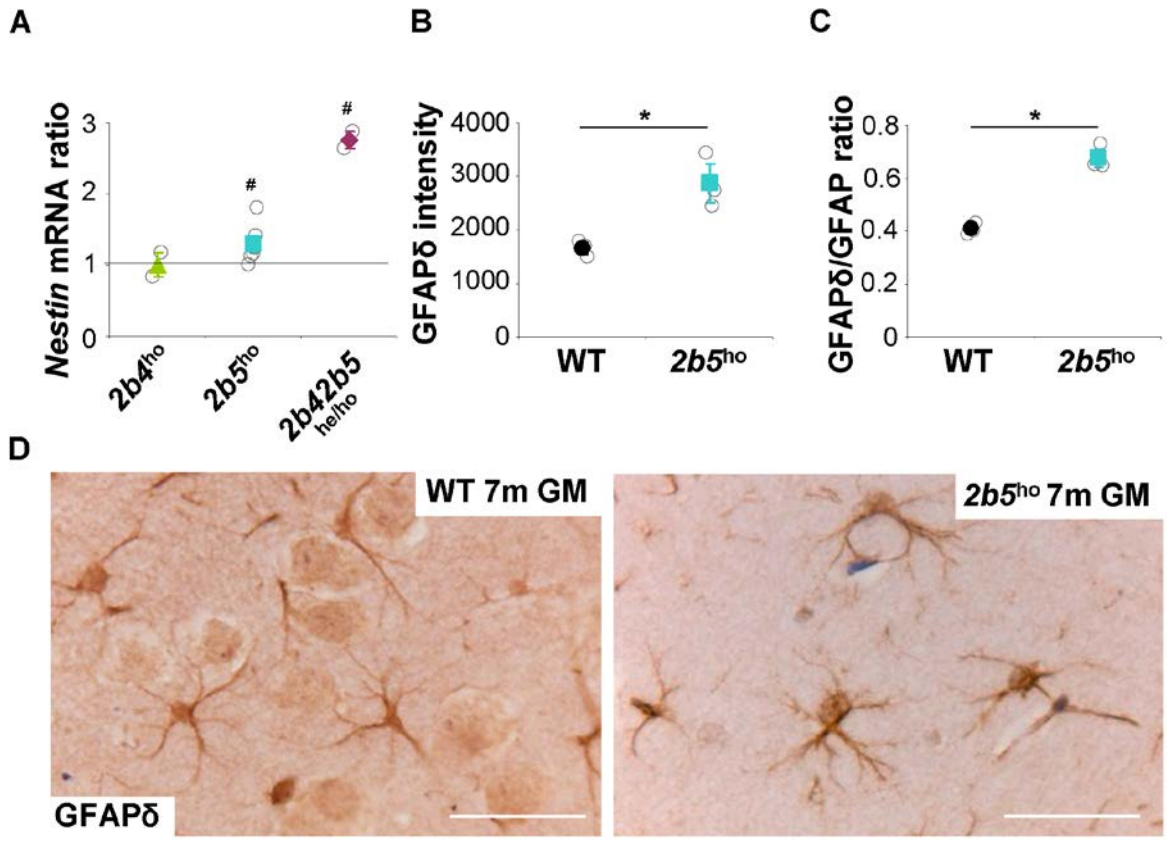

Figure S3. Nestin and GFAP in VWM mouse models. (**A**) In forebrain lysates, *nestin* mRNA levels are increased in 7-month-old *2b5*^{ho} and 4-month-old *2b42b5*$^{he/ho}$ mice, but not in 7-month-old *2b4*^{ho} mice (WT *n*=6; *2b4*^{ho} *n*=2; *2b5*^{ho} *n*=6; *2b42b5*$^{he/ho}$ *n*=2). The intensity of GFAPδ bands on western blot is significantly increased in 2- to 7-months-old *2b5*^{ho} mice (*n*=3) compared to 2- to 7-months-old WT (*n*=3) (**B**). The ratio of the GFAPδ isoform over total GFAP is significantly increased in 2- to 7-months-old *2b5*^{ho} mice (*n*=3) compared to 2- to 7-months-old WT mice (*n*=3) on western blot (**C**). (**D**) Staining for GFAPδ shows normal immunoreactivity in gray matter astrocytes.
(**A**) Data points represent ratio of mutant over WT, with solid data point indicating the mean ratio of mutant over WT ± SEM. (**B**-**C**) Data points represent individual samples with solid data point indicating mean ± SEM. (**A**-**B**) Student's *t*-test. (**C**) Mann-Whitney U test.. Scale bars = 50 µm. # = significant at p<.05 without Bonferroni correction, * = *p*<.05; ** = *p*<.01. Immunostainings are representative images of at least 3 experiments.

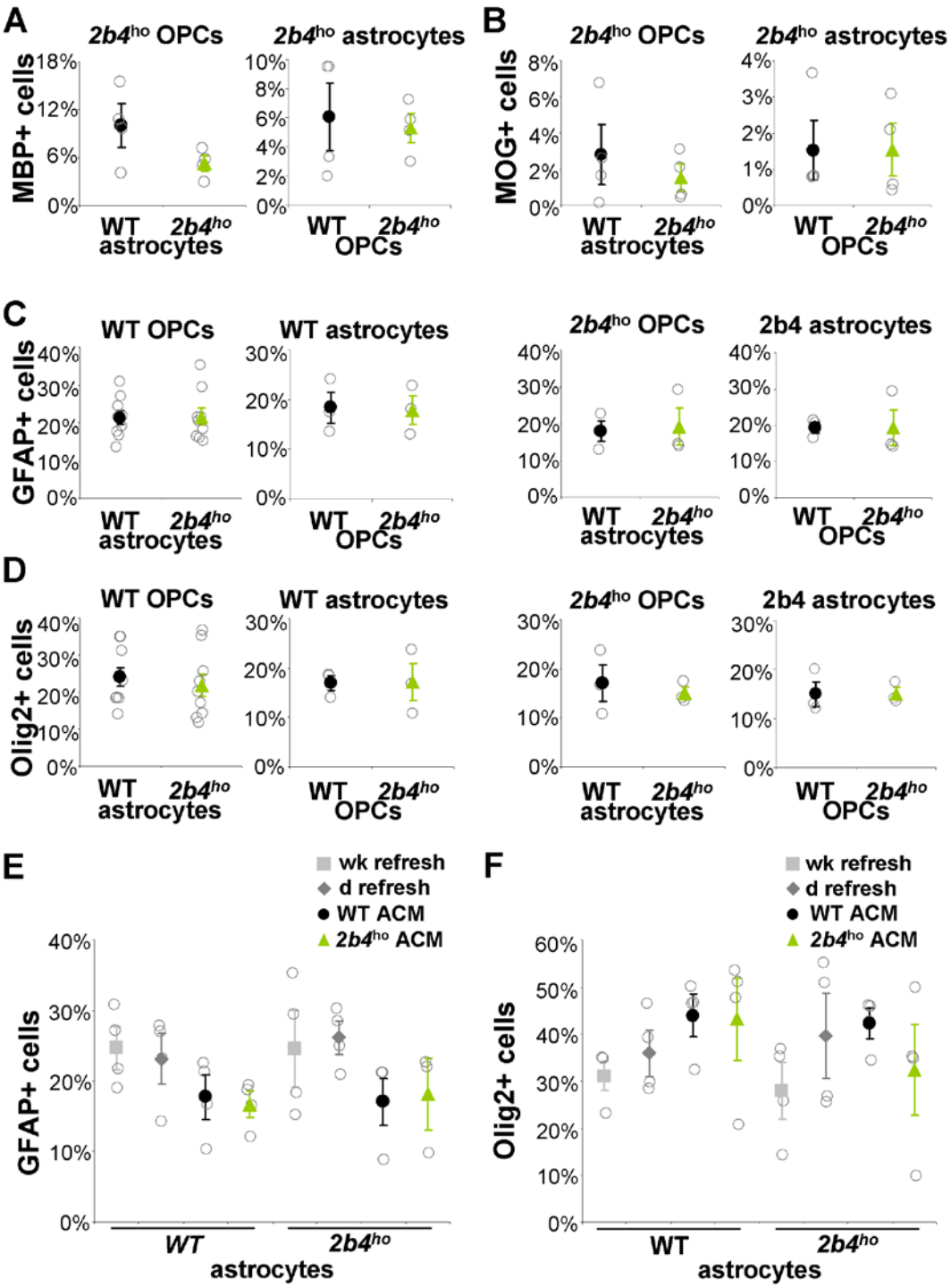

Figure S4. VWM OPCs are capable of normal maturation *in vitro*. (**A**) The number of MBP-positive cells derived from *2b4*^{ho} OPCs is significantly decreased in co-cultures with *2b4*^{ho} astrocytes compared to WT astrocytes (*n*=8). There are no significant differences between cultures with WT or *2b4*^{ho} OPCs on *2b4*^{ho} astrocytes (*n*=6). No significant differences are observed for the number of MOG-positive cells (**B**). Cell counts for GFAP- and olig2-positive cells show no significant differences in any of the conditions when comparing both WT and *2b4*^{ho} OPCs on WT or *2b4*^{ho} astrocytes (**C**-**D**) or in conditioned medium experiments (**E**-**F**) (*n*=7 for WT OPC cultures; *n*=4 for *2b4*^{ho} OPC cultures; *n*=4 for conditioned medium experiments). (**E**-**F**) "wk refresh" indicates a once per week refreshment of the medium, as is done for all other co-cultures. "d refresh" indicates a daily refreshment of the medium, as a control for the daily refreshment in the conditioned medium experiments.
(**A**-**F**) data points represent individual experiments with solid data points indicating mean ± SEM. Paired samples *t*-test. * = *p*<.05

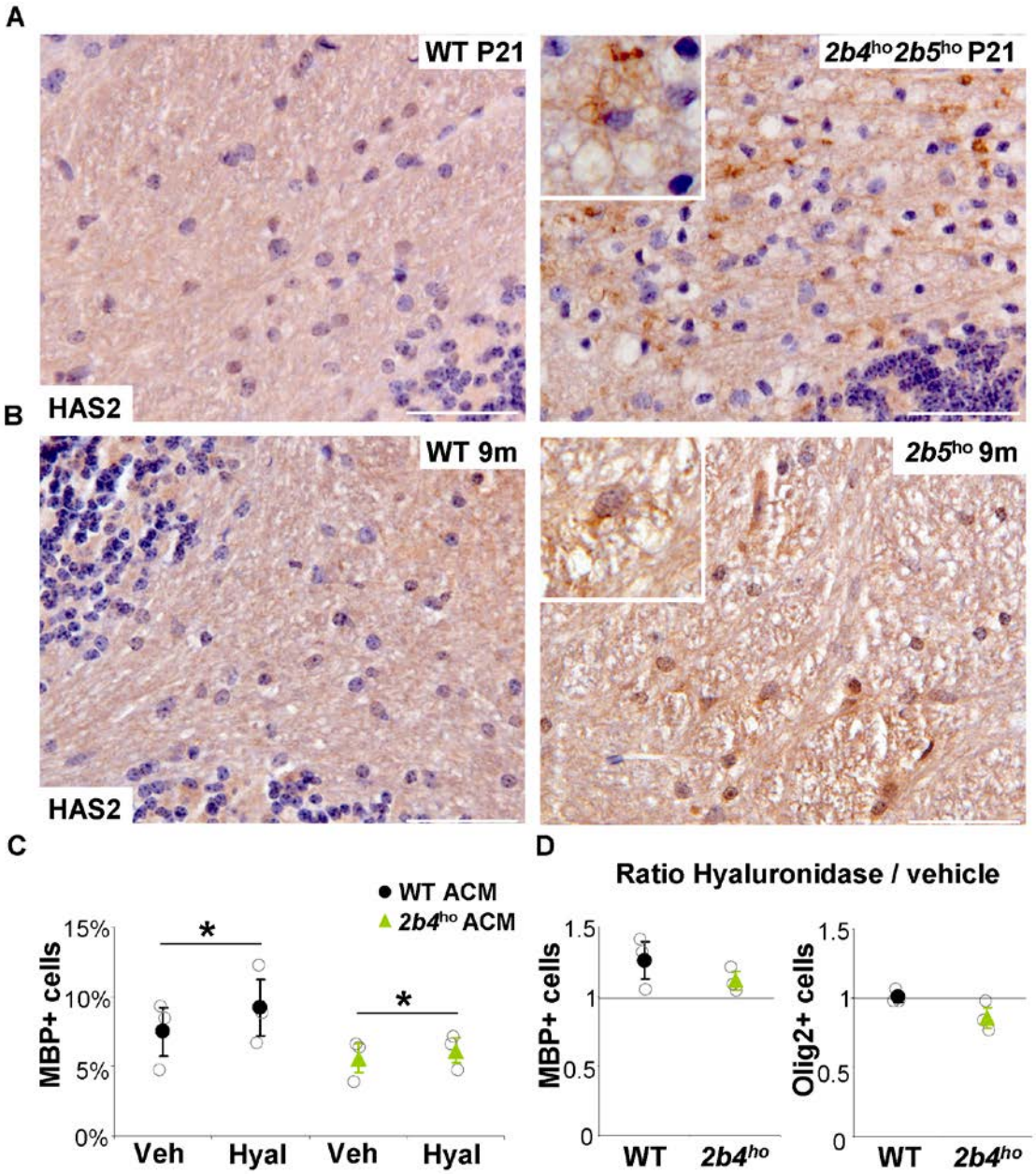

Figure S5. HAS2 in VWM mice and the effect of hyaluronidase on OPC maturation. HAS2 labelling is increased in P21 *2b4*^{ho}*2b5*^{ho} (**A**) and 9-month-old *2b5*^{ho} (**B**) mice compared to age-matched controls. The cells positive for HAS2 show an astrocytic morphology (see insets) and stain double positive for GFAP (not shown). Cell counts of OPC cultures (*n*=3) in WT or *2b4*^{ho} ACM treated with vehicle (control) or hyaluronidase are shown in (**C-D**). The number of MBP positive cells increased in both WT and *2b4*^{ho} ACM upon hyaluronidase treatment (**C**). The effect of the hyaluronidase treatment on the number of MBP positive cells was not significantly different between WT and *2b4*^{ho} ACM (**D**) as is shown by the ratio of MBP+ cells between hyaluronidase and vehicle treated cells. The number of Olig2+ cells was not significantly different in any condition (**D**). The line at 1 indicates no difference between hyaluronidase and vehicle condition; values above 1 indicate a higher number of positive cells after hyaluronidase treatment; values below 1 indicate a lower number of positive cells after hyaluronidase treatment.
(A-B) Scale bars = 50 μM, representative images of at least 3 experiments. (**C-D)** each data point indicates one experiment, solid points show mean ± SEM. paired samples *t*-test. * = *p* <.05.

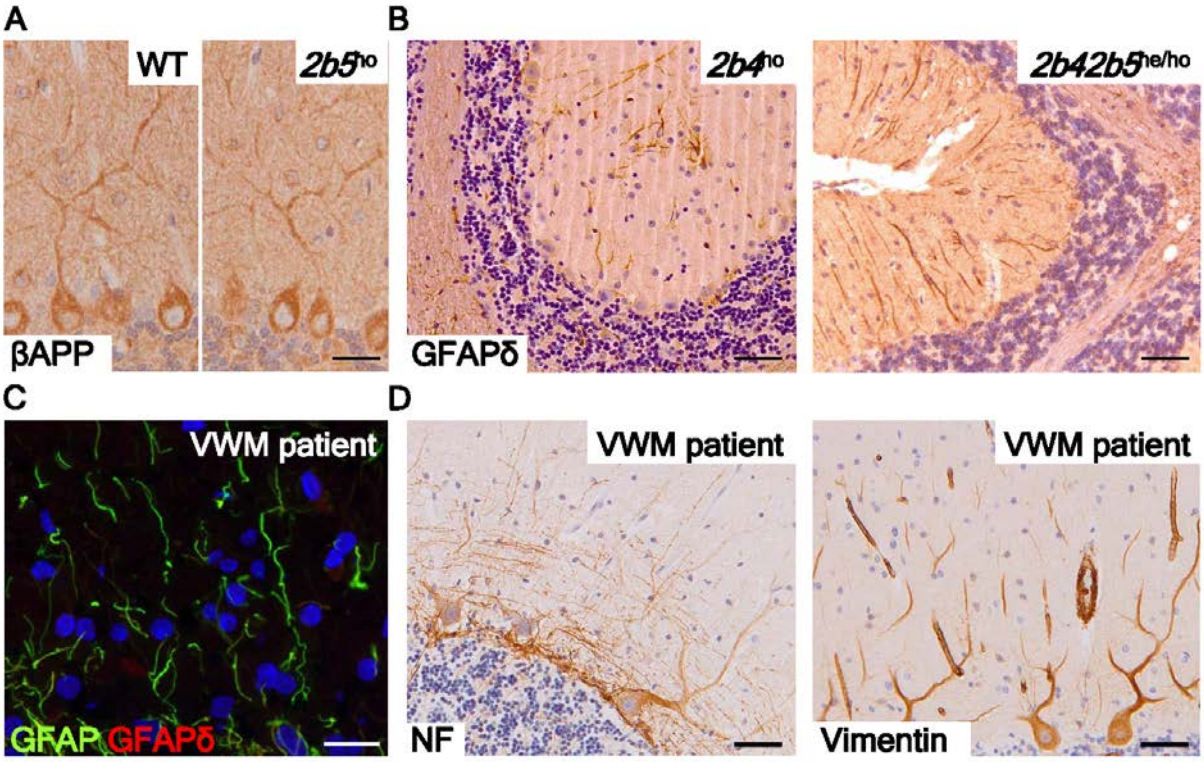

Figure S6. Abnormal Bergmann glia in VWM. (**A**) In the cerebellum of 7-month-old *2b5*^{ho} mice the Purkinje cells and small neurons in the granular layer show no abnormalities. (**B**) GFAPδ-overexpressing ectopic Bergmann glia are present in the cerebellar cortex of 19-month-old *2b4*^{ho} (left) and 4-month-old *2b42b5*$^{he/ho}$ mice (right). (**C**) Normally located Bergman glia in VWM patients are GFAPδ-negative. (**D**) Also in human VWM cerebella, the Purkinje cells show no abnormalities.
Scale bars = (**A-B, D**) 50 µm; (**C**) 20 µm. Immunostainings are representative images of at least 3 experiments.

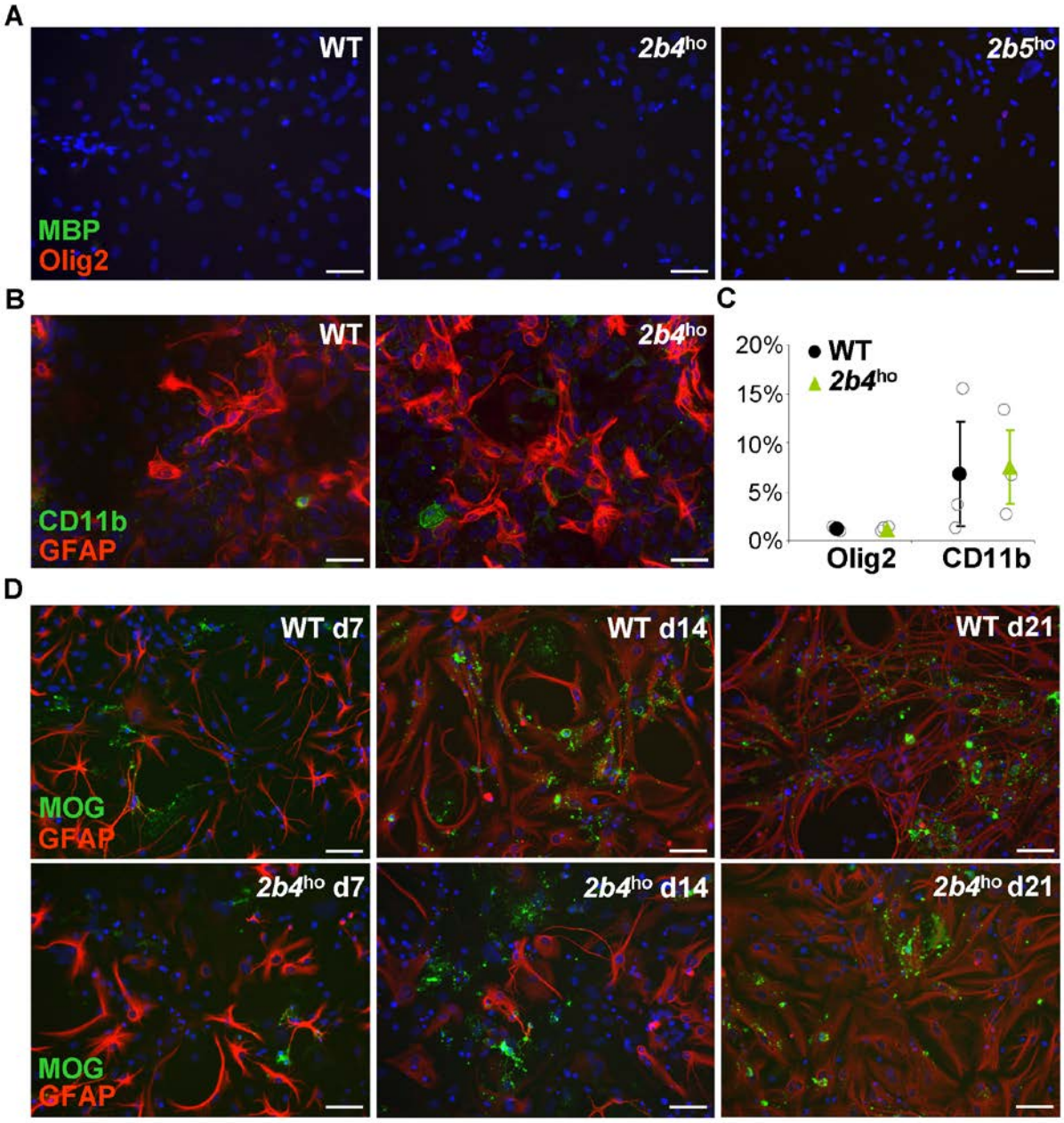

Figure S7. Astrocyte-OPC co-culture system. (**A**) Enriched astrocyte cultures derived from WT (left), *2b4*^{ho} (middle) and *2b5*^{ho} mice (right) show no MBP-positive cells and very little Olig2-positive cells on immunostainings. (**B**-**C**) Astrocyte cultures (*n*=3) contain a low number of CD11b-positive cells (~7%) and very little Olig2-positive cells (~1%). There are no differences between WT and *2b4*^{ho} astrocytes. (**D**) In co-cultures of WT or *2b4*^{ho} astrocytes and WT OPCs fixed after 7 (left), 14 (middle) or 21 days (right), the number of MOG- or MBP-positive cells increases between day 7 and 14 in culture, and decreased slightly at 21 days due to degeneration of the culture.
(**C**) data points indicate individual experiments, with solid data points representing mean ± SEM Scale bars = 50µm. Immunostainings are representative images of at least 3 experiments.

Table S1. Number of animals used per experiment

	# of animals	Ages
Immunohistochemistry		
WT	3	P14, P21, 1m, 2m, 4m, 5m, 7m, 12m, 19m
*2b5*ho	3	P14, P21, 1m, 2m, 4m, 5m, 7m
*2b4*ho	3	1m, 2m, 5m, 7m, 12m, 19m
*2b42b5*he/ho	3	4m
*2b4*ho*2b5*ho	3	P21
***In situ* hybridization**		
WT	3	P21, 1m, 2m, 4m, 5m, 7m
*2b5*ho	3	1m, 2m, 4m, 5m, 7m
*2b42b5*he/ho	3	4m
*2b4*ho*2b5*ho	3	P21
Western blot and qPCR		
WT	2	P0, P7, P14, P21, 1m, 2m, 4m, 7m
*2b5*ho	2	P0, P7, P14, P21, 1m, 2m, 4m, 7m
*2b4*ho	2	7m
*2b42b5*he/ho	2	4m
Electron microscopy		
WT	4	7m
*2b5*ho	4	7m
*2b42b5*he/ho	2	4m
Behavioral tests		
WT	10	2m, 5m
*2b5*ho	10	2m, 5m
Hyaluronan ELISA		
WT	3-5	P21, 1m, 4m, 7m, 19m
*2b5*ho	5	1m, 4m, 7m
*2b4*ho	5	19m
*2b42b5*he/ho	5	4m
*2b4*ho*2b5*ho	3	P21

P = postnatal day; m = months

Table S2. Behavioral screening in home cage by 20 parameters of spontaneous behavior

Behavior	2m		5m	
	WT	*2b5*ho	WT	*2b5*ho
Sheltering behavior				
Short shelter visit threshold	4.67±0.25	4.69±0.28	4.92±0.21	5.00±0.33
Long shelter visit threshold	10.4±0.27	10.1±0.32	10.1±0.24	9.58±0.22
Long shelter visit fraction of total visits	0.06±0.01	0.08±0.01	0.08±0.01	0.12±0.01
Long shelter visit duration - dark	14912±993	16676±2508	18274±2564	22561±1433
Activity				
Activity duration – dark	8220±563	7204±679	6680±648	5255±298
Activity duration – light	2152±1043	909±214	478±114	533±102
Mean activity duration – dark	22.7±1.37	24.3±1.18	22.7±1.55	24.2±1.44
Mean activity duration - light	97.3±78.7	18.2±1.58	17.9±4.28	17.7±2.88
OnShelter zone number - dark	169±42.9	122±26.4	82.4±22.7	41.0±6.78
Kinematic parameters (move and arrest segments)				
Long arrest threshold	4.91±0.17	**5.99±0.30#**	5.32±0.23	**7.03±0.29***
Mean long arrest duration – light	37.5±9.35	38.0±2.66	23.0±1.99	114±72.8
Long movement threshold	1.68±0.11	1.47±0.12	1.33±0.12	1.25±0.08
Long movement max. velocity	20.1±0.41	20.0±0.46	16.9±0.78	16.6±0.64
DarkLight index of activity				
Activity duration – darklight index	0.81±0.07	0.89±0.02	0.93±0.01	0.91±0.02
Habituation across three days in the home cage				
Activity duration – habituation ratio dark	0.98±0.07	1.15±0.10	0.6±0.05	0.60±0.03
Activity duration – habituation ratio light	2.91±1.96	0.78±0.13	1.06±0.27	1.14±0.18
Change in activity surrounding dark/light phase transitions				
Activity change in anticipation of dark	-0.06±0.06	0.01±0.01	0±0	0.01±0.01
Activity change in anticipation of light	0.11±0.02	0.08±0.04	0.09±0.04	0.09±0.03
Activity change in response to dark	0.31±0.04	0.25±0.02	0.2±0.02	**0.12±0.01***
Activity change in response to light	-0.12±0.02	-0.10±0.03	-0.1±0.04	-0.07±0.02

Mean +- SEM are indicated. Significant values are given in bold. # = $p<.05$; *= significant at Bonferroni corrected $p<.0025$

Table S3. P-values of behavioral tests

Test	Parameter	P-value 2m	P-value 5m
Body Weight		**.0004**	**.0001**
Grip Strength test	Front Paws only	.0563	.2905
	Front and hind paws	.0938	**.0372**
Open Field	Distance moved	**.0017**	.0706
	Average Velocity	**.0015**	.0716
	Distance moved in center	.2584	.2622
	Time spent in center	.2463	.469
	Visits in center	.1639	.3174
Balance Beam	Number of slips	.6671	**.0005**
	Latency	.31	**.0008**

m = months; values in bold are significant at $p<.05$

Table S4. Statistical analyses other than behavioral tests

Sample	N	Center ± distribution	Test	Test-statistic	p-value	Effect size
Body weight	**N**	**Median ± range**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT 2-7m	11	34.8 ± 5.50				
2b5^{ho} 2-7m	13	20.3 ± 5.45	Mann-Whitney U test	*U* = 0	**<.001***	r = .85
WT 2-19m	18	37.88 ± 6.58				
2b4^{ho} 2-19m	24	23.34 ± 4.9	Mann-Whitney U test	*U* = 5	**<.001***	r = .81
WT 4m	5	34.6 ± 8.34				
2b42b5$^{he/ho}$ 4m	3	16.14 ± 2.14	Mann-Whitney U test	*U* = 0	**.025#**	r = .79
qPCR	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
Mbp WT P7	2	1.76 ± 0.31				
Mbp 2b5^{ho} P7	2	0.50 ± 0.22	Student's *t* test	*t*(1.78) = 4.67	.053	r = .96
Mbp WT P14	2	17.1 ± 0.85				
Mbp 2b5^{ho} P14	2	13.90 ± 0.40	Student's *t* test	*t*(1.43) = 4.83	.075	r = .96
Mbp WT P21	2	36.32 ± 1.53				
Mbp 2b5^{ho} P21	2	28.67 ± 1.92	Student's *t* test	*t*(1.91) = 4.42	.052	r = .95
Mbp WT P28	2	34.56 ± 0.21				
Mbp 2b5^{ho} P28	2	29.41 ± 0.30	Student's *t* test	*t*(1.81) = 19.96	**.004***	r > .99
Mbp WT 2-7m	6	17.22 ± 1.65				
Mbp 2b5^{ho} 2-7m	6	12.25 ± 4.54	Student's *t* test	*t*(6.29) = 2.52	**.044#**	r = .62
Plp WT 2-7m	6	10.02 ± 1.68				
Plp 2b5^{ho} 2-7m	6	5.83 ± 2.75	Student's *t* test	*t*(10) = 3.18	**.010***	r = .71
Mog WT 2-7m	6	0.32 ± 0.06				
Mog 2b5^{ho} 2-7m	6	0.20 ± 0.07	Student's *t* test	*t*(10) = 2.80	**<.001***	r = .72
Olig2 WT 2-7m	6	0.02 ± 0.00				
Olig2 2b5^{ho} 2-7m	6	0.02 ± 0.00	Student's *t* test	*t*(10) = 1.15	.278	r = .34
Pdgfrα WT 2-7m	6	0.05 ± 0.01				
Pdgfrα 2b5^{ho} 2-7m	6	0.04 ± 0.01	Student's *t* test	*t*(10) = 1.29	.226	r = .38
Gfap WT 2-7m	5	0.02 ± 0.00				
Gfap 2b5^{ho} 2-7m	4	0.02 ± 0.01	Student's *t* test	*t*(7) = -0.03	.978	r = .01
Gfapα WT 2-7m	5	0.18 ± 0.03				
Gfapα 2b5^{ho} 2-7m	4	0.25 ± 0.07	Student's *t* test	*t*(7) = -2.05	.079	r = .61
Gfapδ WT 2-7m	5	0.01 ± 0.00				

Gfapδ 2b5^{ho} 2-7m	4	0.02 ± 0.00	Student's *t* test	*t*(7) = -2.14	.070	r = .63
nestin WT 2-7m	6	0.06 ± 0.01				
nestin 2b5^{ho} 2-7m	6	0.07 ± 0.02	Student's *t* test	*t*(10) = -2.24	**.049#**	r = .58
Mbp WT	2	0.06 ± 0.01				
Mbp 2b4^{ho}	2	0.05 ± 0.00	Student's *t* test	*t*(2) = 1.93	.194	r = .81
Plp WT	2	22.91 ± 0.03				
Plp 2b4^{ho}	2	15.5 ± 0.71	Student's *t* test	*t*(1.003) = 14.67	**.043#**	r > .99
Mog WT	2	0.01 ± 0.00				
Mog 2b4^{ho}	2	0.01 ± 0.00	Student's *t* test	*t*(1.06) = 0.82	.556	r = .50
Gfap WT	5	0.02 ± 0.00				
Gfap 2b4^{ho}	4	0.01 ± 0.00	Student's *t* test	*t*(7) = -2.47	**.043#**	r = .68
Gfapα WT	5	0.18 ± 0.03				
Gfapα 2b4^{ho}	4	0.18 ± 0.01	Student's *t* test	*t*(7) = 0.35	.735	r = .13
Gfapδ WT	5	0.01 ± 0.00				
Gfapδ 2b4^{ho}	4	0.01 ± 0.00	Student's *t* test	*t*(7) = -2.08	.841	r = .08
nestin WT	2	0.03 ± 0.00				
nestin 2b4^{ho}	2	0.03 ± 0.01	Student's *t* test	*t*(1.64) = 0.0	1	r = .00
Olig2 WT	2	0.34 ± 0.01				
Olig2 2b4^{ho}	2	0.29 ± 0.04	Student's *t* test	*t*(2) = 1.74	.225	r = .78
Pdgfrα WT	2	0.11 ± 0.01				
Pdgfrα 2b4^{ho}	2	0.11 ± 0.00	Student's *t* test	*t*(1.01) = 0.25	.844	r = .24
Mbp WT	2	0.06 ± 0.01				
Mbp 2b42b5$^{he/ho}$	2	0.04 ± 0.01	Student's *t* test	*t*(1.03) = 4.86	**.040#**	r = .96
Plp WT	2	22.91 ± 0.03				
Plp 2b42b5$^{he/ho}$	2	9.98 ± 0.88	Student's *t* test	*t*(1,002) = 20.86	**.030#**	r > .99
Mog WT	2	0.12 ± 0.01				
Mog 2b42b5$^{he/ho}$	2	0.04 ± 0.01	Student's *t* test	*t*(2) = 11.31	**.008***	r > .99
Gfap WT	5	0.02 ± 0.00				
Gfap 2b42b5$^{he/ho}$	4	0.01 ± 0.00	Student's *t* test	*t*(7)= 1.65	.144	r = .53
Gfapα WT	5	0.18 ± 0.03				
Gfapα 2b42b5$^{he/ho}$	4	0.20 ± 0.06	Student's *t* test	*t*(7) = -0.54	.609	r = .20
Gfapδ WT	5	0.01 ± 0.00				
Gfapδ 2b42b5$^{he/ho}$	4	0.01 ± 0.00	Student's *t* test	*t*(7) = -0.43	.682	r = .16

nestin WT	2	0.25 ± 0.01				
nestin 2b42b5$^{he/ho}$	2	0.69 ± 0.04	Student's *t* test	*t*(1,22) = -13.91	**.027#**	r = .99
Olig2 WT	2	0.34 ± 0.01				
Olig2 2b42b5$^{he/ho}$	2	0.50 ± 0.06	Student's *t* test	*t*(2) = -3.54	.071	r = .93
Pdgfrα WT	2	0.11 ± 0.01				
Pdgfrα 2b42b5$^{he/ho}$	2	0.11 ± 0.01	Student's *t* test	*t*(1.99) = 0.43	.709	r = .29
WB semi-quantitative analysis	**N**	**Median ± IQR**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT P7 MBP	**4**	100 ± 0.9				
2b5^{ho} P7 MBP	**4**	30.5 ± 16.5	Mann-Whitney U test	*U* = 0	**.020**	r = .82
WT P14 MBP	**4**	100 ± 0.9				
2b5^{ho} P14 MBP	4	33.05 ± 41.95	Mann-Whitney U test	*U* = 0	**.020**	r = .82
WT P21 MBP	4	100 ± 0.9				
2b5^{ho} P21 MBP	4	43.15 ± 9.83	Mann-Whitney U test	*U* = 0	**.020**	r = .82
WT P28 MBP	3	100 ± 0.9				
2b5^{ho} P28 MBP	3	50 ± 31	Mann-Whitney U test	*U* = 0	**.050**	r = .80
WT 2-7m MBP	6	100 ± 0.9				
2b5^{ho} 2-7m MBP	6	57.85 ± 8.08	Mann-Whitney U test	*U* = 0	**.002**	r = .85
WT 2-7m MOG	3	207.25 ± 112.64				
2b5^{ho} 2-7m MOG	3	44.57 ± 12.69	Mann-Whitney U test	*U* = 0	**.050**	r = .80
WT 2-7m GFAPδ/GFAP	3	0.40 ± 0.04				
2b5^{ho} 2-7m GFAPδ/GFAP	3	0.65 ± 0.08	Mann-Whitney U test	*U* =0	.**050**	r = .80
	N	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT 2-7m GFAP	3	4098.83 ± 601.19				
2b5^{ho} 2-7m GFAP	3	4289.09 ± 983.48	Student's *t* test	*t*(4) = -0.29	.789	r = .14
WT 2-7m GFAPδ	3	1657.67 ± 155.05				
2b5^{ho} 2-7m GFAPδ	3	2869.33 ± 516.47	Student's *t* test	*t*(4) = -3.89	**.018**	r = .89
Cell counts - *Pdgfar*	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT 2-7m	14	5.97 ± 2.18				
2b5^{ho} 2-7m	13	5.72 ± 1.70	Student's *t* test	*t*(25) = 0.32	.749	r = .06
WT 2-7m	14	5.97 ± 2.18				
2b42b5$^{he/ho}$ 4m	6	7.17 ± 0.59	Student's *t* test	*t*(16.59) = -1.91	.074	r = .42
WT P21	3	12.8 ± 1.47				

2b4^{ho}*2b5*^{ho} P21	3	19.67 ± 0.83	Student's *t* test	*t*(4) = -7.03	**.002***	r = .96
Cell counts - *Plp*	**N**	**Median ± IQR**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT 2-7m	5	20.00 ± 2.55				
2b5^{ho} 2-7m	6	8.97 ± 1.00	Student's *t* test	*t*(9) = 16.14	**<.001***	r = .98
2b42b5$^{he/ho}$ 4m	4	11.75 ± 0.60	Mann-Whitney U test	*U* = 0	**.014***	r = .82
WT P21	3	20.60 ± 0.20				
2b4^{ho}*2b5*^{ho} P21	3	12.30 ± 6.50	Mann-Whitney U test	*U* = 0	**.046#**	r = .81
Cell counts - nestin	**N**	**Median ± IQR**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT P14	3	4.80 ± 0.90				
2b5^{ho} P14	3	6.20 ± 2.40	Mann-Whitney U test	*U* = 0	**.050#**	r = .80
WT P21	3	0.32 ± 0.20				
2b5^{ho} P21	3	7.30 ± 1.60	Student's *t* test	*t*(4) = -15.13	**<.001***	r = .99
WT 2-19m	24	0.57 ± 2.27				
2b5^{ho} 2-7m	15	5.85 ± 5.62	Mann-Whitney U test	*U* = 19	**<.001***	r = .75
WT 2-19m	24	0.57 ± 2.27				
2b4^{ho} 2-19m	16	6.1 ± 5.34	Mann-Whitney U test	*U* = 43	**<.001***	r = .65
WT 2-19m	24	0.57 ± 2.27				
2b42b5$^{he/ho}$ 4m	6	5.30 ± 3.05	Mann-Whitney U test	*U* = 10	**.001***	r = .59
		Mean ± SD	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT P21	5	0.38 ± 0.32				
2b4^{ho}*2b5*^{ho} P21	3	8.23 ± 1.67	Student's *t* test	*t*(2.09) = -8.08	**.013***	r = .98
Cell counts -Nkx2.2	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT 2-7m	17	5.43 ± 1.92				
2b5^{ho} 2-7m	9	5.02 ± 1.52	Student's *t* test	*t*(24) = 0.55	.589	r = .11
Electron microscopy	**N**	**Median ± IQR**	**Test**	**Test-statistic**	**p-value**	**Effect size**
Mean axonal diameter WT 7m	126	0.61 ± 0.37				
Mean axonal diameter *2b5*^{ho} 7m	446	0.33 ± 0.15	Mann-Whitney U test	*U* = 10135	**<.001***	r = .46
Mean axonal diameter mean *2b42b5*$^{he/ho}$ 4m	404	0.37 ± 0.18	Mann-Whitney U test	*U* = 11434.5	**<.001***	r = .41
Distribution axonal diameter WT 7m	92	0.61 ± 0.37				
Distribution axonal diameter *2b5*^{ho} 7m	100	0.33 ± 0.15	Pearson Chi-Square	$X^2(5)$ = 86.80	**<.001***	V = .67
Distribution axonal diameter *2b42b5*$^{he/ho}$ 4m	99	0.37 ± 0.18	Pearson Chi-Square	$X^2(5)$ = 68.64	**<.001***	V = .60
g-ratio WT 7m	126	0.78 ± 0.07				

g-ratio *2b5*^{ho} 7m	446	0.66 ± 0.11	Mann-Whitney U test	*U* = 9096	**<.001***	r = .49
g-ratio *2b42b5*$^{he/ho}$ 4m	404	0.72 ± 0.11	Mann-Whitney U test	*U* = 15738	**<.001***	r = .28
Astrocyte culture cell counts	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT astrocytes GFAP	3	47,19 ± 5.70				
2b4^{ho} astrocytes GFAP	3	43.81 ± 2.75	Student's *t* test	*t*(4) = 0.93	.406	r = .42
WT astrocytes CD11b	3	6.82 ± 7.56				
2b4^{ho} astrocytes CD11b	3	7.56 ± 5.34	Student's *t* test	*t*(4) = -0.14	.897	r = .07
WT astrocytes olig2	3	1.21 ± 0.19				
2b4^{ho} astrocytes olig2	3	1.20 ± 0.24	Student's *t* test	*t*(4) = 0.03	.976	r = .02
Astrocyte-OPC cultures cell counts	**N**	**Median ± IQR**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT astrocytes MOG	8	5.50 ± 5				
2b4^{ho} astrocytes MOG	8	3.33 ± 4	Wilcoxon signed rank	*Z* = -2.243	**.025**	r = .79
WT astrocytes MBP	8	11.7 ± 10				
2b4^{ho} astrocytes MBP	8	6 ± 4	Wilcoxon signed rank	*Z* = -2.38	**.017**	r = .84
WT astrocytes Olig2	7	23.21 ± 1.7				
2b4^{ho} astrocytes Olig2	7	20.02 ± 0.22	Wilcoxon signed rank	*Z* = -0.73	.463	r = .28
WT astrocytes GFAP	7	21.71 ± 9				
2b4^{ho} astrocytes GFAP	7	20.6 ± 13	Wilcoxon signed rank	*Z* = -0.09	.933	r = .03
		Mean ± SD	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT OPCs MOG	8	2.21 ± 2.41				
2b4^{ho} OPCs MOG	8	2.17 ± 2.14	Paired samples *t* test	*t*(7) = 0.10	.923	r = .04
WT OPCs MBP	8	8.81 ± 5.48				
2b4^{ho} OPCs MBP	8	7.66 ± 4.14	Paired samples *t* test	*t*(7) = 0.89	.405	r = .32
WT OPCs Olig2	6	15.99 ± 3.42				
2b4^{ho} OPCs Olig2	6	16.05 ± 4.46	Paired samples *t* test	*t*(5) = -0.05	.965	r = .02
WT OPCs GFAP	6	18.96 ± 3.84				
2b4^{ho} OPCs GFAP	6	18.65 ± 6.31	Paired samples *t* test	*t*(5) = 0.15	0,89	
Conditioned medium cell counts	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT ACM MOG	8	4.27 ± 4.15				
2b4^{ho} ACM MOG	8	0.96 ± 1.30	Paired samples *t* test	*t*(7) = 3.12	**.017**	r = .76
WT ACM MBP	8	8.84 ± 3.88				
2b4^{ho} ACM MBP	8	4.36 ± 3.04	Paired samples *t* test	*t*(7) = 4.94	**.002**	r = .78
WT ACM Olig2	8	43.06 ± 6.37				

2b4^{ho} ACM Olig2	8	37.96 ± 15.91	Paired samples *t* test	*t*(7) = 1.40	.205	r = .47
WT ACM GFAP	8	17.33 ± 5.30				
2b4^{ho} ACM GFAP	8	15.07 ± 7.91	Paired samples *t* test	*t*(7) = 0.96	.372	r = .34
Hyaluronidase treatment versus vehicle	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
Hyal treated ACM MBP	6	7.67 ± 2.58				
Vehicle treated ACM MBP	6	6.53 ± 2.07	Paired samples *t* test	*t*(5) = -2.66	**.045**	r = .77
Hyal treated ACM Olig2	6	22.75 ± 5.52				
Vehicle treated ACM Olig2	6	24.20 ± 3.85	Paired samples *t* test	*t*(5) = 1.43	.214	r = .54
Hyaluronidase difference scores	**N**	**Median ± IQR**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT ACM MOG	6	0.76 ± 9.87				
2b4^{ho} ACM MOG	6	2.92 ± 13.85	Wilcoxon signed rank	*Z* = -1.15	.249	r = .47
WT ACM MBP	6	1.27 ± 0.28				
2b4^{ho} ACM MBP	6	1.23 ± 0.2	Wilcoxon signed rank	*Z* = -.67	.500	r = .30
WT ACM Olig2	6	1.01 ± 0.63				
2b4^{ho} ACM Olig2	6	0.94 ± 0.27	Wilcoxon signed rank	*Z* = -.41	.686	r = .18
WT ACM GFAP	6	0.99 ± 0.31				
2b4^{ho} ACM GFAP	6	1.01 ± 0.11	Wilcoxon signed rank	*Z* =-1.26	.207	r = .51
Astrocyte-OPC cultures qPCR	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT astrocytes *Mog*	**6**	2.64 ± 0.50				
2b4^{ho} astrocytes *Mog*	6	1.72 ± 0.65	Paired samples *t* test	*t*(5) = 7.36	**.001**	r = .96
WT astrocytes *Mbp*	6	0.78 ± 0.36				
2b4^{ho} astrocytes *Mbp*	6	0.26 ± 0.28	Paired samples *t* test	*t*(5) = 5.03	**.004**	r = .91
WT astrocytes *Olig2*	6	0.02 ± 0.01				
2b4^{ho} astrocytes Olig2	6	0.01 ± 0.00	Paired samples *t* test	*t*(5) = 1.38	.228	r = .52
WT astrocytes *Gfap*	6	0.51 ± 0.18				
2b4^{ho} astrocytes Gfap	6	0.80 ± 0.45	Paired samples *t* test	*t*(5) = -2.35	.065	r = .72
Elisa	**N**	**Mean ± SD**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT P21 brain lysate	3	2103.33 ± 98.15				
2b4^{ho}*2b5*^{ho} P21 brain lysate	3	3405 ± 807.79	Student's *t* test	*t*(4) = -2.77	**.050#**	r = .82
WT 1m brain lysate	5	3129.55 ± 324.21				
2b5^{ho} 1m brain lysate	5	2548.73 ± 516.15	Student's *t* test	*t*(8) = 1.95	.092	r = 59
WT 4m brain lysate	5	1682.58 ±				

		651.32				
2b42b5$^{he/ho}$ 4m brain lysate	5	1942.38 ± 308.67	Student's *t* test	*t*(8) = -0.81	.444	r = 28
2b5^{ho} 4m brain lysate	5	1729.93 ± 526.10	Student's *t* test	*t*(8) = -0.11	.910	r = 04
WT 7m brain lysate	5	2118.22 ± 127.05				
2b5^{ho} 7m brain lysate	5	3584.81 ± 905.27	Student's *t* test	*t*(8) = -3.46	**.009***	r = .77
WT 19m brain lysate	5	1948.95 ± 690.74				
2b4^{ho} 19m brain lysate	5	2405.42 ± 532.47	Student's *t* test	*t*(8) = 1.17	.276	r = 38
	N	**Median ± IQR**	**Test**	**Test-statistic**	**p-value**	**Effect size**
WT ACM	6	21.75 ± 26.40				
2b4^{ho} ACM	6	24.25 ± 70.54	Mann-Whitney U test	U = 15	.631	r = .14

Significant values are given in bold. # = p<.05; * = significant at Bonferroni corrected p value (p<.017 for all tests performed on WT, *2b5*^{ho}, *2b4*^{ho} and *2b42b5*$^{he/ho}$; p<.013 for all tests performed on WT, *2b5*^{ho}, *2b4*^{ho}, *2b42b5*$^{he/ho}$ and *2b4*^{ho}*2b5*^{ho})

Table S5. Primer sequences for polymerase chain reactions

	Forward primer	Reverse primer
Gfap	AAGCCAAGCACGAAGCTAACGA	TTGAGGCTTTGGCCCTCC
Gfapα	GGAGATGCGGGATGGTGAG	ACCACGTCCTTGTGCTCCTG
Gfapδ	TCTCCAACCTCCAGATCCGA	TGACTTTTTGGCCTTCCCCT
nestin	CTACAGAGTCAGATCGCTCAG	AGCAGAGTCCTGTATGTAGC
Mbp	AAGGGAAGGGAGGAAGAG	GCAGTTATATTAAGAAGCCGAG
Plp	CTTCAATACCTGGACCACCT	GGGAGAACACCATACATTCTG
Mog	ACTTGTGCCTACGATCCTC	GGAGATTCTCTACTTCTGCAC
Olig2	TGTGGATGCTTATTACAGACC	ATCTAAGCTCTCGAATGATCC
Pdgfrα	CTGGAGAAGTGAGAAACAAAGG	TGGACAGAAATGGTGACTC
Cyp-b	AAGGACTTCATGATCCAGGG	TGAAGTTCTCATCTGGGAAG
Gapdh	GTGCTGAGTATGTCGTGGAG	TCGTGGTTCACACCCATCAC
Akt	AAGAAGGAGGTCATCGTCGC	GGTCGTGGGTCTGGAATGAG
Rps14	CAGGACCAAGACCCCTGGA	ATCTTCATCCCAGAGCGAGC

Movie S1. *2b5*^{ho} mice

Movie S2. *2b4*^{ho} mice

Movie S3. *2b4*^{ho}*2b5*^{ho} mice