Supplemental Table 1: Table of Antibodies

Antibody	Dilution	Provider	Product Number
Rabbit α Olig2	IHC: 1:1000	Millipore	ab9610
Rabbit α Ng2	IHC: 1:100	Millipore	MAB5384
Rat α MBP	IHC: 1:100	AbD Serotec	MCA409S
Rabbit α Lmnb1	IHC: 1:1000 WB: 1:5000	Abcam	ab16048
Rabbit α Pdgfra	IHC: 1:500 FC: 1:500	Cell Signalling	3174
Goat α Lmna/c	IHC: 1:100 WB:1:7500	Santa Cruz	sc-20681
Mouse α Actin	WB: 1:20000	Sigma	556321
Mouse α CC1	IHC: 1:300	Millipore	MABC200
Goat α Sox10	IHC: 1:100	Santa Cruz	sc-17342
Rabbit α Piezo1	IHC: 1:100 WB:1:500	Proteintech	15939-1-AP
Rabbit α Ha-Tag	IHC: 1:500	Cell Signalling	#3724
Mouse α Anti-Chondroitin Sulfate	IHC: 1:100	Sigma	C8035
Chicken α GFP	IHC: 1:300	Abcam	ab13970
Alexa Fluor 594 Goat α Mouse IgM	IHC: 1:500	Thermo Fisher	A-21044
Alexa Fluor 488 Donkey α Rabbit	IHC: 1:500	Millpore	A21206
Alexa Fluor 594 Donkey α Rabbit	IHC: 1:400	Molecular Probes	A21207
Alexa Fluor 594 Donkey α Goat	IHC: 1:500	Thermo Fisher	A-11058
Alexa Fluor 647 Donkey α Mouse	IHC: 1:500	Thermo Fisher	A-31571
IRDye800CW D anti-Goat	WB: 1:10000	Li-Cor	926_32214
IRDye800CW D anti-Mouse	WB: 1:10000	Li-Cor	926_32212
IRDye600CW D anti-Rabbit	WB: 1:10000	Li-Cor	926_38073
Goat α Olig2	IHC: 1:400 FC: 1:500	R & D	AF2418

Supplemental Table 2: Table of small molecules

Small Molecule	Concentration	Provider	Product Number
Y-27632	10-50µM	Sigma	Y0503
blebbistatin	5uM	Sigma	B0560
Bapta-AM	5uM	Sigma	A1076

Supplemental Table 3: Table of Primers

Gene	Purpose	Forward	Reverse	Provider
LMNB1	qPCR	5'-cagattgagtatgagtacaagc – 3'	5'-agtggaagtgttcatctctg - 3'	Sigma
LMNA	qPCR	5' – ctacagcaaacactaaggaac – 3'	5'- tttttcgggatggaaacaac -3'	Sigma
TBP	qPCR	5'- catcatgagaataagagagcc – 3'	5' – ggattgttcttcactttgg – 3'	Sigma
LMNB1	cDNA Amplification	5'-taatacgactcactatagggtacct tcggt-3'	5'-gaagatcgaccatgtcttgacaagt- 3'	Sigma
LMNA	cDNA Amplification	5'-taatacgactcactatagggccga ggtgcgccagcgcc -3'	5'-tggcattccaaaacactttaatgaaaa gactttggcatggaggc-3'	Sigma
Minicircle Backbone	Minicircle Production	5'-ggcccgccccaactggggtaacct ttga-3'	5'-gaatcatgggaaataggccctccgccg agtgaagtcagcatgaggggcggcgc ccggggagcccaa-3'	Sigma. Plasmid from addgene #87114
U6	Minicircle	5'-gagggcctatttcccatgattcc-3'	5'-ccggtgtttcgtcctttcc -3'	Sigma. Plasmid from addgene #48138
shPiezo1 Construct	Minicircle	5'-tggaaaggacgaaacaccggtgg atgtgtgtggaagacattcaagag atgtcttccacacacatccatttttt ctagagggtaccggggcccggtcg ac -3'	5'-aactagtcaataatcaatgtcggaact ccatatatgggctatgaactaatgaccc cgtaattgattactattaataactagtc gaccgggccccggta-3'	Sigma
shControl	Minicircle	5'-gtggaaaggacgaaacaccggca acaagatgaagagcaccaactcg agttggtgctcttcatcttgttgtttt tctagagggtaccggggcccggtc gac-3'	same as above	Sigma

CMV	Minicircle	5′-gacattgattattgactagttattaatagtaatcaattacggggtcattagttca-3′	5′-ggtgaacagctcctcgcccttgctcaccatggtggcgctagcctgcttatatagacctcccaccgtacacgc-3′	Sigma. Plasmid from addgene #48138
GFP Polya	Minicircle	5′-atggtgagcaagggcgaggagctgtt-3′	5′-gttaccccagttggggcgggccctccccagcatgcctgctattctcttcccaatc-3′	Sigma. Plasmid from addgene #48138
Gfp	qPCR	5′-aagctgaccctgaagttcatctgc-3′	5′-cttgtagttgccgtcgtccttgaa-3′	Sigma
Cas9	qPCR	5′-aaacagcagattcgcctgga-3′	5′-tcatccgctcgatgaagctc-3′	Sigma
Cspg4 Promoter	Cloning	5′-cctgaagctgttaggtaggagc-3′	5′-cggtaccttctcgaaactcc -3′	Sigma
Piezo1 Locus	Surveyor Assay	5′-acacagacccagacgctgct-3′	5′-atactggaaaagagctccgacacact-3′	Sigma
GFP-Pdgfra PCR	Positional PCR	5′-aagctgaccctgaagttcatctgc-3′	5′-aatgctggagttgtctgcagtacaag-3′	Sigma
Off-target site 1	Surveyor Assay	5′-catcagatctcttggaaagt-3′	5′-ccactgtgagatcaaacc-3′	Sigma
Off-target site 2	Surveyor Assay	5′-cagaagggtttaattgaag-3′	5′-atatgcttttgtagtacaggag-3′	Sigma
Off-target site 3	Surveyor Assay	5′-gcactttactgacttagctatc-3′	5′-gcagtacagagaagttattgac-3′	Sigma
Off-target site 4	Surveyor Assay	5′-cgtaagactattggaatatgtc-3′	5′-catatagaacctttaggagatg-3′	Sigma
Off-target site 5	Surveyor Assay	5′-agagtgtcactagtgctttaga-3′	5′-tgtgtcaagagtaaactcagat-3′	Sigma
Piezo1 mRNA for *in vivo* crispr	qpcr	5′-ctggtcaccggcatctacgtca-3′	5′-gaagaggaacatgtagacgattttgtagaccacc -3′	sigma
gRNA reverse transcription	gRNA qPCR	5′- aagcaccgactcggtgccac-3′	N/A	Sigma

Supplemental Table 4: Gene fragments

Description	Sequence	Supplier
U6, Pdgfra	tgtacaaaaaagcaggctttaaaggaaccaattcagtcgactggatccggtaccaaggtcgggcaggaagagggcctatttcccatgattccttcatatttgcatatacgatacaaggctgttagagagataattagaattaatttgactgtaaacacaaagatattagtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggacgaaacaccgcgatctgaactcacagtgggttttagagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttctagacccagctttcttgtacaaagttggcgtttaaac	IDT
Ribozyme, non-TargetgRNA	gcggccgcaaaggtttttctttttcctgagaaatttctcaggttttgctttttaaaaaaaaagcaaaagacgctggtggctggcactcctggtttccaggacggggttcaagtccctgcggtgtctttgcttgaattcaaatcgctgatgagtccgtgaggacgaaacgagtaagctcgtctgtattactgatattggtgggtttagagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttgccggcatggtcccagcctcctcgctggcgccggctgggcaacatgcttcggcatggcgaatgggacgaataaaagatctttattttcattagatctgtgtgttggttttttgtgtgccgccactgtgagttcagatcgc	IDT
Ribozyme, Piezo1gRNA	gcggccgcaaaggtttttctttttcctgagaaatttctcaggttttgctttttaaaaaaaaagcaaaagacgctggtggctggcactcctggtttccaggacggggttcaagtccctgcggtgtctttgcttgaattcaaatcgctgatgagtccgtgaggacgaaacgagtaagctcgtctcgattttgtagaccaccaggggttttagagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgccggcatggtcccagcctcctcgctggcgccggctgggcaacatgcttcggcatggcgaatgggacgaataaaagatctttattttcattagatctgtgtgttggttttttgtgtgccgccactgtgagttcagatcgcz	IDT
Nested CRISPR System	tacaaagttggcgtttaaacccgccactgtgagttcagatcgcgaattcaaatcgctgatgagtccgtgaggacgaaacgagtaagctcgtctcgattttgtagaccaccagggttttagagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgccggcatggtcccagcctcctcgctggcgccggctgggcaacatgcttcggcatggcgaatgggacgaataaaagatctttattttcattagatctgtgtgttggttttttgtgtgcgattttgtagaccaccaggcgg	IDT

Supplemental Table 5: Figure 1

GFP+,EdU+/Olig2	Neonate to Neonate Transplantation	Aged to Neonate Transplantation
Animal 1	0.222222222	0.152380952
Animal 2	0.141104294	0.135135135
Animal 3	0.122641509	0.157407407
Animal 4	0.134920635	0.152866242

GFP+,EdU+/Olig2	Neonate to Neonate Transplantation	Aged to Neonate Transplantation
Animal 1	0.182692308	0.207920792
Animal 2	0.190839695	0.207207207
Animal 3	0.166666667	0.192771084
Animal 4	0.193133491	0.213211085

EdU+Olig2+/mm^2	+Pencillinase	+chABC
Animal 1	3.16	33
Animal 2	15.77	29
Animal 3	6.71	49
Animal 4	14	36

CC1+Olig2+/mm^2	+Pencillinase	+chABC
Animal 1	28.479	130.4
Animal 2	51.26	128.85
Animal 3	31.87	180
Animal 4	44.51	72.17

Stiffness (Pa)	Neonate	Young Adult	Aged
Animal 1	272.1431628	364.0049495	443.3943284
Animal 2	229.3041935	375.464321	482.7785185
Animal 3	243.3769333	367.8861731	488.7230769

Supplemental Table 6: Figure 2

EdU+/Olig2+	shControl	shPiezo1
Animal 1	0.019607843	0.144230769
Animal 2	0.027522936	0.223300971
Animal 3	0.03960396	0.130841121

	Neonate				Adult			Aged	
OPC, Olig2+CC1-	0.642857143	0.774193548	0.717391304	0.677419355	0.571428571	0.782608696	0.838709677	0.766666667	0.733333333
Oligo, Olig2+CC1+	0.75	0.857142857	0.866666667	0.053763441	0.2	0.196428571	0.04109589	0.028169014	0.131147541

Supplemental Table 7: Figure 4

EdU+GFP+/Olig2+	NT Control	Piezo1 Knockout
Animal 1	0.106382979	0.402877698
Animal 2	0.095890411	0.30625
Animal 3	0.209459459	0.285714286

Oig2+CC1+/mm^2	NT Control	Piezo1 Knockout
Animal 1	72.91608392	117.3287645
Animal 2	63.213	129.602845
Animal 3	55.94405594	118.5499632

EdU+/Pdgfra+	NT Control	Piezo1 Knockout
Animal 1	0.009174312	0.047619048
Animal 2	0.014814815	0.044334975
Animal 3	0.009174312	0.048611111

Supplemental Table 8: Extended Data 4

	cortex-bleb	cortex-DMSO
Max	1050.4	752.68
Min	76.877	93.99
Median	278.68	335.265
Mean	284.8131439	339.7734862
Lower Quartile	194.4225	245.87
Upper Quartile	353.8025	426.42

EdU+/Olig2+,CC1-	**DMSO**	**+5µM Bleb**
Amimal 1	0.052631579	0.06779661
Animal 2	0	0.058823529
Animal 3	0	0.1

CC1+Olig2+/mm2	**DMSO**	**+5µM Bleb**
Animal 1	192.3076923	431.372549
Animal 2	57.3172381	233.7541846
Animal 3	163.56	320.6509003

Supplemental Table 9: Extended data 8

Cerebellum		Corpus Callosum		Grey Matter	
GFP+/Olig2+	Olig2-/GFP+	GFP+/Olig2+	Olig2-/GFP+	GFP+/Olig2+	Olig2-/GFP+
0.358108108	0.037735849	0.484042553	0.010989011	0.307291667	0
0.338582677	0.093023256	0.563876652	0	0.276785714	0.129032258
0.253623188	0.114285714	0.537931034	0	0.3125	0

Supplemental Table 10: Extended Data 9

Fluoromyelin (area covered)	**GFP**	**KD**
Animal 1	0.157099464	0.214621912
Animal 2	0.055895848	0.308789912
Animal 3	0.031865962	0.349963891

	GFP	**KD**
Max	342.52	316.845
Min	1.01	7.142
Median	51.515	90.69126101
Mean	58.17954379	114.545587
Lower Quartile	30.83825	51.491
Upper Quartile	82.90854379	130.2826305

Supplemental Table 11: Extended Data 10

Pdgfra+,Olig2+/mm^2	**Control**	**Piezo1**
Animal 1	231.4755061	465.4169934
Animal 2	272.6782635	550.9310275
Animal 3	206.5609921	397.2654443

CC1+,Olig2+/mm^2	**Control**	**Piezo1**
Animal 1	231.4755061	465.4169934
Animal 2	272.6782635	550.9310275
Animal 3	206.5609921	397.2654443

Supplemental Notes:

Models of demyelination-remyelination

The ethidium bromide model is a well-established model of demyelination-remyelination in which low dose ethidium bromide (EtBr) in injected into the large white matter tracts of the caudal cerebellar peduncle (CCP). In this model, the peak of OPC recruitment occurs around day 7 after lesion induction, but thereafter there is delayed differentiation into Olig2+, CC1+ remyelinating oligodendrocytes in aged animals compared to young adult animals[1]. As such, all small molecules/enzymes throughout the text injected into the lesion were injected at day 7 so as to target the highest number of OPCs. An alternative model we have used is to inject lysolecithin into the spinal cord white matter of adult mice. As with the EtBr model, demyelination induced in this way follows a stereotypic temporal pattern of remyelination, with remyelination ongoing at 14 days after lesion-induction making this a suitable time point to examine interventions that alter the rate of remyelination.

Hydrogel substrate effects

The loss of proliferation was surprising given that neonatal OPCs can proliferate on poly d-lysine (PDL)-coated tissue culture plastic (Extended Data Fig. 1b-c). However, we observed that the long-term activity of neonatal OPCs on PDL-coated plastic was largely isolated to spheres that detached from the substrate, suggesting that PDL-coated plastic itself may not be sufficient for maintenance of OPC activity but relies on the instability of ECM attachment. The hydrogels on the other hand have covalently bound ECM, and are therefore more stable, and do not have floating spheres even after 12 days in culture. The stiffness-mediated activation of aged progenitor cells.

In vivo perturbation of actomyosin contractility

To determine the effect of actin contractility on the stiffness of the CNS, we bathed freshly vibratomed aged CNS in 5μM of blebbistatin for 30 minutes. We found that blebbistatin treatment significantly softened the aged brain compared to brains treated with only DMSO (Extended Data Fig. 4f). As blebbistatin alone softened the aged CNS, we next wanted to test the role of actin contractility on the adult CNS *in vivo*. To do so, we first injected blebbistatin into un-lesioned forebrain of 14 month old female rats. In the homeostatic aged CNS, there are almost no cells proliferating 10 days following a control delivery of DMSO, while in the blebbistatin injected brain, a small but significant proportion of OPCs were labelled with EdU (Extended Data 4g-h). To determine the effect of blebbistatin following oligodendrocyte loss, we injected 5μM blebbistatin into areas of experimentally-induced demyelination to perturb OPC contractility (Extended Data 4i). We note that blebbistatin will affect all cells in the area of the lesion. At 14 days post-lesion, we observed more than 3 times the number of Olig2+/CC1+ differentiated oligodendrocytes in lesions treated with blebbistatin than in controls in each aged animal (N=3) (Extended Data 4j-k). Taken together with the *in vitro* data of blebbistatin-treated OPCs, these data suggest that inhibiting actomyosin contractility-mediated mechanotransduction has a positive effect on the activation and subsequent differentiation of aged OPCs.

Nuclear Lamina Changes

Nuclear Lmna is mutated in Hutchinson Gilford Progeria Syndrome[2], a disease in which aging is accelerated. Using qPCR, Western blot, and immunocytochemistry we found that the expressions of Lmna and Lamin A/C in OPCs progressively and significantly increase with OPC aging (Extended Data Fig. 5a-c). The changes are dramatic, with Lmna decreasing by 16-fold. We correspondingly found that aged OPCs on soft substrates had significantly lower levels of Lmna (Lamin A/C) compared to aged OPCs plated on stiff hydrogels (Extended Data 5d-g).

Our qPCR data showed an age-correlated increase in expression of *Lmna* as well as Lamin A/C. We subsequently knocked down *Lmna* of aged OPCs on stiff hydrogels, with negligible effects on aged progenitor cells (Extended Data 5h-k). However, mRNA overexpression of Lamin C, the dominant *Lmna* splice variant in OPCs, in neonatal OPCs on soft hydrogels led to a significant loss of neonate OPC activation (Extended Data 5l-p), suggesting that Lamin A/C plays a role in mediating mechanically induced aging in OPCs.

Piezo1 in multiple organisms

Additional comment is warranted about Piezo1 and its expression in OPCs across multiple organisms. Here, we have shown that OPCs express Piezo1 in human and rat, along with mouse. Nevertheless, several sources have suggested that Piezo1 is not expressed in mouse OPCs. Using RNA-scope we found that Piezo1 is indeed widely expressed in Pdgfra+ OPCs in both white and grey matter. Moreover, using new nuclear sequencing techniques, multiple groups have detected abundant Piezo1 mRNA in human gray and white matter We believe that the discrepancy in these findings is the low number of OPCs and shallow sequencing depth captured in most whole brain murine single cell sequencing studies[5,6], with many studies detecting far less than 1000 expressed genes in adult OPCs. In unpublished single-cell sequencing of our own, we have identified abundant Piezo1 mRNA in adult rodent OPCs. Our protein and qPCR analysis, along with newer studies with more sensitive adult single cell-sequencing studies of adult human cortex (Extended Data Fig. 6h) overwhelmingly confirm that Piezo1 is abundantly expressed in adult OPCs.

Cas9-Mediated Knock-in

OPCs are primary cells that cannot grow clonally, are slowly dividing, do not readily undergo homologous recombination (data not shown), and have a small cytoplasm to nucleus ratio, making them hard to transfect; as such, common-use genetic and viral approaches are inefficient in OPCs. To overcome these limitations to efficiently perturb gene expression in OPCS, we generated a system using Cas9 mRNA, transcribed gRNAs, and HITI-mediated minicircle vectors to efficiently knock in a Piezo1 shRNA-GFP over expression cassette into the unused Tubb3 locus (Extended Data Fig. 7a-e)[7]. gRNA-mediated knockdowns give rise to heterogeneous levels Indels of the target-gene expression across a population of cells, whereas the shRNA-GFP provides for a characterizable monotonic knock-down across the pool of cells expressing the GFP.

In vivo CRISPR overview

To knock-down Piezo1 in OPCs in the post-natal CNS, we developed two dual-AAV CRISPR systems. Using ribozymes, we were able to drive the expression of Piezo1 gRNA from cell-type specific promoters and efficiently knock-down Piezo1 levels in OPCs in both the neonatal and aged CNS. To efficiently target the CNS, we made use of the novel PHP-EB AAV serotype.

OPC-Specific Piezo1 protein knock-down

In order to test the CRISPR constructs used to knock down Piezo1 in OPCs *in vivo*, we first confirmed that the constructs were able to knock down Piezo1 protein *in vitro*. To do so, we needed to use an *in vitro* cell line that would yield enough protein for a quantitative Western blot and would be easy to transfect. We found that acutely isolated OPCs from AAV-infected animals yielded insufficient material for quantitative protein analysis. Moreover, *in vitro* neonatal OPCs were inefficiently transfected with our large CRISPR constructs using lipid-based transfection methods. As such, we required an additional, easy-to-manipulate *in vitro* cell-type to model the capacity of our CRISPR constructs to reduce Piezo1 protein levels. Our *in vivo* CRISPR constructs are dependent on Pdgfra or Cspg4 expression for gRNA transcription, so we required a specific cell-type that expressed both these genes along with Piezo1 for the modelling of our *in vivo* CRISPR efficacy. Mouse Embryonic Fibroblasts (MEFs) express all three of these genes and are efficiently transfected by nucleofection, as we first showed by electroporation of a control GFP-overexpression cassette. Using MEFs, we electroporated our *in vivo* CRISPR constructs and found that our *in vivo* CRISPR systems efficiently reduced Piezo1 protein levels in MEFs *in vitro* after 5 days. Together, these results confirm our *in vivo* DNA and RNA analysis that show that Piezo1 is efficiently targeted and knocked-down with our *in vivo* CRISPR constructs.

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