Young CSF restores oligodendrogenesis and memory in aged mice via Fgf17

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Abstract: Recent understanding of how the systemic environment shapes the brain throughout life has led to numerous intervention strategies to slow brain ageing1,2,3. Cerebrospinal fluid (CSF) makes up the immediate environment of brain cells, providing them with nourishing compounds4,5. We discovered that infusing young CSF directly into aged brains improves memory function. Unbiased transcriptome analysis of the hippocampus identified oligodendrocytes to be most responsive to this rejuvenated CSF environment. We further showed that young CSF boosts oligodendrocyte progenitor cell (OPC) proliferation and differentiation in the aged hippocampus and in primary OPC cultures. Using SLAMseq to metabolically label nascent mRNA, we identified serum response factor (SRF), a transcription factor that drives actin cytoskeleton rearrangement, as a mediator of OPC proliferation following exposure to young CSF. With age, SRF expression decreases in hippocampal OPCs, and the pathway is induced by acute

injection with young CSF. We screened for potential SRF activators in CSF and found that fibroblast growth factor 17 (Fgf17) infusion is sufficient to induce OPC proliferation and long-term memory consolidation in aged mice while Fgf17 blockade impairs cognition in young mice. These findings demonstrate the rejuvenating power of young CSF and identify Fgf17 as a key target to restore oligodendrocyte function in the ageing brain.

One Sentence Summary: Direct brain infusion of young CSF to aged mice restores memory and rejuvenates oligodendrocyte progenitor cells.

Main Text: Brain aging underlies dementia and neurodegenerative diseases, imposing an immense societal burden. Systemic interventions in model organisms have shown great promise in reversing aging related decline of various tissues, including the brain[1]. For example, heterochronic parabiosis and young plasma transfer rejuvenated the aged brain and restored memory function [2-5]. Nevertheless, the brain is protected with barriers which may limit access of these factors, presumably impeding their rejuvenation potential. Cerebrospinal fluid (CSF), which is in close association with brain cells, carries signals that instruct neuronal progenitor proliferation and specification during development [6]. However, CSF protein composition changes with human aging [7], marked by an increase in inflammatory proteins [8] and decrease in growth factors such as BDNF [9]. Whether these CSF changes contribute to age-related cognitive decline is unknown. Testing this functionally by performing CSF transfers *in vivo* has been difficult due to technical limitations in CSF collection and direct CSF infusion to the brain. We hypothesized that intracerebroventricular (i.c.v) administration of young CSF to aged mice will have rejuvenating effects on the brain (Extended data Fig. 1).

We first sought to test if young CSF infusion to aged mice could improve aging-related impairments in hippocampal dependent learning and memory tasks [10]. 20-month-old mice received three-foot shocks associated with a tone and a flashing light. Mice were then randomly split to two groups and infused with either artificial CSF (aCSF) or young mouse CSF (YM-CSF) for one week and remote memory recall was tested three weeks after memory acquisition. YM-CSF infusion resulted in higher average freezing rates following exposure to the tone and light, suggesting better preservation of the remote fear memory (Fig. 1a). Because the hippocampus is central to age-related cognitive decline and is in close apposition to CSF, we measured the effect of young CSF infusion on the hippocampal transcriptome by RNA sequencing. Differential gene expression analysis between aCSF and YM-CSF identified 271 differentially expressed genes (DEGs) significantly altered (FDR<0.1) after young CSF treatment with 115 down- and 156 upregulated (Supplementary Table 1). Strikingly, oligodendrocyte genes were highly upregulated identifying this cell type as a major cellular substrate for CSF. Specifically, young CSF promoted upregulation of transcription factors driving oligodendrocyte differentiation and major myelin protein components (for example, *Olig1, Myrf, Mag, Mbp* and *Mobp*) (Fig.1b).

Neuronal activity, induced by optogenetic tools or by learning tasks, was shown to promote OPC proliferation and differentiation, as well as to regulate myelin plasticity in mature oligodendrocytes (termed activity-dependent myelination) [11,12]. To determine whether OPC proliferation could be underlying the transcriptomic signature observed following young CSF infusion, we labeled dividing cells in the last 2 days of young CSF infusion with the thymidine analogue EdU. Interestingly, while overall cell proliferation was very low in these aged brains, we discovered a surge in overall cell proliferation specifically in the hippocampus, relatively distant

from the infusion site (Extended data Fig. 2). Young CSF induced a 2.35-fold increase in the percentage of proliferating OPCs (EdU$^+$ Pdgfrα^+/ Pdgfrα^+) in the CA1 region of the hippocampus but not in the cortex (Fig. 1c and Extended data Fig. 3). In a different cohort, we allowed these proliferating cells to mature for 3 weeks to assess effects of young CSF on hippocampal myelination by MBP staining. We found an increase in MBP intensity in the molecular layer of the hippocampus (Fig. 1d-e). This indicates that young CSF contains activators of cell growth for the oligodendrocyte lineage or substances that neutralize inhibitory factors. Similar to activity-dependent myelination, re-exposure of the aged brain to young factors induced hippocampal myelination.

To assess if young CSF can stimulate OPC proliferation and differentiation directly, we used an established primary rat OPC culture system [13,14]. Cells were grown in full proliferation media supplemented with human CSF pools (three pools as replicates, each pool from three young adult healthy males (YH-CSF (mean age 24.6) or aCSF as control). We used human instead of rodent CSF because we can obtain larger volumes for these studies. Similar to the *in vivo* infusions, BrdU pulsing confirmed a dose-dependent increase in OPC proliferation (Fig. 1f-g). Excitingly, when OPCs were deprived of mitogens to promote cell differentiation over 4 days, YH-CSF not only induced a 2-fold increase in cell survival – a process in which a significant fraction of cells typically undergo apoptotic cell death [15,16] – but also promoted a prominent expansion of the more differentiated mature cell morphology [13] with an overall increase in MBP intensity per cell (Fig. 1h-i, Extended data Fig. 3h and Supplementary Video S1-2). These results are in line with previous work showing that human CSF from young healthy patients induced neuronal viability [17,18] while CSF from multiple sclerosis patients was toxic to neuronal [19] and OPC cultures [20].

To gain a deeper mechanistic understanding of the cellular processes induced by young CSF in OPCs we metabolically labeled nascent mRNA with 4-thiouridine (s^4U) using thiol(SH)-linked alkylation and sequenced RNA (SLAMseq) [21] from cultured OPCs 1- or 6 hr after exposure to YH-CSF (Fig. 2a, Extended data Fig. 4a-e and Supplementary Table 2). The top gene induced after 1 hr was Serum Response Factor (SRF) (Fig. 2b), a transcription factor studied extensively in skeletal muscle [22], heart [23] and in neurons in the brain [24]. It binds to the serum response element (SRE) promoter sequences to induce cell motility, proliferation and differentiation through modulation of immediate early genes and the actin cytoskeleton [25]. We also noted a dramatic downregulation of the negative regulator of Wnt signaling and pro-apoptotic factor Bcl7b [26] as well as the DNA repair protein Rpa3, in line with an overall pro-survival response following YH-CSF exposure. Nascent SRF mRNA transcripts peaked at 1 hr and returned to baseline by 6 hr (Fig. 2c). Strikingly, many of the DEGs peaking at 6 hr are known SRF target genes (Fig. 2d, marked in red). Indeed, the most strongly increased genes were enriched as "target genes of SRF" based on gene set enrichment analysis (GSEA) and the TRANSFAC database [27] (Extended data Fig. 4f). The combined log2FC of the TRANSFAC SRF predicted targets (423 genes), validated SRF targets (74 genes, curated list from literature) and actin genes (212 genes) in the dataset indicated an overall activation of the SRF pathway and actin cytoskeleton transcripts (Extended data Fig. 4g and Supplementary Table 3). To validate these findings at the protein level, we exposed OPCs to YH-CSF in the presence of SiR-actin, a fluorescent probe used to label actin filaments with live imaging or fixed and stained with the actin filament dye phalloidin [28]. Within hours of YH-CSF stimulation, SiR-actin intensity increased, without change in total area, indicating an increase in cellular actin filament levels (Extended data Fig. 5a-c). In fixed cells,

OPCs exposed to YH-CSF for 6 hrs had double phalloidin mean intensity staining per cell than controls (Fig. 2e). Because SRF is necessary for the formation of actin filaments in axonal growth cones in neurons [29], we tested if it has a similar role in OPCs and quantified the number of growth cones per OPC. We found YH-CSF induced significantly more growth cones per cell (Extended data Fig. 5d). Lastly, using OPC primary cultures from SRF-floxed mice, infected *ex-vivo* with an AAV expressing CRE-GFP to create SRF-KO OPCs or a deleted version of CRE-GFP as control, we repeated the YH-CSF proliferation experiment and showed that CSF induced proliferation is dependent on SRF (Fig, 2f-g). These results shed light on the possible mechanisms underlying the effect of young CSF on OPCs, and point towards SRF and actin cytoskeleton regulation, as potential mediators of young CSF effects *in vivo*.

Deletion of SRF signaling specifically in muscle cells was shown to lead to accelerated aging phenotypes in skeletal muscle of mice [30-32] and worms [33]. We thus asked whether SRF signaling may be downregulated in OPCs in the aging brain. Indeed, the fraction of Srf-positive oligodendrocytes (Srf^{+} $Pdgfr\alpha^{+}$/ $Pdgfr\alpha^{+}$) in the CA1 region of the hippocampus detected by in situ hybridization decreased dramatically with age (Fig. 3a). To expand this analysis to other SRF targets and cellular processes, we sorted hippocampal OPC and OL nuclei by expression of the oligodendrocyte transcription factor Olig2 ($Olig2^{high}$ for OPCs and $Olig2^{low}$ for OLs) from young (3m) and aged (25m) mice and performed bulk RNAseq (Fig. 3b-c). The top pathways downregulated in OPCs with aging were related to oligodendrocyte cell markers, regulation of glial cell differentiation, cellular respiration and metabolism and protein folding. Conversely, immune-related pathways and microglia-specific genes were upregulated as previously reported for OPCs in aging [34] and multiple sclerosis [35] (Fig. 3d-e and Extended data Fig. 6a-b). A focused analysis of SRF TRANSFAC genes indicated an overall downregulation with age (Fig. 3f, left graph).

We next aimed to test if young CSF induces SRF pathway activation in the aged brain. Due to the transient nature of SRF induction in the SLAMseq experiment, we designed an acute injection paradigm where CSF was injected into the lateral ventricle of 18 month-sold mice and hippocampi were dissected 1 and 6 hours post injection for OPC and OL nuclei RNAseq. We found again many predicted target genes of SRF (based on TRANSFAC) in OPCs were upregulated in both timepoints (Fig. 3f and Extended data Fig. 7a) although SRF itself was not among them. These genes were predicted to function both upstream and downstream of SRF in "Regulation of GTPase activity" and "protein kinase activity" or "chromatin organization", "transcription factor binding", "cell cycle" and "regulation of cytoskeleton organization", respectively (Fig. 3g-h and Extended data Fig. 7b-d). Among the notable genes linked to these pathways are the transcription factor Stat3, which was previously shown to mediate remyelination [36], and Kras which was linked to downstream Srf activation [37] (Fig. 3i-j). Interestingly, among the top-regulated genes at the 1hr timepoint was CD68 which is a lysosome-endosome gene involved in phagocytic activities in immune cells, potentially indicating that OPCs are phagocytosing and processing young CSF proteins (Fig. 3k). Notably, SRF target genes were also downregulated in other published human and mouse datasets of OPCs in aging [38] and Alzheimer's disease (AD) [39,40] (Extended data Fig. 6c). Altogether, these experiments indicate that SRF signaling is downregulated with aging and induced following *in vivo* CSF infusion.

CSF contains hundreds of proteins that could potentially induce SRF signaling. We noticed that several SRF target genes are also known upstream inducers of SRF itself such as BDNF and IGF1 [41,42]. Based on this observation, we cross-referenced two published CSF proteomic datasets [9,43] with the predicted TRANSFAC SRF target list and generated a list of 35 potential SRF inducers (Table 1). To test their activity, we transfected HEK293 cells with an SRE-GFP reporter and added the proteins at different concentrations (Fig. 4a). Fibroblast growth factor 8 (Fgf8) and Fgf17 induced the strongest dose-dependent responses (Fig. 4b-d and Extended data Fig. 8a). To study which receptor could mediate this effect reporter cells were pre-incubated with blocking antibodies for Fgfr1, Fgfr2 or Fgfr3 before incubation with 200 ng/ml Fgf17. We found that Fgf17 SRE-activation was dependent on signaling through Fgfr3 (Fig 4e). When added to primary rat OPCs, Fgf8 (20 ng/ml) and Fgf17 (40 ng/ml) induced OPC proliferation (Fig. 4f-g and Extended data Fig. 8b) and Fgf17 induced the transcription of genes associated with cell morphogenesis, migration and cytoskeleton organization (Fig. 4h). We further demonstrate that Fgf17 induced proliferation of WT but not SRF-KO mouse OPCs, indicating that, as with young CSF, the boost in proliferation is dependent on SRF (Fig. 4i). Interestingly, FGF17 levels decrease with age in human CSF [9] (Fig. 4j). To determine the *in vivo* activity of Fgf8 and Fgf17, we infused recombinant proteins acutely or over 7 days exactly as with CSF administration (Fig 1-3) into aged mice. We showed that Fgf17 induces OPC proliferation in the aged hippocampus but Fgf8 does not (Fig. 4k and extended data Fig. 8c). We next tested the effect of Fgf17 infusion on cognition and found that it improves long-term memory performance in the remote memory recall paradigm (Fig. 4l). These experiments demonstrate that Fgf17 is sufficient to mimic the effect of young CSF on OPCs in the hippocampus and memory in aged mice.

In summary, we uncover that hippocampal OPCs proliferate and differentiate in response to young CSF-derived cues in the aged brain, coinciding with beneficial effects on remote memory consolidation. Moreover, young CSF induces expression of the transcription factor SRF, and its actin cytoskeleton target genes to promote OPC proliferation. SRF is a versatile regulator of neuronal development [29], activity dependent plasticity [44,45] and regeneration [46,47]. Yet, we find that it is widely expressed by OPCs, where it is downregulated with aging. Lastly, we show that Fgf17, a growth factor decreased in human CSF with aging, induces OPC proliferation in the aged brain. It is interesting to speculate that SRF orchestrates plasticity in various cell types and its loss with aging results in neurodegeneration. Expanding our examination of SRF signaling in oligodendrocytes with aging to other cell types could illuminate complex regulatory interactions within parenchymal cells and with the environment during development, aging and aging-related diseases.

The CSF proteome consists of proteins secreted by the choroid plexus or transferred through it from the blood plasma, as well as proteins secreted from parenchymal and immune cells. Improper signaling cues derived from the aging choroid plexus were shown to lead to neuronal stem cell quiescence with aging [48]. However, OPCs, which account for the largest population of stem cells in the aged brain, were mostly overlooked. Emerging studies in young rodents are revealing that oligodendrogenesis, the formation of myelinating oligodendrocytes from OPCs, is essential for consolidation of newly formed memories, implicating their active role in cognitive function [11,49-51]. A recent study showed that hippocampal oligodendrogenesis is dramatically inhibited with age and that boosting it was sufficient to improve performance in learning and memory tasks [52]. This is in line with studies showing that aged OPCs in white matter regions, are

slow to proliferate and to differentiate following demyelination in diseases such as multiple sclerosis [53], and that local or systemic environmental manipulations restored their myelination capacity [54,55]. It is possible that these systemic therapeutic strategies affect OPCs through changes in the CSF composition. As a proof of concept, we infused Fgf17 to the CSF of aged mice and found that it partially phenocopied the effects of young CSF on OPC proliferation and long-term memory recall. Interestingly, young mice lacking Fgf17 have an array of social behavior abnormalities coinciding with lower *c-fos* expressing cells in the prefrontal cortex following a novel social interaction test [56]. Further research is needed to demonstrate if SRF, which regulates *c-fos*[57] expression, is involved in those circuits and potentially in neuropsychiatric disorders. Fgf17 is critical for embryonic brain development [58,59] but not much is known about its function in the adult nervous system. Searches we performed in recent transcriptomic and proteomic datasets suggest that Fgf17 is a brain derived protein, which in the adult mouse and human brain is expressed by a small subset of cortical neurons and by the choroid plexus epithelial cells (Extended data Fig. 9). This strengthens the notion that studying the CSF, the media that has adapted to the unique needs of the brain, holds the potential to expand our understanding of its intricate mechanisms and cellular interactions.

Combined, our results suggest that targeting hippocampal myelination through factors present in young CSF might be a therapeutic strategy to prevent or rescue cognitive decline associated with aging and neurodegenerative diseases.

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Figure Legends:

Fig. 1. Young CSF improves memory consolidation and promotes OPC proliferation and differentiation.

a, Percentage of freezing of 20-month old mice in the cued trial of the remote recall contextual fear conditioning test (day 22). aCSF or YM-CSF infusion initiated after memory acquisition for a week. (aCSF n=10, YM-CSF n=8; two-sided *t*-test; mean ± s.e.m.). **b,** Gene set enrichment analysis identifies oligodendrocytes genes as highly upregulated with YM-CSF infusion (asterisk indicates FDR<0.1) (aCSF n=8, YM-CSF n=7). **c**, Quantification of OPC proliferating cells (EDU$^+$ Pdgfrα^+/ Pdgfrα^+ cells) in the CA1 region of the hippocampus following a week of aCSF or YM-CSF infusion to 20-month-old mice (aCSF n=7, YM-CSF n=8; two-sided *t*-test; mean ± s.e.m.). Representative images in Extended data Fig 3a. **d,** Hippocampal MBP stain following

the long-term paradigm of aCSF or YM-CSF infusion. MBP mean intensity was quantified in the molecular layer of the hippocampus (area marked with dashed lines in panel e). (n=7; two-sided *t*-test; mean ± s.e.m.). **e**, Representative images of experiment in panel d. Scale bar, 200µm. **f**, Ratio of percentage of $BRDU^+$/DAPI primary rat OPCs treated with 1 / 2.5 / 5/ 10% YH-CSF over matching aCSF as control (n=3; one-way ANOVA followed by Tukey's post-hoc test; mean ± s.e.m.). **g**, Representative images of OPCs treated with 10% aCSF or YH-CSF and pulsed with BRDU for 6 hours to label proliferating cells (quantified in panel f). Scale bar, 20µm. **h,** Stacked bar plot of average number of cells in each differentiation state at day 4 of differentiation with 10% aCSF or 10% YH-CSF (aCSF n=281 cells analyzed in 3 coverslips, YH-CSF n=454 cells analyzed in 2 coverslips). **i,** Representative images of experiment in panel h. Scale bar, 20µm. See Supplementary movies S1-2 for live imaging of differentiation experiment. Data in (e and f) were replicated in at least 2 independent experiments.

Fig. 2. Serum response factor is induced by young CSF and mediates CSF-induced OPC proliferation. a, Schematic of SLAMseq experiment and extended SLAM-Dunk bioinformatics pipeline. **b,** Volcano plot of DEGs at 1hr following YH-CSF addition (SRF targets marked in red). **c,** Normalized expression levels of SRF in nascent mRNA counts and total counts showing higher expression of SRF at 1hr detected in total reads as well as nascent mRNA reads (aCSF 1hr n=4, all rest of groups n=5; Two-way ANOVA Sidak's post-hoc test (nascent and total reads separately)). **d,** Volcano plot of DEGs at 6hr following YH-CSF addition (SRF targets marked in red). **e,** Mean phalloidin intensity 6hr following YH-CSF exposure. Scale bar, 20µm. (n=3 coverslips per condition, total of 100 single cells measured per condition; two-sided *t*-test; mean ± s.e.m.). **f,** Mouse OPC primary cultures from SRF-fl/fl pups infected with CRE-GFP and △CRE-GFP AAVs to induce recombination. Representative images of infected cells (green) after 48 hours and SRF mRNA levels. Scale bar, 100µm (n=3). **g,** percentage of proliferating cells ($BRDU^+$/GFP^+ cells) in SRF-WT and SRF-KO cells treated with 10% aCSF or YH-CSF. (SRF-WT aCSF n=2, all other groups n=3; two-way ANOVA followed by Sidak's post-hoc test; mean ± s.e.m.). Data in (e and g) were replicated in at least 2 independent experiments.

Fig. 3. SRF signaling is downregulated in hippocampal OPCs with aging and induced following acute young CSF injection. a, Srf mRNA quantified in OPC (Pdgfrα^+ nuclei) in the CA1 region of the hippocampus of young (3 months) and aged (22 months) mice. Scale bar, 10µm and 5µm in insert. (aCSF n=6, YM-CSF n=7; two-sided *t*-test; mean ± s.e.m.). **b,** Diagram of sorting strategy of hippocampal OPC and OL nuclei. **c,** Heatmap of expression OPC and OL specific genes across young and aged OPC and OL samples (aged OL n=3, rest n=4). **d,** Volcano plot of DEGs of aged vs. young hippocampal OPCs (n=4). **e,** Pathways enriched (red) or depleted (blue) in hippocampal OPCs with age. **f,** Box plot of effect size of Srf targets (TRANSFAC database) in hippocampal OPCs from aged vs. young, YM-CSF vs. aCSF at 1hr and 6hr timepoints (n=4; genes pre-filtered by p<0.05 cutoff; Wilcoxon rank sum test). **g,** Pathways enriched (red) or depleted (blue) in hippocampal OPCs following 6 hrs of aCSF or YM-CSF injection (n=4). **h,** Box plot of effect size of cell cycle genes (from category in panel h) in hippocampal OPCs from aged vs. young, YM-CSF vs. aCSF at 1hr and 6hr timepoints (genes pre-filtered by p<0.05 cutoff; Wilcoxon rank sum test). **i-k,** Normalized counts of the genes (i) Stat3, (j) Kras and (k) Cd68 in hippocampal OPCs 1hr and 6hrs after aCSF or YM-CSF injection (n=4; p-adj value by Wald test in Deseq2; mean ± s.e.m.).

Fig. 4. Fgf17 activates SRF signaling *in vitro*, induces OPC proliferation and improved remote memory recall in aged mice. a, Diagram of SRE-GFP reporter. **b,** SRE-GFP activation by the highest dose (500 ng/ml) of each of the CSF ligands (n=3; One-way ANOVA with Dunnett's multiple comparisons test; mean ± s.e.m.). **c,** Dose-dependent activation of SRE-GFP reporter by increasing concentrations of Fgf17 (n=3; one-way ANOVA followed by Tukey's post-hoc test; mean ± s.e.m.). **d,** Representative images of experiment in c. Scale bar, 400μm. **e,** SRE-GFP activation with 200 ng/ml Fgf17 following 30 min pre-treatment with blocking antibodies for FgfR1, FgfR2, FgfR3 (all 50 μg/ml) or FgfR3 alone (n=3; One-way ANOVA with Sidak's multiple comparisons test; mean ± s.e.m.). **f,** Percentage of $BRDU^+$/DAPI primary rat OPCs treated with 20, 40, 80 ng/ml Fgf17 (n=3; one-way ANOVA followed by Tukey's post-hoc test; mean ± s.e.m.). **g,** Representative images of experiment in f. Scale bar, 20μm. **h,** Pathways enriched (red) or depleted (blue) in primary rat OPCs following treatment with 40 ng/ml Fgf17 (n=4). **i,** Percentage of $BRDU^+$/DAPI SRF-WT and SRF-KO mouse OPCs treated with 40 ng/ml Fgf17 or control. (n=4; two-way ANOVA followed by Sidak's post-hoc test; mean ± s.e.m.). **j,** Meta-analysis of Fgf17 levels in CSF collected from healthy human donors in 3 age categories (Li, G. et al). (ages 20-40 n=30, ages 40-60 n=23, ages 60-85 n=36; one outlier excluded (see methods), One-way ANOVA with Dunnett's multiple comparisons test; mean ± s.e.m.). **k,** Quantification of OPC proliferating cells (EDU^+ $Pdgfr\alpha^+$/ $Pdgfr\alpha^+$ cells) in the CA1 region of the hippocampus of 20-months old mice following a week of aCSF or Fgf17 infusion. (aCSF n=8, Fgf17 n=6; two-sided *t*-test; mean ± s.e.m.). **l,** Percentage of freezing of 20-month-old mice in the cued trial of the remote recall contextual fear conditioning test (day 21). aCSF or Fgf17 infusion initiated after memory acquisition for a week. (aCSF n=10, Fgf17 n=11; two-sided *t*-test; mean ± s.e.m.). Data in (b,c,e,f, and i) were replicated in at least 2 independent experiments.

Extended data figure legends

Extended data Fig. 1 Overview of the experimental paradigm.
CSF was collected from young mice (10 weeks) and slowly infused to aged mice through miniosmotic pump. Aged mice were then assessed in a remote memory test and their hippocampi characterized at the transcriptomic and cellular levels.

Extended data Fig. 2. Infusion site details and overall overview of proliferating cells
a, Location of infusion site and **b,** location of analysis site. Image source: Allen Institute, Mouse brain atlas (coronal). **c,** Hippocampal slice of 10-month-old mice given an EDU pulse prior to surgery showing low baseline proliferation and three pulses of BRDU at day 5 and 6 of infusion showing an overall increase in proliferating cells following young mouse CSF infusion (n=4 per group; repeated measures two-way ANOVA followed by Sidak's post-hoc test; Means ± SEM). **d,** Representative images of EDU (red) and BRDU (green) cells in mice with no surgery or infused with aCSF or YM-CSF. Scale bar, 500μm.

Extended data Fig. 3. Increase in OPC proliferation but not overall numbers in the hippocampus following CSF infusions.
a, Representative images of graph in Fig. 1c. YM-CSF induces PDGFRα (green) proliferation by EDU (red) incorporation in the CA1 region of the hippocampus. Arrowheads pointing at proliferating OPCs ($EDU^+Pdgfr\alpha^+$/ $Pdgfr\alpha^+$). Scale bar, 50μm. **b,** Hippocampal density of $EDU^+Pdgfr\alpha^+$ cells per mm^2 (aCSF n=7, YM-CSF n=8; two-sided *t*-test; mean ± s.e.m.). **c,**

Hippocampal density of Pdgfrα$^+$ cells per mm^2 (aCSF n=7, YM-CSF n=8; two-sided *t*-test; mean ± s.e.m.). **d,** Location of region of interest in the cortex. Scale bar, 100μm. **e,** Percentage of EDU$^+$Pdgfrα$^+$ / Pdgfrα$^+$ cells showing very low proliferation rates of OPCs in the cortex (n=4; two-sided *t*-test; mean ± s.e.m.). **f,** Cortical density of EDU$^+$Pdgfrα$^+$ cells per mm^2 (n=4; two-sided *t*-test; mean ± s.e.m.). **g,** Cortical density of Pdgfrα$^+$ cells per mm^2 (n=4; two-sided *t*-test; mean ± s.e.m.). **h,** Quantification of MBP intensity of day 4 differentiated OLs. (aCSF n=3 coverslips, YH-CSF n=2 coverslips; two-sided *t*-test; mean ± s.e.m).

Extended data Fig. 4. SLAMseq QC and principle component analysis
a, Overall conversion rates in all SLAMseq samples, showing an enrichment for T>C mutation rate (orange bar) which increases with longer incubation time (6hr). **b-c,** Distribution of T>C mutations across **b,** read position and **c,** 3'UTR position indicating an equal distribution of s^4U incorporation along the positive strand. **d-e,** UMAP of aCSF and YH-CSF samples in both time points by all genes detected in the **d,** total and **e,** nascent mRNA counts. (aCSF 1hr n=4, all the rest n=5). **f,** Gene set enrichment analysis (GSEA) of 6hr genes sorted by log2FC showing an enrichment for SRF target genes by TRANSFAC [27]. **g,** Overall log2FC enrichment indicating upregulation of SRF target genes (TRANSFAC and curated list) and actin cytoskeleton genes in YH-CSF treated OPCs over aCSF. (SRF TRANSFAC (423 genes), validated SRF targets from literature (74 genes) and actin genes (212 genes); Wilcoxon rank sum test).

Extended data Fig. 5. YH-CSF induces actin cytoskeleton alterations *in vitro*.
a-b, Actin filament content measured by live imaging using SiR-actin (red) throughout 4hr of aCSF and YH-CSF exposure. Average SiR-actin **a**, intensity and **b**, area in rat OPC cultures exposed to aCSF or YH-CSF (n=4-6 wells per condition; Means ± SEM). **c,** Representative images of experiment quantified in panel a and b. Scale bar 200μm. **d,** OPC coverslips were treated with YH-CSF for 6 hrs and stained for phalloidin. Histogram of the percentage of OPC with the indicated number of growth cones per cell. YH-CSF treated cells show a shift towards more growth cones per cell (n=3 coverslips per condition, total of 200 cells analyzed per condition; two-way ANOVA followed by Sidak's post-hoc test; Means ± SEM). Scale bar 20μm. Data were replicated in at least 2 independent experiments.

Extended data Fig. 6. Bulk RNAseq of hippocampal OPC and OL nuclei from young and aged mice.
a, Volcano plot showing OL genes up and downregulated with age. **b,** Pathways enriched (red) or depleted (blue) in hippocampal OLs with age. **c,** Meta-analysis of log2FC of SRF target genes (TRANSFAC) in human AD vs. control and mouse aged vs. young aging datasets (genes pre-filtered by p<0.05 cutoff; Wilcoxon rank sum test).

Extended data Fig. 7. Bulk RNAseq of hippocampal OPC and OL nuclei from aged mice acutely injected with YM-CSF or aCSF.
a, Box plot of effect size of Srf targets (TRANSFAC database) in hippocampal OLs from aged vs. young, YM-CSF vs. aCSF at 1hr and 6hr timepoints (n=4; genes pre-filtered by p<0.05 cutoff; Wilcoxon rank sum test. **b,** Pathways enriched (red) or depleted (blue) in hippocampal OPCs 1hr following injection of aCSF or YM-CSF. **c,** Volcano plot showing OPC genes up and down regulated 1hr following CSF injection. **d,** Volcano plot showing OPC genes up and down regulated 6hr following CSF injection.

Extended data Fig. 8. Fgf8 induces SRF reporter and OPC proliferation *in vitro*.
a, Dose-dependent activation of SRE-GFP reporter by increasing concentrations of Fgf8 and representative images of the experiment at 15.5 hrs. Scale bar, 400µm. (n=3; one-way ANOVA followed by Sidak's post-hoc test; mean ± s.e.m.). **b,** Percentage of BRDU^{+}/DAPI primary rat OPCs treated with 10, 20, 40 ng/ml Fgf8. (n=3; one-way ANOVA followed by Tukey's post-hoc test; mean ± s.e.m.). **c,** Quantification of OPC proliferating cells (EDU^{+} Pdgfrα^{+}/ Pdgfrα^{+} cells) in the CA1 region of the hippocampus of 20-months old mice following a week of aCSF or Fgf8 infusion. (aCSF n=8, Fgf8 n=4; two-sided *t*-test; mean ± s.e.m.).

Extended data Fig. 9. Fgf17 is predominantly expressed in the brain by a subset of neurons and choroid plexus epithelial cells.
a, Fgf17 is predominantly expressed in the brain based on the human protein atlas. **b,** Fgf17 is lowly expressed by neurons but not glial cells in the adult human cortex (Allen brain atlas). **c,** Fgf17 is lowly expressed by neurons and choroid plexus epithelial cells in a human COVID19 brain and choroid plexus dataset.

Video S1. OPCs growing under differentiation conditions with 10% aCSF. OPCs treated with 10% aCSF under differentiation conditions (with T3) for 4 days and imaged in the IncuCyte every 2 hours for 4 days. Scale bar, 200µm.

Video S2. OPCs growing under differentiation conditions with 10% YM-CSF. OPCs treated with 10% YH-CSF under differentiation conditions (with T3) for 4 days and imaged in the IncuCyte every 2 hours for 4 days. Scale bar, 200µm.

Data S1. Log2FC and summary statistics of middle-aged bulk RNAseq of aged hippocampi infused with aCSF or YM-CSF for 6 days (linked to Fig. 1b).

Data S2. SLAMseq gene counts and statistics – nascent and total mRNA counts in 1hr and 6hr (linked to Fig. 2).

Data S3. Gene lists used in the study. (a-b) SRF targets –by the human TRANSFAC database (aligned to rat and mouse), (c) curated list of SRF targets from literature, (d) GO term actin cytoskeleton (rat).

Data S4. Log2FC and summary statistics of sorted OPC and OL from young and aged hippocampi (linked to Fig. 3).

Data S5. Log2FC and summary statistics of hippocampal OPC and OL from aged mice infused with aCSF or YM-CSF for 1hr or 6hr (linked to Fig. 3).

Data S6. Log2FC and summary statistics of rat OPCs treated with Fgf17 or control (linked to Fig. 4).

Materials and Methods

Animals. Aged C57BL/6 mice (18-22 months old) were obtained from the National Institute on Aging rodent colony. Young male C57BL/6 mice (2 months old) were obtained from Charles River Laboratories. All experiments used male mice. All animal care and procedures complied with the Animal Welfare Act and were in accordance with institutional guidelines and approved by the V.A. Palo Alto Committee on Animal Research and the institutional administrative panel of laboratory animal care at Stanford University.

Young mouse CSF collection. CSF was collected as previously described with several adaptations [60]. Briefly, ten-week-old mice were anesthetized intraperitoneally with Ketamine (120mg/kg) and xylazine (8mg/kg), and then placed in a stereotactic instrument (KOPF) with the head secured at a 45-degree angle facing downwards. An incision was made above the neck and muscles were held separated with microretractors allowing exposure of the cisterna magna by blunt forceps without any bleeding. CSF was pulled out of the cisterna magna cavity with a 20µl pipettor connected through an aspirator tube assembly (Sigma, A5177) to a pooled glass capillary (Borosilicate glass tubes, ID 1.30 mm, OD 1.70 mm, Length 4.00 in, type 8250, King precision glass) held secure by Model 1769 90° Electrode Holder (KOPF). CSF was kept in a low-protein bind tube on ice and spun in a cold centrifuge for 10 min at 1500 rpm to exclude CSF immune cells. Supernatant was collected and kept in -80 until use. Pellet was resuspended in 6ul of milli-q ultra-pure water for blood contamination quality control using the Nanodrop UV-vis setting with a 415nm wavelength for detection of oxyhemoglobin [61]. A cutoff of below than 0.02 AU was used for CSF infusions.

Human CSF. CSF samples of nine young heathy individuals (ages 24-26) were obtained through a collaboration with Dr. Henrik Zetterberg, University of Gothenburg, Sweden. The samples were baseline (normal sleep) lumbar CSF samples, collected in the morning, from healthy volunteers who took part in a study on sleep restriction-induced changes of CSF composition[62]. For *in vitro* experiments, three pools consisting of three individuals each were made for each experiment, two pools from six male samples and one of female samples were each used in 3-4 technical triplicates.

Osmotic pump intracerebroventricular infusion. To minimize the volume of mouse CSF infused per mouse, young CSF or artificial CSF were loaded to a coiled polyethylene (PE-60) catheter prepared in house following the lynch coil technique [63]. In brief, the total length of the coil needed was calculated by 4.56ul internal volume per cm tube. Usually, 20cm were wound around a syringe of the same outside diameter as the pump and secured with tape. The syringe was submerged in boiling water for 1 min and immediately immersed in ice cold water for 1 min. Coils were disassembled and left to dry overnight. 90 µl of pooled young CSF or artificial CSF (Tocris) were loaded to the coiled catheter connected to 100ul osmotic pumps (Alzet, 1007D) with a 7-day infusion at a rate of 0.5 µl/hr. Osmotic pumps were connected to a cannula (Brain infusion kit III, Alzet) and incubated overnight in a 37°C water bath. Cannula was inserted at +1 mm medio-lateral, 0 mm anterior–posterior, and −3mm dorso-ventral relative to bregma in order to target the right lateral ventricle. The pump was placed subcutaneously and mice received post-surgical buprenorphine and Baytril. Mice were split to groups to achieve an equal average body weight in all groups. Recombinant carrier free human /mouse Fgf8b (423-F8/CF, R&D) and mouse Fgf17 (7400-FG-025/CF, R&D) were resuspended in aCSF (Tocris) to a concentration of

25 µg/ml and loaded to an osmotic pump (Alzet, 1007D) with a 7-day infusion at a rate of 0.5ul / hr.

Contextual Fear Conditioning. The fear-conditioning paradigm was performed as previously described with some modifications [10,64]. In brief, mice were trained to associate cage context or an audiovisual cue with an aversive stimulus (foot shock). On day 1, mice were placed in a cage and exposed to three periods of 30 s of paired cue light and 1,000-Hz tone followed by a 2s foot shock (0.6 mA), with a 60s interval. On day 2 and day 22, mice were subjected to two trials. In the first trial assessing contextual memory, mice were re-exposed to the same cage context, and freezing behavior was measured during minute 390s using a FreezeScan tracking system (Cleversys). In the second trial measuring cued memory, mice were placed in a novel context and exposed to the same cue light and tone from day 1 on min 2, 3 and 4 of the trial. Freezing behavior was averaged across min 3-5. No significant differences in contextual fear conditioning were observed between groups at day 22. All experiments were performed by a blinded researcher.

***In vivo* BRDU and EDU pulses.** To assess baseline proliferation, mice received one EdU injection 15 mins before the stereotactic surgery (Invitrogen, E10415, 100 mg / kg intraperitoneally). To assess cell proliferation post infusion, mice were pulsed three times with BrdU to label proliferating cells; twice on day 5 of the infusion 10 hrs apart and once on day 6 two hours before perfusion (100 mg /kg intraperitoneally; B5002-5G, Sigma-Aldrich).
In subsequent cohort, in which young mouse CSF or Fgfs were infused to aged mice, mice received post-infusion pulses of EDU instead of BRDU in the same paradigm described above, with no baseline labeling prior to surgery.

Tissue processing prior to immunostaining. Mice were anesthetized with Avertin (2,2,2-tribromoethanol: T48402, Sigma-Aldrich; 2-methyl-2-butanol: 240486, Sigma-Aldrich) (0.018 ml (2.5%) per gram of body weight) and perfused with 20 ml cold PBS. Brains were collected and divided sagittally. One hemisphere was used to dissect the hippocampus for RNA sequencing, which was snap frozen and stored at −80 °C. The second hemisphere was fixed in phosphate-buffered 4% paraformaldehyde overnight at 4 °C before transfer to 30% sucrose in PBS at 4 °C until sectioning. Brains were frozen at −30 °C and cryosectioned coronally at 40 µm with a microtome (Leica, SM2010R). Brain sections were stored in cryoprotectant (40% PBS, 30% glycerol, 30% ethylene glycol) and kept at −20 °C until staining.

Immunostaining.
Brain slices immunofluorescent staining. Brain sections were washed three times for 10 mins in TBST and then blocked in TBS++ (TBS + 3% donkey serum (130787, Jackson ImmunoResearch) + 0.25% Triton X-100 (T8787, Sigma-Aldrich)) for 1 hr, followed by primary antibody incubation overnight on a rocking platform at 4 ˚C. The following primary antibodies were used in this study; PDGF Receptor α (D1E1E) XP® Rabbit mAb (1:500, Cell Signaling, 3174S), Rabbit-anti-MBP (1:100, MAB386, Millipore). For secondary staining, brain sections were washed three times for 10 min in TBST, followed by incubation for 1.5 hrs in Alexa Fluor-conjugated secondary antibodies (1:500). Brain sections were washed and mounted on Superfrost microscope slides (12-550-15, Fisher Scientific) with Vectashield Hardset Antifade Mounting Medium with DAPI (Vector labs, H-1500/ NC9029229). For MBP stains an additional step of

tissue de-lipidation was added before blocking: tissues were incubated in 100% EtOH for 10min in room temperature and then washed twice with PBS.

EdU staining. Brain sections were washed three times for 10 min in PBS, then permeabilized for 20 min in 0.1% Triton X-100 (T8787, Sigma-Aldrich), washed again three times and then blocked in TBS++ for 1hr. EdU staining was performed following the Click-iT® Plus EdU Alexa Fluor® 555 Imaging Kit instructions (C10638, Life Technologies). Sections were washed and stained with primary and secondary antibodies as described above.

BrdU staining- brain slices and 384-well plates. Following staining with other primary and secondary antibodies, sections were incubated in 2N HCl for 30 min at 37 °C and then washed three times for 10 min in TBST. Sections were blocked for 1.5 hr in TBS++ and then transferred to primary antibody with Rat anti-BRDU antibody (1:500, ab6326, Abcam) overnight at 4 °C. Secondary staining started with three washes for 10 min in TBST, followed by incubation with secondary antibody mix for 1.5 h. After three 10-min washes in TBST, sections were mounted as described above. For 384-well plates, nuclei were stained and with Hoechst 33342 (1:2000, H3570, Thermo) and immediately imaged on a Keyence microscope (BZ-X800). In cases were BRDU and EDU staining was performed on the same sections, the sequence was; permeabilization, HCL antigen retrieval, EDU Click-it reaction, blocking, primary and secondary antibody stain as described above in detail.

In situ RNA hybridization (RNAScope)
RNAScope was performed on fresh frozen coronal brain sections (10µm thick) using the Multiplex Fluorescence v.2 kit (Advanced Cell Diagnostics) according to the manufacturer's protocol with minor modifications. Tissue fixation with 4% PFA was extended to 60 min at RT, and Protease IV treatment was shortened to 20 min to better preserve the hippocampal tissue. Probes for mouse Pdgfrα and SRF were commercially available from the manufacturer and secondary Opal 690 and 520 reagents (FP1497001KT and FP1487001KT, Akoya Biosciences) were diluted at 1:1500 in TSA buffer.

Image analysis (IMARIS)
Brain slices. Confocal z stacks of four coronal brain sections spanning the dorsal hippocampus were captured on a Zeiss confocal LSM880 microscope for each brain sample using a 20x magnification. Max projection files (at least 4 hippocampal coronal slices per mouse, 400µm apart) were analyzed in IMARIS by generating masks of two main regions of interest: (1) cornu ammonis area 1 (CA1) - stratum oriens, stratum pyramidale and stratum lacunosum-moleculare (SLM) combined, and and (2) cortex. The percentage of newly proliferated OPCs was analyzed by dividing cell counts of $BRDU^+$ or EDU^+ cells by $PDGFR\alpha^+$ cells. For cell density, $BRDU^+$ or $PDGFR\alpha^+$ cell counts were divided by the corresponding area of the mask per slice.
For RNAscope analysis, similar hippocampal tiled z-stacks were taken (at least 4 hippocampal coronal slices per mouse, 100µm apart). Percentage of SRF^+ OPCs was calculated by dividing the number of SRF^+ $PDGFR\alpha^+$ nuclei by $PDGFR\alpha^+$ nuclei in the CA1 region of the hippocampus. For MBP analysis, epifluorescent tiled images were taken using the 20x magnification (Keyence microscope model BZ-X800) to cover the whole hippocampus. MBP intensity measures of the defined molecular layer were collected using the Keyence analysis

software with a set threshold for all images. Representative images of MBP intensity were made with Fire LUT in IMAGEJ. All analyses were performed by a blinded observer.

In vitro cell culture. Three random 20x images of each well were analyzed using IMARIS batch by setting similar surfaces to automatically count $BRDU^+$ and $Hoechst^+$ nuclei. For each image percentage of proliferating cells was calculated by dividing $BRDU^+$ counts by total $Hoechst^+$ counts. Cellular phalloidin intensity was measured by manually delineating cell borders and measuring intensity, then all cell measurements averaged per coverslip. Representative images of phalloidin intensity were made with Fire LUT in IMAGEJ. All other quantifications we done using IMAGEJ and manual cell counts. In differentiation experiments, cell morphology state was assessed manually as previously described [13]. All analyses were performed by a blinded observer.

SRF reporter assay

HEK293 cells were plated at 50K cells/ well in a 96-well plate in full media (DMEM, 10% FCS and 1% P/S) and transfected on day 2 with Cignal SRE Reporter Assay Kit (GFP) (CCS-010G, Qiagen) using lipofectamine P3000 in experimental media (DMEM, 0.5% FCS, 1% non-essential amino acids (M7145, Sigma)), following the manufacturer's instructions. On day 3 media was changed to 90µm fresh experimental media and supplemented with 10µm of 10x concentration of indicated concentrations of recombinant carrier free Recombinant Human KGF/FGF-7 (251-KG-010, R&D Systems), human /mouse Fgf8b (423-F8/CF, R R&D Systems), mouse Fgf17 (7400-FG-025/CF, R&D Systems), Recombinant Human/Murine/Rat BDNF (450-02, Peprotech), Recombinant Human CX3CL1/Fractalkine (365-FR-025, R&D Systems), Recombinant Human Dhh protein (ab78682, Abcam) and Ckm (9070-CK-050, R&D Systems). Plates were incubated in the IncuCyte and imaged every hour for 24 hours. In FgfR blocking antibody experiments, on day 3 media was changed to 80µm fresh experimental media and pre-incubated at 37ºC with 10ul of anti-FgfR1 (NBP2-12308), anti FGFR2 (MAB684-100) and anti-FGFR3 (MAB7661-100, all from Novus Biologicals) final concentration of 50 µg/ml, before adding 10µl of Fgf17 at a final concentration of 200 ng/ml. Plates were incubated in the IncuCyte and imaged every hour for 24 hours.

OPC primary cultures.

Rat OPC cultures

OPCs were isolated from P7-P8 Sprague-Dawley (Charles Rivers) rat brains by immunopanning and grown in serum-free defined medium, as previously described [65]. Cell culture for proliferation and differentiation experiments was done following the protocol with several modifications. To use the least possible CSF of young healthy human subjects, we minimized the culture conditions to 384 well plates. In addition, to account for inter-subject variability, we pooled CSF of 3 subjects with similar ages, and used three such pools in each experiment in triplicates. Following initial growth of 4 days in 10cm dishes, cells were trypsinized and split to 384-well (Falcon® 384 Well Optilux, 353962), PDL-covered (PDL; Sigma-Aldrich P6407; molecular weight 70–150 kDa) plates. For proliferation experiments, 2,500 cells were plated in a total volume of 50µl of full proliferation medium supplemented with 10 ng/ml PDGF (Peprotech 100-13A), 10 ng/ml CNTF Peprotech 450-02, 4.2 µg/ml forskolin (Sigma-Aldrich F6886) and 1ng/ml NT3 (Peprotech 450-03) with 10% of YH-CSF or aCSF. Actin filaments were visualized by live imaging by addition of 500nM of SiR-Actin (Cytoskeleton, CY-SC002) added with 10%

YH-CSF. Wells were imaged every hr for the remaining 6hrs of the experiment. In BRDU experiments, 18hrs after plating in 384-well plates (with 10% CSF), 5ul of 200µM BRDU (20µM final concentration) was added for a pulse of 6 hrs followed by fixation with 4% PFA for 20 min. In experiments that required phalloidin staining, 10,000 OPCs were plated on PDL-covered 12mm coverslips in a 24-well dish in 90% full proliferation medium (315µl) and let to adhere overnight, then supplemented with 10% CSF (35µl) for 6 hs. Coverslips were fixed with 4% PFA for 20 mins, washed and stained with 555-phalloidin in PBS (1:143, Invitrogen) for 15 min. Coverslips were washed and mounted with Vectashield Hardset Antifade Mounting Medium with DAPI (Vector labs, H-1500/ NC9029229). For differentiation experiments, 10,000 OPCs were plated on PDL-covered 12mm coverslips in a 24-well dish in full proliferation medium overnight. Proliferation medium was completely changed to differentiation media (basal growth media supplemented with 40 ng/ml T3 (Sigma-Aldrich T6397)) with 10% CSF with a 50% media change (with 10% CSF) on day 2 of differentiation. At day 4 of differentiation coverslips were fixed with 4% PFA for 20 mins, washed with PBS, permeabilized with 0.1% Triton X-100 for 3 mins followed by wash and blocking in 3% BSA for 1 hr. Primary antibodies; Rabbit-anti-MBP (1:100, abcam, ab7349, knock-out validated [13]) and mouse anti-GFAP (1:500, Chemicon, MAB360) were incubated overnight at 4 °C. Coverslips were washed, stained with Alexa Fluor-conjugated secondary antibodies (1:500) followed by a 15 min stain with Cell mask (1:1000, Invitrogen, C10046) mounted and set on a coverslip before imaging on a Keyence microscope (BZ-X800) or confocal laser-scanning microscope (Zeiss LSM880).

Mouse OPC cultures

Mouse OPCs were purified from brains of SRF floxed mice (generated by David Ginty and kindly provided by Eric Small) by immunopanning as described above for rat OPCs [65].
On day 3 of the culture, SRF-f/f OPCs were split and plated in 384-well plates for proliferation experiments. When the cells were in suspension in proliferation media before plating, $1*10^{10}$ viral genomes of AAV DJ-CMV eGFP-deleted cre (GVVC-AAV-62) or AAV DJ-CMV eGFP-cre (GVVC-AAV-63) (both generated by the Stanford Gene Vector and Virus Core). The following day, media was fully replaced and 48hrs after infection 10% aCSF or YH-CSF, or 40 ng/ml Fgf17 were added to proliferation media with BRDU (20µM final concentration) for 16 hrs. Cells were fixed and cell proliferation was assessed as indicated above in the BRDU experiment.

SLAMseq experiment and data analysis. Optimal s^4U concentration was assessed following SLAMseq Explorer Kit – Cell Viability Titration Module (061, Lexogen) following manufacturer's recommendations. In brief, OPCs were incubated with increasing doubling concentrations of s^4U (1.95-2000µM) and viability was assessed with live-dead ratio as described above and with ATP incorporation following manufacturer's recommendations (Promega, G7571). A final concentration of 62.5uM was found as the highest concentration without compromising on cell viability within 12 hrs (twice the duration of the intended experiment). SLAMseq experiment was conducted following SLAMseq Kinetics Kit - Anabolic Kinetics Module protocol (061, Lexogen). In brief, following initial growth of 4 days in 10cm dishes, 30,000 OPCs were plated in 315µl full proliferation medium on PDL-covered 24-well plates (one plate per timepoint) overnight. 35µl of pooled YH-CSF or aCSF spiked in with 625µM S4U (10x concentration) were added gently to minimize confounding induction of gene expression. One- or six-hours later the medium was removed, and cells were scraped with 1ml

Trizol (Thremo, 15596018), transferred to foil-covered tubes and frozen until RNA extraction. RNA extraction was done following anabolic kit protocol. All cell culture and RNA extraction steps were done in the dark under red-light following manufacturer's recommendations. Library preparation was done with QuantSeq 3' mRNA-Seq Library Prep Kit for Illumina (FWD) (015, Lexogen) with the indicated modifications to adjust to low-RNA input. After normalization and pooling, libraries were sequenced on a Nextseq550 (Illumina) using single-end 75-bp reads. Libraries were sequenced to a mean depth of ~30 million reads per sample. Raw sequencing files were demultiplexed with bcl2fastq and resulting FASTQ files combined across lanes and per sample. Sequencing quality control was performed using FastQC v0.11.8 and summary reports were generated with MultiQC v1.7. FASTQ files were then analyzed using the SLAM-DUNK pipeline v0.3.4 and the related alleyoop toolchain. The pipeline comprises 1) read mapping, 2) alignment filtering, 3) SNP calling and correction, and 4) 3'-UTR sequence counting. Using NextGenMap reads were aligned against the genome of *Rattus norvegicus* (release 6.0), which was downloaded in FASTA format from Ensembl release v97. For mapping the following parameters were set; '-5 12, -a 4, -n 1, -ss' leaving rest at default. Resulting Binary sequence Alignment/Map (BAM) format files were filtered to remove low-quality alignments using the parameters '-mq 2, -mi 0.95, -nm -1' and rest at default. Next, SNPs in alignments, in particular the T to C (T>C) conversions, were called with parameters '-c 10, -f 0.8' and others remaining at default. Statistical independence of distributions of true SNP-callings identified by VarScan2 in relation to the number of T>C reads was assessed with a Mann-Whitney-U test for each sample as initially described in the alleyoop snpeval toolchain module. For the reference set of 3'-UTRs, a genome feature file (GFF3) for the *Rattus norvegicus* genome (release 6.0) was downloaded from Ensembl release v97, filtered to retain only *three_prime_utr* features and converted to Browser Extensible Data (BED) format using bedops v2.4.36. The BED file was used in combination with parameters '-c 1, -q 27' for the SLAMDUNK count command, leaving other parameters at default. Resulting 3'-UTR T>C and total read counts were added up on gene-level along all transcripts and alternative 3'-UTRs available per transcript. Entire down-stream analysis was performed using R v3.6.1, data.table v1.12.2, and common tidyverse packages (tidyr, dplyr, purrr, stringr, ggplot2). Differential gene expression analysis was carried out using DESeq2 v1.26.0 on the T>C count and total count matrices separately with standard settings with alpha level set to 0.05. In total, six DESeq2 experiments were performed, two with pooled timepoints (1hr + 6hr) per read-type (design variables *Treatment* and *Time*) and four with separate timepoint per read-type (design variable *Treatment* only). Effect size was calculated using the implementation of cohen's d from the effsize v0.7.6 package. Gene set enrichment and over-representation analyses were performed using GeneTrail 3.0 [66]. Category source databases were adjusted independently, and P-values were corrected using the false discovery rate controlling procedure by Benjamini-Hochberg and considered significant if smaller than 0.05.

Oligodendrocyte nuclei isolation and sorting.

Nuclei isolation from frozen dissected hippocampi was done as previously described [67] using the Nuclei EZ Prep Kit (Sigma-Aldrich, St. Louis, USA). Following the final PBS wash, nuclei were pelleted and resuspended in 100 µl of antibody mix (1:100, Anti-NeuN antibody-Alexa Fluor® 647, EPR12763, and 1:100, Anti-Olig2 antibody-Alexa Fluor® 488) with 0.2U/µl RNAse Inhibitor (Takara, 23138) in FACS buffer (0.5% BSA in PBS) and incubated on ice with intermittent shaking for 30 min. Nuclei were washed with 1ml FACS buffer and pelleted by centrifugation at 500 r.c.f. for 5 min, resuspended in FACS buffer with 0.2U/µl RNAse Inhibitor

and Hoechst 33342 (1:2000, H3570, Thermo) and sorted on a Sony Sorter (SH800) based on Houechst^{+} NeuN^{-} Olig2^{+high} (OPC) and Houechst^{+} NeuN^{-} Olig2^{+low} (OL) gating to 350µl of RTL buffer and stored in -80°C until RNA extraction. Data were analyzed using FlowJo software (TreeStar).

RT-qPCR. Oligodendrocyte nuclei were isolated by FACS and RNA was extracted with the RNeasy Plus Micro kit (Qiagen, 74034). cDNA was generated with qScript™ cDNA SuperMix (QuantaBio, 95048). Samples were diluted and mixed with SYBR green master mix before loading as technical triplicates for qPCR on a LightCycler 480 (Roche). ΔΔC T values normalized to Gapdh were used to assess relative gene expression between samples. The following validated primer pairs for mouse SRF were used: 5′- GGC CGC GTG AAG ATC AAG AT-3′ (forward) and 5′- CAC ATG GCC TGT CTC ACT GG-3′ (reverse).

Bulk RNA sequencing.

Hippocampal RNAseq

Frozen dissected hippocampi were thawed on ice and homogenized in 350µl RLT buffer by 20 strokes using a manual homogenizer and total RNA was isolated with the RNeasy Plus Micro kit (Qiagen, 74034). RNA quantity and quality were assessed by Agilent 2100 Bioanalyzer (Agilent Technologies). All samples passed a quality-control threshold (RIN ≥ 9) to proceed to library preparation and RNA-seq on Hiseq 4000 (Illumina) using paired-end 100-bp reads. Libraries were sequenced to a depth of >20 million reads per sample. Raw sequencing files were demultiplexed with bcl2fastq, reads were aligned using STAR, and counts of technical replicates were summed up using DESeq2 before performing normalization and differential expression analysis with standard settings.

Sorted OPC RNAseq

RNA was extracted with the RNeasy Plus Micro kit (Qiagen, 74034). cDNA and library synthesis were done inhouse using the Smart-seq2 protocol as previously described [68] (detailed protocol at https://doi-org.laneproxy.stanford.edu/10.17504/protocols.io.2uvgew6) with several modifications. Due to the low input RNA content, 2µl of RNA extracted from sorted nuclei was reversed transcribed using 16 cycles for OL samples and 18 cycles for OPC samples. Following bead cleanup using 0.7x ratio with AMPure beads (A63881, Fisher), cDNA concentration was measured using the Qubit 1x dsDNA HS kit (Q33231) and normalized to 0.4 ng/µl as input for library prep. 0.4 µl of each normalized sample was mixed with 1.2 µl of Tn5 Tagmentation mix (0.64 µl TAPS-PEG buffer (PEG 8000, V3011 , PROMEGA, and TAPS-NaOH pH8.5, BB-2375, Boston Bioproducts), 0.46 µl H2O and 0.1 µl Tn5 enzyme (20034198, Illumina)), then incubated at 55 °C for 10 min. The reaction was stopped by adding 0.4 µl 0.1% sodium dodecyle sulfate (Fisher Scientific, BP166-500). Indexing PCR reactions were performed by adding 0.4 µl of 5 µM i5 indexing primer (IDT), 0.4 µl of 5 µM i7 indexing primer (IDT), and 1.2 µl of KAPA HiFi Non-Hot Start Master Mix (Kapa Biosystems) using 12 amplification cycles. Libraries were purified using two purification rounds with a ratio of 0.8x and 0.7x AMPure beads. Library quantity and quality was assessed using a Bioanalyzer (Agilent) and Qubit. All steps were done manually using 8-strip PCR tubes and PCR reactions were carried out on a 96-well plate thermal cycler (Biorad). Libraries were pooled and sequenced on a Nextseq550 sequencer (Illumina) using single end 63bp for Read 1 and 12bp for index 1 with a high output 75bp kit (20024906, Illumina).

Libraries were sequenced to a depth of at least >10 million reads per sample. Raw sequencing files were demultiplexed and known adapters were trimmed with bcl2fastq. Data analysis of raw sequencing data was performed using the nextflow-core RNA-seq pipeline v3.0. Briefly, the core workflow of the pipeline maps filtered reads against the species reference genome using STAR and computes transcript counts using RSEM. For nuclear RNA-seq data, a custom reference genome was created where exon sequences in GTF files were modified to include all introns per transcript and used for the mapping instead. For mouse and rat sequencing data the reference genome GRCm38 and Rnor 6.0 provided by Illumina igenomes were used, respectively. All gene annotations were based on the ensembl database. Obtained raw gene transcript counts per sample were loaded into DESeq2, performing normalization for transcript length and sequencing depth, and differential expression analysis with standard settings. Effect sizes for each gene were computed based on normalized counts computed by DESeq2 using the function cohen.d of the R package effsize. Gene set enrichment analysis was performed using GeneTrail 3 using BH-FDR p-value adjustment with all remaining parameters kept at default.

Meta-analysis of Srf targets in single-cell data sets from literature. For each data set gene counts, fold-changes and adjusted p-values calculated between either case and control or old and young age groups were acquired from the publicly available supplement tables of the corresponding publications. Next, the list of Srf targets known in Homo sapiens (by TRANSFAC dataset) were mapped to orthologous gene identifiers in Mus musculus using the Ensembl database at release 100. The process described in the following was performed independently for oligodendrocyte precursor and mature oligodendrocyte clusters. For each data set, the list of genes was intersected with the organism matched list of Srf targets. Next, genes that did not pass the significance threshold for the adjusted p-values at cut-off 0.05 were discarded from subsequent analysis. Finally, fold-changes were normalized for each data set to obtain a comparable scale and to mitigate project-dependent fluctuations of fold-changes due to varying sample counts.

Statistical analysis. All non-RNAseq analysis was done using GraphPad Prism 8 with the indicated statistical tests. Gene set enrichment and over-representation analyses were performed using GeneTrail 3.0 [69]. Category source databases were adjusted independently, and P-values were corrected using the false discovery rate controlling procedure by Benjamini-Hochberg and considered significant if smaller than 0.05.