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RESEARCH ARTICLE

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G protein-coupled receptor GPR37-like 1 regulates adult oligodendrocyte generation

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Abstract

Oligodendrocytes (OLs) continue to be generated from OL precursors (OPs) in the adult mammalian brain. Adult-born OLs are believed to contribute to neural plasticity, learning and memory through a process of "adaptive myelination," but how adult OL generation and adaptive myelination are regulated remains unclear. Here, we report that the glia-specific G protein-coupled receptor 37-like 1 (GPR37L1) is expressed in subsets of OPs and newly formed immature OLs in adult mouse brain. We found that OP proliferation and differentiation are inhibited in the corpus callosum of adult *Gpr37l1* knockout mice, leading to a reduction in the number of adult-born OLs. Our data raise the possibility that GPR37L1 is mechanistically involved in adult OL generation and adaptive myelination, and suggest that GPR37L1 might be a useful functional marker of OPs that are committed to OL differentiation.

KEYWORDS
adaptive myelination, adult myelination, GPR37L1, myelin, oligodendrocyte

1 | INTRODUCTION

In the vertebrate central nervous system (CNS), oligodendrocytes (OLs) synthesize myelin, the multilayered lipid-rich sheath surrounding neuronal axons, allowing swift transmission of action potentials along axons and thereby enabling high-speed communication in neural circuits (Li & Richardson, 2016). Myelination is the culmination of a sequence of developmental events, beginning with OL precursor (OP) specification from neural stem cells around mid-embryogenesis (Li et al., 2011), followed by OP proliferation and differentiation into newly forming/immature OLs (imOLs) and finally OL maturation and myelin formation. The vast majority of mature OLs are generated in the early postnatal period in mammals, for example, in the first 6–8 postnatal weeks in mice (Psachoulia et al., 2009; Young et al., 2013).

Strikingly, unlike other neural precursors, OPs persist in the adult CNS, accounting for ~5% of all neural cells in mice and continuing to proliferate and differentiate into OLs into adulthood, though at a gradually declining rate with age (Richardson et al., 2011; Young et al., 2013). The function of adult-born OLs is still not completely clear. An instinctive explanation is that they might be required to replace some existing OLs over the lifetime, for the purpose of maintaining myelin integrity. Moreover, emerging evidence implies a role for adult-born OLs in neural plasticity via "adaptive myelination," in which new OLs are likely required to refine

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myelination patterns, and hence the properties of neural circuits, in response to neuronal activity throughout adult life (Bechler et al., 2018; Bergles & Richardson, 2015). Consistent with the latter idea, magnetic resonance imaging (MRI) scans of human subjects have revealed alterations of white matter microstructure—possibly reflecting altered myelination—while learning complex motor skills such as playing the piano or juggling (Bengtsson et al., 2005; Scholz et al., 2009). Our own earlier studies demonstrated that adult OL generation is required for motor learning in mice (McKenzie et al., 2014; Xiao et al., 2016). In addition, new OL production and myelin formation have been linked to neural plasticity in other contexts, such as the consolidation of long-term fear memory and spatial memory in mice (Pan et al., 2020; Steadman et al., 2020).

Despite the growing interest in adaptive myelination, there are large gaps in our understanding of how adult OL genesis is regulated. Platelet-derived growth factor A-chain homodimers (PDGF-AA), acting through the PDGF receptor (alpha-subunit, PDGFRα), is known to regulate OP proliferation and differentiation during development (Calver et al., 1998; Richardson et al., 1988; van Heyningen et al., 2001) and is also believed to regulate OP proliferation and differentiation in the adult brain (Hill et al., 2013; Zhou et al., 2020). OL generation and myelination appear to depend on neuronal activity in the adult brain. For instance, stimulation of neuronal activity by optogenetic or chemogenetic means has been shown to promote OP differentiation/OL generation and activity-dependent myelination in adult mouse brain (Gibson et al., 2014; Mitew et al., 2018).

We recently identified a glia-specific G protein-coupled receptor, GPR37-like 1 (GPR37L1), which is expressed in a subset of astrocytes as well as in a subpopulation of OL lineage cells (Jolly et al., 2018). We found that GPR37L1 in astrocytes plays a neuroprotective role in ischemia (Jolly et al., 2018). In the present study, we focus on the role of GPR37L1 in OL lineage cells and, using *Gpr37l1* knockout mice, investigate its influence on adult OL generation.

2 | MATERIALS AND METHODS

2.1 | Animals

Gpr37l1 knockout (*Gpr37l1*$^{-/-}$) mice were imported from the NIH Mutant Mouse Resource and Research Centers and bred by intercrossing heterozygotes in a UCL animal facility. *Gpr37l1*$^{-/-}$ mice were maintained in a mixed genetic background (C57BL/6, 129S5) and genotyped as previously described (Jolly et al., 2018). Mouse husbandry and experimentation followed protocols approved by UK Home Office and complied with UCL Research Ethics Committee guidelines and UK laws and regulations.

2.2 | Tissue preparation

Mice were subjected to cardiac perfusion fixation with cold 4% (w/v) paraformaldehyde (PFA; Sigma) in diethyl pyrocarbonate (DEPC, Sigma)-treated PBS. Dissected brain tissues underwent post-fixation in 4% PFA at 4˚C overnight, immersion in 20% (w/v) sucrose (Sigma) for cryoprotection and embedding in Tissue-Tek OCT (Sakura Finetek) on dry ice before being stored at −80˚C. After cryosectioning, 25 μm brain coronal sections were collected onto SuperFrost Plus Adhesion slides (Thermo Fisher Scientific) for histological analysis.

2.3 | RNA in situ hybridization (ISH) combined with immunostaining

Fluorescence RNA in situ hybridization (ISH) followed by immunostaining was performed as described previously (Jolly et al., 2016). Briefly, digoxigenin (DIG)- or fluorescein isothiocyanate (FITC)-labeled riboprobes were synthesized in vitro using DNA templates of mouse *Pdgfra* (Kasuga et al., 2019), *Pcdh17it* (Kasuga et al., 2019), and *Bmp4* (IMAGE clone IRAVp968B09166D, Source Bioscience) for RNA ISH in brain sections. Hybridization signals were visualized using horseradish peroxidase (HRP)-conjugated anti-FITC or anti-DIG antibody (Roche) with the help of a TSA Tyramide Reagent Pack (PerkinElmer). For subsequent immunostaining, brain sections were incubated with blocking buffer, 10% (v/v) fetal bovine serum with 0.3% (v/v) Triton-X100 in PBS, for 1 h at 20–25˚C and then with rabbit anti-OLIG2 antibody (1:400; Merck Millipore) at 4˚C overnight, followed by detection with Alexa Fluor 568-conjugated secondary antibody (1:750; Thermo Fisher Scientific). For double RNA ISH, FITC- and DIG-labeled riboprobes were applied to brain sections simultaneously. After FITC signal detection with HRP-conjugated anti-FITC antibody plus TSA FITC reagents, 3% (w/v) H_2O_2 was used to inactivate the HRP and then DIG signal was detected using HRP-conjugated anti-DIG antibody together with TSA Cy5 reagents. For single and double immunostaining, mouse anti-OLIG2 antibody (1:400; Merck Millipore), rabbit anti-PDGFRA antibody (1:500; Cell Signaling Technology) and rat anti-MBP antibody (1:200; Abcam) were used. Nuclei were counterstained with Hoechst 33258 (Sigma) for 5 min and then slides were mounted with DAKO Mounting Medium (Agilent).

2.4 | EdU incorporation and detection

5-ethynyl-2′-deoxyuridine (EdU) was used to label dividing cells during S-phase of the cell cycle. EdU was dissolved in sterile saline (0.9% NaCl) and administered to mice at 50 mg/kg via intraperitoneal injection. Brain tissues were

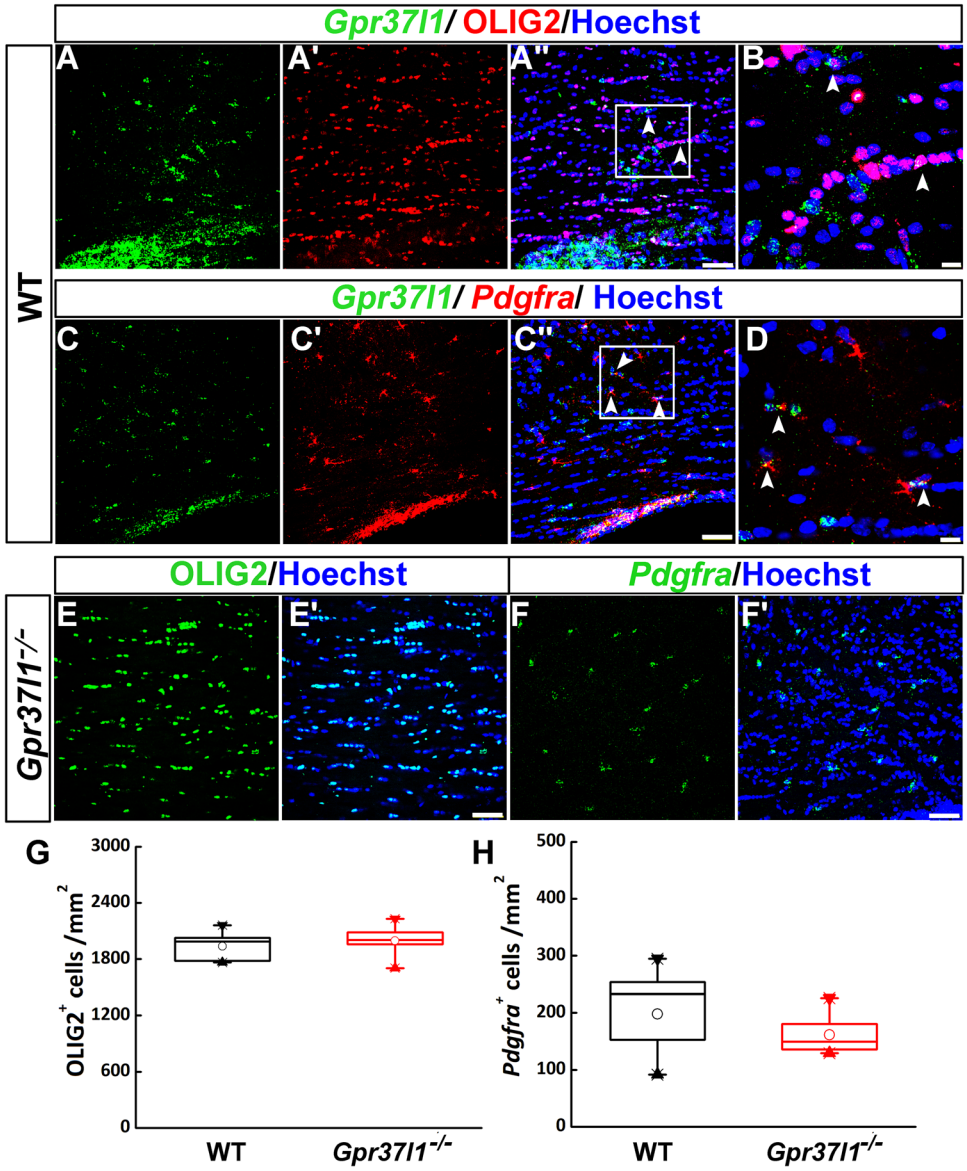

FIGURE 1 *Gpr37l1* is expressed in a subpopulation of OL lineage cells, predominantly OPs. Analysis of *Gpr37l1* expression in OLIG2$^+$ OL lineage cells and *Pdgfra*$^+$ OPs in P60 corpus callosum. Colocalization of *Gpr37l1* (green) with OLIG2 (red, a, b) or *Pdgfra* (red, c, d) was analyzed by RNA ISH combined with immunostaining, or double RNA ISH, in corpus callosum tissues of P60 WT mice. b and d are higher magnification images of the white boxes in a′′ and c′′, respectively. *Gpr37l1*$^+$/OLIG2$^+$ and *Gpr37l1*$^+$/*Pdgfra*$^+$ double positive cells are indicated with arrowheads. Examination of OLIG2$^+$ (green; e, e′) OL lineage cells and *Pdgfra*$^+$ (green; f, f′) OPs in the corpus callosum of P60 *Gpr37l1*$^{-/-}$ mice. Numbers of OLIG2$^+$ (g) and *Pdgfra*$^+$ (h) cells in WT and *Gpr37l1*$^{-/-}$ mice (mean ± SEM, $n = 3$, unpaired *t*-test), showing no significant differences. Nuclei were counterstained with Hoechst dye (Blue). Scale bars: 50 μm for a, c, e, and f and 10 μm for b and d

collected 4 h after the injection and EdU detection was performed with an AlexaFluor-647 Click-iT EdU Cell Proliferation Assay Kit (Invitrogen) immediately following RNA ISH.

2.5 | Statistical analysis

Statistical significance was determined with SPSS software. A group of 3 mice (WT or *Gpr37l1*$^{-/-}$) was used for each histological examination and all labeled cells in the region under study were counted. Student's *t*-test was used to compare two groups and the threshold for significant difference was taken as $p < .05$. Charts were made with OriginPro software (Originlab). Data are presented as mean ± SEM.

3 | RESULTS

3.1 | Gpr37l1$^+$ OL lineage cells are comprised OPs and imOLs

Our previous study revealed that GPR37L1 is expressed in some PDGFRA$^+$ OPs but not in CC1$^+$ mature OLs (Jolly

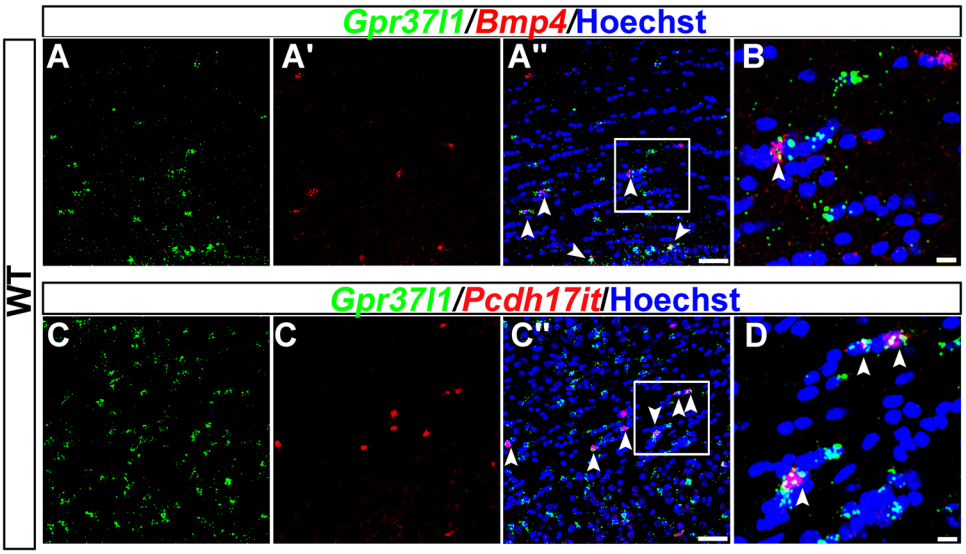

FIGURE 2 *Gpr37l1* is expressed in a significant proportion of imOLs. Coexpression of *Gpr37l1* (green) with *Bmp4* (red, a, b) or *Pcdh17it* (red, c, d) was analyzed by RNA ISH in sections of corpus callosum from P60 WT mice. *Gpr37l1*$^+$/*Bmp4*$^+$ and *Gpr37l1*$^+$/*Pcdh17it*$^+$ double-positive cells are indicated with arrowheads. b and d are higher magnification images of the white boxes in a′′ and c′′, respectively. Nuclei were stained with Hoechst dye (Blue). Scale bars: 50 μm for a and c and 10 μm for b and d

et al., 2018). To define *Gpr37l1*-expressing OL lineage cells more precisely, we performed RNA ISH combined with immunostaining as well as double RNA ISH in sections of corpus callosum from wild type (WT) mice at postnatal day 60 (P60), using OLIG2 and *Pdgfra* to mark OL lineage cells and OPs, respectively. A small proportion, 8.7% ± 1.6%, of OLIG2$^+$ OL lineage cells were found to be *Gpr37l1*$^+$. For *Pdgfra*$^+$ OPs, the proportion that was *Gpr37l1*$^+$ rose to 40% ± 2% (Figure 1a–d). Among *Gpr37l1*$^+$ cells, 36% ± 5% and 26% ± 3% of them were OLIG2$^+$ and *Pdgfra*$^+$, respectively (Figure 1a–d), suggesting that OPs make up the majority (~70%) of *Gpr37l1*$^+$ OL lineage cells.

imOLs are premyelinating OLs at an intermediate stage between OPs and mature OLs (Marques et al., 2016); their prevalence in adult brain can reflect the contemporaneous rate of adult OL generation (Fard et al., 2017; Kasuga et al., 2019). Employing two newly identified imOL markers, *Pcdh17it* and *Bmp4* (Fard et al., 2017; Kasuga et al., 2019; Marques et al., 2016; Fudge et al., unpublished), we examined *Gpr37l1* expression in imOLs by double RNA ISH. We found that 4.4% ± 0.7% and 12% ± 2% of *Gpr37l1*$^+$ cells were *Bmp4*$^+$ and *Pcdh17it*$^+$, respectively (Figure 2a–d). From the perspective of imOLs, 65% ± 5% of *Pcdh17it*$^+$ and 60% ± 6% of *Bmp4*$^+$ cells expressed *Gpr37l1*. Taken together with the fact that GPR37L1 is not expressed in CC1$^+$ mature OLs (Jolly et al., 2018), these results indicate that ~40% of OPs and ~60% of imOLs express GPR37L1. As OPs are more numerous than imOLs, the majority (~70%) of GPR37L1-expressing OL lineage cells are OPs.

3.2 | Gpr37l1 ablation does not alter the numbers of OL lineage cells and OPs in corpus callosum at P60

Although *Gpr37l1* can first be detected around P8 in mouse brain, we found that germline knockout of *Gpr37l1* has no influence on early OL development (Jolly et al., 2018 and Figure S1). To assess the influence of GPR37L1 on overall OL generation, we examined the expression of OLIG2 and *Pdgfra* in brains of *Gpr37l1* germline knockout (*Gpr37l1*$^{-/-}$) mice. We found no differences between *Gpr37l1*$^{-/-}$ mice and WT controls in the number of OLIG2$^+$ OL lineage cells (Figure 1a, e) or *Pdgfra*$^+$ OPs (Figure 1c, f) present in the corpus callosum at P60 (Figure 1g, h), implying that loss of GPR37L1 does not affect overall production of OLs during development. In addition, CC1$^+$ mature OL numbers and myelin density (as illustrated by MBP staining) were also unchanged in the corpus callosum of *Gpr37l1*$^{-/-}$ mice at P60 (Figures S2 and S3).

3.3 | Gpr37l1 ablation leads to decreased imOL numbers in adult corpus callosum

A convenient and reliable way to estimate the rate of adult OL generation is by monitoring imOLs (Kasuga et al., 2019). We found by RNA ISH that the number of *Pcdh17it*$^+$ cells was significantly decreased in the corpus callosum of *Gpr37l1*$^{-/-}$ mice relative to WT controls at P60 (*Gpr37l1*$^{-/-}$ vs. WT:

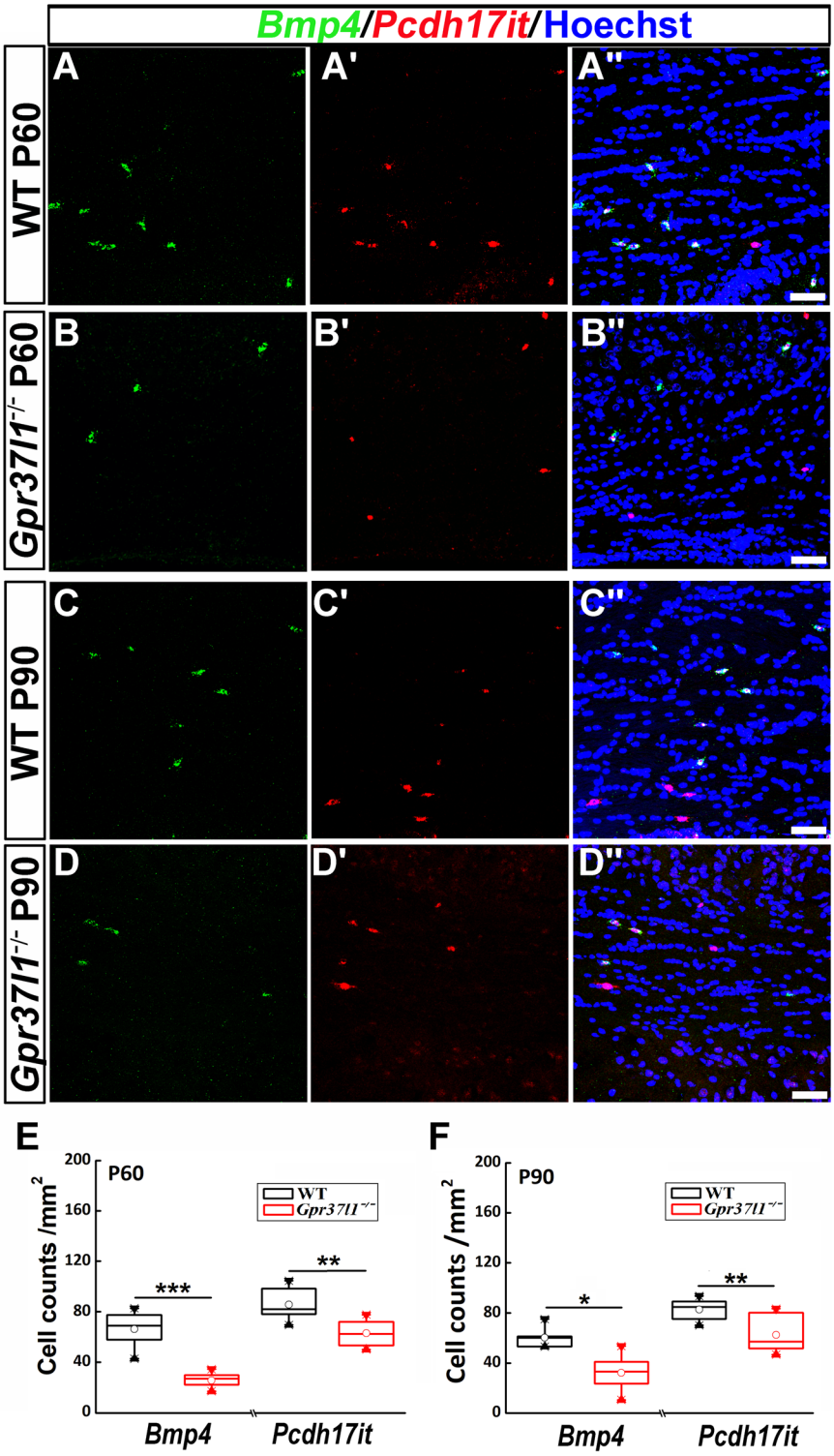

FIGURE 3 *Gpr37l1* knockout leads to decreased imOL numbers in adult corpus callosum. Examination of *Bmp4*$^{-}$ (green) and *Pcdh17it*$^{-}$ (red) expressing imOLs in the corpus callosum of *Gpr37l1*$^{-/-}$ mice versus WT controls at P60 (a, b) and P90 (c, d) by RNA ISH. Nuclei were counterstained with Hoechst dye (Blue). Comparison of *Bmp4*$^{+}$ and *Pcdh17it*$^{+}$ cell numbers in *Gpr37l1*$^{-/-}$ mice versus WT controls at P60 (e) and P90 (f) (mean ± SEM, $n = 3$, unpaired *t*-test). *$p < .05$, ** $p < .01$ ***, $p < .001$. Scale bars: 50 μm

63 ± 4 per mm^2 vs. 86 ± 5 per mm^2, $p < .01$, $n = 3$) (Figure 3a–e). This result was mirrored by the number of *Bmp4*$^{+}$ cells (*Gpr37l1*$^{-/-}$ vs. WT: 25 ± 3 per mm^2 vs. 66 ± 6 per mm^2; $p < .001$. $n = 3$) (Figure 3a–e). Similar results were obtained at P90 (*Gpr37l1*$^{-/-}$ vs. WT: 57 ± 5 per mm^2 vs. 83 ± 5 per mm^2 for *Pcdh17it*$^{+}$ cells, $p < .01$ and 32 ± 9 per mm^2 vs. 60 ± 5 per mm^2 for *Bmp4*$^{+}$ cells, $p < .05$, $n = 3$) (Figure 3c–f). Together, these data reveal that there are decreased numbers of imOLs in adult brain as a result of *Gpr37l1* knockout, hinting at a role for GPR37L1 in stimulating adult OL generation.

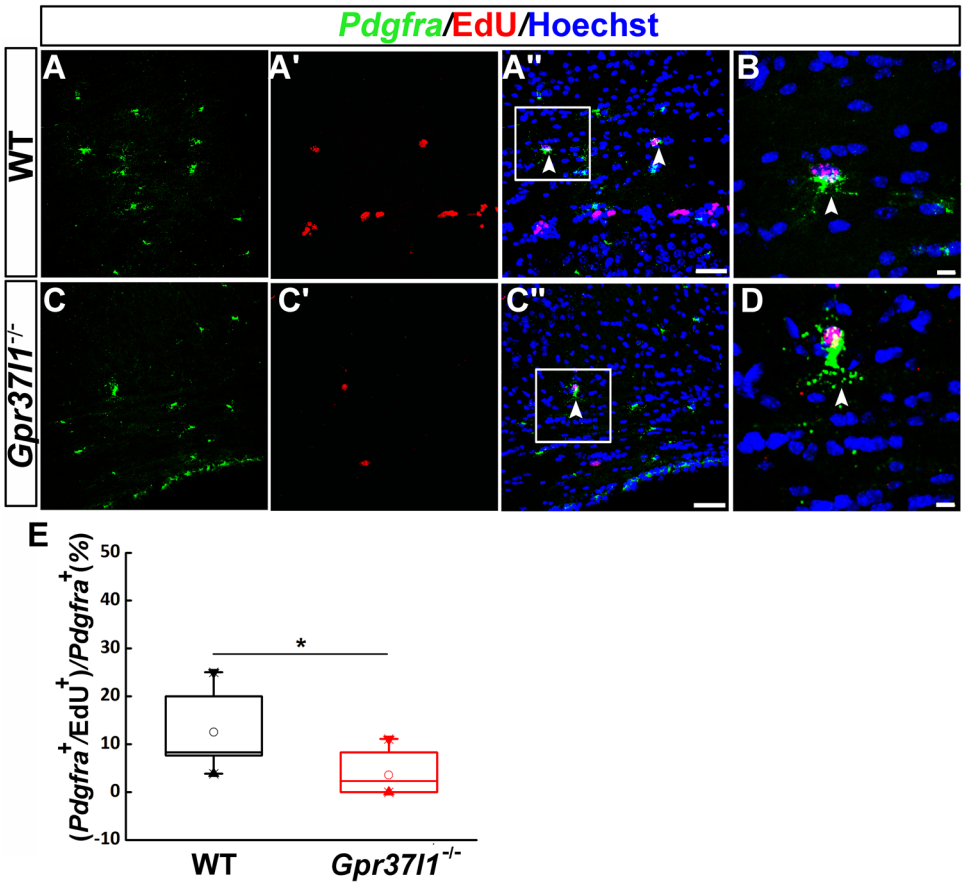

FIGURE 4 *Gpr37l1* ablation reduces OP proliferation in P60 corpus callosum. Corpus callosum tissues were collected from P60 mice 4 h after EdU injection for examination by *Pdgfra* RNA ISH and EdU staining. Representative images of cells labeled by *Pdgfra* (green) and EdU (red) in the corpus callosum of WT (a–b) and *Gpr37l1*$^{-/-}$ mice (c–d) at P60. *Pdgfra*$^{+}$/EdU^{+} double positive cells are indicated with arrowheads. Nuclei were stained with Hoechst dye (Blue). b and d are higher magnification images of the white boxes in a" and c", respectively. (e) Numbers of *Pdgfra*$^{+}$/EdU^{+} cells, plotted as a percentage of total *Pdgfra*$^{+}$ cells, comparing WT and *Gpr37l1*$^{-/-}$ mice (mean ± SEM, $n = 3$, *$p < .05$ for unpaired *t*-test). Scale bars: a and c: 50 μm; b and d: 10 μm

3.4 | Gpr37l1 ablation inhibits OP proliferation and differentiation in P60 corpus callosum

In adult brain, most OP divisions give rise to one replacement OP and one differentiated OL (Hughes et al., 2013). To detect OP proliferation in vivo, we administered EdU to P60 mice and collected brain tissues for analysis 4 h later. We found that the number of EdU^{+}/*Pdgfra*$^{+}$ double-positive cells in the corpus callosum was lower in *Gpr37l1*$^{-/-}$ mice than in WT controls (*Gpr37l1*$^{-/-}$ vs. WT as a percentage of total *Pdgfra*$^{+}$ cells: 3.6% ± 1.8% vs. 13% ± 3%, $p < .05$, $n = 3$) (Figure 4), suggesting that lack of GPR37L1 causes diminished OP proliferation in adult brain.

We next estimated the numbers of newly generated imOLs by counting EdU^{+}/*Pcdh17it*$^{+}$ and EdU^{+}/*Bmp4*$^{+}$ cells. EdU^{+}/*Pcdh17it*$^{+}$ cells were less frequent in the corpus callosum of P60 *Gpr37l1*$^{-/-}$ mice compared to WT littermate controls (*Gpr37l1*$^{-/-}$ vs. WT: 2.6 ± 1.1 vs. 6.9 ± 1.4 per mm^2, $p < .05$, $n = 3$), and the same was true for EdU^{+}/*Bmp4*$^{+}$ cells (*Gpr37l1*$^{-/-}$ vs. WT: 1.3 ± 0.9 vs. 4.5 ± 1.2 per mm^2, $p < .05$, $n = 3$) (Figure 5). These results point to reduced OL differentiation or survival in the adult brain in the absence of GPR37L1 and suggest that GPR37L1 stimulates adult OL generation.

4 | DISCUSSION

OPs, also known as NG2 glia because of their expression of the proteoglycan NG2, are the main proliferating cell population in the healthy adult CNS (Dawson et al., 2003; Young et al., 2013). Almost all OPs continue to divide in adult white matter, though their division rate decreases with age (Young et al., 2013). It has been suggested that adult OPs could be a heterogeneous group, considering their various developmental origins and different local environmental cues at their resting sites - for example, in gray matter versus white matter, or in dorsal versus ventral brain regions (Bostrand &

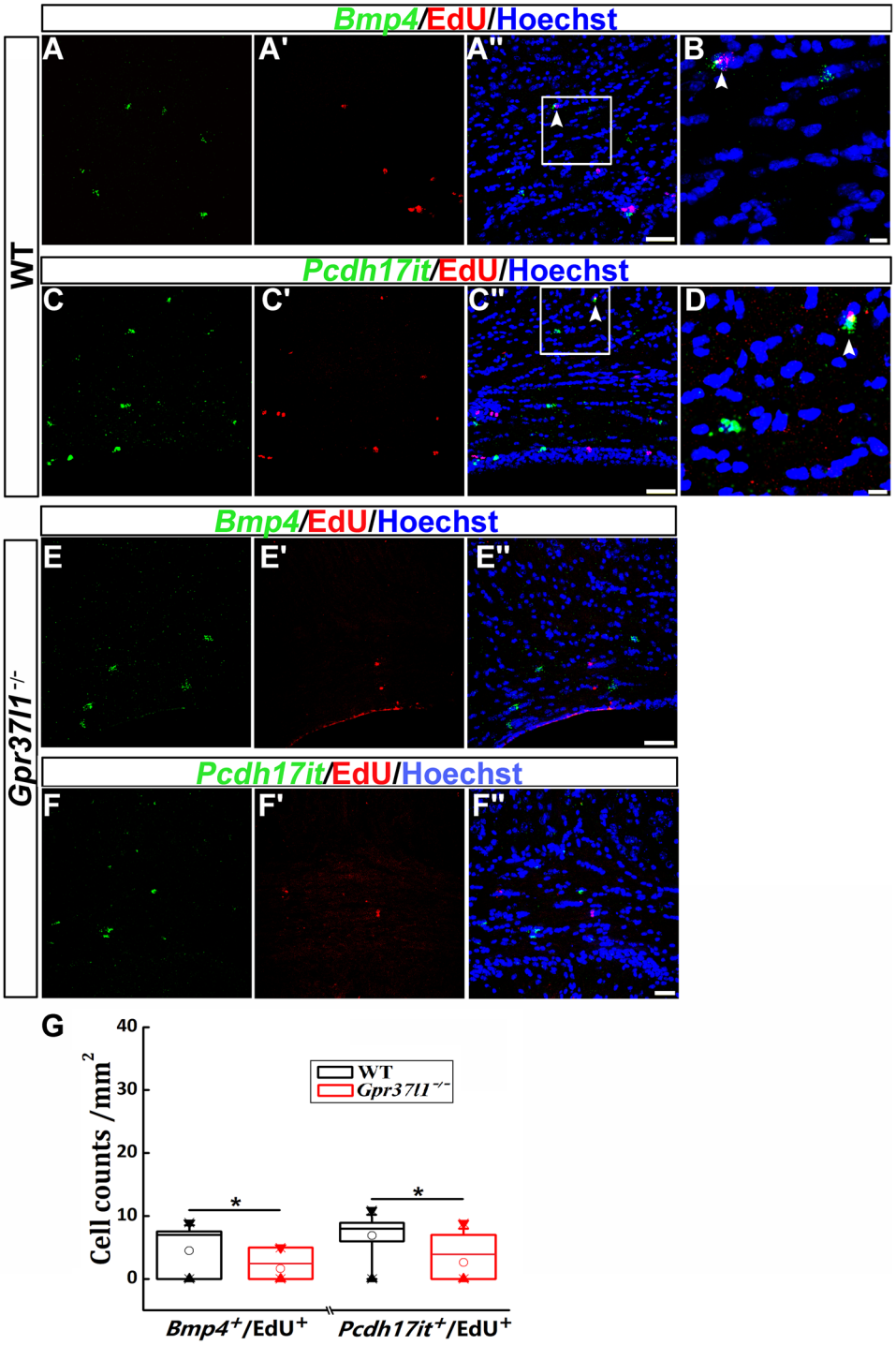

FIGURE 5 *Gpr37l1* ablation inhibits OP differentiation in P60 corpus callosum. Representative images of cells labeled by *Bmp4* (green, a–b, e) or *Pcdh17it* (green, c–d, f) and EdU (red, a–f) in the corpus callosum of WT (a–d) and *Gpr37l1*$^{-/-}$ mice (e–f) at P60. *Bmp4*$^{+}$/EdU^{+} and *Pcdh17it*$^{+}$/EdU^{+} cells are indicated with arrowheads. Nuclei were stained with Hoechst dye (Blue). b and d are higher magnification images of the white boxes in a" and c", respectively. (g) Graph showing reduced numbers of *Bmp4*$^{+}$/EdU^{+} and *Pcdh17it*$^{+}$/EdU^{+} cells in *Gpr37l1*$^{-/-}$ mice compared to WT littermate controls (mean ± SEM, $n = 3$, $^{*}p < .05$ for unpaired *t*-test). Scale bars: a, c, e, and f: 50 μm; b and d: 10 μm

Williams, 2021; Crawford et al., 2016; Kim et al., 2019). A recent electrophysiological study showed that OPs evolve from an initially homogeneous population to a functionally heterogeneous population with increasing age, as reflected by the varying differentiation potentials among individual OPs in different brain regions (Spitzer et al., 2019). We found that a substantial proportion of OPs and imOLs form a subpopulation of *Gpr37l1*-expressing OL lineage cells (Figures 1 and 2). Moreover, our new evidence from *Gpr37l1*$^{-/-}$ mice points to GPR37L1 as a regulator of OP proliferation and differentiation/survival in adult brain (Figures 3–5). Therefore, as with astrocytes (Jolly et al., 2018), it is possible that GPR37L1 could be a molecular marker of OP functional state, perhaps indicating that GPR37L1^{+} OPs have made the decision to differentiate, prior to exiting the cell cycle. Future RNA profiling analysis of GPR37L1^{+} versus GPR37L1^{-} OPs might provide useful information on OP functional heterogeneity and/or differentiation mechanisms.

OP divisions in adult CNS are most likely self-renewing—that is, one OP generates one replacement OP, plus another one destined to differentiate into an OL (Hughes et al., 2013; Kang et al., 2013), which could explain the unchanged number of OPs in adult *Gpr37l1*$^{-/-}$ mice despite the suppressed OP proliferation (Figure 1). In this study, OP proliferation and differentiation were shown to be reduced in the corpus callosum of *Gpr37l1*$^{-/-}$ mice (Figures 4 and 5), hinting at GPR37L1's involvement in adult OL generation. Taking into consideration the fact that GPR37L1 in astrocytes exerts function in response to a cellular stress (ischemia) (Jolly et al., 2018), it is possible that the role of GPR37L1 in OPs could be to drive adaptive myelination in response to cognitive challenge, for example, as occurs during motor learning. We cannot be completely certain that *Gpr37l1* ablation acts cell-autonomously within the OL lineage; it is also conceivable that loss of GPR37L1 from astrocytes indirectly influences OL lineage development. Given that GPR37L1 is a transmembrane receptor protein, it is likely that its loss does induce a cell-intrinsic phenotype in OPs and imOLs, but to be certain of this would require conditional, OL lineage-specific *Gpr37l1* knockout.

Although there is disagreement regarding the ligand of GPR37L1 (Coleman et al., 2016; Smith, 2015), we and others have identified prosaposin as a potential ligand for both GPR37L1 and its close relative GPR37 (Jolly et al., 2018; Meyer et al., 2013; Liu et al., 2018; Sala et al., 2020). Currently it is believed that prosaposin-GPR37L1 signaling confers neural protection during CNS injury (Meyer et al., 2014; Jolly et al., 2018; Li et al., 2017; Liu et al., 2018). Prosaposin can be proteolytically cleaved to generate mature saposins (A–D), which act as sphingolipid activators, required for the lysosomal hydrolysis of sphingolipids (O'Brien & Kishimoto, 1991; O'Brien et al., 1988). Moreover, prosaposin itself can function as a secreted neurotrophic factor, able to promote cell survival, neurite outgrowth and neuronal differentiation in cultured neuroblastoma cells (Meyer et al., 2014; O'Brien et al., 1994; O'Brien et al., 1995). Full-length prosaposin and prosaptides (peptides mimicking the neurotrophic region in prosaposin) have been shown to confer protection on cells of an OL cell line and to stimulate myelin lipid synthesis (Harzer et al., 2001; Hiraiwa et al., 1997). In addition, prosaposin deficiency is known to result in hypomyelination and demyelination across the nervous system as well as loss of OLs both in mice and in humans (Elleder et al., 2005; Fujita et al., 1996; Hulkova et al., 2001; Meyer et al., 2014). These data, taken together with the findings of our present study, suggest several questions that warrant further investigation: (1) Is prosaposin a ligand for GPR37L1 (as well as its paralog GPR37) in OL lineage cells? (2) If so, how does prosaposin-GPR37L1 signaling pathway exert its influence on adult OL generation? (3) Can prosaposin stimulate adaptive myelination in the adult brain and/or remyelination following demyelinating injury or disease?

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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