Enhancement of MBP expression in human iPSC-derived oligodendrocyte-like cells by optimising db-cAMP concentration in media

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Introduction

Oligodendrocytes are specialised glial cells that originate from oligodendrocyte progenitor cells (OPCs) present in the developing and adult central nervous system (CNS). They wrap myelin sheaths around axons of neurons, enabling rapid conduction of action potentials. Loss of myelin sheaths results in neuronal dysfunction and causes neurological disorders like multiple sclerosis (MS).
The function of the myelin basic protein (MBP) in the formation and maintenance of the myelin sheaths has been strongly associated with MS. Therefore, it is critical that cell models to study demyelinating disease express significant levels of MBP.

Human iPSC-derived ioOligodendrocyte-like cells (ioOLCs) powered by opti-ox™ exhibit transcriptional and morphological characteristics of a pre-myelinating oligodendrocyte state. Upon thawing and culturing under defined conditions, ioOLCs undergo rapid maturation within 8 days, developing branched processes and expressing key oligodendrocyte markers—including MBP.

Systematic analysis of the effect of media components on ioOLC cultures revealed that dibutyryl cyclic adenosine monophosphate (db-cAMP) can further enhance MBP expression and positively affects ioOLC maturation as well as the long-term stability of the cultures. Interestingly, primary oligodendrocyte cultures treated with cAMP analogues or a phosphodiesterase-4 (PDE4) inhibitor, which elevate cell intrinsic cAMP levels, also accelerate cellular maturation and promote expression of myelin-associated genes such as MBP[1,2], showing that ioOLCs recapitulate critical aspects of oligodendrocyte biology.

In summary, we demonstrate that db-cAMP enhances MBP expression in a concentration-dependent manner and promotes morphological maturation, offering a refined and optimised culture strategy for studying myelination biology and screening remyelination-promoting compounds.

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Results

A systematic evaluation of medium components aimed at enhancing the robustness of MBP expression in ioOLCs identified db-cAMP as a key modulator of maturation. To test the effects of db-cAMP, ioOLCs were cultured for 10 days in the recommended medium (User Manual NPI-0013-UM V-02), supplemented with a broad range of db-cAMP concentrations (10-500 µM), and assessed via immunofluorescence staining, RT-qPCR and morphological analysis.
Here, we report a detailed evaluation of the most optimal db-cAMP concentrations (50 and 100 µM) for promoting MBP expression and advancing ioOLC maturation.

MBP immunostaining reveals concentration-dependent enhancement of maturation

Immunofluorescence staining for O4 and MBP revealed that, as expected, ioOLCs did not express MBP on day 1 post-thaw, consistent with an immature, OPC-like phenotype at this early stage. By day 8, MBP-positive cells began to emerge, with their abundance strongly correlating with db-cAMP concentration. Elevated db-cAMP levels (50 µM and 100 µM) resulted in a higher proportion of MBP-expressing cells compared to the 1 µM condition. This effect was further enhanced by day 10, with 100 µM db-cAMP yielding the highest number of MBP-positive cells (**Fig. 1A**).

Quantitative image analysis confirmed the concentration-dependent effect of db-cAMP on MBP expression. Treatment with 50 µM db-cAMP led to a moderate increase in the proportion of MBP-positive cells at days 8 and 10, while 100 µM db-cAMP induced a more substantial response. Under the 100 µM condition, approximately 9% of cells were MBP-positive at day 8, increasing to nearly 25% by day 10 (**Fig. 1B**).

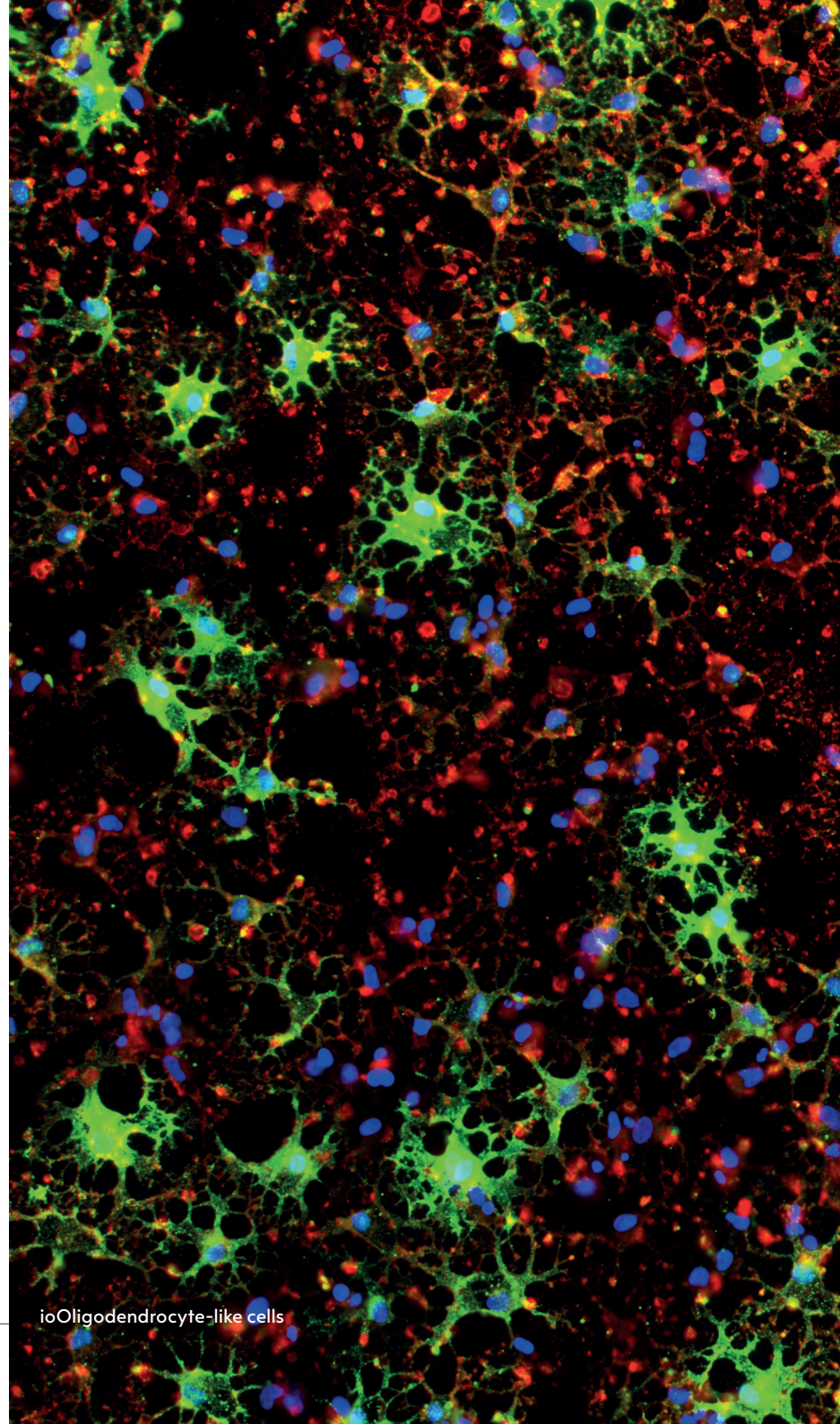
ioOligodendrocyte-like cells

Figure 1A

Day 1

1 µM db-cAMP
As per the User Manual

50 µM db-cAMP

100 µM db-cAMP

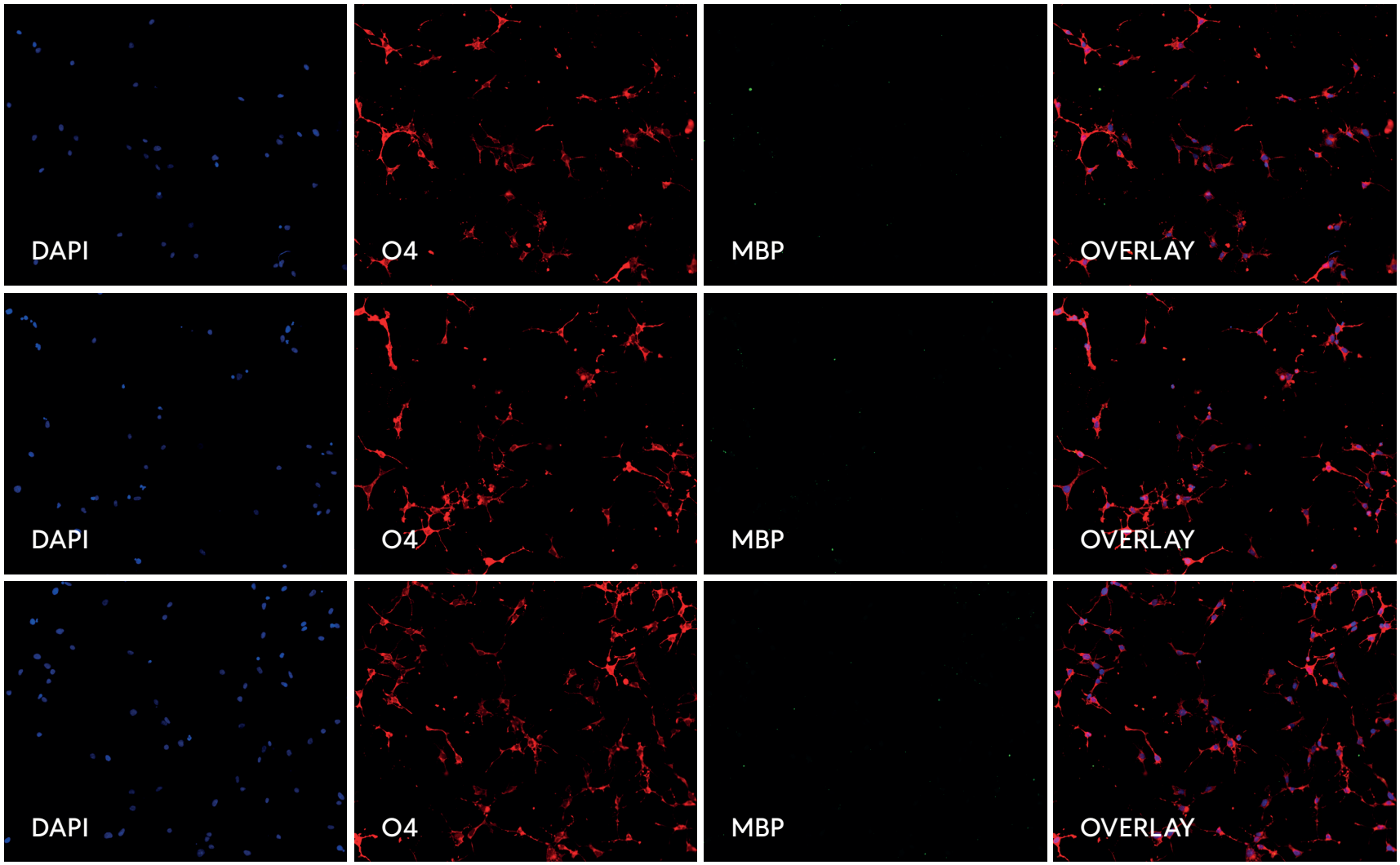

Figure 1A

Day 8

1 µM db-cAMP
As per the User Manual

50 µM db-cAMP

100 µM db-cAMP

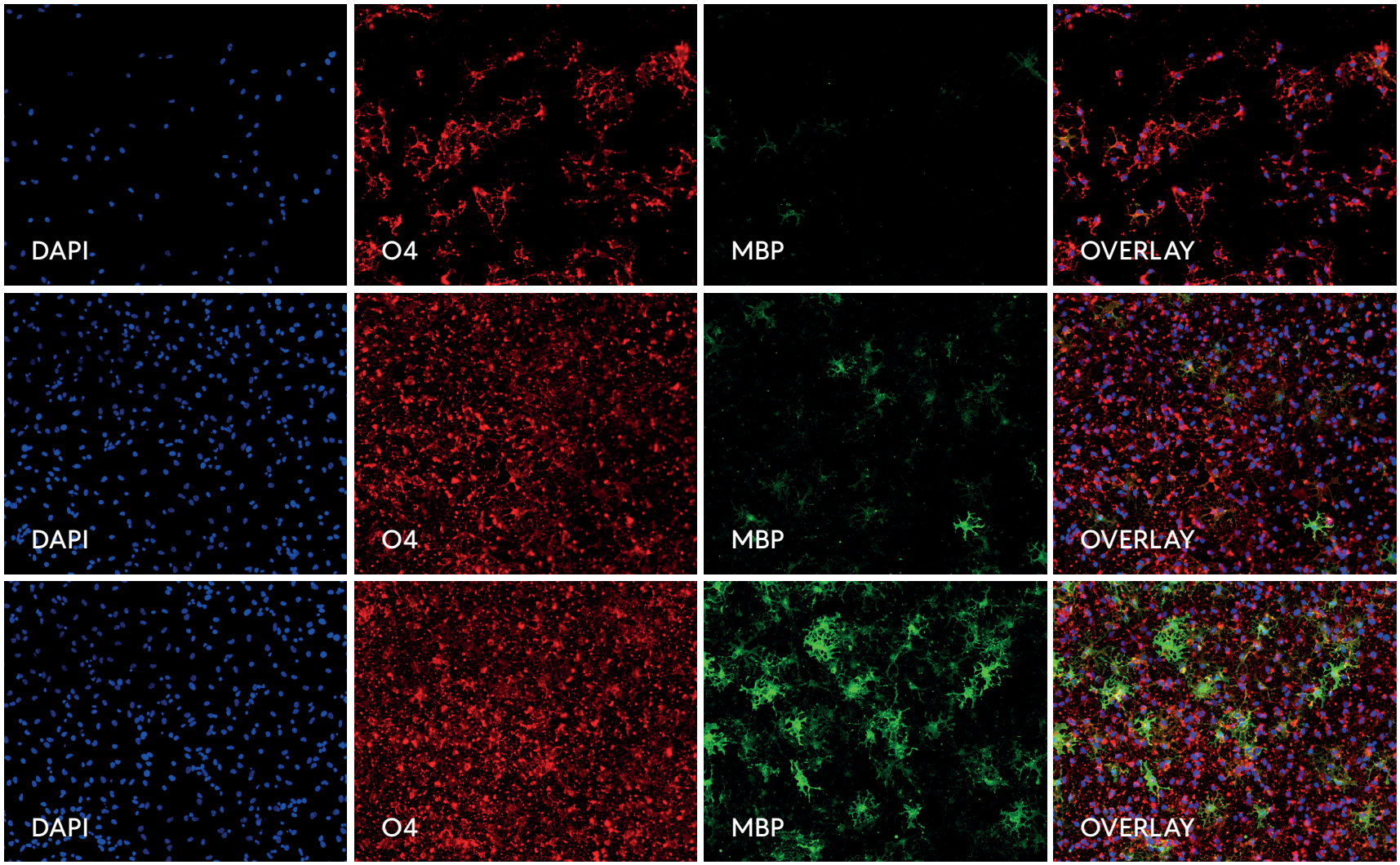

Figure 1A

Day 10

1 µM db-cAMP
As per the User Manual

50 µM db-cAMP

100 µM db-cAMP

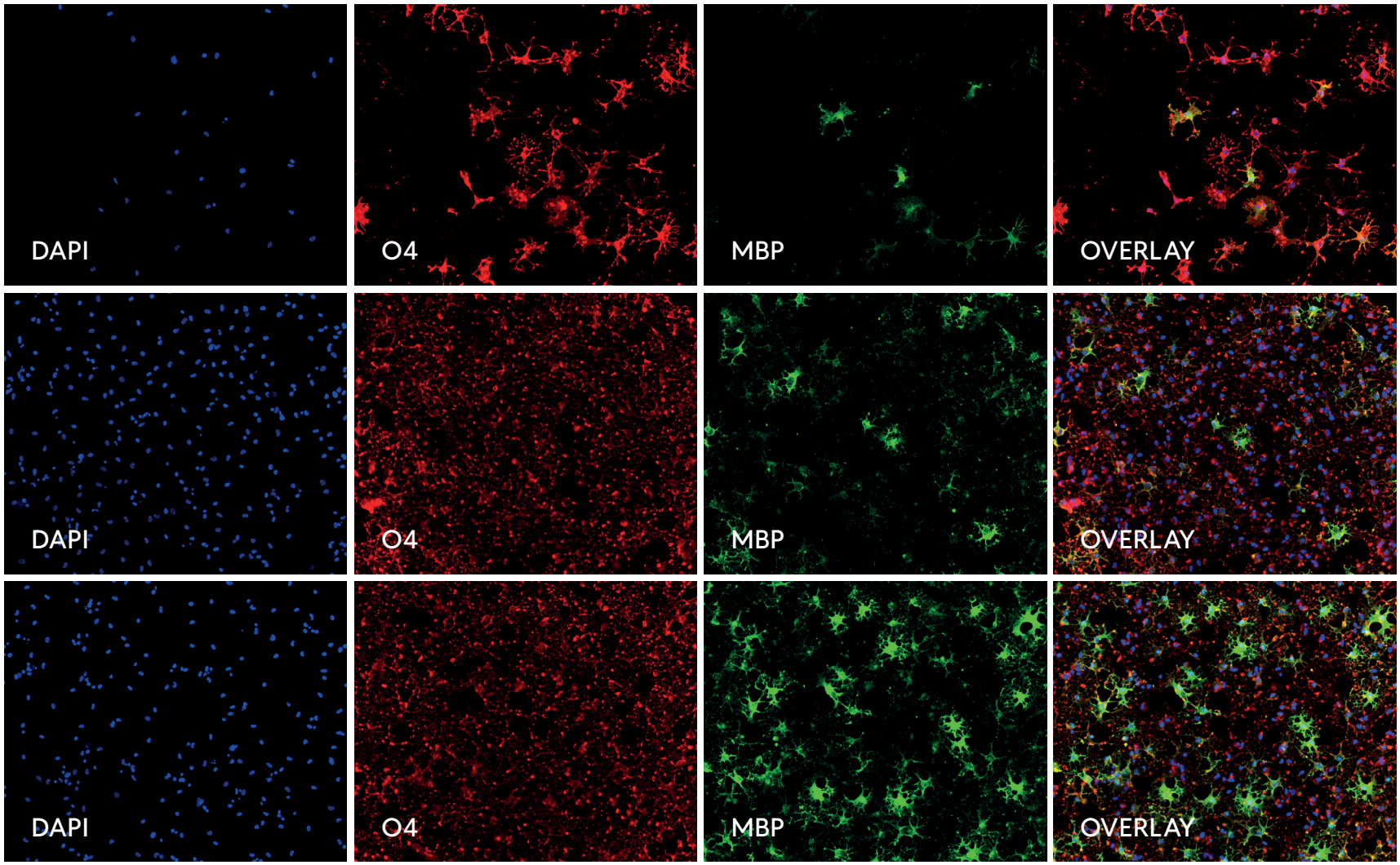

Figure 1B

Percentage of MBP-positive cells across varying db-cAMP levels

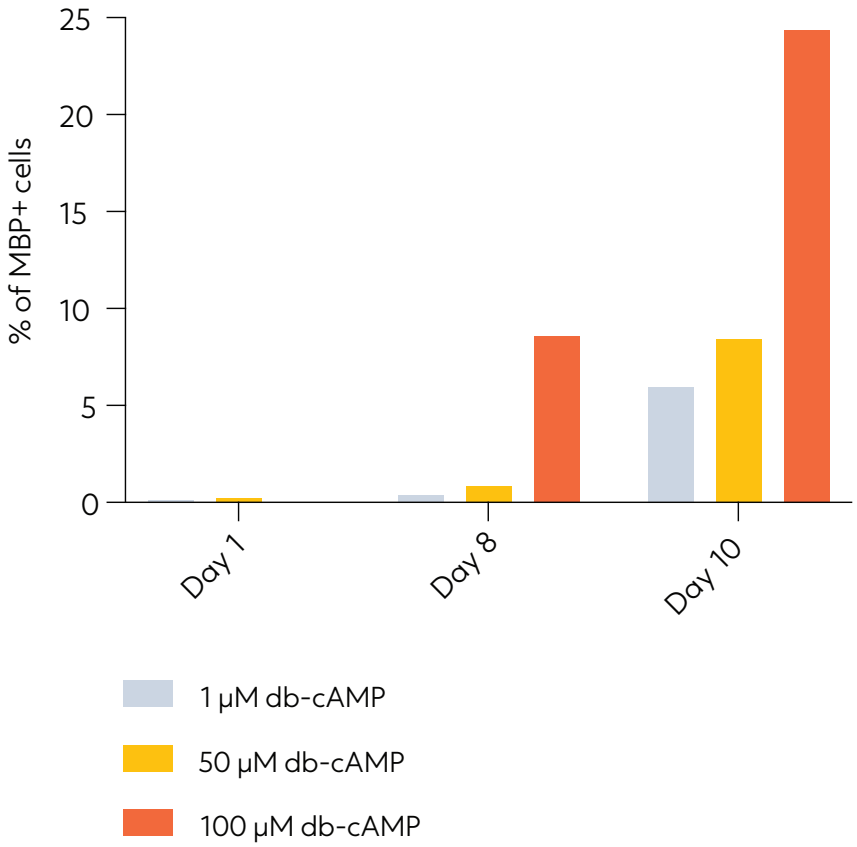

Figure 1: Increasing db-cAMP enhances the number of MBP-positive cells in oligodendrocyte-like cell cultures in a concentration-dependent manner.

ioOLCs were cultured with three different concentrations of db-cAMP (1 µM, 50 µM or 100 µM), as indicated.

A) Immunofluorescent staining of cells shown at day 1 (page 3), day 8 (page 4) and day 10 (page 5) post-revival. At day 1, the cells are positive for the oligodendrocyte marker O4 (red), and counterstained with DAPI (blue). By days 8 and 10, ioOligodendrocyte-like cells display increased morphological complexity and co-expression of O4 (red) and myelin basic protein (MBP; green), alongside nuclear staining (DAPI; blue). Images were acquired at 20X magnification.

B) Quantification of MBP-positive cells at day 1, day 8, and day 10 of cultures with different db-cAMP concentrations. Increased db-cAMP concentration correlated with a higher proportion of MBP-expressing cells over time, suggesting enhanced oligodendroglial maturation. The graph shows the average percentage of MBP-positive cells from 25 fields of view from one well, acquired at 10X magnification.

Morphological changes reflect advanced ioOLC maturation

In addition to MBP expression, cellular morphology was monitored throughout the culture period to assess morphological changes and maturation. By day 8, clear morphological differences were observed between different conditions (**Fig. 2**).

At 1 µM db-cAMP, only a subset of cells exhibited complex branching. In contrast, in cultures with 50 µM or 100 µM db-cAMP, the majority of cells has a more mature phenotype, displaying complex and highly branched processes. This effect was concentration-dependent, with 100 µM db-cAMP resulting in the most uniform and morphologically advanced culture.

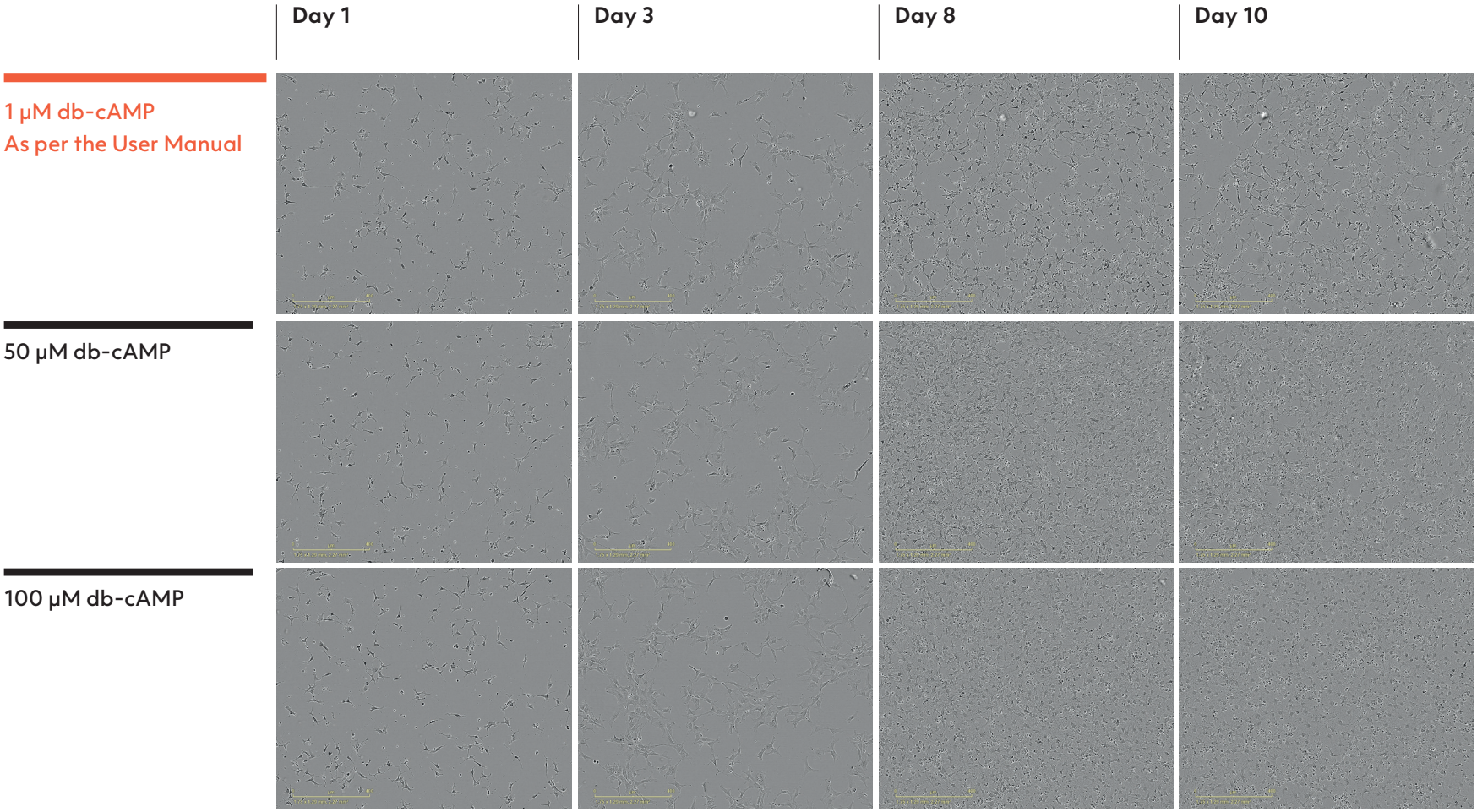

Figure 2: Both 50 and 100 µM db-cAMP concentrations increase proliferation rates and promote uniform morphology.

Brightfield images showing the morphological progression of the cells at days 1, 3, 8, and 10 post-thawing under varying db-cAMP concentrations (1 µM, 50 µM or 100 µM). Cells exhibit an OPC-like morphology by day 1 post-thaw. Over time, cells mature into oligodendrocyte-like cells with increased complexity and multiple branched processes. Higher concentrations of db-cAMP (50 µM and 100 µM) promote maturation and lead to increased cell density at later timepoints (days 8 and 10), suggesting a concentration-dependent effect on cell proliferation or survival during maturation. Scale bar: 400 µm.

Transcript analysis confirms upregulation of maturation markers

RT-qPCR was performed to quantify the expression of mature lineage-specific markers. Mature oligodendrocyte markers were not expressed or were detected at low levels at day 1 and day 3 across all conditions. By day 8, late-stage maturation markers MBP, MAG, and PLP1 became detectable. Elevated db-cAMP levels enhanced MBP and MAG expression, while db-cAMP did not affect PLP1 expression (**Fig. 3**).

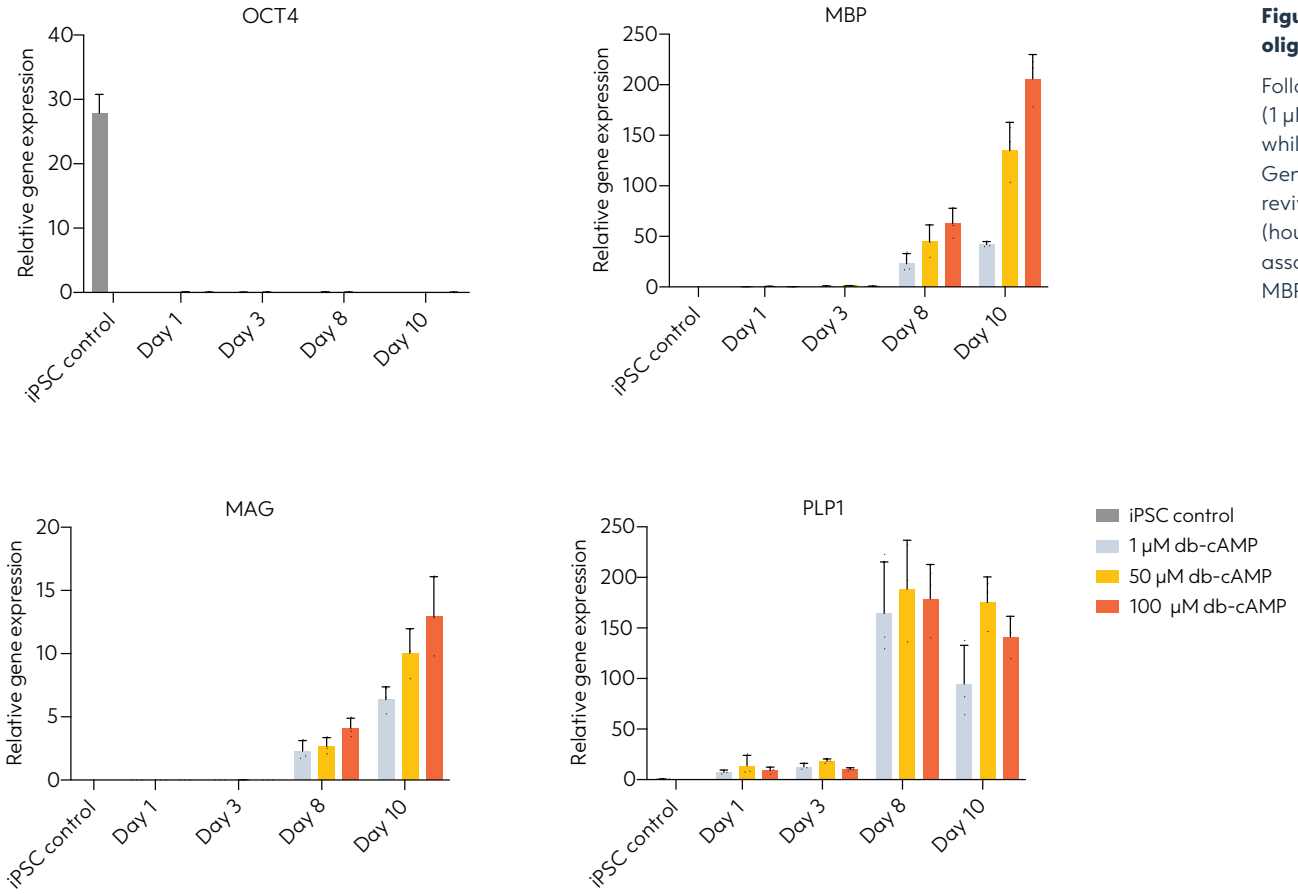

Figure 3: db-cAMP either increases or does not affect the expression of key oligodendroglial genes in ioOligodendrocyte-like cells.

Following deterministic programming under varying db-cAMP concentrations (1 µM, 50 µM or 100 µM), cells downregulate expression of the pluripotency gene OCT4, while upregulating key oligodendroglial lineage markers including MBP, MAG and PLP1. Gene expression was assessed by RT-qPCR at day 1, day 3, day 8, and day 10 post-revival, as well as in the iPSC control, with expression levels normalised to the reference (housekeeping) gene HMBS (n = 3 biological replicates). Increased db-cAMP levels were associated with elevated expression of mature oligodendroglial markers, particularly MBP and MAG, over time.

Methods

User manual instructions were followed for coating plates, cell revival, seeding, and culture of ioOligodendrocyte-like cells (User Manual NPI-0013-UM V-02), with the exception of modified db-cAMP concentrations in the stabilisation (comp:OM1) and maturation (comp:OM2) media.

Instead of preparing db-cAMP stock solution and volumes as per User Manual, for this protocol, two db-cAMP stock solutions were prepared as described in **Table 2**.
The comp:OM1 and comp:OM2 media were prepared according to the User Manual, with the only difference being the use of the db-cAMP stock solution and adjusted volumes indicated in **Table 3**.

Table 1: db-cAMP reagent information

Reagent	Stock solution	Cat no	Storage
db-cAMP	Santa Cruz Biotechnology	sc-201567	-80°C

Table 2: Recommended preparation and storage of db-cAMP stock solutions

Working concentration	Stock solution	Media preparation	Storage
100 µM db-cAMP	100 mM To prepare, reconstitute 20 mg in 407 µL of sterile water	1 µL of 100 mM stock solution per 1 mL of medium (final concentration 100 µM)	-80°C
50 µM db-cAMP	50 mM To prepare, reconstitute 20 mg in 814 µL of sterile water	1 µL of 50 mM stock solution per 1 mL of medium (final concentration 50 µM)	-80°C

Table 3: comp:OM1 and comp:OM2 media preparation with increased db-cAMP concentrations

	Reagent	For 10 mL	For 100 mL
For comp:OM1 & comp:OM2 with 100 µM db-cAMP	db-cAMP (from 100 mM stock)	10 µL	100 µL
For comp:OM1 & comp:OM2 with 50 µM db-cAMP	db-cAMP (from 50 mM stock)	10 µL	100 µL

Conclusions

This study demonstrates that elevating db-cAMP levels in the culture medium is an effective strategy to promote maturation of ioOLCs, as measured by MBP expression. Increasing db-cAMP concentration resulted in a concentration-dependent enhancement of both cellular morphology and MBP expression. At 100 µM db-cAMP, the proportion of MBP-expressing cells improved approximately 4-5 fold compared to the 1 µM condition by day 10.

We have provided this optional optimised medium formulation with higher db-cAMP concentrations for researchers seeking higher MBP expression, faster maturation and extended culture duration of ioOLCs. This adjustment promotes enhanced expression of late-stage oligodendrocyte markers such as MBP and MAG, and contributes to the relevance of ioOLCs in demyelinating disease studies, offering a simple, quick human oligodendroglial model.

References

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