



Overview

A crucial aspect of accurately modeling the central nervous system involves the concurrent differentiation of neurons and glial cells in a 3D organoid organization, including astrocytes and the oligodendrocyte lineage pathway, which consists of oligodendrocyte precursor cells (OPCs), oligodendrocytes (OLs), and myelinating OLs. AxoSim's 3D BrainSim® model addresses this need by differentiating neuron and glial cell types from iPSCs over a 12-week culture period. In addition to baseline media, AxoSim has tested a modified media as well as media supplemented with the pro-myelinating drug clemastine to assess the modulability of the OPC/OL lineage.

Methods

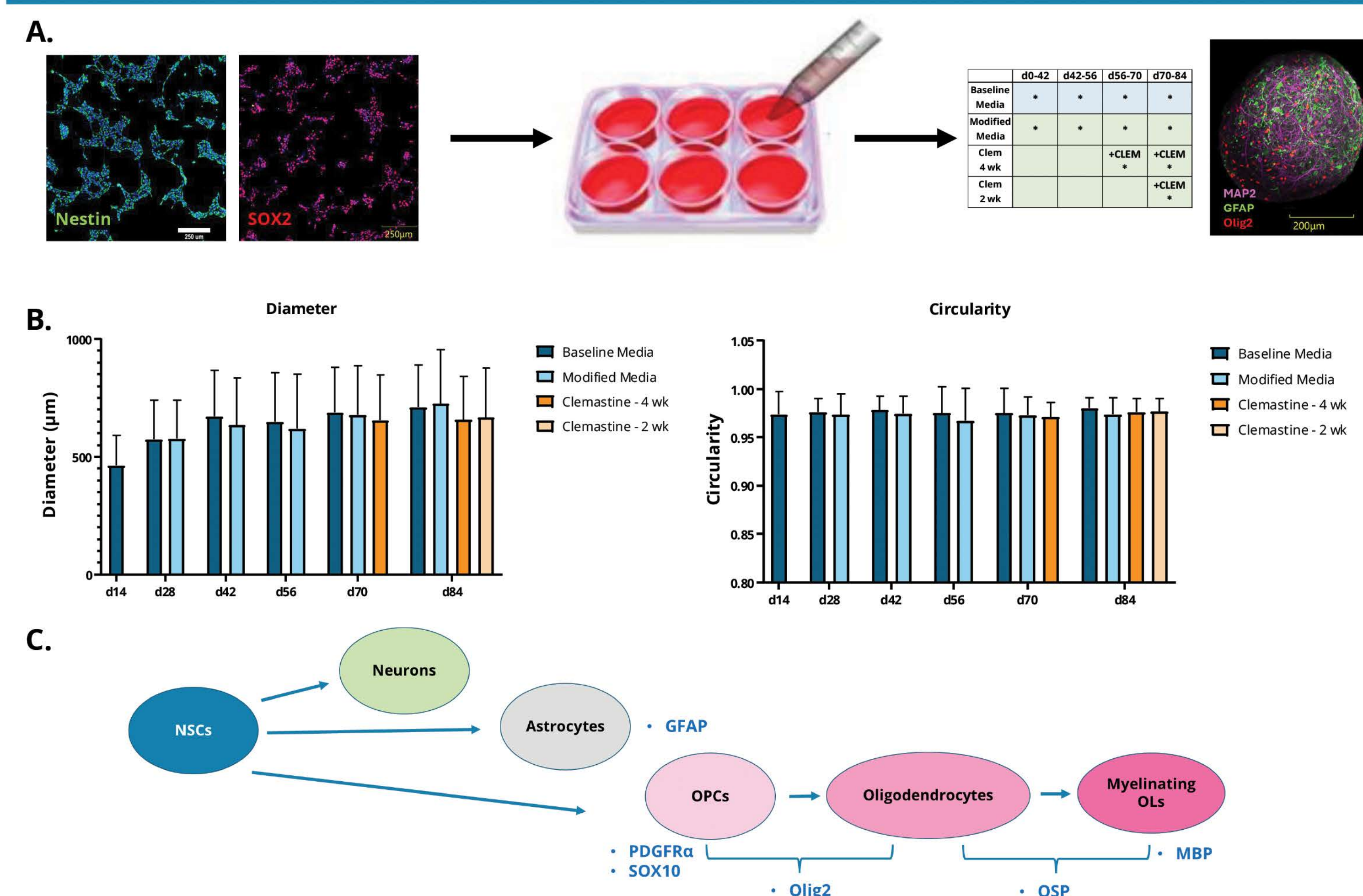


Figure 1. (A) iPSC-derived NSCs are plated in 6-well plates and cultured as spheroids over 12 weeks. Spheroids were cultured in baseline media, modified media, or modified media supplemented by 2- or 4-week clemastine treatments. **(B)** Spheroid diameter and circularity were consistent among the four test groups. **(C)** Markers for glial differentiation pathways were measured at d42, d56, d70, and d84.

OPC/OL Differentiation

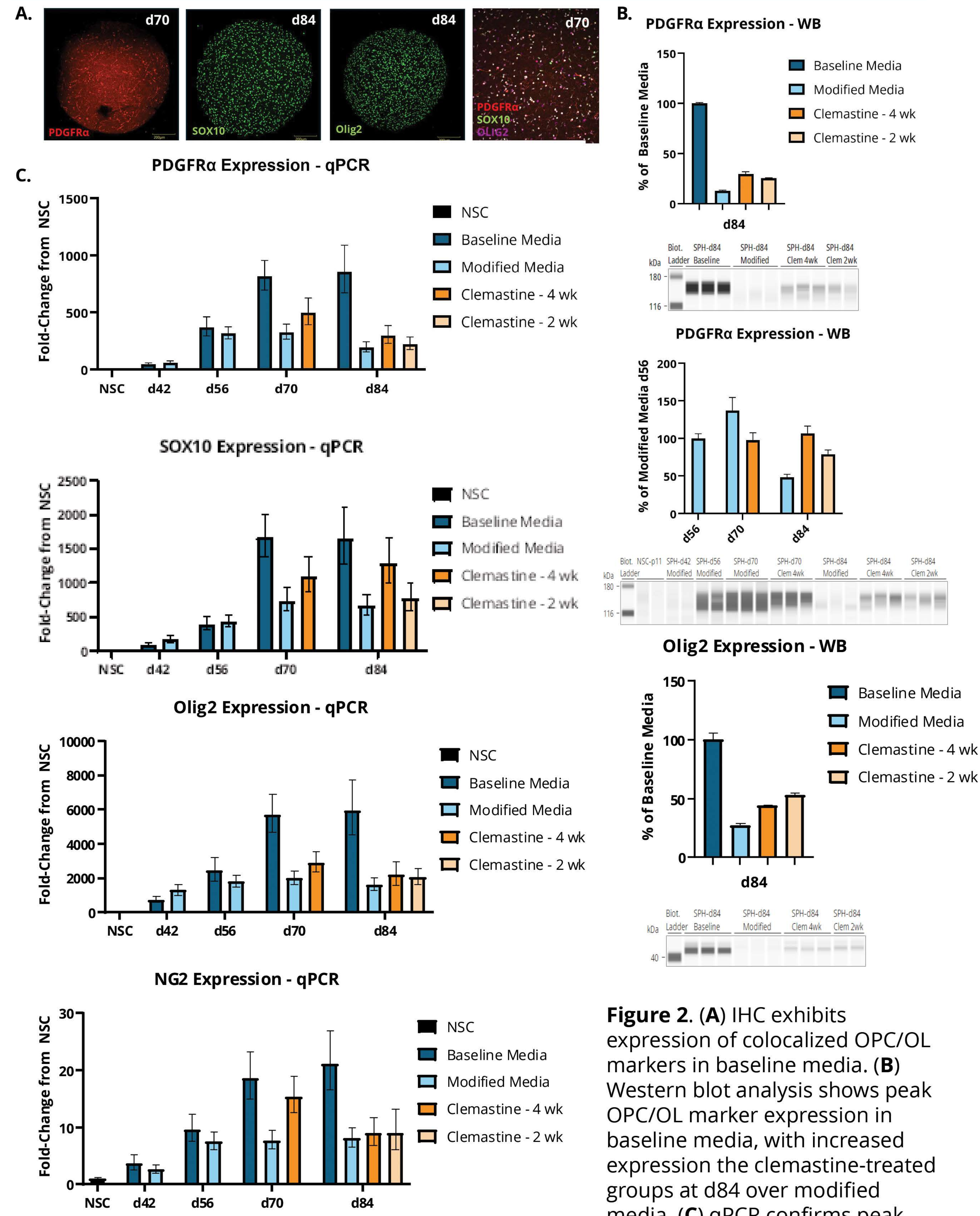
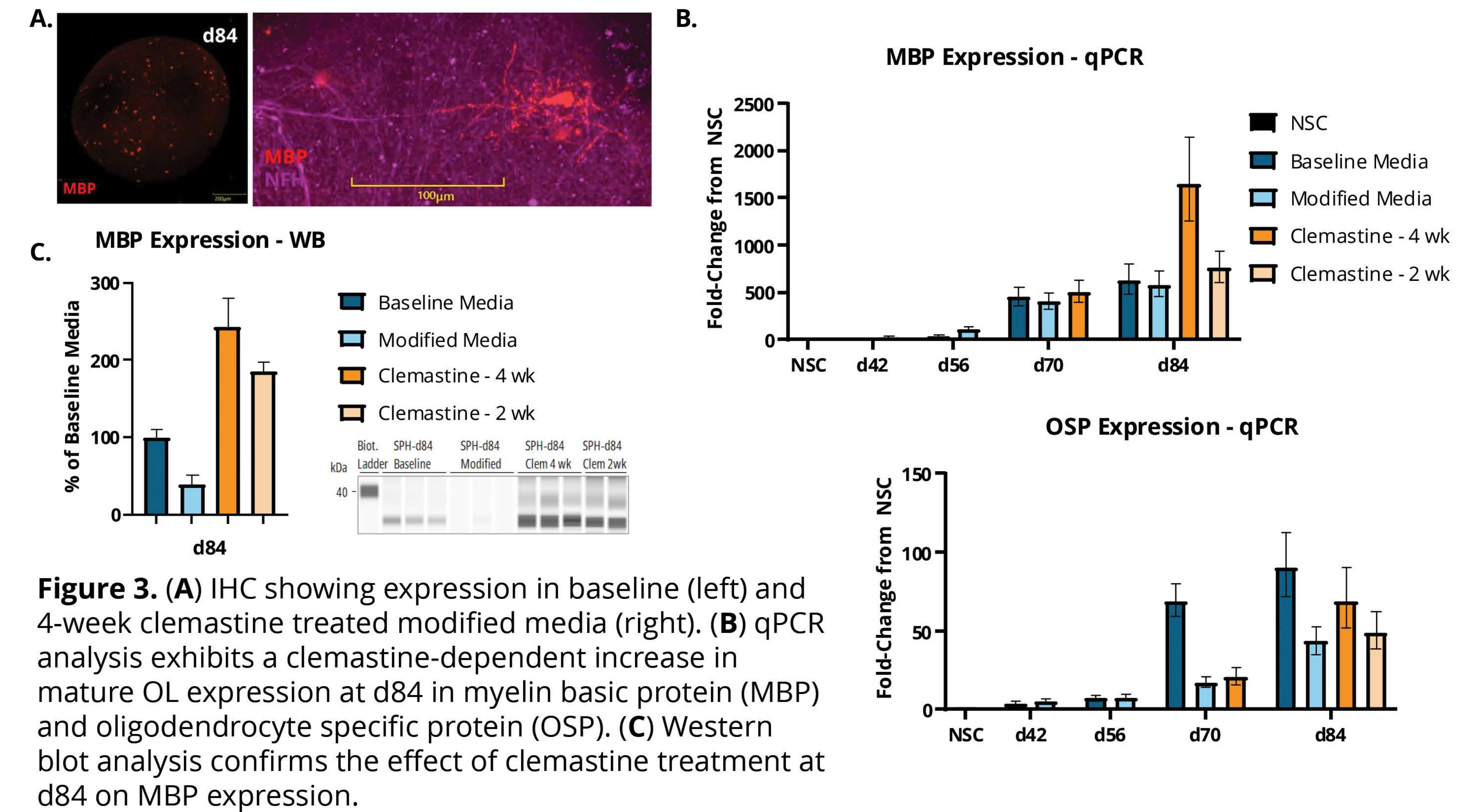
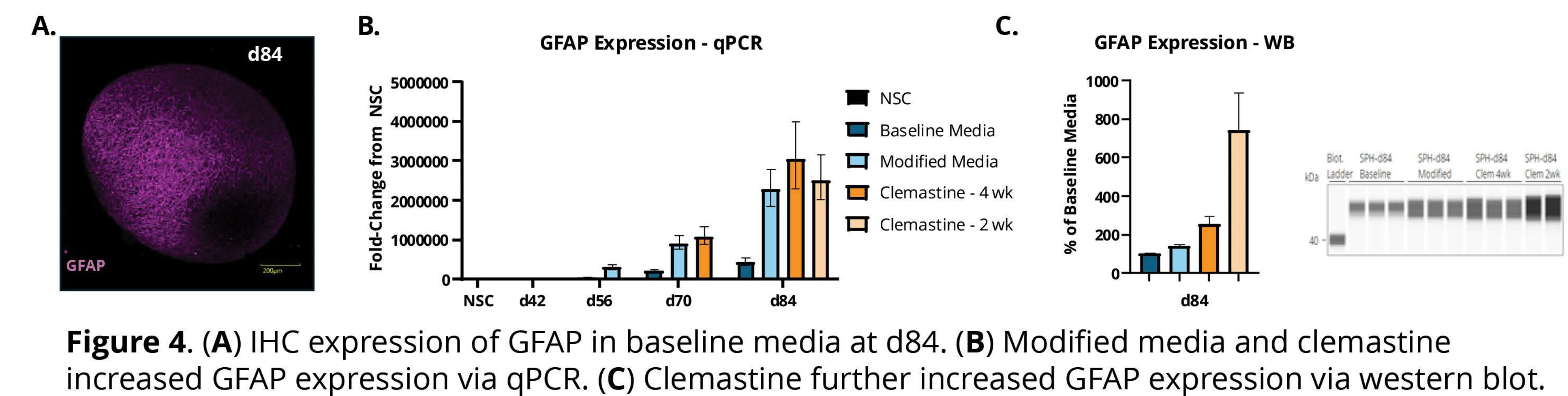


Figure 2. (A) IHC exhibits expression of colocalized OPC/OL markers in baseline media. **(B)** Western blot analysis shows peak OPC/OL marker expression in baseline media, with increased expression the clemastine-treated groups at d84 over modified media. **(C)** qPCR confirms peak OPC/OL transcript expression in baseline media, with an increase in clemastine-treated media over modified media alone.

Myelinating OL Differentiation



Astrocyte Differentiation



Conclusions

- While all conditions tested successfully differentiated glial cells, the baseline media proved to be superior to the modified media in the differentiation of OPCs and OLS.
- By d84, the 4-week clemastine treatment had a robust effect on various OPC and OL markers, most notably MBP.
- The successful modulation of the OPC/OL differentiation pathway by clemastine demonstrates the potential for BrainSim® to assess drugs targeting this pathway, such as those aiming to treat demyelinating disorders like multiple sclerosis.