Glial Cell Type Composition in a 3D Model of the Central Nervous System

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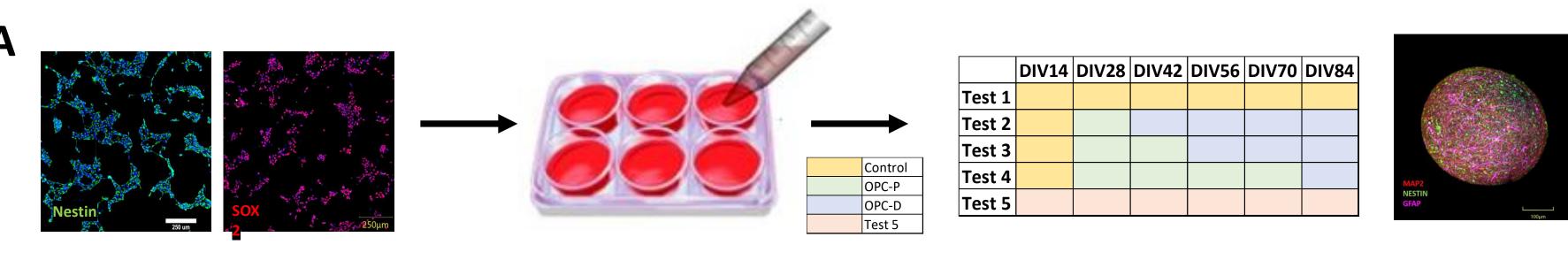


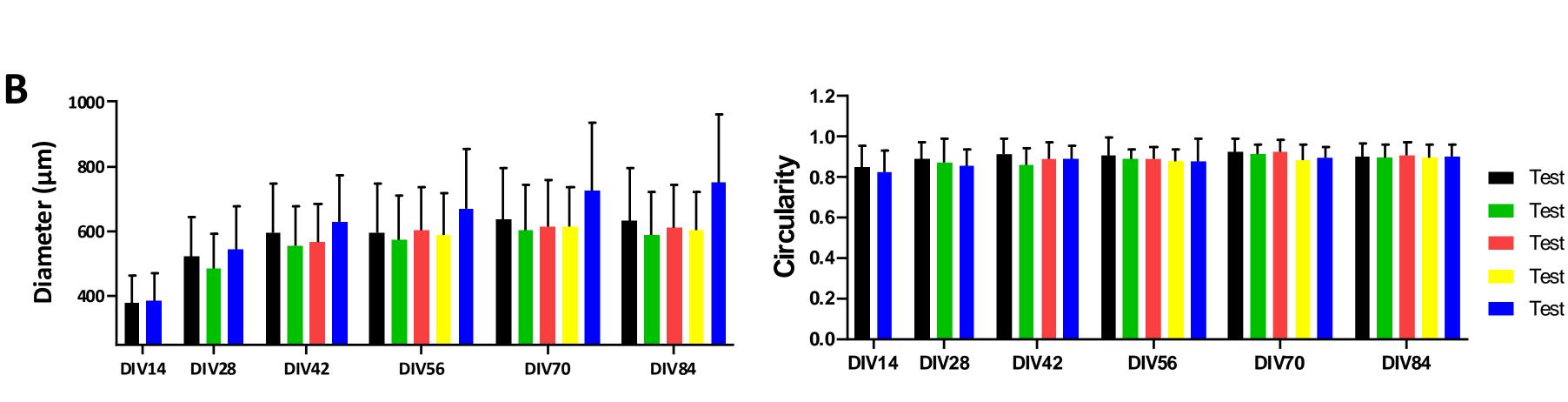
Overview

We successfully cultured iPSC-derived neurons for 12 weeks using variable growth factor formulations, reaching variable levels of glial differentiation.

Differentiation of astrocytes, oligodendrocyte precursor cells (OPCs), and oligodendrocytes were assessed via the presence of cell-type specific markers in IHC, qPCR, and Western blot. Of the five media timelines tested, Test 5 proved to have the highest abundance of all glial cell types, with considerable evidence of glia as early as DIV42.

Methods





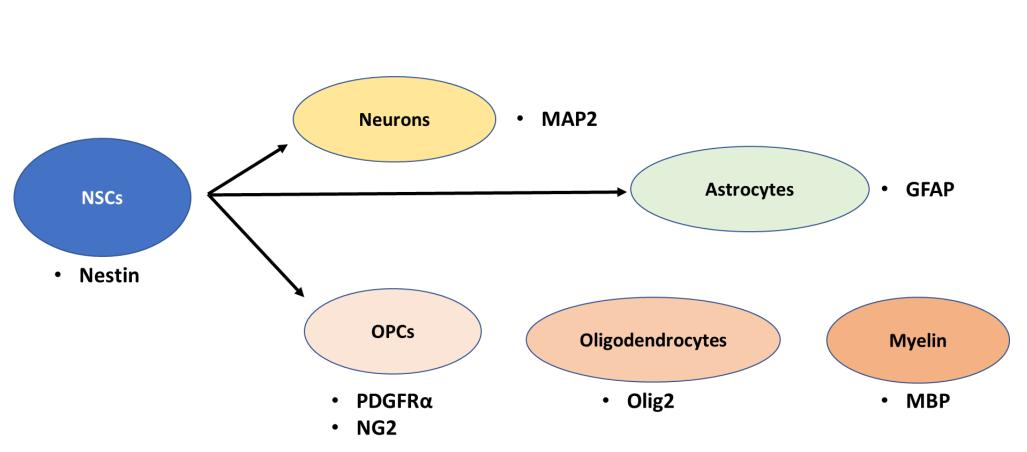
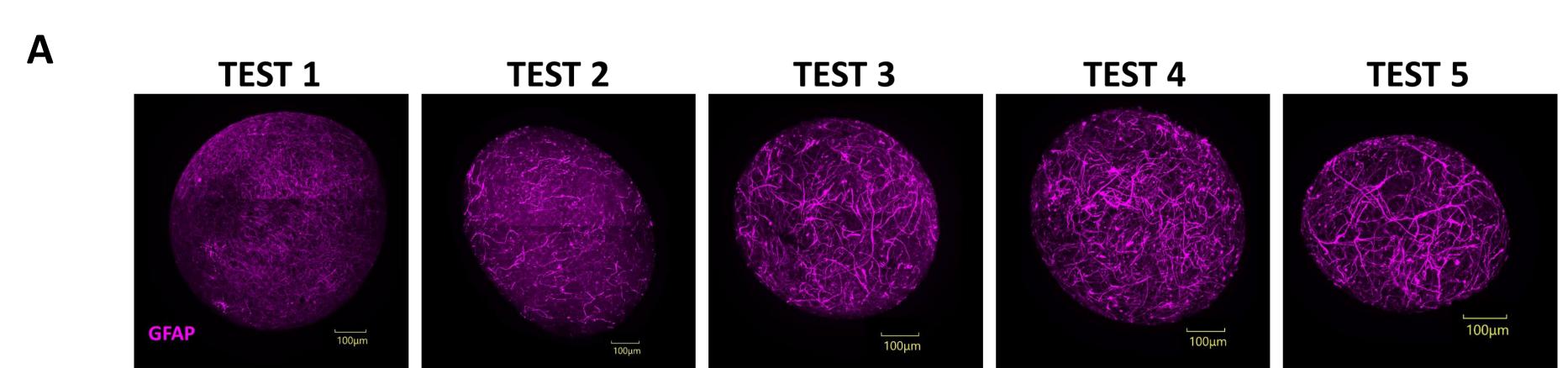
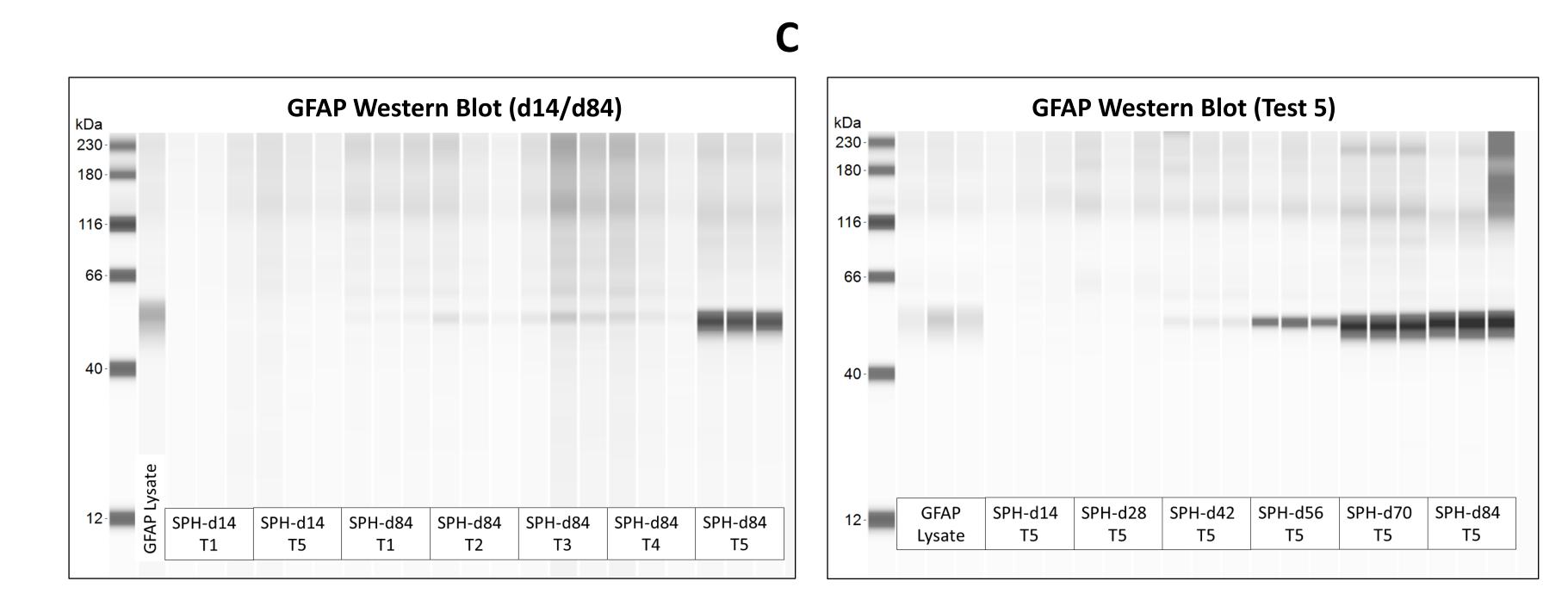


Figure 1: A) Neural stem cells (NSCs) were plated in a 6-well plate and incubated on a rocker for 12 weeks with various media formulations, leading to spheroids expressing a mixture of NSC (Nestin), neuron (MAP2), and glial (GFAP) markers at d84. B) Spheroid size trended towards an increase in Test 5, while circularity was consistent throughout the culture period in all test groups. C) An outline of the differentiation pathways and markers used in the study.

Astrocyte Differentiation





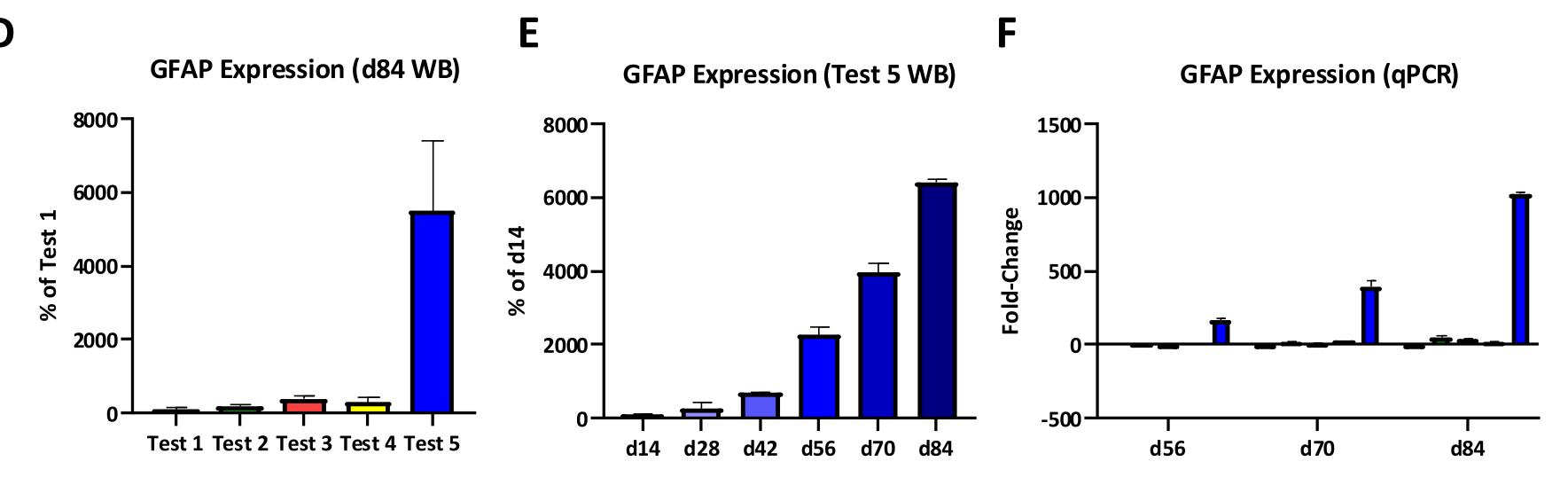


Figure 2. A) IHC expression of astrocyte marker GFAP at d84. **B/D)** GFAP Western blot images from all test groups at d14/d84. **B/D)** GFAP Western blot for all test groups at d14 and d84 with quantitation indicates a large increase in expression in Test 5 at d84 relative to Test 1. **C/E)** GFAP Western blot for Test 5 at all timepoints shows a steady increase over time. **F)** GFAP qPCR indicates increased expression in Test 5, with a steady increase over time between d56 and d84.

OPC and Oligodendrocyte Differentiation

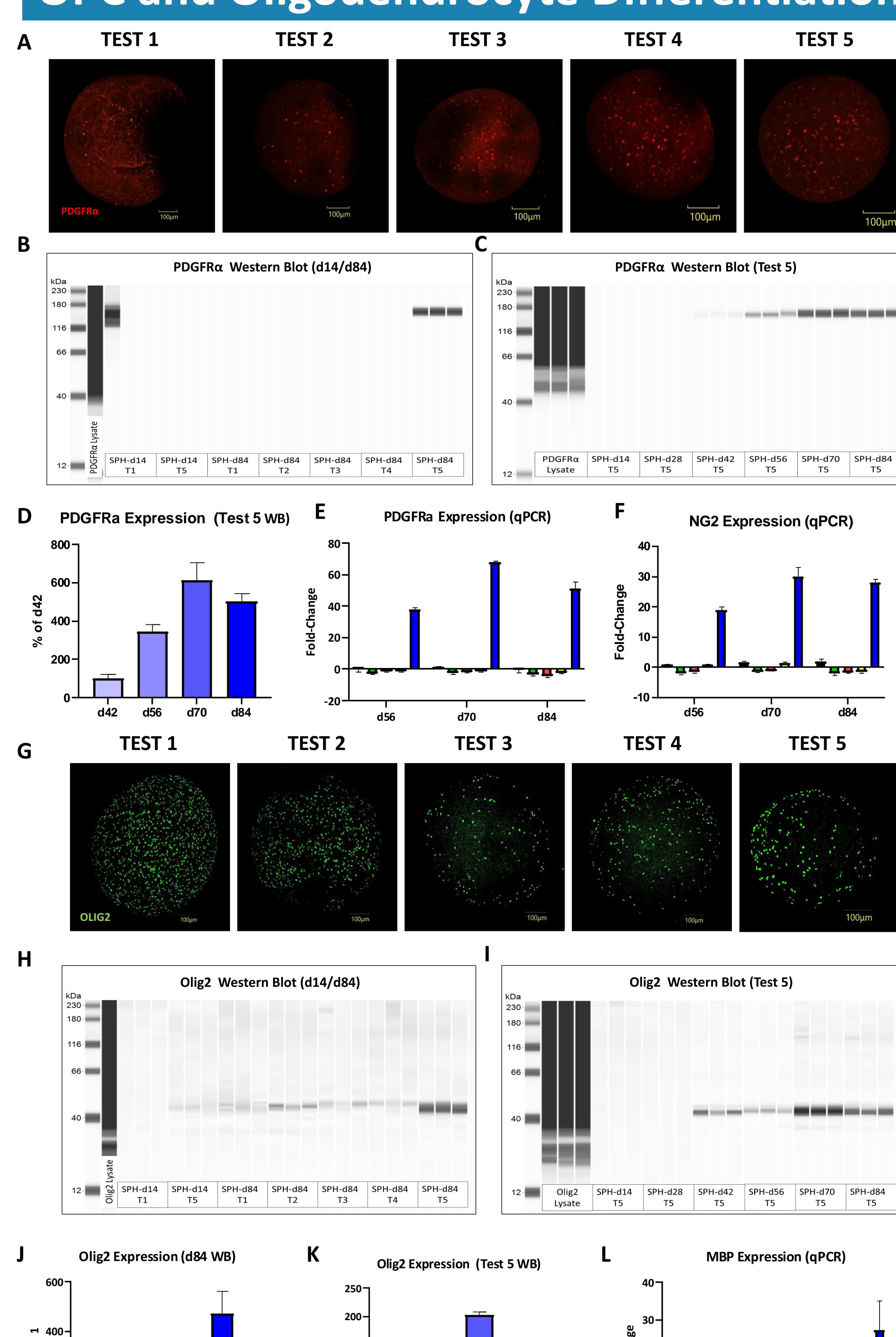


Figure 3. A) IHC images of OPC marker PDGFRα at d84. B) PDGFRα Western blot at d14/d84 in all test groups yielded evidence of expression only in Test group 5 at d84. C/D) PDGFRα Western blot in Test 5 indicates protein expression at d42-d84, with peak expression at d70. E) qPCR analysis from d56-d84 shows increased expression of PDGFRα in Test 5, with peak expression occurring at d70. F) qPCR from d45-d84 for OPC marker NG2 shows increased expression in Test 5, with peak expression occurring at d70. G) IHC for oligodendrocyte marker Olig2 demonstrates expression in every test group at d84. H/J) Quantitative Olig2 Western blot at d14/d84 in all test groups shows a marked increase in Test 5 expression. I/K) Quantitative Olig2 Western blot in Test 5 indicates protein expression at d42-d84, with peak expression at d70. L) qPCR analysis from d56-84 indicates increased expression in Test 5, with a steady increase over time.

Test 1 Test 2 Test 3 Test 4 Test 5

Conclusions

- Test 5 shows a peak in OPC and non-myelinating oligodendrocyte markers at DIV70, and a steady increase in astrocyte and myelin markers across 12 weeks of development.
- Future work will aim to detect the effects of demyelinating and promyelinating drugs on oligodendrocyte development and differentiation.