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Supplemental Information

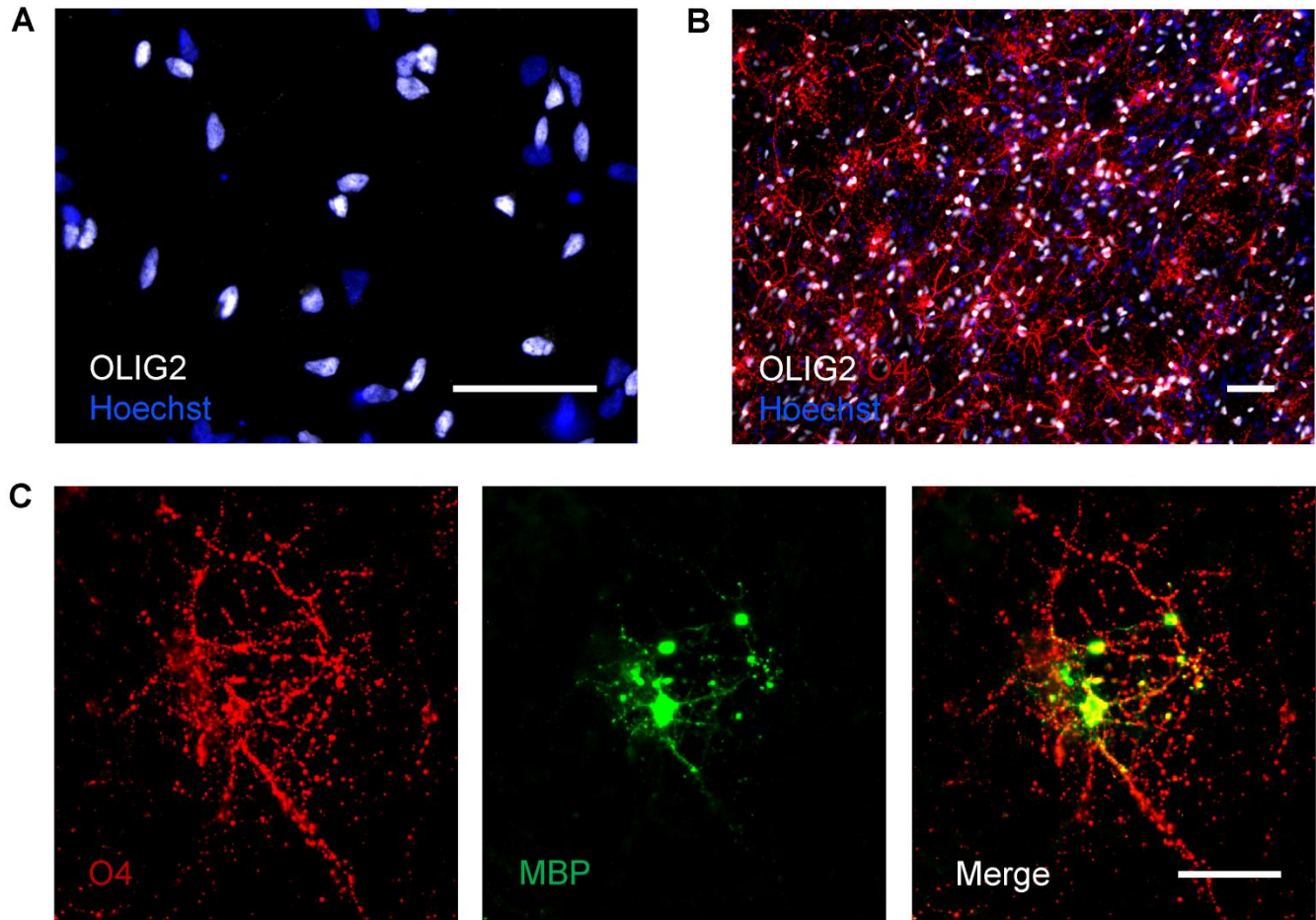
High Yields of Oligodendrocyte Lineage Cells from Human Embryonic Stem Cells at Physiological Oxygen Tensions for Evaluation of Translational Biology

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Inventory of Supplementary Information

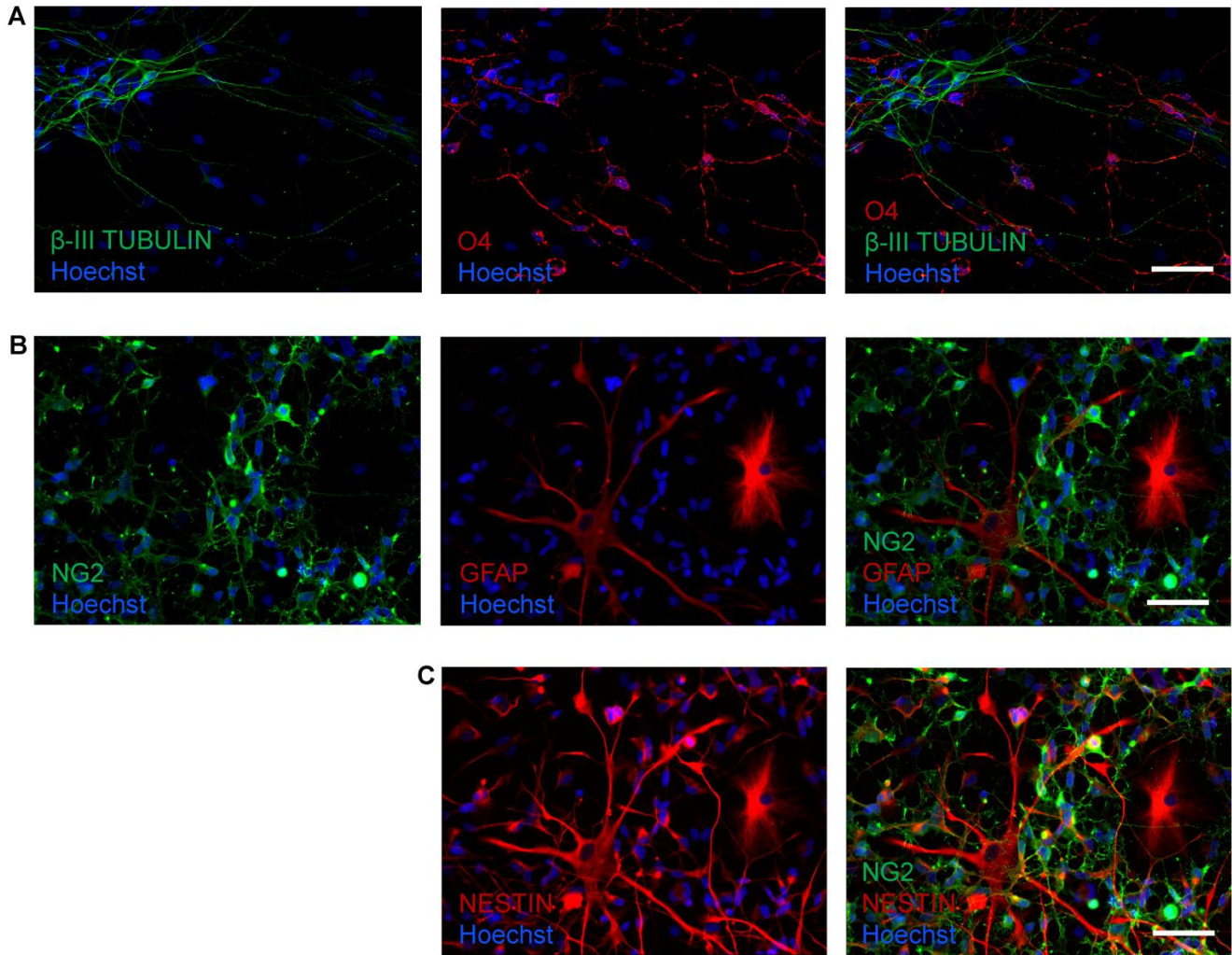
1. Supplementary Figure 1 – oligodendrocyte lineage cells were derived from the HUES-9 cell line with similar efficiency; linked to figures 2 and 3
2. Supplementary Figure 2 – hESC-NPC derived OPCs do not express neuronal or astrocytic markers; linked to figure 2
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Supplementary Information:



Supplementary Figure One: Oligodendrocyte lineage cells were derived from the HUES-9 cell line with similar efficiency; related to Figures 2 and 3.

(a) OLIG2 induction at D28-35 in HUES-9 hESC-derived NPCs via the FGF-2/SHH ventral forebrain route was equivalent to that achieved with the H9 cell line ($53.1 \pm 4.2\%$). (b) After 5 weeks of terminal differentiation of D100 hESC-NPCs, large numbers of O4⁺ oligodendrocytes were generated from the HUES-9 line ($46 \pm 6\%$) (c) MBP expression was also observed in oligodendrocytes derived from HUES-9 hESCs. Scale bar = 50 μm .



Supplementary Figure Two: hESC-NPC derived OPCs do not express neuronal or astrocytic markers; related to Figure 2.

(a&b) There was no overlap in expression between β -III tubulin and O4, nor of NG2 and GFAP, indicating that cells labeled with NG2 or O4 were indeed oligodendroglial lineage cells rather than neurons or astrocytes. (b&c) NG2 expression did overlap with NESTIN, but only in the NESTIN+ population that did not express GFAP. b&c show the same field of view for clarity of comparison of marker co-expression. Scale bar = 50 μ m.

Supplementary Table 1: PCR Primers; related to methods and Figure 1.

| | |
|-------------------|--------------------------------|
| <i>β-ACTIN</i> F: | 5'-GTTACAGGAAGTCCCTTGCCATCC-3' |
| <i>β-ACTIN</i> R: | 5'-CACCTCCCCTGTGTGGACTTGGG-3' |
| <i>GSX2</i> F: | 5'-AGCCTGAGCCGAGCGGTACTC-3' |
| <i>GSX2</i> R: | 5'-CTGGTCCTCACGTCCCCCGC-3' |
| <i>HOXB4</i> F: | 5'-GTGAGCACGGTAAACCCCAAT-3' |
| <i>HOXB4</i> R: | 5'-CGAGCGGATCTTGGTGTTG-3' |
| <i>NKX2.1</i> F: | 5'-AACCAAGCGCATCCAATCTCAAGG |
| <i>NKX2.1</i> R: | 5'-TGTGCCAGAGTGAAGTTTGGTCT -3' |
| <i>PAX6</i> F: | 5'-ATGTGTGAGTAAAATTCTGGGCA-3' |
| <i>PAX6</i> R: | 5'-GCTTACAACCTTCTGGAGTCGCTA-3' |

Supplementary Table 2: Details of Primary Antibodies used for Immunolabelling; related to methods.

| Primary Antibody | Isotype | Concentration | Manufacturer |
|----------------------------|----------------------|----------------------|-------------------------|
| β-III TUBULIN | Mouse IgG2b | 1 in 1000 | Sigma T8660 |
| GALC | Mouse IgG3 | 1 in 100 | Chemicon MAB342 |
| GFAP | Rabbit polyclonal | 1 in 1000 | Dako Z0334 |
| MBP | Rat | 1 in 100 | Abcam AB7349 |
| NESTIN (human specific) | Mouse IgG1 | 1 in 500 | Chemicon MAB5326 |
| NG2 | Mouse IgG2a | 1 in 250 | BD Pharmingen 554275 |
| NG2 | Rabbit polyclonal | 1 in 200 | Chemicon AB5320 |
| NKX2.2 | Mouse IgG2b | 1 in 75 | DSHB 74.5A5 |
| O1 | Mouse IgM | 1 in 1000 | R&D systems MAB1327 |
| O4 | Mouse IgM | 1 in 1000 | R&D systems MAB1326 |
| OLIG2 | Rabbit polyclonal | 1 in 500 | Chemicon AB9610 |
| PDGF-R α | Rabbit polyclonal | 1 in 250 | Santa Cruz SC-338 |
| VIMENTIN | Chick | 1 in 500 | Chemicon AB5733 |