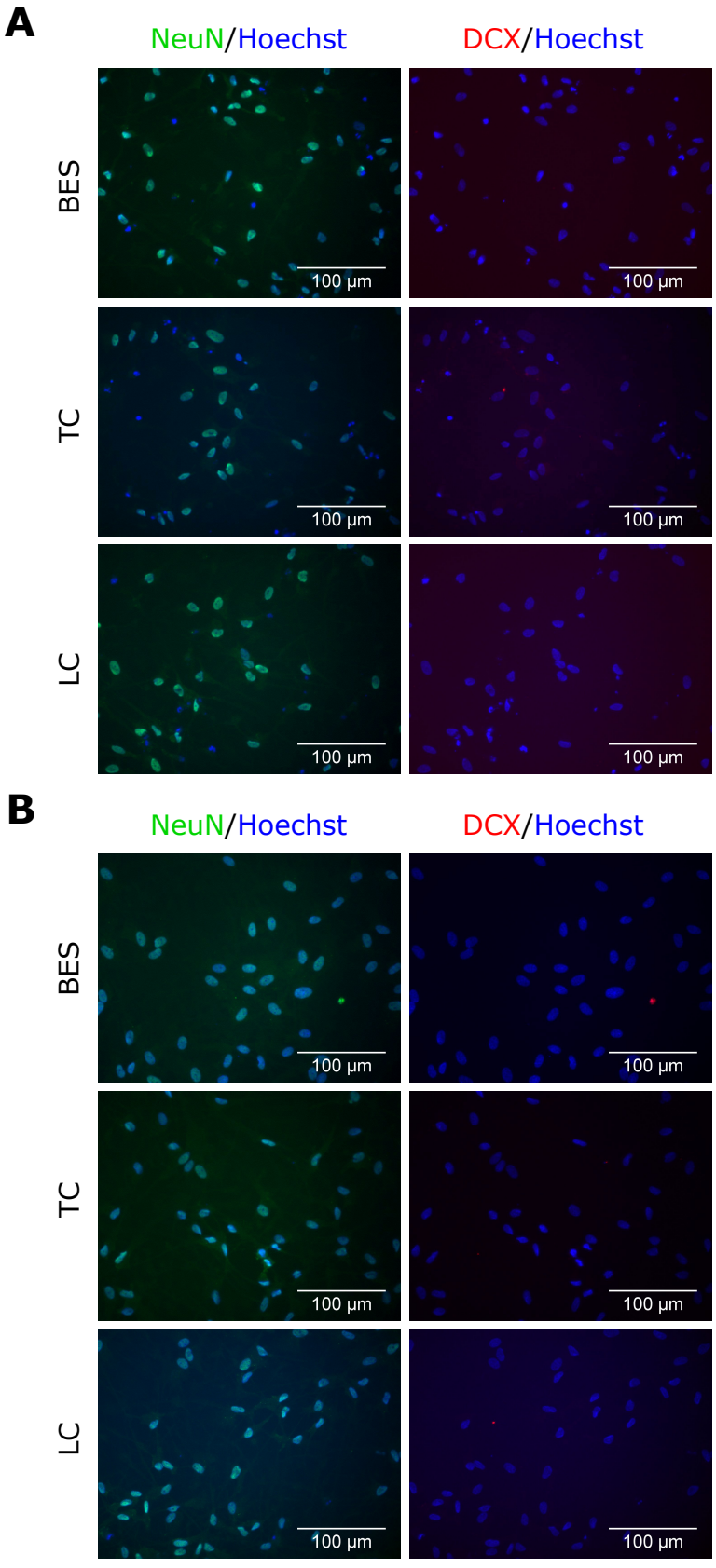
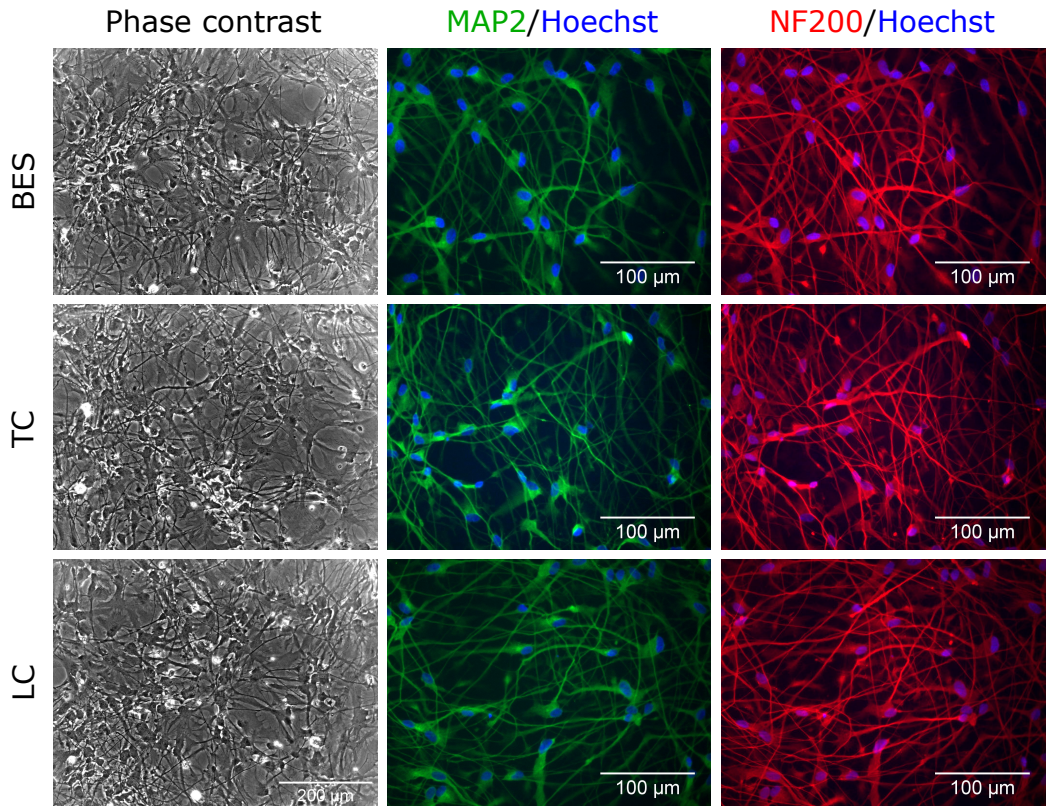


Supplementary Figure 1. Expression of neuronal markers in hNSCs after 4 weeks of differentiation. A -B) Neuronal nuclear protein (NeuN) and doublecortin (DCX) in sprayed (BES) and control (TC: taken to the BES laboratory but non-sprayed; LC: not moved from the tissue culture laboratory) in two hNSC lines, Cs17, passage 22 (A) and Cs23, passage 20 (B). Nuclei are counterstained with Hoechst 33258 (blue).



Supplementary Figure 2. Neuronal differentiation of hNSCs after bio-electrospray assessed by phase contrast imaging and double-labelling for neuronal markers. Sprayed (BES) and control (TC: taken to the BES laboratory but non-sprayed; LC: not moved from the tissue culture laboratory) hNSCs (Cs 23, passage 20) differentiated for 4 weeks. Note typical neuronal morphology and neurite extension and expression of the neuronal markers, MAP2, microtubule-associated protein 2 (green), and NF200, neurofilament 200 (red). Nuclei are counterstained with Hoechst 33258 (blue). All phase contrast images are at the same magnification.

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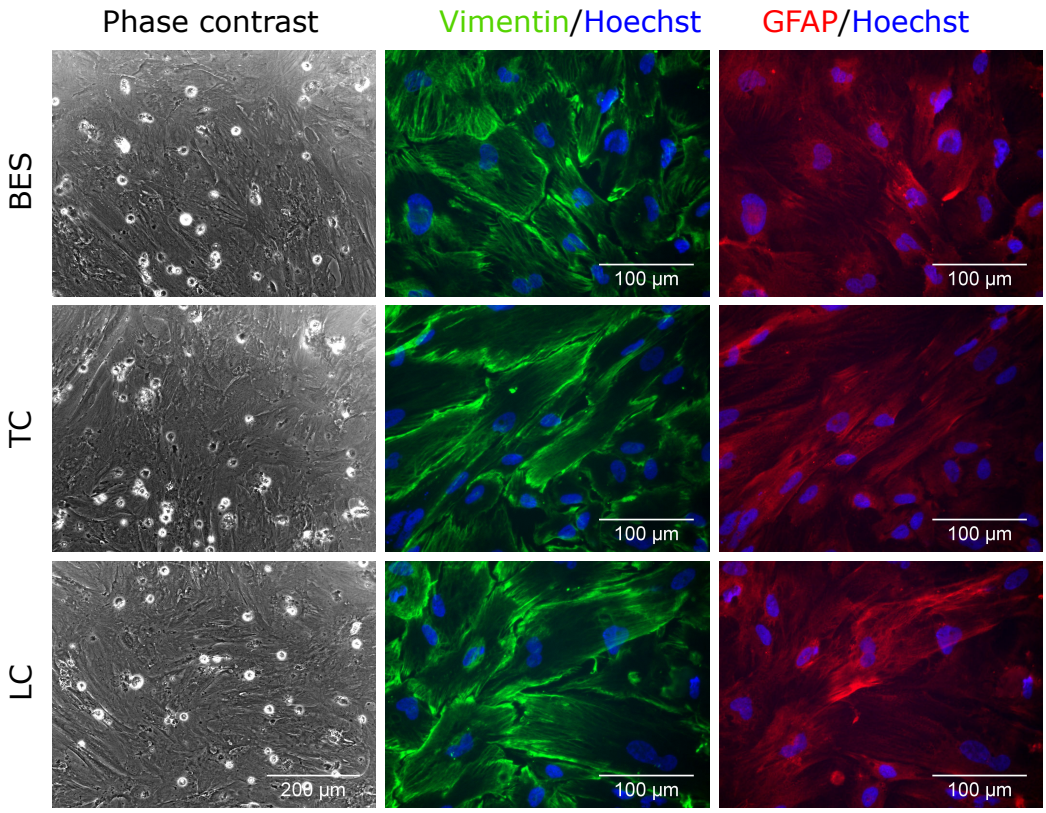

Supplementary Figure 3. Astrocyte differentiation of hNSCs after bio-electrospray assessed by phase contrast imaging and double-labelling for astrocyte markers. Sprayed (BES) and control (TC: taken to the BES laboratory but non-sprayed; LC: not moved from the tissue culture laboratory) hNSCs (Cs23, passage 20) differentiated for 2 weeks. Note the flatten morphology typical of astrocyte morphology and expression of astrocyte markers, Vimentin (green) and GFAP (glial fibrillary acidic protein; red). Nuclei are counterstained with Hoechst 33258 (blue). All phase contrast images are at the same magnification.

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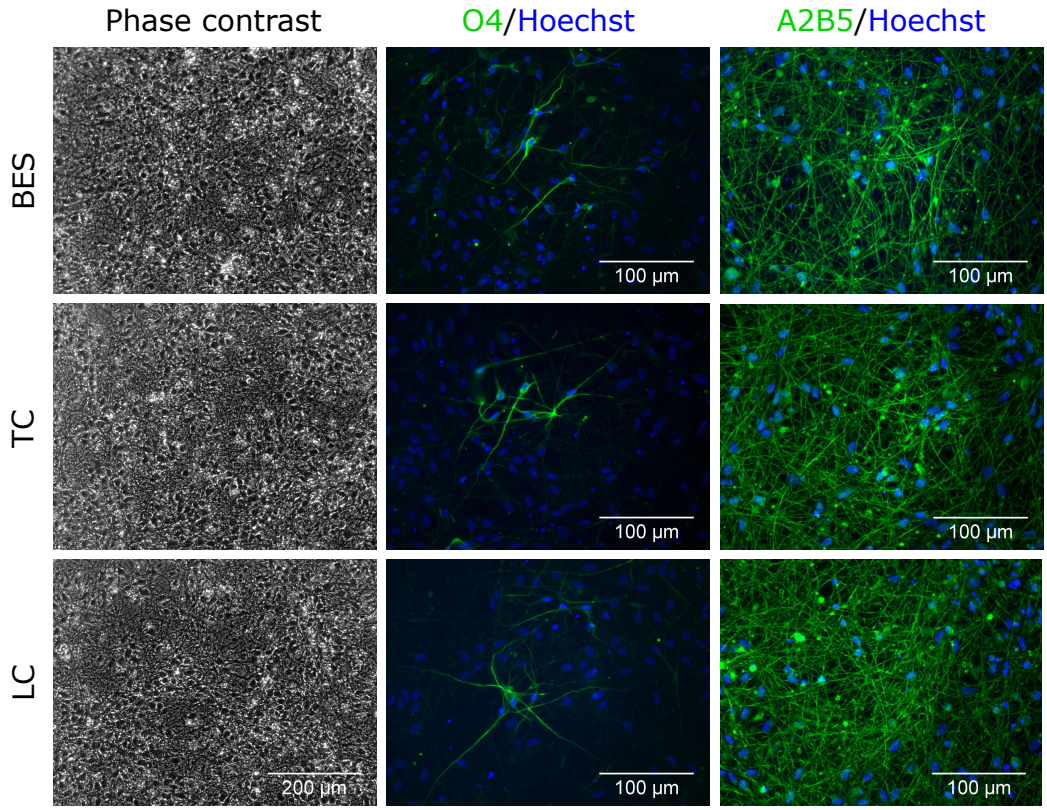

Supplementary Figure 4. Oligodendrocyte differentiation of hNSCs assessed after bio-electrospray by phase contrast imaging and immunostaining. Sprayed (BES) and control (TC: taken to the BES laboratory but not sprayed; LC: not moved from the tissue culture laboratory) hNSCs (Cs23, passage 20) differentiated for 5 weeks. Note the presence of cells with different morphologies with a few expressing the oligodendrocyte marker, O4, and a larger proportion the glial precursor marker, A2B5. Nuclei are counterstained with Hoechst 33258 (blue). All phase contrast images are the same magnification.

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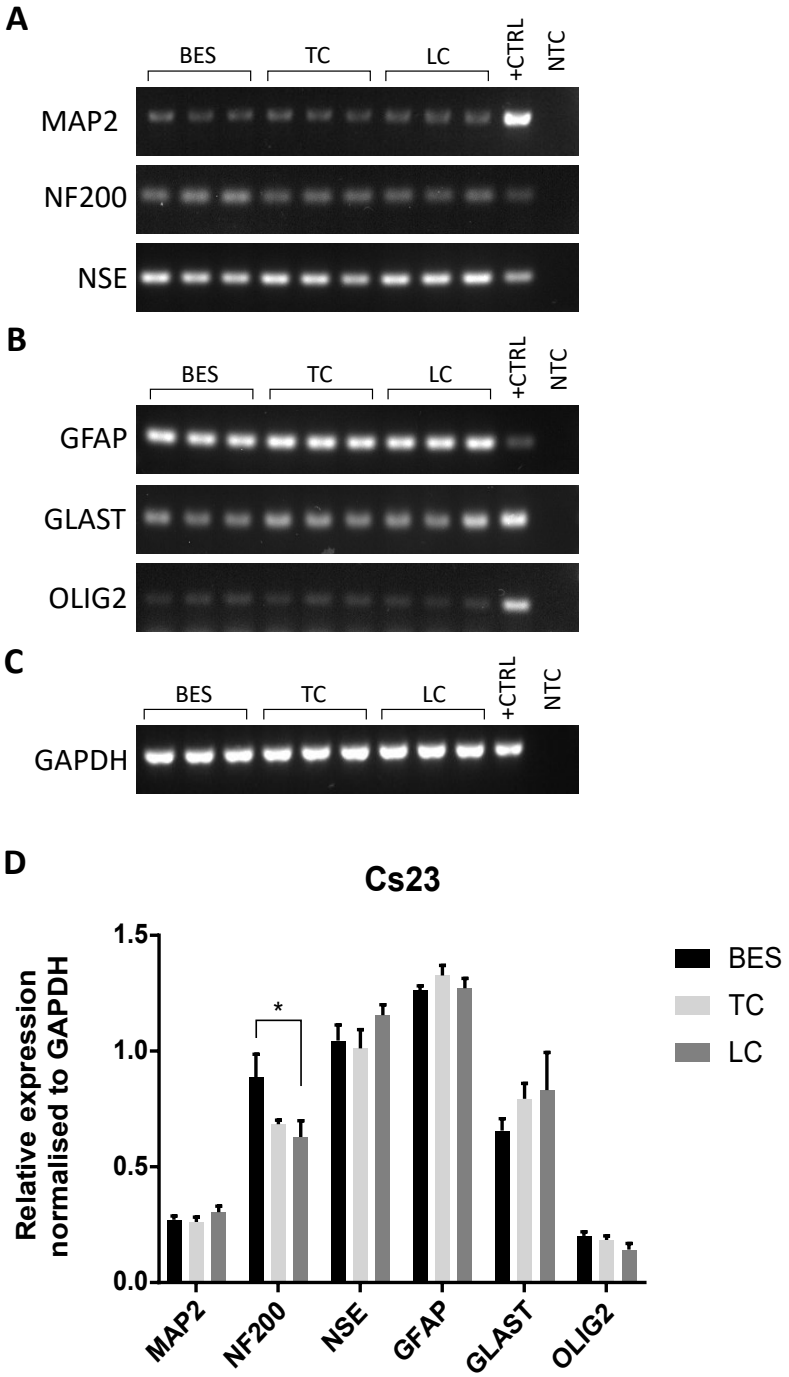

Supplementary Figure 5. Expression of neural markers in hNSCs neuronally differentiated for 4 weeks after bio-electrospray assessed by RT-PCR. A-C. Expression of neuronal markers (A), glial markers (B), and a reference house-keeping gene (C) in biological triplicates of sprayed (BES) and control (TC: taken to the BES laboratory but non-sprayed; LC: not moved from the tissue culture laboratory) hNSCs (Cs 23, passage 22). MAP2: microtubule-associated protein 2; NF200 neurofilament 200; NSE: neuron-specific enolase; GFAP: glial fibrillary acidic protein; GLAST: glutamate aspartate transporter; OLIG2: oligodendrocyte transcription factor 2; GAPDH: glyceraldehyde 3-phosphate dehydrogenase. +CTRL: human embryonic brain cDNA used as a positive control (22 weeks post conception); NTC: no template control using water instead of cDNA. **D)** Relative expression of neuronal and glial markers assessed by densitometry. Data are means ± SEM of band intensity normalised to GAPDH. Increased NF200 expression (* $p \leq 0.05$) is observed in the BES group (two way ANOVA with Tukey's multiple comparisons test).