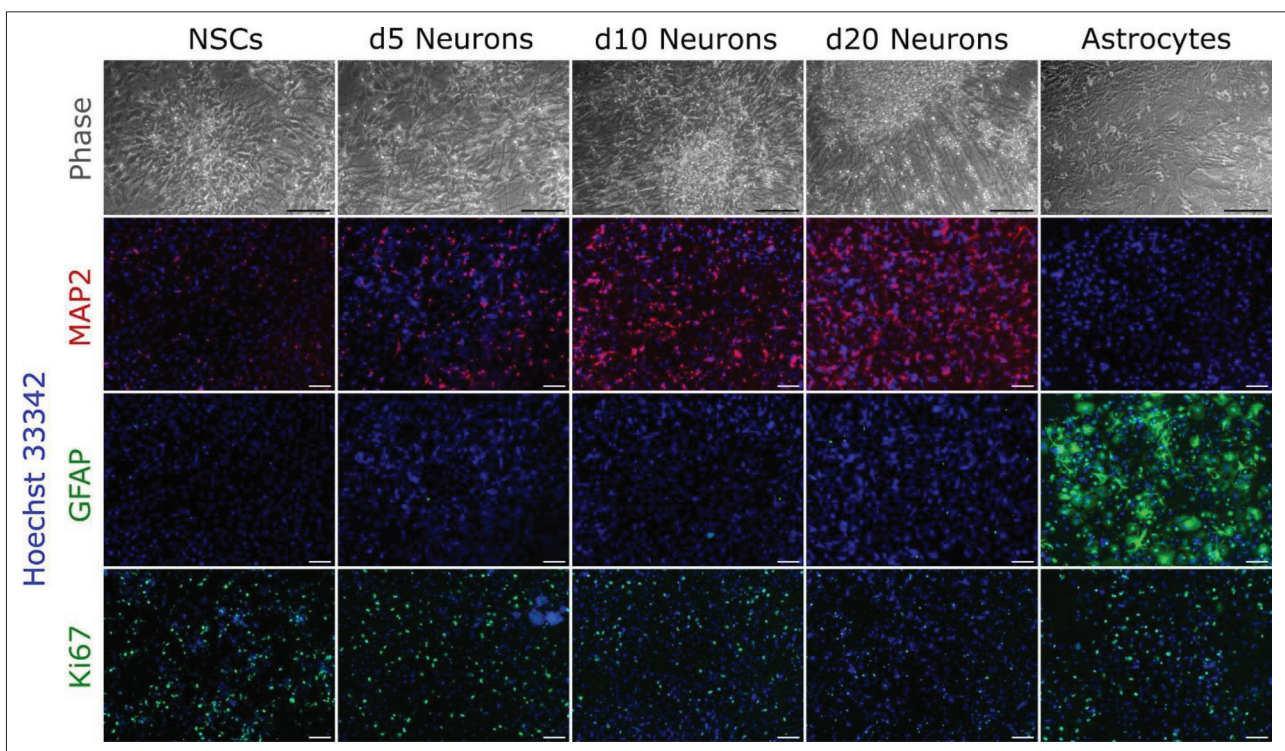


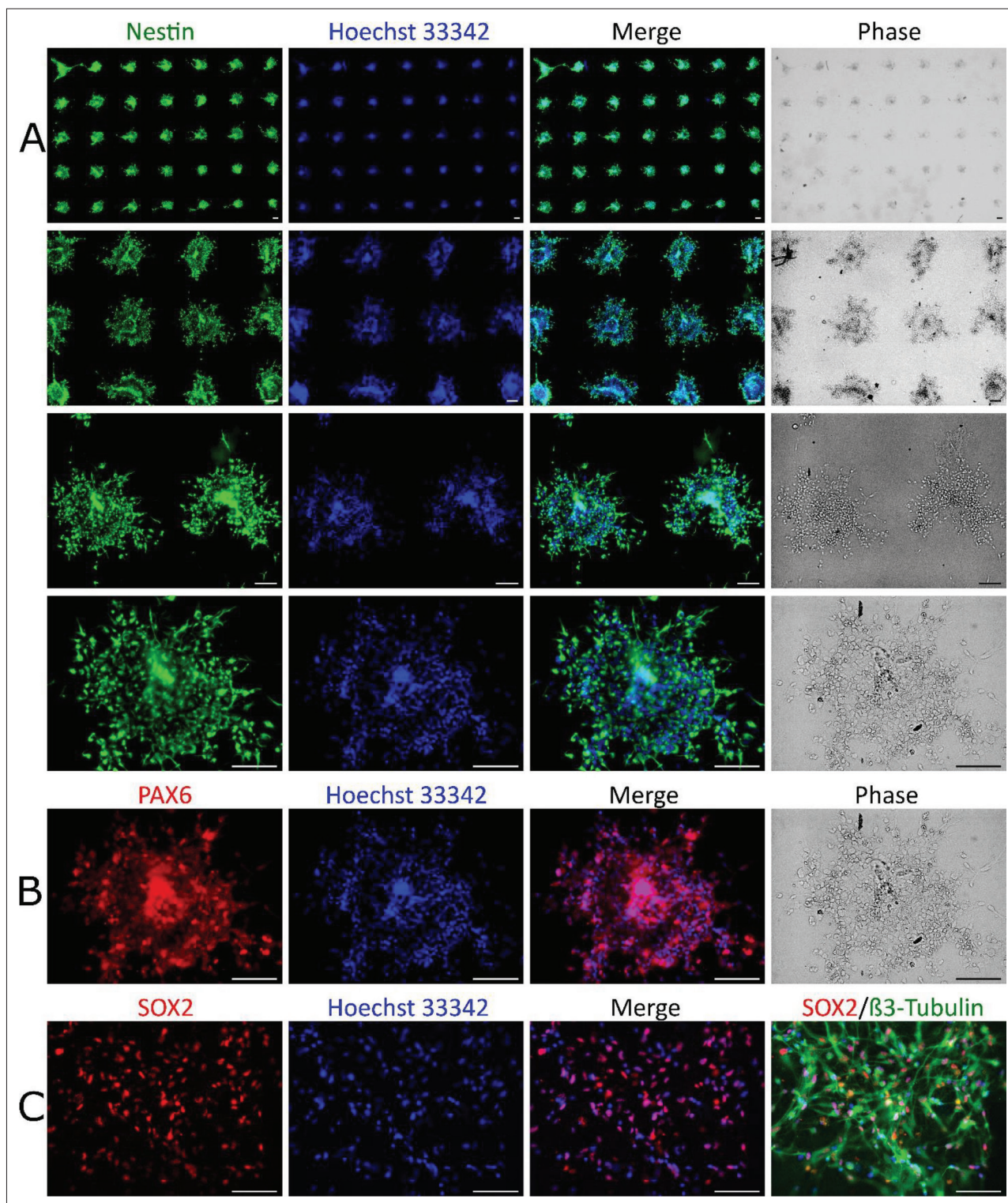
RESEARCH ARTICLE

# Laser bioprinting of human iPSC-derived neural stem cells and neurons: Effect on cell survival, multipotency, differentiation, and neuronal activity

## Supplementary File



**Figure S1.** Microscopic images for quantitative investigation of NSC's differentiation toward neurons and astrocytes. Phase contrast microscopic images and fluorescent microscopic images of d5, d10, and d20 neurons, compared to NSCs and astrocytes before being dissociated for printing; cells were stained for mature neuron marker MAP2, astrocyte marker GFAP, proliferation marker Ki67, and cell nuclei marker Hoechst 33342. NSCs exhibited cortical rosettes and neurons increasingly formed dendritic extensions during pre-differentiation. Some cells with dendritic extensions, however, can also be seen in the NSC culture, demonstrating spontaneous differentiation of NSCs to neurons.



**Figure S2.** Maintenance of multipotency of NSCs in printed patterns. (A) Fluorescent and phase contrast microscopic images with different magnifications of printed NSCs after 2 days. Cells were stained with stem cell marker nestin and cell nuclei marker Hoechst 33342. (B and C) Maintenance of multipotency of printed NSCs 2 days post-printing demonstrated by staining of stem cell marker PAX6 (B) and SOX2 (C) and neuronal marker  $\beta$ 3-tubulin (C), combined with cell nuclei marker Hoechst 33342 (A–C). Scale bars are 100  $\mu$ m, and distance between droplets in printed patterns is 1 mm.

**Table S1. List of antibodies used for immunostaining and Western blotting**

Antibody	Host	Company	Cat. no	Dilution immunostaining	Dilution Western blotting
Nestin	mouse	Santa Cruz	sc-23927	1:500	
PAX6	rabbit	Biolabs/Cellsignaling	60433	1:200	
SOX2	goat	Santa Cruz	sc-17320	1:500	
Ki67	rabbit	Acris Antibodies	DRM004	1:200	
β3-Tubulin	mouse	Sigma/Aldrich	T8578	1:2,000	1:12,000 (55 kDa)
β3-Tubulin	rabbit	Sigma/Aldrich	T2200	1:2,000	
Doublecortin (DCX)	rabbit	Biolabs/Cellsignaling	4604	1:400	1:2,000 (45 kDa)
MAP2	mouse	Sigma/Aldrich	M4403	1:2,000	
MAP2	rabbit	Synaptic Systems	188 002	1:1,000	1:5,000 (70, 280 kDa)
GFAP	rabbit	Abcam	ab7260	1:10,000	150,000 (48, 55 kDa)
S100B	mouse	Novus Biologicals	NBP1-41373SS	1:200	
Synaptophysin	mouse	Santa Cruz	sc-17750	1:50	1:1,000 (34 kDa)
Synaptophysin	rabbit	Santa Cruz	sc-9116	1:50	
Synapsin-1	rabbit	Abcam	ab8	1:500	1:5,000 (80 kDa)
PSD95	mouse	Santa Cruz	sc-32290		1:500 (95 kDa)
PSD95	mouse	Novus Biologicals	NB300-556	1:500	
vGLUT1	mouse	Sigma/Aldrich	AMAB91041	1:500	
GABA	rabbit	Sigma/Aldrich	A2052	1:500	
O4	mouse	R&D Systems	MAB1326	1:1,000	
TBR1	rabbit	Biolabs/Cellsignaling	49661	1:400	1:1,000 (74 kDa)
β-Actin-HRP	mouse	Sigma/Aldrich	A3854		1:12,000 (42 kDa)
Anti-goat Alexa 488	donkey	Invitrogen	SA5-10086	1:200	
Anti-goat Alexa 555	donkey	Invitrogen	A21432	1:400	
Anti-rabbit Alexa 488	donkey	Invitrogen	A21206	1:400	
Anti-rabbit Alexa 555	donkey	Invitrogen	A31572	1:400	
Anti-mouse Alexa 488	donkey	Invitrogen	A21202	1:400	
Anti-mouse Alexa 555	donkey	Invitrogen	A31570	1:400	
Anti-mouse HRP	goat	Dianova	115-035-062		1:5,000
Anti-rabbit HRP	goat	Dianova	111-35-144		1:5,000

**Other files:**

**Videoclip S1.** Calcium fluorescence imaging of NSCs at day 12 (day 12 pp, diff 0/10) (MP4)

**Videoclip S2.** Calcium fluorescence imaging of NSCs at day 27 (day 27 pp, diff 0/25) (MP4)

**Videoclip S3.** Calcium fluorescence imaging of NSCs at day 39 (day 39 pp, diff 0/37) (MP4)

**Videoclip S4.** Calcium fluorescence imaging of d20 neurons at day 26 (day 26 pp, diff 20/26) (MP4)