

SUPPORTING INFORMATION FOR:

A Defined and Scalable Peptide-Based Platform for the Generation of hPSC-Derived Astrocytes

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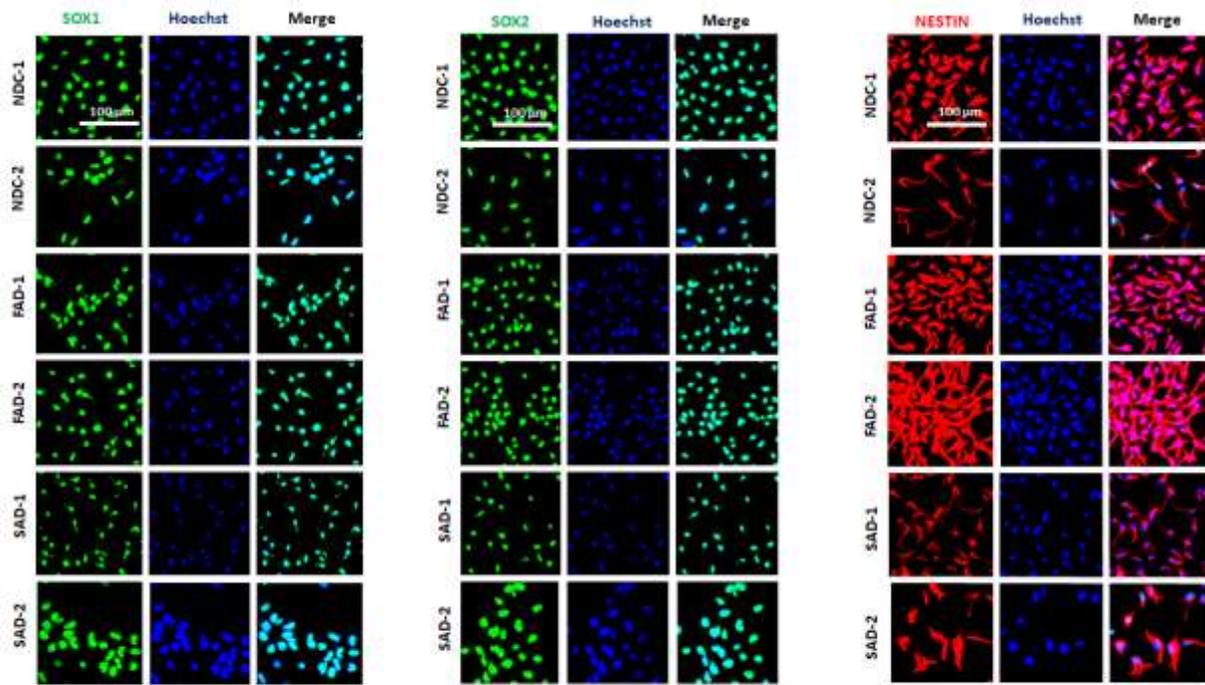
*These authors contributed equally to this work.

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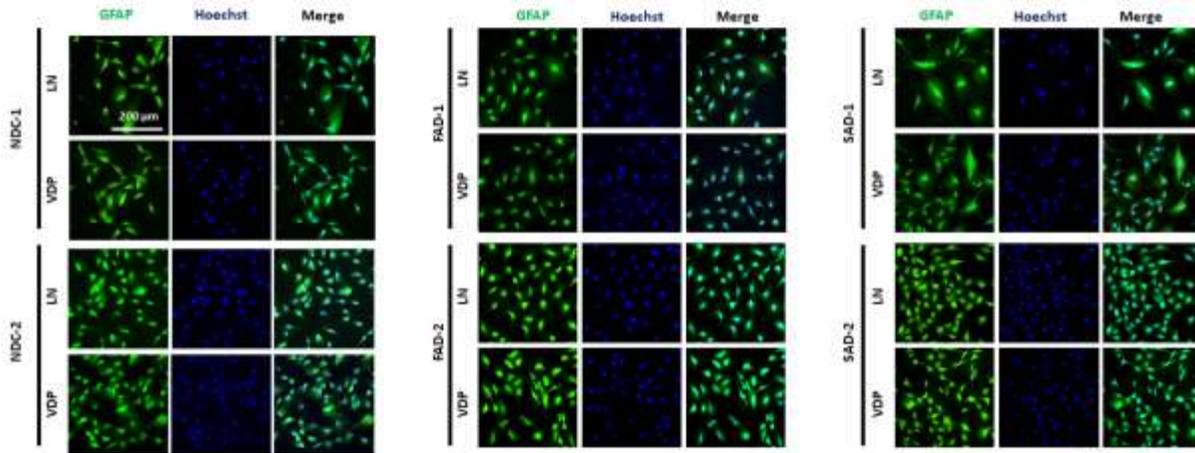
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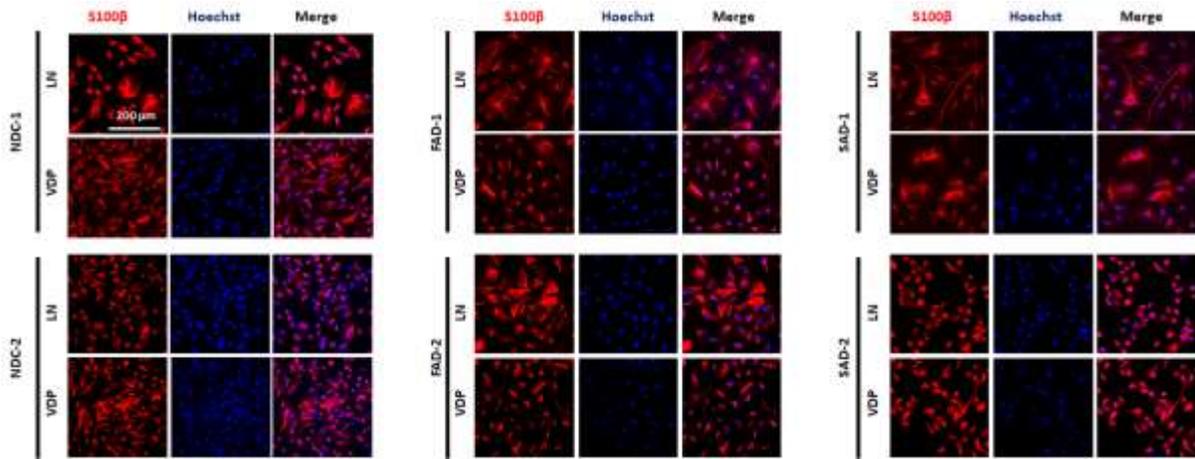
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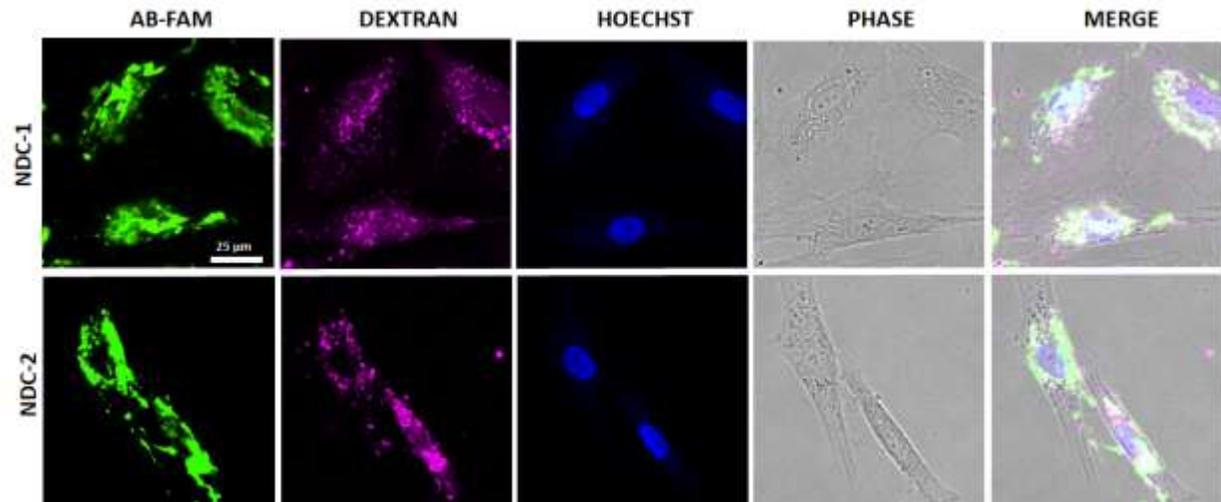
Supplemental Figure 1. Characterization of hNPCs used in this study. Immunofluorescence of hNPC markers SOX1, SOX2, and NESTIN.



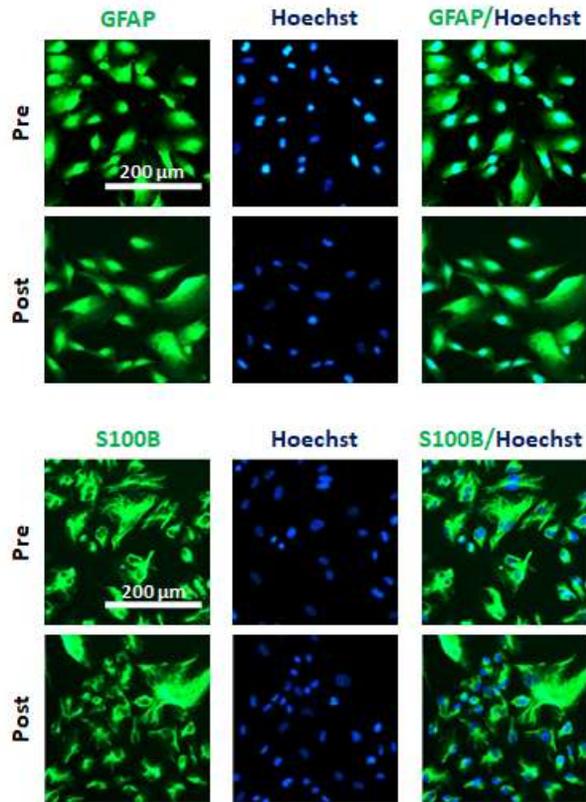
Supplemental Figure 2. Immunofluorescence analysis for expression of GFAP in D50+ cultures. Single channel images for immunofluorescent analysis for expression of GFAP presented in Figure 1D.



Supplemental Figure 3. Immunofluorescence analysis for expression of S100β in D50+ cultures. Single channel images for immunofluorescent analysis for expression of S100β presented in Figure 1D.



Supplemental Figure 4. Fluorescent image analysis of β -amyloid ($A\beta$) uptake in astrocytes. Fluorescent imaging analysis of FITC- $A\beta$ and Alexa 647-dextran uptake in NDC-1 and NDC-2 astrocytes on VDP.



Supplemental Figure 6. Immunofluorescent images of GFAP and S100 β in pre- and post-cryopreserved astrocytes. Single channel images for immunofluorescent analysis for expression of GFAP and S100 β presented in Figure 7B.

Antibody	Vendor	Catalog #	Concentration Used
Goat anti-SOX2	Santa Cruz	SC-17320	1:50
Rabbit anti-GFAP	Abcam	AB7260	1:200
Mouse PE anti-CD44	BD Biosciences	550989	20uL per test (1 × 10 ⁶ cells)
Mouse anti-NESTIN	BD	560341	1:50 (IF), 1:10 (FC)
Mouse anti-S100β	Sigma	S2532	1:500
Mouse anti-SOX1	BD	560749	1:50
Mouse PE IgG1 isotype control	BD Biosciences	555749	20uL per test (1 × 10 ⁶ cells)
Alexa 647 Donkey anti-Goat	Life Technologies	A-21447	1:200
Alexa 647 Donkey anti-Mouse	Life Technologies	A-31571	1:200
Alexa 647 donkey anti-Rabbit	Life Technologies	A-31573	1:200
Alexa 488 Donkey anti-Goat	Life Technologies	A-11055	1:200
Alexa 488 Donkey anti-Mouse	Life Technologies	A-21202	1:200
Alexa 488 Donkey anti-Rabbit	Life Technologies	A-21206	1:200

Supplemental Table 1. List of antibodies used in this study.

<i>Gene</i>	Forward (5' --> 3')	Reverse (5' --> 3')	Product (bp)
<i>18S</i>	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG	151
<i>GFAP</i>	GGCCCGCCACTTGCAGGAGTACCAGG	CTTCTGCTCGGGCCCCTCATGAGACG	328
<i>VIM</i>	GAGAACTTTGCCGTTGAAGC	TCCAGCAGCTTCCTGTAGGT	170

Supplemental Table 2. List of qPCR primers used in this study.

Cell Line	Disease Status	Reference
NDC-1	Non-demented control	Neurosci Lett. 2011 Sep 20; 502(3): 219–224.
NDC-2	Non-demented control	Stem Cell Res. 2017 Dec;25:266-269
FAD-1	Familial AD	Nature. 2012 Jan 25;482(7384):216-20
FAD-2	Familial AD	https://biomanufacturing.cedars-sinai.org/product/cs40ifad-nxx/
SAD-1	Sporadic AD	Stem Cell Res. 2017 Dec;25:266-269
SAD-2	Sporadic AD	Stem Cell Res. 2017 Oct;24:160-163.

Supplemental Table 3. Description of hPSC lines used in this study.

This table can be downloaded on the ACS publications website.

Supplemental Table 4. Complete RNA-seq data set for hNPCs, neurons, and astrocytes generated on VDP- and LN-coated surfaces.

This table can be downloaded on the ACS publications website

Supplemental Table 5. List of genes that are expressed at statistically (FDR <0.05, Fold change > 1.5) different levels in RNA-seq data set.

	This Study	Zhao et al. Hum Mol Genet. 2017 Jul 15;26(14):2690-2700.	Tcw et al. Stem Cell Reports. 2017 Aug 8;9(2):600-614.	Santos et al. Stem Cell Reports. 2017 Jun 6;8(6):1757-1769.	Lundin et al. Stem Cell Reports. 2018 Mar 13;10(3):1030-1045.
Morphology	Flat, star shaped (Figure 1A, Figure 7A)	Not assayed	Flat, star shaped	Flat, star shaped	Flat, star shaped
CD44	Positive (Flow cytometry; Figure 1B)	Not assayed	Not assayed	Positive (Immunofluorescence)	Positive (Immunofluorescence, qPCR)
S100β / GFAP Expression	Positive (Immunofluorescence, Flow cytometry, qPCR; Figures 1C-E, 6C-D, 7B-C)	Positive (Immunofluorescence)	Positive (Flow Cytometry)	Positive (Immunofluorescence)	Positive (Immunofluorescence, qPCR)
RNA-Seq	Upregulation of genes and GO-terms related to astrocytic maturation, immune system process, cytokine-mediated signaling pathway, response to stress, regulation of immune system processes; Downregulation of genes and GO-terms related to neuronal maturation and functionality (Figure 2).	Not assayed	Upregulation of genes related to signals promoting extracellular cell adhesion and interaction were upregulated; Downregulation of genes regulating neuronal maturation, such as synapse or ion channel formation.	Upregulation of genes and GO terms related to inflammatory response, immune response, chemo- kine activity, and cytokine activity.	Upregulation of canonical genes related to mature astrocytes.
APOE Secretion	25-200 ng APOE / mg total protein (Figure 3A, 7D)	50-100 ng APOE /mg total protein	Not assayed	Not assayed	5-15 ng APOE / 1000 cells
Inflammatory Response	Elevated IL-6, MCP-1, IL-8 in response to inflammatory stimuli (Figure 3B-C, 7E-F).	Not assayed	Elevated IL-6 in response to inflammatory stimuli.	Elevated IL-6, IL-8 expression in response to inflammatory stimuli.	Elevated IL-8 expression in response to inflammatory stimuli.
Aβ Uptake	Robust uptake of A β (Figure 4)	Not assayed	Not assayed	Not assayed	Not assayed
Spontaneous Calcium Transient Activity	Spontaneous waves of calcium transients (Figure 5, 6E, 7G)	Not assayed	Spontaneous waves of calcium transients	Spontaneous waves of calcium transients	Spontaneous waves of calcium transients

Supplemental Table 6. Comparison of phenotypic characterization of astrocytes in this study with astrocytes generated in previous studies using undefined, xenogeneic substrates.

The following Supplemental Movies can be downloaded on the ACS publications website.

Supplemental Movie 1. Representative recording of fluorescent calcium transients in NDC-1 astrocytes derived on VDP-coated substrates.

Supplemental Movie 2. Representative recording of fluorescent calcium transients in NDC-1 astrocytes derived on LN-coated substrates.

Supplemental Movie 3. Representative recording of fluorescent calcium transients in FAD-1 astrocytes derived on VDP-coated substrates.

Supplemental Movie 4. Representative recording of fluorescent calcium transients in FAD-1 astrocytes derived on LN-coated substrates.

Supplemental Movie 5. Representative recording of fluorescent calcium transients in SAD-1 astrocytes derived on VDP-coated substrates.

Supplemental Movie 6. Representative recording of fluorescent calcium transients in SAD-1 astrocytes derived on LN-coated substrates.

Supplemental Movie 7. Representative recording of fluorescent calcium transients in NDC-1 astrocytes derived on VDP-coated MCs.

Supplemental Movie 8. Representative recording of fluorescent calcium transients in NDC-2 astrocytes derived on VDP-coated MCs.

Supplemental Movie 9. Representative recording of fluorescent calcium transients in NDC-1 astrocytes pre-cryopreservation.

Supplemental Movie 10. Representative recording of fluorescent calcium transients in NDC-1 astrocytes post-cryopreservation.