

## Supplementary Information

### **A microfiber scaffold-based 3D *in vitro* human neuronal culture model of Alzheimer's disease**

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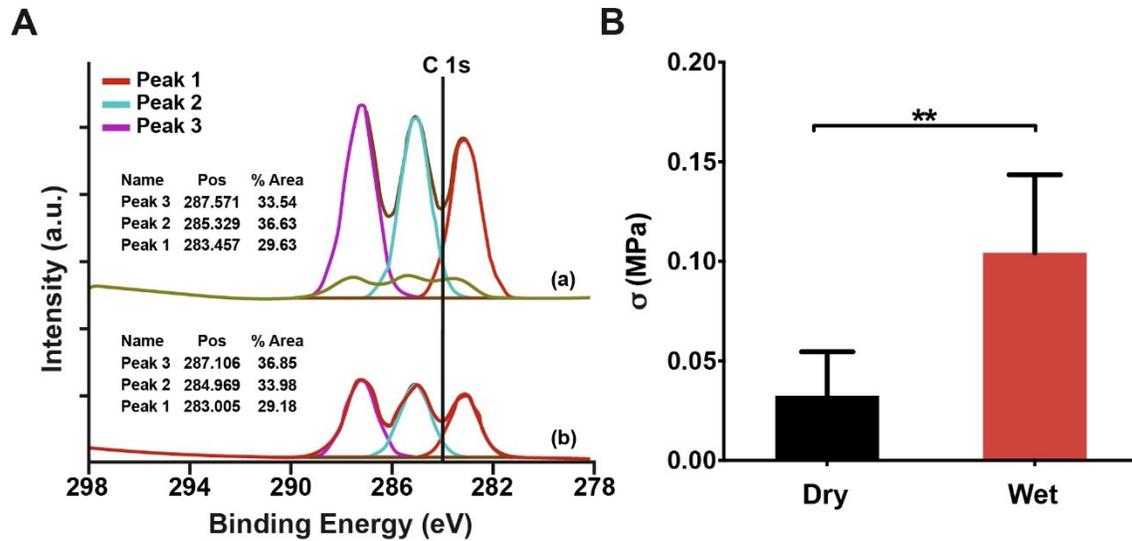
**# The authors contributed equally to this work.**

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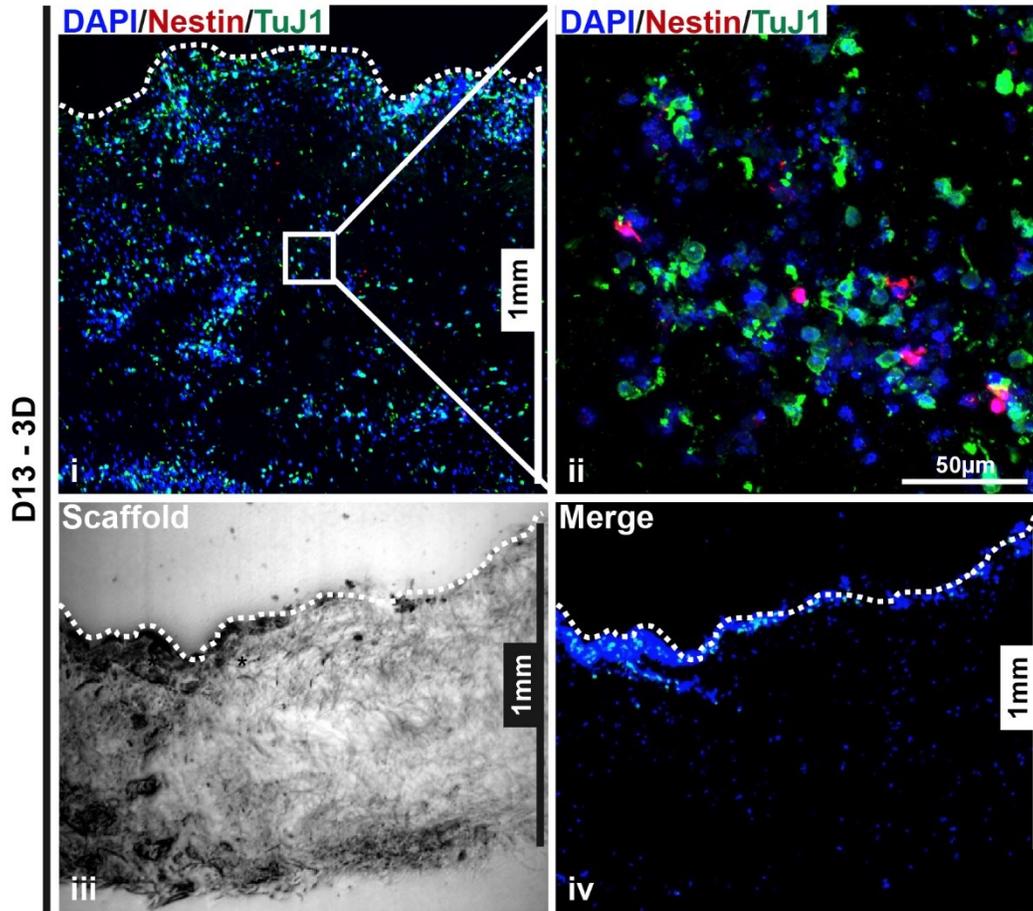
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**Table S1.** Primer sequences used for specific gene targets for qPCR

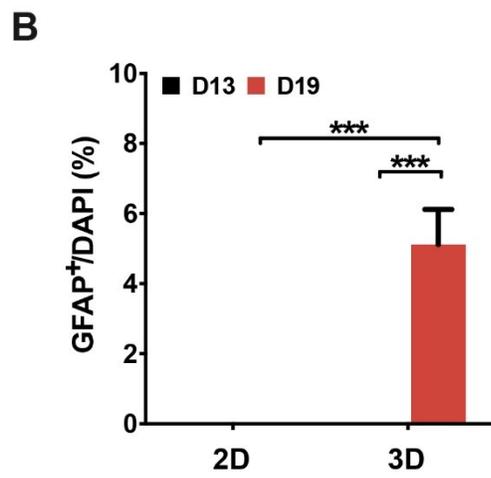
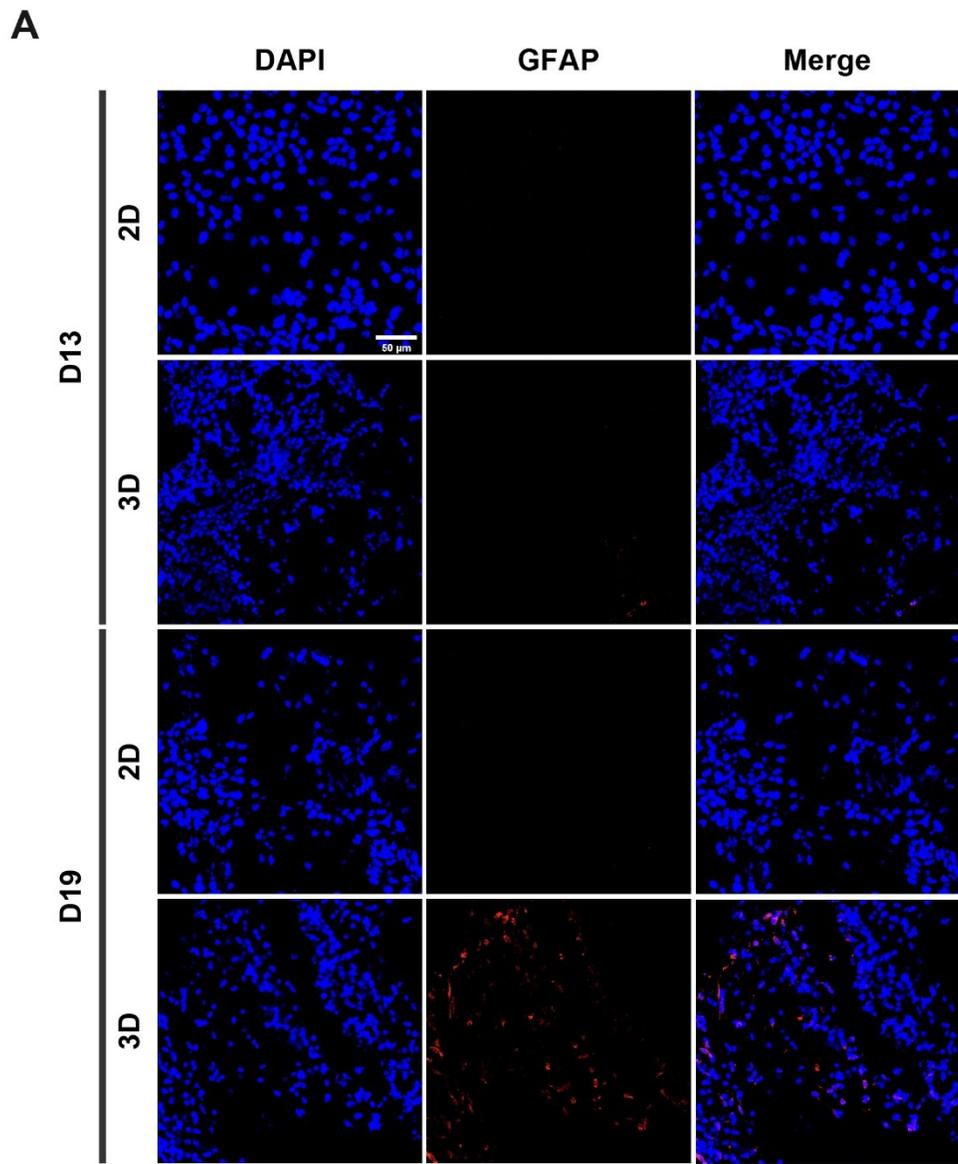
<b>Target Gene</b>	<b>Primer sequence (5'-3')</b>	
<b>Ki67</b>	F	ACGCCTGGTTACTATCAAAAGG
	R	CAGACCCATTTACTTGTGTTGGA
<b>TuJ1</b>	F	CACCCAGCAGATGTTTCGATG
	R	CTTCACGTTGTTGGGGATCC
<b>NeuN</b>	F	GCGGCTACACGTCTCCAACATC
	R	ATCGTCCCATTTCAGCTTCTCCC
<b>PSEN1</b>	F	ACAGGTGCTATAAGGTCATCCA
	R	CAGATCAGGAGTGCAACAGTAAT
<b>ADAM17</b>	F	GGCAAATGTGAGAAAC
	R	TGGACAAGAATGCTGAAAGGA
<b>Actin</b>	F	GCGCAAGTTAGGTTTTGTCA
	R	AGATGTGGACAGCAAGCAG



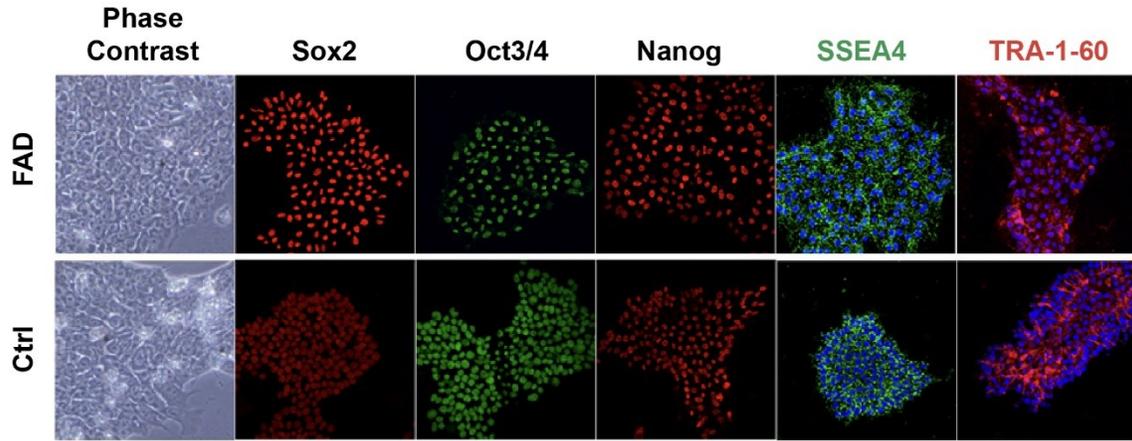
**Figure S1. Characterization of 3D PLGA microfiber scaffolds.** (A) High-resolution XPS spectra of C1s region of PLGA microfiber surface (a) without and (b) with atmospheric air plasma treatment (300 s); (B) Ultimate compressive strengths ( $\sigma$ ) of 3D PLGA microfibrous scaffolds obtained for mechanical testing under dry and wet conditions; Data is expressed as mean  $\pm$  SD (n = 5), \*\* $p < 0.01$ .



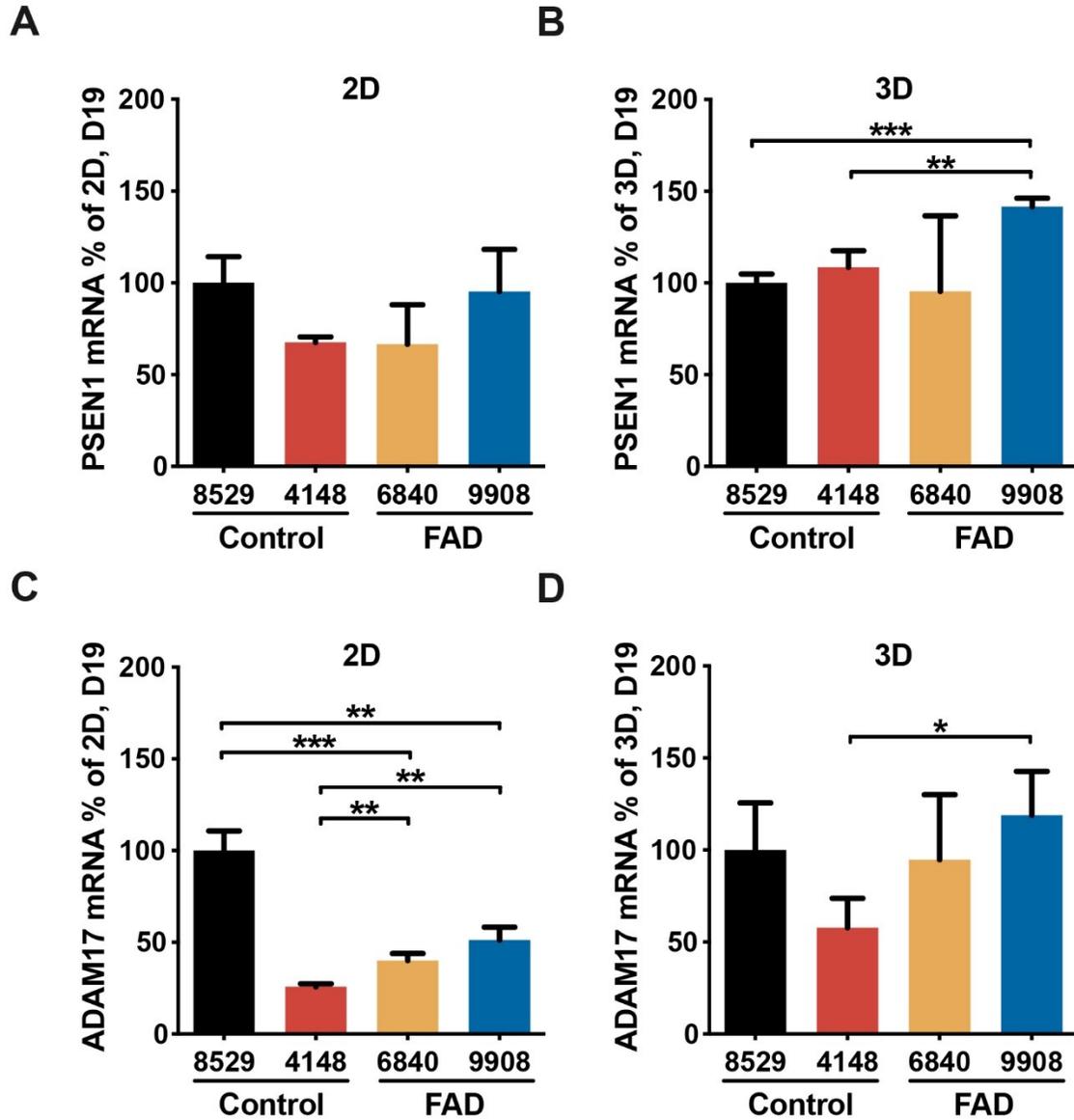
**Figure S2. Encapsulation and characterization of iPSC-derived NPC inside 3D PLGA microfiber scaffolds.** Confocal fluorescent microscopy images indicating (i-ii) differentiation of iPSC-derived NPCs (8529 cell line) inside 3D scaffold as assessed via staining for TuJ1 (green) and Nestin (red) markers on D13; (iii) cross-section of 3D microfiber scaffold after sectioning (dotted line indicates top surface of 3D scaffold); (iv) cell infiltration and distribution of D13 differentiated NPC inside 3D scaffold without orbital shaking as assessed via staining for Ki67 (green) and DAPI (blue) markers.



**Figure S3. Comparison of glial differentiation between 2D and 3D cultures.** (A) Confocal fluorescent microscopy images indicating glial differentiation in 2D and 3D cultures stained for GFAP (red) marker on D13 and D19 respectively; Nuclei were counterstained with DAPI (blue); (B) Quantification of immunostaining results showing percentage positive staining of glial differentiation markers normalized to DAPI for D13 and D19; Data is expressed as mean  $\pm$  SD (n = 3), \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; Scale bar: 50  $\mu\text{m}$ .



**Figure S4. Confocal fluorescent microscopy images indicating pluripotency of iPSCs.** Fibroblast-derived iPSCs were immunostained and positive for various iPSC markers such as SOX2, OCT4, Nanog, SSEA4, and TRA-1-60; Nuclei were counterstained with DAPI (blue).



**Figure S5. 3D culture elevates expression of specific genes linked to the APP pathway.** qPCR analysis of APP-linked pathway genes in D19 FAD-iPSC derived neurons; PSEN1 expression in (A) 2D culture and (B) 3D culture; ADAM17 expression in (C) 2D culture and (D) 3D culture; Data is expressed as mean  $\pm$  SD (n = 3), \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001.