Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information for

Promotion of neurite outgrowth by rationally designed NGF-β binding peptide nanofibers

Zeynep Okur [a,b], Oya I. Senturk [a], Canelif Yilmaz [a,b], Gulcihan Gulseren [a], Busra Mammadov [a], Mustafa O. Guler [c,d], Ayse B. Tekinay [a,b,d]

[a] *National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, 06800, Turkey*

[b] *Neuroscience Graduate Program, Bilkent University, Ankara, 06800, Turkey*

[c] *Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA*

[d] *Corresponding author*

A

Number	Sequence	Frequency
1	WHYVRPP	4/24
2	LGSPMSN	4/24
3	NERALTL	4/24
4	FSRPAFL	2/24
5	NHSTQMY	2/24
6	WHWRYAP	1/24
7	VHKTDYL	1/24
8	IGNTPAI	1/24
9	ANHQSAN	1/24
10	EHTRSMN	1/24
11	TFASHDQ	1/24
12	ITSTFTT	1/24
13	HWSTTIS	1/24

B

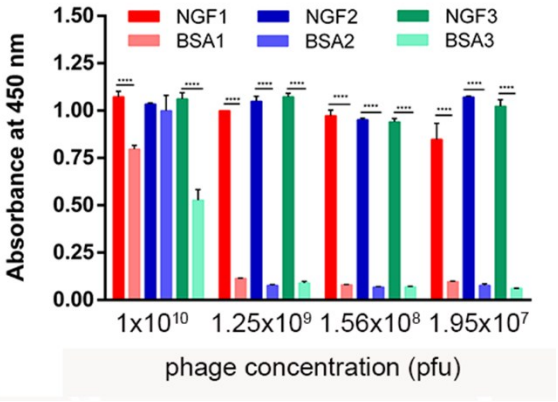

Fig. S1. Identification of phage-displayed peptide sequences binding to NGF-β and affinity analysis of selected phage colonies for NGF-β. Summary of consensus NGF-β binding peptides (A). ELISA of binding ability of selected serially diluted (with 1:8 ratio) phage clones (number 1, number 2 and number 3 are represented as NGF1, NGF2 and NGF3, respectively) to NGF $^{****}P<$ 0.0001 versus blocking buffer sample (BSA) (B). Data presented are the mean OD values (± SEM) of triplicate samples.

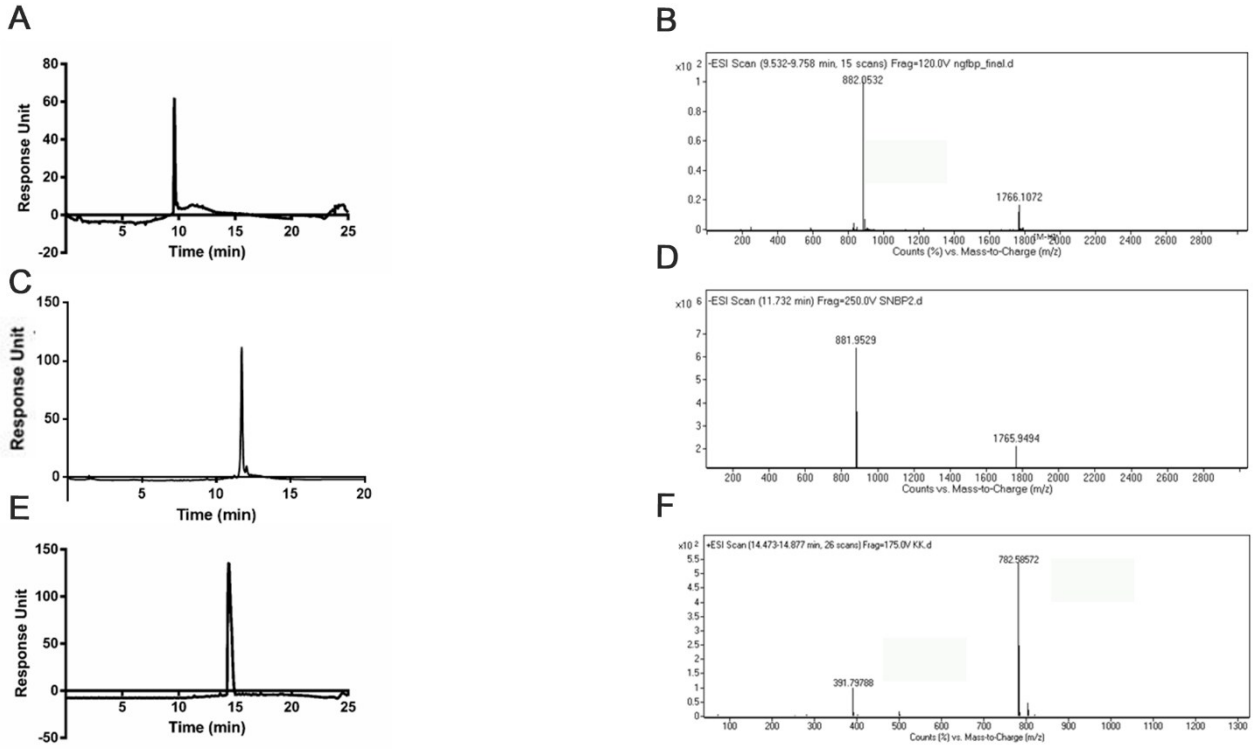

Figure S2. Liquid chromatography and mass spectrometry analysis of peptide amphiphile (PA) molecules. HPLC chromatogram of purified NGFB-PA (A), scrNGFB-PA (C) and KK-PA (E) molecules at 220 nm. Mass spectra of peptides; for NGFB-PA $[M-H]^-$ (calculated) = 1766.03, $[M-H]^-$ (observed) = 1766.10 (A), $[M/2-H]^-$ (calculated) = 883.01, $[M/2-H]^-$ (observed) = 882.05 (B), for scrNGFB-PA $[M-H]^-$ (calculated) = 1766.03, $[M-H]^-$ (observed) =1765.94, $[M/2-H]^-$ (calculated) = 883.01, $[M/2-H]^-$ (observed) = 881.95 (D), for KK-PA $[M+H]^+$ (calculated) = 781.58, $[M+H]^+$ (observed) = 782.58, $[M/2+H]^+$ (calculated) = 390.79, $[M/2+H]^+$ (observed) = 391.79 (F).

A

Peptide sequence	Nomenclature	Net charge (*)
NERALTL-GEEGAVVK(Lauryl)-Am	NGFB-PA	-2
RNTLLAE-GEEGAVVK(Lauryl)-Am	scrNGFB-PA	-2
Lauryl-VVAGKK-Am	KK-PA	+2
Peptide Mixtures		
NGFB-PA/KK-PA	NGFB-PA nanofiber	0
scrNGFB-PA/KK-PA	scrNGFB-PA nanofiber	0

(*) Theoretical net charge at pH 7.4

B

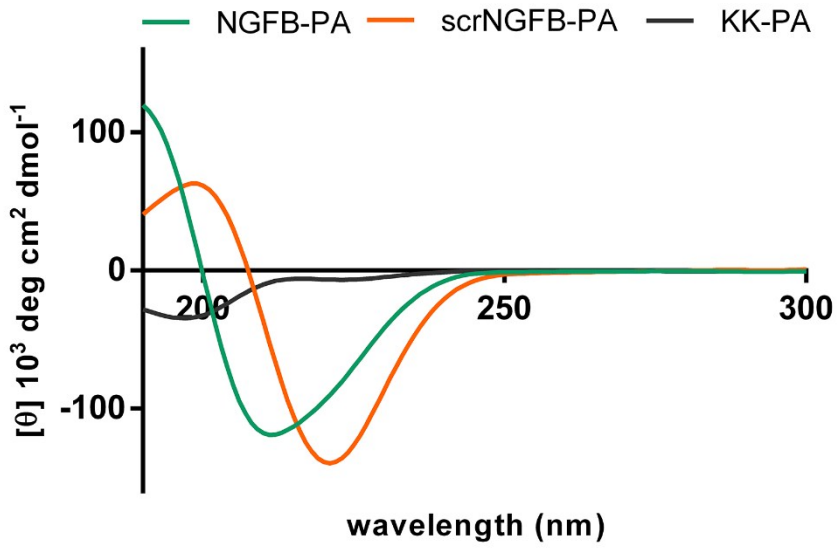

Figure S3. Abbreviations and charges of peptide sequences and peptide mixtures (A). The secondary structure analysis of peptide amphiphiles were analyzed by circular dichroism at pH 7.4 (B).

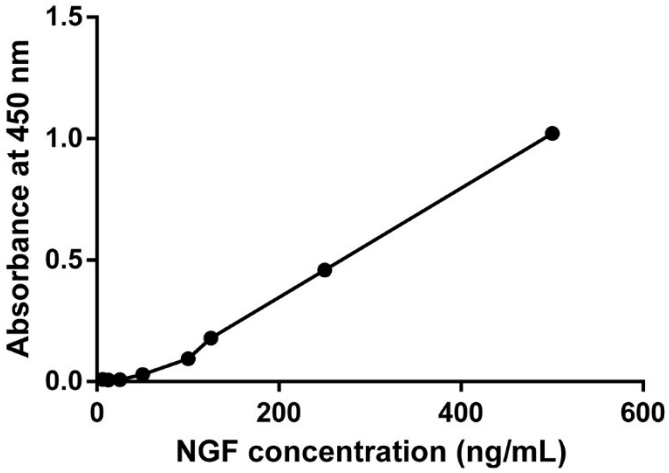

Figure S4. ELISA standard graph of human NGF-β antibody against NGF-β shows that the affinity of the antibody for NGF-β is concentration dependent.

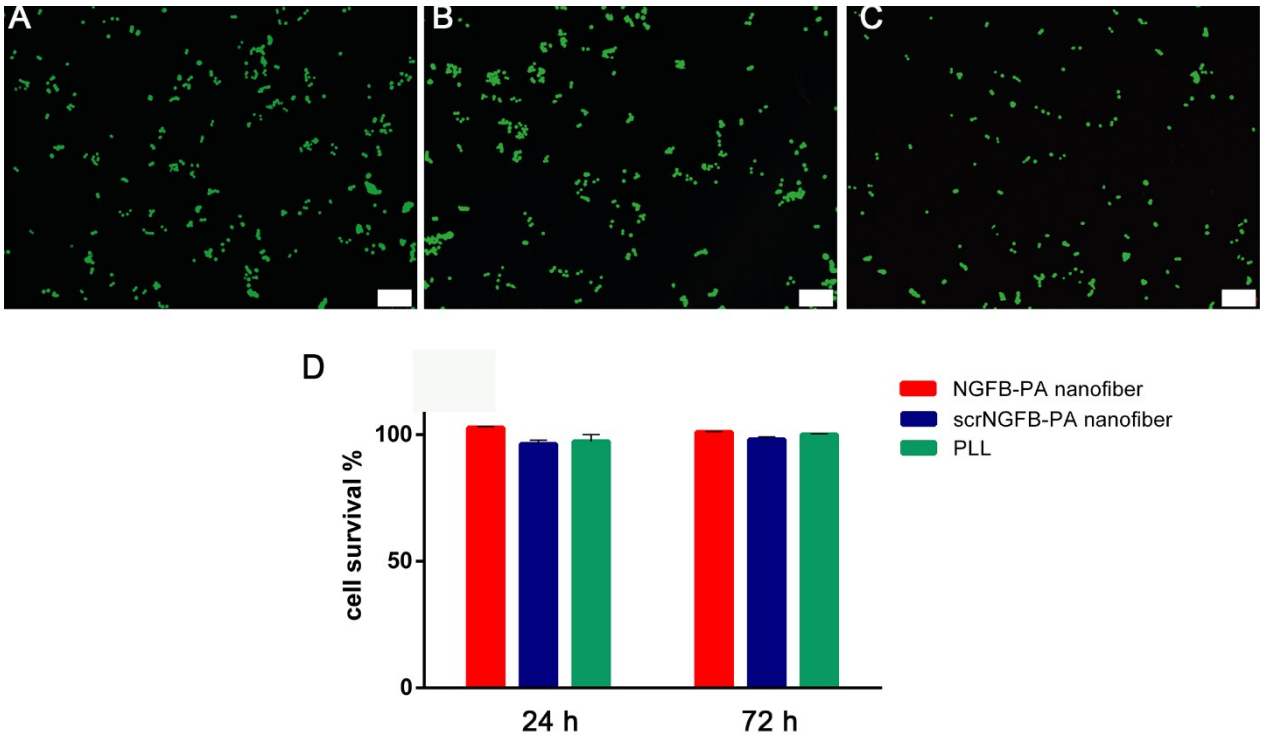

Figure S5. Viability of PC-12 cells cultured on NGFB-PA nanofiber **(A)**, scrNGFB-PA nanofiber **(B)** and PLL **(C)** was analyzed by Alamar Blue Assay for 24 h and 72 h **(D)**. Representative images were taken after cells were co-stained with 2 µM calcein-AM (green) and 4 µM ethidium

homodimer (red) in 1X PBS. Qualitative and quantitative results showed that peptide nanofibers are biocompatible for PC-12 cells. No significant differences exist between cell survival percentages of cells cultured on different scaffolds. Scale bar is 50 μm.