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Supplementary information

Silk Nanofiber Hydrogels with Tunable Modulus to Regulate Nerve Stem Cell Fate

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Fig. S1 SEM images of silk nanofiber films from hydrogels with different treatments. (a) silk nanofiber hydrogel, SN-H; (b) water-annealed silk nanofiber hydrogel, WA-SN-H; (c) 50% methanol-annealed silk nanofiber hydrogel, MA50-SN-H; (d) 80% methanol-annealed silk nanofiber hydrogel, MA80-SN-H.



Fig. S2 Neurospheres cultured for 24 h under differentiation conditions were stained for SOX2 and DAPI (A). Scale bars: 50 μm. Images with higher magnification shown in (B). Scale bars: 25 μm.

WA-SN-H SN-H Α **BrdU/DAPI** MA50-SN-H MA80-SN-H 25µm WA-SN-H SN-H В Caspase-3/DAPI MA50-SN-H MA80-SN-H 25µm

Fig. S3 The high magnification images of the proliferation and apoptosis of NSCs on silk nanofiber hydrogels (SN-H) with different mechanical properties. NSCs on silk nanofiber hydrogels with different mechanical properties were incorporated BrdU for 4 h and stained for BrdU and DAPI (A). NSCs on silk nanofiber hydrogels with different mechanical properties stained for active Caspase3 and DAPI (B).

SN-H WA-SN-H Α TUJ1/DAPI MA50-SN-H MA80-SN-H 25µm SN-H WA-SN-H В **GFAP/DAPI** MA80-SN-H MA50-SN-H 25µm

Fig. S4 The high magnification images of the differentiation of NSCs on silk nanofiber hydrogels (SN-H) with different mechanical properties. NSCs seeded on silk nanofiber hydrogels with different mechanical properties were cultured for 3~5 days *in vitro*. The cells were stained for TUJ1 (A) or GFAP (B) and DAPI.