## **Supplementary Information**

Precise design strategy of cell-derived extracellular matrix based on CRISPR/Cas9 for regulating neural stem cell function

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## The detail information for essential reagents in this study:

DMEM (11995065, Gibco, USA), FBS (FBS, 10099141, Gibco, USA), NeuroCult Proliferation Supplement (05700, Stemcell, USA), human recombinant EGF (78016.1, Stemcell, USA), human recombinant bFGF (78003.1, Stemcell, USA), Accutase (00-4555-56, Gibco, USA), B27 (A3582801, Gibco, USA), Penicillin-streptomycin (15140122, Gibco, USA), L-glutamine (A2916801, Gibco, USA), Trypsin (25200056, Gibco, USA), Puromycin (A1113803, Gibco, USA), G418 (supplied by Genechem), gelatin (G1393, Sigma, USA), L-ascorbic acids (A4544, Sigma, USA), sodium ascorbate (A4403, Sigma, USA), Triton X-100 (T8787, Sigma, USA), DNase I (18047019, Invitrogen, USA), FGF2 (bFGF) Mouse ELISA Kit (EMFGF2, Invitrogen, USA), RIPA lysis buffer (P0013B, Beyotime, China), protease inhibitor (87786, Invitrogen, USA), BCA kit (P0010, Beyotime, China), HSPG2 (ab2501, Abcam, USA), vimentin (ab92547, Abcam, USA), Nestin (ab134017, Abcam, USA), Sox-2 (ab92494, Abcam, USA), Ki67 (ab15580, Abcam, USA), MAP-2 (ab5392, Abcam, USA) and GFAP (ab7260, Abcam, USA), β-actin (ab8226, Abcam, USA), Goat anti-Rat Secondary Antibody Alexa Fluor 488 (A-11006, Invitrogen, USA), Goat anti-Mouse Secondary Antibody Alexa Fluor 594 (A-11005, Invitrogen, USA), Goat Anti-Mouse HRP-conjugated secondary antibody (A0216, Beyotime, China), Goat Anti-Rabbit HRP-conjugated secondary antibody (A0208, Beyotime, China), Pierce@ ECL Substrate (2209, Thermo Scientific, USA), Cell-Light EdU Apollo488 In Vitro Kit (C10310-3, RiboBio, China), DAPI (D9542, Sigma, USA), TRIzol reagent (15596018, Thermo, USA), First Strand cDNA Synthesis Kit (K1622, Thermo, USA), SYBR

Mixture (4364344, Applied Biosystems, USA), PVDF membrane (IPVH00010,

Millipore, USA).

Name	Sequence					
sgRNA						
sgRNA-2 (Serial number: PCA08642)	GGGCGGAACCTGGTCTTGTG					
sgRNA-3 (Serial number: PCA08643)	CTCCTGAACTTGGCGCCTCG					
sgRNA-4 (Serial number: PCA08644)	TGGCTAAATGTTTGGAGGTG					
Primers						
HSPG2 (1)	Forward	TTCCAGATGGTCTATTTCCGGG				
	Reverse	CTTGGCACTTGCATCCTCC				
HSPG2 (2)	Forward	TGGAGCCCGAATACAGGAAGA				
	Reverse	AGATCCGTCCGCATTCCCT				
HSPG2 (3)	Forward	TGCTGCATACAGTGGTCTCC 20				
	Reverse	CCAGGCGTCGGAACTTGAA				
MAP-2	Forward	AGACCTTCCTCCATCCTCCC				
	Reverse	ATTTGTACATTTCCGCCCCCA				
GFAP	Forward	GGGACAACTTTGCACAGGAC				
	Reverse	GCTTCATCTGCCTCCTGTCT				
GAPDH	Forward	AGGTCGGTGTGAACGGATTTG				
	Reverse	TGTAGACCATGTAGTTGAGGTCA				

Table S1 Se	quences of the s	gRNA and	real-time PCR	primers used	l in the study.
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**Figure S1**. (A) The mRNA expression of HSPG2 in three sgRNAs transfected MEFs was detected by real-time PCR with three pairs of primer (n=3). (B) Amplification curve and (C) melt curve in the real-time PCR.



Figure S2.

**Figure S2**. (A) Western blot of HSPG2, fibronectin, vimentin and  $\beta$ -actin protein in Cell<sup>*NC*</sup>, ECM<sup>*NC*</sup>, ECM<sup>*HSPG2*</sup>, ECM<sup>*HSPG3*</sup> and ECM<sup>*HSPG4*</sup>. (B) bFGF concentration encapsulated in ECM<sup>*NC*</sup>, ECM<sup>*HSPG2*</sup>, ECM<sup>*HSPG3*</sup> and ECM<sup>*HSPG4*</sup> (n=3). (C) The mRNA expression of fibronectin and bFGF in three sgRNAs transfected MEFs (n=3).



**Figure S3**. The protein relative expression of HSPG2, Fibronectin and Vimentin in ECM<sup>*NC*</sup> and ECM<sup>*HSPG2*</sup> (n=3). Data are presented as mean  $\pm$  SD, \*\**p* < 0.01 *vs*. ECM<sup>*NC*</sup>

group.



**Figure S4**. (A) DNA content present in the ECM before and after decellularization. (n=3). Data are presented as mean  $\pm$  SD, \*\*p < 0.01 vs. pre-decellularization group. (B) Western blot of  $\beta$ -actin in ECM structural proteins before and after decellularization.



Figure S5. (A) Morphologies of NSCs neurosphere under phase-contrast microscopy.



(B) NSCs neurosphere immunofluorescence staining against nestin and Sox2.

**Figure S6**. (A) Percentages of GFAP-positive cells (n=10). (B) The GFAP mRNA expression of NSCs cultured on ECM<sup>NC</sup> or ECM<sup>HSPG2</sup> (n=3). (C) The GFAP protein expression of NSCs cultured on ECM<sup>NC</sup> or ECM<sup>HSPG2</sup>. Data are presented as mean  $\pm$  SD.