Supplementary Information

High-efficiency brain-targeted intranasal delivery of BDNF mediated by engineered exosomes to promote remyelination

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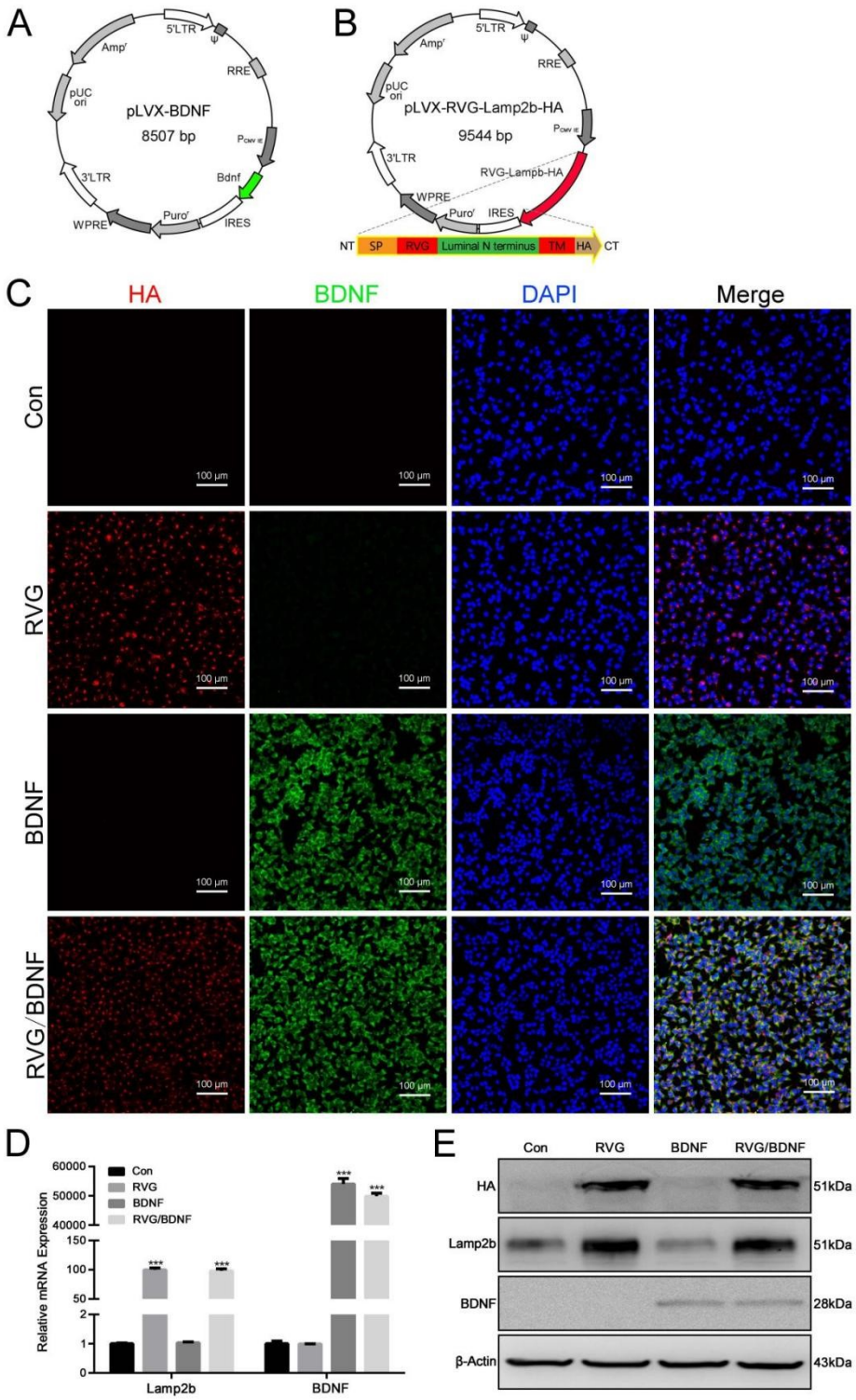

Fig. S1. Stable cell lines construction. (A) Structure of recombinant plasmid pLVX-BDNF. (B) Structure of recombinant plasmid pLVX-RVG-Lamp2b-HA. NT: N terminus, SP: signal peptide, RVG: rabies virus glycoprotein peptide, TM: transmembrane domain, HA: HA tag, CT: C terminus. (C) Immunofluorescence staining of RVG-Lamp2b-HA and BDNF. (D) Relative mRNA expression of Lamp2b and BDNF by qPCR. (E) Western blot detection of RVG-Lamp2b-HA and BDNF.

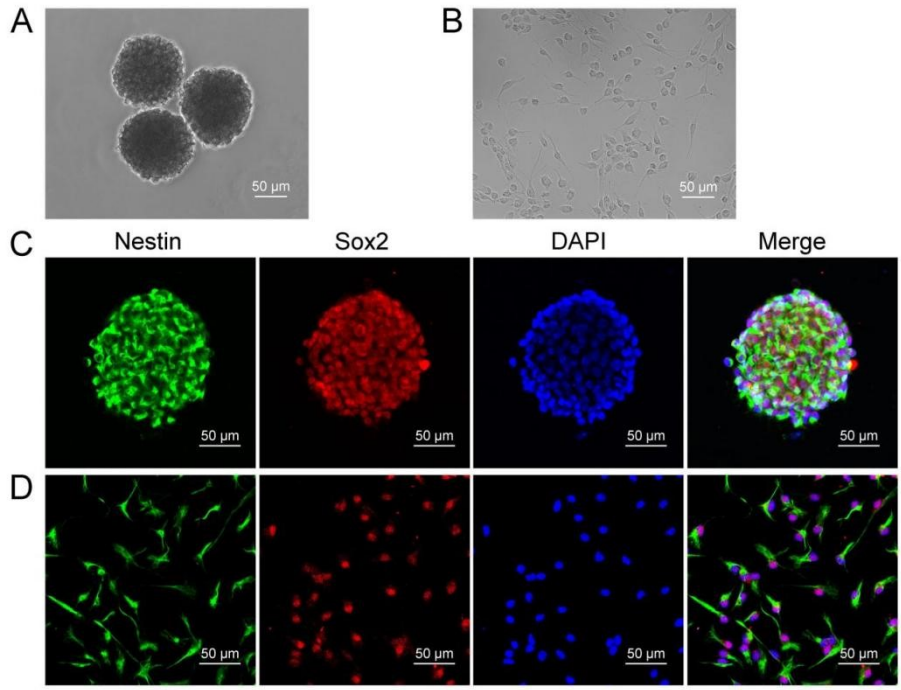

Fig. S2. NPC culture and identification. (A) Bright field image of NPC neurosphere. (B) Bright field image of adherent NPC. (C) NPC neurosphere immunofluorescence staining of Nestin and Sox2. (D) Adherent NPC immunofluorescence staining of Nestin and Sox2.

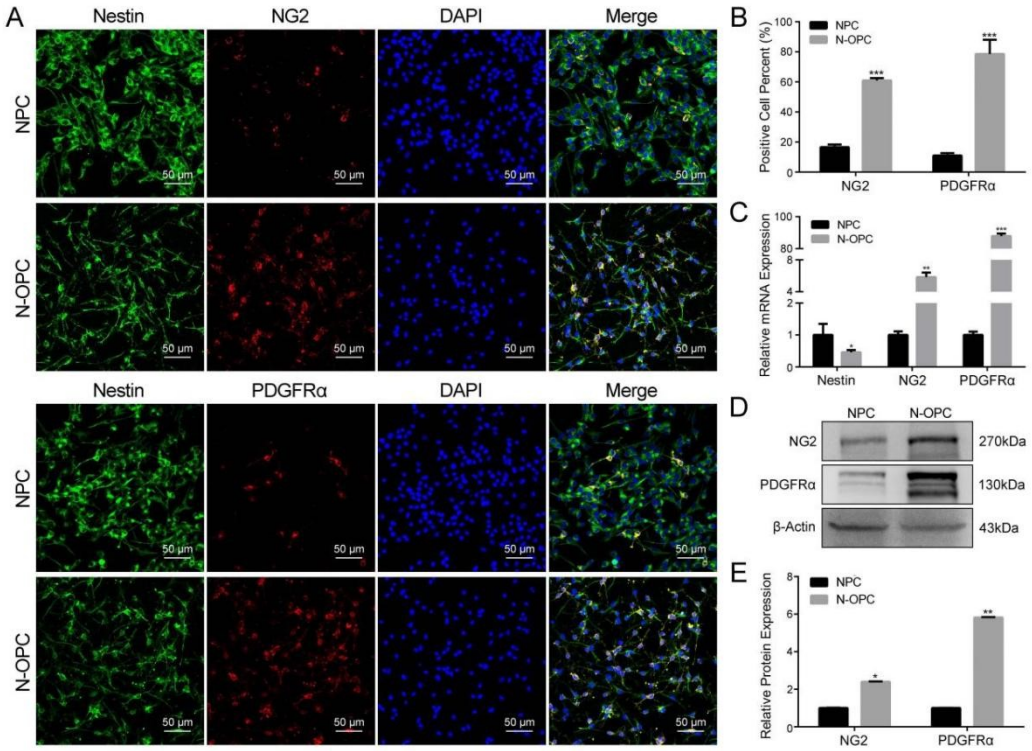

Fig. S3. Identification of N-OPC. (A) Immunofluorescence staining of Nestin, NG2 and PDGFRα. (B) The proportion of NG2+ positive cell and PDGFRα+ positive cell. (C) Relative mRNA expression of Nestin, NG2 and PDGFRα by qPCR. (D) Western blot detection of NG2 and PDGFRα. (E) Densitometric analysis of Western blot bands of NG2 and PDGFRα.

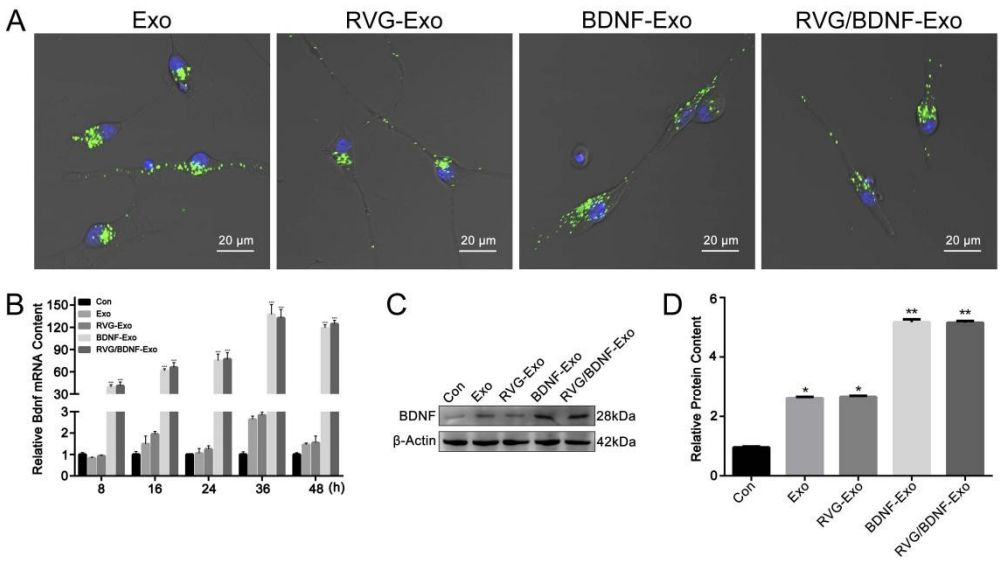

Fig. S4. Exosomes cell uptake. (A) DiO-labeled exosomes cell uptake. (B) The relative BDNF mRNA changes in cells at different time after adding exosomes. (C) BDNF protein was detected by Western blot. (D) Densitometric analysis of Western blot bands of BDNF.

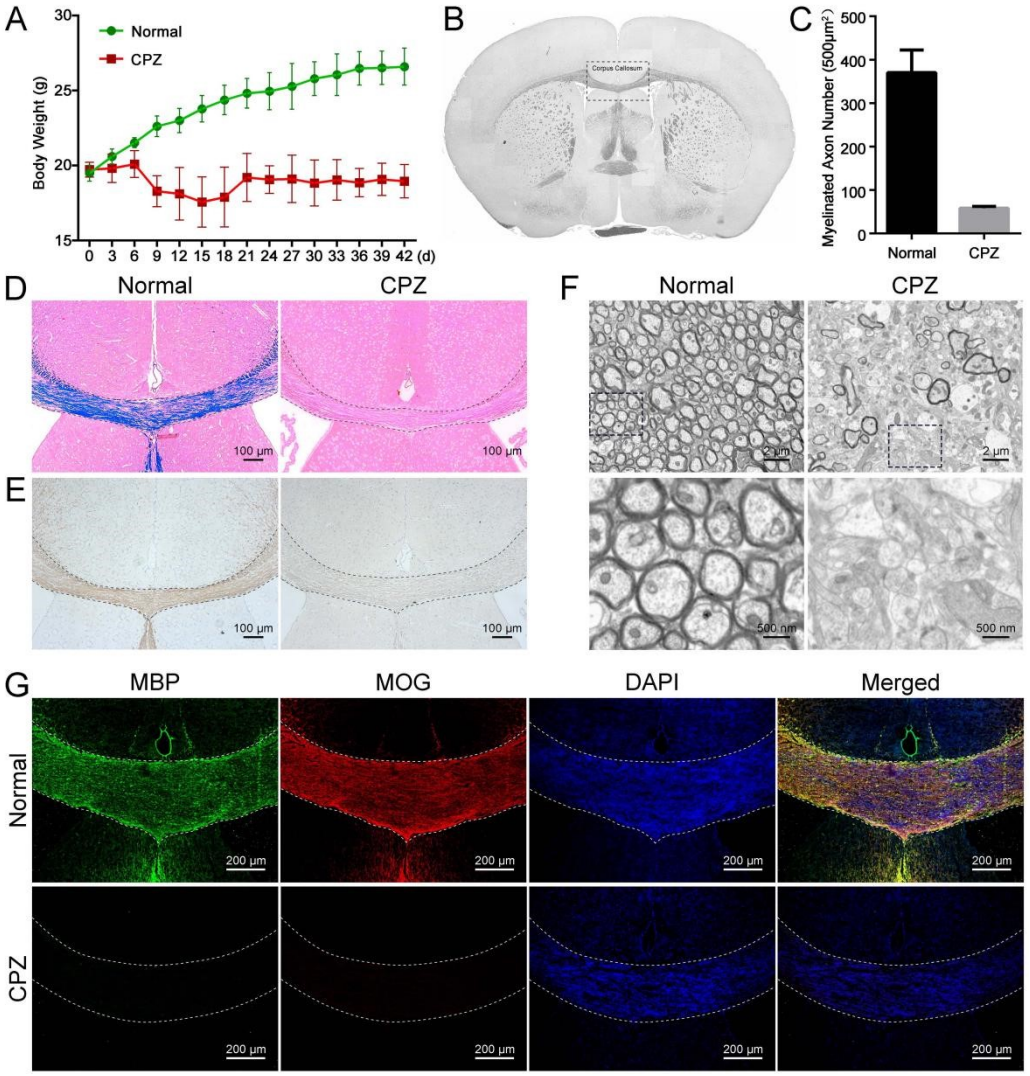

Fig. S5. Identification of CPZ mouse. (A) Body weight changes during CPZ mouse construction. (B) The position diagram of corpus callosum detected by section. (C) Number of myelinated axons. (D) LFB staining of corpus callosum. (E) Immunohistochemical staining of MBP. (F) TEM detection of myelin sheath in corpus callosum. (G) Immunofluorescence staining of MBP and MOG of the corpus callosum.

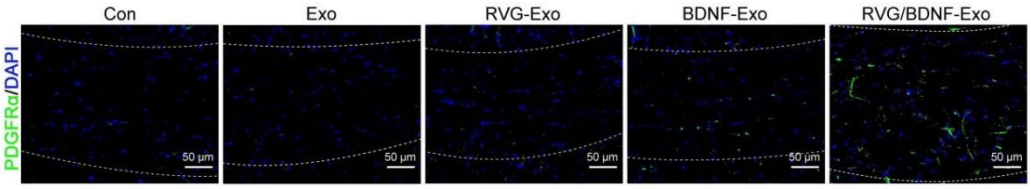

Fig. S6. PDGFRα detection of the corpus callosum.

Talbe S1 qPCR Primers

Gene		Primer sequences（5’-3’）	Product length
Gapdh	Forward	AGGTCGGTGTGAACGGATTTG	123 bp
	Reverse	TGTAGACCATGTAGTTGAGGTCA	
BDNF	Forward	CTACGAGACCAAGTGCAATCC	147 bp
	Reverse	AATCGCCAGCCAATTCTCTTT	
Lamp2b	Forward	GAAAATGCCACTTGCCTTTATGC	184 bp
	Reverse	AGGAAAAGCCAGGTCCGAAC	
MBP	Forward	TCACAGCGATCCAAGTACCTG	125 bp
	Reverse	CCCCTGTCACCGCTAAAGAA	
MOG	Forward	ACCTCTACCGAAATGGCAAGG	120 bp
	Reverse	TCACGTTCTGAATCCTAAGGGT	
Ki67	Forward	ATCATTGACCGCTCCTTTAGGT	104 bp
	Reverse	GCTCGCCTTGATGGTTCCT	
Tuj1	Forward	GCGCCTTTGGACACCTATTCA	92 bp
	Reverse	GCCCTCCGTATAGTGCCCT	
GFAP	Forward	ACCAGCTTACGGCCAACAG	198 bp
	Reverse	CCAGCGATTCAACCTTTCTCT	
NG2	Forward	ACCATGCTACTCCGCAACAG	78 bp
	Reverse	CCGGTGAACATCTATGTGTACG	
PDGFRα	Forward	ATGAGAGTGAGATCGAAGGCA	130 bp
	Reverse	CGGCAAGGTATGATGGCAGAG	

Table S2 Primer and probe sequences for Droplet Digital PCR

Gene	BDNF
Forward primer	CATGGGTTACACAAAAGAAG
Reverse primer	TGGTCAATGTACATACACAA
Probe sequence	HEX-CTATCCTTATGAATCGCCAGCCAAT-BHQ1
Product length	164 bp