

Supporting Information

Black phosphorus nanosheets enhance differentiation of neural progenitor cells for improved treatment in spinal cord injury

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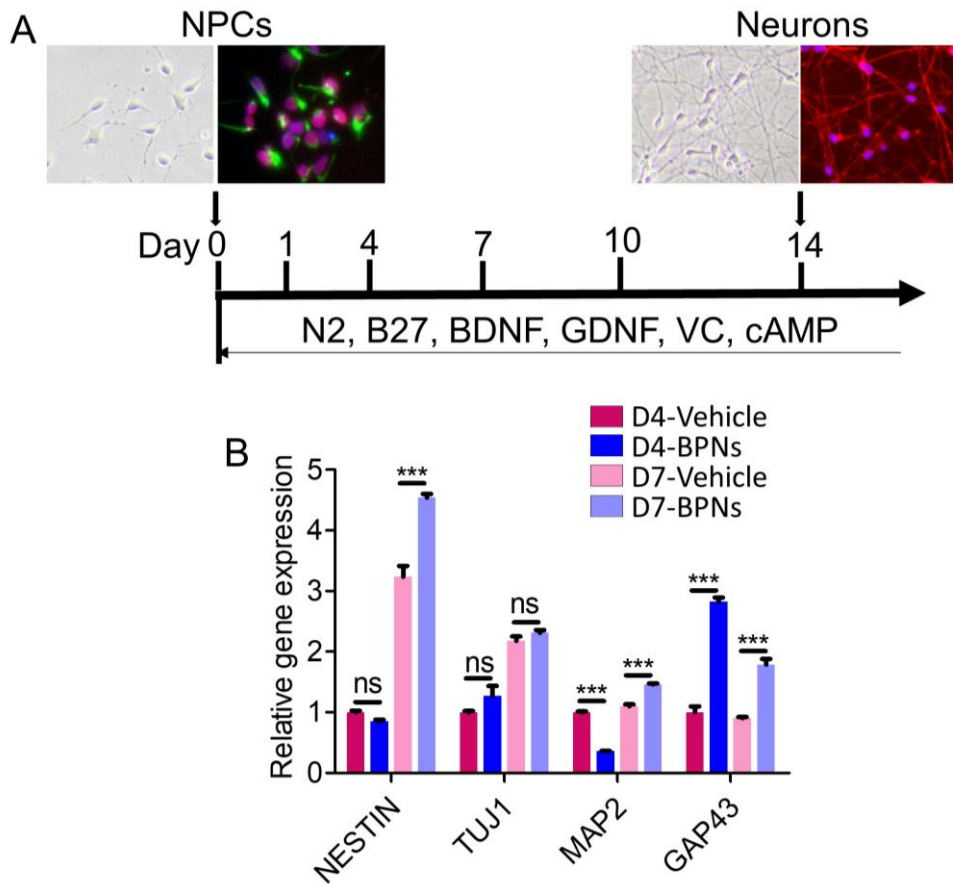
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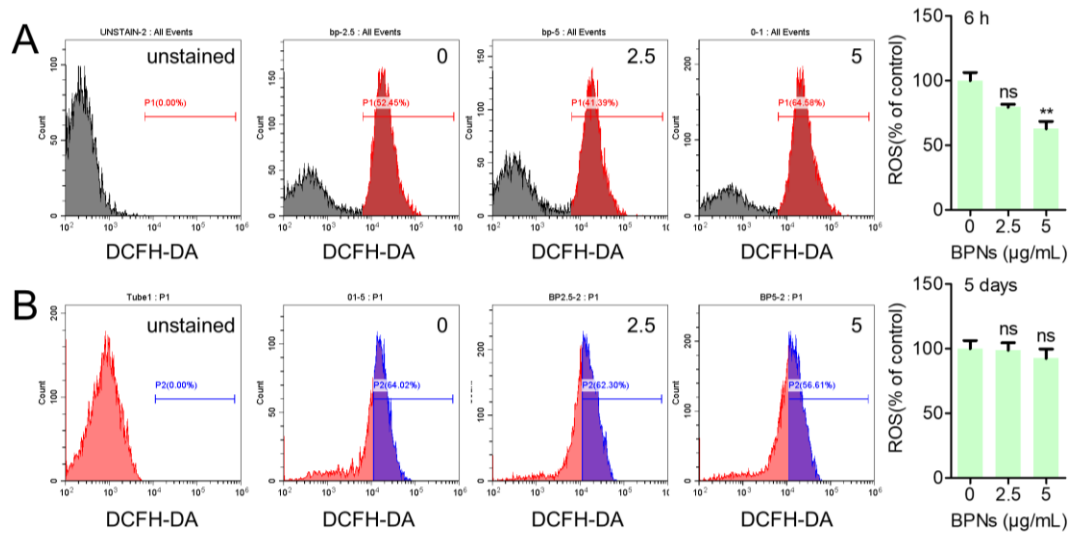


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25 **Fig. S1.** A) Schematic diagram of differentiating NPCs into neurons. B) On the 4th and
 26 7th day of neuronal differentiation, qPCR was used to verify gene expression ($n = 3$).

27 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns. indicates nonsignificant difference.

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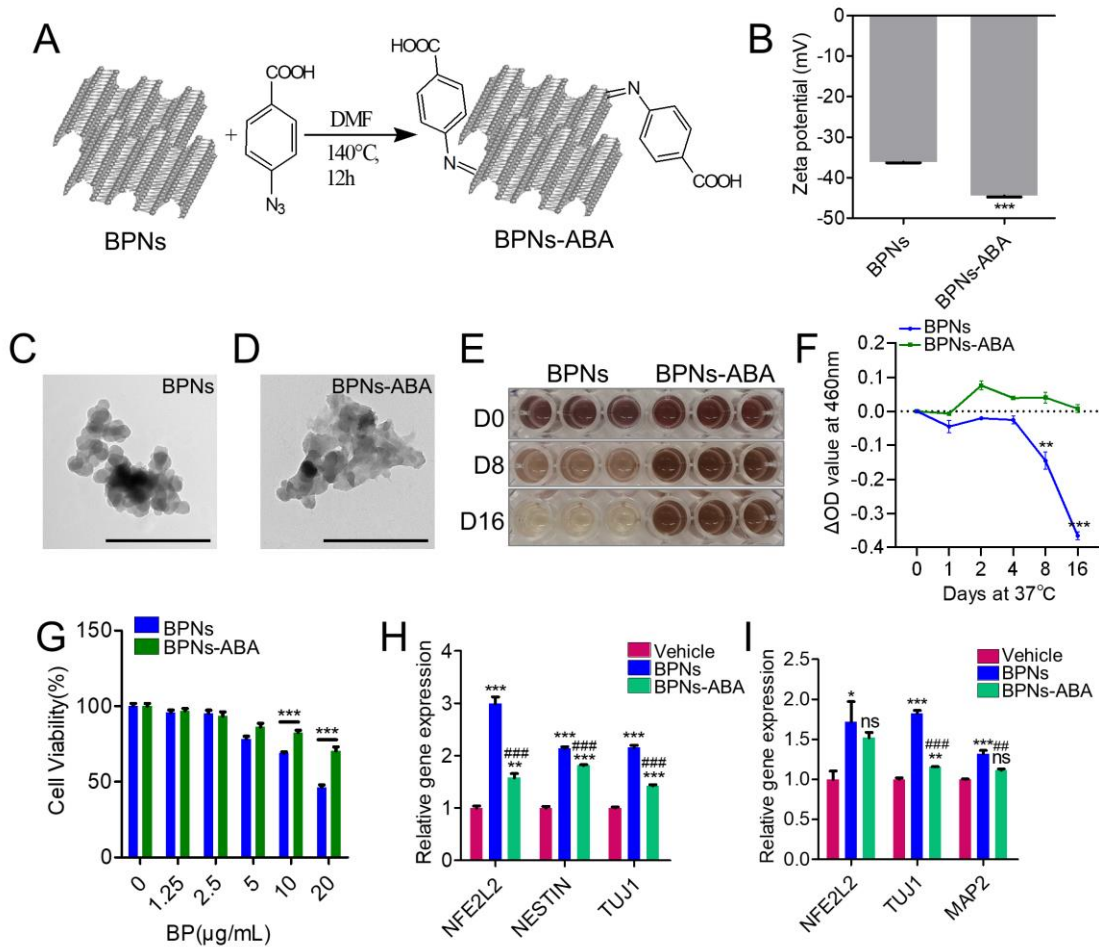


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30 **Fig. S2.** ROS content after incubation of NPCs with different concentrations of
 31 BPNs for 6 h (A) and 5 days (B). $n = 4$. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, ns.

32 indicates nonsignificant difference.

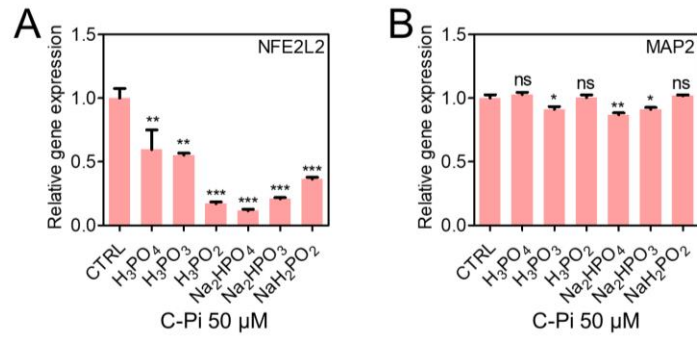
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35 **Fig. S3.** A) Schematic representation of BPNs covalently modified with azobenzoic
 36 acid. B) Zeta potential of BPNs and BPNs-ABA. C-D) TEM images of BPNs and
 37 BPNs-ABA. E-F) Degradation curves of BPNs and BPNs-ABA at 37°C ($n = 3$). G)
 38 Different concentrations of BPNs and BPNs-ABA were incubated with NPCs for 5 days,
 39 and cell viability assay was performed using CCK8 ($n = 5$). H) qPCR validation of
 40 relevant genes after 5 days of co-culture of NPCs with BPNs and BPNs-ABA in
 41 maintenance medium ($n = 3$). I) qPCR to detect the expression of relevant genes in BP-
 42 and BPNs-ABA-treated NPCs after 5 days of culture in neuronal differentiation
 43 medium ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

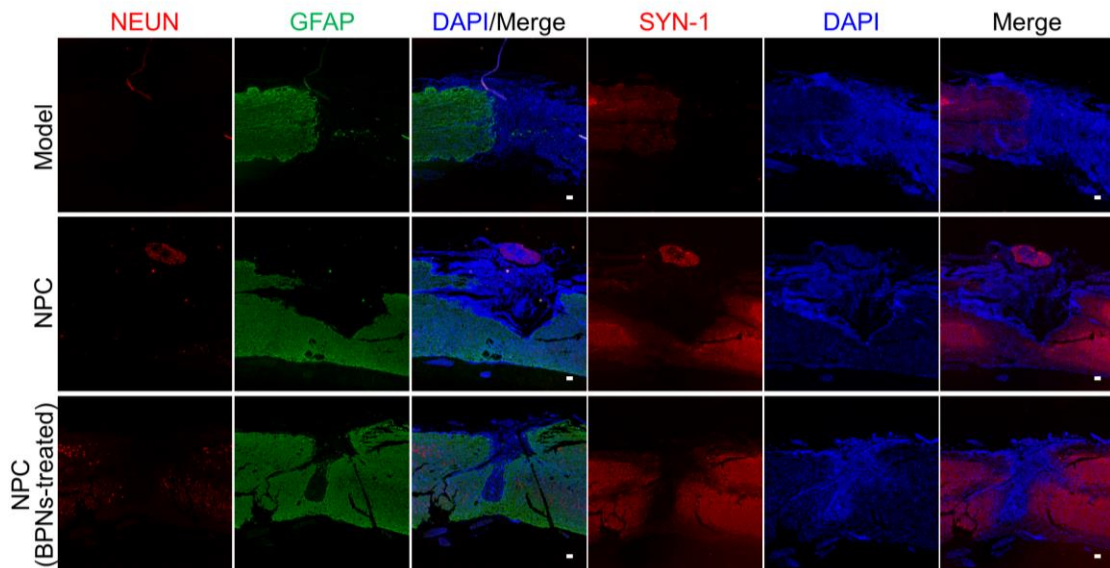
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46 **Fig. S4.** Gene expression of NFE2L2 and MAP2 at 5 days after neural differentiation
 47 of NPCs treated with different species of phosphate at a final concentration of 50 μM
 48 (n = 3). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns. indicates nonsignificant difference.

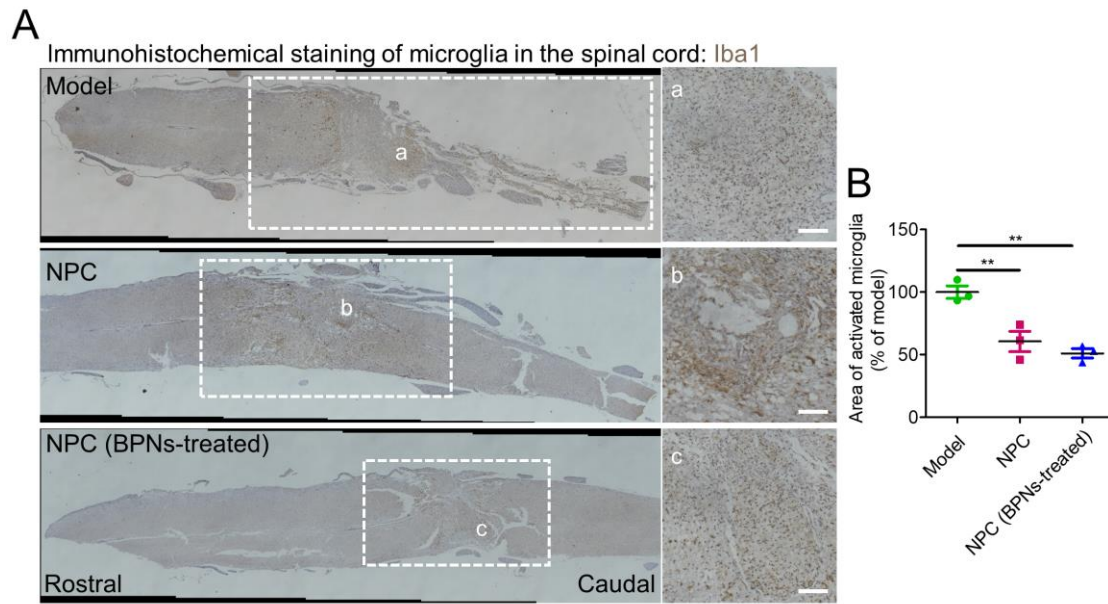
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51 **Fig. S5.** Immunofluorescence staining targeting neurons (NEUN), astrocytes (GFAP),
 52 and neural synapses (SYN1) were performed at 14 days after cell transplantation. Scale
 53 bar: 100 μm.

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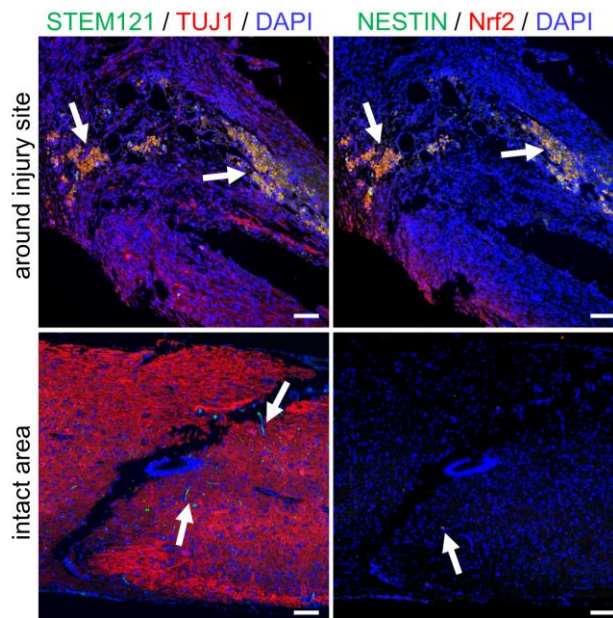


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56 **Fig. S6.** Immunohistochemical staining and quantification of microglia (Iba1) at 14

57 days after cell transplantation ($n = 3$). Scale bar: 100 μm . * $P < 0.05$, ** $P < 0.01$, *** P

58 < 0.001 .



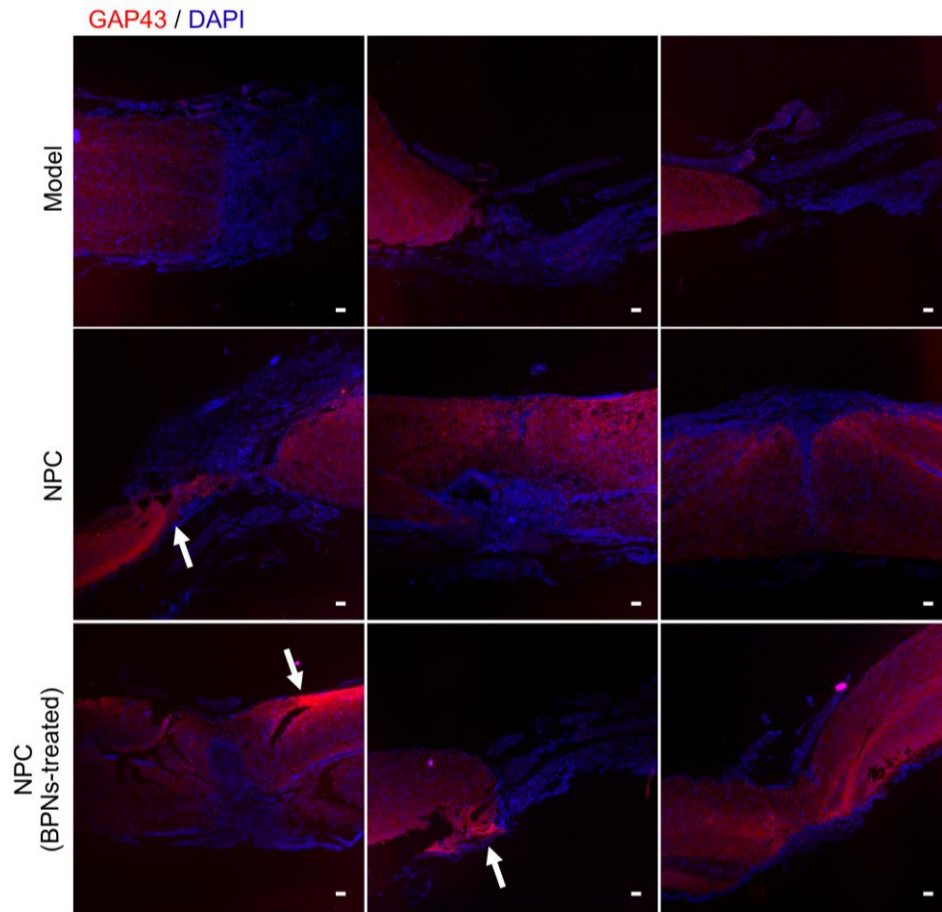
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60 **Fig. S7.** Immunofluorescence staining for STEM121, TUJ1, NESTIN, and Nrf2 were

61 performed at 42 days after cell transplantation in areas with severe spinal cord injury

62 and in areas with intact neural tissue. Scale bar: 100 μ m.

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65 **Fig. S8.** Immunofluorescence staining of axonal membrane protein (GAP43) at 28 days

66 after transplantation; white arrows indicated regions of high expression. Scale bar: 100

67 μ m.

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69 **Table S1.** Primers used for qPCR were as follows:

Gene	Forward Primer	Reverse Primer
<i>ACTIN</i>	CACCATTGGCAATGAGCGGTTTC	AGGTCTTTGCGGATGTCCACGT
<i>GAPDH</i>	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

<i>NESTIN</i>	GGAAGAGAACCTGGGAAAGG	CTTGGTCCTTCTCCACCGTA
<i>PAX6</i>	TGGGCAGGTATTACGAGACTG	ACTCCCGCTTATACTGGGCTA
<i>SOX2</i>	GCCGAGTGGAAACTTTTGTCG	GGCAGCGTGTACTTATCCTTCT
<i>MAP2</i>	GAGAATGGGATCAACGGAGA	CTGCTACAGCCTCAGCAGTG
<i>TUJ1</i>	GGTGTCCGAGTACCAGCAGT	TTCGTACATCTCGCCCTCTT
<i>GAP43</i>	GGCCGCAACCAAAATTCAGG	CGGCAGTAGTGGTGCCTTC
<i>Notch1</i>	GAGGCGTGGCAGACTATGC	CTTGTACTCCGTCAGCGTGA
<i>HES1</i>	TCAACACGACACCGGATAAAC	GCCGCGAGCTATCTTTCTTCA
<i>Jagged1</i>	GTCCATGCAGAACGTGAACG	GCGGGACTGATACTCCTTGA
<i>NFE2L2</i>	TCAGCGACGGAAAGAGTATGA	CCACTGGTTTCTGACTGGATGT
