Supplementary Information

Black Phosphorus Nanosheets Inhibit Glioblastoma Cell Migration and Invasion through Modulation of *WNT/β-catenin* and *NOTCH* Signaling Pathways

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Supplementary Figures

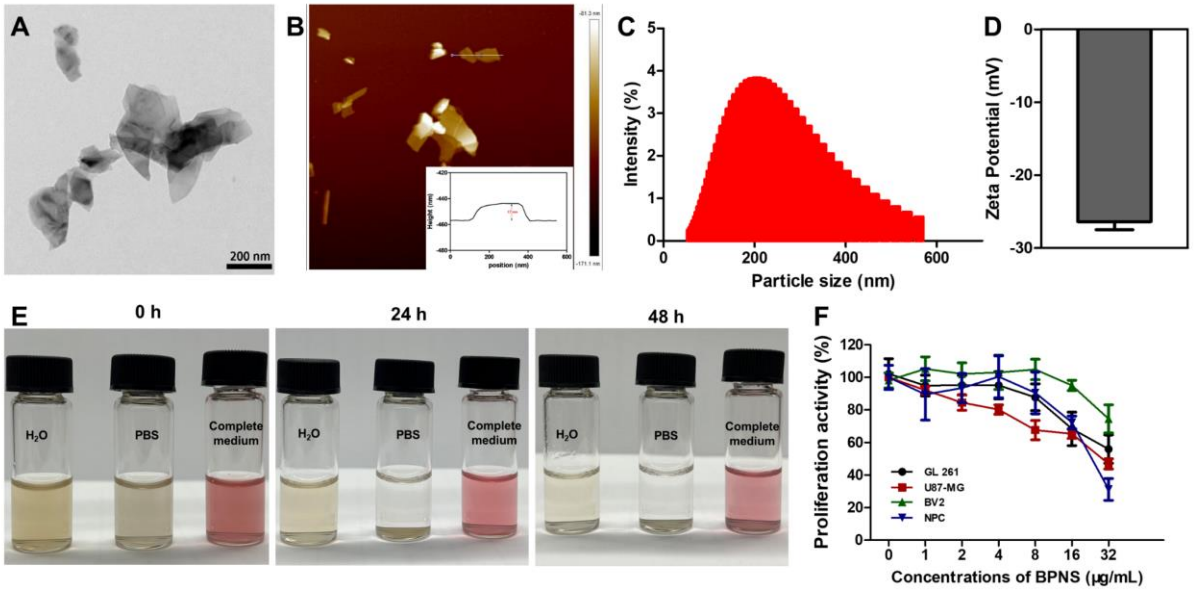


Fig. S1 The characterization and anti-proliferation effects on brain cells of BPNS. (**A**) TEM image of BPNS. (**B**) AFM image and its height profiles along the white lines in the AFM image of BPNS. (**C**) The particle size distribution of BPNS. (**D**) The surface charge of BPNS, $n = 3$. (**E**) The results of dispersibility and stability of BPNS in different dispersive systems (H_2O, PBS, Complete medium) in different time point. (**F**) The proliferation ability of U87-MG cells, GL261 cells, BV2 cells, and NPC cells after being treated with BPNS for 24 h, $n = 5$. Scale bar: 200 nm.

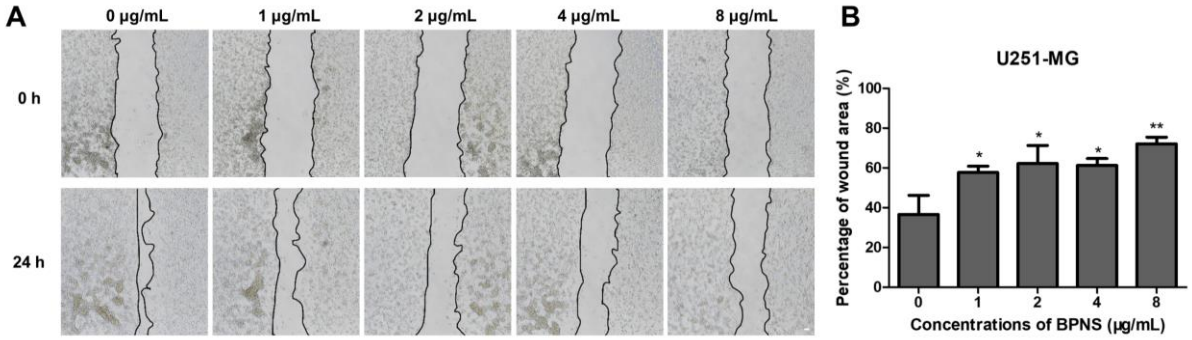


Fig. S2 BPNS suppressed the migration of U251-MG cells. (**A**) U251-MG cells were treated with BPNS (0 - 8 μg/mL) to evaluate cell migration ability by wound healing assay. (**B**) The quantitative statistical results of wound area after 24 h. Scale bar: 100 μm. Error bar, mean ± SD, $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$.

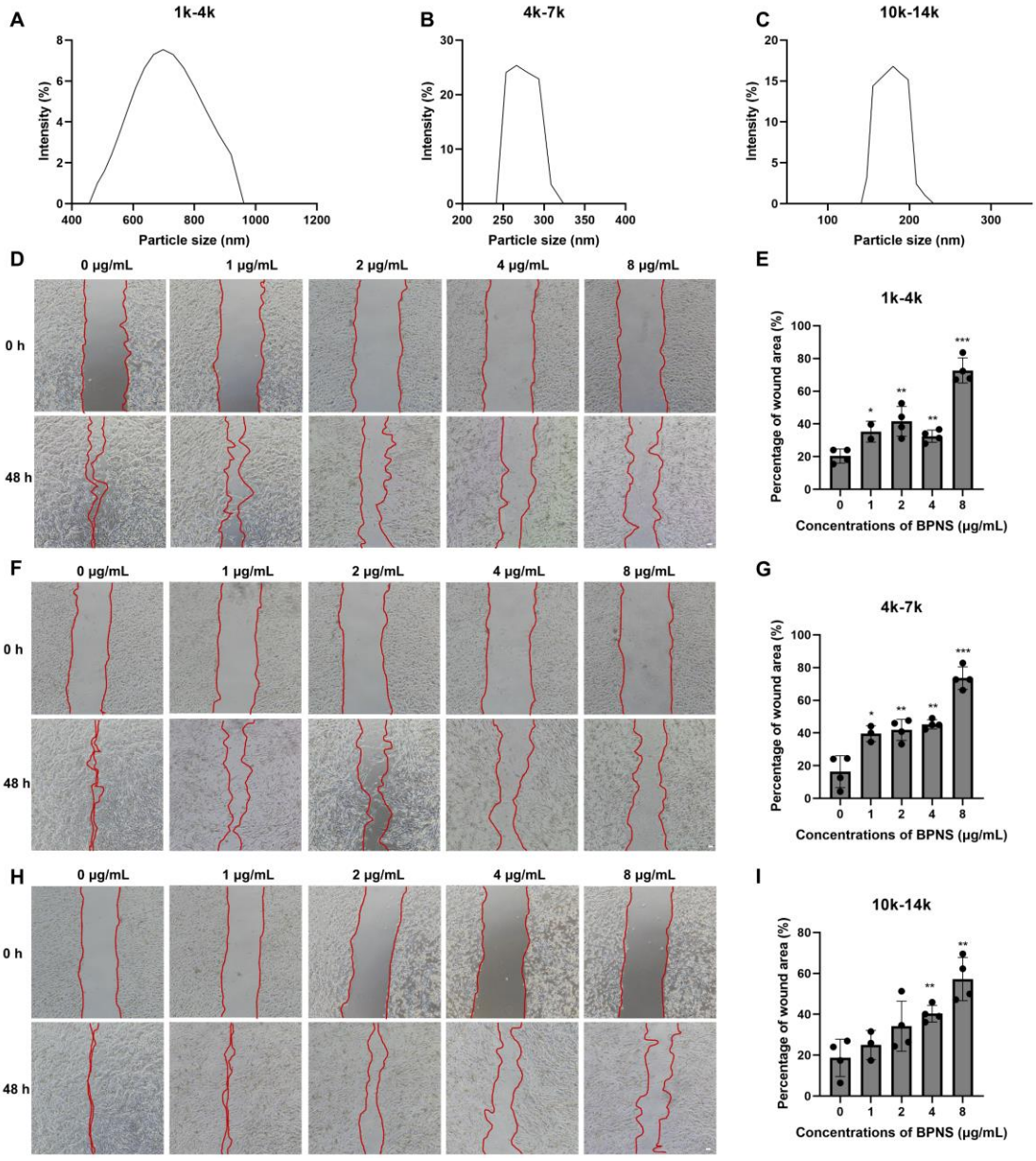


Fig. S3 The impact of different sizes of BPNS on the migration ability of glioblastoma cells. (A) The size of BPNS obtained from gradient centrifugation at 1000 rpm-4000 rpm (1k-4k) was approximately distributed around 650 nm. (B) The size of BPNS obtained from gradient centrifugation at 4000 rpm-7000 rpm (4k-7k) was approximately distributed around 260 nm. (C) The size of BPNS obtained from gradient centrifugation at 10000 rpm-14000 rpm (10k-14k) was approximately distributed around 190 nm. (D-E) Assessing the impact of BPNS (0 - 8 μg/mL), obtained through centrifugation at 1k-4k rpm, on the migration ability of U87-MG cells via scratch experiments and quantitatively analyzing the

experimental results after 48 h. (F-G) Assessing the impact of BPNS (0 - 8 μg/mL), obtained through centrifugation at 4k-7k rpm, on the migration ability of U87-MG cells via scratch experiments and quantitatively analyzing the experimental results after 48 h. (H-I) Assessing the impact of BPNS (0 - 8 μg/mL), obtained through centrifugation at 10k-14k rpm, on the migration ability of U87-MG cells via scratch experiments and quantitatively analyzing the experimental results after 48 h. Scale bar: 100 μm. Error bar, mean ± SD, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

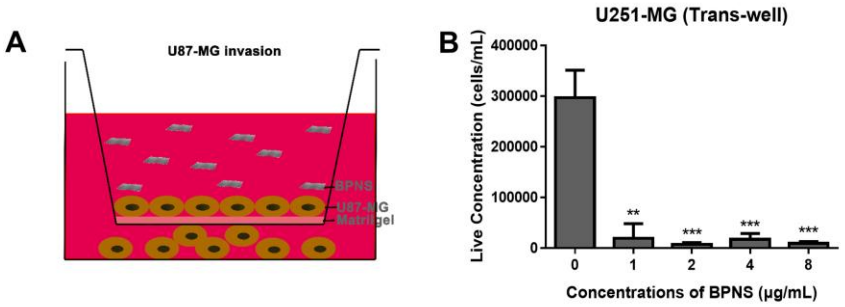


Fig. S4 BPNS suppressed the invasion of U251-MG cells. (**A**) The schematic of the trans-well experiment. (**B**) The trans-well results of U251-MG cells after treated with BPNS in different concentrations. Error bar, mean ± SD, $n = 3$, $^{**}P < 0.01$, $^{***}P < 0.001$.

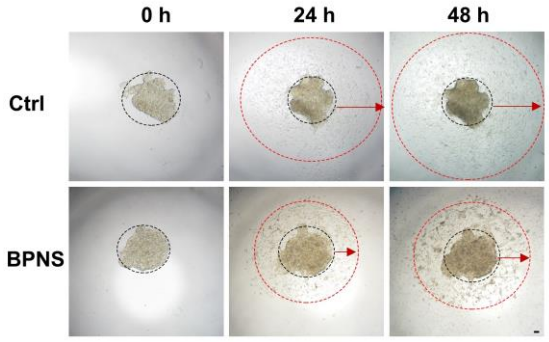
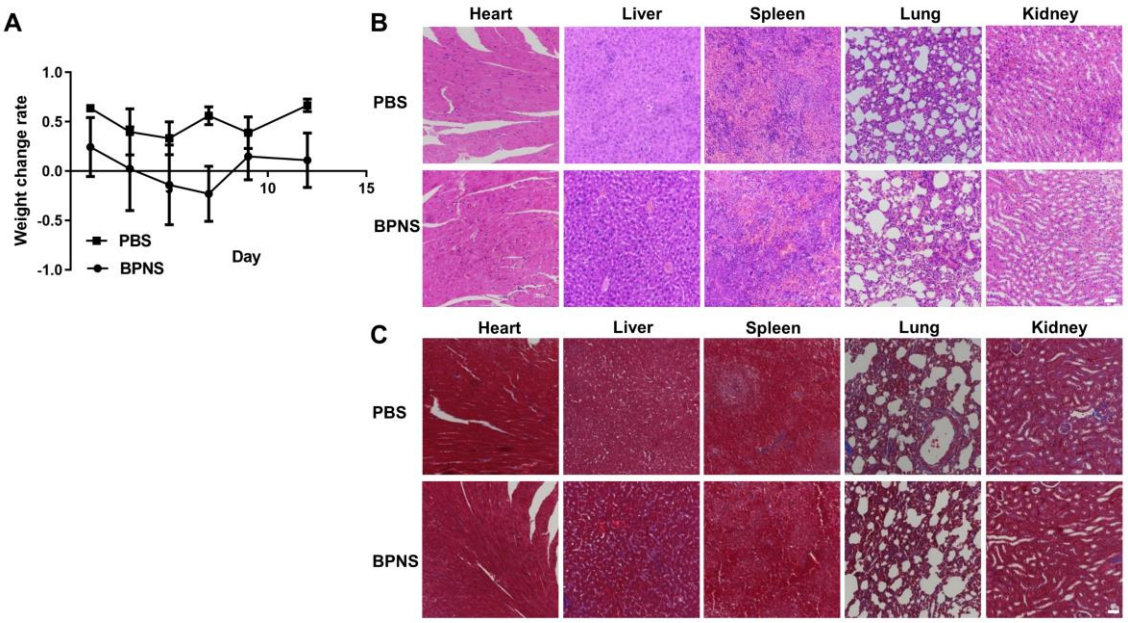


Fig. S5 The 3D cell spheroid invasion assay results of U251-MG cells after being treated with BPNS for 24 h and 48 h. Scale bar: 100 μm.



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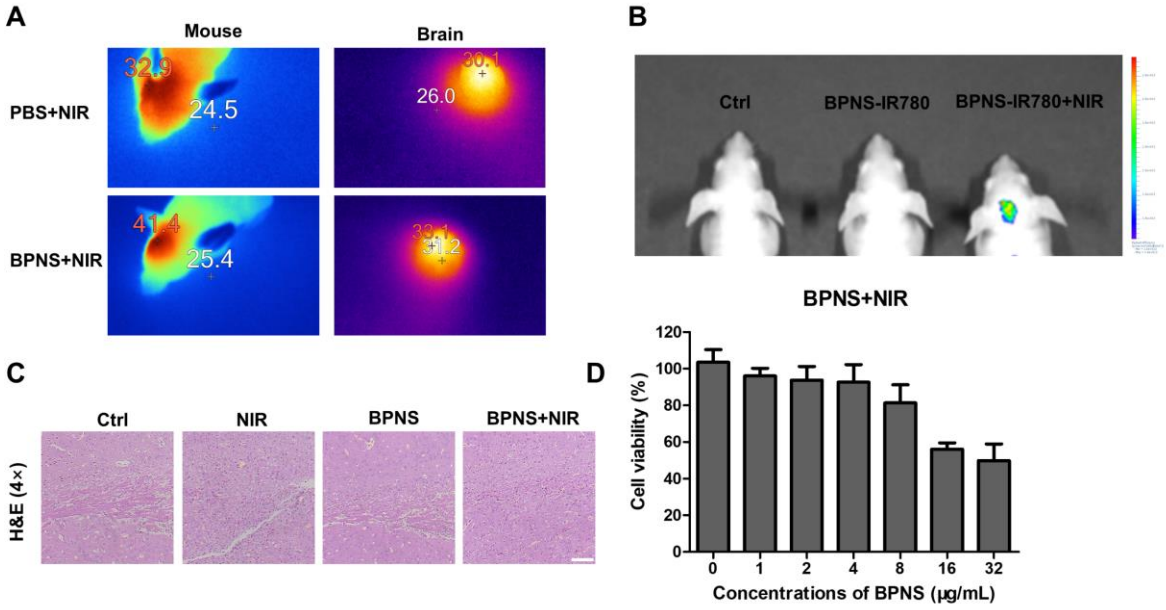
53 **Fig. S6** (**A**) The statistical chart of the weight changes of the PBS and BPNS group of nude mice. (**B**)

54 The results of H&E staining of main organs in PBS and BPNS-treated group, $n = 3$. (**C**) The results of

55 MASSON staining of main organs in PBS and BPNS-treated group. Scale bar: 100 μm.



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57 **Fig. S7 (A)** Healthy mice were intravenously injected with BPNS and PBS separately, and then

58 irradiated with NIR (1 W/cm^2). The brain temperature was monitored using a thermal imager to ensure

59 that BPNS crossed the BBB. After obtaining the mice's brains, under NIR (1 W/cm^2) irradiation, the

brain temperature of mice in the BPNS+NIR group was significantly higher than that of the control group, indicating that BPNS successfully crossed the BBB and remained in the brain with the assistance of NIR. (**B**) The living fluorescent imaging of the mouse after BPNS labeled with IR780 to verify whether BPNS could cross BBB in different groups: Ctrl, BPNS-IR780, BPNS-IR780 + NIR. (**C**) The H&E staining results of brain in different experimental groups showed that NIR-assisted BPNS crossing the BBB would not cause pathological damage to the brain. (**D**) The cell viability of GL261 cells was assessed using CCK-8 assay after treatment with BPNS, and irradiated with NIR (1 W/cm^2), $n = 5$. Scale bar: 100 μm.

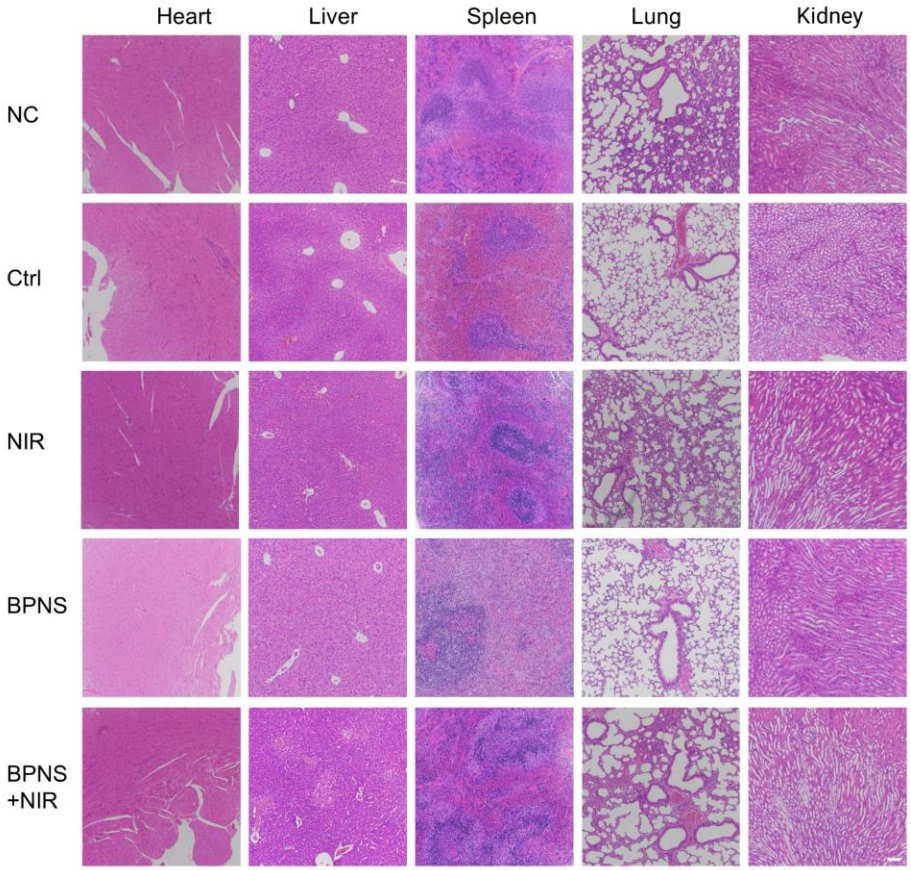


Fig. S8 The H&E staining of major organs in different treatment groups (Normal control, Ctrl group, NIR group, BPNS, and BPNS+NIR group) of orthotopic glioma. Scale bar: 100 μm.

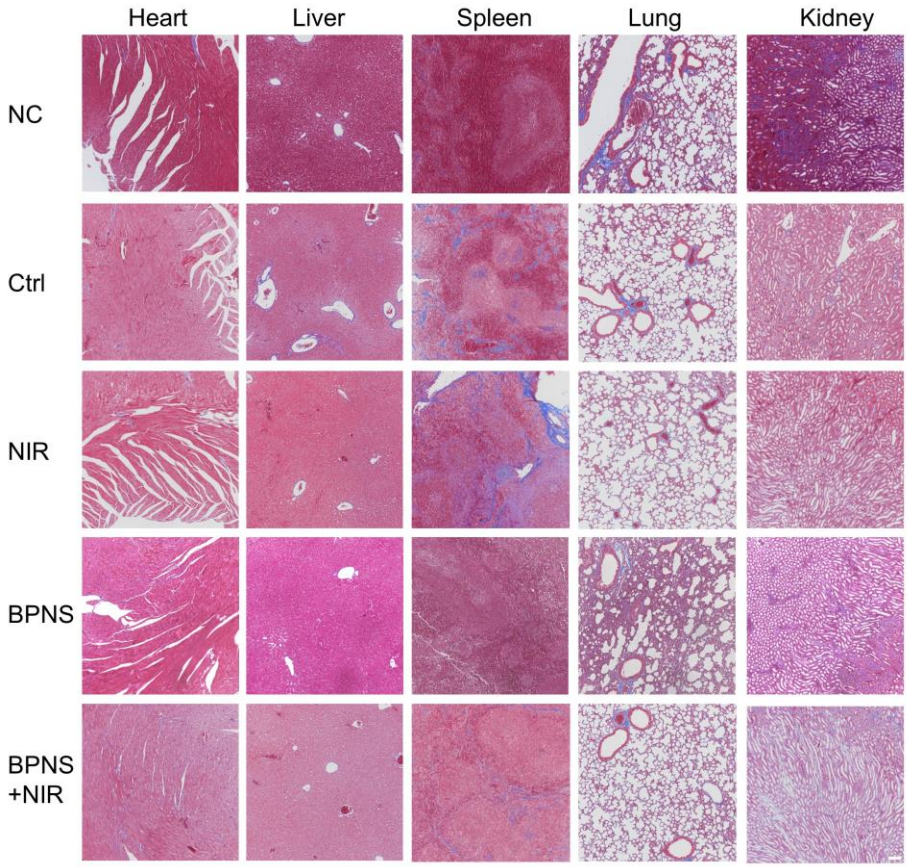


Fig. S9 The MASSON staining of major organs in different treatment groups. Scale bar: 100 μm.

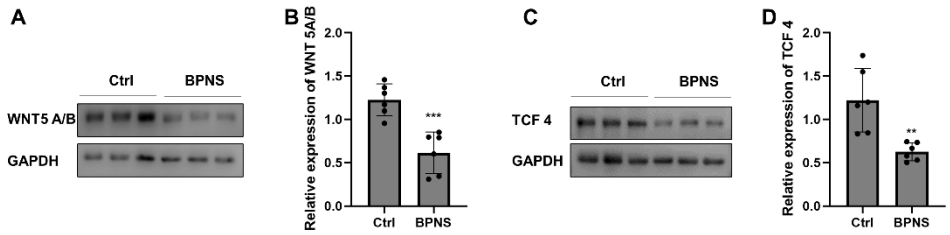
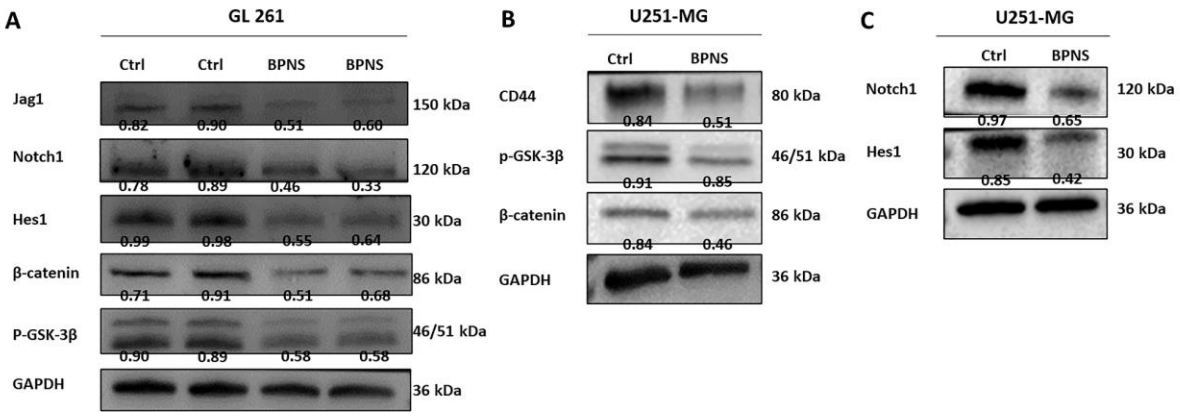


Fig. S10 The changes in the protein levels of *WNT/β-catenin* signaling pathway-related proteins and transcription factors. (A-B) Protein level changes and semi-quantitative analysis of WNT 5A/B in U87-MG cells after BPNS treatment. (C-D) Protein level changes and semi-quantitative analysis of TCF 4 in U87-MG cells after BPNS treatment. Error bar, mean ± SD, $n = 6$, $^{**}P < 0.01$, $^{***}P < 0.001$.



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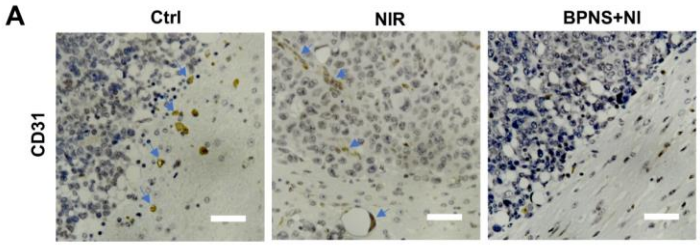
79 **Fig. S11** BPNS down-regulated protein expression in *WNT/β-catenin* and *NOTCH* signal pathway of

80 GL261 and U251-MG cells. (**A**) The western blot results of p-GSK-3β, β-catenin, Hes1, Notch1, and

81 Jag1 of GL261 cells after being treated with BPNS for 24 h. (**B**) The western blot results of p-GSK-3β,

82 β-catenin, CD44 of U251-MG cells after being treated with BPNS for 24 h. (**C**) The western blot results

83 of Notch1, Hes1 of U251-MG cells after being treated with BPNS for 24 h.



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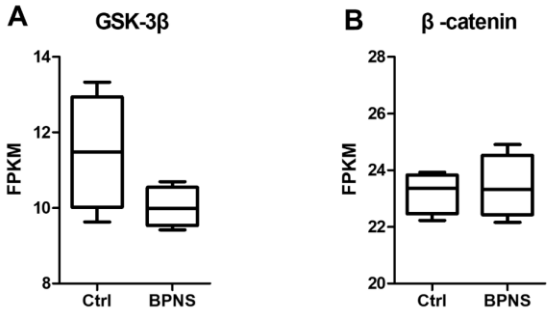
85 **Fig. S12** (**A**) Immunohistochemical staining results of CD31 in orthotopic tumor tissue sections. Scale

86 bar: 50 μm.



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88 **Fig. S13** RNA-Sequencing statistical data graph of *GSK-3β* (**A**) and *β-catenin* (**B**), $n = 4$.

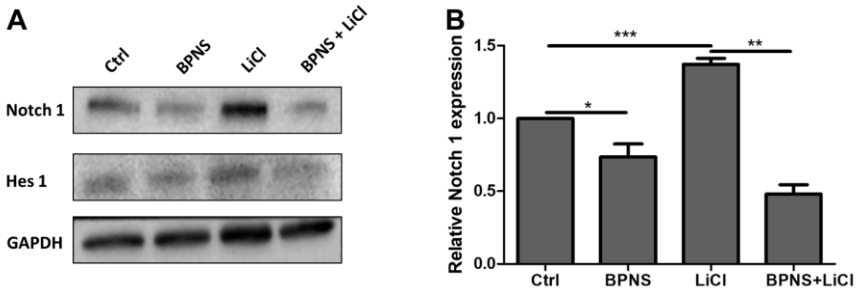

Fig. S14 The impact of *WNT/β-catenin* signaling pathway activators on the *NOTCH* signaling pathway was examined. (**A**) The protein level changes of Notch 1 and HES 1 after different treatments (Ctrl, BPNS, LiCl, BPNS + LiCl). (**B**) The quantification statistical graph of the protein level changes of Notch 1. Error bar, mean ± SD, $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

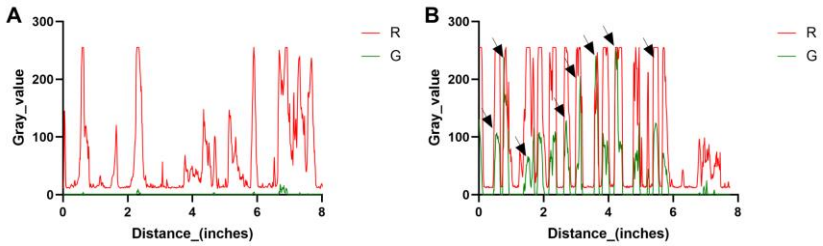

Fig. S15 The results of colocalization analysis. (**A**) The colocalization map analysis of fluorescent molecules C6 and CSNK2A2 in the control group. (**B**) The colocalization map analysis of BPNS-C6 and CSNK2A2 (R: CSNK2A2, G: BPNS-C6).

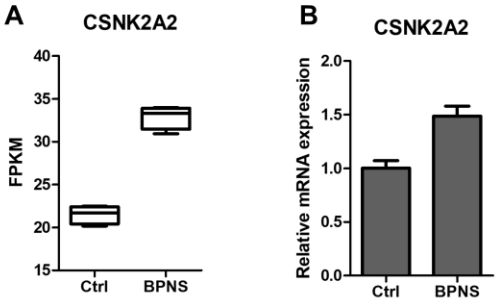

Fig. S16 (**A**) RNA-Sequencing statistical data graph of CSNK2A2, $n = 4$. (**B**) The qRT-PCR results of *CSNK2A2*, $n = 3$.

Supplementary Table

Table S1 The information of primary and secondary antibodies.

Antibodies	Source
1. anti-c-myc (WB)	Santa Cruz Biotechnology
2. anti-cyclin D1 (WB)	Santa Cruz Biotechnology
3. anti-cyclin D3 (WB)	Santa Cruz Biotechnology
4. anti-Sox 2 (WB)	Santa Cruz Biotechnology
5. anti-Jagged1 (WB)	Santa Cruz Biotechnology
6. anti-Hes 1 (WB)	Cell Signaling Technology
7. anti-Notch 1 (WB and IF)	Cell Signaling Technology
8. anti-β-catenin (WB and IF)	R&D System
9. anti-GSK-3β (WB)	Zen BioScience (221162)
10. anti-p-GSK-3β (S21/S9) (WB)	R&D System
11. anti-p-β-catenin (Ser33) (WB)	Zen BioScience (310053)
12. anti-CD44 (WB and IF)	Cell Signaling Technology
13. anti-GAPDH (WB)	Cell Signaling Technology
14. anti-CD31 (IHC)	ServiceBio (GB113151)
15. anti-Rabbit-HRP	SAB
16. anti-Mouse-HRP	SAB
17. anti-CSNK2A2	Proteintech
18. anti-WNT5A/B	Proteintech
19. anti-TCF4	Proteintech
20. Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 594	Invitrogen
21. Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488	Invitrogen

Table S2 Primers described in the article.

Gene	Forward primer sequence 5'→3'	Reverse primer sequence 5'→3'
Sox2	5'-GCCGAGTGGAAACTTTTGTCG-3'	5'-GGCAGCGTGTACTTATCCTTCT-3'
c-Myc	5'-GGCTCCTGGCAAAAGGTCA-3'	5'-CTGCGTAGTTGTGCTGATGT-3'
Axin2	5'-TACACTCCTTATTGGGCGATCA-3'	5'-TTGGCTACTCGTAAAGTTTTGGT-3'
cyclin D1	5'-GCTGCGAAGTGGAAACCATC-3'	5'-CCTCCTTCTGCACACATTTGAA-3'
Notch1	5'-GAGGCGTGGCAGACTATGC-3'	5'-CTTGTACTCCGTCAGCGTGA-3'
Hes1	5'-TCAACACGACACCGGATAAAC-3'	5'-GCCGCGAGCTATCTTTCTTCA-3
β-catenin	5'- CATCTACACAGTTTGATGCTGCT -3'	5'- GCAGTTTTGTCAGTTCAGGGA -3'
GSK-3β	5'- GGCAGCATGAAAGTTAGCAGA -3'	5'- GGCGACCAGTTCTCCTGAATC -3'
ZEB 1	5'-CGCTCTACTAAGGAGGCTGC-3'	5'-GAACCGGGATGGGAAGTGAC-3'

Jagged1	5’-GTCCATGCAGAACGTGAACG-3’	5’ GCGGGACTGATACTCCTTGA-3’
DDR 1	5’-TGCCAGCTTCTCCTTGTTCT-3’	5’-CGCCGTTCTCCATGTAGTCA-3’
DDR2	5’-TTAACGGCTGCTTTCTGACC-3’	5’-AGAGCTAGTGTTGAGTCAGTGT-3’
COL5A2	5’-AGCTGGGACCATCCCAAAAG-3’	5’-CCACTGACATGACAAAAGCGT-3’
COL1A2	5’-AGGGGTCTCCATGGTGAGTT-3’	5’-CCTCGGCTTCCAATAGGACC-3’
COL4A5	5’-TTCTCCTGAGAGACCGGCTT-3’	5’-GACAGTGAGGCTTGGGTGAA-3’
COL4A6	5’-CCAGCTGCTCACAGAACAGA-3’	5’-AGGCACAACGTAACCAGGAG-3’
FURIN	5’-CCTGGTTGCTATGGGTGGTAG-3’	5’-AAGTGGTAATAGTCCCCGAAGA-3’
MMP 1	5’-AAAATTACACGCCAGATTTGCC-3’	5’-GGTGTGACATTACTCCAGAGTTG-3’
MMP 2	5’-TACAGGATCATTGGCTACACACC-3’	5’-GGTCACATCGCTCCAGACT-3’
PRKCA	5’-GTCCACAAGAGGTGCCATGAA-3’	5’-AAGGTGGGGCTTCCGTAAGT-3’
SH3PXD2A	5’-GGACCCCAAGCAAAGGATCAT-3’	5’-TGCCCGGCAGTATTCATCG-3’
TIMP 2	5’-AAGCGGTCAGTGAGAAGGAAG-3’	5’-GGGGCCGTGTAGATAAACTCTAT-3’
BMI1	5’-TGGACTGACAAATGCTGGAGA-3’	5’-GAAGATTGGTGGTTACCGCTG-3’
CALM1	5’-TTGACTTCCCCGAATTTTTGACT-3’	5’-GGAATGCCTCACGGATTTCTT-3’
ADAM12	5’-TCTCATTGCCAGCAGTTTCAC-3’	5’-CGTGTAATTTCGAGCGAGGG-3’
ADAM19	5’-GGGAGCCTGGATGGACAAG-3’	5’-AGCTTTGAGTGGATGCTTTTCTC-3’
MMP 14	5’-GGCTACAGCAATATGGCTACC-3’	5’-GATGGCCGCTGAGAGTGAC-3’
MMP 15	5’-AGGTCCATGCCGAGAACTG-3’	5’-GTCTCTTCGTCGAGCACACC-3’
MMP 16	5’-AGCACTGGAAGACGGTTGG-3’	5’-CTCCGTTCCGCAGACTGTA-3’
MMP 17	5’-CACTCATGTACTACGCCCTCA-3’	5’-TGGAGAAGTCGATCTGGATGTC-3’
MAPK 1	5’-TCTGGAGCAGTATTACGACCC-3’	5’-CTGGCTGGAATCTAGCAGTCT-3’
CD44	5’-CATTTCAACCACACCACGGG-3’	5’-AGCAGTGGTGCCATTTCTGT-3’
GAPDH	5’-GGAGCGAGATCCCTCCAAAAT-3’	5’-GGCTGTTGTCATACTTCTCATGG-3’
CSNK2A2	5’-TCCCGAGCTGGGGTAATCAA-3’	5’-GTTCCACCACGAAGGTTCTCC-3’