Supplementary Data

Title：PRL-3 is a potential glioblastoma prognostic marker and promotes glioblastoma progression by enhancing MMP7 through the ERK and JNK pathways

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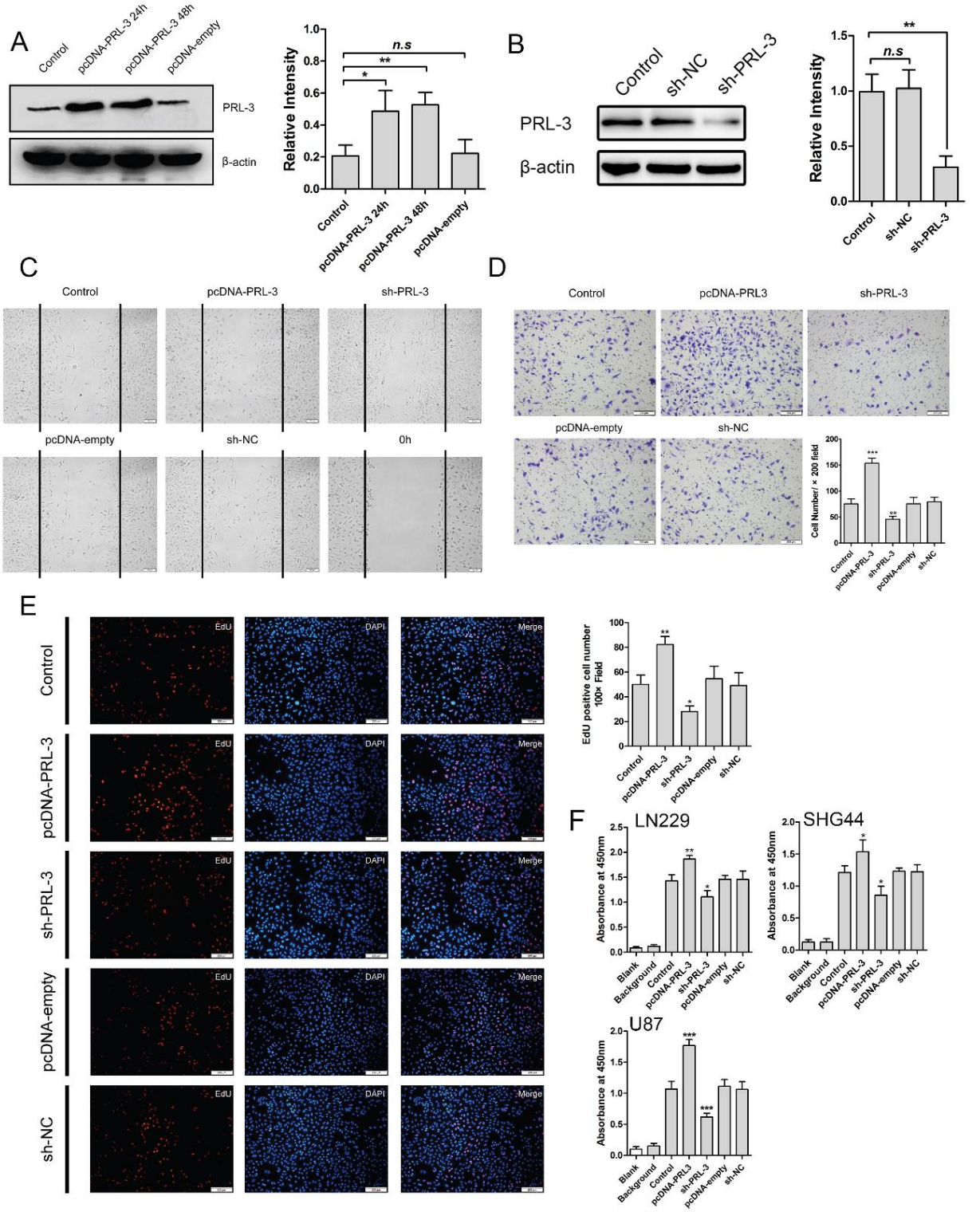
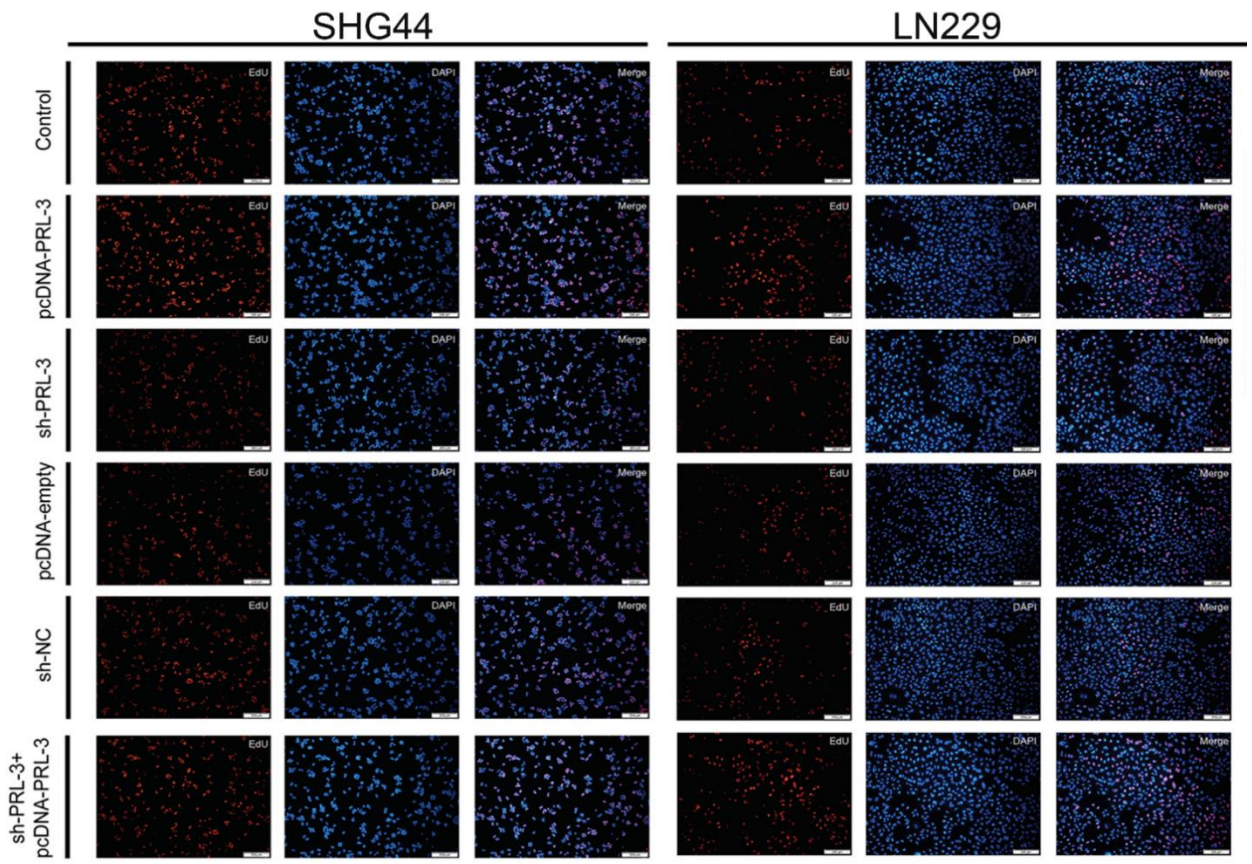
Figure S1


A
Control
pcDNA-PRL-3 24h
pcDNA-PRL-3 48h
pcDNA-empty
PRL-3
β-actin
Relative Intensity
n.s
**
*
B
Control
sh-NC
sh-PRL-3
PRL-3
β-actin
Relative Intensity
n.s
**
C
Control
pcDNA-PRL-3
sh-PRL-3
pcDNA-empty
sh-NC
0h
D
Control
pcDNA-PRL3
sh-PRL-3
pcDNA-empty
sh-NC
Cell Number/ × 200 field
E
Control
pcDNA-PRL-3
sh-PRL-3
pcDNA-empty
sh-NC
EdU
DAPI
Merge
EdU positive cell number 100× Field
F
LN229
SHG44
U87
Absorbance at 450nm
Blank
Background
Control
pcDNA-PRL-3
sh-PRL-3
pcDNA-empty
sh-NC

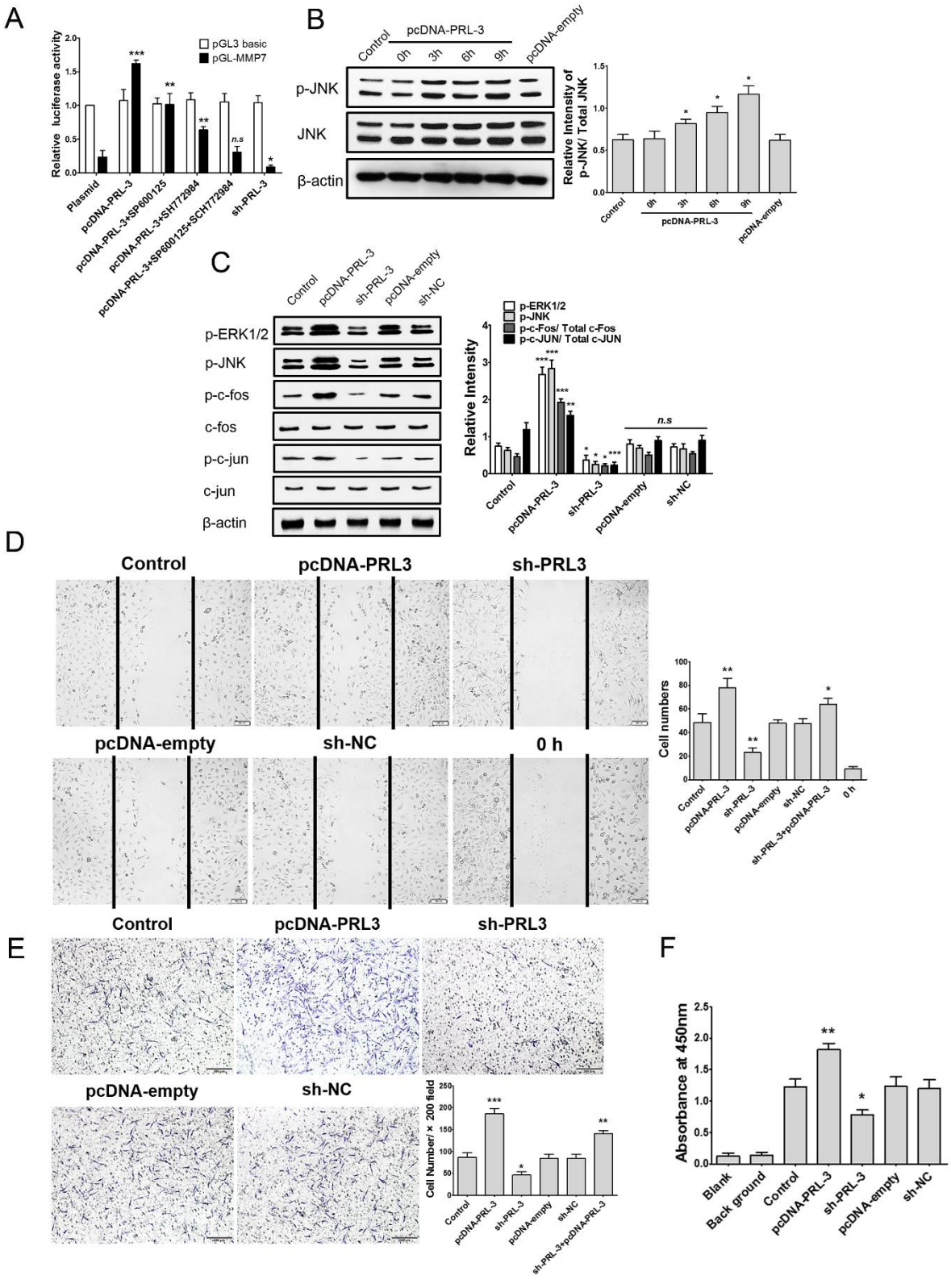
Supplementary Figure S1. PRL-3 promotes U87 cell proliferation, invasion, and migration. A. PRL-3 protein expression was significantly up-regulated in U87 cells transfected with pcDNA-PRL-3. **B.** Levels of PRL-3 protein in the control, sh-NC and sh-PRL-3 groups. **C.** The wound-healing assay was used to investigate the migration capacity of U87 cells. **D.** The effect of PRL-3 on the invasion of U87 cells were evaluated by the transwell assay. **E.** EdU positive cell number of U87 cells (100×). **F.** Cells in each experimental group were cultured in 96-well plates and stained with CCK8 at 48 h. The results were assessed by measuring the absorbance at 450 nm with an ultraviolet spectrophotometer. Data represent the mean±SD of 3 individual experiments. *P<0.05, **P<0.01, ***P<0.001.

Figure S2



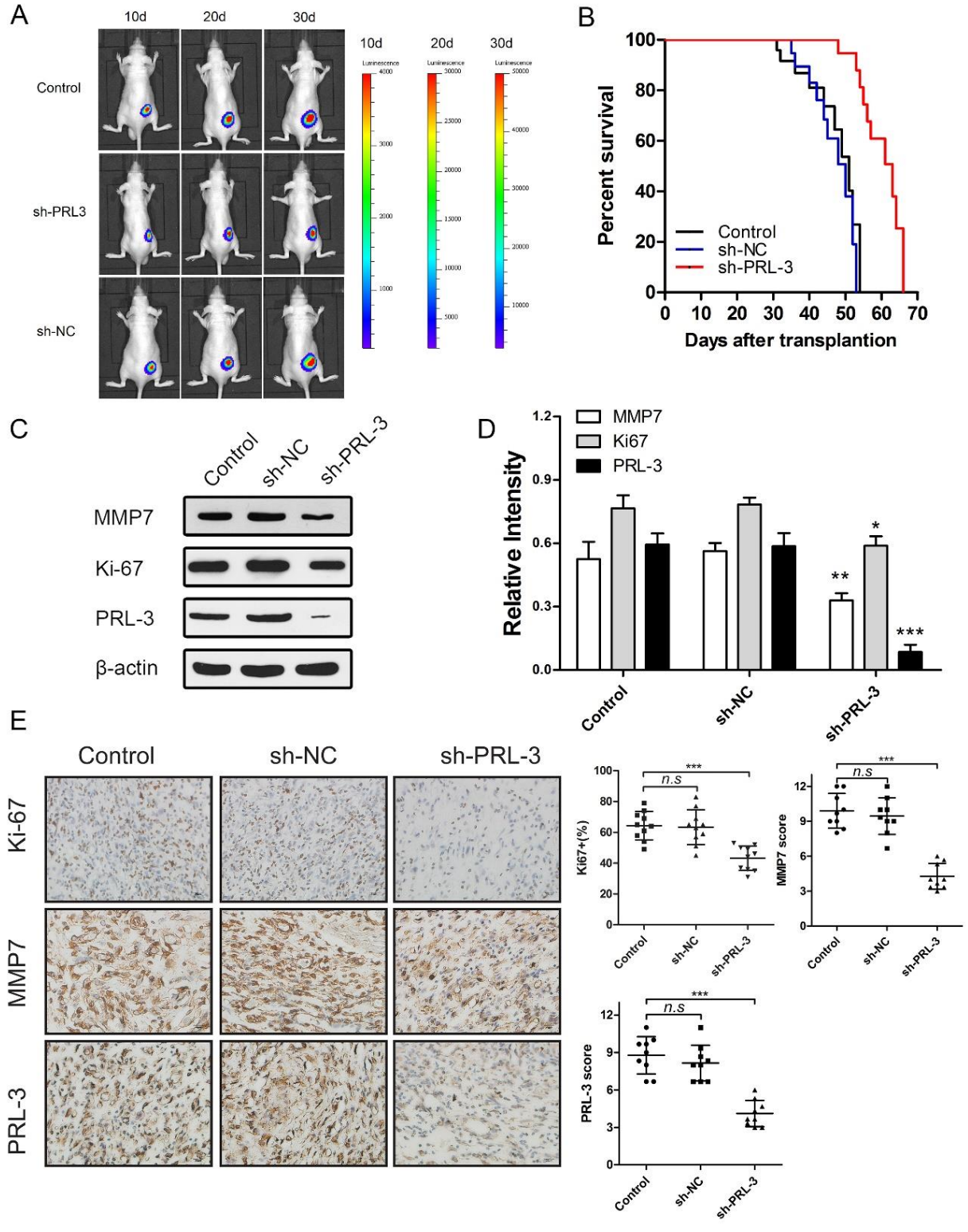
Supplementary Figure S2. PRL-3 promotes SHG44 and LN229 cells proliferation. Apollo staining and DNA staining were performed according to the manufacturer's instructions to detect the number of EdU-labeled cells. The staining images were acquired under a microscope (OLYMPUS, IX73, ×100).

Figure S3


A
Relative luciferase activity
pGL3 basic
pGL-MMP7
Plasmid
pcDNA-PRL-3
pcDNA-PRL-3+SP600125
pcDNA-PRL-3+SH772984
pcDNA-PRL-3+SP600125+SCH772984
sh-PRL-3
B
Control
pcDNA-PRL-3
0h
3h
6h
9h
pcDNA-empty
p-JNK
JNK
β-actin
Relative Intensity of p-JNK/ Total JNK
C
Control
pcDNA-PRL-3
sh-PRL-3
pcDNA-empty
sh-NC
p-ERK1/2
p-JNK
p-c-fos
c-fos
p-c-jun
c-jun
β-actin
p-c-Fos/ Total c-Fos
p-c-JUN/ Total c-JUN
Relative Intensity
n.s
D
Control
pcDNA-PRL3
sh-PRL3
pcDNA-empty
sh-NC
0 h
Cell numbers
sh-PRL-3+pcDNA-PRL-3
E
Cell Number/ × 200 field
F
Absorbance at 450nm
Blank
Back ground

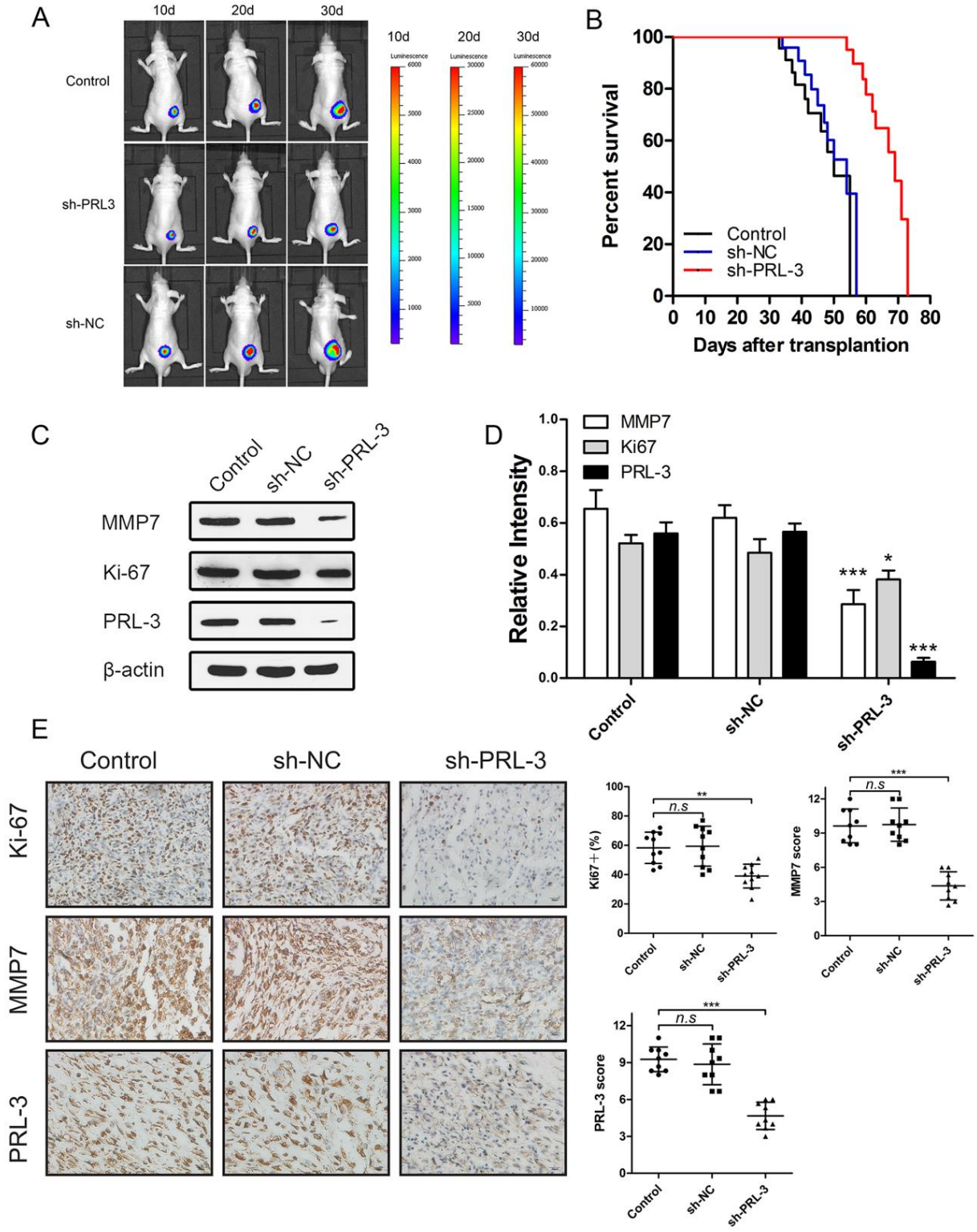
Supplementary Figure S3. ERK and JNK mediate the effect of PRL-3 by enhancing c-Fos/p-c-Fos and c-Jun/p-c-Jun, which promote MMP7 expression by binding on its promoter region directly. A. The luciferase reporter plasmid which contains MMP7 promoter region was used and ERK/JNK inhibitor (SCH772984 and SP600125) to investigate which pathway is the direct causation of higher MMP7 in GMB02 cells. **B.** Cells were transfected with pcDNA-PRL-3, and the levels of JNK and phosphorylated JNK (p-JNK) were detected by western blotting. **C.** Both c-Fos/p-c-Fos and c-Jun/p-c-Jun can be affected by PRL-3. **D.** The wound-healing assay was used to investigate the migration capacity of the patient-derived glioblastoma cells (GBM02). **E.** The effect of PRL-3 on the invasion of GBM02 cells were evaluated by the Transwell assay. **F.** GBM02 Cells in each experimental group were cultured in 96-well plates and stained with CCK8 at 48 h. The results were assessed by measuring the absorbance at 450 nm with an ultraviolet spectrophotometer. The values are the mean±SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 compared with the control group.

Figure S4


A
10d
20d
30d
Control
sh-PRL3
sh-NC
Luminescence
B
Percent survival
Days after transplantion
Control
sh-NC
sh-PRL-3
C
Control
sh-NC
sh-PRL-3
MMP7
Ki-67
PRL-3
β-actin
D
MMP7
Ki67
PRL-3
Relative Intensity
E
Control
sh-NC
sh-PRL-3
Ki-67
MMP7
PRL-3
Ki67+(%)
MMP7 score
PRL-3 score
n.s
***

Supplementary Figure S4. Down-regulation of PRL-3 suppresses glioma growth and prolong survival time in xenograft tumors. A. A total of 5×10^6 U251 cells stably transfected with sh-NC or sh-PRL-3 lentivirus were injected into the right flanks of nude mice, and tumor growth and invasion were assessed by bioluminescence imaging. Changes in the bioluminescent signal were measured at days 10, 20 and 30 after implantation. **B.** Kaplan–Meier survival analysis was performed to evaluate the overall survival time. P values were determined by the log-rank test ($P<0.001$). **C-D.** Total proteins were extracted from the dissected tumor samples. The expression of PRL-3, Ki67 and MMP7 were analyzed by western blotting. **E.** Representative IHC staining of Ki67, MMP7, and PRL-3 for xenografts with indicated administrations. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with the control group.

Figure S5


A
10d
20d
30d
Control
sh-PRL3
sh-NC
Luminescence
B
Percent survival
Days after transplantion
Control
sh-NC
sh-PRL-3
C
Control
sh-NC
sh-PRL-3
MMP7
Ki-67
PRL-3
β-actin
D
Relative Intensity
MMP7
Ki67
PRL-3
E
Control
sh-NC
sh-PRL-3
Ki-67
MMP7
PRL-3
Ki67+(%)
MMP7 score
PRL-3 score
n.s

Supplementary Figure S5. Down-regulation of PRL-3 suppresses glioma growth and prolong survival time in xenograft tumors. A. A total of 5×10^6 U87 cells stably transfected with sh-NC or sh-PRL-3 lentivirus were injected into the right flanks of nude mice, and tumor growth and invasion were assessed by bioluminescence imaging. Changes in the bioluminescent signal were measured at days 10, 20 and 30 after implantation. **B.** Kaplan–Meier survival analysis was performed to evaluate the overall survival time. P values were determined by the log-rank test ($P<0.001$). **C-D.** Total proteins were extracted from the dissected tumor samples. The expression of PRL-3, Ki67 and MMP7 were analyzed by western blotting. **E.** Representative IHC staining of Ki67, MMP7, and PRL-3 for xenografts with indicated administrations. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with the control group.