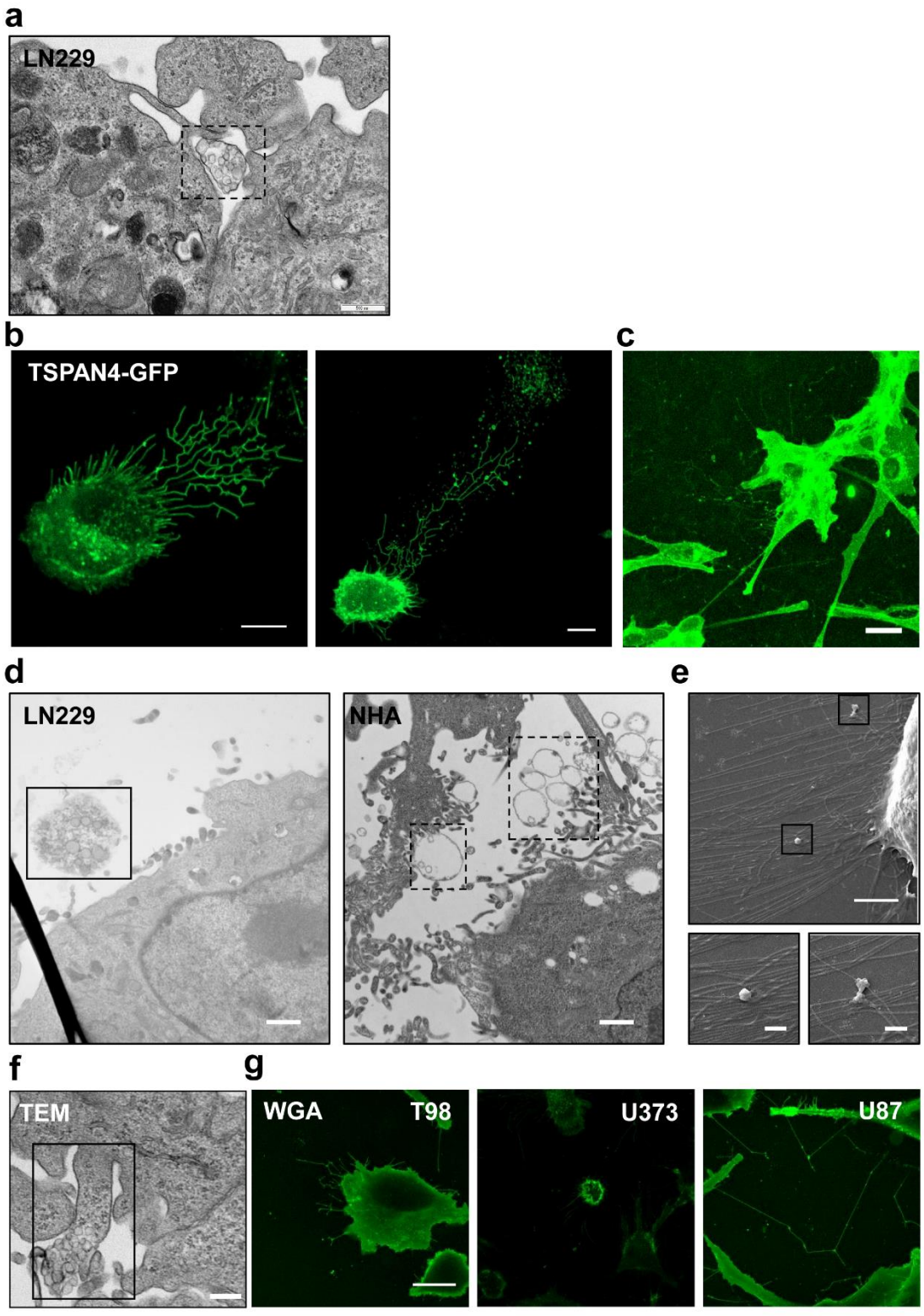
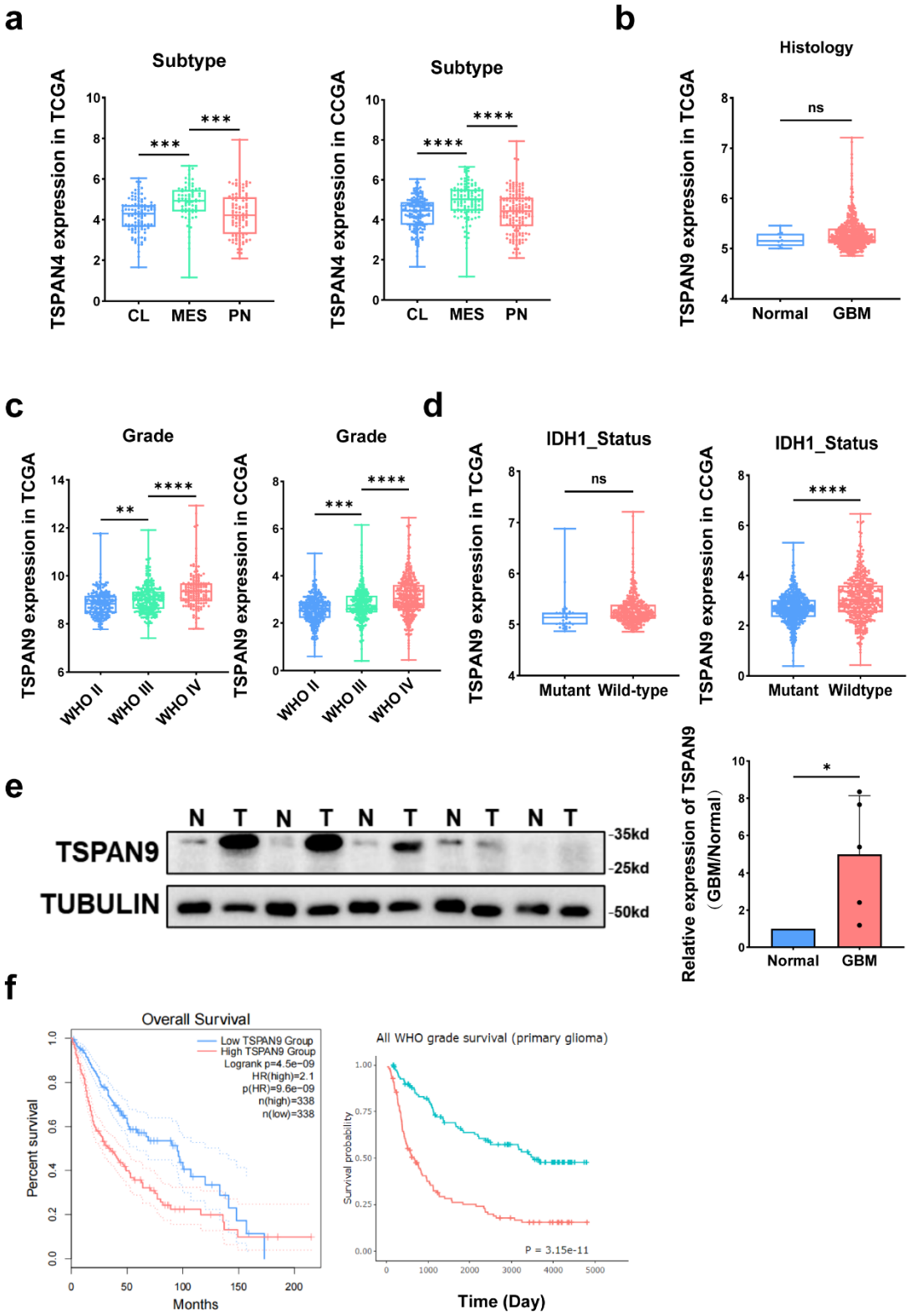


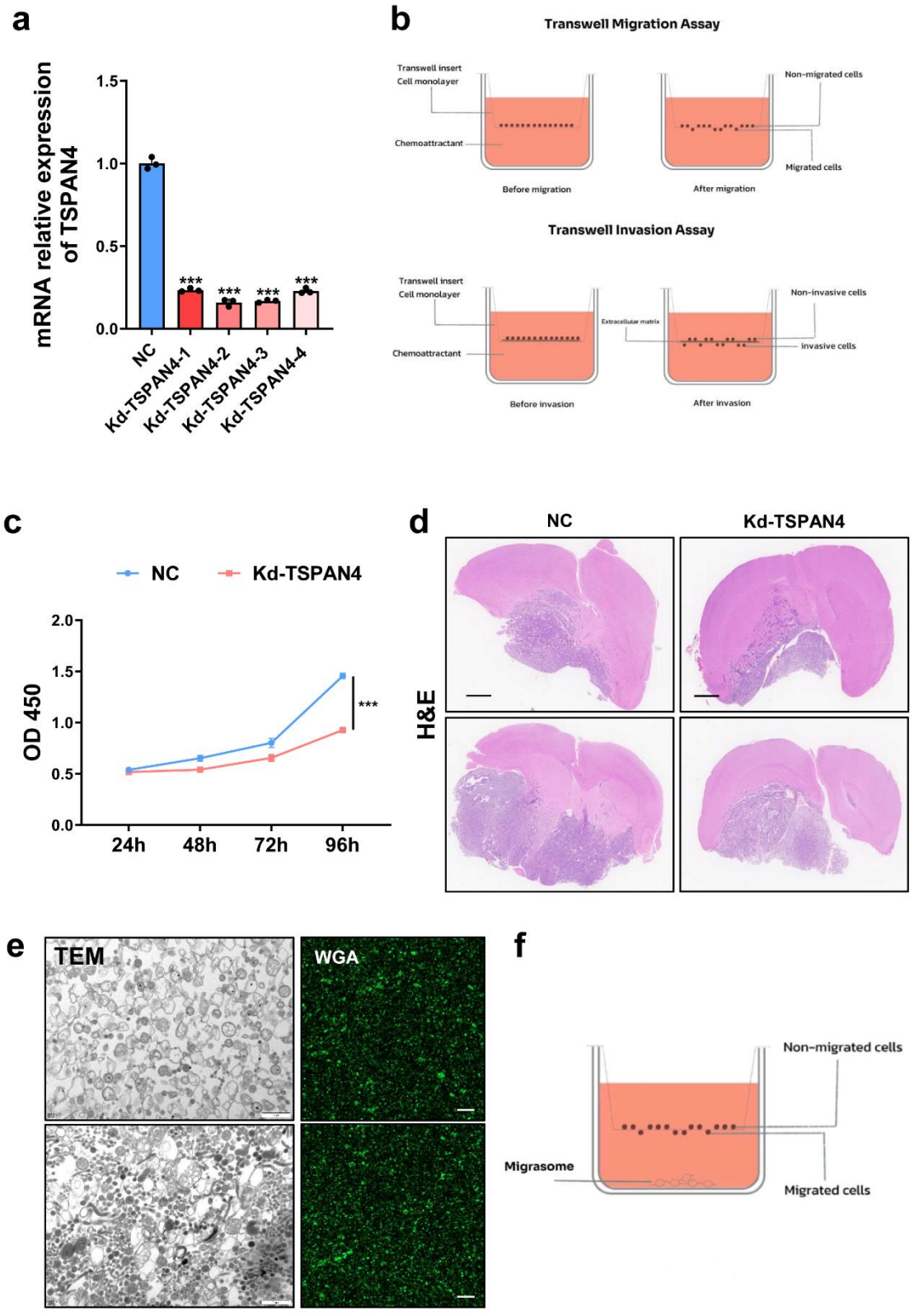
Supplementary Figure 1. Detection of migrasomes in GBM. (a) Vesicles in the extracellular space observed by TEM. Scale bar, 500nm. (b) Confocal images of LN229 cells transfected with TSPAN4-GFP. Scale bar, 10 μm. (c) Confocal images of primary glioma cells with WGA staining. Scale bar, 20 μm.

(d) TEM observation of vesicles in the extracellular space of LN229and NHA cells. (left) Scale bar, 500 nm; (right) Scale bar, 1μm. (e) LN229 cells were grown on cover slips and observed by SEM. Scale bar, 5 μm. Boxed regions in the top left panel are enlarged in the small panels. Scale bar, 1 μm. (f) TEM image of LN229 cell in the process of forming a migrasome. Scale bar, 200nm. (g)T98, U373 and U87 cells were stained with WGA and then visualized. Scale bar, 20 μm.

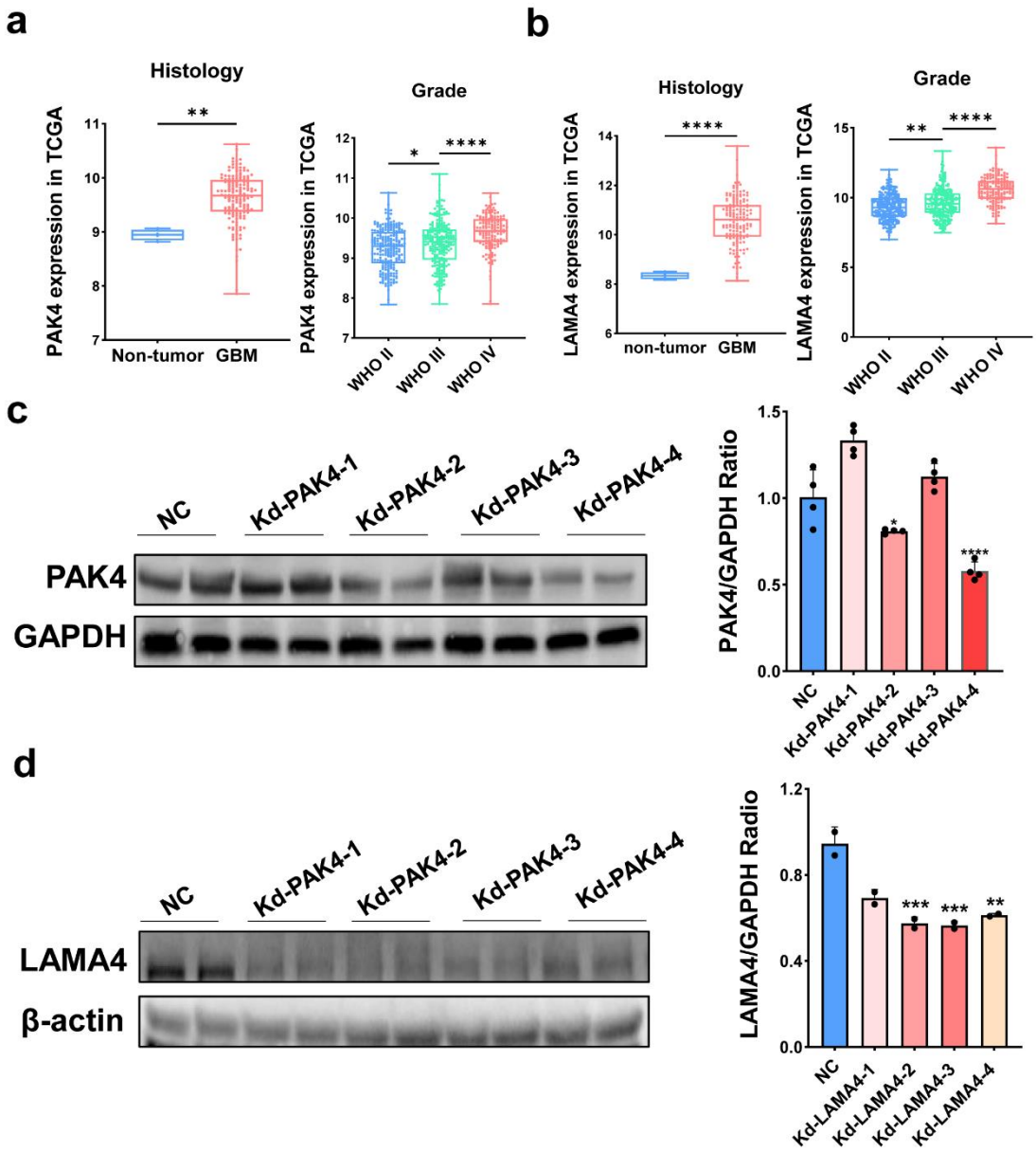


Supplementary Figure 2. Correlation of increased *TSPAN9* expression with glioma malignancy and poor patient prognosis. (a) Expression features of *TSPAN4* in gliomas with different molecular phenotypes in the TCGA (n=287) and CGGA (n=435) databases. ***$P < 0.001$. ****$P < 0.0001$. (b) Differential mRNA levels of *TSPAN9* in normal (n=10) and GBM (n=528)

samples based on the TCGA dataset. $^{ns}P > 0.05$. (c) Levels of *TSPAN9* in gliomas of different clinicopathological grades in the TCGA (n=1013) and CGGA (n=201) databases. $^{**}P < 0.01$. $^{***}P < 0.001$. $^{****}P < 0.0001$. (d) Expression features of *TSPAN9* in gliomas with IDH status in the TCGA (n=538) and CGGA (n=324) databases. $^{ns}P > 0.05$. $^{****}P < 0.0001$. (e) Protein levels of TSPAN4 were analyzed in human glioma patient samples (n=6) and normal peritumor brain tissues (n=6) using Western blot analysis. β-Actin was used as a loading control. $^{***}P < 0.001$. (f) Kaplan–Meier analysis for correlation between *TSPAN4* mRNA levels and survival of patients with gliomas in the TCGA (left) and CGGA (right) datasets.

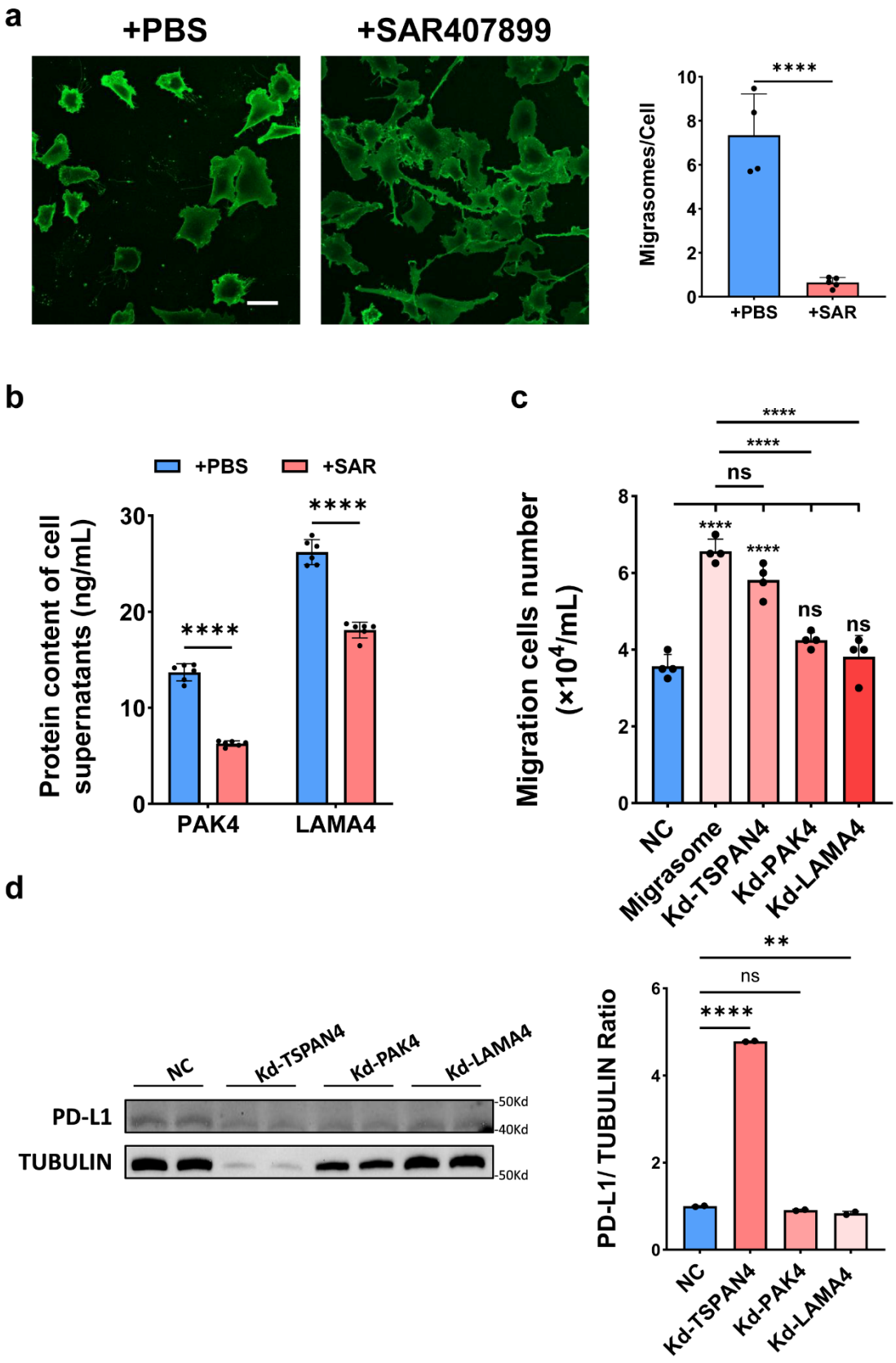

a
mRNA relative expression of TSPAN4
1.5
1.0
0.5
0.0
***
NC
Kd-TSPAN4-1
Kd-TSPAN4-2
Kd-TSPAN4-3
Kd-TSPAN4-4
b
Transwell Migration Assay
Transwell insert
Cell monolayer
Chemoattractant
Non-migrated cells
Migrated cells
Before migration
After migration
Transwell Invasion Assay
Transwell insert
Cell monolayer
Chemoattractant
Extracellular matrix
Non-invasive cells
invasive cells
Before invasion
After invasion
c
NC
Kd-TSPAN4
OD 450
2.0
1.5
1.0
0.5
0.0
***
24h
48h
72h
96h
d
NC
Kd-TSPAN4
H&E
e
TEM
WGA
f
Non-migrated cells
Migrasome
Migrated cells

Supplementary Figure 3. Enhancement of GBM migration and invasion by migrasomes. (a) TSPAN4 mRNA levels were detected by qRT-PCR. ***$P < 0.001$. (b) Schematic representation of the Transwell experiment. (c) Cell proliferation was assessed at various time points following *TSPAN4* knockdown using the CCK-8 assay. ***$P < 0.001$. (d)Representative hematoxylin and eosin-stained sections of mouse brain after inoculation with NC or Kd-TSPAN4 cells; Scale bar, 1 mm. (e) Left: TEM image of migrasomes isolated from LN229 cells; scale bar, 0.2 μm**.** Right: Confocal image of purified migrasomes stained by WGA; scale bar, 10 μm. (f) Schematic diagram of the purified migrasome rescue experiment.



Supplementary Figure 4. ECM-related factors contained in GBM migrasomes. (a) Differential mRNA levels of *PAK4* in GBM (*n*=528) and normal tissues (*n*=10), as well as in GBM of different pathological grades (*n*=620) based on TCGA datasets. (b) Differential mRNA levels of *LAMA4* n GBM (*n*=528) and normal tissues (*n*=10), as well as in GBM of different pathological grades (*n*=620) based on TCGA datasets. (c) Knockdown of *PAK4* in LN229 cells. LN229 were transfected with lentivirus. Western blot analysis of the abundances of PAK4 after LN229 cells were transfected. GAPDH was used as a loading control. **P* < 0.05, **** *P*< 0.0001. (d) Knockdown of *LAMA4* in LN229 cells. LN229 were transfected with lentivirus.

Western blot analysis of the abundances of LAMA4 after LN229 cells were transfected. β-actin was used as a loading control. ***$P < 0.001$.



Supplementary Figure 5. Promotion of GBM migration by migrasomes via PAK4 and LAMA4. (a) Cells were treated with PBS and SAR407899, followed by labeling of migrasomes with WGA and visualization with confocal microscopy.

The number of migrasomes was quantified. Scale bar, 50 μm. ****P < 0.0001. (b) Quantification of PAK4/LAMA4 was released into the supernatant after treating the cells with PBS and SAR407899. ****P < 0.0001. (c) The number of cells that migrated to the lower chamber was quantified by counting the cells in suspensions from the lower chamber of the Transwell. $^{ns}P > 0.05$. ****P < 0.0001. (d) The protein levels of PD-L1 were analyzed using Western blot analysis. Tubulin was used as a loading control. *P < 0.05. $^{ns}P > 0.05$, **P < 0.01, ****P < 0.0001.

Supplementary Table 1. The primer sequences and shRNA sequences.

primer sequences	
TSPAN4-FP	5'-GTACCTGACTCCTGCTGCTT-3'
TSPAN4-RP	5'-AGCAGGTTCTCCTGAAGC-3'
LAMA4-FP	5'-AAGCAGAGTCTCTGTGATGGCAG-3'
LAMA4-RP	5'-GTCCTGTTCAACTCGATGAAAGC-3'
PAK4-FP	5'-ATCTGGTCGCTGGGGATAATG-3'
PAK4-RP	5'-CAGGTTGTCCCGAATCATCTTC-3'
shRNA sequences	
Sh-TSPAN4	CCGGGGACAAGATTGACAGGTATGCCTCGAGGCATACCTG TCAATCTTGTCCTTTTTG
Sh-PAK4	CCGGGACCAGCACGAGCAGAAGTTCCTCGAGGAACTTCTG CTCGTGCTGGTCTTTTTG
Sh-LAMA4	CCGGGCCTAAAGCAAGTCAGAATAACTCGAGTTATTCTGAC TTGCTTTAGGCTTTTTG
scrambled negative control	CCGGTTCTCCGAACGTGUCACGTTTCTCGAGAAACGTGAC ACGTTCGGAGAATTTTTG

Fig. 2D

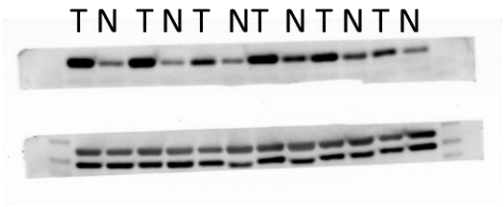


Fig. S2F

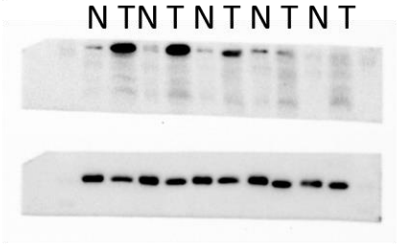


Fig2

Fig. 3A

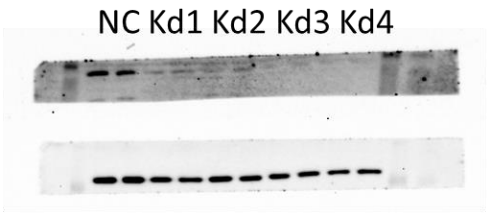
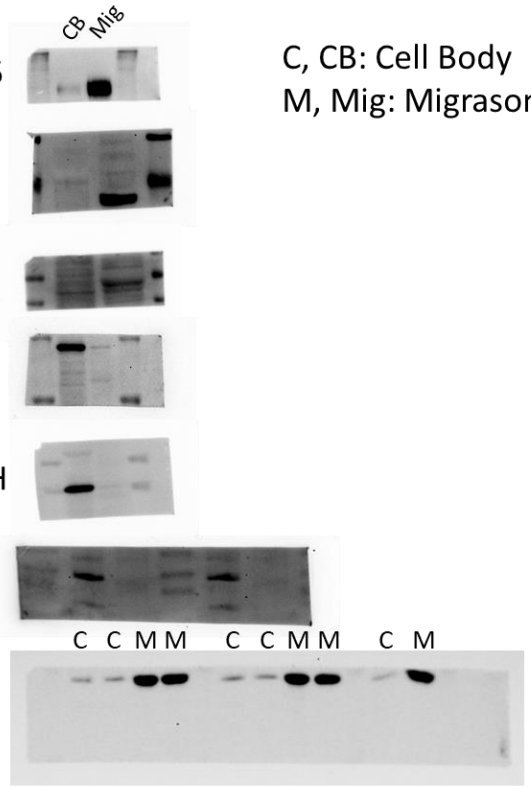


Fig3

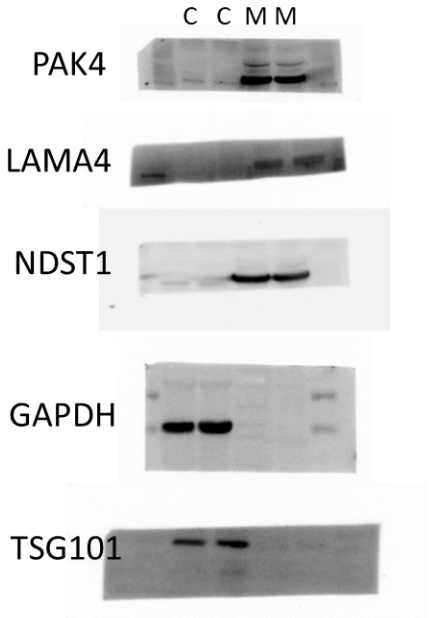
Fig. 4C



C, CB: Cell Body
M, Mig: Migrasome

Fig4

Fig. 5E



C: Cell Body
M: Migrasome

Fig5

Fig. S4C

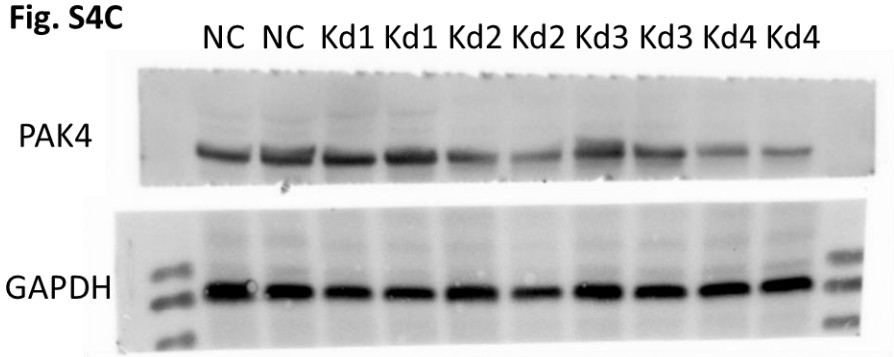
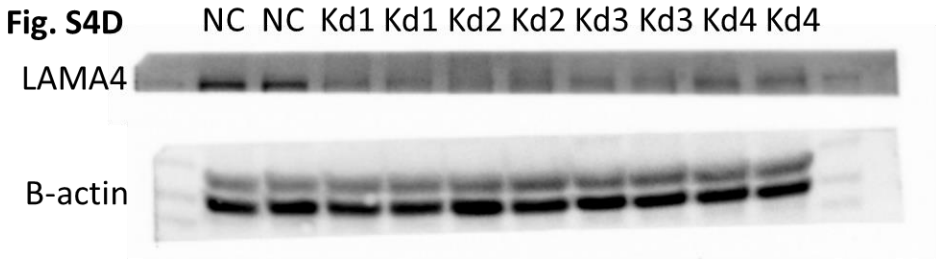


Fig. S4D



FigS4

Fig. S5D