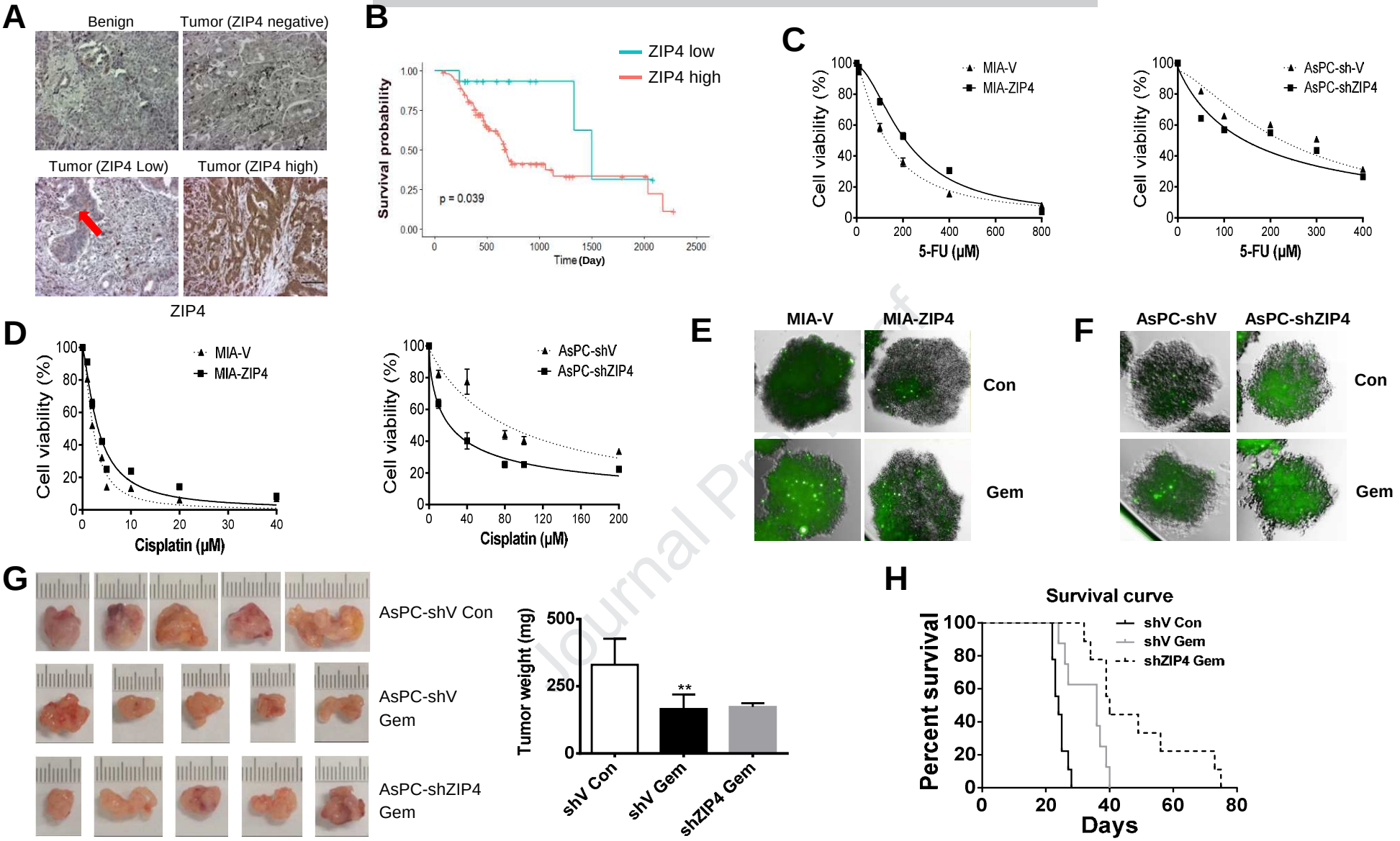
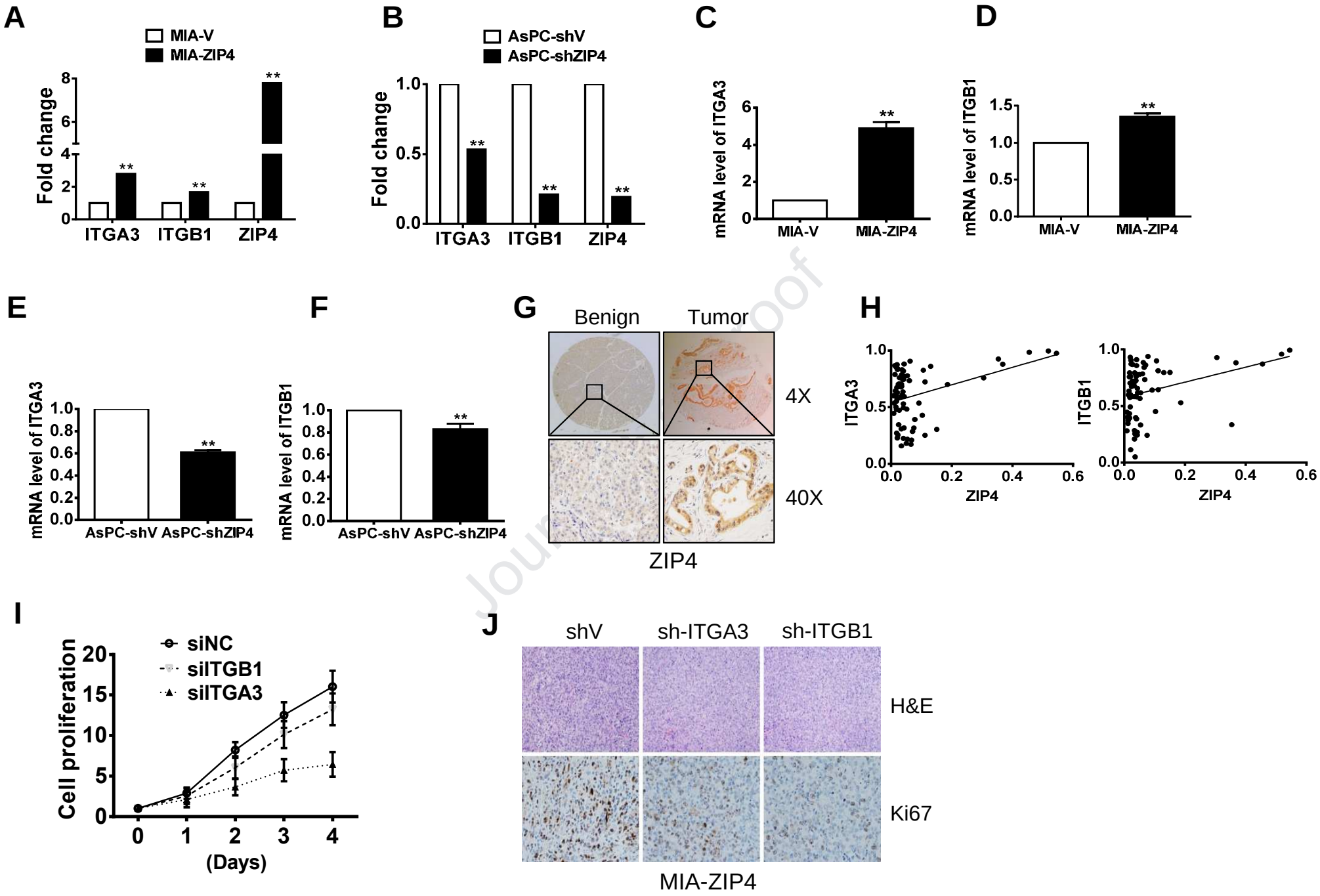
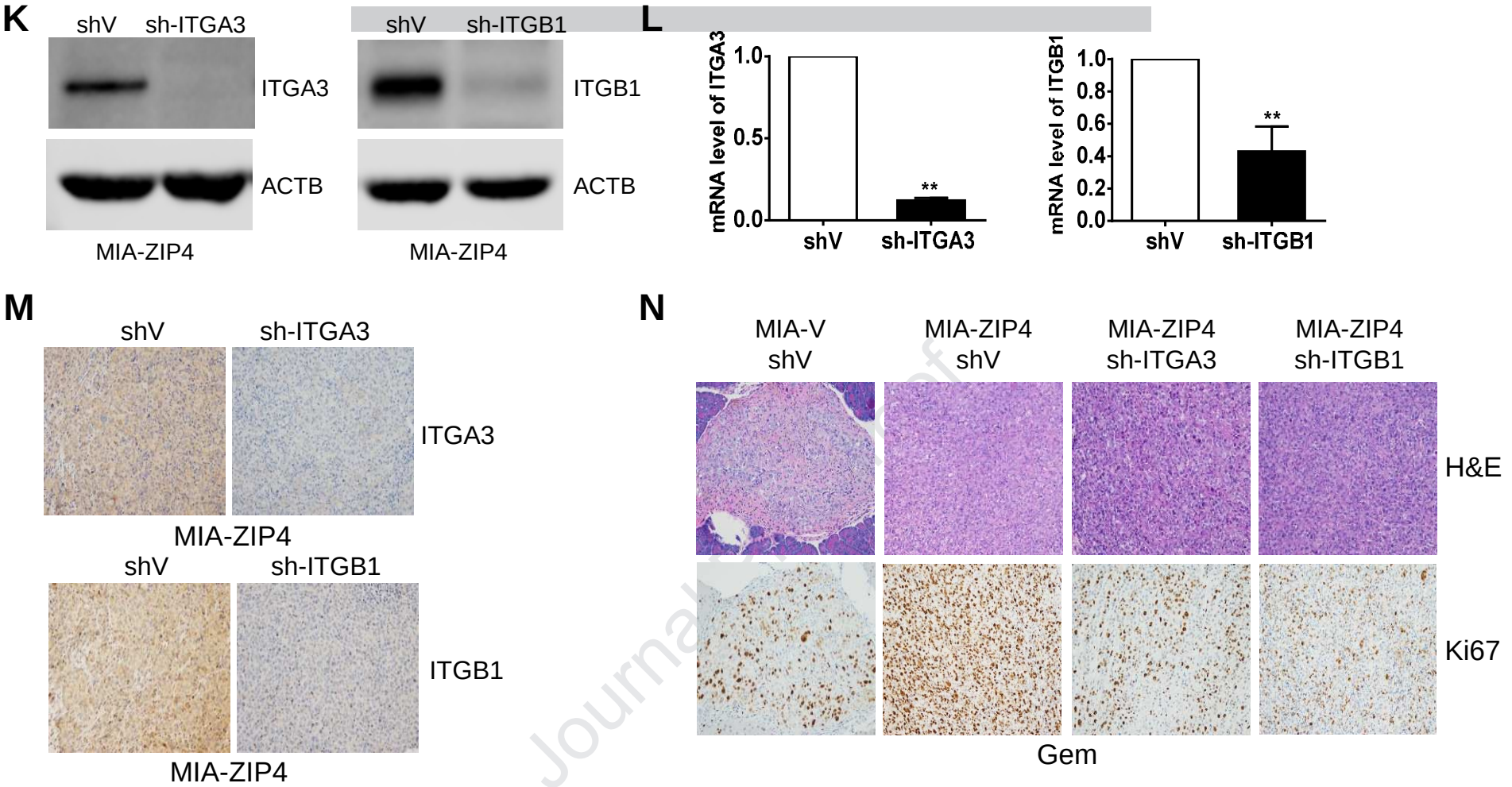


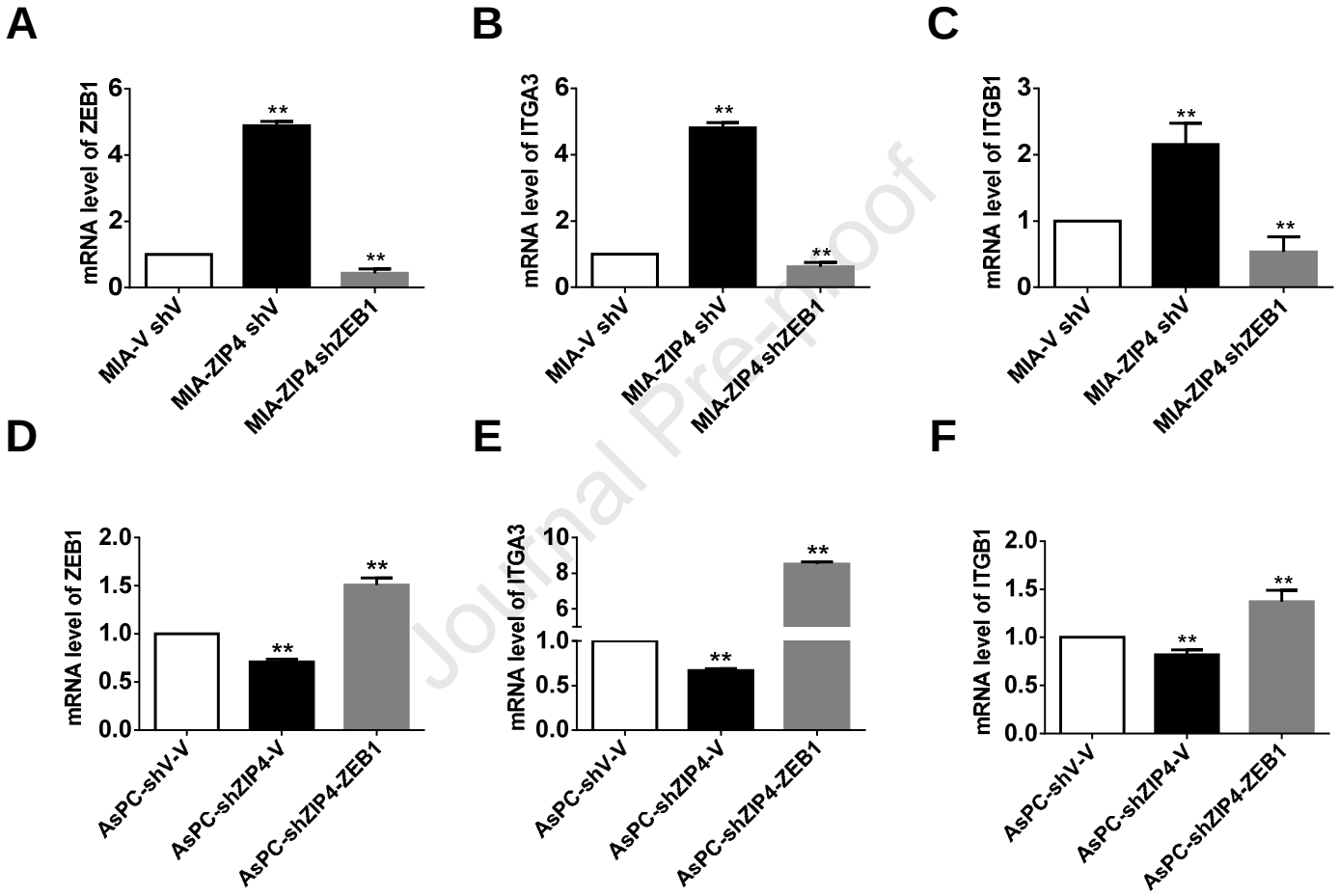
Supplementary Fig. S1. ZIP4 predicts survival and promotes pancreatic cancer cell growth and chemoresistance to gemcitabine. (**A**). Representative image of ZIP4 staining in 93 human pancreatic cancer specimens and adjacent benign tissues. Red arrow indicates positive signal. (**B**). Correlations between ZIP4 and survival time were analyzed in 117 pancreatic cancer patients from TCGA database using Kaplan-Meier survival analysis (*P*= .039). The optimal cut-off value of ZIP4 was determined by the maximally selected rank statistics using R package "maxstat". (**C**). Cell viability of pancreatic cancer cells following 5-FU treatment for 48h. (**D**). Cell viability of pancreatic cancer cells following cisplatin treatment for 48h. (**E-F**). TUNEL staining in spheroids derived from MIA PaCa-2 and AsPC-1 cells under gemcitabine treatment. (**G**). Orthotopic tumor growth (*n*=5) (*P*= *.01*). (**H**). Survival curve (*n=9*).



Supplementary Fig. S2. (continued on next page, legend follows)



Supplementary Fig. S2. ZIP4 enhances pancreatic cancer growth and gemcitabine resistance through integrin α3β1. (**A-B**). Fold change of ITGA3, ITGB1 and ZIP4 expression from Fig. 2A. (**C-D**). mRNA level of ITGA3 and ITGB1 in MIA-ZIP4 cells and (**E-F**). AsPC-shZIP4 cells ($P< .05$). (**G**). Representative image of ZIP4 staining in human pancreatic cancer and adjacent benign tissues. (**H**). Correlations between ZIP4 and ITGA3 or ITGB1 positivity in human pancreatic cancer tissues ($R= .14$ and $.11$ separately). (**I**). Cell proliferation assay in MIA-ZIP4 cells with ITGA3 or ITGB1 blocked. (**J**). H&E and Ki67 staining of xenograft tumor tissues from MIA-ZIP4 sh-ITGA3 and MIA-ZIP4 sh-ITGB1 groups ($n=5$). The scale bar is 100 μm. (**K**). Protein level of ITGA3 and ITGB1 in MIA-ZIP4 sh-ITGA3 and MIA-ZIP4 sh-ITGB1 cells. (**L**). mRNA level of ITGA3 and ITGB1 in MIA-ZIP4 sh-ITGA3 and MIA-ZIP4-ITGB1 cells. (**M**). Protein level of ITGA3 and ITGB1 in xenograft tumor tissues. The scale bar is 100 μm. (**N**). H&E and Ki-67 staining of xenograft tumor tissues from MIA-ZIP4 sh-ITGA3 and MIA-ZIP4 sh-ITGB1 groups ($n=5$). All the mice were treated with gemcitabine for 5 weeks. The scale bar is 100 μm.



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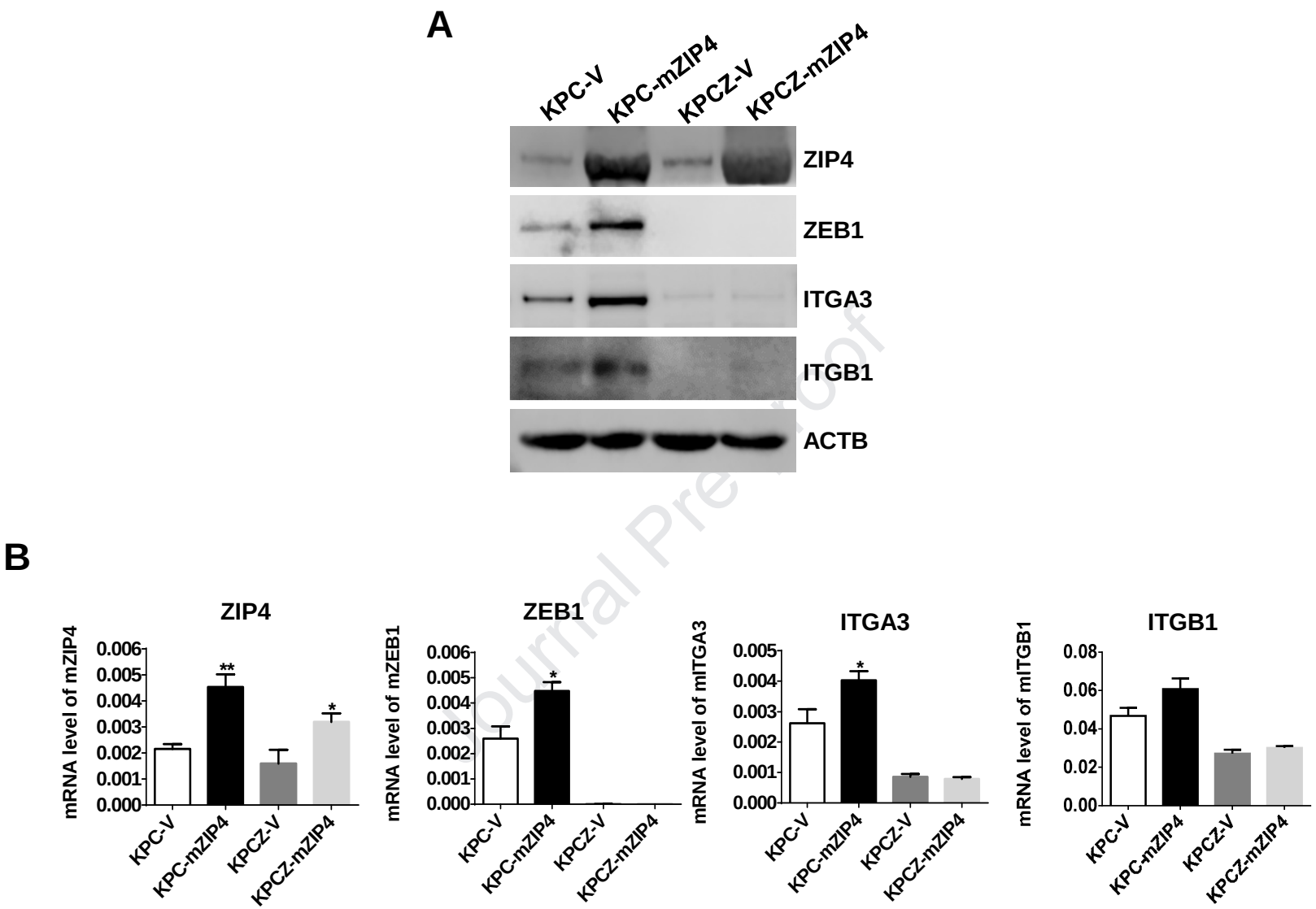
Supplementary Fig. S3. ZIP4 upregulated integrin α3β1 expression is dependent on the EMT like transcription factor ZEB1 in pancreatic cancer cells. (**A-C**). mRNA level of ZEB1, ITGA3 and ITGB1 in MIA-V shV. MIA-ZIP4 shV and MIA-ZIP4 shZEB1 cells (*P*< .05). (**D-F**). mRNA level of ZEB1, ITGA3 and ITGB1 in AsPC-shV-V, AsPC-shZIP4-V, AsPC-shZIP4-ZEB1 cells. (*P*< .05).



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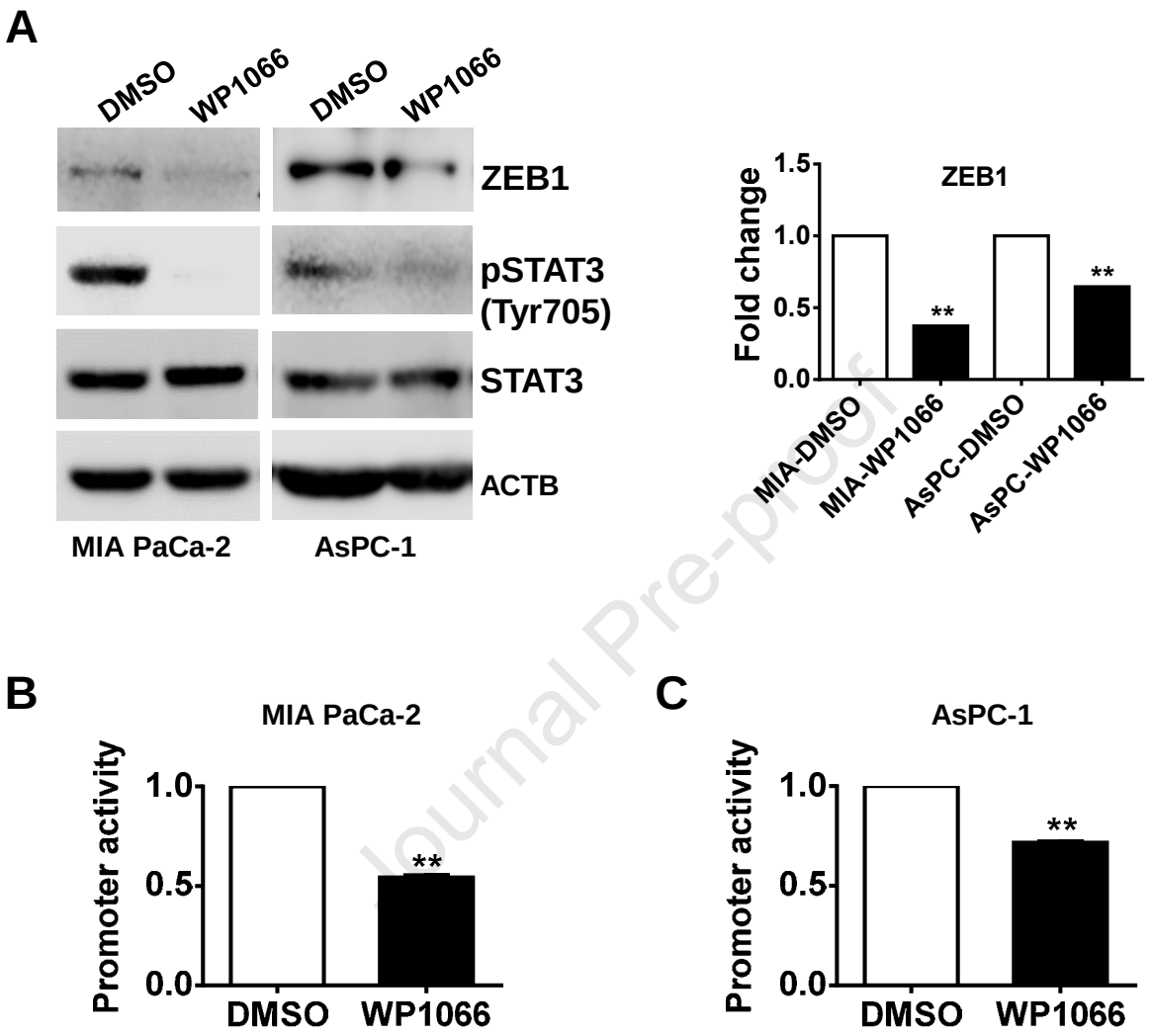
Supplementary Fig. S4. ZEB1 is indispensable for integrin α3β1 upregulation in KPC mouse derived cell lines. (**A**). Protein level of ZIP4, ZEB1, ITGA3 and ITGB1 in KPC-V, KPC-mZIP4, KPCZ-V, KPCZ-mZIP4 cell lines. (**B**). mRNA level of ZIP4, ZEB1, ITGA3 and ITGB1 in KPC-V, KPC-mZIP4, KPCZ-V, KPCZ-mZIP4 cell lines.



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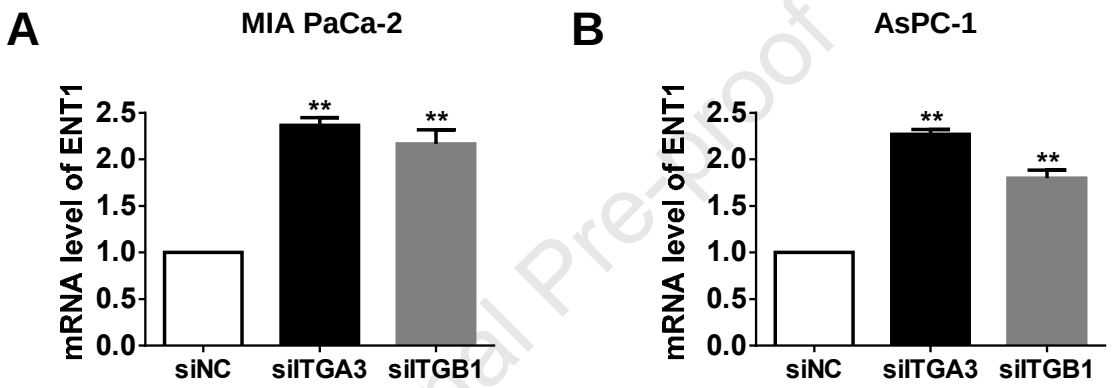
Supplementary Fig. S5. ZIP4 upregulates ZEB1 through activating STAT3 in pancreatic cancer cells. (**A**). Protein level of STAT3 and ZEB1 in MIA PaCa-2 and AsPC-1 cells treated with 10 μM WP1066 (STAT3 inhibitor) for 48h. (**B-C**). Promoter activity of ZEB1 in MIA PaCa-2 and AsPC-1 cells treated with10μM WP1066 (STAT3 inhibitor) for 48h.



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Supplementary Fig. S6. (**A-B**). mRNA level of ENT1 in MIA-PaCa-2 and AsPC-1 cells with ITGA3 and ITGB1 blocked with siRNA.

Supplementary Methods

Cell viability assay.

Pancreatic cancer cells were seeded in 96-well plates and treated with various concentrations of gemcitabine for 2 days. Cell viability was detected with 2 μl alamarblue (Bio-Rad) reagent mixed with 98 μl 10% FBS medium. Absorbance was recorded at 560, 590 nm with microplate reader (Bio-Tek Instruments).

ChIP assay.

The chromatin immunoprecipitation assay was performed in MIA PaCa-2 and AsPC-1 cells by using the anti-STAT3 and anti-ZEB1 antibody (Cell Signaling) with the MAGnif Chromatin Immunoprecipitation System (Life Technologies) following the standard protocol.

Promoter activity assay.

The 930 bp promoter region of ITGA3 and 1.3 kb promoter region of ITGB1 were cloned into pGL4.10□basic reporter vector. The ITGA3 and ITGB1 promoter reporter vectors were cotransfected with control plasmid pRL-TK into MIA PaCa-2 and AsPC-1 cells. ZEB1 and ENT1 promoter vectors were purchased from Genecopoeia and those two promoter reporter vectors were transfected into MIA PaCa-2 and AsPC-1 cells. The promoter activity was determined by a Dual-Luciferase Reporter Assay (Promega).

Immunohistochemical (IHC) staining.

Human pancreatic adenocarcinoma and surrounding benign tissues were collected and processed into 5-μm slides. Fixed tissue slides were incubated in 3% hydrogen peroxide solution to quench endogenous peroxidase activity for 15 m, and incubated in blocking buffer for 30 m at room temperature and stained with anti-hZIP4 antibody (Proteintech, 1:500), ITGA3 (Proteintech, 1:200),

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ITGB1 (Proteintech, 1:200) and incubated overnight at 4°C. After washing with PBS, the section was incubated with polymer secondary antibody for 30 m (Vector Laboratories). Immune complexes were detected with diaminobenzidine (DAB) under a phase-contrast microscope. The sections were then mounted and observed under a phase-contrast microscope. The IHC staining slides were scanned using Aperio scanning software and the positivity was generated automatically. The correlations between ZIP4, ITGA3, and ITGB1 were analyzed using GraphPad Prism software.

TUNEL staining.

Spheroids derived from MIA-V, MIA-ZIP4, AsPC-shV, AsPC-shZIP4 cells were fixed in 24 well plate with 4% PFA and then incubated in permeabilization solution for 2 m on ice. The spheroids were resuspended in 50μl/well TUNEL reaction mixture (In Situ Cell Death Detection Kit, Roche) and incubated for 60 m at 37°C in a humidified atmosphere in the dark. Samples in 24 well plate were washed and analyzed under a fluorescence microscope using an excitation wavelength in the range of 520-560 nm (green) and detection in the range of 570-620 nm (red).

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Supplementary Table 1. IC50 of gemcitabine in pancreatic cancer cell lines

	MIA-V	MIA-ZIP4	AsPC-shV	AsPC-shZIP4
Gemcitabine (nM)	15.6±1.549	32.68±3.554	173.3±20.23	91.68±7.818

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Supplementary Table 2. IC50 of 5-FU and cisplatin in pancreatic cancer cell lines

IC50 of 5-FU and cisplatin	MIA-V	MIA-ZIP4	AsPC-shV	AsPC-shZIP4
5-FU(μM)	129.6±2.749	216.6±6.340	241.6±17.67	153.6±18.61
Cisplatin(μM)	2.219±0.094	3.230±0.198	84.21±7.933	21.54±1.565

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