Supplementary

Table S1 Lists of oligonucleotides used in this study

Name	Sequences (5'-3')	Purpose
hPAIP1-F	ATTGCTGAATGCCCTGTTT	siRNA
hPAIP1-R	GGATTATCCTACT CTATCA	siRNA
sh*PAIP1*	CCGGGAATTGCTGAATGCCCTGTTTCTCG AGAAACAGGGCATTCAGCAA TTCTTTTT	lentivirus vector
PLK1-TurboID-F	CAACCGTCTCAAGGCCTCCGGCTCATCTGGCT CTAG AAAAGACAATACAGTGCCGCTC	expression vector
PLK1-TurboID-R	TAATACG ACTCACTATAGCTCGAGTCACAG ATCTTCCTCAGAGATGAGC	expression vector

Table S2 Lists of antibodies used in this study

Name	Cat. No.	Company
anti-PAIP1, rabbit	ab175211	Abcam
anti-PLK1, rabbit	20894	Cell Signaling Technology
anti-Caspase-3, rabbit	A19664	ABclonal
anti-Caspase-7, rabbit	A1524	ABclonal
anti-JUN, rabbit	ab32137	Abcam
anti-ITGA2, rabbit	ab133557	Abcam
anti-Skp2, rabbit	ab183039	Abcam
anti-CDKN1B, rabbit	ab32034	Abcam
anti-GAPDH, mouse	sc-32233	Santa Cruz Biotechnology
anti-beta-Actin, mouse	6008-1-Ig	Proteintech
HRP-conjugated goat anti-rabbit IgG	7074	Cell Signaling Technology
HRP-conjugated goat anti-mouse IgG	7076	Cell Signaling Technology

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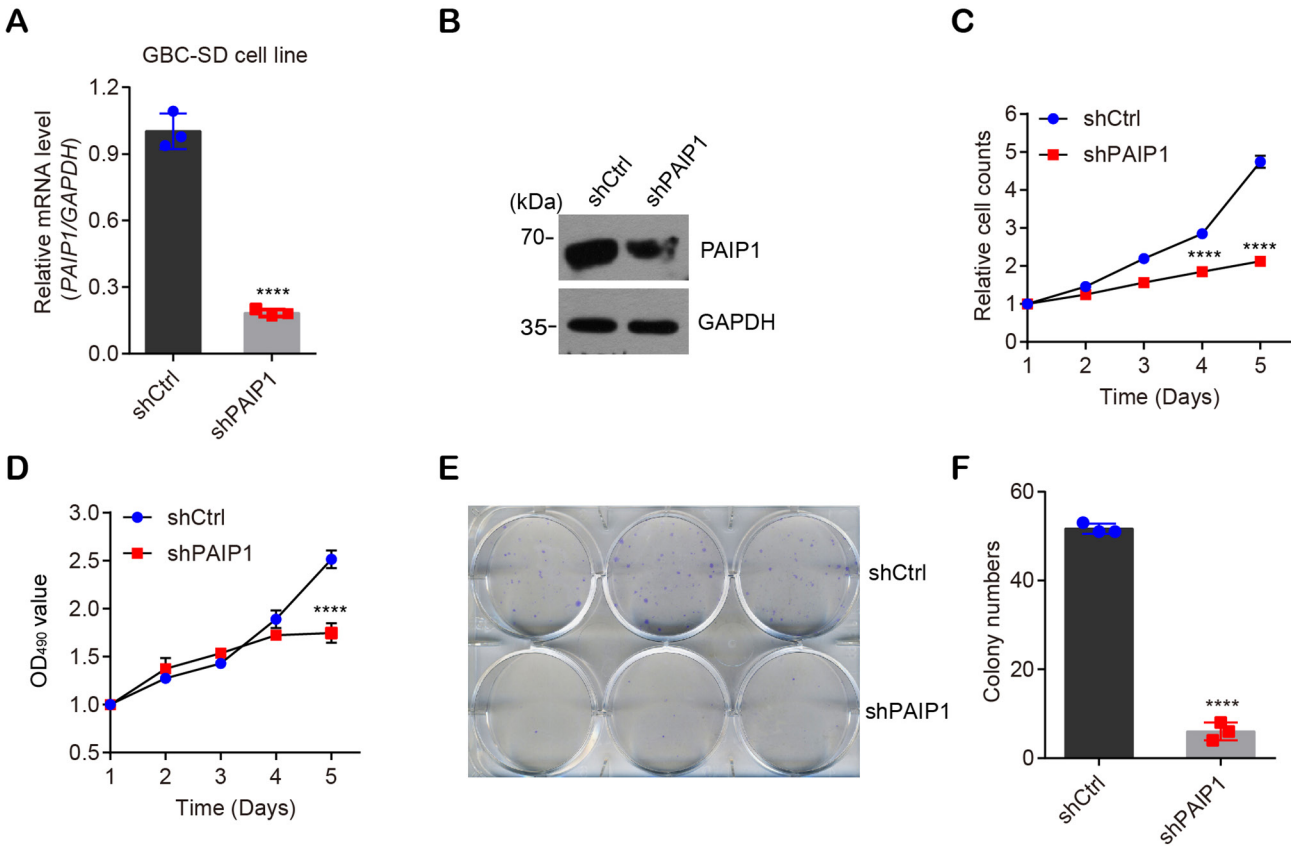

Figure S1 Reduction of *PAIP1* expression suppressed gallbladder GBC-SD cell growth and colony formation in vitro. (A,B) PAIP1 mRNA or protein levels in GBC-SD cells infected with sh*Ctrl* or sh*PAIP1* lentivirus construct were examined by qPCR (A) and western blotting (B), respectively. (C,D) Knockdown of *PAIP1* retarded cell proliferation, as evidenced through cell count numbers observed over time (C) and measurements of optical density values using the CCK-8 kit (D). (E,F) Colony formation assays were performed upon knockdown of *PAIP*. The representative images from 3 independent plates are displayed by staining with methylthionine chloride shown in (E), and the quantifications of colony numbers are plotted (F). Data are presented as mean ± SD. ****P<0.0001.

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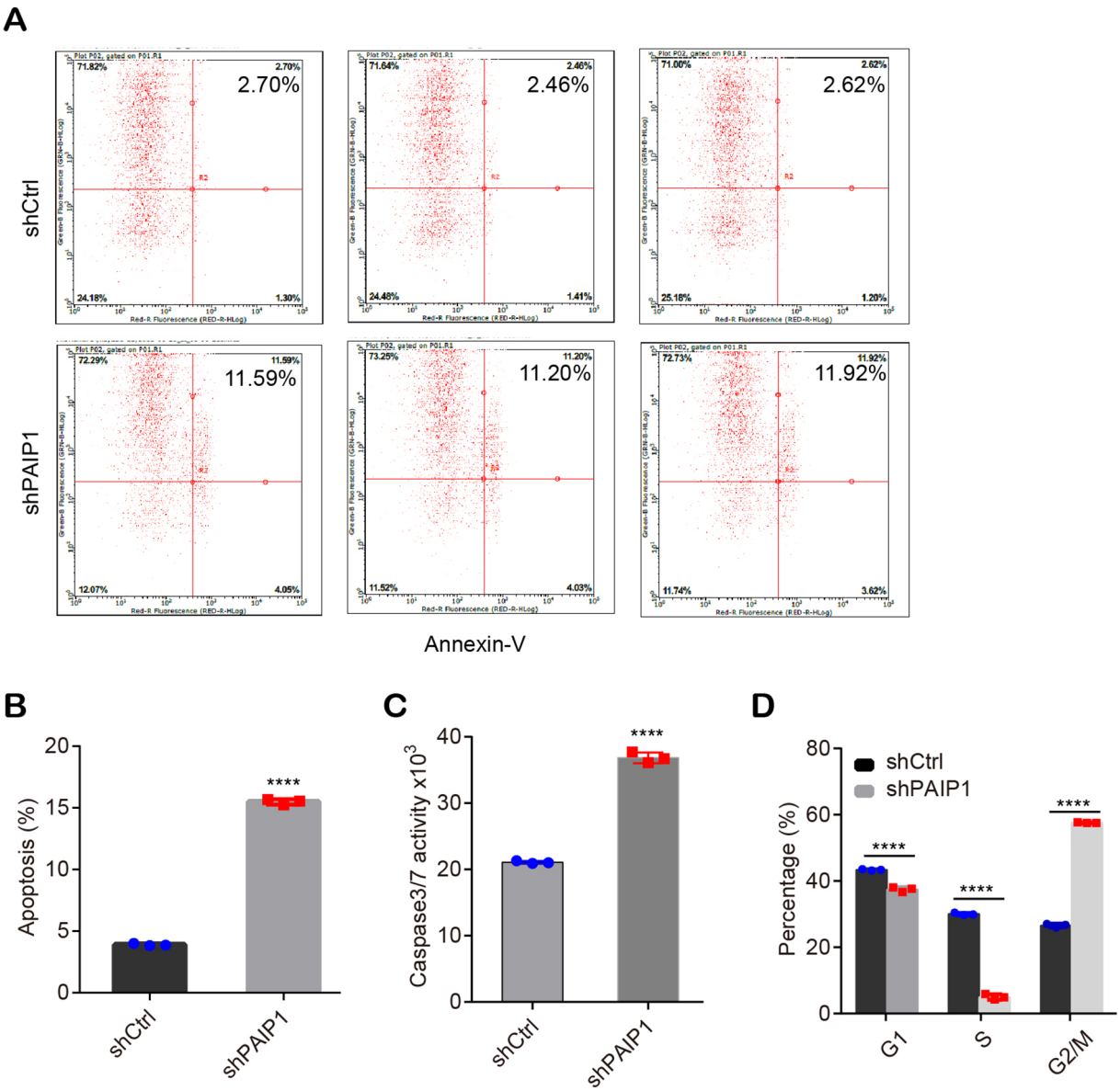

Figure S2 Reduction of *PAIP1* expression promoted gallbladder GBC-SD cell apoptosis and caused cell cycle arrest. (A,B) Flow cytometry analysis was performed to examine the percentage of apoptotic cells upon knockdown of *PAIP1*. The numbers in the top right corner represent apoptotic cells. Three independent images in each group are shown (A), and quantitative data are plotted (B). (C) Caspase-Glo 3/7 assays were performed in cells expressing sh*Ctrl* or sh*PAIP1*. The caspase 3/7 activity was measured by luminescence. (D) Flow cytometry was performed to examine the cell populations at different cell cycle stages. Data are presented as mean ± SD. ****P<0.0001.

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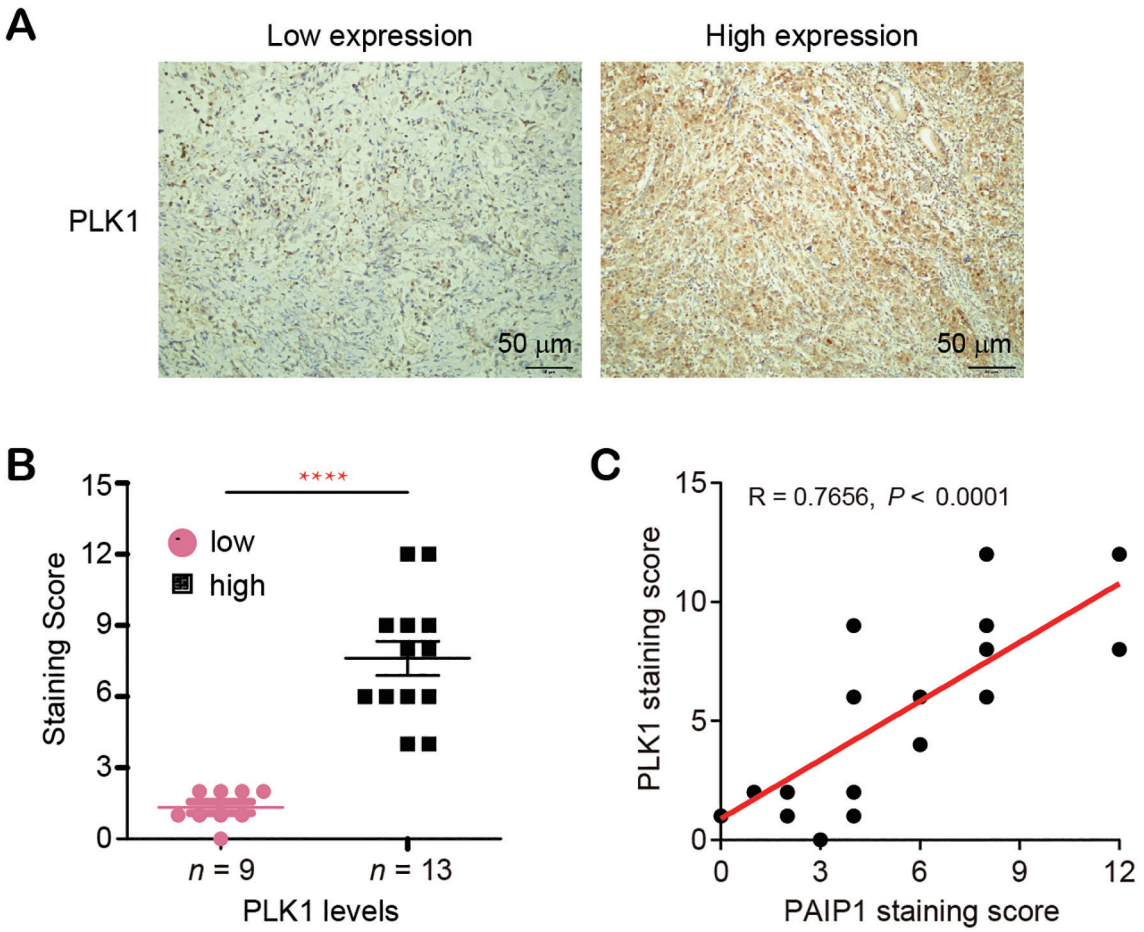

Figure S3 PLK1 levels were positively correlated with PAIP1 levels in GBC patient tissues. (A,B) The protein levels of PLK1 from different GBC patients were examined by IHC staining. The representative images with low or high expression of PLK1 are shown (A). Scores of PLK1 staining in different types of GBC tissue samples are plotted (B). Data are presented as mean ± SD. ****P<0.0001. (C) The relative expression levels of PAIP1 were plotted with the relative expression levels of PLK1 in 22 GBC tissue samples evaluated by IHC staining. The correlation coefficient R value and statistical P value are shown.

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