

Supplementary Information

Manuscript:

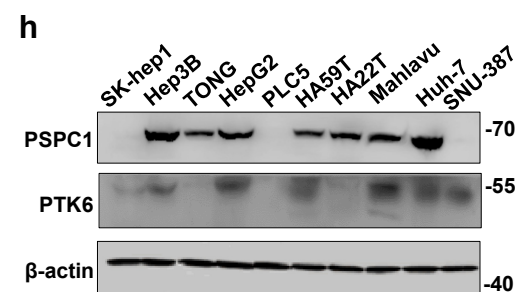
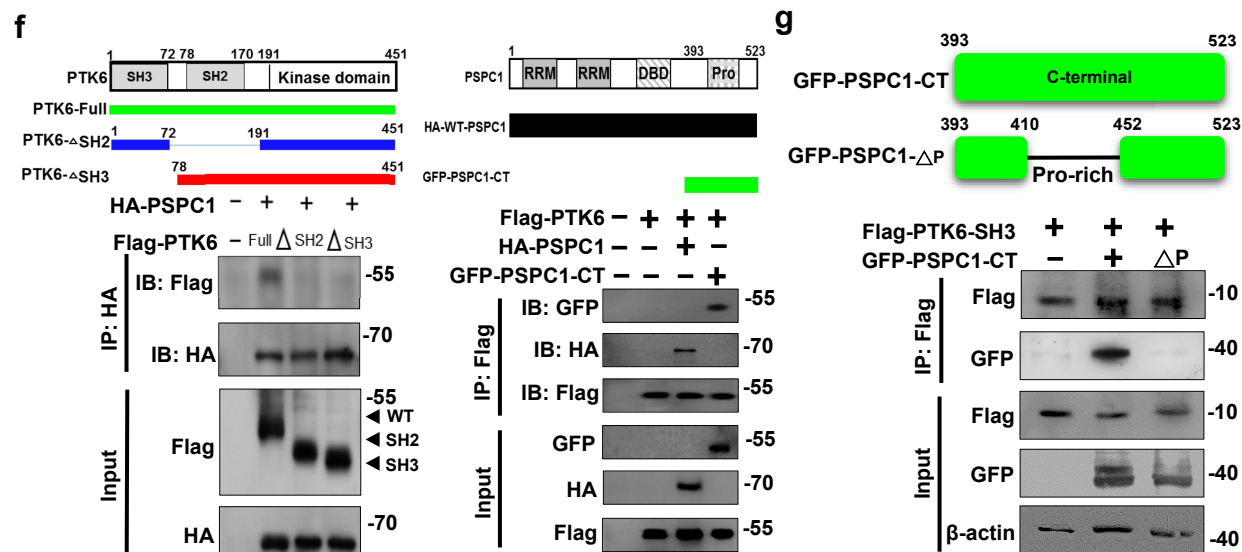
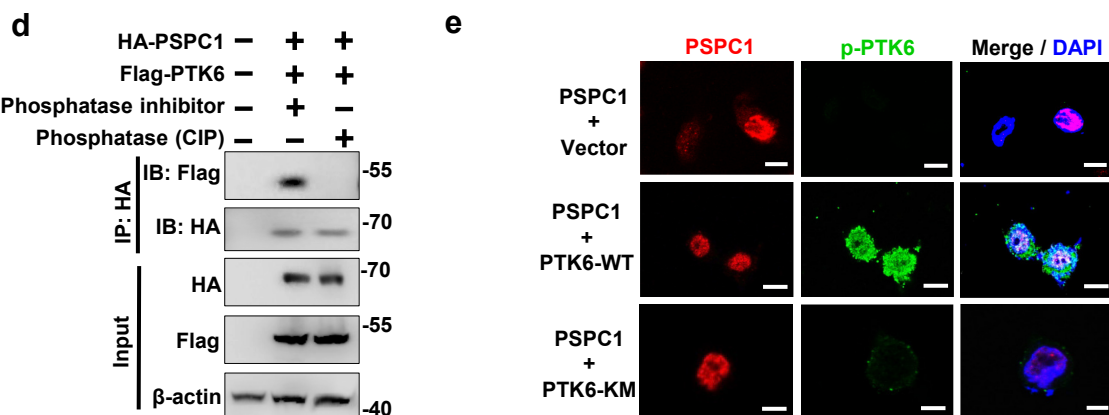
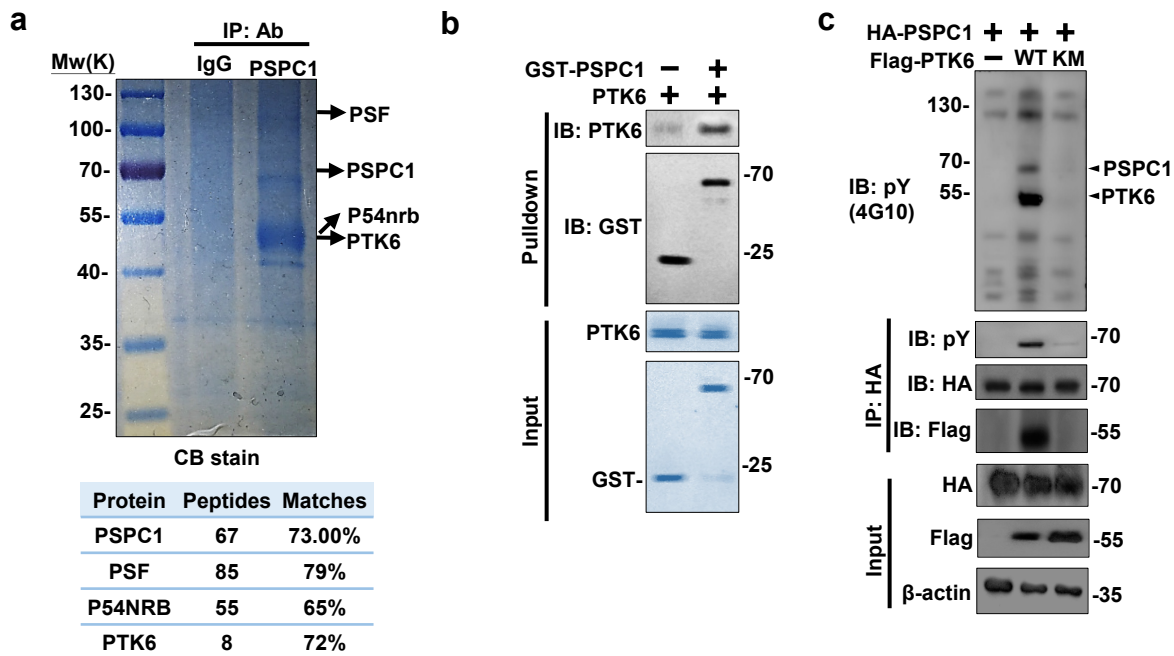
PSPC1-interchanged interactions with PTK6 and β -catenin synergize oncogenic subcellular translocations and tumor progression

Lang et al.,

Contents:

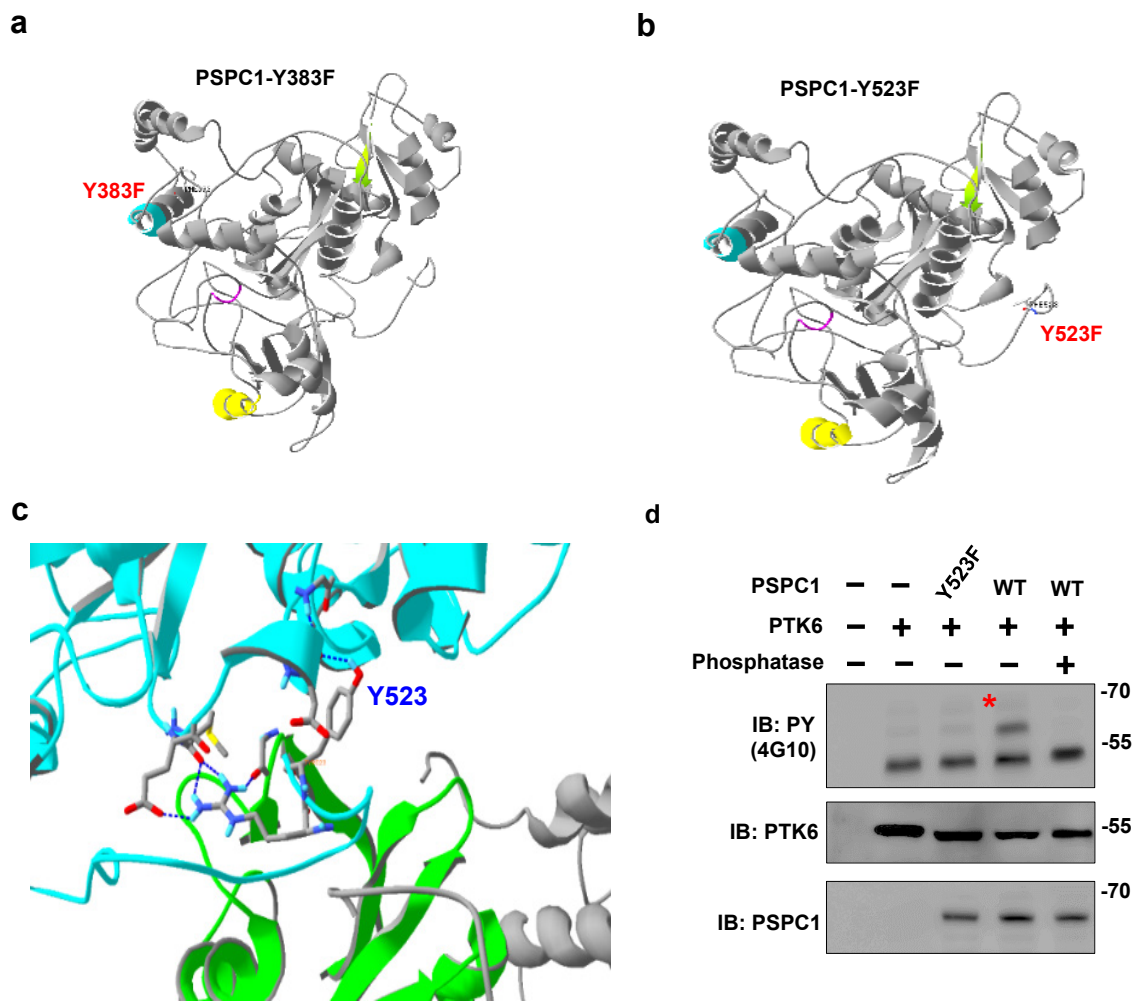
Supplementary Figures 1-8

Supplementary Tables 1-5



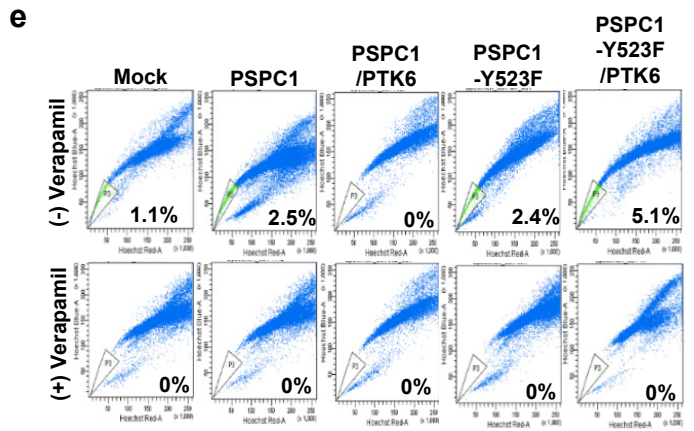
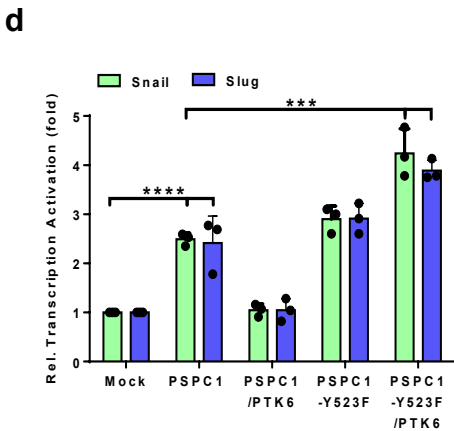
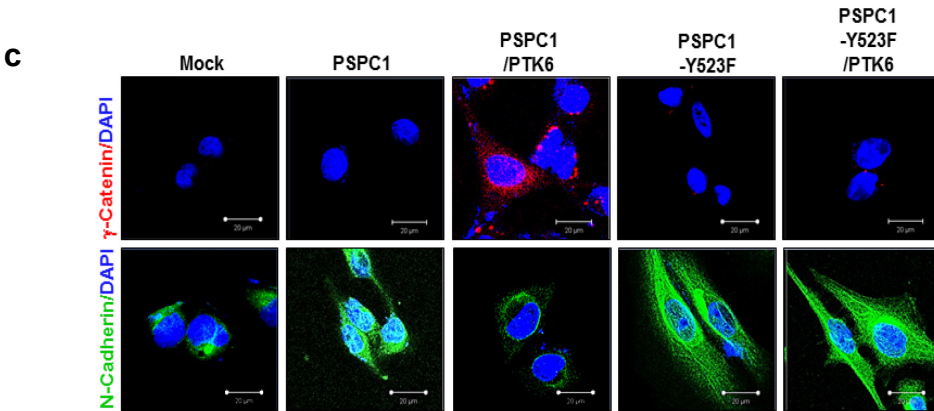
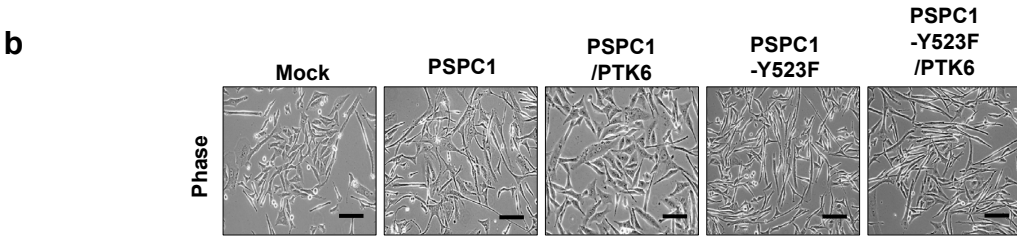
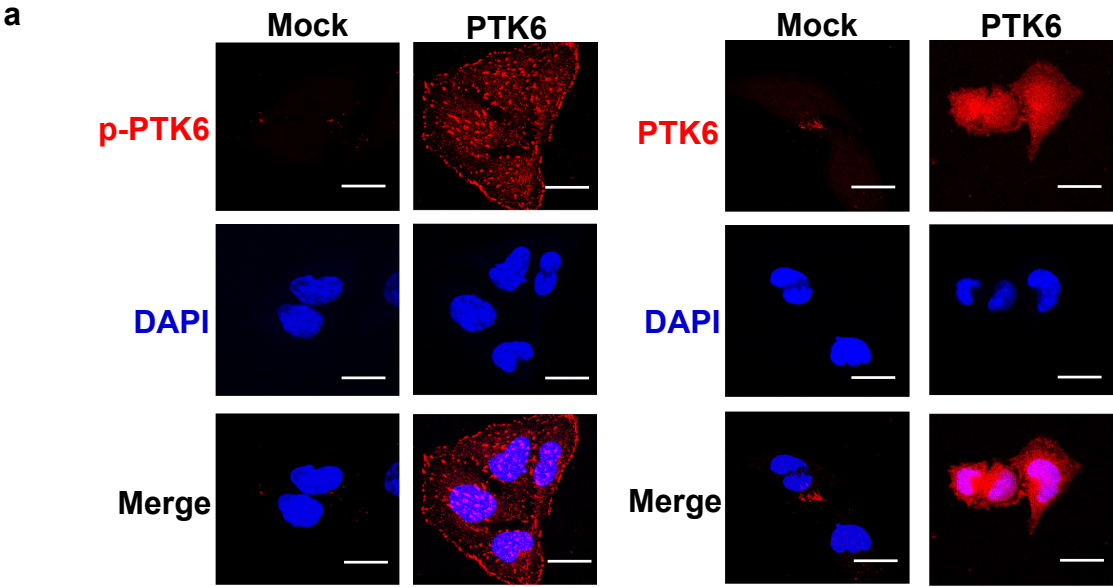
Supplementary Figure 1 Interactions between PSPC1 and PTK6 via the C-terminal proline-rich domain of PSPC1 and the SH2 and SH3 domains of PTK6.

a Coomassie blue staining of the PSPC1 immunoprecipitated (IP) complex of Huh-7 cell lysates with anti-PSPC1 antibody separated by SDS-PAGE (upper panel). The three bands were excised and subjected to LC-MS/MS analysis. Preimmune IgG served as an IP control, and four interacting proteins were identified including matching percentage and the number of peptides (lower panel). Detailed information on IP peptides is included in Supplementary Table 1. **b** Direct interaction of PSPC1 and PTK6 by GST pull-down assay performed by using purified recombinant GST-PSPC1 and the active form of recombinant protein PTK6, followed by IB with anti-PTK6 antibody (upper panel). Coomassie blue staining indicates equal inputs of GST-PSPC1 and PTK6 (lower panel). **c** PSPC1 interacting with PTK6 is dependent on tyrosine kinase phosphorylation demonstrated by IP/Western blotting analysis with 4G10 antibody and indicated protein antibodies. HA-tagged PSPC1, Flag-tagged PTK6 WT and Flag-tagged PTK6 kinase-dead (KM) proteins were expressed in 293T cells. **d** Treatment with alkaline phosphatase (CIP) in contrast to treatment with a phosphatase inhibitor disrupted HA-tagged PSPC1 and Flag-tagged PTK6 protein interaction demonstrated via IP/Western analysis of SK-hep1 cells lysates. **e** IF analysis of subcellular localization of PSPC1 and PTK6 by expressing HA-tagged PSPC1 with wild-type or kinase-dead (KM) mutants of Flag-tagged PTK6 in SK-hep1 cells. Colors are PSPC1 (red), PTK6 (green) and nuclei (DAPI, blue). The scale bar represents 20 μm . **f, g**, Protein interaction domains of PSPC1 and PTK6. **(f)** Left: Top, schematic domain deletion constructs of PTK6. Bottom, the SH2 and SH3 domains are required for PSPC1 and PTK6 interaction demonstrated by expressing HA-tagged PSPC1 and coexpressing full-length and deletion constructs of Flag-tagged PTK6 in 293T cells and IP/Western analysis. Right: Top, schematic domain deletion constructs of PSPC1. Bottom, the proline-rich C-terminal domain of PSPC1 is required for PSPC1 and PTK6 interaction demonstrated by IP/Western analysis of 293T cells expressing Flag-tagged PTK6 and coexpressing full-length or GFP-tagged PSPC1 C-terminal (CT). **g** Top, schematic domain deletion constructs of GFP-tagged PSPC1 C-terminus (CT). The proline-rich C-terminal domain of PSPC1 is essential for PSPC1 and PTK6 interaction demonstrated by IP/Western analysis of 293T cells expressing Flag-tagged PTK6-SH3 and coexpressing GFP-tagged PSPC1-CT and the proline deletion construct of GFP-tagged PSPC1-CT. **h** Endogenous expression of PSPC1 and PTK6 proteins in ten hepatocellular carcinomas cell lines determined by Western blotting analysis. β -actin is the loading and internal control.



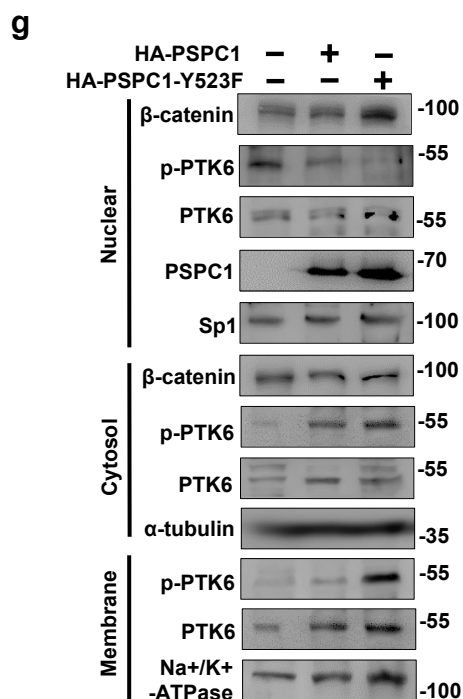
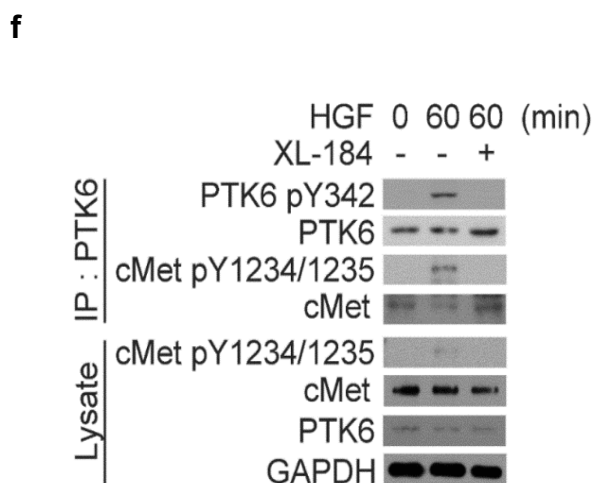
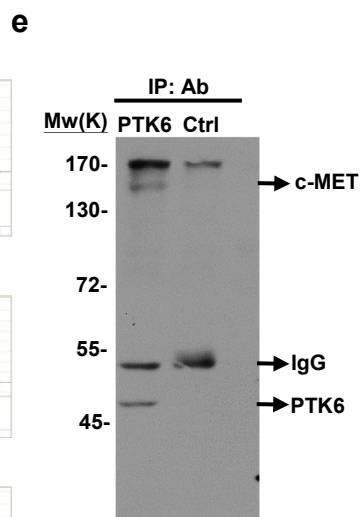
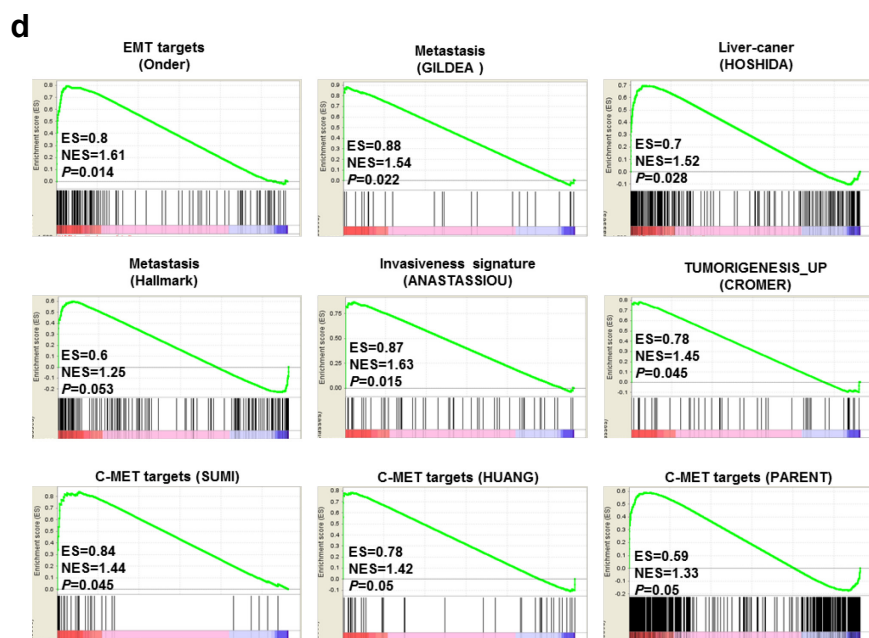
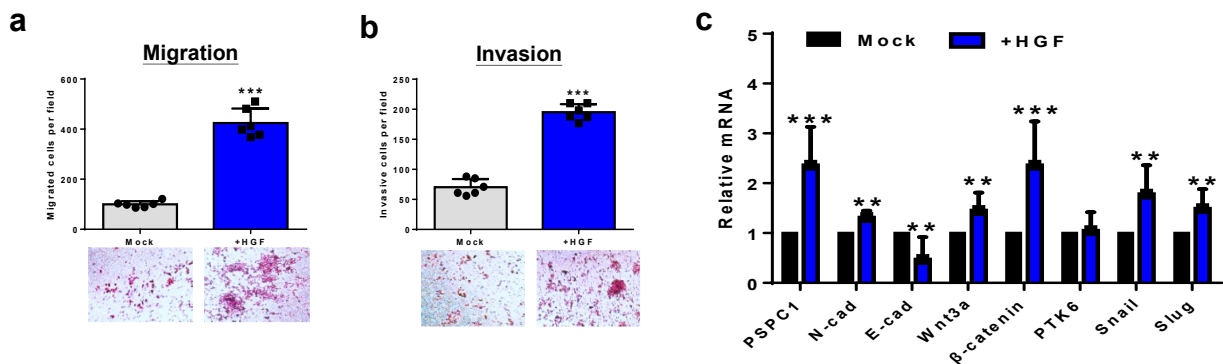
Supplementary Figure 2 The simulated protein structure prediction of the dynamic interactions of PSPC1, PSPC1-Y523F and PTK6.

a-c The predicted 3D protein structure of PSPC1 with tyrosine to phenylalanine mutations at Y383 (**a**) and Y523 (**b**) as well as the predicted docking interface of PSPC1 and PTK6 (**c**). The Y383F and Y523F mutations of PSPC1 have similar protein 3D structure changes (labeled in green/yellow/purple/cyan) compared to wild-type PSPC1. (**c**) The interface of the PSPC1 and PTK6 interaction. The left side (Caribbean blue color) is PSPC1, the right side (green color) is PTK6, and the blue dotted lines represent the hydrogen bonds near the interface. **d** *In vitro* kinase assays were performed by using anti-pY (4G10), anti-PSPC1 and anti-PTK6 antibodies. Purified recombinant PSPC1, PSPC1-YF (Y523F) and PTK6 proteins were incubated in kinase buffer. Asterisks denote the tyrosine-phosphorylated protein.



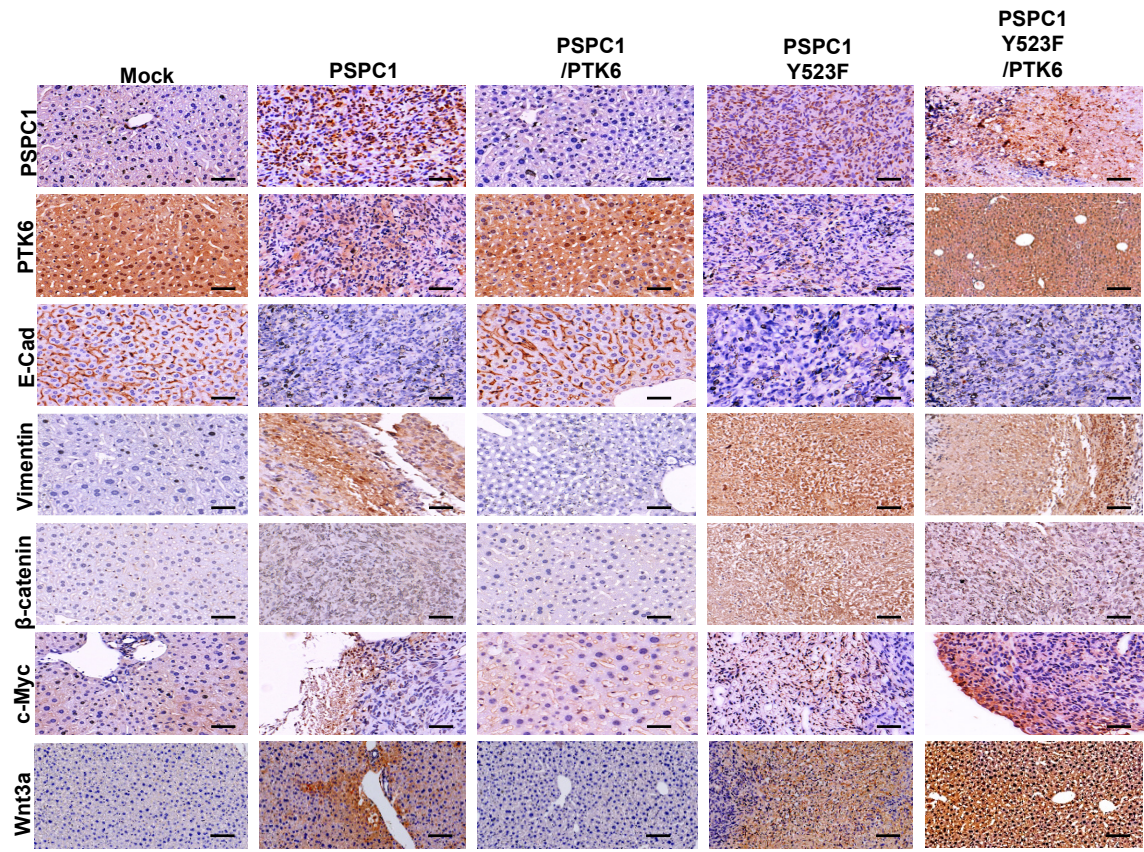
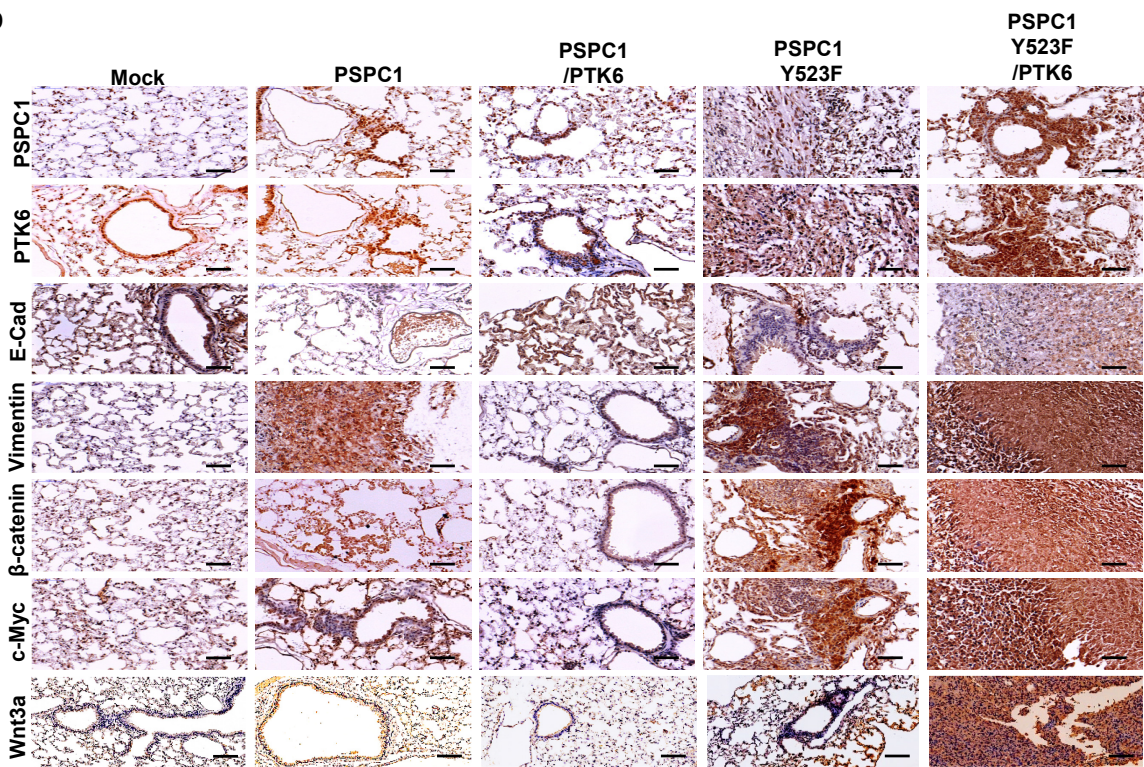
Supplementary Figure 3 PSPC1 deficient, upregulation and expression of the PSPC1-Y523F mutant modulated PTK6 from nuclear sequestration to synergize the oncogenic effects of PSPC1 and cytoplasmic PTK6.

a Immunofluorescence analysis for the expression of phospho-PTK6 (p-PTK6) and PTK6. SK-hep1 (deficient of PSPC1) cells were transfected with the indicated plasmids. Colors are p-PTK6 and PTK6 in red, and nuclei with DAPI in blue. The scale bar represents 25 μ m. **b** Analysis of cell morphology in phase image of the indicated PSPC1/PTK6 SK-hep1 cell transfectants. The scale bar represents 50 μ m. **c** The expression of epithelial (r-catenin) and mesenchymal markers by IF in the indicated PSPC1/PTK6 SK-hep1 cell transfectants. Colors are as follows: r-catenin (red), N-cadherin (green) and nuclei (DAPI, blue). The scale bar represents 20 μ m. **d** Luciferase activity of Snail and Slug promoter reporters after cotransfection of different expression constructs. The luciferase activity/ β -galactosidase of 293T cells cotransfected with the pcDNA3 control vector was used as the baseline control. Data are represented as the mean $\pm$ SEM (n=3). All data statistics were based on *p < 0.05, **p < 0.01, and ***p < 0.001 calculated by Brown-Forsythe test. **e** Flow cytometry analyses of the side population fraction of PSPC1/PTK6 SK-hep1 cell transfectants in the presence and absence of Verapamil.



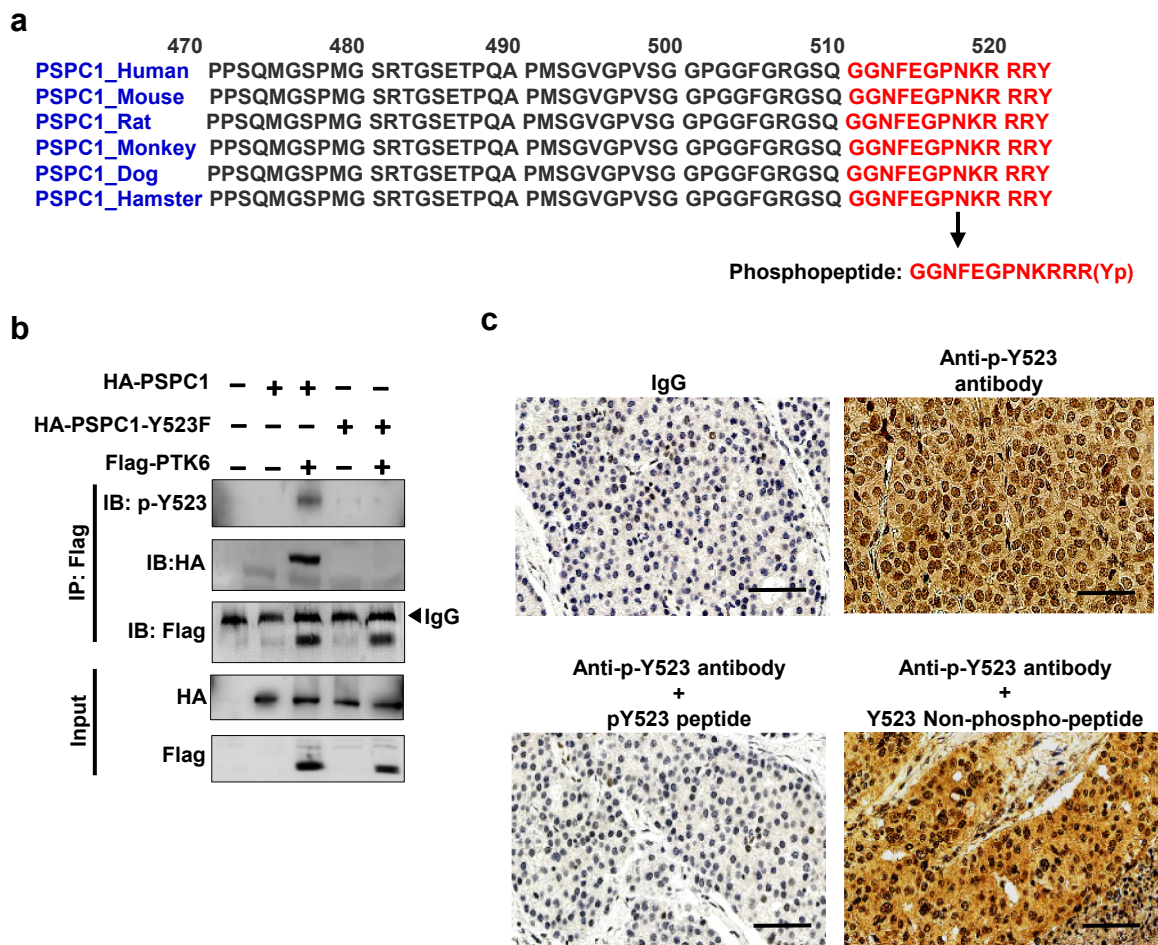
Supplementary Figure 4 HGF-activated oncogenic PSPC1/Wnt3a/c-Met/p-PTK6 axis augments downstream oncogenic signaling.

a, b Migration (**a**) and Matrigel cell invasion (**b**) assays of Huh7 cells treated with 10 ng/ml HGF for 48 h. Data represent the mean $\pm$ SEM (n=6). Data statistics are based on *p < 0.05, **p < 0.01, and ***p < 0.001 calculated by one-way ANOVA with Brown-Forsythe test. **c** mRNA expression of PSPC1/PTK6/Wnt pathway-related genes under HGF stimulation with PSPC1, E-cadherin, N-cadherin, Wnt3a, β -catenin, PTK6, Snail and Slug normalized with GAPDH in Huh-7 cells detected by qRT-PCR. Data are the means $\pm$ SEM (n=3). All data statistics were based on: *p < 0.05, **p < 0.01, and ***p < 0.001 calculated by Student's t-test compared to the Mock control. **d** GSEA profiling of metastasis, EMT and HGF/c-MET signaling in HGF-treated Huh-7 cells after transcriptome and GSEA analysis. ES, enrichment score; NES: normalized enrichment score in HGF high compared to control population. **e** Interaction of PTK6 with c-Met in HGF-treated Huh-7 cell lysate analyzed by IP/Western with anti-PTK6 antibody or control mouse IgG. **f** IP/Western analysis for detection of PTK6 phosphorylation of pY342 and activated phospho-c-Met at pY1234/1235 upon HGF stimulation. HGF-treated Huh-7 cells pretreated with 100 nM XL-184 (c-Met inhibitor) 24 h before serum starvation and then stimulated with 50 ng/ml HGF for 60 min. **g** Dynamic PSPC1 and PSPC1-Y523F interactions with PTK6 and β -catenin altering their subcellular localization in SNU-387 cells demonstrated by Western blotting analysis with corresponding markers for including nuclear (Sp1), cytosolic (α -tubulin) and membrane (Na⁺/K⁺-ATPase) localizations.

a**b**

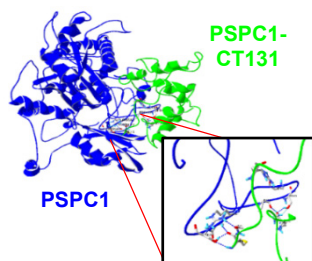
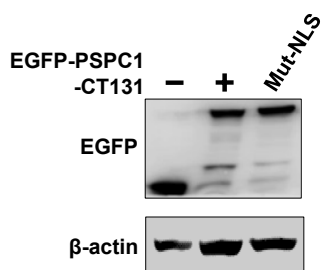
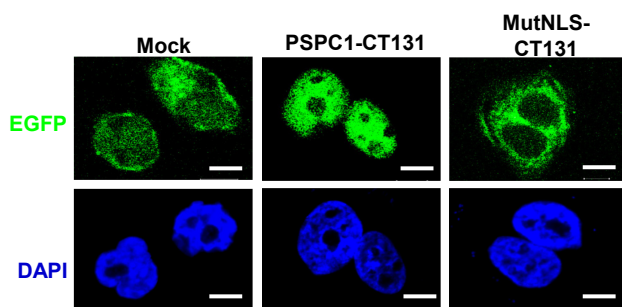
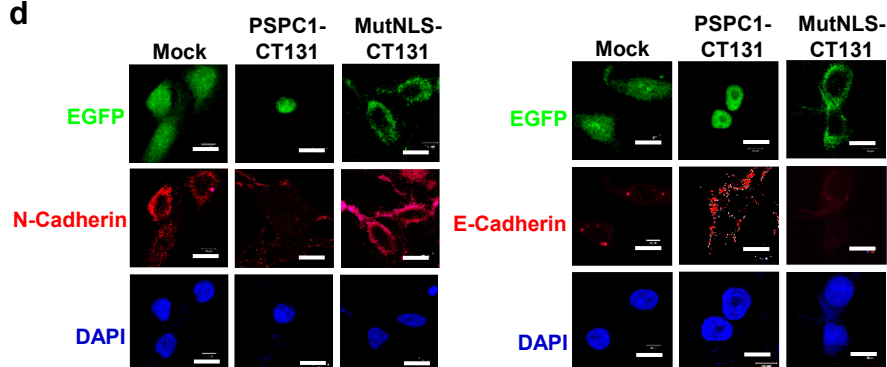
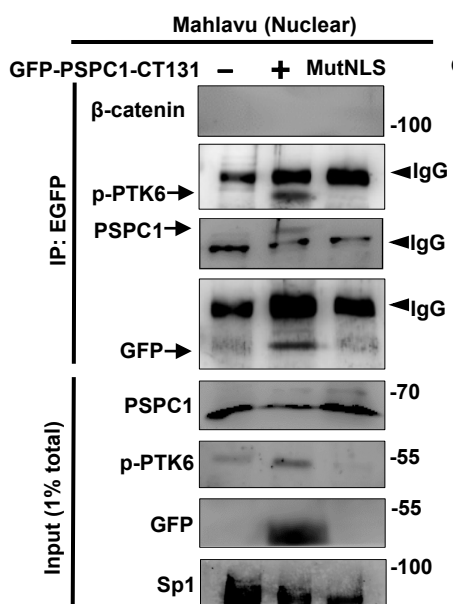
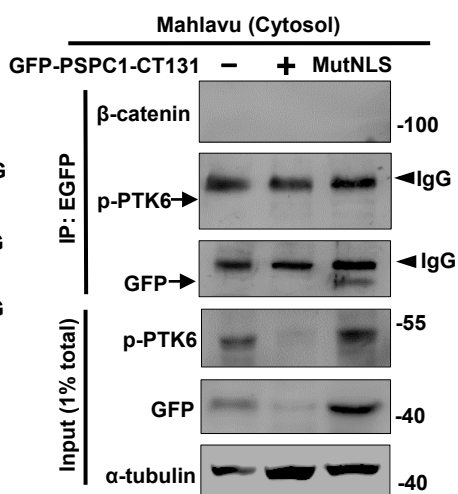
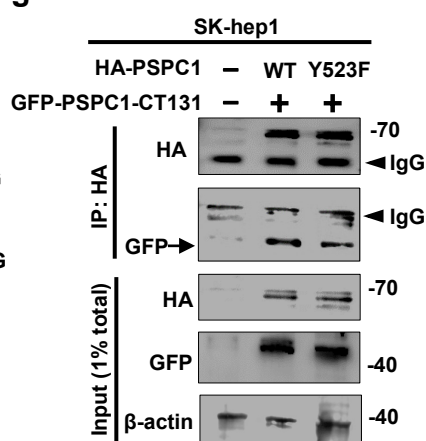
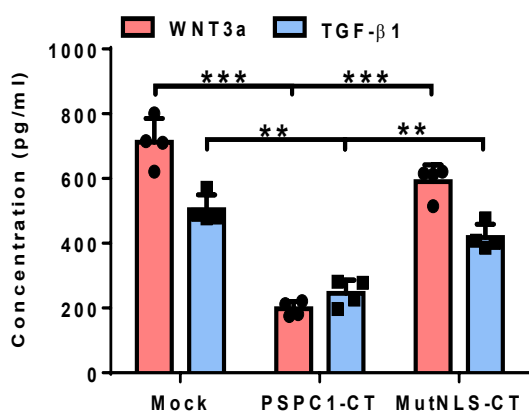
Supplementary Figure 5 The PSC1/PTK6/ β -catenin axis is critical for tumor growth and metastasis in HCC.

a, b Representative IHC staining of PSC1, PTK6, E-cadherin, vimentin, β -catenin, c-Myc and Wnt3a from tumor tissues in the liver (**a**) and lung (**b**) isolated from the mice of orthotopic murine hepatocellular carcinoma model injected with different PSC1/PTK6 SK-hep1 cell transfectants. Scale bars, 50 μ m.



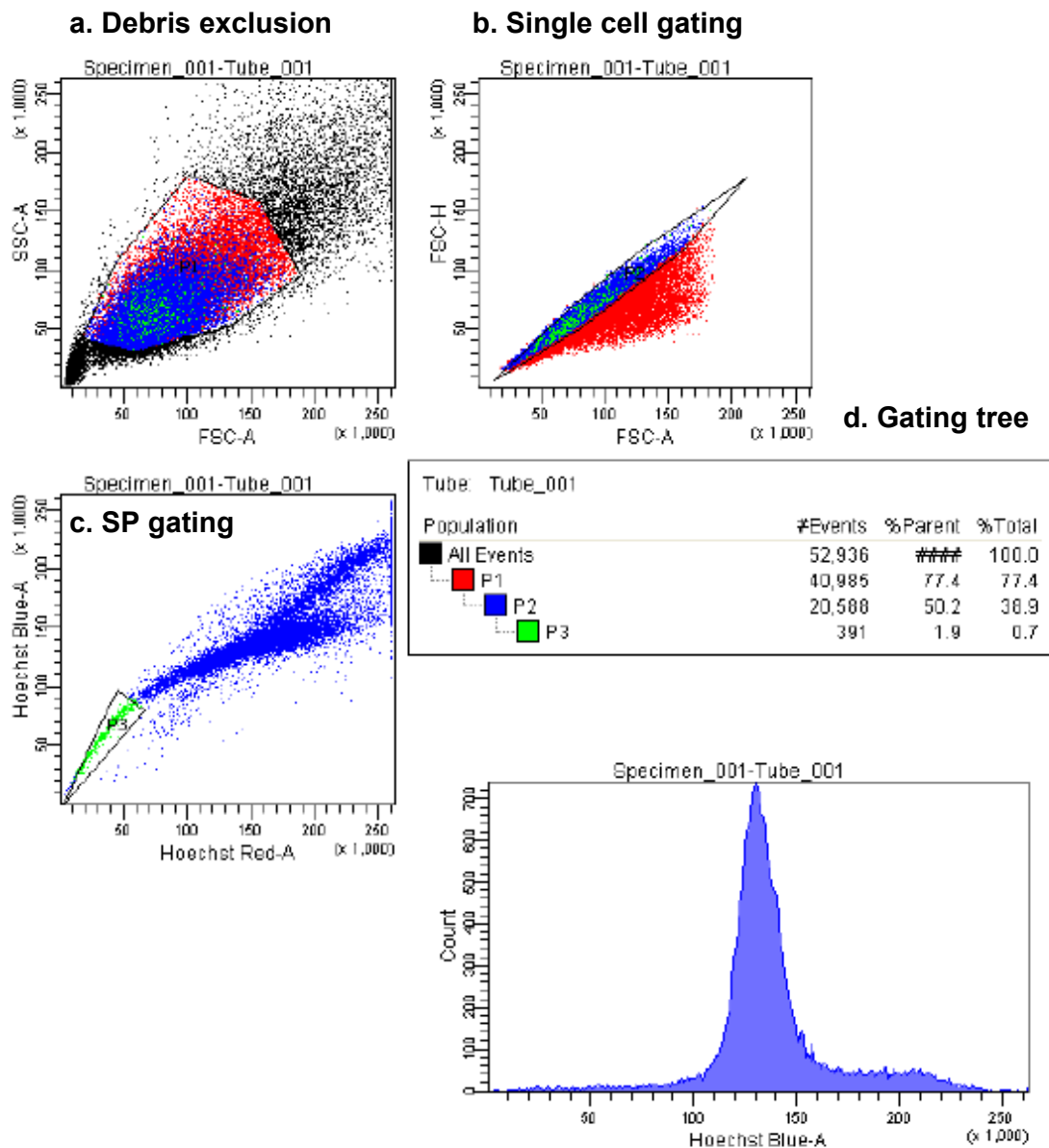
Supplementary Figure 6 Validation of the antibody specificity and the expression intensity of p-Y523-PSPC1 in 215 human HCC tissues.

a Conserved sequences of mammalian PSPC1 orthologs surrounding the Y523 residue of human PSPC1. The arrow indicates that the phosphopeptide (red text) was used as the antigen to raise a phospho-Y523-PSPC1-specific polyclonal antibody. **b** IP/Western analysis using the anti-phospho-PSPC1 Y523 antibody (PSPC1 p-Y523) in 293T cells transfected with PSPC1, PSPC1-Y523F and PTK6 constructs. **c** Validating the specificity of the anti-phospho-PSPC1 Y523 antibody for IHC staining of HCC tumor tissues. Representative IHC images of human HCC tumor tissue stained with the anti-phospho-PSPC1 Y523 antibody competing with the corresponding phosphopeptides and nonphosphopeptides. Scale bar = 25 μ m.

a**b****c****d****e****f****g****h**

Supplementary Figure 7 PSPC1-CT131 is a dual inhibitor suppressing PSPC1- and PTK6-mediated reciprocal PTK6 and β -catenin subcellular translocations as well as autocrine signaling of Wnt3a and TGF- β 1.

a The structural docking of PSPC1-CT131 (green) with PSPC1 (blue). Structures were predicted by using I-TASSER. The protein-protein docking predictions were performed by using ClusPro. **b** Expression of EGFP-PSPC1-CT131 and Mut-NLS-CT131 (nuclear localization sequence (NLS) mutant of PSPC1-CT) in Mahlavu cells demonstrated by Western blotting analysis. **c** Subcellular localization of enhanced green fluorescent protein (EGFP)-conjugated PSPC1-CT131 and Mut-NLS-CT131 in Mahlavu cells. The scale bar represents 10 μ m. **d** Expression of PSPC1-CT131 but not Mut-NLS-CT enhanced the epithelial phenotype and E-cadherin expression and reduced the mesenchymal phenotype and N-cadherin expression in Mahlavu cells determined by IF analysis. Colors are N-Cadherin and E-Cadherin in red, EGFP in green and nuclei with DAPI in blue. The scale bar represents 10 μ m. **e, f**, Interactions and subcellular localization of PSPC1-CT131 with PSPC1 and p-PTK6 in the nucleus (**e**) but not the cytoplasm (**f**) in Mahlavu cells demonstrated by IP/Western blotting analysis. **g** Interaction of PSPC1-CT131 with wild-type PSPC1 and Y523F mutant PSPC1 in SK-hep1 cells shown by IP/Western blotting analysis. **h** Expression of PSPC1-CT131 but not Mut-NLS-CT in Mahlavu cells decreased the levels of cytokines TGF- β 1 and Wnt3a in the conditioned medium demonstrated by ELISA. Data represent the mean $\pm$ SEM (n=4). All data statistics were based on: *p < 0.05, **p < 0.01, and ***p < 0.001 calculated by one-way ANOVA with Brown-Forsythe test.



Supplementary Figure 8 Gating Strategy for side population (SP) Analysis

a Cells are isolated from debris on the flow-cytometric profile based on the Forward Scatter (FSC) and Side Scatter (SSC). **b** Cell aggregates and doublets are gated out and display on the SSC area (SSC-A) v.s height (SSC-H) dot plot. **c** Side population cells are recognized as a dim tail extending toward the lower Hoechst Blue signal (P3). **d** The gating tree indicates the sequential procedure applied to select out the final population for SP discrimination and the percentage of cells (gated events) from each gating step. “% Parent” indicates the percentage of gated events relative to the preceding gate, and “% Total” indicates the percentage of gated events relative to all events recorded.

Supplementary Table 1 Identification of peptide fragments after IP of PSPC1-interacting protein complex of Huh7 cells and LC/MS studies.

Supplementary Table 1: Identification of peptide fragments after IP of PSPC1-interacting protein complex of Huh7 cells and LC/MS studies												
Database : NCBI and SwissProt												
gi 109240550 Mass: 58706 Score: 42348 Matches: 2138(1560) Sequences: 44(32) emPAI: 1241.36												
paraspeckle component 1 [Homo sapiens]												
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide		
210	390.163	778.311	778.31	0.42	0	42	0.0044	1	U	R.MGDMGPR.G		
253	398.16	794.305	794.305	-0.34	0	-34	0.022	1	U	R.MGDMGPR.G		
298	408.24	814.466	814.466	0.34	1	35	0.14	1		R.RLEELR.N		
330	415.728	829.441	829.441	0.67	1	20	5.5	3		R.RQEELR.R		
351	418.197	834.38	834.379	0.39	0	35	0.1	1		K.ALDEMEK.Q		
437	426.195	850.375	850.374	0.62	0	-27	0.4	1		K.ALDEMEK.Q		
542	436.746	871.478	871.476	2.17	0	48	0.0072	1		K.AVVVVDDR.G		
653	453.258	904.502	904.502	-0.13	1	39	0.037	1		K.SFLKPGEK.T		
690	456.238	910.462	910.462	-0.25	1	22	1.6	1		K.EREQPPR.F		
926	480.274	958.533	958.534	-0.33	0	53	0.0026	1		K.AELDGTLK.S		
1026	484.259	966.504	966.504	0.21	1	29	0.32	1		R.DRGFGFIR.L		
1181	493.778	985.542	985.542	-0.16	2	39	0.052	1		R.RQEELRR.L		
1329	510.245	1018.48	1018.48	-0.22	0	-64	1.60E-04	1		K.LEAEAMEAAR.H		
1421	518.242	1034.47	1034.47	-1.08	0	65	9.70E-05	1		K.LEAEAMEAAR.H		
1637	362.54	1084.6	1084.6	-0.08	1	-36	0.087	1		K.AVVVVDDRGR.A		
1640	543.308	1084.6	1084.6	2.41	1	55	0.00091	1		K.AVVVVDDRGR.A		
1746	366.188	1095.54	1095.54	-0.28	1	42	0.027	1		R.HRQEELR.R		
1748	548.778	1095.54	1095.54	0	1	-21	3.3	2		R.HRQEELR.R		
1938	558.314	1114.61	1114.61	-0.32	0	65	0.00017	1		R.FATHGAALTVK.N		
2038	559.79	1117.57	1117.57	-0.15	2	-25	1.9	1		R.RREEEMIR.H		
2039	373.529	1117.57	1117.57	0.22	2	42	0.034	1		R.RREEEMIR.H		
2118	378.861	1133.56	1133.56	-0.04	2	-29	0.69	1		R.RREEEMIR.H		
2121	567.788	1133.56	1133.56	0.14	2	-27	1.3	1		R.RREEEMIR.H		
2229	575.284	1148.55	1148.55	-0.1	1	29	0.82	1		R.WKALDEMEK.Q		
2424	596.273	1190.53	1190.53	-1.04	0	60	0.00025	1	U	R.GSQGGNFEGPNK.R		
2430	596.764	1191.51	1191.52	-1.25	0	-45	0.0058	1	U	R.GSQGGNFEGPNK.R		
2431	596.765	1191.52	1191.52	0.08	0	-55	0.00065	1	U	R.GSQGGNFEGPNK.R		
2450	398.867	1193.58	1193.58	-0.62	0	-45	0.014	1		R.HEHQLMLMR.Q		
2552	601.841	1201.67	1201.67	-0.13	1	41	0.03	1		K.AELDGTLKSR.P		
2553	401.563	1201.67	1201.67	0.66	1	-23	2	2		K.AELDGTLKSR.P		
2620	404.199	1209.57	1209.57	-0.39	0	-38	0.053	1		R.HEHQLMLMR.Q		
2665	404.533	1210.58	1210.56	14.8	0	-22	2.4	1		R.HEHQLMLMR.Q 2666		
2724	409.526	1225.56	1225.57	-11.16	0	-35	0.099	1		R.HEHQLMLMR.Q		
2900	416.539	1246.6	1246.6	-1.76	1	-22	2.9	1		K.ALDEMEKQQR.E		
2904	624.797	1247.58	1247.58	-1.79	1	46	0.0088	1		K.ALDEMEKQQR.E		
3128	638.814	1275.61	1275.61	-0.25	1	54	0.0026	1		K.EKLEAEAMEAAR.H		
3396	645.354	1288.69	1288.69	-0.06	1	64	0.00019	1		K.GFVEFAAKPPAR.K		
3469	431.543	1291.61	1291.61	-0.96	1	-34	0.2	1		K.EKLEAEAMEAAR.H		
protein-tyrosine kinase 6 isoform 1 [Homo sapiens]												
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide		
925	450.779	899.544	899.544	-0.15	0	55	0.00084	1	U	K.GSLLLELR.D		
1769	514.266	1026.52	1026.52	0.08	0	30	0.27	1	U	K.YVGLWDFK.S		
1795	515.769	1029.52	1029.52	-0.41	0	47	0.0072	1	U	K.WTAPALSR.G		
5593	517.278	1548.81	1548.81	-0.93	1	41	0.041	1	U	R.VSEKPSADYVLSVR.D		
625	450.779	1613.8	1613.8	-0.11	0	55	0.04418	1	U	K.GSLLLELLR.D		
1562	514.266	1808.88	1808.89	0.08	0	25	0.029948	1	U	K.YVVGSLWDFK.S		
1425	515.769	2003.97	2003.97	-0.32	0	41	0.015716	1	U	K.WTAPALSSSR.G		
6712	517.278	2199.05	2199.05	-0.12	1	48	0.001484	1	U	R.VCSEKPSADYEVLSVR.D		
non-POU domain-containing octamer-binding protein isoform 1 [Homo sapiens]												
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide		
66	376.203	750.391	750.391	0.27	0	30	0.3	1	U	K.TFNLEK.Q 65		
263	415.728	829.442	829.441	1.47	1	21	4.4	2		R.RQEELR.R		
270	417.226	832.437	832.436	1	0	34	0.19	1		K.ALIEMEK.Q 269		
282	418.73	835.444	835.444	0.56	0	31	0.24	1		K.GIVEFSGK.P		
321	425.223	848.431	848.431	-0.09	0	-22	2.6	1		K.ALIEMEK.Q		
443	443.753	885.491	885.492	-0.84	0	43	0.017	1		R.AVVIVDDR.G 444		
528	456.238	910.462	910.462	0.09	1	23	1.3	1		K.EREQPPR.F		
633	470.255	938.496	938.497	-1.37	1	36	0.058	1		K.DKGFGFIR.L		
971	493.778	985.542	985.542	-0.02	2	24	1.6	1		R.RQEELRR.M		
1341	536.775	1071.53	1071.53	-0.22	0	55	0.001	1		R.AAPGAEFAPNK.R		
1365	540.247	1078.48	1078.48	0.4	0	52	0.0017	1		K.LEMEMEAAR.H		
1485	544.277	1086.54	1086.54	0.47	0	-54	0.0016	1		K.VELDNMPLR.G		
1510	548.244	1094.47	1094.47	-0.54	0	-35	0.075	1		K.LEMEMEAAR.H		
1553	551.781	1101.55	1101.55	-2.02	0	-56	0.0011	1		K.VELDNMPLR.G		
1612	556.241	1110.47	1110.47	-1.01	0	-47	0.0039	1		K.LEMEMEAAR.H		
1859	380.502	1138.48	1138.49	-0.84	1	25	0.78	1		R.RQEEEMMR.R		
1888	381.877	1142.61	1142.61	-0.65	1	24	1.8	1		K.AGEVFIHKDK.G		
2079	590.79	1179.57	1179.56	0.9	0	-39	0.051	1		R.HEHQVLMR.Q		
2139	596.295	1190.57	1190.59	-10.24	0	-40	0.061	1		R.FACHSASLTVR.N		
2176	598.787	1195.56	1195.56	-0.38	0	-36	0.1	1		R.HEHQVLMR.Q		
2432	614.825	1227.64	1227.64	-0.82	1	-46	0.01	1		R.AAPGAEFAPNK.R 2431		
2435	615.316	1228.62	1228.62	-1.91	1	80	4.50E-06	1		R.AAPGAEFAPNK.R		
2458	616.343	1230.67	1230.67	-1.36	1	47	0.0094	1		K.GIVEFSGKPAAR.K		
splicing factor, proline- and glutamine-rich [Homo sapiens]												
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide		
24	358.221	714.428	714.428	0.21	0	51	0.0035	1		R.ALAEIAK.A		
261	394.237	786.459	786.46	-0.6	0	-53	0.0025	1		K.ANLSLLR.R		
531	425.737	849.459	849.46	-0.41	0	38	0.047	1		K.GIVEFASK.P		
535	426.195	850.374	850.374	0.26	0	36	0.048	1		K.SLDEMEK.Q		
547	427.25	852.486	852.486	0.39	1	34	0.057	1		K.GKGFGFIK.L		
618	435.186	868.358	868.357	0.59	0	51	0.00081	1		R.MGYMDPR.E		
684	441.714	881.414	881.413	0.97	0	44	0.0083	1		K.ISDSEGFK.A		
701	443.183	884.352	884.352	-0.3	0	-49	0.00062	1		R.MGYMDPR.E		
717	443.753	885.492	885.492	-0.02	0	40	0.031	1		R.AVVIVDDR.G		
978	469.21	936.404	936.404	-0.06	0	-24	1.1	1		R.EEEMMIR.Q 977		
1064	476.697	951.379	951.379	0.35	0	34	0.038	1		R.EEMEEQMR.R		
1071	477.207	952.399	952.399	-0.49	0	24	0.63	1		R.EEEMMIR.Q		
1280	492.692	983.369	983.369	-0.18	0	-23	0.23	1		R.EEMEEQMR.R 1281		
1762	532.24	1062.46	1062.47	-0.21	0	-37	0.043	1		K.AELDDTPMR.G		
1989	547.259	1092.5	1092.51	-1.76	1	38	0.056	1		R.EEEDMMIR.Q 1991		
1990	365.176	1092.51	1092.51	-0.54	1	-25	1.3	1		R.EEEMMIR.Q		
2041	550.315	1098.61	1098.61	-0.19	1	48	0.0046	1		R.AVVIVDDRGR.S		
2107	555.257	1108.5	1108.5	-1.44	1	-31	0.22	1		R.EEEMMIR.Q		
2108	555.257	1108.5	1108.5	-0.56	1	-34	0.13	1		R.EEEMMIR.Q 2109		
2313	568.761	1135.51	1135.51	0.09	0	-32	0.23	1	U	R.GMGPGTAPGYGR.G		
2418	381.881	1142.62	1142.62	0.26	0	-47	0.0094	1		R.FATHAAALSVR.N		
2494	385.217	1152.63	1152.63	-0.1	1	-35	0.085	1		K.GGFGIKLESRA 2493		
2495	577.322	1152.63	1152.63	-0.01	1	40	0.027	1		K.GGFGIKLESRA		
2576	583.281	1164.55	1164.55	-0.24	1	48	0.0076	1		R.WKSLDEMEK.Q 2578		
2577	389.19	1164.55	1164.55	-0.1	1	-37	0.085	1		R.WKSLDEMEK.Q		
2676	591.278	1180.54	1180.54	-1	1	-31	0.25	1		R.WKSLDEMEK.Q		
2677	394.521	1180.54	1180.54	-0.81	1	-29	0.49	1		R.WKSLDEMEK.Q		
2865	404.202	1209.58	1209.59	-0.4	2	27	0.83	1		R.RQREESYSR.M		

Supplementary Table 2 GSEA analysis after sets of transcriptome analysis of PSPC1/PTK6 transfectants

SPC1 vs Mck					SPC1Y52F vs Mck					SPC1Y52F/PTK6 vs Mck					SPC1-CT131 vs mck				
SPC1 downstream gene dataset					SPC1 downstream gene dataset					SPC1 downstream gene dataset					SPC1 downstream gene dataset				
GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin
Metastasis-related signatures					Metastasis-related signatures					Metastasis-related signatures					Metastasis-related signatures				
Metastasis genes (WINPEPENNICK)	0.9	1.34	0.01	WINPEPENNICK	Metastasis genes (WINPEPENNICK)	-0.89	-1.21	0.01	WINPEPENNICK	Metastasis genes (WINPEPENNICK)	0.83	1.35	0.06	WINPEPENNICK	Metastasis genes (WINPEPENNICK)	0.92	1.33	0.02	WINPEPENNICK
Metastasis genes (Rickman)	0.9	1.22	0.07	Rickman	Metastasis genes (Rickman)	-0.89	-1.32	0.07	Rickman	Metastasis genes (Rickman)	0.76	1.09	0.76	Rickman	Metastasis genes (Rickman)	0.87	1.35	0.03	Rickman
EMT targets vs CDH1 (Onder)	0.8	1.25	0.05	Onder	EMT targets vs CDH1 (Onder)	-0.89	-1.36	0.01	Onder	EMT targets vs CDH1 (Onder)	0.86	1.24	0.04	Onder	EMT targets vs CDH1 (Onder)	0.85	1.31	0.03	Onder
Metastasis genes (Tavazois)	0.6	0.96	0.07	Tavazois	Metastasis genes (Tavazois)	-0.76	-1.12	0.12	Tavazois	Metastasis genes (Tavazois)	0.83	1.37	0.04	Tavazois	Metastasis genes (Tavazois)	0.97	1.40	0.005	Tavazois
Metastasis genes (Provenzano)	0.6	0.87	0.07	Provenzano	Metastasis genes (Provenzano)	0.65	-1.00	0.050	Provenzano	Metastasis genes (Provenzano)	0.63	1.03	0.30	Provenzano	Metastasis genes (Provenzano)	0.91	1.35	0.022	Provenzano
Stemness-related signatures					Stemness-related signatures					Stemness-related signatures					Stemness-related signatures				
Cancer stemness genes (Ramallo)	0.9	1.28	0.01	Ramallo	Cancer stemness genes (Ramallo)	-0.76	-1.12	0.32	Ramallo	Cancer stemness genes (Ramallo)	0.88	1.4	0.00	Ramallo	Cancer stemness genes (Ramallo)	0.87	1.35	0.025	Ramallo
PS stemness (Mikkelsen)	0.9	1.22	0.040	Mikkelsen	PS stemness (Mikkelsen)	-0.87	-1.36	0.01	Mikkelsen	PS stemness (Mikkelsen)	0.84	1.3	0.118	Mikkelsen	PS stemness (Mikkelsen)	0.9	1.33	0.040	Mikkelsen
Stemness genes (Gussmann)	0.8	0.98	0.64	Gussmann	Stemness genes (Gussmann)	-0.92	-1.34	0.01	Gussmann	Stemness genes (Gussmann)	0.79	1.33	0.082	Gussmann	Stemness genes (Gussmann)	0.84	1.27	0.089	Gussmann
Stemness genes (Besporah)	0.8	1.28	0.04	Besporah	Stemness genes (Besporah)	-0.63	-0.99	0.30	Besporah	Stemness genes (Besporah)	0.78	1.14	0.309	Besporah	Stemness genes (Besporah)	0.65	1	0.477	Besporah
Stemness genes (Lim)	0.9	1.15	0.2	Lim	Stemness genes (Lim)	-0.6	-1.01	0.460	Lim	Stemness genes (Lim)	0.84	1.35	0.05	Lim	Stemness genes (Lim)	0.9	1.39	0.014	Lim
Myo-related pathways					Myo-related pathways					Myo-related pathways					Myo-related pathways				
Myo targets (Schlosser)	0.9	1.34	0.01	Schlosser	Myo targets (Schlosser)	-0.8	-1.22	0.046	Schlosser	Myo targets (Schlosser)	0.83	1.32	0.095	Schlosser	Myo targets (Schlosser)	0.9	1.32	0.043	Schlosser
Myo pathway (Lee liver cancer)	1	1.31	0.01	Lee liver cancer	Myo pathway (Lee liver cancer)	-0.86	-1.20	0.04	Lee liver cancer	Myo pathway (Lee liver cancer)	0.83	1.26	0.07	Lee liver cancer	Myo pathway (Lee liver cancer)	0.96	1.36	0.01	Lee liver cancer
Myo targets (Boylan)	1	1.36	0.01	Boylan	Myo targets (Boylan)	-0.92	-1.35	0.01	Boylan	Myo targets (Boylan)	0.6	0.83	0.741	Boylan	Myo targets (Boylan)	0.94	1.38	0.011	Boylan
TGF-β-related pathway					TGF-β-related pathway					TGF-β-related pathway					TGF-β-related and Wnt-related pathways				
TGF-β targets (Lalbe)	0.9	1.33	0.02	albe	TGF-β targets (Lalbe)	-0.8	-1.19	0.094	albe	TGF-β targets (Lalbe)	0.84	1.33	0.051	albe	TGF-β targets (Lalbe)	0.93	1.32	0.038	albe
TGF-β pathway (GSE21670)	0.8	1.22	0.144	GSE21670	TGF-β pathway (GSE21670)	-0.88	-1.38	0.014	GSE21670	TGF-β pathway (GSE21670)	0.73	1.24	0.169	GSE21670	TGF-β pathway (GSE21670)	0.92	1.38	0.015	GSE21670
TGF-β targets (Blatt)	0.8	1.26	0.07	Blatt	TGF-β targets (Blatt)	-0.83	-1.22	0.126	Blatt	TGF-β targets (Blatt)	0.77	1.25	0.149	Blatt	TGF-β targets (Blatt)	0.78	1.12	0.31	Blatt
Wnt-CTNNB1 pathway					Wnt-CTNNB1 pathway					Wnt-CTNNB1 pathway					Wnt-CTNNB1 pathway				
WNT signaling (Samson)	0.9	1.27	0.045	Samson	WNT signaling (Samson)	-0.82	-1.33	0.01	Samson	WNT signaling (Samson)	0.58	0.9	0.607	Samson	WNT signaling (Samson)	0.78	1.2	0.143	Samson
WNT signaling (Schaeffer)	0.8	1.47	0.05	Schaeffer	WNT signaling (Schaeffer)	-0.79	-1.31	0.015	Schaeffer	WNT signaling (Schaeffer)	0.8	1.07	0.454	Schaeffer	WNT signaling (Schaeffer)	0.78	1.17	0.183	Schaeffer
WNT targets (Smid)	0.9	1.27	0.05	Smid	WNT targets (Smid)	-0.84	-1.35	0.05	Smid	WNT targets (Smid)	0.86	1.13	0.362	Smid	WNT targets (Smid)	0.83	1.24	0.106	Smid
WNTA targets (Lalbe)	0.6	0.85	0.1	albe	WNTA targets (Lalbe)	-0.8	-1.2	0.209	albe	WNTA targets (Lalbe)	0.79	1.37	0.045	albe	WNTA targets (Lalbe)	0.91	1.32	0.032	albe
WNT pathways (Boylan)	0.6	1.16	0.324	Boylan	WNT pathways (Boylan)	-0.85	-1.2	0.104	Boylan	WNT pathways (Boylan)	0.95	1.31	0.07	Boylan	WNT pathways (Boylan)	0.86	1.27	0.030	Boylan
WNT targets vs GFR1(Accardo)	0.8	1.18	0.16	Accardo	WNT targets vs GFR1(Accardo)	-0.72	-1.16	0.205	Accardo	WNT targets vs GFR1(Accardo)	0.86	1.5	0.026	Accardo	WNT targets vs GFR1(Accardo)	0.83	1.26	0.08	Accardo
PTK6 downstream gene dataset					PTK6 downstream gene dataset					PTK6 downstream gene dataset					PTK6 downstream gene dataset				
GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin
Focal adhesion	0.9	1.27	0.113	KEGG	Focal adhesion	-0.84	-1.36	0.021	KEGG	Focal adhesion	0.78	1.3	0.087	KEGG	Focal adhesion	0.87	1.35	0.027	KEGG
PKA pathway(cAMP)	0.8	1.46	0.00	KEGG	PKA pathway(cAMP)	-0.89	-1.47	0.00	KEGG	PKA pathway(cAMP)	0.84	1.49	0.010	KEGG	PKA pathway(cAMP)	0.84	1.26	0.094	KEGG
Rac/Rho pathway	0.9	1.32	0.064	KEGG	Rac/Rho pathway	-0.91	-1.48	0.00	KEGG	Rac/Rho pathway	0.91	1.34	0.057	KEGG	Rac/Rho pathway	0.86	1.3	0.050	KEGG
Dynactin kinase pathway	0.8	1.46	0.07	KEGG	Dynactin kinase pathway	-0.92	-1.32	0.01	KEGG	Dynactin kinase pathway	0.84	0.8	0.743	KEGG	Dynactin kinase pathway	0.91	1.14	0.882	KEGG
GPCR pathway (GO)	1	1.35	0.00	GO	GPCR pathway (GO)	-0.88	-1.36	0.015	GO	GPCR pathway (GO)	0.73	1.3	0.068	GO	GPCR pathway (GO)	0.91	1.37	0.01	GO
Receptor clustering	0.8	1.27	0.069	KEGG	Receptor clustering	-0.88	-1.45	0.00	KEGG	Receptor clustering	0.97	1.44	0.01	KEGG	Receptor clustering (GO)	0.82	1.36	0.021	GO
Integrin-linked pathway	0.8	1.2	0.27	PD	Integrin-linked pathway	-1	-1.3	0.02	PD	Integrin-linked pathway	0.84	1.27	0.125	PD	Integrin-linked pathway	0.85	1.31	0.04	PD
β3MAPK pathway	0.6	1	0.42	Phong	β3MAPK pathway	-0.82	-1.32	0.02	Phong	β3MAPK pathway	0.55	0.94	0.550	Phong	β3MAPK pathway	0.61	0.94	0.608	Phong
HGF vs Cnt					HGF downstream gene dataset					HGF downstream gene dataset					HGF downstream gene dataset				
GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin	GSEA	ES	NES	P-val	Data origin
Metastasis-related signatures					Metastasis-related signatures					Metastasis-related signatures					Metastasis-related signatures				
EMT targets vs CDH1	0.88	1.61	0.01	Onder	EMT targets vs CDH1	-0.88	-1.61	0.01	Onder	EMT targets vs CDH1	0.88	1.61	0.01	Onder	EMT targets vs CDH1	0.88	1.61	0.01	Onder
Metastasis genes	0.9	1.54	0.02	ILDEA	Metastasis genes	-1.52	-0.93	0.02	ILDEA	Metastasis genes	0.9	1.54	0.02	ILDEA	Metastasis genes	0.9	1.54	0.02	ILDEA
Liver-cancer genes	0.7	1.52	0.03	HOSHIDA	Liver-cancer genes	-1.52	-0.93	0.03	HOSHIDA	Liver-cancer genes	0.7	1.52	0.03	HOSHIDA	Liver-cancer genes	0.7	1.52	0.03	HOSHIDA
EMT-related signatures					EMT-related signatures					EMT-related signatures					EMT-related signatures				
Metastasis genes	0.6	1.25	0.05	Rallmark	Metastasis genes	-1.25	-0.05	0.05	Rallmark	Metastasis genes	0.6	1.25	0.05	Rallmark	Metastasis genes	0.6	1.25	0.05	Rallmark
INVASIVENESS, SIGNATOR	0.9	1.65	0.02	ANASTASSIOU	INVASIVENESS, SIGNATOR	-1.65	-0.02	0.02	ANASTASSIOU	INVASIVENESS, SIGNATOR	0.9	1.65	0.02	ANASTASSIOU	INVASIVENESS, SIGNATOR	0.9	1.65	0.02	ANASTASSIOU
TUMORIGENESIS,UP	0.8	1.45	0.05	ROMER	TUMORIGENESIS,UP	-1.45	-0.05	0.05	ROMER	TUMORIGENESIS,UP	0.8	1.45	0.05	ROMER	TUMORIGENESIS,UP	0.8	1.45	0.05	ROMER
HGF-c-MET-related pathway					HGF-c-MET-related pathway					HGF-c-MET-related pathway					HGF-c-MET-related pathway				
c-MET targets (HNA4)	0.8	1.44	0.05	UM	c-MET targets (HNA4)	-1.44	-0.05	0.05	UM	c-MET targets (HNA4)	0.8	1.44	0.05	UM	c-MET targets (HNA4)	0.8	1.44	0.05	UM
c-MET targets (FOXA2)	0.8	1.42	0.05	GUANG	c-MET targets (FOXA2)	-1.42	-0.05	0.05	GUANG	c-MET targets (FOXA2)	0.8	1.42	0.05	GUANG	c-MET targets (FOXA2)	0.8	1.42	0.05	GUANG
c-MET targets (mTOR)	0.6	1.35	0.05	ARENT	c-MET targets (mTOR)	-1.35	-0.05	0.05	ARENT	c-MET targets (mTOR)	0.6	1.35	0.05	ARENT	c-MET targets (mTOR)	0.6	1.35	0.05	ARENT

Supplementary Table 3 detail IHC results of HCC patients with clinical features

Supplementary Table 3. detail IHC results of HCC patients with clinical features																									
USM	pathologic	gender	TNM	Stage	survival	Page1				Page1 V323				p-Rib				Wnt 3a							
						L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4				
1	41M	ENH03	R	41aT	1																				
2	52M	ENH03	R	52aT	1																				
3	50M	ENH04	R	50aT	1																				
4	73M	ENH01	R	73aT	1																				
5	73M	ENH03	R	73aT	1																				
6	65M	ENH03	R	65aT	1																				
7	72M	ENH03	R	72aT	1																				
8	68M	ENH03	R	68aT	1																				
9	55M	ENH04	R	55aT	1																				
10	55M	ENH04	R	55aT	1																				
11	60M	ENH04	R	60aT	1																				
12	52M	ENH03	R	52aT	1																				
13	20M	ENH03	R	20aT	1																				
14	60M	ENH03	R	60aT	1																				
15	47M	ENH03	R	47aT	1																				
16	67M	ENH03	R	67aT	1																				
17	63M	ENH04	R	63aT	1																				
18	60M	ENH04	R	60aT	1																				
19	62M	ENH03	R	62aT	1																				
20	64M	ENH03	R	64aT	1																				
21	64M	ENH03	R	64aT	1																				
22	64M	ENH03	R	64aT	1																				
23	64M	ENH03	R	64aT	1																				
24	61M	ENH03	R	61aT	1																				
25	63M	ENH04	R	63aT	1																				
26	63M	ENH04	R	63aT	1																				
27	60M	ENH03	R	60aT	1																				
28	64M	ENH03	R	64aT	1																				
29	60M	ENH03	R	60aT	1																				
30	64M	ENH03	R	64aT	1																				
31	52M	ENH03	R	52aT	1																				
32	64M	ENH03	R	64aT	1																				
33	52M	ENH03	R	52aT	1																				
34	73M	ENH03	R	73aT	1																				
35	64M	ENH03	R	64aT	1																				
36	64M	ENH03	R	64aT	1																				
37	64M	ENH03	R	64aT	1																				
38	64M	ENH03	R	64aT	1																				
39	64M	ENH03	R	64aT	1																				
40	64M	ENH03	R	64aT	1																				
41	64M	ENH03	R	64aT	1																				
42	64M	ENH03	R	64aT	1																				
43	64M	ENH03	R	64aT	1																				
44	64M	ENH03	R	64aT	1																				
45	64M	ENH03	R	64aT	1																				
46	64M	ENH03	R	64aT	1																				
47	64M	ENH03	R	64aT	1																				
48	64M	ENH03	R	64aT	1																				
49	64M	ENH03	R	64aT	1																				
50	64M	ENH03	R	64aT	1																				
51	64M	ENH03	R	64aT	1																				
52	72M	ENH03	R	72aT	1																				
53	64M	ENH03	R	64aT	1																				
54	64M	ENH03	R	64aT	1																				
55	64M	ENH03	R	64aT	1																				
56	64M	ENH03	R	64aT	1																				
57	64M	ENH03	R	64aT	1																				
58	64M	ENH03	R	64aT	1																				
59	64M	ENH03	R	64aT	1																				
60	64M	ENH03	R	64aT	1																				
61	64M	ENH03	R	64aT	1																				
62	72M	ENH03	R	72aT	1																				
63	64M	ENH03	R	64aT	1																				
64	64M	ENH03	R	64aT	1																				
65	64M	ENH03	R	64aT	1																				
66	64M	ENH03	R	64aT	1																				
67	64M	ENH03	R	64aT	1																				
68	64M	ENH03	R	64aT	1																				
69	64M	ENH03	R	64aT	1																				
70	64M	ENH03	R	64aT	1																				
71	64M	ENH03	R	64aT	1																				
72	64M	ENH03	R	64aT	1																				
73	64M	ENH03	R	64aT	1																				
74	64M	ENH03	R	64aT	1																				
75	64M	ENH03	R	64aT	1																				
76	64M	ENH03	R	64aT	1																				
77	64M	ENH03	R	64aT	1																				
78	64M	ENH03	R	64aT	1																				
79	64M	ENH03	R	64aT	1																				
80	64M	ENH03	R	64aT	1																				
81	64M	ENH03	R	64aT	1																				
82	64M	ENH03	R	64aT	1																				
83	64M	ENH03	R	64aT	1																				
84	64M	ENH03	R	64aT	1																				
85	64M	ENH03	R	64aT	1																				
86	64M	ENH03	R	64aT	1																				

Supplementary Table 4 List of antibodies with their sources and experimental conditions

Protein	Assay	Antibody	Origin	Dilution	Protein	Assay	Antibody	Origin	Dilution
PSPC1	WB	sc-374181, Santa Cruz Biotechnology, Inc	mouse	1/1000	Anti-phospho-PTK6 (Tyr342)	WB	#09-144, EMD Millipore	mouse	1/1000
		sc-84576,Santa Cruz Biotechnology, Inc	rabbit	1/500	Snail	WB	#3879, Cell Signaling Technology, Inc.	rabbit	1/1000
	IP	sc-374181, Santa Cruz Biotechnology, Inc	mouse	1/100	Slug	WB	#9585, Cell Signaling Technology, Inc.	rabbit	1/1000
	IF	sc-84576, Santa Cruz Biotechnology, Inc	mouse	1/500	Twist	WB	25465-1-AP, proteintech	rabbit	1/1000
		SAB4200068, Sigma-Aldrich	rabbit	1/500	Nanog	WB	#4903, Cell Signaling Technology, Inc.	rabbit	1/1000
	IHC	sc-374181, Santa Cruz Biotechnology, Inc	mouse	1/250	Oct4	WB	#2840, Cell Signaling Technology, Inc.	rabbit	1/1000
PTK6	WB	sc-166171, Santa Cruz Biotechnology, Inc	mouse	1/1000	Sox2	WB	#3579, Cell Signaling Technology, Inc.	rabbit	1/1000
		18697-1-AP, Proteintech	rabbit	1/1000	β-catenin	WB	#8480, Cell Signaling Technology, Inc.	rabbit	1/1000
	IP	sc-166171, Santa Cruz Biotechnology, Inc	mouse	1/100	c-myc	WB	sc-40, Santa Cruz Biotechnology, Inc	mouse	1/1000
	IF	sc-166171, Santa Cruz Biotechnology, Inc	mouse	1/200	Anti-Phosphotyrosine (4G10)	WB	#05-321, EMD Millipore	mouse	1/1000
	IHC	sc-166171, Santa Cruz Biotechnology, Inc	mouse	1/100	Vimentin	WB	#5741, Cell Signaling Technology, Inc.	rabbit	1/1000
N-cadherin	WB	#13116, Cell Signaling Technology, Inc.	rabbit	1/1000	ZEB1	WB	#3396, Cell Signaling Technology, Inc.	rabbit	1/1000
	IF	#13116, Cell Signaling Technology, Inc.	rabbit	1/200	p-SMAD2	WB	#3108, Cell Signaling Technology, Inc.	rabbit	1/1000
E-cadherin	WB	#3195, Cell Signaling Technology, Inc.	rabbit	1/1000	p-SMAD3	WB	#9520, Cell Signaling Technology, Inc.	rabbit	1/1000
	IF	#3195, Cell Signaling Technology, Inc.	rabbit	1/100	SMAD2/3	WB	#8685, Cell Signaling Technology, Inc.	rabbit	1/1000
Sp1	WB	sc-420, Santa Cruz Biotechnology, Inc	mouse	1/1000	His Tag	WB	34670, QIAGEN	mouse	1/1000
	WB	21962-1-AP,Proteintech	rabbit	1/1000	Anti-6X His tag	IP	ab18184, Abcam	mouse	1/100
HA	WB	H3663, Sigma-Aldrich	mouse	1/1000	β-actin	WB	A5316, Sigma-Aldrich	mouse	1/10000
	IP	H3663, Sigma-Aldrich	mouse	1/100	α-tubulin	WB	A5316,Sigma-Aldrich	mouse	1/10000
Flag	WB	F3165, Sigma-Aldrich	mouse	1/1000	Survivin	WB	sc-17779, Santa Cruz Biotechnology, Inc	mouse	1/1000
	IP	F3165, Sigma-Aldrich	mouse	1/100	Cyclin D1	WB	sc-8396, Santa Cruz Biotechnology, Inc	mouse	1/1000
Living Colors® EGFP	WB	#632569, Clontech	mouse	1/1000	goat anti-mouse IgG-HRP	WB	sc-516102, Santa Cruz Biotechnology, Inc	goat	1/5000
	IF	#632569, Clontech	mouse	1/100	goat anti-rabbit IgG-HRP	WB	sc-2030, Santa Cruz Biotechnology, Inc	goat	1/5000
v-catenin	WB	#2309, Cell Signaling Technology, Inc.	rabbit	1/1000	Abbreviations: IF, immunofluorescence; IHC,immunohistochemistry; IP, immunoprecipitation; mmab, mouse monoclonal antibody; WB, Western blot				
	IF	#2309, Cell Signaling Technology, Inc.	rabbit	1/100					

Supplementary Table 5 Sequences of the oligonucleotides for shRNA,real-time PCR, and site-mutagenesis.

Assay		Name		Sequence	
shRNA-52		PTK6		TACCTCTCCCATGACCACAAT	
				GCTCCGCGACTCTGATGAGAA	
shRNA-53		PTK6		CCGGAGTCGCAGAATTACATCCA	
				CCCTCGAGGGTGGATGTAATTCTGCGACTTTTTT	
Real-Time PCR	human	TGFβ1	F	CGTGGAGCTGTACCAGAAATA	
			R	TCCGGTGACATCAAAAGATAA	
		c-Myc	F	GCCACGTCTCCACACATCAG	
			R	TCTTGGCAGCAGGATAGTCCTT	
		Axin2	F	CTGGCTTTGGTGAAGTGTG	
			R	AGTTGCTCACAGCCAAGACA	
		CCND1	F	GCTGCGAAGTGGAACCATC	
			R	CCTCCTTCTGCACACATTTGAA	
		GAPDH	F	TGTTCGACAGTCAGCCGC	
			R	GGTGTCTGAGCGATGTGGC	
		β-catenin	F	CTGAGGAGCAGCTTCAGTCC	
			R	GAGTAGCCATTGTCCACGCT	
		PTK6	F	CACAACTACCTGGCCGAGAG	
			R	GCTTCTCGCTGACCCTGAT	
		Snail	F	GGCCTAGCGAGTGGTTCTTC	
			R	GTTAGGCTTCCGATTGGGGT	
		Slug	F	TTCAACGCCTCCAAAAAGCC	
			R	GATGGGGCTGTATGCTCCTG	
		Wnt3a	F	CCCTGGAGCTAGTGTCTCCTCT	
			R	CCAATCTGTAGCCCCGCCTC	
Site-mutagenesis	human	PSPC1 Y523F	F	CCCTAATAAGCGTCGTAGATTTTAATCTAGAGGGCCCTATT	
			R	C	
			R	GAATAGGCCCTCTAGATTAATAATCTACGACGCTTATTAGGG	