Supplementary Materials and Methods

siRNAs, Plasmids and constructions

The following siRNAs targeting human genes were used: SMARTpool ON-TARGETplus PRC1 siRNA (L-019491-00-0050), Non-targeting pool scramble siRNA (D-001810-10-50), β-catenin siRNA (L-003482-00-0010), TCF-4 siRNA (L-004594-00-0005), APC siRNA (L-003869-00-0005), GAPDH siRNA (L-004253-00-0010), KIF11 siRNA (L-003317-00-0005), SPC25 siRNA (L-017297-00-0010), FANCI siRNA (L-022320-01-0005) and KIF23 siRNA (L-004956-00-0005) from GE health Dharmacon, PRC1-3'UTR siRNA targeting sequence: 5'-CGCUGUUUACUCAUACAGU-3'. Sequences are available as requested. The Lipofactamine RNAiMax (Life Technologies) was used for siRNA transfection.

Following plasmids constructs were used: M50 Super 8 × TOPFlash, M51 Super 8 × FOPFlash were gifts from Randall Moon (Addgene), human beta-catenin pcDNA3 and BcatMutS33/S37.T41/S45 were gifts from David Rimm (Addgene). EGFP fusion expression vector pEGFP-C2 (Clontech) was used to derive the PRC1 truncated constructs.

In-fusion HD cloning kit (NEB) was used to clone the following gene fragments: PRC1-FL (full length 1-620), PRC1-ΔC (1-1863bp), PRC1-CC (1-1023bp) and PRC1-ΔCC (1021-1863bp) in to pEGFP-C2 (Clontech) vector digested with Xho I and Sal I (NEB).The NLS mutations (NLS-2A and NLS-3A) was done by using the Q5® Site-Directed Mutagenesis Kit (NEB). Primers used for these constructs were listed in the Supplementary Table 4.

Stable cells, Soft agar colony formation assay and Oncosphere assay

The MISSION sh lentivirus system was used for stable knockdown of HCC cells in our experiments. Lentiviral cloning vectors pLKO.1-hPGK-Puro-CMV-tGFP-PRC1 (Sequence: CCGGCCTGAAGGAAAGACTCATCAACTCGAGTTGATGAGTCTTTCCTTCAGGTTTTTG) and pLKO.1-hPGK-Puro-CMV-mCherry-shScramble were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lentivirus was prepared following the Supplier's instructions and used to infect cells three times. The cells were sub-cultured to 10% confluence in a medium containing puromycin (Sigma-Aldrich, St. Louis, MO, USA; Hep3B: 1.5μg/ml, HuH-7: 1.5–2.0μg/ml, and HCCLM3: 2.5μg/ml). Antibiotic-resistant clones were picked and passaged in medium containing puromycin. The knockdown protein level was assessed by Western blot assay.

Stable knockdown cells were counted and seeded in six-well plates (for Hep3B and HuH-7, 8,000 cells were seeded; for HCCLM3, 1,000 cells were seeded) in growth medium containing 0.7% agar (2ml per well) on top of a layer of growth medium containing 1.4% agar (1ml per well). Growth medium (1ml) with 10% FBS (Fetal bovine serum) was added on top of the agar. The cell suspension was plated and cultured in a 37°C incubator for approximately 20 days. After that, the colonies were fixed with 4% PFA, stained with 0.005% (w/v) crystal violet in 25% (v/v) methanol, and the colonies were pictured and counted.

For oncosphere formation assay, 20,000 HCCLM3 or 5,000 Huh-7 stable cells were seeded on ultra-low attachment culture dishes (Corning) in serum-free medium. DMEM/F12 serum-free medium (Invitrogen) contained 2mM L-glutamine, 1% sodium pyruvate (Invitrogen), 100μg/ml penicillin G, and 100U/ml streptomycin supplemented with 20ng/ml epithelial growth factor (Invitrogen), 10ng/ml fibroblast growth factor-2 (Invitrogen), N2 (Invitrogen), and B27 (Invitrogen). Cells were incubated in a CO_2 incubator for one to two weeks, and numbers of

oncosphere cells (diameter >100μm) were counted under a stereomicroscope (NIKON, Tokyo, Japan).

RNA, Real-time PCR, Western blotting and Chromatin Immunoprecipitation (ChIP)

Cells were collected by trypsin digestion. RNA was prepared by RNA purification kit (QIAGEN). cDNAs were prepared by Script™ cDNA Synthesis Kit (#170-8891, BIO-RAD) and processed for real-time PCR (SsoAdvanced™ Universal Supermixes, BIO-RAD) using the primers listed in Supplementary Table 3.

Cell lysate was prepared in RIPA buffer with the addition of protease inhibitor cocktail, phosphatase inhibitor cocktail, DTT (Dithiothreitol), and Benzonase (Sigma-Aldrich, St. Louis, MO, USA). Protein concentration was calculated by the BCA Protein assay kit (Thermo Scientific Pierce, UK). NuPAGE@Novex 4-12% Bis-Tris protein gels (Novex, Life Technologies) were used for all Western blotting assays.

Chromatin Immunoprecipitation was performed using the Millipore Magna ChIP G Kit (17-611) with the protocol provided. In brief, HCCLM3 cells were stimulated with 6.25ng/ml Wnt3a. After 2h, cells were cross-linked with 37% Formaldehyde (Sigma F8775) solution (Final concentration 1%) for 10 minutes. Crosslinking reaction was quenched by adding glycine solution. After washing twice with ice cold PBS, cells were suspended in lysis buffer containing protease inhibitors, at 4°C for 15 minutes with intermittent vortexing. The lysate was pelleted by spinning at 800*g* for 5 minutes. Pellet was resuspended in nuclei lysis buffer and sonicated using the Misonix S-4000 sonicator at 50% amplitude 15 sec pulse with 90 sec rest between each pulse (10 cycles). Sonicated chromatin was analyzed for size by running in gel. Sonicated chromatin was

immunoprecipitated with either 3μg anti-TCF4 or 3μg anti-IgG (Millipore 17-10109) overnight at 4°C. Real-time PCR (Kapa SYBR Fast Master Mix KK4601) was used to detect isolated ChIP fragments with the following primers PRC1: F – CCAAACAAGGAAATGCCAGT, R – CACCGGAAAAGTACCCTCCT; Survivin: F – GGGGCGCTAGGTGTGGG, R – TTCAAATCTGGCGGTTAATGGC; Negative: F –ATGGTTGCCACTGGGGATCT, R – TGCCAAAGCCTAGGGGAAGA.

Nuclear protein extraction and Cell Fractionation

To detect β-catenin nuclear translocation, NE-PER@ Nuclear and Cytoplasmic Extraction kit (Thermo Scientific Pierce, UK) was used for HCCLM3 cells with or without wnt3a treatment. HCCLM3 cells were transfected with PRC1 siRNA or scramble control for 24h and wnt3a was added into the medium. Fresh cells (5 x 10^6 cells) in 10cm dish were collected at different time points and processed for cell fractionation analysis by the Qproteome Cell Compartment Kit (Cat no: 37502, QIAGEN). Briefly, cells were washed by 2ml ice-cold PBS by pipetting and repeated two times. Cell pellet was washed in 1ml ice-cold Extraction Lysis Buffer by pipetting and incubated for 10 min at 4°C on an end-over-end shaker. After incubation, the lysate was centrifuged at 1000 x g for 10 min at 4°C. The supernatant (fraction 1) was carefully transferred into a fresh microcentrifuge tube and stored in ice. This fraction primarily contains the cytosolic proteins. The pellet was resuspended in 1ml ice-cold Extraction Buffer CE2 by pipetting and incubated for 30 min at 4°C on an end-over-end shaker. The suspension was then centrifuged at 6000 x g for 10 min at 4°C. The supernatant (fraction 2) was transferred into a fresh microcentrifuge tube and stored on ice. This fraction primarily contains membrane proteins. 7μl

Benzonase® Nuclease and 13µl distilled water was added to resuspend the pellet by gently flicking the bottom of the tube and incubated for 15 min at ambient temperature. 500µl ice-cold Extraction Buffer CE3 was pipetted into the tube and mixed by pipetting. The mixture was incubated for 10 min at 4°C on an end-over-end shaker and the insoluble materials were removed by centrifugation at 6800 x g for 10 min at 4°C. The supernatant (fraction 3) was transferred into a fresh sample tube and store on ice. This fraction primarily contains nuclear proteins. The pellet was resuspended in 500µl Extraction Buffer CE4 and used as suspension fraction 4. This fraction primarily contains cytoskeletal proteins. The purified membrane, cytoplasmic, nuclear and cytoskeleton proteins were precipitated by acetone and protein concentration was determined by the BCA Protein Assay Kit. Western blot was employed to detect the level of protein expression.

Immunofluorescence, TUNEL assay and Caspase-3/7 assay

For immunofluorescence, cells were seeded on coverslips in six-well plates and fixed with 4% paraformaldehyde (PFA) for 20 min at ambient temperature. After three PBS-T (PBS with 0.1% tween-20) washes, cells were blocked with 5% BSA in PBS-T for 30 min at 37°C , then blocked with the Image-It @FX Signal Enhancer Ready Probes Reagent (Life Technologies) 30 min at 37°C. Cells were incubated with the primary antibodies (Supplementary Table 1) in PBS-T with 1% BSA overnight at 4°C. After three washes (10 min each), the slides were either incubated with Alexa Fluor® 594 goat anti-rabbit or Alexa Fluor® 488 goat anti-mouse (Molecular Probes) prepared in PBS-T with 2% BSA. The slides were counterstained with Hoechst 33342 (Molecular Probes) and imaged using the confocal laser-scanning microscope LSM510 Meta (Carl Zeiss).

Cells were seeded on coverslips at 30% density. After the second day, siRNAs were added to the cells. Forty-eight hours post-transfection, cells were washed once gently and fixed by 4% PFA and processed for TUNEL assay using the DeadEcd^{TM} Fluorometric TUNEL System (Promega). The nuclei were stained with 7-AAD (Life Technologies) and pictures were taken using confocal laser-scanning microscope LSM510 Meta (Carl Zeiss). The caspase-3/7 activity following PRC1 siRNA treatment was detected by the Caspase-Glo® 3/7 Assay kit (G8090, Promega).

BrdU proliferation, Cell Counting Kit-8 (CCK-8) CCK8 proliferation, migration, invasion, and wound healing assays

The HCC cell lines were treated with siRNAs targeting PRC1 or scramble control genes. After 10h, the cells were digested with trypsin and 10,000-20,000 cells were seeded in 96-well plates. The BrdU proliferation assay kit (#6813, Cell Signalling Technology) was used. 10μl CCK-8 solution (Sigma) was added directly to the cells in 1% FBS DMEM medium for 2h and then measured at OD450nm to determine viability.

Migration and invasion assays were carried out in 24-well plates using Boyden chambers with an 8mm pore size PET membrane (Falcon). Briefly, cells were transfected with PRC1 siRNA; 24h post-transfection, cells were counted and processed for transwell assay. After 48h incubation, any non-invasive cells on the upper surface of the Matrigel membrane were gently removed using a cotton-tipped swab. The invasive cells were fixed in 100% methanol and stained with 1% toluidine blue. The stained invasive cells that had passed through to the lower surface of the membrane were photographed under an inverted light microscope with a 40× objective and

quantified by manual counting in three randomly selected areas. Results were obtained from three independent experiments performed in duplicate.

The wound healing assay was performed either with shPRC1- or shScramble-stably knockdown HCCLM3 cells with or without wnt3a treatment (1.5625ng /ml); or with scramble control, GAPDH, PRC1, KIF11, DTL, SPC25 siRNA-treated HCCLM3 cells. Briefly, when cells were grown to confluent, 100μl yellow tip was used to scratch the middle of the well and washed gently once with PBS. 1% FBS serum reduced DMEM was then added to limit cell proliferations during the assays. After culture, photos of different wound region were taken and the size of the wound was calculated by image-J software (NIH, USA) to determine the relative wound closure. The real migration ability of the HCC cells was calculated by analyzing the ratio of the relative wound closure with the relative proliferation of the cells by the CCK8 assay.

TOP/FOP luciferase reporter assay

Transcriptional activity assays were performed using the Luciferase Assay System (Promega) according to the manufacturer's instructions. Briefly, cells were transfected with siRNAs targeting PRC1 or scramble control. Twenty-four hours post-transfection, cells were co-transfected with the M50 Super 8 × TOPFlash or M51 Super 8 × FOPFlash with pRL at the ratio 50:1 by Lipofectamine 3000. In some of the experiments, cells were also co-transfected with β-catenin-expression vector, pEGFP-PRC-NLS-2A, or pEGFP-PRC-NLS-3A, for nuclear Wnt signalling studies. In some experiments, the cells were also treated with XAV939, LiCl or NaCl overnight. The luciferase activity of each sample was normalized against Renilla luciferase activity to monitor transfection efficiency. Twenty-four hours after transfection of plasmids, cells

were lysed and luciferase activity was measured using the Dual-Luciferase Reporter (DLR) Assay Kit (Promega). Firefly and Renilla luciferase activities were determined using a luminometer (Lumat LB9507, Berthold, Bad Wildbad, and Germany) and normalised.

Immunoprecipitation and Cycloheximide (CHX) chase assay

Protein G Dynabeads were pre-cleaned and incubated with antibodies (Listed in Supplementary Table 1) overnight in 5% BSA IP buffer at 4°C. After which, the beads were washed with IP buffer (20mM Tris pH8, 10% glycerol, 150mM NaCl, 0.1% NP-40, 0.1mM EDTA) three times on a magnet, where the beads migrate to the side of the tube facing the magnet. HCCLM3 cells were grown in a 15-cm dish to a density of 70-90% after different treatments and then lysed on ice with IP buffer for 30 min. Protein concentration was determined and 500-1000mg of protein lysate was added to the beads and incubated for 2h at 4°C. After which, the beads were washed six times. Finally, the proteins on the beads were boiled with 1× SDS loading buffer on a 95°C heat block for 10 min. The samples were analyzed by Western blotting and proteins were detected using anti-mouse (ab99697, Abcam) or anti-rabbit (ab99617, Abcam) secondary antibodies that specifically recognise the light chain. For EGFP-PRC1 truncates overexpression

HCCLM3 cells were transfected with scramble or PRC1 siRNAs; 24 h post treatment, the cells were incubated with 10% FBS DMEM containing 100μg/ml Cycloheximide (CHX). Then, at different time point post CHX treatment, cells were collected by trypsin treatment and lysates were prepared for Western blot assay.

Immunohistochemistry (IHC) analysis

Paraffin-embedded tissue samples from consented patients or xenograft mouse tissues derived from HCCLM3 were cut into 5μm sections and placed on poly-lysine-coated slides. Samples were deparaffinised in xylene and rehydrated using a series of graded alcohol. Antigen retrieval was performed by heat mediation in citrate buffer (pH 6; Dako). Samples were blocked with 10% goat serum before incubation with primary antibody. The samples were incubated overnight using following primary antibodies: rabbit anti-PRC1 (H70; 1:50), rabbit-anti-Survivin (Cell Signaling Technology, Danvers, MA, USA; 1:100), mouse anti-β-catenin (Clone 14, BD biosciences) (1:50), Mouse Anti-Glutamine Synthetase (GS, Clone 6, BD biosciences) (1:200) or an isotype-matched IgG as a negative control in a humidified container at 4℃. Immunohistochemical staining was performed with the Dako Envision plus System (Dako, Carpinteria, CA, USA) according to the manufacturer's instructions. The intensity of staining was evaluated on a scale of 1 to 4 according to the percentage of positive tumours.

Statistical analysis

Experimental data are presented as mean ± standard deviation (SD). All statistical analyses were performed using analysis of a two-tailed Student's t test with either GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) or Partek Genomics Suite software (Partek Incorporated, St. Louis, MO, USA). Survival curves were calculated using the Kaplan-Meier method. Differences were considered statistically significant when P values were less than 0.05. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ for all the analysis.

Supplementary Tables

Supplementary Table 1. List of antibodies used in the study.

Antibodies used for western blot				
Cat. No	**Antibody**	**Species**	**dilution**	**Company**
NB100-74508	PRC1(16F2)	Mouse	1/1000	Novus Biologicals
#2118	GAPDH (14C10)	Rabbit	1/5000	Cell Signaling Technology
sc-7199	β-Catenin (H-102)	Rabbit	1/10000	Santa Cruz Biotechnology
ab15196	Cyclin D1	Rabbit	1/200	Abcam
17-10109	TCF4	Mouse	1/1000	Millipore
#2568	P-Ser1490-LRP6	Rabbit	1/1000	Cell Signaling Technology
#3395	LRP6 (C47E12)	Rabbit	1/1000	Cell Signaling Technology
#8814	Active β-Catenin (ABC)	Rabbit	1/2000	Cell Signaling Technology
#2808	Survivin (71G4B7)	Rabbit	1/1000	Cell Signaling Technology
sc-24	HSP70 (W27)	Mouse	1/5000	Santa Cruz Biotechnology
#9322	P-Ser9-GSK3β(D3A4)	Rabbit	1/1000	Cell Signaling Technology
#9832	GSK3β(3D10)	Mouse	1/2000	Cell Signaling Technology
#9561	P-β-Catenin(S33/37/T41)	Rabbit	1/1000	Cell Signaling Technology
#2087	Axin1(C76H11)	Rabbit	1/1000	Cell Signaling Technology
ab75732	LGR5	Rabbit	1/1000	Abcam
#8198	Met(D1C2)	Rabbit	1/1000	Cell Signaling Technology
#3801	MMP-7(D4H5)	Rabbit	1/1000	Cell Signaling Technology
ab3280	β-Actin	Mouse	1/1000	Abcam
ab15270	APC	Rabbit	1/500	Abcam
ab109307	Axin2	Rabbit	1/1000	Abcam
ab137097	DUT	Rabbit	1/10000	Abcam
ab17792	LAMC1	Rat	1/500	Abcam
#5605	c-Myc(D84C12)	Rabbit	1/1000	Cell Signaling Technology
#9165	c-Jun(60A8)	Rabbit	1/1000	Cell Signaling Technology
#2203	TCF1(C63D9)	Rabbit	1/1000	Cell Signaling Technology
ab109416	DKK1	Rabbit	1/2000	Abcam
ab16048	Lamin B1	Rabbit	1/2000	Abcam
T6199	α-Tubulin(DM1A)	Mouse	1/3000	Sigma
3H9	GFP(3H9)	Rat	1/1000	ChromoTek
#3936	Ubiquitin(P4D1)	Mouse	1/5000	Cell Signaling Technology
NBP2-03654	β-TrCP(3D5)	Mouse	1/500	Novus Biologicals
PA5-30933	Tankyrase	Rabbit	1/2000	Thermofisher Scientific

Antibodies used for IP				
Cat. No	**Antibody**	**Species**	**Company**	
sc-8356	PRC1 (H-70)	Rabbit	Santa Cruz Biotechnology	

ab15270	APC	Rabbit	Abcam	
sc-7199	β-Catenin (H-102)	Rabbit	Santa Cruz Biotechnology	
gtm-100	GFP-Trap-M	NA	ChromoTek	
I5006	IgG control	Rabbit	Sigma	
ab99617	Rat monoclonal [187.1] Secondary Antibody to Mouse kappa - light chain (HRP)	Rat	Abcam	
ab99697	Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP)	Mouse	Abcam	

Antibodies used for IF

Cat. No	Antibody	Species	Company	Dilution
sc-8356	PRC1 (H-70)	Rabbit	Santa Cruz Biotechnology	1/200
T6199	α-Tubulin(DM1A)	Mouse	Sigma	1/1000
ab6528	Pan-Cadherin	Mouse	Abcam	1/200
sc-7199	β-Catenin (H-102)	Rabbit	Santa Cruz Biotechnology	1/500
05-1579	Axin1(A5)	Mouse	Millipore	1/50
#9832	GSK3β(3D10)	Mouse	Cell Signaling Technology	1/50
sc-14029	Axin	Rabbit	Santa Cruz Biotechnology	1/100
#3936	Ubiquitin	Mouse	Cell Signaling Technology	1/100
sc-9998	APC (F-3)	Mouse	Santa Cruz Biotechnology	1/50
ab15270	APC	Rabbit	Abcam	1/100
PA5-29265	GSK3β	Rabbit	Thermofisher Scientific	1/100
ab16048	Lamin B1	Rabbit	Abcam	1/1000
558606	Alexa Fluor® 647 Mouse anti-β-Tubulin Clone 5H1	Mouse	BD Biosciences	1/40, 37°C, 30min

Supplementary Table 2. Summary of patient characteristics for the survival analysis.

Group	**PRC1 low** ***(n=33)***	**PRC1 high** ***(n=32)***
Gender		
Male	29	29
Female	4	3
Age		
≤60	13	19
>60	20	13
Tumor venous Infiltration		
VI	10	14
NI	23	18
Cirrhosis status		
Yes	15	18
No	16	14
Not available	2	0
Tumor size		
≤5cm	21	16
>5cm	12	16
AJCC Stage		
I	23	17
II & III	10	15
Child's grade		
A	26	23
B	7	9

Supplementary Table 3. Univariate and multivariate analyses showing that PRC1 expression could serve as an independent prognostic factor for the early recurrence of HCC.

Variables	Univariate Analysis		Multivariate Analysis	
	RR (95% CI)	P-value	RR (95% CI)	P-value
Age				
≤60 years (n = 32) vs. >60 years (n = 33)	0.513 (0.25-0.10)	0.059		n.s.
Gender				
Male (n = 58) vs. Female (n = 7)	0.407 (0.097-1.708)	0.219		n.s.
Cirrhosis				
Yes (n= 33) vs. no (n = 30)	2.02 (0.99-4.09)	0.050		n.s.
AJCC Stage				
I (n = 40) vs. II & III (n = 25)	1.95 (1.00-4.00)	0.049*		n.s.
Tumor venous Infiltration				
VI (n = 24) vs. NI (n = 41)	2.41 (1.20-4.83)	0.013*		n.s.
Child-Pugh Score				
A (n= 49) vs. B (n = 16)	2.05 (1.01-4.18)	0.046*		n.s.
Tumor size				
≤5 cm (n = 37) vs. >5 cm (n = 28)	2.11 (1.07-4.16)	0.029*		n.s.
PRC1 expression				
High (n= 33) vs. low (n = 32)	4.16 (1.92-9.01)	0.000**	3.95 (1.82-8.56)	0.000**

NOTE: n.s. is not significant; P < 0.05 is significant

Univariate and multivariate Cox regression analysis was performed to determine whether PRC1 expression could serve as an independent survival prognostic factor for the early recurrence of HCC. In univariate analysis, the factors like AJCC Stage, Tumor venous Infiltration, Child-Pugh Score, Tumor size and PRC1 expression was significantly associated with the early recurrence of HCC. In Multivariate survival analysis using the Cox's regression model also showed that PRC1 alone was statistically significant factor for identifying early HCC recurrence. In both Univariate and Multivariate analysis, PRC1 expression has a P-value of 0.000, suggesting that it could serve as an independent survival prognostic factor for the early recurrence of HCC.

Supplementary Table 4. Primers used in the study.

Primers for In-fusion construction	
Name	**Sequence**
EGFP-PRC1-F (FL 1-620)	GGACTCAGAT**ctcgag**Caggagaagtgaggtgctg
EGFP-PRC1-R (FL 1-620)	CCGCGGTACC**gtcgac**TCAGGACTGGATGTTGGTT
EGFP-PRC1-R (CC 1-341)	CCGCGGTACC**gtcgac**TCATAACCGCACAATCTCA
EGFP-PRC1-R (ΔC1-486)	CCGCGGTACC**gtcgac**TCAACGTGCTTTGCCCGGT
EGFP-PRC1-F (ΔCC 341-620)	GGACTCAGAT**ctcgag**Cttaaaaaactactatgaag
NLS-2A-F	AACACCTAGCgcggcgCGAGGACTGGCTCC
NLS-2A-R	CGAGGAGCGCTGCCATAC
NLS-3A-F	cccaatacaccgggcgcAGCACGTAAGCTGAACAC
NLS-3A-R	agccagtcctcgcgccgcGCTAGGTGTTCGAGGAGC
Primers for Real-time PCR	
Name	**Sequence**
PRC1 F	CCTATTCTGAGTTTGCGAAGGA
PRC1 R	TGATCAGGGCTTCTCAGGAC
DUT F	TTGACTTCAAACTTTTCTTTGCC
DUT R	GCTTGGCTGCAAAACACTTT
POLD3 F	GGTAACATGCAGTTGGGCTC
POLD3 R	AGTTCGTCACGGACCAAAAC
LAMA3 F	GATCAGGGCGAGGAGAATTT
LAMA3 R	CCTCAGGCACACAGTACAACA
PLXNC1 F	GAACCCTCAGTTTGTCTTTG
PLXNC1 R	AGCTTATTAGTTGGTGCTTC
LEF1 F	ACTCTAAAAGAAAGTGCAGC
LEF1 R	ACCATAATTGTCTCTTGCAG
TCF1 F	AGACTATGCTCATCACCG
TCF1 R	GTCTGAGGTGAAGACCTG
MYC F	TGAGGAGGAACAAGAAGATG
MYC R	ATCCAGACTCTGACCTTTT
AXIN2 F	ACAACAGCATTGTCTCCAAGCAGC
AXIN2 R	GCGCCTGGTCAAACATGATGGAAT
DKK1 F	TCCGAGGAGAAATTGAGGAA
DKK1 R	CCTGAGGCACAGTCTGATGA
SLC13A3 F	ACCAGTACACCGCCATGAG
SLC13A3 R	CTGCTGGTGCTGCTGTTC
RELN F	TGGGTTGAATGTAACAACTG
RELN R	CATATGGTTCACAGAAGGTG

LGR5 F	AAATGCCTTATGCTTACCAG
LGR5 R	ATCTTGAGCCTGAAACATTC
RHBG F	CCTCAAGTGAAATGATGCTG
RHBG R	ATTTTGATTCAAGGATGGGC
MMP7 F	GGGATTAACTTCCTGTATGC
MMP7 R	GATCTCCATTTCCATAGGTTG
DTL F	GATGGGTTTTATAGGCAAGTG
DTL R	GCAAGTCCTTTTGAATTCTG
SURVIVIN F	CATCTCTACATTCAAGAACTGG
SURVIVIN R	CCTTGAAGCAGAAGAAACAC
B-ACT F	AATCTGGCACCACACCTTCTA
B-ACT R	ATAGCACAGCCTGGATAGCAA
CYCD1 F	GGCGGAGGAGAACAAACAGA
CYCD1 R	TGGCACAAGAGGCAACGA
LAMC1 F	GTAAATGGCAAAGCTCTCCG
LAMC1 R	CAGTACCCCAGCTCCATCAA
KIF11 F	GGAAACTCTGAGTACATTGG
KIF11 R	GAGTTTCTGATTCACTTCAGG
SPC25 F	GTTCCTACAAAGGACATGAAC
SPC25 R	TTTAAAGACACATGCACACG
GAPDH F	ATGGGGAAGGTGAAGGTCG
GAPDH R	GGGGTCATTGATGGCAACAATA
18S F	GTAACCCGTTGAACCCCATT
18S R	CCATCCAATCGGTAGTAGCG
KIF23 F	CCAAATGGTAGTCGAAAACGAAGA
KIF23 R	GGGACTGTCAGTTCATGGCT
FANCI F	GAGCTATTGGATGTTGTCAC
FANCI R	TTCTCTGCCTAGTTCATAGTC

Supplementary Table 5. Summary of patient characteristics of the 25 participants employed for the IHC assay in Figure 7.

Group	PRC1 low *(n=14)*	PRC1 high *(n=11)*
Gender		
Male	10	10
Female	4	1
Age		
≤ 60	10	8
>60	4	3
AFP		
≤20 ng	3	4
>20 ng	11	7
Cirrhosis status		
Yes	10	9
No	4	2
Tumor size		
≤5 cm	7	6
>5 cm	7	5
TNM Stage		
I	12	9
II & Above	2	2
Early Recurrence		
Yes	9	10
No	5	1

Supplementary Table 6. Summary of patient characteristics of the 14 participants employed for the western blot in vivo assay in Figure 7.

Group	Recurrence *(n=7)*	Non-Recurrence *(n=7)*
Gender		
Male	7	7
Female	0	0
Age		
≤60	7	5
>60	0	2
AFP		
≤20 ng	3	5
>20 ng	4	2
Cirrhosis status		
Yes	3	3
No	4	4
Tumor size		
≤5 cm	3	4
>5 cm	4	3
TNM Stage		
I	5	7
II & Above	2	0
BCLC Stage		
A	5	7
B&C	2	0

Supplementary Table 7. Summary of patient characteristics of the 20 HCC patients employed for the Glutamine Synthetase (GS) staining.

Group	Recurrence *(n=10)*	Non-Recurrence *(n=10)*
Gender		
Male	9	9
Female	1	1
Age		
≤60	9	6
>60	1	4
AFP		
≤20 ng	3	7
>20 ng	7	3
Cirrhosis status		
Yes	6	3
No	4	7
Tumor size		
≤5 cm	5	7
>5 cm	5	3
TNM Stage		
I	7	7
II & Above	3	3
BCLC Stage		
O&A	9	9
B&C	1	1

Supplementary Figure Legends

Supplementary Figure 1 PRC1 expression is associated HCC recurrence, vascular invasion, tumor stage, and PRC1 is highly expressed in HCC cell lines. The real-time PCR validation of PRC1 expression in (A) 20 pairs of HCC patients' tissues (HCC= HCC tumors; MN= HCC matched adjacent normal tissues, or in (B) HCC recurrent tumors (HCC-R, n=10) and non-recurrent tumors (HCC-NR, n=7). PRC1 gene expression in TCGA RNAseq database indicates that higher PRC1 expression samples are correlated with (C) more positive vascular invasion and (D) higher AJCC tumor stage. (E) PRC1 expression in a panel of HCC cell lines and two MN tissues, GAPDH was used as loading control.

Supplementary Figure 2 Depletion of PRC1 inhibits proliferation, induces apoptosis and suppresses migration of HCC cells. (A) The knockdown of PRC1 in four HCC cell lines shown by western blot, GAPDH is used as the loading controls. (B) Representative figures showing TUNEL staining of three HCC cell lines after silencing PRC1. TUNEL positive cells is indicated by green color, DNA was stained by 7-AAD (in red color). (C) TUNEL-positive cells were quantitated as a percentage of total whole cells per field (*n=5*, 20-60 cells were counted for each quantitation), and (D) Caspase 3/7 relative activity (*n=4*) in cells after silencing by PRC1- or Scramble-siRNA. (E) Western blot analysis of PRC1, and cleaved Caspase-3 in PRC1-stably knocked down cells. (F) Modified wound healing migration assay of shPRC1- or shScramble-stably knocked down HCCLM3 cells (in 1% FBS). The modified migration was shown by the ratio of relative wound closure and proliferation using CCK8 assays.

Supplementary Figure 3 PRC1 is directly regulated by β-catenin/TCF4 in HCC cells. The effect of silencing: (A) β-catenin, or (B) TCF4 on PRC1 or Survivin protein expression in HCC cells. (C) DNA gel analysis of the relative fold enrichment of the fragments on PRC1 or Survivin promoters after 2h Wnt3a-induction using ChIP method with TCF4 or IgG antibodies. (D) A diagram sketch of Wnt pathway inhibitors and their potential targets.

Supplementary Figure 4 Silencing PRC1 impairs Wnt signal in HCC cells. (A) The induction of Wnt/TCF luciferase reporter activity in Wnt3a-treated HCCLM3 or HuH-7 cells after knocking down PRC1 expression. (B) Western blot showing expression of the Wnt signaling targets in scramble or PRC1 siRNAs-treated HuH-7 with or without Wnt3a stimulation (1.5625ng/ml) for 2h and 24h. (C) The endogenous Wnt/TCF reporter activity in shScramble- or PRC1-stably knocked down HCCLM3 or HuH-7 cells, *n=3*. (D) Western blot assay showing expression of the endogenous Wnt signaling components following shScramble- or PRC1-stably knocked down HCCLM3 or HuH-7 cells.

Supplementary Figure 5 PRC1 targets the Wnt signaling on the upstream of GSK3β/β-Catenin. (A) The relative Wnt/TCF activity analysis in scramble or PRC1 siRNA-treated HCCLM3 cells after co-transfected with β-catenin or *β-catenin (Constitutively activated β-catenin) overexpression plasmids, or treated with DMSO or iCRT3 inhibitor. (B) Western blot and Wnt/TCF activity analysis in the HepG2 cells treated with scramble or PRC1 siRNAs with or without Wnt3a (1.5625ng/ml, overnight) stimulation. (C) BrdU proliferation assay and transwell migration invasion assays of HepG2 cells treated with scramble or PRC1 siRNAs post-48h (D)

Western blot analysis of the expression of PRC1, Axin1 or Tankyrase and relative changes in Wnt/TCF activity of XAV939-treated HCCLM3 cells at different dosages after silencing with scramble or PRC1 siRNAs. (E) Western blot analysis of the expression of PRC1 and GSK3β and relative changes in Wnt/TCF activity in LiCl- or NaCl-treated HCCLM3 cells at 30mM after silencing PRC1expression.

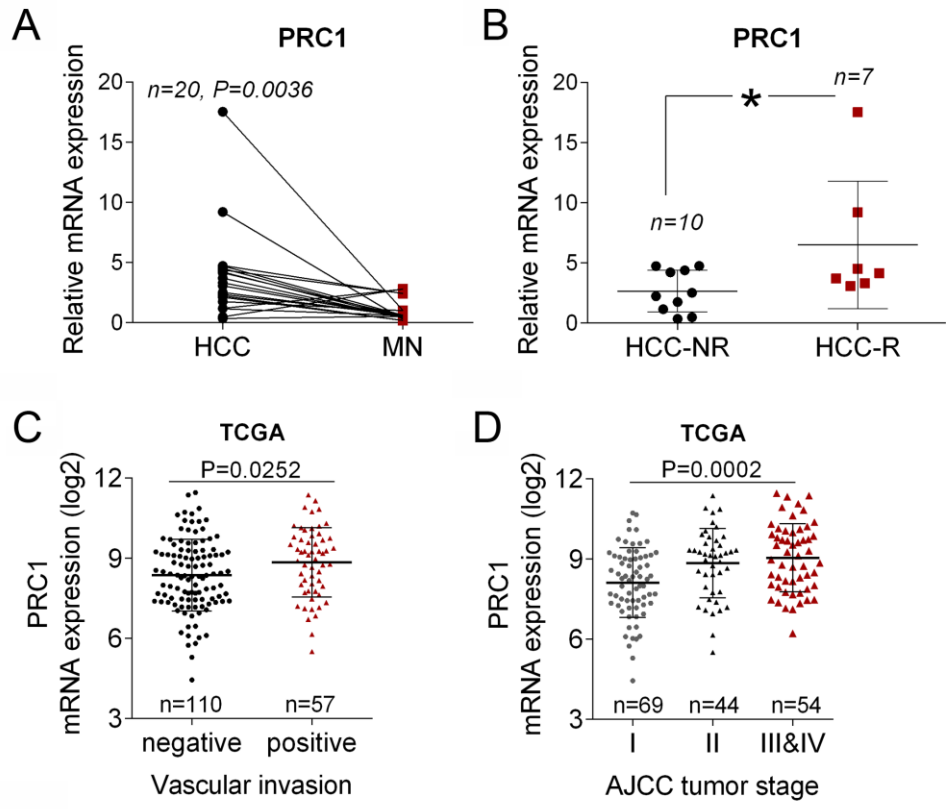
Supplementary Figure 6 PRC1 physically interacts with Axin1/APC/GSK3β destruction complex proteins via its MTB domain and PRC1 regulates Wnt signalling mainly outside of the nucleus. (A) Representative illustrations of the structures and sizes of different PRC1 truncates fused with EGFP, CC, Coiled-coil domain; MTB, MT binding domain; C, C-terminal domain. (B) Immunofluorescence staining of α-Tubulin (in red color) and transiently-expressed GFP-PRC1 truncates in HCCLM3 cells. (C) GFP-Trap beads immunoprecipitation and western blotting assay of the Axin1/APC/GSK3β destruction complex proteins in different GFP-PRC1 truncate-expressing lysates. (D) The NLS sequences of FL, NLS-2A (two point mutations) and NLS-3A (three point mutations), the red letters indicate critical conserved sites in PRC1 NLS. (E) Immunofluorescence staining of the FL-PRC1 and two NLS mutants GFP fusions (green), the nuclear envelop marker-Lamin B1 (red) was used to show the nuclear. Quantitation of the ratio of the nuclear PRC1-positive cells (20-30 GFP-positive cells were counted, *n=4*). (F) Western blot to show PRC1 siRNA targets the 3'-UTR regions of PRC1 mRNA at 48h post-transfection. (G) Wnt luciferase reporter TOP/FOP ratio analysis of siPRC1-UTR and transient expression of FL-PRC1 and two NLS mutants in endogenous PRC1 depletion HCCLM3 cells.

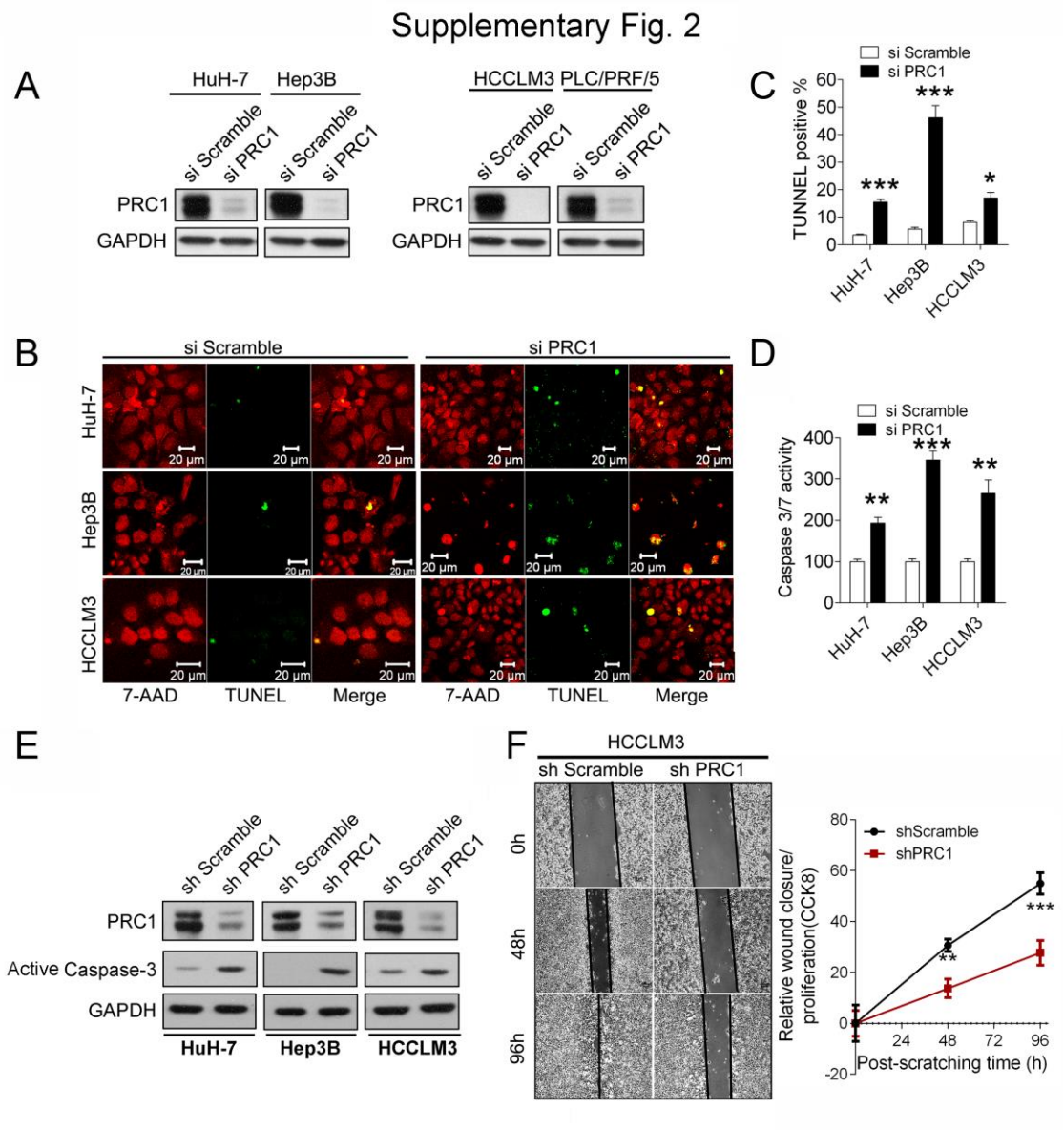
Supplementary Figure 7 PRC1 promotes β-catenin stabilization by blocking β-catenin binding to the APC complex. (A) The effect of depletion of PRC1 on the expression of cytoplasmic stabilized β-catenin during the 2h Wnt3a stimulation. The relative expression of β-catenin was quantitated and indicated on the top of the tracks. (B) The inhibition on the β-catenin and APC interaction in HCCLM3 cells via increased transient expression of EGFP-PRC1-FL or EGFP. The cells were treated with MG132 (10μg/ml) for 2h before samples were collected. (C) The real-time PCR analysis of the APC gene expression in HCCLM3 cells after treating with scramble, APC or PRC1 combined with APC siRNAs.

Supplementary Figure 8 Recurrence-free survivals analysis of eight selected recurrence-specific WRRAGs to confirm that they are recurrence-associated genes.

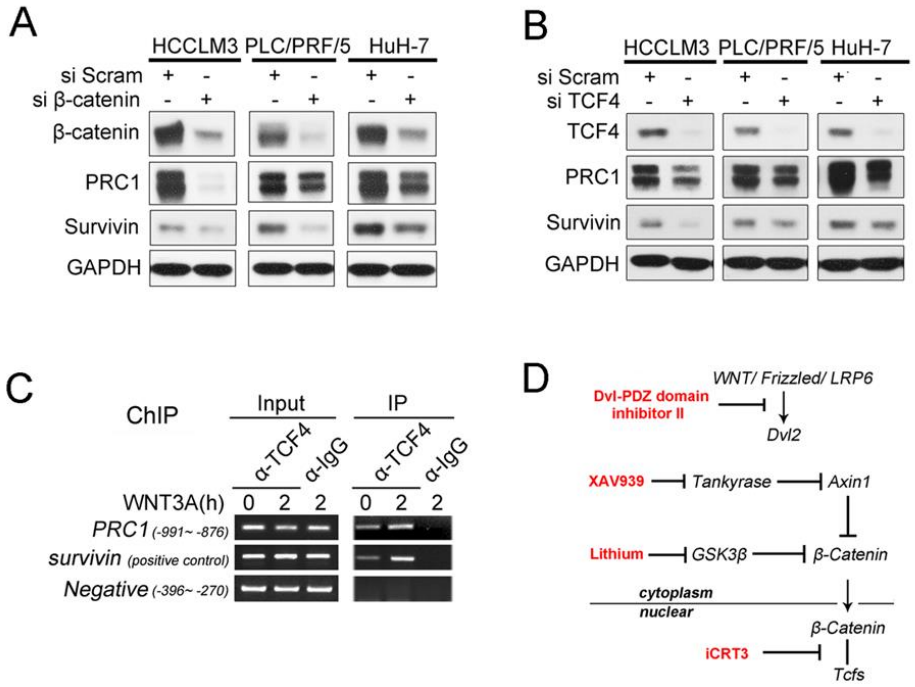
Supplementary Figure 9 Representative illustrations of Glutamine Synthetase (GS) IHC staining of 10 HCC-R and 10 HCC-NR tissues (Summary of patient characteristics is provided in Supplementary Table 7). Quantification of the GS expression between HCC-R and HCC-NR were scored and analyzed.

Supplementary Fig. 1

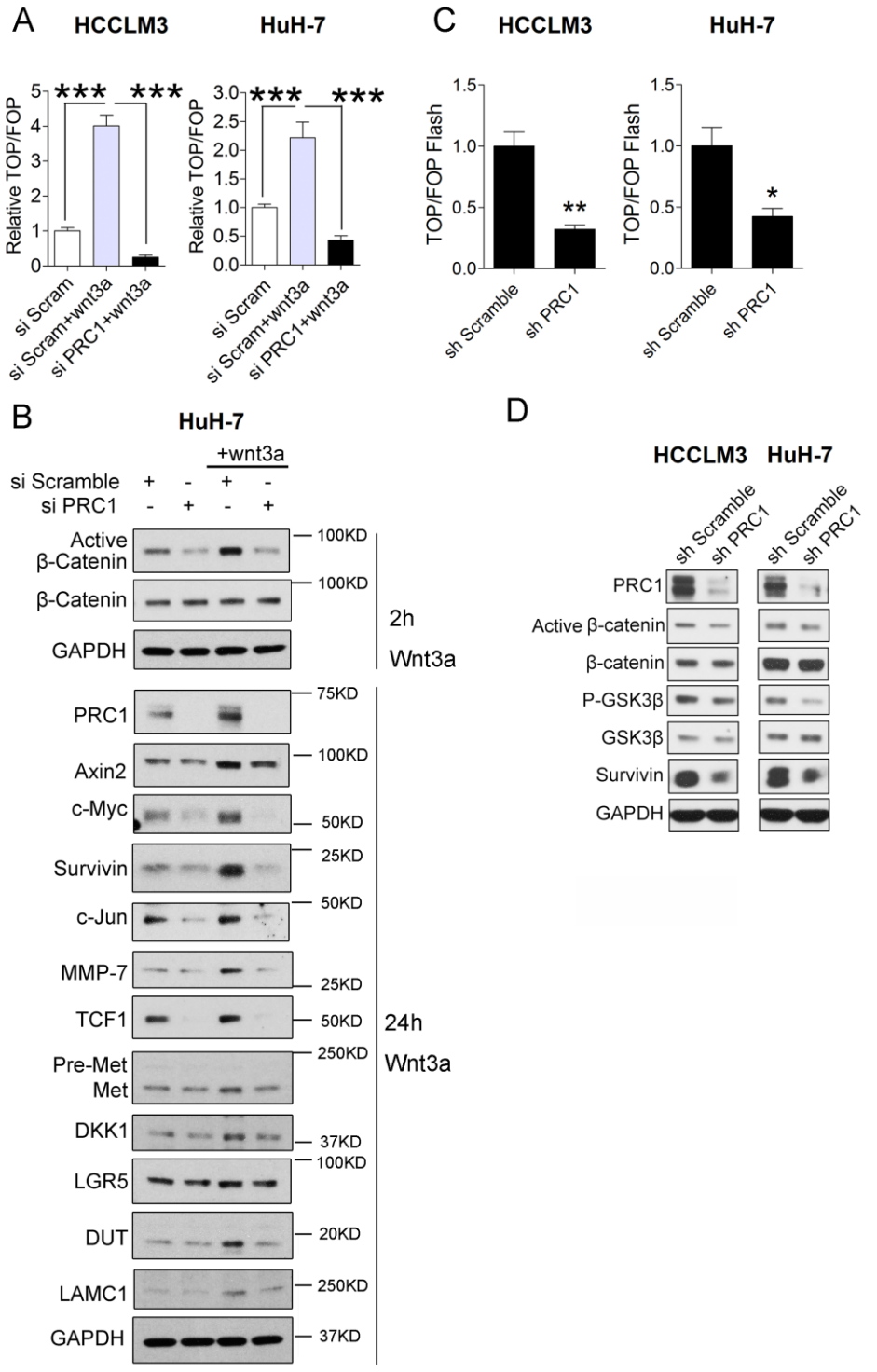



Supplementary Fig. 2
A
HuH-7
Hep3B
HCCLM3
PLC/PRF/5
si Scramble
si PRC1
PRC1
GAPDH
B
si Scramble
si PRC1
HuH-7
Hep3B
HCCLM3
20 μm
7-AAD
TUNEL
Merge
C
si Scramble
si PRC1
TUNEL positive %
D
Caspase 3/7 activity
E
sh Scramble
sh PRC1
PRC1
Active Caspase-3
GAPDH
F
HCCLM3
sh Scramble
sh PRC1
0h
48h
96h
shScramble
shPRC1
Relative wound closure/ proliferation(CCK8)
Post-scratching time (h)

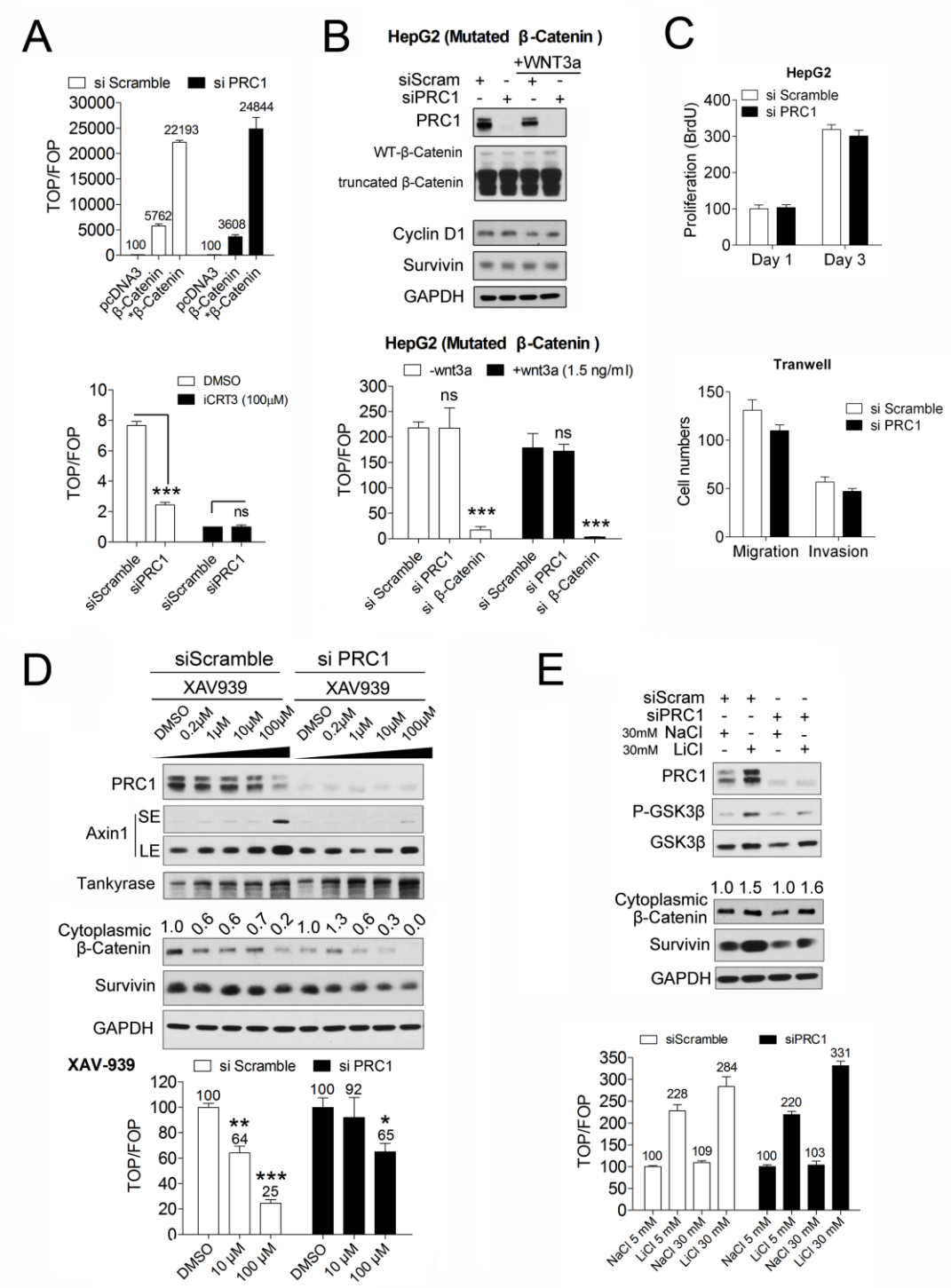
Supplementary Fig. 3



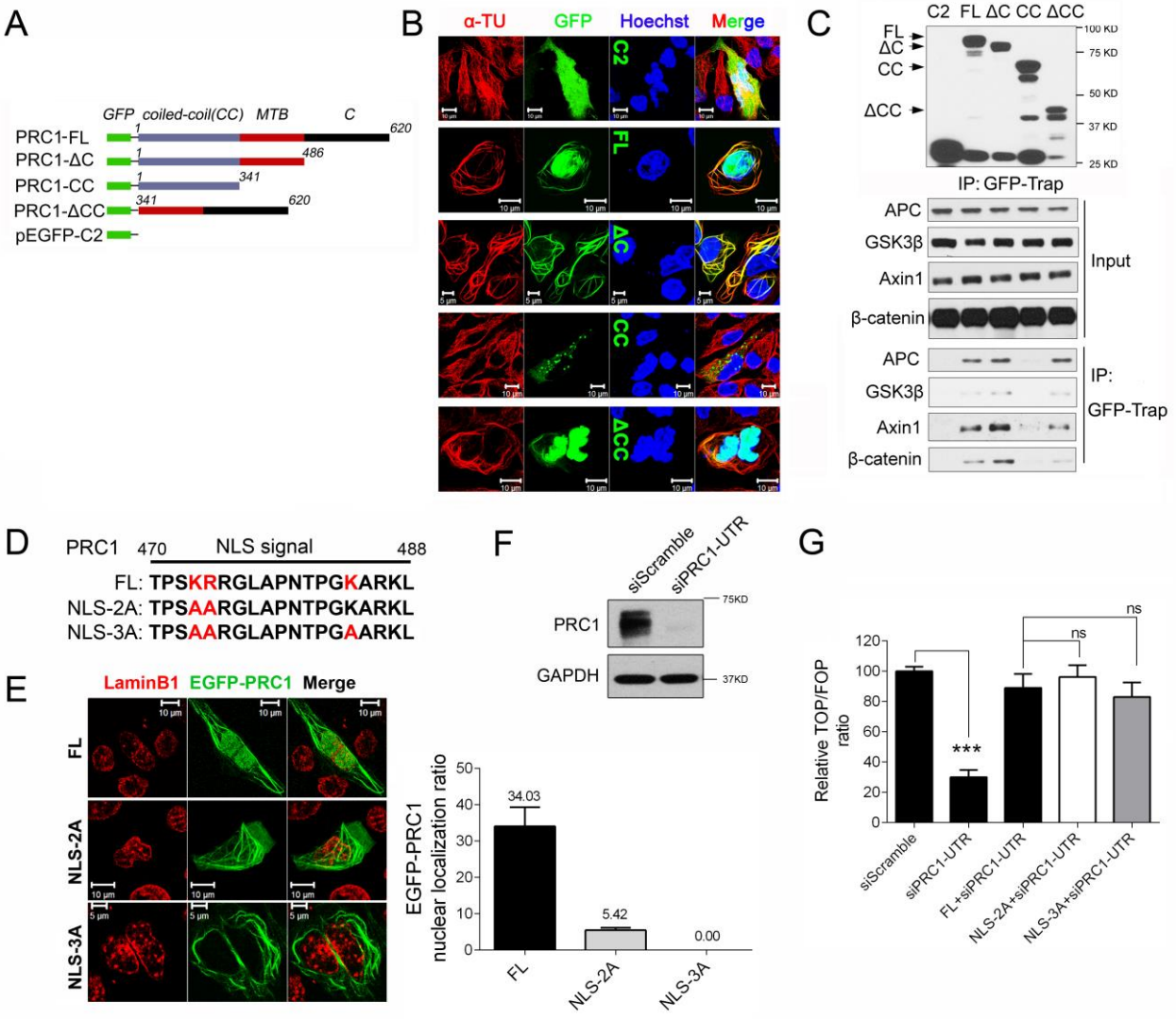
Supplementary Fig. 4



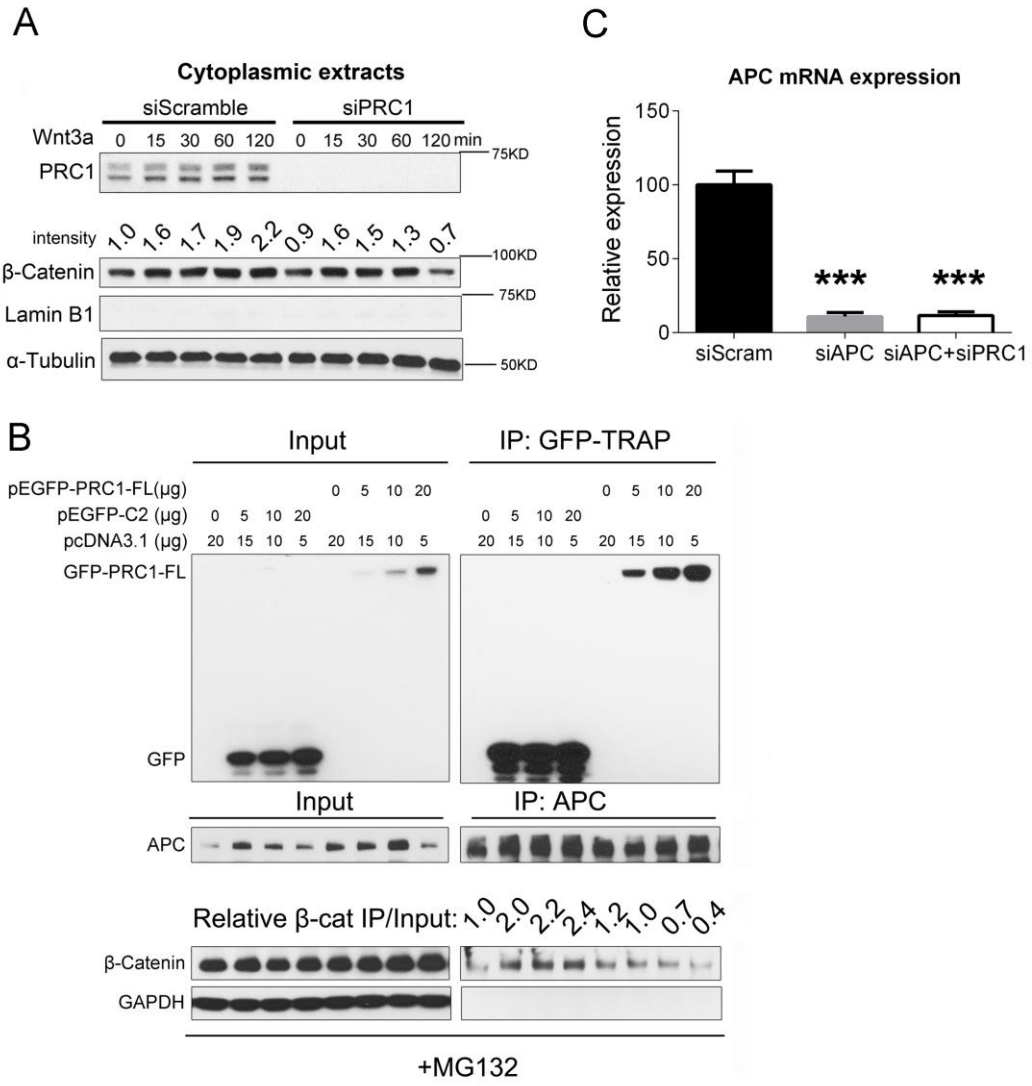
Supplementary Fig. 5



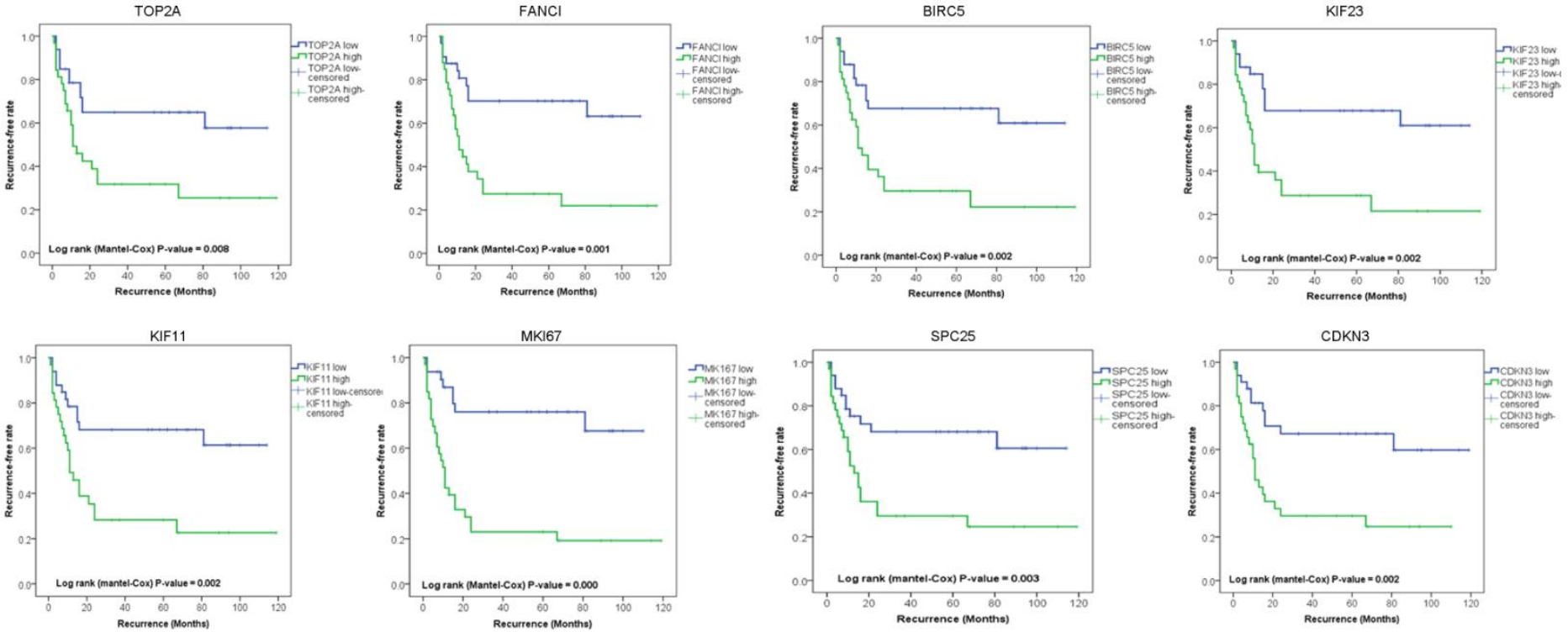
Supplementary Fig. 6



Supplementary Fig. 7



Supplementary Fig. 8



Supplementary Figure 9