

Supplemental Material

Cell cycle–related kinase is a direct androgen receptor–regulated gene that drives β -catenin/T cell factor–dependent hepatocarcinogenesis

Hai Feng, Alfred S.L. Cheng, Daisy P. Tsang, May S. Li, Minnie Y. Go, Yue S. Cheung, Gui-jun Zhao, Samuel S. Ng, Marie C. Lin, Jun Yu, Paul B. Lai, Ka F. To and Joseph J.Y. Sung

Supplemental Figures

Figure S1. Quantitative ChIP-PCR analysis of AR direct target genes in HCC cells.

Figure S2. Effect of bicalutamide on HCC cell growth.

Figure S3. Effects of androgen, AR and protein synthesis inhibitor on *CCRK* gene expression.

Figure S4. Effect of *CCRK* on AR-induced cell cycle progression.

Figure S5. In vitro kinase assay of the phosphorylation of recombinant GSK3 β by recombinant *CCRK*.

Figure S6. Effect of *CCRK* on expression of β -catenin pro-proliferative target genes.

Figure S7. Expression of active β -catenin and its target genes in xenograft tumor tissues.

Supplemental Tables

Table S1. AR direct target genes in Huh7 and PLC5 HCC cells.

Table S2. AR, *CCRK* and β -catenin immunohistochemical scores in HCC tissues.

Table S3. Primers used for ChIP-PCR and RT-PCR in this study.

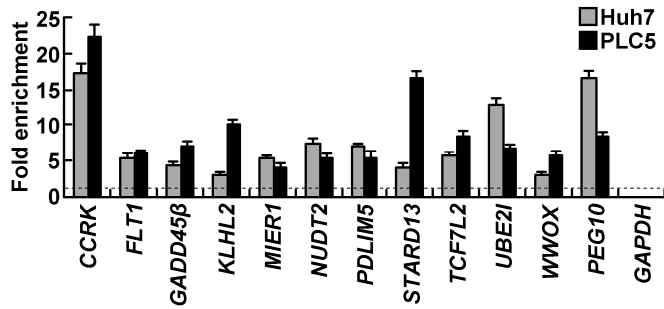


Figure S1. Quantitative ChIP-PCR analysis of AR direct target genes in HCC cells.

Quantitative ChIP-PCR validation of AR occupancy in 10 randomly-selected loci in both Huh7 and PLC5 cells. Fold enrichment represents ratio of DNA amounts pulled-down from AR antibody over IgG control, and each error bar represents standard deviation calculated from triplicates. The fold enrichments of *CCRK* and a known AR target, *PEG10*, are shown. *GAPDH* locus serves as a negative control.

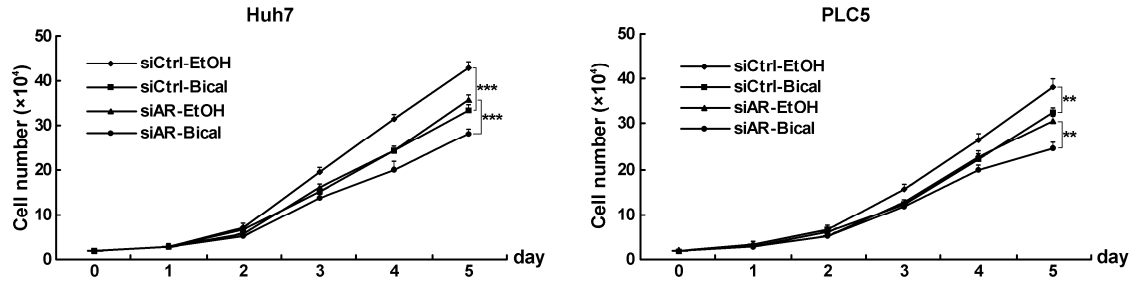


Figure S2. Effect of bicalutamide on HCC cell growth.

Inhibiting AR activity retarded HCC cell growth. Huh7 and PLC5 cells (2×10^4) seeded on 24-well plate were transfected with 25 nM siAR or siCtrl in the presence or absence of $10 \mu\text{M}$ bicalutamide. The cell numbers were determined by trypan blue dye exclusion every 24 hours for 5 consecutive days after transfection.

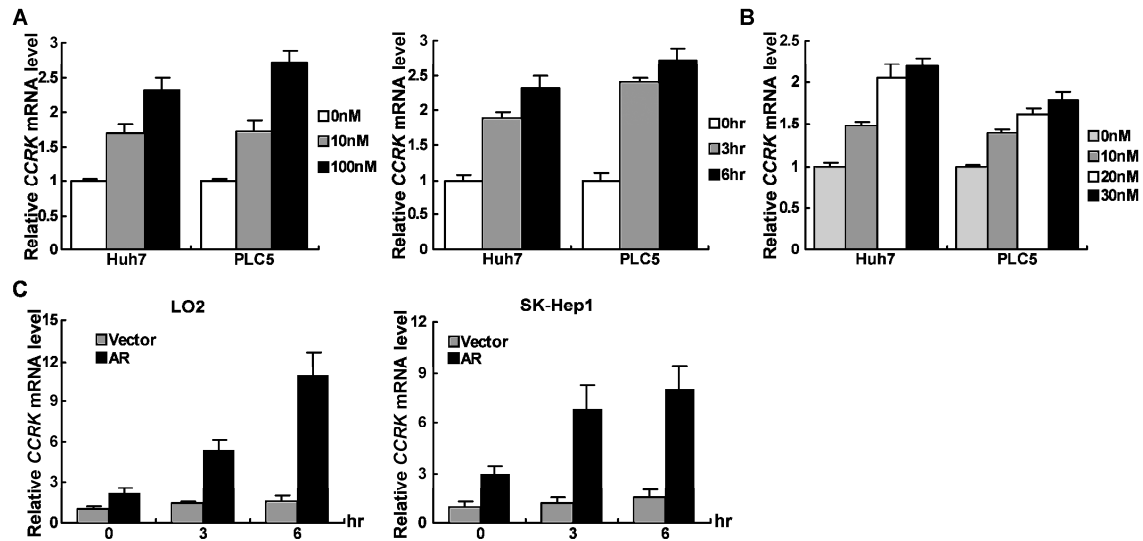


Figure S3. Effects of androgen, AR and protein synthesis inhibitor on *CCRK* gene expression.

(A) R1881 directly affected *CCRK* transcription. Quantitative RT-PCR showed that *CCRK* transcript level was increased by R1881 in a dose- and time-dependent manner in the presence of 50 μM cycloheximide. HCC cells were treated with different doses of R1881 for 6 hours (left) or with 100 nM R1881 for different periods of time (right). *GAPDH* was used as an internal control. (B) Physiological concentration range of testosterone increased *CCRK* transcript levels. Quantitative RT-PCR analysis of *CCRK* transcript level was performed in HCC cells treated with different doses of testosterone for 6 hours. (C) R1881-induced *CCRK* transcription was AR-dependent. Relative transcript level of *CCRK* was measured by quantitative RT-PCR in AR-expressing or control LO2 (left) and SK-Hep1 (right) cells stimulated with 100 nM R1881 for the indicated time.

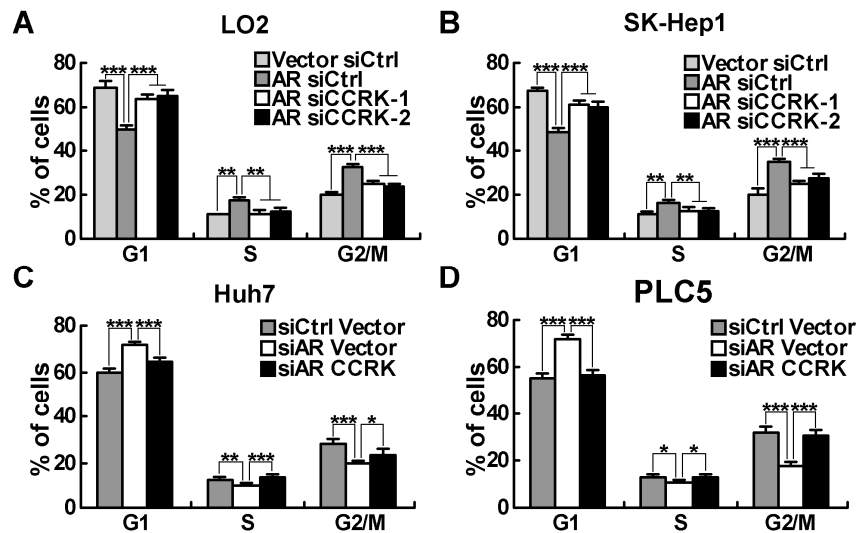


Figure S4. Effect of CCRK on AR-induced cell cycle progression.

(A and B) AR-induced G1-S cell cycle progression was abrogated by CCRK down-regulation. The cell cycle distribution of R1881-stimulated (A) Vec-LO2 and AR-LO2 and (B) Vec-SK-Hep1 and AR-SK-Hep1 cells treated with siCtrl, siCCRK-1 or siCCRK-2 were measured by propidium iodide staining and flow cytometry. (C and D) Ectopic CCRK expression attenuated G1 phase arrest in AR-knockdown cells. Flow cytometry was performed in R1881-stimulated (C) Huh7 and (D) PLC5 cells transiently transfected with siCtrl or siAR and CCRK-expressing or empty vector. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

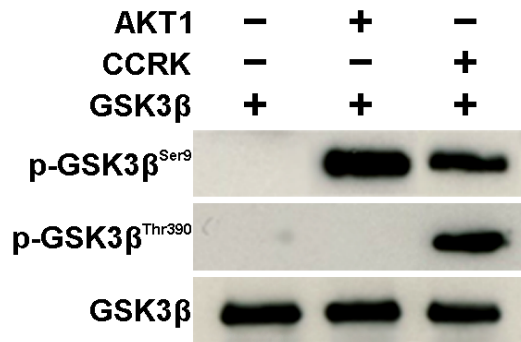


Figure S5. In vitro kinase assay of the phosphorylation of recombinant GSK3β by recombinant CCRK.

Recombinant CCRK directly phosphorylated recombinant GSK3β at Ser9 and Thr390. Proteins were incubated in the presence of ATP, and the reaction products were analyzed by immunoblotting using the same antibodies to Ser9-, Thr390-phosphorylated GSK3β and total GSK3β as that used in Figure 6. AKT1, known to induce phosphorylation of GSK3β at Ser9, was used as a positive control.

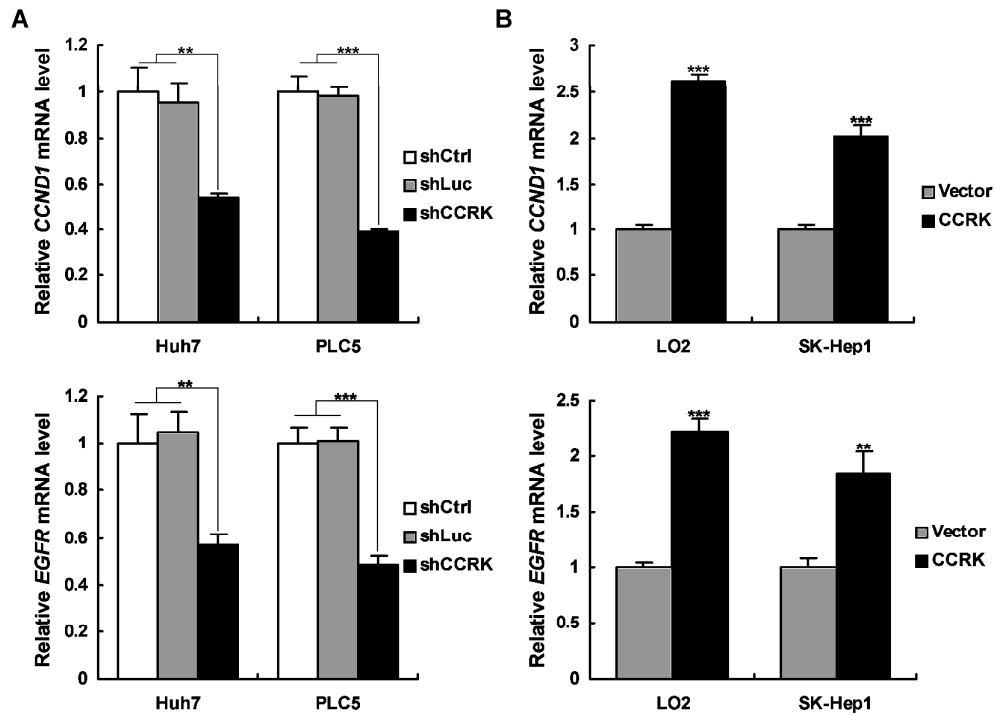


Figure S6. Effect of CCRK on expression of β -catenin pro-proliferative target genes

Expressions of *CCND1* and *EGFR* were modulated by CCRK. (A) Compared with shCtrl- and shLuc-transfectants, *CCND1* and *EGFR* expressions were decreased in Huh7 and PLC5 cells stably expressing shCCRK as detected by quantitative RT-PCR. *GAPDH* was used as an internal control. (B) Stable expression of CCRK in LO2 and SK-Hep1 cells up-regulated *CCND1* and *EGFR* expressions. ** $p < 0.01$, *** $p < 0.001$.

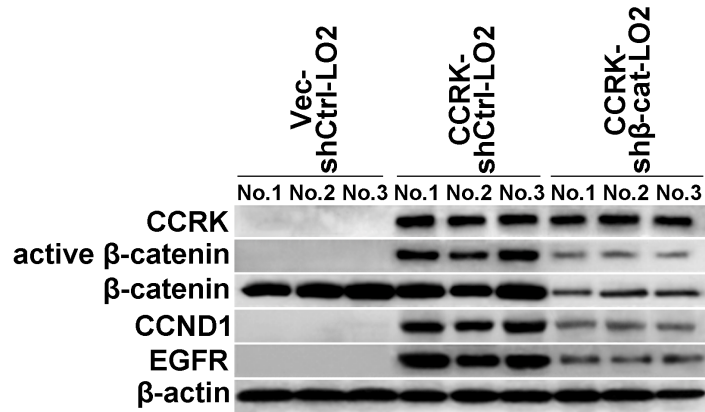


Figure S7. Expression of active β -catenin and its target genes in xenograft tumor tissues. Expressions of CCRK, CCND1, EGFR, active and total β -catenin in the Vec-shCtrl-LO2, CCRK-shCtrl-LO2 and CCRK-sh β -catenin-LO2 xenograft tumor tissues were detected by Western blot. β -actin was used as a loading control.

Table S1. AR direct target genes in Huh7 and PLC5 HCC cells.

Huh7 cell line

¹ Gene	² Chromosome	² Start	² End	³ MinP[x]
ABCA1	chr9	106703644	106735732	9.90E-04
ACE2	chrX	15526648	15534535	1.84E-04
ADAMTSL1	chr9	18458865	18466713	9.59E-04
ADAR	chr1	152844950	152871257	4.40E-03
ADH7	chr4	100573321	100580684	3.58E-04
AMN1	chr12	31770868	31778797	3.59E-03
APAF1	chr12	97563218	97568786	5.20E-03
ARHGEF18	chr19	7346362	7413527	2.60E-03
ARL4D	chr17	38827000	38835034	3.70E-03
ASF1B	chr19	14091536	14111723	2.56E-04
ATP11C	chrX	138739520	138747591	8.75E-03
ATP8A2	chr13	24935793	24942454	7.69E-04
B3GALT6	chr1	1157693	1162786	1.16E-04
BACH2	chr6	91060808	91068712	9.55E-03
BANP	chr16	86537656	86566623	5.29E-03
BCL11B	chr14	98805000	98813103	7.62E-03
BOLA3	chr2	74210871	74233956	5.49E-03
C10orf22	chr10	64229206	64237456	1.43E-03
C11orf58	chr11	16712375	16719418	5.96E-03
C11orf63	chr11	122253347	122264241	5.33E-03
C14orf93	chr14	22526007	22554690	3.59E-03
C21orf45	chr21	32571516	32575919	8.74E-03
C2orf29	chr2	101230597	101237934	5.85E-03
C4orf15	chr4	2200333	2218985	7.94E-04
CABP7	chr22	28440904	28448865	8.56E-03
CCND1	chr11	69159563	69167767	1.06E-04
CCNF	chr16	2414051	2448669	9.97E-04
CCRK	chr9	89777119	89782729	8.74E-04
CD164	chr6	109807871	109815857	5.23E-05
CD3E	chr11	117677776	117683219	1.97E-04
CDH2	chr18	24008571	24016616	3.95E-03
CDH24	chr14	22592434	22600650	6.24E-03
CDH8	chr16	60625209	60633709	9.80E-05

CDK5R2	chr2	219527554	219535227	5.70E-03
CDK6	chr7	92298051	92306286	5.95E-03
CDKN1B	chr12	12756098	12764388	8.54E-03
CGA	chr6	87859667	87866767	2.59E-04
CGNL1	chr15	55450860	55519987	8.17E-06
CHGB	chr20	5834652	5842805	9.21E-05
CHODL	chr21	18533713	18541932	3.42E-05
CIAO1	chr2	96295590	96300874	5.96E-04
CKS1B	chr1	153213960	153217808	6.70E-04
CLIC6	chr21	34958148	34965915	4.87E-03
CLUL1	chr18	584836	609132	5.63E-03
CNTRF	chr9	34577338	34584652	8.74E-03
COL27A1	chr9	115952688	115960507	2.51E-04
CPXCR1	chrX	87883406	87891388	8.75E-03
CPZ	chr4	8640399	8647960	5.75E-03
CSNK1D	chr17	77822118	77830310	9.53E-03
CTGF	chr6	132311731	132319190	8.76E-03
CYR61	chr1	85813751	85821729	7.56E-04
DCTN5	chr16	23560390	23562870	2.14E-04
DDX31	chr9	134535770	134540982	5.33E-05
DHRS12	chr13	51273871	51281605	3.10E-04
DIS3L	chr15	64367247	64375491	3.59E-03
DKK1	chr10	53738570	53746647	8.79E-04
DNAJC5G	chr2	27350115	27357824	6.52E-04
DPPA2	chr3	110515580	110521646	2.88E-04
DPPA3	chr12	7749947	7757668	6.84E-03
DRD3	chr3	115371150	115384135	8.88E-03
DSCR6	chr21	37295531	37303244	8.03E-03
DSCR9	chr21	37497423	37515952	8.46E-03
DTNA	chr18	30321780	30654712	7.76E-04
DUSP21	chrX	44585374	44590838	5.48E-03
DYRK1A	chr21	37656075	37716752	7.46E-03
EGFLAM	chr5	38290135	38483905	2.72E-05
EGR2	chr10	64243403	64251556	3.26E-03
EIF1AX	chrX	20067579	20074596	2.39E-04
EIF4E	chr4	100066990	100075043	4.76E-04
ENDOGL1	chr3	38509729	38515292	8.42E-05
ERH	chr14	68930767	68934716	6.80E-06

ESCO1	chr18	17432281	17435629	8.54E-03
EVI1	chr3	170344352	170352485	4.48E-04
EXOSC3	chr9	37772605	37780549	2.16E-05
FAM45B	chr10	120853685	129457739	5.73E-04
FAM46C	chr1	117944684	117952583	6.85E-04
FAM62A	chr12	54803103	54810819	7.53E-03
FBS1	chr16	30578866	30586483	3.84E-03
FBXO15	chr18	69961442	69965896	3.70E-03
FGF14	chr13	101364838	101857442	3.60E-03
FGFR1	chr8	38432513	38450798	8.76E-03
FLI1	chr11	128063784	128071748	3.84E-03
FLJ21127	chr12	109530895	109538392	9.80E-03
FLT1	chr13	27964543	27972661	2.18E-04
FLT3	chr13	27570504	27576660	2.65E-04
FOS	chr14	74809900	74817771	8.88E-03
FOXA2	chr20	22510624	22519425	8.74E-03
GABRA3	chrX	151308976	151373033	2.25E-04
GABRR1	chr6	89982346	89989297	4.15E-05
GADD45B	chr19	2423856	2429726	1.17E-04
GATA5	chr20	60481532	60489851	4.02E-03
GDPD2	chrX	69557553	69562431	5.61E-04
GFRA2	chr8	21650600	21658150	4.86E-05
GLB1L	chr2	219813005	219818218	2.88E-04
GNAI2	chr3	50238692	50251219	7.57E-03
GPR87	chr3	152514964	152522610	8.45E-03
GRIA4	chr11	104980777	104989227	7.31E-04
GRIN2A	chr16	10179347	10186292	8.34E-03
GRLF1	chr19	52108410	52199363	2.59E-03
GRM1	chr6	146388044	146394853	6.07E-04
GRM7	chr3	6872719	6880482	1.63E-05
GRPR	chrX	16046640	16054432	9.86E-03
GSC	chr14	94303729	94311646	5.92E-03
HAP1	chr17	37142033	37149847	5.87E-03
HDAC6	chrX	48539924	48568152	8.74E-03
HNRPD	chr4	83511336	83518809	9.25E-03
HOXC10	chr12	52659869	52670337	5.47E-04
HOXC13	chr12	52613426	52621431	3.53E-05
HPGD	chr4	175677827	175685698	6.05E-04

hsa-mir-125b-1	chr11	121473461	121481220	8.74E-03
HSCB	chr22	27468154	27472034	3.42E-05
IGBP1	chrX	69265491	69272391	2.53E-04
IL17A	chr6	52153710	52161460	8.63E-05
IL2	chr4	123594562	123600373	4.86E-03
IL27	chr16	28424058	28431137	6.88E-03
IL4I1	chr19	55089433	55097079	2.57E-03
ITCH	chr20	32413474	32562865	8.42E-03
ITFG2	chr12	2786772	2793831	1.06E-03
ITIH1	chr3	52786702	52801103	2.05E-04
ITPKB	chr1	224989444	224996200	1.84E-03
KCNIP4	chr4	20490977	21563714	5.75E-03
KCTD17	chr22	35772056	35780226	8.65E-03
KIAA0182	chr16	84197082	84206865	5.48E-03
KLF10	chr8	103732779	103742600	4.58E-03
KLF4	chr9	109288846	109296864	5.67E-05
KLHL14	chr18	28604543	28612390	2.41E-03
KLHL2	chr4	166342905	166463770	7.56E-03
KLHL30	chr2	238708684	238716605	2.02E-03
KLHL5	chr4	38735230	38743041	8.55E-03
KLHL6	chr3	184753606	184761558	2.47E-04
KRT6C	chr12	51171443	51172589	3.68E-03
L3MBTL4	chr18	6299272	6409076	2.50E-03
LGTN	chr1	204850041	204855737	2.75E-03
LMO4	chr1	87561350	87572781	7.15E-04
LYN	chr8	56949513	56957701	1.96E-04
MAP2K7	chr19	7873549	7877078	3.84E-04
MAP3K5	chr6	137153022	137158517	2.66E-05
MAPRE2	chr18	30870014	30878018	2.31E-03
MARCKS	chr6	114281502	114287957	3.19E-04
MAT1A	chr10	82036829	82044570	8.43E-03
MC2R	chr18	13873037	13910778	8.02E-04
MEIS1	chr2	66510564	66518628	6.31E-04
MEN1	chr11	64327558	64335014	1.64E-04
METAP1	chr4	100170324	100176726	3.18E-04
MGC26733	chr2	98064908	98230344	6.24E-04
MGMT	chr10	131150140	131157952	4.56E-03
MIER1	chr1	67163375	67171046	8.94E-04

MITF	chr3	69865806	70070929	7.73E-05
MMP12	chr11	102248846	102256141	8.55E-03
MORC2	chr22	29691428	29699618	9.12E-03
MSRB2	chr10	23419235	23460940	1.94E-05
NCK2	chr2	105829394	105836285	7.59E-03
NDST4	chr4	116252619	116259865	6.55E-03
NEFM	chr8	24821804	24829520	5.40E-04
NFATC1	chr18	75253029	75263841	1.48E-04
NOL4	chr18	30054784	30061524	1.21E-03
NOX4	chr11	88861718	88869890	9.57E-03
NR2E1	chr6	108588654	108597134	2.38E-04
NRK	chrX	104947942	104955594	7.82E-04
NUDT2	chr9	34314130	34331459	4.21E-04
NUFIP2	chr17	24642755	24649732	8.74E-03
OR9Q1	chr11	57641047	57705597	4.05E-03
OTOA	chr16	21597336	21626076	6.54E-04
PAN3	chr13	27641279	27648649	4.03E-04
PAX2	chr10	102489841	102498514	1.57E-04
PAX7	chr1	18824807	18833022	4.24E-04
PCDH7	chr4	30325765	30333629	6.10E-04
PCDHGA12	chr5	140784943	140792948	7.68E-04
PCID1	chr11	32556607	32564063	2.05E-03
PDLIM5	chr4	95587209	95594593	1.43E-06
PIP5K2A	chr10	23041096	23048726	4.35E-05
PLAGL2	chr20	30239362	30259110	9.04E-04
PLN	chr6	118971500	118987764	7.59E-03
PPP2R3A	chr3	137165644	137226608	2.01E-04
PRC1	chr15	89336939	89342667	2.19E-04
PRDM5	chr4	122060791	122066481	1.84E-04
PRLHR	chr10	120342455	120350300	1.35E-04
PRMT3	chr11	20361912	20367973	6.73E-05
PTGDR	chr14	51798860	51806320	6.02E-04
PTPRT	chr20	41249397	41257020	8.40E-03
PVRL2	chr19	50036396	50083184	5.41E-03
R3HDM1	chr2	136005646	136141865	9.56E-03
RAB28	chr4	13092357	13100571	6.85E-03
RAB30	chr11	82458231	82465717	7.68E-03
RABGAP1	chr9	124743174	124916451	8.45E-03

RABGAP1L	chr1	172390394	172686075	5.16E-03
RASL11B	chr4	53417780	53425823	5.04E-04
RASSF3	chr12	63285471	63292522	6.86E-03
RASSF8	chr12	25998953	26005641	5.73E-04
RICTOR	chr5	39107897	39113613	5.19E-04
RNF103	chr2	86701144	86708020	8.45E-03
RNF122	chr8	33542054	33549671	9.85E-03
RNF130	chr5	179372715	179436732	3.41E-04
RNF17	chr13	24231354	24304582	5.55E-03
RSPO2	chr8	109160982	109168013	2.61E-04
RUNX1T1	chr8	93096291	93182090	3.63E-04
SALL1	chr16	49740247	49748161	3.57E-04
SAMSN1	chr21	14837999	14845840	8.76E-03
SCGB1D1	chr11	61711612	61716640	2.68E-03
SEMA4D	chr9	91282126	91289928	6.75E-04
SERTAD2	chr2	64731894	64739516	1.30E-05
SFRS15	chr21	32023827	32031453	8.52E-03
SFRS3	chr6	36665106	36672411	1.18E-04
SIAH1	chr16	46954747	46982117	2.40E-03
SLC2A4	chr17	7122640	7128427	9.30E-04
SLIT1	chr10	98932959	98940303	3.46E-03
SMAD7	chr18	44728524	44736598	6.51E-05
SNX24	chr5	122205841	122211447	1.48E-04
SORBS1	chr10	97185054	97316243	1.80E-04
SOX5	chr12	23991389	24612164	3.85E-03
SOX6	chr11	16378944	16459282	2.13E-04
SPG20	chr13	35805495	35824248	3.64E-04
SSTR4	chr20	22958743	22966641	4.64E-04
ST6GALNAC3	chr1	76307640	76315346	3.13E-05
STAG2	chrX	122919322	122986516	6.21E-04
STARD13	chr13	32655725	32763172	3.54E-03
STATIP1	chr18	31963993	31968819	6.04E-03
STOM	chr9	123169762	123177476	5.85E-03
TACC3	chr4	1693044	1698204	6.14E-04
TAS2R7	chr12	10844147	10849899	3.51E-05
TCF21	chr6	134246927	134254747	4.90E-05
TCF7L2	chr10	114694749	114702928	2.77E-03
TCL6	chr14	95194152	95203571	4.50E-04

TEX13B	chrX	107109621	107115901	7.52E-04
THBD	chr20	22975907	22982857	9.80E-03
TLE3	chr15	68173699	68182765	5.48E-03
TMED3	chr15	77385707	77392715	3.94E-04
TMEM17	chr2	62584425	62592297	3.29E-04
TSHZ3	chr19	36459632	36467510	9.23E-04
TSPY1	chrY	9270695	9975448	3.65E-03
TTC14	chr3	181797316	181805128	8.74E-03
TWF2	chr3	52237659	52248179	1.88E-07
TXNDC10	chr18	64530956	64538834	3.62E-04
TXNDC3	chr7	37849871	37857133	3.94E-04
UBE2I	chr16	1293821	1306356	9.59E-04
UPF1	chr19	18799619	18806398	2.68E-03
UTS2	chr1	7833210	7841176	7.97E-04
VAX1	chr10	118885157	118893254	9.59E-04
VGLL3	chr3	87120644	87128306	7.50E-03
VPS35	chr16	45275797	45280444	5.68E-03
WWOX	chr16	76685605	76693698	3.03E-03
ZBTB16	chr11	113430193	113636269	5.66E-05
ZBTB2	chr6	151752230	151759706	6.86E-04
ZDHHC6	chr10	114191649	114196563	6.51E-03
ZFP36	chr19	44583835	44591634	2.02E-03
ZIC3	chrX	136470489	136479034	1.55E-04
ZMAT2	chr5	140059049	140062704	4.04E-05
ZNF157	chrX	47110271	47115056	4.66E-05
ZNF44	chr19	12264345	12271193	5.01E-04
ZNF451	chr6	57058090	57142944	1.04E-04
ZNF533	chr2	180432074	180439579	7.82E-05
ZNF667	chr19	61678252	61685739	3.66E-03

Footnotes:

1. AR target genes in Huh7 cells identified by ChIP-chip. Common targets identified in both HCC cell lines are bolded.
2. The chromosome location and genomic coordinates of the probe in the Agilent human ChIP-chip array that shows significant AR binding in HCC cells by the ChIP Analytics software.
3. The minimal $P[Xbar]$ value of the probes corresponding to the AR-bound genes. A gene promoter was considered as positive for AR binding if the probes have $P[Xbar] < 10^{-3}$.

PLC5 cell line

¹ Gene	² Chromosome	² Start	² End	³ MinP[x]
ACA36	chrX	153644412	153646868	1.46E-04
ACE2	chrX	15526648	15534535	7.60E-03
ADAR	chr1	152844950	152871257	4.02E-04
ALAS2	chrX	55066889	55078786	2.74E-05
AMN1	chr12	31770868	31778797	3.62E-04
ANKRD27	chr19	37852866	37857850	9.45E-04
APAF1	chr12	97563218	97568786	5.10E-04
ARHGEF18	chr19	7346362	7413527	8.85E-04
ARL4D	chr17	38827000	38835034	4.76E-04
ASB12	chrX	63360334	63367558	9.29E-05
ATP11C	chrX	138739520	138747591	2.02E-04
ATP8A2	chr13	24935793	24942454	6.45E-05
B3GALT6	chr1	1157693	1162786	5.48E-03
BACH2	chr6	91060808	91068712	6.68E-05
BANP	chr16	86537656	86566623	2.52E-05
BCL11B	chr14	98805000	98813103	7.75E-04
BOLA3	chr2	74210871	74233956	6.07E-04
BTBD12	chr16	3571184	3591118	1.30E-04
C10orf22	chr10	64229206	64237456	4.45E-04
C11orf58	chr11	16712375	16719418	8.88E-04
C11orf63	chr11	122253347	122264241	4.88E-05
C14orf93	chr14	22526007	22554690	2.77E-04
C1R	chr12	7056942	7138134	2.81E-05
C21orf45	chr21	32571516	32575919	2.00E-04
C2orf29	chr2	101230597	101237934	9.98E-04
C4orf15	chr4	2200333	2218985	5.74E-03
CA10	chr17	47588732	47597805	1.49E-04
CABP7	chr22	28440904	28448865	2.53E-04
CABYR	chr18	19967495	19975890	3.41E-06
CBLL1	chr7	107167982	107174249	2.11E-05
CCDC120	chrX	48797960	48805801	7.86E-05
CCND1	chr11	69159563	69167767	7.41E-04
CCNF	chr16	2414051	2448669	7.46E-03
CCRK	chr9	89777119	89782729	2.03E-05

CD164	chr6	109807871	109815857	7.59E-03
CD3E	chr11	117677776	117683219	8.73E-03
CDC42EP4	chr17	68817206	68825145	5.28E-04
CDH2	chr18	24008571	24016616	3.13E-05
CDH24	chr14	22592434	22600650	6.54E-04
CDH8	chr16	60625209	60633709	8.70E-03
CDK5R2	chr2	219527554	219535227	8.79E-05
CDK6	chr7	92298051	92306286	1.88E-04
CDKN1B	chr12	12756098	12764388	9.51E-05
CGNL1	chr15	55450860	55519987	5.70E-03
CHGB	chr20	5834652	5842805	4.87E-04
CHODL	chr21	18533713	18541932	8.37E-03
CIAO1	chr2	96295590	96300874	3.94E-05
CLGN	chr4	141566657	141571070	9.51E-05
CLIC6	chr21	34958148	34965915	8.23E-05
CLUL1	chr18	584836	609132	2.30E-04
CNTRF	chr9	34577338	34584652	2.43E-04
CPXCR1	chrX	87883406	87891388	1.06E-04
CPZ	chr4	8640399	8647960	8.74E-05
CRSP2	chrX	40392486	40484955	9.85E-04
CRYGB	chr2	208718064	208723252	1.16E-04
CSNK1D	chr17	77822118	77830310	3.38E-04
CTGF	chr6	132311731	132319190	7.25E-04
CX3CR1	chr3	39294235	39301590	1.17E-05
CYR61	chr1	85813751	85821729	8.65E-05
DCP1B	chr12	1981466	1989372	4.68E-04
DDX31	chr9	134535770	134540982	4.87E-03
DENND3	chr8	142202490	142210376	7.55E-04
DHRS3	chr1	12597570	12605805	1.12E-04
DHX36	chr3	155522603	155526544	7.18E-04
DIS3L	chr15	64367247	64375491	4.89E-04
DMRT3	chr9	961509	969455	1.16E-04
DNAJC5G	chr2	27350115	27357824	5.88E-03
DPPA3	chr12	7749947	7757668	3.26E-04
DRD3	chr3	115371150	115384135	1.73E-05
DSCR6	chr21	37295531	37303244	4.20E-05
DSCR9	chr21	37497423	37515952	9.23E-05
DTNA	chr18	30321780	30654712	5.99E-03

DUSP21	chrX	44585374	44590838	2.31E-05
DYRK1A	chr21	37656075	37716752	1.09E-04
EGFLAM	chr5	38290135	38483905	7.79E-04
EGR2	chr10	64243403	64251556	3.86E-04
EIF1AX	chrX	20067579	20074596	3.79E-03
ELOVL4	chr6	80711295	80719368	6.59E-04
ENDOGL1	chr3	38509729	38515292	5.50E-03
ERH	chr14	68930767	68934716	4.53E-03
ERN2	chr16	23629830	23637654	3.84E-04
ESCO1	chr18	17432281	17435629	7.74E-04
EVI1	chr3	170344352	170352485	7.82E-03
EXOSC3	chr9	37772605	37780549	5.42E-03
EXOSC5	chr19	46590446	46595066	1.73E-05
FAM112A	chr20	41786315	41794567	4.32E-04
FAM45B	chr10	120853685	129457739	3.28E-06
FAM62A	chr12	54803103	54810819	8.68E-04
FBS1	chr16	30578866	30586483	6.15E-04
FBXO15	chr18	69961442	69965896	1.86E-04
FBXO8	chr4	175442080	175445819	5.98E-04
FCAR	chr19	60071948	60079204	7.94E-04
FGF14	chr13	101364838	101857442	3.12E-05
FGFR1	chr8	38432513	38450798	3.19E-05
FLI1	chr11	128063784	128071748	5.35E-04
FLJ21127	chr12	109530895	109538392	7.24E-04
FLJ45121	chr16	87330769	87335324	2.71E-04
FLT1	chr13	27964543	27972661	7.41E-03
FLT3	chr13	27570504	27576660	4.79E-03
FOS	chr14	74809900	74817771	6.12E-05
FOXA2	chr20	22510624	22519425	4.78E-05
GABRA3	chrX	151308976	151373033	9.83E-03
GADD45B	chr19	2423856	2429726	8.72E-03
GATA5	chr20	60481532	60489851	2.90E-04
GDPD2	chrX	69557553	69562431	7.48E-03
GNAI2	chr3	50238692	50251219	6.61E-04
GPBP1	chr5	56503860	56508171	8.03E-05
GPR87	chr3	152514964	152522610	6.41E-04
GRIA4	chr11	104980777	104989227	8.73E-03
GRIN2A	chr16	10179347	10186292	9.76E-05

GRLF1	chr19	52108410	52199363	8.75E-04
GRM7	chr3	6872719	6880482	3.66E-03
GRPR	chrX	16046640	16054432	2.44E-04
GSC	chr14	94303729	94311646	6.86E-04
GSN	chr9	123064818	123104361	1.20E-04
HAP1	chr17	37142033	37149847	9.82E-04
HARS2	chr20	18512387	18518738	5.31E-04
HDAC6	chrX	48539924	48568152	2.15E-04
HHLA3	chr1	70593103	70598444	9.83E-04
HIST1H2AG	chr6	27209340	27210300	6.67E-05
HNRPD	chr4	83511336	83518809	1.40E-04
HOXA10	chr7	27176821	27187227	9.36E-04
HOXC10	chr12	52659869	52670337	2.57E-05
HOXC13	chr12	52613426	52621431	4.89E-03
HPGD	chr4	175677827	175685698	7.48E-03
HS3ST5	chr6	114488200	114496140	7.06E-05
hsa-mir-125b-1	chr11	121473461	121481220	2.37E-04
HSCB	chr22	27468154	27472034	5.98E-03
HTR2C	chrX	113720089	113965715	1.32E-04
IL17A	chr6	52153710	52161460	8.47E-03
IL2	chr4	123594562	123600373	3.40E-04
IL27	chr16	28424058	28431137	4.45E-05
IL4I1	chr19	55089433	55097079	5.92E-04
ITCH	chr20	32413474	32562865	5.89E-04
ITFG2	chr12	2786772	2793831	9.90E-04
ITGB1BP2	chrX	70437800	70440760	2.02E-04
ITIH1	chr3	52786702	52801103	8.54E-03
ITPKB	chr1	224989444	224996200	2.47E-04
IYD	chr6	150726421	150734303	3.30E-04
KCNIP4	chr4	20490977	21563714	8.79E-04
KCTD17	chr22	35772056	35780226	6.27E-04
KDELC2	chr11	107848242	107879622	2.76E-04
KIAA0182	chr16	84197082	84206865	3.56E-04
KLF10	chr8	103732779	103742600	8.53E-05
KLF4	chr9	109288846	109296864	3.46E-03
KLHL14	chr18	28604543	28612390	1.65E-04
KLHL2	chr4	166342905	166463770	2.94E-04
KLHL30	chr2	238708684	238716605	4.78E-04

KLHL5	chr4	38735230	38743041	3.83E-04
KLHL6	chr3	184753606	184761558	4.57E-03
KRT31	chr17	36805410	36812797	3.43E-05
KRT6C	chr12	51171443	51172589	6.11E-04
L3MBTL4	chr18	6299272	6409076	9.48E-04
LAMA4	chr6	112679622	112687624	7.45E-04
LCE3B	chr1	150847467	150853584	2.45E-04
LGTN	chr1	204850041	204855737	2.51E-04
LMO4	chr1	87561350	87572781	4.43E-05
LPHN2	chr1	82033244	82041119	8.43E-04
LUZP1	chr1	23365888	23373020	1.76E-04
LYN	chr8	56949513	56957701	8.31E-03
MAPRE2	chr18	30870014	30878018	3.18E-04
MAT1A	chr10	82036829	82044570	1.73E-04
MC2R	chr18	13873037	13910778	4.59E-03
MEIS1	chr2	66510564	66518628	5.49E-03
MEN1	chr11	64327558	64335014	4.57E-03
MGC26733	chr2	98064908	98230344	3.54E-03
MGMT	chr10	131150140	131157952	7.50E-04
MIER1	chr1	67163375	67171046	7.45E-03
MITF	chr3	69865806	70070929	8.56E-03
MMP12	chr11	102248846	102256141	2.21E-04
MOCS2	chr5	52438782	52446759	1.81E-04
MORC2	chr22	29691428	29699618	3.46E-04
MOSC2	chr1	218983945	219024131	4.00E-04
MPPED2	chr11	30556225	30568471	5.38E-05
MSR1	chr8	16085978	16099420	2.64E-04
MSRB2	chr10	23419235	23460940	6.59E-03
MX1	chr21	41720178	41728076	8.03E-04
NCK2	chr2	105829394	105836285	1.33E-04
NDST4	chr4	116252619	116259865	2.74E-04
NEFH	chr22	28200788	28208661	9.79E-05
NETO1	chr18	68681471	68690910	7.43E-04
NEURL2	chr20	43953187	43957312	9.53E-05
NFATC1	chr18	75253029	75263841	7.42E-03
NHN1	chr16	87164352	87166556	1.30E-04
NOL4	chr18	30054784	30061524	8.96E-04
NOX4	chr11	88861718	88869890	1.87E-04

NUDT2	chr9	34314130	34331459	8.57E-03
NUFIP2	chr17	24642755	24649732	9.26E-04
OR9Q1	chr11	57641047	57705597	1.53E-04
OSGEPL1	chr2	190334140	190341242	8.69E-04
PAN3	chr13	27641279	27648649	2.94E-05
PARP12	chr7	139406758	139410930	7.39E-04
PAX7	chr1	18824807	18833022	7.99E-03
PCDH7	chr4	30325765	30333629	8.73E-03
PCDHGA12	chr5	140784943	140792948	4.53E-03
PCID1	chr11	32556607	32564063	6.89E-04
PDLIM5	chr4	95587209	95594593	8.74E-03
PEX5	chr12	7231705	7236661	4.01E-04
PIGA	chrX	15248015	15268160	1.02E-04
PIP5K2A	chr10	23041096	23048726	8.02E-03
PLN	chr6	118971500	118987764	8.91E-04
POU4F1	chr13	78073422	78081073	5.39E-04
PRC1	chr15	89336939	89342667	7.84E-03
PRDM5	chr4	122060791	122066481	4.58E-03
PRLHR	chr10	120342455	120350300	5.01E-03
PRMT3	chr11	20361912	20367973	6.92E-03
PRPF4B	chr6	3962299	3968859	2.74E-04
PRRC1	chr5	126876206	126889567	6.30E-04
PSMC3	chr11	47402169	47407571	9.40E-04
PTCHD1	chrX	23256567	23265464	7.09E-04
PTGDR	chr14	51798860	51806320	4.79E-03
PTPN9	chr15	73656287	73663773	2.96E-04
PTPRT	chr20	41249397	41257020	1.48E-04
PVRL2	chr19	50036396	50083184	2.00E-04
R3HDM1	chr2	136005646	136141865	3.63E-05
RAB28	chr4	13092357	13100571	2.03E-04
RAB30	chr11	82458231	82465717	1.52E-04
RAB31	chr18	9692866	9700434	7.96E-04
RABGAP1	chr9	124743174	124916451	1.05E-04
RABGAP1L	chr1	172390394	172686075	3.63E-04
RAI2	chrX	17727596	17735874	3.26E-06
RASL11B	chr4	53417780	53425823	7.33E-03
RASSF3	chr12	63285471	63292522	2.09E-05
RASSF8	chr12	25998953	26005641	7.12E-03

RHOBTB1	chr10	62371394	62432647	1.71E-04
RICTOR	chr5	39107897	39113613	5.70E-03
RNF103	chr2	86701144	86708020	1.38E-04
RNF122	chr8	33542054	33549671	2.07E-04
RNF130	chr5	179372715	179436732	2.37E-03
RNF146	chr6	127624447	127651965	4.20E-04
RNF17	chr13	24231354	24304582	5.57E-05
RORB	chr9	76296737	76305221	2.44E-05
RPL41	chr12	54794071	54797480	1.01E-04
RSPO2	chr8	109160982	109168013	7.33E-03
RUNX1T1	chr8	93096291	93182090	4.24E-03
SALL1	chr16	49740247	49748161	1.76E-06
SAMSN1	chr21	14837999	14845840	3.44E-04
SCGB1D1	chr11	61711612	61716640	2.79E-04
SEMA4D	chr9	91282126	91289928	7.42E-03
SERTAD2	chr2	64731894	64739516	7.87E-04
SETD1A	chr16	30873891	30903504	8.27E-04
SFRS15	chr21	32023827	32031453	2.22E-04
SIAH1	chr16	46954747	46982117	5.85E-04
SLC15A4	chr12	127862393	127880009	2.28E-04
SLC2A4	chr17	7122640	7128427	8.73E-03
SLIT1	chr10	98932959	98940303	4.32E-04
SMAD7	chr18	44728524	44736598	7.85E-03
SNX24	chr5	122205841	122211447	5.04E-03
SOX3	chrX	139412492	139420461	3.35E-04
SOX5	chr12	23991389	24612164	1.80E-04
SPG20	chr13	35805495	35824248	5.69E-03
SPTBN5	chr15	39971213	39978844	6.28E-04
SPTY2D1	chr11	18610334	18616203	5.00E-04
SRGAP3	chr3	9263509	9270751	7.99E-04
SSR3	chr3	157753189	157760735	1.51E-04
SSTR4	chr20	22958743	22966641	8.72E-03
ST6GALNAC3	chr1	76307640	76315346	8.64E-03
STAG2	chrX	122919322	122986516	6.87E-03
STARD13	chr13	32655725	32763172	2.18E-04
STATIP1	chr18	31963993	31968819	7.60E-04
STAU2	chr8	74819094	74826642	4.25E-04
STOM	chr9	123169762	123177476	4.33E-04

STXBP5	chr6	147561475	147569771	7.57E-04
TACC3	chr4	1693044	1698204	7.33E-03
TCF21	chr6	134246927	134254747	8.53E-03
TCF7L2	chr10	114694749	114702928	3.94E-04
TCL1B	chr14	95218064	95224454	1.41E-04
TCL6	chr14	95194152	95203571	5.84E-03
TEX13B	chrX	107109621	107115901	8.35E-03
THBD	chr20	22975907	22982857	6.06E-05
TIGD2	chr4	90249763	90255555	2.76E-04
TLE3	chr15	68173699	68182765	7.76E-04
TMED2	chr12	122630929	122648492	2.63E-04
TMEM17	chr2	62584425	62592297	8.57E-03
TRIM72	chr16	31132568	31134549	6.26E-05
TSPY1	chrY	9270695	9975448	5.56E-05
TTC12	chr11	112685555	112692968	3.32E-07
TTC14	chr3	181797316	181805128	2.50E-04
TWF2	chr3	52237659	52248179	9.86E-03
TXNDC10	chr18	64530956	64538834	8.56E-03
TXNDC3	chr7	37849871	37857133	6.21E-03
UBE1	chrX	46931164	46959474	3.52E-04
UBE2I	chr16	1293821	1306356	4.15E-05
UPF1	chr19	18799619	18806398	1.27E-04
UTS2	chr1	7833210	7841176	8.70E-03
VGLL3	chr3	87120644	87128306	4.06E-04
VPS35	chr16	45275797	45280444	5.17E-04
WDR42B	chrX	27907041	27914981	6.37E-06
WFDC9	chr20	43693628	43698831	3.37E-04
WWOX	chr16	76685605	76693698	4.05E-04
ZBTB16	chr11	113430193	113636269	5.70E-03
ZBTB2	chr6	151752230	151759706	8.72E-03
ZCCHC7	chr9	37105474	37112644	5.69E-04
ZDHHC6	chr10	114191649	114196563	1.99E-04
ZFP36	chr19	44583835	44591634	8.19E-04
ZNF44	chr19	12264345	12271193	5.41E-03
ZNF451	chr6	57058090	57142944	9.24E-03
ZNF667	chr19	61678252	61685739	2.69E-05

Footnotes:

1. AR target genes in PLC5 cells identified by ChIP-chip. Common targets identified in both HCC cell lines are bolded.
2. The chromosome location and genomic coordinates of the probe in the Agilent human ChIP-chip array that shows significant AR binding in HCC cells by the ChIP Analytics software.
3. The minimal $P[Xbar]$ value of the probes corresponding to the AR-bound genes. A gene promoter was considered as positive for AR binding if the probes have $P[Xbar] < 10^{-3}$.

Table S2. AR, CCRK and β -catenin immunohistochemical scores in HCC tissues.

		CCRK			
		1	2	3	Total
AR	0	9	0	0	9
	1	3	4	2	9
	2	1	5	0	6
	3	1	5	1	7
	Total	14	14	3	31

		CCRK			
		1	2	3	Total
β -catenin	0	13	9	1	23
	1	1	1	2	4
	2	0	1	0	1
	3	0	3	0	3
	Total	14	14	3	31

		β -catenin				
		0	1	2	3	Total
AR	0	8	1	0	0	9
	1	8	1	0	0	9
	2	5	0	1	0	6
	3	2	2	0	3	7
	Total	23	4	1	3	31

Immunohistochemistry was performed in the same cohort of HCC specimens used for the Western blot analysis. Based on sample availability, AR, CCRK and β -catenin staining were performed and scored in 31 HCC specimens. Nuclear AR and β -catenin staining reflects the percentage of positive nuclei whereas cytoplasmic CCRK staining reflects a combination of the percentage of positively-stained cells and the intensity of the stain. All 3 comparisons of the immunohistochemical scores (AR-CCRK, CCRK- β -catenin and AR- β -catenin) were significantly correlated ($p = 0.004$, 0.032 and 0.024 , respectively; 2-sided Pearson Chi-Square test).

Table S3. Primers used for ChIP-PCR and RT-PCR in this study.

Gene	Primer	Sequence	Experiment
<i>CCRK</i>	CCRK-F	5'- CGCAACGGCCCAAAGTAG -3'	ChIP-PCR
	CCRK-R	5'- CCACCCTCCGGCTAACG -3'	
<i>FLT1</i>	FLT1-F	5'- CCTACAGTGAGTTCTCAGAAGTTACCA -3'	ChIP-PCR
	FLT1-R	5'- AGACCGTCAGTTTCCAAGTAACAAG -3'	
<i>GADD45β</i>	GADD45β-F	5'- CCACGCCCAATGTTCAAGTC -3'	ChIP-PCR
	GADD45β-R	5'- AATCCAGGAAGTTGCGGAAA -3'	
<i>KLHL2</i>	KLHL2-F	5'- GTCGAACCCCTTAAGCAAAGAA -3'	ChIP-PCR
	KLHL2-R	5'- CCGTCTGACAGCTAGGCACAT -3'	
<i>MIER1</i>	MIER1-F	5'- CATTTGGATACAAAGAAGATATCTGTTGA -3'	ChIP-PCR
	MIER1-R	5'- CTTTTATTATGCCAGAATCTACCTCCTT -3'	
<i>NUDT2</i>	NUDT2-F	5'- ATCGTGGCAGTTATAGCAGAAGTAAA -3'	ChIP-PCR
	NUDT2-R	5'- ACCCGGCCTGCATTGTTT -3'	
<i>PDLIM5</i>	PDLIM5-F	5'- CTCTCCATGCCTGATTTAGTTCTG -3'	ChIP-PCR
	PDLIM5-R	5'- GAAACATTTGGTAATTTAGGGAGAAAAG -3'	
<i>PEG10</i>	PEG10-F	5'- ATGATGACATCGAGCTCCG -3'	ChIP-PCR
	PEG10-R	5'- GCTGGGTAGTTGTGCATCA -3'	
<i>STARD13</i>	STARD13-F	5'- TACCACCACGCCTGGCTAAT -3'	ChIP-PCR
	STARD13-R	5'- AGGCCGGTGGATCACAAG -3'	
<i>TCF7L2</i>	TCF7L2-F	5'- TACAGCACCAGGCAACGAAA -3'	ChIP-PCR
	TCF7L2-R	5'- TGAATCTCTCCTTCCTTAAATCTCTGA -3'	
<i>UBE2I</i>	UBE2I-F	5'- GTTCTGGTAGTTAACGTGCGTAGGA -3'	ChIP-PCR
	UBE2I-R	5'- AACCACCTTCATTCTGCGATTCA -3'	
<i>WWOX</i>	WWOX-F	5'- GAAAGTTCTCTAAAACCAGGAAATCAG -3'	ChIP-PCR
	WWOX-R	5'- TGATTCAACCTTTGTTTTGCA -3'	
<i>BANP</i>	BANP(a)-F	5'-AGAAGACGGACAGGTGCAAGTAG-3'	RT-PCR
	BANP(a)-R	5'-CACGTGATGGATCTGGAGGTT-3'	
<i>CCND1</i>	CCND1(a)-F	5'-CCGTCCATGCGGAAGATC-3'	RT-PCR
	CCND1(a)-R	5'-ATGGCCAGCGGAAGAC-3'	
<i>CCRK</i>	CCRK(a)-F	5'-GCACCAGGCATAGCCACTTT-3'	RT-PCR
	CCRK(a)-R	5'-TCCCTGGGCCTAGACTTCTGT-3'	
<i>CCNF</i>	CCNF(a)-F	5'-CAATGCCATCCTGACACACTTT-3'	RT-PCR
	CCNF(a)-R	5'-GCCTCTTGGTACCTCAGATGT-3'	
<i>CDKN1B</i>	CDKN1B(a)-F	5'-TGCCCTCCCCAGTCTCTCTT-3'	RT-PCR
	CDKN1B(a)-R	5'-CAAGCACCTCGGATTTTTGC-3'	

<i>CDK5R2</i>	CDK5R2(a)-F	5'-CCTTTCCATTTTCCGTTCCCTT-3'	RT-PCR
	CDK5R2(a)-R	5'-ACAGGTGCAGGAGGAGAAACA-3'	
<i>CDK6</i>	CDK6(a)-F	5'-AAAGCCCTCTTGAAGCAAAAGA-3'	RT-PCR
	CDK6(a)-R	5'-TGGGAAAGGAGCAAGAGCAT-3'	
<i>EGFR</i>	EGFR-F	3'-ATGTCCGGGAACACAAAGAC-3'	RT-PCR
	EGFR-R	5'-TTCCGTCATAGTTCTTGGAT-3'	
<i>ESCO1</i>	ESCO1(a)-F	5'-AAAAGGAAAGCTCCAGCTAAATAGG-3'	RT-PCR
	ESCO1(a)-R	5'-ACTTTCTACTTCCTCCTTCCATCAAAG-3'	
<i>EVI1</i>	EVI1(a)-F	5'-TGGGTGAGAAGGGCAGTTG-3'	RT-PCR
	EVI1(a)-R	5'-GTTCCAATCATGTCAGGTGATC-3'	
<i>GADD45β</i>	GADD45 β (a)-F	5'-GCTGCCACAAACAAAAATACAAT-3'	RT-PCR
	GADD45 β (a)-R	5'-GACTCTGCCCCCGATGGT-3'	
<i>GAPDH</i>	GAPDH-F	5'-ACAACAGCCTCAAGATCATCAG-3'	RT-PCR
	GAPDH-R	5'-GGTCCACCACTGACACGTTG-3'	
<i>HPGD</i>	HPGD(a)-F	5'-GGCAGTCAAGGAATAAACTACAGTGTA-3'	RT-PCR
	HPGD(a)-R	5'-AGGAGGCTGAACTGTGAATCAAC-3'	
<i>MAPRE2</i>	MAPRE2(a)-F	5'-GCAGTCTGGTGTAGACTGGAAT-3'	RT-PCR
	MAPRE2(a)-R	5'-GGAGGGCCCTTGTGTTAAAGA-3'	
<i>MEN1</i>	MEN1(a)-F	5'-GTCCAGTGCTCACTTCCAGAGT-3'	RT-PCR
	MEN1(a)-R	5'-GCCGTGAGTTGCAGCTTGAT-3'	
<i>PNN</i>	PNN-F	5'-CCTTTCTGGTCTCTGGTGGAG-3'	RT-PCR
	PNN-R	5'-TGATTCTCTTCTGGTCCGACG-3'	
<i>PRC1</i>	PRC1(a)-F	5'-CAGCGCAACTTCAGCATTAATT-3'	RT-PCR
	PRC1(a)-R	5'-GTTCTCGCTGAAGCCCAACA-3'	
<i>RABGAP1</i>	RABGAP1(a)-F	5'-ATTACGGGAGCTGTGGACAGA-3'	RT-PCR
	RABGAP1(a)-R	5'-TTTACAGACACGCAACTCAGTCTTT-3'	
<i>SIAH1</i>	SIAH1(a)-F	5'-CAGAAAGTAAAGGTGACATCGGAAA-3'	RT-PCR
	SIAH1(a)-R	5'-AGCTCAAAGGTGTGACTCATCTGT-3'	
<i>STAG2</i>	STAG2(a)-F	5'-CCATGCCTTAATATGCTTGCAA-3'	RT-PCR
	STAG2(a)-R	5'-CTACCACAGCCCAATGCTTGA-3'	
<i>TACC3</i>	TACC3(a)-F	5'-AAGACCAACTTACCACAGATCTGAACT-3'	RT-PCR
	TACC3(a)-R	5'-TCTTGCGGTAGCCCTCGAT-3'	
<i>TCF7L2</i>	TCF7L2(a)-F	5'-GCGCGGGATAACTATGGAAA-3'	RT-PCR
	TCF7L2(a)-R	5'-GTGAAAGGCAAGGATTTAGGAAAC-3'	
<i>UBE2I</i>	UBE2I(a)-F	5'-CTCCTGCCGCTCCTCTCTAGA-3'	RT-PCR
	UBE2I(a)-R	5'-ACAAAGGAAGTGGCGCAGAA-3'	
<i>UPF1</i>	UPF1(a)-F	5'-TGCCTGCCGGCAACTTT-3'	RT-PCR
	UPF1(a)-R	5'-CACCTCAGTCCCTTCACACA-3'	