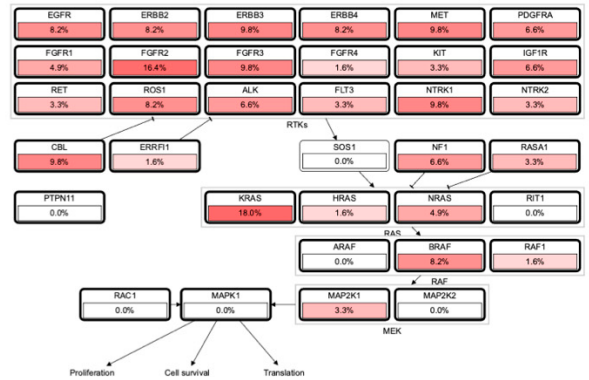
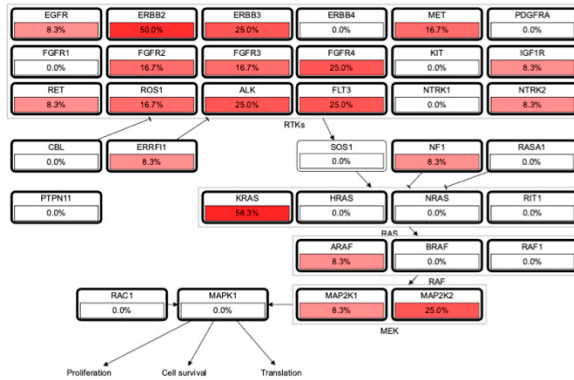


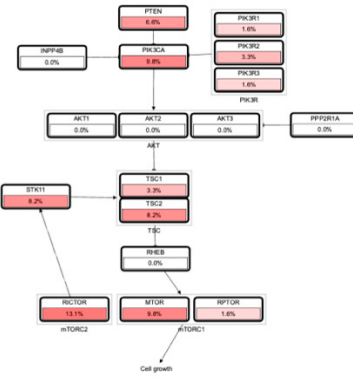
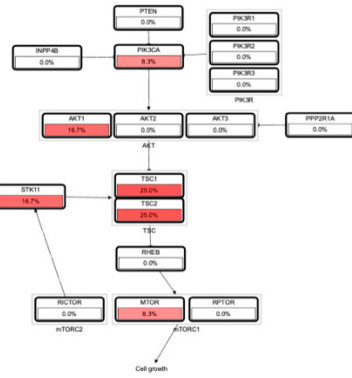
(a) Kegg pathways CCA cell lines

(b) Kegg pathways CCA tissue samples

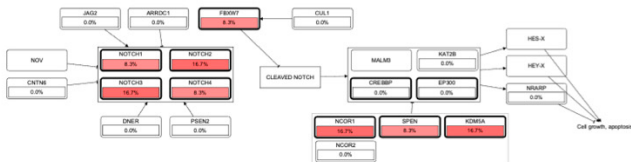
RTK RAS pathway



PI3K pathway



Notch pathway



Cell cycle

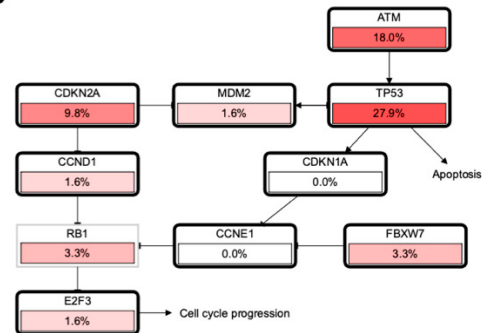
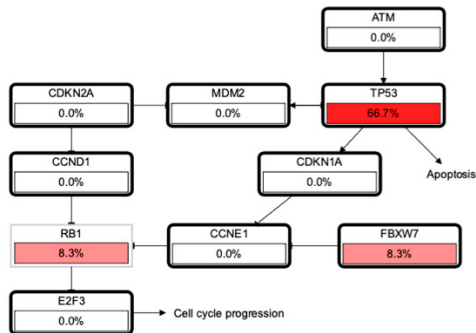
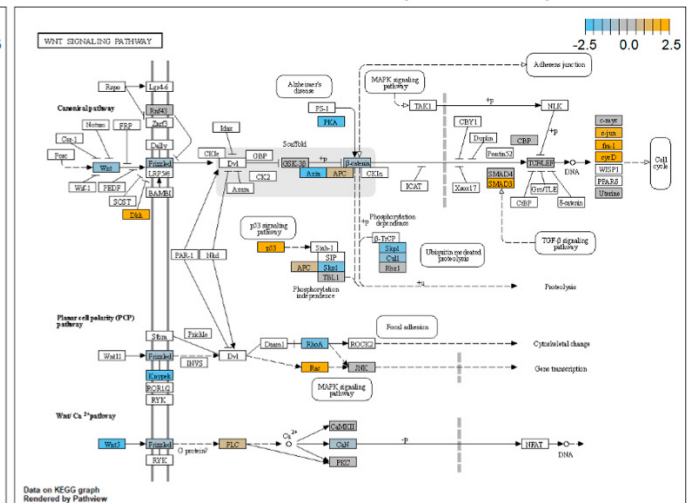


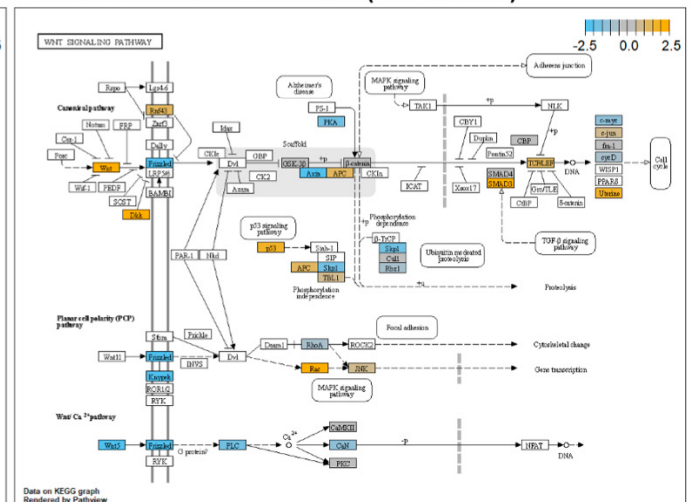
Figure S1. (a) Maps of different Kegg pathways with alterations in CCA cell lines. The mutational frequencies, including putative driver mutations and variants of unknown significance, are given. (b) Mutational frequencies in CCA tissue samples.

(b)

HuCC-A1 vs baseline of KKU-M055 (*APC* mutated)

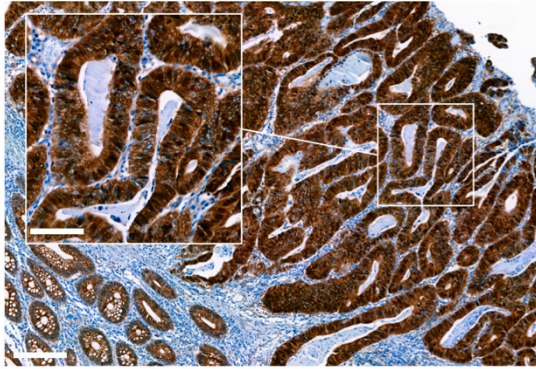


TKKK vs baseline of KKK-M055 (APC mutated)



2

(a) P1- colon cancer



(b)

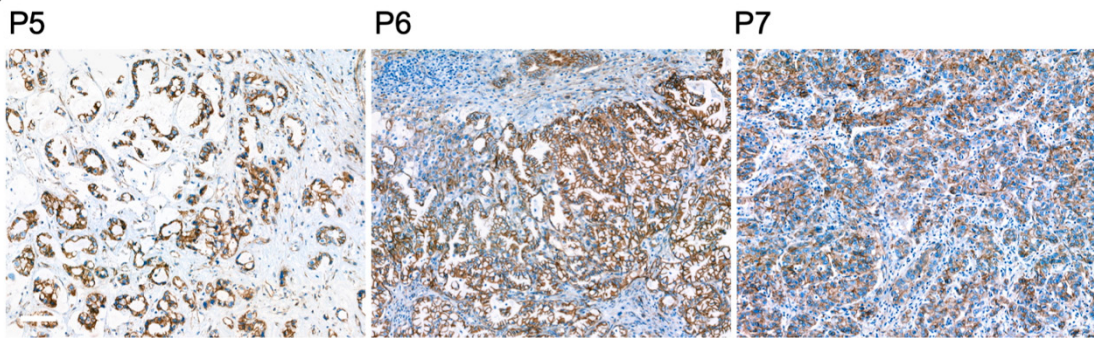


Figure S3. (a) β -catenin immunohistochemistry of metachronous colon cancer in P1. (b) β -catenin immunohistochemistry of WNT-pathway wildtype CCA samples (P5 – P7). Scale bar in (a) 200 μ m, inset 50 μ m, in (b) 100 μ m P, patient identifier.

Table S1. OncoKB driver mutations cell lines overview.

	KRAS	TP53	SMAD4	SMARCA4	IDH1	ARID1A	ARID2	RBM10	RAD51D	TERT	KMT2A	DNMT3A	MAP2K1	APC	NSD1	CTNNB1	EPHA3	TSC1	FBXW7	SETD2	SPEN
Y9CCC	WT	V222M	WT	E457*	R100Q	WT	WT	WT	K111Ifs*13	Promoter	S82*	WT	WT	WT	E531Q	WT	WT	X36_splice	WT	WT	WT
TKKK	WT	Q104*	WT	WT	WT	WT	K569Nfs*12	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
RBE	G12V	WT	WT	WT	R132S	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
OZ	Q61L	WT	WT	WT	WT	WT	WT	WT	WT	WT	G3131S	WT	WT	WT	WT	WT	WT	T899S	WT	T2421A	WT
KKU-M213	G13C	V31I	R361C	WT	WT	G324V	WT	E748*	WT	WT	WT	WT	WT	WT	X1308_splice	WT	WT	WT	WT	WT	WT
KKU-M156	G13C	V31I	R361C	WT	WT	G92A	WT	E748*	WT	WT	WT	WT	WT	WT	WT	WT	WT	F503L	WT	WT	WT
KKU-100	G12D	WT	X380_splice	K1412*	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	P104I	Q743*	Q743*
KKU-M055	WT	WT	R361H	WT	WT	P946Lfs*20 S949P	WT	WT	WT	WT	WT	WT	K57T	D1636Gfs*2	WT	WT	WT	WT	WT	WT	WT
HUCC-T1	G12D	R175H	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	S294*	Q2285*	WT
HuCC-A1	WT	G154V	WT	WT	WT	S1138*	WT	WT	WT	WT	WT	R143*	WT	WT	WT	WT	WT	WT	WT	WT	WT
SG231	G12F	T125P	WT	WT	WT	D2133G	Q1041*	WT	WT	WT	P3076Q	WT	WT	WT	WT	WT	C202*	WT	WT	WT	WT
CC-IP-1	WT	R306*	WT	E590*	WT	WT	WT	WT	WT	WT	WT	WT	WT	A1670V	WT	G34V	WT	WT	WT	WT	WT

Table S2. OncoKB mutations (putative drivers and variants of uncertain significance) in HRK-related genes in cell lines overview.

	ARID1A	ATM	ATRX	BAP1	BLM	BRCA1	BRCA2	BRIP1	CHEK2	CHEK1	FANCA	FANCC	FANCD2	FANCE	FANCF	FANCG	FANCL	MRE11	NBN	PALB2	RAD50	RAD51	RAD51B	BARD1
YSCC	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	S780L	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	S241C
TKKK	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
RBE	WT	WT	WT	WT	WT	WT	I1929V	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
OZ	WT	WT	WT	WT	WT	WT	K2729N	N196S	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
KKU-M213	G324V	WT	WT	WT	WT	SI634C	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
KKU-M156	G92A	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
KKU-100	WT	WT	WT	WT	WT	WT	WT	WT	T576A	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
KKU-M055	P946Lfs*20 S949P	WT	WT	WT	WT	WT	WT	WT	WT	WT	A449T	WT	WT	R89L	P117T	WT	WT	WT	WT	WT	WT	WT	WT	WT
HUCC-T1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
HuCC-A1	S1138*	WT	WT	WT	WT	WT	WT	WT	R223C	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
SG231	D2133G	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	P365R	S761N	
CC-IP-1	WT	WT	WT	C649Y	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	L337S	WT	WT	WT	WT

Table S3. Splice variants in CCA cell lines detected with Illumina TSO500 panel®.

cell line	Gene	Affected Exon	Breakpoint 1	Breakpoint 2	Splice Supporting Reads	Reference Reads Transcript
OZ	MET	2/21	chr7:116312631	chr7:116364746	16	4481
	MET		chr7:116312992	chr7:116339124	13	3586
	MET		chr7:116364218	chr7:116364746	28	72
TKKK	MET	2/21	chr7:116312631	chr7:116364746	15	3735
HuCC-A1	MET		chr7:116381079	chr7:116381487	35	2383
KKU-M156	MET	2/21	chr7:116312631	chr7:116364746	10	6025
	MET		chr7:116312992	chr7:116335803	10	1090
	MET		chr7:116312992	chr7:116339124	35	4782
	MET		chr7:116340338	chr7:116345807	12	4029
	MET		chr7:116342193	chr7:116371721	10	4122
	MET		chr7:116364218	chr7:116364746	26	375
	MET		chr7:116416984	chr7:116417442	19	5400
CC-LP-1	MET		chr7:116381079	chr7:116381487	10	984

Table S4. *CTNNB1/APC* mutated CC-LP-1 + KKU-M055 vs. WT HuCC-A1 and TKKK. Kegg WNT gene set.

Probe Label	Log2 fold change	P-value	Benjamini-Yekutieli adjusted p-value
AXIN2-mRNA	10.9	1.23E-21	3.71E-18
WNT5A-mRNA	7.7	0.0000204	0.000881
GPC4-mRNA	5.37	0.000163	0.00572
PRKX-mRNA	3.08	0.00000365	0.000229
PPP3CB-mRNA	2.17	0.000802	0.0214
PRKCA-mRNA	1.71	0.00383	0.0732
PRKACA-mRNA	1.58	0.00117	0.0293
RAC3-mRNA	1.56	0.00053	0.0156
FZD7-mRNA	1.14	0.000625	0.0178
SKP1-mRNA	0.998	0.0122	0.192
RHOA-mRNA	0.946	0.00154	0.0362
CCND1-mRNA	0.709	0.00226	0.0495
FZD3-mRNA	0.612	0.02	0.292
PLCB4-mRNA	0.561	0.778	1
PPP3CC-mRNA	0.464	0.0393	0.514
RBX1-mRNA	0.458	0.00912	0.154
MYC-mRNA	0.432	0.196	1
APC-mRNA	0.293	0.516	1
CUL1-mRNA	0.118	0.756	1
CTNNB1-mRNA	0.116	0.616	1
EP300-mRNA	0.0203	0.818	1
AXIN1-mRNA	-0.0538	0.532	1
PPP3R1-mRNA	-0.102	0.705	1
GSK3B-mRNA	-0.177	0.285	1
MAPK9-mRNA	-0.226	0.295	1
MAPK8-mRNA	-0.247	0.0762	0.865
CAMK2B-mRNA	-0.296	0.434	1
SMAD4-mRNA	-0.42	0.0563	0.68
PLCB1-mRNA	-0.421	0.647	1
FZD2-mRNA	-0.454	0.546	1
TBL1XR1-mRNA	-0.499	0.00149	0.0358
WNT2B-mRNA	-0.62	0.0193	0.283
CCND3-mRNA	-0.77	0.000122	0.0045
PRKACB-mRNA	-0.821	0.132	1
RAC1-mRNA	-0.859	0.000563	0.0162
JUN-mRNA	-0.877	0.0115	0.186
TCF7L1-mRNA	-0.919	0.0142	0.222
CCND2-mRNA	-1.07	0.71	1
FOSL1-mRNA	-1.15	0.336	1
WNT3-mRNA	-1.16	0.0411	0.532
PPP3CA-mRNA	-1.23	0.00000423	0.000255
MMP7-mRNA	-1.58	0.00796	0.137
SMAD3-mRNA	-2.64	0.000000587	0.0000681
WNT7A-mRNA	-3.79	0.00000774	0.000402
TP53-mRNA	-3.84	0.0983	1
DKK1-mRNA	-4.19	0.114	1
WNT10A-mRNA	-4.52	0.00157	0.0364
RAC2-mRNA	-8.6	0.000000434	0.0000546

Table S5. Overview of genetic alterations and clinical characteristics of P1-P7.

patient identifier	P1	P2	P3	P4	P5	P6	P7	
(likely) pathogenic mutations	TP53	FBXW7	TP53	CTNNB1	TP53	ARID1A	BAP1	c.1182C>A
	c.746G>A			c.133T>C				
	p.R249K			p.S45P				p.Y394*
	c.242_246 delCAGTG	KRAS	FBXW7		Splicing	KRAS	FGFR2	c.827T>G
					RUNX1			
	p.A81fs*8					p.A187T		p.F276C
	APC	NRAS	APC					
	c.2879C>G							
	p.S960*							
	APC	PIK3CA	AXIN2					
	c.1548G>A							
	p.K516K							
	TGFBRI	BARD1						
	c.1007_1024 +11del							
		APC						
		c.3340C>T						
		p.R1114*						
TMB (mut/Mb)	5.8	1.55	10.3	n.d.	3.1	1.6	8.3	
TNM	pT3, R0, G3	pT1b, L0, V0, R0, pN0, G2	pT2, R1, G2	rpT2b, L0, V0, R2, G2	pT4, pN1, cM1, R1, G2	pM1 (HEP)	ypT3, ypN0, cM1	
IPBN	yes	yes	no	no	no	unknown	no	
metachronous cancer	CRC and RCC	CRC	no	no	no	no	no	

CRC, colorectal cancer; RCC, renal cell carcinoma.

Table S6. *CTNNB1/APC* mutated CCA samples (P1-P4) vs. WT (P5-P7). Kegg WNT gene set.

Probe Label	Log2 fold change	P-value	Benjamini-Yekutieli adjusted p-value
WNT11-mRNA	4.43	0.0000175	0.00289
NKD1-mRNA	3.84	0.000000296	0.0000845
AXIN2-mRNA	3.61	9.93E-08	0.0000387
DKK4-mRNA	2.21	0.000222	0.0135
BAMBI-mRNA	1.9	0.0000298	0.00365
RAC3-mRNA	1.88	0.0429	0.669
PRKACG-mRNA	1.81	0.000112	0.00859
PLCB4-mRNA	1.35	0.00566	0.141
CCND1-mRNA	1.14	0.00289	0.0868
DKK2-mRNA	1.06	0.0262	0.461
FZD2-mRNA	1.01	0.0958	1
WNT10A-mRNA	0.989	0.0228	0.415
PLCB1-mRNA	0.894	0.00746	0.175
WNT2-mRNA	0.876	0.0827	1
FZD3-mRNA	0.771	0.0602	0.881
WNT16-mRNA	0.68	0.152	1
WIF1-mRNA	0.663	0.112	1
CAMK2B-mRNA	0.654	0.137	1
PPP3R2-mRNA	0.653	0.18	1
WNT10B-mRNA	0.463	0.263	1
CTNNB1-mRNA	0.416	0.263	1
WNT3-mRNA	0.406	0.313	1
AXIN1-mRNA	0.175	0.517	1
CREBBP-mRNA	0.16	0.603	1
WNT2B-mRNA	0.126	0.794	1
APC-mRNA	0.0298	0.924	1
CCND2-mRNA	-0.204	0.69	1
TCF7L1-mRNA	-0.272	0.259	1
GPC4-mRNA	-0.291	0.33	1
PPP3CC-mRNA	-0.324	0.423	1
MAPK10-mRNA	-0.346	0.389	1
MYC-mRNA	-0.356	0.488	1
CUL1-mRNA	-0.388	0.193	1
PRKCA-mRNA	-0.399	0.123	1
RHOA-mRNA	-0.448	0.0912	1
SMAD3-mRNA	-0.462	0.139	1
PPP3R1-mRNA	-0.514	0.0511	0.767
PPP3CA-mRNA	-0.527	0.207	1
FZD7-mRNA	-0.539	0.448	1
SKP1-mRNA	-0.547	0.0187	0.359

EP300-mRNA	-0.55	0.0561	0.827
TP53-mRNA	-0.553	0.0004	0.0197
MAPK9-mRNA	-0.617	0.0735	1
SFRP1-mRNA	-0.637	0.213	1
SMAD4-mRNA	-0.679	0.0135	0.277
RAC1-mRNA	-0.699	0.0046	0.121
WNT5B-mRNA	-0.73	0.165	1
GSK3B-mRNA	-0.783	0.000229	0.0136
PRKACA-mRNA	-0.834	0.00846	0.192
RBX1-mRNA	-0.841	0.000459	0.0216
JUN-mRNA	-0.948	0.0112	0.24
FOSL1-mRNA	-1.03	0.125	1
FZD8-mRNA	-1.14	0.0403	0.646
TBL1XR1-mRNA	-1.15	0.0000743	0.00664
MAPK8-mRNA	-1.32	0.000645	0.0281
PPP3CB-mRNA	-1.33	0.000661	0.0281
CCND3-mRNA	-1.36	0.000292	0.0161
WNT5A-mRNA	-1.65	0.00209	0.07
PRKX-mRNA	-1.94	0.000308	0.0167
SFRP4-mRNA	-2.1	0.00338	0.0959
RAC2-mRNA	-2.18	0.0000971	0.00771
MMP7-mRNA	-2.57	0.000312	0.0167
PRKACB-mRNA	-2.73	0.000383	0.0191
LEF1-mRNA	-2.91	0.000517	0.0236
SFRP2-mRNA	-3.63	0.000000157	0.000056
DKK1-mRNA	-3.66	2.14E-10	0.000000184