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Supplemental information

**R-2HG downregulates ER α to inhibit
cholangiocarcinoma via the FTO/m6A-methylated
ER α /miR16-5p/YAP1 signal pathway**

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Supplementary Fig. S1. (A) ER α expression in QBC939 and HuCCT1 cell lines. (B) MTT assay analyzed cell growth after over-expressing ER α in HuCCT1 cells. (C) MTT assay analyzed cell growth after knocking down ER α with shER α #1 or shER α #2 in QBC939 cells. (D-E) MTT assay of estrogen promoted cell proliferation in HuCCT1 (D), and estrogen antagonist (MPP) inhibited cell growth in QBC939 cells (E). Quantitations are presented as mean \pm SD. * $P < 0.05$.

Supplementary Fig. S2. (A) YAP1 expression in HuCCT1 cells treated with DMSO or R-2HG. (B) YAP1 expression in QBC939 cells carrying IDH1 wild-type (WT) or mutant (MT). (C) After over-expressing (oe) YAP1 in QBC939 cells, MTT assay was conducted to detect cell proliferation. (D) After knocking down YAP1 (shYAP1#1 and YAP1#2) in QBC939 cells, MTT assay was conducted to detect cell proliferation. Quantitations are presented as mean \pm SD. ** $P < 0.01$.

Supplementary Fig. S3. (A-B) Western blot assay was performed on QBC939 cells transfected with pLKO or shER α (A), and HuCCT1 cells transfected with pWPI or oeER α (B). (C) The qRT-PCR assay was performed on QBC939 cells transfected with Ctrl or miR16-5p inhibitor (right), and HuCCT1 cells transfected with pLKO or oemiR16-5p (left). (D) Cell proliferation and YAP1 expression altered after manipulating miR16-5p. Quantitations are presented as mean \pm SD. * $P < 0.05$, ** $P < 0.01$.

Supplementary Fig. S4. (A) The ER α mRNA level was detected in Argonaute 2 (Ago2) complex using RIP assay in QBC939 cells with/without (w/wo) IDH1^{R132H}. (B) ER α protein stability test in cycloheximide, CHX treated QBC939 cells carrying the IDH1 wild-type (WT) or mutation (MT). (C) After ER α -biotin pull-down, qRT-PCR was conducted to examine ER α expression from ER α -biotin complex. (D) After treating QBC939 cells w/wo R-2HG, anti-m6A antibody was used to pull-down the total m6A methylated RNA from two groups (E) The ER α mRNA level was detected in the anti-m6A complex using RIP assay in QBC939 cells carrying IDH1 WT or MT. (F) After over-expressing FTO (oeFTO) in HuCCT1 cells, MTT was conducted to examine cell growth. (G) After shRNA knockdown of FTO (shFTO#1, left, and shFTO#2, right) in QBC939 cells, MTT assay was conducted to examine cell growth. (H) The ER α mRNA sequence alignment near the stop codons with wild-type versus mutant of potential m6A modification sites. Quantitations are presented as mean \pm SD. * $P < 0.05$ and ** $P < 0.01$.

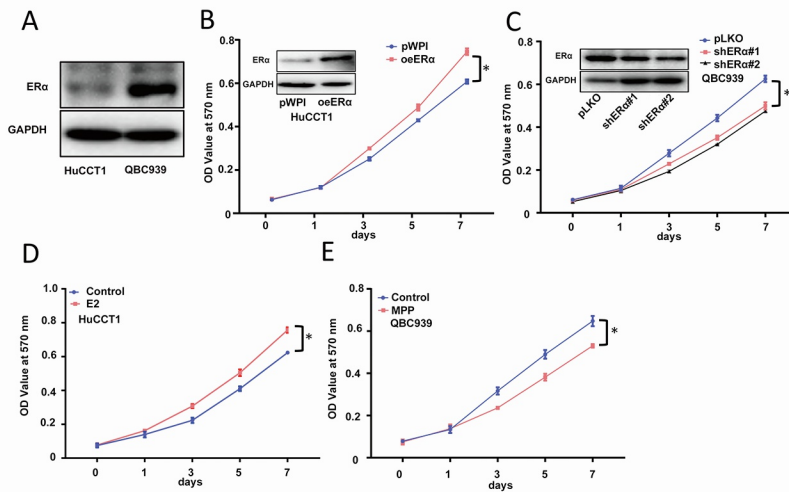
Table S1. Clinical data of Cholangiocarcinoma from TCGA

sampleID	gender	_EVENT	OS.time	histological_type	idh1/2 mutatio n	Amino_A cid_chan ge	neoplasm_h istologic_gr ade	patho logic_ M	patho logic_ N	patho logic_ T	patholo gic_sta ge	Er α	HIF-1 α	GJA1
TCGA-3X-AAV 9-01	MALE	dead	339	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N0	T1	Stage I	6.5 13 6	13.20 1205 01	11.60 3626 34
TCGA-3X-AAV A-01	FEMALE	dead	445	Cholangiocarcinoma; intrahepatic	IDH2	p.R172S	G2	M0	NX	T2b	Stage II	4.0 88 2	12.55 8659 87	9.682 9945 84
TCGA-3X-AAV B-01	FEMALE	alive	402	Cholangiocarcinoma; distal	no	no	G1	M1	N1	T3	Stage IVB	5.6 32 9	13.44 5920 13	12.46 6841 21
TCGA-3X-AAV C-01	FEMALE	alive	709	Cholangiocarcinoma; hilar/perihilar	no	no	G3	M0	N0	T1	Stage I	3.1 97 8	11.84 5097 96	13.64 4982 86
TCGA-3X-AAVE -01	MALE	alive	650	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N0	T2	Stage II	5.5 53 9	12.86 0505 06	9.491 8530 96
TCGA-4G-AAZ O-01	FEMALE	alive	1177	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N0	T2a	Stage II	2.8 10 2	12.61 6778 55	11.56 1287 95
TCGA-4G-AAZ T-01	MALE	alive	420	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N0	T1	Stage I	10.68 3.6 49	10.94 4748 62	10.94 5443 84
TCGA-W5-AA2 G-01	FEMALE	alive	1976	Cholangiocarcinoma; intrahepatic	IDH1	p.R132C	G3	M0	N0	T1	Stage I	7.6 75 7	12.48 6583 66	9.087 4628 41
TCGA-W5-AA2 H-01	FEMALE	alive	1077	Cholangiocarcinoma; distal	no	no	G3	M0	N0	T3	Stage III	6.7 43 43	11.73 2167 43	9.906 8905 96
TCGA-W5-AA2I -01	MALE	dead	1939	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N0	T1	Stage I	3.8 89 7	11.25 8566 03	6.672 4253 42

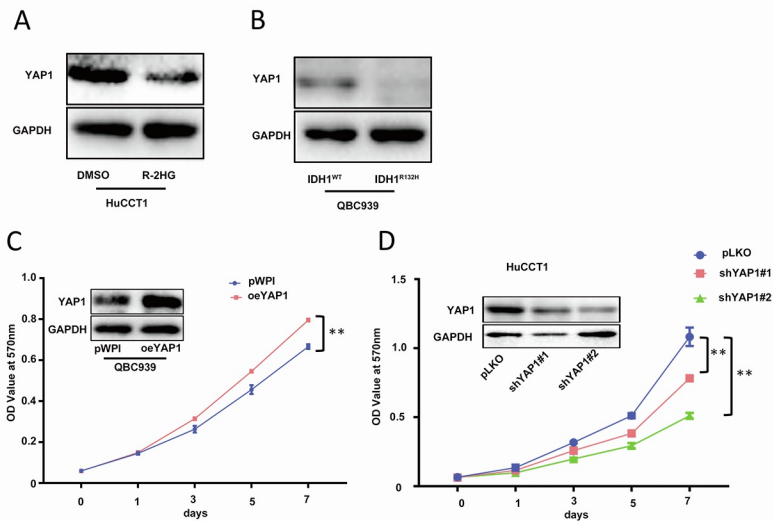
TCGA-W5-AA2												6.0	12.65	8.569
O-01	MALE	dead	640	Cholangiocarcinom a; intrahepatic	IDH1	p.R132C	G3	M0	N0	T1	Stage I	11	6871	8556
												4	73	08
TCGA-W5-AA2												3.5	13.83	10.34
Q-01	MALE	alive	50	Cholangiocarcinom a; intrahepatic	no	no	G2	M0	N0	T2b	Stage II	88	4471	4295
												7	05	91
TCGA-W5-AA2												5.2	12.58	9.231
R-01	FEMALE	alive	1542	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N0	T1	Stage I	30	3552	2211
												5	93	81
TCGA-W5-AA2												2.4	12.23	8.672
T-01	FEMALE	dead	1220	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N0	T2	Stage II	21	9598	4253
												8	53	42
TCGA-W5-AA2												4.2	13.25	10.32
U-01	FEMALE	dead	627	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N0	T1	Stage I	13	5766	3054
												6	26	76
TCGA-W5-AA2												2.8	14.17	9.172
W-01	FEMALE	dead	924	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N1	T2a	Stage IVA	86	6406	4275
												7	93	09
TCGA-W5-AA2												6.2	12.45	11.55
X-01	MALE	dead	271	Cholangiocarcinom a; hilar/perihilar	no	no	G4	M1	N1	T2b	Stage IVB	76	9431	0265
												9	62	81
TCGA-W5-AA2												4.2	14.41	10.72
Z-01	FEMALE	alive	1614	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N0	T2	Stage II	34	7193	7069
													24	56
TCGA-W5-AA3												4.6	12.20	10.38
0-01	MALE	alive	1153	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N0	T1	Stage I	96	5182	1542
												4	33	95
TCGA-W5-AA3												6.7	13.08	10.36
1-01	MALE	alive	10	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N0	T1	Stage I	89	8622	9597
												2	63	35
TCGA-W5-AA3												6.4	13.45	10.77
3-01	MALE	alive	1449	Cholangiocarcinom a; intrahepatic	no	no	G3	M0	N0	T1	Stage I	72	5584	6433
												5	09	03

TCGA-W5-AA3 4-01	FEMALE	dead	555	Cholangiocarcinoma; intrahepatic	IDH1	p.R132C	G2	M0	N0	T1	Stage I	3.2	11.94	8.930
												82	3247	7373
													4	38
TCGA-W5-AA3 6-01	FEMALE	dead	1402	Cholangiocarcinoma; hilar/perihilar	IDH1	p.R132S	G3	M1	N0	T3	Stage IV	1.9	11.94	8.980
												56	6175	1395
												1	24	78
TCGA-W5-AA3 8-01	FEMALE	alive	1471	Cholangiocarcinoma; intrahepatic	no	no	G3	M0	N0	T1	Stage I	3.3	13.41	8.335
												62	2040	3903
													51	55
TCGA-W5-AA3 9-01	MALE	dead	170	Cholangiocarcinoma; intrahepatic	no	no	G4	M0	N0	T2	Stage II	4.7	10.74	7.851
												95	1466	7490
												7	99	41
TCGA-W6-AA0 S-01	FEMALE	alive	808	Cholangiocarcinoma; intrahepatic	no	no	G2	MX	N0	T1	Stage I	3.8	14.58	9.887
												82	3141	2206
												2	55	15
TCGA-WD-A7R X-01	FEMALE	dead	21	Cholangiocarcinoma; intrahepatic	no	no	G2	MX	NX	T2b	Stage II	6.6	12.58	9.519
												18	5432	6362
												3	05	53
TCGA-YR-A95A -01	MALE	dead	26	Cholangiocarcinoma; hilar/perihilar	IDH1	p.R132C	G2	M1	NX	T2	Stage IV	3.0	13.26	13.87
												6.0	8103	6708
												73	34	78
TCGA-ZD-A8I3 -01	FEMALE	dead	169	Cholangiocarcinoma; intrahepatic	no	no	G3	M0	N0	T2	Stage II	3.0	13.67	10.20
												49	3198	8234
												9	64	36
TCGA-ZH-A8Y1 -01	FEMALE	dead	385	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N1	T3	Stage IVA	3.4	13.45	10.83
												00	9303	7627
												2	53	93
TCGA-ZH-A8Y2 -01	FEMALE	dead	701	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	NX	T1	Stage I	3.3	12.48	8.087
												83	0032	4628
												4	81	41
TCGA-ZH-A8Y4 -01	MALE	dead	741	Cholangiocarcinoma; intrahepatic	no	no	G3	M0	N0	T1	Stage I	4.7	13.75	8.851
												11	4365	7490
												2	6	41
TCGA-ZH-A8Y5 -01	MALE	alive	1229	Cholangiocarcinoma; intrahepatic	no	no	G3	M1	N1	T3	Stage IVB	2.9	13.15	9.933
												71	1967	6906

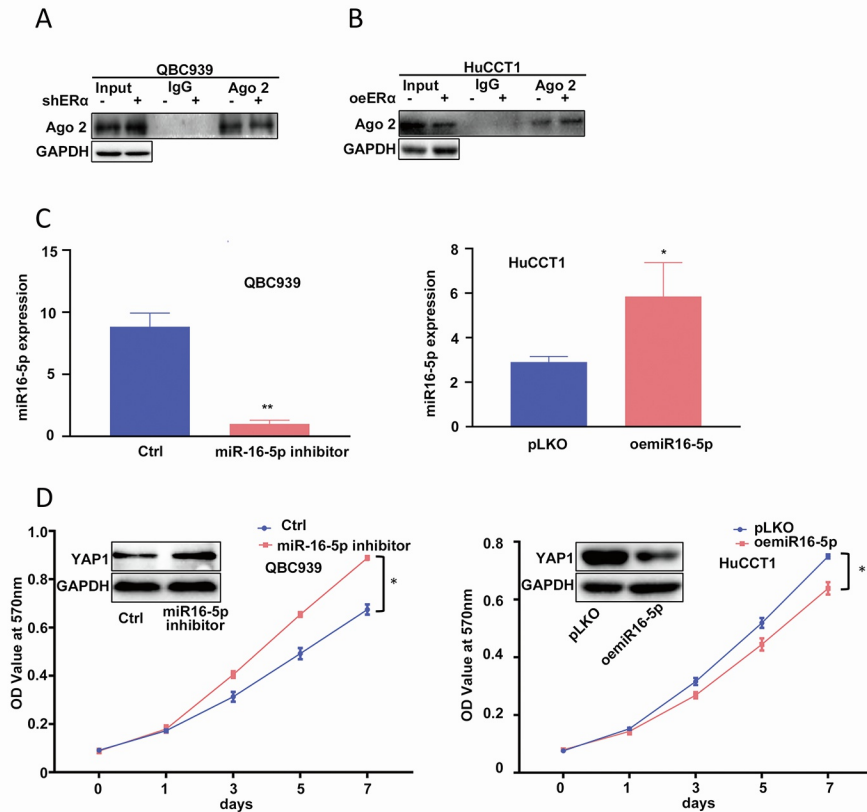
													87	55
TCGA-ZH-A8Y6-01	FEMALE	alive	519	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N0	T1	Stage I	5.1 35 4	16.03 8404 63	10.16 4906 93
TCGA-ZH-A8Y8-01	MALE	alive	602	Cholangiocarcinoma; intrahepatic	no	no	G2	M0	N0	T1	Stage I	5.3 54 7	12.81 4782 72	14.20 3271 52
TCGA-ZU-A8S4-01	MALE	dead	98	Cholangiocarcinoma; intrahepatic	no	no	G3	MX	NX	T1	Stage I	4.6 39 9	13.30 4636 52	13.78 0334 71
TCGA-UB-A7M A-01A	female	alive		Hepatobiliary carcinoma (Mixed)	no	no	G2	M0	N0	T2b	stage ii	5.8 83 6	#N/A #N/A #N/A	#N/A #N/A #N/A
TCGA-ED-A97K-01A	male	alive	6	Hepatobiliary carcinoma (Mixed)	no	no	G2	M0	N0	T3a	stage iiiia	7.1 39 3	#N/A #N/A #N/A	#N/A #N/A #N/A
TCGA-K7-AAU 7-01A	male	alive	359	Hepatobiliary carcinoma (Mixed)	no	no	G2	MX	NX	T2a	stage ii	4.9 41 8	#N/A #N/A #N/A	#N/A #N/A #N/A
TCGA-DD-AA3 A-01A	female	dead	410	Hepatobiliary carcinoma (Mixed)	no	no	G4	MX	N0	T1	stage i	3.4 23 3	#N/A #N/A #N/A	#N/A #N/A #N/A
TCGA-5C-AAP D-01A	male	alive	20	Hepatobiliary carcinoma (Mixed)	no	no	G1	M0	N0	T2	stage ii	4.9 09 4	#N/A #N/A #N/A	#N/A #N/A #N/A
TCGA-FV-A310-01A	female	alive	848	Hepatobiliary carcinoma (Mixed)	no	no	G2	M0	NX	T2	stage ii	2.8 99	#N/A #N/A	#N/A #N/A
TCGA-KR-A7K2-01A	male	alive		Hepatobiliary carcinoma (Mixed)	no	no	G1	M0	N0	T1	stage i	8.7 35 3	#N/A #N/A #N/A	#N/A #N/A #N/A



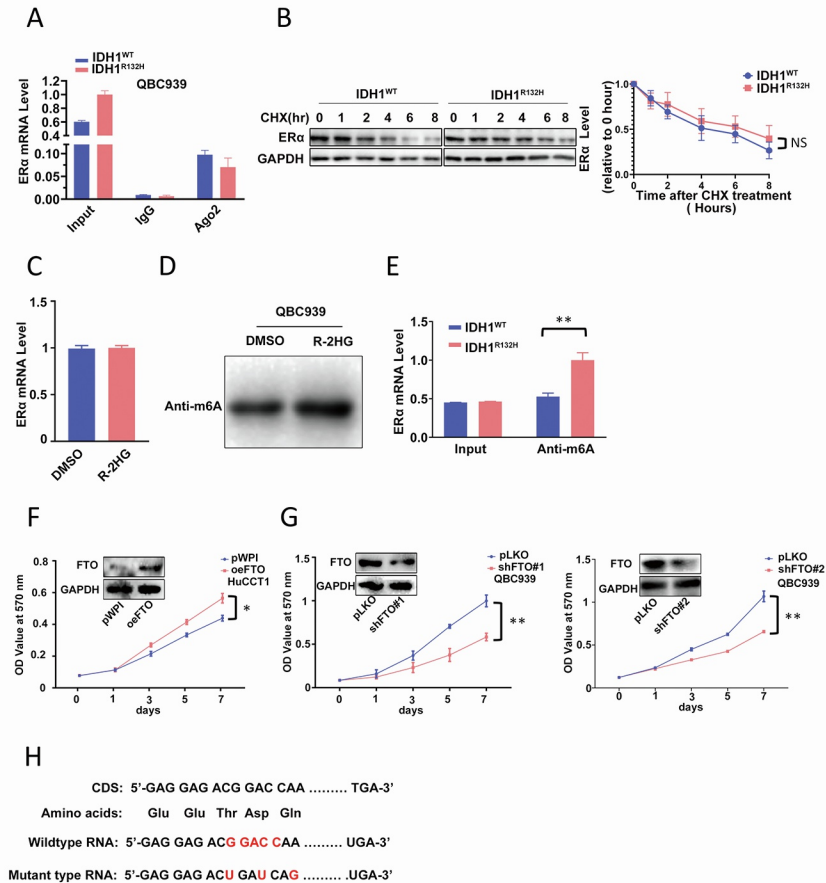
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4