Supplemental Information

LINC01714 Enhances Gemcitabine

Sensitivity by Modulating FOXO3

Phosphorylation in Cholangiocarcinoma

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Supplementary Methods

Pan-cancer expression level of LINC01714

The RNA-seq mRNA levels of LINC01714 in 33 cancer types (including both cancer and adjacent normal samples) were obtained from GDC database (https://portal.gdc.cancer.gov/). Then the expression variations in different cancer types were compared.

5' and 3' RACE assay

The transcriptional initiation and termination sites of LINC0714 was determined by using 5' and 3' RACE assay. The 5' and 3' RACE assays were performed by employing a SMARTer RACE cDNA Amplification kit (Clontech, California, USA) according to the manufacturer's instructions.

Subcellular fractionation

The Nuclear/Cytoplamic Isolation kit (Thermo Fisher Scientific, Carlsbad, California, USA) was used to extract the cytoplasmic and nuclear fraction of CCLP1 cells according to the manufacturer's instructions. β-actin served as cytoplasmic endogenous control and U6 RNA served as nuclear endogenous control.

Gene set enrichment analysis

To further explore the biological processes that LINC01714 might be involved, we performed gene set enrichment analysis of LINC01714-knockdown profiles. The GSEA software (http://software.broadinstitute.org/gsea/index.jsp) [1] was employed to test the most changed genes were enriched in which gene sets of biological processes annotated in the Molecular Signatures Database (MSigDB).

Reference

[1]   Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545–50. doi:10.1073/pnas.0506580102.
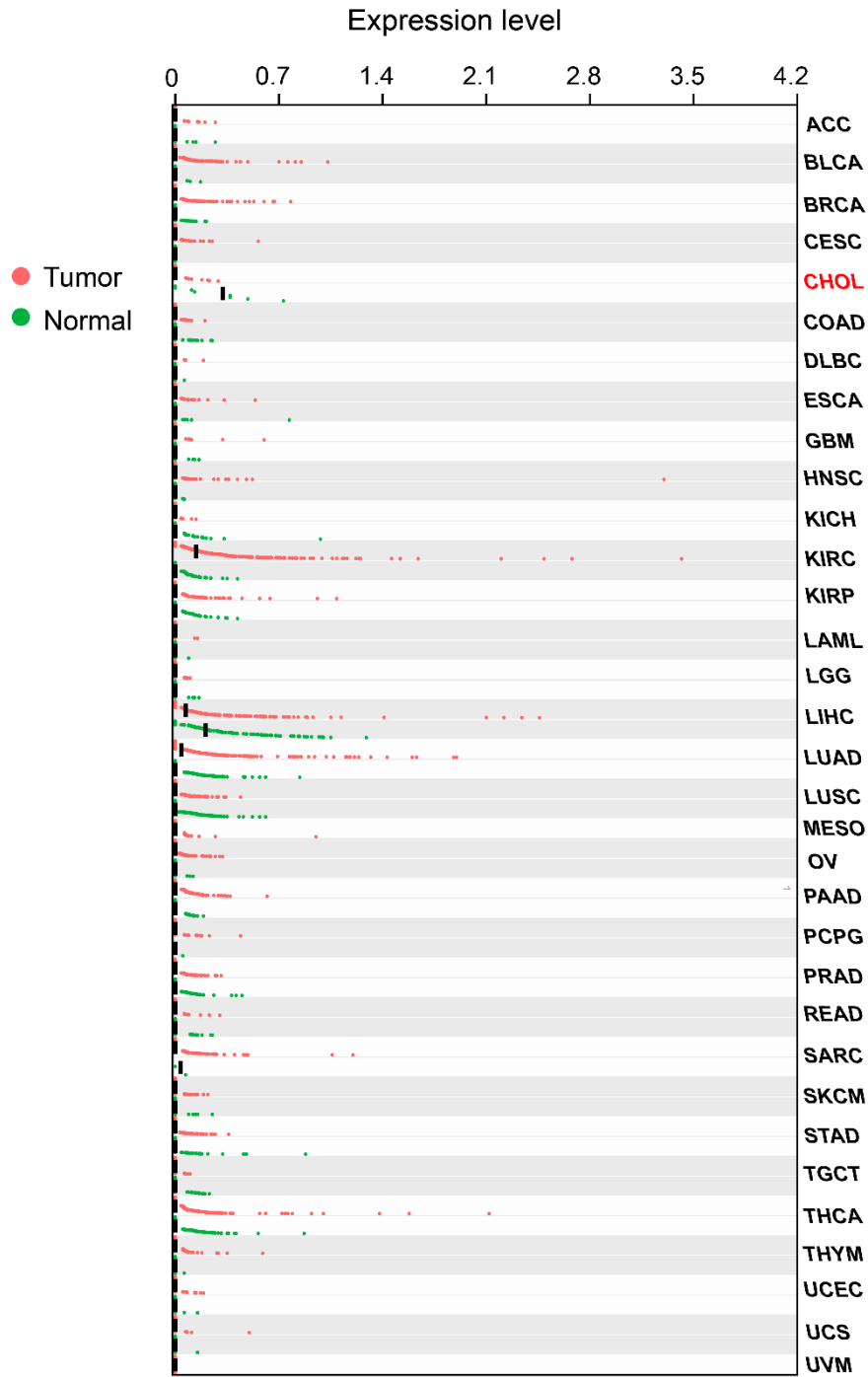
Supplementary Figures

Figure S1. The expression of LINC01714 in tumor and corresponding adjacent normal samples across 33 TCGA tumor types. CCA cohort is marked in red font (CHOL).
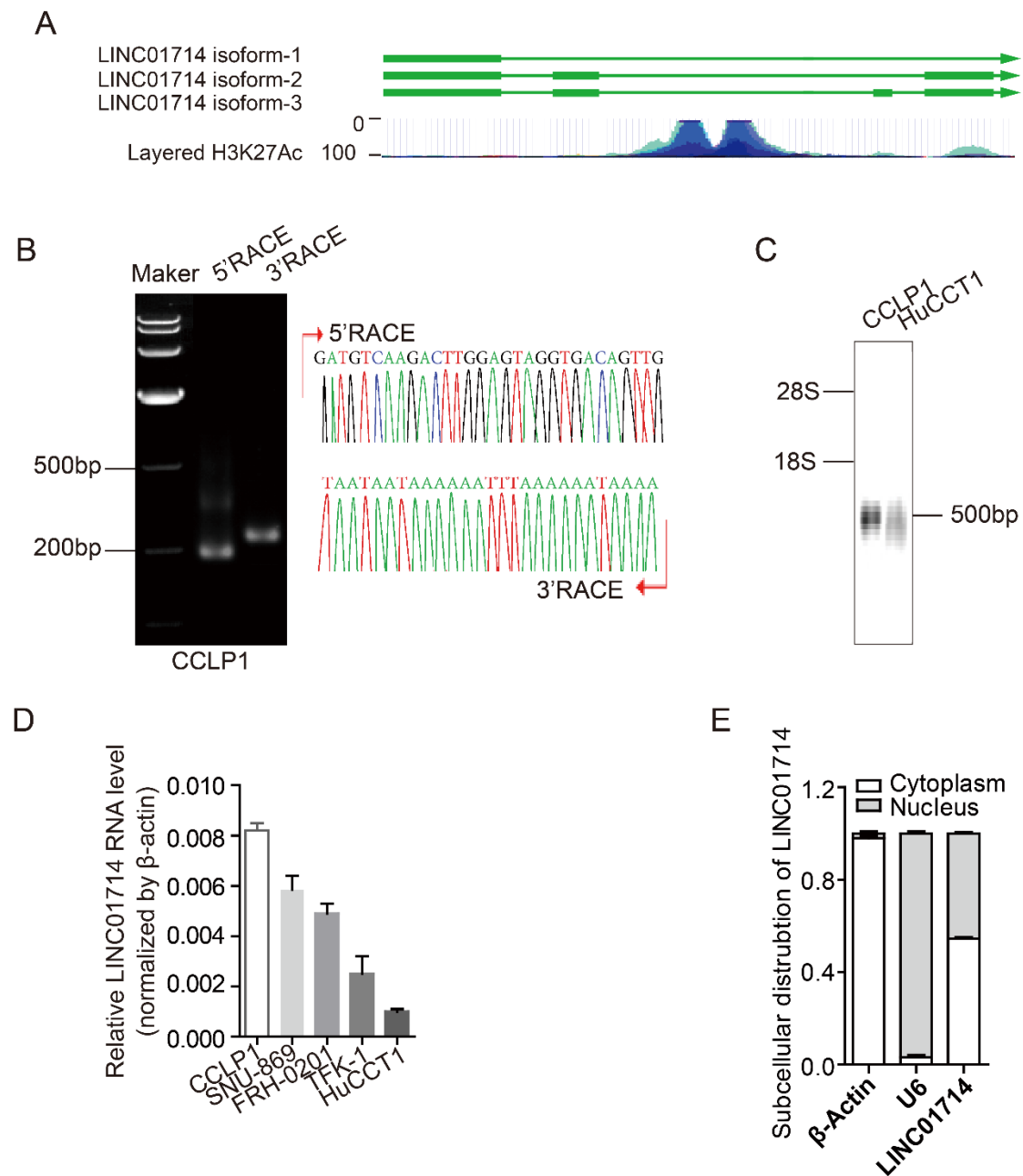
Figure S2. **Characterization of long intergenic non-coding RNA LINC01714 in CCA.** **(A)** Isoform information of LINC01714. **(B)** Identification of full-length LINC01714 in CCLP1 cells. Representative images and the boundary of the PCR products from 5' RACE and 3' RACE were shown. **(C)** Northern blot results for LINC01714 in HuCCT1 and CCLP1 cell lines. **(D)** Relative LINC01714 RNA levels in CCA cell lines. **(E)** The distributon of LINC01714 in cytoplasma ad nucleus of CCA cells. β-actin serves as cytoplasmic marker and U2 serves as nuclear marker. Values are shown as mean ± SEM.

A Protein coding potential

Metric	Raw result	Interpretation
PRIDE reprocessing 2.0	0	non-coding
Lee translation initiation sites	0	non-coding
PhyloCSF score	-46.0469	non-coding
CPAT coding probability	5.78%	non-coding
Bazzini small ORFs	0	non-coding

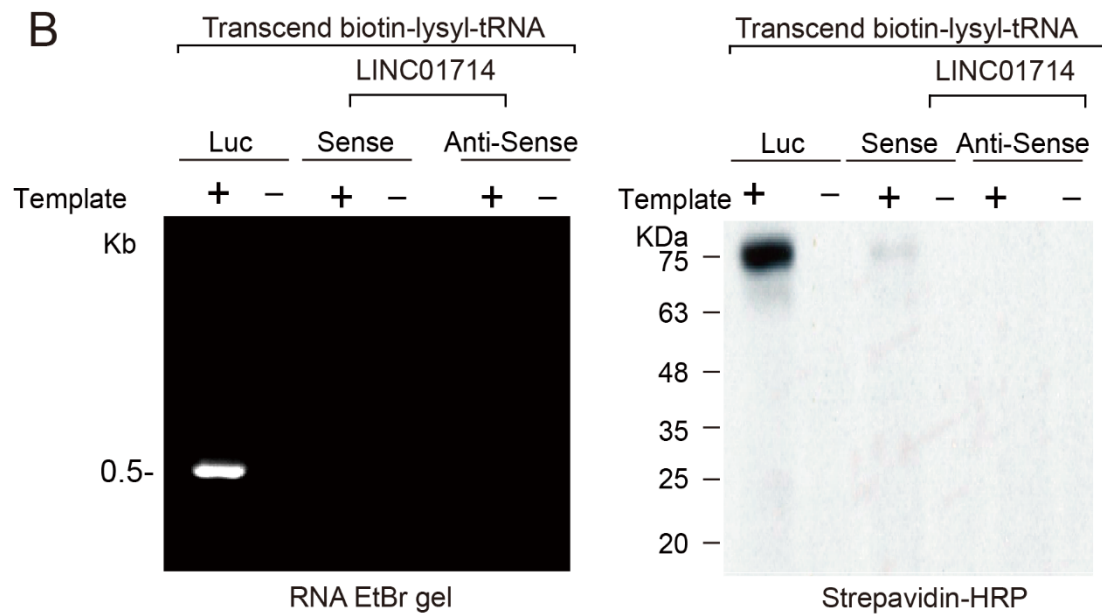https://lncipedia.org/db/transcript/LINC01714:36

B

Figure S3. (**A**) The protein coding potential prediction of LINC01714 from LNCipedia database. (**B**) *In vitro* transcription and translation of LINC01714 sense or antisense transcript. Luciferase (Luc) is used as a positive control.
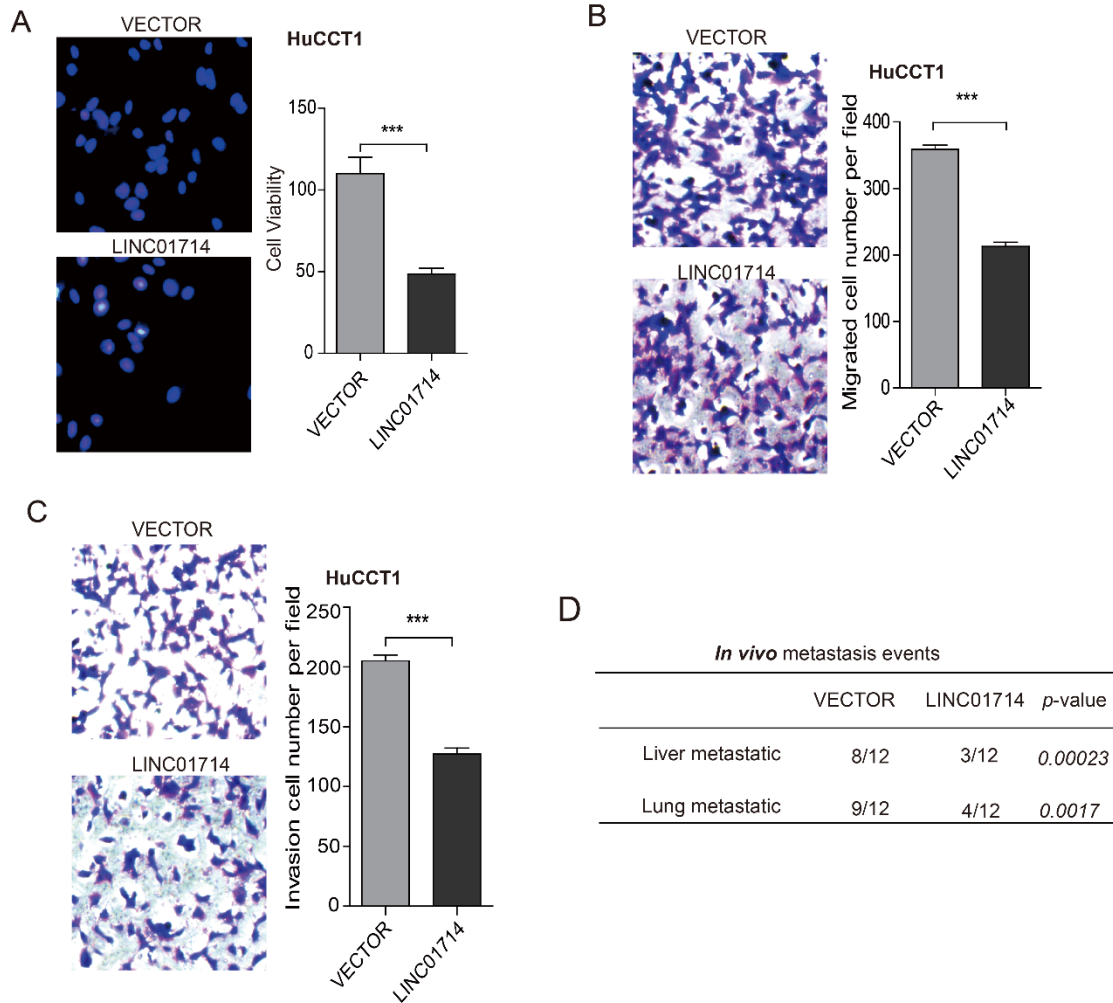
Figure S4. **LINC01714 suppresses cell proliferation, metastasis and invasion *in vitro* and *in vivo*.** **(A)** Cell viability assay shows LINC01714 inhibits CCA cell viability (HuCCT1 cell line). **(B)** The effects of LINC01714 on CCA cell migration (HuCCT1 cell line). **(C)** The effects of LINC01714 on CCA cell invasion (HuCCT1 cell line). **(D)** Table summarizes the liver and lung metastatic events comparing between the LINC01714 and control groups. Values are shown as mean ± SEM, **P < 0.01 and ***P < 0.001.
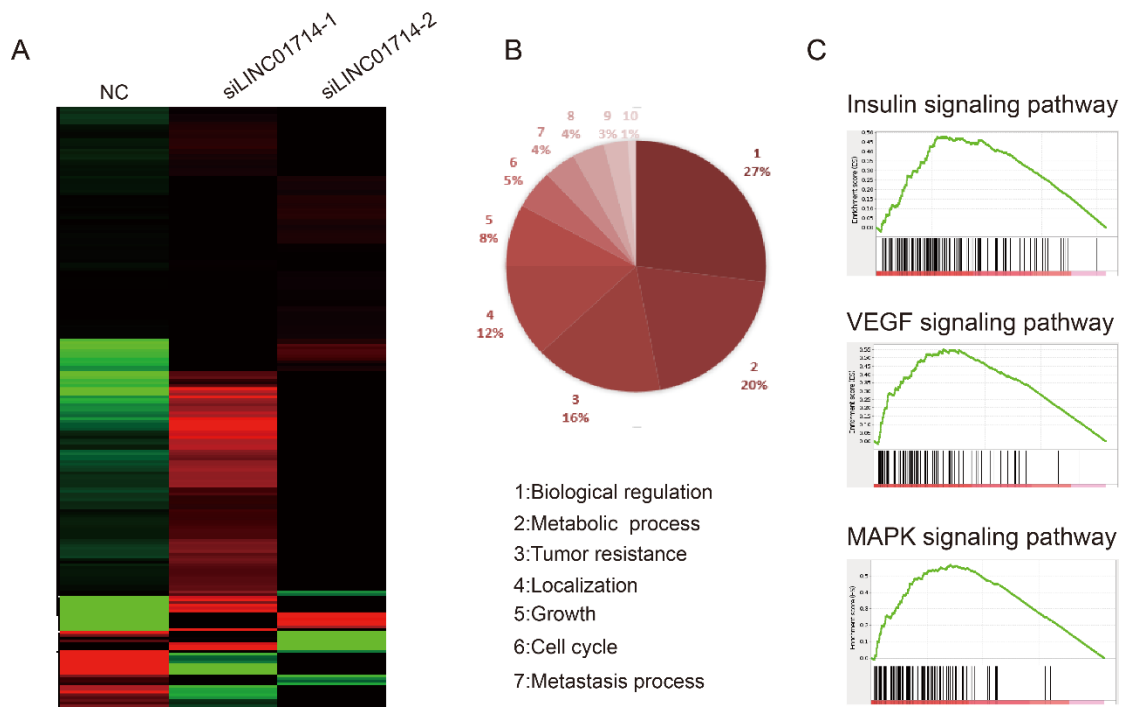
Figure S5. LINC01714 impacts the expression of genes involved in tumor-related pathways. (**A**) Heatmap shows gene expression in CCLP1 cell lines that were transfected with LINC01714 siRNAs or mock controls. (B) Pie chart shows the proportion of differentially expressed genes (si-LINC01714 cells v.s. control cells) in top ranked biological processes. (C) Results of GSEA analysis of differentially expressed genes, including Insulin signaling pathway, VEGF signaling pathway and MAPK signaling pathway.
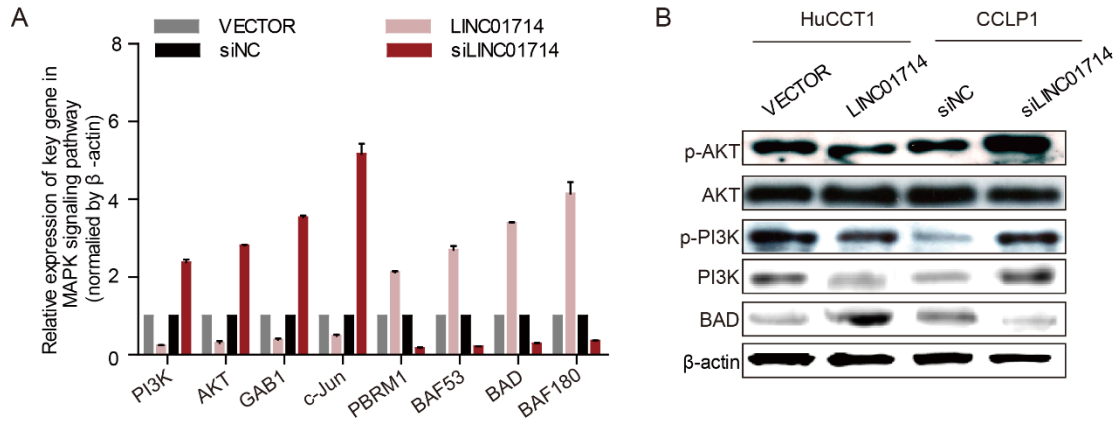
Figure S6. Validation of mRNA and protein change of some key tumor-related genes after knockdown of LINC01714. (**A**) Relative mRNA levels (quantitative real-time PCR) of selected genes from the top ranked pathways affected by LINC01714. (**B**) Western blotting results of selected genes (PI3K and BAD) in HuCCT1 and CCLP1 cell lines, where β-actin serves as control.

Figure S7. LINC01714 enhance Gemcitabine sensitivity in CCA cells. (**A**) Tumor weight of LINC01714 overexpression and control mice groups. (**B**) The change of tumor weights in LINC01714 overexpression and control mice groups. (**C**) The change of tumor volumes in LINC01714 overexpression and control mice groups.

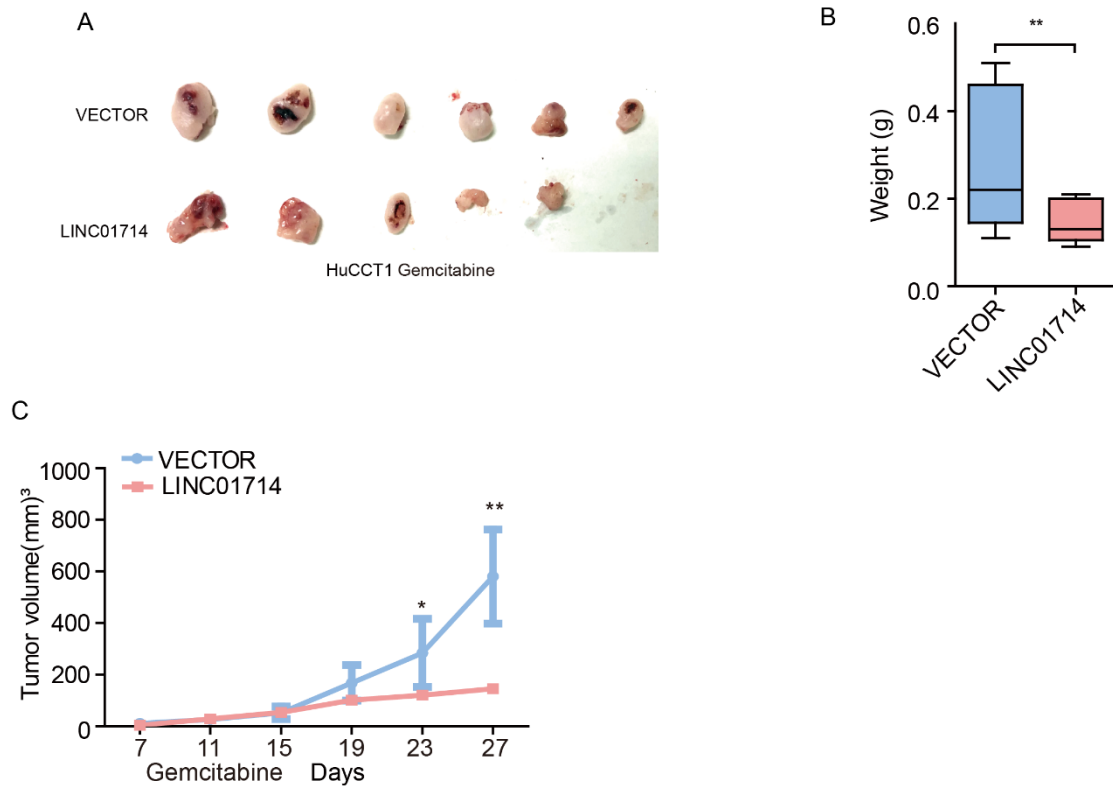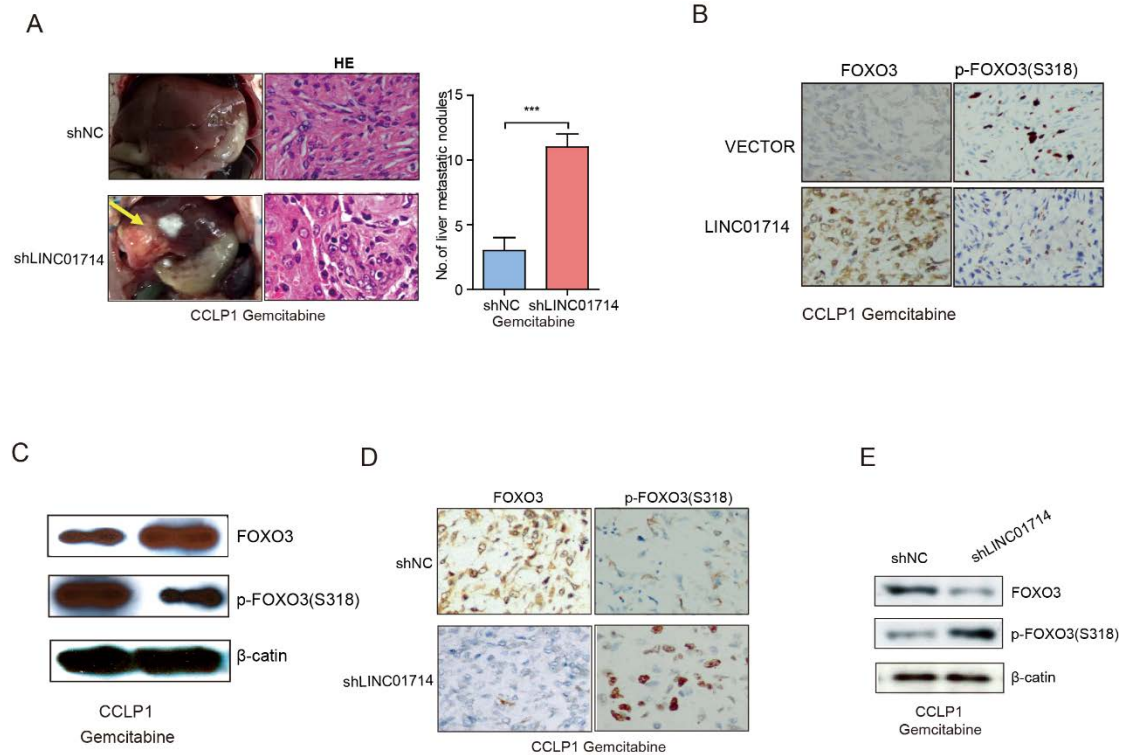**Figure S8.** (A) Representative data showed that knockdown of LINC01714 promoted the liver metastasis of CCA tumor in nude mice bearing subcutaneous tumor xenografts from Gemcitabine treated CCLP1 cells. (B) Representative IHC images of FOXO3 and phosphorylated FOXO3 (S318) protein in samples collected from nude mouse model bearing subcutaneous tumor xenografts from Gemcitabine treated CCLP1 cell lines with overexpressed LINC01714 or control transfection. (C) Western blot results of FOXO3 and phosphorylated FOXO3 (S318) in Gemcitabine treated CCLP1 cells with overexpressed LINC01714 or control transfection. (D) Representative IHC images of FOXO3 and phosphorylated FOXO3 (S318) protein in samples collected from nude mouse model bearing subcutaneous tumor xenografts from Gemcitabine treated CCLP1 cell lines with knockdown LINC01714 or control transfection. (E) Western blot results of FOXO3 and phosphorylated FOXO3 (S318) in Gemcitabine treated CCLP1 cells with knockdown LINC01714 or control transfection.

Supplemental Tables

Supplemental Table 1. lncRNAs that show both differential expression and significant survival difference.

Gene_id	log2FoldChange	de_fdr	logrank_pvalue	Gene_type	Gene_name
ENSG00000281655.1	-6.315765241	3.47E-08	0.035567125	lincRNA	RP11-817J15.3
ENSG00000267629.3	-5.034242992	1.69E-13	0.013442901	processed_transcript	AC138430.4
ENSG00000251164.1	-4.572731764	1.98E-07	0.025410985	lincRNA	HULC
ENSG00000279711.1	-4.086224252	1.37E-07	0.012992475	TEC	AP004782.1
ENSG00000275088.1	-4.064618044	2.40E-20	0.002270004	lincRNA	RP11-442O1.3
ENSG00000228620.1	-3.946917768	6.27E-08	0.012173139	antisense	RP3-434P1.6
ENSG00000227634.1	-3.906270329	1.95E-09	0.047371921	lincRNA	LINC01714
ENSG00000251314.2	-2.856647177	4.86E-06	0.000494717	antisense	CTD-2337A12.1
ENSG00000203307.2	-2.797815894	0.000173984	0.022343837	antisense	RP11-375H19.2
ENSG00000279022.1	-2.577617753	9.66E-08	0.026864054	TEC	RP11-250B2.4
ENSG00000281903.1	-2.55107769	9.89E-06	0.038695937	lincRNA	AF127936.7
ENSG00000250328.4	-2.536761504	0.002095646	0.026862483	antisense	MGC32805
ENSG00000259343.4	-2.513123581	1.82E-08	0.024435357	antisense	TMC3-AS1
ENSG00000240006.1	-2.205091894	2.34E-07	0.023520348	lincRNA	RP11-200A1.1
ENSG00000231680.1	-2.122668031	0.003101925	0.006699473	lincRNA	AP003774.6
ENSG00000225913.2	-1.938365401	0.003866308	0.045482483	lincRNA	RP11-57C13.6
ENSG00000221571.3	-1.862158858	0.007476982	0.024757079	lincRNA	RNU6ATAC35P
ENSG00000272884.1	-1.788725901	0.042063074	0.002694654	processed_transcript	RP11-104H15.10
ENSG00000253701.2	-1.745978679	0.01277356	0.022821766	lincRNA	AL928768.3
ENSG00000278266.1	-1.680893647	0.005573992	0.045220415	lincRNA	RP11-575F12.3
ENSG00000281920.1	-1.655627324	0.025260956	0.037759906	lincRNA	RP11-418H16.1
ENSG00000275995.1	-1.575885757	0.015926017	0.001949641	lincRNA	CTD-2240J17.4
ENSG00000267064.1	-1.549854254	1.44E-05	0.020994026	antisense	UXT-AS1
ENSG00000225335.3	-1.487941018	8.31E-06	0.018339461	antisense	XXbac-B476C20.9
ENSG00000271926.1	-1.397268755	0.024906399	0.014552616	lincRNA	CTD-2376I4.1
ENSG00000215014.4	-1.337914254	0.00052673	0.015535774	lincRNA	RP5-832C2.5
ENSG00000271849.1	-1.327200562	0.013030901	0.015253218	lincRNA	CTC-332L22.1
ENSG00000272402.1	-1.212731718	4.59E-06	0.026000846	antisense	RP1-30M3.6
ENSG00000225177.4	-1.185461501	0.004898683	0.029954382	antisense	RP11-390P2.4
ENSG00000272787.1	-1.124584494	0.022229027	0.037234586	lincRNA	KB-226F1.2
ENSG00000229980.4	-1.097072305	0.001770032	0.022315592	processed_transcript	TOB1-AS1
ENSG00000260296.1	1.07791118	0.029621624	0.047813154	sense_overlapping	RP11-395I6.3
ENSG00000238113.5	1.175596134	0.040278879	0.041349463	lincRNA	LINC01410
ENSG00000262089.1	1.360696622	0.002349923	0.038034976	lincRNA	RP11-589P10.5
ENSG00000279738.1	1.370097106	0.000226011	0.048910858	sense_overlapping	RP5-1014D13.2
ENSG00000259865.1	1.520989486	5.42E-07	0.023038278	lincRNA	RP11-488L18.10

ENSG00000255320.1	1.522062153	0.001875528	0.010398007	antisense	RP11-755F10.1
ENSG00000226091.6	1.534390089	0.034011629	0.040041451	lincRNA	LINC00937
ENSG00000275936.1	1.547541837	0.030146676	0.030988066	antisense	RP1-278C19.8
ENSG00000235852.1	1.963549688	0.041261702	0.047222239	antisense	AC005540.3
ENSG00000261693.1	2.021832009	0.014785295	0.001220763	antisense	RP13-467H17.1
ENSG00000273416.1	2.04661075	0.010155328	0.048350333	lincRNA	RP4-597N16.4
ENSG00000258285.1	2.093888126	0.003431447	0.011215287	lincRNA	TESC-AS1
ENSG00000226510.1	2.174101277	0.030654949	0.039966869	antisense	UPK1A-AS1
ENSG00000267108.1	2.213375137	0.048983664	0.02282787	lincRNA	RP11-861E21.1
ENSG00000228008.1	2.228775111	0.033833091	0.026489778	antisense	CTD-2330K9.3
ENSG00000230438.6	2.231840301	5.15E-06	0.034237866	lincRNA	SERPINB9P1
ENSG00000273424.1	2.244559713	0.00019409	0.031407104	lincRNA	CTA-223H9.9
ENSG00000262001.1	2.333422411	5.96E-07	0.049475712	antisense	DLGAP1-AS2
ENSG00000280167.1	2.377538748	0.021896494	0.004531157	TEC	RP11-867G2.4
ENSG00000227356.2	2.583008589	0.018589351	0.013461062	lincRNA	LINC00866
ENSG00000270035.1	2.595498079	0.003748924	0.02220186	lincRNA	RP11-338N10.2
ENSG00000231057.3	2.614368422	0.004578765	0.046527069	antisense	RP11-122M14.1
ENSG00000255737.2	2.651276946	3.75E-06	0.047021161	antisense	AGAP2-AS1
ENSG00000274414.1	2.693845928	0.000120645	0.010792709	lincRNA	RP5-965G21.4
ENSG00000260989.1	2.808254089	0.00553614	0.001975927	antisense	LA16c-395F10.2
ENSG00000249526.1	2.846937981	0.015883413	0.006047915	lincRNA	CTB-35F21.1
ENSG00000227014.4	2.887439732	0.00331758	0.000655518	antisense	AC007285.6
ENSG00000254987.1	3.113178677	0.009640985	0.021360517	lincRNA	RP11-563P16.1
ENSG00000213793.4	3.137836786	9.60E-09	0.026264348	lincRNA	ZNF888
ENSG00000244502.2	3.248769155	0.042037388	0.044604571	antisense	HDAC11-AS1
ENSG00000279353.1	3.478782697	0.008077798	0.021848457	TEC	RP11-864N7.4
ENSG00000244137.1	3.546325693	0.002389954	0.034715373	antisense	RP11-99J16__A.2
ENSG00000265443.1	3.58841173	0.027800632	0.011484914	lincRNA	CTD-2349P21.6
ENSG00000279721.1	3.661060668	0.019543792	0.018273457	TEC	RP11-416H1.2
ENSG00000250748.5	3.677456099	0.013540169	0.03215293	lincRNA	RP11-230G5.2
ENSG00000261801.4	3.847665589	3.68E-13	0.004038022	antisense	LOXL1-AS1
ENSG00000275484.1	3.852599275	1.30E-09	0.043624511	lincRNA	CTC-1337H24.4
ENSG00000228742.8	3.880234944	0.004543549	0.004777285	lincRNA	RP5-884M6.1
ENSG00000223949.5	4.117953257	0.001911548	0.007306513	antisense	ROR1-AS1
ENSG00000261266.2	4.160071955	0.008908502	0.002689953	antisense	CTD-2196E14.5
ENSG00000272130.1	4.246939793	0.006394118	0.003448526	lincRNA	RP11-360I2.1
ENSG00000230316.5	4.311870046	2.10E-06	0.020371264	antisense	FEZF1-AS1
ENSG00000273786.1	4.397910076	0.006217919	0.004936951	sense_intronic	RP11-133K1.11
ENSG00000183535.8	4.629727144	0.001827452	0.041171516	antisense	COL18A1-AS1
ENSG00000227407.1	4.800923894	0.000146325	0.040765063	antisense	AC008746.3
ENSG00000224481.1	4.965913722	0.001091067	0.010438601	lincRNA	RP11-495P10.3
ENSG00000268496.1	5.547401216	8.79E-08	0.044475934	sense_intronic	CTD-2587H19.2
ENSG00000231290.4	7.09442384	2.66E-10	0.04945905	processed_transcript	APCDD1L-AS1