Supplemental Information

TET1 promotes the malignant progression of cholangiocarcinoma with IDH1 wild-type

Gene name	Species	Forward primer	Reverse primer
CDK4	*Homo sapiens*	ATGGCTACCTCTCGATATGAGC	CATTGGGGACTCTCACACTCT
CDKN1a	*Homo sapiens*	GCAGACCAGCATGACAGATT	CTTCCTGTGGGCGGATTAG
CDKN2b	*Homo sapiens*	TAGTGGAGAAGGTGCGACA	CCATCATCATGACCTGGATCG
GAPDH	*Homo sapiens*	GGTCGGAGTCAACGGATTT	GATGGCAACAATATCCACTTTACC
CCND1	*Homo sapiens*	CCTCGGTGTCCTACTTCAAATG	CACTTCTGTTCCTCGCAGAC
CCNE1	*Homo sapiens*	GTACTGAGCTGGGCAAATAGAG	GAAGAGGGTGTTGCTCAAGAA
CDKN2c	*Homo sapiens*	GGGGACCTAGAGCAACTTACT	CAGCGCAGTCCTTCCAAAT
Cdc25a	*Homo sapiens*	CTCCTCCGAGTCAACAGATTCA	CAACAGCTTCTGAGGTAGGGA
BAK	*Homo sapiens*	GTTTTCCGCAGCTACGTTTTT	GCAGAGGTAAGGTGACCATCTC
BIK	*Homo sapiens*	GACCTGGACCCTATGGAGGAC	CCTCAGTCTGGTCGTAGATGA
HRK	*Homo sapiens*	GGCAGGCGGAACTTGTAGGAAC	TCCAGGCGCTGTCTTTACTCTCC
Bcl2	*Homo sapiens*	GGTGGGGTCATGTGTGTGG	CGGTTCAGGTACTCAGTCATCC
Bcl-XL	*Homo sapiens*	GAGCTGGTGGTTGACTTTCTC	TCCATCTCCGATTCAGTCCCT

Supplemental Table 1. Primers used in this study.

Gene1	Gene2	Correlation	p-value
TET1	TP53	0.501416	0.001837
TET1	IDH1	0.033104	0.848002
TP53	IDH1	0.121159	0.481497
TET1	IDH2	-0.07955	0.644681
TP53	IDH2	-0.05069	0.769076
IDH1	IDH2	0.234794	0.168078
TET1	TP53mut	-0.17063	0.319741
TP53	TP53mut	-0.07258	0.674008
IDH1	TP53mut	0.210263	0.218372
IDH2	TP53mut	-0.19552	0.253127
TET1	IDH1mut	-0.16418	0.338642
TP53	IDH1mut	-0.12237	0.477089
IDH1	IDH1mut	0.036271	0.833656
IDH2	IDH1mut	0.240483	0.157712
TP53mut	IDH1mut	-0.12109	0.481748
TET1	IDH2mut	-0.11978	0.486545
TP53	IDH2mut	0.049861	0.772745
IDH1	IDH2mut	-0.0462	0.789024
IDH2	IDH2mut	0.078675	0.648318
TP53mut	IDH2mut	-0.05096	0.76785
IDH1mut	IDH2mut	-0.06788	0.694034

Supplemental table 2. Correlation analysis for TCGA-CHOL dataset.

Gene1	Gene2	Correlation	p-value
TET1	TP53	0.17646	0.092445
TET1	IDH1	-0.00973	0.926624
TP53	IDH1	0.032646	0.757377
TET1	IDH2	0.069563	0.509959
TP53	IDH2	0.132225	0.208955
IDH1	IDH2	0.349778	0.000631

Supplemental Table 3. Correlation analysis for GSE76297 dataset.

Gene 1	Gene 2	Correlation	p-value
TET1	TP53	0.208656	0.164043
TET1	IDH1	-0.21907	0.143543
TP53	IDH1	-0.03713	0.806471
TET1	IDH2	-0.18427	0.220223
TP53	IDH2	0.066902	0.658662
IDH1	IDH2	-0.13035	0.387894

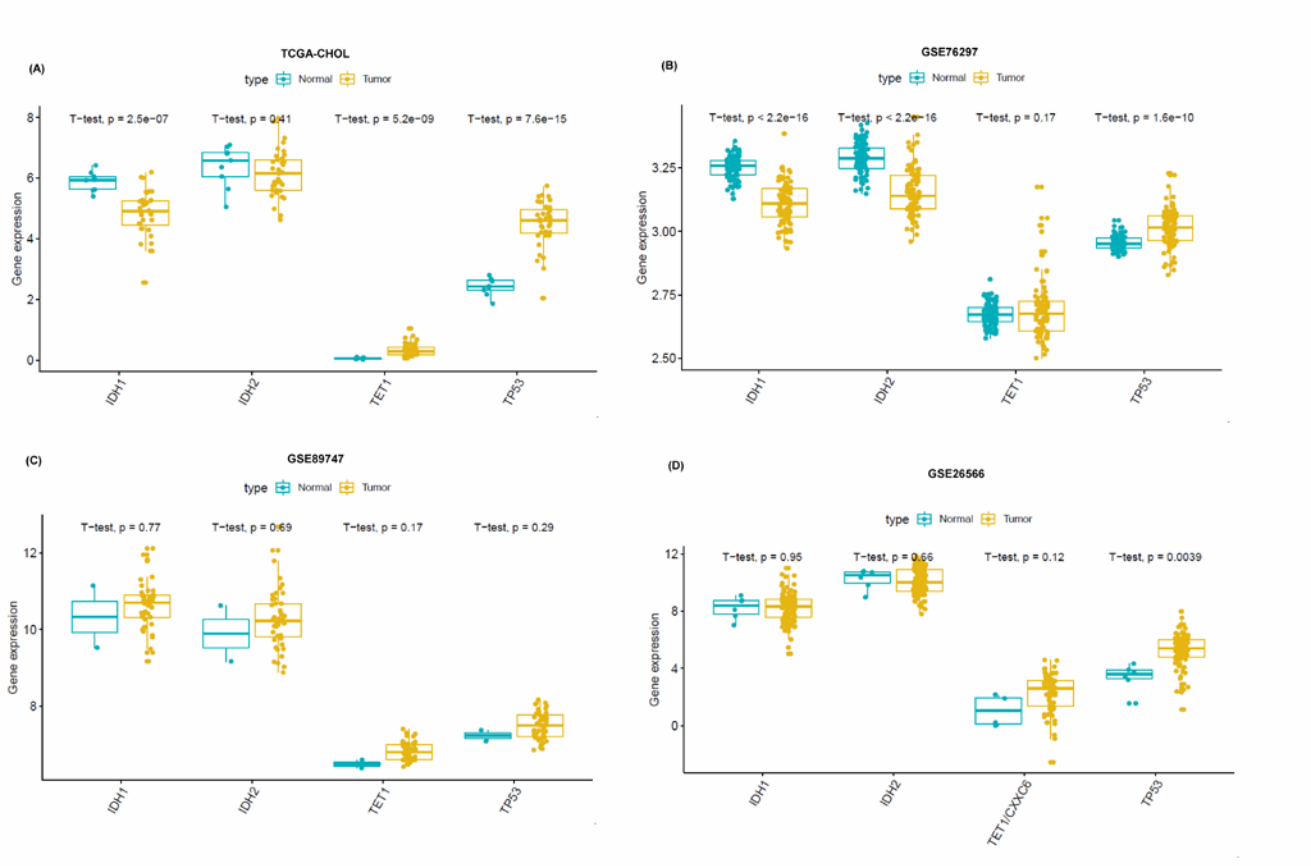
Supplemental Table 4. Correlation analysis for GSE89747dataset.

Gene1	Gene2	Correlation	p-value
TET1/CXXC6	IDH1	-0.03822	0.691792
TET1/CXXC6	IDH2	0.196957	0.039174
IDH1	IDH2	0.399921	1.50E-05
CXXC6	TP53	-0.03325	0.730235
IDH1	TP53	-0.01272	0.895091
IDH2	TP53	-0.0129	0.893604

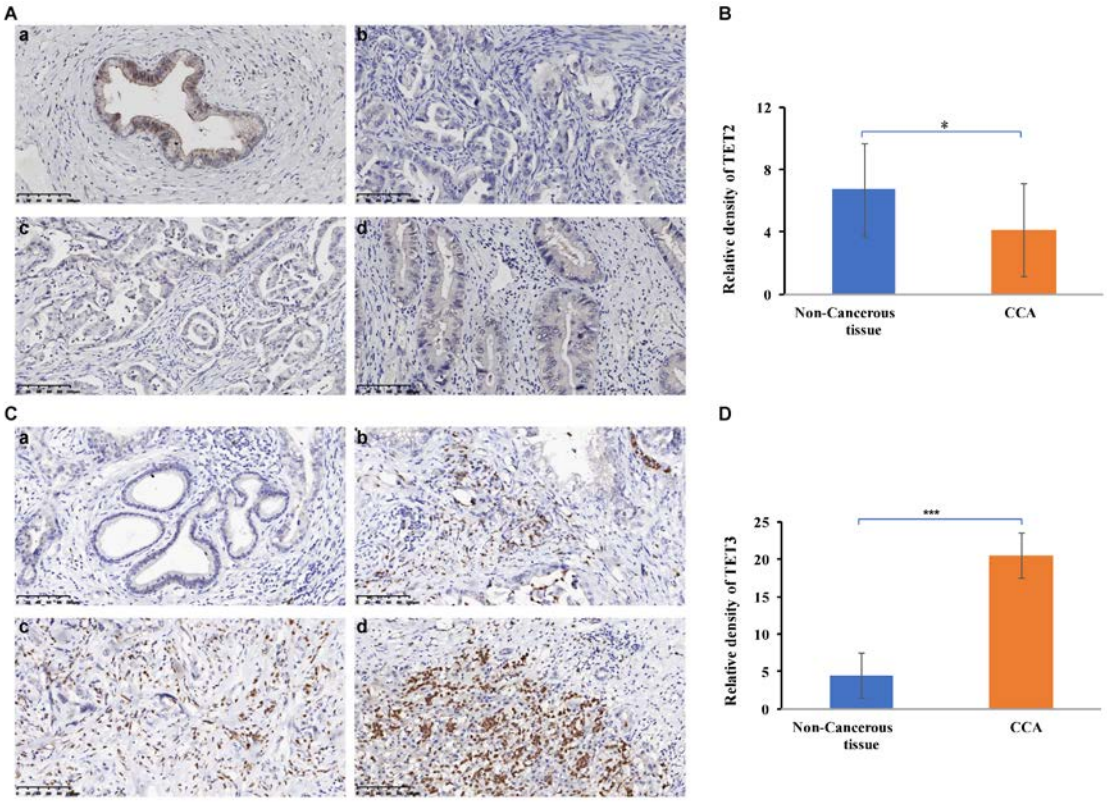
Supplemental Table 5. Correlation analysis for GSE266566 dataset.

NAME	SIZE	ES	NES	p-value
KEGG_NOTCH_SIGNALING_PATHWAY	47	0.610762	1.840032	<0.0001
KEGG_THYROID_CANCER	29	0.45638	1.54861	0.022403
KEGG_SMALL_CELL_LUNG_CANCER	84	0.448934	1.517881	0.026369
KEGG_CHRONIC_MYELOID_LEUKEMIA	73	0.451787	1.467951	0.033465
KEGG_GLIOMA	65	0.414158	1.467408	0.036957
KEGG_PROSTATE_CANCER	89	0.400254	1.463823	0.02045
KEGG_RNA_DEGRADATION	57	0.492549	1.459486	0.046512
KEGG_ERBB_SIGNALING_PATHWAY	87	0.414868	1.45225	0.036437

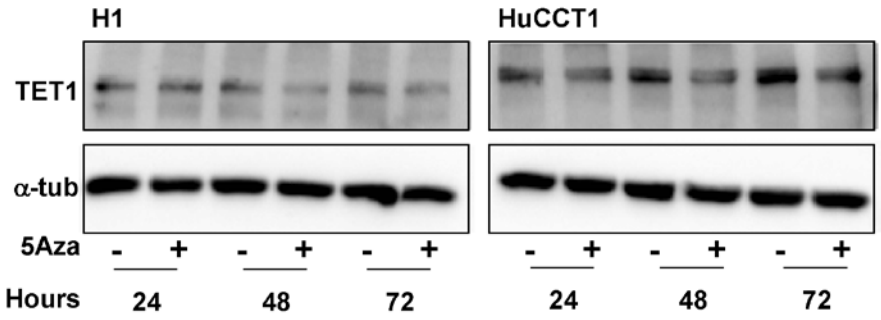
Supplemental Table 6. Enriched KEGG pathways in TET1 high versus TET1 low TCGA-CHOL samples. Size indicates how many genes have been involved in the pathway. ES, enrichment score; NES, normalized enrichment score.



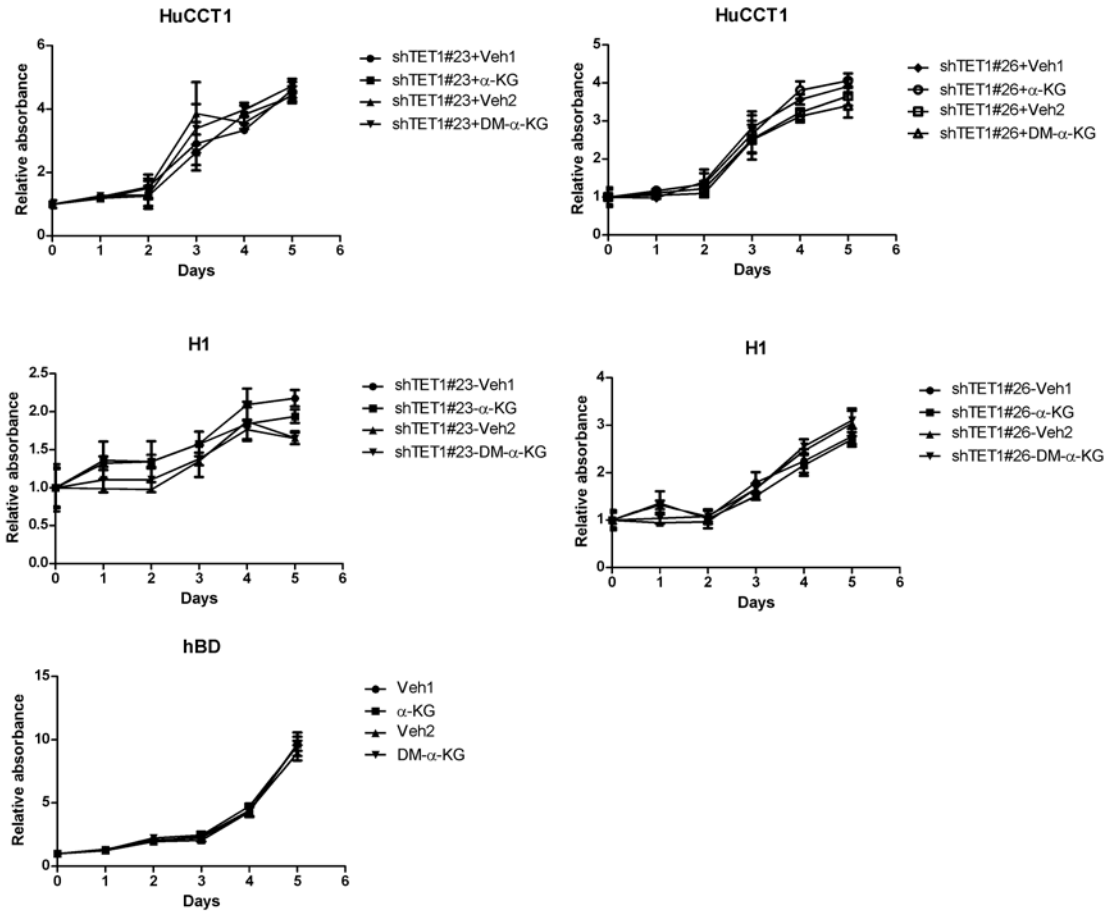
Supplemental Figure 1. The mRNA expression levels of IDH1, IDH2, TET1, and TP53 were analyzed in different datasets including TCGA-CHOL, GSE76297, GSE89747, and GSE26566.



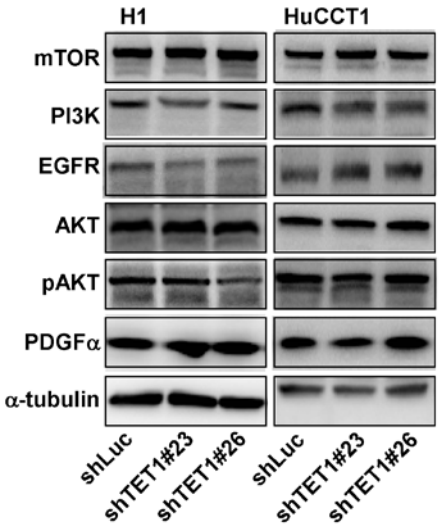
Supplemental Figure 2. The results of TET2 and TET3 protein expression levels in non-cancerous biliary and CCA tissues. (A) The representative IHC images of TET2 in non-cancerous (a) and CCA (b-d) were shown. (B) Quantification of TET2 IHC results. (C) The representative IHC images of TET3 in non-cancerous (a) and CCA (b-d) were shown. (D) Quantification data of TET3 IHC results. *, $p<0.05$; ***, $p<0.001$, when compared with control, n=12 in non-cancerous group and n=91 in CCA group.



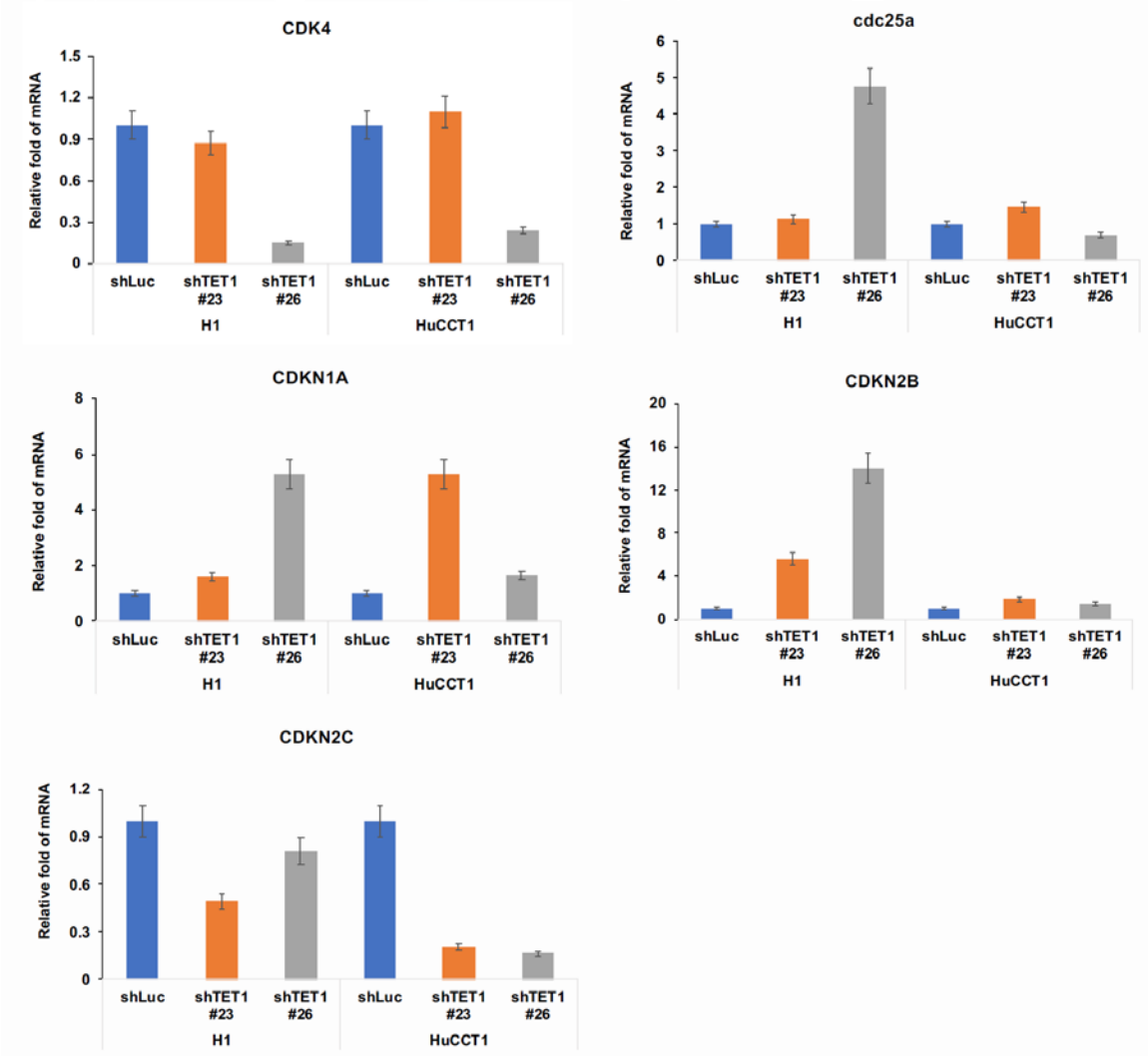
Supplemental Figure 3. TET1 protein expression in H1 and HuCCT1 treated with DNA methylation inhibitor, 5-Aza for 24, 48, and 72 hours. α-Tubulin served as loading control.



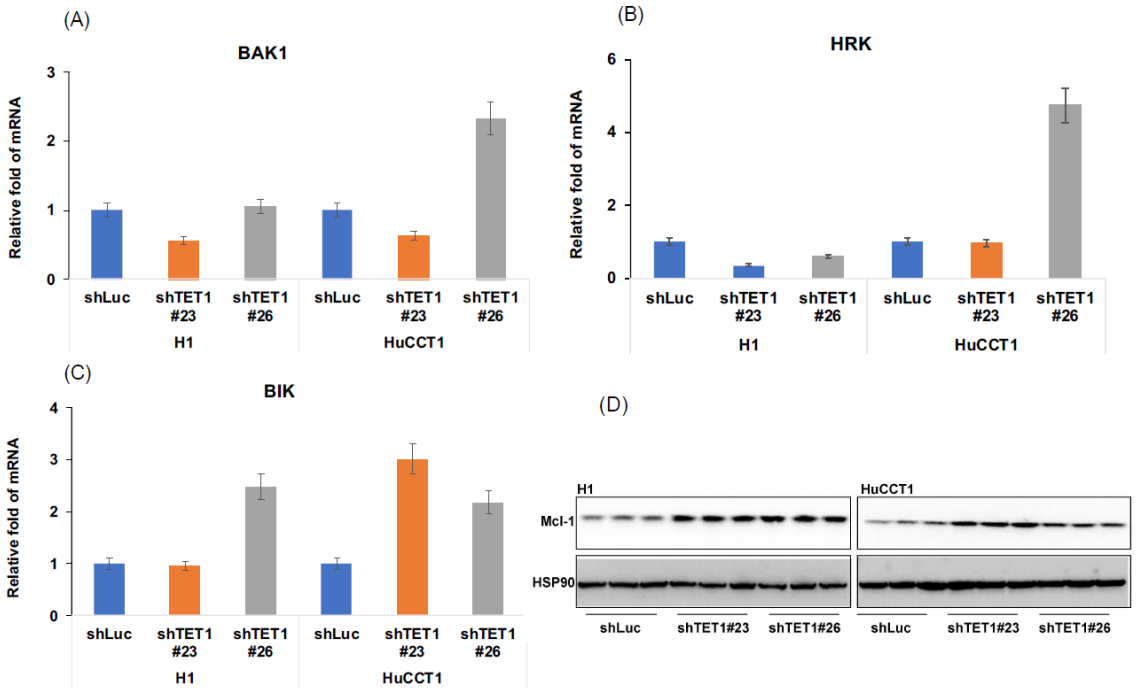
Supplemental Figure 4. MTT results of H1, HuCCT1, and hBD cells. Relative cell growth rates were determined in H1-shTET1#23, H1-shTET1#26, HuCCT1-shTET1#23, HuCCT1-shTET1#26, and hBD cells treated with vehicle #1 (veh1, H2O), α-KG, vehicle #2 (veh2, DMSO), and DM-α-KG for 1, 2, 3, 4, 5 days, n=8.



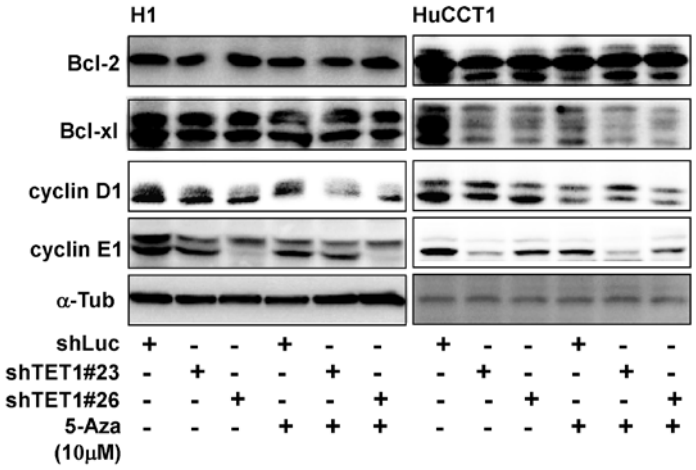
Supplemental Figure 5. The impact of TET1 down-regulation on oncogenic pathways in CCA cells. The protein expression levels of mTOR, PI3K, EGFR, Akt, phosphor-Akt (pAkt) PDGFα, and α-tubulin were determined in H1 and HuCCT1 cells treated with shLuc, shTET1#23, and shTET1#26.



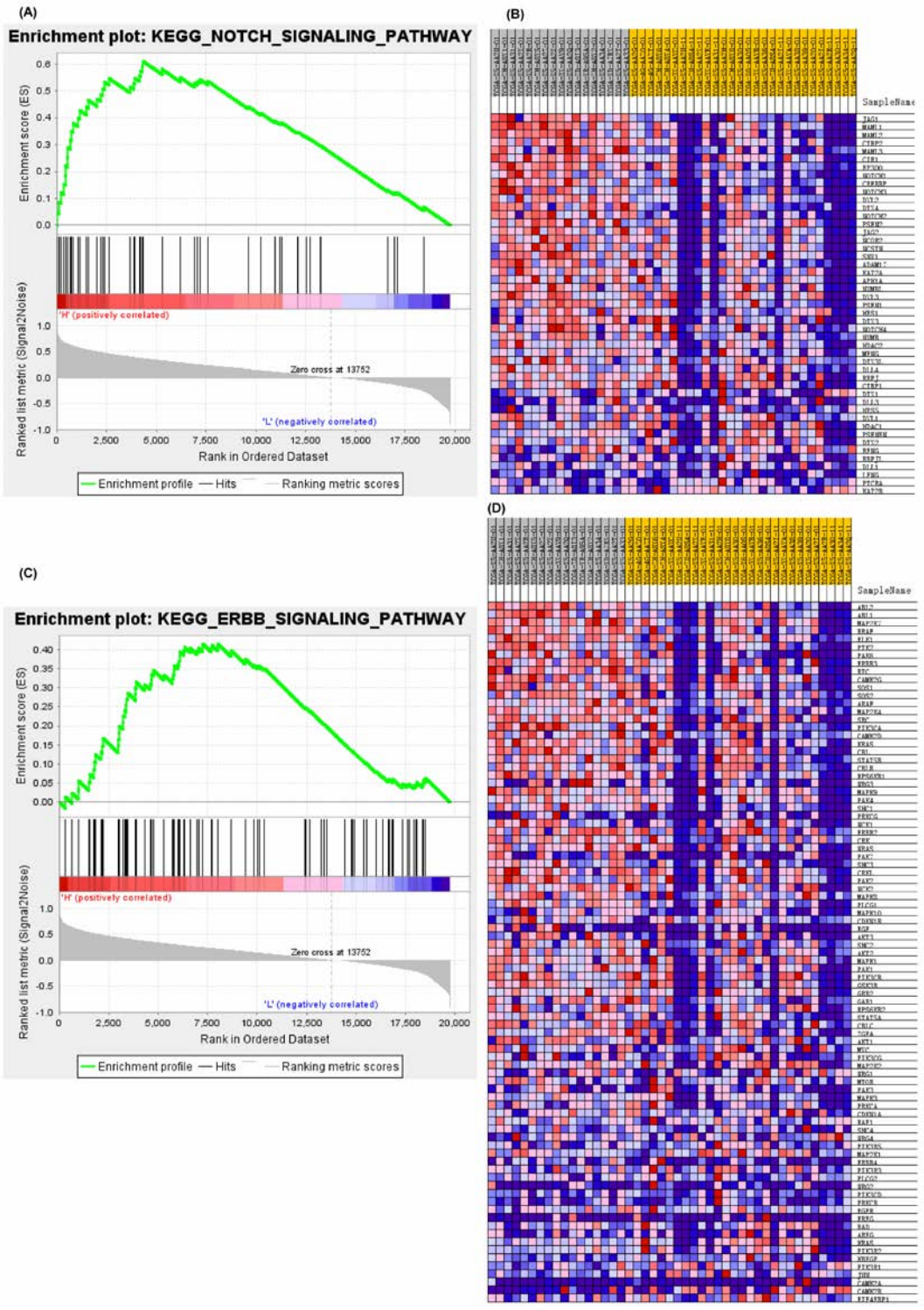
Supplemental Figure 6.The expression levels of cell cycle associated genes, including CDKN2c, CDK4, cdc25a, CDKN1A, and CDKN1B, n=6.



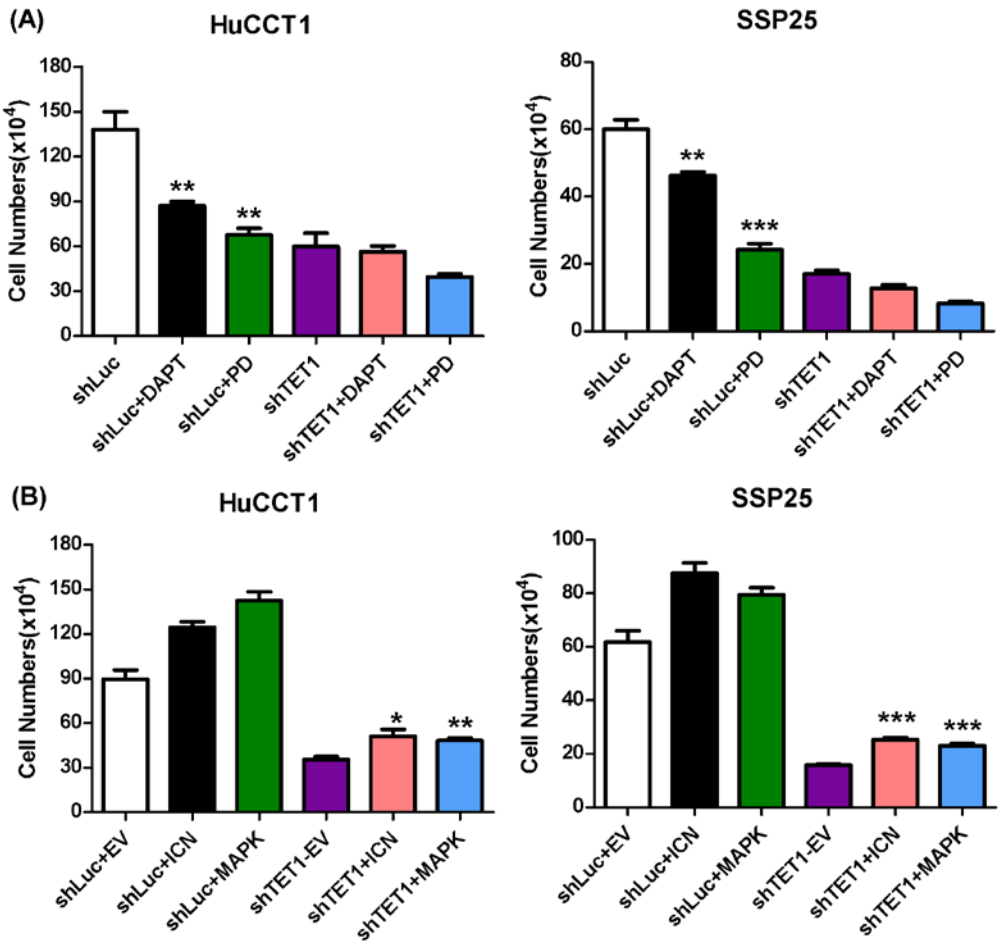
Supplemental Figure 7. The mRNA expression of pro-apoptotic genes, including (A) HRK, (B) BIK and (C) BAK were determined, n=6. (D) The protein expression of Mcl-1 was examined in H1 and HuCCT1 cells treated as indicated.



Supplemental Figure 8. Western blotting results of Bcl-2, Bcl-xl, cyclin D1, and cyclin E1 in H1 and HuCCT1 CCA cells. The protein expression levels of Bcl-2, Bcl-xl, cyclin D1, cyclin E1, and a-Tub were determined in H1-shLuc, H1-shTET1#23, H1-shTET1#26, HuCCT1-shLuc, HuCCT1-shTET1#23, and HuCCT1-shTET1#26 treated with DMSO or 10μM 5-Aza for 48 hours.



Supplemental Figure 9. Notch and ERbB signaling pathways are associated with TET1 expression in CCA tumors. Enrichment plots of (A) Notch signaling pathway and (C) ErbB signaling in TET1 high versus TET1 low CCA tumors. Heatmaps of (B) Notch and (D) ErbB signaling pathways associated molecules in TET1 high (Gray color) and TET1 low (orange color).



Supplemental Figure 10. Involvement of Notch1 and MAPK in TET1-mediated CCA cell growth. (A) Cell numbers were counted in HuCCT1 and SSP25 CCA cells transduced with shLuc, shTET1#23, and shTET1#26 in the presence of DMSO, 10μM DAPT, or 10μM PD98059 (PD) for 5 days. (B) Cell numbers were determined in HuCCT1 and SSP25 cells transduced with shLuc, shTET1#23, and shTET1#26 in the presence of empty vector, Notch1 intracellular domain, or MAPK transfection for 7 days. N=3 per group. **, $p<0.01$; ***, $p<0.001$ when compared with relevant controls.