Supplementary Table 1: Correlation analysis between the clinical features and DTL expression in HCC

Characteristics of patients		DTL Level		P.overall
		Low (N=169)	High (N=40)	
Sex	Male	145 (85.8%)	33 (82.5%)	0.779
	Female	24 (14.2%)	7 (17.5%)	
Age	<50	69 (40.8%)	23 (57.5%)	0.083
	≥50	100 (59.2%)	17 (42.5%)	
Tumor size	<3.0cm	70 (41.4%)	14 (35.0%)	0.572
	≥3.0cm	99 (58.6%)	26 (65.0%)	
Tumor differentiation	I/II	96 (57.4%)	19 (47.5%)	0.339
	III/IV	93 (44.5%)	21 (52.5%)	
Vascular invasion	Present	58 (34.3%)	26 (65.0%)	<**0.001***
	Absent	111 (65.7%)	14 (35.0%)	
Tumor number	<3	162 (95.9%)	35 (87.5%)	0.056
	≥3	7 (4.14%)	5 (12.5%)	
AFP level	<400	124 (73.4%)	23 (57.5%)	0.074
	≥400	45 (26.6%)	17 (42.5%)	
Liver cirrhosis	Present	105 (62.1%)	22 (55.0%)	0.515
	Absent	64 (37.9%)	18 (45.0%)	
Child-pugh class	A	160 (94.7%)	39 (97.5%)	0.691
	B	9 (5.33%)	1 (2.50%)	
TNM	I/II	112 (66.3%)	14 (35.0%)	<**0.001***
	III/IV	57 (33.7%)	26 (65.0%)	

HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; TNM tumor-node-metastasis. Significant values are in bold.

Supplementary Table 2: Multivariate Analyses of Predictors of Overall Survival

Risk Factor	Univariate			Multivariate		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Sex	2.466	0.764 - 7.956	0.131			
Age	0.5239	0.290-0.948	**0.033***	0.649	0.352-1.197	0.166
Liver cirrhosis	1.063	0.586-1.931	0.840			
Child-pugh class	0.857	0.208-3.539	0.831			
AFP level	1.789	0.985-3.248	0.056			
Tumor differentiation	2.281	1.248-4.168	**0.007***	1.823	0.978-3.398	0.059
Tumor size	3.434	1.598-7.376	**0.002***	2.869	1.264-6.514	**0.012***
Tumor number	1.677	0.600-4.684	0.324			
Vascular invasion	0.390	1.215-0.709	**0.002***	/	/	1
TNM	2.623	1.444-4.765	**0.002***	/	/	1
DTL Level	4.333	2.382-7.883	**<0.001***	3.261	1.720-6.185	**<0.001***

Abbreviations: HR, hazard ratio; AFP, Alpha-fetoprotein; TNM tumor-node-metastasis

*P value ≤ 0.05 was considered to indicate statistical significance.

Supplementary Table 3: Multivariate Analyses of Predictors of Disease-free Survival

Risk Factor	Univariate			Multivariate		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Sex	1.294	0.708-2.368	0.402			
Age	1.156	0.771-1.733	0.484			
Liver cirrhosis	0.137	0.768-1.712	0.505			
Child-pugh class	0.739	0.272-2.011	0.554			
AFP level	1.158	0.754-1.778	0.503			
Tumor differentiation	0.267	0.880-1.938	**0.184***	1.869	1.003-3.485	**0.049***
Tumor size	1.647	1.080-2.510	**0.021***	2.806	1.232-6.391	**0.014***
Tumor number	1.355	0.628-2.928	0.439			
Vascular invasion	0.363	0.243-0.542	**<0.001***	/	/	1
TNM	2.675	1.795-3.986	**<0.001***	/	/	1
DTL Level	2.205	1.397-3.480	**<0.001***	3.60	1.942-6.659	**<0.001***

Abbreviations: HR, hazard ratio; AFP, Alpha-fetoprotein; TNM tumor-node-metastasis

*P value ≤ 0.05 was considered to indicate statistical significance.

Supplementary Table 4: Primer sequences for plasmid construction

Primer name	Sequence
DTL WT Forward	5’- GTACTCGAGATGCTCTTCAATTCGGTGCT -3’
DTL WT Reverse	5’- GTAGGATCCCTATAATTCTGTTGAGTGTT -3’
HIF-1α WT Forward	5’-GTAGGTACCAATAAGTGGTGGTTACTCAGCAC-3’
HIF-1α WT Reverse	5’-GTAGGGCCCCCTGGTCCACAGAAGATGTTTA-3’
Notch1 WT Forward	5’- CTCTAGAGATGACTAGTGCCTCGGCC-3’
Notch1 WT Reverse	5’-GGAATTCCTGGAAGCCAGATCACCATCAG-3’
shDTL NC Forward	5’- CCGGACGCATGCATGCTCAGTGCTTCTCG AGAAGCACTGAGCATGCATGCGTTTTTTG -3’
shDTL NC Reverse	5’- AATTCAAAAAACGCATGCATGCTCAGTGC TTCTCGAGAAGCACTGAGCATGCATGCGT -3’
shDTL1 Forward	5’- CCGGCCTGGTGAACTTAAACTTGTTCTCG AGAACAAGTTTAAGTTCACCAGGTTTTTG -3’
shDTL1 Reverse	5’- AATTCAAAAACCTGGTGAACTTAAACTTG TTCTCGAGAACAAGTTTAAGTTCACCAGG -3’
shDTL2 Forward	5’- CCGGGCCTAGTAACAGTAACGAGTACTCG AGTACTCGTTACTGTTACTAGGCTTTTTG -3’
shDTL2 Reverse	5’- AATTCAAAAAGCCTAGTAACAGTAACGAG TACTCGAGTACTCGTTACTGTTACTAGGC -3’
DTL promoter Forward	5’-CTAGCTAGCTAGGTATCAGAATGAGGAGGAAAGTG-3’
DTL promoter Reverse	5’- CCGCTCGAGCGGGGGAGAACTCAGAAGCTGAG-3’
Notch1 promoter Forward	5’-GGCTAGCCATGCAAACCAGGCTCTGCGG-3’
Notch1 promoter Reverse	5’-GGAATTCCACCGGCTGCCCTCTG-3’
DTL-CHIP-P1 Forward	5’- GAGCTCGCTACTTGTTTAGC-3’
DTL-CHIP-P1 Reverse	5’- CAGGAACTGGATGCCAACTC-3’
DTL-CHIP-P2 Forward	5’- GTCAAGCCCTGTTACGCATG-3’
DTL-CHIP-P2 Reverse	5’- CCAAGTTCAGCAGAAATCGC-3’
DTL-P1-mutant Forward	5’- GCTCCCCGCCCAGCGAATGCGCCTATCCGTC-3’
DTL-P1-mutant Reverse	5’- GACGGATAGGCGCATTCGCTGGGCGGGGAGC-3’

DTL-P2-mutant Forward	5’- GACTCTGAGGCGCCTCGGAAGCCAATC-3’
DTL-P2-mutant Reverse	5’- GATTGGCTTCCGAGGCGCCTCAGAGTC-3’
3xFlag-DTL Forward	5’-GTACTCGAGATGGACTACAAAGACCATGACGGTG ATTATAAAGATCATGACATCGACTACAAGGATGACG ATGACAAGATGCTCTTCAATTCGGTGCT-3’
2xHA-SLTM Forward	5’-CTAGCTAGCTAGATGTACCCATACGATGTTCCAGATT ACGCTTACCCATACGATGTTCCAGATTACGCTATG ATTGTGCGCAGCGCTGC-3’
2xHA-SLTM Reverse	5’- GTCGACTTCCAGGTGTGGATTTTTATTTTCAC -3’
1xFlag-DTL 1-398aa Forward	5’- GTAGCTAGCATGGATTACAAGGACGACGATGACAAG ATGCTCTTCAATTCGGTGCT-3’
1xFlag-DTL 1-398aa Reverse	5’- GTAGGATCCCTAGGTTTCTCCTCTAAGCCTCT-3’
1xFlag-DTL 1-448aa Reverse	5’-GTAGGATCCCTATGCGGATGACGGGGAAG-3’
1xFlag-DTL 1-498aa Reverse	5’- GTAGGATCCCTATTGAAAGATGAAGGTGGCTT-3’
1xFlag-DTL 1-598aa Reverse	5’- GTAGGATCCCTAAGGTCTTCCTGGTTACCAGC-3’
1xFlag-DTL 398-730aa Forward	5’- GTAGCTAGCATGGATTACAAGGACGACGATGACAAG GGAGGTGATAAACTTTCCAC-3’
1xFlag-DTL 398-730aa Reverse	5’-GTAGGATCCCTATAATTCTGTTGAGTGTT-3’

Supplementary Table 5: Target sequence of siRNA

Primer name	Sequence
DTL Si-1 Forward	5’- GGUUCCUGGUGAACUUAAACU-3’
DTL Si-1 Reverse	5’- UUUAAGUUCACCAGGAACCCA-3’
DTL Si-2 Forward	5’- GGCUCUACUUUAUUUGCUAAU-3’
DTL Si-2 Reverse	5’- UAGCAAAUAAAGUAGAGCCAG-3’
HIF-1α Si-1 Forward	5’- GGAAAUGAGAGAAAUGCUUDTDT-3’
HIF-1α Si-1 Reverse	5’- AAGCAUUUCUCUCAUUUCCDTDC-3’
HIF-1α Si-2 Forward	5’- GCUGGAGACACAAUCAUAUDTDT-3’
HIF-1α Si-2 Reverse	5’- AUAUGAUUGUGUCUCCAGCDGDG-3’
Notch1 Si-1 Forward	5’-GGAGCAUGUGUAACAUCAADTDT-3’
Notch1 Si-1 Reverse	5’-UUGAUGUUACACAUGCUCCDTDT-3’
Notch1 Si-2 Forward	5’-GUCCAGGAAACAACUGCAADTDT-3’
Notch1 Si-2 Reverse	5’-UUGCAGUUGUUUCCUGGACDTDT-3’
SLTM Si-1 Forward	5’- GCUGUGUGGUAGUGGUUUAAU-3’
SLTM Si-1 Reverse	5’- UAAACCACUACCACACAGCUG-3’
SLTM Si-2 Forward	5’- GGAAGUACAAGUAGUACUAGU-3’
SLTM Si-2 Reverse	5’- UAGUACUACUUGUACUUCCUU-3’

Supplementary Table 6: Primers for Quantitative Real-time PCR

Primer name	Sequence
DTL Forward	5’- CCCTTCAAAACCCAAGAAGA -3’
DTL Reverse	5’- GGTTCTTGTCGATAAGCAGT -3’
HIF-1α Forward	5’-CAGCGAAGCTTTTTTCTCAGAATGA-3’
HIF-1α Reverse	5’- CACAAATCAGCACCAAGC-3’
GAPDH Forward	5’- TGTGTCCGTCGTGGATCTGA -3’
GAPDH Reverse	5’- TTGCTGTTGAAGTCGCAGGAG -3’
NOTCH Forward	5’-GAGGCGTGGCAGACTATGC-3’
NOTCH Reverse	5’-CTTGTACTCCGTCAGCGTGA-3’
Fibronectin Forward	5’-GGAGGAAGCCGAGGTTTTAAC-3’
Fibronectin Reverse	5’-ACGCTCATAAGTGTCACCCA-3’
N-cadherin Forward	5’-TCGATTGGTTTGACCACGG-3’
N-cadherin Reverse	5’-GACGGTTCGCCATCCAGAC-3’
E-cadherin Forward	5’-AGTGGGCACAGATGGTGTGA-3’
E-cadherin Reverse	5’-TAGGTGGAGTCCCAGGCGTA-3’
Vimentin Forward	5’-TGTCCAAATCGATGTGGATGTTTC-3’
Vimentin Reverse	5’-TTGTACCATTCTTCTGCCTCCTG-3’
Slug Forward	5’-GACCCTGGTTGCTTCAAGGA-3’
Slug Reverse	5’-TGTTGCAGTGAGGGCAAGAA-3’
SNAIL Forward	5’-TCGGAAGCCTAACTACAGCGA-3’
SNAIL Reverse	5’-AGATGAGCATTGGCAGCGAG-3’
SLTM Forward	5’-AGACAGGCGAGAAGTACGAG-3’
SLTM Reverse	5’-CTGGTGGGTCTGGAAGGATT-3’
β-actin Forward	5’-CATGTACGTTGCTATCCAGGC-3’
β -actin Reverse	5’-CTCCTTAATGTCACGCACGAT-3’

Supplementary Table 7: List of the primary and secondary antibodies used in this study

Antigen	Species	Source	Catalog
DTL	Rabbit	Abcam	ab174385
HIF-1α	Rabbit	CST	#3716
GAPDH	Rabbit	Proteintech	10494
β-tubulin	Rabbit	CST	#2128
Fibronectin	Mouse	Proteintech	15613
N-cadherin	Rabbit	Affinity	AF5239
E-cadherin	Rabbit	Affinity	AF0131
Vimentin	Rabbit	Proteintech	10366
Slug	Rabbit	Proteintech	12129
Snail	Rabbit	Proteintech	13099
Notch1	Rabbit	Abcam	EP1238Y
FLAG	Mouse	Proteintech	66008
HA	Rabbit	Proteintech	81290
SLTM	Rabbit	Novus	NBP1-47300
Ubiquitin	Rabbit	Proteintech	10201
HRP-conjugated Goat Anti-Rabbit IgG (H+L)	Goat	Proteintech	SA00001-2
HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L)	Goat	Proteintech	SA00001-1
Goat Anti-Rabbit IgG (H+L) Flour 488-conjugated	Goat	Affinity	S0018
Goat Anti-Rabbit IgG (H+L) Flour 594-conjugated	Goat	Affinity	S0006

Supplementary Figure 1: (A) Heatmap of DEGs for GSE102079, GSE112790, and GSE25097 datasets from GEO, respectively. (B-D). Volcano plot of hepatocellular carcinoma (HCC) vs. adjacent nontumor, portal vein tumor thrombosis (PVTT) vs. adjacent nontumor, and PVTT vs HCC, respectively.

Supplementary Figure 2: (A) DTL mRNA levels between HCC samples and matched liver tissue (n=50) from the TCGA database. (B) Kaplan-Meier survival analysis of OS for HCC patients with high and low expression of DTL from TCGA database. (C-E) DTL mRNA levels among different histological grade (C), pathologic T stage (D), and pathologic N stage (E) in the TCGA-LIHC cohort. (F) The capacity to identify tumor or normal tissue sources of DTL in the TCGA-LIHC cohort. *P < 0.05, **P < 0.01, ***P < 0.001, ns, no significance

Supplementary Figure 3: Spearman correlation analysis between DTL and pathway score. [52] The signal pathway positively correlated with the overexpression of DTL.

Supplementary Figure 4: (A) The mRNA level of HIF-1α and DTL was determined after HIF-1α-siRNA transfection or KC7F2 (40 μM) treatment under hypoxia (1% O2) in HCCLM-3 cells. (B) The protein level of HIF-1α and DTL was quantified after HIF-1α-siRNA transfection or KC7F2 (40 μM) treatment under hypoxia (1% O2) in Huh-7 and HCCLM-3 cells. (C) The effectiveness of doxycycline-induced DTL knockdown in Huh-7 and HCCLM-3 cells was detected in various concentrations. Knockdown of

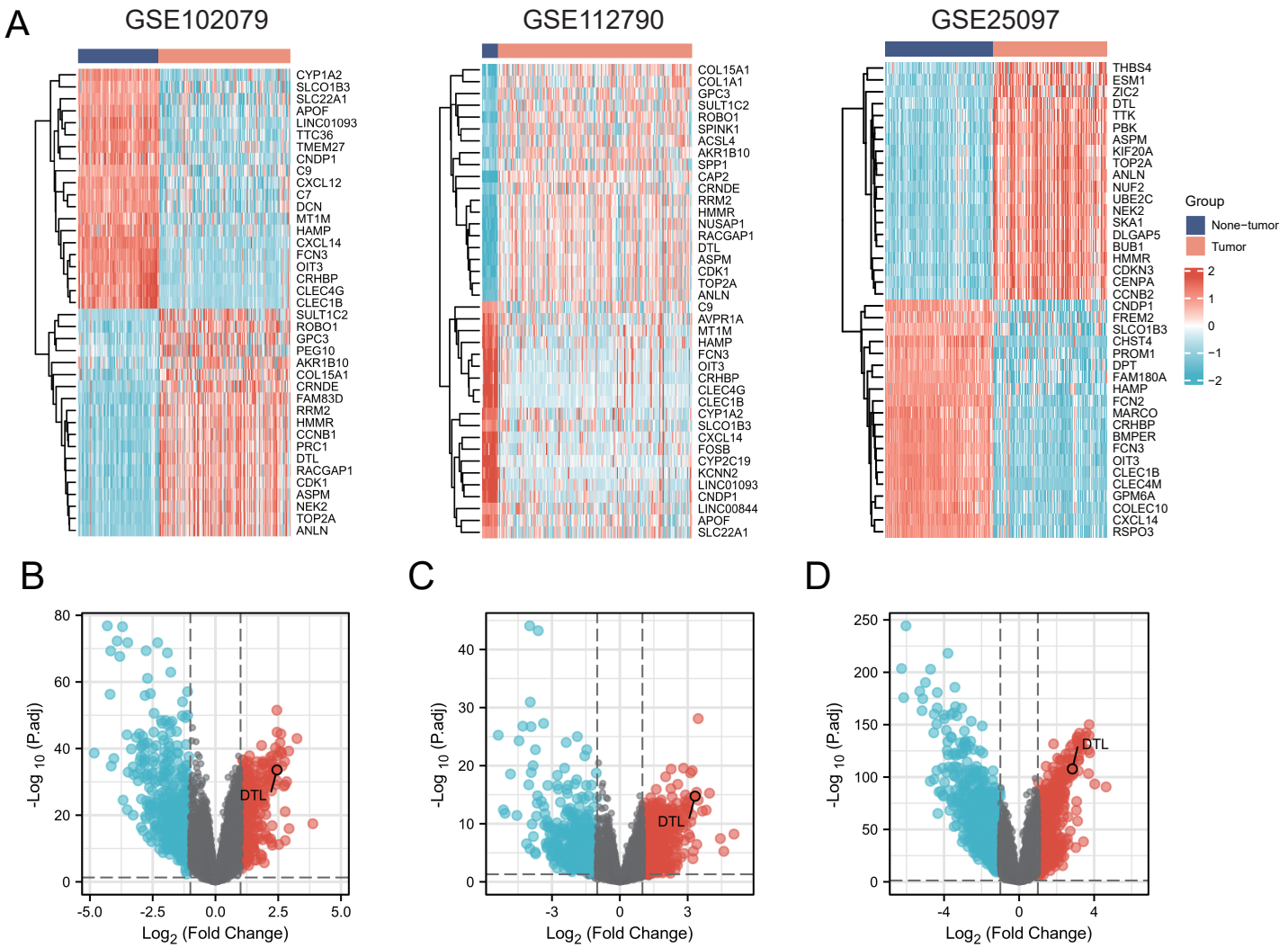
DTL was mediated by treatment with 1.0 μg/mL doxycycline for 48 h in further experiments. (D) Representative Flow Cytometry Analysis of Cell Cycle Distribution in Huh-7 and HCCLM-3 Cells Following Overexpression and Knockdown of DTL.

Supplementary Figure 5: (A) Transwell assays were performed to evaluate the invasion ability of DTL overexpression and knockdown HCC cells. (B) Representative images of wound healing assay in HCC cells. (C) Representative images of lung tissues in tail vein tumor metastasis mouse model from DTL knockdown and negative control (NC) groups. (D) Representative images of liver tissues in orthotopic liver transplantation model from DTL knockdown and negative control (NC) groups. (E) Liver weight in orthotopic liver transplantation model. (F) Representative images of intrahepatic metastasis nodules in HE-stained sections. Left panel: the number of intrahepatic metastasis nodules in the DTL overexpression group. (G and H) Representative images of lung tissues in orthotopic liver transplantation model from DTL overexpression (G) and knockdown (H) groups.

Supplementary Figure 6: (A) The immunofluorescence results of Notch1 and N-cadherin expression in DTL overexpressing or knockdown Huh-7 cells. (B) The knockdown efficiency of Notch1 by siRNA was detected by qRT-PCR in HCCLM-3 cells. (C) The functional role of Notch1 in the DTL-induced proliferation of HCCLM-3 cells was detected by Cell Counting Kit-8 (CCK8) (D). The functional role of Notch1 in DTL-induced metastasis of HCCLM-3 cells was detected by wound-healing assays.

(E) Representative images of transwell assays demonstrating the effect of Notch1 knockdown on the metastasis and invasiveness of DTL-overexpressing Huh-7 Cells. (F) Transwell assays representative images and statistical outcomes assessing the impact of Notch1 knockdown on the metastasis and invasiveness properties of HCCLM-3 cells with DTL overexpression. (G) CCK-8 assay evaluating HCCLM-3 cells viability under DTL overexpression or hypoxia with Notch1 knockdown and sorafenib (3μM) treatment. *P < 0.05, **P < 0.01, ***P < 0.001, ns, no significance

Supplementary Figure 7: (A) Validation of 3xFlag-DTL overexpression in Huh-7 cells using Western blot. (B) The top 20 proteins interacting with DTL, identified through liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.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DCN
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CXCL14
FCN3
OIT3
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GPC3
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FAM83D
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DTL
RACGAP1
CDK1
ASPM
NEK2
TOP2A
ANLN
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ACSL4
AKR1B10
SPP1
CAP2
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RRM2
HMMR
NUSAP1
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DTL
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TOP2A
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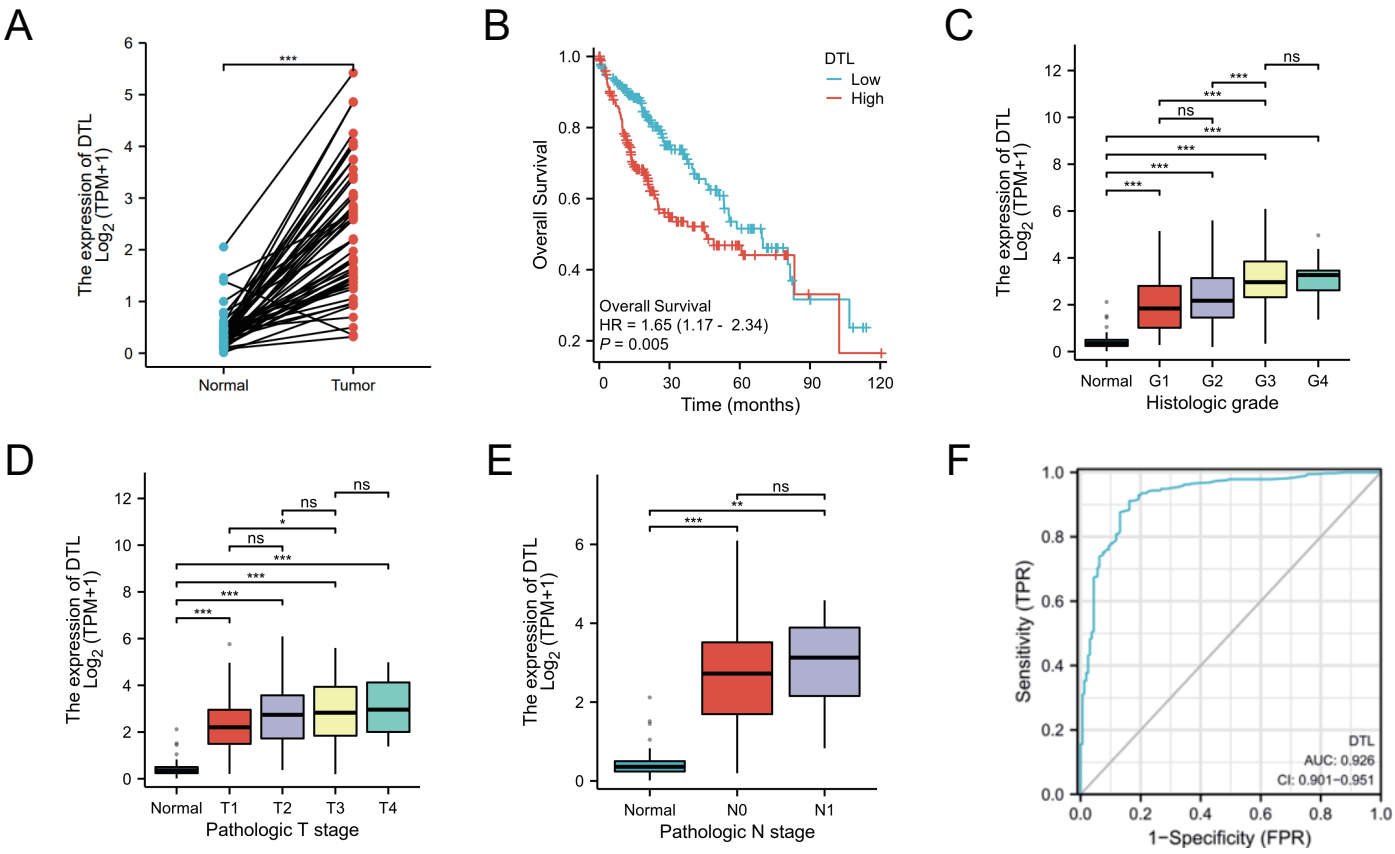
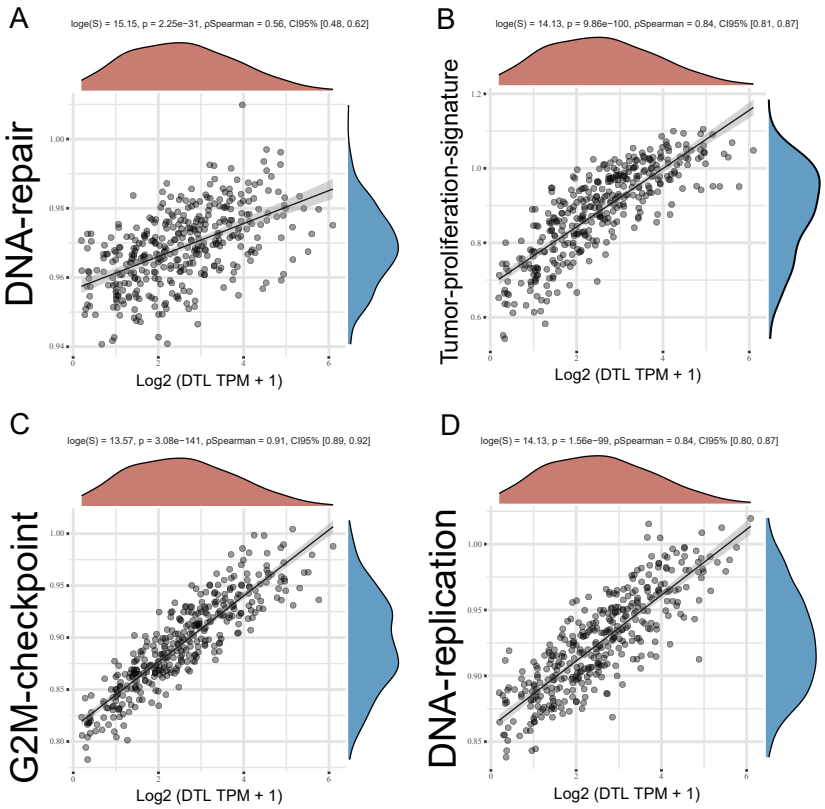
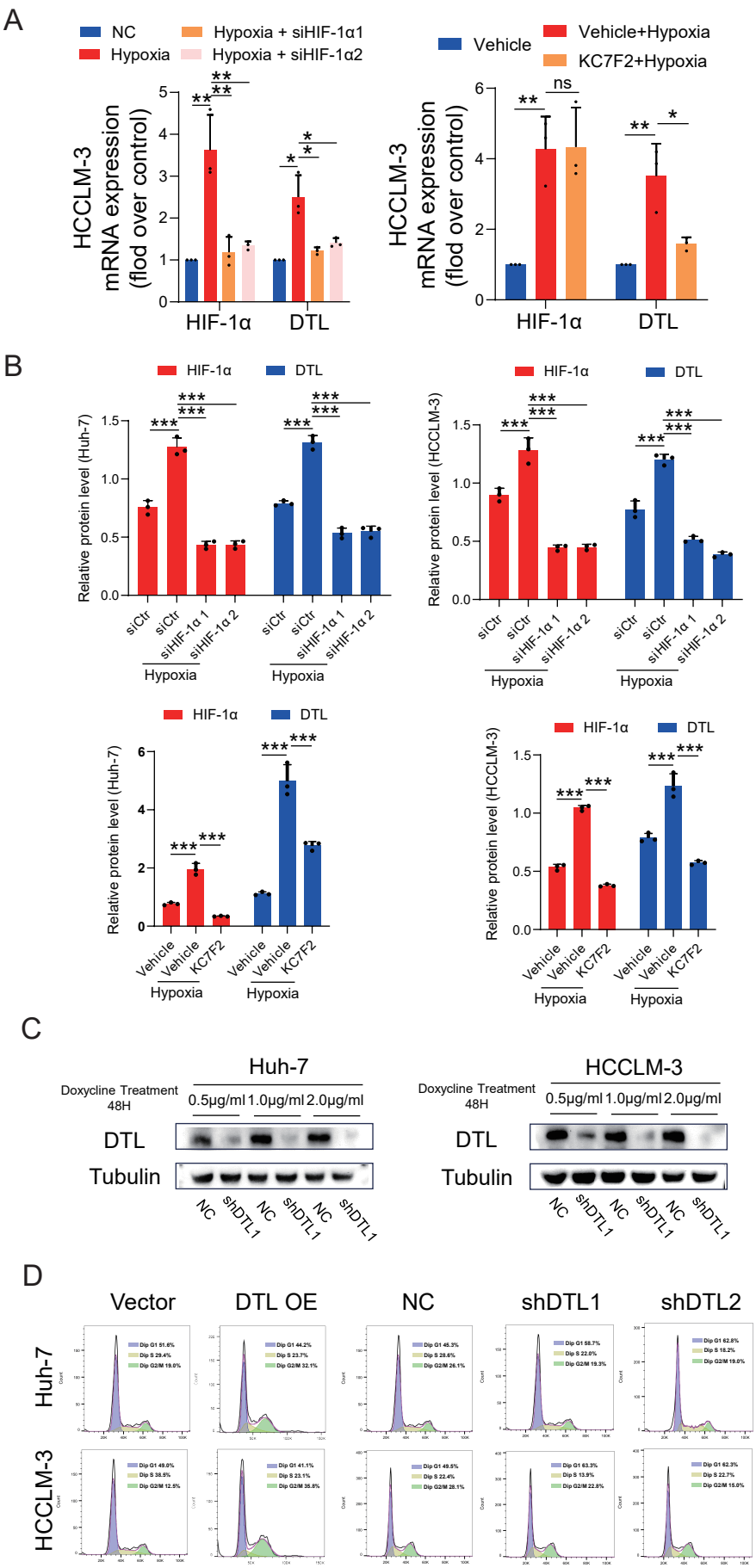
APOF
SLC22A1
GSE25097
THBS4
ESM1
ZIC2
DTL
TTK
PBK
ASPM
KIF20A
TOP2A
ANLN
NUF2
UBE2C
NEK2
SKA1
DLGAP5
BUB1
HMMR
CDKN3
CENPA
CCNB2
CNDP1
FREM2
SLCO1B3
CHST4
PROM1
DPT
FAM180A
HAMP
FCN2
MARCO
CRHBP
BMPER
FCN3
OIT3
CLEC1B
CLEC4M
GPM6A
COLEC10
CXCL14
RSPO3
Group
None−tumor
Tumor
2
1
0
−1
−2
B
-Log 10 (P.adj)
Log2 (Fold Change)
DTL
C
-Log 10 (P.adj)
Log2 (Fold Change)
DTL
D
-Log 10 (P.adj)
Log2 (Fold Change)
DTL


A
B
C
D
E
F
The expression of DTL
Log2 (TPM+1)
Normal
Tumor
***
Overall Survival
DTL
Low
High
HR = 1.65 (1.17 - 2.34)
P = 0.005
Time (months)
ns
G1
G2
G3
G4
Histologic grade
T1
T2
T3
T4
Pathologic T stage
N0
N1
Pathologic N stage
Sensitivity (TPR)
1−Specificity (FPR)
AUC: 0.926
CI: 0.901−0.951


A
loge(S) = 15.15, p = 2.25e−31, ρSpearman = 0.56, CI95% [0.48, 0.62]
DNA-repair
Log2 (DTL TPM + 1)
B
loge(S) = 14.13, p = 9.86e−100, ρSpearman = 0.84, CI95% [0.81, 0.87]
Tumor-proliferation-signature
Log2 (DTL TPM + 1)
C
loge(S) = 13.57, p = 3.08e−141, ρSpearman = 0.91, CI95% [0.89, 0.92]
G2M-checkpoint
Log2 (DTL TPM + 1)
D
loge(S) = 14.13, p = 1.56e−99, ρSpearman = 0.84, CI95% [0.80, 0.87]
DNA-replication
Log2 (DTL TPM + 1)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NC
Hypoxia
Hypoxia + siHIF-1α1
Hypoxia + siHIF-1α2
HCCLM-3 mRNA expression (flod over control)
HIF-1α
DTL
Vehicle
Vehicle+Hypoxia
KC7F2+Hypoxia
ns
B
Relative protein level (Huh-7)
Relative protein level (HCCLM-3)
siCtr
siHIF-1α 1
siHIF-1α 2
Vehicle
KC7F2
Hypoxia
C
Huh-7
HCCLM-3
Doxycline Treatment 48H
0.5μg/ml
1.0μg/ml
2.0μg/ml
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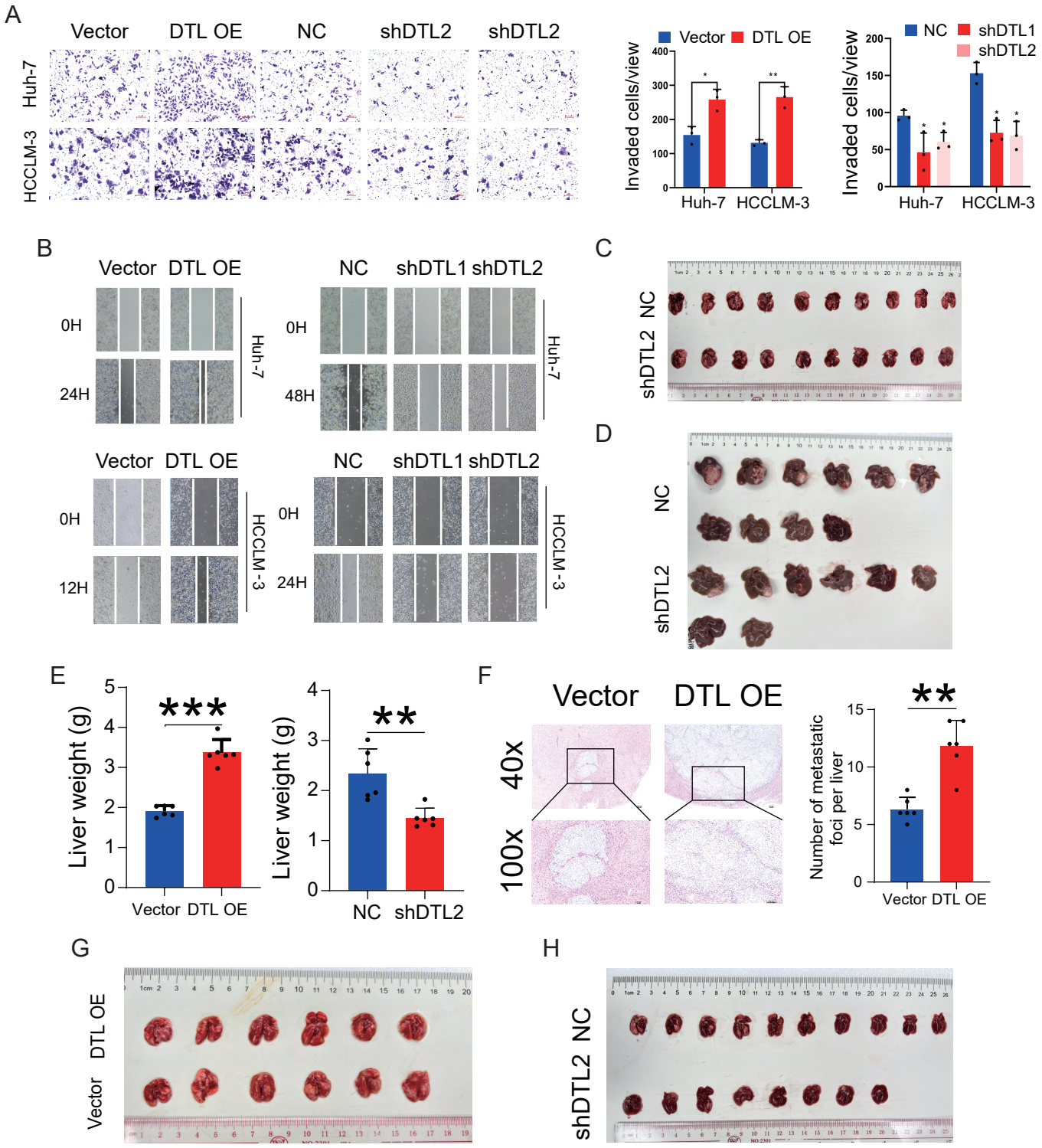
Tubulin
NC
shDTL1
D
Vector
DTL OE
NC
shDTL1
shDTL2
Huh-7
HCCLM-3


A
Vector
DTL OE
NC
shDTL2
shDTL2
Huh-7
HCCLM-3
Vector
DTL OE
Invaded cells/view
Huh-7
HCCLM-3
NC
shDTL1
shDTL2
Invaded cells/view
Huh-7
HCCLM-3
B
Vector
DTL OE
NC
shDTL1
shDTL2
0H
24H
Huh-7
0H
48H
Huh-7
Vector
DTL OE
NC
shDTL1
shDTL2
0H
12H
HCCLM-3
0H
24H
HCCLM-3
C
NC
shDTL2
D
NC
shDTL2
E
Liver weight (g)
Vector
DTL OE
Liver weight (g)
NC
shDTL2
F
Vector
DTL OE
40x
100x
Number of metastatic foci per liver
Vector
DTL OE
G
DTL OE
Vector
H
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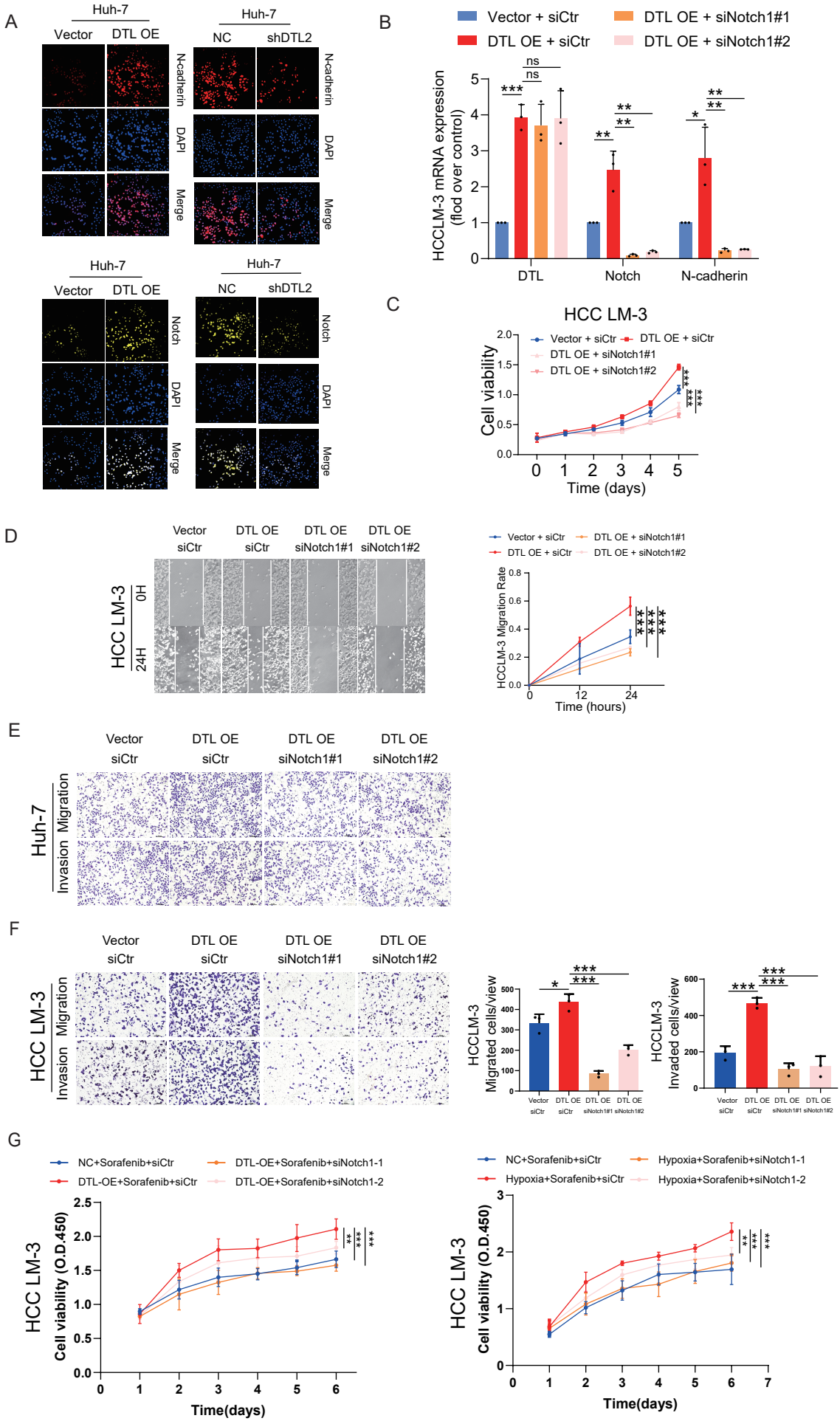
shDTL2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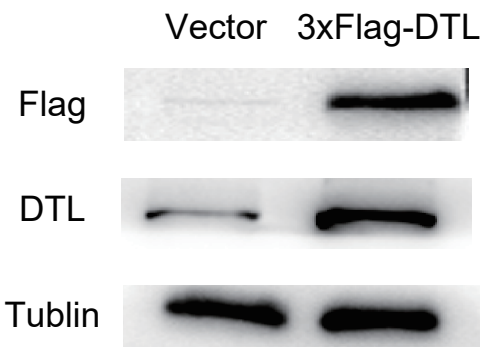
Huh-7
Vector
DTL OE
Huh-7
NC
shDTL2
N-cadherin
DAPI
Merge
Huh-7
Vector
DTL OE
Huh-7
NC
shDTL2
Notch
DAPI
Merge
B
Vector + siCtr
DTL OE + siCtr
DTL OE + siNotch1#1
DTL OE + siNotch1#2
HCCLM-3 mRNA expression (flod over control)
ns
ns
***
**
**
**
**
*
DTL
Notch
N-cadherin
C
HCC LM-3
Vector + siCtr
DTL OE + siCtr
DTL OE + siNotch1#1
DTL OE + siNotch1#2
Cell viability
***
***
***
Time (days)
D
Vector siCtr
DTL OE siCtr
DTL OE siNotch1#1
DTL OE siNotch1#2
HCC LM-3
0H
24H
Vector + siCtr
DTL OE + siCtr
DTL OE + siNotch1#1
DTL OE + siNotch1#2
HCCLM-3 Migration Rate
***
***
***
Time (hours)
E
Vector siCtr
DTL OE siCtr
DTL OE siNotch1#1
DTL OE siNotch1#2
Huh-7
Migration
Invasion
F
Vector siCtr
DTL OE siCtr
DTL OE siNotch1#1
DTL OE siNotch1#2
HCC LM-3
Migration
Invasion
HCCLM-3 Migrated cells/view
*
***
***
HCCLM-3 Invaded cells/view
***
***
***
Vector siCtr
DTL OE siCtr
DTL OE siNotch1#1
DTL OE siNotch1#2
G
NC+Sorafenib+siCtr
DTL-OE+Sorafenib+siCtr
DTL-OE+Sorafenib+siNotch1-1
DTL-OE+Sorafenib+siNotch1-2
HCC LM-3
Cell viability (O.D.450)
**
***
***
Time(days)
NC+Sorafenib+siCtr
Hypoxia+Sorafenib+siCtr
Hypoxia+Sorafenib+siNotch1-1
Hypoxia+Sorafenib+siNotch1-2
HCC LM-3
Cell viability (O.D.450)
**
***
***
Time(days)

A



B Mass spectrometric identification of the proteins bound to DTL.

Number	Citable Accession	Gene name
1	Q14684	RRP1B
2	Q8TDD1	DDX54
3	P46013	MKI67
4	Q9NZJ0	DTL
5	O00560	SDCBP
6	O15213	WDR46
7	P55081	MFAP1
8	Q1ED39	KNOP1
9	Q06787	FMR1
10	Q9NWH9	SLTM
11	Q13595	TRA2A
12	Q7Z6E9	RBBP6
13	Q9NWB6	ARGLU1
14	Q8NC51	SERBP1
15	P46778	RPL21
16	Q9BYG3	NIFK
17	Q13601	KRR1
18	P62910	RPL32
19	P62136	PPP1CA
20	Q8NI27	THOC2