

Supplementary Tables

Supplementary Table 1. Clinical characteristics of 269 LIHC patients from 3 cohorts.

Clinical characteristics	Overall	Verification samples		
		Cohort 1 (n = 27)	Cohort 2 (n = 90)	Cohort 3 (n = 152)
Follow-up time (OS)				
Ranges, months	0.7 - 65.5	N.A	5.0 - 56.0	0.7 - 65.5
Follow-up time (PFS)				
Ranges, months	0.1 - 65.5	N.A	4.0 - 56.0	0.1 - 65.5
Gender				
Mean \pm SD, years	57.0 \pm 12.4	56.1 \pm 12.1	52.4 \pm 10.8	59.9 \pm 12.6
Female, n (%)	47 (17.5)	4 (14.8)	10 (11.1)	33 (21.7)
Male, n (%)	222 (82.5)	23 (85.2)	80 (88.9)	119 (78.3)
Age				
\leq 60 years, n (%)	162 (60.2)	16 (59.3)	71 (78.9)	75 (49.3)
$>$ 60 years, n (%)	107 (39.8)	11 (40.7)	19 (21.1)	77 (50.7)
Pathological grade				
I - II, n (%)	163 (60.6)	19 (70.4)	43 (47.8)	101 (66.4)
III - IV, n (%)	106 (39.4)	8 (29.6)	47 (52.2)	51 (33.6)
Tumor size				
\leq 5 cm, n (%)	121 (45.0)	11 (40.7)	62 (68.9)	48 (31.6)
$>$ 5 cm, n (%)	148 (55.0)	16 (59.3)	28 (31.1)	104 (68.4)
T stage				
T1, n (%)	169 (62.8)	15 (55.6)	63 (70.0)	91 (59.9)
T2-T4, n (%)	100 (37.2)	12 (44.4)	27 (30.0)	61 (40.1)
N stage				
N0, n (%)	261 (97.0)	25 (92.6)	90 (100.0)	146 (96.1)
N1, n (%)	8 (3.0)	2 (7.4)	0 (0.0)	6 (3.9)
M stage				
M0, n (%)	265 (98.5)	25 (92.6)	90 (100.0)	150 (98.7)
M1, n (%)	4 (1.5)	2 (7.4)	0 (0.0)	2 (1.3)
TNM stage				
I, n (%)	165 (61.3)	14 (51.9)	63 (70.0)	88 (57.9)
II - IV, n (%)	104 (38.7)	13 (48.1)	27 (30.0)	64 (42.1)

LIHC, Liver hepatocellular carcinoma; OS, overall survival; DFS, disease-free interval, N.A, not applicable; SD, standard deviation.

Supplementary Table 2. shRNA and siRNA in this study.

	sequence
shALY-1	CCGGCGTGGAGACAGGTGGGAACTCTCGAGAGTTCCACCTGTCTCCACGTTTTTG
shALY-2	CCGGGAACTCTTTGCTGAATTTGGACTCGAGTCCAAATTCAGCAAAGAGTTCTTTTTG
siEGFR-1	GUCGCUAUCAAGGAAUUAATT
siEGFR-2	CCACAAAGCAGUGAAUUUATT

Supplementary Table 3. Sequences of primers used for qRT-PCR in this study.

	qRT-PCR Primers	
Gene	Forward Sequence(5'-3')	Reverse sequence(5'-3')
ALYREF	TCTCAGACGCCGATATTCAGG	GTCTGCTGTTCCCTAAGCTGCG
EGFR	TCCGTGAGTTGATCATCGAATT	TGGAGTCTGTAGGACTTGGCAA
β-actin	CATCGGCAATGAGCGGTTC	ACAGCACCGTGTTGGCGTAG

Supplementary Figures

Supplementary Figure 1. (A) Protein expression level of ALYREF in 7- paired LIHC tissues was examined by western blotting. (B) Protein expression levels of ALYREF in LO2 and LIHC cell lines. (C) The sensitivity and specificity were shown by the ROC curve for the ALYREF expression in predicting normal with LIHC from GEO database.

Supplementary Figure 2. (A-H) Correlation analysis between the MKI67 and ALYREF mRNA expression from the GEO databases. (I) Kaplan-Meier OS curves assessed for LIHC patients with high or low ALYREF expression from TCGA databases. (J) Univariable and multivariable COX regression analyses were performed to detect the association between the OS and the clinical parameters in the LIHC patients from TCGA databases. (K) Kaplan-Meier DFS curves assessed for LIHC patients with high or low ALYREF expression from from TCGA databases. (L) Univariable and multivariable COX regression analyses were performed to detect the association between the DFS and the clinical parameters in the LIHC patients from from TCGA databases. Red lines represent ALYREF significantly related clinicopathologic features with $P < 0.05$.

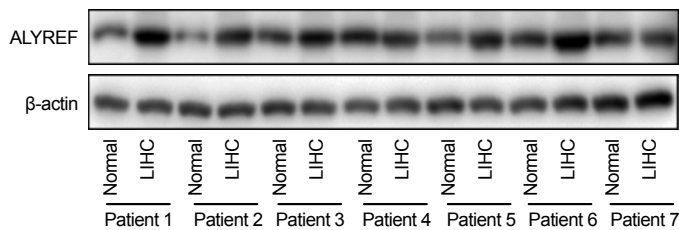
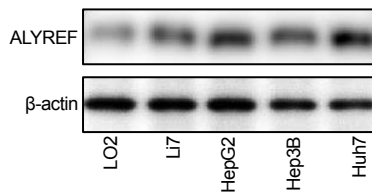
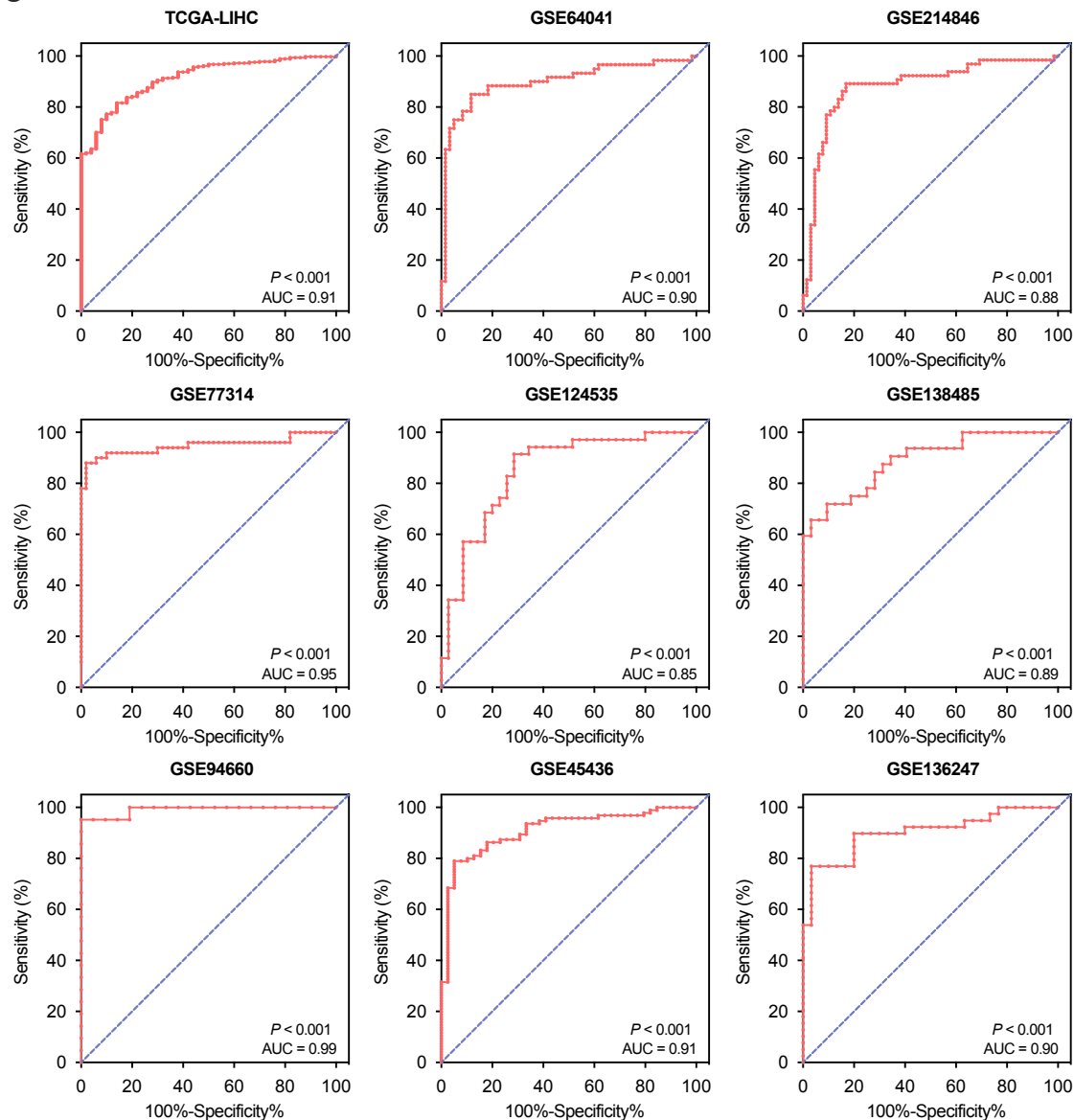
Supplementary Figure 3. (A,B) qPCR analysis and western blotting showing the knockdown efficiency of ALYREF in LIHC cells. (C,D) qPCR analysis and western blotting showing the overexpression efficiency of ALYREF in LIHC cells. (E,F) Representative images of the wound healing assays for ALYREF overexpression LIHC cells. Scale bar, 200 μ m. (G-J) Representative IHC staining of Ki-67 in tumor xenografts were conducted (scale bar: 50 μ m).

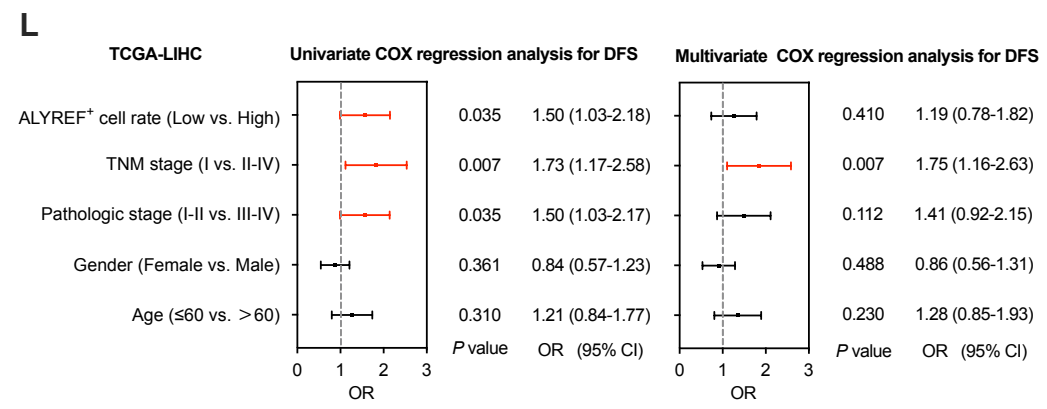
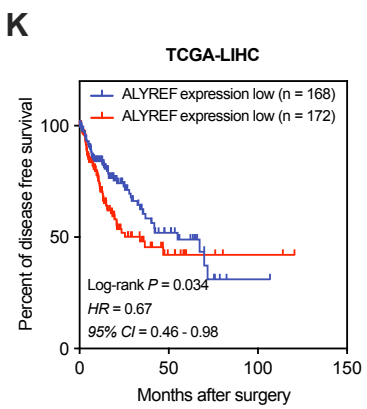
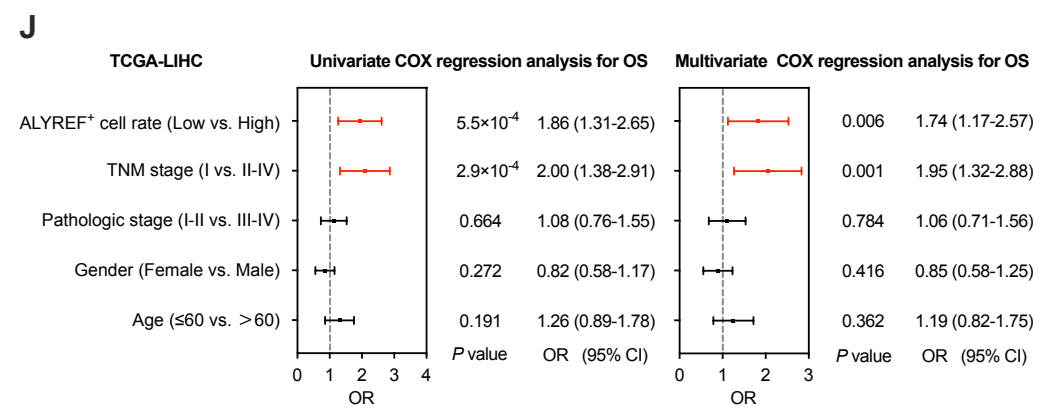
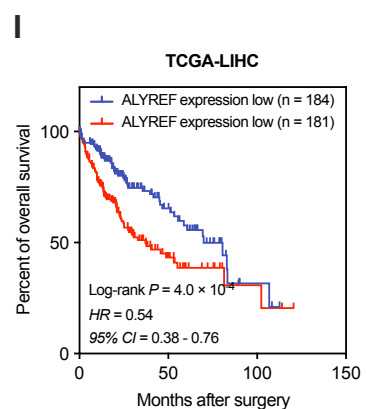
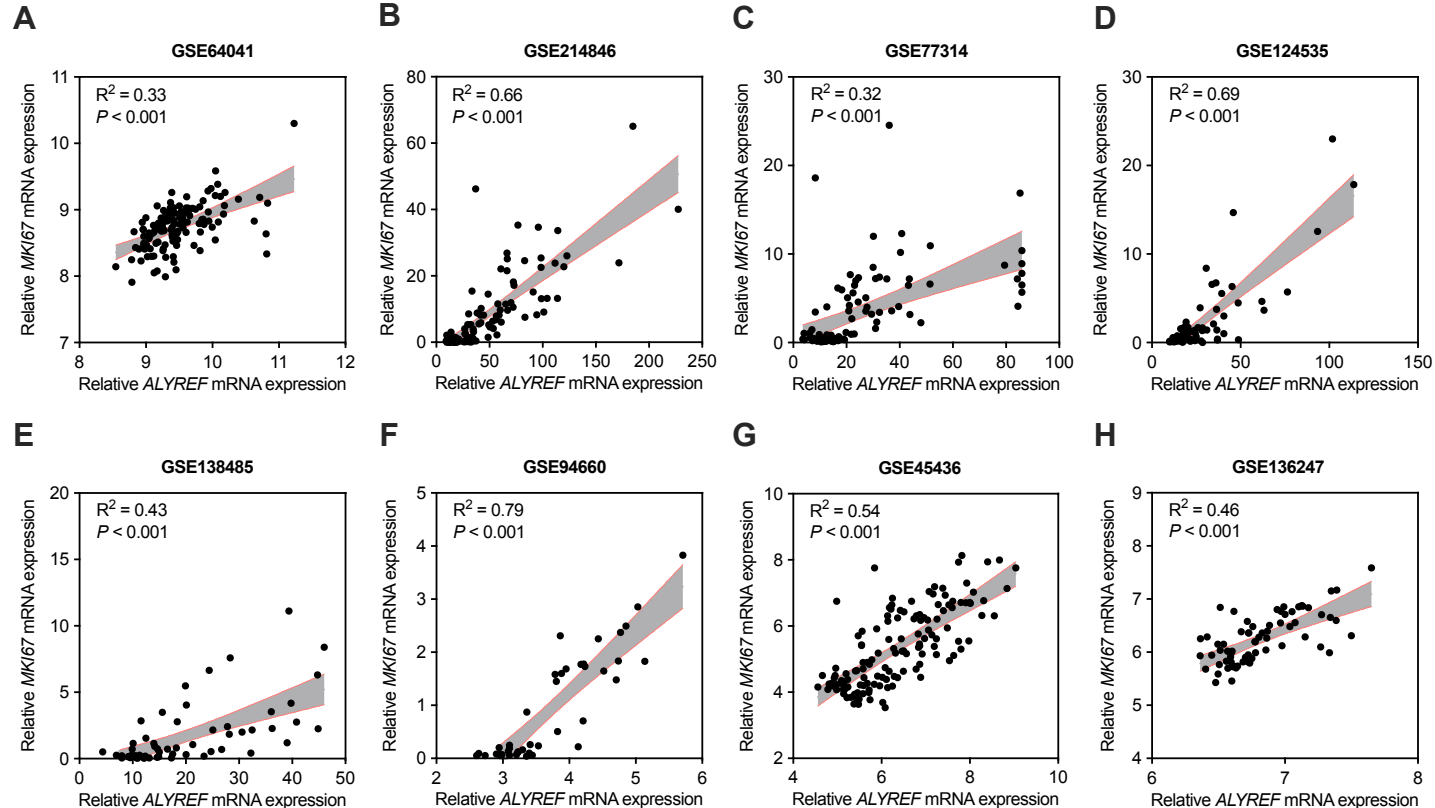
Supplementary Figure 4. Heatmap illustrating the differentially expressed genes in LIHC cells between sh-ALYREF and sh-Con groups.

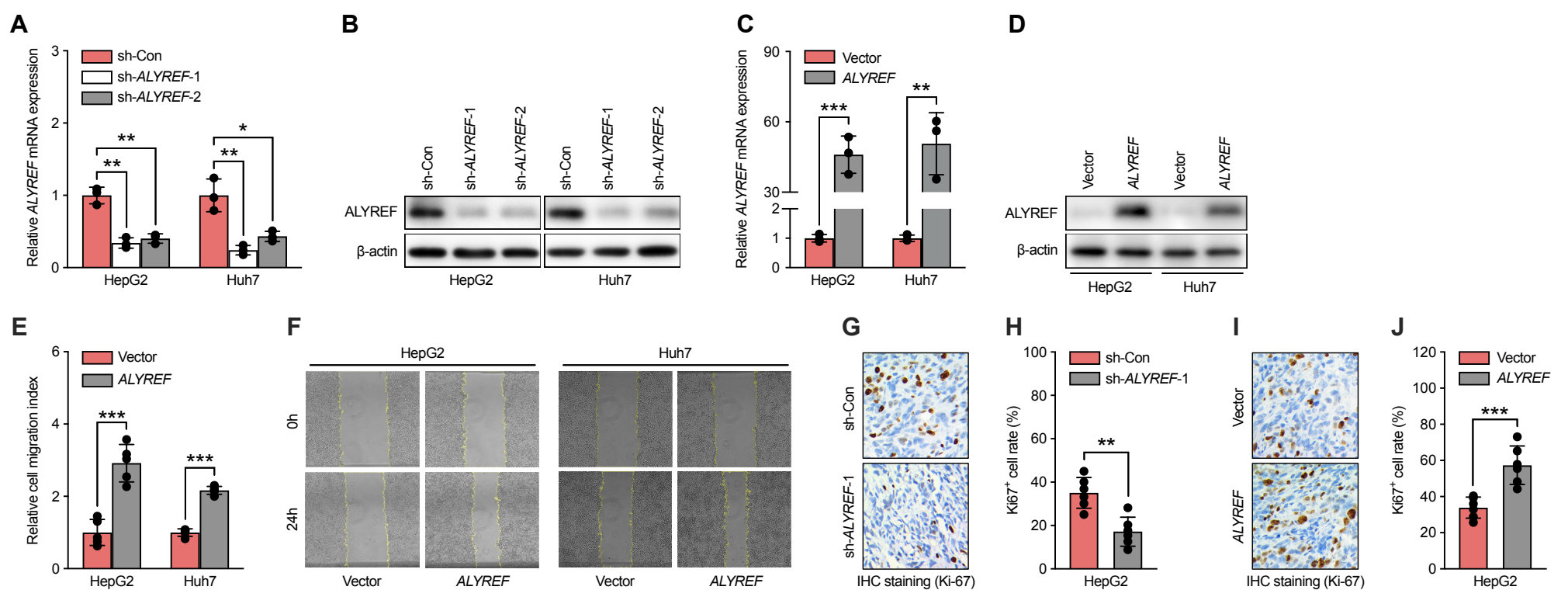
Supplementary Figure 5. (A) IHC staining of EGFR, snail, slug, E-cadherin, N-cadherin in Xenograft tumor transfected with ALYREF shRNA or control shRNA. (B) IHC staining of EGFR, snail, slug, E-cadherin, N-cadherin in Xenograft tumor transfected with ALYREF overexpression vector or control vector.

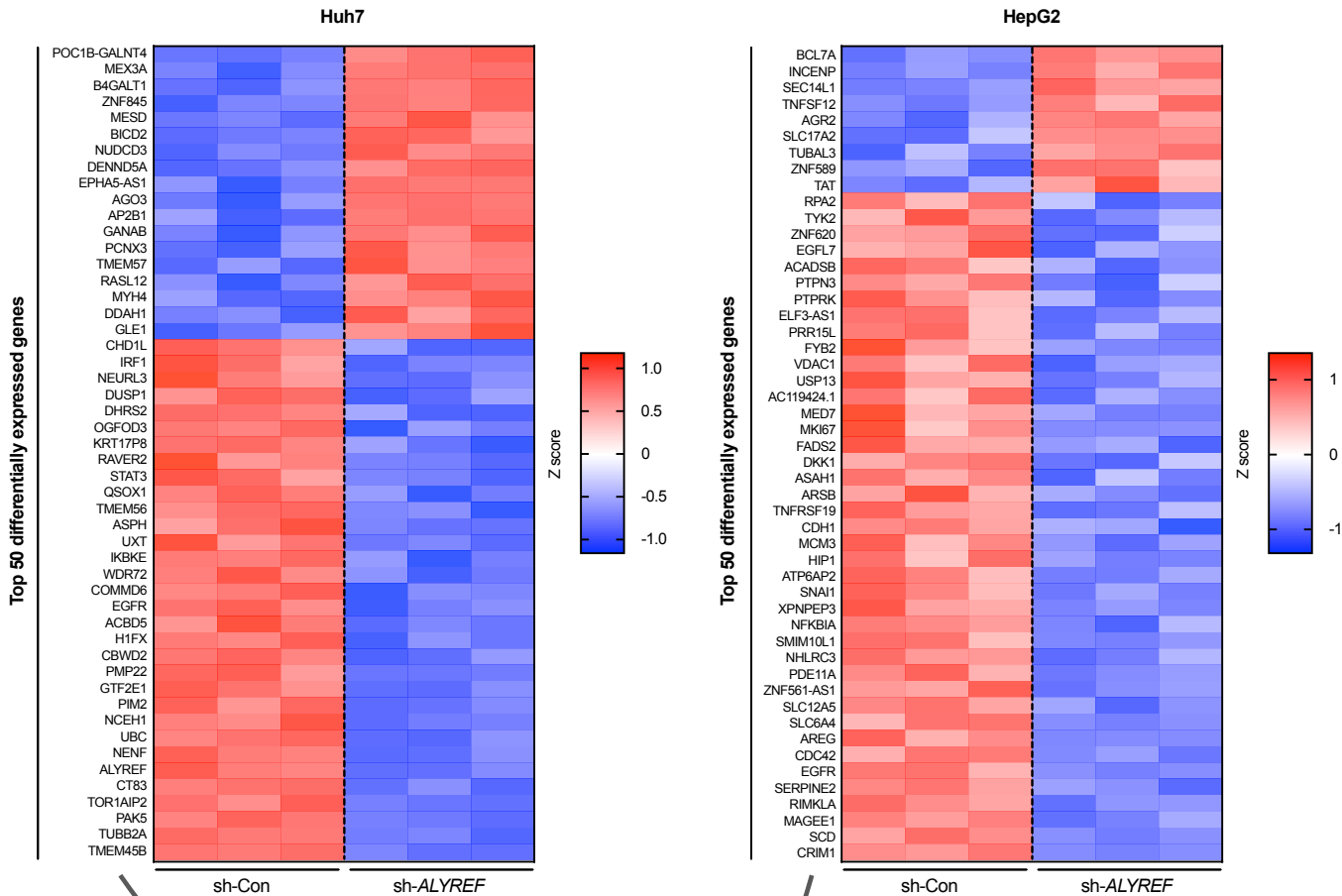
Supplementary Figure 6. (A) Representative IHC staining of EMT-related proteins in tumor xenografts were conducted (scale bar: 50 μm). (B) The IHC staining scores of EMT-related proteins in tumor xenografts.

Supplementary Figure 7. (A) Correlation analysis between the ALYREF mRNA and EGFR mRNA m5C level from LIHC cohort 1. (B) Correlation analysis between the MKI67 mRNA and EGFR mRNA m5C level from LIHC cohort 1. (C) Association between the level of EGFR mRNA m5C and different T stages from LIHC cohort 1. (D) Association between the level of EGFR mRNA m5C and different TNM stages from LIHC cohort 1.

A**B****C**

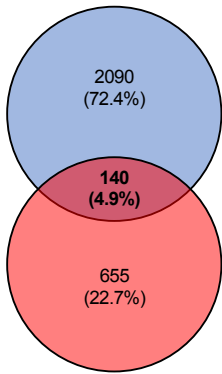






2230 differentially expressed genes ($P < 0.05$) in Huh7 (sh-Con vs. sh-ALYREF)

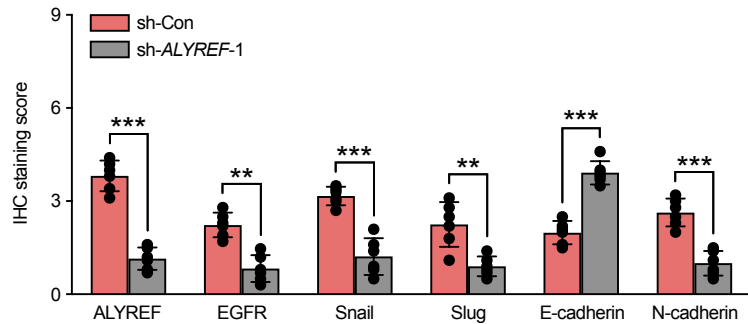
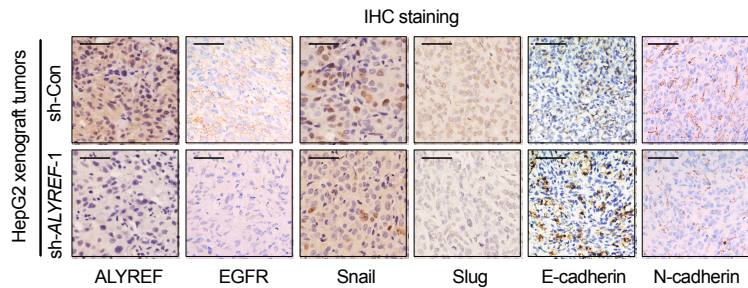
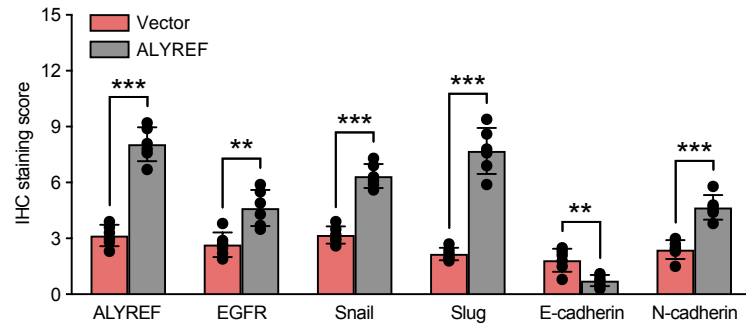
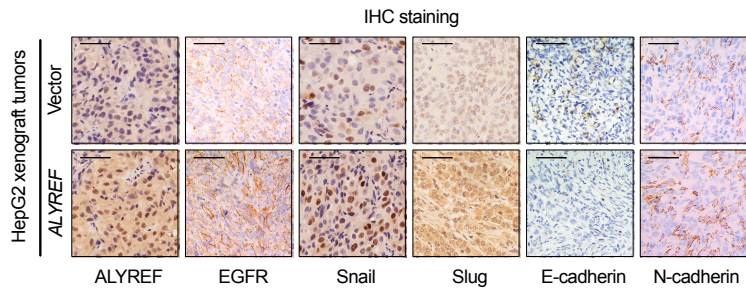
795 differentially expressed genes ($P < 0.05$) in HepG2 (sh-Con vs. sh-ALYREF)

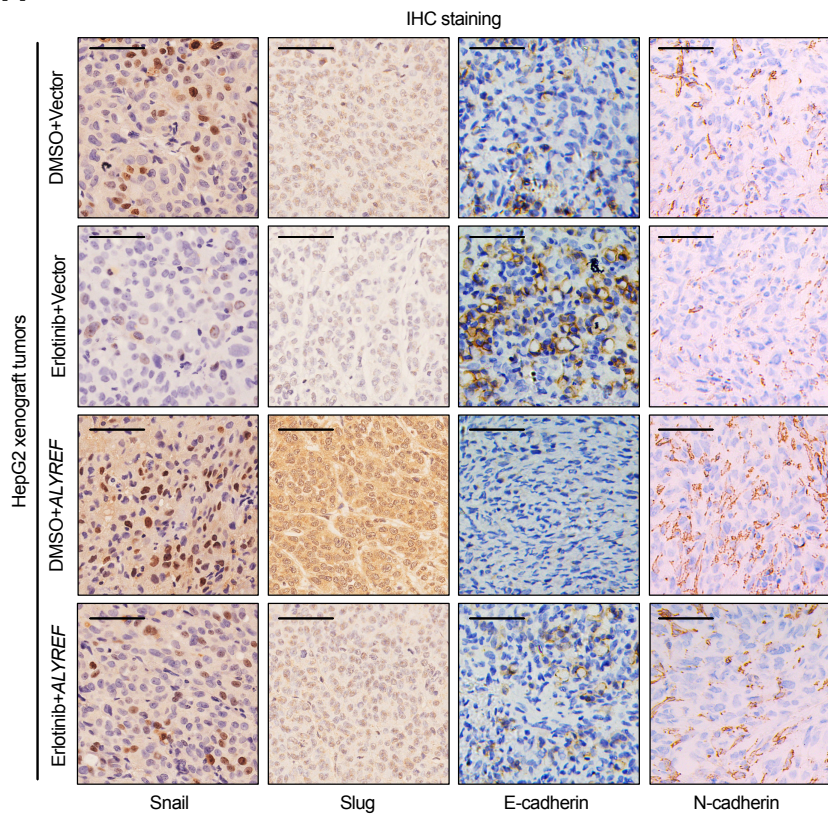
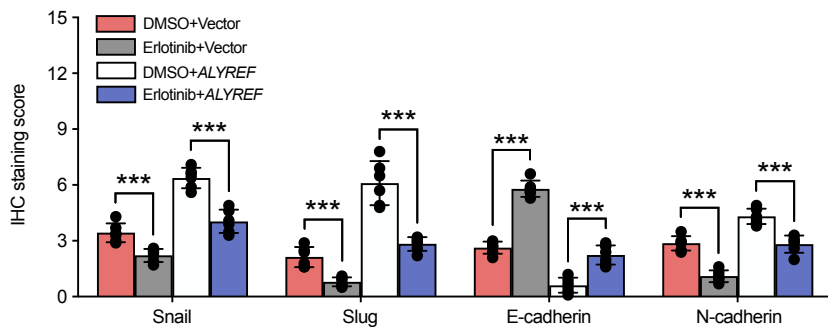


TOP 50 differentially expressed in HepG2 and Huh7 for screening

EGFR

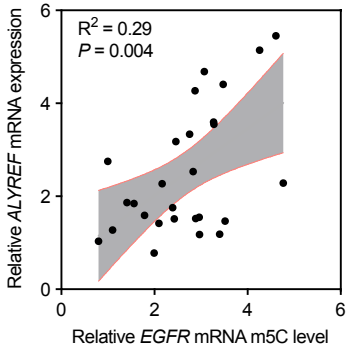
Verification of mechanism

A**B**

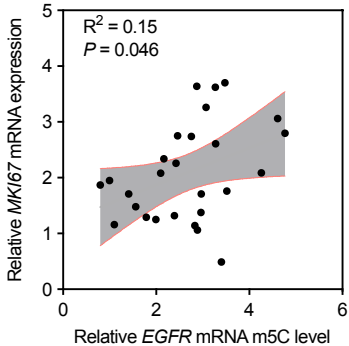
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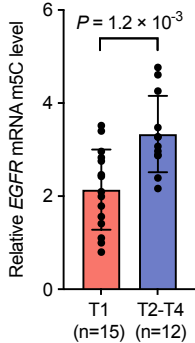
Verification cohort 1

**B**

Verification cohort 1

**C**

Verification cohort 1

**D**

Verification cohort 1

