Supplemental Table 1. Summary of primers used in the study

Primers for plasmid construction (5'-3')	
AY-F1	TGAAACGCTGCCATCAACA
AY-R906	TCAGGGAGGCATAAAAGAAC
AY-F1-kpnI	AAAAGGTACCTGAAACGCTGCCATCAACA
AY-R298-kpnI	CCCCGGTACCGGAGGCCGAGTAGAACTGAA
AY-R371-kpnI	AAAAGGTACCAATGCAACACCGATACCAAA
AY-R401-kpnI	AAAAGGTACCGGCTTATGAGGGTAAGGAGGAT
AY-R522-kpnI	AAAAGGTACCTAAGTGGCAGAGCTGGGAGAT
AY-R671-kpnI	CCCCGGTACCTCCTGCTTCACTGTGGTTCTT
AY-F371-KpnI	AAAAGGTACCTTCCAGGCCATCCTCCTTA
AY-R370	CATGGGTTCCTCTGGGGCATGCAACACCGATACCAAAAC
AY-F523	GTTTTGGTATCGGTGTTGCATGCCCCAGAGGAACCCATG
AYgRNA-53-F	ACACCGCTCCCCAGGACTGTCATAACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTT
AYgRNA-53-R	AACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGTTATGACAGTCCTGGGGAGCGGTGT
AYgRNA-1135-F	ACACCGATGGTACTGGGTTTTAGCGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTT
AYgRNA-1135-R	AACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCGCTAAAACCCAGTACCATCGGTGT
shAY t292	GATCC GGCCTCCCATCAAAGGTTTTTCAAGAGAAAACCTTTGATGGGAGGCC AGA
shAY b292	AGCTTCT GGCCTCCCATCAAAGGTTTTCTCTTGAAAAACCTTTGATGGGAGGCC G
siAY t378	GATCC CCATCCTCCTTACCCTCATTTCAAGAGAATGAGGGTAAGGAGGATGG AGA
siAY b378	AGCTTCT CCATCCTCCTTACCCTCATTCTCTTGAAATGAGGGTAAGGAGGATGG G
ITGAV t2179	GATCCGCTGAGCTCATCGTTTCCATTCAAGAGATGGAAACGATGAGCTCAGCAGA
ITGAV b2179	AGCTTCTGCTGAGCTCATCGTTTCCATCTCTTGAATGGAAACGATGAGCTCAGCG

Primers for PCR (5'-3')	
AY-qPCR-F9	TGCCATCAACAGAACATCAC
AY-qPCR-R199	GAGGAGCAGCCATACCC
GAPDH-qPCR-F	CGGATTTGGTCGTATTGGG
GAPDH-qPCR-R	CGCTCCTGGAAGATGGTGAT
ITGAV-qPCR-F	GACAGTCCTGCCGAGTA
ITGAV-qPCR-R	CTGGGTGGTGTTTGCT
β-actinF	GAGCTACGAGCTGCCTGACG
β-actinR	CCTAGAAGCATTTGCGGTGG
ITGB1F	TGGGAAACTTGGTGGC
ITGB1R	CTCCTTGTAAACAGGCTG
ITGB3F	TCATCACCATCCACGACC
ITGB3R	TCCACATACTGACATTCTCCC
ITGB5F	GTCAGGAAGGGTCGGAGT
ITGB5R	AAGGCATAGCCATCACG

ITGB6F	GCCATCTGTAACAACGG
ITGB6R	TTCTGGGCTGACAAGTAA
ITGB8F	GTGGTGCCCAATGACG
ITGB8R	GCTTCTGATAGGCTTCC
ZNF282-F	CGCAACAGGAACTTCTGGGTC
ZNF282-R	GAGCATCTGGCTTGGGGACT

Primers for CHIP (5'-3')	
ITGAV-exon1-F1	TTCGGCGATGGCTTTTC
ITGAV-exon1-R1	GGGCACGAAGAAATCCAC
ITGAV-exon2-F1	GGATGTTTCTTCTCGTGGGA
ITGAV-exon2-R1	GCGGGTAGAAGACCAGTCA
ITGAV-intron1-F1	AAATCAACAAAGGCTGGTC
ITGAV-intron1-R1	AGACAGAGGCGGAAAGAG
ITGAV-PMT-672F	AGCTCCTGAGCCTGGGT
ITGAV-PMT-492R	CAACAGTCGCACGGAAGT
ITGAV-PMT-894F	TTCTTTGCCCTGCGAATC
ITGAV-PMT-677R	TCACGAACGAAACCCTGTT
ITGAV-PMT-1241F	GCTCATTTCGTCTGCTATTCA
ITGAV-PMT-1128R	AGGACAGGAACCTTGACCTT
AY probe436 (odd)	AAATGCATCGAGCAGTGAGAGTCCATTTAGGAAGGGGCTTATGAGGGTAAG-Biotin
AY probe8 (even)	TGATGTTCTGTTGATGGCAG-Biotin
AY probe873 (even)	AAGAACAATGGAAGCCCCAC-Biotin
LacZ probe	TCACGACGTTGTAAAACGAC-Biotin

Primers for siRNA (5'-3')	
siAY264	CAGUCUUAGAUUUUGUUCAGUUU ACUGAACAAAAUCUAAGACUGUU
siAY292	GGCCUCCCAUCAAAGGUUU AAACCUUUGAUGGGAGGCC
H1FX	ACGUAGAUUUUGUACGGCUUU AGCCGUACAAAAUCUACGUUU
H1.2	CGGCCACUGUAACCAAGAAUU UUCUUGGUUACAGUGGCCGUU
H1.3	AAACACCUGUGAAGAAAAAUU UUUUUCUUCACAGGUGUUUUU
H1.4	UGUCCGAGCUCAUUACUAAUU UUAGUAAUGAGCUCGGACAUU
siITGAV	GGGAUUGACUUCUCAGCAU AUGCUGAGAAGUCAAUCCC

Supplemental Table 2. Summary of antibodies used in the study

Antibody name	Manufacturer	Catalogue Number	Usage and Dilution
ITAGV	Santa Cruz	sc-10719	Western blot(1:500); Immunohistochemistry(1:100)
E-cadherin	Santa Cruz	sc-7870	Western blot(1:500)
N-Cadherin antibody [N2C1]	Gene Tex	GTX112733	Western blot(1:500)
Vimentin antibody	Gene Tex	GTX112661	Western blot(1:500)
ZEB1 antibody [N2C1]	Gene Tex	GTX105278	Western blot(1:500)
Twist1 antibody [10E4E6]	Gene Tex	GTX60776	Western blot(1:500)
beta-Catenin Antibody	Proteintech	17565-1-AP	Western blot(1:2000)
SOX2 Antibody	Proteintech	11064-1-AP	Western blot(1:500)
OCT4 Antibody	Proteintech	11263-1-AP	Western blot(1:500)
αVβ3	Millipore	MAB1976	Immunohistochemistry(1:100)
FITC-ITGAV	Biolegend	327907	Immunofluorescence（1:100）;FACS（1:100）
FITC-β3	Biolegend	336403	FACS（1:100）
FITC-αVβ3	Biolegend	304403	FACS（1:100）
Histone H1.0 Antibody	Proteintech	17510-1-AP	RNA-Pull down(1ug),Western blot(1:500)
Histone H1.1 Antibody	Proteintech	18201-1-AP	RNA-Pull down(1ug),Western blot(1:500)
Histone H1.2 Antibody	Proteintech	19649-1-AP	RNA-Pull down(1ug),Western blot(1:500)
Histone H1.3 antibody	Gene Tex	GTX104481	RNA-Pull down(1ug),Western blot(1:500)
Histone H1.4 Antibody	Thermo Fisher Scientific	702876	RNA-Pull down(1ug),Western blot(1:500)
Histone H1.5 Polyclonal Antibody	Thermo Fisher Scientific	PA5-19487	RNA-Pull down(1ug),Western blot(1:500)
Histone H1Fx antibody	abcam	ab31972	RNA-Pull down(1ug),Western blot(1:500)
Histone H1 Antibody	Proteintech	18201-1-AP	RNA-Pull down(1ug),Western blot(1:500)
HNRNPK Antibody	Proteintech	11426-1-AP	RNA-Pull down(1ug),Western blot(1:1000)
NCL Antibody	Proteintech	10556-1-AP	RNA-Pull down(1ug),Western blot(1:1000)
FUS/TLS Antibody	Proteintech	11570-1-AP	RNA-Pull down(1ug),Western blot(1:1000)
U1 SnRNP70	Santa Cruz	sc-390899	RNA-Pull down(1ug),Western blot(1:1000)
GAPDH	Bioworld Technology Inc	MB001	Western blot(1:500)
HRP-IgG	Jackson ImmunoRes	111-035-003	Western blot(1:10000)
CD31 polyclonal antibody	Bioworld	BS90231	Immunohistochemistry(1:100)

Supplement

AY927503 (AY) sequence was analyzed by CPAT (Coding Potential Assessment Tool) and CPC (coding potential calculator) web. As compared to positive control mRNA coding β-actin, AY transcript showed very low coding probability which was similar to non-coding RNA MALAT1 (Supplemental Figure 1A).

The expression of AY was then investigated in paired samples with HCC and adjacent nontumor tissues from 57 patients by in situ hybridization. Among them, there were 38 cases whose hybridization signals of AY were more extensive in HCC than those in their corresponding adjacent nontumor tissues and only 19 cases showed weaker AY signal in HCC than nontumor tissues (Supplemental Figure 1B).

We further observed that AY expression level was well correlated with ITGAV and AY significantly enhanced *ITGAV* transcription. However ectopic AY overexpression did not stimulated other subtypes of integrin except ITGAV as well as β3 (Supplemental Figure 1C).

To further investigate the roles of AY in HCC, we overexpressed and knocked down AY based on 2 target sequences in HCC cells. As shown in Supplemental Figure 1D, the wound closure rate was significantly accelerated after AY overexpression, but this effect was almost abolished when *ITGAV* was silenced. Wound closure was however retarded after AY knockdown in SMMC-7721 cells, but reversed by ITGAV overexpression. The transmembrane migration ability of BEL-7404 cells overexpressing AY was significantly enhanced compared to that of mock cells, but knockdown of ITGAV completely ablated the stimulatory effect by AY (Supplemental

Figure 1E). Correspondingly, AY silence remarkably attenuated the migration ability of SMMC-7721 cells, but overexpressing ITGAV almost restored the cell migration rate to that of the scramble group (Figure 1E). The activities of lncRNA involved in gene transcriptional regulation is usually through affecting neighboring intrachromosomal genes in cis (1). In the present study, although the genomic loci of AY is located within ZNF282 gene on chromosome 7, overexpression of lncRNA AY did not affect the mRNA level of ZNF282 (Supplemental Figure 1F). Although statistical analysis of TCGA database indicated that there was positive correlation between AY and ZNF282 mRNAs (Pearson correlation coefficient r=0.54, N=200, Supplemental Figure 1G), knockdown of AY did not affect the mRNA level of ZNF282 as well. ZNF282 did expressed higher level in HCC than in nontumor tissues (N=50). But ZNF282 mRNA was poorly correlated with ITGAV mRNA (Pearson correlation coefficient r=-0.13, N=371). This implied that AY regulation of ITGAV transcription was independent of ZNF282.

LncRNAs have also been usually postulated to act as competing endogenous RNA (ceRNA) or ‘‘RNA sponges’’, interacting with miRNAs in a manner that can sequester these molecules and reduce their regulatory effect on target mRNA. This type of lncRNA is found typically distributed in cytoplasm and associated with the RNA-induced silencing complex (RISC). However, our RNA pull-down results showed that there was no direct binding between AY and AGO2 protein, a core member of RISC (Supplemental Figure 2A).

LncRNAs interact with proteins to modulate protein function, regulate or direct

localization within cellular compartments. These interactions are central to determine lncRNA functional effects, yet characterizing protein or lncRNA has presented significant challenges. Through mass spectrometry and protein chip assay, we identified H1FX, a variant of histone 1, as the interacting protein. Gene ontology (GO) analysis of protein arrays showed potential interactions of AY with proteins that were mainly enriched in biological processes such as cell-cell adhesion and RNA processing (Supplemental Figure 2B). Overexpression of AY repelled H1FX binding from the nucleosome and promoter of *ITGAV* gene. H1FX interacted with nucleolin. But this interaction was not enhanced by AY (Supplemental Figure 2C) or reduced by RNase treatment (Supplemental Figure 2D). Comparing the sequence between the unbound H1 variants (H1.0, H1.1, H1.5) and bound H1 variants (H1FX, H1.2, H1.3, H1.4), we noted that there is a significant divergence in the central region from 80 to 150 (Supplemental Figure 2E) constituting the globular domain of linker histone. This suggested that AY might interact with H1FX through the globular domain which is the binding site for linker DNA. Thus interaction of AY with H1fx may hinder H1FX binding to the promoter and nucleosome core particles. This might be another reason for AY repelling H1FX binding from the promoter of *ITGAV* gene.

References

1. Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. Cancer cell. 2016;29:452-63.

Figure legends

Supplemental Figure 1. LncRNA AY analysis in human hepatocellular carcinoma cells.

A. Coding probability prediction for AY927503 by Coding Potential Assessment Tool (CPAT)(http://lilab.research.bcm.edu/cpat/) and CPC (coding potential calculator). β-acting（ACTB）as positive control and long non-coding RNA MALAT1 as negative control.

B. In situ hybridization analysis of AY expression in 57 paired HCC tissues (T) and adjacent nontumor (NT) tissues. The positive signals per cell were comparatively analyzed between HCC tissues and non-tumor tissues.

C. Measurement of integrin subtypes by qPCR in Hep3B cells transfected with AY or control plasmid (Mock).

D. AY-mediated cell migration in wound healing of BEL-7404 cells transfected with pcDNA3.1b-AY and/or pSilencer 4.1-shITGAV, and of SMMC-7721 cells transfected with pSilencer 4.1- shAY and/or pcDNA3.1b-*ITGAV*. Relative wound closure is quantified in low panel. Original magnification: 10×. *,P<0.05; **,P<0.01.

E. Representative micrographs of AY-overexpressing and AY-knockdown cells and their control in transmembrane migration assays (left) and quantitative analysis of migration (right). Original magnification: 10×. *,P<0.05

F. QPCR analysis of ZNF282 in Hep3B cells transfected with plasmid pcDNA3.1b-AY for overexpression of AY and pSilencer4.1-shAY for knockdown of AY respectively. Mock and Scramble served as corresponding control.

G. Pearson correlation analysis of ZNF282, AY, and ITGAV mRNAs expressed in HCC from TCGA database. Comparison analysis of mRNA levels of ZNF282 between HCC and nontumor tissues was also performed.

Supplemental Figure 2. Linker histone H1FX interaction and sequence analysis.

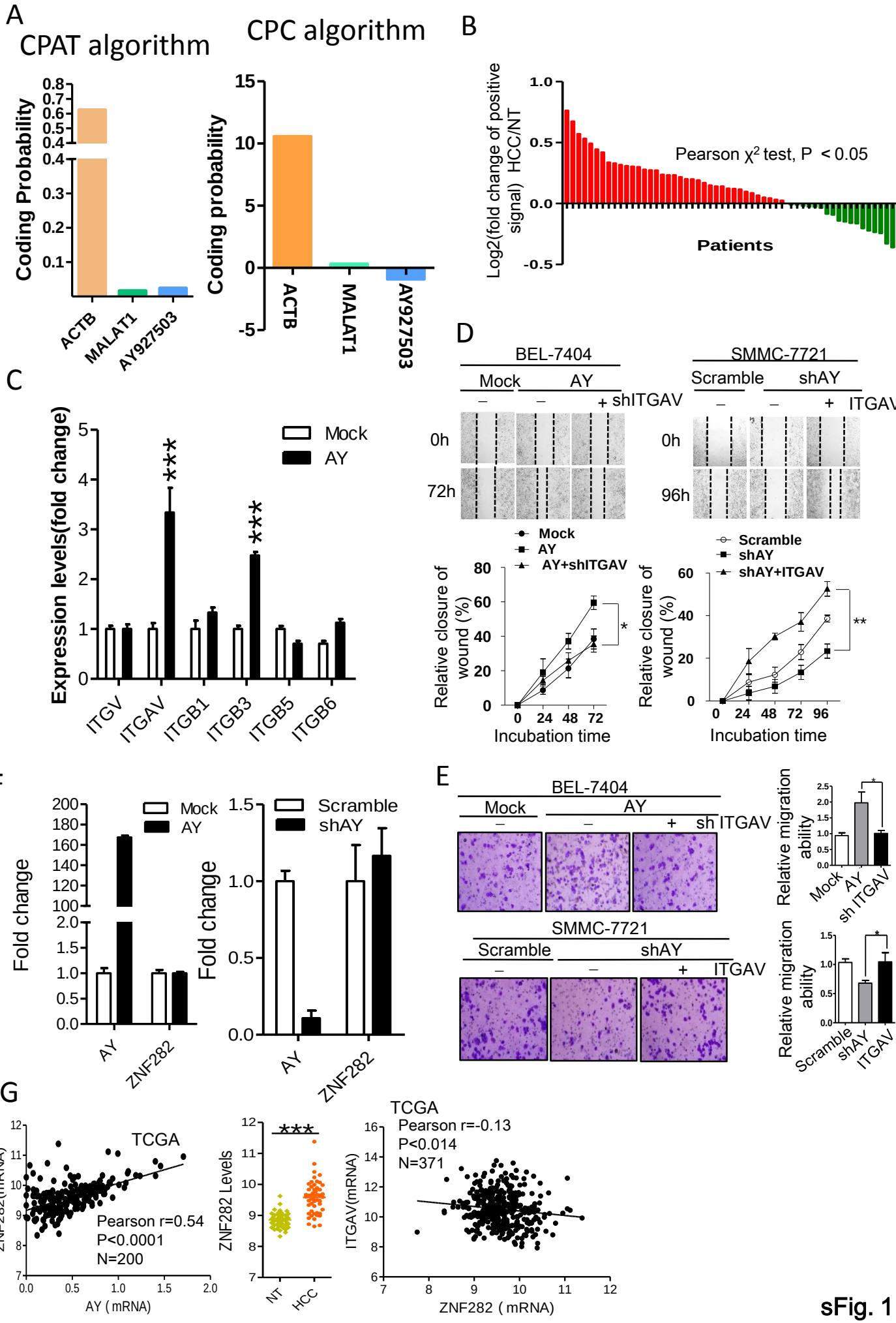
A. RNA pull-down assay by biotinylated AY full length RNA (AY-FL), biotinylated AY antisense RNA (AY-AS), or control biotin (Ctrl). The protein of the complex was analyzed by Western blot using AGO2 antibody.

B. GO (Gene Ontology) analysis of potential proteins associated with AY obtained from protein array studies. BP, Biological Process; MF, Molecular Function.

C. Nucleolin and H1FX proteins were analyzed by Western blotting in the complex Immunoprecipitated (IP) by H1FX antibody and input group.

D. Comparison analysis of nucleolin and H1FX in the immunoprecipitation complex treated with and without RNase (67 ng/μl).

E. Sequence alignment analysis among linker histone variants. The sequence in the framed part from their globular domains shows great divergence.


A
CPAT algorithm
CPC algorithm
Coding Probability
Coding probability
ACTB
MALAT1
AY927503
B
Log2(fold change of positive signal) HCC/NT
Pearson χ2 test, P < 0.05
Patients
C
Expression levels(fold change)
Mock
AY
***
ITGV
ITGAV
ITGB1
ITGB3
ITGB5
ITGB6
D
BEL-7404
Mock
AY
+ shITGAV
SMMC-7721
Scramble
shAY
+ ITGAV
0h
72h
96h
Mock
AY
AY+shITGAV
Scramble
shAY
shAY+ITGAV
Relative closure of wound (%)
Incubation time
*
**
E
BEL-7404
Mock
AY
sh ITGAV
SMMC-7721
Scramble
shAY
ITGAV
Relative migration ability
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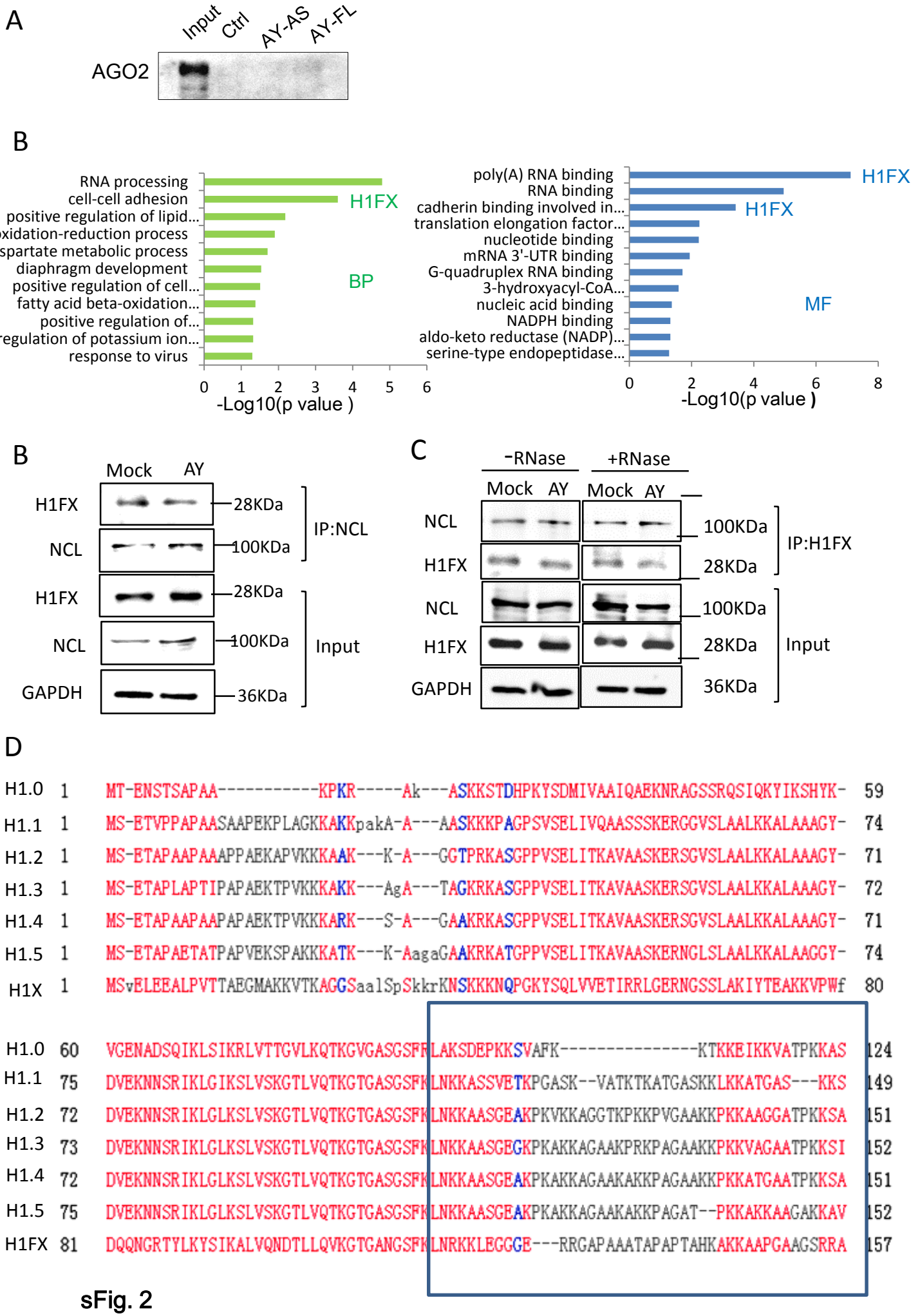
Mock
AY
Scramble
shAY
Fold change
AY
ZNF282
G
TCGA
Pearson r=0.54
P<0.0001
N=200
ZNF282(mRNA)
AY (mRNA)
ZNF282 Levels
NT
HCC
TCGA
Pearson r=-0.13
P<0.014
N=371
ITGAV(mRNA)
ZNF282 (mRNA)

sFig. 1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oxidation-reduction process
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diaphragm development
positive regulation of cell...
fatty acid beta-oxidation...
positive regulation of...
regulation of potassium ion...
response to virus
H1FX
BP
0 1 2 3 4 5 6
-Log10(p value)
poly(A) RNA binding
RNA binding
cadherin binding involved in...
translation elongation factor...
nucleotide binding
mRNA 3'-UTR binding
G-quadruplex RNA binding
3-hydroxyacyl-CoA...
nucleic acid binding
NADPH binding
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H1FX
H1FX
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Mock
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H1FX 81 DQQNGRTYLKYSIKALVQNDTLLQVKGTGANGSFKLNRKKLEGGGE---RRGAPAAATAPAPTAHKAKKAAPGAAGSRRA 157

sFig. 2