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Supplemental Information

The ASH1-miR-375-YWHAZ Signaling Axis Regulates Tumor Properties in Hepatocellular Carcinoma

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Supporting Figures

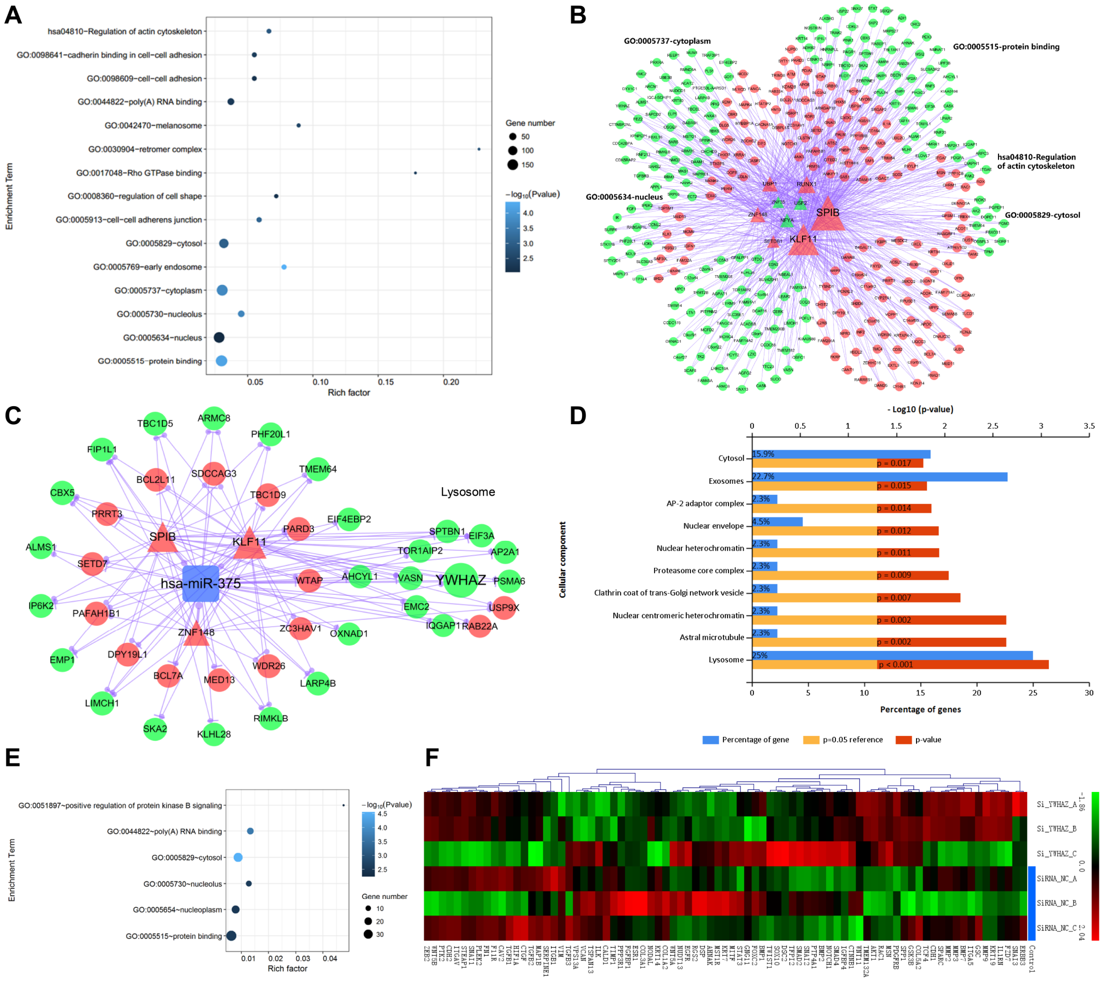

Figure S1 Gene enrichment analysis and network construction about differentially expressed genes. (A) Enrichment Analysis of 553 Differentially Expressed Genes. The vertical axis represents the name of the enriched term, the abscissa is Rich factor (the ratio of the number of genes enriched to the total number of genes contained in the term), and the right graph indicates the number of genes on the term and -log10 (PValue). (B) Regulatory network of si-YWHAZ DEGs. Triangles: TFs; circles: target genes; rounded rectangles: miRNAs. The node size indicates the connectivity (the total number of edges) of the node. Node color: Red represents up-regulation in siYWHAZ samples, green is down-regulation. The large network is categorized and displayed according to enriched terms. (C) A small regulatory network which miR-375 as a core node target to DEGs in figure (B). (D) Enrichment analysis of 44 genes in figure (C). (E) Enrichment Analysis of 78 co-expression genes of YWHAZ in TCGA HCC samples. (F) Heat map of 81 EMT genes in siYWHAZ microarray data.

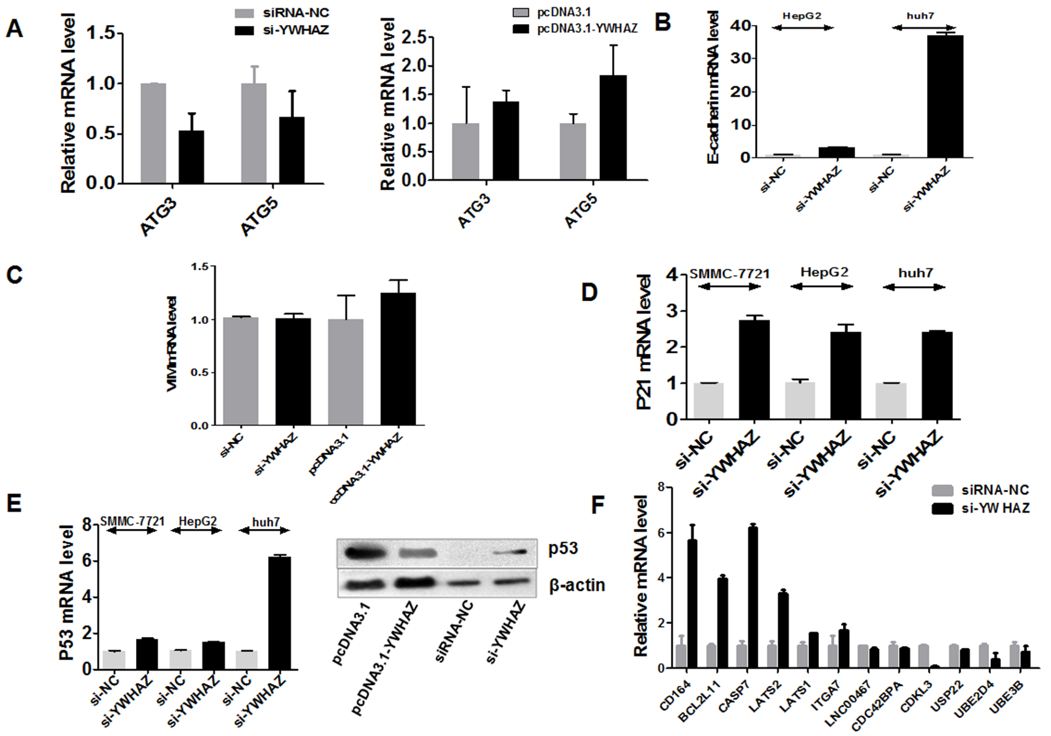

Figure S2 YWHAZ regulates autophagy/EMT and P53-P21 pathway. (A) The expression of ATG3 and ATG5 mRNA was examined by SYBR Green qRT–PCR in SMMC-7721 cell lines transfected with si-YWHAZ, pcDNA3.1-YWHAZ or their relative negative controls. (B) E-cadherin mRNA was examined by SYBR Green qRT–PCR in HepG2 and huh7 cell lines transfected with si-YWHAZ or si-NC. (C) Relative mRNA expression of VIM was detected by SYBR Green qRT–PCR in SMMC-7721 cell lines after transfected with si-NC, si-YWHAZ, pcDNA3.1 or pcDNA3.1-YWHAZ. (D) SYBR Green qRT–PCR was used to detect the expression of P21 in three hepatoma cell lines transfected with si-YWHAZ or si-NC. (E) Left: SYBR Green qRT–PCR was used to detect the expression of P53 in three hepatoma cell lines transfected with si-YWHAZ or si-NC. Right: The expression of p53 protein was detected by Western blot in SMMC-7721 cell lines after transfected with pcDNA3.1-YWHAZ, si-YWHAZ or their relative negative controls. (F) The expression of some other YWHAZ downstream genes was detected by SYBR Green qRT–PCR in SMMC-7721 cell lines after transfected with si-YWHAZ or si-NC.

Supporting Tables

Table S1. Sequences of siRNA

siRNA	Target sequence
si-YWHAZ-1	GCTCGAGAATACAGAGAGA
si-YWHAZ-2	CGCTGGTGATGACAAGAAA
si-YWHAZ-3	GTGAAGAGTCATACAAAGA
si-ASH1-1	CCAACGACTTGAACTCCAT
si-ASH1-2	CCAACAAGAAGATGAGTAA
si-ASH1-3	GCGTCAAGTTGGTCAACCT

Table S2. Primer sequences used in SYBR Green qRT-PCR.

Gene Name	Forward primer	Reverse primer
YWHAZ	TGATCCCCAATGCTTCACAAG	GCCAAGTAACGGTAGTAATCTCC
ASH1	CCCAAGCAAGTCAAGCGACA	AAGCCGCTGAAGTTGAGCC
P53	ACAGCTTTGAGGTGCGTGTTT	CCCTTTCTTGCGGAGATTCTCT
P21	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
E-cadherin	AAAGGCCCATTTCCTAAAAACCT	TGCGTTCTCTATCCAGAGGCT
VIM	AAATGGCTCGTCACCTTCG	TGAGTGGGTATCAACCAGAGG
USP22	GTGTCTTCTTCGGCTGTTT	CCTCCTTGGCGATTATTT
UBE2D4	AGCCTTGCTCTGTCTCC	AACACCGTCTCCACTAAA
UBE3B	CGCTGTCTTACGACGAGG	TGGAACGGAATCCGCTAA
LATS2	GGCGATTCGTTTGCGTCCTA	TTGGGTGGGTGCTGGTGCTG
LATS1	TGGGACAACCTCCTTTCT	AGCACCATTCTTGCCTAA
CDKL3	TATGAAGGTGGACTTGGTC	GTTAGATTGATGGGTGGC
BCL2L11	ACAGAGCCACAAGACAGGAGC	GCCATACAAATCTAAGCCAGTAA
CASP7	TTTGACAGCCCACTTTAGG	GGATCTTGTATCGAGGATTAG
CDC42BPA	CAGTCTTGGCTTCTGATGT	ACTCCCACCCACTTATTCT
CD164	GGTTGACTGAGCGTTGCG	GCCGAGGTTACGTTGGAGA
ITGA7	ACCCTGGGCTCTGCCTTCCT	CGCCGCCTCCTATCCCTACT
LINC00467	GAAGCCAGACAGATTCAAGTA	AGCCCAGTTTCAGTCCCT
ATG3	AGACTCCACGATTATGGTTGT	CTGCATGGGTGAACTGAAC
ATG5	AAAGAATAGCCAGTACAGCA	AATGAACCGACGAATAAAC
ATG7	GGTCAAAGGACGAAGATAACA	TACGGTCACGGAAGCAAA
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

Table S3.The sequences of 55-mer double-stranded oligonucleotides containing the predicted miRNA binding sites.

Targeted Gene	**sequence**
YWHAZ	FS:*5'-CTAGT*GTCATCTTGGAGGGTCGTCTCAAGTATT***GAACAA***AAGACGGAAGGTGCT*A-3'*
	RS:*5'- AGCTT*AGCACCTTCCGTCTT***TTGTTC***AATACTTGAGACGACCCTCCAAGATGAC*A-3'*
Mutated YWHAZ	FS:*5'-CTAGT*GTCATCTTGGAGGGTCGTCTCAAGTATTT***GTATCG***AGACGGAAGGTGCT*A-3'*
	RS:*5'-AGCTT*AGCACCTTCCGTCT***CGATAC***AAATACTTGAGACGACCCTCCAAGATGAC*A-3'*

The sequences highlighted by italic bold type refer to predicted pairing nucleotide with the seeding sequences of corresponding miRNAs. Italic letters refer to overhangs of restriction enzyme sites. FS, forward sequence; RS, reverse sequence.

Table S4. Enrichment analysis of the co-expression genes of YWHAZ in TCGA hepatocellular carcinoma (HCC) samples.

Term	Count	PValue	Co-expression genes of YWHAZ in TCGA HCC samples
GO:0005829~ cytosol	30	2.99E-05	NBN, CHMP4C, POLR2K, KIAA0196, ASAP1, AZIN1, MAF1, ARFGEF1, ARHGAP39, WDYHV1, MTHFD1L, PTK2, RAD21, RPL7, HSF1, EIF3H, AGO2, PABPC1, NSMAF, SUOX, RAN, CCT6A, STK3, VASP, ANXA2, ATP6V1C1, CCT5, RCC2, RIPK2, TCEB1
GO:0044822~ poly(A) RNA binding	15	2.27E-04	UTP23, MTDH, RAN, STIP1, CCT6A, ANXA2, DCAF13, MRPL13, RPL7, RCC2, EIF3H, POP1, AGO2, PABPC1, KIAA1429
GO:0005730~ nucleolus	11	2.52E-03	UTP23, DCAF13, NBN, CCT5, MTDH, RCC2, E2F5, RPL7, RAN, MAF1, ARFGEF1
GO:0005515~ protein binding	39	2.56E-03	NBN, MTDH, E2F5, CHMP4C, MAL2, KIAA0196, ASAP1, TPD52, ARFGEF1, SCRIB, WDYHV1, INTS8, PTK2, MRPL13, RAD21, HSF1, RPL7, EIF3H, AGO2, PABPC1, NSMAF, TRAM1, UTP23, RAN, CCT6A, TATDN1, C8ORF33, VASP, STK3, ANXA2, CHRAC1, CCT5, MED30, RCC2, UBR5, RIPK2, TCEB1, EMC2, DSCC1
GO:0005654~ nucleoplasm	22	2.91E-03	NBN, E2F5, POLR2K, RAN, MAF1, TATDN1, ARFGEF1, SCRIB, INTS8, DCAF13, RAD21, MED30, DCAF11, HSF1, IVD, UBR5, POP1, AGO2, TCEB1, NFIA, DSCC1, KIAA1429
GO:0051897~ positive regulation of protein kinase B signaling	4	5.20E-03	PTK2, MTDH, ARFGEF1, STK3