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Supplemental information

PHF5A promotes colorectal cancer progression by alternative splicing of TEAD2

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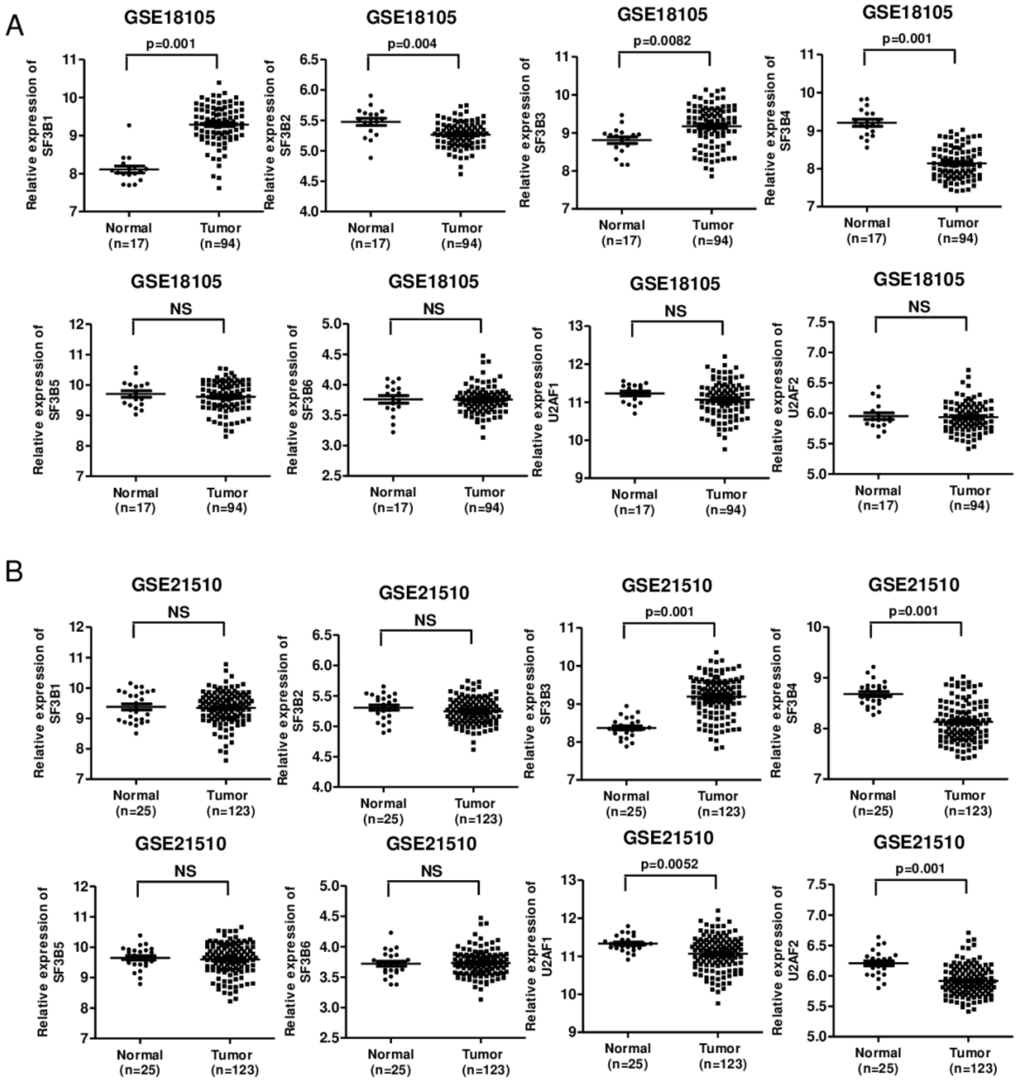
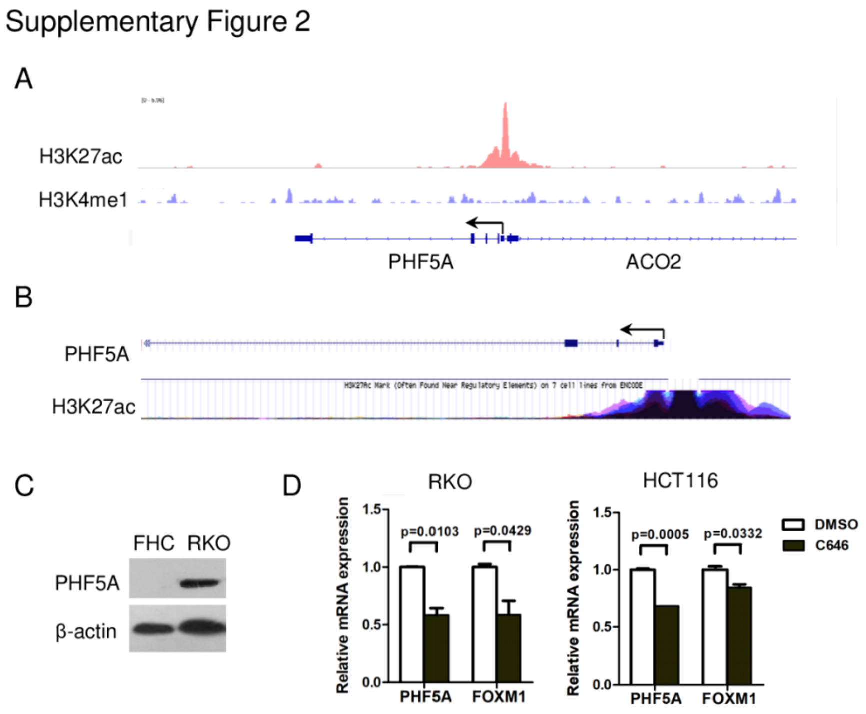
Supplementary Figure 1



Supplementary Figure 1. Expression of of SF3b components from GEO datasets

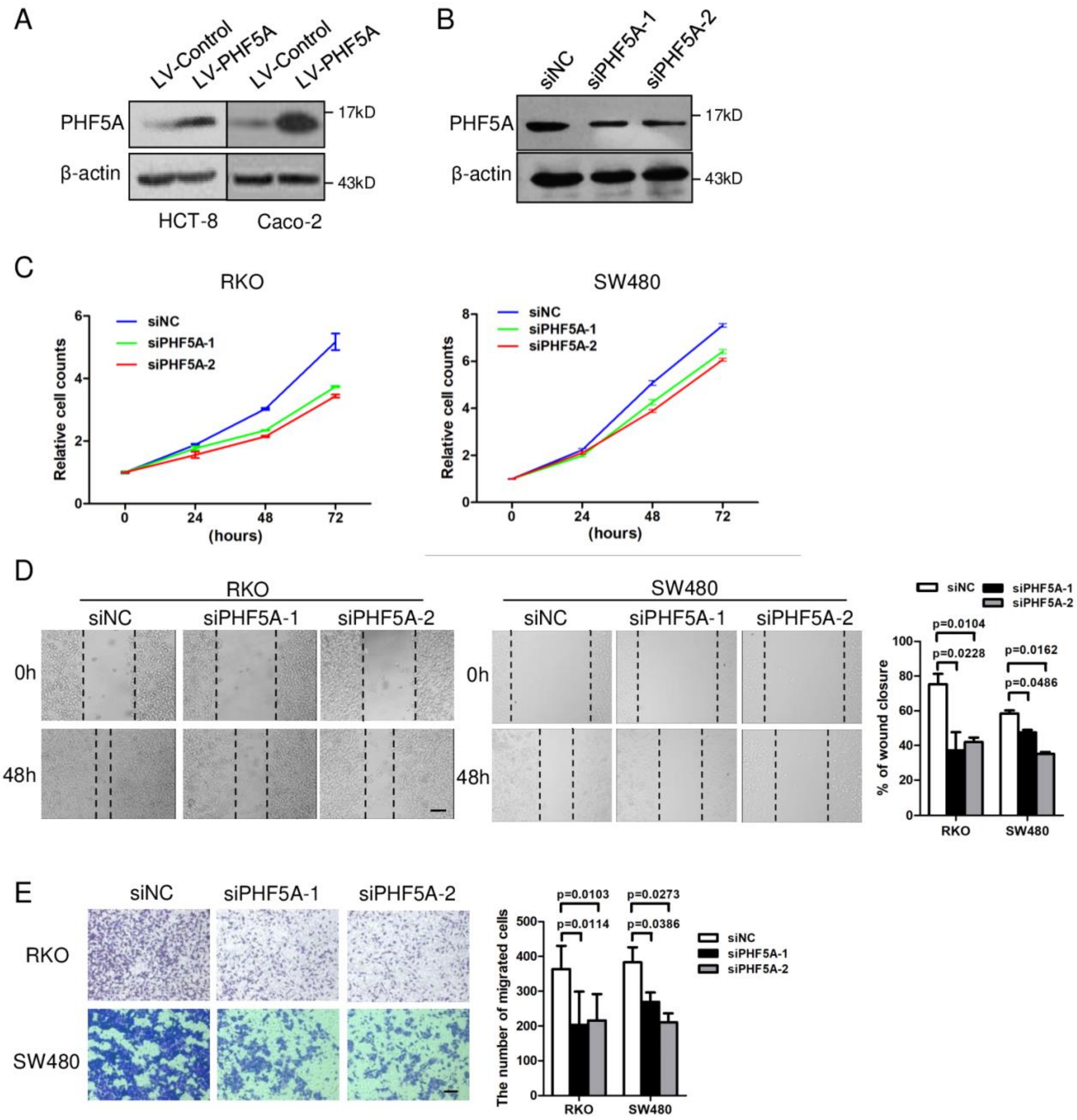
Relative expression of SF3b components of complex in colorectal cancer and normal tissue samples from GSE18105 (A) and GSE21510 (B).



Supplementary Figure 2. H3K27 acetylation activates PHF5A in CRC

(A) Genome browser view of H3K27ac and H3K4me1 ChIP-Seq signals at PHF5A promoters in CRC cells (GSE71510). (B) Enrichment of H3K27ac at the promoter of PHF5A shown in UCSC Genome Bioinformatics Site (http://genome.ucsc.edu/). (C) The protein levels of PHF5A in FHC and RKO cells. (D) The RNA levels of PHF5A in cells treated with C646 (n=3). FOXM1 was used as positive control.

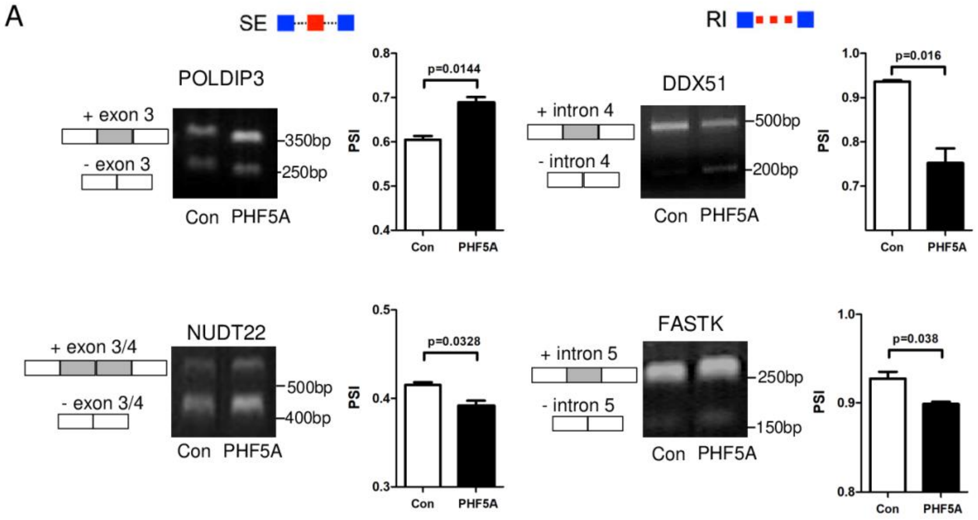
Supplementary Figure 3



Supplementary Figure 3. Knockdown of PHF5A suppresses CRC cell proliferation and migration

(A) The protein levels of PHF5A in HCT-8 or Caco-2 cells stably overexpressing PHF5A. (B) The protein levels of PHF5A in RKO cells transfected with siRNA against PHF5A. (C) Cell proliferations were measured using CCK-8 assays in RKO or SW480 cells transfected with siPHF5A. Wound healing assay (D) and transwell migration assay (E) of CRC cells transfected with siPHF5A. Experiments were repeated at least three times. Data are expressed as mean ±s.d.

Supplementary Figure 4



Supplementary Figure 4. Validation of PHF5A-regulated AS events

(A) RT-PCR validation of representative PHF5A-regulated AS events (n=3).

Supplementary Figure 5

Inserted sequence: exon1-3

CCCACTTTTCCCAAACAAAGCTCCCGGCAACTTTCTCCCTCGCAGCGCCCCGCCCGCCCGCGGCTCCCCAGC
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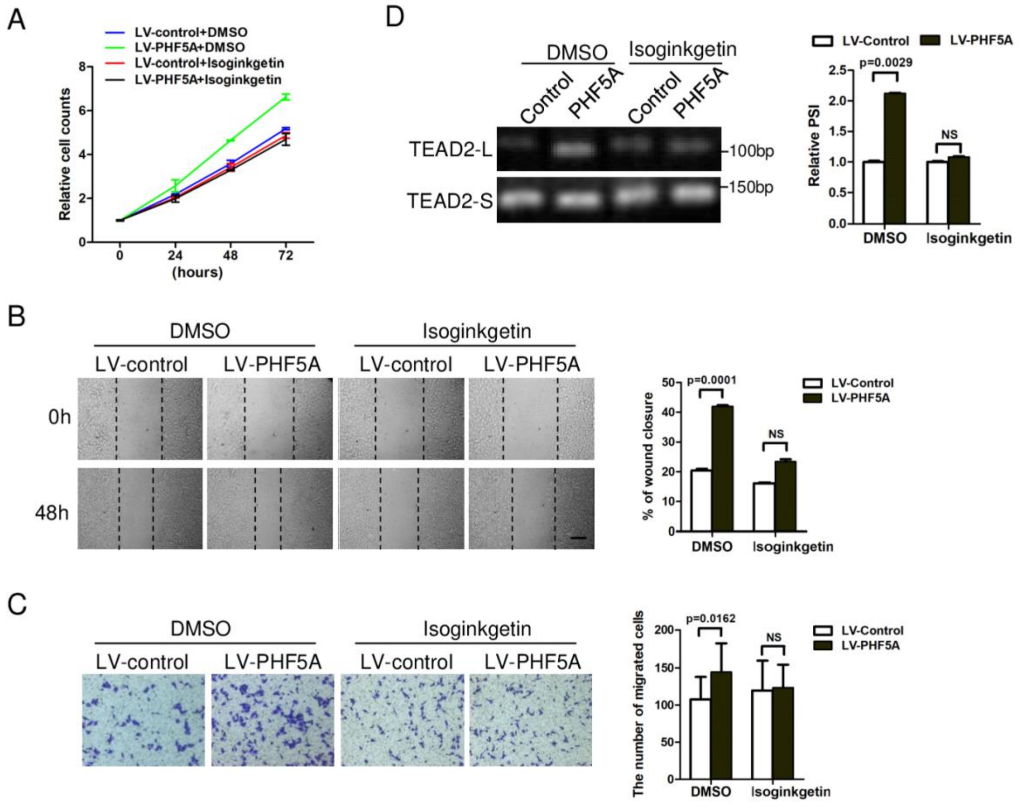
GGGGAAGACCCGAACTCGAAAACAG

Supplementary Figure 5. The schematic diagram of TEAD2 isoforms

(A) The schematic diagram of TEAD2-L and TEAD2-S transcripts. Red arrows indicated the specific primers to detect TEAD2-L or TEAD2-S. (B) The insert sequence of TEAD2 into RG6 minigene. Exons were shown in capital and introns were shown in lowercase. The C-rich polypyrimidine and consensus motifs of BPS were highlighted in red and blue in intron 1, respectively. The underlined sequences were deleted in mutated RG6-TEAD2 minigene. (C) The consensus motifs of BPS in intron 1 of TEAD2. (D) RT-PCR analysis of the effects of PHF5A overexpression on TEAD2-wt minigene and TEAD2-mut minigene.

Supplementary Figure 6



Supplementary Figure 6. Splicing modulator isoginkgetin blocked CRC progression

(A) CCK8 assays of Caco2 cells stably overexpressing PHF5A or control treated with isoginkgetin (15uM). Wound healing (B) and transwell assay (C) of Caco2 cells stably overexpressing PHF5A or control treated with isoginkgetin (15uM). (D) Inclusion of TEAD2 exon 2 was examined by RT-PCR in HCT8 cells stably overexpressing PHF5A upon isoginkgetin treatment (n=3). Experiments were repeated at least three times. Data are expressed as mean ± s.d.

Supplementary Table 1. Detailed clinical characteristics and PHF5A score of 88 CRC patients, which is provided as separate Excel file.

Supplementary Table 2. Relationship Between PHF5A Expression and Clinicopathologic Features.

Variables	**Cases**	**PHF5A Expression**		**P-value**
		High (n=44)	**low (n=44)**	
Gender				0.516
Male	52	28	24	
Female	36	16	20	
Age				0.666
＜55	37	17	20	
≥55	51	27	24	
Tumor size				0.669
＜5 cm	41	22	19	
≥5 cm	47	22	25	
Tumor grade				0.518
Well	9	3	6	
Moderately	64	34	30	
Poorly	15	7	8	
TNM stage				＜0.01
I/II	40	14	26	
III/IV	48	30	18	
Lymph nodal metastasis				＜0.01
No	40	14	26	
Yes	48	30	18	

Supplementary Table 3. Sequences of siRNA against specific target in this study.

siPHF5A-1	Sense (5’-3’)	GCUAAACAUCAUCCUGAUUTT
	Anti-sense (5’-3’)	AAUCAGGAUGAUGUUUAGCTT
siPHF5A-2	Sense (5’-3’)	GCUAGUGUACUGGCAGCUUTT
	Anti-sense (5’-3’)	AAGCUGCCAGUACACUAGCTT
siTEAD2-1	Sense (5’-3’)	UGUAUGGUCGGAAUGAACUTT
	Anti-sense (5’-3’)	AGUUCAUUCCGACCAUACATT
siTEAD2-2	Sense (5’-3’)	GUCUGAUGAAGGCAAGAUGTT
	Anti-sense (5’-3’)	CAUCUUGCCUUCAUCAGACTT

Supplementary Table 4. Sequences of primers used for qRT-PCR in this study.

PHF5A	Forward (5’-3’)	GTTGCCATCGGAAGACTGT
	Reverse (5’-3’)	GCCCCTGGTAAGATCCATAGT
β-actin	Forward (5’-3’)	AATCGTGCGTGACATTAAGGAG
	Reverse (5’-3’)	ACTGTGTTGGCGTACAGGTCTT
BIRC5	Forward (5’-3’)	TTCTCAAGGACCACCGCATCT
	Reverse (5’-3’)	CGCACTTTCTCCGCAGTTTC
ANKRD1	Forward (5’-3’)	GAGGAACTGGTCACTGGAAAGA
	Reverse (5’-3’)	GGGTCACAGGGTGGGCTA
CYR61	Forward (5’-3’)	AGTGCTGCGAGGAGTGGG
	Reverse (5’-3’)	GGTTGTATAGGATGCGAGGCT

Supplementary Table 5. Sequences of primers used for semi-quantitative RT-PCR in this study.

TEAD2-L	Forward (5’-3’)	CCCAGACATTGAGCAGAGC
	Reverse (5’-3’)	TCAGTTCATTCCGACCATACA
TEAD2-S	Forward (5’-3’)	GGGAGGTCGGAATGAACTG
	Reverse (5’-3’)	GTTCAGAGCCTTCAACTTGGAC
POLDIP3	Forward (5’-3’)	TGCCTTCATAAACCCACCCA
	Reverse (5’-3’)	CATGTGGTGGAGAAAGCCG
NUDT22	Forward (5’-3’)	CTGCCTGGCTGCGACA
	Reverse (5’-3’)	TAGTGCTTCCTCACCTGCTC
DDX51	Forward (5’-3’)	GAGCAAAGTTTTCAACATCTACAC
	Reverse (5’-3’)	TCAGCCAAGCAGCGGTA
FASTK	Forward (5’-3’)	CATCTTGATGTCACTGTGCCA
	Reverse (5’-3’)	CAGCAGGGAGAGGTAGCG
RG6	Forward (5’-3’)	AAACGCAAAGTGGAGGACC
	Reverse (5’-3’)	TGTAGTCGGGGATGTCGG