

Original Research

Transcription factor AP-4 reactivates telomerase reverse transcriptase independently of genome alteration in non-HBV associated hepatocellular carcinoma

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Supplementary Materials

Figure S1. Survival analysis upon overexpression of *TFAP4* mRNA in human HCC.

Figure S2. HCC cell proliferation, migratory ability and cell invasiveness upon knockdown of *TFAP4*.

Figure S3. *TFAP4* mRNA expression is not significantly correlated with *TERT* mRNA expression in HCV-associated HCC.

Table S1. Primers used for cloning plasmids.

Table S2. Antibodies used in this study.

Table S3. Primers used for qRT-PCR.

Table S4 Primers used for telomere length measurement.

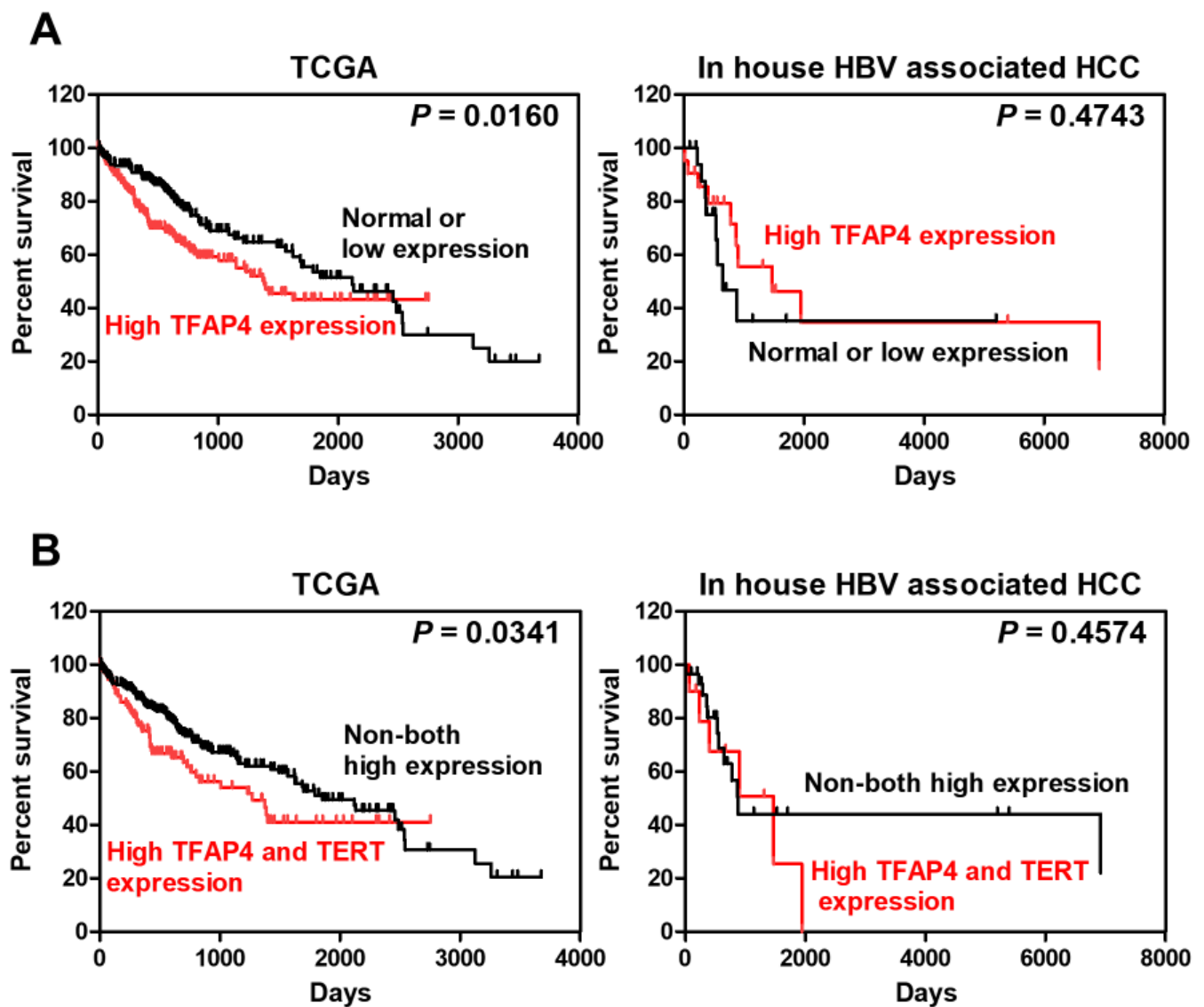


Figure S1. Survival analysis according to expression of *TFAP4* mRNA in human HCC. Survival analysis according to (A) expression of *TFAP4* mRNA and (B) concomitant expression of both *TFAP4* and *TERT* mRNA in TCGA-LIHC cohort and in-house HBV-associated HCC cohort.

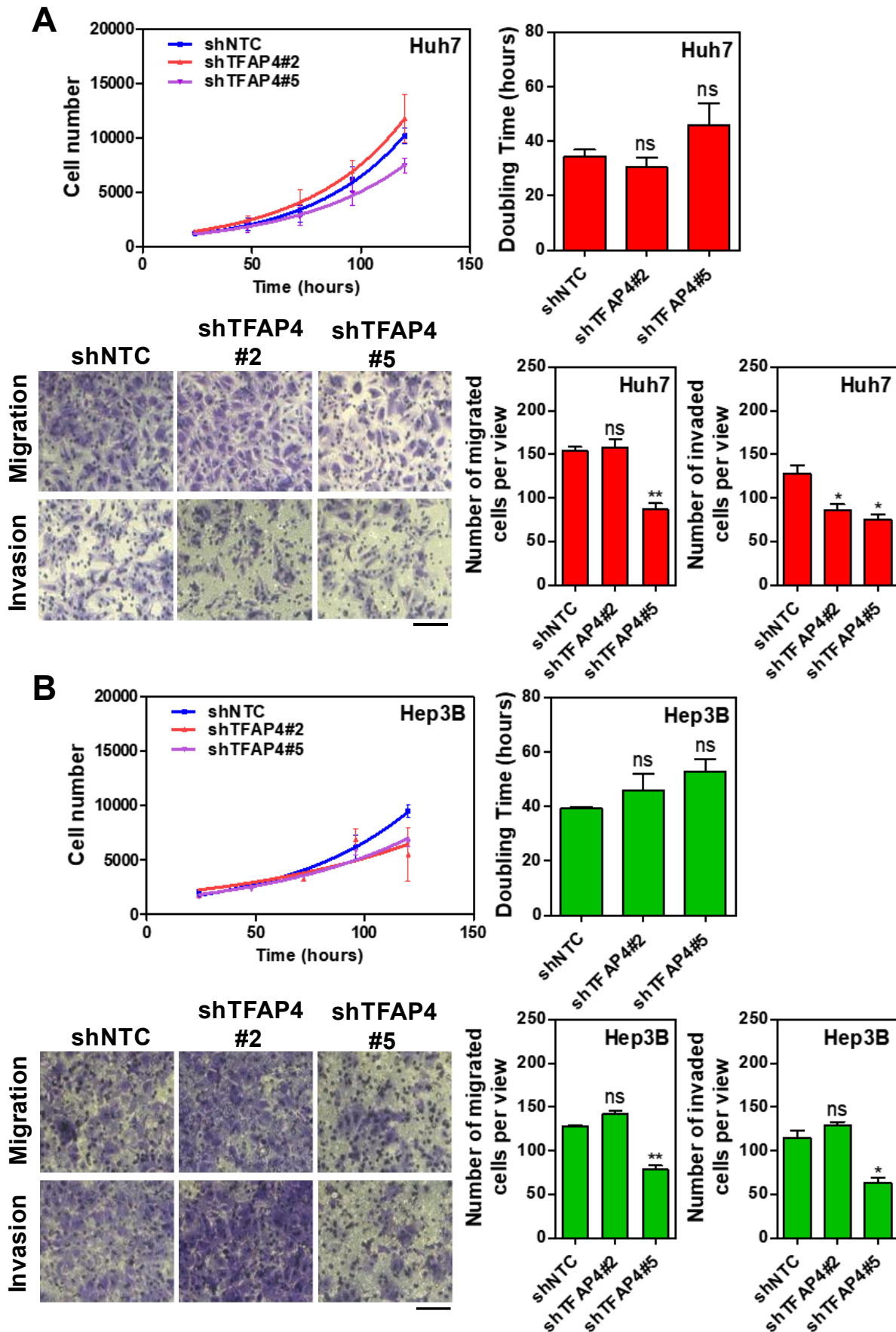


Figure S2. Cell proliferation, doubling time, migration and invasion upon knockdown of *TFAP4* in (A) Huh7 and (B) Hep3B. Scale bar = 100 μ m. (ns = $P > 0.05$, * $P < 0.05$, ** $P < 0.01$)

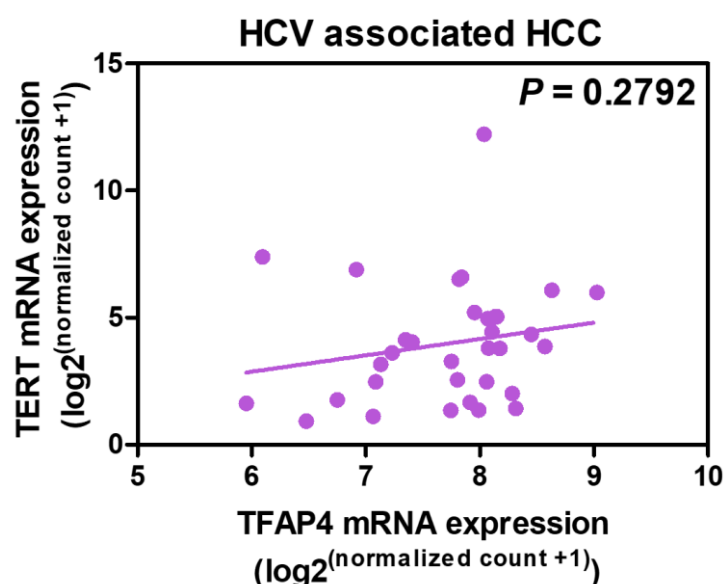


Figure S3. Correlation analysis between *TFAP4* and *TERT* mRNA expression in human HCV-associated HCC (n=33) in TCGA-LIHC cohort showed no significant association.

Table S1. Primers used for cloning plasmids.

Primer Name	Primer Sequence (5' to 3')
CEBPA-F	AAGCTTATGGAGTCGGCCGACTTCTACGAGGC
CEBPA-R	GAATTCACGCGCAGTTGCCCATGGCCTTGACC
CTCF-F	CCCAAGCTTATGGAAGGTGATGCAGTCGAAGCCA
CTCF-R	GCGAATTCTCACCGGTCCATCATGCTGAG
EGRI-F	AAGCTTGCCACCATGGCCGCGGCCAAGGCCGAGATGCAGC
EGRI-R	GAATTCTTA GCAAATTTCAATTGTCCTGGG
ELF1-F	GATATCATGGCTGCTGTTGTCCAACAGAACGACC
ELF1-R	GCGGCCGCTAAAAAGAGTTGGGTTCCAGCAGTTTCG
ELF4-F	CCAAGCTTATGGCTATTACCCTACAGCCCAG
ELF4-R	TTGGATCCTTATATGTCATGGGGCTCC
ELK4-F	GAATTCTATGGACAGTGCTATCACCTGTGGC
ELK4-R	GCGGCCGCTTATGTCTTCTGTAGGTCTGGGGAAAATGGGC
ERF-F	AAGCTTATGAAGACCCCGCGGACAC
ERF-R	GCGGCCGCTCAGGAGTCTCGGTGCTCCAG
ETS2-F	AAGCTTATGAATGATTTTCGGAATCAAG
ETS2-R	GAATTCTCAGTCCTCCGTGTCTGGGCTGG
ETV4-F	AAGCTTATGGAGCGGAGGATGAAAGCCGG
ETV4-R	GAATTCCTAGTAAGAGTAGCCACCCTTGGGGCC
EZH2-F	GGTACCATGGGCCAGACTGGGAAGAAATCTGAG
EZH2-R	GGGCCCTCAAGGGATTTCATTTCTTTTCGATGCCG
FOXA1-F	CCAAGCTTATGTTAGGAACTGTGAAGATGG
FOXA1-F	CCAAGCTTATGTTAGGAACTGTGAAGATGG
FOXA1-R	TTGAATTCCTAGGAAGTGTTTAGGACGGGTCTGG
FOXA1-R	TTGAATTCCTAGGAAGTGTTTAGGACGGGTCTGG
FOXA2-F	AAGCTTGCCACCATGCACTCGGCTTCCAGTATGCTGG
FOXA2-F	AAGCTTGCCACCATGCACTCGGCTTCCAGTATGCTGG
FOXA2-R	GAATTCTTAAGAGGAGTTCATAATGGGCC

FOXA2-R	GAATTCTTAAGAGGAGTTCATAATGGGCC
GABPA-F	AAGCTTATGACTAAAAGAGAAGCAGAGGAGC
GABPA-R	GCGGCCGCTCAATTATCCTTTTCCGTTTGCAGAG
HIF1A-F	GGTACCATGGAGGGCGCCGGCGCGCAACGAC
HIF1A-R	GGGCCCTCAGTTAACTTGATCCAAAGCTCTG
JUN-F	AAGCTTATGACTGCAAAGATGGAAACGACC
JUN-R	GAATTCAAAATGTTTGCAACTGCTGCGTTAGC
MAZ-F	CCCAAGCTTATGTTCCCGGTGTTTCCTTGAC
MAZ-R	GCGAATTCTCACCAGGGTTGGGAGGGAAGTGGC
MYBL2-F	GGAAGCTTATGTCTCGGCGGACGCGCTGCGAGG
MYBL2-R	AATTGGATCCTCAGGACAAGATGAGGGTCCGAG
MYC-F	CCCAAGCTTCTGGATTTTTTTCGGGTAGTGGAACCAGCAG
MYC-R	GCGAATTCTTACGCACAAGAGTTCCGTAGCTG
SOX9-F	GGGAAGCTTATGAATCTCCTGGACCCCTTCATG
SOX9-R	CCCGAATTCAAGGTCGAGTGAGCTGTGTG
SP1-F	AAGCTTATGAGCGACCAAGATCACTCCATGG
SP1-R	GAATTCTCAGAAGCCATTGCCACTGATATTAATGGAC
SPDEF-F	AAGCTTATGGGCAGCGCCAGCCCGGGTC
SPDEF-F	GAATTCAGATGGGGTGACGAACTGGTAGAC
TFAP4-F	CCAAGCTTATGGAGTATTTTCATGGTGCCAC
TFAP4-R	TTGAATTCTCAGGGAAGCTCCCCGTCCCCGAC
TP53-F	AGTCTAGAGCCACCGTCCAG
TP53-R	GTCTGAGTCAGGCCCTTCTG
shNTC F	CCGGTGGTTTACATGTTTTCTGACTCGAGTCAGAAAACATGTAAACCATTTTTG
shNTC R	AATTCAAAAATGTTTACATGTTTTCTGACTCGAGTCAGAAAACATGTAAACCA
shCTCF #1F	CCGGGATGAAGACTGAAGTAATGCTCGAGCATTACTTCAGTCTTCATCTTTTTG
shCTCF #1R	AATTCAAAAAGATGAAGACTGAAGTAATGCTCGAGCATTACTTCAGTCTTCATC
shCTCF #2F	CCGGGGAGAAACGAAGAAGAGTACTCGAGTACTCTTCTTCGTTTCTCCTTTTTG
shCTCF #2R	AATTCAAAAAGGAGAAACGAAGAAGAGTACTCGAGTACTCTTCTTCGTTTCTCC
shTFAP4 #2F	CCGGACACACAGCTCAAGCGCTTCTCGAGAAGCGCTTGAGCTGTGTGTTTTTG
shTFAP4 #2R	AATTCAAAAACACACAGCTCAAGCGCTTCTCGAGAAGCGCTTGAGCTGTGTGT
shTFAP4 #5F	CCGGGCCTTGCCAAACATTCCACTAACTCGAGTTAGTGGAATGTTGGCAAGGCTTTTTG
shTFAP4 #5R	AATTCAAAAAGCCTTGCCAAACATTCCACTAACTCGAGTTAGTGGAATGTTGGCAAGGC
TFAP4p-F	AAGCTAGCTCCTGCTTCAGCCTCCCGAGTAG
TFAP4p-R	CCAAGCTTGTTTCAAGGCGGGAAACAGCTGG
TFAP4-6xF	CTAGCCAGCTGCAGCTCCAGCTGCAGCTCCAGCTGCAGCTCA
TFAP4-6xR	GATCTGAGCTGCAGCTGGAGCTGCAGCTGGAGCTGCAGCTGG
TERTp-F	CCGCTAGCCGACGGGACCGCCCCGGTGG
TERTp-R	CCAAGCTTCAGCGCTGCCTGAAACTC
TERTp-327MF	CCTCTCTCCAATGGGGCCCTCG
TERTp-327MR	CGAGGGCCCCATTGGAGAGAGG
TERTp-286MF	GGGAGCGCGAAAGGCGCGGGG
TERTp-286MR	CCCGCGCGCCTTTCGCGCTCCC
TERTp-234MF	GCCCGGAGCAAATGCGCTGTCG
TERTp-234MR	CGACAGCGCATTTGCTCCGGGC
TERTp-111MF	CACCTTCCAAATCCGCCTCC
TERTp-111MR	GGAGGCGGATTTGGAAGGTG

Table S2. Antibodies used in this study.

Antibodies	Source	Catalog number (used concentration)
TFAP4	Sigma-Aldrich	HPA 001912 (1:2000 Western blot)
CTCF	Santa Cruz Biotechnology	sc-271474 (1:1,000 Western blot)
TERT	Abcam	ab183105 (1:2,000 Western blot)
α -Tubulin	Sigma-Aldrich	T9026 (1:2,000 Western blot)

Table S3. Primers used for qRT-PCR.

Primer Name	Primer Sequence (5' to 3')
TERT-qF	CCTTCAAGAGCCACGTCTCTACCTTG
TERT-qR	CCTGGCACTGGACGTAGGAC
TFAP4-qF	GCCCTTTGATTTCGGATTTCATC
TFAP4-qR	ATGAAGCGCTTGAGCTGTGT
HPRT1-qF	CTTTGCTGACCTGCTGGATT
HPRT1-qR	CTGCATTGTTTTGCCAGTGT

Table S4. Primers used for telomere length measurement.

Primer Name	Primer Sequence (5' to 3')
Telo-F	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT
Telo-R	GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT
RPLP0-F	CAGCAAGTGGGAAGGTGTAATCC
RPLP0-R	CCCATTCTATCATCAACGGGTACAA