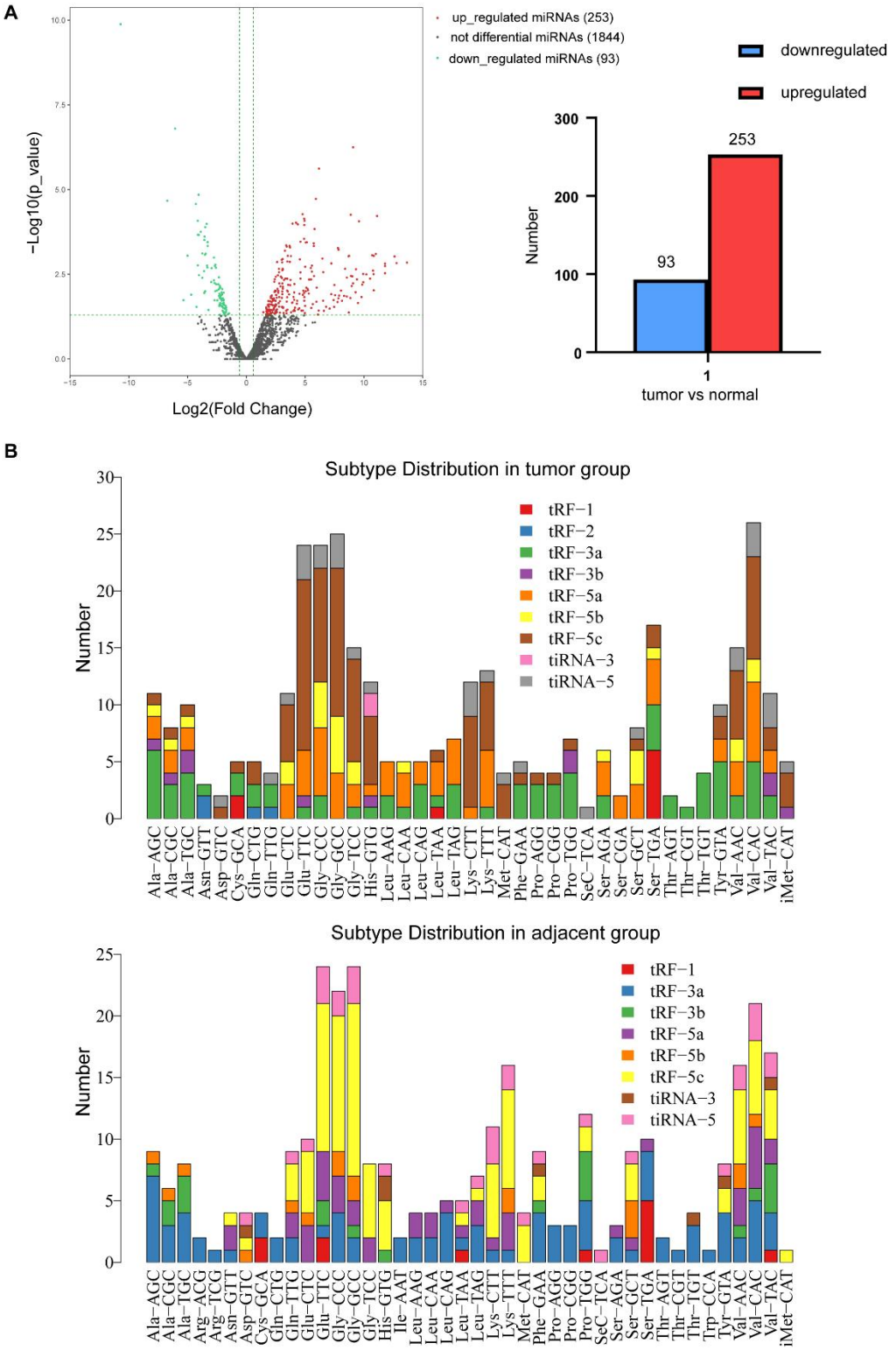
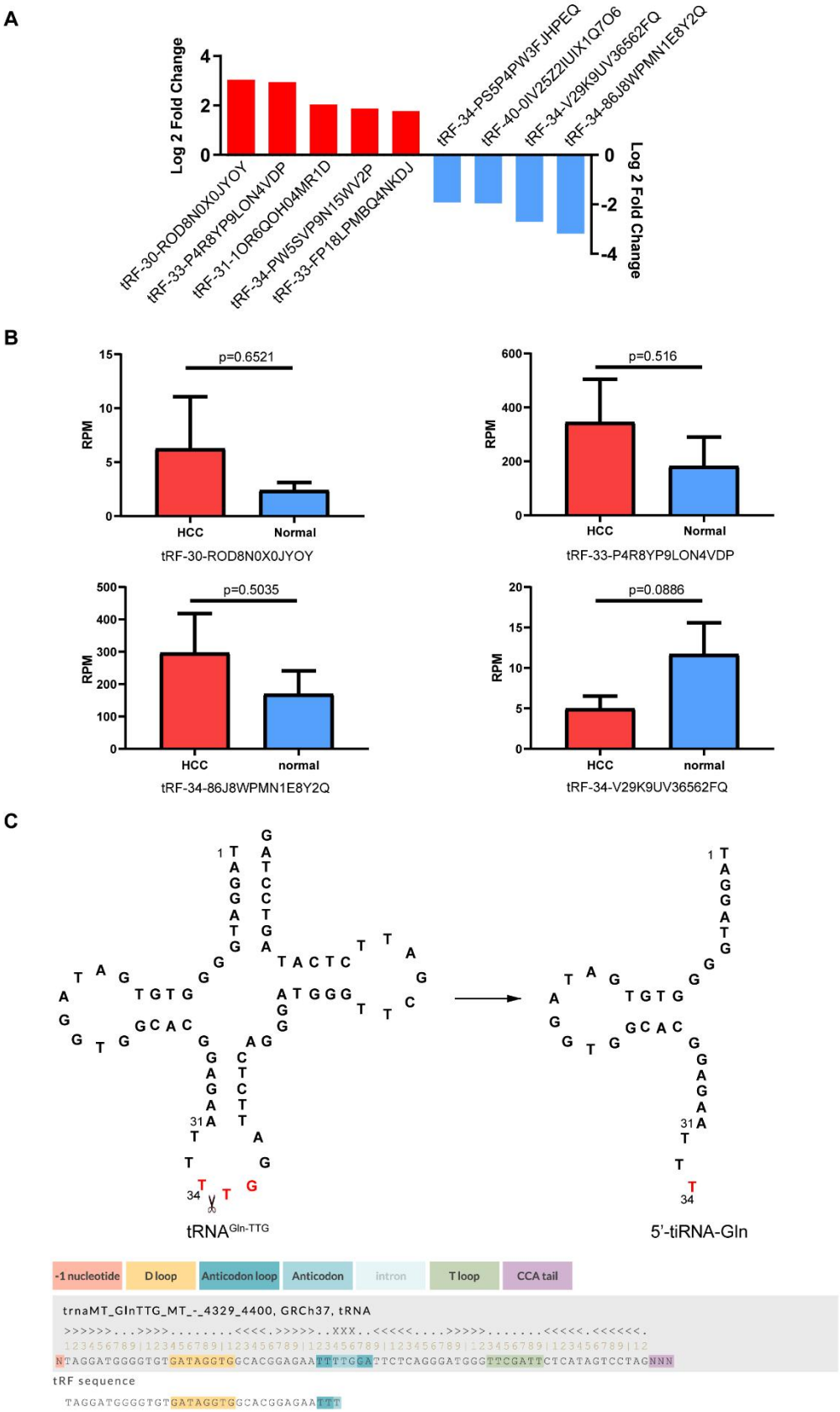
Supplementary Figures and Figure Legends



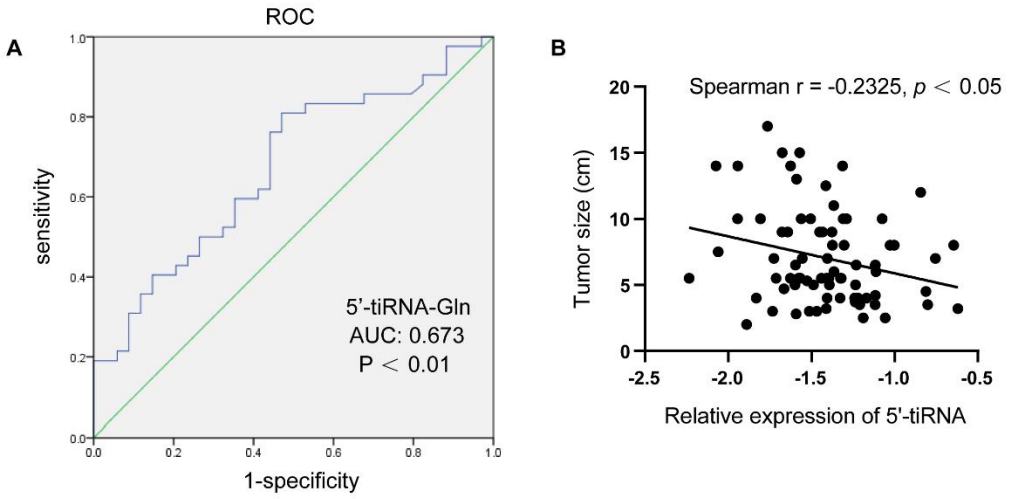
Supplementary Figure S1

Supplementary Figure S1. Differentially expressed miRNAs and subtype tsRNAs against the tRNA isodecoders and isoacceptors. **A**, Left: Volcano plot of differentially expressed miRNAs. Red: upregulated; green: downregulated. Right: The number of down- or upregulated miRNAs in HCC tumor tissues as compared to the adjacent nontumor tissues. Red: upregulated; blue: downregulated. **B**, The number of subtype tsRNAs against the tRNA isodecoders and isoacceptors.



Supplementary Figure S2

Supplementary Figure S2. The sequence information of 5′-tiRNA-Gln and its expression level from MINTbase. **A**, Differential expression of tiRNAs with annotation in MINTbase in 4 pairs of HCC tumor tissues and adjacent nontumorous tissues. **B**, tiRNAs expression data from MINTbase in HCC tumor tissues and normal liver tissues. **C**, The cleavage site is located on the anticodon loop of $tRNA^{Gln\text{-}TTG}$.



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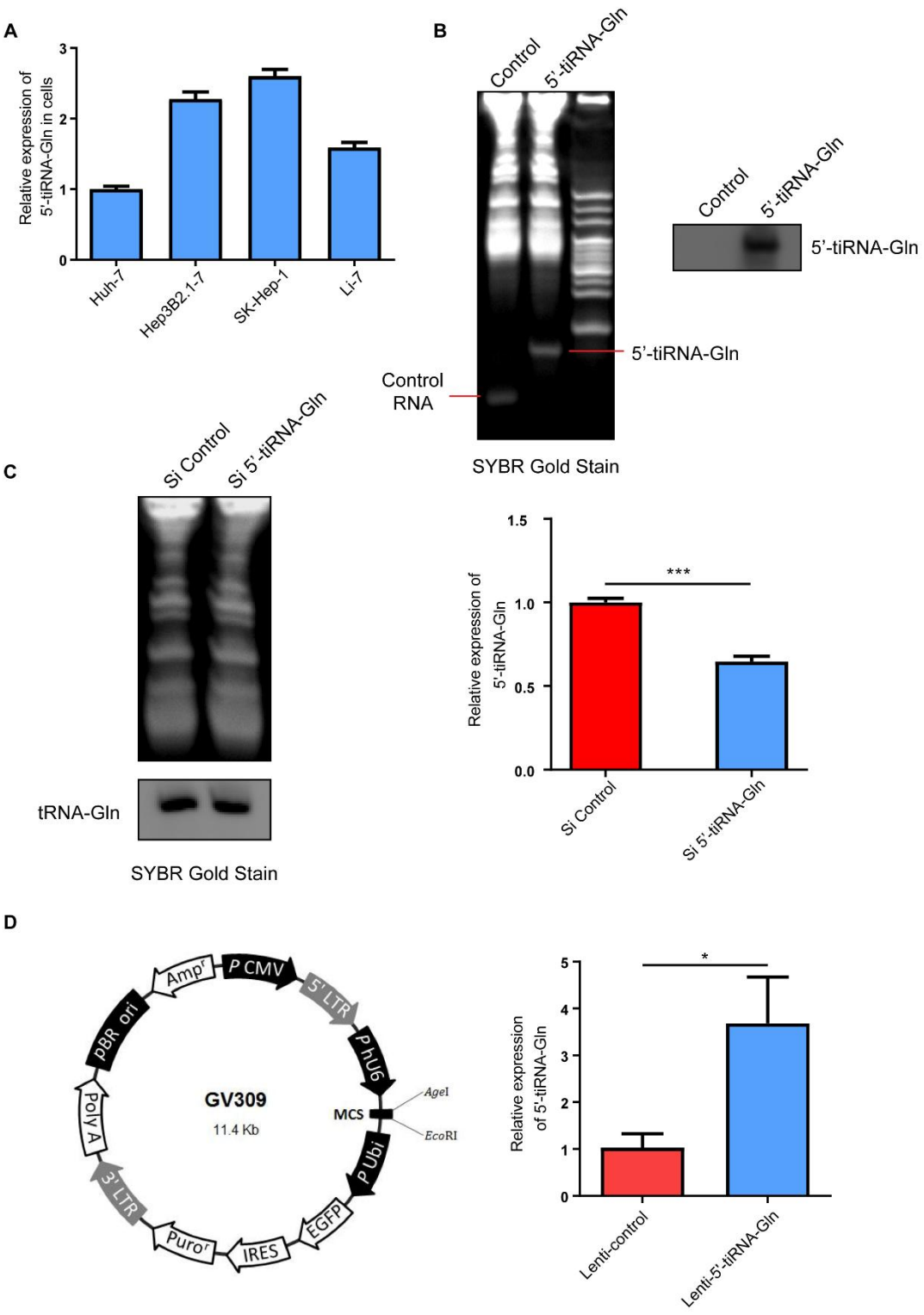
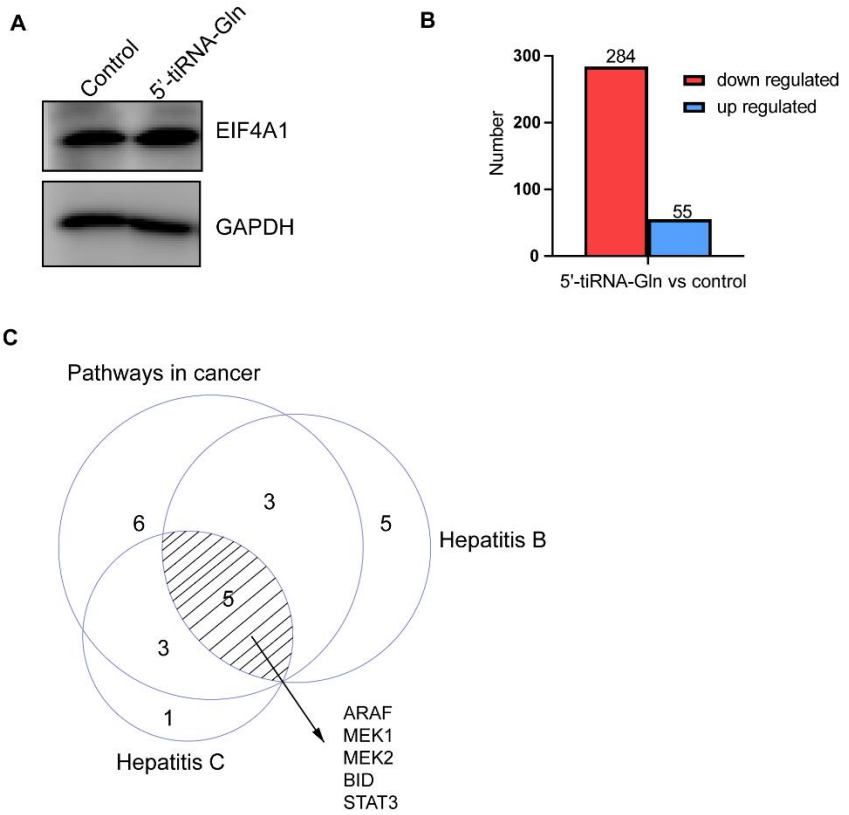
18 **Supplementary Figure S3**

Supplementary Figure S3. The correlation between the expression level of 5′-tiRNA-Gln and the clinical features. **A**, The ROC curve for 5’ -tiRNA-Gln including HCC metastasis patients and non-metastasis patients. **B**, The Spearman’s correlation analysis of the relationship between 5′-tiRNA-Gln and tumor size.



Supplementary Figure S4

Supplementary Figure S4. 5′-tiRNA-Gln expression levels in HCC cells. **A**, Relative 5′-tiRNA-Gln expression levels in HCC cells. **B**, Northern blotting detection of Huh-7 cells transfected with 5′-tiRNA-Gln or control RNA. Left: SYBR Gold staining of 15% urea-TBE PAGE; labels indicate the 5′-tiRNA-Gln and control RNA. Right: DIG-labeled probe detection of 5′-tiRNA-Gln. **C**, Detection of tRNA-Gln and 5′-tiRNA-Gln levels in SK-Hep-1 cells transfected with Si-5′-tiRNA-Gln or Si-control. Short exposure after SYBR Gold staining showed the total RNA loading; the tRNA-Gln level was detected by the DIG-labeled probe. The efficiency of knockdown by Si-5′-tiRNA-Gln was detected by RT-qPCR. **D**, Schematic diagram of the lentivirus vector structure. Bar chart shows the expression level of Huh-7 cells infected with lenti-5′-tiRNA-Gln as compared to the lenti-control. $*P < 0.05$, $***P < 0.001$.



Supplementary Figure S5

Supplementary Figure S5. The downregulated proteins in HCC. **A**, The EIF4A1 quantity in Huh-7 cells transfected with 5′-tiRNA-Gln or control RNA. **B**, The number of down- or upregulated proteins in Huh-7 cells transfected with 5′-tiRNA-Gln as compared to control RNA-transfected cells. **C**, Venn diagram shows the intersection of the hepatitis B, hepatitis C and pathway in cancer in the KEGG enrichment analysis.

A 5'-tiRNA-Gln

Gene Information	
Gene ID:	**Number of Products:** 1
Gene Symbol:	**Number of poly A Signals:**
Gene Size: 34 nt.	**QGRS found:** 1
	QGRS found (including overlaps): 19

000001 UAGGAUGGGG UGUGAUAGGU GGCACGGAGA AUUU

Position	Length	QGRS	G-Score
3	17	GGATGGGGTGTGATAGG	14
3	20	GGATGGGGTGTGATAGGTGG	11
3	20	GGATGGGGTGTGATAGGTGG	13
3	20	GGATGGGGTGTGATAGGTGG	14
3	20	GGATGGGGTGTGATAGGTGG	15
3	25	GGATGGGGTGTGATAGGTGGCACGG	6
3	25	GGATGGGGTGTGATAGGTGGCACGG	14
3	25	GGATGGGGTGTGATAGGTGGCACGG	11
3	25	GGATGGGGTGTGATAGGTGGCACGG	16
3	25	GGATGGGGTGTGATAGGTGGCACGG	13
3	25	**GGATGGGGTGTGATAGGTGGCACGG**	18
3	25	GGATGGGGTGTGATAGGTGGCACGG	14
3	25	GGATGGGGTGTGATAGGTGGCACGG	9
7	16	GGGGTGTGATAGGTGG	14
7	21	GGGGTGTGATAGGTGGCACGG	14
7	21	GGGGTGTGATAGGTGGCACGG	11
7	21	GGGGTGTGATAGGTGGCACGG	13
8	20	GGGTGTGATAGGTGGCACGG	14
9	19	GGTGTGATAGGTGGCACGG	15

B Mutant 5'-tiRNA-Gln

Gene Information	
Gene ID:	**Number of Products:** 1
Gene Symbol:	**Number of poly A Signals:**
Gene Size: 34 nt.	**QGRS found:** 0
	QGRS found (including overlaps): 0

000001 UAUGAUGUUG UGUGAUAGGU GGCACGGAGA AUUU

C 5'UTR of *ARAF*

Gene Information	
Gene ID:	**Number of Products:** 1
Gene Symbol:	**Number of poly A Signals:**
Gene Size: 106 nt.	**QGRS found:** 2
	QGRS found (including overlaps): 14

D 5'UTR of *MEK1*

Gene Information	
Gene ID:	**Number of Products:** 1
Gene Symbol:	**Number of poly A Signals:**
Gene Size: 436 nt.	**QGRS found:** 7
	QGRS found (including overlaps): 271

E 5'UTR of *MEK2*

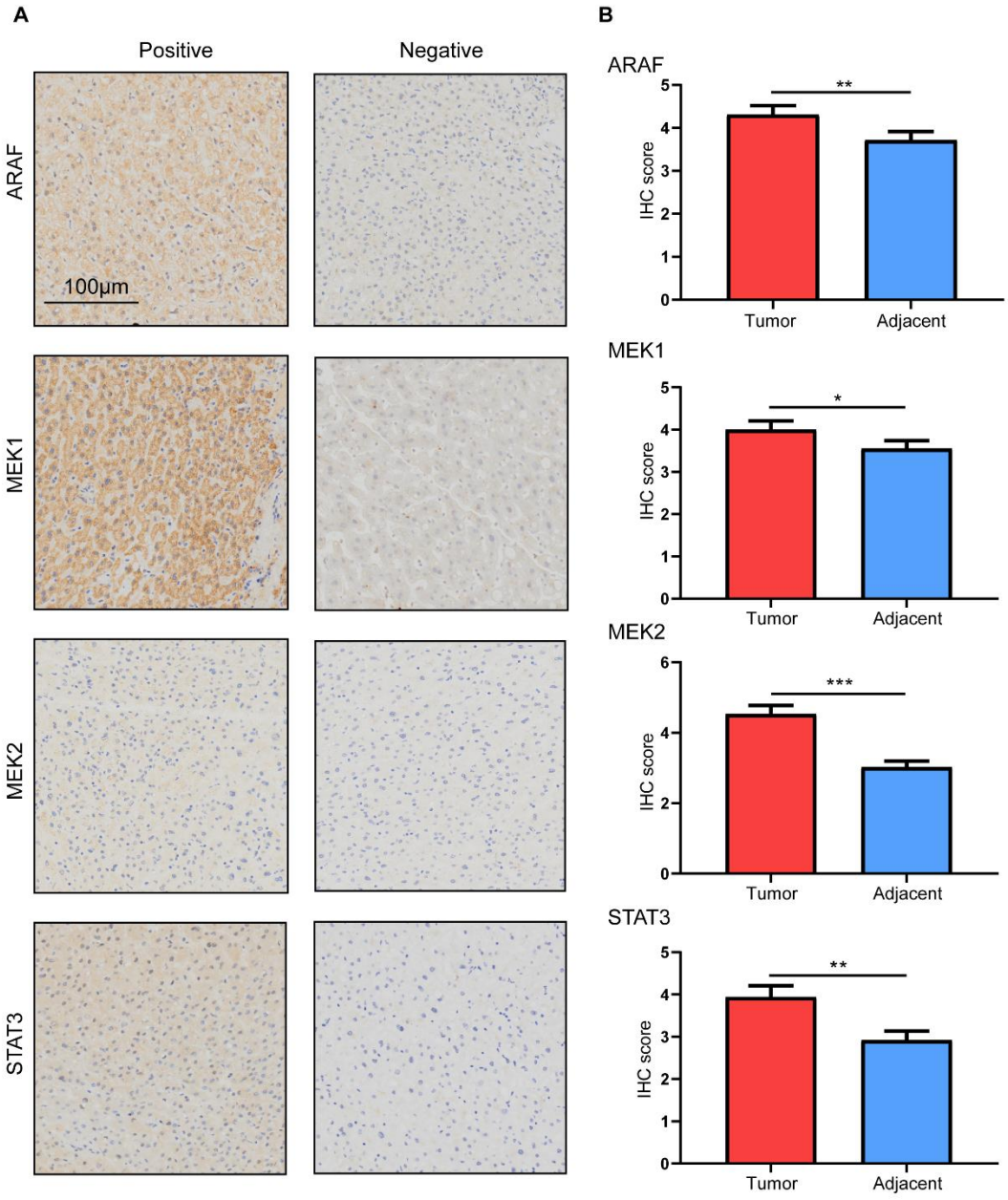
Gene Information	
Gene ID:	**Number of Products:** 1
Gene Symbol:	**Number of poly A Signals:**
Gene Size: 247 nt.	**QGRS found:** 3
	QGRS found (including overlaps): 11

F 5'UTR of *STAT3*

Gene Information	
Gene ID:	**Number of Products:** 1
Gene Symbol:	**Number of poly A Signals:**
Gene Size: 165 nt.	**QGRS found:** 3
	QGRS found (including overlaps): 16

Supplementary Figure S6

Supplementary Figure S6. The G-quadruplex structure predicted by QGRS Mapper. **A**, The potential G-quadruplex structure formed by 5′-tiRNA-Gln (including the position and sequence information of the 19 overlaps). **B**, Mutant 5′-tiRNA-Gln failed to form the G-quadruplex predicted by QGRS Mapper. **C**, **D**, **E**, **F** The number of potential G-quadruplexes formed from the *ARAF* (C) *MEK1*/*2* (D, E) and *STAT3* (F) 5′-UTRs.



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54 **Supplementary Figure S7**

Supplementary Figure S7. ARAF, MEK1/2 and STAT3 expression levels in the adjacent nontumorous tissues. **A**, Representative IHC staining images of adjacent nontumorous tissues in a microarray that included 51 paired HCC and adjacent nontumorous tissue samples (scale bar: 100 μm). **B**, Paired t-test analysis of the IHC score between the paired tumor and adjacent nontumorous tissues. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.