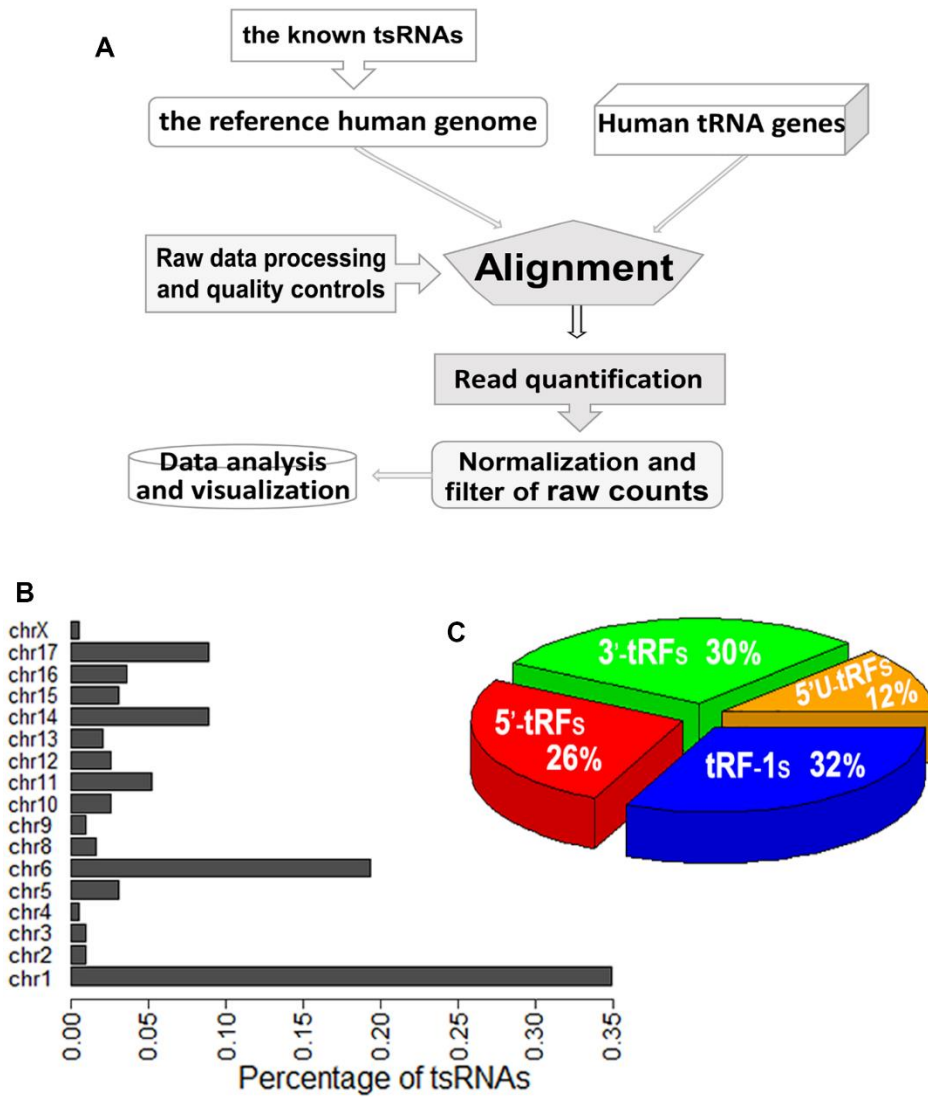
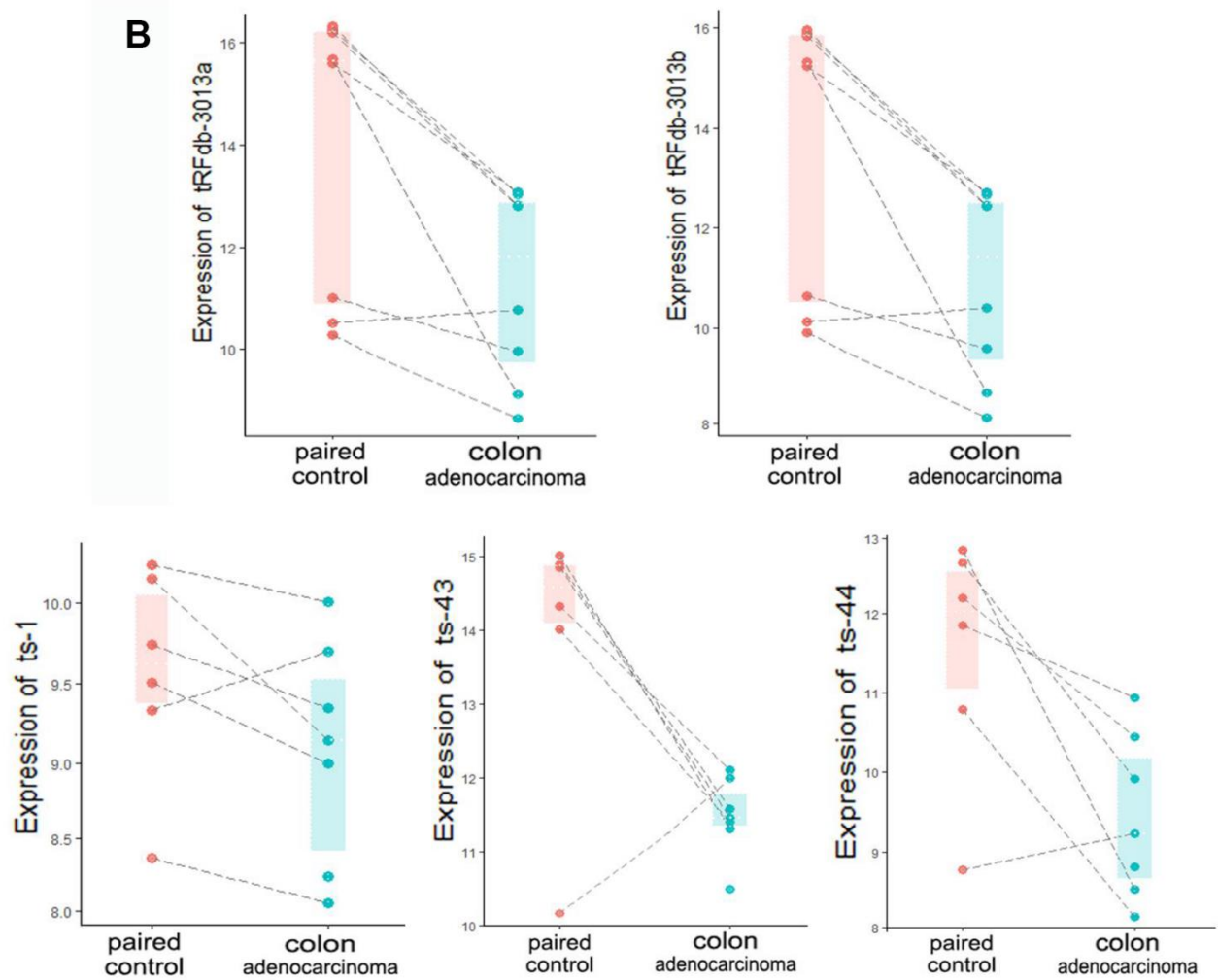
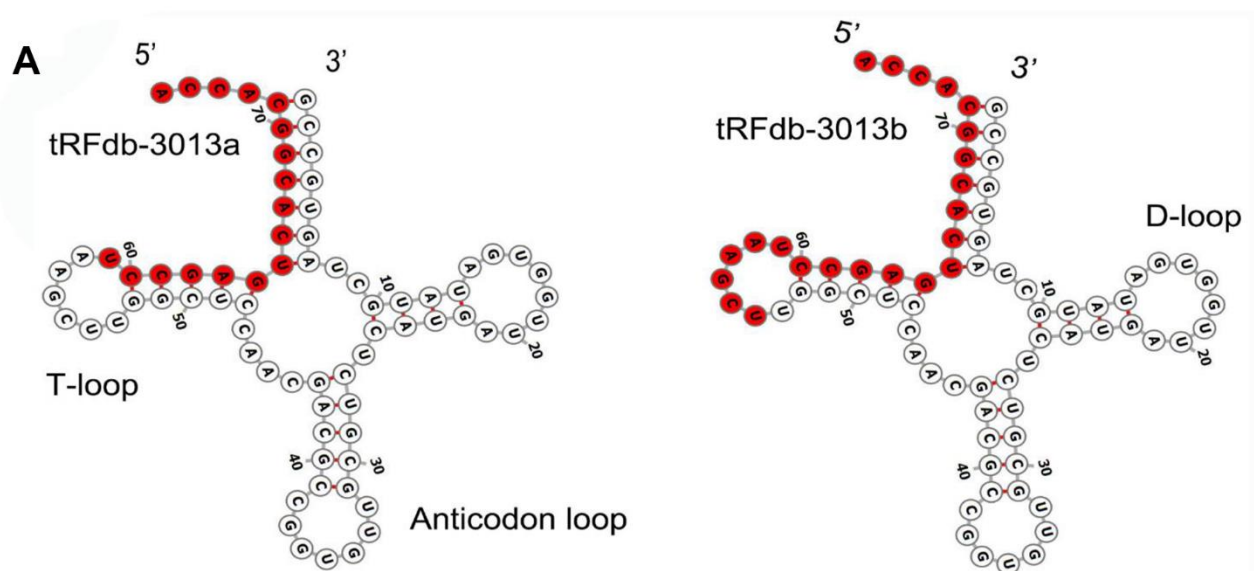


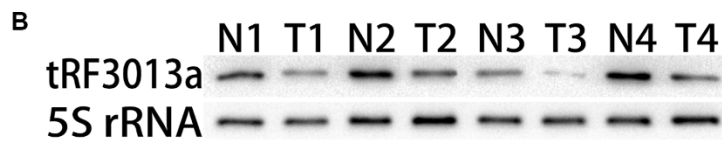
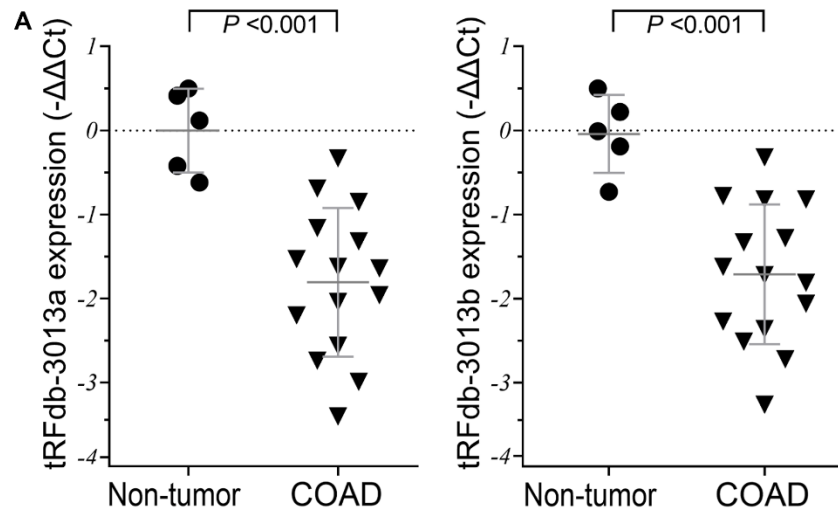
SUPPLEMENTARY FIGURES



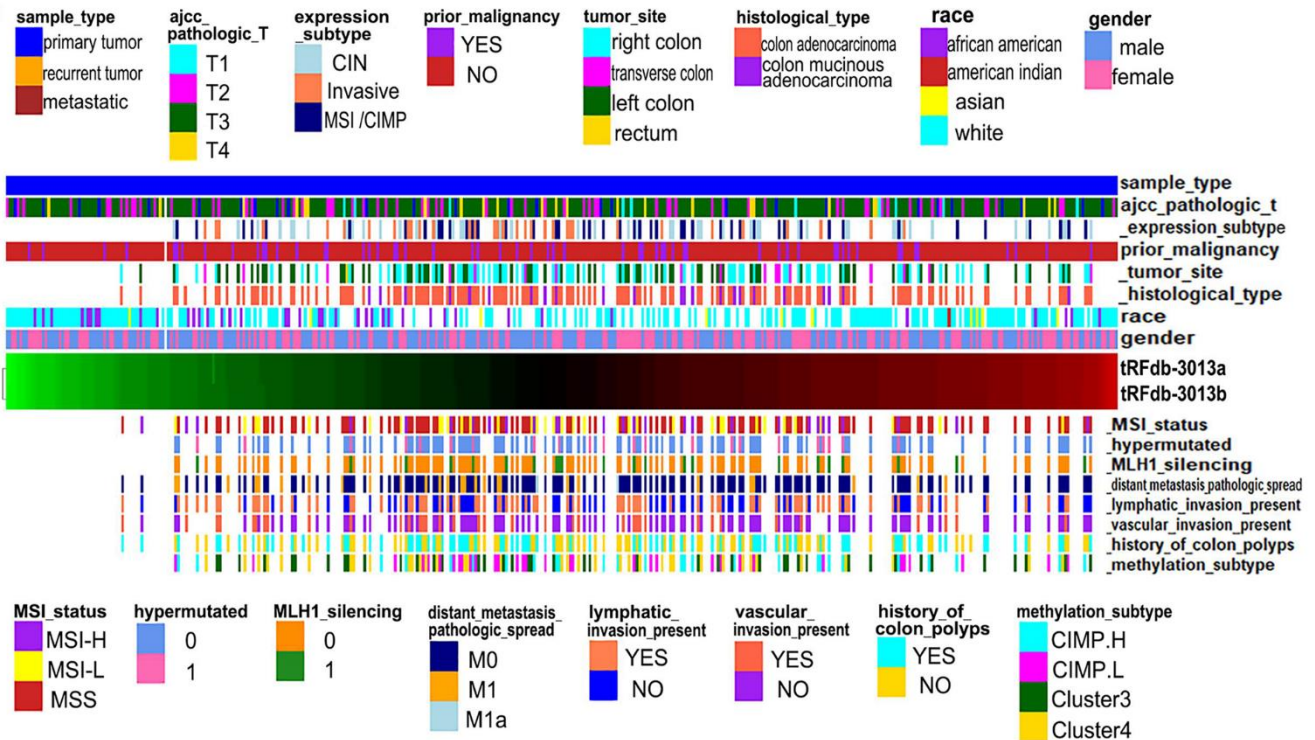
Supplementary Figure 1. A summary of tsRNAs identification and characterization. (A) The flow chart of data processing and tsRNAs identification pipeline. **(C)** The characterization of tsRNA corresponding derived tRNA gene sources and **(B)** the chromosome locations statistical analysis.



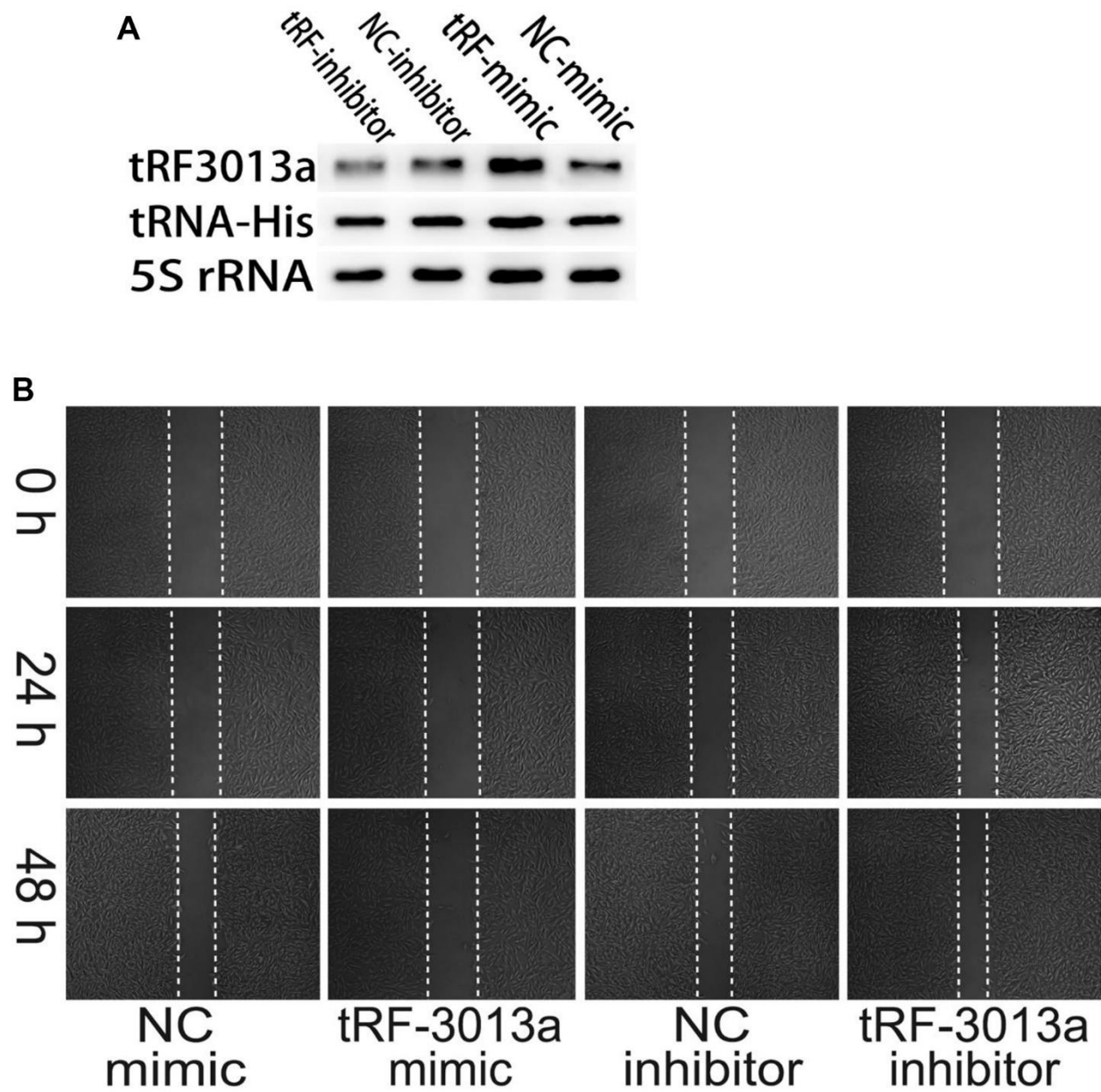
Supplementary Figure 2. (A) Representative secondary structure of tRNA-His-GUG and its derived fragments (tRFdb-3013a and tRFdb-3013b). (B) The expression patterns of several tRNA-His-GTG derived tsRNA fragments (including tRFdb-3013a, tRFdb-3013b, ts-1, ts-43 and ts-44) in paired colon adenocarcinomas and non-tumor controls of TCGA-COAD datasets.



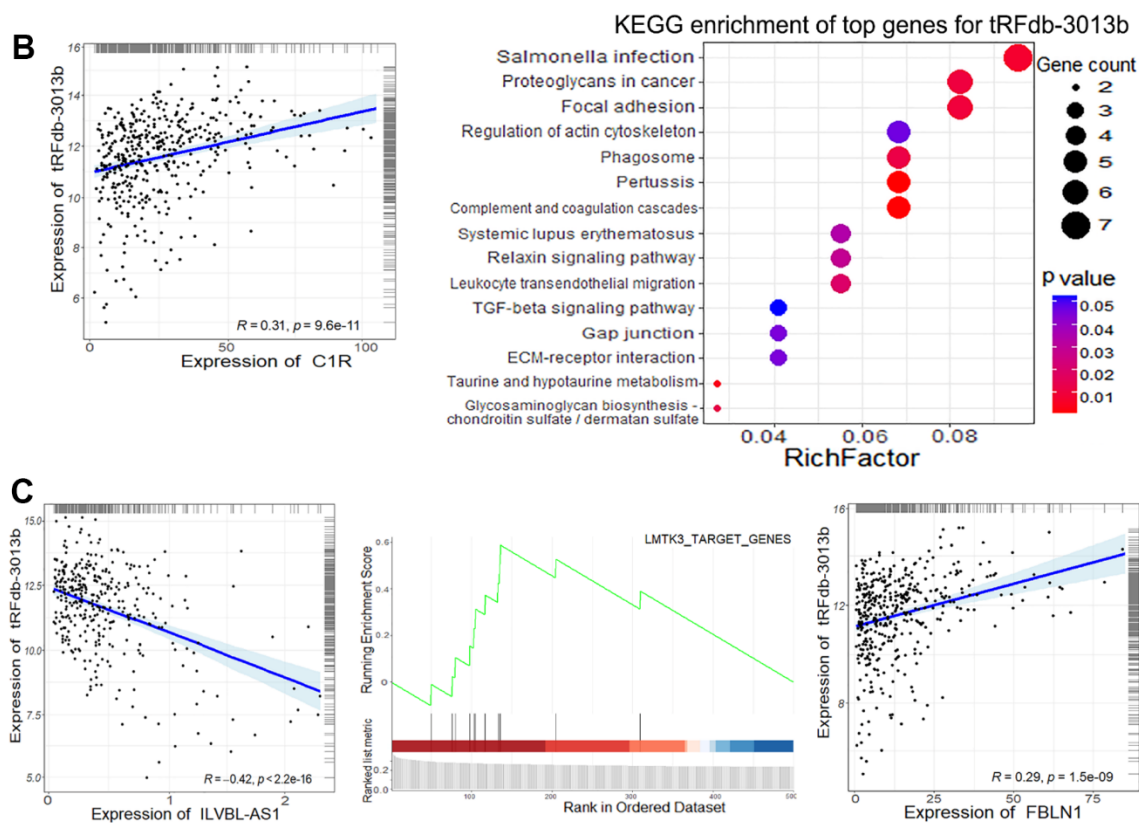
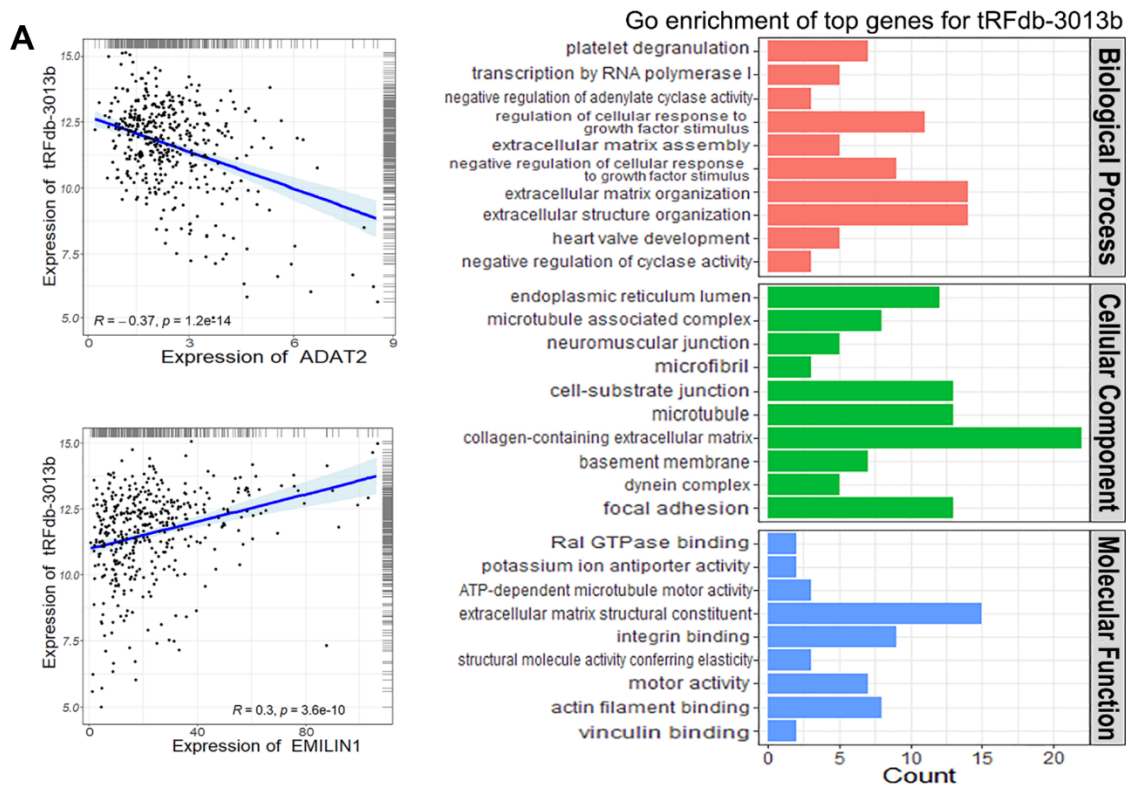
Supplementary Figure 3. (A) The boxplot of tRFdb-3013a/b expression between non-tumor controls and colon adenocarcinomas with the stem-loop tsRNA qRT-PCR analysis. (B) The Northern blotting analysis of tRFdb-3013a (tRF3013a) between non-tumor controls (N1-4) and colon adenocarcinoma (T1-4) samples (all $P < 0.01$).



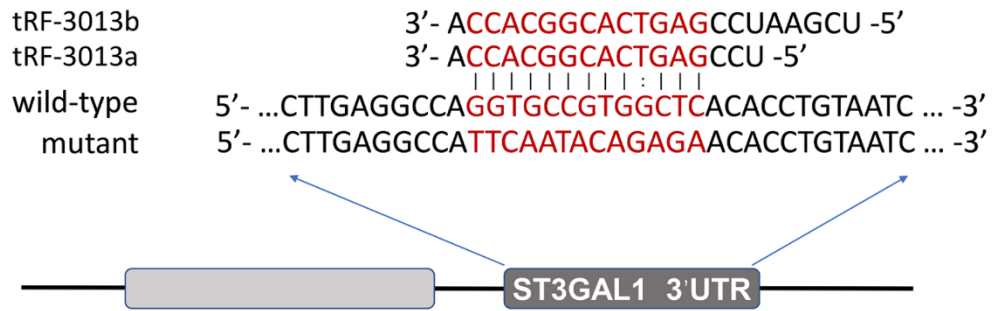
Supplementary Figure 4. The correlation analysis of tRFdb-3013a/b expression with the primary pathology characteristics. Hierarchical clustering heatmap of tRFdb-3013a/b expression and the pathology characteristics parameters.



Supplementary Figure 5. (A) The Northern blotting analysis of tRFdb-3013a (tRF3013a, both $P < 0.05$) and tRNA-His-GUG (tRNA-His) in the cells transfected with tRFdb-3013a mimic (tRF-mimic) or inhibitor (tRF-inhibitor) or negative controls (NC-mimic, NC-inhibitor). (B) Relative gap analysis scratch assay was used to assess the migration ability of SW480 cell.



Supplementary Figure 6. The enrichment analyses of tRFdb-3013b correlated-genes within TCGA-COAD datasets. (A) The top gene ontology (GO) terms, including biological process, cellular component and molecular function. **(B)** The top KEGG pathway for tRFdb-3013b correlated-genes. The correlation scatter-plots of tRFdb-3013b and its correlated-genes (ADAT2, EMILIN1, and C1R). **(C)** GSEA (gene set enrichment analysis) plots of the molecular signatures, the scatter plots for tRFdb-3013b and its correlated-genes (ILVBL-AS1, FBLN1).



Supplementary Figure 7. Schematic diagram of the interaction position between tRFdb-3013a/b and the seed regions within the 3'UTR wide-type region and mutation region of ST3GAL1 gene in the luciferase reporter assay.

A	tDRnamer ID	Fragment sequence	length	MINTbase ID	reads	RPM
	tDR-61:76-His-GTG-1-M2	CCGAGTCACGGCACCA	16	tRF-16-308HP1B	541	32.08
	tDR-60:76-His-GTG-1-M2	TCCGAGTCACGGCACCA	17	tRF-17-8US5652	947	72.07
	tDR-59:76-His-GTG-1-M2	ATCCGAGTCACGGCACCA	18	tRF-18-HS16MFD2	416	18.58
	tDR-58:76-His-GTG-1-M2	AATCCGAGTCACGGCACCA	19	tRF-19-DR5MEJE2	49	6.998
	tDR-57:76-His-GTG-1-M2	GAATCCGAGTCACGGCACCA	20	tRF-20-OML32826	61	17.99
	tDR-56:76-His-GTG-1-M2	CGAATCCGAGTCACGGCACCA	21	tRF-21-LE308HP1B	321	42.75
	tDR-60:75-His-GTG-1-M2	TCCGAGTCACGGCACC -	16	tRF-16-8US565D	162	27.8
	tDR-59:75-His-GTG-1-M2	ATCCGAGTCACGGCACC -	17	tRF-17-HS16MFJ	7	3.955
	tDR-58:75-His-GTG-1-M2	AATCCGAGTCACGGCACC -	18			
	tDR-57:75-His-GTG-1-M2	GAATCCGAGTCACGGCACC -	19	tRF-19-OML328FV	1	2.008
	tDR-56:75-His-GTG-1-M2	CGAATCCGAGTCACGGCACC -	20	tRF-20-LE308HP1	17	6.55
	tDR-60:74-His-GTG-1-M2	TCCGAGTCACGGCAC --	15			
	tDR-59:74-His-GTG-1-M2	ATCCGAGTCACGGCAC --	16	tRF-16-HS16MFD	5	4.889
	tDR-58:74-His-GTG-1-M2	AATCCGAGTCACGGCAC --	17			
	tDR-57:74-His-GTG-1-M2	GAATCCGAGTCACGGCAC --	18	tRF-18-OML328D1	1	1.176
	tDR-56:74-His-GTG-1-M2	CGAATCCGAGTCACGGCAC --	19	tRF-19-LE308HI1	3	5.345
	tDR-59:73-His-GTG-1-M2	ATCCGAGTCACGGCA ---	15			
	tDR-58:73-His-GTG-1-M2	AATCCGAGTCACGGCA ---	16			
	tDR-57:73-His-GTG-1-M2	GAATCCGAGTCACGGCA ---	17	tRF-17-OML3282	1	1.225
	tDR-56:73-His-GTG-1-M2	CGAATCCGAGTCACGGCA ---	18	tRF-18-LE308H DU	5	2.312
B	tDRnamer ID	Fragment sequence	length	MINTbase ID	reads	RPM
	tDR-55:76-His-GTG-1-M2	TCGAATCCGAGTCACGGCACCA	22	tRF-22-WB8US5652	6503	62.09
	tDR-54:76-His-GTG-1-M2	TTCGAATCCGAGTCACGGCACCA	23	tRF-23-YUHS16MFD2	100	35.37
	tDR-53:76-His-GTG-1	GTTTCGAATCCGAGTCACGGCACCA	24	tRF-24-7SDR5MEJE2	86	14.38
	tDR-52:76-His-GTG-1	GGTTCGAATCCGAGTCACGGCACCA	25	tRF-25-R9OML32826	29	5.159
	tDR-51:76-His-GTG-1	CGGTTTCGAATCCGAGTCACGGCACCA	26	tRF-26-MNLE308HP1B	12	3.181
	tDR-50:76-His-GTG-1	TCGGTTTCGAATCCGAGTCACGGCACCA	27	tRF-28-48YUHS16MFD2	8	1.718
	tDR-55:75-His-GTG-1-M2	TTCGAATCCGAGTCACGGCACC -	21	tRF-21-WB8US565D	1541	16.82
	tDR-54:75-His-GTG-1-M2	TTCGAATCCGAGTCACGGCACC -	22	tRF-22-YUHS16MFJ	15	2.22
	tDR-53:75-His-GTG-1	GTTTCGAATCCGAGTCACGGCACC -	23	tRF-23-7SDR5MEJV	4	1.443
	tDR-52:75-His-GTG-1	GGTTCGAATCCGAGTCACGGCACC -	24			
	tDR-51:75-His-GTG-1	CGGTTTCGAATCCGAGTCACGGCACC -	25			
	tDR-50:75-His-GTG-1	TCGGTTTCGAATCCGAGTCACGGCACC -	26			
	tDR-55:74-His-GTG-1-M2	TTCGAATCCGAGTCACGGCAC --	20	tRF-20-WB8US565	113	3.881
	tDR-54:74-His-GTG-1-M2	TTCGAATCCGAGTCACGGCAC --	21	tRF-21-YUHS16MFD	18	5.239
	tDR-53:74-His-GTG-1	GTTTCGAATCCGAGTCACGGCAC --	22	tRF-22-7SDR5MEJ1	5	2.81
	tDR-52:74-His-GTG-1	GGTTCGAATCCGAGTCACGGCAC --	23			
	tDR-51:74-His-GTG-1	CGGTTTCGAATCCGAGTCACGGCAC --	24	tRF-24-MNLE308HI1	1	1.298
	tDR-50:74-His-GTG-1	TCGGTTTCGAATCCGAGTCACGGCAC --	25	tRF-26-48YUHS16MFD	5	18.6
	tDR-55:73-His-GTG-1-M2	TTCGAATCCGAGTCACGGCA ---	19	tRF-19-WB8US5IU	44	5.828
	tDR-54:73-His-GTG-1-M2	TTCGAATCCGAGTCACGGCA ---	20	tRF-20-YUHS16MF	25	12.33
	tDR-53:73-His-GTG-1	GTTTCGAATCCGAGTCACGGCA ---	21	tRF-21-7SDR5MEJB	14	10.81
	tDR-52:73-His-GTG-1	GGTTCGAATCCGAGTCACGGCA ---	22	tRF-22-R9OML3282	2	1.378
	tDR-51:73-His-GTG-1	CGGTTTCGAATCCGAGTCACGGCA ---	23	tRF-23-MNLE308H DU	1	1.608
	tDR-50:73-His-GTG-1	TCGGTTTCGAATCCGAGTCACGGCA ---	24	tRF-25-48YUHS16MF	2	1.075

Supplementary Figure 8. Representative images of the tRFdb-3013a (A) or tRFdb-3013b (B) sequence alignments of some fragments derived tRNA-His-GTG from tRF fragments within tDRnamer and MINTbase v2.0.