

OMTN, Volume 27

Supplemental information

**A tRNA-derived fragment from
Chinese yew suppresses ovarian
cancer growth via targeting *TRPA1***

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LC-MS/MS analysis of tRF-T11 in four kinds of cancer cells

To investigate whether tRF-T11 is present in cancer cells, we extracted and enriched small RNAs (Figure S6A, B) from A2780, SK-OV-3, HepG2, and MCF-7 cells (5×10^6 cells for each) in good quality. The results of LC-MS analysis showed that the $[M-3H]^{3-}$ ion at m/z 2364.0124 of tRF-T11 can be sensitively detected in a range from 0.5 to 1000 ng of tRF-T11, while 0.1 ng of tRF-T11 is undetectable (Figure S6C, D). However, we were not able to detect the $[M-3H]^{3-}$ ion in the LC-MS chromatograms of the extracted small RNA of the four cancer cells (Figure S6E, F). Thus, the above results demonstrated that the absolute content of tRF-T11 in cancer cells is lower than 1×10^{-7} ng/cell. The current data serve as evidence to clarify that tRF-T11 is not present in four kinds of cancer cells.

Table S1. Identified tRNA sequences in Chinese yew by NGS sequencing.

Table S2. IC₅₀ values of tRNA^{Trp(CCA)}, siRNA, negative sequence and taxol on A2780 cells.

Sample	tRNA ^{Trp(CCA)}	siRNA	Negative sequence	Taxol
IC ₅₀ (nM)	14.3	0.25	>200	7.4

Table S3. Synthetic tRF mimics derived from top 9 abundant tRNAs in Chinese yew.

Table S4. Identified *Taxus* tRF that possibly target biosynthetic genes of taxol.

Biosynthesis gene	Genebank number	Identified tRF
Taxadiene synthase	HM113487.1	tRF-T16, tRF-T33
taxane 5-alpha hydroxylase	AY289209.2	NI ^a
taxadien-5-alpha-ol-O-acetyltransferase	AF190130.1	NI ^a
taxane 13-alpha-hydroxylase	NM_001154694.2	tRF-T11, tRF-T16, tRF-T24, tRF-T29, tRF-T32, tRF-T33, tRF-T35, tRF-T39, tRF-T45, tRF-T47, tRF-T48
taxane 10 beta-hydroxylase	AY453403.1	tRF-T29
taxane 14b-hydroxylase	MH497022.1	tRF-T27, tRF-T32, tRF-T36
taxane 2-alpha-hydroxylase	AY518383.2	NI ^a
taxane 7β-hydroxylase	AY307951.1	tRF-T27, tRF-T32, tRF-T36
taxane 2α-O-benzoyl transferase	AF297618.1	tRF-T33
10-deacetyl-baccatin Oacetyltransferase	III-10- AF193765.1	tRF-T16, tRF-T24, tRF-T32, tRF-T33
phenylalanine aminomutase	AY582743.1	NI ^a
13-O-(3-amino-3-phenylpropanoyl) transferase	AY082804.1	NI ^a
3'-N-debenzoyltaxol benzoyltransferase	N- AF466397.1	tRF-T36
Geranylgeranyl diphosphate synthase	AF081514.1	tRF-T16, tRF-T29, tRF-T45

NI^a: not identified.

Table S5. DNA probes for purification of individual tRNAs.

tRNA	Probe (5'-3')
tRNA ^{His(GUG)}	GGCGAACGACGGGGATTGAACCCGCGCGTG
tRNA ^{Glu(UUC)}	TTGCCTCCTTGAAAGAGAGATGTCCTGGGC
tRNA ^{Trp(CCA)}	ACGGCATCAGGTTTTGGAGACCTGCGTTCT
tRNA ^{Leu(CAA)}	ACGCTGTTTAGCACGAGATTTTGAGTCTCG
tRNA ^{Arg(ACG)}	CGTGGTTCGCAACCACGTGCTCTAATCCTC

Table S6. Primers for quantitative real-time PCR.

Gene name	Forward (5'-3')	Reverse (5'-3')
TSPAN17	GAAGGGCGTTCTCTCGAACA	AAAGGCCAGGATCCCTGTTG
TRPA1	TGCATGTTGCATTCCACAGAAG	TTGAGGGCTGTAAGCGGTTTCATA
SCG5	CTCACCAGGCCATGAATCTT	TGTTGTCTCCAGTCAACTCTGC
tRF-T11	GAAGCGGACGTAGCCAAGT	GTGCGTGTCGTGGAGTCG
U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT
<i>β</i> -actin	GGGAAATCGTGCGTGACATTAAGG	CAGGAAGGAAGGCTGGAAGAGTG

Table S7. Oligonucleotides used for western blot assay.

Oligonucleotides	Forward (5'-3')	Reverse (5'-3')
TRPA1_siRNA01	GGUGGGAUGUUAUCCAATT	UAUGGAAUAACAUCCACCTT
TRPA1_siRNA02	GAAGGACGCUCUCCACUUATT	UAAGUGGAGAGCGUCCUUCTT
Negative sequence	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

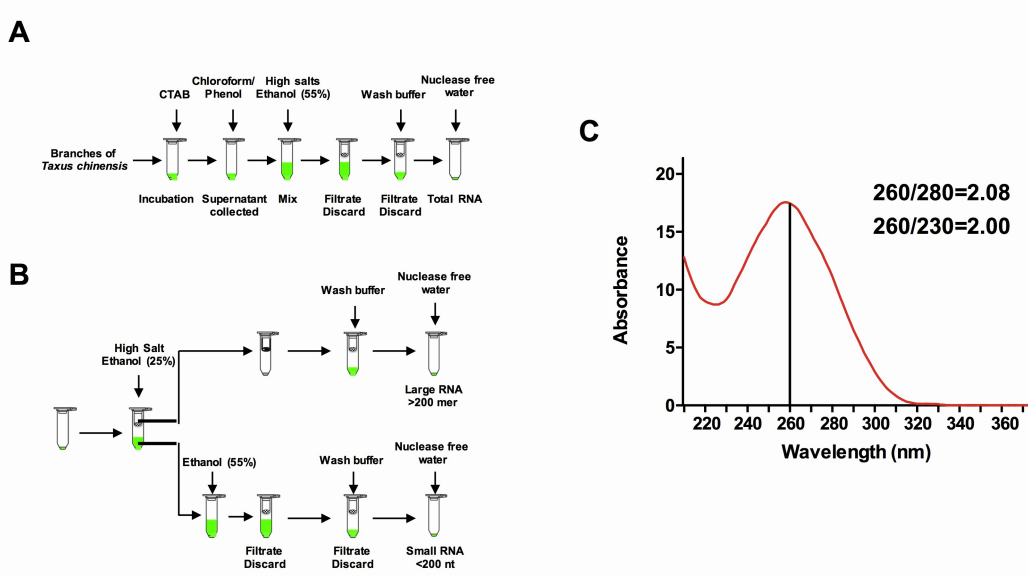


Figure S1. Extraction and separation of small RNA from *Taxus chinensis* (Pilger) Rehd. var. mairei. (A) Workflow of total RNA extraction using a developed CTAB-based method. (B) NanoDrop analysis indicated that the extracted total RNA is in high purity. (C) Separation and enrichment of small RNA from large RNA.

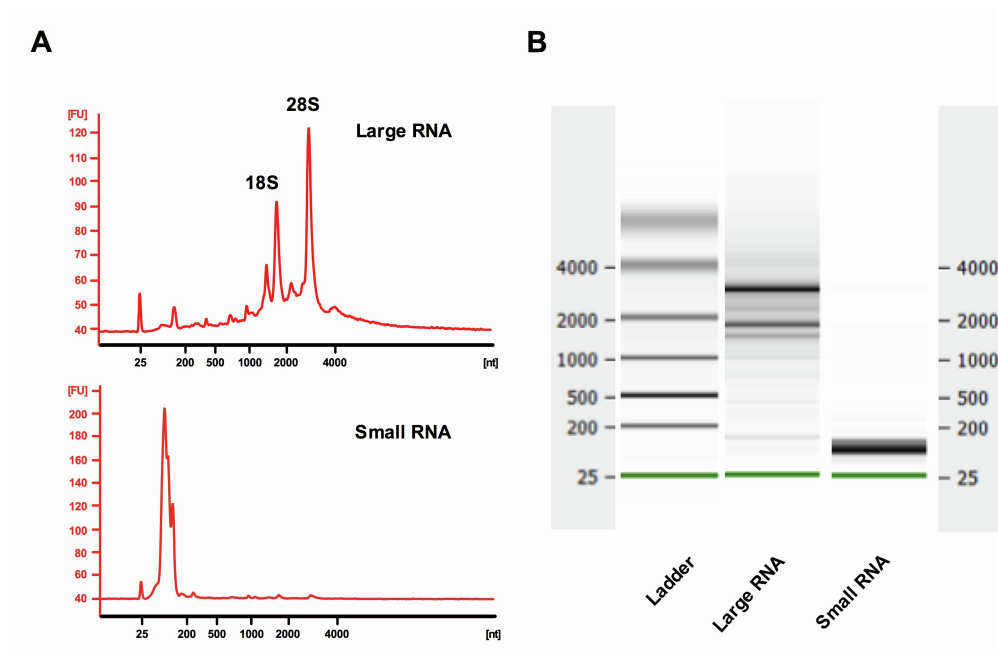


Figure S2. Quality evaluation confirmed that small RNA was well separated from large RNA. (A) Agilent 2100 Bioanalyzer profile of small RNA and large RNA from *Taxus chinensis (Pilger) Rehd. var. mairei*. **(B)** Monitored electrophoresis gram of small RNA and large RNA.

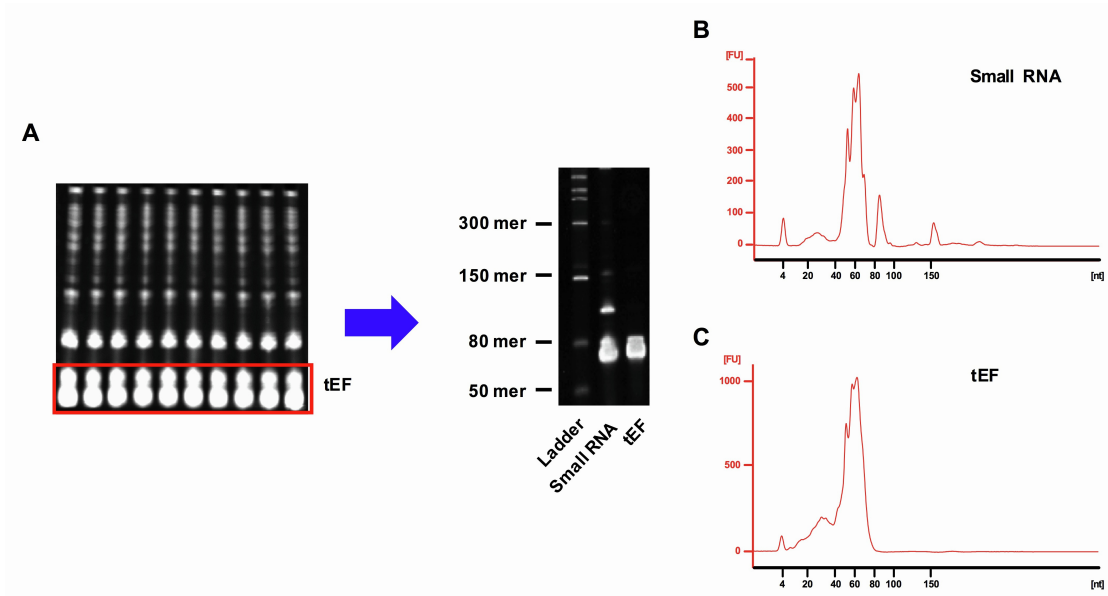


Figure S3. Preparation of tEF from small RNA of Chinese yew. (A) Small RNA was gel-fractionated and the representative urea-PAGE image of prepared tEF. **(B)** Agilent 2100 Bioanalyzer showed that compared to small RNA, 5S, 5.8S rRNA and other RNA species could not be detected in the prepared **(C)** tEF.

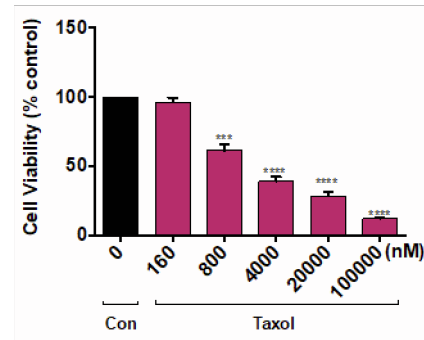
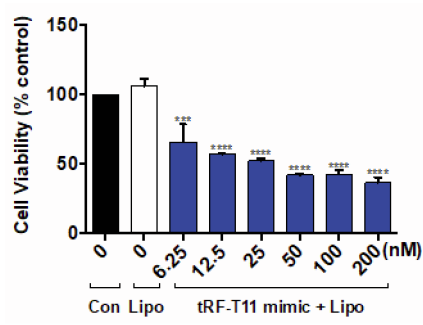


Figure S4. tRF-T11 mimic exhibited significant inhibitory effects on taxol-resistant A2780 strain. Data are presented as the means \pm SDs of three independent experiments. *** $P < 0.001$, **** $P < 0.0001$.



Figure S5. Morphological images of major organs from A2780 xenograft-bearing nude mice.

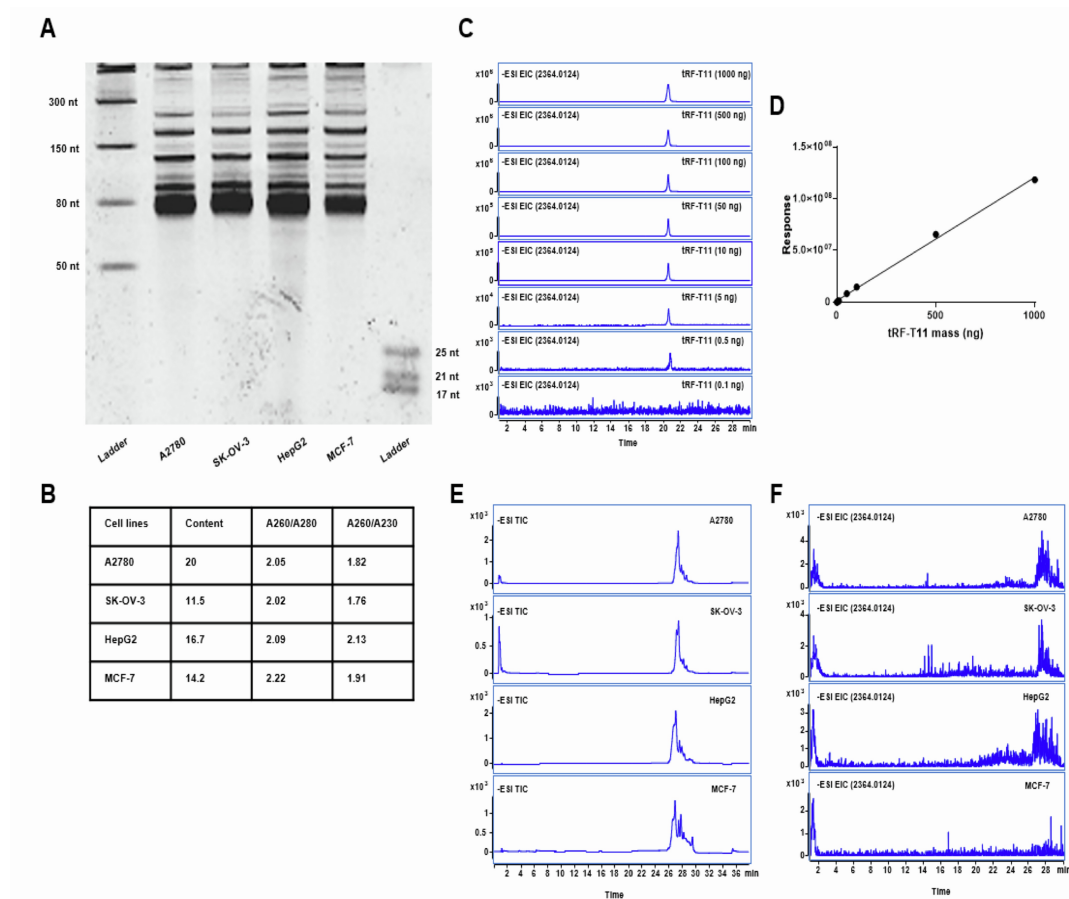


Figure S6. tRF-T11 is not present in four kinds of cancer cells. (A) Urea-PAGE analysis of small RNA extracted from A2780, SK-OV-3, HepG2, and MCF-7. (B) Content and quality of extracted small RNA from 5×10^6 cells. (C) Extracted ion chromatography of $[M-3H]^{3-}$ ion at m/z 2364.0124 of tRF-T11 in a range from 0.5 to 1000 ng. (D) Calibration curves of tRF-T11 in a range from 0.5 to 1000 ng. (E) Total ion chromatography of small RNA from cancer cells. (F) $[M-3H]^{3-}$ ion at m/z 2364.0124 of tRF-T11 were undetected in the extracted ion chromatography of small RNA from four kinds of cancer cells.