1 **Cross-kingdom regulation of tRNAs/tRFs derived from Chinese yew**

2 Kai-Yue Cao^{1,2†}, Tong-Meng Yan^{1,2†}, Ji-Zhou Zhang³, Ting-Fung Chan³, Jie Li⁴,

3 Chong Li⁴, Elaine Lai-Han Leung^{1,2}, Zhi-Hong Jiang^{1,2*}

4

5 ¹State Key Laboratory of Quality Research in Chinese Medicine, Macau University of

6 Science and Technology, Macau SAR, China.

7 ²Macau Institute for Applied Research in Medicine and Health, Macau University of

8 Science and Technology, Macau SAR, China.

9 ³School of Life Sciences, and State Key Laboratory of Agrobiotechnology, The

10 Chinese University of Hong Kong, Shatin, Hong Kong SAR, China.

11 ⁴College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, P. R.

12 China.

13 ***Corresponding author:** Dr. Zhi-Hong Jiang, State Key Laboratory of Quality

14 Research in Chinese Medicine, Macau University of Science and Technology,

15 Avenida Wai Long, Taipa, Macau SAR, China.

16 **E-mail:** zhjiang@must.edu.mo (Zhi-Hong Jiang)

17 [†]These authors contributed equally to this work.

18

19 **Plants containing countless chemical constituent have benefited mankind since**
20 **the origin of life. Although secondary metabolites in plants, such as morphine,**
21 **artemisinin and taxol, have been developed as therapeutic drugs for clinical**
22 **therapy, few study focuses on the pharmacological activities of plant small RNAs**
23 **with function of cross-kingdom regulations. Yew is widely considered as a**
24 **“superstar” plant due to the discovery of paclitaxel, or taxol, which is a**
25 **well-known natural drug for the treatment of multiple types of cancer¹. Here we**
26 **show the surprising finding that an RNA fragment, named tRF-T11, derived**
27 **from tRNA^{His(GUG)} of Chinese yew strongly suppressed human ovarian cancer**
28 **progression. In A2780 cells, tRF-T11 mimic (a double-stranded RNA with**
29 **tRF-T11 as antisense chain) exhibited potent cytotoxicity comparable to that of**
30 **taxol, but no significant cytotoxicity to normal ovarian surface epithelial cells.**
31 **Moreover, the cytotoxicity of tRF-T11 mimic is 80-fold stronger than that of**
32 **taxol in taxol-resistant A2780 cells. Bioinformatic and molecular biological**
33 **studies revealed that tRF-T11 targets transient receptor potential cation channel**
34 **subfamily A member 1 (*TRPA1*) to inhibit its expression levels. In a further *in***

35 ***in vivo* investigation, the growth rate of ovarian tumor xenografts in nude mice was**
 36 **significantly reduced by treatment with tRF-T11 mimic, and the *TRPA1* protein**
 37 **expression in tumors treated with tRF-T11 mimic was also down-regulated. Our**
 38 **findings are the first to provide evidence that plant-derived tRFs can regulate**
 39 **the expression of target genes *in vitro* and *in vivo*, indicating that they may**
 40 **become as a new source of druggable siRNA. Moreover, this discovery**
 41 **demonstrated a pilot example of an innovative approach for not only identifying**
 42 **pharmacologically-active tRFs from plants, but also for improving the efficiency**
 43 **and possibilities of discovering new drug target.**

44 Plants have provided mankind with an important source of medicinal products
 45 for millennia. Throughout ancient history, humans learned to harness herbal
 46 decoctions for disease prevention and treatment. Today, thousands of secondary
 47 metabolites, such as flavonoids, terpenes and alkaloids, from this chemical treasure
 48 have been identified as active compounds. Intriguingly, crude plant extracts were
 49 more effective than the synthetic versions of phytochemicals, implying that whole
 50 plant extracts contain a cocktail of products in which substances like macromolecules

51 other than small molecules may contribute to the overall pharmacological effects².

52 However, few study has been performed on the pharmacological activity of plant

53 macromolecules excluding polysaccharides because they are considered inactive,

54 nonabsorbable and unstable in human body, such as RNA, DNA and proteins. Small

55 RNAs (<200 nt) were found to play critical roles in a wide range of biological

56 processes in microorganisms, plants, animals and human, and to participate in a

57 variety of human diseases by regulating protein-coding genes^{3,4}. Since the first

58 evidence that plant-derived miRNAs can regulate gene expression in mammalian

59 systems was provided by Zhang *et al*⁵ in 2012, many other miRNAs from small RNAs

60 in plants have been extensively studied for their cross-kingdom regulatories⁶⁻⁹. These

61 findings suggested the promise of small RNAs from plants to be developed as

62 therapeutic agents. A recent report by Shekhawat¹⁰ showed that the small RNA of

63 corn exhibited appreciable antiproliferative activity toward HeLa cells, implying the

64 possibility that small RNA species with larger molecular weight than miRNA play

65 regulatory roles. In fact, as the most abundant small RNA species expressed in all

66 living organisms, transfer RNAs (tRNAs) play a well-defined role in protein

67 translation and perform diverse regulatory functions in some important biological
68 processes, such as cell signaling, apoptosis modulation and stress response programs¹¹.
69 Recently, tRNA-derived fragments (tRFs) have also been recognized to play
70 regulatory roles¹². This class of small RNAs is non-randomly generated from tRNAs,
71 and many are specifically and abundantly produced under particular conditions, such
72 as cancer and viral infection^{13,14}. Some studies have also revealed that the expression
73 of several tRFs is positively correlated with cell proliferation in different cancer cell
74 lines¹⁵, suggesting that tRFs and their related signaling pathways could be novel
75 targets for intervention in the treatment of cancers. These findings prompted us to
76 consider the further possibility that in addition to well-established players such as
77 miRNAs and small molecules in plants, tRNAs and their fragments, which account
78 for overwhelming majority of small RNA species in plants, may hold great potential
79 as biological regulators in mammals. Below, we describe the first study of the
80 pharmacological activities of tRNAs and tRFs derived from Chinese yew (*Taxus*
81 *chinensis*) and identification of their molecular target.

82 Total RNA with high integrity (RIN=7.7) and purity (A260/280=2.08,
83 A260/230=2.00; [Supplementary Fig 1a](#)) was isolated from fresh branches of *T.*
84 *chinensis* by using a well-developed CTAB method ([Supplementary Fig 1b, c](#)), in
85 which small RNA was separated from large RNA with high purity (<200 nt,
86 [Supplementary Fig 2](#)). The tRNAs were then gel fractionated and purified
87 ([Supplementary Fig 3a](#)) to successfully delete 5S and 5.8S rRNA and other RNA
88 species ([Supplementary Fig 3b](#)). Sequence information and the proportion of each
89 type of RNA in this tRNA-enriched fraction (tEF) were determined by performing
90 next-generation sequencing (NGS). As shown in [Fig 1c](#) and [Supplementary Table 1](#),
91 over 1.7 million clean reads distributed mainly from 60 to 80 mer were generated, and
92 over 20 individual tRNA sequences were identified, which accounted for
93 approximately 14% of the overall number of reads in the tEF library ([Fig 1d](#)). The
94 cytotoxicity of the tEF was investigated using an MTT assay on the A2780 human
95 ovarian cancer (OVCA) cell line. The IC₅₀ value for dose-dependent growth
96 inhibition of A2780 cells *in vitro* was 128.8 nM when the tEF was well encapsulated
97 and delivered via liposomes, while no significant cytotoxicity of the tEF was observed

without transfection (Fig 1a, b), implying that tRNAs or their fragments in the tEF might act as effectors. Furthermore, when rRNA degradation fragments (Fig 1e) were removed from the tEF samples via a Ribo-Zero rRNA removal kit (Illumina), the retained tRNA species induced the death of A2780 cells with increased efficiency (Fig 1f), indicating the major contribution of tRNA species to the overall cytotoxic effects of the tEF. Subsequently, the five most abundant (>500 reads per million) individual tRNAs were purified from small RNA of *T. chinensis* by using a magnetic solid-phase DNA probe method¹⁶ and were identified by ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (Fig 1g-i). Cytotoxicity of purified tRNAs was then investigated on A2780 cells, HepG2 of liver cancer and MCF-7 of breast cancer cells. As a result, A2780 and HepG2 cells exhibited a proliferation reduction of over 75% when treated with individual tRNAs at 25 nM (Fig 1j, k), while MCF-7 cells were much less sensitive (reduction of less than 30%) to these tRNA molecules. This pattern corresponded to an approximate IC₅₀ value of 14.3 nM for the growth inhibition of A2780 cells caused by treatment with tRNA^{Trp(CCA)} for 48 h, which is close to that of taxol (7.4 nM) used

114 as a positive control drug (Fig 11). This provides the first evidence of pharmacological
115 activities of plant-derived tRNAs, suggesting that they are a new class of therapeutic
116 agents from medicinal plants.

117 Meanwhile, since the previous study has revealed that the tRFs could regulate
118 endogenous gene in breast cancer cells¹⁵, we hypothesized that tRF from the above
119 cytotoxic tRNAs of Chinese yew may be pharmacologically-active. Indeed, tRFs
120 from the 5' terminal and 3' ends (5'-tRF and 3'-tRF, respectively) are the most
121 abundant tRFs associated with the AGO protein, which is a key component of the
122 RNA interference pathway¹⁷. A similar report showed that a 5'-tRF of 19 nucleotides
123 is the most abundant tRF in land plants¹⁸. Thus, 36 tRFs derived from the top 9
124 abundant tRNAs of Chinese yew, including 5'-tRF and 3'-tRF with lengths of 22 and
125 19 nt, respectively, were selected as antisense strands of tRF mimics in siRNA form
126 (Fig 2a, Supplementary Table 2), and the antiproliferative activities of these tRF
127 mimics against A2780 and its taxol-resistant strain (A2780T) cells were screened.
128 The results showed that two of these 36 tRF mimics significantly inhibited the
129 proliferation of the A2780 cells by over 50% at 50 nM (Fig 2b). Among these tRF

130 mimics, tRF-T11 mimic (a double strand RNA with a 22 nt 5'-tRF derived from
131 tRNA^{His(GUG)} as antisense RNA) showed the highest antiproliferative activity against
132 both cell lines. As shown in Fig 2c, the IC₅₀ value of tRF-T11 mimic for inhibiting the
133 growth of A2780 cells was 31 nM, which is comparable to that of taxol (10.2 nM).
134 Surprisingly, high sensitivity to tRF-T11 mimic was also observed in A2780T cells,
135 corresponding to an IC₅₀ value of 32 nM, which is 80-fold lower than that of taxol
136 (2600 nM). In addition, no significant cytotoxicity of tRF-T11 mimic to IOSE80
137 normal ovarian surface epithelial cells was observed. Furthermore, the cytotoxicity of
138 tRF-T11 mimic was confirmed by visualizing and tracking fluorescent dye
139 (FAM)-labeled tRF-T11 mimic in A2780 cells. The images shown in Fig 2d indicated
140 that tRF-T11 mimic was efficiently transfected into the cytoplasm of A2780 cells,
141 where they exhibited functions involved in the biological processes, such as
142 suppressing the expression of endogenous targets¹⁹. These results indicated that
143 *Taxus*-derived tRFs are promising therapeutic agents for drug-resistant OVCA.

144 The divergent activity of tRF mimics derived from individual tRNAs observed in
145 this study suggested that plant-derived tRFs can exhibit bioactivities in a

sequence-specific manner in mammalian cells. It has been reported that functionality of exogenous tRFs possibly overlaps that of miRNAs, a characteristic that has been widely documented for endogenous tRFs in both plants and mammalian cells^{20,21}. To further identify potential target genes of tRF-T11, we performed an *in silico* prediction using two independent bioinformatic tools, IntaRNAv2²² and psRNATarget²³, followed by differential expression analysis based on the gene expression profiles in normal and tumor ovarian tissue to prioritize the candidate targets. As a result, the top three differentially expressed genes, namely transient receptor potential cation channel subfamily A member 1 (*TRPA1*), tetraspanin 17 (*TSPAN17*) and secretogranin V (*SCG5*), with the minimum required energy to hybridize with tRF-T11 at their 3' UTR regions of mRNA, were considered as the potential targets for further experimental validation (Fig 2e). Among these three genes, *TRPA1*, a new target recently unveiled by Takahashi *et al*²⁴ for cancer therapies, which acts by triggering a noncanonical oxidative stress defense mechanism of tumor suppression, was dramatically downregulated in A2780 cells (by approximately 75%) in a dose-dependent manner by tRF-T11 mimic treatment (Fig 2j, g), while the

162 mRNA levels of the other two targets did not vary significantly (Fig 2f). Furthermore,
163 a marked decrease of over 50% in the protein expression level of *TRPA1* was also
164 observed in A2780 cells treated with tRF-T11 mimic (Fig 2h, i), which may act as a
165 translational repressor of the *TRPA1* gene by acting like a miRNA in human OVCA
166 cells. Indeed, previous reports revealed that *TRPA1* overexpression is associated with
167 poor survival probability in patients (Fig 2k). Unfortunately, no clinical therapeutic
168 drug targeting *TRPA1* is available, and our findings provide a novel RNA sequence
169 derived from plant can potently silence human *TRPA1*.

170 Recently, advances in RNA therapeutics have been facilitated by breakthroughs
171 in pharmaceutical delivery systems, including liposomes, nanoparticles, dendrimers
172 and carbon nanotubes have been shown to provide promising application *in vitro* and
173 *in vivo*²⁵. To evaluate the antitumor activity *in vivo*, a histidine-lysine polymer (HKP)
174 nanoparticle system was applied to deliver tRF-T11 mimic to a well-established
175 xenograft mouse model of human OVCA cells. The mass ratio of HKP and tRF-T11
176 mimic was optimized (Fig 3a, b) with an appropriate particle size (164.2 nm) and
177 good dispersion (41.2 mV, Fig 3c). As shown in Fig 3d to g, compared to vehicle

control group, tRF-T11 mimic treatment of xenografts for 20 days significantly diminished the tumor growth rate by approximately 50% at a dose of only 177 nmol/kg, which is more than 16-fold lower than that of taxol (2928 nmol/kg) required to achieve comparable tumor-suppressive effects without significant adverse impacts on body weight. Compared to those from control mice, the protein expression levels of *TRPA1* in the tumors with intratumoral injection of high-dose tRF-T11 mimic exhibited significant reduction (Fig 3h, i), which is consistent with the western blot results *in vitro*. Although taxol is a commonly used first-line drug for cancer patients, chemoresistance is usually observed. Since tRF-T11 mimic exhibited comparable tumor suppression efficacy to taxol at a much lower dose, our study may indicate an alternative treatment option.

In summary, this study provides the first evidence that plant-derived tRNAs and tRFs from Chinese yew exhibit potent antitumor effects against human OVCA, which will greatly expand our knowledge on the active ingredients in natural sources, and provide additional clues for evaluating the active components of medicinal plants or herbal medicines. Although cross-kingdom regulation in human by miRNA uptake

194 from food and medicinal plants has been widely studied, this report emphasizes the
195 potential regulatory roles of plant-derived small RNAs from an abundance of
196 housekeeping noncoding RNA species, such as tRNAs and tRFs. This infinitely vast
197 pool filled with RNA sequences and chemical modifications provided by nature can
198 serve as a new sequence library for the development of novel RNAi-based
199 therapeutics. Furthermore, since chemical modified oligonucleotides could be simply
200 and efficiently synthesized in large amount using the phosphoramidite
201 methodology^{26,27}, this discovery demonstrated a pilot strategy for identifying
202 pharmacologically active tRFs from plants, such as those from the genus *Taxus*.

203 As a promising and rapidly advancing frontier, siRNA can be readily designed
204 based on a well-validated target mRNA. Often, the process of unraveling the
205 pathogenesis of many human diseases to find key molecular targets for drug treatment
206 is time-consuming and difficult without a functional approach with bottom-up
207 guidance. As a result, the effective design of RNAi therapeutic drug candidates will
208 be difficult or impossible due to the lack of reliable targets. As a benefit of the high
209 target specificity and selectivity of RNA drugs, pharmacologically active RNA

210 molecules identified from medicinal plants could, in turn, lead to the improvement of
211 the efficiency and possibilities of discovering new drug target, and shed light on
212 disease etiology.

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221 **Contributions**

222 Z.-H.J. and T.-M.Y. conceived the study. K.-Y.C., T.-M.Y. and T.-F.C. designed
223 the experiments. K.-Y.C., T.-M.Y., J.-Z.Z. and J. L. conducted the experiments.
224 K.-Y.C., J.-Z.Z. and C. L. analyzed the data. K.-Y.C., T.-M.Y. and E.L.L. drafted the
225 manuscript. All authors approved the manuscript.

226 **Methods**

227 **Plant materials and RNA extraction of *Taxus chinensis*.** Plants of *Taxus chinensis*
 228 (*Pilger*) *Rehd. var. mairei* (Lemee et Levl.) Cheng et L. K. Fu used in this study were
 229 collected from Sanming City, Fujian Province, China. Plant materials were freshly cut,
 230 frozen and stored in liquid nitrogen until use.

231 Plant tissues were ground into a fine powder in liquid nitrogen and were then
 232 homogenized (IKA, Germany) in CTAB buffer. After incubation for 2 min at 65°C,
 233 the tissue lysate was cooled immediately on ice for 10 min, followed by
 234 centrifugation at 12,000 ×g for 15 min at 4°C. The supernatant was collected and
 235 extracted with an equal volume of phenol:chloroform:isopentanol (50:48:1). Phases
 236 were separated at 4°C by centrifugation at 12,000 ×g for 15 min. The supernatant was
 237 extracted again as described above with chloroform:isopentanol (24:1) and mixed
 238 with an equal volume of 6 M guanidinium thiocyanate solution (Tokyo Chemical
 239 Industry, Japan) and ethanol (Anaqua Chemicals Supply, U.S.A.) to a final
 240 concentration of 55%. The mixture was passed through a silicon-membrane column
 241 and washed with 80% ethanol. Finally, total RNA was eluted with RNase-free water,

242 and small RNA species were enriched by using a mirVana miRNA isolation kit
243 (Invitrogen, U.S.A.) following the manufacturer's instructions.

244

245 **tEF preparation.** Small RNA was separated by 6% urea-polyacrylamide gel
246 electrophoresis (PAGE). Gel fractions containing the tEF, as visualized by SYBR
247 Gold nucleic acid gel staining (Thermo, U.S.A.), were excised and electroeluted in 3
248 kD molecular weight cut off dialysis tubing (Spectrum, U.S.A.) at 100 V for 1.5 h.
249 The tEF was finally desalted and recovered on a silicon-membrane column.

250

251 **tEF library construction and sequencing.** Sequencing libraries were generated by
252 using a TruSeq small RNA Library Preparation Kit (Illumina, U.S.A.), followed by a
253 round of adaptor ligation, reverse transcription and PCR. PCR products were then
254 purified, and libraries were quantified on an Agilent Bioanalyzer 2100 system
255 (Agilent Technologies, U.S.A.). The library preparations were sequenced at the
256 Novogene Bioinformatics Institute (Beijing, China) on an Illumina HiSeq platform
257 using the 150-bp paired-end (PE150) strategy to generate over 15 million raw paired

258 reads. A total of 1,729,438 clean reads were obtained by removing low quality
259 regions and adaptor sequences. The tRNA genes were identified by using the
260 tRNAscan-SE 2.0 program (<http://lowelab.ucsc.edu/tRNAscan-SE/>) and annotated by
261 searching the Nucleotide Collection (nr/nt) database using the Basic Local Alignment
262 Search Tool (BLAST) program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

263

264 **Purification of individual tRNAs.** Synthetic single-stranded DNA oligonucleotides
265 (BGI, China; [Supplementary Table 3](#)) complementary to the target tRNA were mixed
266 with *Taxus* small RNA and denatured at 95°C for 5 min, followed by incubation at a
267 temperature 5°C lower than the melting temperature (T_m) for 1.5 h. Streptavidin
268 magnetic beads (Beaverbio, China) were then added to the mixture and incubated for
269 30 min at the annealing temperatures. Then, the biotinylated DNA/tRNA-coated
270 beads were separated with a magnet and washed at 40°C. Subsequently, the magnetic
271 beads were resuspended in RNase-free water, and the immobilized tRNA with probes
272 was released by incubation at 65°C for 5 min. The solutions were directly injected
273 using an ACQUITY UPLC OST C₁₈ column (2.1×100 mm i.d., 1.7 μm; Waters,

274 U.S.A.) maintained at 60°C into a UHPLC Agilent 1290 system (Agilent, U.S.A.)
275 equipped with a diode array detector. The flow rate was set at 0.2 mL/min, and a
276 gradient elution was performed with (A) 100 mM HFIP+15 mM TEAA and (B) 50%
277 MeOH in A as follows: 0-1.5 min, 2% B; 1.5-8.3 min, 2%-28% B; and 8.3-16.5 min,
278 28%-34% B. MS data were acquired on an Agilent 6545 Q-TOF mass spectrometer
279 with a gas temperature of 320°C, spray voltage of 3.5 kV, and sheath gas flow and
280 temperature of 12 L/min and 350°C, respectively. For the MS experiment, samples
281 were analyzed in negative polarity over an m/z range of 500 to 3200. Fractions from
282 chromatographic peaks of individual tRNAs were collected and freeze dried.

283

284 **RNA transfection and cytotoxicity determination.** RNA samples dissolved in
285 RNase-free water were stored at -20°C before use. Transfection of RNA samples was
286 performed according to the manufacturer's instructions for Lipofectamine RNAiMAX
287 Transfection Reagents (Thermo, U.S.A.). After treatment for 48 h, an MTT assay was
288 performed, and absorbance values were calorimetrically determined at 570 nm using a
289 SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, U.S.A.). The

290 dose-dependent IC₅₀ values were calculated by GraphPad Prism 5.0 (GraphPad,
291 U.S.A.). A siRNA (Forward: 5'-GGAGCGAUUUAGCCAAGAATT-3', Sense:
292 5'-UUCUUGGC UAAAUCGCUCCTT-3') was used as positive control. Each
293 experiment was carried out three times. The results are expressed as the means ±
294 standard deviations.

295

296 **Acquisition of fluorescence images of transfected FAM-labeled tRF-T11 mimic**
297 **in A2780 cells.** Briefly, 1×10⁵ A2780 cells were seeded in glass bottom cell culture
298 dishes (Nest Biotechnology, China). The medium was discarded after 20 h, and 50
299 nM FAM-labeled tRF-T11 mimic (GenePharma, China) was transfected into the cells.
300 After 6 h, the transfection medium was discarded, and the cells were rinsed 3 times
301 with RPMI 1640 medium. For nuclear staining, cells were incubated with 1 mL of
302 Hoechst 33258 (Sigma, U.S.A.) for 10 min at 37°C, followed by washing with RPMI
303 1640 medium. For mitochondrial staining, cells were incubated with 1 mL of
304 MitoBeacon Red (GeneCopoeia, U.S.A.) for 30 min at 37°C, followed by washing
305 with RPMI 1640 medium. Fluorescence images were acquired with an API

306 DeltaVision Live-cell Imaging System (Applied Precision Inc., GE Healthcare Co.,
307 U.S.A.) and were captured at x60 magnification, scale bar, 25 μ m.
308
309 **tRF-T11 target prediction and prioritization.** Previously described small RNA or
310 tRF target prediction methods and restriction criteria were applied in this study to
311 identify functional target genes of tRF-T11²⁸. Specifically, the 3' UTR sequences of
312 all mRNAs retrieved from GENCODE (v23) and the sequence of tRF-T11 derived
313 from *Taxus* were firstly loaded into IntaRNAv2 and psRNATarget to predict the
314 potential targets. The top 10 overlapping targets predicted were then prioritized and
315 filtered by crosschecking their expression in tumor and normal ovarian tissues.
316 Accordingly, we first obtained the gene expression profiles for 379 OVCA tissues and
317 88 normal ovarian tissues from UCSC Xena, which contains curated comprehensive
318 batch-effect-adjusted pancancer genomic data²⁹. Subsequently, EBSeq³⁰ was
319 employed to filter the target genes differentially expressed between tumor and normal
320 ovarian tissues. The predicted targets satisfying two criteria, fold change
321 (tumor/normal) > 2 and FDR < 0.1.

322

323 **Quantitative real-time PCR.** Total RNA was extracted from A2780 cells treatment
 324 with the tRF-T11 mimic for 48 h using TRIzol (Invitrogen, U.S.A.) on a
 325 silicon-membrane column. RNA was then reverse transcribed using the GoScript
 326 Reverse Transcription System (Promega, U.S.A.). Quantitative real-time PCR was
 327 performed on a high-productivity real-time quantitative PCR ViiATM 7 system (Life
 328 Technologies, U.S.A.) using GoTaq[®] qPCR Master Mix (Promega, U.S.A.) according
 329 to the manufacturers' instructions. The PCR primers were synthesized commercially
 330 (BGI, China, [Supplementary Table 4](#)). All data are presented as the mean fold change
 331 values duplicated and normalized to β -actin expression using the $2^{-\Delta\Delta CT}$ method. Each
 332 experiment was carried out three times. The results are expressed as the means $\pm$
 333 standard deviations.

334

335 **Western blot analysis.** A2780 cell lysates were collected after treatment with
 336 tRF-T11 mimic for 48 h, and protein extraction was carried out using RIPA buffer
 337 (Cell Signaling Technology, U.S.A.) supplemented with protease and phosphatase

338 inhibitor cocktails (Roche, Switzerland). Protein concentrations were then determined
339 by a BCA protein assay (Thermo, U.S.A.). Denatured proteins were separated by 8%
340 SDS-PAGE and transferred to nitrocellulose membranes, followed by blocking with 5%
341 bovine serum albumin (Thermo, U.S.A.) for 2 h. Membranes containing proteins were
342 incubated with primary antibody against *TRPA1* (1:500 dilution, Novus, U.S.A.) or
343 β -actin (1:1000 dilution, Santa Cruz, U.S.A.) overnight, followed by washing in
344 TBST and incubation with a fluorescent secondary antibody (Li-cor, U.S.A.) for
345 detection. Band intensities were measured using ImageJ and were normalized to those
346 of β -actin. The sequences of TRPA1_siRNA01, TRPA1_siRNA02 and the scrambled
347 control (GenePharma) are shown in [Supplementary Table 5](#). Each experiment was
348 carried out three times. The results are expressed as the means $\pm$ standard deviations.
349

350 **Preparation of HKP-tRF-T11 mimic nanoparticles.** HKP (a gift from Dr. Yang Lu,
351 Sirnaomics, Inc., U.S.A.) dissolved in RNase-free water (240 μ g/mL) were first
352 balanced overnight at 4°C. Equal volumes of tRF-T11 mimic solutions at a
353 concentration of 80 μ g/mL were then mixed and incubated at room temperature for 30

354 min. The particle sizes and zeta potential of the resulting nanoparticles were then
355 evaluated by a Malvern Zetasizer Nano ZS90 (Malvern Instruments, U.K.).

356

357 ***In vivo* models for tumor therapy.** A2780 cells (4.0×10^6) were injected
358 subcutaneously into the armpits of 8-week-old BALB/c nude mice (Shanghai SLAC
359 Laboratory Animal Co., Ltd., China). When the tumors reached 50 mm^3 , the
360 tumor-bearing nude mice were treated with different doses (71 nmol/kg, 177 nmol/kg)
361 of tRF-T11 mimic (GeneDesign, Inc. Osaka, Japan) encapsulated with HKP and
362 different doses of taxol (2928 nmol/kg or 5855 nmol/kg) via intratumoral injection
363 once every three days. A group treated with HKP alone was established as a control.
364 The animals were sacrificed on day 20. Tumor diameters were measured at the points
365 of maximum length and maximum width with digital calipers. Tumor volumes were
366 calculated by the following formula: $\text{volume} = (\text{width})^2 \times \text{length} / 2$. The data were
367 statistically analyzed using GraphPad Prism 5.0. Animal care and use protocols were
368 performed in compliance with the guidelines of the Ethics Committee of the College
369 of Pharmaceutical Sciences, Southwest University.

370

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Figure Legends

Figure 1. *Taxus*-derived tRNAs exhibit cytotoxicity *in vitro*. **a**, *Taxus* tEF with liposomal transfection exhibited strong cytotoxicity on A2780 cells. **b**, Representative MTT image of the *Taxus* tEF with or without liposomal transfection. **c**, Read length distribution of the tEF determined by NGS was primarily between 60 and 80 mer. **d**, NGS read content analysis showed that 14% of the tEF was tRNA. **e**, Urea-PAGE analysis of the tEF, rRNA fragment and purified tEF. **f**, The cytotoxicity of purified tEF increased after rRNA fragment deletion. **g**, Representative UHPLC method for purification of tRNA^{His(GUG)}. **h**, Deconvolution mass spectrometry and urea-PAGE analysis of purified tRNA^{His(GUG)}. **i**, Deconvolution mass results of tRNA^{Glu(UUC)}, tRNA^{Trp(CCA)}, tRNA^{Leu(CAA)} and tRNA^{Arg(ACG)}. **j**, MTT assay of the top five abundant tRNAs from Chinese yew on A2780, HepG2 and MCF-7 cells. **k**, Representative MTT image of individual tRNAs on A2780 cells. **l**, Dose-dependent investigation of tRNA^{Trp(CCA)} on A2780 cells. The data are shown as the means $\pm$ SDs of 3 independent experiments. ** P <0.01, *** P <0.001, **** P <0.0001.

Figure 2. *Taxus*-derived tRF-T11 mimic inhibits human OVCA cell proliferation by targeting *TRPA1* *in vitro*. **a**, Design of tRF mimic from 5' and 3' end of tRNA. **b**, Heatmap of the inhibition rate of 36 synthesized tRF mimics against A2780 and A2780T cells. **c**, Investigation of the dose dependency of tRF-T11 mimic and taxol against A2780, A2780T and IOSE80 cells. **d**, Fluorescent images capture of FAM labeled tRF-T11 mimic in A2780 cells. **e**, Summary of hybridization energies and differential expression analysis of predicted target genes identified by the IntaRNA and psRNATarget tools. **f**, qPCR validation of the mRNA levels of predicted targets of tRF-T11 in A2780 cells. **g**, tRF-T11 mimic decreased the mRNA levels of *TRPA1* in a dose-dependent manner. **h**, Western blot assay for the evaluation of *TRPA1* expression in A2780 cells treated with tRF-T11 mimic, siRNA against *TRPA1* (TRPA1_siRNA01, TRPA1_siRNA02) and scrambled control at 25 nM. Band intensities were measured using ImageJ and were normalized to those of β -actin (**i**). **j**, Putative tRF-T11 binding sites in the *TRPA1* 3' UTR. **k**, Overall survival probability of patients in the TCGA OVCA cohort. The data are shown as the means $\pm$ SDs of 3 independent experiments. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

478

479 **Figure 3.** *Taxus*-derived tRF-T11 mimic decreases OVCA tumor growth via targeting

480 *TRPA1* *in vivo*. **a**, Structure of HKP. **b**, Optimization of the mass ratio of tRF-T11

481 mimic and HKP. **c**, The particle sizes and zeta potential of the HKP-tRF-T11 mimic.

482 **d**, Growth rate of tumors treated with tRF-T11 mimic, taxol and HKP alone. **e**,

483 Quantification of body weights in each group. **f**, Images of each tumor. **g**, No

484 significant difference in tumor weight was observed during treatment in the high-dose

485 tRF-T11 mimic group and the low-dose taxol group. **h**, Representative western blot

486 image of *TRPA1* protein levels in tumors of different treatment. **i**, Band intensity ratio

487 of *TRPA1*/ β -actin in tumors treated with different doses of tRF-T11 mimic (n=6 per

488 group). The data are shown as the means $\pm$ SDs of 3 independent experiments

489 excluding those shown in **c** ($\pm$ SEM). * P <0.05, ** P <0.01, *** P <0.001.

Figure 1

Figure 2

Figure 3

a **c** **d** **e** **b** **f** **g** **h** **i** **j** **l** **k**

a **b** **c** **d** **e**

Gene symbol	Hybridization energy		Differential expression analysis	
	IntaRNA	psRNA Target	log ₂ [Fold Change (tumor/normal)]	FDR
TRPA1	-16.6536	20.6330	1.6019	< 0.0001
TSPAN17	-18.6490	22.2300	2.1892	< 0.0001
SCG5	-16.2213	16.8240	7.4252	< 0.0001

h **j** **f** **g** **i** **k**

a **b** **d** **f** **h** **c** **e** **g** **i**