Supplementary Materials and Methods

Cell culture. Human mammary epithelial cells (HMECs) purchased from Invitrogen were cultured in HuMEC Basal Serum-Free Medium (Invitrogen) supplemented with HuMEC Supplement (Invitrogen) and 1% penicillin/streptomycin (P/S). MCF7 breast cancer cells were cultured in DMEM/F-12 medium (Thermo Scientific) supplemented with 10% FBS and 1% P/S. The breast cancer cell lines ZR75-1, HCC1419, and HCC1806 were purchased from ATCC and were cultured in ATCC-formulated RPMI 1640 medium (ATCC) supplemented with 10% FBS and 1% P/S. HEK293 cells were grown in DMEM/high glucose (Thermo Scientific) supplemented with 10% FBS and 1% P/S. AGO2+/+ NIH3T3 cell line and AGO2-/- NIH3T3 cell line which was generated previously using the CRISPR/Cas9 (1) were cultured in DMEM medium (WISENT BIOPRODUCTS) supplemented with 10% bovine calf serum (Sigma) without antibiotics. All cells were cultured at 37°C in a humidified atmosphere of 5% CO_2.

IR-exposure. HMEC cells were grown to 50-60% confluence and exposed to either 30 kVp/0.1 Gy or 80 kVp/2.5 Gy X-rays. Controls were left unexposed. The culture was then continued for 96 h at 37°C in a humidified atmosphere of 5% CO_2.

Transient transfection. The breast cancer cell line HCC1806 and MCF7 cells grown to 80-90% confluence were transfected with either 30 nM hsa-miR-34a mimic or negative control siRNA No. 1 (Ambion) or AllStars negative control siRNA (QIAGEN, scrambled miR) using Lipofectamine 3000 (Invitrogen) for the indicated time points. HCC1806 cells grown to 90% confluence were transfected with either 50 nM wild-type $tRNA_i^{Met}$ DsiRNA (WT tMet DsiRNA) or scrambled $tRNA_i^{Met}$ DsiRNA (Scr tMet DsiRNA) using

Lipofectamine 3000 (Invitrogen). To generate double-stranded DsiRNA, RNA oligos (WT tMet DsiRNA: 5'-rArGrArGrGrUrCrGrArUrGrGrArUrCrGrArArArCrCrArUrCrC-3', 5'-rGrGrArUrGrGrUrUrUrCrGrArUrCrCrArUrCrGrArCrCrUrCrUrGrG-3'; Scr tMet DsiRNA: 5'-rArGrArGrCrUrArCrGrUrCrGrArUrCrGrArArGrGrUrArUrCrC-3', 5'-rGrGrArUrArCrCrUrUrCrGrArUrCrGrArCrGrUrArGrCrUrCrUrGrG-3') were synthesized and annealed (IDT Integrated DNA Technologies).

Real-time RT-PCR. Total RNA was isolated from the indicated cells using TRIzol reagent (Invitrogen) and was subjected to quantitative real-time RT-PCR (qRT-PCR) using a primer set for either has-miR-34a or mm-miR-34a (QIAGEN) or total human $tRNA_i^{Met}$ (Met-F: 5'-AGA GTG GCG CAG CGG AAG-3'; Met-R: 5'-TGG TTT CGA TCC ATC GAC CT-3') or immature human $tRNA_i^{Met}$ (pre-tRNA-Met F: 5'-AGA GTA CTG TGA GAC CGT GTG C-3'; pre-tRNA-Met R: 5'-CCT AGC AGA GGA TGG TTT CG-3') or immature mouse $tRNA_i^{Met}$ (mitiMet-F: 5'-AAC ACC TAG GCA GCT CTA GCA-3'; mitiMet-R: 5'-TTG GTT TTG TGT GAC GAG GA-3') with miScript II RT Kit and SYBR Green PCR Master Mix (QIAGEN) or iScript Select cDNA Synthesis Kit and SsoFast EvaGreen Supermix (Bio-Rad) according to the manufacturers' instructions. Human *RNU6-2* (QIAGEN) and *GAPDH* (2) or mouse *GAPDH* (m*GAPDH*) served as the loading controls. The experiments were done in triplicate.

AGO2 restoration. NIH3T3 AGO2-/- cells grown to 80% confluency were transfected with either mouse AGO2 ORF mammalian expression plasmid (pCMV3-GFPSpark-mAGO2, Sino Biological Inc.) or pCMV3-N-GFPSpark control plasmid (Sino Biological Inc.) using Lipofectamine 3000 Transfection Reagent (Invitrogen) according to the manufacturer's instruction. Forty-eight hours after transfection, the positive cells that

were stably expressing either mouse AGO2 or GFP were selected with 200 μg/ml Hygromycin. The expression of mouse AGO2 was then induced with 1 mg/ml Hygromycin.

Western blot analysis. The cells were rinsed twice with ice-cold PBS and scraped off the plate in radioimmunoprecipitation assay buffer (RIPA). The whole cellular lysates (100 μg per sample) were electrophoresed via 10% SDS-PAGE and electrophoretically transferred to PVDF membranes (Amersham Hybond^{TM}-P, GE Healthcare) at 4°C for 1.5 h. The blots were incubated for 1 h with 5% nonfat dry milk to block the nonspecific binding sites and subsequently incubated with 1:500 dilution of polyclonal antibody against mouse AGO2 (Abcam) at 4°C overnight. Immunoreactivity was detected using a peroxidase-conjugated antibody and visualized by the ECL Plus Western Blotting Detection System (GE Healthcare). The blots were stripped before reprobing with antibody against mouse GAPDH (Santa Cruz Biotechnology) or actin (Abcam).

Standard curves for molecule number assay. Mature miR-34a and $tRNA_i^{Met}$ were synthesized and diluted to a series of concentration, 7×10^0 to 7×10^8 molecules per reverse transcription reaction, and then subjected to qRT-PCR using iScript Select cDNA Synthesis kit and SsoFast EvaGreen Supermix (Bio-Rad) with primer sets of miR-34a and $tRNA_i^{Met}$. Standard curve was generated with two variants Ct value and molecule number using Avogadro's constant (http://en.wikipedia.org/wiki/Avogadro_constant) as described previously (3).

Bioinformatics. The human, rat, and mouse sequences used to compare the similarity among the mature $tRNA_i^{Met}$s were downloaded from the UCSC Genome Browser

(http://genome.ucsc.edu/cgi-bin/hgTables). tRNA datasets were derived from the following versions of the genome assemblies: 1) human, the February 2009 assembly of the human genome (hg19, GRCh37) from the Genome Reference Consortium; 2) rat, the November 2004 update of the rat genome (rn4, version 3.4) from the Rat Genome Sequencing Consortium; and 3) mouse, the December 2011 assembly of the mouse genome (mm10, GRCm38) from the Genome Reference Consortium. Additionally the genome and mRNA Human datasets are from Dec. 2013 (GRCh38/hg38) assembly of the human genome (hg38, GRCh38 Genome Reference Consortium Human Reference 38 (GCA_000001405.2)). The blastn algorithm from the standalone BLAST software package (ver. 2.2.27+) was used to analyze the similarity of the $tRNA^{Met(CAT)}$ sequences. The program settings were adjusted so that the number of results was not restricted.

Generation of plasmid constructs. To generate the $tRNA_i^{Met}$ shRNA expression vector and scrambled control shRNA vector, oligonucleotides (Met-shRNA-sense, 5'-Phos/ GAT CCC AGG TCG ATG GAT CGA AAC CAT TTC AAG AGA ATG GTT TCG ATC CAT CGA CCT TTT TTT CCA AA-3'; Met-shRNA-antisense, 5-Phos/ AGC TTT TGG AAA AAA AGG TCG ATG GAT CGA AAC CAT TCT CTT GAA ATG GTT TCG ATC CAT CGA CCT GG-3'; Neg-sense, 5'-Phos/ GAT CCC AAT TCT CCG AAC GTG TCA CGT TTC AAG AGA ACG TGA CAC GTT CGG AGA ATT TTT TTT CCA AA-3'; Neg-antisense, 5'-Phos/ AGC TTT TGG AAA AAA AAT TCT CCG AAC GTG TCA CGT TCT CTT GAA ACG TGA CAC GTT CGG AGA ATT GG-3') were synthesized and annealed. The sequences were then cloned into the pRNAT-U6.1/Neo vector between *BamH* I and *Hind* III to produce the pRNAT-Met-shRNA and pRNAT-Neg-shRNA

plasmids; the sequence identity was confirmed by automatic sequencing. To generate the hsa-miR-34a expression plasmid, the human miR-34a gene was amplified by PCR using human genomic DNA and cloned into pmRi-ZsGreen1 (Clontech Laboratories) between the *BamH* I and *Hind* III sites to produce pmRi-miR34a; the sequence identity was confirmed by automatic sequencing. The primers bearing *BamH* I and *Hind* III sites designed to amplify hsa-miR-34a were as follows: miR34a Exp F, 5'-CCG GAT CCT CTC CAG TAG CTA GG-3'; miR34a Exp R, 5'-CAC AAG CTT ACG TGC AAA CTT CTC C-3'. To construct the $tRNA_i^{Met}$ expression vector, a 667-bp fragment containing human wild-type pre-$tRNA_i^{Met}$ amplified from genomic DNA was cloned into the pGEM-T easy vector (Promega). The fragment was released by digestion with *Kpn* I and *Xho* I and subcloned into pcDNA 3.1(+) (Invitrogen) to generate the pcDNA-tRNA-Met plasmid; the sequence identity was confirmed by automatic sequencing. The primers bearing the *Kpn* I and *Xho* I sites designed to amplify the pre-$tRNA_i^{Met}$ gene were as follows: Chr1-Met F, 5'-ATG GTA CCC TCC CGC CTT AC-3'; Chr1-Met R, 5'-TTT CTC GAG TGG ATC ACC TGT TT-3'.

Gene copy number assay. Genomic DNA was extracted from parental cell line HCC1806, uninduced [Dox(-)] and induced [Dox(+)] miR-34a-expressing HCC1806 cells, HCC1806 cells stably expressing $tRNA_i^{Met}$, apoptotic and non-apoptotic cells selected from HCC1806 stably expressing $tRNA_i^{Met}$ shRNA with Annexin V Microbead Kit and MACS Separation Columns (MACS Miltenyi Biotec), using Blood & Cell Culture Mini Kit (QIAGEN). 100-200 ng of genomic DNA was then subjected to real-time PCR using SsoFast EvaGreen Supermix (Bio-Rad) with the following primers: Met-shRNA-F: 5'-GCA GAG TGT AGC TTA ACA CAA AG-3', Met-shRNA-R:

CCAGTTTTTTTTTTTTTTTAGTTGGGT-3', which were designed by miRprimer (4). Primers for miR-34a and RPP38 were described previously (ref.17, see main text). The copy number of $tRNA_i^{Met}$ in HCC1806 cell stably expressing $tRNA_i^{Met}$ was measured with Custom TaqMan Copy Number Assay (Applied Biosystems). The copy number level relative to the internal control (*RNase P*/*RPP38*) was calculated with the comparative threshold cycle (Ct) method (5).

***In vitro* pull-down assay.** A biotin-labeled hsa-miR-34a pull-down probe (/5Biosg/TGG CAG TGT CTT AGC TGG TTG T) was synthesized by Exiqon, and the negative control probe (Biotin5/ACG TGA CAC GTT CGG AGA ATT) was synthesized by Eurofins. The binding reactions were performed as described previously (6). Briefly, RNA samples isolated from HCC1806 cells using TRIzol reagent (Invitrogen) were treated with RNase-free DNase I (Fermentas); DNase I was removed using TRIzol reagent according to the manufacturer's instructions. The binding reactions (100-µl final volume) contained 1, 0.2 or 0.04 µM biotin-labeled hsa-miR-34a pull-down probe or negative control probe and 15 µg of DNA-free RNA, and 1 × binding buffer (25 mM Tris-HCl [pH 7.5], 60 mM KCl, 2.5 mM EDTA, 0.2% Triton X-100, and 80 U/ml rRNasin); the binding reactions were incubated at 4°C for 30 min. The purification and elution procedures were performed using a µMACS separator according to the manufacturer's instructions (Miltenyi Biotec). The levels of pulled-down $tRNA_i^{Met}$ were analyzed by real-time RT-PCR and normalized to the total input.

***In vitro* cleavage of $tRNA_i^{Met}$**. $tRNA_i^{Met}$ oligos used in *in vitro* cleavage study were synthesized by Dharmacon (GE Healthcare Dharmacon), and their sequences were as follows. Unmodified $tRNA_i^{Met}$: 5'-AGC AGA GUG GCG CAG CGG AAG CGU GCU GGG

CCC AUA ACC CAG AGG UCG AUG GAU CGA AAC CAU CCU CUG CUA-3'; modified $tRNA_i^{Met}$: 5'-AGC AGA GUm1G GCG CAG CGG AAG CGU GCU GGG CCC AUA ACC CAG AGG DHU5-M-CG AUG GAU CGA AAC CAU CCU CUG CUA-3'; scrambled $tRNA_i^{Met}$: 5'-AGC AGA GUG GCG CAG CGG AAG CCA GCU GGG CGG AAA ACG GAC AGG UCG AUG GAU CGA AAG GAA GCU GAC GAU-3'. The *in vitro* cleavage was performed as described previously (7). Briefly, 1 μM or 2 μM hsa-miR-34a or AllStars negative control siRNA (QIAGEN) was preincubated with 100 ng or 200 ng of human wild-type AGO2 (Sino Biological Inc.) or mutant AGO2 (D597N, a kind gift from Dr. Leemor Joshua-Tor, Watson School of Biological Sciences, Cold Spring Harbor Laboratory, USA), respectively, in 1× AGO cleavage buffer (10 mM Tris, pH 7.5, 100 mM KCl, 2 mM $MgCl_2$, 1× protease inhibitor, 0.5 mM DTT) at 37°C for 30 min. The preincubated mixture was then incubated with 1.5 μM heat denatured $tRNA_i^{Met}$ at 37°C for 2 hours. Cleavage products were resolved by 15% denaturing polyacrylamide gel, stained with RedSafe (iNtRON Biotechnology) and visualized with a Bio Imaging System (SYNGENE).

***In vitro* RNA-RNA interaction.** *In vitro* miR-34a and $tRNA_i^{Met}$ interaction was performed as described previously (ref.20, see main text). 1.5 μM synthesized $tRNA_i^{Met}$ oligos (unmodified, modified and scrambled) were incubated for 3 min at 95ºC in binding buffer (50 mM sodium cacodylate, pH 7.5, 300 mM KCl, and 1 mM $MgCl_2$), immediately leave on ice for 15 min; dilute has-miR-34a in binding buffer to a concentration of 1 μM or 6 μM and incubate with same volume of pre-denatured $tRNA_i^{Met}$ oligos for 3 h at 37ºC; the products were resolved by 15% denaturing polyacrylamide gel, stained with RedSafe (iNtRON Biotechnology) and visualized with a Bio Imaging System (SYNGENE).

Generation of a breast cancer cell line with doxycycline-inducible hsa-miR-34a expression. The procedures were performed as described in the Mir-X^{TM} inducible miRNA Systems User Manual (Clontech Laboratories). Briefly, HCC1806 cells grown to 90% confluence were transfected with pTet-On Advanced (Clontech Laboratories) using Lipofectamine 2000 (Invitrogen). After G418 selection, the positive clones were seeded in 6-well plates and co-transfected with 500 ng of pTRE-Tight-Luc (Clontech Laboratories) and 5 ng of pRL-TK using Lipofectamine 2000. The cells were then exposed to 0.5 μg/ml doxycycline (Dox) at 24 h after transfection. At 48 h after Dox treatment, the luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) with a luminometer (FLUOstar Omega, BMG LABTECH); the firefly luciferase data were normalized to *renilla* luciferase. The clones with the highest fold induction were selected as Tet-On positive cells, grown to 90% confluence, and co-transfected with 6 μg of pmRi-miR34a and 300 ng of Puromycin marker; the positive clones were selected with 50 ng/ml Puromycin at 48 h after transfection. After Dox treatment, the clones with the highest miR-34a induction were sorted twice at the University of Calgary.

Generation of stable $tRNA_i^{Met}$-shRNA-expressing breast cancer cell lines. HCC1806 and MCF7 cells grown to 90% confluence were transfected with either pRNAT-Met-shRNA or pRNAT-Neg-shRNA. At 48 h after transfection, 600 μg/ml or 800 μg/ml G418 (Promega) was added; after G418 selection, the positive clones with lowest levels of $tRNA_i^{Met}$ were then sorted twice at the University of Calgary.

Generation of a stable $tRNA_i^{Met}$-expressing breast cancer cell line. HCC1806 cells grown to 90% confluence were transfected with either pcDNA-tRNA-Met or pcDNA

3.1(+). At 48 h after transfection, 600 μg/ml G418 (Promega) was added to select the pooled positive cells.

RNA immunoprecipitation (RIP)-PCR. RIP-PCR was performed as described previously (8,9). Briefly, 10 days after Dox treatment (untreated cells were used as control), the doxycycline-inducible hsa-miR-34a-expressing HCC1806 cells were cross-linked and lysed in IP buffer containing protease inhibitors, phosphatase inhibitors, and an RNase inhibitor (rRNasin, 160 U/ml, Promega). After sonication, the lysates were incubated overnight with either 3 μl of ChIP-grade anti-Ago2 (Abcam) or rabbit IgG (Millipore) in a 4°C waterbath, followed by incubation with nProtein A Sepharose 4 Fast Flow (GE Healthcare Bio-Sciences). After washing, the eluted RNA samples were further purified with TRIzol reagent and subjected to real-time RT-PCR analysis to detect miR-34a and $tRNAi^{Met}$, with normalization to the total input.

MTT assay. At 24 h after transfection with 30 nM hsa-miR-34a mimic or negative control siRNA No. 1 (Ambion) or AllStars negative control siRNA (QIAGEN) or at 10 days after Dox exposure, 3.0×10^3 HCC1806 or MCF7 cells were plated in 96-well plates. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays were performed using the Cell Proliferation Kit I (Roche Diagnostics GmbH), as described by the manufacturer. The absorbance of the samples was measured at 595 nm using a microtiter plate reader (FLUOstar Omega, BMG LABTECH). The experiments were done in triplicate.

Cell cycle and apoptosis analyses. HCC1806 and MCF7 cells grown to 80-90% confluence were transiently transfected with either 30 nM hsa-miR-34a mimic (QIAGEN)

or negative control siRNA No. 1 (ambion) or AllStars negative control siRNA (QIAGEN). At 96 h after transfection, the cells were harvested, and cell cycle and apoptosis analyses were performed using a BD FACSCanto II Flow Cytometer (BD Biosciences) with propidium iodide staining solution and a FITC Annexin V Apoptosis Detection Kit II (BD Biosciences) according to the manufacturer's instructions. Cell cycle and apoptosis analyses for the HCC1806 cells stably expressing $tRNA_i^{Met}$ were also performed using the BD FACSCanto II Flow Cytometer (BD Biosciences) with propidium iodide staining solution and a FITC Annexin V Apoptosis Detection Kit II (BD Biosciences). For the cell lines stably expressing miR-34a or $tRNA_i^{Met}$ shRNA, the cell cycle and apoptosis analyses were performed at the University of Calgary using the Nuclear-ID^{TM} Red Cell Cycle Kit (GFP-certified, Enzo Life Sciences) and Annexin V-Cy3 (BioVision), respectively, according to the manufacturers' instructions. All the experiments were done in duplicate.

Protein concentration determination. Stable HCC1806 cells expressing either $tRNA_i^{Met}$-shRNA or control shRNA were grown to 90% confluence, digested with 0.25% trypsin-EDTA (Gibco), and washed twice with ice-cold PBS; 5×10^6 cells of each line were resuspended in 150 µl of radioimmunoprecipitation assay buffer (RIPA). The protein concentration was determined with the Bradford reagent (Bio-Rad) in triplicate samples according to the manufacturer's instructions.

Rescue experiments. HCC1806 cells grown to 80% confluency were transiently transfected with either 30 nM miR-34a mimic (QIAGEN) or in combination with modified $tRNA_i^{Met}$ (miR-34a and $tRNA_i^{Met}$ cotransfection, $tRNA_i^{Met}$ rescue). Twenty-four hours after transfection, the cells were replated in 96-well plates for MTT assay, the

experiments were done in triplicate. Seventy-two hours after transfection, the cells were harvested for apoptosis analysis, the experiments were done in duplicate.

Statistical analysis. Student's *t* test was used to determine the statistical significance of the differences in hsa-miR-34a expression, $tRNA_i^{Met}$ expression, luciferase activity, cell growth, cell migration, colony formation, tube formation, apoptosis, and cell cycle between the groups. Apoptosis and cell cycle analyses were done in duplicate, while other experiments were done in triplicate. A Pearson correlation was used to determine the statistical significance between hsa-miR-34a expression and $tRNA_i^{Met}$ expression in the human breast and other cancer specimens. $P < 0.05$ was considered significant.

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SUPPLEMENTARY FIGURE LEGENDS AND FIGURES

Supplementary figure legends

Fig. S1. $tRNA_i^{Met}$ modifications influence reverse detection by real time RT-PCR. Serial dilutions of modified and unmodified synthetic $tRNA_i^{Met}$ molecules were subjected to RT-PCR analysis using primers specific to mature $tRNA_i^{Met}$.

Fig. S2. Dox-inducible miR-34a expression in stable HCC1806 cells. HCC1806 cells with Dox-inducible miR-34a expression were exposed to 200 ng/ml Dox or left unexposed as a control; at 24 h after exposure, miR-34a expression was visualized using a fluorescence microscope (×20).

Fig. S3. Mass spectrometry analysis of unmodified $tRNA_i^{Met}$. The analysis was done by the supplier (Dharmacon).

Fig. S4. miR-34a alone can not cleave $tRNA_i^{Met}$. 1 μM or 6 μM hsa-miR-34a (Upper panel) or negative control siRNA (Lower panel) was incubated with 1.5 μM heat denatured $tRNA_i^{Met}$ in RNA-RNA binding buffer at 37°C for 2 hours. Products were resolved by 15% denaturing polyacrylamide gel electrophoresis.

Fig. S5. Standard curve for calculation of concentrations of endogenous miR-34a and $tRNA_i^{Met}$ molecules. (*A* and *B*) The synthesized mature miR-34a (A) and unmodified $tRNA_i^{Met}$ (B) molecules were serially diluted to and subjected to qRT-PCR, the standard curves were then generated as described in “METHODS”.

Fig. S6. Molecular number of endogenous miR-34a and $tRNA_i^{Met}$. (*A* and *B*) The number of miR-34a and total $tRNA_i^{Met}$ molecules in IR-exposed HMECs and breast cancer cell lines was calculated according to the Ct value obtained from qRT-PCR and presented as column charts. Asterisks indicate $p < 0.05$.

Fig. S7. Expression levels of total $tRNA_i^{Met}$ in IR-exposed HMECs and breast cancer cell lines. (*A* and *B*) Total RNA samples isolated from human mammary epithelial cells (HMECs) exposed to the indicated doses of ionizing radiation (IR) and the indicated breast cancer cell lines were subjected to quantitative real-time RT-PCR (qRT-PCR) using primers specific to total $tRNA_i^{Met}$. Asterisks indicate $p < 0.05$.

Fig. S8. $tRNA_i^{Met}$ levels and miR-34a copy numbers in cells inducibly expressing miR-34a and the effect of doxycycline on HCC1806 cell biology. (*A*) At 10 days after Dox treatment, the levels of immature $tRNA_i^{Met}$ in HCC1806 cells expressing miR-34a were measured by qRT-PCR. (*B*) Genomic DNA extracted from the indicated cell lines was subjected to miR-34a copy number analysis as described in the “METHODS”. (*C*) HCC1806 cells were plated in 96-well plates and treated with 400 ng/ml or 800 ng/ml Doxycycline or left untreated as control, the MTT (34) assay was performed as described in the “METHODS”. (*D*) Total RNA isolated from HCC1806 cells treated with 800 ng/ml Doxycycline for the indicated time points was subjected to qRT-PCR using specific primers to total $tRNA_i^{Met}$. * indicates $p<0.03$; ** indicates $p<0.007$.

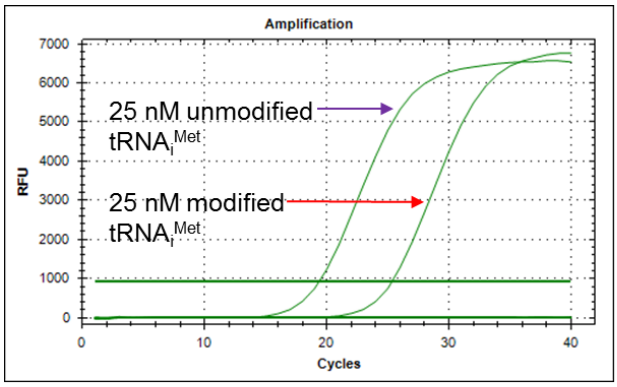
Fig. S9. Ectopic miR-34a slightly induces apoptosis and a cell cycle arrest at G2 in MCF7 cells. (*A*) 96 h after transfection, total RNA isolated from MCF7 cells transfected with either 30 nM hsa-miR-34a mimic or AllStars negative control was subjected to qRT-PCR using primers specific to total $tRNA_i^{Met}$. (*B* and *C*) At 96 h after transfection, the effect of the miR-34a mimic on apoptosis (B) and the cell cycle (C) was detected in MCF7 cells. Asterisks indicate $p < 0.05$.

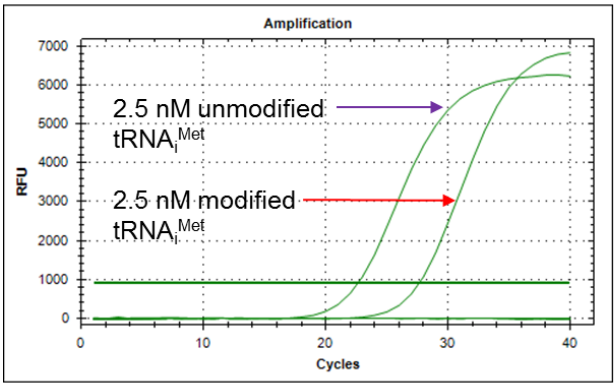
Fig. S10. $tRNA_i^{Met}$ shRNA copy number, aneuploid $tRNA_i^{Met}$-knockdown cells, and off-target effect of $tRNA_i^{Met}$ siRNA. (*A*) Genomic DNA extracted from apoptotic and non-apoptotic cells that were selected from HCC1806 stably expressing $tRNA_i^{Met}$ shRNA was subjected to quantitative PCR with specific primers, *RPP38* served as a reference gene. (*B*) Representatives of aneuploidy in HCC1806 cells expressing $tRNA_i^{Met}$ shRNA visualized with a fluorescence microscope (20×). The arrow indicates aneuploidy. (*C*) HCC1806 cells grown to 90% confluency were transfected with either 50 nM wild-type $tRNA_i^{Met}$ dsiRNA (WT-DsiRNA) or 50 nM scrambled $tRNA_i^{Met}$ dsiRNA (Scr-DsiRNA) or left untransfected as a control. At 48 hours and 96 hours after transfection, total RNA isolated from the transfected and untransfected cells was subjected to qRT-PCR using total $tRNA_i^{Met}$-specific primers. (*D*) 24 hours after transfection, the cells were replated in 96-well plates for MTT assay as described in the “METHODS”. Asterisk indicates $p<0.01$.

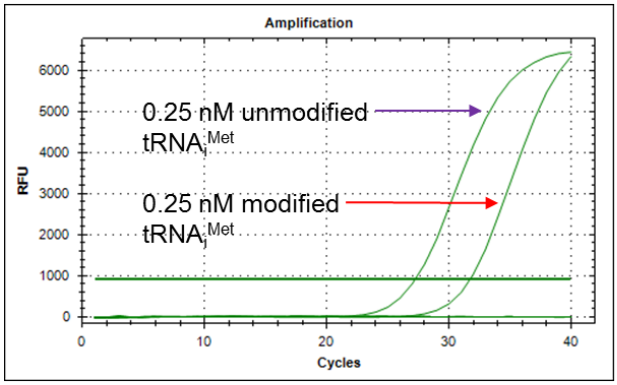
Fig. S11. $tRNA_i^{Met}$ knockdown suppresses breast cancer cell proliferation while inducing cell cycle arrest and apoptosis. (*A*) The effect of $tRNA_i^{Met}$ knockdown on cell proliferation was measured in MCF7 cells expressing $tRNA_i^{Met}$-shRNA. (*B*) The levels of total $tRNA_i^{Met}$ were detected in MCF7 cells expressing $tRNA_i^{Met}$-shRNA by qRT-PCR. (*C* and *D*) The effect of $tRNA_i^{Met}$ knockdown on the cell cycle (C) and apoptosis (D) was determined in MCF7 cells expressing $tRNA_i^{Met}$-shRNA. Asterisks indicate $p < 0.05$.

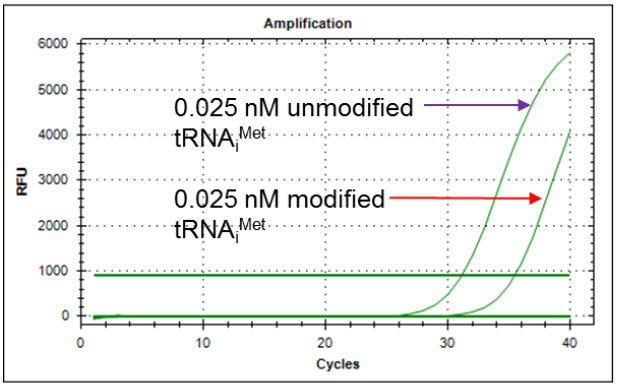
Fig. S12. $tRNAi^{Met}$ copy number in HCC1806 cells stably expressing $tRNA_i^{Met}$. Genomic DNA extracted from HCC1806 cells stably expressing $tRNA_i^{Met}$ and empty vector was subjected to $tRNA_i^{Met}$ copy number analysis as described in the “METHODS”. Asterisks indicate $p < 0.05$.

Fig. S13. Enforced expression of $tRNA_i^{Met}$ promotes cell proliferation, accelerates the cell cycle, and inhibits apoptosis in breast cancer cells. (*A*) The effect of ectopic $tRNA_i^{Met}$ on cell proliferation was detected in HCC1806 cells stably expressing $tRNA_i^{Met}$. (*B*) The levels of total $tRNA_i^{Met}$ were determined in HCC1806 cells expressing $tRNA_i^{Met}$ by qRT-PCR. (*C* and *D*) The effect of ectopic $tRNA_i^{Met}$ on apoptosis (C) and the cell cycle (D) was measured using HCC1806 cells expressing $tRNA_i^{Met}$. Asterisks indicate $p < 0.05$.

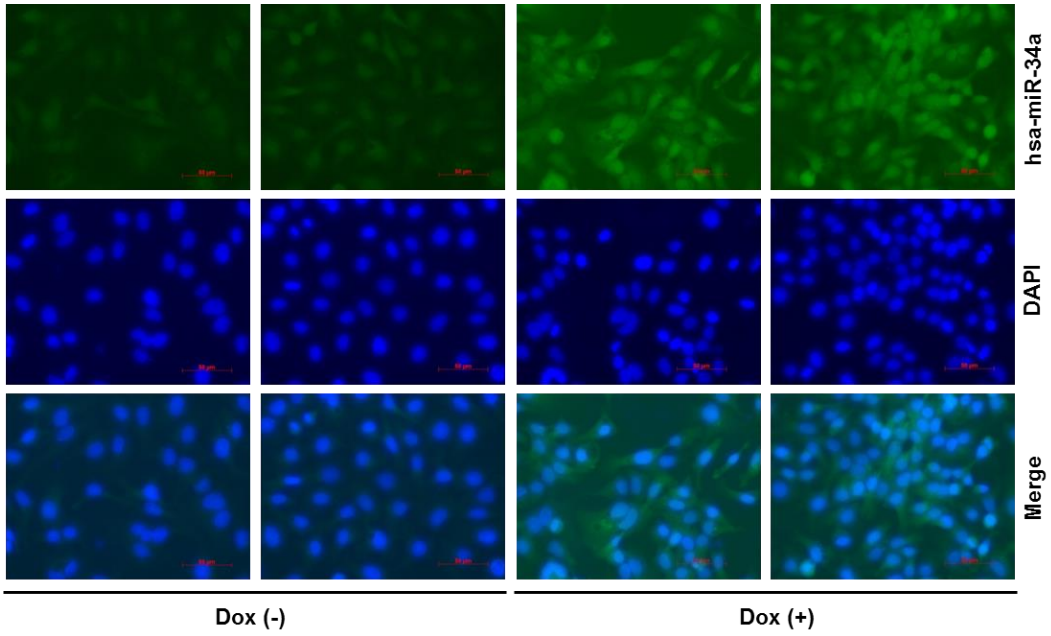








Supplementary Fig. S1

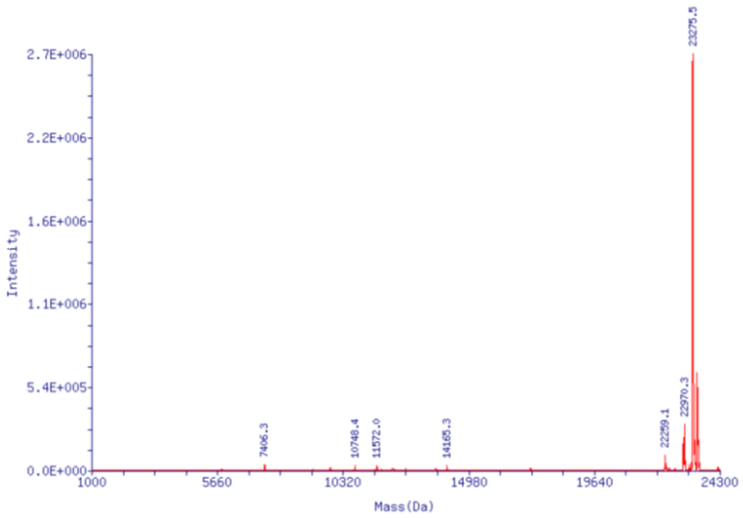


Supplementary Fig. S2

Quality Control Test –
ESI-MS (Electrospray Ionization-Mass Spectrometry)

GE Healthcare Dharmacon, Inc.
2650 Crescent Drive, Suite 100
Lafayette, CO 80026 USA

Sample Name- GETRA-000001
Theoretical Mass- 23275.1
Observed Mass- 23275.5



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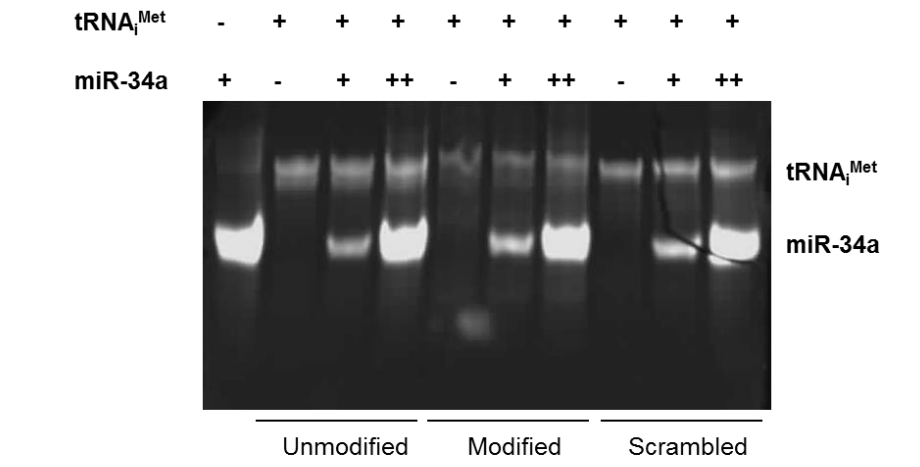
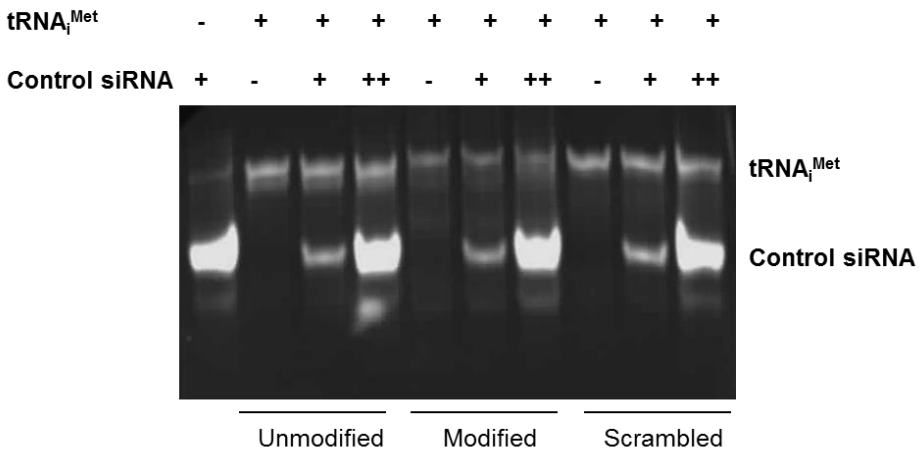
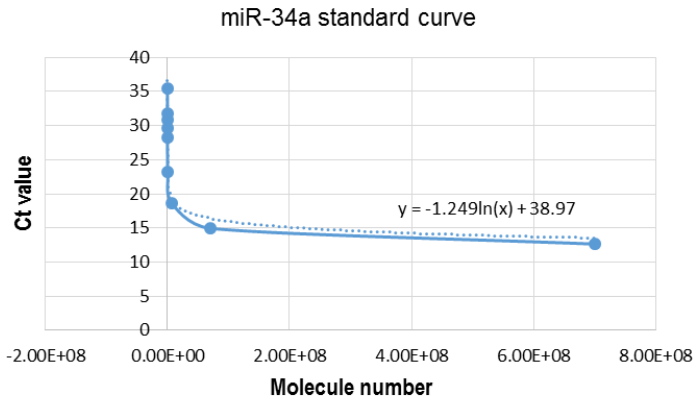
Supplementary Fig. S3





Supplementary Fig. S4

A



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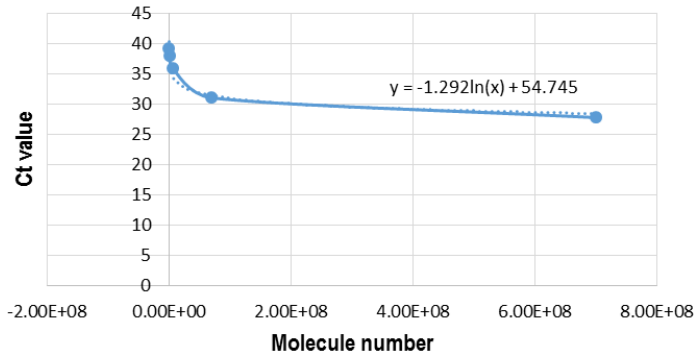
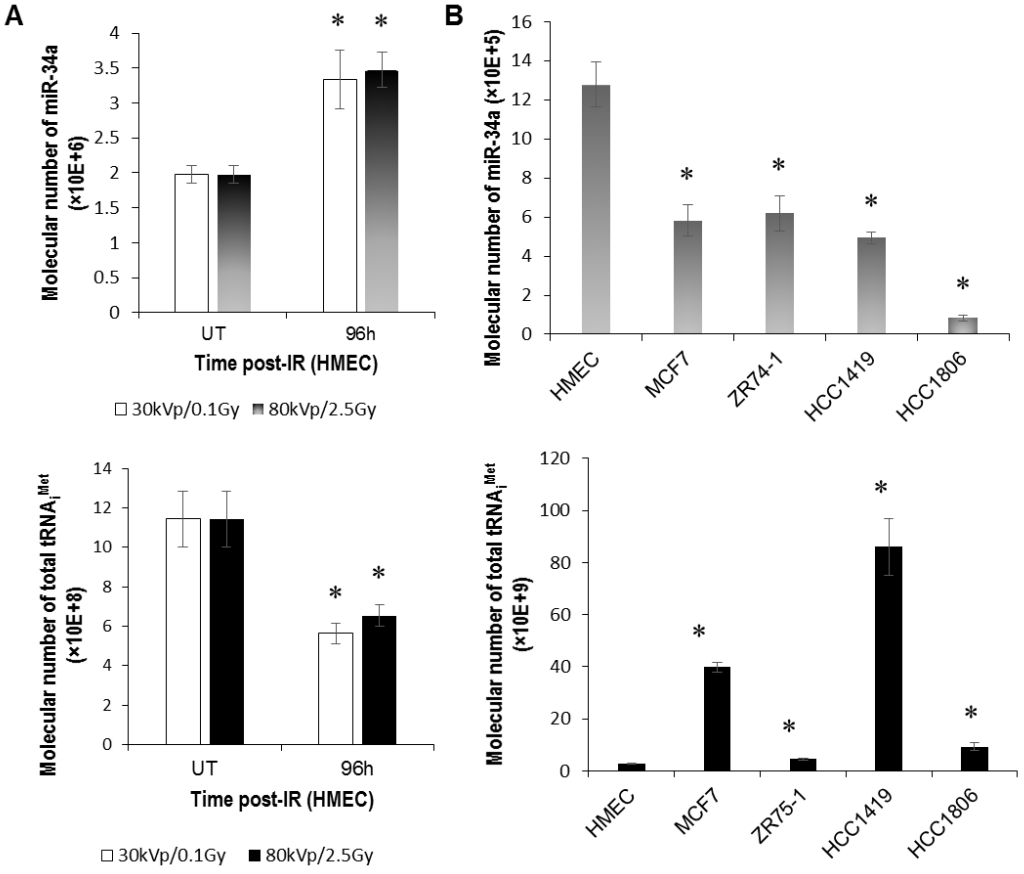
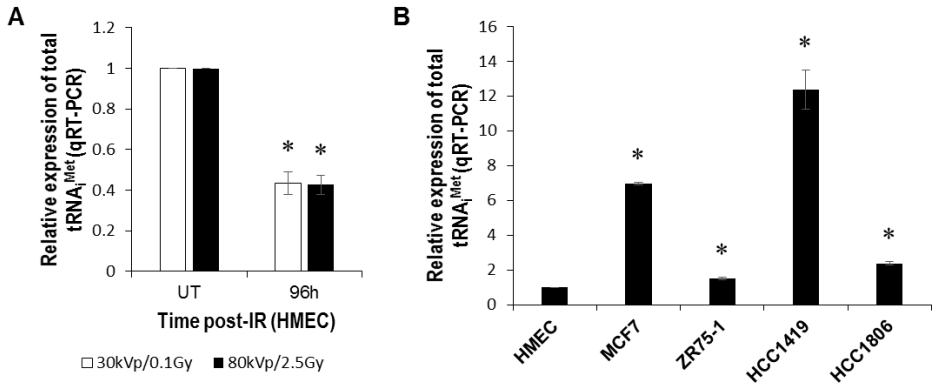


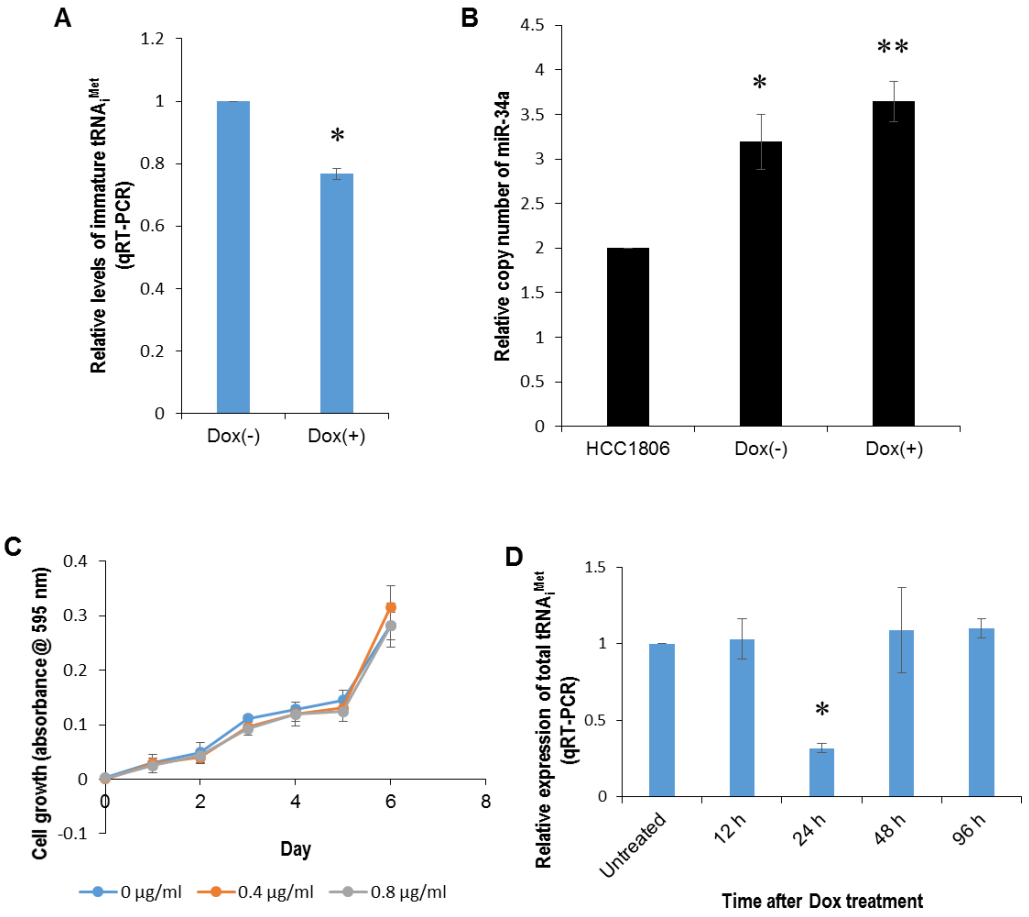
Supplementary Fig. S5



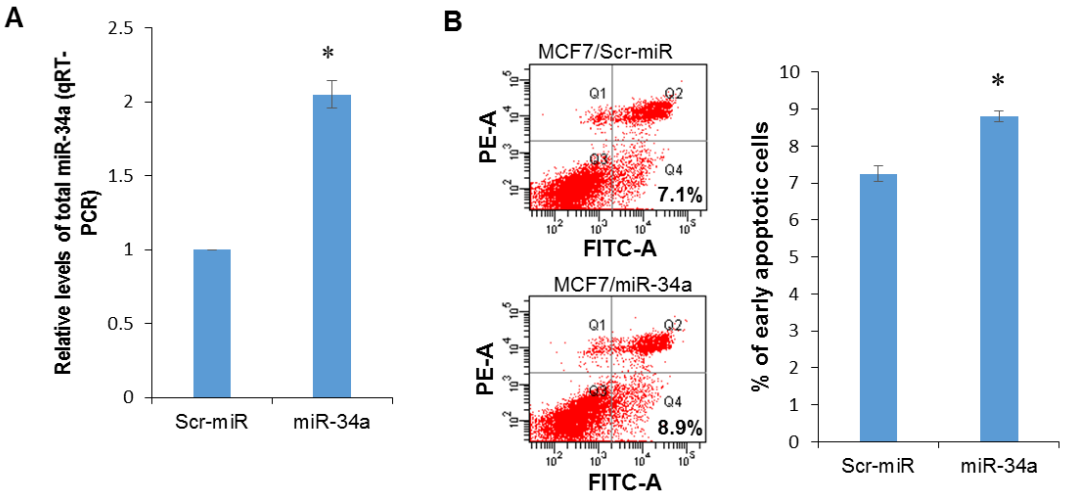
Supplementary Fig. S6

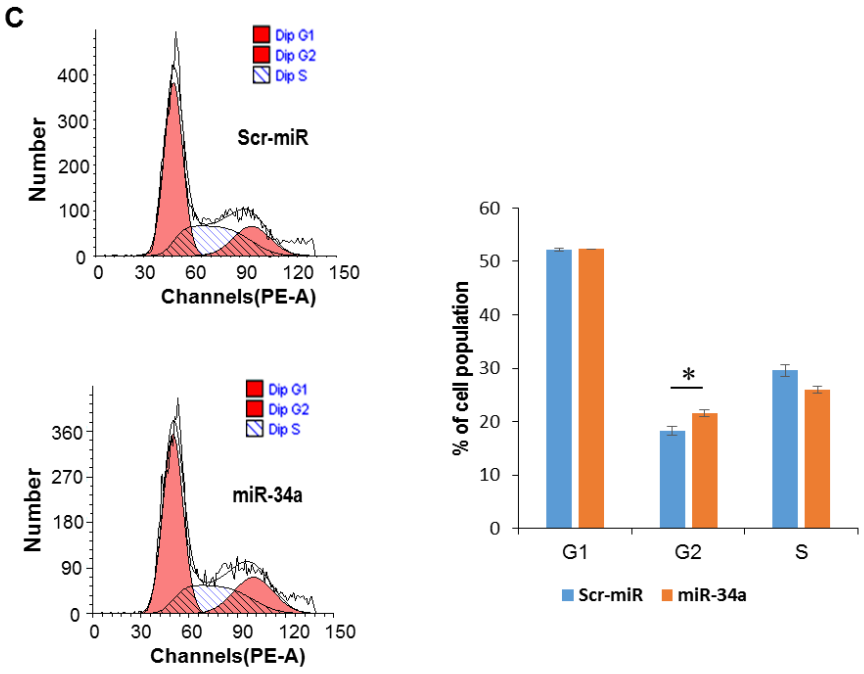


Supplementary Fig. S7

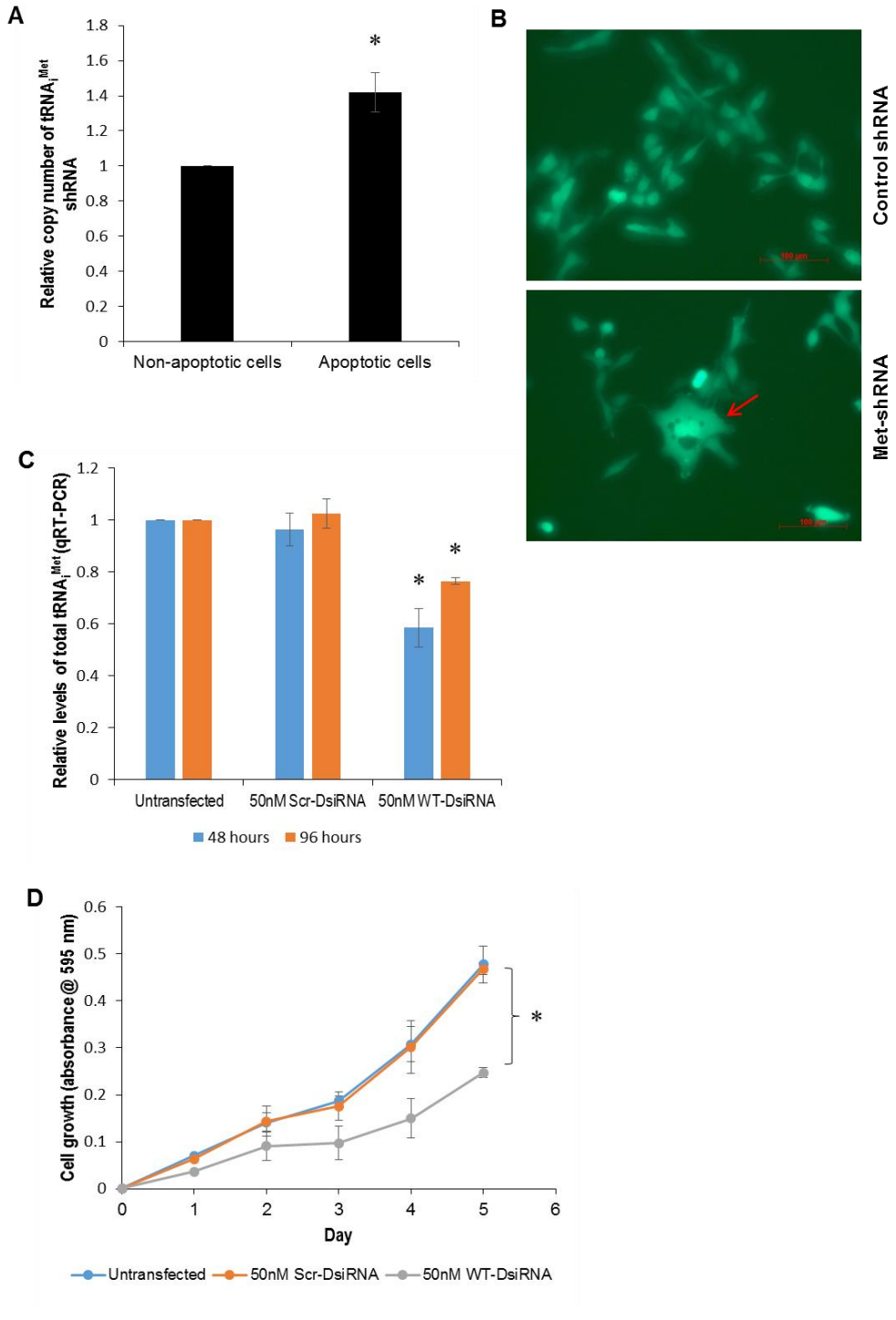


Supplementary Fig. S8

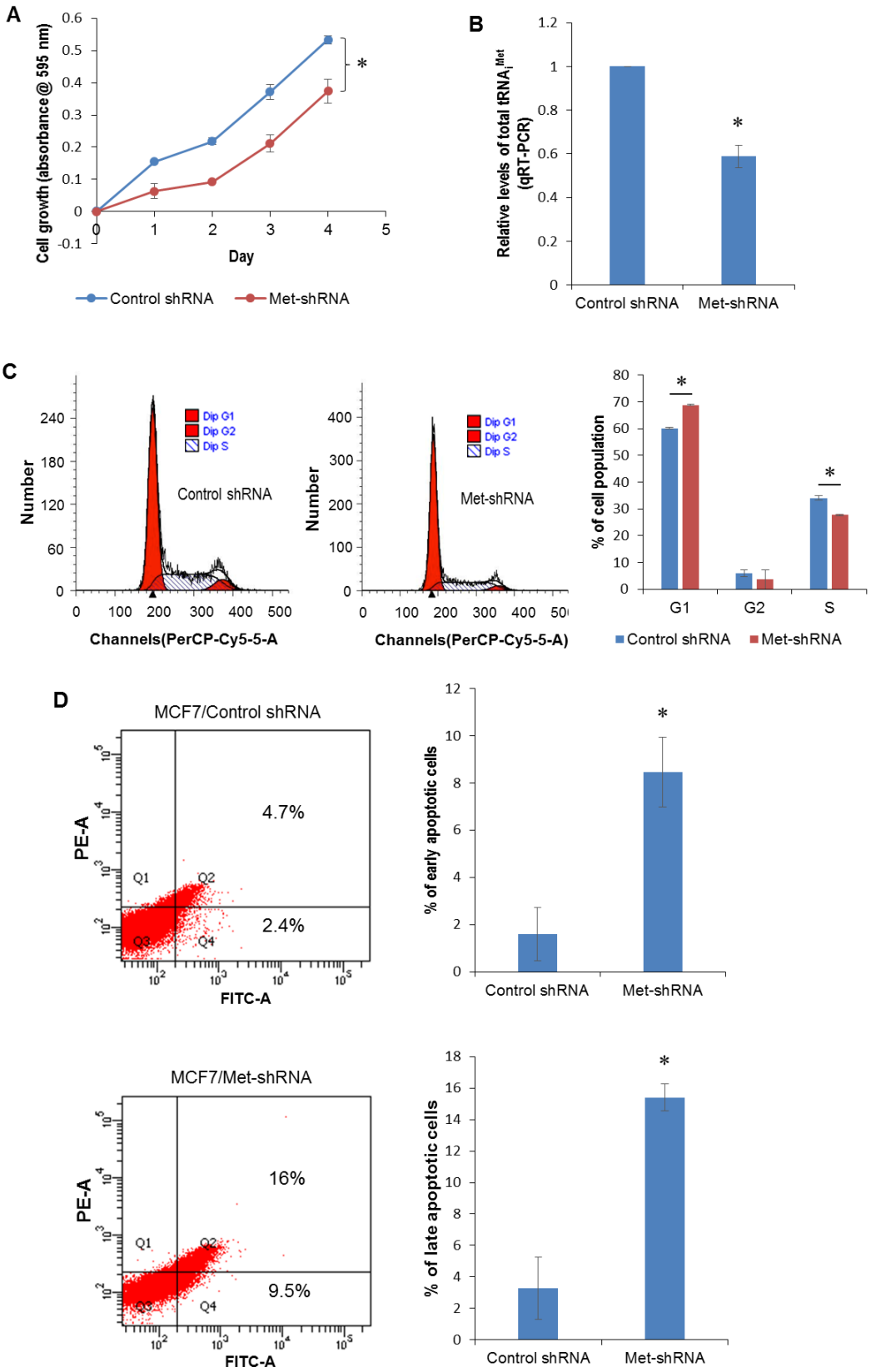




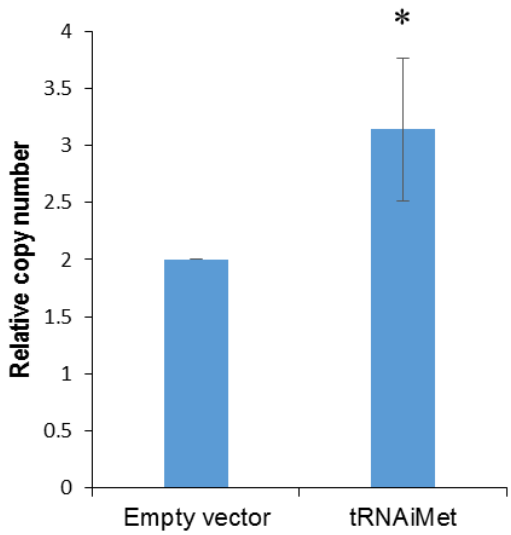
Supplementary Fig. S9



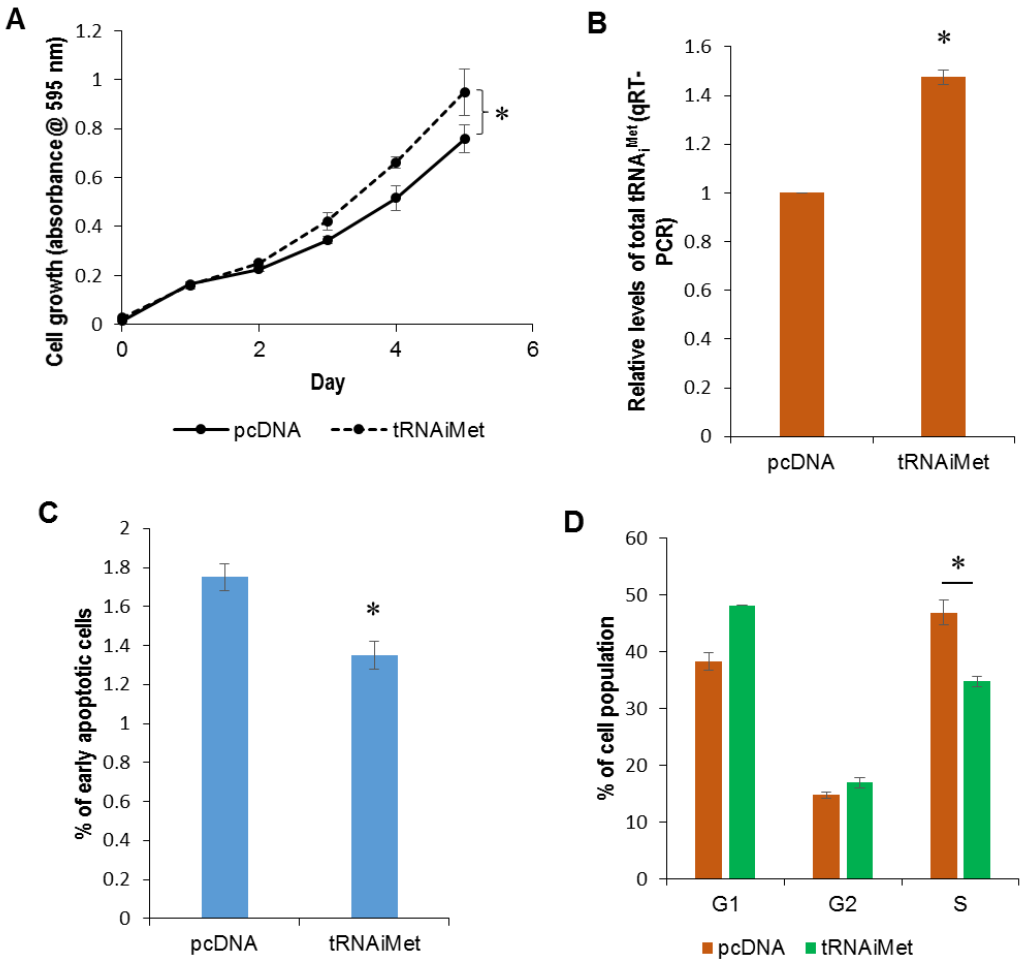
Supplementary Fig. S10



Supplementary Fig. S11



Supplementary Fig. S12



Supplementary Fig. S13

Table S1. Similarity in tRNA$^{Met(CAT)}$ sequence among human, rat, and mouse

Chromosome/tRNA-MetCAT	Mature sequences
mm10_tRNAs_chr13.tRNA959-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr13.tRNA111-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr13.tRNA108-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr13.tRNA90-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr13.tRNA995-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr13.tRNA78-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr3.tRNA792-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr17.tRNA20-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA129-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA142-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA150-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA169-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA171-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA2-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr1.tRNA32-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
rn4_tRNAs_chr17.tRNA3836-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
rn4_tRNAs_chr17.tRNA3845-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
rn4_tRNAs_chr17.tRNA1647-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
rn4_tRNAs_chr17.tRNA3856-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
rn4_tRNAs_chr17.tRNA1583-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
rn4_tRNAs_chr11.tRNA1954-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
rn4_tRNAs_chr2.tRNA7573-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr15.tRNA876-MetCAT	AGCAGAGTGGCGCAGCGGAAGCATGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
mm10_tRNAs_chr13.tRNA82-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATAGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA61-MetCAT	AGCAGAGTGGCGCAGCGGAAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCTAAACCATCCTCTGCTA
rn4_tRNAs_chr17.tRNA3895-MetCAT	AGCAGAGTGGCGCAGCGGGAGCGTGC- - -TGGGCCCATAACCCAGAGGTCGATGGATCGAAACCATCCTCTGCTA
hg19_tRNAs_chr6.tRNA151-ThrCGT	GCAGAGTGGTGCAGCGGAAGCGTGC- - -TGGGCCCGTAACCCAGAGGTCAATGGATCGAAGCCATCCTTGGCTA
hg19_tRNAs_chr9.tRNA1-MetCAT	AGCAGAGTGGTGCAGTGGAAGCATACCTATGGGCCCATAACCCAGAGGTTGATGGATGGAAACCATCCTCTGCTA

Red letters indicate changed nucleotides. Sequences in the purple box indicate the "seed" sequence at 3'-terminal of tRNA$^{Met(CAT)}$ that is predicted to bind miR-34a.

Supplementary Table S1