Supplementary Material

Summary:

The supplementary information includes 1 table (Table S1) and 4 figures (Figure S1 to S4).

Supplementary Figure Legends

Figure S1 RTL-bearing nude mouse model.

(A) Tumor formation rate of P-R/T/L (T) and P-NT (N) inoculated in BALB/c nude mice (n=12, each group). (B) Transplanted RTL cell shows invasive phenotype. Representative metastasis histopathological images of several organs (H & E staining). (C) Electron microscopy analysis of transplanted RTL. Black Bars: 50 μm, Red Bar: 1 μm.

Figure S2 Pathway prediction of identified miRNAs.

The 4 validated most up-regulated miRNAs (miR-762, miR-714, miR-455 and miR-467a) in RTL tissues were subjected to an online pathway prediction by MAS software. Each cellular signaling pathway potentially regulated by these miRNAs is indicated as a predicted p value.

Figure S3 kinetics of miR-467a induction.

(A) Time kinetics of miR-467a induction upon four weekly γ-ray irradiations (1.75-Gy per time). 7 day after each irradiation, miR-467a level in thymus tissues was assessed using q-PCR. The first time point was set as 1 week and by this analogy, and miR-467a level in normal non-irradiated thymus tissues (0 week) was used as control (n=4, each group). (B) Different irradiation-dose response level of miR-467a. The four weekly γ-ray irradiations model were also used, and each irradiation-dose was set as 0.75-Gy, 1.25-Gy and 1.75-Gy (cumulative dose was 3-Gy, 5-Gy and 7-Gy) respectively. 7 day after the last irradiation, miR-467a level in thymus tissues was assessed using q-PCR, and normal non-irradiated thymus tissues (0 Gy) were used as control (n=4, each group). (C)

MiR-467a response to H_2O_2-induced oxidative damage. EL4 cells were treated with 200 ~ 600 μM H_2O_2 for 20 min, and then returned to their conditioned medium, and PBS treatment was used as control (0 μM). 24 h after exposure, miR-467a were assessed by q-PCR (n=4, each group). * $p < 0.05$; ** $p < 0.01$; NS, no significant.

Figure S4 MiR-467a targets Fas and Bax in Molt4 cells.

(A) MiR-467a mimics-transfected Molt4 cells were exposed to 7-Gy irradiation for 5 min using miR-NC mimics as control. 24h after exposure, apoptosis rate was assessed by flow cytometry. The representative scatter diagrams are shown. (B) The values of apoptosis rate are shown in the form of a bargraph (n=3, each group). (C) Predicted wild type (WT) and mutant (MUT) miR-467a binding sites at human Fas and Bax mRNA 3'UTR. (D) Molt4 cells were transfected with miR-467a mimics or miR-NC mimics vector. 24 h later, these cells were exposed to 7-Gy irradiation for 5 min. 24 h after exposure, the protein levels of endogenous Fas and Bax were determined using immunoblotting. (E) Interaction of miR-467a with 3'UTR of human Fas and Bax mRNA. At 48 h after transfection with miR-467a mimics or miR-NC mimics vector, a reporter plasmid containing human Fas / Bax wt-3'UTR or mut-3'UTR and a plasmid expressing renilla luciferase were cotransfected into Molt4 cells. Luciferase activities were measured at 48 h after cotransfection, and normalized data are shown (n=3, each group).* $p < 0.05$; ** $p < 0.01$; NS, no significant.

Supplementary Figures

Figure S1

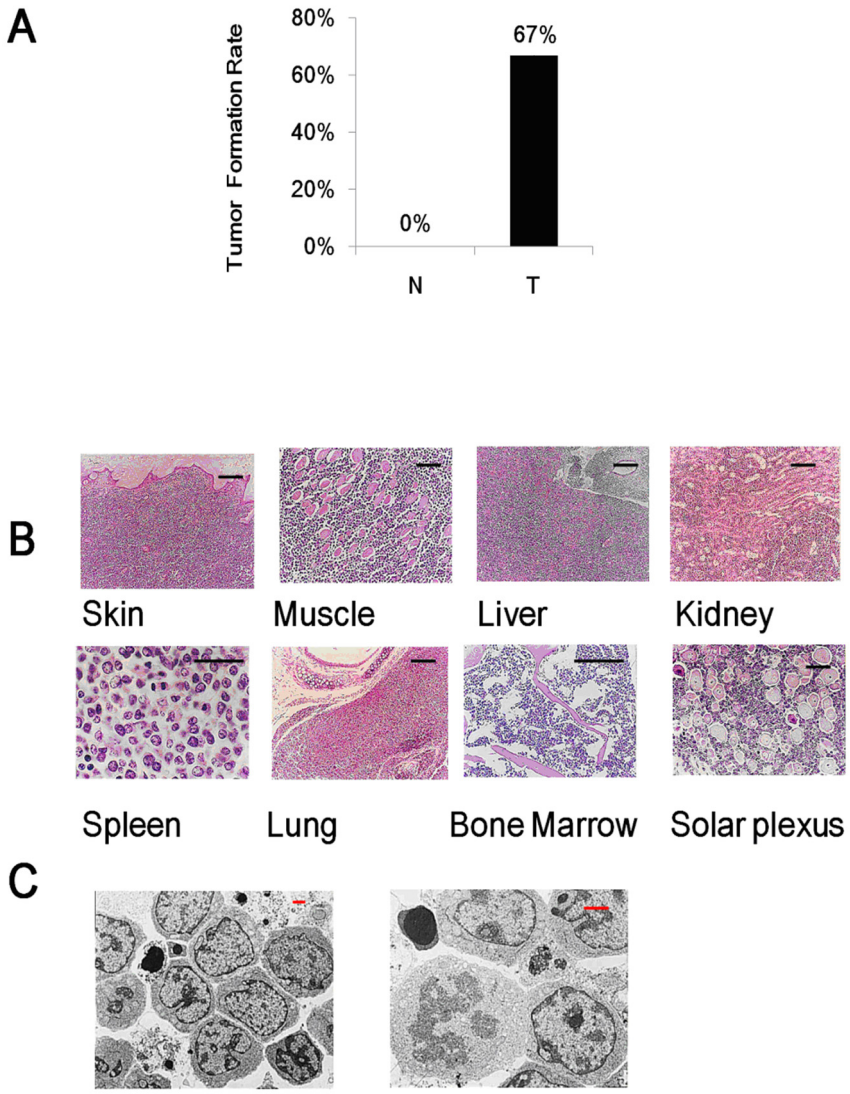


Figure S2

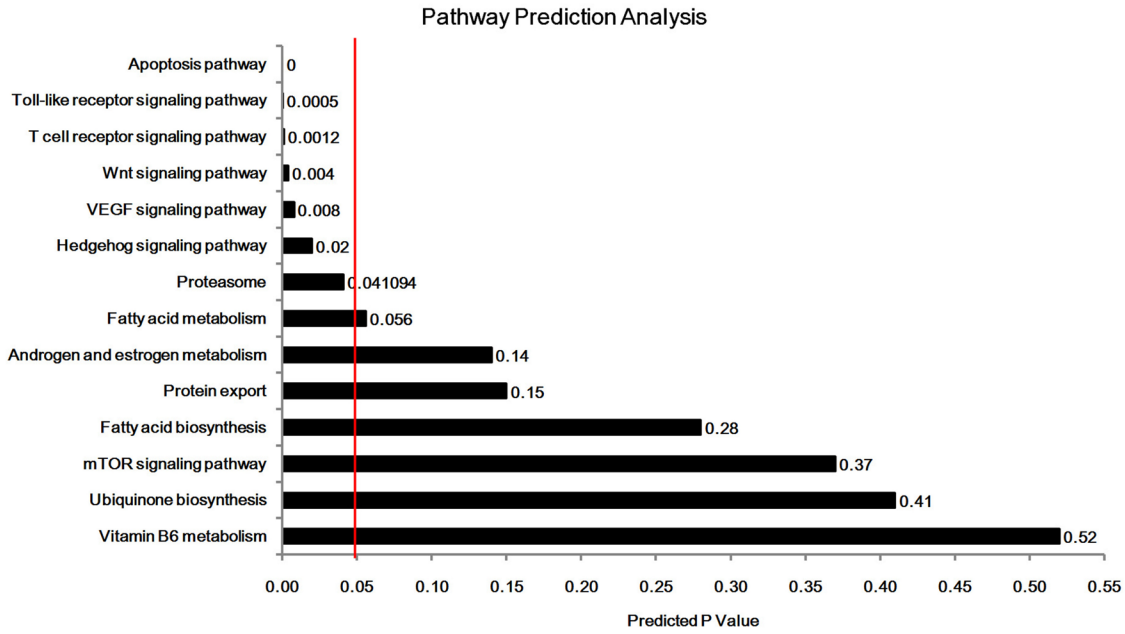

Pathway Prediction Analysis
Apoptosis pathway
Toll-like receptor signaling pathway
T cell receptor signaling pathway
Wnt signaling pathway
VEGF signaling pathway
Hedgehog signaling pathway
Proteasome
Fatty acid metabolism
Androgen and estrogen metabolism
Protein export
Fatty acid biosynthesis
mTOR signaling pathway
Ubiquinone biosynthesis
Vitamin B6 metabolism
0
0.0005
0.0012
0.004
0.008
0.02
0.041094
0.056
0.14
0.15
0.28
0.37
0.41
0.52
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55
Predicted P Value

Figure S3

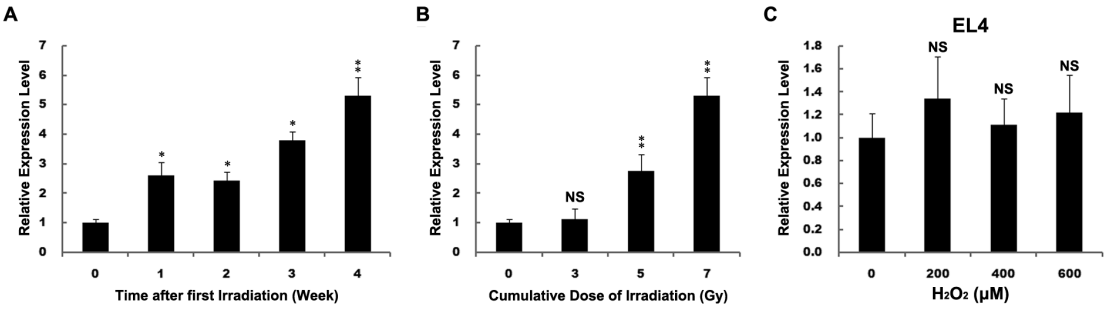
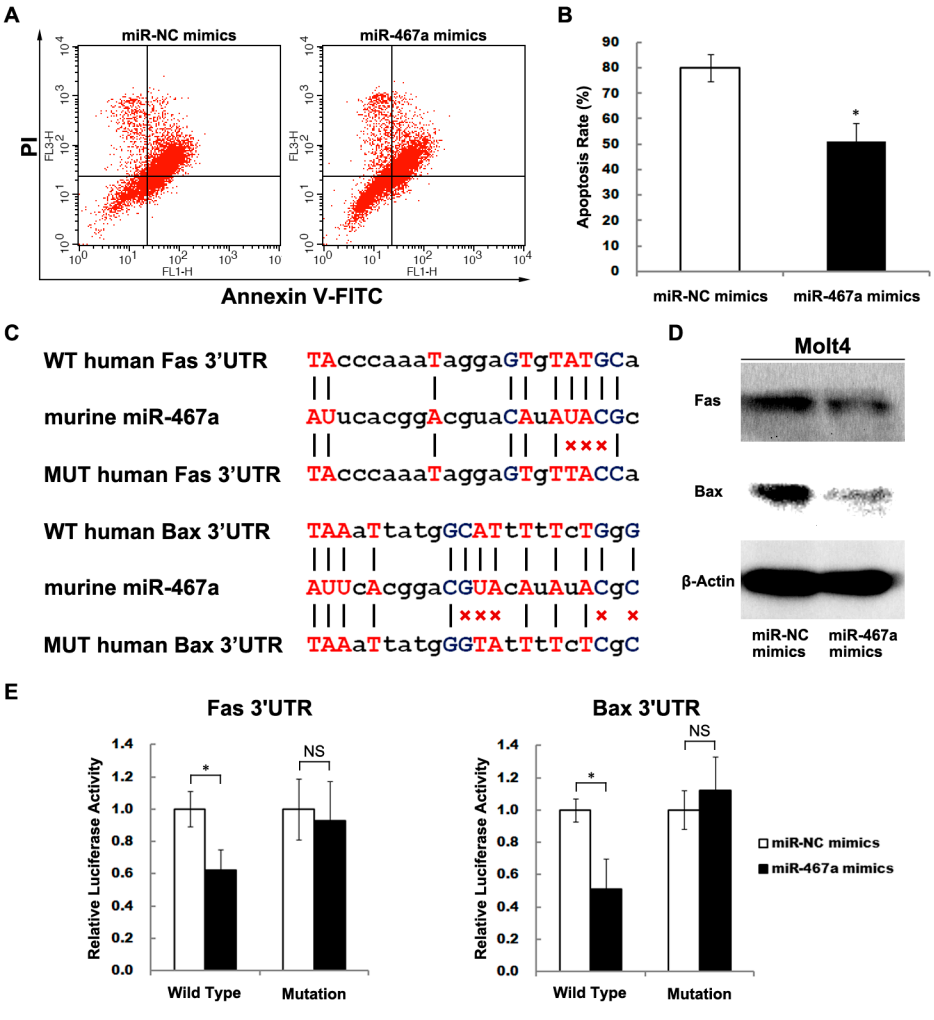

A
Relative Expression Level
Time after first Irradiation (Week)
B
Relative Expression Level
NS
Cumulative Dose of Irradiation (Gy)
C
EL4
Relative Expression Level
NS
NS
NS
H2O2 (μM)

Figure S4


A
miR-NC mimics
miR-467a mimics
PI
FL3-H
FL1-H
Annexin V-FITC
B
Apoptosis Rate (%)
miR-NC mimics
miR-467a mimics
C
WT human Fas 3'UTR
murine miR-467a
MUT human Fas 3'UTR
WT human Bax 3'UTR
murine miR-467a
MUT human Bax 3'UTR
D
Molt4
Fas
Bax
β-Actin
miR-NC mimics
miR-467a mimics
E
Fas 3'UTR
Bax 3'UTR
Relative Luciferase Activity
NS
Wild Type
Mutation
miR-NC mimics
miR-467a mimics

Supplementary Tables: Table S1

Table S1. The predicted apoptosis associated genes targeted by indicated miRNAs

Pathway	P value	Candidate gene	MiRNA
Apoptosis	0.0	Bad	mmu-miR-455
Apoptosis	0.0	Bax	mmu-miR-467a;mmu-miR-455
Apoptosis	0.0	Birc3	mmu-miR-467a;mmu-miR-762
Apoptosis	0.0	Capn1	mmu-miR-762
Apoptosis	0.0	Casp6	mmu-miR-455
Apoptosis	0.0	Cflar	mmu-miR-455;mmu-miR-467a
Apoptosis	0.0	Chuk	mmu-miR-467a
Apoptosis	0.0	Dffa	mmu-miR-455
Apoptosis	0.0	Fas	mmu-miR-467a
Apoptosis	0.0	Fasl	mmu-miR-467a
Apoptosis	0.0	Ikbkg	mmu-miR-467a
Apoptosis	0.0	Il1b	mmu-miR-455
Apoptosis	0.0	Il1rap	mmu-miR-467a
Apoptosis	0.0	Nfkb2	mmu-miR-762
Apoptosis	0.0	Nfkbia	mmu-miR-467a
Apoptosis	0.0	Pik3cd	mmu-miR-467a
Apoptosis	0.0	Rela	mmu-miR-762
Apoptosis	0.0	Tnf	mmu-miR-762;mmu-miR-455
Apoptosis	0.0	Tnfrsf1a	mmu-miR-455
Apoptosis	0.0	Trp53	mmu-miR-762
Apoptosis	0.0	Xiap	mmu-miR-467a

Luciferase reporter plasmids carrying mouse Fas / Bax 3’-UTR

Luciferase-UTR reporter constructs were generated by introducing the full length mouse Fas 3’-UTR into pGL3 promoter vector (Promega). We first amplified the Fas 3’-UTR sequence by PCR using primers Fas-F (5′TGGAtctagaACTACCTCAGTTCCAGCCATGA3') and Fas-R (5' GCTGtctagaGAAATGCAAAAAGAGATACTTTAAT 3') and mouse genome cDNA as a template. The PCR product was ligased into pGL3 promoter vector by the Xba1 site. All PCR products were verified by DNA sequencing.

The sequencing data of WT Fas 3’-UTR were:

TCGACGCAGAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGGAA
AGATCGCCGTGTAATTCTAGAACTACCTCAGTTCCAGCCATGAAGAGAGG
AGAGAGCCTGCCACCCATGATGGAAACAAAATGAATGCCAACTGTATTGA
CATTGGCAACTCCTGGTGTGTTCTCTTTGCCAGCAAATGGTAGTTGATACT
CAGTGAGGGTCAAATGACTAGCAGGTTCCAGGGACTGCTTCTGTTATTCT
CTGCAGTTGCTGAGATGAACCATTTTCTCTGTCTACTGCAATTTTTACATTC
AAATGTCCATGAAATTTGTATTAAATGTGAAGTGGAATCTGCAGTGTTTGT
GTTTATATTCATATACTATGAACTGAGGAGAATTATAAACTGAAACAAATACT
CGCAGTTAATTGAAGACCTTCCATTGATGGACAGTTCTTTTCCTCTCTATG
TGGAAATGTATAATAGAAGAAATAATTTTTAAATTAAAGTATCTCTTTTTGCA
TTTCTCTAGAGTCGGGGCGGCCGGCCGCTTCGAGCAGACATGATAAGAT
ACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCT
TTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGC
AATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGG
GGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGT
AAAATCGATAAGGATCCGTCGACCGATGCCCTTGAGAGCCTTCAACCCAG

TCAGCTCCTTTCTCGTGGGCGCGGGGGATGACTATCGTCGTCGCACCTA
TGACTGTCTTCCCTATCATGCAACTTCGTATGAGAAGAGGCGGGAGCGC
GCTTCGCTTCCTCGCTCACTGACACGATGCGCTCGGTCGGTTCGCTGCG
CGAGCGGGATCAGCTCACTCTAAGCGGTGATACCGGTATCTCTCATCACG
GTACGCAGGAAGACATGTACAAGGCAGCCATGCAGAACGTCAAGCGCGT
GCTGCGGGTC

The mutation Fas 3'-UTR, wild type and mutation Bax 3'-UTR was generated by chemical synthesis and then ligased into pGL3 promoter vector by the Xba1 site. The sequence of mutation Fas 3'-UTR that were ligased into pGL3 promoter vector were:

AAAACTACCTCAGTTCCAGCCATGAAGAGAGGAGAGAGCCTGCCACCCA
TGATGGAAACAAAATGAATGCCAACTGTATTGACATTGGCAACTCCTGGT
GTGTTCTCTTTGCCAGCAAATGGTAGTTGATACTCAGTGAGGGTCAAATG
ACTAGCAGGTTCCAGGGACTGCTTCTGTTATTCTCTGCAGTTGCTGAGAT
GAACCATTTTCTCTGTCTACTGCAATTTTTACATTCAAATGTCCATGAAAT
TTGTATTAAATGTGAAGTGGAATCTGCAGTGTTTGTGTTTATATTCATATA
CTATGAACTGAGGAGAATTATAAACTGAAACAAATACTCGCAGTTAATTG
AAGACCTTCCATTGATGGACAGTTCTTTTCCTCTCTATGAGGAAATCATTA
ATAGAAGAAATAATTTTTAAATTAAAGTATCTCTTTTTGCATTTCTATTGCT
CTGTTGTCTTGCT

(The red highlighted were mutated with wild types)

The sequence of wild type Bax 3'-UTR that were ligased into pGL3 promoter vector were:

GCCTCCCACTGCCTT-GGACTGTGTCTTTTCTTCATAAATTATGACATTTT-C

CTGGGATGAATGGGGGAAGGGGAAAGGCATTTTTCTTACTTTTGTAATTAT

TGGGAGGGGTGGGAATGGTGGCCTGGGGAGGCGCCAATAAACCTCAGG

TCCACTTTGGATTGTA

The sequence of mutation Bax 3'-UTR that were ligased into pGL3 promoter vector were:

GCCTCCCACTGCCTT-GGACTGTGTCTTTTCTTCATAAATTATGACATTTT-C

CTGGGATGAATGGGGGAAGGGGAAACCGATTTTTCTTACTTTTGTAATTAT

TGGGAGGGGTGGGAATGGTGGCCTGGGGAGGCGCCAATAAACCTCAGG

TCCACTTTGGATTGTA

(The red highlighted were mutated with wild types)