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Supplemental information

Activation of long-non-coding RNA NEAT1 sponging microRNA-147 inhibits radiation damage by targeting PDPK1 in troxerutin radioprotection

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Supplementary data

S-Fig.1 Sequencing results of NEAT1 and sgRNAs of miR-147. Related to Fig. 1. A. Sequencing results of NEAT1(miR-147)-GV272 positive recombinant clone. B. Sequencing results of NEAT1(miR-147a)-GV272 positive recombinant clone. C. Sites of sgRNAs and primers in miR-147 gene.

S-Fig2. NEAT1 targets miR-147a and miR-147a can target PDPK1. Related to Fig. 1. A. miR-147a was predicted to be a target miRNA of NEAT1. **B**. The relative luciferase activity in 293T cells co-transfected with miR-147a and NEAT1-WT or NEAT1-MUT. **C**. The relative expression of miR-147a influenced by NEAT1 in L02 cells. U6 is the internal control. **D**. The target area of miR-147a on PDPK1 3'UTR. **E**. The relative luciferase activity in 293T cells co-transfected with miR-147a and PDPK1-3'UTR-WT or PDPK1-3'UT-MUT. **F**. The relative expression of PDPK1 mRNA and protein in L02 cells in Pre-miR group. **G**. After 293T cells were transfected with miR-147 overexpression plasmid for 48 hours, Act D 10 μg/mL was added to each group to inhibit the synthesis of mRNA. After the RNA was extracted at the corresponding time point, the expression of PDPK1 mRNA at each time point was analyzed by RT-PCR, and the PCR amplified product was subjected to 3% agarose gel electrophoresis. n=3; luciferase activity. C, F, G, n=3; B, E, n=5. * $p < 0.01$; NS: not significant. Data were shown as mean±SEM.

S-Fig3. TRT up-regulated PDPK1 by targeting miR-147 in non-irradiated TC and V79 cells and pre-miR-/- mice. Related to Fig. 3. A. The expression of miR-147 in the cells at the different time points after the radiation. At 1 h after the radiation, miR-147 increased significantly. **B.** The expression of PDPK1 mRNA in cells at different time points after the radiation. At 2 h after radiation, PDPK1 mRNA decreased significantly. **C**. The expression of PDPK1 protein in the cells at the different time points after the radiation. At 2 h after the radiation exposure, the expression of PDPK1 protein decreased significantly. **D**. The effect of TRT on miR-147 after miR-147 overexpression. Pre-miR was transfected after TRT treatment, after 48 h, and the total RNA was extracted. TRT significantly reduced the expression of miR-147. **E**. The

effect of TRT on PDPK1 mRNA after miR-147 overexpression. Pre-miR was transfected with TRT treatment, after 48 h, total RNA was extracted. TRT significantly increased the expression of PDPK1 mRNA, compared with pre-miR transfected alone. **F**. The effect of TRT on PDPK1 protein after miR-147 overexpression. Pre-miR was transfected with TRT treatment, after 48 h, and the total protein was extracted. TRT significantly increased the expression of PDPK1 protein, compared with pre-miR transfected alone. n=3. * $p < 0.01$. Data were shown as mean±SEM.

S-Fig.4 TRT down-regulated miR-147 level in irradiated cells. Related to Fig. 3. A-C. Effect of TRT on the expression of miR-147 after the transfection of pre-miR and radiation injury. D-F. Effect of TRT on the expression of miR-147 after transfection of miR-inh and radiation injury. n=3. * $p < 0.01$; NS: not significant. Data were shown as mean±SEM.

S-Fig. 5 TRT up-regulated PDPK1 levels by targeting miR-147 to activate AKT in irradiated TC and V79 cells. Related to Fig. 3. A. Effect of TRT on the expression of PDPK1 mRNA after the transfection of pre-miR and radiation injury. **B**. Effect of TRT on the expression of PDPK1 protein after transfection of pre-miR and the radiation injury. **C**. Effect of TRT on the expression of p-AKT protein after the transfection of pre-miR and the radiation injury. **D**. Effect of TRT on the expression of PDPK1 mRNA after transfection of miR-inh and the radiation injury. **E**. Effect of TRT on the expression of PDPK1 protein after the transfection of miR-inh and radiation injury. **F**. Effect of TRT on the expression of p-AKT protein after transfection of miR-inh and the radiation injury. n=3. * $p < 0.01$; NS: not significant. Data were shown as mean±SEM.

S-Fig.6 Effect of TRT on the apoptosis of the liver, thymus and lung after the radiation injury in pre-miR-/- mice. Related to Fig. 3. Scale bar, 10 μm.

S-Fig.7 TRT up-regulated p-AKT expression by targeting PDPK1 in radioprotection in TC and V79 cells. Related to Fig. 4. A. The effect of TRT on PDPK1 mRNA after knockdown of PDPK1 mRNA in non-irradiated cells. PDPK1 shRNA was transfected, and after 46 h, TRT was added, followed by incubation for

another 2 h, after which the total RNA was extracted. **B**. The effect of TRT on PDPK1 protein after the knockdown of PDPK1 mRNA in non-irradiated cells. **C**. The effect of TRT on AKT protein after the knockdown of PDPK1 mRNA in non-irradiated cells. **D**. The effect of TRT on PDPK1 protein after knockdown of PDPK1 mRNA in irradiated cells. **E**. The effect of TRT on p-AKT protein after knockdown of PDPK1 mRNA in irradiated cells. **F**. The effect of TRT on PDPK1 protein after knockdown of PDPK1 mRNA in irradiated cells.**G.** The effect of TRT on p-AKT protein after knockdown of PDPK1 mRNA in irradiated TC cells. **H.** The effect of TRT on the expression of p-AKT protein after the knockdown of PDPK1 mRNA in irradiated V79 cells. n=3. * $p < 0.01$; NS: not significant. Data were shown as mean±SEM.

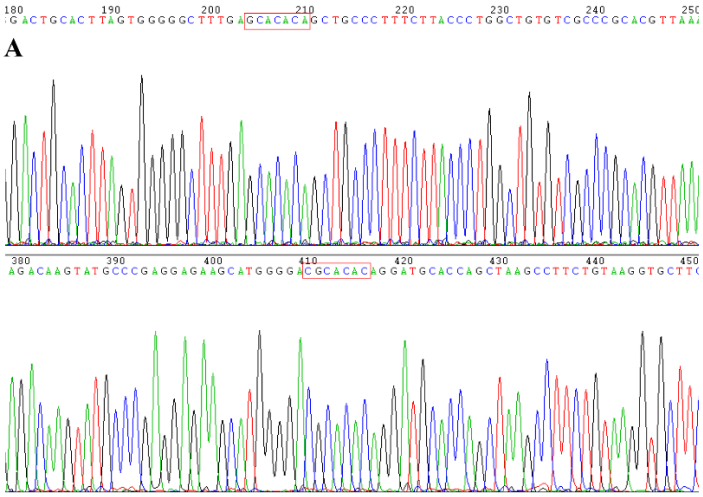
S-Fig. 8 TRT down-regulated p-JNK expression by targeting AKT during the radioprotection in cells. Related to Fig. 6. A. The effect of TRT on p-JNK protein after the knockdown of AKT1 mRNA in irradiated L02 cells. **B**. The effect of TRT on p-JNK protein after knockdown of AKT1 mRNA in irradiated MTEC-1 cells (cells purified from TC). **C**. The effect of TRT on p-JNK protein after knockdown of AKT1 mRNA in irradiated V79 cells. **D**. The effect of TRT on p-JNK protein after overexpression of AKT1 mRNA in irradiated L02 cells. **E**. The impactof TRT on p-JNK protein after overexpression of AKT1 mRNA in irradiated MTEC-1 cells (cells purified from TC). **F**. The effect of TRT on p-JNK protein after overexpression of AKT1 mRNA in irradiated V79 cells. n=3. * $p < 0.01$; NS: not significant. Data were shown as mean±SEM.

S-Fig. 9 TRT up-regulated PDPK1 levels by targeting NEAT1 in L02 cells after the radiation exposure. Related to Fig. 5. A. The relative expression of PDPK1 mRNA decreased in miR-147 group, compared with NC, but increased in miR-147 + NEAT1 group, compared with miR-147 + NC. **B**. The relative expression of PDPK1 protein decreased in miR-147 group compared with NC, but increased in miR-147 + NEAT1 group, compared with miR-147 + NC. **C**. Effect of TRT on the expression of PDPK1 mRNA after transfection of NEAT1 and radiation injury. **D**. Impact of TRT on the expression of PDPK1 protein after the transfection of NEAT1 and the radiation

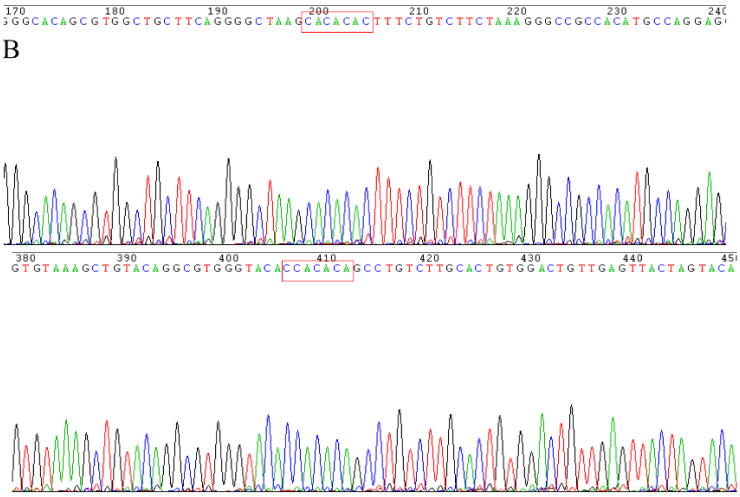
injury. n=3. * $p < 0.01$; NS: not significant. Data were shown as mean±SEM.

S-Fig.10 TRT up-regulated PDPK1 levels by targeting miR-147 during the radioprotection in the mice (A-D: thymus; E: liver). Related to Fig. 6. A. The expression of PDPK1 protein at the different time points in the liver after the radiation. **B**. Effect of TRT on the expression of NEAT1 expression after the radiation in pre-miR -/- mice. **C**. Effect of TRT on the expression of PDPK1, p-AKT and p-JNK proteins after radiation in pre-miR -/- mice. **D**. Effect of TRT on histopathological changes in the thymus after the radiation in pre-miR -/- mice treated with TRT. Scale bar, 100 μm. **E**. The effect of TRT on NEAT1 in the cytoplasm and nucleus in pre-miR -/- mice co-treated with TRT and 6 Gy. n=8. * $p < 0.01$; NS: not significant. Data were shown as mean± SEM.

S-Fig.11 Mechanism of TRT intervention on radiation injury by modulating NEAT1/miR-147/PDPK1 axis. Related to conclusions. Related to Fig. 6.



A



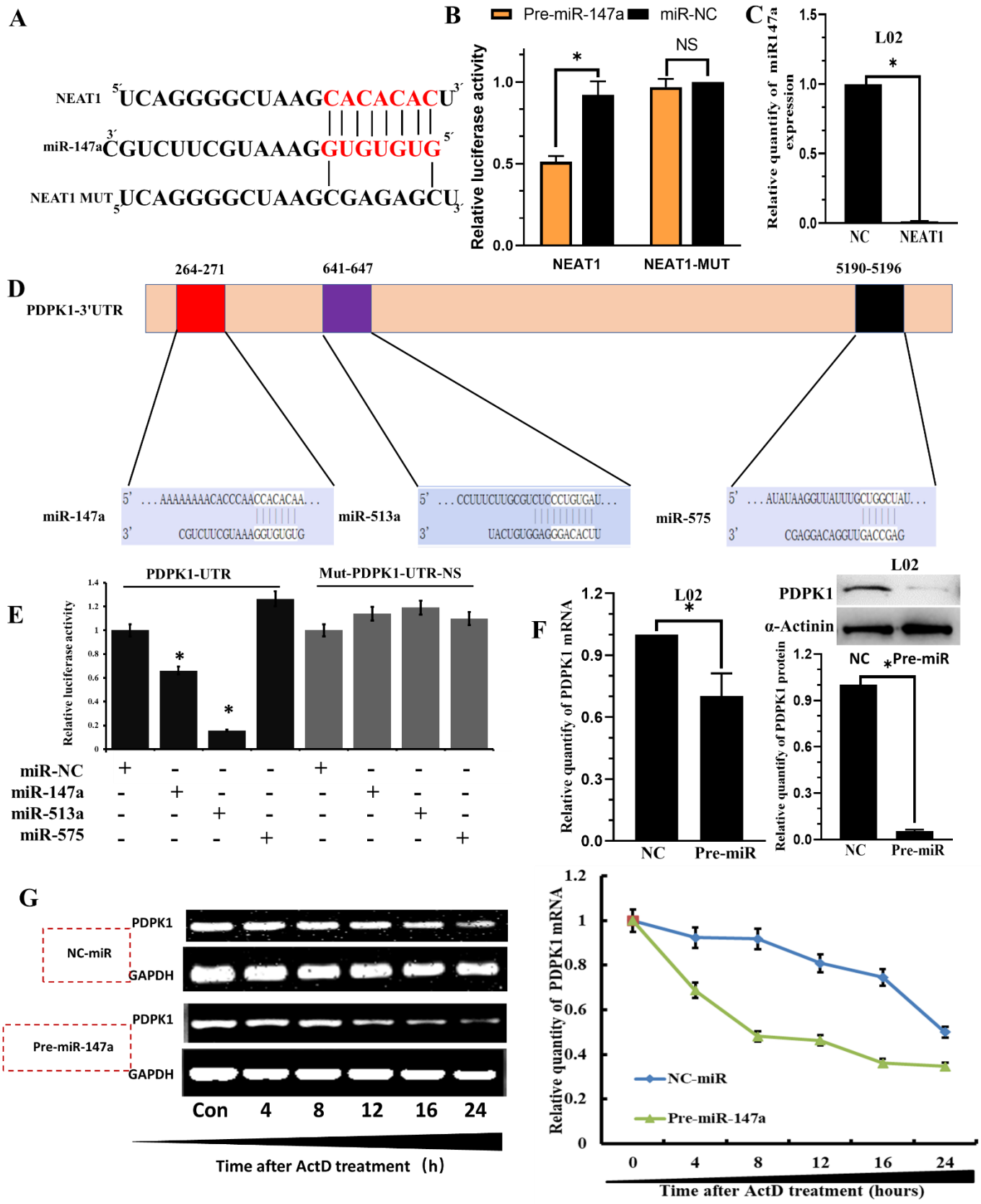
B

C

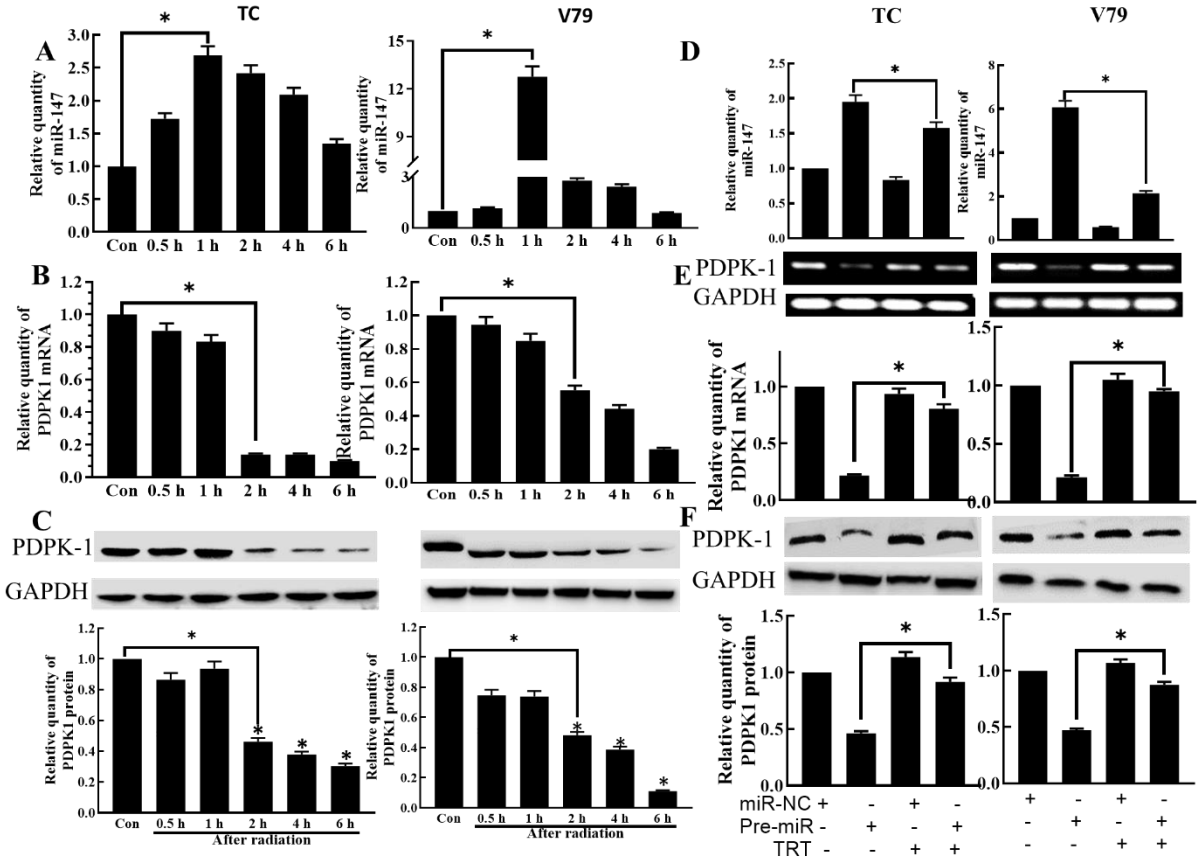
CTCCCAGGGTCTGGGATGAGAGAACACTGATGAGGGCAAATGTCAGGATGTCTGACTGAGAACTGACTTCGGCTTATACCTTCATGAAGCAATTGCAATGAGGAAGT
TTTAAGAGACTATTGTGTGCCTTGCCCTGTGTTTTGACCATTGTACATGCATTTACAAGGAACGAATGTGCCTCTTGAATTTAAATGCTATCTTAGCAAATTAATAATC
TTGAAACTTCTAGTGAAAGCAAGACAGGTGTATGACTGCTCTCCCTGGTCCCGAGACACCTAGAGACCAAGGGATTTTCGGGGACAGACTGGTAGAAACAGGCCCA
GTGTGATATTCTGTGCTTGTTTCAGAGATGAGGAGAAGAGTGTGTGTGTATGTATGTGTTTTGTTTTTTAATTATTGCTATAGTTTTTCCTTAGAGGGGTATAGACGAT
TCCTCCAAGGAAGTGTGAGGCTGGAGAGTAGATGGTTAACACAATGTCTTGCTTTCTGTTTTCAAAGCTTATAACCATCAACCAGCAATGGAAGCCCGTTGAGGAGC
TGCAAAAAGTCGGGAGGGCAACCAGATGATTGCTCACCACTCCTCTCTTCCAAAGAACACTCTATGAATCTAGTGGAAACATTTCTGCACAAACTAGATGTTGAT
GCCAGTGTGCGGAAATGCTTCTGCTACATTTGTAGGGTTTGCCTGCATTCTTTGGATCCTGCATTAGCAAGTGAAGGTAGCACATAGTCTAAAATAGTTTTCTGTG
TTTATTGGTGTAAATTTCAATTTTACAGTTGAAATTTTATGTTTGTGATGCTTGGATATTTTCCTTGAAATGTATAAACATGTAAAAATTAGATTACTGCCTGTAATAA
AATAATTCGATGACTATCTGTAAAACATGATTTGTGCAAGAGATATCAAAAATTACTTACTTTAAAAGTGGGGGGGGACATCTTTCTATGGGAACAAGCACATTTGCA
AGTATAAAACTTCATCTTTTGAATATCAAATGCAGATTTCTAAGTTTTACTTCCAAGTTCATTCACCCTCACCCTAGAAACTTCTTGAGCATTGTTTGCAGTAAAGCAA
AGAAAGGGTGCAAAATTTGCAGGGATGTGGATAGGGTCCGTTCAGGAGGAAAGGGATGTGGAGACAGGTGGGAGGGCTGCTGGAGCATGGCCCGTGACCAGGAAT
CCAGGCATGTGGAGACCAGCCCTTGGAGTGGGAACTCTGCTGCTCCTGGGAGGATGGAGAGAGGAGGCCGAGGAACTCTGGCCAGTAGAGACCAAGTGG

The yellow part is sgRNAs; the highlighted red part is the primer, and the amplified fragment is 435 bp; wine red is the precursor of miR-147.

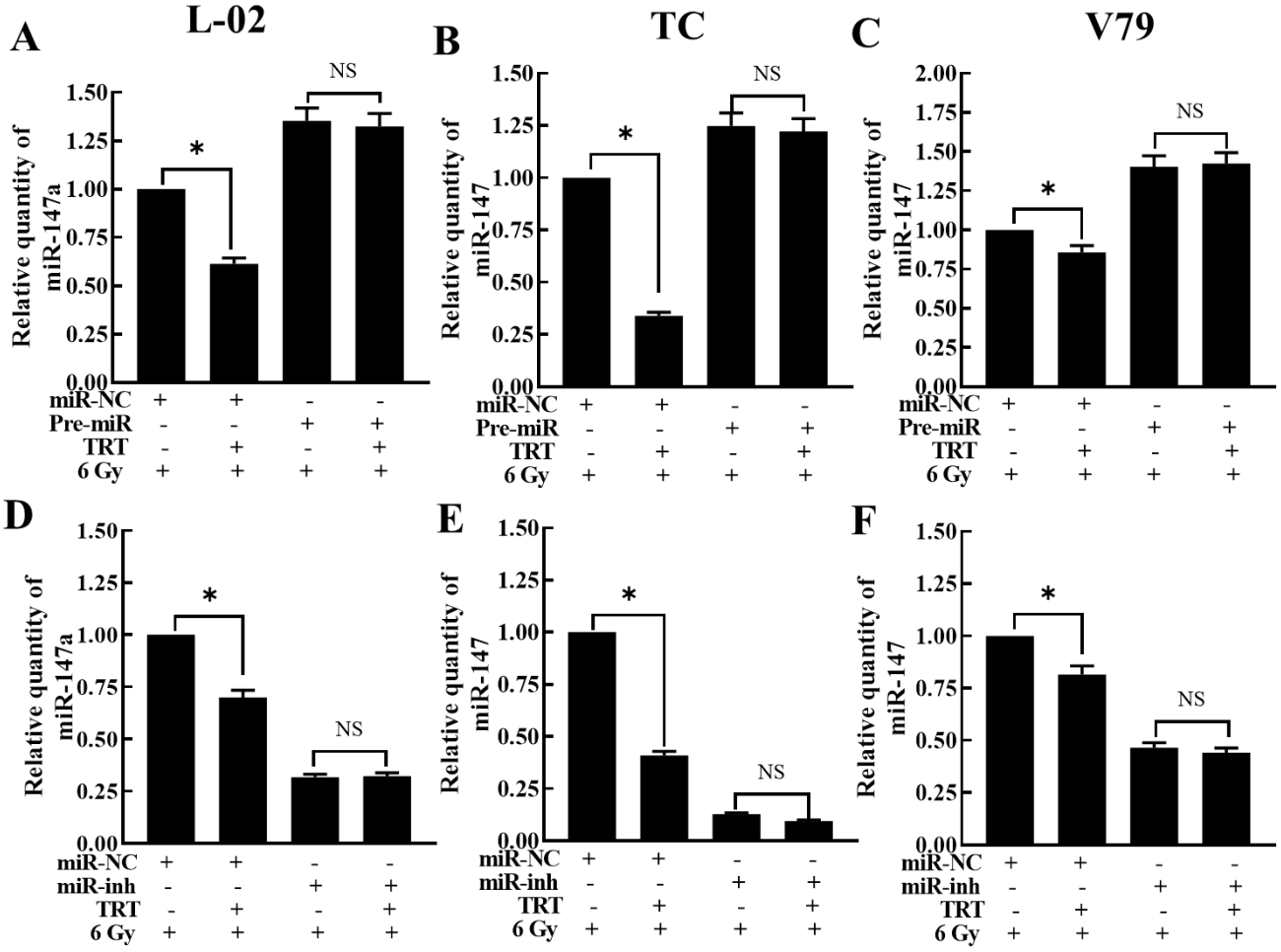
S-fig.1


A
NEAT1 5′UCAGGGGCUAAGCACACACU3′
miR-147a 3′CGUCUUCGUAAAGGUGUGUG5′
NEAT1 MUT 5′UCAGGGGCUAAGCGAGAGCU3′
B
Pre-miR-147a
miR-NC
Relative luciferase activity
NS
NEAT1
NEAT1-MUT
C
L02
Relative quantity of miR147a expression
NC
NEAT1
D
PDPK1-3'UTR
264-271
641-647
5190-5196
miR-147a
5' ...AAAAAAAACACCCAACCACACAA...
3' CGUCUUCGUAAAGGUGUGUG
miR-513a
5' ...CCUUUCUUGCGUCUCCCUGUGAU...
3' UACUGUGGAGGGACACUU
miR-575
5' ...AUAUAAGGUUAUUUGCUGGCUAU...
3' CGAGGACAGGUUGACCGAG
E
PDPK1-UTR
Mut-PDPK1-UTR-NS
Relative luciferase activity
miR-NC
miR-147a
miR-513a
miR-575
F
L02
Relative quantity of PDPK1 mRNA
NC
Pre-miR
PDPK1
α-Actinin
Relative quantity of PDPK1 protein
G
NC-miR
Pre-miR-147a
PDPK1
GAPDH
Con
4
8
12
16
24
Time after ActD treatment (h)
Relative quantity of PDPK1 mRNA
NC-miR
Pre-miR-147a
Time after ActD treatment (hours)

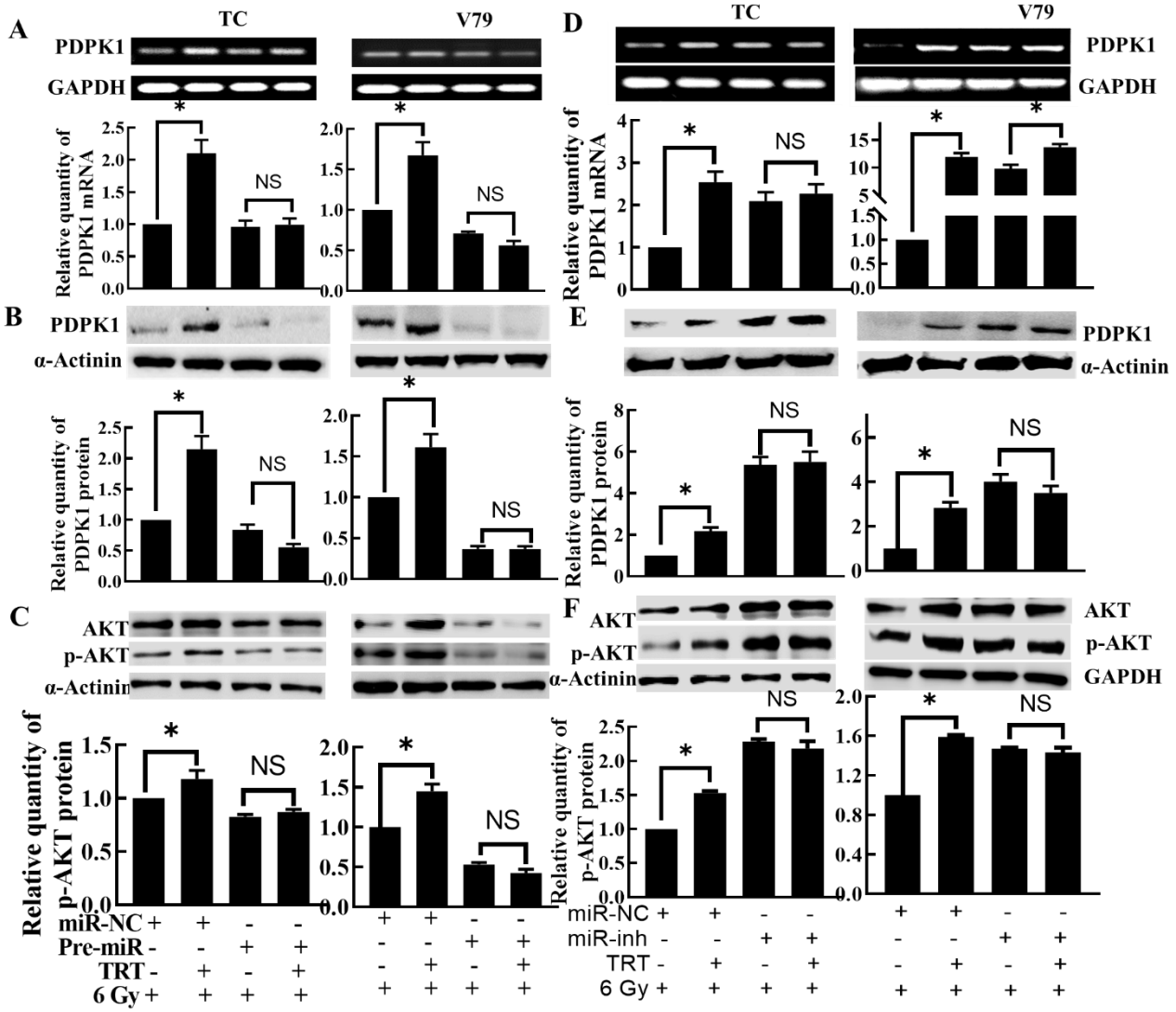
S-fig.2


A
TC
V79
Relative quantity of miR-147
Con 0.5 h 1 h 2 h 4 h 6 h
B
Relative quantity of PDPK1 mRNA
C
PDPK-1
GAPDH
Relative quantity of PDPK1 protein
After radiation
D
E
F
miR-NC +
Pre-miR -
TRT -

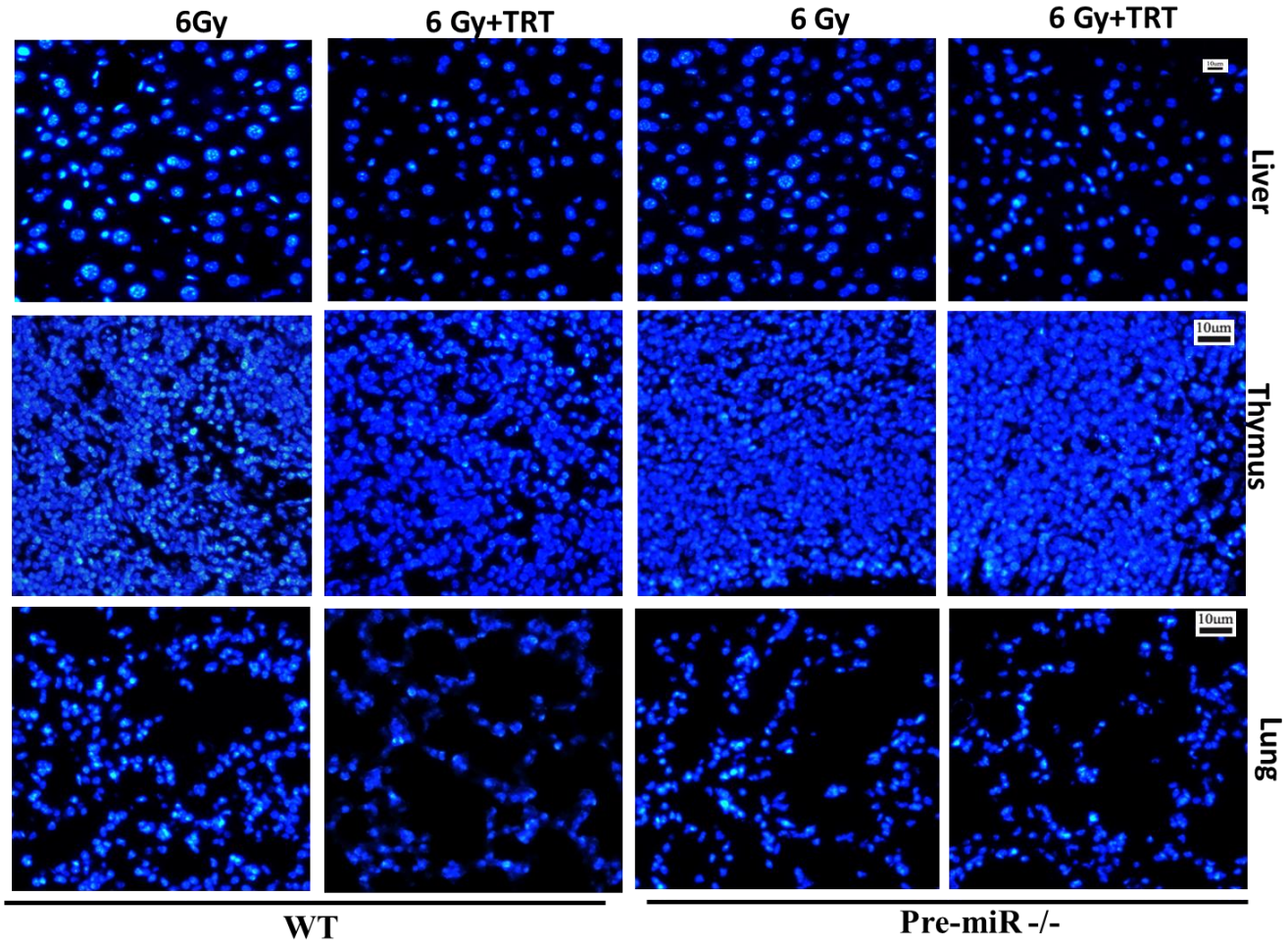
S-fig. 3


A
L-02
B
TC
C
V79
D
E
F
Relative quantity of miR-147a
Relative quantity of miR-147
NS
*
miR-NC
Pre-miR
miR-inh
TRT
6 Gy

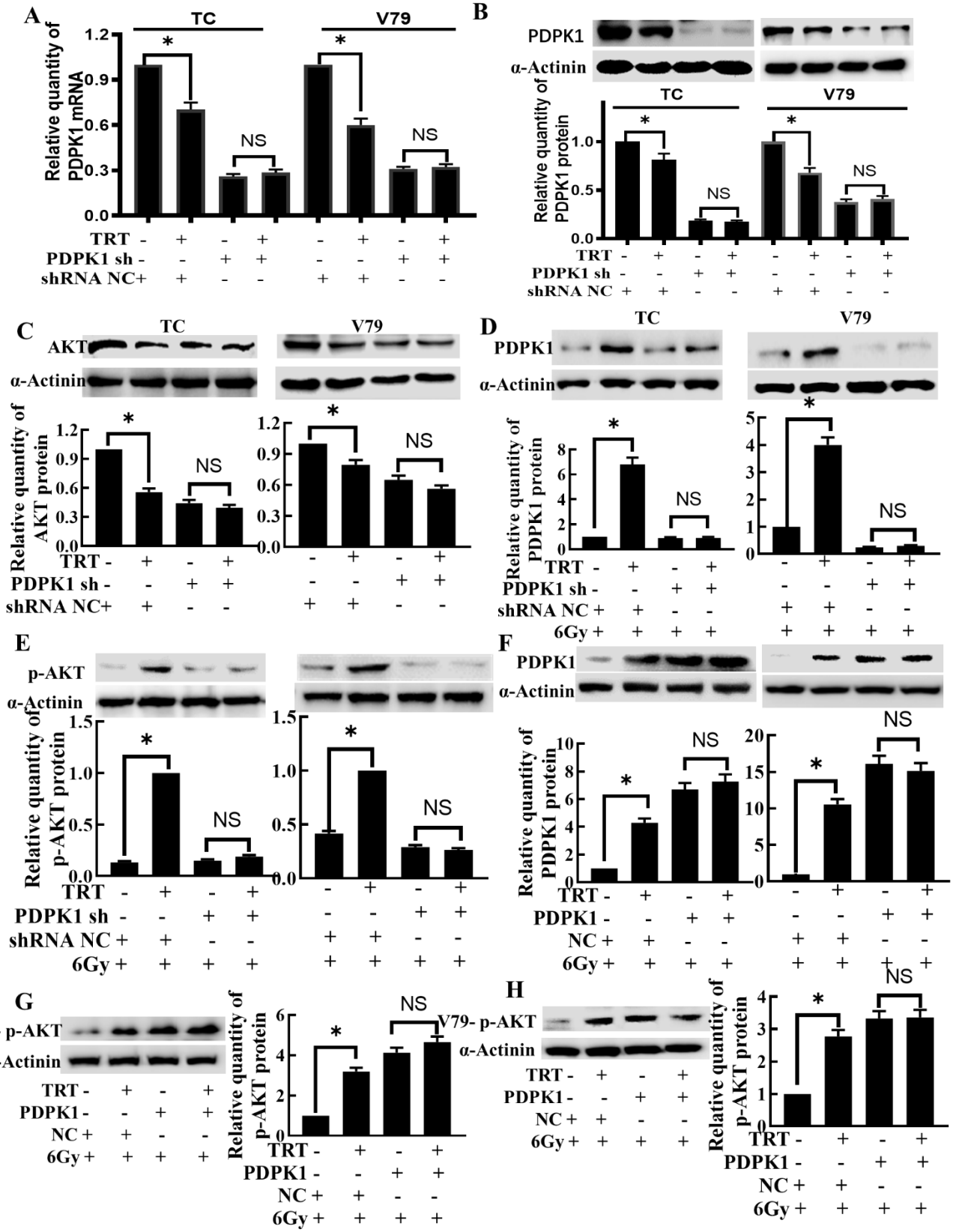
S-fig.4


A
TC
V79
PDPK1
GAPDH
Relative quantity of PDPK1 mRNA
NS
B
PDPK1
α-Actinin
Relative quantity of PDPK1 protein
C
AKT
p-AKT
α-Actinin
Relative quantity of p-AKT protein
miR-NC
Pre-miR
TRT
6 Gy
D
E
F
miR-inh

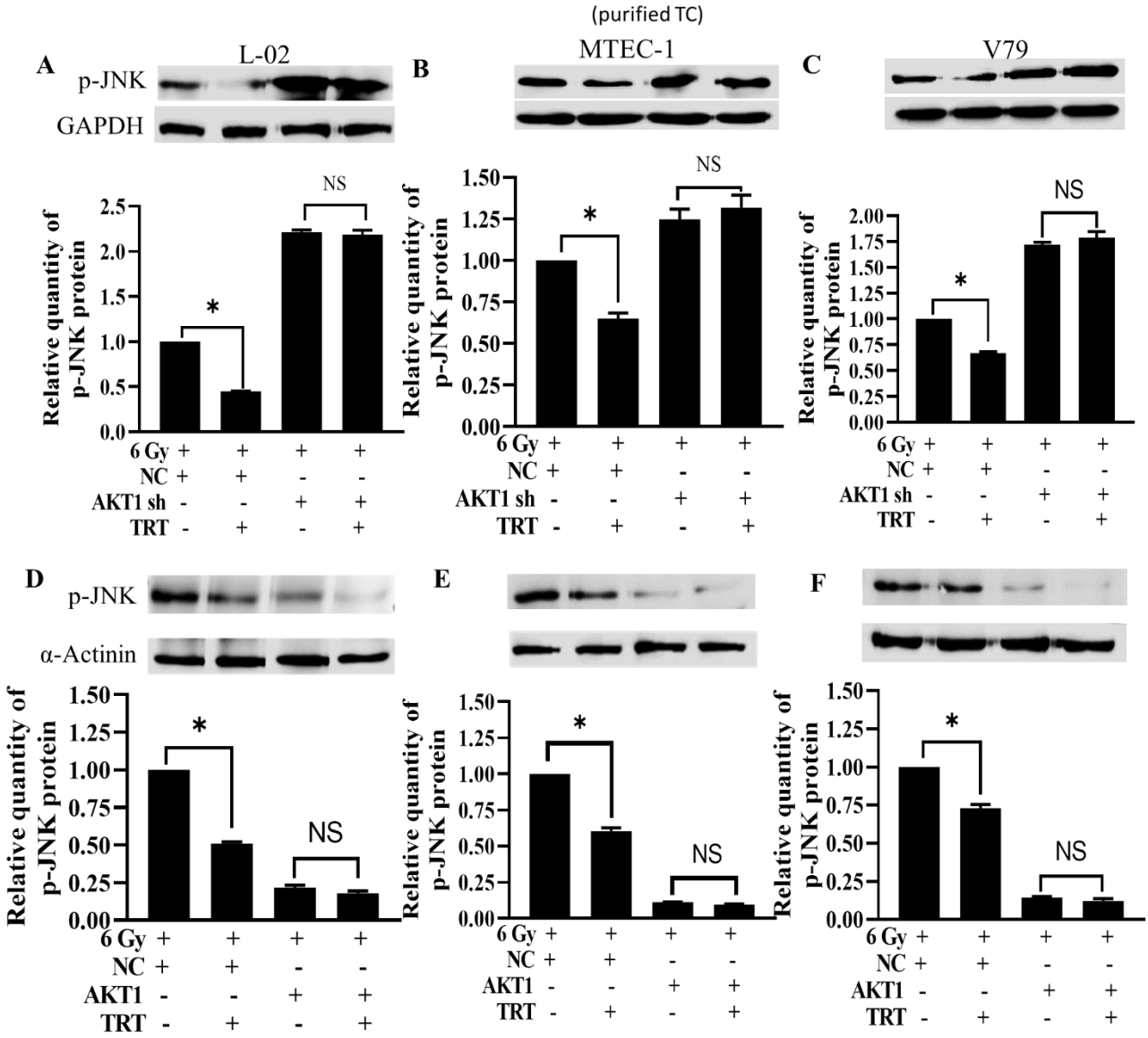
S-fig.5



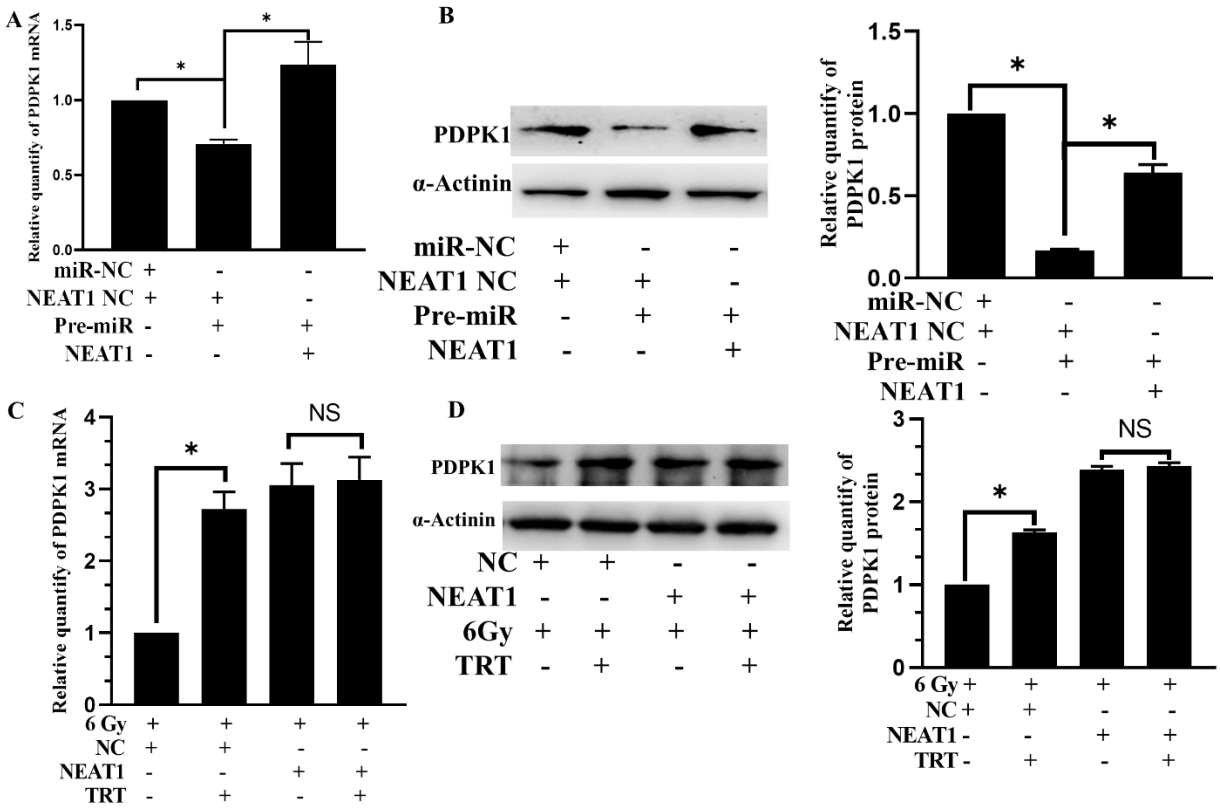
S-fig.6


A
TC
V79
Relative quantity of PDPK1 mRNA
1.2
0.9
0.6
0.3
0.0
*
NS
TRT - + - + - + - +
PDPK1 sh - - + + - - + +
shRNA NC+ + - - + + - -
B
PDPK1
α-Actinin
TC
V79
Relative quantity of PDPK1 protein
1.0
0.5
0.0
*
NS
TRT - + - + - + - +
PDPK1 sh - - + + - - + +
shRNA NC + + - - + + - -
C
TC
V79
AKT
α-Actinin
Relative quantity of AKT protein
1.2
0.9
0.6
0.3
0.0
*
NS
TRT - + - +
PDPK1 sh - - + +
shRNA NC+ + - -
- + - +
- - + +
+ + - -
D
TC
V79
PDPK1
α-Actinin
Relative quantity of PDPK1 protein
8
6
4
2
0
5
4
3
2
1
0
*
NS
TRT - + - +
PDPK1 sh - - + +
shRNA NC + + - -
6Gy + + + +
- + - +
- - + +
+ + - -
+ + + +
E
p-AKT
α-Actinin
Relative quantity of p-AKT protein
1.5
1.0
0.5
0.0
*
NS
TRT - + - +
PDPK1 sh - - + +
shRNA NC + + - -
6Gy + + + +
- + - +
- - + +
+ + - -
+ + + +
F
PDPK1
α-Actinin
Relative quantity of PDPK1 protein
10
8
6
4
2
0
20
15
10
5
0
*
NS
TRT - + - +
PDPK1 - - + +
NC + + - -
6Gy + + + +
- + - +
- - + +
+ + - -
+ + + +
G
TC- p-AKT
α-Actinin
TRT - + - +
PDPK1 - - + +
NC + + - -
6Gy + + + +
Relative quantity of p-AKT protein
6
4
2
0
*
NS
TRT - + - +
PDPK1 - - + +
NC + + - -
6Gy + + + +
H
V79- p-AKT
α-Actinin
TRT - + - +
PDPK1 - - + +
NC + + - -
6Gy + + + +
Relative quantity of p-AKT protein
4
3
2
1
0
*
NS
TRT - + - +
PDPK1 - - + +
NC + + - -
6Gy + + + +

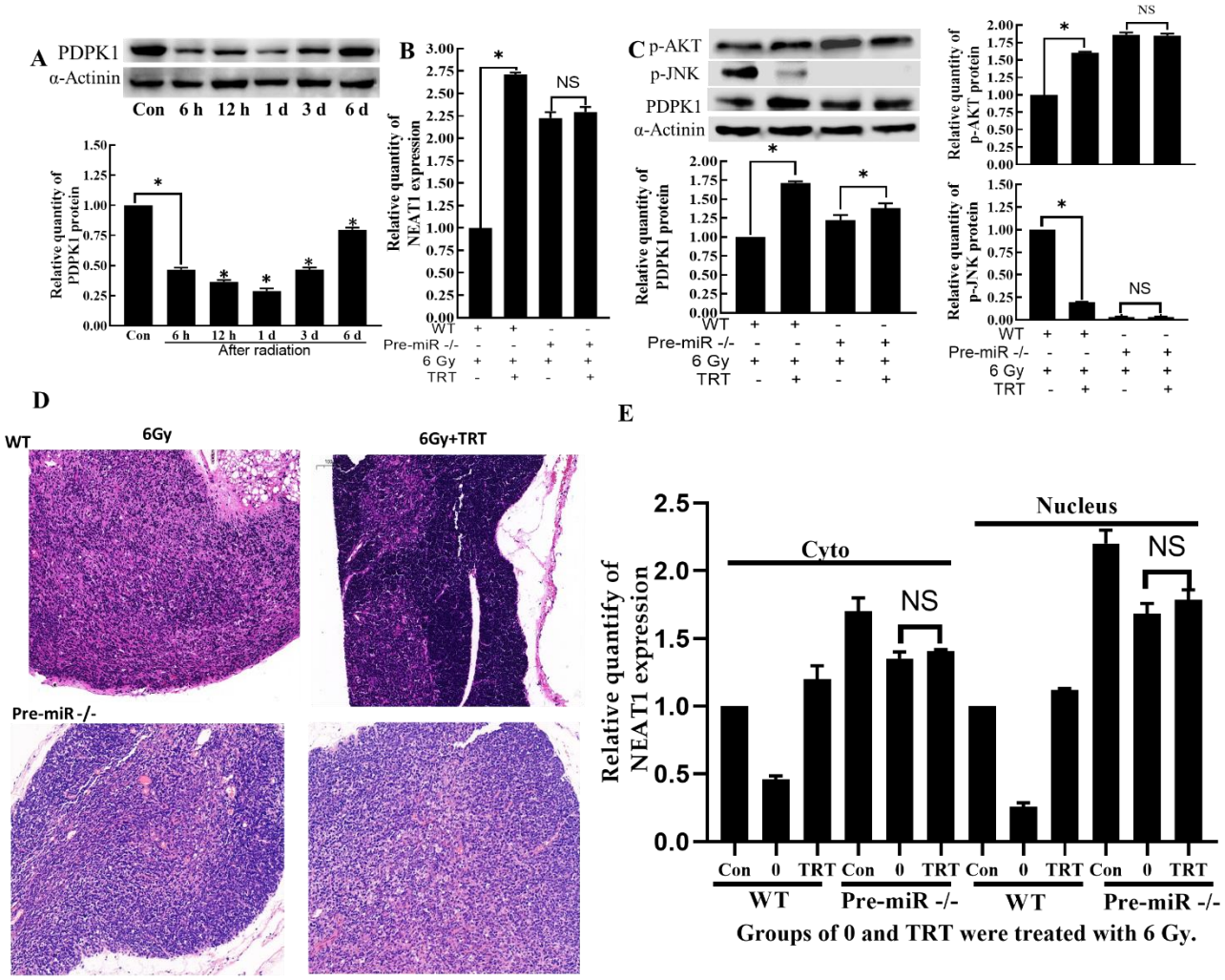
S-fig.7


A
L-02
p-JNK
GAPDH
B
(purified TC)
MTEC-1
C
V79
Relative quantity of p-JNK protein
NS
*
6 Gy
NC
AKT1 sh
TRT
D
p-JNK
α-Actinin
E
F
AKT1

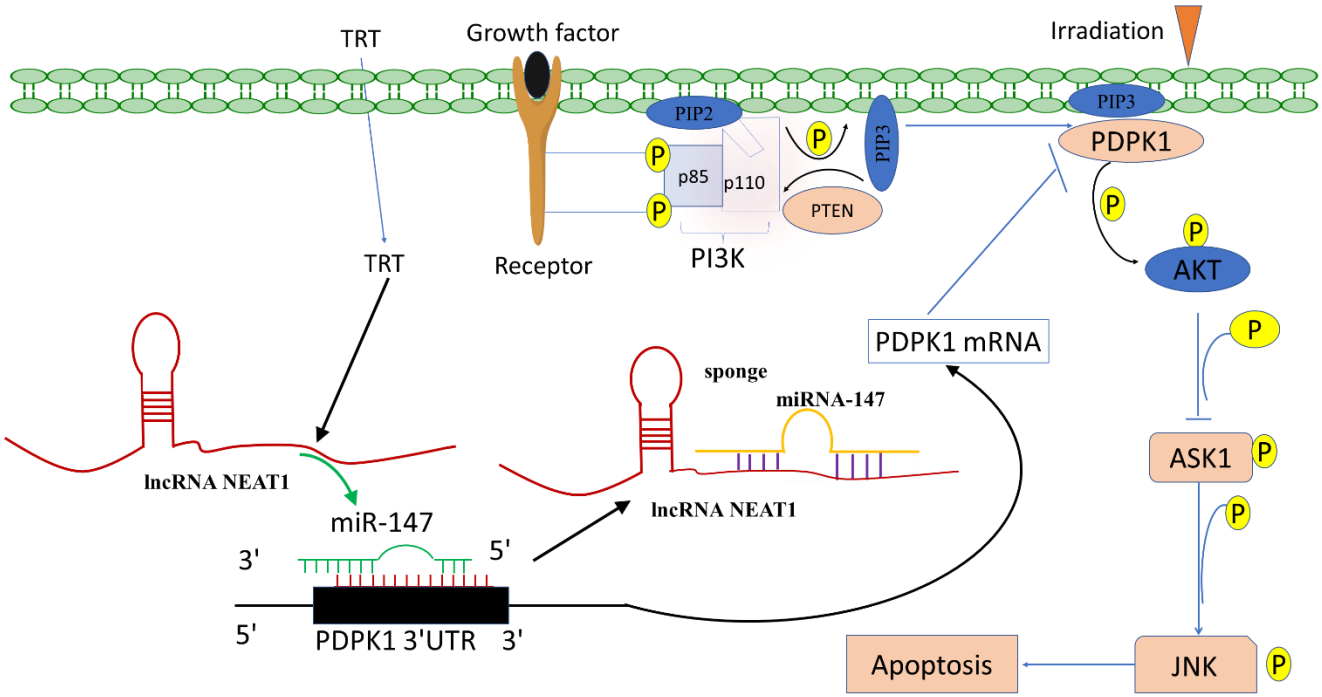
S-fig.8


A
Relative quantify of PDPK1 mRNA
1.5
1.0
0.5
0.0
*
*
miR-NC + - -
NEAT1 NC + + -
Pre-miR - + +
NEAT1 - - +
B
PDPK1
α-Actinin
miR-NC + - -
NEAT1 NC + + -
Pre-miR - + +
NEAT1 - - +
Relative quantify of PDPK1 protein
1.5
1.0
0.5
0.0
*
*
miR-NC + - -
NEAT1 NC + + -
Pre-miR - + +
NEAT1 - - +
C
Relative quantify of PDPK1 mRNA
4
3
2
1
0
*
NS
6 Gy + + + +
NC + + - -
NEAT1 - - + +
TRT - + - +
D
PDPK1
α-Actinin
NC + + - -
NEAT1 - - + +
6Gy + + + +
TRT - + - +
Relative quantify of PDPK1 protein
3
2
1
0
*
NS
6 Gy + + + +
NC + + - -
NEAT1 - - + +
TRT - + - +

S-fig.9


A
PDPK1
α-Actinin
Con 6 h 12 h 1 d 3 d 6 d
Relative quantity of PDPK1 protein
After radiation
B
Relative quantity of NEAT1 expression
NS
WT
Pre-miR -/-
6 Gy
TRT
C
p-AKT
p-JNK
PDPK1
α-Actinin
Relative quantity of PDPK1 protein
Relative quantity of p-AKT protein
Relative quantity of p-JNK protein
D
WT
6Gy
6Gy+TRT
Pre-miR -/-
E
Relative quantify of NEAT1 expression
Cyto
Nucleus
NS
Con 0 TRT Con 0 TRT Con 0 TRT Con 0 TRT
WT
Pre-miR -/-
WT
Pre-miR -/-
Groups of 0 and TRT were treated with 6 Gy.

S-fig.10


TRT
Growth factor
Irradiation
PIP2
PIP3
PDPK1
P
p85
p110
PTEN
PI3K
Receptor
AKT
PDPK1 mRNA
sponge
miRNA-147
lncRNA NEAT1
miR-147
3'
5'
PDPK1 3'UTR
ASK1
Apoptosis
JNK

S-fig.11