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Supplemental Information

Upregulation of Circular RNA circATRNL1

to Sensitize Oral Squamous

Cell Carcinoma to Irradiation

Guanhui Chen, Yiming Li, Yi He, Binghui Zeng, Chen Yi, Chao Wang, Xiliu Zhang, Wei Zhao, and Dongsheng Yu

Supplemental Information

Supplemental Information includes two tables and five figures.

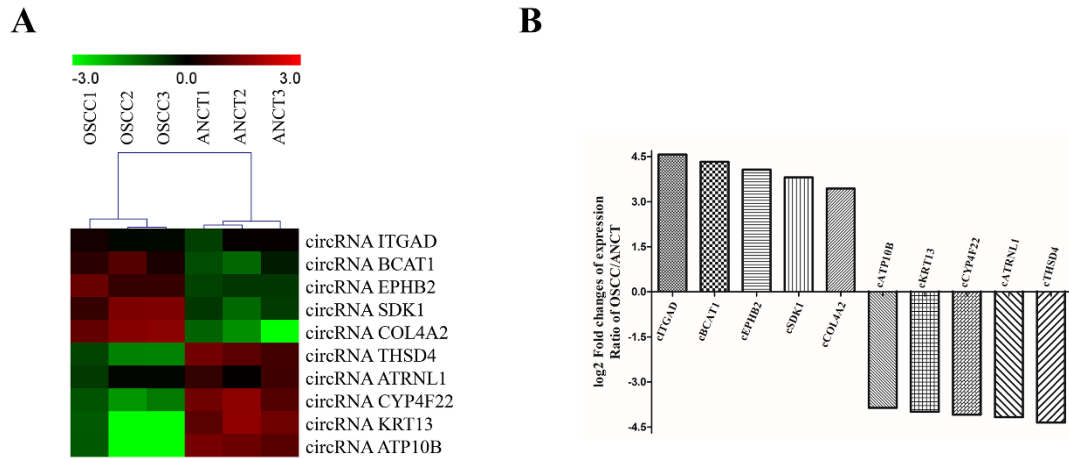


Figure S1. Hierarchical cluster analysis of circRNA expression profiling among the top five up- and downregulated circRNAs in OSCC (A), and the fold changes (B) are shown accordingly.

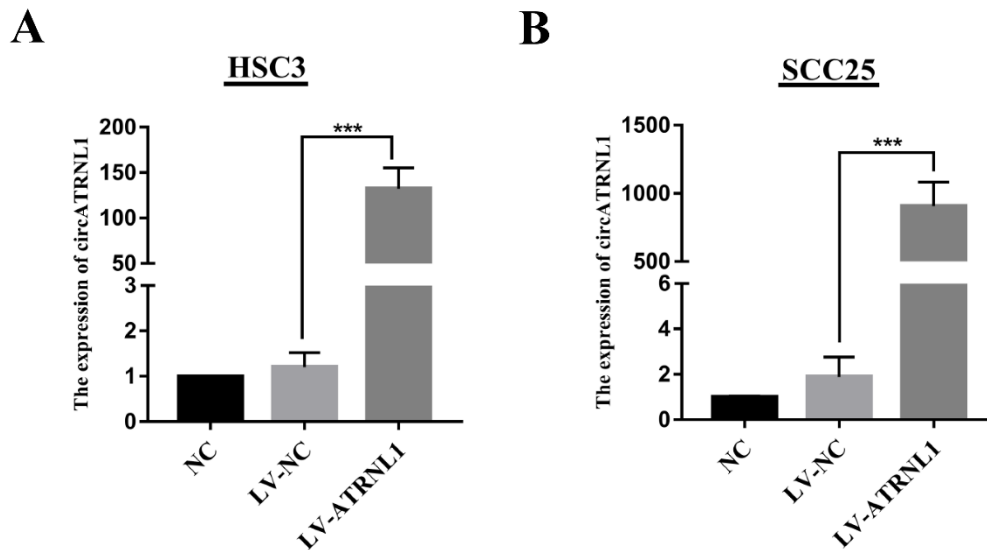


Figure S2. The transfection efficiencies of LV-ATRNL1 in HSC3 (A) and SCC25 (B) cells were measured by qRT-PCR. All results are indicated as mean \pm SD, n = 3. *** p < 0.001.

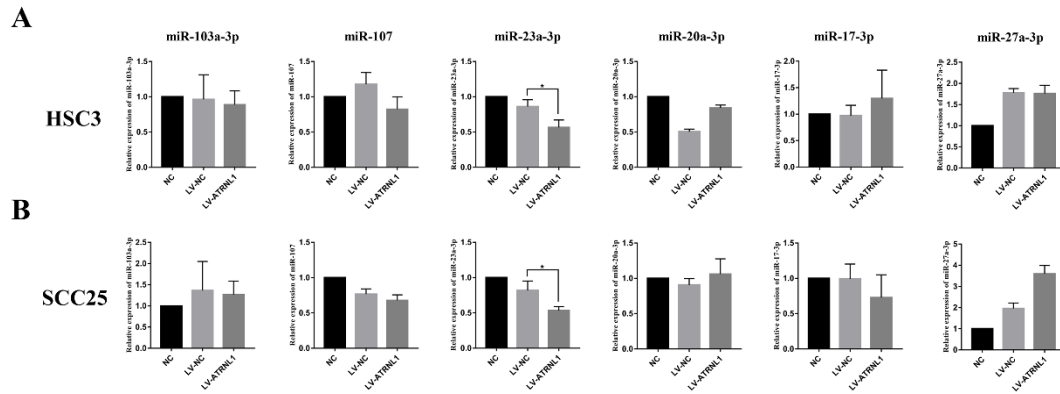


Figure S3. Relative expression of the six indicated miRNAs, including miR-103a-3p, miR-107, miR-23a-3p, miR-20a-3p, miR-17-3p, and miR-27a-3p, was evaluated by qRT-PCR in HSC3 (A) and SCC25 (B) cells. NC: Blank control; LV-NC: Negative control. Values are shown as mean \pm SD of triplicate experiments. * $p < 0.05$.

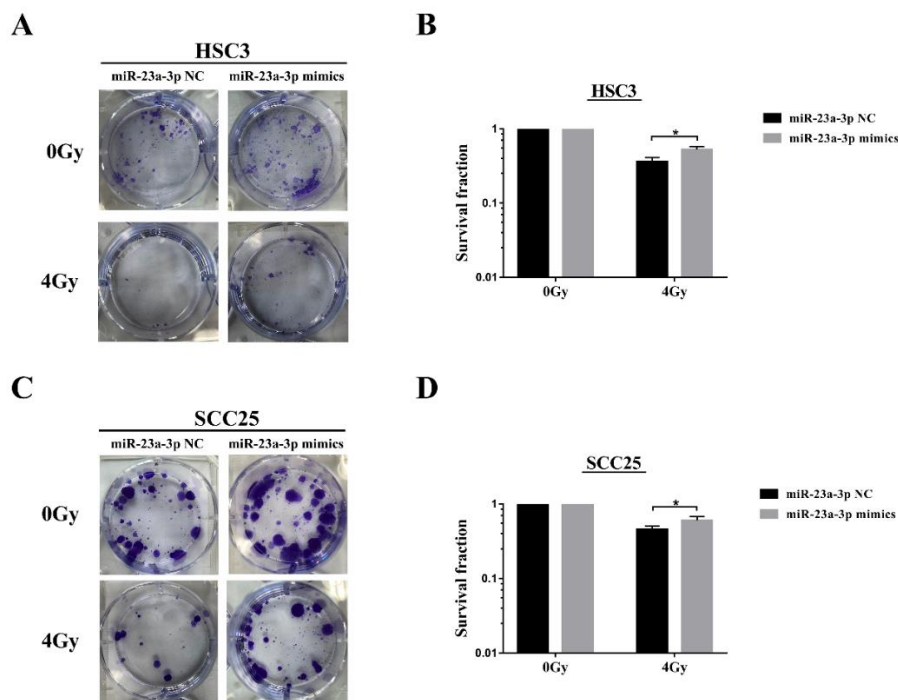


Figure S4. The clonogenic survival ability of transfected miR-23a-3p was determined by colony formation assay under 4Gy irradiation in HSC3 (A and B) and SCC25 (C and D) cells. All results are indicated as mean \pm SD, $n = 3$. * $p < 0.05$.

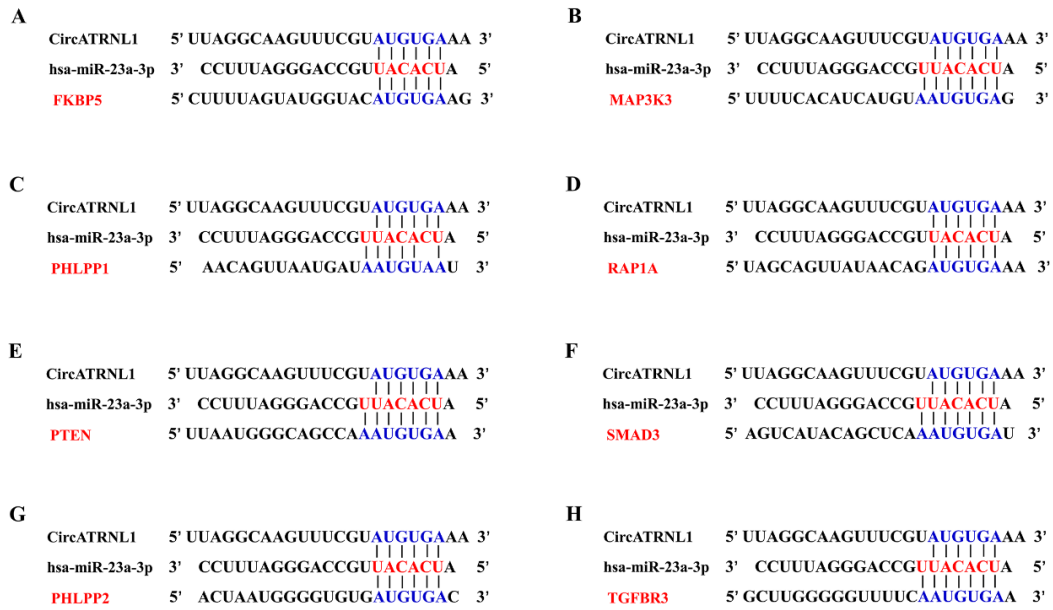


Figure S5. A sequence within the circATRNL1 and eight mRNAs, including FKBP5 (A), MAP3K3 (B), PHLPP1 (C), RAP1A (D), PTEN (E), SMAD3 (F), PHLPP2 (G), and TGFB3 (H), that are complementary to miR-23a-3p was identified by bioinformatics analysis.

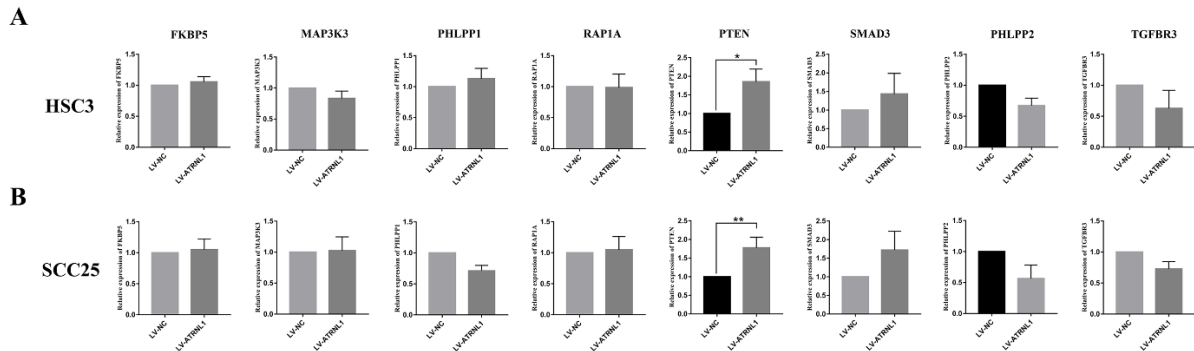


Figure S6. The expression of the eight indicated mRNAs, including FKBP5, MAP3K3, PHLPP1, RAP1A, PTEN, SMAD3, PHLPP2, and TGFB3, was analyzed by qRT-PCR in HSC3 (A) and SCC25 (B) cells transfected with LV-NV or LV-ATRNL1. Data are shown as mean \pm SD, $n = 3$.

* $p < 0.05$, ** $p < 0.01$.

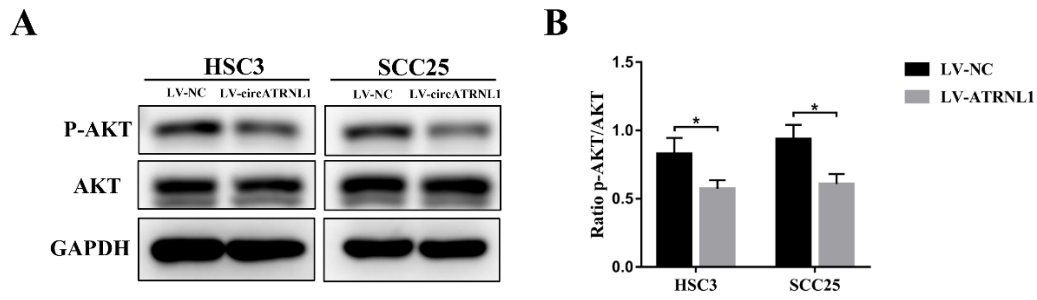


Figure S7. AKT and p-AKT levels of cells were measured by Western blot analysis after circATRNL1 overexpression (A), and quantitative analyses of them are presented (B). All values are presented as mean \pm SD, n = 3. *p < 0.05.

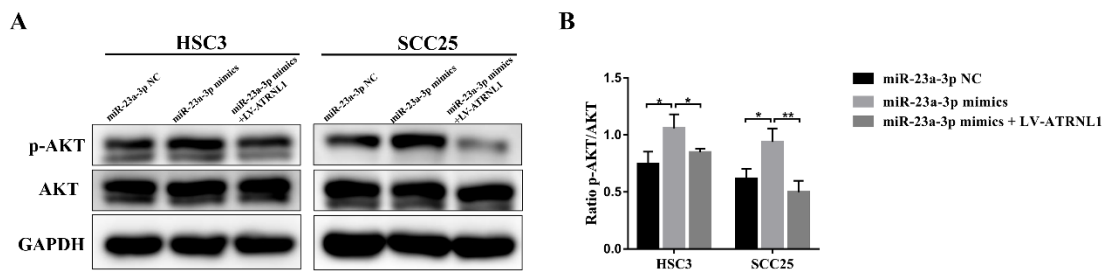


Figure S8. The levels of AKT and p-AKT were evaluated in OSCC cells after transfection (A), and the quantitative data were computed as a ratio to levels of GAPDH (B). Values are shown as mean \pm SD of triplicate experiments. *p < 0.05, **p < 0.01.

Table S1. Primers used for RT-qPCR

Target	Primer (5' to 3')
cITGAD-F	5'-AAGCGAAAACCTCGACAATC-3'
cITGAD-R	5'-CTTCCTGCACCTCGATCATC-3'
cBCAT1-F	5'-GATGTTTGGCTCTGGTACAG-3'
cBCAT1-R	5'-CCTTGTCACCTGGAAGAA-3'
cTHSD4-F	5'-GCATGACCAACCATGTCA-3'
cTHSD4-R	5'-GCTGGAGTCACAGTAACTCTCA-3'
cATRNL1-F	5'-ATCACATCCCAGTCTTTCCT-3'
cATRNL1-R	5'-GAGCCTAACATCCAAACTAAGC-3'
cCYP4F22-F	5'-CCACTGAAGTCATCCAGGAA-3'
cCYP4F22-R	5'-TCCACTTGTCACCTTTGCT-3'
has-miR-103a-3p-F	5'-ACACTCCAGCTGGGAGCAGCATTGTACAGGGC-3'
has-miR-103a-3p-RT	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCATAGCC-3'
has-miR-107-F	5'-ACACTCCAGCTGGGAGCAGCATTGTACAGGGC-3'
has-miR-107-RT	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGATAGCC-3'
has-miR-23a-3p-F	5'-ACACTCCAGCTGGGATCACATTGCCAGGGATTT-3'
has-miR-23a-3p-RT	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGGAAATCC-3'
has-miR-20a-3p-F	5'-ACACTCCAGCTGGGACTGCATTATGAGCACTT-3'
has-miR-20a-3p-RT	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTTTAAGT-3'
has-miR-17-3p-F	5'-ACACTCCAGCTGGGACTGCAGTGAAGGCACTT-3'
has-miR-17-3p-RT	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTACAAGT-3'
has-miR-27a-3p-F	5'-ACACTCCAGCTGGGTTTACAGTGGCTAAGTTCC-3'
has-miR-27a-3p-RT	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCGGAACT-3'
miRNA-R	5'-CTCAACTGGTGTCGTGGA-3'
U6-F	5'-CTCGCTTCGGCAGCACA-3'
U6-R	5'-AACGCTTCACGAATTTGCGT-3'
U6-RT	5'- AACGCTTCACGAATTTGCGT-3'
ATRNL1-F	5'-GTGGCTGCTGTGGTATGGAA-3'
ATRNL1-R	5'-ATGGCCCTCGCAGAACTCT-3'
FKBP5-F	5'-TCTGTTCTGGCGTGAGTTGT-3'
FKBP5-R	5'-AGAGCCATGCTCAATCTGTT-3'
MAP3K3-F	5'-CGAGACTCTGCTGGGAATGT-3'
MAP3K3-R	5'-TGATCACCTCAGGGCTCATC-3'
PHLPP1-F	5'-ACTGAGGATGGCAAGGTGAA-3'
PHLPP1-R	5'-CAACCCCTTACTGCCTAGGA-3'
RAP1A-F	5'-GGCTTGGTGGTATCCCTGAA-3'
RAP1A-R	5'-TTGCAGTGAGCGGAGACTGA-3'
PTEN-F	5'-GTCAGAGGCGCTATGTGTATTA-3'
PTEN-R	5'-TTAGCTGGCAGACCACAA-3'
SMAD3-F	5'-GCACAGCCAGTTCTGAATGT-3'
SMAD3-R	5'-GTGAGTGAAGGCTTCTTGGA-3'
PHLPP2-F	5'-ACAGCCTGAACCTCATTGAA-3'

PHLPP2-R	5'-GTCATGAGGCACAACAAACT-3'
TGFBR3-F	5'-GTTGGGTGAGTCCTATGTGAT-3'
TGFBR3-R	5'-GCTGACACACTGAACAGAGAA-3'
β-actin-F	5'-TGGATCAGCAAGCAGGAGTA-3'
β-actin-R	5'-TCGGCCACATTGTGAACTTT-3'
si-PTEN	5'-CCCACCACAGCTAGAACTT-3'
circATRNL1-WT-F	5'-CCGCTCGAGGTGATTATATGTGGGTGTGATTCATTG-3'
circATRNL1-WT-R	5'-ATAAGAATGCGGCCGCTCAAGCCAGAGCTGGGAAGCTGGAA-3'
circATRNL1-mut-F	5'-AAGTTTCGTCGAGACAAACCTCAGTTTTGAAGATAAGTAAGTGAGGAGAT-3'
circATRNL1-mut-R	5'-GGTTTGTCTCGACGAAACTTGCCTAAACTCCATAAACACATCTGTTAGG-3'
PTEN-WT-F	5'-CCGCTAGAGATTTTTTTTTTATCAAGAGGGATAAAA-3'
PTEN-WT-R	5'-ATAAGAATGCGGCCGCATCAATCAATACTCTATATATCAAT-3'
PTEN-mut-F	5'-ATTGTAAAGCTCCGAGACCGATATTATTA AAAAGGTTTTTTTTTC-3'
PTEN-mut-R	5'-ATCGGTCTCGGAGCTTTACAATAGTAGTTGTACTCCGCTTAAAAT-3'

Table S2. The target microRNAs of CircRNA ATRNL1 predicted by Miranda and TargetScan software

CircRNA	microRNA	Miranda max_score	Miranda max_Energy	TargetScan _score	TargetScan _score_percentile
Circ ATRNL1	hsa-miR-103a-3p	152	-15.54	-0.20	76
Circ ATRNL1	hsa-miR-107	152	-11.47	-0.20	76
Circ ATRNL1	hsa-miR-23a-3p	150	-12.73	-0.19	68
Circ ATRNL1	hsa-miR-20a-3p	151	-15.57	-0.26	84
Circ ATRNL1	hsa-miR-17-3p	143	-17.03	-0.19	78
Circ ATRNL1	hsa-miR-27a-3p	154	-14.80	-0.14	55