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Supplemental Information

Therapeutic Delivery of miR-29b Enhances Radiosensitivity in Cervical Cancer

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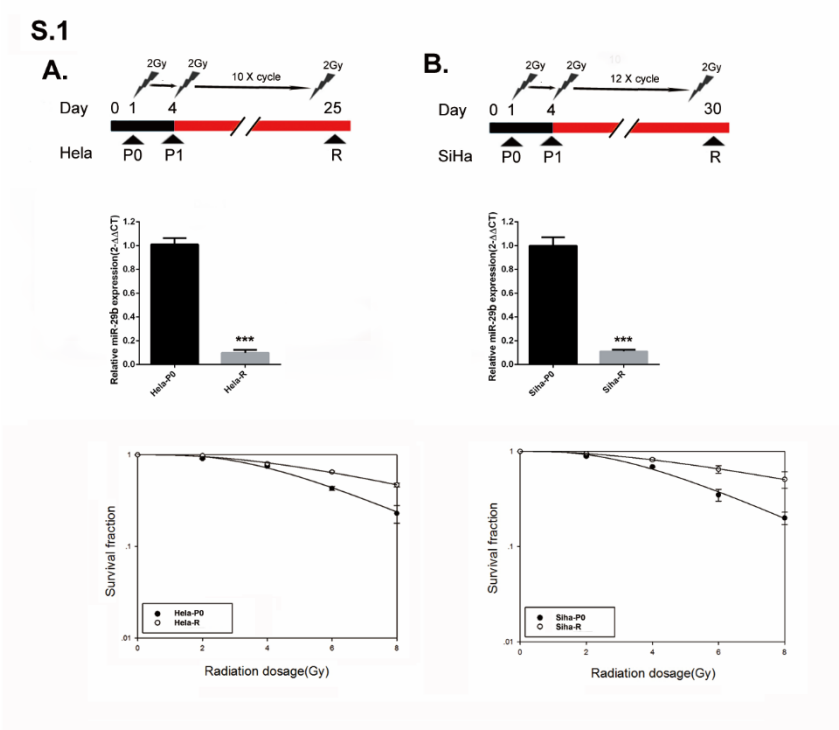
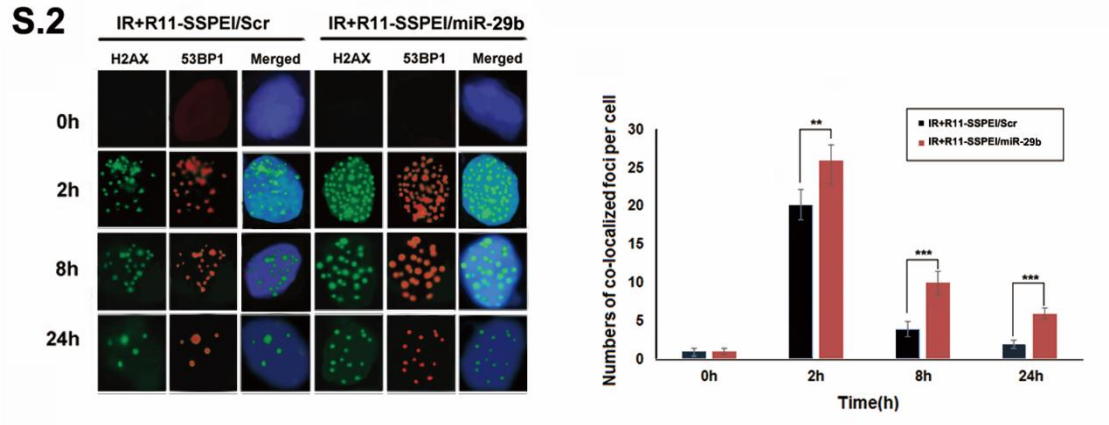
Supplemental figures

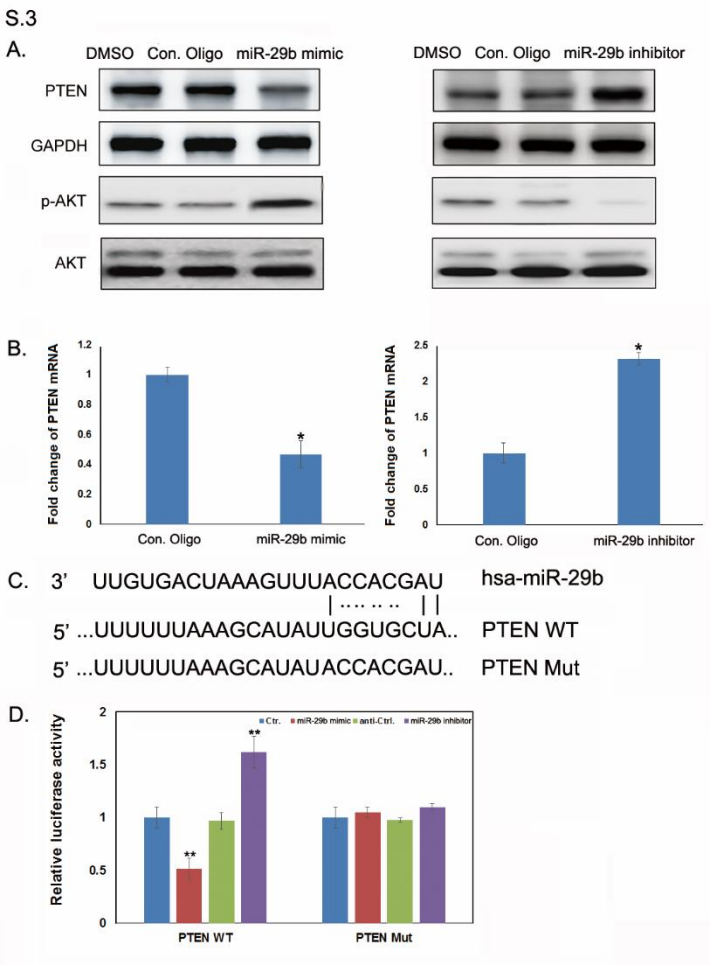


S1 Two radioresistant cervical cancer cell variants were established.
Top: schematic representation of the generation of a radioresistant subline (Hela-R or Siha-R) from the parental cells (Hela-P0 or Siha-P0).
Middle: miR-29b expression profiling of Hela-R or Siha-R cells relative to Hela-P0 or Siha-P0 cells using a qPCR-based miRNA array.
Bottom: miR-29b expression increased radiosensitivity of Hela-R or Siha-R cells. The survival fractions were determined by colony-forming assay as described in "Materials and methods". Data was expressed as mean ± SD of triplicates in one experiment. Shown was representative of 3 independent experiments



S2 Internalization of nanoparticles in Siha-R cells.
The number of co-localized foci (phospho-γ-H2AX and 53BP1) was determined for

each time point in R11-SSPEI/Scr or R11-SSPEI/miR-29b treated cells. The remaining merged foci in the nuclei were counted in 3 independent experiments (50 nuclei each). Statistical significance was evaluated using Student's t-test (*, $P < 0.05$; **, $P < 0.01$).



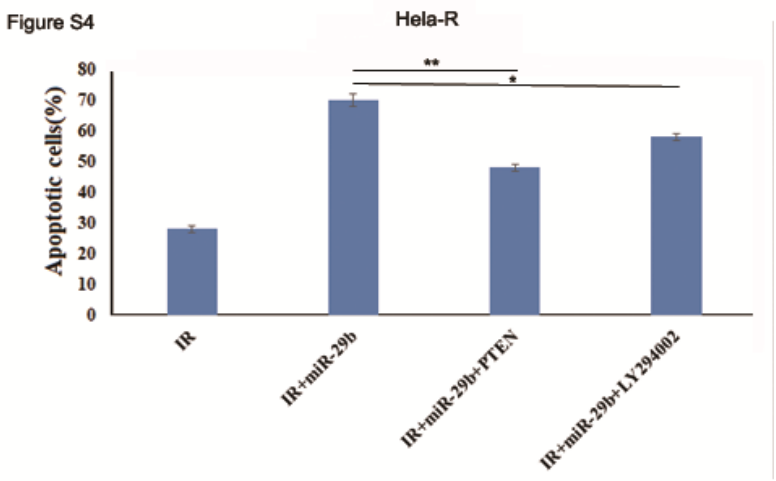
S3 PTEN are targets of miR-29b.

A. PTEN contain predicted miR-29b binding sites. In the figure the alignment of the seed regions of miR-29b with PTEN is shown.

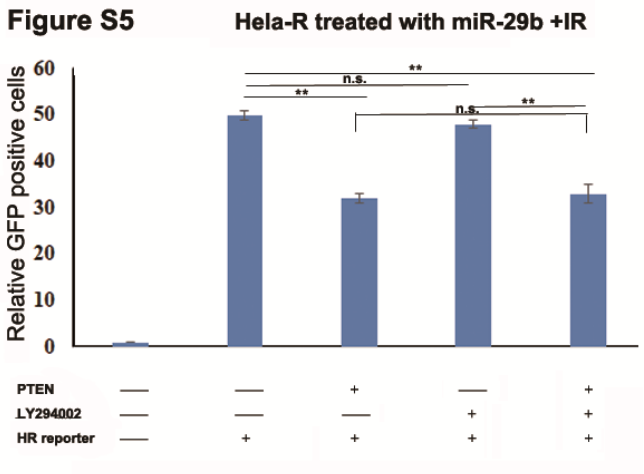
B. The expression levels of PTEN, p-AKT and AKT after the inhibition of miR-29b via lentiviral transduction or the overexpression of the same miRNA by oligonucleotide transfection in Hela-R cells were detected using western blot.

C. The mRNA expression levels of PTEN after the inhibition of miR-29b or the overexpression of the same miRNA in Hela-R cells was detected using qRT-PCR. *$p<0.05$.

D. PTEN 3′UTRs are targets of miR-29b. pluc3-PTEN that contained a wildtype or mutated PTEN 3′UTRs (indicated as WT or mut on the X-axis) was transfected into Hela-R cells. The relative repression of firefly luciferase was standardized to a transfection control. The reporter assays were performed three times with essentially identical results. *$p<0.05$.



S4. Inhibition of PI3K/Akt signaling pathway can partially reverse miR-29b-mediated-PTEN radiosensitivity in Hela-R cells.
Indicated cells were treated with or without 10 μM Ly294002 in the presence of IR. The cells were stained with PI/Annexin V for measuring the percentage of apoptotic cells. The percentage of apoptotic cells in experiment was presented as bar graphs. ns, no significance; *p<0.05, **p<0.03.



S5. The role of PTEN-PI3K-AKT pathway in HR repair assays of Hela-R-GFP cells.
Hela-R-GFP cells transfected with miR-29b under radiation were re-expressed PTEN alone or in combination with LY294002.
Shown are the means ± SEM from three experiments. n = 3 wells per group.