Supplementary Materials:

DNMT3A/*miR-129-2-5p*/Rac1 Is an Effector Pathway for *SNHG1* to Drive Stem Cell-like and Invasive Behaviors of Advanced Bladder Cancer Cells

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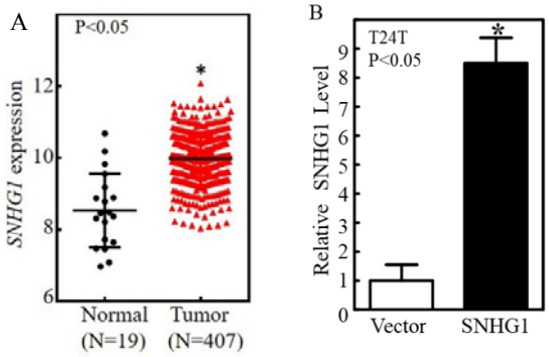

Figure S1. Expression of *SNHG1* in human bladder cancer and enforced expression of *SNHG1* in bladder cancer cell line. (**A**) Comparison of *SNHG1* expression in human bladder tumor vs. normal bladder tissues by RNA-seq (Illumina-Hiseq) based on TCGA database. Long and short horizontal lines denote mean and SD, respectively. (**B**) *SNHG1* overexpression was verified in T24T(*SNHG1*) in comparison to T24T(Vector) cells. The asterisk (*) indicates a statistically significant increase of *SNHG1* in T24T(*SNHG1*) cells than in T24T(Vector) cells (P< 0.05).

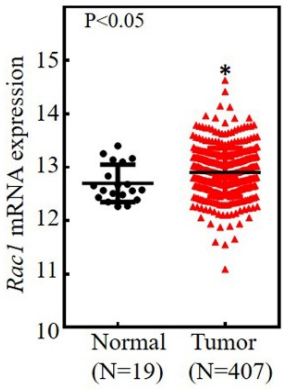

Figure S2. Expression of *Rac1* mRNA in human bladder cancer. *Rac1* mRNA expression in human bladder tumor vs. normal bladder tissues by RNA-seq (Illumina-Hiseq) based on TCGA database.

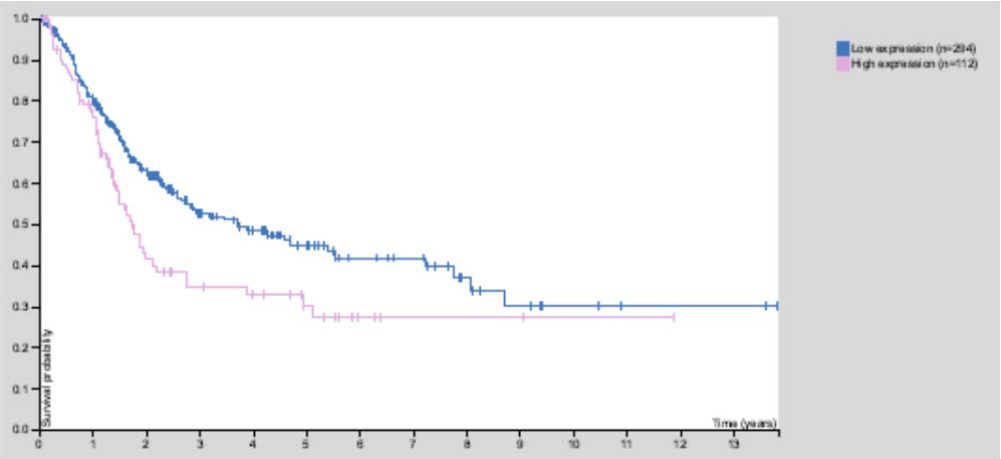

Figure S3. Rac1 mRNA expression in human bladder cancer. Analysis of Human Protein Atlas shows that the high expression of Rac1 in human MIBC is strongly associated with poor survival. N=406. P=0.0087.

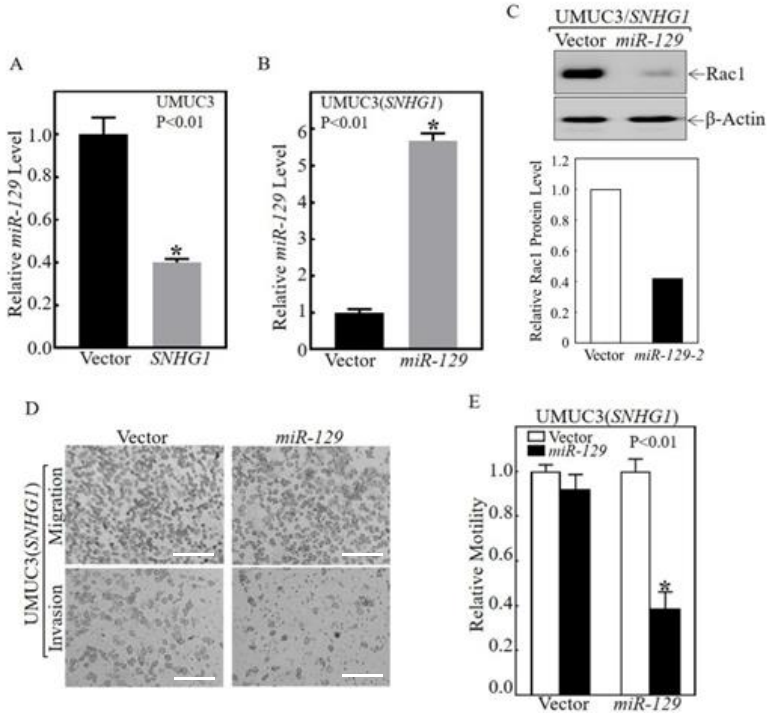

Figure S4. *SNHG1* overexpression inhibited *miR-129-5p* expression in UMUC3 cells. (A) Quantitative real-time PCR was carried out to determine the expression levels of *miR-129-2* in UMUC3 cells. **(B)** Plasmids bearing *miR-129-2* were stably transfected into UMUC3(*SNHG1*) cells. The stable transfectants were identified by real-time PCR. (**C, Top panel**) Protein lysates extracted from the indicated cells were subjected to Western blotting to determine Rac1 expression. β-Actin was used as a loading control. **(C, Bottom panel)** Relative protein levels determined by densitometry and expressed as ratios versus β-Actin. (**D-E**) UMUC3(*SNHG1*) cells and UMUC3(*SNHG1*/*miR-129-2*) cells were subjected to a transwell invasion assay, and the migration and invasion were calculated and presented relative to UMUC3(*SNHG1*) control. The results were presented as mean ± SD from triplicate experiments and asterisk (*) indicated a significant decrease in comparison to UMUC3(*SNHG1*) cells ($p < 0.01$). Bars in (D) equal to 100 μm.

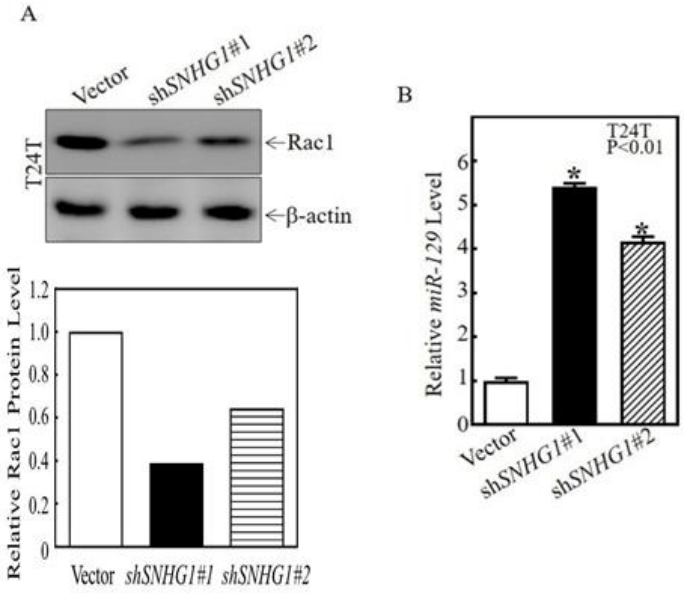

Figure S5. *SNHG1* inhibition decreased Rac1 expression and promoted *miR-129-5p* expression in UMUC3 cells. (A, Top panel) The total protein extracts from the indicated cells were subjected to Western blotting to determine for the expression of Rac1 expression. β-Actin served as a protein loading control. (**A, Bottom panel**) Relative protein levels determined by densitometry and expressed as ratios versus β-Actin. **(B)** Quantitative real-time PCR was carried out to determine the expression levels of *miR-129-5p* in the indicated cells. The results were presented as mean ± SD from triplicate experiments and asterisk (*) indicated a significant increase in comparison to T24T(Vector) cells ($p < 0.01$).

Table S1. The potential *miRNA* binding sites in *Rac1* mRNA 3'UTR region