

Expanded View Figures

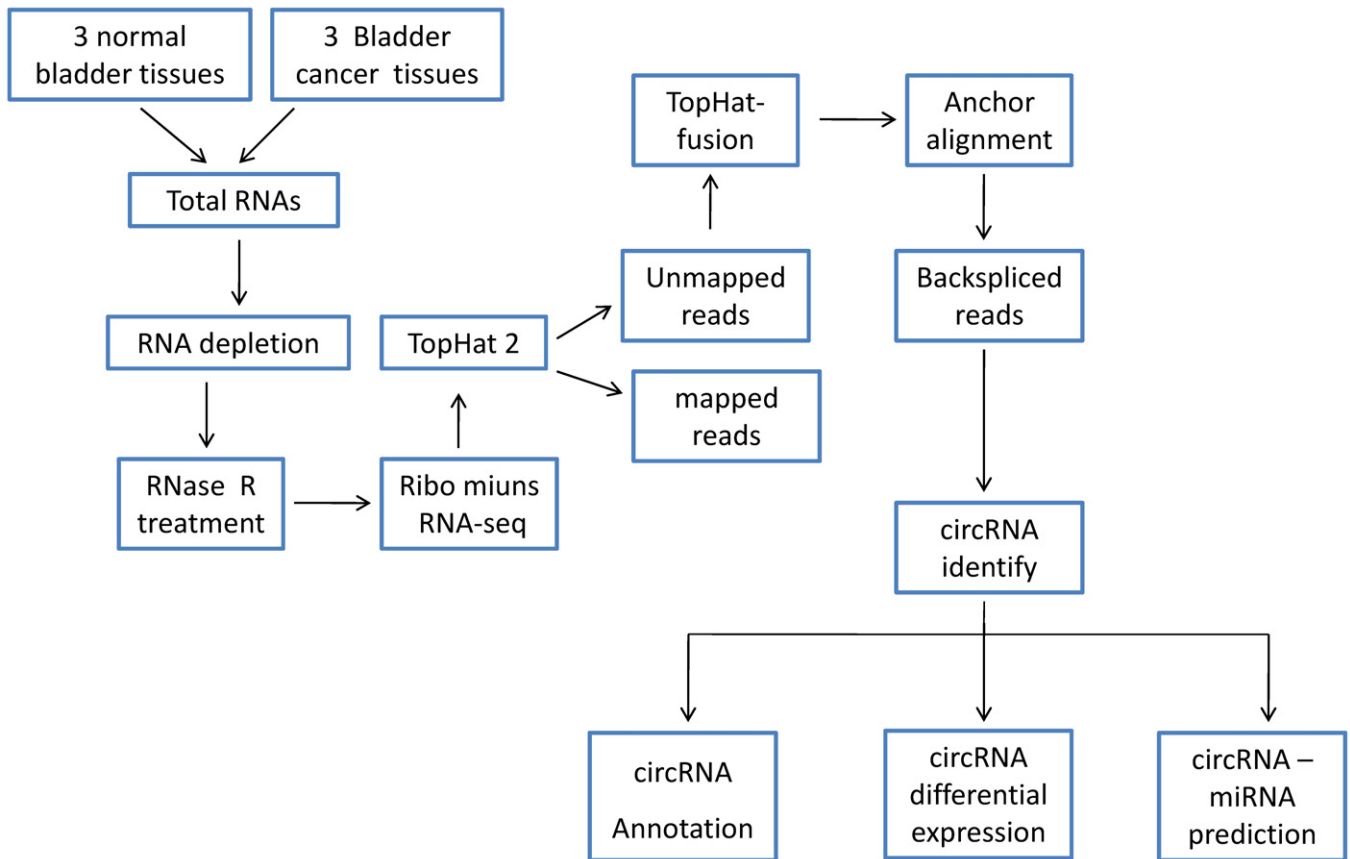


Figure EV1. RNA-seq pipeline.

The RNA-seq pipeline for identifying circRNAs in three pairs of bladder cancer tissues and paired adjacent normal bladder tissues is shown.

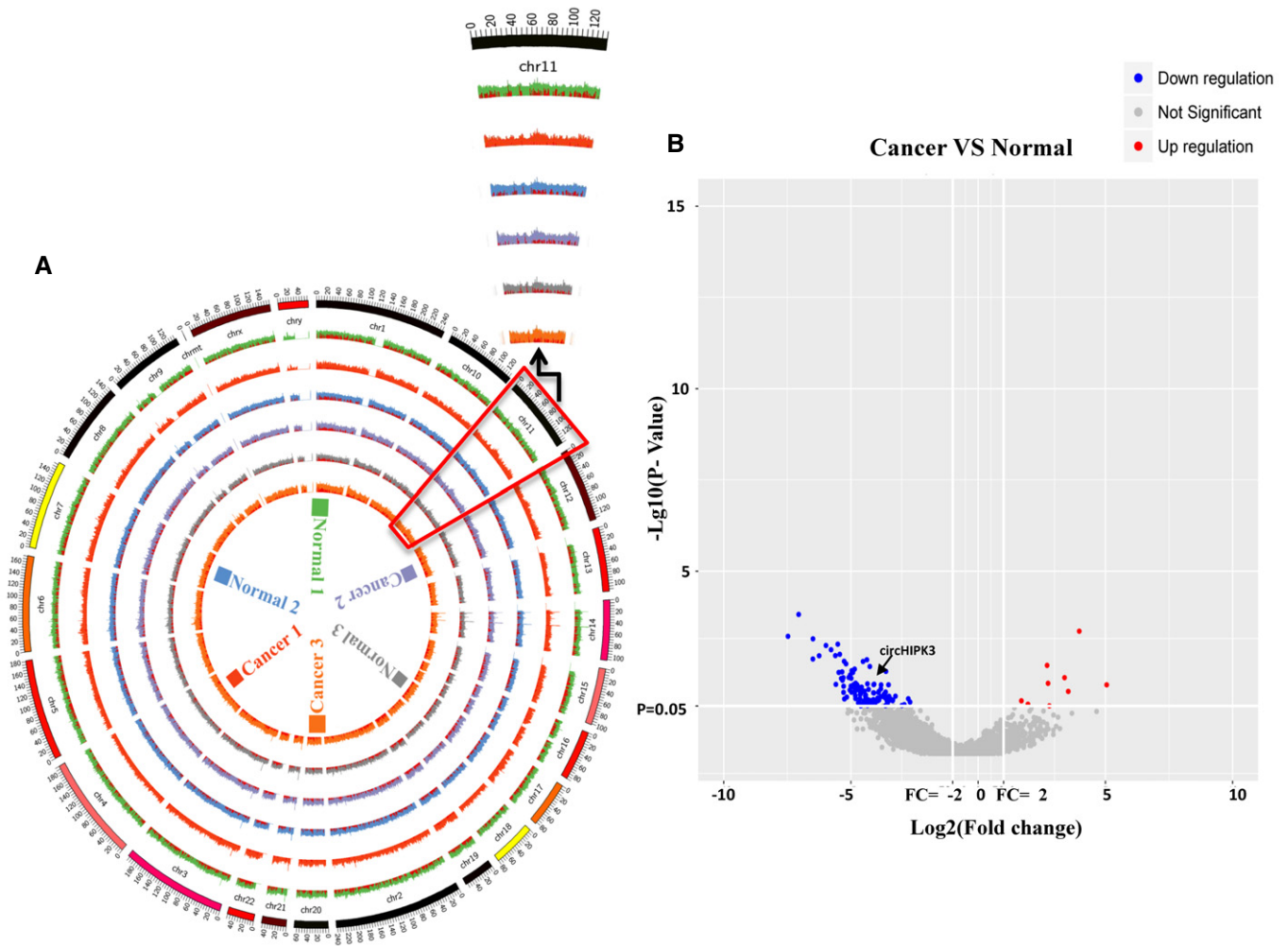


Figure EV2. Mapping results of linear and circular RNA reads on human chromosomes and differentially expressed circRNAs.

A The outside circle represents the genomic DNA, and the red color represents circRNAs reads junction of each sample. Different color represents different sample. The zoomed-in part is chromosome 11, and the locus of circHIPK3 is chr11:33286413|33287511 (–). The scale of the axis is 10^6 bp.

B Volcano plots were constructed for visualizing differentially expressed circRNAs between bladder cancer and normal bladder samples. Differentially expressed circRNAs were filtered by $|\text{FC} (\text{fold change})| \geq 2$ (Log_2 scaled) and $P < 0.05$ ($-\text{Log}_{10}$ scaled). The red points represent significantly up-regulated circRNAs, and blue points represent significantly down-regulated circRNAs.

Target: circHIPK3 Length: 1099

miRNA : hsa-miR-558 Length: 19

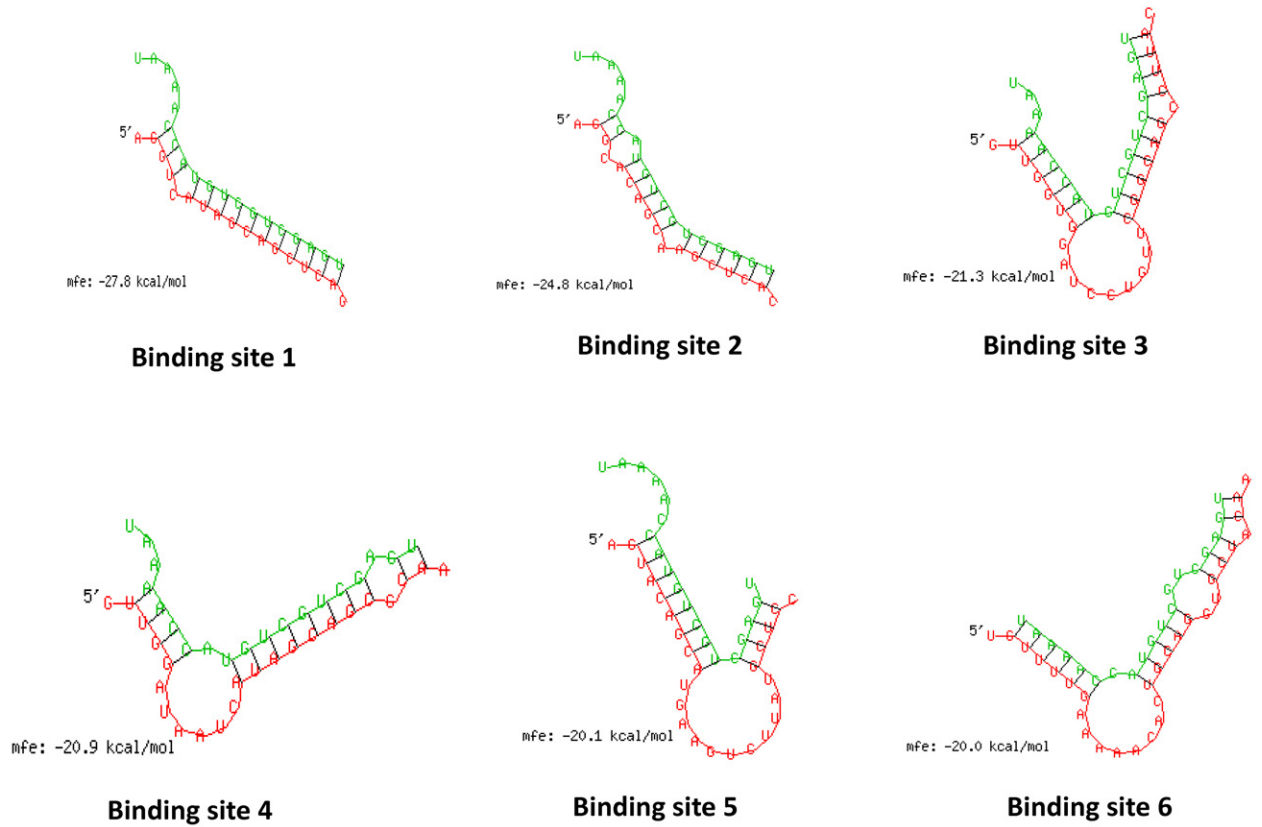


Figure EV3. Binding sites of miR-558 on circHIPK3.

Detailed information of six binding sites of miR-558 on circHIPK3 that were analyzed by the bioinformatics program RNAhybrid.

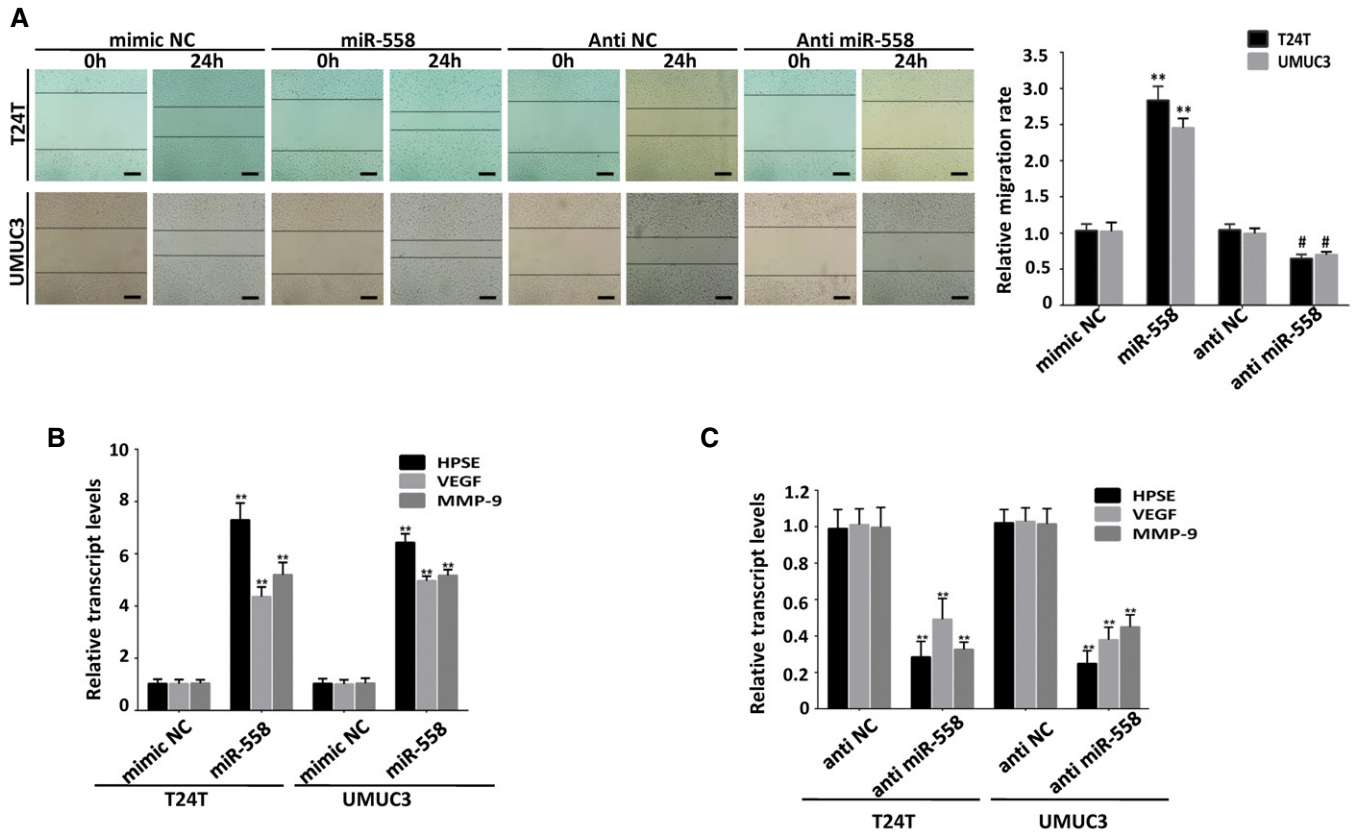


Figure EV4. miR-558 promotes cell migration, invasion, and angiogenesis via up-regulating the expression of HPSE *in vitro*.

- A** Wound healing assay indicating that transfection of miR-558 mimics promoted cell migration, while transfection with anti-miR-558 inhibited cell migration. Data are mean \pm SEM, $n = 3$. ** $P < 0.01$ versus mimic NC; # $P < 0.05$ versus anti-NC (Student's *t*-test). Scale bar, 200 μ m.
- B, C** Real-time PCR assay indicating that transfection of miR-558 mimics up-regulated the expression of HPSE and its downstream targets MMP-9 and VEGF in bladder cancer cells, and anti-miR-558 down-regulated the expression of HPSE, MMP-9, and VEGF. Data are mean \pm SEM, $n = 3$. ** $P < 0.01$ versus mimic NC or anti-NC (Student's *t*-test).

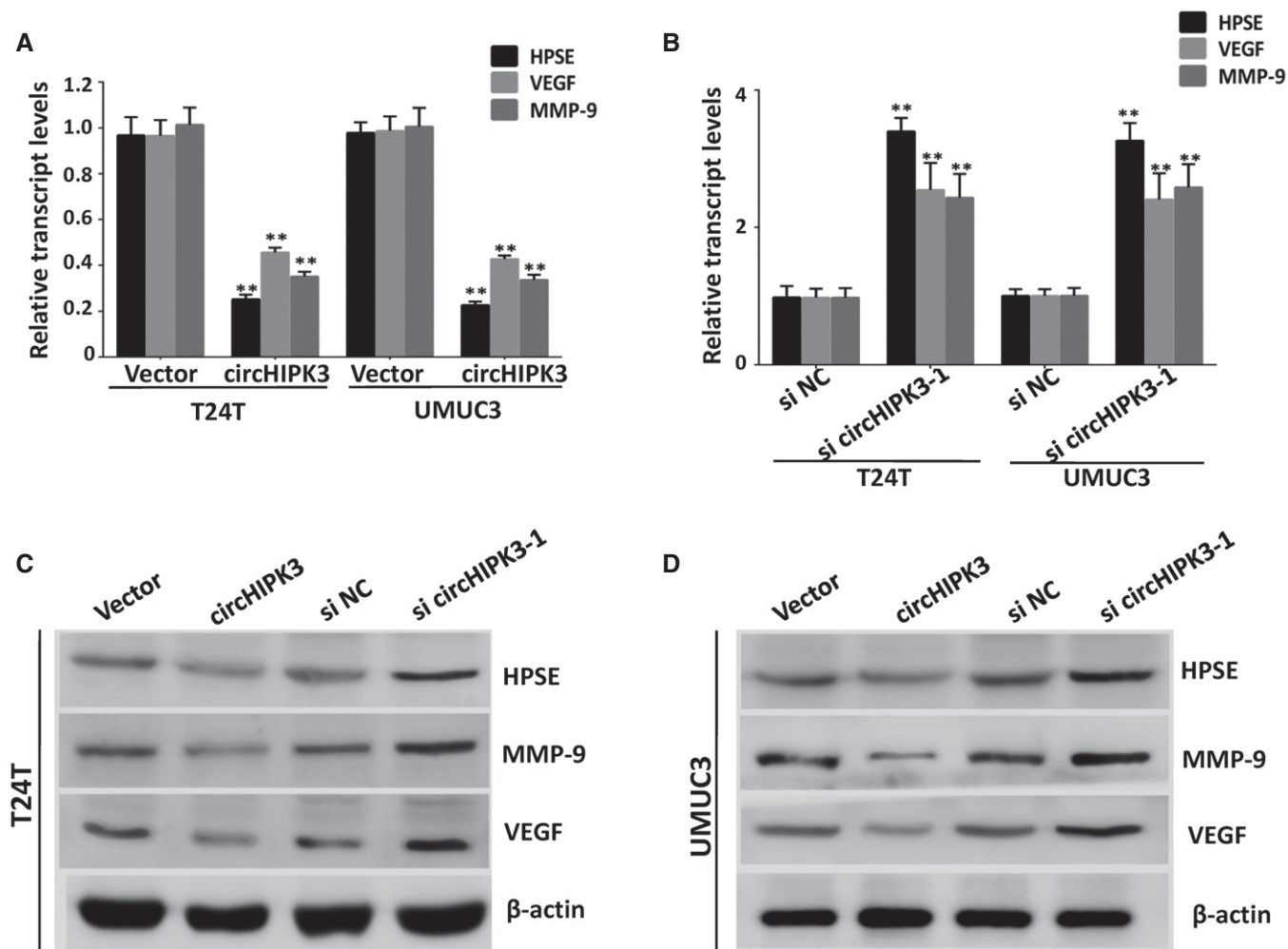


Figure EV5. Over-expression of circHIPK3 down-regulates the expression of HPSE, MMP-9, and VEGF in bladder cancer cells.

A–D Real-time PCR and Western blot assays demonstrated that over-expression of circHIPK3 in bladder cancer cells significantly down-regulates the expression of HPSE, together with its downstream targets MMP-9 and VEGF. In contrast, knockdown of circHIPK3 up-regulates the expression of HPSE, MMP-9, and VEGF. Data are mean ± SEM, $n = 3$. ** $P < 0.01$ versus vector (A) or si NC (B) (Student's t -test).