Figure S1. KIF4A is efficiently knockdown by KIF4A targeted shRNA transfecting in both T24 and 5637 cells. (A) RT-qPCR results showing that KIF4A expression was efficiently decreased in T24 and 5637 BC cells. (B) Western blot analysis revealed that KIF4A expression was markedly decreased in T24 and 5637 cells. Results are presented as the means  $\pm$  SEM; \*\*P<0.01 vs. control. KIF4A, kinesin family member 4A; BC, bladder cancer.



Figure S2. KIF4A depletion induces the arrest of cell cycle in BC cells. (A) T24 and 5637 cells were transfected with control or KIF4A shRNA, and flow cytometric assays were subsequently performed. The percentage of cells in G1, S and G2/M phases between the control and KIF4A ablation groups was determined. (B) Western blot analysis exhibited the expression levels of cyclin D1 and cyclin A in the control- or KIF4A shRNA-transfected T24 and 5637 cells. Results are presented as the mean  $\pm$  SEM; \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001, vs. control; ns, not significant. KIF4A, kinesin family member 4A; BC, bladder cancer.



Figure S3. KIF4A does not affect the regulation of CDCA1 and CDCA8 expression. (A) TCGA data show the correlations between CDCA1, CDCA8 expression and KIF4A expression in BC tissues. (B) Luciferase activity of pGL3-Basic, pGL3-CDCA1 or pGL3-CDCA8 in T24 cells co-transfected with pEnter-KIF4A or pEnter-vector plasmids was analyzed by luciferase reporter assays. (C) PCR amplification of the anti-IgG or anti-KIF4A antibody enriched CDCA1 and CDCA8 promoter fragment in T24 cells performing ChiP assays; ns, means not significant. KIF4A, kinesin family member 4A; BC, bladder cancer.



Figure S4. KIF4A or CDCA3 are efficiently overexpressed by KIF4A or CDCA3 plasmid transfecting in T24 cells. Western blot analysis showing that KIF4A or CDCA3 were efficiently overexpressed by KIF4A or CDCA3 plasmid transfecting in T24 cells. KIF4A, kinesin family member 4A; BC, bladder cancer; CDCA3, cell division cycle-associated protein 3.

