

## Inventory of Supporting Information

**Supplementary Figure S1.** Characterization of *TUBB4A* expression profiling and genetic alteration in human primary prostate cancer.

**Supplementary Figure S2.** CRISPR-Cas9 *TUBB4A* knockout (KO) in prostate cancer cell lines.

**Supplementary Figure S3.** Cell movement and migration of scrambled and *TUBB4A* KO prostate cancer cell lines.

**Supplementary Figure S4.** Ectopic expression of *TUBB4A* rescues the *TUBB4A* KO-reduced proliferation and migration of prostate cancer cells.

**Supplementary Figure S5.** *TUBB4A* KO increases the cell area and the F-actin intensity in prostate cancer cells.

**Supplementary Figure S6.** *TUBB4A* KO does not change  $\alpha$ -tubulin or lamin.

**Supplementary Figure S7.** *TUBB4A*-related signaling pathways and gene network.

**Supplementary Figure S8.** *MYH9*-related GSK3 $\beta$ / $\beta$ -catenin signaling gene network and the effect of *MYH9* knockdown on growth of prostate cancer cells.

**Supplementary Figure S9.** Expression of EMT, DNA damage response and apoptotic markers in scrambled and *TUBB4A* KO DU145 xenograft tumors of NSG mice.

**Supplementary Figure S10.** *TUBB4A* KO reduces tumor colonization in the lungs of NSG mice.

**Supplementary Figure S11.** Establishment of genetically engineered spontaneous prostate cancer mouse models.

**Supplementary Table S1.** Human specimen characteristics

**Supplementary Table S2.** Mass spectrometry analysis for *TUBB4A*-interacting proteins

**Supplementary Table S3.** Specific primary antibodies used in this study

**Supplementary Table S4.** The sequences of primer, sgRNA, siRNA, and ORF used in this study

**Supplementary Video 1.** Automated live-cell two-dimensional random movement assay for scramble control PC3 cells.

**Supplementary Video 2.** Automated live-cell two-dimensional random movement assay for *TUBB4A* KO PC3 cells.

**Supplementary Video 3.** Automated live-cell two-dimensional random movement assay for scramble control DU145 cells.

**Supplementary Video 4.** Automated live-cell two-dimensional random movement assay for *TUBB4A* KO DU145 cells.

**Supplementary Video 5.** Automated live-cell two-dimensional scratch migration assay in scramble control DU145 cells.

**Supplementary Video 6.** Automated live-cell two-dimensional scratch migration assay in *TUBB4A* KO1 DU145 cells.

**Supplementary Video 7.** Automated live-cell two-dimensional scratch migration assay for *TUBB4A* KO2 DU145 cells.

**Source data**