Supplemental figure legends

Supplemental figure 1

Overexpression of ZBRK1 inhibits cells proliferation and anchorage-independent activity in U2OS cells. (**A**) ZBRK1 attenuated the growth rate of cells. Equal amounts of U2OS cells stably expressing EGFP (control) or EGFP-ZBRK1 (ZBRK1) were seeded, and then the viable cell number determined at the indicated times. Data shown are the mean of three independent experiments. (**B**) An increase in ZBRK1 expression attenuated cell proliferation. A focal formation assay was performed with the stable cell lines of U2OS cells. The colonies were detected by methyl blue staining, and then the number of colonies counted for statistical analysis. Values are the relative cell number ± S.E.M. (**C**) Overexpression of ZBRK1 attenuated focal formation of cancer cells. A soft agar assay was performed with U2OS cell lines stably expressing EGFP (C) or EGFP-ZBRK1 (ZBRK1). The colonies were detected by 0.005% crystal violet staining, and then the number of colonies counted for statistical analysis. Values are the relative cell number ± S.E.M. Each result shown here is plotted from three independent experiments. (**D**)(**E**) Verification of ZBRK1-regulated candidates in stable lines of U2OS, HeLa and xenogenic tumor. Transcripts of selected ZBRK1 downstream targets were confirmed

by RT-PCR in U2OS cells stably expressing EGFP or EGFP-ZBRK1. Transcripts of human GAPDH served as a control. ZBRK1 attenuated MMP9 and upregulated ICAM1 expression in EGFP-ZBRK1-expressing HeLa cells and xenogenic nude mice. Left panel: Expression levels of multiple genes in HeLa cells stably expressing EGFP or EGFP-ZBRK1 were confirmed by RT-PCR. Right panel: Expression levels of multiple genes of tumor samples from mice resulting from subcutaneous injections with EGFP (TS-C) or EGFP-ZBRK1#6 (TS-#6) HeLa cells, analyzed by RT-PCR. The transcripts of human GAPDH served as a control.

Supplemental figure 2

Profiling of modulating ZBRK1 in U2OS and HeLa cells. Two profiling of modulating ZBRK1 expression in U2OS and HeLa cells were shown in (A) and (B), respectively. The potential ZBRK1-regulated candidates involved in tumorigenesis were selected to show in the lower panels of (A) and (B).

Supplemental figure 3

ZBRK1 inhibits cell migration in U2OS and HeLa cells. (**A**) Wound-healing migration was performed with control, EGFP- and EGFP-ZBRK1-expressing cells as described in Figure 3. (**B**) The level of cell migration was determined using QCM^{TM}

Haptotaxis 96-well cell migration assays in HeLa (left panel) and U2OS cells (right panel). The plot shows the statistical results from three independent experiments. (**C**) Inactivation of ZBRK1 increased migration of cancer cells. U2OS cells were treated with lentiviral shZBRK1 or shGFP in the QCM^{TM} Haptotaxis cell migration assays as in (B). (**D**) The loss-of-function ZBRK1 enhanced MMP9 transcripts in U2OS cells. Stable ZBRK1-expressing cells were incubated with lentiviral shRNA of ZBRK1 or the control for 24 h. The lysates and total RNA of infected cells were harvested for Western blot and RT-PCR analyses, respectively.

Supplemental figure 4

ZBRK1 binds to putative ZBRK1-binding motifs of candidate genes' 5'-flanking region. EMSA was performed using *in vitro*-translated FLAG/ZBRK1 and individual ^{32}P-labeled probes containing putative ZBRK1-binding motifs. Sequences of the putative ZBRK1-binding motifs on the 5'-flanking regions of interested targets were shown on lower panel.

Supplemental figure 5

Multiple genes are expressed in clinical cervical cancer patients. An RT-PCR of surgical biopsies from 12 cervical cancer patients was performed using specific

primers as indicated. GAPDH transcription level was used as the loading control. “N” and “T” respectively denote “normal” and “tumor” areas of the same patients.