

Figure S1. PLK4 affects proliferation and invasion of CRC cells.

A. The verification of PLK4-control (sh-control), PLK4-knockdown (sh-PLK4) and empty-vector and PLK4-overexpression (PLK4-OE) in CRC cell lines. B. CCK-8 assay revealing the proliferative abilities of SW480 and HCT8 cells in the sh-control and sh-PLK4 groups. C. GSEA showing the relationships between PLK4 expression and proliferation as well as cell cycle genes. D&E. Wound-healing, chemotaxis assays comparing cell migration and invasion differences between the sh-control and sh-PLK4 groups of SW480 and HCT8 cells (scale bar, 1.0 mm). F. The SA- β -gal staining for senescence cells in sh-control and sh-PLK4 groups and the percentage of SA- β -gal positive cells were quantified (scale bar, 150 μ m). ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S2. PLK4 is overexpressed in CRC tissues and associated with a series of malignant behaviors linked to an unfavorable prognosis.

A-F: GSEA was used to analysis the associations of high PLK4 expression in CRC with a series of malignant behaviors linked to an unfavorable prognosis in CRC. G. Enhanced PLK4 expression was observed in tumor tissues compared with adjacent non-tumor tissues in most cancer types, including CRC. H. The expression of PLK4 was examined in CRC tissue samples and adjacent nonmalignant tissue samples from the TCGA and GEO databases (N: normal, T: tumor, and P: polyp). I-L: Correlation analysis between the PLK4 IHC score and lymphatic metastasis, tumor capsule, TNM stage as well as CEA in the CRC cohort. M-P. Kaplan-Meier survival curve showing the correlation between the PLK4 IHC score and OS of the high-risk subgroup in the CRC cohort. **

$p < 0.01$, *** $p < 0.001$.

Figure S3. A. The representative flow cytometry images of cell cycle in Vector, PLK4 OE with or without rapamycin groups. B. The mRNA levels of PLK4 and autophagy-related markers in sh-control, sh-PLK4 with or without BIRB796. ns: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S4. A-C. The lung images (A), lung weights (B), and H&E staining of lung metastasis in tail vein-injected mice (scale bar, 100 μm ; magnification, 4 \times and 40 \times) D. Verification of PLK4 and cell proliferation, cell cycle and autophagy markers in the xenograft model, as assessed by IHC (scale bar, 100 μm ; magnification, 20 \times and 40 \times). ns: $p > 0.05$.







