

**Supplementary figure 1.** **A.** Venn diagram showing the overlap between essential genes and differentiation-associated genes in neuroblastoma. **B.** Kaplan-Meier curves of overall neuroblastoma survival probability between PLK4 high group and low group generated from publicly available neuroblastoma data sets accessible at the TARGET and GSE49711 analysis. **C.** Western blotting analysis of PLK4, E-Cadherin and N-Cadherin expression in NB tissues with different differentiation status. **D.** Western blotting analysis of E-Cadherin and N-Cadherin expression in PLK4-overexpression SH-SY5Y with different dosages of 13-*cis* RA. **E.** GSEA analysis indicates enrichment and upregulation of genes associated with neurogenesis, neuron development and neuron differentiation in samples with low expression levels of PLK4. **F.** Photomicrographs depict five NB cells following a 5-day treatment with either 10  $\mu$ M 13-*cis* RA or 0.1% DMSO. The presented images are representative of three independent samples. **G.** Immunofluorescence staining showing neurite outgrowth in SH-SY5Y cells with DMSO or 13-*cis* RA (Scale bar, 5  $\mu$ m). **H.** Western blotting analysis of PLK4 expression in 5 NB cell lines.

**Supplementary figure 2.** **A.** GO analysis was conducted on differentially expressed genes subsequent to the knockdown of PLK4.  $n = 3$ , Fisher's exact test. **B.** GSEA plot of axon extension, axogenesis, axon guidance, neuron development, neurogenesis, regulation of neurogenesis between the PLK KD group and control group. **C.** Representative photomicrographs for IMR-32, SK-N-SH and SK-N-AS cells showing neurite outgrowths (red arrows) after PLK knockdown. **D.** Cell cycle distribution analysis following PLK4 KD/OE. Data represent mean  $\pm$  SD. Three technical replicates shown. **E.** Significant changes in proliferation of NB cell lines following PLK4 KD/OE.  $**p < 0.01$ ,  $***p < 0.001$ ,  $****p < 0.0001$ , ns: no significance.

**Supplementary figure 3.** **A.** Western blotting analysis demonstrating the effect on different signaling pathway activation following PLK4-knockdown in IMR-32. **B.** PLK4-overexpression SK-N-BE(2) and SH-SY5Y cells were pretreated with PD98059 or BIRB796. Western blotting analysis of the protein levels of pathway and differentiation-associated protein. **C-D.** Tumor development in mice post injection of SK-N-AS cells stably infected with shRNA against the SCR or KD. Tumor volumes were measured every 4 days. Data are presented as the mean  $\pm$  SEM of tumors in 5 mice. **E.** Tumor weights in the two groups. **F.** CXCR4 modulation in PLK4-altered cells influences differentiation marker expression, indicating a regulatory link in NB differentiation. **G.** CXCR4 protein levels were assessed by Western blotting in Vector and PLK4 OE cells treated with 10  $\mu$ g/mL cycloheximide (CHX) for the indicated time points. **H.** CXCR4 protein levels were evaluated in Vector and PLK4 OE cells with or without treatment with the proteasome inhibitor MG132 (2  $\mu$ M). Correlation between PLK4 expression and CCND1(**I**), GAP43, PHOX2B as well as SYN (**J**) in samples obtained from the GSE49711 dataset.  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ,  $****p < 0.0001$ .

**Supplementary figure 4.** **A.** The expression of PLK4 in tissue microarray (TMA) samples was evaluated by immunohistochemistry. **B.** Kaplan-Meier plots displayed overall survival (left) and progress free survival (right) that were associated with PLK4 expression levels. **C.** Kaplan-Meier curve displaying overall survival and progress free survival for patients with different differentiation status. Western blotting (**D**) and RT-PCR (**E**) analysis of PLK4 expression in NB tissue with different stage. **F.** Correlation analysis between PLK4 expression and International Neuroblastoma Staging System (INSS) staging. **G.** Photomicrographs depict five NB cells following a 5-day treatment with either CFI-400945

or 0.1% DMSO. **H.** Immunofluorescence staining showing  $\beta$ -III tubulin expression and neurite outgrowth in SH-SY5Y cells treated with DMSO or CFI-400945 (Scale bar, 5  $\mu$ m). \*\*\* $p < 0.001$ .