

**Fig. S1. KPNA2 was overexpressed in osteosarcoma and correlated with poor prognosis. Related to Figure 1.**

**A** The differential expression of NTR genes between normal and osteosarcoma tissues in the GSE87624 dataset. **B** The correlation between RANBP1 expression and osteosarcoma prognosis by Kaplan-Meier survival analysis. **C** Construction of a prognostic Nomogram model for predicting osteosarcoma individual survival using the GSE21257 dataset. **D** Confirming the accuracy of the prognostic Nomogram model by calibration plot.



**Fig. S2. KPNA2 promoted osteosarcoma growth i*n vitro* and *in vivo*. Related to Figure 2.**

**A and B** KPNA2 expression was detected in osteosarcoma cell lines (U2OS, 143B, HOS, MG63) and a human osteoblast cell line (hFOB1.19) by western blotting. **C** RT-qPCR was performed to examine KPNA2 expression in U2OS and 143B cells after transfection with siNC or siKPNA2 (siKPNA2-1, siKPNA2-2, siKPNA2-3). **D and E** The knockdown efficiency of KPNA2 (siKPNA2-1, siKPNA2-2, siKPNA2-3) was detected in U2OS and 143B cells by immunoblotting. **F and G** Effect of KPNA2 knockdown on the proliferation of U2OS and 143B by Edu assay. Scale bar: 50 μm.



**Fig. S3. KPNA2 promoted osteosarcoma growth and metastasis via the hedgehog/GLI1 pathway. Related to Figure 5.**

**A** Knockdown of GLI1 (siGLI1-3) reversed or partly reversed the effects of oeKPNA2 on the expression of OCT4, SOX2, N-cadherin, PCNA, P21 and BAX proteins in 143B cells using immunoblotting. Knockdown of GLI1 reversed or partly reversed the effects of oeKPNA2 on cell function by CCK8 assay (**B**), cell cycle assay (**C and D**), and apoptosis assay (**E and F**), in 143B cells. **G–J** Knockdown of GLI1 reversed or partly reversed the promotor effects of oeKPNA2 on the cell migration and invasion ability of 143B cells by the Transwell assay or wound healing assay. Scale bar: 50 μm.



**Fig. S4. KPNA2 regulated the hedgehog/GLI1 pathway in a c-Myc- dependent manner. Related to Figure 6.**

**A** Co-IP was performed to exam the endogenous interaction between KPNA2 and c-Myc; cell lysates of 143B cells were precipitated with anti-KPNA2 or anti-c-Myc antibodies, and the precipitates were examined by immunoblotting. **B and C** The siNC- or siKPNA2-depleted 143B cells were subjected to extraction of the cytosol and nuclear fractions. Subsequently, western blot was performed to detect the expression of KPNA2 and c-Myc. Lamin B1 and GAPDH were used as reference proteins for nuclear and cytoplasmic fractions, respectively. **D and E** The 143B cells were prepared for immunofluorescence staining using an anti-KPNA2 antibody to detect the subcellular expression of KPNA2 and c-Myc. Nuclei were stained with DPAI. Scale bar: 20 μm. **F and G** ChIP-PCR and ChIP-qPCR were performed to detect the endogenous interaction between c-Myc BS and the promoter regions of GLI1 or KPNA2 in 143B cells. H Effect of MYC knockdown (siMYC-1 and siMYC-2) on the expression of GLI1 and KPNA2 in 143B cells by RT-qPCR.



**Fig. S5. The knockdown efficiency of GLI1 and MYC. A** The knockdown efficiency of GLI1 (siGLI1-1, siGLI1-2, siGLI1-3) was detected in U2OS and 143B cells by qPCR. **B** The knockdown efficiency of MYC (siMYC-1, siMYC-2, siMYC-3) was detected in U2OS and 143B cells by qPCR.