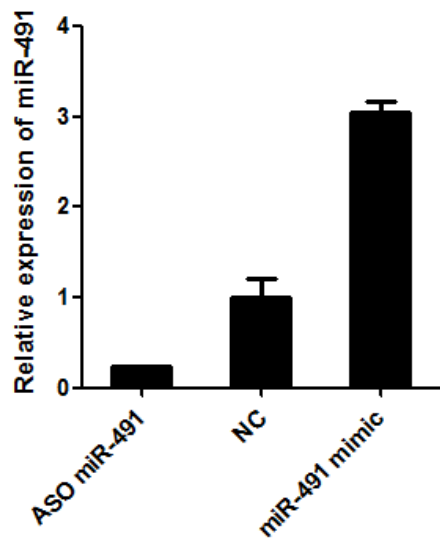
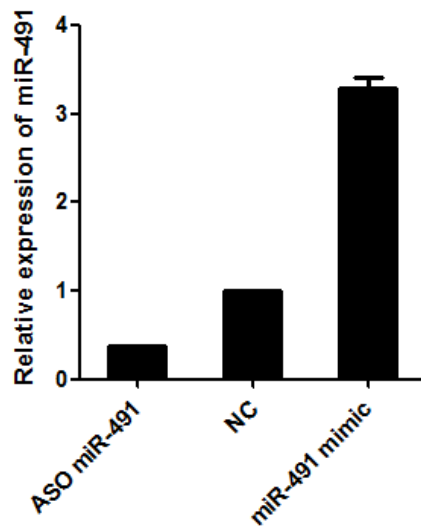
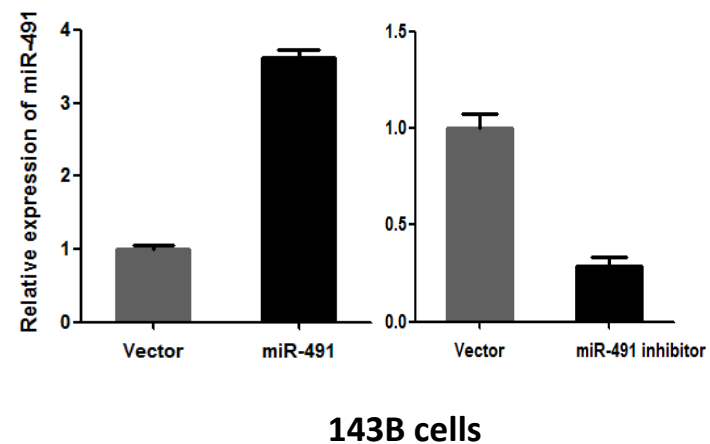
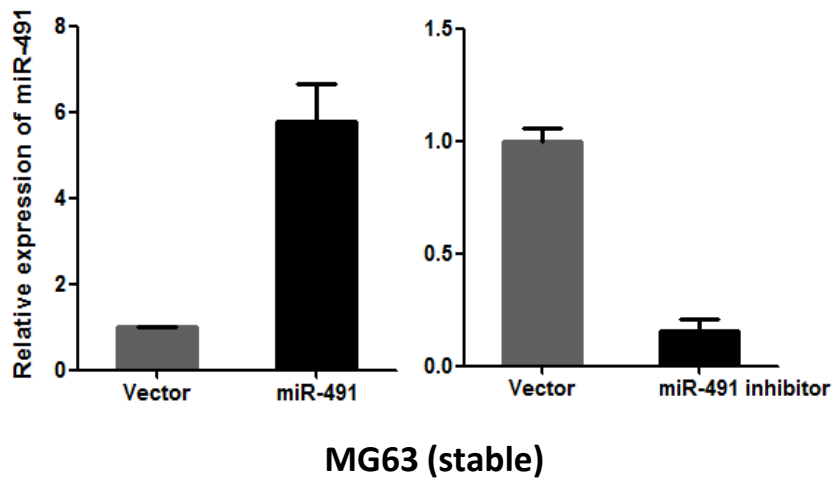
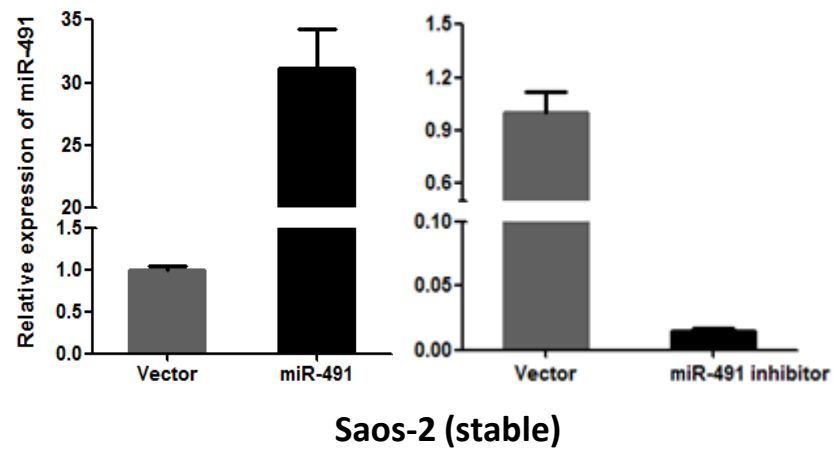
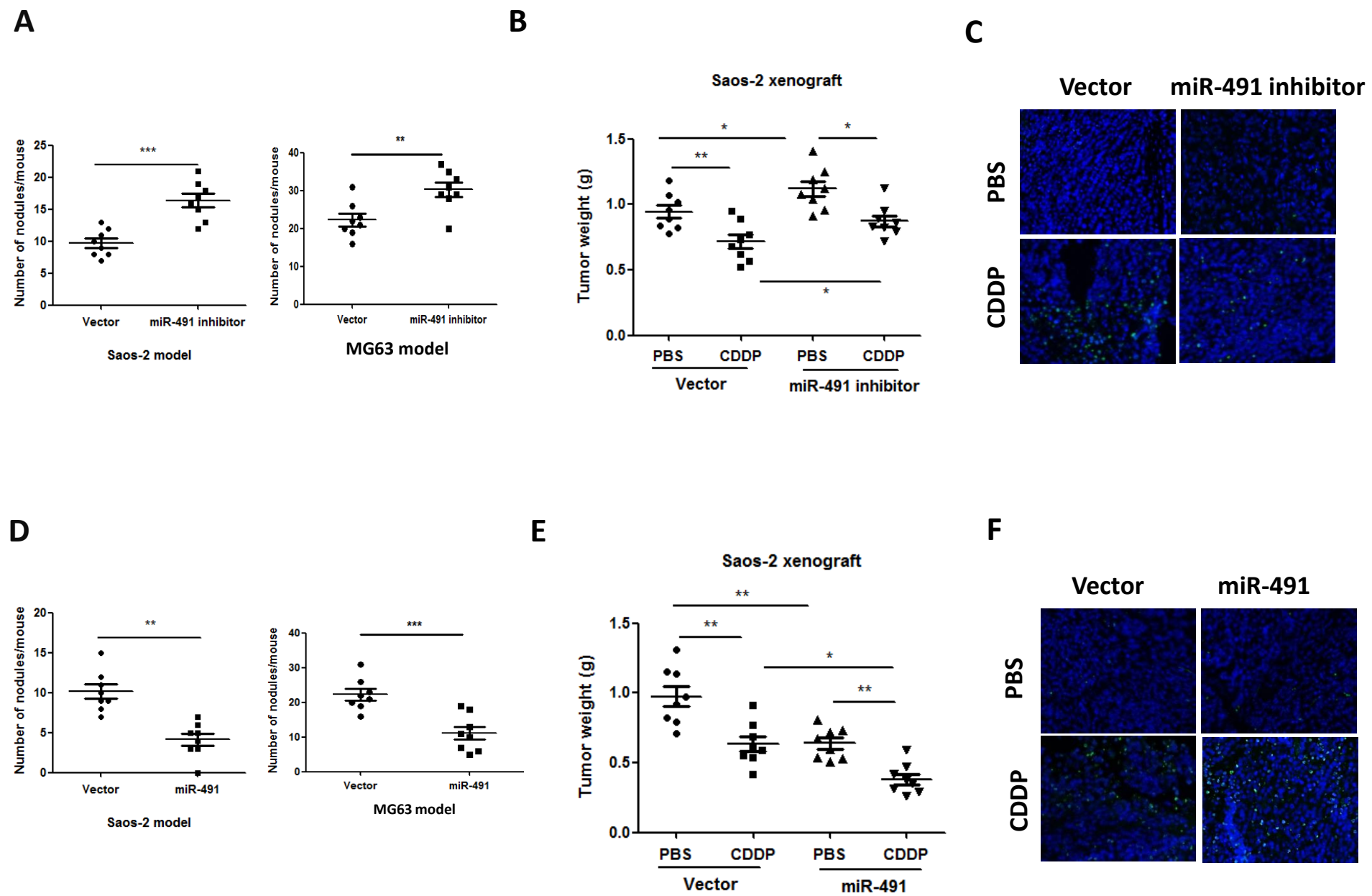


## Supplemental Information

### **miR-491 Inhibits Osteosarcoma Lung Metastasis and Chemoresistance by Targeting $\alpha$ B-crystallin**

**Shu-Nan Wang, Song Luo, Chang Liu, Zhenghao Piao, Wenlong Gou, Yun Wang, Wei Guan, Qing Li, Hua Zou, Zhen-Zhou Yang, Dong Wang, Yan Wang, Meng Xu, Hua Jin, and Cheng-Xiong Xu**

**A****Saos-2****B****U2OS****C****D****E****Figure S1**



**Figure S2.**

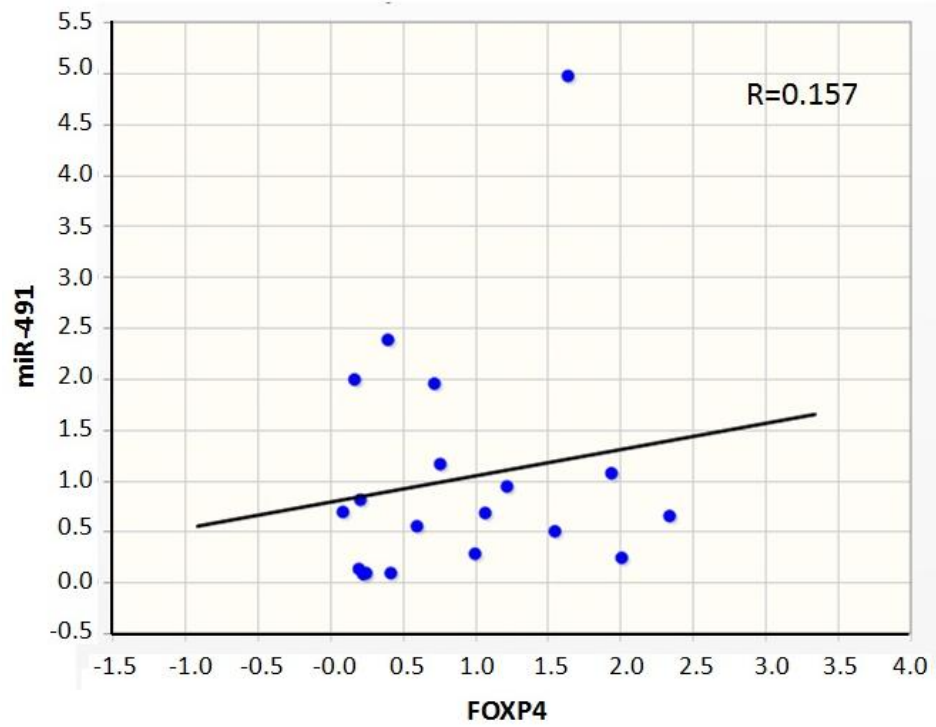
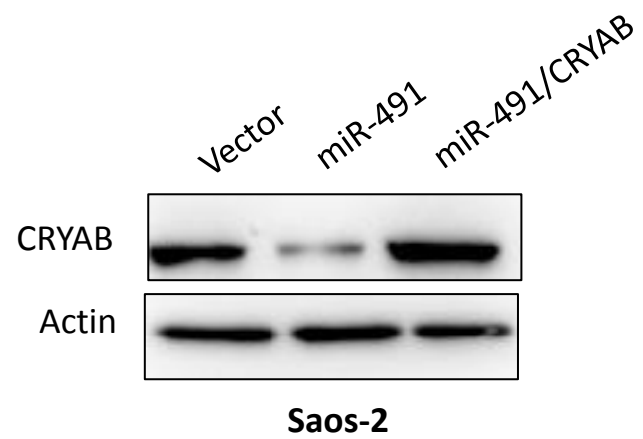
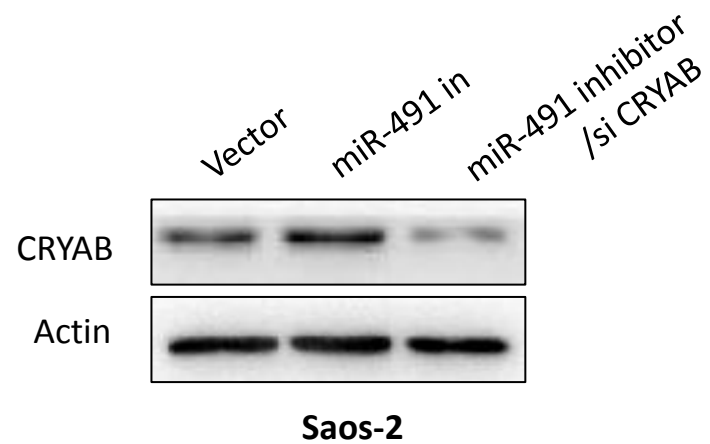


Figure S3.

**A****B****Figure S4.**

## SUPPLEMENTARY FIGURE LEGENDS

**Figure S1. Expression of miR-491 in indicated OS cells.** (A) Saos-2 cells were transfected with the indicated oligonucleotides. After 48 hours of transfection, the cells were subjected to RT-qPCR analysis to detect the miR-491 expression level. (B) U2OS cells were transfected with the indicated oligonucleotides. After 48 hours of transfection, the cells were subjected to RT-qPCR analysis to detect the miR-491 expression level. (C) 143B cells were transfected with the indicated plasmids. After 72 hours of transfection, the cells were subjected to RT-qPCR analysis to detect the miR-491 expression level. (D) The expression level of miR-491 was measured by RT-qPCR in Saos-2 cells stably expressing miR-491 (miR-491) or miR-491-antisense (miR-491 inhibitor). (E) the expression level of miR-491 was measured by RT-qPCR in MG63 cells stably expressing miR-491 or miR-491-antisense. NC: negative control oligonucleotides, ASO miR-491: antisense oligonucleotides of miR-491.

**Figure S2. miR-491 negatively regulates OS cell lung metastasis and chemoresistance *in vivo*.** (A) Inhibition of miR-491 stimulates OS cell lung metastasis in an animal model. Indicated cells that stably expressed miR-491-antisense (miR-491 inhibitor) were injected into the tail vein of 6-week-old nude mice ( $n = 8$  per mice). The mice were sacrificed 6 weeks after the tail vein injection, and the lung surface nodules were counted using microscopy. (B) Inhibition of miR-491 promotes tumor growth and induces resistance to CDDP in Saos-2 xenograft models. Saos-2 cells stably expressing miR-491-antisense were injected subcutaneously into nude mice ( $n = 8$  per group). After the tumor size reached approximately  $100 \text{ mm}^3$ , the mice were started on a treatment of either PBS or CDDP (10 mg/kg body weight). The mice were sacrificed after 3 weeks of CDDP treatment, and the tumor weight was measured. (C) Apoptotic cells were detected using a TUNEL assay in the indicated xenograft tumor samples. (D) Overexpression of miR-491 inhibits OS cell lung metastasis. Indicated cells that stably expressed miR-491 (miR-491) were injected into the tail vein of 6-week-old nude mice ( $n = 8$

per group). The mice were sacrificed 6 weeks after the tail vein injection, and the lung surface nodules were counted using microscopy. (E) Overexpression of miR-491 inhibits tumor growth and enhances the tumor growth inhibition effect of CDDP in animal models. Saos-2 cells stably expressing miR-491 were injected subcutaneously into nude mice ( $n = 8$  per group). After the tumor size reached approximately 100 mm<sup>3</sup>, the mice were started on a treatment of either PBS or CDDP (10 mg/kg body weight). The mice were sacrificed after 3 weeks of CDDP treatment, and the tumor weight was measured. F, Apoptotic cells were detected using a TUNEL assay in the indicated xenograft tumor samples.

**Figure S3. Correlation between miR-491 and FOXP4 expression in OS specimens.**

Using RT-qPCR, we analyzed the expression of miR-491 and FOXP4 in 18 tumor specimens from patients with OS.

**Figure S4. Expression of CRYAB.** (A) Saos-2 cells that stably overexpressed miR-491 were transfected with a CRYAB expression plasmid. After 72 hours of transfection, cells were subjected to Western blot analysis. (B) Stably overexpressing miR-491-antisense Saos-2 cells were transfected with CRYAB siRNA (siCRYAB). After 72 hours of transfection, cells were subjected to Western blot analysis.