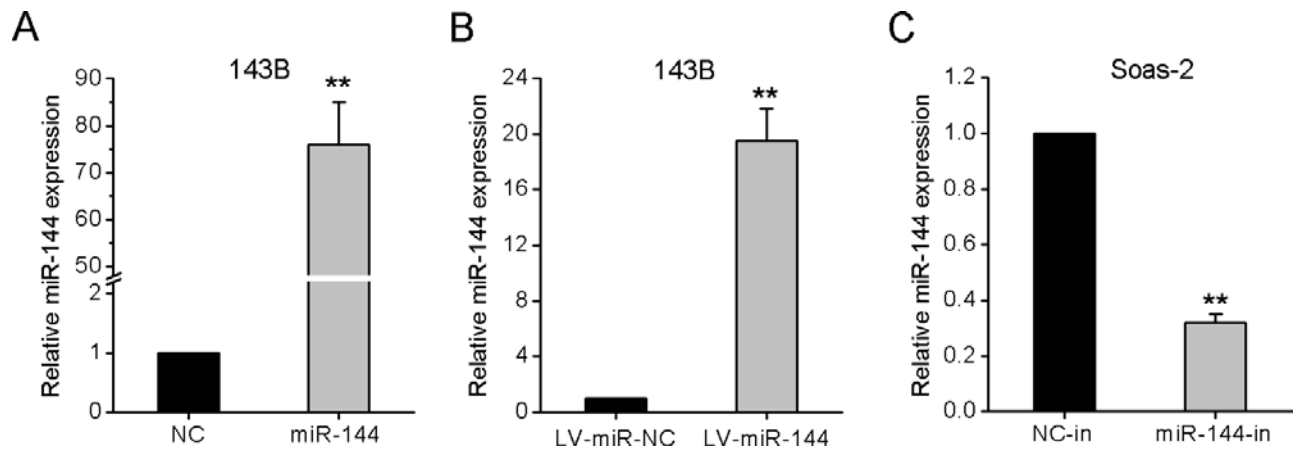
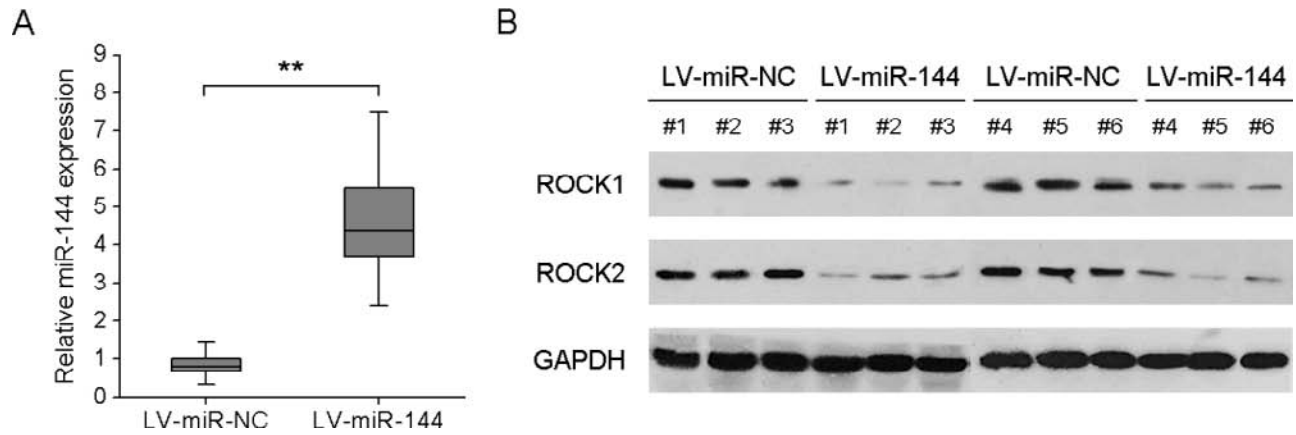


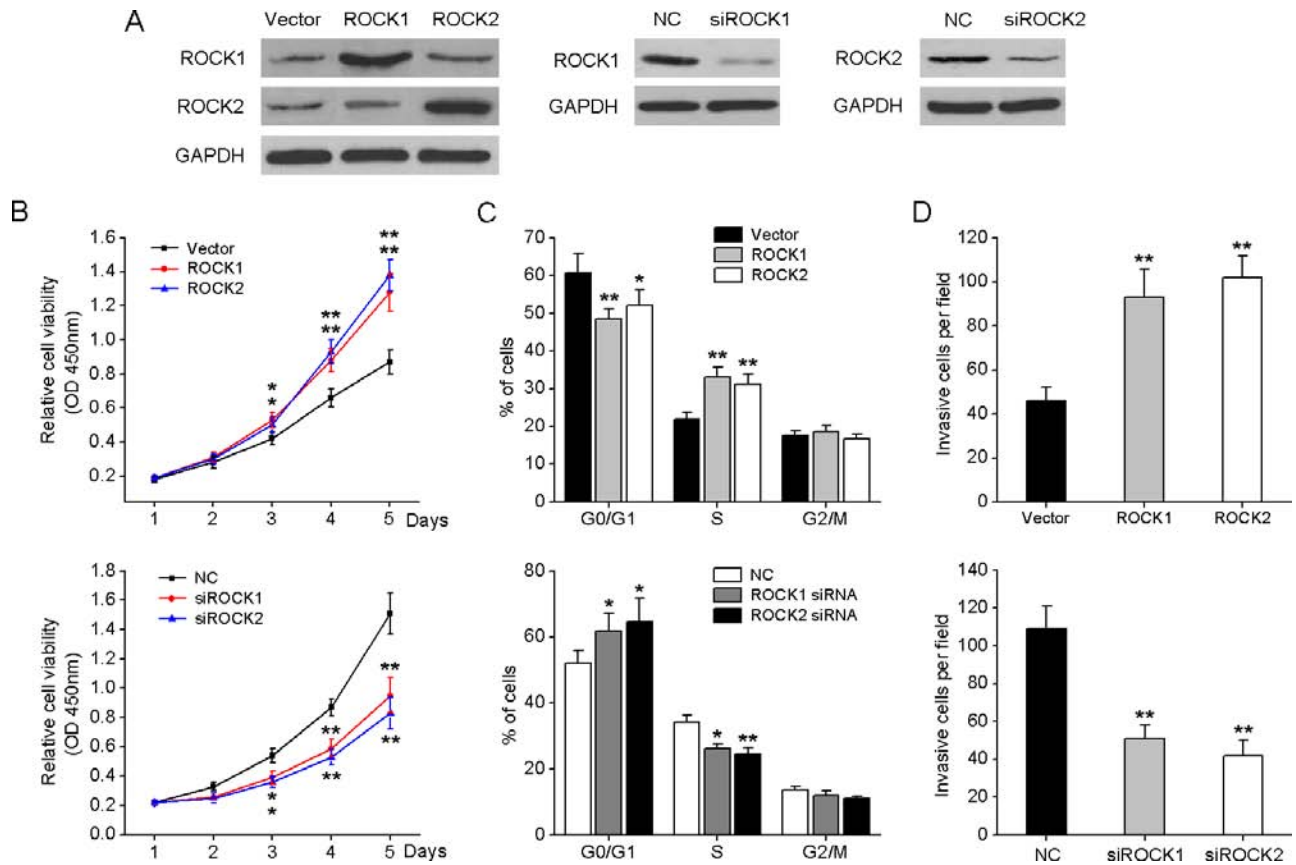
SUPPLEMENTARY FIGURES AND TABLES



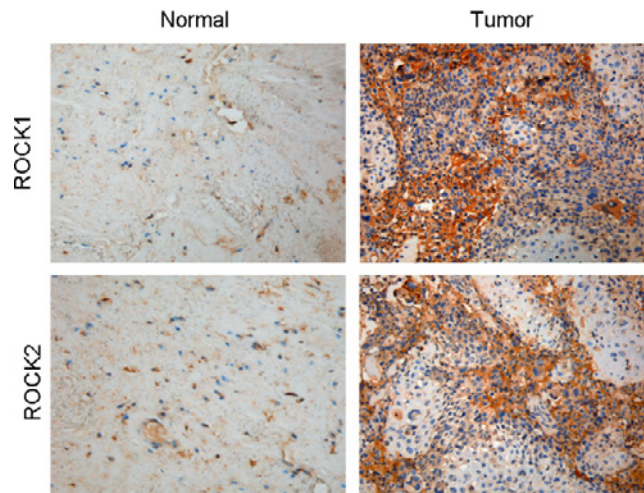
Supplementary Figure S1: Modification of miR-144 levels in the OS cell lines 143B and Soas-2. **A.** Relative expression of miR-144 in 143B cells transfected by miR-144 mimics (100 nM) or NC mimics. **B.** Relative expression of miR-144 in 143B cells stably infected with lentivirus expressing miR-144 or NC. **C.** Relative expression of miR-144 in Soas-2 cells transfected by miR-144 inhibitor or NC inhibitor. ** $P < 0.01$.



Supplementary Figure S2: miR-144 inhibited expression of ROCK1 and ROCK2 in xenograft tumor tissues.
A. Expression of miR-144 in isolated tumors was analyzed by qRT-PCR. **B.** Western blot analysis of ROCK1 and ROCK2 protein levels in isolated tumors. ****** $P < 0.01$.



Supplementary Figure S3: Downregulation of *ROCK1* and *ROCK2* mediates miR-144's function in OS cells. 143B-miR-144 cells were transfected with empty pcDNA3.1 vector, *ROCK1*- or *ROCK2*-containing plasmid (left); 143B cells were transfected with siRNA against *ROCK1* or *ROCK2* (right). Western blot analysis of *ROCK1* and *ROCK2* expression is shown in **A**. Cell viability **B**, cell cycle distribution **C**, and cell invasion **D**, were measured with the CCK-8 assay, flow cytometry and transwell invasion assay, respectively. * $P < 0.05$, ** $P < 0.01$.



Supplementary Figure S4: Immunohistochemistry (IHC) analysis of ROCK1 and ROCK2 protein expression in OS. Representative images from IHC assays of 24 paired OS and adjacent normal bone tissues.

Supplementary Table S1. Summary of clinicopathologic variables

Clinical pathologic parameters	Number of Patients
Gender	
Male	39
Female	28
Age (yrs)	
< 18	31
≥ 18	36
Location	
Femur	43
Tibia	17
Other	7
Tumor size (cm)	
< 8	30
≥ 8	37
Enneking stage	
I	9
II	45
III	13
Histologic grade	
Low grade	9
High grade	58
Metastasis	
Present	13
Absent	54

Supplementary Table S2. Primers used for qRT-PCR

Primers	Nucleotide sequence	
miR-144	RT-Oligo	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGAT ACGACAGTACA-3'
	Sense	5'- TACAGTATAGATGA -3'
	Antisense	5'- GTGCAGGGTCCGAGGT -3'
U6 snRNA	RT-Oligo	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACAAAATATG -3'
	Sense	5'- GCGCGTCGTGAAGCGTTC -3'
	Antisense	5'- GTGCAGGGTCCGAGGT -3'
ROCK1	Sense	5'- AAAAAATGGACAACCTGCTGC -3'
	Antisense	5'- GGCAGGAAAATCCAAATCAT-3'
ROCK2	Sense	5'- CGCTGATCCGAGACCCT -3'
	Antisense	5'- TTGTTTTTCCTCAAAGCAGGA-3'
β -actin	Sense	5'- GCATCCTCACCCCTGAAGTAC -3'
	Antisense	5'- GGCTGGGGTGTTGAAGGTCT -3'

Supplementary Table S3. Relationship between miR-144 expression and clinicopathologic features of OS patients

Clinical pathologic parameters	miR-144 expression		P-Value
	High	Low	
Gender			
Male	21	18	0.624
Female	13	15	
Age (yrs)			
<18	14	17	0.466
≥18	20	16	
Location			
Femur	23	20	0.648
Tibia	7	10	
Other	4	3	
Tumor size (cm)			
<8	19	11	0.087
≥8	15	22	
Enneking stage			
I	3	6	0.091
II	27	18	
III	4	9	
Histologic grade			
Low grade	3	6	0.305
High grade	31	27	
Metastasis			
Present	3	10	0.033
Absent	31	23	