

**YMTHE, Volume 26**

## **Supplemental Information**

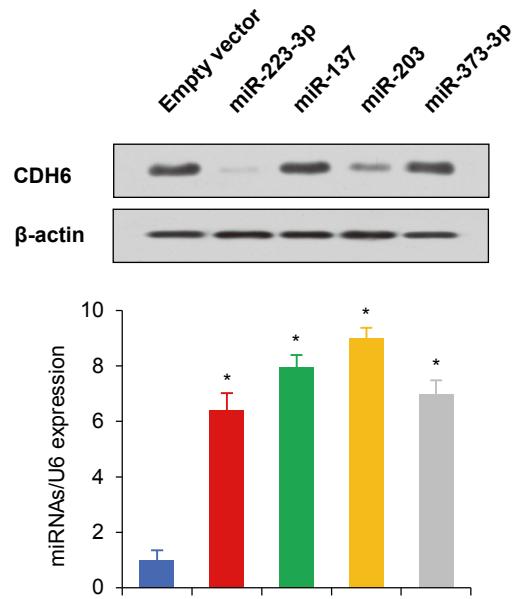
**miR-223-3p Inhibits Human Osteosarcoma**

**Metastasis and Progression by Directly**

**Targeting CDH6**

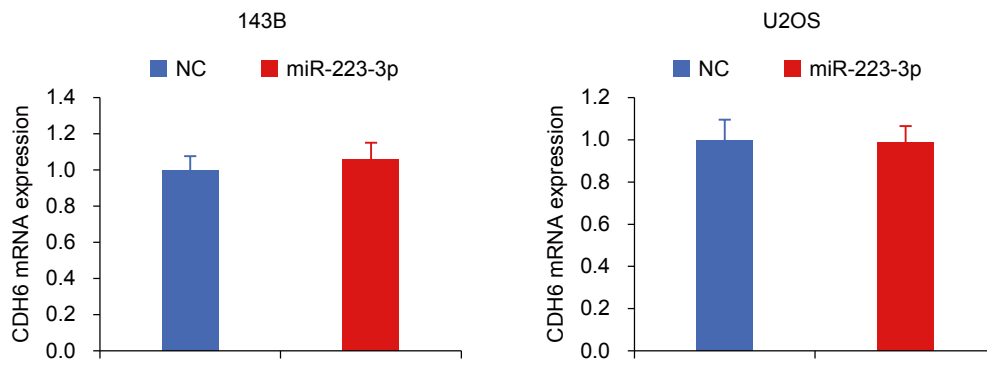
**Quanbo Ji, Xiaojie Xu, Qi Song, Yameng Xu, Yanhong Tai, Stuart B. Goodman, Wenzhi Bi, Meng Xu, Shunchang Jiao, William J. Maloney, and Yan Wang**

**Figure S1**



**Figure S1. Screening for potential CDH6-targeting miRNAs.** Immunoblot analysis of the 143B cells transfected with empty vector, miR-223-3p, miR-137, miR-203, or miR-373-3p. Histograms under the immunoblot graphs show corresponding expression levels of miRNAs by qRT-PCR. \* $p < 0.05$  and \*\* $p < 0.01$ .

## Figure S2



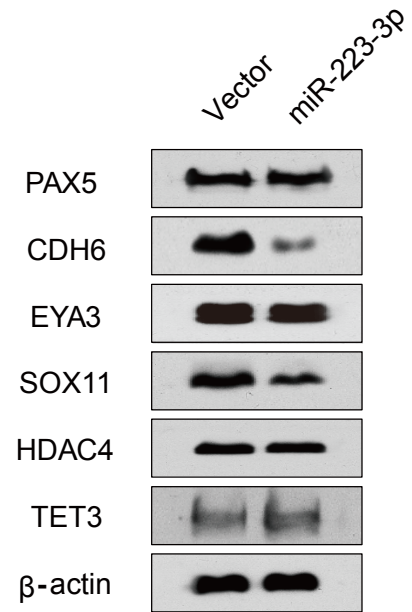
**Figure S2. miR-223-3p regulates CDH6 expression in a posttranscriptional way.** Analysis of 143B, U2OS cells transfected with scramble or miR-223-3p mimics. The corresponding miRNA expression was determined using qRT-PCR. Each bar represents the mean of at least three independent experiments performed in triplicate  $\pm$  standard deviation. \* $p < 0.05$  and \*\* $p < 0.01$ .

## Figure S3

**A**

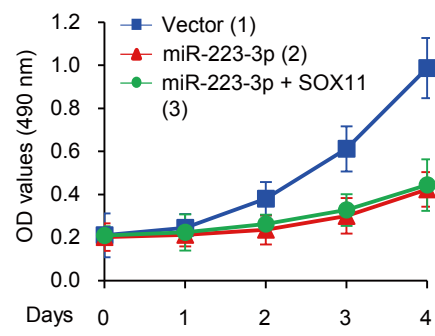
Target gene	ResfSeq Id
PAX5	NM_016734
CDH6	NM_004932
EYA3	NM_001990
SOX11	NM_003108
HDAC4	NM_006037
TET3	NM_001287491

**B**



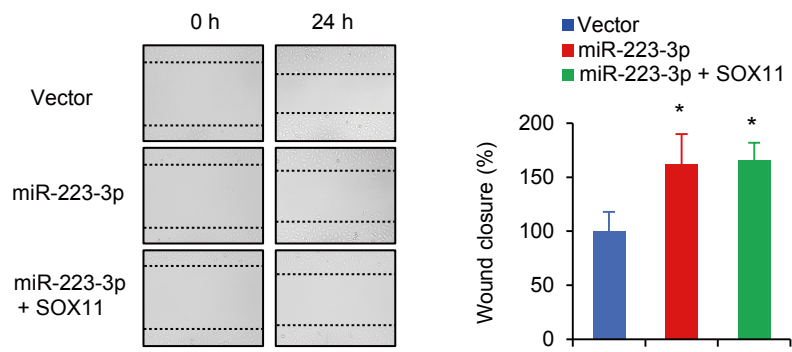
**Figure S3. Potential target genes of miR-223-3p screened.** (A) Candidate target genes of miR-223-3p were found using publicly available databases (TargetScan and miRanda). (B) Immunoblot analysis showing the expression of the candidate target genes in 143B cells transfected with miR-223-3p.

## Figure S4



**Figure S4. miR-223-3p inhibits osteosarcoma cell growth but not through the suppression of SOX11.** 143B cells expressing miR-223-3p or miR-223-3p and SOX11 were cultured in regular medium. At the specified times, cell numbers were determined with the CCK-8 assay.

## Figure S5



**Figure S5. miR-223-3p inhibits osteosarcoma cell migration but not through the suppression of SOX11.** Wound healing assays were conducted in 143B cells transfected with miR-223-3p or miR-223-3p and SOX11. Each bar represents the mean  $\pm$  standard deviation of at least three independent experiments performed in triplicate. \* $p < 0.05$ , \*\* $p < 0.01$ .

**Table S1** Clinicopathological characteristics of osteosarcoma patients

<b>Characteristics</b>	<b>n (%) *Median (range)</b>
Age	17.28 (8.16-17.39)*
Gender	
Male	76 (57.1%)
Female	57 (42.9%)
Tumor size (cm)	
> 7	69 (51.9%)
≤ 7	64 (48.1%)
Location	
Distal femur	57 (42.9%)
Proximal tibia	34 (25.6%)
Proximal humerus	26 (19.5%)
Proximal femur	11 (8.2%)
Others	5 (3.8%)
TNM Stage	
I	57 (42.9%)
II/III	76 (57.1%)
Relapse	
Yes	26 (19.5%)
No	107 (80.5%)
Metastasis	
Lung	49 (36.9%)
Others	16 (12.0%)
No	68 (51.1%)

**Table S2** Primer sequences of Oligonucleotides

<b>Name</b>	<b>Forward (5'→3')</b>	<b>Reverse (5'→3')</b>
<b>Primer sequences for real-time quantitative RT-PCR</b>		
<b>CDH6</b>	AAGGAGGTTTACACAGCCACTGT	CTTGGTACTGCTCCCTGTTTTCT
<b>β-actin</b>	ATCACCATTGGCAATGAGCG	TTGAAGGTAGTTTCGTGGAT
<b>Primers sequences for PCR</b>		
<b>CDH6 3'UTR</b>	TCTGTTGCCTTTTTCATTTCCAA	TTAGAAAAAAACCAGTTTATTAAGT
<b>CDH6 3'UTR Mut</b>	ATGCACATAAATAAACCCAGTCAC	GTGACTGGTTTATTTATGTGCAT
<b>CDH6</b>	ATGAGAACTTACCGCTACTTCTTG	TTAGGAGTCTTTGTCACTGTCCA