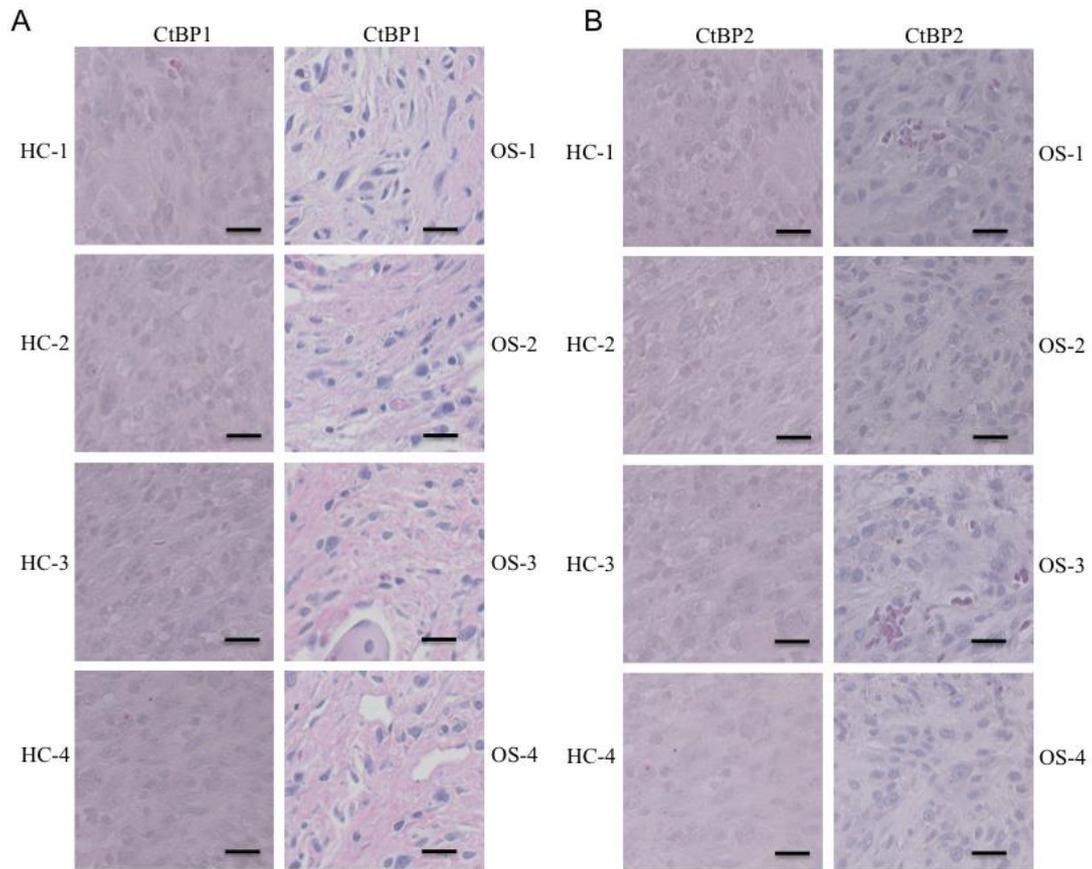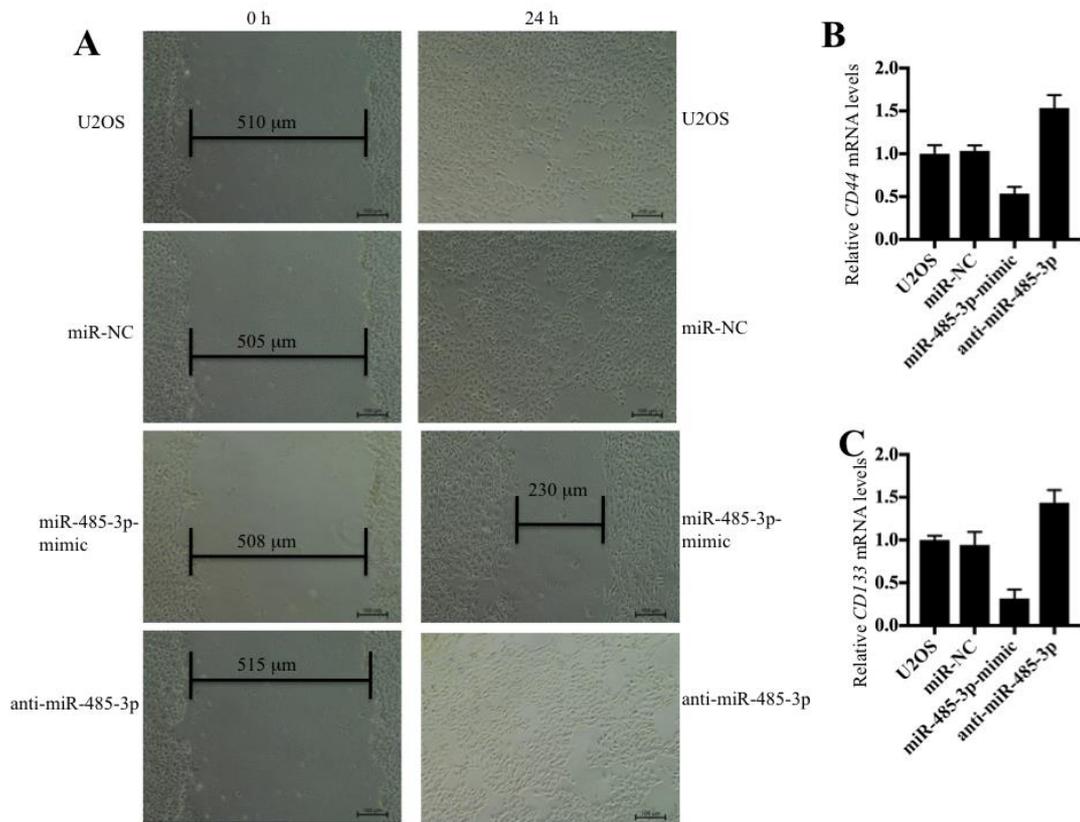**Supplementary Figure 1. The relative mRNA levels of CtBPs in 24 noncancerous and 30 cancerous osteosarcoma tissues.**

The relative mRNA levels of CtBP1 **(A)** and CtBP2 **(B)** in noncancerous (HC, n=24) and cancerous (OS, n=30) tissues determined by qRT-PCR. **$P<0.001$.

Supplementary Figure 2. Elevated CtBP1 level in cancerous osteosarcoma tissues.
(**A**) Representative IHC images of CtBP1 in noncancerous (HC, n=4) and cancerous (OS, n=4) tissues. (**B**) Representative IHC images of CtBP2 in noncancerous (HC, n=4) and cancerous (OS, n=4) tissues. Bars=50 μm.

Supplementary Figure 3. Overexpression of miR-485-3p decreased cell migration ability.

(**A**) Cell used in Figure 4C were subjected to assess cell migration by scratch assay after treatment with mitomycin C for 2 h. Representative images of the cell migration gap distance of 0 and 16 h after treatment were shown. Bars=250 μm. (**B** and **C**) The relative mRNA levels of *CD44* and *CD133*. The miR-NC, miR-485-3p and anti-miR-485-3p were transfected into U2OS cells, respectively. Then, qRT-PCR was performed to examine the mRNA levels of *CD44* (**B**) and *CD133* (**C**).
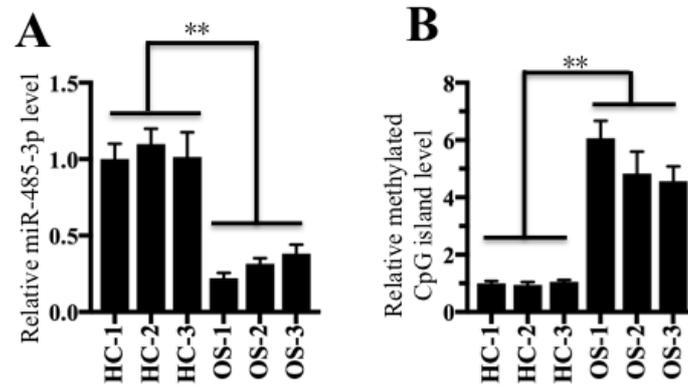
Supplementary Figure 4. The expression of miR-485-3p in cancerous osteosarcoma tissues.

(A) The expression of miR-485-3p in cancerous osteosarcoma tissues. The qRT-PCR was performed to measure miR-485-3p in three noncancerous tissues (HC-1, -2 and -3) and three cancerous tissues (OS-1, -2 and -3). **(B)** The methylation of CpG island in the promoter of miR-485-3p in cancerous osteosarcoma tissues. The qMSP was performed to determine DNA methylation of CpG island in three noncancerous tissues (HC-1, -2 and -3) and three cancerous tissues (OS-1, -2 and -3). **$P<0.001$.

Supplementary Table-1. The basic information of patients

Patient	Age	Sex	MSTS stage	Source of samples
HC-1	9	Female	NA	Knee
HC-2	9	Male	NA	Knee
HC-3	10	Male	NA	Knee
HC-4	10	Female	NA	Knee
HC-5	11	Female	NA	Knee
HC-6	11	Male	NA	Knee
HC-7	11	Female	NA	Knee
HC-8	11	Male	NA	Knee
HC-9	12	Female	NA	Knee
HC-10	13	Female	NA	Knee
HC-11	13	Male	NA	Knee
HC-12	13	Female	NA	Knee
HC-13	15	Male	NA	Knee
HC-14	16	Female	NA	Knee
HC-15	16	Female	NA	Knee
HC-16	17	Female	NA	Knee t
HC-17	17	Female	NA	Knee
HC-18	18	Male	NA	Knee
HC-19	19	Male	NA	Knee
HC-20	19	Male	NA	Knee
HC-21	22	Female	NA	Knee
HC-22	22	Female	NA	Knee
HC-23	23	Male	NA	Knee
HC-24	24	Male	NA	Knee
OS-1	7	Female	III	Knee
OS-2	7	Male	III	Knee
OS-3	7	Male	III	Knee
OS-4	8	Female	III	Knee
OS-5	9	Female	III	Knee
OS-6	9	Male	III	Knee
OS-7	9	Male	III	Knee
OS-8	9	Male	III	Knee
OS-9	10	Female	III	Knee
OS-10	10	Female	III	Knee
OS-11	11	Male	III	Knee
OS-12	12	Female	III	Knee
OS-13	12	Male	III	Knee
OS-14	12	Male	III 9 (CtBP1 low)	Knee
OS-15	14	Female	III	Knee
OS-16	15	Male	IV	Knee
OS-17	15	Male	IV	Knee
OS-18	15	Male	IV	Knee
OS-19	15	Male	IV (CtBP1 low)	Knee
OS-20	16	Male	IV	Knee
OS-21	16	Female	IV	Knee
OS-22	17	Female	IV	Knee
OS-23	17	Male	IV	Knee

OS-24	17	Female	IV (CtBP1 low)	Knee
OS-25	17	Female	IV	Knee
OS-26	18	Female	IV	Knee
OS-27	19	Female	IV (CtBP1 low)	Knee
OS-28	19	Female	IV	Knee
OS-29	21	Male	IV	Knee
OS-30	22	Male	IV	Knee

Supplementary Table-2. Primers used for qRT-PCR analyzes

Gene	Forward Primers	Reverse primers
CtBP1	5'-TTCTGCGACGCGCAGTCCACGCA-3'	5'-TCGATGTTGTCAAAACCACTGCCAA-3'
CtBP2	5'-CCTGCAGGATTTGCTGTATCA-3'	5'-CTTGTGCTAAGGCTTTCTCGT-3'
Bax	5'-GGGGACGAACTGGACAGTAA-3'	5'-CAGTTGAAGTTGCCGTCAGA-3'
Bim	5'-CCGCCCTTATGATGAAGTGT-3'	5'-AAAGCCTGGAGTCAGCAAAA-3'
E-cadherin	5'-AGCGTGTGTGACTGTGAAGG -3'	5'-GCTGGCTCAAGTCAAAGTCC-3'
PUMA	5'-TGTGACCACTGGCATTCATT -3'	5'-TCCTCCCTCTTCCGAGATTT -3'
p16	5'-ATATGCCTTCCCCCACTACC -3'	5'-CGTGAGTGCTCACTCCAGAA-3'
p21	5'-ATGAAATTCACCCCCTTTCC-3'	5'-CCCTAGGCTGTGCTCACTTC-3'
PTEN	5'-ACCAGGACCAGAGGAAACCT -3'	5'-GCTAGCCTCTGGATTTGACG-3'
CD44	5'-CACAAATGGCTGGTACGTCT -3'	5'-GTGTGGTTGAAATGGTGCTGG-3'
CD133	5'-GCCCAGTACAACACTACCAA -3'	5'-GGTCTCCTTGATCGCTGTTGCC-3'
β-Actin	5'-GATGAGATTGGCATGGC-3'	5'-CACCTTCACCGTTCCAGTTT-3'

Supplementary Table-3. Primers used for qMSP analyzes

Gene	Forward Primers	Reverse primers
GAPDH	5'-CGCTTTCTTTCCTTTCGC-3'	5'-TGCCCATTCATTTCCTTCC-3'
miR-485-3p	5'-TTGTGTAGAAGATGTAGGAATGTTG-3'	5'-TCAAATCCAAAAAAACACCTAATAAA-3'