

FOXO3a links the crosstalk of autophagy and apoptosis

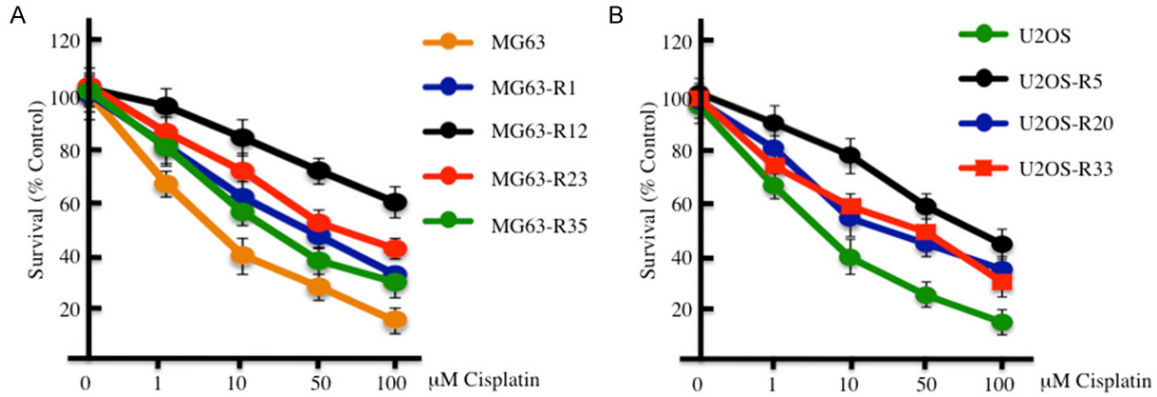
Supplementary Table 1. Primers used for qRT-PCR

| Gene | Forward | Reverse |
|----------------|------------------------------|-------------------------------|
| β -Actin | 5'-CACCAACTGGGACGACAT-3' | 5'-ACAGCCTGGATAGCAACG-3' |
| ATG5 | 5'-TGTTACCATTTGACTGGTCG-3' | 5'-CTGCAGTCAAGAGACAGGTAATC-3' |
| ATG7 | 5'-TGGAGCAGCTCATCGAAAGCC-3' | 5'-TCCAAGGTCCGGTCTCTGGTTG-3' |
| Beclin-1 | 5'-TGGCACAGTGGACAGTTTGG-3' | 5'-TGTCAGAGACTCCAGATATG-3' |
| Hsp110 | 5'-AGGATGAGAAATACAACCAT-3' | 5'-TCAATATTTGGGCCATTTGGA-3' |
| PUMA | 5'-TGCACTGACGGAGATGCGGACT-3' | 5'-AGAGTGAAGGAGCACCCGAGA-3' |
| Caspase-3 | 5'-GAATGGGCTGAGCTGCCTGTAA-3' | 5'-TTCTTACTTGGCGATGGCG-3' |
| Bcl-6 | 5'-GCGCCAGAAGCATGGCGCCAT-3' | 5'-GCTATGATTGCACTAGTGGATG-3' |
| FOXO3a | 5'-CATGTAGAGTGTGTGGAGAGC-3' | 5'-AACGGCTGGCCTGCTCTGAA-3' |

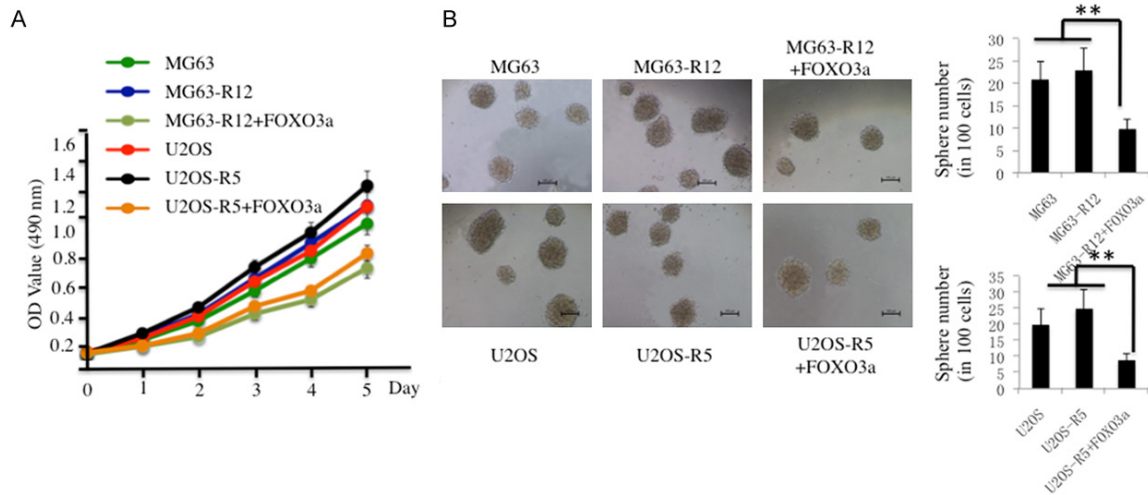
Supplementary Table 2. Primers used for ChIP assay

| Gene | Forward | Reverse |
|-----------|------------------------------|-----------------------------|
| ATG5 | 5'-ACGCCATTCTCCTGCCTCAGAC-3' | 5'-CCATCCAAGAGTACATATCT-3' |
| ATG7 | 5'-AGTGCTGTGATTACAGGCG-3' | 5'-TAATTAAGTACCACCATG-3' |
| Beclin-1 | 5'-ACAAGCGTGAGCCACCATGC-3' | 5'-CCTGGCCAACACAGTGAA-3' |
| LC3 | 5'-AGCATCCTGTTCTATCCTGT-3' | 5'-CACAGTCCCGTGACGTCAT-3' |
| SIRT1 | 5'-GCAACCGACTAAGGAGAAAAG-3' | 5'-GGAATTTCCACGTCTTCCTGA-3' |
| Apaf-1 | 5'-ATGAGCCGTGGCAGGAGTGC-3' | 5'-CTCAAGTCTTCGCGGTC-3' |
| Bax | 5'-GGGGTGGCTCAAGCCTGTAAT-3' | 5'-GTGTTGCCAGGCTGGAG-3' |
| Caspase-3 | 5'-AGAGAGACCTGAGGGTAACC-3' | 5'-CTAGGTCTTCTGAGAGTCC-3' |
| PUMA | 5'-ATATGTCATAATCCATGGTT-3' | 5'-ATGTCAGACTTCTCAAAC-3' |

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Supplementary Figure 1. Cisplatin-resistant osteosarcoma cells are insensitive to cisplatin treatment. MG63, MG63-R1, MG63-R12, MG63-R23 and MG63-R35 cells (A), as well as U2OS, U2OS-R5, U2OS-R20 and U2OS-R33 cells (B), were plated in 96-well plates. After 24 hrs, the culture medium was replaced with 0.1 ml of fresh medium containing 0.5% FBS and the same medium containing the indicated concentrations of cisplatin for another 72 hrs. The percentages of surviving cells in each well were calculated by defining the control (MG63 or U2OS, 0 μM cisplatin) as 100%.



Supplementary Figure 2. The overexpression of FOXO3a results in decreases in the cell proliferation and sphere formation ability of cisplatin-resistant osteosarcoma cells. A: MG63, MG63-R12, MG63-R12+FOXO3a, U2OS, U2OS-R5 and U2OS-R5+FOXO3a cells were plated in 96-well plates containing 0.1 ml of DMEM medium with 0.5% FBS. Cells on each day were subject to a MTT assay to evaluate the degree of cell proliferation, and the cell viability was determined at 490 nm. B: MG63, MG63-R12, MG63-R12+FOXO3a, U2OS, U2OS-R5 and U2OS-R5+FOXO3a cells were plated in 24-well plates at a density of 100 cell/well. Fresh media was added every 2-3 days. After 9 days, the numbers of spheres were counted. Scale bar = 50 μm (**P < 0.001).