Supplementary Tables

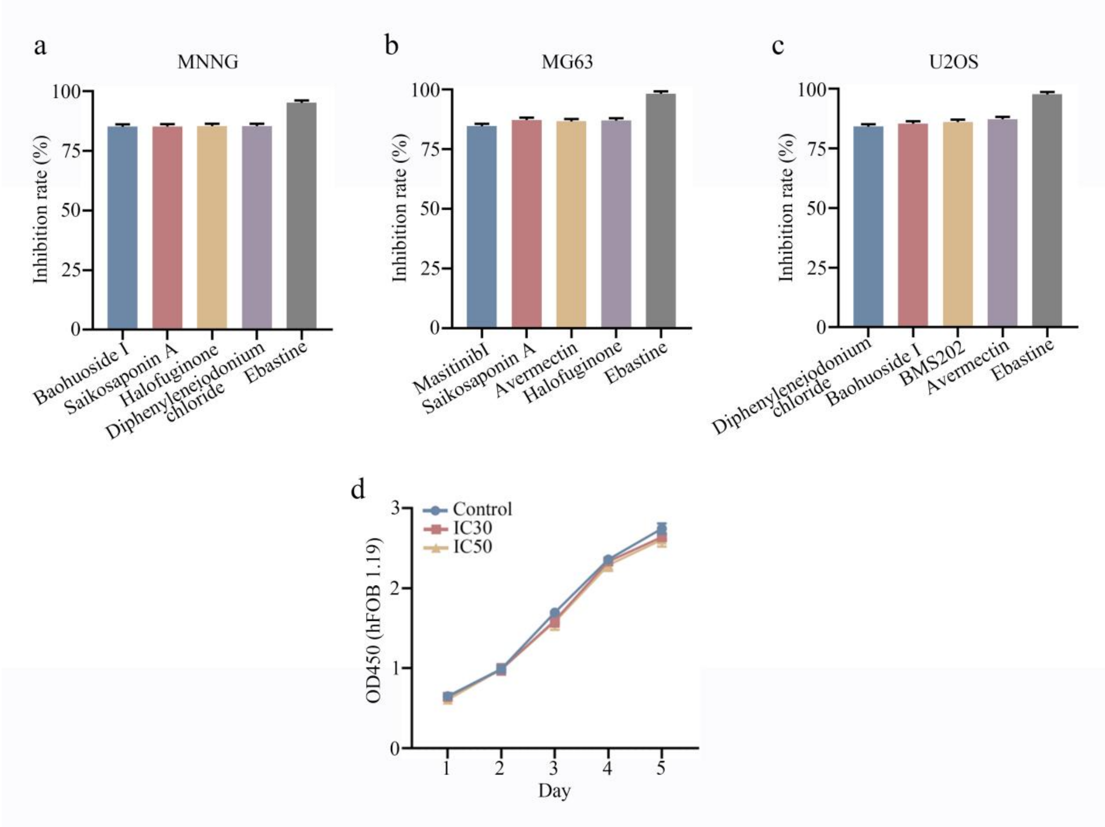
Supplementary Table 1. The primer sequences for qRT-PCR

Gene name		Sequence (5'-3')
FOSB	Forward	CTTCCACCAGCGGAACTACC
	Reverse	ACTGATCTGTCTCCGTCTCCT
FSD2	Forward	GGACGGACCAAGCAGAGTTT
	Reverse	GGACGGACCAAGCAGAGTTT
LCP1	Forward	AGGGGTTCCTGGTCATACAC
	Reverse	TCCATTGCCATCAGTATCAACT
HMGCS1	Forward	GGACTGTCCTTTCGTGGCTC
	Reverse	GGCATGGTGAAAGAGCTGTG
PLA2G4C	Forward	TGCACCATGGGAAGAAAGAAGA
	Reverse	TACGTGACGGCATCCAACAG
CXCL8	Forward	AGCTCTGTGTGAAGGTGCAG
	Reverse	TCTCAGCCCTCTTCAAAAACTTC
MSMO1	Forward	GTTCCGAGGTTGGAACACCT
	Reverse	TCTCTGGCTTATCCTGAACGG
IDI1	Forward	GCTGTTCCCTGCGAAAGGTAT
	Reverse	TGTTGCTTGTCGAGGTGGTT
CLK4	Forward	TGGCATGCATGTAGCAGTGA
	Reverse	GCATCTGGACACATCGGAAGA
BIRC3	Forward	TCTGGGCAGCAGGTTTACAA
	Reverse	CCCGAGATTAGACTAAGTCCCTT
N4BP3	Forward	TGCAGGAGGGTTCAAATCCG
	Reverse	AGGCAGCTGCTTCATGGTG
AGR2	Forward	AGGTGGGTGAGGAAATCCAGA
	Reverse	TCATCAAGGGTTTGTTGCTTGT
DDIT3	Forward	GGAACCTGAGGAGAGAGTGTTC
	Reverse	CTGCCATCTCTGCAGTTGGA
CPEB2	Forward	ACCCTTACAGGTAAGAATGGATCG
	Reverse	ACCTCGTCTTCGCCCATAAC
MYBPH	Forward	GAGAGAACATTGAGGCCCCC
	Reverse	TTGCAGGTTGACCGTCTCTC
CHRND	Forward	GTGTGGATAGAGCACGGCTG
	Reverse	CTGCCAGTCGAAGGGGAAAT
ZFHX2	Forward	CAATGGAAACCCCCTCACGA
	Reverse	GTGACAGCCAGTACCACCTC
PRR15	Forward	CCTGATCTCTCCAAGTCCGC
	Reverse	CAGCGGCCTGCTCGG
KCNK3	Forward	ATCGTGTGCACCTTCACCTA
	Reverse	GTGCCCGTAGCCGATG
EPN3	Forward	CCATGGGACATCCCAGGTTTT
	Reverse	TTCCTGAGGGGATCGGAGAC

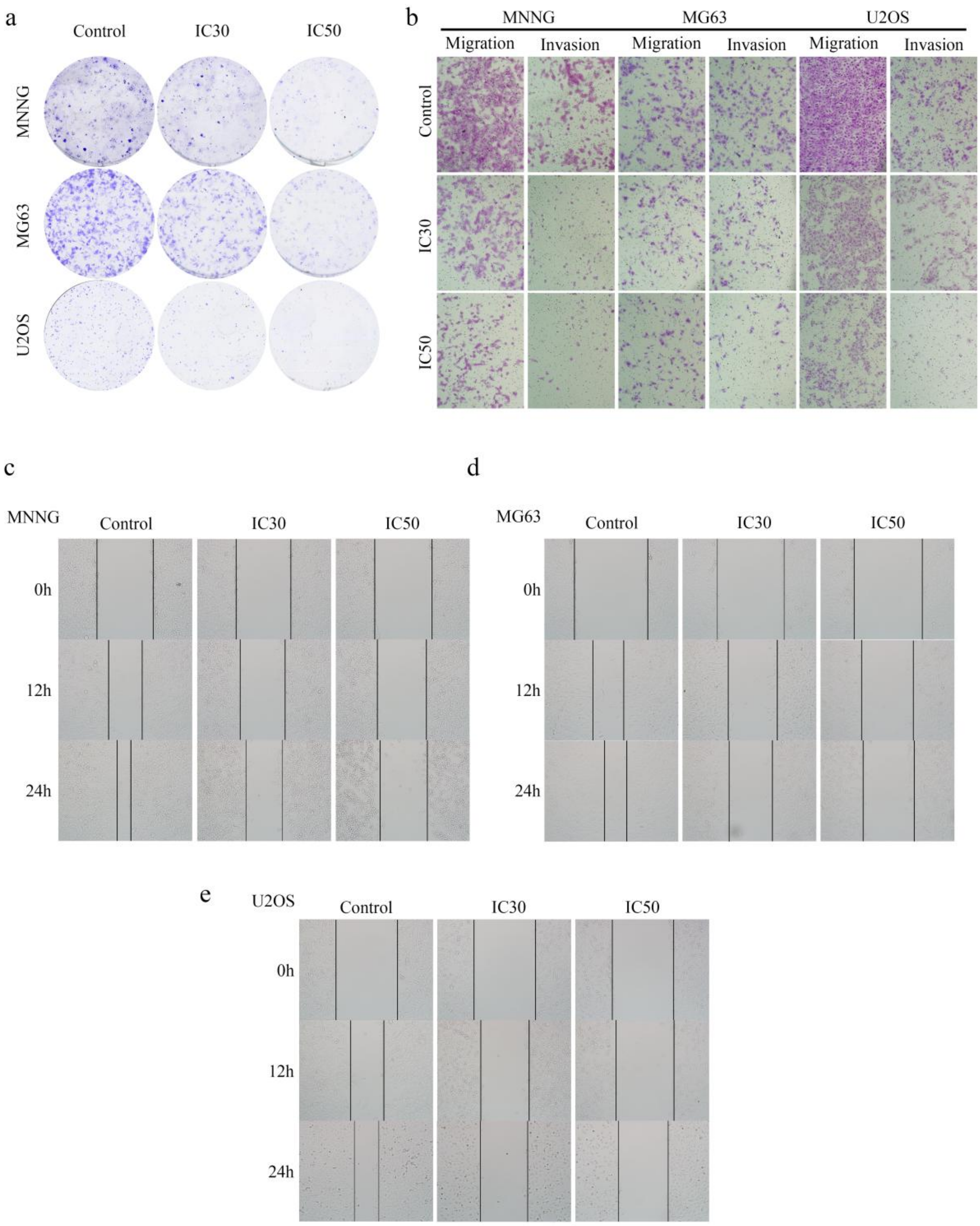
CCR7	Forward	GTCATGGACCTGGGTATGC
	Reverse	TCCACTGTGGTGTTGTCTCC
SLC1A7	Forward	CCGGCCGGGCTCTCAT
	Reverse	TCCGGACATCAAGCTGGAGA
LSP1	Forward	GCAGAGCGGCCAGCAA
	Reverse	GCTTCAGGCTGAGGAGCATC
ADAMTSL2	Forward	TGCAACACCCCTACAAGTGC
	Reverse	GTTGGGCTGTTGTCCGTGG
ALPK2	Forward	ACGGACATGCAAGGTGTAGG
	Reverse	TTCTTTTCGCCTGGGGTCTC
EIF2AK3	Forward	AGAGATTGAGACTGCGTGGC
	Reverse	AGAGATTGAGACTGCGTGGC
PER1	Forward	ACTCCTGCGACCAGGTACT
	Reverse	GCCATGGGGAGAACAGAACA
KLF6	Forward	GCTCCGAGGAACTTTCTCCC
	Reverse	GCTCCGAGGAACTTTCTCCC
GBE1	Forward	AGCCATGATCAGGCATTGGT
	Reverse	GTAGCGAAGAAGGTCGTCGT
ADM	Forward	GTTTCCGTCGCCCTGATGTA
	Reverse	GCATCCGGACTGCTGTCTT
GADD45B	Forward	GTACGAGTCGGCCAAGTTGA
	Reverse	GTGTGAGGGTTCGTGACCA
HERPUD1	Forward	GGGACTTGCTTCCAAAGGAAAA
	Reverse	CCTTGGCGTTGATTTCTGGC
JUN	Forward	CAGCCAGGTCGGCAGTATAG
	Reverse	GGACTCTGCCACTTGTCTCC
ACSS2	Forward	AGGCCCCGCCTCTAGTTC
	Reverse	AGGCCCCGCCTCTAGTTC
NOG	Forward	TCGCCCTGGAGTAATTTCGG
	Reverse	CTGGGTGTTCGATGAGGTCC
HSPA5	Forward	CATCAACGAGCCTACGGCA
	Reverse	TCCATGACACGCTGGTCAAA
HDAC9	Forward	CTCCTCCTCGAAAGATGGCT
	Reverse	TTCCACATGAGGTCAGGCTGT
NR1D1	Forward	CTTGTCTCTGCAGACCGCT
	Reverse	CTTGTCTCTGCAGACCGCT
BBC3	Forward	CAGGATGAAATTTGGCATGGGG
	Reverse	CAGGATGAAATTTGGCATGGGG
TRAF6	Forward	GCGCACTAGAACGAGCAAG
	Reverse	GCGCACTAGAACGAGCAAG
IPMK	Forward	ACTGCAACATCCAGATGGCA
	Reverse	ACTGCAACATCCAGATGGCA
CHMP2B	Forward	AGAAAACCGTGGATGGAATTAGAA
	Reverse	AGAAAACCGTGGATGGAATTAGAA

KLF4	Forward	GGTCAGTCCCGGGGATTTGT
	Reverse	CAGTGGTAAGGTTTCTCACCTGTGT
HSPA1A	Forward	ACATCAGCCAGAACAAGCGA
	Reverse	ACATCAGCCAGAACAAGCGA
PPP1R15A	Forward	CTAAAGGCCAGAAAGGTGCG
	Reverse	ACGAAGGGACAGAGGAGGAA
PIM1	Forward	CTGGGGAGAGCTGCCTAATG
	Reverse	GCTCCCCTTTCCGTGATGAA
β-actin	Forward	GTCATTCCAAATATGAGATGCGT
	Reverse	GCATTACATAATTTACACGAAAGCA

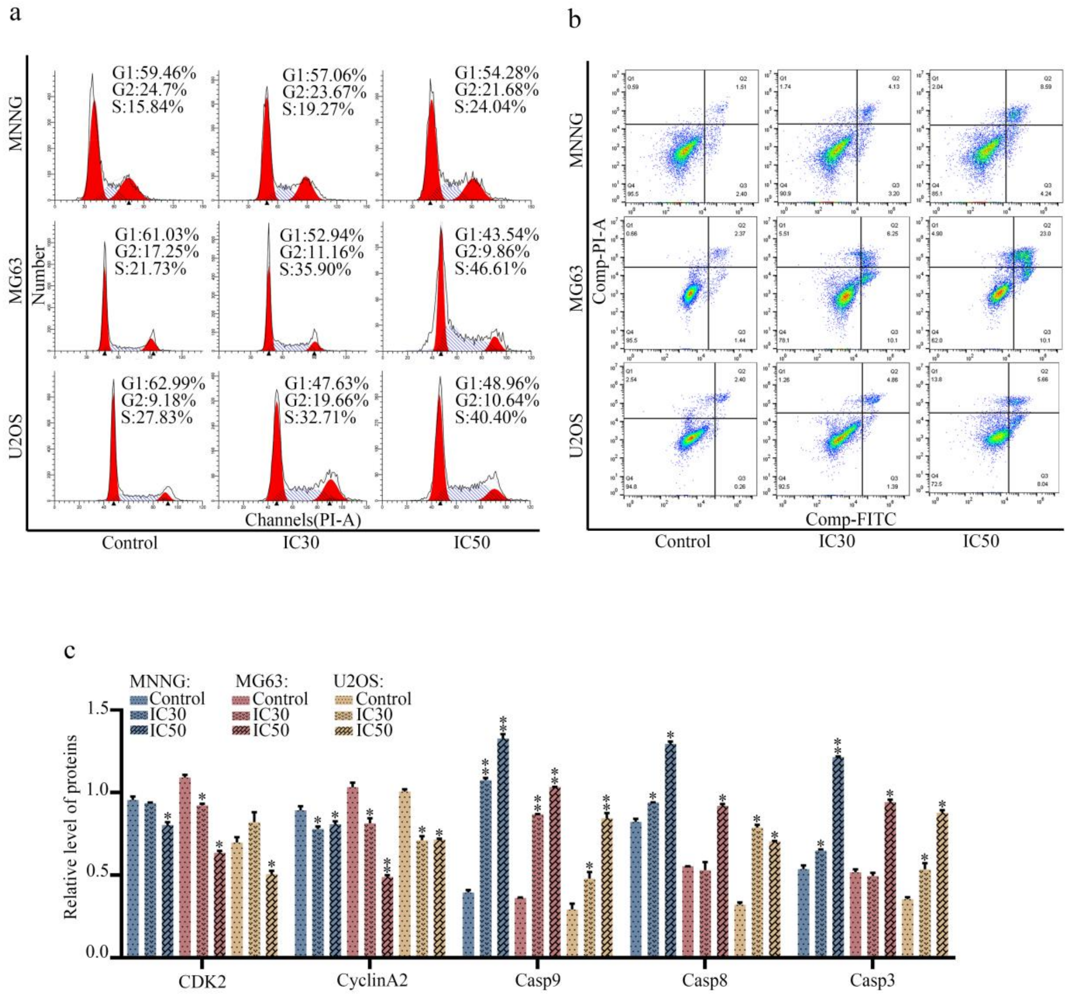
Supplementary Figures and figure legends:



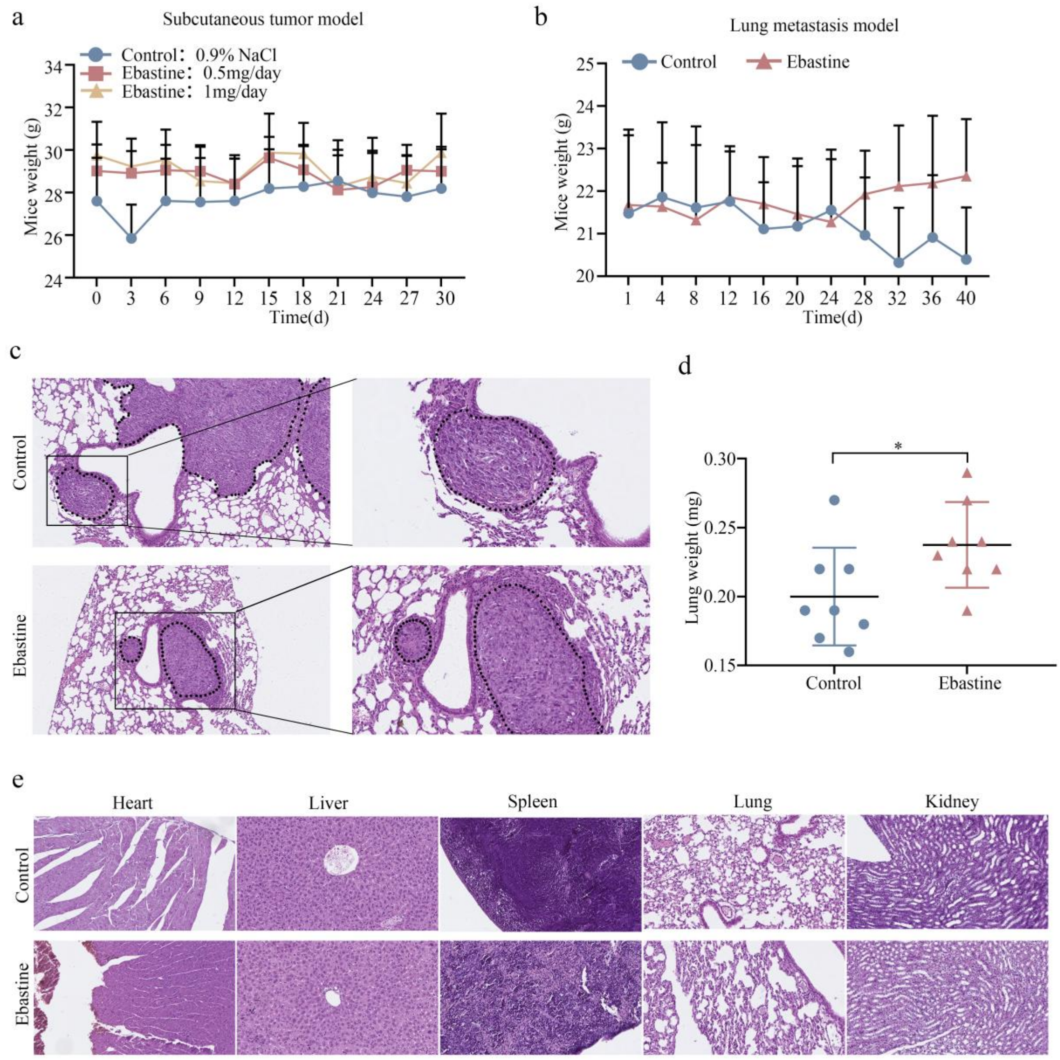
Supplementary Figure 1. (a-c) The inhibition of the first 5 effective drug in all three osteosarcoma cell lines. (d) CCK-8 assay was used to detect the proliferation of osteoblast cell hFOB 1.19 after treatment with the IC30 and IC50 of ebastine.



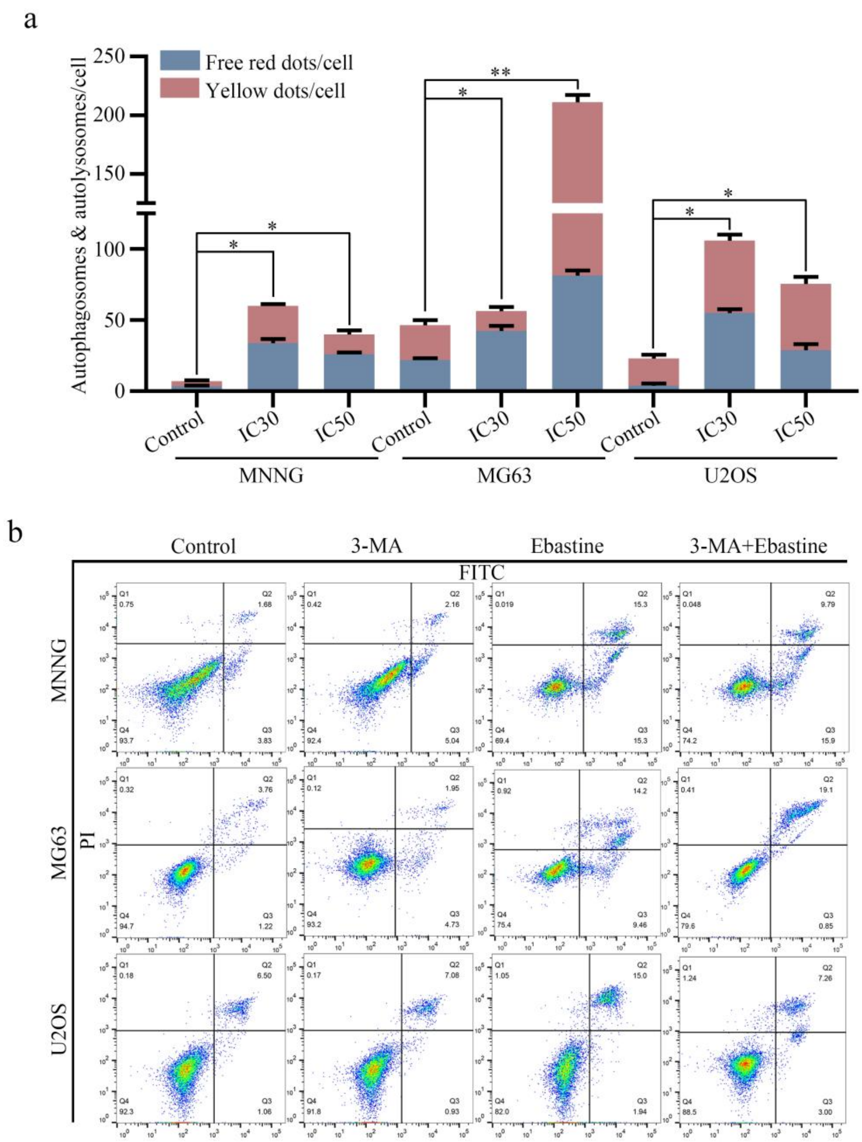
Supplementary Figure 2. (a) Colony formation assays in osteosarcoma cells after treatment with the IC30 and IC50 of ebastine (magnification, ×40). (b) Transwell assays in osteosarcoam cells after treatment with the IC30 and IC50 of ebastine (magnification, ×200). (c-e) A wound-healing assay was used to determine cell migration after treatment with the IC30 and IC50 of ebastine (magnification, ×200). Data are shown as the means ± SD from at least three independent experiments. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. **P*<0.05; ** *P*<0.01; *** *P*<0.001.



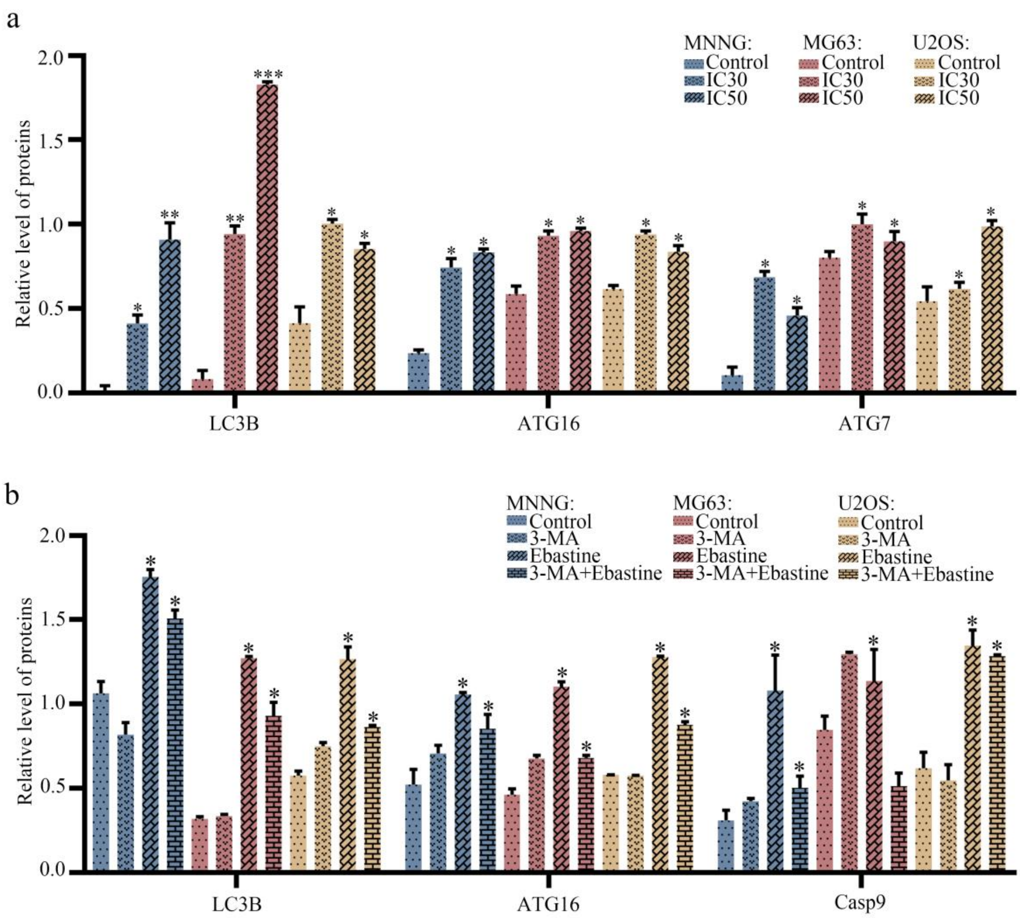
Supplementary Figure 3. (a) Flow cytometry of cell cycle distribution in each phase after treatment with the IC30 and IC50 of ebastine after 48 h. (b) Flow cytometry of cell apoptosis distribution after treatment with the IC30 and IC50 of ebastine for 48 h. (c) Protein levels of cell cycle- and apoptosis-related proteins after treatment with ebastine for 48 h, assayed by Western blot. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. *P<0.05; ** P<0.01.



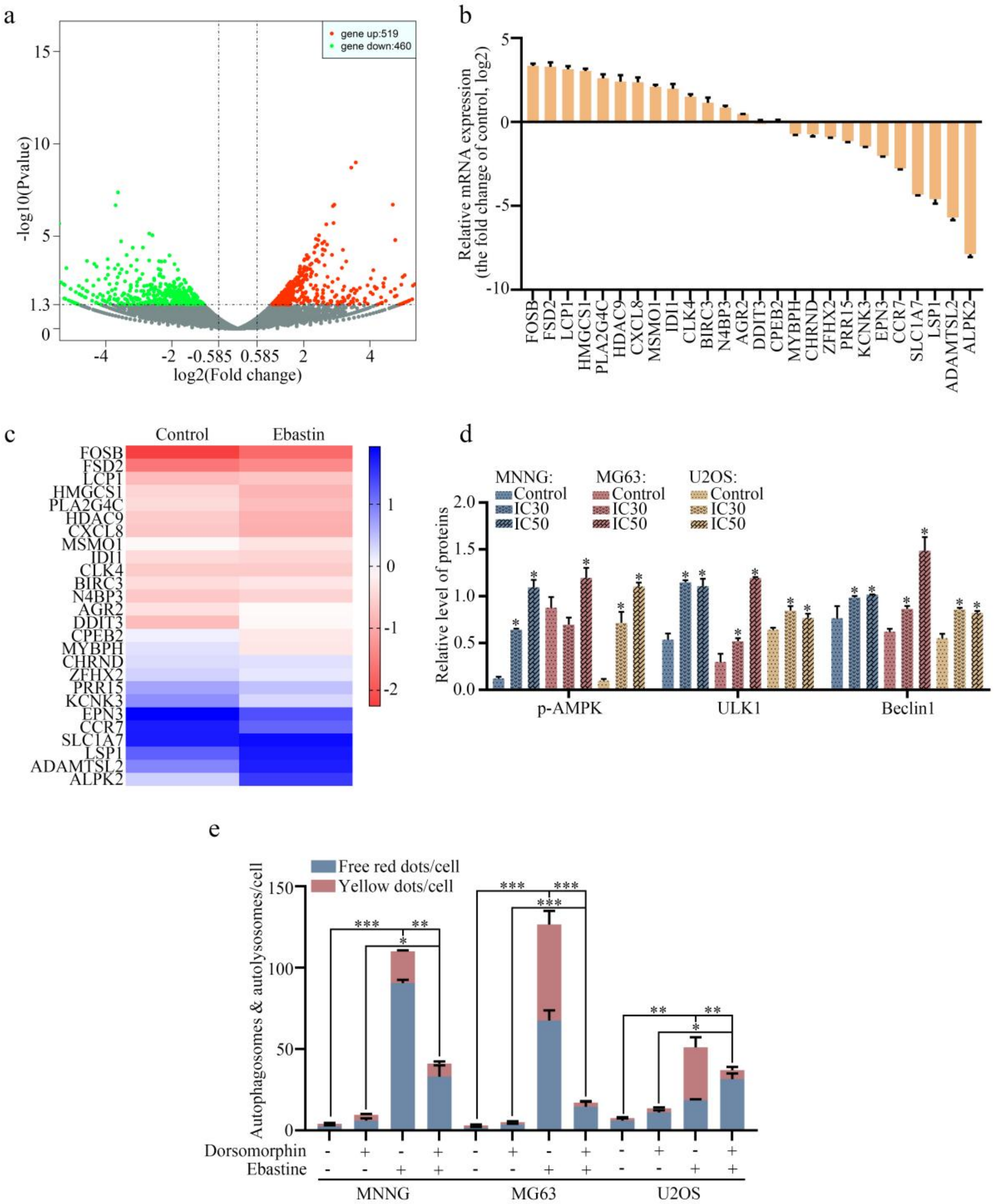
Supplementary Figure 4. (a-b) Growth curve drawn by measuring the weight of mice on the indicated days in subcutaneous tumors and lung metastasis model. (c) HE staining of lung samples obtained from nude mice after injection with MNNG cells. (d) Diagram showing lung weight after treatment with ebastine. (e) HE staining of the heart, liver, spleen, lung, and kidney organs in ebastine-treated MNNG mice and control mice. Data are shown as the means ± SD from at least three independent experiments. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. *P<0.05.



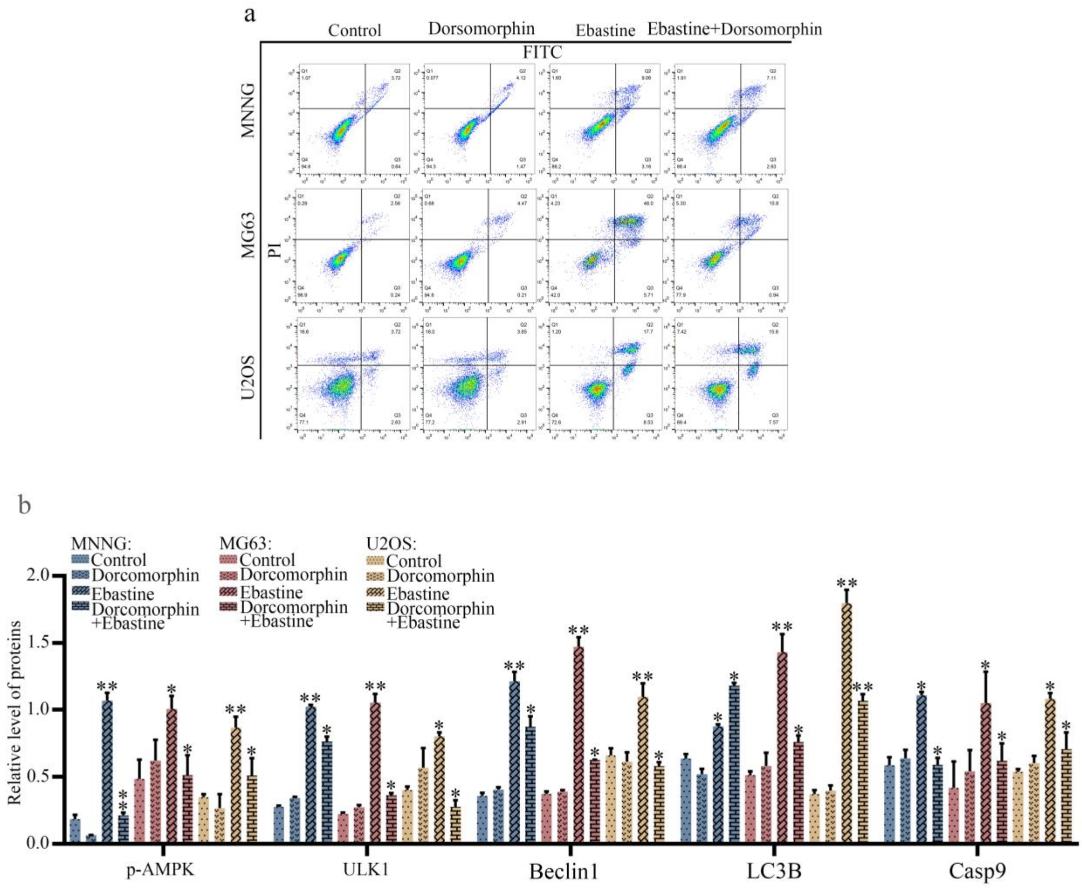
Supplementary Figure 5. (a) LC3 puncta were analyzed using the mRFP-GFP-LC3 construct after treatment with ebastine for 48 h. (b) Flow cytometry was used to analyze changes in apoptosis after treatment with ebastine for 48 h with or without 3-MA. Data are shown as the means ± SD from at least three independent experiments. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. *P<0.05; ** P<0.01.



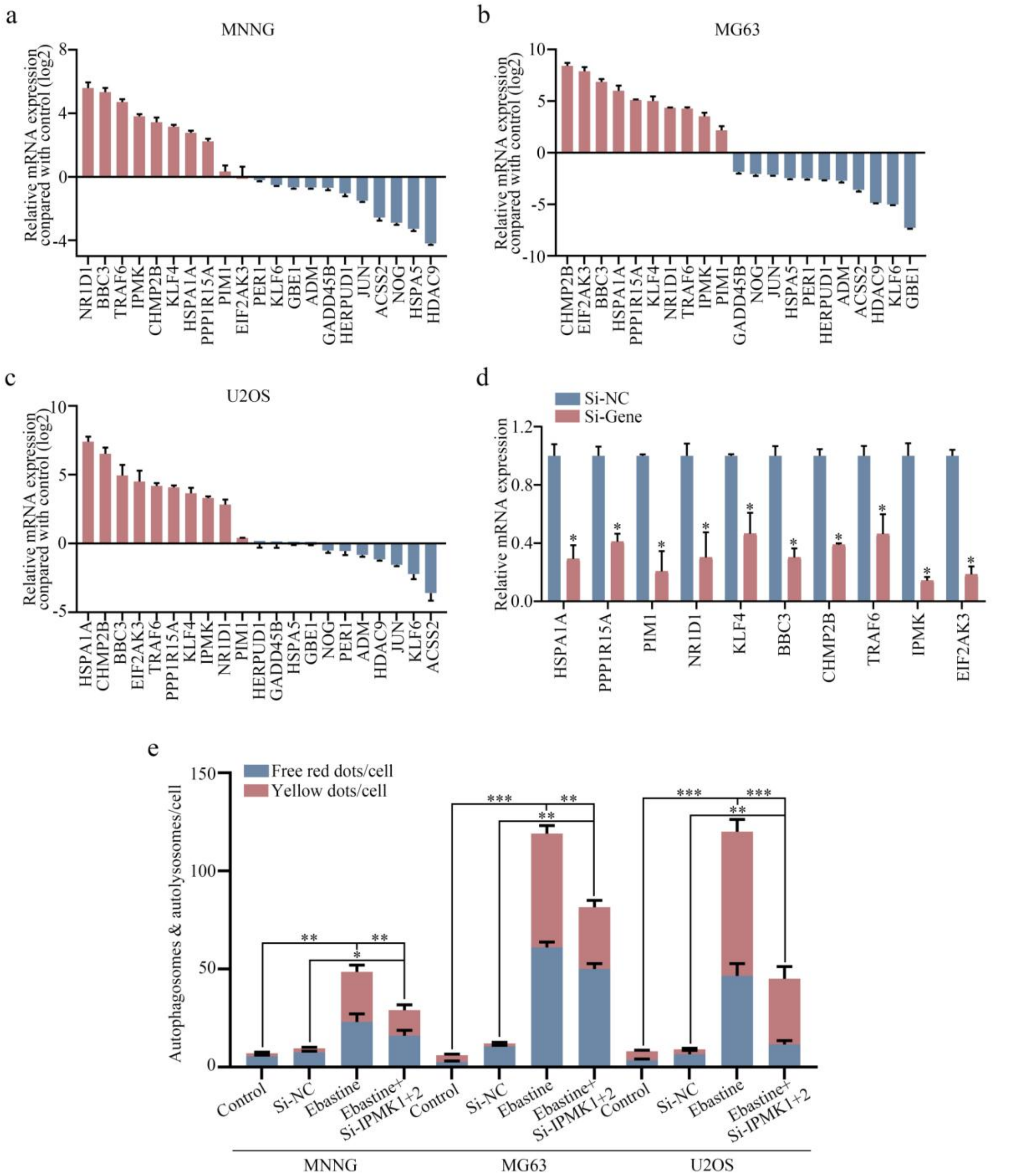
Supplementary Figure 6. (a) Protein levels of LC3B, ATG16 and ATG7 after treatment with the IC30 and IC50 of ebastine for 48 h, assayed by Western blot. (b) Autophagy-related proteins were analyzed by Western blot after treatment with ebastine for 48 h with or without 3-MA. Data are shown as the means ± SD from at least three independent experiments. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. *P<0.05; ** P<0.01; *** P<0.001.



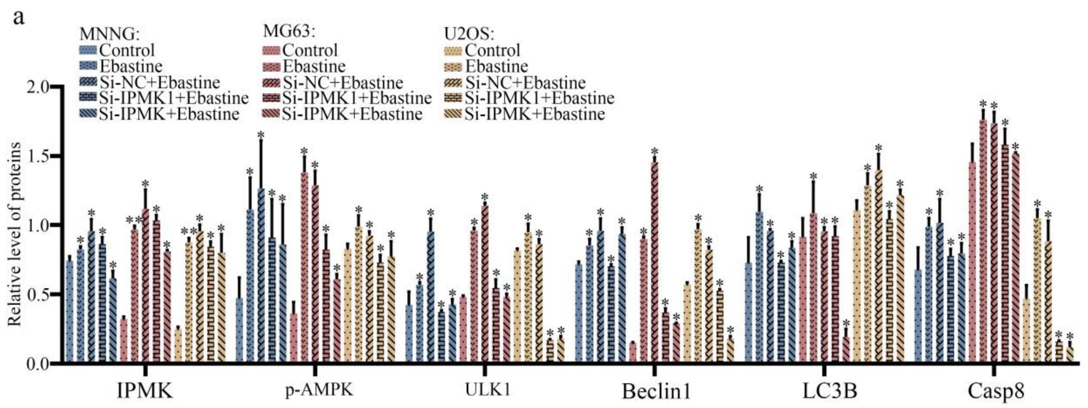
Supplementary Figure 7. (a) A volcano plot analysis revealed 519 up-regulated and 460 downregulated genes. (b) The mRNA expression levels of 15 up-regulated and 11 down-regulated genes in osteosarcoma cells. (c) Heatmap displaying the mRNA expression levels of 15 up-regulated and 11 down-regulated genes in osteosarcoma cells. (d) Western blot showing the protein expression of p-MEK1 and LC3B after treatment with ebastine. (e) LC3 puncta were analyzed by the mRFP-GFP-LC3 construct after treatment with ebastine for 48 h with or without dorsomorphin. Data are shown as the means ± SD from at least three independent experiments. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. **P*<0.05; ** *P*<0.01; *** *P*<0.001.

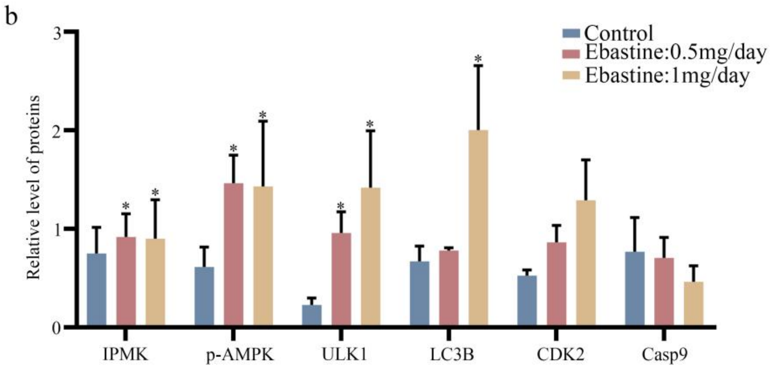


Supplementary Figure 8. (a) Flow cytometry was used to analyze changes in apoptosis after treatment with ebastine for 48 h with or without dorsomorphin. (b) The relative protein levels of p-AMPK, ULK1, Beclin1, LC3B and Casp9 after treatment with ebastine for 48 h with or without dorsomophin. Data are shown as the means ± SD from at least three independent experiments. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. *P<0.05; ** P<0.01.

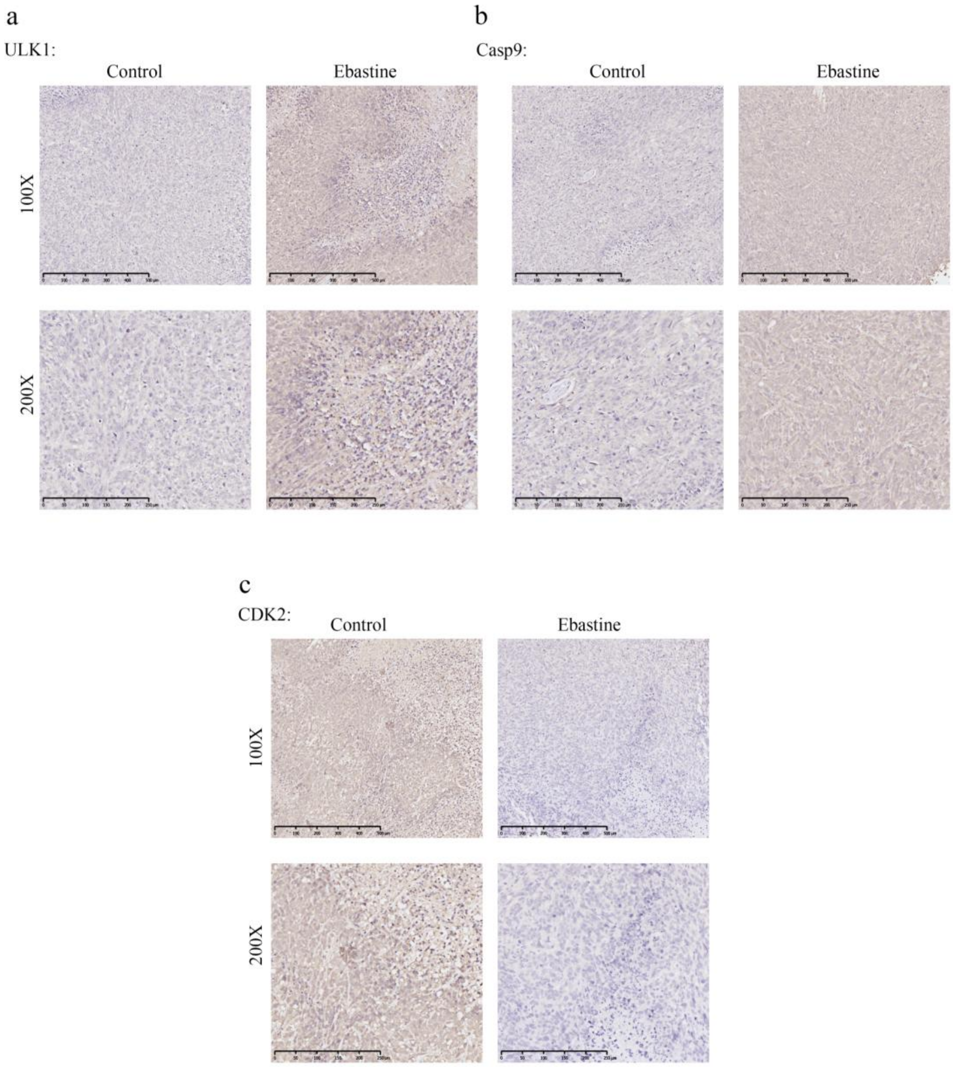


Supplementary Figure 9. (a-c) The mRNA expression of 21 genes after treatment with ebastine in three osteosarcoma cell lines. (d) The relative mRNA expression of the 10 genes was determined by qRT-PCR after transfection with independent siRNAs in MNNG cells. (e) LC3 puncta were analyzed by the mRFP-GFP-LC3 construct after treatment with ebastine for 48 h with or without knockdown of IPMK. Data are shown as the means ± SD from at least three independent experiments. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. *P<0.05; ** P<0.01; *** P<0.001.





Supplementary Figure 10. (a) Protein levels of apoptosis, autophagy, IPMK, and p-AMPKK in osteosarcoma cells treated with ebastine for 48 h with or without knockdown of IPMK. (b) Protein expression of IPMK, p-AMPK, ULK1, LC3B, and CDK2 and cleavage of caspase-9 were analyzed by western blot in tumor tissues. Statistical analysis was performed using Student's t-test. Error bars represent the SEM. **P*<0.05.



Supplementary Figure 11. (a-c) IHC analysis of the related proteins ULK1, CDK2, and caspase-9.