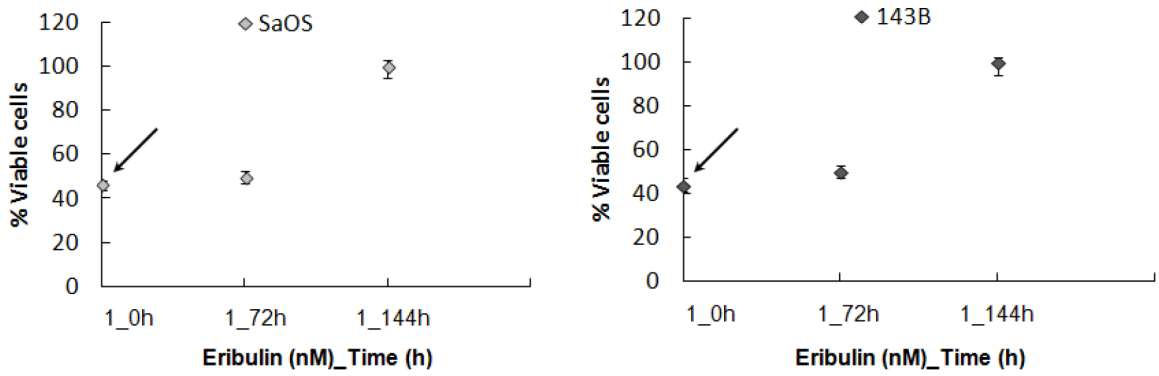
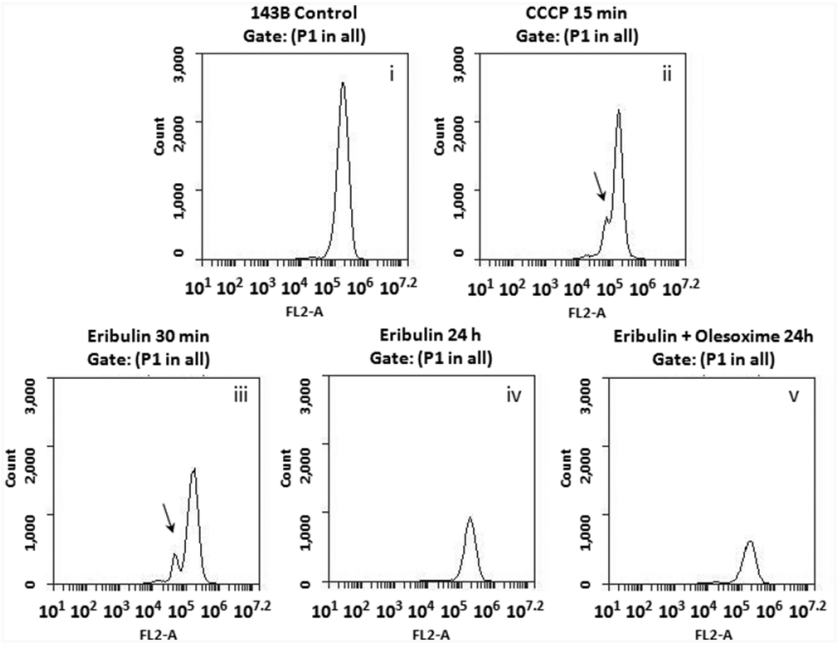
Integrating mechanisms of response and resistance against the tubulin binding agent Eribulin in preclinical models of osteosarcoma

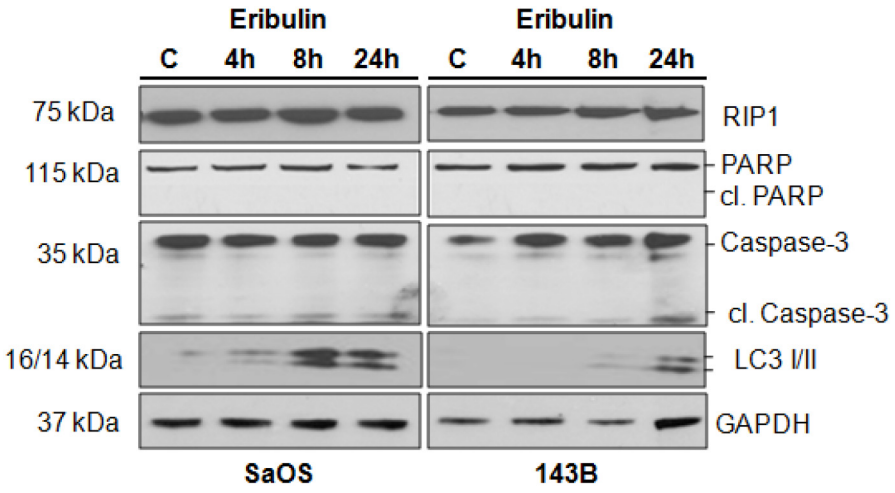
SUPPLEMENTARY FIGURES AND TABLE



Supplementary Figure S1: Regrowth of SaOS and 143B cells following eribulin treatment. Arrow indicates the percentage of viable cells following eribulin treatment. Cells were maintained in 1 nM eribulin and viable cells were measured at indicated time points using the cell titer blue assay. Data are presented as mean absorbance ±SE of six replicates, n = 6.



Supplementary Figure S2: Mitochondrial membrane potential was assayed by flow cytometry. Relative uptake of the cationic dye TMRE for 143B cells is shown for (i) untreated controls (ii) treatment with a mitochondrial membrane potential disruptor, CCCP (carbonylcyanide 3-chlorophenylhydrazone) for 15 min, (iii) treatment with eribulin for 30 min, (iv) treatment with eribulin for 24 h, (v) treated with eribulin and olesoxime for 24 h. Each analysis was performed in triplicate.



Supplementary Figure S3: Immunoblot analysis was performed on lysates of SaOS and 143B cells that were either untreated or treated with eribulin for 4h, 8h and 24h using antibodies against RIP1, PARP, caspase-3 and LC3 I/II. GAPDH was loading control.

Supplementary Table S1: Primer sequences for quantitative PCR

Gene Name	Protein Name	Accession Number	Forward Primer (5'-3')	Reverse Primer (5'-3')
STMN1	Stathmin-1	NM_203401.1	ccccttccccctccaaagaa	tcgcaaacgttccagtttgg
TUBB3	βIII-Tubulin	NM_006086	gcaactacgtgggcgact	cgaggcacgtacttgtgaga
GAPDH	GAPDH	NM_002046.5	agggctgcttttaactctggt	ccccacttgattttggaggga