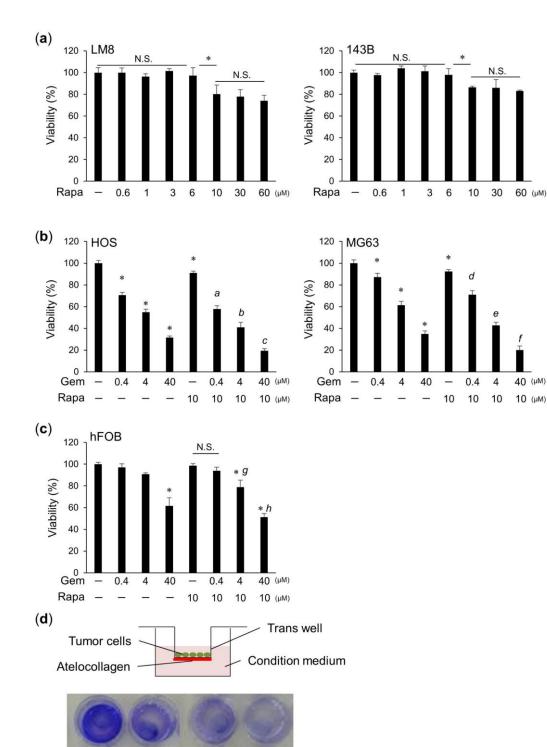
## Supplementary Materials: Gemcitabine and Rapamycin Exhibit Additive Effect Against Osteosarcoma by Targeting Autophagy and

## 4 Apoptosis

## 5 Takashi Ando, Jiro Ichikawa, Taro Fujimaki, Naofumi Taniguchi, Yoshihiro Takayama and 6 Hirotaka Haro

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Gem+Rapa

Vehicle Gem Rapa

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**Figure S1.** (a) LM8 and 143B OS cell lines were treated with Rapa and cell viability was measured using WST-8 assays. (b) HOS and MG63 OS cells and (c) hFOB osteoblast cells were treated with Gem alone or in combination with Rapa and cell viability was measured using WST-8 assays. Data are shown as the mean  $\pm$  SD (n = 4). \*P < 0.05 vs. vehicle; a–f, P < 0.05 vs. Rapa alone. N.S., not significant. (d) Schematic of the cell invasion assay methodology.

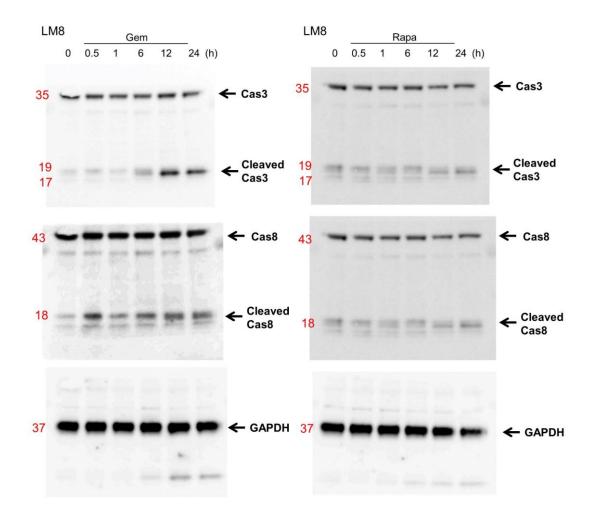


Figure S2. Example uncropped western blots depicting the expression of cleaved caspase 3, cleaved
 caspase 8, and GAPDH in LM8 cell lysates. These blots complement the cropped blots shown in Figure
 2c.

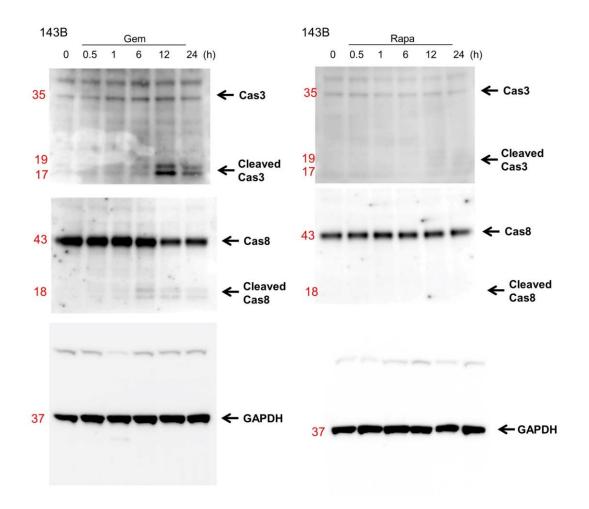


Figure S3. Example uncropped western blots depicting the expression of cleaved caspase 3, cleaved
 caspase 8, and GAPDH in 143B cell lysates. These blots complement the cropped blots shown in
 Figure 2c.

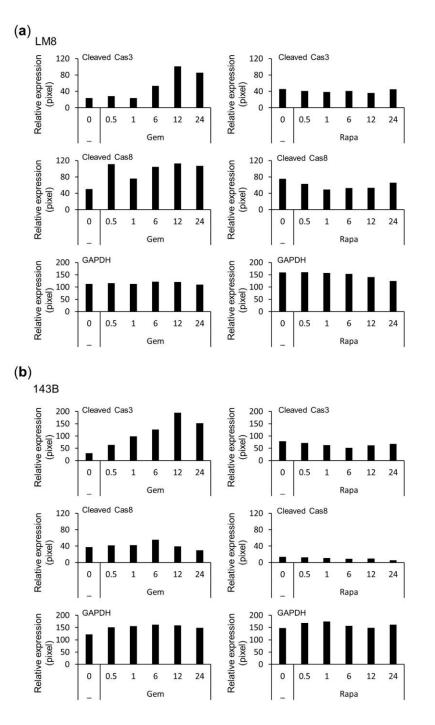


Figure S4. Quantification of cleaved caspase 3, cleaved caspase 8, and GAPDH bands in western blots
 depicted in Figure 2d. (a) LM8 cells. (b) 143B cells.

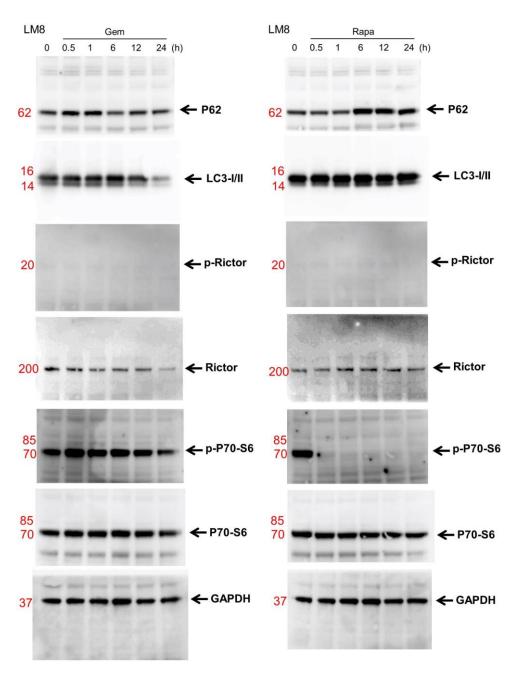


Figure S5. Example uncropped western blots depicting the expression of p62, LC3-I/II, p-RICTOR,
 RICTOR, p-P70-S6, P70-S6, and GAPDH in LM8 cell lysates. These blots complement the cropped
 blots in Figure 3a.

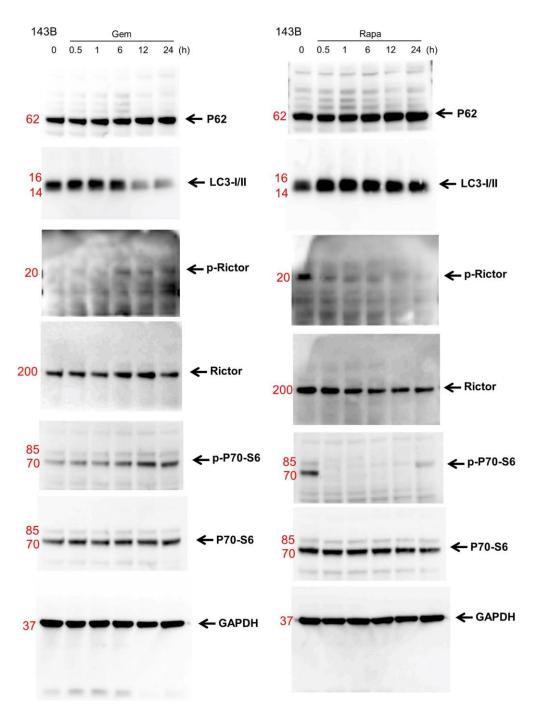
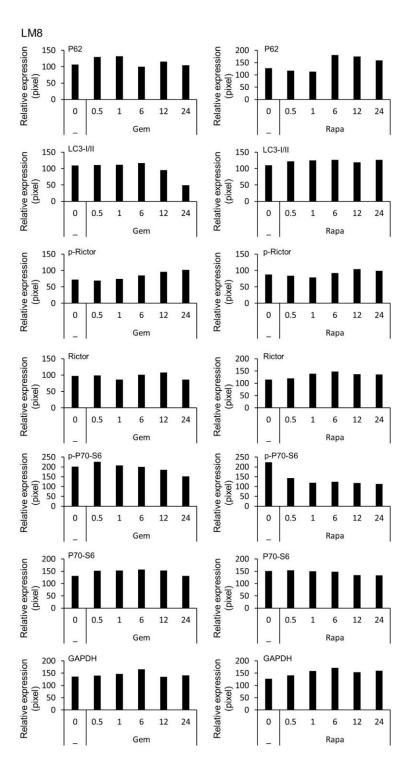


Figure S6. Example uncropped western blots depicting the expression of p62, LC3-I/II, p-RICTOR,
 RICTOR, p-P70-S6, P70-S6, and GAPDH in 143B cell lysates. These blots complement the cropped
 blots in Figure 3a.



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**Figure S7.** Quantification of western blot bands corresponding to p62, LC3-I/II, p-RICTOR, RICTOR, p-P70-S6, P70-S6, and GAPDH in LM8 cell lysates depicted in Figure 3a.

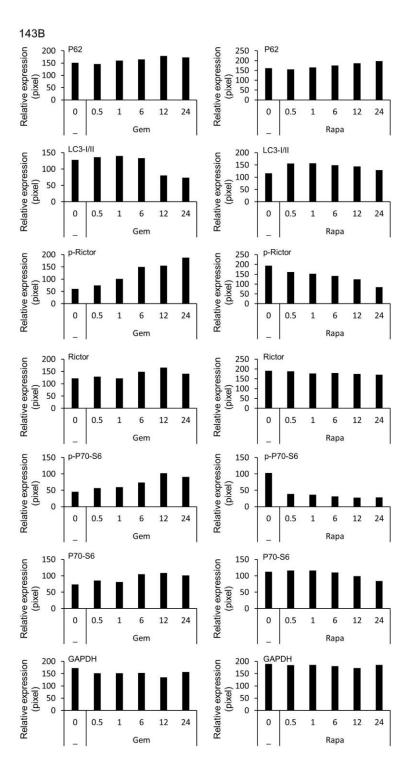


Figure S8. Quantification of western blot bands of p62, LC3-I/II, p-RICTOR, RICTOR, p-P70-S6, P70-S6, and GAPDH in 143B cell lysates depicted in Figure 3a.

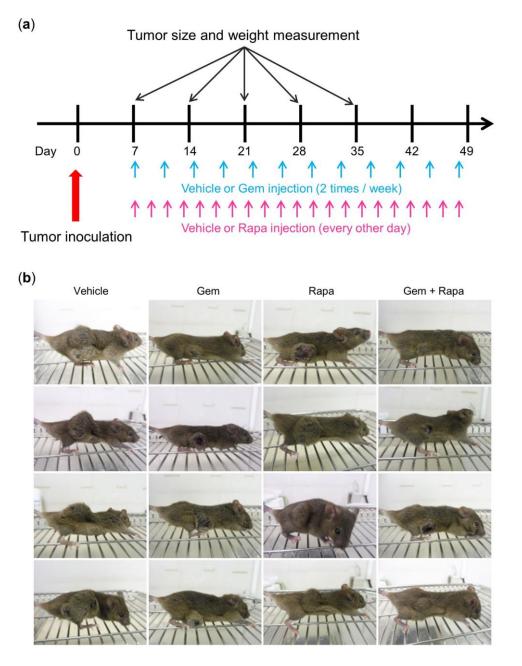


Figure S9. (a) Schematic diagram showing the experimental design for Gem and Rapa treatment of
 mice with allograft or xenograft osteosarcoma cell transplants. (b) Photos of tumor-bearing mice from
 Figure 4.

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