

## Legend to Supplemental Figures

### **Supplemental Figure 1. MG132 increased intracellular calcium 2-fold in HCT116 cells and produced similar results in DU145 Cells.**

**(A)** HCT116 Bax (-) cells were treated with MG132 (1 $\mu$ M) for 16 hours. Cells were then stained with 2.5  $\mu$ M of Fluo-4-AM in calcium-free Hank's buffer for 30 minutes followed by flow cytometry analysis. Fluorescence intensity (FI) is shown. **(B)** DU145 Bax (-) cells were treated with MG132 (1 $\mu$ M) in the presence or absence of BAPTA-AM (10  $\mu$ M) for 16 hours. Cells were then washed and stained with 2.5  $\mu$ M of Fluo-4-AM in calcium-free Hank's buffer for 30 minutes followed by flow cytometry analysis. Representative histogram data are shown.

### **Supplemental Figure 2. BAPTA-AM inhibited MG132-induced cellular vacuolization in DU145 cells.**

DU145 Bax (-) cells were treated with MG132 (1  $\mu$ M) in the presence or absence of BAPTA-AM (10  $\mu$ M) for 12 hours followed by phase-contrast microscopy. Representative images are presented in **(A)**. **(B)** Vacuolated cells were counted, and results are expressed as percent of vacuolated cells (\* $p$ <0.05 vs untreated-control; ^  $p$ <0.05 vs MG132, One Way ANOVA).

### **Supplemental Figure 3. The source of intracellular calcium increase was most likely not ER or extracellular calcium influx.**

HCT116 Bax (-) cells were treated with MG132 (1  $\mu$ M) in the presence or absence of Xec (25 nM) or 2-APB (20  $\mu$ M) for 12 hours followed by phase-contrast microscopy. Representative images are presented in **(A)**. **(B)** Vacuolated cells were counted, and results are expressed as percent of vacuolated cells. Results are from two individual experiments. **(C and D)** HCT116 Bax (-) cells were treated with MG132 (1 $\mu$ M) in the presence or absence of Xec (25 nM) or 2-APB (20  $\mu$ M) for 16 hours. Cells were then washed and stained with 2.5  $\mu$ M of Fluo-4-AM in calcium-free Hank's buffer for 30 minutes followed by flow cytometry analysis. Representative

histogram data are shown. (E) HCT116 Bax (-) cells were treated with MG132 (1  $\mu$ M) in the presence or absence of varying concentrations of EGTA for 12 hours followed by phase-contrast microscopy. Representative images are shown.

**Supplemental Figure 4. MG132 induced ER dilation in DU145 cells.**

DU145 Bax (-) cells were treated with MG132 (1 $\mu$ M) for 16 hours, and cells were fixed with 4% PFA before immunostaining for Calnexin (green) for visualization of ER dilation and DAPI (blue) for visualization of the cell nucleus. Representative fluorescence images are shown.