SUPPLEMENTAL FIGURES

Supplemental Figure 1. Effect of Ca^{2+} influx modulators on clastolactacystin β-lactone induced neuronal death. A. Murine neocortical cultures were treated with clastolactacystin-β-lactone (CL, 3 µM) for 48 hr. (-) Bay K8644 (BayK, 10 µM), kainate (KA, 1 µM) or benzamil (BENZ, 10 µM) were added to the indicated cultures at the beginning of the treatment period. Values shown are mean ± SEM cell death analyzed by PI fluorescence at the end of the 48 hr treatment period. * indicates values for BayK and benzamil treated cultures differed significantly from CL alone (P=0.007 for BayK; P=0.019 for benzamil). The injury reduction in kainate-treated cultures did not reach significance. B. Similar to A. except that cultures were treated with 1 µM clastolactacystin alone (CL) or with the addition of MK-801 (10 µM), D-APV (1 mM) or MK-801 + kainate (5 µM) during the 48 hr treatment with proteasome inhibitor. * indicates significant difference from cultures treated with CL alone; # indicates CL+MK+KA condition differs significantly from CL+MK condition.

Supplemental Figure 2. Proteasome inhibition does not alter basal neuronal Ca^{2+} efflux. Neuronal cultures were loaded with $^{45}Ca^{2+}$ for 48 hr, then cultures were washed and returned to standard media, either with no additions (Sham) or 1 uM or 3 uM MG-132. $^{45}Ca^{2+}$ efflux into the media was measured at 1 hr intervals. Values shown are mean ± SEM % cpm released (cpm in media/cpm in cells at start of efflux period).

Supplemental Figure 3. **Validation of mag-fura 2 fluorescence as a measure of $[Ca^{2+}]_{ER}$.**

A. Murine neocortical cultures were loaded with Mag-fura-2, washed into Ca^{2+}-free buffer and then treated with thapsigargin (2.5 μM for 30 min, Thapsigargin) or sham-washed (Sham). Values shown are mean ± SEM 340/380 ratio for at least 40 cells for each condition. * indicates value in thapsigargin condition differs significantly with that in control condition ($P < 0.01$). B. Similar to A, except cultures were loaded with Mag-fura-2 (black line) or fura-2 (gray line). Images were obtained for 5 min, then digitonin (10 μM) was added and imaging continued for an additional 30-35 min. Values shown are fluorescence intensity (ex360 (isosbestic point)/em510) from a representative tracing for each condition. n= 8 neurons imaged with Mag-fura-2 and 5 for Fura-2.

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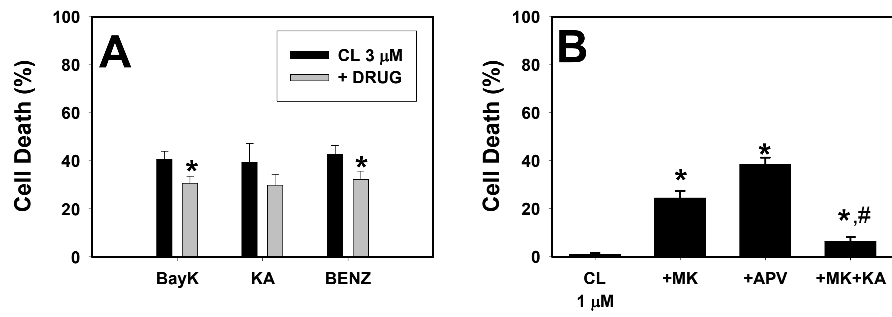
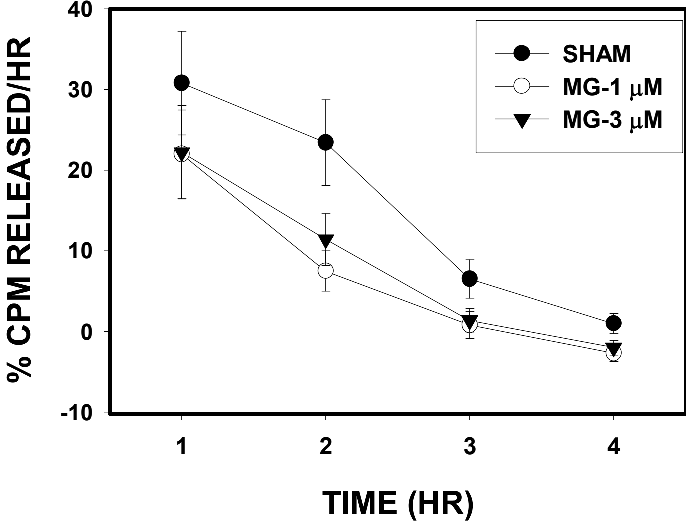


Wu et al., Supplemental Figure 2



Wu et al., Supplemental Figure 3