

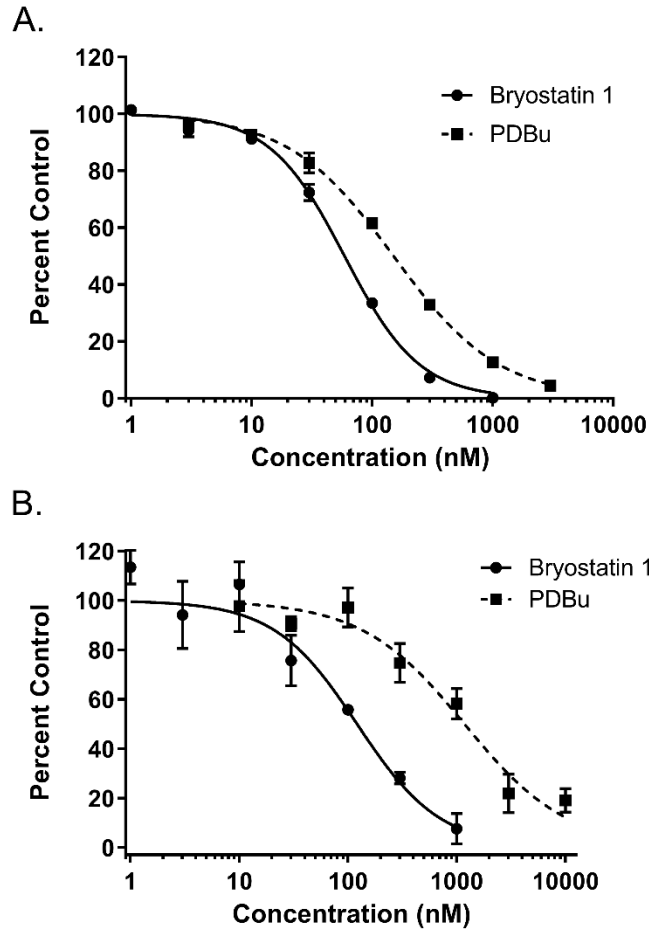
## Supporting Information

### **Munc13 is a molecular target of bryostatin 1**

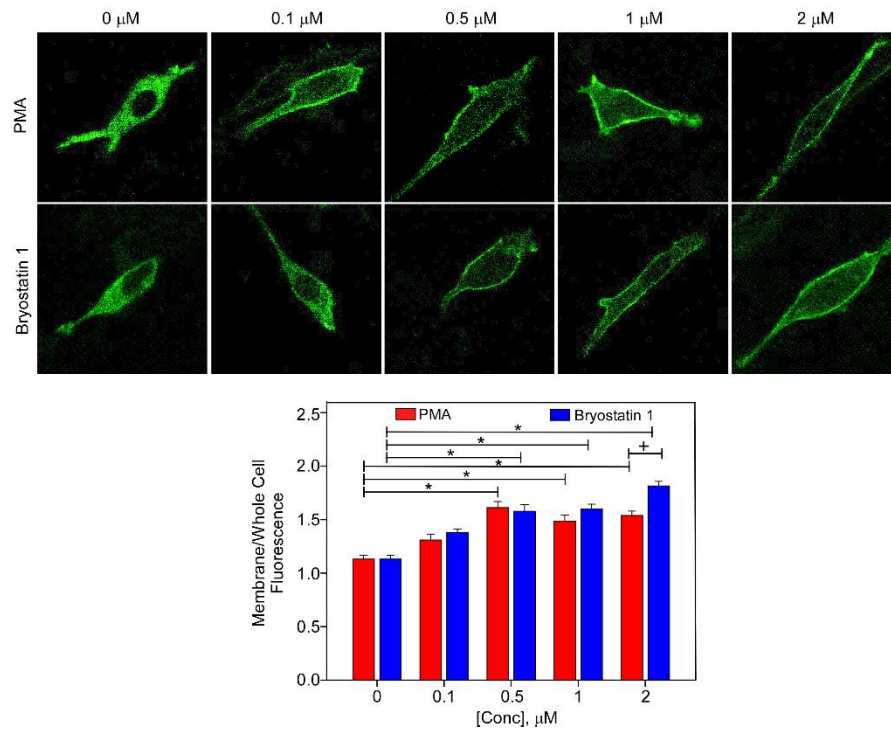
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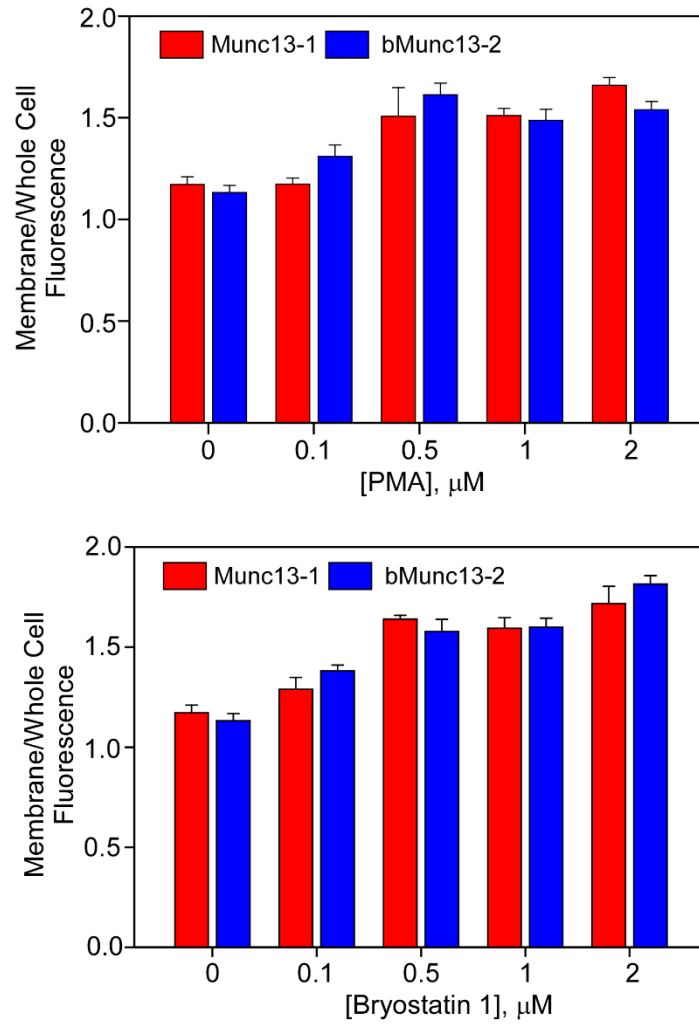
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**Figure S1. Binding of bryostatin 1 to the A) Munc13-1 C1 domain and its B) W588A mutant.** Binding of 500 nM [<sup>3</sup>H]PDBu to the purified Munc 13-1 C1 domain containing the W588A mutation was performed in the presence of 100  $\mu$ g/ml lipid (100% PS), 0.1 mM Ca<sup>++</sup>, and increasing concentrations of bryostatin 1 or nonradioactive PDBu (for comparison). A representative experiment is shown. Data represent the mean  $\pm$  SEM of three experimental values in a single experiment. Where error bars are not visible, they lie within the symbols. Two additional experiments gave similar results.



**Figure S2. Effect of PMA and bryostatin 1 on bMunc13-2 membrane translocation.** Top panel confocal images show HT22 cells expressing bMunc13-2, treated with PMA or bryostatin 1 (0-2 μM) for 5 min. Lower panel bar graph shows normalized membrane to whole cell fluorescence intensity ratio, quantified from upper panel confocal images. Data are expressed as mean  $\pm$  SEM of at least three independent experiments and obtained from 4 to 9 confocal cell images per treatment. Two-way ANOVA, followed by *Tukey's post hoc* test, was used for statistical significance analysis. \*,  $P < 0.05$  compared to 0 μM of PMA and bryostatin 1, +,  $P < 0.05$  compared to 2 μM PMA. HT22 cells were transiently transfected with bMunc13-2 and confocal image analysis was carried out as described in experimental procedures.



**Figure S3. Comparison of effects of PMA and bryostatin 1 on Munc13-1 and Munc13-2.** Top panel bar graph shows the effects of PMA on Munc13-1 and bMunc13-2. Bottom panel bar graph shows effect of bryostatin 1 on Munc13-1 and bMunc13-2. Bar graphs were generated from data obtained from confocal analysis described in Figure 3 and Figure S2. Two-way ANOVA, followed by *Tukey's post hoc* test, was used for statistical significance analysis. Treatments with PMA and bryostatin 1 had similar effects on Munc13-1 and bMunc13-2.