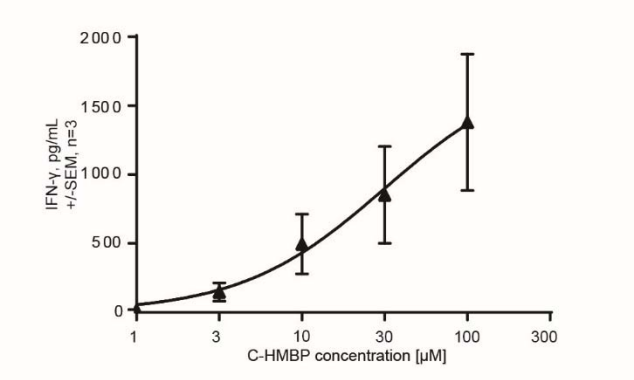
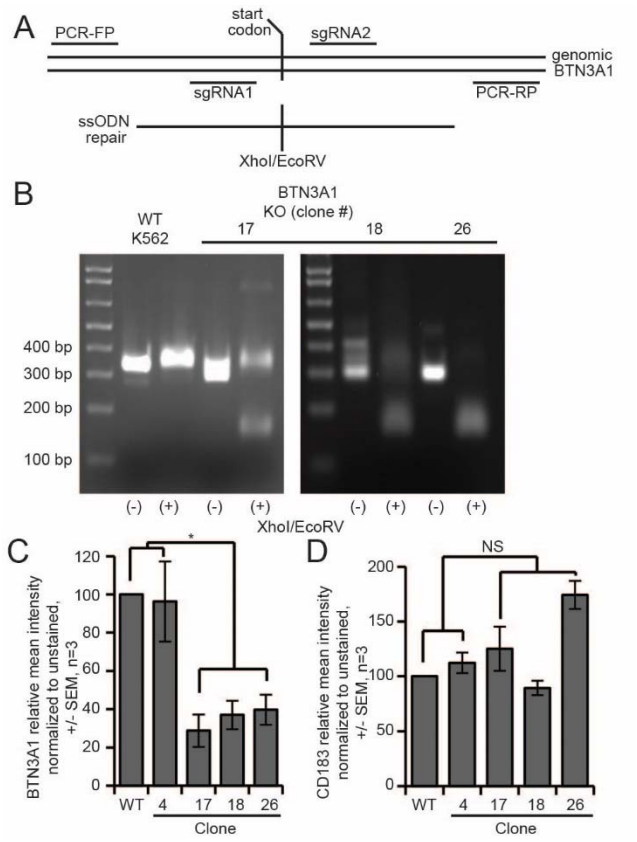
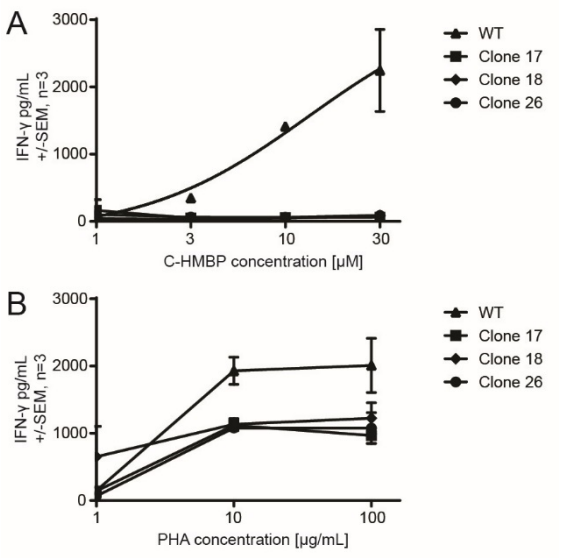


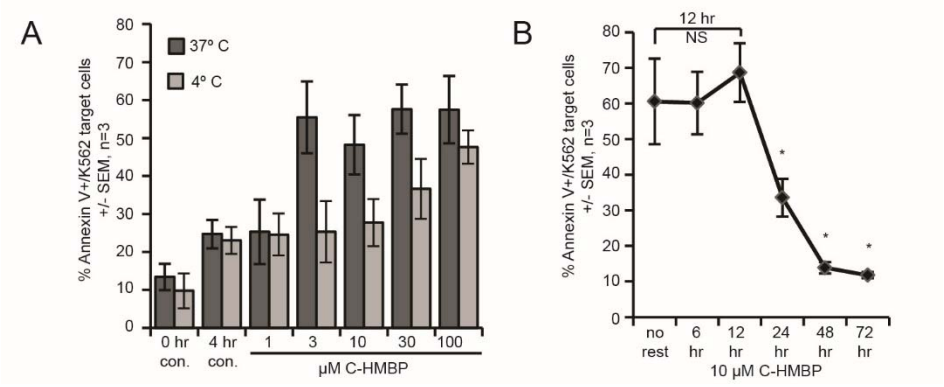
Supplemental Figure 1. IFNγ secretion induced by C-HMBP. Quantification of IFNγ secretion following 2 hour C-HMBP pre-treatment and 24 hour incubation with effector cells.



Supplemental Figure 2. Disruption of BTN3A1 expression in target cells. A) Schematic of design for knockout utilizing CRISPR/Cas9. The start codon was excised from genomic DNA using paired single strand breaks and replaced with a dual restriction site using an ssODN repair template to facilitate genotyping of clones. B) PCR genotyping of wild-type K562 cells and three BTN3A1 CRISPR/Cas9 knockout clones that displayed the desired genotype. C) Quantification of BTN3A1 surface expression in WT, clone 4 and three BTN3A1 CRISPR/Cas9 knockout clones, as analyzed by flow cytometry. D) Quantification of surface expression of CD183 in WT, clone 4 and three BTN3A1 CRISPR/Cas9 knockout clones, as analyzed by flow cytometry.



Supplemental Figure 3. IFNγ secretion induced by C-HMBP is abrogated by depletion of BTN3A1. WT K562 cells or BTN3A1 deficient K562 clones 17, 18 and 26 were preloaded for 2 hours with the indicated concentrations of C-HMBP (Panel A) or PHA-P (Panel B). Loaded cells were washed and exposed to effector Vγ9Vδ2 T cells for 24 hours, then IFN-γ was determined by ELISA.



Supplemental Figure 4. Lysis assays with C-HMBP. A) C-HMBP requires energy dependent uptake to enter target cells. C-HMBP dose response lysis assay at 37ºC and 4ºC. B) Metabolism of C-HMBP. Time course lysis assay of K562 cells pre-treated for 2 hours with 10 µM C-HMBP. K562 cells were rested for 0 hours (no rest), 6 hours, 12 hours, 24 hours, 48 hours or 72 hours. Once rested, cells were incubated with effector cells for 4 hours. *****, significant compared to no rest.