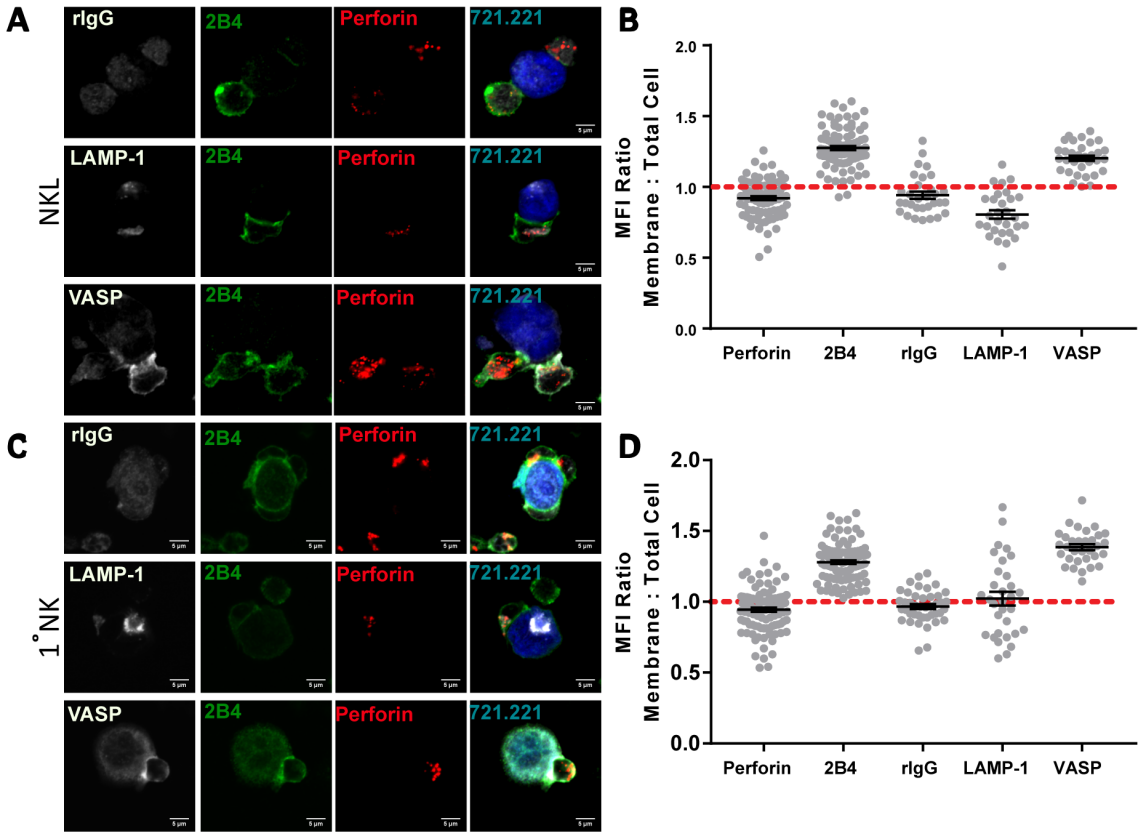
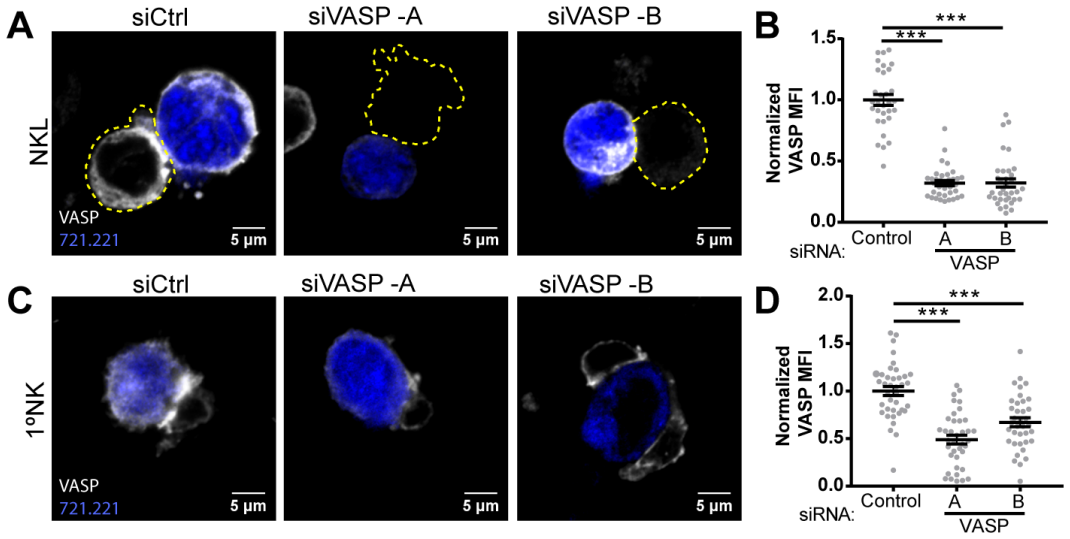
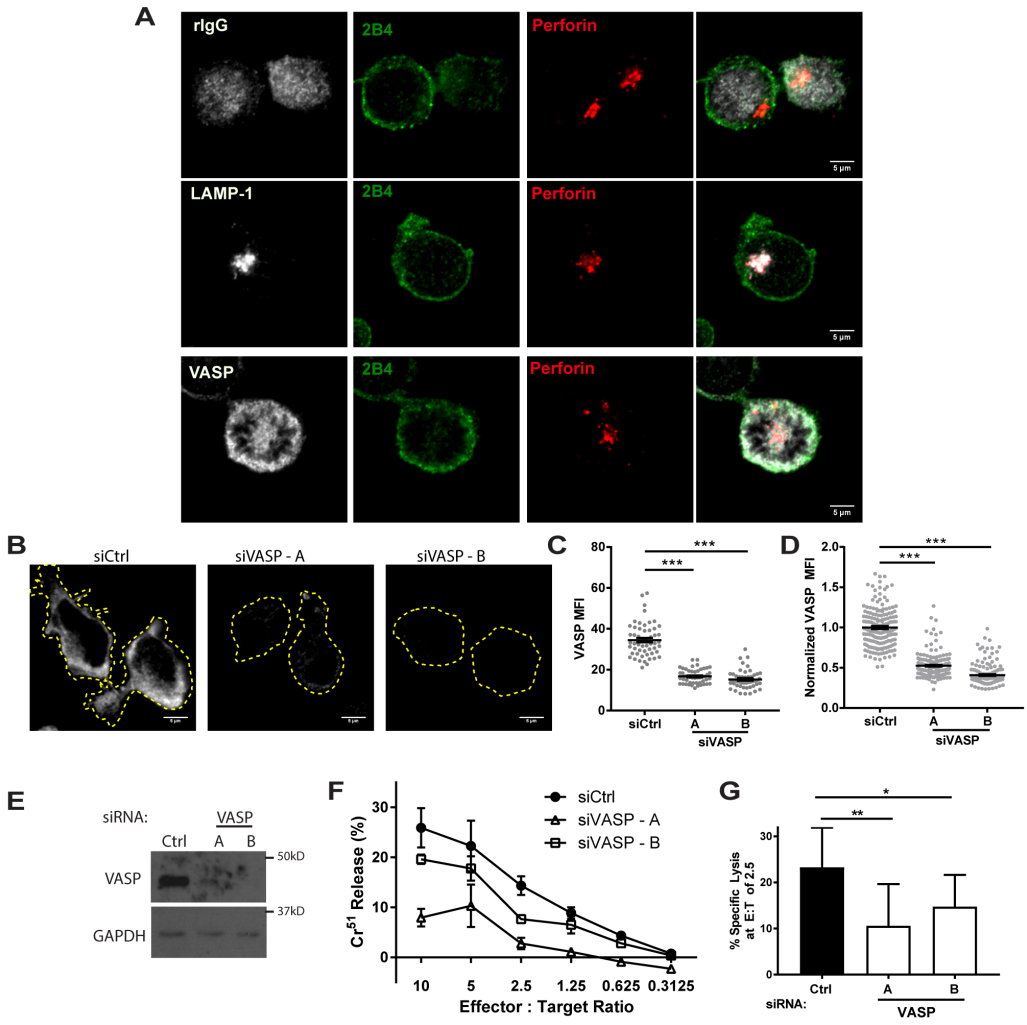


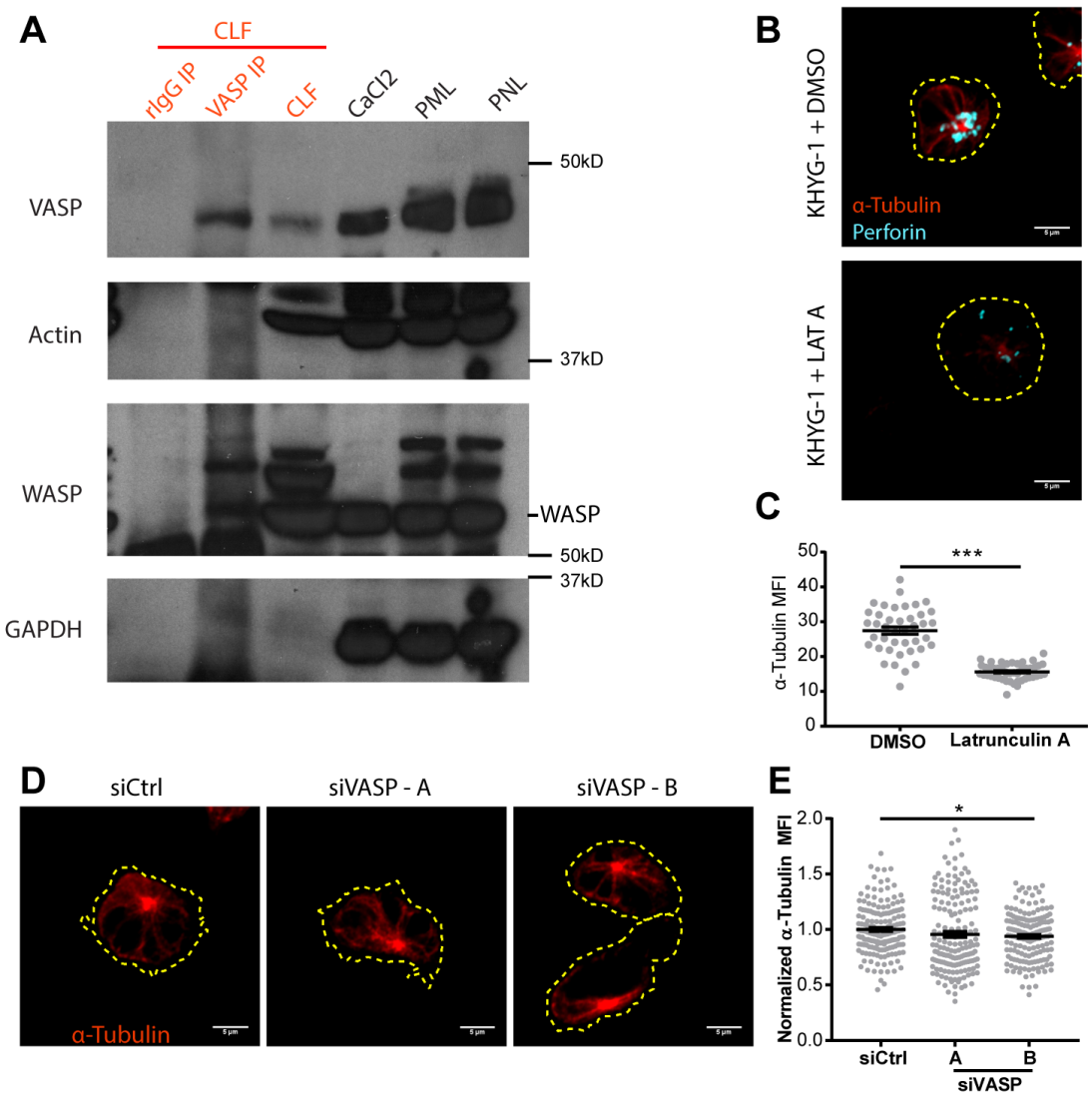
SUPPLEMENTAL FIGURE 1. VASP localizes to the membrane, and specifically synapse of 721.221 target cell activated NK cells. Representative images of (**A**) NKL and (**C**) primary NK cells that were allowed to form conjugates with CMAC-stained 721.221 cells and stained with the indicated antibodies. The amount of protein present at the cell membrane was quantified for NKL (**B**) and primary NK cells (**D**).



SUPPLEMENTAL FIGURE 2. VASP-targeting siRNAs deplete VASP from NKL and primary NK cells. NKL (**A-B**) and primary NK (**C-D**) cells were nucleofected with control or one of two VASP-targeting siRNAs and allowed to form conjugates with CMAC-stained (blue) 721.221 cells. These conjugates were stained and imaged for the presence of VASP (white). (**A, C**) Representative images of conjugates and (**B, D**) representative quantitation of relative Mean Fluorescence Intensity (MFI) from a single example experiment of each is shown. Error bars indicate SEM. *$p < 0.05$, ** $p < 0.005$, and ***$p < 0.0005$ compared with control group. For NKL cells, a dotted line denotes the exterior of the cell, as defined by cortical actin staining (not shown).



SUPPLEMENTAL FIGURE 3. VASP localizes to the membrane and perigranular area in KHYG-1 and VASP knockdown inhibits KHYG-1 cytotoxicity. (**A**) KHYG-1 cells were imaged for the localization of the indicated proteins via immunofluorescence. (**B**) KHYG-1 cells were nucleofected with control siRNA (siCtrl) or one of two siRNAs targeting VASP. The cells were imaged and assessed by immunofluorescence for VASP knockdown. Representative images are shown. (**C**) Quantitation of VASP MFI in KHYG-1 cells nucleofected with siCtrl RNA or VASP-targeting siRNA for a single VASP knock down experiment and (D) for a meta-analysis of all confocal experiments where this control was used, with each normalized to the average VASP MFI for the siCtrl group. (**E**) KHYG-1 cells were nucleofected with control or VASP-targeting siRNAs and knockdown was verified for each experiment using immunoblot. Seventy-two hours after nucleofection, KHYG-1 cells were incubated with ^{51}Cr-labelled 721.221 cells at the specified effector to target ratios and percent specific lysis was determined. Representative image of a single experiment (**F**) and (**G**) average values of 3 independent experiments at a specific effector to target ratio of 2.5, each completed in quadruplicate, are shown. Error bars indicate SEM. *p < 0.05, ** p < 0.005, and ***p < 0.0005 compared with control group.



SUPPLEMENTAL FIGURE 4. VASP purifies with lytic granules but does not impact gross microtubule stability. (**A**) Unstimulated KHYG-1 cells were fractionated and VASP was immunoprecipitated from the crude lysosomal fraction. The resulting fractions and immunoprecipitations were immunoblotted for the proteins shown on multiple membranes. The immunoblot shown is representative of three independent experiments. (**B**) KHYG-1 cells were treated with DMSO or 1 µM Latrunculin A, fixed and imaged for the indicated proteins. Representative images are shown. (**C**) Immunofluorescence of the quantification of the MFI of α-tubulin in cells treated with DMSO and Latrunculin A. These results show one representative experiment of three independent experiments completed. (**D**) KHYG-1 cells were nucleofected with control siRNA (siCtrl) or one of 2 siRNAs targeting VASP. The resulting cells were fixed and stained for α-tubulin. Representative images are shown. (**E**) Quantification of the mean fluorescence intensity of tubulin a meta-analysis of 3 independent experiments, each normalized to the average MFI of α-tubulin in the siCtrl treated cells. Error bars indicate SEM. *$p < 0.05$, ** $p < 0.005$, and ***$p < 0.0005$ compared with control group.