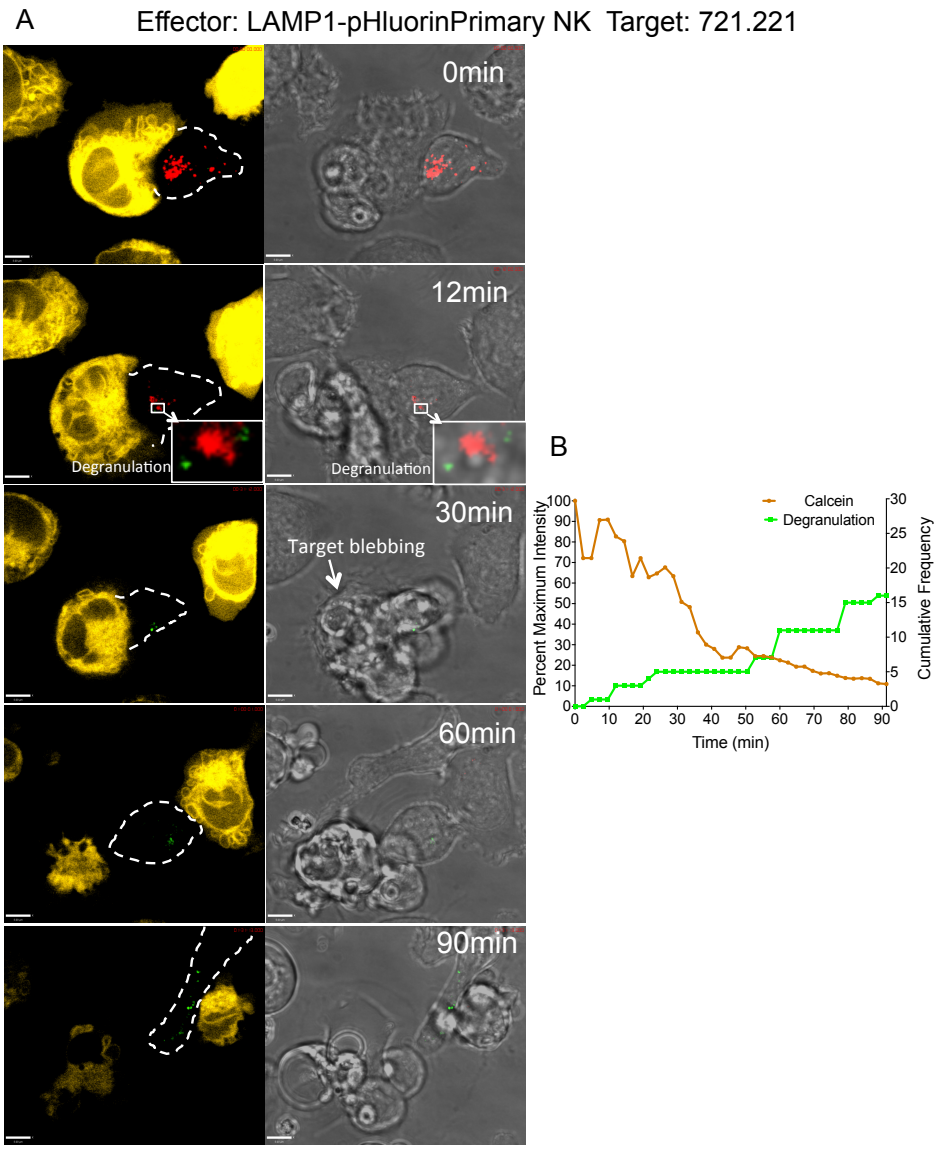
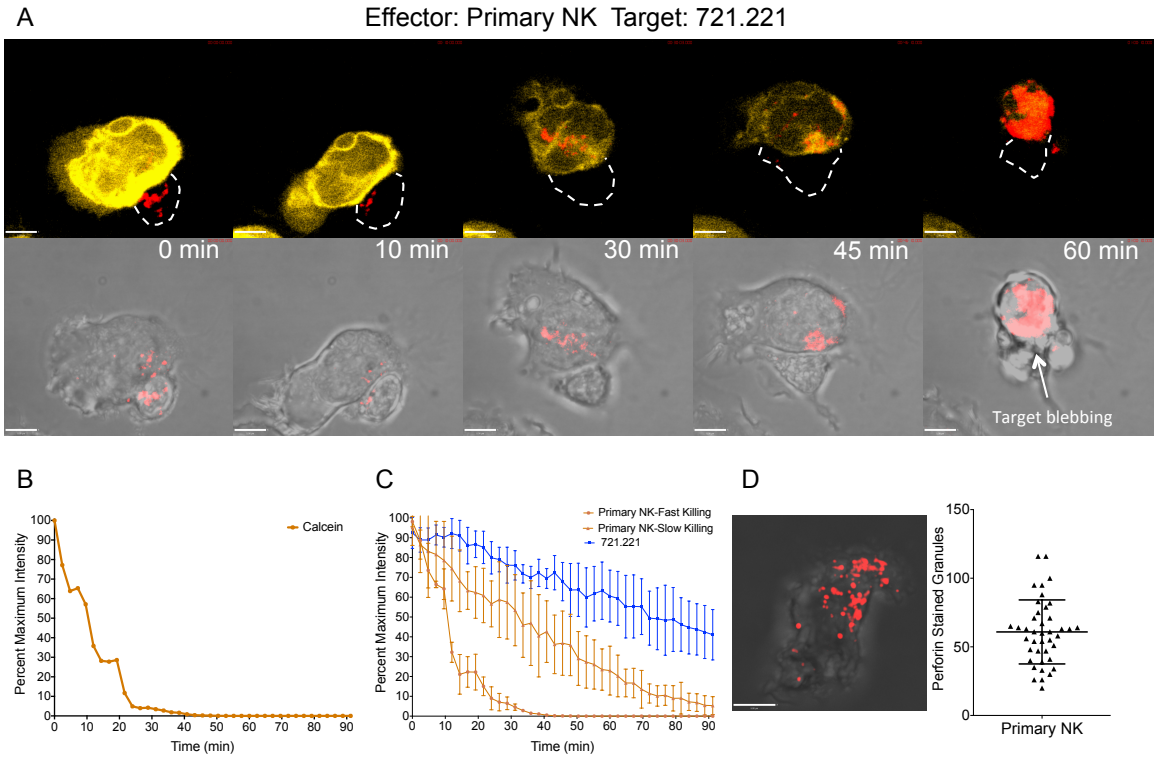


Supplemental Figure 1: Live single-cell Imaging for visualization and measurement of target cell killing by LAMP1-pHluorin primary NK cells. Primary NK cells transduced with LAMP1-pHluorin were loaded with LysoTracker Deep Red (red) and incubated with calcein, AM orange-red labeled 721.221 target cells (yellow). Z stack images of single cell conjugates were acquired by confocal microscopy. Imaging was performed at a frame rate of 1 image every 2.4 minutes for 90 minutes until target cell death was observed. (A) Representative maximum projection time-lapse image sequences of a LAMP1-pHluorin positive primary NK cell conjugated to a single 721.221 target cell. Left panel shows calcein fluorescence (yellow); Right panel shows bright field images of the conjugate with Degranulation events (green) and characteristic apoptotic membrane blebbing in the target cell. Scale bar 5μm. (B) Quantitative analysis of calcein fluorescence and cumulative frequency of degranulation in the representative NK-721.221 conjugate shown in (A). Time-lapse image sequence and analysis is representative of at least 3 independent experiments.



Supplemental Figure 2: Live single-cell imaging for visualization and measurement of target cell killing by primary NK cells. Primary NK cells were loaded with LysoTracker Deep Red (red) and incubated with calcein, AM orange-red labeled 721.221 target cells (yellow). Z stack images of single cell conjugates were acquired by confocal microscopy. Imaging was performed at a frame rate of 1 image every 2.4 minutes for 90 to 120 minutes until target cell death was observed. (A) Representative maximum projection time-lapse image sequences of a single primary NK cell conjugated to a single 721.221 target cell. Top panel shows calcein fluorescence (yellow); Bottom panel shows bright field images of the conjugate with characteristic apoptotic membrane blebbing in the target cell. Scale bar 5μm. (B) Quantitative analysis of calcein fluorescence in the representative NK-721.221 conjugate shown in (A). (C) Analysis of calcein fluorescence in 721.221 target cells conjugated to primary NK cells showed either a slow release or a fast release pattern. Images are representative and analyses are from 8 independent experiments using freshly isolated primary NK cells. (D) Representative extended focus confocal image of perforin positive lytic granules and quantitative analysis of total number of granules in primary NK cells. Lytic granule number analyzed from 40 primary NK cells of 2 donors. Data are represented as mean values +/- SD.