Supplementary Figure 1. Effect of actin depolymerization upon degranulating granule area and LAMP1-pHluorin intensity. Analyses depict the first 20 minutes post-degranulation to demonstrate mean data from multiple events (see text). **A.** Mean sum fluorescent intensity of lytic granules in NK92 cells expressing pHluorin-LAMP1. Cells were treated with DMSO (solid) and Latrunculin A (dashed). **B.** Size of the observed lytic granules in cells treated with DMSO (solid) and LatA (dashed). Time points reflect elapsed time post-degranulation. Differences in sum fluorescent intensity between the two groups are statistically significant by the Mann-Whitney U test ($p>0.05$) whereas there is no significant difference in area. Results shown are from four independent experiments, n = 12 (control), n=18 (LatA).

Supplementary Figure 2. The behavior of long-lived non-degranulating lytic granules at the cell membrane. Comparative measurements of LysoTracker Red motility from Figure 6 (grey) were compared to those granules visible for >55 minutes (black). No significant difference in track length, velocity, displacement, displacement rate or velocity kinetics was observed (A-D). E) No substantive difference was seen in granule kinetics between granules that degranulate (grey), those that don't (black dashed) and those that are visible for >55 minutes (red). F) Time of visibility of granules analyzed in (A-E). Those seen for >55 minutes were still visible at the termination of imaging.

Supplementary Video Legends

Video 1. Lytic granule polarization and target cell death visualized by confocal microscopy. A LysoTracker Red labeled YTS GFP-actin NK cell (right) is conjugated to a CellMask labeled 721.221 (yellow) target cell (right) in the presence of SYTOX Blue (blue) to detect cell death. Conjugates were imaged by confocal microscopy at 1 frame per minute as described in Materials and Methods. Shown is the representative conjugate shown in Figure 1 (imaged for 108 minutes). Time in minutes is shown in the upper right corner and represents the time after imaging began, which was ten minutes after the addition of cells to the imaging chamber. Scale bar = 1 μm.

Video 2. Lytic granule polarization and degranulation visualized by confocal microscopy. NK92 pHluorin-LAMP1 NK cells were loaded with LysoTracker Red (red) and incubated with CellMask labeled (grey) K562 target cells in the presence of SYTOX Blue (blue). Conjugates were imaged for 60 minutes at one frame per minute as described in Materials and Methods. Shown is the representative image from Figure 2 with the NK cell on the right and the target cell in the center. The same image with transmitted light overlay is in the panel on the right. Degranulation events are visible as a change from red to green fluorescence in a granule at the NK cell membrane at minutes 6, 12, 13, 18, 21, and from minute 25 on. Target cell blebbing and subsequent death is seen beginning at 40 minutes and includes some autofluorescence visible in the green channel in the target cell at later time points. Time in minutes is shown in the upper right corner and represents the time after imaging began, which was ten minutes after the addition of cells to the imaging chamber. Scale bar = 6 μm.

Video 3. Lytic granule navigating the cell cortex and subsequent degranulation. NK92 cells expressing pHlourin-LAMP1 were loaded with LysoTracker Red (red) and activated by immobilized antibody to NKp30 and CD18. Cells were imaged by TIRFm at 6 frames per minute as described in Materials and Methods. Shown is the representative granule from Figure 3 with degranulation at approximately 39 minutes marked by a transition from red to green fluorescence. Time in minutes is shown in the upper right corner and represents the time after imaging began, which was 10-15 minutes after the addition of cells to the imaging chamber.

Video 4. Lytic granule navigating the cell cortex without undergoing degranulation. NK92 cells expressing pHlourin-LAMP1 were loaded with LysoTracker Red (red) and activated by immobilized antibody to NKp30 and CD18. Cells were imaged by TIRFm at 6 frames per minute as described in Materials and Methods. Shown is the representative granule from Figure 5 with withdrawal from the cortex at approximately 35 minutes. Time in minutes is shown in the upper right corner and represents the time after imaging began, which was 10-15 minutes after the addition of cells to the imaging chamber.

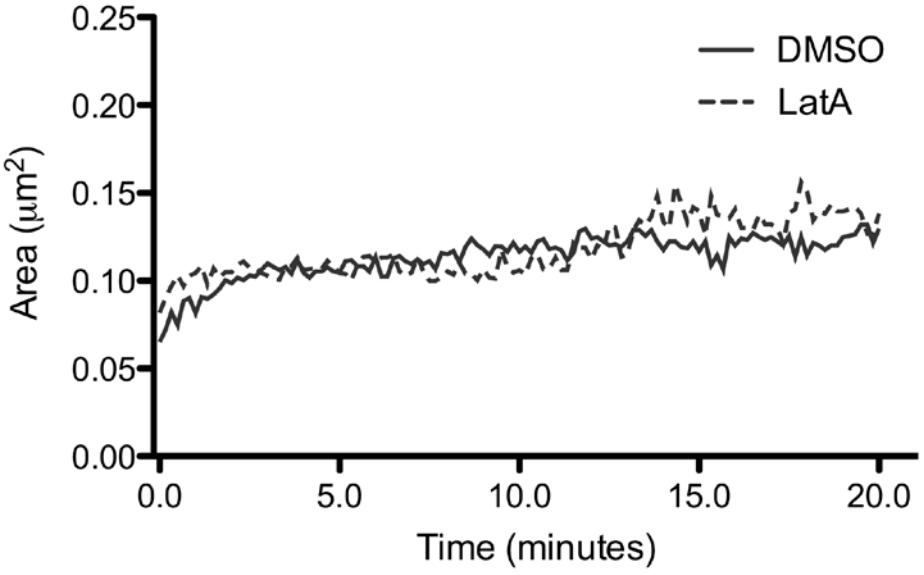
Video 5. Granule from a DMSO-treated cell navigating the cortex and undergoing degranulation. NK92 cells expressing pHlourin-LAMP1 were loaded with LysoTracker Red (red) and activated by immobilized antibody to NKp30 and CD18. Cells were imaged by TIRFm at 6 frames per minute as described in Materials and Methods. Shown is the representative granule from Figure 7A with degranulation at approximately

20 minutes marked by a transition from red to green fluorescence. Time in minutes is shown in the upper right corner and represents the time after imaging began, which was 10-15 minutes after the addition of cells to the imaging chamber. DMSO was added immediately prior to the beginning of imaging and was present throughout the rest of the experiment. Scale bar = 0.5 μm.

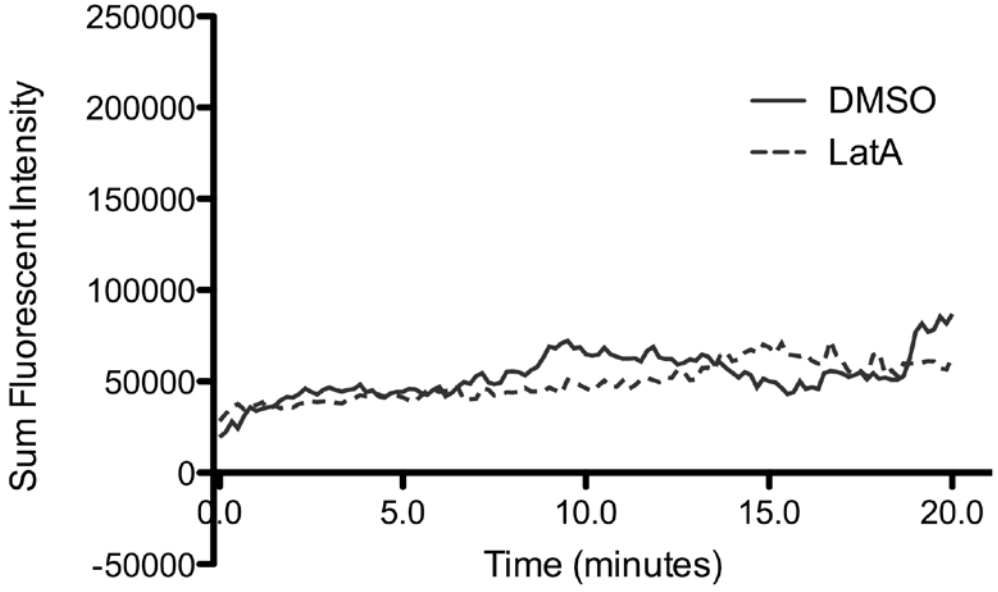
Video 6. Granule from a LatA-treated cell navigating the cortex and undergoing degranulation. NK92 cells expressing pHlourin-LAMP1 were loaded with LysoTracker Red (red) and activated by immobilized antibody to NKp30 and CD18. Cells were imaged by TIRFm at 6 frames per minute as described in Materials and Methods. Shown is the representative granule from Figure 7B with degranulation at approximately 20 minutes marked by a transition from red to green fluorescence. Time in minutes is shown in the upper right corner and represents the time after imaging began, which was 10-15 minutes after the addition of cells to the imaging chamber. 10 μM LatA was added immediately prior to the beginning of imaging and was present throughout the experiment.

Supplementary Figure 1

A



B



Supplementary Figure 2