

Online Supplemental Material

Supplementary Figure Legends

Figure S1: Chromium-release assays evaluating models of cytolytic and non-cytolytic conjugation. Cytolytic activity of NK cells against ^{51}Cr -labeled target cells measured in a 4hr chromium release assay. The different effector and target cell combinations represent those used comparatively in Figures 1 and 2 and include: YTS cells and susceptible 721.221 target cells (red line), NK92 cells and susceptible K562 target cells (blue line), as well as YTS NK cells and non-susceptible K562 target cells (green line). Each line represents the mean from three independent experiments with the error bars demonstrating \pm SD of the independent experiments.

Figure S2: Effect of conjugation time, activation, Taxol treatment, Cytochalasin D treatment, or Latrunculin A treatment on lytic granule area-weighted distance from the MTOC. Quantitative analyses of lytic granule movement relative to the MTOC as measured by mean area-weighted distance \pm SD across all time points in: (A) GFP-tubulin expressing NK cells in isolation (black), or conjugated with target cells (gray) as specified on the x-axis, (B) YTS GFP-tubulin cells in control or antibody-coated imaging chambers as defined in the inset legend, (C) eNK cells in isolation (black) or conjugated to susceptible K562 target cells (gray), and (D) Taxol-, Cytochalasin D-, or Latrunculin A-treated YTS GFP-tubulin cells in isolation (black) or conjugated to susceptible 721.221 target cells (gray). Each data point represents the mean over all time points recorded in 8-10 cells.

Figure S3: Effect of conjugation time, activation, Taxol treatment, Cytochalasin D treatment, Latrunculin A treatment, or dynein disruption on NK cell perimeter. Quantitative analyses of mean NK cell perimeter \pm SD across all time points in: (A) GFP-tubulin expressing NK cells in isolation (black), or conjugated with target cells (gray) as specified on the x-axis, (B) YTS GFP-tubulin cells in control or antibody-coated imaging chambers as defined in the inset legend, (C) Taxol-, Cytochalasin D-, or Latrunculin A-treated YTS GFP-tubulin cells in isolation (black) or conjugated to susceptible 721.221 target cells (gray), and (D) GFP-, p50-GFP-, or CC1-GFP-nucleofected YTS cells conjugated to susceptible 721.221 target cells as defined in the inset legend. Each data point represents the mean over all time points recorded in 5-10 cells.

Figure S4: Effect of conjugation time, activation, Taxol treatment, Cytochalasin D treatment, Latrunculin A treatment, or dynein disruption on lytic granule area. Quantitative analyses of mean total lytic granule area \pm SD across all time points in: (A) GFP-tubulin expressing NK cells in isolation (black), or conjugated with target cells (gray) as specified on the x-axis, (B) YTS GFP-tubulin cells in control or antibody-coated imaging chambers as defined in the inset legend, (C) eNK cells in isolation (black) or conjugated to susceptible K562 target cells (gray), (D) Taxol-, Cytochalasin D-, or Latrunculin A-treated YTS GFP-tubulin cells in isolation (black) or conjugated to susceptible 721.221 target cells (gray), and (E) GFP-, p50-GFP-, or CC1-GFP-nucleofected YTS cells conjugated to susceptible 721.221 target cells as defined in the inset legend. Each data point represents the mean over all time points measured in 5-10 cells.

Figure S5: Quantitative analysis of lytic granule convergence to the MTOC in fixed cell conjugates. Microscopy of YTS cells that were (A) unconjugated, or conjugated to 721.221 cells for 5 min, or 30 min prior to fixation. Images show DIC (left) and fluorescence (right) of the fixed cells in which staining was performed with anti- α -tubulin (green) and anti-perforin (red).

The rightmost images demonstrate an overlay of tubulin and perforin fluorescence. The biotinylated anti-tubulin mAb was detected with Pacific Blue-streptavidin (pseudo-colored green); the anti-perforin antibody was directly FITC-conjugated (pseudocolored red). This particular pseudo-coloring was performed to enable comparison to similarly colored structures in images in other figures. Lytic granule proximity to the MTOC in unconjugated or conjugated NK cells as measured by mean MTOC to granule distance \pm SD (B) and by mean area-weighted distance \pm SD (C). Data are representative of 3 independent experiments in which 50 cells were analyzed per condition. Lytic granule distance from the MTOC in fixed conjugated NK cells is significantly less than in fixed unconjugated NK cells ($p < 0.001$). (D) Lytic granule area in the plane of the MTOC as measured by perforin staining in fixed cells compared to the area of the region defined LysoTracker Red staining in live cells used to generate Figure 2.

Figure S6: Quantification of MTOC-directed lytic granule movement in resting, activated and conjugated NK cells. (A) Mean MTOC to lytic granule distance measured in streaming video of YTS GFP-tubulin or NK92 GFP-tubulin cells unconjugated, or conjugated to susceptible target cells. YTS GFP-tubulin cells were also measured in noncytolytic conjugates with K562 target cells. One image per second for 15 s prior to and 2 min after conjugation were analyzed for conjugated cells. In unconjugated cells a similar number of images were acquired and measured. Each point represents mean data of lytic granule to MTOC distances at a single time point from 3-6 cells. (B-E) Additional measurements of lytic granule tracks identified in Figure 4. Total lytic granule velocity (B and C), or total run length (D and E) over the entirety of individual lytic granule tracks identified in YTS GFP-tubulin cells (B and D), and NK92 GFP-tubulin cells (C and E) from streaming video sequences recorded in control (gray) or activating antibody-coated (black) imaging chambers. Each bar represents the number of lytic granule tracks having a particular mean velocity or run length as obtained from 3 cells. The velocities of lytic granules in activated YTS and NK92 cells were significantly different from that in resting YTS and NK92 cells ($p < 0.0001$, $p = 0.0007$, respectively). The difference in lytic granule run lengths between activated and resting YTS cells was significant ($p < 0.0001$) while the difference in lytic granule run lengths between activated and resting NK92 cells was not ($p = 0.2$).

Figure S7: Effect of Nocodazole on lytic granule convergence. Differential interference contrast (left) and fluorescence (right) time-lapse images of a YTS GFP-tubulin cell pre-treated with Nocodazole in conjugation with a susceptible 721.221 cell. Time shown represents the time after acquisition began, which was approximately 1-4 minutes after YTS cells were added to the imaging chamber. Nocodazole was also present in the imaging chamber media throughout the experiment. While GFP-tubulin signal was present, an intensity corresponding to the MTOC was not found throughout the volume of the cell and thus the images represent a single x,y plane intersecting the IS. Convergence of lytic granules was not identified in this or other time-lapse sequences of Nocodazole-treated cells.

Supplemental Video Legends

Video 1: MTOC polarization in a YTS cell conjugated to a susceptible target cell. Time-lapse movie of MTOC polarization in a YTS GFP-tubulin cell (right) conjugated to a susceptible 721.221 cell (middle, outlined; Fig. 1 B) showing differential interference contrast (top) and fluorescent acquisition sequences (bottom). The 721.221 target cell to which the YTS GFP-tubulin cell (right) is conjugated, is outlined with white dashes every few frames in the fluorescent imaging sequence to facilitate orientation recognition. Green fluorescence represents GFP-tubulin and red LysoTracker-loaded acidified lysosomes. The MTOC is defined as the focus of GFP intensity as defined in the text. Images were obtained at a rate of 4 frames per minute and at least one image per minute is shown from $T = 4$ min to $T = 24$ min.

Video 2: MTOC polarization in an NK92 cell conjugated to a susceptible target cell. Time-lapse movie of MTOC polarization in an NK92 GFP-tubulin cell (top) conjugated to a susceptible K562 cell (bottom; Fig. 1 C). Differential interference contrast imaging is shown on top and fluorescence on the bottom. Green fluorescence represents GFP-tubulin and red LysoTracker-loaded acidified lysosomes. The MTOC is defined as the focus of GFP intensity as defined in the text. In selected frames, the target cell is outlined with a dashed line. In some frames the NK92 cell is extending superior to the target and thus the dashes are discontinuous. Images were obtained at a rate of 4 frames per minute and at least one image per two minutes is shown from T = 1 min to T = 24 min.

Video 3: MTOC positioning in a YTS cell conjugated to a non-susceptible target cell. Time-lapse movie of MTOC dynamics in a YTS GFP-tubulin cell (left) conjugated to a non-susceptible K562 cell (right; Fig. 1 D) showing differential interference contrast (top) and fluorescent acquisition sequences (bottom). Green fluorescence represents GFP-tubulin and red LysoTracker-loaded acidified lysosomes. The MTOC is defined as the focus of GFP intensity as defined in the text. Images were obtained at a rate of 4 frames per minute and at least one image per two minutes is shown from T = 0 min to T = 34 min.

Video 4: Lytic granule movement relative to the MTOC in YTS cells exposed to immobilized antibodies. Time-lapse movies of lytic granule dynamics in a YTS GFP-tubulin cell on an anti-CD28- (left), anti-CD11a- (middle), or anti-CD45-coated (right) imaging chambers (Fig. 3). Green fluorescence represents GFP-tubulin and red LysoTracker-loaded acidified lysosomes. The MTOC is defined as the focus of GFP intensity as defined in the text. Images were obtained at a rate of 6 frames per minute and at least one image per 90 s is shown from T = 0 min to T = 7, 8.5 or 15 min.

Video 5: Lytic granule dynamics in resting YTS cells. Streaming video of a resting YTS GFP-tubulin cell in an uncoated imaging chamber (Fig. 4A). Red fluorescence represents LysoTracker-loaded acidified lysosomes. Images were obtained at a streaming rate of 4 frames per second and the video is shown at 5 times real time.

Video 6: Lytic granule dynamics in resting NK92 cells. Streaming video of a resting NK92 GFP-tubulin cell in an uncoated imaging chamber (Fig. 4B). Red fluorescence represents LysoTracker-loaded acidified lysosomes. Images were obtained at a rate of 4 frames per second and the video is shown at 5 times real time.

Video 7: Lytic granule dynamics in activated YTS cells. Streaming video of an activated YTS GFP-tubulin cell in an anti-CD28- and anti-CD11a-coated imaging chamber (Fig. 4C). Red fluorescence represents LysoTracker-loaded acidified lysosomes. Images were obtained at a rate of 4 frames per second and the video is shown at 5 times real time.

Video 8: Lytic granule dynamics in activated NK92 cells. Streaming video of an activated NK92 GFP-tubulin cell in an anti-NKp30- and anti-CD11a-coated imaging chamber (Fig. 4D). Red fluorescence represents LysoTracker-loaded acidified lysosomes. Images were obtained at a rate of 4 frames per second and the video is shown at five times real time.

Video 9: MTOC and lytic granule dynamics in an ex vivo NK cell conjugating with a susceptible target cell. Time-lapse movie of an eNK cell nucleofected with GFP-tubulin (small cell) shown during conjugation with a susceptible K562 target cell (large cell; Fig. 5). Differential

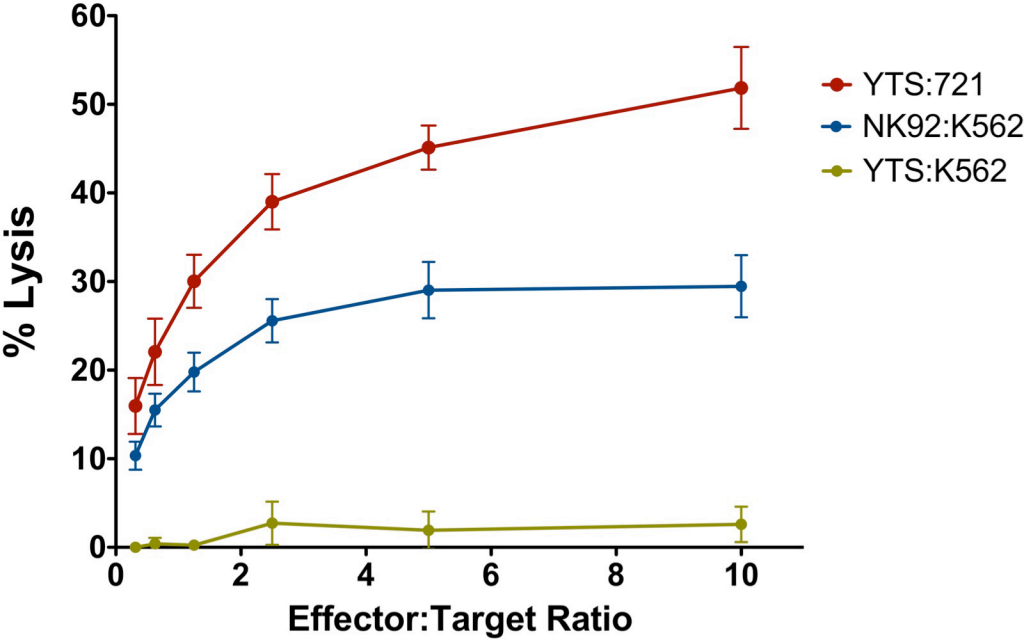
interference contrast imaging is shown on top and fluorescence on the bottom. Green fluorescence represents GFP-tubulin and red LysoTracker-loaded acidified lysosomes. The MTOC is defined as the focus of GFP intensity as defined in the text. Images were obtained at a rate of 4 frames per minute and at least one image per 90 s is shown from T = 0 min to T = 23 min.

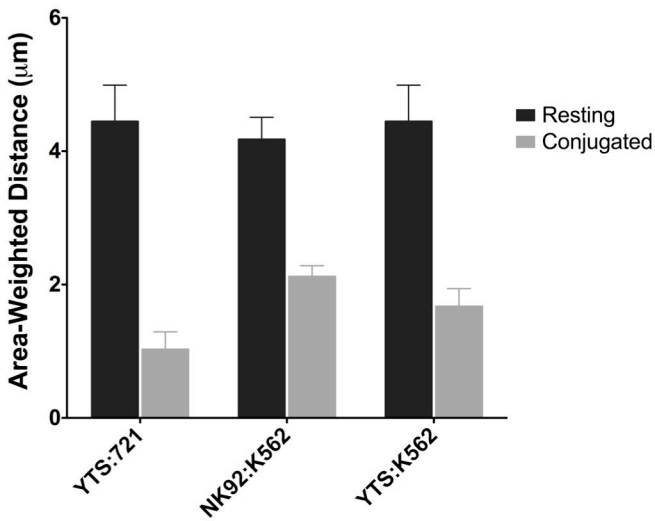
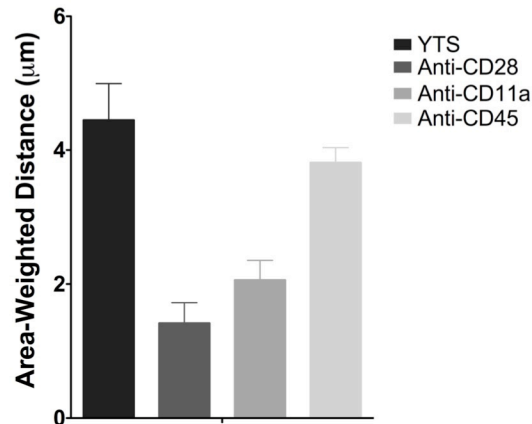
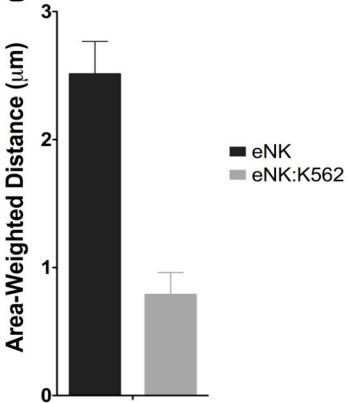
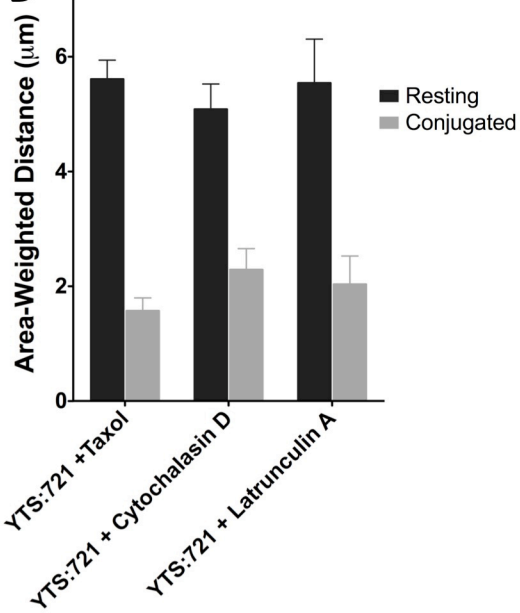
Video 10: Effect of Taxol treatment on MTOC dynamics and lytic granule convergence.

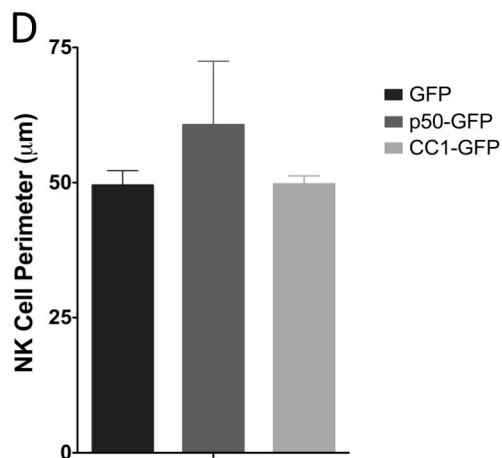
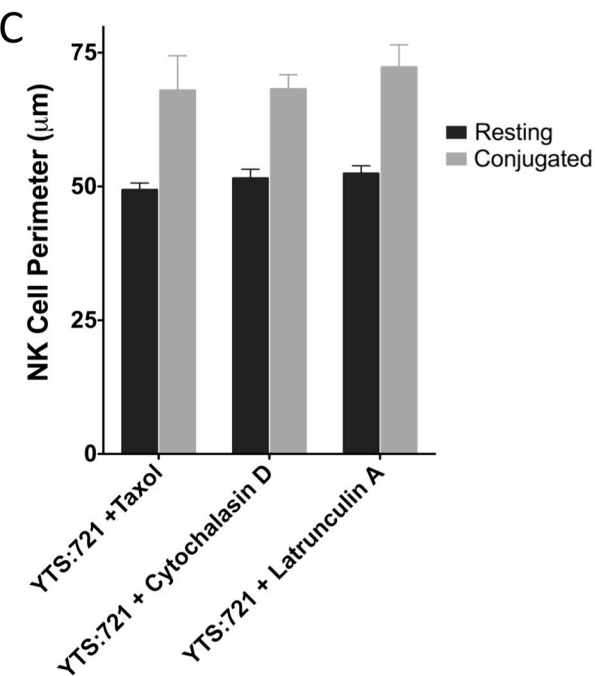
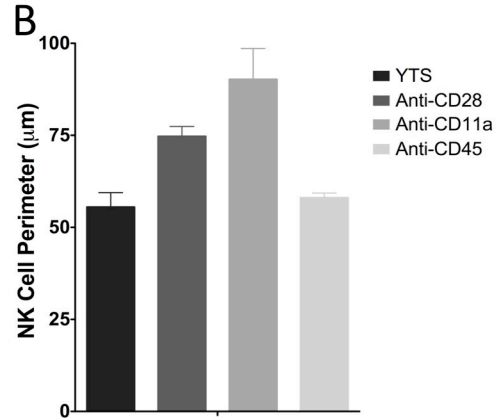
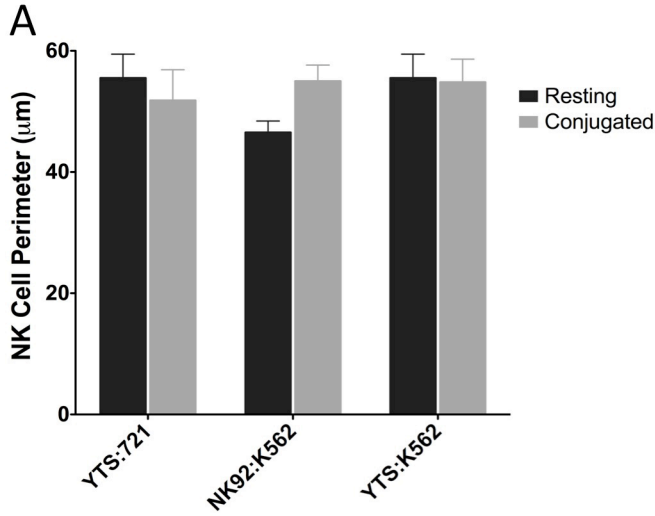
Time-lapse movie of a YTS GFP-tubulin cell pre-treated with Taxol in conjugation with a susceptible 721.221 target cell (Fig. 6, A-C). Differential interference contrast imaging is shown on top and fluorescence on the bottom. Green fluorescence represents GFP-tubulin and red LysoTracker-loaded acidified lysosomes. The MTOC is defined as the focus of GFP intensity as defined in the text. Images were obtained at a rate of 4 frames per minute and at least two images per minute are shown from T = 0 min to T = 31 min. Taxol was present in the imaging chamber media throughout the duration of the experiment.

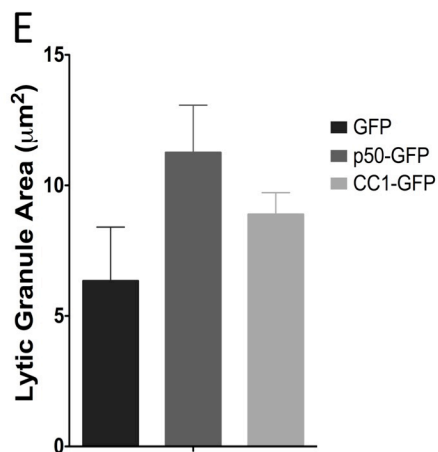
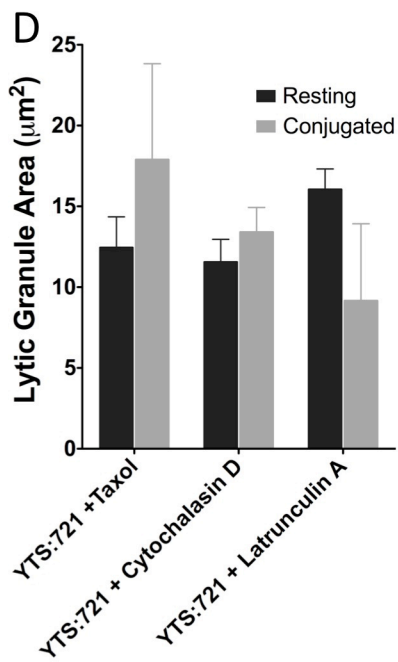
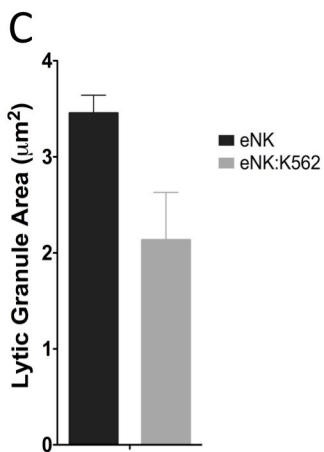
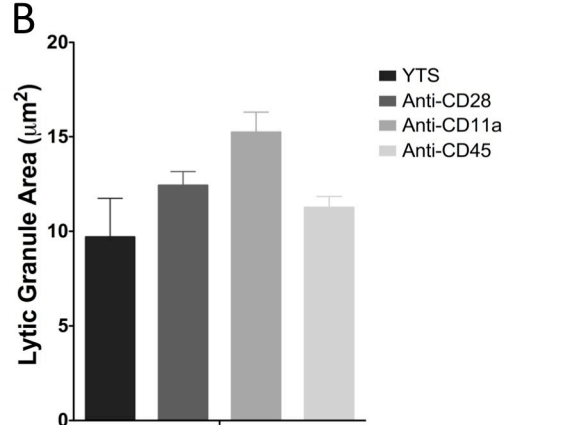
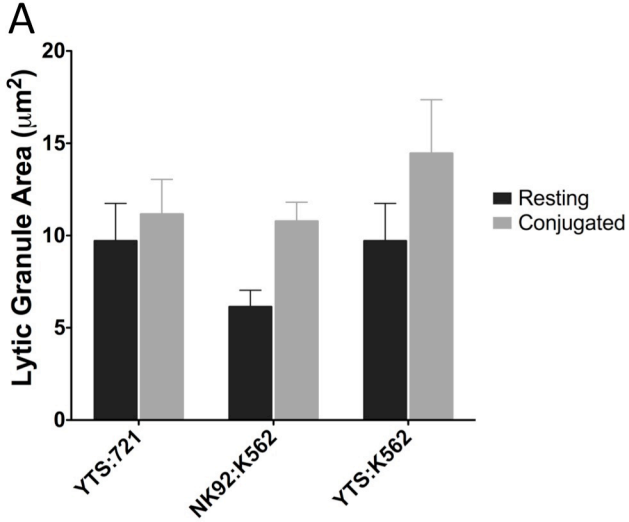
Video 11: Effect of Cytochalasin D treatment on MTOC dynamics and lytic granule convergence. Time-lapse movie of a YTS GFP-tubulin cell pre-treated with Cytochalasin D in conjugation with a susceptible 721.221 target cell (Fig. 6, D-F). Differential interference contrast imaging is shown on top and fluorescence on the bottom. Green fluorescence represents GFP-tubulin and red LysoTracker-loaded acidified lysosomes. The MTOC is defined as the focus of GFP intensity as defined in the text. Images were obtained at a rate of 6 frames per minute and at least one image per two minutes is shown from T = 0 min to T = 12 min. Cytochalasin D was present in the imaging chamber media throughout the duration of the experiment.

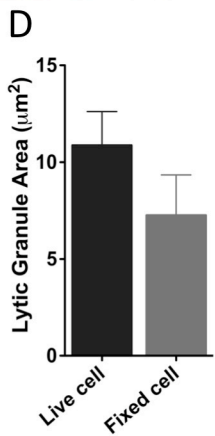
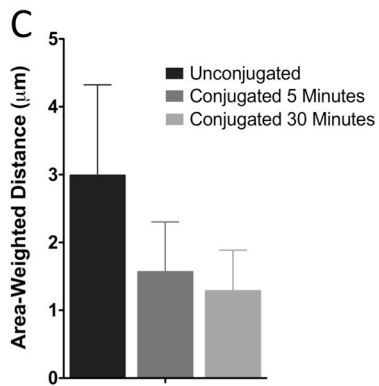
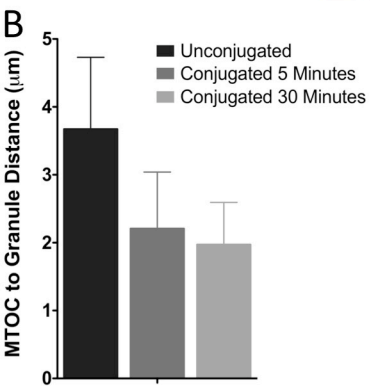
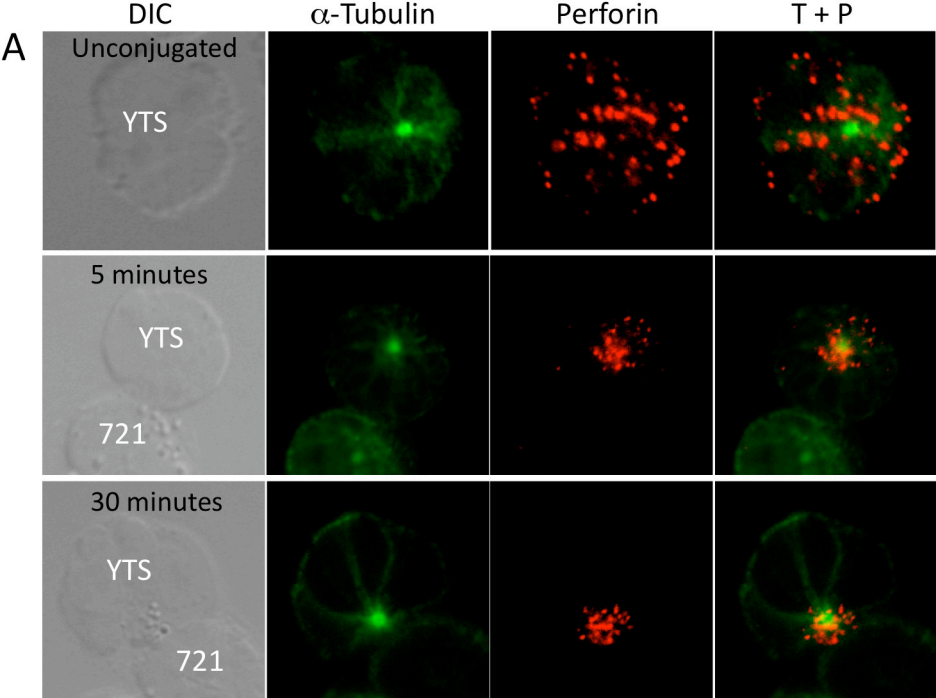
Video 12: Effect of p50 dynamitin, or p150Glued dynactin first coiled-coil domain (CC1) overexpression on lytic granule dynamics relative to the MTOC. Time-lapse movie of a YTS cell nucleofected with p50-GFP (left), or with CC1-GFP (right) in conjugation with a susceptible 721.221 target cell (Fig. 9). Differential interference contrast imaging is shown on top and fluorescence on the bottom. Green fluorescence represents the p50-GFP, or the CC1-GFP fusion protein and red LysoTracker-loaded acidified lysosomes. Images were obtained at a rate of 6 frames per minute and at least one image per minute is shown from T = 0 min to T = 15 min (left) or one image per four minutes from T = 0 min to T = 22 min (right).

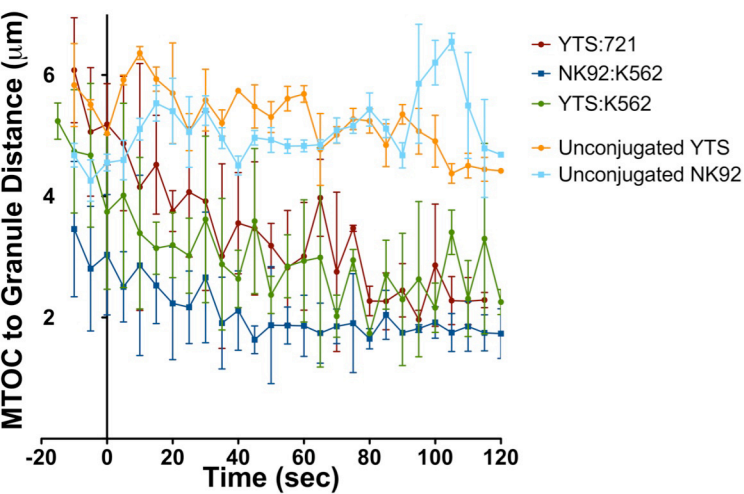
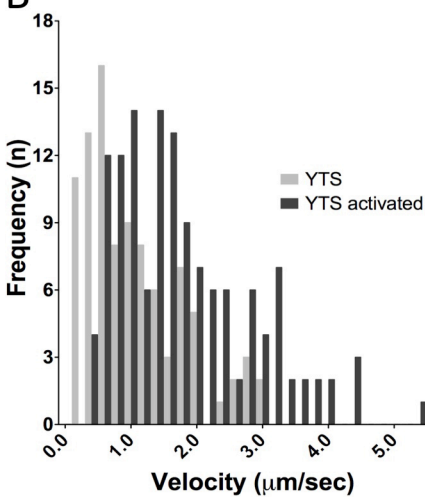
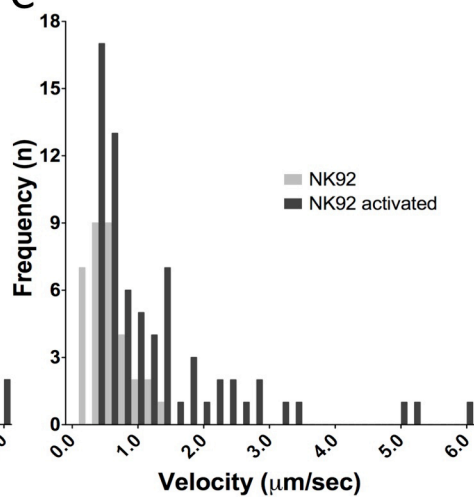
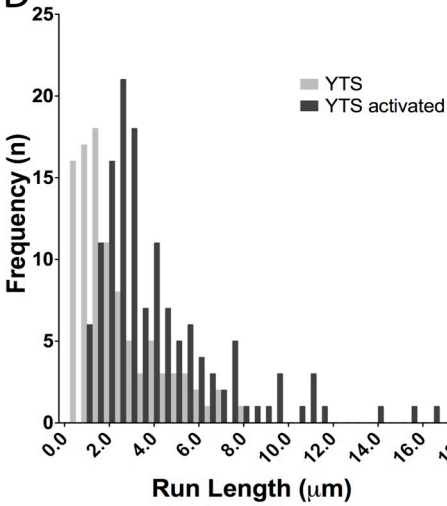
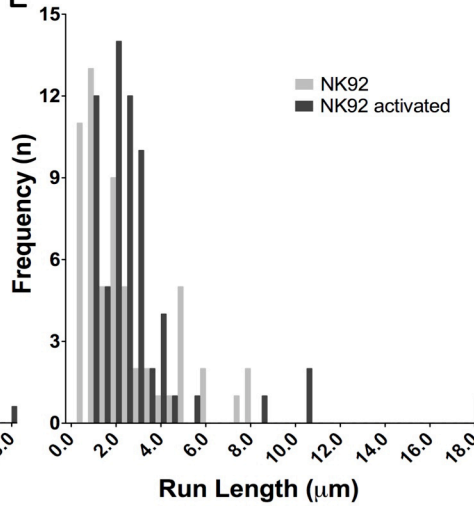


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YTS:721 + Nocodazole

