Hsu et al., https://doi.org/10.1083/jcb.201604136

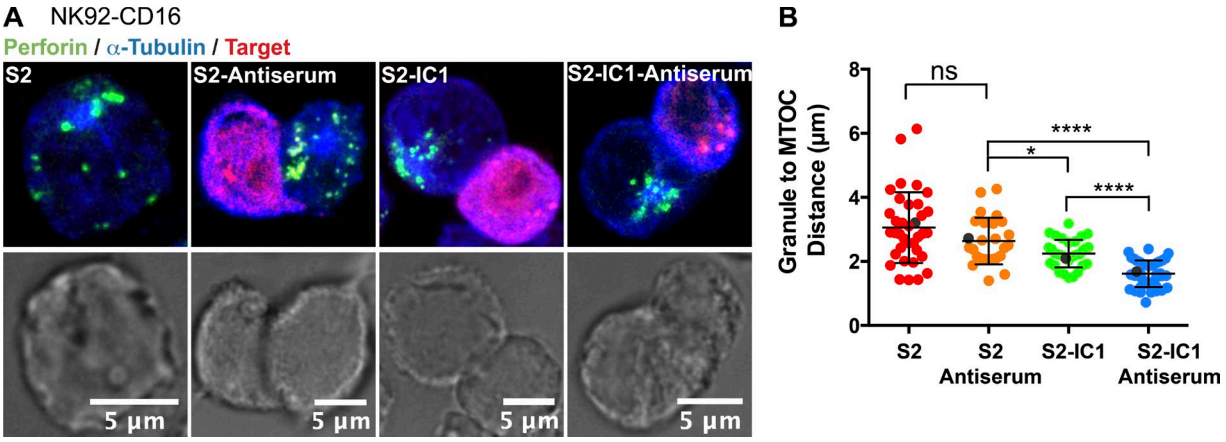


Figure S1. **LFA-1 but not CD16 engagement induces lytic granule convergence in NK92 cells.** Fixed-cell confocal microscopy of NK92-CD16 cells conjugated with differentially labeled S2 cells. Red, CellTracker orange (S2 cells); green, anti-perforin; blue, anti–α-tubulin. Data represent at least 25 cells per group from one experiment (B). Error bars show ± SD. Gray points in each condition indicate the representative cells shown in A. *, $P < 0.05$; ****, $P < 0.0001$; ns, not significant.

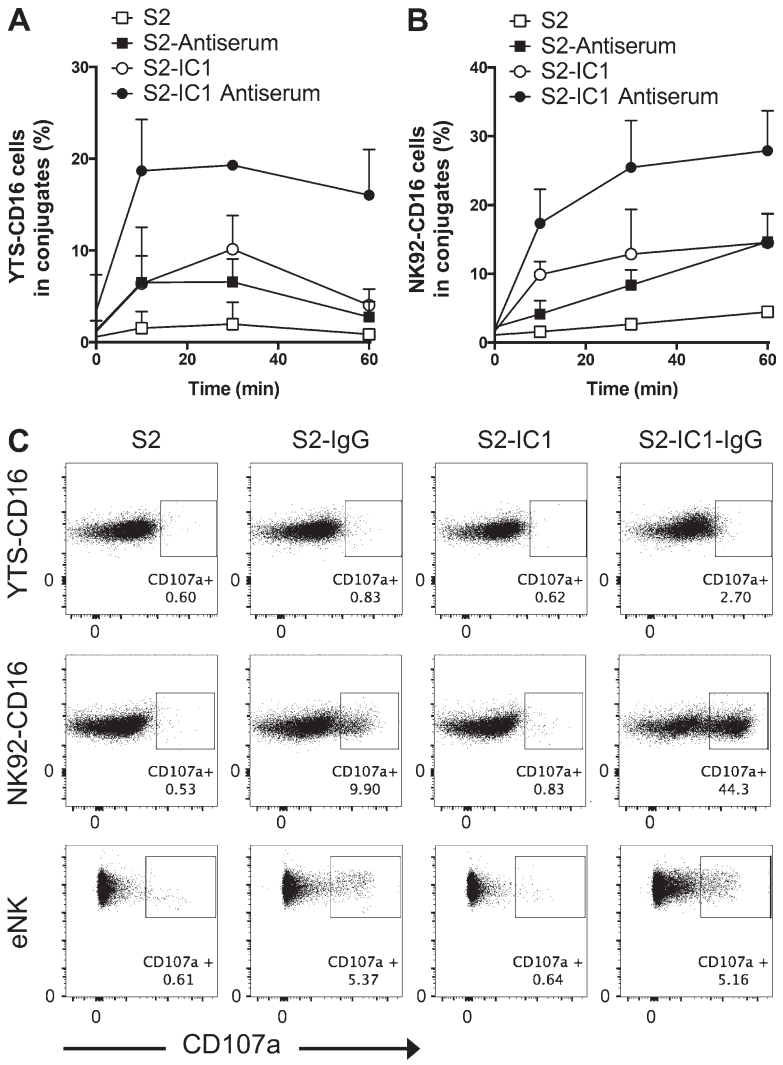


Figure S2. **CD16 engagement induces conjugate formation and degranulation in human NK cells.** For conjugation analysis, YTS-CD16 (A) or NK92-CD16 (B) cells were incubated with S2, S2 antiserum, S2-IC1, or S2-IC1 antiserum cells for 0, 10, 30, or 60 min, vortexed, fixed, and analyzed by flow cytometry to determine the percentage of NK cells in conjugates. Data represent the combined results from three independent experiments. Error bars indicate + SD. For degranulation analysis, YTS-CD16, NK92-CD16, or eNK cells were mixed with S2, S2-IgG, S2-IC-1, or S2-IC1-IgG cells at 37°C for 2 h in the presence of anti-CD107a antibody and GolgiStop (BD) and analyzed using flow cytometry. Numbers in the representative flow cytometry plots indicate the percentage of CD107a-positive NK cells among all NK cells acquired (C).

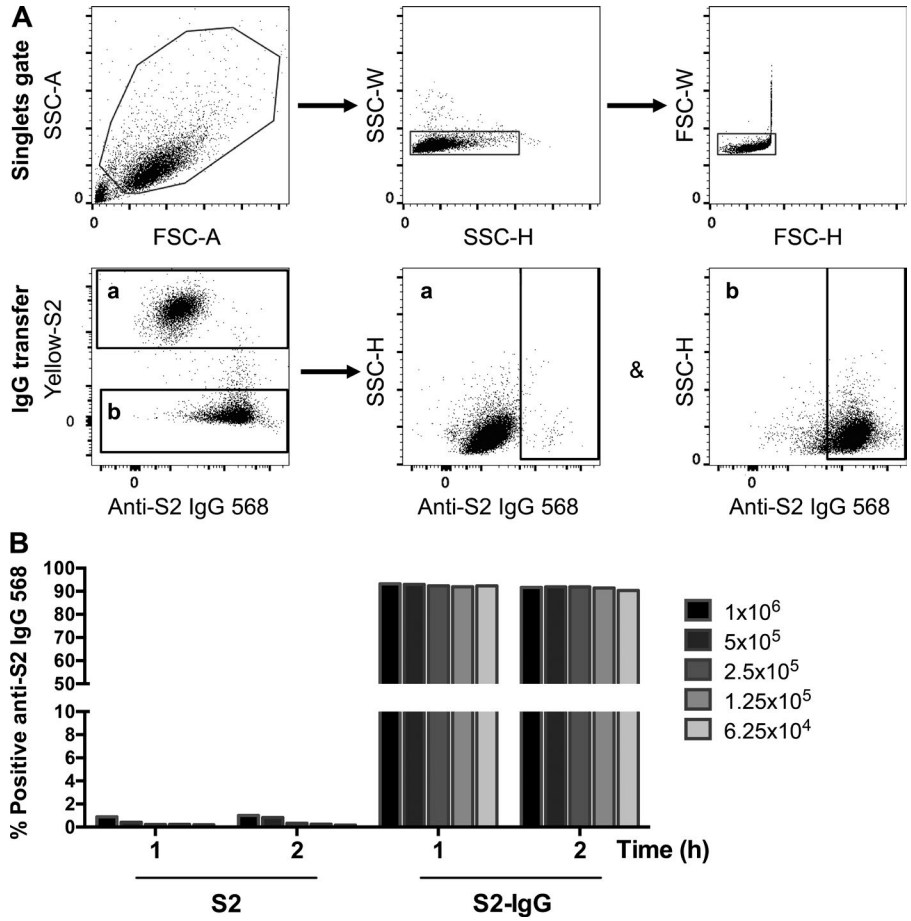


Figure S3. **Transfer rate and labeling efficiency of anti–S2-IgG on *Drosophila* S2 cells.** Plain S2 cells were either stained with yellow vital dye or labeled with anti–S2-IgG directly conjugated with Alexa Fluor 568. The yellow-labeled S2 cells and S2-IgG 568 cells were then mixed at different concentrations at a 1:1 ratio. Cell mixtures were incubated at 37°C and analyzed after 1 and 2 h. (A) Representative flow plots showing the gating strategy for flow cytometry analysis. Cells were first gated for singlets and further analyzed for the transfer rate of anti–S2-IgG 568 from the IgG-labeled S2 to yellow-labeled S2 cells (group a). The labeling efficiency of anti–S2-IgG 568 on the plain S2 cells was also analyzed as a positive control (group b). Representative data from three independent experiments are shown. (B) The percentage of anti–S2-IgG 568–positive yellow-labeled S2 cells is shown on the left, demonstrating the transfer rate of anti–S2-IgG from IgG-labeled to IgG-unlabeled S2 cells during coincubation. The percentage of anti–S2-IgG 568–positive S2-IgG 568 cells is shown on the right, demonstrating the labeling efficiency of anti–S2-IgG antibodies.

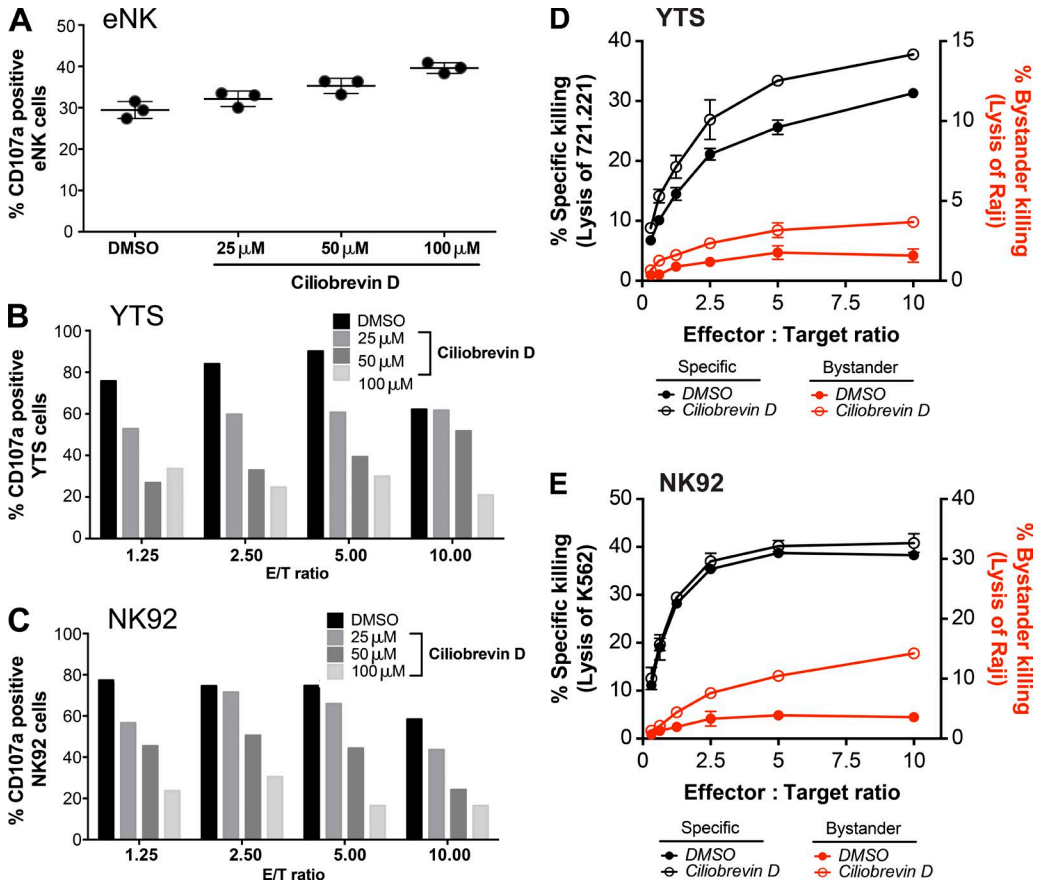


Figure S4. **The effect of ciliobrevin D on degranulation and the increase of bystander killing by NK cells.** Flow cytometry–based degranulation assay of NK cells treated with ciliobrevin D or DMSO as vehicle control. 721.221 cells were used as targets for YTS cells, and K562 cells were used as targets for NK92 and eNK cells. DMSO- or ciliobrevin D–treated NK cells were mixed with their respective target cells, co-cultured at 37°C for 2 h in the presence of anti-CD107a antibody and GolgiStop (BD), and analyzed using flow cytometry. Numbers in the representative flow cytometry plots indicate the percentage of CD107a-positive eNK (A), YTS (B), or NK92 (C) cells. (D and E) Standard 4-h ^{51}Cr cytotoxicity assay of NK cells treated with ciliobrevin D (100 µM) or DMSO control. NK-resistant Raji cells were used as innocent bystander cells. Compared with DMSO, cytotoxic function of YTS against 721.221 cells slightly increased after ciliobrevin D treatment (D, black), whereas lysis of K562 cells by NK92 cells was not affected (E, black). Bystander killing of Raji cells by both YTS (D, red) and NK92 (E, red) cells increased with ciliobrevin D treatment. Representative experiments from three independent experiments for YTS and NK92 cells are shown. Error bars show ± SD.

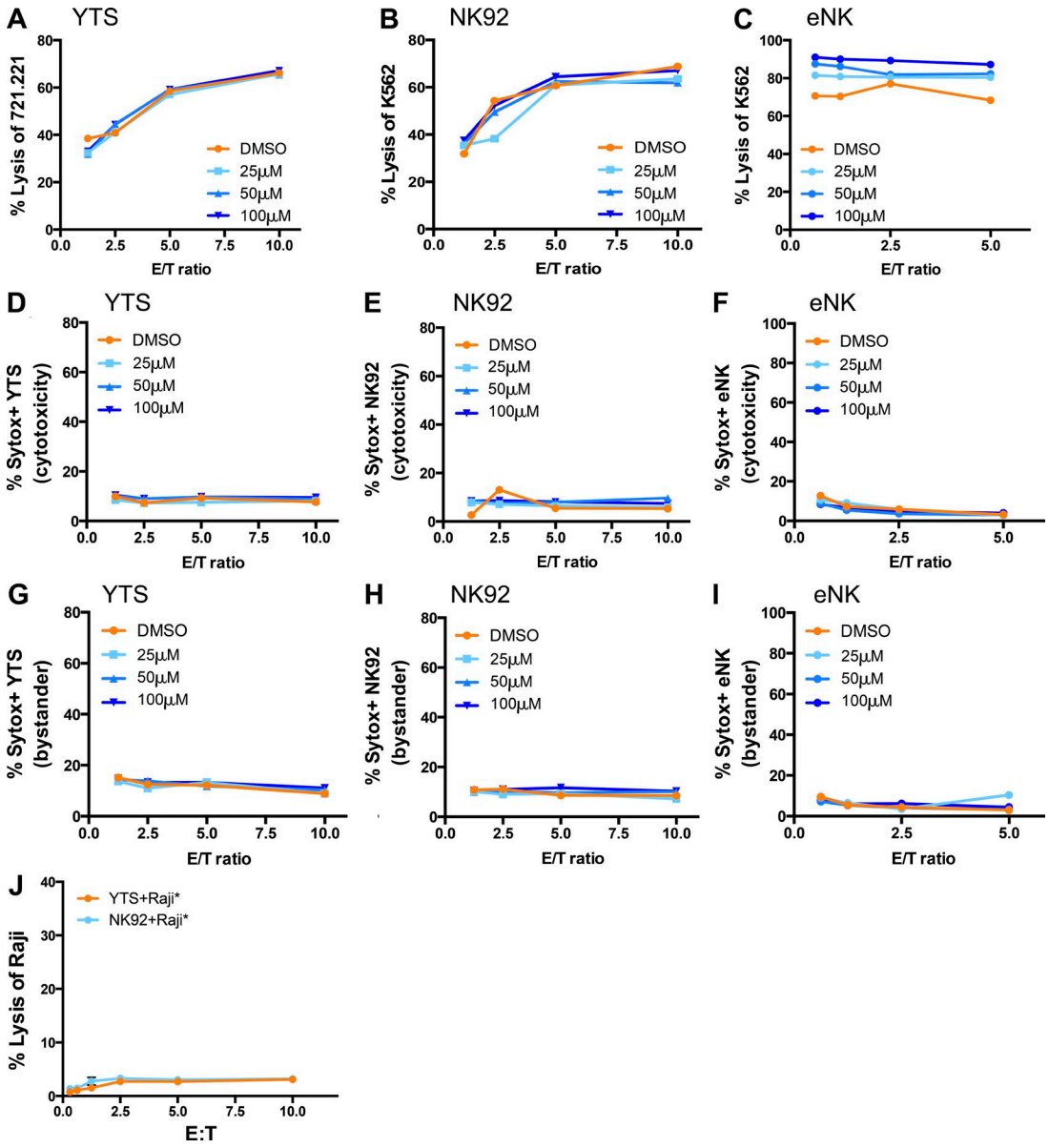
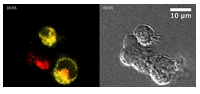
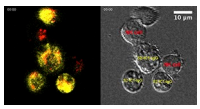


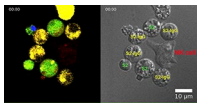
Figure S5. **No effect of ciliobrevin D on the specific killing rate or viability of human NK cells.** Flow cytometry–based cytotoxicity assay of NK cells treated with ciliobrevin D or DMSO vehicle control. With the susceptible targets alone, the cytotoxic functions of YTS (A), NK92 (B), and eNK (C) cells against 721.221 and K562 cells were not affected after ciliobrevin D treatment compared with DMSO control. The viability of YTS (D and G), NK92 (E and H), and eNK (F and I) cells in the standard cytotoxicity assay and the bystander killing assays were not different between ciliobrevin D– and DMSO-treated groups. Representative experiments from three independent experiments for YTS and NK92 cells as well as three healthy donors for eNK cells are shown. YTS or NK92 cells co-cultured with ^{51}Cr-labeled NK cell–resistant Raji B lymphoblastoid cells in the absence of 721.221 or K562 cells showed no spontaneous killing of the Raji cells (J).



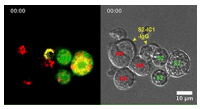
Video 1. **Unconverged lytic granules in one YTS-CD16 cell conjugated with two S2-IgG cells.** Time-lapse image capture of one YTS-CD16 cell (lower left) conjugated with two S2-IgG cells (lower and upper right). S2-IgG target cells were preloaded with and identified using cell dye eFluor 670 and thus are depicted as yellow. YTS-CD16 cells were preloaded with LysoTracker red to denote lytic granules depicted here in red. The viability of cells was monitored using SYTOX blue viability dye and is depicted as blue (nonviable cells). Images were obtained at a rate of 1 frame every 5 min for 2 h.



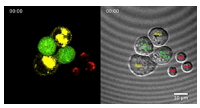
Video 2. **Converged and polarized lytic granules in YTS-CD16 cells conjugated with two S2-IC1-IgG cells.** Time-lapse image capture of two YTS-CD16 cells (upper left and lower right) conjugated with two S2-IC1-IgG cells, respectively. S2-IC1-IgG target cells were preloaded with and identified using cell dye eFluor 670 and thus are depicted as yellow. YTS-CD16 cells were preloaded with LysoTracker red to denote lytic granules depicted in red. The viability of cells was monitored using SYTOX blue viability dye and is depicted as blue (nonviable cells). Images were obtained at a rate of 1 frame every 5 min for 2 h.



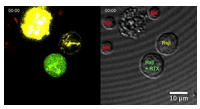
Video 3. **YTS-CD16 cells kill IgG-coated and bystander S2 cells with unconverged lytic granules.** Time-lapse image capture of one YTS-CD16 cell (middle) surrounded by four S2-IgG cells and three S2 cells. S2-IgG target cells were preloaded with and identified using cell dye eFluor 670 and thus are depicted as yellow. S2 bystander cells were preloaded with and identified using cell dye CFSE and thus are depicted as green. YTS-CD16 cells were preloaded with LysoTracker red to denote lytic granules depicted here in red. The viability of cells was monitored using SYTOX blue viability dye and is depicted as blue (nonviable cells). Images were obtained at a rate of 1 frame every 5 min for 2 h.



Video 4. **YTS-CD16 cells kill IgG-coated S2 cells with converged lytic granules.** Time-lapse video of three YTS-CD16 cells conjugated with two S2-IC1-IgG cells adjacent to two S2 cells. S2-IC1-IgG target cells were preloaded with and identified using cell dye eFluor 670 and thus are depicted as yellow. S2 bystander cells were preloaded with and identified using cell dye CFSE and thus are depicted as green. YTS-CD16 cells were preloaded with LysoTracker red to denote lytic granules depicted here in red. The viability of cells was monitored using SYTOX blue viability dye and is depicted as blue (nonviable cells). Images were obtained at a rate of 1 frame every 5 min for 2 h.



Video 5. **Killing of rituximab-coated Raji cells.** Time-lapse video of three eNK cells aggregated with two RTX-coated and two uncoated Raji cells using the UGATm system. RTX-coated Raji cells were preloaded with and identified using CellTracker green dye and thus are depicted as green. Bystander Raji cells were preloaded with and identified using cell proliferation dye eFluor670 and thus are depicted as yellow. eNK cells were preloaded with LysoTracker red to denote lytic granules depicted here in red. The viability of cells was monitored using SYTOX blue viability dye and is depicted as blue (nonviable cells). Images were obtained at a rate of 1 frame every 4 min for 4 h.



Video 6. **Killing of rituximab-coated and bystander Raji cells in the presence of anti–LFA-1 blocking antibody.** Time-lapse video of eNK cells aggregated with RTX-coated and uncoated Raji cells using the UGATm system. RTX-coated Raji cells and bystander Raji cells were preloaded with and identified using CellTracker green dye (depicted in green) and cell proliferation dye eFluor670 (depicted in yellow), respectively. eNK cells were preloaded with LysoTracker red to denote lytic granules depicted in red. eNK cells were pretreated with the murine IgG anti-CD11a mAb (clone TS1/22, 20 µg/ml final concentration) for 15 min at 37°C before being added to the target cells. The viability of cells was monitored using SYTOX blue viability dye and is depicted as blue (nonviable cells). Images were acquired at a rate of 1 frame every 4 min for 4 h.