Supplemental figure legends

Supplemental fig 1: Characterization of the NKL(DAP10-GFP) transfectant. (A) FACS analysis for surface expression of NKG2D and 2B4 in NKL and NKL(DAP10-GFP). FL1/FL2 dot plots are shown. Neg. Secondary reagent alone. (B) Cytotoxic assay of NKL and NKL(DAP10-GFP) against 221 and 221B cells. Antibodies used to block the receptor/ligand interaction are shown. One representative experiment is shown.
(C) DAP10-GFP was recognized by an anti-DAP10 antibody in confocal microscopy experiments. Scale bar 5 μm. (D) Subcellular distribution of DAP10-GFP in living cells**.** NKL(DAP10-GFP) cells were loaded with CTxB Alexa-594 as described in materials and methods and analysed by time-lapse confocal microscopy. Cells were maintained at 37°C in 5% CO2, and confocal images were acquired using the Leica DM IRE2 confocal microscope every minute (numbers indicate minutes). Confocal series of fluorescence and the differential interference contrast (DIC) images were simultaneously obtained by sequential scanning of first the GFP signal, along with the DIC image, and secondly the Alexa-594 signal. The maximal projection of 2 representative confocal sections of the GFP and the alexa-594 channels was obtained in a single image (middle panels).

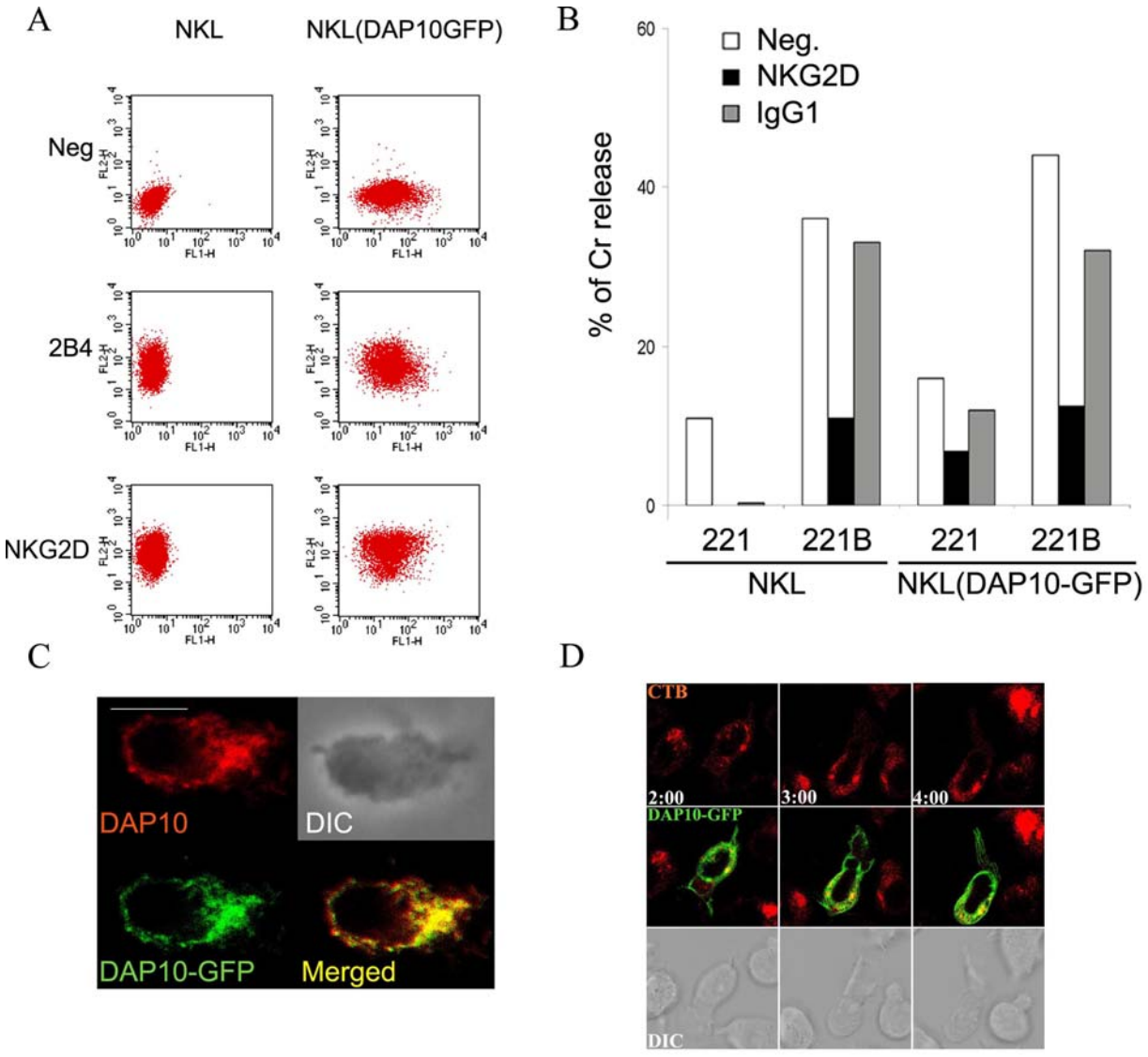
Supplemental fig 2: **DAP10 co-localizes with CD63 at the synapse:** NK/221B conjugates were stained for DAP10 (green) and CD63 (red). DIC images (Squares mark the areas selected for synapse analysis) and merged green and red channels of three confocal sections of the synapse are shown. Co-localization histograms and the Manders coefficients (R) are shown on the right. Yellow arrowheads indicate the synapse interface and white arrows the polarized vesicles. Scale bars 2.5 μm.

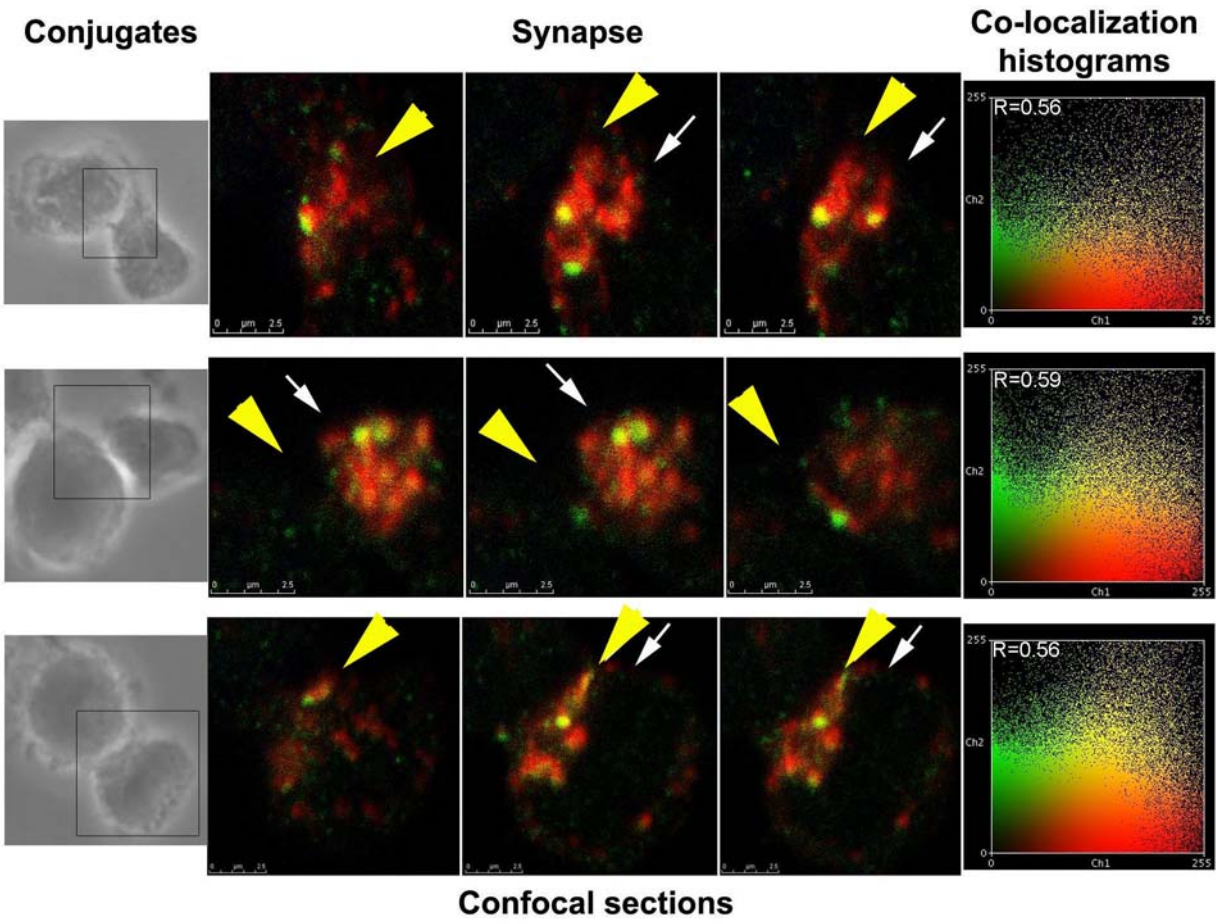
Supplemental fig 3: Ultrastructural analysis of the cNK-IS synapse. NKL (DAP10-GFP)/221B Conjugate samples were processed for immunogold labeling with an anti-

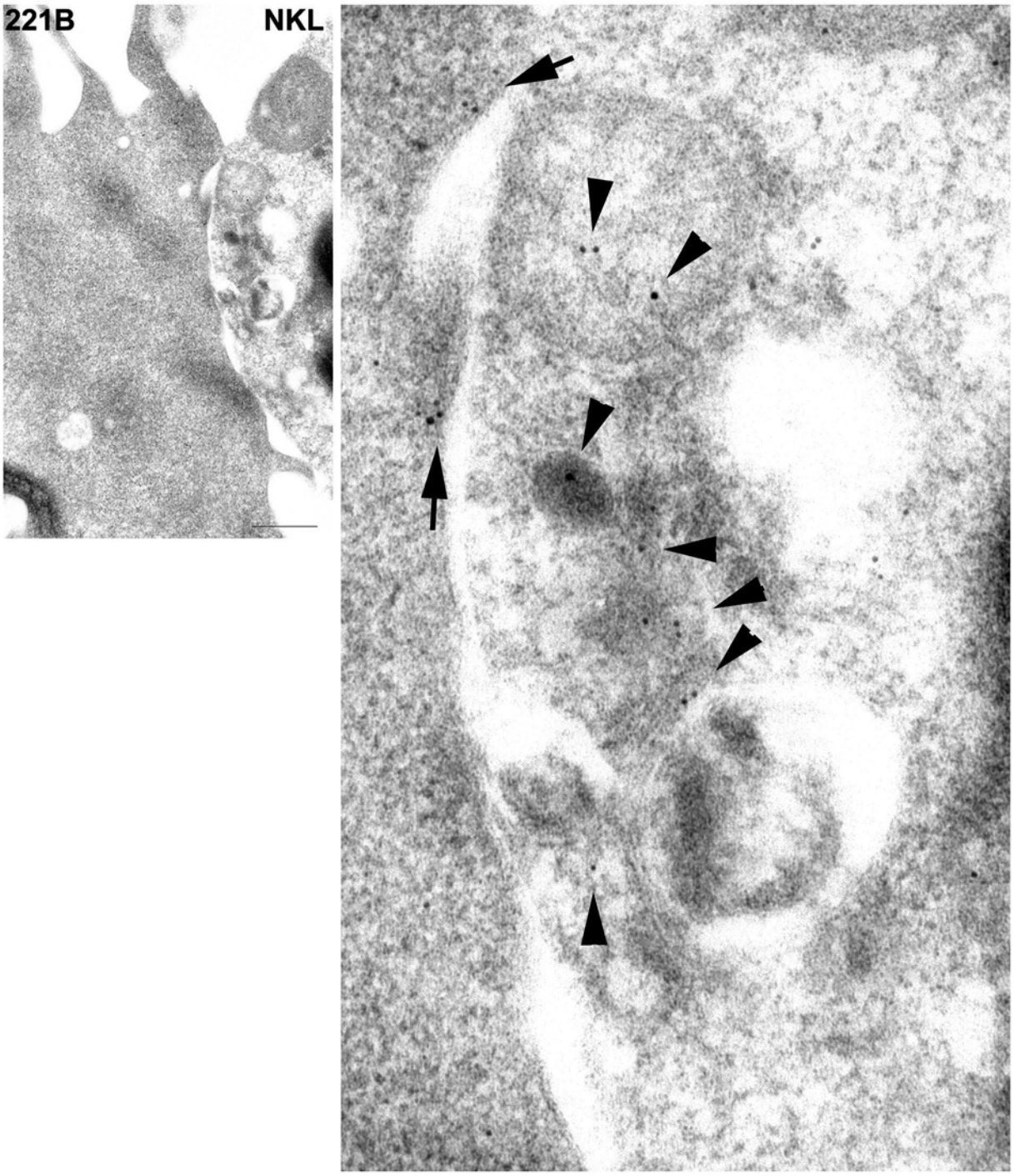
GFP antibody and analysed by electron microscopy. A magnified image of the synapse is shown (right panel). Arrows and arrowheads point gold particles at the surface interface and in polarized SL respectively. Scale bar 500nm.

Supplemental video legends

Video 1: **DAP10-GFP localizes in dynamic vesicles in the NKL cell line.** Time lapse confocal microscopy was performed to study the distribution of DAP10-GFP in live cells. NKL(DAP10-GFP) transfectants were plated on PLL-coated coverslips, maintained at 37°C in 5% CO_2 and one equatorial confocal section was acquired each 10 seconds using a Leica DM IRE2 microscope. The protein is expressed at the cell surface however dynamic intracellular vesicles moving in and out of the confocal section were noted demonstrating the rapid movement of these vesicles inside the cell.


A
NKL
NKL(DAP10GFP)
Neg
2B4
NKG2D
FL1-H
FL2-H
B
Neg.
NKG2D
IgG1
% of Cr release
60
40
20
0
221
221B
221
221B
NKL
NKL(DAP10-GFP)
C
DAP10
DIC
DAP10-GFP
Merged
D
CTB
2:00
3:00
4:00
DAP10-GFP
DIC


Conjugates
Synapse
Co-localization histograms
R=0.56
R=0.59
R=0.56
Ch2
Ch1
0
255
Confocal sections


221B
NKL