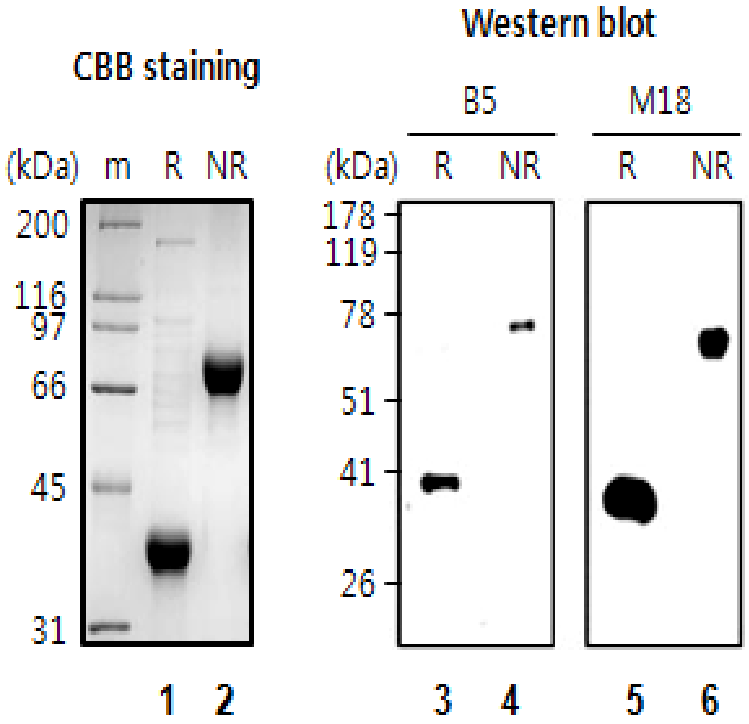
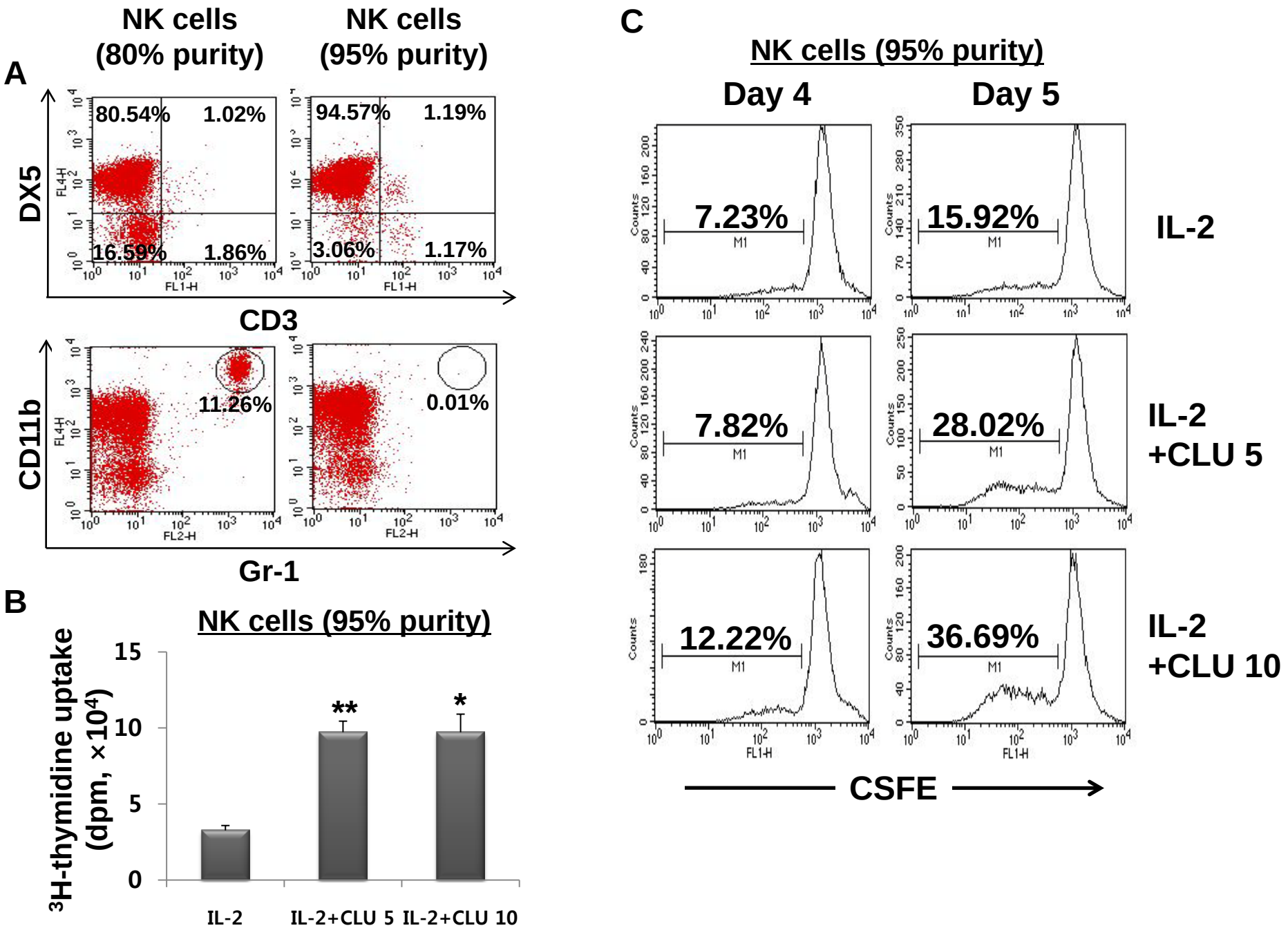
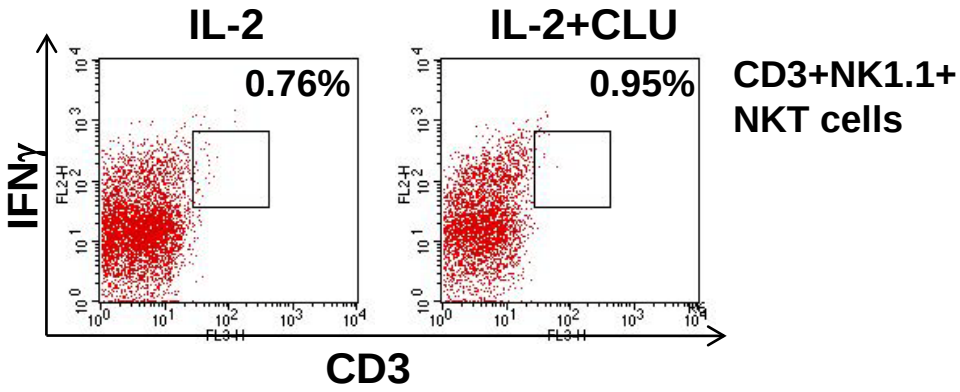
Supplementary Fig 1



Supplementary Fig 2

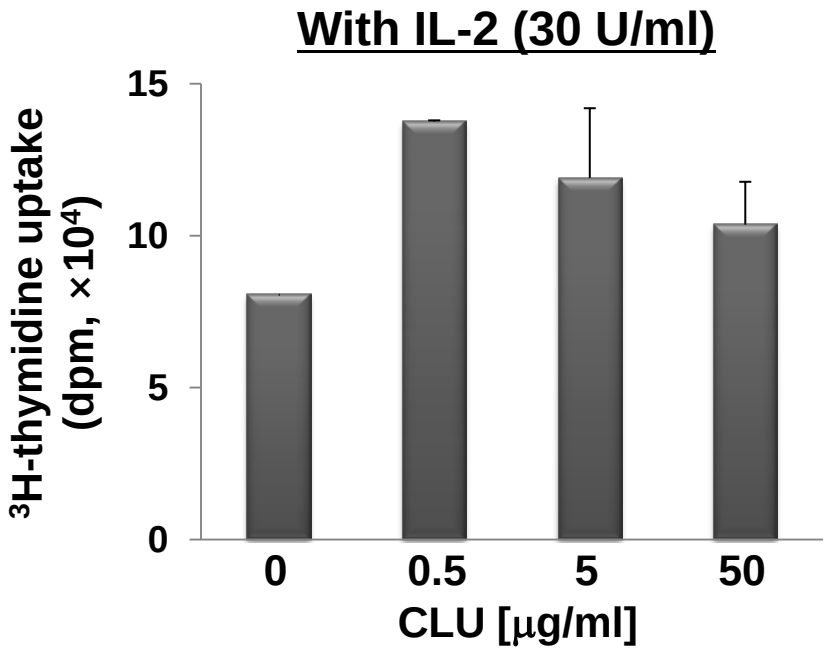


Supplementary Fig 3

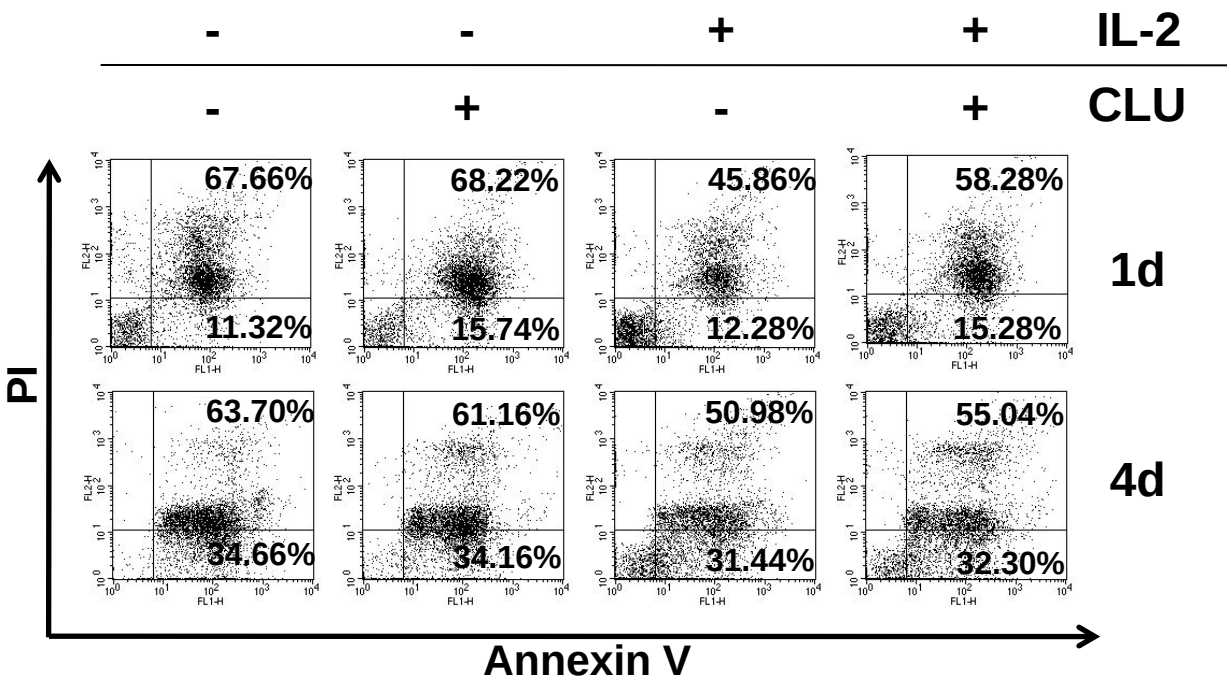


CD3+NK1.1+
NKT cells

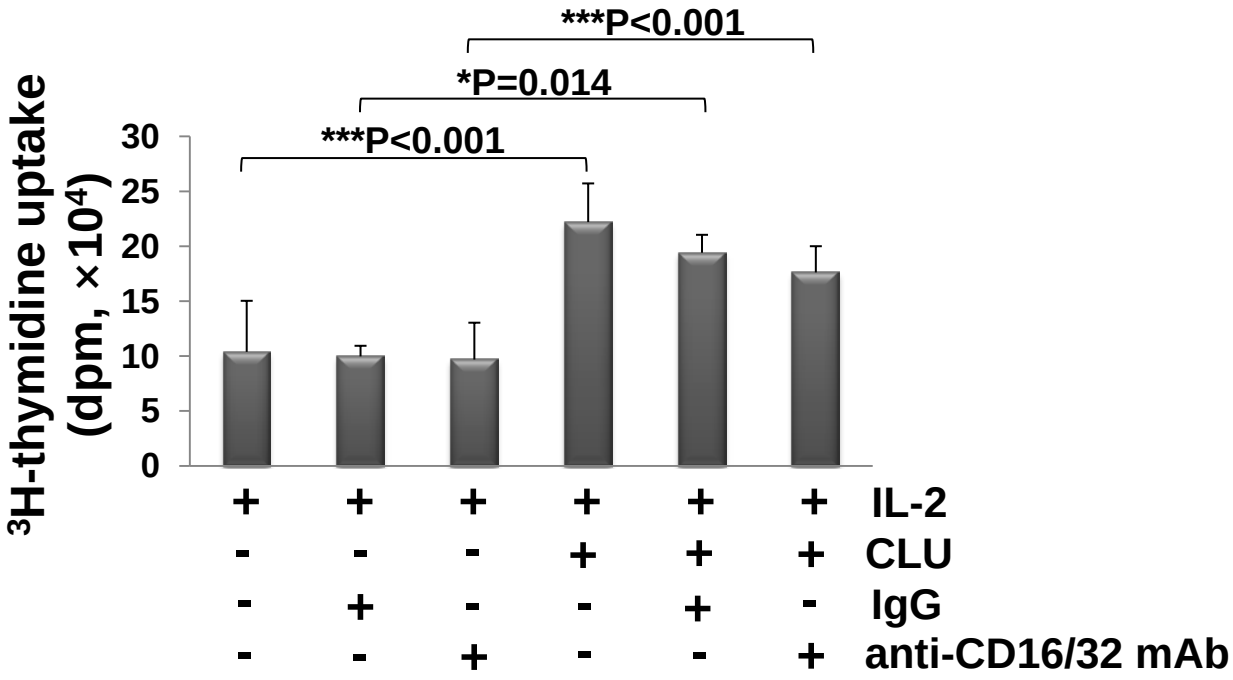
Supplementary Fig 4



Supplementary Fig 5



Supplementary Fig 6



SUPPLEMENTS

Supplementary Fig. 1. Purity and identity of CLU purified from human plasma using SDS-PAGE followed by by Coomassie brilliant blue (CBB) staining and Western blots. 5 μg of purified CLU was separated under the reducing (lane 1) or non-reducing condition (lane 2) and CBB staining was performed to confirm its size and dimeric nature. The identity of the purified CLU was further verified by Western blots using mouse monoclonal anti-CLU (B5, lane 3-4) or goat polyclonal anti-CLU (M-18, lane 5-6) antibodies.

Supplementary Fig. 2. The role of CLU on the proliferation of highly enriched NK cells **A,** Comparison of NK cells isolated using MACS via negative selection (left, <80% purity) and mouse NK isolation kit (right, >95% purity, Miltenyi Biotec) for the presence of Gr-1^{+}CD11b+ granulocytes by FACS. **B,** Purified NK cells (>95%) were plated at 1×10^{5} cells/well and incubated for 3 days with 100 U/ml IL-2 in the presence or absence of 5 or 10 μg/ml of CLU. One μCi of ^{3}H-thymidine was added for additional 12 hours to count radioactivity incorporated into the DNA. **C**, 1×10^{5} purified NK cells (>95%) were labeled with 5 μM of CFSE for 10 min and cell division was analyzed by FACS, as described under Materials and Methods. Numbers in each graph indicates the percentage of cells undergoing cell division.

Supplementary Fig. 3. **CD3+NK1.1+ NKT cells did not contribute significantly to the production of IFN-γ.** The percentage of NKT cells and the population producing IFN-γ was assessed via intracellular staining in both IL-2 alone and IL-2+CLU cultures gated on CD3+NK1.1+ NKT cells.

Supplementary Fig. 4. The role of CLU on the proliferation of enriched human NK cells. Human primary NK cells were isolated from PBMCs of healthy volunteers using a RosetteSep Human NK Enrichment Cocktail (Stem Cell Technologies, Vancouver, Canada) and Ficoll-Plaque (GE Healthcare, Seoul, Korea) following manufacturers' instructions. The purity of $CD3^{-}CD56^{+}$ NK cells was >98%, as determined by flow cytometry (FACScalibur, BD Biosciences, San Jose, CA). **A,** Freshly purified NK cells were cultured for 3 days in RPMI1640 medium (Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS; Lonza, Walkersville, MD), 100u/ml penicillin (Lonza, Walkersville, MD), 100 U/ml streptomycin (Lonza, Walkersville, MD), and 30 U/ml of hrIL-2 in the presence of indicated concentration of CLU at 37°C in 5% CO_2. **B,** Purified NK cells were cultured in the presence of 0, 0.5, 5, or 50 μg/ml of CLU for 3 days. One μCi of ^{3}H-thymidine was added for additional 12 hours to count radioactivity incorporated into the DNA.

Supplementary Fig. 5. **Effect of CLU on the early and late apoptosis of NK cells.** Thirty thousand purified NK cells were cultured with 5 μg/ml of CLU and/or 100 U/ml of IL-2 for 1 or 4 days and subjected to Annexin V/PI staining. Cultured NK cells were harvested and incubated with 5 μl of FITC-Annexin V for 15 min and washed once with FACS buffer. After 5 μl of Propidium iodide (PI) was added, cells were analyzed by flow cytometry. Annexin V+/PI- population represent early apoptotic cells whereas Annexin V+/PI+ cells represent late apoptotic cells. The values represent the percentages of Annexin V+ or Annexin V+/PI+ cells, which are representatives of three independent experiments.

Supplementary Fig. 6. CLU stimulates NK cell proliferation independently from IgG and FcγRIII/II signaling. Purified NK cells were cultured with 100 U/ml of IL-2 in the

presence or absence of 5 μg/ml CLU, 5 μg/ml rat IgG, or anti-CD16/CD32 mAb for 3 days. One μCi of ^{3}H-thymidine was added for additional 12 hours and radioactivity was compared. The data shown are representatives of three independent experiments. Error bar represents SD (*$p<0.05$, ***$p<0.001$).