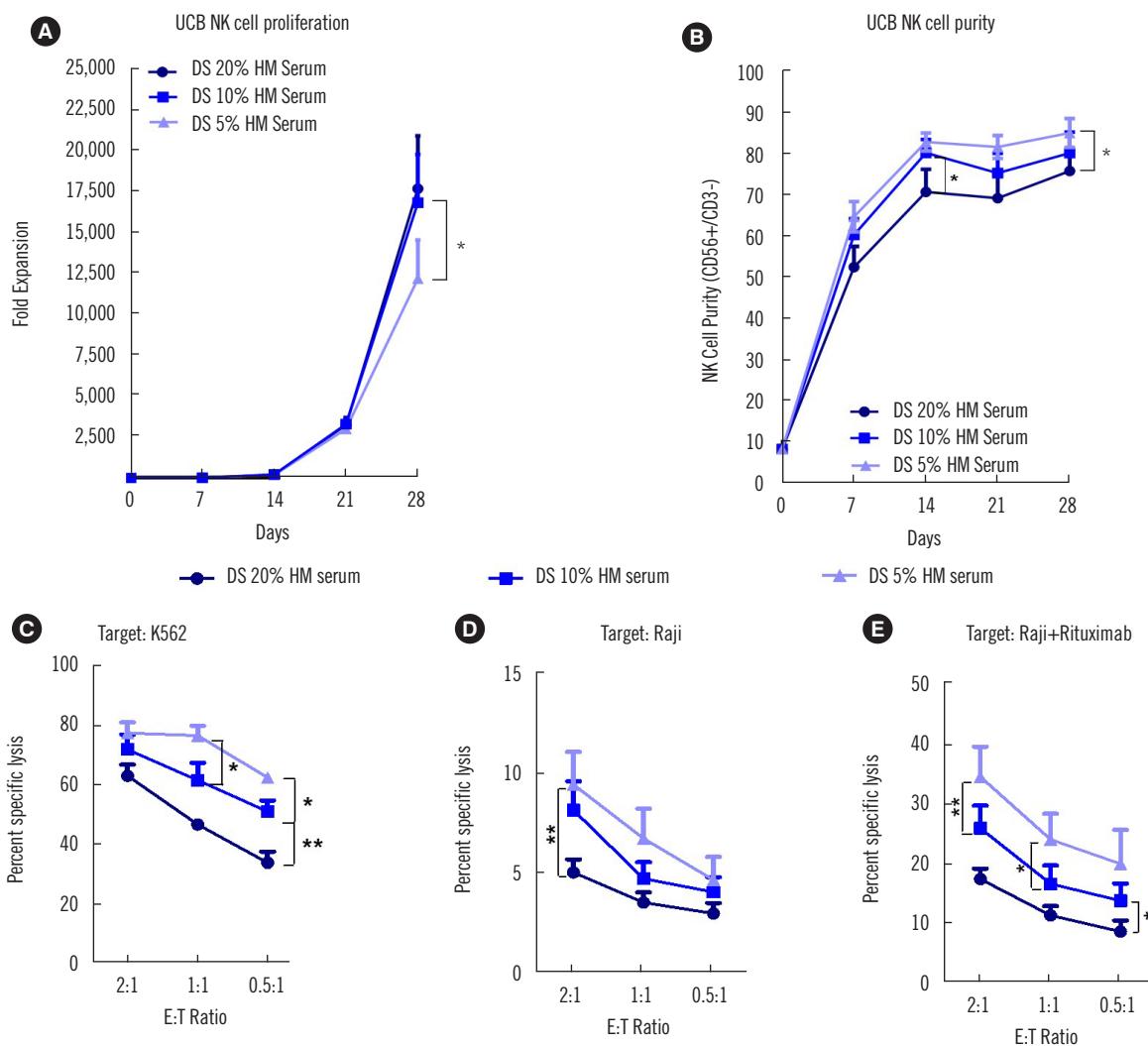


**Supplemental Data Fig. S1.** Enhanced proliferation and relatively low functional potency of NK cells cultured in DS medium are reproducible in different serum batches. (A–C) Fold expansion of AI (red) and DS (blue) NK cells cultured using three different human serum batches on day 28. (D–I) Flow cytometry-based cytotoxicity assay of UC-NK cells cultured in AI (red) and DS (blue) against K562 cells and Raji cells + rituximab using three different human serum batches between days 14 and 28.



**Supplemental Data Fig. S2.** The concentration of human serum significantly affects NK cell expansion and function. (A, B) Fold expansion ( $N=5$ ) and purity ( $N=5$ ) of NK cells cultured in DS medium supplemented with 20% (dark blue), 10% (blue), or 5% (light blue) human serum. (C–E) Day 14 flow cytometry-based cytotoxicity assay of UCB NK cells cultured in DS medium supplemented with 20% (dark blue), 10% (blue), or 5% (light blue) human serum; (C) K562 cells ( $N=5$ ), (D) Raji cells ( $N=5$ ), (E) Raji cells + rituximab ( $N=5$ ).

**Supplemental Data Videos 1.** Representative time-lapse videos of NK cell killing events after target cell contact. K562 cells are in green, and NK cells are unlabeled. (S1A) Representative killing event of NK cells cultured in DS medium. (S1B) Representative killing event of NK cells cultured in AI medium.