

Targeting CD94 for the Depletion of Large Granular Lymphocyte Leukemia Cells via Enhanced Antibody-Dependent Cellular Cytotoxicity

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INTRODUCTION

Large granular lymphocyte leukemia (LGLL)

- Rare type of leukemia that causes neutropenia, anemia, or both, resulting in recurring infections and transfusion dependence¹
- Standard-of-care treatments include immunosuppressive therapies such as methotrexate, cyclosporine, or cyclophosphamide, but modest response rates and treatment durations highlight the critical need for more novel and effective therapies²

CD94

- Type II transmembrane receptor exclusively expressed on natural killer (NK), terminal effector (TE) CD8⁺ T and $\gamma\delta$ T cells, and upregulated on leukemic cell populations in LGLL patients³⁻⁵

DR-01

- Non-fucosylated anti-CD94 antibody hypothesized to cause LGLL cell depletion via enhanced antibody-dependent cellular cytotoxicity (ADCC)

Objective

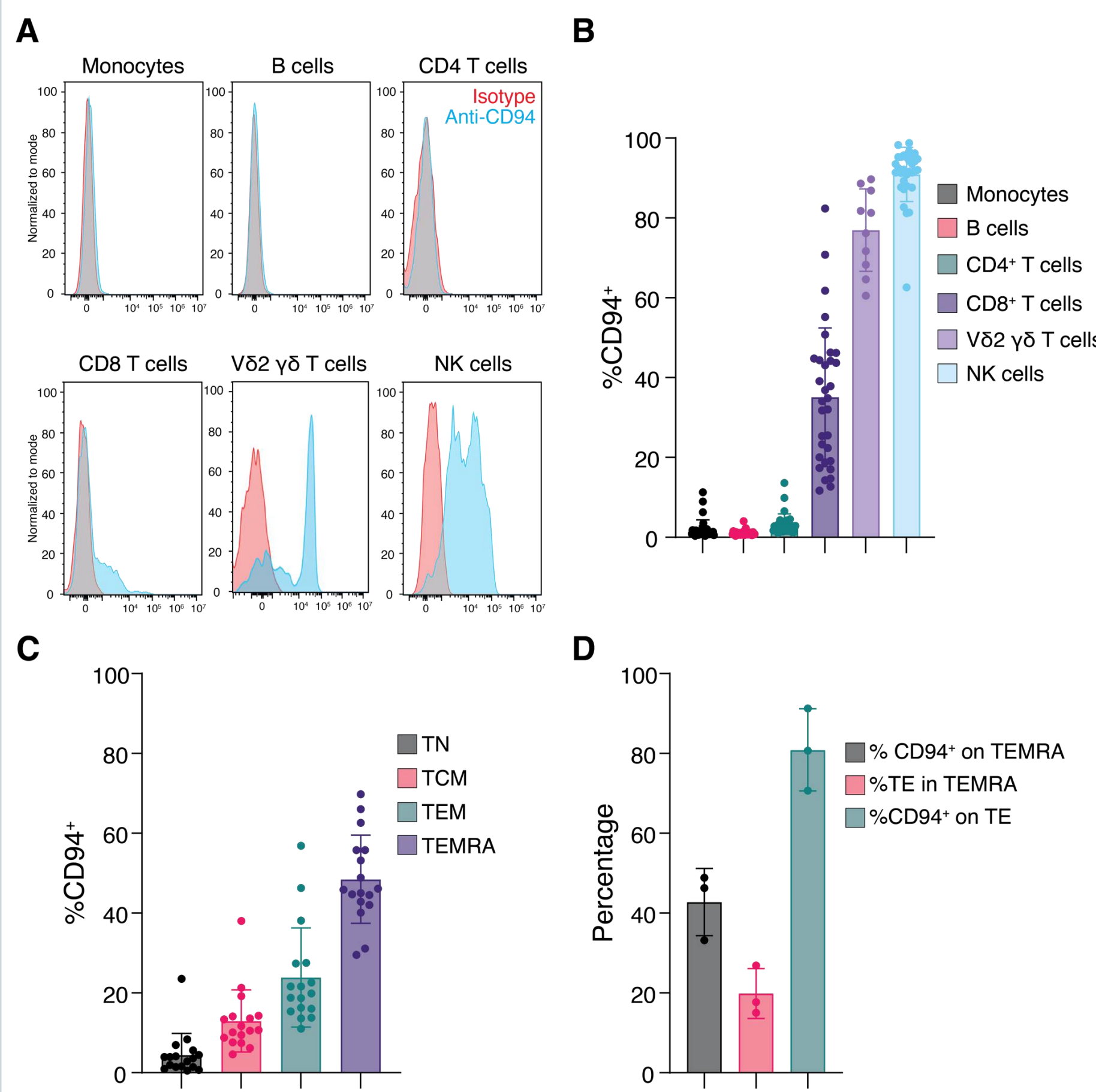
- Characterize the CD94 expression profile of LGLL
- Determine the function and therapeutic potential of DR-01 ex vivo and in vivo

METHODS and RESULTS

CD94 expression is restricted to NK, V δ 2 $\gamma\delta$ T, and terminally differentiated CD8⁺ T cells

- CD94 is expressed predominantly on CD8⁺ T, V δ 2 $\gamma\delta$ T, and NK cells (Fig. 1A)
- CD94 is expressed on ~40% of CD8⁺ T cells, ~80% of V δ 2 $\gamma\delta$ T cells, and ~90% of NK cells (Fig. 1B)
- Within CD8⁺ T cells, CD94 is mainly expressed on ~50% of TEMRA cells and 80% of TE cells (Fig. 1C, D)

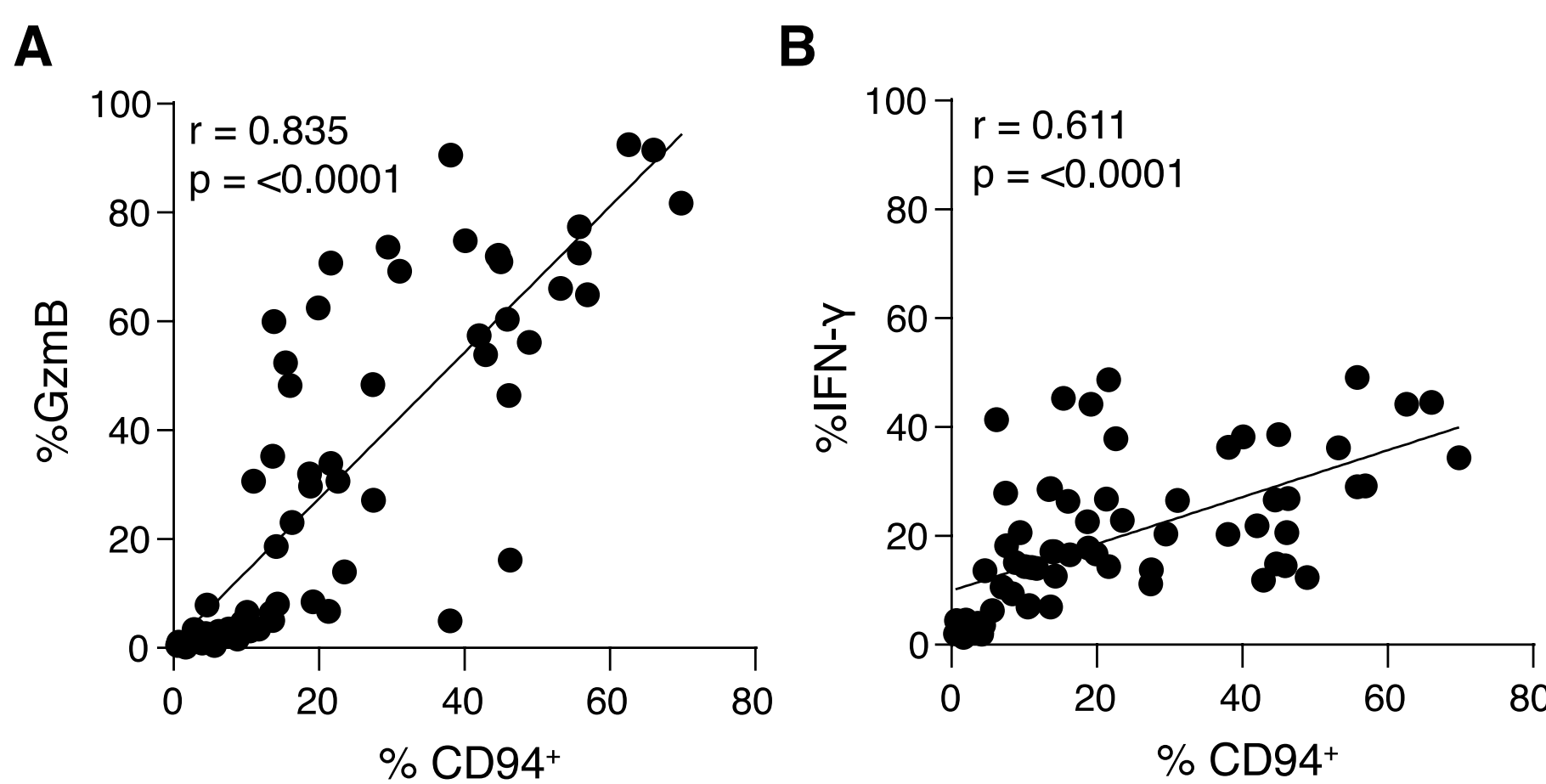
Figure 1. CD94 expression profiling in healthy donor PBMCs by flow cytometry



CD94 correlates with cytotoxic markers in healthy donor CD8⁺ T cells

- Correlation coefficients between CD94 and granzyme B (GzmB), and CD94 and interferon (IFN)- γ , were 0.835 (Fig. 2A) and 0.611 (Fig. 2B), respectively

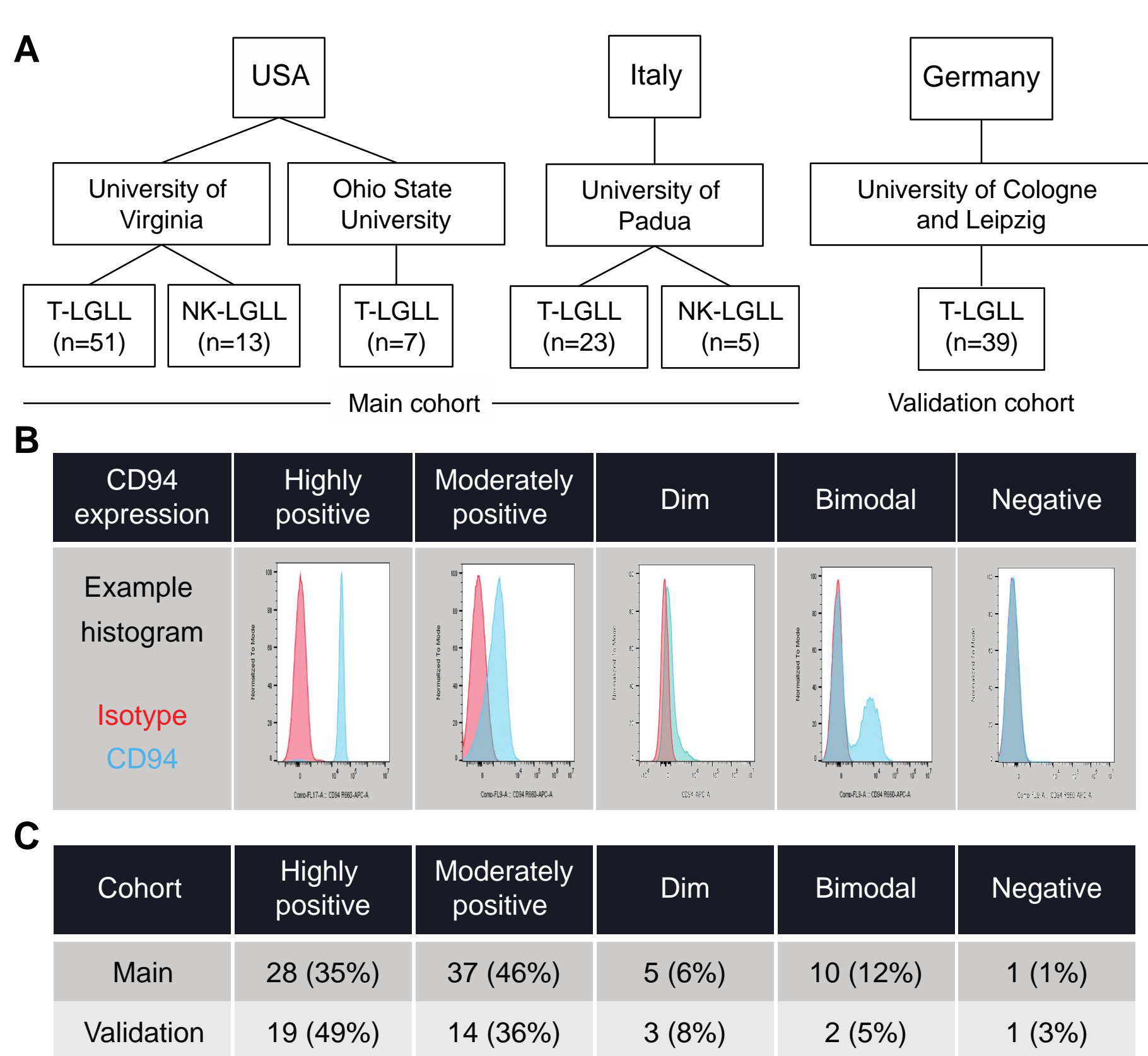
Figure 2. Correlation between CD94, GzmB, and IFN- γ



CD94 expression is variable across T-LGLL patient samples

- Combined data across two patient cohorts (main and validation) showed that CD94 is expressed in 98% (118/120) of T-LGLL samples (Fig. 3)

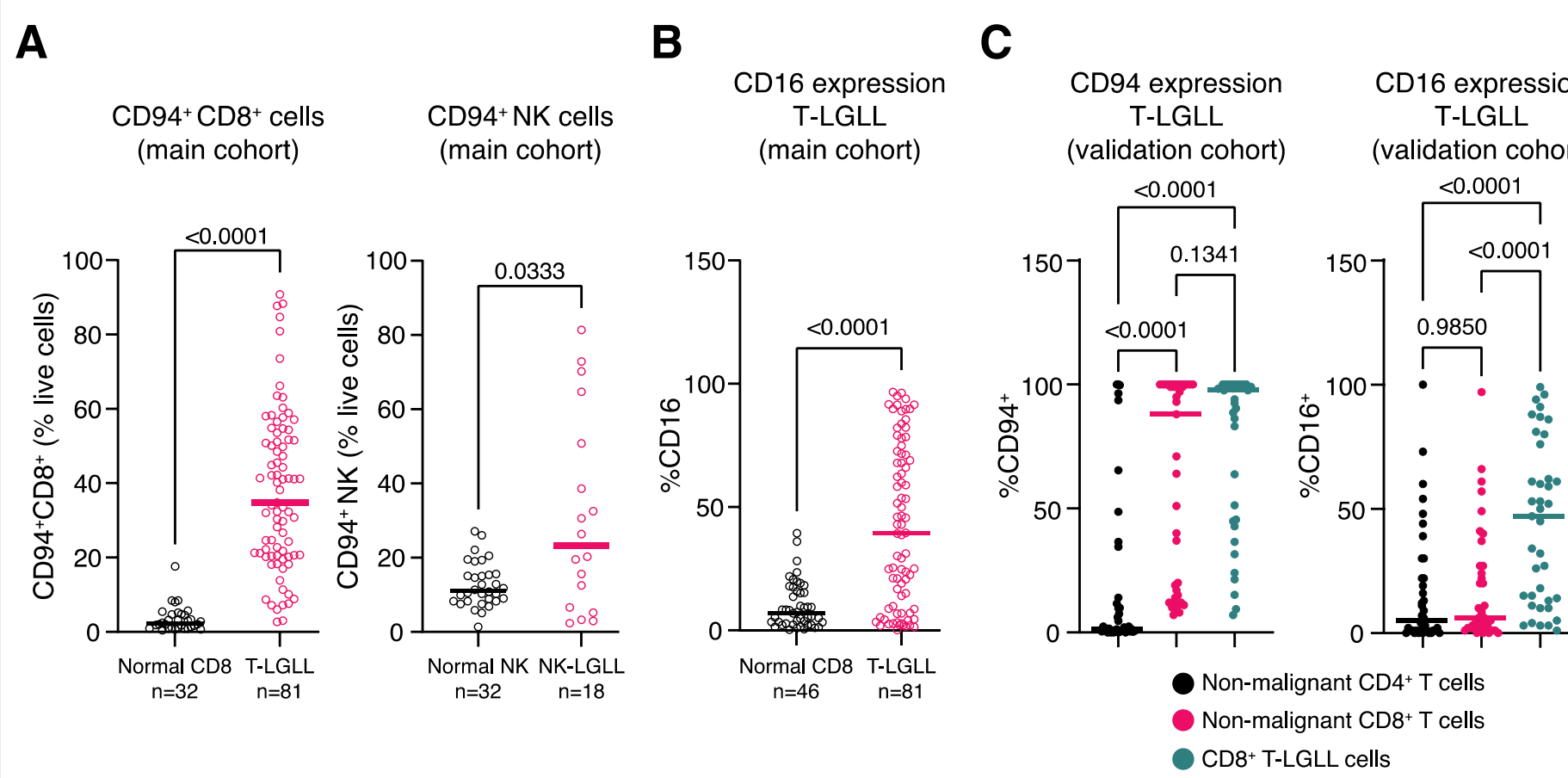
Figure 3. LGLL sample procurement and CD94 expression profile



CD94 and CD16 are upregulated in LGLL

- In the main cohort, CD94⁺CD8⁺ T cells and CD94⁺ NK cells were significantly elevated in LGLL compared to healthy donor CD8⁺ T and NK cells, respectively (Fig. 4A)
- In the main cohort, percentage of CD8⁺ T cells expressing CD16 was significantly higher in T-LGLL compared to healthy donor CD8⁺ T cells (Fig. 4B)
- Validation cohort confirmed the findings above in T-LGLL (Fig. 4C)

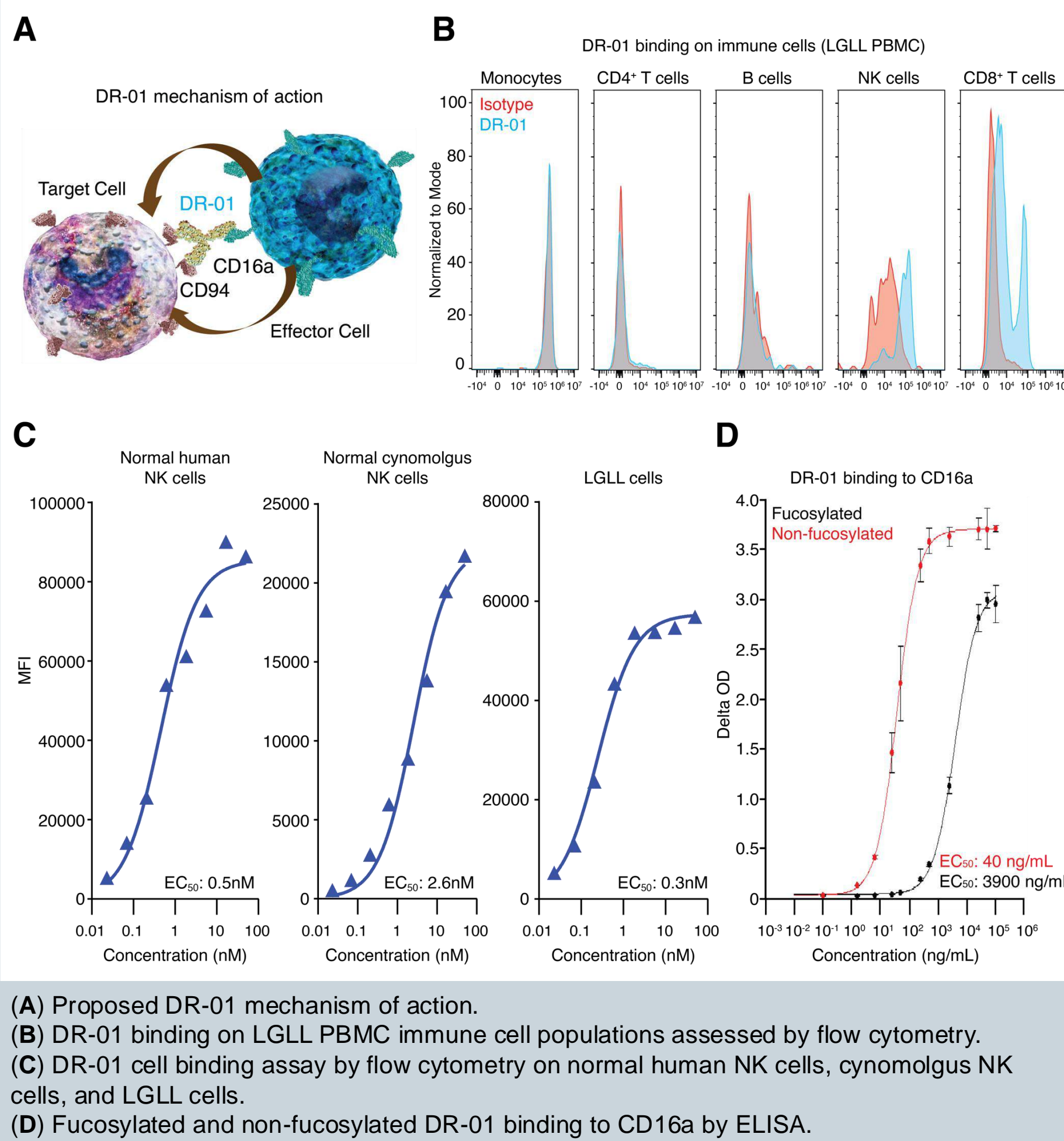
Figure 4. CD94 and CD16 expression levels in LGLL cells



DR-01, a non-fucosylated anti-human CD94 antibody, selectively binds to CD94⁺ cells and CD16a in vitro

- DR-01 Fc engages CD16a on the effector cell and CD94 on the target cell, leading to ADCC depletion (Fig. 5A)
- DR-01 predominantly binds to CD8⁺ T cells and NK cells in a T-LGLL patient sample (Fig. 5B)
- DR-01 binds to human NK cells (EC₅₀: 0.5 nM), cynomolgus NK cells (EC₅₀: 2.6 nM) and LGLL cells (EC₅₀: 0.3 nM) (Fig. 5C)
- Non-fucosylated and fucosylated DR-01 bind to CD16a protein with EC₅₀ of 40 ng/mL and 3900 ng/mL, respectively (Fig. 5D)

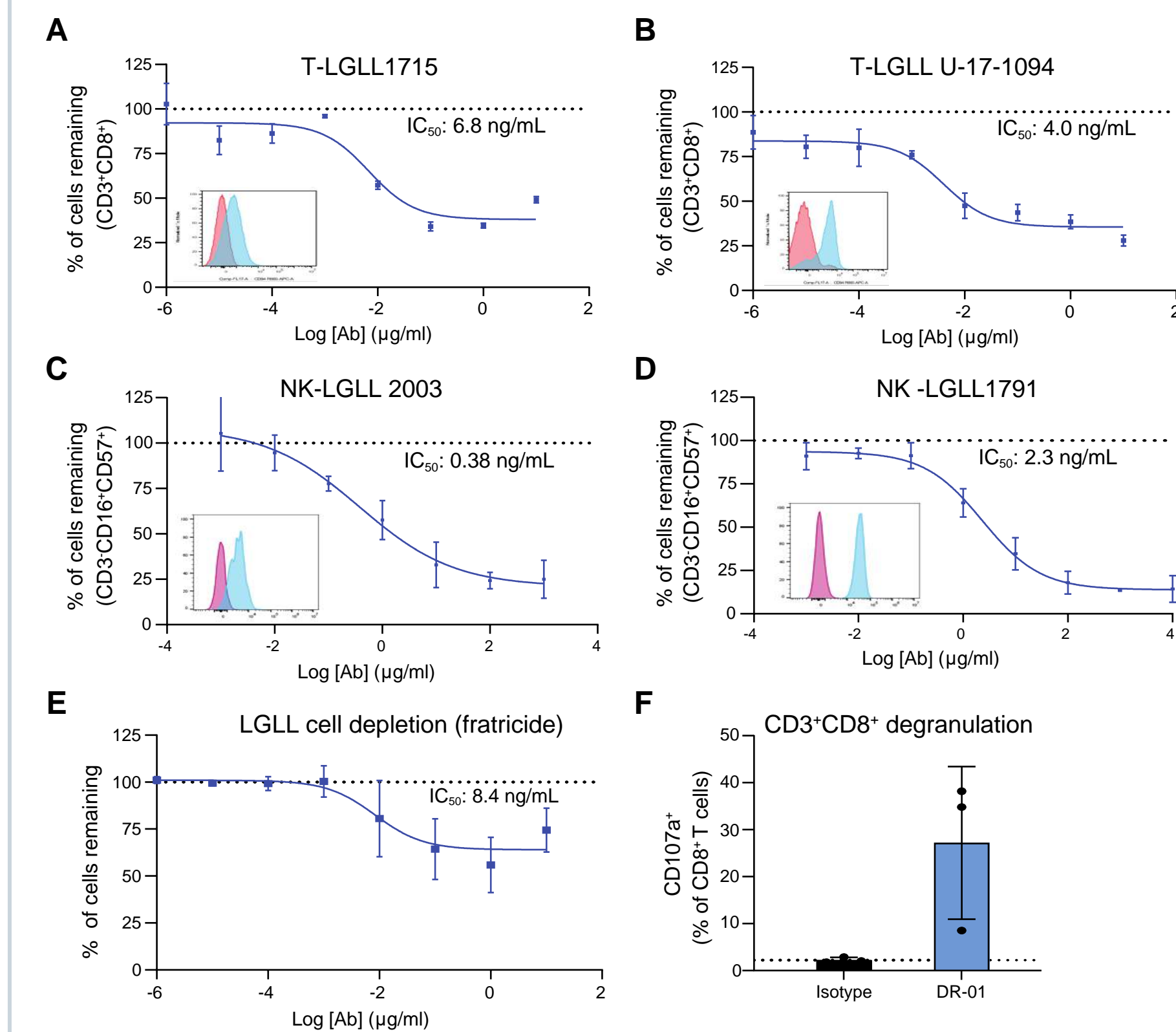
Figure 5. Development and characterization of DR-01



DR-01 depletes LGLL cells ex vivo

- DR-01 eliminated T-LGLL and NK-LGLL cells in a dose-dependent manner with high potency (Fig. 6A-D)
- Depletion of CD8⁺ T cells in isolation suggests that T-LGLL CD8⁺ T cells can deplete each other, demonstrating a novel mechanism of action known as fratricide (Fig. 6E, F)

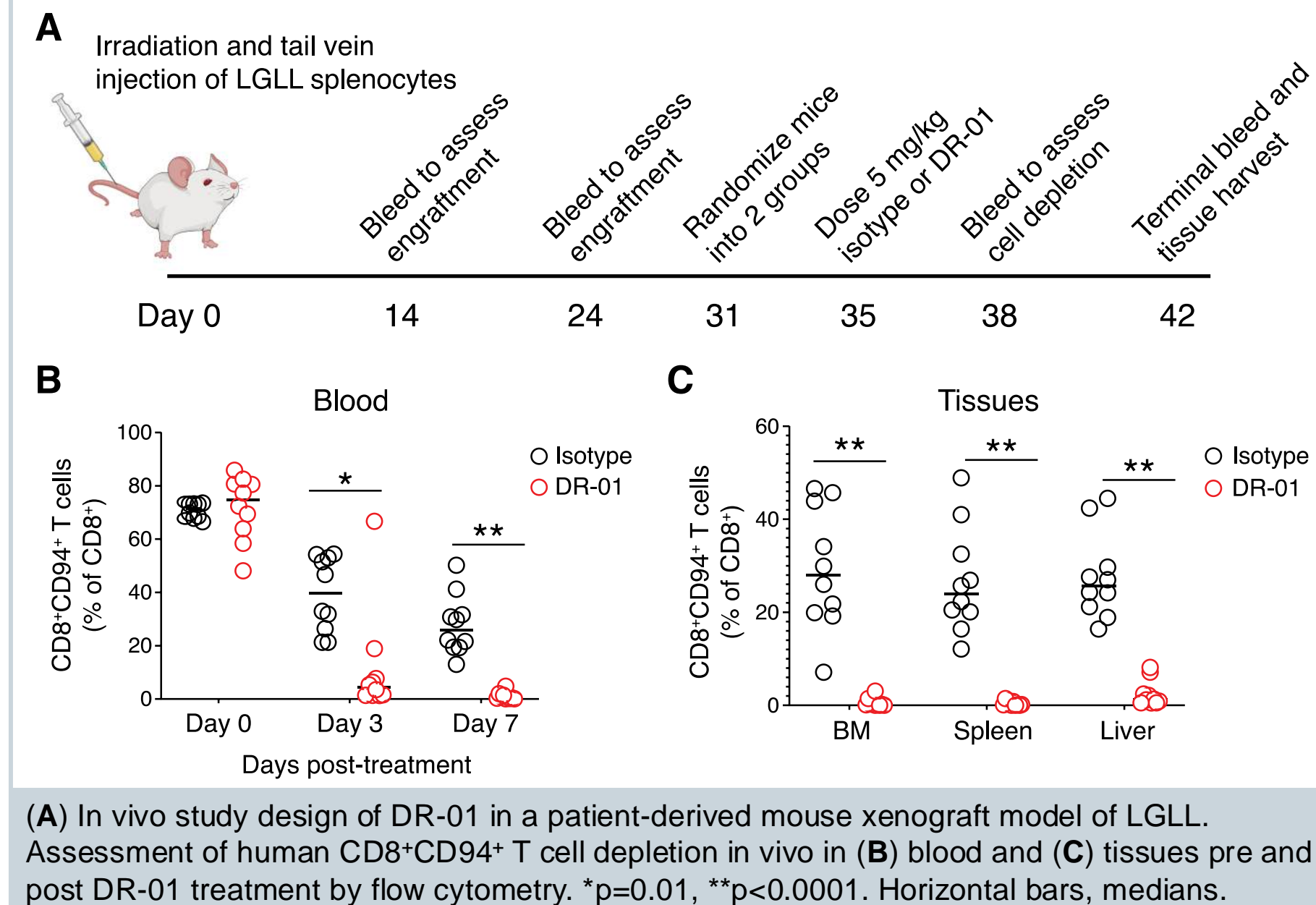
Figure 6. Ex vivo ADCC T-LGLL and NK-LGLL cell depletion assay



DR-01 depletes leukemic cells in a mouse model of LGLL

- DR-01 elicited deep depletion CD94⁺CD8⁺ T cells in blood and tissues in vivo (Fig. 7)

Figure 7. DR-01 in an LGLL in vivo mouse model



CONCLUSIONS

- Targeting CD94 is a novel therapeutic strategy for LGLL
- DR-01, an anti-CD94 antibody with enhanced ADCC activity, exploits elevated CD94 expression on LGLL cells to elicit potent leukemic cell depletion via fratricide
- Our findings support further clinical development of DR-01 for treatment of LGLL, for which treatment options remain extremely limited

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