Supplemental Figures

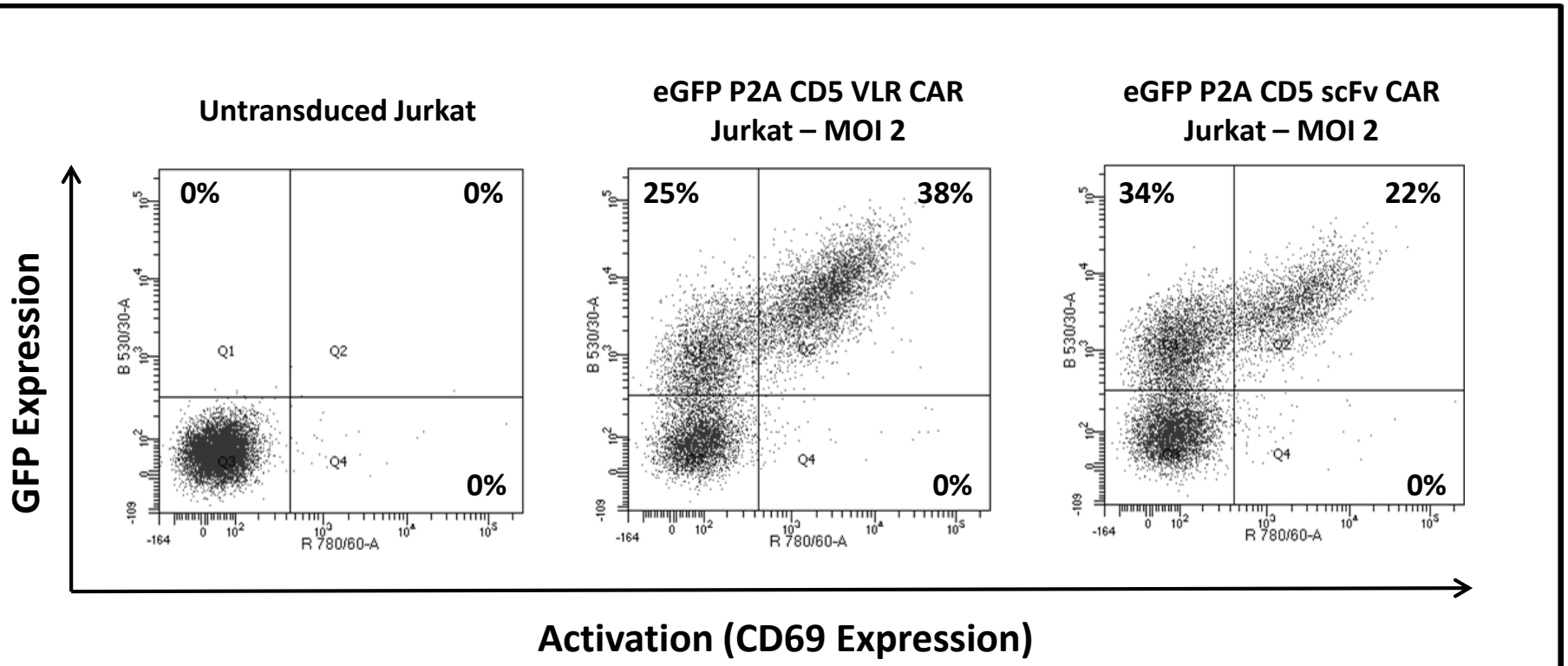


Fig. S1. Activation (CD69 expression) in Jurkat T cells transduced with the eGFP-P2A-CD5-CAR lentiviral vector correlates with eGFP expression in both CD5-CAR groups. Data above shows activation in Jurkat T cells transduced at a multiplicity of infection (MOI) of 2, four days post-transduction. Only GFP positive cells were activated, no activation is seen in the eGFP-negative Jurkat T cells.

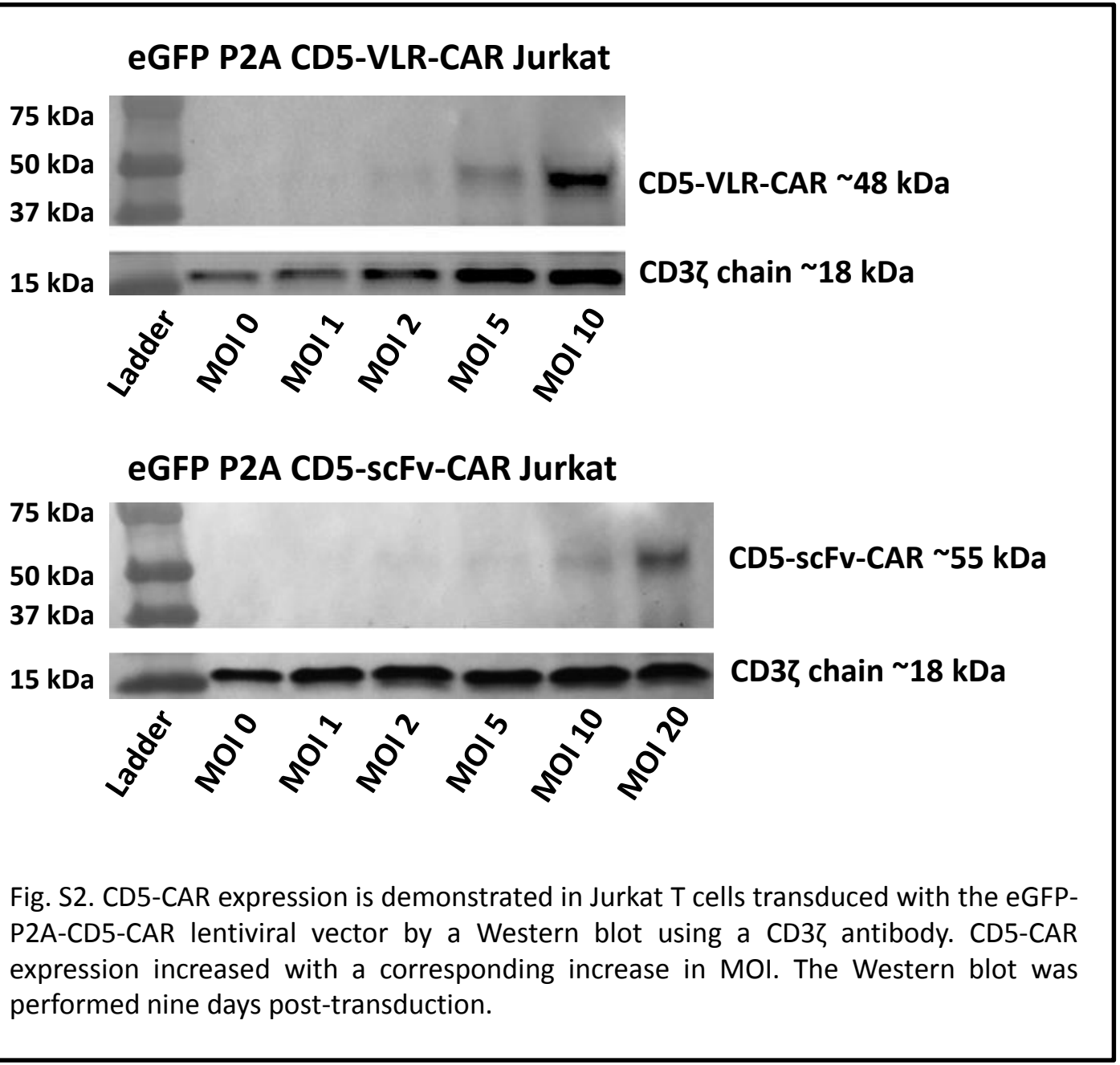


Fig. S2. CD5-CAR expression is demonstrated in Jurkat T cells transduced with the eGFP-P2A-CD5-CAR lentiviral vector by a Western blot using a CD3ζ antibody. CD5-CAR expression increased with a corresponding increase in MOI. The Western blot was performed nine days post-transduction.

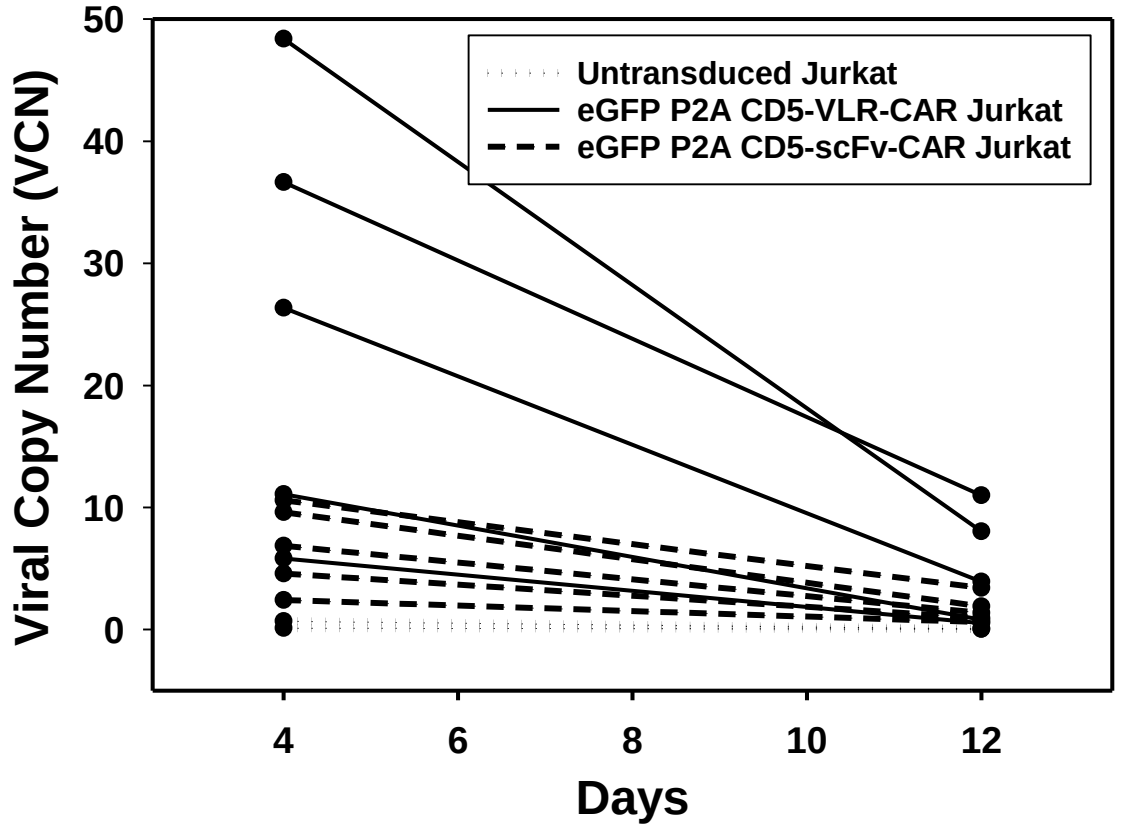


Fig. S3. Transduced proviral vector copy number (VCN) in Jurkat T cells transduced with the eGFP-P2A-CD5-CAR lentiviral vector decreased over time. The decrease in VCN corresponded with a decrease in activation as measured by surface CD69 expression.

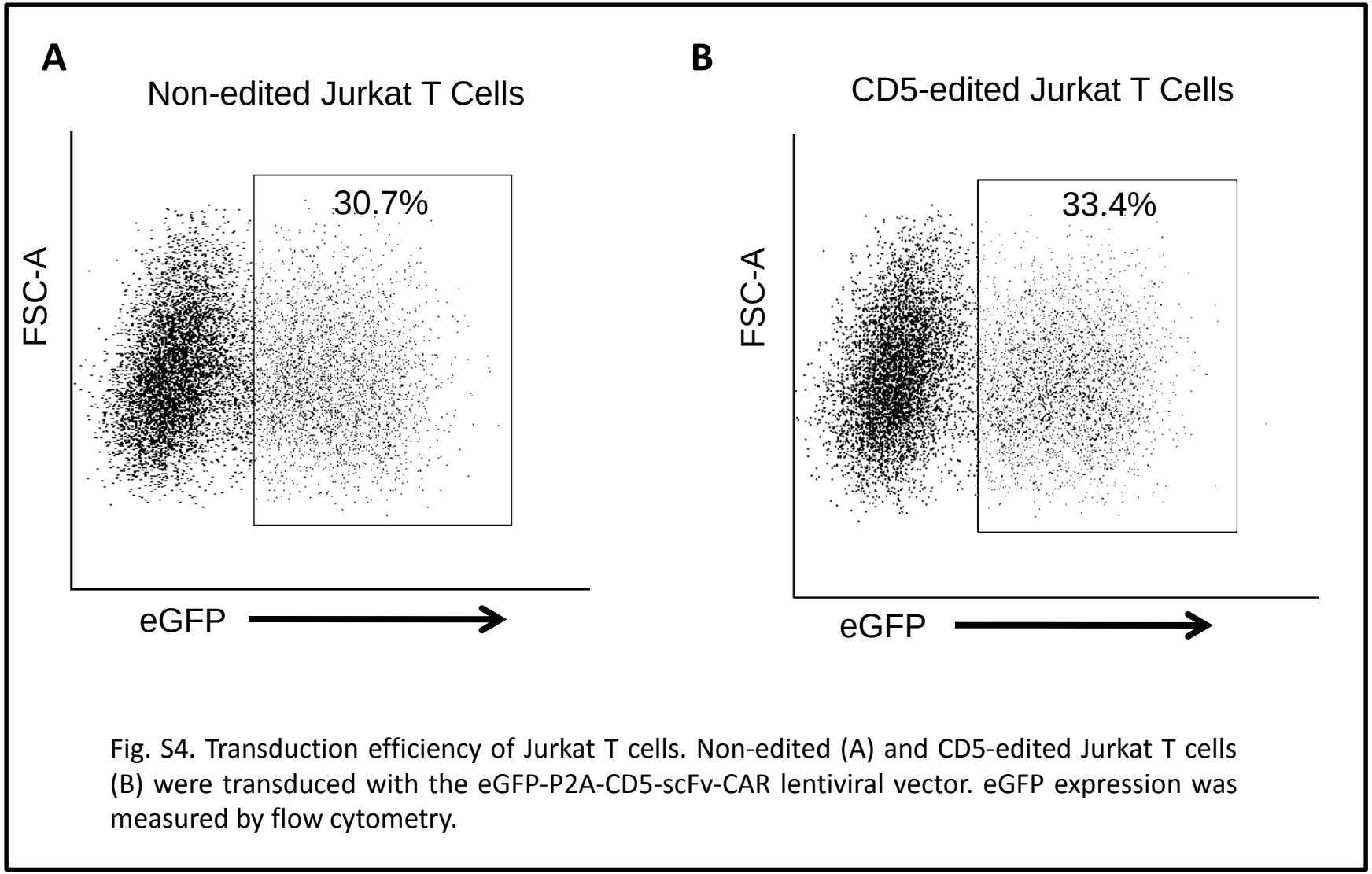
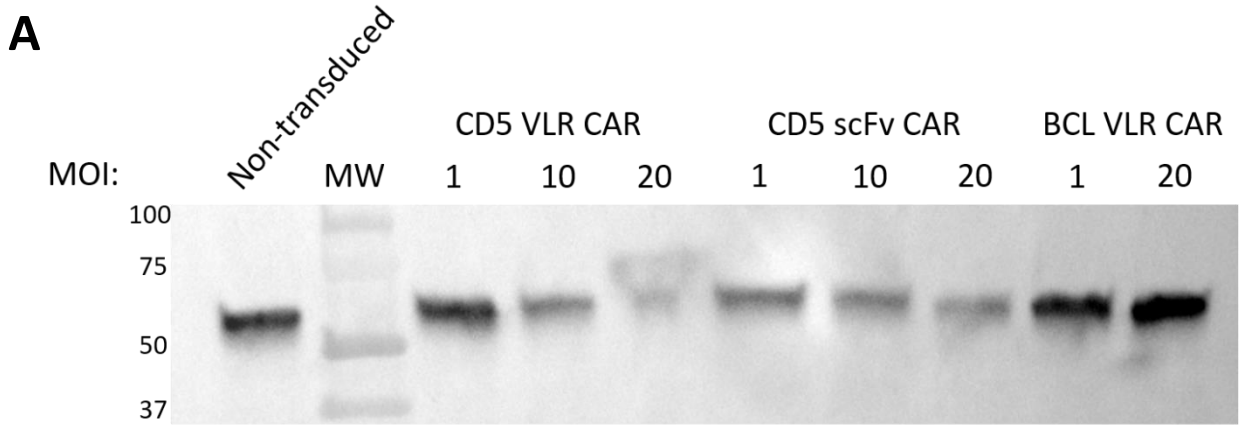


Fig. S4. Transduction efficiency of Jurkat T cells. Non-edited (A) and CD5-edited Jurkat T cells (B) were transduced with the eGFP-P2A-CD5-scFv-CAR lentiviral vector. eGFP expression was measured by flow cytometry.



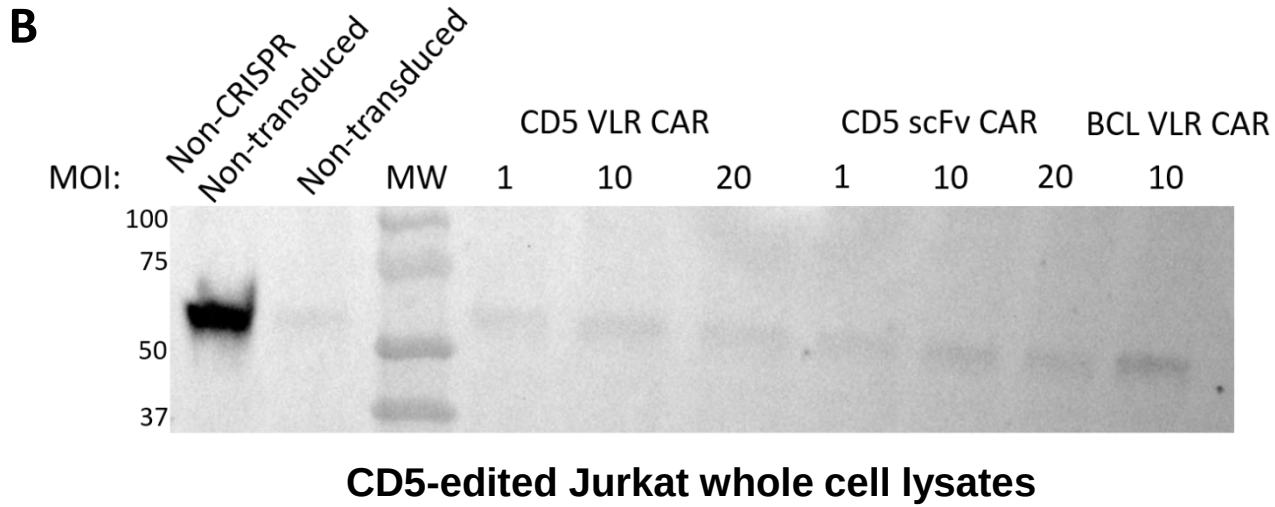


Fig. S5. CD5 protein expression. Western blot using anti-CD5 antibody on whole cell lysates from non-edited (A) and CD5-edited Jurkat T cells (B) transduced with CD5-VLR-CAR, CD5-scFv-CAR or BCL-VLR-CAR lentiviral vector at MOIs 1, 10 and 20.

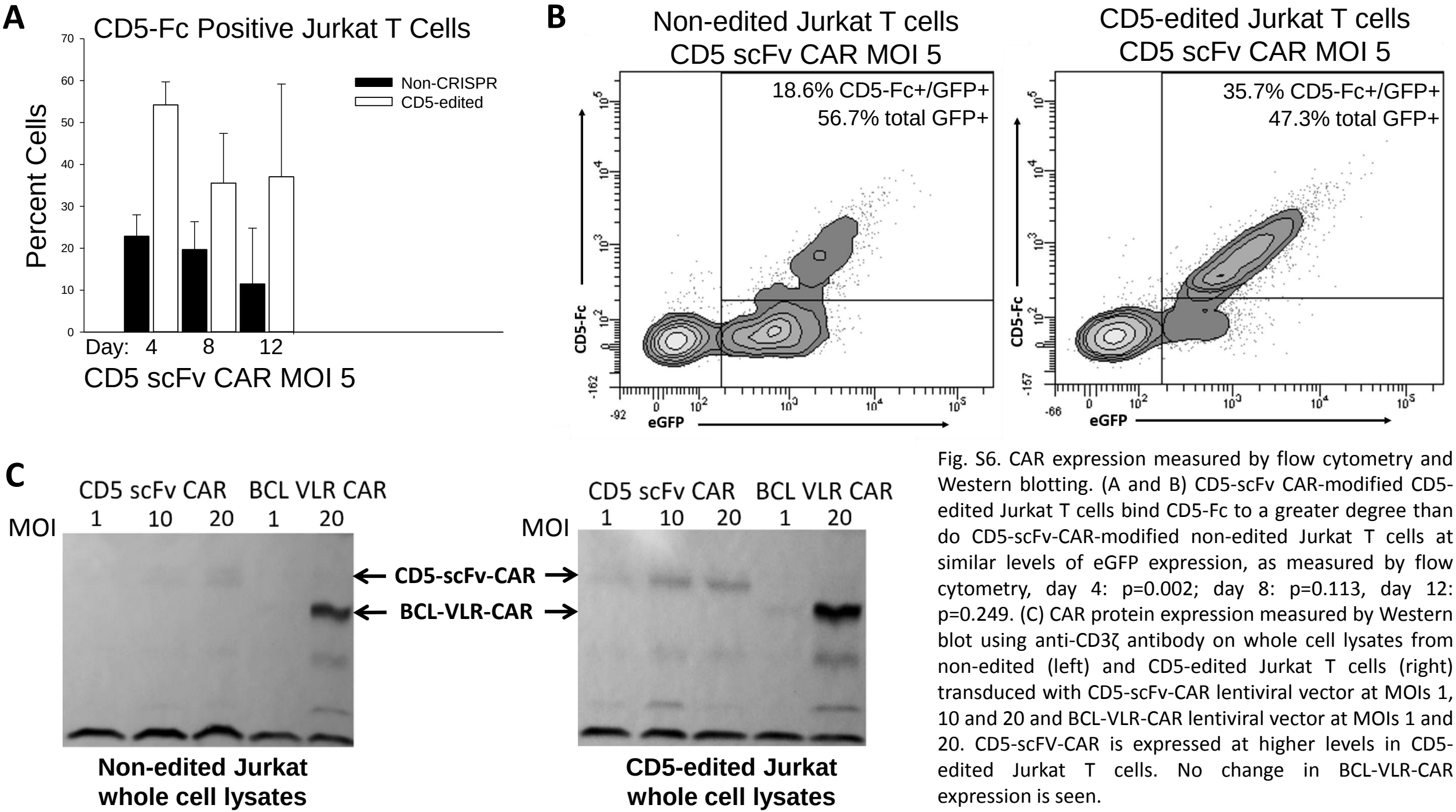


Fig. S6. CAR expression measured by flow cytometry and Western blotting. (A and B) CD5-scFv CAR-modified CD5-edited Jurkat T cells bind CD5-Fc to a greater degree than do CD5-scFv-CAR-modified non-edited Jurkat T cells at similar levels of eGFP expression, as measured by flow cytometry, day 4: p=0.002; day 8: p=0.113, day 12: p=0.249. (C) CAR protein expression measured by Western blot using anti-CD3ζ antibody on whole cell lysates from non-edited (left) and CD5-edited Jurkat T cells (right) transduced with CD5-scFv-CAR lentiviral vector at MOIs 1, 10 and 20 and BCL-VLR-CAR lentiviral vector at MOIs 1 and 20. CD5-scFV-CAR is expressed at higher levels in CD5-edited Jurkat T cells. No change in BCL-VLR-CAR expression is seen.

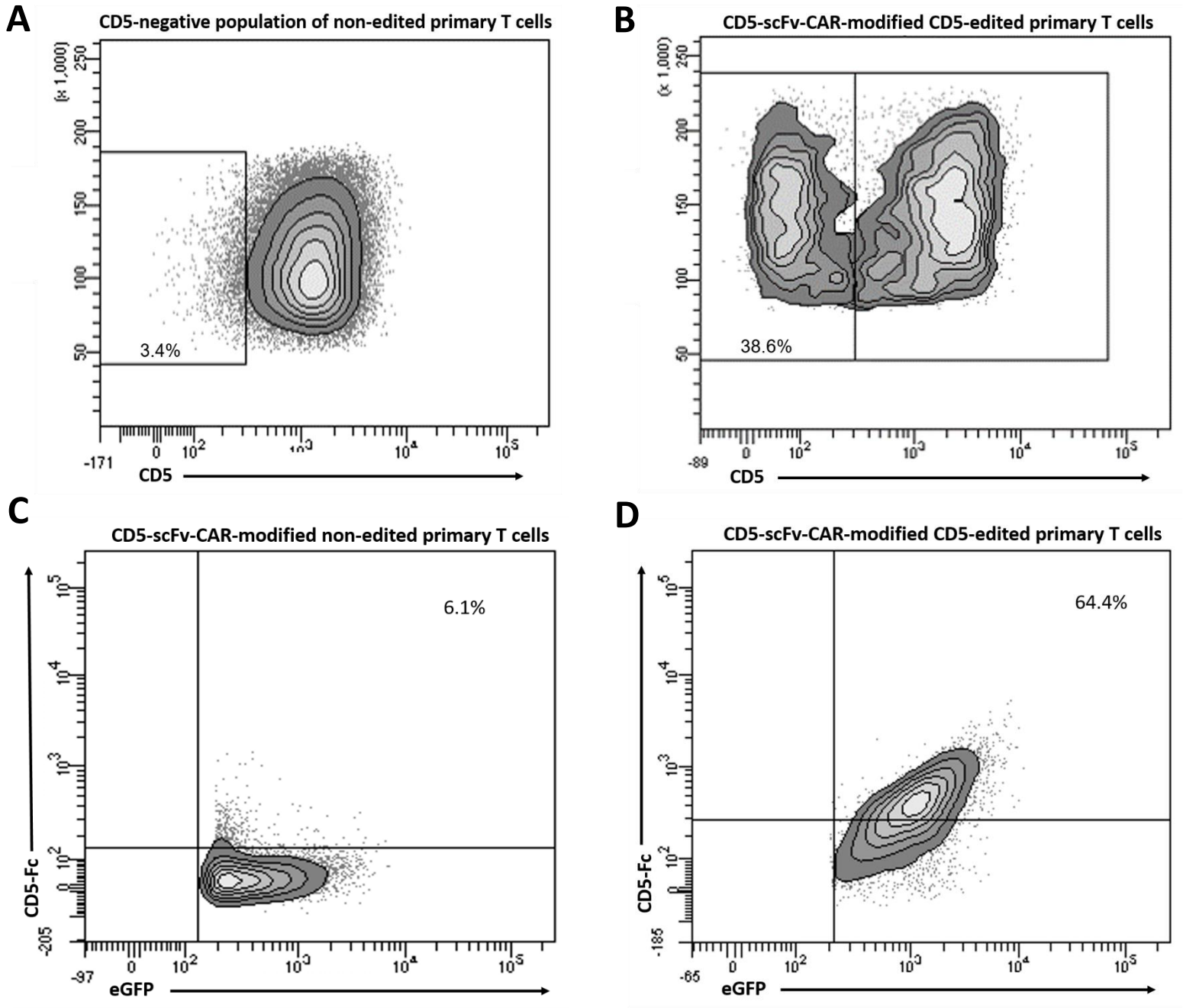


Fig. S7: Primary T cell CD5 and CD5-CAR expression measured by flow cytometry. CD5 expression in non-edited (A) and CD5-edited primary T cells (B). CD5-CAR expression measured by CD5-Fc binding in eGFP-positive non-edited (C) and CD5-edited primary T cells (D).

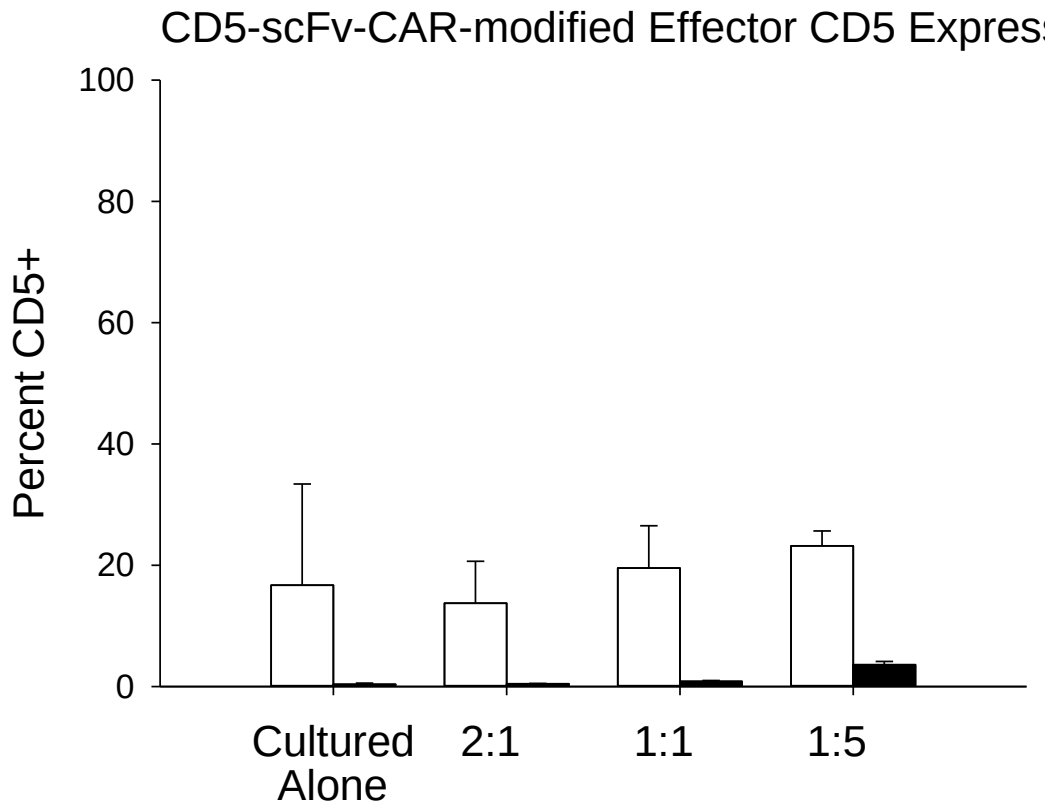


Fig. S8. CD5 surface expression on CD5-scFv-CAR-modified effector Jurkat T cells in culture with naïve Jurkat target T cells. Flow cytometry was used to measure CD5 expression on effector cells cultured alone or at E:T ratios 2:1, 1:1, 1:5. White bars signify non-edited effector cells; black bars signify CD5-edited effector cells.

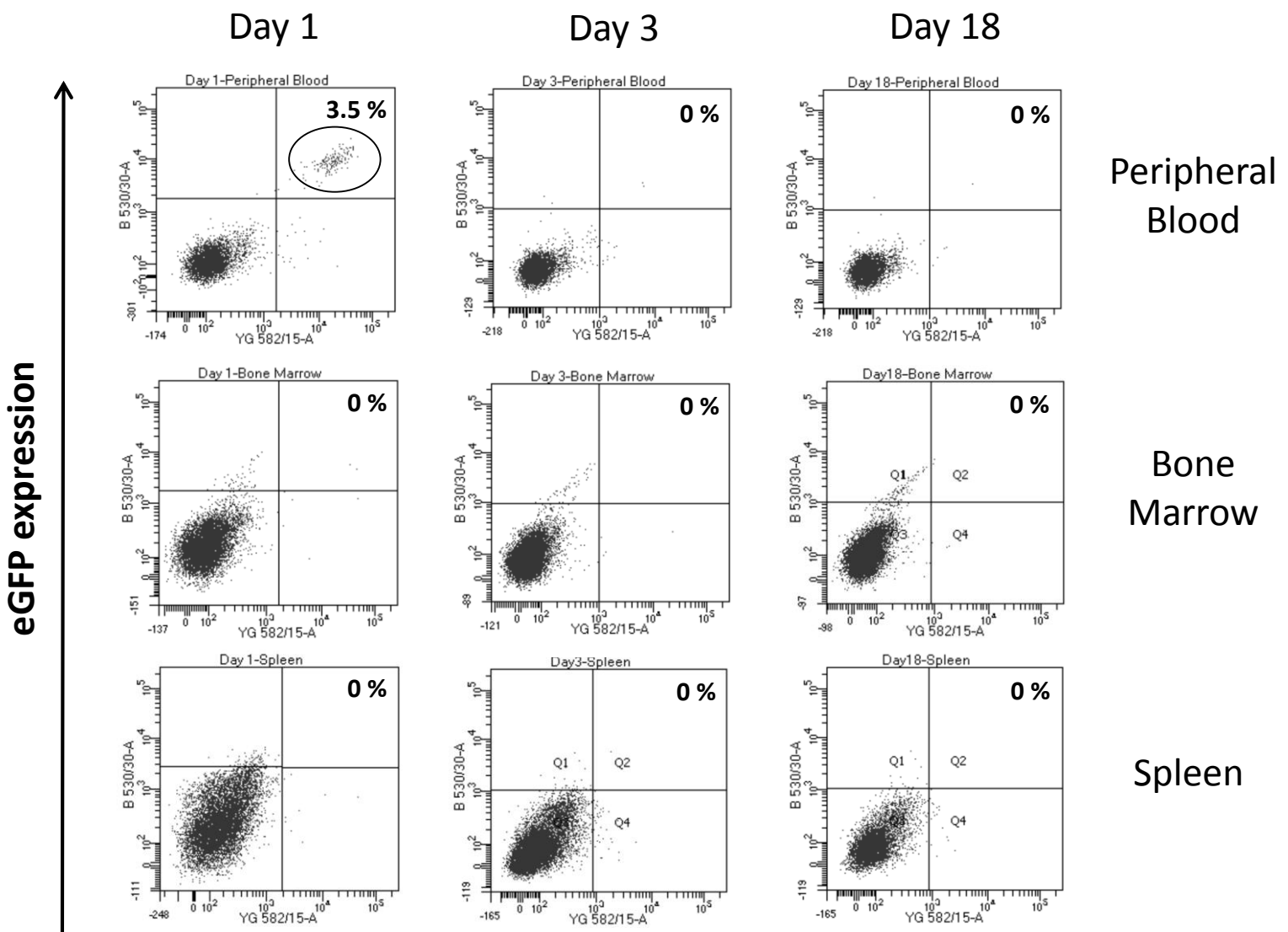


Fig. S9. Persistence of CD5-CAR expressing NK-92 cells in absence of IL-2. 10^7 non-irradiated eGFP-P2A-CD5-scFv-CAR expressing NK-92 cells were injected into NSG mice. Mice were not given IL-2. Peripheral blood, bone marrow and spleen were evaluated for presence of CAR expressing NK-92 cells using eGFP and human CD45 expression. No evidence of CAR-NK-92 cells were seen by day 3. No evidence of engraftment in bone marrow was seen. No evidence of disease from NK-92 cells was seen seven weeks post injection.