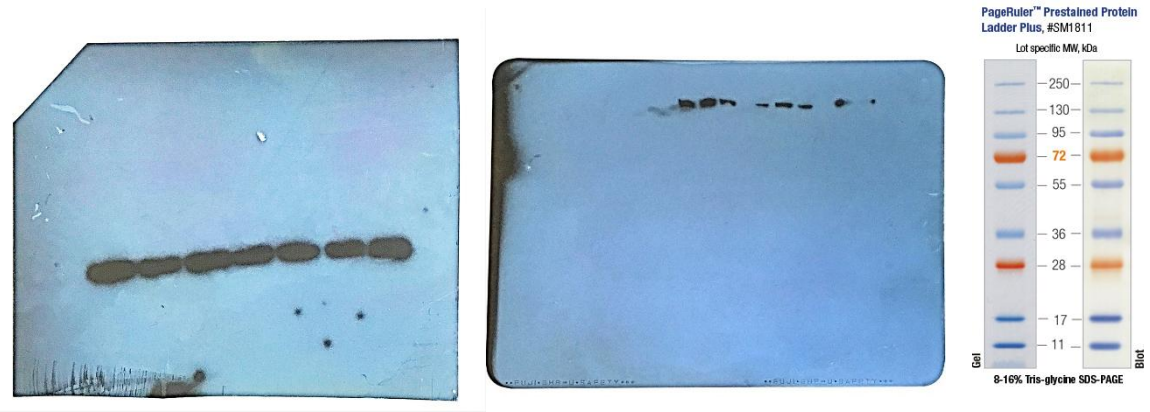
Fig. 1

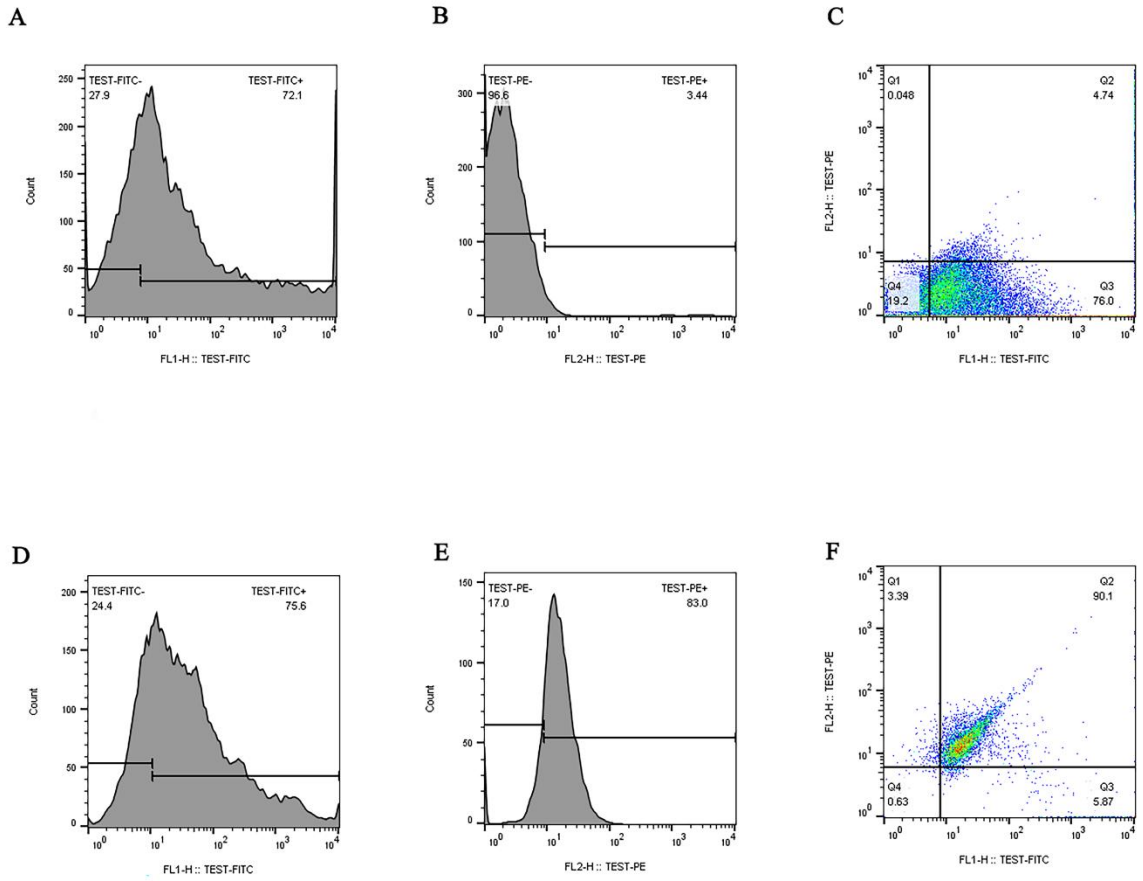
A

B



Original blots presented in this study. (A) Western blot analysis of β-actin protein expression. (B) Detection of STAT5 gene expression. Molecular weight markers are indicated on the right of panels. β-actin serves as a loading control to normalize protein levels across samples.

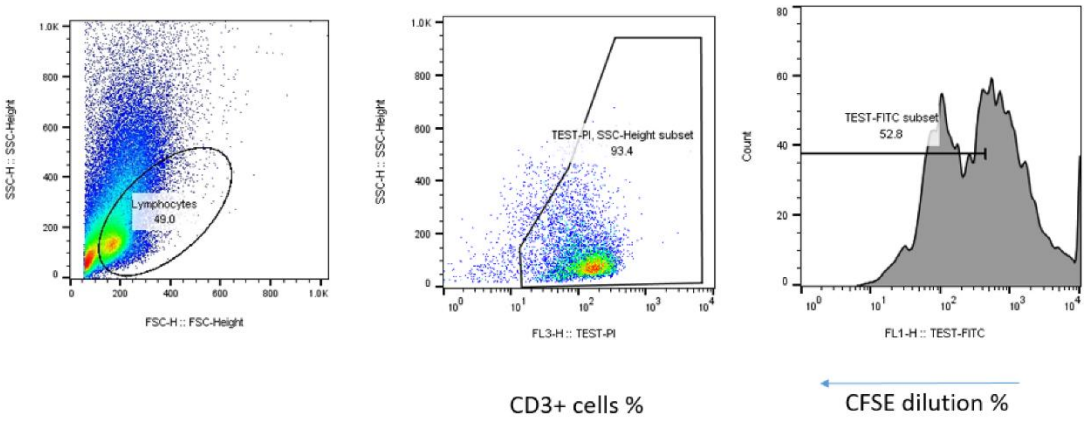
Fig. 2



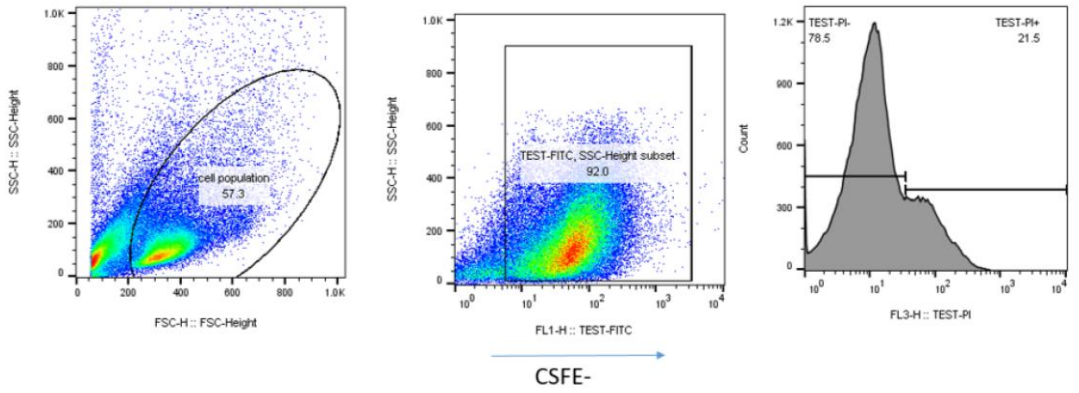
Comparison of GFP and PD1 expression in transfected HEK 293T cells with CAR plasmids. We evaluated the transfection efficiency of HEK 293T cells using flow cytometry. The cells were incubated with a PE-labeled anti-PD1 antibody for 30 minutes and then washed with PBS. Panels **A** and **C** show GFP-positive cells, confirming successful transfection with the MSLN-CAR plasmid. Panels **D** and **F** also display GFP-positive cells, while Panel **E** shows PD1-positive cells, indicating the transfection efficiency of the MSLN-PD1/IL15Rβ construct. Notably, the expression levels of PD1 and GFP within the MSLN-PD1/IL15Rβ construct appear to be comparable, thereby reinforcing the viability of GFP as a reliable indicator for CAR expression in this experimental context.

Fig. 3

A



B



For proliferation and cytotoxicity assessment, we used a gating strategy. **A** To measure the proliferation rate of CAR T cells we labeled effector cells with carboxyfluorescein succinimidyl ester (CFSE) dye. After 72 hours of co-cultivation, we stained the combined population with APC-anti-CD3 antibody and evaluated the percentage of CFSE dilution in the gated CD3-positive cells. This percentage represents the proportion of proliferation. **B** We stained effector cells with CFSE dye and cultured them with target cells for 4 hours. Then, we stained the cells with propidium iodide (PI) to identify dead target cells as CFSE- and PI+.