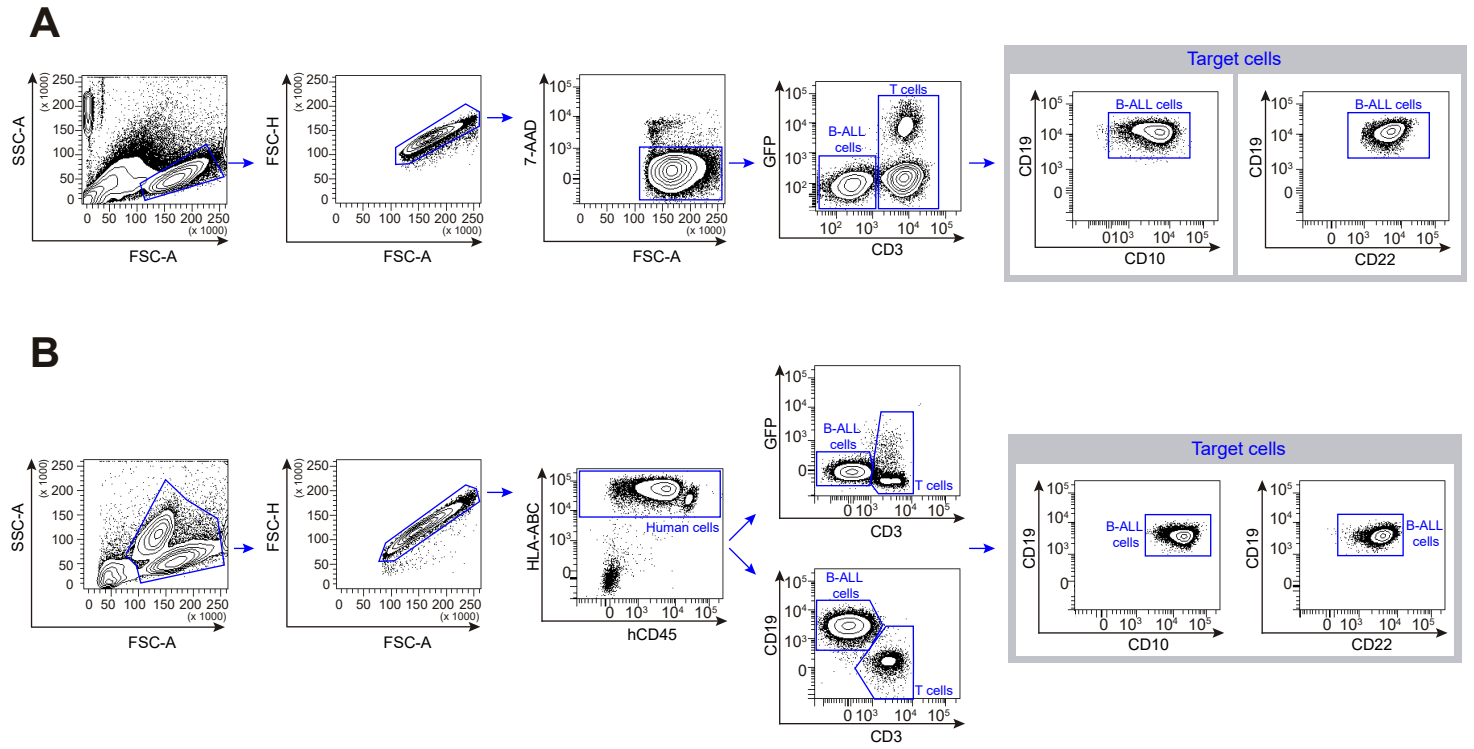


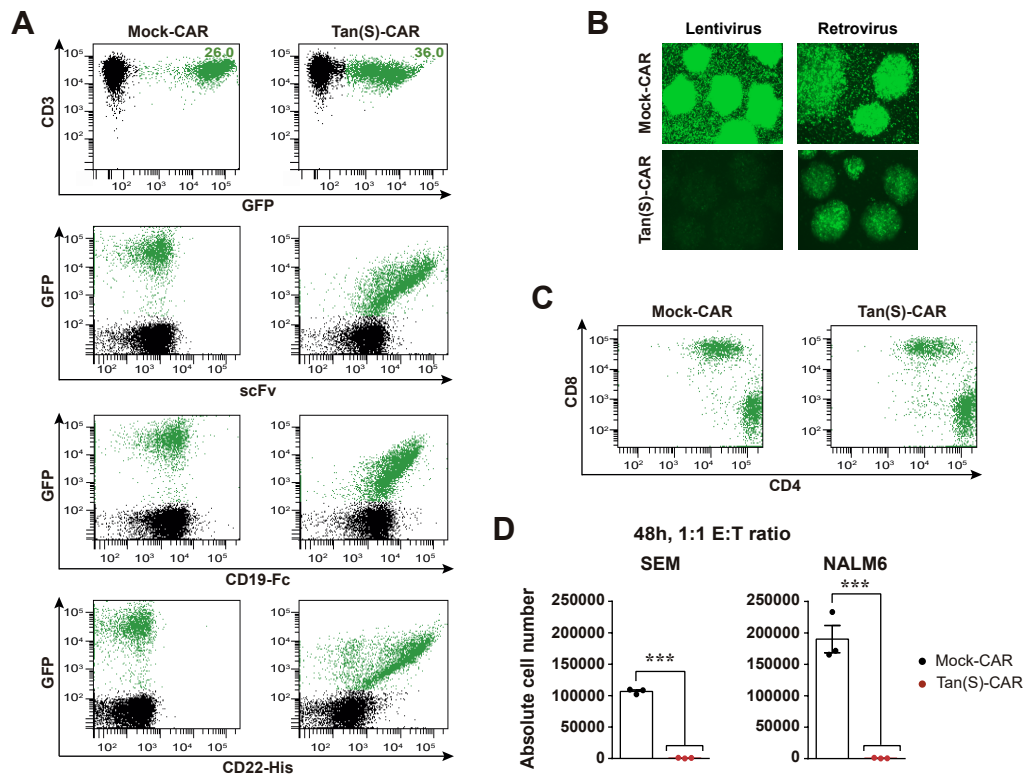
## **Supplemental Information**

### **A novel and efficient tandem CD19- and CD22-directed CAR for B cell ALL**

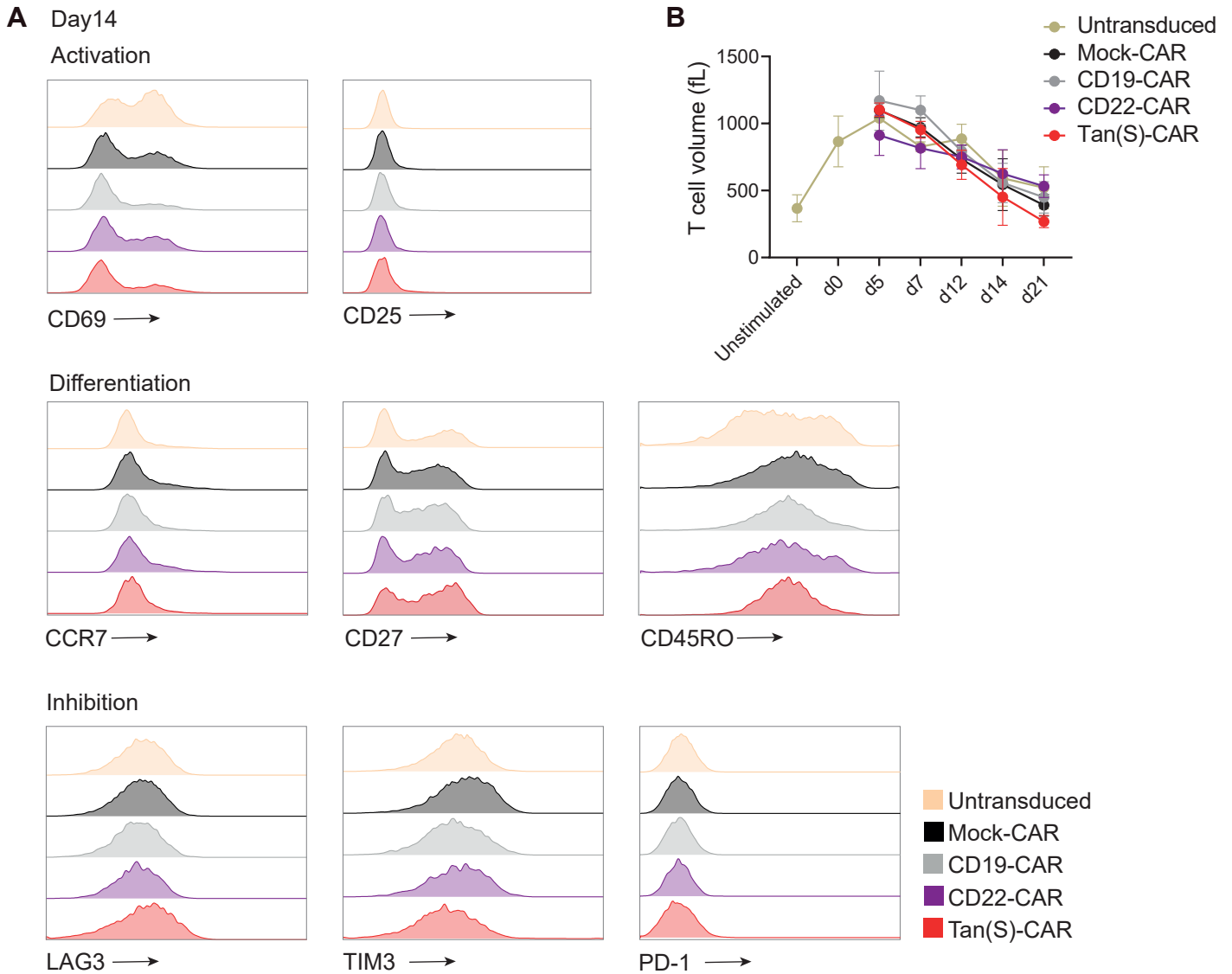
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**Figure S1. Flow cytometry gating strategies.** **A)** Flow cytometry gating strategy used to analyse *in vitro* CAR T cell cytotoxicity. Doublets were removed from the analysis. Live target cells were identified as 7-AAD<sup>-</sup>CD3<sup>+</sup>GFP<sup>+</sup>CD10<sup>+</sup>. Then, within this population of cells, we studied the expression of CD19 and CD22. **B)** Flow cytometry gating strategy used to analyse *in vivo* CAR T cell cytotoxicity. Doublets were removed from the analysis. Live target cells were identified as CD45<sup>+</sup>HLA-ABC<sup>+</sup>CD3<sup>+</sup>GFP<sup>+</sup>CD10<sup>+</sup>. Then, within this population of cells, we studied the expression of CD10 and CD22. Human T cells were identified as CD45<sup>+</sup>HLA-ABC<sup>+</sup>CD3<sup>+</sup>.



**Figure S2. Detection and *in vitro* antileukemic efficacy of Tan(S)-CAR T cells transduced with retrovirus. A)** Representative FACS plots of CAR expression on human T cells detected as GFP<sup>+</sup> (top panels), anti-scFv (second row), CD19-Fc/anti-Fc-PE (third row), and CD22-HisTag/anti-HisTag-APC (bottom row). CAR-transduced T cells are shown in green. Mock-CAR depicts T cells transduced with the GFP-SFG vector. **B)** Fluorescence microscope images of CAR T cells cultures depicting the GFP expression of T cells transduced with the corresponding lentivirus or retrovirus. **C)** Representative CAR detection on human CD4<sup>+</sup> and CD8<sup>+</sup> T cells. **D)** Absolute number of live target cells (SEM or NALM6) after 48 h incubation with the indicated CAR T cells at 1:1 E:T ratio (PBMCs from n=3 independent HD). Data are shown as mean  $\pm$  SEM \*\*\*p<0.001; 2-tailed unpaired Student's t-test.



**Figure S3. Tan(S)-CAR shows no increased tonic signaling than single CARs, Mock or untransduced T cells.** **A)** Tonic signaling of the CAR T cells was determined by analysing the expression of activation (CD69 and CD25), differentiation (CCR7, CD27 and CD45RO) and inhibition (LAG3, TIM3 and PD-1) markers on the indicated CAR T cells throughout *in vitro* expansion (day 7, day 14 and day 21). Representative FACS histograms of the expression of these markers at day 14 are shown. **B)** Kinetics of T cell volume of the CAR T cells throughout the 21-day *in vitro* expansion. (PBMCs from n=3 independent healthy donors). No significant differences were found among the different CAR T cells using two-way ANOVA with the Tukey *post hoc* multiple comparisons test.