Figure S1. Difference of chromium 51 (^{51}Cr) release of tumor cells Nalm-6 (orange), Reh (red), Daudi (brown) and Raji (blue) cells. As previously described (43,44), cytotoxicity was calculated as the percentage of specific lysis according to the following formula: % specific lysis=(^{51}Cr- release in the test well-spontaneous ^{51}Cr release)/Δrelease x100. Nalm-6 had the lowest Δrelease (Δrelease=maximum release-spontaneous release) compared to other tumor cell lines, making chromium release assay to assess the cytotoxicity of CAR T cells on Nalm-6 cells inefficient. Therefore, functionality of CAR T cells towards Nalm-6 cells was assessed via flow cytometric analysis. CPM: Counts per minute. Experiments were performed in triplicate. Results are represented as mean ± standard deviation (SD).

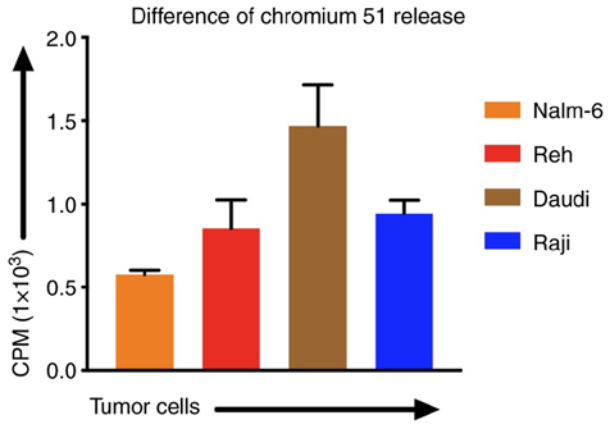

Figure S2. Expression of exhaustion markers LAG-3, PD-1 and Tim-3 on CAR T cells after simultaneous cultivation for 5 days of Daudi cells, CAR T cells and eltanexor (0.1 μM: Orange, 0.5 μM: red) compared to the DMSO control (brown). Experiments were performed in triplicate. Results are represented as mean ± standard deviation (SD).

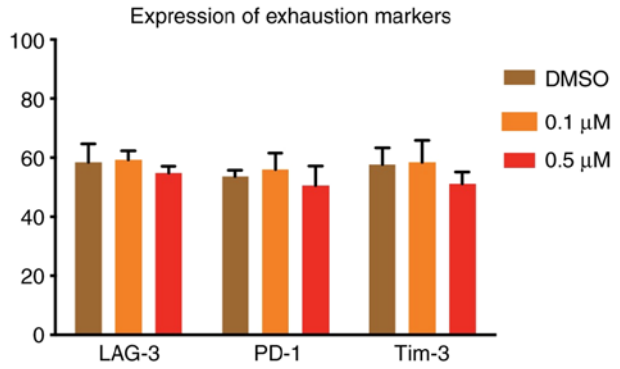

Figure S3. Representative flow cytometry dotplots (one healthy donor, HD) corresponding to Fig. 2A (displaying the cytotoxic effect of CAR T cells towards Nalm-6 cells, E:T: 1:2). Data of CAR T cells ($CD3^+CD10^-$) and Nalm-6 cells ($CD3^-CD10^+$) cultivated with DMSO (left, control), eltanexor 0.1 μM (medial) and eltanexor 0.5 μM (right) are represented. E, effector cells; T, target cells.

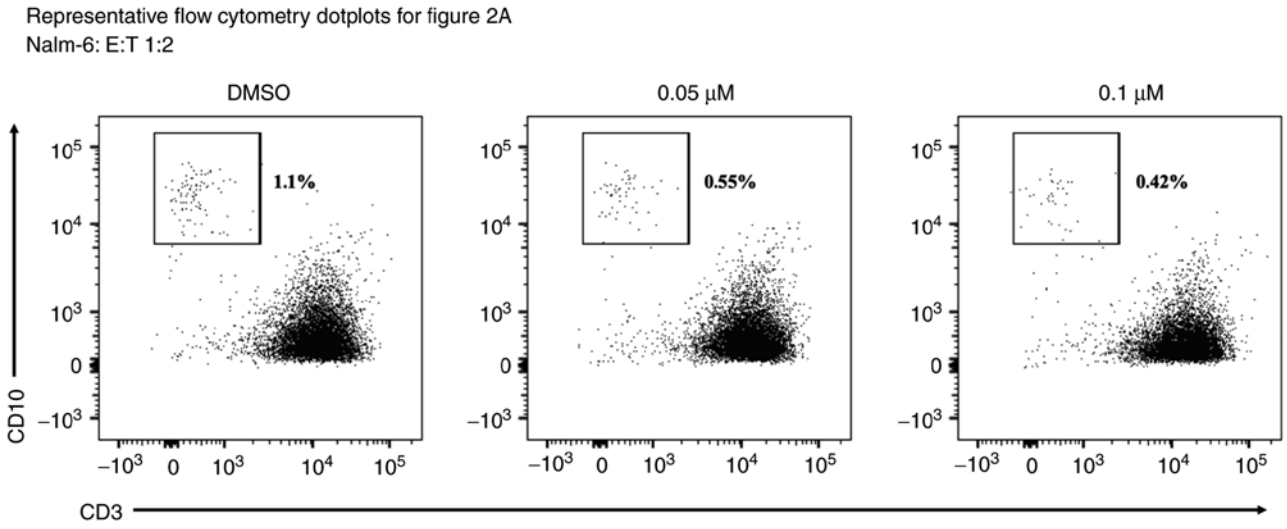

Figure S4. Representative flow cytometry data (one healthy donor, HD) corresponding to Fig. 4A (displaying the effects on cytokine release of co-culturing of eltanexor, CAR T cells and Daudi cells). Cytokine release defined as $CD8^{+}TNF\text{-}\alpha^{+}$ of CAR T cells cultivated with DMSO (left, control), eltanexor 0.1 μM (medial) and eltanexor 0.5 μM (right) is represented.

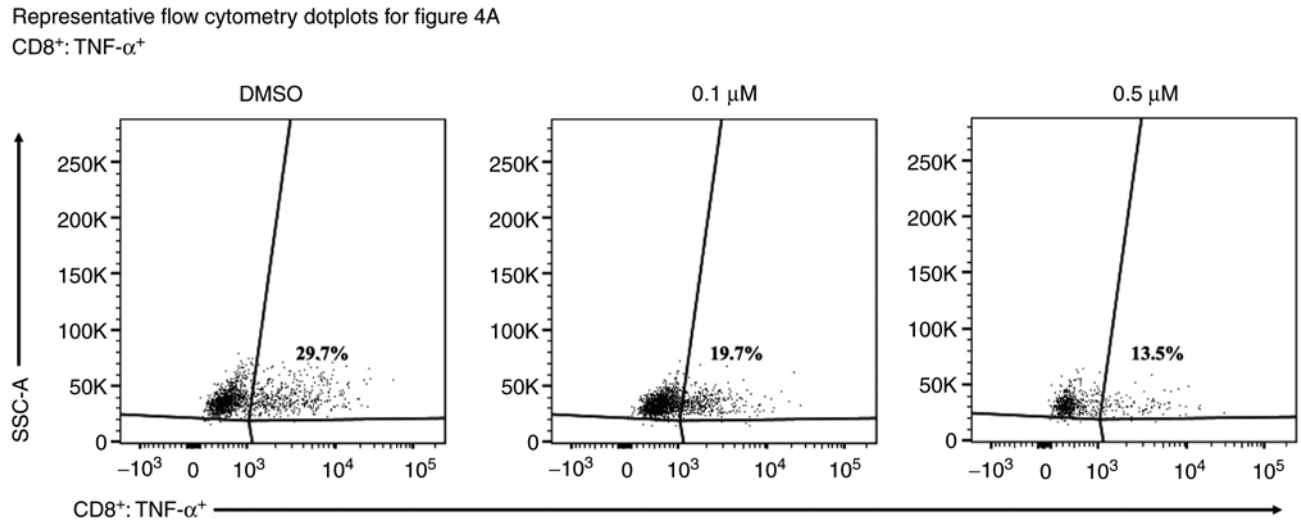

Figure S5. Representative flow cytometry data corresponding to Fig. 4B (level of phosphorylated-STAT3 of CAR T cells). Phosphorylated-STAT3^{+} of CAR T cells cultivated with DMSO (left, control), eltanexor 0.1 μM (medial) and eltanexor 0.5 μM (right) is represented.

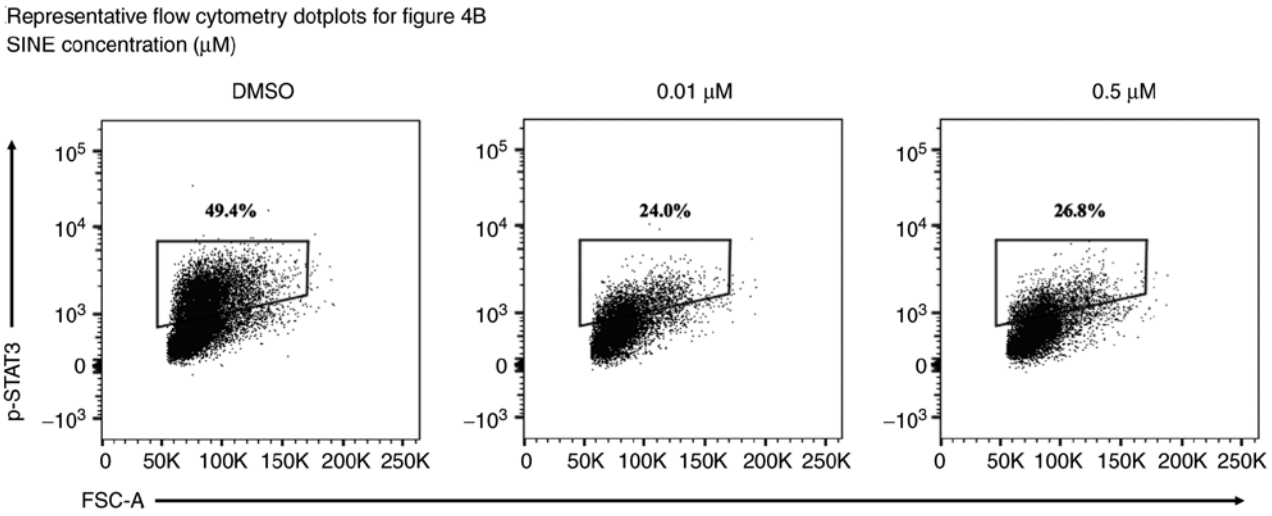

Figure S6. Representative flow cytometry data (one healthy donor, HD) corresponding to Fig. 5A (displaying cytotoxicity of CAR T cells on Nalm-6 cells, E: T: 1:2). Data of CAR T cells ($CD3^+CD10^-$) and Nalm-6 cells ($CD3^-CD10^+$) cultivated with DMSO (left, control), eltanexor 0.1 μM (medial) and eltanexor 0.5 μM (right) are represented. E, effector cells; T, target cells.

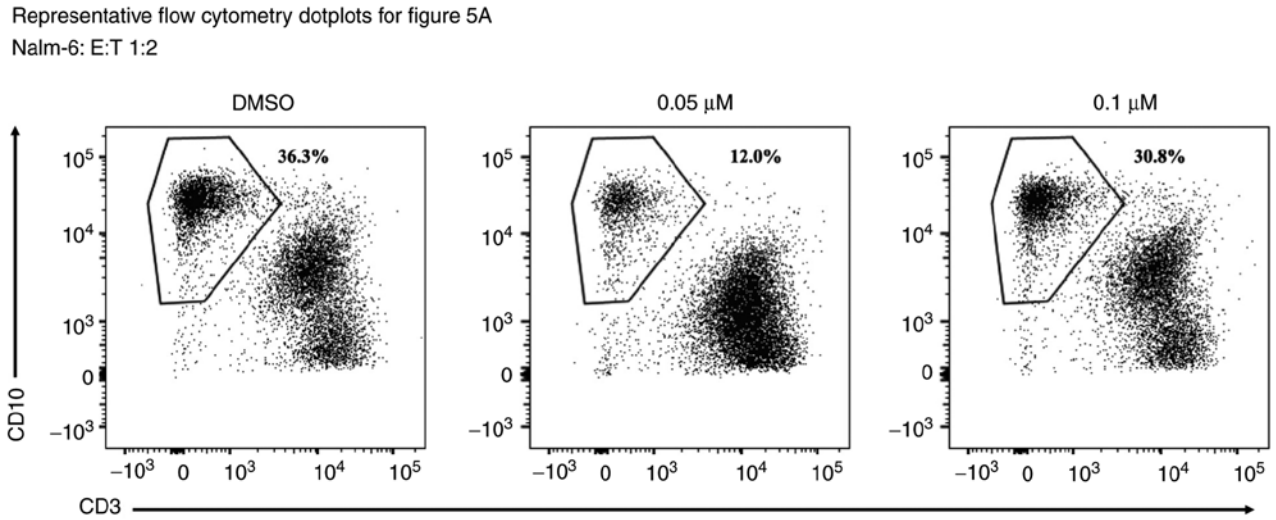

Figure S7. Representative flow cytometry data (one healthy donor, HD) corresponding to Fig. 6A (displaying the effects on cytokine release of coculturing of eltanexor, CAR T cells and Daudi cells). Cytokine release defined as $CD8^{+}TNF\text{-}\alpha^{+}$ of CAR T cells cultivated with DMSO (left, control), eltanexor 0.1 μM (medial) and eltanexor 0.5 μM (right) is represented.

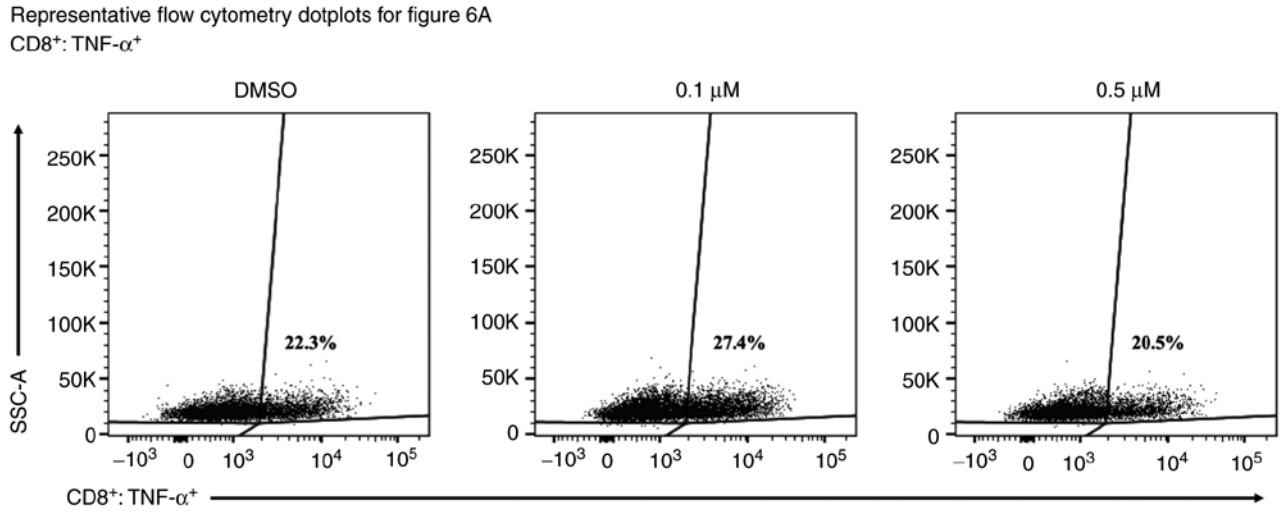

Figure S8. Representative flow cytometry data (one healthy donor, HD) corresponding to Fig. 7 (displaying expression of exhaustion markers on CAR T cells after simultaneous cultivation for 5 days of Nalm-6 cells, CAR T cells and eltanexor). PD-1^{+} CAR T cells cultivated with DMSO (left, control), eltanexor 0.1 μM (medial) and eltanexor 0.5 μM (right) are represented.

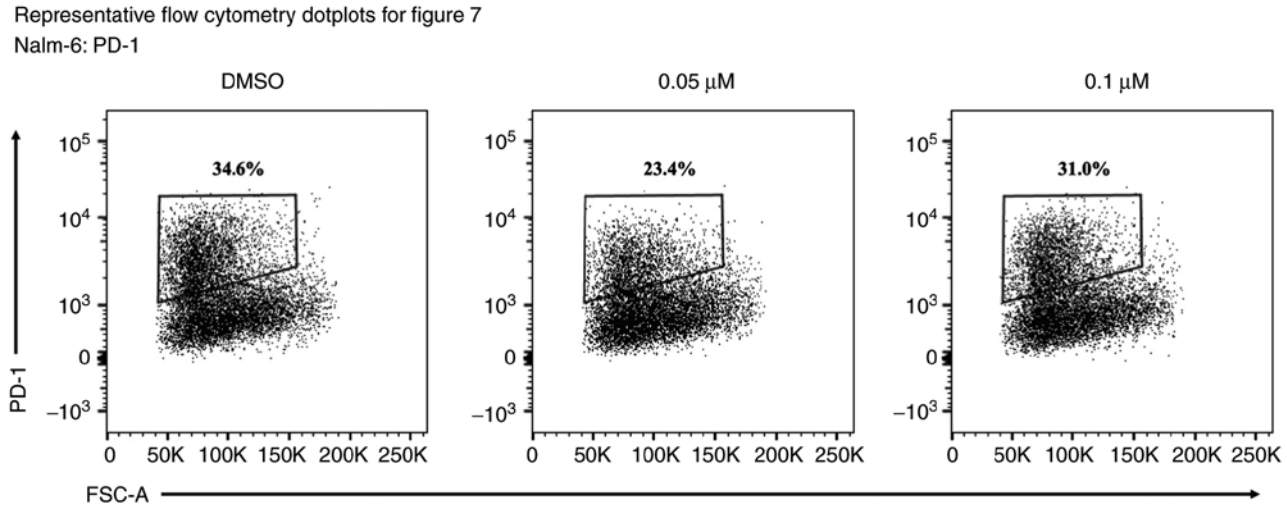

Figure S9. Representative flow cytometry data (one healthy donor, HD) corresponding to Fig. S2 (displaying expression of exhaustion markers on CAR T cells after simultaneous cultivation of Daudi cells, CAR T cells and eltanexor for 5 days). PD-1$^+$ CAR T cells cultivated with DMSO (left, control), eltanexor 0.1 μM (medial) and eltanexor 0.5 μM (right) are represented.

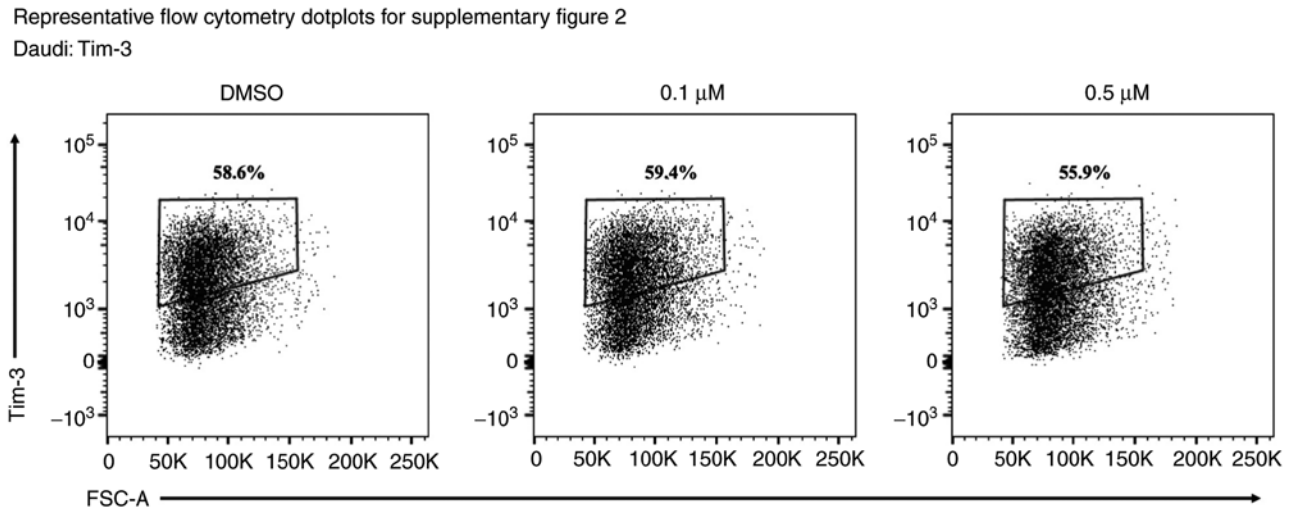

Figure S10. Representation of the gating strategy to assess cytotoxicity of CAR T cells towards Nalm-6 cells. Absolute counting beads were used to quantify vital cells. Live cells were defined as Near-IR^{+}. Single cells were selected by SSC-A and SSC-H. CAR T cells (CD3^{+}CD10^{-}) and Nalm-6 cells (CD3^{-}CD10^{+}) were distinguished using CD3 and CD10 antibodies.

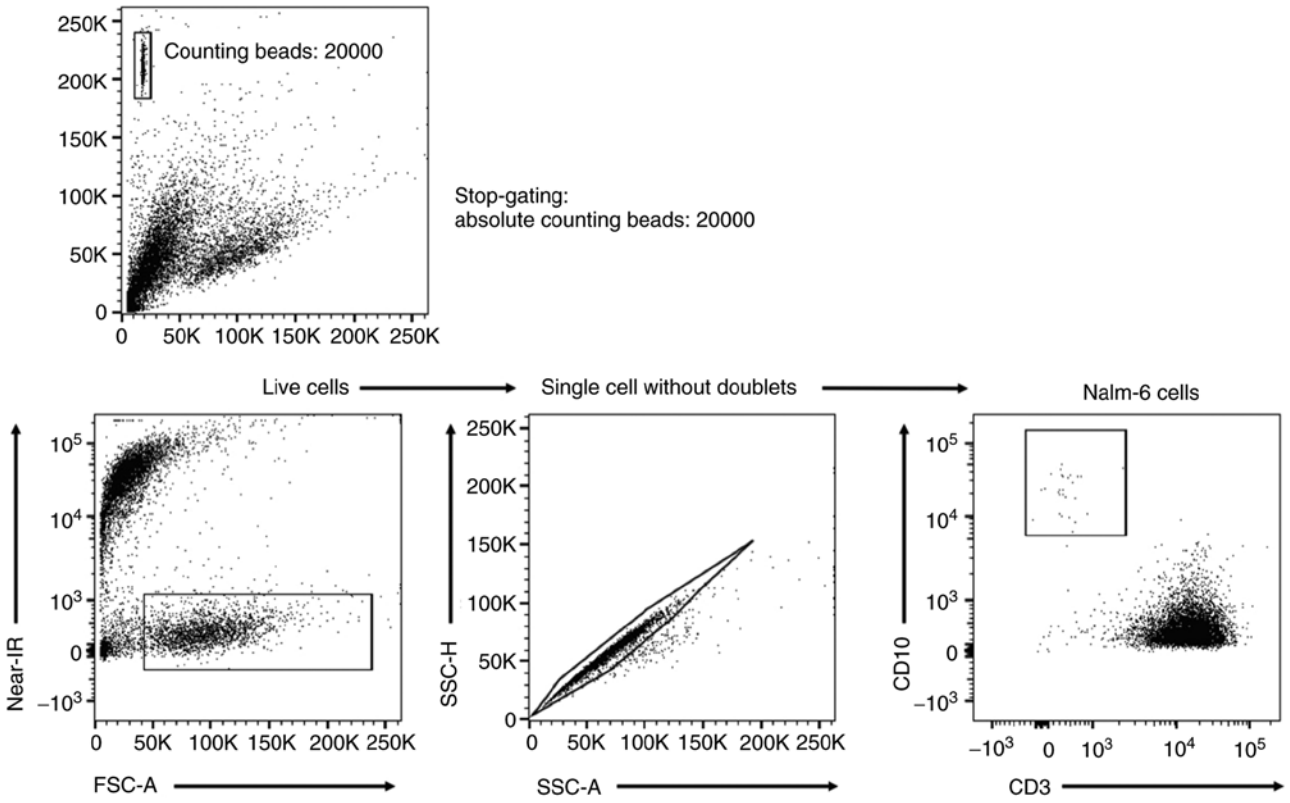

Figure S11. Representative flow cytometry data to distinguish CAR T cells from non-transduced T cells. CAR T cells (CD19 CAR PE, i.e., anti-human goat F(ab) IgG (H+L) PE)$^{+}$ were defined as CAR T cells. The anti-IgG antibody is directed against the extracellular single chain variable fragment (scFv) of the CAR. As neither natural T cells nor untransduced T cells express the CAR on their surface, flow cytometry results prove successful transduction of cells.

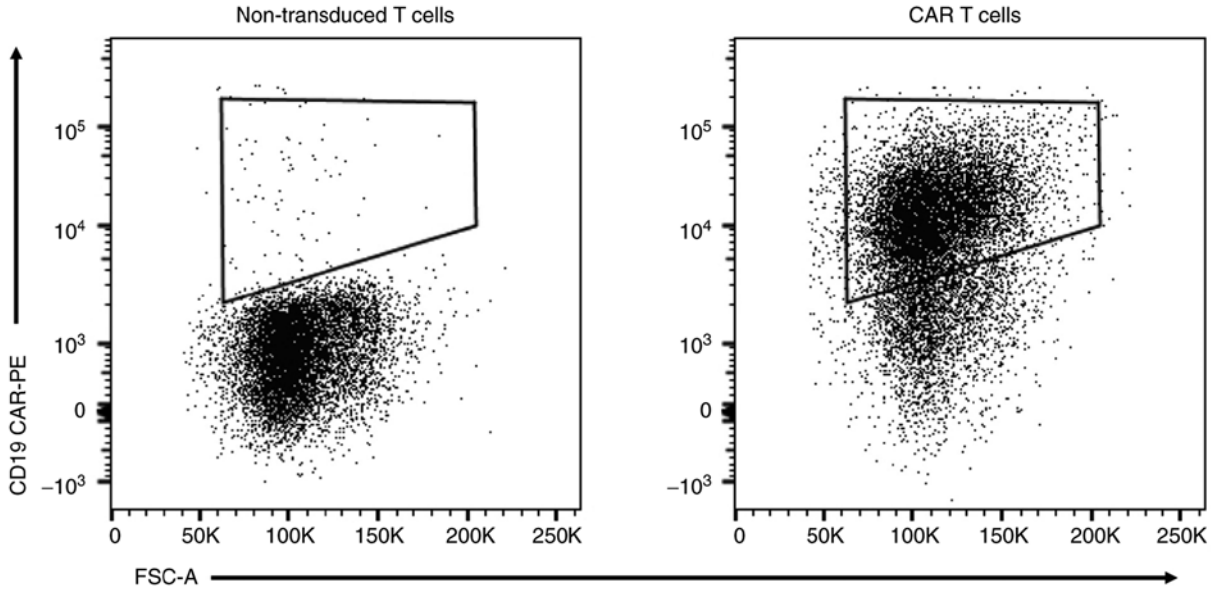

Figure S12. Gating strategy showing the approach to distinguish CAR T cells from non-transduced T cells (Fig. 11), after gating for CD3^{+} T cells. Anti-IgG staining distinguished CAR T cells from non-transduced T cells.