

Figure S1. Expression profile of T cells nucleofected with CAR(2448) mRNA. (a) Representative flow cytometry of CAR(2448) expression on T cells, detected via eGFP reporter. **(b)** Expression of CAR(2448) on T cells over a 48 hour timeframe.

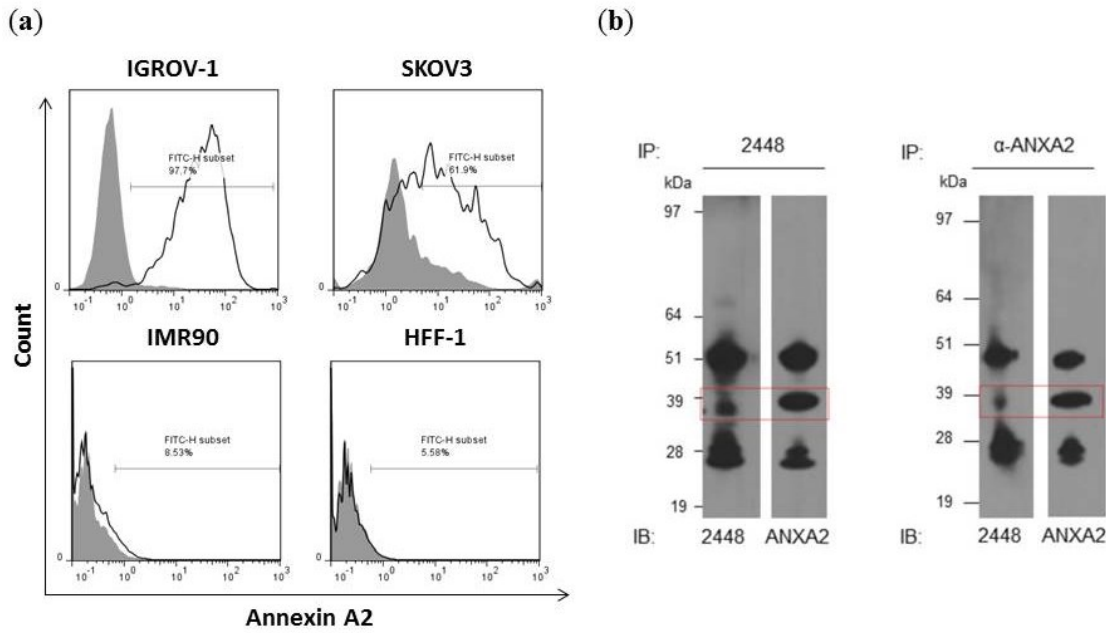


Figure S2. Expression of target antigen, annexin A2, on target cell lines. Detection of ANXA2 via mAb 2448. **(a)** Flow cytometry detection of surface ANXA2 expression. IGROV-1 is a highly ANXA2 positive cell line, while SKOV3 exhibits the heterogeneous expression of ANXA2. IMR90 and HFF-1 are ANXA2 negative. **(b)** Immunoprecipitation of IGROV-1 lysate with mAb 2448 (left panel) and commercial α -ANXA2 antibody (right panel). Cross probing with the different mAbs produce bands of a similar size (red box).

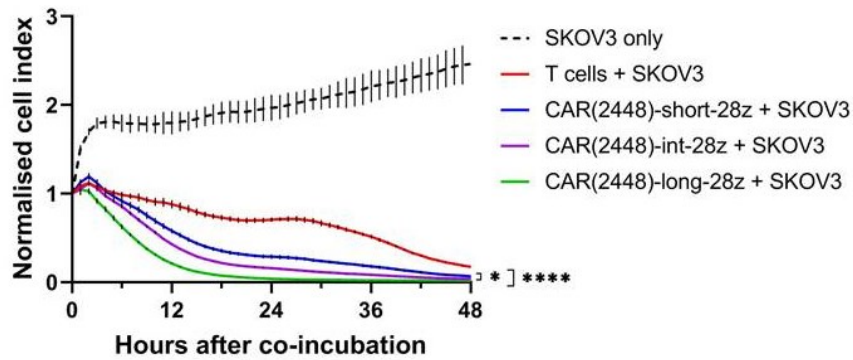


Figure S3. mRNA transfected CAR(2448) T cells mediate cytotoxicity. CAR(2448)-long-28z T cells mediate superior cytotoxicity against SKOV3 cells compared to CAR(2448)-short-28z or CAR(2448)-int-28z T cells.

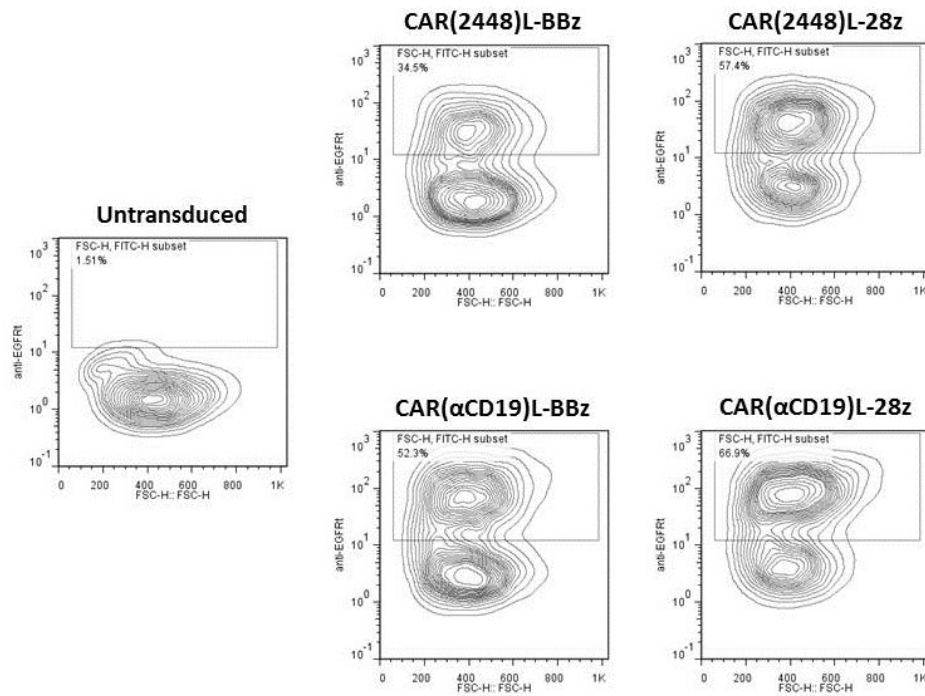


Figure S4. Expression of CAR(2448) by lentivirally-transduced T cells. Representative expression of CAR constructs on T cells, detected via cetuximab on EGFRt reporter gene.

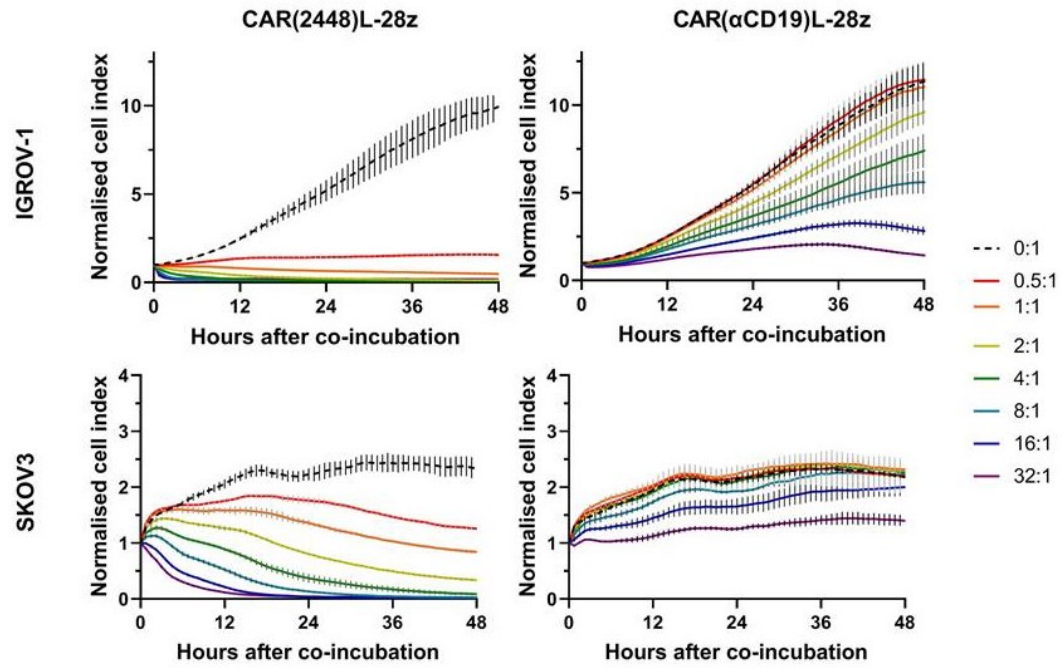


Figure S5. Cytotoxicity of CAR(2448)L-28z. Real-time cytotoxicity of CAR(2448)L-28z and control CAR(αCD19)L-28z T cells against target cells at varying E:T ratios.

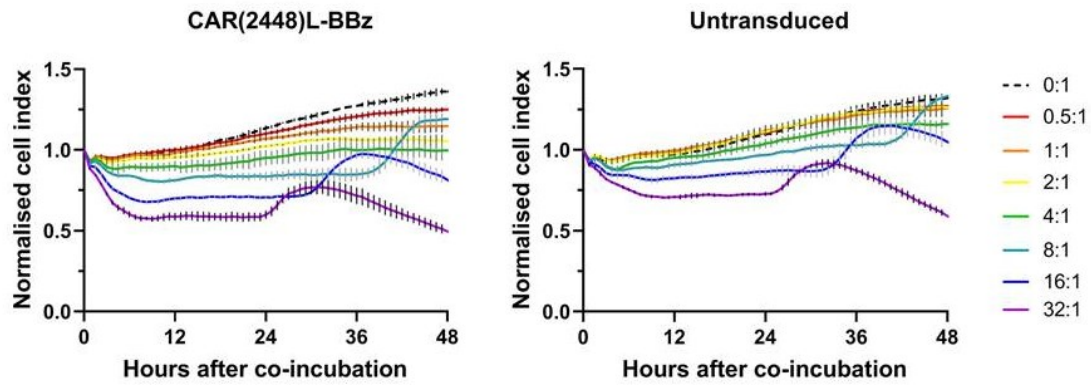


Figure S6. Cytotoxicity of CAR(2448)L-BBz against HFF-1. Real-time cytotoxicity of CAR(2448)L-BBz and control untransduced T cells against ANXA2- HFF-1 cells at varying E:T ratios. Cytotoxic activity of CAR T cells not observable in CAR(2448)L T cells against ANXA2- normal cells

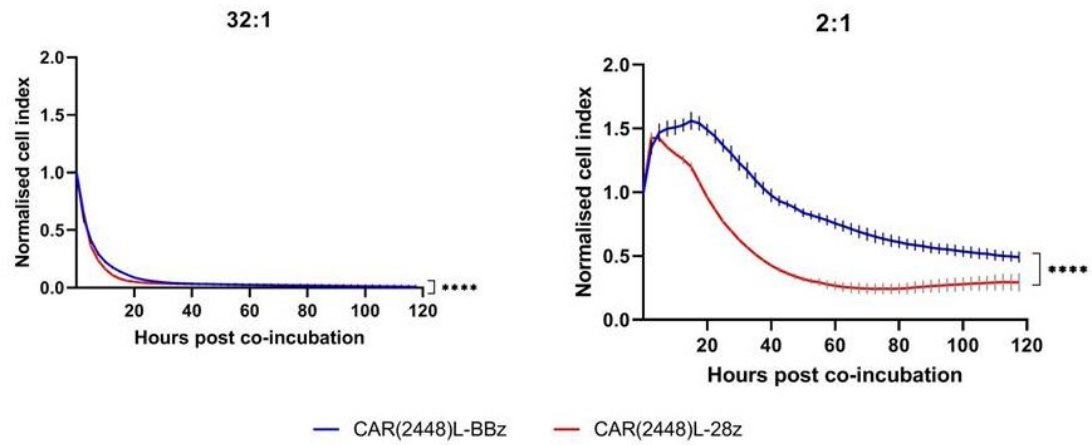


Figure S7. Lentivirally transduced CAR(2448)L T cells mediates dose-sensitive cytotoxicity upon recognition of target cells. Real-time cytotoxicity of CAR(2448)L T cells against SKOV3 target cells at 32:1 and 2:1 E:T ratios.

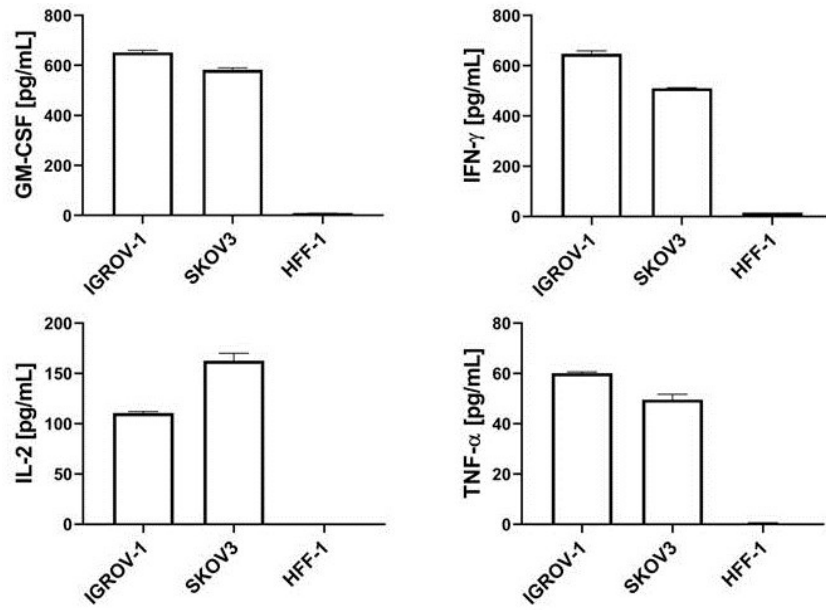


Figure S8. Cytokine release of CAR(2448)L-BBz. CAR(2448)L-BBz T cells secrete inflammatory cytokines upon recognition of ANXA2⁺ target cells, but not against the ANXA2⁻ cell line HFF-1.

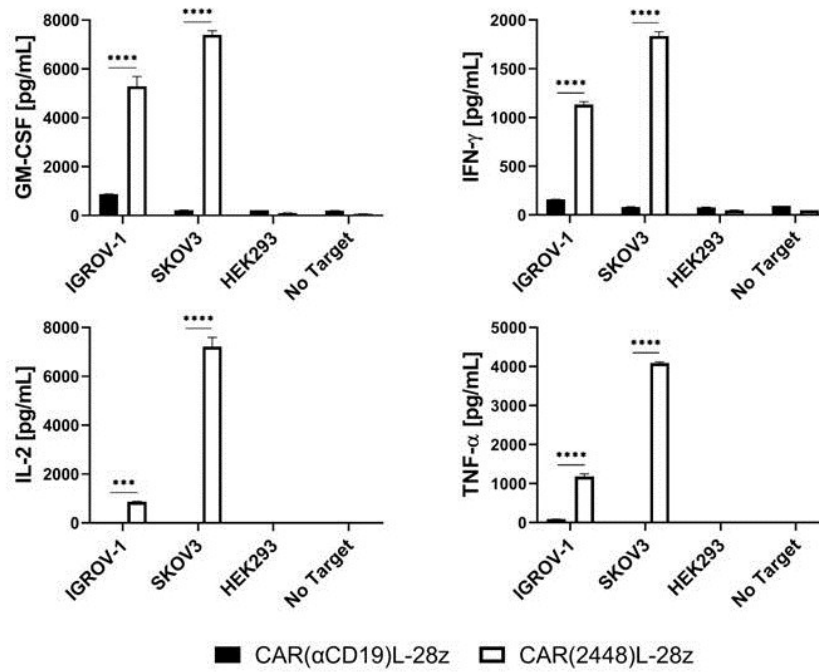


Figure S9. Cytokine release of CAR(2448)L-28z. CAR(2448)L-28z T cells secrete inflammatory cytokines upon recognition of ANXA2+ target cells, but not control CAR(α CD19)L-28z T cells. Cytokine release above basal level is not observed in co-incubation with ANXA2- cell lines.

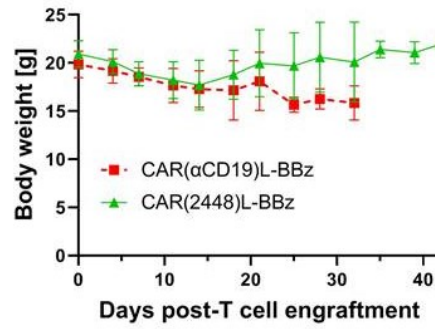


Figure S10. Body weight of mice treated with CAR T cells. Mice treated with the control CAR(α CD19)L-BBz exhibited minor weight loss compare to mice treated with CAR(2448)L-BBz as tumours progressed.