



Supplementary Figure S10. Human T cells expressing 5F9.h28BBz CAR recognize and kill GUCY2C-expressing colorectal cancer cells. A) CAR-T cells expressing a human 5F9 CAR construct (5F9.h28BBz) were stimulated for 6 hours with plate-coated antigen (BSA or hGUCY2C) or PMA and ionomycin (PMA/IONO). The T-cell activation marker CD69 and intracellular cytokines (IFN γ , TNF α , and IL-2) were then quantified by flow cytometry. B-C) Parental (CT26), human GUCY2C-expressing CT26 (CT26.hGUCY2C) mouse colorectal cancer cells, (B) or T84 human colorectal cancer cells (C) cultured in an E-Plate were treated with Control or 5F9.h28BBz CAR-T cells (E:T ratio of 10:1), media, or 2.5% Triton-X 100 and the relative electrical impedance was quantified every 15 min to quantify cancer cell death (normalized to time=0). Percent specific lysis values were calculated using impedance values following the addition of media and Triton for normalization (0% and 100% specific lysis, respectively). ***, $p < 0.001$ (Two-way ANOVA).