



Figure S4. Cytokine and cytotoxicity profile of CD20 CAR T cells with or without re-stimulation during cell expansion. Healthy donor PBMC positively selected for CD4 or CD8 were separately stimulated with anti-CD3/CD28 beads and transduced with lentiviral vector encoding the 1.5.3-NQ-28-BB-z CAR. Cells were either re-stimulated with irradiated CD20⁺ lymphoblastoid cell line (TM-LCL) cells on day 7, or left in culture without re-stimulation. Following expansion, cells were used as effectors in cytokine or cytotoxicity assays. (A and B) CD4⁺ CAR⁺ cells (A) or CD8⁺ CAR⁺ cells (B) were co-cultured with irradiated Granta-519 cells, and supernatants were harvested 24 hours later and the indicated cytokines measured by Luminex assay. (C) CD8⁺ CAR⁺ cells were used in a standard 4-hour ⁵¹Cr-release assay at the indicated effector:target (E:T) ratios, using Granta-ffLuc cells as targets. The data represent the mean (+ SD) of triplicate values.