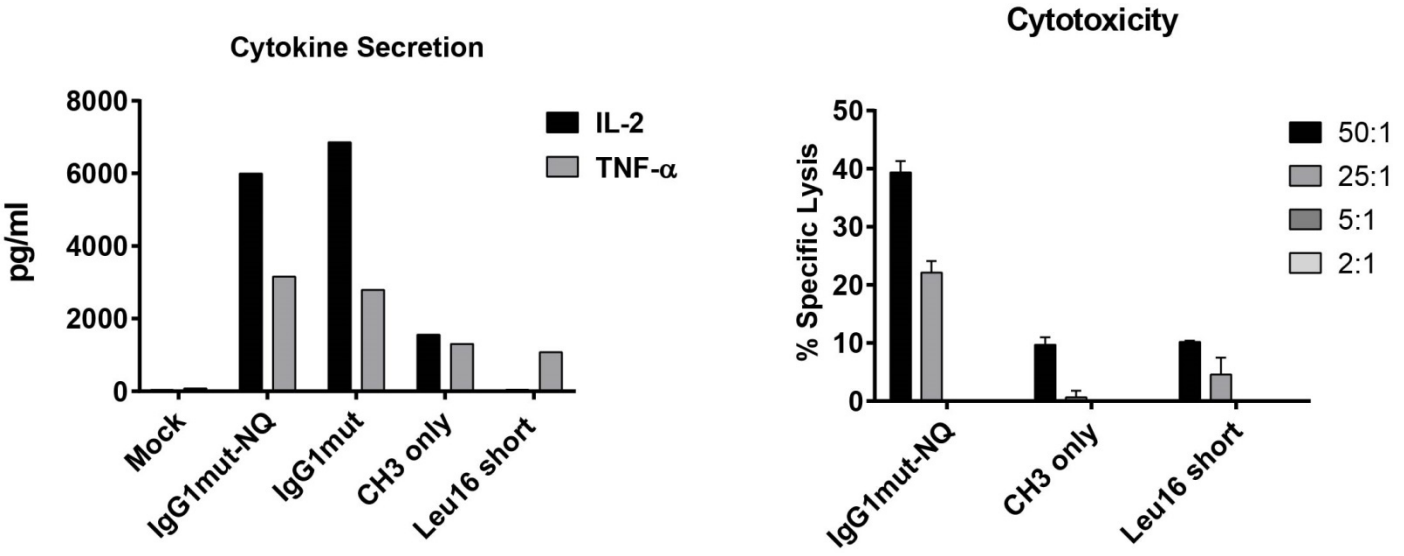

Figure S3. Optimal CD20 CAR activity requires a long spacer. T_{CM}-enriched T cells from a healthy donor were stimulated with anti-CD3/CD28 beads and transduced the next day with lentiviral vectors encoding iC9_1F5-IgG1mut-NQ-28-BB-z (“IgG1mut-NQ”), 1F5-IgG1mut-28-BB-z (“IgG1Mut”), iC9_1F5-CH2-28-BB-z (“CH3 only”), or Leu16-hinge-28-BB-z (“Leu16 short,” which contains only the hinge linker but not C_H2-C_H3), or Mock (untransduced) T cells and were expanded in vitro. Cytokine secretion by Mock, IgG1mut-NQ, IgG1mut, CH3 only and Leu16 short T cells was evaluated after stimulation with Raji-ffLuc cells. Stimulation was performed by co-incubation in a 1:1 ratio with irradiated Raji-ffLuc cells, and supernatant was collected 24 hours later and analyzed for IL-2 and TNF-α levels by Luminex assay. For the cytotoxicity assay, ^{51}Cr-labeled target cells (Raji-ffLuc) were incubated for 4 hours with IgG1mut-NQ, CH3 only, or Leu16 short transduced T cells at effector-to-target ratios of 50:1, 25:1, 5:1, and 2:1, and specific lysis based on ^{51}chromium activity in the supernatant was calculated using triplicate samples.