Supplementary Figures

Supplementary Figure 1: Design of ROR1-CARs with modified spacer length and derived from the 2A2 and R12 scFV with different affinity. (A) Design of lentiviral transgene inserts encoding a panel of ROR1-CARs containing the 2A2 scFV, an IgG4-Fc derived spacer of 'Hinge-CH2-CH3' (long spacer, 229 AA), 'Hinge-CH3' (intermediate, 119 AA), or 'Hinge' only (short, 12 AA), and a signaling module with CD3ζ and CD28. Each CAR cassette contains a truncated EGFR marker encoded downstream of a T2A element. **(B)** Lentiviral transgene inserts encoding ROR1-specific CARs derived from the R12 and 2A2 scFV with short IgG4-Fc 'Hinge' spacer (12 AA), and a signaling module containing CD28 or 4-1BB and CD3ζ respectively (total: 4 constructs).

Supplementary Figure 2: Analysis of cytokine production and proliferation of $CD4^+$ T-cells lines modified with a ROR1-CAR derived from mAb R12 with higher affinity than 2A2. **(A-B)** The 2A2 and R12 ROR1-CARs had the optimal short spacer and a CD28 costimulatory domain. **(A)** Multiplex cytokine analysis from supernatants obtained 24 hours after stimulation of $5x10^4$ $CD4^+$ T-cells expressing the 2A2 and R12 ROR1-CAR with Raji/ROR1 tumor cells. **(B)** Proliferation of $CD4^+$ R12 and 2A2 ROR1-CAR T-cells and tEGFR control T-cells 72 hours after stimulation with Raji/ROR1 cells and without addition of exogenous cytokines was assessed by CFSE dye dilution. Numbers above each histogram indicate the number of cell divisions the proliferating subset underwent, and the fraction of T-cells in each gate that underwent ≥5/4/3/2/1 cell divisions is provided above the histograms.

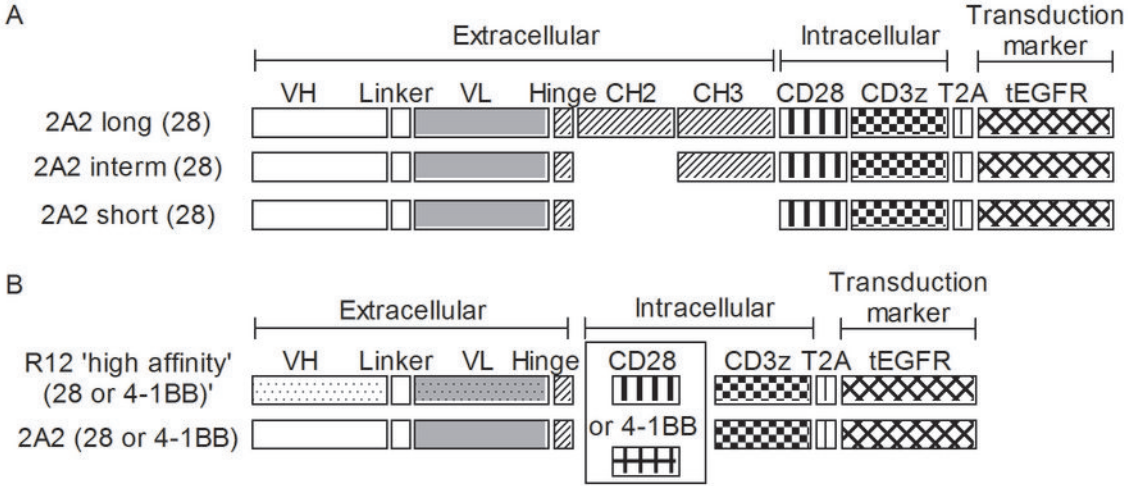
Supplementary Figure 3: *In vitro* function of T-cells expressing CD19-CARs with IgG4-Fc Hinge vs. CD8a Hinge spacer and a signaling module with 4-1BB costimulatory domain. **(A-C)** CD19-CARs contained the FMC63 scFV, a spacer derived from the 'Hinge' domain of IgG4-Fc or CD8a respectively and a signaling module with 4-1BB and CD3ζ. **(A)** Cytolytic activity of CD19-CAR modified T-cells and T-cells modified to express a tEGFR control vector against $CD19^+$ and control target cells analyzed by standard 4-hour chromium release assay. **(B)** ELISA for IFNγ production by $5x10^4$ CD19-CAR T-cells after a 24-hour co-culture with Raji tumor cells. O.D. of 1 corresponds to approximately 500 pg/mL. **(C)** CFSE dye dilution assay to measure proliferation of CD19-CAR and tEGFR control T-cells, 72 hours after stimulation with Raji tumor cells without addition of exogenous cytokines. For analysis, triplicate wells were pooled and the proliferation of live (PI^-), $CD8^+$ T-cells analyzed. Numbers above each histogram indicate the number of cell divisions the proliferating subset underwent, and the fraction of T-cells in each gate that underwent ≥3/2/1 cell divisions is provided next to the plot.

Supplementary Figure 4: The function of ROR1-CAR and CD19-CAR modified $CD8^+$ T-cells against primary CLL is augmented by CAR-modified $CD4^+$ helper T-cells. (A) ELISA for IL-2 production from triplicate co-cultures of $5x10^4$ $CD8^+$ and $CD4^+$ T-cells expressing the R12 ROR1 and CD19-CAR respectively, incubated with primary CLL for 24-hours. O.D. of 1 corresponds to approximately 800 pg/mL. **(B)** Proliferation of CAR-modified $CD8^+$ T-cells in response to primary CLL is enhanced by addition of CAR-modified $CD4^+$ T-cells. CFSE-labeled $CD8^+$ T-cells expressing the 2A2 ROR1, R12 ROR1 and CD19-CAR respectively, were co-cultured with tumor cells and with 2A2 ROR1, R12 ROR1 and CD19-CAR transduced or control untranduced $CD4^+$ T-cells ($CD8^+$:$CD4^+$ = 1:1). Proliferation of the $CD8^+$ subset was analyzed 72

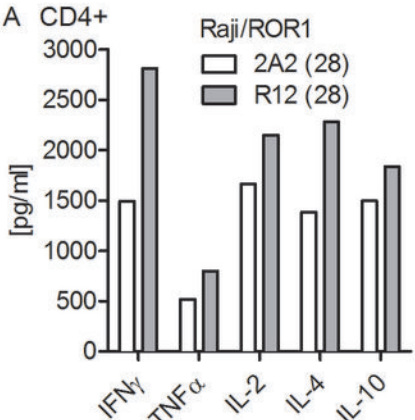
hours after stimulation. Numbers above each histogram indicate the number of cell divisions, and the fraction of T-cells in each gate that underwent ≥3/2/1 cell divisions is provided above each plot.

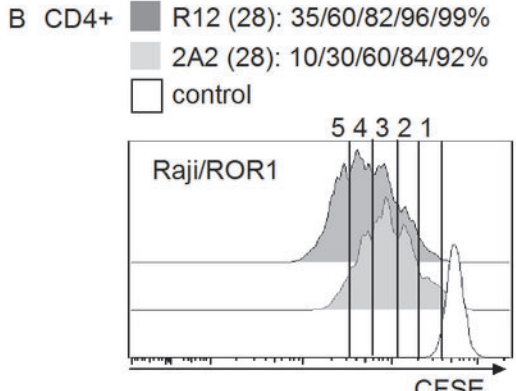
Supplementary Figure 5: Expression of ROR1 and NKG2D ligands on epithelial cancer cell lines. (A) Expression of ROR1 on the triple negative breast cancer cell lines MDA-MB-231 and 468, and the renal cell cancer lines FARP, TREP and RWL (black histograms). Staining with matched isotype control antibody is shown as grey histograms. **(B)** Expression of CD80/86 and the NKG2D ligands MICA/B on MDA-MB-231 and Raji/ROR1 tumor cells, and NKG2D (CD314) on 2A2 and R12 ROR1-CAR T-cells. Staining with matched isotype control mAbs is shown as grey dot plots/histograms.

Supplementary Figure 1

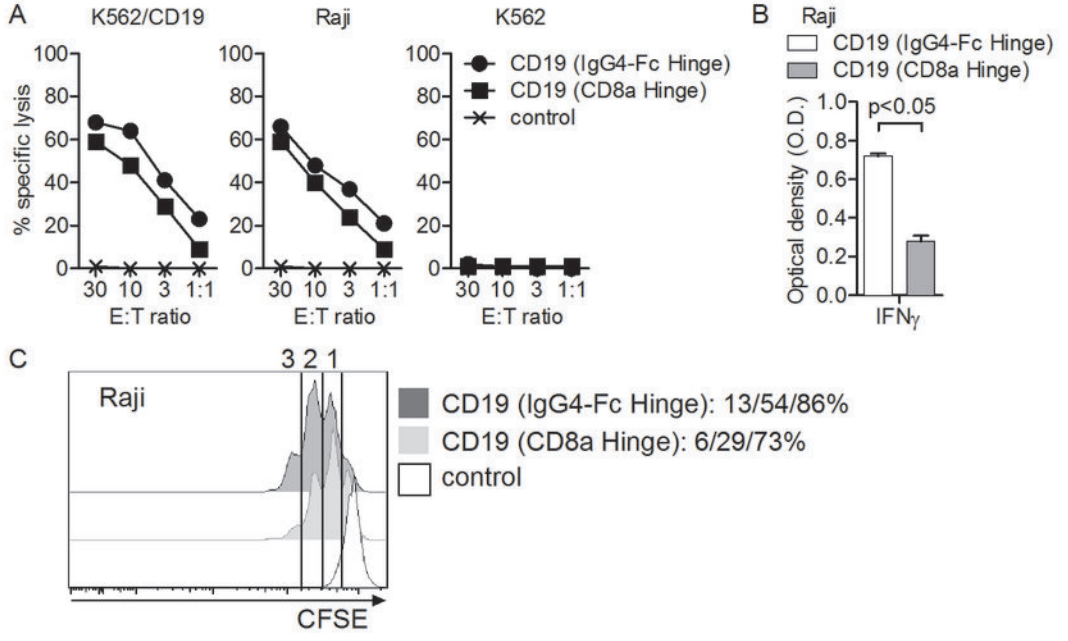


Supplementary Figure 2

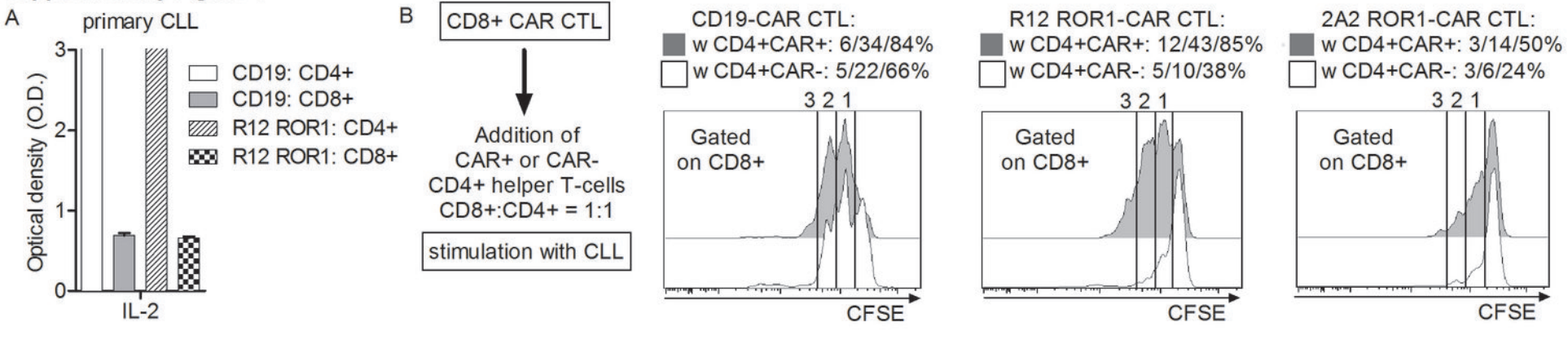




Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5