## Supplementary Material and Methods

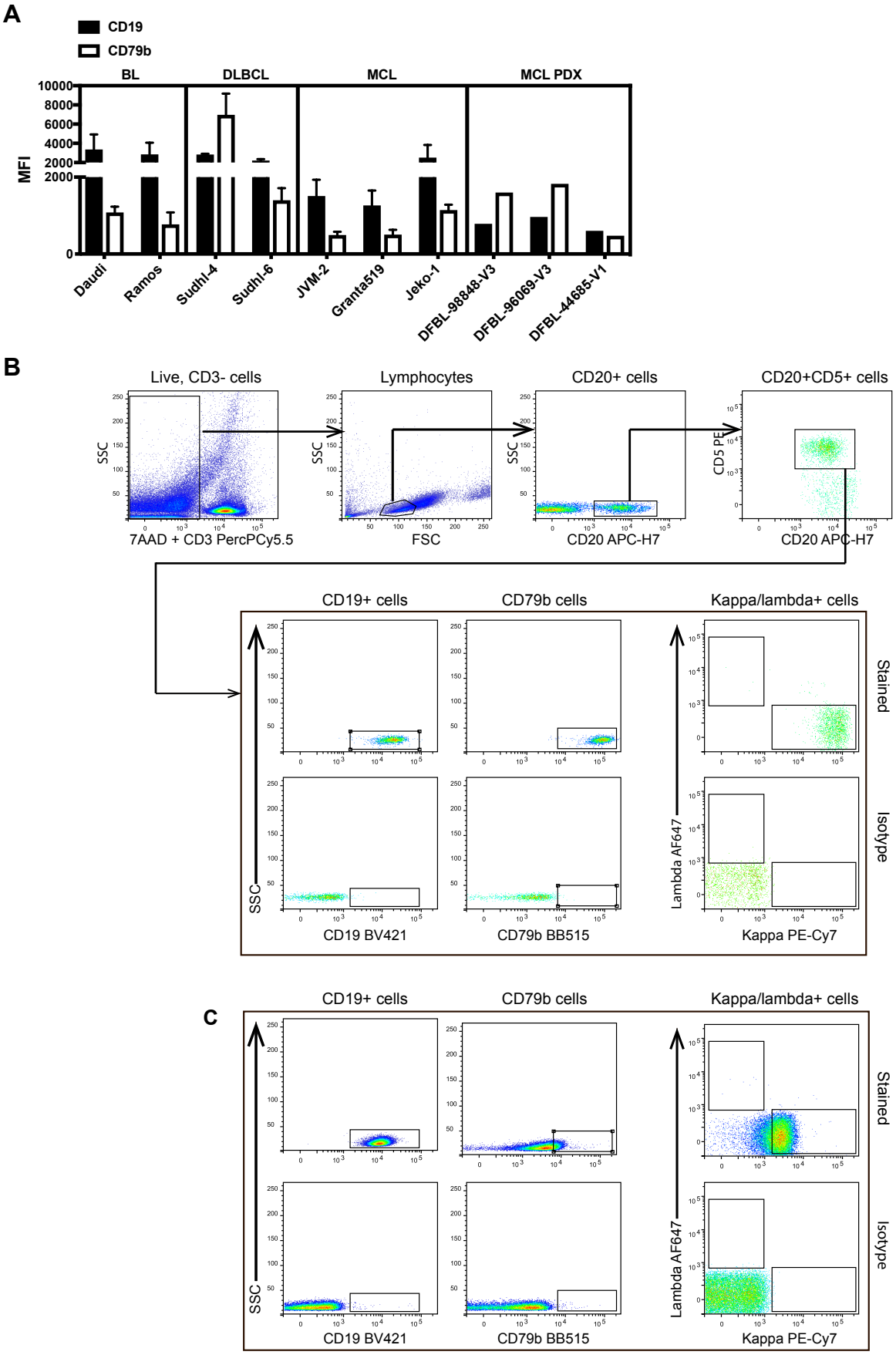
### CD19 shRNA knockdown

pLKO.1 vectors with two different shRNA inserts specific for CD19 (MISSION shRNA, Sigma) as well as empty vector were packed in HEK293T cells into lentiviral particles, which were concentrated using PEG-6000. On day 1, 10e6 Jeko-1 cells were transduced with 50 ul 100x concentrated virus in the presence of 5 µg/ml polybrene for 24h. On day 5, transduction efficiency was analyzed with flow cytometry, and selection with 0.4 µg/ml puromycin was initiated. After 11 days of selection, CD19 and CD79b expression was analyzed again using flow cytometry and SYBR green qPCR.

### SYBR green qPCR

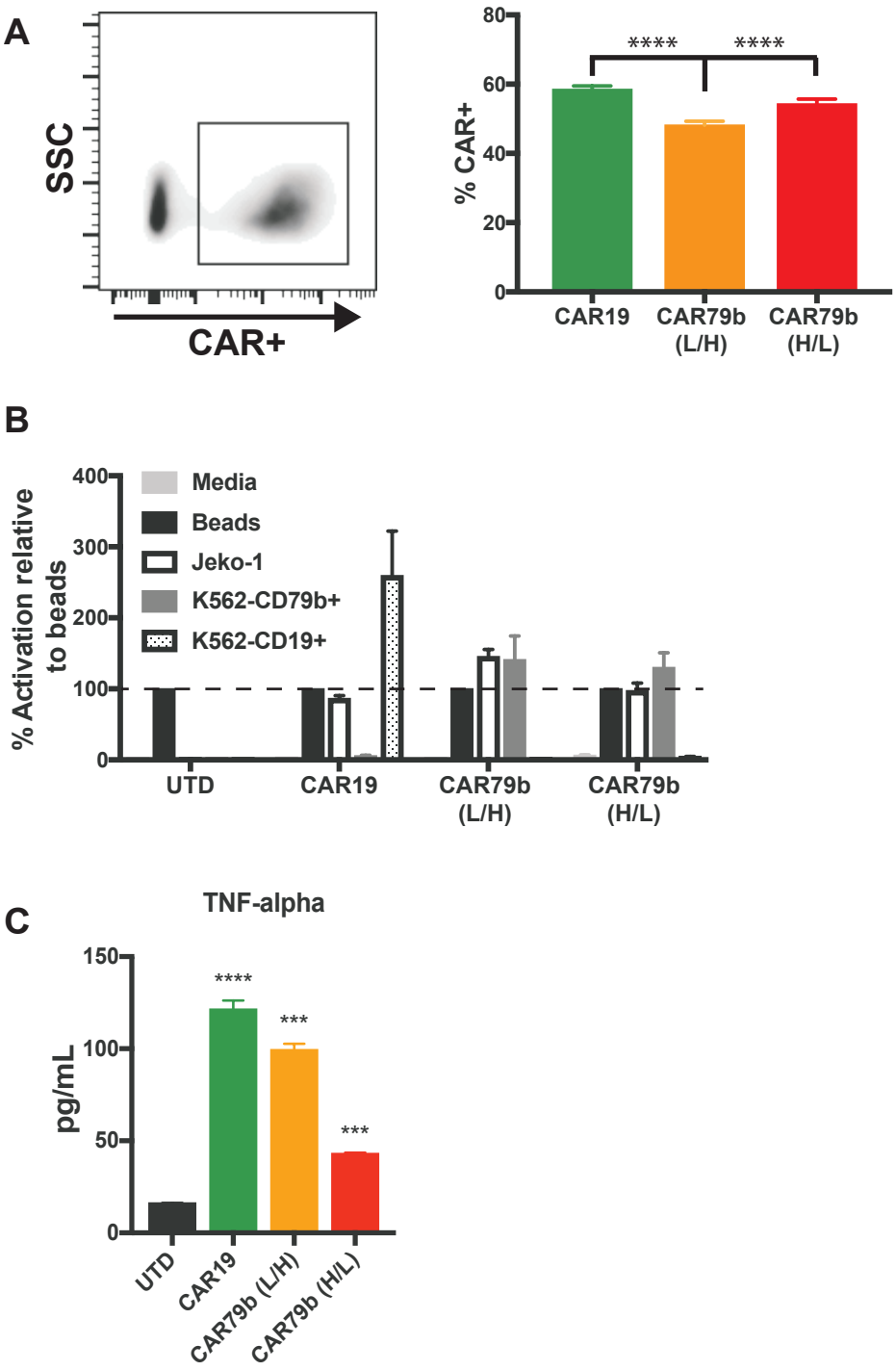
RNA was isolated from jeko-1 cells with RNeasy minikit (Qiagen). cDNA was synthesized using Maxima H Minus Reverse Transcriptase (Thermo Scientific). QuantiTect SYBR green PCR kit (Qiagen) was used for qPCR, which was run on a StepOnePlus Real Time PCR System (Applied Biosystems). CD19 forward primer: 5'AGGGAGATAACGCTGTGCTG-3', CD19 reverse primer: 5'-AGAAGGGTTTAAGCGGGGAC-3', PUM1 forward primer: 5'-GTACTGTCCCCACGATCGGA-3, PUM1 reverse primer: 5-'TGCATCCCTTGGGCCAA ATC-3'.

# Supplementary figure 1



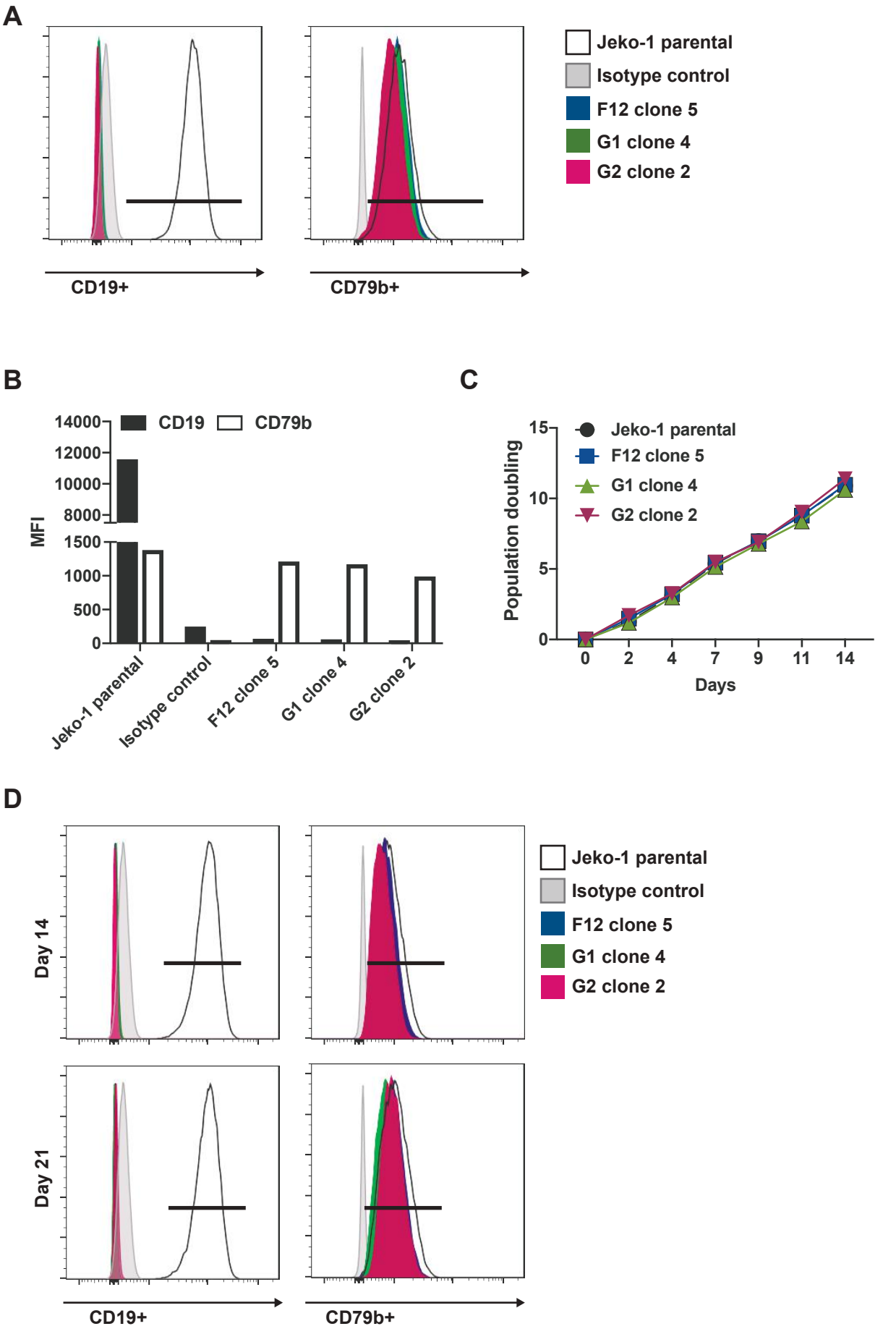

Supplementary figure 1: CD79b and CD19 expression on human lymphomas
(A) Mean fluorescent intensity (MFI), determined by flow cytometry, of CD79b and CD19 surface expression on human tumor B cell lines or mantle cell lymphoma patient derived xenografts. MM: multiple myeloma, BL: Burkitt's lymphoma, DLBCL: diffuse large B cell lymphoma, MCL: mantle cell lymphoma. Peripheral blood mononuclear cells from 6 patients diagnosed with MCL were evaluated for CD19 and CD79b expression using flow cytometry. Malignant B cells were gated as CD3-CD20+CD5+. (B) CD19 and CD79b surface staining on one MCL donor with bright CD79b expression. The CD3-CD20+CD5+CD19+CD79b+ cells are clearly defined as a kappa+ population. (C) CD19 and CD79b surface staining on one MCL donor with dim CD79b expression. The CD3-CD20+CD5+CD19+CD79b+ cells are not clearly defined as either kappa or lambda+. Dim expression of CD79b could not be clearly resolved by the chosen flourochrome thereby giving the impression of being CD79b negative.

Supplementary Figure 2



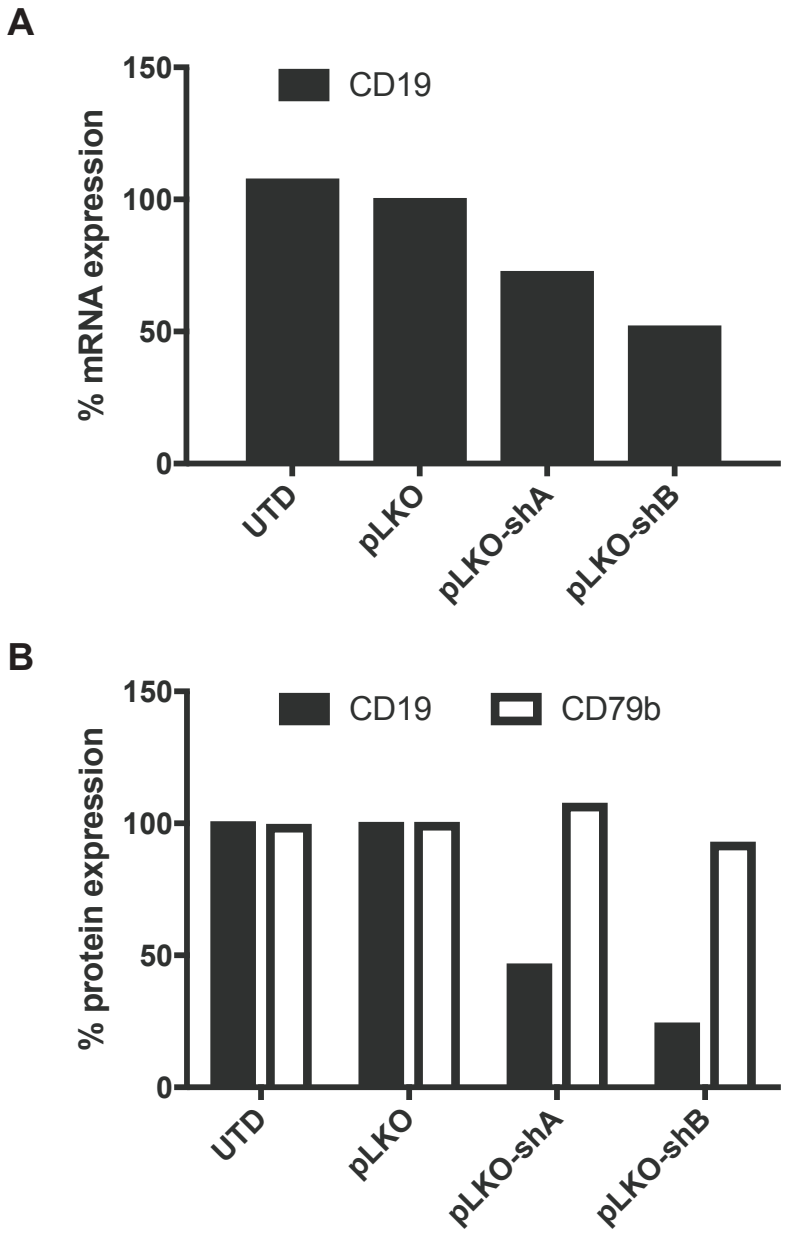

Supplementary figure 2: Transduction efficiency and in vitro activation of CAR T cells
(A) Representative flow plot of human T cells transduced with a chimeric antigen receptor, and transduction efficiency after 10 days of culture (n=3 healthy donors, mean+SEM shown). (B) Activation of Jurkat reporter (NFAT-Luc) T cells, based on luciferase activity, transduced with different CAR constructs and co-cultured overnight with target cells, anti-CD3/CD28 Dynabeads as positive control, or media as negative control (n=3, mean+SEM shown). (C) TNFa production measured by Luminex in collected cell culture supernatants after overnight co-culture of CAR T cells and target cells at a 1:1 ratio (n=2 healthy donors, mean+SEM shown, ***<0.001, ****<0.0001 by Anova).

Supplementary figure 3



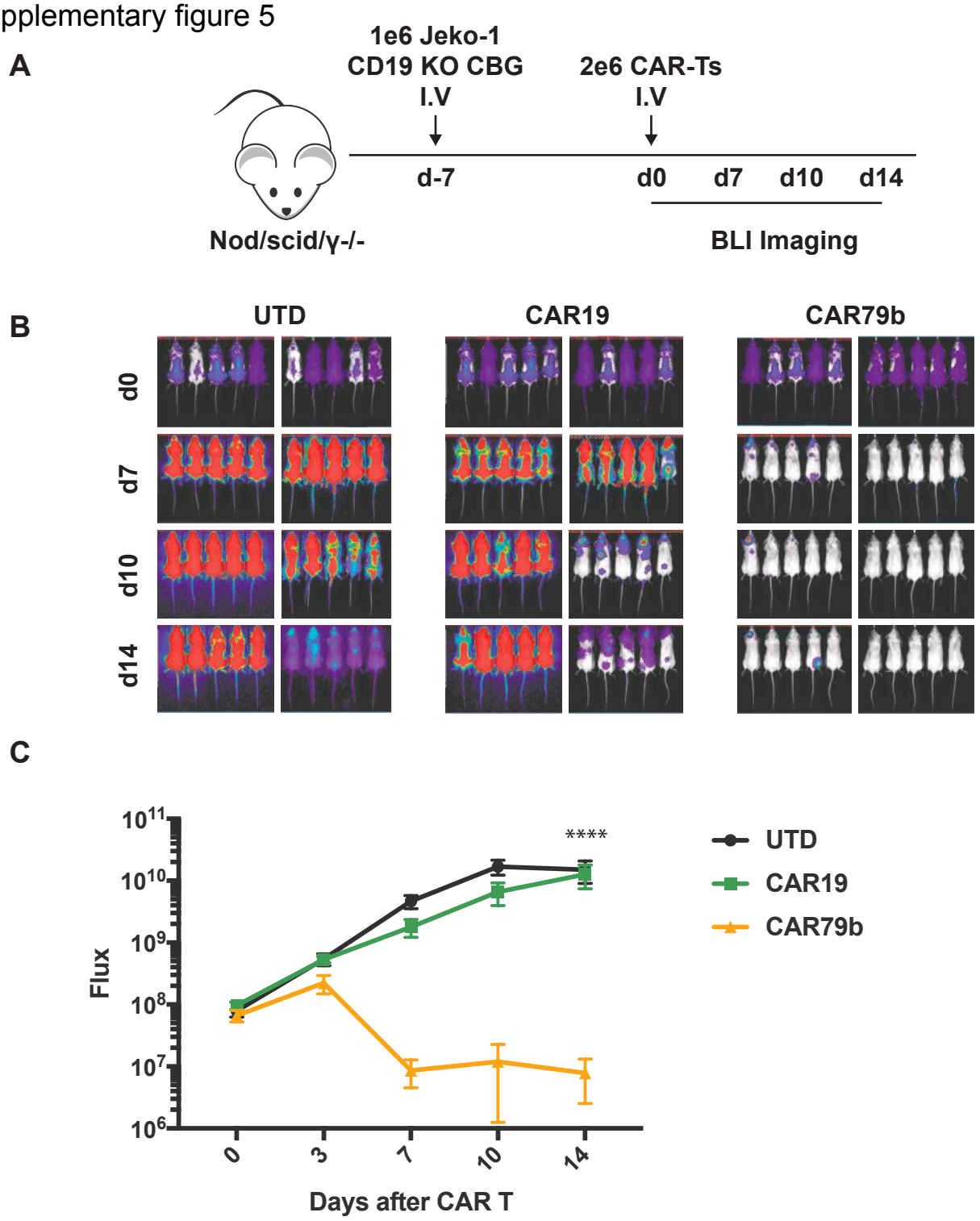

Supplementary figure 3: Loss CD19 does not reduce CD79b surface expression
(A) Representative histograms showing CD19 and CD79b surface expression, determined by flow cytometry, on parental Jeko-1 cells and Jeko-1 cells with CRISPR/Cas9 mediated knockout of CD19. (B) Mean fluorescent intensity (MFI) of surface CD19 and CD79b on parental Jeko-1 cells and CD19 knockout cell lines. (C) Comparison of long-term growth of parental Jeko-1 and CD19 negative Jeko-1 clones. (D) Representative histograms of surface CD19 and CD79b expression, determined by flow cytometry, on parental Jeko-1 or CD19 negative Jeko-1 clones at day 14 and 21 of culturing.

Supplementary figure 4



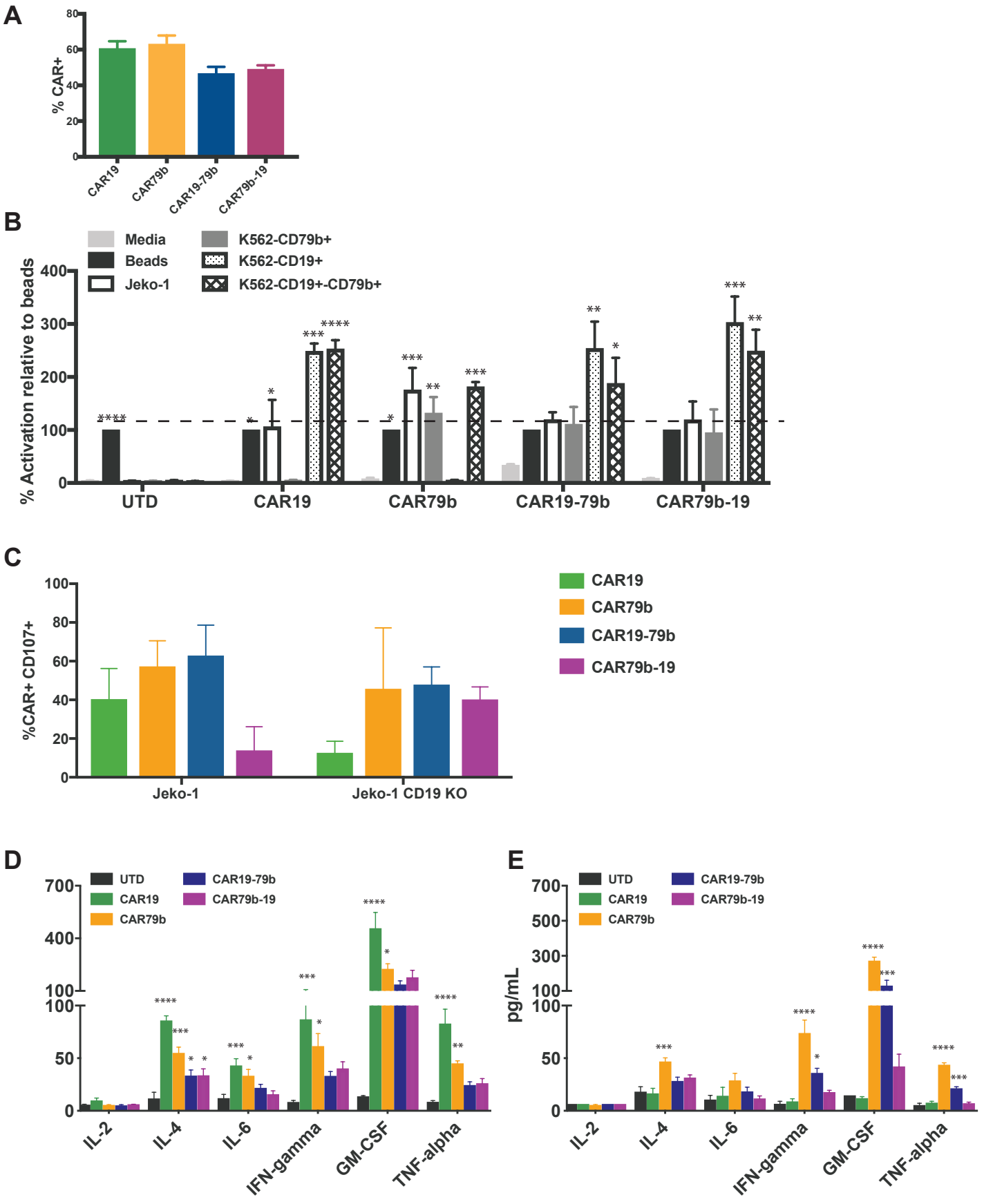

Supplementary figure 4: shRNA knockdown of CD19 does not reduce CD79b surface expression
(A) Efficient knockdown of CD19 was achieved in Jeko-1 cells with two different CD19 directed shRNAs. (B) Flow cytometric analysis of CD19 and CD79b surface expression on Jeko-1 cells after shRNA mediated CD19 knockdown. The graphs are representative of two independent experiments (one shown).

Supplementary figure 5



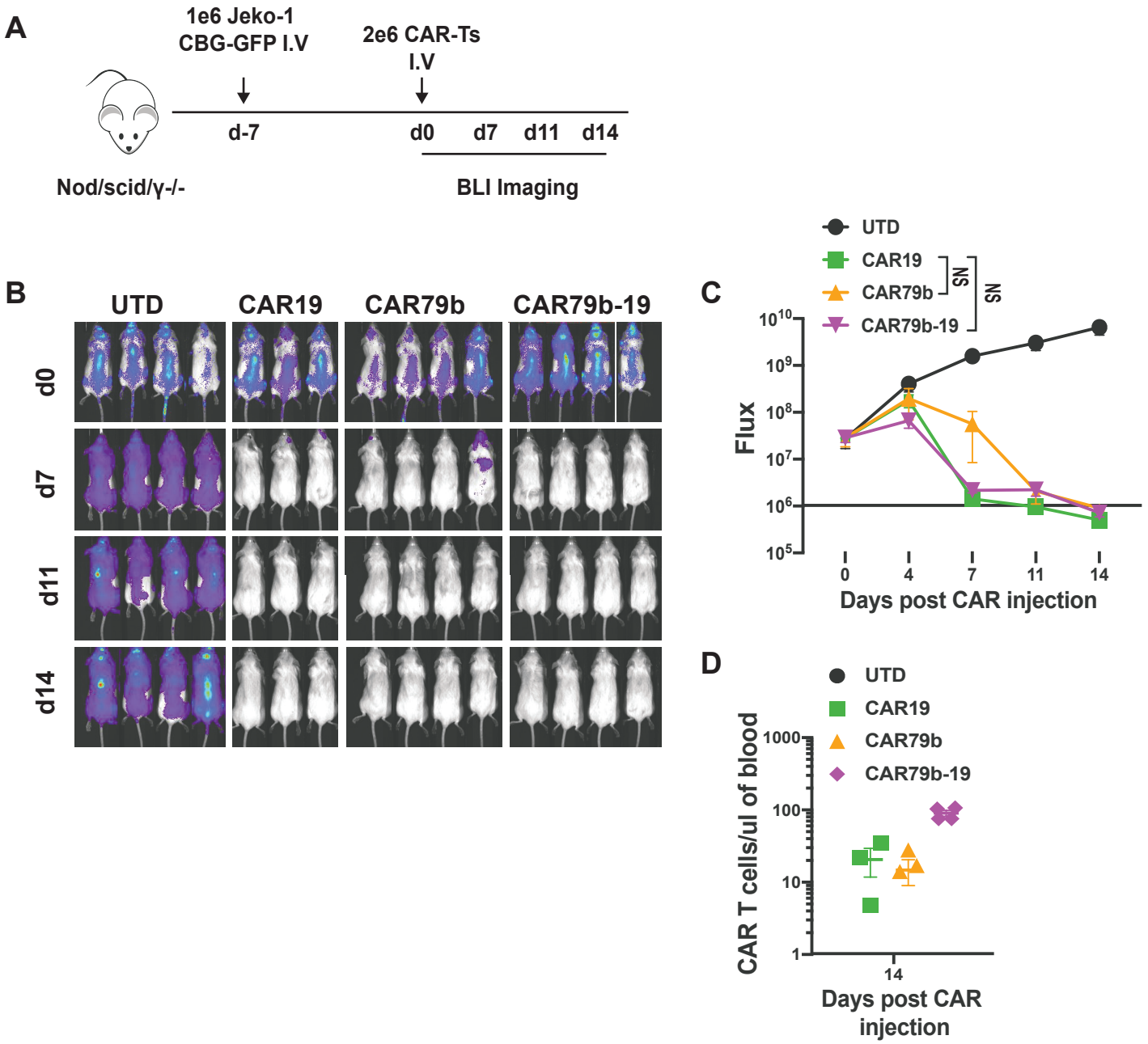

Supplementary figure 5: CD79b CAR T cells eradicate a CD19 negative MCL xenograft tumor
(A) Schematic illustration of the in vivo experiment: NSG mice received an IV injection of a total of 1e6 Jeko-1 CD19 negative CBG-GFP (F12 clone 5) tumor cells. Tumor engraftment was established with BLI imaging. At day 0, mice were grouped according to BLI expression and given a single dose of 2e6 CAR19, CAR79b (L/H) or UTD controls cells. (B) Representative bioluminescent images of CD19 negative tumor growth over time. (C) The average radiance [p/s] FLUX, of whole mice in different treatment groups over time (n=10 mice pr. group, mean±SEM shown, two-way ANOVA). Graphs are based on experiments with T cells from two different healthy donors.

Supplementary figure 6




Supplementary figure 6: Transduction efficiency and In vitro efficacy of tandem CAR T cells
(A) Tandem CAR transduction efficiency of human T cells across multiple donors (n=3, healthy donors, mean+SEM shown, ANOVA). (B) Luciferace activity of Jurkat reporter (NFAT-Luc) T cells, transduced with different CAR constructs, after overnight co-culture with target cells, anti-CD3/CD28 Dynabeads as positive control, or media as negative control (n=3 mean+SEM, ANOVA compared to UTD, *<0.05, **<0.01, ***<0.001, ****<0.0001). (B) Degranulation of CAR T cells in response to parental Jeko-1 or CD19 negative Jeko-1 (F12 clone 5) cells established by CRISPR/Cas9-mediated knockout of CD19. Effector cytokine production measured in cell culture supernatants of CAR T cells stimulated with (C) parental Jeko-1 or (D) CD19 negative (F12 clone 5) Jeko-1 cells. Cytokine analysis was performed using a Luminex array (n=3 healthy donors, mean+SEM shown, *<0.05, **<0.01, ***<0.001, ****<0.0001, ANOVA of each condition compared to UTD).

Supplementary figure 7




Supplementary figure 7: CD79b and CAR79b-19 CAR T cells clear MCL CD79b+CD19+ "upfront" tumors in vivo (A) Schematic illustration of the experimental design indicating tumor cells, timeline, dose, and imaging time points. Mice were grouped according to tumor engraftment and before injection with a single dose of 2e6 CAR19, CAR79b, CAR79b-19 or untransduced control T (UTD) cells. (B) Representative bioluminescent images of tumor growth over time. (C) The average radiance [p/s/cm2/sr] FLUX, of whole mice in different treatment groups over time (n=3-4 mice per group, mean±SEM shown, p=NS, two-way ANOVA). (D) Presence of CD3+mCherry+ cells in peripheral blood at day 14 post CAR T cell injection (n=3-4 mice per group, mean±SEM shown). Graphs are representative of T cells from one healthy donor.