

**Cell Reports Medicine, Volume 5**

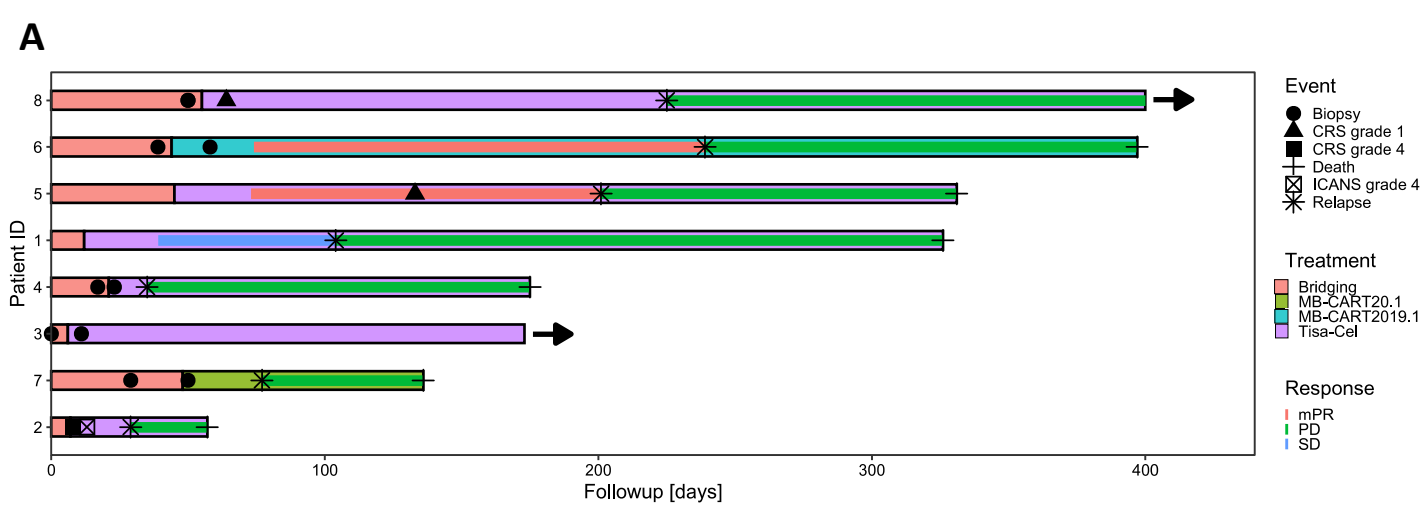
**Supplemental information**

**An anti-CD19/CTLA-4 switch improves efficacy**

**and selectivity of CAR T cells targeting**

**CD80/86-upregulated DLBCL**

**Lars Fabian Prinz, Tobias Riet, Daniel Felix Neureuther, Simon Lennartz, Danuta Chrobok, Hanna Hübbe, Gregor Uhl, Nicole Riet, Petra Hofmann, Marianna Hösel, Adrian Georg Simon, Luis Tetenborg, Paul Segbers, Joji Shimono, Philipp Gödel, Hyatt Balke-Want, Ruth Flümman, Gero Knittel, Hans Christian Reinhardt, Christoph Scheid, Reinhard Büttner, Björn Chapuy, Roland Tillmann Ullrich, Michael Hallek, and Markus Martin Chmielewski**

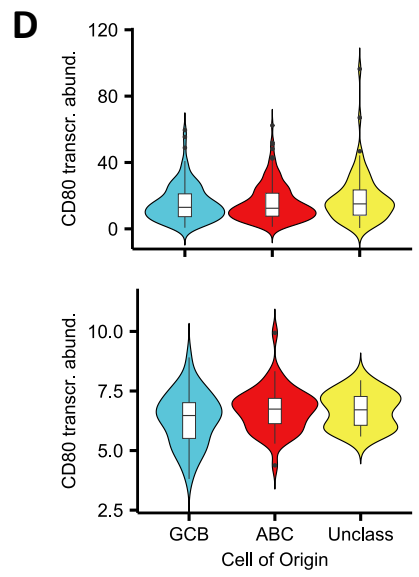


**B**

| Patient               | 1     | 2     | 3     | 4     | 5     | 6   | 7     | 8   |
|-----------------------|-------|-------|-------|-------|-------|-----|-------|-----|
| Age                   | 55    | 38    | 67    | 72    | 67    | 77  | 60    | 48  |
| Sex assigned at birth | M     | F     | M     | F     | M     | F   | M     | M   |
| ECOG                  | 0     | 1     | 0     | 1     | 1     | 1   | 1     | 0   |
| Entity                | DLBCL | DLBCL | DLBCL | DLBCL | DLBCL | tFL | DLBCL | FL  |
| COO                   | ABC   | GCB   | GCB   | UC    | GCB   | GCB | GCB   | n/a |
| DH/TH                 |       | DH    |       |       | DH    |     |       |     |
| ki67 %                | 100   | 90    |       | 80    | 90    | 10  | 60    | 25  |
| IPI                   | 2     | 0     | 4     | 4     | 3     | 1   | 1     | 2   |
| Stage                 | 4     | 2     | 4     | 4     | 4     | 2   | 4     | 4   |
| Bulk                  | -     | -     | -     | -     | -     | -   | -     | -   |
| Extranodal lesion     | +     | +     | -     | +     | +     | -   | +     | +   |
| Relapse               | +     | -     | -     | +     | +     | +   | +     | +   |

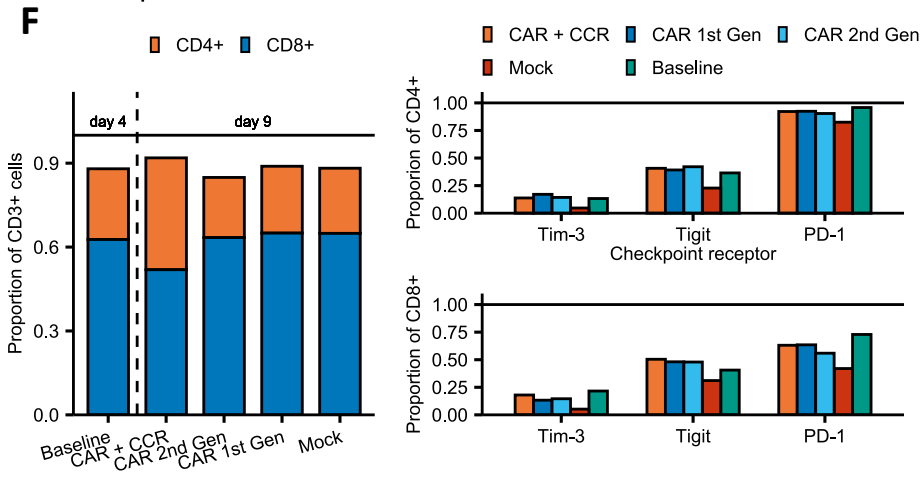
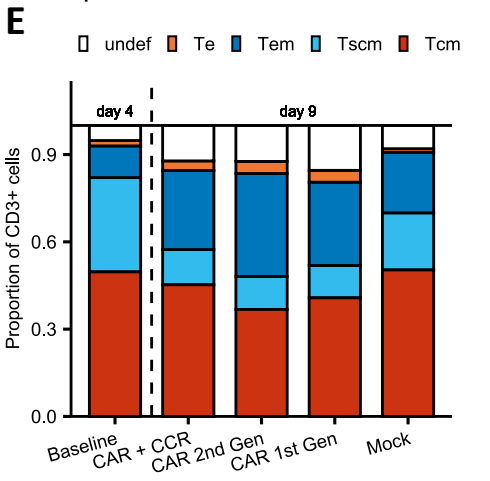
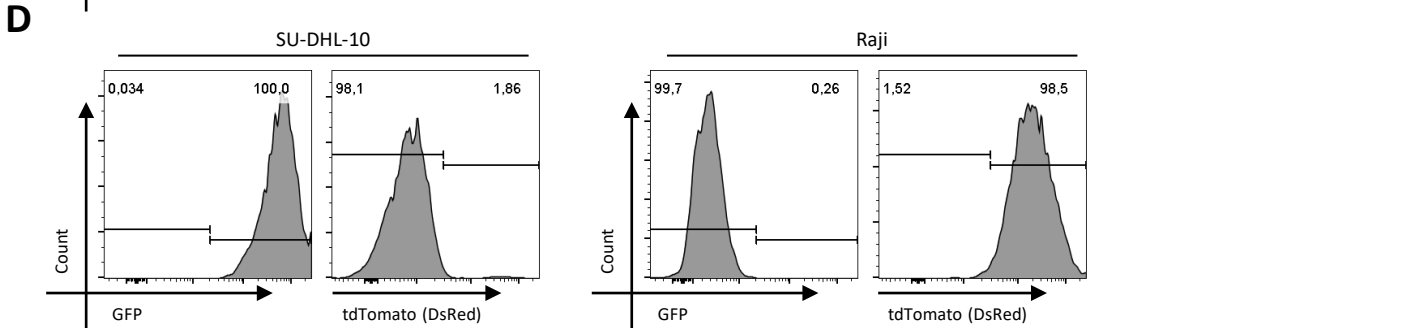
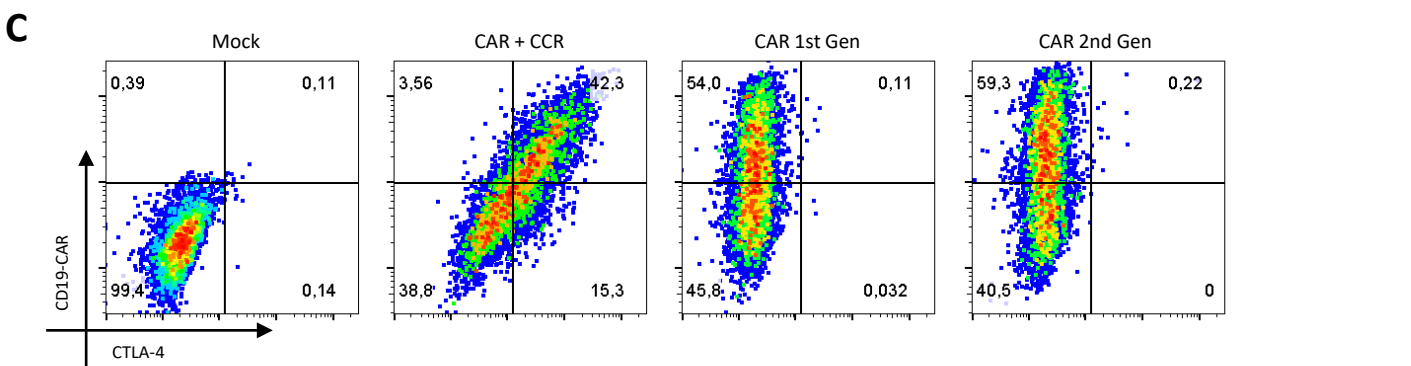
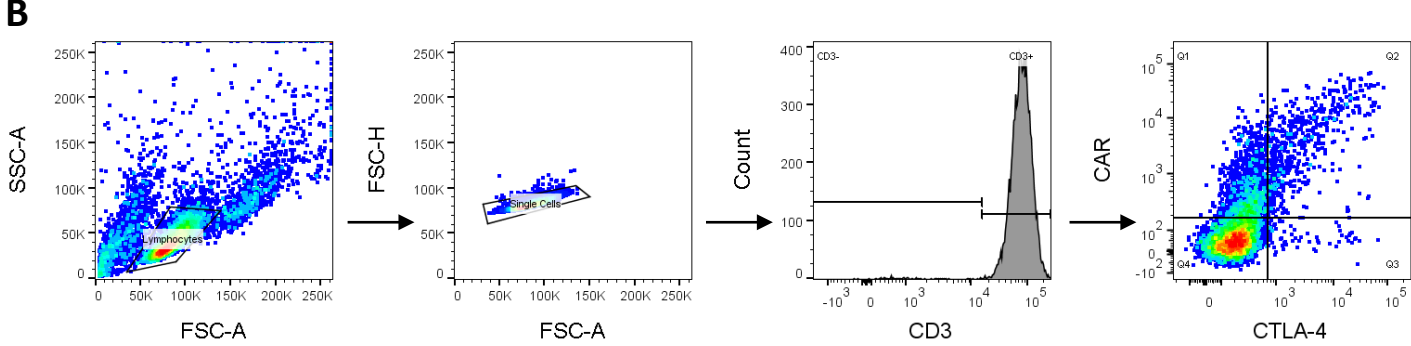
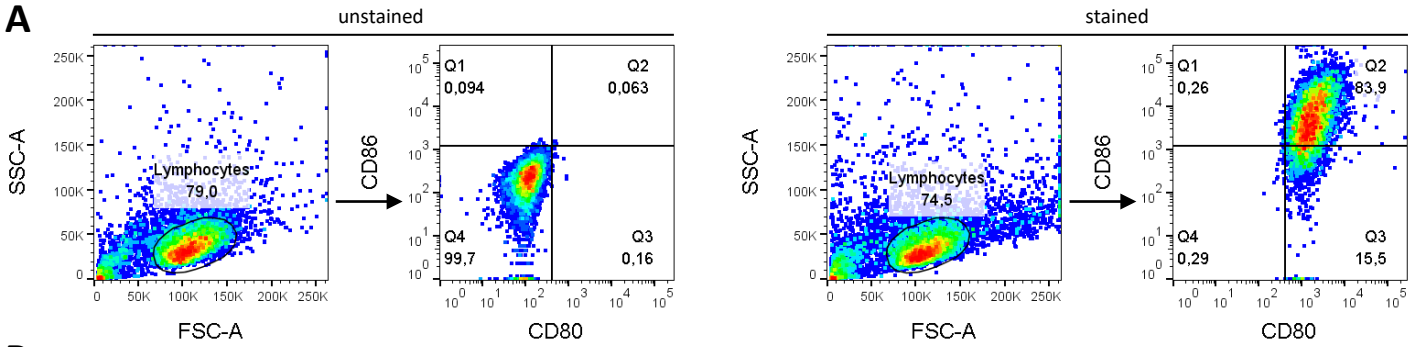
**C**

| Patient                    | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|----------------------------|---|---|---|---|---|---|---|---|
| ICU                        | - | + | - | - | - | - | - | - |
| IMC                        | - | + | - | - | - | - | + | - |
| CRS (Grade)                | - | 4 | - | - | 1 | - | - | 1 |
| ICANS (Grade)              | - | 4 | - | - | - | - | - | - |
| Infection                  | - | + | - | - | - | - | - | - |
| Drugs/Blood products       |   |   |   |   |   |   |   |   |
| Tocilizumab                | - | + | - | - | - | - | - | - |
| Steroids                   | - | + | - | - | - | - | - | - |
| G-CSF                      | - | - | - | - | - | - | - | + |
| Platelets                  | - | + | - | - | + | - | - | + |
| Cytopenias                 |   |   |   |   |   |   |   |   |
| Neutropenia <1000/ $\mu$ l | + | + | - | + | - | - | + | + |
| Neutropenia <500/ $\mu$ l  | - | + | - | - | - | - | + | + |
| Anemia grade 3/4           | - | + | - | - | + | - | - | - |
| B cell aplasia             | + | + | - | - | + | + | + | + |
| IgG <5 g/L                 | + | + | - | - | + | + | + | + |



**Figure S1, related to Fig 1: Clinical and demographic data of IHC sample patients and CD80 expression plots.**

A-C Clinical courses, patient, and tumor characteristics of CAR T patients in IHC analyses. D CD80 transcriptional abundance across COOs in Schmitz set (upper plot) and Chapuy set (lower plot).



**Figure S2, related to Fig 2: Gating strategies and additional plots related to Figure 2 and expression of fluorochromes in lymphoma cell lines**

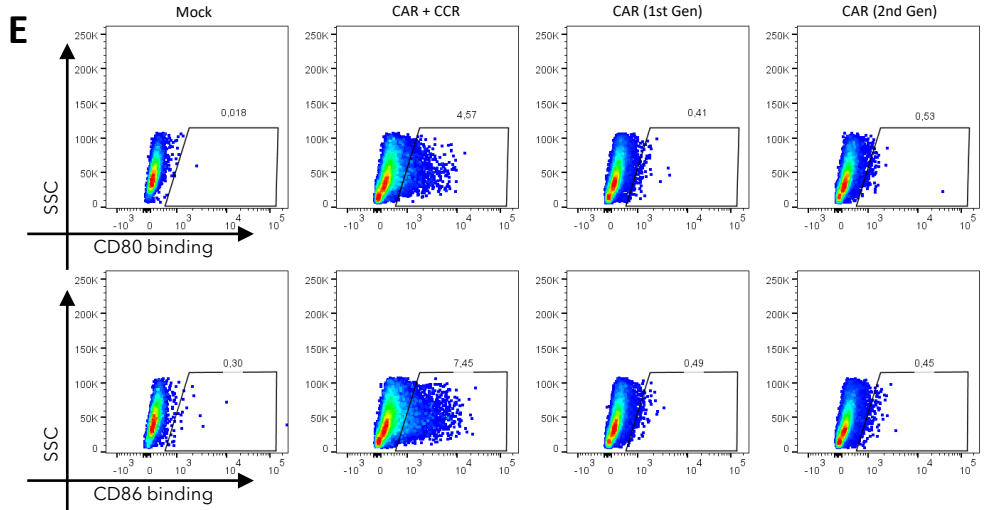
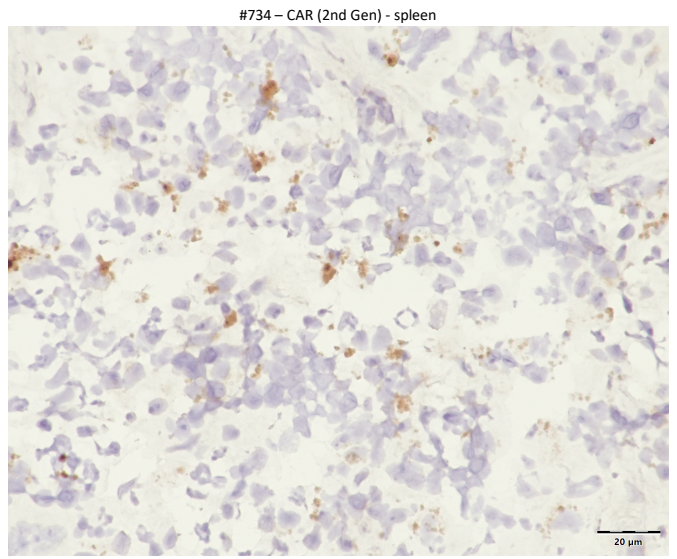
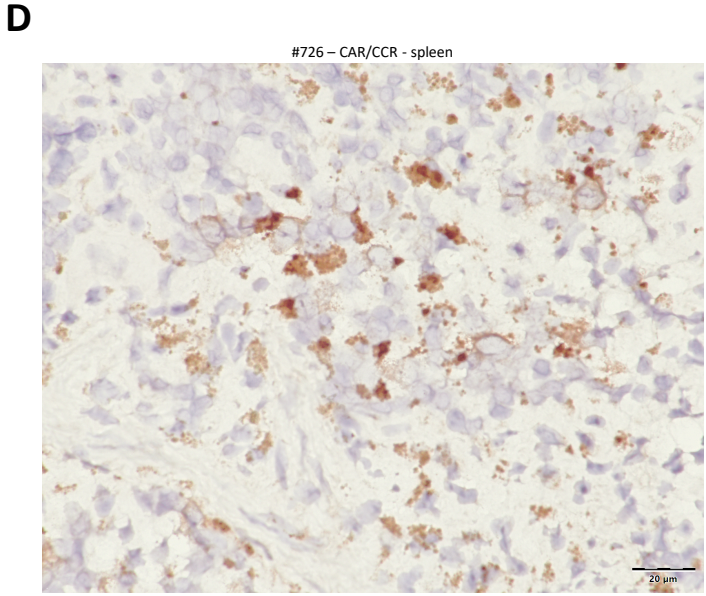
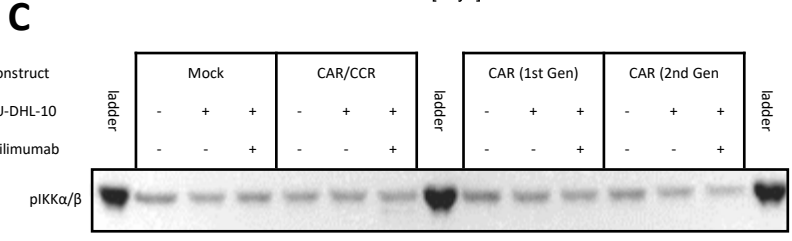
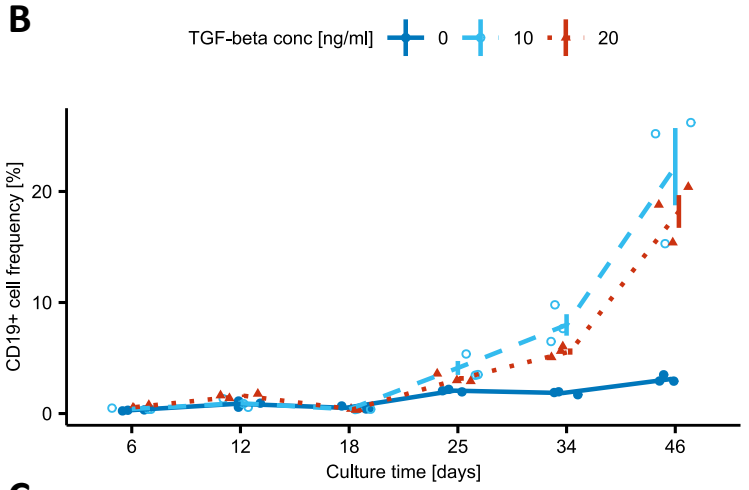
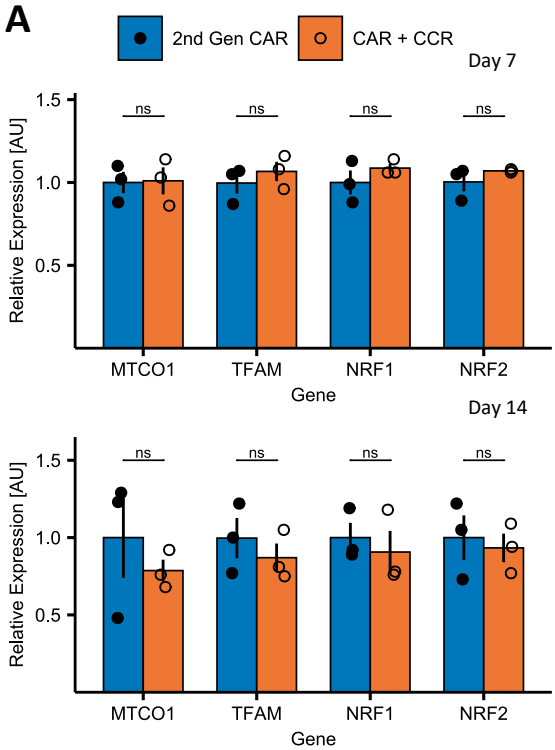
**A** Flow cytometry gating strategy used to obtain CD80/CD86 expression in lymphoma tumor cell lines (Figure 2E) following the example of the Raji cell line. **B** Flow cytometry gating strategy used to obtain flow cytometry plots showing expression of CAR and CCR receptors on primary healthy donor T cells (Figure 2C). **C** CD19 CAR and CTLA-4 CCR expression of transfected HEK293t cells to eliminate possible endogenous CTLA-4 expression on T cells. (Figure 2C) **D** Fluorochrome expression in lymphoma tumor cell lines used for in vitro cytotoxicity assays (Figure 3A) **E** Phenotype of T cells according to their differentiation into effector (Te), effector memory (Tem), central memory (Tcm) and stem cell-like central memory (Tscm) T cells before (day 4) and after (day 9) transduction. **F** Phenotype of T cells according to their expression of CD4 and CD8 and their checkpoint receptor expression.





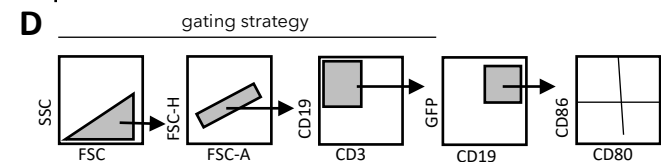
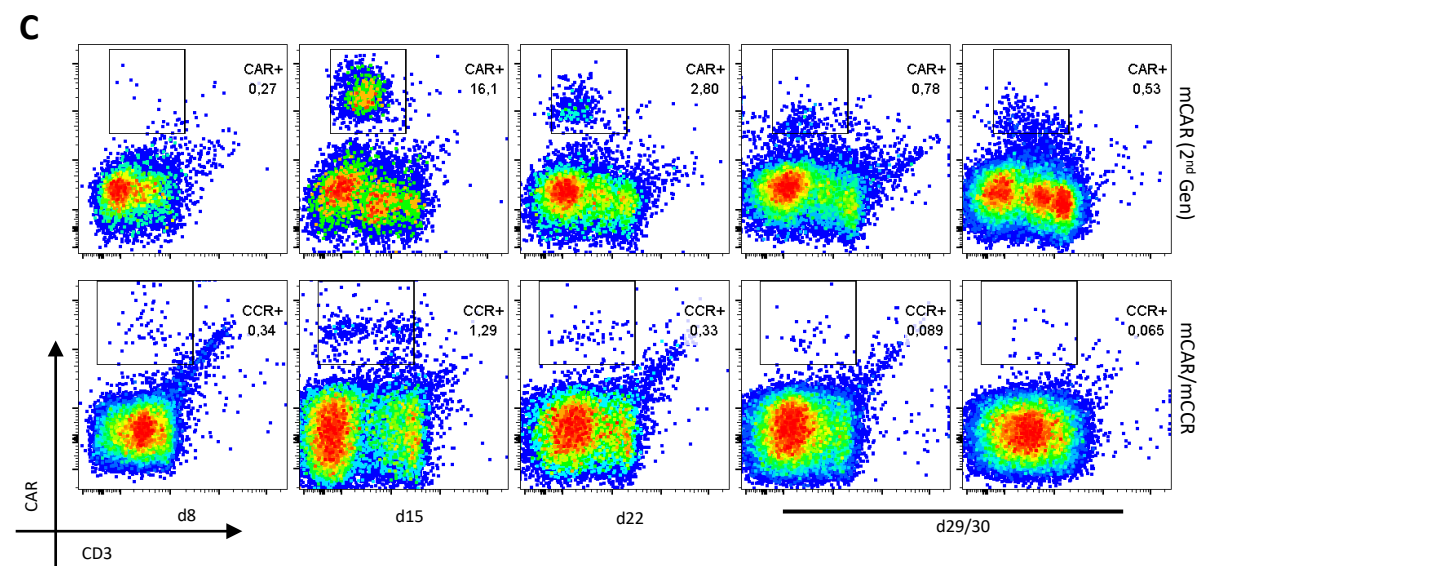
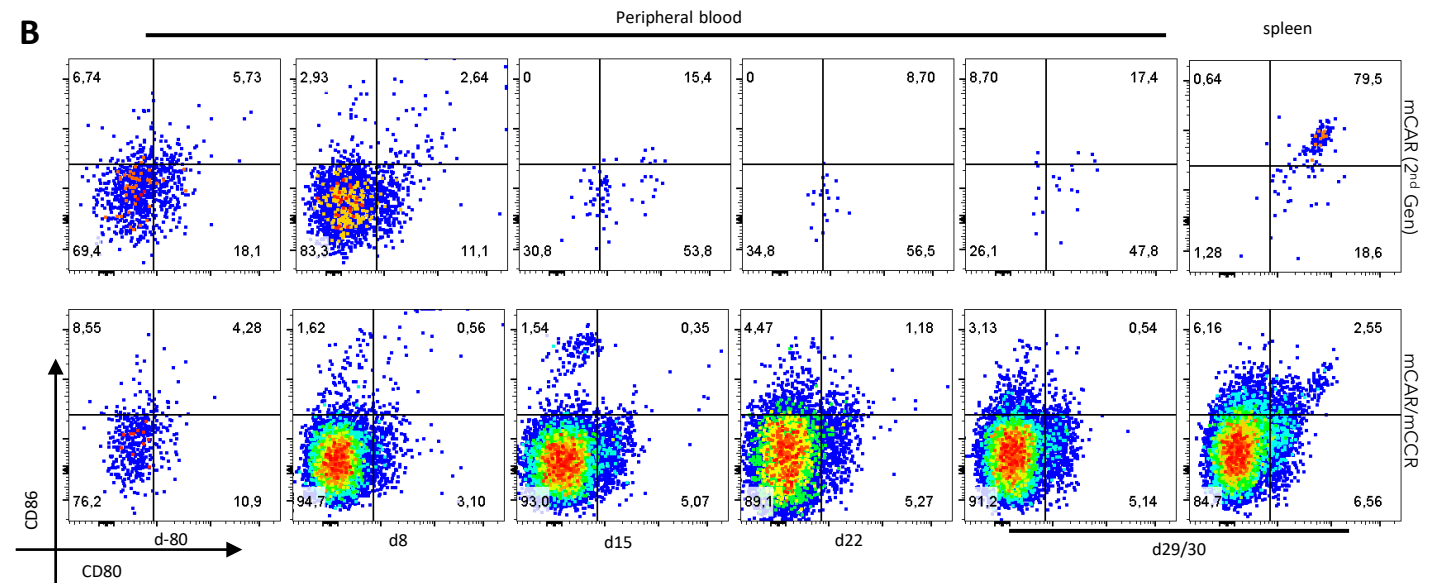
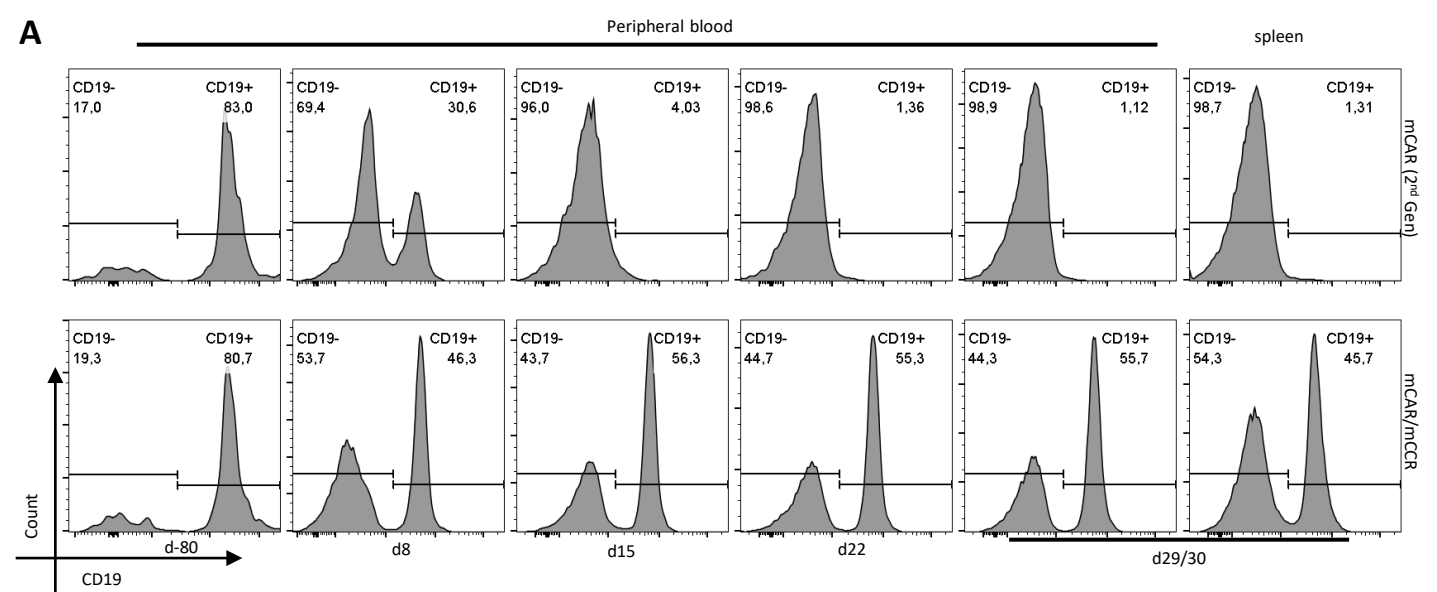
**Figure S3, related to Fig 2: The CAR + CCR concept works even with its binding domains exchanged.**

**A** CD19 and CD20 expression of SU-DHL-10 cell line (blue) compared to isotype control (red). **B** Expression of aCD20CAR and aCTLA4CCR on T cells. **C** Expression of aCD19CAR and CD3 on CAR (2<sup>nd</sup> Gen) T cells and of aCD19CAR and aCD86CCR on CAR/CD86CCR T cells. **D** Killing of CD19+CD20+ SU-DHL-10 cells and associated Interferon- $\gamma$  secretion by aCD20CAR(/CCR) and aCD19CAR/CD86CCR T cells compared with CAR (2<sup>nd</sup> Gen) and Mock.



**Figure S4, related to Fig 3, 5, 6: Additional data on CAR/CCR T cell molecular biology, pericyte differentiation, target persistence in treated mice and target antigen binding by the CCR**

**A** Results of qRT-PCR of mitochondrial genes and pro-mitochondrial transcription factors comparing CAR (2<sup>nd</sup> Gen) and CAR/CCR over 7 and 14 days. **B** Time series of CD19 expression on VW-MSC derived pericytes. (see Figure 5C-E) **C** Western blot stained for phospho-IKKa/b with each lane corresponding to a construct either without target cells, with target cells but without ipilimumab or with both target cells and ipilimumab. (Stained lane at ~64 kD is shown with the rest of the gel only showing ladder staining removed) **D** Representative pictures of spleen slides of mice from second-line xenograft mouse trial (see Figure 5) stained to show human-CD19 expression with DAB chromogen. **E** Binding of CD80 and CD86 on transfected 293t cells detected with recombinant Fc-linked CD80 and CD86 and a FITC-linked anti-human IgG antibody.



**Figure S5, related to Fig 6: Gating strategy for Figure 6D and results of B cell depletion in C57BL/6J mice.**

**A** Proportion of CD19<sup>+</sup> B cells over time in C57BL/6J mice injected with mCAR and mCAR/mCCR. **B** CD80 and CD86 expression of CD19<sup>+</sup> B cells in Supplemental Figure 5A. **C** mCAR and mCAR/mCCR cells over time in peripheral blood and spleen samples of C57BL/6J mice. **D** Gating strategy used to evaluate CD80/86 positivity rate in in vivo selectivity trial (see Figure 6D).