

Supplementary Materials.

Supplementary Tables.

Supplementary Table S1. List of CAR and TALEN® sequences used in this study.

Supplementary Table S2. List of antibodies used in this study.

Supplementary Table S3. Summary of properties for NHL samples used in this study.

Supplementary Table S4. Key features of autologous vs allogeneic CAR T-cells (1,2,11,3–10).

Supplementary Figures.

Supplementary Figure S1. Current clinical trials in www.clinicaltrials.gov identified under CAR T-cell and Lymphoma search. Combination trials, suspended or withdrawn trials were not considered. Cutt-off date December 19, 2022.

Supplementary Figure S2. A. Titration of CD20-His protein from ACRO biosystems.

Supplementary Figure S3. A. Bargraph showing CAR expression at the end of production for the experiments tested. n=15 representative of 3 independent experiments. Mean ± SD shown is shown. **B.** Bar graphs showing MFI (Mean geographic fluorescent intensity) for CD20 and CD22 CAR expression. Mean +/- SD is shown. n=2 is shown, data obtained in 2 independent experiments. **C.** Representative dot plots for CD4 vs CD8 analysis via flow cytometry. **D.** Representative flow cytometry plot showing efficiency of TRACab CD52 TALEN® mediated gene editing in CD20xCD22 CAR T-cells. **E.** Compiled information for 7 different donors in samples that did not undergo TCRab depletion. Mean ± SD shown . **F,G.** Representative flow cytometry plot and bargraph showing efficiency of TRACab knock-out before and after depletion. n=2and mean ± SD shown.

Supplementary Figure S4. A. Bargraphs showing percentage of double and single CAR achieved via co-transduction or bicistronic delivery. **B.** Representative flow cytometry plot showing CD20CAR and CD22CAR expression after co-transfection or bicistronic delivery. Two independent donors and n=6 different conditions were used for this experiment.

Supplementary Figure S5. A. Cytotoxic experiment at different effector:target ratios with indicated Raji cells to evaluate CD20 activity, n=3, mean ± SD shown. **B.** Cytotoxic experiment at different effector:target ratios with Jeko-1 cell line, n=3, mean ± SD shown. **C.** FACS plot showing the levels of CD20 and CD22 in the indicated cell lines. **D,E.** Number of CD22 and CD20 molecules respectively in Raji cells after knocking out CD20 and CD22 genes and measured with QIKIFIT® (Agilent). **F.** Cytotoxic experiment at different effector:target ratios with indicated Raji CD20 negative cells to evaluate CD20 activity. Unpaired t-test was calculated and significant samples are indicated. N=3, mean ± SD shown. **G.** IFN γ release analysis of indicated to evaluate CD20 single CAR. **H.** IFNg released by CAR T-cells non-activated and activated with PMA Ionomycin (PMA: 20ng/uL -Sigma Aldrich P8139, Ionomycin: 1uM Sigma Aldrich I0634) for 24 hours. n=4, mean ± SEM shown.

Supplementary Figure S6. **A.** Flow cytometry plots showing both expression CD20 and CD22 CAR at the end of the serial killing assay. **B.** Bar graph showing CAR+ and CD19+ cells at the end of the serial killing assay. **C.** Flow cytometry plots showing both expression CD19 and CAR (CD22) at the end of the serial killing assay **D.** Flow cytometry plots showing differentiation analysis at the end of the study. Note that CD22 CAR with CD22- cells and NTD samples had a very little amount of cells remaining as shown above.

Supplementary Figure S7. **A.** Schematics representing the antigen proliferation assay performed to evaluate proliferation properties. **B.** Representative dot plot for flow cytometry analysis for CD5+ and CD19+ cells identification. **C.** Absolute cell counts of tumor cells and CD5+ CAR T-cells after incubation at the indicated times. **D.** Absolute cell counts of cells without exposure to antigen. **E.** CAR T-cell activation upon exposure to the antigen measured by CD25 expression. **F.** Representative dot plot for flow cytometry analysis for CD25+ cells identification. **G.** Expression of the activation/exhaustion markers PD-1, TIM-3 and LAG-3 at the end of the assay. **H.** Representative dot plot for flow cytometry analysis to identify CD25+ cells. Experiment was performed with 2 independent donors. Mean ± SEM shown.

Supplementary Figure S8. **A.** Representative bioluminescence dorsal at the indicated days (n=3-5). **B.** Kaplan-Mayer curve showing the survival of the NSG animals treated with 3 millions of the indicated CAR T-cells. **C, D** Flow cytometry showing hCD45 and CD22CAR and CD19 to evaluate CAR T-cells and tumor cells the bone marrow of NSG animals treated with the dual CAR after 100 days of treatment. **E.** Graph showing the number of CD20 and CD22 molecules in Raji and Daudi cells. **F.** Flow cytometry data showing labelling of Raji wild-type and CD22 negative cell lines with a CD22-scFV-IgG1Fc protein (produced for Celllectis by Lakepharma) and a secondary anti-IgG1-Cy3 antibody (Jackson Immunoresearch, 115-165-205) and a anti-CD22 antibody targeting the N-terminal end of CD22 (Biologen, #363504). Log-rank Mantel -Cox test was calculated for survival curves. Significant values are indicated. p-value definition: * <0.05 , ** <0.01 , *** <0.001 , **** <0.00014

Supplementary Figure S9. **A.** Gating strategy to identify B-cell lineage, CD20 and CD22 cells in samples from primary NHL samples. **B.** Bargraph showing the levels of CD19+ cells in the indicated primary samples. **C.** Bargraph showing cytolytic activity based on type of B-NHL subtype. **D.** Bargraph showing cytotoxic activity based on the origin of the tumor sample. Unpaired t-test was used.

Supplementary Figure S10. **A.** Gating strategy and representative dot plots showing the identification of tumor cells ($\text{hCD45}^+\text{hCD19}^+$) and T-cells ($\text{hCD45}^+\text{CD19}^-$) in PDX models 20 days after CAR T-cell treatment.

Supplementary Table S1. CAR and TALEN® sequences used in this study

	TTTAGCCGCTCCGCTGATGCACCTGGCTATCAGCAGGGCAGAATCAGCTGTACAACGAGCTGAATCTGGGACGGAGAGAG GAATACCGACGTGCTGGATAAAAGGCCAGGACGAGATCCAGAAATGGGAGGGAAAGCCCCGACGGAAAAACCCCTCAGGAGGGC CTGTATAATGAAC TG CAGAAGGACAAAATGGCTGAGGCATACTCTGAATCGGAATGAAGGGCAGAGAGAAGGCGCGAAAA GGCCACGATGGCTGTATCAGGACTGAGTACCGCCACAAAGGACACCTACGATGCAC TG CATATGCAGGCCCTGCCACCC CGGTGA
NanoLuc_T2A EGFP	GGAGAAAGTCGACGCCACCATGGCTLEDFTVGDWRQTAGYNDQVLEQGGVSSLFQNLGVSVTPIQRIVLSENGLKDIDH VIIPYEGLSGDQMGIKEIKFKVVYPVDDHHFKVILHYGTLVIDGVTPNMDYFGRPYEGIAVFDGKKITVTGTLWNNGNKII DERLINPDGSSLFRVTINGVTGWRLCERILAAGAGCCGAGGGCAGAGGCAGCTGACCTGCGCGACGTGGAGGAGAA CCCCGGGCCCATGGTGVSKEELFTGVVPILVELDGVNVNGHKFSVSGEGEGLDATYGKLTLKFICTTGLPWPWLTWT YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKE DGNILGHKLEYNN SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDPVLLPDNHYLSTQSALSKDPNEKRDHMVLLFVTAA GITLGMDELYKTGAGGCGGCCCTCGAC

Supplementary Table S2. Antibodies used in this study.

Exhaustion			
Antigen	Fluorochrome	Company	RRID
PD-1	BV421	BioLegend (329920)	AB_10960742
LAG-3	PerCP-eFluor710	eBioscience (46-2239-42)	AB_2573732
TIM-3	APC	BioLegend (345012)	AB_2561718
CD4	PE-Vio770	Miltenyi Biotec (130-113-227)	AB_2726038
CD8	BV510	BD Bioscience (563919)	AB_2722546
Viability	e780	Invitrogen (65086514)	
Differentiation			
Antigen	Fluorochrome	Company	
CD45RA	APCCy7	Miltenyi Biotec (130-113-363)	AB_2726133
CD62L	PE	Miltenyi Biotec (130-113-625)	AB_2733829
CD4	PE-Vio770	Miltenyi Biotec (130-113-227)	AB_2726038
CD8	BV510	BD Bioscience (563919)	AB_2722546
Viability	e780	Invitrogen (65086514)	
Dual CAR staining			
CD22 Protein	Cy3 (secondary)	Lakepharma (custom made)	
CD20 Protein- His	APC (secondary)	AcroBiosystems (CD0-H52H3)	
Anti-His	APC	BioLegend (362605)	AB_2715818
Anti-Fcgamma	Cy3	Jackson ImmunoResearch (115-165-205)	AB_2338694
CD4	APC-Vio770	Miltenyi Biotec (130-113-211)	AB_2726022
CD8	VB510	BD Bioscience (563919)	AB_2722546
Serial Killing staining 1			
CD22-Fc	Cy3 (secondary)	Lakepharma (custom made)	
CD20 Protein- His	APC (secondary)	AcroBiosystems	
Anti-His	APC	BioLegend (362605)	AB_2715818
Anti-Fcgamma	Cy3	Jackson ImmunoResearch (115-165-205)	AB_2338694

CD4	FITC	Miltenyi Biotec (130-114-585)	AB_2726703
CD8	VB510	BD Bioscience (563919)	AB_2722546
CD19	PeCy7	Miltenyi Biotec (130-113-170)	AB_2733209
Viability	e780	Invitrogen (65086514)	
Serial Killing staining 1 (Differentiation profile)			
CD22-Fc-AF647	AF647		
PD-1	VB510	BD Bioscience (563076)	AB_2737990
CD45RA	APCCy7	Miltenyi Biotec (130-113-363)	AB_2726133
CD62L	PE	Miltenyi Biotec (130-113-625)	AB_2733829
CD4	PE-Vio770	Miltenyi Biotec (130-113-227)	AB_2726038
CD19	FITC	Miltenyi Biotec (130-091-328)	AB_244222
CD5	AF700	Biolegend (364025)	AB_256625
CD8	VioBlue	Miltenyi Biotec (130-110-683)	AB_2659239
Bone marrow analysis			
Antigen	Fluorochrome	Company	
CD45	PE	Miltenyi (130-110-632)	AB_2658239
mCD45	BV-421	BD Biosciences (560501)	AB_1645275
CD5	AlexaFluor-700	BioLegend (300632)	AB_2632671
CD8	VB510	BD Bioscience (563919)	AB_2722546
CD4	PerCP-Vio-770	Miltenyi Biotec (130-113-228)	AB_2726039
CD19	PE-Vio700	Miltenyi Biotec (130-113-170)	AB_2733209
CD22 Fc	AlexFluor-647	LakePharma (custom)	
B-NHL samples			
CD45	APC	BD Bioscience (555485)	AB_398600
CD20	BV421	BD Bioscience (562873)	AB_2737857
CD22	PE	BioLegend (363504)	AB_2564610
CD3	PeCy7	BD Bioscience (563423)	AB_2738196
CD56	PeCy7	BD Bioscience (557747)	AB_396853
CD33	PeCy7	BD Bioscience (333952)	AB_333952
Tumor cell lines characterization/Qifit a			
CD22	PE	Biolegend (363504)	AB_2564610
CD22	Biotin	Biolegend (363510)	AB_2564615
CD20	APC	Biolegend (302310)	AB_314258
CD20	Biotin	Biolegend (302350)	AB_2565524

Supplementary Table S3

Sample number	Diagnostic	Tissue	Viability after thawing %	% B-cell lineage	% CD22 ⁺	% CD20 ⁺	% CD20 ⁺ CD22 ⁺
1	MCL	bone marrow	93.9	69.8	98	93.3	92.8
2	MCL	lymph node	91.2	79.3	99.6	99.5	96.3
3	MZL	lymph node	64.3	55.8	91.2	97.6	96.4
4	FL	lymph node	90.7	79.9	99.8	99.4	98.8
5	MCL	bone marrow	55.9	10.5	53.5	8.17	8
6	MCL	Bone marrow	63	7.61	16.8	0.025	0
7	MZL	lymph node	72.9	21.9	91.2	93.6	88.6
8	DLBCL	lymph node	82.3	34.4	83.8	77.1	72.1
9	BL	bone marrow	96.7	75.4	6.46	91.3	6.2
10	DLBCL	lymph node	64.4	13.2	78.5	86.8	73.3
11	BL (like)	lymph node	64.8	78.8	62.7	99.4	64.1
12	MZL	lymph node	83.5	81.5	82.8	98.9	81.3
13	DLBCL	lymph node	81.7	63.3	73.3	96.5	72.1
14	FL	lymph node	90.4	72.1	71	32.5	27
15	MCL	lymph node	90.9	76.6	0.43	91.9	0.34
24	FL > DLBCL	bone marrow	55.8	13.1	52.9	57.5	52.8
26	BL	bone marrow	78.8	61	3.94	92.3	3.94
30	DLBCL	lymph node	71.2	87.6	17.6	74.7	29.8
32	FL	lymph node	75.3	77.8	80.6	97.3	85.8
33	FL	lymph node	72.7	76.3	3.21	55.1	3.33
42	FL	lymph node	81.6	71.4	61.8	99.1	70.4

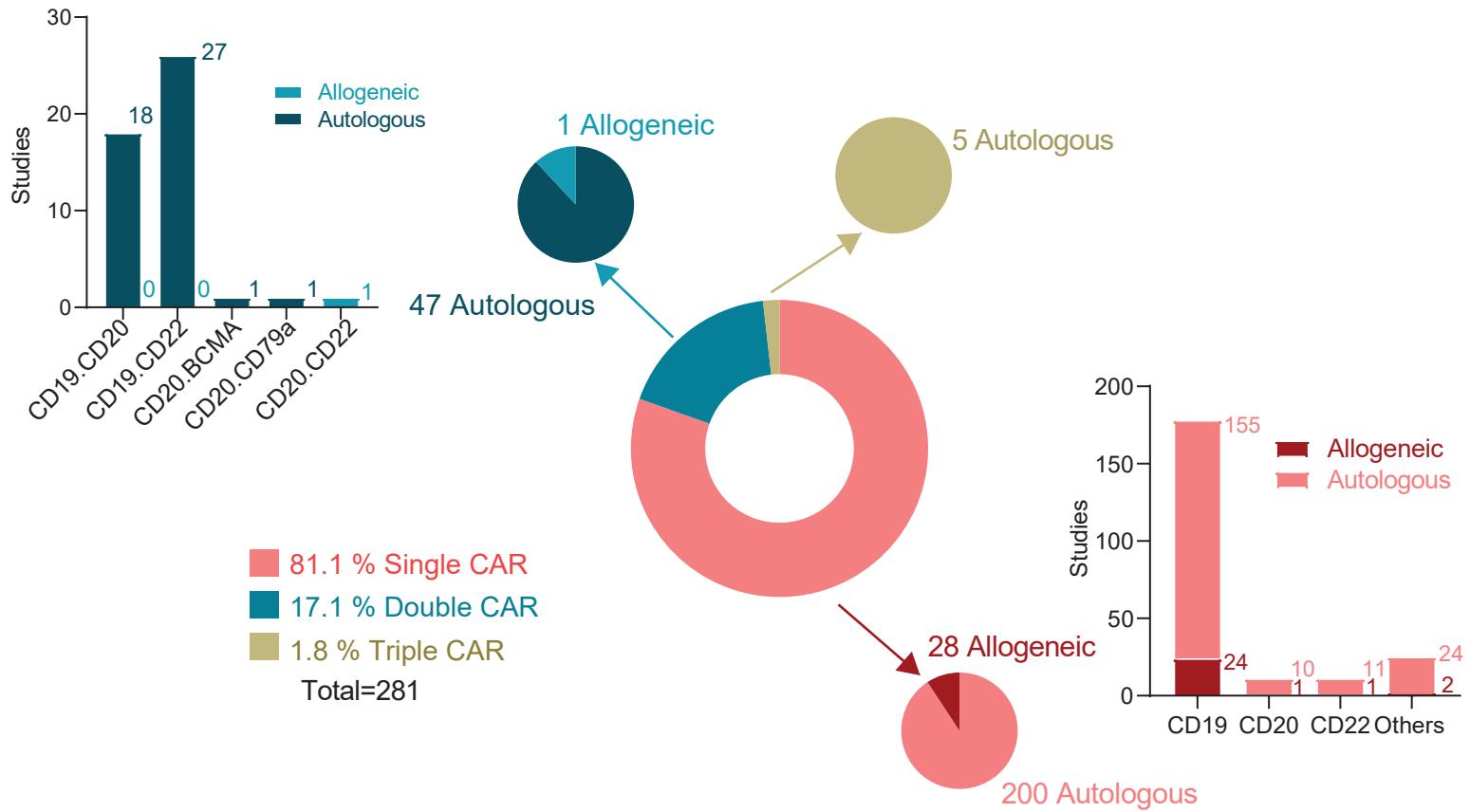
Abbreviations: MCL: Mantle Cell Lymphoma; MZL: Marginal Zone Lymphoma; DLBCL: Diffuse Large B-cell Lymphoma; FL: Follicular Lymphoma; BL; Burkitt Lymphoma

Supplementary Table S4

Feature	Allogeneic	Autologous
Cell source	Healthy donor	Individual patient derived
Time to infusion	Readily available after treatment decision.	Intermediate to long (3 weeks to months)
Manufacturing considerations	Scaled up batch manufacturing, possibility of automation and implementation of standardized controls to ensure efficacy, safety and T-cell characteristics.	High process variability due to patient-individualized manufacturing. Low number of available lymphocytes due to disease or previous treatments. Complicated logistics. Limited in regions lacking modern infrastructure.
T-cell characteristics	Higher inter-patient product homogeneity. Enhanced T-cell fitness and starting material composition.	Reduced T-cell fitness due to previous treatments or disease burden.
Potential adverse events	Graft-vs-host disease, cytokine release syndrome, genomic alterations due to CAR integration/gene editing, neurologic toxicities.	Cytokine release syndrome, genomic alterations due to CAR integration, B-cell aplasia, neurologic toxicities.
Durability	Short to Intermediate (days to months).	Intermediate to long (months to >10 years).
Redosing potential	Possible in the absence of donor specific anti-HLA antibodies.	Can be limited by the number of patient-specific lymphocytes collected.

Supplementary Figure S1

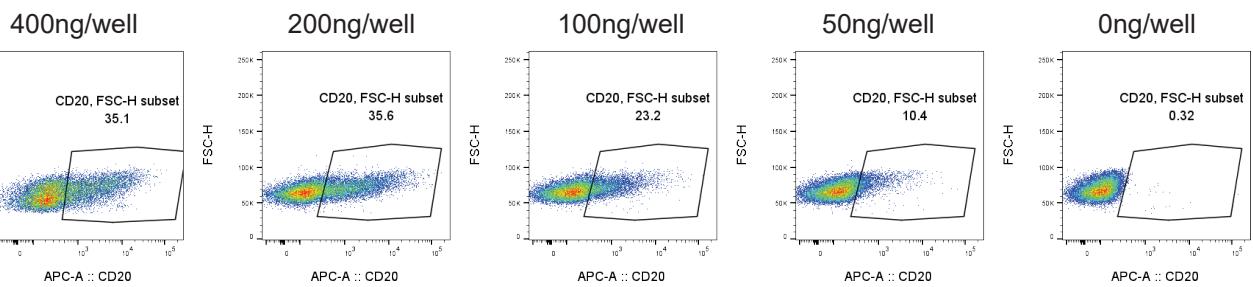
Current landscape CAR-T clinical trials in B-cell lymphoma



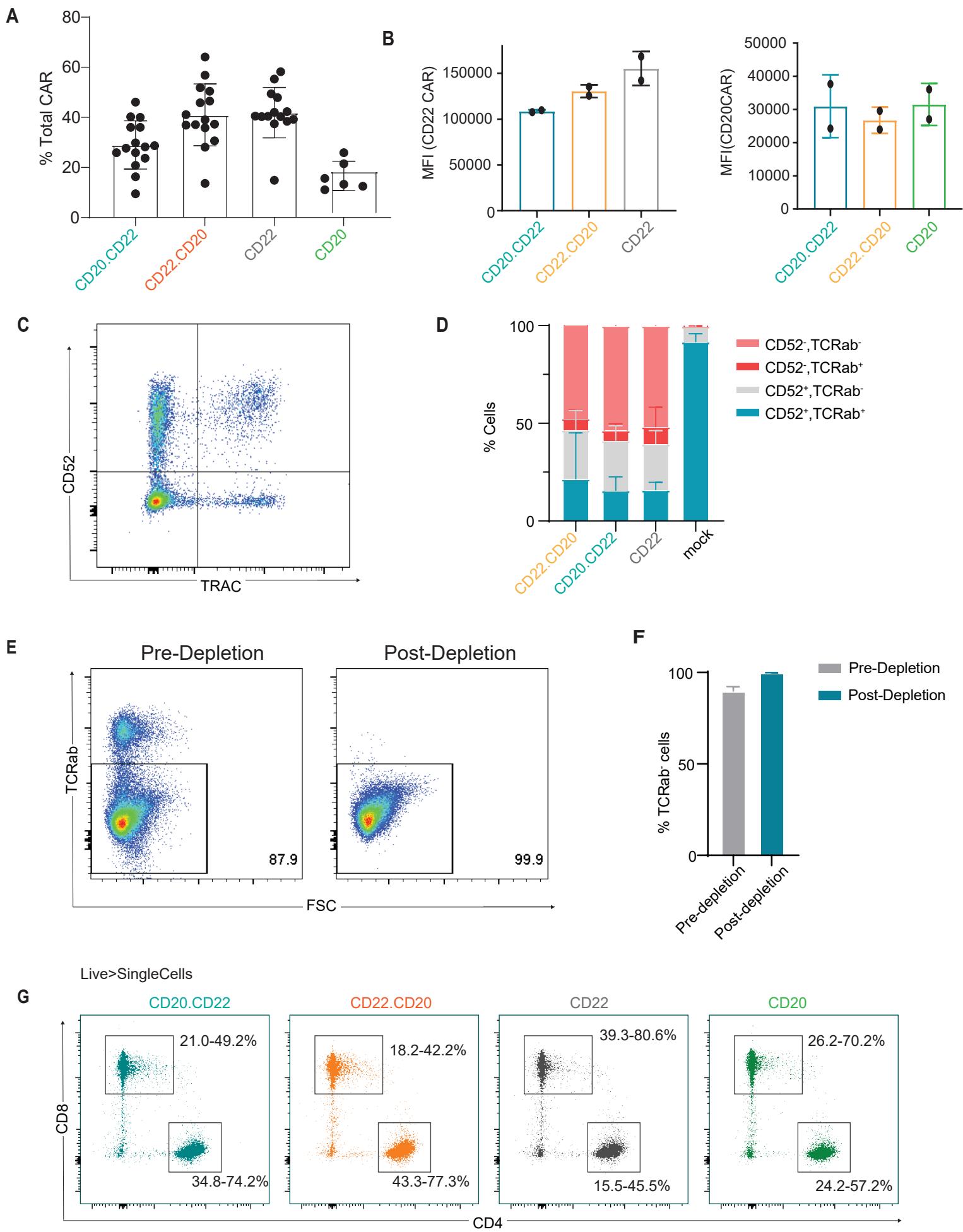
Supplementary Figure S2

A

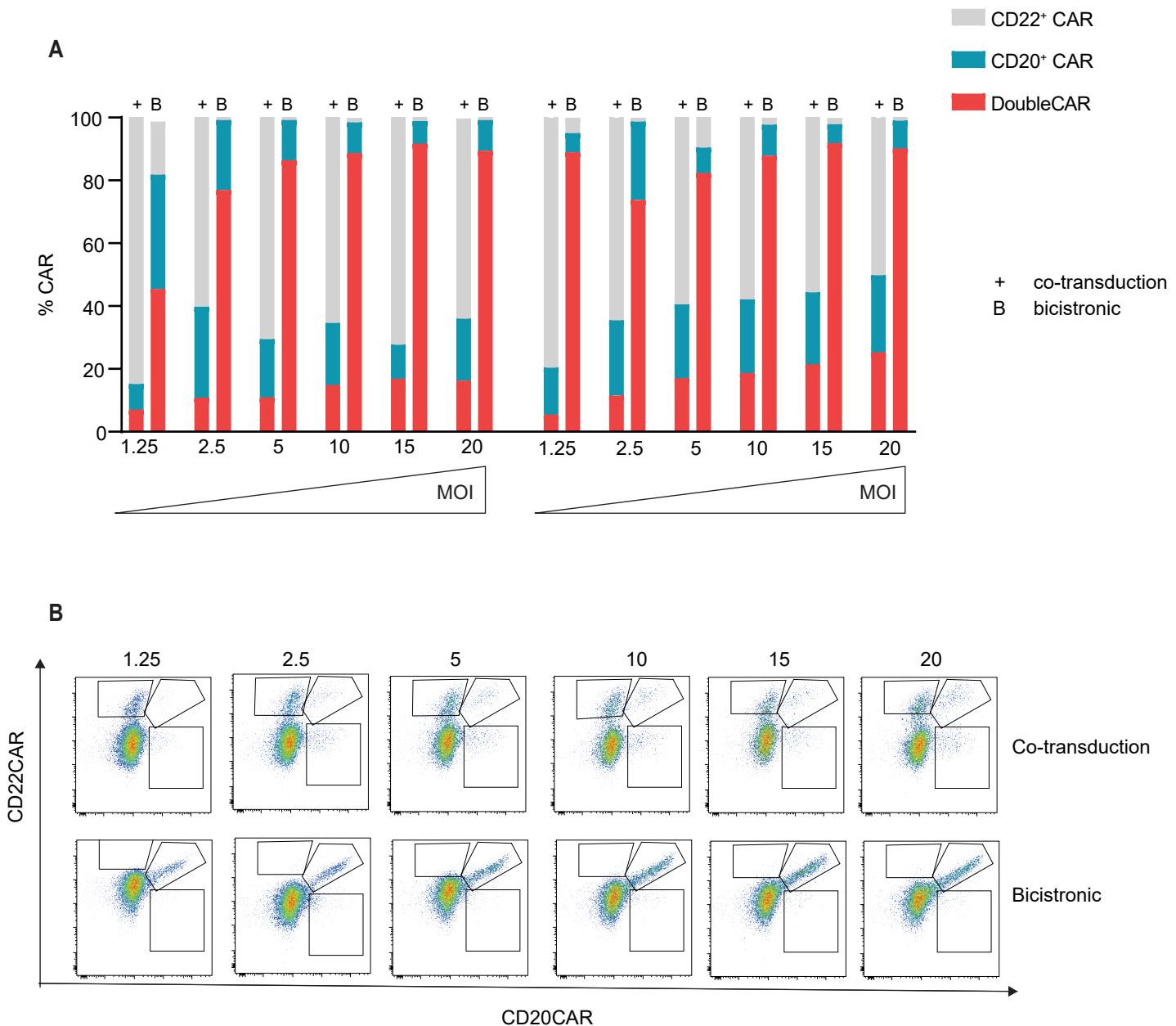
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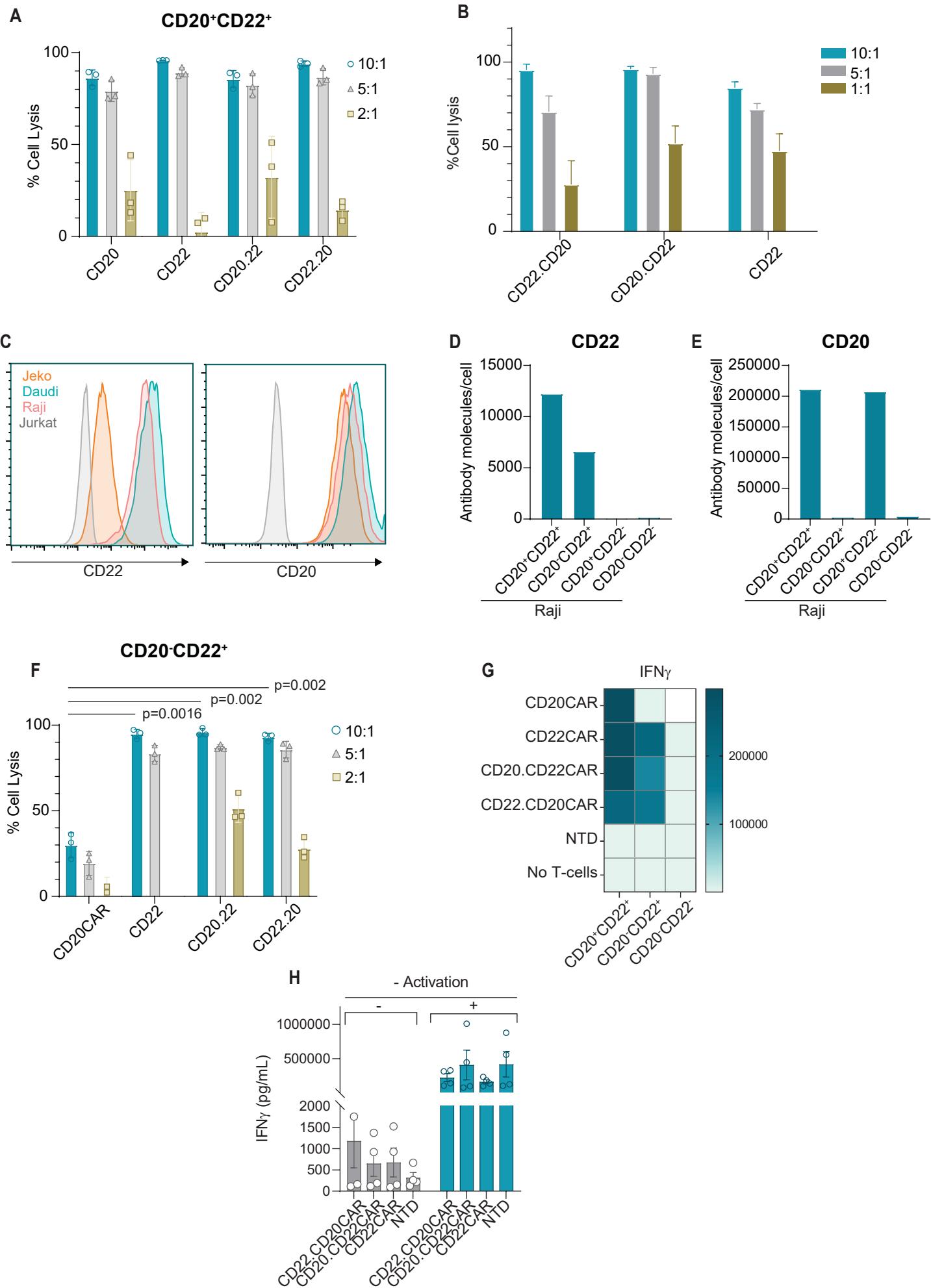
Supplementary Figure S3



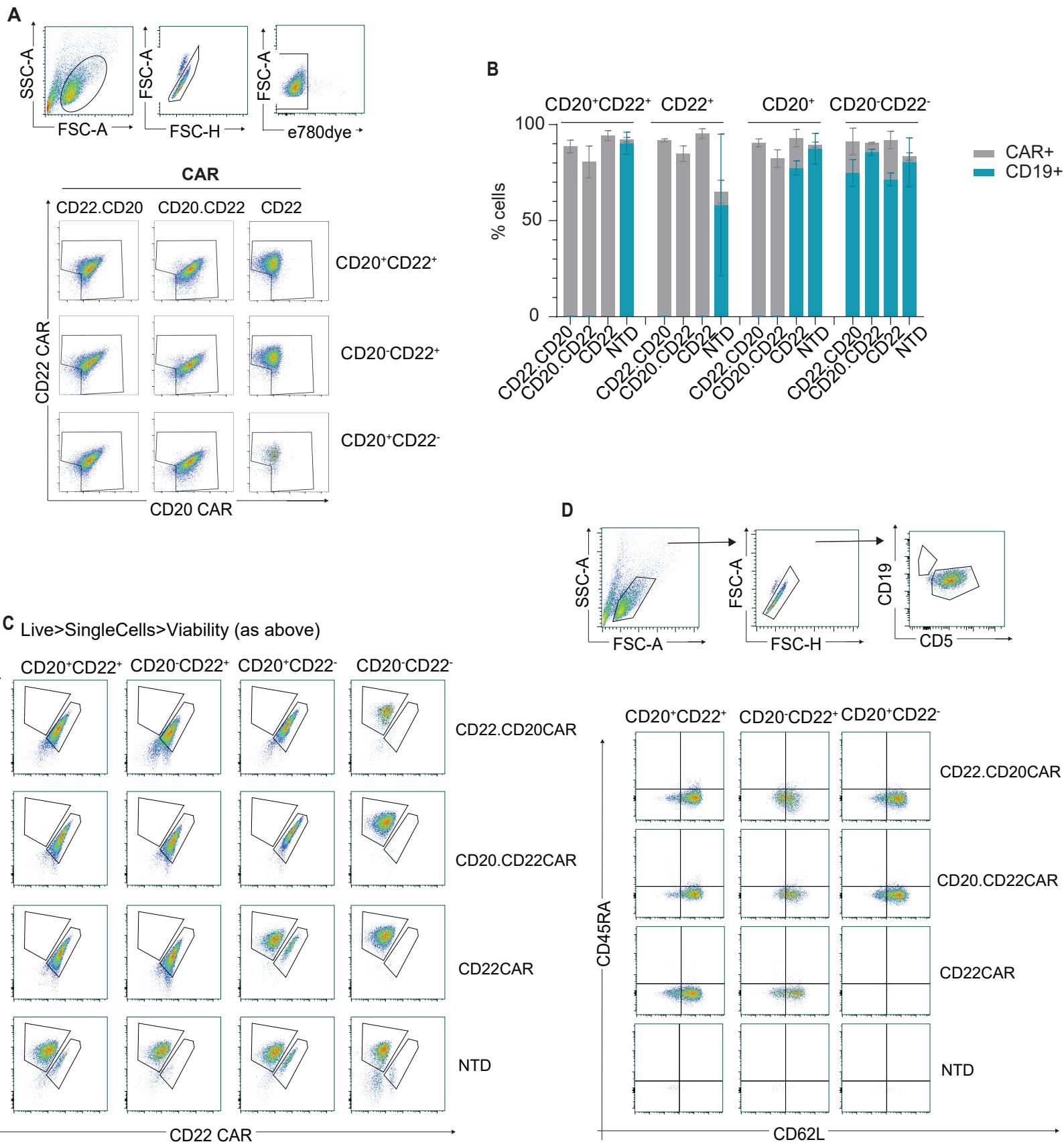
Supplementary Figure S4



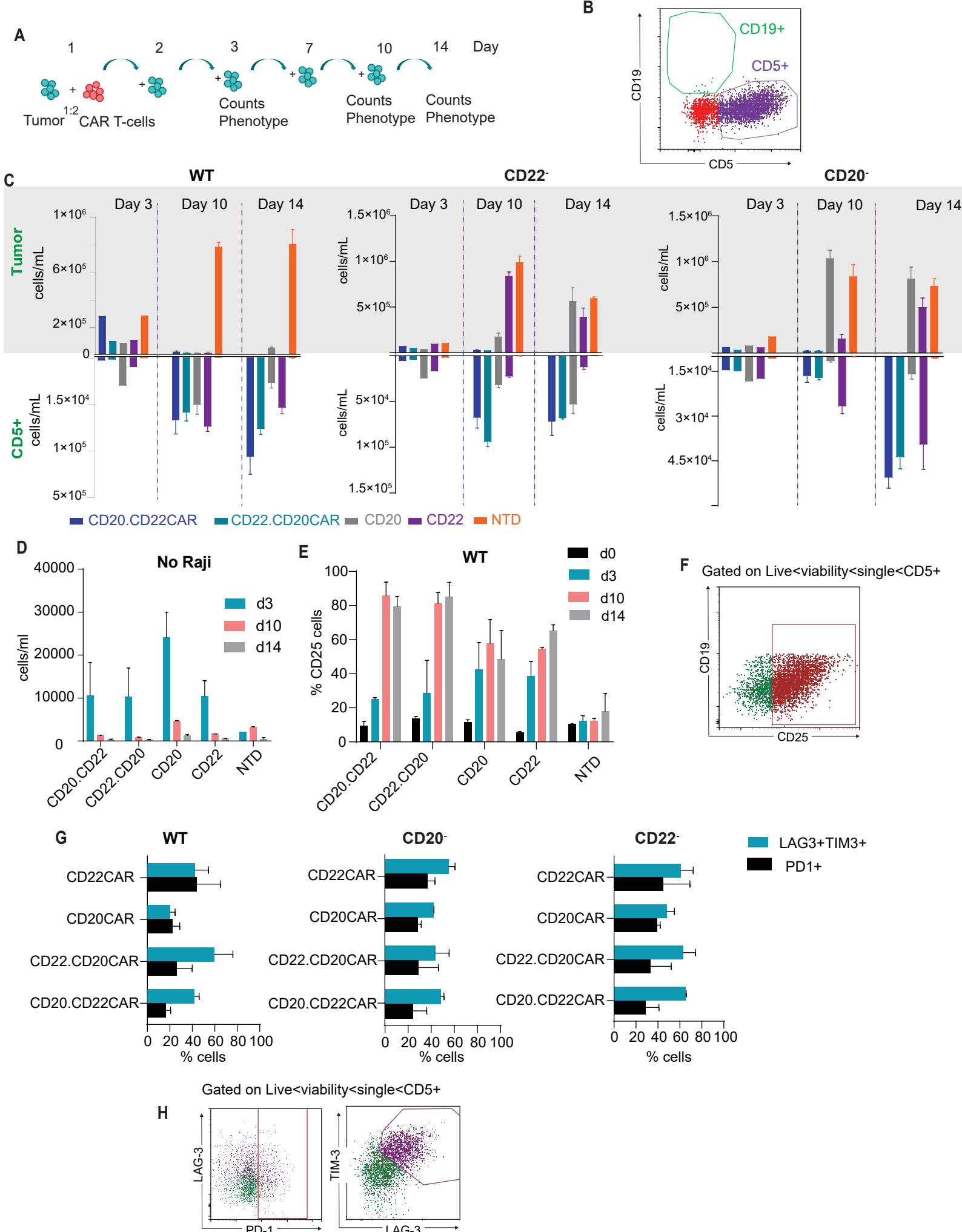
Supplementary Figure S5



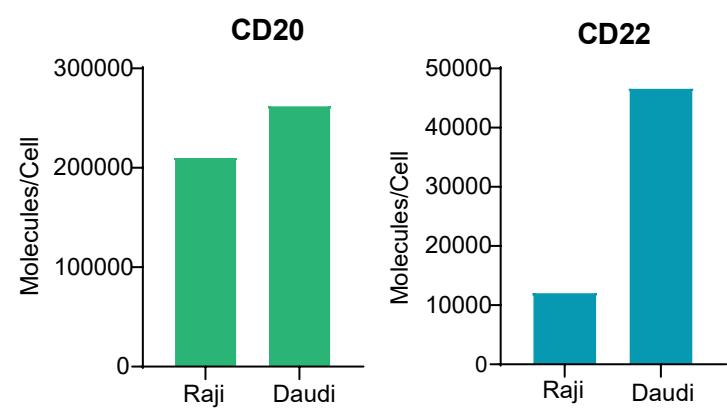
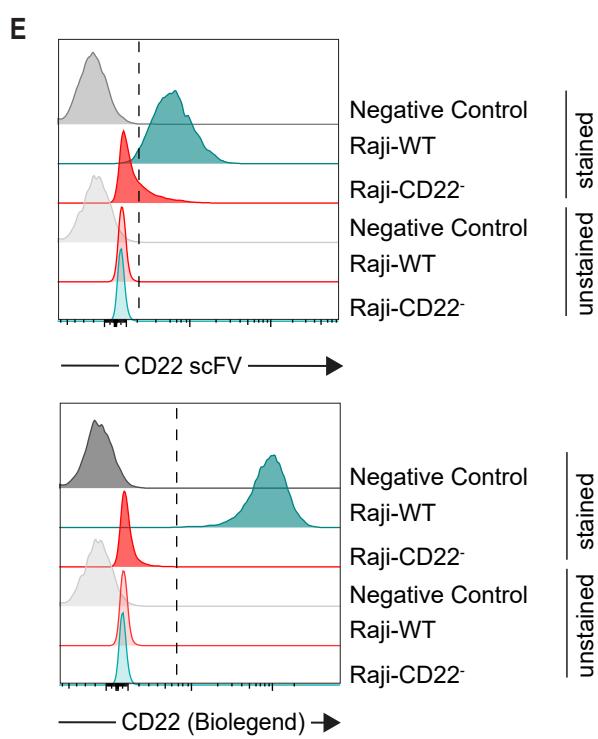
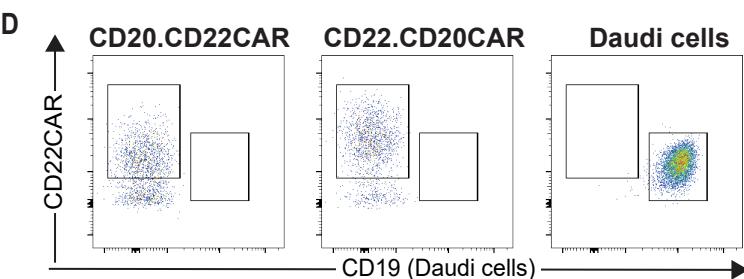
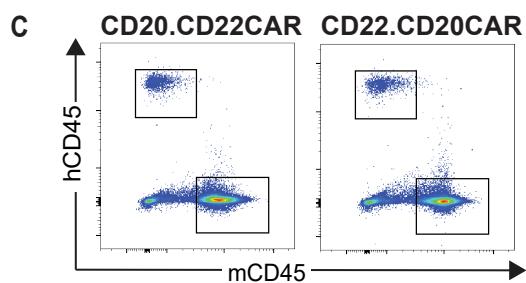
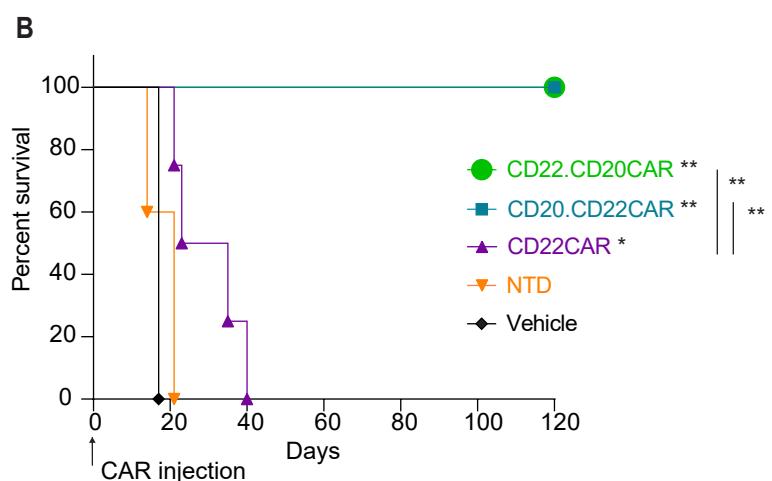
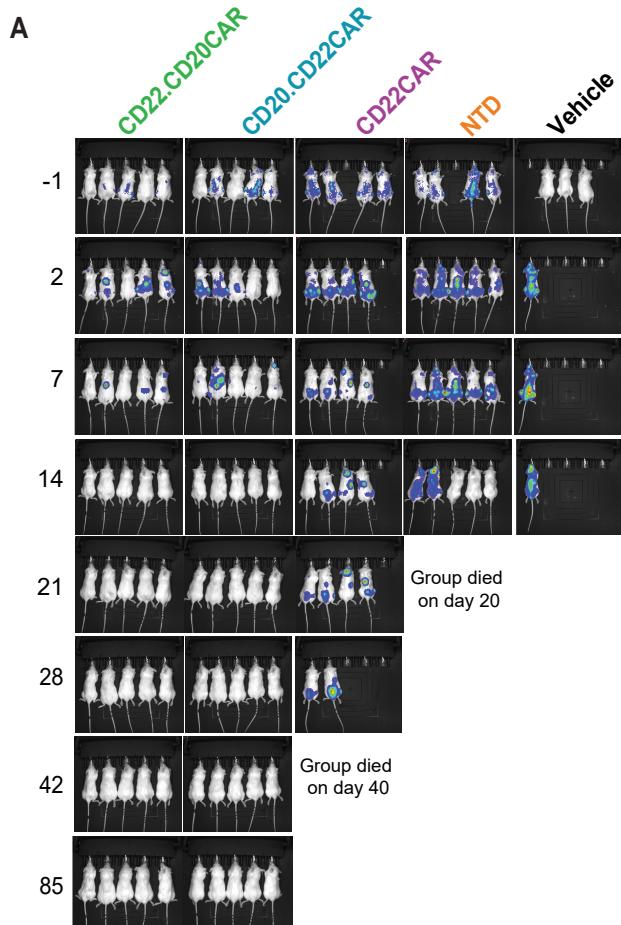
Supplementary Figure S6



Supplementary Figure S7

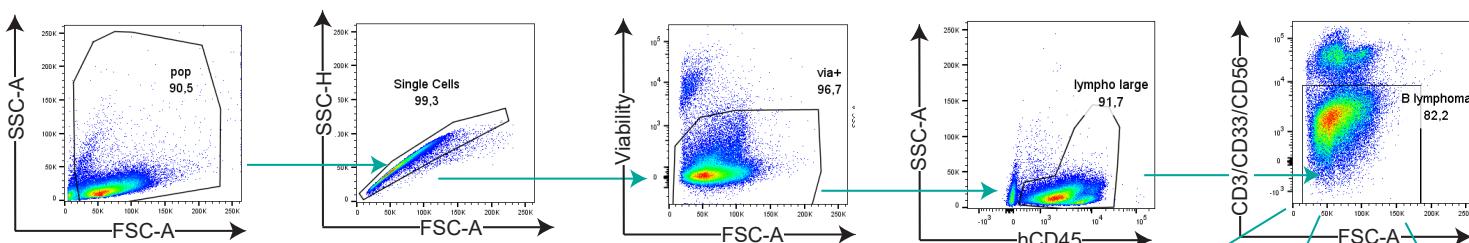


Supplementary Figure S8

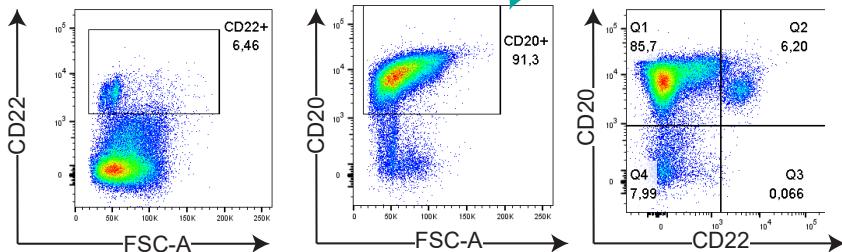
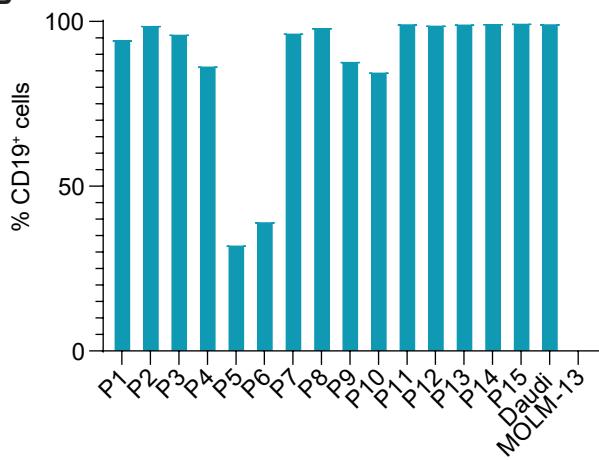


Supplementary Figure S9

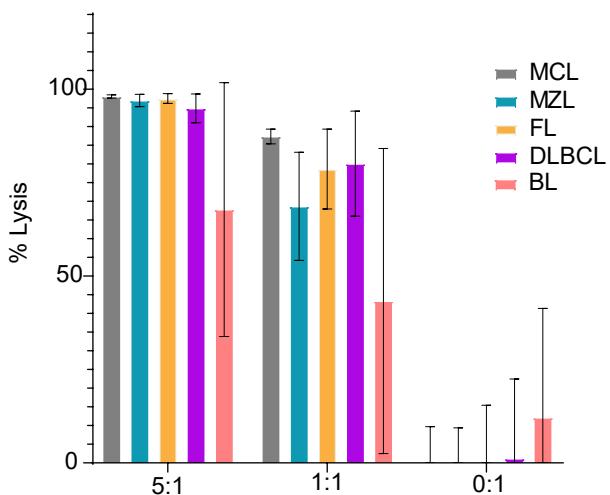
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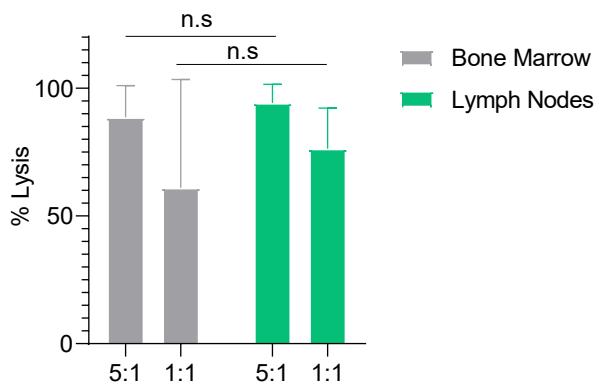
B



C



D



Supplementary Figure S10

A

