SUPPLEMENTARY MATERIAL

Chimeric antigen receptor T cells to target CD79b in B-cell lymphomas

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SUPPLEMENTARY METHODS

Generation and screening of anti-CD79b monoclonal antibody

Immunization and hybridoma generation procedures were conducted at the University of Texas MD Anderson Cancer Center, Monoclonal Antibody Core Facility following established protocols.[1-3] Briefly, human CD79b cloned into a lentiviral vector was transduced into L cells (ATCC), a mouse fibroblast cell line. Two 6-week-old female BALB/c mice were immunized once every 3 days for six injections into the footpad with human CD79b-expressing L cells using 50 µL of solution per injection containing 5×10^6 cells with no adjuvant. After the fifth injection, serum samples were obtained from both mice to confirm by ELISA the presence of serum antibodies against the target. Popliteal draining lymph nodes from the immunized mice footpad were harvested around day 20, and lymphocytes were fused with Sp2/0 myeloma to establish hybridomas, plated under hypoxanthine-aminopterin-thymidine selection media. Screening for selection of positive clones reacting with the protein was performed by cell-based ELISA. After subcloning and positive selection of hybridoma candidates' master cells, monoclonal antibodies were purified using MabSelect SuRe antibody purification resin (GE HealthCare) and eluted with low pH antigen/antibody elution buffer. Validation and quality control tests for the purified anti-CD79b antibody (clone 28B) included confirming specific binding to the target by ELISA and flow cytometry, assessing purity by SDS-PAGE, evaluating endotoxin level (Lonza Endotoxin kit), and

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determining isotype (Sigma-Aldrich) according to the recommendations of the International Working Group for Antibody Validation[4].

Screening for off-target binding of monoclonal antibody

To assess off-target binding, we employed cell microarray technology (Retrogenix) to screen the antibody against 5,828 full-length human plasma membrane proteins and cell surface-tethered human secreted proteins plus a further 398 human heterodimers expressed in human cells as previously described.[5] Briefly, this platform consists of arrays of expression vectors encoding the human proteins spotted onto slides. Human 293-HEK cells grown over the top were reverse-transfected resulting in cell surface expression of each respective protein at distinct slide locations. The antibody was then applied, and specific binding was analyzed and confirmed using a detection system.

Quantitative PCR

Quantitative PCR (qPCR) for CD79B expression was performed using SYBR Green JumpStartTM Taq ReadyMix^{TM} (Sigma-Aldrich) on a StepOne plus instrument (Applied Biosystem) as previously described.[6] Briefly, cells were lysed using RealTime Ready Cell Lysis Kit (Roche), followed by direct reverse transcription using Transcriptor Universal cDNA Master Kit (Roche). The expression of individual genes was normalized to GAPDH using the $\Delta\Delta C_t$ method. To determine whether CD79b was expressed in normal tissues, the FirstChoice Human Total RNA Survey Panel containing 20 normal human tissue total RNA was obtained from Applied Biosystems. Total RNA was extracted from purified B and T cells from human tonsil samples and used as positive and negative controls, respectively. Probes used for detection of CD79b and GAPDH mRNA were as follows:

CD79b L: 5'-AGCAGAAGTGCAACAACACC

CD79b R: 5'-AGGTGCTGAATCCCATGAC

GAPDH L: 5'- CATCAATGGAAATCCCATCA

GAPDH R: 5'-GACTCCACGACGTACTCAGC

Patient-derived xenograft (PDX) lymphoma samples

To generate PDX samples, 6-8-week old NSG mice were anesthetized with 5% isoflurane vaporizer and then injected with 5×10^6 freshly isolated lymphoma cells obtained from patients into the subrenal capsule. Magnetic resonance imaging was used to monitor engraftment and tumor burden. After detection of engraftment, the tumors were transferred to additional NSG mice to create second generation. Single cell suspensions were prepared from freshly isolated PDX samples and were cultured *in vitro* to generate cell lines (PDX203 and PDX300) that were used in *in vitro* functional studies to assess function of CAR T cells. PDX203 cells were also transduced with firefly luciferase and green fluorescent protein and sorted into a single cell clone (PDX203 5D4) for *in vivo* studies.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. CD79b expression is restricted to B-cell lineage amongst normal tissues and in B-cell malignancies amongst cancers. A) Relative expression of CD79b mRNA across various normal human tissues was determined by quantitative PCR. **B)** Expression of CD19, CD20, CD79a, and CD79b mRNA amongst various normal human tissues from publicly available cDNA microarray datasets (http://biogps.org). **C)** Relative expression of CD79b mRNA was determined by quantitative PCR in B-cell lymphoma and T cell leukemia cell lines. **D)** CD79b protein expression as assessed by flow cytometry in various B-cell lymphoma and T cell leukemia cell lines is shown. **E)** Expression of CD79b mRNA in various human lymphoma subtypes as determined from publicly available cDNA microarray datasets from the Oncomine database (https://www.oncomine.org). The number of samples for each subtype is shown in parentheses. **F)** CD79b transcript expression across different cancers from publicly available RNAseq data (https://cbioportal.org). **G)** CD79b expression as determined by flow cytometry in parental and $CD19^{KO}$ lymphoma cell lines.

Supplementary Figure 2. Screening funnel to identify CD79b-binding antibody clone 28B for CAR generation. Clone 28B was selected after a series of screening steps as shown. First, supernatants from hybridoma fusions plated in 2,496 wells (26 plates x 96 wells/plate) with multiple hybridoma clones/well were tested for binding to human CD79b-transduced L cells but not wild type L cells using a cell-based ELISA as previously described.[3] Hybridomas from wells with positive readouts (91 wells) were then plated to isolate single cell hybridoma clones by limiting dilution. The supernatants from these single cell clones were retested in the above cell-based ELISA and 41 clones were identified. Further screening of these 41 clones to assess binding to human CD79b-transduced L cells but not wild type L cells by flow cytometry resulted in 37 clones. These 37 clones were then tested by flow cytometry for binding to five CD79b+ lymphoma cell lines. Consistent binding was observed for 12 clones. CAR constructs were

generated from these 12 clones but only 4 of the CARs bound to recombinant CD79b-Fc protein when the antibodies were expressed in single chain variable fragment (scFv) format in the CAR. These four CARs were screened in *in vitro* cytotoxicity assays against lymphoma cell lines and clone 28B was identified based on superior cytotoxic activity for further testing (data not shown).

Supplementary Figure 3. Specificity of anti-CD79b monoclonal antibody (clone 28B). Fluorochrome-labeled anti-CD79b monoclonal antibody (clone 28B) was used to stain isogenic cell lines with human CD79b knock-in (A) or knock-out (B) and binding was assessed by flow cytometry. **A)** Binding of anti-CD79b monoclonal antibody (clone 28B) to parental L cells and L cells transduced with human CD79b in the presence or absence of polatuzumab is shown. **B)** Binding of anti-CD79b monoclonal antibody (clone 28B) to parental Jeko-1 cells or Jeko-1 cells with $CD19^{KO}$ or $CD79b^{KO}$ is shown. Mean fluorescence intensity values for each histogram are shown.

Supplementary Figure 4. CAR expression, *in vitro* expansion, and phenotypic profile of different CAR T cells. A) CAR constructs were transduced lentivirally into healthy donor primary human T cells and transduction efficiency was determined by flow cytometry by assessing eGFP expression on days 4 and 7 after transduction. **B)** Expansion of T cells transduced with different CAR constructs is shown over a period of 11 days. **C)** Proportion of $CD4^+$ and $CD8^+$ T cells in total T cells and CAR^+ and CAR^- T-cells at the end of *in vitro* expansion. **D)** Proportion of naïve (Tn, $CD45RA^+CD62L^+$), central memory (Tcm, $CD45RA^-CD62L^+$), effector memory (Tem, $CD45RA^-CD62L^-$), and terminal effector (Teff, $CD45RA^+CD62L^-$) T cell subsets on day 7 and day 10 during generation of different CD79b CAR T cells.

Supplementary Figure 5. Survival, weight change and *in vivo* CAR T cells persistence in Daudi tumor-bearing mice treated with various CAR T cells. These data derived from the experiment shown in Figure 4 in main text. **A)** Kaplan-Meier survival curves of groups of mice

treated with CD79b CAR T cells containing CD28 hinge/transmembrane domain. Survival of mice treated with untransduced T cells and CD19 CAR T cells is shown for comparison. **B)** Weight change in mice over time following treatment with untransduced T cells or various CAR T cells. Each line indicates an individual mouse. **C)** Blood sample was obtained on day 94 from a NSG mouse free of tumor following treatment with 79b-8α-OX40 CAR T cells (see Figure 4 in main text) and was analyzed by flow cytometry for persistence and phenotype of CAR T cells. CAR T cells were detected by dual expression of human CD3 and eGFP and consisted of both $CD4^+$ and $CD8^+$ T cells. Expression of various co-inhibitory molecules on $CD4^+$ and $CD8^+$ CAR T cells assessed by flow cytometry is shown.

Supplementary Figure 6. Phenotypic profile of CD79b CAR T cells. CD79b-targeting CAR T cells were generated from a lymphoma patient relapsing after autologous CD19 CAR T-cell therapy and phenotypic profile was assessed by flow cytometry. CD79b CAR T cells generated from a normal donor (ND) is shown for comparison. **A)** Transduction efficiency was determined by assessing eGFP and surface CAR expression by flow cytometry, after staining with fluorescently labeled recombinant human CD79b-Fc conjugated protein. **B)** Proportion of $CD4^+$ and $CD8^+$ T cells and naïve ($CD45RA^+CD62L^+$), central memory ($CD45RA^-CD62L^+$), effector memory ($CD45RA^-CD62L^-$), and terminal effector ($CD45RA^+CD62L^-$) T cell subsets is shown. **C)** Expression of various co-inhibitory, co-stimulatory, and memory markers in normal donor- and patient-derived CD79b CAR T cells.

Supplementary Figure 7. Antitumor activity of CD79b CAR T cells against PDX-derived lymphoma tumor cells. CD79b CAR T cells generated from healthy donor T cells were co-cultured with PDX203 5D4 tumor cells and cytotoxic activity (A) was assessed after 96 h by flow cytometry by counting live tumor cells using TruCOUNT^{TM} beads as shown in Figure 3 in main text. Cytokine production was determined after 24 h (B). Untransduced T cells were used as controls. **A)** Summary results of cytotoxicity assay from replicate wells (N=2) showing absolute

live tumor cell count. **B)** Cytokine production by CAR T cells in response to tumor cells in replicate wells (N=2) is shown. Data represents mean ± standard deviation and are representative of at least three separate experiments. ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05; (ns) P>0.05.

Supplementary Figure 8. Expression of CD19 and CD79b on isogenic cell lines of SUDHL6. CD19 and CD79b expression was evaluated by flow cytometry on isogenic SUDHL6 cell lines (parent, $CD19^{KO}$, $CD79b^{KO}$, and $CD19^{KO}CD79b^{KO}$) and shown as histogram plots. Mean fluorescence intensity (MFI) for CD19 and CD79b expression in each cell line is shown.

Supplementary Figure 9. Signaling in CD79b CAR T cells. CAR T cells were cultured alone or in the presence of anti-CD3/CD28 antibodies or recombinant human CD79b-Fc protein for 15 min and then signaling molecules were assessed by phosflow analysis in CAR^+ and CAR^- T cell population. Representative flow cytometric plots (*top panels*) showing expression of phosphorylated CD3ζ (A) and phosphorylated ERK1/2 (B) are shown along with MFI. Summary results (*bottom panels*) showing MFI of phosphorylated CD3ζ (A) and phosphorylated ERK1/2 (B) from replicate wells (N=2) with mean ± standard deviation. Data is representative of two separate experiments with CAR T cells from two different normal donors. **P<0.01; *P<0.05; (ns) P>0.05.

Supplementary Figure 10. Specific cytotoxic activity of CD79b CAR T cells. Cytotoxic activity of CD79b CAR T cells derived from two healthy donors (HD) against Jeko-1 and PDX203 5D4 (both $CD19^+CD79b^+$) or NALM6 acute lymphoblastic leukemia ($CD19^+CD79b^-$) cells at an E:T ratio of 1:1 as compared to the corresponding untransduced T cells (UTD) is shown. Absolute number of live tumor cells present in each condition after 4 days of culture is shown. The percent specific lysis calculated from this experiment is shown in Figure 7F in the main manuscript. Data represents mean ± standard deviation from two replicate wells from two different healthy donors. ****P<0.0001; ***P<0.001; **P<0.01; *P<0.05; (ns) P>0.05.

Supplementary Figure 11. Expression of costimulatory molecules on tumor cells. Expression of various costimulatory molecules (CD40, CD58, CD80, CD86, and CD137L) was assessed by flow cytometry on various B-cell lymphoma tumor cell lines and PDX samples used to evaluate antitumor activity of CD79b CAR T cells. Jurkat T cell leukemia tumor cell line and unstained Daudi tumor cells were used as controls. Mean fluorescence intensity (MFI) for each of the markers is shown.

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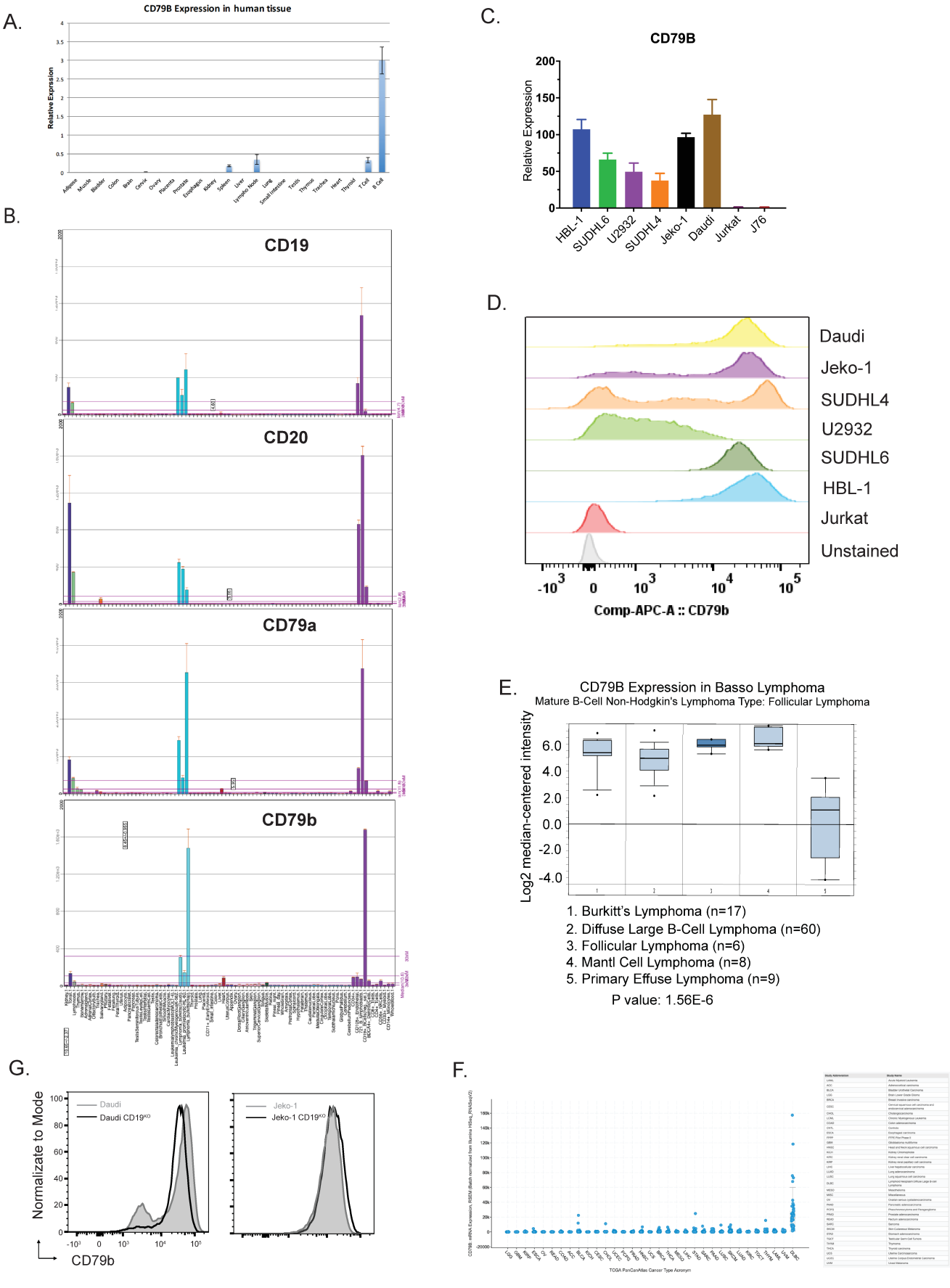
Supplementary Figure 1



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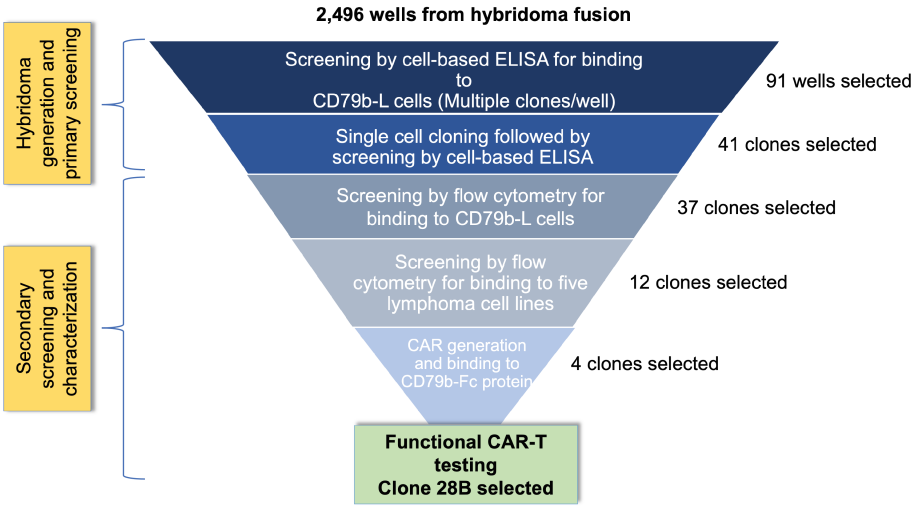

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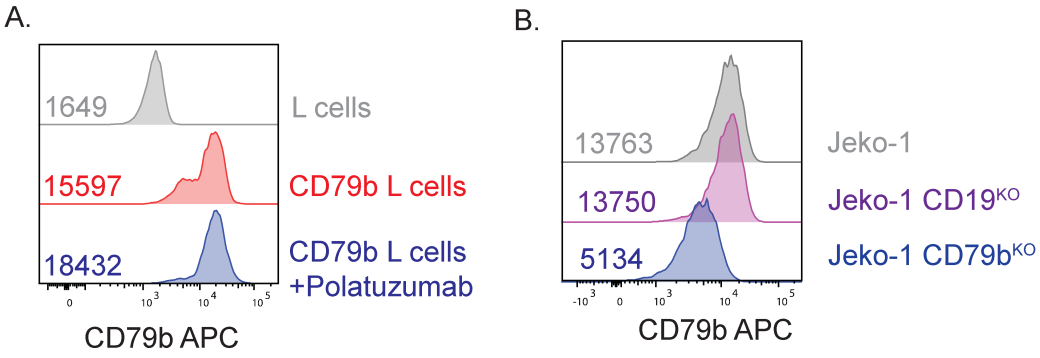

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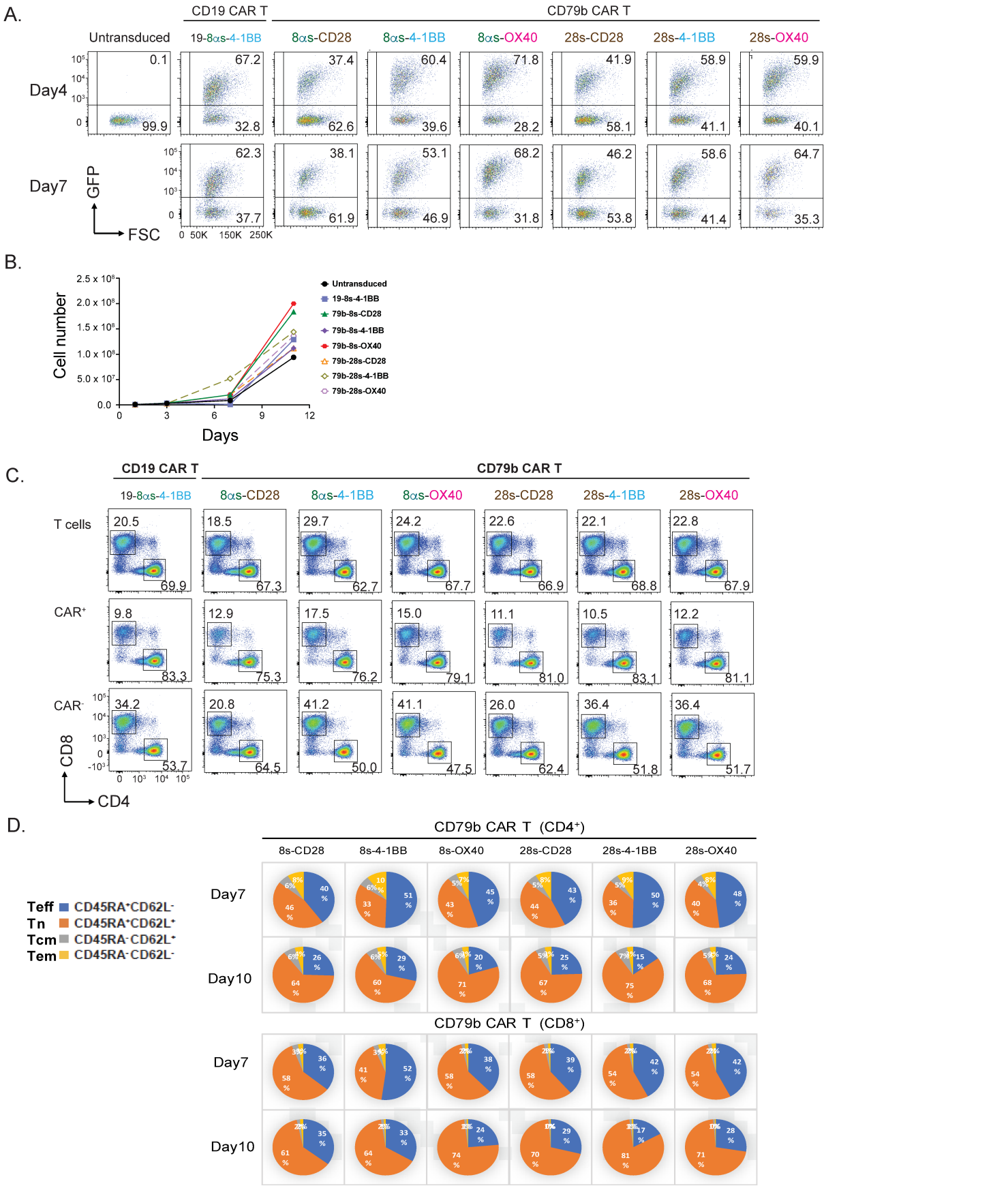

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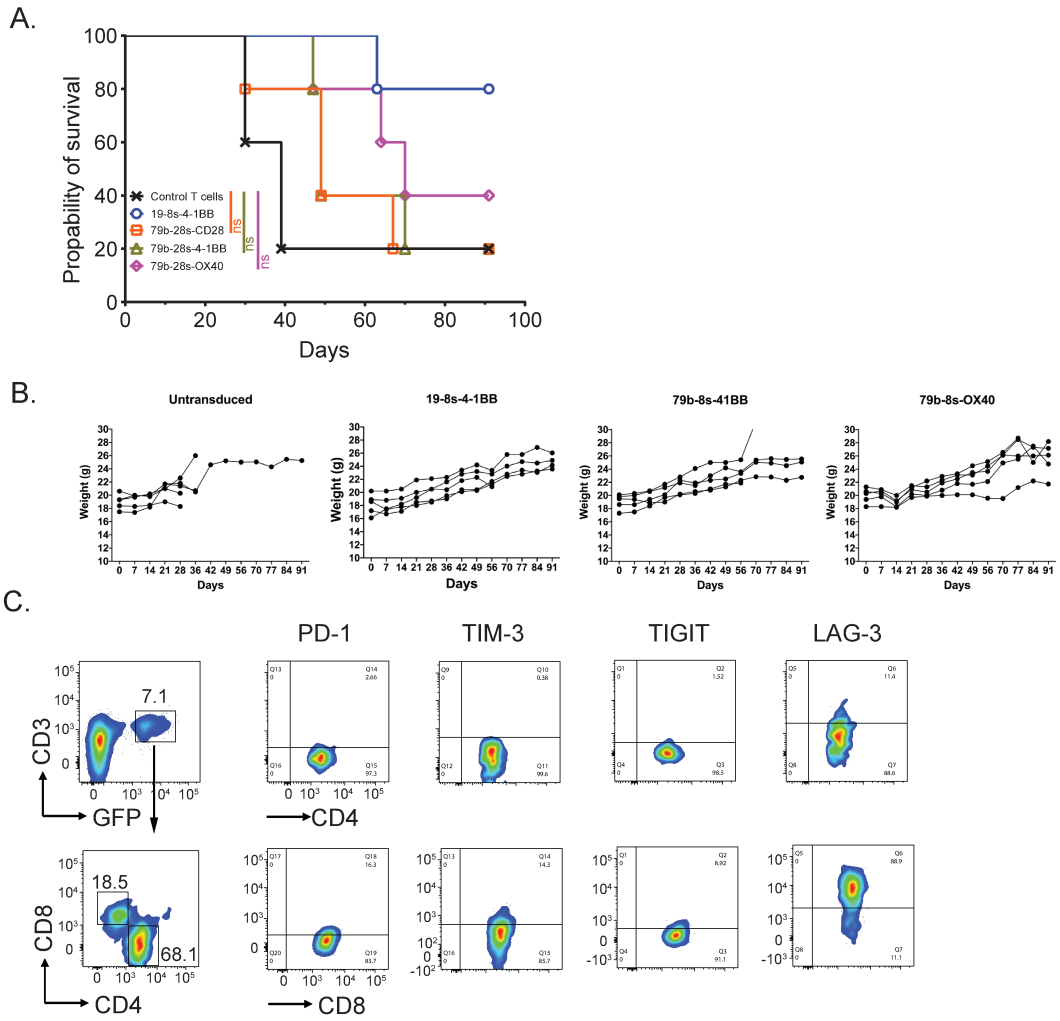

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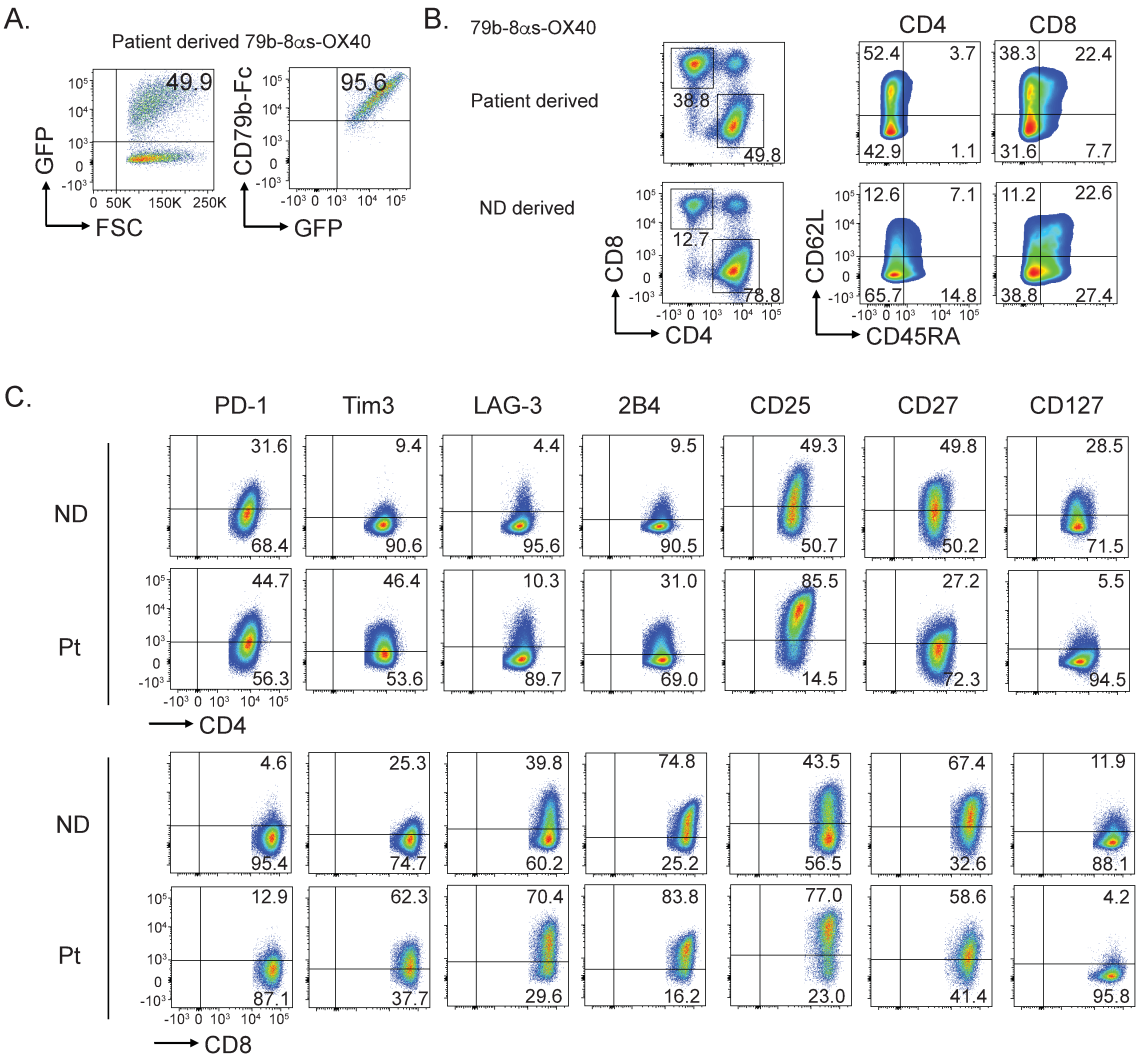

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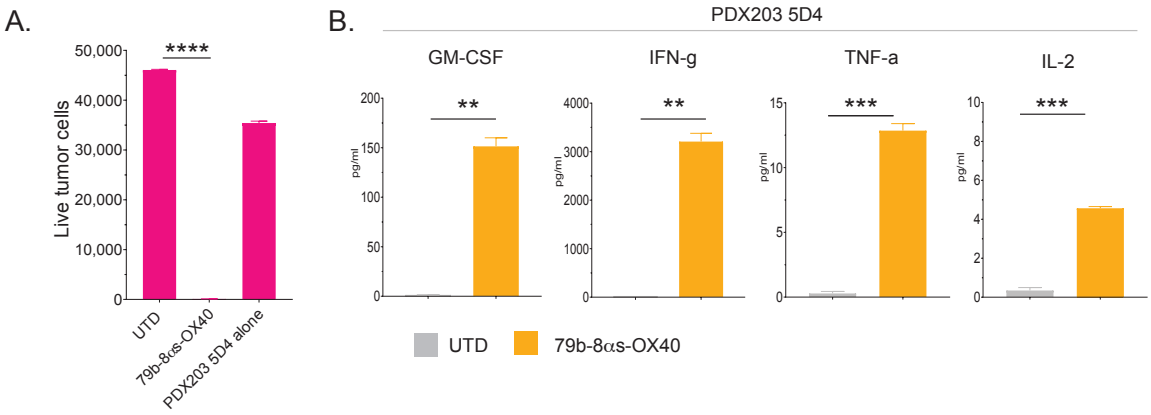

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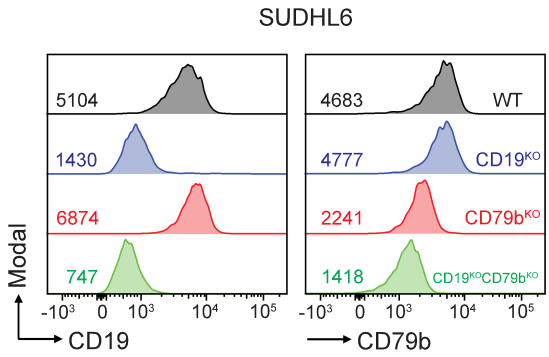

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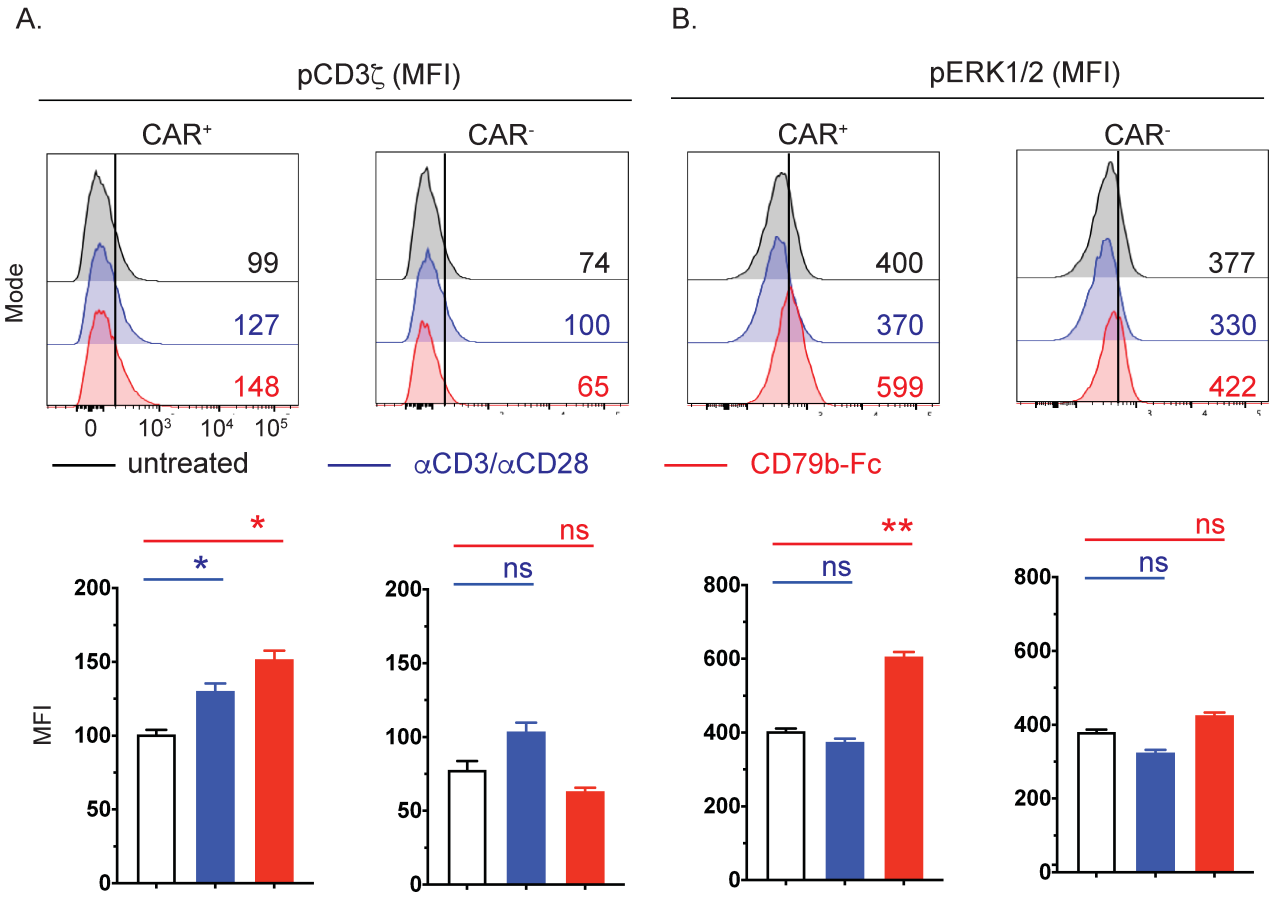

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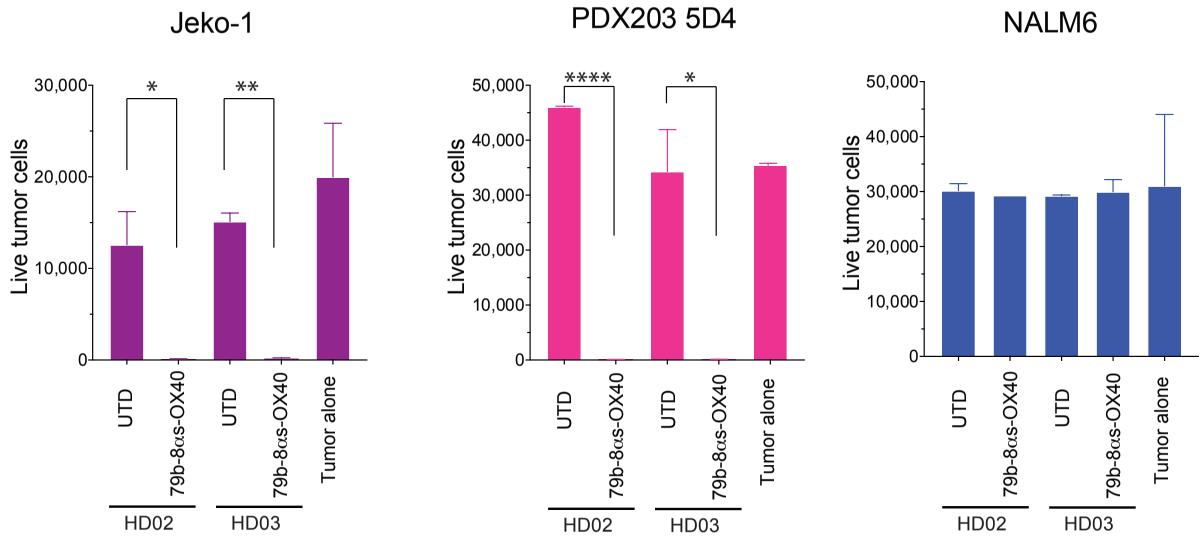

Supplementary Figure 11