Supplementary Materials

Title: Overcoming the Immunosuppressive Tumor Microenvironment of Hodgkin Lymphoma Using Chimeric Antigen Receptor T Cells

Running title: CART123 for Hodgkin Lymphoma and the environment

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Supplementary Figures

Figure S1

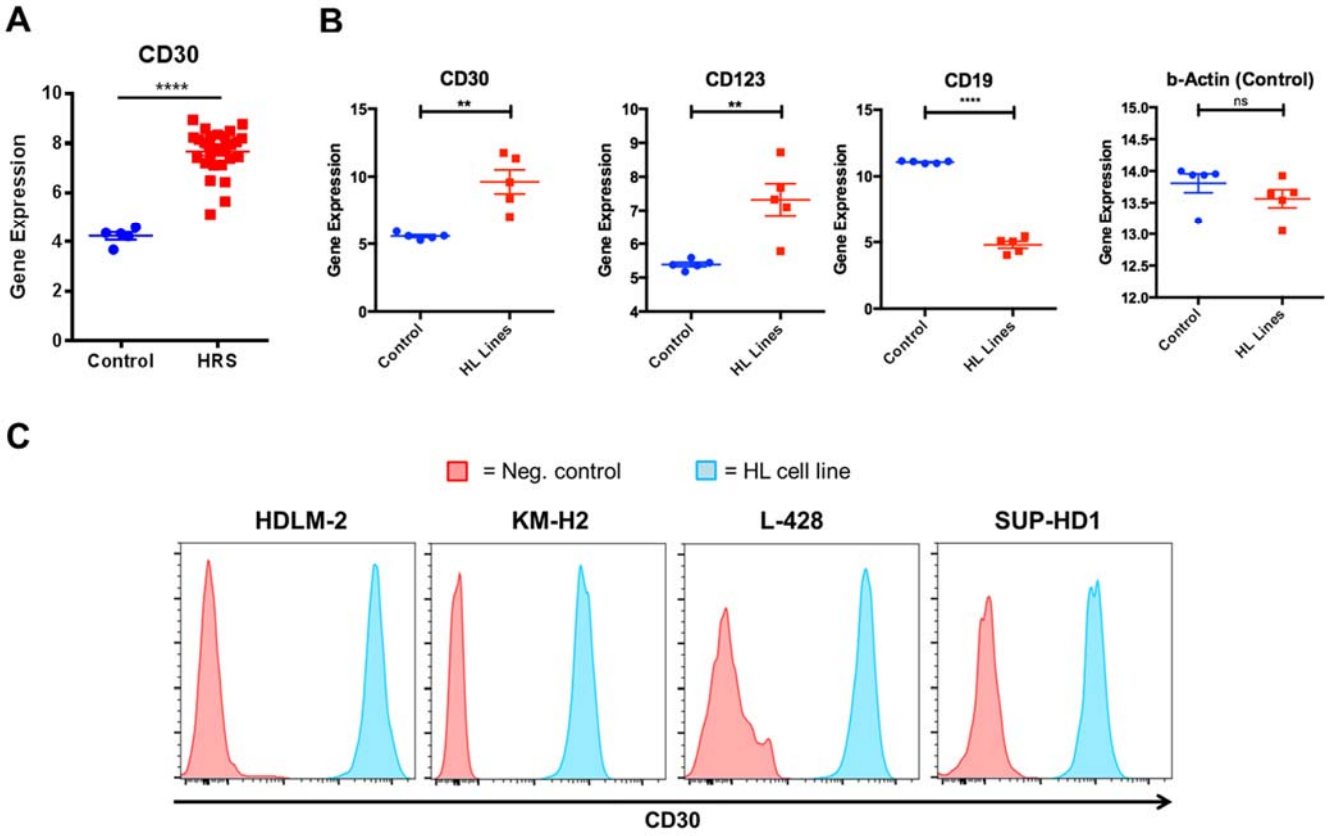

Figure S1. CD123 and CD30 expression in Hodgkin lymphoma. A. Affymetrix array gene expression analysis of microdissected HRS (GSE-39133, accessible via www.biogps.org) reveals high expression of CD30 in 29 HL patients as compared to 5 controls (microdissected germinal center B cells) **B.** CD30 and CD123, were highly expressed also in HL cell lines by RNA expression analysis (GSE-39132). CD19 and beta-actin are used as controls. **C.** High CD30 protein expression by flow cytometry on 4 standard HL cell lines (HDLM-2, KM-H2, SUP-HD1 and L428).

Figure S2

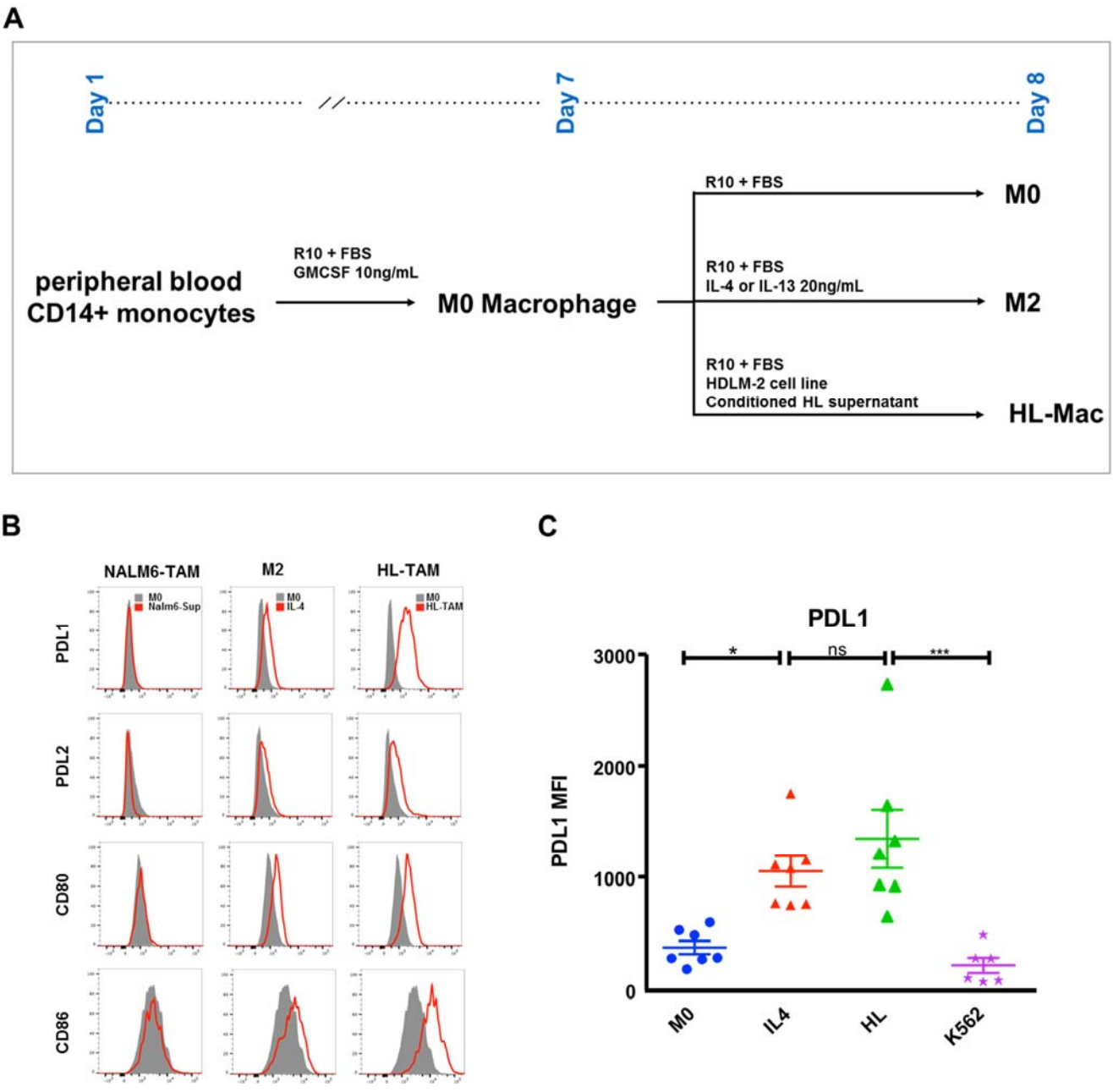

Figure S2. Hodgkin Lymphoma polarizes normal macrophage to an immunosuppressive phenotype. A. Macrophage differentiation protocol: human macrophages (M0) were generated by differentiating positively selected CD14+ normal donor monocytes for 7 days in RPMI-1640 supplemented with 10% fetal bovine serum, 1x Glutamax, 10ng/mL recombinant human GM-CSF and 1x penicillin/streptomycin. Macrophages were polarized to M2 by adding

either 20ng/mL human IL-4 or IL-13 to the differentiation media for an additional 24 hours. HL-TAMs were generated by culturing macrophages with HDLM-2 cells or using HDLM2 conditioned media for 24 hours post-differentiation. **B.** Expression of CD80, CD86, PD-L1 and PD-L2 in macrophages polarized using IL-4 (M2), NALM-6 (control) or HDLM-2 cells. HL-polarized macrophages share similar phenotype as the IL-4 polarized macrophages. **C.** PD-L1 is highly expressed on M2 and HL-polarized macrophages as compared to M0 or macrophages co-cultured with a non-HL cell line (K562).

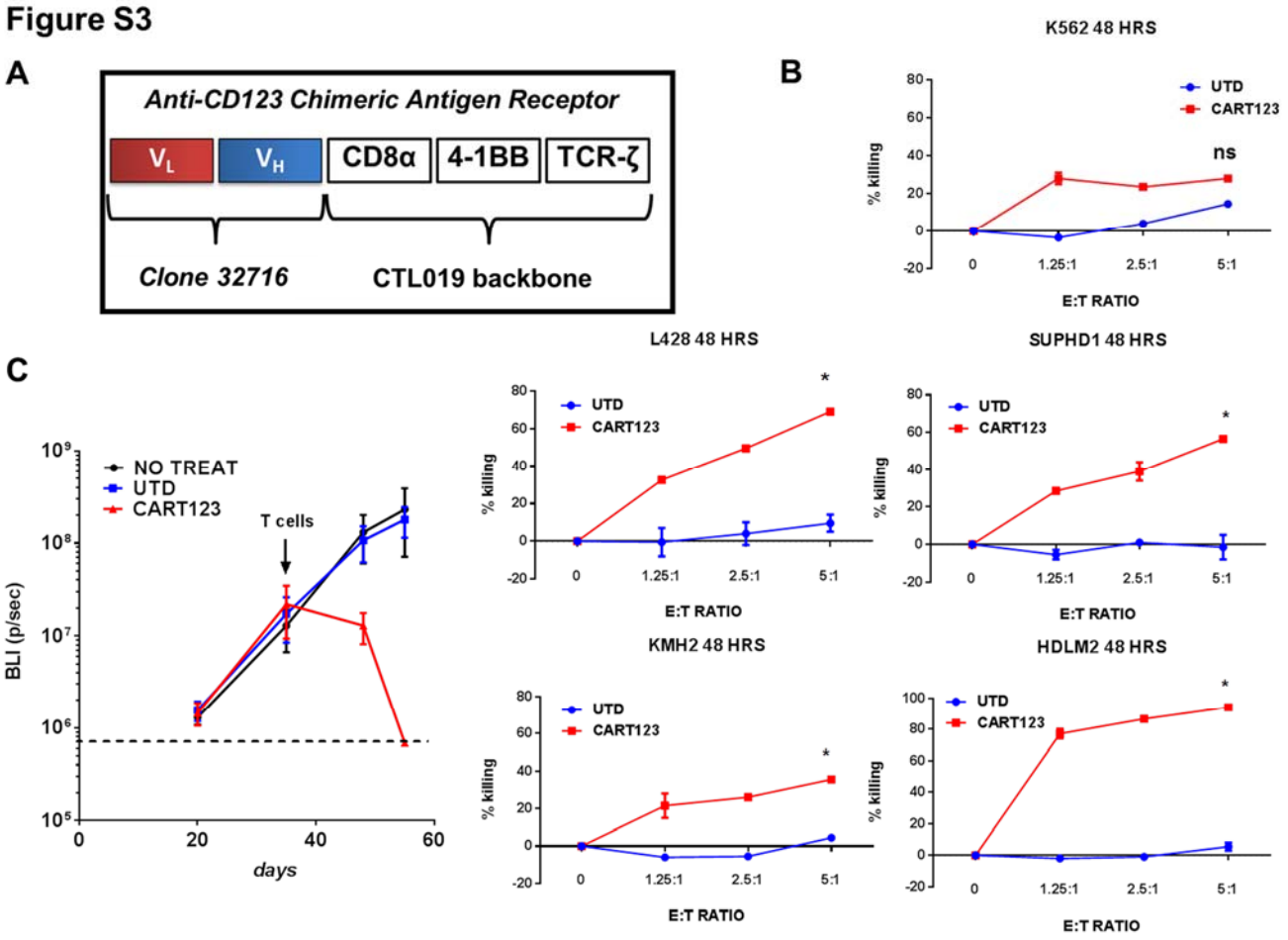

Figure S3. Anti-lymphoma activity of CART123 A. The anti-CD123 chimeric antigen receptor construct: we used a 2nd generation anti-CD123 chimeric antigen receptor (CAR123) that features an anti-CD123 scFv (clone 32716), CD8 hinge, 4-1BB costimulatory domain and

CD3-ζ signaling domain. (1) **B.** CART123, but not UTD, exert potent cytotoxicity (luciferase-based killing assay) against 4 HL cells (HDLM-2, KM-H2, SUP-HD1 and L428) in a dose-dependent manner. **C.** CART123 exert potent anti-HL activity in vivo. Experiment schema: 2 x10^6 Luciferase-positive SUP-HD1 cells were injected i.v. in NSG mice and tumor engraftment was monitored by bioluminescence imaging. At day 21 mice were randomized to receive no treatment, 4 x10^6 control untransduced T cells (UTD) or 4 x10^6 CART123 (10 mice per group) Mice receiving CART123, but not controls, experienced complete response.

Figure S4

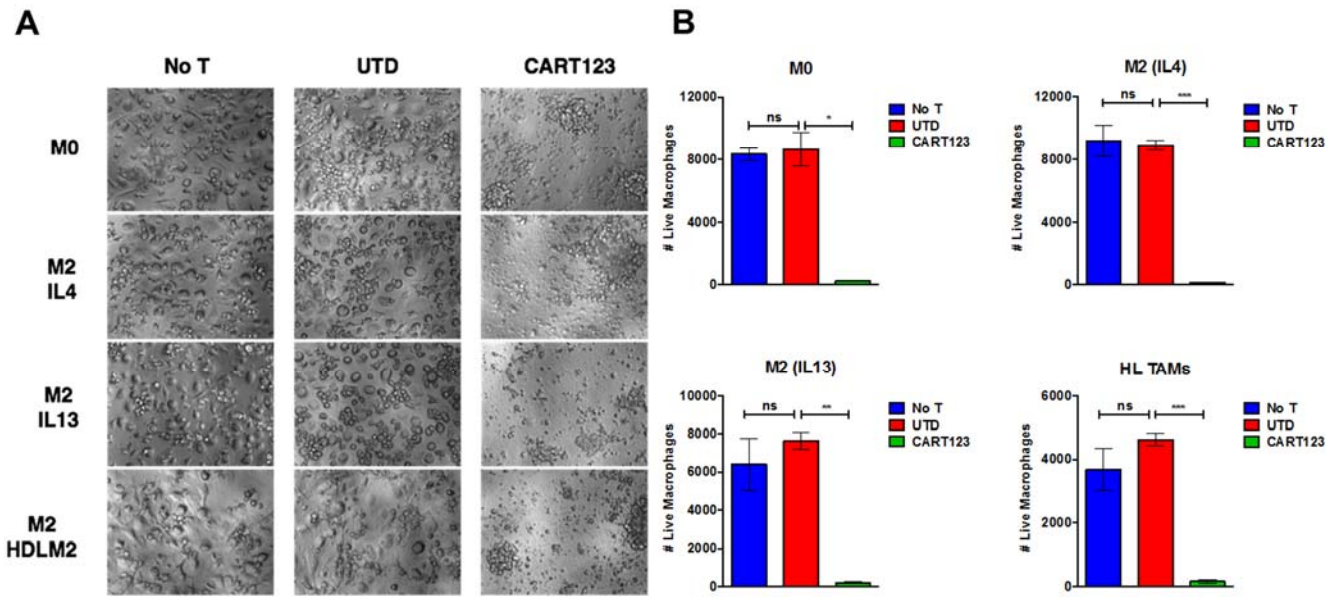

Figure S4. CART123 efficiently kill macrophages. A. Primary human monocyte derived macrophages were differentiated and polarized as described above. Autologous macrophages were co-cultured with untransduced (UTD) T cells, CART123, or media alone for 7 days at an E:T ratio of 1:1 in a 96-well plate. All conditions were tested in triplicate. At the end of a 7 day co-culture period, killing was assessed by 10x phase microscopy. **B.** Absolute macrophage counts were determined by flow cytometry using CountBright Absolute Counting Beads (Thermo Fisher). Live macrophages were identified as CD11b+ and LiveDeadAqua-negative.

References

1. Gill S, Tasian SK, Ruella M, Shestova O, Li Y, Porter DL, et al. Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor-modified T cells. Blood. 2014;123:2343-54.