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Supplemental Information

Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma

Avery D. Posey, Jr., Robert D. Schwab, Alina C. Boesteanu, Catharina Steentoft, Ulla Mandel, Boris Engels, Jennifer D. Stone, Thomas D. Madsen, Karin Schreiber, Kathleen M. Haines, Alexandria P. Cogdill, Taylor J. Chen, Decheng Song, John Scholler, David M. Kranz, Michael D. Feldman, Regina Young, Brian Keith, Hans Schreiber, Henrik Clausen, Laura A. Johnson, and Carl H. June

Supplemental Figures

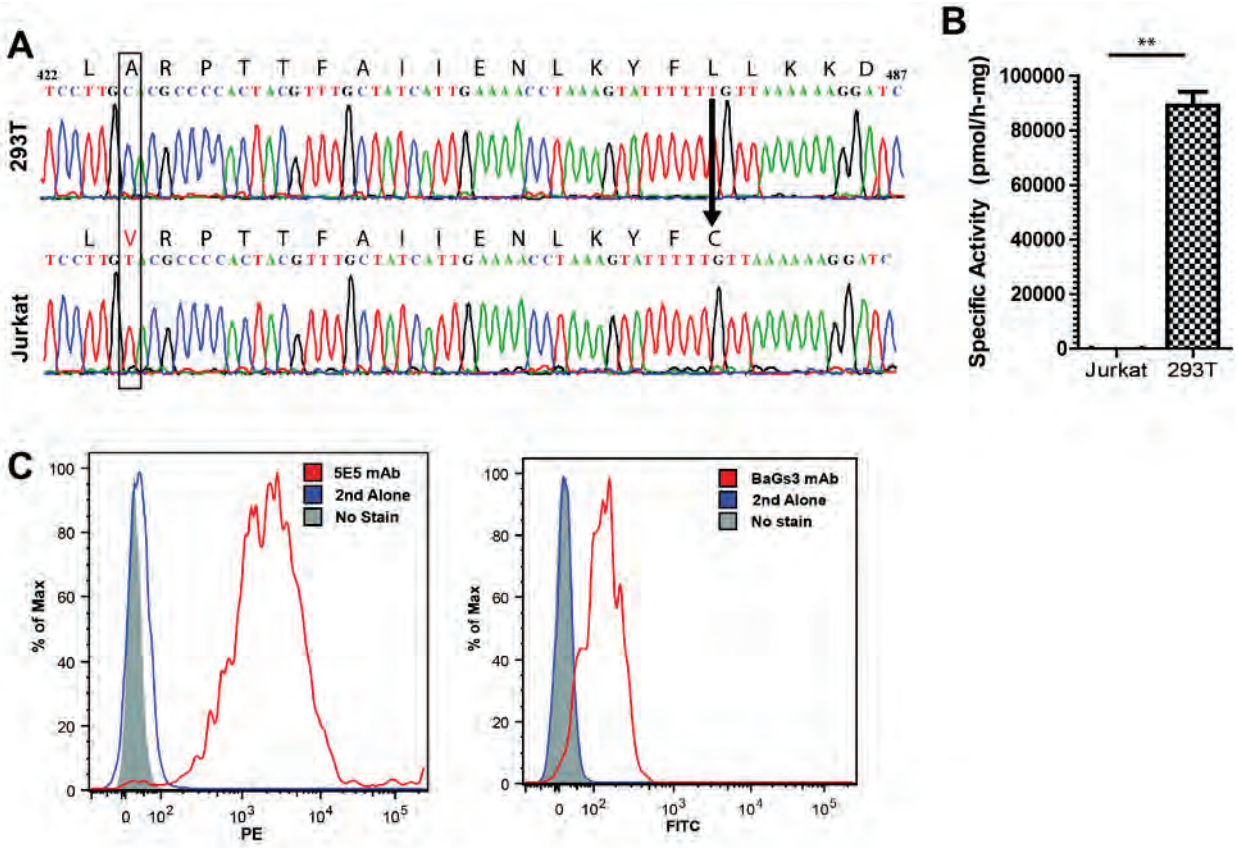

Figure S1, related to Figure 2 and Figure 3. Jurkat E6-1 cells have decreased O-linked glycosylation because of a truncation within *Cosmc* that eliminates T synthase activity.

A. DNA sequencing chromatogram from *Cosmc* genomic DNA amplicon (bases 422-487 of the reference coding sequence). Note the deletion of base 473 in the Jurkat sample (also the conversion of C to T at base 428 in the Jurkat sample, compared with the 293T sample).
B. T synthase activity of cell extracts from Jurkat and 293T cell lines. Reactions were performed twice in duplicate. p= 0.0032. Data are plotted as mean ± SEM.
C. Flow cytometric analysis of Jurkat cell line immunostained with Tn-antigen specific antibodies 5E5 (left histogram) and BaGs3 (right histogram).

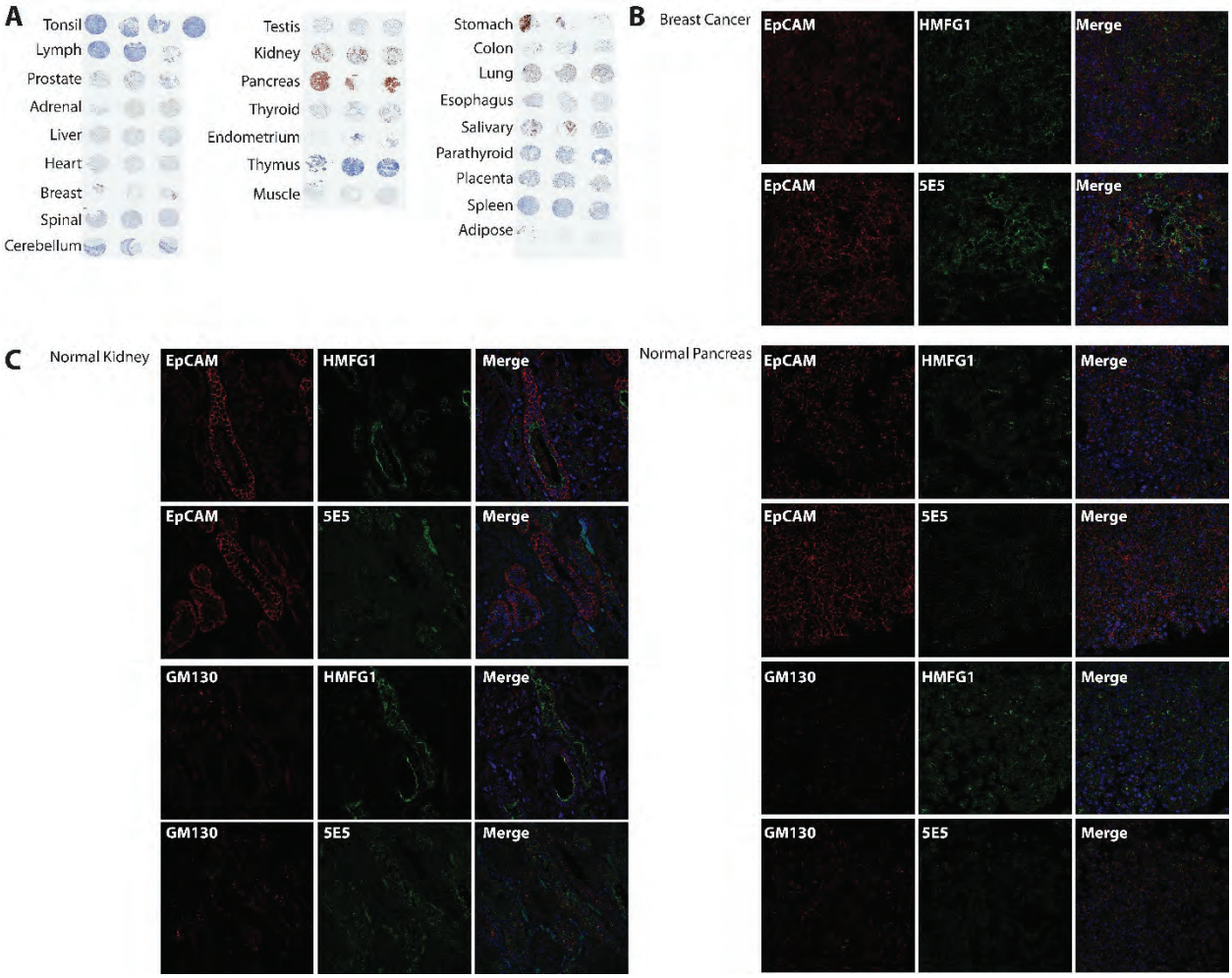

Figure S2, related to Figure 2. 5E5 mAb demonstrates intracellular, golgi-like staining within normal human tissue and membrane staining on malignant tissue.

A. Normal human tissue microarray stained with anti-Tn-MUC1 5E5 mAb.
B. Human breast cancer stained with anti-MUC1 HMFG1 or 5E5 mAb (green) and EpCAM (red).
C. Normal human pancreas and kidney immunostained with HMFG1 or 5E5 mAb (green), and epithelial membrane marker EpCAM or cis-golgi marker GM130 (red).

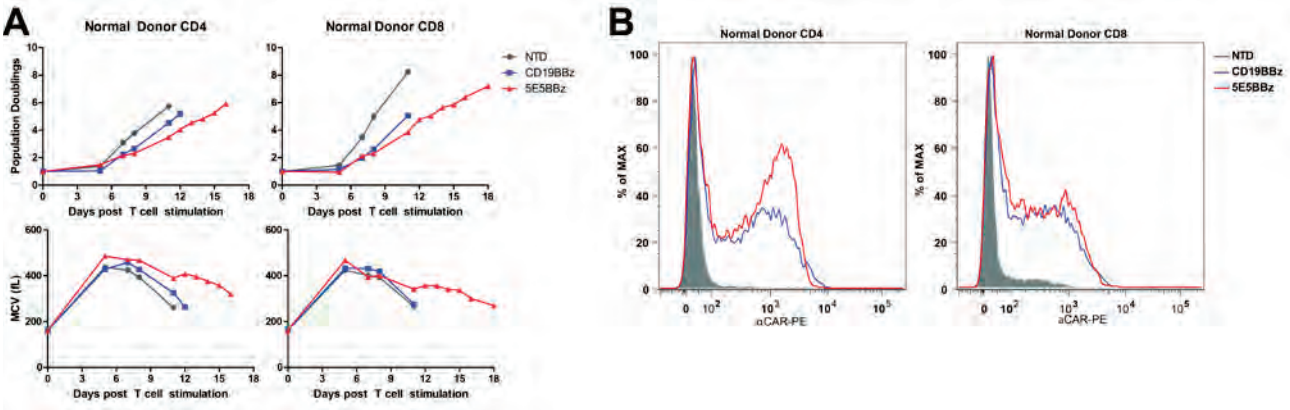

Figure S3, related to Figure 1B. Representative expansion and transduction of normal human donor T cells.

A. Normal donor CD4 and CD8 T cells were stimulated with anti-CD3 and anti-CD28 magnetic beads on Day 0. Number of cells and mean cell volume (MCV) were measured serially on indicated days.
B. Normal donor CD4 and CD8 T cells, transduced and expanded as in A, were frozen when MCV reached 300fL. Aliquots of cells were thawed and stained 18 hours after thawing for CAR using goat-anti-mouse $F(ab)_2$ fragments.

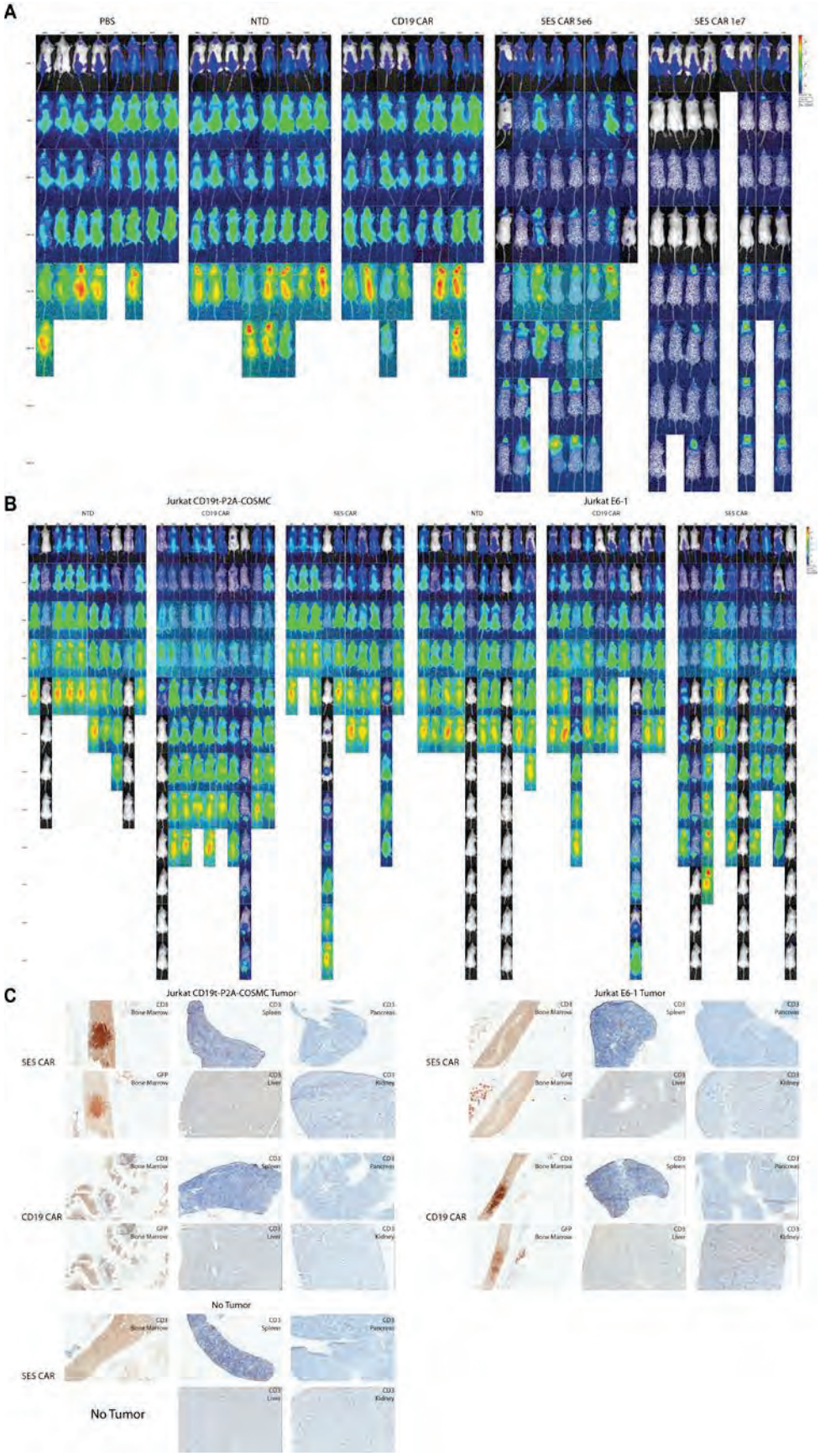
A
PBS
NTD
CD19 CAR
SES CAR 5e6
SES CAR 1e7
B
Jurkat CD19t-P2A-COSMC
NTD
CD19 CAR
SES CAR
Jurkat E6-1
NTD
CD19 CAR
SES CAR
C
Jurkat CD19t-P2A-COSMC Tumor
Jurkat E6-1 Tumor
SES CAR
CD19 CAR
No Tumor
SES CAR
No Tumor
CD3 Bone Marrow
CD3 Spleen
CD3 Pancreas
GFP Bone Marrow
CD3 Liver
CD3 Kidney

Figure S4, related to Figures 3C and 4D. 5E5 CAR T cell-treated mice demonstrated an increase in survival and reduced tumor progression, which is specific to COSMC-deficient aberrant glycosylation.

A. Mice were injected with $5x10^6$ CBG^+ Jurkat E6-1 cells and imaged 1 day prior to T cell infusion and serially after treatment until the end of the experiment.
B. Mice were injected with $5x10^6$ CBG^+ Jurkat CD19t-P2A-COSMC (left half) and Jurkat E6-1 (right half) cells and imaged 1 day prior to T cell infusion and serially after treatment until the end of the experiment.
C. Immunohistochemistry of bone marrow, spleen, pancreas, liver, and kidney from mice bearing Jurkat tumor and treated with CD19 CAR or 5E5 CAR. Staining for human CD3 revealed human T cells in the bone marrow and spleen of all mice, including mice without tumor, with considerably more T cells in the bone marrow of 5E5 CAR mice bearing Jurkat CD19t-P2A-COSMC tumor and CD19 CAR mice bearing Jurkat E6-1 tumor. Similarly, these mice also demonstrated the presence of Jurkat leukemia cells in the bone marrow, stained with anti-GFP.

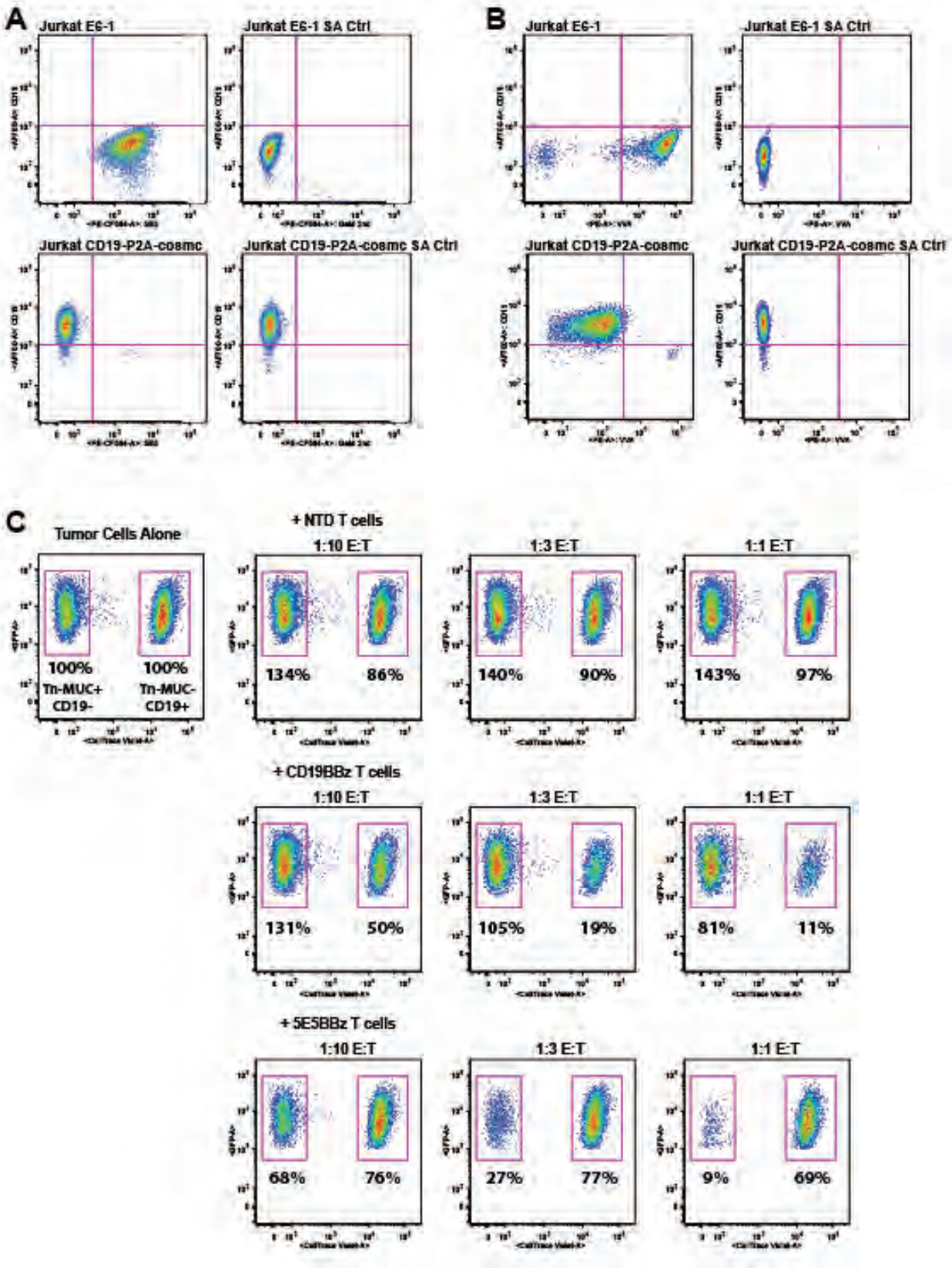
A
Jurkat E6-1
Jurkat E6-1 SA Ctrl
Jurkat CD19-P2A-cosmc
Jurkat CD19-P2A-cosmc SA Ctrl
B
Jurkat E6-1
Jurkat E6-1 SA Ctrl
Jurkat CD19-P2A-cosmc
Jurkat CD19-P2A-cosmc SA Ctrl
C
Tumor Cells Alone
100%
Tn-MUC+
CD19-
100%
Tn-MUC-
CD19+
+ NTD T cells
1:10 E:T
134%
86%
1:3 E:T
140%
90%
1:1 E:T
143%
97%
+ CD19BBz T cells
1:10 E:T
131%
50%
1:3 E:T
105%
19%
1:1 E:T
81%
11%
+ 5E5BBz T cells
1:10 E:T
68%
76%
1:3 E:T
27%
77%
1:1 E:T
9%
69%

Figure S5, related to Figure 4A and 4C. COSMC overexpression in Jurkat cells reduces the presentation of αGalNac on surface proteins as measured by 5E5 mAb and VVA lectin staining, and cytotoxicity with 5E5 CAR T cells is abrogated by COSMC overexpression (Tn-MUC1$^-$ cells) even in presence of Tn-MUC1$^+$ cells.

A. Flow cytometric analysis of GFP$^+$ Jurkat cells and GFP$^+$ Jurkat CD19t-P2A-COSMC cells stained with goat-anti-mouse-PE alone or 5E5 mAb + GAM-PE and anti-CD19 AF700.
B. Flow cytometric dot plots of Jurkat E6-1 and Jurkat CD19t-P2A-COSMC cells stained with anti-CD19 AF700 and biotinylated VVA lectin + streptavidin PE. The right panels are stained with anti-CD19 AF700 and streptavidin-PE.
C. Flow cytometric dot plots of Jurkat E6-1 (Tn-MUC1$^+$) cells (left side of plots) and Jurkat CD19t-P2A-COSMC (Tn-MUC1$^-$, CellTrace Violet-stained) cells (right side of the plots) cultured without T cells or with NTD, CD19 CAR, or 5E5 CAR T cells at the indicated effector-to-target ratios. Percentages beneath each population represent the total count of cells compared to the same population in the tumor alone control group.