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Supplemental Information

The Art and Science of Selecting a CD123-Specific Chimeric Antigen Receptor for Clinical Testing

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8.41BBz

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8.28z

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716.8.28z

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28.28z

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28.28.41BBz

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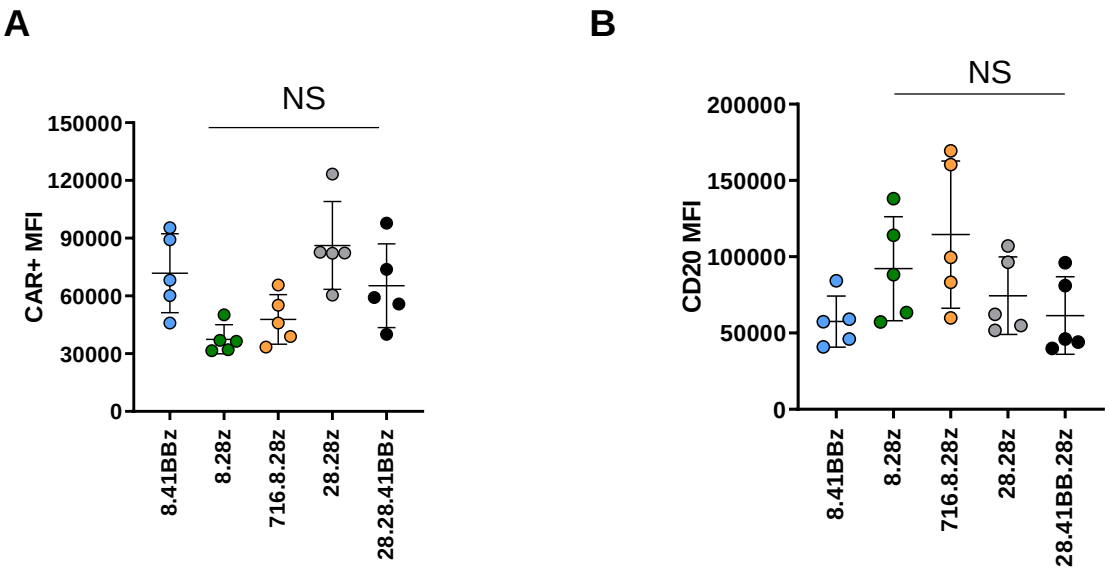
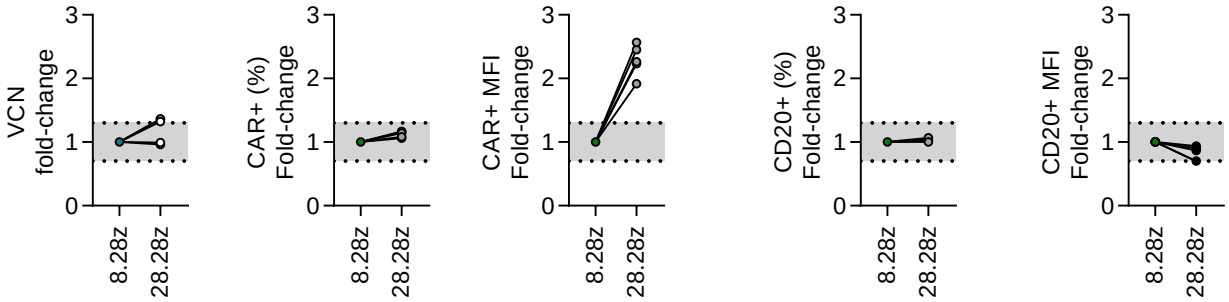
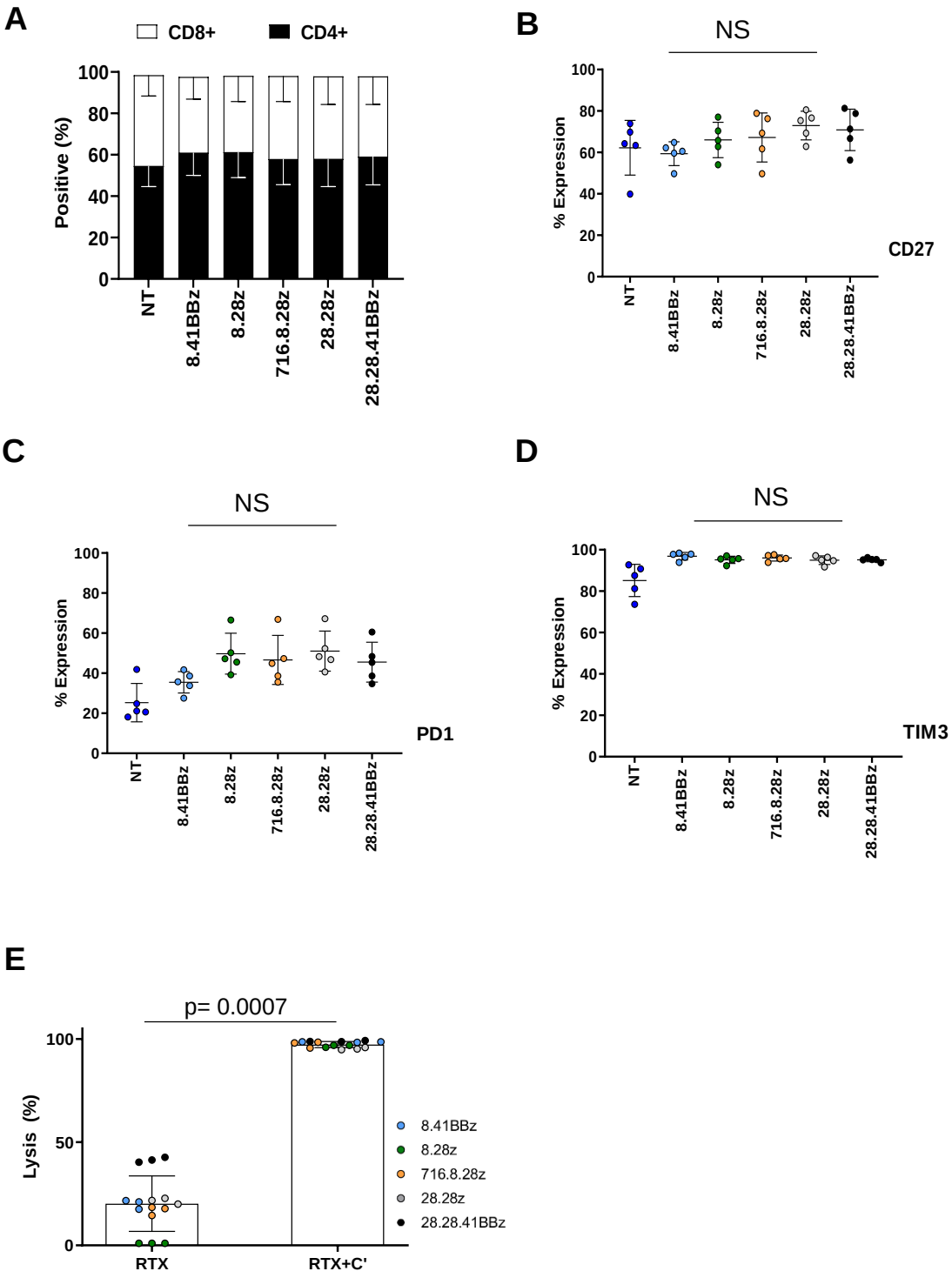
Supplemental Figure 1: Amino acid sequence of genes encoding CD20, 2A, and individual CD123-CARs. One letter code amino acid sequence of used constructs.



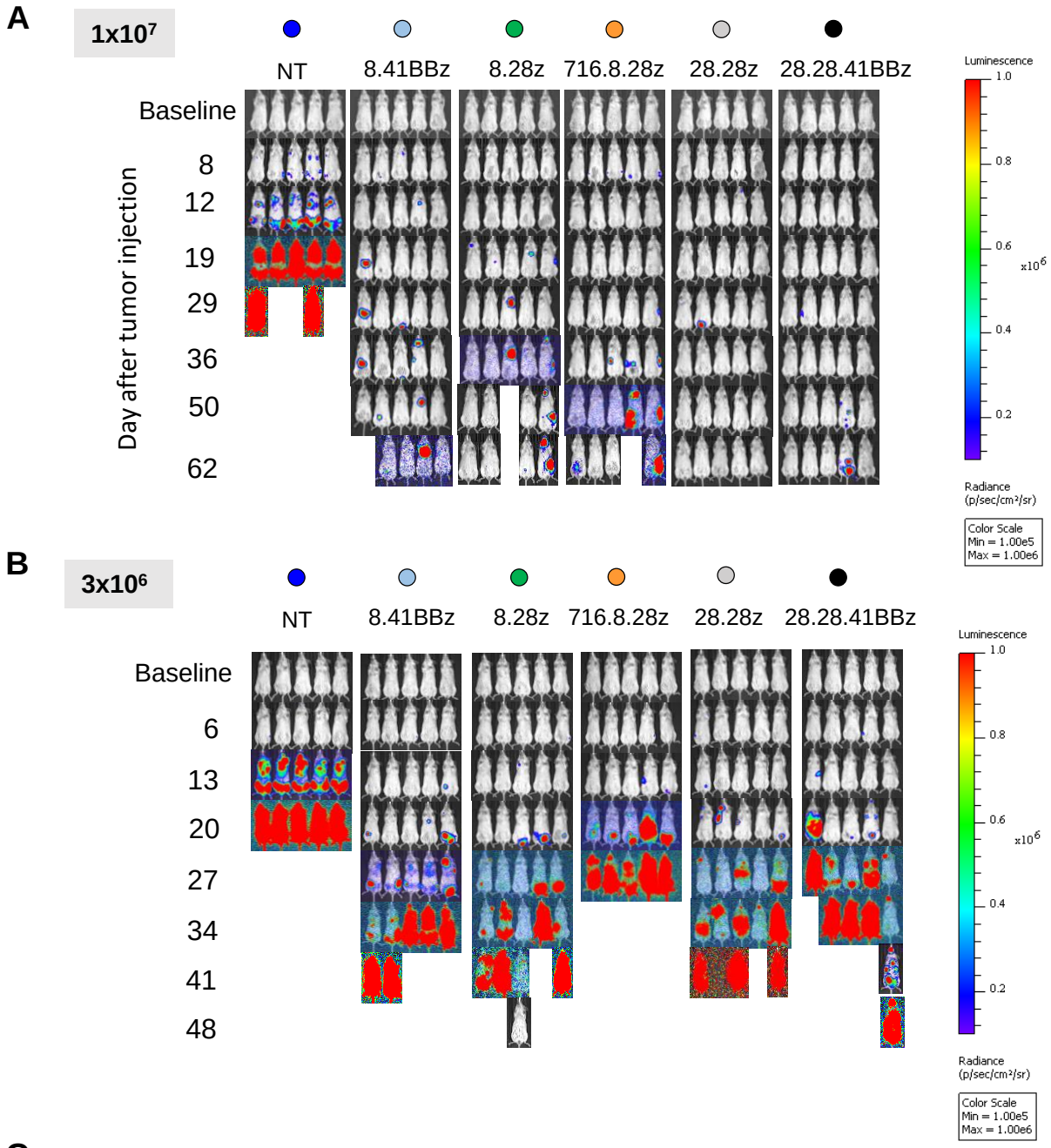
Supplemental Figure 2: CD123-CAR^{CD20} T-cells have similar transduction efficiencies. (A,B) CD123-CAR ^{CD20} T-cells were stained and analyzed by flow cytometry for % CAR and CD20 expression and mean fluorescence intensity (MFI). **(A)** CAR MFI (N=5, p=NS). **(B)** CD20 MFI (N=5, p=NS).



Supplemental Figure 3: T cells transduced with 28.28z-CARs express higher MFI levels of CARs than T cells transduced with 8.28z-CARs. Fold-change of 28.28z- and 8.28z-CAR T cells using the data presented in Figure 1 and Supplemental Figure 2 (n=5; shaded area: 0.7- to 1.3-fold change).

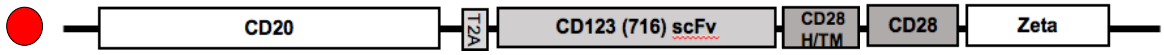


Supplemental Figure 4: CD123-CAR^{CD20} T-cells have similar CD4:CD8 ratios, expression of CD27, PD1 and TIM3 and are effectively eliminated by rituximab in vitro. (A) CD4:CD8 ratio distribution as assessed by flow cytometry. **(B-D**) Evaluation of CD27, PD1 and TIM3 expression using flow cytometry assay. **(E)** T-cells treated with rituximab (RTX) alone or rituximab plus baby rabbit complement (RTX+C') were analyzed by flow cytometry to determine the percent of CD20+ cells lysed (N=15).



Supplemental Figure 5: CD123-CAR^{CD20} T-cells have antitumor activity *in vivo* without any weight changes. This is supplemental data for the animal experiment shown in Figure 5. **(A, B)** Representative images of animal experiments using 1x10^7 and 3x10^6 T-cells as treatment (scale: 1x10^5-1x10^6). **(C)** Weights of animals.

A



B

716.28.28z

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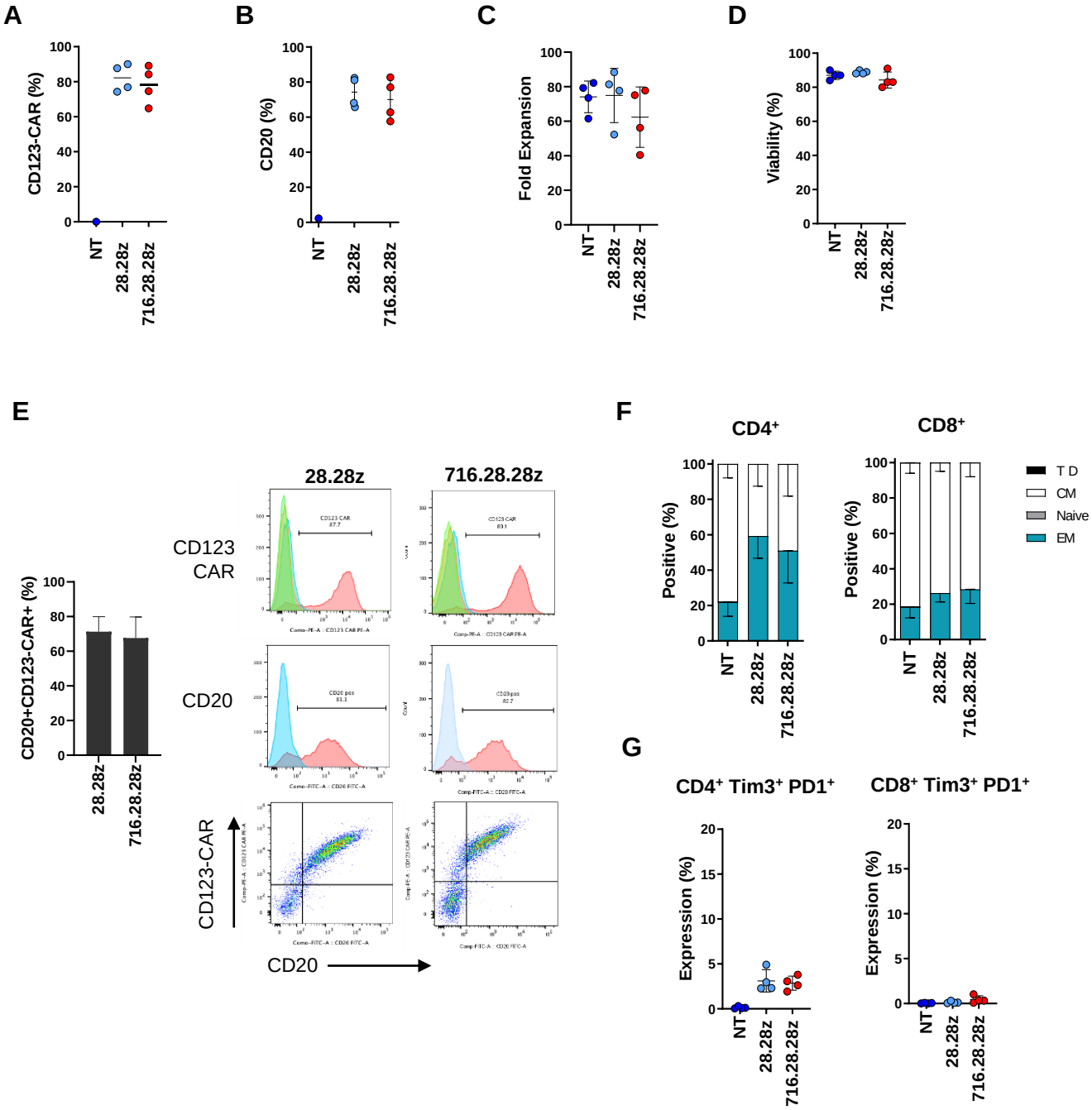
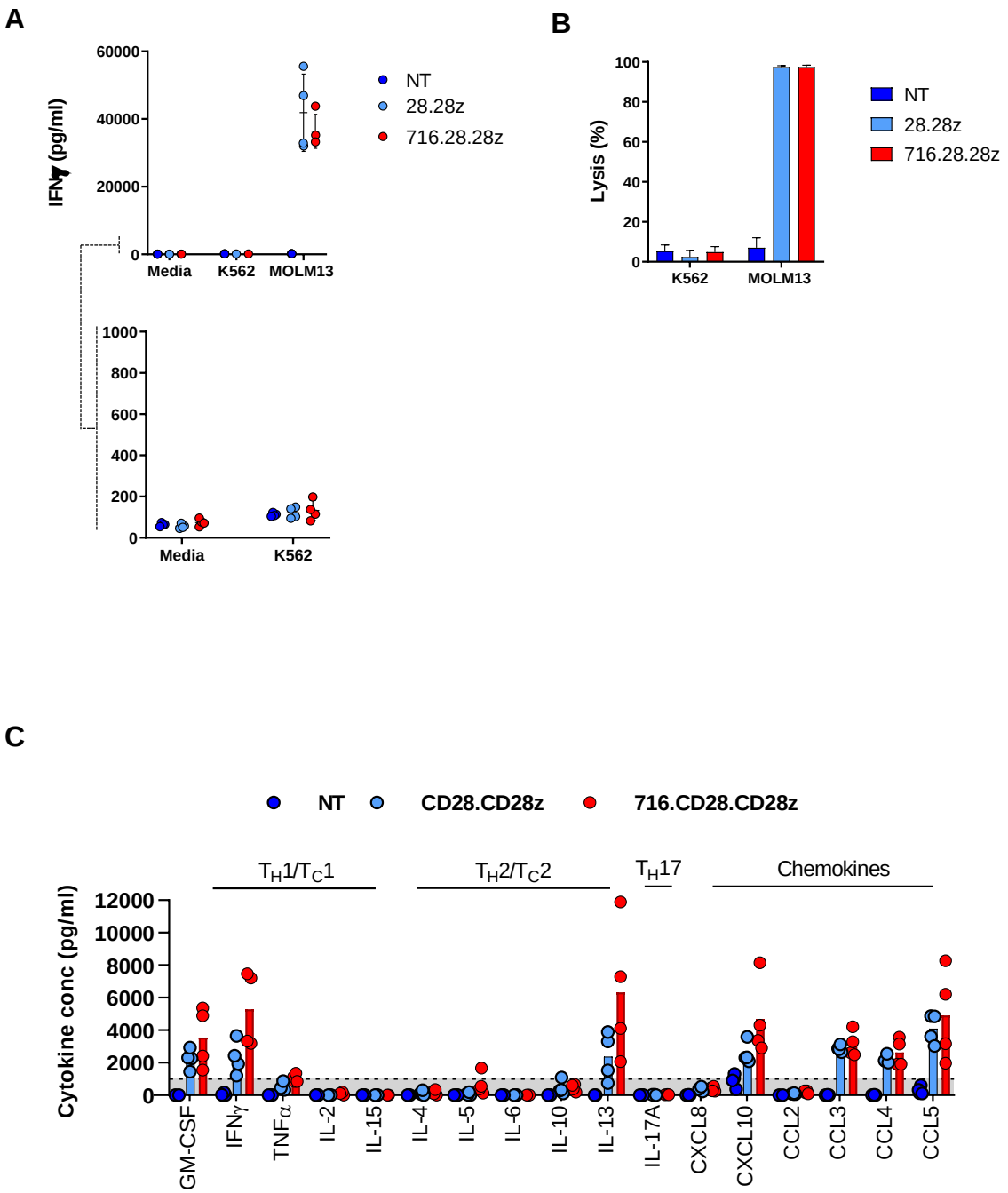
Supplemental Figure 6: Scheme and sequence of 716.28.28z CAR. (**A**) Scheme, (**B**) amino acid sequence.



Supplemental Figure 7: Comparison of CD28z based CD123-CAR^{CD20} T-cells. CD123-CAR^{CD20} T-cells were generated by transduction with lentiviral vectors encoding 292 scFV-based (CD28.CD28z) or 716 scFV-based (716.CD28.CD28z) CARs and CD20. (**A**) CAR expression, (**B**) CD20 expression, (**C**) Fold expansion, (**D**) viability, (**E**) Percentage of double transduced T cells, (**F**) CD4:CD8 ratio, (**G**) Tim3/PD1 expression (n=4; no significance difference between CD28.CD28z vs 292.CD28.z for all analyzed parameters).



Supplemental Figure 8: Comparison of CD28z based CD123-CAR^{CD20} T-cells. (**A**) Effector cells were grown in cocultures with media, K562 (CD123^{-}), or Molm13 (CD123^{+}) at an E:T ratio of 2:1 for 24 h. Supernatants were collected and evaluated for IFN-γ content by ELISA (n = 4; $p < 0.0001$ for nontransduced [NT] vs both CD123-CAR^{CD20} T-cell groups, and $p > 0.05$ for comparison 28.28z vs 716.28.28z). (**B**) Target cell populations were labeled with CFSE, incubated with effector T cells at the indicated ratios overnight and analyzed by flow cytometry by using absolute counting beads to determine cytotoxicity. n = 4; $p > 0.05$ for comparison on K562 targets and $p < 0.0001$ for CD123-CAR^{CD20} T-cell groups as compared with NT on Molm13. (**C**) Effector cells were grown in cocultures with media, K562 (CD123^{-}), or Molm13 (CD123^{+}) at an E:T ratio of 2:1 for 24 h. Supernatants were collected and evaluated by Multiplex analysis. High level of cytokine production was defined as >1,000 pg/ml (indicated by dotted line).