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Supplemental Information

Preclinical Development of Bivalent Chimeric Antigen Receptors Targeting Both CD19 and CD22

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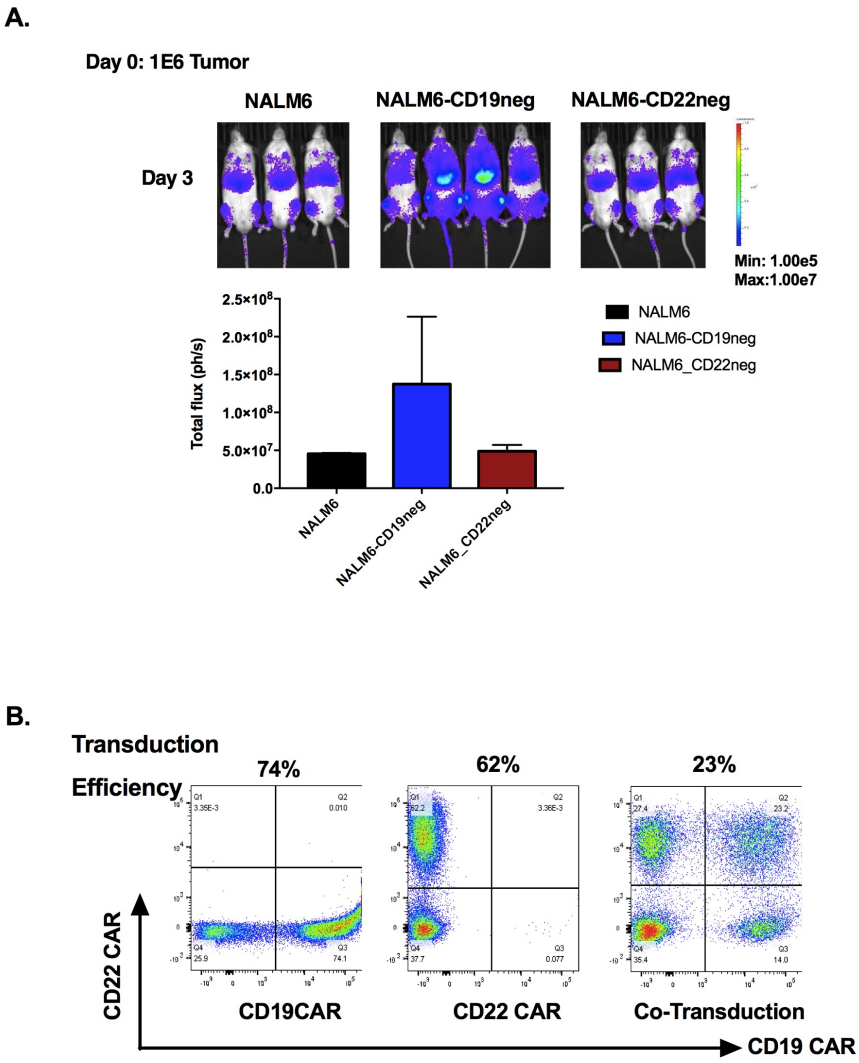
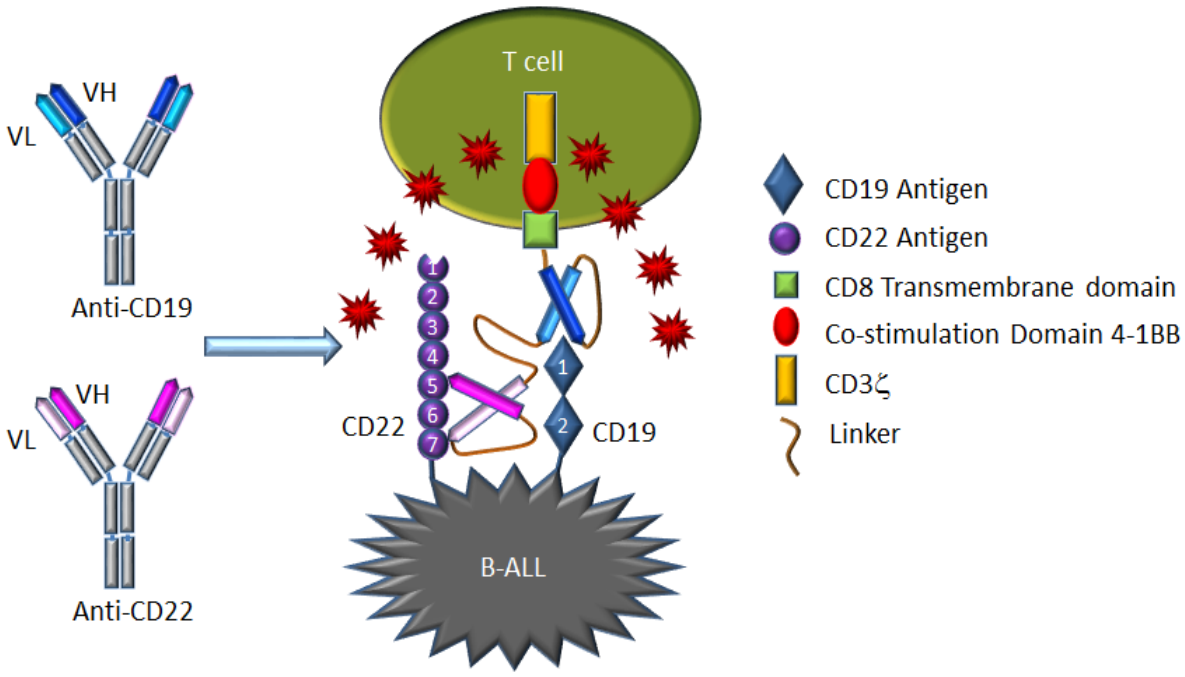
Supplementary Information

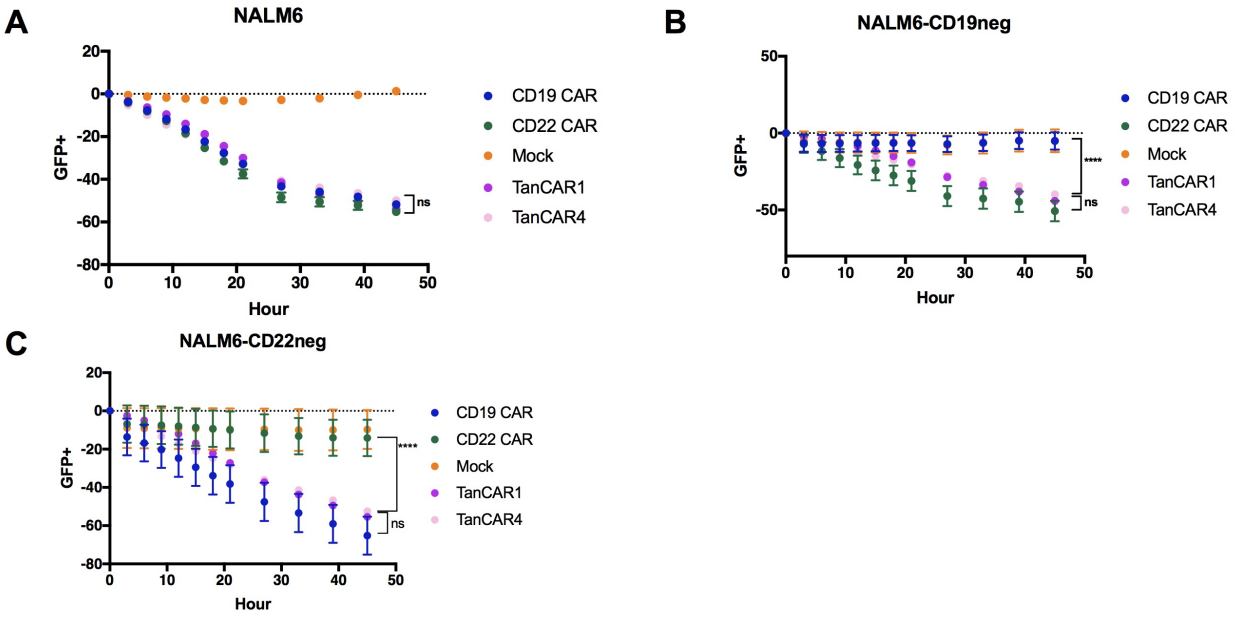


Supplemental Figure 1: *In vivo* progression of CD19neg and CD22neg Leukemia and Co-Transduction of CD19 and CD22 CAR.

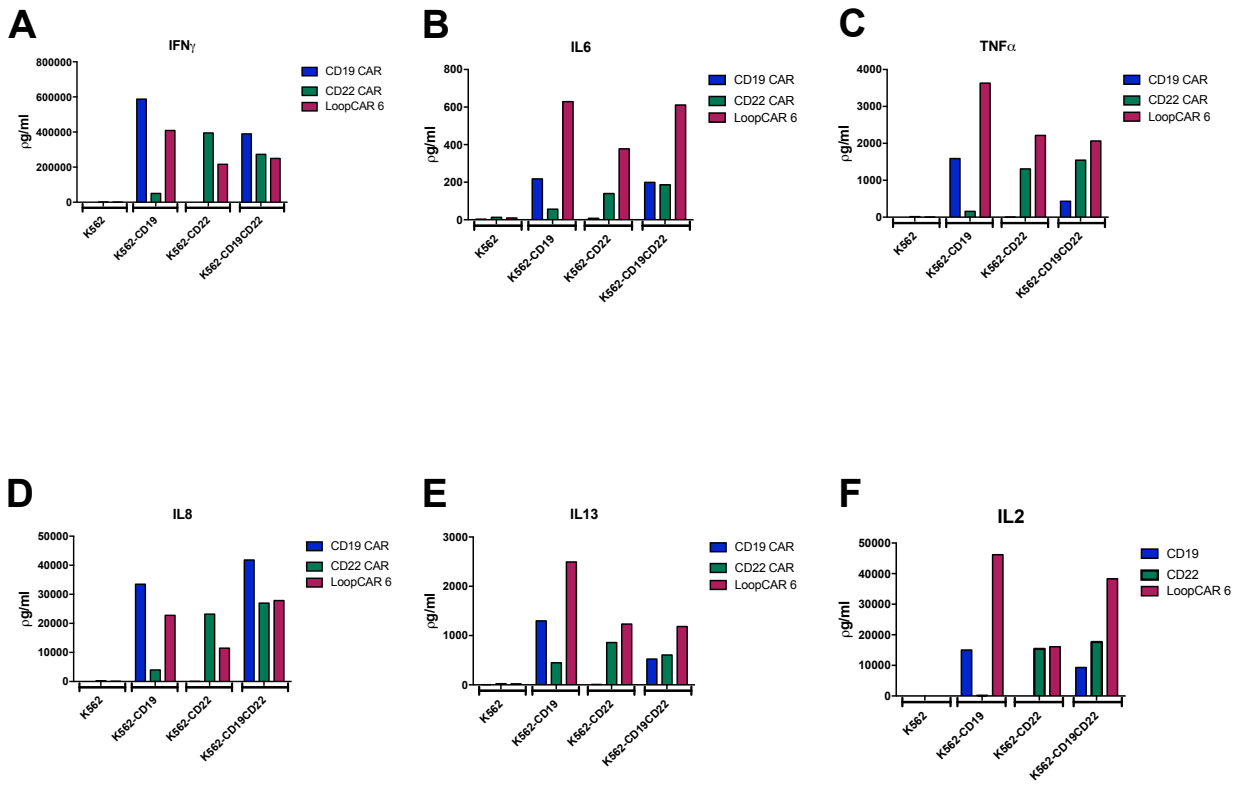
(A) NSG mice were challenged with 1E6 of NALM6, or NALM6-CD19neg, or NALM6-CD22neg leukemia on day 0 and imaged on day 3. (C)Co-transduction with both CD19 and CD22 vectors. The vectors for CD19 and CD22 CAR were produced by transient transfection of the 293T lenti packaging cell line. Human PBMCs from a healthy donor were activated with CD3/CD28 microbeads for 24 hours. Activated T cells were then transduced with the vector individually or co-transduced with both CD19 and CD22 vectors. Surface expression of CD19 CAR and CD22 CAR were analyzed on day 8.



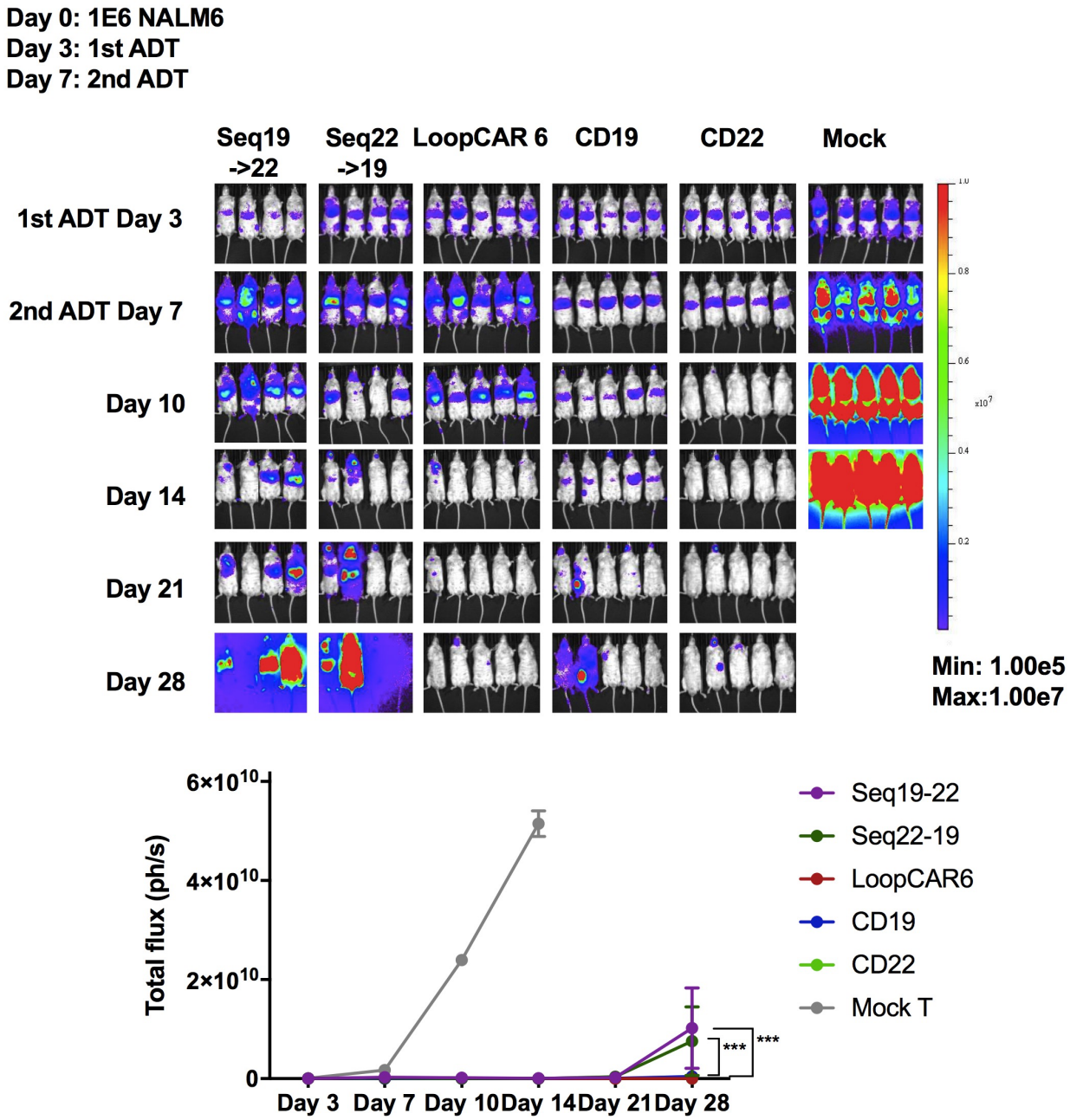
Supplemental 2: Schematic illustration of the Bispecific CAR Activity



Supplemental 3: Killing of GFP+ tumor cells assay upon co-incubation of 5E4 of CD19 and CD22 monovalent CARs, TanCAR1, TanCAR4 T cells with 5E4 of (A) NALM6, (B) NALM6-CD19^{neg}, or (C) NALM6-CD22^{neg} cells. ****p<0.0001

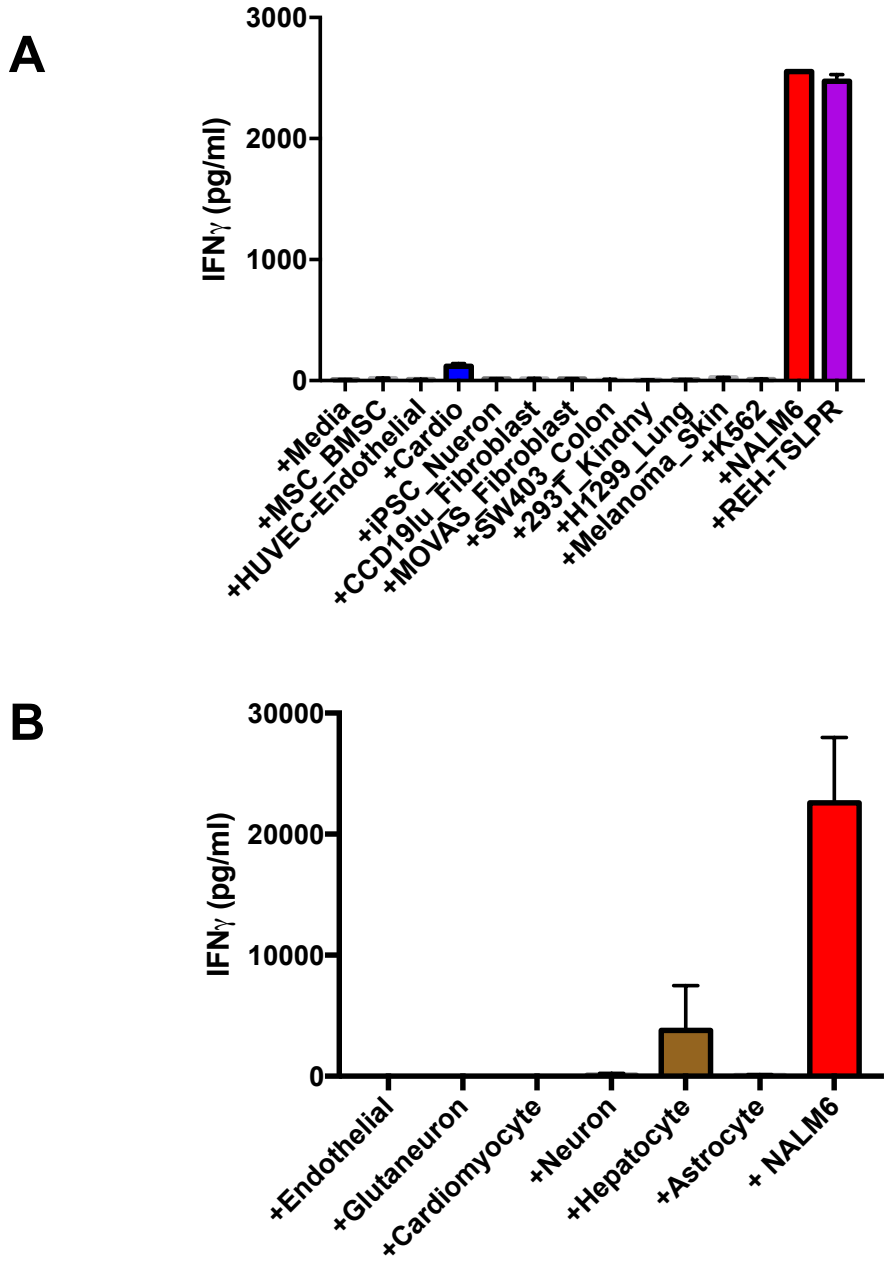


Supplemental 4: Cytokine Production by Bivalent LoopCAR6 upon stimulation via one or both binding domains. Equal number (1E5) of loopCAR6 CAR+ cells were co-incubated with CD19 or CD22, or CD19CD22 expression K562. (A) IFNγ, (B)IL6, (C)TNFa, (D) IL8, (E) IL13, and (F) IL2 production in the culture supernatant were detected with multiplex assay.



Supplemental 5: Treatment with LoopCAR6 CAR is superior to sequential admission of monovalent CARs and may reduce the likelihood of leukemic relapse

NSG mice were challenged with 1E6 of NALM6 leukemia on day 0. On day 3, mice in group 3 to 6 received 3E6 of CAR^+ T cells. Mice in group 1 and group 2 received sequential treatment with 1E6 CD19 or CD22 CAR^+ T cells on day 3 and followed by 3E6 CAR^+ T cells on day 7. Quantification of luminescence is shown below. ***p<0.001.



Supplemental 6: LoopCAR6 demonstrates no or minimal reactivity against iPS-derived cell lines. 1E5 of LoopCAR6 CAR$^+$ T cell were co-incubated with equal number of iPS-derived cells. IFNγ in the culture supernatant was measured by ELISA at 24 hours.

Construct Name	Structure	Surface Expression	In Vitro Efficacy	In Vivo Efficacy
TanCAR1	CD22VH-Linker1-CD22VL-Linker5-CD19VL-Linker6-CD19VH-BBZ	60% of both anti-CD19 and anti-CD22	CD19 ++ CD22	++
TanCAR2	CD19VL-Linker6-CD19VH-Linker5-CD22VH-Linker1-CD22VL-BBZ	29% anti-CD19 and anti-CD22	CD19 +	+
TanCAR3	CD22VH-Linker6-CD22VL-Linker5-CD19VL-Linker6-CD19VH-BBZ	None		
TanCAR4	CD22VH-Linker1-CD22VL-Linker4-CD19VL-Linker6-CD19VH-BBZ	56% of both anti-CD19 and anti-CD22	CD19 ++ CD22 ++	+++
LoopCAR1	CD19VL-Linker3-CD22VH-Linker1-CD22VL-Linker3-CD19VH-BBZ	19% of both anti-CD19 and anti-CD22	CD19 + CD22	
LoopCAR2	CD19VL-Linker3A-CD22VH-Linker6-CD22VL-Linker3B-CD19VH-BBZ	42% of both anti-CD19 and anti-CD22	CD19 ++ CD22	
LoopCAR3	CD19VL-Linker2-CD22VH-Linker6-CD22VL-Linker2-CD19VH-BBZ	24% of both anti-CD19 and anti-CD22	CD19 ++ CD22 ++	
LoopCAR4	CD22VH-Linker2-CD19VL-Linker2-CD19VH-Linker2-CD22VL-BBZ	63% of both anti-CD19 and anti-CD22	CD19 ++ CD22 +++	+++
LoopCAR5	CD19VL-Linker3C-CD22VH-Linker2-CD22VL-Linker3D-CD19VH-BBZ	49% of both anti-CD19 and anti-CD22	CD19 + CD22	
LoopCAR6	CD19VL-Linker1-CD22VH-Linker6-CD22VL-Linker1-CD19VH-BBZ	82% of both anti-CD19 and anti-CD22	CD19 +++ CD22 ++	++++

Linker annotation:

Linker 1: GGGGS
Linker 2: GGGGSGGGGS
Linker 3: GGGGSGGGGSGGGGS
Linker 3A: GGCGSGGGGSGGGGS
Linker 3B: GGGGSGGGGSGGCGS
Linker 3C: GGGGSGGGGSCGGGS
Linker 3D: GGGGCGGGGSGGGGS
Linker 4: GGGGSGGGGSGGGGSGGGGS
Linker 5: GGGGSGGGGSGGGGSGGGGSGGGGS
Linker 6: GSTSGSGKPGSGEGSTKG

Supplemental Table 1: Summary of CD19/CD22 Bivalent CAR Sturctures and Activity.