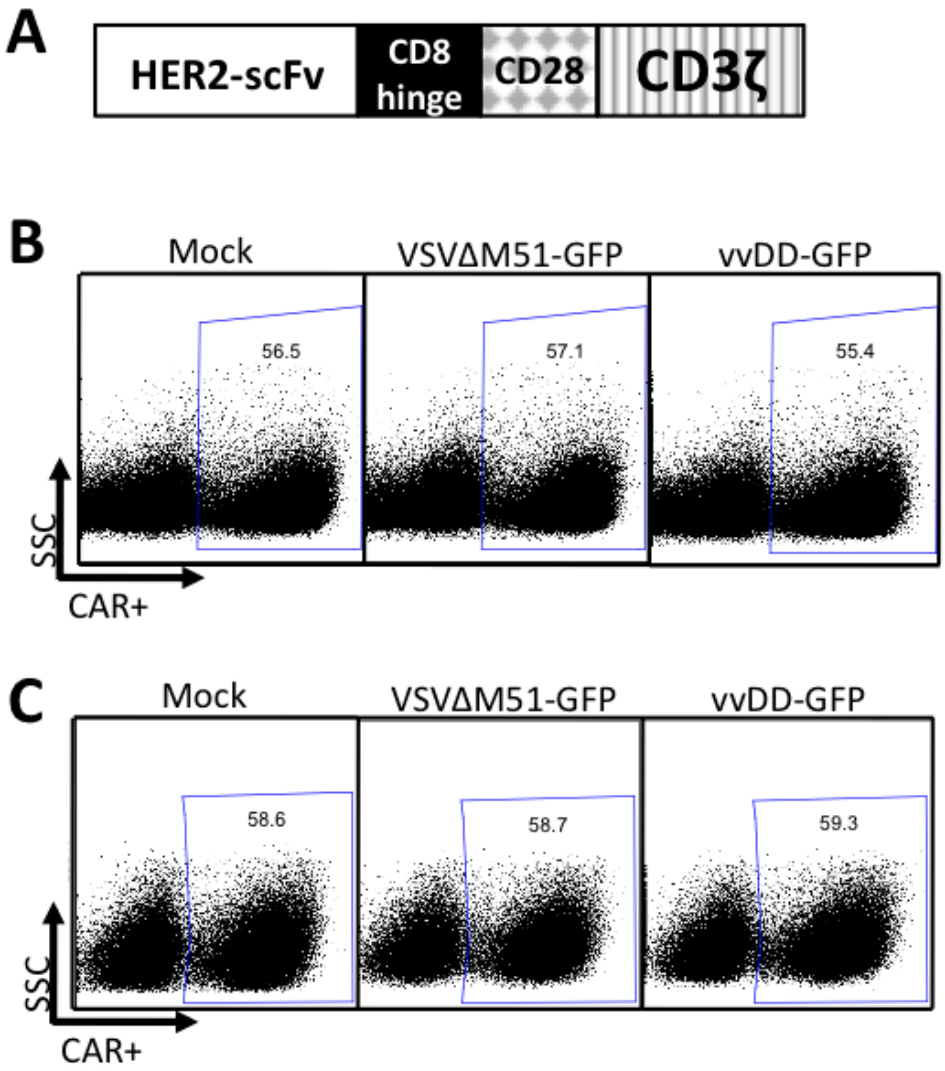
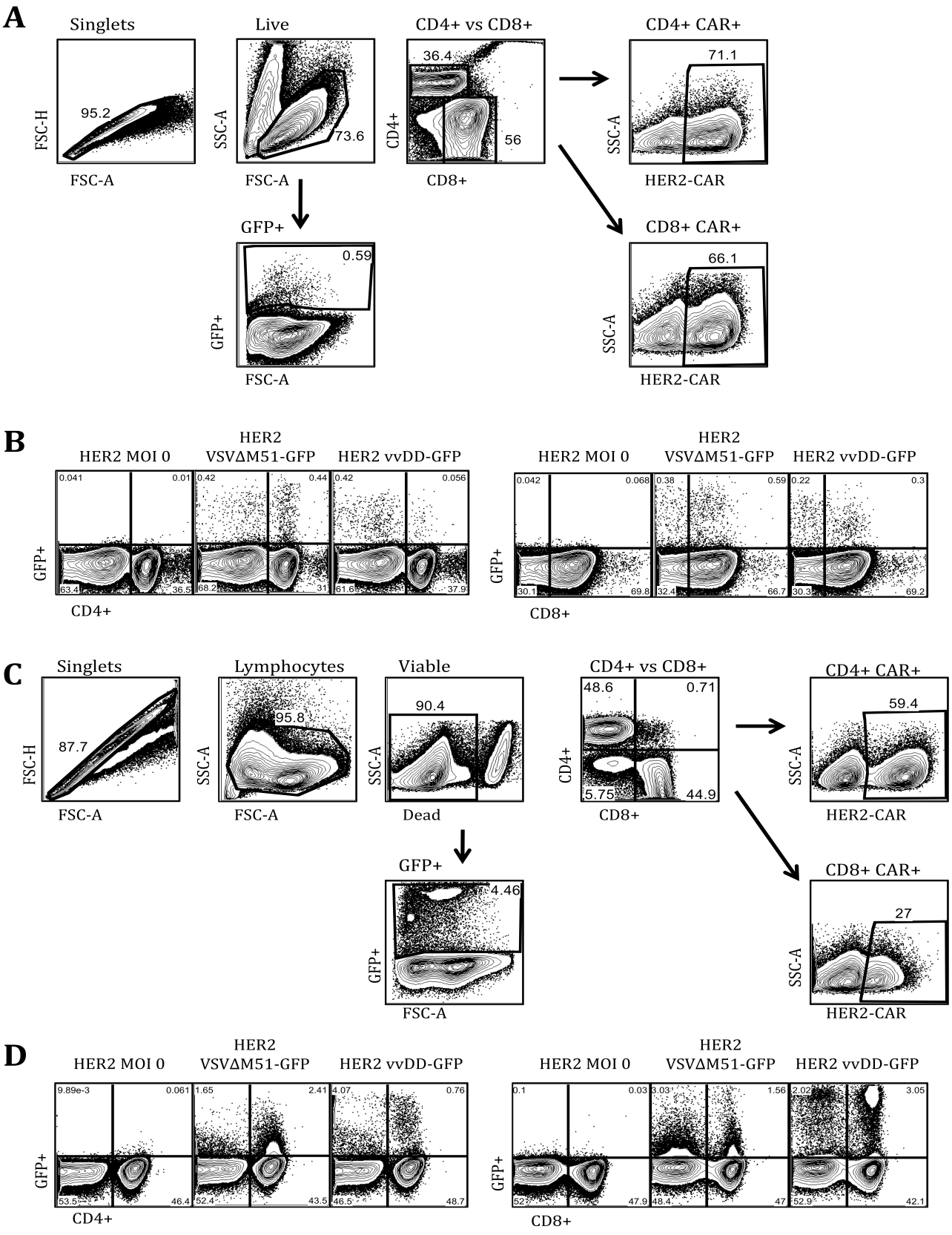
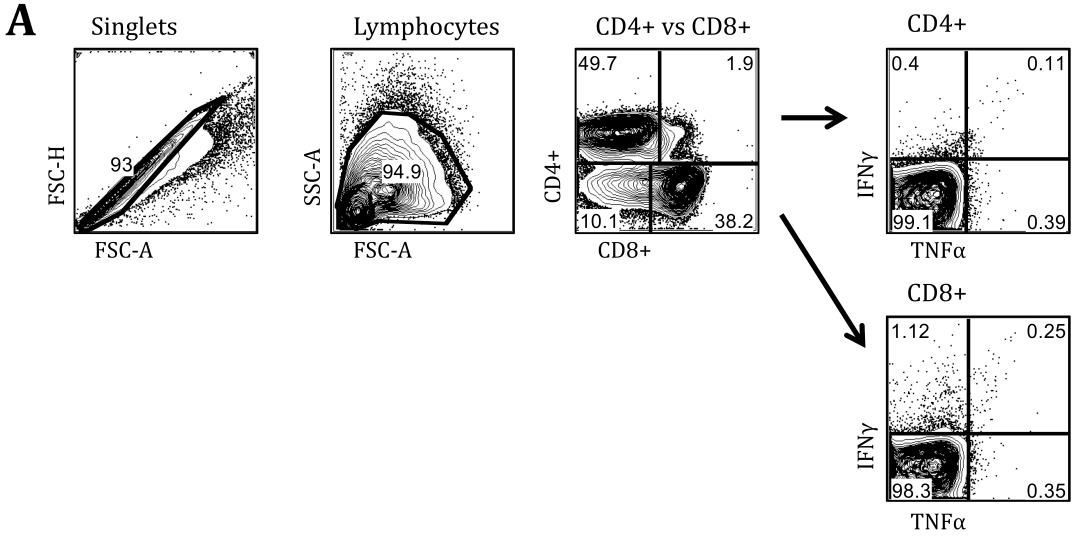
Supplemental Material

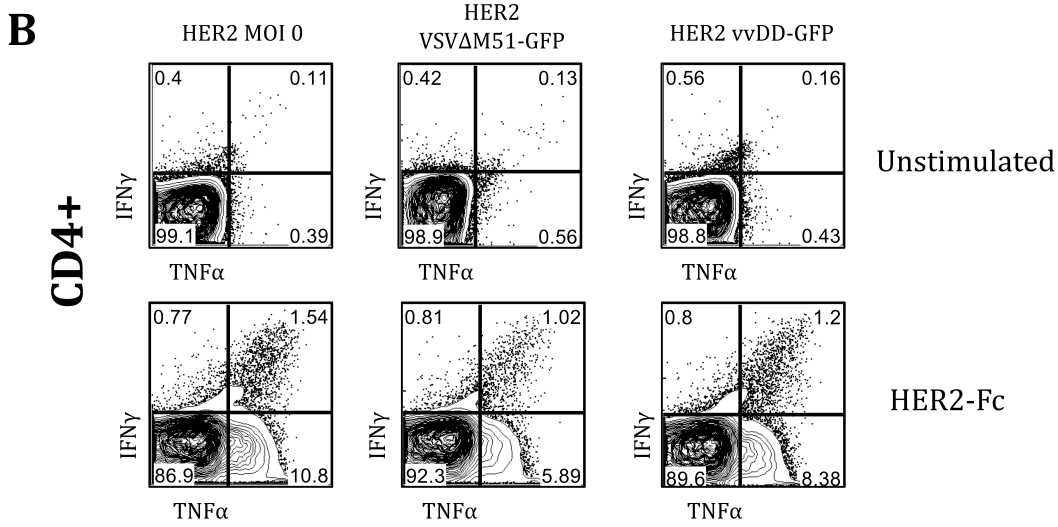


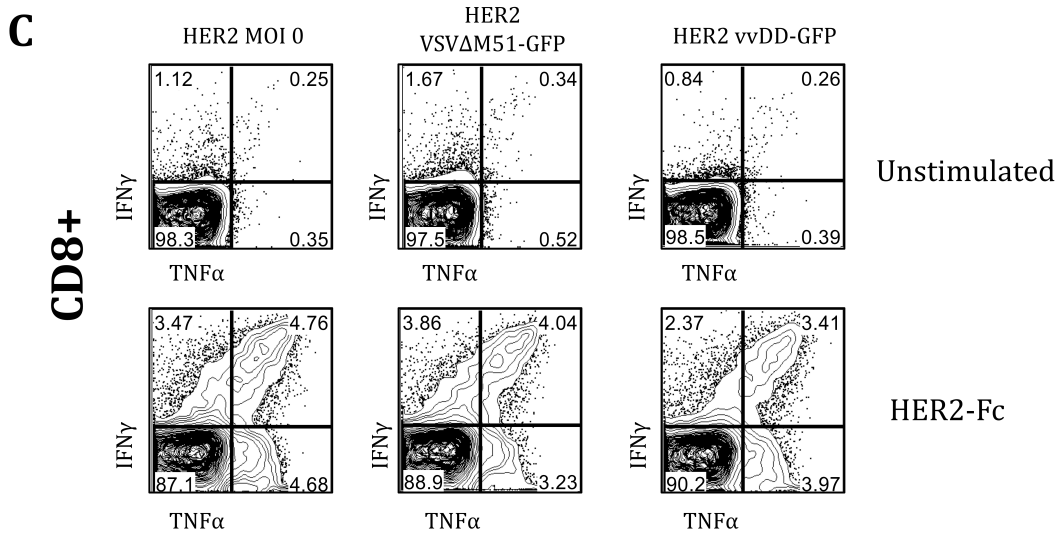
Supplemental Figure 1: Engineering T cells with CARs. (a) Schematic of the CAR construct used in both murine and human T cell engineering. (b-c) Human T cells were engineered with the HER-2-CAR with or without subsequent OV-loading, and stained for CAR expression using a HER2-Fc fusion protein, and detected using an anti-human IgG secondary antibody. Human (b) CD8+ T cells and (c) CD4+ T cells showing representative flow cytometry plots of CAR+ staining.


A
Singlets
FSC-H
95.2
FSC-A
Live
SSC-A
73.6
FSC-A
CD4+ vs CD8+
36.4
CD4+
56
CD8+
CD4+ CAR+
71.1
SSC-A
HER2-CAR
GFP+
0.59
GFP+
FSC-A
CD8+ CAR+
66.1
SSC-A
HER2-CAR
B
HER2 MOI 0
HER2 VSVΔM51-GFP
HER2 vvDD-GFP
0.041
0.01
63.4
36.5
0.42
0.44
68.2
31
0.42
0.056
61.6
37.9
GFP+
CD4+
HER2 MOI 0
HER2 VSVΔM51-GFP
HER2 vvDD-GFP
0.042
0.068
30.1
69.8
0.38
0.59
32.4
66.7
0.22
0.3
30.3
69.2
GFP+
CD8+
C
Singlets
FSC-H
87.7
FSC-A
Lymphocytes
SSC-A
95.8
FSC-A
Viable
SSC-A
90.4
Dead
GFP+
4.46
GFP+
FSC-A
CD4+ vs CD8+
48.6
0.71
CD4+
5.75
44.9
CD8+
CD4+ CAR+
59.4
SSC-A
HER2-CAR
CD8+ CAR+
27
SSC-A
HER2-CAR
D
HER2 MOI 0
HER2 VSVΔM51-GFP
HER2 vvDD-GFP
9.89e-3
0.061
53.5
46.4
1.65
2.41
52.4
43.5
4.07
0.76
46.5
48.7
GFP+
CD4+
HER2 MOI 0
HER2 VSVΔM51-GFP
HER2 vvDD-GFP
0.1
0.03
52
47.9
3.03
1.56
48.4
47
2.02
3.05
52.9
42.1
GFP+
CD8+

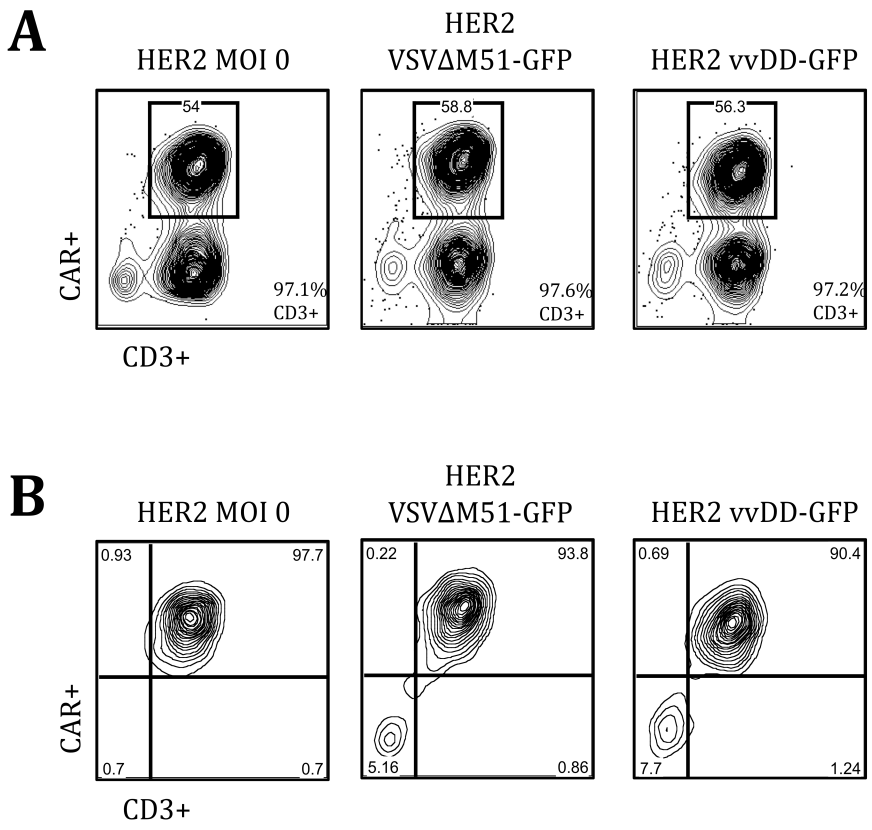
Supplemental Figure 2: Flow cytometry gating strategies and GFP expression on T cell subsets. (a) Murine or (c) human engineered T cell cultures loaded with vvDD-GFP indicating the gating strategy used to define OV-infected T cells, as well as levels of CAR expression on CD4+ and CD8+ T cells. Data is presented from day 1 post OV-load, and is representative of several independent experiments and T cell donors. Murine (b) and human (d) T cells examined for GFP+ staining following loading with either mock, VSVΔM51-GFP or vvDD-GFP showing the presence of GFP+ cells within the CD4+ and CD8+ T cell populations.


A
Singlets
FSC-H
93
FSC-A
Lymphocytes
SSC-A
94.9
FSC-A
CD4+ vs CD8+
49.7
1.9
CD4+
10.1
38.2
CD8+
CD4+
0.4
0.11
IFNγ
99.1
0.39
TNFα
CD8+
1.12
0.25
IFNγ
98.3
0.35
TNFα


B
HER2 MOI 0
HER2 VSVΔM51-GFP
HER2 vvDD-GFP
CD4+
Unstimulated
0.4
0.11
99.1
0.39
0.42
0.13
98.9
0.56
0.56
0.16
98.8
0.43
HER2-Fc
0.77
1.54
86.9
10.8
0.81
1.02
92.3
5.89
0.8
1.2
89.6
8.38
IFNγ
TNFα


C
HER2 MOI 0
HER2 VSVΔM51-GFP
HER2 vvDD-GFP
CD8+
Unstimulated
1.12
0.25
98.3
0.35
1.67
0.34
97.5
0.52
0.84
0.26
98.5
0.39
HER2-Fc
3.47
4.76
87.1
4.68
3.86
4.04
88.9
3.23
2.37
3.41
90.2
3.97
IFNγ
TNFα

Supplemental Figure 3: OV-loaded CAR- T cell cytokine staining. (a) Flow cytometry gating strategies for T cell cytokine production assays. Depicted data shows unstimulated, mock-loaded human HER2-CAR T cells as reference for where gates were set. (b-c) Human HER2-CAR T cells loaded with the indicated viruses (at day 1 post OV-load) were either unstimulated (PBS) or stimulated with plate-bound HER2-Fc antigen for 4 hours at 37°C in the presence of brefeldin A. Cytokine production was measured using flow cytometry. Data is gated on either (b) CD4+ or (c) CD8+ cells as in panel (a). Data is representative of several independent experiments and T cell donors.



Supplemental Figure 4: Flow cytometry sorting of CD3+ CAR+ following virus loading. (a) Pre-sorting gating of HER2-CAR engineered human T cells, showing CD3+ and CAR+ staining used for purifying cells for use in viral titration assays. (b) Purity of CD3+ CAR+ T cells following flow cytometry sorting. These cells were collected, frozen and used to titrate the virus on the surface of the purified T cells as found in Table S3.

Table S1: Frequency of CAR+ and CAR- subpopulations infected with OV (as indicated by GFP+). Data presented as mean frequency of CAR+ CD8+ T cells ± SEM.

	VSVΔM51-GFP		vvDD-GFP	
Days post load	**%GFP+ of CAR+**	**%GFP+ of CAR-**	**%GFP+ of CAR+**	**%GFP+ of CAR-**
1	4.52± 0.18	3.14± 0.22	2.9±0.18	1.19±0.05
2	4.12±0.16	2.75±0.31	3.94±0.35	1.45±0.02
3	1.81±0.07	1.24±0.11	6.82±1.96	2.84±0.64
5	1.33±0.06	0.64±0.09	5.94±0.95	2.89±0.32
7	1.07±0.07	0.63±0.05	5.43±0.89	2.76±0.31

Table S2: Cytokine production from GFP+ and GFP- subsets of vvDD-GFP-loaded HER2-CAR T cells following HER2 stimulation. Data is normalized to unstimulated cells, and presented as mean frequency ± SEM.

			Days Post Load				
	Cytokine(s)		**1**	**2**	**3**	**5**	**7**
CD4+	**IFNγ+**	*GFP+*	0.80±0.11	0.30±0.25	0.77±0.89	0.51±0.02	0.48±0.03
		GFP-	1.97±0.64	2.31±0.79	1.96±0.80	0.60±0.84	1.78±1.36
	TNFα+	*GFP+*	2.76±0.89	5.78±0.90	7.67±2.74	3.87±0.26	3.94±0.09
		GFP-	8.45±0.15	12.38±0.13	12.11±1.34	7.91±0.11	7.61±0.73
CD8+	**IFNγ+**	*GFP+*	2.24±0.06	0.37±0.01	0.32±0.11	0.47±0.03	0.38±0.05
		GFP-	5.15±0.50	3.57±0.59	2.57±0.58	1.38±0.48	2.85±1.83
	TNFα+	*GFP+*	4.97±0.05	2.70±0.28	2.28±0.61	1.17±0.24	2.18±0.47
		GFP-	6.44±0.20	5.41±0.18	4.41±0.26	3.16±0.26	3.86±1.22

Table S3: Viral titers from purified CD3+ CAR+ T cells loaded with VSVΔM51-GFP or vvDD-GFP. T cells were purified using flow cytometric sorting on CD3+ CAR+ cells (as depicted in Figure S4). Purified cells were frozen and used in viral plaque assays to assess viral titer on purified, CAR+ T cells.

	Virus Titer (PFU)	
	VSVΔM51-GFP	**vvDD-GFP**
Purified HER2-CAR+, CD3+ T cells	6.14×10^2 (±1.41)	2.09×10^4 ($\pm 6 \times 10^2$)