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Supplemental information

Effective chimeric antigen receptor T cells against SARS-CoV-2

Xueyang Guo, Alexandra Kazanova, Stephanie Thurmond, H. Uri Saragovi, and Christopher E. Rudd

Supplemental Figures

SFigure 1: viSNE profiles of anti-Flag and EGFP expression on CR3022-8a-28Z T cells, related to Figure 1B. Anti-Flag staining shows the surface expression of the Flag-tagged CARs in islands i, ii and iii (following expansion and prior to incubation with 293-ACE2 cells), while GFP shows the expression of EGFP by the pHIV bicistronic lentiviral construct containing IRES-EGFP (n=4).

SFigure 2: **CD4 and CD8 CAR-Ts generated within the lentiviral transduced human peripheral T-cell population, related to Figure 1B.** Flow cytometric staining with anti-CD4 and anti-CD8 shows similar proportions of CD8+ and CD4+ cells amongst the CR3022-28Z, CR3022-8a-28Z, CR3022-CH3-28Z or CR3022-IgG4-28Z CAR-Ts.

SFigure 3: **Flow cytometric profile of human ACE2 expression on 293 cells, related to Figure 1C.** 293 cells were transduced to stably express the hACE2 receptor. Light blue: isotype control; pink: anti-h ACE2 receptor.

SFigure 4: Changes in the expression of CD69 in subsets of CAR-Ts following incubation with 293-ACE2 or 293-ACE2-RBD cells, related to Figure 1E. From Figure 1e, the viSNE is subdivided into Islands i ii and and iii based on the surface expression of the activation marker CD69. Fig. 1e, right panels shows the changes in expression for island i. SFigure 4 shows that change in the percent representation amongst CAR-Ts for islands ii and and iii (Panel A); MFI changes in expression (Panel B).

SFigure 5: Response of transduced Jurkat T-cells expressing CARs, related to Figure 1. Induction of CD69 on Jurkat cells expressing different CARs in response to Vero cells loaded with RBD peptide. Data are shown as mean ± SD (n=3). * represents $p < 0.05$. ** represents $p < 0.01$. **** represents $p < 0.0001$. (compared with CAR-Jurkat only group by one-way ANOVA, n=3).

Supplemental Figures

SFigure 6: Examples of the cytolytic response of SARS-CoV-2 CAR-Ts with different donor to the RBD and S1 peptides, related to Figure 2. The killing of 293-ACE2 target cells that had been pre-coated with either the RBD (upper panels: donors B and C) or S1 peptide (lower panels: donors A and E) **.** Data are shown as mean ± SD (n=3). * represents $p < 0.05$. ** represents $p < 0.01$. *** represents $p < 0.001$. **** represents $p < 0.0001$ (compared with CR3014-28Z by two-way ANOVA).

SFigure 7: CAR-Ts do not specifically block SARS-CoV-2 pseudotyped viral particle entry into cells, related to Figure 7. Recombinant pseudotyped viral particles containing SARS-CoV-2 spike protein were used to mimic SARS-CoV-2 cell infection and cell entry. The SARS-CoV-2 pseudo-virus particles encode DsRed in the viral genome. The DsRed gene will be strongly expressed after the SARS-CoV-2 pseudo-virus entry into ACE2-expressing cells. It shows a trend in the blockade of pseudotyped viral particle entry by both CR3014 and CR3022 at different ratios.

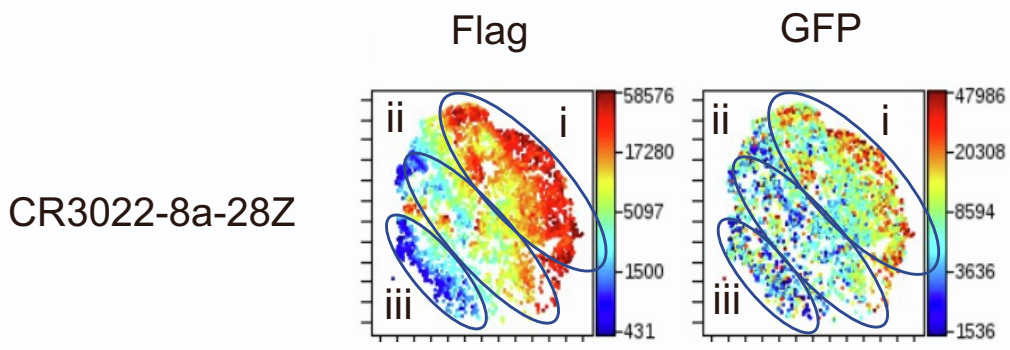
SMovies

SMovie 1: Panels of time-lapse movies of co-culturing of CAR-Ts with 293-ACE2 cells, related to Figure 3. CR3022-8a-28Z CAR-Ts were co-cultured with the 293-ACE2 cells and tracked for 20 hours. EGFP+ CAR-T cells failed to form clusters with 293-ACE2.

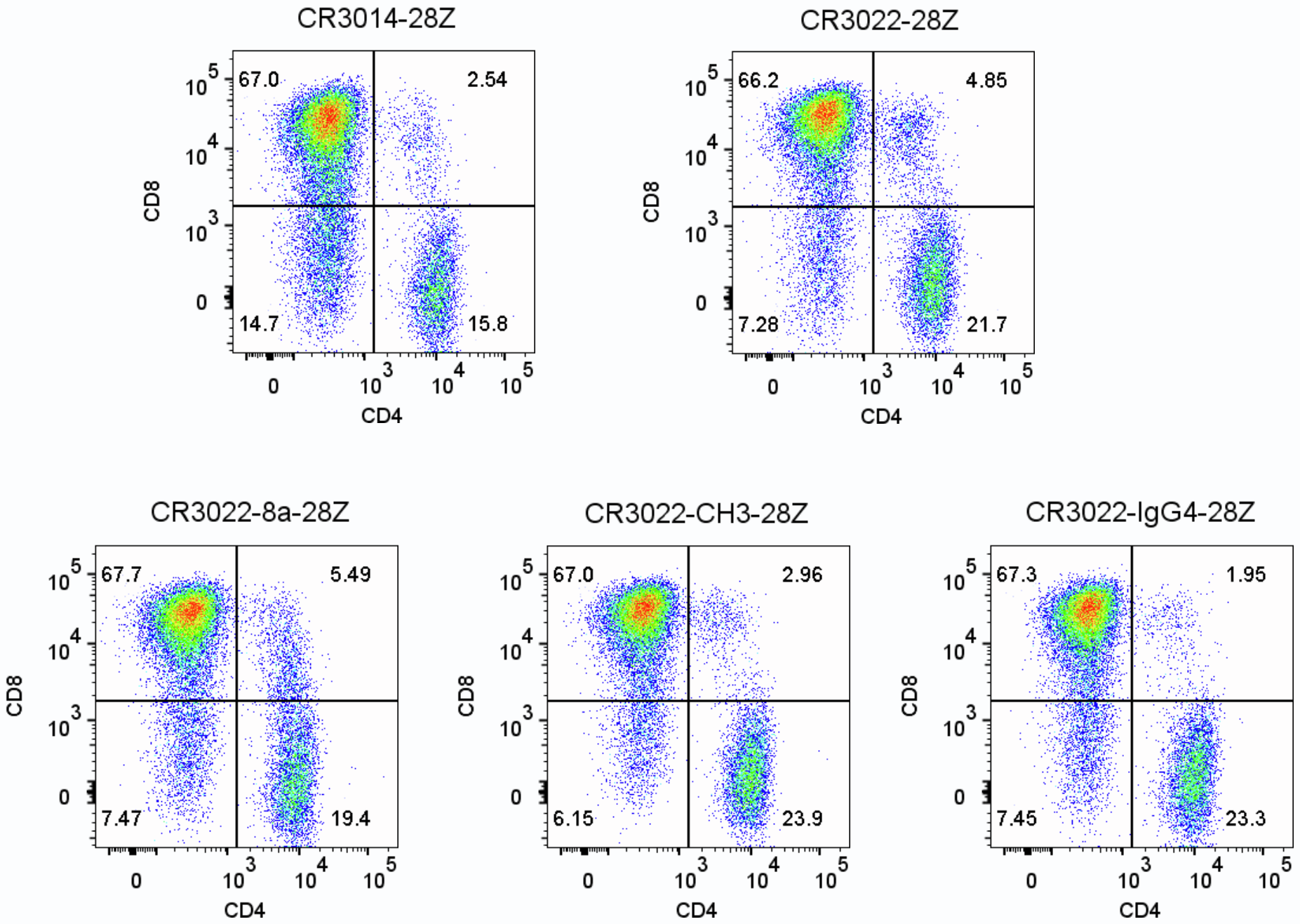
SMovie 2: Panels of time-lapse movies of co-culturing of CAR-Ts with RBD peptide loaded 293-ACE2 cells, related to Figure 3. CR3022-8a-28Z CAR-Ts were co-cultured with 293-ACE2 that had been pre-incubated with RBD peptide and tracked for 20 hours. EGFP+ CAR-T cells were observed to form clusters with 293-ACE2+RBD cells.

SMovie 3: Panels of time-lapse movies of co-culturing of CAR-Ts with S1 peptide loaded 293-ACE2 cells, related to Figure 3. CR3022-8a-28Z CAR-Ts were co-cultured with 293-ACE2 that had been pre-incubated with S1 peptide and tracked for 20 hours. EGFP+ CAR-T cells were observed to form clusters with 293-ACE2+S1 cells.

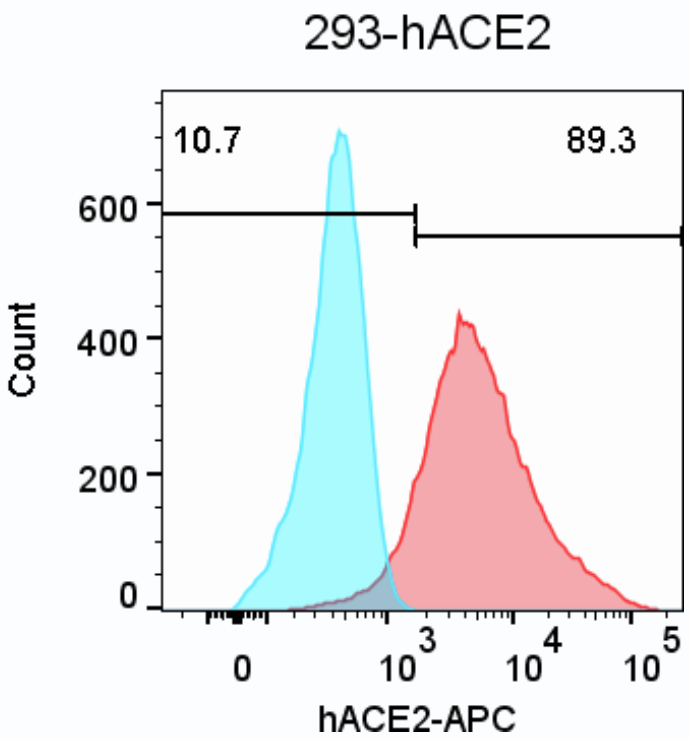
SFigure 1


Flag
GFP
CR3022-8a-28Z
ii
i
iii
58576
17280
5097
1500
431
ii
i
iii
47986
20308
8594
3636
1536

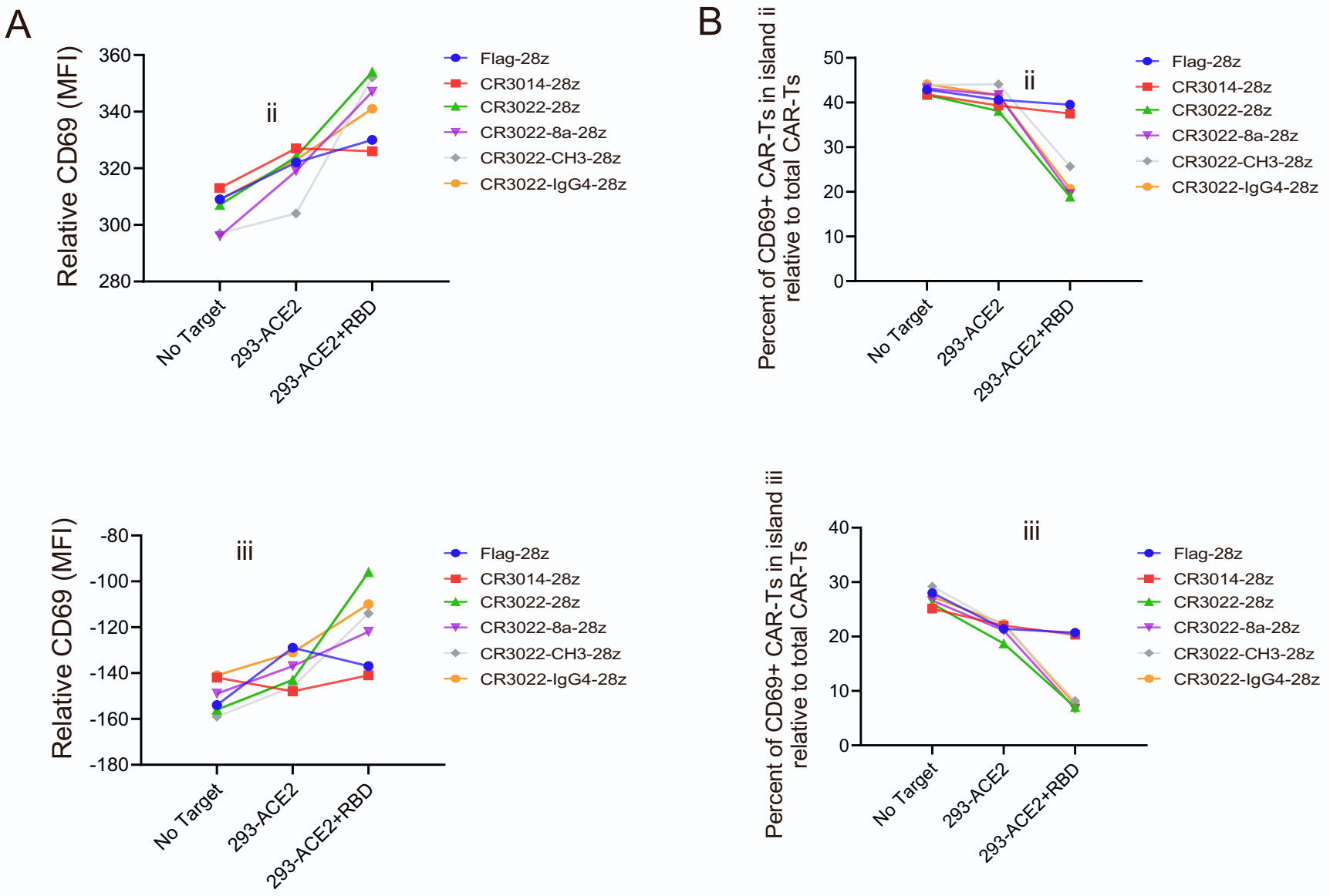
SFigure 2


CR3014-28Z
67.0
2.54
14.7
15.8
CR3022-28Z
66.2
4.85
7.28
21.7
CR3022-8a-28Z
67.7
5.49
7.47
19.4
CR3022-CH3-28Z
67.0
2.96
6.15
23.9
CR3022-IgG4-28Z
67.3
1.95
7.45
23.3
CD8
CD4
0
10^3
10^4
10^5

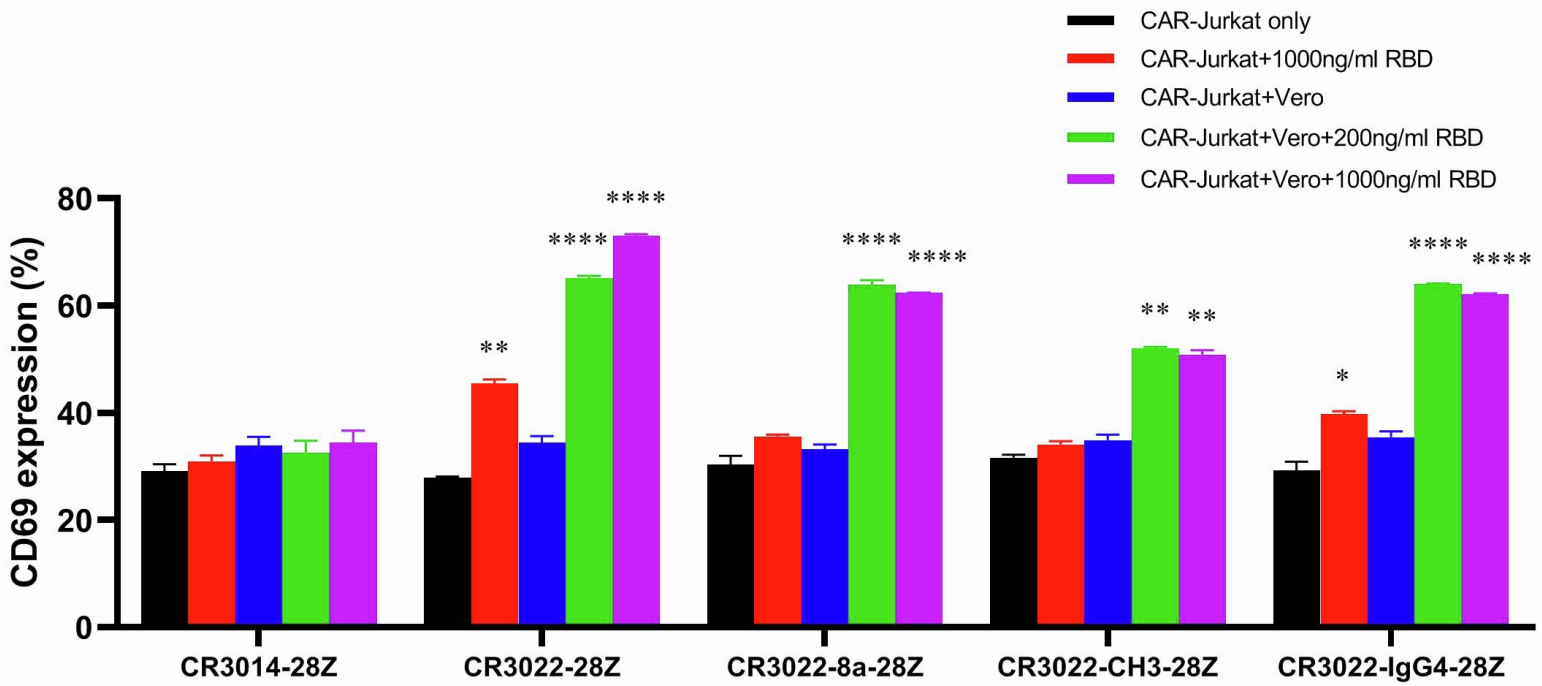
SFigure 3



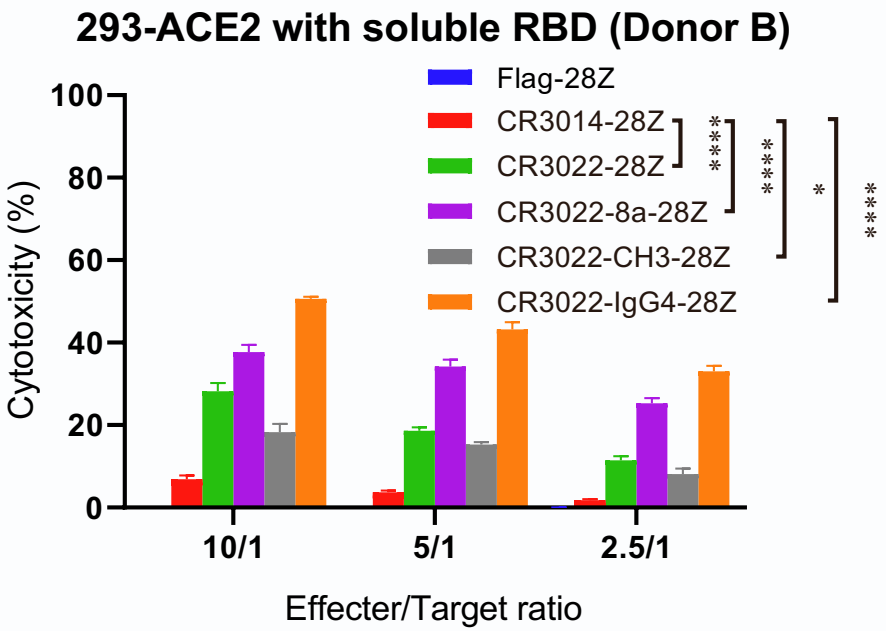
SFigure 4

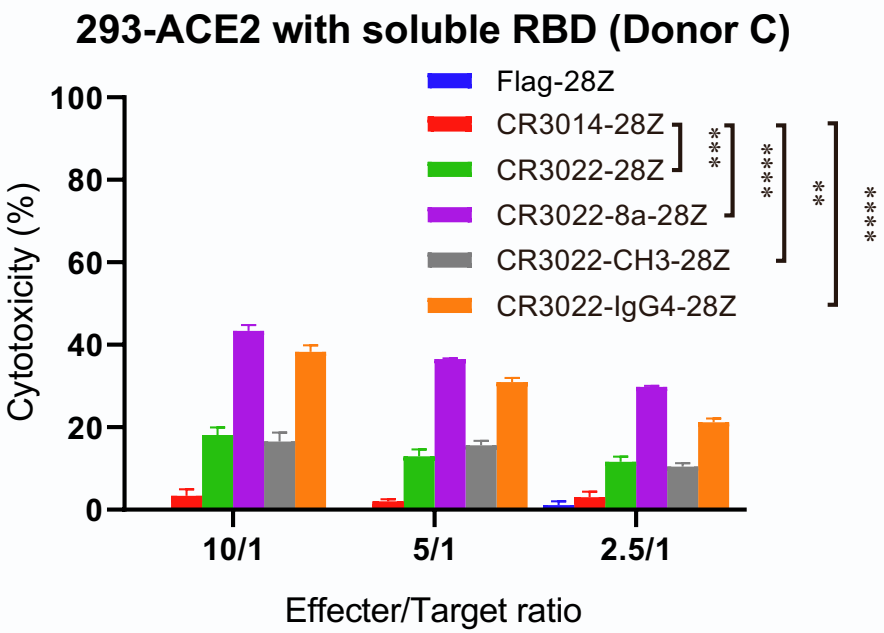

A
Relative CD69 (MFI)
ii
360
340
320
300
280
No Target
293-ACE2
293-ACE2+RBD
Flag-28z
CR3014-28z
CR3022-28z
CR3022-8a-28z
CR3022-CH3-28z
CR3022-IgG4-28z
B
Percent of CD69+ CAR-Ts in island ii relative to total CAR-Ts
ii
50
40
30
20
10
0
No Target
293-ACE2
293-ACE2+RBD
Flag-28z
CR3014-28z
CR3022-28z
CR3022-8a-28z
CR3022-CH3-28z
CR3022-IgG4-28z
Relative CD69 (MFI)
iii
-80
-100
-120
-140
-160
-180
No Target
293-ACE2
293-ACE2+RBD
Flag-28z
CR3014-28z
CR3022-28z
CR3022-8a-28z
CR3022-CH3-28z
CR3022-IgG4-28z
Percent of CD69+ CAR-Ts in island iii relative to total CAR-Ts
iii
40
30
20
10
0
No Target
293-ACE2
293-ACE2+RBD
Flag-28z
CR3014-28z
CR3022-28z
CR3022-8a-28z
CR3022-CH3-28z
CR3022-IgG4-28z

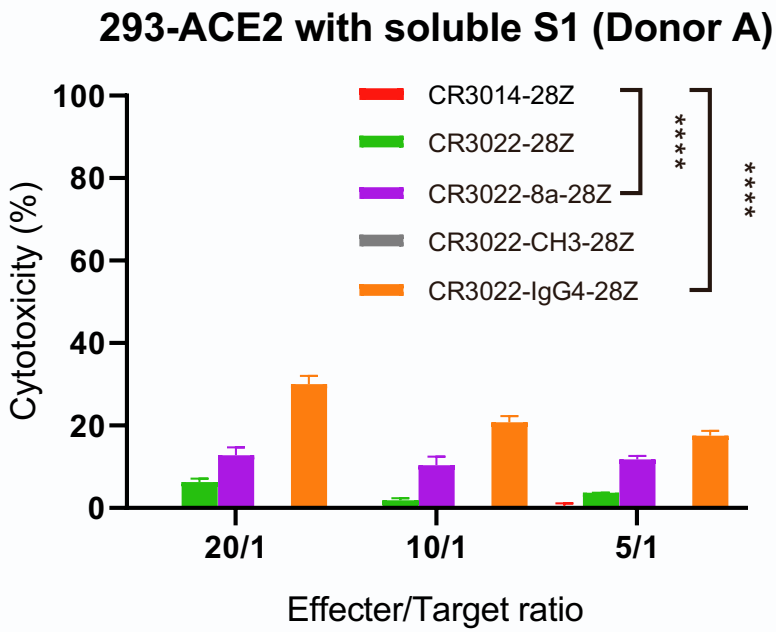
SFigure 5

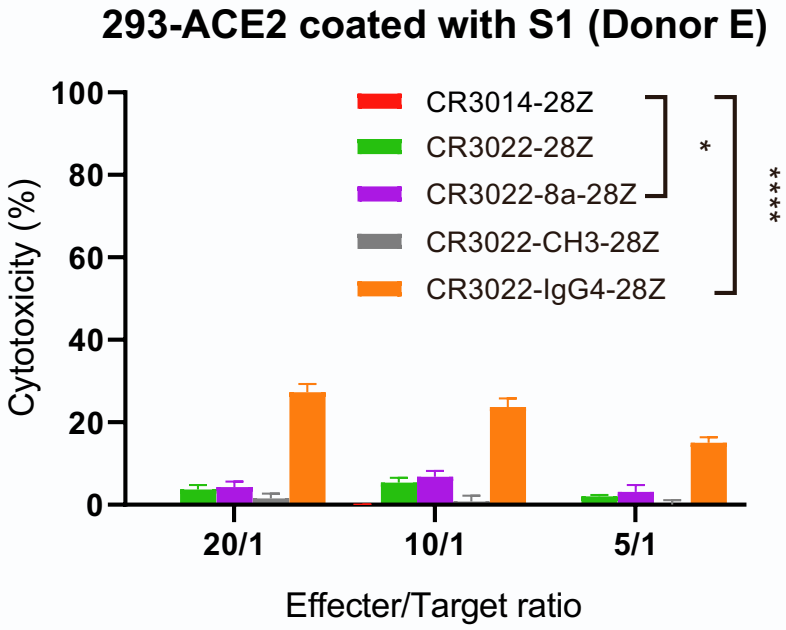

CAR-Jurkat only
CAR-Jurkat+1000ng/ml RBD
CAR-Jurkat+Vero
CAR-Jurkat+Vero+200ng/ml RBD
CAR-Jurkat+Vero+1000ng/ml RBD
CD69 expression (%)
80
60
40
20
0
**
****
****
****
****
**
**
*
****
****
CR3014-28Z
CR3022-28Z
CR3022-8a-28Z
CR3022-CH3-28Z
CR3022-IgG4-28Z

SFigure 6

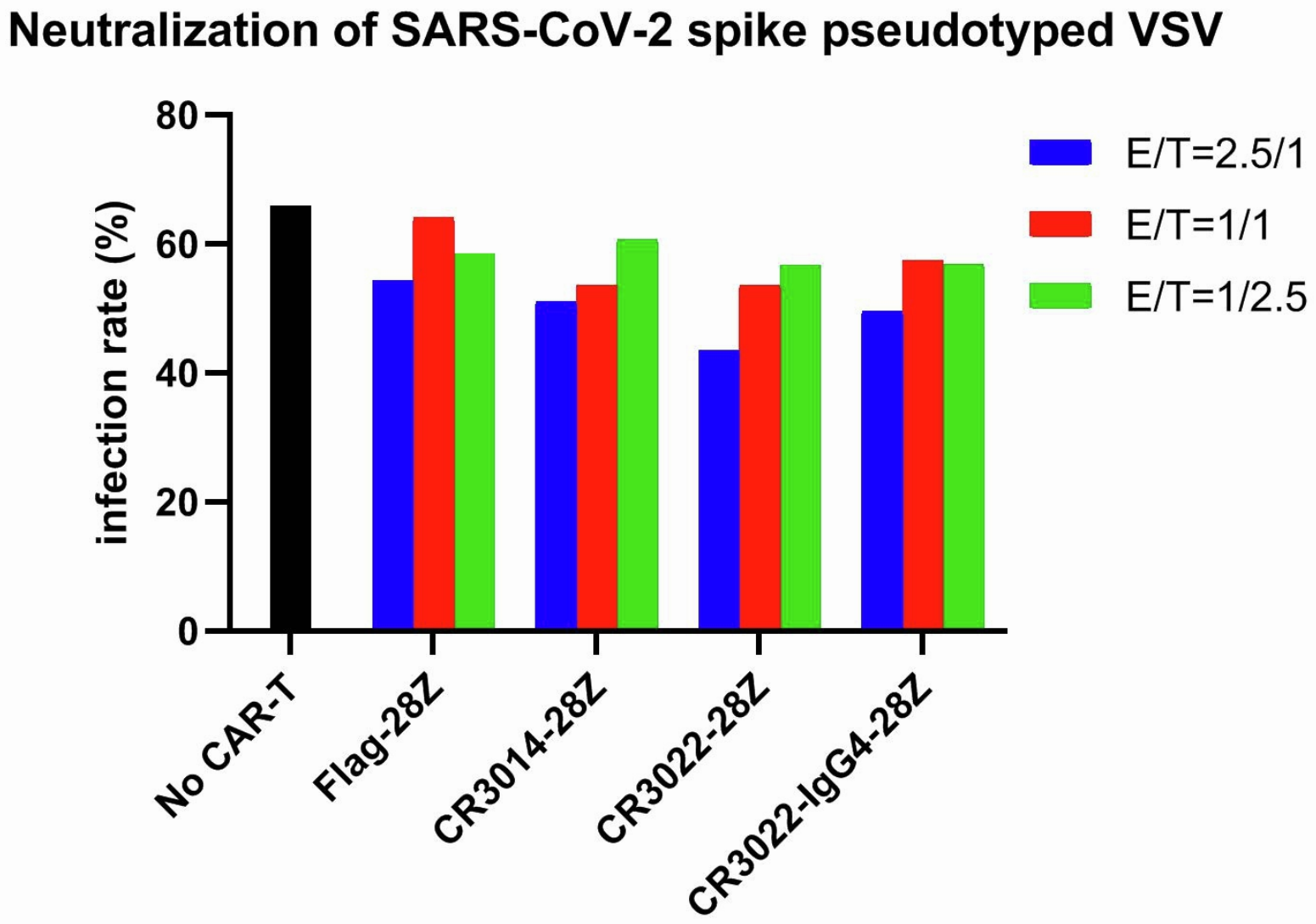

293-ACE2 with soluble RBD (Donor B)
Flag-28Z
CR3014-28Z
CR3022-28Z
CR3022-8a-28Z
CR3022-CH3-28Z
CR3022-IgG4-28Z
Cytotoxicity (%)
100
80
60
40
20
0
10/1
5/1
2.5/1
Effecter/Target ratio
****
****
*
****


293-ACE2 with soluble RBD (Donor C)
Flag-28Z
CR3014-28Z
CR3022-28Z
CR3022-8a-28Z
CR3022-CH3-28Z
CR3022-IgG4-28Z
Cytotoxicity (%)
100
80
60
40
20
0
10/1
5/1
2.5/1
Effecter/Target ratio
****
****
**
****


293-ACE2 with soluble S1 (Donor A)
CR3014-28Z
CR3022-28Z
CR3022-8a-28Z
CR3022-CH3-28Z
CR3022-IgG4-28Z
Cytotoxicity (%)
100
80
60
40
20
0
20/1
10/1
5/1
Effecter/Target ratio
****
****


293-ACE2 coated with S1 (Donor E)
CR3014-28Z
CR3022-28Z
CR3022-8a-28Z
CR3022-CH3-28Z
CR3022-IgG4-28Z
Cytotoxicity (%)
100
80
60
40
20
0
20/1
10/1
5/1
Effecter/Target ratio
*
****

SFigure 7


Neutralization of SARS-CoV-2 spike pseudotyped VSV
infection rate (%)
80
60
40
20
0
E/T=2.5/1
E/T=1/1
E/T=1/2.5
No CAR-T
Flag-28Z
CR3014-28Z
CR3022-28Z
CR3022-IgG4-28Z