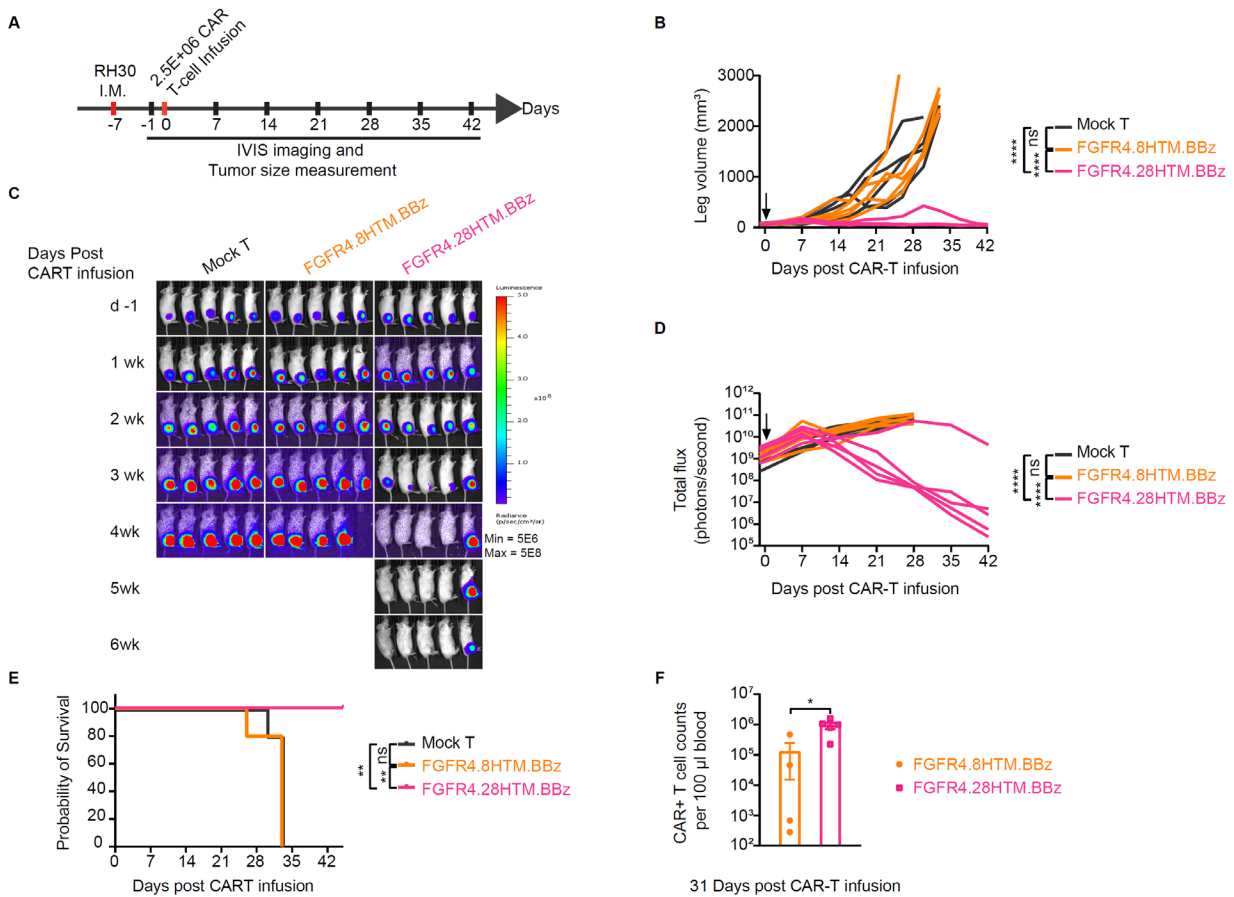


## **Supplementary Information**

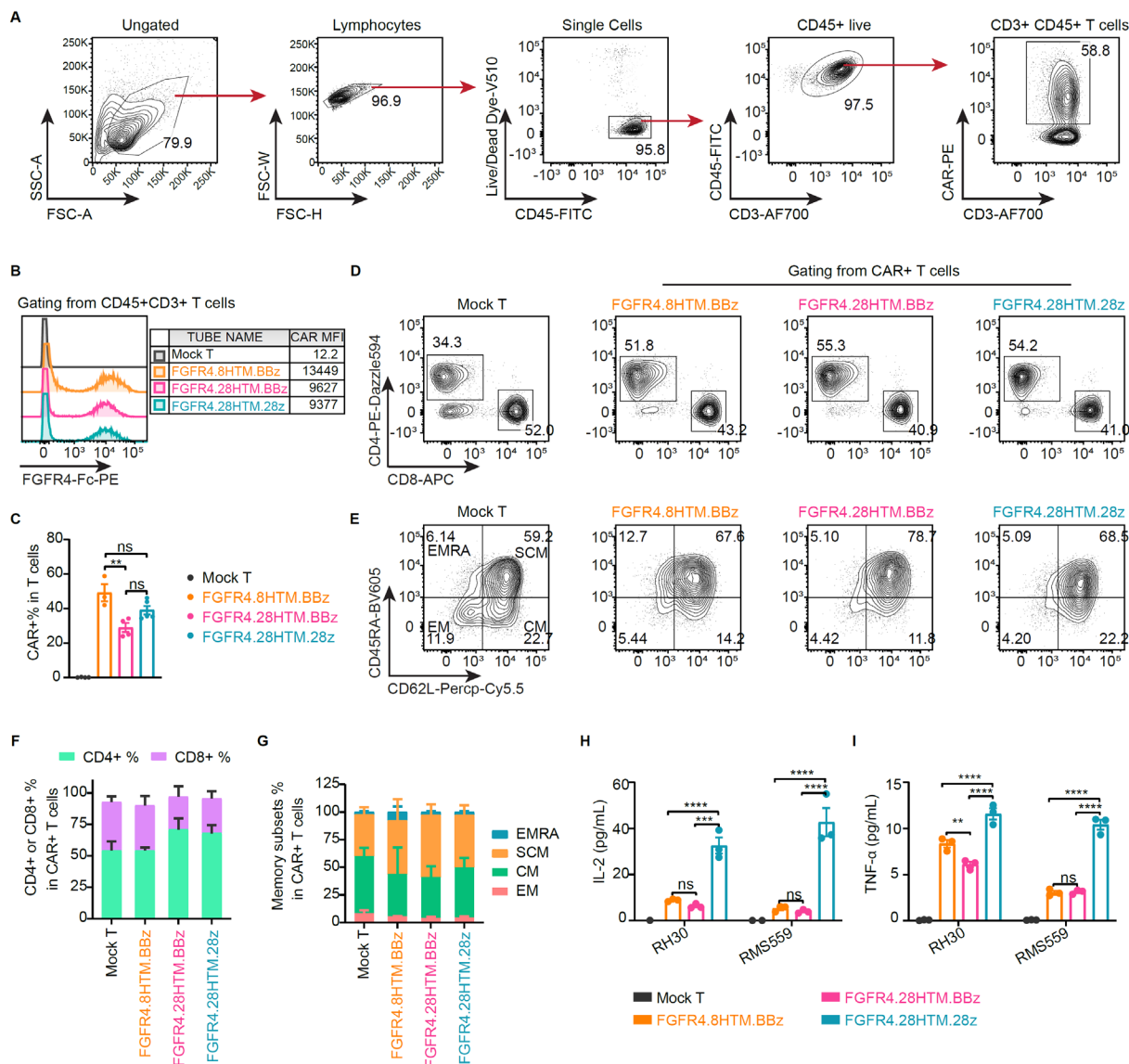
### **CAR T-cells targeting FGFR4 and CD276 simultaneously show potent antitumor effect against childhood rhabdomyosarcoma**

Meijie Tian, Jun S. Wei, Adam Tai-Chi Cheuk, David Milewski, Zhongmei Zhang, Yong Yean Kim, Hsien-Chao Chou, Can Liu, Sherif Badr, Eleanor G. Pope, Abdelrahman Rahmy, Jerry T. Wu, Michael C. Kelly, Xinyu Wen, and Javed Khan



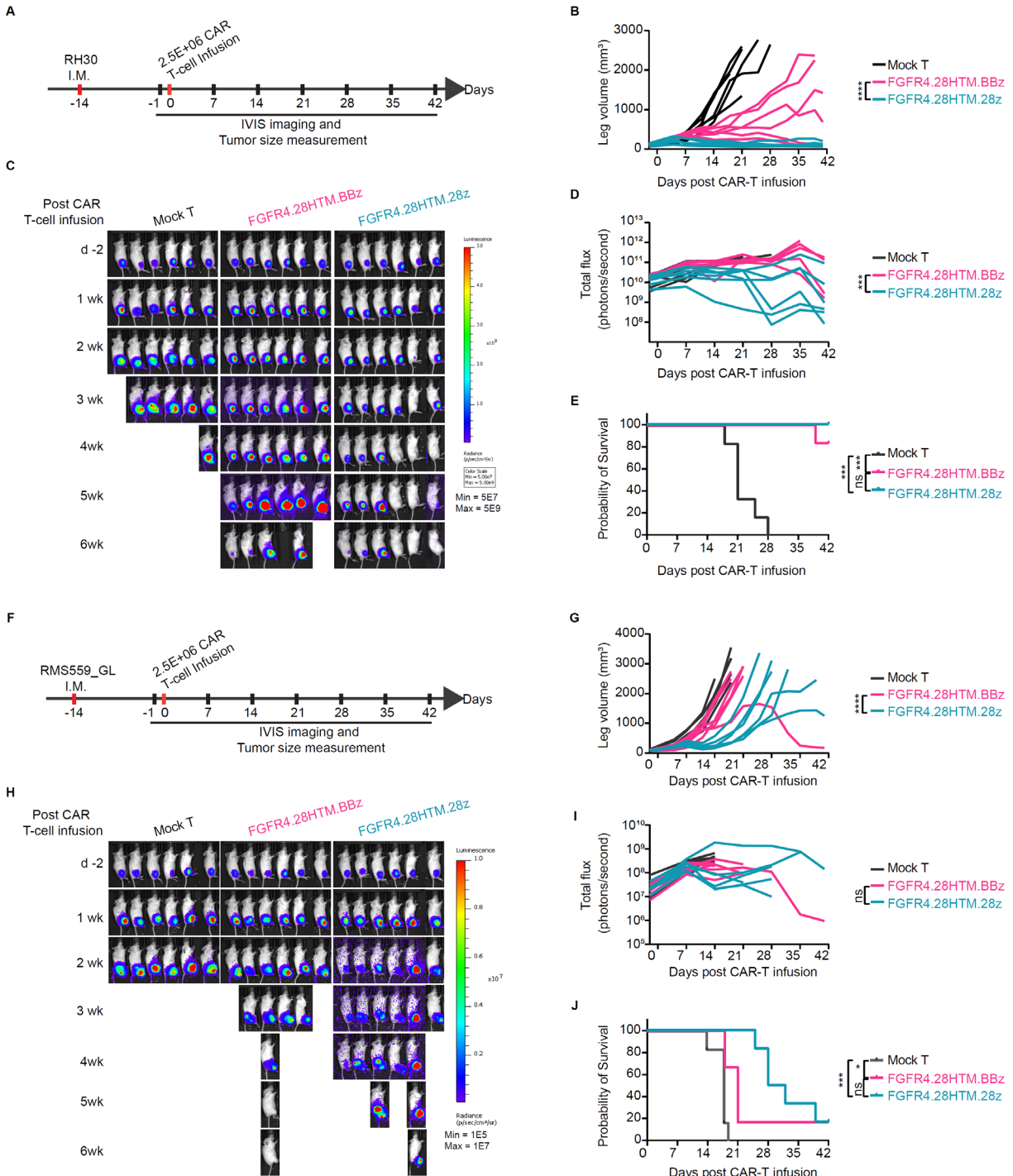
**Supplementary Fig. 1 (related to Fig. 1). Low dose (2.5E+6) of FGFR4-CAR T-cells containing a CD28 HTM outperformed the original design with a CD8 HTM in RH30 orthotopic xenografts.**

(A) Schema of RH30 orthotopic, intramuscular, xenograft model infused with mock, or 2.5E6 FGFR4.8HTM.BBz, or FGFR4.28HTM.BBz CAR T-cells on day 7 post tumor inoculation. (B) tumor size, (C) Bioluminescent images, and (D) total flux of RH30 intramuscular xenografts growth before (day -1) and after infusion with mock T-cells, FGFR4.8HTM.BBz, or FGFR4.28HTM.BBz CAR T-cells. Each replicate per group is shown,  $n = 5$ . Mixed-effects analysis or Two-way repeated measures (RM) ANOVA analysis is used to calculate the  $p$  values between two groups in Figures B and D. \*\*\*\* $p < 0.0001$ , ns, not significant. (E) Kaplan-Meier survival analysis of mice receiving different treatments is shown. \*\* $p = 0.0035$ ; ns, not significant; by log-rank test. (F) FGFR4 CAR T-cell counts per 100µl in blood from mice of RH30 orthotopic model at day 31 post-treated with 2.5E+6 CAR T-cells. Statistics for comparing two FGFR4 CAR T-cell counts represent two-tailed unpaired Mann-Whitney test. \* $p = 0.0317$ . Source data are provided as a Source Data file.



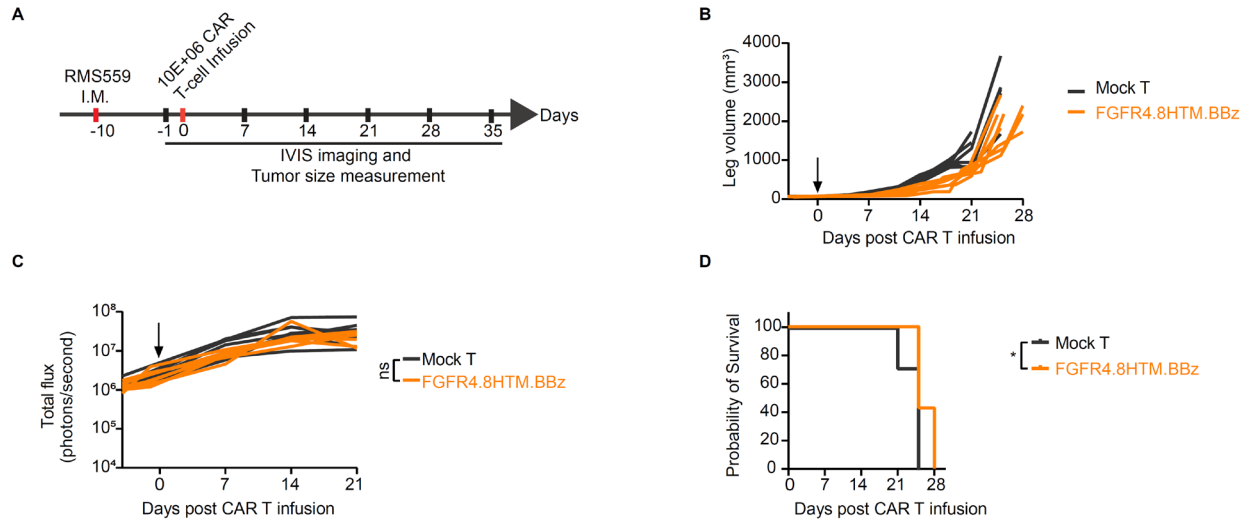
**Supplementary Fig. 2 (related to Fig. 1). Phenotypic characterization of three FGFR4 CAR T-cells before infusion into tumor-bearing mice. (A)** Flow cytometry-based gating strategy for analyzing the phenotypes of CAR+ T cells presented on Fig. 1L, S1F, 3K, 4F, 4J, 4K, S2, S5, S6, and S7G. **(B)** Representative histogram of 3 FGFR4 CAR expressions on T-cell surface assessed by binding to FGFR4-Fc protein after 9 days post-transduction. Mean fluorescent intensity (MFI) of CAR expression in all three FGFR4-CAR constructs and mock T-cells (in black) shown in the table. **(C)** Percentage of CAR positive in all CD45<sup>+</sup>CD3<sup>+</sup> T-cells measured by flow cytometry at day 9 after manufacturing. Data represent independent experiments with 4 different T-cell donors, mean  $\pm$  SEM. Statistics represent one-way ANOVA with Tukey's multiple comparisons. \*\* $p = 0.0058$ ; ns, not significant. **(D and E)** Representative flow cytometry plots characterize phenotypes of CAR-transduced T-cells, including CD4<sup>+</sup> and CD8<sup>+</sup> T-

cells, stem cell memory (SCM, CD45RA<sup>+</sup> and CD62L<sup>+</sup>), central memory (CM, CD45RA<sup>-</sup> and CD62L<sup>+</sup>), effector memory (EM, CD45RA<sup>-</sup> and CD62L<sup>-</sup>) and terminally differentiated effector memory (EMRA, CD45RA<sup>+</sup> and CD62L<sup>-</sup>) on day 10 after CAR transduction. (**F** and **G**) Mean frequencies were plotted for CD4<sup>+</sup> and CD8<sup>+</sup> T-cells for panel D, or memory subsets for panel E with SEM for 4 independent experiments. (**H** and **I**) IL-2 (H) and TNF-  $\alpha$  (I) released by three FGFR4 CAR T-cells following a 72-hour coculture with RH30 or RMS559 cells. Data means are plotted with SEM for 3 independent cocultures. \*\*\* $p$  = 0.0002, \*\*\*\* $p$  < 0.0001 in H; \*\* $p$  = 0.0018, \*\*\*\* $p$  < 0.0001 in I, and ns, not significant, by two-way ANOVA with Tukey's multiple-comparison test. Source data are provided as a Source Data file.

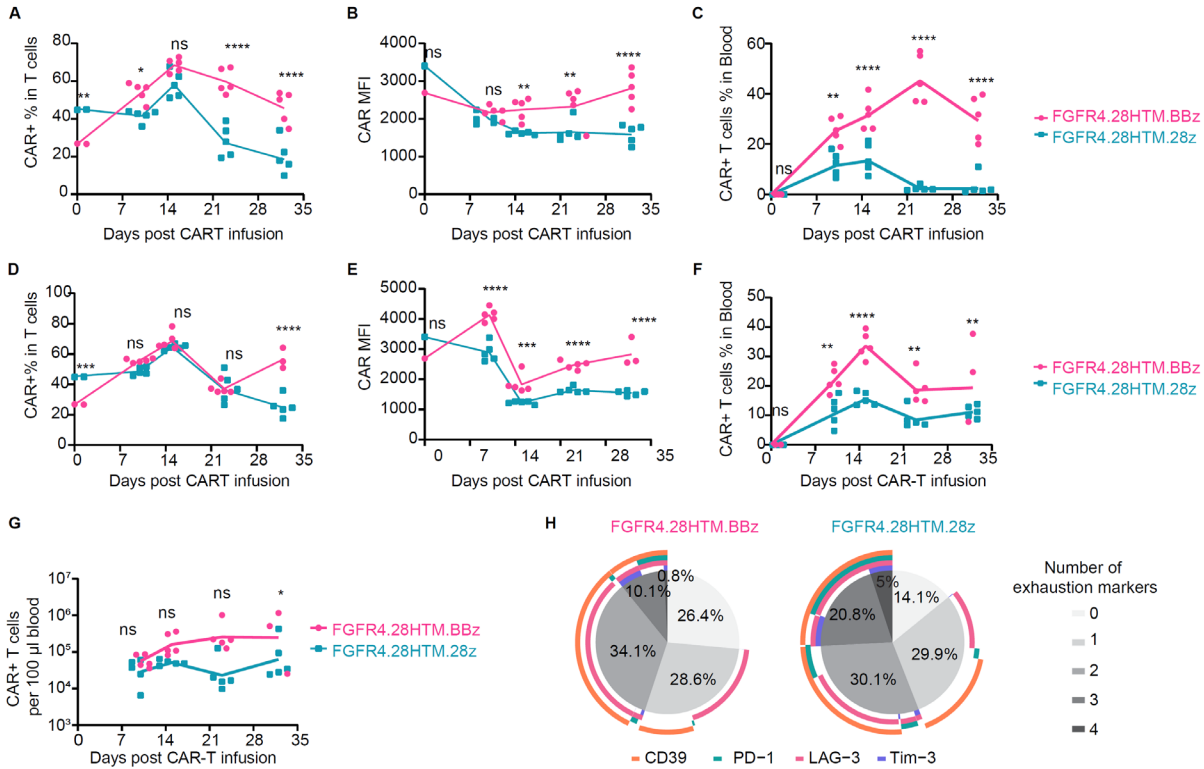


**Supplementary Fig. 3 (related to Fig. 1). Altering the HTM and CSD significantly improves the killing ability of FGFR4 CAR T-cells from donor 5 against two RMS orthotopic xenografts. (A)** Schema of testing CAR T-cells in an RH30 intramuscular (I.M.) xenograft model infused with mock or 2.5E6 FGFR4 CAR T-cells derived from donor 5, 14 days after tumor inoculation. **(B)** Leg volume for testing CAR T-cell efficacy in an RH30 I.M. xenograft model. Each line represents an individual mouse ( $n = 6$  per group).

Mixed effects analysis is used to calculate the P values between two groups. \*\*\*\* $p < 0.0001$ . (C and D) RH30 tumor burden, represented by bioluminescent images (C) or total flux (photons/second, D), was assessed by an IVIS imaging system. Mixed effects analysis was used to calculate the  $p$ -value between two groups. \*\*\* $p = 0.0002$ . (E) Kaplan-Meier survival analysis of RH30-bearing mice treated with mock or FGFR4 CAR T-cells ( $n = 6$  mice/group). \*\*\* $p = 0.0005$ , ns for not significant, by log-rank test. (F) Schema of testing the activity of 2.5E6 FGFR4 CAR T-cells from donor 5 in an RMS559 I.M. xenograft mouse model. (G) Tumor size was measured as leg volume. Each line represents an individual mouse ( $n = 6$  per group). \*\*\*\* $p < 0.0001$ ; ns, not significant, by mixed-effects analysis between two groups. (H and I) RMS559 tumor burden, represented by bioluminescent images (H) and total flux (photons/second, I), was assessed by an IVIS imaging system. Mixed-effects analysis was used to calculate  $p$ -values between the two groups, respectively. (J) Kaplan-Meier survival analysis of mice treated with mock T or FGFR4 CAR T-cells ( $n = 6$  mice/group) by log-rank test. \* $p = 0.0166$ , \*\*\* $p = 0.0013$ ; ns, not significant. Source data are provided as a Source Data file.

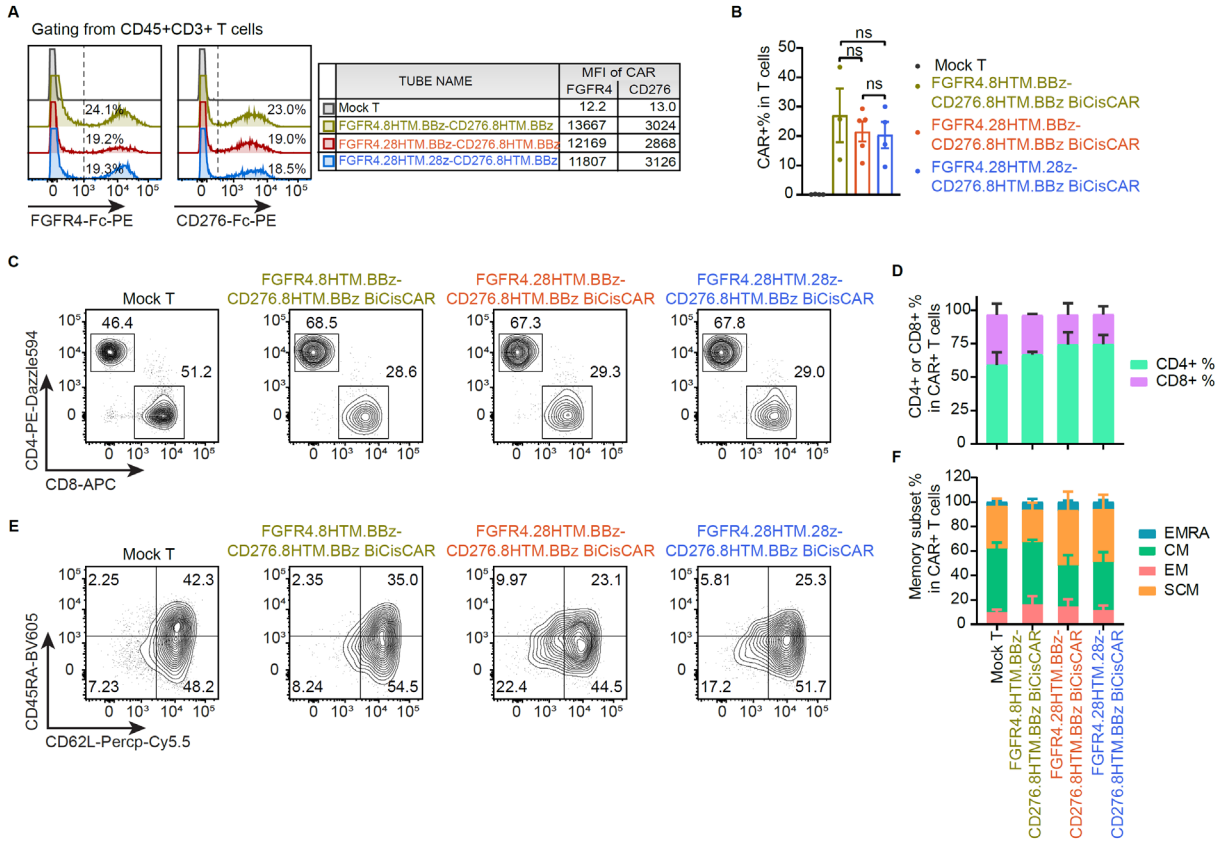


**Supplementary Fig. 4 (related to Fig. 1). 10E+6 FGFR4-CAR T-cells incorporating a CD8 HTM and 4-1BB CSD showed minimal activity in RMS559 orthotopic xenografts. (A)** Schema of the RMS559 intramuscular xenograft model infused with mock or 10E+6 FGFR4.8HTM.BBz CAR T-cells on day 10 post-tumor inoculation. **(B)** Tumor size and **(C)** total flux of RMS559 intramuscular xenografts growth before (day -1, day -4) and after infusion with mock T-cells or FGFR4.8HTM.BBz CAR T-cells. Each replicate per group is shown,  $n = 7$ . Two-way repeated measures (RM) ANOVA analysis is used to calculate the  $p$  values between two groups: ns, not significant. **(D)** Kaplan-Meier survival analysis of mice receiving different treatments is shown.  $*p = 0.0287$ , by log-rank test. Source data are provided as a Source Data file.

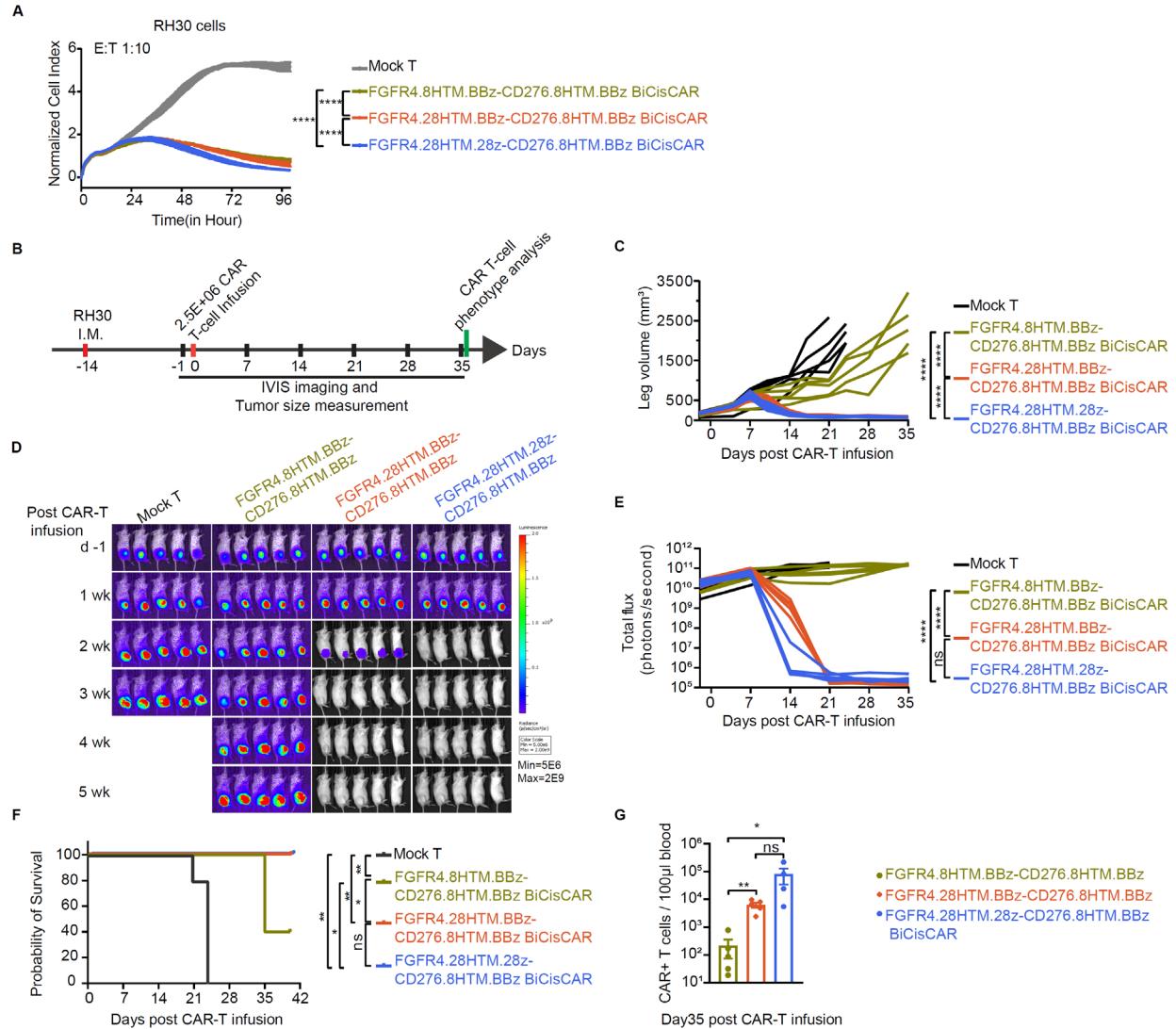


**Supplementary Fig. 5 (related to Fig. 1). Phenotypic characterization of FGFR4 CAR T-cells in vivo.**

(A-C) Percentage of CAR<sup>+</sup> in CD45<sup>+</sup>CD3<sup>+</sup> T-cells from blood (A), MFI of surface CAR expression (B), and the percentage of CAR<sup>+</sup> T-cells in blood singlets (C) from mice of RH30 I.M. model treated with 2.5E6 CAR T-cells analyzed by flow cytometry at day 10, day 15, day 23, and day 32 ( $n = 5$ ; each replicate is shown). \* $p = 0.0166$ , \*\* $p = 0.0049$  in A; \*\* $p = 0.0096$  for day 15, \*\* $p = 0.0037$  for day 23 in B; \*\* $p = 0.0022$  in C; \*\*\*\* $p < 0.0001$ , ns, not significant, by two-way ANOVA with Sidak's multiple comparisons test. (D-F) Percentage of CAR<sup>+</sup> in CD45<sup>+</sup>CD3<sup>+</sup> T-cells (D), MFI of T-cell surface CAR expression (E), and the percentage of CAR<sup>+</sup> T-cells in the blood (F) at day 10, 15, 23, or 32 after CAR T-cell infusion into RMS559 I.M xenografts bearing mice ( $n = 5$  mice per group). Two-way ANOVA with Sidak's multiple comparisons test is used to calculate the above  $p$  values. \*\*\* $p = 0.0004$  in D; \*\*\* $p = 0.0009$  in E; \*\* $p = 0.0068$  for day 10, \*\* $p = 0.01$  for day 23, \*\* $p = 0.0074$  for day 32 in F; \*\*\*\* $p < 0.0001$ , ns, not significant. (G) Expansion dynamics of FGFR4 CAR T-cell counts per 100µl in blood from mice of RMS559 I.M. model treated with 2.5E6 CAR T-cells by flow cytometry. Statistics for comparing two FGFR4 CAR T-cell counts at each time point represent two-way ANOVA with Sidak's multiple-comparison test. \* $p = 0.0348$ ; ns, not significant. (H) Percentage of CAR T-cells expressing or co-expressing CD39, PD-1, LAG-3, and TIM-3 at day 32 after T-cell infusion into RMS559-bearing mice. Source data are provided as a Source Data file.



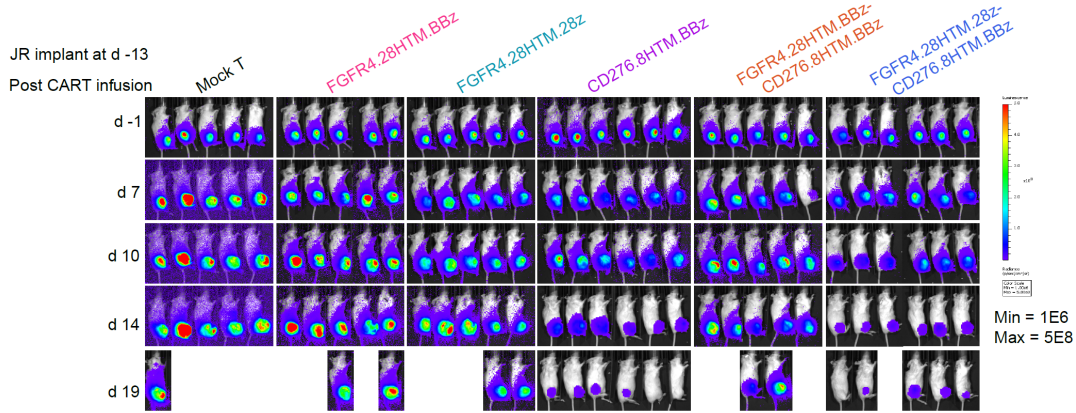
**Supplementary Fig. 6 (related to Fig. 3). Phenotypic characterization of three BiCisCAR T-cells before infusion into orthotopic RH30-bearing mice. (A)** Representative histogram of CAR cell surface expression among different BiCisCAR constructs assessed by binding to FGFR4-Fc or CD276-Fc protein after manufacturing for 10 days. Mean fluorescent intensity (MFI) of CAR expression in all three BiCisCAR constructs, and mock T-cells (shown in black) are shown in the right table. **(B)** Mean percentage  $\pm$  SEM of CAR positive in all CD45<sup>+</sup>CD3<sup>+</sup> T-cells measured by flow cytometry at day 10 after manufacturing. Data represent independent experiments with 3 or 5 different T-cell donors. Statistics represents one-way ANOVA with Tukey's multiple comparison tests. Ns, not significant. **(C and E)** Representative flow cytometry plots characterize phenotypes of CAR-transduced T-cells, including CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, stem cell memory (SCM, CD45RA<sup>+</sup> and CD62L<sup>+</sup>), central memory (CM, CD45RA<sup>-</sup> and CD62L<sup>+</sup>), effector memory (EM, CD45RA<sup>-</sup> and CD62L<sup>-</sup>) and terminally differentiated effector memory (EMRA, CD45RA<sup>+</sup> and CD62L<sup>-</sup>) on day 10 after CAR transduction. **(D and F)** Mean frequencies were plotted for CD4<sup>+</sup> and CD8<sup>+</sup> T-cells for panel C, or memory subsets for panel E with SEM for 3 or 5 independent experiments. Source data are provided as a Source Data file.



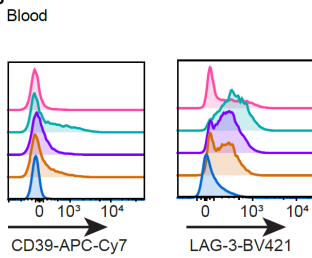
**Supplementary Fig. 7 (related to Fig. 3). Dual targeting CAR T-cells using the same CD8 HTM and 4-1BB CSD construct for FGFR4 and CD276 exhibit reduced tumor killing against RH30 orthotopic model.** (A) Cytolytic activity of three BiCisCAR T-cells against RH30 at an E:T ratio of 1:10 by an xCELLigence RTCA. Statistical analysis was performed with two-way RM ANOVA. \*\*\*\* $p < 0.0001$ . (B) Schema of an *in vivo* model testing the activity of 2.5E6 BiCisCAR T-cells against an RH30 I.M. xenograft. (C) Tumor volumes following CAR T-cell infusion. Data indicates the tumor volume of each mouse ( $n = 5$ ). \*\*\*\* $p < 0.0001$ , as determined by two-way RM ANOVA. (D and E) Bioluminescence images (D) and total flux (E) of RH30 tumor growth assessed by IVIS imaging post three BiCisCAR T-cells treatment. A two-way RM analysis was used to calculate the  $p$ -value between every two BiCisCAR T-cells groups respectively. \*\*\*\* $p < 0.0001$ . (F) In the above model, the Kaplan-Meier survival curve of mice ( $n = 5$  mice per group); comparisons of survival curves was determined by log-rank test, \* $p = 0.0495$ , \*\* $p = 0.0035$ . (G) Cell counts of three BiCisCAR T-cells per 100µl blood from mice treated with 2.5E6 CAR T-cells were

analyzed by flow cytometry at day 35 ( $n = 5$ ). Statistics for every two BiCisCAR T-cell counts represent a two-tail nonparametric test using the Mann-Whitney test.  $*p = 0.0159$ ,  $**p = 0.0079$ , ns, not significant. Source data are provided as a Source Data file.

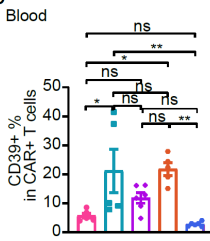
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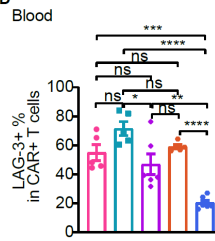
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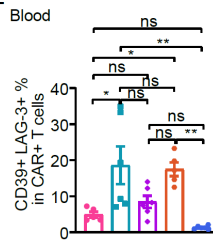
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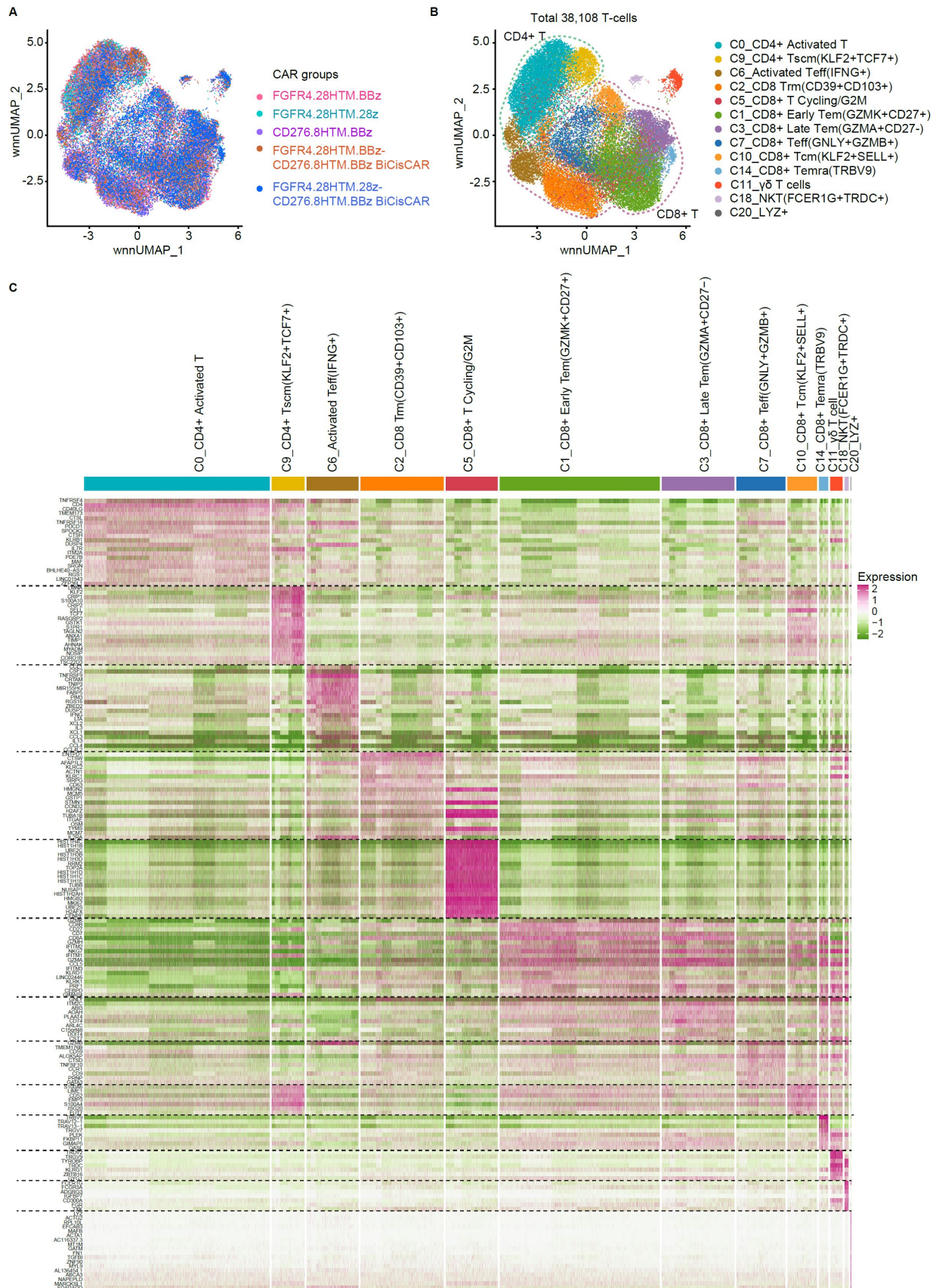
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E

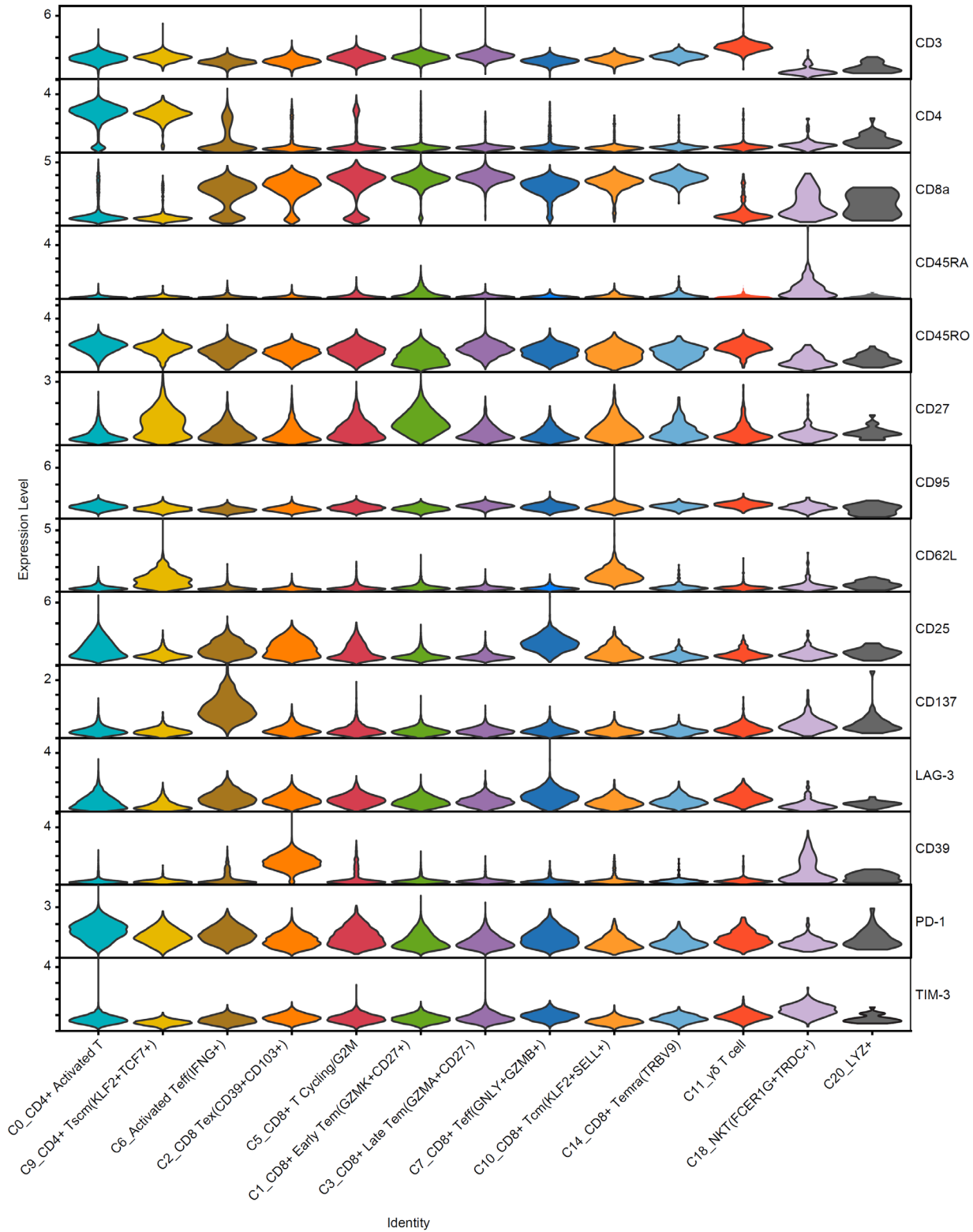


**Supplementary Fig. 8 (related to Fig. 4) Dual targeting BiCisCAR with two different CSDs exhibited rapid tumor shrinkage and limited exhaustion.** (A) Representative tumor BLI images of JR\_Luc tumor growth in the orthotopic xenograft model shown in Fig. 4. (B) Representative flow cytometry illustrating CD39 and LAG-3 expression in CAR<sup>+</sup> T-cells in the blood of mice from the JR I.M xenograft model, 21 days after infusion of 2.5E+6 CAR T-cells. (C - E) Percentages of CD39<sup>+</sup> (C), LAG-3<sup>+</sup> (D), and CD39<sup>+</sup> LAG-3<sup>+</sup> (E) in CAR<sup>+</sup> T-cells circulating in the blood from mice 21 days after CAR T-cells infusion ( $n = 5$  or 6, mean  $\pm$  SEM). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ , by one-way ANOVA, with Tukey's multiple comparison tests, the full list of  $p$  values can be found in the Source Data. Source data are provided as a Source Data file.

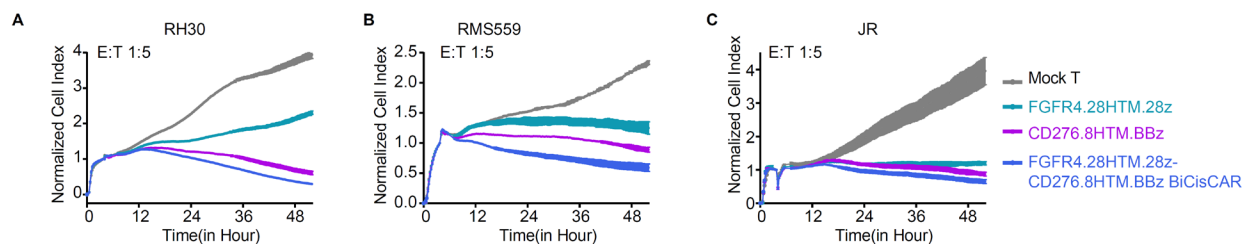


**Supplementary Fig. 9 (related to Fig. 5). Cluster annotation based on the top genes of each cluster.**

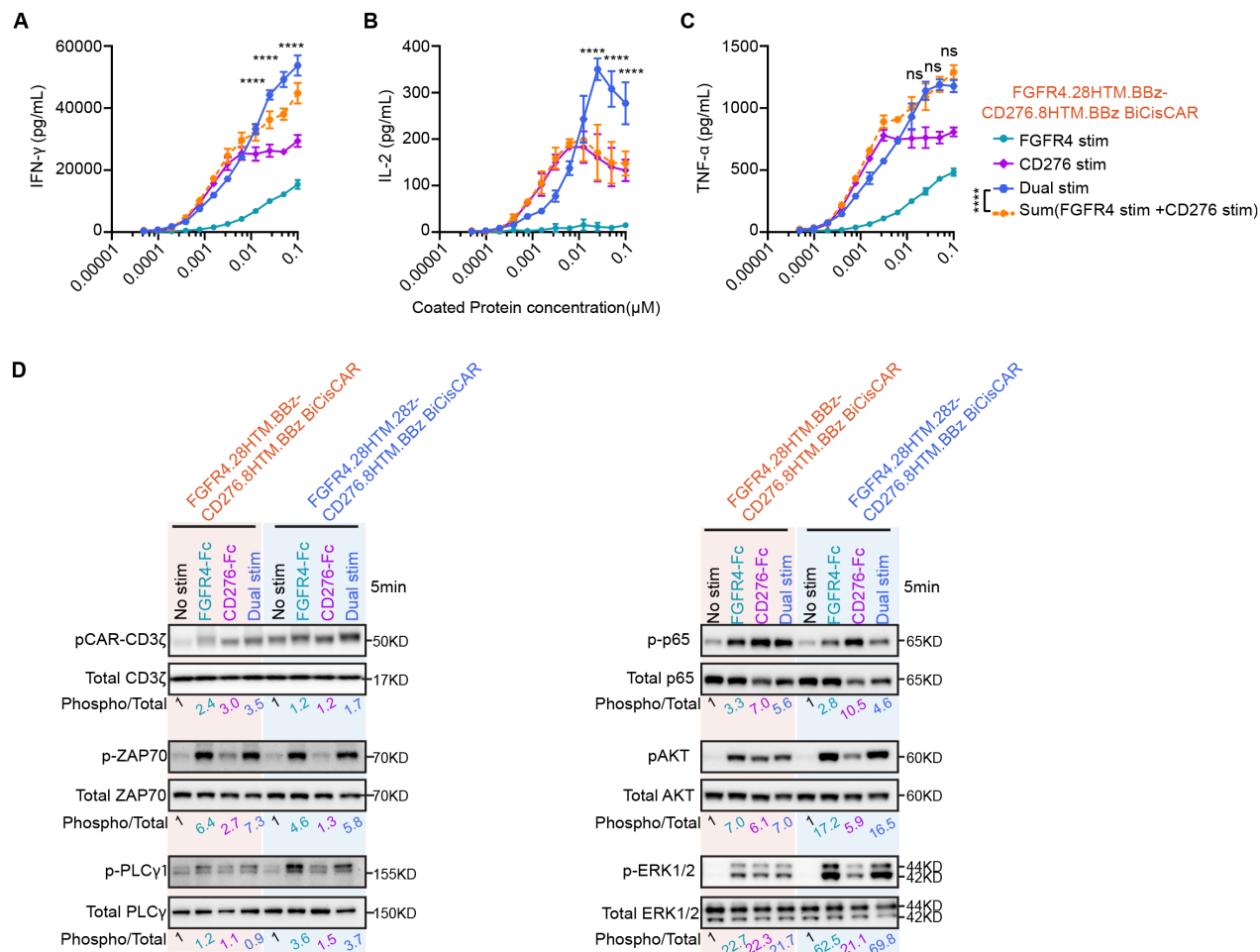
(A) UMAP visualization of single cells from five CAR T-cell groups based on WNN assay after removing batch effect among sequencing lanes by SCT. Cells are colored according to CAR T-cell groups. (B) WNN UMAP visualization of a total of 38,108 tumor-infiltrating T-cells from the above model, revealed 13 clusters with different transcriptome and protein profiles. The green dash circle gates out the CD4<sup>+</sup> T-cells and the purple dash circle frames out CD8<sup>+</sup> T-cells. (C) Single cell heatmap showing the top 20 differentially expressed genes of each cluster for cell type annotation.



**Supplementary Fig. 10 (related to Fig. 5). Violin plots of surface protein markers expression levels among 13 clusters were used for cluster annotation.**



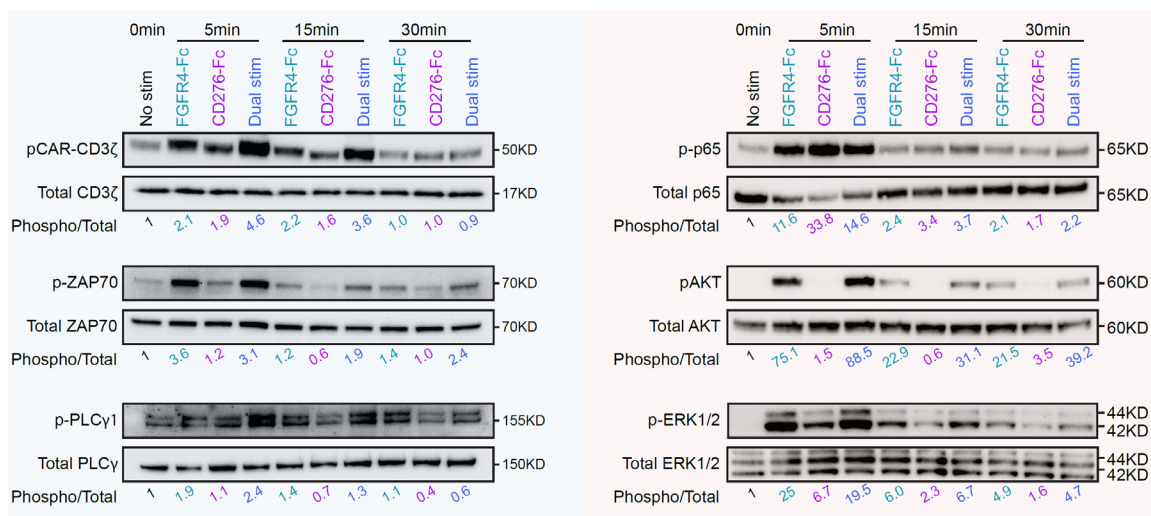
**Supplementary Fig. 11 (related to Fig. 7). FGFR4.28HTM.28z-CD276.8HTM.BBz BiCisCAR T-cells exhibited an additive or synergistic effect on cytolytic activity. (A to C)** Cytolytic activities of FGFR4.28HTM.28z, CD276.8HTM.BBz CARs or FGFR4.28HTM.28z-CD276.8HTM.BBz BiCisCAR T-cells were evaluated in vitro with RTCA against RH30 (A), RMS559 (B), or JR (C) cells. Source data are provided as a Source Data file.



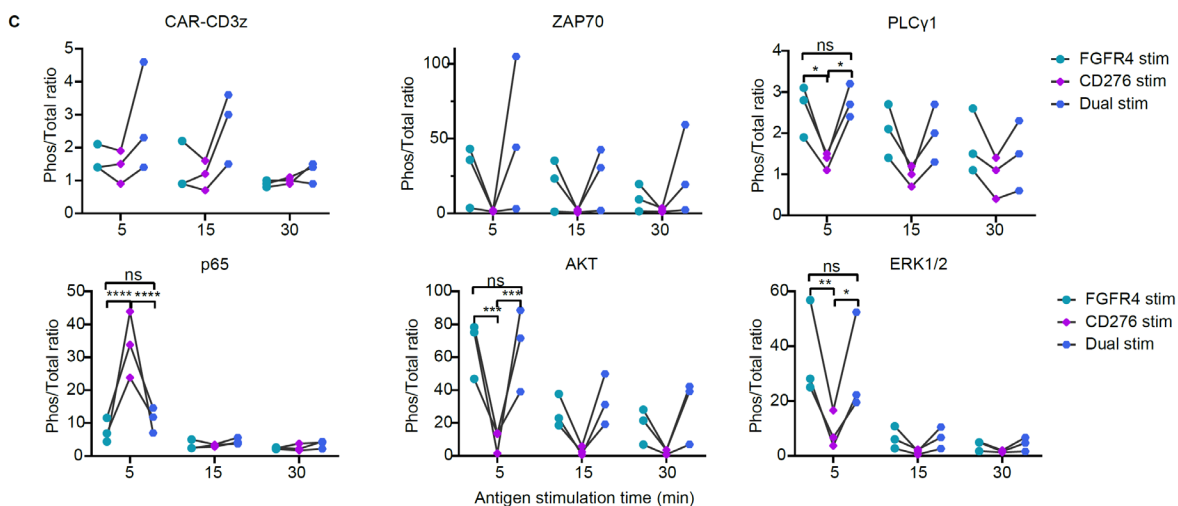
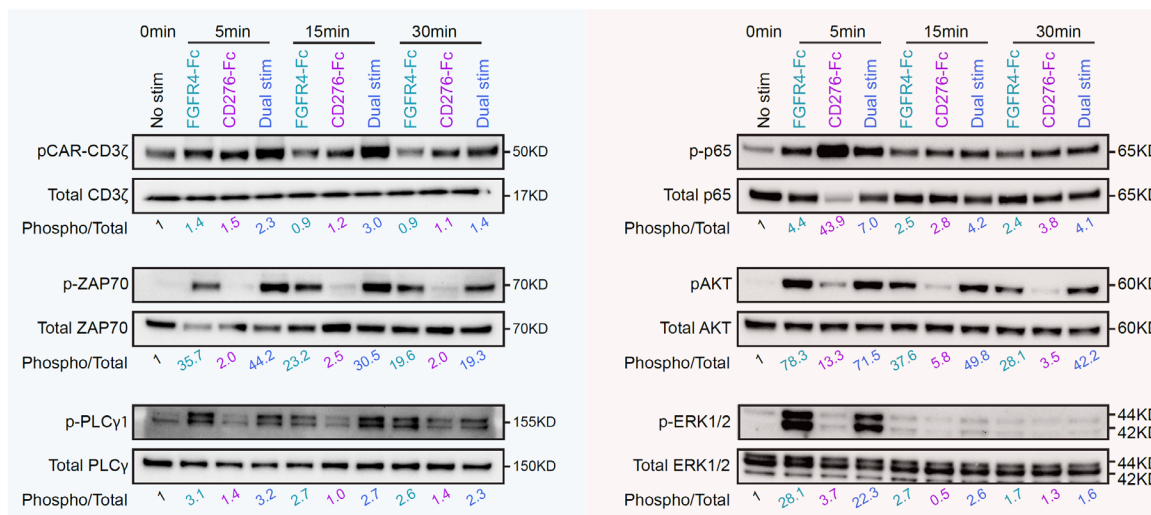
**Supplementary Fig. 12 (related to Fig. 7) FGFR4 and CD276 dual-targeting CAR T-cells with 4-1BB CSDs demonstrate suboptimal downstream T-cell activation signaling.** (A) IFN- $\gamma$ , (B) IL-2 (B), and (C) TNF- $\alpha$  release by FGFR4.28HTM.BBz-CD276.8HTM.BBz BiCisCAR T-cells following a 20-hour stimulation with plate-coated FGFR4-Fc, CD276-Fc, or both proteins. Data are shown as the mean  $\pm$  SD;  $n = 3$  independent stimulation with CAR T-cells. The green dotted line shows the sum of cytokine released after single protein stimulation. Two-way ANOVA Sidak's multiple comparisons test was performed to statistic the difference between two proteins' dual stimulation and the sum of single stimulation. \*\*\*\* $p < 0.0001$ ; ns, not significant. (D) Time course of CAR-CD3 $\zeta$ , ZAP70, PLC $\gamma$ 1, p65, Akt and Erk1/2 phosphorylation in FGFR4.28HTM.BBz-CD276.8HTM.BBz BiCisCAR or FGFR4.28HTM.28z-CD276.8HTM.BBz BiCisCAR T-cells after CAR cross-linking (FGFR4-Fc protein for FGFR4 CAR, CD276-Fc protein for B7-H3, or both proteins for dual CAR cross-linking) measured by Western blot analysis. Numbers under the gels represent the ratio of the intensity of the signal obtained with phospho-specific antibodies relative to the total. Relative values were normalized to one of the unstimulated controls. Source data are provided as a Source Data file.

**A** Donor 1

FGFR4.28HTM.28z-  
CD276.8HTM.BBz BiCiCAR



**B** Donor 3



**Supplementary Fig. 13 (related to Fig. 7). Western blot analyses of dual-targeting CAR T-cells using different CSDs demonstrate elevated downstream T-cell activation signaling by exposure to both antigens.** (A and B) Time course of CAR-CD3 $\zeta$ , ZAP70, PLC $\gamma$ 1, p65, Akt and Erk1/2 phosphorylation in FGFR4.28HTM.28z-CD276.8HTM.BBz BiCisCAR T-cells after stimulation by indicated antigens using T cells from another 2 independent donors. (C) Dot plots depict the ratio of immune band intensities between phospho-protein and total protein from three independent experiments using T-cells from 3 donors. Data from the same donor are connected by black lines. Two-way ANOVA Tukey's multiple comparison tests were performed between two paired conditions.  $*p \leq 0.05$ ,  $**p \leq 0.01$ ,  $***p \leq 0.001$ ,  $****p \leq 0.0001$ , the full list of  $p$  values can be found in the Source Data. Source data are provided as a Source Data file.

## Supplementary Table 1

Antibody-oligo conjugates directed against T-cell antigens and cell hashing antibodies were used in CITE-seq assay

| CITE-seq Antibody name                               | Surface marker         | isotype        | Clone    | Catalog # |
|------------------------------------------------------|------------------------|----------------|----------|-----------|
| TotalSeq™-C0034 anti-human CD3 Antibody              | CD3                    | Mouse IgG1, κ  | UCHT1    | 300479    |
| TotalSeq™-C0072 anti-human CD4 Antibody              | CD4                    | Mouse IgG1, κ  | RPA-T4   | 300567    |
| TotalSeq™-C0046 anti-human CD8 Antibody              | CD8α                   | Mouse IgG1, κ  | RPA-T8   | 344753    |
| TotalSeq™-C0063 anti-human CD45RA Antibody           | CD45RA                 | Mouse IgG2b, κ | HI100    | 304163    |
| TotalSeq™-C0087 anti-human CD45RO Antibody           | CD45RO                 | Mouse IgG2a, κ | UCHL1    | 304259    |
| TotalSeq™-C0154 anti-human CD27 Antibody             | CD27                   | Mouse IgG1, κ  | O323     | 302853    |
| TotalSeq™-C0156 anti-human CD95 (Fas) Antibody       | CD95                   | Mouse IgG1, κ  | DX2      | 305651    |
| TotalSeq™-C0147 anti-human CD62L Antibody            | CD62L(L-selection)     | Mouse IgG1, κ  | DREG-56  | 304851    |
| TotalSeq™-C0085 anti-human CD25 Antibody             | CD25                   | Mouse IgG1, κ  | BC96     | 302649    |
| TotalSeq™-C0355 anti-human CD137 (4-1BB) Antibody    | CD137                  | Mouse IgG1, κ  | 4B4-1    | 309839    |
| TotalSeq™-C0152 anti-human CD223 (LAG-3) Antibody    | LAG-3                  | Mouse IgG1, κ  | 11C3C65  | 369335    |
| TotalSeq™-C0176 anti-human CD39 Antibody             | CD39                   | Mouse IgG1, κ  | A1       | 369335    |
| TotalSeq™-C0088 anti-human CD279 (PD-1) Antibody     | PD-1                   | Mouse IgG1, κ  | EH12.2H7 | 329963    |
| TotalSeq™-C0169 anti-human CD366 (Tim-3) Antibody    | TIM-3                  | Mouse IgG1, κ  | F38-2E2  | 345049    |
| TotalSeq™-C0090 Mouse IgG1, κ isotype Ctrl Antibody  | Mouse IgG1, κ isotype  | Mouse IgG1, κ  | MOPC-21  | 400187    |
| TotalSeq™-C0091 Mouse IgG2a, κ isotype Ctrl Antibody | Mouse IgG2a, κ isotype | Mouse IgG2a, κ | MOPC-173 | 400293    |
| TotalSeq™-C0092 Mouse IgG2b, κ isotype Ctrl Antibody | Mouse IgG2b, κ isotype | Mouse IgG2b, κ | MPC-11   | 400381    |

\*Above antibodies were purchased from BioLegend.

| Hashtag Antibody                              | Reactivity | Barcode Sequence | Manufactory | Catalog # |
|-----------------------------------------------|------------|------------------|-------------|-----------|
| TotalSeq™-C0255 anti-human Hashtag 5 Antibody | Human      | AAGTATCGTTTCGCA  | BioLegend   | 394669    |
| TotalSeq™-C0256 anti-human Hashtag 6 Antibody | Human      | GGTTGCCAGATGTCA  | BioLegend   | 394671    |

## Supplementary Table 2

### Clusters Annotation

| Cluster | Cell subtype Annotation                                            | Top Markers                                     | Reference                                                         |
|---------|--------------------------------------------------------------------|-------------------------------------------------|-------------------------------------------------------------------|
| C0      | CD4 <sup>+</sup> activated T-cells                                 | CD25 <sup>+</sup> CD40LG <sup>+</sup>           | (Laidlaw et al., 2016)                                            |
| C9      | CD4 <sup>+</sup> stem memory T-cells (Tscm)                        | KLF2 <sup>+</sup> TCF7 <sup>+</sup>             | (Gattinoni et al., 2017; Gautam et al., 2019)                     |
| C6      | Activated effector T-cells (IFNG <sup>+</sup> Teff)                | TNFRSF9 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> | (Hamann et al., 1997; Sallusto et al., 1999)                      |
| C2      | CD8 <sup>+</sup> resident memory T-cell (Trm)                      | CD39 <sup>+</sup> CD103 <sup>+</sup>            | (Duhon et al., 2018)                                              |
| C5      | CD8 <sup>+</sup> T Cycling/G2M                                     | TOP2A <sup>+</sup> MKI67 <sup>+</sup>           | (Kowalczyk et al., 2015)                                          |
| C1      | CD8 <sup>+</sup> Early effector memory T-cells (Tem)               | GZMK <sup>+</sup> CD27 <sup>+</sup>             | (Hamann et al., 1997; Mahnke et al., 2013; Sallusto et al., 1999) |
| C3      | CD8 <sup>+</sup> Late Tem                                          | GZMA <sup>+</sup> CD27 <sup>-</sup>             | (Hamann et al., 1997; Mahnke et al., 2013; Sallusto et al., 1999) |
| C7      | CD8 <sup>+</sup> effector T-cells (Teff)                           | GNLY <sup>+</sup> GZMB <sup>+</sup>             | (Kaeche and Cui, 2012)                                            |
| C10     | CD8 <sup>+</sup> central memory T-cells (Tcm)                      | KLF2 <sup>+</sup> SELL <sup>+</sup>             | (Hamann et al., 1997; Mahnke et al., 2013; Sallusto et al., 1999) |
| C14     | CD8 <sup>+</sup> terminally differentiated effector memory (Temra) | GZMH <sup>+</sup> KLRG1 <sup>+</sup>            | (Henson and Akbar, 2009; Kaeche and Cui, 2012)                    |
| C11     | $\gamma\delta$ T-cells                                             | TRDV2 <sup>+</sup> TRGV9 <sup>+</sup>           | (Ribot et al., 2021)                                              |
| C18     | natural killer (NK)-like T-cells                                   | FCER1G <sup>+</sup> TRDC <sup>+</sup>           |                                                                   |
| C20     | LYZ <sup>+</sup> cells                                             | LYZ <sup>+</sup>                                |                                                                   |

### Supplementary Data 1

**Conserved gene list for each cluster used for cluster annotation.** The 'FindAllMarkers()' function was used to compare each cluster against all other clusters to identify potential marker genes. A two-sided non-parametric Wilcoxon rank sum test was performed in this analysis.