

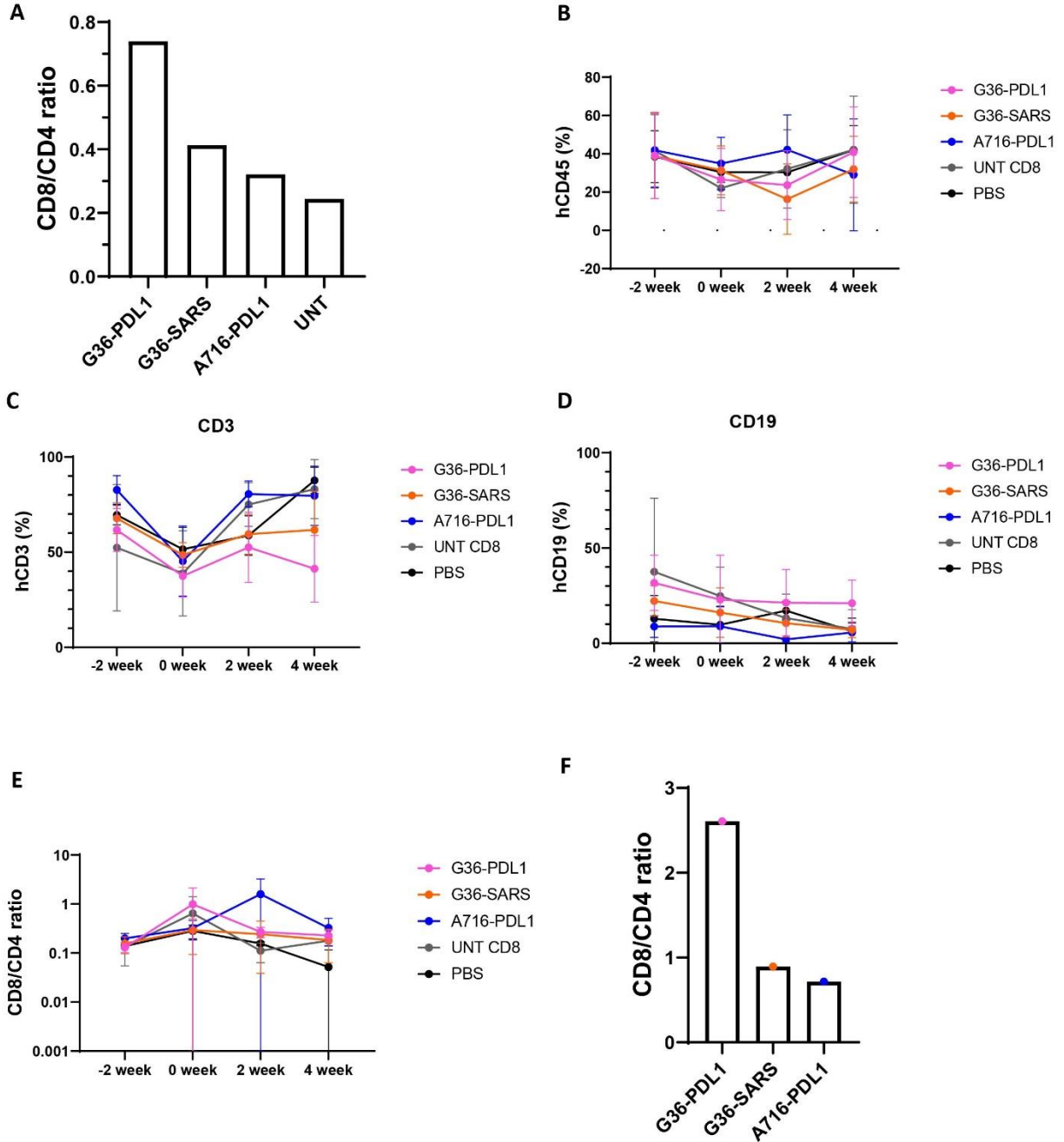
Supplemental information

Immune-restoring CAR-T cells display antitumor activity and reverse immunosuppressive TME in a humanized ccRCC mouse model

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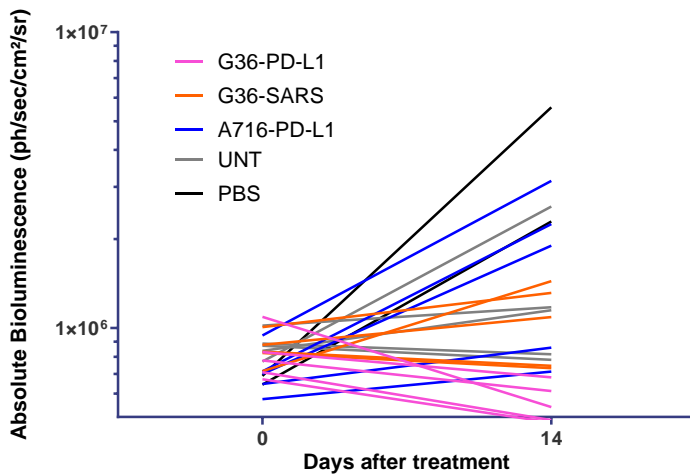
Supplementary Materials

Supplementary Figures



Supplementary Figure 1. *In vitro* and *in vivo* characterize CAR-T cells, peripheral circulating T cells and tumor infiltrating T cells, related to Figures 1 and 2. (A) CD8/CD4 ratio of T cells

after 48h co-incubation with skrc-59 tumor cells *in vitro*. **(B)** Human CD45 percentage in peripheral blood at -2, 0, 2, 4 weeks. **(C)** Human CD3 percentage in CD45+ peripheral circulating cells at -2, 0, 2, 4 weeks. **(D)** Human CD19 percentage in CD45+ peripheral circulating cells at -2, 0, 2, 4 weeks. **(E)** CD8/CD4 ratio of peripheral circulating T cells at -2, 0, 2, 4 weeks. **(F)** CD8/CD4 ratio of tumor infiltrating T cells 4 weeks after the treatment.



Supplementary Figure 2. Tumor growth curve of each individual mouse treated with one million CD8:CD4=1:2 G36-PDL1 (pink), G36-SARS (orange), A716-PDL1 (blue) CAR-T cells, UNT cells (grey) or PBS (black), related to Figure 2. BLI was performed on Day 0 and 14 after CAR-T infusion.

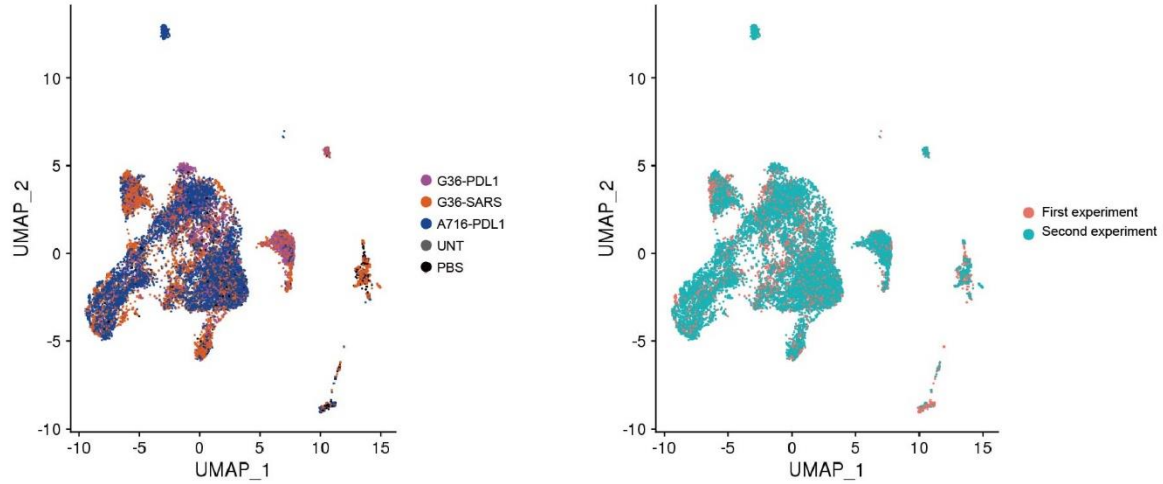
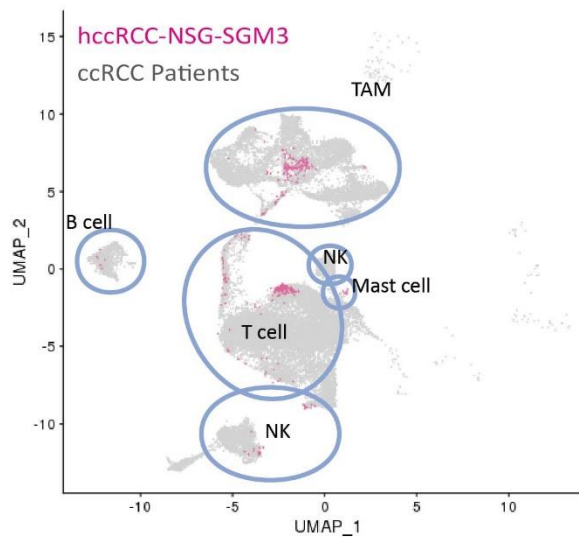


Figure S3. The overview of scRNA-seq data, related to Figure 3. UMAP plot of scRNA-seq data by each group (left) and by batch (right). Group1 G36-PDL1 is shown in pink, Group2 G36-SARS in orange, Group3 A716-PDL1 in blue, Group4 Untransduced T cells (UNT) in grey, and Group5 PBS in black. First batch of scRNAseq is shown in salmon, and the second batch is in cyan.

A



B

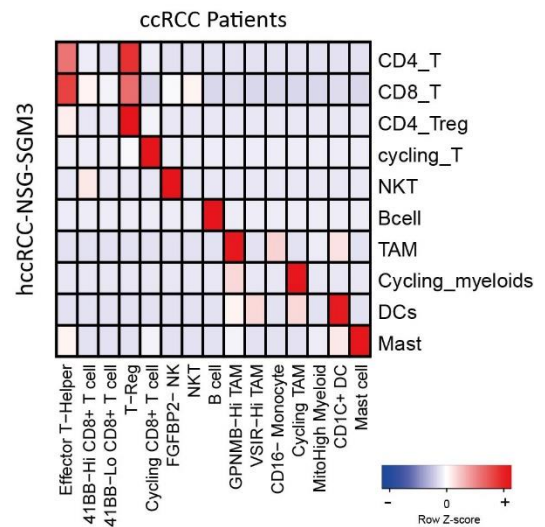


Figure S4. The immune cells from hccRCC-NSG-SGM3 could recapitulate the immune cells in ccRCC patients, relate to Figure 3. (A) UMAP overlay of TILs recovered from hccRCC-NSG-SGM3 (treatment naïve Group 5 PBS) (in pink) and TILs recovered from ccRCC patients (in grey). Three hundred forty-nine TILs recovered from Group 5 were shown here. (B) The cell type mapping ratio between humanized mouse model (treatment naïve Group 5 PBS) and public human data. Row indicates the cell type from humanized mouse model while column indicates the cell type from public human data. Row indicates the cell type from humanized mouse model while column indicates the cell type from public human data.

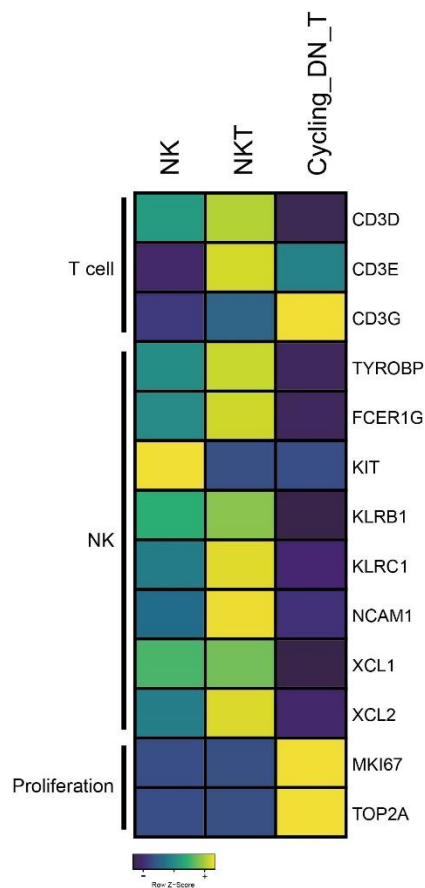
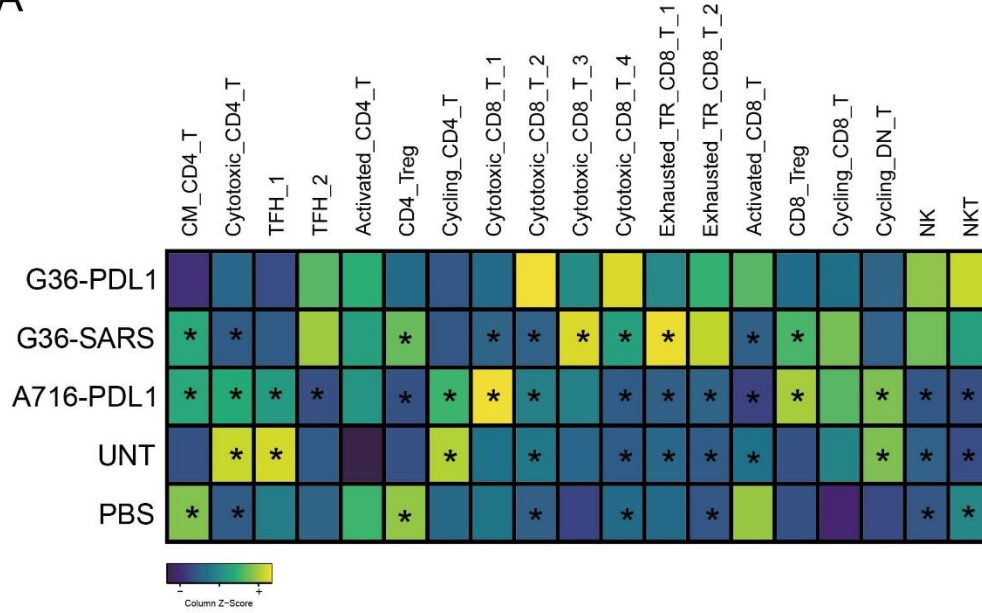


Figure S5. NK and NKT marker gene expression, related to Figure 4. Heatmap of mean expression for genes characterizing NK, NKT, and cycling_DN_T.

A



B

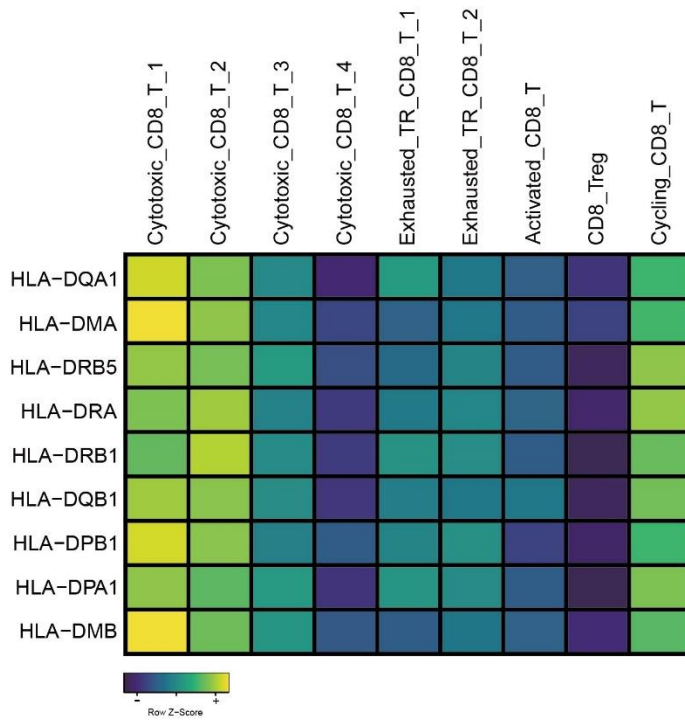


Figure S6. Characterization of T cell, related to Figure 4. (A) Heatmap of relative cell abundance of T cell subsets between groups. * indicates the significant difference between group 1

and other groups (adj.p.value < 0.01). **(B)** The expression level of MHC class II molecules in CD8 T cell subsets.

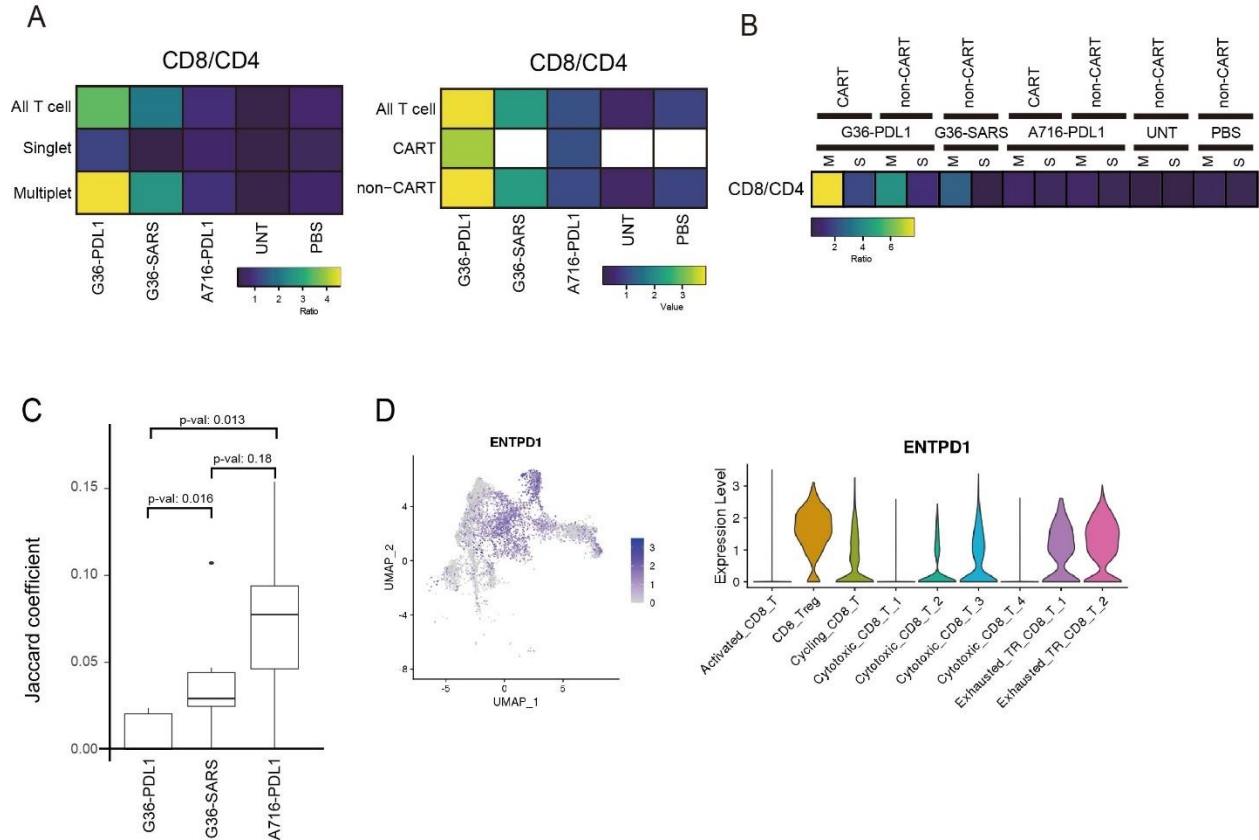


Figure S7. The clonality analysis of T cell, related to Figure 5. (A) Heatmap of CD8 / CD4 T cell ratio in all the T cells, only singlets, and only multiplets (left panel). Here, singlet indicates T cells with a unique clonotype while multiplet indicates T cells that share clonotype with other T cells. Heatmap of CD8 / CD4 T cell ratio of CAR-seq expressing T cells or non-CAR-seq expressing T cells from each group (right panel). Clonality was defined by considering only CAR-seq expressing T cells or only non-CAR-seq expressing T cells independently. White color indicates no value. **(B)** Heatmap of CD8 / CD4 T cell ratio in multiplets (M) or singlets (S) of CAR-seq expressing T cells or non-CAR-seq expressing T cells from each group. Clonality was defined by considering only CAR-seq expressing T cells or only non-CAR-seq expressing T cells

independently. **(C)** The Jaccard coefficient of clonotype between CD4 Treg and the other CD4 T cell subsets from each group in second experiment. Wilcoxon-rank sum test was performed for statistical test. **(D)** UMAP plot of ENTPD1 in CD8 T cells (left panel) and Violine plot of ENTPD1 in each subset of CD8 T cell (right panel).

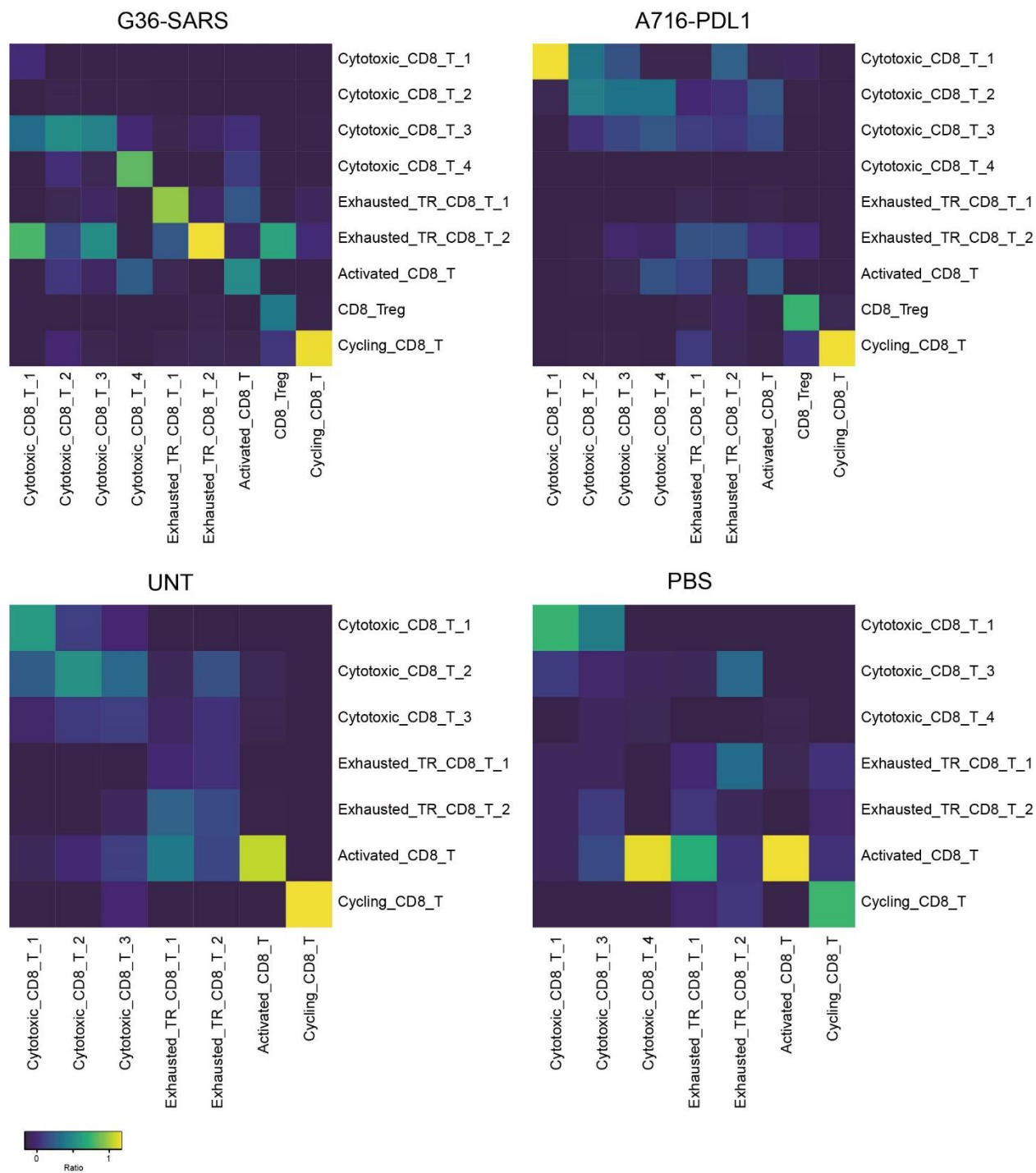


Figure S8. RNA velocity analysis of CD8 T cell, related to Figure 5. Heatmap of transition ratio from celltypes in column to celltypes in row from G36-SARS, A716-PDL1, UNT, PBS groups.

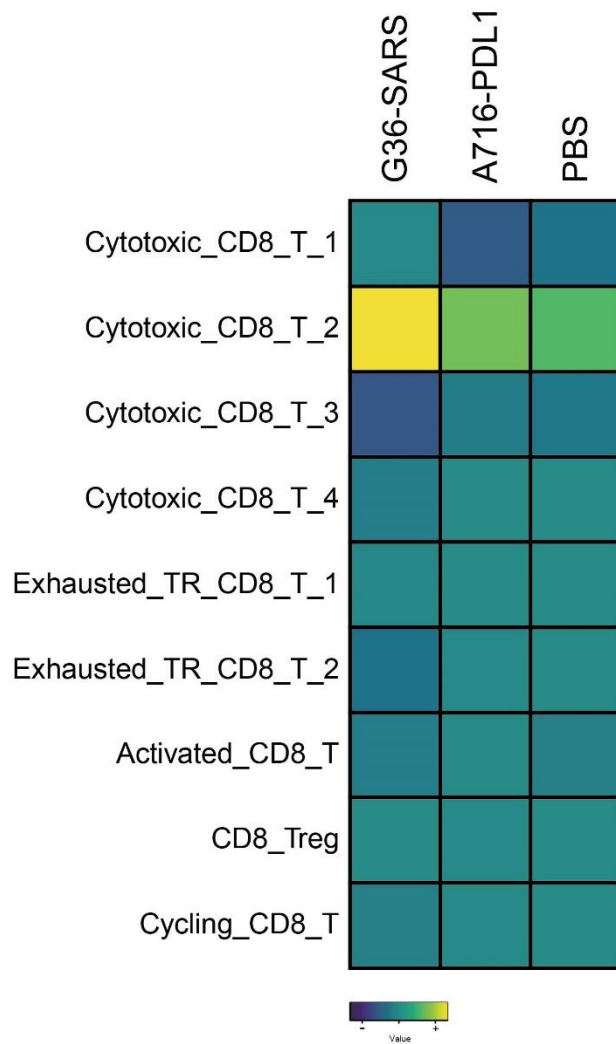


Figure S9. Comparison of state transition, related to Figure 5. Heatmap of a transition ratio difference between G36-PDL1 to a given group.

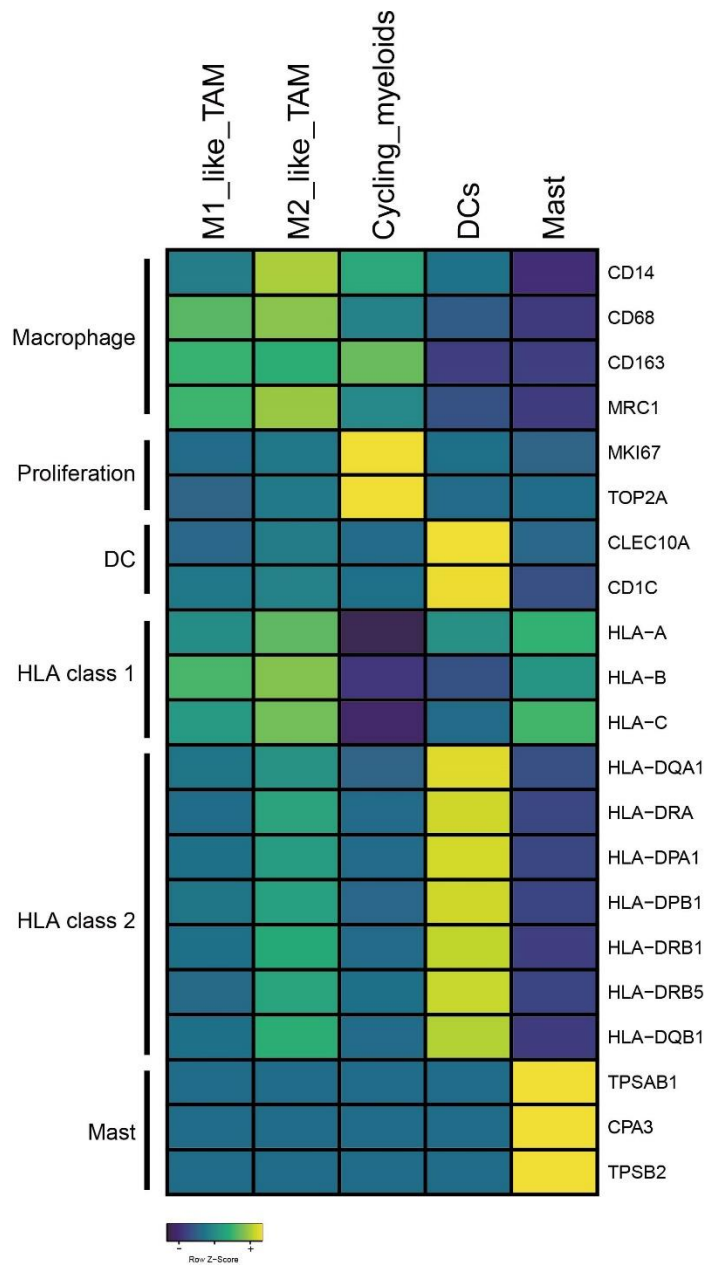


Figure S10. Characterization of myeloids, related to Figure 6. Heatmap of mean expression for characterizing myeloid subsets.

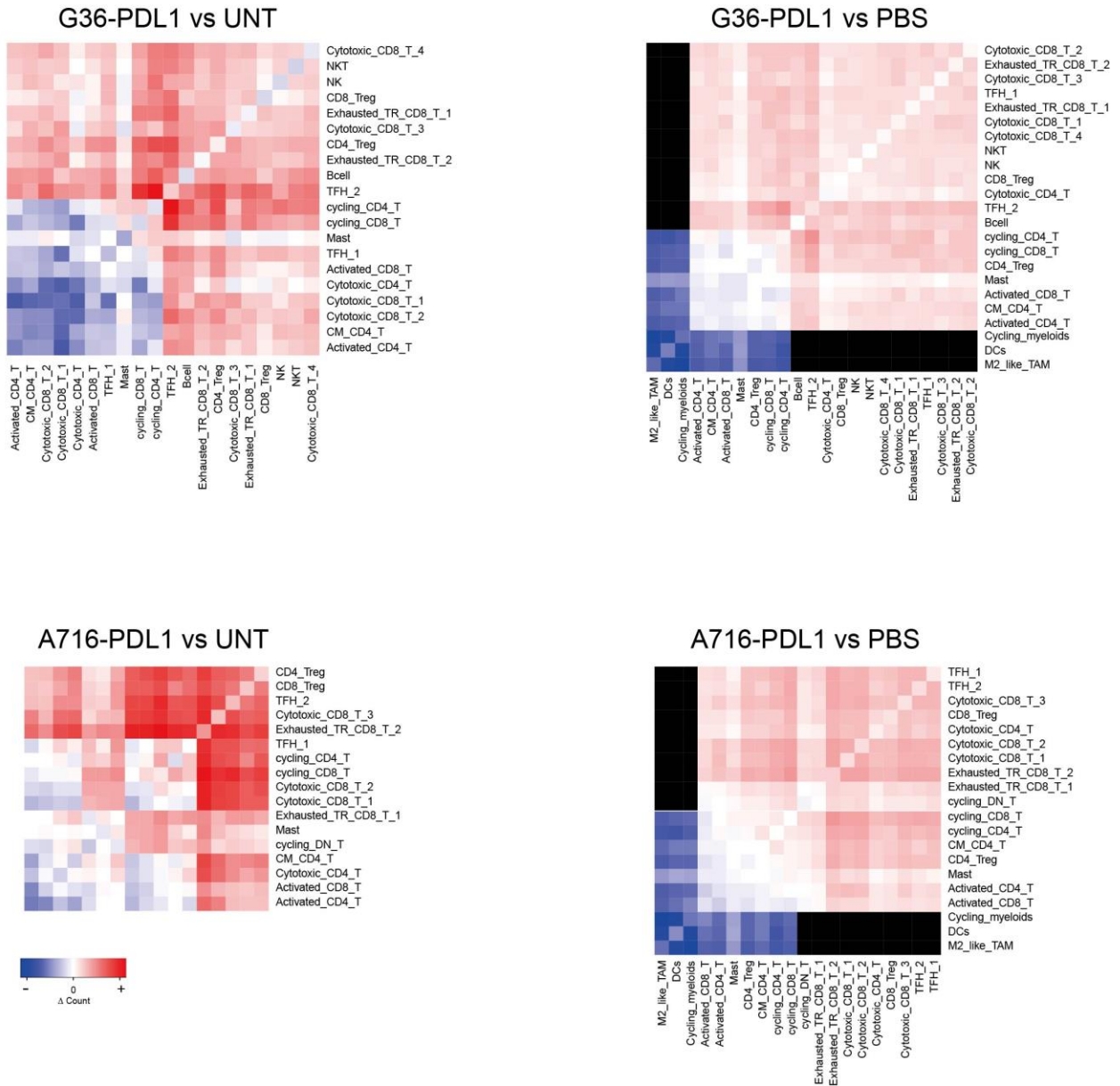


Figure S11. The cell-cell interaction in the TME, related to Figure 6. The difference of Cell-Cell interaction counts between two groups from CellChat package. The positive (red) sign indicates a higher value from G36-PDL1.

Supplementary Tables

	CAR sequence
G36	<p>ATGAAACATCTGTGGTTCTTCCTTCTCCTGGTGGCAGCGGCCAGCCGGCC ATGGCCGAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTACAGCCTGG GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCCCTTTAGCAGCTA TGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGCTAATGGTGGTACCACATACTACGCAGACTCCGTGAAG GGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCA AATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAATA ATGGGAACTATCGCGGTGCTTTTGATATCTGGGGCCAAGGGACAATGGTC ACCGTCTCTTCAGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGT GGCAGCCAGTCTGTGCTGACTCAGCCACCCTCAGTGTCTGGGGCCCCAGG GCAGAGGGTACCATCTCCTGCACTGGGAGCAGCTCCAACATCGGGGCAG GTTTTGATGTACACTGGTACCAGCAGCTTCCAGGAACAGCCCCAAACTCC TCATCTACGGTAACACCAATCGACCCTCAGGGGTCCCTGACCGATTCTCTG GCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCACTGGGCTCCAGGCTG AGGATGAGACTGATTATTACTGCCAGTCCTATGACAGTAGACTGAGTGCTT GGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAG</p>
A716	<p>ATGAAACATCTGTGGTTCTTCCTTCTCCTGGTGGCAGCGGCCAGCCGGCCA TGGCCCAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTACAGCCTGGCA GGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTGATGATTATGC CATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCTCAGG TATTAGTTGGAATAGTGGTAGCATAGGCTATGCGGACTCTGTGAAGGGCCG ATTCACCATCTCCAGAGACAACGCCAAGAAGTCCCTGTATCTGCAAATGAA CAGTCTGAGAGCTGAGGACACGGCCTTGTATTACTGTGCGAGAACAGGAGG GGATGGTGATTATATTGGTGCTTTTGATATGTGGGGCCAAGGCACCCTGGTC ACCGTCTCCTCAGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTG GCATCCAGCCTGTGCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACA GTCGATCACCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGTTATAAC TATGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCAAACTCATGATTT ATGAGGTCAGTAAGCGGCCCTCAGGGGTCCCTGATCGTTCTCTGGCTCCAA GTCTGGCAACACGGCCTCCCTGACCGTCTCTGGGCTCCAGGCTGAGGATGA GGCTGATTATTACTGCAGCTCATATGCAGGCAGCACCCCTGTGGTATTCGGC GGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCCGCCCCCTCG</p>

Table S1. CAR sequence of G36 and A716, related to Figure 1.