**Supplementary data**

**Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition**

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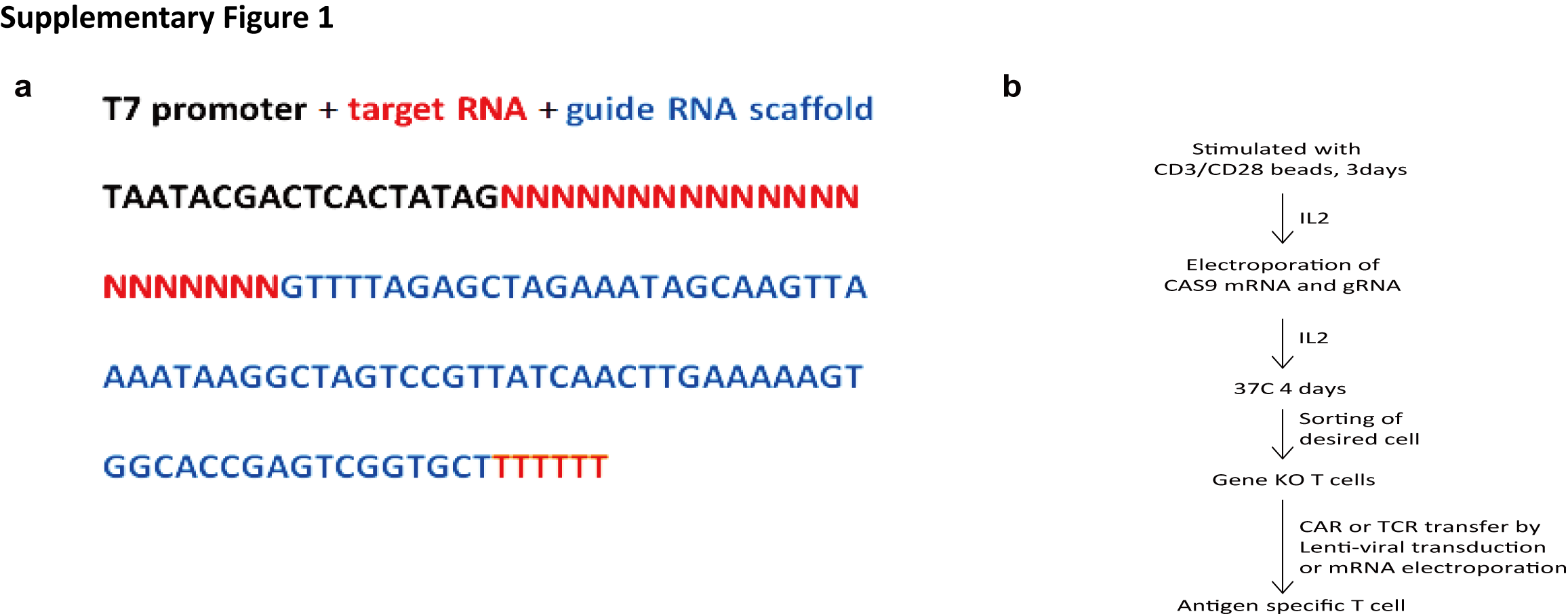
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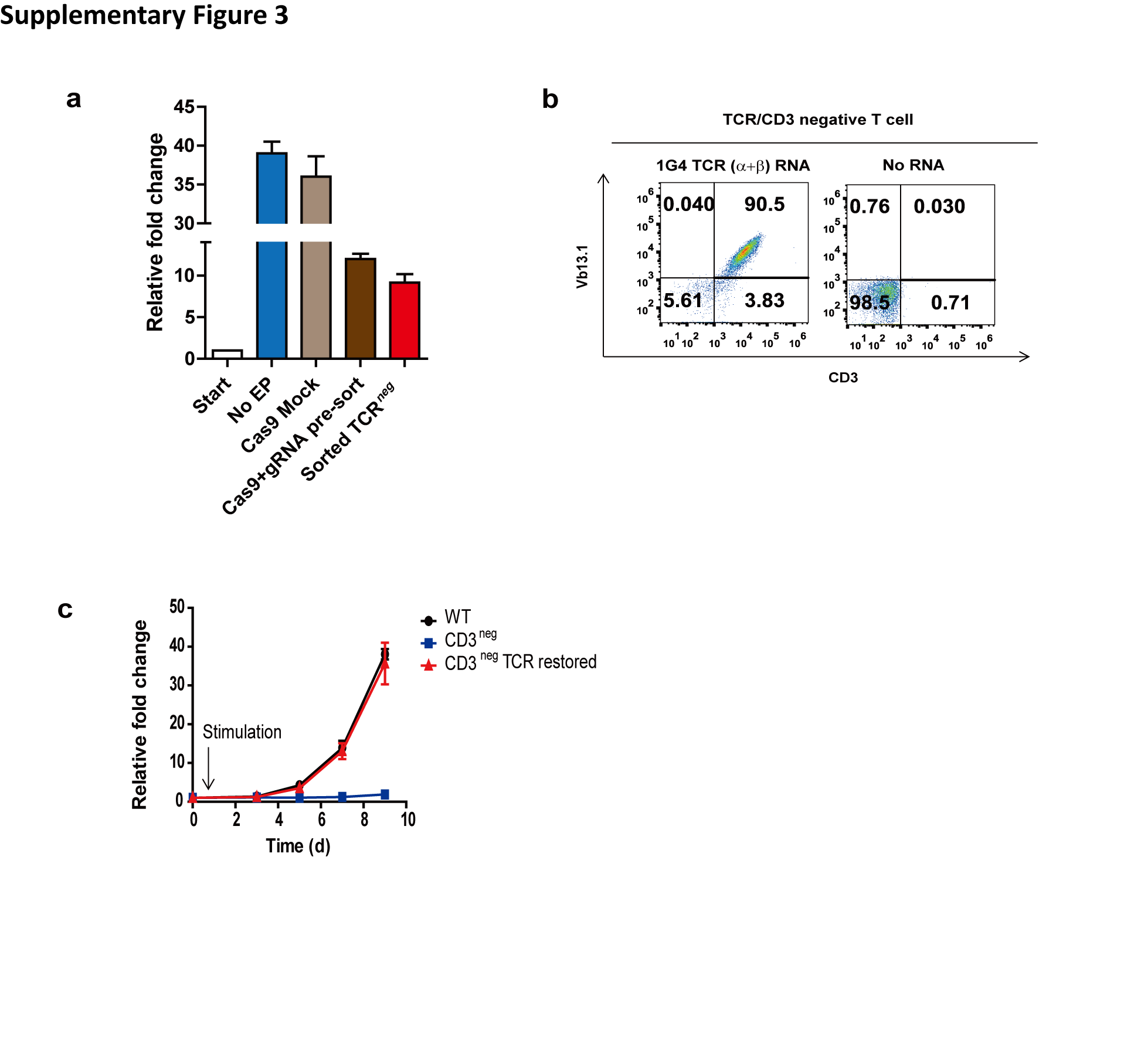
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**Supplementary Figure 1. Strategy of introducing CRISPR/Cas9 into T cells.** (a) Schematic representation of gRNAs driven by the T7 promoter. (b) Schematic representation of the generation of gene-edited antigen-specific T cells using the CRISPR system. T cells were stimulated by CD3/CD28 Dynabeads for three days prior to RNA electroporation. Then T cells were washed three times with OPTI-MEM and re-suspended in OPTI-MEM (Invitrogen) at a final concentration of 1-3x108 cells/ml. Subsequently, 0.1 ml of the cells was mixed with IVT RNA and electroporated in a 2 mm cuvette. 20 µg of Cas9 mRNA and 10 µg of gRNA were electroporated into the cells using a BTX830 (Harvard Apparatus BTX) at 360 V and 1 ms; this process was followed by a second electrotransfer of 5 µg of gRNA 12 to 24 hours later. Following electroporation, the cells were immediately placed in 2 ml of pre-warmed culture media and cultured in the presence of IL-2 (100 IU/ml) at 37°C and 5% CO2. Specific gene-disrupted T cells were sorted on day 8 and redirected with CAR or TCR by lentiviral transduction or mRNA electroporation gene transfer.

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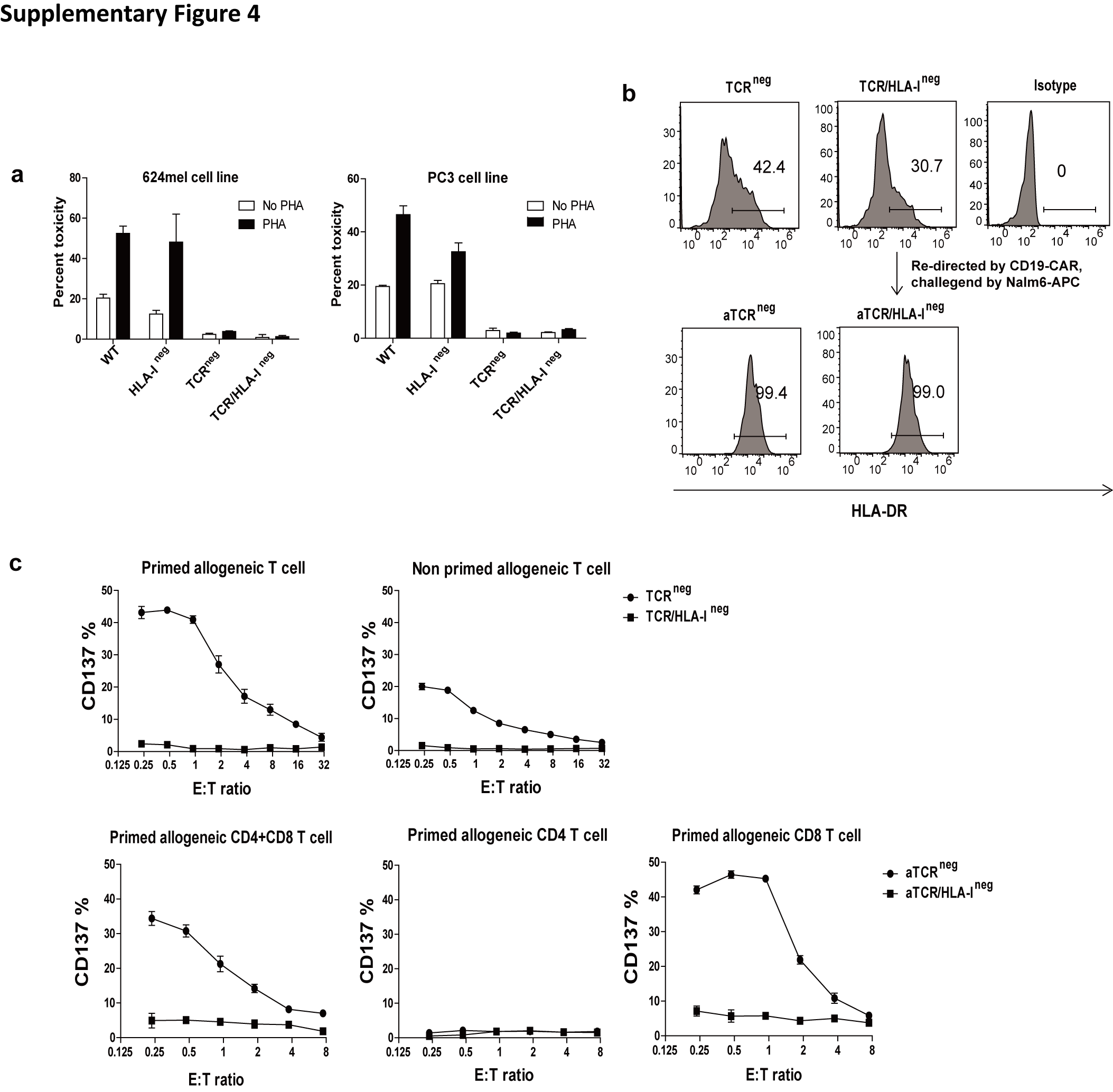
**Supplementary Figure 2. Multiple deliveries of gRNAs disrupts genes in human primary T cells with high efficiency without impairing effector function.**

(a) CD3 disruption under different Cas9 mRNA and gRNA amount and ratios. (b) Targeting events validated by cloning PCR. PCR products flanking TRBC-gRNA targeting sites were cloned into TOPO vector and performed Sanger sequencing.

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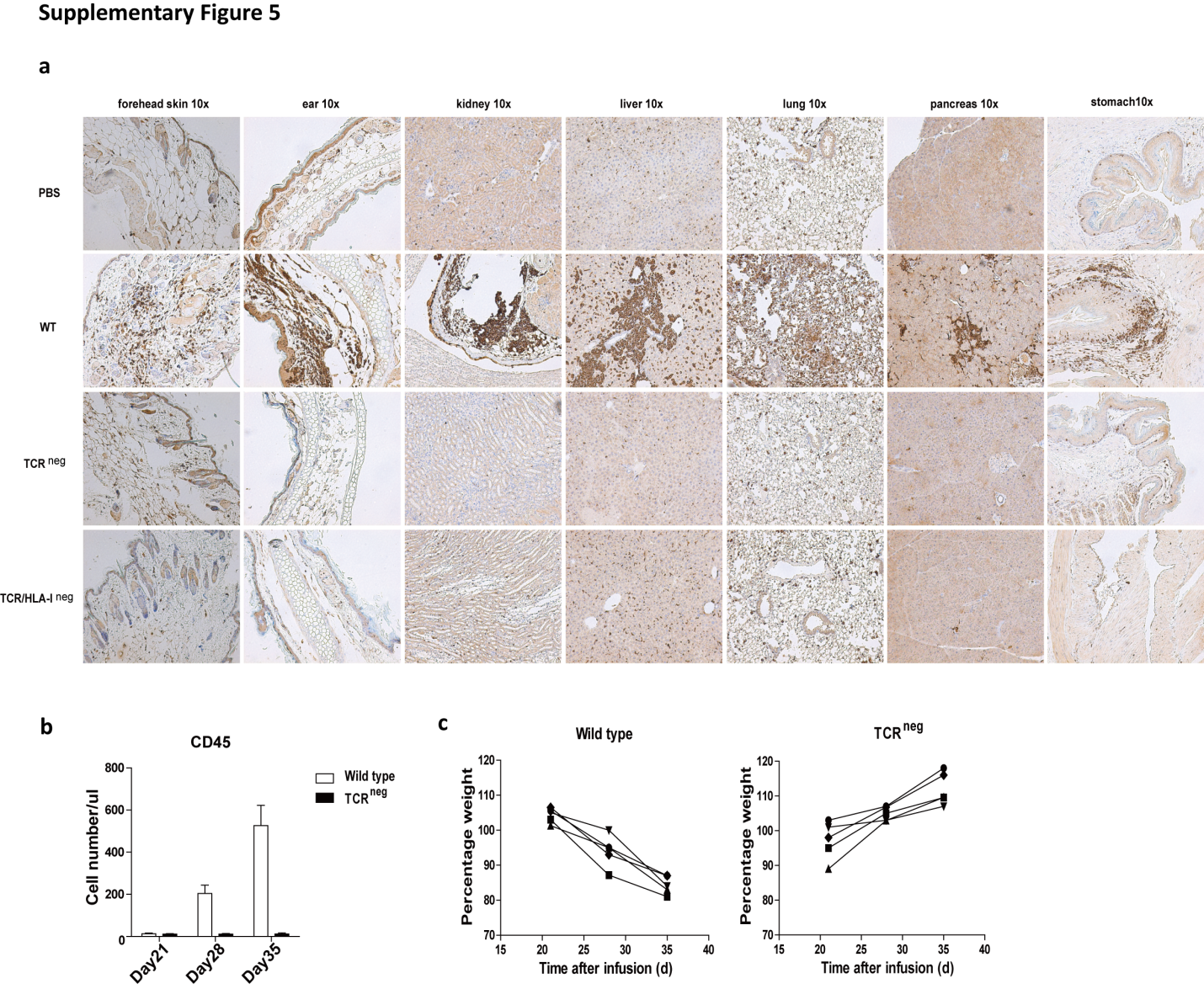
**Supplementary Figure 3. Proliferation test of CD3neg T cells.**

(a) Relative fold expansion of T cells with or without CRISPR editing. EP, electroporation. (b)CD3 expression measured by flow cytometry. (c) Proliferation of TCR restored CD3neg T cells.

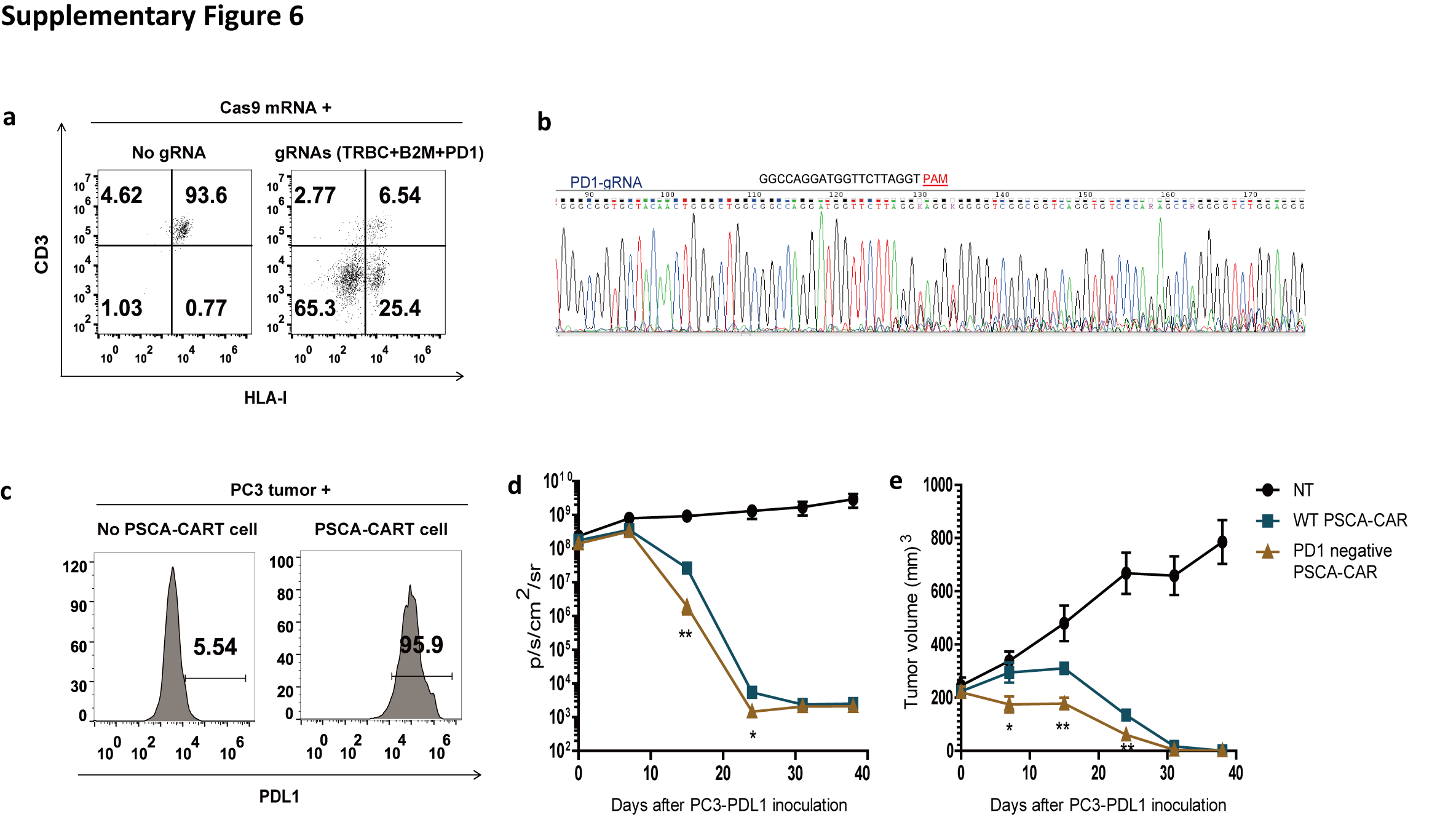
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**Supplementary Figure 4. Reduced alloreactivity of TCR and B2M double-disrupted T cells.**

(a) TCR and B2M double-knockout T cells abrogated the allogeneic killing of HLA unmatched tumor cell lines. Gene-edited T cells were overnight co-cultured with A624-CBG and PC3-CBG tumor, toxicity was calculated by measuring luciferase activity of live cells, Bars, SE, n=3. (b) Gene-edited T cells were re-directed with CD19-CAR mRNA by electroporation, and then incubated with Nalm6 target cells at an E:T ratio of 1:1. HLA-DR expression was measured by flow cytometry. a, activated. (c)Abolishment of target recognition of HLA-I disrupted T cells by allogeneic effector T cells. To generate dendritic cells to prime allogeneic T cells, donor PMBCs were incubate at 37°C, 5% CO2 for 2~3 hours, discarded non-adherent cells, then cultured in10 mL culture medium with Recombinant GM-CSF and IL-4 at a final concentration of 20 ng/mL at 37°C, 5% CO2. Recombinant TNF was added to a final concentration of 20 ng/mL on the fifth day of incubation. The mature dendritic cells were harvested and co-cultured with allogeneic T cells (50% each of CD4 and CD8) at a ratio of 1:10. The primed T cells were harvested at day 9 and CD4 and CD8 were separated for alloreactivity test against HLA-Ineg T cells generated from different donors. The alloreactivity of allogeneic T cells was determined by limited dilution assay performed in round-bottomed microplates seeded with different numbers of primed allogeneic CD4 or CD8 T cells and 2X104 irradiated TCRneg or TCR/ HLA-Ineg T cells. Cultures were incubated for 2 days and the activation of CD3 positive cells was measured by CD137 staining by flow cytometry. Allogeneic effector CD4 and CD8 T cells were challenged by resting or activated gene-edited T cells at different ratios. Activation of allogeneic T cells by gene-edited T cells was measured by detecting the upregulation of CD137 with flow cytometry (n=3).

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**Supplementary Figure 5. GVHD effect of gene-modified CAR T cells** (a) Organs of mice from different treatment groups were collected and used for CD8 immunohistochemistry staining. PBS, or 10x106 non-manipulated, TCR ablated, TCR/HLA-I double ablated T cells were injected into 8- to 10-week-old NSG mice that had been conditioned with 175 cGy irradiation. After T cell infusion (Day0), mice were monitored 3 times per week for GvHD study. Mice with weight loss of ~20% were sacrificed for ethical reasons, other mice were sacrificed at day 62. Wild type and TCRneg T cells were infused into sub-lethal irradiated mice, (b) presence of CD45 T cells by a FACS Trucount assay, and (c) weight loss in mice were recorded. Results are expressed as the mean absolute count per µl of peripheral blood±SD with *n*=5 for all groups. ns, \*\*\*\**P<0.001* by Mann-Whitney test.

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**Supplementary Figure 5.** **Generation of universal CART cells deficient of PD1.**

(a) Expression of CD3 and HLA-I expression was measured by flow cytometry on CD19 CART cells treated with Cas9 mRNA alone or treated with Cas9 mRNA and gRNAs targeting TRBC, B2M and PD1. CD3 and HLA-I double negative cells were sorted by negative selection. (b) Sanger sequencing of PCR products flanking PD1-gRNA targeting site. (c) Expression of PDL1 on PC3 tumors. PC3 tumors were co-cultured with PSCA-CART cells at a ratio of 1:1 for 18 hours and PDL1 was measured by flow cytometry. (d) Bioluminescence and tumor burden of mice receiving different treatment, *n*=6. Imaging commenced 1 day before the start of T cell treatment. \**P*<0.05, \**P*<0.01, by Mann-Whitney test. (e) Tumor volume of mice. Results are expressed as the mean tumor volume (mm3±SE) with *n*=6 for all groups. \**P*<0.05, \**P*<0.01, by Mann-Whitney test.

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| **Supplementary table 1** |  |  |  |  |
| TRAC-OT1-Chr5-f1 | CTTGGCTGGAAAGGGTACTTAGGAA | | | |
| TRAC-OT1-Chr5-r1 | CCCACCTCCAACACTAGAGACCACA | | | |
| TRAC-OT2-Chr22-f1 | TTTGAGAGGTACAGCTCCCTTTTGC | | | |
| TRAC-OT2-Chr22-r1 | AGACCACAGCTCTTCAACAGCCAAA | | | |
| TRAC-OT3-Chr13-f1 | TATGTCAACCCAGAACCAGAGCAGC | | | |
| TRAC-OT3-Chr13-r1 | TGTGCCTTTAATTCCCACAACTGGA | | | |
| TRAC-OT4-Chr2-f1 | TGGATGCATTACACATCAGCATACC | | | |
| TRAC-OT4-Chr2-r1 | GGCAGCAAAATGAAGCTTTGCTTAA | | | |
| TRAC-OT5-Chr18-f1 | CTCAGATAAATGGAGCGCAGCCTT | | | |
| TRAC-OT5-Chr18-r1 | GATCATGCCATCCAACTTTCTTCAG | | | |
| TRAC-OT6-Chr5-f1 | TCCCATCTTCTTCCAAGAGCTGCTA | | | |
| TRAC-OT6-Chr5-r1 | AAGTAAAATCACCCAAACACAAAAGGAT | | | |
| TRAC-OT7-Chr10-f1 | TTGATGAGGGCTAGCAATTTCTTCA | | | |
| TRAC-OT7-Chr10-r1 | GAAATCCTAACACCCAAGGTGATGG | | | |
| TRAC-OT8-Chr4-f1 | AGAGACGCGGTTTCACTGTGTTAGC | | | |
| TRAC-OT8-Chr4-r1 | TTCTTGGCTCGGTGTTCAATCTTTT | | | |
| TRAC-OT9-Chr22-f1 | TCATGGCTCACTGCAACCTTAAATT | | | |
| TRAC-OT9-Chr22-r1 | ATGAAGCACAGCCTGTGCTTACAAG | | | |
| TRAC-OT10-Chr6-f1 | GGAGGTGGTCTGTTTTGCATATGGA | | | |
| TRAC-OT10-Chr6-r1 | TTTCACCTGCTATAACCAAGCTCCA | | | |
| TRBC-OT1-Chr8-f1 | ACAGGACATGGTCTATCCTTGCAGG | | | |
| TRBC-OT1-Chr8-r1 | CTGGCTACAGAGCGAGAGCTCAGAG | | | |
| TRBC-OT2-Chr1-f1 | CCTTCCTGTCAGAAGATTTATCGGG | | | |
| TRBC-OT2-Chr1-r1 | GTGAAACCCCGTCTCTACCAAAAAT | | | |
| TRBC-OT3-Chr9-f1 | TCGCCTCCTCATTCCAGAGTACTCA | | | |
| TRBC-OT3-Chr9-r1 | AAAACACTAGGTGGCTTCTGGTCAA | | | |
| TRBC-OT4-Chr16-f1 | TGCATCAGGTATTTAGAACAGTGCCTG | | | |
| TRBC-OT4-Chr16-r1 | TTTACTGATAATGGGTGGCCAGGAG | | | |
| TRBC-OT5-Chr3-f1 | TCAAATCTAGTCATCCGTGCATCTAAGTA | | | |
| TRBC-OT5-Chr3-r1 | CAAAAATGTCTTCTCCACATAGGCCA | | | |
| TRBC-OT6-Chr20-f1 | GTTAACTTCTTCCTCCTCCCAGCCC | | | |
| TRBC-OT6-Chr20-r1 | GCAACAGAGCAAGACCCTGTCTCTT | | | |
| TRBC-OT7-Chr21-f1 | CCTCGATAAGACTGTGTGCCTGAGA | | | |
| TRBC-OT7-Chr21-r1 | AGAGAATGGACCAACAGGCATACAA | | | |
| TRBC-OT8-Chr4-f1 | TCCTTGAAGGTAAATTTGCCCAACA | | | |
| TRBC-OT8-Chr4-r1 | TTGTTTAATCGTCACAGGACATCCC | | | |
| TRBC-OT9-Chr2-f1 | GAGAGTCTGAGTAAGAAGCAGGGCC | | | |
| TRBC-OT9-Chr2-r1 | AAATCCAGAGTCAAAATCCCTGAGC | | | |
| TRBC-OT10-ChrX-f1 | GCAACAAACATGGGAGTGCAGATAT | | | |
| TRBC-OT10-ChrX-r1 | TGTGTGCAGCTTGTTAACTGCACAG | | | |