

Supporting Information for

CAR-T cells co-expressing IL-7 and CCL19 promote epitope spreading to enhance antitumor immunity

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Supporting Information Text

Materials and Methods

Mice and cell lines

Male and female C57BL/6, DBA/2, and B6D2F1 (a cross between female C57BL/6 and male DBA/2) mice, aged 7–10 weeks, were obtained from SLC (Shizuoka, Japan). OT-I transgenic mice, in which T cells express a TCR specific for the SIINFEKL epitope of chicken OVA [1], were kindly provided by Professor Mamoru Harada (Shimane University, Japan), with permission from Professor William Heath (The University of Melbourne, Australia). Congenic C57BL/6 mice expressing CD90.1 and firefly luciferase, and lacking CD90.2, were purchased from The Jackson Laboratory (Bar Harbor, ME). XCR1-DTRvenus mice, engineered to express a fusion protein of the human diphtheria toxin receptor and Venus fluorescent protein, were obtained from the RIKEN BioResource Research Center (Tsukuba, Japan) [2, 3]. For selective depletion of XCR1⁺ dendritic cells (DCs) in XCR1-DTRvenus mice, diphtheria toxin (25 ng/g body weight; Merck/Sigma-Aldrich, 322326) was administered intraperitoneally every 3 days. All mice were housed under specific pathogen-free conditions at Yamaguchi University. T cells were isolated from spleens and lymph nodes using the Pan T Cell Isolation Kit II (Miltenyi Biotec, 130-095-130). Tumor-draining lymph nodes (TDLNs; right inguinal, axillary, and brachial) were enzymatically digested with Liberase TL and DNase I

(Roche Diagnostics) to generate single-cell suspensions for flow cytometry. DCs were further purified from TDLN suspensions using the Pan Dendritic Cell Isolation Kit (Miltenyi Biotec, 130-100-875). For tumor studies, the P815 (mastocytoma, DBA/2 origin), 3LL (Lewis lung carcinoma, C57BL/6 origin), and MC38 (colon carcinoma, C57BL/6 origin) cell lines were transduced with a retroviral vector encoding human CD20 to generate P815-hCD20, 3LL-hCD20, and MC38-hCD20 clones, respectively. The B16F10 melanoma cell line (C57BL/6 origin) was transduced with vectors encoding human CD20, TagGFP, and an OVA fragment containing the SIINFEKL epitope, yielding the B16.20-cOVA-TagGFP clone.

For flow cytometric analysis of tumor-infiltrating cells, tumors with a major axis of approximately 1 cm were excised, digested with Liberase TL and DNase I, and processed into single-cell suspensions. To enrich for immune cells and reduce tumor cell contamination, samples were treated with CD45 MicroBeads (Miltenyi Biotec, 130-110-618, RRID: AB_3224404) prior to analysis of T cells and DCs. All T cell, DC, and tumor cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin, 100 µg/mL streptomycin, 50 µM 2-mercaptoethanol, 25 mM HEPES, and 2 mM L-glutamine, or in DMEM supplemented with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin.

Animal study

All mouse experiments were performed in accordance with the national guidelines for the humane care and use of laboratory animals and with ARRIVE guidelines. The Institutional Animal Care and Use Committee of Yamaguchi University (the Committee on the Ethics of Animal Experiments of Yamaguchi University; 14-001, 14-003, and 14-S02) approved the study.

Retroviral vectors for gene transduction into mouse T cells

The anti-human CD20 scFv construct was designed based on previously reported sequences [4]. The CAR construct was generated by linking the scFv to the transmembrane domain of mouse CD8 α and the intracellular signaling domains of mouse CD28, 4-1BB (CD137), and CD3 ζ (Suppl. Fig. 1). This construct was subsequently cloned into the MSGV1 vector to produce retroviral vectors for gene delivery [4, 5]. To enable co-expression of murine IL-7 and CCL19 with the CAR, a foot-and-mouth disease virus 2A self-cleaving peptide sequence was inserted between the coding regions of the three genes [6, 7]. All sequences were synthesized by Thermo Fisher Scientific (Gaithersburg, MD).

Retroviral transduction of mouse T cells was performed as described previously [4, 5]. Briefly, GP2-293 packaging cells (Clontech, RRID: CVCL_WI48) were co-transfected

with the CAR-encoding plasmid and the pCL-Eco packaging plasmid using Lipofectamine[®] reagent (Thermo Fisher Scientific). Culture supernatants containing the retroviral particles were collected and used for T cell transduction. Mouse T cells were activated with plate-bound anti-CD3 ϵ antibody (clone 145-2C11, Thermo Fisher Scientific, 16-0031-85, RRID: AB_468848), anti-CD28 antibody (clone 37.51, BD Biosciences, 553294, RRID: AB_394763), and recombinant IL-2 (PeproTech or Miltenyi Biotec), followed by retroviral infection in the presence of RetroNectin[®] (Takara Bio).

Flow cytometry

For cell surface staining, the following antibodies were used: Allophycocyanin (APC)/Cyanine-7 (Cy7)-conjugated anti-CD3 ϵ (clone 145-2C11, BioLegend, 100330, RRID: AB_1877170), Fluorescein isothiocyanate (FITC)-conjugated anti-CD3 ϵ (clone 145-2C11, BioLegend, 100306, RRID: AB_312671), APC/Cy7-conjugated anti-CD4 (clone GK1.5, BD Biosciences, 552051, RRID: AB_394331), Brilliant Violet 510 (BV510)-conjugated anti-CD4 (clone RM4-5, BD Biosciences, 563106, RRID: AB_2687550), FITC-conjugated anti-CD8 α (clone 53-6.7, Thermo Fisher Scientific, 11-0081-85, RRID: AB_464916), Phycoerythrin (PE)/Cy7-conjugated anti-CD8 α (clone 53-6.7, BioLegend, 100722, RRID: AB_312761), APC-conjugated anti-CD8 α (clone 53-6.7, BioLegend, 100712, RRID: AB_312751), Peridinin-chlorophyll-protein complex

(PerCP)/Cy5.5-conjugated anti-CD11b (clone M1/70, BioLegend, 101228, RRID: AB_893232), APC-conjugated anti-CD11c (clone N418, BioLegend, 117310, RRID: AB_313779), APC/Cy7-conjugated anti-CD19 (clone 6D5, BioLegend, 115530, RRID: AB_830707), FITC-conjugated anti-CD19 (clone 6D5, BioLegend, 115506, RRID: AB_313641), FITC-conjugated anti-CD40 (clone HM40-3, BioLegend, 102906, RRID: AB_312949), PerCP/Cy5.5-conjugated anti-CD44 (clone IM7, BioLegend, 103031, RRID: AB_2076206), APC-conjugated anti-CD45 (clone 30-F11, Tonbo Biosciences, 20-0451, RRID: AB_2621573), BV421-conjugated anti-CD45 (clone 30-F11, Invitrogen, 404-0451-82, RRID: AB_2925507), PE/Cy7-conjugate anti-CD62L (clone MEL-14, BioLegend, 104418, RRID: AB_313103), BV421-conjugated anti-CD90.1 (clone OX-7, BD Biosciences, 563770, RRID: AB_2738416), PerCP/Cy5.5-conjugated anti-CD90.2 (clone 30-H12, BioLegend, 105338, RRID: AB_2571945), BV650-conjugated anti-CD197 (CCR7, clone 4B12, BD Biosciences, 564356, RRID: AB_2738766), BV421-conjugated anti-CD274 (PD-L1, clone MIH5, BD Biosciences, 564716, RRID: AB_2738911), APC-conjugated anti-H-2K^d (clone SF1-1.1, BioLegend, 116620, RRID: AB_10645328), PE-conjugated anti-I-A^b (clone AF6-120.1, BD Biosciences, 553552, RRID: AB_394919), APC/Cy7-conjugated anti-NK1.1 (clone PK136, BD Biosciences, 560618, RRID: AB_1727569), FITC-conjugated anti-NK1.1 (clone PK136, BioLegend, 108706, RRID: AB_313393), FITC-conjugated anti-XCR1 (clone ZET, BioLegend,

148210, RRID: AB_2564366), APC/Cy7-conjugated anti-XCR1 (clone ZET, BioLegend, 148224, RRID: AB_2783118), and BV421-conjugated anti-human CD20 (clone 2H7, BioLegend, 302330, RRID: AB_10965543), PE-conjugated anti-human CD20 (clone 2H7, BioLegend, 302306, RRID: AB_314254). For the detection of PD-1 expression, anti-PD-1 (clone G4), which was purified in-house from hybridoma supernatant or purified hamster IgG1 control (clone G235-2356; BD Biosciences, 554709, RRID: AB_395522), was utilized in combination with a FITC-conjugated anti-hamster IgG secondary antibody (goat polyclonal IgG; BioLegend, 405502, RRID: AB_315020).

Detection of anti-human CD20 CAR expression was performed using biotinylated recombinant protein L (GenScript), followed by APC-conjugated streptavidin (BioLegend, 405243), as previously described [4, 8]. Anti-CD16/CD32 antibody (clone 2.4G2), purified from hybridoma supernatant in-house, was used to block non-specific binding via Fc γ receptors. Dead cells were excluded using Fixable Viability Dye eFluor™ 780 (Thermo Fisher Scientific, 65-0865-14) or Zombie Yellow™ Fixable Viability Kit (BioLegend, 423104). Flow cytometry data were acquired using a CytoFLEX S system (Beckman Coulter) and analyzed with FlowJo software (BD Biosciences/FlowJo, LLC, RRID: SCR_008520). Fluorescence minus one (FMO) controls were used to define marker positivity or negativity where appropriate.

***In vitro* stimulation of T cells with tumor peptides**

Antigenic peptides corresponding to the tumor-associated antigens used in this study were synthesized by Scrum (Tokyo, Japan) for T cell stimulation. The peptide sequences were as follows: GP100, EGSRNQDWL; TRP-1, TAPDNLGYA; TRP-2, SVYDFFVWL [9]; OVA, SIINFEKL [10]; Dpagt1, SIIVFNLL; Adpgk, ASMTNMELM; REPS1, AQLANDVVL [11]; and P1A, LPYLGWLVF [12]. Single-cell suspensions were prepared from the spleens and lymph nodes of tumor-bearing mice treated with CAR-T cells or from untreated, tumor-free control mice. Cells were stimulated with peptides at concentrations of 0, 0.1, 0.5, or 1 µg/mL for 2–3 days. Concentrations of IFN-γ, TNF-α, or granzyme B in the culture supernatants were quantified using ELISA kits (IFN-γ; Thermo Fisher Scientific, 88-7314-88, RRID: AB_2575070, TNF-α; R&D Systems, DY410-05, granzyme B; R&D Systems, DY1865-05). Values obtained from unstimulated samples derived from naive control mice were used as background. Peptide-specific reactivity was defined as a response exceeding both the background level and that of the unstimulated control within the same treatment group.

***In vivo* murine tumor models**

In the mixed tumor model, DBA/2 mice were subcutaneously (s.c.) inoculated in the right flank on day 0 with 4.17×10^5 P815-hCD20 cells, 0.833×10^5 parental P815 cells, or a

5:1 mixture of CAR target-positive to -negative cells. Cyclophosphamide (CPA; 100 mg/kg; Shionogi) was administered intraperitoneally (i.p.) on day 11, followed by intravenous (i.v.) injection of 1×10^6 conventional anti-human CD20 CAR-T cells (Conv.) or IL-7/CCL19-producing anti-human CD20 CAR-T cells (7×19) on day 14.

In the lung cancer model, on day 0, C57BL/6 mice were s.c. inoculated in the right flank with 3LL-hCD20 cells, parental 3LL cells, or a mixture at a 3:1 ratio (1.875×10^6 3LL-hCD20; 0.625×10^6 3LL) or 11.5:1 ratio (2.3×10^6 3LL-hCD20; 0.2×10^6 3LL). CPA (100 mg/kg) was administered i.p. on day 7, followed by i.v. injection of 1×10^6 anti-human CD20 CAR-T cells on day 10. In certain experiments, CAR-T cells were administered on day 3 without CPA pretreatment.

In the melanoma model, C57BL/6 mice were s.c. inoculated in the right flank on day 0 with either 9.2×10^5 , 7.5×10^5 , or 5×10^5 B16.20-cOVA-TagGFP cells; 0.8×10^5 , 2.5×10^5 , or 5×10^5 parental B16F10 cells; or mixtures at 11.5:1, 3:1, or 1:1 ratios. CPA (100 mg/kg) was administered i.p. on day 4, followed by i.v. injection of 1×10^6 anti-human CD20 CAR-T cells on day 7. In a separate model, C57BL/6 mice were s.c. inoculated on day 0 with a 4.6:1 mixture of 9.2×10^5 B16.20-cOVA-TagGFP and 2×10^5 3LL cells, treated with CPA on day 4, and administered 1×10^6 CAR-T cells on day 7.

In the unmixed tumor model, C57BL/6 mice were s.c. inoculated with 1×10^6 B16.20-cOVA-TagGFP cells in the right flank, followed by CPA treatment on day 4 and

administration of 1×10^6 anti-human CD20 CAR-T cells on day 7. In some experiments, 1×10^7 conventional anti-human CD20 CAR-T cells were used following CPA. To evaluate DC and T cell recruitment, treatment schedules were adjusted to allow for sufficient tumor growth and immune cell infiltration. In this setting, mice were inoculated with 1×10^6 B16.20-cOVA-TagGFP cells, followed by CPA and CAR-T cell administration on days 10 and 13, respectively. In the repetitive CAR-T cell injection model, allogeneic 7×19 CAR-T cells derived from B6D2F1 mice were administered once weekly for a total of three doses.

In additional models, C57BL/6 mice were s.c. inoculated with 1.5×10^6 MC38-hCD20 cells and treated with CPA (30 mg/kg) and 1×10^6 anti-human CD20 CAR-T cells on days 11 and 14, respectively. In the P815-hCD20 model, DBA/2 mice were inoculated with 5×10^5 tumor cells, followed by CPA (100 mg/kg) on days 11, and either 1×10^6 or 4×10^6 anti-human CD20 CAR-T cells on days 14. For experiments involving XCR1-DTRvenus mice, recipients were inoculated with 1×10^6 B16.20-cOVA-TagGFP cells, followed by CPA (100 mg/kg) and 1×10^6 CAR-T cells on days 4 and 7. To deplete XCR1⁺ DCs, diphtheria toxin was administered i.p. every 3 days starting on day 7.

In certain experimental settings, an anti-PD-1 monoclonal antibody (150 µg per mouse, clone G4) was administered. Treatment commenced on day 10 following allogeneic CAR-T cell infusion, corresponding to days 17 and 24 post-tumor inoculation in the

B16.20-cOVA-TagGFP and P815-hCD20 models, respectively, and was continued twice weekly thereafter. In cases where tumor masses remained nonpalpable for a continuous period of 14 days, further administration of the antibody was discontinued.

The tumor size was measured twice weekly using digital calipers, and the tumor volume was calculated using the formula: $(\text{major axis}) \times (\text{minor axis})^2 / 2$. Mouse survival was monitored throughout the duration of each experiment.

The anesthesia and euthanasia protocols were designed in accordance with the guidelines of the American Veterinary Medical Association (AVMA) and were implemented following approval by the Committee on the Ethics of Animal Experiments of Yamaguchi University. Anesthesia was intraperitoneally administered as needed using a combination of three agents: medetomidine (0.3 mg/kg, Kyoritsu Seiyaku), butorphanol (4 mg/kg, Meiji Animal Health), and midazolam (5 mg/kg, Sandoz). Mice were humanely euthanized by CO₂ inhalation in accordance with predefined humane endpoints, which included the tumor size reaching 20 mm or the presence of clinical signs of distress, such as reduced food or water intake, weight loss, hunched posture, or unkempt fur. All animal experiments were conducted in accordance with the ARRIVE guidelines.

Statistics

Statistical analyses for *in vitro* assays were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test, or two-sided unpaired Student's *t*-tests, as appropriate. ANOVA and HSD tests were conducted using online tools based on R (<https://www.statskingdom.com/180Anova1way.html>; <https://www.socscistatistics.com/tests/anova/default2.aspx>), and *t*-tests were performed using Microsoft Excel for Mac. Survival analyses were carried out using the two-sided log-rank test implemented in R-based online tools (<https://www.statskingdom.com/kaplan-meier.html>; <https://www.evanmiller.org/ab-testing/survival-curves.html>). When comparisons involved more than three groups, *P*-values were adjusted using the Bonferroni correction method. Effect sizes, defined as normalized χ^2 statistics indicating the relative magnitude of differences between survival curves, were also calculated. *P*-values < 0.05 were considered statistically significant. The median survival times and 95% confidence intervals (CIs) were calculated using the JMP® Pro 16 software (SAS Institute, RRID: SCR_014242).

References

1. Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR (1994) T cell receptor antagonist peptides induce positive selection. *Cell* 76:17-27. [https://doi.org/10.1016/0092-8674\(94\)90169-4](https://doi.org/10.1016/0092-8674(94)90169-4)

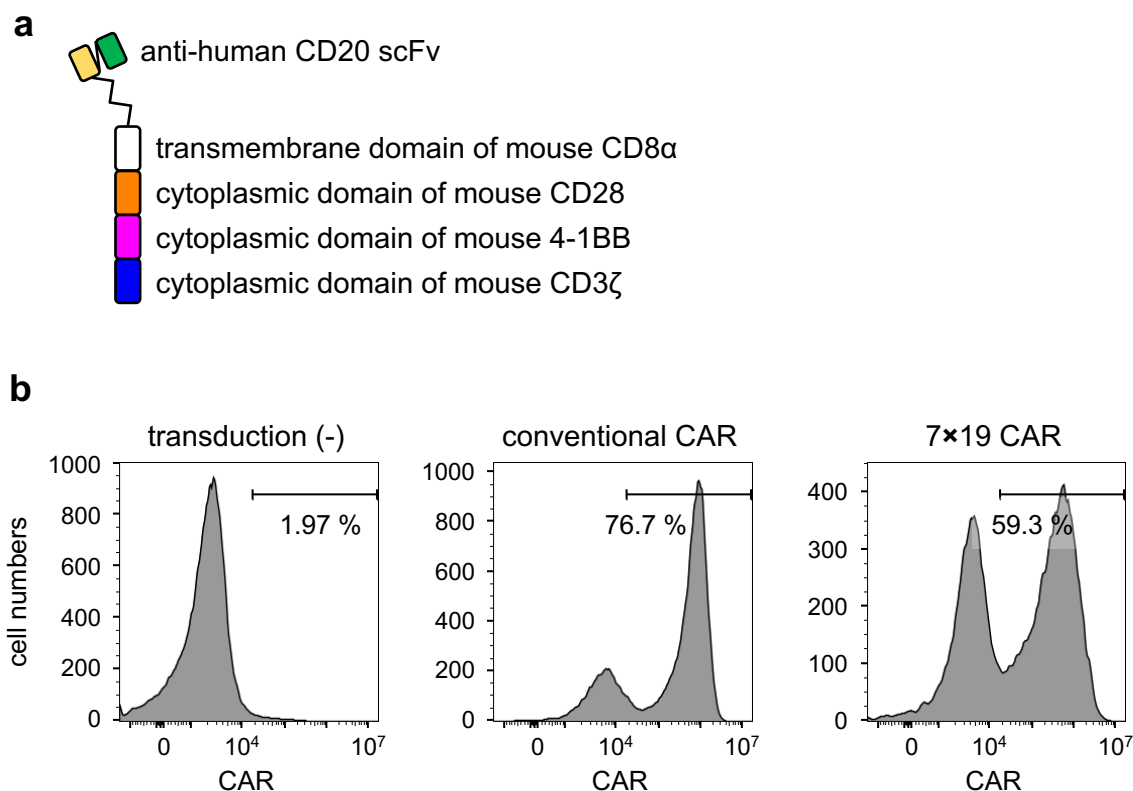
2. Yamazaki C, Sugiyama M, Ohta T, Hemmi H, Hamada E, Sasaki I, Fukuda Y, Yano T, Nobuoka M, Hirashima T, Iizuka A, Sato K, Tanaka T, Hoshino K, Kaisho T (2013) Critical roles of a dendritic cell subset expressing a chemokine receptor, XCR1. *J Immunol* 190:6071-6082. <https://doi.org/10.4049/jimmunol.1202798>
3. Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A (2002) A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat Biotechnol* 20:87-90. <https://doi.org/10.1038/nbt0102-87>
4. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K (2018) IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol* 36:346-351. <https://doi.org/10.1038/nbt.4086>
5. Tamada K, Geng D, Sakoda Y, Bansal N, Srivastava R, Li Z, Davila E (2012) Redirecting gene-modified T cells toward various cancer types using tagged antibodies. *Clin Cancer Res* 18:6436-6445. <https://doi.org/10.1158/1078-0432.ccr-12-1449>
6. Deng W, Yang D, Zhao B, Ouyang Z, Song J, Fan N, Liu Z, Zhao Y, Wu Q, Nashun B, Tang J, Wu Z, Gu W, Lai L (2011) Use of the 2A peptide for generation of multi-transgenic pigs through a single round of nuclear transfer.

PLoS One 6:e19986. <https://doi.org/10.1371/journal.pone.0019986>

7. Kim JH, Lee SR, Li LH, Park HJ, Park JH, Lee KY, Kim MK, Shin BA, Choi SY (2011) High cleavage efficiency of a 2A peptide derived from porcine teschovirus-1 in human cell lines, zebrafish and mice. PLoS One 6:e18556. <https://doi.org/10.1371/journal.pone.0018556>
8. Zheng Z, Chinnasamy N, Morgan RA (2012) Protein L: a novel reagent for the detection of chimeric antigen receptor (CAR) expression by flow cytometry. J Transl Med 10:29. <https://doi.org/10.1186/1479-5876-10-29>
9. Ly LV, Sluijter M, van der Burg SH, Jager MJ, van Hall T (2013) Effective cooperation of monoclonal antibody and peptide vaccine for the treatment of mouse melanoma. J Immunol 190:489-496. <https://doi.org/10.4049/jimmunol.1200135>
10. Lipford GB, Hoffman M, Wagner H, Heeg K (1993) Primary in vivo responses to ovalbumin. Probing the predictive value of the Kb binding motif. J Immunol 150:1212-1222
11. Yadav M, Jhunjunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, Franci C, Cheung TK, Fritsche J, Weinschenk T, Modrusan Z, Mellman I, Lill JR, Delamarre L (2014) Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 515:572-576.

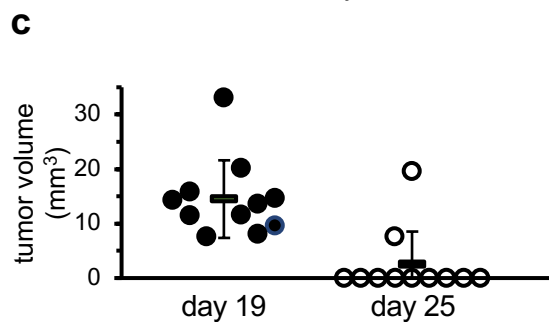
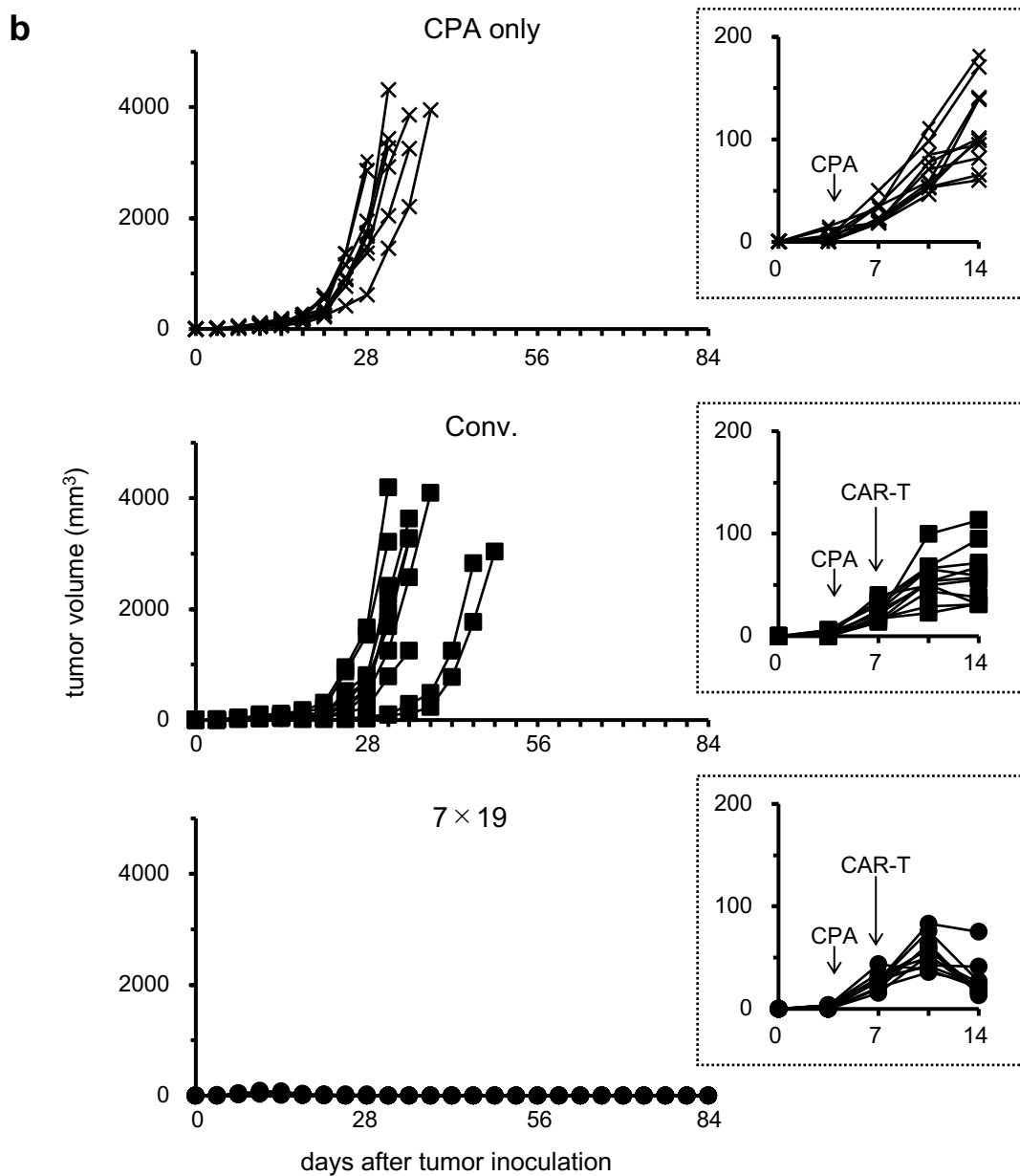
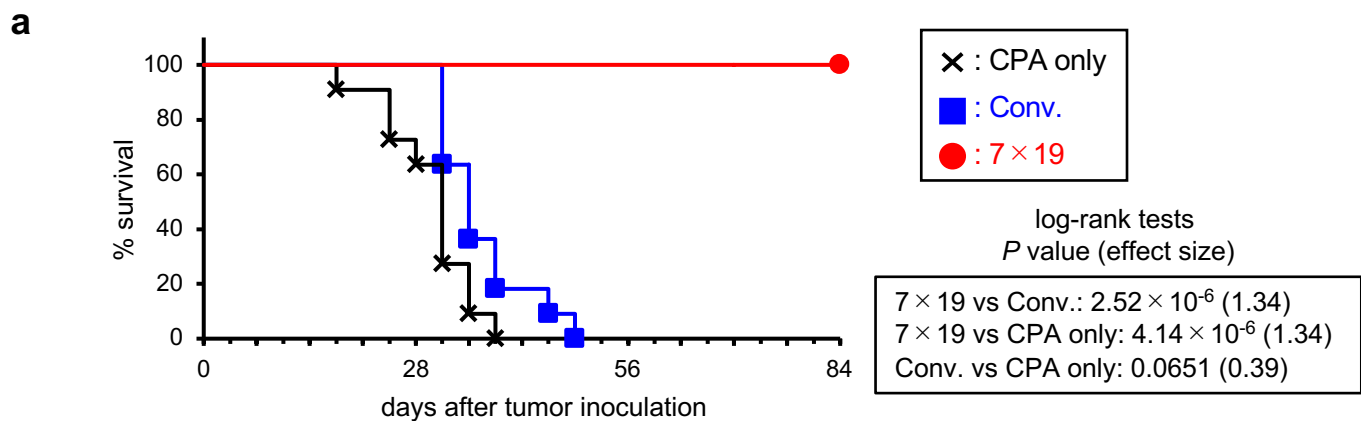
<https://doi.org/10.1038/nature14001>

12. Ramarathinam L, Sarma S, Maric M, Zhao M, Yang G, Chen L, Liu Y (1995)
Multiple lineages of tumors express a common tumor antigen, P1A, but they are
not cross-protected. J Immunol 155:5323-5329



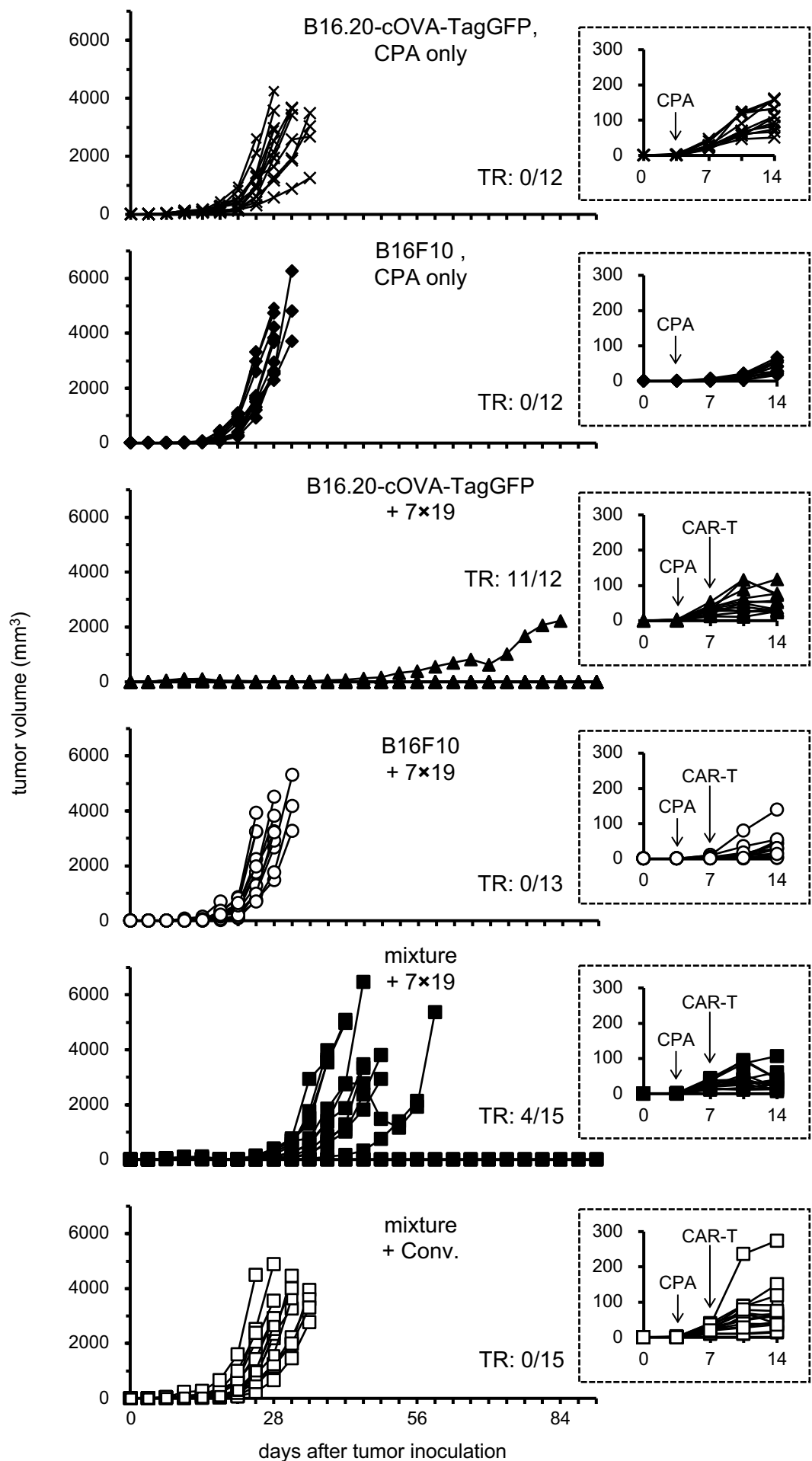
Supplementary Figure 1.

CAR used in this study. (a) Schema of the CAR construct. (b) Mouse T cells were transduced with either a conventional anti-hCD20 or 7×19 anti-hCD20 CAR via retroviral vectors. Flow cytometry confirmed CAR expression, with the histogram values indicating the percentage of CAR-positive cells. Similar results were obtained from ten independent experiments.



Supplementary Figure 2.

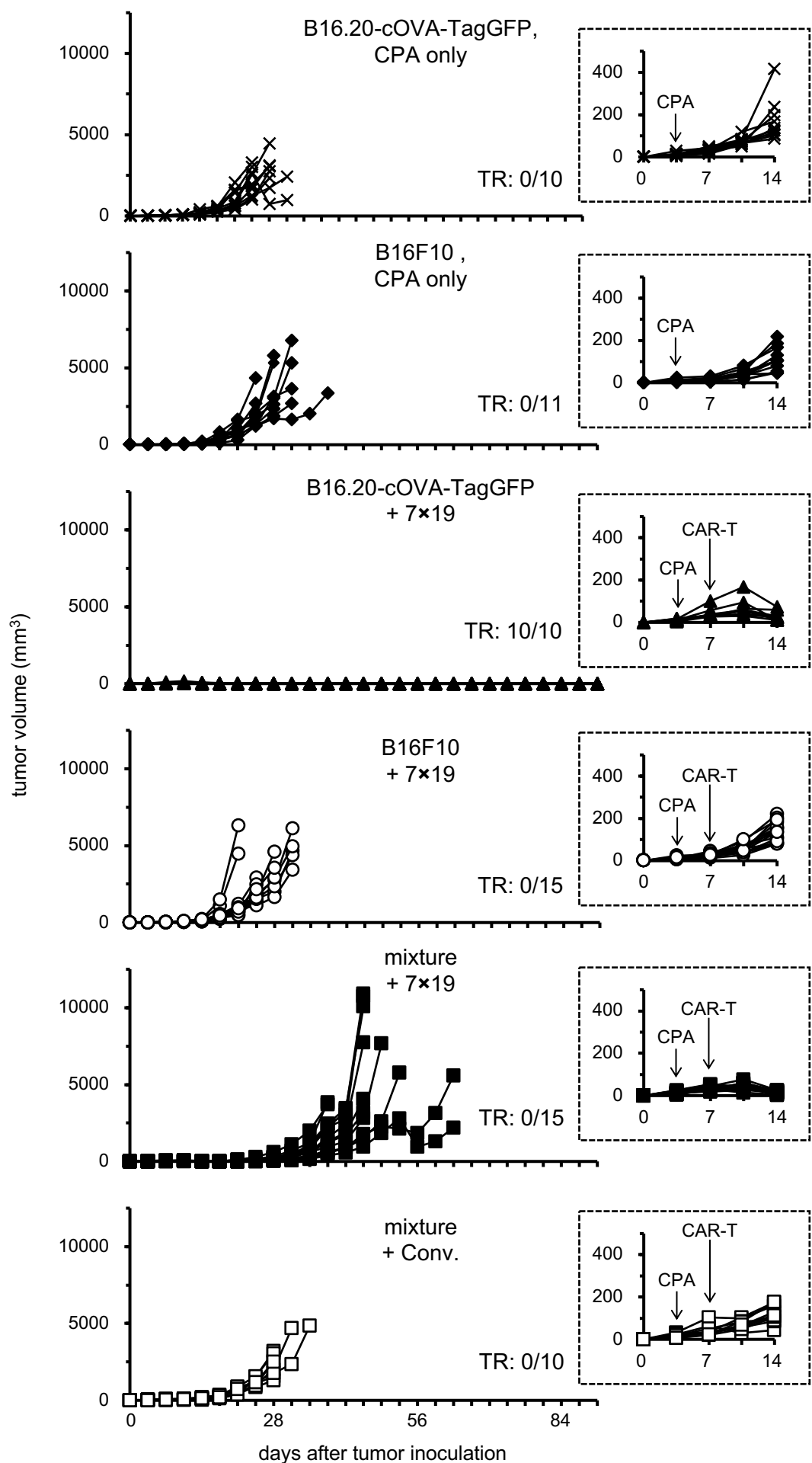
Complete regression of B16.20-cOVA-TagGFP tumors induced by 7×10^6 CAR-T cell treatment. (a–c) C57BL/6 mice were s.c. inoculated with B16.20-cOVA-TagGFP tumors, followed by an i.p. injection of CPA on day 4 and an i.v. infusion of either Conv. or 7×10^6 CAR-T cells on day 7. Control mice received CPA only (CPA only). $n = 11$ mice per group. (a) Mouse survival and (b) individual tumor volumes were monitored, with the insets showing the tumor volumes during the first 14 days. (c) Tumor volumes on days 19 and 25 are shown for the 7×10^6 group; no mice exhibited tumor rejection on day 19, whereas nine mice had complete rejection by day 25. Median survival time (days) and 95% CI were: CPA only, 31 (24–35); Conv., 35 (31–38); 7×10^6 , not reached (NR) (not estimated [NE]–NE). Pairwise P values were calculated using two-sided log-rank tests and adjusted by the Bonferroni method. Numbers in parentheses indicate effect sizes.



Supplementary Figure 3, K. Adachi, K. Tamada

Supplementary Figure 3.

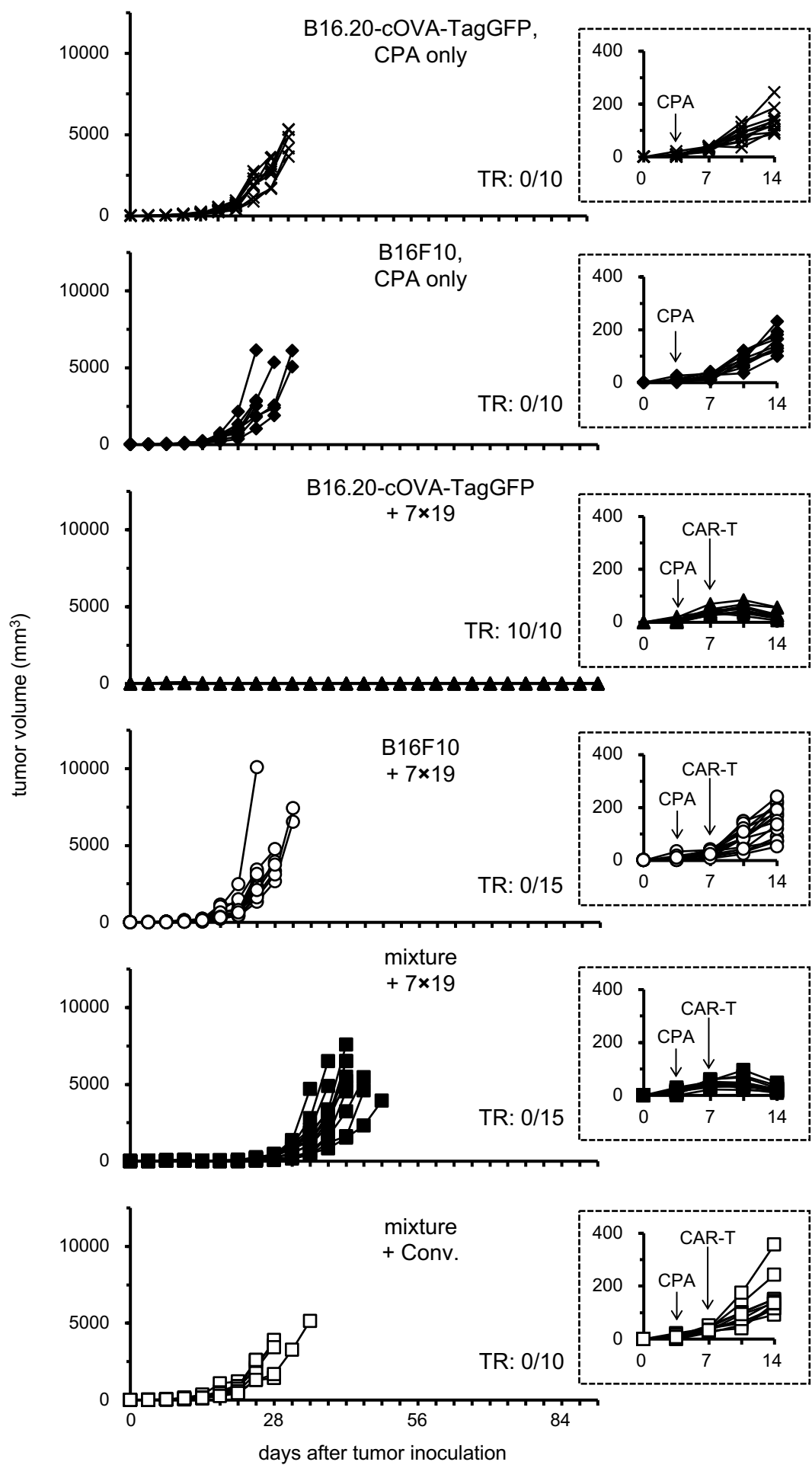
Anti-tumor effects of 7×19 CAR-T cells in mice inoculated with a mixture of B16.20-cOVA-TagGFP and parental B16F10 at an 11.5:1 ratio. C57BL/6 mice were inoculated with B16.20-cOVA-TagGFP plus parental B16F10 (11.5:1 ratio) and treated as shown in Fig. 1a. Treatment groups: mixture (inoculated with both cell lines), CPA only (no CAR-T), Conv. (Conv. CAR-T following CPA), and 7×19 (7×19 CAR-T following CPA). The tumor volumes were monitored, with the first 14 days shown in the inset. The number of mice exhibiting tumor rejection (tumor-rejected [TR], numerator) relative to the total number of mice in each group (denominator) is shown. Pairwise *P*-values (two-sided log-rank analyses, Bonferroni-corrected), together with effect sizes and median survival times with 95% CI are detailed in Suppl. Table 1.



Supplementary Figure 4, K. Adachi, K. Tamada

Supplementary Figure 4.

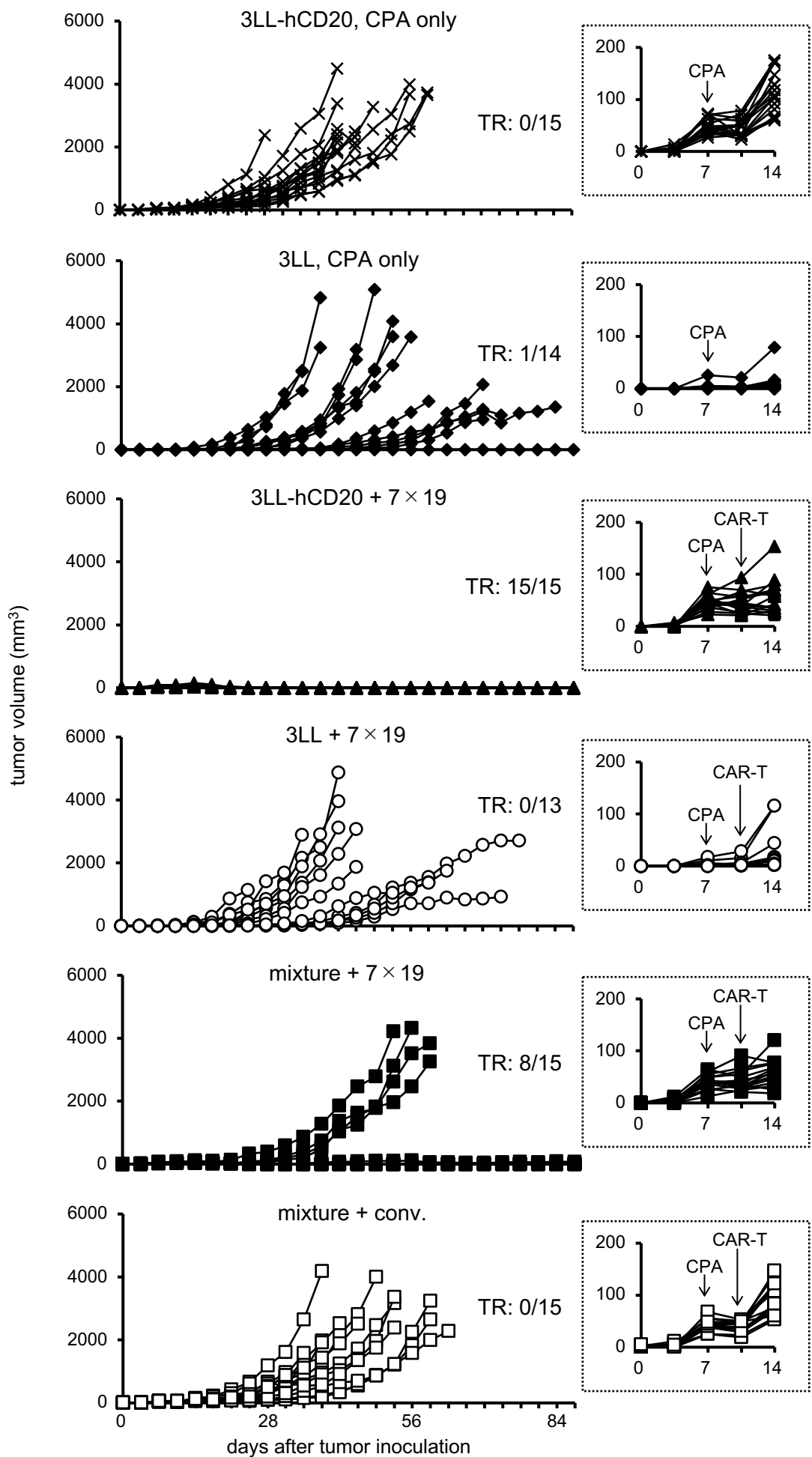
Anti-tumor effects of 7×19 CAR-T cells in mice inoculated with a 3:1 mixture of B16.20-cOVA-TagGFP and parental B16F10. C57BL/6 mice were inoculated with B16.20-cOVA-TagGFP and B16F10 at a 3:1 ratio, as shown in Figure 1b. Treatment groups: mixture (3:1), CPA only, Conv. (Conv. CAR-T + CPA), and 7×19 (7×19 CAR-T + CPA). The tumor volumes were recorded, with the first 14 days shown in the inset. The total mice per group (denominator) and those achieving tumor rejection (TR, numerator) are shown. Pairwise *P*-values from two-sided log-rank analyses adjusted by the Bonferroni method, together with effect sizes and median survival times with 95% CI are in Suppl. Table 2.



Supplementary Figure 5, K. Adachi, K. Tamada

Supplementary Figure 5.

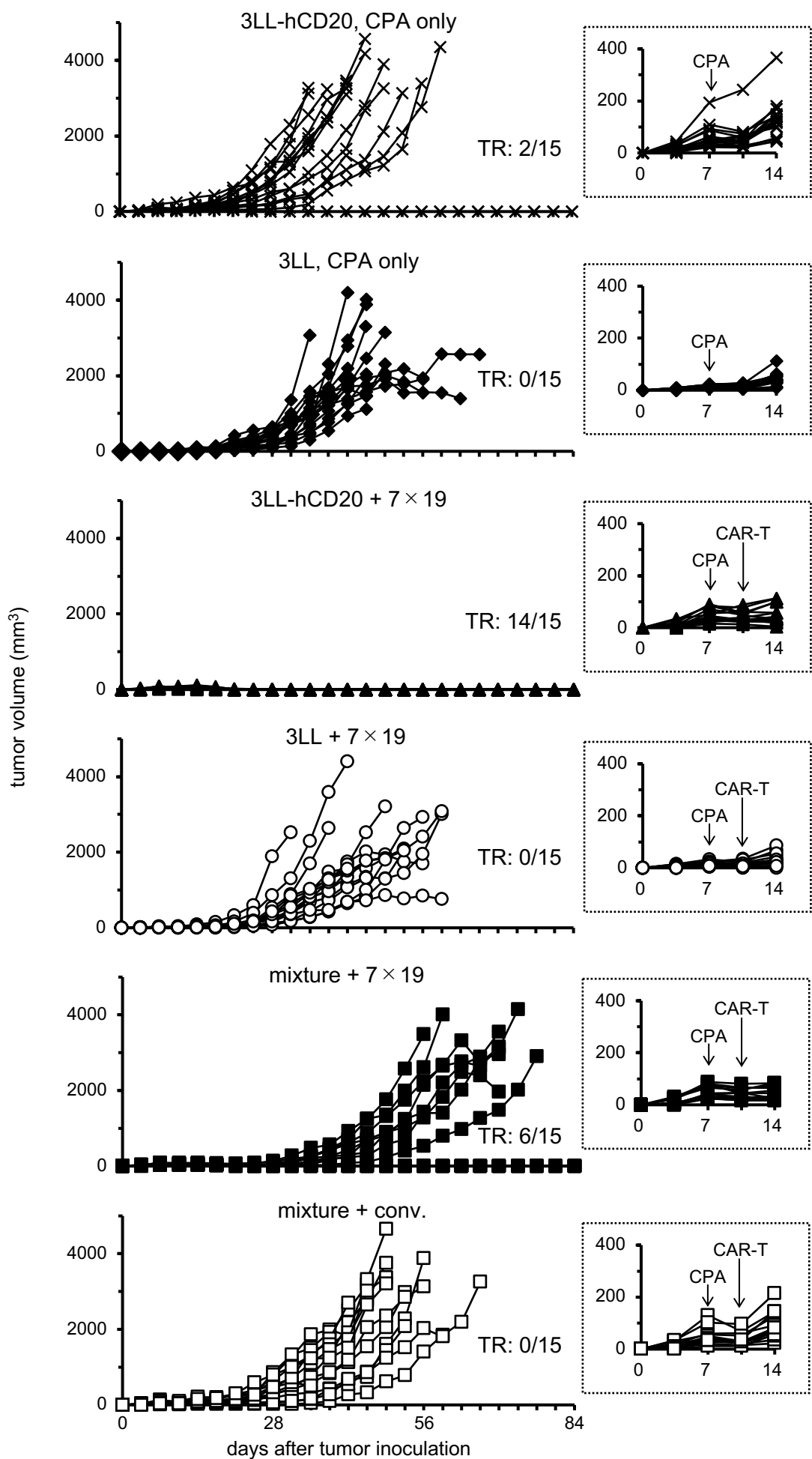
Anti-tumor effects of 7×10^6 CAR-T cells in mice inoculated with a 1:1 mixture of B16.20-cOVA-TagGFP and parental B16F10. C57BL/6 mice were inoculated with a 1:1 mixture of B16.20-cOVA-TagGFP and parental B16F10 cells, then treated as described in Fig. 1c. Groups were: mixture, CPA only, Conv. (Conv. CAR-T post-CPA), and 7×10^6 (7×10^6 CAR-T post-CPA). The tumor volumes were monitored, with data from the first 14 days shown in the inset. Numbers in the graphs denote the total mice (denominator) and those rejecting tumors (TR, numerator). Pairwise *P*-values from log-rank tests (Bonferroni-adjusted), together with effect sizes and median survival times with 95% CI are included in Suppl. Table 3.



Supplementary Figure 6, K. Adachi, K. Tamada

Supplementary Figure 6.

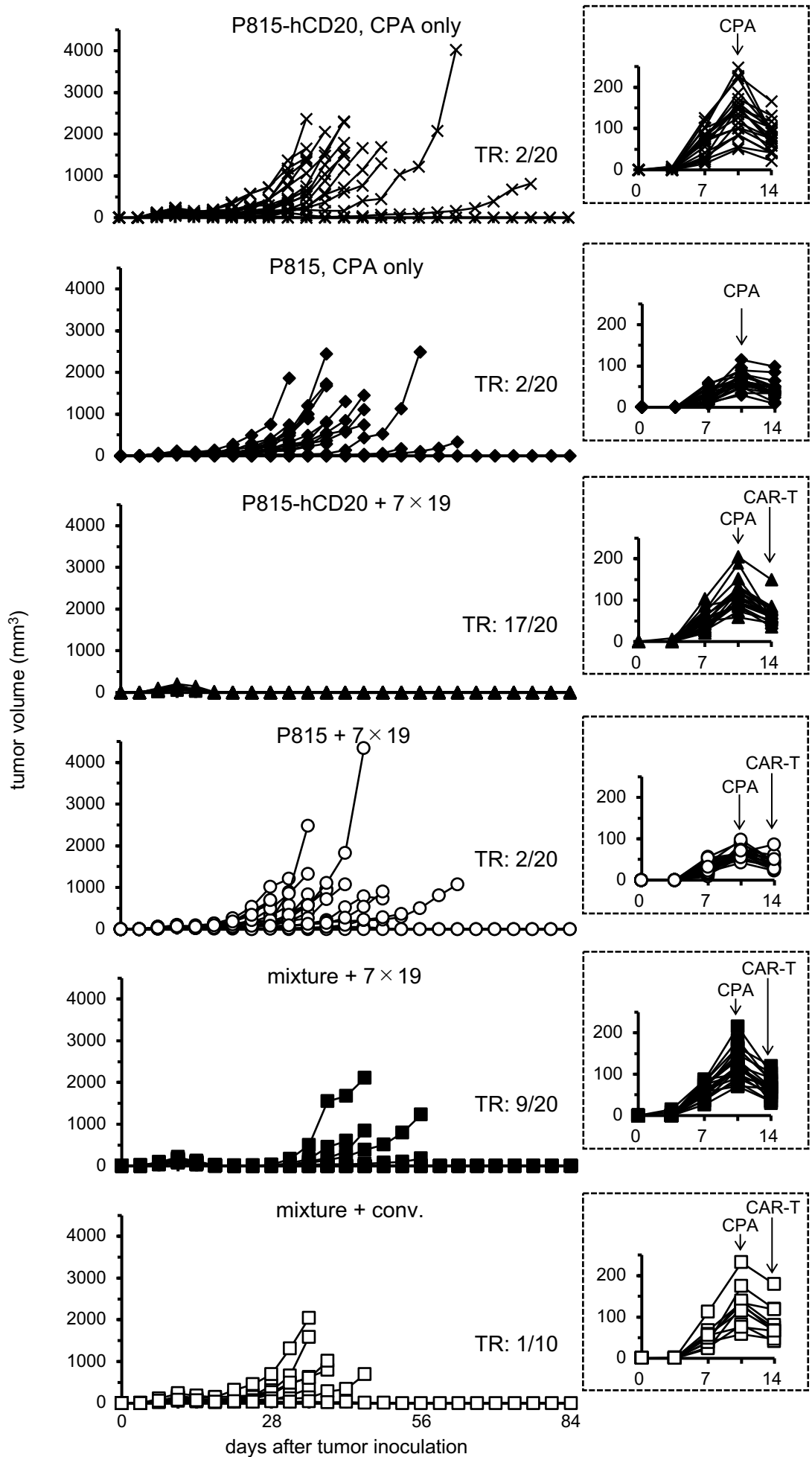
Anti-tumor effects of 7×19 CAR-T cells in mice inoculated with a mixture of 3LL-hCD20 and parental 3LL at an 11.5:1 ratio. C57BL/6 mice were s.c. inoculated with 3LL-hCD20, parental 3LL, or an 11.5:1 mixture ("mixture"), as shown in Fig. 1d. CPA was administered i.p. before i.v. infusion of either Conv. or 7×19 CAR-T cells. Control mice (CPA only) received no CAR-T therapy. The tumor volumes were measured, with the first 14 days in the inset. The total number of mice (denominator) and those rejecting tumors (TR, numerator) are indicated. Pairwise *P*-values from log-rank tests adjusted by the Bonferroni method, together with effect sizes and median survival times (95% CI) appear in Suppl. Table 4.



Supplementary Figure 7, K. Adachi, K. Tamada

Supplementary Figure 7.

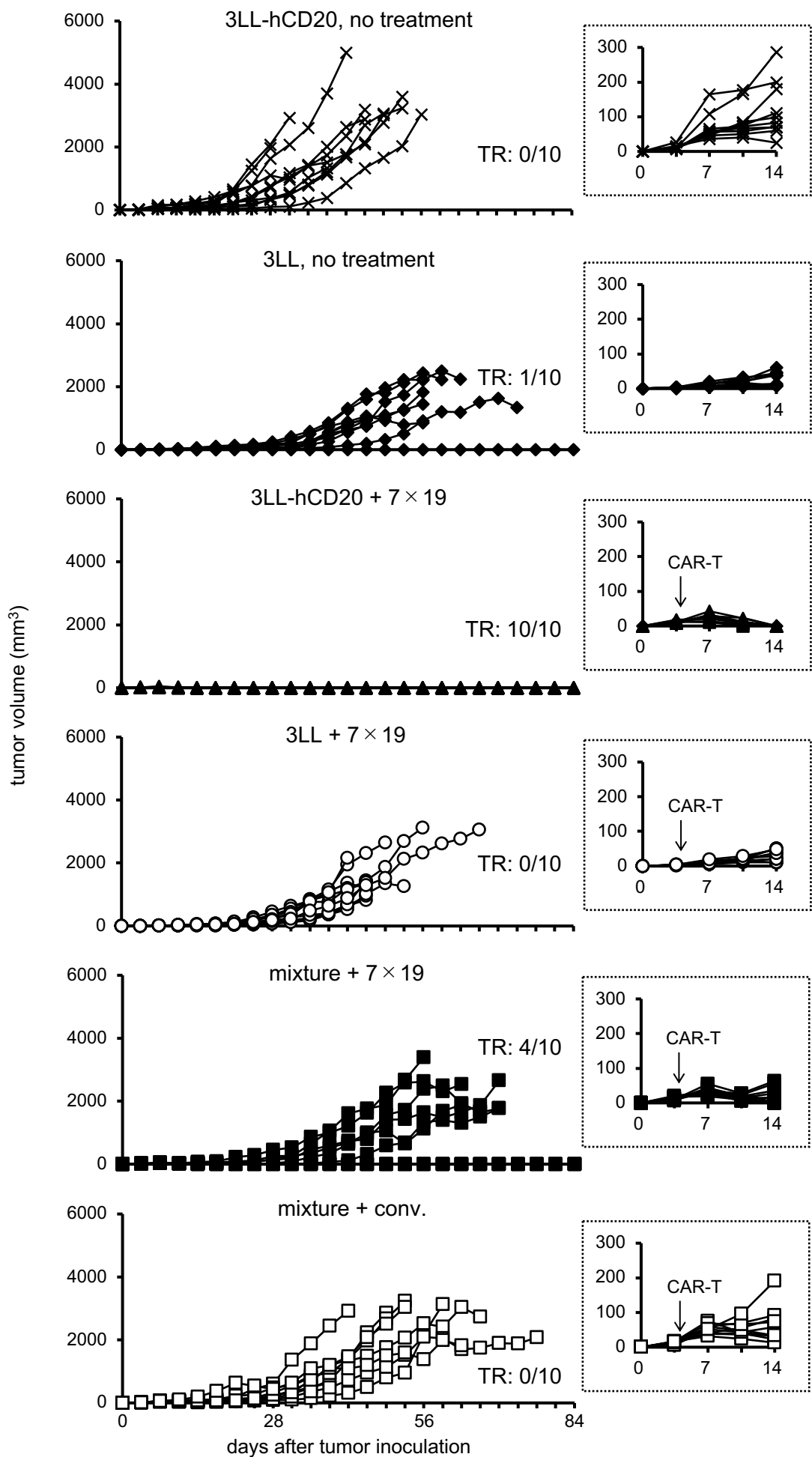
Anti-tumor effects of 7×19 CAR-T cells in mice inoculated with a 3:1 mixture of 3LL-hCD20 and parental 3LL. C57BL/6 mice were s.c. inoculated with 3LL-hCD20, parental 3LL, or a 3:1 mixture ("mixture"), according to Fig. 1e. Following CPA injection (i.p.), mice received either Conv. or 7×19 CAR-T cells (i.v.). Control mice (CPA only) did not receive CAR-T cells. Tumor volumes were monitored, with the first 14 days shown in the inset. The Figs. present total mice (denominator) and those rejecting tumors (TR, numerator). Pairwise *P* values from log-rank tests (Bonferroni-adjusted), together with effect sizes, median survival times, and 95% CI, are detailed in Suppl. Table 5.



Supplementary Figure 8, K. Adachi, K. Tamada

Supplementary Figure 8.

Anti-tumor effects of 7×19 CAR-T cells in mice inoculated with a P815-hCD20 and parental P815 mixture. DBA/2 mice were s.c. inoculated with either P815-hCD20, parental P815, or a 5:1 mixture of these cells ("mixture"), as outlined in Fig. 1f. After tumor inoculation, mice received an i.p. injection of CPA, followed by an i.v. infusion of either Conv. or 7×19 anti-hCD20 CAR-T cells (7×19). Control mice (CPA only) received no further treatment. The tumor volumes were monitored, with data for the first 14 days shown in the insets. In each graph, the total number of mice per group (denominator) and the number achieving tumor rejection (tumor-rejected: TR, numerator) are indicated. Pairwise *P*-values from log-rank tests adjusted with the Bonferroni method, together with effect sizes and median survival times (95% CI) appear in Suppl. Table 6.

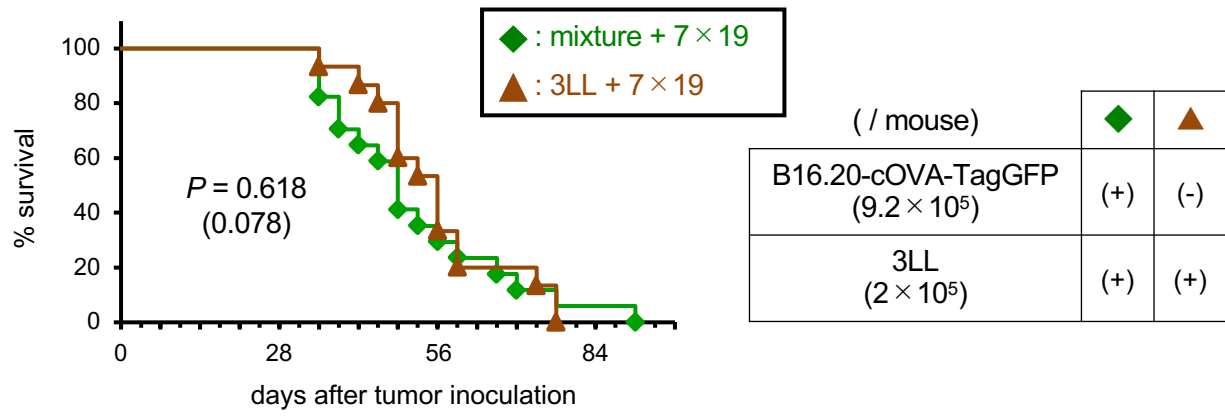


Supplementary Figure 9, K. Adachi, K. Tamada

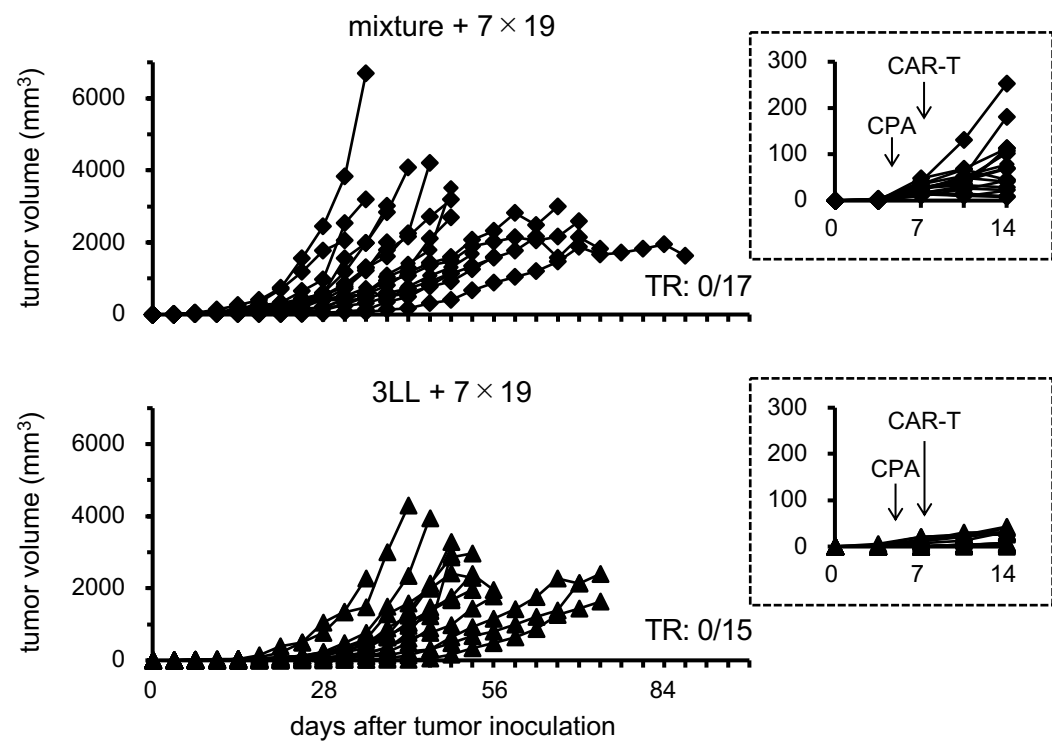
Supplementary Figure 9.

Anti-tumor effects of 7×19 CAR-T cells in mice inoculated with a mixture of 3LL-hCD20 and parental 3LL without CPA pretreatment. C57BL/6 mice were s.c. inoculated with 3LL-hCD20, parental 3LL, or a 3:1 mixture of these cells (“mixture”), as described in Fig. 1g. Mice then received either Conv. or 7×19 anti-hCD20 CAR-T cells (7×19) i.v. without CPA-induced lymphodepletion. Control mice received no post-inoculation treatment (no treatment). The tumor volumes were measured, with data from the first 14 days shown in the insets. The numbers in the graphs represent total mice per group (denominator) and those that rejected the tumor (tumor-rejected: TR, numerator). Pairwise *P*-values from log-rank tests (Bonferroni-adjusted), together with effect sizes and median survival times (95% CI) are listed in Suppl. Table 7.

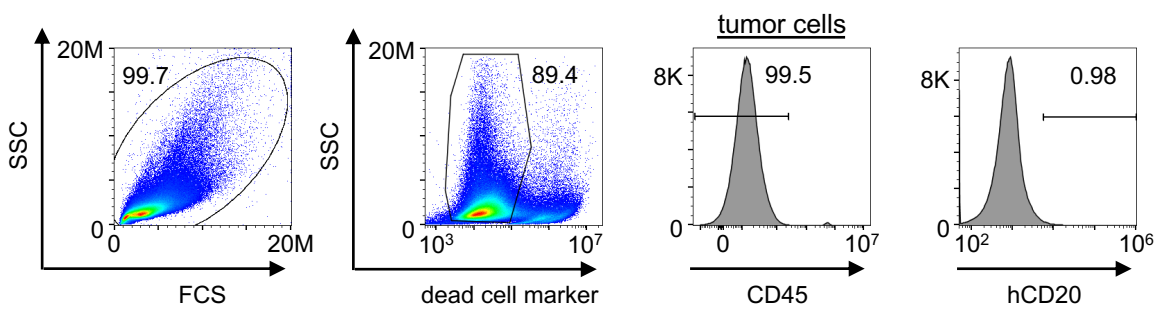
a



b

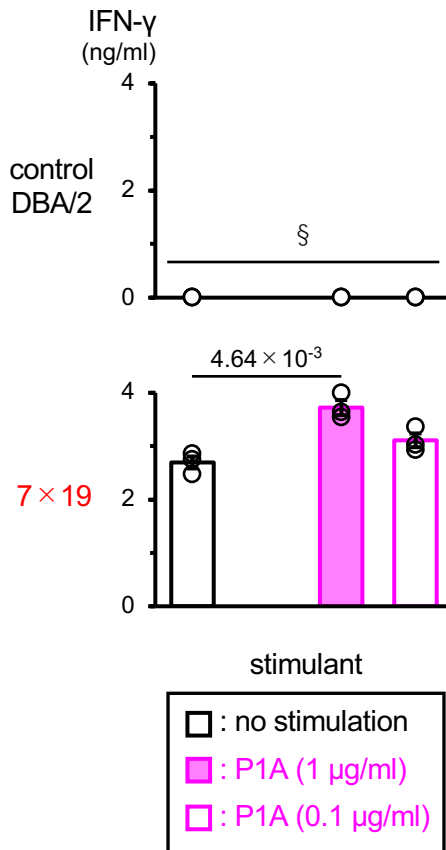


c



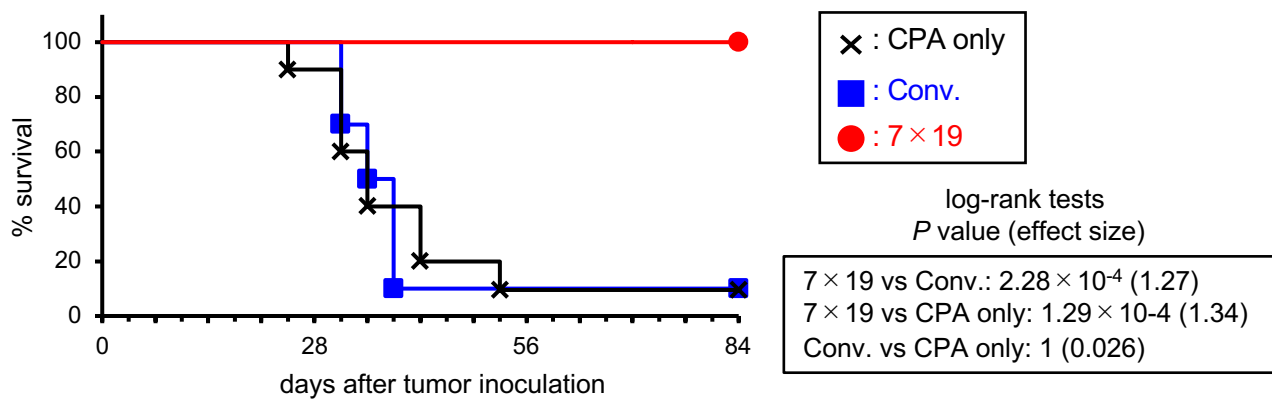
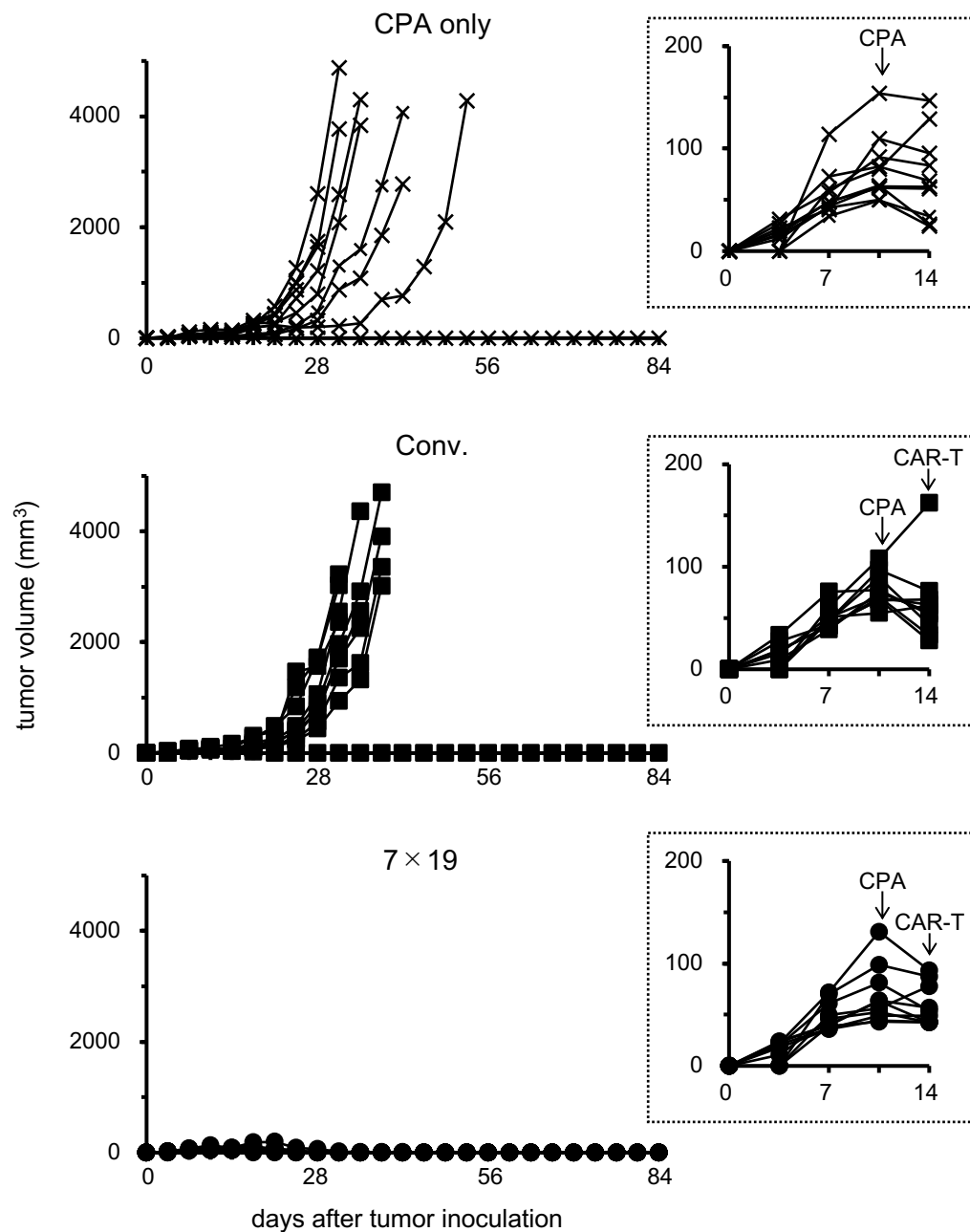
Supplementary Figure 10.

Requirement for shared antigens between CAR target-positive and -negative tumors in the antitumor efficacy mediated by 7×19 CAR-T cell-induced epitope spreading. (a, b) C57BL/6 mice were s.c. inoculated with either 3LL cells alone or a 4.6:1 mixture ("mixture") of B16.20-cOVA-TagGFP (9.2×10^5 cells) and 3LL (2×10^5 cells), matching the cell numbers used in Fig. 1. Mice received CPA (i.p.) on day 4, followed by 7×19 anti-hCD20 CAR-T cells (7×19) on day 7 (i.v.). (a) Mouse survival and (b) tumor volumes were tracked; insets show the initial 14-day tumor volume data. Median survival times (days) with 95% CI were as follows: mixture + 7×19 : 49 (38–59); 3LL + 7×19 : 56 (45–59). (b) The numbers in the graphs denote the total mice per group (denominator) and those that rejected the tumor (tumor-rejected: TR, numerator). Pairwise *P* value was calculated using two-sided log-rank test. Number in parentheses indicates effect size. (c) Tumors from mice inoculated with the mixture were harvested post- 7×19 treatment, digested, and analyzed by flow cytometry. Tumor cells were identified as CD45⁺ live cells (third column from the left), and hCD20 expression was examined (rightmost column). Representative data from one of five mice are shown.



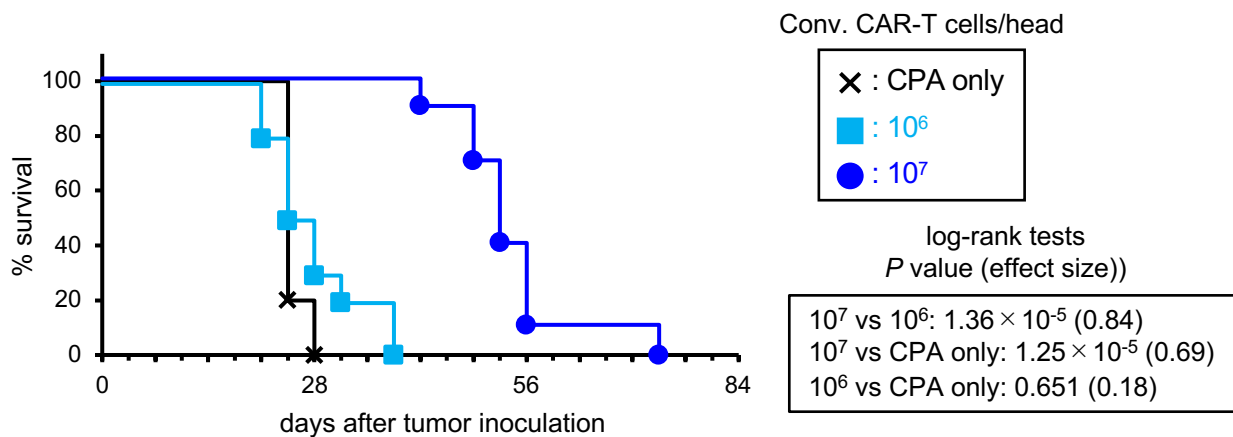
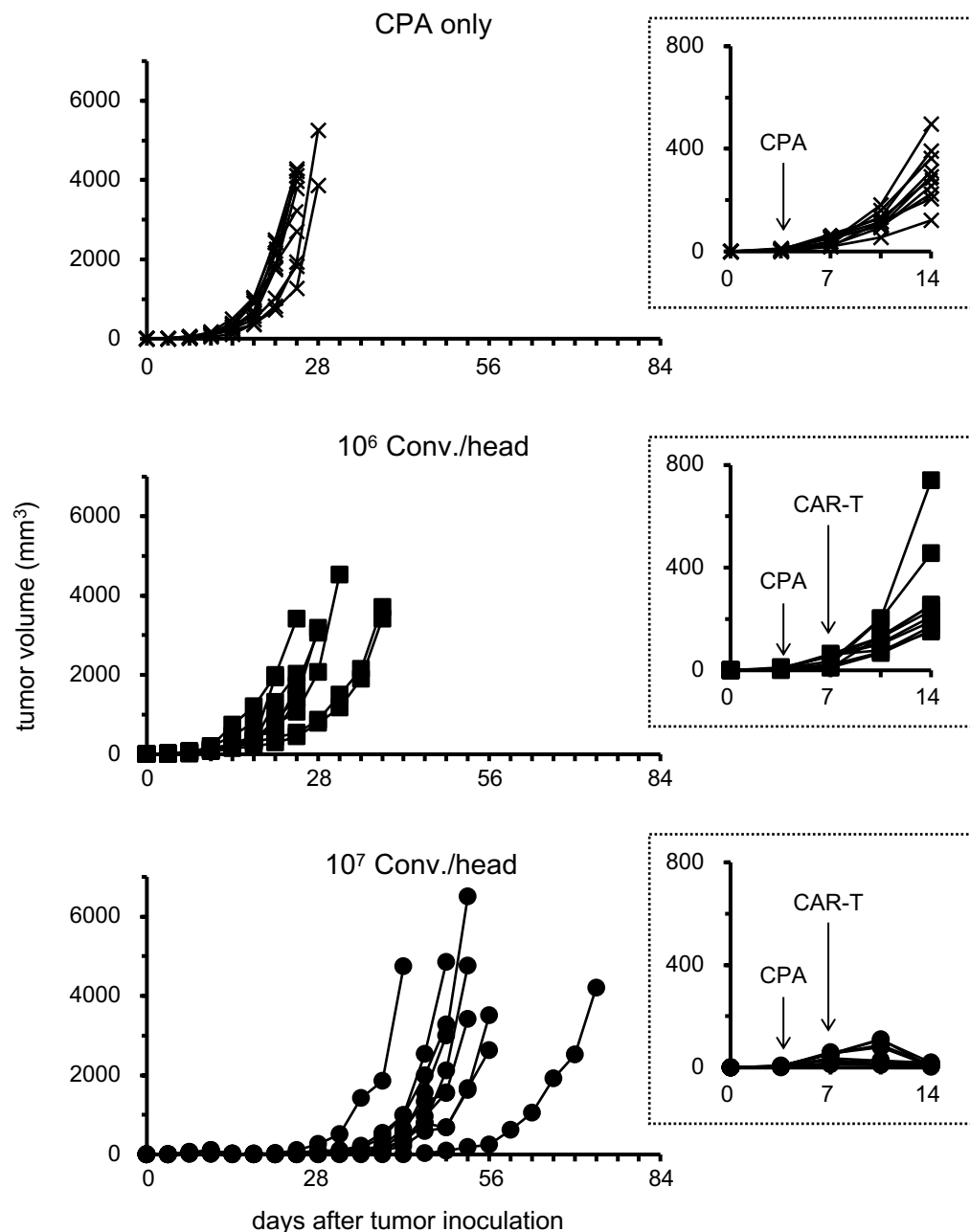
Supplementary Figure 11.

Induction of P1A-specific T cells after 7×19 CAR-T cell treatment. Experimental details follow Suppl. Fig. 8. Single-cell suspensions were prepared from the spleen and tumor-draining lymph nodes (TDLNs) of mice that rejected the 5:1 P815 mixture following 7×19 CAR-T cell treatment (7×19 group). Cells were stimulated with 1 µg/mL (filled bar) or 0.1 µg/mL (blank bar) of P1A peptide. Control cell suspensions were obtained from the spleen and lymph nodes (LNs) of naïve DBA/2 mice. IFN-γ concentrations in the supernatants were measured by ELISA and compared with unstimulated controls. Data are expressed as mean $\pm$ SEM, with each open circle representing a single well. § denotes values below the detection limit. *P*-values were calculated by two-sided *t*-tests. $n = 4$ for the 7×19 group, $n = 3$ for the control group. Representative data from each group are shown.

a**b**

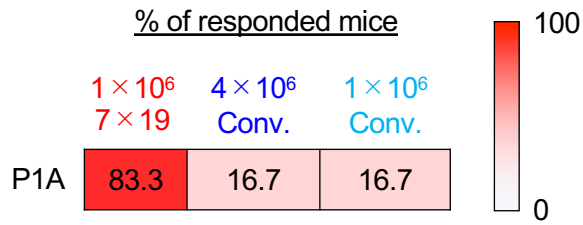
Supplementary Figure 12.

Complete regression of MC38-hCD20 tumors induced by 7×19 CAR-T cell treatment. (a, b) C57BL/6 mice were s.c. inoculated with MC38-hCD20 cells, treated with CPA (i.p.) on day 11, and received either Conv. or 7×19 CAR-T cells (i.v.) on day 14. Control mice received CPA only. (a) The survival time and (b) individual tumor volumes were tracked; the insets in (b) show tumor volumes during the first 14 days. Median survival times (days) with 95% CI were: CPA only, 35 (24–42); Conv., 36.5 (31–38); and 7×19 , NR (NE–NE). Pairwise *P* values were calculated using two-sided log-rank tests and adjusted by the Bonferroni method. Numbers in parentheses indicate effect sizes. *n* = 10 mice per group.

a**b**

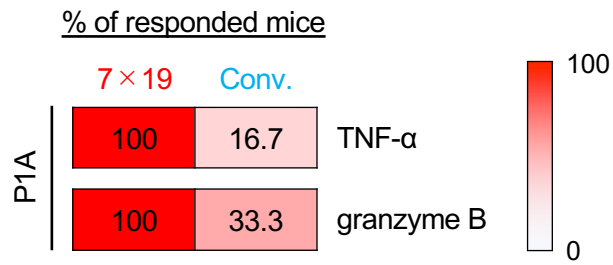
Supplementary Figure 13.

Increased doses of conventional CAR-T cells prolong survival in B16.20-cOVA-TagGFP-inoculated mice. (a, b) C57BL/6 mice were inoculated s.c. with B16.20-cOVA-TagGFP cells, treated with CPA (i.p.) on day 4, and infused (i.v.) on day 7 with either 1×10^6 (10^6) or 1×10^7 (10^7) conventional anti-hCD20 CAR-T cells. Control mice received CPA only. (a) Survival and (b) individual tumor volumes were assessed; the insets highlight tumor volumes over the first 14 days. Median survival times (days) with 95% CI were: CPA only, 24 (NE–NE); 10^6 , 26 (21–31); 10^7 , 52 (42–56). Pairwise *P* values were calculated using two-sided log-rank tests and adjusted by the Bonferroni method. Numbers in parentheses indicate effect sizes. *n* = 10 mice per group.



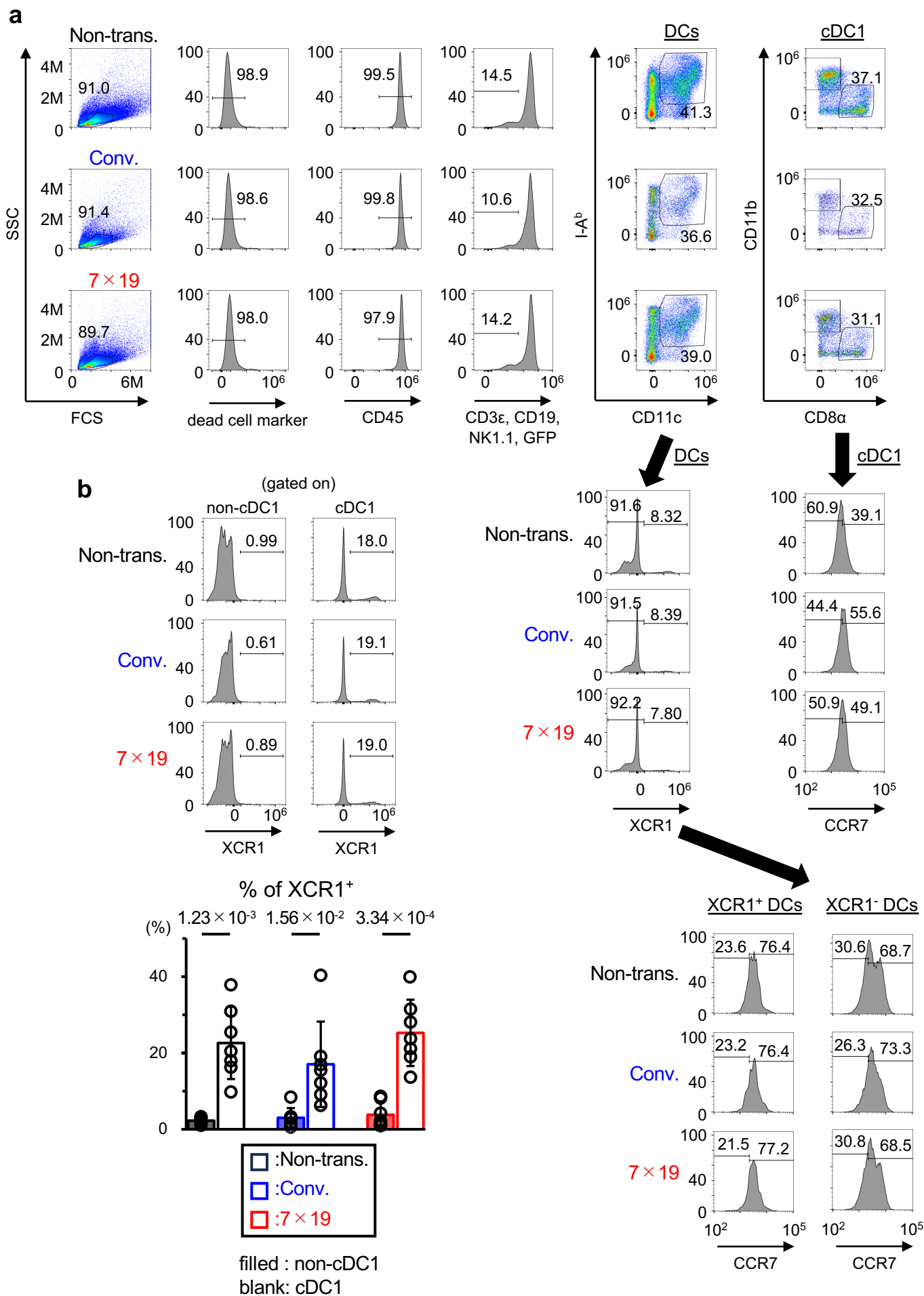
Supplementary Figure 14.

Extent of epitope spreading in P815-hCD20 tumor-bearing mice following high-dose conventional CAR-T cell treatment. DBA/2 mice were s.c. inoculated with P815-hCD20 cells. On day 11, mice received CPA, followed on day 14 by either Conv. or 7×19 CAR-T cells at 1×10^6 cells/mouse or 4×10^6 cells/mouse (high-dose Conv.). Spleens and tumor-draining lymph nodes were collected on days 26–28, by which time both the high-dose Conv. and 7×19 groups had rejected tumors. Single-cell suspensions were stimulated with P1A peptide, and IFN- γ production was quantified by ELISA. The tables indicate the percentage of mice reactive to P1A in each group. $n = 6$ for all groups.



Supplementary Figure 15

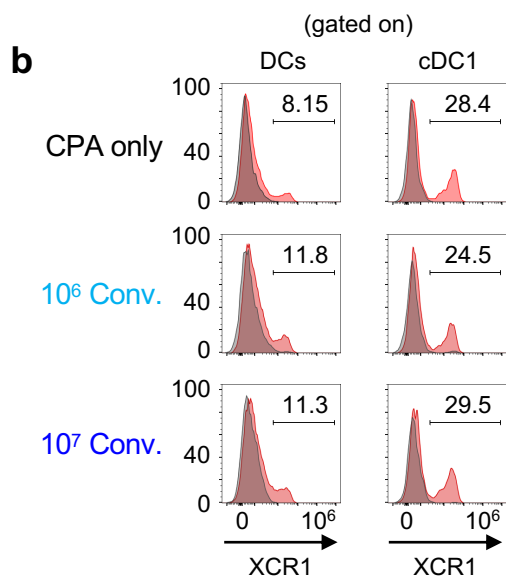
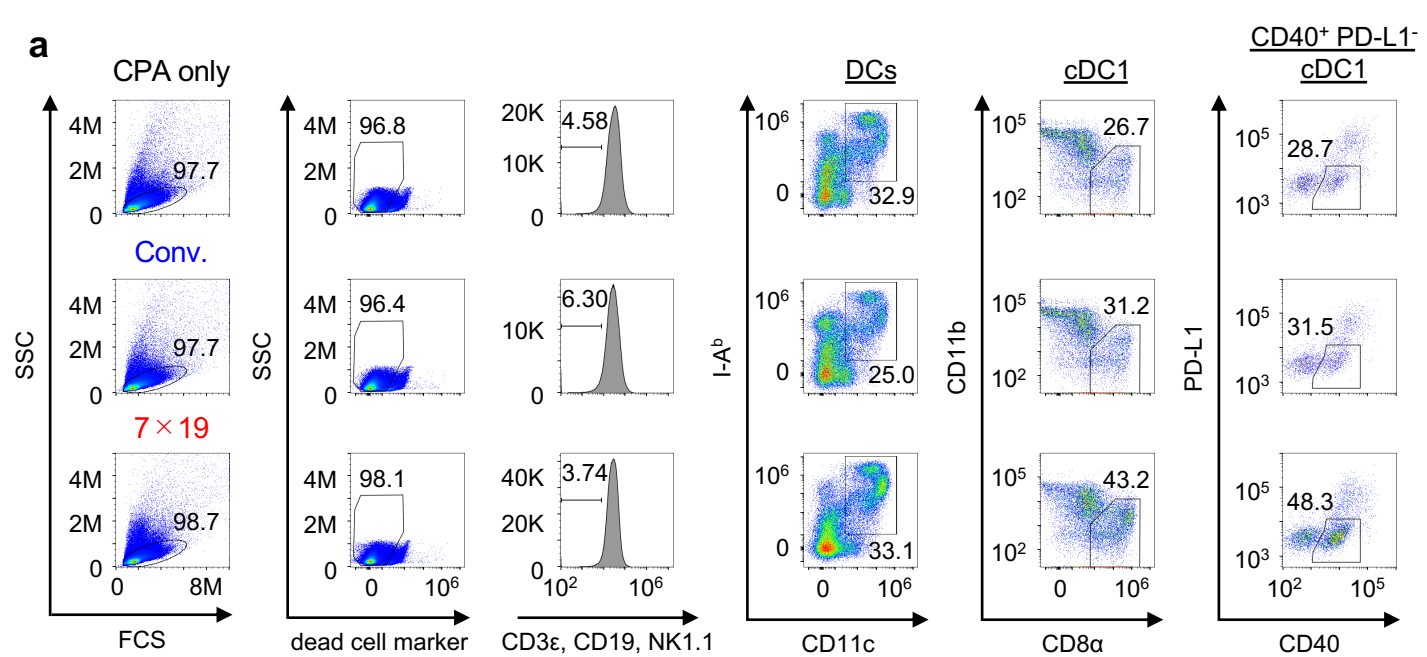
Evaluation of epitope spreading using indicators other than IFN- γ . DBA/2 mice were inoculated s.c. with P815-hCD20. Mice were treated with CPA and either Conv. or 7×19 CAR-T cells (1×10^6 cells/mouse) on day 11 and 14, respectively. Single-cell suspensions were prepared with spleens and tumor-draining lymph nodes on days 26–28, and were stimulated with P1A peptide. TNF- α and granzyme B production was measured by ELISA. The tables indicate the percentage of mice reactive to P1A in each group. $n = 6$ for both groups.



Supplementary Figure 16, K. Adachi, K. Tamada

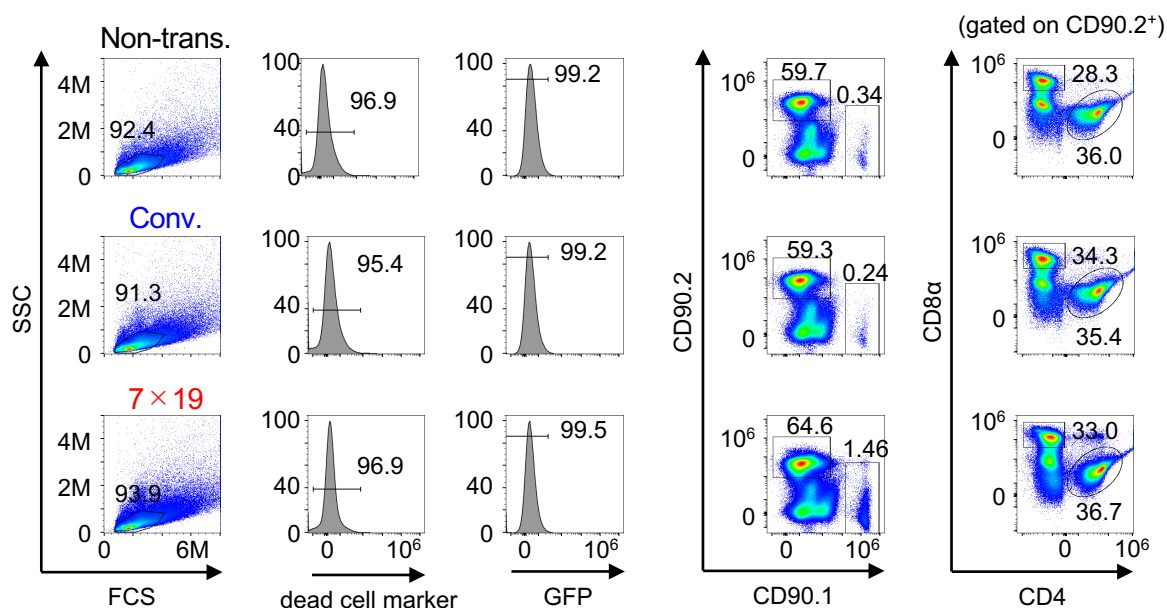
Supplementary Figure 16.

Flow cytometric analysis of cross-presenting DCs in tumor-draining lymph nodes, corresponding to Fig. 3a and 3b. (a, b) C57BL/6 mice were s.c. inoculated with B16.20-cOVA-TagGFP, treated with CPA (i.p.) on day 10, and infused (i.v.) on day 13 with either Conv. or 7×19 anti-hCD20 CAR-T cells (7×19), both derived from CD90.1⁺ CD90.2⁻ luciferase⁺ congenic C57BL/6 donors. Control mice received non-transduced T cells (Non-trans.) after CPA treatment. On day 20 or 21, tumor-draining lymph nodes were excised, enzymatically digested, and analyzed by flow cytometry. DCs were defined as CD11c⁺ I-A^b⁺ cells within the CD45⁺ CD3ε⁻ CD19⁻ NK1.1⁻ live cell population (fifth column from the left). Subsets were further characterized by CD8α, CD11b, CCR7, and XCR1. Cross-presenting cDC1 and non-cDC1 subsets were defined as CD8α⁺ CD11b⁻ and CD8α⁻ CD11b⁺, respectively, as shown in the rightmost columns. (b) XCR1 expression in cDC1 (open bars) and non-cDC1 (filled bars) are shown as mean $\pm$ SD; each open circle denotes an individual mouse. *P*-values were calculated with two-sided Student's *t*-tests. Representative data from one of seven mice are shown.



Supplementary Figure 17.

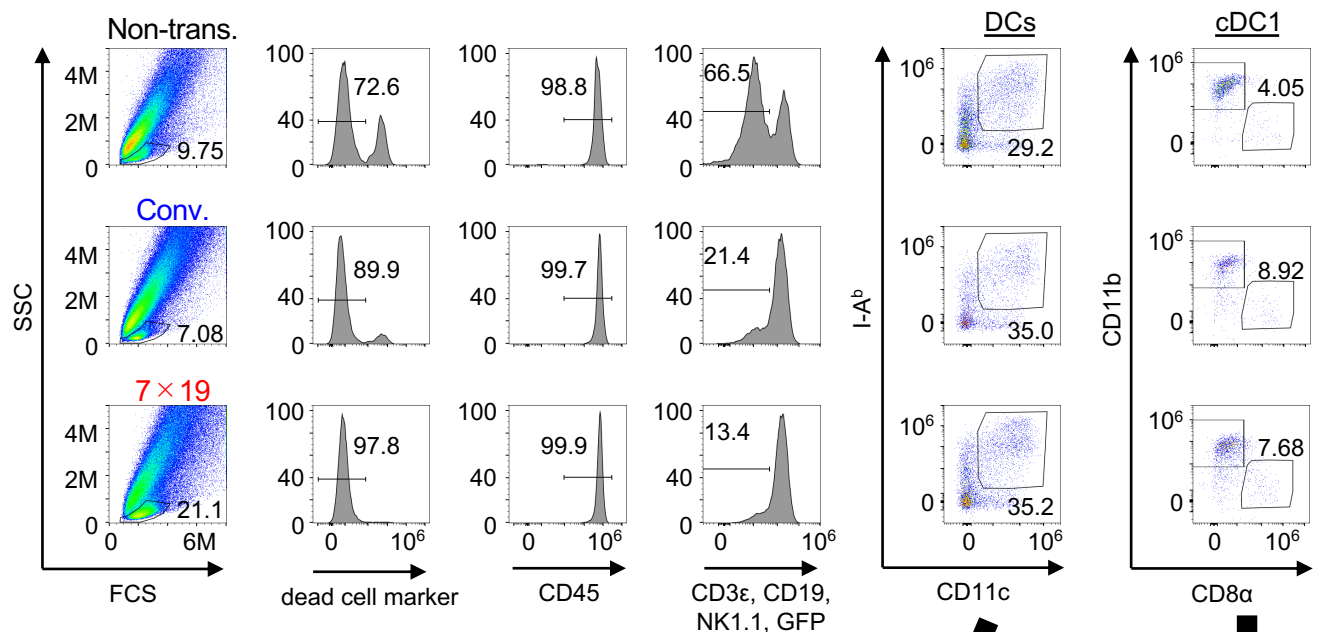
Flow cytometric analysis of cross-presenting DCs in tumor-draining lymph nodes, corresponding to Fig. 3c and 3d. Tumor inoculation and treatment details appear in the legends for Suppl. Figs. 2 and 13. As a control, mice received CPA alone. Tumor-draining lymph nodes were collected on day 27 (a) or days 25–27 (b), enzymatically digested, and processed for flow cytometry. DCs were identified as CD11c⁺ I-A^b⁺ cells within the live CD3ε⁻ CD19⁻ NK1.1⁻ fraction (a, b). In (a), additional markers included CD8α, CD11b, CD40, and PD-L1; (b) we also examined XCR1. cDC1 were defined as the CD8α⁺ CD11b⁻ subset (a). Percentages within each gate are indicated in dot plots or histograms. Representative data are shown.



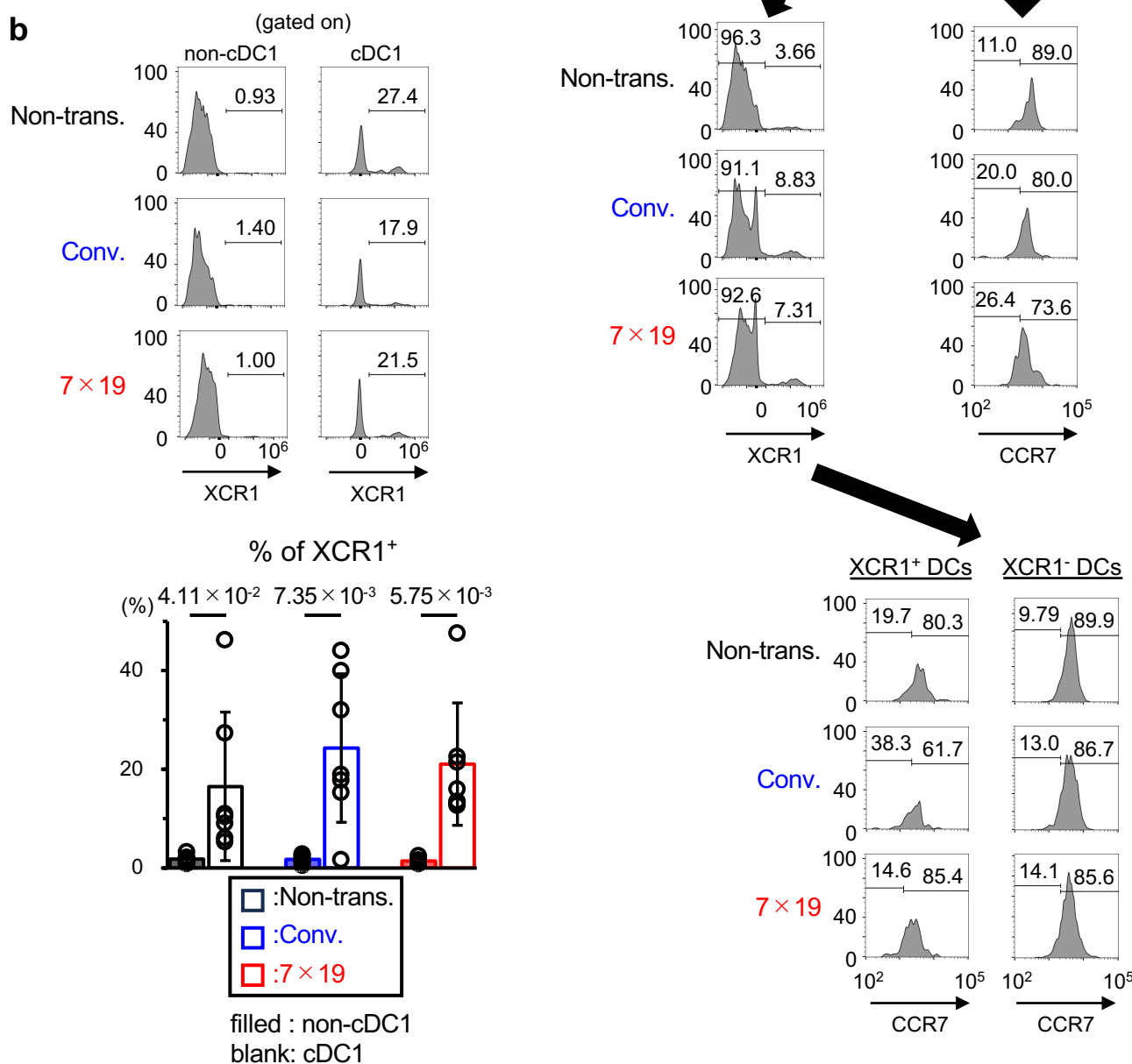
Supplementary Figure 18.

Flow cytometric analysis of T cells in tumor-draining lymph nodes, corresponding to Fig. 3e and 3f. See Suppl. Fig. 16 for tumor inoculation and treatment protocols. Single-cell suspensions of tumor-draining lymph nodes were analyzed by flow cytometry. Donor T cells (CD90.1⁺) and recipient T cells (CD90.2⁺) are shown in the fourth column from the left, with further gating on CD8α and CD4. Percentages in each gate appear on the dot plots or histograms. Data from one representative mouse out of seven are shown.

a

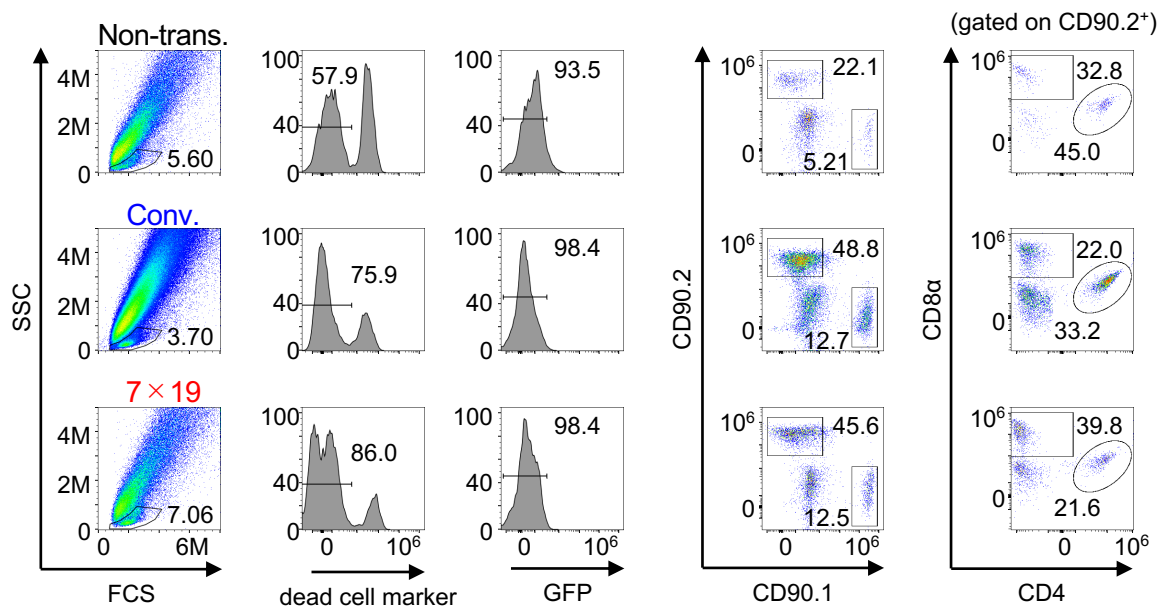


b



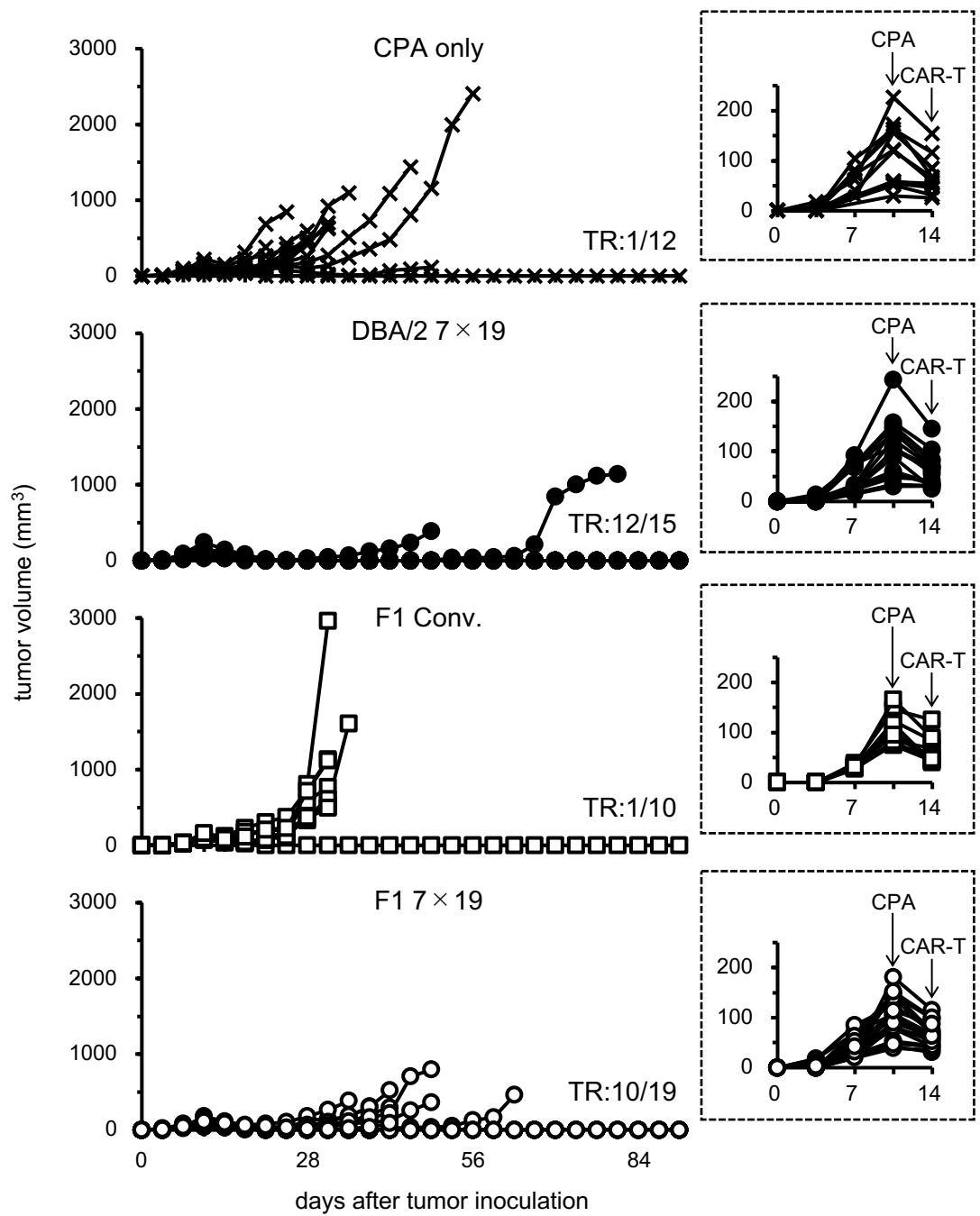
Supplementary Figure 19.

Flow cytometric analysis of cross-presenting DCs in tumor tissue, corresponding to Fig. 4a and 4b. (a, b) Following the procedures outlined in Suppl. Fig. 16, tumor tissues were excised, enzymatically digested, and enriched for CD45⁺ cells using MicroBeads to generate single-cell suspensions. Gating strategies are described in Suppl. Fig. 16a. (b) The percentages of XCR1-positive cells in cDC1 (open bars) and non-cDC1 (filled bars) are shown as mean $\pm$ SD, with each open circle representing an individual mouse. Two-sided, unpaired Student's *t*-tests were used to determine *P*-values. Percentages in each gate are indicated in dot plots or histograms. Representative data from one of seven mice are displayed.



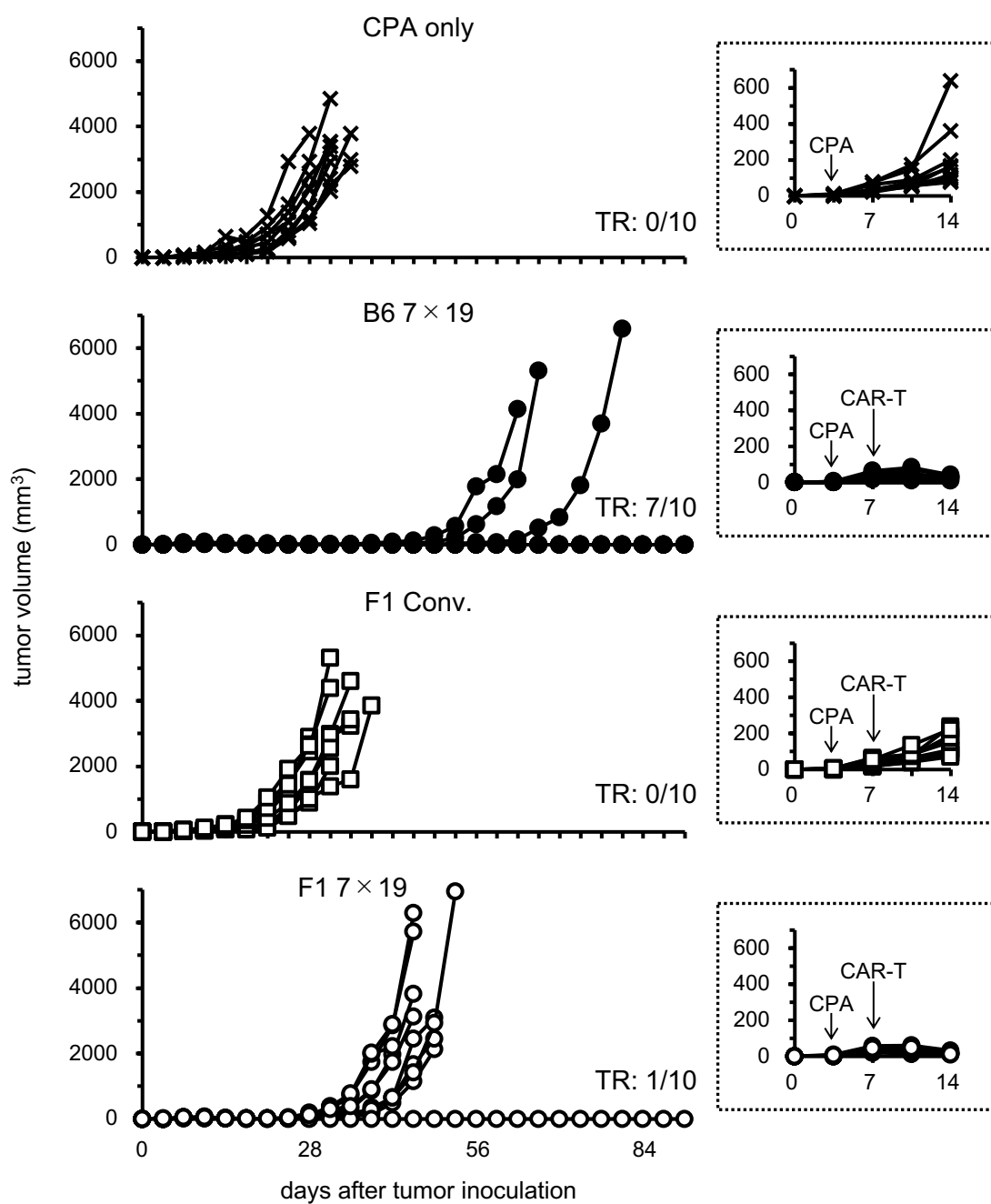
Supplementary Figure 20. Flow cytometric analysis of T cells within tumor tissue.

Flow cytometric analysis of T cells within tumor tissue, corresponding to Fig. 4c and 4d. Tumor inoculation, treatments, and single-cell suspension protocols follow the methods in Suppl. Figs. 16 and 19. Gating strategies are detailed in Suppl. Fig. 18. Percentages of cells in each gate are shown in the dot plots or histograms. Data from one of seven mice are provided.



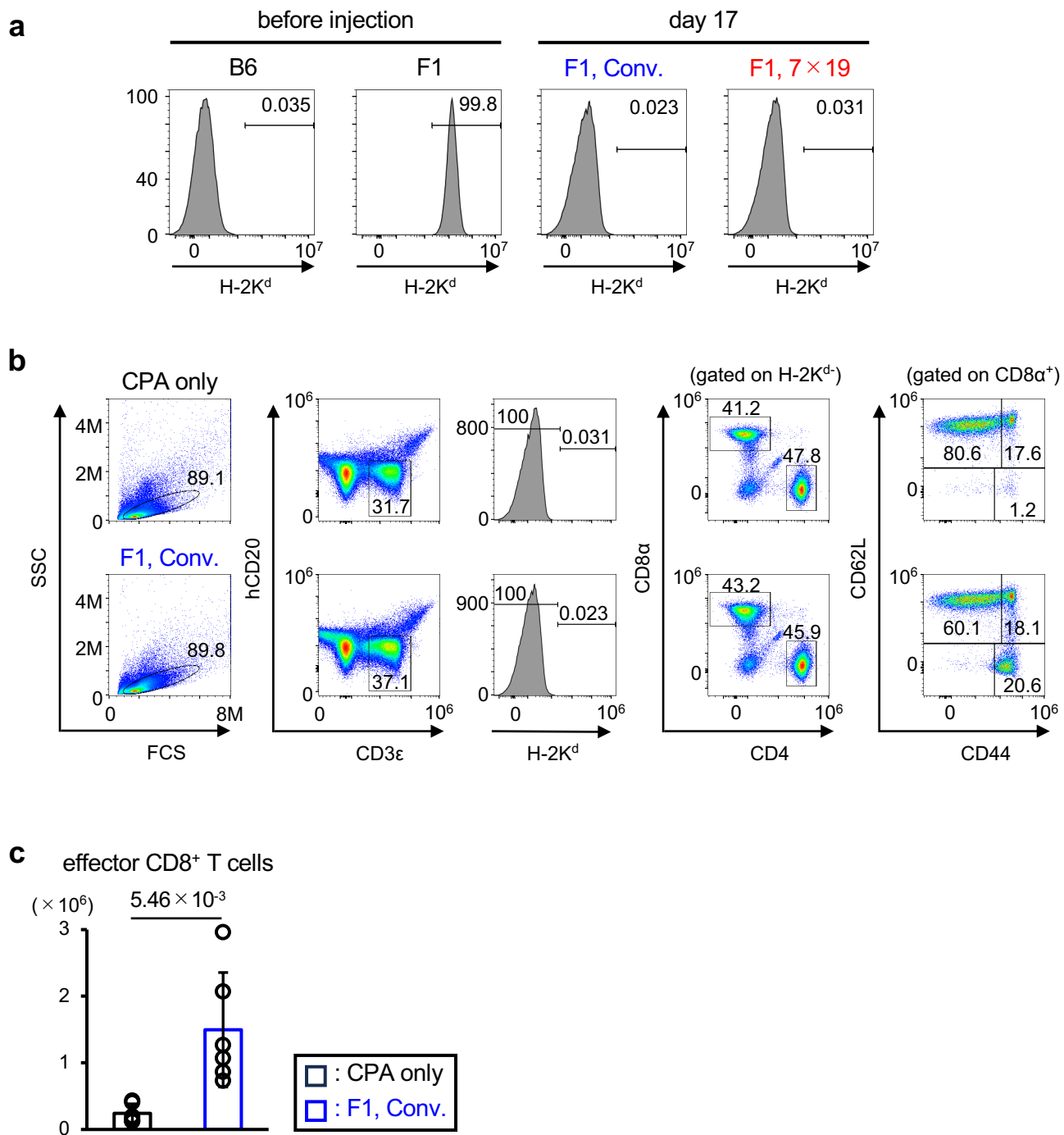
Supplementary Figure 21.

Prolonged survival was observed in P815-hCD20-bearing DBA/2 mice treated with allogeneic 7×10^6 CAR-T cells. DBA/2 mice were s.c. inoculated with P815-hCD20 cells, as described in Fig. 6a. Following tumor inoculation, mice received an i.p. injection of CPA and i.v. infusion of either conventional (Conv.) or 7×10^6 anti-hCD20 CAR-T cells (7×10^6). Control mice (CPA only) received no further treatment after CPA pretreatment. CAR-T cells were derived from either syngeneic DBA/2 mice or allogeneic B6D2F1 (F1) mice. The tumor volumes were monitored longitudinally. Insets depict tumor volumes during the initial 14 days post-inoculation. Pairwise *P*-values were calculated using log-rank tests (Bonferroni-adjusted) together with effect sizes; the median survival times (days) and 95% CIs are provided in Suppl. Table 8. Graphs indicate the total number of mice per group (denominator) and the number of mice achieving complete tumor rejection (tumor-rejected [TR], numerator).



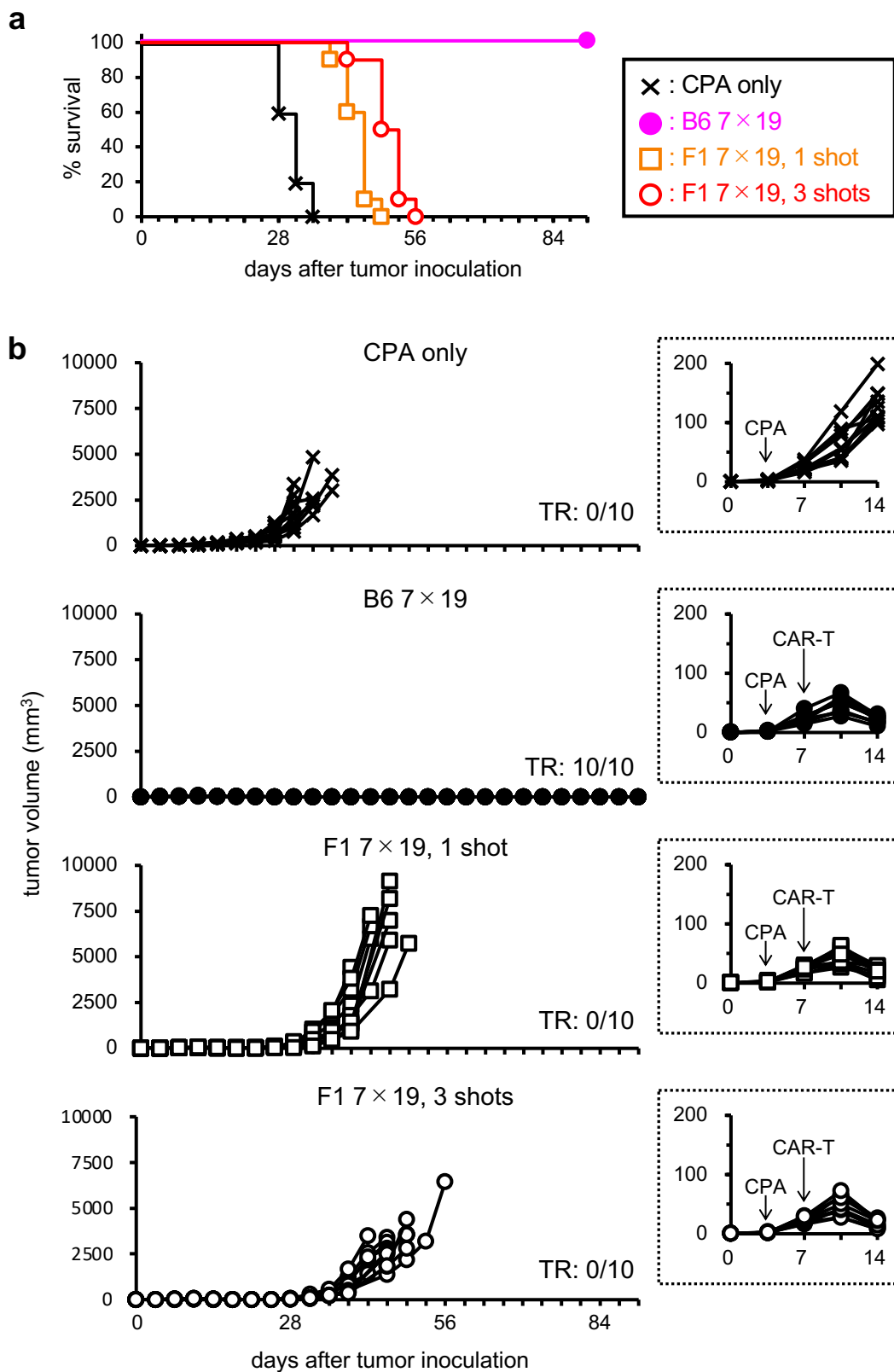
Supplementary Figure 22.

Prolonged survival and epitope spreading in B16.20-cOVA-TagGFP-bearing C57BL/6 mice treated with allogeneic 7×10^6 CAR-T cells. C57BL/6 (B6) mice were s.c. inoculated with B16.20-cOVA-TagGFP cells, as delineated in Figure 6b. Subsequent to the establishment of tumors, mice received an i.p. injection of CPA and were subsequently administered an i.v. injection of either conventional (Conv.) or 7×10^6 anti-hCD20 CAR-T cells (7×10^6). Mice that received only CPA treatment (CPA only) served as controls. CAR-T cells were generated from either syngeneic B6 or allogeneic B6D2F1 (F1) mice. The growth of the tumors was monitored over time, with insets highlighting those during the initial 14 days post-inoculation. Pairwise *P*-values and effect sizes were calculated using log-rank tests and adjusted by the Bonferroni methods. The median survival times (days) and the corresponding 95% CIs are summarized in Suppl. Table 9. The graphs present two values: the total number of mice per group (denominator) and the number of mice that achieved complete tumor rejection (tumor-rejected [TR], numerator).



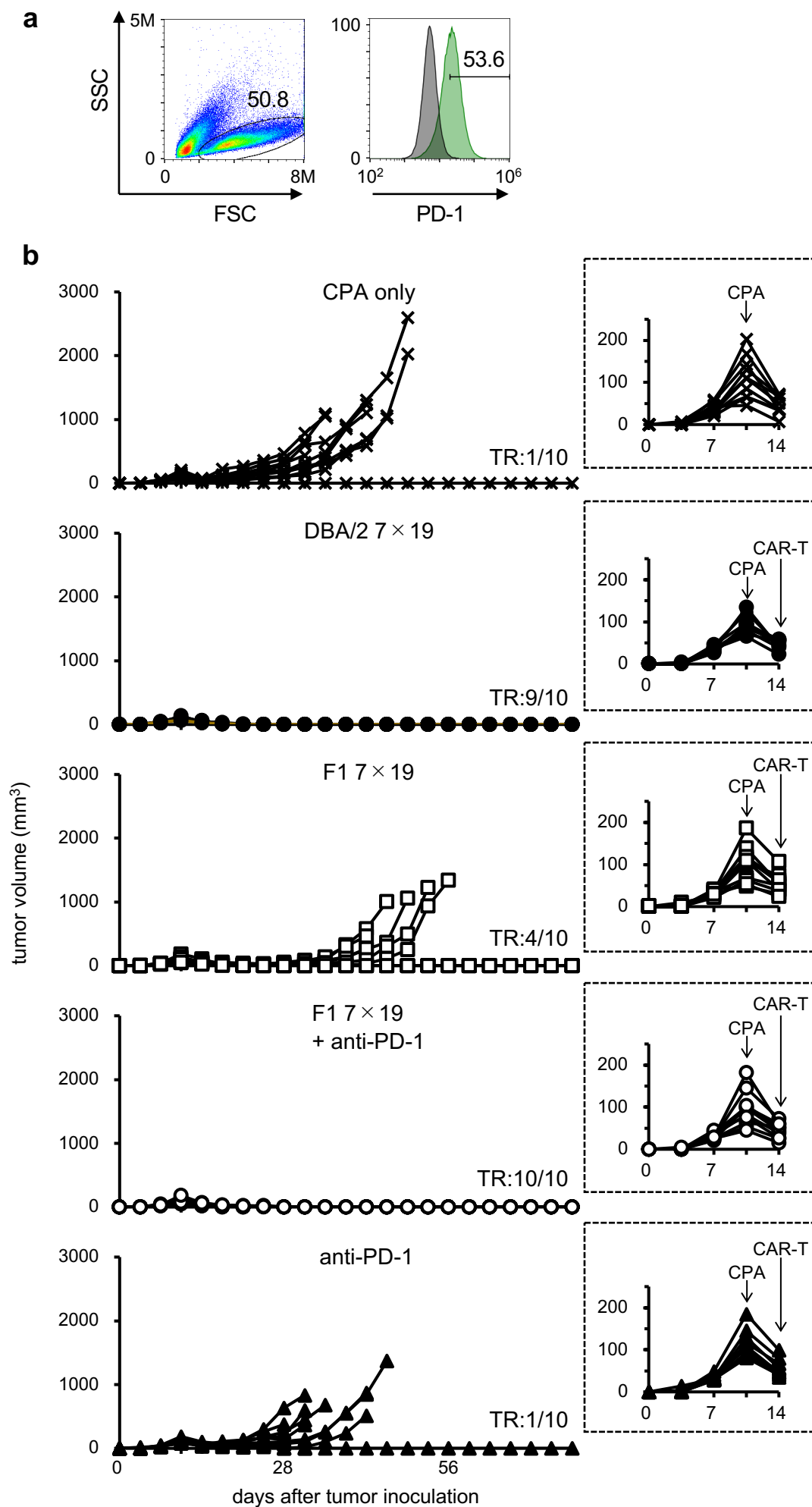
Supplementary Figure 23.

Rejection of allogeneic donor cells by day 10 post-infusion. C57BL/6 mice were inoculated with tumor cells and treated with CAR-T cells as shown in Fig. 6b. CAR-T cells were derived from either C57BL/6 (B6) or B6D2F1 (F1) donors. (a-c) On day 17 following tumor inoculation (i.e., 10 days after CAR-T cell infusion), spleens and tumor-draining lymph nodes were collected for flow cytometric analyses. (a) H-2K^d expression on T cells was examined, and pre-infusion CAR-T cell products were analyzed in parallel as controls. (b) Recipient T cells (H-2K^d) are shown in the third columns from the left, and were subsequently gated on CD8 α and CD4. Within the CD8 α ⁺ T-cell population, effector cells were defined as CD44⁺ CD62L⁻ and are displayed in the rightmost columns. (c) The numbers of effector CD8⁺ T cells were compared between control group (CPA only) and the mice treated with F1-derived conventional CAR-T cells (F1, Conv.). Two-sided, unpaired Student's *t*-tests were used to determine *P*-values. (a, b) Percentages in each gate appear on the dot plots or histograms. Representative data are shown.



Supplementary Figure 24.

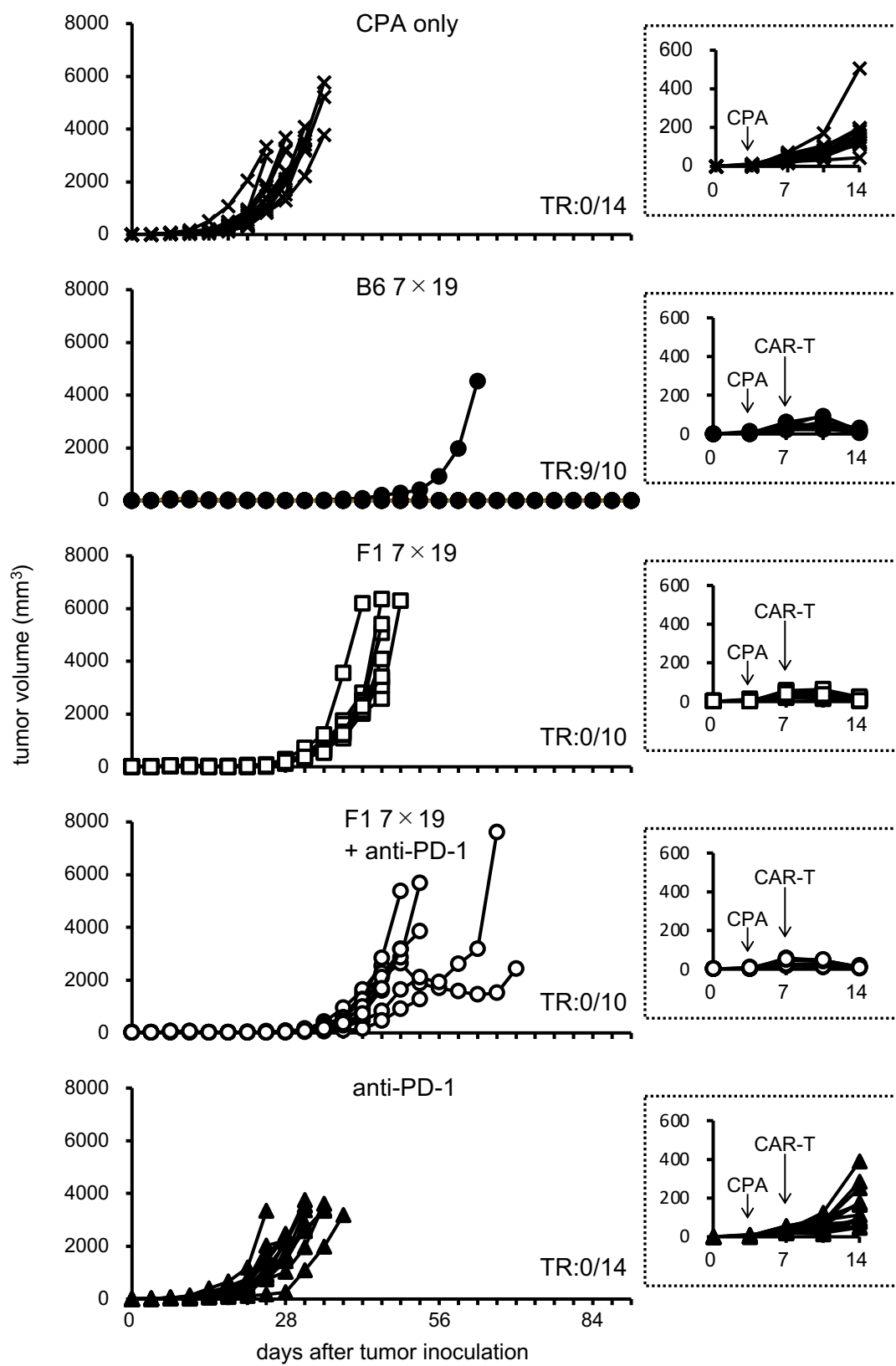
Repetitive infusion of allogeneic 7×10^6 CAR-T cells resulted in a modest extension of survival in tumor-bearing mice. C57BL/6 (B6) mice were inoculated s.c. with B16.20-cOVA-TagGFP cells and pretreated with CPA, as described in Suppl. Fig. 2. Administration of 7×10^6 anti-hCD20 CAR-T cells (7×10^6) which were derived from syngeneic B6 mice or allogeneic B6D2F1 (F1) mice was performed either once on day 7 post-inoculation (1 shot) or three times on days 7, 14, and 21 (3 shots). (a) The survival and (b) individual tumor volumes were monitored over time, with insets highlighting the tumor volumes during the first 14 days post-inoculation. (b) The total mice per group (denominator) and those achieving tumor rejection (TR, numerator) are shown. Pairwise *P*-values from log-rank analyses (Bonferroni-adjusted), together with effect sizes and median survival times with 95% CI are in Suppl. Table 10.



Supplementary Figure 25, K. Adachi, K. Tamada

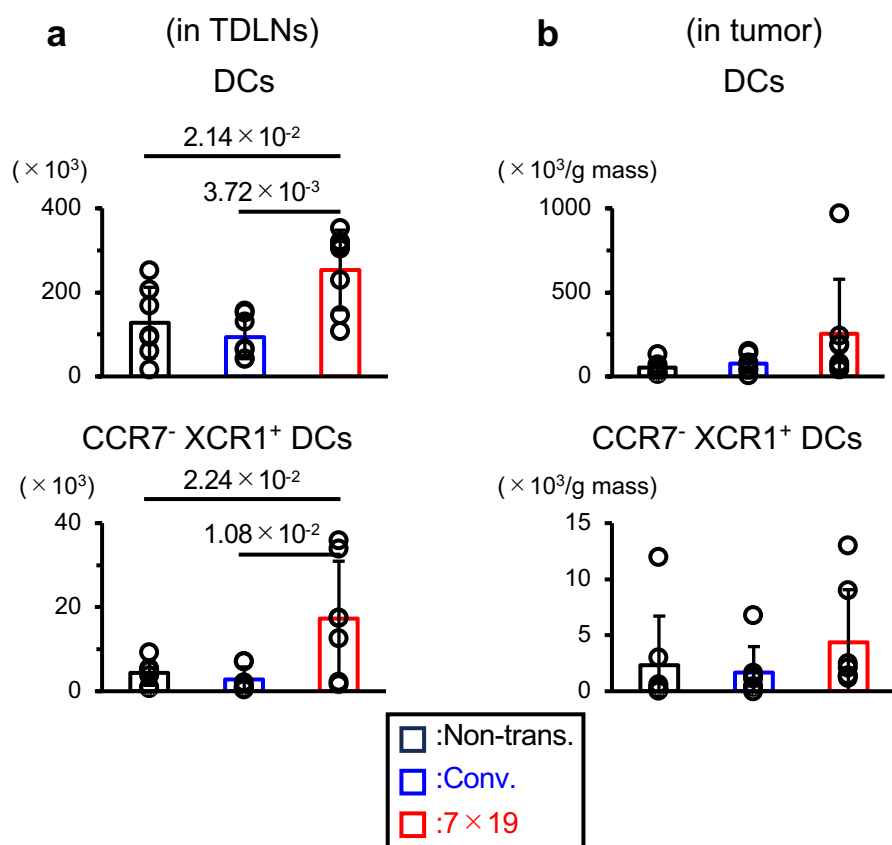
Supplementary Figure 25.

Combination therapy with PD-1 blockade enhances the antitumor efficacy of allogeneic 7×19 CAR-T cells against P815-hCD20. (a) T cells stimulated with anti-CD3 mAb, anti-CD28 mAb, and IL-2 for two days were treated with either an anti-PD-1 mAb prepared from the supernatant of a hybridoma (green histogram, right panel), which was also utilized in the subsequent in vivo experiments, or a control mAb (gray histogram, right panel), followed by staining with a FITC-conjugated secondary Ab. (b) DBA/2 mice were s.c. inoculated with P815-hCD20 cells, preconditioned with cyclophosphamide (CPA), and subsequently infused with 7×19 anti-hCD20 CAR-T cells (7×19) as detailed in Suppl. Fig. 8 and 14. CAR-T cells were derived either from syngeneic DBA/2 mice or from allogeneic B6D2F1 (F1) mice. I.p. administration of an anti-PD-1 mAb (anti-PD-1, 150 μ g per mouse) was initiated on day 24 post-inoculation and continued twice a week thereafter. For mice in which the tumor volume remained undetectable for 14 consecutive days, further antibody administration was discontinued. The tumor volumes were measured over time, with the early phase (first 14 days post-inoculation) highlighted in inset graphs. In each panel, the total number of mice per group (denominator) and the number achieving complete tumor rejection (tumor-rejected; TR, numerator) are indicated. Pairwise *P*-values obtained from log-rank tests (Bonferroni-adjusted), together with effect sizes and median survival times with 95% confidence intervals are provided in Supplementary Table 11.



Supplementary Figure 26.

Combination therapy with PD-1 blockade enhanced the antitumor efficacy of allogeneic 7×19 CAR-T cells against B16.20-cOVA-TagGFP. C57BL/6 mice were s.c. inoculated with B16.20-cOVA-TagGFP cells, pretreated with CPA, and subsequently infused with 7×19 anti-hCD20 CAR-T cells (7×19) as described in Suppl. Fig. 2. CAR-T cells were generated from either syngeneic B6 mice or allogeneic B6D2F1 (F1) mice. I.p. administration of anti-PD-1 mAb (anti-PD-1, 150 μ g per mouse) was initiated on day 17 post-inoculation and continued twice weekly thereafter. Individual tumor volumes were monitored over time, with insets highlighting the tumor volumes during the first 14 days post-inoculation. In each graph, the total number of mice per group (denominator) and the number achieving tumor rejection (tumor-rejected: TR, numerator) are indicated. Pairwise *P*-values from log-rank tests (Bonferroni-adjusted), together with effect sizes and median survival times (95% CI) appear in Suppl. Table 12.



Supplementary Figure 27.

Flow cytometric analysis of total DCs and CCR7⁻ XCR1⁺ DCs in tumor-draining lymph nodes (TDLNs) and tumors. (a, b) The treatment regimens and flow cytometric gating strategies are described in the legends of Suppl. Figure 16 (for a) and Suppl. Figure 19 (for b). The numbers of unfractionated total DCs and CCR7⁻ XCR1⁺ DCs (upper and lower bar graphs, respectively) in tumor-draining lymph nodes (a) and tumors (b) are shown as mean $\pm$ SD; each open circle represents an individual mouse. *P*-values were calculated using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test.

	B16.20-cOVA-TagGFP + 7 × 19	B16F10 + 7 × 19	mixture + 7 × 19	mixture + Conv.
B16.20-cOVA-TagGFP, CPA only	5.95 × 10 ⁻⁶ (1.25)	0.169 (0.37)	1.26 × 10 ⁻⁶ (0.94)	1 (0.054)
B16F10, CPA only	1.06 × 10 ⁻⁵ (1.15)	1 (0.092)	1.00 × 10 ⁻⁶ (0.88)	0.970 (0.24)
B16.20-cOVA-TagGFP + 7 × 19		7.78 × 10 ⁻⁶ (1.17)	7.15 × 10 ⁻³ (0.97)	2.51 × 10 ⁻⁶ (1.14)
		B16F10 + 7 × 19	6.01 × 10 ⁻⁷ (0.91)	0.312 (0.32)
			mixture + 7 × 19	4.24 × 10 ⁻⁷ (0.89)

Supplementary Table 1.

Data for Fig. 1a and Suppl. Fig. 3. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% confidence intervals (CI) were: B16.20-cOVA-TagGFP, CPA only ($n = 12$): 31 (28–35); B16F10, CPA only ($n = 12$): 28 (24–31); B16.20-cOVA-TagGFP + 7 × 19 ($n = 12$): not reached (NR) (not estimated [NE]–NE); B16F10 + 7 × 19 ($n = 13$): 28 (24–28); mixture + 7 × 19 ($n = 15$): 52 (42–59); mixture + Conv. ($n = 15$): 31 (28–31).

	B16.20-cOVA- TagGFP + 7 × 19	B16F10 + 7 × 19	mixture + 7 × 19	mixture + Conv.
B16.20-cOVA- TagGFP, CPA only	8.96×10^{-5} (1.25)	1 (0.13)	1.64×10^{-6} (0.86)	1 (0.030)
B16F10, CPA only	4.51×10^{-5} (1.29)	0.796 (0.28)	1.56×10^{-6} (0.88)	1 (0.15)
B16.20-cOVA- TagGFP + 7 × 19		4.30×10^{-5} (1.07)	8.38×10^{-6} (1.08)	6.94×10^{-5} (1.30)
		B16F10 + 7 × 19	4.90×10^{-7} (0.81)	1 (0.16)
			mixture + 7 × 19	1.01×10^{-6} (0.89)

Supplementary Table 2.

Data for Fig. 1b and Suppl. Fig. 4. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: B16.20-cOVA-TagGFP, CPA only ($n = 10$): 28 (24–28); B16F10, CPA only ($n = 11$): 31 (24–35); B16.20-cOVA-TagGFP + 7 × 19 ($n = 10$): NR (NE–NE); B16F10 + 7 × 19 ($n = 15$): 28 (21–31); mixture + 7 × 19 ($n = 15$): 45 (42–45); mixture + Conv. ($n = 10$): 28 (24–31).

	B16.20-cOVA- TagGFP + 7 × 19	B16F10 + 7 × 19	mixture + 7 × 19	mixture + Conv.
B16.20-cOVA- TagGFP, CPA only	2.10×10^{-4} (1.22)	0.475 (0.29)	4.25×10^{-6} (0.84)	1 (0.13)
B16F10, CPA only	1.01×10^{-4} (1.29)	1 (0.096)	1.42×10^{-5} (0.89)	1 (0.058)
B16.20-cOVA- TagGFP + 7 × 19		2.06×10^{-5} (1.07)	1.44×10^{-5} (1.07)	4.99×10^{-5} (1.29)
		B16F10 + 7 × 19	2.49×10^{-7} (0.80)	1 (0.15)
			mixture + 7 × 19	1.56×10^{-6} (0.87)

Supplementary Table 3.

Data for Fig. 1c and Suppl. Fig. 5. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: B16.20-cOVA-TagGFP, CPA only ($n = 10$): 29.5 (24–31); B16F10, CPA only ($n = 10$): 26 (17–31); B16.20-cOVA-TagGFP + 7 × 19 ($n = 10$): NR (NE–NE); B16F10 + 7 × 19 ($n = 15$): 24 (24–28); mixture + 7 × 19 ($n = 15$): 42 (38–42); mixture + Conv. ($n = 10$): 28 (24–28).

	3LL-hCD20 + 7 × 19	3LL + 7 × 19	mixture + 7 × 19	mixture + Conv.
3LL-hCD20, CPA only	1.25×10^{-7} (1.37)	1 (0.18)	3.18×10^{-6} (0.99)	1 (0.038)
3LL, CPA only	1.92×10^{-6} (1.40)	1 (0.23)	0.025 (0.69)	0.244 (0.39)
	3LL-hCD20 + 7 × 19	1.25×10^{-7} (1.50)	0.098 (1.08)	1.22×10^{-7} (1.39)
		3LL + 7 × 19	7.48×10^{-4} (0.87)	1 (0.20)
			mixture + 7 × 19	6.93×10^{-6} (0.99)

Supplementary Table 4.

Data for Fig. 1d and Suppl. Fig. 6. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: 3LL-hCD20, CPA only ($n = 15$): 45 (42–49); 3LL, CPA only ($n = 14$): 54 (38–73); 3LL-hCD20 + 7 × 19 ($n = 15$): NR (NE–NE); 3LL + 7 × 19 ($n = 13$): 45 (35–63); mixture + 7 × 19 ($n = 15$): NR (63–NE); mixture + Conv. ($n = 15$): 45 (35–52).

	3LL-hCD20 + 7 × 19	3LL + 7 × 19	mixture + 7 × 19	mixture + Conv.
3LL-hCD20, CPA only	2.69×10^{-5} (1.21)	1 (0.027)	0.019 (0.63)	1 (0.014)
3LL, CPA only	3.85×10^{-7} (1.29)	1 (0.030)	2.37×10^{-5} (0.85)	1 (0.018)
	3LL-hCD20 + 7 × 19	2.52×10^{-7} (1.32)	0.038 (0.93)	2.67×10^{-7} (1.30)
		3LL + 7 × 19	3.91×10^{-5} (0.84)	1 (0.018)
			mixture + 7 × 19	1.25×10^{-5} (0.87)

Supplementary Table 5.

Data for Fig. 1e and Suppl. Fig. 7. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: 3LL-hCD20, CPA only: 45 (38–52); 3LL, CPA only: 49 (42–52); 3LL-hCD20 + 7 × 19: NR (NE–NE); 3LL + 7 × 19: 52 (42–56); mixture + 7 × 19: 73 (59–NE); mixture + Conv.: 49 (42–52) ($n = 15$ per group).

	P815-hCD20 + 7 × 19	P815 + 7 × 19	mixture + 7 × 19	mixture + Conv.
P815-hCD20, CPA only	1.25×10^{-6} (1.09)	1 (0.098)	4.58×10^{-3} (0.62)	1 (0.21)
P815, CPA only	8.88×10^{-7} (1.10)	1 (0.075)	2.27×10^{-3} (0.65)	1 (0.16)
	P815-hCD20 + 7 × 19	6.71×10^{-7} (1.12)	0.109 (0.68)	2.55×10^{-6} (1.42)
		P815 + 7 × 19	1.61×10^{-3} (0.67)	1 (0.079)
			mixture + 7 × 19	5.94×10^{-4} (0.85)

Supplementary Table 6.

Data for Fig. 1f and Suppl. Fig. 8. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: P815-hCD20, CPA only: 45 (38–52); P815, CPA only: 42 (38–49); P815-hCD20 + 7 × 19: NR (NE–NE); P815 + 7 × 19: 40 (35–52); mixture + 7 × 19: 66 (52–NE); mixture + Conv.: 38 (31–42) ($n = 20$ in all groups except mixture + Conv., where $n = 10$).

	3LL-hCD20 + 7 × 19	3LL + 7 × 19	mixture + 7 × 19	mixture + Conv.
3LL-hCD20	6.13×10^{-5} (1.37)	1 (0.24)	1.67×10^{-4} (0.94)	0.882 (0.36)
3LL	6.30×10^{-4} (1.26)	0.218 (0.45)	0.497 (0.50)	1 (0.21)
	3LL-hCD20 + 7 × 19	6.08×10^{-5} (1.29)	0.059 (1.14)	4.13×10^{-5} (1.40)
		3LL + 7 × 19	1.81×10^{-3} (0.80)	1 (0.15)
			mixture + 7 × 19	0.035 (0.71)

Supplementary Table 7.

Data for Fig. 1g and Suppl. Fig. 9. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: 3LL-hCD20, no treatment: 45 (31–52); 3LL, no treatment: 59 (49–66); 3LL-hCD20 + 7 × 19: NR (NE–NE); 3LL + 7 × 19: 49 (42–56); mixture + 7 × 19: 73 (56–NE); mixture + Conv.: 52 (38–66) (*n* = 10 per group).

	DBA/2 7 × 19	F1 Conv.	F1 7 × 19
CPA only	2.03×10^{-5} (1.16)	1 (0.039)	3.42×10^{-3} (0.73)
	DBA/2 7 × 19	9.47×10^{-5} (1.10)	0.413 (0.51)
		F1 Conv.	2.65×10^{-4} (0.86)

Supplementary Table 8.

Data for Fig. 6a and Suppl. Fig. 21. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: CPA only ($n = 12$): 35 (28–53); DBA/2 7 × 19 ($n = 15$): NR (84–NE); F1 Conv. ($n = 10$): 35 (18–35); F1 7 × 19 ($n = 19$): NR (46–NE).

	B6 7 × 19	F1 Conv.	F1 7 × 19
CPA only	6.32 × 10 ⁻⁵ (1.00)	1 (0.024)	6.32 × 10 ⁻⁵ (0.77)
	B6 7 × 19	2.98 × 10 ⁻⁵ (1.07)	2.92 × 10 ⁻⁵ (0.88)
		F1 Conv.	2.98 × 10 ⁻⁵ (0.83)

Supplementary Table 9.

Data for Fig. 6b and Suppl. Fig. 22. Numbers in parentheses indicate effect sizes. Median survival times (days) and 95% CI were: CPA only: 32 (28-35); B6 7 × 19: NR (63-NE); F1 Conv.: 32 (28-35); F1 7 × 19: 51 (46-53) (*n* = 10 per group).

	B6 7×19	F1 7×19 , 1 shot	F1 7×19 , 3 shots
CPA only	3.37×10^{-5} (1.27)	3.37×10^{-5} (0.78)	3.37×10^{-5} (0.78)
	B6 7×19	4.35×10^{-5} (1.25)	3.55×10^{-5} (1.27)
		F1 7×19 , 1 shot	3.35×10^{-3} (0.59)

Supplementary Table 10.

Data for Suppl. Fig. 24. Numbers in parentheses indicate effect sizes. The median survival times (days) and 95% CI were as follows: CPA only: 32 (28–32); B6 7×19 : NR (NE–NE); F1 7×19 , 1 shot: 46 (39–46); F1 7×19 , 3 shots: 51 (42–53) ($n = 10$ mice per group).

	DBA/2 7 × 19	F1 7 × 19	F1 7 × 19 + anti-PD1	anti-PD-1
CPA only	4.87×10^{-3} (1.02)	0.220 (0.52)	6.67×10^{-4} (1.24)	1 (0.22)
	DBA/2 7 × 19	0.267 (0.81)	1 (1.00)	1.93×10^{-3} (1.08)
		F1 7 × 19	0.039 (1.16)	0.076 (0.62)
			F1 7 × 19 + anti-PD1	4.07×10^{-4} (1.26)

Supplementary Table 11.

Data corresponding to Fig. 6d and Suppl. Fig. 25b. Numbers in parentheses indicate effect sizes. The median survival times (days) and 95% CI were as follows: CPA only: 46 (35–49); DBA/2 7 × 19: NR (46–NE); F1 7 × 19: 58 (46–NE); F1 7 × 19 + anti-PD-1: NR (NE–NE); anti-PD-1: 37 (32–46) (*n* = 10 mice/group).

	B6 7 × 19	F1 7 × 19	F1 7 × 19 + anti-PD1	anti-PD-1
CPA only	2.61×10^{-5} (1.06)	2.61×10^{-5} (0.75)	2.61×10^{-5} (0.75)	1 (0.16)
	B6 7 × 19	6.43×10^{-5} (1.04)	1.38×10^{-4} (1.21)	1.56×10^{-5} (1.05)
		F1 7 × 19	2.42×10^{-3} (0.55)	1.56×10^{-5} (0.74)
			F1 7 × 19 + anti-PD1	1.56×10^{-5} (0.74)

Supplementary Table 12.

Data for Fig. 6e and Suppl. Fig. 26. Numbers in parentheses indicate effect sizes. The median survival times (days) and 95% CI were as follows: CPA only (*n* = 14): 30 (25–32); B6 7 × 19 (*n* = 10): NR (63–NE); F1 7 × 19 (*n* = 10): 46 (42–46); F1 7 × 19 + anti-PD-1 (*n* = 10): 53 (46–56); anti-PD-1 (*n* = 14): 32 (28–35).