Supplemental Material

Supplemental Materials and Methods:

Cell lines

The MM cell line MM1.S, mantle cell lymphoma cell line JeKo-1, T cell lymphoma cell line Jurkat, chronic myelogenous leukemia cell line K562, and fibroblast cell line WI-38 were purchased from ATCC, Manassas, VA, USA, and the MM cell line OPM-2 was purchased from DSMZ, Braunschweig, Germany. The MM cell line ALMC-2 was gifted by Dr. Diane Jelinek. The mouse melanoma cell line B16 was gifted by Dr. Michael Barry. These cell lines were transduced with a luciferase-ZsGreen lentivirus (Addgene, Cambridge, MA, USA) and sorted to 100% purity, as previously described[1,2]. Cell lines were cultured in R10 medium made with Roswell Park Memorial Institute (RPMI) 1640 (Gibco, Gaithersburg, MD, USA), 10% Fetal Bovine Serum (FBS, Sigma, St. Louis, MO, USA), and 1% Penicillin-Streptomycin-Glutamine (Gibco, Gaithersburg, MD, USA). Flow cytometric analysis of SLAMF7 expression on MM1.S, OPM-2, ALMC-2 cells showed similar pattern (Supplemental Figure S13). All cell lines used were regularly tested negative for mycoplasma contamination throughout the whole duration of this study.

Multi-parametric flow cytometry

Anti-human antibodies were purchased from Biolegend, eBioscience, or BD Biosciences (San Diego, CA, USA). The preparation of samples for flow cytometry was described previously[2]. Countbright beads (Invitrogen, Carlsbad, CA, USA) were used for cell number quantitation, as previously described[2].

A goat anti-mouse F(ab') antibody (Invitrogen, Carlsbad, CA, USA) was used for detecting a single transduced BCMA, SLAMF7, or FAP CAR. To assess dual transduced CARs, labelled proteins for the specific CAR were used. For BCMA CAR, human BCMA/TNFRSF17 protein, Fc Tag (BC7-H5254-100ug, ACRO Biosysytems, Newark, DE) was used for primary staining, followed by Alexa Fluor 647-conjugated anti-human IgG Fc antibody (109-606-170, Jackson ImmunoResearch, West Grove, PA). For SLAMF7 CAR and FAP CAR staining, human SLAMF7/CRACC/CD319 protein, His Tag (SL7-H5225-100ug, ACRO Biosystems, Newark, DE) or recombinant human FAP, His tagged (FAP-1141H, Creative BioMart, Shirley, NY) was used for primary staining, respectively, followed by FITC conjugated anti-6X His tag antibody (Cambridge, CB2 0AX, UK). The following antibodies were also used: anti-human CD45 (clone HI30) BV421 (cat# 304032, BioLegend, San Diego, CA, USA), anti-mouse CD45 (clone 30-F11) APC- eFluor 780 (cat# 103116, BioLegend, San Diego, CA, USA), CD3 (clone UCHT1) PE-Cy7 (cat# 300420, BioLegend, San Diego, CA, USA), CD3 (clone UCHT1) APC (cat# 17-0038-42, eBiosciences, San Diego, CA, USA), and CD3 (clone SK7) APC-H7 (cat# 560176, BD Pharmingen, San Diego, CA, USA), CD107a (clone H4A3) FITC (cat# 555800, BD Pharmingen, San Diego, CA, USA), IL-2 (clone 5344.111) PE-CF594 (cat# 562384, BD Pharmingen, San Diego, CA, USA), GM-CSF (clone BVD2-21C11) BV421 (cat# 562930, BD Pharmingen, San Diego, CA, USA), IFN-γ (clone 4S.B3) APC-eFluor 780 (cat# 47-7319-42, Invitrogen, Carlsbad, CA, USA), MIP1-β (clone D21-1351) PE-Cy7 (cat# 560687, BD Pharmingen, San Diego, CA, USA), BCMA (clone 19F2) PE-Cy7 (cat# 357507, BioLegend, San Diego, CA, USA), SLAMF7 (clone 162) PE (cat# 12-2229-

42, Invitrogen, Carlsbad, CA, USA), SLAMF7 (clone 162) APC (cat# 331809, BioLegend, San Diego, CA, USA), FAP (clone 427819) PE (cat# FAB3715P-025, R&D Systems, Minneapolis, MN, USA), SMA (clone 1A4) APC (cat# IC1420A, R&D Systems, Minneapolis, MN, USA), human CD45 (clone 2D1) PerCP (cat# 340665, BD Pharmingen, San Diego, CA, USA), PD-1 (clone EH12.2H7) BV421 (cat# 329920, BioLegend, San Diego, CA, USA), PD-L1 (clone 29E.2A3) BV421 (cat# 329714, BioLegend, San Diego, CA, USA). For murine FAP staining, rat anti-mouse FAP (clone 983802) (cat# MAB9727, R&D Systems, Minneapolis, MN, USA), followed by phycoerythrin-conjugated anti-rat IgG secondary antibody (cat# F0105B, R&D Systems, Minneapolis, MN, USA). For murine SLAMF7 staining, rat anti-mouse SLAMF7 (clone 983802) (cat# MAB46281 , R&D Systems, Minneapolis, MN, USA), followed by phycoerythrin-conjugated anti-rat IgG secondary antibody.Flow cytometry was performed on three-laser CytoFLEX (Beckman Coulter, Chaska, MN, USA). All analyses were performed using FlowJo X10.0.7r2 software (Ashland, OR, USA).

Bone marrow-derived mesenchymal stem cells (BM-MSCs)

BM-MSCs were obtained under a Mayo Clinic IRB approved protocol (IRB# 1062-00). Whole normal bone marrow aspirates from patients with nonmalignant disease which requires an orthopedic procedure (e.g. hip replacement) resulting in waste of normal bone marrow. Samples are only identified with a laboratory assigned sequence number. Whole bone marrow aspirates were first filtered through a 70 μm nylon cell strainer (Falcon/Corning Inc., cat.# 352350). To lyse the red blood cells

(RBCs), ACK lyse buffer was added to the bone marrow samples. MEM-alpha medium was used to culture bone marrow samples. Once confluent, supernatant was discarded, and adherent cells were trypsinized and cryopreserved.

Primary cells and CART cells

The use of recombinant DNA in the laboratory was approved by the Mayo Clinic Institutional Biosafety Committee (IBC), IBC number HIP00000252.20. Peripheral blood mononuclear cells (PBMC) were isolated from de-identified normal donor blood apheresis cones obtained under a Mayo Clinic IRB approved protocol, using SepMate tubes (STEMCELL Technologies, Vancouver, Canada). T cells were separated with negative selection magnetic beads using EasySep™ Human T Cell Isolation Kit (STEMCELL Technologies). Second generation BCMA, SLAMF7, or FAP CAR constructs were synthesized *de novo* (IDT, Coralville, IA, USA) and cloned into a third-generation lentivirus under the control of the EF-1α promotor. Several constructs were synthesized using different co-stimulation as indicated below and in the described specific experiments. The BCMA CAR construct (C11D5.3-41BBz) included 4-1BB costimulation and a single chain variable fragment (scFv) derived from an anti-human BCMA antibody clone C11D5.3 [3]. The SLAMF7 CAR construct (Luc90-CD28z) included CD28 costimulation and an scFv derived from a mouse anti-human SLAMF7 antibody clone Luc90[4,5]. The FAP CAR construct (FAP05-41BBz) included 4-1BB costimulation and an scFv derived from anti-FAP clone FAP-05. These constructs are presented in Supplemental Figure S14A. BCMA, SLAMF7, or FAP CART cells were then generated through the lentiviral transduction of normal

donor T cells. The generation of lentiviral particles was described previously [2]. The titers and subsequently multiplicity of infection (MOI) were analyzed and calculated by flow cytometry as described previously[2]. T cells isolated from normal donors were stimulated using Cell Therapy Systems Dynabeads CD3/CD28 (Life Technologies, Oslo, Norway) at a 1:3 ratio (cells:beads) and then transduced 24 hours after stimulation with lentivirus particles at a MOI of 5.0. Magnetic bead removal and the evaluation of CAR expression on T cells by flow cytometry were performed on day 6. The representative flow plots of CART cells are shown in Supplemental Figure S14B. CART cells were harvested and cryopreserved on day 8 for future experiments. CART cells were thawed and rested in T cell medium 6-12 hours prior to their use in experiments, as specified in each experiment.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNAs were extracted with QIAzol lysis reagent (Qiagen, Hilden, Germany) and miRNeasy Micro Kit Reagent (Qiagen) according to the manufacturer's protocol. RT-qPCR analysis of BCMA, SLAMF7, and FAP was performed with 1 µg of total RNA and iScript Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad, Hercules, California, USA). The primer sequences used were as follows: BCMA forward primer, 5'-TGTTCTTCTAATACTCCTCCTCT-3' and reverse primer, 5'-AACTCGTCCTTTAATGGTTC-3'; SLAMF7 forward primer, 5'-AGCAGCCCTCCACCCAGGAG-3' and reverse primer, 5'-AGGGCCTTCCAGGTATAAATCAC-3'; and FAP forward primer, 5'-

GGAAGTGCCTGTTCCAGCAATG-3' and reverse primer, 5'-TGTCTGCCAGTCTTCCCTGAAG-3'. Primers specific for TBP forward, 5'-GCCAGCTTCGGAGAGTTCTGGGATT-3' and reverse, 5'-CGGGCACGAAGTGCAATGGTCTTTA-3'). RT-qPCR was performed in an S1000 Thermal Cycler (Bio-Rad). PCR conditions consisted of the following: 95°C for 3 min for denaturation; 95°C for 30 sec for annealing; and 62°C for 20 sec for extension, for 49 cycles. The threshold cycle for each sample was selected from the linear range and converted to a starting quantity by interpolation from a standard curve generated on the same plate for each set of primers. The BCMA, SLAMF7, and FAP messenger (m) RNA levels were normalized for each well to the TBP mRNA levels using the $2^{-\Delta\Delta Cq}$ method.

Immunohistochemistry

The bone marrow samples were harvested, fixed and embedded in paraffin. The blocks were sliced into 5 mm sections and then dewaxed by zylene. The slides were dehydrated using increasing concentrations of alcohol. The sections were immunostained with primary antibodies: anti-CD3 (clone LN10, Leica Biosystems, Buffalo Grove, IL), anti-CD138 (clone MI15, Leica Biosystems), anti-FSP-1 (clone 15E2E2, Millipore Sigma) followed by reaction with BOND Polymer Refine Detection Kit (Leica Biosystems). Images were taken of at least three randomly selected fields per sample. Percentage of FSP-1-positive area by calculating the ratio of positive staining area to total area using ImageJ software (National Institute of Health).

Statistics

Prism Graph Pad (La Jolla, CA, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA) were used to analyze the experimental data. The high cytokine concentrations in the heat map were normalized to “1” and low concentrations normalized to “0” via Prism. Statistical tests are described in the figure legends. Briefly, normally distributed data were tested by one- and two-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparisons test, and unpaired and paired two-sample Student’s *t*-test or Mann–Whitney U test for two-group comparisons. Log-rank test was used to test the hypotheses for *in vivo* survival.

Supplemental Figure Legends

Supplemental Figure S1 | Potent anti-tumor activity of BCMA-CART cells against BCMA expressing cells *in vitro*. A, BCMA-CART-cells were co-cultured at different E:T ratios with luciferase^{+} MM1.S. At 24 hours, cell killing was assessed by luminescence relative to controls (**** $p<0.0001$, two-way ANOVA; n=3, 2 replicates). **B,** CFSE-labeled BCMA-CART were co-cultured with lethally irradiated MM1.S for 5 days. Cells were then analyzed for CFSE dilution to detect cell proliferation, and absolute number of CART cells was counted by flow cytometry (5 ng/mL phorbol 12-myristate 13-acetate (PMA) and 0.1 µg/mL ionomycin stimulation was used as a positive control; n=3, 2 replicates). **C,** BCMA-CART-cells exhibited more antigen-specific proliferation compared to untransduced T cells (UTD). BCMA-CART or UTD derived from the same donor were co-cultured with lethally irradiated MM1.S for 5 days. Antigen-specific proliferation was measured by flow cytometry after 5 days using absolute counts with counting beads. **D,** BCMA-CART or UTD were co-cultured with lethally irradiated K562, MM1.S, or OPM-2 cells for 5 days. Absolute number of T cells were counted via flow cytometry (mean and SEM, **** $p<0.0001$, one-way ANOVA; n=3, 2 replicates). **E,** BCMA-CART cell degranulation and cytokine release assay. BCMA-CART degranulation assay and cytokine production. BCMA-CART or UTD were co-cultured with MM1.S for 4 hours. Medium was used as a negative control (mean and SEM, ****$p<0.0001$, **$p<0.005$, one-way ANOVA; n=3, 2 replicates). **F,** Representative flow plots of BCMA-CART or UTD CD107a degranulation and intracellular cytokine assays.

Supplemental Figure S2 | Potent anti-tumor activity of BCMA-CART-cells against BCMA-expressing cells *in vivo*. A, Experimental schema of BCMA-CART-cells in MM xenograft model. NSG mice were engrafted with luciferase^{+}OPM-2 ($1x10^6$ cells/mouse, IV, 6 mice per group). On day 28, mice were randomized according to tumor burden, which was accessed by BLI, to receive $1x10^6$ UTD, $1x10^6$ BCMA-CART-cells, $0.5x10^6$ BCMA-CART-cells, or $0.25x10^6$ BCMA-CART-cells. **B,** BLI curve of *in vivo* BCMA-CART-cell dose-finding assay. **C-D,** Anti-myeloma activity of BCMA-CART-cells in OPM-2 xenograft mice, which was shown by bioluminescence imaging (mean and SEM, **p=0.006 at day 21, unpaired, two-sided, Student's t-test). **E**, Kaplan-Meir survival curve is shown [hazard ratio = 0.03320; 95% confidence interval (CI) = 0.004605 to 0.2393, ***p=0.0007, log-rank test]. **F,** Immunohistochemical analysis of BM samples harvested from OPM-2 xenograft mice treated with BCMA-CART-cells (magnification 10). **G,** Immunohistochemical analysis of bone marrow from OPM-2 xenograft models, which were treated with UTD (H&E, *upper left and right,* magnification 10 and 40, respectively. CD138, *lower left*, magnification 10), and FSP-1 (*lower right*) staining revealed the absence of BM-CAFs in this model.

Supplemental Figure S3 | Bone marrow-derived cancer-associated fibroblasts (BM-CAFs) accelerate MM1.S cell growth *in vivo*. NSG mice were intravenously injected with $1x10^6$ of luciferase^{+} MM1.S cells or in combination with $1x10^6$ of BM-CAFs. Tumor growth was assessed with a bioluminescence imager (mean and SEM, **** p<0.0001, two-way ANOVA; n=3).

Supplemental Figure S4 | BM-CAFs inhibit BCMA-CART effector functions. A, CFSE-labeled BCMA-CART or UTD were co-cultured with lethally irradiated $BCMA^+SLAMF7^+$MM1.S and BM-CAFs for 5 days (mean and SEM, ****p<0.0001, two-way ANOVA; n=3, 2 replicates). **B,** Antigen-specific BCMA-CART CD107a degranulation assay in the presence of BM-CAFs. OPM-2 cells were used as a stimulator (**p<0.01, unpaired, two-sided, Student's t-test; n=3, 2 replicates). **C,** CTLA-4 expression on BCMA-CART in the presence or absence of CAFs (Student's t-test; n=3, 2 replicates). **D**, Representative flow plots of surface PD-1 expression on BCMA-CART cells when co-cultured with OPM-2 (*upper panels*) or MM1.S (*lower panels*) in the presence or absence of CAFs.

Supplemental Figure S5 | Bone barrow MSCs derived from healthy donor do not inhibit BCMA-CART proliferation. BCMA-CART cells were co-cultured with lethally irradiated $BCMA^+SLAMF7^+$MM1.S (*left*) or $BCMA^+SLAMF7^+$OPM-2 (*right*) in the presence or absence of MSCs for 5 days. Absolute number of $CD3^+$ T cells were counted via flow cytometer (mean and SEM, t-test; n=6, 2 replicates).

Supplemental Figure S6 | Actual values of cytokines from the multiplex assay. Cytokines were analyzed by multiplex using supernatant from the co-culture of BCMA-CART and irradiated MM1.S with or without BM-CAFs (mean and SEM, *p<0.05, **p<0.01, ****p<0.0001, Student's t-test; n=3, 2 replicates).

Supplemental Figure S7 | Inhibitory cytokines and growth factors were

increased, and effector cytokines were decreased in the presence of BM-CAFs. BCMA-CART-cells were co-cultured with irradiated OPM-2 **(A)** or ALMC-2 **(B)** for 3 days and supernatants were analyzed via multiplex. Bar graphs represent the raw values of cytokines (n.s. not significant, *p<0.05, **p<0.01, ***p<0.005, unpaired, two-sided, Student's t-test; n=3 experiments, 2 replicates).

Supplemental Figure S8 | TGF-β depletion does not overcome BM-CAF induced impairment of BCMA CART cytotoxicity. BCMA-CART were co-cultured with MM1.S and BM-CAFs in the presence or absence of anti TGF-β antibody. At 24 hours, cytotoxicity was assessed by luminescence relative to controls (*** p<0.001, two-way ANOVA; n=3, 2 replicates).

Supplemental Figure S9 | Flow cytometric gating for assessment of inhibitory receptor-ligand expression. BCMA-CART were co-cultured with lethally irradiated MM1.S and BM-CAFs. Cells were gated on FSC/SCC followed by singlet and live cell discrimination. CD3, CD38, CD45, and FSP-1 were used to distinguish CAFs from CART-cells. The surface PD-L1 or PD-L2 expression on BM-CAFs were assessed by flow cytometry. Isotype controls are shown as gray peaks.

Supplemental Figure S10 | CAF inhibition of BCMA-CART-cell proliferation cannot be reversed by blocking PD-1/PD-L1 axis and/or TGF-β neutralization. BCMA-CART cells were co-cultured with lethally irradiated OPM-2 (A) or MM1.S (B) in the presence or absence of CAFs for 5 days. PD-L1 blocking antibody (20 μg/mL)

and/or TGF-β neutralizing antibody (1 μg/mL) were also added to some conditions. Absolute number of $CD3^+$ T cells were assessed by flow cytometry on day 5 (mean and SEM, *p<0.05, **p<0.001, t-test; n=3, 2 replicates).

Supplemental Figure S11 | Flow cytometry gating for BM-CAFs and CD45 negative fraction derived from bone marrow of patients with multiple myeloma. **A,** Gating of BM-CAFs samples from patients with MM. The bone marrow was first isolated with $CD138^+$ microbeads. Then, $CD138^-$ fraction was cultured for two weeks and CAFs were isolated with anti-fibroblasts microbeads. BM-CAFs were defined as the live, $CD45^-CD38^-FSP\text{-}1^+$ fraction. **B,** Representative flow plots of BM-CAFs. Isotype control of IgG1-APC and IgG1-PE were used for left flow plots. SLAMF7-APC and FAP-PE were used for right flow plots.

Supplemental Figure S12 | Flow cytometric analysis for BM-MSCs and qPCR analysis of BM-MSCs and BM-CAFs. A, Flow cytometric analysis for BM-MSCs derived from healthy donor. MSCs were defined as $CD38^-$, $CD45^-$, and FSP^+ cells. Grey peaks are fluorescence minus one (FMO) controls. Purple peaks are the stained samples. Representative histograms are shown; n=3. **B,** qPCR analysis for BM-MSC derived from healthy donors and BM-CAFs from MM patients (** p<0.01, **** p<0.0001, Student's t-test; n=3, 3 replicates).

Supplemental Figure S13 | Flow cytometric analysis of BCMA, SLAMF7, and FAP expression on OPM-2, MM1.S, and ALMC-2 cells. Grey peaks represent the isotype

controls.

Supplemental Figure S14| Constructs of the BCMA-, SLAMF7-, and FAP-CAR vector, and surface CAR expression on human CD3 T-cells. A, Schematic representation of the BCMA-, SLAMF7-, and FAP-CAR constructs. BCMA-CAR consisted of anti-BCMA single chain variable fragment (scFv) linked to CD3 zeta and a 4-1BB costimulatory domain. H, hinge; TM, transmembrane. SLAMF7-CAR consisted of anti-SLAMF7 scFv linked to CD3 zeta and a CD28 costimulatory domain. FAP-CAR consisted of anti-FAP scFv linked to CD3 zeta and a 4-1BB costimulatory domain. **B**, Representative flow plots of UTDs, BCMA-, SLAMF7-, and FAP-CART. Goat anti-mouse F(ab')2 antibody (GAM) was used with live/dead aqua to detect CAR expression on CART-cells. Cells were gated on FSC/SSC followed by singlet discrimination and live cells. Negative gates for CAR expression were set based on untransduced (UTD) T cells.

Supplemental Figure S15 | Representative flow plots of FAP-CART degranulation assay. FAP-CART stimulated with FAP^{+}WI-38 or FAP^{-}JeKo-1 cells. CD3 was used to distinguish CART-cells from target cells.

Supplemental Figure S16 | Representative flow plots of SLAMF7-CART-cell degranulation assay. SLAMF7-CART-cells stimulated with $SLAMF7^{+}$MM1.S or $SLAMF7^{-}$Jurkat cells. CD3 was used to distinguish CART-cells from target cells.

Supplemental Figure S17 | SLAMF7- or FAP-CART cytotoxicity assay against BM-CAFs. SLAMF7- or FAP-CART were co-cultured with BM-CAFs at 1:1 ratio. At 24 hours, cytotoxicity was assessed relative to controls. CD105 and CD3 were used to differentiate CAF and T cells (mean and SEM, ** p<0.01, *** p<0.001, one-way ANOVA; n=3, 2 replicates).

Supplemental Figure S18 | SLAMF7 or FAP-CART inhibit CAF growth *in vivo*. A, Experimental schema. Four weeks after the injection of $1x10^6$ luciferase positive OPM-2 and $1x10^6$ BM-CAFs, mice were randomized into two groups: 1) SLAMF7-CART or 2) FAP-CART. **B,** Tumor burden was assessed by BLI curve (mean and SEM, two-way ANOVA; n=3). **C,** Mice were euthanized when they reached an endpoint due to the high tumor load. Bone marrow was harvested and immunohistochemical staining was performed (H&E magnification 10, CD138 magnification 10, and FSP-1 magnification 20).

Supplemental Figure S19 | BCMA-, dual BCMA-SLAMF7-, or BCMA-FAP-CART degranulation and intracytoplasmic cytokine assay. CART were co-cultured with OPM-2 and BM-CAFs for 4 hours and stained for CD107a and intracytoplasmic cytokines (mean and SEM, ****p<0.0001, ***p<0.001, **p<0.01, one-way ANOVA; n=3, 2 replicates).

Supplemental Figure S20 | Cytokine and chemokine analysis of BCMA- or dual CART in the presence of BM-CAFs. A and B, BCMA-, dual BCMA-SLAMF7- or

BCMA-FAP-CART were co-cultured with irradiated MM1.S (A) or OPM-2 (B) for 3 days in the presence of BM-CAFs and supernatant were analyzed for cytokines using multiplex (mean and SEM*p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001, one-way ANOVA; n=2, 2 replicates).

Supplemental Figure S21 | Anti-tumor efficacy of BCMA- and dual BCMA-SLAMF7-CART against OPM-2 and MM1.S cells *in vitro* and *in vivo*. A and B, BCMA or BCMA-CS1 CART cells equally lysed OPM-2 cells **(A)** or MM1.S cells **(B)** within 24 hours (two-way ANOVA; n=3, 2 replicates). **C and D,** CART CD107a degranulation assay upon stimulation of OPM-2 **(C)** or MM1.S **(D)** cells (mean and SEM, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, one-way ANOVA), n=3, 2 replicates. **E-G,** The head-to-head comparison of single BCMA-CART and dual BCMA-SLAMF7-CART in an OPM-2 xenograft mouse model. NSG mice were injected with $1x10^6$ of luciferase$^+$ OPM-2 cells on day -28. On day -1, tumor burden was assessed with bioluminescence imaging (BLI) and mice were randomized according to the tumor burden. On day 0, mice received $1x10^6$ of 1) UTD, 2) BCMA-CART, or 3) BCMA-SLAMF7-CART (mean and SEM, * p<0.05 at day 13, two-way ANOVA; n=5 per group). **G,** Kaplan-Meier curve of OPM-2 xenograft mouse model is shown [BCMA-CART vs. BCMA-SLAMF7-CART hazard ratio = 0.0630; 95% confidence interval (CI) = 0.005903-0.6722, *p=0.05].

Supplemental Figure S22 | Dual BCMA-SLAMF7 targeting CART cells overcome BM-CAF-induced impairment of BCMA-CART killing. UTD, BCMA-CART, or

BCMA-SLAMF7-CART-cells were co-cultured with luciferase positive OPM-2 cells at different E:T ratio, in the presence or absence of BM-CAFs (ratio of OPM-2: CAFs of 1:0.1; at 0.6:1 ratio, mean and SEM, * p<0.05, ** p<0.01, one-way ANOVA); n=3, 2 replicates.

Supplemental Figure S23 | BCMA-SLAMF7- or BCMA-FAP-CART demonstrate a long-term durable response and improve overall survival in the MM-TME mouse model. A, Experimental schema. Three weeks after the injection of $1x10^6$ luciferase$^+$ MM1.S and $1x10^6$ BM-CAFs, mice were randomized into four groups: 1) UTD, 2) BCMA-CART, 3) BCMA-SLAMF7-CART, or 4) BCMA-FAP-CART. Tumor burden was assessed by BLI (3 mice per group). **B** and **C**, Bioluminescent imaging of mice treated with UTD or CART (mean and SEM, *p<0.05, two-way ANOVA at day 27). **D**, Kaplan-Meier survival curve is shown. [BCMA-CART vs. BCMA-SLAMF7-CART hazard ratio = 0.06518; 95% confidence interval (CI) = 00.006025 to 0.7051, *p=0.02, BCMA-CART vs. BCMA-FAP-CART hazard ratio = 0.06518; 95% CI = 0.006025 to 0.7051, *p=0.02].

Supplemental Figure S24 | CART-cell expansion and the composition of CART at day 8 of the generation. A, T cells were isolated from the healthy-donor-derived PBMCs. T cells were then stimulated with anti-CD3/CD28 beads at 1:3 (T cells:beads). CARs were lentivirally tranduced on day 1 at an MOI of 3. T cells were counted on days 0, 3, 5, 6, and 8. Beads were removed from T cells on day 6. **B,** Representative flow plots of UTD, BCMA-, FAP-, SLAMF7-, BCMA-FAP-, and BCMA-SLAMF7-CART-

cells (n=3).

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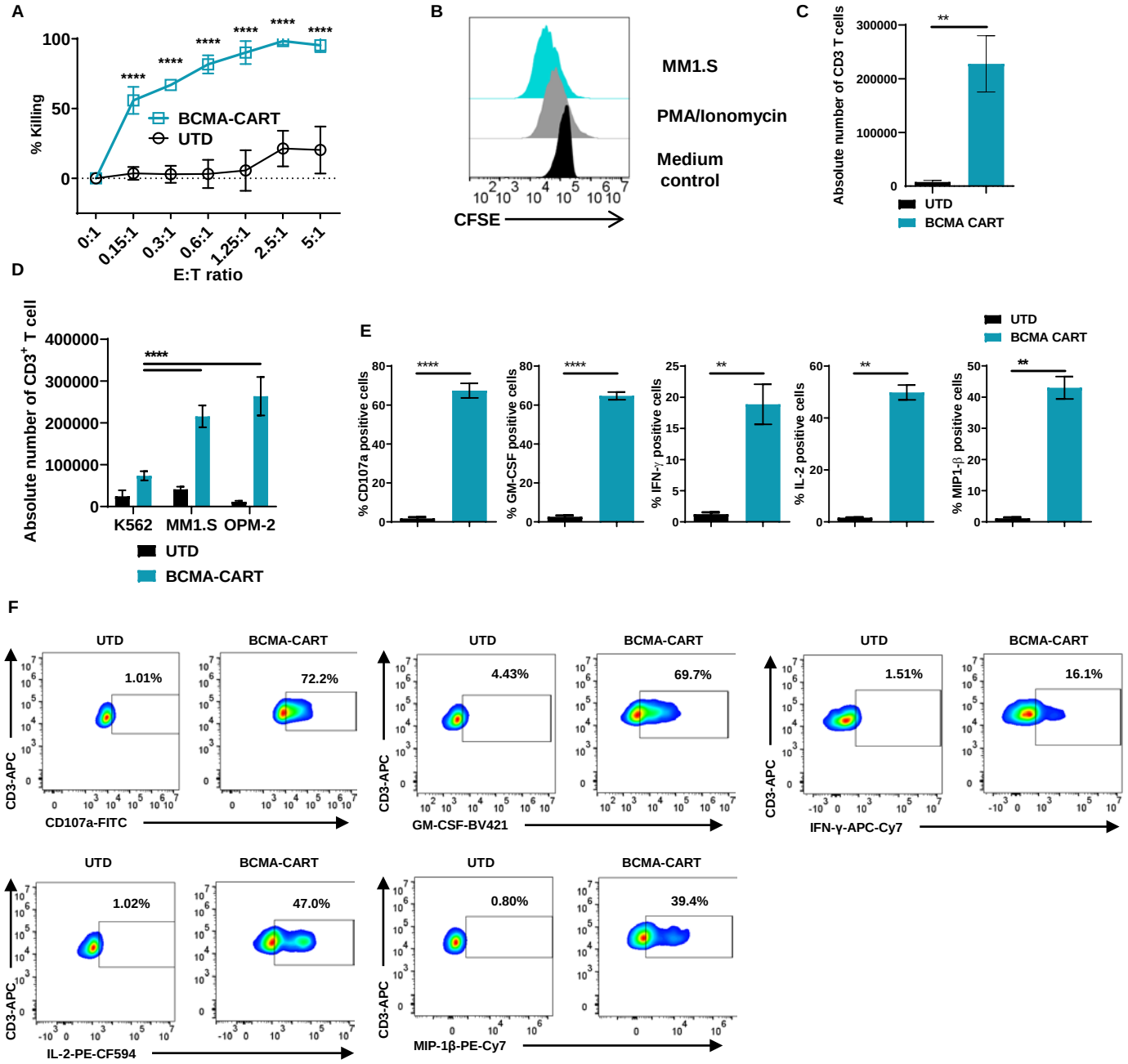
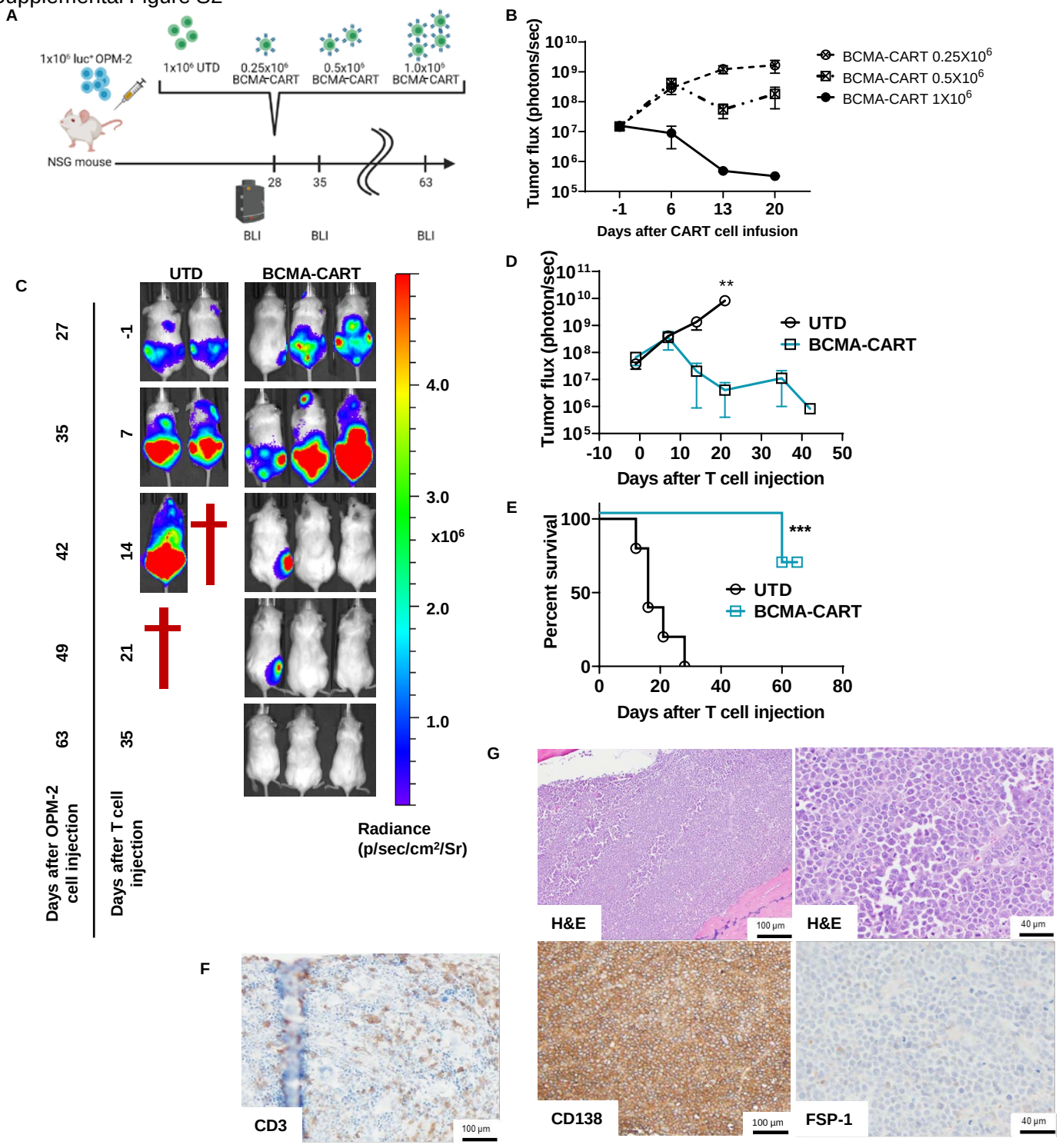
Supplemental Figure S1



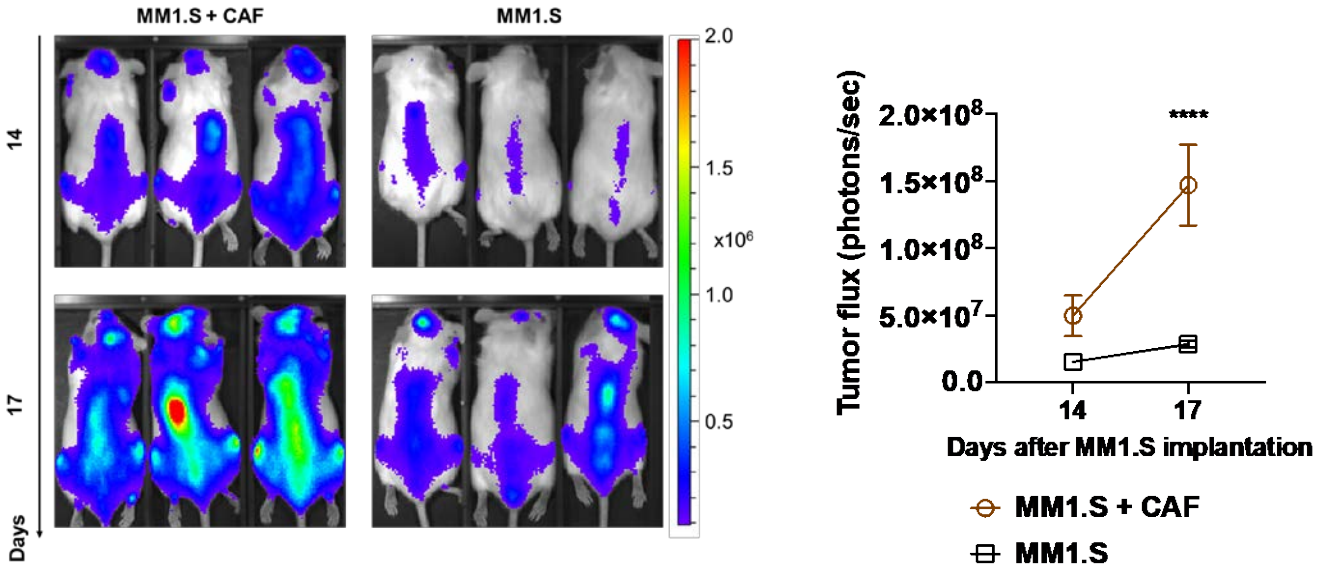
Supplemental Figure S1 | Potent anti-tumor activity of BCMA-CART cells against BCMA expressing cells *in vitro*. **A**, BCMA-CART-cells were co-cultured at different E:T ratios with luciferase$^+$ MM1.S. At 24 hours, cell killing was assessed by luminescence relative to controls (**** p<0.0001, two-way ANOVA; n=3, 2 replicates). **B,** CFSE-labeled BCMA-CART were co-cultured with lethally irradiated MM1.S for 5 days. Cells were then analyzed for CFSE dilution to detect cell proliferation, and absolute number of CART cells was counted by flow cytometry (5 ng/mL phorbol 12-myristate 13-acetate (PMA) and 0.1 µg/mL ionomycin stimulation was used as a positive control; n=3, 2 replicates). **C,** BCMA-CART-cells exhibited more antigen-specific proliferation compared to untransduced T cells (UTD). BCMA-CART or UTD derived from the same donor were co-cultured with lethally irradiated MM1.S for 5 days. Antigen-specific proliferation was measured by flow cytometry after 5 days using absolute counts with counting beads. **D**, BCMA-CART or UTD were co-cultured with lethally irradiated K562, MM1.S, or OPM-2 cells for 5 days. Absolute number of T cells were counted via flow cytometry (mean and SEM, **** p<0.0001, one-way ANOVA; n=3, 2 replicates). **E,** BCMA-CART cell degranulation and cytokine release assay. BCMA-CART degranulation assay and cytokine production. BCMA-CART or UTD were co-cultured with MM1.S for 4 hours. Medium was used as a negative control (mean and SEM, ****p<0.0001, **p<0.005, one-way ANOVA; n=3, 2 replicates). **F**, Representative flow plots of BCMA-CART or UTD CD107a degranulation and intracellular cytokine assays.

Supplemental Figure S2



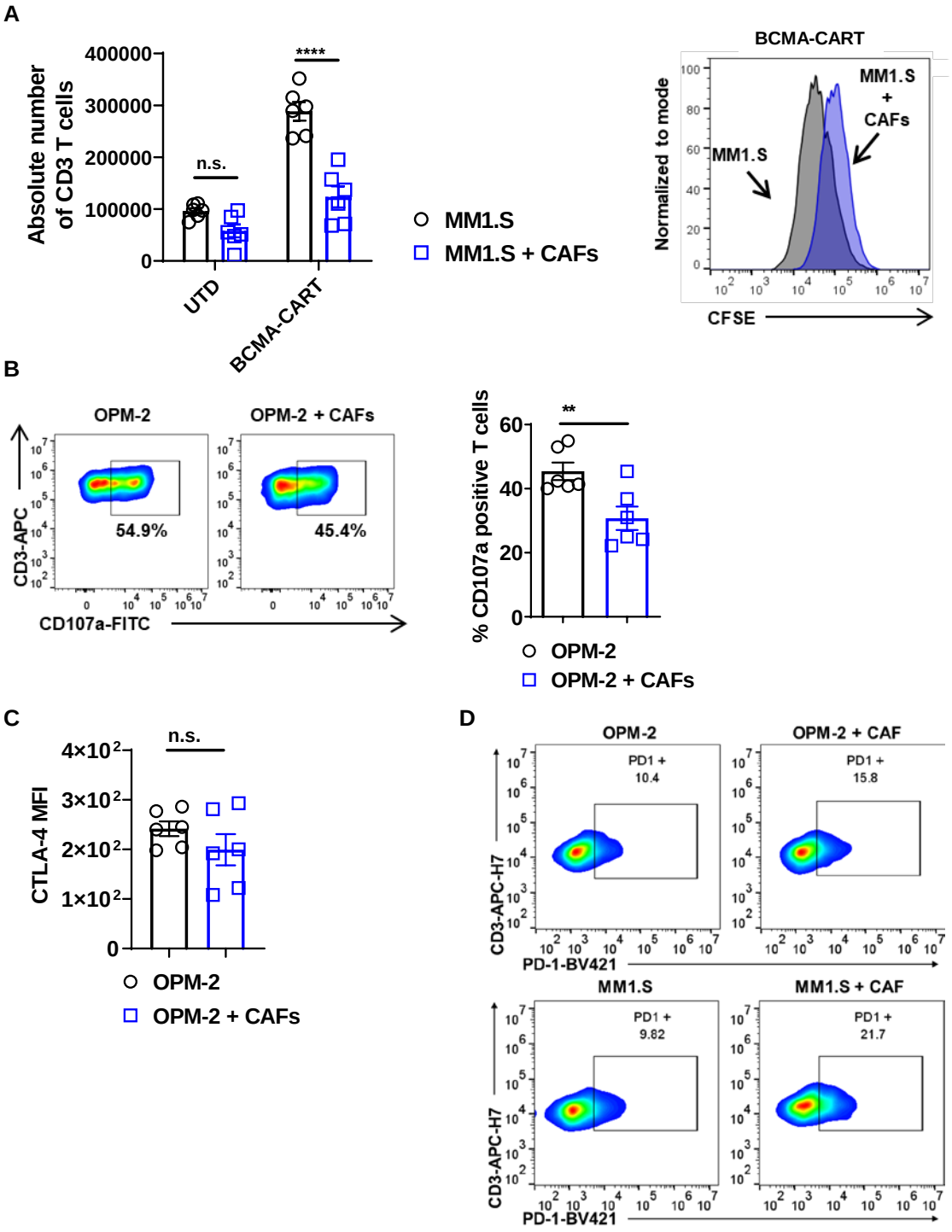
Supplemental Figure S2 | Potent anti-tumor activity of BCMA-CART-cells against BCMA-expressing cells *in vivo*. A, Experimental schema of BCMA-CART-cells in MM xenograft model. NSG mice were engrafted with luciferase^{+}OPM-2 (1x10^{6} cells/mouse, IV, 6 mice per group). On day 28, mice were randomized according to tumor burden, which was accessed by BLI, to receive 1x10^{6} UTD, 1x10^{6} BCMA-CART-cells, 0.5x10^{6} BCMA-CART-cells, or 0.25x10^{6} BCMA-CART-cells. **B,** BLI curve of *in vivo* BCMA-CART-cell dose-finding assay. **C-D,** Anti-myeloma activity of BCMA-CART-cells in OPM-2 xenograft mice, which was shown by bioluminescence imaging (mean and SEM, **p=0.006 at day 21, unpaired, two-sided, Student's t-test). **E**, Kaplan-Meir survival curve is shown [hazard ratio = 0.03320; 95% confidence interval (CI) = 0.004605 to 0.2393, ***p=0.0007, log-rank test]. **F,** Immunohistochemical analysis of BM samples harvested from OPM-2 xenograft mice treated with BCMA-CART-cells (magnification 10). **G,** Immunohistochemical analysis of bone marrow from OPM-2 xenograft models, which were treated with UTD (H&E, *upper left and right,* magnification 10 and 40, respectively. CD138, *lower left*, magnification 10), and FSP-1 (*lower right*) staining revealed the absence of BM-CAFs in this model.

Supplemental Figure S3



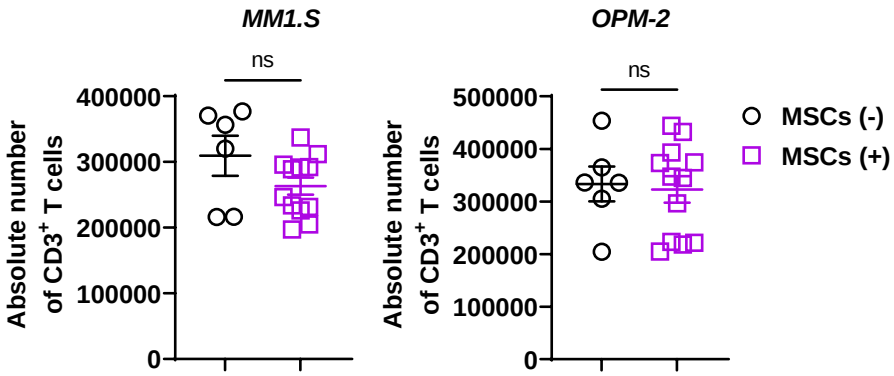
Supplemental Figure S3 | Bone marrow-derived cancer-associated fibroblasts (BM-CAFs) accelerate MM1.S cell growth *in vivo*. NSG mice were intravenously injected with $1x10^6$ of luciferase$^+$ MM1.S cells or in combination with $1x10^6$ of BM-CAFs. Tumor growth was assessed with a bioluminescence imager (mean and SEM, **** $p<0.0001$, two-way ANOVA; n=3).

Supplemental Figure S4



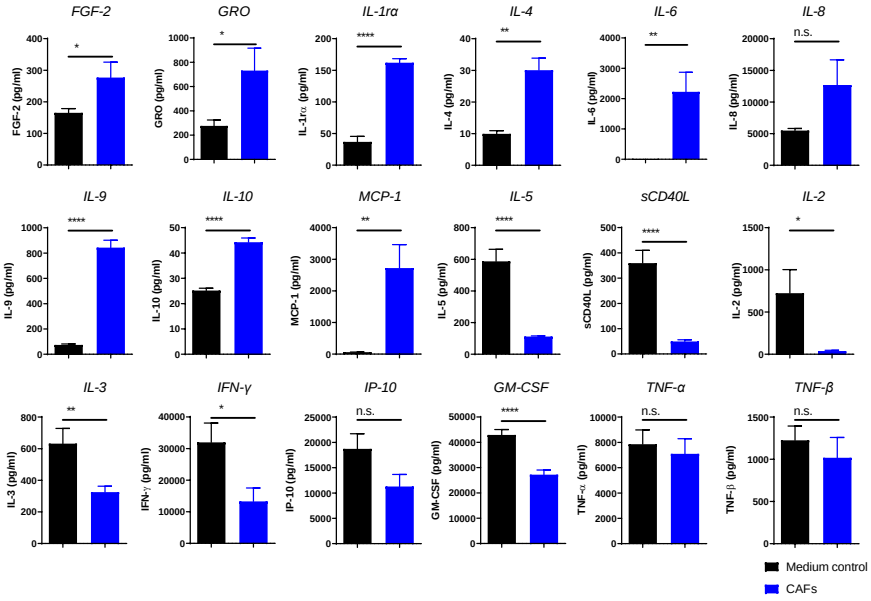
Supplemental Figure S4 | BM-CAFs inhibit BCMA-CART effector functions. A, CFSE-labeled BCMA-CART or UTD were co-cultured with lethally irradiated BCMA^{+}SLAMF7^{+}MM1.S and BM-CAFs for 5 days (mean and SEM, ****p<0.0001, two-way ANOVA; n=3, 2 replicates). **B,** Antigen-specific BCMA-CART CD107a degranulation assay in the presence of BM-CAFs. OPM-2 cells were used as a stimulator (**p<0.01, unpaired, two-sided, Student's t-test; n=3, 2 replicates). **C,** CTLA-4 expression on BCMA-CART in the presence or absence of CAFs (Student's t-test; n=3, 2 replicates). **D**, Representative flow plots of surface PD-1 expression on BCMA-CART cells when co-cultured with OPM-2 (*upper panels*) or MM1.S (*lower panels*) in the presence or absence of CAFs.

Supplemental Figure S5



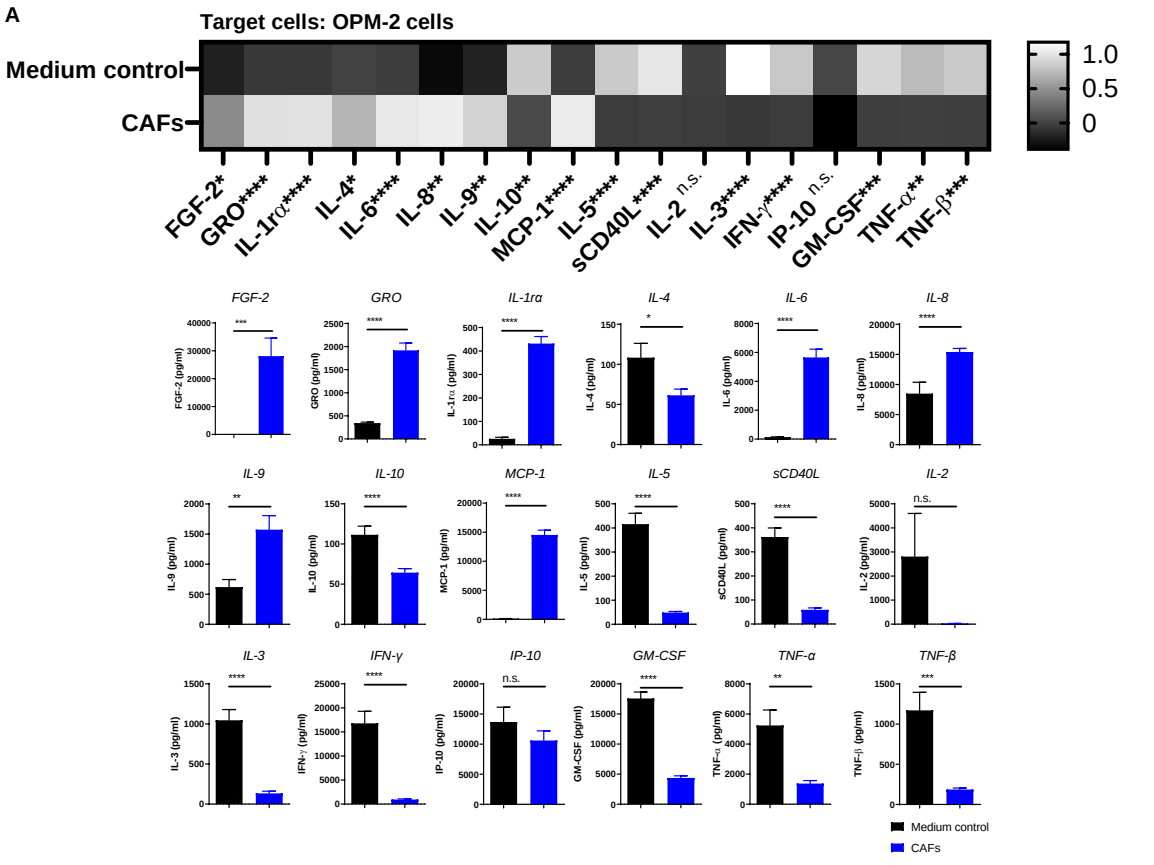
Supplemental Figure S5 | Bone barrow MSCs derived from healthy donor do not inhibit BCMA-CART proliferation. BCMA-CART cells were co-cultured with lethally irradiated BCMA$^+$SLAMF7$^+$MM1.S (*left*) or BCMA$^+$SLAMF7$^+$OPM-2 (*right*) in the presence or absence of MSCs for 5 days. Absolute number of CD3$^+$ T cells were counted via flow cytometer (mean and SEM, t-test; n=6, 2 replicates).

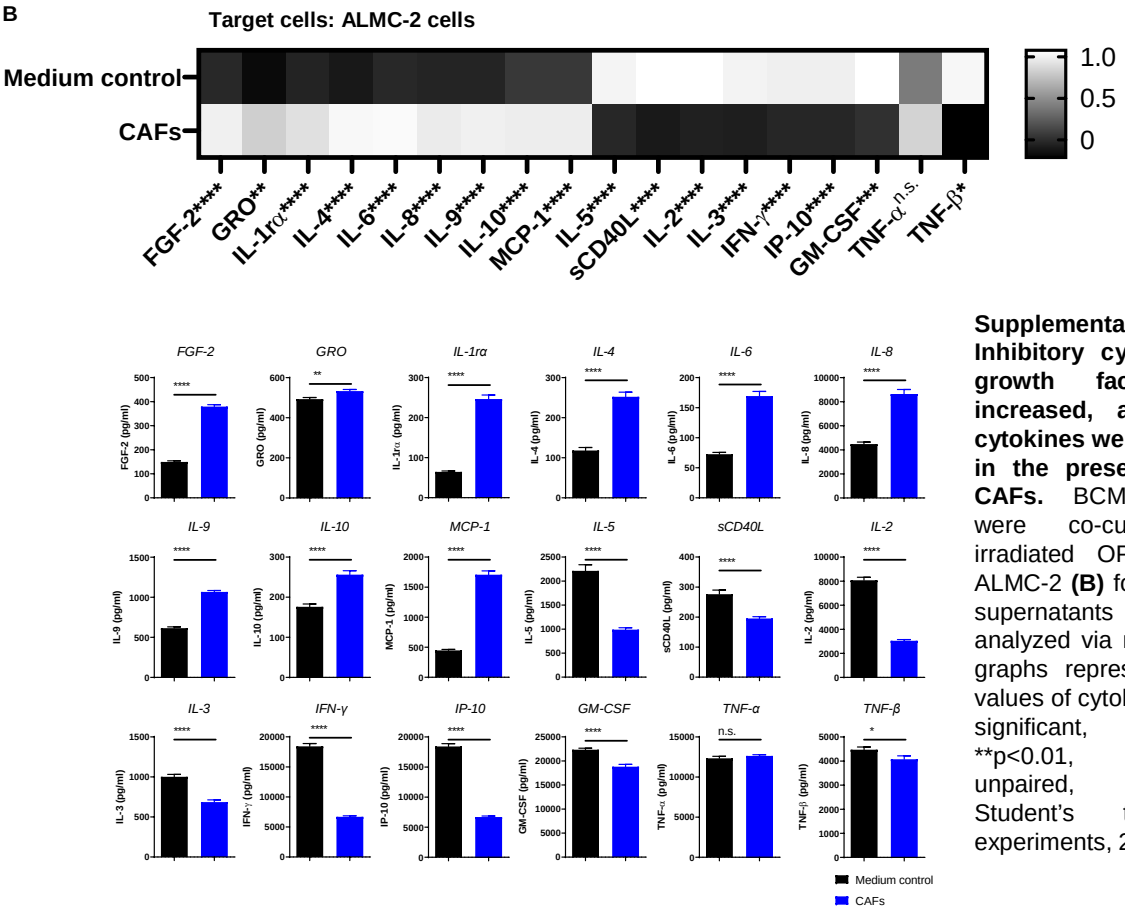
Supplemental Figure S6



Supplemental Figure S6 | Actual values of cytokines from the multiplex assay. Cytokines were analyzed by multiplex using supernatant from the co-culture of BCMA-CART and irradiated MM1.S with or without BM-CAFs (mean and SEM, *p<0.05, **p<0.01, ****p<0.0001, Student's t-test; n=3, 2 replicates).

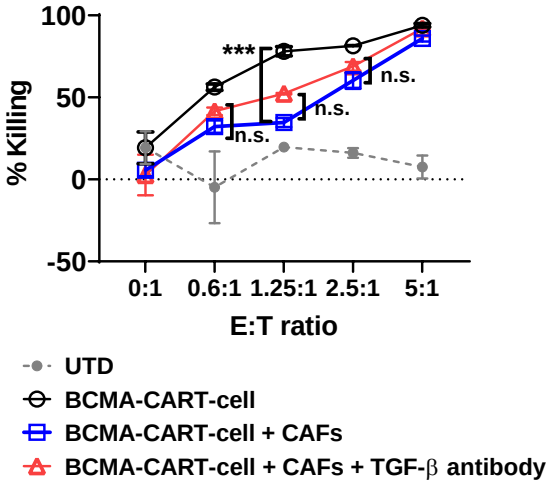
Supplemental Figure S7





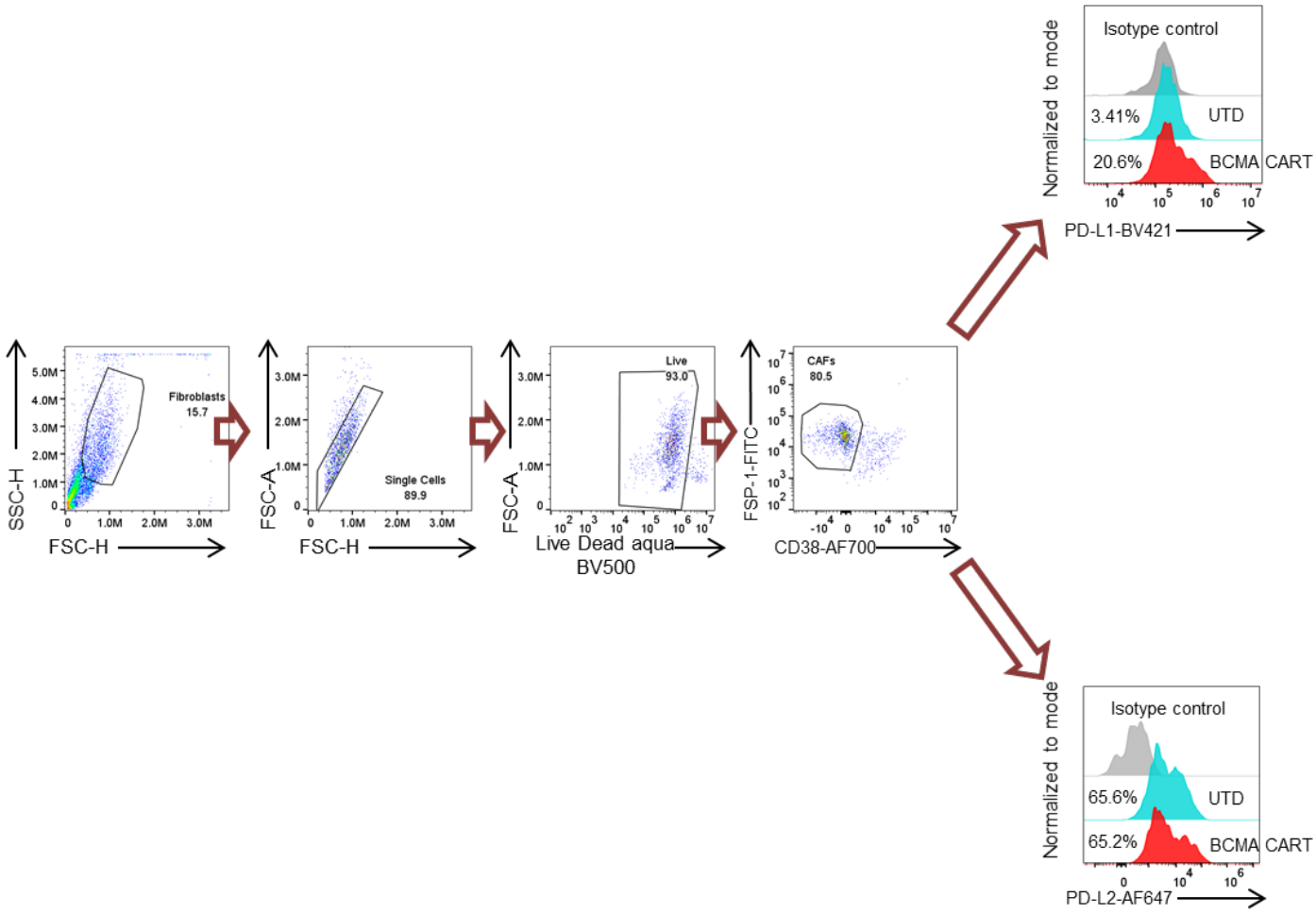
Supplemental Figure S7 | Inhibitory cytokines and growth factors were increased, and effector cytokines were decreased in the presence of BM-CAFs. BCMA-CART-cells were co-cultured with irradiated OPM-2 **(A)** or ALMC-2 **(B)** for 3 days and supernatants were analyzed via multiplex. Bar graphs represent the raw values of cytokines (n.s. not significant, *p<0.05, **p<0.01, ***p<0.005, unpaired, two-sided, Student's t-test; n=3 experiments, 2 replicates).

Supplemental Figure S8



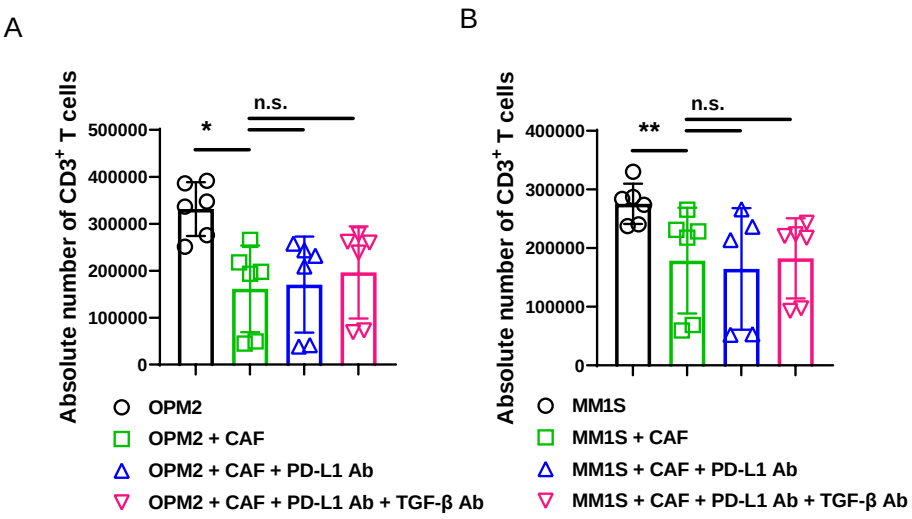
Supplemental Figure S8 | TGF-β depletion does not overcome BM-CAF induced impairment of BCMA CART cytotoxicity. BCMA-CART were co-cultured with MM1.S and BM-CAFs in the presence or absence of anti TGF-β antibody. At 24 hours, cytotoxicity was assessed by luminescence relative to controls (*** $p<0.001$, two-way ANOVA; n=3, 2 replicates).

Supplemental Figure S9



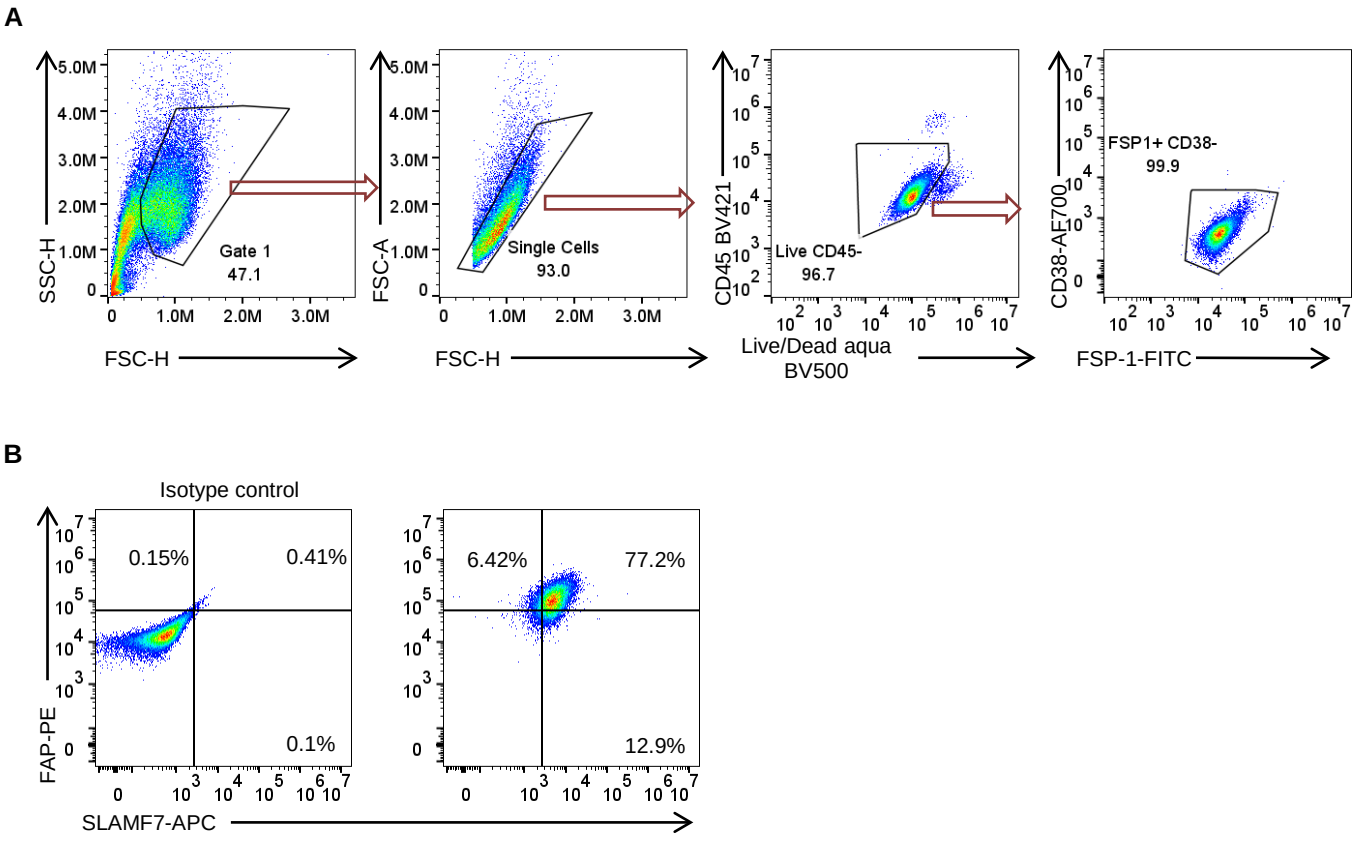
Supplemental Figure S9 | Flow cytometric gating for assessment of inhibitory receptor-ligand expression. BCMA-CART were co-cultured with lethally irradiated MM1.S and BM-CAFs. Cells were gated on FSC/SCC followed by singlet and live cell discrimination. CD3, CD38, CD45, and FSP-1 were used to distinguish CAFs from CART-cells. The surface PD-L1 or PD-L2 expression on BM-CAFs were assessed by flow cytometry. Isotype controls are shown as gray peaks.

Supplemental Figure S10



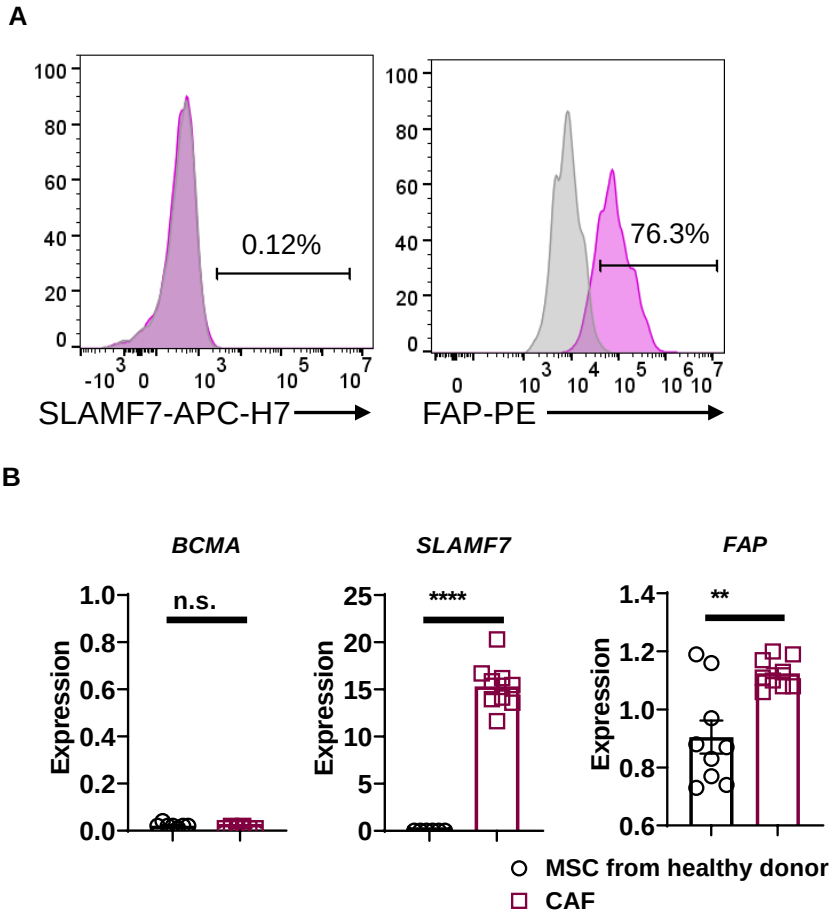
Supplemental Figure S10 | CAF inhibition of BCMA-CART-cell proliferation cannot be reversed by blocking PD-1/PD-L1 axis and/or TGF-β neutralization. BCMA-CART cells were co-cultured with lethally irradiated OPM-2 (A) or MM1.S (B) in the presence or absence of CAFs for 5 days. PD-L1 blocking antibody (20 μg/mL) and/or TGF-β neutralizing antibody (1 μg/mL) were also added to some conditions. Absolute number of $CD3^+$ T cells were assessed by flow cytometry on day 5 (mean and SEM, *p<0.05, **p<0.001, t-test; n=3, 2 replicates).

Supplemental Figure S11



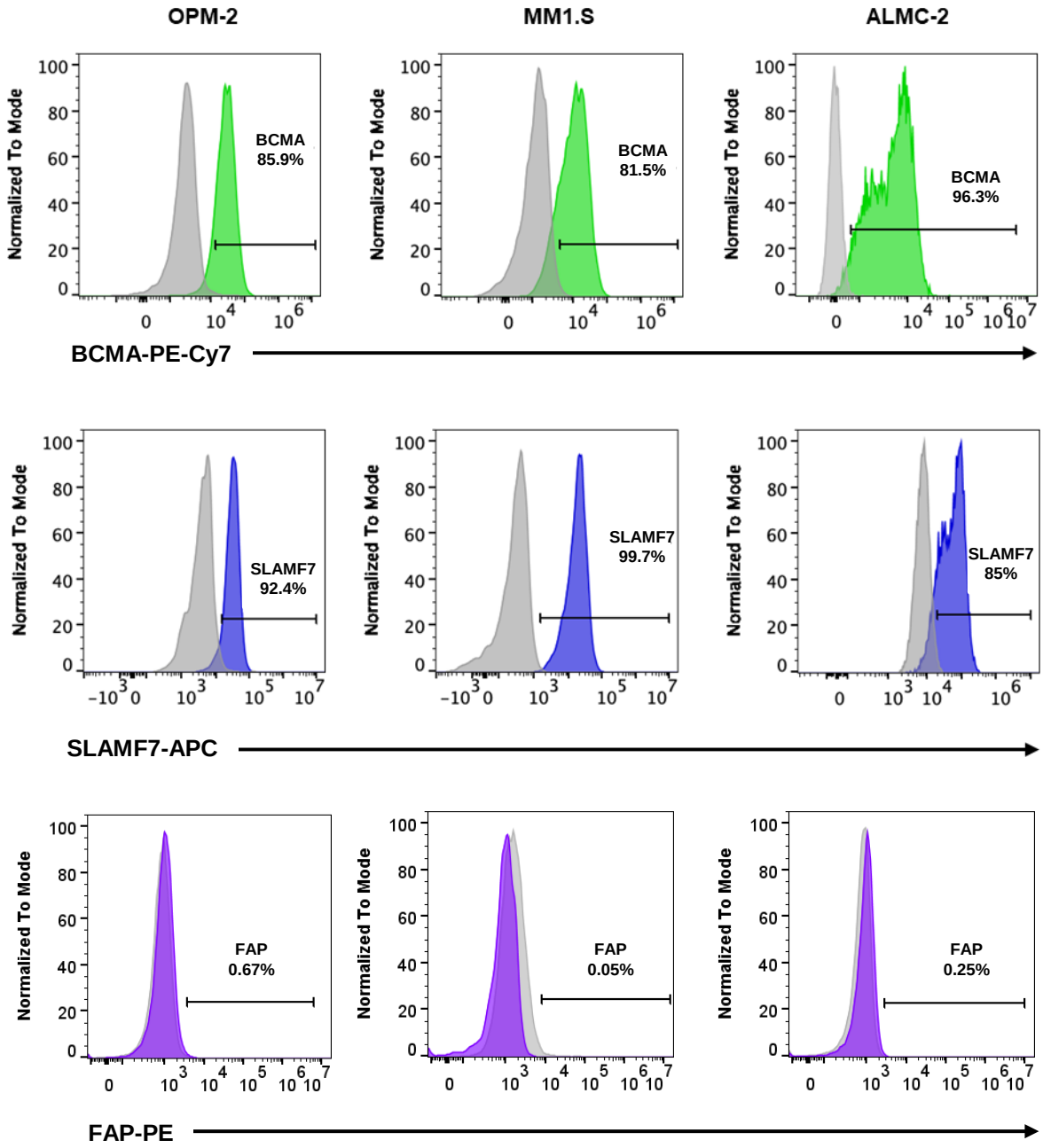
Supplemental Figure S11 | Flow cytometry gating for BM-CAFs and CD45 negative fraction derived from bone marrow of patients with multiple myeloma. A, Gating of BM-CAFs samples from patients with MM. The bone marrow was first isolated with CD138^{+} microbeads. Then, CD138^{-} fraction was cultured for two weeks and CAFs were isolated with anti-fibroblasts microbeads. BM-CAFs were defined as the live, CD45^{-}CD38^{-}FSP-1^{+} fraction. **B,** Representative flow plots of BM-CAFs. Isotype control of IgG1-APC and IgG1-PE were used for left flow plots. SLAMF7-APC and FAP-PE were used for right flow plots.

Supplemental Figure S12



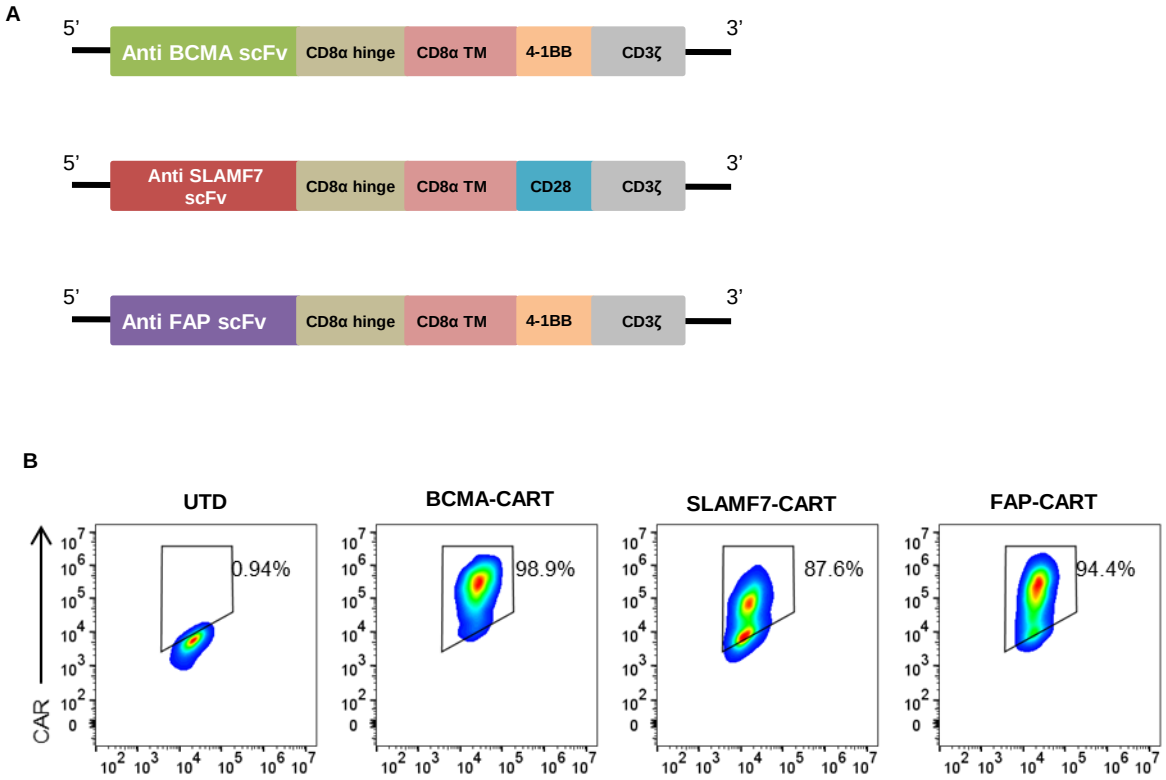
Supplemental Figure S12 | Flow cytometric analysis for BM-MSCs and qPCR analysis of BM-MSCs and BM-CAFs. A, Flow cytometric analysis for BM-MSCs derived from healthy donor. MSCs were defined as $CD38^-$, $CD45^-$, and FSP^+ cells. Grey peaks are fluorescence minus one (FMO) controls. Purple peaks are the stained samples. Representative histograms are shown; n=3. **B,** qPCR analysis for BM-MSC derived from healthy donors and BM-CAFs from MM patients (** $p<0.01$, **** $p<0.0001$, Student's t-test; n=3, 3 replicates).

Supplemental Figure S13



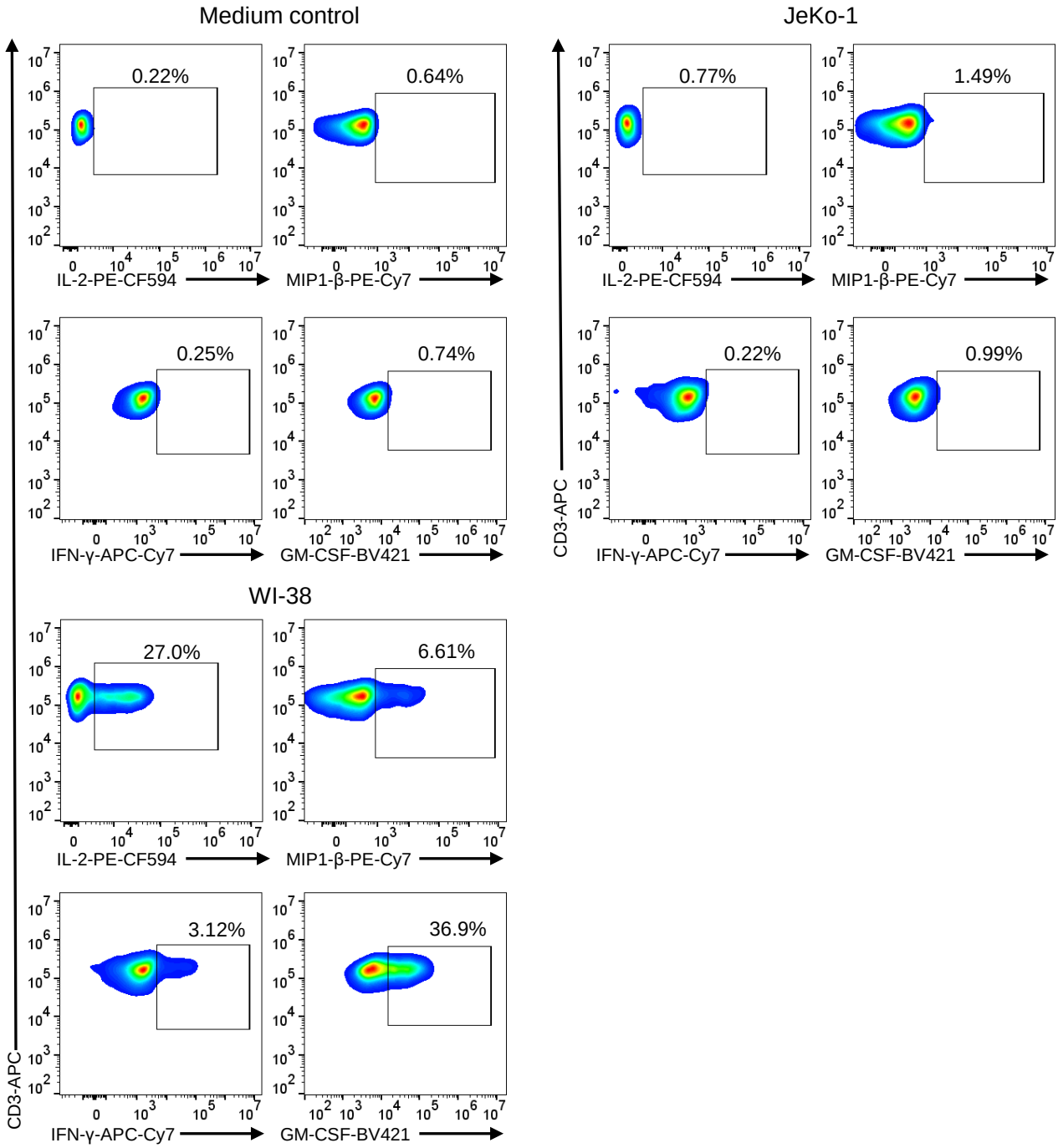
Supplemental Figure S13 | Flow cytometric analysis of BCMA, SLAMF7, and FAP expression on OPM-2, MM1.S, and ALMC-2 cells. Grey peaks represent the isotype controls.

Supplemental Figure S14



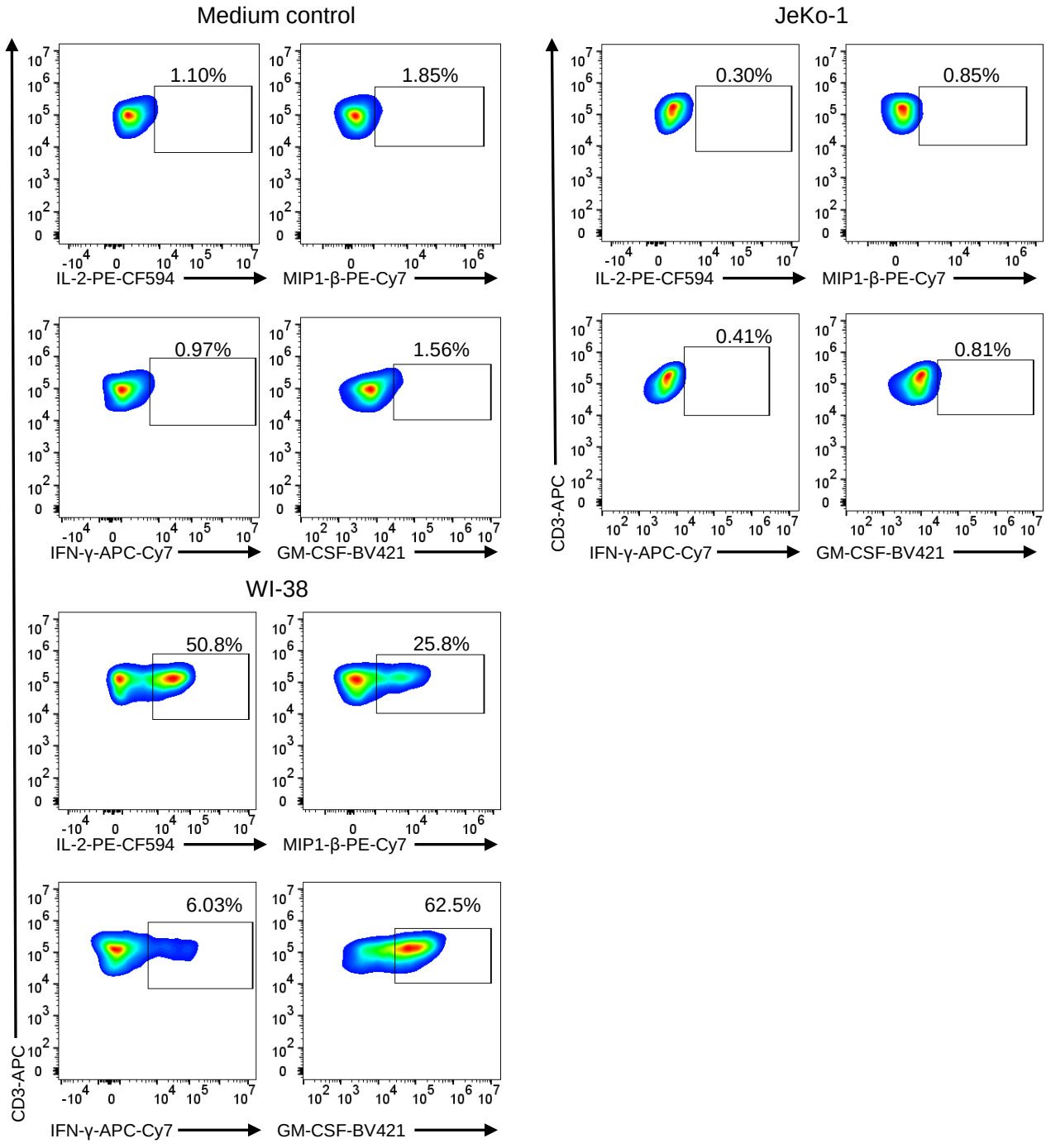
Supplemental Figure S14| Constructs of the BCMA-, SLAMF7-, and FAP-CAR vector, and surface CAR expression on human CD3 T-cells. A, Schematic representation of the BCMA-, SLAMF7-, and FAP-CAR constructs. BCMA-CAR consisted of anti-BCMA single chain variable fragment (scFv) linked to CD3 zeta and a 4-1BB costimulatory domain. H, hinge; TM, transmembrane. SLAMF7-CAR consisted of anti-SLAMF7 scFv linked to CD3 zeta and a CD28 costimulatory domain. FAP-CAR consisted of anti-FAP scFv linked to CD3 zeta and a 4-1BB costimulatory domain. **B**, Representative flow plots of UTDs, BCMA-, SLAMF7-, and FAP-CART. Goat anti-mouse F(ab')2 antibody (GAM) was used with live/dead aqua to detect CAR expression on CART-cells. Cells were gated on FSC/SSC followed by singlet discrimination and live cells. Negative gates for CAR expression were set based on untransduced (UTD) T cells.

Supplemental Figure S15



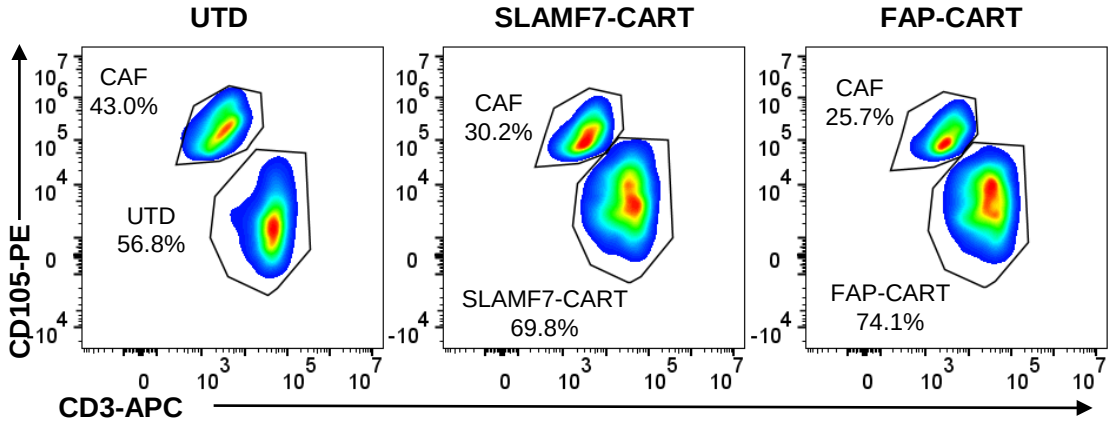
Supplemental Figure S15 | Representative flow plots of FAP-CART degranulation assay. FAP-CART stimulated with FAP^{+}WI-38 or FAP^{-}JeKo-1 cells. CD3 was used to distinguish CART-cells from target cells.

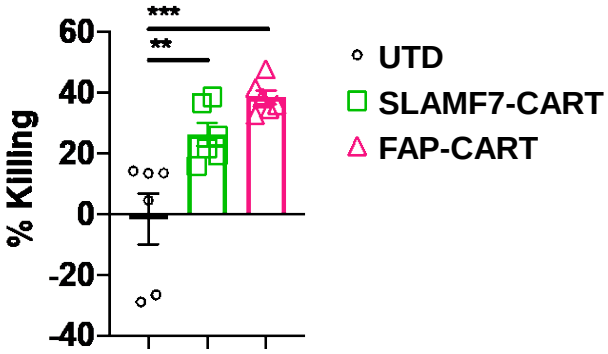
Supplemental Figure S16



Supplemental Figure S16 | Representative flow plots of SLAMF7-CART-cell degranulation assay. SLAMF7-CART-cells stimulated with SLAMF7^{+}MM1.S or SLAMF7^{-}Jurkat cells. CD3 was used to distinguish CART-cells from target cells.

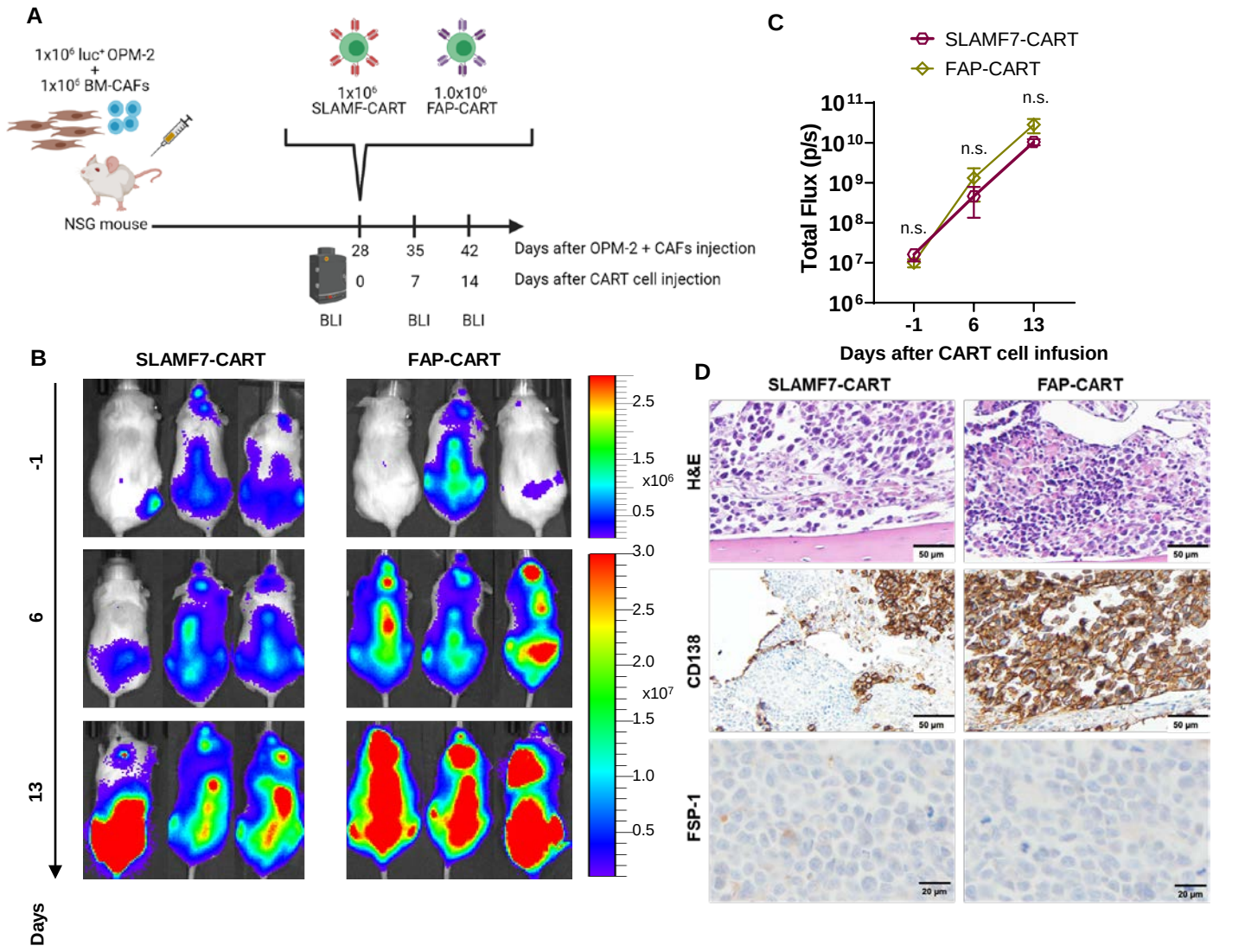
Supplemental Figure S17





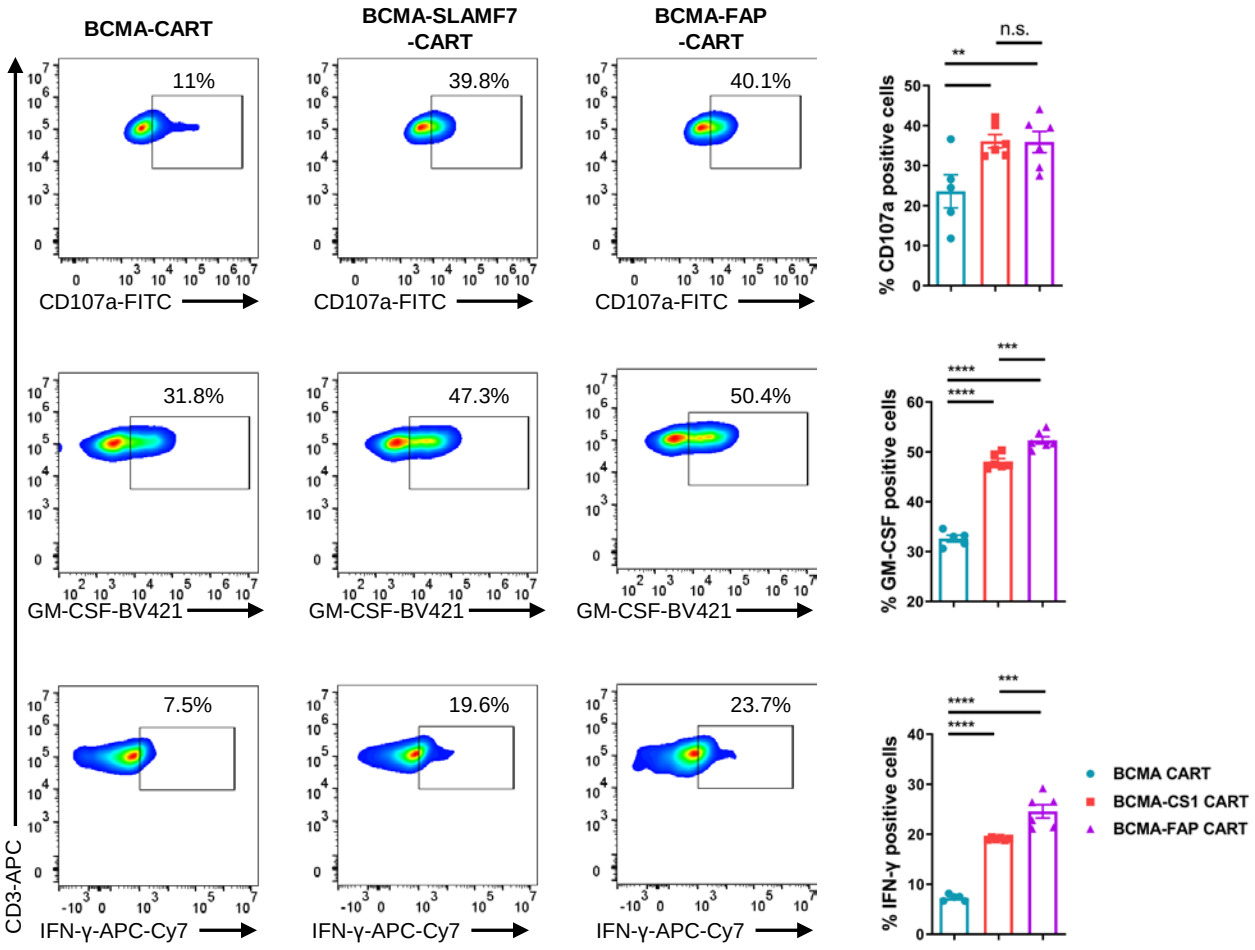
Supplemental Figure S17 | SLAMF7- or FAP-CART cytotoxicity assay against BM-CAFs. SLAMF7- or FAP-CART were co-cultured with BM-CAFs at 1:1 ratio. At 24 hours, cytotoxicity was assessed relative to controls. CD105 and CD3 were used to differentiate CAF and T cells (mean and SEM, ** p<0.01, *** p<0.001, one-way ANOVA; n=3, 2 replicates).

Supplemental Figure S18



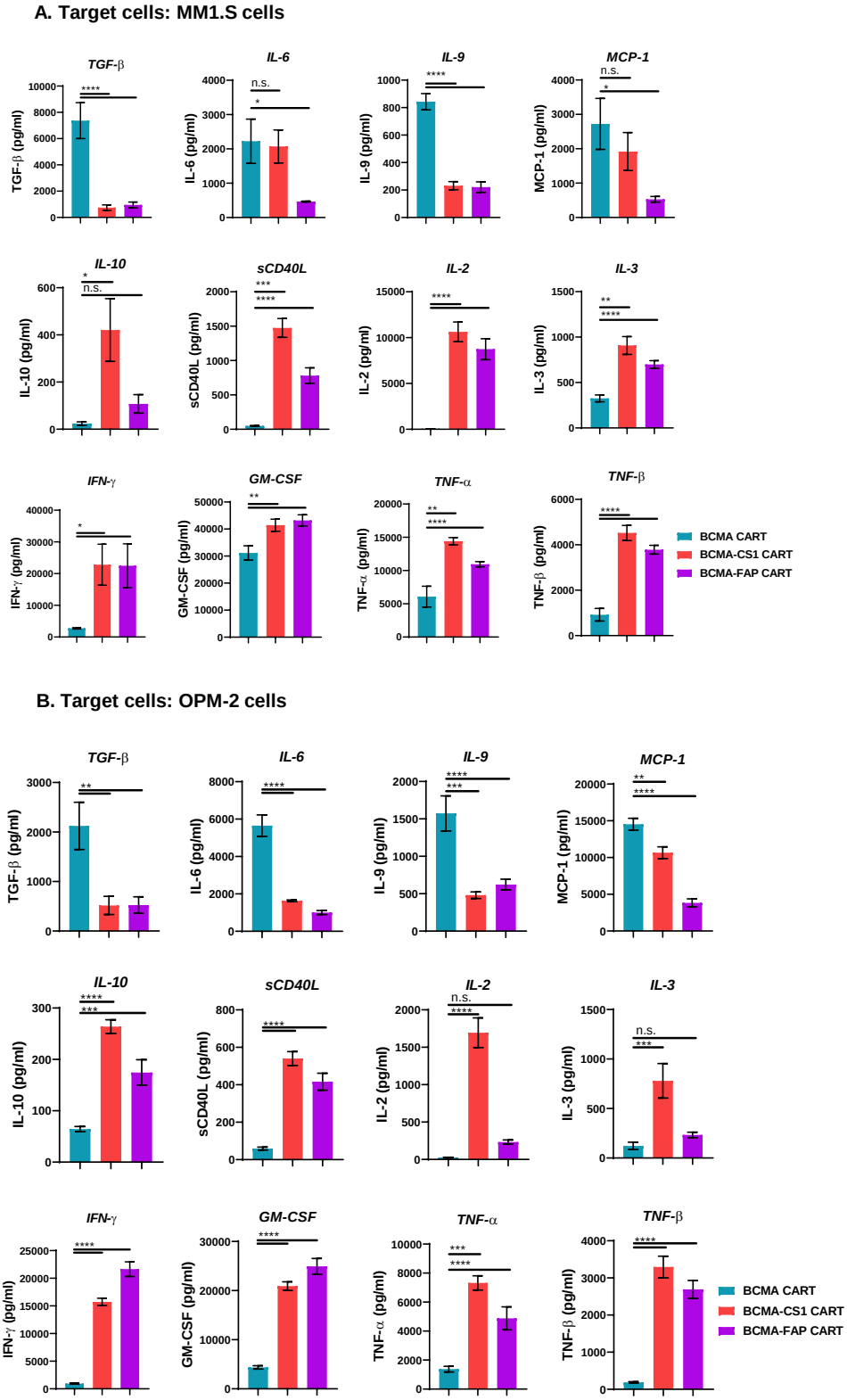
Supplemental Figure S18 | SLAMF7 or FAP-CART inhibit CAF growth *in vivo*. A, Experimental schema. Four weeks after the injection of 1x10^6 luciferase positive OPM-2 and 1x10^6 BM-CAFs, mice were randomized into two groups: 1) SLAMF7-CART or 2) FAP-CART. **B,** Tumor burden was assessed by BLI curve (mean and SEM, two-way ANOVA; n=3). **C,** Mice were euthanized when they reached an endpoint due to the high tumor load. Bone marrow was harvested and immunohistochemical staining was performed (H&E magnification 10, CD138 magnification 10, and FSP-1 magnification 20).

Supplemental Figure S19



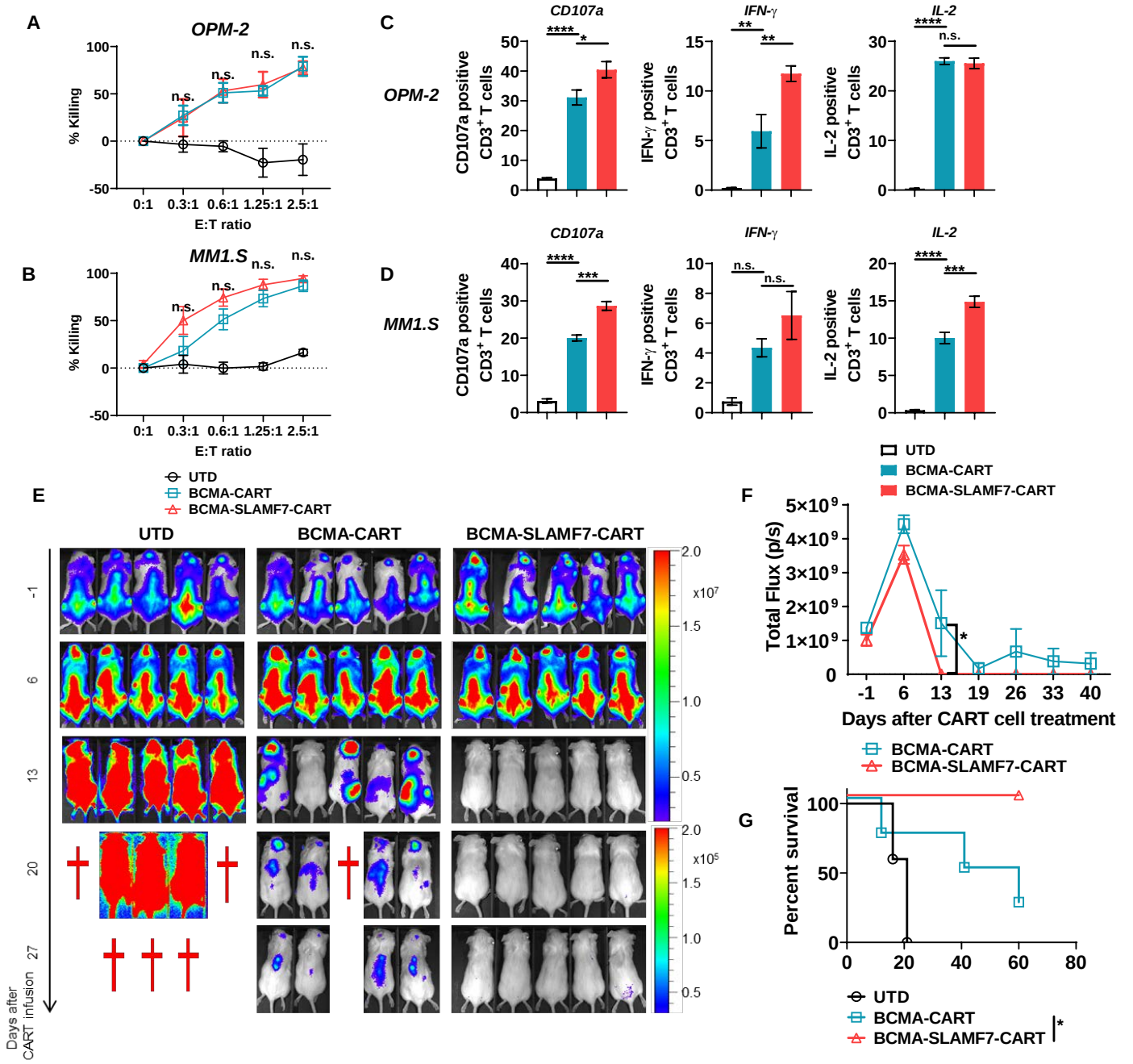
Supplemental Figure S19 | BCMA-, dual BCMA-SLAMF7-, or BCMA-FAP-CART degranulation and intracytoplasmic cytokine assay. CART were co-cultured with OPM-2 and BM-CAFs for 4 hours and stained for CD107a and intracytoplasmic cytokines (mean and SEM, ****p<0.0001, ***p<0.001, **p<0.01, one-way ANOVA; n=3, 2 replicates).

Supplemental Figure S20



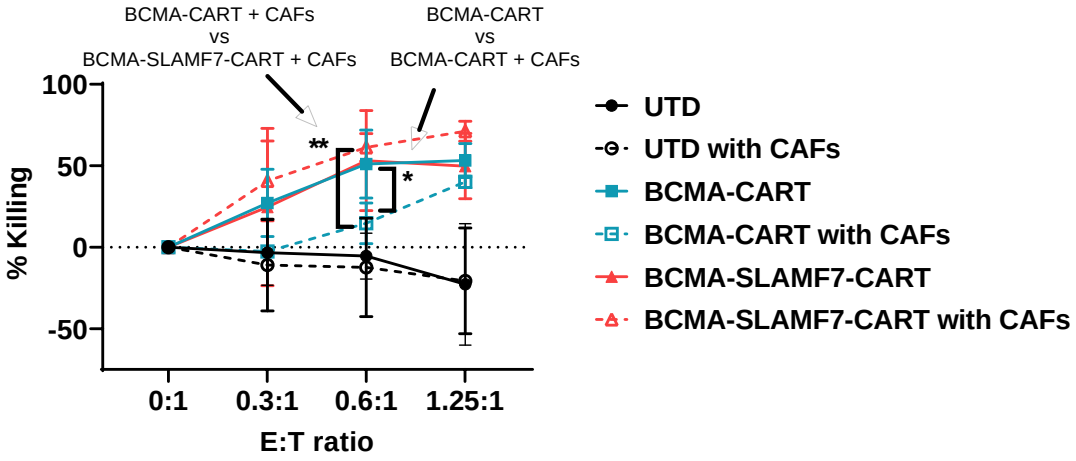
Supplemental Figure S20 | Cytokine and chemokine analysis of BCMA- or dual CART in the presence of BM-CAFs. A and B, BCMA-, dual BCMA-SLAMF7- or BCMA-FAP-CART were co-cultured with irradiated MM1.S (A) or OPM-2 (B) for 3 days in the presence of BM-CAFs and supernatant were analyzed for cytokines using multiplex (mean and SEM*p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001, one-way ANOVA; n=2, 2 replicates).

Supplemental Figure S21



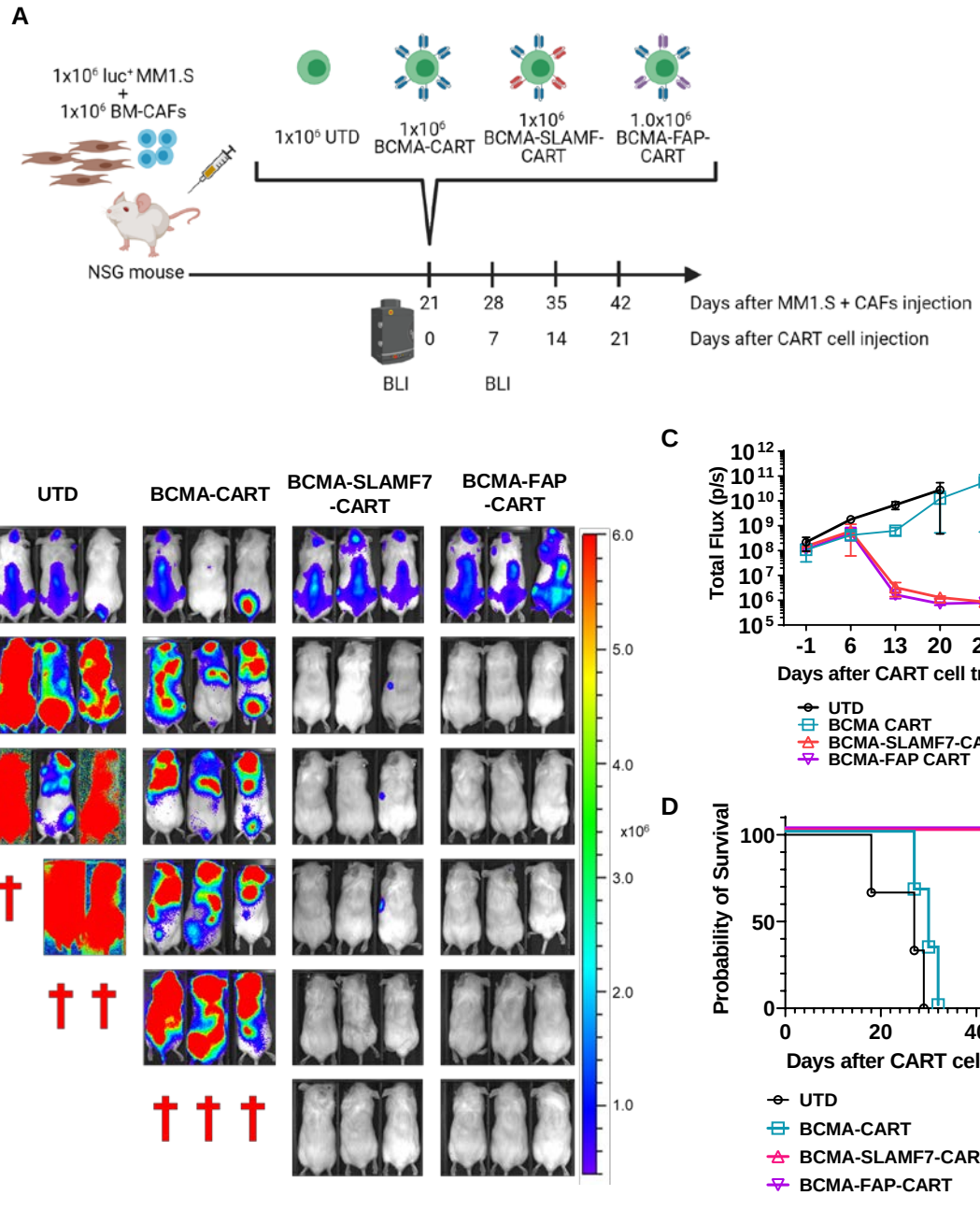
Supplemental Figure S21 | Anti-tumor efficacy of BCMA- and dual BCMA-SLAMF7-CART against OPM-2 and MM1.S cells *in vitro* and *in vivo*. A and B, BCMA or BCMA-CS1 CART cells equally lysed OPM-2 cells **(A)** or MM1.S cells **(B)** within 24 hours (two-way ANOVA; n=3, 2 replicates). **C and D,** CART CD107a degranulation assay upon stimulation of OPM-2 **(C)** or MM1.S **(D)** cells (mean and SEM, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, one-way ANOVA), n=3, 2 replicates. **E-G,** The head-to-head comparison of single BCMA-CART and dual BCMA-SLAMF7-CART in an OPM-2 xenograft mouse model. NSG mice were injected with 1x10^6 of luciferase$^+$ OPM-2 cells on day -28. On day -1, tumor burden was assessed with bioluminescence imaging (BLI) and mice were randomized according to the tumor burden. On day 0, mice received 1x10^6 of 1) UTD, 2) BCMA-CART, or 3) BCMA-SLAMF7-CART (mean and SEM, * p<0.05 at day 13, two-way ANOVA; n=5 per group). **G,** Kaplan-Meier curve of OPM-2 xenograft mouse model is shown [BCMA-CART vs. BCMA-SLAMF7-CART hazard ratio = 0.0630; 95% confidence interval (CI) = 0.005903-0.6722, *p=0.05].

Supplemental Figure S22



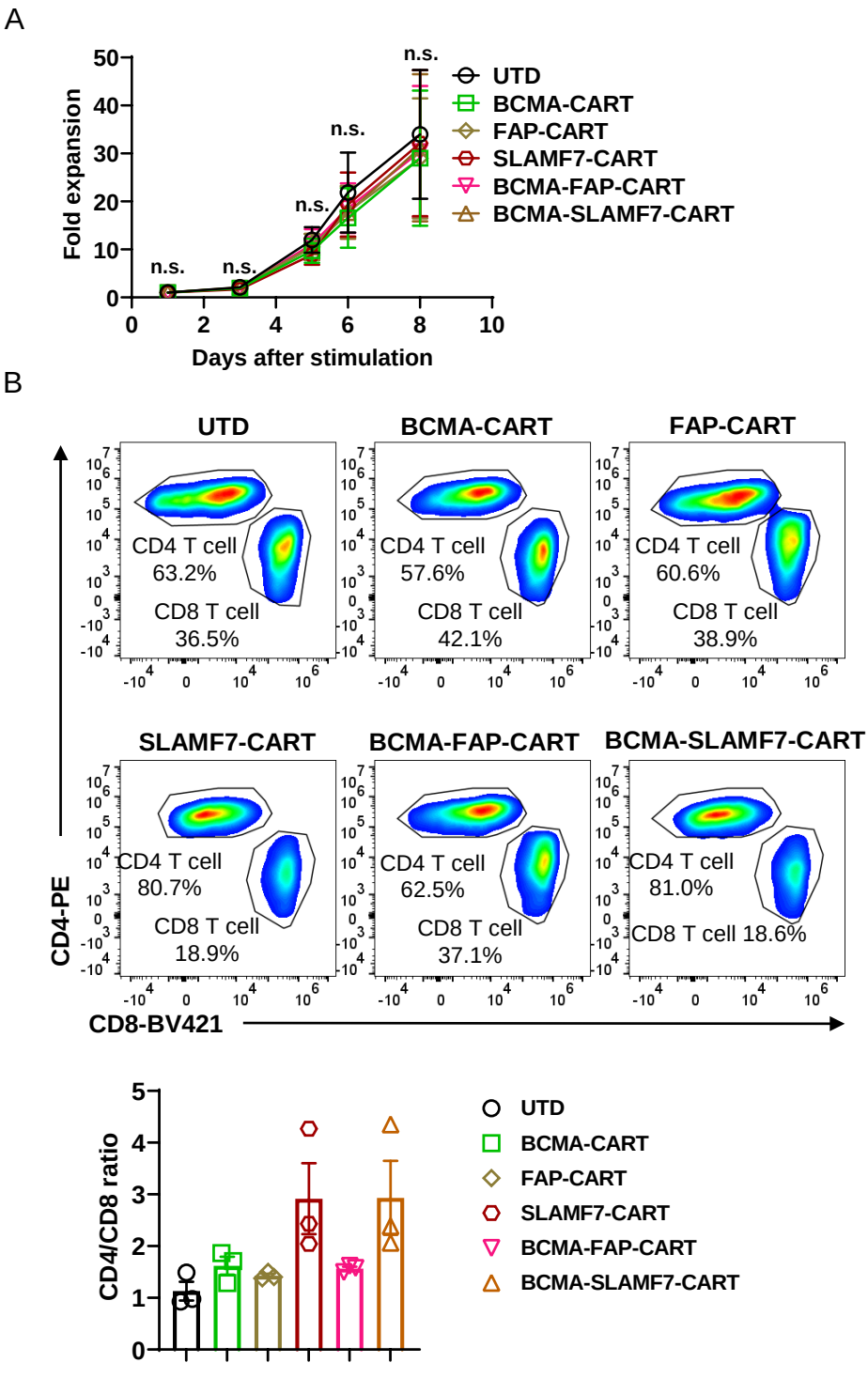
Supplemental Figure S22 | Dual BCMA-SLAMF7 targeting CART cells overcome BM-CAF-induced impairment of BCMA-CART killing. UTD, BCMA-CART, or BCMA-SLAMF7-CART-cells were co-cultured with luciferase positive OPM-2 cells at different E:T ratio, in the presence or absence of BM-CAFs (ratio of OPM-2: CAFs of 1:0.1; at 0.6:1 ratio, mean and SEM, * $p<0.05$, ** $p<0.01$, one-way ANOVA); n=3, 2 replicates.

Supplemental Figure S23



Supplemental Figure S23 | BCMA-SLAMF7- or BCMA-FAP-CART demonstrate a long-term durable response and improve overall survival in the MM-TME mouse model. A, Experimental schema. Three weeks after the injection of 1x10^6 luciferase$^+$ MM1.S and 1x10^6 BM-CAFs, mice were randomized into four groups: 1) UTD, 2) BCMA-CART, 3) BCMA-SLAMF7-CART, or 4) BCMA-FAP-CART. Tumor burden was assessed by BLI (3 mice per group). **B** and **C**, Bioluminescent imaging of mice treated with UTD or CART (mean and SEM, *p<0.05, two-way ANOVA at day 27). **D**, Kaplan-Meier survival curve is shown. [BCMA-CART vs. BCMA-SLAMF7-CART hazard ratio = 0.06518; 95% confidence interval (CI) = 00.006025 to 0.7051, *p=0.02, BCMA-CART vs. BCMA-FAP-CART hazard ratio = 0.06518; 95% CI = 0.006025 to 0.7051, *p=0.02].

Supplemental Figure S24



Supplemental Figure S24 | CART-cell expansion and the composition of CART at day 8 of the generation. A, T cells were isolated from the healthy-donor-derived PBMCs. T cells were then stimulated with anti-CD3/CD28 beads at 1:3 (T cells:beads). CARs were lentivirally tranduced on day 1 at an MOI of 3. T cells were counted on days 0, 3, 5, 6, and 8. Beads were removed from T cells on day 6. **B,** Representative flow plots of UTD, BCMA-, FAP-, SLAMF7-, BCMA-FAP-, and BCMA-SLAMF7-CART-cells (n=3).