

Supplementary Information

Chimeric antigen receptor T-cell therapy for atherosclerosis

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Materials and Methods

Mice

Eight-week-old male *Apoe*^{-/-} mice (B6/JGpt-Apoeem1Cd82/Gpt), *Ldlr*^{-/-} mice (B6/JGpt-Ldlrem1Cd82/Gpt), and C57BL/6JGpt mice were purchased from GemPharmatech Company. All mice were housed under specific pathogen-free conditions at the Laboratory Animal Center of Shenzhen Bay Laboratory. Animal facilities were maintained at 24°C with 40-70% relative humidity under a 12 h light/dark cycle, with free access to water and food. All animal procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at Shenzhen Bay Laboratory, Shenzhen, China (Ethical No. AEZZ202501).

Atherosclerosis studies

***Apoe*^{-/-} mouse models.** Eight-week-old male *Apoe*^{-/-} were fed a high-fat diet (HFD, Regular casein and 1.25% added Cholesterol; Research Diets, #D12108C) for a total of 16 or 20 weeks. After 12 weeks of HFD feeding, mice were randomized to receive a single intravenous (IV) injection of 5×10^6 CAR T cells in 100 μ L PBS or 5×10^6 untransduced T cells in 100 μ L PBS. Mice remained on the HFD for an additional 4 or 8 weeks before tissue collection and analysis.

***Ldlr*^{-/-} mouse models.** Eight-week-old male *Ldlr*^{-/-} mice were HFD for a total of 20 weeks. After 16-week HFD feeding, mice were randomized to receive a single IV injection of 5×10^6 CAR T cells in 100 μ L PBS or 5×10^6 untransduced T cells in 100 μ L PBS. Mice remained on the HFD for an additional 4 weeks before tissue collection and analysis.

AAV8-PCSK9 mouse model. Eight-week-old male C57BL/6JGpt mice received a single tail vein injection of AAV8-PCSK9 (5×10^{11} viral genomes in 100 μ L PBS; WZ Biosciences, #D377Y) and were

then fed HFD for a total of 20 weeks. After 16-week HFD feeding, mice were randomized to receive a single IV injection of 5×10^6 CAR T cells in 100 μ L PBS or 5×10^6 untransduced T cells in 100 μ L PBS. Mice remained on the HFD for an additional 4 weeks before tissue collection and analysis.

Single-cell RNA-seq analysis.

Raw sequencing data were obtained from the Gene Expression Omnibus (GEO). The Seurat package was used to generate integrated meta-datasets and conduct downstream analyses. Human arterial vessel datasets included GSE225650, GSE235436, GSE260657, GSE224273, GSE131778, GSE159677, GSE179159, GSE247238, GSE155512, GSE234077, and GSE253903. Mouse aortic datasets included GSE264253, GSE252243, GSE248394, GSE225773, GSE209525, GSE205931, and GSE155513.

Standard quality-control filtering was applied to exclude low-quality and dead cells. Cells with mitochondrial gene content greater than 15% were removed. Principal component analysis (PCA) was conducted on the merged Seurat object, and uniform manifold approximation and projection (UMAP) was conducted using the first 25 principal components for two-dimensional visualization of cellular heterogeneity.

Identification of plaque macrophages and foamy macrophages

In the human arterial single-cell datasets, clustering was conducted using the FindNeighbors and FindClusters functions in Seurat at a resolution of 0.4. Based on expression of canonical macrophage markers, including *CD68*, *CSF1R*, and *CD14*, macrophages were annotated. These macrophages were subsequently subsetted and re-analyzed independently in Seurat, generating 7 subclusters. Among them, foamy macrophages were annotated based on high expression of *PLAC8*, *ITGAX*, *MRC1*, *MMP12*, *GPXMB*, and *TREM2*.

In the mouse aortic samples, clustering was conducted at a resolution of 0.2. Macrophage populations were defined by the expression of *Spp1*, *Trem2*, *Ccl8*, *Cd63*, and *Ccl7*. These macrophage clusters were then extracted and re-analyzed, generating 10 subclusters. Among them, foamy macrophages were annotated based on high expression of *Plac8*, *Itgax*, *Mrc1*, *Mmp12*, *Gpnmb*, and *Trem2*.

Construction of a Trem2 CAR lenti/retroviral vector

The Trem2 CAR lentiviral and retroviral vectors were constructed with the following components arranged from 5' to 3': an Igκ leader sequence, Trem2 single-chain variable fragment (scFv), V5 tag, the co-stimulatory domain of human or mouse CD28, and intracellular signaling domain of human or mouse CD3ζ. The CAR1-TREM2 scFv (RS9.F6) sequence was obtained from the international patent application of Denali Therapeutics (#DNL919). The CAR2-TREM2 scFv (237920) sequence was obtained from the international patent application of R&D Systems (#MAB17291). The CAR3-TREM2 scFv (PI37012) sequence was obtained from the international patent application of Pionyr Immunotherapeutics (#PY314). The final constructs were cloned into a Retro-EF1α-AmpR vector and packaged into viral particles using HEK293T cells.

Lenti/retrovirus packaging

Lentivirus and retrovirus were produced in HEK293T cells using similar procedures. For each 10-cm dish, 6×10^6 HEK293T cells were seeded in 10 mL complete DMEM (Gibco, #21063029) containing 10% fetal bovine serum (FBS; Sigma, #F8687). After 18 h, cells were transiently transfected with polyethylenimine (PEI; Polysciences, #24765-1; prepared at 1 µg/µL in water).

For lentivirus production, each dish was transfected with 12 µg gene-of-interest plasmid, 6 µg pMD2.G envelope plasmid (Addgene, #12259), and 7.5 µg psPAX2 packaging plasmid (Addgene, #12260). For retrovirus production, each dish was transfected with 10 µg gene-of-interest plasmid and 10 µg pCL-Eco

packaging plasmid (Addgene, #12371). Six hours after transfection, the medium was replaced with fresh DMEM containing 2% FBS for lentivirus production or fresh DMEM containing 10% FBS for retrovirus production.

Seventy-two hours later, virus-containing supernatants were collected and centrifuged at $400 \times g$ for 10 min at 4 °C to remove cellular debris, and passed through a 0.45- μ m PES filter (Biosharp, BS-PES-45). Viral particles were then concentrated by ultracentrifugation at $19,800 \times g$ for 2 h at 4 °C. Pellets from each dish were resuspended in 500 μ L RPMI 1640 medium and stored at -80 °C until use.

T cell isolation, activation, and viral transduction

Leukopaks from healthy donors were purchased from Milesbio. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using Ficoll (Cytiva, #17544203). Briefly, whole blood was diluted 1:1 with PBS and centrifuged at $400 \times g$ for 30 min at 18°C. The PBMC layer was collected and washed twice with PBS. Cells were either cryopreserved in CryoStor® CS10 at 2.5×10^7 cells/mL or used immediately for downstream experiments. For immunophenotyping, PBMCs were stained with FITC-conjugated anti-CD3 antibody and analyzed by flow cytometry to determine the percentage of CD3⁺ T cells.

Primary human T cells were activated using Dynabeads™ Human T-Expander CD3/CD28 (Invitrogen, #11141D) at a 1:1 bead-to-cell ratio. Cells were cultured in T cell medium containing 45% RPMI1640, 45% Click's medium (Sigma, #C5572), 10% FBS, and 1% penicillin/streptomycin (Gibco, #15140122), supplemented with 100 U/mL IL-2 (Proteintech, #HZ-1015). After 48 h of activation, the beads were removed.

For lentiviral transduction, 24-well plates were coated with RetroNectin (Takara, #T100AC) for 4 h. Lentiviral supernatant was then added and centrifuged at $2,600 \times g$ for 1.5 h at 4°C to facilitate viral binding. Activated human T cells were seeded at 0.5×10^6 cells per well and culture medium was replaced every two days.

Mouse T cells were isolated using the EasySep™ Mouse T Cell Isolation Kit (Stemcell Technologies, #19851), according to the manufacturer's instructions. Briefly, mice were euthanized by cervical dislocation under isoflurane anesthesia. Lymph nodes and spleen were collected, mechanically dissociated through a 40-μm cell strainer, and centrifuged at $600 \times g$ for 5 min at 4°C. Red blood cells were lysed using red blood cell lysis buffer (Biosharp, #BL503A) for 1 min at room temperature, followed by centrifugation at $600 \times g$ for 5 min at 4°C. The cell pellet was resuspended in 1 mL RPMI 1640, followed by the addition of 20 μL FcR blocker and 50 μL isolation cocktail. After mixing and incubation for 10 min at room temperature, 75 μL RapidSpheres™ were added and the mixture was incubated for additional 2.5 min. After that, the supernatant containing total T cells was collected.

For activation, 1×10^6 mouse T cells were cultured in a 24-well plate in medium containing 45% RPMI 1640 and 45% Click's medium (Sigma, #C5572) supplemented with 10% FBS, 1% Penicillin/Streptomycin (Solarbio, #P1400), L-glutamine (Gibco, #25030-081), β-mercaptoethanol (Sigma, #M6250), 1000U/mL human IL-2 (Proteintech, #HZ-1015-GMP), 0.25 ng/μL anti-CD3 antibody (Bio X Cell, #BE0002), and 1 ng/μL anti-CD28 antibody (Bio X Cell, #BE0015-1) for 48 h. Following activation, cells were transduced with retrovirus using the similar transduction protocol as described for human T cell transduction.

On the day of injection, CAR expression was quantified by anti-V5 flow cytometry. The percentage of CAR-positive cells was determined by flow cytometry using a V5 tag-PE antibody (Invitrogen, #12-6796-42, 1:100). No additional sorting was performed prior to injection, as the transduction efficiency

routinely achieved 60%–90%. Based on the measured CAR⁺ rate, the total number of T cells was adjusted to deliver 5×10^6 CAR⁺ T cells per mouse via tail-vein injection in 100 μ L PBS.

***In vitro* cytotoxicity and cytokine secretion**

THP1 cells were cultured in RPMI 1640 containing 10% FBS and 1% penicillin/streptomycin (complete medium of THP1 cells), whereas RAW264.7 cells were cultured in DMEM containing 10% FBS and 1% penicillin/streptomycin (complete medium of RAW264.7 cells). Target cells were seeded separately in 96-well plates at 1×10^4 cells per well in 100 μ L complete medium. After 4 h, CAR T cells were added at effector-to-target (E:T) ratios of 0.25:1, 0.5:1, 1:1, 2:1, or 4:1. Supernatants were collected after 6 or 24 h of co-culture and stored at -80°C. TNF- α and IFN- γ were measured by ELISA according to the manufacturers' instructions. Human TNF- α and IFN- γ kits (Invitrogen, #88-7346-77 and #88-7316-86) were used for THP1 co-cultures, and mouse TNF- α and IFN- γ kits (Invitrogen, #88-7324-77 and #88-7314-88) were used for RAW264.7 co-cultures.

Cytolytic activity was quantified by bioluminescence. D-luciferin (100 μ L, 1 mM; MedChemExpress, #HY-12591) was added to each well, and luminescence was measured at the indicated time points as relative luminescence units (RLU). RLUMax was defined as the signal from target cells cultured without effector cells. Specific lysis was calculated as $1 - (\text{RLU}_{\text{sample}}/\text{RLU}_{\text{max}})$. To normalize CAR T-cell input across constructs, total T-cell numbers were adjusted according to the measured CAR-positive fraction.

Flow cytometry-based cytotoxicity assay

THP1 and RAW264.7 cells were seeded in 24-well plates at a density of 1×10^5 cells per well and cultured as described above. After 4 h, CAR T cells were added at the E:T ratio of 1:20. Following a 4-day co-culture, all cells were collected and stained with CD3 APC-Cy7 anti-human (BD, #557832, 1:100) or CD3 APC anti-mouse (Gene Baygene 4Abio, FMA003-01, 1:100). Samples were analyzed by flow

cytometry. Absolute cell counts were determined using counting beads (eBioscience, #01-1234-42). Target cell killing was calculated as: Killing efficiency = $1 - (\text{Target cell count in treated group} / \text{Target cell count in target-alone control})$

Flow cytometry

Cells were centrifuged at $400 \times g$ for 5 min at room temperature. The cell pellets were resuspended with 100 μL RPMI 1640 and incubated with the indicated antibodies for 30 min at room temperature. Flow cytometric analysis was conducted on a BD LSRFortessa cytometer. Data were analyzed using FlowJo (Tree Star). The following antibodies were used: V5 tag-PE, Trem2 APC anti-mouse (R&D, #AF1729, 1:100), CD3 APC anti-mouse (Gene Baygene 4Abio, FMA003-01, 1:100), CD3 APC-Cy7 anti-human (BD, #557832, 1:100).

Generation, co-culture and imaging of human blood vessel organoids

To generate human blood vessel organoids, U-bottom ultra-low attachment 96-well plates were coated with anti-adherence rinsing solution (Stemcell, #0-7010), according to the manufacturer's instructions. Human induced pluripotent stem cells (hiPSCs) were suspended in E8 medium (ThermoFisher Scientific, #A1517001) supplemented with 10 μM ROCK inhibitor (Selleck, #S1049) at a concentration of 3,000 cells per 100 μL . A total of 100 μL of the cell suspension was seeded into each well. Plates were centrifuged at $300 \times g$ for 3 min and then incubated at 37°C in a humidified incubator. After 24 h, the culture medium was changed to E8 medium. Vessel organoid differentiation was induced in a chemically defined medium (CDM). The CDM was prepared by mixing 245 mL of DMEM/F-12 (Gibco, #10565018), 245 mL of IMDM (Gibco, #C12440500BT), 5 mL of chemically defined lipid concentrate (Gibco, #11905031), 5 mL of GlutaMAX (Gibco, #35050-061), 500 μL of Insulin-Transferrin-Selenium (Abmole, #M19989), and 20 μL of α -monothioglycerol (Sigma-Aldrich, #M6145). hiPSCs were induced for vascular lineage cell differentiation by continuous treatments with 12 μM CHIR99021 (LC

Laboratories, #C-6556) and 30 ng/mL BMP4 (PeproTech, #120-05ET) for three days. On day 3, medium was replaced by a cocktail of 100 ng/mL VEGF165 (PeproTech, #100-20), 100 ng/mL FGF2 (PeproTech, #100-18B), and 10 μ M SB431542 (Selleck, #S1067) in CDM for two days. Vessel organoids were then embedded into a 4:1 mixture of Collagen I-Matrigel solution. The embedded organoids were then incubated with CDM medium supplemented with 100 ng/mL VEGF165 and 100 ng/mL FGF2. The medium was replaced every three days throughout the differentiation period until further use.

For co-culture experiments, vessel organoids were incubated for 4 days with mCherry-expressing THP1 cells and either Mock T cells or hCAR T cells (1×10^5 cells of each population). Live Z-stack images were acquired on an Olympus FV3000 confocal microscope, with THP1-associated red fluorescence visualized together with brightfield images.

Following co-culture, organoids were collected, fixed in 4% paraformaldehyde (PFA), and cryo-sectioned. Sections were then permeabilized with 0.5% Triton X-100 in PBS for 20 min, followed by blocking with 10% FBS and 0.05% Triton X-100 in PBS for 2 h. Sections were incubated with rat anti-CD68 (BioLegend, #137002, 1:300), mouse anti-V5 tag (ABclonal, #AE017, 1:100), and rabbit anti-CD31 (Proteintech, #28083-1-AP, 1:200), followed by goat anti-rat Alexa Fluor 488 (Cell Signaling, #4416S), goat anti-mouse Alexa Fluor 555 (Cell Signaling, #4409S), and goat anti-rabbit Alexa Fluor 647 (Thermo Fisher Scientific, #A21245). Nuclei were counterstained with Hoechst 33342 (Sigma, #B2261, 1:5,000), and images were acquired on an Olympus FV3000 confocal microscope.

For whole organoid staining and clearing: Organoids were fixed in 4% PFA and blocked in PBS containing 10% FBS and 0.2% Triton X-100 for 12 h at room temperature. They were then incubated with mouse anti-CD31 (R&D Systems, #BBA7, 1:400) at room temperature for 12 h, followed by goat anti-mouse Alexa Fluor 488 (Cell Signaling, #4408S, 1:400) at room temperature for 12 h. Nuclei were

counterstained with Hoechst 33342 (Sigma, #B2261, 1:5,000). For optical clearing, organoids were dehydrated through a methanol/H₂O series (20%, 40%, 60%, 80%, 100%, 100%; 1 h each at RT, with optional overnight incubation at RT in 100% methanol). Samples were then incubated in 66% dichloromethane (DCM) / 33% methanol for 3 h with shaking, followed by two 15-min washes in 100% DCM (Sigma, #270997) with shaking to remove methanol. Finally, organoids were transferred to dibenzyl ether (DBE, Sigma, #108014) without shaking; the tube was filled almost completely with DBE to prevent air oxidation of the sample. Before imaging, tubes were inverted several times to ensure complete mixing. Images were acquired on an Olympus FV3000 confocal microscope. All hiPSC-related work was approved by the Shenzhen Bay Laboratory (SZBL) Medical Ethics Committee (YL 2023-002 and YL 2025-017).

***In vivo* cytotoxicity assessment of CAR T cells using a THP1-luciferase xenograft model**

Six- to eight-week-old male NCG mice (GemPharmatech, #T001475) were used as the immunodeficient host for mass engraftment. For xenograft establishment, THP1 cells stably expressing firefly luciferase (THP1-luc) were harvested during logarithmic growth phase, washed twice with sterile PBS, and resuspended in a 1:1 mixture of PBS and Matrigel (Corning, #356237). Each mouse received a subcutaneous injection of 2×10^4 THP1-luc cells in 150 μ L of this suspension into the right flank using a 26G needle. Three days after mass cell inoculation, successful engraftment was verified by bioluminescence imaging (IVIS Spectrum, PerkinElmer). Mice with detectable and homogeneous signal distribution were enrolled and randomly allocated to treatment groups. The experimental groups included: Mock T cell (Untransduced T cell) control and hCAR1-3 T cell treatment group. On the day of treatment, Mock or CAR T cells were harvested, washed twice with PBS, and resuspended at an appropriate concentration. Each mouse received a single intravenous injection via the tail vein of 5×10^6 Untransduced T cells or 5×10^6 CAR+ T cells suspended in 150 μ L of PBS containing 12 mg/mL

IVISbrite™ D-Luciferin Potassium Salt (PerkinElmer, #122799). The volume was delivered slowly using a 26G needle to ensure complete administration.

Mass burden and CAR T cell-mediated anti-mass activity were monitored longitudinally by two complementary approaches at baseline (pre-treatment) and at regular intervals (Day 3, Day 10, Day 17, Day 24, Day 31, Day 38, and Day 45) post-injection. For mass measurement, the length (major axis, L) and width (minor axis, W) of each subcutaneous mass were measured using a digital caliper. Mass volume (V) was calculated according to the formula: $V = (L \times W^2) / 2$. For bioluminescence imaging, each mouse received an intravenous injection of D-Luciferin (12 mg/mL) prior to image acquisition. Animals were anesthetized with 2% isoflurane in oxygen, and bioluminescent signals were captured using the IVIS Spectrum system. Photon flux (photons/sec) was quantified within consistent regions of interest (ROIs) encompassing the entire thoracic and abdominal region of each mouse using Living Image® Software (Caliper). Background luminescence was subtracted using ROIs from non-mass-bearing areas. Mice were monitored daily for general health status, body weight, and signs of discomfort throughout the study duration. Any animal exhibiting severe distress (e.g., body weight loss exceeding 20%, mass volume exceeding 2,000 mm³, persistent hunched posture, or impaired mobility) was humanely euthanized.

Serum lipid, cytokine, and biochemical analyses

Blood was allowed to clot for 1 h at room temperature and then centrifuged at $10,000 \times g$ for 10 min at 4°C. Serum was collected for biochemical and cytokine measurements. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA-S), and urea (UREA) were measured using colorimetric assay kits (Elabscience, #E-BC-K109-M, #E-BC-K261-M, and #E-BC-K205-M; Mindray Animal Care, #105-020579-00, #105-020580-00, #105-020587-00, and #105-020583-00, respectively).

Serum IFN- γ , TNF- α , tartrate-resistant acid phosphatase 5b (TRAP5b), procollagen type I N-terminal propeptide (PINP), and C-terminal telopeptide of type I collagen (CTX-I) were measured using mouse ELISA kits (Invitrogen, #88-7314-88 and #88-7324-77; Elabscience, #E-EL-M3100, #E-EL-M0233, and #E-EL-M3023, respectively) according to the manufacturers' protocols.

Aortic root histology

Mice were euthanized by cervical dislocation under isoflurane anesthesia. The heart and aorta were exposed and perfused with PBS in situ. Aortic roots were harvested and embedded in Tissue-Tek[®] O.C.T. Compound (Sakura Finetek, #4583). Serial cryosections (8 μ m) were prepared through the aortic sinus using a cryostat (Leica, #CM1950). Sections were fixed in 4% PFA for 5 min at room temperature. Hematoxylin–eosin (H&E), Masson's trichrome, and Oil Red O staining were conducted using commercial kits (Solarbio #G1120, Solarbio #G1340, and Beyotime #C0157S). Slides were scanned using an Olympus SLIDEVIEW VS200 system. Necrotic core area, lesion area and plaque area were quantified using ImageJ.

Immunofluorescence

For immunofluorescence staining, sections were fixed with 4% PFA, permeabilized with PBS containing 0.5% Triton X-100 for 20 min, and blocked by PBS containing 10% FBS and 0.05% Triton X-100 for 2 h. Sections were then incubated with rat anti-mouse CD68 (BioLegend, #137002, 1:300), rabbit anti-mouse Trem2 (HuaBio, #ER1918-04, 1:200), and mouse anti-V5-Tag (ABclonal, #AE017, 1:100). After washing with PBS, sections were stained with goat anti-rat Alexa Fluor 488 (Cell Signaling, #4416S) and goat anti-rabbit Alexa Fluor 555 (Cell Signaling, #4413S). Nuclei were counterstained with Hoechst 33342 (Sigma, #B2261, 1:5,000). Images were captured using a confocal microscope (Olympus FV3000) and analyzed using ImageJ.

Aortic plaque quantification

The entire aorta was dissected and fixed in 4% PFA for 24 h. After removal of perivascular adipose tissue, the aorta was longitudinally opened. The aorta was briefly treated with 60% isopropanol for 3 s and then stained with Oil Red O solution (Servicebio, #G1015) at 37 °C for 1 h in a light-protected environment. The tissue was differentiated in 60% isopropanol until plaques appeared clearly red. Images were captured using a Canon 70D camera, and plaque area was quantified using ImageJ software.

Histology of major organs

Four weeks after CAR T-cell injection, the liver, brain, kidney, spleen, lung, intestine, pancreas, testis, and skin were harvested and embedded in Tissue-Tek O.C.T. Compound. Cryosections were stained with H&E and imaged as described above.

Kinetic analysis of CAR T cells in the peripheral blood

To determine the number of V5+ CAR T cells in the peripheral blood, blood samples were collected from the retro-orbital sinus of mice at day-1 (baseline) and 1, 3, 7, and 14 days after CAR T-cell injection in EDTA-coated tubes. Approximately 50 μ L of whole blood was collected at each time point. For each sample, 30 μ L of whole blood was incubated with 1 μ L of V5 tag-PE antibody (Invitrogen, #12-6796-42, 1:100) for 30 min at room temperature. Erythrocytes were then lysed by adding 450 μ L of red blood cell lysis buffer for 15 min at room temperature protected from light. The reaction was terminated by adding 900 μ L of serum-free RPMI 1640 medium, followed by centrifugation at $400 \times g$ for 5 min. The supernatant was discarded, and the cell pellet was washed once. Finally, cells were resuspended in serum-free RPMI 1640 medium. Samples were analyzed by flow cytometry. Absolute cell counts were determined using counting beads (eBioscience, #01-1234-42).

Cognitive and behavioral testing

Behavioral testing was performed during the light phase (9:00 AM-12:00 PM) in a dedicated sound-attenuated room. Different behavioral paradigms were separated by at least 24 h to minimize carryover effects. Apparatuses were cleaned with 70% ethanol between animals and allowed to dry completely before reuse.

Novel object recognition (NOR) test.

The novel object recognition test was performed over two consecutive days. On day 1 (familiarization), mice were placed in a 40 × 40 × 40 cm open-field arena containing two identical objects and allowed to explore for 10 min. On day 2 (test), one object was replaced with a novel object, and mice were allowed to explore for 10 min. Exploration was defined as directing the nose toward an object at a distance of <2 cm. The discrimination index (DI) was calculated as $(T_{\text{novel}} - T_{\text{familiar}})/(T_{\text{novel}} + T_{\text{familiar}})$. Time spent exploring the novel object and total object-exploration time were also recorded.

Elevated plus maze (EPM) test.

The elevated plus maze consisted of two open arms and two closed arms elevated 50 cm above the floor. Mice were placed on the central platform facing an open arm and allowed to explore for 5 min. An arm entry was scored when all four paws entered an arm. The percentage of time spent in the open arms, percentage of entries into the open arms, and total arm entries were recorded.

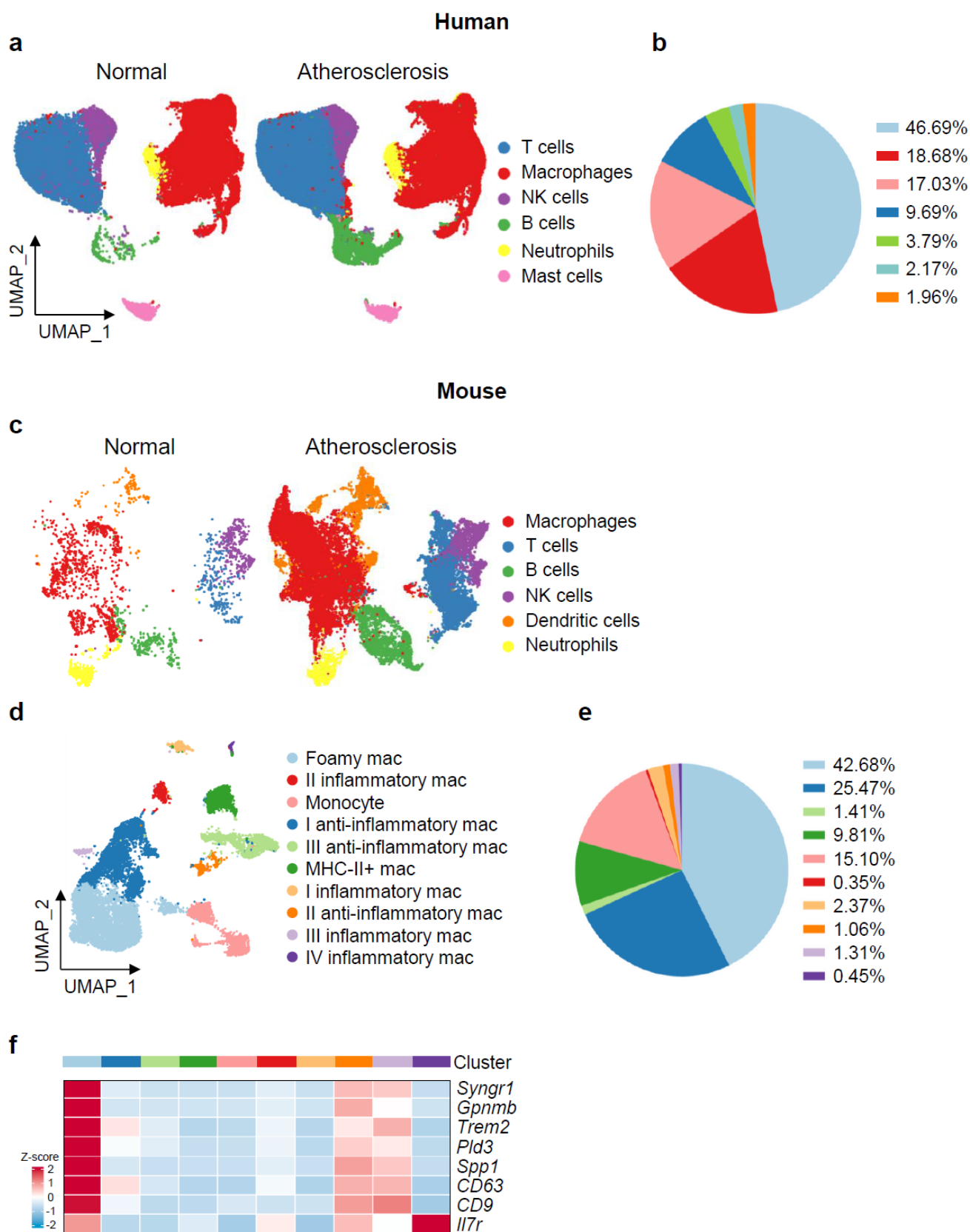
Vascular permeability assay

Four weeks after CAR T-cell injection, mice received an intravenous injection of 200 μL 0.5% Evans blue dye (Sigma, #E2129) in sterile PBS. After 30 min of circulation, mice were euthanized and perfused with 50 mL PBS. Brain, liver, kidney, spleen, lung, testis, and heart were collected, weighed, and incubated in formamide (Macklin, #F832010) at 37°C for 48 h to extract the dye. After centrifugation,

absorbance was measured at 620 nm using a microplate reader. Evans blue content was calculated from a standard curve and expressed as μg dye per g tissue.

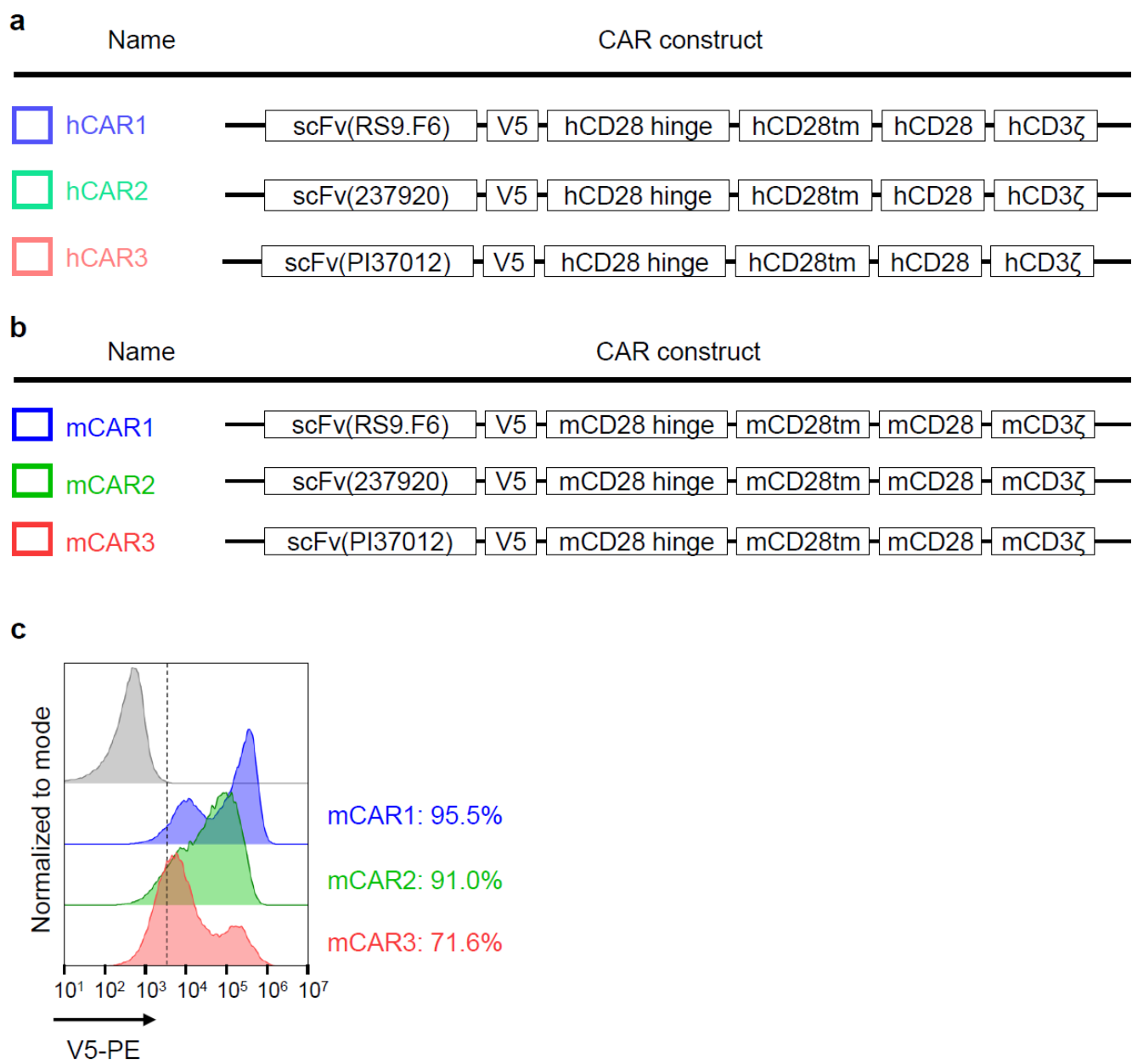
Statistics

Statistical analyses and graphing were performed using GraphPad Prism version 8.4.3. Two-group comparisons were analyzed using an unpaired two-tailed Student's t-test. One-Way ANOVA with Dunnett's multiple comparisons test was conducted for *in vitro* cytotoxicity studies and *Apoe*^{-/-} mouse studies. Two-Way ANOVA with Tukey's multiple comparisons test was conducted for THP1-luc xenograft model, as specified in the corresponding figure legends. Data are shown as mean $\pm$ SEM. $P < 0.05$ was considered statistically significant. Significance is denoted as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; n.s., not significant. Sample sizes are provided in the relevant figure legends.

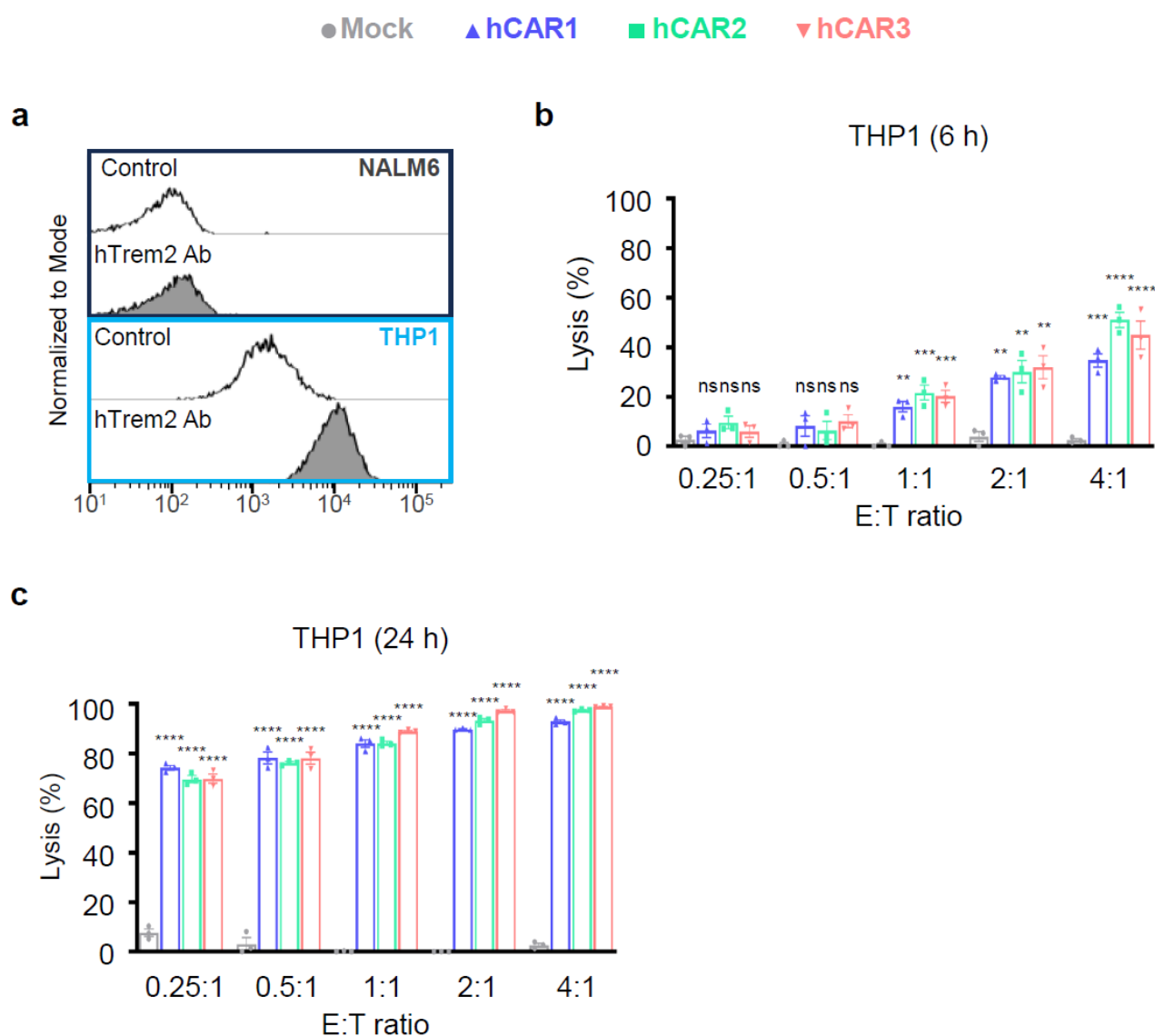


Supplementary Fig. S1. Immune-cell composition and macrophage heterogeneity in human and

mouse atherosclerotic arteries. **a** and **c.** UMAP plots of immune cells from normal and atherosclerotic human arterial datasets (**a**) and mouse aortic datasets (**c**), colored by major immune-cell type. **b.** Relative proportions of the major macrophage populations in human atherosclerotic samples. **d.** UMAP visualization of mouse monocyte/macrophage subclusters after re-clustering of the macrophage population. **e.** Relative proportions of the major macrophage populations in mouse atherosclerotic samples. **f.** Heatmap showing representative genes enriched across the mouse monocyte/macrophage subclusters, including the foamy macrophage-associated genes *Gpnmb* and *Trem2*.



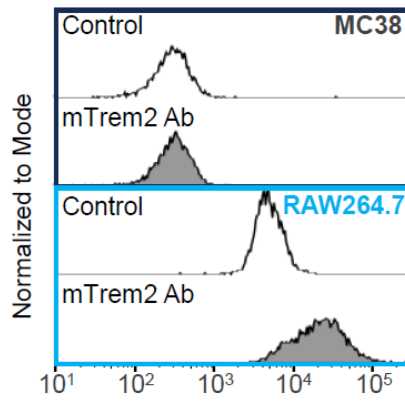
Supplementary Fig. S2. Generation of human and mouse Trem2-targeted CARs. a and b. Schematics of the human **(a)** and mouse **(b)** CAR constructs. Three Trem2-binding scFvs were used: RS9.F6 (CAR1), 237920 (CAR2), and PI37012 (CAR3). Each construct contains a V5 tag followed by species-matched CD28 hinge, transmembrane, and co-stimulatory domains and a CD3 ζ signaling domain. **c.** Flow-cytometric detection of V5-tagged CAR expression in primary mouse T cells 4 days after retroviral transduction. The indicated values show the percentage of CAR-positive cells for mCAR1, mCAR2, and mCAR3.



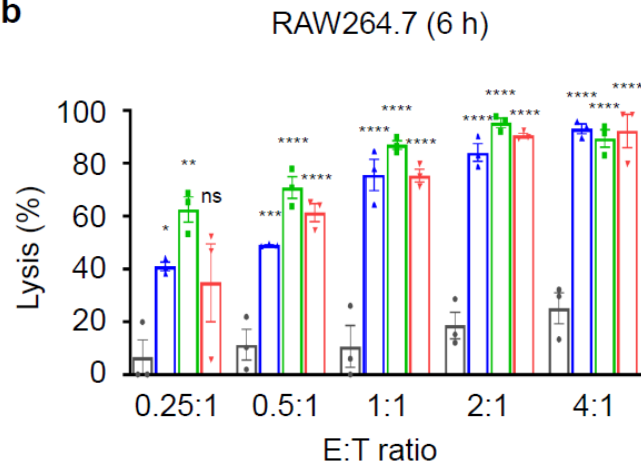
Supplementary Fig. S3. Trem2-dependent cytotoxicity of human CAR T cells *in vitro*. **a.** Flow-cytometric analysis of Trem2 expression in NALM6 and THP1 cells, showing low/undetectable Trem2 in NALM6 cells and high Trem2 expression in THP1 cells. **b** and **c.** Cytotoxicity of mock T cells and hCAR1-3 T cells against THP1 cells at E:T ratios of 0.25:1, 0.5:1, 1:1, 2:1, and 4:1 after 6 h (**b**) or 24 h (**c**) of co-culture. n = 3 independent experiments. ns, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. One-way ANOVA with Dunnett's multiple-comparisons test.

● Mock ▲ mCAR1 ■ mCAR2 ▼ mCAR3

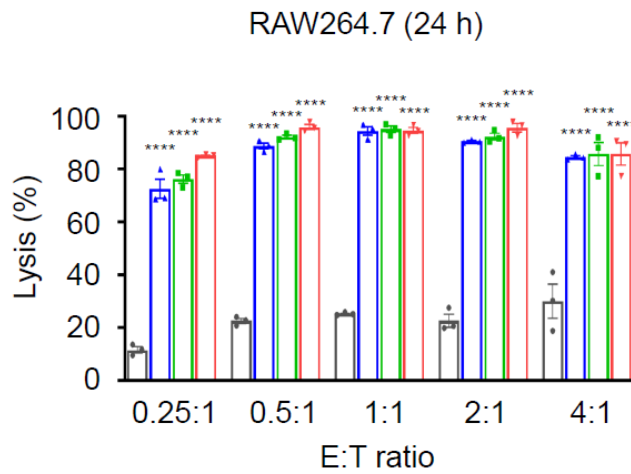
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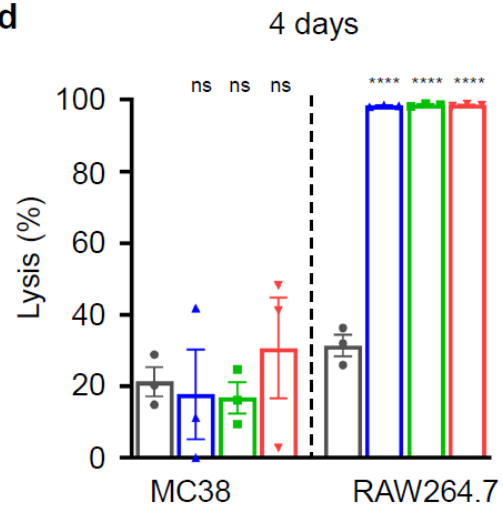
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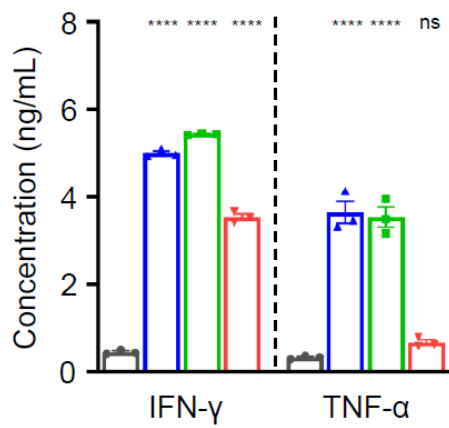
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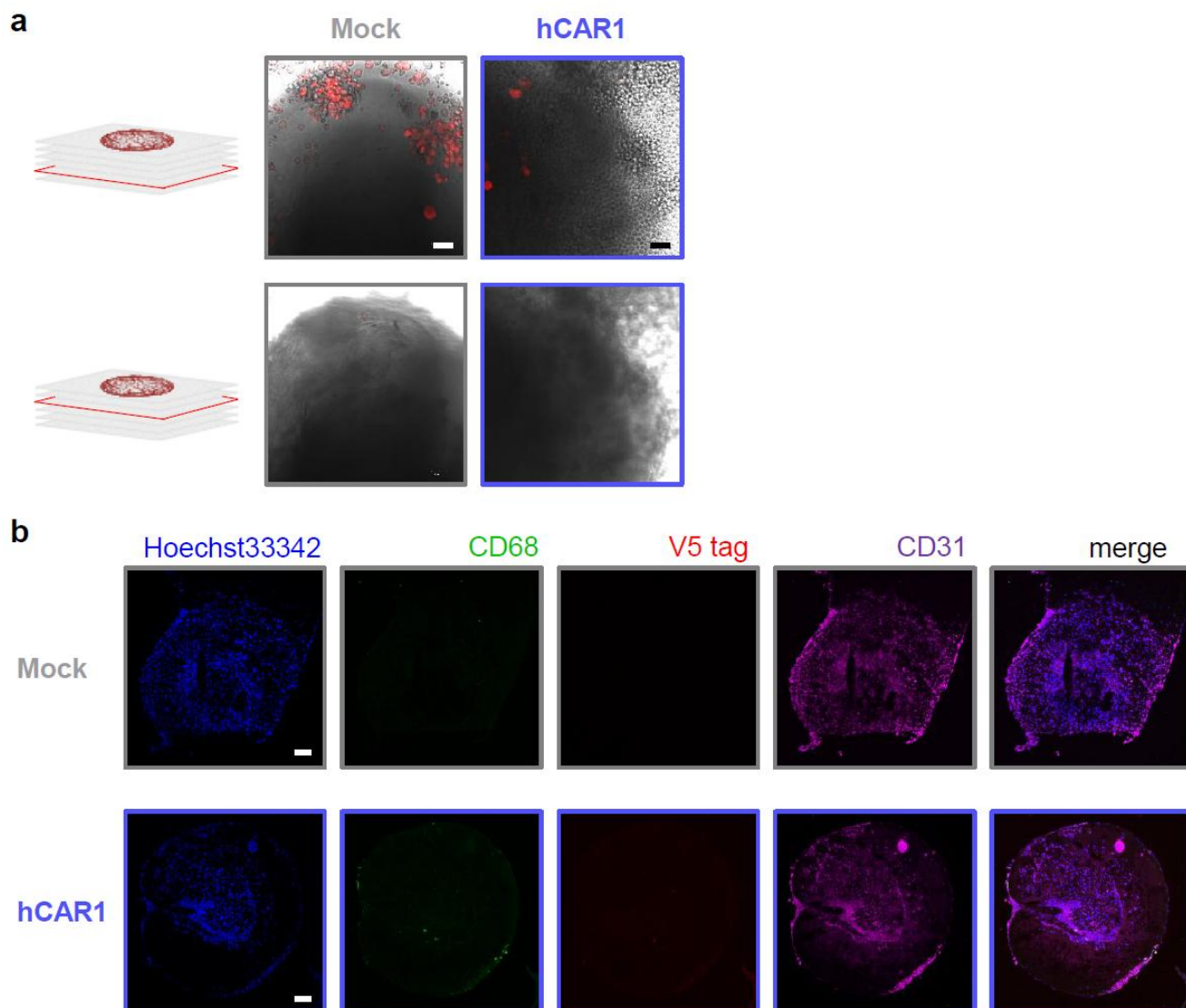
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e

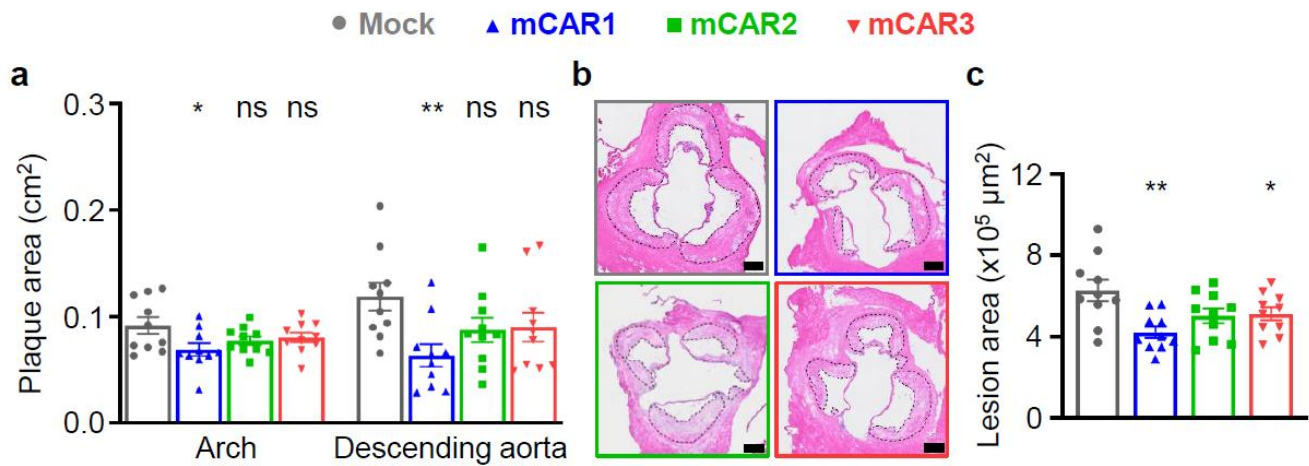


Supplementary Fig. S4. Trem2-dependent cytotoxicity of mouse CAR T cells *in vitro*. **a.** Flow-cytometric analysis of Trem2 expression in MC38 and RAW264.7 cells, showing low/undetectable Trem2 in MC38 cells and high Trem2 expression in RAW264.7 cells. **b** and **c.** Cytotoxicity of mock T cells and mCAR1-3 T cells against RAW264.7 cells at E:T ratios of 0.25:1, 0.5:1, 1:1, 2:1, and 4:1 after 6 h (**b**) or 24 h (**c**) of co-culture. **d.** Target-cell killing after 4 days of co-culture with Trem2-negative MC38 cells or Trem2-positive RAW264.7 cells at an E:T ratio of 1:20. **e.** IFN- γ and TNF- α concentrations in supernatants from RAW264.7-CAR T-cell co-cultures. n = 3 independent experiments. ns, not significant; ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. One-way ANOVA with Dunnett's multiple-comparisons test.

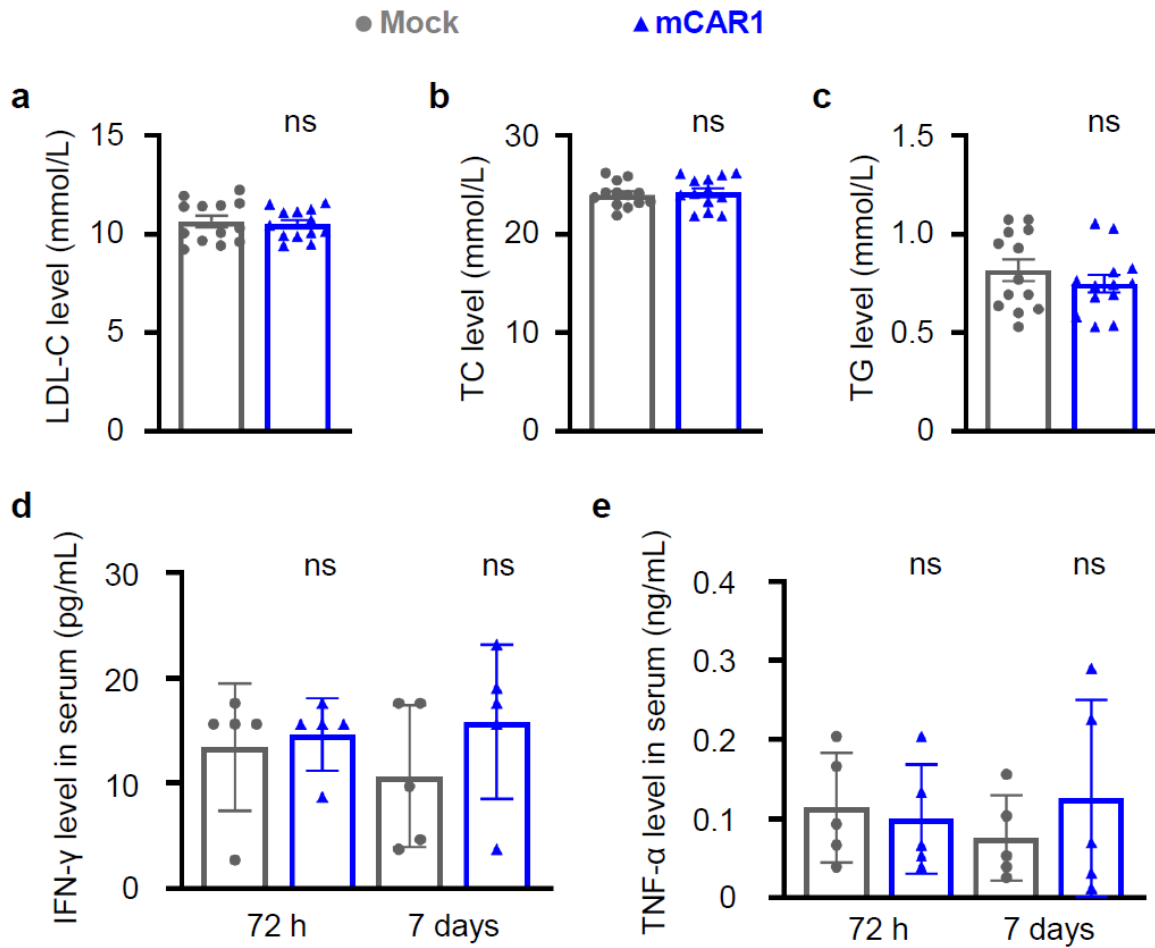


Supplementary Fig. S5. Localization of THP1 and CAR T cells in human blood vessel organoids.

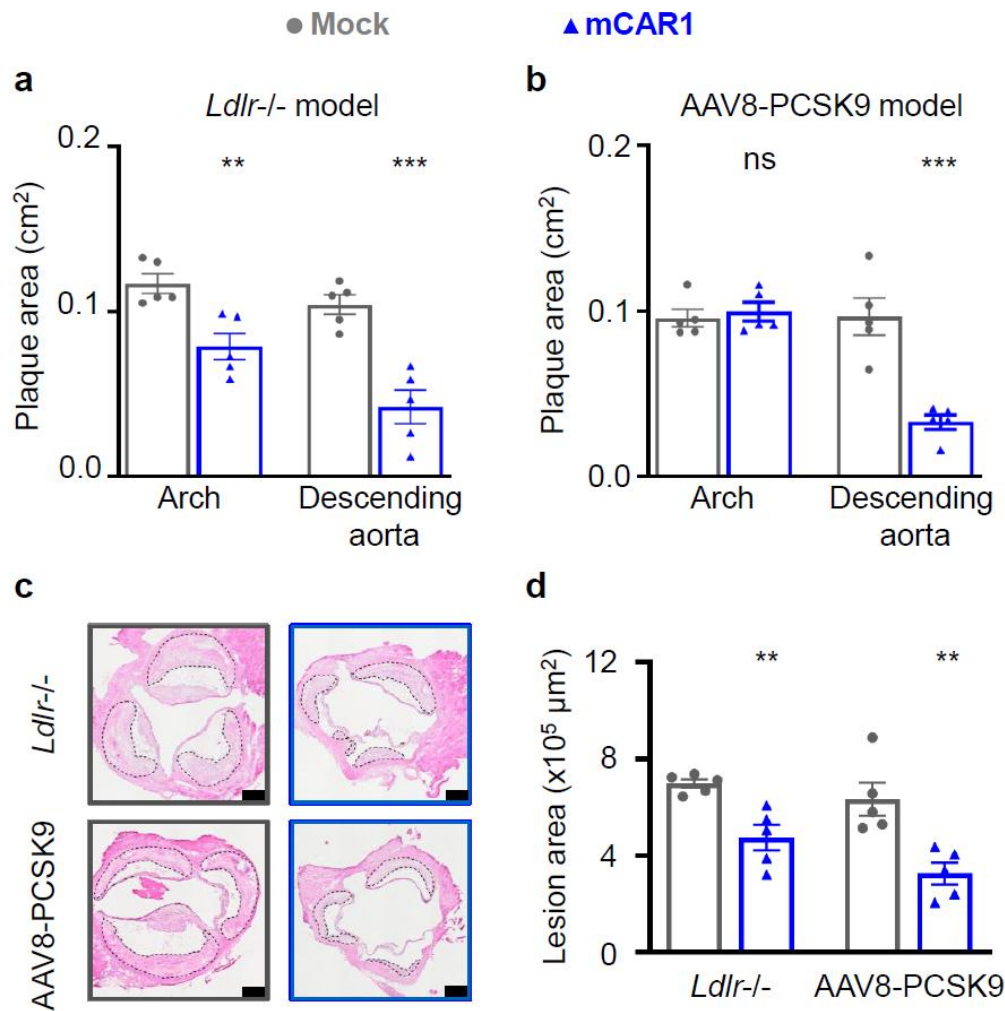
a. Live Z-stack imaging of vessel organoids after 4 days of co-culture with THP1 cells and either mock T cells or hCAR1 T cells. Representative optical sections at different focal planes are shown; mCherry-expressing THP1-associated red fluorescence is overlaid on brightfield images. **b.** Representative immunofluorescence images of organoid cryosections stained for CD68 (THP1 cells, green), V5 tag (CAR T cells, red), CD31 (vascular structures, violet), and Hoechst 33342 (nuclei, blue) showing only occasional CD68⁺ THP1 cells and no readily detectable V5⁺ CAR T cells within the organoids. Scale bars, 100 μ m.



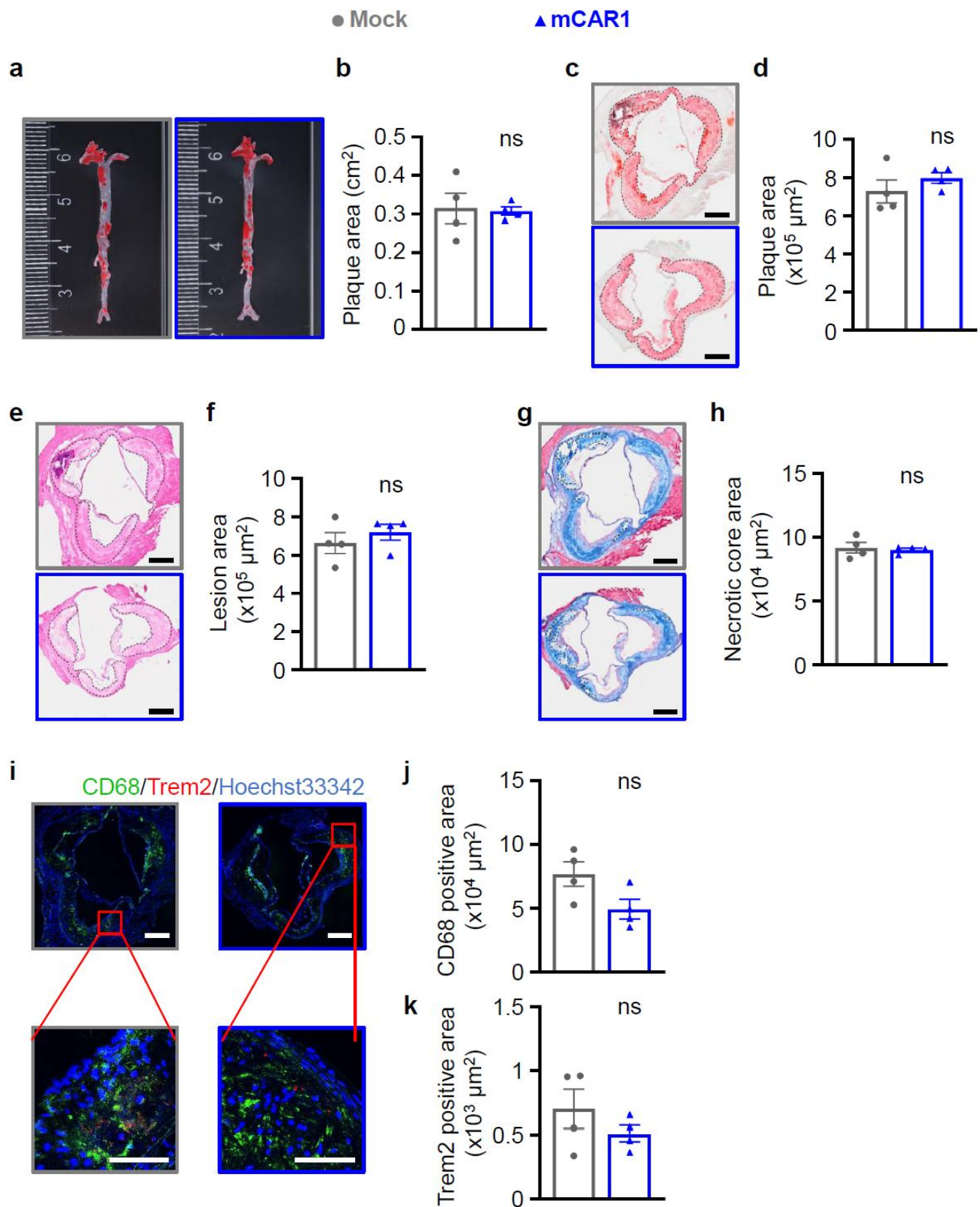
Supplementary Fig. S6. Trem2-targeted CAR T cells reduce atherosclerotic plaque burden in *Apoe*^{-/-} mice. *Apoe*^{-/-} mice were maintained on HFD for 12 weeks before injection of control T cells or mCAR1-3 T cells and analyzed 4 weeks later. **a.** Quantification of plaque area in the aortic arch and descending aorta from en face Oil Red O-stained aortas. **b.** Representative H&E-stained aortic-root sections from control, mCAR1, mCAR2, and mCAR3 groups. **c.** Quantification of aortic-root lesion area from H&E-stained sections. n = 10 mice per group. ns, not significant; **P* < 0.05, ***P* < 0.01. One-way ANOVA with Dunnett's multiple-comparisons test. Scale bars, 200 μm.



Supplementary Fig. S7. Serum lipid and inflammatory cytokine levels after mCAR1 treatment in *Apoe*^{-/-} mice. **a-c.** Serum LDL-C (**a**), total cholesterol (TC; **b**), and triglyceride (TG; **c**) levels in HFD-fed *Apoe*^{-/-} mice treated with control T cells or mCAR1 T cells. n = 13 mice per group. **d** and **e.** Serum IFN-γ (**d**) and TNF-α (**e**) measured 72 h and 7 days after T-cell injection. n = 5 mice per group. ns, not significant. Unpaired two-tailed Student's t-test.

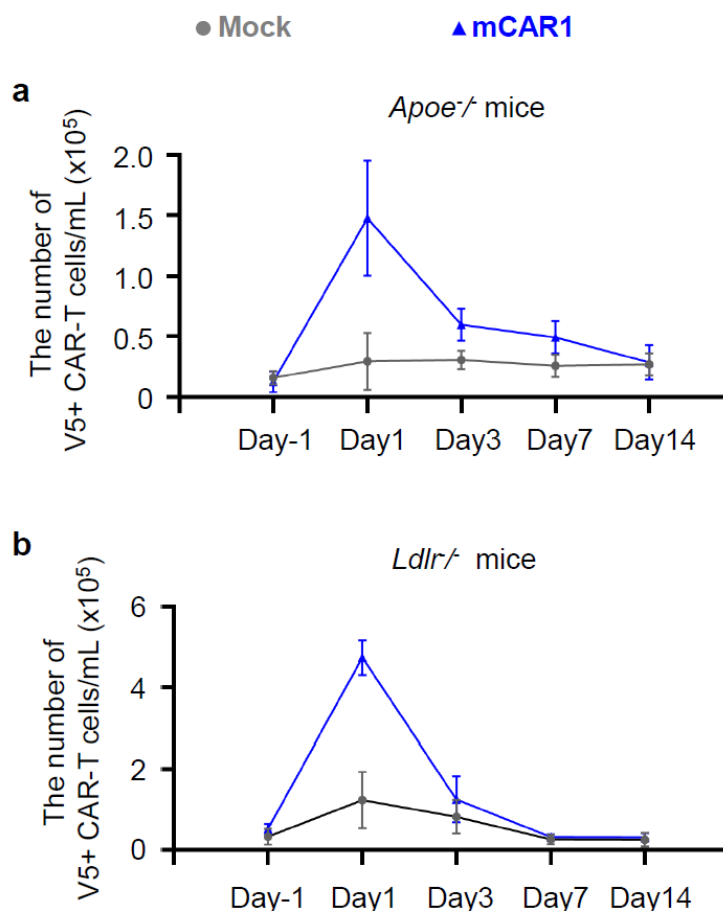


Supplementary Fig. S8. mCAR1 reduces atherosclerotic plaque burden in *Ldlr*^{-/-} and AAV8-PCSK9 mouse models. *Ldlr*^{-/-} mice and AAV8-PCSK9-treated mice were maintained on HFD for 16 weeks before injection of control T cells or mCAR1 T cells and analyzed 4 weeks later. **a** and **b**. Quantification of plaque area in the aortic arch and descending aorta after en face Oil Red O staining in *Ldlr*^{-/-} mice (**a**) and AAV8-PCSK9-treated mice (**b**). **c**. Representative H&E-stained aortic-root sections from control and mCAR1-treated *Ldlr*^{-/-} mice (top) and AAV8-PCSK9-treated mice (bottom). **d**. Quantification of aortic-root lesion area from H&E-stained sections. n = 5 mice per group. ns, not significant; **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Unpaired two-tailed Student's t-test. Scale bars, 200 μm.



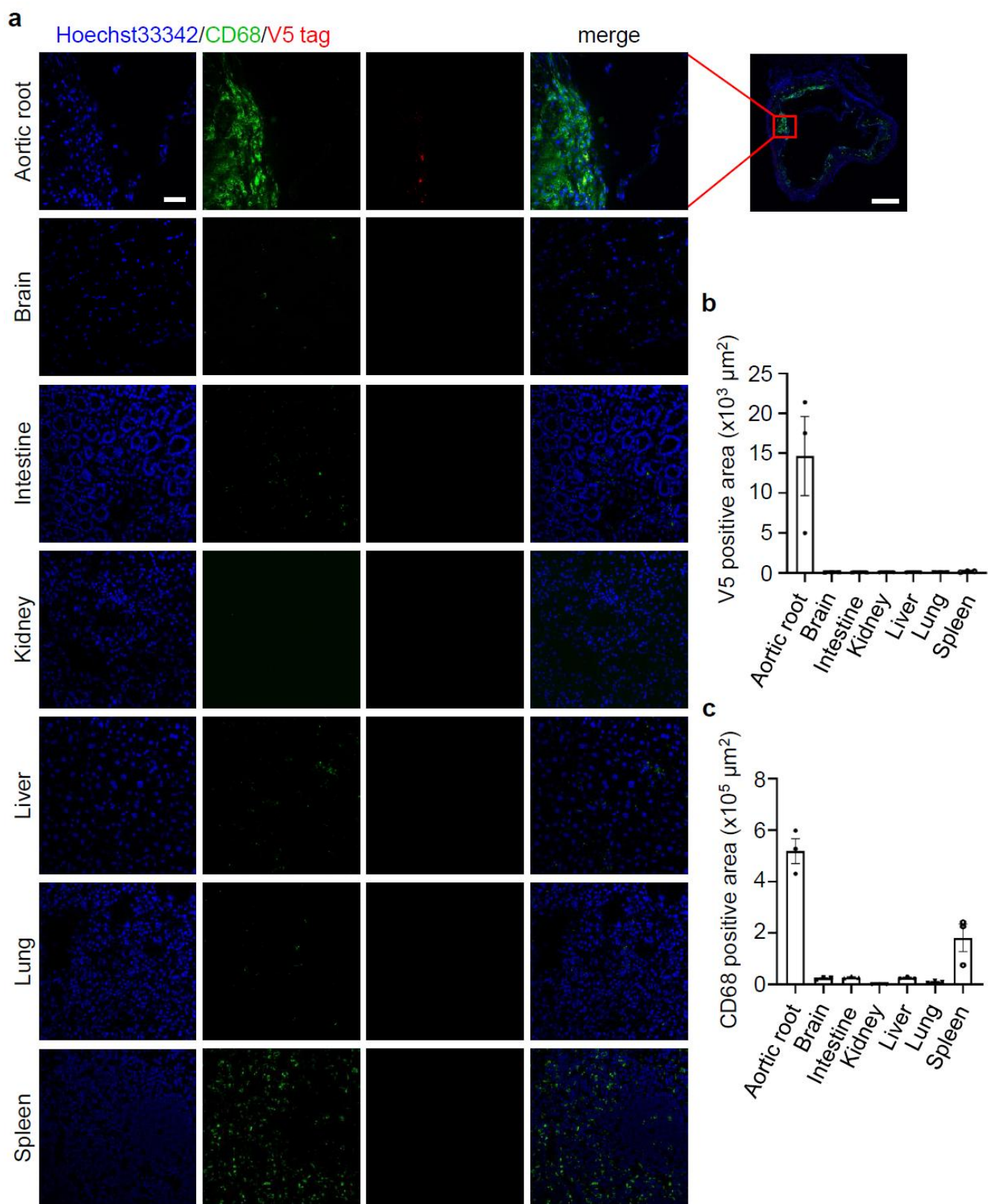
Supplementary Fig. S9. Long-term treatment outcomes of mCAR1 T cells in *Apoe*^{-/-} atherosclerosis model. *Apoe*^{-/-} mice were fed with HFD for 12 weeks before receiving 5 x 10⁶ mCAR1 T cells

intravenously. Mice were continuously fed with HFD for additional 8 weeks before being sacrificed for analysis. **a-b.** Representative En face ORO staining of whole aortas (**a**) and quantification of plaque area (**b**) in *Apoe*^{-/-} mice. **c-d.** Representative ORO staining of aortic roots (**c**) and quantification of plaque area (**d**) in *Apoe*^{-/-} mice. **e-f.** Representative H&E-stained aortic-root sections (**e**) and quantification of aortic-root lesion area (**f**) from H&E-stained sections. **g-h.** Masson's trichrome staining of aortic roots (**g**) and quantification of necrotic core area (**h**) in *Apoe*^{-/-} mice. **i-k.** Immunofluorescence staining of aortic roots for CD68 and Trem2 (**i**) and quantification of CD68+ (**j**) and Trem2+ (**k**) areas. n = 4 mice per group. ns, not significant. Unpaired two-tailed Student's t-test. Scale bars for aortic-root overview images, 300 μ m; magnified images in i, 50 μ m.



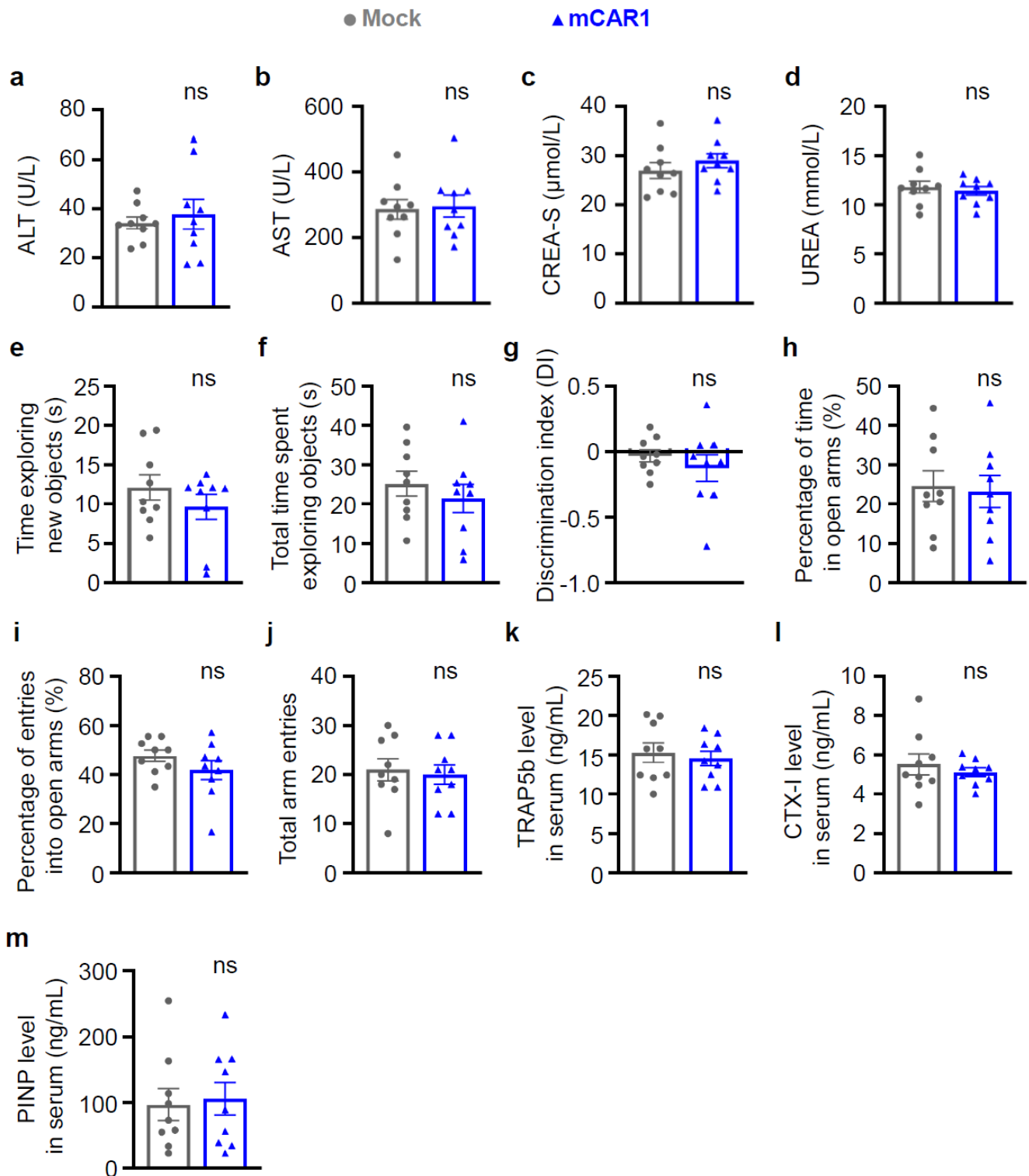
Supplementary Fig. S10. Kinetics of circulating mCAR1 T cells. Longitudinal quantification of mCAR1 T cells in peripheral blood from *Apoe*^{-/-} (**a**) and *Ldlr*^{-/-} (**b**) mice fed with HFD for 12 weeks and

16 weeks, respectively. Blood was collected from the retro-orbital sinus before injection (day -1) and on days 1, 3, 7, and 14 after mCAR1 T-cell injection. mCAR1 T cells were quantified by flow cytometry. n = 3 mice per group.



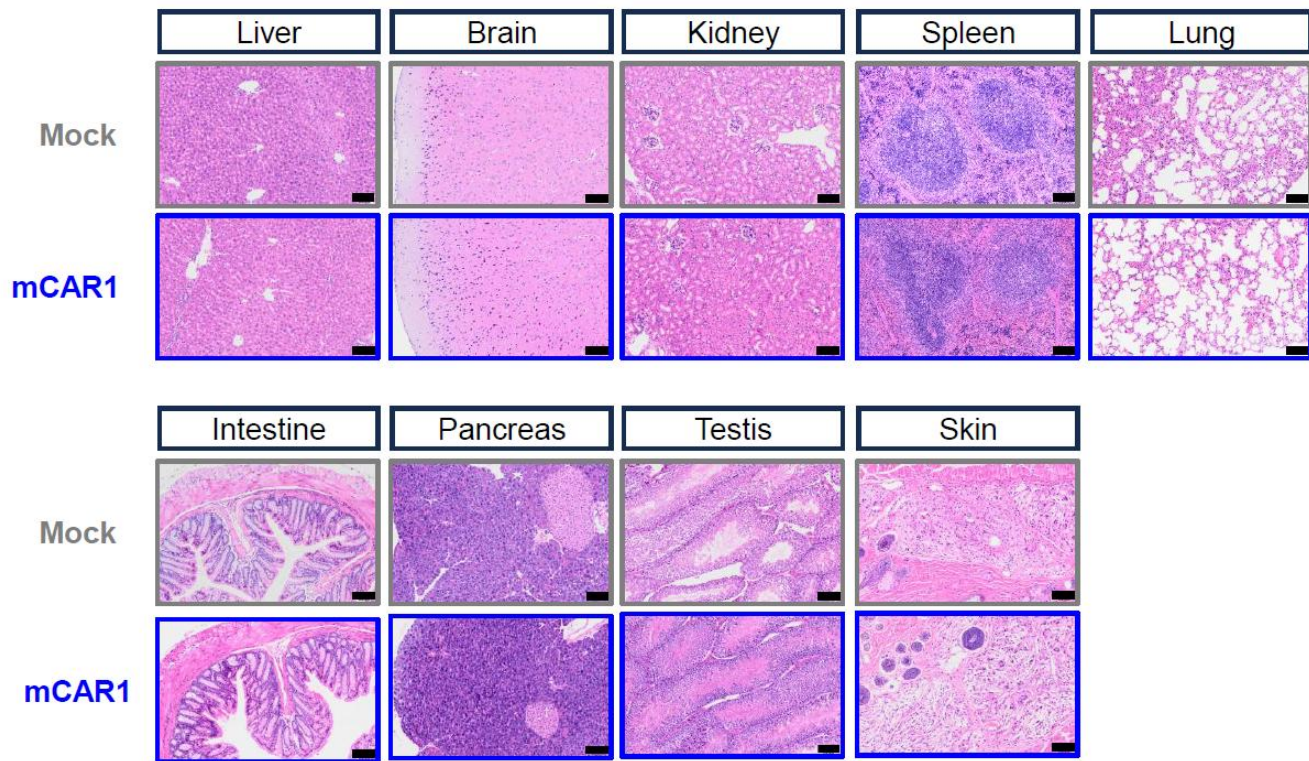
Supplementary Fig. S11. Tissue distribution of mCAR1 T cells 7 days after injection. *Apoe*^{-/-} mice were fed with HFD for 12 weeks and injected intravenously with mCAR1 T cells; aortic-root and major-

organ tissues were collected 7 days later. **a.** Representative immunofluorescence images of aortic-root, brain, intestine, kidney, liver, lung, and spleen sections stained for CD68 (green), V5 tag (red), and Hoechst 33342 (blue). The V5 tag identifies the injected mCAR1 T cells. Sparse V5⁺ cells were detectable in the aortic-root plaque, whereas V5 signal was not readily detected in the examined major-organ sections. **b** and **c.** Quantification of V5⁺ (**b**) and CD68⁺ (**c**) areas in the indicated tissues. Each dot represents one analyzed tissue section from an individual mice. n = 3 mice per group. Scale bars, 40 μ m for tissue sections and 200 μ m for the whole-aortic-root overview.



Supplementary Fig. S12. Safety assessment of mCAR1 T cell treatment in *Apoe*^{-/-} mice 4 weeks after injection of CAR T cells. **a** and **b**. Serum alanine aminotransferase (ALT; **a**) and aspartate aminotransferase (AST; **b**). **c** and **d**. Serum creatinine (CREA-S; **c**) and urea (UREA; **d**). **e-g**. Novel

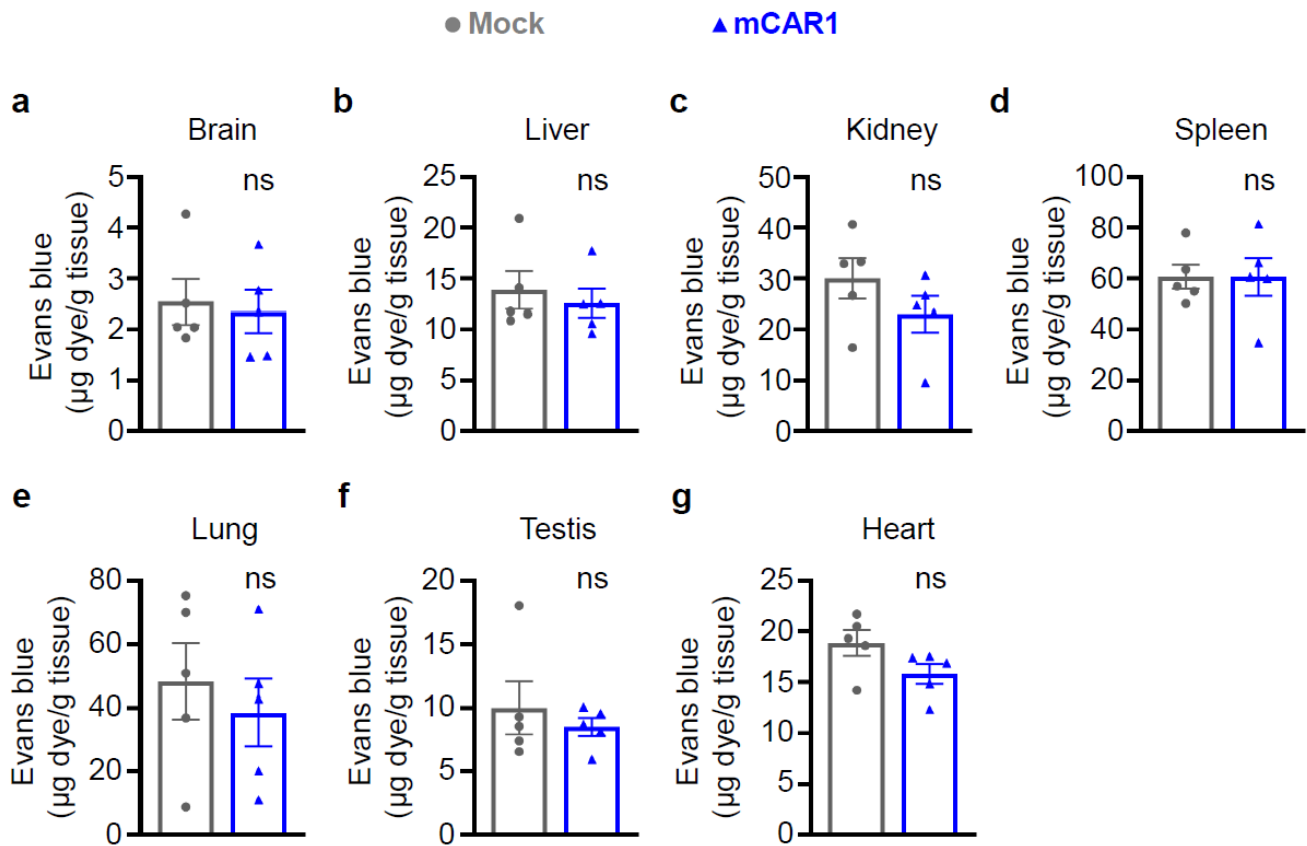
object recognition test showing time spent exploring the novel object (e), total object-exploration time (f), and discrimination index (DI; g), calculated as $(T_{\text{novel}} - T_{\text{familiar}})/(T_{\text{novel}} + T_{\text{familiar}})$. **h-j**. Elevated plus maze showing percentage of time in the open arms (h), percentage of entries into the open arms (i), and total arm entries (j). **k-m**. Serum tartrate-resistant acid phosphatase 5b (TRAP5b; k), C-terminal telopeptide of type I collagen (CTX-I; l), and procollagen type I N-terminal propeptide (PINP; m). HFD-fed Apoe^{-/-} mice received control T cells or mCAR1 T cells. No significant differences were detected between groups for the measured parameters. n = 9 mice per group. ns, not significant. Unpaired two-tailed Student's t-test.



Supplementary Fig. S13. Major-organ histology after Trem2-targeted CAR T-cell treatment.

Representative H&E-stained sections of liver, brain, kidney, spleen, lung, intestine, pancreas, testis, and skin collected from HFD-fed Apoe^{-/-} mice 4 weeks after injection of control T cells or mCAR1 T cells.

Scale bars, 100 μ m.



Supplementary Fig. S14. Vascular permeability in major organs after mCAR1 treatment. a-g. Evans blue extravasation in brain (a), liver (b), kidney (c), spleen (d), lung (e), testis (f), and heart (g) 4 weeks after injection of control T cells or mCAR1 T cells in HFD-fed *Apoe*^{-/-} mice. Evans blue content was normalized to tissue weight and expressed as µg dye per g tissue. n = 5 mice per group. ns, not significant. Unpaired two-tailed Student's t-test.