Cellular senescence is characterized by stable cell-cycle arrest and a secretory program that modulates the tissue microenvironment. Physiologically, senescence serves as a tumour-suppressive mechanism that prevents the expansion of premalignant cells and has a beneficial role in wound-healing responses. Pathologically, the aberrant accumulation of senescent cells generates an inflammatory milieu that leads to chronic tissue damage and contributes to diseases such as liver and lung fibrosis, atherosclerosis, diabetes and osteoarthritis. Accordingly, eliminating senescent cells from damaged tissues in mice ameliorates the symptoms of these pathologies and even promotes longevity. Here we test the therapeutic concept that chimeric antigen receptor (CAR) T cells that target senescent cells can be effective senolytic agents. We identify the urokinase-type plasminogen activator receptor (uPAR) as a cell-surface protein that is broadly induced during senescence and show that uPAR-specific CAR T cells efficiently ablate senescent cells in vitro and in vivo. CAR T cells that target uPAR extend the survival of mice with lung adenocarcinoma that are treated with a senescence-inducing combination of drugs, and restore tissue homeostasis in mice in which liver fibrosis is induced chemically or by diet. These results establish the therapeutic potential of senolytic CAR T cells for senescence-associated diseases.

Upregulation of uPAR during senescence

To identify cell-surface proteins that are broadly and specifically upregulated in senescent cells, we compared RNA-sequencing (RNA-seq) datasets derived from three independent and robust models of senescence: 1) therapy-induced senescence in mouse lung adenocarcinoma KrasG12D:p53−/− (KP) cells (p53 is also known as Trp53) that are triggered to senescence by a combination of MEK inhibition and CDK4 and CDK6 (CDK4/6) inhibition; 2) oncogene-induced senescence in mouse hepatocytes, mediated by the in vivo delivery of NrasG12V through hydrodynamic tail vein injection (HTVI); and 3) culture-induced senescence in mouse hepatic stellate cells (HSCs) (Extended Data Fig. 1a). We focused on transcripts that encode molecules that are located in the plasma membrane (as determined by UniProtKB) and that were upregulated in all datasets (Extended Data Fig. 1b, c). Eight transcripts were identified, which encode proteins related to extracellular matrix remodelling or the coagulation cascade (Extended Data Fig. 1d).

Given that ideal antigens for the engagement of CAR T cells should be highly expressed on target cells but not in vital tissues, we ranked each transcript according to its magnitude of upregulation (log expression in senescent cells/expression in non-senescent cells), and then excluded those that were highly expressed in vital tissues (as determined by the Human Protein Atlas and the Human Proteome Map). This process identified PLAUR, which encodes the urokinase-type plasminogen activator receptor (uPAR).
Fig. 1 uPAR is a cell-surface and secreted biomarker of senescence. *a* Left, flow cytometry analysis of uPAR expression on primary human melanocytes after induction of senescence by treatment with MEK and CDK4/6 inhibitors (CDK4/6i + MEKi) as compared to controls. FMO, fluorescence minus one control. Representative results of n = 3 independent experiments. Right, levels of suPAR as determined by enzyme-linked immunosorbent assay (ELISA) in the supernatant of senescent (passage 15; P15) or proliferating (passage 2; P2) primary human melanocytes. Representative results of n = 2 independent experiments. *b* Left, flow cytometry analysis comparing uPAR expression on primary human melanocytes after induction of senescence by continuous passage (P) with proliferating controls. Representative results of n = 2 independent experiments. Right, levels of suPAR in the supernatant of senescent (passage 15; P15) or proliferating (passage 2; P2) primary human melanocytes. Representative results of n = 2 independent experiments.
Extended Data Fig. 4d). m.uPAR-h.28z—but not h.19-h.28z—CAR T cells (h.19-h.28z) when targeting uPAR-expressing NALM6 cells (Fig. 2a, CAR T cells incorporating human CD28 and CD3ζ signalling elements Fig. 4c). m.uPAR-h.28z CAR T cells showed no cytotoxicity towards lymphoblastic leukaemia (B-ALL) cell line NALM6 (Extended Data CD1929, mouse uPAR was introduced into the human CD19+ pre-B acuteulatory and CD3ζ (h.28z) signalling domains (m.uPAR) single-chain variable fragment linked to human CD28 costimulatory and CD3ζ (h.28z) signalling domains (m.uPAR-h.28z), transduced human T cells and performed cytotoxicity assays using target expression of NrasG12V-luciferase used above4. Although these senescent cells normally undergo SASP-mediated immune clearance4, they are efficiently eliminated senescent KP cells that express endogenous uPAR, and this was accompanied by antigen-specific secretion of granzyme B and interferon γ (IFNγ) (Fig. 2b, Extended Data Fig. 4e). Hence, m.uPAR-h.28z CAR T cells can selectively and efficiently target senescent cells.

To study whether m.uPAR-h.28z CAR T cells could function as a bona fide senolytic agent in vivo, we took advantage of the well-characterized model of oncogene-induced senescence triggered by the hepatic overexpression of NrasG12V-luciferase used above4. Although these senescent cells normally undergo SASP-mediated immune clearance4, they are retained in the livers of immunodeficient NOD scid gamma (NSG) mice4. Successful transfection of NrasG12V into hepatocytes of NSG mice was confirmed by bioluminescence imaging, and was followed by administration of 0.5 × 10⁶ m.uPAR-h.28z CAR T cells or untransduced T cells as controls (Extended Data Fig. 4f).

**Senolytic activity of uPAR CAR T cells**

We constructed a uPAR-specific CAR comprising an anti-mouse uPAR (m.uPAR) single-chain variable fragment linked to human CD28 costimulatory and CD3ζ (h.28z) signalling domains (m.uPAR-h.28z), transduced human T cells and performed cytotoxicity assays using target expression of CD62L and CD45RA (group). Scale bar, 50 μm. g–i. Number of liver-infiltrating CAR T cells (g), expression of CD62L and CD45RA (h) and percentage of PD-1 TIM3 LAG3+ CAR T cells (i) among m.uPAR-h.28z CAR T cells as determined by flow cytometry (n = 4 mice per group). Representative results of n = 2 independent experiments (c–f). Data are mean ± s.e.m.; two-tailed unpaired Student’s t-test (c–e).

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**Fig. 3** Senolytic CAR T cells show therapeutic efficacy in CCl4-induced liver fibrosis. 

**a.** Cytotoxicity of mouse CAR T cells as determined by an 18-h bioluminescence assay using luciferase-expressing wild-type EμALL01 cells (WT) or Eμ-ALL01 cells that overexpress mouse uPAR (Eμ-ALL01-m.uPAR) as targets. Data are representative of n = 3 independent experiments, each performed in triplicate. **b.** Cytotoxic T cell activity as determined by an 18-h bioluminescence assay using KP cells as targets in which senescence was induced by MEK and CDK4/6 inhibition. Data are representative of n = 3 independent experiments, each performed in triplicate. **c.** Representative levels of fibrosis as evaluated by Sirius red staining and SAβ-gal expression (top) and respective quantifications (bottom) (UT and m.19-m.28z, n = 3; m.uPAR-m.28z, n = 4; m.uPAR-m.28z at 0.5 × 10⁶, n = 5 mice). Scale bars, 500 μm (top); 50 μm (bottom). **d.** Fold change difference in the serum levels of suPAR 20 days after (day 20) compared to 1 day before (day −1) infusion of T cells. **e, f.** Levels of serum AST (e) and ALT (f) 20 days after infusion of T cells (UT, m.19-m.28z and m.uPAR-m.28z, n = 3; m.uPAR-m.28z at 0.5 × 10⁶, n = 5 mice (d–f)). **g.** Mice with CCl4-induced liver fibrosis were injected with 0.5 × 10⁶ or 1 × 10⁶ m.uPAR-m.28z CAR T cells, 1 × 10⁶ m.19-m.28z CAR T cells or control T cells that were transduced to express click beetle red luciferase. **g.** Luciferase signal (average radiance) of treated mice after administration of T cells, reflecting the expansion of T cells (control T cells and m.19-m.28z, n = 3; m.uPAR-m.28z, n = 4; m.uPAR-m.28z at 0.5 × 10⁶, n = 3 mice). **h.** Representative bioluminescence images of mice at different time points after injection. T cells were initially detected in the lungs in all treated mice; m.uPAR-m.28z CAR T cells showed trafficking to the liver area followed by a short period of expansion and a rapid contraction. The signal in control mice at day 10 indicates abdominal peritonitis induced by CCl4 injections, as confirmed by pathology. For the colour scales on the right (measured in p s⁻¹ cm⁻² sr⁻¹), the minimum value and maximum values are 1.43 × 10⁶ and 8.00 × 10⁸, respectively (top) and 1.50 × 10⁵ and 3.63 × 10⁶, respectively (bottom). Results of n = 1 independent experiment (c–h). Data are mean ± s.e.m.; two-tailed unpaired Student’s t-test (c–f).
Previous studies suggest that the combination of a senescence-inducing cancer therapy and a senolytic agent can improve treatment outcome in mouse models. We thus treated mice that had orthotopic KP lung adenocarcinomas with combined MEK and CDK4/6 inhibitors, and then administered uPAR- or CD19-specific CAR T cells or untransduced T cells (Extended Data Fig. 5a). Treatment with uPAR-targeted CAR T cells significantly prolonged survival without eliciting signs of toxicity (Extended Data Fig. 5b–d). Lungs that were collected from mice treated with uPAR-specific CAR T cells showed a substantial decrease in senescent tumour cells, accompanied by enhanced infiltration of adoptively transferred CD4+ and CD8+ T cells that expressed activation markers (Extended Data Fig. 5e, f). In addition to confirming the senolytic properties of uPAR-directed CAR T cells, these results indicate that combinatorial strategies using senolytic CAR T cells could be used to treat solid tumours.

Besides cancer, senescence contributes to a range of chronic tissue pathologies, including liver fibrosis—a condition that can evolve into cirrhosis and produces a microenvironment that favours the development of hepatocellular carcinoma. As genetic ablation of senescent cells ameliorates liver fibrosis, we performed dose-escalation studies using m.uPAR-m.28z CAR T cells in the well-defined mouse model of CCl4-induced liver fibrosis, in which treatment with CCI4, leads to the accumulation of senescent HSCs, fibrosis and liver damage within six weeks. m.uPAR-m.28z CAR T cells, m.19-m.28z CAR T cells or untransduced T cells were infused at either the previously effective dose of 0.5–1 × 10^6 CAR T cells or a higher dosage (2–3 × 10^6)) into mice with established liver fibrosis (Extended Data Fig. 6a). In some experiments, mice were treated with m.uPAR-m.28z, m.19-m.28z or control T cells that express click beetle red luciferase to track T cells in vivo using bioluminescence (Extended Data Fig. 6b).

At either dosage, treatment with m.uPAR-m.28z CAR T cells produced a marked reduction in liver fibrosis compared to treatment with m.19-m.28z or untransduced T cells. Hence, liver samples obtained from mice 20 days after treatment with m.uPAR-m.28z CAR T cells had fewer senescent cells and less fibrosis (as assessed by SA-β-gal and Sirius red staining) than controls (P < 0.001), and this was associated with an accumulation of adoptively transferred T cells (Fig. 3c, Extended Data Fig. 6c, d). Consistent with on-target activity and a therapeutic benefit, mice that were treated with m.uPAR-m.28z CAR T cells showed reduced serum levels of suPAR and of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Fig. 3d–f, Extended Data Fig. 6e–g), indicating efficient elimination of pro-inflammatory senescent HSCs and a reduction in liver damage, respectively. Bioluminescence imaging revealed that transferred T cells at first transited through the lungs as expected. Eventually, uPAR-specific CAR T cells—but not CD19-directed CAR T cells or untransduced T cells—accumulated in the livers of CCl4-treated mice, showing expansion over a few days followed by rapid contraction (Fig. 3g, h). The high senolytic activity of uPAR CAR T cells was corroborated by an efficient reduction of fibrosis under the aggravated conditions produced by prolonged exposure to CCl4, as well as a sustained resolution of fibrosis in long-term follow-up studies (Extended Data Fig. 6h, i).

Mice treated at the lower effective dose remained highly active and did not display observable signs of morbidity, changes in temperature or weight or relevant alterations in cell blood counts (Extended Data Fig. 7a–c, e). A moderate infiltration of macrophages was noted in the lungs after 20 days, which also occurred in mice treated with m.19-m.28z CAR T cells or untransduced T cells (Extended Data Fig. 7d). Mice treated at the supertherapeutic dose presented with hypothermia and weight loss, which was accompanied by a rise in serum cytokines including IL-6, GM-CSF, G-CSF and IFNγ (Extended Data Fig. 8a–e). Similar to CAR T cell-associated cytokine-release syndrome (CRS), this early toxicity was transient, associated with local accumulation and activation of macrophages and could be mitigated by lower doses of CAR T cells or treatment with CRS-preventing inhibitors of IL-6R and IL-1R.
Figs. 8f–i, 9). Altogether, these findings indicate that uPAR-directed CAR T cells at an appropriate dosage can deplete senescent cells without inducing severe CRS-like symptoms, and highlight the potential of short-acting CD28- and CD3ζ-based CAR T cells in senescence-associated indications.

We also tested whether CAR T cells that target uPAR could be effective against fibrosis induced by non-alcoholic steatohepatitis (NASH)—a condition that is increasing in incidence and for which effective therapeutic options are lacking. Although the contribution of cellular senescence to the pathology of NASH is poorly understood, its role in other fibrosis settings prompted us to test two well-established mouse models of NASH for the presence of senescent cells. Indeed, senescent cells were prevalent around the fibrotic areas (Fig. 4a, Extended Data Fig. 10a) and co-expressed uPAR together with either a marker of HSCs (desmin) or a marker of macrophages (F4/80) (Fig. 4b). Accordingly, treatment of mice with diet-induced NASH using 0.5 × 10^9 m.uPAR-m.28z CAR T cells—but not untransduced control T cells—efficiently eliminated senescent cells, reduced fibrosis and improved liver function (as assessed by serum albumin levels) without eliciting detectable toxicity (Fig. 4c, d, Extended Data Fig. 10b–f). Thus, senolytic CAR T cells are effective against liver fibrosis of different aetiologies.

### Perspectives
Here we identify uPAR as a protein that is broadly induced on the surface of senescent cells, and we show that uPAR-targeted CAR T cells can eliminate senescent cells in vitro and in vivo. Owing to its secretion, suPAR serves as a plasma biomarker to assess the senolytic activity of CAR T cells in vivo. Whereas a previous report investigated uPAR as a CAR target in ovarian cancer 

10, our results provide proof-of-principle of the therapeutic potential of senolytic CAR T cells in senescence-associated pathologies. Although further work is needed to determine whether uPAR-targeting CAR T cells have the required safety profile to be developed clinically, appropriately dosed senolytic CAR T cells can infiltrate the areas of senescence, efficiently target senescent cells and produce a therapeutic benefit without notable toxicity in mice. Future iterations of this approach could target other cell-surface molecules that are specific to particular senescence contexts, incorporate safety switches

36, or use combinatorial strategies to maximize efficacy while minimizing side-effects 

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Methods

RNA extraction, RNA-seq library preparation and sequencing
Total RNA was isolated from three different models of senescence. (1) 
Kras<sup>G12D</sup>;p53<sup>−/−</sup> cells after 8 days of treatment with vehicle (dimethyl sulfoxide (DMSO)) or combined treatment with the MEK inhibitor trametinib (25 nM) and the CDK4/6 inhibitor palbociclib (500 nM). (2) Oncogene-induced senescent hepatocytes generated in C57BL/6 mice by HTVI. For each mouse, 25 μg of pt3-EFα-Nras<sup>G12V</sup>-IREs-GFP-P2A-luciferase plasmid (or pt3-EFα-Nras<sup>G12V</sup>-IREs-GFP-P2A-luciferase plasmid as control) and 5 μg CMV-SB13 were suspended in saline solution at the volume of 10% of mouse body weight for administration. Six days after HTVI, mice were anaesthetized and placed on the platform for liver perfusion. Sequential perfusions of Hank’s balanced salt solution (HBSS) containing EGTA and HBSS containing collagenase IV were performed, followed by passing the dissociated liver cells through a 100-μm cell strainer. The hepatocytes were then washed again using low-glucose Dulbecco’s modified Eagle’s medium (DMEM) and centrifuged at a low speed. DAPI-negative and GFP-positive hepatocytes, indicating successful transduction of mutant Nras expression, were isolated through low-pressure fluorescence-activated cell sorting (FACS). 3) Senescent or proliferating HSCs (datasets were obtained from a previous study<sup>28</sup>) and proliferating, quiescent or senescent IMR-90 cells (datasets were obtained from a previous study<sup>29</sup>). Sequencing and library preparation were performed at the Integrated Genomics Operation (IGO) at the Memorial Sloan Kettering Cancer Center (MSKCC). RNA-seq libraries were prepared from total RNA. After RiboGreen quantification and quality control by Agilent BioAnalyzer, 100–500 ng of total RNA underwent poly(A) selection and TruSeq library preparation according to the instructions provided by Illumina (TruSeq Stranded mRNA LT Kit, RS-122-2102), with 8 cycles of PCR. Samples were barcoded and run on a HiSeq 4000 or HiSeq 2500 in a 50 bp–50 bp paired-end run, using the HiSeq 3000/4000 SBS Kit or TruSeq SBS Kit v.4 (Illumina) at MSKCC’s IGO core facility.

RNA-seq read mapping, differential gene expression analysis and heat map visualization
The resulting RNA-seq data were analysed by removing adaptor sequences using Trimmomatic<sup>30</sup>. RNA-seq reads were then aligned to GRCm38.91 (mm10) with STAR<sup>31</sup> and the transcript count was quantified using featureCounts<sup>32</sup> to generate a raw-count matrix. Differential gene expression analysis and adjustment for multiple comparisons were performed using the DESeq2 package<sup>33</sup> between experimental conditions, with two independent biological replicates per condition, implemented in R (http://cran.r-project.org/). Genes were determined to be differentially expressed on the basis of a greater than two-fold change in gene expression with an adjusted P value of less than 0.05. For heat map visualization of differentially expressed genes, samples were normalized by z-score and plotted using the pheatmap package in R. Transcripts encoding molecules that were determined to be located in the plasma membrane with a confidence score higher than 3 (range 0–5) as determined by UniProtKB were considered cell-surface molecules.

Functional annotations of gene clusters
Pathway enrichment analysis was performed in the resulting gene clusters with the Reactome database using Enrichr<sup>34</sup>. The significance of the tests was assessed using a combined score, described as c = log(p) × z, in which c is the combined score, p is the Fisher’s exact test P value and z is the z-score for deviation from expected rank.

Cell lines and compounds
The following cell lines were used in this study: mouse Kras<sup>G12D</sup>;Trp53<sup>−/−</sup> (KP) lung cancer cells (provided by T. Jacks and expressing luciferase–GFP as described<sup>35</sup>), and NALM6 and Eμ-ALL01 cells expressing firefly luciferase–GFP<sup>36</sup>. Cells were maintained in a humidified incubator at 37 °C with 5% CO₂. KP cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS) and 100 μg ml<sup>−1</sup> penicillin–streptomycin. NALM6 and Eμ-ALL01 cells were grown in complete medium composed of RPMI supplemented with 10% FBS, 1% l-glutamine, 1% MEM non-essential amino acids, 1% HEPES buffer, 1% sodium pyruvate, 0.1% β-mercaptoethanol and 100 IU ml<sup>−1</sup> penicillin–streptomycin. Human primary melanocytes were grown in dermal cell basal medium (ATCC, 200-030) supplemented with the adult melanocyte growth kit (ATCC, 200-042), 10% FBS and 100 IU ml<sup>−1</sup> penicillin–streptomycin. All cell lines used were negative for mycoplasma.

For drug-induced senescence experiments in vitro, trametinib (S2673) and palbociclib (S1116) were purchased from Selleck Chemicals and dissolved in DMSO to yield 10 mM stock solutions, which were stored at −80 °C. Cells were treated with MEK inhibitor (25 nM) and CDK4/6 inhibitor (500 nM). The growth medium was changed every two days. For in vivo experiments trametinib was dissolved in a 5% hydroxypropyl methylcellulose and 2% Tween-80 solution (Sigma) and palbociclib was dissolved in sodium lactate buffer (pH 4) (as described previously<sup>37</sup>). Mice were treated with 1 mg per kg body weight of trametinib and 150 mg per kg body weight of palbociclib as previously described<sup>38</sup>. Caerulein was purchased from Bachem. Anakinra was purchased from Sobi and administered intraperitoneally at a dose of 30 mg per kg body weight twice a day for 8 days starting 24 h before transfer of CAR T cells. Anti-mouse IL-6R (clone MPs-20F3) was purchased from BioXCell and administered intraperitoneally once per day at 25 mg per kg body weight for the first dose and 12.5 mg per kg body weight for subsequent doses for 8 days starting 24 h before transfer of CAR T cells as previously described<sup>39</sup>.

SA-β-gal staining
SA-β-gal staining was performed as previously described<sup>29</sup> at pH 6.0 for human cells and tissue and pH 5.5 for mouse cells and tissue. Fresh frozen tissue sections or adherent cells plated in 6-well plates were fixed with 0.5% glutaraldehyde in phosphate-buffered saline (PBS) for 15 min, washed with PBS supplemented with 1 mM MgCl₂ and stained for 5–8 h in PBS containing 1 mM MgCl₂, 1 mg ml<sup>−1</sup> X-gal, 5 mM potassium ferricyanide and 5 mM potassium ferrocyanide. Tissue sections were counterstained with eosin. Five high power fields per well or section were counted and averaged to quantify the percentage of SA-β-gal<sup>+</sup> cells.

Quantitative PCR with reverse transcription
Total RNA was isolated using the RNeasy Mini Kit (Qiagen) and cDNA was obtained using TaqMan reverse-transcription reagents (Applied Biosystems). Quantitative PCR (qPCR) was performed in triplicates using SYBR green PCR master mix (Applied Biosystems) on the Viia7 Real-Time PCR System (Invitrogen). GAPDH or ACTB served as endogenous normalization controls for mouse and human samples.

Mice
All mouse experiments were approved by the MSKCC Internal Animal Care and Use Committee. All relevant animal use guidelines and ethical regulations were followed. Mice were maintained under specific pathogen-free conditions, and food and water were provided ad libitum. The following mice were used: C57BL/6N background, NOD-scid IL2R<sup>γc</sup> (NSG) mice (purchased from The Jackson laboratory) and B6.SJL-Ptcr<sup>−/−</sup>/BoyAItac (CD45.1 mice) (purchased from Taconic). Mice of both sexes were used at 8–12 weeks of age (5–7 weeks old for the xenograft experiments and 6–10 weeks old for T cell isolation) and were kept in group housing. Mice were randomly assigned to the experimental groups.

Transposon-mediated intrahepatic gene transfer
Transposon-mediated intrahepatic gene transfer was performed as previously described<sup>40</sup>. In brief, 8–12-week-old C57BL/6j mice received a saline solution at a final volume of 10% of their body weight containing 30 μg of total DNA composed of a 5:1 molar ratio of transposon-encoding DNA and 5 μg CMV-SB13.
vector (containing either the sequence for NrasG12D or the sequence for the GTPase-dead form NrasG12D/D38A) to transposase-encoding vector (Sleeping Beauty 13) through HTVLI. For CAR T cell studies, NSG mice were intravenously injected with 5 × 10^6 human CAR T cells or untransduced T cells 10 days after HTVLI delivery and monitored by bioluminescence imaging using the IVIS Imaging System (PerkinElmer) with Living Image software (PerkinElmer). At day 15 after CAR injection, mice were euthanized and livers were removed and used for further analysis.

**Generation of mouse pancreatic intraepithelial neoplasias**

The mouse strain has been previously described49. To induce pancreatic intraepithelial neoplasias, KC:RIK (p48-Cre;RIK;LSLKrasG12D) male mice were treated with eight (one per hour) intra-peritoneal injections of 80 μg kg⁻¹ caraeulin (Bachem) for two consecutive days. Mice were then euthanized 21 weeks later and their pancreas were used for further analysis. Age-matched C57BL/6 mice (expressing wild-type Kras) injected with PBS were used as controls for normal pancreas.

**In vivo induction of CCl₄-induced liver fibrosis**

C57BL/6N mice were treated twice a week with 12 consecutive intra-peritoneal injections of 1 ml kg⁻¹ tetrachloride (CCl₄) to induce liver fibrosis44,45. For mouse CAR T cell studies, cyclophosphamide (200 mg kg⁻¹) was administered 16–24 h before T cell injection. Mice received 0.5–1×10⁶ or 2–3×10⁶ CAR T cells or untransduced T cells (same total numbers of T cells) and CCl₄ was continuously administered at the same dose and interval until day 20 after CAR T cell injection, when mice were euthanized 48–72 h after the last CCl₄ injection. Blood was collected by facial vein puncture or cardiac puncture.

**In vivo induction of NASH-induced liver fibrosis**

C57BL/6N mice were fed with a NASH-inducing diet (Teklad TD.160785, which contains 10.2% kcal from protein, 37.3% kcal from carbohydrate and 52.6% kcal from fat) and fructose-containing drinking water which contains 10.2% kcal from protein, 37.3% kcal from carbohydrate and 52.6% kcal from fat. Mice were then euthanized 21 weeks later and their livers were removed. In vivo induction of NASH-induced liver fibrosis46,47. For mouse CAR T cell studies, cyclophosphamide (200 mg kg⁻¹) was administered 16 h before T cell injection. Mice received 0.5–1×10⁶ or 2–3×10⁶ CAR T cells or untransduced T cells (same total numbers of T cells) and they received the same NASH diet until day 20 after CAR T cell injection, when they were euthanized. Blood was collected by facial vein puncture or cardiac puncture.

**Histological analysis**

Tissues were fixed overnight in 10% formalin, embedded in paraffin and cut into 5-μm sections. Sections were subjected to haematoxylin and eosin (H&E) staining, and to Sirius red staining for fibrosis detection. For fibrosis quantification, at least three whole sections from each mouse were scanned and the images were quantified using NIH ImageJ software. The amount of fibrotic tissue was calculated relative to the total analysed liver area as previously described. Immunohistochemical and immunofluorescence staining was performed following standard protocols. The following primary antibodies were used: anti-human uPAR (R&D, AF807, lot BB0315071, 1:50), anti-mouse uPAR (R&D, AF534, lot DCL0418021, 1:50), anti-mouse NRAS (Santa Cruz, SC-31, lot A1020, 1:50), anti-mouse SMA (Abcam, ab5694, lot GR283004-16, 1:50), anti-mouse KATE (Evrogen, ab233, lot 2330120167, 1:1,000), anti-human CD3 (Abcam, ab5690, lot GR3220039-4, 1:50), Myc-tag (Cell Signalling, 2276S, lot 24, 1:50), anti-mouse Ki-67 (Abcam, ab16667, lot GR3305281-1, 1:200), anti-mouse IL-6 (Abcam, ab6672, lot GR3195128-19, 1:50), p16INK4A (Proteintech, 10883-1-AP, lot 00057396, 1:50), anti-mouse P-ERK202/Y204 (Cell Signalling, 4370, lot 1:800), desmin (Thermo Fisher Scientific, RB-9014, lot 9014p1806Q, 1:200), AF488 donkey anti-rabbit (Invitrogen, A21206, lot 1874771, 1:500), AF488 donkey anti-mouse (Invitrogen, A21202, lot 1820538, 1:50), AF594 donkey anti-rabbit (Invitrogen, A21207, lot 1607280, 1:50), AF594 donkey anti-mouse (Invitrogen, A21203, lot 1163990, 1:50), AF594 donkey anti-goat (Invitrogen, A1058, lot 2045324, 1:50), AF594 goat anti-rat (Invitrogen, A1107, lot 1903506, 1:500).

**Flow cytometry**

For analysis of uPAR expression in cell lines after induction of senescence, KP cells were treated with trametinib (25 nM) and palbociclib (500 nM) or with vehicle (DMSO), and human primary melanocytes were continuously passaged for 15 passages and then trypsinized, resuspended in PBS supplemented with 2% FBS and stained with the following antibodies for 30 min on ice: PE-conjugated anti-mouse uPAR (R&D, FAB531P) or APC-conjugated anti-human uPAR (Thermo Fisher Scientific, 17-3879-42). The following fluorophore-conjugated antibodies were used for in vitro and in vivo experiments in the indicated dilutions (‘h’ prefix denotes anti-human; ‘m’ prefix denotes anti-mouse): hCD4 APC-Cy7 (clone 2D1, BD, 557833, lot 9081815, 1:100), hCD4 BV395 (clone SK3, BD, 563550, lot 6252529, 1:100), hCD4 BV480 (clone SK3, BD, 566104, lot 8092993, 1:50), hCD62L BV421 (clone DREG-56, BD, 563862, lot 8194954, 1:100), hCD45RA BV650 (clone HI100, BD, 536936, lot 9057952, 1:100), hPD-1 BV408 (clone EH12.1, BD, 566112, lot 8235507, 1:100), hCD9 BV737 (clone SJ25C1, BD, 563403, lot 8130572, 1:100), hCD271 PE (clone C40-1457, BD, 566104, lot 8130572, 1:100), hCD271 PE-Cy7 (clone SK3, BD, 566104, lot 8130572, 1:100), hCD3 BV517 (clone 2D1, BD, 557833, lot 9081815, 1:100), hCD4 BV395 (clone SK3, BD, 563550, lot 6252529, 1:100), immunofluorescence staining was performed following standard protocols. The following primary antibodies were used: anti-human TP53 (Cell Signaling, 2233S, lot 24, 1:50), anti-mouse P-ERKT202/Y204 (Cell Signaling, 4370, lot 1:800), desmin (Thermo Fisher Scientific, RB-9014, lot 9014p1806Q, 1:200), AF488 donkey anti-rabbit (Invitrogen, A21206, lot 1874771, 1:500), AF488 donkey anti-mouse (Invitrogen, A21202, lot 1820538, 1:50), AF594 donkey anti-rabbit (Invitrogen, A21207, lot 1607280, 1:50), AF594 donkey anti-mouse (Invitrogen, A21203, lot 1163990, 1:50), AF594 donkey anti-goat (Invitrogen, A1058, lot 2045324, 1:50), AF594 goat anti-rat (Invitrogen, A1107, lot 1903506, 1:500).

**Patient-derived xenografts**

Experiments with patient-derived xenografts were performed as described47, using 6–7-week-old female NSG mice. MSK-LX27 was derived from a lung adenocarcinoma containing KRASG12D and P53 (P53 is also known as TP53) mutations and a deletion in CDKN2A and was cut into pieces and inserted in the subcutaneous space. Mice were monitored daily, weighed twice weekly and caliper measurements began when tumours became visible. Tumours were measured using the formula: tumour volume = (D × d^2)/2 (in which D is the longer diameter and d is the shorter diameter) and when they reached a size of 100–200 mm³, mice were randomized on the basis of the starting tumour volume and treated with vehicle or trametinib (3 mg per kg body weight) and palbociclib (150 mg per kg body weight) orally for 4 consecutive days followed by 3 days off treatment. Experimental end points were achieved when tumours reached a size of 2,000 mm³ or became ulcerated. Tumours were collected at the experimental end point and tissue was divided evenly for 10% formalin fixation and optimal cutting temperature (OCT) compound frozen blocks.

**Patient samples**

De-identified human samples from liver biopsies of patients with liver fibrosis from viral (hepatitis B or C), alcoholic and non-alcoholic fatty liver disease were obtained through the Department of Pathology at Mount Sinai Hospital. Human pancreatic intraepithelial neoplasia samples were obtained through the Department of Pathology at MSKCC. Human atherosclerosis samples were obtained through the Department of Pathology at Weill Medical College of Cornell University. All human studies complied with all relevant guidelines and ethical regulations and were approved by the Institutional Review Board at Mount Sinai, Weill Medical College or MSKCC.
Serum cytokines were measured using cytometric bead arrays (BD) as per the manufacturer’s instructions. Detection of suPAR levels

suPAR levels from cell culture supernatant or mouse plasma were evaluated by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s protocol (R&D systems, DY531 (mouse) or DY807 (human)).

Liver function tests

The levels of ALT, AST and albumin in mouse serum were measured according to the manufacturer’s protocol, using the EALT-100 (ALT), EASTR-100 (AST) and DIAG-250 (albumin) kits from BioAssay systems.

Isolation, expansion and transduction of human T cells

All blood samples were handled following the required ethical and safety procedures. Peripheral blood was obtained from healthy volunteers and buffy coats from anonymous healthy donors were purchased from the New York Blood Center. Peripheral blood mononuclear cells were isolated by density gradient centrifugation. T cells were purified using the human Pan T Cell Isolation Kit (Miltenyi Biotec), stimulated with CD3/CD28 T cell activator Dynabeads (Invitrogen) as described27 and cultured in X-VIVO 15 (Lonza) supplemented with 5% human serum (Gemini Bio-Products), 5 ng/ml interleukin-7 and 5 ng/ml interleukin-15 (PeproTech). T cells were counted using an automated cell counter (Nexcelom Bioscience).

Forty-eight hours after initiating T cell activation, T cells were transduced with retroviral supernatants by centrifugation on RetroNectin-coated plates (Takara). Transduction efficiencies were determined four days later by flow cytometry and CAR T cells were adoptively transferred into mice or used for in vitro experiments.

Isolation, expansion and transduction of mouse T cells

B6.129P2-Tcrd/Tcrd mice (CD45.1 mice) were euthanized and spleens were collected. After tissue dissection and red blood cell lysis, primary mouse T cells were purified using the mouse Pan T cell Isolation Kit (Miltenyi Biotec). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (HyClone), 10 mM HEPES (Invitrogen). Purified T cells were cultured in RPMI-1640 (Invitrogen). CAR T cells were transduced with retroviral supernatants by centrifugation on RetroNectin-coated plates (Takara). Transduction efficiencies were determined four days later by flow cytometry and CAR T cells were adoptively transferred into mice or used for in vitro experiments.

Genetic modification of T cells

The human and mouse SFG γ-retroviral m.uPAR-28z plasmids were constructed by stepwise Gibson assembly (New England Biolabs) using the SFG-1928z backbone as previously described34–56. The amino acid sequence for the single-chain variable fragment (scFv) specific for mouse uPAR was obtained from the heavy and light chain variable regions of a selective monoclonal antibody against mouse uPAR (R&D MAB531-100) through mass spectrometry performed by Bioinformatics Solutions. In the human SFG-m.uPAR-h.28z CARs, the anti-mouse uPAR scFv is thus preceded by a human CD8A leader peptide and followed by CD28 hinge–transmembrane–intracellular regions, and CD3ζ intracellular domains linked to a P2A sequence to induce co-expression of truncated LNGFR. In the mouse SFG-m.uPAR-m.28z CARs, the anti-mouse uPAR scFv is preceded by a mouse CD8A leader peptide and followed by the MHC-I tag sequence (EQLKISEEDL), mouse CD28 transmembrane and intracellular domain and mouse CD3ζ intracellular domain33.

Plasmids encoding the SFG retroviral vectors were used to transfect gpg29 fibroblasts (H29) to generate VSVG-pseudotyped retroviral supernatants, which were used to construct stable retrovirus-producing cell lines as described33. For T cell imaging studies, mouse T cells were transduced with retroviral supernatants encoding SFG-GFP–click beetle red luciferase42.
Cytotoxicity assays
The cytotoxicity of CAR T cells was determined by standard luciferase-based assays or by calcein-AM-based cytotoxicity assays.

For luciferase-based assays, target cells expressing firefly luciferase (FFLuc-GFP) were co-cultured with T cells in triplicate at the indicated effector:target ratios using black-walled 96-well plates with 5 × 10^4 (for NALM6 and Eμ-ALL01) or 1.5 × 10^5 (for KP) target cells in a total volume of 100 μl per well in RPMI or DMEM medium, respectively. Target cells alone were plated at the same cell density to determine the maximum luciferase expression (relative light units (RLU)) and maximum release was determined by addition of 0.2% Triton-X100 (Sigma). Either 4 or 18 h later, 100 μl luciferase substrate (Bright-Glo, Promega) was directly added to each well. Emitted light was detected in a luminescence plate reader. Lysis was determined as (1−(RLU_{sample}/(RLU_{max}))) × 100. For calcein-AM-based assays, target cells (NALM6) were loaded with 20 μM calcein-AM (Thermo Fisher Scientific) for 30 min at 37 °C, washed twice and co-incubated with T cells in triplicate at the indicated effector:target ratios in 96-well round-bottomed plates with 5 × 10^4 target cells in a total volume of 200 μl per well in complete medium. Target cells alone were plated at the same cell density to determine spontaneous release and maximum release was determined by incubating the targets with 0.2% Triton-X100 (Sigma). After a 4 h co-culture, supernatants were collected and free calcein was quantitated using a Spark plate reader (Tecan). Lysis was calculated as: ((experimental release − spontaneous release)/(maximum release − spontaneous release)) × 100.

Statistical analysis and figure preparation
Data are presented as mean ± s.e.m. Statistical analysis was performed by Student’s t-test using GraphPad Prism v.6.0 or 7.0 (GraphPad software). P-values of less than 0.05 were considered to be statistically significant. Survival was determined using the Kaplan–Meier method. No statistical methods were used to predetermine sample size in the mouse studies, and mice were allocated at random to treatment groups. The investigators were not blinded to allocation during experiments and outcome assessment. Figures were prepared using BioRender.com for scientific illustrations and Illustrator CC 2019 (Adobe).

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
The RNA-seq data have been deposited in the Gene Expression Omnibus under the accession number GSE145642. Source data are provided with this paper. All other data supporting the findings of this study will be made available upon reasonable request to the corresponding authors. Source data

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Genes encoding surface molecules that are commonly upregulated in senescence. a, Heat map of genes upregulated in therapy-induced senescence (TIS), oncogene-induced senescence (OIS) or replication-induced senescence (RIS) in HSCs. b, Venn diagram showing the number of common genes upregulated in the three datasets in a. c, Fold change (log₂(expression in senescent cells/expression in non-senescent cells)) of the eight commonly upregulated genes in the three different datasets in a. d, Combined enrichment score of significantly enriched gene sets among the eight commonly upregulated genes in senescence. ECM, extracellular matrix; GPI, glycosylphosphatidylinositol. e, Heat map showing the expression profile of uPAR (PLAUR) in human vital tissues (as determined by the Human Proteome Map) compared to the expression profiles of other targets of CAR T cells in clinical trials. NK cells, natural killer cells. f, Immunohistochemical staining of mouse uPAR (m.uPAR) in vital tissues of C57BL/6j mice. Representative results of n = 2 independent experiments. g, Reads per kilobase (RPKM) of PLAUR mRNA in proliferating, quiescent (induced by serum starvation) or senescent (triggered by overexpression of HRASG12V) human IMR-90 fibroblasts. Results of one independent experiment with n = 3 replicates for proliferating, quiescent and senescent conditions. Data are mean ± s.e.m.; two-tailed unpaired Student’s t-test.
Extended Data Fig. 2 | uPAR is a cell-surface and secreted biomarker of senescence. **a, b,** qPCR of SASP-associated gene expression in senescent versus proliferating mouse KP tumour cells (a) or human primary melanocytes (b) and representative SA-β-gal staining; a.u. arbitrary units. **c, d,** Co-immunofluorescence staining and quantifications of uPAR (red) and Ki-67 (green) (c) or uPAR (red) and IL-6 (green) (d). **e,** Immunohistochemical staining of uPAR or phosphorylated ERK (P-ERK) in serial sections of mouse livers six days after transfection by HTVI with a plasmid encoding NrasG12V. Representative results of two independent experiments (n = 3 mice per group). **f–i,** Mice expressing endogenous KrasG12D in pancreatic epithelial cells were treated with caerulein (Cr) and euthanized 21 weeks afterwards when they had developed pancreatic intraepithelial neoplasias. Age-matched C;Rlk mice (expressing wild-type Kras) injected with PBS were used as controls. **f,** Co-immunofluorescence staining of KATE (red) and uPAR (green). Representative results of two independent experiments (n = 3 mice per group). **g,** Levels of suPAR in the mice in f. Representative results of two independent experiments (n = 2 mice per group). **h,** Co-immunofluorescence staining and quantification of uPAR (red) and Ki-67 (green). Representative results of two independent experiments (n = 3 mice per group). **i,** Representative SA-β-gal staining. Representative results of one independent experiment (n = 3 mice per group). **j–m,** Mice were treated with either vehicle or CCl4 twice weekly for six weeks to induce liver fibrosis. **j,** Fold change in serum levels of suPAR. Representative results of two independent experiments (vehicle, n = 4; CCl4, n = 9 mice per group). Two-tailed unpaired Student’s t-test. **k,** Co-immunofluorescence staining and quantification of uPAR (red) and Ki-67 (green). Representative results of two independent experiments (n = 2 mice per group). **l,** Co-immunofluorescence staining and quantification of uPAR (red) and IL-6 (green). Representative results of two independent experiments (n = 3 mice per group). **m,** Representative SA-β-gal staining. Representative results of two independent experiments (n = 3 mice per group). Data are mean ± s.e.m. (c, d, h, j, l).
Extended Data Fig. 3 | uPAR is a marker of senescence in senescence-associated human pathologies. a, Left, immunohistochemical expression of human uPAR (h.uPAR) and SA-β-gal in human samples of hepatitis-induced liver fibrosis (n = 7 patients). Right, co-immunofluorescence staining and quantification of uPAR (red) and p16 (green) or uPAR (red) and IL-6 (green) in human samples of hepatitis-induced liver fibrosis (n = 3). b, Left, immunohistochemical expression of uPAR and SA-β-gal in human samples from patients with eradicated hepatitis C virus (HCV) and residual liver fibrosis (n = 7 patients). Right, co-immunofluorescence staining and quantification of uPAR (red) and p16 (green) or uPAR (red) and IL-6 (green) in human samples of HCV-induced liver fibrosis (n = 3). Data are mean ± s.e.m. (a, b). c, Immunohistochemical staining of uPAR in human carotid endarterectomy samples (n = 5 patients). d, Immunohistochemical staining of uPAR in human pancreas bearing pancreatic intraepithelial neoplasia (PanIN) compared to normal pancreas controls (n = 3 patients).
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | m.uPAR-h.28z CAR T cells selectively target uPAR-positive cells. 

a, Construct maps encoding human m.uPAR-h.28z and h.19-h.28z CARs or mouse m.uPAR-m.28z and m.19-m.28z CARs. 

b, Flow cytometry analysis showing the expression levels of CAR and LNGFR in m.uPAR-h.28z and h.19-h.28z CAR T cells compared to untransduced T cells. Representative results of \( n = 4 \) independent experiments. 

c, Flow cytometry analysis of mouse uPAR and human CD19 expression on wild-type NALM6 cells and NALM6-m.uPAR cells. Representative results of \( n = 3 \) independent experiments. 

d, Cytotoxic activity of m.uPAR-h.28z, h.19-h.28z and untransduced T cells as determined by 4-h calcein assay with firefly luciferase (FFL)-expressing NALM6 wild-type or NALM6-m.uPAR cells as targets. Representative results of \( n = 3 \) independent experiments performed in triplicate. Data are mean ± s.e.m. 

e, Granzyme B (GrB) and IFNγ expression of CD4+ and CD8+ m.uPAR-h.28z CAR T cells 18 h after co-culture with wild-type NALM6, NALM6-m.uPAR or senescent KP cells as determined by intracellular cytokine staining. Results of \( n = 1 \) independent experiment (no target and NALM6 WT, \( n = 2 \); NALM6-m.uPAR and KP senescent, \( n = 3 \) replicates). Data are mean ± s.e.m. 

f, Experimental layout for Fig. 2c–i. Mice were injected with a plasmid encoding NrasG12V-GFP-luciferase and treated with \( 0.5 \times 10^6 \) m.uPAR-h.28z CAR T cells or untransduced T cells 10 days after injection. Mice were euthanized 15 days after CAR administration and livers were used for further analysis. Images were created with BioRender.com. 

g, Flow cytometry analysis of mouse uPAR and human CD19 expression on wild-type Eμ-ALL01 cells and Eμ-ALL01-m.uPAR cells. Representative results of \( n = 3 \) independent experiments. 

h, Flow cytometry staining of Myc-tag and mouse uPAR on m.uPAR-m.28z CAR T cells, m.19-m.28z CAR T cells and untransduced T cells as compared to FMO control. Representative results of \( n = 2 \) independent experiments.
Extended Data Fig. 5 | Senolytic CART cells target senescent cells in a 
\textit{Kras}^{G12D}\textsuperscript{-driven model of lung cancer.} \textbf{a}. Experimental layout. C57BL/6N mice were intravenously injected with 10,000 \textit{Kras}^{G12D};\textit{p53}^{−/−} cells. Treatment with combined MEK inhibitor (1 mg per kg body weight) and CDK4/6 inhibitors (100 mg per kg body weight) was started seven days later, followed by adoptive transfer of 2 × 10^6 CD45.1\textsuperscript{+} T cells (m.uPAR-m.28z CART cells, m.19-m.28z CART cells or untransduced T cells) one week later. A subset of mice received a second infusion of 1 × 10^6 m.uPAR-m.28z CART cells, m.19-m.28z CART cells or untransduced T cells seven days after the first injection of T cells. The images of the mouse, tumour cells and CART cells were created with BioRender.com. Cp, cyclophosphamide. \textbf{b}, Kaplan–Meier curve showing survival of mice (one-sided log-rank (Mantel–Cox) test). Results of two independent experiments (UT, \(n = 16\); m.19-m.28z, \(n = 14\); m.uPAR-m.28z, \(n = 18\); UT reinjection, \(n = 6\); m.19-m.28z reinjection, \(n = 7\); m.uPAR-m.28z reinjection, \(n = 7\) mice). \textbf{c}, Weight (c) and temperature (d) measured 24 h before and at different time points after CART cell infusion. \(P\) values (ns, not significant) refer to the comparison between untransduced and m.uPAR-m.28z injected mice at 48 h (weight, \(P = 0.9329\); temperature, \(P = 0.1534\)). Results of one independent experiment (UT, \(n = 5\); m.19-m.28z, \(n = 5\); m.uPAR-m.28z, \(n = 8\); UT reinjection, \(n = 5\); m.19-m.28z reinjection, \(n = 7\); m.uPAR-m.28z reinjection, \(n = 7\) mice). \textbf{d}, Cell counts of CD45.1\textsuperscript{+} T cells and expression of the activation markers CD25 and CD69 (UT, \(n = 4\); m.19-m.28z, \(n = 5\); m.uPAR-m.28z, \(n = 5\) mice) on CD45.1\textsuperscript{+} T cells in the lungs of mice seven days after administration of m.uPAR-m.28z CART cells, m.19-m.28z CART cells or untransduced T cells. \textbf{f}, Representative SA-β-gal staining and quantification in the lungs of mice seven days after treatment with m.uPAR-m.28z CART cells compared to mice that were treated with m.19-m.28z CART cells or untransduced T cells (\(n = 3\) mice per group). Data are mean ± s.e.m.; two-tailed unpaired Student’s \(t\) test. (\(c–f\)).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Senolytic CAR T cells show therapeutic activity in CCl4-induced liver fibrosis. a, Layout for experiments performed using the CCl4-induced liver fibrosis model: C57BL/6N mice received intraperitoneal infusions of CCl4 twice weekly for six weeks and were intravenously infused with 0.5–1 × 10⁶ (Fig. 3) or 2–3 × 10⁶ (c–i) mouse m.uPAR-m.28z CAR T cells, m.19-m.28z CAR T cells or untransduced T cells 16–24 h after administration of cyclophosphamide (200 mg kg⁻¹). Mice were euthanized 20 days after CAR T cell infusion to assess liver fibrosis. Images were created with BioRender.com. b, Expression of GFP-tagged click beetle red (CBR) luciferase and Myc-tag in m.uPAR-m.28z and m.19-m.28z CAR T cells that were used for T cell imaging experiments (Fig. 3g, h) compared to control T cells. Representative results of n = 2 independent experiments. c, Sirius red and SA-β-gal staining and quantifications in livers from treated mice (n = 6 mice per group). d, Co-immunofluorescence of uPAR (red) and SMA (green) or Myc-tag (red) and SMA (green) in the livers of treated mice. e, Fold change difference in serum levels of suPAR 20 days after compared to 1 day before (day −1) injection of CAR T cells (UT, n = 18; m.19-m.28z, n = 6; m.uPAR-m.28z, n = 17 mice). f, g, Levels of serum ALT (f) and AST (g) 20 days after CAR treatment (UT, n = 10; m.19-m.28z, n = 8; m.uPAR-m.28z, n = 10 mice). h, Co-immunofluorescence staining of desmin (red) and Ki-67 (green) in the livers of mice 15, 20 and 77 days after treatment with CAR T cells. CCl4 treatment was stopped 20 days after T cell infusion (n = 3 mice per group). i, Mice were treated with CCl4 for 10 weeks. Sirius red staining in the livers of mice before (day −1) and 20 days after T cell administration (UT, n = 4; m.uPAR-m.28z, n = 2 mice). Representative results of n = 2 independent experiments (c–i). Data are mean ± s.e.m.; two-tailed unpaired Student’s t test (c, e–g).
Extended Data Fig. 7 | Safety profile of m.uPAR-m.28z CAR T cells at therapeutic doses of T cells. a–e, C57BL/6N mice received intraperitoneal infusions of CCl4, twice weekly for six weeks and were intravenously injected with 0.5–1 × 10^6 m.uPAR-m.28z CAR T cells, 1 × 10^6 m.19-m.28z CAR T cells or untransduced T cells 16 h after administration of cyclophosphamide (200 mg kg⁻¹). Mice were euthanized 20 days after T cell administration to assess potential toxicities and lung histopathology. a, Kaplan–Meier curve showing survival of mice after treatment with m.uPAR-m.28z CAR T cells (n = 16 mice), m.19-m.28z CAR T cells (n = 6 mice) or untransduced T cells (n = 6 mice). b, c, Weight (b) and temperature (c) of mice measured before and at different time points after CAR T cell infusion (UT and m.19-m.28z, n = 6; m.uPAR-m.28z, n = 7 mice). The P value in b refers to differences in weight at 48 h. d, e, Representative H&E staining of lungs (d) and complete blood counts (e) of treated mice 20 days after T cell infusion (UT and m.19-m.28z, n = 3 or 4; m.uPAR-m.28z, n = 4 mice). An increased accumulation of macrophages was observed in the immunodeficient setting. Representative results of n = 1 independent experiment (a–e). Data are mean ± s.e.m. (b, c, e); two-tailed unpaired Student’s t test (b, e).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Safety profile of m.uPAR-m.28z CAR T cells at supratherapeutic doses of T cells. C57BL/6N mice received intraperitoneal infusions of CCl₄ twice weekly for six weeks followed by intravenous infusion of 2–3 × 10⁶ m.uPAR-m.28z CAR T cells or untransduced T cells 16–24 h after administration of cyclophosphamide (200 mg kg⁻¹). A subset of mice (as specified in the figure) received additional treatment with IL-6R-blocking antibodies (IL6Ri) and the IL-1R antagonist anakinra (IL1Ri), starting 24 h before T cell infusion and continuing daily until 6 days after T cell infusion. Mice were euthanized 12 weeks after CAR infusion to assess potential toxicities. a, Kaplan–Meier curve showing survival of mice after injection of CAR T cells (UT, n = 19; UT + IL6Ri/IL1Ri, n = 7; m.uPAR-m.28z, n = 30; m.uPAR-m.28z + IL6Ri/IL1Ri, n = 19 mice). b, c, Temperature (b) and weight (c) of treated mice (UT, n = 7; UT + IL6Ri/IL1Ri, n = 8; m.uPAR-m.28z, n = 11; m.uPAR-m.28z + IL6Ri/IL1Ri, n = 10 mice). d, Weight of mice 120 h after infusion with either m.uPAR-m.28z or m.uPAR-m.28z CAR T cells and additional treatment with IL6Ri and IL1Ri (m.uPAR-m.28z, n = 11; m.uPAR-m.28z + IL6Ri/IL1Ri, n = 10 mice). e, Serum levels of IL-6, GM-CSF, G-CSF and IFNγ in mice that were treated with either m.uPAR-m.28z or untransduced T cells 72 h or 20 days after T cell infusion (UT, n = 5; m.uPAR-m.28z, n = 4 mice at 72 h; n = 5 mice at 20 days). f, g, Number of adoptively transferred CD45.1⁺ T cells (f) and number of macrophages, uPAR⁺ and iNOS⁺ macrophages (g) in the lungs of mice that were treated with m.uPAR-m.28z CAR T cells, m.19-m.28z CAR T cells or untransduced T cells alone or in combination with treatment with IL6Ri and IL1Ri three days after T cell infusion (n = 4 mice per group). h, i, Number of macrophages (h) and uPAR⁺ macrophages (i) in the lungs, liver, bone marrow (BM) and spleen of untreated mice or mice treated with either m.uPAR-m.28z CAR T cells or untransduced T cells 12 weeks after T cell infusion (n = 3 mice per group). Representative results of n = 3 independent experiments (a–d) or n = 1 independent experiment (e–i). All data are mean ± s.e.m.; two-tailed unpaired Student’s t-test (d, e).
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Therapeutic intervention with IL-6R and IL-1R inhibitors does not decrease the therapeutic efficacy of senolytic CAR T cells in CCl₄-induced liver fibrosis. **a**, Experimental layout. C57BL/6N mice received intraperitoneal infusions of CCl₄ twice weekly for six weeks and were intravenously infused with 2–3 × 10⁶ m.uPAR-m.28z CAR T cells or untransduced T cells 24 h after administration of cyclophosphamide (200 mg kg⁻¹). IL-6R-blocking antibodies (IL6Ri) and anakinra (IL1Ri) were first administered 24 h before T cell infusion followed by daily (IL6Ri) or twice daily (IL1Ri) injections for the first six days until treatment was stopped. Mice were euthanized 20 days after T cell infusion to assess liver fibrosis. Images were created with BioRender.com. **b**, Fold change difference in serum levels of suPAR 20 days after compared to 1 day before (day −1) CAR T cell treatment (UT, n = 4; UT + IL6Ri/IL1Ri, n = 8; m.uPAR, n = 5; m.uPAR + IL6Ri/IL1Ri, n = 8 mice). **c, d**, Levels of serum ALT (c) and AST (d) in treated mice 20 days after T cell infusion (UT, n = 3; UT + IL6Ri/IL1Ri, n = 5; m.uPAR-m.28z, n = 5 (ALT) and n = 3 (AST); m.uPAR-m.28z + IL6Ri/IL1Ri, n = 5 mice). **e**, Representative levels of fibrosis evaluated by Sirius red staining and SA-β-gal staining in livers from treated mice and quantification of liver fibrosis and SA-β-gal⁺ cells in the respective livers 20 days after treatment (UT, n = 4; UT + IL6Ri/IL1Ri, n = 4; m.uPAR-m.28z, n = 4; m.uPAR-m.28z + IL6Ri/IL1Ri, n = 5 mice). **f**, Co-immunofluorescence staining of uPAR (red) and SMA (green) or Myc-tag (red) and SMA (green) in the livers of treated mice. Representative results of n = 1 independent experiment (b–f). Data are mean ± s.e.m.; two-tailed unpaired Student’s t test (b–e).
Extended Data Fig. 10 | Safety profile of senolytic CAR T cells at therapeutic doses in a mouse model of NASH-induced liver fibrosis.

a, Immunohistochemical expression of uPAR in samples from the ‘STAM’ model$^{52,58}$ (n = 3 mice). b, Experimental layout for experiments performed using the model of diet-induced NASH (Fig. 4, this figure). C57BL/6N mice were treated with a chow or a NASH-inducing diet$^{50}$ for three months, followed by intravenous infusion with $0.5 \times 10^6$ m.uPAR-m.28z CAR T cells or untransduced T cells 16 h after administration of cyclophosphamide (200 mg kg$^{-1}$). Mice were euthanized 20 days after CAR infusion to assess liver fibrosis. Images were created with BioRender.com. c, Kaplan–Meier curve showing survival of mice after treatment with either m.uPAR-m.28z CAR T cells or untransduced T cells (m.uPAR-m.28z, n = 16; UT, n = 10 mice). d, e, Weight (d) and temperature (e) of mice 24 h before and at different time points after T cell infusion (m.uPAR-m.28z, n = 11; UT, n = 9 mice). Data are mean ± s.e.m. 

f, Representative H&E staining of the lungs of treated mice (m.uPAR-m.28z, n = 6; UT, n = 4 mice). Representative results of n = 2 independent experiments (c–f).
Extended Data Fig. 11 | Gating strategies, summary and potential applications of senolytic CAR T cells. a, b, Representative flow cytometry staining of m.uPAR-h.28z CAR T cells (a) or untransduced T cells (b) obtained from the livers of mice that had undergone HTVI (as depicted in Fig. 2). Representative results of one independent experiment (n = 4 mice per group). c, Summary of the key points of our findings. uPAR-28z CAR T cells (red) infiltrate fibrotic livers that contain senescent cells (blue) and efficiently eliminate them, leading to fibrosis resolution and improved liver function. The therapeutic action of senolytic uPAR-28z CAR T cells might be extended to other senescence-associated diseases such as atherosclerosis, diabetes or osteoarthritis. Images were created with BioRender.com.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a | Confirmed

☐ ☑ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐ ☑ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ ☑ The statistical test(s) used AND whether they are one- or two-sided

Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ ☑ A description of all covariates tested

☐ ☑ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ ☑ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ ☑ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

Give P values as exact values whenever suitable.

☐ ☑ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ ☑ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ ☑ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

BD LSR-II and BD LSR-Fortessa cytometer, Cytek Aurora (CYTLK), Xenogen IVIS Imaging System living image V4.4, Microsoft Excel for Mac 2011.

Data analysis

FlowJo 10.1, GraphPad Prism V6 and V7, Living Image 4.4, Image J, version 2.0.0-rc-43/1.51h. RNA seq analysis was performed with the following software: HTSeq v0.5.3, picard tools v1.124, R v3.2.0, STAR v2.5.0a, samtools v0.1.19, Microsoft Excel for Mac 2011.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-seq data has been deposited in the Gene Expression Omnibus (GEO) under the accession number GSE145642.

The datasets generated during the current study are available from the corresponding author upon reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- ✔ Life sciences
- ☐ Behavioural & social sciences
- ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- Sample size: No statistical methods were used to pre-determine sample size. Sample sizes were estimated based on preliminary experiments, with an effort to achieve a minimum of n=5 mice per treatment group which proved to be sufficient to reproducibly observe a statistically significant difference.
- Data exclusions: No data were excluded throughout the studies.
- Replication: All in vitro and in vivo experiments were repeated in replications and/or from different subjects in independent experiments. All attempts at replication were successful. Efficacy of CAR T cell treatment may vary between donors.
- Randomization: Senescence burden for HTLV was determined by bioluminescent imaging one day prior to CAR T cell transfer and by suPAR measurement in the liver fibrosis model. Since senescent burdens were very even, mice were randomly assigned into treatment groups. Buffy coats were obtained from anonymous donors.
- Blinding: Mouse conditions were observed by an operator who was blinded to the treatment groups in addition to the main investigator who was not blinded to group allocation. Analysis of data was not performed in blinded fashion. Data analysis are based on objectively measurable data (fluorescence intensity, cell count, blood tests).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology |
| ☐ | Animals and other organisms |
| ☐ | Human research participants |
| ☒ | Clinical data |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☐ | ChiP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

Antibodies

The following fluorophore-conjugated antibodies were used ("h" prefix denotes anti-human, "m" prefix denotes anti-mouse):

- hCD45 APC-Cy7 (clone 2D1, BD, #557833, Lot: 9081815, 1:100), hCD4 BV539 (clone SK3, BD, #563550, Lot: 6252529, 1:100), hCD4 BV480 (clone SK3, BD, #561104, Lot: 8092993, 1:50), hCD62L BV421 (clone DREG-56, BD, #563862, Lot: 8194954, 1:100), hCD45RA BV650 (clone HI100, BD, #563963, Lot: 9057952, 1:100), hPD1 BV480 (clone EH12.1, BD, #566112, Lot: 8235507, 1:100), hCD19 BV737 (clone SJ25C, BD, #564495, Lot: 8130572, 1:100), hCD271 PE (clone C4D-1457, BD, #557196, Lot: 7008645, 1:100), hIL2 PE-Cy7 (clone M21-17H12, Invitrogen, #25-7029-42, Lot: 4336833, 1:50), hTNFa BV650 (clone Mab11, BD, #563148, Lot: 7082890, 1:50), hIFNg BV650 (clone B27, BD, #563563, Lot: 6320936, 1:50), hIL2 BM7 BV785 (clone F39-262, Biolegend, #345032, Lot: 8265346, 1:100), hCD8 PE-Cy7 (clone SK1, BD, #567834, Lot: 7110951, 1:50), hCD223 PerCP-eFlour710 (clone 30S224H, eBioscience, #46-2229-42, Lot: 4321735, 1:100), hGFAP APC (clone GB12, Invitrogen, #MV2G05, Lot: 1884625, 1:67), HMVC-tag AF647 (clone 9811, Cell Signaling Technology, #22335, Lot: 23, 1:50), hCD19 PE (clone SJ25-C1, Invitrogen, #MHC1928, 1:100), hCD87 APC (clone V5M, eBioscience, #17-3879-42, Lot: 17-3879-42, 1:50), hCD87 PerCP-eFlour710 (clone V5M, eBioscience, #46-3879-42, Lot: 46-2229-42, 1:50), mIgPAR PE (R&D Systems, #FAB531P, Lot: AHB20140598, 1:50), mIgPAR AF700 (R&D Systems, #FAB531N, Lot: 1552229, 1:50), mCD45.1 APC-Cy7 (clone A20, Biolegend, #110716, Lot: 8286865, 1:2000), mCD45.1 BV715 (clone A20, Biolegend, #110743, Lot: B270183, 1:100), mCD45.2 PE (clone A20, Biolegend, #110982B, Lot: B271929, 1:1000), mCD45.2 AF700 (clone 104, Biolegend, #109822, Lot: B252126, 1:2000), mSiglec-F PerCP-Cy5.5 (clone ES-2440, BD, #565526, Lot: B232650, 1:200), mIa/I-E Bv605 (clone M5.114.5.2, Biolegend, #107639, Lot: B259222, 1:50), mIa/I-E BV421 (clone 45-3242, BD, #565411, Lot: 8330026, 1:200), mCD11b BV539 (clone 12570, BD, #565353, Lot: 8339988, 1:2000), mCD311c BV580 (clone N41E, Biolegend, #117395, Lot: B253523, 1:200), mLY6G BV510 (clone 1A8, Biolegend, #127633, Lot: B266675, 1:2000), mLY6G
Validation

All used antibodies were titrated. All the antibodies are validated for use in flow cytometry or immunohistochemistry or immunofluorescence. Data are available on the manufacturer's website. The following primary antibodies have been validated by the manufacturer in the mentioned species: CD45 APC-Cy7 (clone 2D1, BD, #557833, Human), CD44 BV395 (clone SK3, BD, #563550, Human), CD44 BV480 (clone SK3, BD, #566104, Human), CD62L BV421 (clone DREG-56, BD, #563662, Human), CD45RA BV650 (clone H100, BD, #563963, Human), CD122 BV480 (clone E12.1, BD, #566112, Human), CD19 BV737 (clone SJ25C1, BD, #564303, Human), CD27 PE (clone CD1457, BD, #557196, Human), HI22 PE-CY7 (clone MO3:17H12, Invitrogen, #25-7029-42, Human), hTNFα BV650 (clone Mab11, BD, #563418, Human), hIFNg BV395 (clone 2B7, BD, #563563, Human), HTIM3 BV785 (clone F38-2E2, Biologene, #345032, Human), CD8 PE-Cy7 (clone SK1, BD, #557834, Human), CD223 PerCP-Cy5.5 (clone 5D2533H, eBioscience, #45-2239-42, Human), hGrB APC (clone G812, Invitrogen, #MIB05D, Human), hMyeloid tag AF647 (clone 9H11, Cell Signaling Technology, #2233S, Transfected mammalian cells), HCD19 PB (clone SJ25-CI, Invitrogen, #MHC1928, Human), HCD8 APC (clone V55, eBioscience, #57-2870-42, Human), HCD8 PerCP-Cy5.5 (clone V55, eBioscience, #45-2870-42, Human), muPAR PE (R&D Systems, #AB931P, Mouse), muPAR AF700 (R&D Systems, #AB531N, Mouse), mCD45.1 APC-Cy7 (clone A20, Biologene, #110716, Mouse), mCD45.1 BV785 (clone A20, Biologene, #110714, Mouse), mCD45.2 PE (clone 104, Biologene, #110808, Mouse), mCD45.2 AF700 (clone 104, Biologene, #110892, Mouse), muSgcG-F PerCP-Cy5.5 (clone E50-2440, BD, #565526, Mouse), muA-E BV605 (clone M5/114.15.2, Biologene, #107639, Mouse), m4/80 BV421 (clone T4-3424, BD, #565411, Mouse), mCD11b BV395 (clone M1/70, BD, #563353, Mouse), mCD11c BV650 (clone N418, Biologene, #117339, Mouse), mIgG1 BV510 (clone 1A8, Biologene, #112633, Mouse), mIgG1 AF488 (clone 1A8, Biologene, #112632, Mouse), mIgG2b PE-Cy7 (clone CN58, eBioscience, #75-9920-82, Mouse), CD19 PE (clone 1D3, Biologene, #152408, Mouse), mCD25 BV605 (clone PE-Cy7, #566120, Mouse)

Eukaryotic cell lines

Policy information about: cell lines

Cell line source(s)

ATCC, (NAMM5, human melanocytes), KP cells were a gift from Tyler Jacks. Eu-ALL01 were a gift from Renier J. Brentjens.

Authentication

COA with short tandem repeat was provided with cell line by ATCC. No other authentication was performed. Morphology and properties of all the cell lines pertinent to the experiments (e.g. antigen expression or GFP-Luciferase expression) were routinely confirmed by flow cytometry.

Mycoplasma contamination

All cell lines were tested for mycoplasma and were found to be negative.

Commonly misidentified lines

(See ITAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about: studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

NSG (NOD.Cg-Prkdcscid/Il2rgtm1WjlSglc91) mice male, 8-12 weeks old and obtained from the Jackson Laboratory. C57BL/6N mice were males and females, 8-12 weeks old and obtained from the Jackson Laboratory. B6.SJL-Ptprca/BoyJ/tac were females, 6-8 weeks old and obtained from Taconic.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.
Human research participants

Policy information about studies involving human research participants

Population characteristics

Buffy coats from anonymous healthy donors were purchased from the New York Blood Center. The researchers were blind to any covariate characteristics. Samples from liver fibrosis were obtained from patients with a diagnosis of hepatitis C or B, alcoholic hepatitis or non-alcoholic steatohepatitis from the Biorepository and Pathology Core (ICahn School of Medicine at Mount Sinai). Samples from normal pancreas and pancreatic tissue with PanIN were obtained from cases with a confirmed diagnosis of pancreatic ductal adenocarcinoma from the Department of Pathology at Memorial Sloan Kettering Cancer Center. Samples from human carotid sections were obtained from patients undergoing endarterectomy through the Department of Pathology at Weill Medical College of Cornell University.

Recruitment

Buffy coats were purchased from the New York Blood Center. Samples from liver fibrosis were obtained from patients with a confirmed diagnosis of hepatitis C or B, alcoholic hepatitis or non-alcoholic steatohepatitis. Samples were selected by pathologists at the Biorepository and Pathology Core (ICahn School of Medicine at Mount Sinai) from their archives. Samples from normal pancreas and pancreatic tissue with PanIN were selected from cases with a confirmed diagnosis of pancreatic ductal adenocarcinoma. Samples were selected by pathologists at the Department of Pathology at Memorial Sloan Kettering Cancer Center from their archives. Samples from human carotid sections were obtained from patients undergoing endarterectomy as described in Peerschke, E.I. & al. Molecular Immunology, 41; 759-766 (2004): these were all patients with atherosclerotic lesions of type V according to the classification of the American Heart Association. No systematic bias likely to impact results were known at the time of data analysis for any of the samples.

Ethics oversight

All human studies were approved by Mount Sinai, or Weill Medical College of Cornell, or Memorial Sloan-Kettering Institutional Review Board.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Buffy coats from anonymous healthy donors and peripheral blood from healthy volunteers were isolated and purified as described in Methods. For analysis of T cells in the livers, livers were dissociated using MACS Miltenyi Biotec Liver dissociation kit (130-1-5-S-807), filtered through a 100μm strainer, washed with PBS, and red blood cell lysis was achieved with an ACK (Ammonium-Chloride-Potassium) lysis buffer (Lonza). Cells were washed with PBS, resuspended in PBFI buffer and used for subsequent analysis. Lungs were minced and digested with 1mg/ml collagenase type IV and DNase type IV in RPMI at 37°C and 200rpm for 45 minutes, filtered through 100μm strainer, washed with PBS, and red blood cell lysis was achieved with an ACK lysis buffer (Lonza). Cells were washed with PBS, resuspended in FACS buffer and used for subsequent analysis. For bone marrow samples, samples and femurs were mechanically disrupted with a mortar in PBS/2mM EDTA, filtered through 40μm strainer, washed with PBS/2mM EDTA and red blood cell lysis was achieved with an ACK lysis buffer (Lonza). Cells were washed with PBS/2mM EDTA, resuspended in FACS buffer and used for subsequent analysis. Spleens were mechanically disrupted with the back of a 5-ml syringe, filtered through 40μm strainer, washed with PBS/2mM EDTA and red blood cell lysis was achieved with an ACK lysis buffer (Lonza). Cells were washed with PBS/2mM EDTA, resuspended in FACS buffer and used for subsequent analysis.

Cells were subsequently washed, resuspended in FACS Buffer with FcR blocking reagent; antibodies were added and washed off after the incubation time. If intracellular staining was performed, cells were fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) or Intracellular Fixation & Permeabilization Buffer Set Kit (eBioscience, #88-8824-00) according to the manufacturer’s instructions. If cells were counted, counting beads (Invitrogen) were added in the final cell suspension to quantify cells. For analysis of live cells, 7-AAD (BD, #555925, Lot: 9031656, 1,400), Fixable Viability Dye eFlour 560 (65-0866-18, eBioscience, Lot: 209542, 1:200) and LIVE/DEAD Fixable Violet (L34963, Invitrogen, Lot: 1985351, 1:100) were used.

Instrument

LSRII, BD.
Fortessa 3, BD.
Cytex Aurora (CytEX).

Software

Collection: FACS DIVA.
| Software     | Analysis: Flowjo 10.1 |
|--------------|-----------------------|
| Cell population abundance | The purity was verified by flow cytometry. |
| Gating strategy | The starting cell population was gated on a SSC-A/FSC-A plot. Cell singlets were identified by FSC/SSC gating. Positive/Negative populations were determined by FMO controls. |

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.