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Supplementary Materials for

Title: CAR T cells produced *in vivo* to treat cardiac injury

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Materials and Methods:

Mice

C57BL/6N mice were procured from Charles River Laboratories (Willmington, MA). Ai6 mice (Rosa$^{CAG\text{-}LSL\text{-}ZsGreen}$) were obtained from the Jackson Laboratory (Bar Harbor, ME). All animal studies were conducted ethically under protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) in facilities accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Reagents and antibodies

Phoenix-ECO retroviral packaging cells (CRL-3214) and HEK293T (CRL-3216) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). ACH2 cells were obtained from Farida Shaheen (University of Pennsylvania, CFAR). All immortalized cell lines are tested quarterly for mycoplasma contamination. Angiotensin II (A9525) and phenylephrine hydrochloride (P6126) were purchased from Millipore Sigma (St. Louis, MO). Osmotic mini-pumps (Alzet model 2004) were obtained from Durect Corporation (Cupertino, CA). Sterile saline (0.9% sodium chloride) was purchased from Hospira (Lake Forest, IL). Luciferase assay (E151A) and pGL3-control plasmid were obtained from Promega Corporation (Madison, WI). Murine FAP expression plasmid was synthesized by Twist Bioscience (South San Francisco, CA) using the NCBI reference sequence NM_007986.3. Td_Tomato was fused in-frame to the N-terminus (intracellular fragment) of FAP for the *in vitro* trogocytosis experiment. Goat anti-Mouse IgG F(ab')$_2$ (115-065-072) was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). His-tagged, recombinant mouse FAP (ab271506) was obtained from Abcam (Cambridge, MA). Rabbit anti-His-tag-PE monoclonal antibody (clone D3I10, 15024) was obtained from Cell Signaling Technology (Danvers, MA). CD3-APC (clone 17A2, 20-0032-U100) was obtained from Tonbo Biosciences (San Diego, CA). CD3-APC-Cy7 (clone 17A2, 100222), CD4-PerCP-Cy5.5 (clone GK1.5, 100434), CD8-PacificBlue (clone 53-6.7, 100725), CD19-PE-Cy7 (clone 6D5, 115520), CD25-PE-Dazzle594 (clone 3C7, 101920), CD44-AlexaFluor700 (clone IM7, 103026), CD62L-BrilliantViolet605 (clone MEL-14, 104445), and NK1.1-APC (clone S17016D, 156506) were obtained from Biolegend (San Diego, CA).

RNA synthesis and encapsulation in lipid nanoparticles

The FAPCAR construct contained the scFv fragment from mouse-specific FAP monoclonal antibody (clone 73.3) with mouse CD3ζ and CD28 cytoplasmic signaling domains (*25*). A small peptide conferring resistance to adenosine- and prostaglandin E2-mediated suppression is included (RISR-RIAD) (*33*). The FAPCAR and RISR-RIAD coding sequences are separated by a P2A self-cleaving peptide coding sequence, and after codon-optimization for expression in mammalian cells, the full sequence was cloned into an IVT template plasmid carrying a T7 promoter, 5' and 3' UTR elements, and poly(A) tail. Cloning and endotoxin-free plasmid preparation service was provided by GenScript (Piscataway, NJ). mRNA was produced with MEGAScript T7 kit (Invitrogen AMB13345) using m1Ψ-5'-triphosphate (TriLink N-1081) instead of UTP and contained 101 nucleotide-long poly(A) tails. Capping of the *in vitro* transcribed mRNAs was performed co-transcriptionally using the trinucleotide cap1 analog, CleanCap (TriLink, San Diego, CA). mRNA was purified by cellulose purification, as previously described (*34*). All mRNAs were analyzed by agarose gel electrophoresis and were stored at -20°C. Cellulose purified m1Ψ-containing RNAs were encapsulated in LNP using a self-assembly process as previously described (*35*), briefly an ethanolic lipid mixture of ionizable cationic lipid, phosphatidylcholine, cholesterol and polyethylene glycol-lipid was rapidly mixed with an aqueous solution containing the mRNA at acidic pH. The RNA-loaded particles were characterized by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern UK) and a Ribogreen assay. The mean hydrodynamic diameter of these LNP-mRNAs was approximately 80nm with a polydispersity index of 0.02-0.06 and an encapsulation efficiency of ~95%. LNP used in this study are proprietary to Acuitas Therapeutics.

To prepare antibody-targeted LNP-mRNA, LNP-mRNA were conjugated with purified rat anti-mouse CD5, clone 53-7.3 (BioLegend) or mouse anti-human CD5, clone UCHT2 (BioLegend), and control isotype-matched IgG via SATA–maleimide chemistry, as described previously (*24*). Briefly, LNP was modified with maleimide functioning groups (DSPE-PEG-mal) by a post-insertion technique. The antibody was functionalized with SATA (N-succinimidyl *S*-acetylthioacetate) (Millipore Sigma) to introduce sulfhydryl groups allowing conjugation to maleimide. SATA was deprotected using 0.5 M hydroxylamine followed by removal of the unreacted components by G-25 Sephadex Quick Spin Protein columns (Roche Applied Science, Indianapolis, IN). The reactive sulfhydryl group on the antibody was then conjugated to maleimide moieties using thioether conjugation chemistry. Purification was carried out using Sepharose CL-4B gel filtration columns (Millipore Sigma). mRNA content was calculated by performing a modified Quant-iT RiboGreen RNA assay (Invitrogen). After addition of the targeting ligand, all the targeted and non-targeted LNP preparations were kept at 4°C and were used within three days of preparation.

Animal Experiments

Adult male mice were randomized to intervention and injured with a constant infusion of (1.5μg/g/day) angiotensin II and (50μg/g/day) phenylephrine via implanted, 28-day osmotic mini-pump (Alzet model 2004). Female mice were excluded because they do not develop severe fibrosis in the AngII/PE injury model (*36*). Control mice were sham injured with pumps containing sterile saline (0.9% sodium chloride). LNP were injected intravenously under 2-3% isoflurane general anesthesia via the retro-orbital venous sinus after seven days of AngII/PE injury. Experimental number was estimated by power analysis. One small cohort of LNP-treated mice was excluded from the data contained in this report for multiple reasons: first, no positive control group was included. Second, half of the animals had significant left ventricular posterior wall abnormalities indicative of excessive injury and obfuscating echocardiographic quantification. Left ventricular function was assayed by echocardiography two weeks after LNP administration using a Fujifilm VisualSonics Ultrasound System (VisualSonics Inc, Toronto, ON, Canada) equipped with a MS400 (18-38 MHZ) transducer. Mice were lightly anesthetized with an intraperitoneal injection of 0.005 mL/g of 2% Avertin (2,2,2-Tribromoethanol, Millipore Sigma). Left ventricular (LV) systolic function: two-dimensional long-axis, short-axis M-Mode images were obtained. LV diastolic function: trans-mitral inflow pattern and tissue Doppler were obtained in modified 4 chamber apical view. Images were analyzed for LV structure and function using Vevo Lab software (Visual Sonics Inc, Toronto, ON, Canada). The stenographer was blinded to treatment condition. Bioluminescence images were acquired with an IVIS Spectrum (Perkin Elmer, Waltham, MA) immediately after intraperitoneal injection of luciferin substrate.

Generation of FAPCAR T cells

Mouse T cells with genomic integration of the FAPCAR sequence were generated as previously described (*9*, *25*). Briefly, mouse T cells were isolated from wild type 10–14-week-old male mice using negative selection (StemCell Technologies 19851), activated using CD3/CD28 Dynabeads (Gibco 11453D) and expanded using 100 Units/mL of recombinant mouse interleukin-2 (R&D Systems 402-ML). T cells were grown in RPMI 1640 (Invitrogen 11875085) containing 10% FBS (Atlanta Biologicals S11150), 4mM L-glutamine (Invitrogen 25030081), penicillin/streptomycin (Invitrogen 15140122), 1mM sodium pyruvate (Invitrogen 11360079), and 50μM 2-mercaptoethanol (Gibco 21985023). FAPCAR retroviral particles were packaged using Phoenix-ECO viral production cells (ATCC CRL-3214). T cell infection with the FAPCAR retrovirus was facilitated by plates coated with 0.5μg/cm^2 Retronectin (Takara T100B).

T cells with transient mRNA expression of the FAPCAR construct were generated *in vitro* by mixing LNP with isolated and activated T cells (as described above). 5µg LNP per 1 million T cells were combined in T cell media and assayed for FAPCAR expression via flow cytometry at the indicated time points.

Flow cytometry

T cells isolated from mouse spleens via negative selection (StemCell Technologies 19851) were stained for FAPCAR expression with His-tagged recombinant FAP (Abcam ab271506) and Rabbit-anti-His-PE (Cell Signaling Tech 15024S). Cells were assayed using an Accuri C6 Plus or BD LSR II (BD Biosciences San Jose, CA). The gating strategy for all plots was first to select lymphocytes (FSC-A/SSC-A) and single cells using FSC-A/FSC-H (supplemental figure 1A). Preliminary experiments using *in vitro* cultures included live-dead fixable green (Invitrogen L34970) where it was determined that lymphocytes analyzed with this gating strategy were ≥98% living. Flow cytometry experiments from whole spleens included live-dead fixable aqua (Invitrogen L34957). Plots were generated using FlowJo software (version 10.7.1, BD Ashland, OR)).

***in vitro* killing assay**

Target cells were made by transfecting HEK293T cells (ATCC CRL-3216) with murine FAP and firefly luciferase plasmids using Lipofectamine 2000 (Invitrogen 11668027) according to the manufacturer recommendations. Three thousand target cells were re-plated 48 hours after transfection into 96-well plates and co-cultured with the indicated ratio of FAPCAR T cells overnight. Cells were washed with DPBS before being lysed and assayed for luciferase luminescence according to the manufacturer's recommendations (Promega E151A) on a PerkinElmer Victor X3 plate reader (Waltham, MA). A decrease in luciferase indicates the FAP expressing HEK293T target cells were eliminated by functional FAPCAR T cells. Killing efficiency was equal to 100 – ((test RLU / no T cell average RLU)*100), where RLU is relative luminescent units.

Histology and Immunohistochemistry

Tissues were fixed overnight in 4% paraformaldehyde and gradually dehydrated with ethanol. Hematoxylin and eosin (H&E) was performed according to a standard protocol. Picrosirius red (PSR) staining was completed as previously described by the Pathology Core Laboratory at the Children's Hospital of Philadelphia Research Institute (*9*). Immunohistochemistry for CD3 (Abcam Ab16669), FAP (Abcam Ab207178) and IgG [$F(ab')_2$] (Jackson ImmunoResearch 115-065-072) was performed with overnight antibody incubations following antigen retrieval with either EDTA pH9.0 (FAP or CD3) or citrate buffer (GAM). DAPI (Sigma 32670) was used to counterstain some sections. Slides were mounted with Vectashield (Vector Labs H-1000). Images were obtained using a DMi8S widefield microscope (equipped with 4X/0.13 HC PL FLUOTAR and 20X/0.80 PH2 HC PL APO objectives) using a DFC7000 T camera or an SP8 confocal (equipped with HydD detectors, 20X/0.5 HC LP FLUOTAR and 63X/1.40 HC PL APO CS2 objectives) and deconvolved with Lightning using the default settings (Leica Microsystems, Buffalo Grove, IL). $CD3^+$/$FAPCAR^+$ T cells were counted on the DMi8S at 40X. Percent fibrosis of both ventricles and septum was quantified blindly based on color deconvolution of PSR staining in ImageScope (Aperio) software in at least four cardiac sections per mouse.

Statistics

Differences between two conditions were assessed using Students t-test. Significance was determined for multiple conditions using one-way analysis of variance (ANOVA). Significant ANOVA results ($p<0.05$) were analyzed further using Tukey's post-hoc multiple comparisons test. Error bars indicate the standard error of the mean (SEM). Statistics were calculated and graphs generated in R (version 4.0.5, www.R-project.org) using RStudio (version 1.4.1106, Boston, MA) and ggplot2 (version 3.3.3). Cartoons were drawn and figures compiled in Illustrator (version 25.3.1, Adobe Inc. San Jose, CA).

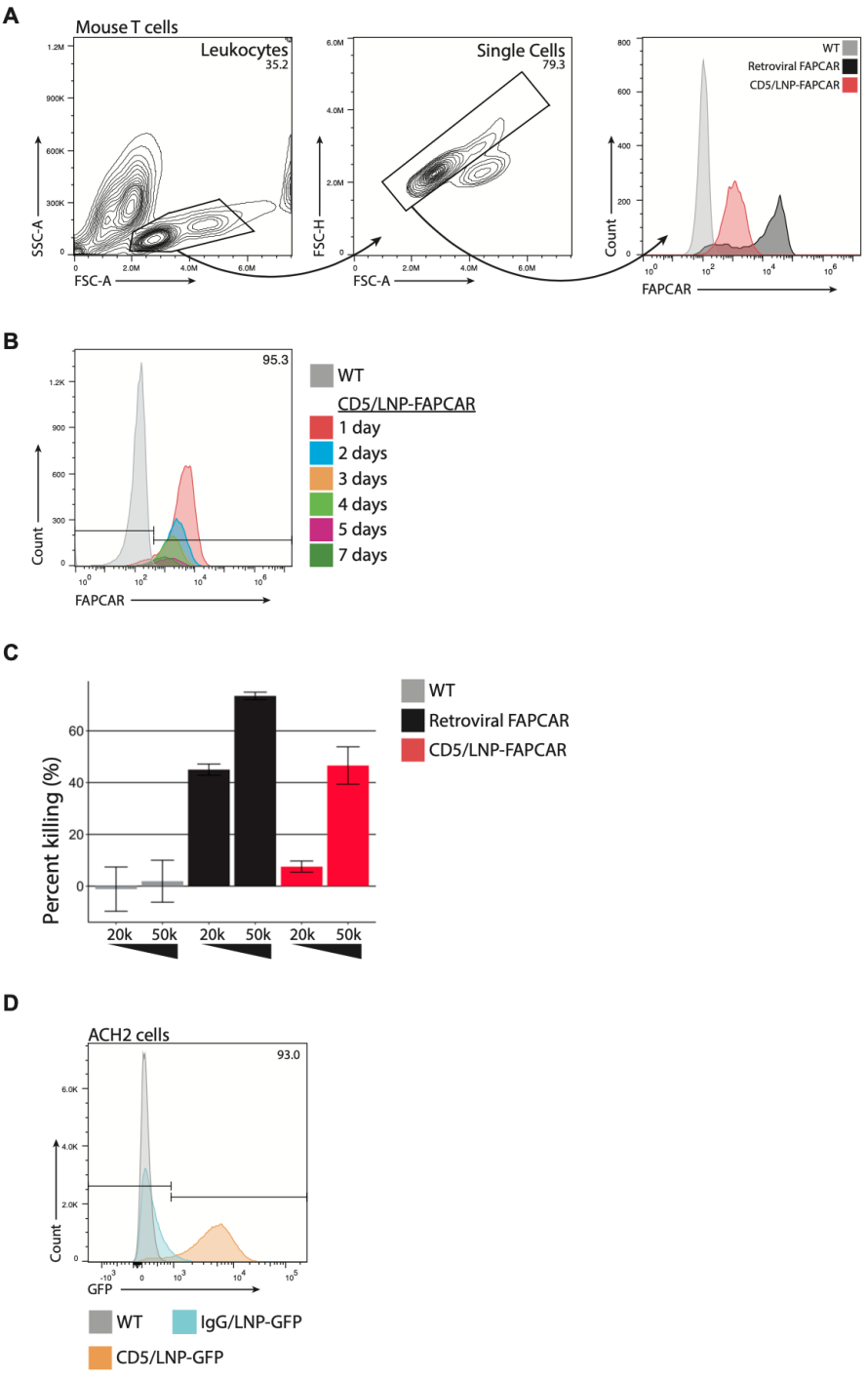

Fig. S1. CD5/LNP-FAPCAR produce functional, mRNA-based FAPCAR T cells *in vitro*. **(A)** Representative gating strategy for flow cytometry. **(B)** *In vitro* time course of FAPCAR expression in freshly isolated, activated mouse T cells after mixing with CD5/LNP-FAPCAR. **(C)** Increased killing of target FAP-expressing HEK293T cells assay is seen with increasing number of FAPCAR T cells from two biologically independent replicates. Data are mean +/- s.e.m. **(D)** Human ACH2 immortalized T cells express GFP, 24 hours after mixing with CD5/LNP-GFP *in vitro*.

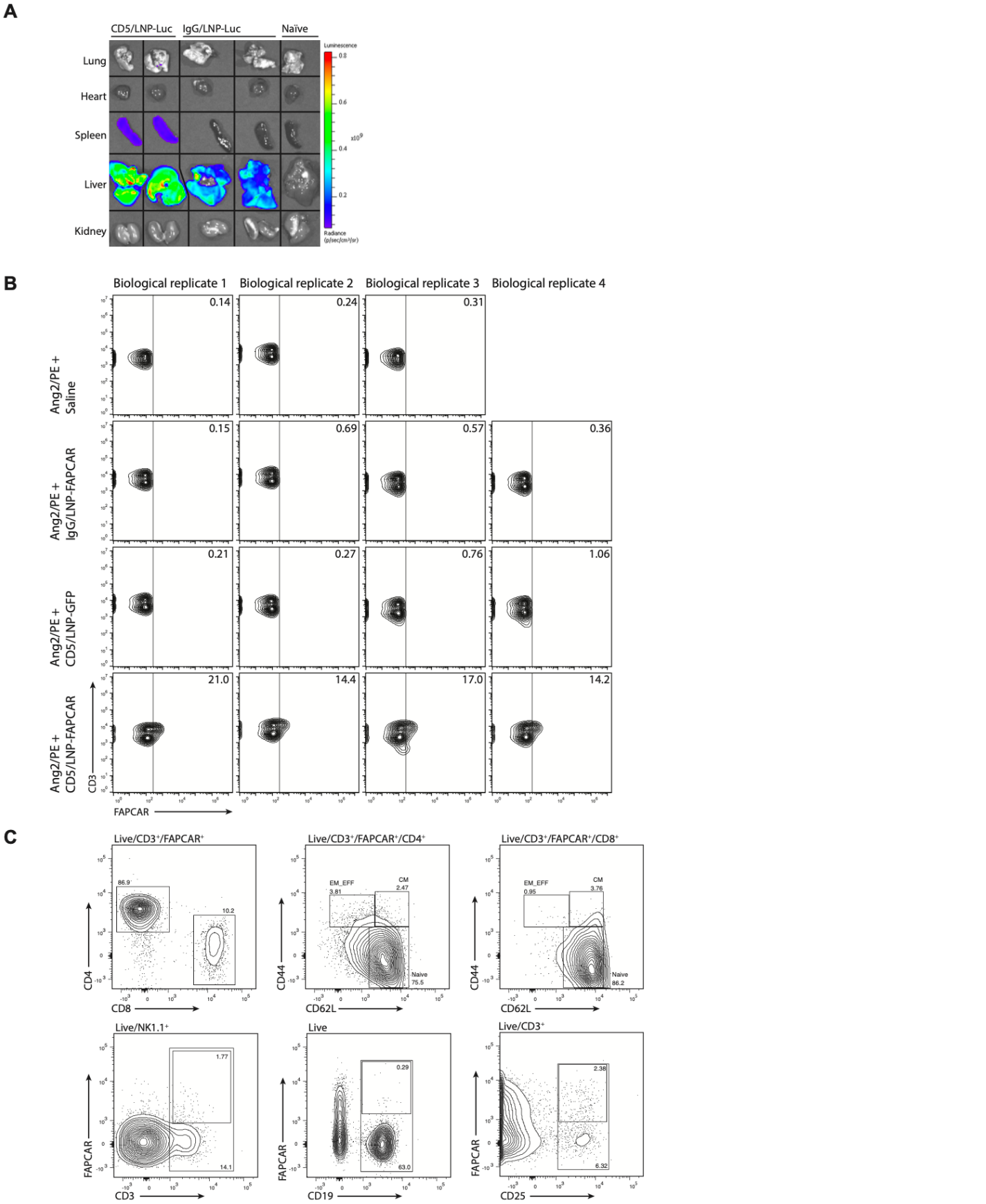
A
CD5/LNP-Luc
IgG/LNP-Luc
Naïve
Lung
Heart
Spleen
Liver
Kidney
Luminescence
Radiance (p/sec/cm²/sr)
B
Biological replicate 1
Biological replicate 2
Biological replicate 3
Biological replicate 4
Ang2/PE + Saline
Ang2/PE + IgG/LNP-FAPCAR
Ang2/PE + CD5/LNP-GFP
Ang2/PE + CD5/LNP-FAPCAR
0.14
0.24
0.31
0.15
0.69
0.57
0.36
0.21
0.27
0.76
1.06
21.0
14.4
17.0
14.2
CD3
FAPCAR
C
Live/CD3⁺/FAPCAR⁺
86.9
10.2
CD4
CD8
Live/CD3⁺/FAPCAR⁺/CD4⁺
EM_EFF
3.81
CM
2.47
Naive
75.5
CD44
CD62L
Live/CD3⁺/FAPCAR⁺/CD8⁺
EM_EFF
0.95
CM
3.76
Naive
86.2
Live/NK1.1⁺
1.77
14.1
FAPCAR
CD3
Live
0.29
63.0
CD19
Live/CD3⁺
2.38
6.32
CD25

Fig. S2. CD5-targeted lipid nanoparticles produce mRNA-based FAPCAR T cells *in vivo*. **(A)** Bioluminescent imaging 24 hours after injection of IgG/LNP-Luc or CD5/LNP-Luc reveals splenic expression of luciferase only in animals injected with CD5-targeted animals. **(B)** Flow cytometry scatter plots from splenic T cells isolated from animals injured for 1 week with AngII/PE and 48 hours after LNP administration show FAPCAR staining only with CD5/LNP-FAPCAR injection and not in saline, non-targeted (IgG/LNP-FAPCAR) or CD5-targeted, unrelated mRNA containing (CD5/LNP-GFP) LNP controls. **(C)** Splenocyte flow cytometry 24 hours after injection of CD5/LNP-FAPCAR into AngII/PE injured animals. The gated population is indicated above each plot, with the percent of parent population listed for each gate. EM_EFF is effector memory/effector and CM is central memory. For cell counts see table S1.

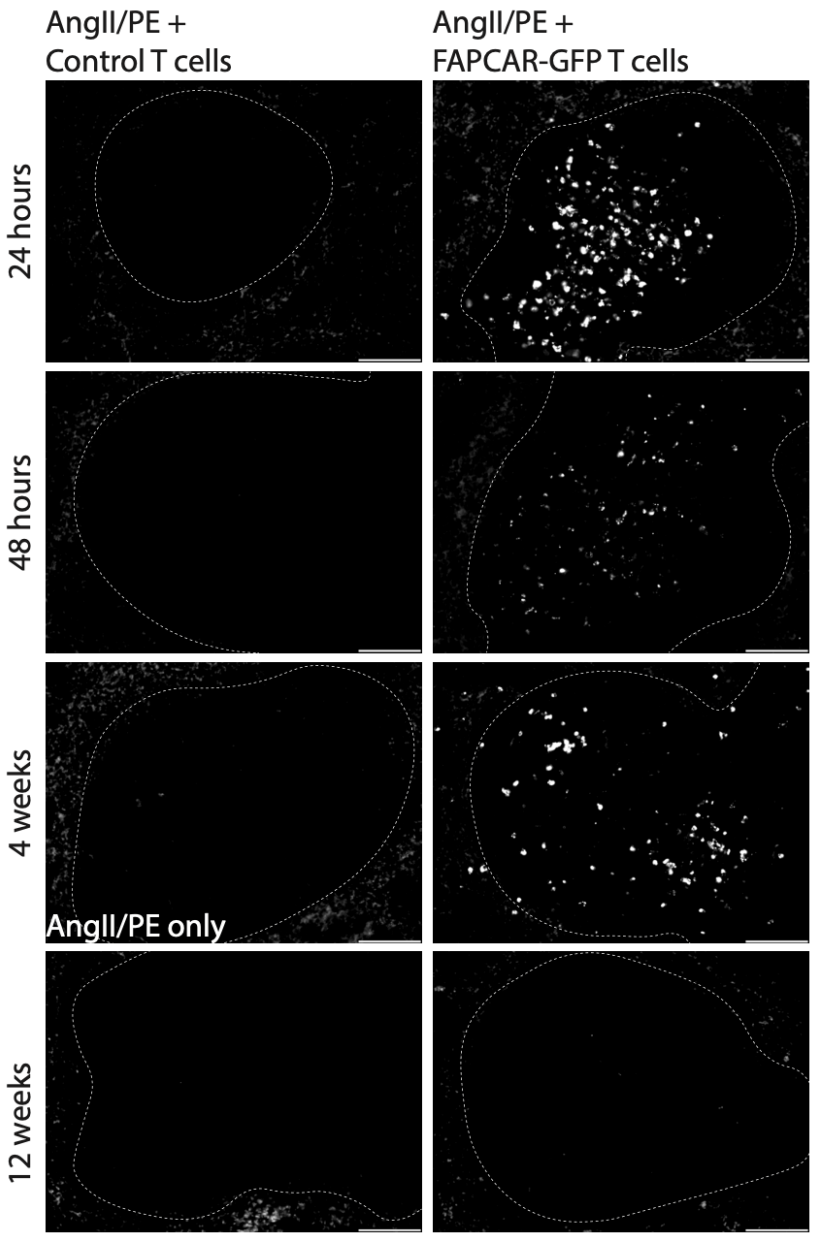

Fig. S3 - FAPCAR T cells trogocytose FAP from activated cardiac fibroblasts and return FAP to the spleen only in AngII/PE-injured, FAPCAR-treated animals. Evidence of trogocytosis is restricted to the splenic white pulp of AngII/PE-injured (7 days) animals at 1, 2, and 28 days after adoptive transfer of virally engineered 10^7 FAPCAR-GFP T cells (but not injection of an equal number of MigR1-control T cells). Twelve weeks after adoptive transfer, the number of FAP^+ T cells in the spleen is dramatically reduced, however rare cells can be observed.

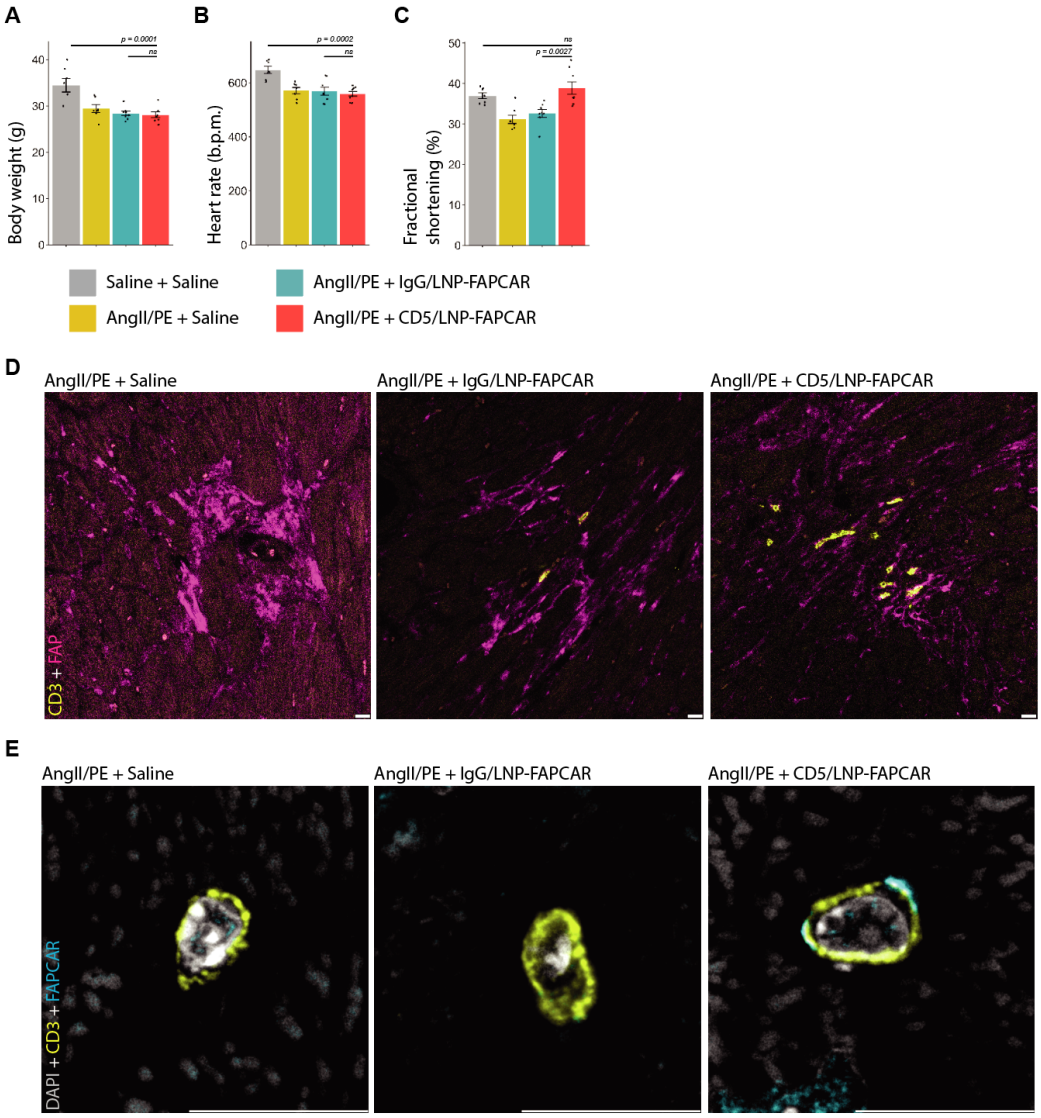

Fig. S4 - *In vivo* produced, transient FAPCAR T cells improve cardiac function after injury. (**A**) Mouse body weight and (**B**) heart rate measured under Avertin anesthesia. (Body weight and heart rate was decreased in all conditions exposed to AngII/PE compared to controls likely due to loss of appetite and reflex bradycardia induced by phenylephrine.) **(C)** Fractional shortening (%) demonstrates systolic improvement only in CD5/LNP-FAPCAR treated animals and not in control animals. Data represent n = 7, 7, 8, 8 biologically independent mice per condition, spread over three cohorts. **(D)** Cardiac sections stained with FAP (magenta) and CD3 (yellow) show an accumulation of T cells adjacent to activated fibroblasts 48 hours after CD5/LNP-FAPCAR injection into AngII/PE injured animals. Scale bar: 10µm. **(E)** The $CD3^+$ T cells in the injured myocardium of (D) stain positive for the FAPCAR variable fragment $F(ab')_2$ (cyan). Scale bar: 10µm.

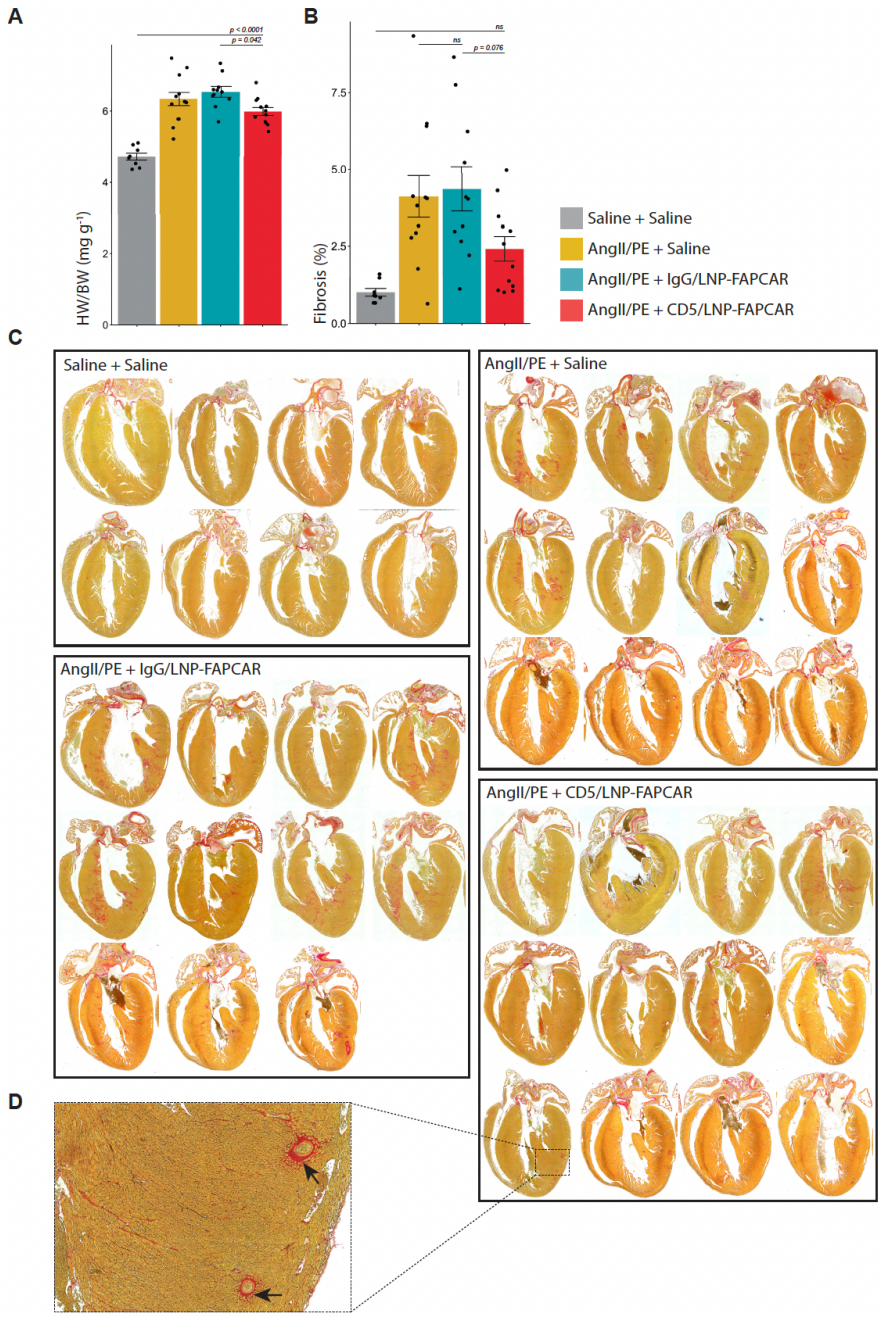

Fig. S5. *In vivo* produced FAPCAR T cells alter cardiac fibrosis after injury. **(A)** Heart weight to body weight ratio (HW/BW, mg/g) increases upon AngII/PE injury and is partially resolved following CD5/LNP-FAPCAR treatment. **(B)** Quantification of percent fibrosis of the ventricles seen in (C) demonstrates fibrosis resolution only in CD5/LNP-FAPCAR treated animals. *n* = 8, 11, 12, 12 biologically independent mice per condition, spread over five cohorts. **(C)** Representative picrosirius red-stained hearts from all dosed animals described in figure 4. **(D)** Inset of AngII/PE injured, CD5/LNP-FAPCAR treated animal with perivascular fibrosis, without interstitial fibrosis. Data are mean +/- s.e.m. Displayed *p*-values are from Tukey's post-hoc test following one-way ANOVA $p<0.05$.

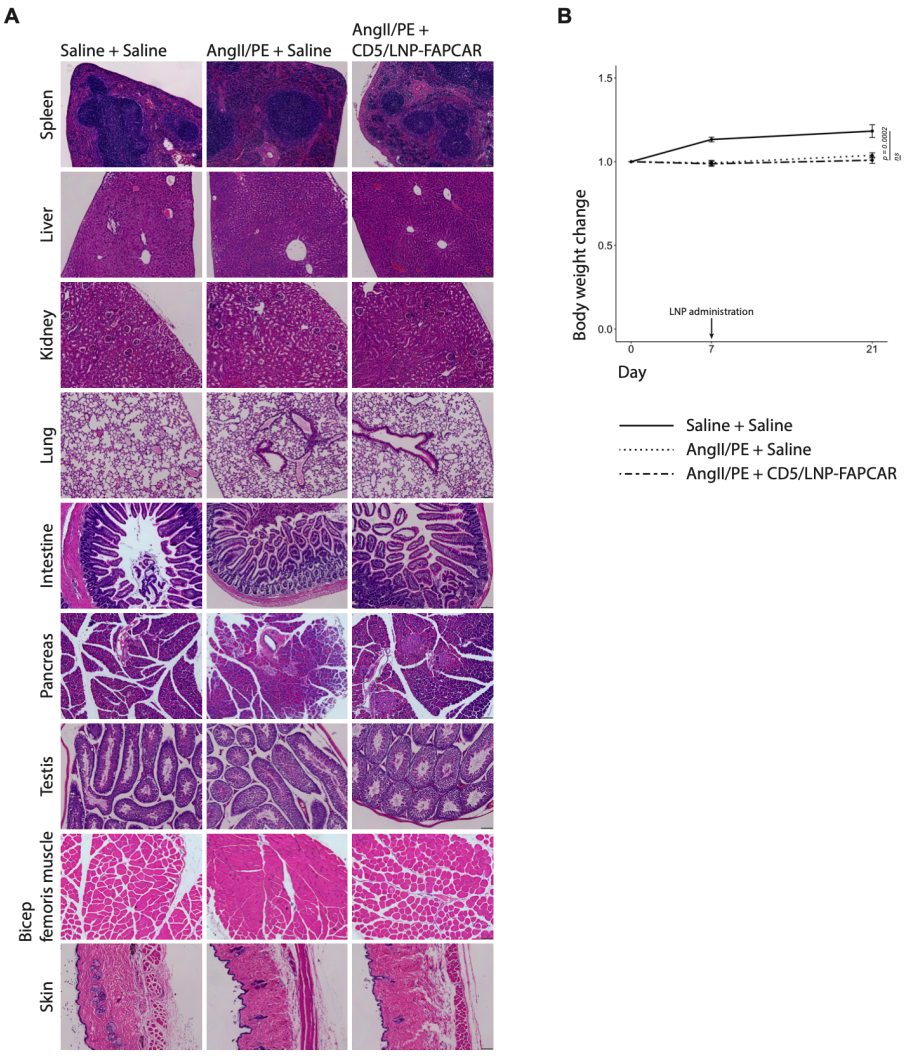

Fig. S6. Systemic impact of *in vivo* produced FAPCAR T cells in injured mice. **(A)** H&E staining of various organs after three weeks of saline or AngII/PE pressure-overload injury and two weeks after the indicated injection (saline or CD5/LNP-FAPCAR). **(B)** Body weight of AngII/PE injured, CD5/LNP-FAPCAR treated animals does not decrease after injection. *n* = 8, 11, 12 biologically independent mice per condition, spread over five cohorts.

Movie S1 – Time-lapse of confocal micrographs taken every 5 minutes after adding FAPCAR T cells to target HEK293T cells overexpressing RFP-FAP (magenta). Two individual FAPCAR T cells can be seen first forming immunological synapse then trogocytosing RFP-FAP from the HEK293T cell (magenta punctae).

Movie S2 – Parasternal long-axis 2D echocardiograph taken two weeks after saline injection, three weeks after saline pump mock injury (healthy control).

Movie S3 – Parasternal long-axis 2D echocardiograph taken two weeks after saline injection, three weeks after AngII/PE pump injury (injury control).

Movie S4 – Parasternal long-axis 2D echocardiograph taken two weeks after 10μg of IgG/LNP-FAPCAR injection, three weeks after AngII/PE pump injury (non-targeted LNP control).

Movie S5 – Parasternal long-axis 2D echocardiograph taken two weeks after 10μg of CD5/LNP-FAPCAR injection, three weeks after AngII/PE pump injury (test treatment).

AngII/PE + CD5/LNP-FAPCAR

Cell type	Markers/Gating	Animal: JE222 (24hr) Count	JE235 (24hr) Count	JE234 (7day) Count	JE237 (7day) Count
Live lymphocytes	Live/Dead-aqua	273588	273145	351126	336346
T cells	CD3+	75556	62195	117919	109183
CD4+ T cells	CD3+/CD4+	55585	45051	61407	56861
CD8+ T cells	CD3+/CD8+	16502	13448	48352	44650
Effector/Effector Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L-	3365	2479	12898	11229
Central Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L+	1512	736	4276	4029
Naïve CD4+ T cells	CD3+/CD4+/CD44-/CD62L+	38355	27017	35269	32817
Effector/Effector Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L-	250	547	1793	2133
Central Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L+	1652	610	8978	9565
Naïve CD8+ T cells	CD3+/CD8+/CD44-/CD62L+	12766	9868	29723	25870
FAPCAR+ T cells	**CD3+/FAPCAR+**	**20654**	**9645**	**98**	**140**
FAPCAR+ CD4+ T cells	**CD3+/CD4+/FAPCAR+**	**17946**	**8419**	**31**	**47**
FAPCAR+ CD8+ T cells	**CD3+/CD8+/FAPCAR+**	**2103**	**835**	**18**	**18**
FAPCAR+ Effector/Effector Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L-/FAPCAR+	683	357	21	30
FAPCAR+ Central Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L+/FAPCAR+	444	124	3	3
FAPCAR+ Naïve CD4+ T cells	CD3+/CD4+/CD44-/CD62L+/FAPCAR+	13547	5476	4	4
FAPCAR+ Effector/Effector Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L-/FAPCAR+	20	74	4	6
FAPCAR+ Central Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L+/FAPCAR+	79	33	6	5
FAPCAR+ Naïve CD8+ T cells	CD3+/CD8+/CD44-/CD62L+/FAPCAR+	1813	582	2	2
Treg	CD3+/CD25+	4778	4523	6802	6558
FAPCAR+ Treg	CD3+/CD25+/FAPCAR+	1795	1214	82	105
FAPCAR+ Non-T cells	CD3-/FAPCAR+	3377	3890	428	756
NK cells	NK1.1+	5256	6477	16255	12762
NK T cells	CD3+/NK1.1+	739	682	861	761
FAPCAR+ NK cells	NK1.1+/FAPCAR+	195	181	20	40
FAPCAR + NK T cells	NK1.1+/CD3+/FAPCAR+	93	61	13	17
B cells	CD19+	172278	187956	180985	182626
FAPCAR+ B cells	CD19+/FAPCAR+	786	1064	394	541

AngII/PE + Saline

Cell type	Markers/Gating	Animal: JE223 (24hr) Count	JE231 (24hr) Count	JE224 (7day) Count	JE226(7day) Count
Live lymphocytes	Live/Dead-aqua	319135	298237	369794	349258
T cells	CD3+	122779	120059	138212	120406
CD4+ T cells	CD3+/CD4+	71683	72474	71035	66361
CD8+ T cells	CD3+/CD8+	44776	45287	59061	47169
Effector/Effector Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L-	9210	8670	13121	11390
Central Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L+	4263	3679	5623	5400
Naïve CD4+ T cells	CD3+/CD4+/CD44-/CD62L+	51145	50973	46181	42689
Effector/Effector Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L-	742	800	1297	1107
Central Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L+	5891	4774	12691	8298
Naïve CD8+ T cells	CD3+/CD8+/CD44-/CD62L+	35492	37595	43484	33384
FAPCAR+ T cells	**CD3+/FAPCAR+**	**145**	**43**	**139**	**96**
FAPCAR+ CD4+ T cells	**CD3+/CD4+/FAPCAR+**	**76**	**28**	**32**	**30**
FAPCAR+ CD8+ T cells	**CD3+/CD8+/FAPCAR+**	**8**	**2**	**21**	**11**
FAPCAR+ Effector/Effector Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L-/FAPCAR+	15	13	19	21
FAPCAR+ Central Memory CD4+ T cells	CD3+/CD4+/CD44+/CD62L+/FAPCAR+	5	0	1	4
FAPCAR+ Naïve CD4+ T cells	CD3+/CD4+/CD44-/CD62L+/FAPCAR+	43	2	8	1
FAPCAR+ Effector/Effector Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L-/FAPCAR+	0	1	6	5
FAPCAR+ Central Memory CD8+ T cells	CD3+/CD8+/CD44+/CD62L+/FAPCAR+	1	1	6	4
FAPCAR+ Naïve CD8+ T cells	CD3+/CD8+/CD44-/CD62L+/FAPCAR+	3	0	6	1
Treg	CD3+/CD25+	4185	449	7637	7004
FAPCAR+ Treg	CD3+/CD25+/FAPCAR+	37	1	101	80
FAPCAR+ Non-T cells	CD3-/FAPCAR+	765	149	656	627
NK cells	NK1.1+	17143	12893	14967	12654
NK T cells	CD3+/NK1.1+	1105	1037	810	786
FAPCAR+ NK cells	NK1.1+/FAPCAR+	27	17	24	28
FAPCAR + NK T cells	NK1.1+/CD3+/FAPCAR+	20	14	11	12
B cells	CD19+	156206	137311	189196	188986
FAPCAR+ B cells	CD19+/FAPCAR+	464	182	393	401

Table S1. Count matrix of splenocytes analyzed by flow cytometry, 24 hours and 7 days after injection of either saline or CD5/LNP-FAPCAR into AngII/PE injured animals.

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