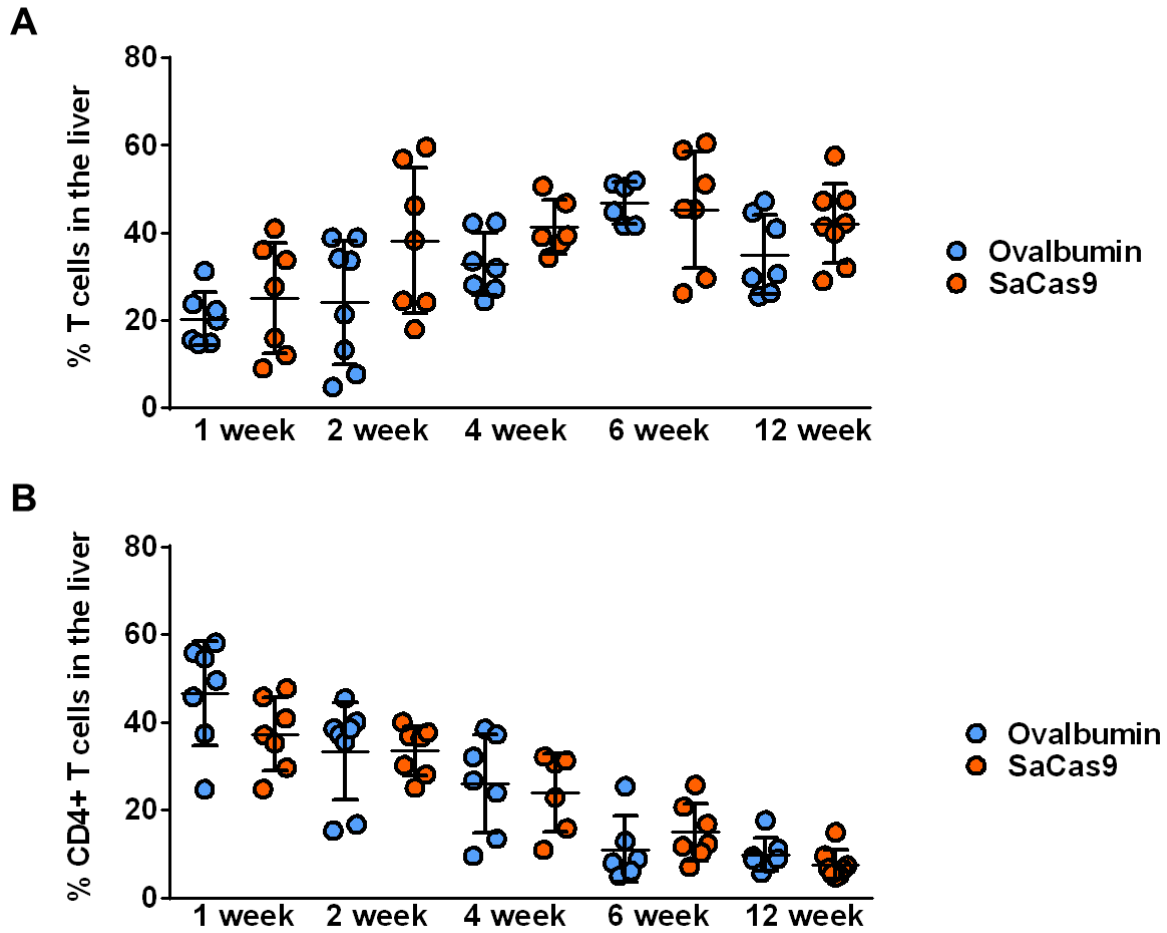## Supplemental Information

AAV-CRISPR Gene Editing Is Negated
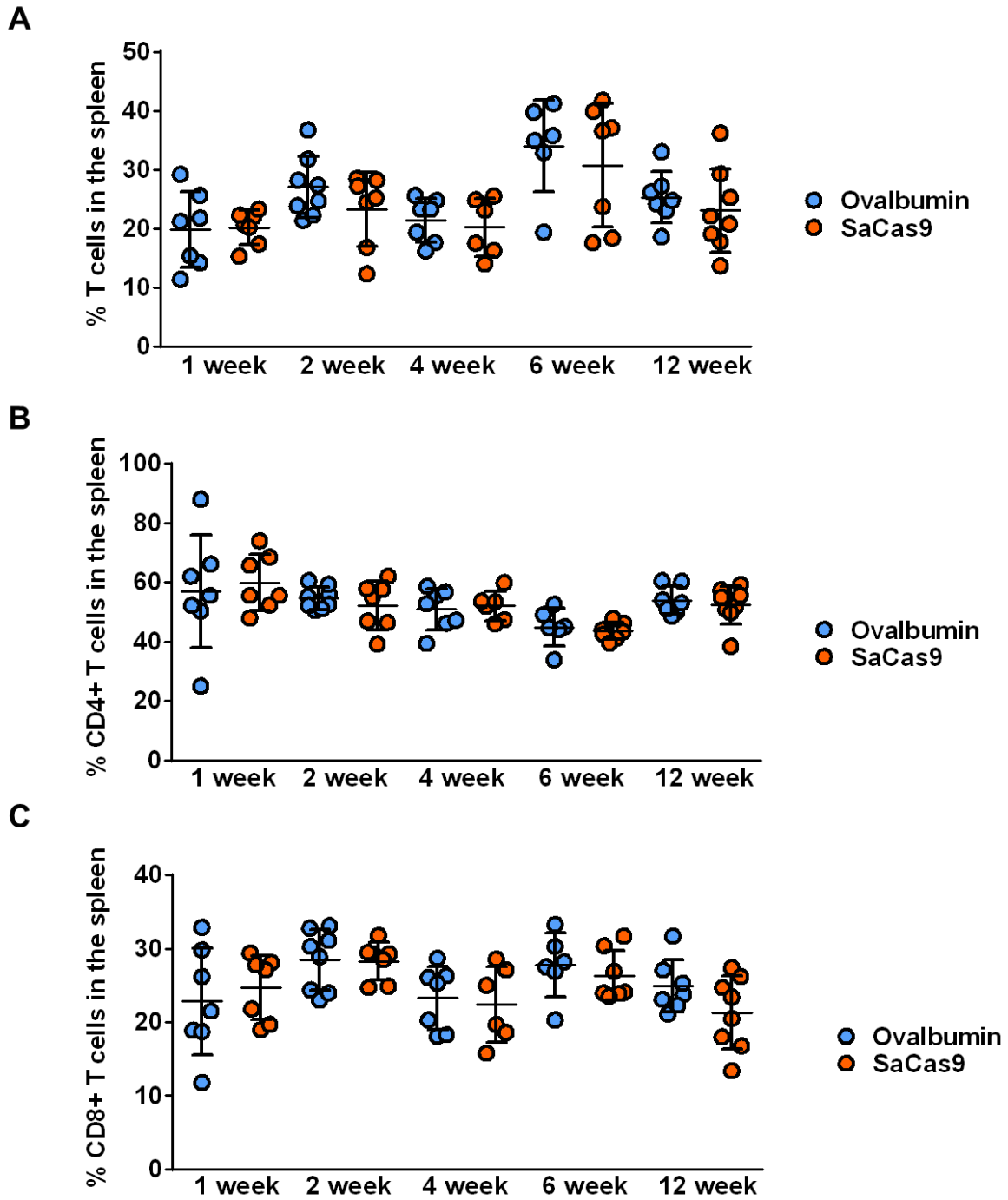
by Pre-existing Immunity to Cas9

Ang Li, Mark R. Tanner, Ciaran M. Lee, Ayrea E. Hurley, Marco De Giorgi, Kelsey E. Jarrett, Timothy H. Davis, Alexandria M. Doerfler, Gang Bao, Christine Beeton, and William R. Lagor

SUPPLEMENTAL FIGURE S1

A

B

Supplemental Figure S1. T-cell populations in the liver. *A)* T-cells in the liver as a percentage of total lymphocytes measured by flow cytometry. ***B)*** CD4$^+$ T-cells as a percentage of total lymphocytes in the liver measured via flow cytometry.
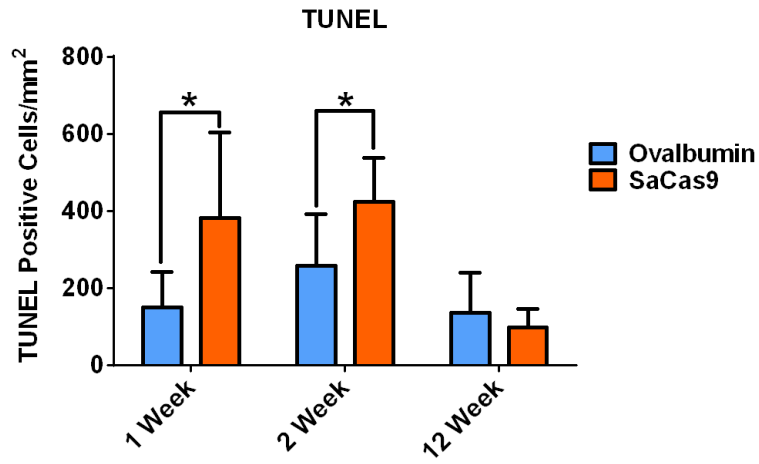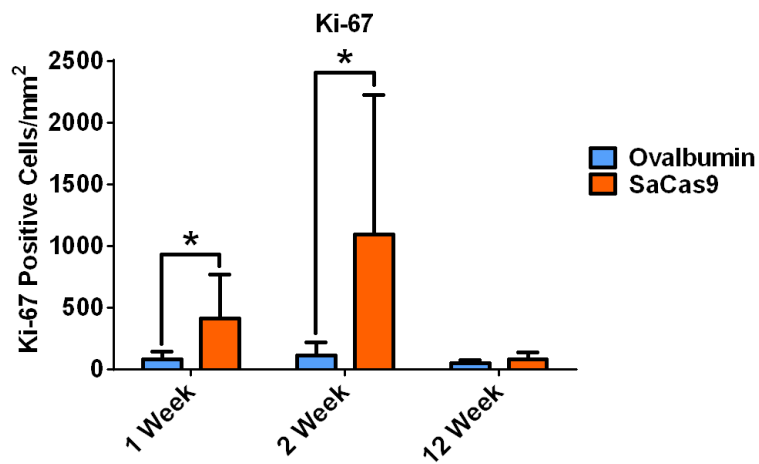
A

B

C

Supplemental Figure S2. T-cell populations in the spleen. *A)* T-cells as a percentage of total lymphocytes in the spleen measured by flow cytometry. *B)* CD4^{+} T-cells as a percentage of total lymphocytes in the spleen measured by flow cytometry. *C)* CD8^{+} T-cells as a percentage of total lymphocytes in the spleen measured by flow cytometry.
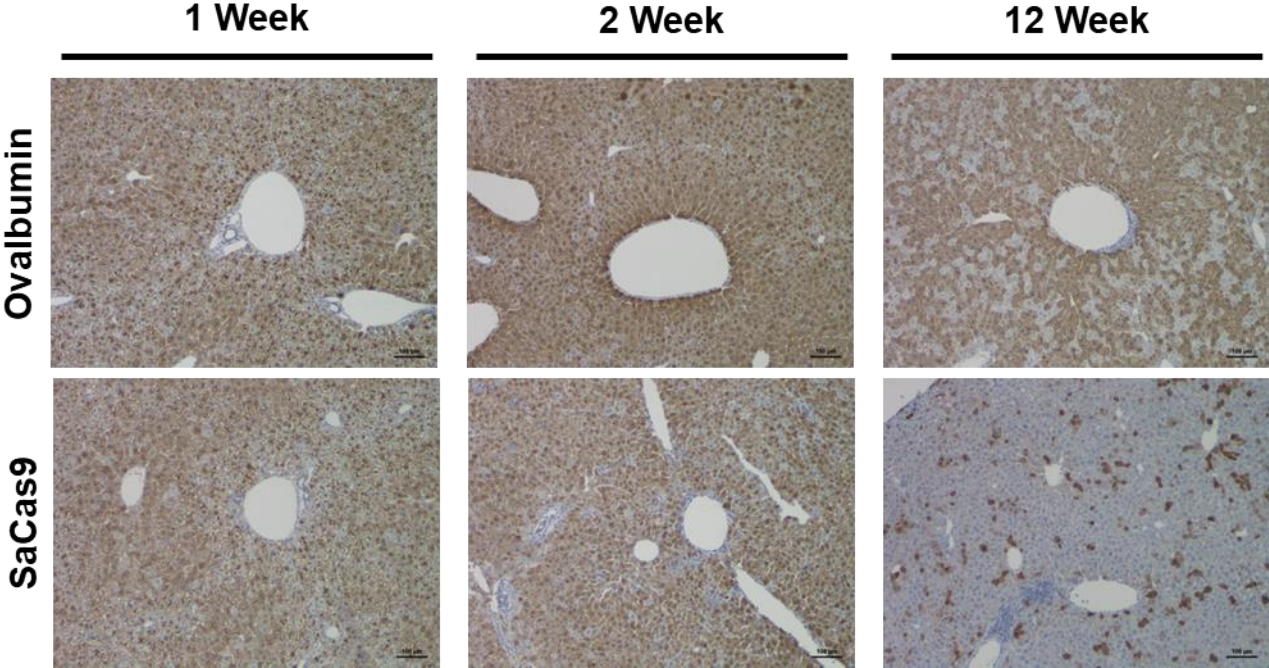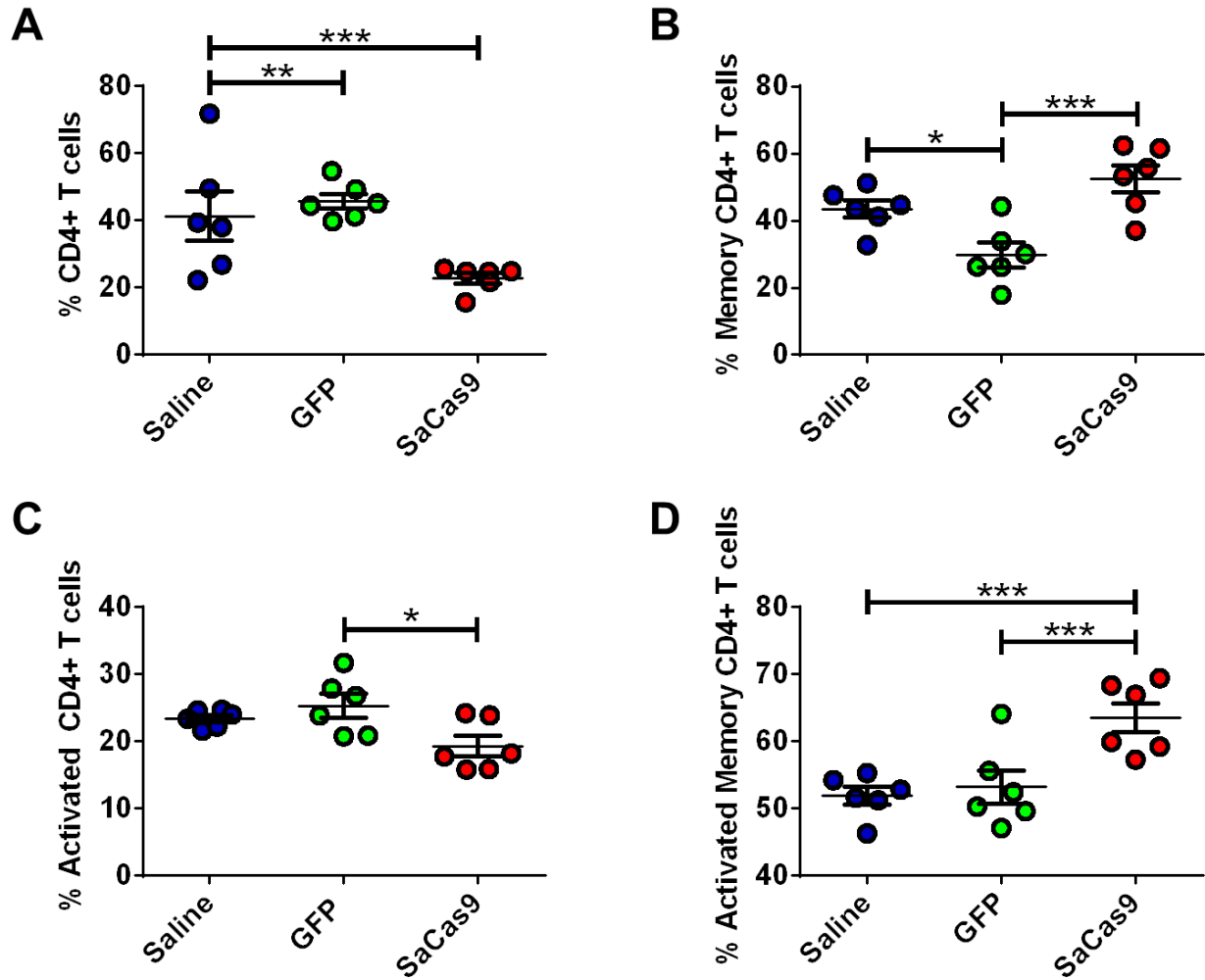
SUPPLEMENTAL FIGURE S3

Supplemental Figure S3. Apoptosis and proliferation analysis of the liver via immunohistochemistry. A) Total cells positive for TUNEL staining per square millimeter of tissue area. **B)** Total positive cells for Ki-67 staining per square millimeter of tissue area.

SUPPLEMENTAL FIGURE S4

Supplemental Figure S4. Immunohistochemistry of GFP in mouse liver. Representative immunohistochemistry staining for GFP in mouse liver sections at 1, 2, and 12 weeks in mice pre-immunized with ovalbumin or SaCas9
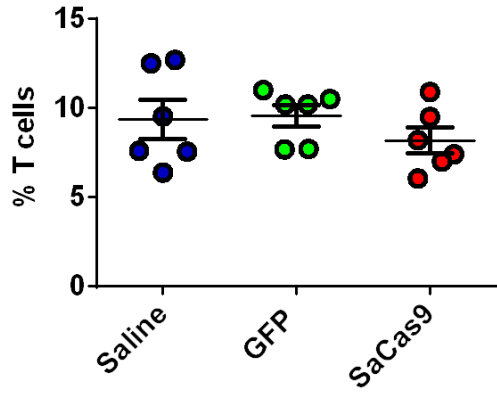
Supplemental Figure S5. CD4$^+$ T-cell responses in the liver. A) CD4$^+$ T-cells as a percentage of total lymphocytes in the liver measured via flow cytometry. **B)** Memory CD4$^+$ T-cells as a percentage of total CD4$^+$ T-cells. **C)** Activated CD4$^+$ T-cells as a percentage of total CD4$^+$ T-cells. **D)** Activated memory CD4$^+$ T-cells as a percentage of memory CD4$^+$ T-cells.

SUPPLEMENTAL FIGURE S6
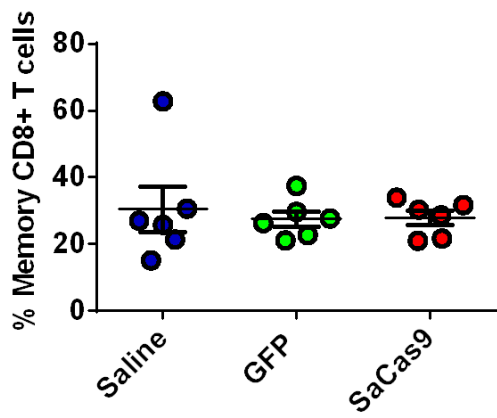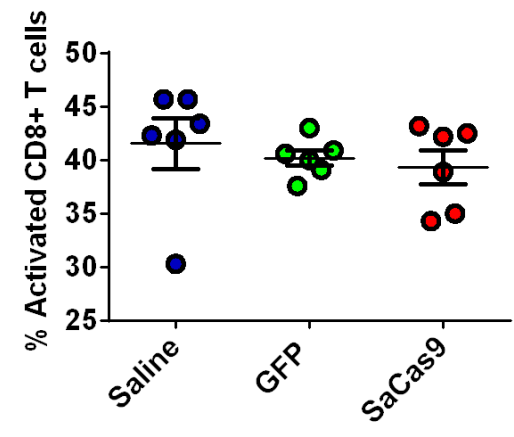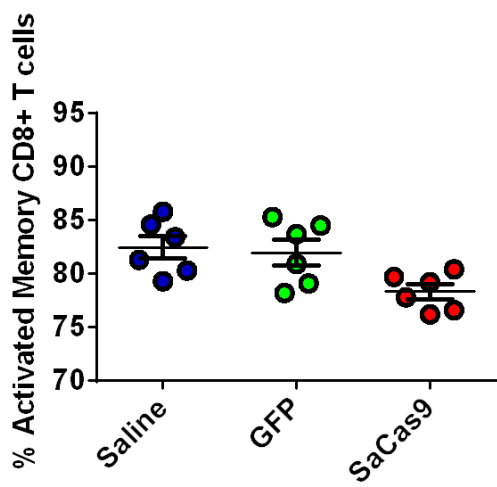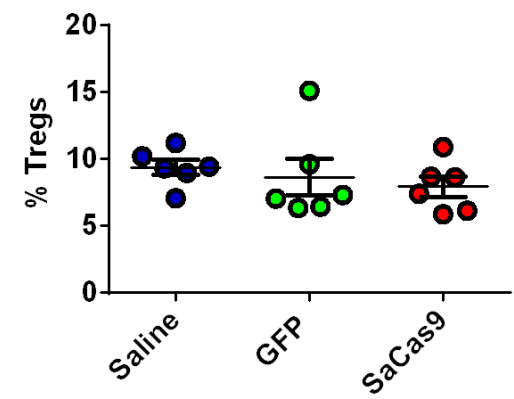
Supplemental Figure S6. CD8⁺ T-cell and Treg responses in the spleen. *A)* T-cells as a percentage of total lymphocytes in the spleen measured via flow cytometry. *B)* CD8⁺ T-cells as a percentage of T-cells *C)* Percentage of memory CD8⁺ to total CD8⁺ T-cells. *D)* Percentage of activated CD8⁺ T-cells to total CD8⁺ T-cell population. *E)* Percentage of activated memory CD8⁺ T-cells to total CD8⁺ T-cell population. *F)* Tregs as a percentage of total lymphocytes in the spleen measured via flow cytometry. N=6 per condition.
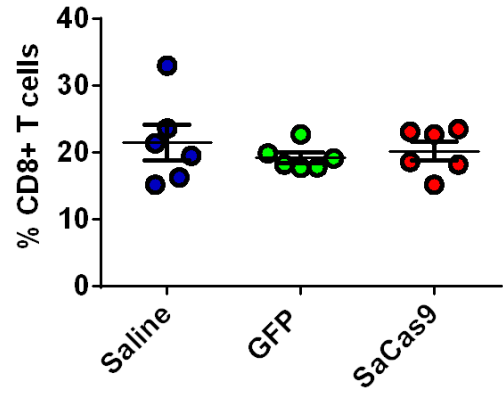
SUPPLEMENTAL FIGURE S7

Supplemental Figure S7. CD4⁺ T-cell responses in the spleen. *A)* CD4⁺ T-cells as a percentage of total lymphocytes in the spleen measured via flow cytometry. *B)* Memory CD4⁺ T-cells as a percentage of total CD4⁺ T-cells. *C)* Activated CD4⁺ T-cells as a percentage of total CD4⁺ T-cells. *D)* Activated memory CD4⁺ T-cells as a percentage of memory CD4⁺ T-cells.
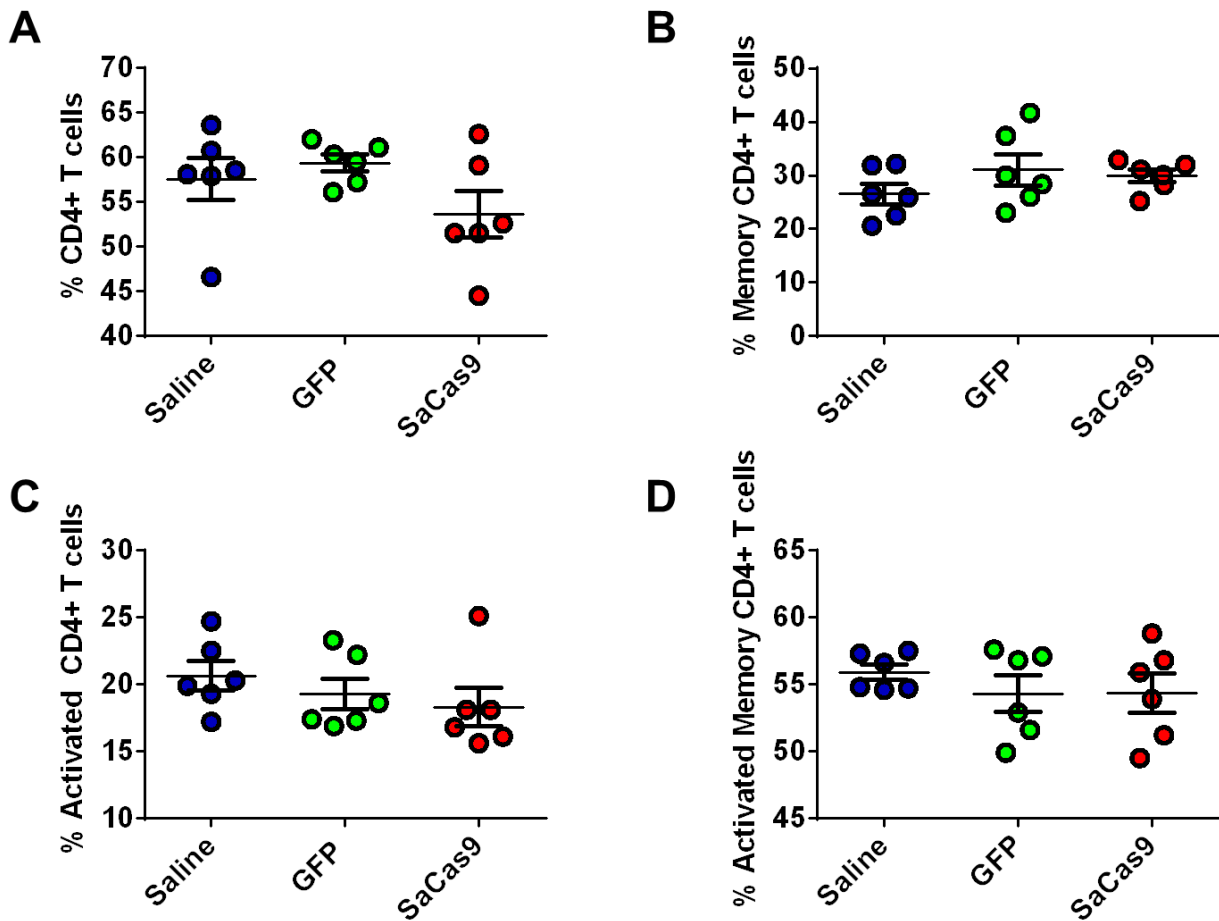
SUPPLEMENTAL TABLE S1

Description	Vendor	Catalog Number	Figures
Anti-CD3 conj. to Pacific Blue	BD Pharmigen	558214	2B, 4, S1, S2, S5-S7
Anti-CD4 conj. to Phycoerythrin	Invitrogen	12-0041-83	2B, S1, S2
Anti-CD4 conj. to Pacific Orange	Thermo Fisher	79-0042-82	4, S5, S7
Anti-CD-8 conj. to Alexa Fluor 647	Novus Biologicals	AP-MAB 0708	2B, 4, S1, S2, S6
Anti-CD25 conj. to Alexa Fluor 750	Novus Biologicals	FAB2438S	4, S5-S7
Anti-CD62L conj. to FITC	Sino Biological	50045-R414-F	4, S5-S7
Anti-Foxp3 conj. to PE-Cy5	eBioscience	15-5773-82	4, S6

Supplemental Table S1. List of antibodies used for flow cytometry analysis with vendors, catalogue numbers, and figures using the corresponding antibody

SUPPLEMENTAL TABLE S2

Description	Vendor	Catalogue Number	Dilution
Rabbit Anti HA-Tag	Cell Signaling	3350S	1 to 1000
Rabbit Anti GFP	Fisher Scientific	A-11122	1 to 3000
Mouse Anti Beta-Tubulin	Hybridoma Bank	E7	1 to 250
Rabbit Anti Ldlr	Gene Ness	N/A	1 to 5000
Goat Anti-Rabbit (680 nm)	RockLand Immunochemical	RL6111440020.5	1 to 10000
Goat Anti-Mouse (800 nm)	RockLand Immunochemical	RL6111450020.5	1 to 10000

Supplemental Table S2. List of antibodies used for western blot analysis with corresponding vendors, catalogue numbers, and dilutions used in this study.

SUPPLEMENTAL TABLE S3

Name	Sequence (5' to 3')	Purpose
EmGFP_F1	CAAGCTGACCCTGAAGTTCATC	EmGFP TIDE Sequencing
PolA_R	CACACAAAAAACCAACACACAGATCTAATG	EmGFP, SaCas9 TIDE Sequencing
HLP_F	CCCTGTTTGCTCCTCCGATAAC	SaCas9 TIDE Sequencing
mTTP_F	GACCTCTGCTTGACCTATAGTCACATGAC	mMttp Exon 2 Forward Primer
mTTP_R	GCAGCCTGGAGCACGTATC	mMttp Exon 2 Reverse Primer
Ldlr_F	CCGGAGGACATTGTCCTGTTC	mLdlr Exon 14 Forward TIDE Primer
Ldlr_R	CTCAGGGAATCTGCTTCAGCAAC	mLdlr Exon 14 Reverse TIDE Primer
ApoE_F	GGAAGGAGAGAGCCAATTCCCTC	mApoE Exon 2 Forward TIDE Primer
ApoE_R	CGCTTGTGTTCCCAGAAGTTGAG	mApoE Exon 2 Reverse TIDE Primer
EmGFP_F2	GCATCGACTTCAAGGAGGAC	EmGFP qPCR Forward Primer
EmGFP_R1	TGCACGCTGCCGTCCTCGATG	EmGFP qPCR Reverse Primer
SaCas9_F	CCGTCGTGAAGAGAAGCTTCATC	SaCas9 qPCR Forward Primer
SaCas9_R	CCACCTCATAGTTGAAGGGGTTG	SaCas9 qPCR Reverse Primer

Supplemental Table S3. List of qPCR primers with corresponding targets used in this study.