YMTHE, Volume *29*

Supplemental Information

Long-term stable reduction of low-density lipoprotein in nonhuman primates following *in vivo* genome editing of PCSK9

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Supplemental Figures S1–S7

Supplemental Tables S1–S5

Supplemental Data S1-S2

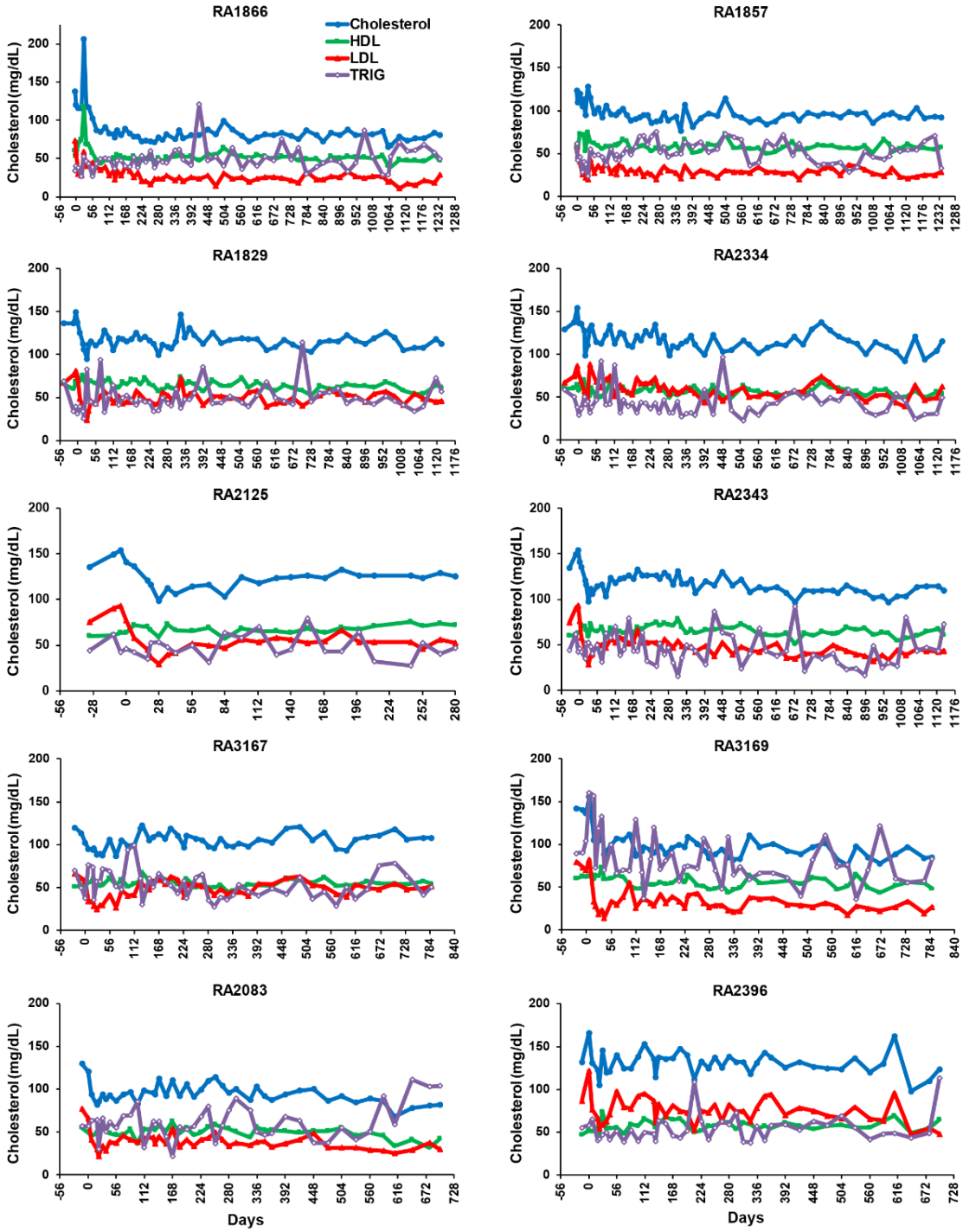

Figure S1. Long-term follow-up of serum lipid profiles of macaques in this study. Serum samples collected from each animal before and after AAV vector treatment were assayed for total cholesterol, high-density lipoprotein (HDL), LDL, and triglyceride (TRIG) levels. The results of early time points for RA1866, RA1857, RA1829, RA2334, RA2125, and RA2343 have been previously published and are included here for completeness of the data set.[24]

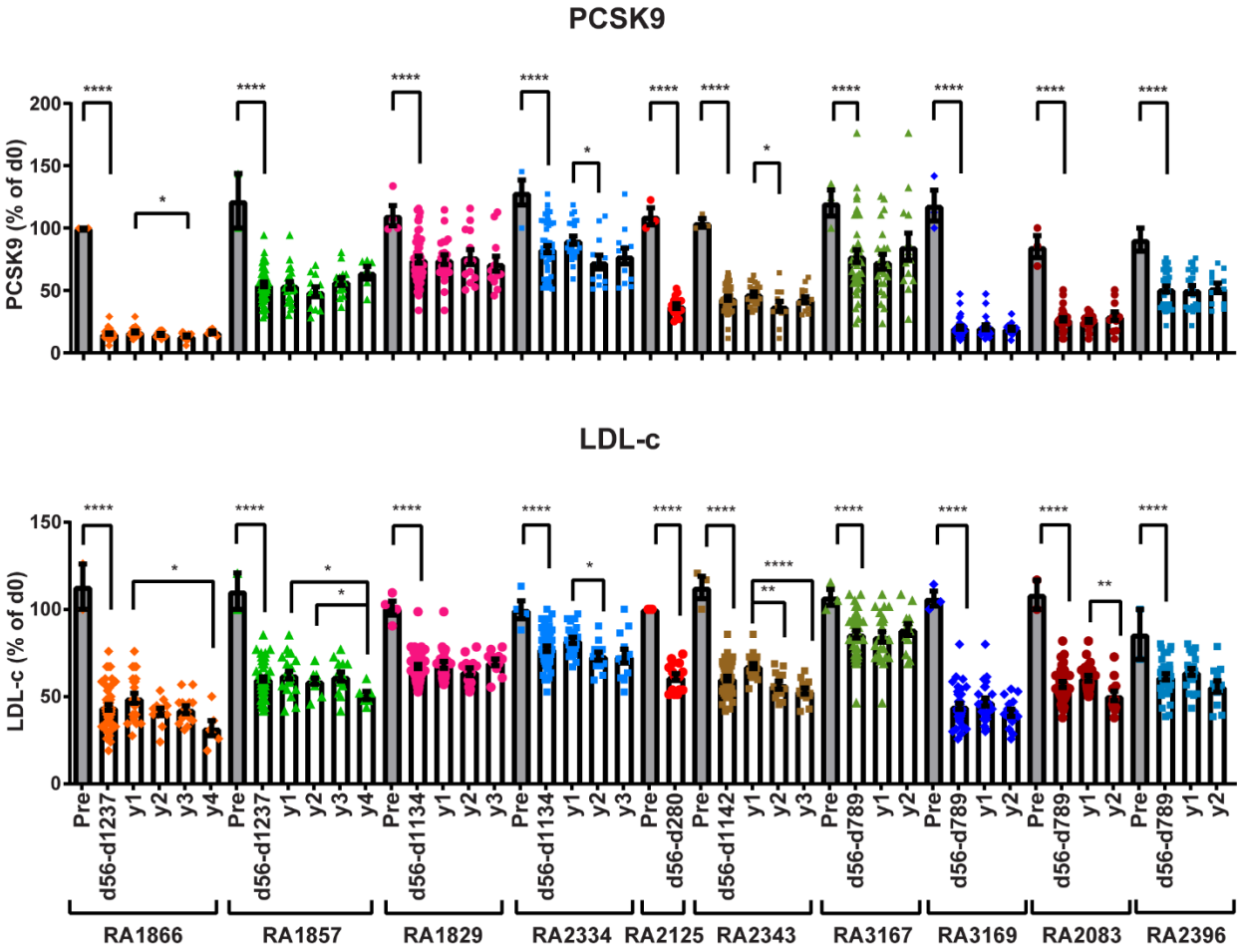

Figure S2. Significant, long-term reduction in serum PCSK9 and LDL-c in macaques following a single infusion of AAV-meganuclease vectors. A, Serum PCSK9 levels. **B**, Low-density lipoprotein cholesterol (LDL-c) levels. All data are shown as the percentage of day-0 levels. The data for each animal are segregated as pre-dosing data (pre) and all data points post-dosing starting from day 56 (d56 to the most recent time point), year 1 (y1), year 2 (y2), year 3 (y3, if applicable), and year 4 (y4, if applicable). Individual data points and the mean ± SEM are shown. Pre- and post-dosing levels were analyzed by a one-sided one-sample *t*-test using R Statistical Software (version R.4.0.0). Changes in levels between each year were analyzed by a two-sided Wilcoxon rank-sum test using R Statistical Software (version R.4.0.0). *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$.

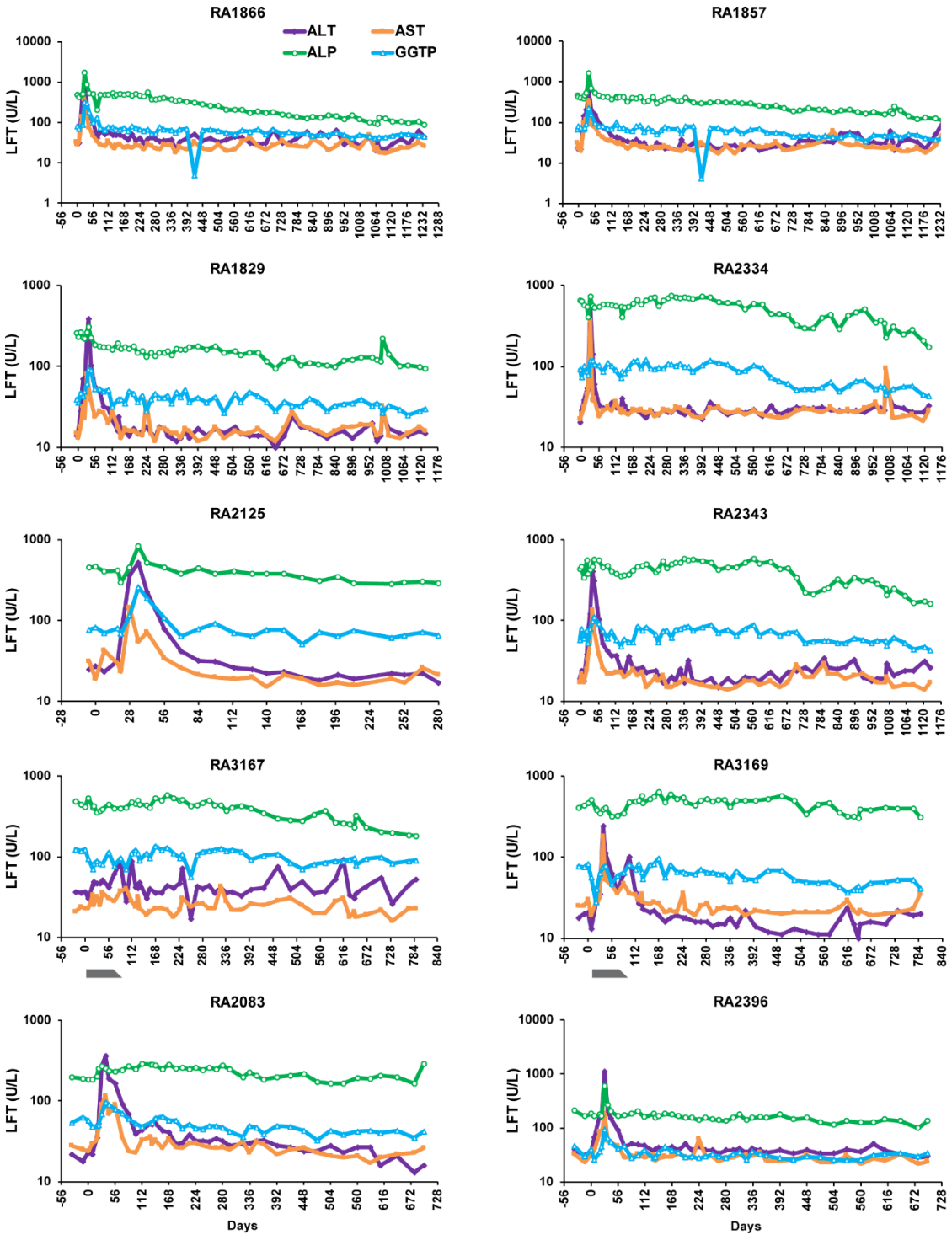

Figure S3. Long-term follow-up of liver function tests (LFTs) of macaques in this study. LFTs, including alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and gammaglutamyl transpeptidase (GGTP) tests, were performed on serum samples collected from each animal before and after AAV vector treatment. The grey bar indicates the period of steroid treatment and tapering course. The results of early time points for RA1866, RA1857, RA1829, RA2334, RA2125, and RA2343 have been previously published and are included here for completeness of the data set.[24]

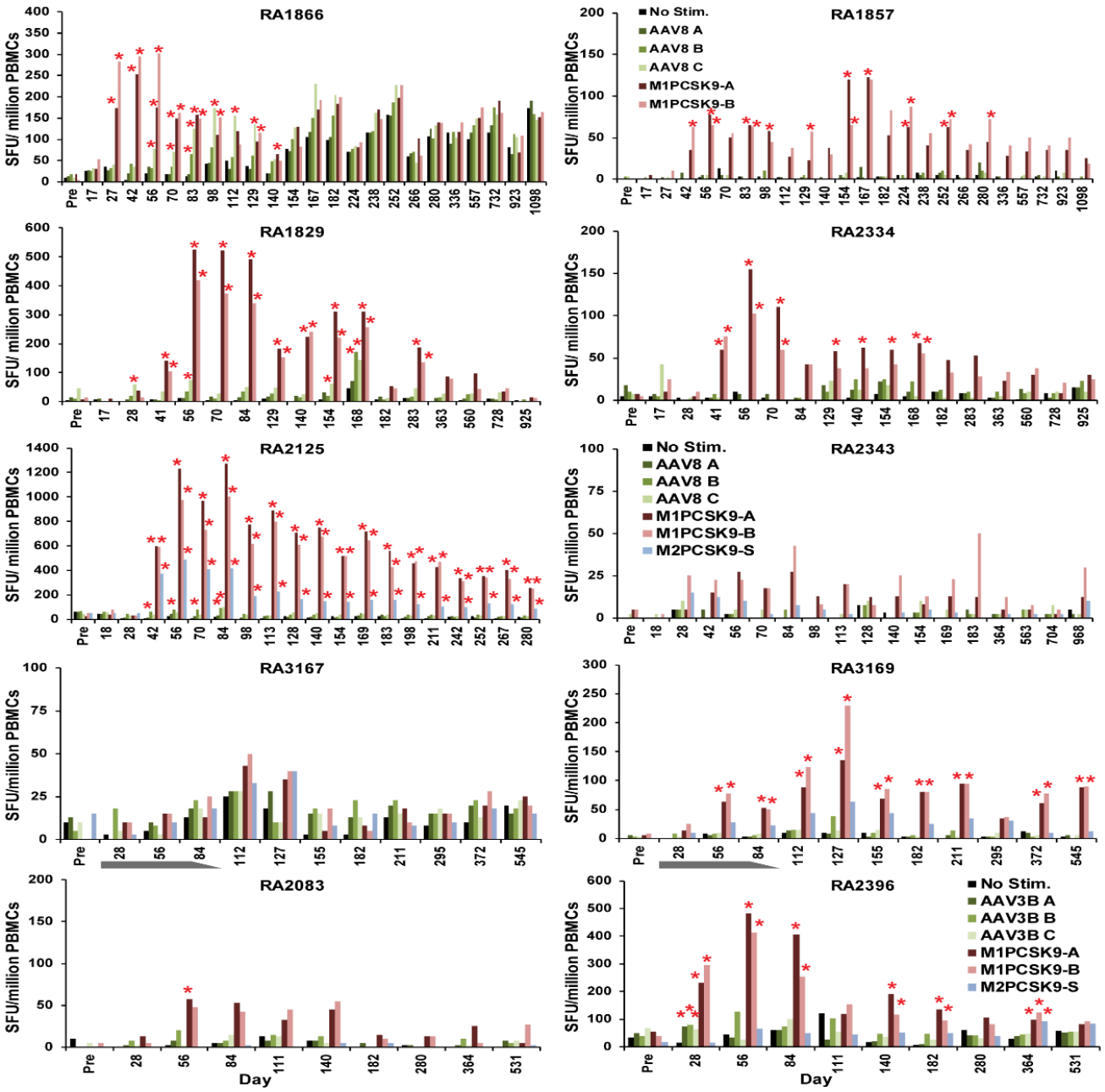

Figure S4. Long-term follow-up of T-cell responses to AAV capsid and meganuclease by IFN-γ ELISpot assay. Peripheral blood mononuclear cells (PBMCs) were isolated at various time points throughout the study and were stimulated with peptide libraries of AAV capsid (pools AAV8 A, B, C or pools AAV3B A, B, C) and M1PCSK9 (pool A, B) or M2PCSK9 (M2PCSK9-S). The red asterisk indicates a positive T-cell response against a particular peptide library, which is arbitrarily defined as > 55 spot-forming units (SFUs) per million PBMCs and an SFU value more than three-fold greater than that of the medium control. The grey bar indicates the period of steroid treatment and tapering course. The results of early time points for RA1866, RA1857, RA1829, RA2334, RA2125, and RA2343 have been previously published and are included here for completeness of the data set.[24]

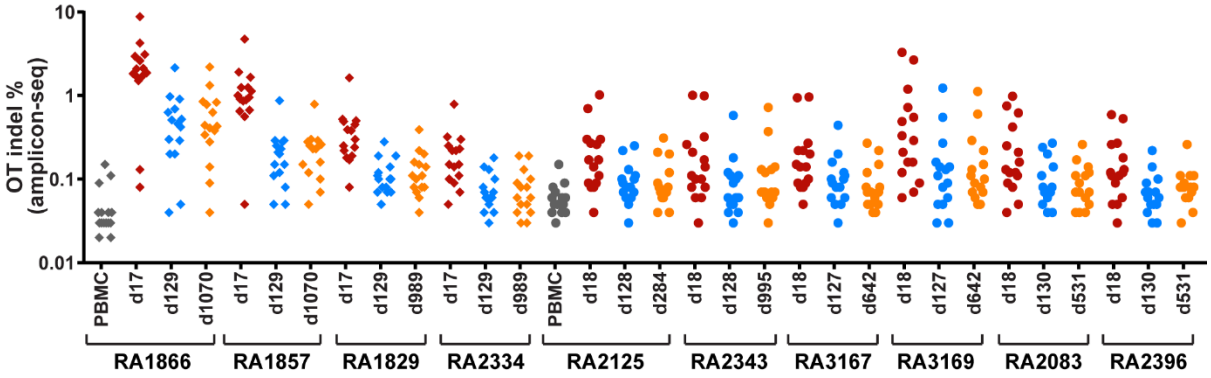

Figure S5. Validation of OT editing by amplicon-seq for the top 15 OT sites predicted *in vitro* by GUIDE-seq. Data for DNA sample set A are shown. Details for the OT sites and indel% for each sample are shown in Tables S2-S4.

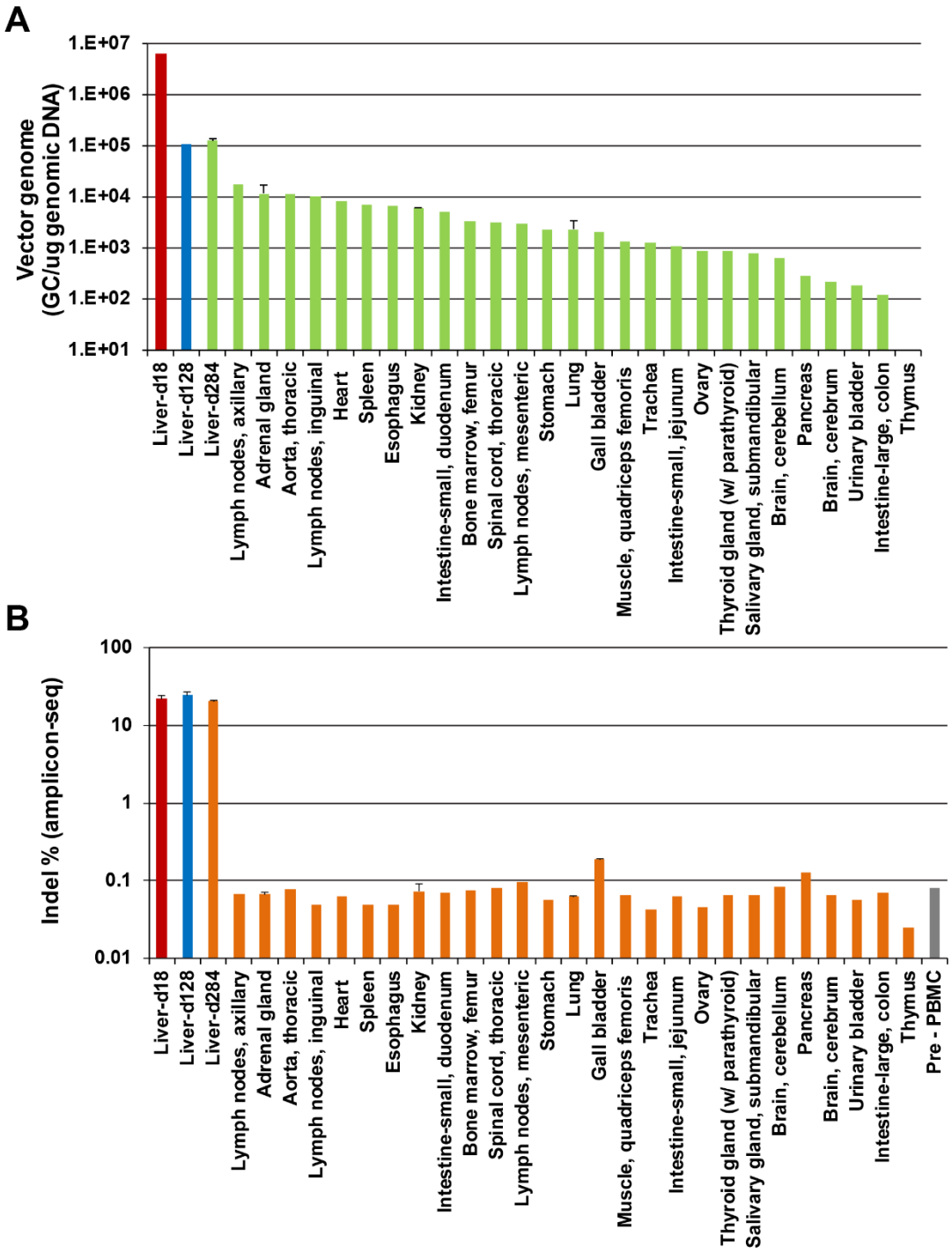

Figure S6. Vector biodistribution and indel analysis of tissue samples collected at necropsy of RA2125 on day 284 post-vector administration. A, Vector biodistribution in tissues analyzed by qPCR. **B**, On-target indels analyzed by amplicon-seq. Data from Pre - PBMC and liver biopsies obtained on days 18 and 128 are included for reference. The means ± SEM are shown for the liver-284 (n = 8), liver-d18, liver-d128, adrenal gland, kidney, and lung (n = 2). GC: genome copy.

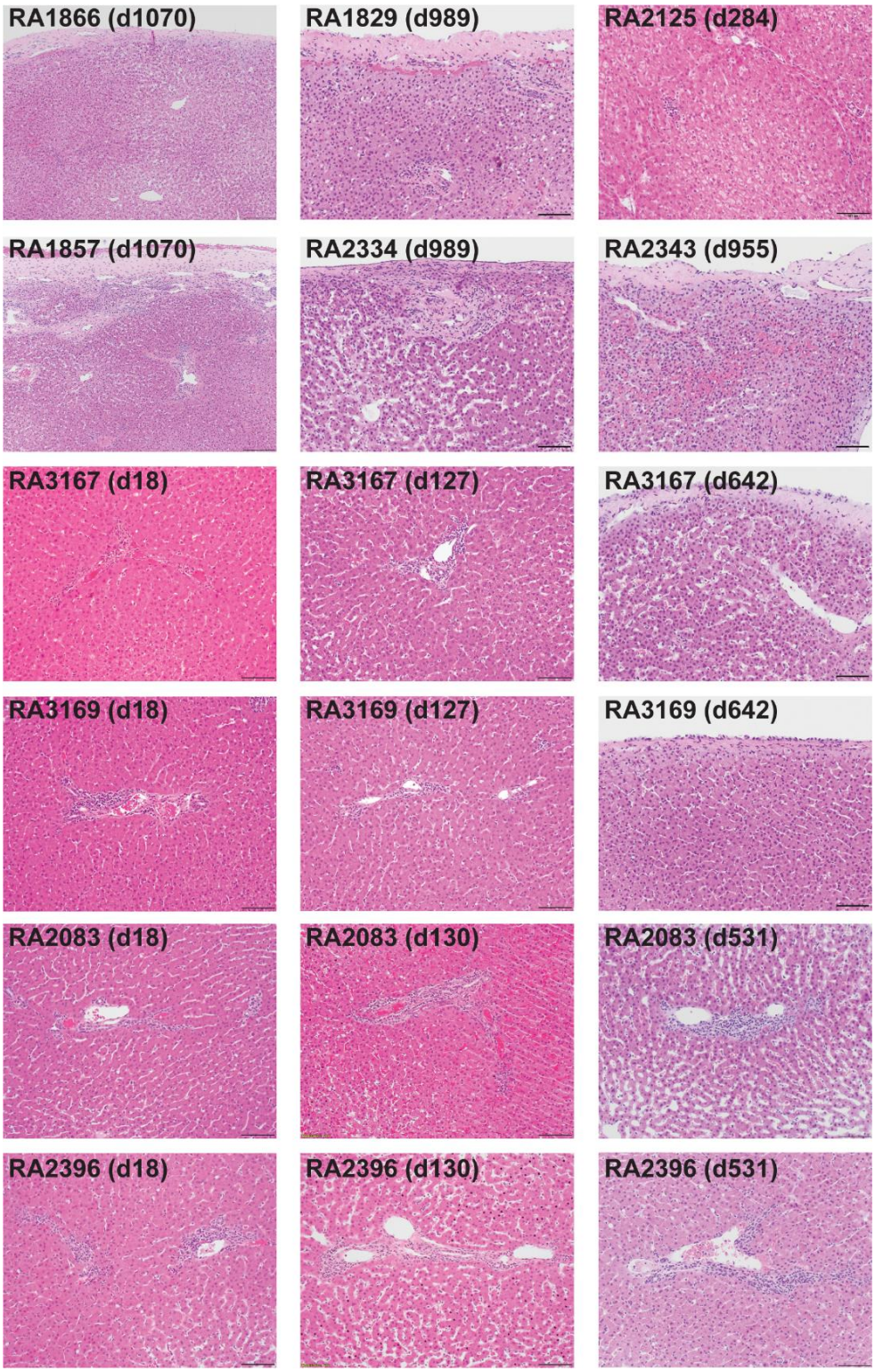

Figure S7. Examination of liver histopathology in biopsy or necropsy samples collected at different time points following vector administration. Representative images of hematoxylin and eosin staining are shown. The results of early time points for RA1866, RA1857, RA1829, RA2334, RA2125, and RA2343 have been previously published and are included here for reference and comparison.[24] Scale bar: 50 µm.

Table S1. Summary of histology findings in non-liver tissues from RA2125 collected at necropsy (day 284 post-dosing).

Tissue	Histology findings
Heart	Minimal mononuclear cell infiltrates (grade 1)
Kidneys	Minimal interstitial mononuclear cell infiltrates (grade 1)
Lung	Minimal mononuclear cell infiltrates with and without pigment, multifocal (grade 1)
Thymus	Multiple congenital cysts
Thyroid/Parathyroid	Multiple congenital cysts Ectopic thymus, focal
Summary	No significant treatment-related findings. All findings listed above are incidental and considered background. Organs without abnormalities: adrenal glands, lymph nodes (axillary, inguinal, and mesenteric), pancreas, parathyroid gland, small intestine (duodenum), and spleen. No histologic evidence of infection with *Mycobacterium tuberculosis* (TB) in any tissue examined.

Table S2. OT validation by amplicon-seq in liver biopsy samples from AAV8-M1PCSK9 treated macaques.

	AAV8-M1PCSK9															
Location	**Indel %**															
	RA1866				**RA1857**				**RA1829**				**RA2334**			
High rank	**Pre**	**d17**	**d129**	**d1070**	**Pre**	**d17**	**d129**	**d1070**	**Pre**	**d17**	**d129**	**d989**	**Pre**	**d17**	**d129**	**d989**
Chr5:112049529	0.04	**1.87**	**0.69***	**0.63**	0.04	**0.67**	**0.28***	**0.26**	0.03	**0.22**	**0.14***	**0.08***	0.04	**0.14**	0.07*	**0.09**
Chr20:69811042	0.03	**1.51**	**0.29***	**0.41***	0.04	**1.13**	**0.20***	**0.29***	0.03	**0.17**	**0.07***	**0.07**	0.04	**0.10**	0.03*	0.04
Chr7:123575698	0.03	**0.13**	**0.05***	**0.09**	0.06	0.05	0.05	0.05	0.05	0.08	0.05	0.04	0.05	0.05	0.04	0.05
Chr12:10658914	0.11	**2.09**	**0.91***	**1.32***	0.09	**1.00**	**0.29***	**0.30**	0.46	0.52	0.19*	0.20	0.10	**0.22**	0.13*	0.13
Chr12:51647755	0.04	**8.81**	**2.15***	**2.20**	0.04	**4.75**	**0.87***	**0.79**	0.03	**1.63**	**0.28***	**0.39***	0.03	**0.79**	**0.18***	**0.19**
Chr13:92389310	0.15	**1.83**	**0.51***	**0.44**	0.17	**0.89**	**0.23***	**0.23**	0.14	**0.39**	**0.19***	**0.22**	0.11	**0.25**	**0.14***	**0.19**
Chr16:49265525	0.02	**2.61**	**0.63***	**0.85***	0.03	**0.56**	**0.08***	**0.07**	0.03	**0.30**	**0.12***	**0.14**	0.01	**0.07**	**0.06**	**0.03**
Chr13:43000760	0.02	**3.10**	**0.52***	**0.78***	0.02	**1.66**	**0.21***	**0.25**	0.02	**0.45**	**0.10***	**0.11**	0.02	**0.23**	**0.07***	**0.04**
Chr6:2022570	0.03	**1.67**	**0.97***	**0.83***	0.02	**0.65**	**0.25***	**0.28**	0.03	**0.19**	**0.10***	**0.15***	0.02	**0.15**	**0.06***	**0.08**
Chr5:139700784	0.09	**2.12**	**0.20***	**0.28**	0.09	**0.95**	0.11*	**0.23**	0.15	**0.50**	0.11*	0.16*	0.11	**0.32**	0.10*	0.10
Chr9:114398062	0.03	**1.75**	**0.42***	**0.43**	0.03	**0.86**	**0.15***	**0.16**	0.03	**0.25**	**0.08***	**0.06**	0.03	**0.11**	**0.08***	**0.06**
Chr9:53019653	0.03	**4.26**	**0.46***	**0.34***	0.04	**1.91**	**0.15***	**0.12**	0.02	**0.49**	**0.07***	**0.08**	0.02	**0.30**	**0.06***	**0.08**
Chr10:22429622	0.04	**1.81**	**0.20***	**0.14**	0.03	**0.91**	**0.05***	**0.15***	0.02	**0.18**	**0.07***	**0.11**	0.03	**0.09**	0.04*	0.03
Chr12:46743016	0.04	**2.94**	**0.30***	**0.38**	0.05	**1.25**	**0.12***	**0.10**	0.03	**0.38**	**0.08***	**0.08**	0.03	**0.15**	0.06*	0.05
Chr10:72232623	0.03	0.08	0.04	0.04	0.03	**1.25**	**0.29***	**0.25**	0.04	**0.24**	**0.10***	**0.10**	0.04	**0.22**	0.05*	0.06

DNA from macaque liver biopsies treated with AAV8-M1PCSK9 vector was used as a template for PCR amplification of a subset of genomic regions predicted to be an OT site of M1PCKS9 by GUIDE-seq. DNA from PBMCs before dosing (Pre) serves as control for each NHP. Highlighted in boldface: Indel percentage increases that are statistically higher ($p < 0.05$) from those of the Pre- and post-treatment samples (d17, d129, d989, or d1070, respectively) based on a one-sided Fisher's exact test (fisher.test in R Statistical Software version R.4.0.0). *: Statistically significant difference ($p < 0.05$) between first biopsies (d17) and second biopsies (d129), or between second biopsies and third biopsies (d989, or d1070) based on a two-sided Fisher's exact test (fisher.test in R Statistical Software version R.4.0.0). The results for Pre and the first two liver biopsy samples have been previously published and were reanalyzed for consistency and included here for completeness of the data set.[24] RA1829 has a single nucleotide deletion at position 10658919 on chromosome 12 in pre PBMC sample and is excluded from indel analysis. NHP: nonhuman primate. OT: off-target.

Table S3. OT validation by amplicon-seq in liver biopsy or necropsy samples from AAV8-M2PCSK9 treated macaques.

AAV8-M2PCSK9																
Location	**Indel %**															
	RA2125				**RA2343**				**RA3167**				**RA3169**			
High rank	**Pre**	**d18**	**d128**	**d284**	**Pre**	**d18**	**d128**	**d995**	**Pre**	**d18**	**d127**	**d642**	**Pre**	**d18**	**d127**	**d642**
Chr10:72232623	0.05	**0.70**	**0.25***	**0.31**	0.04	**1.01**	**0.58***	**0.72***	0.03	**0.96**	**0.44***	**0.27***	0.06	**3.29**	**1.23***	**1.12**
Chr5:112049529	0.05	**0.17**	**0.09***	**0.12**	0.04	**0.10**	**0.10**	**0.07**	0.04	**0.22**	**0.10***	0.07	0.06	**0.33**	0.05*	**0.11***
Chr19:51609207	0.07	0.08	0.08	0.08	0.07	0.06	0.06	0.07	0.09	0.10	0.08	0.07	0.09	0.07	0.06	0.06
Chr19:31971930	0.06	**0.11**	0.05*	0.00	0.07	**0.11**	0.06	0.06	0.07	0.08	0.05	0.05	0.07	**0.21**	0.09*	0.09
Chr16:48383076	0.09	**0.30**	0.11*	0.09	0.04	**0.26**	**0.12***	**0.14**	0.14	**0.27**	0.16	0.15	0.08	**0.72**	0.14*	**0.22**
Chr16:41164165	0.08	0.09	0.10	0.20	0.11	0.14	0.11	0.12	0.17	0.14	0.12	0.12	0.16	0.16	0.11	0.13
Chr9:53019653	0.04	**1.02**	**0.22***	**0.21**	0.03	**0.99**	**0.18***	**0.37***	0.05	**0.94**	**0.20***	**0.22**	0.04	**2.67**	**0.55***	**0.60**
Chr14:11716320	0.06	**0.17**	**0.10***	0.06	0.07	0.08	0.05	0.07	0.08	**0.15**	0.06*	0.08	0.08	**0.29**	0.08*	0.07
Chr14:69311382	0.15	**0.27**	0.13*	0.07	0.04	**0.32**	**0.09***	**0.13**	0.09	**0.20**	0.08*	0.08	0.09	**0.49**	0.14*	0.10
Chr7:123575698	0.05	0.04	0.06	0.04	0.04	0.03	0.03	0.05	0.04	0.05	0.06	0.05	0.04	0.06	0.03	0.05
Chr3:169340141	0.05	0.08	0.06	0.08	0.09	0.08	0.05	0.06	0.05	0.08	0.11	0.06	0.06	**0.09**	0.03	0.07
Chr5:178494103	0.06	**0.14**	0.08*	0.07	0.05	**0.10**	0.04*	0.07	0.07	**0.14**	0.10	0.07	0.07	**0.12**	**0.16**	0.08*
Chr12:51647755	0.04	**0.26**	**0.07***	0.06	0.03	**0.17**	**0.06***	**0.07**	0.02	**0.22**	**0.09***	**0.05***	0.04	**0.55**	**0.13***	**0.15**
Chr16:49265525	0.04	**0.30**	**0.07***	0.07	0.04	**0.21**	**0.11***	**0.13**	0.04	**0.09**	0.03*	0.04	0.05	**1.19**	**0.27***	**0.29**
Chr6:2022570	0.03	**0.09**	0.03*	0.04	0.02	**0.06**	**0.04**	0.03	0.04	**0.09**	0.05*	0.04	0.05	**0.16**	0.05*	0.05

DNA from macaque liver biopsies, or RA2125 necropsy, treated with AAV8-M2PCSK9 vector was used as a template for PCR amplification of a subset of genomic regions predicted to be an OT site of M2PCSK9 by GUIDE-seq. DNA from PBMCs before dosing (Pre) serves as control for each NHP. Highlighted in boldface: Indel percentage increases that are statistically higher ($p < 0.05$) from those of Pre- and post-treatment samples (d18, d127, d128, or d284, d642, d995, respectively) based on a one-sided Fisher's exact test (fisher.test in R Statistical Software version R.4.0.0). *: Statistically significant difference ($p < 0.05$) between first biopsies (d18) and second biopsies (d127 or d128), or between second biopsies and necropsy (RA2125) or third biopsies (d642 or d995) based on a two-sided Fisher's exact test (fisher.test in R Statistical Software version R.4.0.0). The results for Pre and the first two liver biopsy samples for RA2125, and RA2343 have been previously published and were reanalyzed for consistency and included here for completeness of the data set.[24] RA2125 has a single nucleotide deletion at position 69311389 on chromosome 14 in pre PBMC sample and is excluded from indel analysis. NHP: nonhuman primate. OT: off-target

Table S4. OT validation by amplicon-seq in macaque liver biopsy samples and xenograft FRG mouse liver samples following treatment with AAV3B-M2PCSK9.

AAV3B-M2PCSK9													
NHP study									Humanized FRG mice study				
Location	Indel %								Location	Indel %			
	RA2083				RA2396					Ctrl 1	Ctrl 2	FRG #1	FRG #2
High rank	Pre	d18	d130	d531	Pre	d18	d130	d531	High rank	d28	d28	d49	d49
Chr10:72232623	0.02	**0.75**	**0.27***	**0.26**	0.07	**0.59**	**0.22***	**0.26**	Chr19:53812462	0.12	0.04	**1.52**	**1.28**
Chr5:112049529	0.06	0.11	0.07	0.07	0.06	0.12	0.04*	0.08*	Chr22:46843754	0.01	0.02	**7.84**	**11.81**
Chr19:51609207	0.04	0.09	0.05	0.06	0.09	0.06	0.06	0.08	Chr19:2614048	0.16	0.14	**5.70**	**4.21**
Chr19:31971930	0.06	0.16	0.07*	0.11	0.04	**0.11**	0.05*	0.03	Chr7:70210115	0.16	0.07	**4.53**	**7.46**
Chr16:48383076	0.08	0.21	0.20	0.11	0.03	**0.18**	0.10	0.08	Chr19:36036134	0.07	0.06	**0.43**	**0.42**
Chr16:41164165	0.11	0.12	0.11	0.14	0.09	0.11	0.07	0.11	Chr6:166346623	0.06	0.07	**6.75**	**6.71**
Chr9:53019653	0.02	**0.98**	**0.24***	**0.17**	0.02	**0.53**	**0.14***	**0.11**	Chr5:168303807	0.07	0.06	**2.19**	**2.29**
Chr14:11716320	0.06	0.13	0.07	0.04	0.02	**0.12**	0.03*	0.08	Chr11:78099338	0.05	0.03	**3.64**	**4.52**
Chr14:69311382	0.04	**0.42**	0.04*	0.07	0.07	**0.26**	0.07*	0.09	ChrX:123413838	0.01	0.05	**2.81**	**2.57**
Chr7:123575698	0.01	0.04	0.06	0.04	0.04	0.05	0.03	0.04	Chr2:45967389	0.03	0.06	**1.98**	**2.18**
Chr3:169340141	0.03	0.08	0.08	0.05	0.06	0.05	0.05	0.08	Chr17:81556274	0.05	0.03	**0.86**	**0.44**
Chr5:178494103	0.07	0.05	0.08	0.09	0.07	0.09	0.06	0.06	Chr11:36256974	0.10	0.07	**0.35**	**0.41**
Chr12:51647755	0.12	0.25	0.07*	0.07	0.00	0.13	0.05*	0.06	Chr16:60132284	0.06	0.08	**0.21**	**0.29**
Chr16:49265525	0.05	**0.62**	**0.14***	0.12	0.02	**0.27**	**0.09***	**0.11**	Chr11:62596750	0.05	0.05	**0.23**	**0.53**
Chr6:2022570	0.03	0.12	0.04*	0.04	0.03	0.03	0.07	0.07	Chr1:108873560	0.04	0.03	**3.15**	**3.48**
									Chr10: 103181012	0.05	0.06	**2.42**	**1.40**
									Chr9: 119938961	0.08	0.08	**1.46**	**1.48**
									Chr6: 42112864	0.04	0.02	**1.16**	**1.41**
									Chr22: 19062361	0.07	0.05	**2.10**	**2.88**
									Chr15: 74372153	0.05	0.05	0.04	0.09
									Chr20: 64112204	0.16	0.15	**2.11**	**1.65**
									Chr4: 186170668	0.07	0.08	**1.12**	**1.74**

DNA from macaque liver biopsies, or the liver of xenograft mice treated with control AAV3B and AAV3B-M2PCSK9 vectors was used as a template for PCR amplification of a subset of genomic regions predicted to be an OT site of M2PCSK9 by GUIDE-seq. DNA from PBMCs before dosing (Pre) serves as control for each NHP. Highlighted in boldface: Indel percentage increases that are statistically higher ($p < 0.05$) from those of the control samples (Pre or Ctrl) and post-treatment samples (d18, d49, d130, or d531, respectively) based on a one-sided Fisher's exact test (fisher.test in R Statistical Software version R.4.0.0). *: Statistically significant difference ($p < 0.05$) between first biopsies (d18) and second biopsies (d130), or between second biopsies or third biopsies (d531) based on a two-sided Fisher's exact test (fisher.test in R Statistical Software version R.4.0.0). FRG: $Fah^{-/-}Rag2^{-/-}Il2rg^{-/-}$; NHP: nonhuman primate; OT: off-target

Table S5. Summary of histology findings in liver biopsy and necropsy samples.

Animal No. (biopsy/necropsy day)	Liver histology findings
RA1866 (d1070)	Mild capsular and subcapsular fibrosis, diffuse (grade 2)
RA1857 (d1070)	Minimal mononuclear cell infiltrates (grade 1); Moderate capsular and subcapsular fibrosis, diffuse (grade 3)
RA1829 (d989)	Minimal mononuclear cell infiltrates (grade 1); Mild capsular and subcapsular fibrosis, diffuse (grade 2)
RA2334 (d989)	Minimal mononuclear cell infiltrates (grade 1); Minimal capsular and subcapsular fibrosis, diffuse (grade 1)
RA2125 (d284, necropsy)	Minimal mononuclear cell infiltrates (grade 1); Moderate cytoplasmic vacuolation (grade 3, consistent with incidental glycogen accumulation)
RA2343 (d955)	Minimal mononuclear cell infiltrates (grade 1); Mild capsular and subcapsular fibrosis, diffuse (grade 2)
RA3167 (d18)	Minimal mononuclear cell infiltrates (grade 1)
RA3167 (d127)	Minimal mononuclear cell infiltrates (grade 1)
RA3167 (d642)	Minimal mononuclear cell infiltrates (grade 1) Mild capsular fibrosis with minimal mesothelial cell hypertrophy, regional (grade 2)
RA3169 (d18)	Minimal mononuclear cell infiltrates (grade 1)
RA3169 (d127)	Minimal mononuclear cell infiltrates (grade 1)
RA3169 (d642)	Minimal mononuclear cell infiltrates (grade 1) Minimal capsular fibrosis with minimal mesothelial cell hypertrophy, regional (grade 1)
RA2083 (d18)	Minimal mononuclear cell infiltrates (grade 1)
RA2083 (d130)	Minimal mononuclear cell infiltrates (grade 1)
RA2083 (d531)	Mild mononuclear cell infiltrates (grade 2)
RA2396 (d18)	Mild mononuclear cell infiltrates (grade 2)
RA2396 (d130)	Minimal mononuclear cell infiltrates (grade 1)
RA2396 (d531)	Minimal mononuclear cell infiltrates (grade 1)