

Supplementary Table 1. sgRNA sequences for generation of *F9^{Δ130/Δ130}* rat.

gRNA ID	Score	Location of gRNA	Target Sequence (5'-3')	Protospacer Adaptor Motif
gRNA-rF9 1	59.9	chrX:+143131648	CGTCCAAAGAGATATAACTC	AGG
gRNA-rF9 2	64	chrX:-143131783	ATATTAATCAGGTATGATGT	GGG
Primers for genotyping in rats			Length (bp)	
ID	Sequence (5'-3')			
rF9-KO-ID -F	TCATTATTGACACTCAACCGAT		747	
rF9-KO-ID -R	GATGTGGACTCCTTTCCTTTT			

Supplementary Table 2. Primer sequences for targeted integration analysis in the intron 13 of rAlb gene.

ID	Sequence (5'-3')	Length (bp)
Primers for genotyping in rats		
P-L-F	TGTGACGCATACGAACATGG	527
P-L-R	CTCGTGCTTCTTCAAACTACACT	
P-R-F	GAAGATGCCAAACCAGGTCAAT	1300
P-R-R	TGGCTCTTGGCAAATGCTTATCG	
Hitom- <i>Alb-F9p</i> -KI-5-F	GGAGTGAGTACGGTGTGCTGCCCTGAAATGCTACTTTTTGAA	188
Hitom- <i>Alb-F9p</i> -KI-5-R	GAGTTGGATGCTGGATGGCAAGGTTTGGCCCTCCGC	
Hitom- <i>Alb-F9p</i> -KI-3-F	GGAGTGAGTACGGTGTGCCATTGTCTGAGTAGGTGTCATTCT	190
Hitom- <i>Alb-F9p</i> -KI-3-R	GAGTTGGATGCTGGATGGTCCATTGGAATTCTTAAAACAGGCT	

Supplementary Table 3. Potential off-target sites of sgAlb7 in rat genome

Match Name	Score	Spacer + PAM	coordinate	# of mismatch
sgAlb7	100	GAATCATTTACATTCCCTC CGG	chr14: 19084511	0
<i>F9_off_1</i>	1.531335456	AAGTCATTTACATTCTCTC AGG	chr13: 99525284	3
<i>F9_off_2</i>	1.445401982	TCACCATTTTACATTCCCTC CAG	chr17: 16290752	4
<i>F9_off_3</i>	1.240720994	GAAACATTTTACATTCACTC AGG	chr15: 81461028	3
<i>F9_off_4</i>	1.240350575	GAATCATCTGTCATTCCCTC AGG	chr9: 101189192	3
<i>F9_off_5</i>	1.19437619	CAATCATTTGACCTTCCCTC AGG	chr4: 242284766	3
<i>F9_off_6</i>	1.001311728	CTATGAATTCACATTCCCTC TGG	chr9: 97607931	4
<i>F9_off_7</i>	0.94878	GATTCTTTTCCCATTCCCTC CAG	chr11:1531776	3
<i>F9_off_8</i>	0.901804393	CAACCCTCTCACATTCCCTC AGG	chr11: 36751925	4
<i>F9_off_9</i>	0.894654977	CTAACATTTCCCATTCCCTC TAG	chr18: 58108152	4
<i>F9_off_10</i>	0.859852881	GATTCATTTAGATTCCCTA AGG	chr1: 167588939	3
<i>F9_off_11</i>	0.858813601	GTGTAATTTCTCATTCCCTC CAG	chr20: 36749557	4

Supplementary Table 4. Primers used for deep sequencing of on-target and potential off-target sites

Primers for off target sites analysis in rats	
ID	Sequence (5'-3')
sgAlb7-OT1-F	GGAGTGAGTACGGTGTGC CCAGTCCAGGTGCTGCTAAAT
sgAlb7-OT1-R	GAGTTGGATGCTGGATGG AACTTAAGCCCTCGGATGC
sgAlb7-OT2-F	GGAGTGAGTACGGTGTGC GCTAGGCAAGTGCTCTAC
sgAlb7-OT2-R	GAGTTGGATGCTGGATGG AAGGAAAATTGAAGTCACAA
sgAlb7-OT3-F	GGAGTGAGTACGGTGTGC TATCTGAACAATCACCTT
sgAlb7-OT3-R	GAGTTGGATGCTGGATGG CCTGAAAGTAGTCTGTAA
sgAlb7-OT4-F	GGAGTGAGTACGGTGTGC TGAGGAACCACAGTAATTAAGCCA
sgAlb7-OT4-R	GAGTTGGATGCTGGATGG CAGCGTTGAACACGTCCTTT
sgAlb7-OT5-F	GGAGTGAGTACGGTGTGC TCTGCAGGTTTGTAAGGGGAA
sgAlb7-OT5-R	GAGTTGGATGCTGGATGG CAGCCTGTCCTTGGATTTGC
sgAlb7-OT6-F	GGAGTGAGTACGGTGTGC ACAGAGTAGCTGTGAGGTCAA
sgAlb7-OT6-R	GAGTTGGATGCTGGATGG GTCTCCAGGCAGTTCTACCG
sgAlb7-OT7-F	GGAGTGAGTACGGTGTGC CCAAGGCTCAGTGAAATACTACAT
sgAlb7-OT7-R	GAGTTGGATGCTGGATGG AAATGTGGAATGCCTGTTATTCA
sgAlb7-OT8-F	GGAGTGAGTACGGTGTGC GTGACTGGAATATCCCCAGAA
sgAlb7-OT8-R	GAGTTGGATGCTGGATGG CCCATGGGCCTGCTTGTTTA
sgAlb7-OT9-F	GGAGTGAGTACGGTGTGC ACGTCATTTGCTGGGCTTAGA
sgAlb7-OT9-R	GAGTTGGATGCTGGATGG TCAGACCCCTACTTCTTGTACCT
sgAlb7-OT10-F	GGAGTGAGTACGGTGTGC CCGCCTGTCTAGTCCACTGT
sgAlb7-OT10-R	GAGTTGGATGCTGGATGG GTTGTCAAGCACCGCTTTCC
sgAlb7-OT11-F	GGAGTGAGTACGGTGTGC TCAGGAACAGGTGTGAGTCAA
sgAlb7-OT11-R	GAGTTGGATGCTGGATGG TGATGGTAGTGGTGACAGCC

Supplementary Table 5. The target sequences of sgRNAs and primer sequences for sequencing and T7E1 assay

gRNA ID	Score	Location of gRNA	Target Sequence (5'-3')	Protospacer Adaptor Motif
sgAlb4	28.47	chr14: +19177821	GTTCAAAAAGTAGCATTTCA	GGG
sgAlb6	24.55	chr14: +19177765	GAAATGATTCAGACATTGAA	TGG
sgAlb7	36.69	chr14: -19177741	GAATCATTTACATTCCCTC	CGG
sgAlb10	41.79	chr14: +19177733	ATAAACTGTTGTTAGGGCAC	CGG
sgAlb11	34.68	chr14: +19177727	AAAAGGATAAACTGTTGTTA	GGG
sgAlb15	32.48	chr14: -19177667	GCCTGTTTTAAGAATTCCAA	TGG
Primers for genotyping in rats			Length (bp)	
ID	Sequence (5'-3')			
rAlb-T7E1-F	TTGATTTGTGAGCCTTCC		487	
rAlb-T7E1-R	AGCCATCGGTGATGATAC			

Supplementary Table 6. Primers for qPCR and qRT-PCR.

ID	Sequence (5'-3')	Length (bp)
AAV-hF9p-qPCR-F	AAAATGGACTATCATATGCTTACCG	175
AAV-hF9p-qPCR-R	GACTCGGTGCCACTTTTCAA	
AAV-Cas9-qPCR-F	GGCGTGGTTTAGGTAGTGTG	229
AAV-Cas9-qPCR-R	GTCCCTCGTCCGTATTAAAGC	
<i>rβ-actin</i> -qPCR-F	TGCTATGTTGCCCTAGACTTCG	200
<i>rβ-actin</i> -qPCR-R	AGCATTGGTCACCTTTAGATGGA	
<i>rIL6</i> -RT-PCR-F	GTCTTCTGGAGTTCCGTTTC	224
<i>rIL6</i> -RT-PCR-R	GATGGTCTTGGTCCTTAGCC	
<i>rIL10</i> -RT-PCR-F	CAGGACTTTAAGGGTTACTTGG	220
<i>rIL10</i> -RT-PCR-R	CATTCTTCACCTGCTCCACT	
<i>rIFN-β1</i> -RT-PCR-F	TGCCATTCAAGTGATGCTCC	169
<i>rIFN-β1</i> -RT-PCR-R	CACCCAAGTCAATCTTTCCTCT	
<i>rAlb</i> -RT-PCR-F	GTGAGCGAGAAGGTCACCAA	198
<i>rAlb</i> -RT-PCR-R	TTTCACCAGCTCAGCGAGAG	
<i>rF9</i> -RT-PCR-F	TGCAGTTTTGAAGAAGCACGA	161
<i>rF9</i> -RT-PCR-R	CCAGCTTGGCACCAACATTC	
<i>hF9</i> -RT-PCR-F	TCCATGTTTAAATGGCGGCAG	177
<i>hF9</i> -RT-PCR-R	TCAGTACAGGAGCAAACCACC	
<i>rAlb</i> -hF9-RT-PCR-F	AGGCTGCCGACAAGGATAAC	203
<i>rAlb</i> -hF9-RT-PCR-R	GGTGATGAGGCCTGGTGATT	
<i>rβ-actin</i> -RT-PCR-F	GTACGCCAACACAGTGCTG	212
<i>rβ-actin</i> -RT-PCR-R	CGTCATACTCCTGCTTGCTG	

Supplementary Table 7. The primer and probe sequences of ddPCR.

Primer	Sequence (5'-3')	Length (bp)	Tm
P5F	AACTCATTTAAGCCTTGCCC	166	56.28
P5R	CTGTGGGAGGAAGAGAAGAG		56.65
P3F	GGAAGAGAATAGCAGGCATG	166	56.28
P3R	CCTGGCCTTGCTTAATTACA		56.65
Probe	Sequence (5'-3')		Tm
Ins5	Vic-TGTCTGAATCATTTTC-MGB		64
Ref3	cy5-ACACAACAGATGTCAGAGAGCC-BHQ3		64.8
Ref52	Fam-AGCTTTATCCTCTCTC-MGB		65
Ins32	AF594-AGCTCCGGGTCATTCTAACTAGT-BHQ2		65.1

Supplementary Table 8. Primers used for PEM-seq analysis

ID	Sequence	Note
LY069	5 Biotin -cttgtaaagcccagaatcgctaactc-3	Forward Biotin-primer
LY069a	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTAGCGcctgaaatgctacttttgaactg-3	Forward Red-primer
LY069b	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTGCC Tctgaaatgctacttttgaactg-3	
LY069c	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTAGGAcctgaaatgctacttttgaactg-3	
LY069d	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTTCAGcctgaaatgctacttttgaactg-3	
LY070	5 Biotin -ggtaaatgcatgtgcctggccttc-3	Reverse Biotin-primer
LY070a	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTAGCGcattggaattcttaaacaggctc-3	Reverse Red-primer
LY070b	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTGCC Tcattggaattcttaaacaggctc-3	
LY070c	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTAGGAcattggaattcttaaacaggctc-3	
LY070d	5 -ACTCTTTCCCTACACGACGCTCTTCCGATCTTCAGcattggaattcttaaacaggctc-3	
BA-up	5 PO4 -CCACGCGTGCTCTACANNNTNNNTNNNAGATCGGAAGAGCACACGT CTGAACTCCAGT-NH2 C7 3'	Bridge adapter
BA-down	5 -TG TAGAGCACGCGTGGNNNNNN-NH2 C7 3'	
I7-B01	5 -CAGAAGACGGCATAACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC-3	I7-index primer Nested PCR
P5-I5	5 -AATGATACGGCGACCACCGAGATCTACACAACTCTTCCCTACACGACGC-3	Tagged PCR Primers
P7-Tag	5 -CAAGCAGAAGACGGCATAACGAGAT-3	