

Supplementary Materials

Multiplex gene editing reduces oxalate production in primary hyperoxaluria type 1

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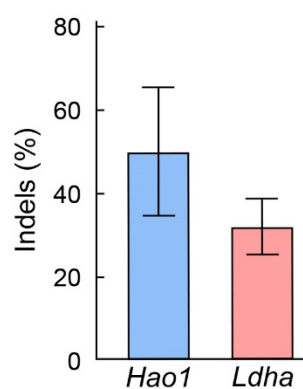
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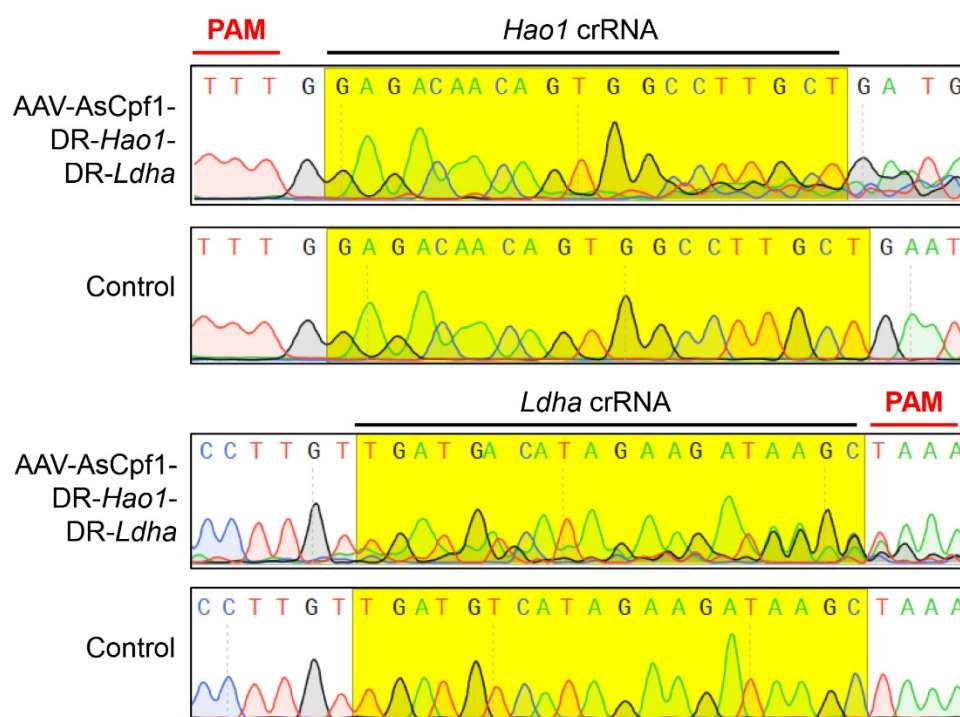
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A



B



Supplementary Figure S1. (A) Targeting efficiency of all-in-one AAV-AsCpf1 system in PC12 cells. (B) Representative sanger sequencing results of target sites in *Hao1* and *Ldha* genes in rats.

Hao1 PAM

↓

GGGGAATT**TTT**GAGACAACAGTGGCCTTGCTTGAATATGTGGCACAAGCCA WT

GGGGAATTTTGGAGACAACA-----GTGGCACAAGCCA -17

GGGGAATTTTGGAGACAACAG---CTTGCTGAATATGTGGCACAAGCCA -4

GGGGAATTTTGGAGACAACAGTGGCCT--TGAATATGTGGCACAAGCCA -3

GGGGAATTTTGGAGACAAC-----AGCCA -26

GGGGAATTTTGGAGACAACAGTG-----GAATATGTGGCACAAGCCA -8

GGGGAATTTTGGAGACAACAGTGGCCTTGCTGA--ATGTGGCACAAGCCA -2

GGGGAATTTTGGAGACAACAGTGGCC-----TATGTGGCACAAGCCA -8

GGGGAATTTTGGAGACAACAGTG----GCTGAATATGTGGCACAAGCCA -5

GGGGAATTTTGGAGACAACAG-----TGAATATGTGGCACAAGCCA -9

GGGGAATTTTGGAGACAACAG-----TGTGGCACAAGCCA -15

Ldha

↓

GGCTGATGAGCTTGCCCTTGTTGATGTCATAGAAGATAAGCTAAAGGGAG PAM WT

GGCTGATGAGCTTGCCCT-----TGTCATAGAAGATAAGCTAAAGGGAG -6

GGCTGATGAGCTTGCCCTTGT--TGTCATAGAAGATAAGCTAAAGGGAG -3

GGCTGATG-----AGCTAAAGGGAG -30

GGCT-----GATAAGCTAAAGGGAG -30

GGCTGATGAGCTTGCCCTTGTTG-----ATAGAAGATAAGCTAAAGGGAG -5

GGCTGATGAGCTTGCCCTTGTT-----ATAGAAGATAAGCTAAAGGGAG -6

GGCTGATGAGCTTGCCCTTGTTGA---CATAGAAGATAAGCTAAAGGGAG -3

GGCTGATGAGCTTGCCCTTGTTG-----TAGAAGATAAGCTAAAGGGAG -6

GGCTGATGAGCTTGCCCTT-----TGTCATAGAAGATAAGCTAAAGGGAG -5

GGCTGATGAGCTT-----GCTAAAGGGAG -26

Supplementary Figure S2. Sequencing of *Hao1* and *Ldha* loci after AsCpf1-mediated gene disruption. Top 10 most frequent NHEJ products are displayed in descending frequency. Sequences of crRNA and PAM are marked in blue and red, respectively. DNA break sites are indicated with arrows above sequences.

Supplemental Table S1. Primers and crRNAs used in this study.

Primers for PCR amplification			
Primer ID	Primer sequence	Primer ID	Primer sequence
Hitom- <i>Hao1</i> -on-F	tgaaccaacgatttggcattt	Hitom- <i>Hao1</i> -on-R	cctttacaacaatgggcagtgag
Hitom- <i>Ldha</i> -on-F	tgctggagacaatttttgggtgc	Hitom- <i>Ldha</i> -on-R	cctttacaacaatgggcagtgag
Hitom- <i>Hao1</i> -OT1-F	ggagtgagtacggtgtgctgggtcgtgctcctgaaaaa	Hitom- <i>Hao1</i> -OT1-R	gagttggatgctggatggtgttctgacatacctcaacaaga
Hitom- <i>Hao1</i> -OT2-F	ggagtgagtacggtgtgctctccttcgttatgtgtgagcc	Hitom- <i>Hao1</i> -OT2-R	gagttggatgctggatgggggaccaacccctggaatga
Hitom- <i>Hao1</i> -OT3-F	ggagtgagtacggtgtgcatttggtatgtgctctgtggtaa	Hitom- <i>Hao1</i> -OT3-R	gagttggatgctggatggtatgagataaggggtggagcaagaa
Hitom- <i>Hao1</i> -OT4-F	ggagtgagtacggtgtgctgtgcctcaagtgtggtgtt	Hitom- <i>Hao1</i> -OT4-R	gagttggatgctggatgggaggtgtctgcggaaggaa
Hitom- <i>Hao1</i> -OT5-F	ggagtgagtacggtgtgctgttctccactgagccat	Hitom- <i>Hao1</i> -OT5-R	gagttggatgctggatgggtgcctgctggaatcgaatgt
Hitom- <i>Hao1</i> -OT6-F	ggagtgagtacggtgtgcggcctcatccttactctgcc	Hitom- <i>Hao1</i> -OT6-R	gagttggatgctggatgggtggtgattcagaccatttgc
Hitom- <i>Hao1</i> -OT7-F	ggagtgagtacggtgtgcgctgaggtgttctccaggt	Hitom- <i>Hao1</i> -OT7-R	gagttggatgctggatggggctttaccacaggaggagg
Hitom- <i>Hao1</i> -OT8-F	ggagtgagtacggtgtgctcgaaacaggtcggttccag	Hitom- <i>Hao1</i> -OT8-R	gagttggatgctggatgggaggtgctctctggcgtag
Hitom- <i>Hao1</i> -OT9-F	ggagtgagtacggtgtgctattacttcaatctgtttgcctgc	Hitom- <i>Hao1</i> -OT9-R	gagttggatgctggatgggaacaaaacattacacagcagaac
Hitom- <i>Hao1</i> -OT10-F	ggagtgagtacggtgtgcgcagctgcagtcactctcta	Hitom- <i>Hao1</i> -OT10-R	gagttggatgctggatggatggggaacgcactggatt
Hitom- <i>Ldha</i> -OT1-F	ggagtgagtacggtgtgcacaggtccccagaacaaga	Hitom- <i>Ldha</i> -OT1-R	gagttggatgctggatggaaggctgccatgctgaagat
Hitom- <i>Ldha</i> -OT2-F	ggagtgagtacggtgtgcgaccagcttgagtttgcag	Hitom- <i>Ldha</i> -OT2-R	gagttggatgctggatggaatgaaggacttgctgatga
Hitom- <i>Ldha</i> -OT3-F	ggagtgagtacggtgtgcggctgtgccatccgtatctt	Hitom- <i>Ldha</i> -OT3-R	gagttggatgctggatgggccgtgataatgaccagcgt
Hitom- <i>Ldha</i> -OT4-F	ggagtgagtacggtgtgcaccagcttgagtttgcattc	Hitom- <i>Ldha</i> -OT4-R	gagttggatgctggatggtcagttaccttctggccgatg
Hitom- <i>Ldha</i> -OT5-F	ggagtgagtacggtgtgctaaggacttgccgatgagc	Hitom- <i>Ldha</i> -OT5-R	gagttggatgctggatggaccagcttgagtttgcagt
Hitom- <i>Ldha</i> -OT6-F	ggagtgagtacggtgtgcaccagcttgagtttgcagt	Hitom- <i>Ldha</i> -OT6-R	gagttggatgctggatggttggcatggctgtgtcatca
Hitom- <i>Ldha</i> -OT7-F	ggagtgagtacggtgtgctgttgaggtgatgtttggc	Hitom- <i>Ldha</i> -OT7-R	gagttggatgctggatggcttgagtttctgtcacgc
Hitom- <i>Ldha</i> -OT8-F	ggagtgagtacggtgtgcaatgaaggacttgctgatga	Hitom- <i>Ldha</i> -OT8-R	gagttggatgctggatggagctctctcccttctgtga

<i>Hao1</i> -qPCR-F	atgtggcacaagccatagacc			<i>Hao1</i> -qPCR-R	ctgggcatcatcac
<i>Ldha</i> -qPCR-F	tcttgacctacgtggcttgg			<i>Ldha</i> -qPCR-R	cgggttcagagacttcaggg
Rat-actin-F	gggaaatcgtgcgtgacatt			Rat-actin-R	gcggcagtggccatctc
<i>Hao1</i> -Exon1-F	cggaattgtccgggcataga			<i>Hao1</i> -Exon1-R	tcacttttgcacccatcca
<i>Hao1</i> -Exon2-F	tcacaaggagtacaagcagca			<i>Hao1</i> -Exon2-R	aaagcatcatgggaagggca
<i>Hao1</i> -Exon3-F	acagaacaaatcatttccagtgcc			<i>Hao1</i> -Exon3-R	ctagtcttagcagggggagc
<i>Hao1</i> -Exon4-F	aatgatagggcatctgccatgaa			<i>Hao1</i> -Exon4-R	tgcagacaaccaacccgtaag
<i>Ldha</i> -Exon3/4-F	gcaaactccaagctgggtcat			<i>Ldha</i> -Exon3/4-R	acagaacaaatcatttccagtgcc
<i>Ldha</i> -Exon2-F	ccatggcaagtctctgagaaga			<i>Ldha</i> -Exon2-R	actcacaccctaattcggaga
SgRNAs					
Target gene	Target site	nuclease		crRNA Sequence	PAM
<i>Hao1</i>	Exon1	AsCpf1	crRNA1	GCTGATAATATCAGAGCATT	TTTG
<i>Hao1</i>	Exon2	AsCpf1	crRNA2	GGACAGAGAGTGAGCATGCC	TTTA
<i>Hao1</i>	Exon3	AsCpf1	crRNA3	TAGATGTAGAGTTGCATCCA	TTTG
<i>Hao1</i>	Exon3	AsCpf1	crRNA4	TGACTGTGGACACCCCTTAC	TTTG
<i>Hao1</i>	Exon4	AsCpf1	crRNA5	AAACCAACGATTTGGCATT	TTTG
<i>Hao1</i>	Exon4	AsCpf1	crRNA6	CAACAATGGGCAGTGAGGTC	TTTA
<i>Hao1</i>	Exon4	AsCpf1	crRNA7	ATATCATCCCAGCTGAGAGA	TTTA
<i>Hao1</i>	Exon4	AsCpf1	crRNA8	GAGACAACAGTGGCCTTGCT	TTTG
<i>Ldha</i>	Exon2	AsCpf1	crRNA1	GCTTATCTTCTATGACATCA	TTTA
<i>Ldha</i>	Exon3	AsCpf1	crRNA2	GCTGGACCAAATTGAGCCGG	TTTC
<i>Ldha</i>	Exon3	AsCpf1	crRNA3	GAATGATGAACCTGAAGATG	TTTG
<i>Ldha</i>	Exon3	AsCpf1	crRNA4	ACAACATTTGGAATGATGAA	TTTC
<i>Ldha</i>	Exon3	AsCpf1	crRNA5	CACTGTGGACTGTATTTAC	TTTG
<i>Ldha</i>	Exon3	AsCpf1	crRNA6	AGACGATGAGCAGTTTGCAC	TTTG

<i>Ldha</i>	Exon4	AsCpfI	crRNA7	GGGAAGCCGCTGATCTTCCA	TTTG
<i>Ldha</i>	Exon4	AsCpfI	crRNA8	TCCCATCAGGTAACGGAACC	TTTC

Supplemental Table S2. Potential off-target sites for *Hao1* and *Ldha* crRNA.

Potential off-target sites predicted by Benchling software for the on-target sgRNA. Mismatched base pairs are labeled in red.

Target ID	Sequence	PAM	Chromosome	Position	Direction	Mismatches
<i>Hao1_off_1</i>	AAGACAACAGTGGCCATGAT	TTTG	chr14	61292163	+	4
<i>Hao1_off_2</i>	GAGAGAACAGCGGCCCTTCCT	TTTG	chr3	175260445	+	3
<i>Hao1_off_3</i>	GAGACAGCAGTGGCCTTGAA	TTTT	chr5	81209829	+	3
<i>Hao1_off_4</i>	GTGACATCAGTGACCTTGCT	TTTG	chr5	165157770	-	4
<i>Hao1_off_5</i>	GTGAGAACAGTGGCCTTGAA	TTTA	chr2	60146773	-	3
<i>Hao1_off_6</i>	CAGACAAAGTGGTCTTGTT	TTTG	chr20	9145890	-	5
<i>Hao1_off_7</i>	AAGACAACAGTGACCTCGTT	TTTG	chr1	132699489	-	4
<i>Hao1_off_8</i>	GAGAACACAGTGGCCTTCC	TTTG	chr18	26720546	-	4
<i>Hao1_off_9</i>	GACCCAACAGTGGCCATTCT	TTTG	chr2	126854866	+	4
<i>Hao1_off_10</i>	CAGACAGCAGTGTCCTTCCT	TTTG	chr8	75662648	-	4
<i>Ldha_off_1</i>	GCTTATCTTCTATGACATCA	TTTA	chr8	33075366	-	0
<i>Ldha_off_2</i>	GTTTATCTTCTATGACATCA	TTTA	chr7	14762784		1
<i>Ldha_off_3</i>	GCTTGTCTTCCATGACATCA	TTTA	chr1	166727858	+	2
<i>Ldha_off_4</i>	GCTTGTCTTCCATGACATCA	TTTA	chr20	50599058	+	2
<i>Ldha_off_5</i>	GCTTGTCTTCCATGACATCA	TTTA	chr20	37910730	-	2
<i>Ldha_off_6</i>	GCTTGTCTTCCATGACATCA	TTTA	chrX	63692362	+	2
<i>Ldha_off_7</i>	GCTTGTCTTCCATGACATCA	TTTA	chr5	55829896	-	2
<i>Ldha_off_8</i>	GCTTGTCTTCCATGACATCA	TTTA	chr19	17968242	-	2