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Supplemental Information

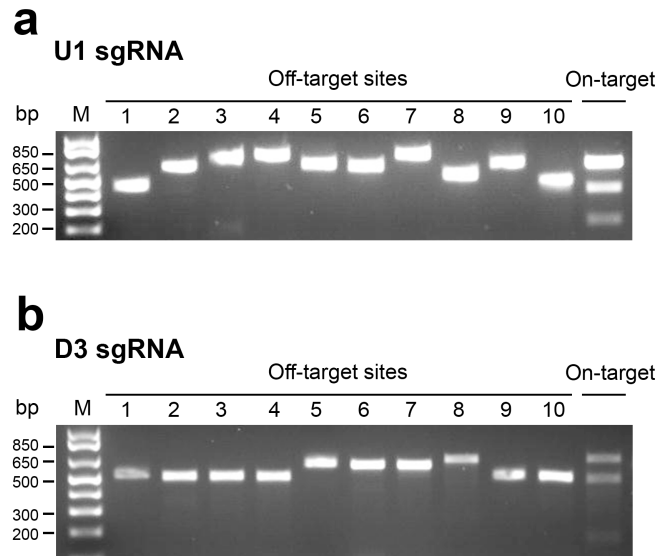
CRISPR/Cas9-Mediated Genome Editing

as a Therapeutic Approach for Leber

Congenital Amaurosis 10

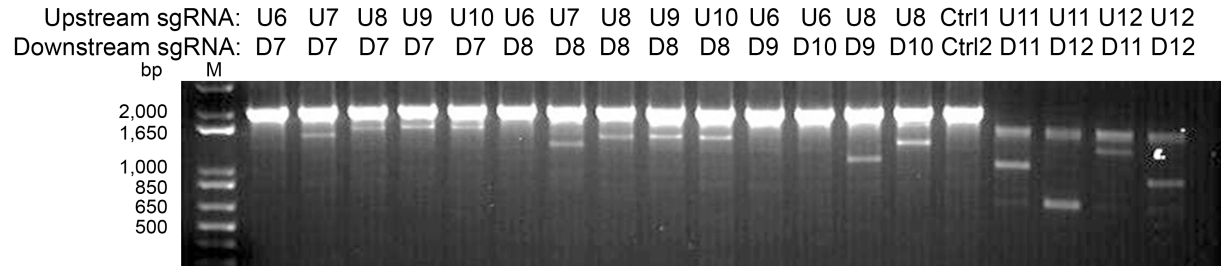
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Supplementary Figure S1



Supplementary Figure S1 T7E1 assay for ten potential off-target sites for the U1 and D3 sgRNAs. Human 293FT cells were transfected with either pSpCas9(BBU)-U1 sgRNA or pSpCas9(BBD)-D3 sgRNA. The DNA fragment including the on- and off-target sequence was amplified using primers listed in Supplementary Table S5, and then subjected to T7E1 digestion. In the on-target lanes, the two lower bands correspond to DNA bands cleaved by mismatch-sensitive T7E1. M, 1-kb DNA ladder.

Supplementary Figure S2



Supplementary Figure S2 Screening of sgRNA pairs for targeted genomic deletion of intron 25 of the mouse *Cep290* gene. Mouse Neuro-2a cells were transfected with the indicated pairs of sgRNA and SpCas9 using NeuroMag (OZ Biosciences). Primers were designed to bind outside of the regions to be deleted. The upper bands represent PCR products amplified from intron 25 of wild-type *Cep290*, whereas the lower bands represent PCR products amplified from the *Cep290* allele following genomic deletion. M, 1-kb DNA ladder.

Supplementary Table S1 – Protospacer sequences for the sgRNAs used in the current study

sgRNA name	Protospacer sequence
sgRNA for HDR	AAGACACTGCCAATAGGGAT
U1	GGCGGGTGGATCACGAGTTC
D1	AAAGCTACCGGTTACCTGAA
D2	TCATTCTTGTGGCAGTAAGG
D3	GGAGTCACATGGGAGTCACA
aU1	TTTAACGTTATCATTTCCTCA
aU2	AGTTTCATTCTGTCACCCAGG
aU3	AAAAATTAGCCGGGCATGATG
aD1	TGTAAGACTGGAGATAGAGAC
aD2	CTTTTGACAGTTTTTAAGGCG
Control sgRNA 1	GCACTACCAGAGCTAACTCA
Control sgRNA 2	GTGCGAATACGCCACGCGAT
U6	CTTAAAATCTCTAATACTGA
U7	AGAATAGCCTTGAAATACTG
U8	TATACAGTCTAGGCTAGCAA
U9	GCCTAGACTGTATACGAATG
U10	GCCTCATTCGTATACAGTCT
U11	GCATAAGGACTAAAGACCTA
U12	ATAACAACCTTCCATCCAAA
D7	GCTCCTACTTGCCTGCATCG
D8	ATTGCTTTCAGTACAGGCGA
D9	AACACACATCCACATCCACA
D10	GTTAGGTTAGGAAGAATCGA
D11	GGTAGTGGTTGAACTCACAA
D12	ACTGAAGTAATCTTTCACAG

Supplementary Table S2 – Primer sequences

Name	Sequence
<i>CEP290</i> Intron 26_F	GGTCCCTGGCTTTTGTTCCT
<i>CEP290</i> Intron 26_R	CAGGAGGCTGAGGGTGTTTT
<i>CEP290</i> Intron 26 sequencing primer	AGTAGAGATGGGGTTTCACC
Wild-type <i>CEP290</i> _F	TGACTGCTAAGTACAGGGACATCTTG
Wild-type <i>CEP290</i> _R	AGGAGATGTTTTCACACTCCAGGT
Mutant <i>CEP290</i> _F	CTGGCCCCAGTTGTAATTTGTGA
Mutant <i>CEP290</i> _R	CTGTTCCCAGGCTTGTTCAATAGT
<i>PPIA</i> _F	TTCATCTGCACTGCCAAGAC
<i>PPIA</i> _R	TCGAGTTGTCCACAGTCAGC
BbsI mutation_Top	GGGAGGATTGGGAAGAGAATAGCAGGCATGCTG
BbsI mutation_Bottom	CAGCATGCCTGCTATTCTCTTCCCAATCCTCCC
pSpCas9(BBD)_F	ATAACATGTGGTCTCACTCTAGAGGCATGTGAGGGCC TATTCCC
pSpCas9(BBD)_R	TATGGTACCGGTCTCATAGAGCCATTTGTCTGCAGA
m <i>Cep290</i> Intron 25_F	CCCCTCGCCTGTACTGAAAG
m <i>Cep290</i> Intron 25_R	GCACATCATCTGAGGCAGGT
BGH_P	6-FAM-TCCCCCGTGCCTTCCTTGACC-TAMRA
BGH_F	TCTAGTTGCCAGCCATCTGTTGT
BGH_R	TGGGAGTGGCACCTTCCA
SV40_P	6-FAM- AGCATTTTTTTCCTGCATTCTAGTTGTGGTTTGTGTC- TAMRA
SV40_F	AGCAATAGCATCACAAATTTACAA
SV40_R	GCAGACATGATAAGATACATTGATGAGTT

Supplementary Table S3 – Other sequences

ssODN for HDR with c.2991+1655A>G (LCA10 mutation) and c.2991+1666C>G (PAM mutation) underlined:

CCACCCGCTCGGCCTCCTAAAGTGCTGGGATTACAGATGTGAGCCACCGCACCT
GGCCCCAGTTGTAATTGTGAGTATCTCATACGTATCCCTATTGGCAGTGTCTTAGT
TTTATTTTTTATTATCTTTATTGTGGCAGCCATTATTCCTGTCTCTATCTC

U6 promoter-BbsI:BbsI-sgRNA scaffold for constructing pSpCas9(BB):

CACATGTGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGG
CTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACA
AAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTA
TGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCTT
GGCTTTATATATCTTGTGGAAAGGACGAAACACCGGGTCTTCGAGAAGACCTGTT
TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGCACCGAGTCGGTGCTTTTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAAT
AAGGCTAGTCCGTTTTTAGCGCGTGCGCCAATTCTGCAGACAAATGGCTCTAGAG
GTACCC

minCMV promoter sequence:

GACTCACGGGGATTTCCAAGTCTCCACCCCATGACGTCAATGGGAGTTTGTTTTG
GCACCAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACG
CAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAG
TGAACCGTCAGATC

U1 sgRNA recognition sequence (U1T):

GGCGGGTGGATCACGAGTTCAGG

D3 sgRNA recognition sequence (D3T):

GGAGTCACATGGGAGTCACAGGG

Supplementary Table S4 – Top 10 off-target cleavage sites for the U1 and D3 sgRNAs

	#	Sequence	PAM	Score	Chromosome	Strand	Position
U1 sgRNA	ON	GGCGGGTGGATCACGAGTTC	AGG	100.00	chr12	1	88101266
	OFF1	GGTGGGTGGATCATGAGTTC	AGG	1.37	chr16	1	81828887
	OFF2	GCTGGGTAGACCACGAGTTC	TAG	0.88	chr3	1	127784508
	OFF3	GGTGAGTGGATCACGAGGTC	AGG	0.74	chr1	1	13910293
	OFF4	GGCTGGTGGTTCACGAGGTC	AGG	0.66	chr22	1	48558795
	OFF5	GGTGGCTGGGTCACGAGTTG	GGG	0.47	chr17	-1	79716222
	OFF6	GATGTGTGGATCACAAGTTC	TAG	0.46	chr5	1	74778050
	OFF7	GACAGGTGGGTCACAAGTTC	CAG	0.45	chr2	-1	96950573
	OFF8	GGGTGGTGGTTCACAAGTTC	TAG	0.43	chr10	1	23896895
	OFF9	TGCGGCTGGCTCCCGAGTTC	TGG	0.37	chr3	-1	37641510
	OFF10	GGCAGGTGGATCACAAGTAC	AGG	0.32	chr7	1	64273295
D3 sgRNA	ON	GGAGTCACATGGGAGTCACA	GGG	100.00	chr12	1	88101036
	OFF1	GCAGTGACATGGGAGTCACA	TGG	3.64	chr14	-1	97826379
	OFF2	GCAGCCACAGGGGAGTCACA	CAG	2.64	chr16	1	5027699
	OFF3	GGAGACATAGGGGAGTCACA	GGG	2.38	chr2	1	240564643
	OFF4	GCAGGCTCATGGGAGTCACA	AAG	1.77	chr9	1	97605919
	OFF5	TTAGTCACATGGGAGTCAGA	CAG	1.41	chr5	1	66775081
	OFF6	GTCCCACATGGGAGTCACA	GAG	1.33	chr21	-1	45971854
	OFF7	TTAGTCTCAAGGGAGTCACA	TGG	1.01	chr2	-1	98389517
	OFF8	GAACTCAACTGGGAGTCACA	AAG	0.93	chr4	1	116584817
	OFF9	CCAGTGAGATGGGAGTCACA	GAG	0.92	chr2	1	180622519
	OFF10	GGATTCTGAAGGGAGTCACA	AAG	0.91	chr6	1	52106176

Supplementary Table S5 – Primer sequences used to amplify the on- and off-target sites for the U1 and D3 sgRNAs

	#	Forward primer	Reverse primer
U1 sgRNA	ON	GGAGTCACATGGGAGTCACAG	GCACGATCTCGGCTTAACGC
	OFF1	AGAGGGGGCAGTCCTTGTC	CAGTTTTGATCAGCGAGTCCTTGC
	OFF2	GAAAAGCTCCCCAGTGGCTC	GCAATGCGAAGTTTCTGGAAGGC
	OFF3	GGGCAGAAGGCAAATGGGGA	CAAATGTTGAGGCTCATGGAGGC
	OFF4	CCCTCATGTAGCAGTGAGAGGGA	CGCTCTTTAATGTCTGTGCGACC
	OFF5	CCAGGAGGCCCATGGTAAGA	GACAACGAGGATTTATACCCAGC
	OFF6	ACACATACAGACGCTTAGGAAAAGAAA GGC	AGCAGACTCCAGAACACTACCTAC
	OFF7	GCCCCACTGTTCCCCATTAC	TCACCAGCGATCACAGGTCC
	OFF8	CAGCCAAACTTGGATGACACTTGC	GCTGACAAGGGCATTTCGAATCTG
	OFF9	GTGAGGGATCTAGATTGTGCCG	AGAGCTGGCCACGGGAAAAG
	OFF10	TAAGAGGCATACACTGTCCACAGG	GTTGGCTACGGTTAGGTGAGTAAG
D3 sgRNA	ON	CCTGGCCCCAGTTGTAATTGTG	GCTTGAACCTCTGTGCCAAACAGG
	OFF1	GCAACCTTGCTGAAATCCTCTTATGCAT TTTGT	CTTTGATGCCGAAACTAGAAAAGAAA CTGTGAG
	OFF2	TGGACTAGCCCCTGAACGTG	TGTGGCCCCTCTAACTGCAG
	OFF3	CTGCGCATCAGACCACATGGA	CTATGTGACCTTAAGTTGGGGTGC
	OFF4	GAATTGGTAGATCTCCCCATTCCC	TGGGGCTAAGCAGCCTTGG
	OFF5	GGTTATGTGCCTAGGCGTTATCAG	GGTGTAGGGCTGGAATTCATCCTT
	OFF6	CTGCGTCTCGCTCCAGTTT	TGACCACACCGTGGCTCAGA
	OFF7	ACCAGTTATAGAAGACAGGCAGGG	GGTTCTGGAGGGAATACTGTGG
	OFF8	CACAGGTCTCCTAGATTGCTGATG	CTTGCACATCACATCATAAAGTGAGCA CTG
	OFF9	GACAAACCTTGTATCCGGTGAGC	CTTCAGGCAGACAACACTTCTACC
	OFF10	CCTTCTCAGCTATGTGGAGGAAAC	AGGCAGTTCATGTGCAATGGGC