Sense and pCMV Δ with sgRNA A &	В	
Forward sequencing primer	Ds	DSB by sgRNA A
	CACCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	асселентестсерселенталиансел
AAGTTCACCCTCGCGCACTACTTGAAG	CTCCTGCCGCCGCACCGCTGGCAC	TGGGTCCTGAGGAGGGACGTCCATACAATTATACCT
		PAM sgRNA A
Intron	Se	equence with branch site
CTAAAGGAGGCTTTTCTCAGGTCGACT	CTAGACGCGTAGGATCCCCCGGGT	ACCGAGCTCGAATTTTTACTAACAAATGGTATTATT
GATTTCCTCCGAAAAGAGTCCAGCTGA	GATC TGCGCATCCTAGGGGGGCCCA	TGGCTCGAGCTTAAAAATGATTGTTTACCATAATAA
DSB by sgRNA B	D. J	
татесасассассестсеттеатетас		י ח חר 3/
ATAGGTGTCCTGCCGACGAAGTAGATG	TTCCACTTCAAGTAGCCGCACTTC	AAGG 5'
PAM sgRNA B	·	
	Reverse sequencing p	primer
Branch Δ with sgRNA A & B		
	Ds	DSB by sgRNA A
TTCAAGTGGGAGCGCGTGATGAACTTC	GAGGACGGCGGCGTGGCGACCGTG	
AAGTTCACCCTCGCGCACTACTTGAAG	DEP by coDNA P	
Introp	DSB by Sgrina B	Bod
CTAAAGGAGGCTTTTTCTCAGGTCGACT	CTAGTTATCCACAGGACGGCTGCT	KEG TCATCTACAAGGTGAAGTTCATCGGCGTGAACTTCC
GATTTCCTCCGAAAAGAGTCCAGCTGA	GATCAATA <mark>GGT</mark> GTCCTGCCGACGA	AGTAGATGTTCCACTTCAAGTAGCCGCACTTGAAGG
	PAM sgRNA	B
Sense, Branch Δ and pCMV Δ with se	zRNA E & J	
		DSP by caPNA F
Forward sequencing primer		DSB DY SERIA E
CTTCAAGGTGCGCATGGAGGGCACCGT	GAACGGCCACGAGTTCGAGATCGA	GGGCGAGGGCGAGGGCCGCCCTACGAGGGCCACAA
GAAGTTCCACGCGTACCTCCCGTGGCA	CTTGCCGGTGCTCAAGCTCTAGCT	CCCGCTCCCGCTCCCGGCGGGGATGCTCCCGGTGTT
		PAM sgRNA E
		_
CACCGTGAAGCTGAAGGTGACCAAGGG	CGGCCCCTGCCCTTCGCCTGGGA	CATCCTGTCCCCCAGTTCCAGTACGGCTCCAAGGT
GTGGCACTTCGACTTCCACTGGTTCCC	GCCGGGGGACGGGAAGCGGACCCT	GTAGGACAGGGGGGGTCAAGGTCATGCCGAGGTTCCA
DSB by sgRNA J		
	003 003 03 3 03 3 00000000000000000000	
	CGACTACAAGAAGCTGTCCTTCCC	
	NA I	GUICUGAAGIICACUCICGCGCACIA 5
PAIVI SgR	INPA J	Devenue conversing primer

Reverse sequencing primer

Antisense with sgRNA C & C)		DSB by	SORNA D
Forward sequencing primer	Ds			
TTCAAGTGGGAGCGCGTGATG	AACTTCGAGGACGGC	GGCGTGGCGACCGTGACCO	CAGGACTCCTCC	CTGTGGATAAATAATACCATT
AAGTTCACCCTCGCGCACTAC	TTGAAGCTCCTGCCG	CCGCACCGCTGGCACTGG	GTCCTGAGGAGG	GACACCTATTTATTATGGTAA
TGTTAGTAAAAATTCGAGCTC	GGTACCCGGGGGGATC	CTACGCGTTAGGGATAAC	AGGGTAATACGC	GTCTAGAGTCGACCTGAGAAA
ACAATCATTTTTAAGCTCGAG	CCATGGGCCCCCTAG	GATGCGCAATCCCTATTG	TCCCATTATGCG	CAGATCTCAGCTGGACTCTTT
	DSB by sgRN	IA C northi		
	sgRNA C P.			
TCGCAGGAAATCAGGTATAAT	TGTATGGACGTCCTG	CCGACGAAGTAGATGTTC		CCGCACTTGAAGG 5'
5'	-solice site	Pod		
5		Reu	Reverse s	sequencing primer
5'-Splicing∆ with sgRNA C' &	& D		DSB by	serna d
Forward sequencing primer	Ds		sgRNA D	
TTCAAGTGGGAGCGCGTGATG	AACTTCGAGGACGGC	GGCGTGGCGACCGTGACCO	CAGGACTCCTCC	CTGTGGATAAATAATACCATT
AAGTTCACCCTCGCGCACTAC	TTGAAGCTCCTGCCG	CCGCACCGCTGGCACTGG	GTCCTGAGGAGG	GACACCTATTTATTATGGTAA
ͲϹͲͲϪϹͲϪϪϪϪϪͲͲϹϹϪϹϹͲϹ	CCTACCCCCCCATC	СТАССССТТАСССАТАСС		стстасастссасстсасааа
ACAATCATTTTTAAGCTCGAG	CCATGGGCCCCCTAG	GATGCGCAATCCCTATTG	ICCCATTATGCG	CAGATCTCAGCTGGACTCTTT
ſ	DSB by <mark>sgRNA C'</mark>	Intron		
sgRNA C	<u>PAM</u>			
AGCCTCCTTTAGTCCATATTA	CTGCAGGACGGCTGC	TTCATCTACAAGGTGAAG	TTCATCGGCGTG	AACTTCC 3'
TUGGAGGAAATUAGGTATAAT	GAUGTCUTGUUGAUG	AAGTAGATGTTCCACTTC	AAGTAGCCGCAC	TTGAAGG D'
	I	Red	everse sequenc	ing primer

Supplementary Figure 1 | DNA sequence of the *DsRed* loci of the sense and antisense constructs.

Blue sequence, exons of the *DsRed* gene; green sequence, intron; underlined green sequence, canonical GT- and -AG splice sites of the intron; underlined dark green sequence, intron sequence containing the branch site (Sense and pCMV Δ) or the 5'-splice site (Antisense). Black arrows, sequencing primers; yellow highlighted sequence, PAM site; orange highlighted sequence, sequence of sgRNA A, C or C'; purple highlighted sequence, sequence of sgRNA B or D; brown highlighted sequence, sequence of sgRNA J. Vertical black bar, site of DSB by sgRNA.

а

Plasmid	spliced transcript frequency	non-spliced transcript frequency	alt-spliced transcript frequency	non-canonical alt-spliced frequency	unaligned frequency
Sense	0.96	0.0048	0.00043	0.0077	0.026
Branch∆	0.0034	0.95	0.000015	0.010	0.035
pCMVΔ	0.98	0.0043	0.000076	0.0032	0.014
Antisense	0.16	0.70	0.092	0.0068	0.043
5'-Splicing∆	0.0042	0.92	0.033	0.0075	0.034

b





Plasmid	spliced transcript frequency	non-spliced transcript frequency	alt-spliced transcript frequency	non-canonical alt-spliced frequency	unaligned frequency
Sense	0.99	0.010	0.000	0.000	0.0034
Branch∆	0.0021	0.97	0.015	0.0014	0.014

Supplementary Figure 2 | The RNA transcribed from the Branch Δ and the 5'-Splicing Δ constructs has no intron splicing, and the one transcribed from the pCMV Δ is in low amount.

a. Table of RNA-sequencing results of *DsRed* transcripts from the Sense, Branch Δ , and pCMV Δ , as well as the Antisense and the 5'-Splicing∆ constructs in HEK293T cells. RNA-sequencing reads for each transcript of the different constructs were categorized after alignment to the corresponding DNA sequence (details in Methods). Spliced transcript, a transcript in which the intron was spliced out from the canonical GT---AG-splice sites (see Supplementary Figure 1); alt-spliced transcript, a transcript in which splicing happened at alternative GT---AG sites; non-canonical alternative splicing, a transcript sequence that had splicing at sites different from the GT---AG sites. Splicing frequencies were calculated by dividing the number of the spliced-transcript reads by the total number of reads within each RNA-sequencing library, N=1. b, Results of RT-qPCR of the DsRed transcripts generated from the sense and antisense constructs. Shown on the left is the relative amount of the DsRed transcripts from the Branch Δ (green bar) and the pCMV Δ (yellow bar) constructs compared to the amount of the Sense (red bar) transcripts. Shown on the right is the relative amount of the DsRed transcripts from the 5'-Splicing Δ construct (green bar) compared to the amount of the Antisense construct (red bar). (Left) Plotted data are the mean fold change \pm s.d. of the 4 biological replicates with the individual values shown as dots; N=4. The mean value is shown above each bar. (Right) N=1. c, Table of RNAsequencing results of *DsRed* transcripts from the Sense and Branch∆ constructs in HEK293 cells, N=1. Source data are provided as a Source Data file.



2-DSB	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	Avg.
Sense	0.890	0.891	0.883	0.879	0.891	0.882	0.884	0.885	0.876	0.882	0.889	0.885
Branch∆	0.884	0.878	0.879	0.876	0.870	0.851	0.880	0.879	0.873	0.873		0.874



DSB, sgRNA	1st	2nd	3rd	4th	Average
1-DSB, sgRNA E	0.933	0.895	0.899		0.909
1-DSB, sgRNA J	0.844	0.844	0.844	0.857	0.847
2-DSB, sgRNA E+J	0.816	0.838	0.832		0.829



C)							
sgRNA D		1st	2nd	3r	ď	4th	Average
Antisense	0.836		0.824	0.8	46	0.849	0.839
5'-Splicing∆	ng∆ 0.864		0.861	0.8	55	0.861	0.860
2-DSB	1st	2nd	3rd	4th	5th	6th	Avg.
Antisense	0.835	0.822	0.791	0.844	0.824	0.843	0.826
5'-Splicing∆	0.835	0.853	0.846	0.860	0.854		0.849

Supplementary Figure 3 | Constructs with splicing have similar cleavage efficiency by Cas9 to those without splicing.

Scheme of PCR fragments for **a**, **b**, the Sense or pCMV Δ construct, and **c**, the Antisense construct used for the *in vitro*-cleavage assay. Cas9 cleavage was done using sgRNA A, B, A and B, E, J and E and J for the sense (Sense/pCMV Δ and Branch Δ) constructs, and by using sgRNA C/C', D or C/C' and D for the antisense (Antisense and 5'-Splicing Δ) constructs. The formulas used to calculate the DSB efficiencies from the molarity of each DNA fragment detected by the Bioanalyzer following cleavage by Cas9 with sgRNA A, B, or A and B for the sense constructs (a), sgRNA E, J or E and J for the sense constructs (b) or sgRNA C/C', D, or C/C' and D for the antisense constructs (c) are shown. The bp sizes in the formulas are those for the Sense/pCMV Δ and Branch Δ (in parenthesis when different) in (a, b), and those for the sense constructs (a, b), and the antisense constructs (c) are shown in the tables underneath the construct schemes.





С



b

Supplementary Figure 4 | Characterization of RNase H2A KO in HEK-293T cells.

a, Mutated sequences of the *RNASEH2A* alleles from the RNase H2A KO HEK-293T cells. **b**, Result of Western blot for RNase H2A in HEK-293T RNase H2A wild-type and KO cells. **c**, Double-stranded Cy5-labeled 25-mers oligos were used in the *in vitro* cleavage assay to evaluate RNase H2 activity in protein extracts from HEK-293T RNase H2A wild-type and KO cells, schemes shown on the top of each gel image. L, ladder for 25-mers oligonucleotide; N, negative control with the double-stranded oligonucleotide treated by water; HII, *Escherichia coli* RNase HII was used as a positive control cleaving 5' of the rGMP embedded in the double-stranded DNA oligonucleotides; WT, protein extract from HEK-293T RNase H2A wild-type cells; KO, protein extract from HEK-293T RNase H2A KO cells. The cleavage % is shown underneath the image. Source data are provided as a Source Data file.





Wild type



RNase H2A KO

Supplementary Figure 5 | In/dels are sequence variations specific to DSB repair by NHEJ.

Variation-position histograms showing the distribution of sequence variations in the 20-bp DSB-sequence windows categorized by the position of the variations and the number of variations. The histograms are arranged in a grid: rows specify the sgRNA(s) used to induce the DSB(s), including the controls (No-DSB and DSB-sequence windows 30 bp downstream from the DSB); columns specify the construct and the type of variation (insertion in orange, deletion in blue, or substitution in gray). The *x*-axis indicates the position of the variations relative to the DSB site on the reference sequence. Figures with reverse-strand data have their *x*-axis coordinates reversed so that they correspond to forward-strand coordinates. The *y*-axis indicates the total number of variations in the DSB-sequence windows with *y* variations including position *x*. If a DSB-sequence window has more than one variation (say *k*) at the same position (which can only happen if the DSB-sequence window contains insertions), this window contributes *k*-fold to the corresponding *z*-value. The *x*, *y*, *z* axes have been limited to the ranges [-10, 10], [0, 20], and $[10^{-5}, 1]$, respectively, and values outside these ranges have been cropped. **a**, Variation-position histograms for experiments conducted in the wild-type cells, and **b**, in the RNase H2A KO cells.



sgRNA A, 3 days

sgRNA A, 3 days



Supplementary Figure 6 | Transcript RNA affects the repair of a DSB in a sequence-dependent manner via NHEJ and MMEJ

Pie charts showing frequencies of sequencing reads in the categories Error-free NHEJ/uncut sequence (green), NHEJ with in/dels (red), or MMEJ exon-exon (blue) following a DSB by the sgRNA A, B or E in the Sense, Branch Δ , and pCMV Δ constructs of wild-type (a, c) and RNase H2A KO (b) cells. Percentages of the exon-intron MMEJ were 0.07% (wild type, sgRNA A, Sense, \pm 0.003%), 0.12% (wild type, sgRNA A, Branch Δ , \pm 0.006), 0.07% (wild type, sgRNA A, pCMV Δ , \pm 0.013%), 0.03% (wild type, sgRNA B, Sense, \pm 0.003%), 0.07% (wild type, sgRNA B, BranchA, \pm 0.020%), 0.02% (wild type, sgRNA B, pCMVA, ± 0.004%), 0.03% (RNase H2A KO, sgRNA A, Sense, ± 0.002%), 0.06% (RNase H2A KO, sgRNA A, BranchA, ± 0.008%), 0.03% (RNase H2A KO, sgRNA A, pCMVA, ± 0.004%), 0.02% (RNase H2A KO, sgRNA B, Sense, ± 0.002%), 0.03% (RNase H2A KO, sgRNA B, BranchΔ, ± 0.002%), 0.0009% (RNase H2A KO, sgRNA B, pCMV Δ , \pm 0.004%). Percentages represent the average of 4 repeats with standard deviations in parenthesis; N=4. *, P = 0.029 comparing frequencies of the Branch Δ or the pCMV Δ with those of the Sense construct via the two-tailed Mann-Whitney U test. The frequencies have been normalized to only consider sequencing reads classified in one of the three categories (see Methods for an analysis of the unclassified reads). d, e, f, g, Pie charts showing frequencies of sequencing reads in each category for No DSB controls without Cas9 expression (see Method) in HEK293T cells with sgRNA E (d), sgRNA A (e, right) or sgRNA E and J (f) or in HEK293 cells with sgRNA A (e, left) or sgRNA A and B (g), N=1. Source data are provided as a Source Data file.

Variation-distance graph key



Inserted sequence with first nucleotide A, C, G, or T



Supplementary Figure 7 | Variation-distance graph key.

Variation-distance graph key showing the placement of the vertices representing the analyzed DSBsequence windows. Vertex placement depends on both the type of variation (insertion or deletion) and the number of variations of the corresponding DSB-sequence window compared to the reference sequence. Insertion vertices are placed above the reference vertex (center), while deletion vertices are placed below it. For insertions, the alphabetical order of the inserted sequence, from A on the left to T on the right, are indicated by the *x*-coordinate. Each insertion box is labeled with the inserted nucleotides of the DSBsequence window placed in that location. Insertions of size 3 or more have vertices on multiple lines, staggered vertically to reduce overlap. The *x*-coordinate of deletions indicates the position of the first deleted nucleotide in the given DSB-sequence window, from the most upstream (left-most) to the most downstream (right-most). Each deletion box is labeled with the range of the deleted nucleotide sequence, relative to the DSB site, of the DSB-sequence window placed in that location. The *y*-coordinate indicates the number of variations in the DSB-sequence windows, with higher variations placed further from the reference.





Supplementary Figure 8 | Transcript RNA enhances DSB repair by NHEJ in a sequence dependent manner – all data for the sense constructs.

a, Individual variation-distance graphs illustrating sequence variations within DSB-sequence windows observed after DSB induction by sgRNA A (top) or sgRNA B (bottom) in the Sense, BranchA, and pCMVA constructs of wild-type cells. An edge between two vertices indicates that the two corresponding DSBsequence windows differ by a single nucleotide insertion or deletion (in/del). Insertion vertices (orange circles) are placed above the reference vertex (white circle with a green outline), while deletion vertices (blue circles) are placed below it. The vertex size shows the log of the mean frequency of the corresponding DSB-sequence window in the four repeats of the considered experiment. For insertions, the alphabetical order of the inserted sequences, from A on the left to T on the right, are indicated by the x-coordinate. Insertions of size 3 or more have vertices on multiple lines, staggered vertically to reduce overlap. The xcoordinate of deletions indicates the position of the first deleted nucleotide, from the most upstream (leftmost) to the most downstream (right-most). The y-coordinate indicates the number of variations in the DSBsequence windows, with higher variations placed further from the reference. See Supplementary Fig. 7 for the variation-distance graph key. b, Comparison variation-distance graphs of the DSB-sequence windows obtained after DSB induction by sgRNA A (top) or sgRNA B (bottom) for the Sense vs. the Branch Δ construct (left) or for the Sense vs. the pCMV Δ construct (right) of wild-type cells. The vertices represent the same DSB-sequence windows as for the individual graphs while the vertex colors specify the relative frequency in the Sense (red) vs. the Branch Δ (green) construct or in the Sense (red) vs. the pCMV Δ (yellow) construct; the vertex sizes show the log of the maximum of the two mean frequencies of the corresponding DSB-sequence windows in the two analyzed constructs. c, Individual variation-distance graphs illustrating sequence variations within DSB-sequence windows observed after DSB induction by sgRNA A (top) or sgRNA B (bottom) in the Sense, BranchA, and pCMVA constructs of RNase H2A KO cells. **d**, Comparison variation-distance graphs of the DSB-sequence windows obtained after DSB induction by sgRNA A (top) or sgRNA B (bottom) for the Sense vs. the Branch Δ construct (left) or for the Sense vs. the pCMVA construct (right) of RNase H2A KO cells.

а



Exon1-Intron, 1 DSB by sgRNA A



Exon1-Exon1, 1 DSB by sgRNA E



Exon2-Exon1, 1 DSB by sgRNA B



Exon2-Intron, 1 DSB by sgRNA B



С

b Mic

Microhomology scheme: Sense/pCMVA, sgRNA A, forward strand

		DSB										
	5 10 15 20 25 30 35 40 45 50 55 60	65 70	75 80 85 90	95 100 105 110	115 120 125	130 135 140	145 150	155 160 165 170	175 180	185 190	195 200 205	210 215 220 225
	DS Red					intron					DS I	Red
	Primer Exon1			Intron		Intr	ron (branch site)		Intron		Exon2	Primer
EE1	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGC GTGAACTTCC
EE2	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE3	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE4 *	TTCANGTGGGAGCGCGTGATGAACTTCGAG <mark>GACGGC</mark> GGCGTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE5 *	TTCANGTGGGAGCGCGTGATGAACTTCGAGGACGG <mark>CGGC</mark> GTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE6 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGC <mark>GTG</mark> GCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE7 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGG <mark>ACGGCGGCGT</mark> GACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GAC <mark>GGC</mark> TGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE8 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACG <mark>GCGGCGTGGC<mark>GAC</mark>CGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCA TCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE9 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGG <mark>CGTGGCGACC<mark>GTGA</mark>CCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GA AGTTCATCGGCGTGAACTTCC
EE10 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCG <mark>TGGCGACCGT</mark> GACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCA TCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EE11 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCG <mark>ACCGTGACCC</mark> AGG <mark>ACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCAT CGGCGTGAACTTCC
EE12 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGAC <mark>CGTGACCCAG</mark> GAC <mark>TCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GAC <mark>GGCTGCT</mark>	TCA TCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI1 *	TTCAAGTGGGAG <mark>CGCGTGATGA<mark>ACT</mark>TCGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	G G T A T G T T A A T A T G G <mark>A C T</mark> <mark>A A A G</mark>	GAGGCT TTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI2	TTCAAGTGGGAG <mark>CGCGTGATGA<mark>ACT</mark>TCGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCG <mark>A</mark>	CT CTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI3 *	TTCAAGTGGGAGC <mark>GCGTGATGAACTT</mark> CGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGG <mark>CTT</mark> TTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI4 *	TTCAAGTGGGAGCG <mark>CGTGATGAAC<mark>TTC</mark>GAGGACGGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTT <mark>TTC</mark> TCAGGTCGA	C T C T A G A C G C G T A G	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI5 *	TTCAAGTGGGAGCGC <mark>GTGATGAACT<mark>TCGA</mark>GGACGGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGG <mark>TCGA</mark>	C T C T A G A C G C G T A G	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI6 *	TTCAAGTGGGAGCGCGT <mark>GATGAACTTC<mark>GAGG</mark>ACGGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAG GTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI7 *	TTCAAGTGGGAGCGCGTG <mark>ATGAACTTCGAGG</mark> ACGGCGGCGTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTC <mark>AGG</mark> TCGA	CTCTAG <mark>ACGCGTAG</mark>	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI8 *	TTCAAGTGGGAGCGCGTG <mark>ATGAACTTCG<mark>AGGA</mark>CGGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GA GGCTTTTCTC <mark>AGG</mark> TCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI9 *	TTCAAGTGGGAGCGCGTGA <mark>TGAACTTCGA<mark>GGAC</mark>GGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATAT <mark>GGAC</mark> TAAAG	GAGGC TTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI10 *	TTCAAGTGGGAGCGCGTGAT <mark>GAACTTCGAG<mark>GAC</mark>GGCGGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTC <mark>GA</mark>	C T C T A G A C G C G T A G	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI11 *	TTCAAGTGGGAGCGCGTGATGAA <mark>CTTCGAGGAC<mark>GGC</mark>GGCGTGGCGACCGTGACCCAGGACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GA <mark>GGC</mark> TTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI12 *	TTCAAGTGGGAGCGCGTGATGAACTT <mark>CGAGGACGGC</mark> GTGGCGACCGTGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATGGACTAAAG	GA <mark>GGC</mark> TTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI13 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAG <mark>GACGGCGGCG<mark>TGG</mark>CGACCGTGACCCAGGACTCC</mark>	CCCTGCA	G G T A T G T T A A T A <mark>T G G</mark> A C T A A A G	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI14 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGG <mark>ACGGCGGCGT</mark> GGC <mark>GACCGTGACCCAGGACTCC</mark>	CCCTGCA	G G T A T G T T A A T A T G G A C T A A A G	GA <mark>GGC</mark> TTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI15 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACG <mark>GCGGCGTGGC</mark> GTCGACCCAGGACTCC	CCCTGCA	GGTATGTTAATATG <mark>GAC</mark> TAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI16 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGAC <mark>GGCGGCGTGG<mark>CGAC</mark>CGTGACCCAGGACTCC</mark>	CCCTGCA	g g t a t g t t a a t a t g g a c t a a a g	GAGGCTTTTCTCAGGT <mark>CGA</mark>	C T C T A G A C G C G T A G	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI17 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCG ^T GGCGACCGT <mark>GAC</mark> CAGGACTCC	CCCTGCA	GGTATGTTAATATG <mark>GAC</mark> TAAAG	GAGGC TTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI18 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCG ^T GGCGACCGT <mark>GAC</mark> CAGGACTCC	CCCTGCA	G G T A T G T T A A T A T G G A C T A A A G	GAGGCTTTTCTCAGGTC <mark>GA</mark>	C T C T A G A C G C G T A G	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI19 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGGGGGGG	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI20 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGC <mark>GACCGTGACC</mark> CAGG <mark>ACTCC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCT <mark>CAGG</mark> TCGA	CTCTAG <mark>ACGCGTAG</mark>	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI21 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGGGGGGG	CCCTGCA	GGTATGTTAATATGGACTAAAG	GA GGCTTTTCTC <mark>AGGTCGA</mark>	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI22 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGGGGGGG	CCCTGCA	GGTATGTTAATAT <mark>GGACT</mark> AAAG	GAGGCT TTTCTCAGGTCGA	CTCTAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI23 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCG <mark>TGACCCAGGACTC</mark> C	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTT <mark>CTC</mark> AGGTCGA	CTC TAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI24 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGAC <mark>GGGCCCAG</mark> GACTC <mark>C</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTC <mark>GA</mark>	CTC TAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI25 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCGTGA <mark>CCCAGGACTC</mark>	CCCTGCA	G G T A T G T T A A T A T G G A C T A A A G	GAGGCTTTT <mark>CTC</mark> AGGTCGA	CTC TAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC
EI26 *	TTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCGTGA <mark>CCCAGGACTC</mark>	CCCTGCA	GGTATGTTAATATGGACTAAAG	GAGGCTTTTCTCAGGTCGA	CTC TAGACGCGTAG	GATCCCCCGGGTACCG	GAGCTCGAATTT	TACTAACAAATGGTAT	TATTTATCCACA	GGACGGCTGCT	TCATCTACAAGGT	GAAGTTCATCGGCGTGAACTTCC

Microhomology scheme: Sense/pCMVA, sgRNA B, reverse strand

		U	58						
	5 10 15 20	0 25 30 35 40 45	50 55	60 65 70 75 80 85 90 9	5 100 105 110	115 120 125 130 135 140 145	150 155 160	165 170 175 180 185 190 195 200 20	5 210 215 220 225
		DS Red			Intron			DS Red	
	Primer	Exon2	Intron	Intron (branch site)		Intron		Exon1	Primer
EE1R *	GGAAGTTCACGCCGATGAAC	TTC ACCTTGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	.G <mark>TTC</mark> ATCACGCGCTCCCACTTGA#
EE2R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGTAGAT <mark>GAAG</mark> CAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTC	GTTCATCACGCGCTCCCACTTGA#
EE3R *	GGAAGTTCACGCCG <mark>ATGAAC</mark>	TTCACCT TGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGT <mark>CCT</mark> CGAA	.GTTCATCACGCGCTCCCACTTGA#
EE4R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGT <mark>AGATGAAGCA</mark> GCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCC <mark>GCCGTC</mark> CTCGAA	.GTTCATCACGCGCTCCCACTTGA
EE5R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGT <mark>AGATGAAGCA<mark>GCCG</mark>TC</mark>	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	TAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCAC <mark>GCCGCCGTCCTCGA</mark> A	GTTCATCACGCGCTCCCACTTGA
EE6R *	GGAAGTTCACGC<mark>CGATGAAC</mark>	TT CAC CTTGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGC <mark>CAC</mark> GCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EE7R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGT <mark>AGATGAAGCA<mark>GCC</mark>GTC</mark>	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTC <mark>GCC</mark> ACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EE8R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGTAGA <mark>TGAAGCAGCCGTC</mark>	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACG <mark>GTC</mark> GCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EE9R *	GGAAGTTCACG <mark>CCGATGAAC</mark>	TTCACCTTGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGG <mark>TCAC</mark> GGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EE10R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGTAGA <mark>TGAAGCAGCC</mark> GTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGG <mark>GTC</mark> ACGGTCGCCA ^C GCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EE11R *	GGAAGTTCACGCCG <mark>ATGAAC</mark>	TTCACCT TGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGT <mark>CCT</mark> GGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EE12R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGTAGA <mark>TGAAGCAGCC</mark> GTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGA <mark>GTC</mark> CTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EE13R *	GGAAGTTCACGCCGATGAAC	TTCACCT <mark>TGTAGATGAA<mark>GCAG</mark>CCGTC</mark>	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCT <mark>GC</mark>	AGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
E127R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGTAGA <mark>TGAAGCAGCC</mark> GTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTTTA <mark>GTC</mark> CATA	ATTAACA TACCTGC	AGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI28R	GGAAGTTCACGCCGATGAAC	TTCACCTTG <mark>TAG</mark> ATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCTCCTT <mark>TAG</mark> TCCATA	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI29R *	GGAAGTTCACGCCG <mark>ATGAAC</mark>	TTCACCTTGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAGCCT <mark>CCTT</mark> TAGTCCATA	AT TAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI30R *	GGAAGTTCACGCCG <mark>ATGAAC</mark>	TTCA <mark>CCT</mark> TGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAAAG <mark>CCT</mark> CCTTTAGTCCAT#	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI31R *	GGAAGTTCACGCCGATGAAC	TTCACCTTG TAGATGAAGC AGCC GTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAAA <mark>AGCC</mark> TCCTTTAGTCCAT#	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI32R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGTAGATG <mark>AAGC</mark> AGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGAGAA <mark>AAGC</mark> CTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI33R *	GGAAGTTCACGCCGATGAAC	TTCA <mark>CCTTGTAGAT<mark>GAA</mark>GCAGCCGTC</mark>	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTGA <mark>GAA</mark> AAGCCTCCTTTAGTCCATA	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI34R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGT <mark>AGA</mark> TGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACCTG <mark>AGA</mark> AAAGCCTCCTTTAGTCCAT <i>i</i>	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI35R *	GGAAGTTCACGCCGATGAAC	TTC <mark>ACCTTGTAGA<mark>TGA</mark>AGCAGCCGTC</mark>	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCGACC <mark>TGA</mark> GAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI36R *	GGAAGTTCACGCCGATGAAC	TTCACCT TGTAGATGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGAGTCG <mark>ACCT</mark> GAGAAAAGCCTCCTTTAGTCCAT <i>I</i>	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI37R *	GGAAGTTCACGCCGATGAAC	TTCACCTTGTAGA TGAAGCAGCC GTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	CTAGA <mark>GTC</mark> GACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA
EI38R *	GGAAGTTCACGCCGATGAAC	TTCACCTTG TAGA TGAAGCAGCCGTC	CTGTGGATAA	ATAATACCATTTGTTAGTAAAAATTCGAGCTCGGTACCC	GGGGGATCCTACGCGT	TAGAGTCGACCTGAGAAAAGCCTCCTTTAGTCCAT	ATTAACATACCTGC	AGGGAGGAGTCCTGGGTCACGGTCGCCACGCCGCCGTCCTCGAA	GTTCATCACGCGCTCCCACTTGA

Microhomology scheme: Sense/pCMV Δ /Branch Δ , sgRNA E, forward strand

ł	Micro	bhomology scheme: Sense/pCMVΔ/BranchΔ, sgRNA E, forward strand
	5 10 15 20 25 30 35 40 45 50 55 60 65 70	75 elo e5 elo 165 160 165 120 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 240 245 DS.Red
•	Primer CTTCAAGGTGC <mark>GCATGAAGGCCACGGCCACGAGTCGAGATCGAGGCGAGG</mark>	Exon1 Primer CTACGAGGGCCACAACAACGTGAAGGTGAACGAGGGGGGCCCCCTGGCCTTGCCCGGGGACATCCCGGGGGGCGCCCAAGGTGTACGGGGGGGG
	CTTCAAGGTGCGCATGGAGGGC <mark>ACC</mark> BTGAACGGCCACGAGTTCGAGAGGCGAGGGCGAGGGCGAGGCCGCC CTTCAAGGTG <mark>CGCATGGAGG<mark>CGACC</mark>BTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGCCGCC</mark>	ΤΑ Ε Ο ΜΟΤΟΓΙΑ ΤΑ Ε Ο ΜΟΤΟΓΙΑ ΤΑ Ο ΜΟΤΟ Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο Ο
	CTTCAAGGTGCGCATGGAGGGCA <mark>CCG</mark> TGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCC	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$
	CTTCANGGTGCGCATGGAGGGCA <mark>CCG</mark> TGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC	The shear called a c
	CTTCAAGGTGCGCATGGAGGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGGGGGG	
	CTTCANGGTGCGCATGGAGGGCACCG <mark>TGAA</mark> CGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCCGCC CTTCAAGGTGC <mark>GCATGGAGGGCACCGTGAAC</mark> GGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCCGCC	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$
•	CTTCANGGTGCGCATG <mark>GAGGGCACCGTGAA</mark> CGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGACG	THE SAGE CALLAR ACCOUNT AND OF SACE AND SOCIECT COCCUTE COCCUTE COCCULATE CAST CONTRACTOR CALL CAST COCCULATE CAST COCCULATE CAST COCCULATE CAST COCULATE CAST
•	CTTCAAGGTGCGCATGGAGGGCACGGGTGAACGGCCACGAGTTCGAGATCGAGGGGAGGGGGGGG	
	CTTCANGGTGCGCATGGAGGGCACCGTG <mark>AAC</mark> GGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCCGCC CTTCAAGGTGCGCATGGAG <mark>GGCACCGTGAACG</mark> GCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCCGCC	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$
	CTTCANGGTGCGCATGGAG <mark>GGCACCGTGA<mark>ACG</mark>GCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGACGGCCGCC</mark>	
	CTTCARGETGCGCATGGAG ^{GG} CACCGTGA <mark>ACGGC</mark> CACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC	The GAGGGE CACAACACCOTOAAGT TAAGT TAACTAGE COCCCTTCCCCTTCCCCTTCCCCTTCCCCCACTCCATTCCATTCCACTACT
	CTTCANGGTGCGCATGGAGGG <mark>CACCGTGAAC<mark>GGC</mark>CACGAGTTCGAGATCGAGGCGAGGGCGAGGGCGAGGCCGCC CTTCAAGGTGCGCATGGAGG<mark>CACCGTGAAC<mark>GGCCA</mark>CGAGTTCGAGATCGAGGCGAGGGCGAGGGCGAGGCCGCC</mark></mark>	The challed calk a case of a second
	CTTCAAGGTGCGCATGGAGGGCACCGTGAACG <mark>bCC</mark> ACGAGTTCGAGACCGAGGGCGAGGGCGAGGGCGAGGGCGACGGCGCGCCCC	
	CTTCANGGTGCGCATGGAGGGCACCGTGAACG <mark>CCC</mark> ACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC	TX CANGG CC C C A C X C X C X C X C X C X C X C
•	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGGAGGGGGAGGGGCGAGGGCGACGGGCGACGGGCGAGGGGCGAGGGGGG	TA CANAGG CANACCATCANG TA ACTA AGA AGA CAGA CONTROL TA CATA CAGA CANACATA CAGA CAGA CAGA CAGA CAGA CAGA CAGA
	CTTCANGGTGCGCATGGAGGGCACCGTGAACGG <mark>CCA</mark> CGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC CTTCANGGTGCGCATGGAGGGCACCGTGAACGG <mark>CCA</mark> CGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCC	The characteristic constant of the constant
*	CTTCANGGTGCGCATGGAGGGCACCGTGAAC<mark>GGCCAC</mark>GAGTTCGAGATCGAGGGCGAGGGCGAGGGCG AGGGCCGCC	TACAN <mark>09CCCC</mark> ALCHACKCTOAAGCTOACCTACCCGTCCCCCTCCCCCTCCCCCTCCCCCCCATTCCAGTACGGCTCCAAGCGTOTACGTOACCCCACCCC
)	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGGCGCCCC	
1	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGACGGCCGCC CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCC	Τλ ε αλαφασε λαλακ τα ταλασταλασταλαστακεί το μασσασε στο στο στο στο στο στο στο από τη αναγματική στο στο από τη αναγματική στο
3	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCA <mark>CGA</mark> GTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGACG	T X C 8 0 8 0 C C C L L X X X C 7 0 A X C 7 A X 8 0 C C C C T C C C C T C C C T C C C C X A T C C X T C X A X C 8 C T C X A X C 8 C T C X X X X X X X X X X X X X X X X X
5 *	CTTCARGETGCGCATGGAGGGCACCG <mark>ETGAACGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC</mark> CTTCAAGGTGCGCCAAGGGCGAGGGCCGAGGGCCGCC	
5 *	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCA <mark>CGAG</mark> TTCGAGATCGAGGGCGAGGGCGAGGGCGAGGCCGCC CTTCAAGGTGCGCATGGAGGGCACCGTG <mark>AACGGCCACGAGT</mark> TCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGACG	ΤΑ ταλασσο καλικά καταλασταλασταλαστακά τα καταγμάτερα το
3 *	CTTCANGGTGCGCATGGAGGGCACCGTG <mark>AACGGCCACGAGTTC</mark> GAGATCGAGGGCGAGGGCGAGGGCGAGGGCCGACG	T 1 A GAGGE CALLAR CE CALAGE C
•	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAG <mark>TTCG</mark> AGATCGAGGGCGAGGGCGAGGGCCGCCC	
•	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGGCGCGCGC	The GAGGGC CALA CALCE THAT GO THAT CALL AND
:	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGACGAGGGCGAGGGCGAGGGCGAGGGCGACGAGGCCGACGA	
5 *	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCC	
5 * 7 *	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGACGCCCC CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAG <mark>ATC</mark> GAGGGCGAGGGCGAGGGCGAGGGCGACGGCCGCC	The change called a constraint of the constrai
3	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCCAAGGGCGAGGGCGAGGGCCGCC CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGCCGAGGGCGAGGGCCGCC	
0	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACG <mark>AGTTCGAGAT</mark> CGAGGGCGAGGGCGAGGGCGACGGCGAGGGCGACG	The charge of Linke carbon of the charge sector to compare the compare the charge of t
2	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGGCGAGGGCCGCCC	
3 * 4 *	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGA <mark>GGC</mark> GAGGGCGAGGGCCGCC CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACG <mark>AGTTCGAGATCGAGGCC</mark> AGGGCGAGGGCCGCC	The 0 hadded calked and that other and the consistency of the construction of the co
5 * 4	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGAACGAGGGCGAGGGCGAGGGCCGCC	
7 *	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACG <mark>AGTTCGAGAT<mark>CGAGGGC</mark>GAGGGCGAGGGCCGCCC</mark>	TX CARGAGECC2.CX CX C
8 ★ 9	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAG <mark>TTCGAGATCGAGGCCAGGGCGAGGGCGACGGCCGCCCCCCCC</mark>	TA COMBRGE CALARCACET AND TAXES AND ADDRESS CONTROL TO TOTAL CONTROL AND TAXES AND ADDRESS CONTROL AND ADDRESS
) L *	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCC CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCC	T 1 C 0.0 4 G C C 0 4 G C C C C C C C C C C C C C C C C C C
2	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAG <mark>ATCGAGGGCG<mark>AGG</mark>CCGAGGGCCGCCC</mark>	T A C 9A 98 C C L C A C A C T O A A 6 T O A C A A 95 C 9 C C C C T C C C T C C C T C C C C A G 1 C C A T A C 9 C A C T C A A A C T O A A G C A C C C C C C C C C A C A C T C C C A C A
•	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGG <mark>CGAGGGCGAGGGCGCGCGC</mark> GAGGGCCGCCG	
*	CTTCARGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAG <mark>GGCGAGGGCGCGCCCCCCCTCAAGGGCGAAGGGCGGGGGG</mark>	The characteristic construction and the construction of the cons
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	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGCGCG	
٠	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCG <mark>AGGGCAGGGGCGAGGGGGCGAGGGCGAGGGCGAGGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGCGAGGGGGCGAGGGCGAGGGCGAGGGGGG</mark>	The challed call a calced that the face of a degree correct of the correct of the calced
•	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGACGCCCCC	
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•	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGGAGGGGGGGG	
:	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGACGGCCCCCCCC	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$
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•	CTTCARGETGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCCGCCC	
•	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGCGCCCCCCCC	Τλε όλα σο σελλελοτικά σταλα σταλα σταλα σταλα στα στο σταστα στα στα στα στα στα στα στα στα
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•	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGA <mark>GGGCGAGGCGAGGGCGAGGCGAGGCGAGGGGGG</mark>	
:	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCG <mark>aGGGCGAGGGCCGCC</mark> CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAG <mark>GGCGAGGGCC</mark>	TA COM GOLO CONTRACTA CONTRACT
•	CTTCANGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGAGGGCGACGCCGCCCCCCGAGTTCGAGATCGAGGGCGAGGGGGGGG	
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:	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGA CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGGTCGAGGGCGAGG <mark>GCGAGGGCGG</mark>	ΤΑ Ο 1999 στο Αλακό τα πατά τα το το το 1990 στο το 1990 στο 1990
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:	CTTCAAGGTGCGCATGGAGGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGG <mark>GCGAGGGCCG</mark> CC	1 / C ANG C C C A C A C A C A C A C A C A C A C
	CITCARGUIGUEATUUAUUEACEUTUAAEUUEEACGAGITEGAGATEGAGGGGGGGGGGGGGGGGGGGGGGGGGG	
, * 20 *	CTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGG <mark>GCGAGGGCCGCCCCCCCCCC</mark>	TA consider can execute an action considered and considered and construction and construction and construction construction and construction





2.5

Sense BranchApCMV

Sense BranchApCM

0.5





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Sense BranchApCh

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Sense Branch4pcM



Sense BranchApCMV

RNase H2A KO, sgRNA B, reverse strand



е

f	Wild ty	pe, sgRN/	A E, forw	ard strand	d						
Geographics of MMG	EE1_CAC	EE3_GCACC	EE9 GTGA	EE10 CACCGTGAA	EE11 TGAA	EE12 CGTGAA	EE15 ACG	EE17 GGC	EE20 CGGCC	EE24 CCA	EE28 GGCCAC
	EE35_ACGAG	EE36_CGAG	EE38_AGTTC	EE40_TTCG	EE41_CGA	EE43_CGAG	EE44_CGAG	EE45_AGA	EE46_ATC	EE47_ATC	EE51_AGG
	EE53 GGG	EES4 CGAGGGC	EE55 GGC	EE57 CGAGGGC	EE58 AGGGCG	EE61 AGG	EE64 CGAGGGC	EE65 GGC	EE67 CGAGGGC	EE68 AGGGCG	EE71 AGG 20 15 15 16 0.5 5ense Brancha pCMVA
	EE73_GGG	EE74_AGGGC	EE75_GGC	EE76_CGAGGGC E	sense granch power	EE78_GGCC	EE79_GCC	EE80_GCC	EE81_CCG	EE82_GCCG	EE85_GCC
	EE86 CGCC	EE87 CCGCC	EE88 GCCC	EE89 CCC	EE90 CCC	EE91 GCCC	EE92 CCC	EE93 CCC	EE94 CCC	EE95 CCC	EE96 CCC
	EE97 CCC	EE98 CCC	EE99 CCC	EE100 CCC 1.5 1.0 0.5 5ense grancha pcMVb	EE101 CCC						



Ratio of MMEJ frequency [Sum of MMEJ freq using the exon-exon microhomology] / [Sum of MMEJ freq using the exon-intron microhomology]

HEK-293T RNase H2A KO



Ratio of MMEJ frequency

[Sum of MMEJ freq using the exon-exon microhomology] / [Sum of MMEJ freq using the exon-intron microhomology]

Supplementary Figure 9 | The spliced transcript facilitates MMEJ between exon-exon microhomologies, while the non-spliced transcript facilitates MMEJ between exon-intron microhomologies.

a, Schemes of regions in which microhomology pairs were identified within the sequenced area to determine the frequency of MMEJ following a DSB by the sgRNA A, B or E. The microhomology pairs were categorized into two groups for a DSB induced by sgRNA A or B: exon-exon and exon-intron. Light blue lines indicate examples of microhomologies; the black-parallel lines show the DSB site; the dark green box shows the region containing the branch site. **b**, Schemes showing all microhomology pairs 3 bp or longer between the two primer sequences in the Sense and pCMVA constructs, sgRNA A, forward strand. The x-axis shows nucleotide positions from the 5'-primer sequence. First row: DsRed gene exons (solid blue), intron (solid green). Second row: primer sequence (purple outline), Exon1/Exon2 (blue outline), Intron (green outline), branch site (dark green outline). DSB position is indicated with a red vertical line. Left margin labels are microhomology IDs (EE, exon-exon; EI, exon-intron); a bold asterisk indicates MMEJ products detected in the sequencing data. Each following row shows the nucleotide sequence of the construct between primer sequences; region deleted by MMEJ repair (gray highlight); microhomology pair (light blue highlight); ± 10-bp flanking region of microhomology pair used for MMEJ detection in the sequencing data (light green highlight). c, As in (b) for Sense and pCMV∆ constructs, sgRNA B, reverse strand. d, As in (b) for Sense, pCMV Δ , and Branch Δ constructs, sgRNA E, forward strand. e, MMEJ frequencies from each microhomology pair detected in the sequencing libraries following a DSB by the sgRNA A or B in the Sense (red), Branch Δ (green), and pCMV Δ (yellow) constructs of wild-type and RNase H2A KO cells. The ID of the microhomology pair being analyzed is shown on top of each bar graph. Plotted data are the mean \pm s.d. of the 4 biological replicates with the individual values shown as dots; N=4. The 'ns' on the bar graphs means a non-significant difference (P-value > 0.05, two-tailed Mann-Whitney U test). f, MMEJ frequencies from each microhomology pair detected in the sequencing libraries following a DSB by the sgRNA E. Plotted data are the mean \pm s.d. of the 4 biological replicates with the individual values shown as dots; N=4. The 'ns' on the bar graphs means a non-significant difference (P-value > 0.05, two-tailed Mann-Whitney U test). g, Ratio of MMEJ frequencies for the exon-exon and exon-intron following a DSB by the sgRNA A or B in the Sense (red), Branch Δ (green), and pCMV Δ (yellow) constructs of wild-type (top) and RNase H2A KO (bottom) cells. The ratio was calculated by dividing the sum of MMEJ frequencies from the exon-exon microhomology pairs by the sum of MMEJ frequencies from the exon-intron microhomology pairs in each sequencing library, N=4. The median of the points is shown as the middle line of the box. The first and third quartiles are indicated by the box frames and the whiskers represent the largest point not more than 1.5 interquartile range (IQR) beyond the box frame. All data points outside the whiskers are classified as outliers and shown as diamond points. *, P = 0.029 (two-tailed Mann-Whitney U test). Source data are provided as a Source Data file.







Supplementary Figure 10 | Transcript RNA promotes double-strand gap repair in a sequencedependent manner via NHEJ – all data for the sense and antisense constructs.

a, Individual variation-distance graphs illustrating sequence variations within DSB-sequence windows observed after gap induction by sgRNA A and sgRNA B in the Sense, Branch Δ , and pCMV Δ constructs of wild-type cells. Data obtained by sequencing the forward (top) and the reverse (bottom) strands. b, Comparison variation-distance graphs of the DSB-sequence windows obtained after gap induction by sgRNA A and sgRNA B for the Sense vs. the Branch Δ construct (left) or for the Sense vs. the pCMV Δ construct (right) of wild-type cells. The vertices represent the same DSB-sequence windows as for the individual graphs while the vertex colors specify the relative frequency in the Sense (red) vs. the Branch Δ (green) construct, or the Sense (red) vs. the pCMV Δ (yellow) construct; the vertex sizes show the log of the maximum of the two mean frequencies of the corresponding DSB-sequence window in the two compared constructs. Data obtained by sequencing the forward (top) and the reverse (bottom) strands. c, Same as in (a) for constructs of RNase H2A KO cells. d, Same as in (b) for constructs of RNase H2A KO cells. e, Individual variation-distance graphs illustrating sequence variations within DSB-sequence windows observed after gap induction by sgRNA C/C' and sgRNA D in the Antisense and 5'-Splicing∆ constructs of wild-type cells. Data obtained by sequencing the forward (top) and the reverse (bottom) strands. f, Comparison variation-distance graphs of the DSB-sequence windows obtained after gap induction by sgRNA C/C' and sgRNA D for the Antisense vs. the 5'-Splicing∆ construct of wild-type cells. The vertices represent the same DSB-sequence windows as for the individual graphs while the vertex colors specify the relative frequency for the Antisense (red) vs. the 5'-Splicing (green) construct; the vertex sizes show the log of the maximum of the two mean frequencies of the corresponding DSB-sequence window in the two compared constructs. Data obtained by sequencing the forward (left) and the reverse (right) strands. Refer to Supplementary Figure 7 for the description of variation-distance graphs. For graphs showing reversestrand data, the sequences are reverse-complemented prior to computing xy-coordinates so that they correspond to forward-strand sequence coordinates.



Exon1-Exon2, sense system 2 DSBs by sgRNA A & B



Exon1-Exon2, antisense system 2 DSBs by sgRNA C(C') & D



Exon1-Exon1, sense system 2 DSBs by sgRNA E & J



EE10R

EE11R *



Microhomology scheme: Sense/pCMV Δ /Branch Δ , sgRNA E & J, forward strand С 80 05 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 105 200 205 210 215 220 225 10 15 230 235 240 EE1 * EE2 * EE3 EE4 EE5 * EE6 * EE7 * EE8 * EE9 * EE10 * EE11 * EE12 * EE13 EE14 EE15 * EE16 * FF17 * CTICLASSET COLOGICAL CONTRACTOR CONTRACTOR CONCERCE CONCERCENCE CONCERCE CONCERCENCE CONCE EE18 * EE19 * EE20 * EE21 * EE22 * EE23 Microhomology scheme: Sense/pCMV Δ /Branch Δ , sgRNA E & J, reverse strand 10 15 20 25 30 35 40 45 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 190 195 200 205 210 215 220 225 230 235 240 245 50 185 55 EE1R EE2R

1411 J 11	
EE4R *	ATCACGCGCTCCCACTTGAAGCCCTCCGCGGAAGGCACGGGAAGGCACGGGGGGGG
EE5R *	ATCACGCGCTCCCACTTGAAGCCCTCGGGGAAGGACGGCCCTCGCCCTCGACGCCGAAGGGCAGGGGGGGG
EE6R *	ATCACGCGCTCCCACTTCAAGCCCTCCCCCTCGACTTCAAGGCGAAGGCCCCCCCGAACTGGAGGCCCCCCCGACGCCCTCGACCTCCACGGGGGCCCCCCGACGCCCTCGACCTCACGGGGGCCCCCCGACGCCCTCGACCCCCCGACGCCCTCGACCCCCCGACGCGAAGGGGGGGG
EE7R *	ATCACCCCCCCCTCCAACTTCAAGCCCTCCGGGCAACGCCCCCCGAACTCCTCAAGCTCTCACGGGGGCCCCTCGAACTCCCCGAACTCCCCGGGGGGCCCCCCGAACTCCCCGGGGGGGG
EE8R *	ATCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
EE9R *	ATCACCCCCCCACTTCAAGCCCTCCCCCCCCCCCCCCCC
EE10R *	ATCACCCCCCCACTTGAAGCCCTCCCCCCCCCCCCCCCC
EE11R *	ATCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
EE12R *	ATCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
EE13R *	ATCACCCCCTCCCACTTCAAGCCCTCCGGGGAAGGACCGGGCCCTCCGACCTCCACGGGGGACCTCACGGGGGGCCCTCCACGGGGGGGG
EE14R *	ATCACCCCCCCCTCCAACCTCCAACCTCCCCCCCCCCC
EE15R *	ATCACCCCCTCCACTTCAACCTCCCCCCCCCCCCCCCC
EE16R *	ATCACCCCCCCCACTAGAAGCCCCCCGGGGAAGGACGGCCCCCCCGACCCCCCCC
EE17R *	ATCACCCCCTCCCACTTCAAGCCCTCCGGGGAAGGACGGGCCCTCCGGGGGAAGGGGGGGG
EE18R *	ATCACCCCCCCCCTCCAGEGGAAGGCCCCCCGAGGGAAGGCCCCCCGAACTGGAGGGGCCCCCCCC
EE19R *	ATCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
EE20R *	ATCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
EE21R *	ATCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
EE22R *	ATCACCCCCCCCCTCCACCTCOAGCCCCCCCCCCCCCCCC
EE23R *	ATCACCCCCCCCCTCGACCCTCGACCCTCGACCCTCCGACCTCCTCGACCCTCCCCCCCC



Microhomology scheme: Antisense, sgRNA C & D, reverse strand

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	5	10 1	15 20	25	30	35 4	0 45	50	55	60	65 7	0 75	5 80	85	90	95	100	105	110	115	120	125 13	0 13	5 140	145	150	155	160	165 17	175	180	185 :	190 :	195 20	10 205	210	215	220	225 230	235	240	245	250
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		Primer				Exon2			5' splice												ntron	I														Exon1		_			Pr	imer	
EE1R *	GGAAGTTO	ACGCCG	ATGAACT	TCACCI	TGTAGA	TGAAGC	GCCGTC	CTGCAG	GTATGT	T A A T A T	GGACT	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	STTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAGGG	GAGGAG	TCCTG	GTCACG	GTCGCC	ACGCCC	SCCGTC	CTCGARG	TTC <mark>ATC</mark>	ACGCGC	TCCCA	CTTGAR
EE2R *	GGAAGTTO	ACGCCG	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	CTGCAG	GTATGT	TAATAT	GGACT	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	STTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAGGG	AGGAG	TCCTG	GTCACG	GTCGCC	ACGCCC	SCCGTC	CTCGARG!	TTCATO	ACGCGC	TCCCA	CTTGAR
EE3R *	GGAAGTTO	ACGCCG	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	CTGCAG	GTATGT	TAATAT	GGACTJ	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	TTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAG <mark>GG</mark>	GAGGAG	TCCTG	GTCACG	GTCGCC	ACGCC	SCCGT <mark>C</mark>	CTCGARG!	TTCAT	ACGCGC	TCCCA	СТТБАА
EE4R *	GGAAGTTO	ACGCCG	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	TGCAG	GTATGT	TAATAT	GGACTJ	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	TTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAG <mark>GG</mark>	GAGGAG	TCCTG	GTCACG	GTCGCC	ACGCC	SCCGTC	CTCGARG!	TTCATO	ACGCGC	TCCCA	СТТБАА
EE5R *	GGAAGTTO	ACGCCG	атсалст	TCACCT	TGTAGA	TGAAGC	GCCGTC	CTGCAG	GTATGT	TAATAT	GGACTJ	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	STTATC	CCTARC	GCGTAG	GATCO	сссббб	TACCG	GCTCG	AATTTT	тастал	CAAATO	GTATTA	TTTATC	C A C A G G G	GAGGAG	TCCTG	GTCACG	GTCGCC	ACGCCC	CCGTC	CTCGAAG!	TTCATO	ACGCGC	TCCCA	СТТБАА
EE6R *	GGAAGTTO	ACGCCG	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	CTGCAG	GTATGT	TAATAT	GGACTJ	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	TTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAG <mark>GG</mark>	GAGGAG	TCCTG	GTCACG	GTCGC	. AC GCCC	SCCGTC	CTCGARG!	TTCATC	ACGCGC	TCCCA	СТТБАА
EE7R *	GGAAGTTC	ACGCCG	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	CTGCAG	GTATGT	TAATAT	GGACTI	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	STTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAN	CAAATO	GTATTA	TTTATC	CACAG <mark>GG</mark>	GAGGAG	TCCTG	GTCACG	GTCGCC	ACGCCC	3 C C G T C	CTCGAAG	TTCATC	ACGCGC	TCCCA	CTTGAA
EE8R *	GGAAGTTO	ACGCCG	атсалст	TCACCT	TGTAGA	TGAAGC	AGCC GTC	CTGCAG	GTATGT	TAATAT	GGACTJ	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	TTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAG <mark>GG</mark>	GAGGAG	TCCTG	GTCACG	GTCGCC	ACGCCC	SC CGTC	CTCGAAG	TTCATC	ACGCGC	TCCCA	СТТБАА
EE9R *	GGAAGTTO	ACGCCG	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	CTGCAG	GTATGT	TAATAT	GGACTJ	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	STTATC	CCTARC	GCGTAG	GATCO	сссббб	TACCG	GCTCG	AATTTT	тастал	CAAATO	GTATTA	TTTATC	C A C A G G G	GAGGAG	TCCTG	GTCAC	GTCGCC	ACGCCC	CCGTC	CTCGAAG	TTCATC	ACGCGC	TCCCA	СТТБАА
EE10R *	GGAAGTTO	ACGCCGI	ATGAACT	TCACCT	TGTAGA	TGAAGC	AGCC GTC	C T G C A G	GTATGT	TAATAT	GGACT	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	STTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAG <mark>GG</mark>	GAGGAG	TCCTG	GTCACG	GTCGCC	. ACGCCC	JCCGTC	CTCGARG	TTCATC	ACGCGC	TCCCA	CTTGAA
EE11R *	GGAAGTTO	ACGCCG	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	C T G C A G	GTATGT	TAATAT	GGACT	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	STTATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAG <mark>GG</mark>	GAGGAG	TCCT GO	GTCACG	GTCGCC	ACGCCC	JCCGTC	CTCGARG	TTCATC	ACGCGC	TCCCA	CTTGAA
EE12R *	GGAAGTTC	ACGCCGI	ATGAACT	TCACCT	TGTAGA	TGAAGC	GCCGTC	TGCAG	GTATGT	TAATAT	GGACT	AAAGGA	GGCTTT	TCTCA	GGTCG	ACTCTA	GACGC	GTATT	ACCCTG	TATC	CCTARC	GCGTAG	GATCO	ccccc	TACCG	GCTCG	AATTT	TACTAR	CAAATO	GTATTA	TTTATC	CACAGGG	AGGAG	TCCTGG	GTCACG	GTCGCC	ACGCCC	SCCGTC	CTCGAAG	TTCATC	ACGCGC	TCCCA	CTTGAB

е



Wild type, sgRNA E and J (2 DSBs), reverse strand

0⁻³ *

1.0

1.5 ×10⁻³ *

x10⁻⁴ ns 1.5

1

ns_____ns____

ns

ns

+



ns ____ns

÷

0⁻³ *

- -

×10⁻³ *

ns Ť

ns_____ns____

ns

Wild type, sgRNA A and B (2 DSBs), forward strand

0.50 0.25

+







1.0 0.5 0.5





Supplementary Figure 11 | Transcript RNA promotes double-strand gap repair in a sequence-dependent manner via MMEJ – all data for the sense and antisense constructs.

a, Schemes of regions in which Exon1-Exon2 microhomology pairs were identified within the sequenced area to determine the frequency of MMEJ following a gap by the sgRNAs A and B in the sense constructs (left), or by the sgRNAs C/C' and D in the antisense constructs (right), or Exon1-Exon1 microhomology pairs identified following a gap by the sgRNAs E and J in the sense constructs (bottom). Light blue lines indicate examples of microhomologies; the black, parallel lines show the DSB sites; the dark, green box shows the region containing the branch site for the Sense and pCMVA constructs, and the region with the 5'-splice site for the Antisense construct. b, Schemes showing all microhomology pairs 3 bp or longer between the two primer sequences in the Sense and pCMVA constructs, sgRNAs A and B, forward strand (top), reverse strand (bottom). The x-axis shows nucleotide positions from the 5'-primer sequence. First row: DsRed gene exons (solid blue), Intron (solid green). Second row: primer sequence (purple outline), Exon1/Exon2 (blue outline), intron (green outline), branch site (dark green outline). DSB positions are indicated with two red vertical lines. Left margin labels are microhomology IDs (EE, exon-exon); bold/asterisk indicates MMEJ products detected in the sequencing data. Each following row shows the nucleotide sequence of the construct between primer sequences; region deleted by MMEJ repair (gray highlight); microhomology pair (light blue highlight); \pm 10-bp flanking region of microhomology pair used for MMEJ detection in the sequencing data (light green highlight). c, As in (b) for sense constructs Sense, pCMV Δ , and Branch Δ , sgRNAs E and J, forward strand (top), reverse strand (bottom). **d**, As in (b) for Antisense construct, sgRNAs C and D, forward strand (top), reverse strand (bottom). The reversed intron in the antisense constructs is indicated with an upside-down label; 5'-splice site (dark green outline). e, f, Mean of frequencies of MMEJ repair from each microhomology pair detected in the sequencing libraries following a gap by the sgRNAs A and B (e) or sgRNAs E and J (f) in the Sense (red), Branch (green), and pCMVA (yellow) constructs of wild-type and RNase H2A KO cells, and g, following a gap by the sgRNAs C/C' and D in the Antisense (red) and 5'-Splicing Δ (green) constructs of wild-type cells. Plotted data are the mean \pm s.d. of the 4 biological replicates with the individual values shown as dots. The ID of the microhomology pair being analyzed is on top of each bar graph; N=4. The 'ns' on the bar graphs means a non-significant difference (P-value > 0.05, two-tailed Mann-Whitney U test). *, P = 0.029 (two-tailed Mann-Whitney U test). Source data are provided as a Source Data file.

а

0

sense

0

sense

BranchA

BranchA



Supplementary Figure 12 | Impact of transcript RNA on MMEJ and flipped intron repair following overexpression of RNase H1.

a, Mean of frequencies of MMEJ repair from each microhomology pair detected in the sequencing libraries following a gap by the sgRNAs A and B in the Sense (red) and Branch Δ (green) constructs of Control and H1 OX. Plotted data are the mean \pm s.d. of the 4 biological replicates with the individual values shown as dots. The ID of the microhomology pair being analyzed is on top of each bar graph; N=4. The 'ns' on the bar graphs means a non-significant difference (*P*-value > 0.05, two-tailed Mann-Whitney *U* test). *, *P* = 0.029 (two-tailed Mann-Whitney *U* test). b, Frequency of intron flipping caused by re-capture of the intron via NHEJ following a gap by the sgRNAs A and B in the Sense (red) and Branch Δ (green) constructs of Control and H1 OX. Plotted data are the mean \pm s.d. of the 4 biological replicates with the individual values shown as dots; N=4. *, *P* = 0.029 (two-tailed Mann-Whitney *U* test). Source data are provided as a Source Data file.



HEK293 WT, sgRNA A, Individual Exon-Exon MMEJ

HEK293T WT, sgRNA A, Individual Exon-Exon MMEJ



HEK293 WT, sgRNA A, Individual Exon-Intron MMEJ





HEK293T WT, sgRNA A, Individual Exon-Intron MMEJ



HEK293 WT, 2DSB, Individual Exon-Exon MMEJ



Supplementary Figure 13 | RNA-mediated DNA double-strand break/gap repair by MMEJ is independent of DNA replication.

MMEJ frequencies from each microhomology pair detected in the sequencing libraries following a DSB by the sgRNA A or double-strand gap by the sgRNAs A and B in the Sense (red) and Branch Δ (green) constructs of HEK293 and HEK293T cells. The ID of the microhomology pair being analyzed is shown on top of each bar graph. Plotted data are the mean \pm s.d. of the 4 biological replicates with the individual values shown as dots; N=4. The 'ns' on the bar graphs means a non-significant difference (*P*-value > 0.05, two-tailed Mann-Whitney *U* test). Source data are provided as a Source Data file.

Antisense	
Forward sequencing primer	DSB by sgRNA C
GCCTCTTT AAAAGCTTGACCGAGAGCAATCCCGCAGTCTTCAGTGTGGTGATGGTCGTCTATGTGTAAGTCACCAATGCACT CGGAGAAATTTTCGAACTGGCTCTCGTTAGGGCGTCAGAAGTCACCACACTACCAGCAGATACACATTCAGTGGTTACGTGA £5	CAACGATTAGCGACCAGCCGGAATGCTT GTTGCTAATCGCTGGT <mark>CGGCCTTACGA</mark> A PAM
Intron	
GGGTATGTTAATATGGACTAAAGGAGGCTTTTCTGCAGGTCGACTCTAGAACCACTCTACAAAACCAAAACCAGGGTTTATAA CCCATACAATTA TACCTGATTTCCTCCGAAAAGACGTCCAGCTGAGATCTTGGTGAGATGTTTTGGTTTTGGTCCCAAATATT SGRNAC	AATTATACTGTTGCGGAAAGCTGAAAC TTAATATGACAACGCCTTTCGACTTTG
Sequence w	ith branch site
TAAAAAGAAAAAACCCGACTATGCTATTTTAATCATTGAAAAACGAATTTATTT	CTCGAATTTTTACTAACAAATGGTATT
ATTTTCTTTTTGGGCTGATACGATAAAATTAGTAACTTTTGCTTAAATAAA	<u>GAGCTTAAAAATGATTGTTTACCATAA</u>
TAAAGGTTGTCGGTCTCGTACATAGTATACCAGGTCTTTGGGATATGGACACCTGCAATTAGTGAACGCTAACACACCGG. PAM sgRNA D !!!	ACAAGACGATGAC 5'
Branch	
Forward sequencing primer	DSB by sgRNA C
GCCTCTTTAAAAGCTTGACCGAGAGCAATCCCGCAGTCTTCAGTGTGGTGGTGGTGGTCGTCTATGTGTAAGTCACCAATGCACTG CGGAGAAATTTTCGAACTGGCTCTCGTTAGGGCGTCAGAAGTCACCACACTACCAGGAGATACACATTCAGTGGTTACGTGAC <i>ES</i>	AAC GAT TAG CGA CCA GCC GGA A TG CTT TTG CTA A TC GCT GGT CGG CCT TAC GAA PAM
CCCATACAATATCGCACTGATTTCCTCCGAAAAGACGTCCAGCTGAGATCTTGGTGAGATGTTTTGGTTTTGGTCCCAAATATC SgRNA C	TTAATATGACAACGCCTTTCGACTTTG by sgRNA D
TAAAA GAAAAACCCGACTATGCTATTTTAATCATTGAAAACGAATTTATTT	AAC AGCCAGAGCATGTATCATATGGTC
at tit cti tit gggctgatacgataaaat tag taa cti tig cti aaa taa atc tag ggg cat gt c cti tita cca taa taa ag PA	TTGTC GGTCTCGTACATAGTATACCAG
CAGAAACCCTATACCTGTGTGGACGTTAATCACTTGCGATTGTGTGGCCTGTTCTGCTACTG 3' GTCTTTGGGATATGGACACACCTGCAATTAGTGAACGCTAACACCGGACAAGACGATGAC 5'	
iy Reverse sequencing primer	

Supplementary Figure 14 | DNA sequence of the *his3* loci of the yeast constructs.

Blue sequence, exons of the *his3* gene; green sequence, intron; underlined green sequence, canonical GTand -AG splice sites of the intron; underlined dark green sequence, intron sequence containing the branch site. Black arrows, sequencing primers; yellow highlighted sequence, PAM site; orange highlighted sequence, sequence of sgRNA C; purple highlighted sequence, sequence of sgRNA D. Vertical black bar, site of DSB by sgRNA.

Construct	spliced transcript frequency	non-spliced transcript frequency	alt-spliced transcript frequency	non-canonical alt-spliced frequency	unaligned frequency
WT, antisense	0.23	0.73	0	0.029	0.0066
WT, branch∆	0.00057	0.99	0	0.0020	0.0058
spt3, antisense	0.16	0.82	0	0.0160	0.0020
<i>spt3</i> , branch∆	0.0001	0.9981	0	0.0001	0.0017
<i>spt3 rnh1 rnh201,</i> antisense	0.21	0.76	0	0.0258	0.0028
<i>spt3 rnh1 rnh201,</i> branch∆	0.0001	0.9969	0	0.0006	0.0023



С

Construct	spliced transcript frequency	non-spliced transcript frequency	alt-spliced transcript frequency	non-canonical alt-spliced frequency	unaligned frequency	
WT, antisense	0.077	0.91	0	0.0096	0.0013	
WT, branch∆	0.0007	0.998	0	0.0003	0.0012	
ku70, antisense	0.15	0.82	0	0.028	0.0070	-
<i>ku70,</i> branch Δ	0.0007	0.98	0	0.011	0.0061	

Supplementary Figure 15 | The RNA transcribed from the Branch∆ yeast construct has no intron splicing in all genomic backgrounds used in this study.

a, Table of RNA-sequencing results of *his3* transcripts from the Antisense and Branch Δ in wild-type, *spt3*, *spt3 rnh1 rnh201* KO cells. RNA-sequencing reads for each transcript of the different constructs were categorized after alignment to the corresponding DNA sequence (details in Methods). Spliced transcript, a transcript in which the intron was spliced out from the canonical GT---AG-splice sites; alt-spliced transcript, a transcript in which splicing happened at alternative GT---AG sites; non-canonical alternative splicing, a transcript sequence that had splicing at sites different from the GT---AG sites. Splicing frequencies were calculated by dividing the number of the spliced-transcript reads by the total number of reads within each RNA-sequencing library, N=1. **b**, Results of RT-qPCR of the *his3* transcripts generated from each construct. The bar shows the relative amount of the *his3* transcripts. Plotted data are the mean fold change \pm s.d. of the 6 biological replicates with the individual values shown as dots; N=6. The mean value is shown above each bar. **c**, Table of RNA-sequencing results of *his3* transcripts from the Antisense and Branch Δ in wild-type and *ku70* KO cells, N=1. Source data are provided as a Source Data file.



2-DSB	1st	2nd	3rd	4th	5th	Avg.
Antisense	0.891	0.894	0.885	0.890	0.891	0.890
Branch∆	0.885	0.886	0.895	0.890	0.891	0.890

Supplementary Figure 16 | The yeast construct with splicing has similar cleavage efficiency by Cas9 to that without splicing.

Scheme of PCR fragments for the yeast Antisense and Branch Δ used for the *in vitro*-cleavage assay. Cas9 cleavage was done using sgRNA C, D or C and D for the Antisense and Branch Δ constructs. The formulas used to calculate the DSB efficiencies from the molarity of each DNA fragment detected by the Bioanalyzer following cleavage by Cas9 with sgRNA C, D, or C and D are shown. The bp sizes in the formulas are those for the Antisense and Branch Δ (in parenthesis when different). Results of the *in vitro*-cleavage assay are shown in the tables underneath the construct schemes.



Wild type



Supplementary Figure 17 | In/dels are sequence variations specific to DSB repair by NHEJ detected in the yeast constructs.

Variation-position histograms showing the distribution of sequence variations in the 20-bp DSB-sequence windows categorized by the position of the variations and the number of variations. The histograms are arranged in a grid: rows specify the sgRNA(s) used to induce the DSB(s), including the No-DSB controls; columns specify the construct and the type of variation (insertion in orange, deletion in blue, or substitution in gray). The x-axis indicates the position of the variations relative to the DSB site on the reference sequence. Figures with reverse-strand data have their x-axis coordinates reversed so that they correspond to forward-strand coordinates. The y-axis indicates the total number of variations in the DSBsequence windows. The z-axis indicates the total sum of frequencies (log-scale) of DSB-sequence windows with y variations including position x. If a DSB-sequence window has more than one variation (say *k*) at the same position (which can only happen if the DSB-sequence window contains insertions), this window contributes k-fold to the corresponding z-value. The x, y, z axes have been limited to the ranges [-10, 10], [0, 20], and $[10^{-5}, 1]$, respectively, and values outside these ranges have been cropped. Like in the 2-DSB experiments with sgRNAs C & D, in the No-DSB experiments with sgRNAs C & D the reads were aligned to the 2-DSB reference sequence, which is the sequence of the construct between the two sequencing primers with the nucleotides between the two DSB sites deleted. Since the No-DSB reads had small frequency of gap deletion (i.e. intron pop-out, 0.02% to 1.0%), the reads mostly fail to align with the reference sequence, which caused variations (especially substitutions) to be nearly undetectable in many cases. The variations that are detected in the No-DSB controls are likely in reads with gap removal due to leakiness of the pGAL promoter causing Cas9 to be expressed in the cells. a. Variation-position histograms for experiments conducted in the wild-type yeast cells, b, in the spt3 KO cells (wild-type RNase H cells), and **c**, in the *rnh1 rnh201 spt3* KO cells (*rnh1 rnh201*).





Supplementary Figure 18 | RNA-mediated DSB repair following a DNA double-strand gap is promoted in the absence of RNase H function in yeast cells.

a, Pie charts showing frequencies of sequencing reads displaying intron retention or pop-out following a double-strand gap by the sgRNAs C and D in the Antisense and BranchA constructs of yeast spt3 knockout (left, WT) and *rnh1 rnh201 spt3* triple knock-out (right, *rnh1 rnh201*) cells. The fraction of sequences containing the intron is shown in green, and the fraction without the intron is shown in blue. Within the blue fraction of sequences without the intron, the red dotted line marks a small fraction of repaired sequences having an identical sequence with the spliced RNA, which could be the result of either NHEJ or RNA-templated DSB repair (R-TDR). The frequencies of the repair events having the same sequence as the spliced RNA are 0.03% for WT, Antisense ($\pm 0.01\%$), 0.03% for WT, Branch Δ ($\pm 0.04\%$), 2.29% for *rnh1 rnh201*, Antisense ($\pm 1.15\%$), and 0.02% for *rnh1 rnh201*, Branch Δ ($\pm 0.02\%$). Percentages represent an average of 4 repeats with standard deviation in parenthesis; N=4. The percentages of sequences with and without intron are bolded. *, P = 0.029 comparing frequencies of the Branch Δ with those of the Antisense construct via the two-tailed Mann-Whitney U test. **b**, Antisense/Branch Δ frequency ratios for two types of repaired sequences, with intron (green) and without intron (blue), detected in the sequencing libraries following a double-strand gap by the sgRNAs C and D in the spt3 knock-out (WT) and rnh1 rnh201 spt3 triple knock-out (*rnh1 rnh201*) cell types. Plotted data are the mean \pm s.d. of the ratios of the 4 biological replicates with the individual values shown as dots; N=4. *, P = 0.029 (two-tailed Mann-Whitney U test). c, Pie charts showing frequencies of sequencing reads displaying intron retention or pop-out for no DSB controls without Cas9 expression (see Method) in the Antisense and BranchA constructs of yeast WT (left) and ku70 knock-out (right), N=1. Source data are provided as a Source Data file.