

SUPPLEMENTARY FIGURES

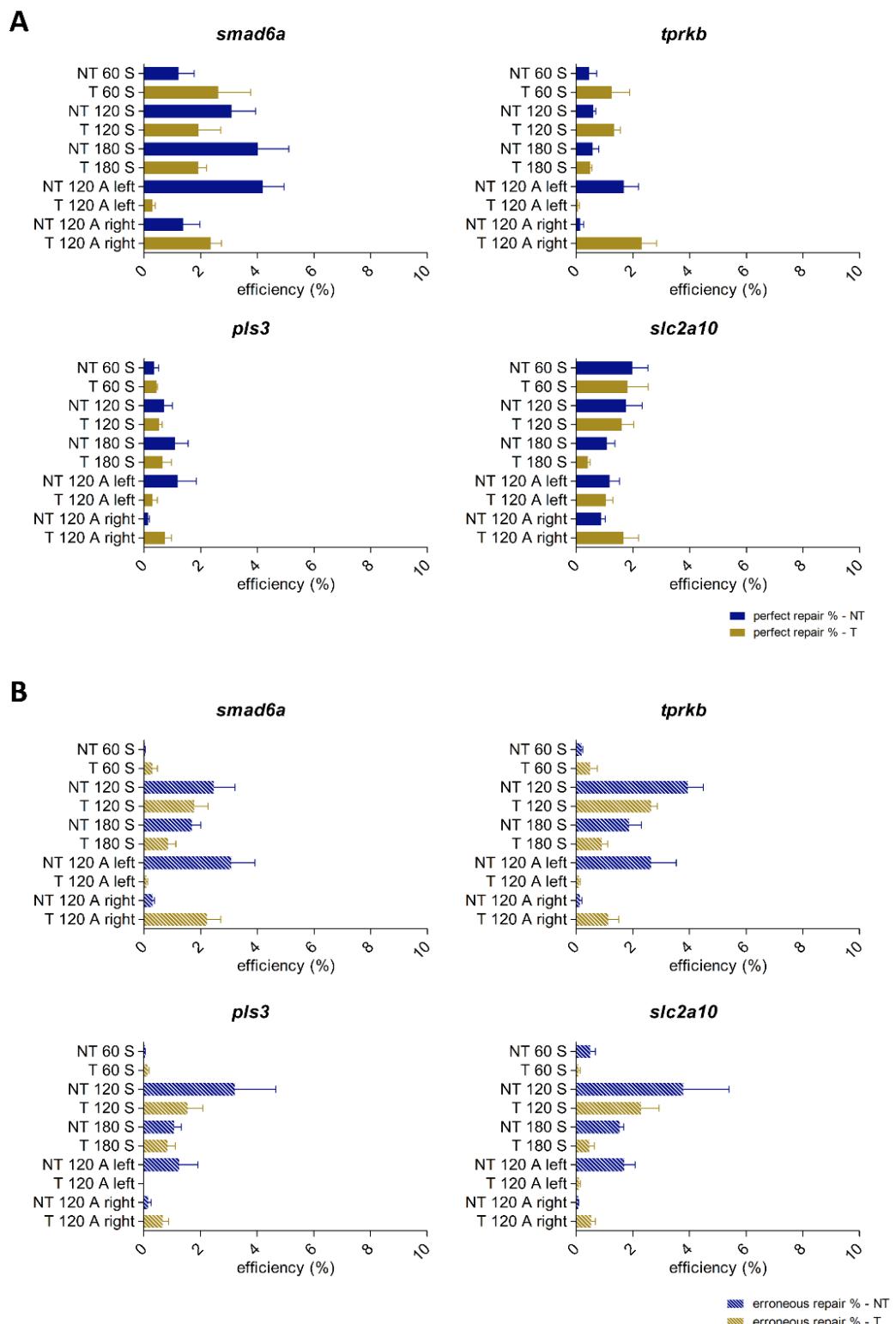


Fig. S1: Impact of repair template homology arm length, strand complementarity and symmetry on HDR efficiency. For each target site and each repair template type, average HDR efficiencies resulting from 5 repetitive experiments were plotted as perfect repair events (panel A) or erroneous repair events (panel B). NT: non-target, T: target, 60-120-180: total repair template length, S: symmetrical, A: asymmetrical. Error bars represent the SEM for 5 independent biological replicates each consisting of a pooled sample of 20 embryos.

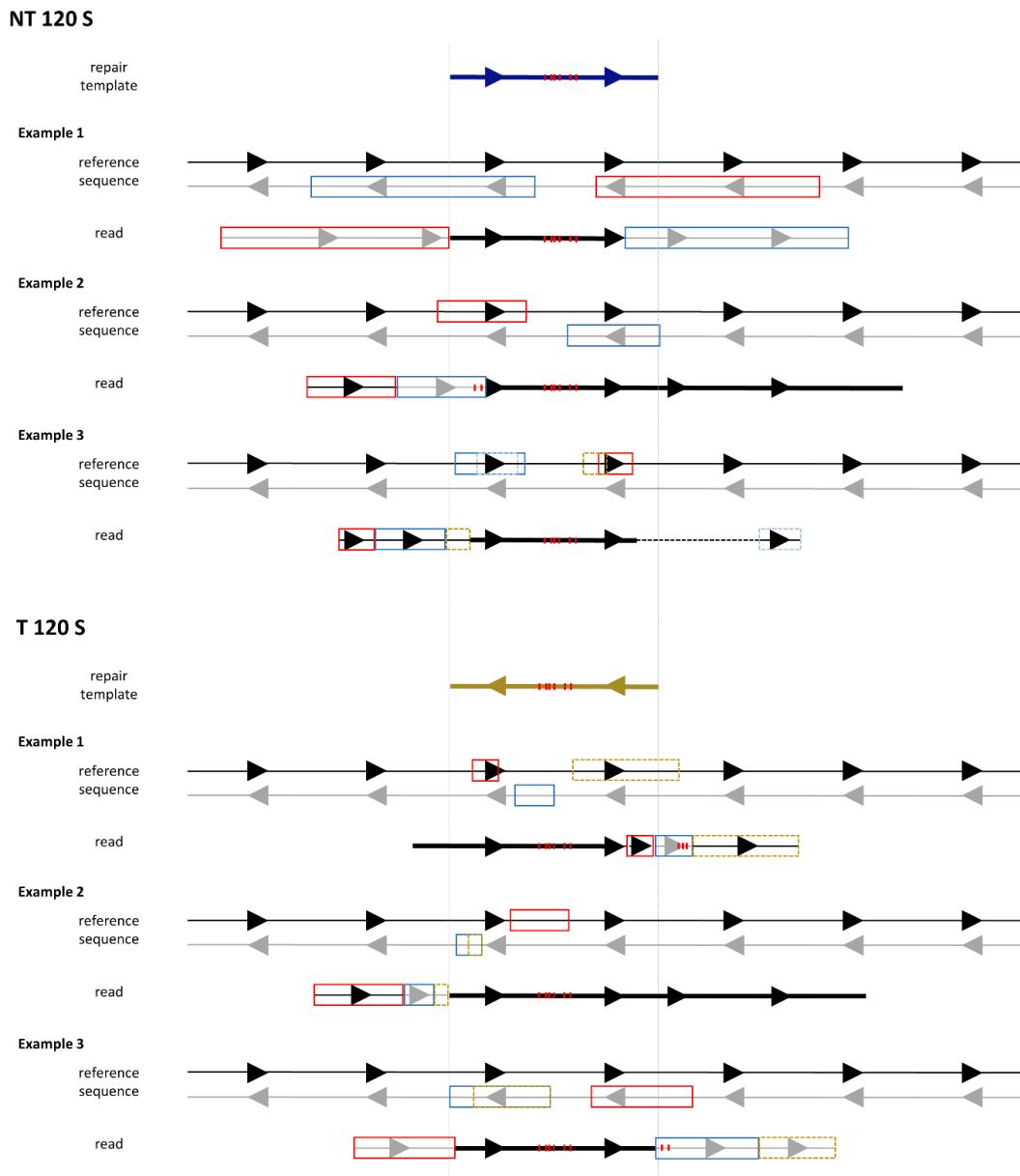


Fig. S2: Schematic representation of erroneous repair template integration events in the *smad6a* zebrafish gene with the NGS reads sequence as reference point. For both the 'NT 120 S' and 'T 120 S' repair templates, 3 examples of NGS reads are schematized to clarify the erroneous integration patterns that were encountered. For each 'Example' in the graph, both the reference sequence and the actual NGS read are depicted. The reference sequence consists of a black and a grey arrowed strand, representing the sense and antisense DNA strand, respectively. These strands serve as a reference frame used to estimate the size of the erroneous integration events and to indicate to which part of the reference sequence the integrated part of the repair template corresponds. The NGS read consists of both bold and thin arrowed segments. The bold segments correspond to the part of the read that actually mapped to the reference sequence during the NGS data analysis workflow. The thin lines correspond to the segments identified as non-matching (categorized as 'clipped off' by the CIGAR algorithm, see Fig. S4). Dashed segments represent an integration event, not corresponding to the sequence of the targeted gene. In both the repair template and the NGS read, precise base pair substitutions are indicated with red marks. Sequences corresponding to these examples are listed in Table S2.

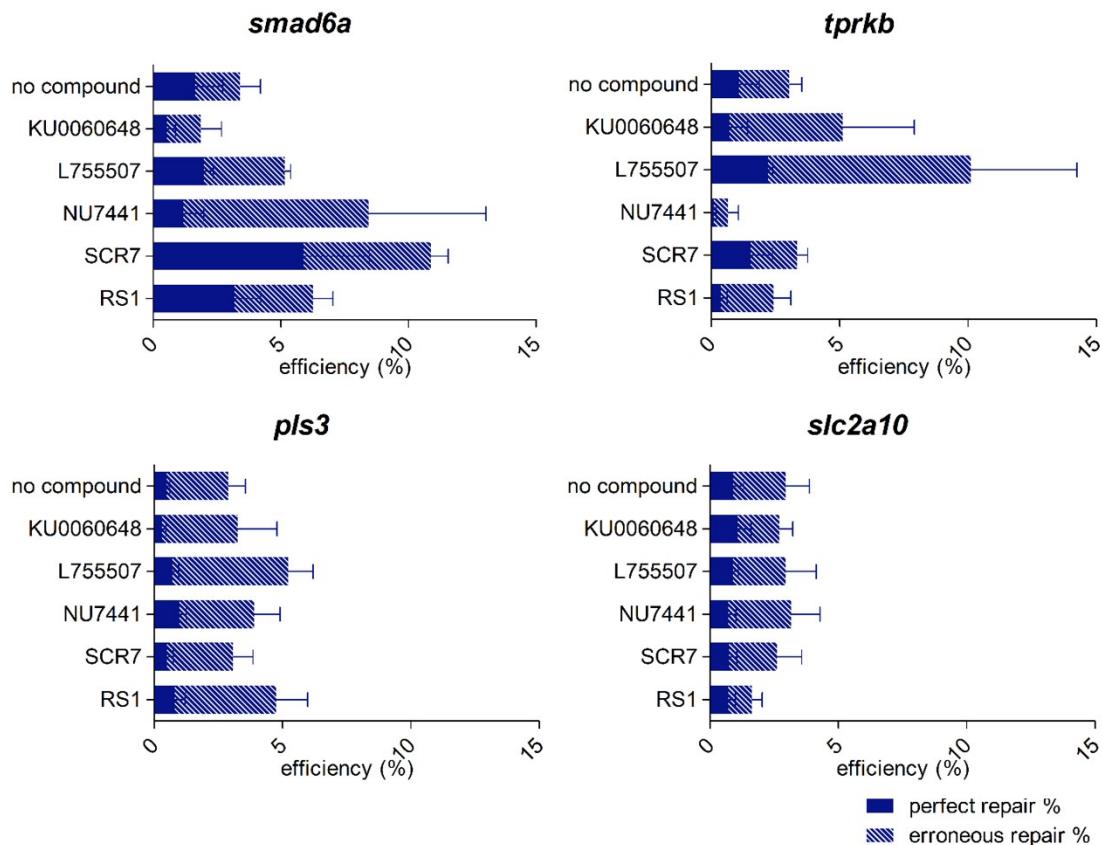


Fig. S3: Impact of chemical compound administration through incubation on HDR efficiency. Twenty embryos injected with mixes containing the NT 120 S repair template were incubated in screening medium supplemented with a chemical compound that either inhibit specific components of the NHEJ pathway, including SCR7, NU7441 and KU0060648 or that were shown to stimulate the HDR pathway, including RS1 and L755507. Three independent experiments were carried out and average total HDR rates are shown, split up in two categories: perfect repair % (plain bars) and erroneous repair % (dashed bars). Error bars represent the SEM for 3 independent biological replicates each consisting of a pooled sample of embryos. Indel rates depicted in this graph, are listed in Table S4. Statistical tests performed: independent samples t-test for normal distributed groups.

1 DSB formation

5' CCTGAGGTAGCTGAGAGATGCTGAGAGGGCCTCGAACCTGGTGACAGGCCACACGGTGCAGCTCCAGTAAGCCACATTACACCAGTTCTGGCCAGCGAGGAGG 3'

GAGCTCCATCCAGCATCTTCTACGACTCTCTCCGCAGCACTTGACCCATGTCCGCCGGGTGTGCCACGCTTCGAGGGTCATTGGTGTAAATGTGGTCACCAAGACGCCGTGCTCCCTCC
3' 5'

2 End resection

5' CCTGAGGTAGGTCGTAAGAGTGTGAGAGAGGGCGTCGTGAACCTGGGTACAGGC GGCCCA-----3' AGG

GGA-----GTGCCACGCTCGAGGGTATTGGTGTAAATGGTCAACCAAGACGGTCGCTCC

3 T 120 S template binding and DNA synthesis – round 1

5' CCTGAGGTAGTCGTAGAA**GAT**TGCTGAGAGGGCGTGTAACTGG**A**TACA**AT**CGTCCA**CCG**TGTGCCAAGCTCCCAGTAAGCCACATTACACCAGTGGTTCTGCGCCAGCAGGA
3' **GGACT**CCATCAGCATCTTCA**G**ACTCTCTCCGAGCACTTGACCT**T**ATGTTAGCAGGGT**G**CGC**C**ACAGCTTCGAGGGT**C**ATTGGTGTAA**T**GTGGT**C**ACCAAGACGGT**C**G**C**TC**C**
3' 5'

GGA-----GTGCCACGCTTCAGGGTCATTGGTGTAAATGGTCACCAAGACGGCGTCGCTCCCTC

4 Template switching (mechanism c in figure 6c) and DNA synthesis – round 2

5'

3'

3'

5'

5 Template switching (mechanism a in figure 6c) and DNA synthesis – round 3

5' CCTGAGGTAGTCCTAGAA**GAT**GCCTGAGAGAGGGCGTGTGA**ACT**TGG**AT**ACA**AT**CGTCCCACGCCGTGTGCCAGCTCCAGTAAGCCACATT
 3' **CA**CGACAGTGTTGCAGCAGAG**GA**TGCTGAGAG**AT**GG**AT**TGC**AT**CGAT**CA**GT**AC**GC**AC**


7 Resulting NGS read sequence

ACATAGACCTGTACCCGAGGTAGGTCGTAGAATGCTGAGAGAGGCCTCGTGAACGGATAACAATCGTCCCACCGTGTGCGAA
GCTCCCAGTAAGCCACATTACACCAGTGGTTCTGCGCCAGCGAGGAAGATGCTGAGGAAGAGATTGTATCCAGTTCACGACGCACC
TACCCCTACACGGGTGCGAACGCTCCAGTAAGCCACATTACACCAGTGGTTCTGCGCCAGCGAGGAGGGAGACATACTGGCAT

Fig. S4: Multiple template switching events occur during SDSA-dependent DSB repair. The origin of the complex mutational patterns encountered in this study is depicted, using an NGS read sequence, obtained from the analysis of the T 120 S repair template at the *smad6a* sgRNA target site. DSB formation (step 1) by Cas9 is followed by DNA end resection (step 2). Binding of the repair template (in yellow), is followed by DNA synthesis (blue arrow), leading to the lengthening of the 3' single-stranded DNA tail (blue bases) (step 3). Note the introduction of the intended precise base pair

substitutions, (underlined in the repair template). The complex dissociates and the 3' DNA tail, reanneals to the repair template, using a 2 bp microhomology pattern (step 4). This mechanism corresponds to 'mechanism c' depicted in Fig. 6C. From here DNA synthesis (green arrow) and further lengthening of the 3' single-stranded DNA tail (green bases) takes place. Red-colored bases are introduced via a non-identified template switching and DNA synthesis step. Complex dissociation and a reannealing step, using a 7 bp microhomology pattern, corresponding to 'mechanism a' depicted in Fig. 6C, leads to further lengthening of the 3' single-stranded DNA tail by DNA synthesis in the opposite direction (pink arrow and bases) (step 5). Again, brown-colored bases are introduced via a non-identified template switching and DNA synthesis step. Finally, reannealing according to 'mechanism d' depicted in Fig. 6C, using a 3 bp microhomology pattern, leads to a final DNA synthesis step (light green arrow and bases), followed by the resolution step (step 6). The resulting NGS read sequence, with color codes, is depicted at the bottom of the graph (step 7). The black-colored bases at the edges perfectly match the endogenous DNA sequence flanking the 120 depicted base pairs.

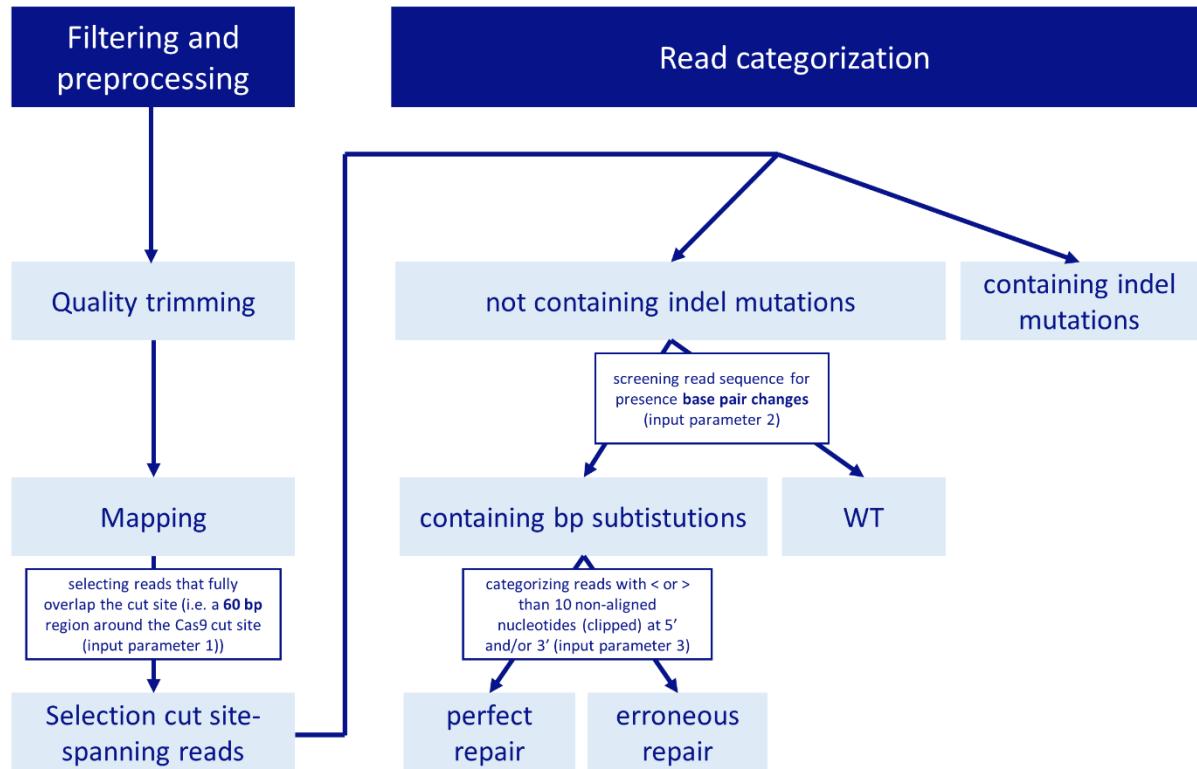


Fig. S5: Adapted BATCH-GE Next-Generation Sequencing data analysis pipeline for indel, perfect and erroneous repair rate calculation. Following quality trimming and read mapping, reads that fully overlap the cut site were divided into four categories (indel mutations, wild type, perfect repair and erroneous repair). Discrimination between perfect and erroneous repair is based on the number of nucleotides that are clipped from the alignment, i.e. 'S' or 'H' in the CIGAR specification for respectively soft and hard clipping. The BATCH-GE script needs specification of certain parameters. First, the precise screening region which is used in the last filtering step and the first categorizing step where the reads are divided into reads that do or do not contain any indels, can be modified (30 bp up and downstream of the theoretical cut site in our analyses – input parameter 1). Second, the BATCH-GE analysis method requires a repair template sequence as an input parameter for the HDR analysis. In our analyses, we supplied the repair template sequence fragment running from the first to the last base pair substitution (input parameter 2). A third input parameter is required as well, stating that an analysis discriminating between perfect and erroneous repair is desired (input parameter 3). Input parameters are also listed in Table S12.

SUPPLEMENTARY TABLES

Table S1: perfect and erroneous repair rates presented in Fig. 1B

sgRNA target	strand complementarity	length/symmetry	perfect repair (%)					erroneous repair (%)				
			1	2	3	4	5	1	2	3	4	5
<i>smad6a</i>	non-target	60 S	2,86	0,00	1,18	0,07	2,01	0,00	0,00	0,00	0,07	0,06
		120 S	5,97	1,54	2,12	4,04	1,78	0,16	2,56	1,98	2,69	4,89
		180 S	2,86	4,26	2,79	2,01	8,14	1,17	1,54	2,85	1,15	1,74
		120 A left	4,80	6,16	3,47	1,68	4,83	3,50	5,24	0,69	1,68	4,29
		120 A right	1,91	0,41	3,51	0,37	0,70	0,18	0,43	0,10	0,46	0,35
	target	60 S	7,05	0,90	0,94	1,52	2,68	0,07	0,00	0,94	0,46	0,00
		120 S	1,77	5,01	1,01	0,78	1,05	1,77	3,45	2,02	0,88	0,70
		180 S	2,25	2,43	2,47	0,90	1,48	1,29	0,29	1,51	0,13	1,02
		120 A left	0,29	0,65	0,21	0,31	0,00	0,00	0,16	0,21	0,00	0,00
		120 A right	1,70	1,38	3,27	3,15	2,28	1,61	3,55	2,87	2,32	0,78
<i>tprkb</i>	non-target	60 S	0,00	0,07	1,40	0,22	0,59	0,00	0,28	0,11	0,28	0,22
		120 S	0,39	0,78	0,68	0,55		3,92	5,49	3,40	2,94	
		180 S	1,42	0,28	0,48	0,44	0,25	2,85	0,83	2,71	2,07	0,87
		120 A left	1,68	1,09	0,75	3,64	1,24	1,68	0,54	1,50	4,55	4,97
		120 A right	0,00	0,06	0,65	0,00	0,00	0,00	0,25	0,11	0,33	0,00
	target	60 S	0,35	0,34	3,51	0,22	1,83	0,14	0,40	0,19	0,22	1,50
		120 S	1,48	1,89	0,85	1,08		2,63	2,43	3,28	2,28	
		180 S	0,63	0,32	0,61	0,41	0,48	0,76	1,62	0,79	0,93	0,48
		120 A left	0,26	0,00	0,00	0,00	0,00	0,20	0,24	0,00	0,00	0,00
		120 A right	2,68	1,04	4,20	1,85	1,74	2,01	0,00	1,87	1,11	0,63
<i>pls3</i>	non-target	60 S	0,28	0,91	0,36	0,26	0,00	0,00	0,08	0,09	0,06	0,00
		120 S	0,49	1,78	0,77	0,51	0,00	4,87	8,13	1,28	1,52	0,25
		180 S	1,18	2,82	0,26	0,80	0,46	0,39	1,69	1,55	1,20	0,46
		120 A left	0,00	1,81	3,50	0,33	0,32	0,00	1,81	3,50	0,00	0,96
		120 A right	0,13	0,33	0,14	0,08	0,00	0,00	0,57	0,00	0,16	0,00
	target	60 S	0,39	0,45	0,43	0,58	0,29	0,26	0,23	0,00	0,19	0,00
		120 S	0,39	0,52	0,34	0,37	1,00	0,20	0,77	1,37	3,18	2,23
		180 S	0,57	0,30	0,54	0,00	1,87	1,72	0,76	0,00	0,92	0,80
		120 A left	0,95	0,12	0,41	0,00	0,00	0,00	0,00	0,00	0,00	0,00
		120 A right	0,97	0,00	1,02	0,38	1,29	0,00	0,53	1,32	0,76	0,74
<i>slc2a10</i>	non-target	60 S	1,37	1,78	0,51	3,68	2,57	0,81	0,11	0,07	0,99	0,51
		120 S	0,65	3,74	0,76	1,40	2,20	0,76	9,57	1,76	1,90	4,93
		180 S	0,88	1,00	1,42	1,89	0,23	1,12	1,55	1,78	1,89	1,32
		120 A left	1,29	2,26	0,65	0,20	1,45	1,93	3,11	1,30	1,01	1,09
		120 A right	0,57	0,62	1,26	1,15	0,78	0,05	0,06	0,17	0,06	0,00
	target	60 S	1,77	0,49	4,47	0,38	1,91	0,00	0,05	0,32	0,00	0,00
		120 S	0,46	1,46	0,99	2,85	2,23	1,84	1,46	3,81	0,62	3,74
		180 S	0,48	0,28	0,19	0,60	0,49	0,10	0,46	0,19	1,09	0,49
		120 A left	1,90	0,53	0,79	0,84	1,16	0,35	0,10	0,00	0,00	0,00
		120 A right	3,74	0,70	1,13	1,26	1,48	1,10	0,35	0,32	0,58	0,29

Table S2: sequence NGS reads depicted in Fig. 2 and Fig. S2

Repair template	Example	Read sequence
NT 120 S	1	TTAACATGTCGCTTCCACCTCTTCAAGATGCCAGTATGACTCCCTCTCGCTGGCGCAGAACCCTGGTGAA TGTGGCTTACTGATGCTGAGAGAGGCCTCGTAAGTGGATAACATCGTCCCACCGCTGTGCGAAGCTCCAGT AAGCCACATTACACCGGTGGTCTCGGCCCTGTACCCAGTTACGACGCCCTCTCAGCATCTCTACGACCT ACCTCAGGGTACAGGCCTATGTTGGG
	2	TACCCCTGAGGTAGGTCGTAGAGGATGCTGAGAGAGGCCTCGTAAGTGGGTACAGGCCCTCTCGCTGG GCGCAGAACCCTGGTGAATGTGGTACTGGAGCTCGCACCGCTGGGAGGCGTCGTGAACTGGATA CAATCGTCCCACCGCGGTGCGAAGCTCCAGTAAGCCACATTACACCAGTGGTCTCGGCCAGCGAGGAGGG AGACATACTGGCATCTGAAAGAGAGGTGGGAACG
	3	TTACACCACTGGTCTGAGAAGATGCTGAGAGAGGCCTCGTAAGTGGTAACACATTACACATCGTAGAAGATGCTG AGAGAGCGCTCGTAAGTGGATAACATCGTCCCACCGCTGTGCGAAGCTCCAGTAAGCCGCAATTACACAGT GGTCTCGGCCCTGTGGTCTAACCGTCGGATATCGGAAGCTGAAGCTCGACAGGTCTCACAGTGCAG GTCCTACTAATGTGAGAGAGGCCTCGTGAAG
T 120 S	1	ACATAGACCTGACCCGAGGTAGGTCGTAGAATGCTGAGAGAGGCCTCGTAAGTGGATAACATCGTCCC CGCGTGTGCGAAGCTCCAGTAAGCCACATTACACCGTGGTCTCGGCCAGCGAGGAAGATGCTGAGGAAG ATTGTATCCAGTTACGACGCACCTACCCCTACCGGTGCGAAGCTCCAGTAAGCCACATTACACCGTGGT CTCGCCAGCGAGGAGGGAGACATACTAGCATCTGAAAGAGAGGTGAAACGACATGTTAAGCATAAATT AAAATAGAGAACACGAATAAAATAAAAT
	2	CGTGAACCTGGTACAGGCCAGCATCTTACGAGCATCTTGAGGTAGGTCGTAGAAGATGCTGAGA GAGGCCTCGTAAGTGGATAACATCGTCCCACCGCTGTGCGAAGCTCCAGTAAGCCACATTACACCGTGGT TCTCGCCAGCGAGGAGGGAGACATACTAGCATCTGAAAGAGAGGTGAAACGACATGTTAAGCATAAATT AAAATAGAGAACACGAATAAAATAAAAT
	3	TATGTCTCCCTCTCGCTGGCGCAGAACCCTGGTGAATGTGGCTTACTGAGGTAGGTCGTAGAAGATGCTG AGAGAGCGCTCGTAAGTGGATAACATCGTCCCACCGCTGTGCGAAGCTCCAGTAAGCCACATTACATTGTA TCCAGTTACGACGCCTCTCAGCATCTACGACCTACCTCAGGTACCCAGTACCCAGTTACGACGCCCTCT CTCAGCATCTCTACGAC

Table S3: perfect repair rates presented in Fig. 3

		perfect repair (%)																																		
Experiment		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5					
Position bp substitution		-14					-9					-8					-5					-2					5									
<i>smad6a</i>	NT	60 S	0,00	0,00	0,08	0,00	0,45	0,00	0,00	0,00	0,07	0,65	0,00	0,00	0,00	0,07	0,65	0,00	0,00	1,18	0,07	0,71	2,86	0,00	1,18	0,07	2,01	2,86	0,00	1,26	0,07	1,82				
		120 S	5,97	0,52	1,39	2,70	1,77	5,97	1,54	1,47	3,03	1,78	5,89	1,54	1,44	3,03	1,78	5,97	1,54	1,38	3,03	1,78	5,97	1,54	2,12	4,04	1,78	5,97	1,54	1,92	4,04	1,78				
		180 S	0,93	2,87	1,69	1,43	8,14	0,99	3,43	2,79	1,72	8,14	0,79	3,43	2,92	1,65	8,14	0,94	4,12	2,86	1,80	8,14	2,86	4,26	2,79	2,02	8,14	2,65	4,26	2,72	1,73	6,94				
		120 A left	4,28	5,85	3,48	1,40	4,29	4,75	5,86	3,48	1,68	4,29	4,75	6,01	3,48	1,68	4,29	4,75	6,16	3,48	1,68	4,29	4,81	6,16	3,48	1,68	4,82	4,53	5,99	2,09	0,84	1,87				
		120 A right	0,49	0,02	3,29	0,05	0,18	0,85	0,02	3,31	0,05	0,53	0,80	0,02	3,27	0,05	0,53	0,76	0,12	3,24	0,06	0,53	1,92	0,41	3,51	0,37	0,70	1,43	0,25	3,34	0,26	0,53				
	T	60 S	0,15	0,21	0,00	0,61	0,19	6,52	0,28	0,32	0,91	1,72	6,52	0,28	0,32	0,91	1,72	6,52	0,28	0,62	0,91	1,72	7,04	0,90	0,94	1,51	2,68	6,67	0,55	0,62	1,36	2,68				
		120 S	0,81	1,11	1,00	0,88	0,26	1,13	1,71	1,01	0,78	0,44	1,29	1,71	1,01	0,78	0,35	1,13	1,71	1,01	0,78	0,44	1,77	5,01	1,01	0,78	1,05	1,45	4,92	1,01	0,78	0,78				
		180 S	0,24	0,44	1,51	0,26	0,78	0,80	1,33	2,36	0,45	1,05	0,80	1,25	2,36	0,45	1,05	0,88	1,33	2,36	0,48	1,02	2,25	2,43	2,47	0,90	1,47	1,85	1,98	2,47	0,55	1,41				
		120 A left	0,00	0,41	0,21	0,31	0,37	0,00	0,41	0,21	0,31	0,00	0,41	0,21	0,31	0,00	0,41	0,21	0,31	0,00	0,29	0,65	0,21	0,31	0,00	0,00	0,73	0,21	0,31	0,00	0,00					
		120 A right	0,75	0,78	0,98	1,87	0,67	1,22	0,78	1,42	2,25	1,55	1,32	0,98	1,42	2,20	1,55	1,22	1,18	1,42	2,25	1,55	1,70	1,38	3,27	3,15	2,28	1,70	1,19	2,64	2,93	2,23				
Position bp substitution		-14					-8					2					5					8					11									
<i>tprkb</i>	NT	60 S	0,06	0,07	1,26	0,03	0,30	0,00	0,07	1,30	0,06	0,37	0,00	0,07	1,41	0,22	0,60	0,06	0,07	1,48	0,22	0,60	0,00	0,07	1,48	0,19	0,60	0,00	0,07	0,97	0,16	0,60				
		120 S	0,47	0,76	0,79	0,37	0,39	0,78	0,69	0,55	0,39	0,78	0,68	0,55	0,39	0,78	0,79	0,37	0,39	0,76	0,69	0,37	0,39	0,53	0,68	0,18	0,39	0,53	0,68	0,18						
		180 S	0,28	0,42	0,32	0,37	0,13	0,28	0,28	0,48	0,29	0,25	1,43	0,28	0,48	0,44	0,25	1,71	0,42	0,55	0,44	0,38	1,43	0,28	0,48	0,44	0,25	1,41	0,28	0,32	0,44	0,38				
		120 A left	0,84	0,54	0,75	2,73	0,00	1,05	1,08	0,75	3,64	1,24	1,68	1,08	0,75	3,64	1,24	1,68	1,08	0,75	3,64	1,24	1,68	1,08	0,75	3,64	1,24	0,84	0,55	0,75	0,90	1,22				
		120 A right	0,20	0,00	0,11	0,00	0,00	0,00	0,00	0,11	0,05	0,00	0,00	0,06	0,65	0,00	0,00	0,00	0,00	0,76	0,00	0,00	0,00	0,00	0,65	0,00	0,00	0,00	0,00	0,00	0,00					
	T	60 S	0,28	0,13	2,28	0,17	0,21	0,00	0,07	2,37	0,22	0,45	0,36	0,34	3,50	0,22	1,83	0,21	0,34	3,50	0,22	1,75	0,00	0,20	3,22	0,17	1,71	0,00	0,14	3,22	0,22	1,59				
		120 S	0,52	0,54	0,48	0,12	0,62	0,54	0,61	0,23	1,48	1,89	0,85	1,08	1,37	1,62	0,98	0,96	1,48	1,62	0,98	0,96	1,48	1,36	0,98	0,84	1,48	1,36	0,98	0,84	1,48	0,38				
		180 S	0,38	0,54	0,53	0,41	0,35	0,38	0,00	0,44	0,10	0,14	0,63	0,32	0,62	0,42	0,49	0,88	0,32	0,78	0,31	0,62	0,38	0,32	0,62	0,31	0,55	0,51	0,32	0,53	0,49					
		120 A left	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,26	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00					
		120 A right	0,67	0,00	2,15	0,37	0,48	0,67	0,00	2,15	0,55	0,78	2,68	1,04	4,20	1,85	1,74	2,68	1,04	4,05	1,85	1,74	2,67	1,04	4,20	1,65	1,74	1,35	1,04	4,20	1,85	1,74				
Position bp substitution		-15					-12					-3					1					10					1					6				
<i>pls3</i>	NT	60 S	0,14	0,49	0,09	0,06	0,17	0,78	0,74	0,27	0,58	0,87	0,28	0,99	0,36	0,32	0,17	0,28	0,91	0,36	0,39	0,17	0,21	0,58	0,27	0,27	0,13	0,00	0,00	0,00	0,00	0,00	0,00			
		120 S	0,49	1,34	0,51	0,76	0,25	0,56	1,56	0,51	0,76	0,51	0,63	1,66	0,77	0,51	0,25	0,49	1,77	0,77	0,51	0,00	0,21	0,85	0,77	0,26	0,51	0,00	0,00	0,00	0,00	0,00	0,00			
		180 S	0,79	1,69	0,51	0,40	0,46	0,79	2,82	0,51	1,19	0,92	1,19	1,69	0,51	0,79	0,46	1,19	2,82	0,26	0,80	0,46	0,79	1,69	0,26	0,40	0,00	0,00	0,00	0,00	0,00	0,00				
		120 A left	0,00	1,30	2,09	0,33	0,32	0,00	1,81	2,09	0,66	0,32	0,00	1,55	3,49	0,33	0,32	0,00	1,81	3,49	0,33	0,32	0,00	1,29	0,69	0,00	0,32	0,00	0,00	0,00	0,00	0,00	0,00			
		120 A right	0,00	0,19	0,29	0,04	0,00	0,54	0,29	0,21	0,40	0,00	0,27	0,34	0,14	0,08	0,00	0,27	0,34	0,14	0,08	0,00	0,13	0,19	0,14	0,12	0,00	0,00	0,00	0,00	0,00	0,00				
	T	60 S	0,00	0,11	0,00	0,10	0,00	0,13	0,68	0,86	0,49	0,96	0,26	0,23	0,29	0,58	0,07	0,39	0,45	0,43	0,58	0,29	0,00	0,11	0,29	0,68	0,07	0,00	0,00	0,00	0,00	0,00				
		120 S	0,20	0,52	0,00	0,44	0,59	0,52	0,00	0,00	0,66	0,00	0,52	0,35	0,18	1,01	0,39	0,52	0,35	0,38	1,01	0,20	0,52	0,35	0,38	0,89	0,00	0,00	0,00	0,00	0,00	0,00				
		180 S	0,19	0,15	0,00	0,00	0,27	0,95	0,90	0,27	0,00	0,80	0,77	0,30	0,27	0,00	0,54	0,57	0,30	0,54	0,00	1,87	0,77	0,46	0,27	0,00	0,27	0,00	0,00	0,00	0,00					
		120 A left	0,00	0,00	0,20	0,00	0,00	0,47	0,41	0,00	0,00	0,00	0,12	0,41	0,00	0,00	0,00	0,95	0,12	0,41	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00				
		120 A right	0,32	0,00	0,15	0,19	0,18	0,65	1,60	0,58	0,75	1,09	1,30	0,00	1,16	0,38	0,92	0,97	0,00	1,03	0,38	1,29	0,32	0,53	0,73	0,38	0,92	0,00	0,07	0,06	0,11					

Table S4: perfect and erroneous repair rates presented in Fig. 4 (compound injection)

gene	compound	perfect repair (%)					erroneous repair (%)				
		1	2	3	4	5	1	2	3	4	5
<i>smad6a</i>	no compound	5,97	1,54	2,12	4,04	1,78	0,16	2,56	1,98	2,69	4,89
	KU0060648	5,92	1,76	9,41	0,81	1,58	4,86	0,93	1,75	1,94	2,06
	L755507	5,34	1,48	2,59	3,48	5,96	1,93	6,49	2,65	2,45	2,55
	NU7441	3,38	5,71	1,19	2,67	1,37	0,75	1,27	4,07	2,03	0,68
	SCR7	2,16	1,07	0,89	2,65	0,68	3,72	1,16	0,78	4,76	0,68
	RS1	1,20	2,35	3,65	2,22	3,99	0,00	4,51	0,00	2,22	5,52
<i>tprkb</i>	no compound	0,39	0,78	0,68	0,55		3,92	5,49	3,40	2,94	
	KU0060648	0,87	2,35	0,79	0,58	2,34	5,81	7,06	6,15	0,00	2,34
	L755507	0,55	1,85	1,14	0,00	0,00	2,89	4,61	6,26	0,00	2,94
	NU7441	0,00	0,00	1,29	0,00	0,00	2,15	3,25	3,38	0,68	1,64
	SCR7	0,00	0,24	0,50	0,00	0,00	2,49	0,47	1,00	11,25	8,51
	RS1	0,00	1,95	0,00	0,00	1,89	0,00	3,25	1,69	2,61	1,89
<i>pls3</i>	no compound	0,49	1,78	0,77	0,51	0,00	4,87	8,13	1,28	1,52	0,25
	KU0060648	1,75	0,66	1,42	0,58	1,23	5,24	7,23	8,78	3,69	3,70
	L755507	1,40	0,55	0,54	1,23	1,54	4,72	4,98	4,70	2,87	1,54
	NU7441	1,16	2,52	0,88	0,37	0,41	1,45	9,88	11,82	1,12	2,06
	SCR7	1,73	1,46	9,43	1,28	1,72	10,64	5,33	5,66	4,70	0,00
	RS1	2,42	0,44	2,16	0,00	0,00	2,42	3,95	4,58	6,28	0,76
<i>slc2a10</i>	no compound	0,65	3,74	0,76	1,40	2,20	0,76	9,57	1,76	1,90	4,93
	KU0060648	0,79	2,40	0,99	1,20	3,06	1,64	2,99	1,54	3,23	7,87
	L755507	0,09	2,02	0,42	0,97	4,02	0,89	2,26	0,60	1,74	2,68
	NU7441	0,00	0,00	0,90	1,23	2,07	0,00	1,21	3,01	4,53	2,53
	SCR7	1,03	1,07	0,80	0,76	0,00	3,55	3,13	1,84	0,95	1,83
	RS1	0,81	3,62	1,62	1,44	4,06	2,03	1,88	1,98	3,31	6,35

Table S5: perfect and erroneous repair rates presented in Supplementary Fig. S3 (compound incubation)

gene	compound	perfect repair (%)			erroneous repair (%)		
		1	2	3	1	2	3
<i>smad6a</i>	no compound	1,33	3,64	0,00	2,80	2,27	0,17
	KU0060648	1,19	0,00	0,39	2,83	0,00	1,17
	L755507	1,33	1,97	2,63	2,87	3,03	3,64
	NU7441	0,88	2,68	0,00	4,27	1,17	16,30
	SCR7	2,47	4,30	10,93	5,07	3,75	6,11
	RS1	1,45	4,98	3,14	4,55	1,87	2,79
<i>tprkb</i>	no compound	2,63	0,61	0,00	2,63	1,02	2,26
	KU0060648	0,00	2,12	0,00	1,53	1,69	10,00
	L755507	1,89	2,58	2,15	16,04	2,58	5,12
	NU7441	0,30	0,00	0,00	0,30	0,00	1,36
	SCR7	0,00	1,74	2,89	1,52	2,62	1,27
	RS1	0,87	0,23	0,00	0,87	3,17	2,17
<i>pls3</i>	no compound	0,67	0,26	0,54	3,19	2,93	1,08
	KU0060648	0,56	0,35	0,00	1,83	5,98	1,04
	L755507	1,12	0,25	0,75	4,31	2,97	6,29
	NU7441	1,08	0,47	1,39	2,94	1,17	4,65
	SCR7	0,88	0,00	0,60	4,09	1,42	2,19
	RS1	1,43	0,95	0,00	2,04	6,20	3,68
<i>slc2a10</i>	no compound	1,62	0,25	0,87	3,62	0,39	2,08
	KU0060648	0,18	1,13	1,96	0,57	2,04	2,24
	L755507	1,29	0,57	0,82	4,42	0,64	1,12
	NU7441	0,94	0,08	1,09	3,11	0,23	4,00
	SCR7	1,07	0,14	1,04	3,38	0,07	2,13
	RS1	0,16	0,95	1,03	0,10	1,28	1,37

Table S6: perfect and erroneous repair rates presented in Fig. 5

gene	fish #	perfect repair (%)	erroneous repair (%)
<i>smad6a</i>	1	13,75	0,13
	2	22,97	0,00
	3	8,51	0,00
	4	0,26	0,26
	5	0,70	22,06
	6	0,48	0,00
	7	10,65	21,82
	8	0,14	0,14
<i>slc2a10</i>	1	0,00	1,82
	2	10,33	6,04
	3	0,15	0,00
	4	0,04	0,00
	5	0,05	0,00
	6	0,19	0,00
	7	0,27	0,40
	8	0,17	0,08

Table S7: gBlock designs for *tprkb*, *pls3*, *slc2a10* and *smad6a*

gene	exon	strand	protospacer + PAM	double-stranded DNA (gBlock) sequence (5'-3')
<i>tprkb</i>	2	+	GGATGCATTCCAGATCCTGG <u>TGG</u>	CCGCTAGCTAATACGACTCACTATAGGATGCATTCCAGATCCT GGGTTTAGAGCTAGAAATAGCAAGTAAAATAAGGCTAGTC CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTT
<i>pls3</i>	7	+	GGCTGTCACGTGGTCAACATT <u>GG</u>	CCGCTAGCTAATACGACTCACTATAGGCTGTACGTGGCAA CATGTTTAGAGCTAGAAATAGCAAGTAAAATAAGGCTAGT CCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTT
<i>slc2a10</i>	2	+	AAAGCAAAGATAACATGCGG <u>AGG</u>	CCGCTAGCTAATACGACTCACTATAGGAGCAAAGATAACATG CGGGTTTAGAGCTAGAAATAGCAAGTAAAATAAGGCTAGT CCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTT
<i>smad6a</i>	4	-	GGGTACAGGCCGCCACAC <u>GGG</u>	ACCGCTAGCTAATACGACTCACTATAGGGTACAGGCCGCCA CACGTTTAGAGCTAGAAATAGCAAGTAAAATAAGGCTAGT CCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTT

Table S8: single-stranded oligodeoxynucleotide (ssODN) designs for *tprkb*, *pls3*, *slc2a10* and *smad6a*

gene	orientation	length (nt)	ssODN sequence (5'-3')
<i>tprkb</i>	non-target	60	TAATAAAGGTTGGACGCATTCAGATCCTCGTAGGCCACCAATAAGCAGTCACCTTC
		120	TGTACGTATATTACCTTTTATTTTTAAATAAAGGTTGGACGCATTCAGATCCTCGTAGGCCACCAATAAGCAGTCACCTTC
		180	AAATAATAGTCAAATATATTCTAAAACATGTACGTATATTACCTTTATTTTTAAATAAAGGTTGTGGACGCATTCAGATCCTCGTAGGCCACCAATAAGCAGTCACCTTCATAAAATCGGGAAAATGAAA
		left	AAATAATAGTCAAATATATTCTAAAACATGTACGTATATTACCTTTATTTTTAAATAAAGGTTGTGGACGCATTCAGATCCTCGTAGGCCACCAATAAGCAGTCACCTTC
		right	TAATAAAGGTTGGACGCATTCAGATCCTCGTAGGCCACCAATAAGCAGTCACCTTCATAAAATCGGGAAAATGAAA
	target	60	GAAGGTGAACTGCTTATTGGTGGCTACGAGGATCTGAAATCGTCCACAACCTTATTAA
		120	GGCTCTGGTTTCTATTTCCGATTTGAGGTGAACTGCTTATTGGTGGCTACGAGGATCTGAAATCGTCCACAACCTTATTAA
		180	GTGAAAGATTGAAAATGATTTCAGAGTAAAGGCTCTGGTTTCTATTTCCGATTTGAGGTGAACTGCTTATTGGTGGCTACGAGGATCTGAAATCGTCCACAACCTTATTAA
		left	GTGAAAGATTGAAAATGATTTCAGAGTAAAGGCTCTGGTTTCTATTTCCGATTTGAGGTGAAATCGTACATGTTTAA
		right	GTGAAAGATTGAAAATGATTTCAGAGTAAAGGCTCTGGTTTCTATTTCCGATTTGAGGTGAAATCGTCCACAACCTTATTAA
<i>pls3</i>	non-target	60	AGCCTCTGCCATTGGGTGCCACGTGGTAATTTGGAGCATTAGACCTTCGAGAAGGGAA
		120	GCAGGAGAACCTAACTTGCTCAATTAGCCTCTGCCATTGGGTGCCACGTGGTAATTTGGAGCATTAGACCTTCGAGAAGGGAA
		180	GTCTATATTGAAAAACATTCTTCCATCCTCGAGGAGAACCTCATCTGGTTAGGGCTTGTGGCA
		left	GTCTATATTGAAAAACATTCTTCCATCCTCGAGGAGAACCTCATCTGGTTAGGGCTTGTGGCA
		right	AGCCTCTGCCATTGGGTGCCACGTGGTAATTTGGAGCATTAGACCTTCGAGAAGGGAAACCTCATCTGGTTAGGGCTTGTGGCA
	target	60	TTCCCTCTCGAAGGCTAAATGCTCAATTACCCACGTGGCACCAATGGCAGAGGCT
		120	TGCCACAGAACCCCTAAACCAGATGAGGTTCCCTCTGAAGGTCTAAATGCTCCAATTACCAACG
		180	ATGTCAAGAACAGACCAATTGATGATCTGCCACAGAACCCCTAAACCAGATGAGGTTCCCTCTGAAGGTCTAAATGCTCCAATTACCAACG
		left	ATGTCAAGAACAGACCAATTGATGATCTGCCACAGAACCCCTAAACCAGATGAGGTTCCCTCTGAAGGTCTAAATGCTCCAATTACCAACG
		right	ATGTCAAGAACAGACCAATTGATGATCTGCCACAGAACCCCTAAACCAGATGAGGTTCCCTCTGAAGGTCTAAATGCTCCAATTACCAACG
<i>slc2a10</i>	non-target	60	TCTGACCTTTAAATCAAAGGATAACATGAGGAGAAGAACGGTGATTGGTGGTTGGTTGGTTGGTTGGTTGGTGCTGAGTCACAGGTCACCA
		120	CAGCATCAGCAAAGTGGAAATATGGTGGTGGTGCTGAGTCAGCAGTCACAGGTCACCA
		180	AGATTAACAGAGGAAACGGAAACATCAAATCAGCATCAGCAAAGTGGAAATATGGTGGTGGTGCTGAGTCACAGGTCACCA
		left	AGATTAACAGAGGAAACGGAAACATCAAATCAGCATCAGCAAAGTGGAAATATGGTGGTGGTGCTGAGTCACAGGTCACCA
		right	TCTGACCTTTAAATCAAAGGATAACATGAGGAGAAGAACGGTGATTGGTGGTGGTGCTGAGTCACAGGTCACCA
	target	60	CAACCCAAACACCAATCACGGTCTCTCTCATGTTACCGTCTCTCTCATGTTAAAGGTCAGA
		120	TGGTTGACCTGTGAACTGCTGACTCAGCACCAACCCAAACCAATCACGGTCTCTCTCATGTTAAAGGTCAGA
		180	AAGTATAGTTGAGGCGTAAAGAGCACATTGGTGACCTGTGAACTGCTGACTCAGCACCAACCCAA

		left	CAACCCAACACCAATCACCGTTCTTCCTCATGTTATCCTTGATTAAAAGGTAGAAACACCATAT TTCTCACTTGCTGATGCTGATTTGATGTTCCGTTCTGTTAACAT
		right	AAGTATAAGTTGAGGCAGAAAGAGCACATTGGGTGACCTGTAAGCTGACTCAGCACCAACCAA CACCAATCACCGTTCTTCCTCATGTTATCCTTGATTAAAAGGTAGA
<i>smad6a</i>	non-target	60	AGGCGTCGTGAACGGATAACATCGTCCCACCGCGTGTGCGAAGCTCCAGTAAGCCACAT
		120	CCTGAGGTAGGTCGTAGAAGATGCTGAGAGAGGCCGTCGTGAACGGATAACATCGTCCCACCGCGTGT GCGAAGCTCCAGTAAGCCACATTACACCAGTGGTCTGCGCAGCGAGGAGG
		180	GGGGCAGCAGGCCAAACATAGGCGTGTACCGTGGTAGGTCGTAGAAGATGCTGAGAGAGGCCGTC GTGAACGGATAACATCGTCCCACCGCGTGTGCGAAGCTCCAGTAAGCCACATTACACCAGTGGTCT GCGCCAGCGAGGAGGGAGACATACTGGCATCTGAAAGAGAGGTGG
		left	GGGGCAGCAGGCCAAACATAGGCGTGTACCGTGGTAGGTCGTAGAAGATGCTGAGAGAGGCCGTC GTGAACGGATAACATCGTCCCACCGCGTGTGCGAAGCTCCAGTAAGCCACATTACACCAG
		right	AGGCGTCGTGAACGGATAACATCGTCCCACCGCGTGTGCGAAGCTCCAGTAAGCCACATTACACCAG TGGTCTGCGCAGCGAGGAGGGAGACATACTGGCATCTGAAAGAGAGGTGG
	target	60	ATGTGGCTTACTGGGAGCTTCGCACACCGCGTGGGACGATTGTATCCAGTTACGACGCCT
		120	CCTCCTCGCTGGCGCAGAACCACTGGTGTAAATGTGGCTTACTGGGAGCTTCGCACACCGCGTGGGACGA TTGTATCCAGTTACCGACGCCCTCTCAGCATCTTACGACCTACCTCAGG
		180	CCACCTCTTCAGATGCCAGTATGTCTCCCTCTCGCTGGCGAGAACCACTGGTGTAAATGTGGCTTA CTGGGAGCTTCGCACACCGCGTGGGACGATTGTATCCAGTTACGACGCCCTCTCAGCATCTTACGCA CCTACCTCAGGGTACAGGCCATTGTTGGGCTGCTGCC
		left	ATGTGGCTTACTGGGAGCTTCGCACACCGCGTGGGACGATTGTATCCAGTTACGACGCCCTCTCAGCA TCTTCTACGACCTACCTCAGGGTACAGGCCATTGTTGGGCTGCTGCC
		right	CCACCTCTTCAGATGCCAGTATGTCTCCCTCTCGCTGGCGAGAACCACTGGTGTAAATGTGGCTTA CTGGGAGCTTCGCACACCGCGTGGGACGATTGTATCCAGTTACGACGCC

Table S9: injection mix compositions

Component	Injection mixes					
	No compound	SCR7	NU7441	KU0060648	RS1	L755507
Cas9 (1000 ng/μl)	0,7 μl	0,7 μl	0,7 μl	0,7 μl	0,7 μl	0,7 μl
sgRNA (100 ng/μl)	0,7 μl	0,7 μl	0,7 μl	0,7 μl	0,7 μl	0,7 μl
phenol red (2%)	1 μl	1 μl	0,8 μl	0,8 μl	1 μl	0,8 μl
ssODN (10 μM)	0,8 μl	0,8 μl	0,8 μl	0,8 μl	0,8 μl	0,8 μl
compound	0 μl	0,4 μl (10 mM)	1 μl (2 mM)	1 μl (0,5 mM)	0,2 μl (20 mM)	1 μl (2,5 mM)
nuclease-free water	0,8 μl	0,4 μl	0 μl	0 μl	0,6 μl	0 μl

Table S10: Selected concentrations compound incubation

Compound	Administered concentration (μM)
SCR7	5
NU7441	2
KU0060648	2
RS1	100
L755507	2

Table S11: PCR primers for *tprkb*, *pls3*, *slc2a10* and *smad6a*

gene	primer sequence (5'-3')	
	forward	reverse
<i>tprkb</i>	TCACCCCAAGCACATAAACAA	CGCCAACATAGGGAGAACAT
<i>pls3</i>	ACCTGTTGTTTCCGTA	TTGTCATTACCATGCCAAG
<i>slc2a10</i>	TCACGGTGGCATCTTGATA	AAGTGCATCGTTCGTTG
<i>smad6a</i>	GCCAGGACTCAAAACAAGC	CACACTGTTGGGTGATG

Table S12: input parameters BATCH-GE

gene	Input parameter 1			Input parameter 2	Input parameter 3
	Chromosome	Start position	End position		
<i>tprkb</i>	chr4	28383726	28383785	(C)GCATT(T)CAGATCCT[C]GT(A)GC(C)AC(C)	yes
<i>pls3</i>	chr14	13159801	13159860	(G)TG(C)CACGTGGT(G)AA[T]ATTGGAGC(A)	yes
<i>slc2a10</i>	chr11	2348500	2348559	(T)(C)(A)AA(G)GATAACATG[A]GGAG(A)	yes
<i>smad6a</i>	chr7	33912937	33912996	(A)TACA(A)(T)CG(T)CCAC[G]CG(T)G	yes