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Supplemental Information

CRISPR-Pass: Gene Rescue of Nonsense

Mutations Using Adenine Base Editors

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Supplementary Information

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Supplementary Figure 1. Coding or noncoding targeting depends on the *EGFP* sequence and the PTC position. The sequence of the *EGFP* gene is shown. ABE target sequences are underlined. Depending on the target strand, codons for Lys53 or Asn213 were mutated such that they became STOP codons. The codons that are mutated are shown in blue and the PAM sequences are shown in red.

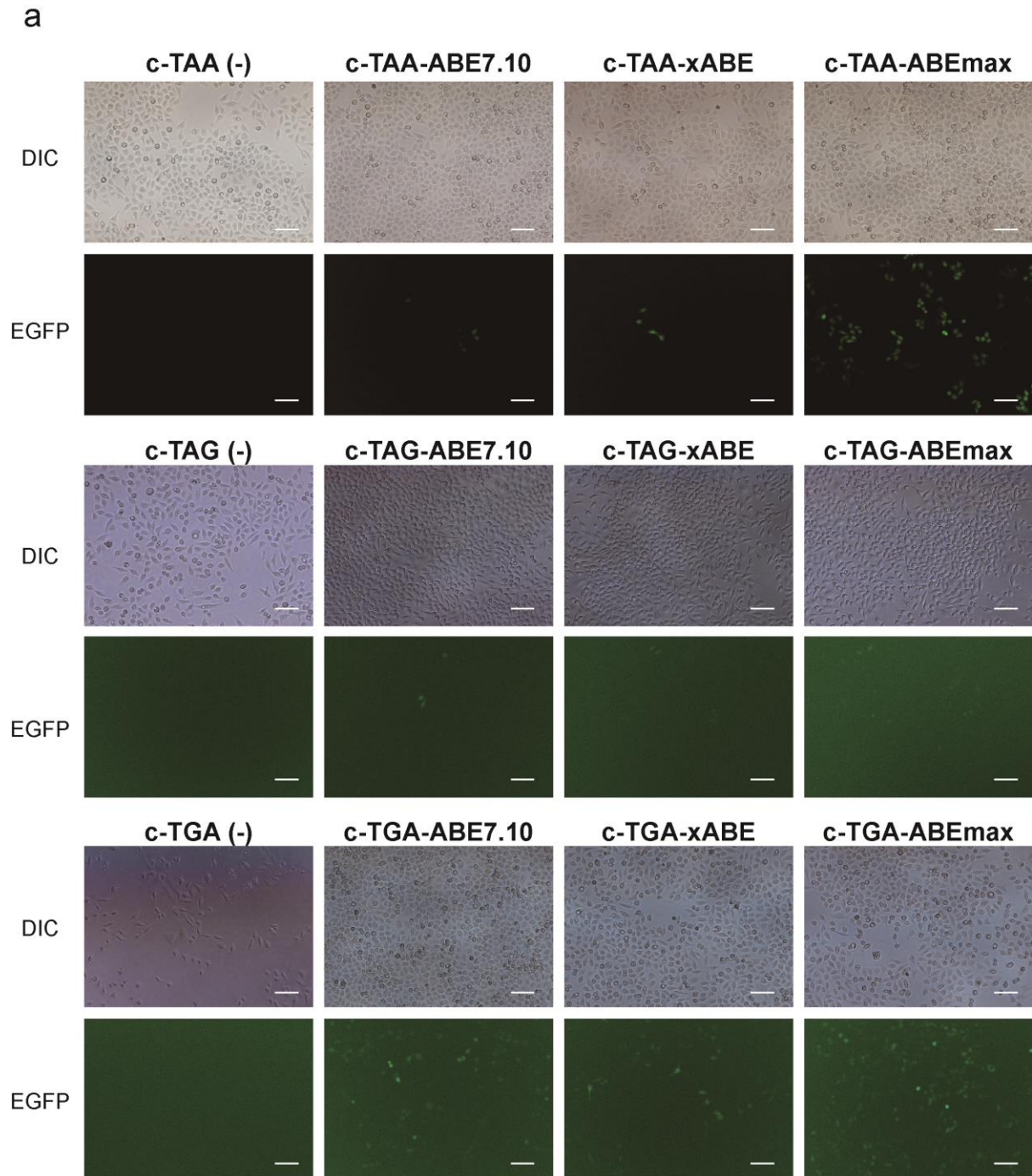
EGFP-PTCs-knockin DNA sequences

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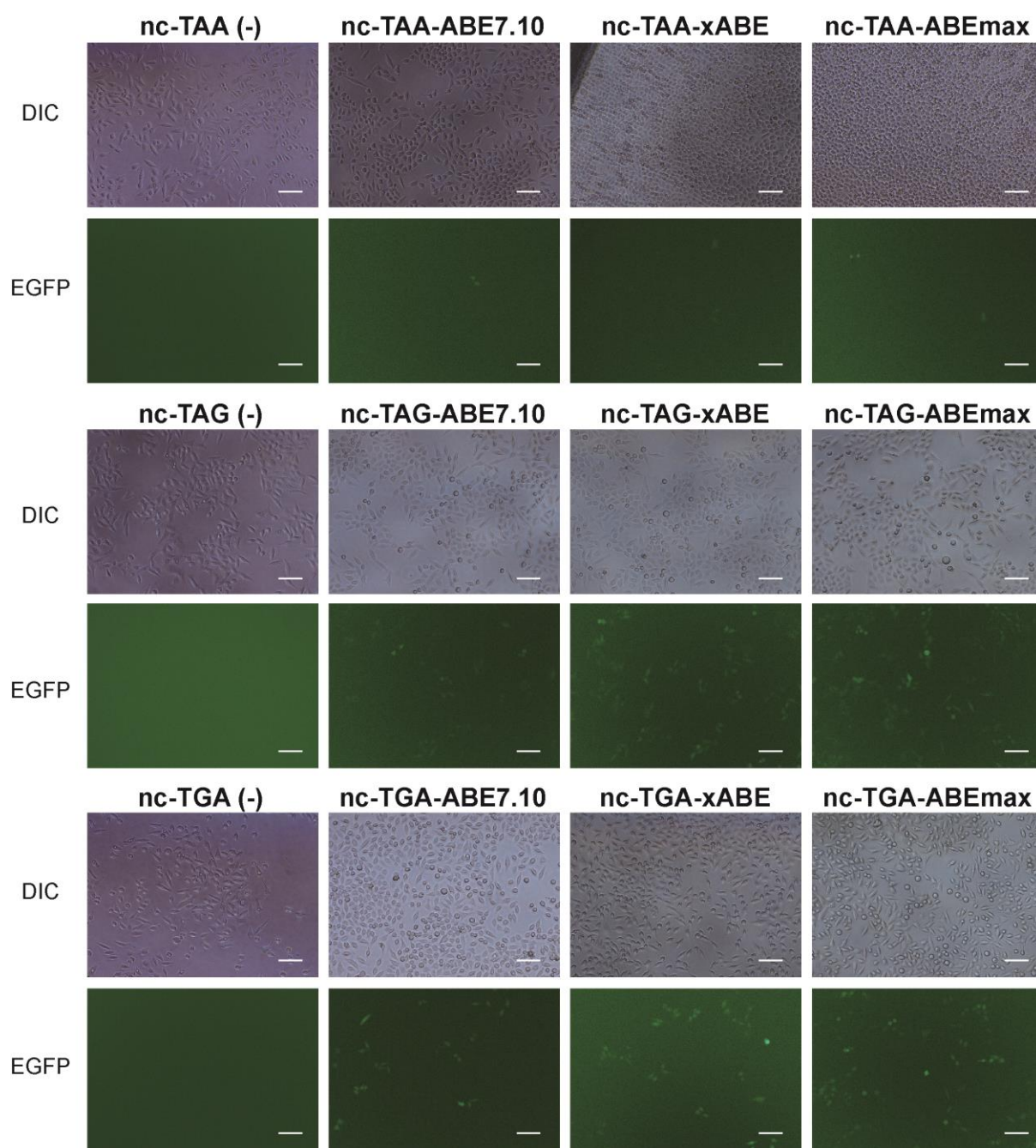
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78 caagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccg
   coding strand target sgRNA (53rd Lysine -> STOP)
   ↓
155 gcaagctgcccgtgccctggccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccccgac
232 cacatgaagcagcagcacttcttcaagtcgcatgccgaaggctacgtccaggagcgcaccatcttcttcaagga
309 cgacggcaactacaagaccgcccgcgaggtgaagttcgagggcgacaccctggtgaaccgcatcgagctgaagggca
386 tcgacttcaaggaggacggcaacatcctggggcacaagctggagtacaactacaacagccacaacgtctatatcatg
463 gccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgc
540 cgaccactaccagcagaacacccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagcaccagt
   noncoding strand target sgRNA (213th Asparagine-> STOP)
   ↓
617 ccgccctgagcaaagaccccaacgagaagcgcgatcacatgggtcctgctggagtctcgtgaccgccgcccggatcact
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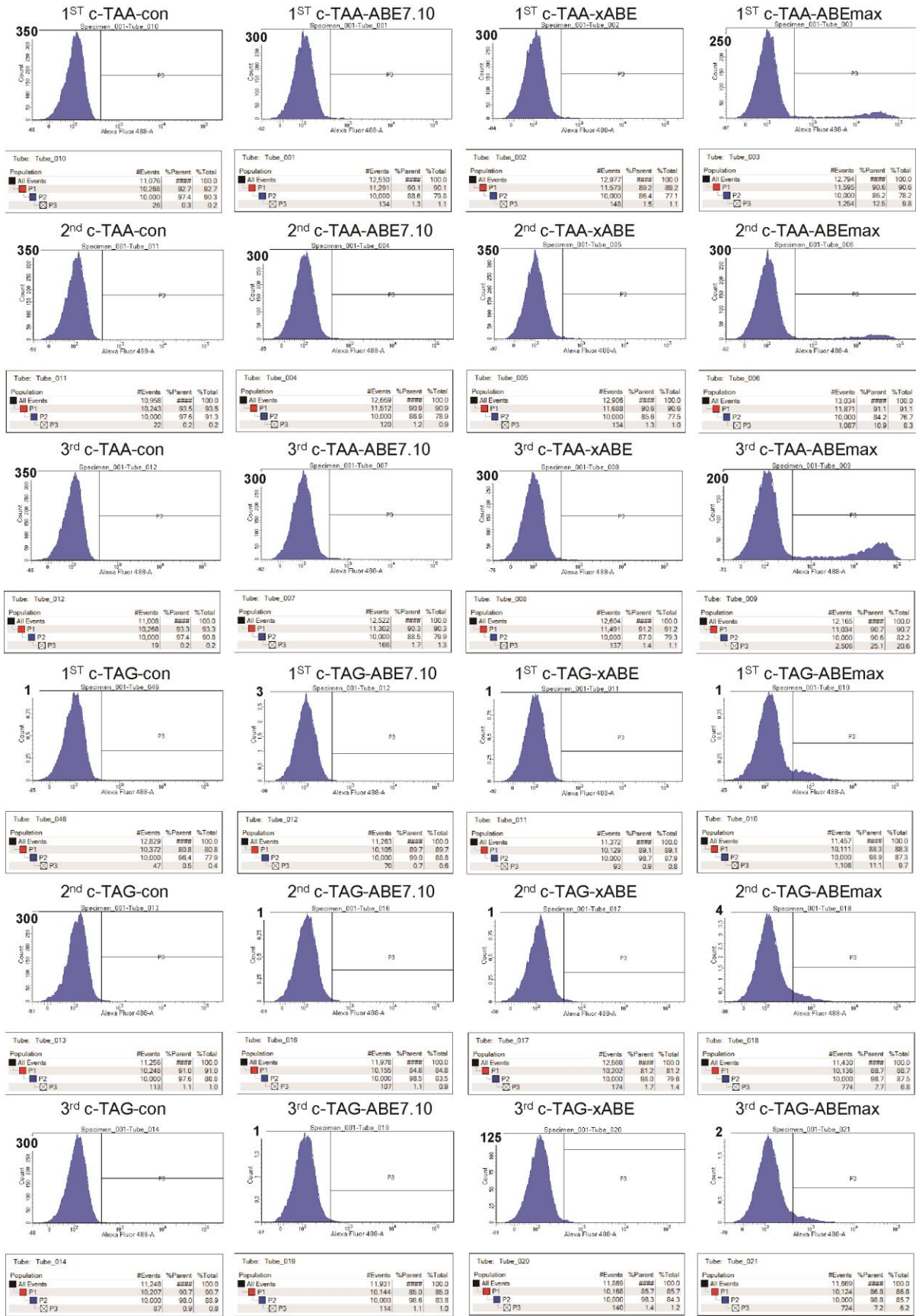
Supplementary Figure 2. Rescued EGFP expression after treatment with ABEs. Rescued EGFP expression in EGFP-PTC-KI cell lines in which the coding strand (a) or noncoding strand (b) is targeted for PTC bypass. All scale bars are 100 μ m.

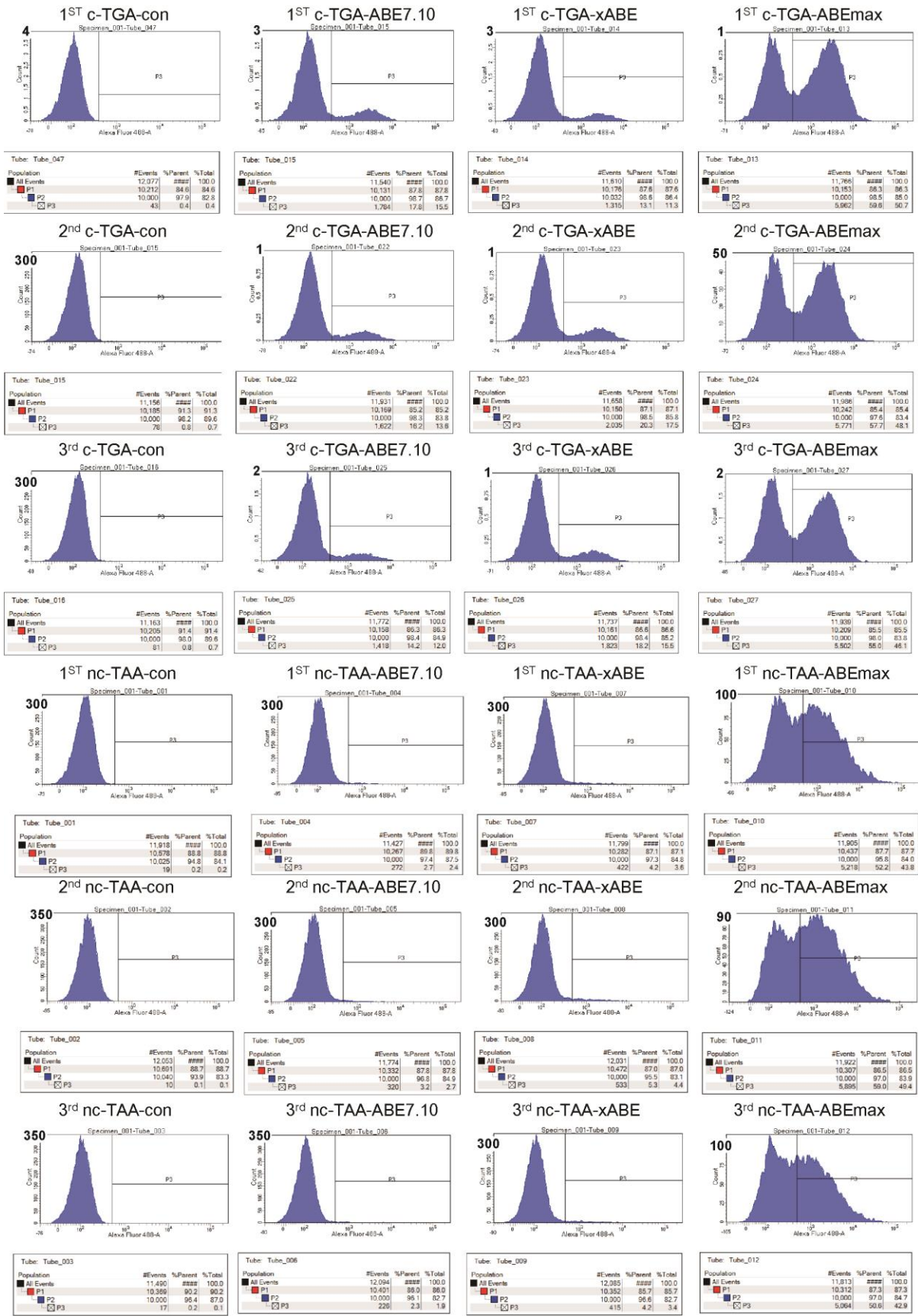


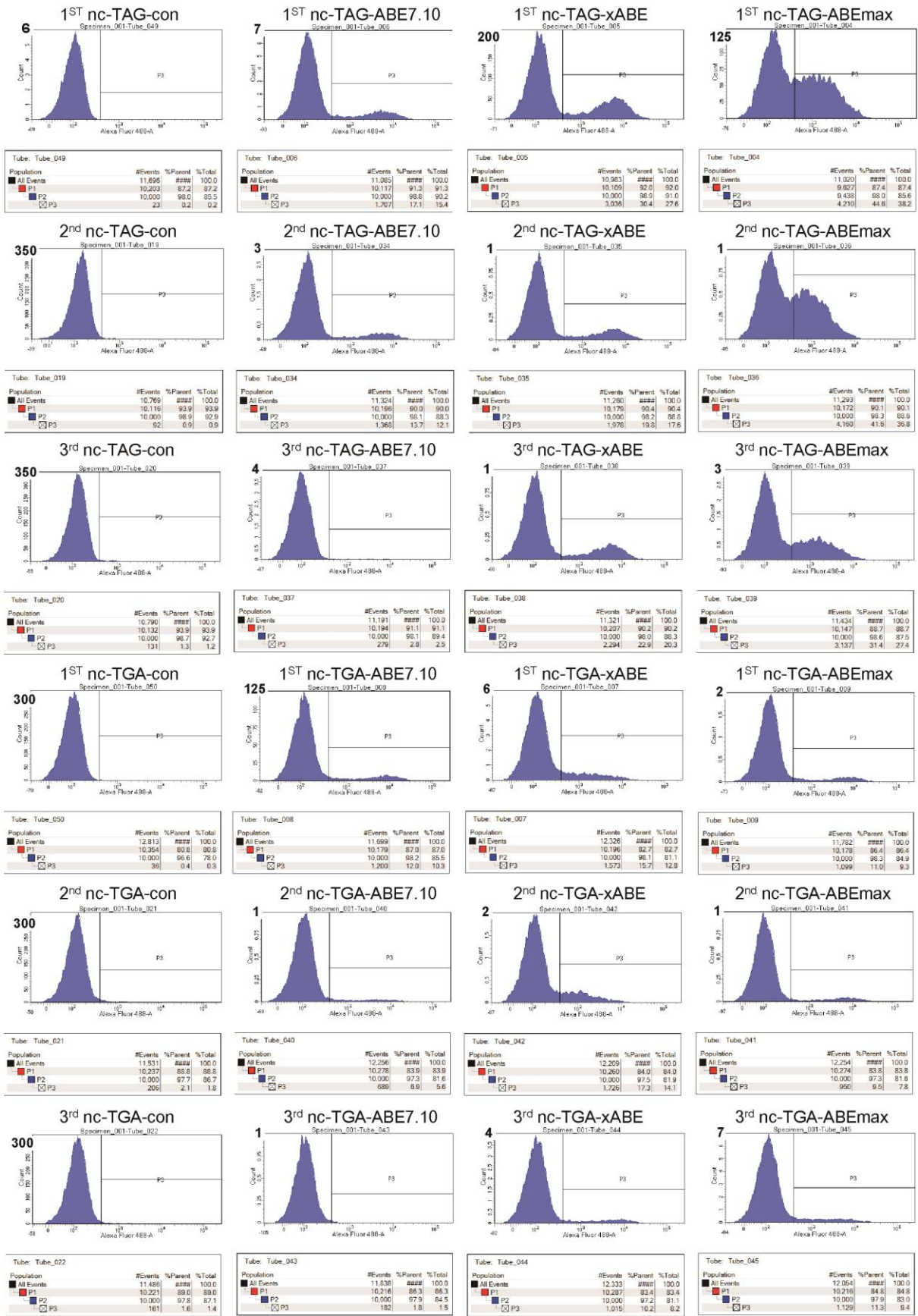
b



Supplementary Figure 3. Flow cytometry results.







Supplementary Figure 4. CRISPR-pass for XPC patient-derived fibroblasts. Next generation sequencing (NGS) results from (a) untreated GM14867 fibroblasts, (b) ABE7.10-treated GM143867 fibroblasts, and (c) xABE-treated GM143867 fibroblasts. (d) Image of complete SDS-PAGE gel that is shown in part in Figure 3c.

a

XPC_wt

	Trp			Val			STOP			Asp			Val			Thr			Gln			Arg		
	G	G	G	T	C	T	G	A	G	A	T	G	T	C	A	C	A	C	A	G	A	G	G	
A	0.1	0.1	0.1	0.0	0.0	0.0	0.0	99.7	0.0	99.7	0.0	0.1	0.0	0.0	99.7	0.0	99.8	0.0	99.8	0.0	0.0	0.0	0.0	
T	0.0	0.0	0.0	99.6	0.0	99.8	0.0	0.0	0.0	0.0	99.7	0.0	99.6	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
G	99.9	99.9	99.9	0.1	0.0	0.0	100	0.2	100	0.2	0.0	99.9	0.0	0.0	0.2	0.0	0.2	0.0	0.2	100	0.3	99.9	99.9	
C	0.0	0.0	0.0	0.3	100	0.2	0.0	0.0	0.0	0.0	0.3	0.0	0.4	99.9	0.0	99.9	0.0	100	0.0	0.0	0.0	0.0	0.0	

b

XPC_ABE7.10

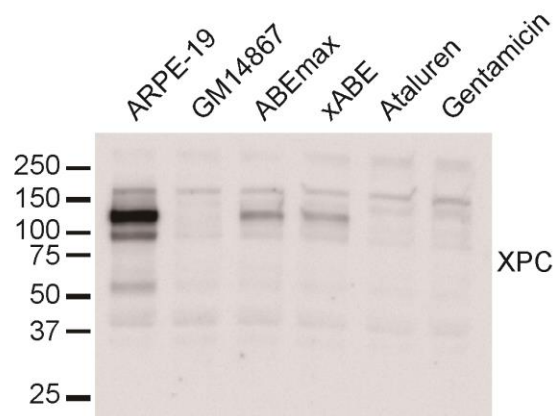
	Trp			Val			STOP			Asp			Val			Thr			Gln			Arg		
	G	G	G	T	C	T	G	A	G	A	T	G	T	C	A	C	A	C	A	G	A	G	G	
A	0.1	0.1	0.1	0.0	0.0	0.0	0.1	96.5	0.1	99.3	0.0	0.0	0.0	0.0	99.6	0.0	99.7	0.0	99.8	0.0	99.7	0.1	0.1	
T	0.0	0.0	0.0	99.5	0.4	99.8	0.0	0.1	0.0	0.0	99.7	0.0	99.5	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
G	99.9	99.9	99.9	0.0	0.0	0.0	99.9	3.4	99.9	0.7	0.0	99.9	0.0	0.0	0.3	0.0	0.3	0.0	0.2	99.9	0.3	99.9	99.9	
C	0.0	0.0	0.0	0.4	99.6	0.2	0.0	0.0	0.0	0.0	0.3	0.0	0.4	99.9	0.0	99.9	0.0	99.9	0.0	0.0	0.0	0.0	0.0	

c

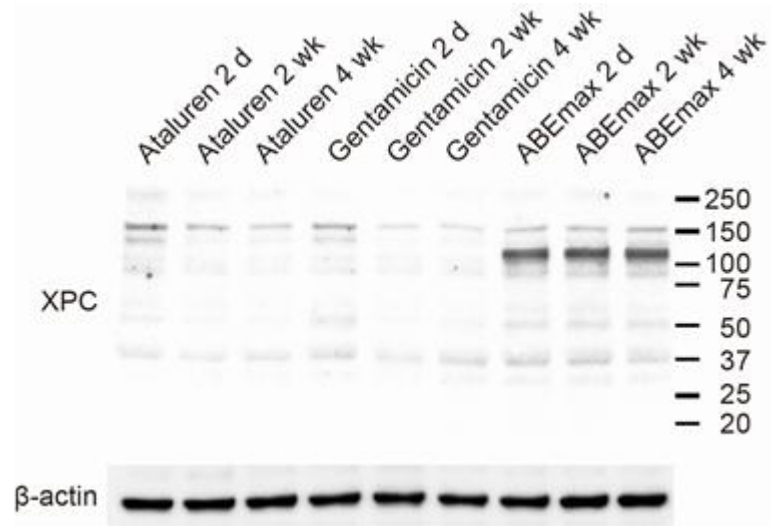
XPC_xABE

	Trp			Val			STOP			Asp			Val			Thr			Gln			Arg		
	G	G	G	T	C	T	G	A	G	A	T	G	T	C	A	C	A	C	A	G	A	G	G	
A	0.1	0.1	0.1	0.0	0.0	0.0	0.0	86.2	0.0	99.3	0.0	0.0	0.0	0.0	99.7	0.0	99.7	0.0	99.8	0.0	99.6	0.1	0.1	
T	0.0	0.0	0.0	99.5	0.7	99.7	0.0	0.1	0.0	0.0	99.7	0.0	99.5	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	
G	99.9	99.9	99.9	0.0	0.1	0.0	99.9	13.7	99.9	0.7	0.0	99.9	0.0	0.0	0.3	0.0	0.2	0.0	0.2	100	0.4	99.9	99.9	
C	0.0	0.0	0.0	0.4	99.2	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.5	99.9	0.0	99.9	0.0	99.9	0.0	0.0	0.0	0.0	0.0	

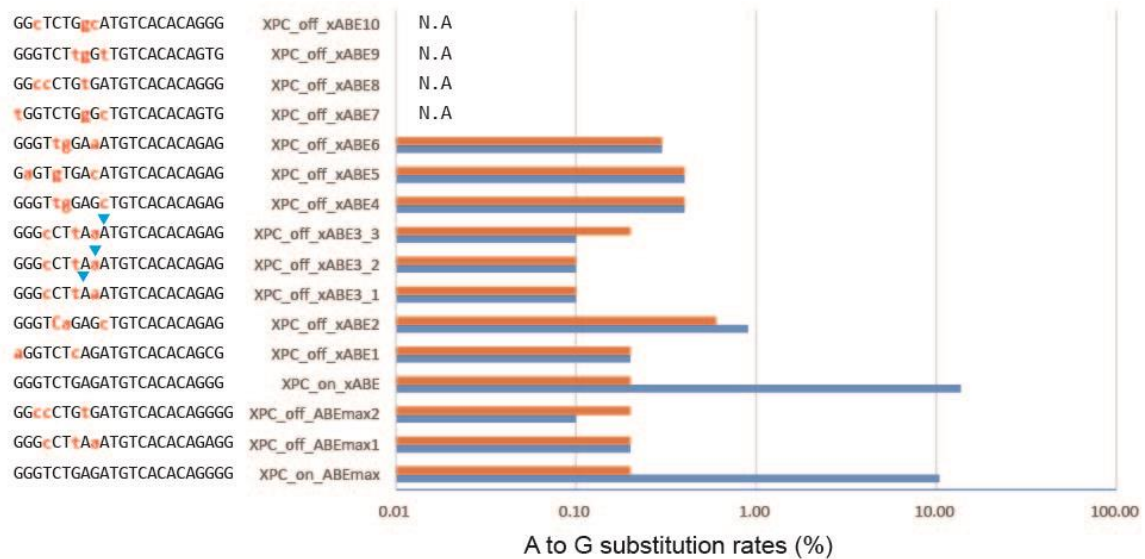
d



Supplementary Figure 5. Prolonged expression of the XPC protein after ABE treatment. Image of complete SDS-PAGE gel that is shown in part in Figure 3e.



Supplementary Figure 6. Off-target analysis for CRISPR-pass targeting XPC. A to G substitution rates at off-target sites are displayed. The percentages of substitutions at each site are summarized in Supplementary Table 3. The red bars depict A to G substitution rates in untreated samples, whereas the blue bars depict A to G substitution rates in ABE-treated samples. Blue arrowhead indicates a target “A” which shows the A to G substitution rates (%).



Supplementary Table 1. Flow cytometry results. The percentages of EGFP (+) cells in populations of ABE-treated EGFP-PTC-KI cells. Each experiment was repeated 3 times.

	(-)			ABE7.10			xABE			ABEmax		
	1 st trial	2 nd trial	3 rd trial	1 st trial	2 nd trial	3 rd trial	1 st trial	2 nd trial	3 rd trial	1 st trial	2 nd trial	3 rd trial
c-TAA	0.3%	0.2%	0.2%	1.3%	1.2%	1.7%	1.5%	1.3%	1.4%	12.5%	10.9%	25.1%
c-TAG	0.5%	1.1%	0.9%	0.7%	1.1%	1.1%	0.9%	1.7%	1.4%	11.1%	7.7%	7.2%
c-TGA	0.4%	0.8%	0.8%	17.8%	16.2%	14.2%	13.1%	20.3%	18.2%	59.6%	57.7%	55.0%
nc-TAA	0.2%	0.1%	0.2%	2.7%	3.2%	2.3%	4.2%	5.3%	4.2%	52.2%	59.0%	50.6%
nc-TAG	0.2%	0.9%	1.3%	17.1%	13.7%	2.8%	30.4%	19.8%	22.9%	44.6%	41.6%	31.4%
nc-TGA	0.4%	2.1%	1.6%	11.0%	6.9%	1.8%	12.0%	9.5%	10.2%	15.7%	17.3%	11.3%

Supplementary Table 2. NGS results. The percentages of A to G substitutions in populations of ABE-treated EGFP-PTC-KI cells. Each experiment was repeated 3 times.

	(-)			ABE7.10			xABE			ABEmax		
	1 st trial	2 nd trial	3 rd trial	1 st trial	2 nd trial	3 rd trial	1 st trial	2 nd trial	3 rd trial	1 st trial	2 nd trial	3 rd trial
c-TAA	0.0%	0.1%	0.0%	0.2%	0.2%	0.0%	0.0%	0.0%	0.9%	11.2%	9.7%	43.5%
c-TAG	0.5%	0.6%	0.4%	5.3%	6.5%	2.7%	9.1%	9.5%	4.0%	51.2%	52.2%	54.1%
c-TGA	0.3%	0.3%	0.0%	6.2%	5.9%	5.4%	5.1%	4.4%	2.4%	37.5%	34.0%	41.3%
nc-TAA	0.0%	0.0%	0.0%	6.7%	8.6%	7.2%	11.2%	14.0%	11.8%	80.9%	84.2%	81.6%
nc-TAG	0.3%	0.1%	0.2%	12.7%	1.8%	14.3%	16.3%	22.2%	28.7%	41.9%	33.1%	44.8%
nc-TGA	0.2%	0.3%	0.4%	9.1%	2.3%	15.2%	15.0%	12.1%	16.0%	37.4%	19.2%	26.9%

Supplementary Table 3. A-to-G substitution rates (%) in potential ABE off-target sites.
 N.A., not available; these sites are a Cas9 or xCas9 off-target site but do not contain an A targetable by ABEs. Blue colored letter means a target “A” which shows the A to G substitution rates (%)

Name	Target sequences	ABE treated	wt
XPC_ABEmax_on	GGGTCTGAGATGTCACACAGNGG	10.40%	0.20%
XPC_ABEmax_off_1	GGGcCTtAaATGTCACACAGAGG	0.20%	0.20%
XPC_ABEmax_off_2	GGccCTGtGATGTCACACAGGGG	0.10%	0.20%
XPC_xABE_on	GGGTCTGAGATGTCACACAGNG	13.70%	0.20%
XPC_xABE_off_1	aGGTCTcAGATGTCACACAGCG	0.20%	0.20%
XPC_xABE_off_2	GGGTCaGAGcTGTCACACAGAG	0.90%	0.60%
XPC_xABE_off_3_1	GGGcCTtAaATGTCACACAGAG	0.10%	0.10%
XPC_xABE_off_3_2	GGGcCTtAaATGTCACACAGAG	0.10%	0.10%
XPC_xABE_off_3_3	GGGcCTtAaATGTCACACAGAG	0.10%	0.20%
XPC_xABE_off_4	GGGTtgGAGcTGTCACACAGAG	0.40%	0.40%
XPC_xABE_off_5	GaGTgTGAcATGTCACACAGAG	0.40%	0.40%
XPC_xABE_off_6	GGGTtgGAaATGTCACACAGAG	0.30%	0.30%
XPC_xABE_off_7	tGGTCTGgGcTGTCACACAGTG	N.A	N.A
XPC_xABE_off_8	GGccCTGtGATGTCACACAGGG	N.A	N.A
XPC_xABE_off_9	GGGTCTtgGtTGTCACACAGTG	N.A	N.A
XPC_xABE_off_10	GGcTCTGgcATGTCACACAGGG	N.A	N.A

Supplementary Table 4. List of oligomers encoding sgRNAs.

Name	sequences
psg-nc-TAA-up	CACCGTCTCTTAGGGGTCTTTGCTC
psg-nc-TAG-up	CACCGTCTCCTAGGGGTCTTTGCTC
psg-nc-TGA-up	CACCGTCTCTCAGGGGTCTTTGCTC
psg-nc-TAA-bo	AAACGAGCAAAGACCCCTAAGAGAC
psg-nc-TAG-bo	AAACGAGCAAAGACCCCTAGGAGAC
psg-nc-TGA-bo	AAACGAGCAAAGACCCCTGAGAGAC
psg-c-TAG_1up	CACCGCCGGCTAGCTGCCCGTGCCC
psg-c-TAA_2up	CACCGCCGGCTAACTGCCCGTGCCC
psg-c-TGA_3up	CACCGCCGGCTGACTGCCCGTGCCC
psg-c-TAG_1bo	AAACGGGCACGGGCAGCTAGCCGGC
psg-c-TAA_2bo	AAACGGGCACGGGCAGTTAGCCGGC
psg-c-TGA_3bo	AAACGGGCACGGGCAGTCAGCCGGC
psg-XPC-up	CACCGGGTCTGAGATGTCACACAG
psg-XPC-bo	AAACGACACACTGTAGAGACTGGGC
psg-AAVS1-up	CACCGTAAGCAAACCTTAGAGGTTC
psg-AAVS1-bo	AAACCTTGAGATTCCAACGAATC

Supplementary Table 5. PCR primers used in this study.

Name	Sequences
EGFP-1stF	gacatatccacgccctccta
EGFP-1stR	ctgacaattccgtggtgttg
EGFP_c_PTC_Deep_F	ACACTCTTTCCTACACGAC GCTCTCCGATCT acgtaaacggccacaagttc
EGFP_c_PTC_Deep_R	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT tcgtccttgaagaagatggtg
EGFP_nc_PTC_Deep_F	ACACTCTTTCCTACACGAC GCTCTCCGATCT gaacggcatcaaggtgaact
EGFP_nc_PTC_Deep_R	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT cttgtacagctcgtccatgc
inf_sacl_Cgo_add_F	GGTCTATATAAGCAGAGCTC TCGTCGACGAGCTCGTTTAGTG
inf_sacl_Cgo_add_R	CTCACCATGGCGGCGAGCTC GGTACCCTGGACACCTGTGG
inf_ccn_n2_TAA_2F	CTGAGCAAAGACCCCTgagagaagcgcgatcacatgg
inf_ccn_n2_TAA_1R	tcaGGGGTCTTTGCTCAGGGCG
inf_ccn_n2_TAG_2F	CTGAGCAAAGACCCCaagagaagcgcgatcacatgg
inf_ccn_n2_TAG_1R	ttgGGGGTCTTTGCTCAGGGCG
inf_ccn_n2_TGA_2F	CTGAGCAAAGACCCCcagagaagcgcgatcacatgg
inf_ccn_n2_TGA_1R	tcgGGGGTCTTTGCTCAGGGCG
inf_go_F1-1	TGGGAGGTCTATATAAGCAGAGCTCATGGTGAGCAAGGGCGAGG
inf_go_R1-TAG	CCATGTGCTAGCGCTTCTCGTTGGGGTC
inf_go_R1-TAA	CCATGTGTTAGCGCTTCTCGTTGGGGTC
inf_go_R1-TGA	CCATGTGTCAGCGCTTCTCGTTGGGGTC
inf_go_F1-2TAG	CGAGAAGCGCTAGcacatggtcctgctggagtt
inf_go_F1-2TAA	CGAGAAGCGCTAAcacatggtcctgctggagtt
inf_go_F1-2TGA	CGAGAAGCGCTGAcacatggtcctgctggagtt
inf_go_R1-2	TGAGATGTCTCTGTGCGGCTCACTTGTACAGCTCGTCCATGC
inf_go_R2-1TAG	CGGGCAGCTAGCCGGTGGTGCAGATGAAC
inf_go_R2-1TAA	CGGGCAGTTAGCCGGTGGTGCAGATGAAC
inf_go_R2-1TGA	CGGGCAGTCAGCCGGTGGTGCAGATGAAC
inf_go_F2-2TAG	CACCACCGGCTAGCTGCCCGTGCCCTGGCCC
inf_go_F2-2TAA	CACCACCGGCTAACTGCCCGTGCCCTGGCCC
inf_go_F2-2TGA	CACCACCGGCTGACTGCCCGTGCCCTGGCCC

Name	Sequences
XPC_1stF	ccaggagacaagcaggagaa
XPC_1stR	cgcggcagttcatctttcaa
XPC_deepF	ACACTCTTTCCTACACGAC GCTCTCCGATCT gtgagcaggaggaaaagtgg
XPC_deepR	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT gtatggtetcaaggtctcggc
XPC_off_2nd_F1	ACACTCTTTCCTACACGAC GCTCTCCGATCT CACATGCTCCTGGAAGGGAA
XPC_off_2nd_R1	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT AGGAGTGCCTACAGATGGGT
XPC_off_2nd_F2	ACACTCTTTCCTACACGAC GCTCTCCGATCT TTCACAGGCTGGCATTGAGT
XPC_off_2nd_R2	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT TGCCAGACAGAAGTTTGCT
XPC_off_2nd_F3_NGG_F1	ACACTCTTTCCTACACGAC GCTCTCCGATCT TGGAAGTGAAAGGGTTGTCT
XPC_off_2nd_R3_NGG_R1	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT TCCATCTTTCACAGAGCTTCCA
XPC_off_2nd_F4	ACACTCTTTCCTACACGAC GCTCTCCGATCT GCATTTCCAGGCACACAGTG
XPC_off_2nd_R4	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT CAGAGGATGCAAGGAAACACC
XPC_off_2nd_F5	ACACTCTTTCCTACACGAC GCTCTCCGATCT TCCATTTAGCTCGGGATGGC
XPC_off_2nd_R5	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT TGCCTCATTGTTTCATTAGTGTCT
XPC_off_2nd_F6	ACACTCTTTCCTACACGAC GCTCTCCGATCT AGTCATAATATTTCAAGGCAGAAAAGA
XPC_off_2nd_R6	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT ACGCTCTTTTCAGACATTCTTGT
XPC_off_2nd_F7	ACACTCTTTCCTACACGAC GCTCTCCGATCT TGGCAGCAAGAGAAAGGAGG
XPC_off_2nd_R7	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT GTGACCTTCTCCTTCCGTG
XPC_off_2nd_F8_NGG_F1	ACACTCTTTCCTACACGAC GCTCTCCGATCT GACCTGTACTATGGGCTGCC
XPC_off_2nd_R8_NGG_R1	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT TCATCATCCCCTCCCTGTGT
XPC_off_2nd_F9	ACACTCTTTCCTACACGAC GCTCTCCGATCT ACCTCCCTCCTGAAGAAGTGA
XPC_off_2nd_R9	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT TGGGCAGGACTGATATCCCT
XPC_off_2nd_F10	ACACTCTTTCCTACACGAC GCTCTCCGATCT CCTCCTAAGGAACAACATGGTGT
XPC_off_2nd_R10	GTGACTGGAGTTCAGACGTGT GCTCTCCGATCT TGCAATTTCTTCTTGTCTGAGT
XPC_off_1st_F1	TGCAAACCCCTTCTGTCTGT
XPC_off_1st_R1	TGCAGTGAGCTGAGATTGGG
XPC_off_1st_F2	AATGGGGGTACAGGCATTGG
XPC_off_1st_R2	AGCTGGCTGCAGAAATTTGC
XPC_off_1st_F3_NGG_F1	GAGGTTGCAGTGAGCCAAGA
XPC_off_1st_R3_NGG_R1	GGAGGGAGAGAGGAGTGGAG
XPC_off_1st_F4	GCCTTCTCAACAATCCCCCA
XPC_off_1st_R4	CCACTGTTTTGTGCAGCCTC
XPC_off_1st_F5	TGAGGCGTGAAGTGTGTAC
XPC_off_1st_R5	TCAGCTCACTGCAACCTCTG
XPC_off_1st_F6	CTTACCAGCGGCTCTTGGAA
XPC_off_1st_R6	CATCTGCTAAAGGGCTGGCT
XPC_off_1st_F7	CCTCACAGCCAATCCCATGT
XPC_off_1st_R7	AGGAGTGGCTCATCAAAGGC
XPC_off_1st_F8_NGG_F1	ATGTGGACCCAGGCATTCTG

XPC_off_1st_R8_NGG_R1	CAGAGGGAGCACCAAGGAAG
XPC_off_1st_F9	GCAAGGGAGAAAGGAGGGTC
XPC_off_1st_R9	CTCCTTCTTGTCGTGGGGAC
XPC_off_1st_F10	TTCAAACCCCAAGGAAGTT
XPC_off_1st_R10	TCAGCCATACCACCAAGA

Supplementary Table 6. List of off-target sites.

Name	On-target sequences	Off-target sequences	chr no.	position	direction	no. of mismatches	sequence ID	features
XPC_ABE _{max} _off_1	GGGTCTGAGATGTCACACAGNGG	GGccCTGtGATGTCACACAGGGG	chr1	55008670	-	3	NC_000001.11	intergenic region
XPC_ABE _{max} _off_2	GGGTCTGAGATGTCACACAGNGG	GGGcCTtAaATGTCACACAGAGG	chr1	199005822	+	3	NC_000001.11	intergenic region
XPC_xABE_off_1	GGGTCTGAGATGTCACACAGNG	aGGTCTcAGATGTCACACAGCG	chr7	24217661	-	2	NC_000007.14	intragenic region; intron
XPC_xABE_off_2	GGGTCTGAGATGTCACACAGNG	GGGTcAGAGcTGTCACACAGAG	chr13	47873206	-	2	NC_000013.11	intergenic region
XPC_xABE_off_3	GGGTCTGAGATGTCACACAGNG	GGGcCTtAaATGTCACACAGAG	chr1	199005822	+	3	NC_000001.11	intergenic region
XPC_xABE_off_4	GGGTCTGAGATGTCACACAGNG	GGGTtgGAGcTGTCACACAGAG	chr2	102917030	-	3	NC_000002.12	intergenic region
XPC_xABE_off_5	GGGTCTGAGATGTCACACAGNG	GaGTgTGAcATGTCACACAGAG	chr17	48043863	+	3	NC_000017.11	intergenic region
XPC_xABE_off_6	GGGTCTGAGATGTCACACAGNG	GGGTtgGAaATGTCACACAGAG	chr6	148983560	+	3	NC_000006.12	intragenic region; intron
XPC_xABE_off_7	GGGTCTGAGATGTCACACAGNG	tGGTCTGgGcTGTCACACAGTG	chr16	66513305	-	3	NC_000016.10	intragenic region; intron
XPC_xABE_off_8	GGGTCTGAGATGTCACACAGNG	GGccCTGtGATGTCACACAGGG	chr1	55008671	-	3	NC_000001.11	intergenic region
XPC_xABE_off_9	GGGTCTGAGATGTCACACAGNG	GGGTCTtgGtTGTCACACAGTG	chr21	35580798	+	3	NC_000021.9	intergenic region
XPC_xABE_off_10	GGGTCTGAGATGTCACACAGNG	GGcTCTGgcATGTCACACAGGG	chr10	9927098	+	3	NC_000010.11	intergenic region