

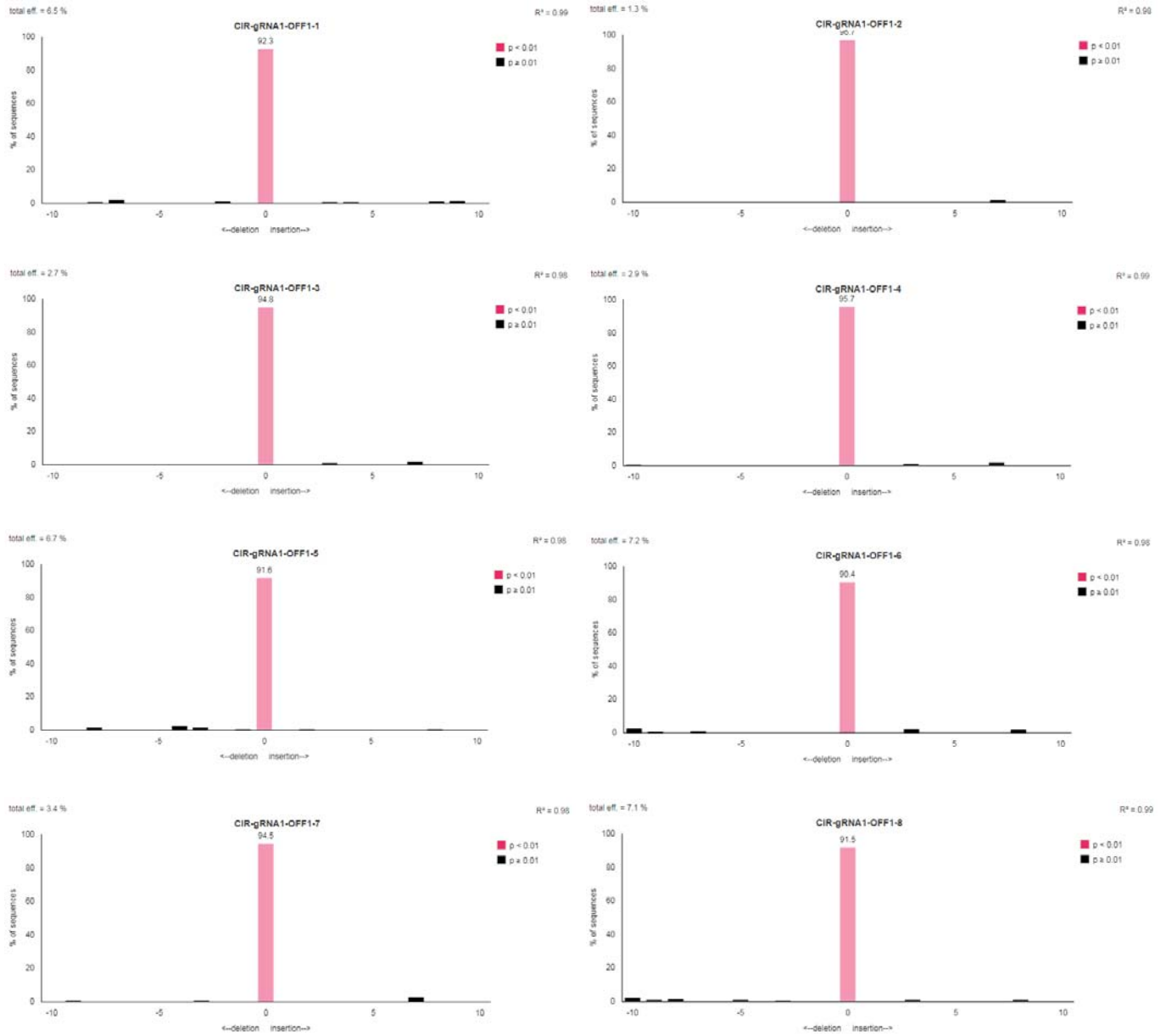
Data S1. Off-target mutagenesis analysis

A844B (gRNA1) T1 sample information

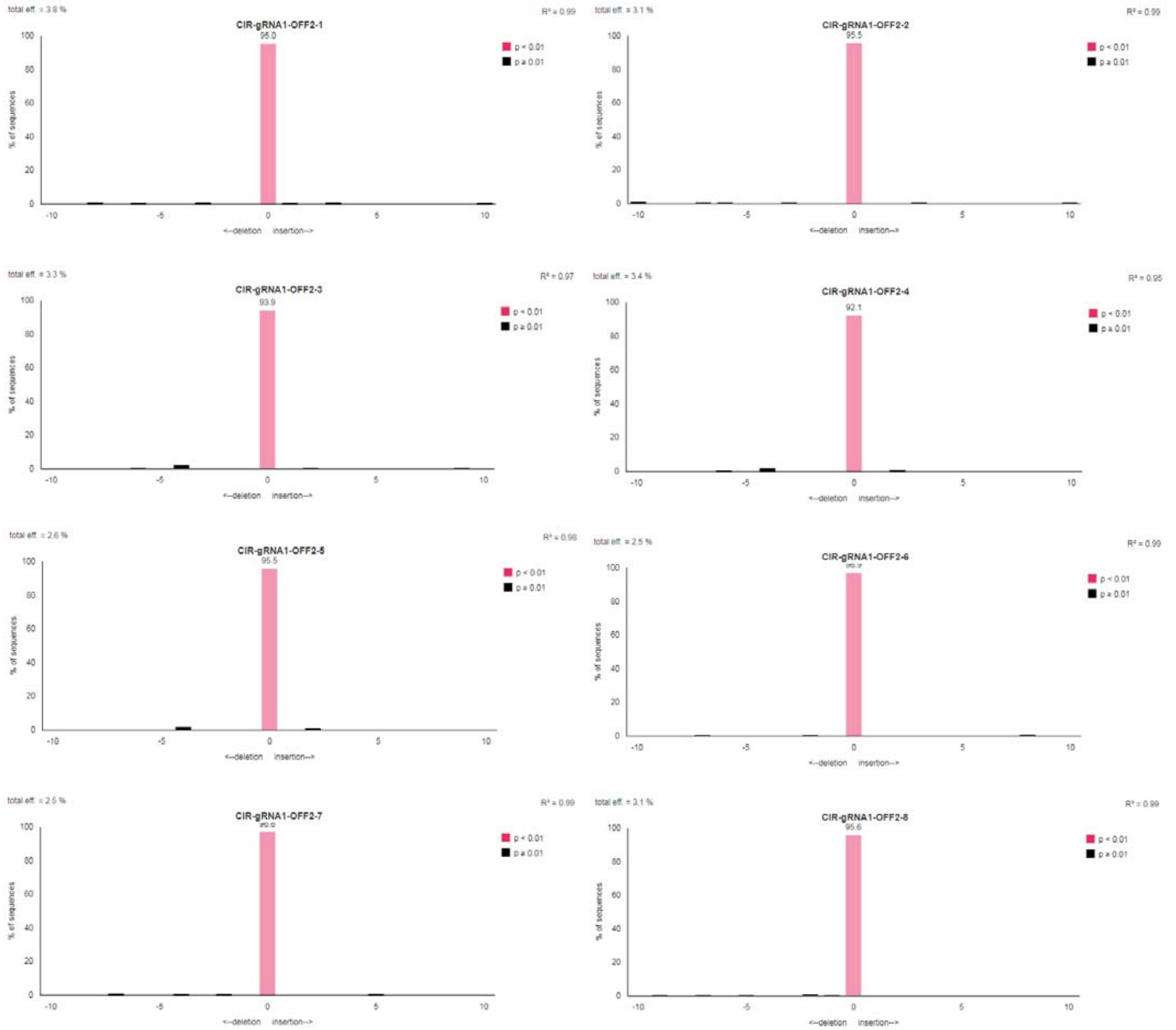
A total of 32 T1 plants derived from seven different T0 lines bearing on-target mutations were screened for off-target mutagenesis analysis. Twenty four plants were T-DNA positive (+), which constitutively expresses Cas9 nuclease, whereas eight were negative segregants (-). PCR was carried for each gDNA sample, and then four reactions were pooled (Sample pool) for Sanger sequencing. Thus, eight Sanger sequencing reactions were carried out per off-target. Fifteen CIRCLE-seq found (gRNA1-OFF1-10, 13-17) and two Cas-OFFinder predicted off-targets (gRNA1-OFF11-12) were analyzed, and there was no detectable level of mutations in 32 T1 lines. HM, homozygous; BI, biallelic; HT, heterozygous; MO, mosaic; WT, wild type; MT, *gl2* mutant.

T0 genotype	A844B	T-DNA	T1 phenotype	Sample pool	Off-targets with mutation	
(-7, -7)	HM	1-4-1	-	MT	Pool 1	None
(-7, -7)	HM	1-4-2	-	WT	Pool 2	None
(-7, -7)	HM	1-4-3	-	WT	Pool 3	None
(-7, -7)	HM	1-4-4	-	WT	Pool 4	None
(-7, -7)	HM	1-4-5	+	MT	Pool 5	None
(-7, -7)	HM	1-4-6	+	MT	Pool 6	None
(-7, -7)	HM	1-4-7	+	MT	Pool 7	None
(-7, -7)	HM	1-4-8	+	MT	Pool 8	None
(+1, +1)	HM	2-5-1	-	WT	Pool 1	None
(+1, +1)	HM	2-5-2	+	WT	Pool 2	None
(+1, +1)	HM	2-5-3	+	WT	Pool 3	None
(+1, +1)	HM	2-5-4	-	WT	Pool 4	None
(-2, -2)	HM	3-2-1	+	MT	Pool 5	None
(-2, -2)	HM	3-2-2	+	MT	Pool 6	None
(-2, -2)	HM	3-2-3	+	MT	Pool 7	None
(-2, -2)	HM	3-2-4	+	MT	Pool 8	None
(-2, -2)	HM	3-4-1	+	MT	Pool 1	None
(-2, -2)	HM	3-4-2	+	MT	Pool 2	None
(-2, -2)	HM	3-4-3	+	MT	Pool 3	None
(-2, -2)	HM	3-4-4	+	MT	Pool 4	None
(0, +1)	HT	6-1-1	+	MT	Pool 5	None
(0, +1)	HT	6-1-2	-	MT	Pool 6	None
(0, +1)	HT	6-1-3	+	MT	Pool 7	None
(0, +1)	HT	6-1-4	-	MT	Pool 8	None
(0, +1)	HT	7-2-1	+	MT	Pool 1	None
(0, +1)	HT	7-2-2	+	WT	Pool 2	None
(0, +1)	HT	7-2-3	+	MT	Pool 3	None
(0, +1)	HT	7-2-4	+	MT	Pool 4	None
(+1, -2)	BI	8-2-1	+	MT	Pool 5	None
(+1, -2)	BI	8-2-2	+	MT	Pool 6	None
(+1, -2)	BI	8-2-3	+	MT	Pool 7	None
(+1, -2)	BI	8-2-4	+	MT	Pool 8	None

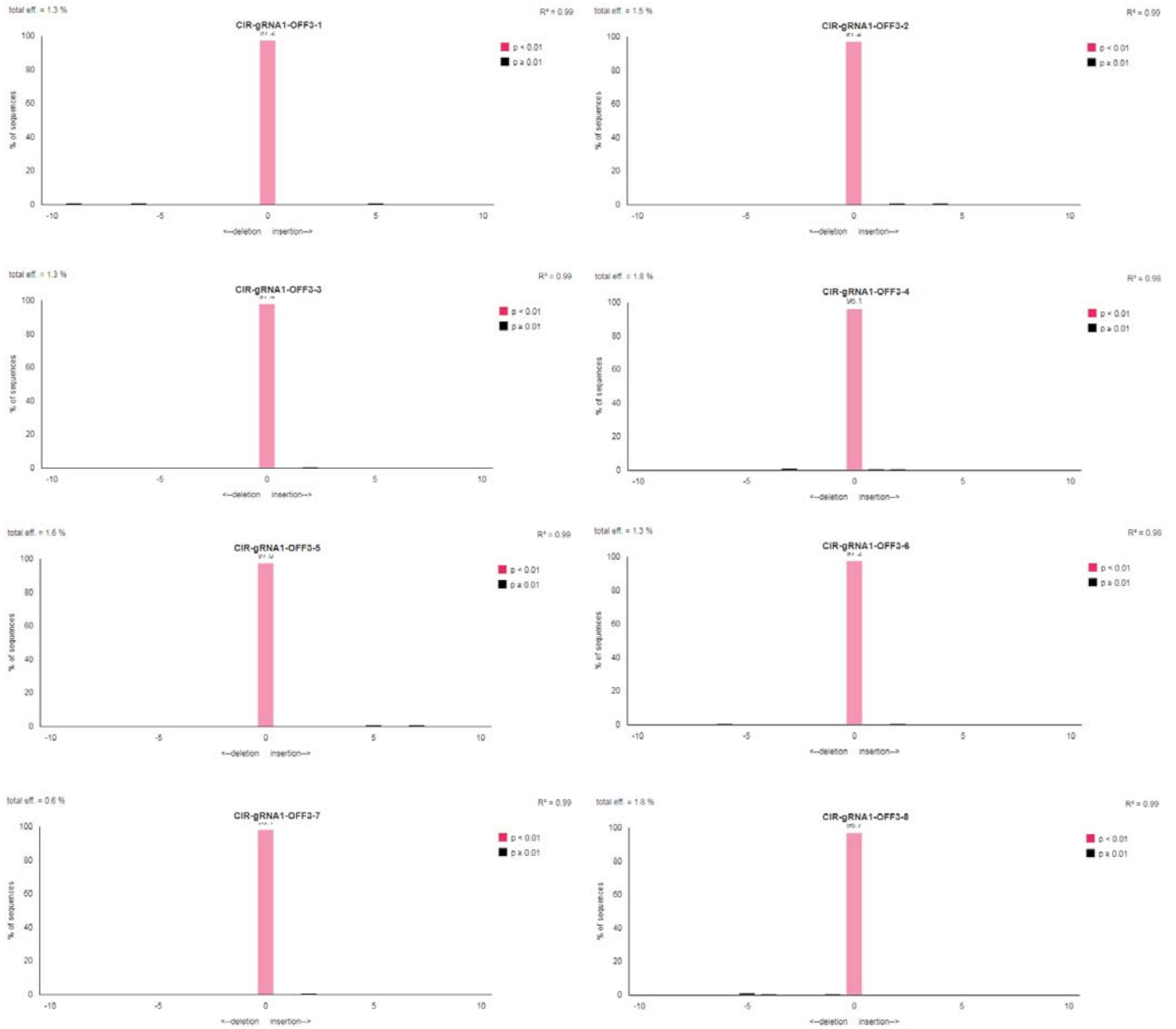
gRNA1-OFF1



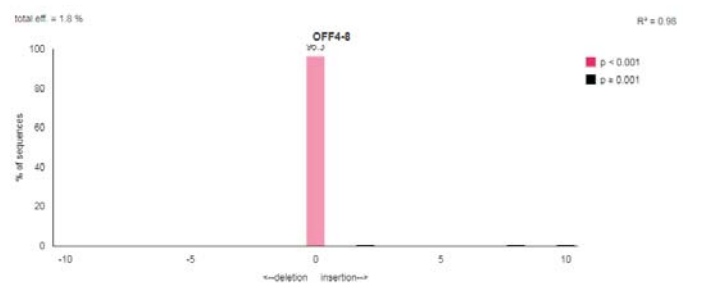
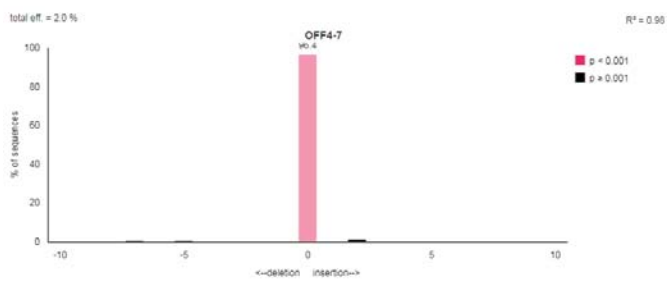
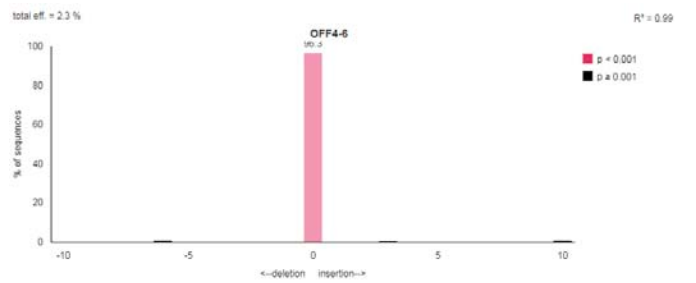
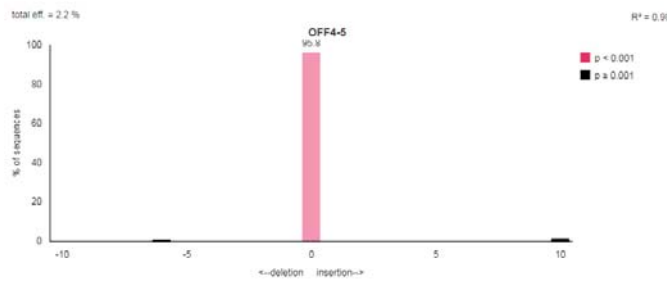
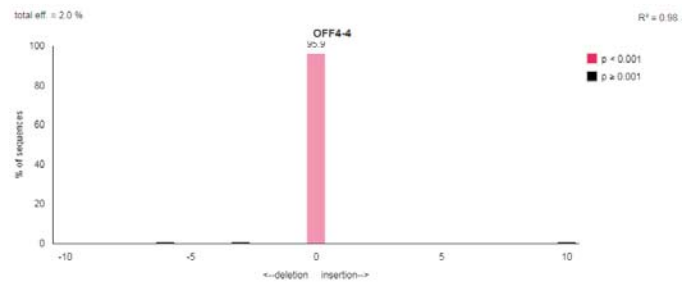
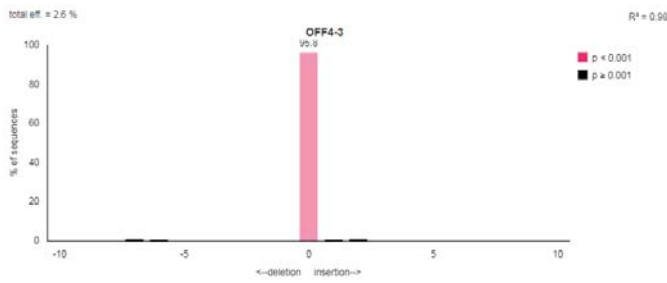
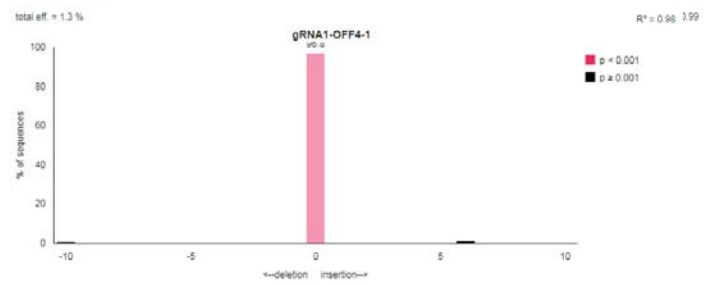
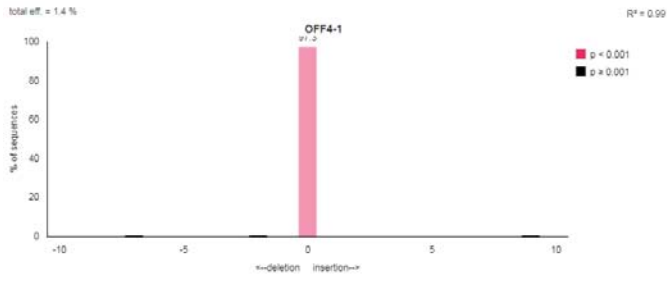
gRNA1-OFF2



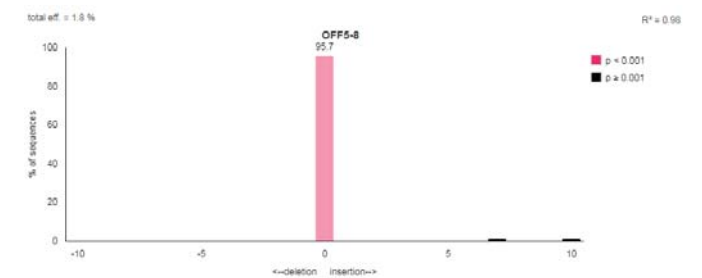
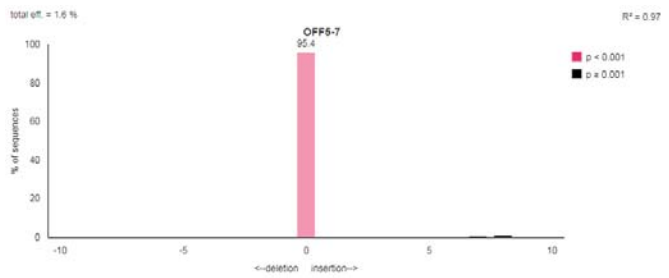
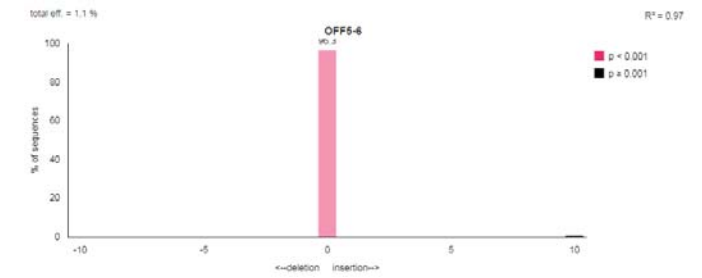
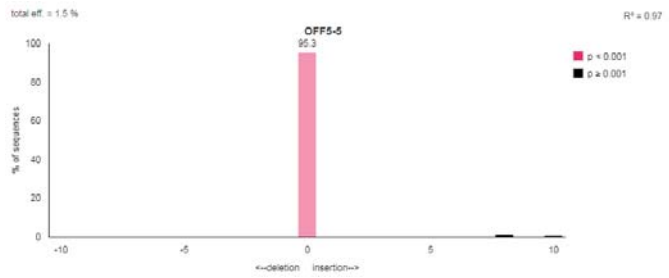
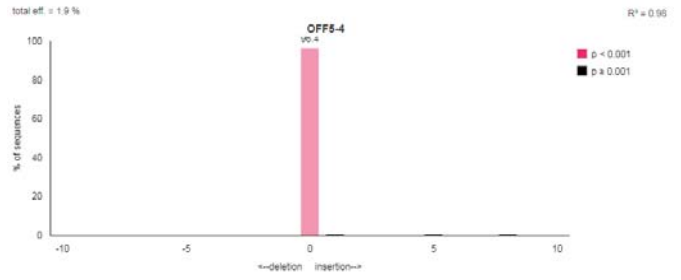
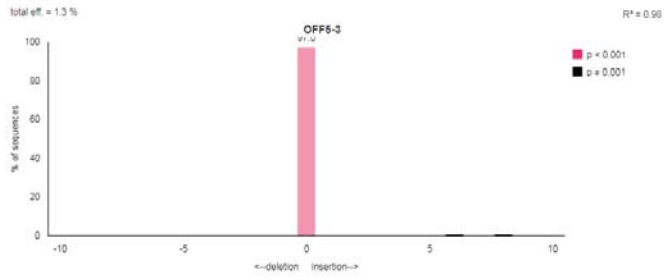
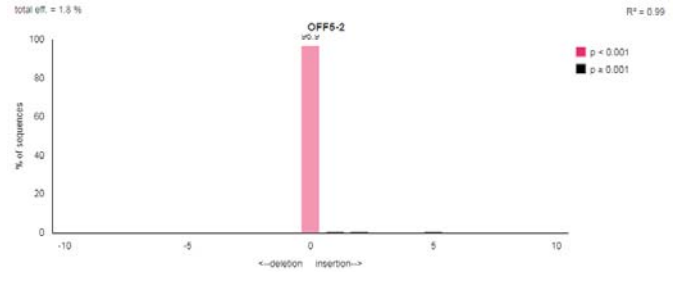
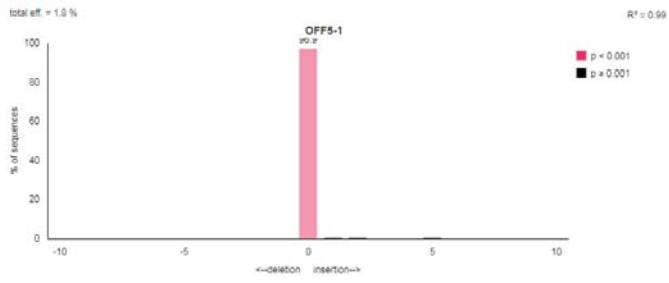
gRNA1-OFF3



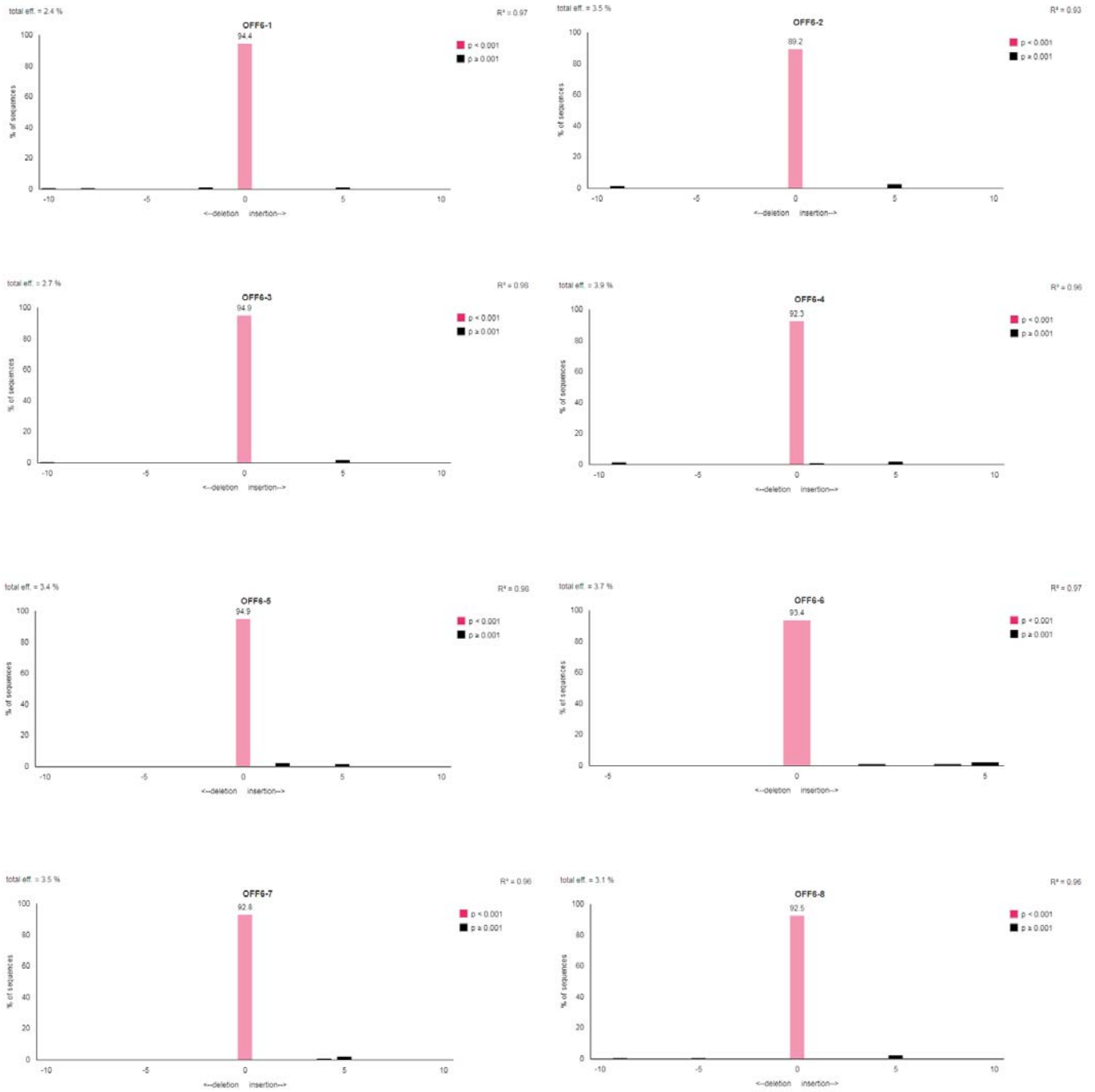
gRNA1-OFF4



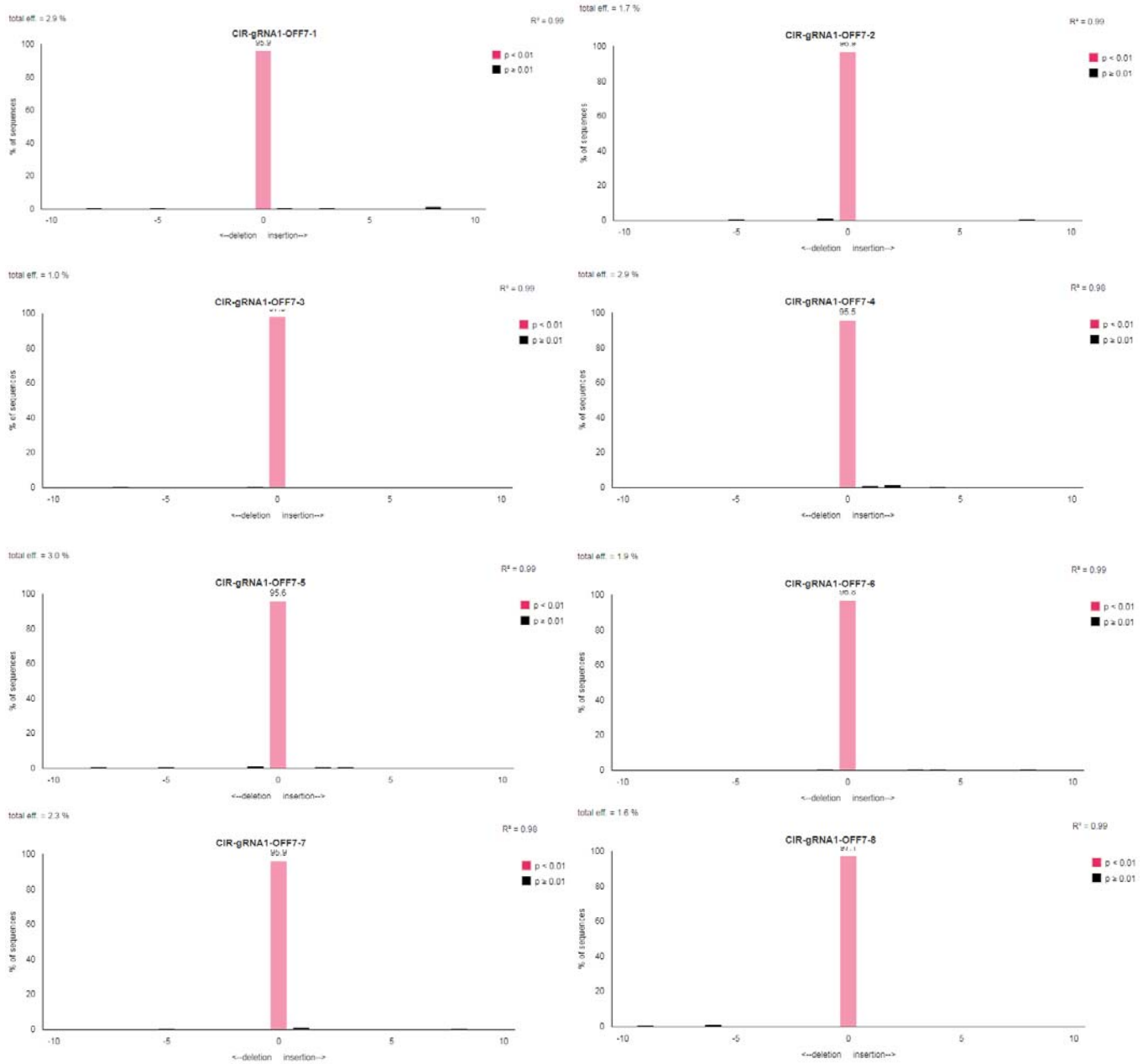
gRNA1-OFF5



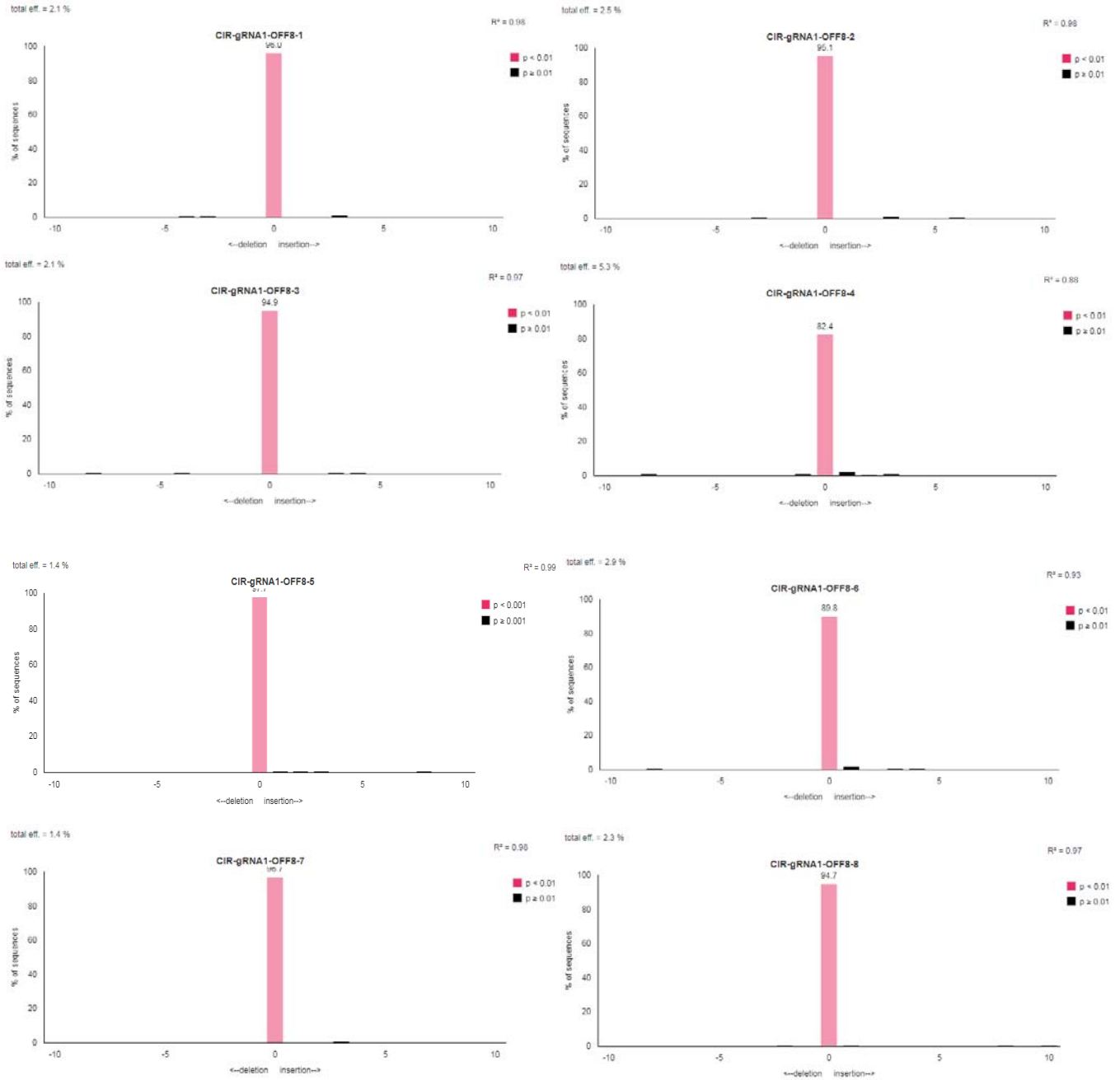
gRNA1-OFF6



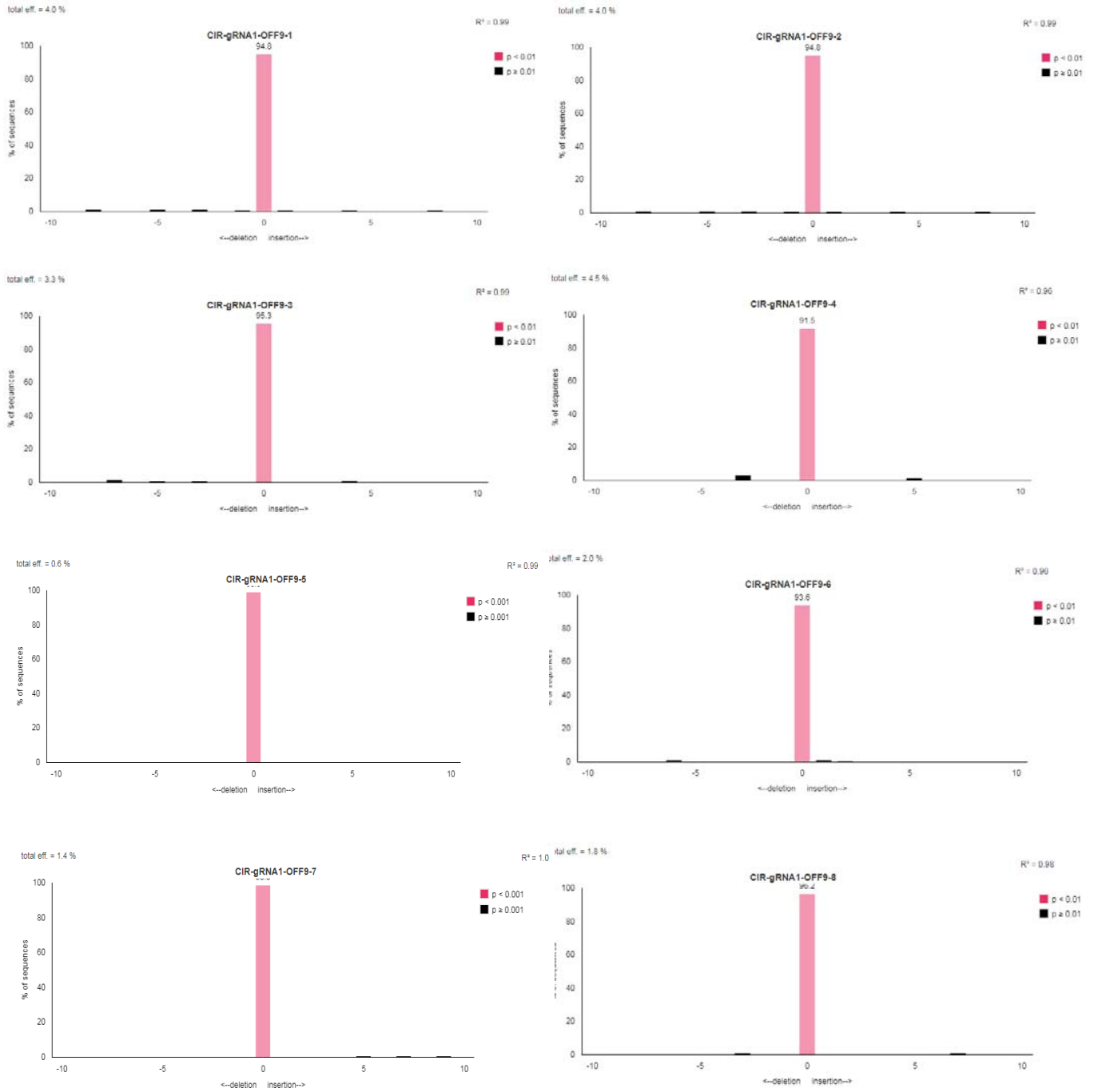
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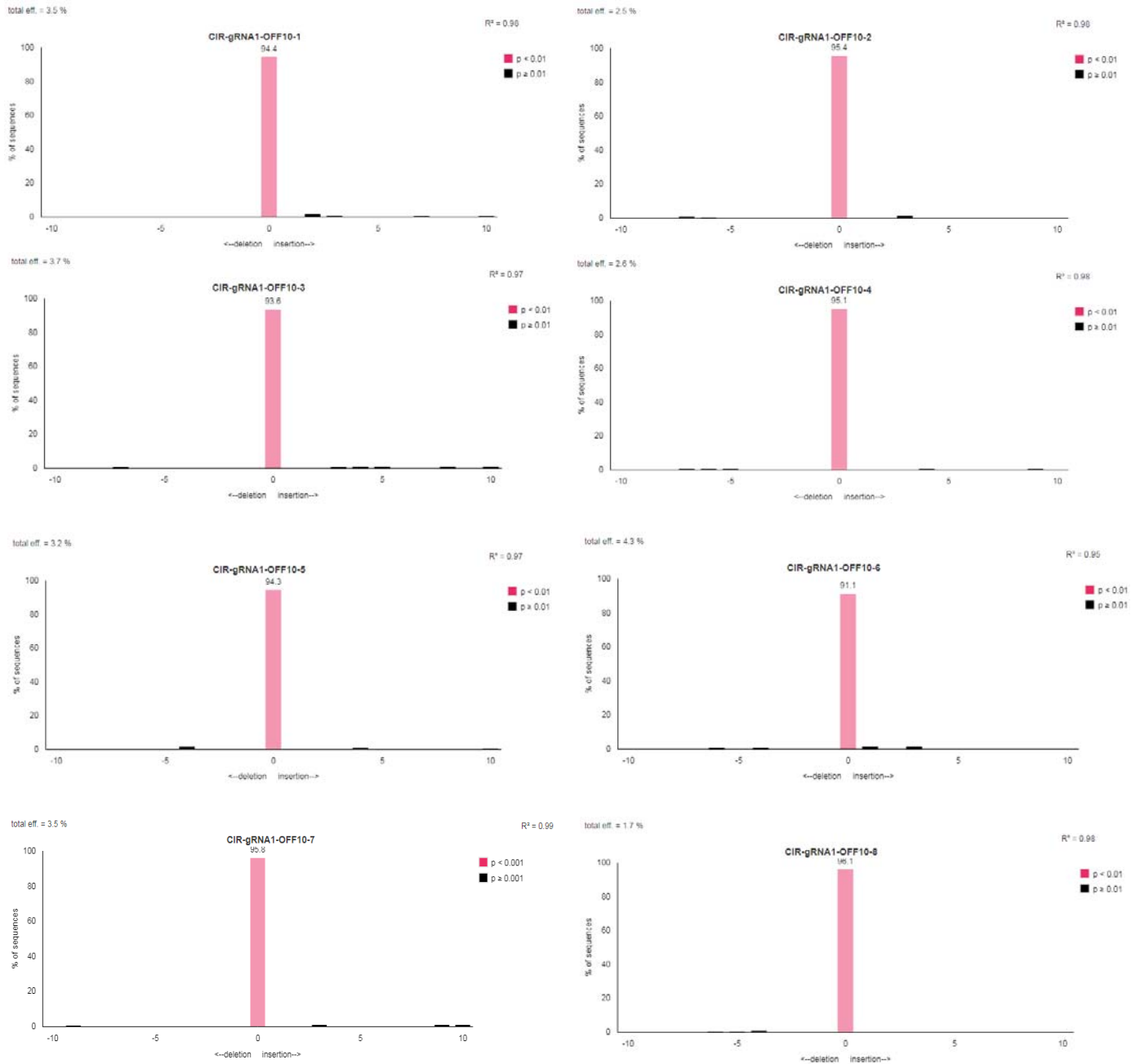
gRNA1-OFF8



gRNA1-OFF9

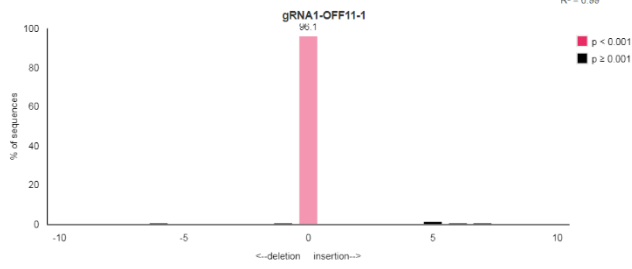


gRNA1-OFF10

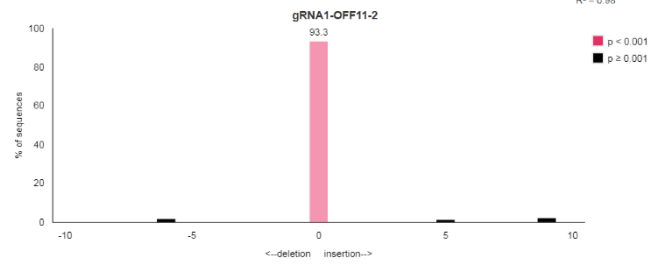


gRNA1-OFF11

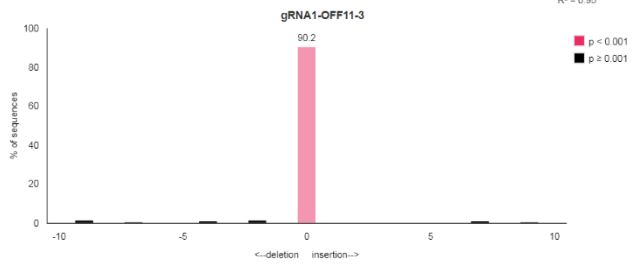
total eff. = 3.1 %



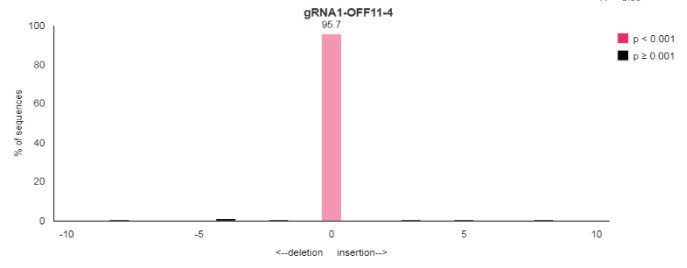
total eff. = 4.9 %



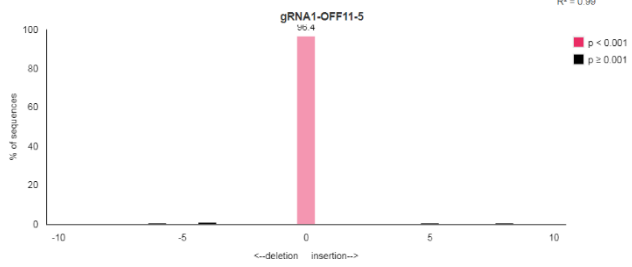
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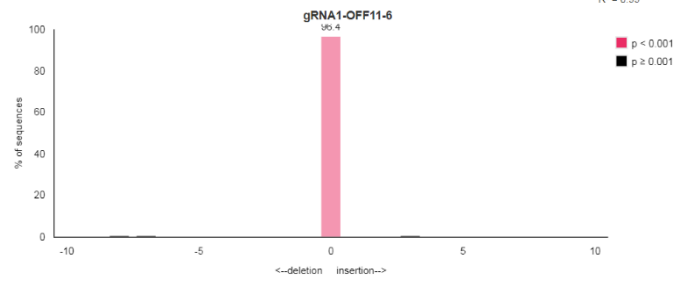
total eff. = 2.9 %



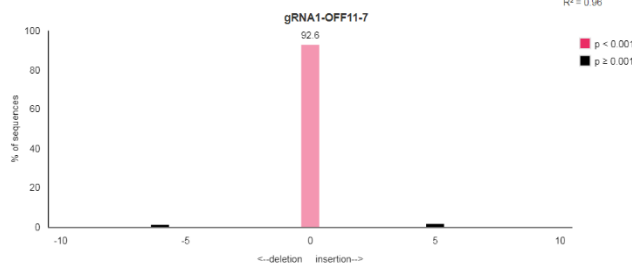
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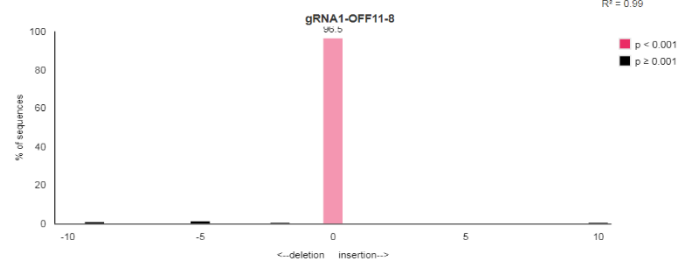
total eff. = 2.4 %



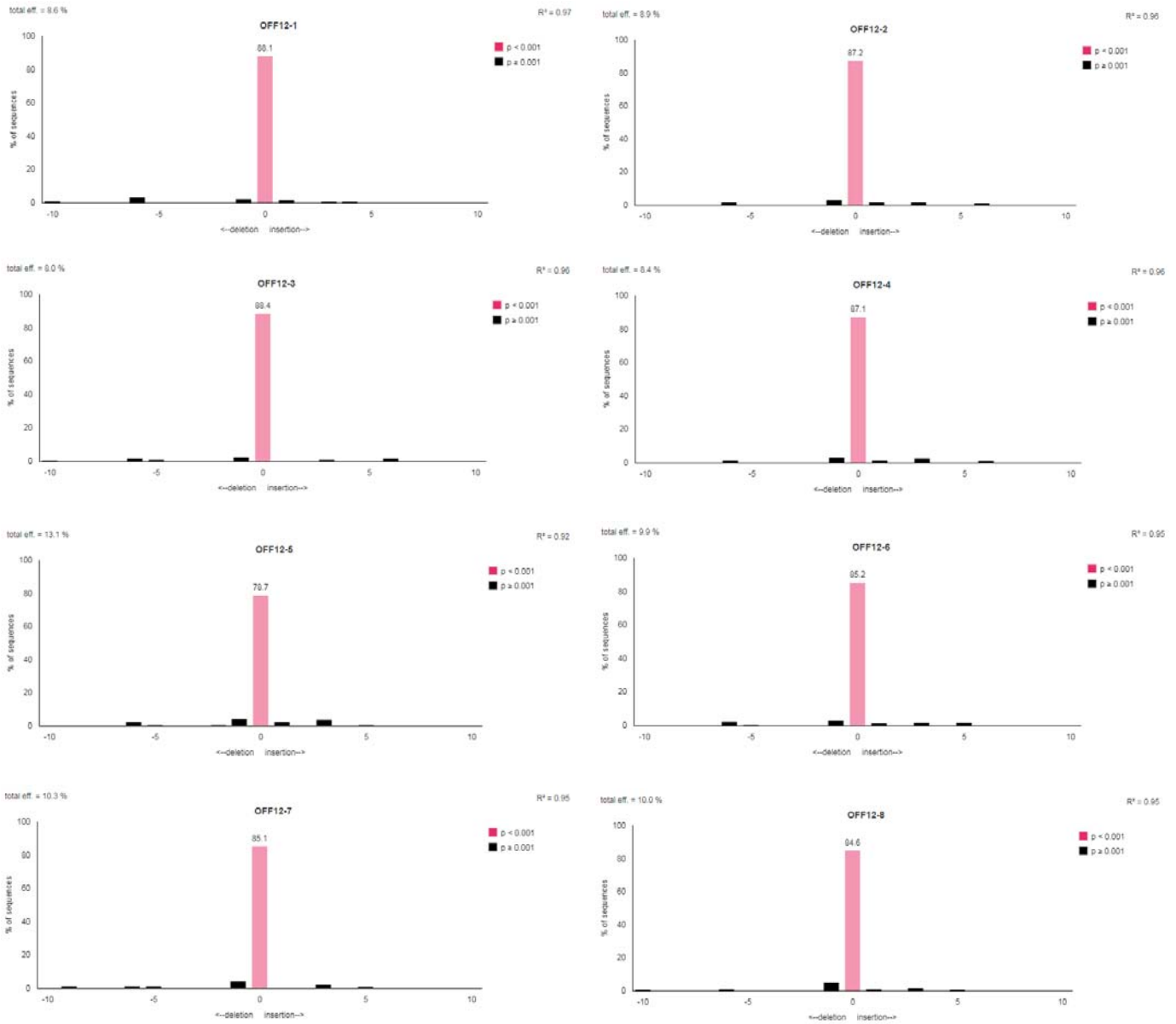
total eff. = 3.4 %



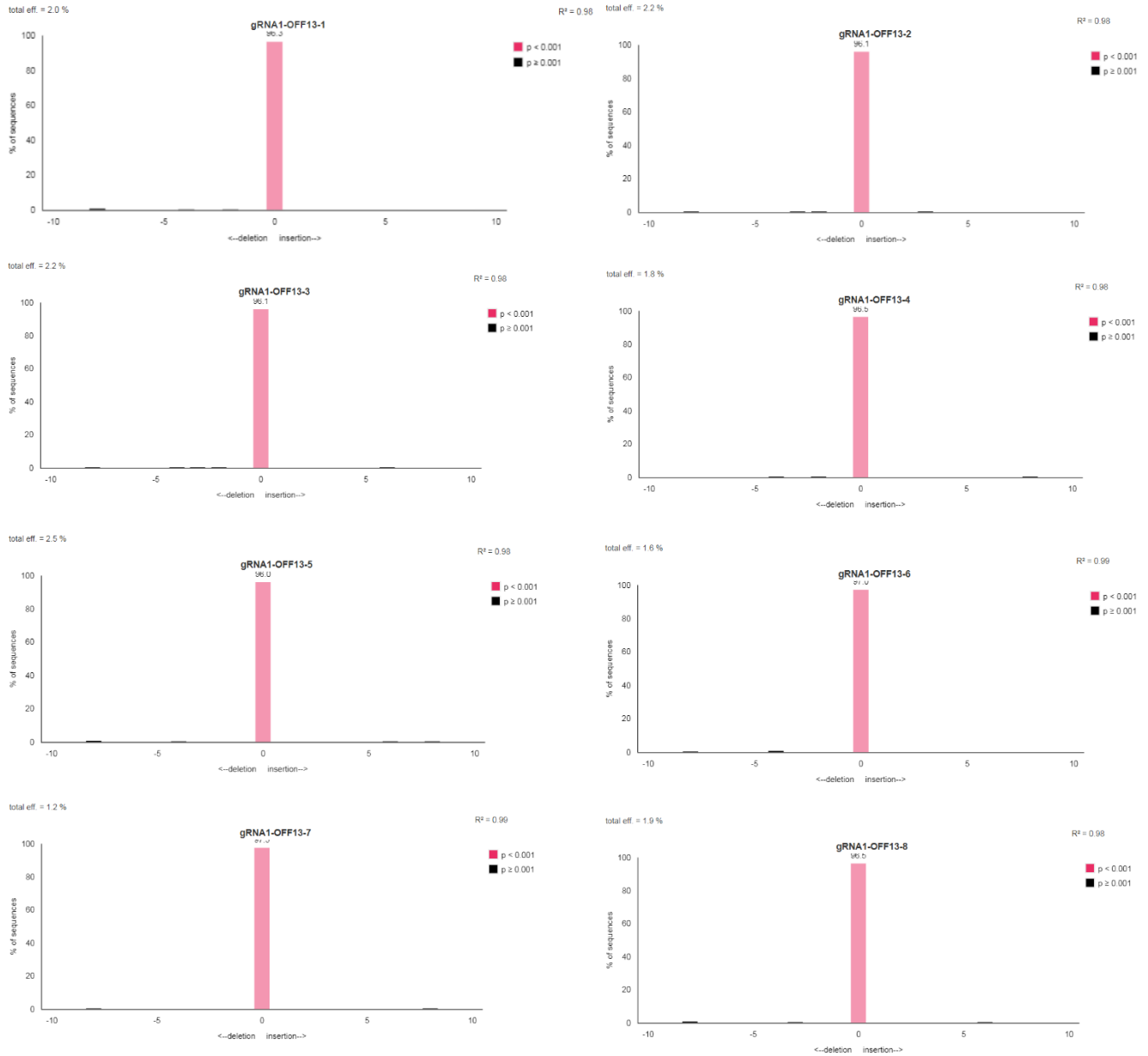
total eff. = 2.7 %



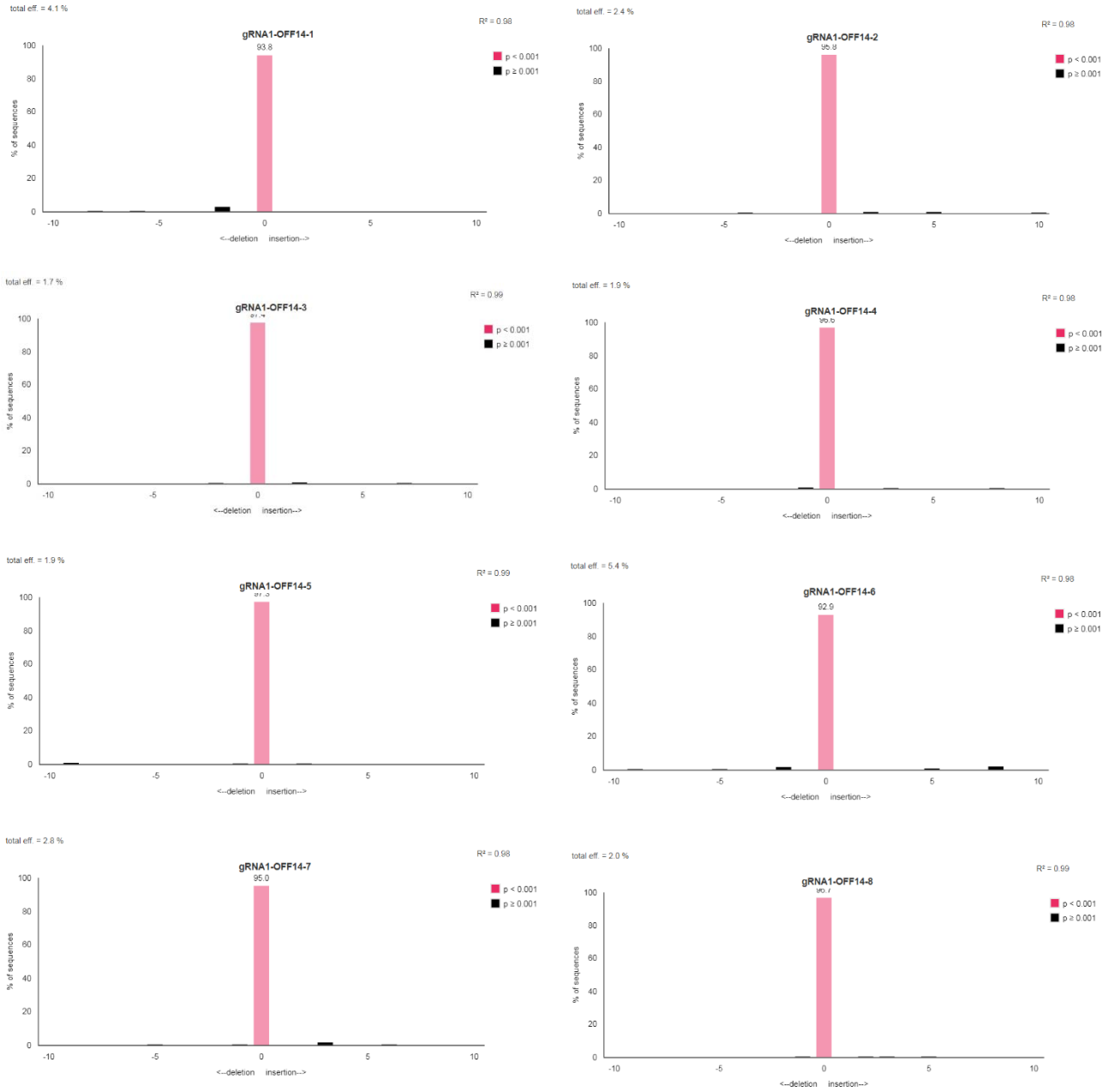
gRNA1-OFF12



gRNA1-OFF13

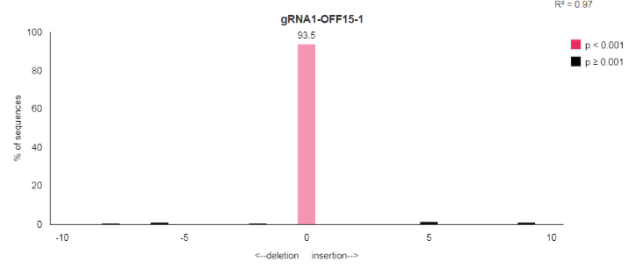


gRNA1-OFF14

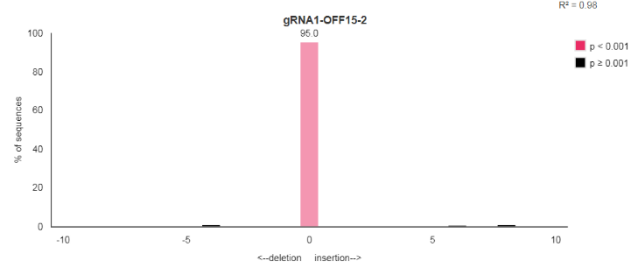


gRNA1-OFF15

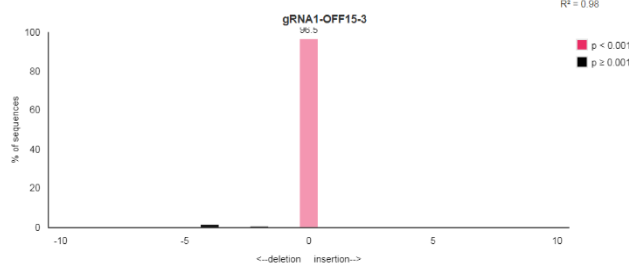
total eff. = 4.0 %



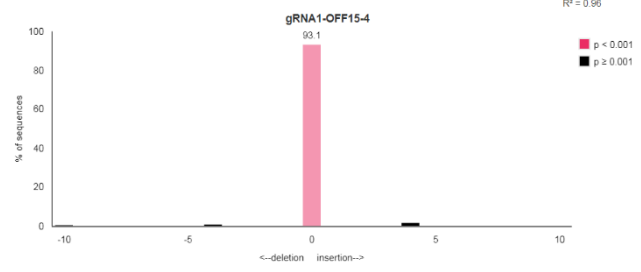
total eff. = 2.5 %



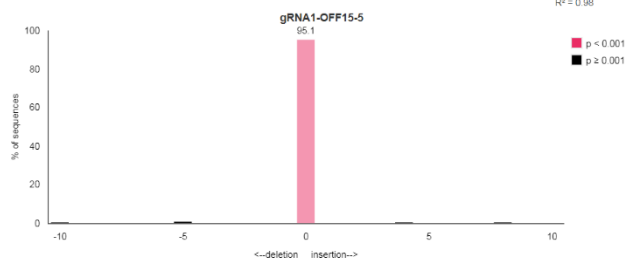
total eff. = 2.0 %



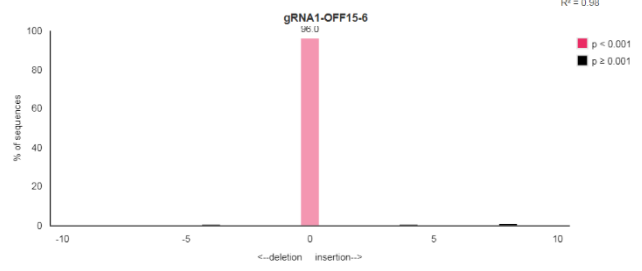
total eff. = 3.0 %



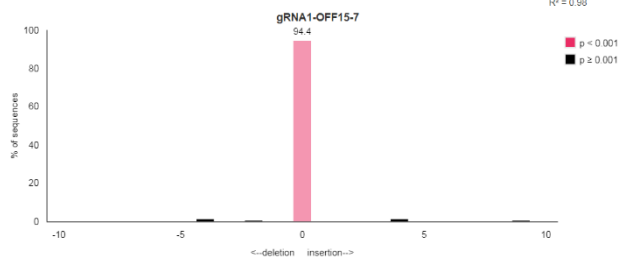
total eff. = 2.5 %



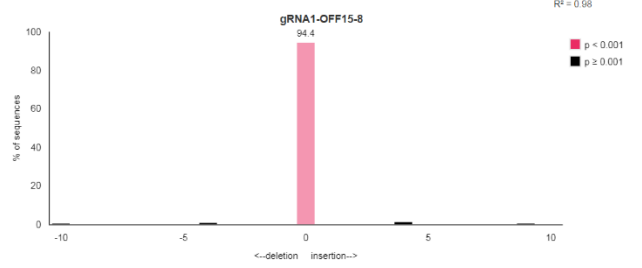
total eff. = 1.8 %



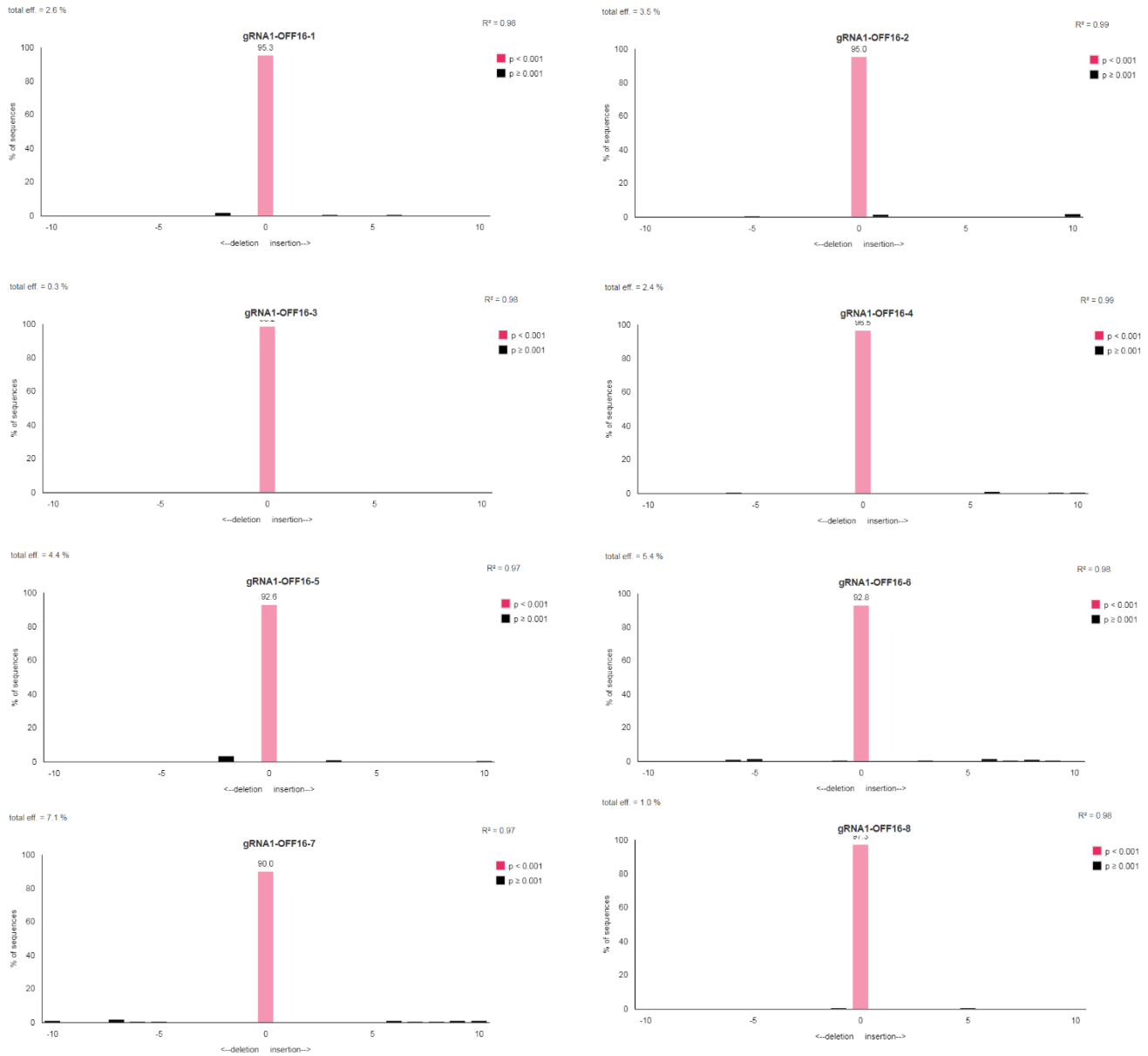
total eff. = 3.1 %



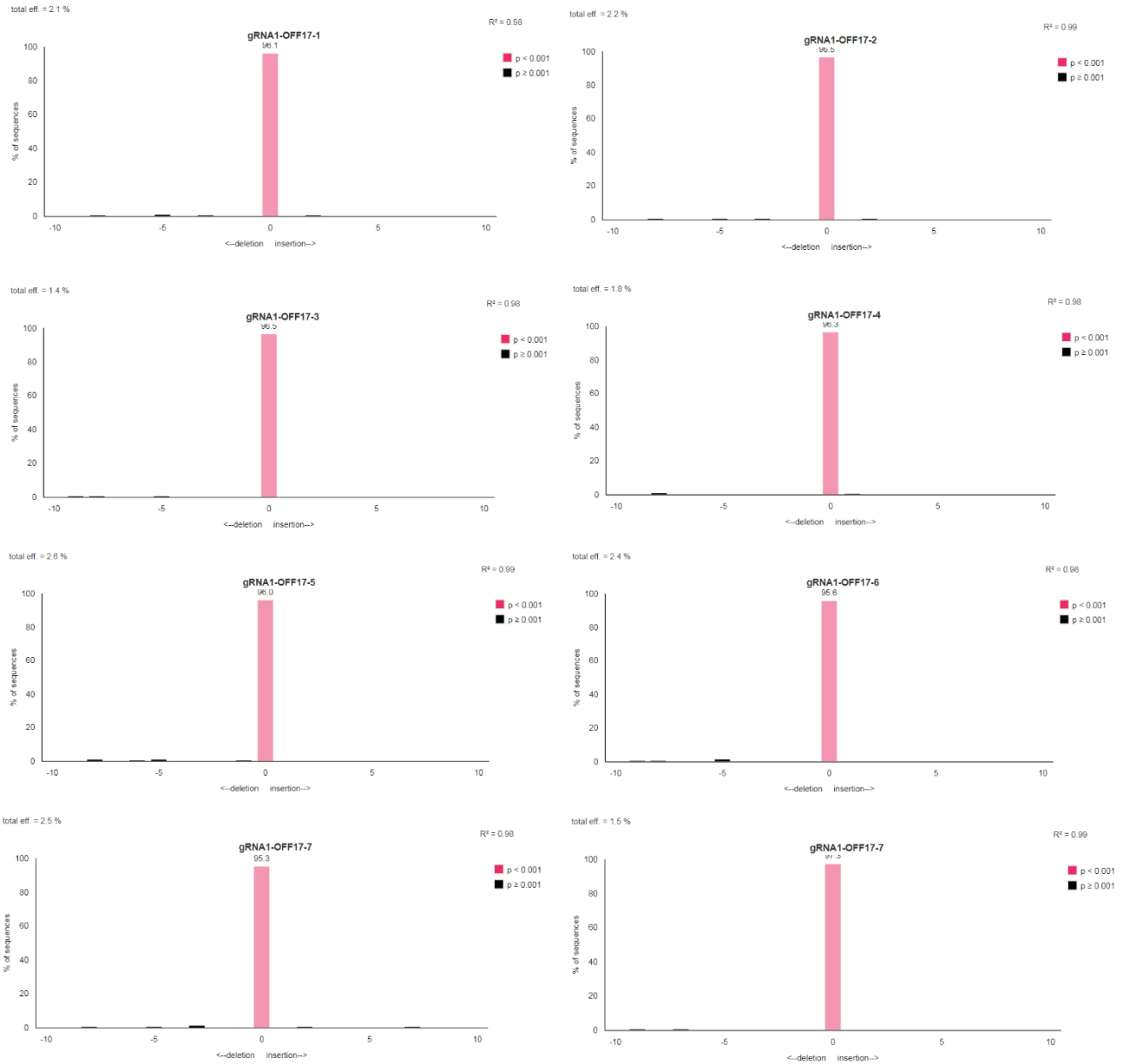
total eff. = 3.6 %



gRNA1-OFF16



gRNA1-OFF17

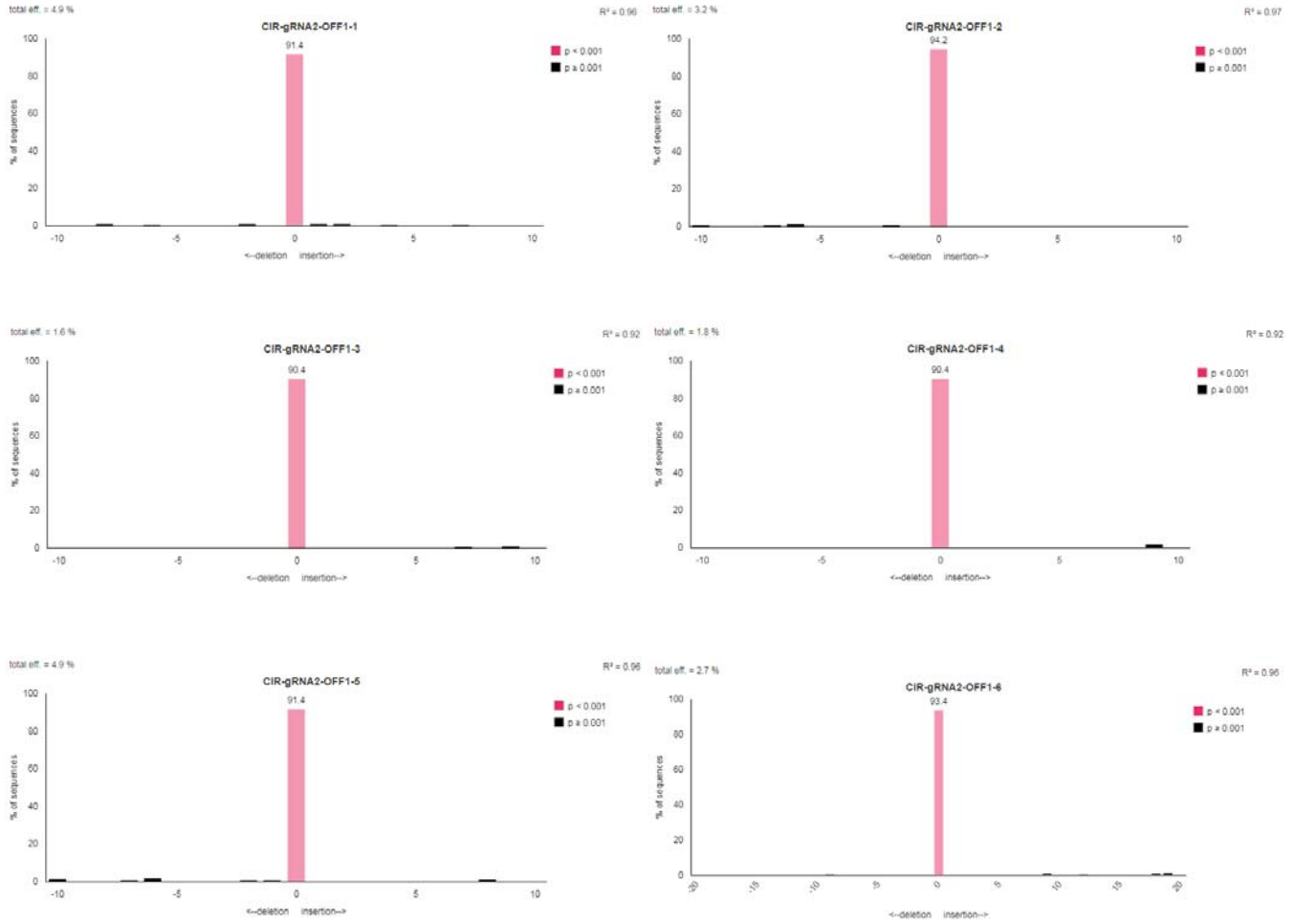


A845B (gRNA2) T1 sample information

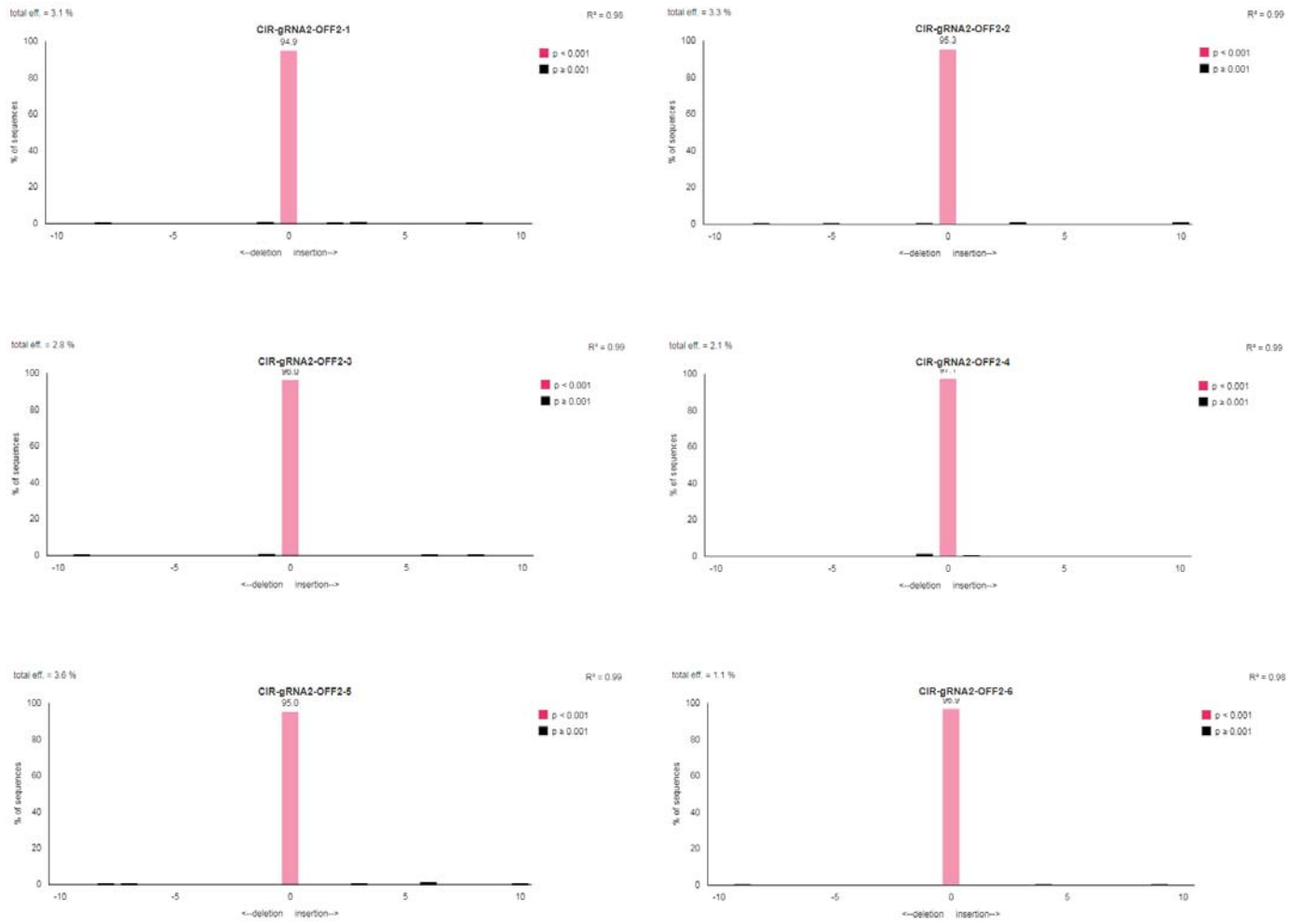
A total of 24 T1 plants derived from 10 different T0 lines were screened for off-target mutagenesis analysis. Seventeen plants were T-DNA positive (+), which constitutively expresses Cas9 nuclease, whereas seven were negative segregants (-) without Cas9 gene. PCR was carried for each gDNA sample, and then four reactions were pooled (Sample pool) for Sanger sequencing. Thus, six sanger sequencing reactions were carried out per off-target. Eighteen CIRCLE-seq found (gRNA2-OFF1-16, 19, 20) and two Cas-OFFinder predicted off-targets (gRNA2-OFF17,18) have been screened, but no evident off-target mutations were detected in the 24 T1 lines. HM, homozygous; BI, biallelic; HT, heterozygous; MO, mosaic; WT, wild type; MT, *gl2* mutant.

T0 genotype	A845B	T-DNA	T1 phenotype	Sample pool	Off-targets with mutation
(-6, -4+1)	BI	2-4-5	-	WT	
(-6, -4+1)	BI	2-4-12	-	WT	Pool 1 None
(-2, -5)	BI	3-3-6	+	MT	
(-2, -5)	BI	3-3-7	+	MT	
(-2, -5)	BI	3-3-10	+	MT	Pool 2 None
(-2, -5)	BI	3-4-14	+	MT	
(-2, -4)	BI	4-1-1	-	MT	
(-2, -4)	BI	4-1-12	-	MT	Pool 3 None
(-2, -4)	BI	4-3-12	+	MT	
(-2, -4)	BI	4-3-13	+	MT	
(-2, -3)	BI	5-3-2	+	WT	Pool 4 None
(-2, -3)	BI	5-3-5	+	WT	
(-2, -3)	BI	5-3-11	+	WT	
(-2, -3)	BI	5-3-13	+	WT	Pool 5 None
(-6, -6)	HM	6-4-1	+	MT	
(-6, -6)	HM	6-4-2	+	MT	
(-6, -6)	HM	6-4-9	+	MT	Pool 6 None
(-6, -6)	HM	6-4-13	-	WT	
(-1, -6)	BI	7-3-7	+	MT	
(-1, -6)	BI	7-3-10	+	MT	None
(-1, -6)	BI	7-3-15	+	MT	
(0, 0)	WT	8-3-5	-	WT	
(0, -6)	HT	10-2-2	+	WT	None
(0, -6)	HT	10-2-10	-	WT	

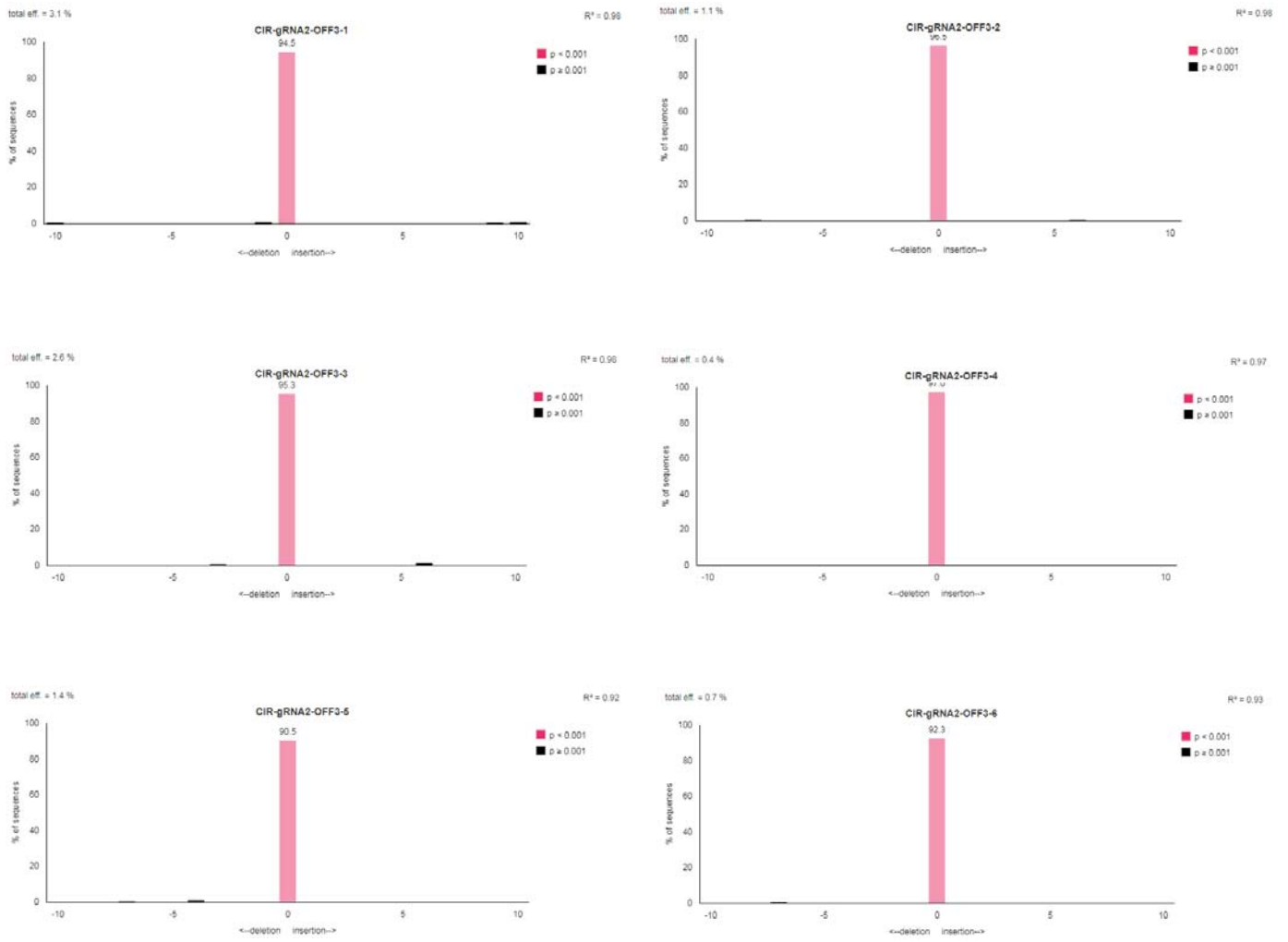
gRNA2-OFF1



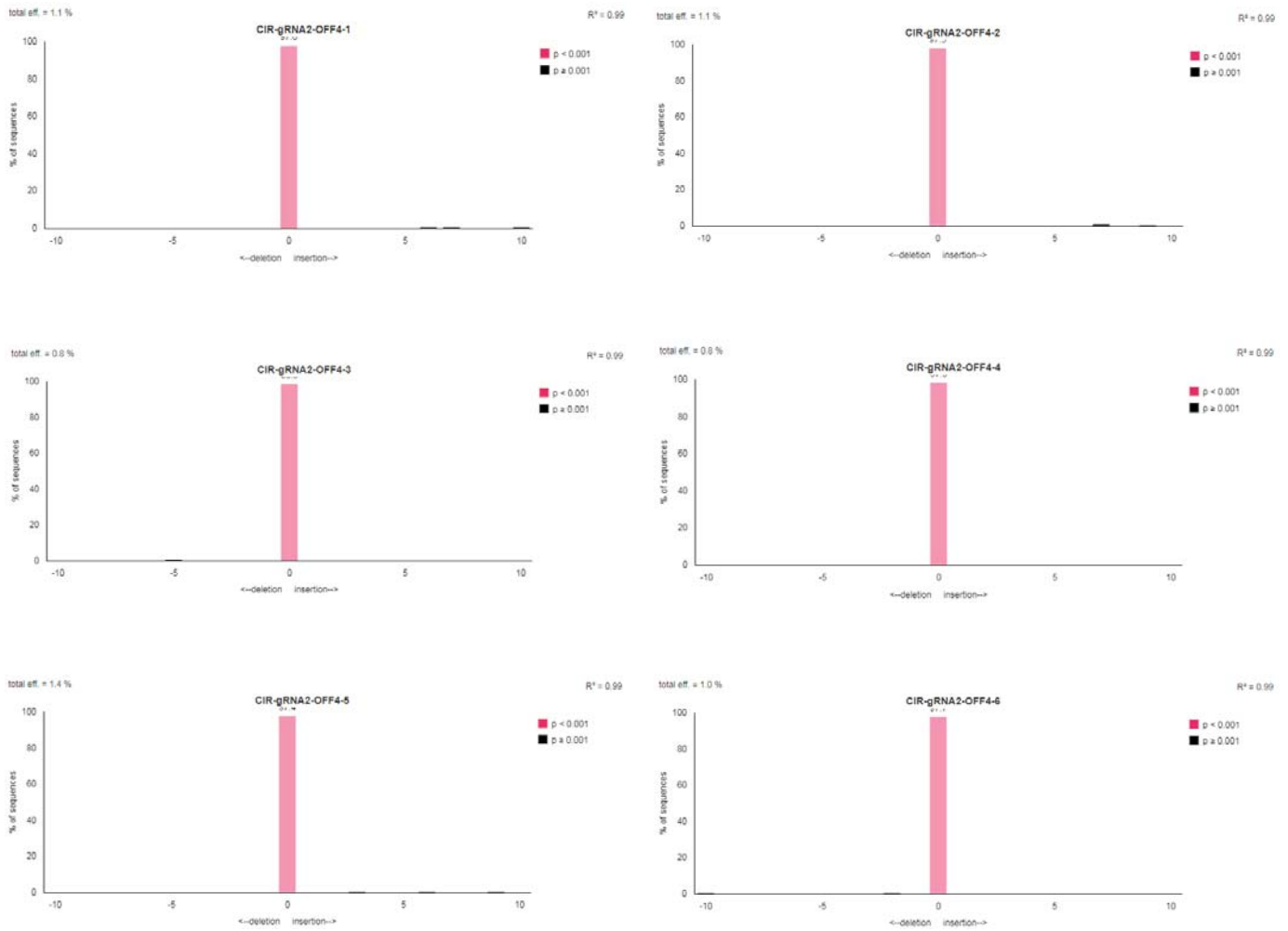
gRNA2-OFF2



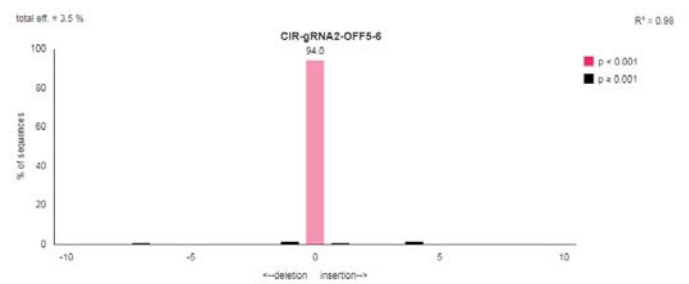
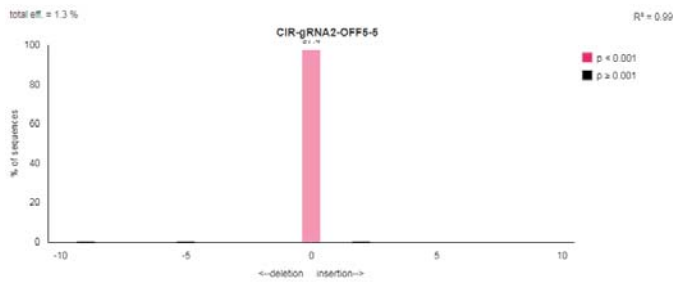
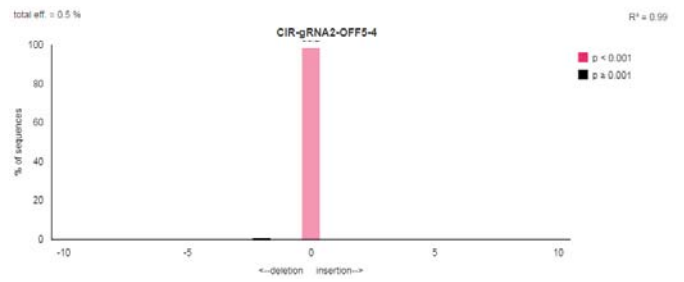
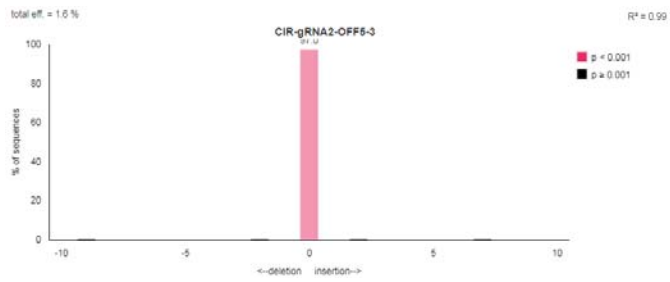
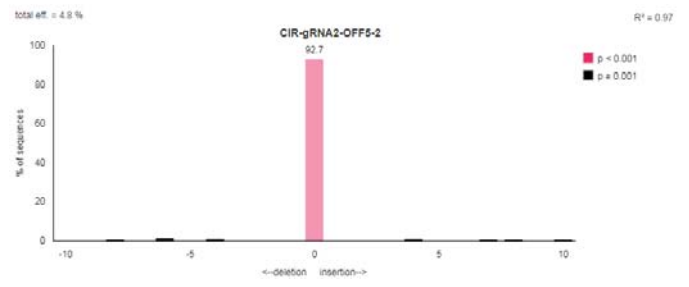
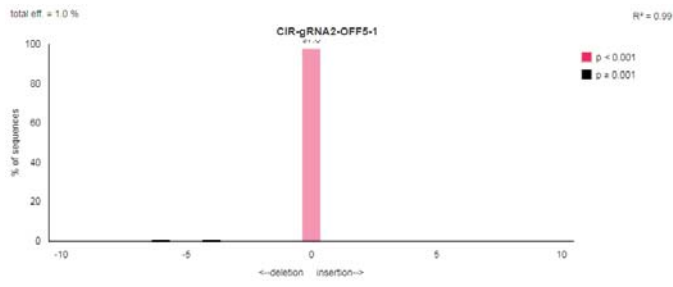
gRNA2-OFF3



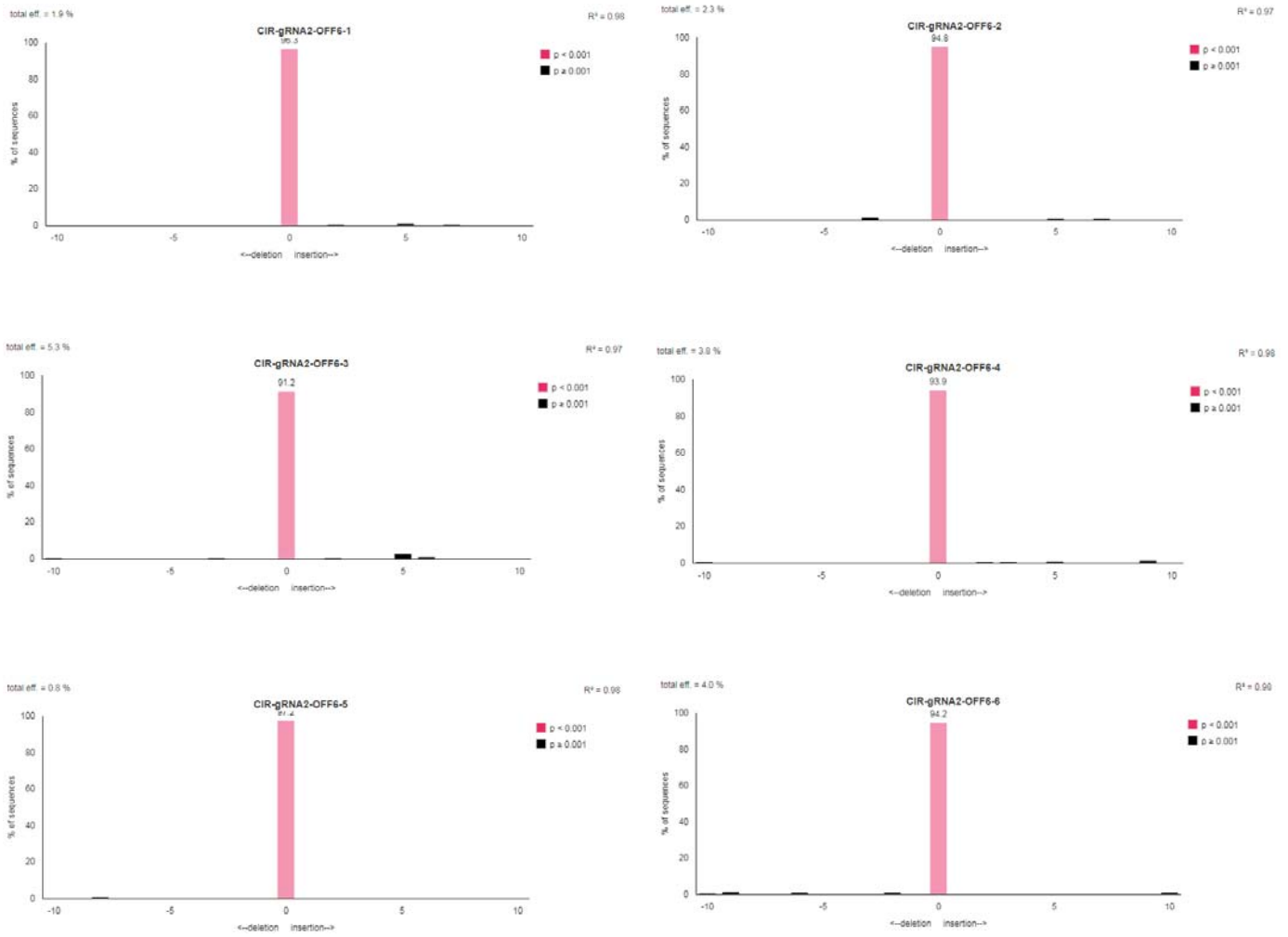
gRNA2-OFF4



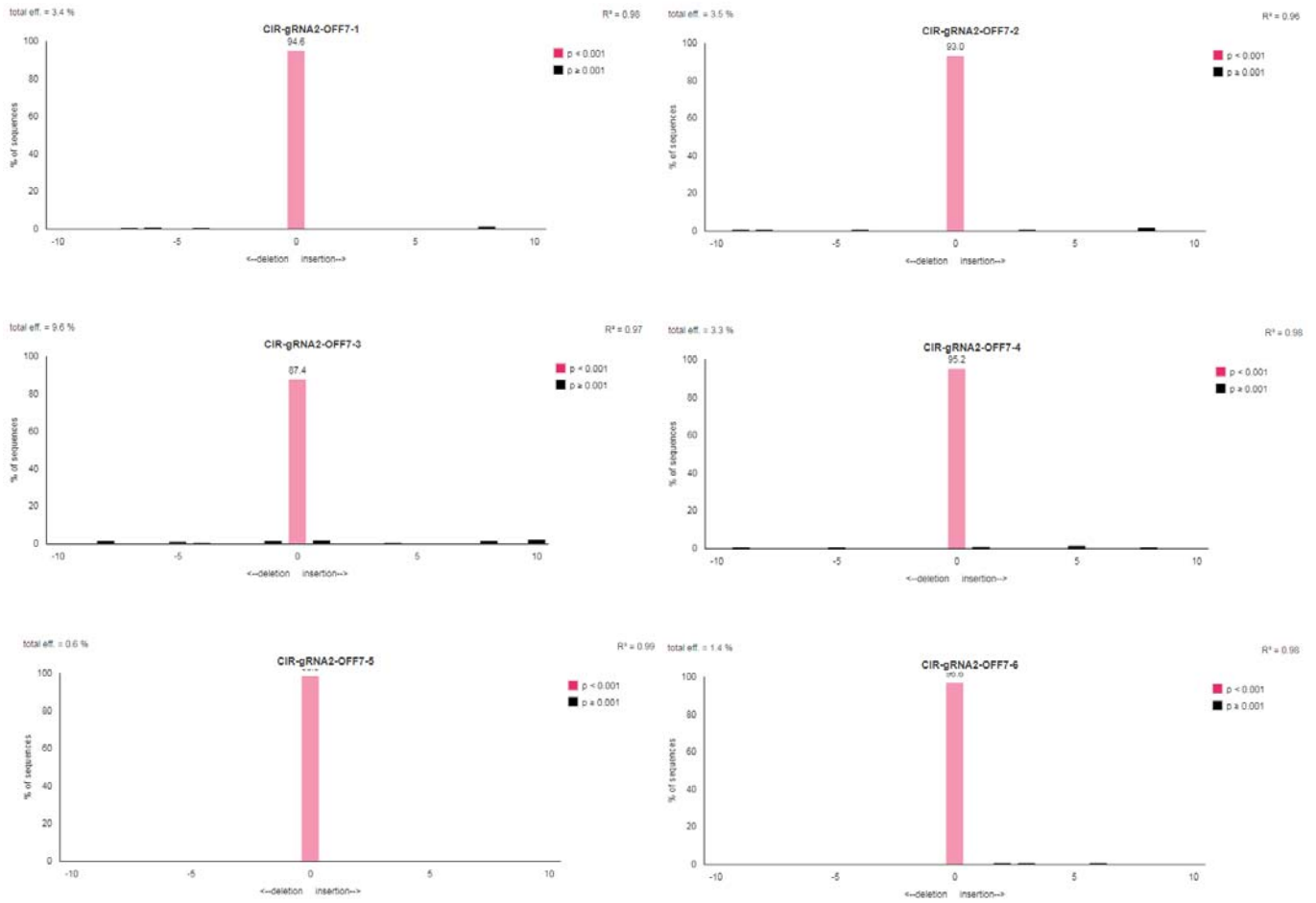
gRNA2-OFF5



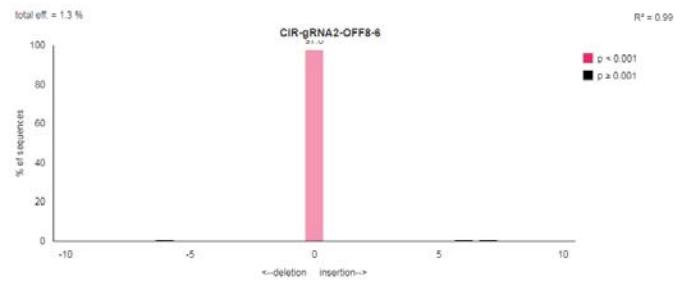
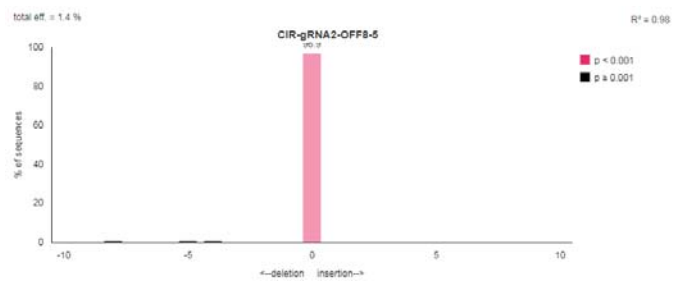
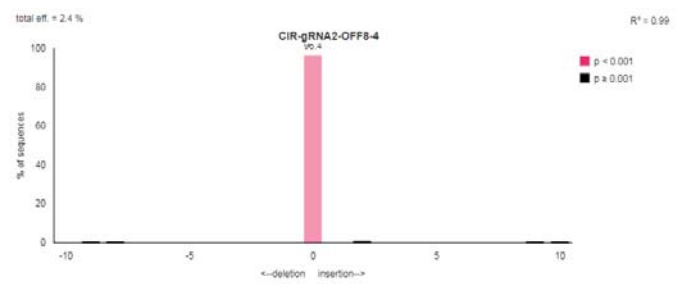
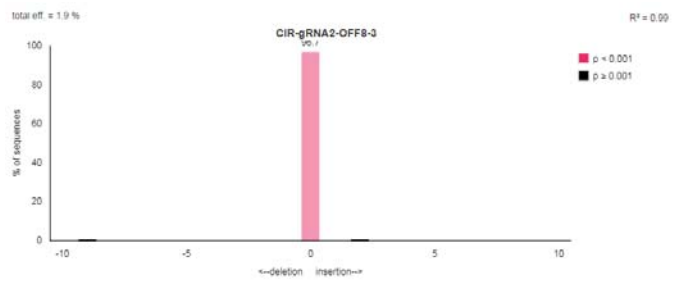
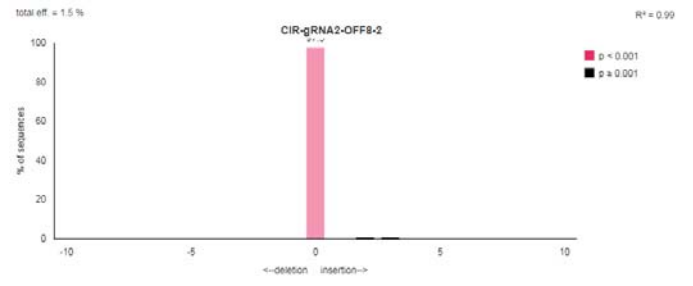
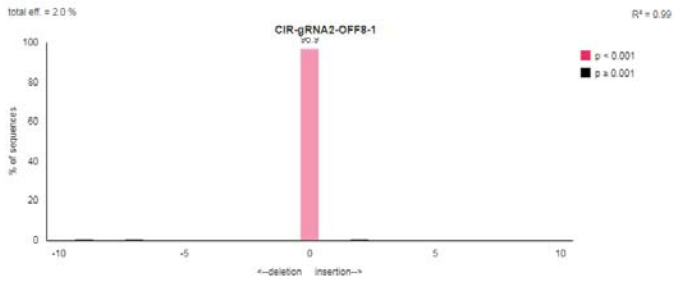
gRNA2-OFF6



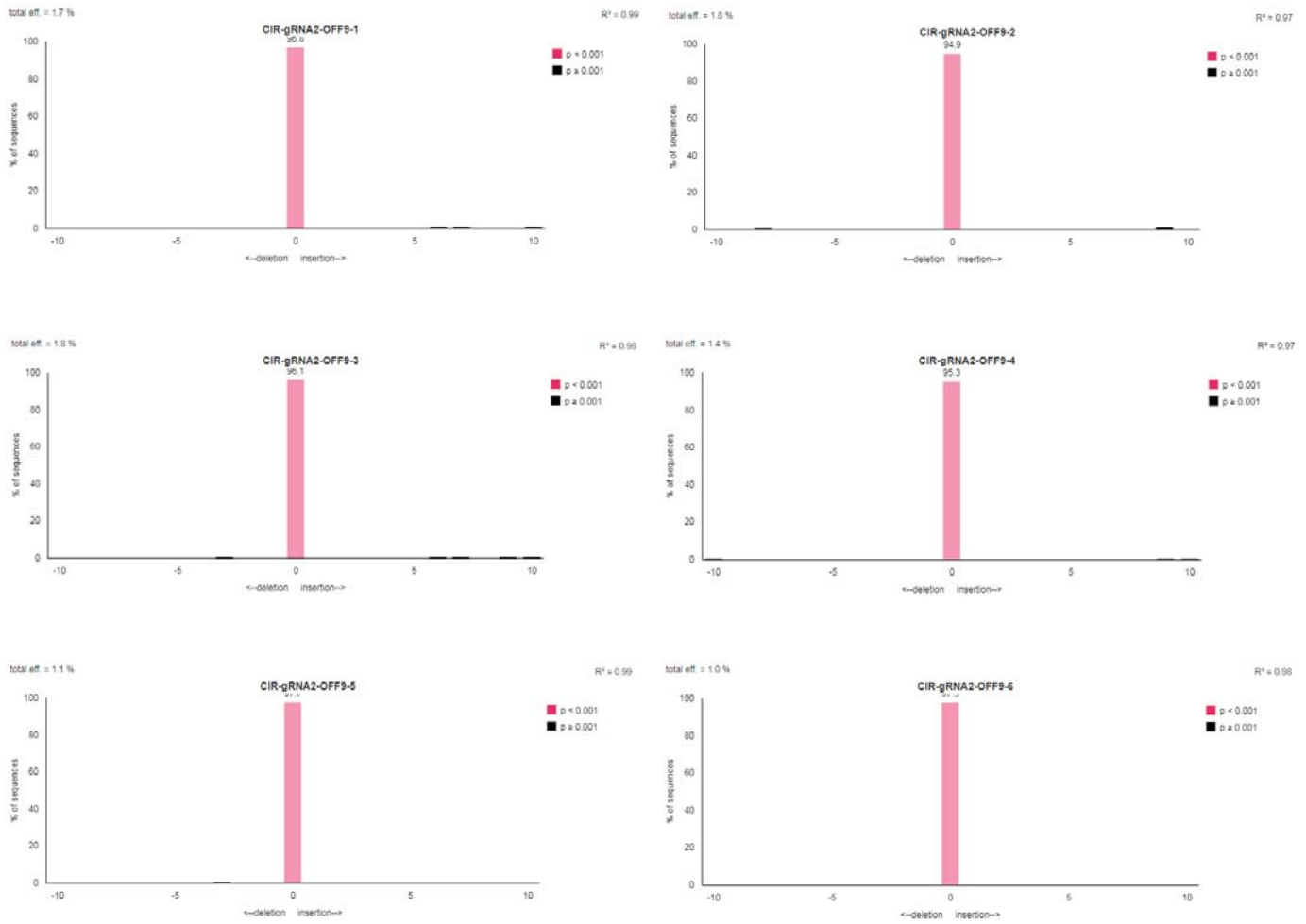
gRNA2-OFF7



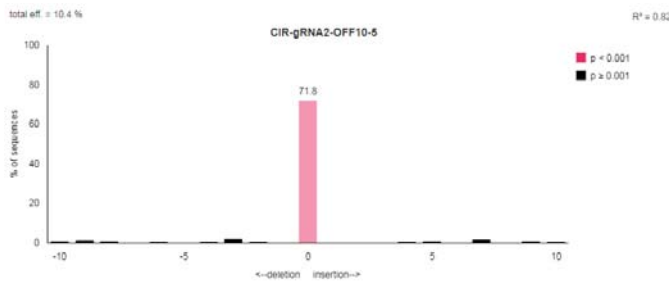
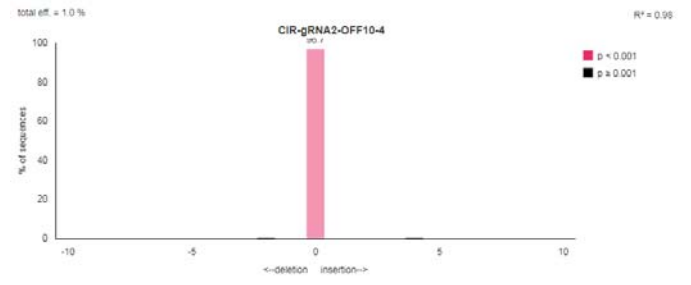
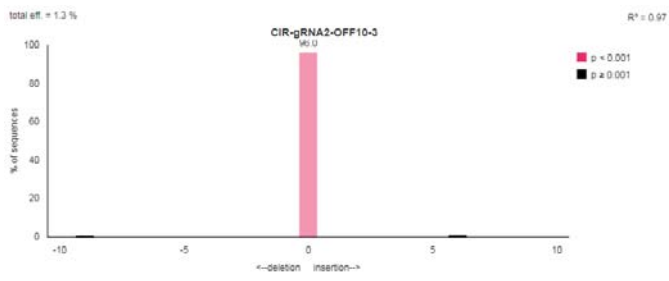
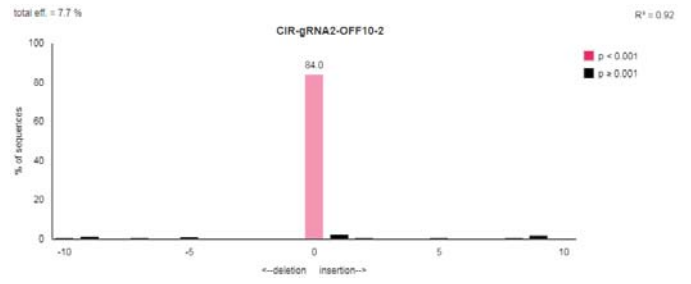
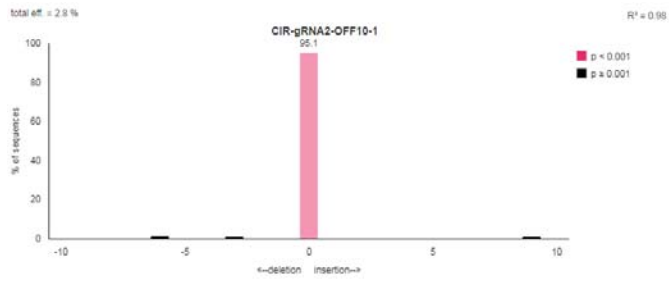
gRNA2-OFF8



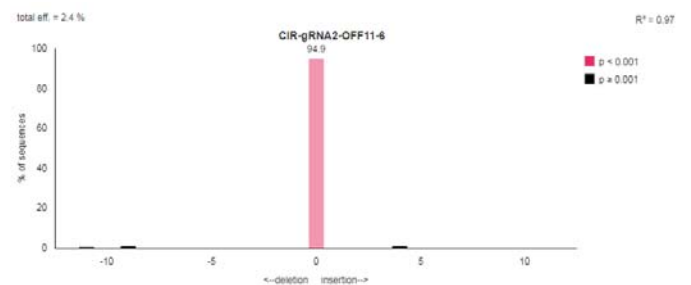
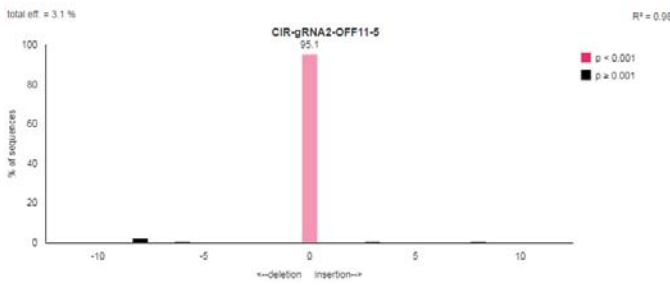
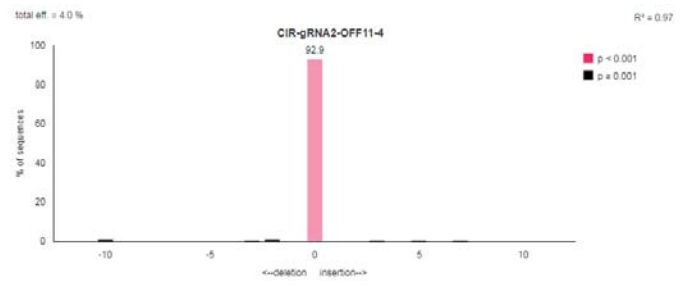
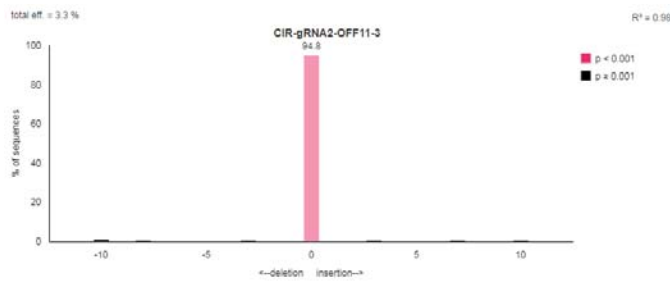
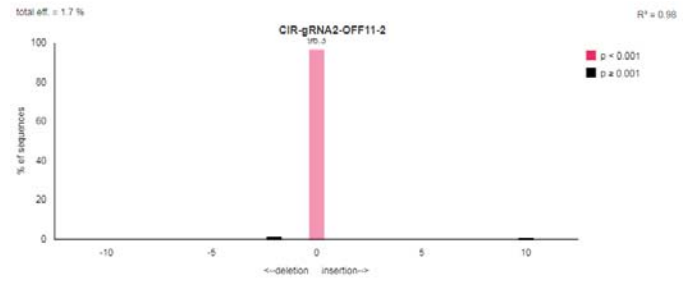
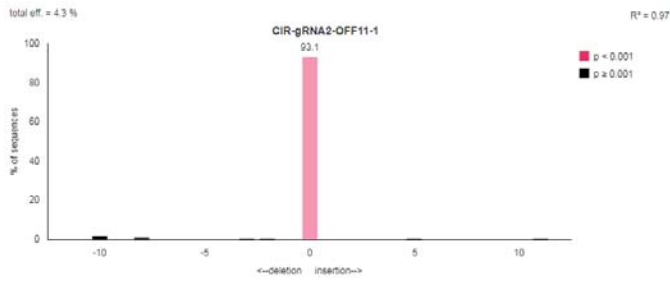
gRNA2-OFF9



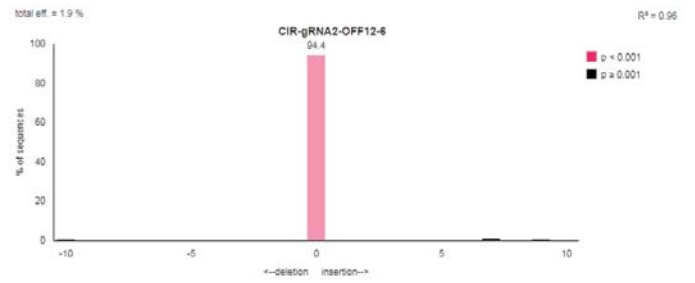
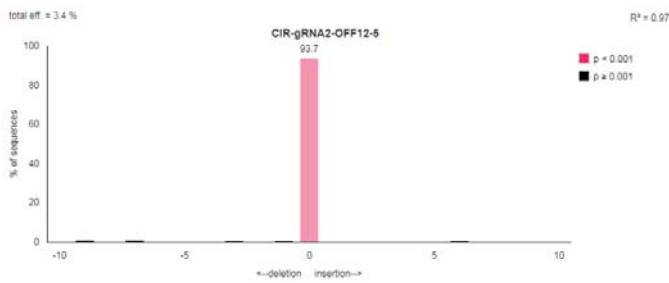
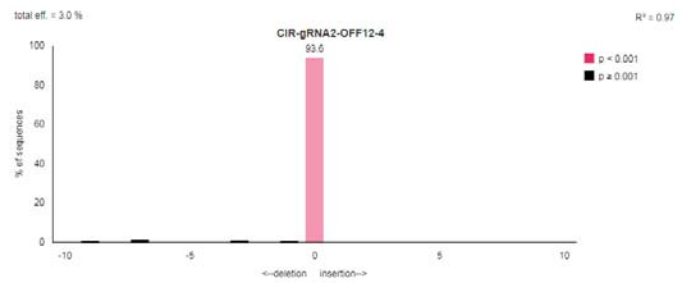
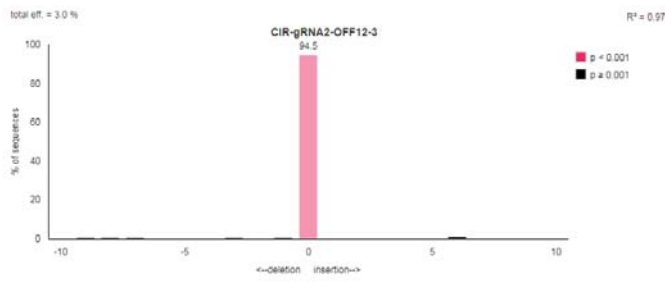
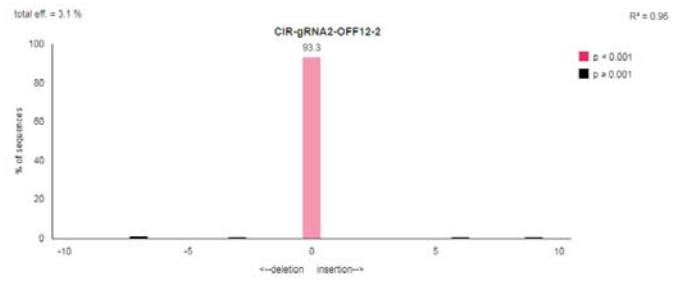
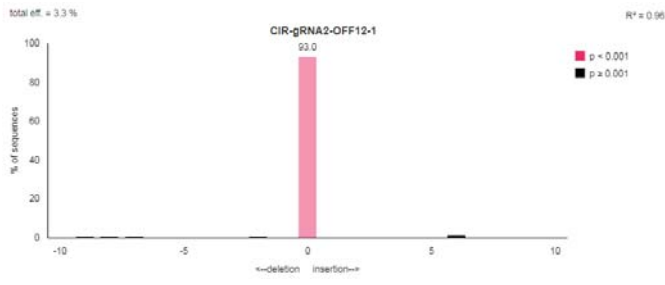
gRNA2-OFF10



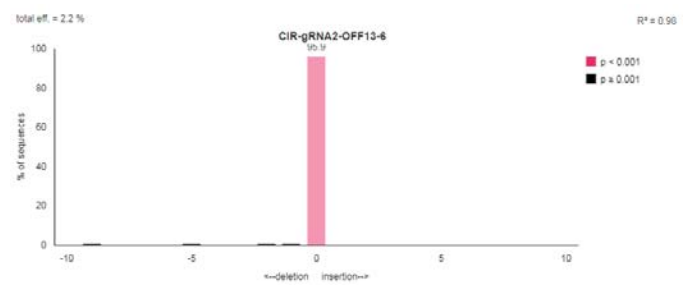
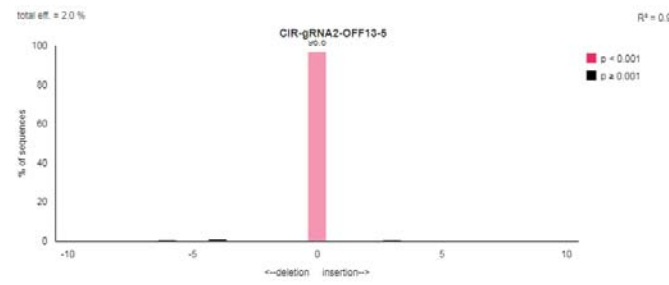
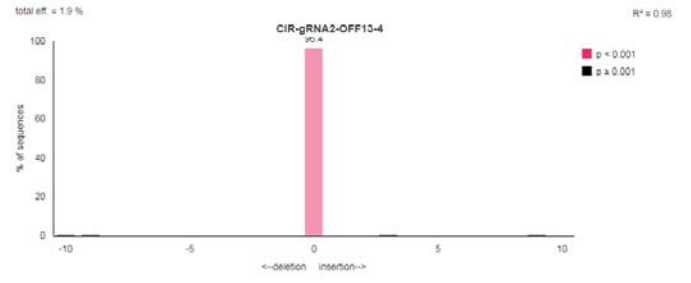
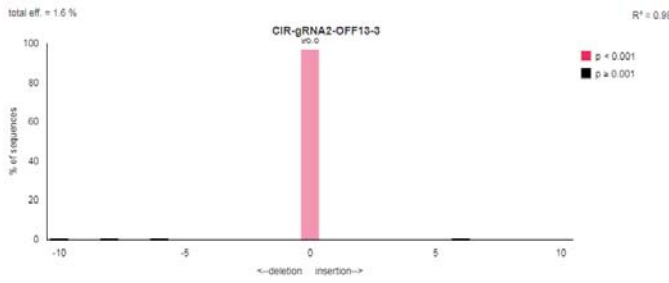
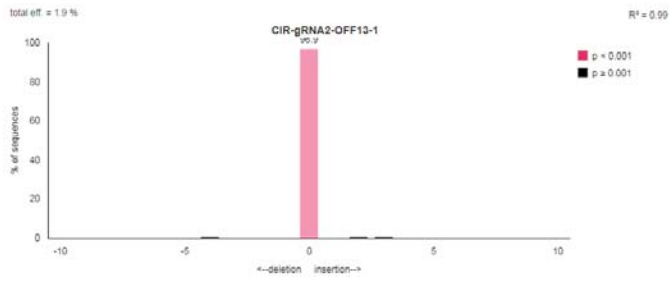
gRNA2-OFF11



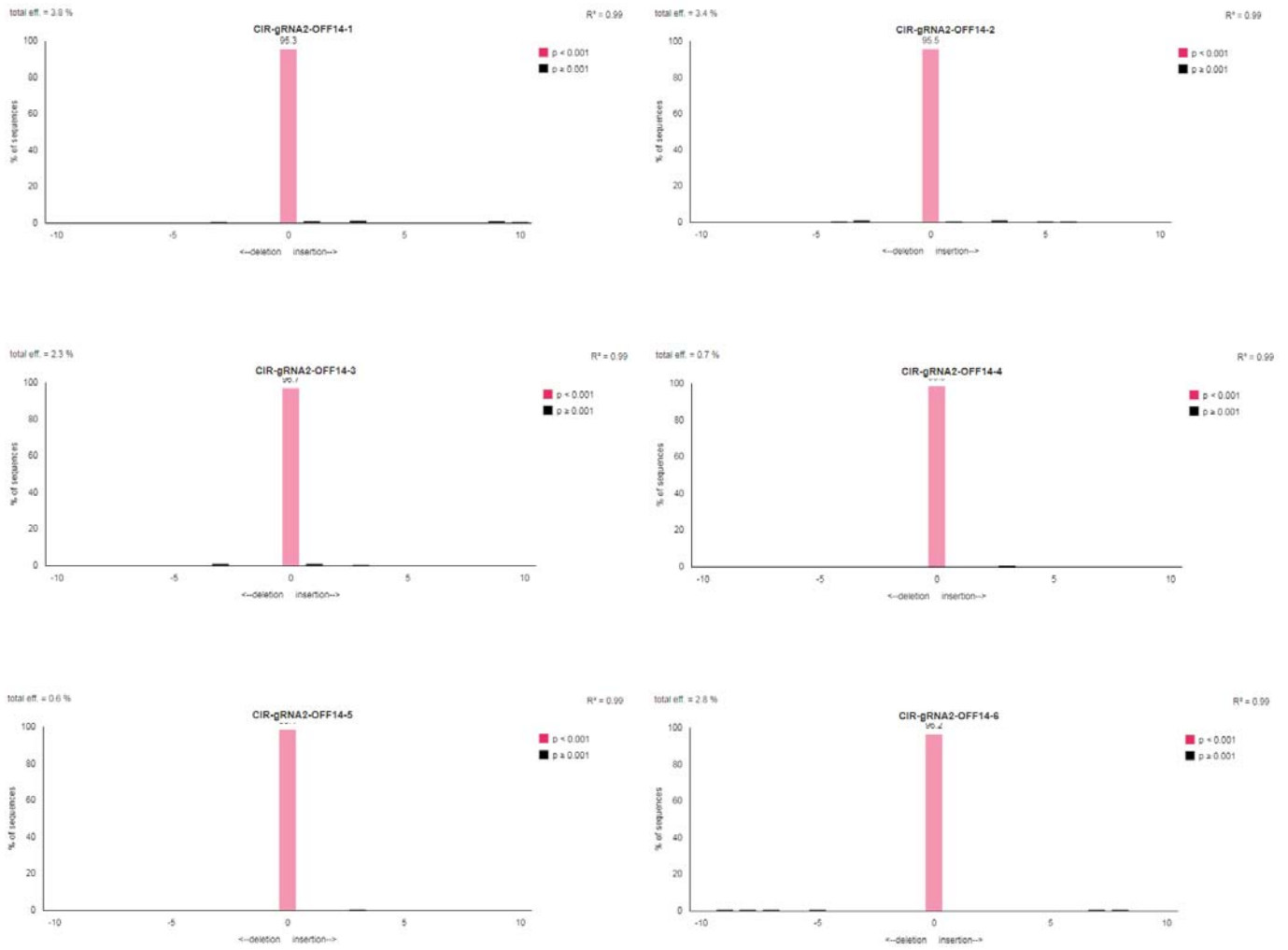
gRNA2-OFF12



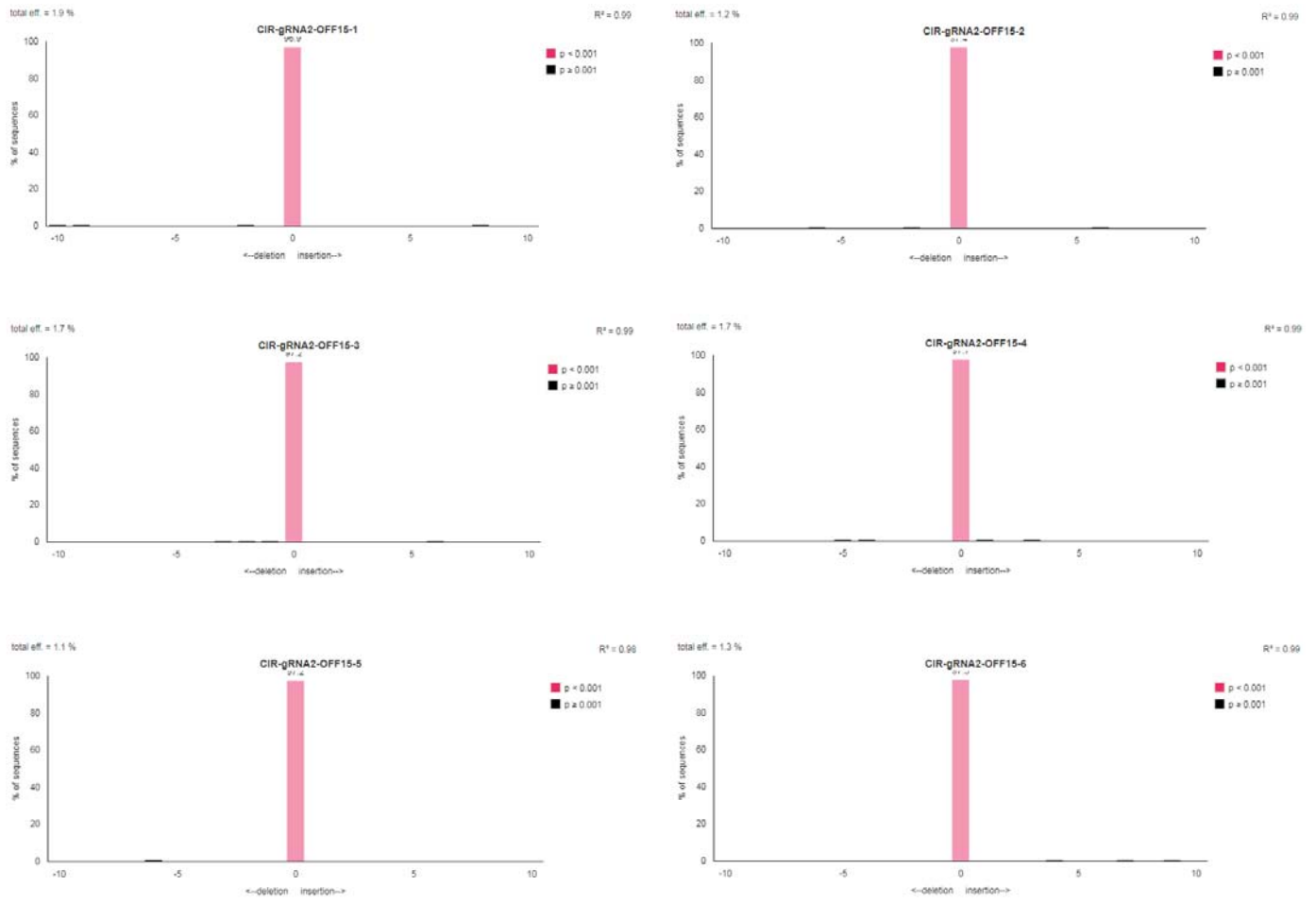
gRNA2-OFF13



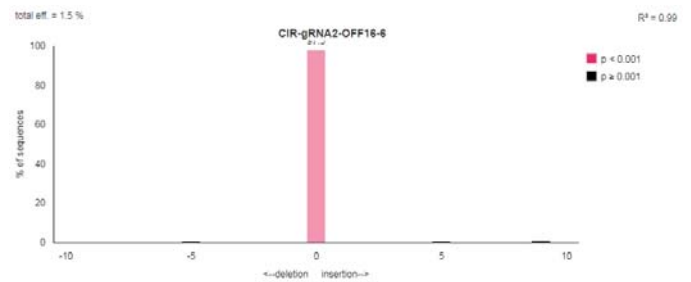
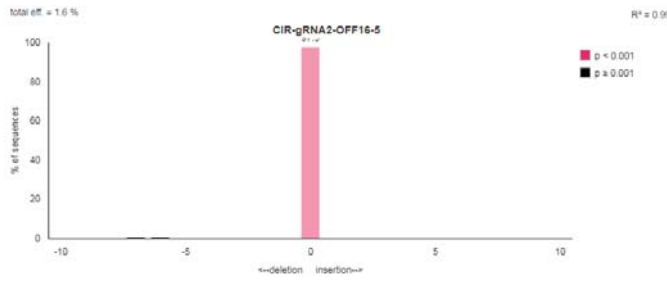
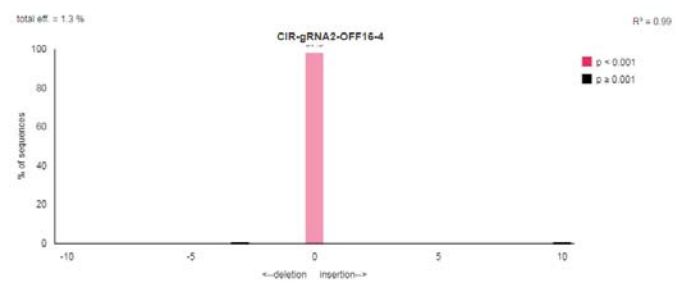
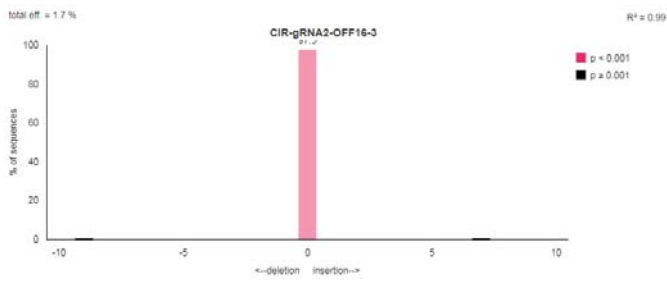
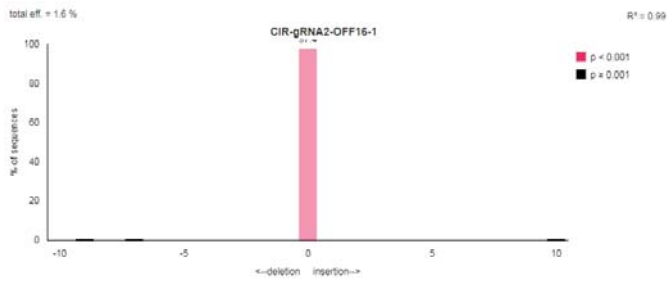
gRNA2-OFF14



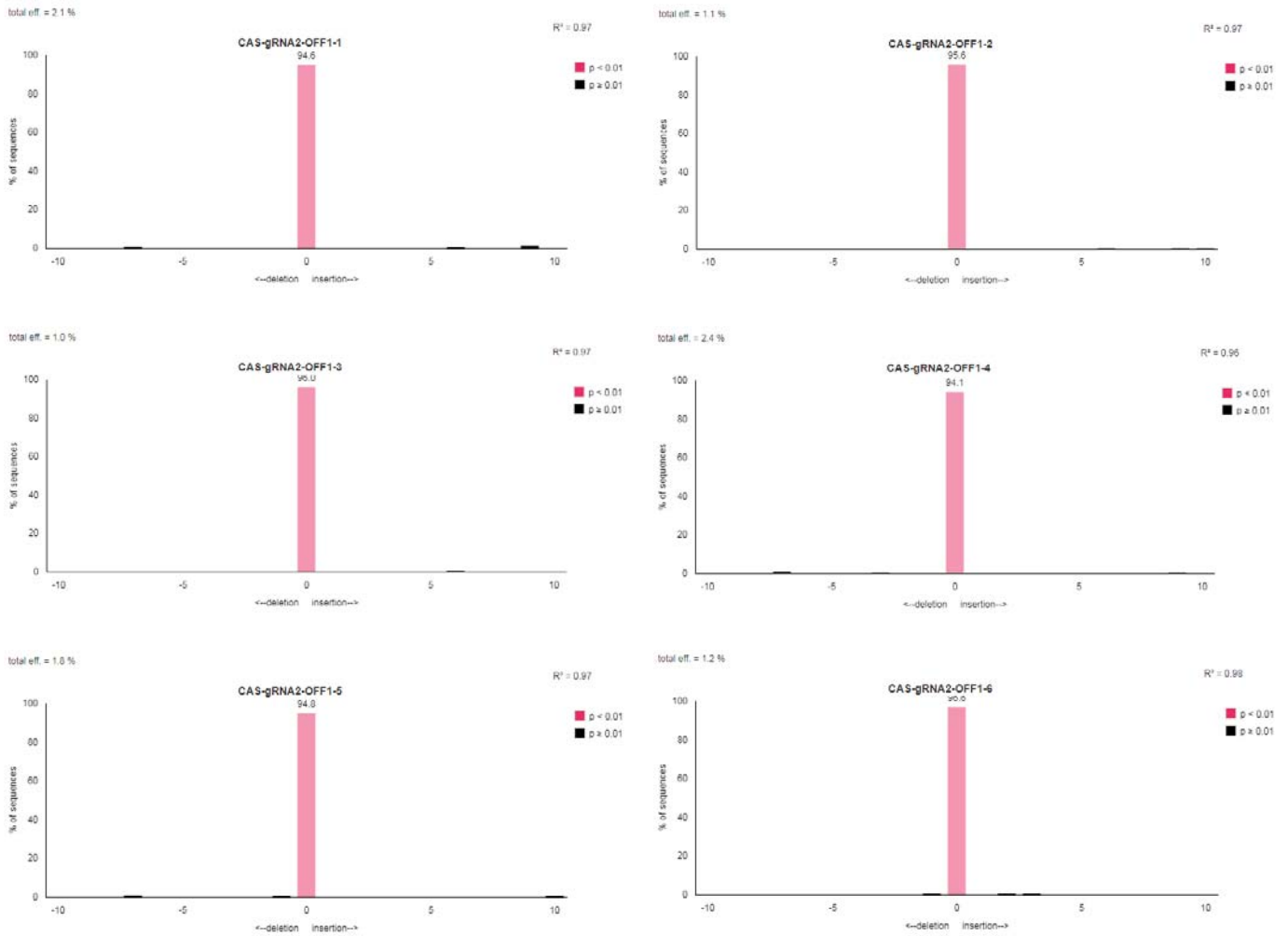
gRNA2-OFF15



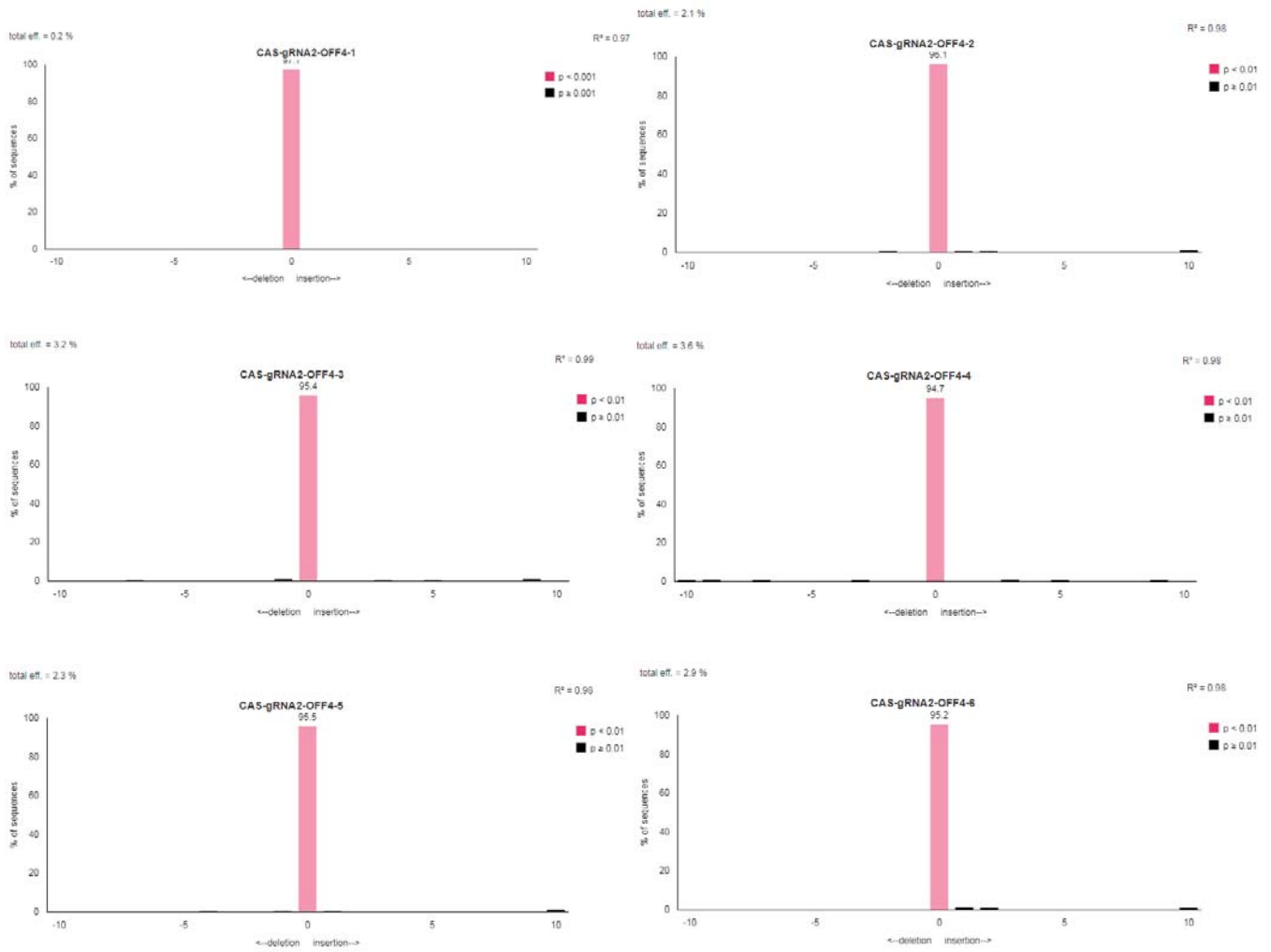
gRNA2-OFF16



gRNA2-OFF17

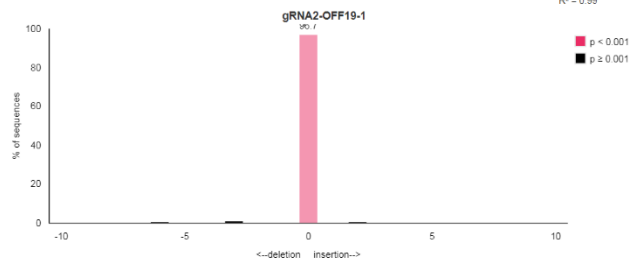


gRNA2-OFF18

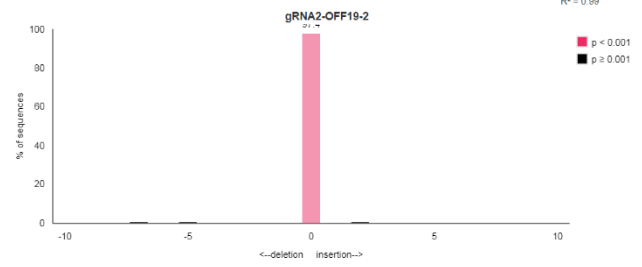


gRNA2-OFF19

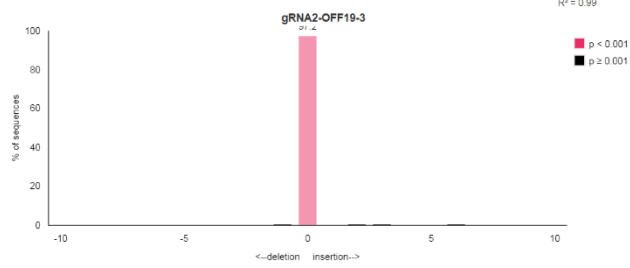
total eff. = 2.0 %



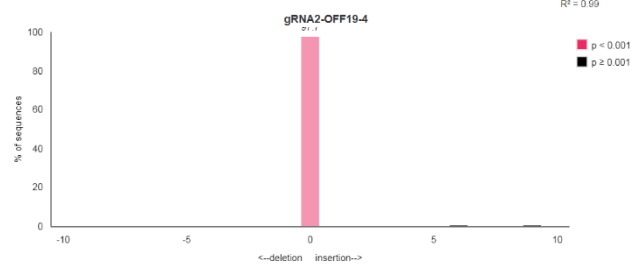
total eff. = 1.3 %



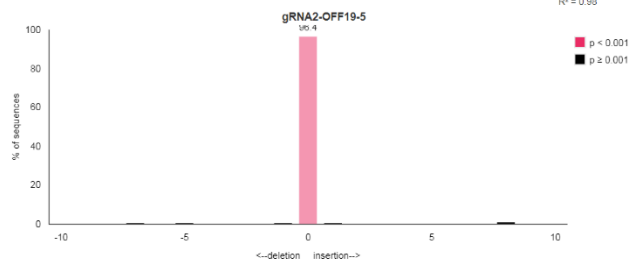
total eff. = 1.6 %



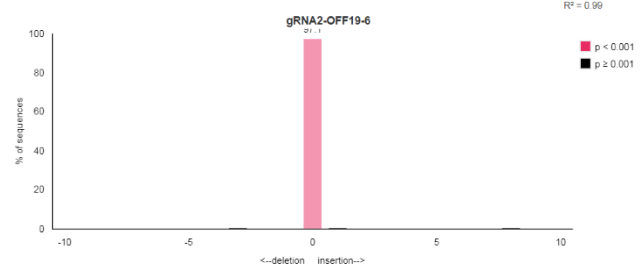
total eff. = 1.2 %



total eff. = 2.1 %

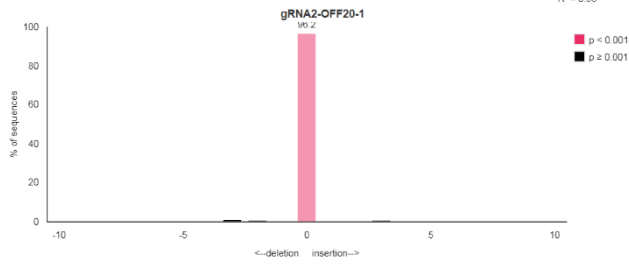


total eff. = 1.6 %

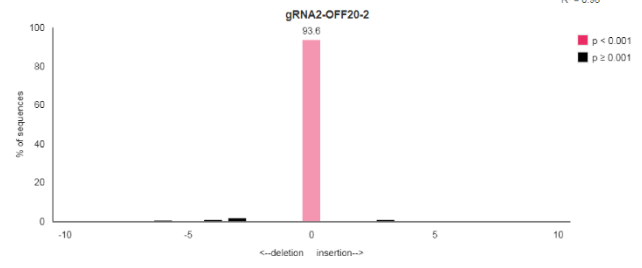


gRNA2-OFF20

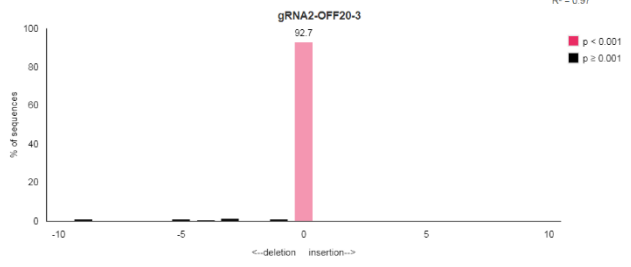
total eff. = 2.0 %



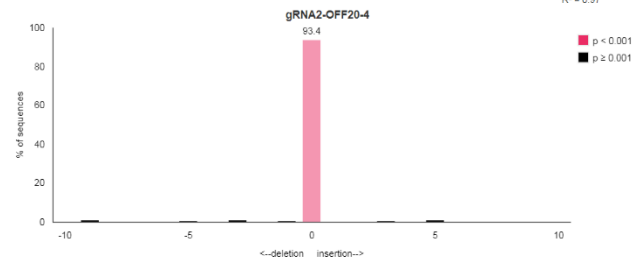
total eff. = 4.1 %



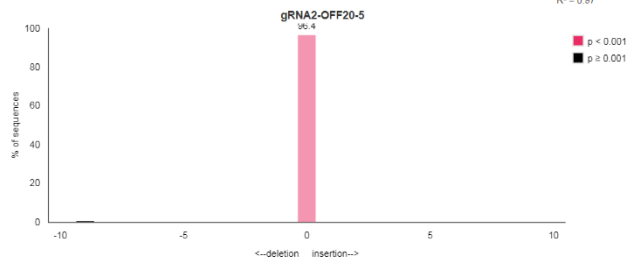
total eff. = 4.6 %



total eff. = 3.7 %



total eff. = 0.6 %



total eff. = 1.8 %

