

S3 Table. No signs for off-targeting activity of anti-HCMV and anti-EBV gRNAs

Off-target site	Off-target sequence ^a	# mismatches + location ^b	gRNA-positive cells			gRNA-negative cells			gRNA-pos minus gRNA-neg ^e
			# sequences total ^c	# mutated ^d	% mutated	# sequences total ^c	# mutated ^d	% mutated	
EBNA1 #1 OFF1	GCCTCTG GT TCCTGAGCCGCC AGG	3MMs [2:4:8]	1930	5	0,26	1024	3	0,29	-0,03
EBNA1 #1 OFF2	TGCCCT CG TCCTGAGCCGCC GGG	3MMs [1:7:8]	2558	6	0,23	1300	5	0,38	-0,15
EBNA1 #1 OFF3	TGCC TTA ATCCTGAGCCGCC AAG	3MMs [1:5:7]	1665	14	0,84	1110	10	0,90	-0,06
EBNA1 #2 OFF1	GT TT GA GC CATGTCTGACGAG GGG	3MMs [3:7:8]	1628	52	3,19	904	33	3,65	-0,46
EBNA1 #2 OFF2	GTGAGAA GC CATGTCTGAC AGAG	3MMs [4:8:19]	1755	20	1,14	953	17	1,78	-0,64
EBNA1 #2 OFF3	CT GTGAA AC CATGTCTGAG GATGG	3MMs [1:8:18]	2449	13	0,53	1182	12	1,02	-0,48
OriP #2 OFF1	GT AT TAC TC CACATAGAA AATTTGG	3MMs [4:8:17]	2085	13	0,62	1089	11	1,01	-0,39
OriP #2 OFF2	GTACCAC CC TCATAGAGAG GTTAG	3MMs [5:10:19]	2414	19	0,79	1133	13	1,15	-0,36
OriP #2 OFF3	GAAAGG CCACATAGAGAT TTAG	4MMs [2:4:5:6]	1749	10	0,57	937	5	0,53	0,04
UL44 #2 OFF1	G CC GATGAAG GCG TACAAGAA AAG	3MMs [3:5:11]	2348	20	0,85	1186	8	0,67	0,18
UL44 #2 OFF2	G AGG CTG GGG CCGTACAAG GCGG	4MMs [2:8:9:20]	603	2	0,33	353	2	0,57	-0,23
UL44 #2 OFF3	G GGG CT TC AG CC TTACAAGAG GAG	4MMs [2:7:8:13]	1229	10	0,81	617	2	0,32	0,49
UL57 #3 OFF1	GT AG CT TC TAC CT AGGATCAC GAG	3MMs [3:8:13]	2132	11	0,52	1009	7	0,69	-0,18
UL57 #3 OFF2	GTGG GT TT CACT GAGGATCAC GAG	3MMs [5:9:12]	2303	11	0,48	1158	10	0,86	-0,39
UL57 #3 OFF3	ATGC TT ATAC AGAGGATCAT AG	4MMs [1:4:8:12]	2326	16	0,69	1231	11	0,89	-0,21
UL105 #3 OFF1	G G CTGAG GGAGG GAAACCAC AGG	3MMs [2:7:12]	597	4	0,67	438	6	1,37	-0,70
UL105 #3 OFF2	GA AT GTATG CAGG GAAACCAC TAG	3MMs [3:9:12]	2205	17	0,77	1027	8	0,78	-0,01
UL105 #3 OFF3	TACT GTATGGAT TG GAAACCAC AGG	3MMs [1:11:12]	1451	11	0,76	668	8	1,20	-0,44

^apotential off-target sequence. Mismatches as compared to the gRNA are indicated in bold and red whereas the PAM sequence is indicated in bold

^bnumber of mismatches (MM) and location of mismatch as compared to the gRNA is indicated

^cNumber of sequences analyzed

^dNumber of sequences displaying alterations as compared to the reference sequence. A sequence scored as 'mutated' when there was a mismatch/indel 2bp up and downstream of the Cas9 cleavage site

^ePercentage of mismatches that could have occurred by CRISPR/Cas9 mediated gene editing. The percentage of edited sequences in gRNA-expressing cells was subtracted from the percentage of gRNA-negative cells. A negative value denotes a higher percentage of mutations in gRNA-negative cells as compared to gRNA-expressing cells.