Supplementary Table 1. Predicted number of ERV target sites for Myr- and PPYP-specific sgRNAs in the CHO-K1 genome.

			Number of mismatches allowed				
sgRNA	**sgRNA sequence (5'-3')**	**PAM sequence**[1]	**0**	**1**	**2**	**3**	**Total**
Myr2	TCCTAAGCCTAGAAACTATG	Canonical	**59**	29	16	26	147
		Non-canonical	-	-	1	16	
PPYP6	GCCACTGCCGCCCCCACCAG	Canonical	**55**	16	9	36	133
		Non-canonical	1	-	-	16	

[1] The canonical PAM sequence of SpCas9 is NGG

Supplementary Table 2a. Sequences of the sgRNAs and corresponding primers used in this study.

sgRNA	Orientation	5'-3 targetsite (without PAM)	Addition of G at 5' end for better U6 expression	Oligo 1	Oligo 2
Myr2	Forward strand	TCCTAAGCCTAGAAACTATG	GTCCTAAGCCTAGAAACTATG	ACACCGTCCTAAGCCTAGAAACTATGG	AAAACCATAGTTTCTAGGCTTAGGACG
PPYP6	Forward strand	GCCACTGCCGCCCCCACCAG	–	ACACCGCCACTGCCGCCCCCACCAGG	AAAACCTGGTGGGGGCGGCAGTGGCG

Output from Zifit software (http://zifit.partners.org/ZiFiT/ChoiceMenu.aspx)

Supplementary Table 2b. Sequences of the PCR and Illumina sequencing primers used to characterize corresponding genomic loci of edited CHO cells.

Primer Name	Full sequence (5'-3')	Ilumina Adapter	Spacer	Gene-specific Primer	Primer ratio according to expected % of ERVs (total =1)
Myr Forward Primers					
Myr_Fa_3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACCGCTTGAAGGATTTGCAATC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	ACC	GCTTGA**A**GGATTTG**C**AATC	0.15
Myr_Fb_0	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTTGAGGGATTTGCAATC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG		GCTTGA**G**GGATTTG**C**AATC	0.2
Myr_Fb_1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCTTGAGGGATTTGCAATC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	T	GCTTGA**G**GGATTTG**C**AATC	0.2
Myr_Fb_2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGCTTGAGGGATTTGCAATC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	TT	GCTTGA**G**GGATTTG**C**AATC	0.2
Myr_Fb_3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTGCTTGAGGGATTTGCAATC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	ACT	GCTTGA**G**GGATTTG**C**AATC	0.2
Myr_Fc_2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCTTGAGGGATTTGTAATC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	GT	GCTTGA**G**GGATTTG**T**AATC	0.05
					1
Myr Reverse Primers					
Myr_R_0	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACAAAGAGTAATCCATTTGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG		ACAAAGAGTAATCCATTTGCG	0.25
Myr_R_1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACAAAGAGTAATCCATTTGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	G	ACAAAGAGTAATCCATTTGCG	0.25
Myr_R_2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGACAAAGAGTAATCCATTTGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	CG	ACAAAGAGTAATCCATTTGCG	0.25
Myr_R_3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGACAAAGAGTAATCCATTTGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	AAG	ACAAAGAGTAATCCATTTGCG	0.25
					1
PPYP Forward Primers					
PPYP_Fa_0	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTCCAGCCTTTACCCTAC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG		A**C**TCCA**G**CCTTTACCCTAC	0.1
PPYP_Fa_1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACTCCAGCCTTTACCCTAC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	A	A**C**TCCA**G**CCTTTACCCTAC	0.1
PPYP_Fb_0	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTCCAACCTTTACCCTAC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG		A**T**TCCA**A**CCTTTACCCTAC	0.2
PPYP_Fb_1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGATTCCAACCTTTACCCTAC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	G	A**T**TCCA**A**CCTTTACCCTAC	0.2
PPYP_Fb_2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGATTCCAACCTTTACCCTAC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	TG	A**T**TCCA**A**CCTTTACCCTAC	0.2
PPYP_Fb_3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTATTCCAACCTTTACCCTAC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	CTT	A**T**TCCA**A**CCTTTACCCTAC	0.2
					1
PPYP Reverse Primers					
PPYP_Ra_1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGGTCTGATGCTGAGAATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	G	GGTC**T**GATGCTGAGAATG	0.04
PPYP_Rb_0	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTCCGATGCTGAGAATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG		GGTC**C**GATGCTGAGAATG	0.24
PPYP_Rb_1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGTCCGATGCTGAGAATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	T	GGTC**C**GATGCTGAGAATG	0.24
PPYP_Rb_2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGGTCCGATGCTGAGAATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	GT	GGTC**C**GATGCTGAGAATG	0.24
PPYP_Rb_3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGGGTCCGATGCTGAGAATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	AAG	GGTC**C**GATGCTGAGAATG	0.24
					1

Supplementary Table 2c. Sequences of the PCR and qPCR primers used in this study to characterize and validate ERV locis and expression

Amplification type	Oligo 1	Oligo 2
Locus ERV type 1 validation1	CTCTGGTTCTTGCCTGCTGAGCT	TGGTCAATGTATATGAGGCGCT
qPCR Type1 ERV specific LTR	GGGAATTGAGTCTGCTGTACCA	ACAGAGTCTTTCAAATGAGGCG
qPCR ref GAPDH	GCGACTTCAACAGTGACTCCCA	TGAGGTCCACCACTCTGTTGCT
qPCR Type2 ERV specific	GAATAAAAGGTCAGGGCGTTGG	CTGACTTGGCTCTATCTTGGGT
qPCR Type1 ERV specific Gag	TGACGATATAAGCCACTTGA	ACCCCCAGACTATATTCCAGATA
qPCR Type1 ERV specific Env	CTATGTGCTGCCCTCAAGGA	GCCTCTCCCTAAGTTTGGCC
Locus ERV type 1 validation2	CTCTGGTTCTTGCCTGCTGAGCT	TAAGCCATTGGTGAAGGGTCA
Locus ERV type 1 validation3	CTCTGGTTCTTGCCTGCTGAGCT	TGACGATATAAGCCACTTGA
Locus ERV type 1 validation4	TTTTCTGGTGCCCTCTTGCCTGG	TAAGCCATTGGTGAAGGGTCA
Locus ERV type 1 validation without ERV	CTCTGGTTCTTGCCTGCTGAGCT	TTGTGGAGCTGTGTGAGTGGTGG
Locus ERV ETC386F validation	GTGTGTGCGTCCCTGAATTTGCC	GCGTGTCAGGATCTTTGGGGATGT
Locus ERV ETC506F validation	AGGAAAAGAGTGTGCTTTGTTTGG	ACAGGTCTTTGTCACCAGGCCAG

Supplementary Table 3. Detection of CRISPR-mediated mutations in the group 1 type-C ERV mRNA sequences of edited CHO-K1 clones.

Sample	# screened clones	# mutated clones	Mutation frequency	Loss-of-function mutation frequency[1]
Myr2 sgRNA	95	18	19%	11%
PPYP6 sgRNA	181	14	8%	79%
Total	276	32	12%	45%

[1] Includes translation inhibition and frameshift mutations and is expressed relative to the number of mutated clones.

Supplementary Table 4. Analysis of repair junctions of edited CHO-K1 clones by Sanger sequencing of the expressed group 1 ERVs or by targeted Illumina DNA amplicon sequencing

Analysis	Level	sgRNA	Clone	ID	Locus	RefSeq	MutSeq	Type	Insertion	DelSeq	InsSeq	DelSize	InsSize	Length	Index	MHLength	MHSeq	Connection	PercentageReads	NumberAlleles T	Pathway	altEJ	SDMMEJ	Effect	Detected At mRNALevel	Cluster	ClusterGroup	Predicted Cleavage
Sanger	mRNA	Myr2	C02			5'-TGTCATT	5'-TGTCATT	Deletion		TA		2		2	-2						CNHEJ			TranslationInhibition				
Sanger	mRNA	Myr2	D12			5'-CGACTCT	5'-CGACTCT	Deletion		TTCCTTCTGTCGACTCT		114		114	-114	3	CAA				altEJ	MMEJ		TranslationInhibition				
Sanger	mRNA	Myr2	G09			5'-TCTTTGT	5'-TCTTTGT	Deletion		ATTTGTGCCCTCCTAAG		27		27	-27	2	AT				altEJ	MMEJ		OutsideERVCodingRegion				
Sanger	mRNA	Myr2	H02			5'-AGCTGT	5'-AGCTGT	Deletion		AAC		3		3	-3						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	A04			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	A09			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	A12			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	B05			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	B10			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	B11			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	C06			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	C07			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	D06			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	E07			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	E11			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	F11			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	F12			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	Myr2	H04			5'-CTCCTAA	5'-CTCCTAA	Insertion	Smaller5		T		1	1	1						CNHEJ			OutsideERVCodingRegion				
Sanger	mRNA	PPYP6	A02			5'-CCATATC	5'-CCATATC	Indel	Templated_t	CACCAG AG	ACTGCTTC	20	10	10	-10						HR_altEJ	SDMMEJ	Unknown	Frameshift				
Sanger	mRNA	PPYP6	A07			5'-CCCCCG	5'-CCCCCG	Deletion		CCAGAGG		7		7	-7	2	CA				Unknown			Frameshift				
Sanger	mRNA	PPYP6	B11			5'-CGCCAT	5'-CGCCAT	Insertion	Smaller5		C		1	1	1						CNHEJ			Frameshift				
Sanger	mRNA	PPYP6	D10			5'-CGCCAT	5'-CGCCAT	Insertion	Smaller5		C		1	1	1						CNHEJ			Frameshift				
Sanger	mRNA	PPYP6	K7			5'-CGCCAT	5'-CGCCAT	Insertion	Smaller5		C		1	1	1						CNHEJ			Frameshift				
Sanger	mRNA	PPYP6	D08			5'-GGAGAA	5'-GGAGAA	Deletion		CACCAGAGG		9		9	-9						altEJ	MMEJ		InFrameMutation				
Sanger	mRNA	PPYP6	E10			5'-AGCCCC	5'-AGCCCC	Deletion		TCCGCCACTGCCGCCCC		37		37	-37						altEJ	MMEJ		Frameshift				
Sanger	mRNA	PPYP6	G12			5'-CCCCCG	5'-CCCCCG	Deletion		CCA		3		3	-3	3	CCA				altEJ	MMEJ_SDMMEJ	LoopOut	InFrameMutation				
Sanger	mRNA	PPYP6	H03			5'-CCCCCG	5'-CCCCCG	Deletion		CCA		3		3	-3	3	CCA				altEJ	MMEJ_SDMMEJ	LoopOut	InFrameMutation				
Sanger	mRNA	PPYP6	K03			5'-ATATCCG	5'-ATATCCG	Deletion		CGCCCCCACCAGAGGCAGAAGC				22	-22	2	GC				altEJ	MMEJ_SDMMEJ	SnapBack	Frameshift				
Sanger	mRNA	PPYP6	K09			5'-CCCCCG	5'-CCCCCG	Deletion		C		1		1	-1						CNHEJ			Frameshift				
Sanger	mRNA	PPYP6	K19			5'-CCCCCG	5'-CCCCCG	Deletion		C		1		1	-1						CNHEJ			Frameshift				
Sanger	mRNA	PPYP6	K12			5'-TTAACGG	5'-TTAACGG	Indel	Smaller5	CAG	A	3	1	2	-2						Unknown			Frameshift				
Sanger	mRNA	PPYP6	K14			5'-AGGAGC	5'-AGGAGC	Deletion		GCCCCCACCAGAG		13		13	-13	2	GC				altEJ	MMEJ_SDMMEJ	LoopOut	Frameshift				
Illumina	DNA	Myr2	C02	1_1	1	5'-TTGTAG	5'-TTGTAG	Insertion	Smaller5		T		1	1	1			NA	0.2722458	1	CNHEJ			OutsideERVCodingRegion		Cluster16	Group1	yes
Illumina	DNA	Myr2	C02	3_9	2	5'-GTCATTT	5'-GTCATTT	Indel	Templated_d	A	GTCACCAC	1	8	7	7			NA	0.3008392	1	altEJ	SDMMEJ	LoopOut	TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	C02	-4_7	3	5'-TTGTAG	5'-TTGTAG	Deletion		AAACTATGGGGC		12		12	-12	5	AAACT	NA	0.3306494	1	altEJ	MMEJ		TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	C02	0_1	4	5'-TGTCATT	5'-TGTCATT	Deletion		TA		2		2	-2			NA	0.347988	1	CNHEJ			TranslationIn	yes	Cluster21	Group1	yes
Illumina	DNA	Myr2	D12	1_1	1A	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	0.8389501	3	CNHEJ			OutsideERVCodingRegion		Cluster8	Group1	yes
Illumina	DNA	Myr2	D12	1_1	1B	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	0.8389501	3	CNHEJ			OutsideERVCodingRegion		Cluster16	Group1	yes
Illumina	DNA	Myr2	D12	1_1	1C	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	0.8389501	3	CNHEJ			OutsideERVCodingRegion		Cluster21	Group1	yes
Illumina	DNA	Myr2	D12	1_17	2	5'-TGTGTTA	5'-TGTGTTA	Indel	Templated_d	CT	TAGGCTTA	2	19	17	17			NA	0.3176607	1	HR_altEJ	SDMMEJ	SnapBack	OutsideERVCodingRegion		Cluster18	Group1	yes
Illumina	DNA	Myr2	D12	13_14_1	3_1	5'-TTGTAG	5'-TTGTAG	Deletion		AAACTATGGGGC		12		2	2	5	AAACT	D12_3_2	0.3632735	1	altEJ	MMEJ		TranslationInhibition		Cluster17	Group1	yes
Illumina	DNA	Myr2	D12	13_14_2	3_2	5'-TTGTAG	5'-TTGTAG	Insertion	Templated_cis		TTTGTCCCTAACAC		14	2	2			D12_3_1	0.3632735	1	HR_altEJ	SDMMEJ	LoopOut	TranslationInhibition		Cluster17	Group1	yes
Illumina	DNA	Myr2	D12	-107_6	4	5'-CGACTCT	5'-CGACTCT	Deletion		TTCCTTCTGTCGACTCT		114		114	-114	3	CAA	NA	0.3111446	1	altEJ	MMEJ		TranslationIn	yes	Cluster21	Group1	yes
Illumina	DNA	Myr2	D12	-23_-1	5	5'-AAGTCTG	5'-AAGTCTG	Deletion		GTGTCCTCCTAAGCCTA		23		23	-23			NA	0.2920035	1	altEJ	SDMMEJ	LoopOut	OutsideERVCodingRegion		Cluster19	Group1	yes
Illumina	DNA	Myr2	D12	62_111	6_1	5'-TTGTAG	5'-TTGTAG	Deletion		TAGAAACT		8		42	42			D12_6_2	0.292818	1	Unknown			InFrameMutation		Cluster21	Group1	yes
Illumina	DNA	Myr2	D12	-7_0	6_2	5'-TTGTAG	5'-TTGTAG	Insertion	Templated_trans		TGTCACCACTCCTTT		50	42	42			D12_6_1	0.292818	1	HR_altEJ	SDMMEJ	LoopOut	InFrameMutation		Cluster21	Group1	yes
Illumina	DNA	Myr2	D12	-3_27	7	5'-CTTTGTC	5'-CTTTGTC	Deletion		AACTATGGGGCAAACTG		31		31	-31			NA	0.6756399	2	altEJ	SDMMEJ	SnapBack	TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	D12	-3_27	7	5'-CTTTGTC	5'-CTTTGTC	Deletion		AACTATGGGGCAAACTG		31		31	-31			NA	0.6756399	2	altEJ	SDMMEJ	SnapBack	TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1A	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1A	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1A	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1A	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1B	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster17	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1C	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster15	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1D	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster8	Group1	yes
Illumina	DNA	Myr2	G09	1_1	1E	5'-CGGTCT	5'-CGGTCT	Insertion	Smaller5		T		1	1	1			NA	2.5733213	8	CNHEJ			OutsideERVCodingRegion		Cluster4	Group1	yes
Illumina	DNA	Myr2	G09	-33_8	2	5'-TGTTCTG	5'-TGTTCTG	Deletion		ACTGTCATTTGTGCCCT		42		42	-42			NA	0.4286549	1	altEJ	SDMMEJ	LoopOut	TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	-26_0	3	5'-TCTTTGT	5'-TCTTTGT	Deletion		ATTTGTGCCCTCCTAAG		27		27	-27	2	AT	NA	0.3947893	1	altEJ	MMEJ		OutsideERV	yes	Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	-4_8	4	5'-TTGTAG	5'-TTGTAG	Deletion		AAACTATGGGGCA		13		13	-13	2	AA	NA	0.3715937	1	altEJ	MMEJ		TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	-4_7	5	5'-TTGTAG	5'-TTGTAG	Deletion		AAACTATGGGGC		12		12	-12	5	AAACT	NA	0.343759	1	altEJ	MMEJ		TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	0_1	6A	5'-TGTTCTG	5'-TGTTCTG	Indel	Smaller5	TAT	A	3	1	2	-2			NA	0.404670695	1	CNHEJ			TranslationInhibition		Cluster21	Group1	yes
Illumina	DNA	Myr2	G09	0_1	6B	5'-TTGTAG	5'-TTGTAG	Deletion		AT		2		2	-2			NA	0.331094205	1	CNHEJ			TranslationInhibition		Cluster14	Group1	off-target
Illumina	DNA	PPYP6	A02	-2_5	1	5'-CCATATC	5'-CCATATC	Indel	Templated_t	CACCAG AG	ACTGCTTC	20	10	10	-10			NA	0.4012993	1	HR_altEJ	SDMMEJ		Frameshift	yes	Cluster7	Group1	yes
Illumina	DNA	PPYP6	E10	-13_20	1	5'-AGCCCC	5'-AGCCCC	Deletion		TCCGCCACTGCCGCCCC		37		37	-37	6	TCCGCC	NA	0.4190737	1	altEJ	MMEJ		Frameshift	yes	Cluster7	Group1	yes
Illumina	DNA	PPYP6	K03	-29_-18	1	5'-ATTAACT	5'-ATTAACT	Insertion	Templated_cis		GCCATATCTGCC		12	12	12			NA	0.2352734	1	altEJ	SDMMEJ	LoopOut	InFrameMutation		Cluster12	Group2	off-target
Illumina	DNA	PPYP6	K03	-25_5	2	5'-GGAGCC	5'-GGAGCC	Deletion		GCCATATCCGCCACTGC		31		31	-31	2	GC	NA	0.389	1	altEJ	MMEJ		Frameshift		Cluster4	Group1	yes
Illumina	DNA	PPYP6	K03	2_20	3	5'-ATATCCG	5'-ATATCCG	Deletion		CGCCCCCACCAGAGGC		22		22	-22	2	GC	NA	0.3537365	1	altEJ	MMEJ_SDMMEJ	SnapBack	Frameshift	yes	Cluster7	Group1	yes
Illumina	DNA	PPYP6	K03	-9_7	4	5'-GGTTGAT	5'-GGTTGAT	Deletion		CCGCCCCCACCAGAGG		17		17	-17			NA	0.2628229	1	altEJ	SDMMEJ	LoopOut	Frameshift		Cluster8	Group1	yes
Illumina	DNA	PPYP6	K03	-6_-4	5	5'-CCGCCAT	5'-CCGCCAT	Deletion		CCA		3		3	-3	3	CCA	NA	0.1686034	0.5	altEJ	MMEJ		InFrameMutation		Cluster15	Group2	off-target
Illumina	DNA	PPYP6	K03	0_0	6	5'-CCCCCG	5'-CCCCCG	Deletion		C		1		1	-1			NA	0.3135142	1	CNHEJ			Frameshift		Cluster7	Group1	yes
Illumina	DNA	PPYP6	K14	-6_17	1	5'-ATATCCG	5'-ATATCCG	Indel	Templated_t	GCCCCCACC	ACATCCTC	13	91	78	78			NA	0.26705875	1	HR_altEJ	SDMMEJ		Frameshift		Cluster3	Group1	yes
Illumina	DNA	PPYP6	K14	-7_5	2	5'-AGGAGC	5'-AGGAGC	Deletion		GCCCCCACCAGAG		13		13	-13	2	GC	NA	0.3488253	1	altEJ	MMEJ_SDMMEJ	LoopOut	Frameshift	yes	Cluster7	Group1	yes
Illumina	DNA	PPYP6	K14	5_18	3	5'-CCCCCG	5'-CCCCCG	Deletion		ACCAGAGGCAGAAGCG		17		17	-17	2	AC	NA	0.2957876	1	altEJ	MMEJ_SDMMEJ	LoopOut	Frameshift		Cluster7	Group1	yes
Illumina	DNA	PPYP6	K14	3_5	4	5'-CGCCAT	5'-CGCCAT	Indel	Smaller5	CAGA	G	4	1	3	-3			NA	0.4096828	1	CNHEJ			InFrameMutation		Cluster3	Group1	yes

Supplementary Table S5. Sequence analysis of the expressed mRNA ERV sequences of mutated CHO-K1 clones.

Clone[1]		Sequence[2]	Features[3]	Mutation type[4]	Repair pathway[5]	Pattern score[6]	FORECasT[7]
Myr2 sgRNA (n=18)							
C02	Genomic	5′-TGTCATTTGTGCCCTCCTAAGCCTAGAAACTATGGGGCAAACTGTC ACCACTCCTTTGTCCCTAACACTCTCCCACTGGAA-3′	2 bp deletion, 1 bp MH	Translation inhibition	C-NHEJ	-	2.6%
	Junction	5′-TGTCATTTGTGCCCTCCTAAGCCTAGAAAC--TGGGGCAAACTGTC ACCACTCCTTTGTCCCTAACACTCTCCCACTGGAA-3′					
D12	Genomic	5′-CGACTCTCTCTCAATTCCT-75bp-GAAACTATGGGGCAAACTGTC ACCACTCCTTTGT-3′	114 bp deletion, 3 bp MH	Translation inhibition	MMEJ	NA	-
	Junction	5′-CGACTCTCTCTCAA------95bp----------ACTGTC ACCACTCCTTTGT-3′					
G09	Genomic	5′-TCTTTGTCTTGTAGCTGTCATTTGTGCCCTCCTAAGCCTAGAAACT ATGGGGCAAACTGTCACCACTCCTTTGTCCCTAACACTCTCCCACTGGA AAGATGTACAGGAATATGCTCATAACCAATCT-3′	27 bp deletion, 2 bp MH	Outside ERV coding region	MMEJ	51.8	-
	Junction	5′-TCTTTGTCTTGTAGCTGTC---------- ATGGGGCAAACTGTCACCACTCCTTTGTCCCTAACACTCTCCCACTGGA AAGATGTACAGGAATATGCTCATAACCAATCT-3′					
H02	Genomic	5′-AGCTGTCATTTGTGCCCTCCTAAGCCTAGAAACTATGGGGCAAACT GTCACCACTCCTTTGTCCC-3′	3 bp deletion, no MH	Outside ERV coding region	C-NHEJ	-	-
	Junction	5′-AGCTGTCATTTGTGCCCTCCTAAGCCTAGA---TATGGGGCAAACT GTCACCACTCCTTTGTCCC-3′					
A04 (n=14)	Genomic	5′-CTCCTAAGCCTAGAAACT-ATGGGGCAAACTGTCACCACTCC-3′	1 bp insertion, 1 bp MH	Outside ERV coding region	C-NHEJ[8]	-	17.8%
	Junction	5′-CTCCTAAGCCTAGAAACTTATGGGGCAAACTGTCACCACTCC-3′					

PPYP6 sgRNA (n=14)							
A02	Genomic	5′-CCCCCGCCATATCCGCCACTGCCGCCCC*CACCAGAGGCAGAAGCGG* *AC*TCCGCCGCTGCCTTGGCGGAAGC-3′	Deletion + Insertion (Replace-ment of 20 bp with 10 bp; net=10 bp deletion); inverted templated insertion from three possible ERV alleles	Frameshift mutation	HR	-	-
	Junction	5′-CCCCCGCCATATCCGCCACTGCCGCCCC*ACTGCTTCTG*--------TCCGCCGCTGCCTTGGCGGAAGC-3′					
A07	Genomic	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCACCAGAGGCAGAAGCGG ACTCCGCCGCTGCCTTGGC-3′	7 bp deletion, 2 bp MH flanking DSB	Frameshift mutation	Unknown[9]	-	1.1%
	Junction	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCA-------CAGAAGCGG ACTCCGCCGCTGCCTTGGC-3′					
B11 (n=3)	Genomic	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCAC-CAGAGGCAGAAGCG GACTCCGCCGCTGCCTTGGC-3′	1 bp insertion, 1 bp MH	Frameshift mutation	C-NHEJ[8]	-	3.1%
	Junction	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCACCCAGAGGCAGAAGCG GACTCCGCCGCTGCCTTGGC-3′					
D08	Genomic	5′-GGAGAAGACCAGTTGGTTGATCTATTAACGGAGGAGCCCCCCGCCA TATCCGCCACTGCCGCCCCCACCAGAGGCAGAAGCGGACTCCGCC-3′	9 bp deletion, 2 bp MH	In-frame mutation	MMEJ	191.4	2%
	Junction	5′-GGAGAAGACCAGTTGGTTGATCTATTAACGGAGGAGCCCCCCGCCA TATCCGCCACTGCCGCCCC---------CAGAAGCGGACTCCGCC-3′					
E10	Genomic	5′-AGCCCCCGCCATATCCGCCACTGCCGCCCCCACCAGAGGCAGAAGC GGACTCCGCCGCTGCCTTG-3′			MMEJ	172.7	-

	Junction	5′-AGCCCCCGCCATATCCGCC----------GCTGCCTTG-3′	37 bp deletion, 6 bp MH	Frameshift mutation			
G12 (n=2)	Genomic	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCACCAGAGGCAGAAGCGG ACTCCGCCGCTGCCTTGGC-3′	3 bp deletion, 3 bp MH or 4 bp MH (4 bp downstream)	In-frame mutation	MMEJ or SD-MMEJ (loop-out)	258.3	14.8%
	Junction	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCA---GAGGCAGAAGCGG ACTCCGCCGCTGCCTTGGC-3′					
K3	Genomic	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCACCAGAGGCAGAAGCGG ACTCCGCCGCTGCCTTGGCGGAAGCGG-3′	22 bp deletion, 2 bp MH or 5 bp MH (6 bp upstream)	Frameshift mutation	MMEJ or SD-MMEJ (snap-back)	133.1	-
	Junction	5′-CCCCCGCCATATCCGCCACTGC----------GG ACTCCGCCGCTGCCTTGGCGGAAGCGG-3′					
K9 (n=2)	Genomic	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCACCAGAGGCAGAAGCGG ACTCCGCCGCTGCC-3′	1 bp deletion, 1 bp MH	Frameshift mutation	C-NHEJ	-	19.5%
	Junction	5′-CCCCCGCCATATCCGCCACTGCCGCCCCCAC-AGAGGCAGAAGCGG ACTCCGCCGCTGCC-3′					
K12	Genomic	5′-TTAACGGAGGAGCCCCCGCCATATCCGCCACTGCCGCCCCCACCAG AGGCAGAAGCGGACTCCGC-3′	Deletion + Insertion (Replace-ment of 3 bp with 1 bp; net= 2 bp deletion)	Frameshift mutation	Unknown	-	-
	Junction	5′-TTAACGGAGGAGCCCCCGCCATATCCGCCACTGCCGCCCCCAC--A AGGCAGAAGCGGACTCCGC-3′					
K14	Genomic	5′-AGGAGCCCCCGCCATATCCGCCACTGCCGCCCCCACCAGAGGCAGA AGCGGACTCCCCCGCCATATCCGCGGAAGCGGCCCCTGACCCTT-3′	13 bp deletion, 2 bp	Frameshift mutation		208.8	-

	Junction	5′–AGGAGCCCCCGCCATATCCGCCACTGCC----------GCAGA AGCGGACTCCGCCGCTGCCTTGGCGGAAGCGGCCCCTGACCCTT–3′	MH or 5 bp MH (15 bp downstream)		MMEJ or SD-MMEJ (loop-out)		

[1] The table shows mRNA Sanger sequencing data of the expressed ERV repair junctions of CHO-K1 clones treated with wild-type Cas9 nuclease and the Myr2 or PPYP6 sgRNAs. The sequences are derived from Sanger sequencing of cDNA PCR amplicons. If the same repair junction was detected more than once, the number is indicated below each sample name as (n=). The lines above each mutated sequence termed 'Genomic' indicates the corresponding unmutated CHO-K1 cell genome sequence.

[2] Predicted blunt-ended DSB sites induced by the two sgRNAs and the wild-type Cas9 nuclease are highlighted in yellow, PAM sites are shown in bold blue, and the ATG translation initiation codon is depicted in brown letters. Myr and PPYP target motifs are highlighted in turquoise and purple, respectively. Pre-existing microhomologies (MH) of the microhomology-mediated end-joining (MMEJ) repair mechanism are shown in bold green, while *de novo* MH of the synthesis-dependent microhomology-mediated end-joining (SD-MMEJ) mechanism are underlined with a double line. Inserted bases are represented in bold red letters, deleted bases with a "-" sign, and replacements in italic underlined with a single bold line.

[3] Size of mutation and MH length (in bp). The distance between priming site and the break site for *de novo* MH are shown in parenthesis.

[4] ERV mutation types include in-frame mutations, out-of-frame mutations, translation inhibition (mutation of the ATG translation initiation codon) or mutations locating outside of the ERV coding region. Out-of-frame mutations and translation inhibition are likely, while in-frame mutations and mutations outside of the coding region are less likely to influence ERV expression and VLP formation.

[5] Most probable DSB repair mechanism based on manual junction analysis. Possible repair mechanisms include C-NHEJ, MMEJ, SD-MMEJ (snap-back), SD-MMEJ (loop-out), single strand annealing (SSA), homologous recombination (HR), and unknown. For snap-back SD-MMEJ mechanism, *de novo* priming sites are inverted repeats, while loop-out SD-MMEJ mechanisms uses priming sites with direct repeats (Khodaverdian et al., 2017). If the observed junction sequence is compatible with more than one mechanism and both appear equally likely, all potential pathways are listed. Junctions were verified for homologies at break site and templated insertions (SD-MMEJ) using program described in (Schimmel et al., 2017).

[6] Score of each repair pattern according to the MH size and the deletion length. Pattern score was calculated using the RGenome "Microhomology-Predictor" tool (http://www.rgenome.net/mich-calculator/) described in (Bae et al., 2014). The higher the score, the more likely the predicted mutation should be observed. The pattern score is only valid for repair junctions having MHs at the break site (MMEJ-mediated repair). NA: not available.

[7] Predicted frequencies of CRISPR-Cas9 editing outcomes using the online tool FORECasT (Favoured Outcomes of Repair Events at Cas9 Targets; https://partslab.sanger.ac.uk/FORECasT) as described in (Allen et al., 2018). The higher the frequency, the more junctions are expected to contain the predicted mutation pattern. Only the frequencies of the predicted ten most frequent mutations are listed.

[8] Frequent 1 bp insertions consisting of a duplication of the 4th nucleotide were also observed previously (Lemos et al., 2018)

[9] Unknown mechanism but similar junction pattern was described in (Shin et al., 2017)

Supplementary Table 6a. Cluster analysis of the Myr motif flanking sequences of the CHO type-C ERVs following genomic DNA Illumina deep sequencing.

Myr	Illumina deep sequencing				Whole genome reference		
Cluster	Abolute reads	% reads[1]	Predicted CRISPR-Cas9 cleavage	Presence of Myr motif	ERV number	% reads[1]	Type-C ERV group[2]
Cluster1	16911	4.5		yes	3	3	Group2
Cluster2	30056	8.0		yes	7	6	Group1
Cluster3	1674	0.4		yes	1	1	Group2
Cluster4	3304	0.9	yes	yes	2	2	Group1
Cluster5	6775	1.8	yes	yes	3	3	Group1
Cluster6	3330	0.9		yes	1	1	Group1
Cluster7	2074	0.6		yes	1	1	Group2
Cluster8	6383	1.7	yes	yes	3	3	Group1
Cluster9	6260	1.7	yes	yes	3	3	Group1
Cluster10	4503	1.2	yes	yes	2	2	Group1
Cluster11	3869	1.0		yes	2	2	Group2
Cluster12	3578	1.0		yes	0	0	Group2
Cluster13	6480	1.7		yes	1	1	Group2
Cluster14	2673	0.7		yes	1	1	Group1
Cluster15	10236	2.7	yes	yes	4	4	Group1
Cluster16	16877	4.5	yes	yes	9	8	Group1
Cluster17	9703	2.6	yes	yes	4	4	Group1
Cluster18	2465	0.7	yes	yes	1	1	Group1
Cluster19	1976	0.5	yes	yes	1	1	Group1
Cluster20	2677	0.7	yes	yes	1	1	Group1
Cluster21	136722	36.3	yes	yes	33	30	Group1
Cluster22	2348	0.6	yes	yes	1	1	Group1
Cluster23	1603	0.4		yes	3	3	Group1
Cluster24	1231	0.3		yes	0	0	Group1
Cluster25	1559	0.4		yes	0	0	Group3
Cluster26	1345	0.4		yes	1	1	Group3
Cluster27	16035	4.3		yes	2	2	Group3
Cluster28	1403	0.4		yes	3	3	Group3
Cluster29	1897	0.5		yes	0	0	Group3
Cluster30	28691	7.6		yes	11	10	Group3
Cluster31	1526	0.4		yes	1	1	Group3
Cluster32	3274	0.9			2	2	Group3
Cluster33	3787	1.0		yes	1	1	Group3
Cluster34	2003	0.5		yes	1	1	Group3
Total	376471	92	13	33	109	100	

[1] Illumina Gag sequence clusters are compared to the CHO whole genome reference assembly to show consistency of the estimations

[2] Comparison of cluster sequences with phylogenetic groups obtained using the whole genome reference assembly as shown in Figure 1

Supplementary Table 6b. Cluster analysis of the PPYP motif flanking sequences of the CHO type-C ERVs following genomic DNA Illumina deep sequencing.

PPYP	Illumina deep sequencing				Whole genome reference		
Cluster	Abolute reads	% reads[1]	Predicted CRISPR-Cas9 cleavage	Presence of Myr motif	ERV number	% reads[1]	Type-C ERV group[2]
Cluster1	41087	10.7	yes	yes	10	8	Group1
Cluster2	1857	0.5		yes	1	1	Group2
Cluster3	3387	0.9	yes	yes	7	6	Group1
Cluster4	3406	0.9	yes	yes	2	2	Group1
Cluster5	4073	1.1	yes	yes	2	2	Group1
Cluster6	1514	0.4		yes	1	1	Group2
Cluster7	169956	44.1	yes	yes	48	39	Group1
Cluster8	2688	0.7	yes	yes	2	2	Group1
Cluster9	5497	1.4	yes	yes	3	2	Group1
Cluster10	5038	1.3	yes	yes	1	1	Group1
Cluster11	2466	0.6			1	1	Group2
Cluster12	12156	3.2			6	5	Group2
Cluster13	5412	1.4		yes	2	2	Group2
Cluster14	3173	0.8		yes	2	2	Group2
Cluster15	1556	0.4		yes	1	1	Group2
Cluster16	5251	1.4		yes	1	1	Group2
Cluster17	2100	0.5		yes	1	1	Group2
Cluster18	1797	0.5		yes	1	1	Group2
Cluster19	2089	0.5		yes	1	1	Group2
Cluster20	1969	0.5		yes	1	1	Group2
Cluster21	1367	0.4		yes	1	1	Group2
Cluster22	3449	0.9		yes	3	2	Group3
Cluster23	27482	7.1		yes	12	10	Group3
Cluster24	6739	1.8			8	6	Group3
Cluster25	2410	0.6		yes	1	1	Group3
Cluster26	1493	0.4		yes	1	1	Group3
Cluster27	3839	1.0			3	2	Group3
Cluster28	2030	0.5		yes	1	1	Group3
Total	384960	84	8	24	124	100	

[1] Illumina Gag sequence clusters are compared to the CHO whole genome reference assembly to show consistency of the estimations

[2] Comparison of cluster sequences with phylogenetic groups obtained using the whole genome reference assembly as shown in Figure 1