Endogenous Retroviruses (ERV)

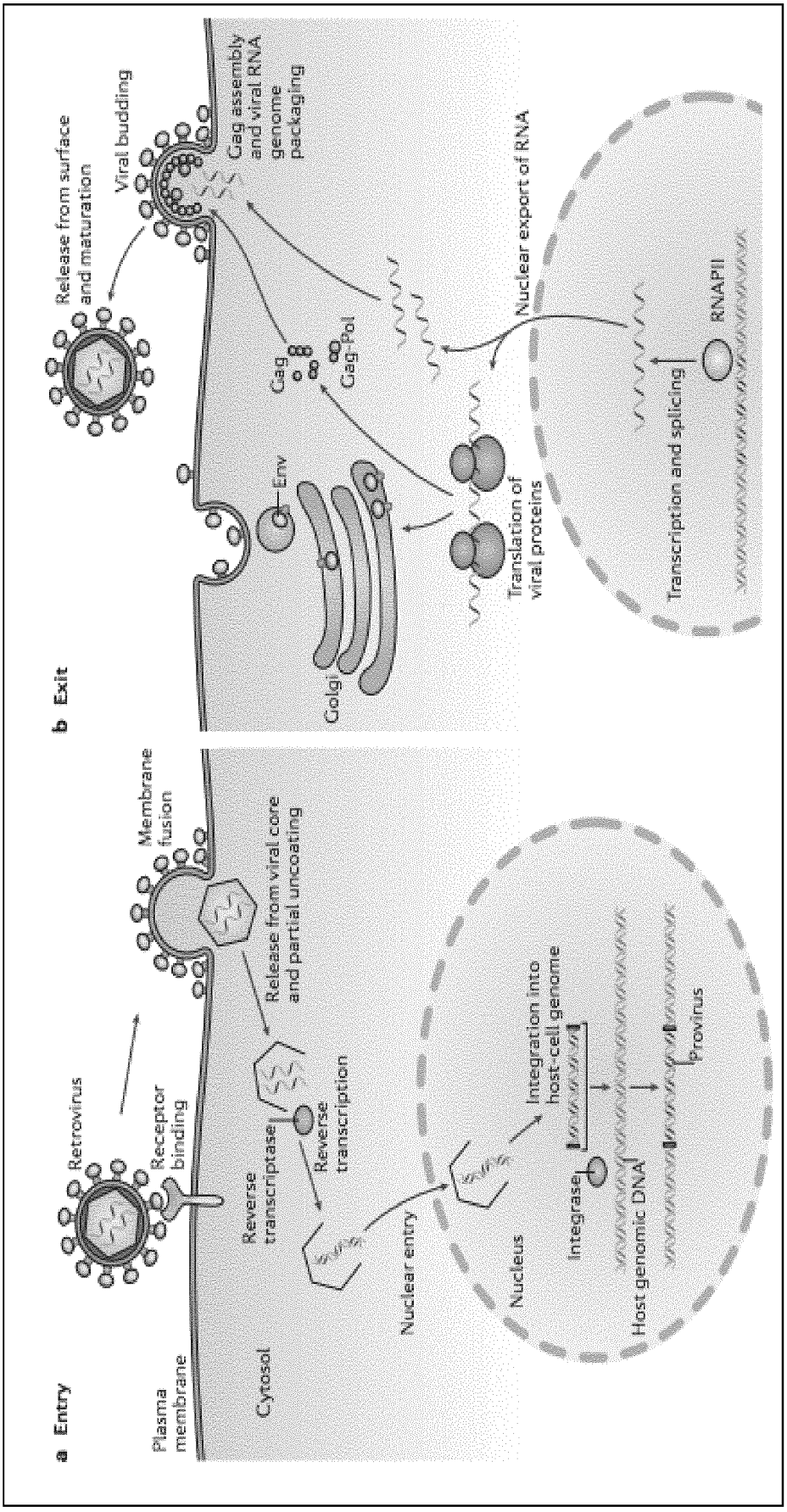


Figure 1

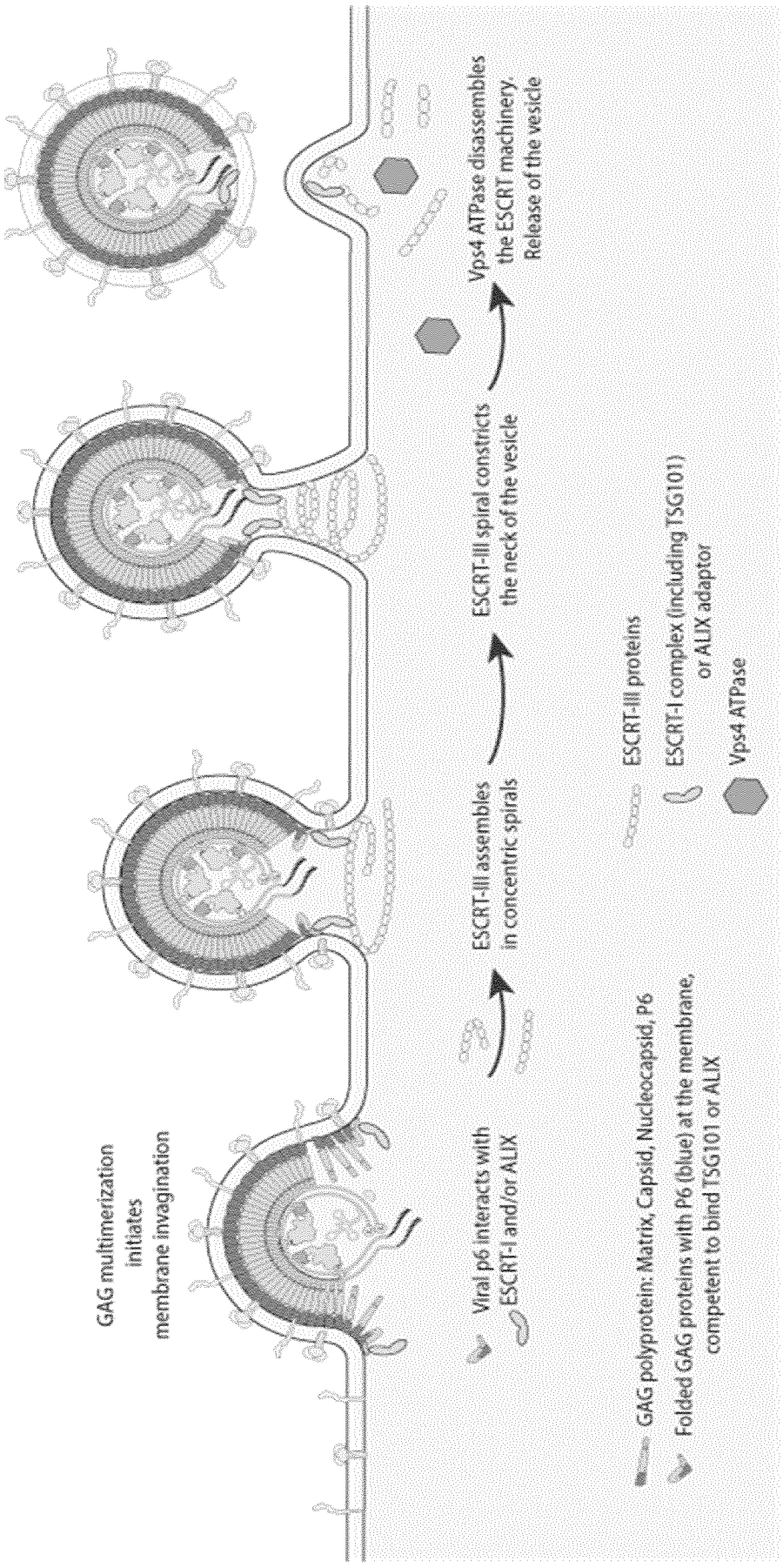

GAG multimerization
initiates
membrane invagination
Viral p6 interacts with
ESCRT-I and/or ALIX
ESCRT-III assembles
in concentric spirals
ESCRT-III spiral constricts
the neck of the vesicle
Vps4 ATPase disassembles
the ESCRT machinery.
Release of the vesicle
GAG polyprotein: Matrix, Capsid, Nucleocapsid, P6
Folded GAG proteins with P6 (blue) at the membrane,
competent to bind TSG101 or ALIX
ESCRT-III proteins
ESCRT-I complex (including TSG101)
or ALIX adaptor
Vps4 ATPase

Figure 2A

Motif

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Motif[1]	111 - 114	4	PTAP/PSAP motif			
Motif[1]	130 - 134	5	LYPX(n)L motif			
Motif[1]	162 - 165	4	PPXY motif			

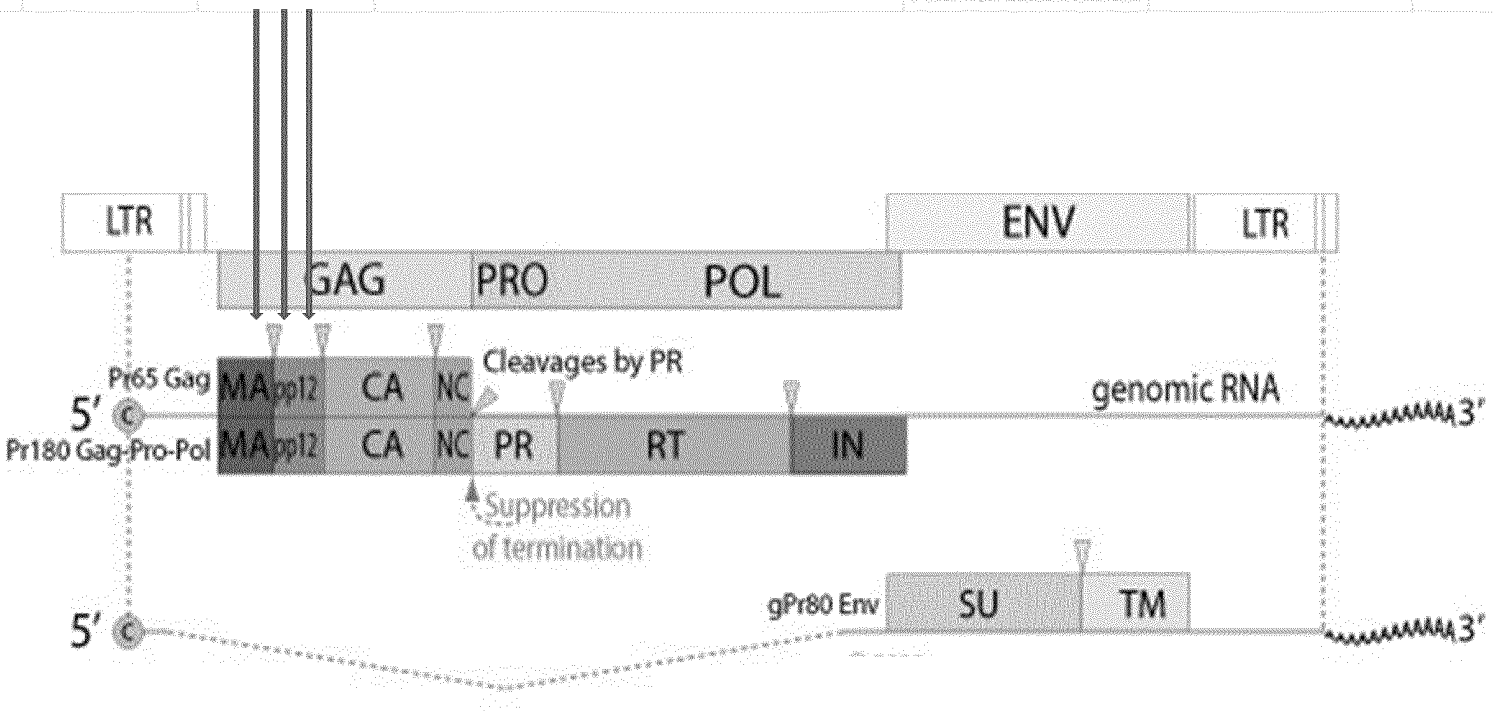
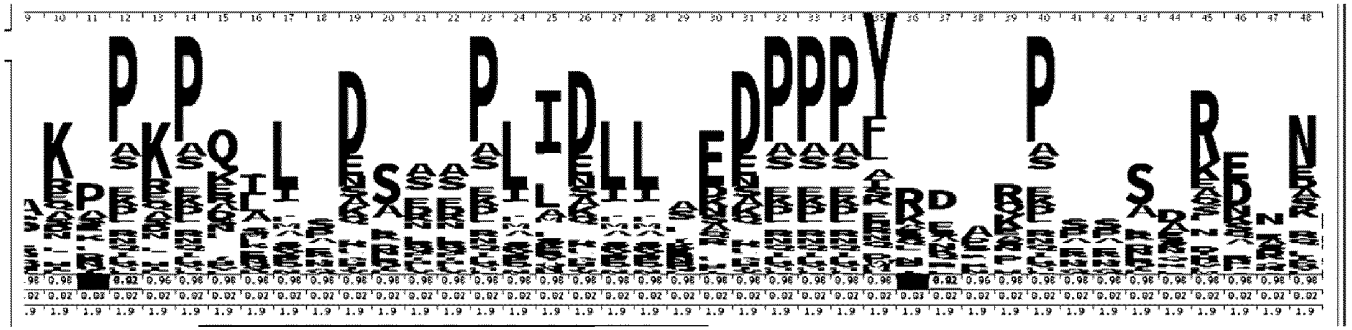


Figure 2B

HMM logo

Figure 3



```
P03332 GAG_MLVMS          155
Q27ID9 GAG_XMRV6          154
P03336 GAG_MLVAV          154
P03341 GAG_BAEVM          152
P27460 GAG_MLVCB          153
P23090 GAG_MLVDU          151
P10262 GAG_FLV            224
P03340 GAG_FSVMD          227
P26807 GAG_MLVF5          156
P26806 GAG_MLVFF          155
P26805 GAG_MLVFP          155
P21416 GAG_GALV           132
P21435 GAG_MLVHO          153
Q9TTC2 GAG_KORV           135
P03334 GAG_MSVMO          155
P32594 GAG_MSVMT          155
P29167 GAG_MLVBM          154
P29168 GAG_MLVDE          154
P11269 GAG_MLVRD          154
P03330 GAG_WMSV           135
Q2F7J2 GAG_XMRV3          154
Q2F7I9 GAG_XMRV4          154
```

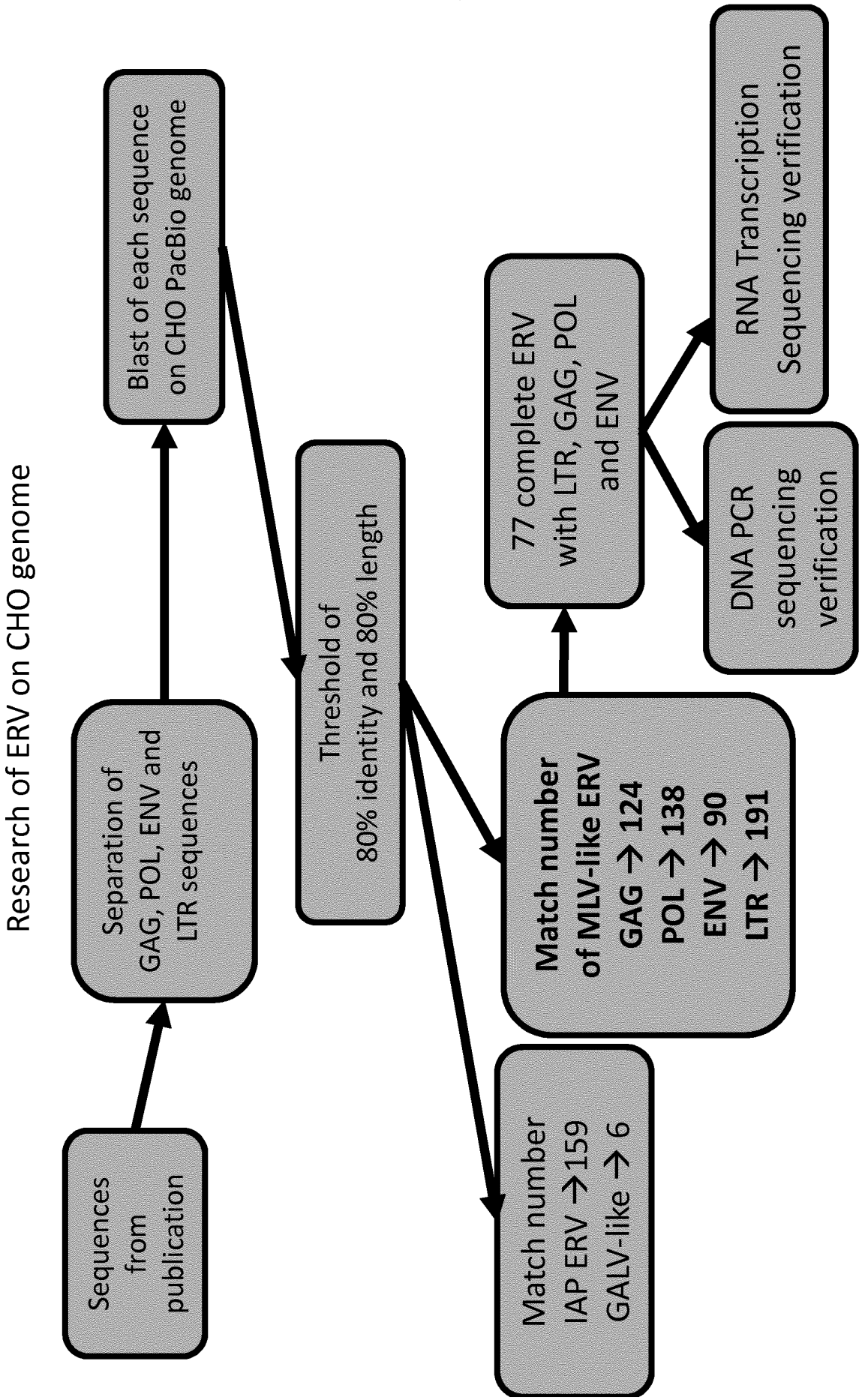
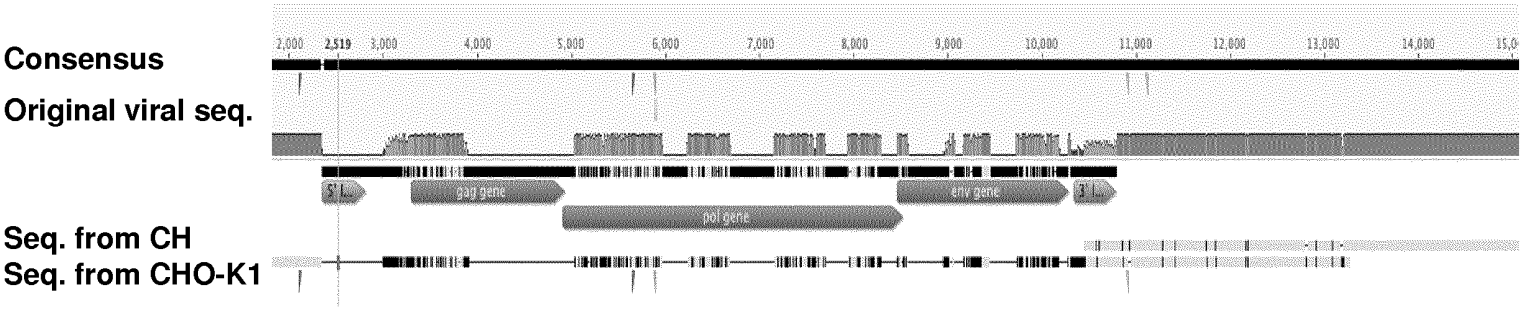
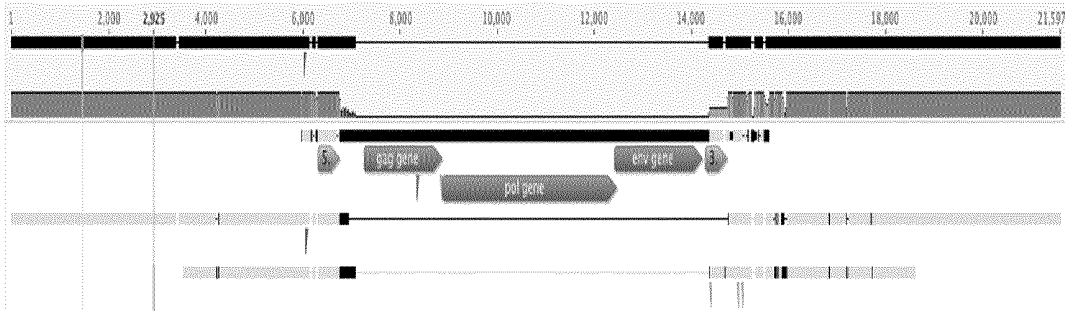

Research of ERV on CHO genome
Sequences from publication
Separation of GAG, POL, ENV and LTR sequences
Blast of each sequence on CHO PacBio genome
Threshold of 80% identity and 80% length
Match number IAP ERV →159 GALV-like → 6
Match number of MLV-like ERV GAG → 124 POL → 138 ENV → 90 LTR → 191
77 complete ERV with LTR, GAG, POL and ENV
RNA Transcription Sequencing verification
DNA PCR sequencing verification

FIGURE 4A

Search for Murine retrovirus type C sequences



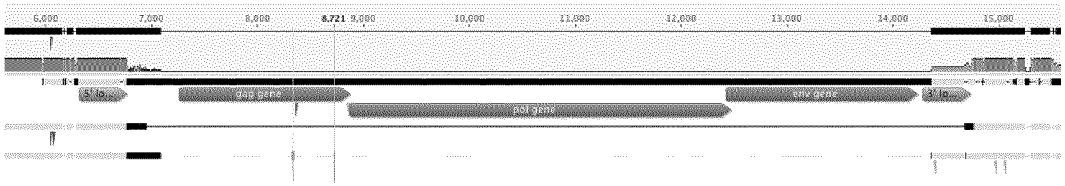
Work on the sequences

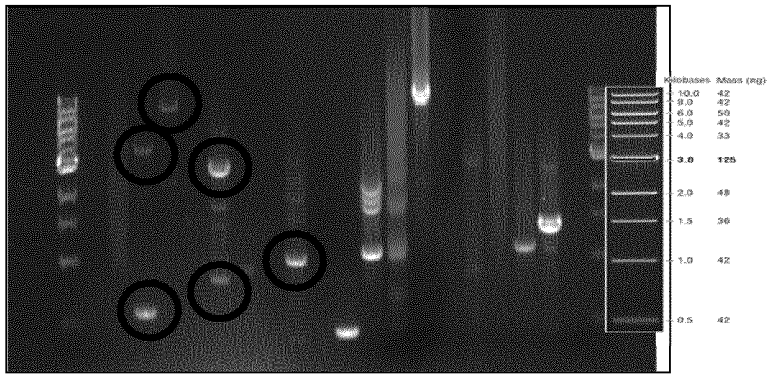


- Manual alignment
- Annotation
- Design of primers
- Search for corresponding sequences
- Search in the bordering sequences for genes, specific integration markers, expression, etc

Figure 4B

- PCR amplification and sequencing





- Long and/or Nested PCR

Figure 4C

Regulation of ERV in CHO cells

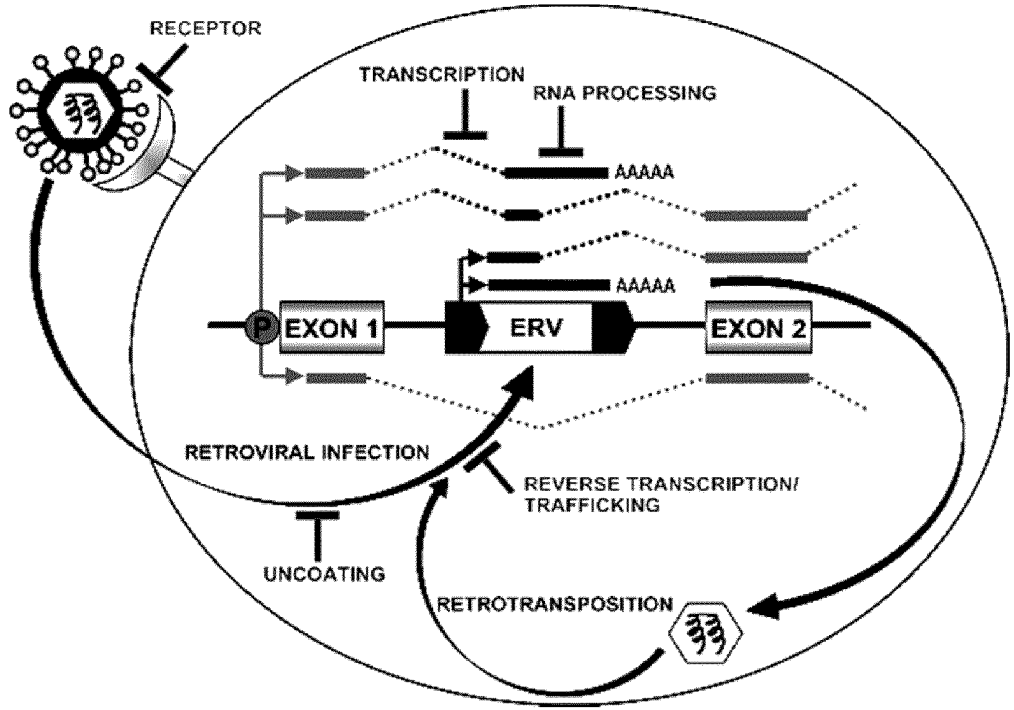
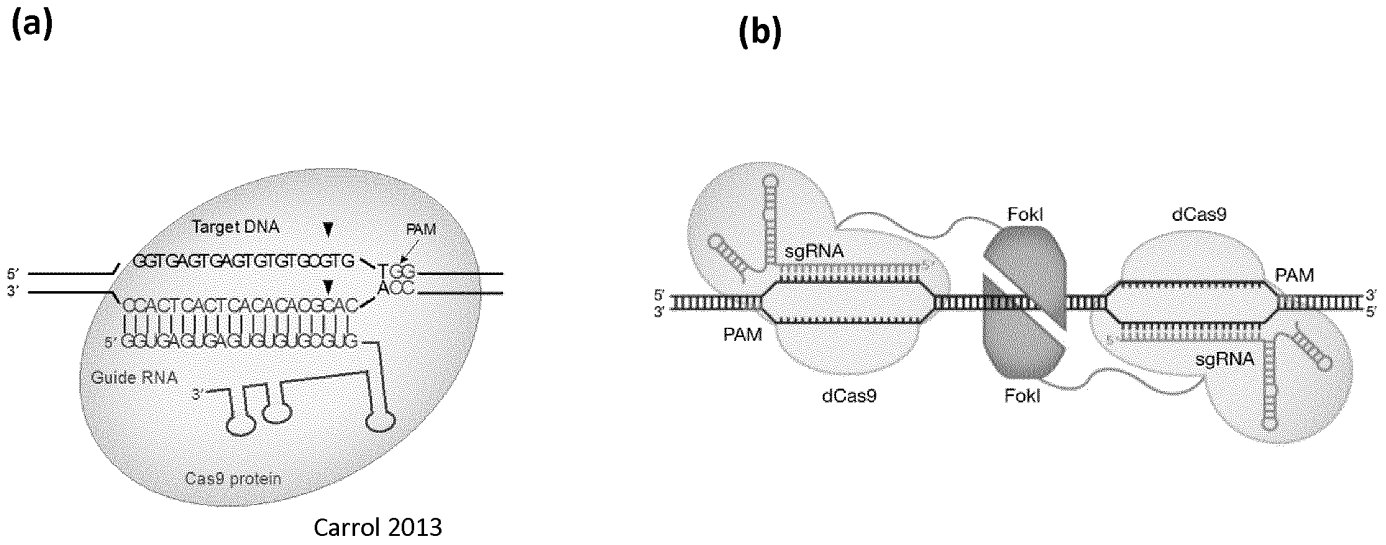


Figure 5

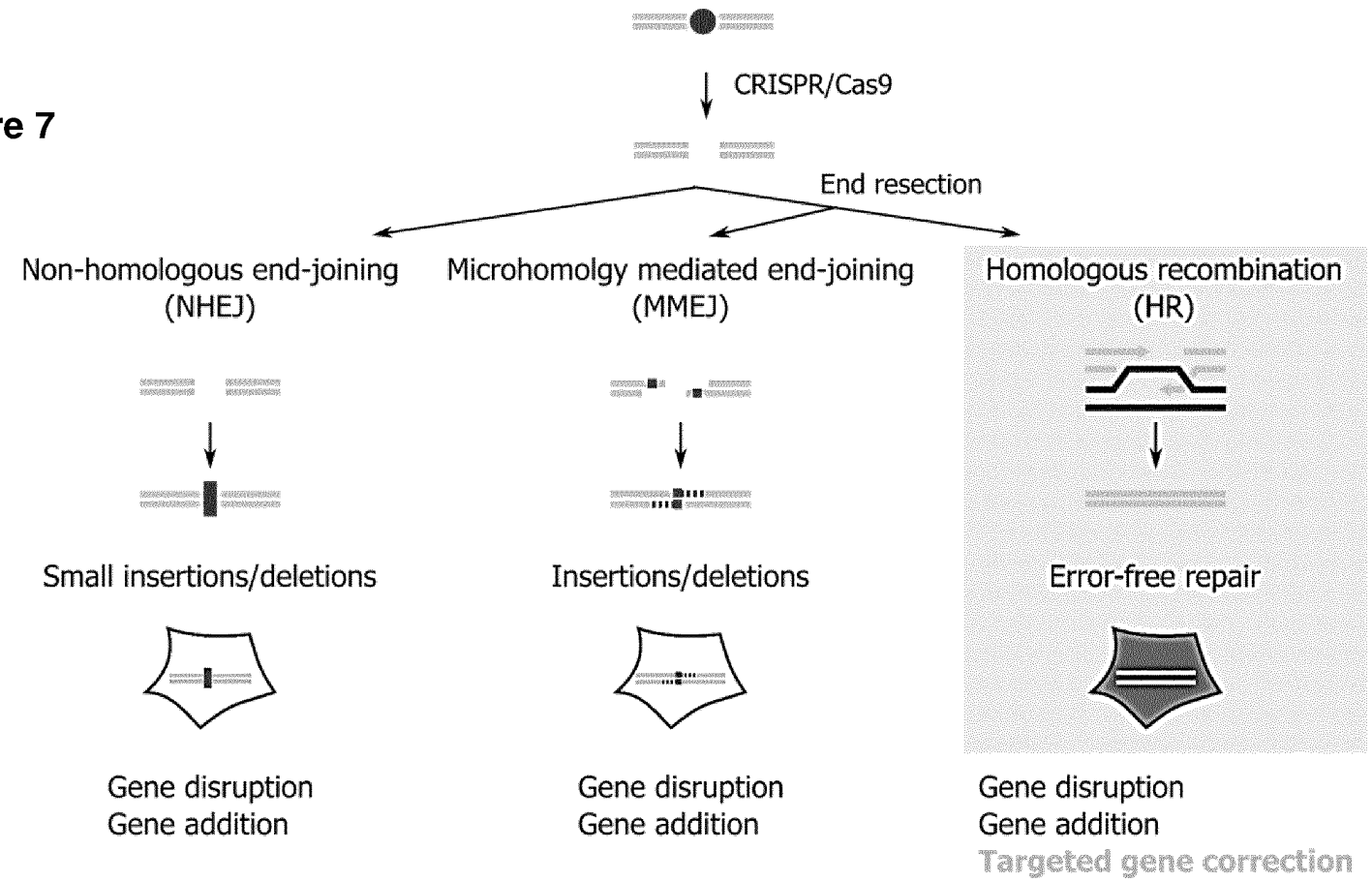
Nucleases facilitated gene targeting: CRISP/Cas9 system



Carrol 2013

Figure 6

Figure 7


Double Strand Break (DSB) repair pathways in genome editing
CRISPR/Cas9
End resection
Non-homologous end-joining (NHEJ)
Microhomolgy mediated end-joining (MMEJ)
Homologous recombination (HR)
Small insertions/deletions
Insertions/deletions
Error-free repair
Gene disruption
Gene addition
Gene disruption
Gene addition
Gene disruption
Gene addition
Targeted gene correction

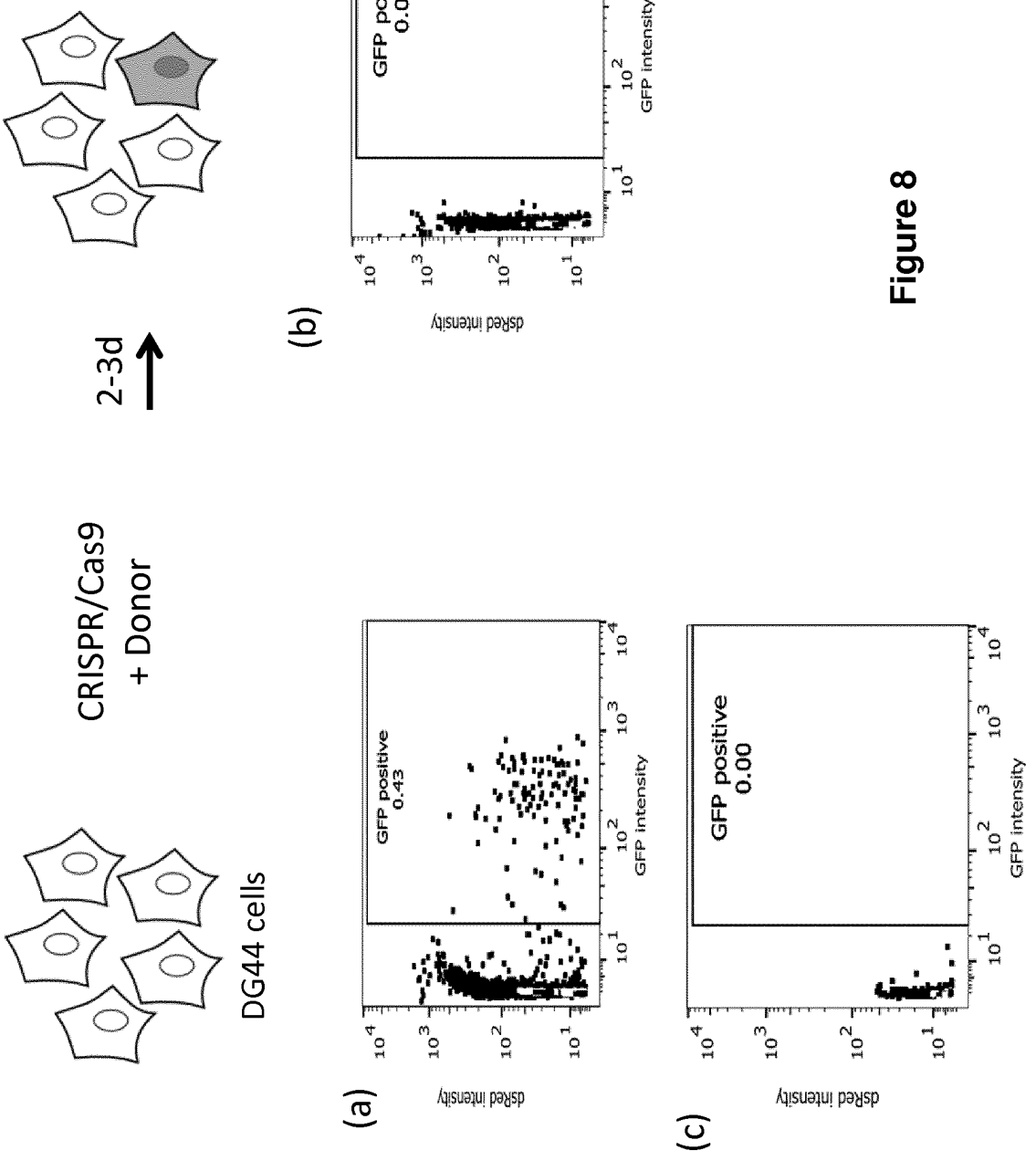

DG44 cells
CRISPR/Cas9
+ Donor
2-3d
(a)
GFP positive
0.43
dsRed intensity
GFP intensity
(b)
GFP positive
0.00
dsRed intensity
GFP intensity
(c)
GFP positive
0.00
dsRed intensity
GFP intensity

Figure 8

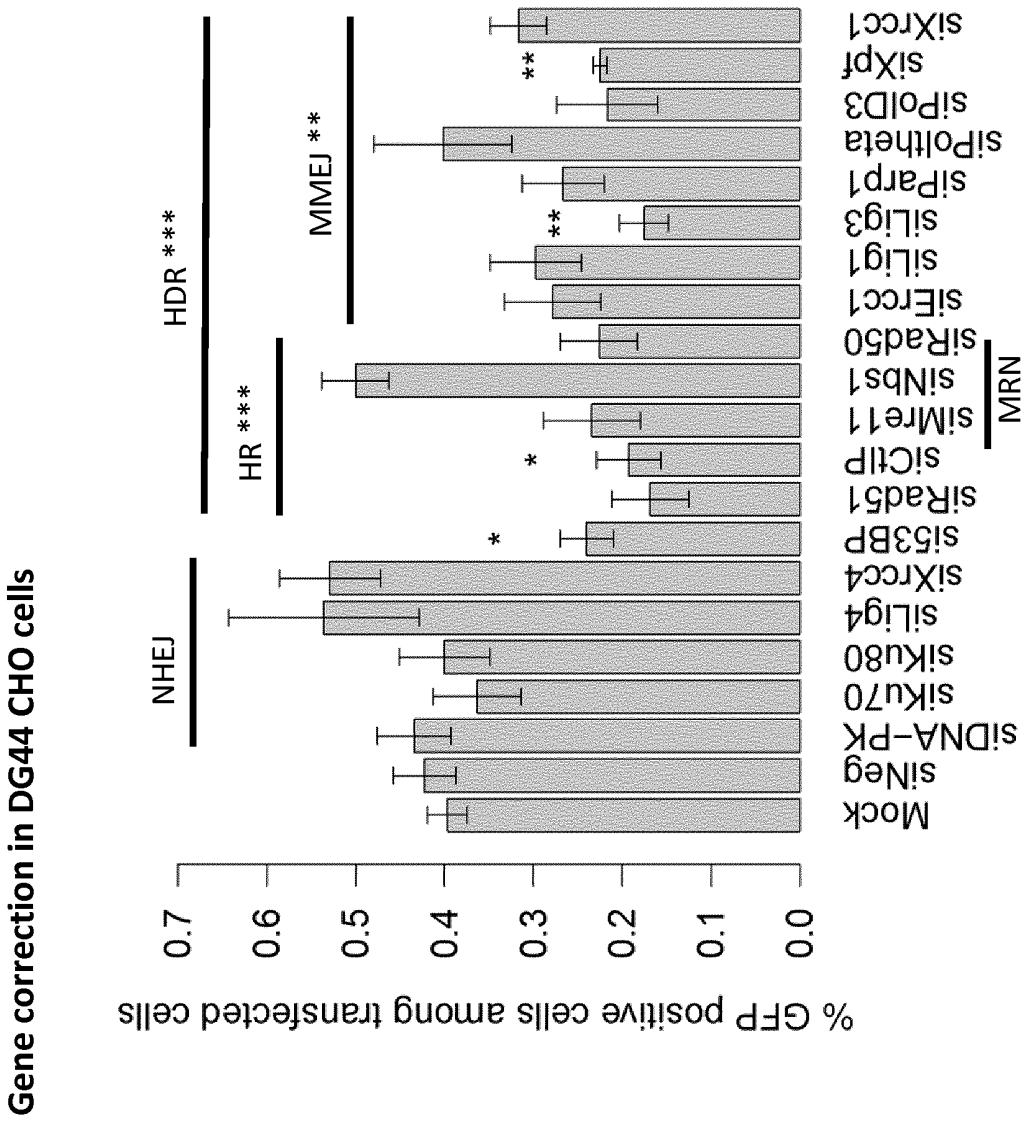

Gene correction in DG44 CHO cells
% GFP positive cells among transfected cells
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
NHEJ
HR ***
HDR ***
MMEJ **
Mock
siNeg
siDNA-PK
siKu70
siKu80
siLig4
siXrcc4
si53BP
siRad51
siCtIP
siMre11
siNbs1
siRad50
siErcc1
siLig1
siLig3
siParp1
siPoltheta
siPolD3
siXpf
siXrcc1
MRN complex
n≥3, mean ± SEM
*
*
**
**

Figure 9

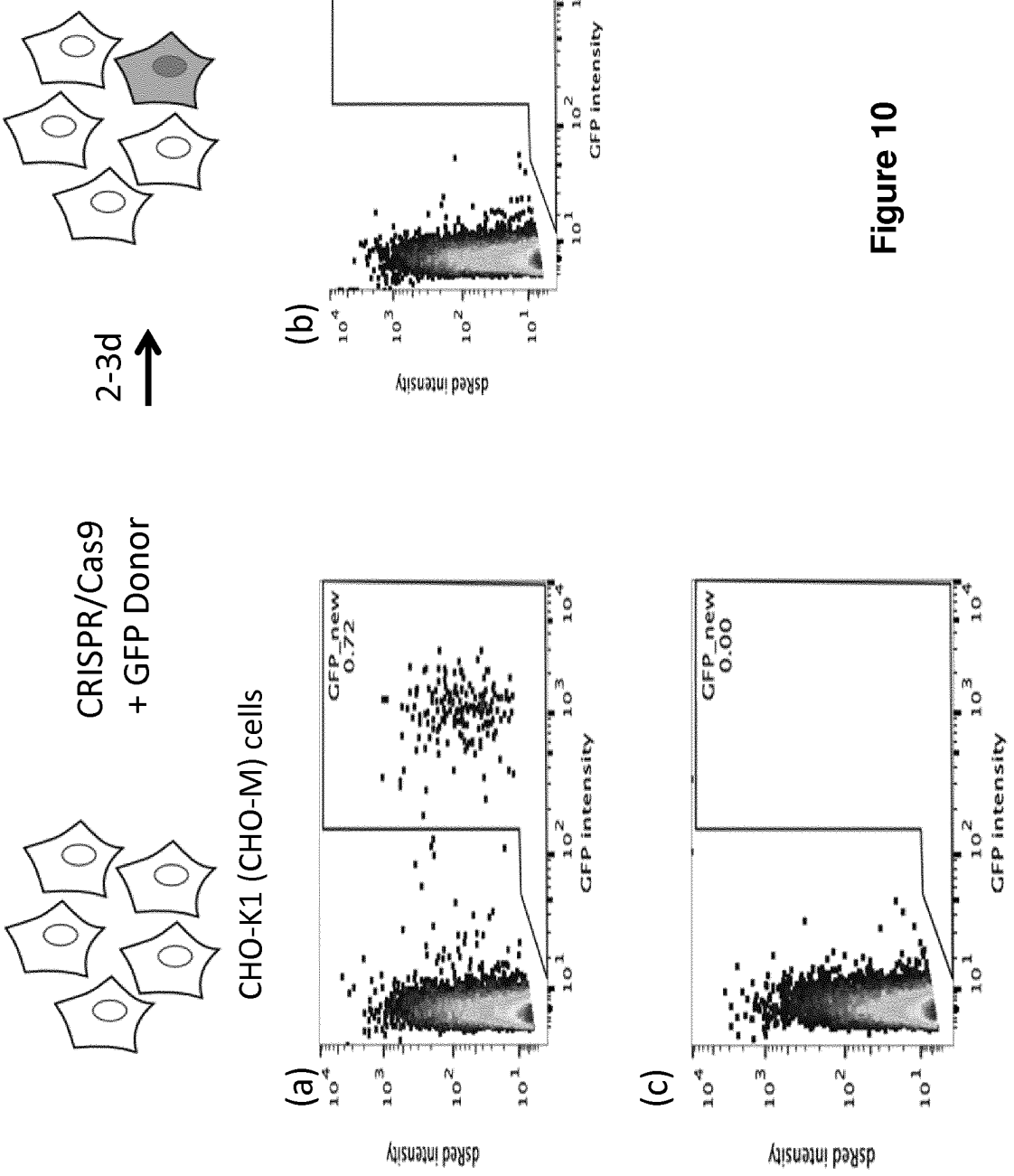
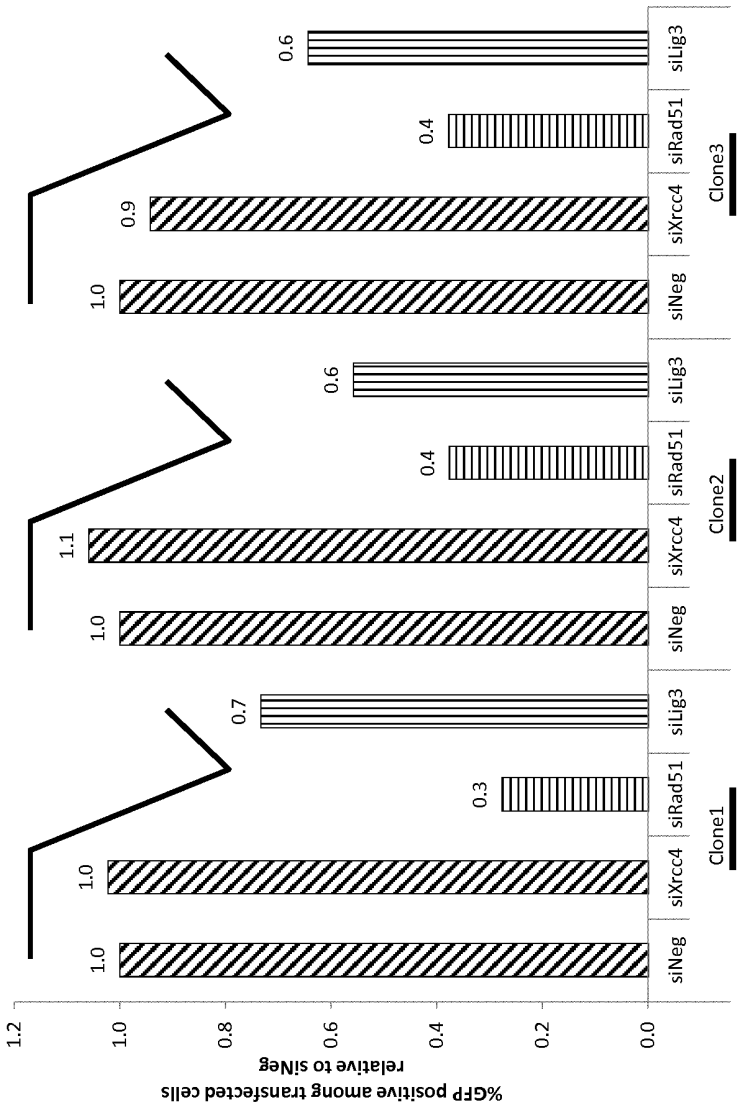

CHO-K1 (CHO-M) cells
CRISPR/Cas9
+ GFP Donor
2-3d
(a)
(b)
(c)
GFP_new
0.72
GFP_new
0.00
GFP_new
0.00
dsRed intensity
GFP intensity

Figure 10

Figure 11


%GFP positive among transfected cells
relative to siNeg
0.0
0.2
0.4
0.6
0.8
1.0
1.2
siNeg
siXrcc4
siRad51
siLig3
Clone1
1.0
1.0
0.3
0.7
siNeg
siXrcc4
siRad51
siLig3
Clone2
1.0
1.1
0.4
0.6
siNeg
siXrcc4
siRad51
siLig3
Clone3
1.0
0.9
0.4
0.6

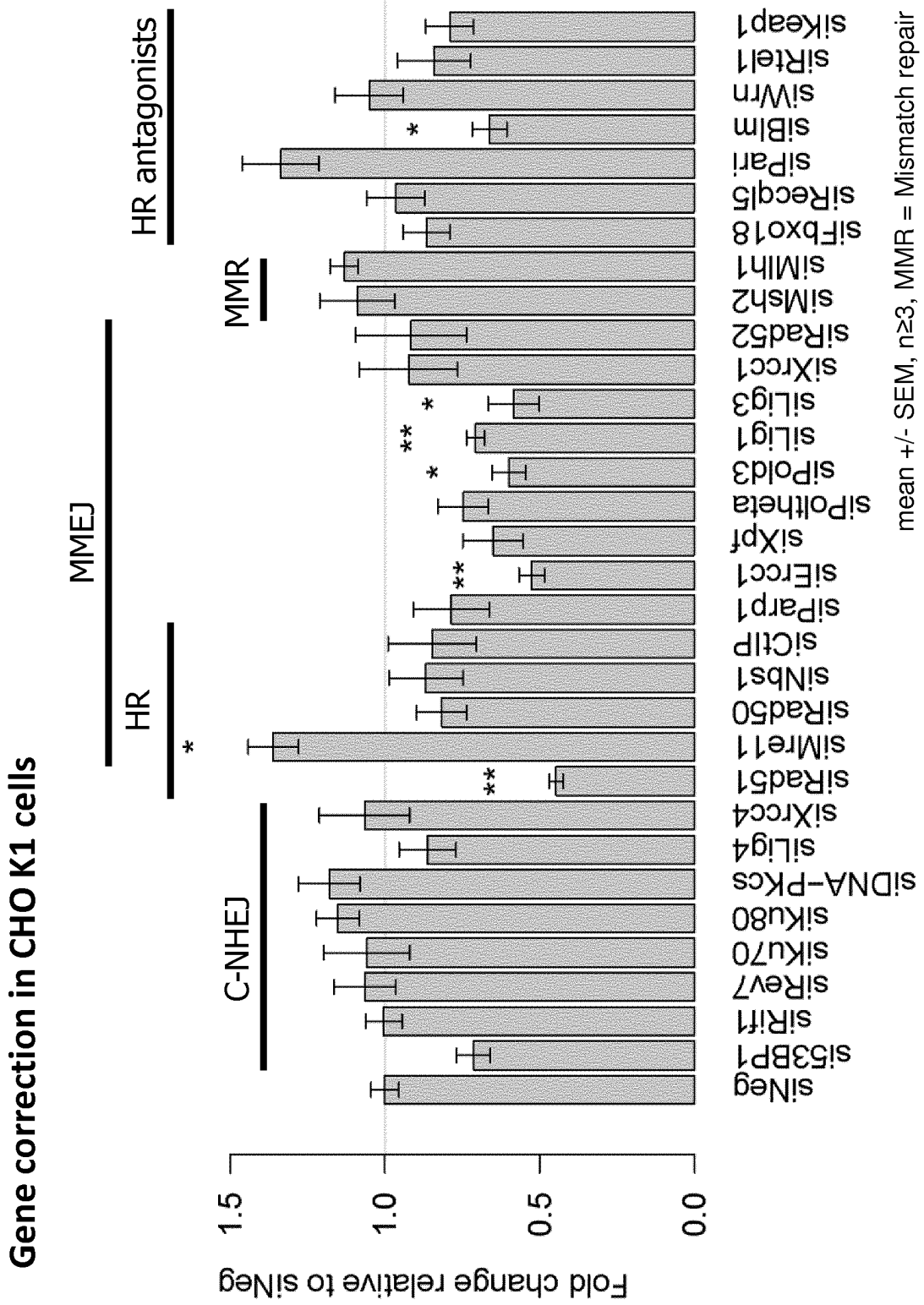
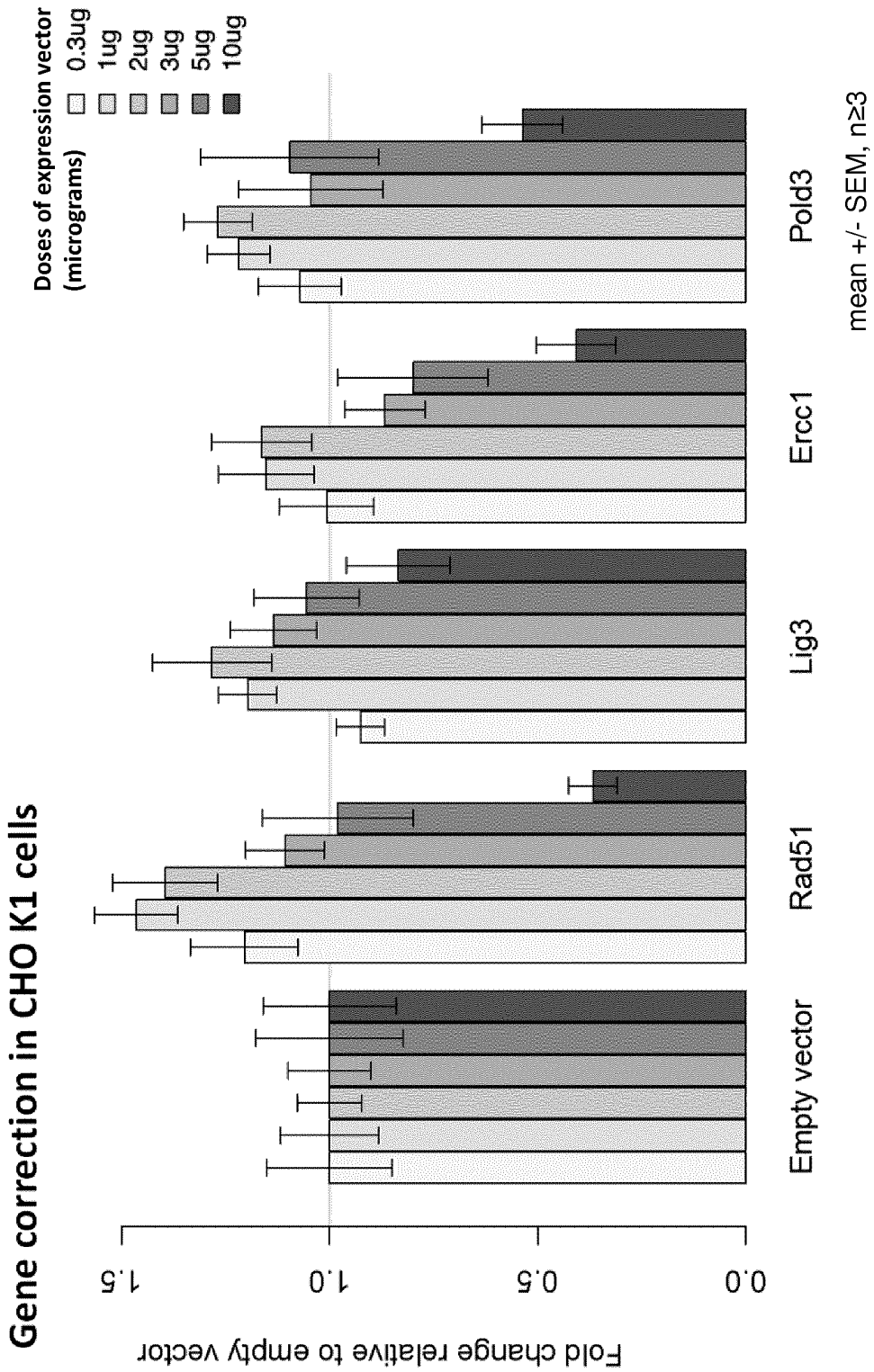

Gene correction in CHO K1 cells
C-NHEJ
MMEJ
HR
MMR
HR antagonists
Fold change relative to siNeg
1.5
1.0
0.5
0.0
siNeg
si53BP1
siRif1
siRev7
siKu70
siKu80
siDNA-PKcs
siLig4
siXrcc4
siRad51
siMre11
siRad50
siNbs1
siCtIP
siParp1
siErcc1
siXpf
siPoltheta
siPold3
siLig1
siLig3
siXrcc1
siRad52
siMsh2
siMlh1
siFbxo18
siRecql5
siPari
siBlm
siWrn
siRtel1
sikeap1
mean +/- SEM, n≥3, MMR = Mismatch repair

Figure 12A



Increased expression of rate-limiting HR and/or MMEJ factors stimulates gene correction in a dose-dependent manner

Figure 12B

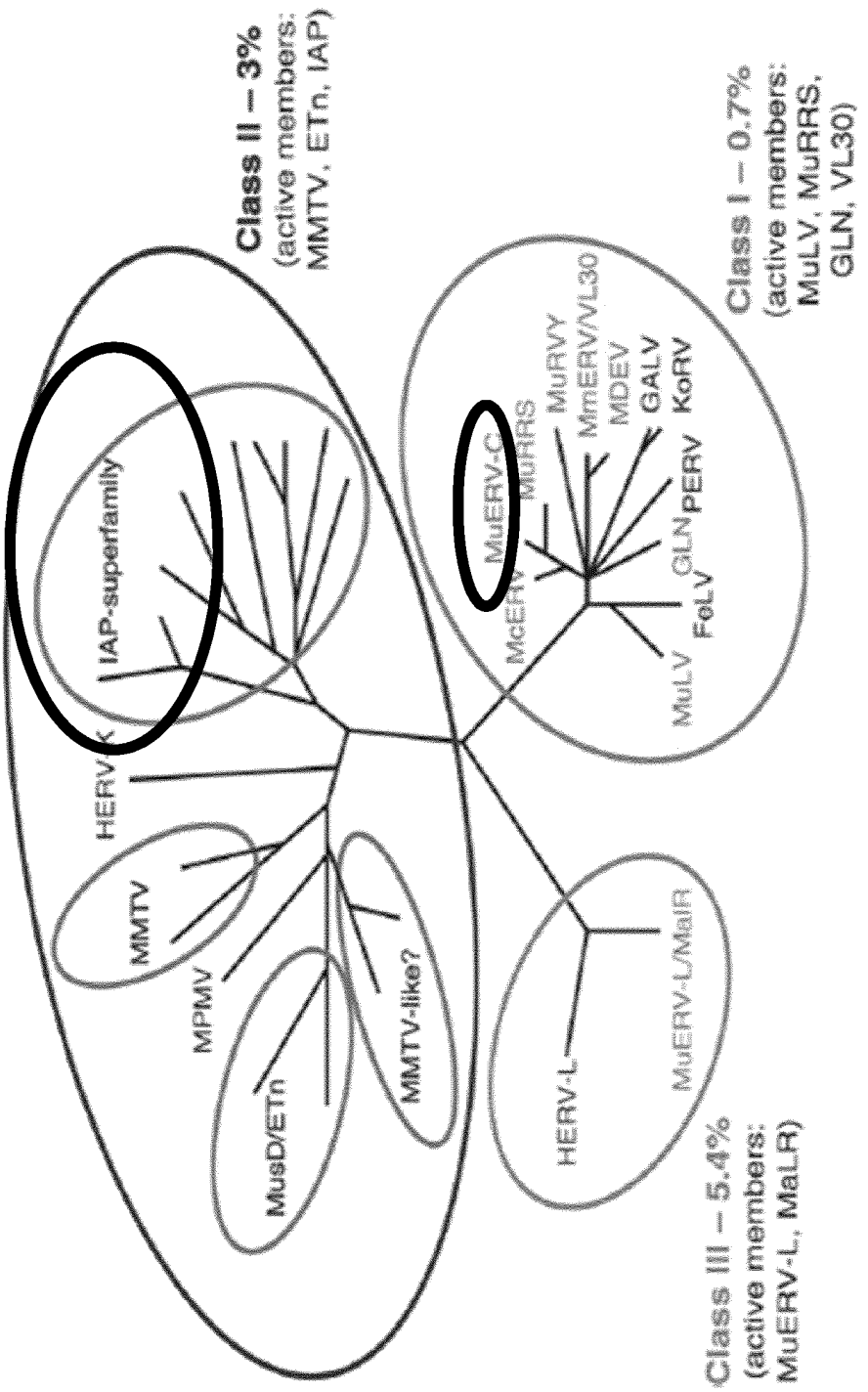

Class II – 3%
(active members:
MMTV, ETn, IAP)
HERV-K
IAP-superfamily
MMTV
MPMV
MusD/ETn
MMTV-like?
Class I – 0.7%
(active members:
MuLV, MuRRS,
GLN, VL30)
MuERV-C
McERV
MuRRS
MuRVY
MmERV/VL30
MDEV
GALV
KoRV
PERV
GLN
FeLV
MuLV
Class III – 5.4%
(active members:
MuERV-L, MaLR)
HERV-L
MuERV-L/MaIR

Figure 13

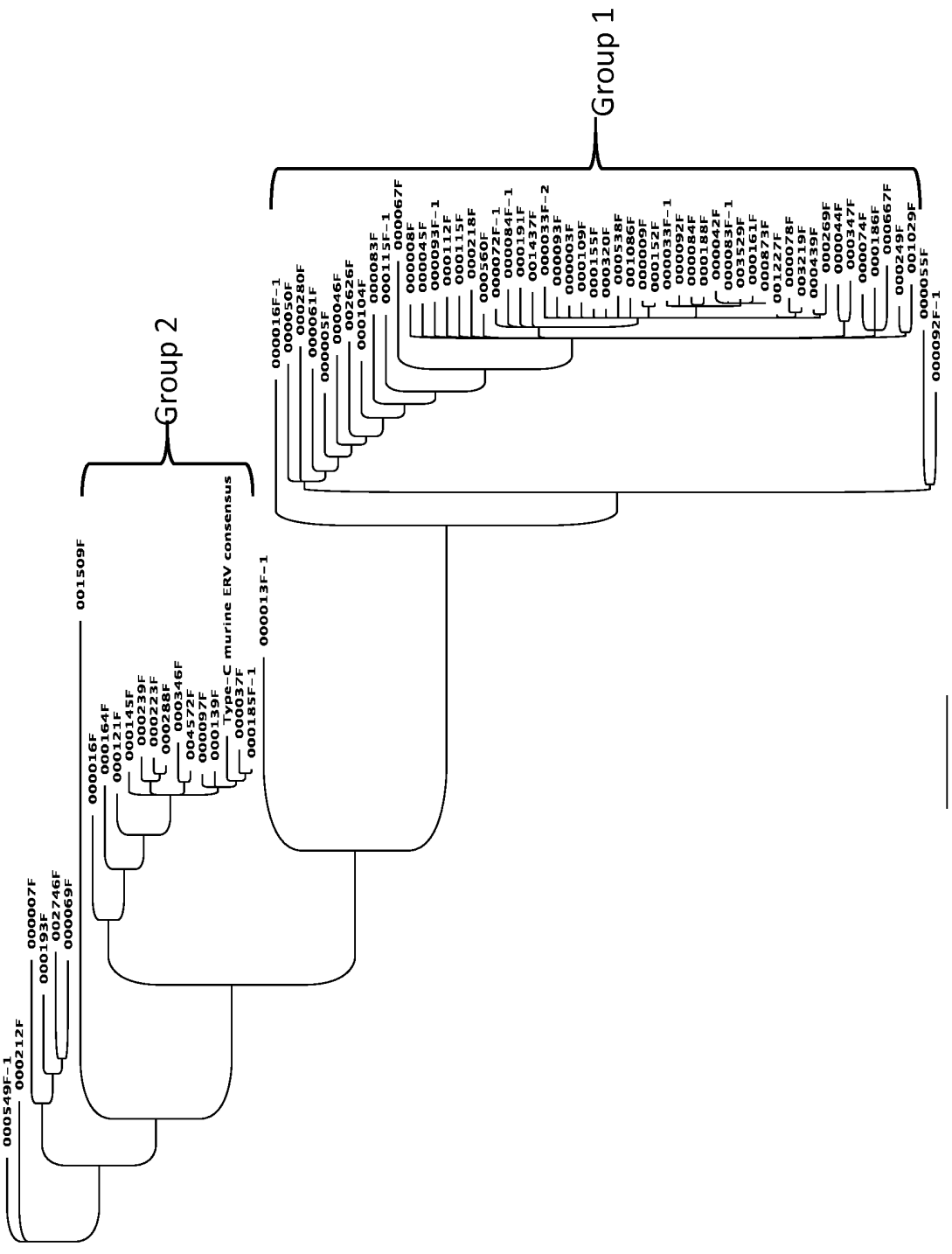
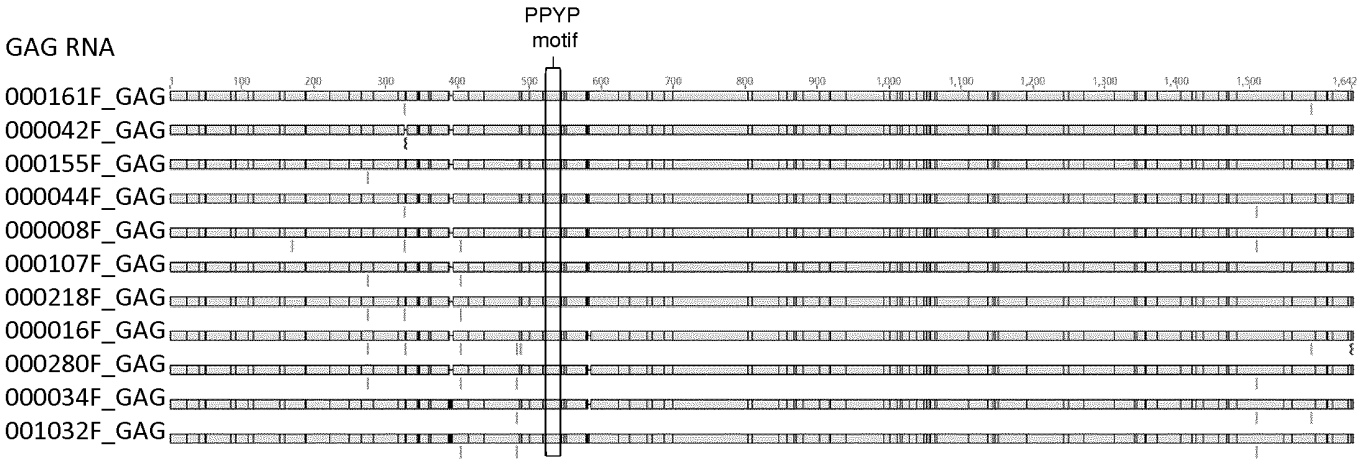

Group 1
Group 2
000549F-1
000212F
000007F
000193F
002746F
000069F
001509F
000016F
000164F
000121F
000145F
000239F
000223F
000288F
000346F
004572F
000097F
000139F
Type-C murine ERV consensus
000037F
000185F-1
000013F-1
000016F-1
000050F
000280F
000061F
000005F
000046F
002626F
000104F
000083F
000115F-1
000067F
000008F
000045F
000093F-1
000112F
000115F
000218F
000560F
000072F-1
000084F-1
000191F
001437F
000033F-2
000093F
000003F
000109F
000155F
000320F
000538F
001086F
000009F
000152F
000033F-1
000092F
000084F
000188F
000042F
000083F-1
003529F
000161F
000873F
001227F
000078F
003219F
000439F
000269F
000044F
000347F
000074F
000186F
000667F
000249F
001029F
000055F
000092F-1
0.007

Figure 14



Alignment of GAG RNAs from selection of CHO Gammaretrovirus-like ERV. The marks under the alignments represent mutations.

Figure 15A

Retroviral element CpG DNA methylation in CHO cells

Examples of 5' LTR-hypomethylated ERVs

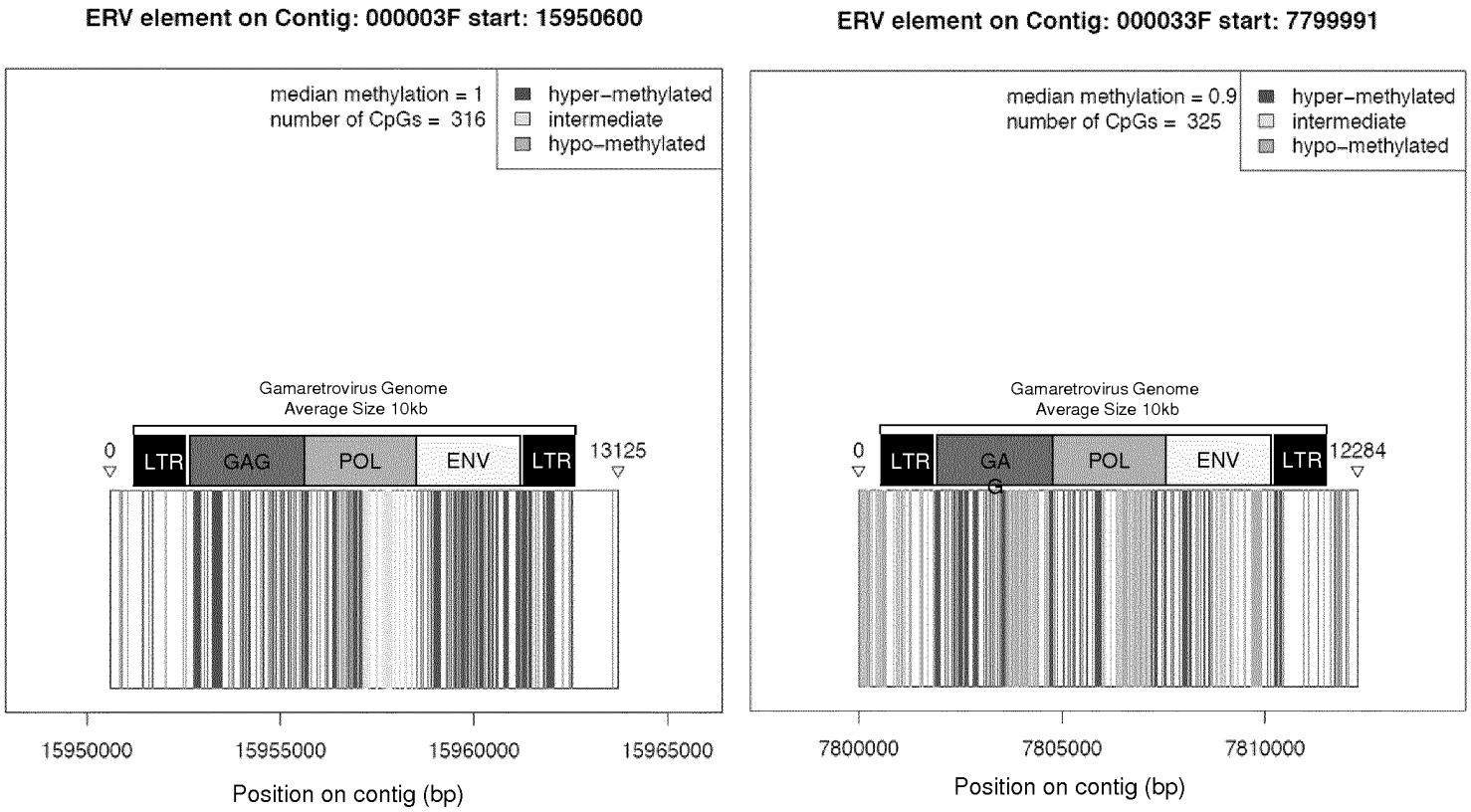


Figure 16

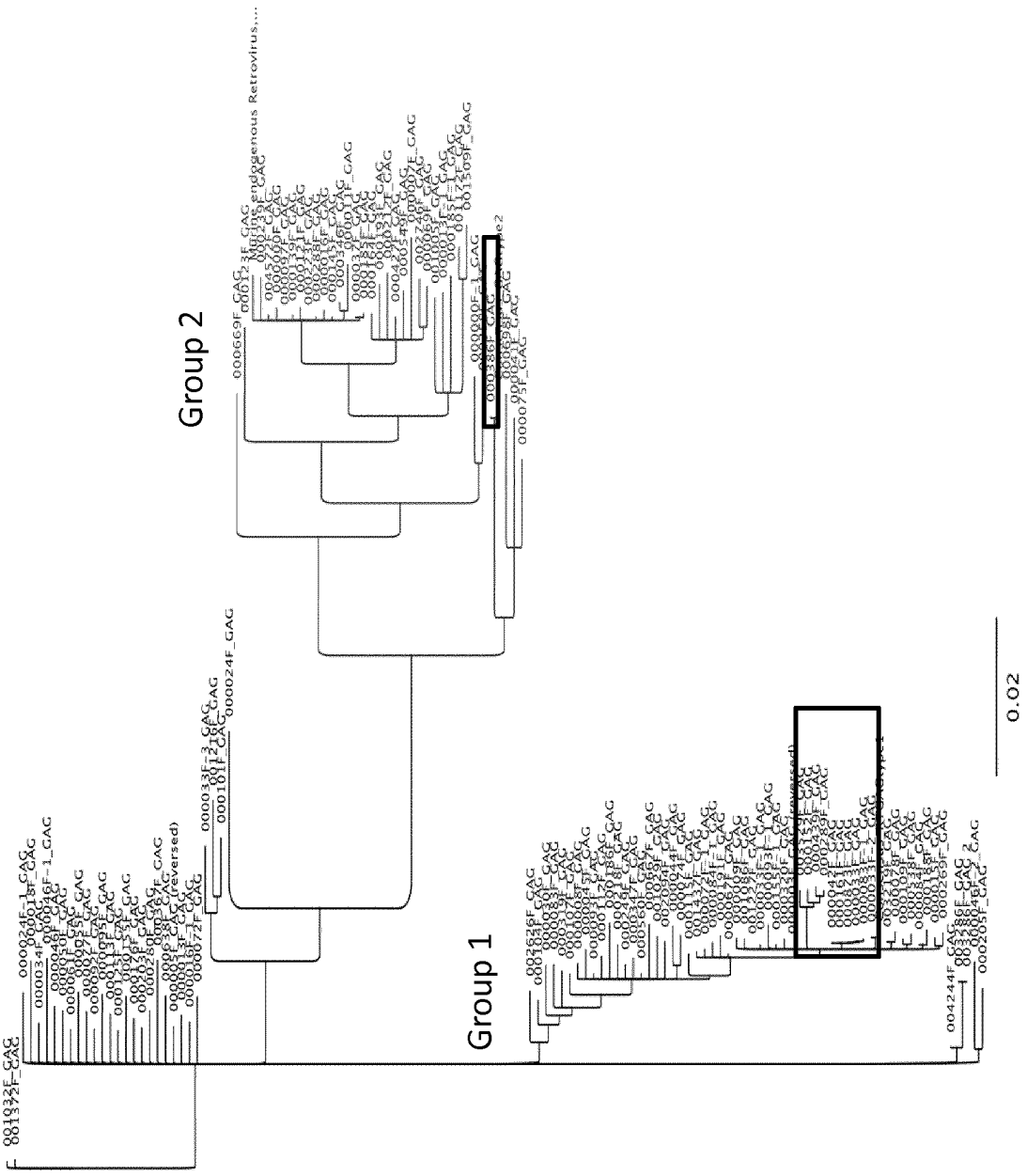
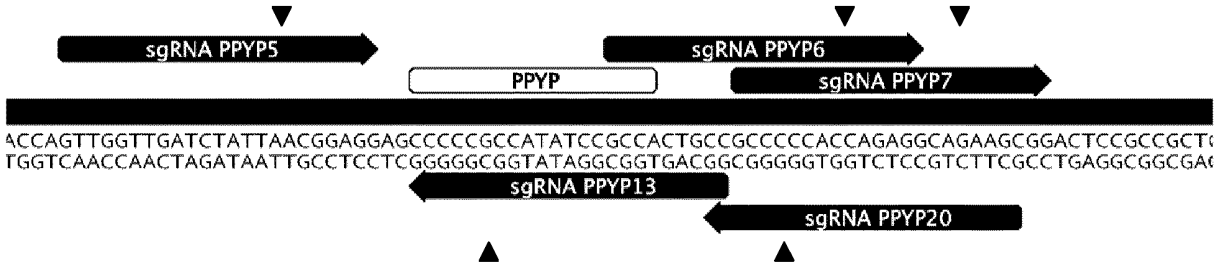


Figure 17

CRISPR/Cas9 mediated ERV inactivation

Target 1:
PPYP motif - ERV budding
Strategy: **knockout** (frameshift via C-NHEJ/alt-EJ repair) using 5 different sgRNAs



Target 2:
Myristoylation motif - membrane binding
Strategy: **dominant-negative mutation** (point mutation via HR repair) from 3 sgRNAs

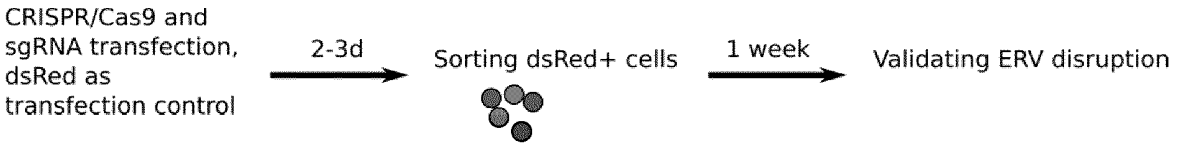


▲ = double strand breaks

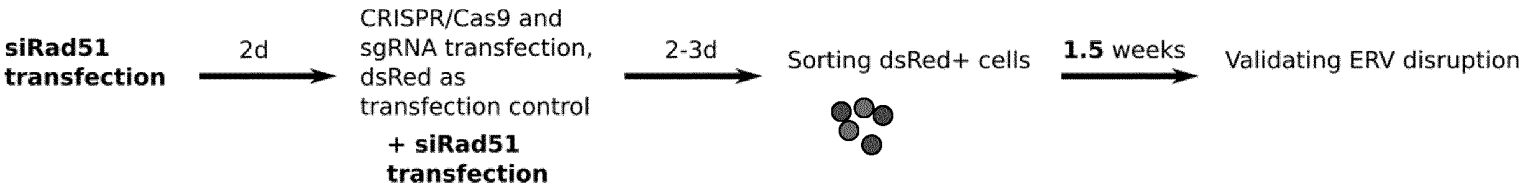
Figure 18

Experimental set-up for ERV inactivation

1. Do sgRNAs cleave ERV sequences efficiently?



2. Increasing knockouts by blocking HR using Rad51 siRNAs



-> mRNA (i.e. cDNA) assays by endpoint PCR

-> mRNA (i.e. cDNA) reading frame assays by sequencing

Figure 19

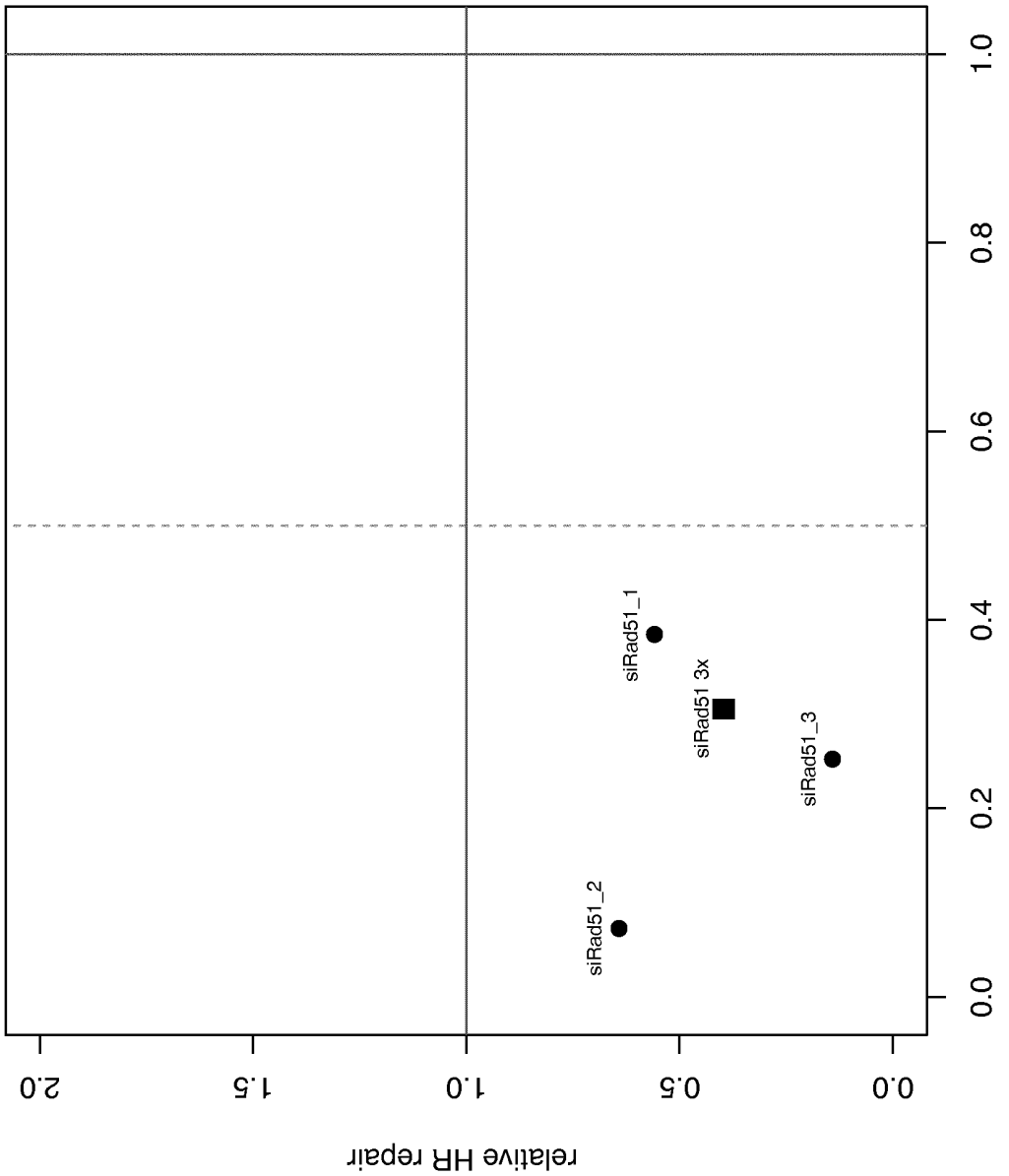
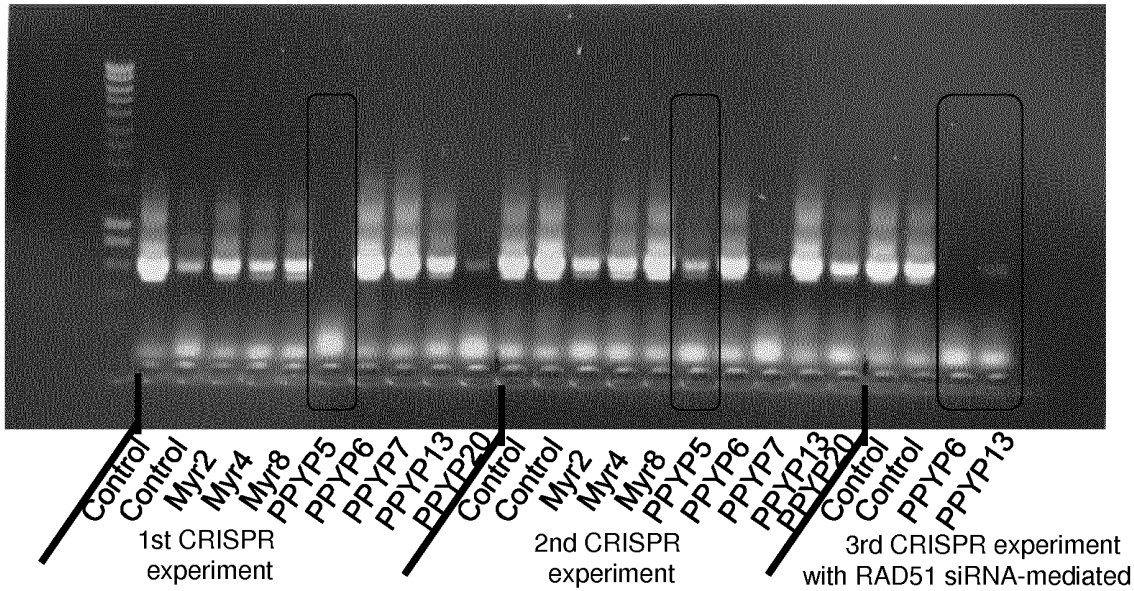

siRNA Rad51 deconvolution
relative HR repair
0.0
0.5
1.0
1.5
2.0
siRad51_2
siRad51_1
siRad51 3x
siRad51_3
0.0
0.2
0.4
0.6
0.8
1.0
relative mRNA level

Figure 20

Detection of CRISPR-Cas9 effects



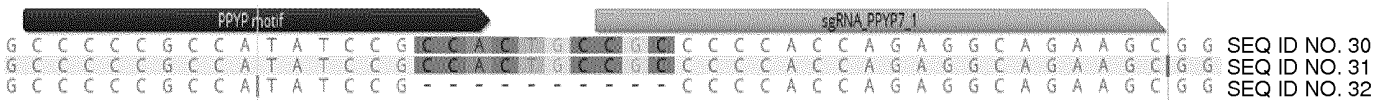


Figure 21

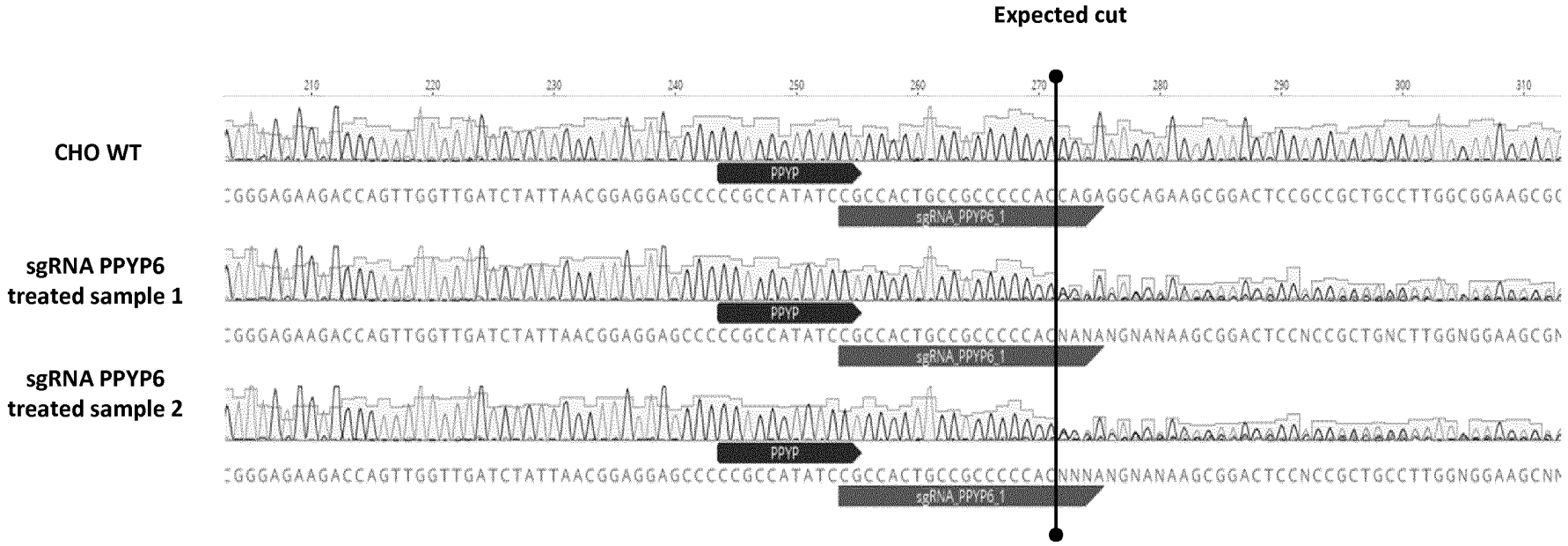

Expected cut
CHO WT
sgRNA PPYP6
treated sample 1
sgRNA PPYP6
treated sample 2
210
220
230
240
250
260
270
280
290
300
310
PPYP
sgRNA_PPYP6_1

Figure 22