CLAIMS

1. An engineered cell, preferably of a mammalian cell line such as an engineered CHO cell, including an engineered CHO-K1 cell, an engineered porcine cell or an engineered human cell comprising:

within the genome of the cell:

at least one locus comprising an insertion site of an endogenous retrovirus (ERV) sequence or a LTR-retrotransposon (LTR-RT) sequence, wherein said at least one locus comprise(s):

(i) a) the ERV sequence or the LTR-RT sequence, or b) the insertion site and, optionally, parts of the sequence of a), and/or

(ii) an allelic wild type counterpart sequence of (i), and

at least one transgene encoding at least one transgene expression product integrated into the at least one locus.

2. The engineered cell of claim 1, wherein the cell comprises (i) and (ii) on different chromosomes, such as chromosome 15 and 9 of a CHO cell.

3. The engineered cell of claim 1 or 2, wherein cell comprises (i) and (ii), and the at least one transgene is integrated into (ii).

4. The engineered cell of claim 3, wherein the at least one transgene is not integrated into (i).

5. The engineered cell of claim 3, wherein the at least one transgene is also integrated into (i).

6. A cell population comprising engineered cells according to any one of the preceding claims, wherein the at least one transgene is integrated into more than 20%, 30% or even more than 40% of (i) and/or (ii) of cells within said cell population.

7. The cell population of claim 6, wherein the cell comprises (i) and (ii), wherein (i) comprises: at least nucleotides 29021 to 40247 of SEQ ID NO: 1 or a sequence having 95%, 98% or 99% sequence identity with nucleotides 29021 to 40247 of SEQ ID NO: 1 and (ii) comprises at least nucleotides 29020 to 31020 of SEQ ID NO: 2 or a sequence having 95% 98%, or 99% sequence identity with nucleotides 29020 to 31020 of SEQ ID NO: 2, or

at least nucleotides 29521 to 39747 of SEQ ID NO: 1 or a sequence having 95%, 98% or 99% sequence identity with nucleotides 29521 to 4247 of SEQ ID NO: 1 and (ii) comprises at

least nucleotides 29520 to 30520 of SEQ ID NO: 2 or a sequence having 95% 98%, or 99% sequence identity with nucleotides 29520 to 30520 of SEQ ID NO: 2.

8. The cell of any one of claims 1 to 5, or the cell population of any one of claims 6 or 7, wherein the cell or cell population lacks expressed endogenous retroviral sequences (ERV) and/or there are no detectable viral particles comprised in the cell population culture supernatant.

9. The cell of any one of claims 1 to 5, or the cell population of any one of claims, 6, 7 or 8, wherein the at least one transgene expression product is a protein of interest and the cell or cell population optionally expresses the protein of interest per unit of time, in an amount (such as picograms per cell and per day, µg/l or mg/l) that exceeds the amount of a protein of interest when the at least one transgene is integrated into the genome outside the at least one locus, by at least 1.5 fold, 2 fold, 2.5 fold, 3 fold or more.

10. The engineered cell or cell population of any one of the preceding claims, wherein the ERV or LTR-RT is selected from the group consisting of a type C endoretroviral element (ERV C), MLV (murine leukemia virus), XMRV (xenotropic murine leukemia virus-related virus), MMTV (mouse mammary tumor virus), MERV-L (mouse ERV with L-tRNA PBS), VL30 (virus like 30), IAP (intracisternal A-type particle), MusD (Mus type-D related retrovirus), PERVs (porcine endogenous retroviruses), KoRV (koala retrovirus), enJSRV (Jaagsiekte sheep retrovirus), MaLR (mammalian apparent LTR retrotransposons), HERV (Human endogenous retroviruses) such as HERV-E (human ERV with E-tRNA PBS), HERV-H (human ERV with H-tRNA PBS), HERV-K (human ERV with K-tRNA PBS), HERV-L (human ERV with L-tRNA PBS), HERV-W (human ERV with W-tRNA PBS) and combinations thereof.

11. The engineered cell or cell population of any one of the preceding claims, wherein the ERV or LTR-RT sequence comprises at least one ERV subsequence selected from the group consisting of a gag (group-specific antigen) gene, a pol (polymerase) gene, a env (envelope) gene, a sequence encoding a MA (matrix), a CA (capsid), a NC (nucleocapsid), a sequence encoding a SP1 (Spacer peptide 1), a sequence encoding a SP2 (Spacer peptide 2) or a further domain encoding proteins such as pp12 or p6, are long terminal repeats (LTRs) of a ERV and combinations thereof and wherein the transgene is optionally integrated into one of the subsequences.

12. The engineered cell or cell population of any one of the preceding claims, wherein the cell is transfected with one or more vectors comprising one or more genes of Table 2 or

SEQ ID Nos: 25-28, 38-58 and/or 59 or sequences having at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with SEQ ID Nos: 25-28, 38-58 and/or 59, wherein the cytoplasm of the cell(s) optionally further comprise(s) exogenous chemical inhibitors and/or stimulators of one or more DNA Repair Pathways (DRPs), such as NHEJ inhibitors selected from the group consisting of NU7441, Olaparib, DNA Ligase IV inhibitor, Scr7 KU-0060648 anti-EGFR-antibody C225 (Cetuximab), Compound 401 (2-(4-Morpholinyl)-4H-pyrimido[2,l-a]isoquinolin-4-one), Vanillin, Wortmannin, DMNB, IC87361, LY294002, OK- 1035, CO 15, NK314, PI 103 hydrochloride and combinations thereof, MMEJ inhibitors selected from the group of Mirin, derivatives of Mirin, inhibitors of PolQ, inhibitors of CtIP and combinations thereof, HR inhibitors such as RI-1 and BO2, HR stimulators such as RS-1, NHEJ stimulators, such as IP6 and combinations of any one of the above inhibitors and/or stimulators.

13. The engineered cell of any one of the preceding claims, wherein the locus has at least 80%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity with a sequence selected from SEQ ID Nos. 1 and/or SEQ ID No. 2.

14. The engineered cell of any one of the preceding claims, wherein the transgene is a landing pad.

15. The engineered cell of any one of the preceding claims, wherein the cell is a Chinese Hamster Ovary (CHO) cell, a human cell or a porcine cell.

16. A method for transgene integration into a genome of a cell, preferably of a mammalian cell line comprising:
(a) providing at least one transgene as part of a vector, such as a plasmid or viral vector, comprising the at least one transgene, wherein the vector integrates the transgene into a least one locus of the cell comprising an insertion site of an endogenous retrovirus (ERV) sequence or a LTR-retrotransposon (LTR-RT) sequence, or
(b) providing at least one transgene, optionally as part of a vector, and at least one nuclease and/or nickase, wherein the nuclease and/or nickase is preferably encoded by at least one vector, wherein the nuclease and/or nickase introduces, for integration of said transgene therein, double and/or single strand breaks into a least one locus of the cell comprising an insertion site of an endogenous retrovirus (ERV) sequence or a LTR-retrotransposon (LTR-RT) sequence and optionally, providing at least one vector encoding at least one targeting element guiding said least one nuclease and/or nickase,

optionally, upmodulating, in particular stimulating at least one first DNA Repair Pathway (DRP) of the cell and optionally downmodulating, in particular stimulating at least one second DRP of the cell, or vice versa,
transfecting the cell with the at least one transgene; and
optionally isolating an engineered cell that comprises the at least transgene integrated into the locus.

17. The method of claim 16, wherein the cell is also:
- transfected, preferably as part of one or more further vector(s), with one or more genes of Table 2 or SEQ ID Nos: 25-28, 38-58 and/or 59 or sequences having at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with SEQ ID Nos: 25-28, 38-58 and/or 59 and/or
- brought in contact with a chemical affecting the DNA Repair Pathway (DRP) of the cell.

18. The method of claim 16, wherein the cell is transfected with one or more further vector(s) comprising and expressing SEQ ID Nos: 25-28, 38-58 and/or 59, preferably SEQ ID Nos: 25-28.

19. The method of claim 16 and any one of the subsequent claims, wherein the at least one nuclease and/or nickase is:
a transposase, an integrase, a recombinase such as a site-specific recombinase, a nickase, or a nuclease such as a site-specific nuclease, a fusion protein comprising a programmable DNA-binding domain and a nuclease domain or any combinations thereof, or
a homing endonuclease, a restriction enzyme, a zinc-finger nuclease or a zinc-finger nickase, a meganuclease or a meganickase, a transcription activator-like effector nuclease or a transcription activator-like effector nickase, an RNA-guided nuclease or an RNA-guided nickase, a DNA-guided nuclease or a DNA-guided nickase, a megaTAL nuclease, a BurrH-nuclease, an ARCUS nuclease, a modified or chimeric version or variant thereof, and combinations thereof, in particular a zinc-finger nuclease or a zinc-finger nickase, a transcription activator-like effector nuclease or a transcription activator-like effector nickase, a RNA-guided nuclease or an RNA-guided nickase, wherein the RNA-guided nuclease or an RNA-guided nickase is optionally part of a CRISPR-based system, restriction enzyme and combinations thereof.

20. The method of claim 19, wherein the recombinase is a Cre recombinase, FLP recombinase, lambda integrase, PhiC31 integrase, Dre recombinase, xb1 integrase, gamma delta resolvase, R4 integrase, Tn3 resolvase, or TP901 -1 recombinase.

21. The method of claim 19, wherein said nuclease is a transcription activator-like effector nuclease or a RNA-guided nuclease.

22. The method of claim 16 and any one of the subsequent claims, wherein the viral vector is an AAV vector.

23. The method of claim 16 and any one of the subsequent claims, wherein the first and/or second DRP is selected from the group consisting of resection, canonical homology directed repair (canonical HDR), homologous recombination (HR), alternative homology directed repair (Alt-HDR), double-strand break repair (DSBR), single-strand annealing (SSA), synthesis-dependent strand annealing (SDSA), break-induced replication (BIR), alternative end-joining (Alt-EJ), microhomology mediated end-joining (MMEJ), DNA synthesis-dependent microhomology-mediated end-joining (SD-MMEJ), canonical non-homologous end-joining repair (C-NHEJ), alternative non-homologous end joining (A-NHEJ), translesion DNA synthesis repair (TLS), base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), DNA damage responsive (DDR), blunt end joining, single strand break repair (SSBR), interstrand crosslink repair (ICL), Fanconi Anemia (FA) Pathway and combinations thereof.

24. The method of claim 16 and any one of the subsequent claims, wherein:
the at least one first DRP is homologous recombination (HR) and the at least one second DRP is one or more non-homologous end joining (NHEJ) DNA Repair pathway(s);
the at least one first DRP is an Alt-EJ pathway such as MMEJ, and the at least one second DRP is one or more non-homologous end joining (NHEJ) DNA Repair pathway(s);
the at least one first DRP is an Alt-EJ pathway such as MMEJ, and the at least one second DRP is the homologous recombination (HR) DNA Repair pathway(s); or
the at least one first DRP is an Alt-EJ pathway such as MMEJ, and the at least one second DRP is one or more alternative DNA repair pathway.

25. The method of claim 16 and any one of the subsequent claims, wherein the upmodulating comprises:
a) expressing, including causing overexpression of at least one component of the DRP in said cell,
b) introducing into said cell, at least one component of the said DRP, and/or

c) contacting said cell, with at least one stimulator such as a chemical stimulator of a component of the DRP, such as HR stimulators such as RS-1 and/or NHEJ stimulators, such as IP6.

26. The method of claim 16 and any one of the subsequent claims, wherein the downmodulating comprises:

a) contacting said cell, with at least one inhibitor such as a chemical inhibitor, such as NHEJ inhibitor selected from the group of NU7441, Olaparib, DNA Ligase IV inhibitor, Scr7 KU-0060648 anti-EGFR-antibody C225 (Cetuximab), Compound 401 (2-(4-Morpholinyl)-4H-pyrimido[2,l-a]isoquinolin-4-one), Vanillin, Wortmannin, DMNB, IC87361, LY294002, OK-1035, CO 15, NK314, PI 103 hydrochloride and combinations thereof, MMEJ inhibitors selected from the group of Mirin, derivatives of Mirin, inhibitors of PolQ, inhibitors of CtIP and combinations thereof, HR inhibitors such as RI-1 and/or BO2, of a component of the DRP,

b) inactivating or downregulating at least one component of the DRP, by contacting or expressing in said cell, at least one inhibitory nucleic acid such as a miRNA, a siRNA, a shRNA, and/or

c) expressing in said cell a protein that inhibits the said DRP, or any combination thereof.

27. A engineered cell produced by the method of claim 16 and any one of the subsequent claims.

28. A kit for introducing at least one transgene into a cell comprising:
in one container a vector encoding a nuclease and/or nickase targeting at least one locus comprising an insertion site of an endogenous retrovirus (ERV) sequence or a LTR-retrotransposon (LTR-RT) sequence, such as SEQ ID NOs: 1 and 2, preferably a locus comprising (i) nucleotides 29021 to 40247 of SEQ ID NO: 1 or a sequence having 95%, 98% or 99% sequence identity with nucleotides 29021 to 40247 of SEQ ID NO: 1 and (ii) at least nucleotides 29020 to 31020 of SEQ ID NO: 2 or a sequence having 95% 98%, or 99% sequence identity with nucleotides 29020 to 31020 of SEQ ID NO: 2, or a locus comprising nucleotides 29521 to 39747 of SEQ ID NO: 1 or a sequence having 95%, 98% or 99% sequence identity with nucleotides 29521 to 4247 of SEQ ID NO: 1 and (ii) comprising nucleotides 29520 to 30520 of SEQ ID NO: 2 or a sequence having 95% 98%, or 99% sequence identity with nucleotides 29520 to 30520 of SEQ ID NO: 2, including an ERV sequence or a LTR-RT sequence integrated into the insertion site, such as SEQ ID NO: 3 and, optionally at least one vector encoding at least one targeting element guiding said least one nuclease and/or nickase,

optionally, in a separate container at least one vector encoding at least one targeting element guiding said least one nuclease and/or nickase,

in a separate container: at least one stimulator and/or inhibitor a DNA Repair Pathway (DRP), and/or

one or more vectors one or more genes encoding one or more of the DRP proteins of Table 2 or SEQ ID Nos: 25-28, 38-58 and/or 59 or sequences having at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with SEQ ID Nos: 25-28, 38-58 and/or 59;

and instructions how to transfect the cell with the at least one transgene using the at least one nuclease and/or nickase and the at least one stimulator and/or inhibitor.