Dever *et al* (2016, Nature 539(7629): 384-389) discuss homologous recombination at the HBB gene in human stem cells (HSC) by using the combination of Cas9 ribonucleoproteins and recombinant AAV6 homologous recombination donor delivery. Dever *et al* (2016) disclose AAV vector plasmids comprising tNGFR. Dever *et al* (2016) discuss using tNGFR as a marker to enrich HBB targeted HSPCs and anti-NGFR magnetic-bead separation.

Bak *et al* (2018, Nat Protoc 13(2):358-376) discuss the use of CRISPR/Cas9 technology and recombinant AAV6 homologous donor delivery for editing human hematopoietic stem cells by homologous recombination. Bak *et al* (2018) disclose reporter genes such as GFP or truncated growth factor receptor (tNGFR). Bak *et al* (2018) discuss flow cytometry to enrich for cells with targeted integration.

US2015/0191744 discusses methods for modifying the transcriptional regulation of stem or progenitor cells using a lentiviral vector encoding a nuclease deficient Cas9 effector domain fusion protein and a lentiviral vector comprising at least one sgRNA gene complementary to a genomic target. US2015/0191744 discloses a Cas9-fluorescent protein fusion protein.

The present inventors have developed a platform to achieve gene editing and enrichment of edited cells before *in vivo* administration, thus increasing the efficacy of gene therapy, and potentially expanding its application to a wide spectrum of genetic diseases, including those in which no proliferative growth advantage is conferred to corrected cells.

SUMMARY

The present inventors have developed methods for selecting edited cells that couple particular donor DNA vector designs (the first component) with the use of engineered transcriptional transactivators (ETTs) (the second component) combined with a nuclease system (the third component) to drive the selection of edited cells. The present inventors have exploited transient expression of neutral genes as selectable markers, such as the cell-surface-expressed mutated Low-affinity Nerve Growth Factor Receptor (ΔLNGFR), upon on-site recombination, and specific selection of edited cells.

The present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell (i.e. the starting cell) or a population of cells (i.e. a starting population of cells) at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI);

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells; and

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector (and, optionally, a minimal promoter) and the NOI into a target locus and wherein the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector.

Transient expression or transient upregulation of a nucleotide sequence encoding a selector enables the specific selection of cells in which the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) has been inserted into the target locus from those cells in which the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) have not been inserted into the target locus.

In one aspect, the present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and, optionally, a minimal promoter operably linked to a regulatory element;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells; and

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector and the NOI and, optionally, the minimal promoter operably linked to the regulatory element into a target locus and wherein the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector optionally when a modulator is present in the cell or population of cells or optionally when a modulator is not present in the cell or population of cells.

The present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and a minimal promoter operably linked to a regulatory element;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells, and

the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector, the NOI and the minimal promoter operably linked to the regulatory element into a target locus and wherein the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector when a modulator is present in the cell or population of cells.

The present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and a minimal promoter operably linked to a regulatory element;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells, and

the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector, the NOI and the minimal promoter operably linked to the regulatory element into a target locus and wherein the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector when a modulator is not present in the cell or population of cells.

The present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and a minimal promoter operably linked to a regulatory element;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector, the NOI and the minimal promoter operably linked to the regulatory element into a target locus and wherein the transient presence of a modulator in the cell or population of cells enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector when a modulator is present in the cell or population of cells.

The present disclosure provides a method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and a minimal promoter operably linked to a regulatory element;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector, the NOI and the minimal promoter operably linked to the regulatory element into a target locus and wherein the transient presence of a modulator in the cell or population of cells enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector when the modulator is not present in the cell or population of cells.

Without wishing to be bound by theory, the transient presence of the modulator in the cell or population of cells may occur by the addition of the modulator to the media followed by subsequent washing of the cell or population of cells.

In one example, (herein referred to as Selection by Means of Artificial Transactivators [SMArT]), the first component comprises a donor reporter cassette, in which the expression of a selector (such as the mutated Low-affinity Nerve Growth Factor Receptor [ΔLNGFR]) is driven by a minimal promoter (such as cytomegalovirus [CMV] or synthetic promoter [T6-SK] (Loew, Heinz, Hampf, Bujard, & Gossen, 2010)), that should putatively limit basal expression of the selector. The first component comprises homologous arms (HAs) comprising nucleotide sequences homologous to the target locus, these HAs enable the donor reporter cassette to be inserted into the target locus by homology-driven repair (HDR). The donor reporter cassette further comprises a NOI operably linked to a promoter. ETT binding sites are outside the homology arms and upstream of the minimal promoter. Insulator elements might be also present in the cassette in order to limit the possible enhancer activity of the fully efficient promoter on the minimal promoter. In this case, insulator elements might be derived from CTCF-dependent or independent binding sites (Phillips-Cremins & Corces, 2013) (**Figure 1A**). This selection strategy is useful for targeted integration of a transgene expression cassette into a neutral region of the genome (such as the Adeno-Associated Virus Integration Site 1 [AAVS1]), where sustained therapeutic transgene expression can be achieved without perturbing the neighbor gene regulation and the epigenetic landscape (Lombardo et al., 2011). Other possible neutral regions of the genome can be common integration sites (CIS) of lentiviral vectors (such as those identified by Biffi et al., 2013) or other regions possibly defined in the future as “safe harbors” or neutral areas of the genome.

In one example, (herein referred to as Selection by Means of Artificial Transactivators [SMArT] gene correction), the first component comprises a donor reporter cassette, in which

the expression of a selector (such as the mutated Low-affinity Nerve Growth Factor Receptor [ΔLNGFR]) is driven by a minimal promoter (such as cytomegalovirus [CMV] or synthetic promoter [T6-SK] (Loew, Heinz, Hampf, Bujard, & Gossen, 2010)), that should putatively limit basal expression of the selector. The first component comprises homologous arms (HAs) comprising nucleotide sequences homologous to the target locus, these HAs enable the donor reporter cassette to be inserted into the target locus by homology-driven repair (HDR). The donor reporter cassette further comprises a NOI. (Optionally, the cassette further comprises a splicing acceptor site and/or a nucleotide sequence encoding a self-cleaving 2A peptide or, alternatively, an internal ribosome entry site (IRES) element.) The insertion of the donor reporter cassette into the target locus corrects a genetic defect and/or introduces a new function into the endogenous gene. ETT binding sites are outside the homology arms and downstream of the minimal promoter. Insulator elements might be also present in the construct in order to limit the possible enhancer activity of the fully efficient promoter on the minimal promoter. In this case, insulator elements might be derived from CTCF-dependent or independent binding sites (Phillips-Cremins & Corces, 2013) (**Figure 1A**). This selection strategy is useful for targeted integration of a transgene expression cassette into a neutral region of the genome (such as the Adeno-Associated Virus Integration Site 1 [AAVS1]), where sustained therapeutic transgene expression can be achieved without perturbing the neighbor gene regulation and the epigenetic landscape (Lombardo et al., 2011). Other possible neutral regions of the genome can be common integration sites (CIS) of lentiviral vectors (such as those identified by Biffi et al., 2013) or other regions possibly defined in the future as “safe harbors” or neutral areas of the genome. In gene correction a full or partial wild-type sequence is used to correct a genetic defect or introduce a new function into an endogenous gene (Schiroli et al., 2017).

In another example, (herein referred to as Selection by Means of Artificial Transactivation of Endogenous Receptors [SMArTER]) the donor reporter cassette comprises: (i) a nucleotide sequence of interest; ii) optionally a splicing acceptor site and/or a nucleotide sequence encoding a self-cleaving 2A peptide or, alternatively, an internal ribosome entry site (IRES) element; iii) the cDNA encoding for the selector protein (such as ΔLNGFR), optionally the nucleotide sequence encoding the selector is operably linked to a minimal promoter; and iv) homology arms (HAs) for the intended target site(s). ETT binding sites are situated close to the promoter of the endogenous gene in order to transiently boost (i.e. transiently upregulate) the expression of the selector (and the edited gene) upon targeted integration (**Figure 5A**). This strategy can be applied to select cells that have undergone correction of specific genes. In this case, basal expression of the selector protein is dependent from the transcriptional regulation of the edited gene. To reduce the risk of

constitutive expression of the selector, the selector can be fused (in frame) with destabilizer domains (DDs), which are able to induce proteasomal degradation of the selector in absence of specific stabilizer ligands (Rakhit, Navarro, & Wandless, 2014). In particular, destabilizer domains can be based on the FKBP domain (Banaszynski et al., 2006), bacterial DHFR protein (Iwamoto, Björklund, Lundberg, Kirik, & Wandless, 2010) or from the Human Estrogen Receptor (Miyazaki et al., 2012; PMID: 22332638; Journal of the American Chemical Society 134(9):3942-3945). This strategy allows the use of biological selectors that specifically boost growth or engraftment of HDR-edited cells. Constitutive and prolonged expression of proteins enhancing homing and engraftment capacity of corrected cells might lead to undesired and exacerbate expansion of edited clones. Coupling ETT-mediated transactivation and DD-based post-translational regulation would allow transient and temporary overexpression of the biological selector (e.g. CXCR4, CD47).

In another example, (herein referred to as Selection by Means of Artificial Transactivation with Doxycycline regulation [SMArT-D]) the first component comprises a donor reporter cassette, in which the expression of a selector (such as GFP) is driven by a minimal promoter (such as synthetic promoter [T6-SK] (Loew, Heinz, Hampf, Bujard, & Gossen, 2010)), that should putatively limit basal expression of the selector, wherein the minimal promoter is operably linked to a regulatory element (such as a tetracycline operator (TetO) sequence). The regulatory element is typically upstream of the minimal promoter. ETT binding sites are within the regulatory element. Without wishing to be bound by theory, in the Tet-Off system, the ETT binds to the regulatory element and activates the minimal promoter when the selector is inserted into the target locus; the binding of a modulator (such as tetracycline or deoxycycline) prevents the binding of the ETT to the regulatory element when a donor reporter cassette has not been inserted into the target locus. Without wishing to be bound by theory, in the Tet-On system, the ETT binds to the regulatory element and activates the minimal promoter when the selector is inserted into the target locus and when a modulator (such as tetracycline or deoxycycline) is present; the binding of a modulator (such as tetracycline or deoxycycline) enables the binding of the ETT to the regulatory element when a donor reporter cassette has been inserted into the target locus. The first component further comprises homologous arms (HAs) comprising nucleotide sequences homologous to the target locus, these HAs enable the donor reporter cassette to be inserted into the target locus by homology-driven repair (HDR). Insulator elements might be also present in the cassette in order to limit the possible enhancer activity of the fully efficient promoter on the minimal promoter. In this case, insulator elements might be derived from CTCF-dependent or independent binding sites (Phillips-Cremins & Corces, 2013) (**Figure 1A**). This selection strategy is useful for targeted integration of a transgene expression cassette into a neutral

region of the genome (such as the Adeno-Associated Virus Integration Site 1 [AAVS1]), where sustained therapeutic transgene expression can be achieved without perturbing the neighbor gene regulation and the epigenetic landscape (Lombardo et al., 2011). Other possible neutral regions of the genome can be common integration sites (CIS) of lentiviral vectors (such as those identified by Biffi et al., 2013) or other regions possibly defined in the future as “safe harbors” or neutral areas of the genome.

In one example, the present disclosure provides a population of genome edited cells produced by the method according to the present disclosure.

The population of genome edited cells produced by the method according to the present disclosure comprise the nucleotide sequence encoding the selector. The nucleotide sequence encoding the selector is transiently expressed such that the nucleotide sequence encoding the selector is no longer expressed after the cells have been selected.

The population of genome edited cells produced by the method according to the present disclosure is enriched for cells which are capable of expressing the nucleotide of interest (NOI).

In a further aspect, the present disclosure provides a pharmaceutical composition comprising the population of genome edited cells according to the present disclosure.

In another aspect, the present disclosure provides a kit comprising a first component, a second component and a third component as disclosed herein and, optionally, a cell population.

In a broad example, the present disclosure provides a population of genome edited cells according to the present disclosure for use in therapy.

In another aspect, the present disclosure provides a population of genome edited cells as disclosed herein for use in gene therapy. In another example, the present disclosure provides a population of genome edited cells according to the present disclosure for use in gene therapy, hematopoietic stem cell transplantation, cancer treatment and/or tissue repair.

In another aspect, the present disclosure provides a use of a population of genome edited cells as disclosed herein for the manufacture of a medicament for the treatment or prevention of X-linked Severe Combined Immunodeficiency (SCID –X1). In another aspect, the present disclosure provides a use of a population of genome edited cells as disclosed herein for the manufacture of a medicament for hematopoietic stem cell transplantation (HSCT). In another aspect, the present disclosure provides a use of a population of genome edited cells as disclosed herein for the manufacture of a medicament for tissue repair.

The present disclosure provides, in a further example, a population of genome edited cells according to the present disclosure for use in the treatment or prevention of X-linked Severe Combined Immunodeficiency (SCID –X1), a skin disease and/or a retinal disease.

In a further example, the present disclosure provides a population of genome edited cells according to the present disclosure for use in the treatment or prevention of a monogeneic disorder such as epidermolysis bullosa and/or retinitis pigmentosa and/or Hyper-IgM (HIGM) syndrome.

In a further example, the present disclosure provides a population of genome edited cells according to the present disclosure for use in the treatment or prevention of Hyper-IgM (HIGM) syndrome.

In a broad example, the present disclosure provides use of a population of genome edited cells according to the present disclosure for therapy.

The present disclosure provides in another example, use of a population of genome edited cells according to the present disclosure for gene therapy, hematopoietic stem cell transplantation, cancer treatment and/or tissue repair.

In a further example, the present disclosure provides use of a population of genome edited cells according to the present disclosure for the manufacture of a medicament for the treatment or prevention of X-linked SCID, a skin disease and/or a retinal disease.

In a further example, the present disclosure provides use of a population of genome edited cells according to the present disclosure for the manufacture of a medicament for the treatment or prevention of a monogeneic disorder such as epidermolysis bullosa and/or retinitis pigmentosa and/or Hyper-IgM (HIGM) syndrome.

In a further example, the present disclosure provides use of a population of genome edited cells according to the present disclosure for the manufacture of a medicament for the treatment or prevention of Hyper-IgM (HIGM) syndrome.

In a broad example, the present disclosure provides a method of therapy comprising the step of administering a population of genome edited cells according to the present disclosure to a subject.

In another example, the present disclosure provides a method of gene therapy, hematopoietic stem cell transplantation, cancer treatment and/or tissue repair comprising the step of administering a population of genome edited cells according to the present disclosure to a subject.

The present disclosure provides, in another example, a method for the treatment or prevention of X-linked SCID, a skin disease and/or a retinal disease comprising the step of

administering a population of genome edited cells according to the present disclosure to a subject wherein the subject has X-linked SCID, a skin disease and/or a retinal disease.

The present disclosure provides, in another example, a method for the treatment or prevention of a monogeneic disorder such as epidermolysis bullosa and/or retinitis pigmentosa and/or Hyper-IgM (HIGM) syndrome comprising the step of administering a population of genome edited cells according to the present disclosure to a subject wherein the subject has a mongeneic disorder.

The present disclosure provides, in another example, a method for the treatment or prevention of Hyper-IgM (HIGM) syndrome comprising the step of administering a population of genome edited cells according to the present disclosure to a subject wherein the subject has Hyper-IgM (HIGM) syndrome.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Transient transactivation of a reporter gene stably integrated in the *AAVS1* locus by means of engineered transcriptional transactivators. A) Fig. 1Ai: description of SMArT strategy to enrich for *AAVS1*-edited cells; Fig. 1Aii: description of SMArTER strategy for *IL2RG*-edited cells; Fig. 1Aiii: description of SMArT strategy for gene correction. **B)** Isolation and characterization of a single cell-derived clone harboring minimally expressing ΔLNGFR cassette in *AAVS1* locus. (Top left) Experimental workflow for clone isolation and characterization. (Bottom left) Molecular analyses to screen for clones with intact 5' donor-genome junction (5'-TI). Clones positive for 5'-TI were successively screened for intact 3' donor-genome junction and mono-allelic integration (amplification of the wild-type allele) (middle) and basal expression of ΔLNGFR. Clone H1 was selected for further experiments. (Right) Flow cytometry of the selected clone (clone H1). **C)** Percentage of NHEJ-mediated editing with gRNAs spanning from 100 to 600 bp from the minimal promoter transcriptional start site (TSS). **D)** (Top) Schematic of the panel of sgRNAs (single guide RNAs) tested for reporter gene transactivation in K562 clone H. (Bottom) Representative FACS plot of transactivated clone H cells 24 hours upon electroporation. **E)** Percentage (top) and relative fluorescence intensity (RFI) on untreated clone H cells (UT) (bottom) of ΔLNGFR+ cells at 1, 2 and 14 days after electroporation of plasmid expressing either dCas9-VP160 or dCas9-VPR and selected gRNAs from **A**. sgRNAs # 4, 5, 9, 10, 12, 13, 26, 28 from **C** has been selected from further transactivation experiments. sgRNAs are ordered from the closest to the farthest from the TSS. The last treatment of each panel of sgRNA is the combination of the first and fifth sgRNAs, respectively. **F)** (Top) Schematic of the panel of TALE-TA tested for reporter gene transactivation in K562 clone H. Percentage (left) and mean fluorescence

CLAIMS

1. A method for selecting genome edited cells and/or for the enrichment of genome edited cells in a population of cells comprising:

(a) introducing into a cell or a population of cells at least one first component, at least one second component and at least one third component; and

(b) selecting the genome edited cells which transiently express or transiently upregulate a nucleotide sequence encoding a selector;

wherein the first component is a donor reporter cassette comprising the nucleotide sequence encoding the selector and a nucleotide sequence of interest (NOI) and, optionally, a minimal promoter operably linked to a regulatory element;

wherein the second component is an engineered transcriptional transactivator (ETT) polypeptide or a nucleotide sequence encoding an ETT polypeptide; wherein the ETT polypeptide comprises a DNA binding domain (DBD) and at least one transcription activator (TA) domain;

wherein the third component is a nuclease system comprising a genome targeted nuclease and, optionally, a guide RNA (gRNA) comprising at least one targeted genomic sequence;

wherein the ETT polypeptide is transiently present in the cell or population of cells or the nucleotide sequence encoding the ETT polypeptide is transiently expressed in the cell or population of cells; and

wherein the presence of the nuclease system in the cell or the population of cells enables the insertion of the nucleotide sequence encoding the selector and the NOI and, optionally, the minimal promoter operably linked to the regulatory element into a target locus and wherein the transient presence of the ETT polypeptide or the transient expression of the nucleotide sequence encoding the ETT polypeptide enables transient expression or transient upregulation of the inserted nucleotide sequence encoding the selector optionally when a modulator is present in the cell or population of cells or optionally when a modulator is not present in the cell or population of cells.

2. The method according to claim 1 wherein the donor reporter cassette sequentially comprises:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;

(ii) the nucleotide sequence encoding the selector operably linked to a minimal promoter;

(iii) the NOI operably linked to a promoter; and

(iv) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus.

3. The method according to claim 1 wherein the donor reporter cassette sequentially comprises:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;

(ii) optionally, a splicing acceptor site (SA);

(iii) the NOI;

(iv) the nucleotide sequence encoding the selector operably linked to a minimal promoter; and

(v) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus.

4. The method according to claim 1 wherein the donor reporter cassette sequentially comprises:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;

(ii) optionally, a splicing acceptor site (SA);

(iii) the NOI;

(iv) optionally, a nucleotide sequence encoding a 2A self-cleaving peptide (2A) or an internal ribosome entry site (IRES) element;

(v) the nucleotide sequence encoding the selector, optionally the nucleotide sequence encoding the selector is operably linked to a minimal promoter; and

(vi) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component activates an endogenous promoter in the target locus.

5. The method according to claim 1 wherein the donor reporter cassette sequentially comprises:

(i) a left homology arm (HA) comprising a nucleotide sequence homologous to a target locus;

(ii) the NOI, optionally operably linked to a promoter;

(iii) the nucleotide sequence encoding the selector, wherein the nucleotide sequence encoding the selector is operably linked to a minimal promoter and the minimal promoter is operably linked to a regulatory element; and

(iv) a right homology arm (HA) comprising a nucleotide sequence homologous to the target locus;

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component binds to the regulatory element and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus and when a modulator is present in the cell or population of cells; or

wherein the ETT polypeptide of the second component or the ETT polypeptide expressed by the second component binds to the regulatory element and activates the minimal promoter when the nucleotide sequence encoding the selector is inserted into the target locus and when a modulator is not present in the cell or population of cells.

6. The method according to any one of claims 1 to 4 wherein the DBD is a Transcriptional Activator-Like Effector (TALE) DBD, a Zinc finger, catalytically inactive Cpf1 or catalytically inactive Cas (dCas) and wherein the TA domain is selected from the group consisting of VP16, VP64, VP128, VP160, VPR, p65, Rta, HSF1, SAM, and SunTag.

7. The method according to claim 5 wherein the DBD is a TetR or reverseTetR (rTetR) and wherein the TA domain is selected from the group consisting of VP16, VP64, VP128, VP160, VPR, p65, Rta, HSF1, SAM, and SunTag.

8. The method according to any one of the preceding claims wherein the gRNA is capable of binding to one or more of the nucleotide sequences selected from the group consisting of SEQ ID NOs 1 to 31 and sequences having at least 75% identity thereto.

9. The method according to any one of the preceding claims wherein the target locus is a safe harbour.

10. The method according to claim 9 wherein the target locus is adeno-associated virus integration site 1 (AAVS1), a common integration site (CIS) of lentiviral vectors, IL2RG, gp91phox, HBB, RAG1, CD40LG, TRAC, TRBC, STAT, PRF1, a gene encoding for a protein expressed in the skin (such as collagen, keratin, laminin, desmocolin,

desmoplachine, desmoglein, placoglobin, placophylline, integrin or other proteins that are involved in desmosomes and hemidesmosomes) or another safe harbour genomic locus.

11. A kit comprising a first component, a second component and a third component as defined in any one of the preceding claims and, optionally, a cell population.

12. A population of genome edited cells produced by the method according to any one of the preceding claims.

13. A pharmaceutical composition comprising the population of genome edited cells according to claim 12.

14. A population of genome edited cells according to claim 12 for use in gene therapy.

15. Use of a population of genome edited cells according to claim 12 for the manufacture of a medicament for the treatment or prevention of X-linked Severe Combined Immunodeficiency (SCID –X1).

16. Use of a population of genome edited cells according to claim 12 for the manufacture of a medicament for hematopoietic stem cell transplantation (HSCT).

17. Use of a population of genome edited cells according to claim 12 for the manufacture of a medicament for tissue repair.