

Transposons, Vectors and Genetically Engineered Cells

Technical Field

The invention relates to transposons comprising a Ubiquitous Chromatin Opening Element (UCOE) nucleic acid sequence and a nucleic acid sequence encoding a gene of interest under control of a promoter, flanked by Piggybac™ transposon-specific inverted terminal repeat sequences PB 5' ITR and PB 3' ITR, vectors comprising such transposons, cells comprising such transposons or vectors and cells in which such transposons have been integrated, to methods for genetic manipulation and differentiation of iPSC cells comprising such transposons or vectors to partially or fully mature cells such as macrophage and to uses of such cells.

Background to the Invention

US20200157567A1 describes a viral vector comprising a transposon and a nucleic acid encoding a transposase. The transposon comprises a transgene, or insertion site for a transgene, for integration into the genome of a target cell. The expression of the transposase is controlled such that the transposase is not expressed during production or packaging of the viral vector. Furthermore, the transposon comprises a packaging signal for the virus genome, thus preventing the packaging of any viral genome from which the transposon has been removed. Also disclosed are processes for producing a modified mammalian cell and for producing a mammalian cell with a modified genome, using a viral vector of the invention.

US 20220033845 A1 describes expression vectors for expressing recombinant proteins (e.g., biologics) in mammalian cells, host cells comprising the expression vectors, methods of producing the recombinant proteins, and methods of propagating the expression vectors.

AU2019250224B2 describes constructs and methods for expressing DNAs of interest in particular in non-primate eukaryotic host cells that display advantages with regard quantity and quality of expression including high stability of expression and transport of the expression product out of the cell.

WO2022104155A1 describes cells, tissues, organs, and/or animals having one or more modified genes for enhanced xenograft survival and/or tolerance. Also disclosed are methods of making and using the cells, tissues, organs, and/or animals having one or more of the modified genes.

EP2417263B1 describes compositions for the *in vitro* or *in vivo* production of specific proteins that comprise components of vectors, such as a vector backbone, a promoter, and a gene of interest that encodes for the protein of interest, and the transposon-based vectors comprising these components. Also described are methods of making these compositions and methods of using these compositions for the production of desired proteins *in vivo* or in transfected cells *in vitro*.

KR20210143897A describes matrix attachment regions (MARs), recombinant vector comprising the MAR, transformants transformed with the recombinant vector, and methods of culturing the transformant to produce a target protein.

WO2020164702 A1 describes a polypeptide comprising a transposase and at least one heterologous chromatin reader element (CRE), polynucleotide encoding the polypeptide and vector comprising the polynucleotide. An artificial transposable element comprising the polynucleotide can be targeted to active chromatin via a transposase coupled with a heterologous chromatin reader element.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5473066/pdf/tmh-0044-0135.pdf> Ackermann, M. *et al.* (2017) used genetically modified iPSC lines to address transgene stability during hematopoietic differentiation towards macrophages, using lentiviral vectors equipped with a CBX3-UCOE or ZFN-mediated targeting of an expression cassette into the human AAVS1 safe harbor to provide a tool for stable transgene expression in iPSCs and differentiated macrophages. Using different iPSC lines which expressed reporter genes either from randomly integrated lentiviral vector expression systems or the safe harbor AAVS1 allowed for the expression of reporter genes in pluripotent cells as well as in macrophages derived therefrom, indicating their suitability to efficiently express therapeutic transgenes in iPSCs.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4330381/> Müller-Kuller, U. *et al.* (2015) introduced a functional, 0.7 kb minimal CBX3-UCOE and constitutive or tissue-specific promoters to counteract epigenetic silencing of transgene expression in multipotent and pluripotent stem cells. In addition to preventing promoter silencing, the chromatin opening function of the CBX3-UCOE element was able to spread to adjacent heterologous DNA regions. This feature of the CBX3-UCOE was potentially of concern, as integrated CBX3-UCOE containing vectors could alter the chromatin status and therefore the expression pattern of cellular genes located nearby the integration site. The chromatin opening function of the endogenous *CBX3* gene appeared to be centered at the promoter region and spanned a DNA region of approximately 1.5 kb, as defined by the transition from unmethylated to methylated

DNA and the presence of active histone marks. Within the lentiviral vectors used, the distance of the CBX3-UCOE to the left border of the 5'-LTR was 1.7 kb, so in theory the minimal distance of cellular sequences to the CBX3-UCOE was beyond the distance covered by the chromatin opening effect of the element.

- 5 <https://pubmed.ncbi.nlm.nih.gov/31706316/> Skipper KA *et al.* (2019) used a core-fragment of the HNRPA2B1-CBX3 UCOE in a sleeping beauty transposon vector to modify CHO Cells and compared four different protective strategies in CHO-K1 cells, finding robust protection from silencing of transgene cassettes mediated by the ubiquitous chromatin-opening element (UCOE) derived from the HNRPA2B1-CBX3 locus. Using a bioinformatic approach, a shorter
- 10 HNRPA2B1-CBX3 UCOE core fragment was defined and demonstrated to maintain transgene expression after extended passaging of CHO-K1 cells carrying DNA transposon vectors equipped with the shorter HNRPA2B1-CBX3 UCOE core fragment.

Studies involving human macrophages have been hampered by technical difficulties related to their isolation/derivation in sufficient quantities, the inability of expanding their numbers *in*

15 *vitro*, and the difficulty of genetically modifying the cells (due to their expression of viral and transgene restriction factors). Human induced pluripotent stem cells (iPSCs) can be easily genetically modified *via* viral and non-viral vectors, and then used to produce macrophages in large quantities. However, upon differentiation from iPSC to macrophages, transgene expression from currently-used vectors often undergoes strong or complete transcriptional

20 silencing/repression¹. Thus, novel vectors that permit the persistent expression of the transgenes from the iPSC through the mature macrophage stages would be an invaluable tool in the field.

Statement of Invention

25 The invention provides:

1. A transposon comprising a Ubiquitous Chromatin Opening Element (UCOE) nucleic acid sequence, a nucleic acid sequence of a gene encoding a protein of interest or a fragment thereof and optionally a 3' polyadenylation sequence under control of a promoter, flanked by
- 30 Piggybac™ transposon-specific inverted terminal repeat sequences PB 5' ITR and PB 3' ITR.

In its simplest form, the transposon will have a structure as follows, with the promoter, coding sequence and polyadenylation sequence (if present) being operably linked for expression of the gene / protein of interest or fragment thereof.

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PB 5' ITR-UCOE-Promoter-gene sequence or fragment thereof-[optionally PolyA]-PB 3' ITR

2. A transposon of clause 1, wherein the UCOE is a CBX3 UCOE (UCOE_{CBX3}) nucleic acid sequence.

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3. A transposon of clause 1 or clause 2, wherein the UCOE is a nucleic acid sequence of SEQ ID NO: 1.

4. A transposon of any preceding clause, wherein the Piggybac™ transposon-specific inverted terminal repeat sequences are PB 5' ITR (SEQ ID NO: 2) and PB 3' ITR of (SEQ ID NO: 3).

5. A transposon of any preceding clause wherein the promoter, gene of interest and optionally a 3' polyadenylation sequence are comprised within an expression cassette.

6. A transposon of any preceding clause wherein the expression cassette comprises

- (a) a promoter,
- (b) at least one cloning site (restriction enzyme cleavage site) or a multiple cloning site downstream of the promoter,
- (c) a gene of interest or a fragment thereof downstream of and operably linked to the promoter,
- (d) a polyadenylation sequence positioned downstream of and operably linked to the gene of interest or fragment thereof,
- (e) optionally an internal ribosome entry site,
- (f) optionally a second promoter sequence positioned downstream of the gene of interest and polyadenylation sequence,
- (g) optionally a fluorescent protein sequence positioned downstream of an operably linked to the second promoter,
- (h) a self-cleaving peptide sequence,
- (i) optionally a gene for selection, e.g., for antibiotic selection, and / or
- (j) optionally a fluorescent protein gene,
- (k) optionally a second a polyadenylation sequence.

In embodiments of the invention, a transposon and / or expression cassette may comprise one or more optional components, e.g., selected from (e) to (g) and (i) to (k); in preferred embodiments of the invention, a transposon and / or expression cassette may comprise all of the optional components (e) to (g) and (i) to (k). Component sequences of a transposon and / or expression cassette of the invention will generally be present in the order listed.

7. A transposon of clause 6 wherein the expression cassette comprises
- (a) a CMV promoter,
 - (b) a multiple cloning site downstream of the promoter,
 - (c) a gene of interest downstream of and operably linked to the promoter, optionally wherein
- 5 the gene of interest is a human SIGLEC10 gene of SEQ ID NO: 4,
- (d) a bovine growth hormone polyadenylation signal (bGH poly(A) signal) (SEQ ID NO: 5) positioned downstream of and operably linked to the gene of interest,
 - (e) optionally an internal ribosome entry site,
 - (f) an EF1-alpha (short version) (SEQ ID NO: 6) sequence positioned downstream of the gene
- 10 of interest and polyadenylation sequence, and
- (g) a fluorescent protein (CopGFP (SEQ ID NO: 7)) sequence positioned downstream of the second promoter,
 - (h) a T2A self-cleaving peptide sequence (SEQ ID NO: 8) positioned downstream of the fluorescent protein sequence,
- 15 (i) a Puromycin resistance gene and
- (j) a SV40 Poly(A): Simian virus 40 polyadenylation sequence.
8. A transposon of clause 6 wherein the expression cassette comprises:
- (a) a CAG promoter,
- 20 (b) a multiple cloning site downstream of the promoter,
- (c) a gene of interest downstream of and operably linked to the promoter, optionally wherein the gene of interest is a dCas9-KRAB sequence of SEQ ID NO: 9 (a dead/deactivated Cas9 with the repression domains and a nuclear localization signal sequence),
 - (d) a T2A self-cleaving peptide sequence (SEQ ID NO: 8) positioned downstream of the gene
- 25 of interest, e.g., dCas9-KRAB sequence of SEQ ID NO: 9, and
- (e) a fluorescent protein gene sequence encoding eGFP (enhanced green fluorescent protein) positioned downstream of the T2A self-cleaving peptide sequence (SEQ ID NO: 8), and
 - (f) an rBG polyadenylation sequence.
- 30 9. A transposon of clause 6 wherein the expression cassette comprises:
- (a) a CAG promoter,
 - (b) a multiple cloning site downstream of the promoter,
 - (c) a gene of interest downstream of and operably linked to the promoter, optionally wherein the gene of interest is a dCas9-VPR sequence of SEQ ID NO: 10,
- 35 (d) a T2A self-cleaving peptide sequence positioned downstream of the gene of interest, e.g., dCas9-VPR sequence of SEQ ID NO: 10, and

(e) a fluorescent protein gene sequence encoding eGFP (enhanced green fluorescent protein) positioned downstream of the T2A self-cleaving peptide sequence (SEQ ID NO: 8), and
(f) an rBG polyadenylation sequence.

5 10. A vector comprising a transposon of any preceding clause, preferably wherein the vector is a PiggyBac™ vector, such as a plasmid vector of SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO:14.

11. A cell comprising transposon and / or vector of any one of clauses 1 to 10, optionally
10 comprising a second vector capable of expressing a transposase, preferably wherein the transposase is a PiggyBac™ transposase.

12. A cell comprising transposon of any one of clauses 1 to 11, wherein the transposon is integrated into the cell genome.

15 13. A cell according to clause 11 or clause 12 wherein the cell is selected from an undifferentiated cell, a partially differentiated cell and a fully differentiated cell, optionally wherein differentiation status is assessed by using a panel of flow cytometry markers.

20 14. A cell according to any one of clauses 11 to 13, wherein the cell is selected from an induced pluripotent stem (iPSC), a partially or fully differentiated iPSC-derived macrophage, iPS-derived neutrophil, NK, monocyte, myocyte, hepatocyte, neuron and a progenitor cell.

15. A method for:
25 (a) preventing silencing of a gene of interest (transgene)
(b) promoting expression of a gene of interest,
(c) expressing a product of a gene of interest, and / or
(d) expressing a larger (≥ 7.0 Kb) expression cassette,
30 comprising culturing a cell according to any one of clauses 11 to 14.

16. A method for:
(a) preventing silencing of a gene of interest (transgene) and / or
(b) maintaining expression of a gene of interest during differentiation,
35 comprising culturing a cell according to any one of clauses 11 to 14 in the presence of a differentiation stimulus.

17. A method for knockdown (downregulation) of expression of a target gene comprising culturing a cell according to any one of clauses 11 to 14 comprising dCas9-KRAB in the presence of the target gene-directed gRNA (e.g., delivered via lentiviral vector).
- 5 18. A method for inducing or upregulating expression of a target gene comprising culturing a cell according to any one of clauses 11 to 14 comprising dCas9-VPR in the presence of the target gene-directed gRNA (e.g., delivered via lentiviral vector).
19. A composition comprising a transposon, vector and / or cell of any one of clauses 1 to 14
10 and a carrier.
20. A composition comprising a transposon, vector and / or cell of any one of clauses 1 to 14 and a vector capable of expressing transposase.
- 15 21. A gene transfer system comprising a transposon, vector, and / or cell of any one of clauses 1 to 14 and a transposase.
22. A gene transfer system of clause 21, wherein the transposase is a Piggybac™ transposase.
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23. A method for modulating expression of a gene of interest in a cell, comprising
(a) introducing a vector of clause 10 to a cell,
(b) introducing a vector encoding a transposase, preferably a Piggybac™ transposase to the cell and
25 (c) introducing a vector, containing a guide RNA capable of controlling the expression of the target gene, to the cell.
24. A method for integrating a gene of interest into the genome of a cell comprising contacting a cell with the gene transfer system of clause 21, thereby integrating the gene of interest into
30 the cell.
25. A method for knockdown (downregulation) of a target gene in a cell comprising contacting a cell with the gene transfer system of clause 21 thereby to introduce and integrate dCas9-KRAB and introducing a gRNA (preferably delivered via lentiviral vector) to the cell to
35 knockdown expression of the target gene.

26. A method for upregulation of a target gene in a cell comprising contacting a cell with the gene transfer system of clause 21 thereby to introduce and integrate dCas9-VPR and introducing a gRNA (delivered via lentiviral vector) to the cell to upregulate expression of the target gene.

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Detailed Description of the Invention

Cells of the invention are useful in research, drug discovery and development, for example in methods of screening, such as CRISPR screening, to identify novel targets, or in screening for antibody or small molecule inhibitors of a target gene. Transposons, expression cassettes, vectors, cells, compositions, gene transfer systems and methods of the invention may be used in selection of lead candidate molecules, in target validation for large and small molecules, in biology, safety and toxicological studies.

Transposons, expression cassettes, vectors, cells, compositions, gene transfer systems and methods of the invention may be used in biomarker discovery.

Transposons, expression cassettes, vectors, cells, compositions, gene transfer systems and methods of the invention may be used in cell therapy, for example in CRISPR knock down of HLA of iPSC, to provide universal-HLA-silent knockdown cells.

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Transposons, expression cassettes, vectors, cells, compositions, gene transfer systems and methods of the invention may be used in autologous iPSC gene editing to engineer a subject's cells, prior to administration of engineered cells back to patient.

The invention provides a safe, robust, and rapid approach to produce genetically engineered iPSC-macrophages *via* a modified transposon-based vector that ensures sustained transgene expression.

Sustained transgene expression in iPSC-differentiated cells can be achieved through a targeted gene engineering approach using Zinc Finger Nucleases, TALENs or CRISPR/Cas9 that allows for transgene integration into safe harbor loci, such as the AAVS1 locus^{2,3}. Although this method is safe (it introduces a transgene into one site in the genome that is known), and robust; there are three main disadvantages: 1) It is very time consuming as the efficiency of introducing a transgene into one site means a lower efficiency of successful genetic engineering. 2) Introducing large transgenes/DNA fragments significantly reduces the efficiency of this approach. 3) There is a limit to how many transgenes can be inserted into the genome in one round of targeting (this approach does not allow for bigger, more

sophisticated multi-transgene approaches, for example, inserting a library of sgRNAs for a genome wide-CRISPR Based screen).

One way to enable sustained transgene expression in a random integration fashion is the use of lentiviral vectors equipped with elements that are able to actively remodel the local chromatin environment and activate transcription such as ubiquitous chromatin opening elements (UCOE)⁴. Viral vectors containing a 1.5 kb methylation-free CpG island from the human HNRPA2B1-CBX3 housekeeping genes (A2UCOE) or a minimal 0.7 kb sequence encompassing the CBX3 gene only, are able to prevent transgene silencing and variegation in cell lines, multipotent and pluripotent stem cells, and their differentiated progeny (macrophages included)⁵. This approach circumvents the disadvantage of not being able to insert several transgenes/DNA fragments/libraries in one round of targeting. It also decreases the time needed to generate genetically modified iPSC lines. However, the main disadvantages of this approach are twofold: lentiviral vectors have a genetic cargo limit, preventing the introduction of large transgenes/fusion gene sequences and laboratories need to have specialized biohazard containment procedures for viral packaging and viral-based gene delivery.

Vectors based on DNA transposons such as PiggybacTM or *Sleeping Beauty* (SB) are effective non-viral tools for gene therapy and genetic engineering of cells. These vectors provide a safer, faster, and less costly approach to generate genetically modified iPSC lines, than their viral counterparts. This is because there is no need to package virus, nor the need of cumbersome quality-assurance procedures, such as titration of vectors and testing for replication-competent virus. This method only involves the manipulation of plasmids, so it can be easily performed in a biosafety level 1/2 laboratory with basic equipment, without requiring complex biohazard containment procedures. Moreover, it has been reported that PiggybacTM and SB transposon-based gene transfer is considered a safer tool when compared with viral tools due to their integration pattern⁶. Another advantage is that these vectors do not have a cargo limit, circumventing the problem of not being able to insert large transgenes into the host's genome.

Promoter DNA methylation and a near-random integration profile of transposon based-systems often results in transgene integration into heterochromatin, which renders such vectors vulnerable to transcriptional repression/silencing (Skipper *et al.*, 2019, *ibid*) . Therefore, we reasoned that to secure persistent transgene expression it may be necessary to protect transposon-embedded transgenes with anti-transcriptional silencing elements. We hypothesized that integrating the minimal 0.7 kb sequence encompassing the CBX3 gene in

a Piggybac™ vector could present a tool for the genome editing of iPSCs that would allow for sustained transgene expression in differentiated progeny, such as macrophages.

The PiggyBac™ Transposon System (System Biosciences (SBI), Palo Alto, California, USA; Hera BioLabs, Inc. Lexington, Kentucky, USA) consists of a PiggyBac Vector and the Super
5 PiggyBac Transposase which recognizes transposon-specific inverted terminal repeats (ITRs) and efficiently integrates the ITRs and intervening DNA into the genome at ttaa sites. The Super PiggyBac Transposase is delivered to the cell via the Super PiggyBac Transposase Expression Vector (SEQ ID NO: 24), which is co-transfected with one or more PiggyBac Vectors. The PiggyBac Transposon System uses a cut-and-paste mechanism to transfer DNA
10 from the PiggyBac Vector into the genome.

The present invention uses the transposon machinery of the “ttaa” specific PiggyBac® transposons to insert a targeted functional heterologous DNA sequence (gene of interest) into the genome of the host cell and uses a CBX3 UCOE to prevent silencing of expression of the
15 gene of interest. The resulting transformed cell or group of cells are stable transformants that will pass the introduced gene to all subsequent progeny. The targeted functional heterologous DNA “gene of interest” for purposes of this invention is any heterologous DNA capable of being expressed in a host cell.

20 The transformation system of the present invention includes a vector that includes the modified piggyback - CBX3 UCOE transposon (PB-UCOE_{CBX3} transposon), the vector may include a marker construct, such as the enhanced green fluorescent protein gene (EGFP) operably linked to a suitable promoter. This vector can be used to transform and detect transgenic organisms based on expression of the marker, e.g., green fluorescent protein marker under
25 ultraviolet light. After chromosomal integration and inheritance of the vector, expression of the marker will occur in all progeny cells. The transformation system of the present invention also includes a PiggyBac transposase vector. The creation of a transformed cell requires that the vector containing the functional heterologous DNA first be physically placed within the host cell. A variety of transformation techniques may be used to introduce the vector DNA into a
30 cell. The DNA sequence flanked by the transposon inverted repeats will be inserted into the genome of the cells. This DNA will then be passed on to the progeny cells. Transformed cells can be selected from untransformed cells, for example by ultraviolet light when the transformation system includes an enhanced green fluorescent protein gene that produces an altered visible phenotype under ultraviolet light. Using standard techniques known to those
35 familiar with the field, techniques such as, for example, Southern blotting and polymerase

chain reaction, DNA can be isolated from transformed cells to confirm that the introduced DNA has been inserted.

To our knowledge, this invention represents the first time that a PiggyBac system containing a UCOE CBX3 sequence has been used to produce genetically modified iPSC lines and most importantly, to produce genetically engineered human iPSC-derived Macrophages. The integration of the minimal 0.7 kb UCOE CBX3 sequence in a PiggyBac vector and use to transform iPSCs, allows production of human genetically-engineered macrophages in a reduced-time frame when compared to prior art methods.

The new vectors prevent silencing of transgenes in differentiated cells from iPSCs (macrophages). These vectors are advantageous when compared to viral vectors because they present a safer, faster, and lower-cost approach to generate genetically-modified iPSC lines. There is no need to package virus, nor the need of cumbersome quality-assurance procedures, such as titration of vectors and testing for replication-competent virus. It only involves the manipulation of plasmids, so it can be easily performed in a biosafety level 1/2 laboratory with basic equipment, without requiring complex biohazard containment procedures. PiggyBac and SB transposon-based gene transfer is considered safer when compared with viral tools due to its integration pattern. These vectors do not have a cargo limit, so they circumvent the problem of not being able to insert large transgenes into the host's genome.

List of Figures

Figure 1. A. PB-UCOE-SIGLEC10 plasmid (SEQ ID NO: 11) map. PB: PiggyBac, ITR: inverted tandem repeat, UCOE: Ubiquitous Chromatin Opening Element, CMV: cytomegalovirus, hSIGLEC10: human Sialic Acid Binding Ig Like Lectin 10 codon optimized sequence, bgH Poly(A): bovine growth hormone polyadenylation sequence, EF1 α : Elongation factor 1-alpha, CopGFP: copepod Pontellina plumate green fluorescent protein, T2A: 2A self-cleaving peptide, PuroR: Puromycin Resistance, SV40 Poly(A): Simian virus 40 polyadenylation sequence

Figure 2. A. Representative flow cytometry histogram of CopGFP expression for un-transfected SFCi55 wild type iPS cells and PB-UCOE-SIGLEC10 + Super PiggyBac Transposase transfected SFCi55-iPSC cells. B. Representative flow cytometry histograms of SIGLEC10 protein expression for un-transfected SFCi55-iPS cells and PB-CBX3-SIGLEC10 + Super PiggyBac Transposase transfected SFCi55-iPSC cells. C. Representative flow cytometry histograms of CopGFP expression for iPSC-derived macrophages derived from un-

transfected SFCi55-iPS cells and macrophages derived from PB-CBX3-SIGLEC10 + Super PiggyBac Transposase transfected SFCi55-iPSC cells. D. Representative flow cytometry histograms of SIGLEC10 protein expression for iPSC-derived macrophages derived from un-transfected SFCi55-iPS cells (left) and macrophages derived from PB-CBX3-SIGLEC10 + Super PiggyBac Transposase transfected SFCi55-iPSC cells.

Figure 3. A. Representative flow cytometry histograms for SIGLEC10 expression for iPSC cells (stem cell stage) from the parental line SFCi55 WT (wild type) and three PB-UCOE-SIGLEC10 engineered clonal lines: SIGLEC10 1.3, SIGLEC10 2.19, SIGLEC10 2.39. SIGLEC10 OE stands for SIGLEC10 over-expressing B. Representative flow cytometry histograms for SIGLEC10 expression for cells (Macrophage stage) from the parental line SFCi55 Wild type line and three engineered clonal lines (SIGLEC10 1.3, SIGLEC10 2.19, SIGLEC10 2.39). C. Compilation of delta MFI (SIGLEC10 antibody-stained sample median fluorescence intensity minus median fluorescent intensity of IgG1 isotype-stained sample) for macrophages derived from the SFCi55 parental line and three modified clonal lines (SIGLEC10 1.3, SIGLEC10 2.19, SIGLEC10 2.39) n=3.

Figure 4. Expression profile of the SFCi55 parental wild type line compared to three different SIGLEC10 over-expressing (SIGLEC10 OE) iPSC Clonal lines: 1.3, 2.19 and 2.39 for CD45, CD93, 25F9, CD14, CD163, and CD169 markers. Flow Cytometry histograms representative of one experiment out 3 independently performed replicas.

Figure 5. Phagocytosis level of macrophages from the parental line, and SIGLEC10 over-expressing macrophages (from Clonal lines SIGLEC10 OE 1.3, 2.19 and 2.39). Phagocytosis is quantified as the Area under the curve (AUC) of red fluorescence measured at 20 min intervals for 5h. Engineered Jurkat cells that express CD24 were labelled with pHrodo red labelling dye, and were given as bait at a 3 Jurkat cell: 1 macrophage ratio. (n = 6 biologically independent samples) Kruskal–Wallis test with Dunn's post-test). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure 6. A. PB-UCOE-dCas9KRAB-eGFP plasmid map. PB: PiggyBac, ITR: inverted tandem repeat, UCOE: Ubiquitous Chromatin Opening Element, CAG promoter: Hybrid construct consisting of the cytomegalovirus (CMV) enhancer fused to the chicken beta-actin promoter cytomegalovirus, dCas9 KRAB (ns): dead Cas9 with the Krüppel associated box (KRAB) domain and nuclear signalling sequence, T2A: 2A self-cleaving peptide, eGFP: Enhanced green fluorescent protein, rBG Poly(A): rabbit beta-globin polyadenylation sequence. B. Representative histogram for eGFP expression of SFCi55 WT iPSC-derived

macrophages and dCas9-KRAB-GFP iPSC-derived macrophages. C. LV-UCOE-U6gRNA plasmid map. LTR: lentivirus long-terminal repeat, RRE: Rev Response Element, cPPT: central polypurine tract sequence, UCOE: Ubiquitous Chromatin Opening Element, U6 promoter: type III RNA polymerase III promoter, Stuffer: random DNA sequence to aid in cloning of gRNAs, sgRNA scaffold: single guide RNA scaffold, EF1 α : Elongation factor 1-alpha, mTagBFP2: basic (constitutively fluorescent) blue fluorescent protein derived from *Entacmaea quadricolor*, T2A: 2A self-cleaving peptide, PuroR: Puromycin Resistance, WPRE: Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element sequence, SV40 Poly(A): Simian virus 40 polyadenylation sequence. D. Representative histogram for eGFP (Top) and mTagBFP2 (bottom) of dCas9-KRAB-GFP iPSC-derived macrophages either uninfected (left), or infected at the iPSC stage with LV-UCOE-U6gRNA lentivirus: NTCsgRNA (non-targeting single guide RNA (middle)) and B2MsgRNA (single guide RNA targeting human β 2-microglobulin (right)).

Figure 7. A. Representative histogram for B2M expression (β 2-microglobulin) of SFCi55 WT, dCas9-KRAB-GFP iPSC-derived macrophages uninfected or dCas9-KRAB-GFP iPSC derived macrophages infected at the iPSC stage with LV-UCOE-U6gRNA NTCsgRNA (non-targeting single guide RNA or B2MsgRNA (single guide RNA targeting human β 2-microglobulin. B. Representative histogram for MHC-I expression (from top to bottom: HLA-ABC, HLA-G, HLA-E, HLA-F) of dCas9-KRAB-GFP iPSC-derived macrophages either uninfected or infected at the iPSC stage with LV-UCOE-U6gRNA (NTC gRNAs and B2M gRNAs).

Figure 8. A. Expression profile of the SFCi55 WT macrophages compared to macrophages from a dCas9-KRAB-GFP iPSC line for CD45, CD93, 25F9, CD14, CD163, and CD169 markers B. Phagocytic index measure of SFCi55 WT macrophages and dCas9-KRAB-GFP iPSC-derived macrophages according to polarisation state. Macrophages from both lines were polarised with LPS + IFN γ , IL-4, IL-10 or left un-polarised. Phagocytosis is quantified as the Area under the curve (AUC) of red fluorescence measured at 20 min intervals for 5h. Zymosan beads were used as bait; they are transparent at neutral pH but fluoresce red when ingested by macrophages (n = 8 biologically independent samples) Kruskal–Wallis test with Dunn's post-test; ns stands for non-statistically significant.

Figure 9. Representative histograms of B2M (β 2-microglobulin) expression on SFCi55 WT macrophages and dCas9-KRAB iPSC-derived macrophages. From left to right: untransduced, transduced with NTC gRNA (non-targeting single guide RNA) or B2MsgRNA (single guide RNA targeting human β 2-microglobulin).

Figure 10. A. PB-UCOE-dCas9VPR-eGFP plasmid map. PB: PiggyBac, ITR: inverted tandem repeat, UCOE: Ubiquitous Chromatin Opening Element, CAG promoter: Hybrid construct consisting of the cytomegalovirus (CMV) enhancer fused to the chicken beta-actin promoter cytomegalovirus, dCas9+ VPR (VP64, p65+ Rta), ns: dead Cas9 with VPR domains (VP64, p65, Rta) and nuclear signalling sequence, T2A: 2A self-cleaving peptide, eGFP: Enhanced green fluorescent protein, rBG Poly(A): rabbit beta-globin polyadenylation sequence. B. Representative flow cytometry histograms for CD4 expression of dCas9VPR-A18 clonal line derived macrophages transduced with NTC sgRNA (non-targeting single guide RNA) or CD4gRNA (single guide RNA targeting CD4). C. Representative flow cytometry histograms for SIGLEC10 expression of dCas9VPR-A18 line derived macrophages transduced with NTC gRNA or SIGLEC10 gRNA. D. Flow Cytometry histograms representative of one experiment for macrophages from the dCas9-VPR A18 line for pan macrophage markers: Expression of CD45, CD93, 25F9, CD11b, CD14, CD163, and CD169. E. Phagocytic index measure of SFCi55 WT macrophages and dCas9-VPR A18 iPSC-derived macrophages according to polarisation state. Macrophages from both lines were polarised with LPS + IFN γ , IL-4, IL-10 or left un-polarised. Phagocytosis is quantified as the Area under the curve (AUC) of red fluorescence measured at 20 min intervals for 5h. Zymosan beads were used as bait; they are transparent at neutral pH but fluoresce red when ingested by macrophages (n = 6 biologically independent samples for all, except for untreated macrophages: n=11) Kruskal–Wallis test with Dunn's post-test; ns stands for non-statistically significant.

Figure 11: Graphical description of the improvements to the iPSC derived macrophage genetic engineering process, showing milestones and time-scales. Traditional approaches take from 20-24 weeks on average, whereas the lines described in this invention can reduce the time-scale to as little as 2 weeks.

Examples

Materials and Methods

Cell Culture

30 *Human iPSC Maintenance*

The human induced pluripotent stem cell (iPSC) line SFCi55 (parental control) was generated as described in (Yang et al., 2017). In brief, the SFCi55 iPSC line was originally generated using fibroblasts obtained from blood group O Rhesus negative individuals by R Biomedical under REC 1/AL/0020 ethical approval and programmed to iPSCs using Yamanaka factors on episomal vectors. The line has been confirmed to be pluripotent and have normal karyotype.

Human induced pluripotent stem cells (hiPSCs) from SFCi55 and all derivatives were cultured in mTESR™ Plus medium (Stem Cell Technologies, Cat #100-0276) on 0.5 µg/cm² vitronectin (Invitrogen, Cat#A31804) coated tissue culture treated 6-well plates. The cells were passaged routinely every 5-7 days using ReLeSR™ (Stem Cell Technologies Cat# 05872) at a 1:10-1:20
5 split ratio.

Genome Edited iPSC line derivation (PiggyBac)

Reactions of DNA-lipid complex were prepared by diluting 10µl Lipofectamin STEM (ThermoFisher Cat# STEM00008) in 100µl of Opti-MEM™ (Gibco Cat #31985062). On a
10 separate reaction, 2.5ug total DNA (2ug expression plasmid + 0.5ug transposase plasmid) was added to 100ul of Opti-MEM™. Both reactions were mixed 1:1 and then incubated for 10 minutes.

SFCi55 wild-type iPSC cells were washed once with PBS (without magnesium and calcium),
15 followed by incubation with Accutase (Gibco, Cat# A1110501) for 3 minutes. 1ml of mTESR media was added and the cells were detached, centrifuged and counted. The cells were seeded at 1 x 10⁴ cells/well in a 6-well plate containing 2ml cloning medium: [(mTESR Plus medium supplemented with CloneR™2 (1:10, Stem Cell Technologies Cat# 100-0691)]. DNA-lipid complex (180ul) was then added to each well in a drop-wise manner, leaving one well as
20 an un-transfected control. After 2 days, the medium was replaced with fresh cloning medium. From day 4 onwards the medium was replaced with mTESR Plus medium according to normal iPSC culture procedures.

To sort genome edited cells, once expanding cells had reached desired confluency, they were
25 detached using Accutase and sorted for the main GFP population by flow cytometry. Sorted cells were seeded into 96-well plates (1 cell per well), or into 10cm dishes at a density of 600 cells/dish, pre-coated with vitronectin and containing cloning medium. After 2 days the medium was replaced with fresh cloning medium. From day 4 onwards the medium was replaced with mTESR Plus according to normal iPSC culture procedures. On day 7, the colonies were
30 picked and transferred to 24-well plates pre-coated with vitronectin, and expanded for another ~7 days before being transferred to a 6-well plate using ReLeSR.

iPSC differentiation to macrophages

Differentiation of macrophages was carried out using as base protocols the ones reported in
35 (Lopez-Yrigoyen et al., 2018, 2019, 2020). Maintenance media on one confluent well of iPSCs in a 6-well plate was replaced with 1 ml of Mix1, which consisted of mTESR Plus (Stem Cell Technologies Cat#100-0276) supplemented with 50ng/ml of BMP4 (R&D Cat# 314-BP-050) ,

50ng/ml of VEGF (R&D Cat# 293-VE-050 and 20ng/ml of SCF (Invitrogen Cat# PHC2111). Colonies were cut using a StemPro™ EZPassage™ tool (Invitrogen Cat#23181010) and transferred into two wells containing 2.5 ml of Mix1 cultured in Ultralow Attachment 6-well plates (Greiner, Cat# 657970) to induce embryoid body (EB) formation. After 2 days the medium was topped up with 500µl concentrated fresh Mix1 cytokines [(BMP4 (300 ng/ml), VEGF (300 ng/ml) and SCF (120 ng/ml)]. A full media change was performed on the EBs on day 4 by first collecting the EBs, letting them settle by gravity in a 50ml falcon tube and resuspending them in fresh Mix1 medium (3 ml/well). On day 6, tissue culture treated 6-well plates were coated with 1 ml/well of EmbryoMax® 0.1% Gelatin Solution (Merck, Cat# ES-006-B) for 30 minutes. The gelatin was then replaced with 3ml of Mix2 [(X-VIVO15 (Lonza, Cat# BE02-060F media supplemented with 100ng/ml of CSF1 (Biolegend, Cat# 574808), 25ng/ml IL-3 (Peprotech, Cat# 200-03), 1% Penicillin-Streptomycin (Gibco, Cat# 15140122), 2nM Glutamax (Gibco, Cat# 35050061) and 0.55Mm β-mercaptoethanol (Gibco Cat# 31350010)]. EBs were collected into a 50ml tube, and once settled at the bottom by gravity, they were resuspended in a small amount of Mix2 medium. 10-15 EBs were added per well to the gelatin-coated 6-well plate. Non-adherent macrophage precursor cells were harvested starting from 2-3 weeks after EB plating every 3-4 days for up to 3 months: media from EB plates was collected and filtered using a 40µm cell strainer. EB plate was re-fed with 3ml of Mix 2 and returned to the incubator. Macrophage precursors were plated at a density of 3-4 x 10⁵ cells/well in a tissue culture treated 6-well plate in Mix3 (X-VIVO15 media supplemented with 100ng/ml of CSF1, 2nM Glutamax and 1% Penicillin-Streptomycin). Macrophage precursors in Mix3 were left for at least 7 days to mature into fully differentiated macrophages. On day 7, either mature macrophages were used for assays or re-fed with Mix-3 and kept in culture for assays. No assays were performed beyond day 15 after macrophage precursor collection.

Macrophage polarisation/activation

Adherent iPSC-derived macrophages (iPSC-DMs) were activated *in vitro* to three different phenotypes: M(LPS+IFN-γ), M(IL-4), and M(IL-10) by treating with 20ng/ml LPS (Sigma, Cat# L2654) and 20ng/ml IFN-γ (Peprotech, Cat# 300-02), 20ng/ml IL-4 (Peprotech, Cat#200-04) or 5ng/ml IL-10 (Peprotech, Cat# 200-10) respectively. For the VPR A18 line experiment, IL4 concentration was 10ng/ml. Cytokine treatments were left for 48h.

Jurkat cell culture

Jurkat E6 cells were purchased from ATCC (Cat #TIB-152), and Jurkat CD24 Over-expressing (CD24-OE) cells were derived in house. Both cell lines were cultured in RPMI-1640 + Glutamax Medium (Thermofisher Cat no: 61870-010) supplemented with foetal bovine serum

(FBS) (Thermofisher Cat no: 10500064) to a final concentration of 10%. Cells were passaged every 2-3 days, so that a cell density between 1×10^5 and 1×10^6 viable cells/mL was maintained. Of note, CD24-OE cells were kept under G418 (Thermofisher Cat#10131035) selection at a final concentration of 2mg/ml.

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For the derivation of CD24-OE cells the plasmid pcDNA3.1+C-(K)-DYK hCD24 NM_013230.3 was purchased from Genescript (Cat # OHu23288D). The pcDNATM3.1(+) vector contains the neomycin resistance gene for selection of stable cell lines using neomycin (Geneticin® or G418). To obtain stable transfectants, plasmid pcDNA3.1+C-(K)-DYK hCD24 NM_013230.3 was linearised with Pvu-I HF (NEB Cat# R3150L) restriction enzyme according to the manufacturer's instructions. 1×10^5 cells were transfected with 0.5µg of DNA diluted into 100µl of Opti-MEM® I Reduced Serum Medium without serum (Gibco Cat# 31985070), and 2.75µl of Lipofectamine® LTX (Thermofisher Cat# 15338100). Plasmid and transfection reagent solutions were mixed and incubated for 25 minutes at room temperature and then added directly to the cells in a 12 well-plate. Media was changed 24h after transfection. Antibiotic selection was started 72h after transfection: G418 was added at a final concentration of 2mg/ml and kept under selection all the time since then. CD24 expression was tested by flow-cytometry before each phagocytosis assay to make sure that at least 90% of the cells expressed the transgene.

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HEK293 Culture

Lenti-X HEK293T cells were purchased from Takara Clontech (Cat# 632180) and were cultured in Iscove's Modified Dulbecco's Medium: IMDM (Sigma Cat# I3390) supplemented with Glutamax (1:100) (Gibco Cat# 35050-038) and FBS (Thermofisher Cat no: 10500064) to a final concentration of 10%. Cells were passaged every 2 to 3 days at a ratio of 1:8 or 1:10 by washing once with PBS and using trypsin (Gibco Cat# 15050065).

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Flow Cytometry

iPSC derived macrophages were detached from cell culture plates by 4 minutes' incubation with cell dissociation buffer [5mM EDTA in PBS without calcium and magnesium]. Cells were dislodged from the vessels using a P1000 pipette, followed by cell counting. Cells were spun at 200g for 3 min, and $<10^7$ cells were resuspended in 80µl FACS buffer [Cell dissociation buffer with 2% BSA] and FcR Blocking Reagent (20µl for $<10^7$ cells) for 20 min on ice. The cells were then spun at 200g for 3 min, and resuspended at 2×10^6 cells/mL. The cells were added to a U-bottomed 96-well plate at a density of 2×10^5 cells/specimen (100µl). Corresponding primary antibodies and isotype controls were added to the wells and mixed gently, followed by incubation at 4°C in the dark for 30 min. All final concentrations of primary

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antibodies were 10ug/ml (unless otherwise stated). FACS buffer (100µl) was then added to each well, the plate was spun down at 200g for 3min, and the supernatant was removed. Cells were resuspended in FACS buffer, and a viability dye was added (DAPI or Propidium Iodide at a 1:1000 dilution). Flow cytometry was performed using a 6 Laser Fortessa flow cytometer and FACS Diva software.

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Antibody table

Antibody	Isotype	Fluorophore	Dilution	Cat#	Source
CD45	Mouse IgG1, κ	APC	1/20	304012	Biolegend
CD163	Mouse IgG1, κ	APC	1/100	333610	Biolegend
CD169	Mouse IgG1, κ	APC	1/100	346008	Biolegend
CD93	Mouse IgG1, κ	APC	1/200	336120	Biolegend
CD11b	Mouse IgG1, κ	APC	1/200	301310	Biolegend
Mouse IgG1, κ Iso. Ctrl (Fc)	-	APC	1/200	400122	Biolegend
25F9	Mouse IgG1, κ	eFluor 660	1/20	50-0115-42	Thermo Fisher
Mouse IgG1, κ Iso. Ctrl	-	eFluor 660	1/800	50-4714-82	Thermo Fisher
CD14	Mouse IgG2a, κ	APC	1/200	301808	Biolegend
Mouse IgG2a, κ Iso. Ctrl	-	APC	1/200	400220	Biolegend
CD85j (ILT2, LILRB1)	Mouse IgG2b, κ	APC	1/200	333720	Biolegend
Mouse IgG2b, κ Iso. Ctrl	-	APC	1/100	400322	Biolegend
CD4	Mouse IgG1, κ	APC	1/25	300514	Biolegend
Siglec10	Mouse IgG1, κ	APC	1/100	347606	Biolegend

PDL1	Mouse IgG1, κ	APC	1/100	393610	Biolegend
Mouse IgG1, κ Iso. Ctrl	-	APC	1/100	400120	Biolegend
Mouse IgG1, κ Iso. Ctrl (Fc)	-	APC	1/100	400122	Biolegend

Phagocytosis assay

Cancer cell phagocytosis assay

iPSC derived macrophages from SFCi55 parental line, as well as SIGLEC10 over-expressing lines were plated 2-3 days before live imaging, at a density of 2×10^4 per well of an imaging TC Treated 96 well plate (CellCarrier-96 Ultra Microplates, Perkin Elmer Cat# 6055300) in 100 μ l of macrophage maturation media: X-VIVO15 media (Lonza, Cat# BE02-060F) supplemented with 100ng/ml recombinant human CSF1 (Biolegend Cat# 574808), 2mM Glutamax (Invitrogen Cat# 35050038) and 1% Penicillin-Streptomycin (Gibco Cat# 15140-122).

On the day of the assay, 48h/72h post macrophage seeding, Jurkat WT cells or Jurkats-CD24 over-expressing cells were stained with IncuCyte® pHrodo® Orange Cell Labelling Kit for Phagocytosis (Sartorius Cat# 4766). Briefly, cells were collected, centrifuged for 4 minutes at 200g, and washed with IncuCyte pHrodo Cell Wash Buffer (1×10^6 cells/ml of wash buffer). Cells were spun down for 4 minutes at 200g and pellet was then resuspended in IncuCyte pHrodo Cell Labelling Buffer to a density of 1×10^6 cells/ml. Solubilized IncuCyte pHrodo Orange Cell Labelling Dye was added to the cell suspension at a final concentration of 600ng/ml. Cell suspension was mixed and then incubated 1hr at 37 °C (cells were mixed every 20min of incubation time). To remove excess IncuCyte pHrodo Labelling Dye, cells were centrifuged at 1300 rpm for 7 minutes. The cell pellet was then re-suspended at a density of 1.2×10^6 cells/ml in macrophage maintenance media. 50 μ l of cell suspension (60,000 cells) were placed into each macrophage containing well (macrophage: target cell ratio was 1:3). The plate was immediately placed into the Incucyte S3 Live imaging system. Acquisition of 3 fields per well was carried out every 30 minutes for 5 hours, at 20X magnification. Image analyses were carried out using the 'Cell by Cell' pipeline from Incucyte. Briefly, macrophages were segmented based on size and morphology. Total orange fluorescence in the macrophage population per time-point was taken as a first output. Then, the area under the curve was calculated per treatment and plotted for further statistical analyses.

Zymosan Beads Phagocytosis assays.

iPSC derived macrophages from SFCi55 parental line, as well as dCas9-KRAB 1.8 and dCas9-VPR-A18 clonal lines were plated 2-3 days before live imaging, at a density of 2×10^4 per well of an imaging TC Treated 96 well plate (CellCarrier-96 Ultra Microplates, Perkin Elmer Cat# 6055300) in 100ul of macrophage maturation media: X-VIVO15 media (Lonza, Cat# BE02-060F) supplemented with 100ng/ml recombinant human CSF1 (Biolegend Cat# 574808), 2mM Glutamax (Invitrogen Cat# 35050038) and 1% Penicillin-Streptomycin (Gibco Cat# 15140-122).

On the day of the assay, a vial of pHrodo™ Red Zymosan Bio particles (Invitrogen Cat# P35364) was re-suspended in 10 ml of PBS and vortexed at high speed for 10 minutes. 50µl of bead suspension was added to each well of iPSC-derived macrophages. Plate was immediately placed into the Incucyte S3 Live imaging system. Acquisition of 3 fields per well was carried out every 20 or 30 minutes for 5 hours, at 20X magnification. Image analyses were carried out using the Cell-by-Cell pipeline from Incucyte. Briefly, macrophages were segmented based on size and morphology. Total red/orange fluorescence in the macrophage population per time-point was taken as a first output. Then, the area under the curve was calculated per treatment and plotted for further statistical analyses.

Lentivirus preparation/packaging

2.3x10⁵ HEK293T cells were plated in 1 ml of IMDM in a well of a 12-wp (about 30% confluency). At 24 hours after plating, a media change was performed, and cells were transfected with desired lentiviral plasmid (transfer plasmid). Transfection reactions were prepared by mixing 100 µl of Opti-MEM® I Reduced Serum Medium without serum (Gibco Cat# 31985070), 250 ng of transfer plasmid, 750ng of pC-Pack2 (Cellecta Cat# CPCP-K2A) and 3 µl of Transit293 (Mirus Cat# MIR 2704) and incubating at room temperature for 25 minutes. Transfection reactions were added dropwise to the wells. At 24 hours after transfection media was replaced. Media with lentiviral particles was collected and stored at -80 °C.

Vpx-Viral like particles (VLPs) preparation

Vpx-containing VLPs were produced by transfection of HEK293T cells as described in the previous section. In this case, transfer plasmid is pSIV3+ (VLP Vpx+) (Nègre *et al.*, 2000).

Lentivirus infection

Human iPSCs

iPSCs need to be reverse transduced. An 80% confluent well of iPSCs was washed with PBS, followed by an incubation with 0.5 ml of Stempro-Accutase (Gibco Cat#A1110501) at 37 °C to

detach the cells and get a single cell suspension. 1 ml of mTSER plus media was added, and cells were collected and counted. A total of 30,000 cells were seeded per well in a 6-well plate previously coated with vitronectin and containing 2 ml cloning medium (mTESR Plus medium supplemented with CloneR™2 (1:10) Stemcell Technologies Cat# 100-0691). Lentiviral particles were added dropwise to desired wells. Lentiviral particle volume was added calculating for an MOI of 10. Cells were incubated at 37 °C and 5 % CO₂ for 2 days. A full media change was performed with cloning medium 48 hours after infection. Cells were then grown and passaged as described in the maintenance section.

10 ***Human iPSC-derived macrophages***

iPSC-derived macrophages were seeded at a density of 2.5×10^5 cells/ml in 1 ml (12-well plate) or at 1.33×10^5 cells/ml in 3 ml (6-well plate) of macrophage maturation media: X-VIVO15 media (Lonza, Cat# BE02-060F) supplemented with 100 ng/ml recombinant human CSF1 (Biolegend Cat# 574808), 2 mM Glutamax (Invitrogen Cat# 35050038) and 1 % Penicillin-Streptomycin (Gibco Cat# 15140-122). At 24 or 48 hours after seeding, macrophages were transduced by adding Vpx-VLP particles produced in house and lentiviral particles, aiming for an MOI of 3 to 5.

Plasmid synthesis/cloning

20 The PL-21.0001 PB-UCOE-SIGLEC10 (SEQ ID NO: 11) plasmid vector was derived by cloning the UCOE sequence (SEQ ID NO: 1) , the full length human SIGLEC10 ORF (codon optimised) and CopGFP by restriction enzyme digests into the base vector PL-20.0002 PiggyBacDualProm (SEQ ID NO: 12) (PB-CMV-Restriction enzyme fragment-EF1alpha short/truncated-copGFP-T2A-PuroR), which was purchased from Gentaur. Cloning was performed by Genewiz.

The PL-22.003 PB-UCOE-dCas9-KRAB-eGFP plasmid (SEQ ID NO: 13) was designed in house and then synthesized from scratch by Vector builder.

The PL-22.0071 PB-UCOE-dCas9-VPR-eGFP plasmid (SEQ ID NO: 14) was derived in house, by cloning in a gene block containing the dCas9-VPR sequence into the PB-UCOE-dCas9-KRAB-eGFP plasmid (SEQ ID NO: 13) by Gibson assembly. The Gibson PCR products were added to the restricted PiggyBac backbone (restricted with AgeI and AvrII) using Gibson assembly protocol from NEB. The Enzymatic Digest PCR products were added to the restricted PiggyBac backbone (restricted with AgeI and AvrII) using T4 ligase protocol from NEB.

35 The PL-22.0050 LV-UCOE_{CBX3}-U6gRNA-mTagBFP2 (SEQ ID NO: 15) was produced in house by cloning in the mTagBFP2 sequence by Gibson Assembly into base vector PL-21.0067 LV-

UCOE_{CBX3}-pU6-stuffer-pEF1a-eGFP-T2A-Puro-WPRE (SEQ ID NO: 16) which was designed in house but synthesised from scratch by Vector builder. Full plasmid sequences are provided.

Single gRNA cloning into PL-22.0050 LV-UCOE_{CBX3}-U6gRNA-mTagBFP2 (SEQ ID NO: 15) was done by all-in one digestion-ligation. LV-UCOE_{CBX3}-U6gRNA-mTagBFP2 has a stuffer region which can be cleaved by TypeIIIS restriction enzymes AarI or PaeCI, leaving 5'-GTTG and GTTT-3' overhangs with which to ligate in sgRNA oligos. TypeIIIS restriction enzymes allow for digestion of stuffer containing vector and ligation of annealed sgRNA oligos into cut vector at the same time.

- 10 sgRNA sequences were taken from the Weissman Lab CRISPRi and CRISPRa annotated libraries (<https://elifesciences.org/articles/19760/figures#SD3-data>) and ordered as DNA oligos with the adapters for cloning with PaeCI restriction enzyme.

sgRNA sequences

CRISPRi

- 15 SEQ ID NO: 17 NTC#3i gtgtgcaacctccgccgttg (CRISPRi.v2 Weissman library)
 SEQ ID NO: 18 B2M#1i ggcgagcacagctaaggcca (CRISPRi.v2 Weissman library)
 SEQ ID NO: 19 B2M#3i ggccacggagcgagacatct (CRISPRi.v2 Weissman library)

CRISPRa

- SEQ ID NO: 20 NTC#1a tgctgtgatgcgtagacgg (CRISPRa.v2 Weissman library)
 20 SEQ ID NO: 21 SIGLEC10#1a aaggtgggccggagagtgt (CRISPRa.v2 Weissman library)
 SEQ ID NO: 22 CD4#1a gtgaaatgccaaagtcaa (CRISPRa.v2 Weissman library)
 SEQ ID NO: 23 PDL1#1a cggcggaagctttcagttt (CRISPRa.v2 Weissman library)

- Oligo annealing and phosphorylation were carried out by setting the following reaction: 6.5 µL
 25 H₂O, 0.5 µL 10x T4 DNA ligase buffer, 1 µL forward oligo (100 µM), 1 µL Reverse oligo (100 µM) and 1 µL T4PNK enzyme. Tubes were placed in a PCR machine with the following program: 37 °C for 30 min, 95 °C for 5 min, and then a temperature ramp down 0.1°C/s to 4°C. Annealed oligos were diluted to 200 µL with H₂O and kept at 4 degrees. The digestion and ligation of plasmid and annealed sgRNA oligos was carried out by setting the following
 30 reaction: 1 µL 10x CutSmart buffer, 1 µL diluted annealed oligos (+ H₂O negative control), 100 ng plasmid, 1 µL 10 mM DTT, 1 µL 10 mM ATP, 0.5 µL PaeCI, 0.25 µL PaeCI Activator, 0.25 µL T4 ligase and H₂O up to 10 µL. Reaction was then placed in a PCR machine and the following protocol was run. 6 cycles of (37°C – 5 min, 16°C – 5 min), then 1 cycle of 60°C for 5 min.

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Ligated plasmids were then transformed into *E.coli* and plated on LB-Ampicillin plates and incubated at 30°C over-night. gRNA cloning was confirmed by Sanger Sequencing.

Statistical analyses

- 5 Data were expressed as mean $\pm$ standard deviation (SD). Statistical tests that were used are indicated in figure legends and were performed using Graph Pad software version 6.0c. P-values <0.05 were considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns non-significant).

10 Example 1: Macrophages over-expressing SIGLEC10

- A novel PiggyBac plasmid PB-UCOE-SIGLEC10 (SEQ ID NO: 11) (Figure 1A) was designed and synthesized for expression of proteins in mammalian cells. The main features of this plasmid are: the two transposon-specific inverted terminal repeat sequences (PB 5' ITR and PB 3' ITR), the 0.7kb CBX3 UCOE (Ubiquitous chromatin opening element sequence)
 15 sequence (SEQ ID NO: 1) just upstream of a CMV Promoter, Multiple cloning sites, human SIGLEC10 gene (gene of interest), the bovine growth hormone polyadenylation signal (bGH poly(A) signal), a second promoter: EF1-alpha (short version), a CopGFP sequence immediately upstream of a T2A self-cleaving peptide sequence and a puromycin resistance gene.

- 20 To test this new plasmid, the full length human SIGLEC10 ORF was cloned into PB-CBX3 (SEQ ID NO: 12) (obtaining PB-UCOE-SIGLEC10 plasmid, (SEQ ID NO: 11, Figure 1A), and then human iPSCs were co-transfected with PB-UCOE-SIGLEC10 and a plasmid containing the sequence for the Super PiggyBac-Transposase (SEQ ID NO: 24). Stably transfected cells
 25 were selected with 0.5 $\mu\text{g/ml}$ of puromycin in complete medium, starting from 48 hours after transfection for 1.5 weeks. At this stage, the expression of SIGLEC10 and CopGFP was examined by flow-cytometry. 99.65 % showed detectable levels of CopGFP protein (Figure 2A), while 93.9 % of iPSCs from the transfected pool expressed SIGLEC10 (Figure 2B). The pool of Puromycin resistant cells was differentiated to macrophages and again tested for the
 30 expression of CopGFP and SIGLEC10 by flow-cytometry (Figure 2C and D). SIGLEC10 was detected in over 58.85 % of iPSC derived macrophages, however, CopGFP fluorescence was not detected in the cells. The CMV promoter-SIGLEC10 gene cassette being directly downstream of the UCOE sequence was protected from gene silencing upon iPSC differentiation to macrophages. The EF1-alpha promoter/copGFP gene cassette, however,
 35 being further distanced away from the UCOE sequence was completely silenced.

Next, three clonal iPSC lines that express SIGLEC10 protein were derived (Figure 3A). This was done firstly, to be able to generate a homogeneous population of macrophages expressing SIGLEC10 at comparable levels, and secondly, to estimate whether this approach of obtaining clonal cells was quicker than a UCOE-based lentiviral vector method. To this end, human iPSCs were co-transfected as a single cell suspension with the PB-UCOE-SIGLEC10 and plasmid Super PiggyBac Transposase plasmids and then seeded at a low density (0.8×10^6 cells/10 ml in a 10 cm dish). At 48 hours after transfection, antibiotic selection was initiated and Puromycin selection was continued for 1 week. Well-separated colonies were then picked and seeded individually into the wells of a 24-well plate. When confluent, the cells were moved to 6-well plates and were then tested for SIGLEC10 and CopGFP expression by flow cytometry. Three SIGLEC10 over-expressing (OE) iPSC clones were selected: 1.3, 2.19 and 2.39 with differing levels of SIGLEC10 expression (Figure 3A). These clonal lines were then differentiated into macrophages. SIGLEC10 expression was retained at this differentiated level (Figure 3B and 3C). These results confirmed that having a UCOE sequence directly upstream of a CMV promoter and a gene of interest in a PiggyBac based vector, permits the sustained transgene expression in the differentiated progeny of iPSC-cells (in this case, macrophages). Furthermore, this system allowed the production of clonal lines with different levels of transgene expression (Figure 3B and 3C). This is useful for interrogating the function of certain genes in terms of expression level.

As a next step, we checked whether the genome editing approach had an impact on macrophage phenotype. SIGLEC10-expressing macrophages from three different clonal lines express major lineage markers, such as CD45, 25F9, CD163 and CD169 (at similar levels of the SFCi55 parental line), and do not express/express low levels of CD93, similar to the macrophages derived from the parental iPSC cells (Figure 4). SIGLEC10 is a type-I lectin belonging to the immunoglobulin superfamily, and it has been found to be expressed by tumor associated macrophages. SIGLEC10 binds to CD24 via its V-set domain and this binding negatively regulates phagocytosis of tumor cells⁸. To test the function of the SIGLEC10-expressing macrophages, a cancer cell phagocytosis assay was performed. The hypothesis was that SIGLEC10 expression on iPSC-derived macrophages would inversely correlate with the ability of the macrophage to phagocytose CD24-expressing tumor cells. CD24-expressing Jurkat cells were stained with pHrodo orange labelling dye. When labelled Jurkat cells are phagocytosed, the dye fluoresces bright orange and allows the quantification of phagocytosis. Images were taken every 20 minutes for 5 hours and then the area under the curve was calculated for every treatment. Macrophages from the parental line (without SIGLEC10 expression) had the highest level of phagocytosis, while the macrophages with highest SIGLEC10 expression, had the lowest level of phagocytosis (Figure 5). This showed that the

cell lines were functional and corroborated reported data on the macrophage-tumor cell SIGLEC10-CD24 “do not eat” me signal.

Importantly, a pure clonal population of transgene-expressing macrophages was produced
5 considerably faster than when using a viral vector approach. The lentiviral approach required
5 weeks from plasmid design to infection of iPSCs (this time encompassed plasmid expansion
and purification, as well as lentiviral packaging and viral transduction quality control checks),
whereas the PiggyBac approach of the invention required less than a week from plasmid
design to transfection of iPSCs (only expansion and purification of the plasmids was required).
10 Furthermore, PiggyBac transfection caused less cell death than lentiviral infection, resulting
in a shorter time between transgene insertion in iPSCs and colony picking.

**Example 2: Generation of a CRISPRi iPSC-DM system (UCOE dCas9KRAB iPSC line to
generate knock-downs in iPSC-derived macrophages)**

15 CRISPR/Cas9 technology has transformed our ability to elucidate mammalian cell biology and
represents a valuable tool to interrogate gene function in different settings. Large CRISPR-
Cas9 based genetic screens have been conducted in cancer cell lines, rather than in more
biological relevant systems, like healthy, differentiated macrophages. Deriving a clonal iPSC
20 line that expresses Cas9 can facilitate large genetic screens in different cell types. Recently
developed screens have used Cas9 systems that avoid the double strand cut to bypass cell
toxicity effects, or other on-target effects such as allele loss, chromosomal abnormalities and
p53 activation due to DNA damage response. An example of such system is CRISPR
interference (CRISPRi). This system uses a deactivated/dead Cas9 (dCas9) that binds to the
25 target genomic region with the same efficiency as Cas9 but cannot generate a double strand
break. The dCas9 is fused to repressor domains, and when it uses a guide RNA, it targets the
promoter region of a specific gene and drives its transcriptional repression, or knockdown. To
our knowledge, there is one approach that has been successful in iPSC-differentiated cells so
far (neurons and microglia)^{9,10}, whereby a dCas9-KRAB iPSC line (CRISPRi system) was
30 derived by targeting a safe harbour locus: *CYBL*. After the long genome editing cell line
derivation process, the cells are differentiated into neurons or microglia (6 and 8 days
respectively) by the genetic enforcement of transcription factors (an artificial differentiation
system). Biologically relevant iPSC macrophage differentiation systems take an average of 4
weeks. This *CYBL* genome editing approach needs to be tested for silencing in longer
35 differentiation protocols.

We pursued a different strategy. We derived a clonal line that expressed a deactivated Cas9 with a KRAB domain (Krüppel associated box repressor domain) and a reporter (eGFP: enhanced green fluorescent protein), using our UCOE-plasmid approach (Figure 6A). Of note, it would be very difficult to package the dCas9-KRAB cassette into a virus, as the cargo size exceeds the 7kb recommended cargo to allow for good viral titers. To solve this problem, it has been proposed in the art that dCas9 protein be split into different domains and integrated inside the cells.¹¹ This approach would involve co-infection of two cassettes and more cumbersome cell selection processes, plus it would still need a ubiquitous chromatin opening element in both cassettes to ensure transgene expression at differentiated cell states. We reasoned that the UCOE Piggy Bac system of the invention would allow for a dCas9-KRAB transgene, as well as a reporter, such as eGFP to be delivered into iPSC cells without the need for splitting any sequence. To try and avoid silencing of the reporter like the one we observed for the SIGLEC10 example, we used a T2A self-cleaving peptide downstream the dCas9-KRAB sequence, so dCas9 KRAB and eGFP would be in the same transcript (Figure 6A). The main features of this plasmid are: the two transposon-specific inverted terminal repeat sequences (PB 5' ITR (SEQ ID NO: 2) and PB 3' ITR (SEQ ID NO: 3)), the 0.7kb CBX3 sequence just upstream of a CAG Promoter, multiple cloning sites, dCas9-KRAB (SEQ ID NO: 9), a dead/deactivated Cas9 with the repression domains and a nuclear localisation signal sequence (gene of interest), a T2A (self-cleaving peptide) (SEQ ID NO: 8) and eGFP (enhanced green fluorescent protein).

Human iPSCs were co-transfected with PB-UCOE_{CBX3}-dCas9KRAB-GFP ((SEQ ID NO: 13) PL-22.003 *PB-UCOE_{CBX3}-pCAG-dCas9-KRAB-T2A-eGFP-pA (CRISPRi plasmid)*) (Figure 6A) and the plasmid containing the sequence for the Super PiggyBac-Transposase (SEQ ID NO: 24)... One week after transfection, the cells were sorted based on GFP expression and they were seeded at a clonal density. Clonal lines were then differentiated to macrophages. The dCas9-KRAB-GFP-engineered macrophages expressed GFP (Figure 6B), so no silencing of the transgene occurred at differentiated cell states. To test the functionality of dCas9-KRAB-GFP iPSC line, delivery of a sgRNA was required. A lentiviral vector (LV-UCOE_{CBX3}-U6gRNA-mTagBFP2, SEQ ID NO: 15) was designed with the CBX3 UCOE upstream of a U6 promoter to drive the expression of a sgRNA. A separate promoter was used to drive the expression of an mTagBFP2 reporter and a puromycin resistance gene (Figure 6C). A LV approach was chosen for this particular plasmid as the cargo fits (3.8kb size). Moreover, lentivirus with small cargos can be used to infect cells at different stages: at the iPSC-level and at the iPSC-derived macrophage stage. iPSCs were infected with different versions of the LV-UCOE_{CBX3}-U6gRNA: a non-targeting gRNA (NTC) and (β2-microglobulin gene, B2M). Both the eGFP from the dCas9-KRAB-eGFP line and the mTagBFP2 from the 2

versions of the LV-UCOE_{CBX3}-U6gRNA lines were expressed at the macrophage level (Figure 6D). This suggested that both dCas9-KRAB and the sgRNA would also be expressed at the macrophage level. Macrophages that expressed the sgRNA against B2M knocked down the expression of the gene (Figure 7A). This showed that the system was functional and that
5 knock-down of genes in iPSC-derived macrophages could be achieved.

Generating universal human induced pluripotent stem cells and their differentiated products with hallmarks that prevent immune rejection is desirable for clinical applications. B2microglobulin is part of the polypeptide chain of the HLA-Class I antigen complex and is
10 found on the surface of all nucleated human cells. Mutations in B2M or knockouts of B2M have been performed to produce cells with the capability of preventing immune rejection in several hosts. We hypothesized that the CRISPRi dCas9 KRAB macrophages containing a B2M gRNA would also have low levels of HLA-Class I molecules. Indeed, dCas9KRAB macrophages have low levels (isotype comparable fluorescence) of HLA-ABC, HLA-G, HLA-
15 E and HLA-F (Figure 7B). With this approach, we were able to generate an MHC-I null/low iPSC-derived macrophages.

Cell-surface marker expression in the dCAS9 KRAB iPSC-derived macrophages was checked. Macrophages expressed pan-macrophage markers at comparable levels to the wild
20 type WT-Macrophages. (Figure 8A), showing that the insertion of dCas9-KRAB-eGFP transgene has no profound effect on macrophage phenotype. Furthermore, macrophages from the dCas9 KRAB Line, were able to phagocytose Zymosan™ beads (phagocytosis levels were the same as the SFCi55 WT macrophages) (Figure 7B). Engineered macrophages from the dCas9 KRAB Line also responded to stimuli. Upon IL-10 stimulation, they were able to
25 phagocytose more Zymosan Beads, while stimulation with LPS + IFN γ down-modulates their phagocytic ability. Again, this was comparable to the responses shown by SFCi55 WT macrophages (Figure 8B).

This dCas9 KRAB line and sgRNA delivery at the iPSC stage significantly reduced the time
30 required to produce genetically engineered macrophages. All that is required is to design gRNAs, clone them and package them. After packaging, iPSCs can be infected and differentiated into iPSC-derived macrophages in 4 weeks.

Example 3: dCas9KRAB iPSC line, infection of gRNA at the macrophage stage (genome-engineered macrophages can be obtained in 2 weeks).
35

dCas9KRAB macrophages transduced with a B2M gRNA containing lentivirus and viral like particles containing VPX (Vpx-VLPs) were able to downmodulate B2M compared to SFCi55-

WT macrophages and dCas9KRAB macrophages transduced with lentivirus containing NTCgRNA. (Figure 9). Having a dCas9-KRAB iPSC macrophages in culture now allows to genetically modify these cells in a span of 2 weeks. This line, and this macrophage stage infection system opens up the possibility to do larger scale screens by co-infecting
5 macrophages with multiple gRNAs against different genes and evaluating macrophage phenotype.

Example 4: Generation of a CRISPRa iPSC-DM system (UCOE dCas9VPR iPSC line to activate/up-regulate genes in iPSC-derived macrophages)

10 A CRISPR activation system (dCas9-VPR line) was generated to up-regulate genes that are not normally expressed in iPSC-derived macrophages, and/or further increase the expression of genes that are already expressed in the system. This tool can improve current *in vitro* models (by activating genes to change the phenotype of culture macrophages, for example, steer them to a more tumor associated macrophage behavior). Since gene activation is faster
15 than gene down-modulation, using this system allows to obtain genetically modified macrophages in 1 week. CRISPRa systems use a deactivated/dead Cas9 (dCas9) that binds to the target genomic region with the same efficiency as Cas9 but cannot generate a double strand break. The dCas9 is fused to activator domains, and when it uses a guide RNA, it targets a region before the promoter of a specific gene and drives its transcriptional activation.

20 Using the PL-22.0071 *PB-UCOE_{CBX3}-pCAG-dCas9-VPR-T2A-GFP-Pa* SEQ ID NO: 14 (CRISPRa plasmid) we derived a clonal line that contained a dCas9-VPR cassette (Figure 10A): the dCas9 VPR A18 line. The genetic cargo comprises about 10.4kb, which is very large. It would be very challenging to produce lentivirus at a good titer with such a cargo. dCas9VPR
25 macrophages transduced with a CD4 gRNA or SIGLEC10gRNA containing lentivirus and viral like particles (Vpx-VLPs) were able to up-regulate their respective genes when compared to dCas9VPR macrophages transduced with lentivirus containing NTCgRNA. (Figure 10B, 10C).

Cell-surface marker expression in the dCAS9 VPR iPSC-derived macrophages was checked
30 and the dCas9VPR differentiated cells expressed pan-macrophage markers (Figure 10D). Furthermore, macrophages from the dCas9VPR Line were able to phagocytose Zymosan™ beads (phagocytosis levels were the same as the SFCi55 WT macrophages) (Figure 10E). Engineered macrophages from the dCas9 VPR Line also responded to stimuli. Upon IL-10 stimulation, they were able to phagocytose more Zymosan Beads, while
35 stimulation with LPS + IFN γ down-modulates their phagocytic ability. Again, this was comparable to the responses shown by SFCi55 WT macrophages (Figure 10E)

Of note, up-regulating genes via this dCas9-VPR system in macrophages represents gives several advantages over traditional genetic over-expressing systems: 1; Activation of genes in as little as one-week, 2: Larger scale screens are enabled by co-infecting macrophages with multiple gRNAs against different genes and activating multiple genes in one-go, 3: Activation
5 of endogenous gene transcription and translation. This system of gene activation is less artificial than over-expressing genes via an exogenous promoter and a codon optimized gene sequence.

References

1. Luo, Y. *et al.* Stable Enhanced Green Fluorescent Protein Expression After Differentiation and Transplantation of Reporter Human Induced Pluripotent. *Stem Cells Trans Med* **3**, 821–835 (2014).
- 5 2. Lopez-Yrigoyen, M., Fidanza, A., Cassetta, L., Axton, R. A., Taylor, A. H., Meseguer-Ripolles, J., Tsakiridis, A., Wilson, V., Hay, D. C., Pollard, J. W., & Forrester, L. M. (2018). A human iPSC line capable of differentiating into functional macrophages expressing ZsGreen: a tool for the study and in vivo tracking of therapeutic cells. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1750). <https://doi.org/10.1098/RSTB.2017.0219>
- 10 3. Lopez-Yrigoyen, M., Yang, C. T., Fidanza, A., Cassetta, L., Taylor, A. H., McCahill, A., Sellink, E., von Lindern, M., van den Akker, E., Mountford, J. C., Pollard, J. W., & Forrester, L. M. (2019). Genetic programming of macrophages generates an in vitro model for the human erythroid island niche. *Nature Communications* 2019 10:1, 10(1), 1–11. <https://doi.org/10.1038/s41467-019-08705-0>
- 15 4. Ackermann, M. *et al.* Ex vivo Generation of Genetically Modified Macrophages from Human Induced Pluripotent Stem Cells. *Transfusion Medicine and Hemotherapy* **44**, 135–142 (2017).
5. Müller-Kuller, U. *et al.* A minimal ubiquitous chromatin opening element (UCOE) effectively prevents silencing of juxtaposed heterologous promoters by epigenetic remodeling in multipotent and pluripotent stem cells. *Nucleic Acids Research* **43**, 1577–1592 (2015).
- 20 6. Yant, S. R. *et al.* High-Resolution Genome-Wide Mapping of Transposon Integration in Mammals. *Molecular and Cellular Biology* **25**, 2085–2094 (2005).
7. Lopez-Yrigoyen, M., May, A., Ventura, T., Taylor, H., Fidanza, A., Cassetta, L., Pollard, J. W., & Forrester, L. M. (2020). Production and characterization of human macrophages from pluripotent stem cells. *Journal of Visualized Experiments*, 2020(158). <https://doi.org/10.3791/61038>
- 25

8. Barkal, A. et al. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature*, 572, 392–396 (2019)
9. Tian R. et al. CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons. *Neuron*, **104**(2):239-255 (2019)
- 5 10. Drager, Nina et al. A CRISPRi/a platform in iPSC-derived microglia uncovers regulators of disease states Biorxiv, preprint (2022)
11. Huang, Xinbo et al. A Light-Inducible Split-dCas9 System for Inhibiting the Progression of Bladder Cancer Cells by Activating p53 and E-cadherin. *Front. Mol. Biosci* **7**: (2021)
- 10 12. Skipper KA, Hollensen AK, Antoniou MN, Mikkelsen JG. Sustained transgene expression from sleeping beauty DNA transposons containing a core fragment of the HNRPA2B1-CBX3 ubiquitous chromatin opening element (UCOE). *BMC Biotechnol.* (2019).
13. Nègre, D., Mangeot, P. E., Duisit, G., Blanchard, S., Vidalain, P. O., Leissner, P., Winter, A. J., Rabourdin-Combe, C., Mehtali, M., Moullier, P., Darlix, J. L., & Cosset, F. L. (2000).
- 15 Characterization of novel safe lentiviral vectors derived from simian immunodeficiency virus (SIVmac251) that efficiently transduce mature human dendritic cells. *Gene Therapy* 2000 **7**:19, 7(19), 1613–1623. <https://doi.org/10.1038/sj.gt.3301292>
14. Yang, C. T., Ma, R., Axton, R. A., Jackson, M., Taylor, A. H., Fidanza, A., Marenah, L., Frayne, J., Mountford, J. C., & Forrester, L. M. (2017). Activation of KLF1 Enhances the
- 20 Differentiation and Maturation of Red Blood Cells from Human Pluripotent Stem Cells. *Stem Cells (Dayton, Ohio)*, **35**(4), 886–897. <https://doi.org/10.1002/STEM.2562>

Sequence Listing Information

25 SEQ ID NO: 1 UCOE

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SEQ ID NO: 5 bovine growth hormone polyadenylation signal (bGH poly(A) signal)

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SEQ ID NO: 9 dCas9-KRAB (a dead/deactivated Cas9 with the repression domains and a nuclear localisation signal sequence)

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ccc

5

SEQ ID NO: 10 dCas9-VPR sequence

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Plasmid sequences

SEQ ID NO: 11 PL-21.0001 PB- UCOE_{CBX3}-DUO-CMV-SIGLEC10-EF1short-copGFP-PURO

Base vector was PiggyBacDualProm (SEQ ID NO: 12). Human SIGLEC10 codon optimized
sequence was inserted immediately after CMV promoter and UCOE sequence was inserted
30 immediately upstream of the CMV promoter.

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45 **SEQ ID NO: 12** PL-20.0002 PiggyBacDualProm: Base vector PB-CMV-Restriction enzyme fragment-EF1alpha short/truncated-copGFP-T2A-PuroR.

Purchased from Gentaur (not used for deriving lines as is, but used to derive first Macomics over-expression plasmids).

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35 **SEQ ID NO: 13** PL-22.003 *PB-UCOE_{CBX3}-pCAG-dCas9-KRAB-T2A-eGFP-pA (CRISPRi plasmid)*

PiggyBac expression vector containing UCOE_{CBX3} to protect expression of dCas9-KRAB on
 iPSC derived macrophages with eGFP reporter fused via T2A cleavable peptide linker.
 Designed by Macomics and synthesized by vector builder

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SEQ ID NO: 14 PL-22.0071 PB-UCOE_{CBX3}-pCAG-dCas9-VPR-T2A-GFP-Pa (CRISPRa plasmid)

30 Macomics produced. This is a derivative of PL-22.003 (SEQ ID NO: 13). dCAS9 KRAB has been swapped for dCas9 VPR sequence to enable CRISPRa. eGFP is kept as selectable marker.

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43

44

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SEQ ID NO: 15 PL-22.0050 LV-UCOE_{CBX3}-pU6-stuffer-pEF1a-mTagBFP2-T2A-Puro-dWPRE

Macomics produced. Derivative of PL-22.008 with WPRE swapped to introduce point mutations inactivating any potential expression of oncogenic protein X.

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SEQ ID NO: 16 PL-21.0067 *LV-UCOE_{CBX3}-pU6-stuffer-pEF1a-eGFP-T2A-Puro-WPRE*

Macomics designed and vector Builder synthesized. Lentiviral expression vector containing
 UCOE_{CBX3}, a human U6 promoter to drive sgRNA expression and EF1a promoter driving
 30 eGFP-T2A-Puro_R expression. sgRNA are cloned into this base vector by restriction digest to
 remove stuffer and ligation of sgRNA DNA sequences.

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15 *sgRNA sequences*

CRISPRi

SEQ ID NO: 17 *NTC#3i* gtgtgcaacctccgcttg (CRISPRi.v2 Weissman library)

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SEQ ID NO: 19 *B2M#3i* ggccacggagcgagacatct (CRISPRi.v2 Weissman library)

20 **CRISPRa**

SEQ ID NO: 20 *NTC#1a* tgtctgatgcgtagacgg (CRISPRa.v2 Weissman library)

SEQ ID NO: 21 *SIGLEC10#1a* aaggtggccggagagtgt (CRISPRa.v2 Weissman library)

SEQ ID NO: 22 *CD4#1a* gtgaaatgccaaagtcaa (CRISPRa.v2 Weissman library)

SEQ ID NO: 23 *PDL1#1a* cggcggaagctttcagttt (CRISPRa.v2 Weissman library)

25

SEQ ID NO: 24 PL-20.004 Super PiggyBac Transposase

Purchased from Gentaur. Co-transfected to mediate genomic integration of PiggyBac expression vectors

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35 Plasmid used to derive CD24+ Jurkat cells

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Claims

1. A transposon comprising a Ubiquitous Chromatin Opening Element (UCOE) nucleic acid sequence, a nucleic acid sequence of a gene of interest or a fragment thereof and optionally a 3' polyadenylation sequence under control of a promoter, flanked by Piggybac™ transposon-specific inverted terminal repeat sequences PB 5' ITR and PB 3' ITR.
2. A transposon of claim 1, wherein the UCOE is a CBX3-UCOE nucleic acid sequence that precedes CBX3.
3. A transposon of claim 1 or claim 2, wherein the UCOE is a nucleic acid sequence of SEQ ID NO: 1.
4. A transposon of claim 1, wherein the Piggybac™ transposon-specific inverted terminal repeat sequences are PB 5' ITR (SEQ ID NO: 2) and PB 3' ITR (SEQ ID NO: 3).
5. A transposon of any preceding claim wherein the promoter, gene of interest or fragment thereof and optionally 3' polyadenylation sequence are comprised within an expression cassette.
6. A transposon of any preceding claim wherein expression cassette comprises:
- (a) a promoter,
 - (b) at least one cloning site (restriction enzyme cleavage site) or a multiple cloning site downstream of the promoter,
 - (c) a gene of interest downstream of and operably linked to the promoter,
 - (d) a polyadenylation sequence positioned downstream of and operably linked to the gene of interest,
 - (e) optionally an internal ribosome entry site,
 - (f) optionally a second promoter sequence positioned downstream of the gene of interest and polyadenylation sequence,
 - (g) optionally a fluorescent protein sequence positioned downstream of the second promoter,
 - (h) a self-cleaving peptide sequence,
 - (i) optionally a gene for selection, e.g., for antibiotic selection,
 - (j) optionally a fluorescent protein gene, and
 - (k) optionally a second polyadenylation sequence.
7. A transposon of claim 6 wherein the expression cassette comprises:

- (a) a CMV promoter,
- (b) a multiple cloning site downstream of the promoter,
- (c) a gene of interest downstream of and operably linked to the promoter, optionally wherein the gene of interest is a human SIGLEC10 gene of SEQ ID NO: 4,
- 5 (d) a bovine growth hormone polyadenylation signal (bGH poly(A) signal) (SEQ ID NO: 5) positioned downstream of and operably linked to the gene of interest,
- (e) optionally an internal ribosome entry site,
- (f) an EF1-alpha (short version) (SEQ ID NO: 6) sequence positioned downstream of the gene of interest and polyadenylation sequence, and
- 10 (g) a fluorescent protein (CopGFP (SEQ ID NO: 7)) sequence positioned downstream of the second promoter,
- (h) a T2A self-cleaving peptide sequence (SEQ ID NO: 8) positioned downstream of the fluorescent protein sequence,
- (i) a Puromycin resistance gene, and
- 15 (j) a SV40 Poly(A): Simian virus 40 polyadenylation sequence.

8. A transposon of claim 6 wherein the expression cassette comprises:

- (a) a CAG promoter,
- (b) a multiple cloning site downstream of the promoter,
- 20 (c) a gene of interest downstream of and operably linked to the promoter, optionally wherein the gene of interest is a dCas9-KRAB sequence of SEQ ID NO: 9 (a dead/deactivated Cas9 with the repression domains and a nuclear localisation signal sequence),
- (d) a T2A self-cleaving peptide sequence (SEQ ID NO: 8) positioned downstream of the gene of interest, e.g., dCas9-KRAB sequence of SEQ ID NO: 9, and
- 25 (e) a fluorescent protein gene sequence encoding eGFP (enhanced green fluorescent protein) positioned downstream of the T2A self-cleaving peptide sequence (SEQ ID NO: 8),
- (f) an rBG polyadenylation sequence.

9. A transposon of claim 6 wherein the expression cassette comprises:

- 30 (a) a CAG promoter,
- (b) a multiple cloning site downstream of the promoter,
- (c) a gene of interest downstream of and operably linked to the promoter, optionally wherein the gene of interest is a dCas9-VPR sequence of SEQ ID NO: 10,
- (d) a T2A self-cleaving peptide sequence positioned downstream of the gene of interest, e.g.,
- 35 dCas9-VPR sequence of SEQ ID NO: 10, and

- (e) a fluorescent protein gene sequence encoding eGFP (enhanced green fluorescent protein) positioned downstream of the T2A self-cleaving peptide sequence (SEQ ID NO: 8),
- (f) an rBG polyadenylation sequence.

5 10. A vector comprising a transposon of any preceding claim, preferably wherein the vector is a PiggyBac® vector, such as a plasmid vector of SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 14.

10 11. A cell comprising transposon and / or vector of any one of claims 1 to 10, optionally comprising a second vector capable of expressing a transposase, preferably wherein the transposase is a PiggyBac™ transposase, (e.g., Super PiggyBac™ transposase plasmid vector of SEQ ID NO: 24).

15 12. A cell comprising transposon of any one of claims 1 to 11, wherein the transposon (expression construct / transgene) is integrated into the cell genome.

13. A cell according to claim 11 or claim 12 wherein the cell is selected from an undifferentiated cell, a partially differentiated cell and a fully differentiated cell, optionally wherein differentiation status is assessed by using a panel of flow cytometry markers.

20 14. A cell according to any one of claims 11 to 13, wherein the cell is selected from an induced pluripotent stem (iPSC), a partially or fully differentiated iPSC-derived macrophage, iPSC-derived neutrophil, NK, monocyte, myocyte, hepatocyte, neuron and a progenitor cell.

25 15. A method for:
(a) preventing silencing of a gene of interest (transgene)
(b) promoting expression of a gene of interest,
(c) expressing a product of a gene of interest, and / or
(d) expressing a larger (≥ 7.0 Kb) expression cassette,
30 comprising culturing a cell according to any one of claims 11 to 14.

16. A method for:
(a) preventing silencing of a gene of interest (transgene) and / or
(b) maintaining expression of a gene of interest during differentiation,
35 comprising culturing a cell according to any one of claims 11 to 14 in the presence of a differentiation stimulus.

17. A method for knockdown (downregulation) of expression of a target gene comprising culturing a cell according to any one of claims 11 to 14 comprising dCas9-KRAB in the presence of the target gene-directed gRNA (e.g., delivered via lentiviral vector).

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18. A method for inducing or upregulating expression of a target gene comprising culturing a cell according to any one of claims 11 to 14 comprising dCas9-VPR in the presence of the target gene-directed gRNA (e.g., delivered via lentiviral vector).

10 19. A composition comprising a transposon, vector and / or cell of any one of claims 1 to 14 and a carrier.

20. A composition comprising a transposon, vector and / or cell of any one of claims 1 to 14 and a vector capable of expressing transposase.

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21. A gene transfer system comprising a transposon, vector, and / or cell of any one of claims 1 to 14 and a transposase.

22. A gene transfer system of claim 21, wherein the transposase is a Piggybac™ transposase.

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23. A method for modulating expression of a gene of interest in a cell, comprising

(a) introducing a vector of claim 10 to a cell,

(b) introducing a vector encoding a transposase, preferably a Piggybac™ transposase to the cell and

25 (c) introducing a vector containing a guide RNA capable of controlling the expression of the target gene to the cell.

24. A method for integrating a gene of interest into the genome of a cell comprising contacting a cell with the gene transfer system of claim 21, thereby integrating the gene of interest into
30 the cell.

25. A method for knockdown (downregulation) of a target gene in a cell comprising contacting a cell with the gene transfer system of claim 21 thereby to introduce and integrate dCas9-KRAB and introducing a gRNA (preferably delivered via lentiviral vector) to the cell to
35 knockdown expression of the target gene.

26. A method for upregulation of a target gene in a cell comprising contacting a cell with the gene transfer system of claim 21 thereby to introduce and integrate dCas9-VPR and introducing a gRNA (preferably delivered via lentiviral vector) to the cell to upregulate expression of the target gene.

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