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Lab Resource: Genetically-Modified Single Cell Line

Generation of a genetically encoded voltage indicator MARINA reporter human iPS cell line using Cas9 (VULSCi002-A-2)

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ABSTRACT

Fluorescent protein-based Genetically Encoded Voltage Indicators (GEVI) offer a remarkable system for high-throughput screening of membrane potential phenotypes. The GEVI MARINA is a derivative from ArcLight, which conversely to ArcLight increases its fluorescence intensity alongside depolarization. Here we created knock-in reporter human iPS cell lines carrying the *MARINA* reporter using *Sp*Cas9 programmable nuclease and characterize a heterozygous clone.

1. Resource table

Unique stem cell line identifier	Cell line 1: VULSCi002-A-2
Alternative name(s) of stem cell line	Cell line 1: CIGLi001-A-2
Institution	Vilnius University Life Science Center – EMBL Partnership Institute for gene editing technologies
Contact information of the reported cell line distributor	Head of laboratory, nuclease-enabled cell therapies jonathan.arias@gmc.vu.lt
Type of cell line	Human iPS cell
Origin	Human
Additional origin info	Age: 30 Gender: Female Ethnicity: Hispanic
Cell Source	Peripheral blood-derived endothelial progenitor cells (EPCs)
Method of reprogramming	mRNA
Clonality	clonal
Evidence of the reprogramming transgene loss	Non-integrative reprogramming
The cell culture system used	StemFit complete media (defined culture conditions)
Type of the Genetic Modification	Transgene generation: GNAT family puromycin N-acetyltransferase VULSCi002-A-2: Heterozygous knock-in of MARINA reporter
Associated disease	None reported
Gene/locus modified in the reported transgenic line	Gene of insertion: *PPP1R12C* (Gene ID: 54776) Gene inserted: *MARINA* (Gene ID: PQ094238) including a variant of *Ciona intestinalis* Voltage-Sensitive Phosphatase D389A, D390A, Y442V and A469D

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(*continued*)

	(NP_001028998.1) containing the inwardly rectifying potassium channel KIR2.1 channel Golgi to plasma membrane split trafficking signal (NP_000882.1), and the super ecliptic pHluorin containing positive relation mutations D389A, H390A and Y442V and A469D.
Method of modification and user-customizable nuclease (UCN) used	CRISPR Cas9-mediated homology directed repair (Addgene ID 223323 carrying T2 sgRNA, Addgene ID 223321 CM^r ccdB empty, Addgene ID 223328 donor)
User-customizable nuclease (UCN) delivery method	Encoding plasmid electroporation
All double-stranded DNA genetic material molecules introduced into the cells	Gene-editing plasmids: 1. CRISPR *Sp*Cas9 plasmid carrying T2 sgRNA (Addgene plasmid ID 223323) 2. Donor plasmid (Addgene plasmid ID 223328)
Evidence of the absence of random integration of any plasmids or dsDNA introduced into the cells	WGS analysis: wgs_S11105Nr2 – VULSCi002-A-2 wgs_S11105Nr6 – parental CIGLi001-A
Analysis of the nuclease-targeted allele status	PCR genotyping, Sanger sequencing and WGS
Homozygous, Heterozygous allele status validation	Genotyping
Method of the off-target nuclease activity prediction and surveillance	COSMID, CCTop, and Cas-OFFinder prediction for up to 4 bp off-target site analyzed by WGS
Descriptive name of the transgene	*MARINA* depolarization fluorescent reporter (GENE ID: PQ094238)
Eukaryotic selective agent resistance cassettes	Constitutive GNAT family puromycin N-acetyltransferase (GENE ID: WP_335902160.1)
Expression system details	Constitutive – CAG
Date archived/stock creation date	22 April 2024
Cell line repository/bank	Cell line 1: https://hpscreg.eu/cell-line/CIGLi001-A-2
Ethical/GMO work approvals	Cell source was acquired from https://hpscreg.eu/cell-line/CIGLi001-A Ethical approval 2023/6–1524-984
Addgene public access repository recombinant DNA sources	Plasmids created in this study: Addgene 223321 Addgene 223323 Addgene 223328

2. Resource utility

Human-induced Pluripotent Stem (iPS) cells can be differentiated into the entirety of adult cell types and can be used as a platform for phenotype rescue in therapeutic screening. Genetically encoded reporters such as MARINA allow evaluating depolarization phenotypes without the need for electrodes or external staining agents during screening campaigns.

3. Resource details

The movement of ions across the plasma membrane can be affected by genetic causes and pharmaceutical side-effects. Depolarization abnormalities can stratify cardiac and neuronal lineage pathologies. The transient expression of reporters and the use of membrane-permeable dyes to monitor depolarization is limited due to their low efficiency, short expression time or associated reduction in viability. These aspects make them incompatible with long-term assays as differentiation. GEVI reporter lines overcome these challenges.

MARINA GEVI was the first positively correlated depolarization reporter (Platisa et al., 2017). Here, we generated a knock-in human iPS cell line with heterozygous (VULSCi002-A-2) transgene dose using the programmable Cas9 nuclease (Table 1). We cloned an sgRNA targeting the first intron of the *PPP1R12C* gene (Mali et al., 2013) (Fig. 1A) into a dual plasmid expressing *Sp*Cas9 (Addgene 223321), which resulted in Addgene 223323. We created a Homology Directed Repair (HDR) donor template expressing the MARINA reporter and with homology to PPP1R12C (Addgene 223328) as shown in Fig. 1A. A model of MARINA reporter protein and its topology is shown in Fig. 1A.

The parental healthy genetic background human iPS cells, CIGLi001-A, were electroporated with the nuclease-containing and donor vectors. After electroporation, the human iPS cells were seeded on feeder-free culture conditions laminin-511, and HDR events selected with puromycin. Emerging clones were isolated (50 clones) using a stereomicroscope, expanded and genotyped (12 clones). From the various heterozygous (11 clones) and homozygous (1 clone) lines identified, one heterozygous (VULSCi002-A-2) clone was selected (Fig. 1B). We characterized the second allele of the heterozygous clone through sanger sequencing and confirmed its wildtype status (Fig. 1C). Off-target sites were predicted with COSMID, CCTop, and Cas-OFFinder, and screened on Whole Genome Sequencing (WGS) reads. The clone VULSCi002-A-2 was found to be free from indels in all predicted off-targets on coding sequences.

VULSCi002-A-2 iPS cell line exhibited morphology of primed pluripotency (Fig. 1D). Nuclear localization of pluripotency transcriptional factors NANOG, OCT4 and SOX2 was confirmed by immunocytochemistry (Fig. 1E). Primed pluripotency markers CD90, CD24 and SSEA4 (Fig. 1F), as well as dual primed-naive pluripotency PDPN and TRA1-81 were positive (Fig. 1F). Microarray karyotype analyses of the edited lines did not reveal differences to the parental healthy iPS cell line CIGLi001-A (Fig. 1G). The edited line retained the capacity to differentiate into double positive PAX6 and SOX2 ectoderm, CXCR4(CD184) positive endoderm, as well as CD140b and CD144 positive mesoderm populations when evaluated through flow cytometry (Fig. 1H). Absolute quantification of genomic DNA confirmed the genetic dose of the MARINA GEVI reporter was 1.16 copies when compared to two somatic loci in *EOMES* (2.01) and one locus in *PAX6* (2.00) (Fig. 1I). Gene dose was consistent when compared with parental line CIGLi001-A and unrelated control SK006.4. Expression of the reporter was confirmed by the nuclear localization of the T2A cleaved SV40 NLS-mCherry (Fig. 1J).

4. Materials and methods

4.1. Molecular biology

The *MARINA* gene (Platisa et al., 2017) (Addgene 85843) is a derivative from ArcLight (Jin et al., 2012), co-expressing T2A-NLS-mCherry. *MARINA* was cloned into a donor plasmid (Arias et al., 2019) with deleted sgRNA target site (Addgene 223328). The sgRNA-T2 (Mali et al., 2013) was cloned in plasmid Addgene 223321, derived from pX330 (Ran et al., 2013), resulting in plasmid Addgene 223323.

4.2. Human iPS cell culture, electroporation, selection and genotyping

The human iPS cell line CIGLi001-A was cultured on Stemfit media (Ajinomoto – AK04C) on laminin 511 (Nippi – 892021). Cells were passaged as single cells using accutase and cultured with 10μM of Y27632 (Tocris – 1254) for 24h after passaging. For electroporation, 1M cells were resuspended in Opti-MEM with 3μg of donor (Addgene 223328) and 1μg of nuclease plasmid (Addgene 223323) and pulsed using a NEPA21 system (Nepagene) (Table 2). The cells were selected with puromycin (0.25 to 1μg/mL) (Tocris – 4089). Colonies were picked using a stereomicroscope (Leica M165C). Genomic DNA was purified (Qiagen – 69504) and genotyped using the primers indicated in Table 2.

Table 1
Characterization and validation.

Classification	Output type	Result	Data
Transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification	A visual representation of edited allele, showing exon structure and protein product showing important functional elements.	Fig. 1A
Morphology	Microscopy	Normal primed pluripotent human iPS cell morphology	Fig. 1D
Pluripotency status evidence for the described cell line	Qualitative analysis (Immunocytochemistry)	Nuclear expression of transcriptional factor: OCT3/4, SOX2 and NANOG	Fig. 1E
	Quantitative analysis (Flow cytometry)	SSEA4: 99.5 %, TRA(1–81): 85.6 %, PDPN: 98.9 %, CD90: 83.1 %, CD24: 71.5 % *All gates positioned regarding matching isotype controls	Fig. 1F
Karyotype	Microarray-based karyotype	VULSCi002-A-2: 46XX CIGLi001-A: 46XX *Resolution 450–500 bps	Fig. 1G
Genotyping for the desired genomic alteration/allelic status of the gene of interest	Targeted allele-specific PCR	PCR specific to desired knock-in donor cargo, right homology arm junction, left homology arm junction and wild type junction	Fig. 1B and C
	Evaluation of the homozygous or heterozygous status of introduced genomic alteration(s)	Absolute qPCR of transgene dose in relation to somatic genes *EOMES* and *PAX6*.	Fig. 1B and I
	Transgene-specific PCR	Absolute qPCR product encompassing the transgene	Fig. 1I
Verification of the absence of random plasmid integration events	WGS	WGS data analyses Absolute quantification of PCR product encompassing the transgene	wgs_S11105Nr2 – VULSCi002-A-2 wgs_S11105Nr6 – parental CIGLi001-A Fig. 1I
Parental and modified cell line genetic identity evidence	STR analysis	Matched electropherogram including D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, AMEL, vWA, D8S1179, TPOX and FGA	Supplementary material
Mutagenesis / genetic modification outcome analysis	Genomic DNA PCR, Sequencing, qPCR of transgene expressed and transgene expression validation	Confirmation of the precise nature of the introduced *MARINA* reporter, also heterozygous status, status of second allele in heterozygous clone, genetic dose through absolute quantification of transgene, functional validation of reporter through microscopy	Fig. 1B, C, I and J
	PCR-based analyses	Detection of correctly targeted and randomly integrated selectable targeting construct status	Fig. 1B and I
	WGS	Number of insertions in the genome, off-target effects	sequencing deposited at repository: wgs_S11105Nr2 – VULSCi002-A-2 wgs_S11105Nr6 – parental CIGLi001-A
Off-target nuclease activity analysis	WGS	Demonstration of the lack of NHEJ-caused mutagenesis in the top predicted off-target Cas9 nuclease binding sites for relevant sgRNA	COSMID, CCTop, and Cas-OFFinder in-silico analysis over wgs_S11105Nr2 and wgs_S11105Nr6
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by RT-PCR	Supplementary material
Multilineage differentiation potential	Directed lineage differentiation	Quantitative differentiation capacity by flow cytometry. Ectoderm: PAX6 and SOX2 double positive 47.5 %. Endoderm: CXCR4 positive 94 %. Mesoderm: CD144 single positive 12.3 % and CD140b single positive 83.9 %.	Fig. 1H
Outcomes of gene editing experiment	Brief description of the outcomes in terms of clones generated/establishment approach/screening outcomes	Total of 50 clones established and targeted by HDR	Clones established 50, Clones characterized 12, heterozygous 11 (1 selected: VULSCi002-A-2), homozygous 1
Genotype – additional histocompatibility information	HLA genotyping	NGS *HLA* typing class I and class II	VULSCi002-A-2, and CIGLi001-A: *HLA-A* 23:01:01, 29:02:01; *HLA-B* 44:03:01, 44:03:01; *HLA-C* 04:01:01, 16:01:01; *HLA-E* 01:01:01, 01:03:02; *HLA-F* 01:01:01, 01:03:01; *HLA-G* 01:01:01, 01:04:04; *HLA-H* 02:02:01, 02:02:01 *DPA1* 01:03:01, 02:01:01; *DPB1* 04:01:01, 17:01:01; *DQA1* 02:01:01, 03:03:01; *DQB1* 02:02:01, 03:02:01, *DRB1* 04:05:01, 07:01:01; *DRB4* 01:01:01G, –:–:–;

4.3. Immunocytochemistry

Engineered human iPS cells were plated on ibidi chamber slides (Ibidi – 80826) and fixed with paraformaldehyde 4%. Cells were permeabilized with Triton-X 0.3% and stained with the antibodies listed in Table 2. Nuclei were stained using DAPI. Cells were imaged on a TCS SP8 laser scanning confocal microscope. Images were analyzed using ImageJ software (version 2.1.0/1.53c).

4.4. Flow cytometry for pluripotency and differentiation

Human iPS cell lines (0.25M) were stained with primary-conjugated

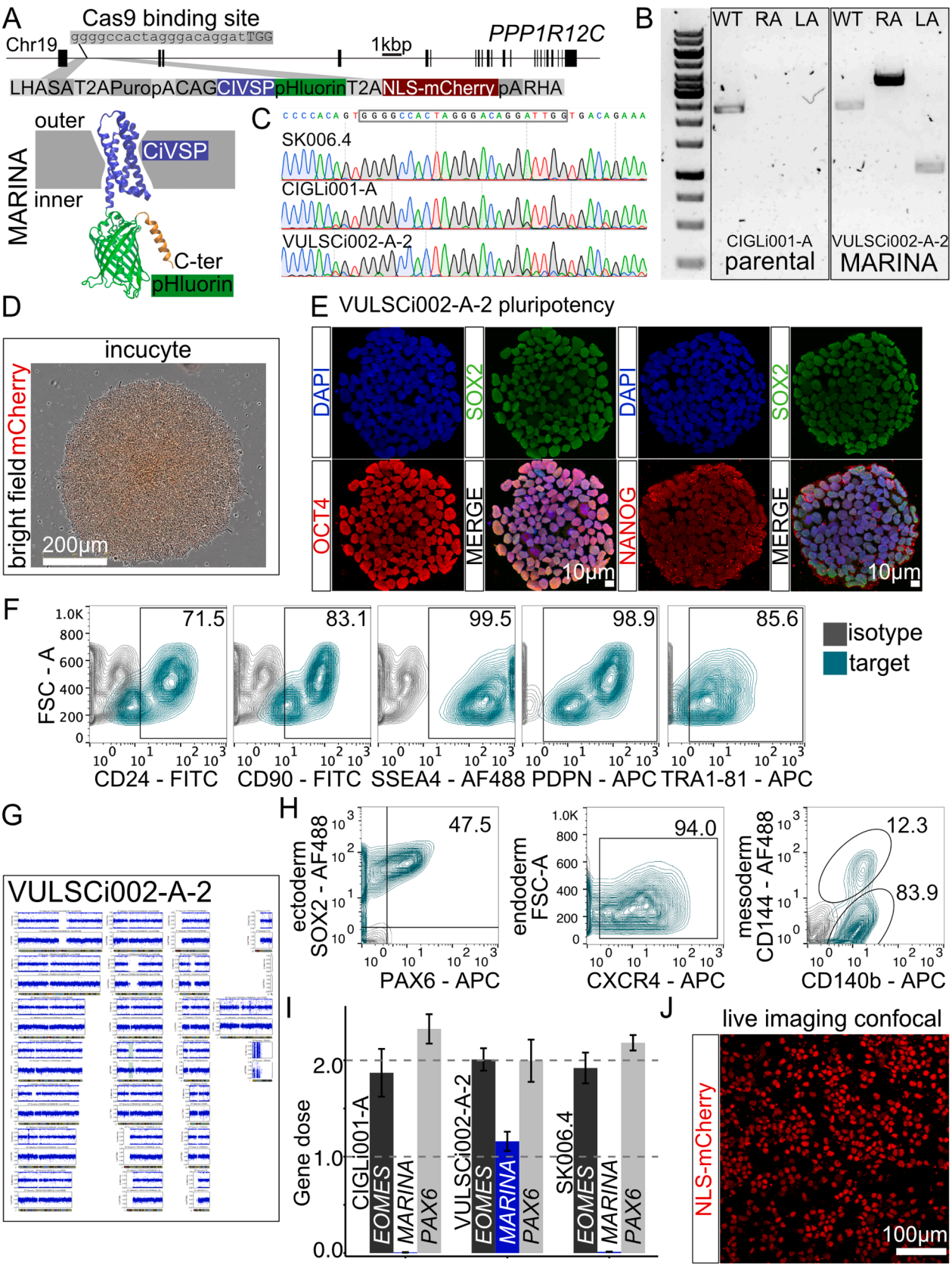

Fig. 1. Generation of genetically encoded *MARINA* human iPS cell line using Cas9.

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry and flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Anti-NANOG clone 1E6C4	unreported concentration (1:100 dilution)	Thermofisher cat no. MA5-15469, RRID AB_10980986
	Anti-OCT4 clone 9B7	at 1μg per well (1:100 dilution)	Thermofisher cat no. MA1-104, RRID: AB_2536771
	Anti-SOX2 clone Btjce	at 0.5μg per well (1:100 dilution)	Thermofisher cat no. 14–9811-82, RRID: AB_11219471
Secondary antibodies	AF488-conjugated goat anti-rat IgG	at 0.4μg per well (1:500 dilution)	Thermofisher cat no. A-11006, RRID: AB_2534074
	APC-conjugated goat anti-mouse IgG	at 0.2μg per well (1:500 dilution).	Thermofisher cat no. A-865, RRID: AB_2536211
Nuclear staining	DAPI	at 0.1μg per well	ThermoFisher cat no. 62247
Flow cytometry			
Primed and naive pluripotency markers	Anti-Podoplanin clone NZ-1.3, APC	0.125μg per reaction (dilution 1:20)	Thermofisher cat no. 17–9381-42, RRID: AB_10801951
	Anti-TRA-1–81 clone TRA-1–81, APC	0.5μg per reaction (dilution 1:10)	Thermofisher cat no. 17–8883-42, RRID: AB_10597905
Primed pluripotency markers	Anti-CD24 clone eBioSN3 (SN3 A5-2H10), FITC	0.25μg per reaction (dilution 1:20)	Thermofisher cat no. 11–0247-42, RRID: AB_10854886
	Anti-CD90 clone eBio5E10 (5E10), FITC	1μg per reaction (dilution 1:20)	Thermofisher cat no. 11–0909-42, RRID: AB_10668828
	Anti-SSEA4 clone eBioMC-813–70 (MC-813–70), Alexa Fluor 488	0.5μg per reaction (dilution 1:40)	Thermofisher cat no. 53–8843-42, RRID: AB_10609335
Isotype controls	rat IgG2a kappa-APC clone eBR2a	0.32μg per reaction (dilution 1:62.5)	Thermofisher cat no. 17–4321-81, RRID: AB_470181
	mouse IgG1 kappa-FITC clone P3.6.2.8.1	1μg per reaction (dilution 1:50)	Thermofisher cat no. 11–4714-82, RRID: AB_470022
	mouse IgG3 kappa-Alexa Fluor 488 clone J606	0.5μg per reaction (dilution 1:40)	BD Biosciences cat no. 563636, RRID: AB_2818997
	mouse IgM-APC clone 11E10	0.5μg per reaction (dilution 1:40)	Thermofisher cat no. 17–4752-80, RRID: AB_10670203
	human IgG1-APC REA control clone REA293	0.3μg per reaction (dilution 1:50)	Miltenyi Biotec cat no. 130–113-446, RRID: AB_2733446
	rat IgG2a kappa- Alexa Fluor 488 clone R35-95	0.5μg per reaction (dilution 1:40)	BD Biosciences cat no. 557676, RRID: AB_396787
	mouse IgG1 kappa- Alexa Fluor 488 clone X40	0.25μg per reaction (dilution 1:80)	BD Biosciences cat no. 567121, RRID: AB_2918008
	mouse IgG1 kappa-APC clone X40	0.25μg per reaction (dilution 1:80)	(BD Biosciences cat no. 567155, RRID: AB_2918009
	mouse IgG2a kappa-APC clone G155-178	0.25μg per reaction (dilution 1:80)	BD Biosciences cat no. 550882, RRID: AB_10055999
Ectodermal differentiation markers	Anti-SOX2 clone Btjce, AF488	0.5μg per reaction (dilution 1:100)	Thermofisher cat no. 53–9811-82, RRID: AB_2574479
	Anti-PAX6 clone REA507, APC	unreported concentration (dilution 1:50)	Miltenyi Biotec cat no. 130–123-328, RRID: AB_2819477
Mesodermal differentiation markers	Anti-CD144 (VE-cadherin) clone 16B1, AF488	0.25μg per reaction (dilution 1:20)	Thermofisher cat no. 53–1449-42, RRID: AB_10753926
	Anti-CD140b (PDGFRB) clone 18A2, APC	unreported concentration (dilution 1:10)	Thermofisher cat no. A15719, RRID: AB_2534499)
Endodermal differentiation marker	Anti-CD184 (CXCR4) clone 12G5, APC	0.25μg per reaction (dilution 1:20)	Thermofisher cat no. 17–9999-42, RRID: AB_1724113
Cell viability	SYTOX Blue	1μM	Thermofisher cat no. S34857
Site-specific nuclease			
Nuclease information	*Sp*Cas9		
Delivery method	Electroporation conditions: Poring pulse: Voltage: 125V, Pulse width: 5ms, Pulse interval: 50ms, Frequency: 2, Decay rate: 10%, Polar character: +/- Transfer pulse: Voltage: 20V, Pulse width: 50ms, Pulse interval: 50ms, Frequency: 5, Decay rate: 40%, Polar character: +/-		
Selection/ enrichment strategy	Selection based on puromycin selection and clonal expansion		
Primers and Oligonucleotides used in this study			
	Target	Forward/Reverse primer (5′-3′)	
Knock-in genotyping	Right homology arm junction spanning cargo to genome	FW: GCCTACCCTGGTCACAACTCT RE: AACCCCCTAGCCACTAAGGC	
Knock-in genotyping	Left homology arm junction spanning cargo to genome	FW: CCTGAGTCCGGACCACTTTG RE: CGTGGGCTTGTACTCGGT	
WT allele genotyping	Outside of homology arms	FW: CCTGAGTCCGGACCACTTTG RE: AACCCCCTAGCCACTAAGGC	
Sequencing of nuclease binding site	SpCas9 cutting site	FW: TGTCATGGCATCTTCCAGGG	
Absolute qPCR gene dose	*EOMES*	FW1: CGGTACTACCTCCAGTCCCC RE1: CCTGGTACGGGAAGAGTGAG FW2: CTGCTCACTCTTCCCGTACC RE2: AGCCGGGTACACAGGTCC	

(*continued on next page*)

Table 2 (*continued*)

Antibodies and stains used for immunocytochemistry and flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
	PAX6	FW1: GTGGTGTCTTTGTCAACGGG RE1: GCTCTACAATCTTCTGCCGGG	
	MARINA	FW: CACTCCTCAGGTGCAGGCTG RE: GGGCTTCATGATGTCCCCAT	
sgRNA target sequence	Provide gRNA seed genomic sequence, or full gRNA/crRNA	Name: T2 target (Mali et al., 2013) FW: CACCGGGGGCCACTAGGGACAGGAT RE: AAACATCCTGTCCCTAGTGGCCCCC	
Genomic target sequence(s)	Including PAM and other sequences likely to affect UCN activity	Including exact position in the reference genome (e.g. GRCh38 for human cell lines)	GGGGCCACTAGGGACAGGATTGG 19q13.42 NC_000019 (55115749–55115771)
Plasmids used as templates for HDR	Addgene 223328		

antibodies (Table 2) for 30min at 4°C. Samples with matching isotypes were used to calibrate gates. Cell viability staining SYTOX Blue (Thermofisher – S34857) was used. Single cells were prepared with 35μm strainers (Corning – 352235). The iPS cells were differentiated into the three germ layers using the tri-lineage differentiation kit (Miltenyi – 130–115-660) for seven days, and their marker expression evaluated using primary-conjugated antibodies (Table 2). The samples were analyzed using a MQ10 flow cytometer (Miltenyi). Fcs files were analyzed with FLowJo software.

4.5. Karyotyping, short Tandem Repeat (STR), and qPCR analysis

Genomic DNA was analyzed using an Illumina microarray at Life&-Brain. STR loci were amplified using PowerPlex 16 HS System (Promega) and fragment analysis performed on ABI3730xl (Life Technologies) at Microsynth. Results were visualized with GeneMarker HID (Softgenetics). The qPCR was performed with PowerUp SYBR Green Master Mix (4334973) and primers in Table 2, using a QuantStudio Real-Time PCR 3 System (Applied Biosystems).

4.6. NGS HLA-typing and Whole genome sequencing (WGS)

Genomic DNA was analyzed with NGS on AlloSeq Tx17 kit (CareDx) and haplotype identified with AlloSeq Assign software. A mass of 100ng of genomic DNA was used for WGS on an Illumina Novaseq 6000 at CeGaT (Germany). The parental genome was used as reference. The read depth was $\geq$ 50x in all samples. The reads were mapped to *MARINA* to confirm the knock-in. COSMID, CCTop, and Cas-OFFinder tools were used to predict off-target sites. IGV and BaseSpace were used to visualize and interpret genome variants at the predicted sites.

5. Contributions

Molecular biology (A.P., U.S., S.K., C.M., B.R., A.V.), Cell biology (A.P., U.S., C.M., S.K., P.M.), Microscopy (A.P., C.M., B.R.), Flow cytometry (A.P., U.S., S.K.), Genomics (A.P., R.N., B.V.), Infrastructure accessibility (U.N., J.I., I.N., M.S., A.J., A.B.), Ethics (D.N., J.A.), Supervision (D.B., L.G., J.A.), Article writing and Figures preparation (A.P., S.K., C.M., J.A.). All authors read, understood and approved the last version of the manuscript.

CRediT authorship contribution statement

Aistė Petruškevičiūtė: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Ugnė Šimuliūnaitė:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Catalina M. Polanco:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Barbara Rojas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Methodology, Formal analysis, Data curation. **Simonas Kuras:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Beatričė Valatkaitė-Rakštienė:** Investigation, Formal analysis. **Rimvydas Norvilas:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Akshay Kumar Vijaya:** Methodology. **Patricia Muñoz:** Methodology, Investigation. **Urtė Neniškytė:** Resources. **Artūras Jakubauskas:** Resources. **Aurelijus Burokas:** Resources. **Ivan Nalvarte:** Writing – review & editing, Supervision, Resources. **Jose Inzunza:** Writing – review & editing, Supervision, Resources. **Daniel Naumovas:** Resources, Investigation. **Mindaugas Stoškus:** Supervision, Resources. **Laimonas Griškevičius:** Supervision, Resources. **Daiva Baltriukienė:** Writing – review & editing, Supervision, Resources. **Jonathan Arias:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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