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Lab Resource: Genetically-Modified Multiple Cell Lines

Engineering STRAIGHT-IN single and dual lines in the male iPS11 parental line for programmable DNA integration

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A B S T R A C T

STRAIGHT-IN is a genome engineering platform that enables precise integration of DNA payloads into mammalian genomes, including hiPSCs. In this study, we generated three hiPSC acceptor lines containing either one (single) or two (dual) landing pads. These landing pads support efficient, seamless integration of DNA cargos with single-copy control and a near-scarless genomic footprint. All landing pads were targeted to the *CLYBL* genomic safe harbor locus in the male hiPSC line iPS11. The resulting acceptor lines offer a versatile resource for the controlled genomic integration of diverse transgenes, making them broadly applicable to a wide range of applications.

1. Resource Table

Unique stem cell line identifiers	MITi001-A MITi002-A MITi003-A
Alternative name(s) of stem cell lines	*iPS11 STRAIGHT-IN Single-GT* *iPS11 STRAIGHT-IN Single-GA* *iPS11 STRAIGHT-IN Dual*
Institution	*Massachusetts Institute of Technology*
Contact information of the reported cell line distributor	*Kate E. Galloway (katiegal@mit.edu)*
Type of cell lines	*iPSC*
Origin	*Human*
Additional origin info *(applicable for human ESC or iPSC)*	*Age: N/A* *Sex: male* No disease known
Cell Source	*Fibroblasts*
Method of reprogramming (if applicable)	*Episomal plasmid transfection*
Evidence of the reprogramming transgene loss (including genomic copy absence/removal/silencing, if applicable) (if applicable)	*N/A*
The cell culture system used	Feeder-free cell culture. The hiPSCs were maintained in StemFlex™ Medium (ThermoFisher) on rhLaminin-521 (ThermoFsher or BioLamina)-coated plates

(*continued on next column*)

(*continued*)

Genetic Features: Introduced and Pre-Existing	Targeted heterozygous (Single) and homozygous (Dual) insertion of STRAIGHT-IN landing pads
Associated disease	*N/A*
Gene/locus modified in the reported transgenic lines	*The* Citrate lyase beta-like (*CLYBL*) *genomic safe harbor loci located in* chromosome 13q
Method of modification / user-customizable nucleases (UCN) used, *in silico* resources used for design optimization	*TALENs*
User-customizable nuclease (UCN) delivery method	*Plasmid transfection*
All double-stranded DNA genetic material molecules are administered to the cells	*CLYBL_CAG-TALEN-L1 (Addgene #252226)* *CLYBL_CAG-TALEN-R1 (Addgene #252227)* *CLYBL_Bxb1-GT_CAG-EBFP_LP_TC (Addgene #252223)* *CLYBL_Bxb1-GA_CAG-GFP_LP_TC (Addgene #252224)* *CLYBL_Bxb1-GA_CAG-mSc-ALFA_LP_TC (Addgene #252225)*
Evidence of the absence of random integration of any plasmids or dsDNA introduced into the cells	Droplet digital PCR (ddPCR)
Analysis of the resulting nuclease-targeted allele status	PCR from genomic DNA, Sanger sequencing of the targeted loci, and ddPCR

(*continued on next page*)

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(*continued*)

Homozygous allele status validation	PCR from genomic DNA, Sanger sequencing of the targeted and non-targeted loci, and ddPCR
Method of the off-target nuclease activity prediction and surveillance	*N/A*
Descriptive name of the transgene	*EBFP2, EGFP, mScarlet-ALFA,* Bleomycin resistance gene (*BleoR*), and Puromycin resistance gene (PuroR). Both antibiotic selection markers are lacking the ATG initiation codon
Eukaryotic selective agent resistance cassettes (including inducible, gene/cell type-specific)	Positive (Zeocin and Puromycin)
Inducible/constitutive expression system details	*EBFP2, EGFP and mScarlet-ALFA* driven by CAG constitutive promoter; *BleoR* and PuroR expressed following targeted integration of Donor plasmid; iRFP670 and Tet-On driven by CAG constitutive promoter and NKX3.1 transcription factor driven by doxycycline-inducible TRE-3G promoter
Date archived/stock creation date	*N/A*
Cell line repository/bank	*https://hpscreg.eu/cell-line/MITi001-A* *https://hpscreg.eu/cell-line/MITi002-A* *https://hpscreg.eu/cell-line/MITi003-A*
Ethics/GMO work approvals	*iPS11 parental line was obtained from ALSTEM* *https://www.alstembio.com/web/product_details.php?id=iPS11*
Disclaimers for Addgene/ repository-sourced used recombinant DNA resources (if applicable)	*CLYBL_CAG-TALEN-L1 (Addgene #252226)* *CLYBL_CAG-TALEN-R1 (Addgene #252227)* *CLYBL_Bxb1-GT_CAG-EBFP_LP_TC (Addgene #252223)* *CLYBL_Bxb1-GA_CAG-GFP_LP_TC (Addgene #252224)* *CLYBL_Bxb1-GA_CAG-mSc-ALFA_LP_TC (Addgene #252225)* *Bxb1-GA-CAG-iRFP670 (Addgene #252235)* *Bxb1-GA-Dox-NKX3-1_Div (Addgene #252237)* *STRAIGHT-IN kit (Addgene #1000000283)*

2. Resource utility

STRAIGHT-IN enables rapid, efficient, and single-copy DNA cargo integration into landing pads at CLYBL loci in hiPSCs. Previously, STRAIGHT-IN has been established in two female donors. Here, we engineer and characterize male hiPSCs, thereby extending the platform to a male background and providing a resource for global distribution.

3. Resource details

The generation of human and mouse cell lines harboring landing pads containing site-specific recombination sites has proven highly valuable (Zhu et al. 2014; Duportet et al. 2014; Matreyek et al. 2017; Gaidukov et al. 2018). These platforms address major challenges, including limitations in DNA cargo size, clonal variability, and low efficiency. By ensuring that all derived clones share an identical genetic background, they improve reproducibility, while the incorporation of positive selection markers enables rapid and efficient isolation of correctly targeted cells. In addition, the use of counterselection markers and orthogonal recombinase systems allows for near-scarless genome engineering and integration of multiple payloads (Brosh et al. 2021; Blanch-Asensio et al. 2022; Roelle et al. 2023). We previously established the STRAIGHT-IN platform in two female donor lines (Blanch-Asensio et al. 2022, 2023); however, its applicability to male donors was lacking. To address this limitation, we engineered the male iPS11 line to carry either one or two landing pads inserted at the genomic safe harbor locus *CLYBL* (Fig. 1A), which supports stable transgene expression (Cerbini et al. 2015).

To enrich for targeted cells, we employed DASIT, an antigen-specific selection strategy that obviates the need for physical cell sorting (Wang et al. 2026). Following enrichment, we isolated a clone for each line expressing the fluorescent reporter (Fig. 1B). Genomic PCR screening revealed monoallelic targeting in the Single-GT and Single-GA lines and biallelic targeting in the Dual line (Fig. 1C). Droplet digital PCR (ddPCR) analysis further confirmed single-copy integration of the two transgenes present in the landing pad (Fig. 1D). Sanger sequencing verified precise homologous recombination at the *CLYBL* locus, although indels were detected at the non-targeted allele (Fig. S1A). All three engineered lines retained expression of pluripotency markers following clonal isolation (Fig. 1E,F and Fig. S1B). Moreover, each line maintained the ability to differentiate into all three germ layers (Fig. 1G,H). Karyotype analysis showed a normal 46XY karyotype for all clones (Fig. S1C) (Table 1).

We next validated the downstream STRAIGHT-IN workflow in the Single-GA line, including site-specific integration of DNA cargoes into the landing pad and subsequent excision of auxiliary elements. Two payloads were integrated: a constitutively expressed fluorescent reporter, *iRFP670*, driven by CAG promoter, and an all-in-one doxycycline-inducible gene circuit with divergent syntax (Johnstone et al. 2026), encoding the transcription factor *NKX3.1*, which promotes rapid differentiation into mural cells (Fig. 1I)(Ng et al. 2021; Gong et al. 2025). DNA integration was mediated by eeBxb1 modRNA, an evolved serine recombinase derived from Bxb1 (Pandey et al. 2025), followed by excision using the Flp recombinase (Blanch-Asensio et al., 2026b). Following these steps, >95 % of cells expressed *iRFP670* and had excised the *EGFP* reporter originally present in the landing pad (Fig. 1J). A similar excision efficiency was observed for the doxycycline-inducible *NKX3.1* construct. Upon three days of induction, nearly all cells differentiated into mural-like cells, expressing mural markers, and staining positive for Transgelin (SM22)(Fig. 1K and Fig. S1D).

In addition, we generated a STRAIGHT-IN kit available on Addgene containing the three novel landing pads used in this study, TALEN vectors targeting *CLYBL*, DASIT constructs for efficient enrichment of targeted cells, and GT- and GA-Donor plasmids that are either empty or engineered for constitutive (CAG) or doxycycline-inducible expression of GOIs. The kit also includes IVT templates for mRNA-mediated expression of eeBxb1 and p53DD for donor plasmid integration, and Cre and Flp-T2A-BleoR for auxiliary element excision; an expression plasmid for DNA-mediated expression of eeBxb1; and donor plasmids expressing fluorescent proteins or transcription factors as positive controls (Fig. S2). Together, these components enable seamless establishment of STRAIGHT-IN (Dual) in virtually any hiPSC line and potentially other cell lines.

4. Materials and methods

4.1. hiPSC culture

hiPSCs (iPS11, ALSTEM) were maintained in StemFlex™ Medium (ThermoFisher) on rhLaminin-521–coated plates (ThermoFisher) at 37 °C with 5 % CO_2, as previously described (Blanch-Asensio et al., 2026a). Cells were passaged twice weekly using Gentle Cell Dissociation Reagent (STEMCELL Technologies).

4.2. Generation of acceptor hiPSC lines

For stable integration of landing pads at *CLYBL*, both TALENs were co-delivered with the targeting construct into hiPSCs. Briefly, 600 ng of each plasmid was transfected into 1×10^5 cells seeded in a 24-well plate the day prior, using Lipofectamine™ Stem Transfection Reagent (ThermoFisher). Cells were passaged upon reaching 80 % confluency, and 5×10^5 cells were seeded in a 6-well plate. The next day, cells were

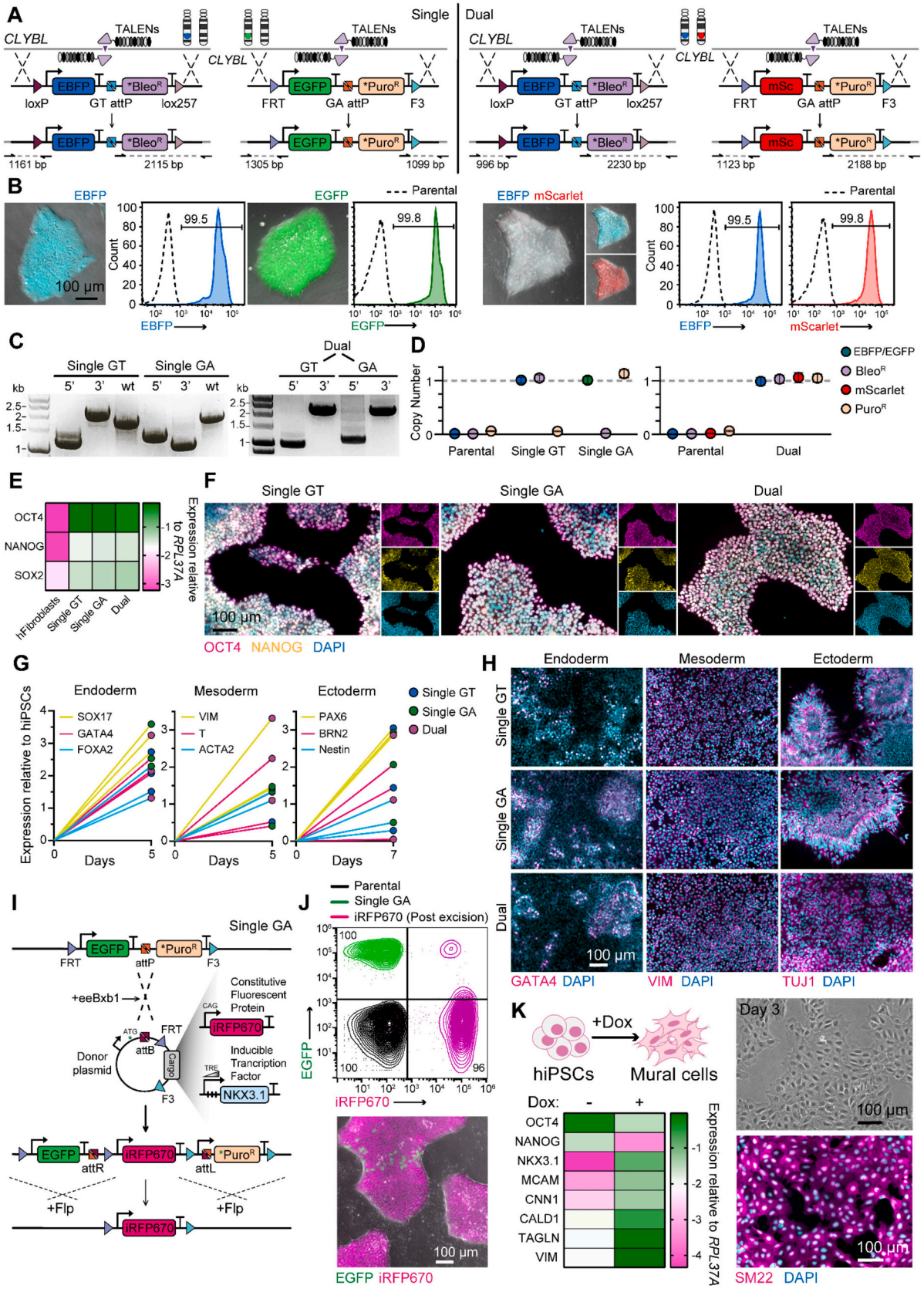

Fig. 1. Generation, characterization and functional validation of the iPS11 STRAIGHT-IN Single-GT, Single-GA and Dual lines.

Table 1
Characterisation and validation.

Classification	Output type	Result	Data (suggested placement)
Schematic of a transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification	Schematic of the edited *CLYBL* alleles	Fig. 1 panel A
Morphology	Photography	Phase contrast image of typical colony morphology	Fig. 1 panel B
Characterization of the pluripotent/ undifferentiated state	Expression of pluripotent markers analyzed with RT-qPCR and immunofluorescence at passage 4 for all lines.	Positive for pluripotency markers (OCT4, NANOG and SOX2) normalized to housekeeping gene RPL37A, compared to human fibroblasts and detected with RT-qPCR	Fig. 1 panel E
		Positive for pluripotency markers (OCT4, NANOG and SSEA-4) detected with immunofluorescence	Fig. 1 panel F and Sup. Fig. 1 panel B
Karyotypic characterization	Karyotyping by G-banding at passage 10 (Single-GT and −GA) and passage 4 (Dual)	46, XY (all lines) Normal (all lines)	Sup. Fig. 1 panel C
Genotyping for the desired genomic alteration/allelic status of the gene of interest	Genomic DNA PCR specific for homology-directed insertion including both 5′ and 3′ junctions	Specific integration in either one of the *CLYBL* alleles (Single-GT and −GA) or both (Dual) verified by genomic DNA PCR and Sanger sequencing	Fig. 1 panel C Sup. Fig. 1 panel A
	Evaluation of the − (homo-/hetero-/hemi-) zygous status of introduced genomic alteration(s)	ddPCR CNV analysis confirming a single copy of the two transgenes present in each of the landing pads (all lines)	Fig. 1 panel D
	Transgene-specific PCR	Genomic DNA PCR for the transgene presence	Fig. 1 panel C
Verification of the absence of random plasmid integration events	ddPCR	CNV analysis confirming a single copy of the two transgenes present in each of the landing pads (all lines)	Fig. 1 panel D
Evidence of genetic identity for the parental and the reported genetically-modified cell lines	STR analysis	STR profiles match iPS11 parental line for all three lines	The results are available on hPSCreg.eu and submitted in archive with the journal
Mutagenesis / genetic modification outcome analysis	Sanger sequencing (genomic DNA PCR)	Sanger sequencing chromatograms verifying the integration of either one LP in one of the *CLYBL* alleles or both LPs in both alleles. In both Single lines, the other allele presented indels	Sup. Fig. 1 panel A
	PCR-based analyses	PCR confirming the presence of one LP in the single edited *CLYBL* allele and absence of integration in the other allele (both Single-GT and −GA lines) while both allele were modified for the Dual line	Fig. 1 panel C
Specific pathogen-free status	Mycoplasma	Mycoplasma testing at passage 10 (Single-GT and −GA) and 6 (Dual) using the MycoAlert™ PLUS Assay (Lonza BioSciences) Result: Negative (for all lines)	Sup. Fig. 1 panel E
Characterization of the differentiation potential	Directed trilineage differentiation analyzed with RT-qPCR and immunofluorescence at passage 4 for all lines	Upregulation of three germ-layer specific markers normalized to housekeeping gene RPL37A and hiPSCs: endoderm (SOX17, GATA4 and FOXA2), mesoderm (Vimentin, T and ACTA2) and ectoderm (PAX6, BRN2 and Nestin) – all lines	Fig. 1 panel G
		Positive immunofluorescence for three germ-layer specific markers: endoderm (GATA4), mesoderm (Vimentin) and ectoderm (TUBB3/TUJ1) – all lines	Fig. 1 panel H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	–
Genotype − additional histocompatibility info (OPTIONAL)	Blood group genotyping	N/A	–
	HLA tissue typing	N/A	–

transfected with 2 μg of DASIT modRNA using the same reagent (Wang et al. 2026). 6 h post-transfection with DASIT modRNA, medium was replaced with StemFlex™ containing either zeocin (15 μg/mL, ThermoFisher), puromycin (1 μg/mL, InvivoGen), or both. Medium was refreshed daily for 3 days, after which cells were maintained in StemFlex™ without antibiotics. For clonal isolation, multiple colonies were manually picked per condition for further genotype screening to identify correctly targeted clones. Genotyping was performed by ddPCR and PCR as previously described (Blanch-Asensio et al., 2026a), followed by Sanger sequencing. Primer and probe sequences are provided in Table 2.

4.3. Donor vector integration and enrichment

600 ng of Donor plasmid together with 400 ng of eeBxb1 modRNA were transfected into the hiPSCs using Lipofectamine™ Stem Transfection Reagent. Two days post-transfection, integrated cells were enriched witn StemFlex™ containing either zeocin (15 μg/mL) or puromycin (1 μg/mL) for 3 days. For excision, 400 ng of Cre modRNA (Miltenyi Biotec) or Flp-T2A-BleoR was transfected. In the latter case, cells expressing Flp were selected by adding zeocin (15 μg/mL) to the medium for 3 days, beginning 6 h post-transfection.

4.4. ddPCR

Copy number was determined as previously described (Blanch-Asensio et al., 2026a) with primer and probe sequences listed in Table 2.

4.5. Karyotype and STR analysis

Karyotypes were determined by G-banding (Cell Line Genetics), with 20 metaphase spreads analyzed per clone. STR analysis was evaluated using the PowerPlex® 16 HS (Promega), including 15 autosomal loci and 1 gender determination marker.

4.6. RNA extraction, cDNA synthesis, and RT-qPCR

RNA was extracted using Monarch® Total RNA Purification Kit (NEB) and reverse transcribed with ProtoScript® II First Strand cDNA

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	*Mouse anti-OCT4*	*1:200*	*Cell Signaling Technology Cat# 75463S, RRID: AB_2799870*
	Rabbit anti-NANOG	*1:200*	*Cell Signaling Technology Cat# 4903T, RRID: AB_10559205*
	Mouse anti-SSEA-4	*1:200*	*BioLegend Cat# 330402, RRID:AB_1089209*
Differentiation Markers	*Mouse anti-GATA4*	*1:10*	*DSHB Cat# PCRP-GATA4-1A7, RRID:AB_2618645*
	Mouse anti-VIM	*1:10*	*DSHB Cat# 3CB2, RRID: AB_531888*
	Mouse anti-TUBB3	*1:100*	*BioLegend Cat# 801202, RRID:AB_2313773*
Mural Marker	*Rabbit anti-SM22*	*1:200*	*Abcam Cat# ab14106, RRID:AB_443021*
Secondary antibodies	*Donkey anti-mouse IgG (H + L) Secondary Antibody, Alexa Fluor 647*	*1:500*	*ThermoFisher Cat# A31571, RRID:AB_162542*
	Donkey anti-rabbit IgG (H + L) Secondary Antibody, Alexa Fluor 488	*1:500*	*ThermoFisher Cat# A21206, RRID: AB_2535792*
Nuclear stain	*DAPI*	1 μg/mL	*Tocris Cat # 5748*
Site-specific nuclease			
Nuclease information	*TALENs*	*CLYBL_CAG-TALEN-L1 (Addgene #252226)* *CLYBL_CAG-TALEN-R1 (Addgene #252227)*	
Plasmids used as templates for HDR-mediated site-directed mutagenesis.	Plasmid	*CLYBL_Bxb1-GT_CAG-EBFP_LP_TC (Addgene #252223)* *CLYBL_Bxb1-GA_CAG-GFP_LP_TC (Addgene #252224)* *CLYBL_Bxb1-GA_CAG-mSc-ALFA_LP_TC (Addgene #252225)* *STRAIGHT-IN Dual kit (Addgene #1000000283)*	
Delivery method	*Lipofection*	*Lipofectamine Stem Transfection Reagent (ThermoFisher)*	
Selection/enrichment strategy	*DASIT*	(Wang et al. 2026)	
Primers and Oligonucleotides used in this study			
	Target	**Forward/Reverse primer (5′-3′)**	
House-Keeping Gene (qPCR)	*RPL37A*	GTGGTTCCTGCATGAAGACAGTG TTCTGATGGCGGACTTTACCG	
Pluripotency Markers (qPCR)	*OCT4*	GTGGAGGAAGCTGACAACAA ATTCTCCAGGTTGCCTCTCA	
	NANOG	AGCAGATGCAAGAACTCTCCAA TGAGGCCTTCTGCGTCACAC	
	SOX2	GCTACAGCATGATGCAGGACCA TCTGCGAGCTGGTCATGGAGTT	
Endoderm Markers (qPCR)	*SOX17*	CGCACGGAATTTGAACAGTA GGATCAGGGACCTGTCACAC	
	GATA4	GGCCTGTCATCTCACTACGG ATGGCCAGACATCGCACT	
	FOXA2	GGAGCGGTGAAGATGGAA TACGTGTTCATGCCGTTCAT	
Mesoderm Markers (qPCR)	*VIM*	TGGACCAGCTAACCAACGAC GCCAGAGACGCATTGTCAAC	
	T	TGTTTATCCATGCTGCAATCC CCGTTGCTCACAGACCACAG	
	ACTA2	GTGATCACCATCGGAAATGAA TCATGATGCTGTTGTAGGTGGT	
Ectoderm Markers (qPCR)	*PAX6*	CGAGATTTCAGAGCCCCATA AAGACACCACCGAGCTGATT	
	BRN2	ACCCGCTTTATCGAAGGCAA CCTCCATAACCTCCCCCAGA	
	Nestin	TCAAGATGTCCCTCAGCCTGGA AAGCTGAGGGAAGTCTTGGAGC	

Table 2 (*continued*)

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Mural Markers (qPCR)	*NKX3.1*		CGCAGAACGACCAGCTGAGCA CCTGAAGTGTTTTCAGAGTCCAAC
	MCAM		ATCGCTGCTGAGTGAACCACAG CTACTCTCTGCCTCACAGGTCA
	CNN1		TCATCAAGGCCATCACCAAGT AGGGTGGACTGCACCTGTGTA
	CALD1		CTGTTCCTGCTGAAGGTGTACG CCTACCTTCAAGCCAGCAGTTTC
	TAGLN		CGAAGTGCAGTCCAAAATCGAGAA AATCACGCCATTCTTCAGCCAGAC
Genotyping (desired allele/transgene presence detection)	*CLYBL* targeted allele with Bxb1-GT (EBFP2) and Bxb1-GA (EGFP) LPs		5′ junction: *AGATCATCCAGCCCTAGTCAAG ATGTAACGGGCCTAACTTCG* 3′ junction: *AGCAAAGACCCCAACGAGAA TGGAGCAGTGGATGACAACTT*
	CLYBL targeted allele with Bxb1-GA (mScarlet-ALFA) LP		5′ junction: *AGATCATCCAGCCCTAGTCAAG CAGACTGCCTTGGGAAAAGCG* 3′ junction: *GACATCACCTCCCACAACGA TGGAGCAGTGGATGACAACTT*
	CLYBL non-targeted allele		*AGATCATCCAGCCCTAGTCAAG TGGAGCAGTGGATGACAACTT*
Targeted mutation analysis/ sequencing	*Sequencing data from both alleles*		5′ junction and non-targeted allele: GGGGCGTACTTGGCATATGA 3′ junction and non-targeted allele: GATCTCATGCTGGAGTTCTTCG (Blanch-Asensio, Grandela, et al. 2026)
Potential random integration detection using ddPCR	*EGFP/EBFP2* *mScarlet* *BleoR* *PuroR*		

Synthesis Kit (NEB). qPCR was performed using iQ SYBR Green Supermix (Bio-Rad) on a CFX Opus 384 Real-Time PCR System (Roche). Data were analyzed by the ΔCt method, normalized to *RPL37A*, and $\log_{10}$-transformed. Primer sequences are listed in Table 2.

4.7. Immunofluorescence analysis

Cells were fixed with 4 % PFA for 10 min at room temperature, blocked and permeabilized in PBS containing 0.05 % Tween™20 (ThermoFisher) and 5 % FBS for 1 h at 4 °C. Cells were incubated with primary antibodies (Table 2) in PBS containing 0.05 % Tween™ 20 and 1 % FCS overnight at 4 °C, washed 3x with PBS, and incubated with secondary antibodies (Table 2) in the same solution supplemented with DAPI (1 μg/mL) for 1 h at room temperature. After three PBS washes, images were acquired using a BZ-X800 Live-Cell Imaging Microscope (KEYENCE).

4.8. Trilineage differentiation

Cells were seeded on rhLaminin-521-coated plates and differentiated using the STEMdiff™ Trilineage Differentiation Assay Kit (STEMCELL Technologies).

5. Materials availability

All plasmids generated in this study have been deposited on Addgene, including the STRAIGHT-IN Dual kit (#1000000283) containing 24 plasmids (https://www.addgene.org/kits/galloway-straight-in). The hiPSC lines are available under an MTA. Requests should be directed to Dr Kate. E. Galloway (katiegal@mit.edu).

CRediT authorship contribution statement

Albert Blanch-Asensio: Writing – review & editing, Writing –

original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Derin R. Gumustop:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Nathan B. Wang:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emma L. Peterman:** Writing – review & editing, Methodology, Data curation. **Deon S. Ploessl:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Kate E. Galloway:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kate E. Galloway reports financial support was provided by National Institute of General Medical Sciences of the National Institutes of Health award R35-GM143033. Kate E. Galloway reports financial support was provided by National Science Foundation CAREER award grant 2339986. Kate E. Galloway reports financial support was provided by Institute for Collaborative Biotechnologies W911NF-19-2-0026. Kate E. Galloway reports financial support was provided by Air Force Research Laboratory MURI FA9550-22-1-0316. Kate E. Galloway reports financial support was provided by Pershing Square Foundation MIND Prize. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2026.104068.

Data availability

Data will be made available on request.

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