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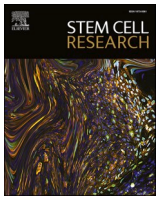

Lab Resource: Multiple Cell Lines

Generation of a Flattop-T2A-H2B-Venus x C-peptide-mCherry double reporter human iPSC line to monitor WNT/Planar cell polarity pathway activity

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A B S T R A C T

Deriving functional β-cells from human induced pluripotent stem cells (hiPSCs) holds potential for cell replacement therapy, disease modeling, and drug testing in diabetes research. Wnt/Planar cell polarity (PCP) signaling is crucial for endocrine cell development and β-cell maturation in murine models and can be tracked by the expression of the tissue-specific effector gene *Flattop*. Here, we report the generation of a human fluorescent *FLTP/CFAP126* (Flattop-T2A-H2B-Venus) and FLTP-Insulin (Flattop-T2A-H2B-Venus x C-peptide-mCherry) double reporter by CRISPR/Cas9 gene editing. These hiPSC reporter lines allow monitoring of WNT/PCP signaling during endocrine cell formation and studying its role in β-cells in a human model system.

1. Resource Table

Unique stem cell line identifier	HMGUi001-A-55 HMGUi001-A-56
Alternative name(s) of stem cell line	Flattop-T2A-H2B-Venus; FVR Flattop-T2A-H2B-Venus x C-peptide-mCherry-hiPSC; FVR-CPEP-mCherry
Institution	Institute of Diabetes and Regeneration Research, Helmholtz Center Munich, 85,764 Neuherberg, Germany
Contact information of the reported cell line distributor	Heiko Lickert, heiko.lickert@helmholtz-munich.de
Type of cell line	hiPSC
Origin	Human, HMGUi001-A hiPSC described in Wang et al. 2018
Additional origin info *(applicable for human ESC or iPSC)*	Age: N/A Sex: female Ethnicity: Caucasian
Cell Source	Fibroblasts
Method of reprogramming	N/A
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
The cell culture system used	Feeder free

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Unique stem cell line identifier	HMGUi001-A-55 HMGUi001-A-56
Type of the Genetic Modification	Homozygous (Flattop-T2A-H2B-Venus) and heterozygous (C-peptide-mCherry) insertion of fluorescence proteins under the endogenous promoter
Associated disease	N/A
Gene/locus modified in the reported transgenic line	Flattop gene (CFAP126)/1q23.3 Insulin gene (INS)/11p15.5
Method of modification / user-customisable nucleases (UCN) used, the resource used for design optimisation	CRISPR/Cas9
User-customisable nuclease (UCN) delivery method	Plasmid lipofection
All double-stranded DNA genetic material molecules introduced into the cells	pU6-(BbsI)sgRNA_CAG-Cas9-venus-bpA; Addgene #86986 pBlueScript FVR- T2A-H2B-Venus donor plasmid pBlueScript Ins-C-pep-mCherry Fusion; (Siehler et al., 2021)
Evidence of the absence of random integration of any plasmids or DS DNA introduced into the cells.	PCR from genomic DNA for the plasmid backbone

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Table 1
Characterization and validation.

Classification (optional *italicized*)	Output type	Result	Data
Schematic of a transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification		Fig. 1A,C
Morphology	Bright-Field imaging	Typical colony shaped morphology of hiPSC	Fig. 1E, sale bar = 200 μm
Pluripotency status evidence for the described cell line	Qualitative analysis(Immunocytochemistry)	Expression of undifferentiated state markers OCT3/4 and SOX2	Fig. 1F, scale bar = 50 μm
	Quantitative analysis (Flow cytometry)	Expression of cell surface markers SSEA4 and Tra1-60: 99.5 % double positives	Fig. 1G
Karyotype	Karyotype (G-banding) and resolution	46XX, Resolution 475–600 bands	Fig. 1I
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	KI and WT specific PCR at knock-in region	Fig. 1B,D Supp Fig. 1A,B
	Evaluation of the − (homo-/hetero-/hemi-) zygous status of introduced genomic alteration(s)	N/A	
	Transgene-specific PCR (when applicable)	PCR for the (intact) transgene presence	Fig. 1B,D Supp Fig. 1A,B
Verification of the absence of random plasmid integration events	PCR/Southern	PCR detection	Supp Fig. 1E
Parental and modified cell line genetic identity evidence	STR analysis	DNA Profiling	Supplementary file submitted in the archive with journal
		All analyzed loci matched: AMEL, CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179,FGA, TH01, TPOX, vWA	
Mutagenesis / genetic modification outcome analysis	Sequencing of the genomic DNA PCR product	Homozygous integration at the Flattop locus and heterozygous integration at the Insulin locus with normal integration sites	Supp Fig. 1A,B
	PCR-based analyses	Homozygous integration at the Flattop locus and heterozygous integration at the Insulin locus	Fig. 1 B, D
	Southern Blot or WGS; western blotting (for knock-outs, KOs)	N/A	N/A
Off-target nuclease activity analysis	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	Top 3 predicted off-targets for each targeting approach (in total 6) amplified by PCR and sequencing of the product showed no alterations	Supp Fig. 1C
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by luminescence with MycoAlert PLUS Mycoplasma Detection Kit: Negative	Supp Fig. 1D
Multilineage differentiation potential	Directed differentiation	Cell successfully differentiated into all three lineages	Fig. 1 H
List of recommended germ layer markers	Immunofluorescence	Expression of lineage markers assessed by immunofluorescence. Endoderm: FOXA2 and SOX17Mesoderm: SM22-a and SNAIL1Ectoderm: TUBB3 and PAX6	Fig. 1 H
Outcomes of gene editing experiment (OPTIONAL)	Brief description of the outcomes in terms of clones generated/establishment approach/ screening outcomes	N/A	N/A
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype − additional histocompatibility info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

(*continued*)

Unique stem cell line identifier	HMGUi001-A-55 HMGUi001-A-56
Analysis of the nuclease-targeted allele status	PCR and Sanger sequencing of the targeted and untargeted alleles
Homozygous allele status validation	N/A
Method of the off-target nuclease activity prediction and surveillance	Off-target prediction with CRISPOR webtool (https://crispor.gi.ucsc.edu/) and PCR combined with Sanger sequencing for the top 3 off-targets for each sgRNA, in total 6 off-targets.
Descriptive name of the transgene	Flattop-T2A-H2B-Venus Flattop-T2A-H2B-Venus x C-peptide-mCherry
Eukaryotic selective agent resistance cassettes (including inducible, gene/cell type-specific)	N/A
Inducible/constitutive expression system details	N/A
Date archived/stock creation date	May 27, 2025

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Unique stem cell line identifier	HMGUi001-A-55 HMGUi001-A-56
Cell line repository/bank	https://hpscreg.eu/cell-line/HMGUi001-A-55 https://hpscreg.eu/cell-line/HMGUi001-A-56
Ethical/GMO work approvals	The use of the iPSCs in scientific investigations was covered by the positive votes of the Ethics Committee of the Medical Faculty of the Eberhard Karls University, Tübingen (file number 130/2018BO2) and of the Medical Faculty of the Technical University Munich (file number 219/20 S). The study design followed the principles of the Declaration of Helsinki, including informed consent of donors of cellular material.

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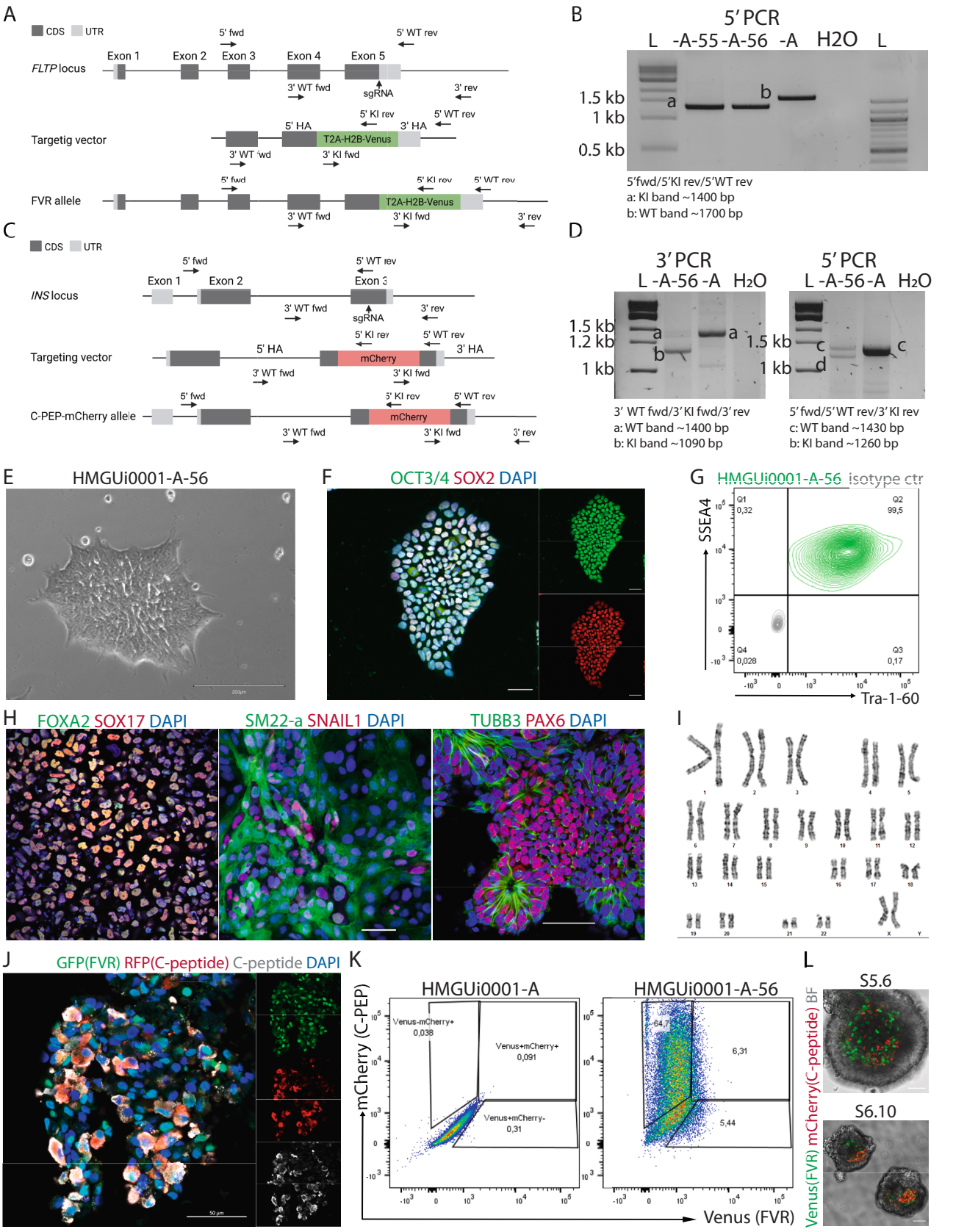

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Fig. 1. Generation and characterization of Flattop-T2A-H2B-Venus (FVR, HMGUi-001-A-55) single and Flattop-T2A-H2B-VenusxC-Peptide-mCherry (FVR-Cpep-mCherry, HMGUi-001-A-56) double reporter hiPSC lines. A) Targeting strategy and scheme for the insertion of a T2A-H2B-Venus sequence at the Flattop locus with primers for verification. B) PCR amplification from genomic DNA, confirming the homozygous integration of the targeting vector at the Flattop locus and the parental line as a comparison. C) Targeting strategy as described in Siehler et al., 2021. D) PCR amplification from genomic DNA confirming the heterozygous integration of the targeting vector into the Insulin locus. E) Phase contrast image of a pluripotent HMGUi-001-A-56 colony. Scale bar = 200 μm. F) Immunofluorescence staining of undifferentiated state markers OCT3/4 and SOX2 in HMGUi-001-A-56. Scale bar = 50 μm. G) Flow cytometry analysis of HMGUi-001-A-56 stained for surface proteins SSEA4 and Tra-1–60 as markers for pluripotency. H) Immunofluorescence staining for markers of endoderm (FOXA2, SOX17), mesoderm (SM22-α, SNAIL1), and ectoderm (TUBB3, PAX6) upon directed trilineage differentiation. Scale bars = 50 μm. I) Karyotype analysis by G-banding confirming a normal 46XX karyotype. J) Immunofluorescence staining of immature β-cells generated according to Rezania et al., 2014 at day 10 of stage 6 for GFP (Venus), RFP (mCherry), and C-Peptide confirms expression of the fluorescent proteins as well as endocrine identity. Scale bar = 50 μm. K) Flow cytometry detection of mCherry and Venus in live cells generated according to Rezania et al., 2014, at day 10 of stage 6. L) Detection of mCherry and Venus in live aggregates generated according to Velazco-Cruz et al., 2019 at day 6 of the endocrine induction stage 5 and day 10 of the β-cell maturation stage 6.

(*continued*)

Unique stem cell line identifier	HMGUi001-A-55 HMGUi001-A-56
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	pU6-(BbsI)sgRNA_CAG-Cas9-venus-bpA was a gift from Ralf Kuehn (Addgene plasmid #86986; *https://n2t.net/addgene:86986*; RRID: Addgene_86986)

2. Resource utility

The Flattop-T2A-H2B-Venus-hiPSC (HMGUi-001-A-55) line expresses a nuclear H2B-Venus upon expression of the WNT/PCP effector gene *FLTP* and enables monitoring of active WNT/PCP signaling. Adding the fusion of mCherry to C-peptide, the Flattop-T2A-H2B-Venus x C-peptide-mCherry double reporter hiPSC line permits detection and isolation of WNT/PCP active β-cells during pancreatic endocrine differentiation. Table 1.

3. Resource details

Wnt/PCP signaling, a pathway that regulates cytoskeletal dynamics, is crucial for the development and functionality of endocrine cells (Cortijo et al. 2012; Yoshihara et al. 2020; Böttcher et al. 2021). Our studies identified *Flattop* (*Cfap126*) as a Wnt/PCP reporter and effector gene that distinguishes mature from immature, proliferative β-cells (Bader et al. 2016; Gegg et al. 2014) in the murine pancreas. To address the role of WNT/PCP signaling in human pancreatic endocrine formation, we generated a Flattop-T2A-H2B-Venus (FVR) hiPSC reporter line. To investigate WNT/PCP signaling in human β-cell maturation, we applied the previously published targeting strategy for C-peptide (Siehler et al. 2021), a byproduct of proinsulin processing, to generate the Flattop-T2A-H2B-Venus x C-peptide-mCherry double reporter.

We targeted the human Flattop locus for insertion of a T2A-H2B-Venus sequence by CRISPR/Cas9 gene editing in front of the stop codon (Fig. 1A). The successful homozygous insertion of the reporter cassette was confirmed by PCR (Fig. 1B) and subsequent Sanger Sequencing of the amplified products (Supplementary Fig. 1A) to validate sequence integrity at the insertion site. In a second targeting step, the generated homozygous clone was used for targeting of the insulin gene locus (Fig. 1C) as previously described (Siehler et al., 2021). Heterozygous integration of the reporter sites was confirmed by PCR (Fig. 1D) and the integrity of both wild-type and knock-in allele by Sanger Sequencing (Supplementary Fig. 1B).

Typical colony morphology of pluripotent cells was confirmed by microscopy (Fig. 1E). We assessed the expression of the nuclear undifferentiated state markers OCT3/4 and SOX2 by immunofluorescence (Fig. 1F) and expression of the surface markers SSEA4 and TRA-1–60 in pluripotent cells by flow cytometry (Fig. 1G). To further validate the pluripotent capacity, differentiation to all three germ layers was performed. The cells expressed the key markers for endoderm FOXA2 and SOX17, SM22-α and SNAIL1 for mesoderm, as well as TUBB3 and PAX6 for ectoderm (Fig. 1H). Furthermore, chromosomal aberrations were ruled out by karyotype analysis (Fig. 1I). Upon differentiation towards stem cell-derived islets (sc-islets) in a seven-stage protocol (Rezania et al. 2014), expression of both fluorescent reporters was detected at day 10 of the immature β-cell stage 6 (S6.10) (Fig. 1J) as well as in live cells of the same stage by flow cytometry (Fig. 1K). We could confirm accurate co-localization of mCherry with C-peptide (Fig. 1J). We observed cells single positive for the FVR or C-peptide-mCherry but also double-positive cells (Fig. 1J,K) and could also detect the fluorescent proteins in live aggregates generated according to Velazco-Cruz et al. 2019 at endocrine induction stage S5 (Fig. 1L). To summarize, the generated cell lines can be used to track the expression of the WNT/PCP marker gene *FLTP* as well as C-peptide and enable the isolation of β-cells with active WNT/PCP signaling by flow cytometry. This will greatly help to deepen our understanding of the role of WNT/PCP signaling during the emergence and maturation of β-cells.

4. Materials and Methods

4.1. HiPSC culture and differentiation to β-cells

HiPSC were cultured under feeder-free conditions on Geltrex (Life Technologies, Cat# A1413302) coated plates in StemMACS™ iPSBrew XF medium (Miltenyi Biotec, Cat# 130–104-368) at 37 °C and 5 % CO_2. Cells were passaged at 80 % confluency as single cells by Accutase (Sigma-Aldrich, Cat# A6964) treatment. 10 μM ROCK inhibitor (Y-27632, Santa Cruz Biotechnology, Cat# sc-281642A) was added for the first 24 h and mycoplasma tests using the MycoAlert® PLUS Mycoplasma Detection Kit (Lonza, Cat# LT07-710) were performed. For the generation of β-like cells, hiPSCs were differentiated according to Rezania et al. 2014 with transfer to air–liquid interface after stage 4 or as 3D culture in flasks on magnetic stirrer platforms according to Velazco-Cruz et al. 2019.

4.2. Three germ layer differentiation

Three germ layer differentiation was performed as a monolayer culture with the StemMACS Trilineage Differentiation Kit (Miltenyi Biotec, Cat# 130–115–660) following the manufacturer's protocol.

4.3. Cloning of targeting constructs and transfection of hiPSC

For the Flattop-T2A-H2B-Venus donor plasmid, the T2A-H2B-Venus sequence was amplified from a pCAG-T2A-H2B-Venus plasmid, and the flanking 944 bp 3′ homology as well as the 1093 bp 5′ homology arms were amplified from genomic DNA extracted from HMGUi-001-A. For the cloning of the FLTP-sgRNA-Cas9-Venus vector, the pU6-(BbsI) sgRNA-CAG-Cas9-venus-bpA Addgene plasmid #86986 was digested with BbsI and a sgRNA targeting *FLTP* was inserted. The sgRNA sequence was designed using the CRISPOR web tool (https://crispor.tefor.net). Transfection was performed using the Lipofectamine™ Stem Transfection Reagent (Fisher Scientific, Cat# STEM00003). 0.4 x 10^6 HMGUi001-A cells were seeded into 6-well plates. The next day the

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Undifferentiated state Markers	Rabbit anti-SOX2	1:1000	Cell Signaling Technology Cat# 3579S, RRID:AB_2195767
	Mouse anti-OCT3/4	1:1000	Santa Cruz Cat# sc-5279, RRID:AB_628051
	Human anti-SSEA4-FITC	1:11	Miltenyi Biotec, Cat# 130–098-371, RRID:AB_2653517
	Human anti-TRA-1–60-PE	1:11	Miltenyi Biotec, Cat# 130–100-347, RRID:AB_2654227
	Human anti-TRA-1–60-PE	1:11	Miltenyi Biotec, Cat# 130–100-347, RRID:AB_2654227
	Human anti-TRA-1–60-PE	1:11	Miltenyi Biotec, Cat# 130–100-347, RRID:AB_2654227
Isotype control	REA Control (S)-PE-Vio615	1:11	Miltenyi Biotec, Cat# 130–107-146, RRID:AB_2661694
	REA Control (S)-FITC	1:11	Miltenyi Biotec, Cat# 130–104-610, RRID:AB_2661688
Germ Layer Differentiation Markers	Rabbit anti-FOXA2	1:500	Cell Signaling Technology Cat# 8186, RRID:AB_10891055
	Goat anti-SOX17	1:400	R and D Systems Cat# AF1924, RRID:AB_355060
	Rabbit anti-SM22-α	1:100	Abcam Cat# ab14106, RRID:AB_443021
	Goat anti-Snail	1:300	R and D Systems Cat# AF3639, RRID:AB_2191738)
	Mouse anti-PAX6	1:100	DSHB Hybridoma, Cat# PAX6, RRID:AB_528427
	Rabbit anti- Tubulin beta III	1:1000	Abcam, Cat# ab18207, RRID: AB_444319
Pancreatic Differentiation Markers	Goat anti-PDX1	1:300	R and D Systems Cat# AF2419, RRID:AB_355257
	Rabbit anti-NKX6.1	1:300	Novus Cat# NBP1-82553, RRID:AB_11023606
	Guinea pig anti-C-Peptide	1:100	Abcam Cat# ab30477, RRID:AB_726924
	Guinea pig-anti-Insulin	1:300	Bio-Rad Cat# 5330-0104G, RRID:AB_1605150
	Mouse anti-Glucagon	1:500	Sigma-Aldrich Cat# G2654, RRID:AB_259852
mCherry	Rat anti-RFP	1:1000	ChromoTek Cat# 5f8-100, RRID:AB_2336064
Venus	Chicken anti-GFP	1:500	Aves Labs Cat# GFP-1020, RRID:AB_10000240
Secondary antibodies	Donkey anti-rabbit IgG 488	1:800	Thermo Fisher Scientific Cat# A-21206, RRID:AB_2535792
	donkey anti-rabbit IgG 555	1:800	Thermo Fisher Scientific Cat# A-31572, RRID:AB_162543
	Donkey anti rabbit IgG 647	1:500	Thermo Fisher Scientific Cat# A-31573, RRID:AB_2536183
	Donkey anti-goat IgG 555	1:800	Thermo Fisher Scientific Cat# A-21432, RRID:AB_2535853
	Donkey anti-Goat 647	1:800	Jackson ImmunoResearch Labs Cat# 705–605-147, RRID:AB_2340437
	Donkey anti-chicken IgY Cy2	1:800	Jackson ImmunoResearch Labs Cat# 703–225-155, RRID:AB_2340370
	Donkey anti-rat IgG (H + L)-Cy3	1:500	Jackson ImmunoResearch Labs Cat# 712–165-153, RRID:AB_2340667
	Donkey anti-guinea pig 649	1:200	Jackson ImmunoResearch Labs Cat# 706–605-148, RRID:AB_2340476
	donkey anti-mouse IgG 488	1:800	Thermo Fisher Scientific Cat# A-21202, RRID:AB_141607
	donkey anti-mouse IgG 555	1:800	Thermo Fisher Scientific Cat# A-31570, RRID:AB_2536180
	Donkey anti-mouse, Alexa Fluor 647	1:500	Jackson ImmunoResearch Labs Cat# 715–605-151, RRID:AB_2340863
Nuclear Stain	DAPI	2 μg/mL	Carl Roth Cat# 6335.1
Site-specific nuclease			
Nuclease information	Cas9	SpCas9-Venus Fusion	
Delivery method	Lipofection	Lipofectamine Stem Transfection Reagent (Fisher Scientific, Cat# STEM00003)	
Selection/enrichment strategy	FACS	Sorting of GFP positive cells with FACS Aria™ III (BD Biosciences)	
Primers and Oligonucleotides used in this study			
	Target	**Forward/Reverse primer (5′-3′)**	
Cloning targeting vector C-peptide	5′ Homology Arm	GGGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCACCTGGCCTTCAGCCTGCCTCAGC/ GTTATCCTCCTCGCCCTTGCTCACCATTGCCCTTTTTTGGAGGGACCCCTCCAGGGCCAAGGG	
	3′ Homology Arm	GCCACTCCACCGGCGGCATGGACGAGCTGAAGCGTGGCATTGTGGAACAATGCTGTAC/ GCTGGGTACCGGCCCCCCCTCGAGGTTCCCTGCTTCTCCTGGGCTGCAATC	
Cloning targeting vector Flattop	5′ Homology Arm	GAACAAAAGCTGGAGCTCCACCGCGGTGGCCTTCCGGCCAGCTTACCCAGCAGGATAG/ CCGCATGTTAGTAAAGAGCCTCTACCTTCAAAGGATTTGGCTGGTCTTTGGGGACCTGGAG	
	3′ Homology Arm	CTCGGCATGGACGAGCTGTACAAGTAAAGAGCCCACCTGGAAGTCCACGTATGC/ GAATTGGGTACCGGGCCCCCCCTCGAGGTGGAATGAGGGACAAGTGATCATGACAGAAC	
Oligo for sgRNA cloning C-Peptide		CACCGGGGAGGGGTCCCTGCAGAAGCG/AAACCGCTTCTGCAGGGACCCCTCCCC	
Oligo for sgRNA cloning Flattop		CACCG CCAGGTGGGCTCTTAGGATT/AAACAATCCTAAGAGCCCACCTGGC	
Genotyping	C-peptide 5‘ knock-in allele (1260 bp)	GTCAGGTGGGCTCAGGATTCCAG/ TGTTATCCTCCTCGCCCTTGCTC	
	C-peptide 5‘ wt allele (1433 bp)	GTCAGGTGGGCTCAGGATTCCAG/ TCACAACAGTGCCGGGAAGTGGG	
	C-peptide 3‘ knock-in allele (1088 bp)	ACCTCCCACAACGAGGACTAC/TAGCAAAGGAAGCCAGCCAAGTCAC	
	C-peptide 3‘ wt allele (1404 bp)	GGCAGCTCCATAGTCAG/TAGCAAAGGAAGCCAGCCAAG	
	Flattop 5‘ knock-in allele	TCGTGGTCATCTACTGCCTTC/CCTTTCTTCTGCGCCTTAGTC	
	Flattop 5‘ wt allele	TCGTGGTCATCTACTGCCTTC/GGTTGAGGGACTGGTTCTTTG	
	Flattop 3‘ knock-in allele	TCGGCATGGACGAGCTGTACAAG/TTTACTAGAGGGGCCCTGGGAAG	
	Flattop 3‘ wt allele	AGGTCATACTCCAGGTCCCCAAAG/TTTACTAGAGGGGCCCTGGGAAG	
Random integration-detecting PCR	Backbone plasmid (571 bp)	CATTGGAAACCGTTCTTCGGGGC/TGGCCCACTACGTGAACCATC	
Genotyping & sequencing off-targets C–Peptide	RCAN3	CTGTGAGCGGGAAACTATGC/GTCTTGGCCTCCCAAATTGC	
	PRSS27	CTGCTATGGACCATGTCTTCAC/AGGGCTACTTTAGGAAGGAAGG	
	CYTH4	CTCATGGAGCCGAGAGTCTAGC/GGCCACACTGTCTGTTGACC	
Genotyping & sequencing off-targets Flattop	RARRES2P5	TGAACTGTGCAGGGTAGCAG/AAAGCTGAGGGGTGCATACC	
	PSTPIP2	CACAGTTTGACTCTGTCACC/ACGTCTGCAACTTAGATTTCC	
	SCLY	GTCTCCCAGGAAAGGAAGGC/TATCCACTGCCCACTCAGGA	
Primers for Sanger sequencing	C-peptide sgRNA target site in wt allele	TCACAACAGTGCCGGGAAGTGGG	
	C-peptide 3′ recombination border knock-in allele	TCACAACAGTGCCGGGAAGTGGG	
	C-peptide 5′ recombination border knock-in allele	GCGGGCACTGTGTCTCCCTGACTG	
	RCAN3	CTGTGAGCGGGAAACTATGC	
	PRSS27	AGGGCTACTTTAGGAAGGAAGG	
	CYTH4	CTCATGGAGCCGAGAGTCTAGC	

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Table 2 (*continued*)

Antibodies and stains used for immunocytochemistry/flow-cytometry		
	Antibody	**Dilution Company Cat # and RRID**
Primers for Sanger sequencing	Flattop 3′ recombination border knock-in allele	TTTACTAGAGGGGCCCTGGGAAG
	Flattop 5′ recombination border knock-in allele	TCGTGGTCATCTACTGCCTTC
	RARRES2P5	AAAGCTGAGGGGTGCATACC
	PSTPIP2	ACGTCTGCAACTTAGATTTCC
	SCLY	GTCTCCCAGGAAAGGAAGGC

lipofection mix, containing 1.25 μg FVR donor plasmid and 1.25 μg Fltp-sgRNA-Cas9-Venus vector, was added overnight and cells FACsorted for GFP expression 48 h later. Clonal isolation and expansion was performed as described in Yumlu et al. 2017. Clone HMGUi001-A-55 was selected for further targeting of the insulin locus as described in Siehler et al. 2021, resulting in the generation of HMGUi001-A-56.

4.4. STR analysis

STR analysis was performed on extracted DNA by the Genomics Core Facility of the Helmholtz Zentrum München utilizing the AmpFℓ-STR™Identifiler™ PCR Amplification Kit (Applied Biosystems).

4.5. Karyotyping

Karyotyping was performed by the Institute of Human Genetics, Technical University of Munich, by standard G-banding technique and analysis of 20 mitotic events as described in Wang et al. 2018.

4.6. Flow cytometry

For live cell analysis, aggregates were dissociated with Accutase into single cells and analyzed immediately BD FACS Aria III. For analysis of surface markers, 1 x 10^6 cells were stained with the conjugated antibodies SSEA4-FITC and TRA-1–60-PE or the isotype controls REA-Control (S)-PE-Vio615 and REA-Control (S)-FITC according to the manufacturer's protocol.

4.7. Immunocytochemistry

Aggregates were fixed with 4 % paraformaldehyde, embedded in cryo blocks, sectioned, and immunofluorescence stainings performed as described in Bastidas-Ponce et al. 2017. Monolayer cells were fixed with 4 % PFA and stained as described in Wang et al. 2018. Details about primary and secondary antibodies can be found in Table 2. Images were acquired by confocal microscopy with the Zeiss LSM 880 Airy Scan.

CRediT authorship contribution statement

Tobias Greisle: Data curation, Formal analysis, Investigation, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ines Kunze:** Investigation, Resources. **Xianming Wang:** Investigation, Supervision, Validation. **Andrzej R. Malinowski:** Investigation, Methodology, Resources. **Anika Böttcher:** Funding acquisition, Supervision, Writing – review & editing. **Heiko Lickert:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Ingo Burtscher:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Heiko Lickert reports financial support was provided by European Union. Heiko Lickert reports financial support was provided by Federal Ministry of Education and Research Bonn Office. 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.2025.103838.

Data availability

Data will be made available on request.

References

Bader, E., Migliorini, A., Gegg, M., et al., 2016. Identification of proliferative and mature beta-cells in the islets of langerhans. Nature 535 (7612), 430–444. https://doi.org/10.1038/nature18624.

Bastidas-Ponce, A., Roscioni, S.S., Burtscher, I., et al., 2017. Foxa2 and Pdx1 cooperatively regulate postnatal maturation of pancreatic β-Cells. Mol. Metab. 6 (6), 524–534. https://doi.org/10.1016/j.molmet.2017.03.007.

Böttcher, A., Büttner, M., Tritschler, S., et al., 2021. Non-canonical Wnt/PCP signalling regulates intestinal stem cell lineage priming towards enteroendocrine and paneth cell fates. Nat. Cell Biol. 23 (1), 23–31. https://doi.org/10.1038/s41556-020-00617-2.

Cortijo, C., Gouzi, M., Tissir, F., Grapin-Botton, A., 2012. Planar cell polarity controls pancreatic beta cell differentiation and glucose homeostasis. Cell Rep. 2 (6), 1593–1606. https://doi.org/10.1016/j.celrep.2012.10.016.

Gegg, M., Böttcher, A., Burtscher, I., et al., 2014. "Flattop regulates basal body docking and positioning in mono- And Multiciliated Cells". Elife 3 (October), e03842. https://doi.org/10.7554/eLife.03842.

Rezania, A., Bruin, J.E., Arora, P., et al., 2014. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat. Biotechnol. 32 (11), 1121–1133. https://doi.org/10.1038/nbt.3033.

Siehler, J., Blöchinger, A.K., Akgün, M., et al., 2021. Generation of a Heterozygous C-Peptide-mCherry Reporter Human iPSC Line (HMGUi001-A-8). Stem Cell Res. 50 (January), 102126. https://doi.org/10.1016/j.scr.2020.102126.

Velazco-Cruz, L., Song, J., Maxwell, K.G., et al., 2019. Acquisition of dynamic function in human stem cell-derived beta cells. Stem Cell Rep. 12 (2), 351–365. https://doi.org/10.1016/j.stemcr.2018.12.012.

Wang, X., Sterr, M., Burtscher, I., et al., 2018. Genome-wide analysis of PDX1 target genes in human pancreatic progenitors. Mol. Metab. 9 (March), 57–68. https://doi.org/10.1016/j.molmet.2018.01.011.

Yoshihara, E., O'Connor, C., Gasser, E., et al., 2020. Immune evasive human islet-like organoids ameliorate diabetes. Nature 586 (7830), 606–611. https://doi.org/10.1038/s41586-020-2631-z.

Yumlu, S., Stumm, J., Bashir, S., et al., 2017. Gene editing and clonal isolation of human induced pluripotent stem cells using CRISPR/Cas9. Methods, CRISPR-Cas Systems for Genome Engineering and Investigation 121–122 (May), 29–44. https://doi.org/10.1016/j.ymeth.2017.05.009.