Ex vivo correction of severe coagulation Factor VII deficiency in patient-derived 3D liver organoids

Giacomo Roman,[1-3] Knut H. Lauritzen,[1-3] Sean P. Harrison,[4] Anindita Bhattacharya,[1-3] Marianne Seierstad Andresen,[2,3] Marie-Christine Mowinckel,[2,3] Barbora Smolkova,[4] Carola E. Henriksson,[1,5] Heidi Glosli,[4,6] Nina Iversen,[7] Bernd Thiede,[8] Gareth J. Sullivan,[4,9] Runar Almaas,[1,4] Per Morten Sandset,[1-3] Benedicte Stavik[2,3] and Maria E. Chollet[1-3]

[1]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [2]Department of Hematology, Oslo University Hospital, Oslo, Norway; [3]Research Institute of Internal Medicine, Oslo University Hospital, Oslo, Norway; [4]Department of Pediatric Research, Oslo University Hospital, Oslo, Norway; [5]Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway; [6]Center for Rare Disorders, Oslo University Hospital, Oslo, Norway; [7]Department of Medical Genetics, Oslo University Hospital, Oslo, Norway; [8]Department of Biosciences, University of Oslo, Oslo, Norway and [9]School of Medicine, University of St Andrews, St Andrews, UK

Correspondence: M.E. Chollet
m.e.c.dugarte@ous-research.no

Received: April 23, 2025.
Accepted: September 29, 2025.
Early view: October 9, 2025.

https://doi.org/10.3324/haematol.2025.288046

©2026 Ferrata Storti Foundation
Published under a CC BY-NC license

Supplementary information

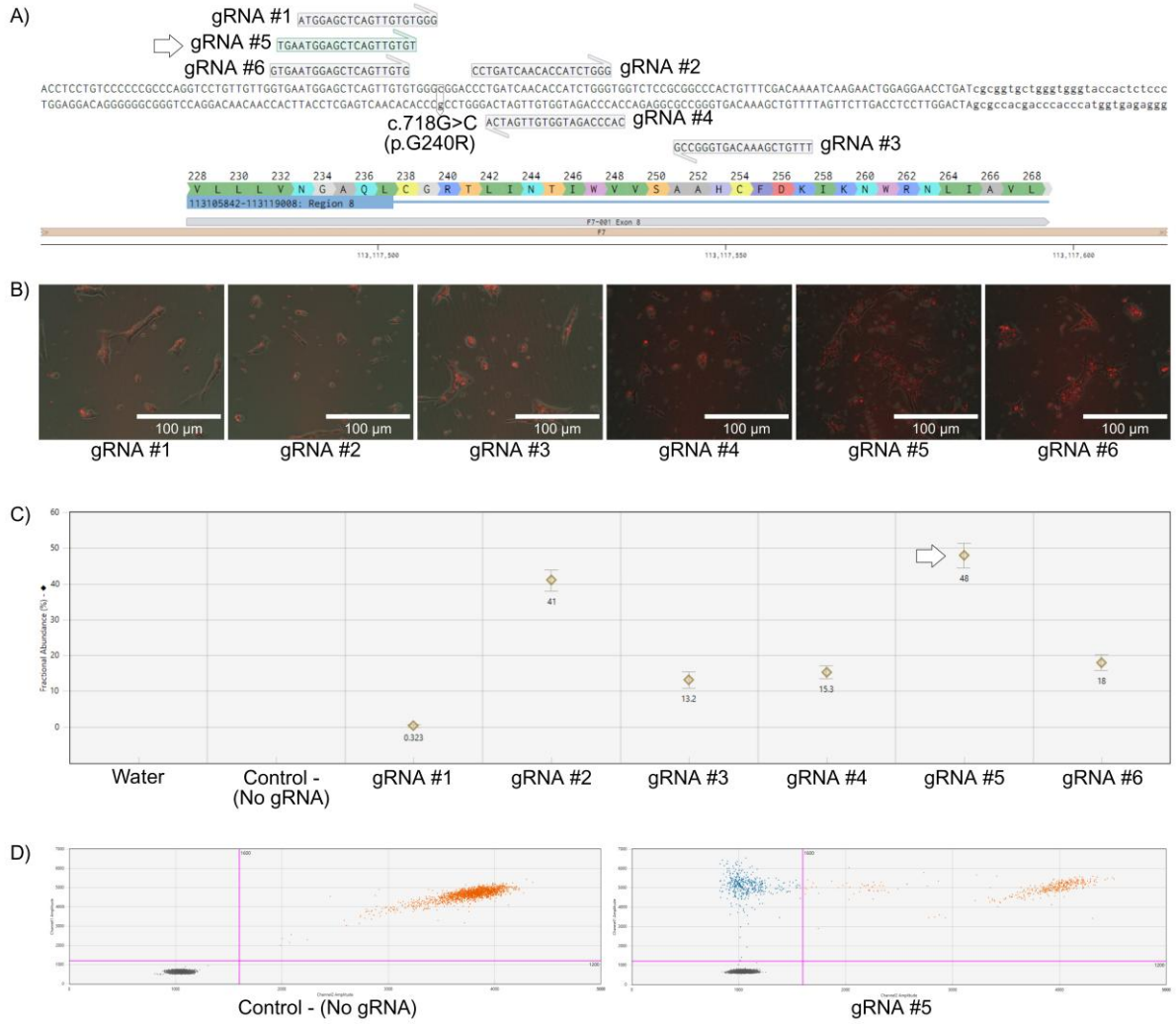
Ex vivo correction of severe coagulation Factor VII deficiency in patient-derived 3D liver organoids

This document includes:

- Supplementary figures and figure legends
- Supplementary table file legends
- Supplementary video file legend
- Supplementary materials and methods
- References

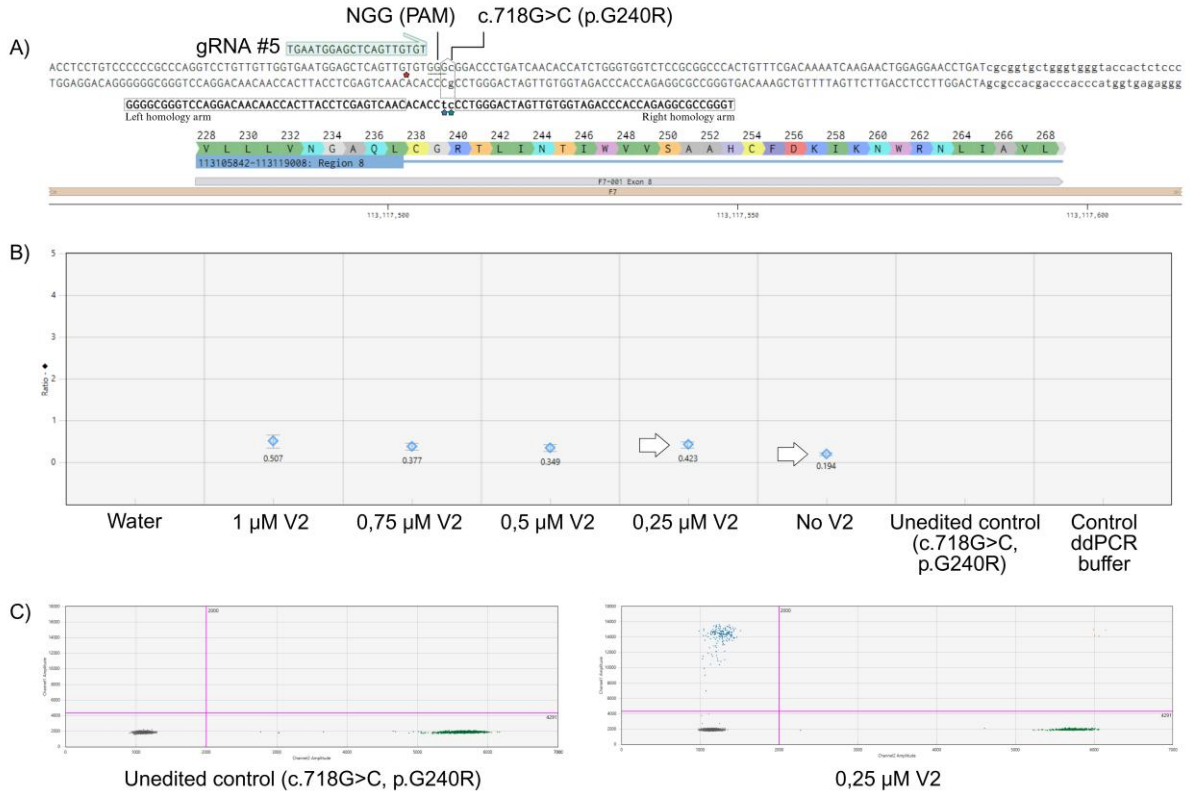
Supplementary figures

Supplementary figure 1



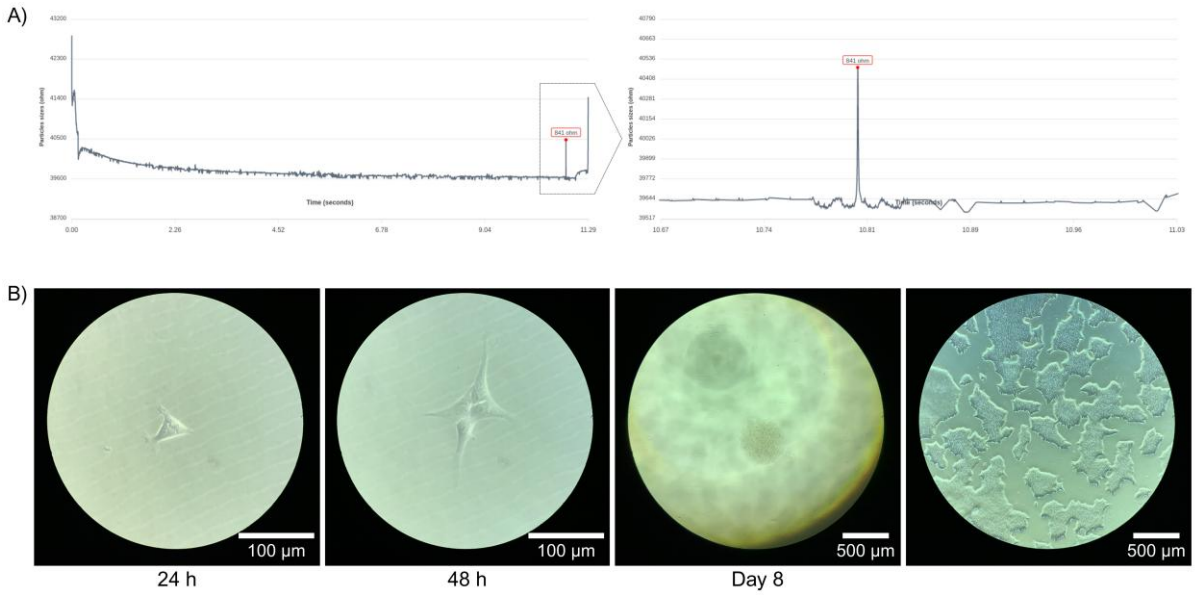
Supplementary figure 1. gRNA design and screening. Selected gRNA candidate (gRNA #5) is indicated with an arrow. (A) Summary of the gRNA panel. Created with Benchling. (B) Merge of brightfield and ATTO 550 (red) fluorescence, for each nucleofected RNP complex. (100 µm scale bar). (C, D) ddPCR NHEJ Genome Edit Detection Assays. On-target efficiency is displayed as fractional abundance (%), calculated as NHEJ edited alleles/(WT + NHEJ edited alleles), i.e. edited alleles/total alleles. (E) 2D drop-off amplitude view of c.718G>C, p.G240R control (left) and gRNA #5 (right). HEX amplitude on x-axis, FAM amplitude on y-axis. FAM^{+}/HEX^{+} cluster is represented in orange, FAM^{+}/HEX^{-} cluster in blue.

Supplementary figure 2



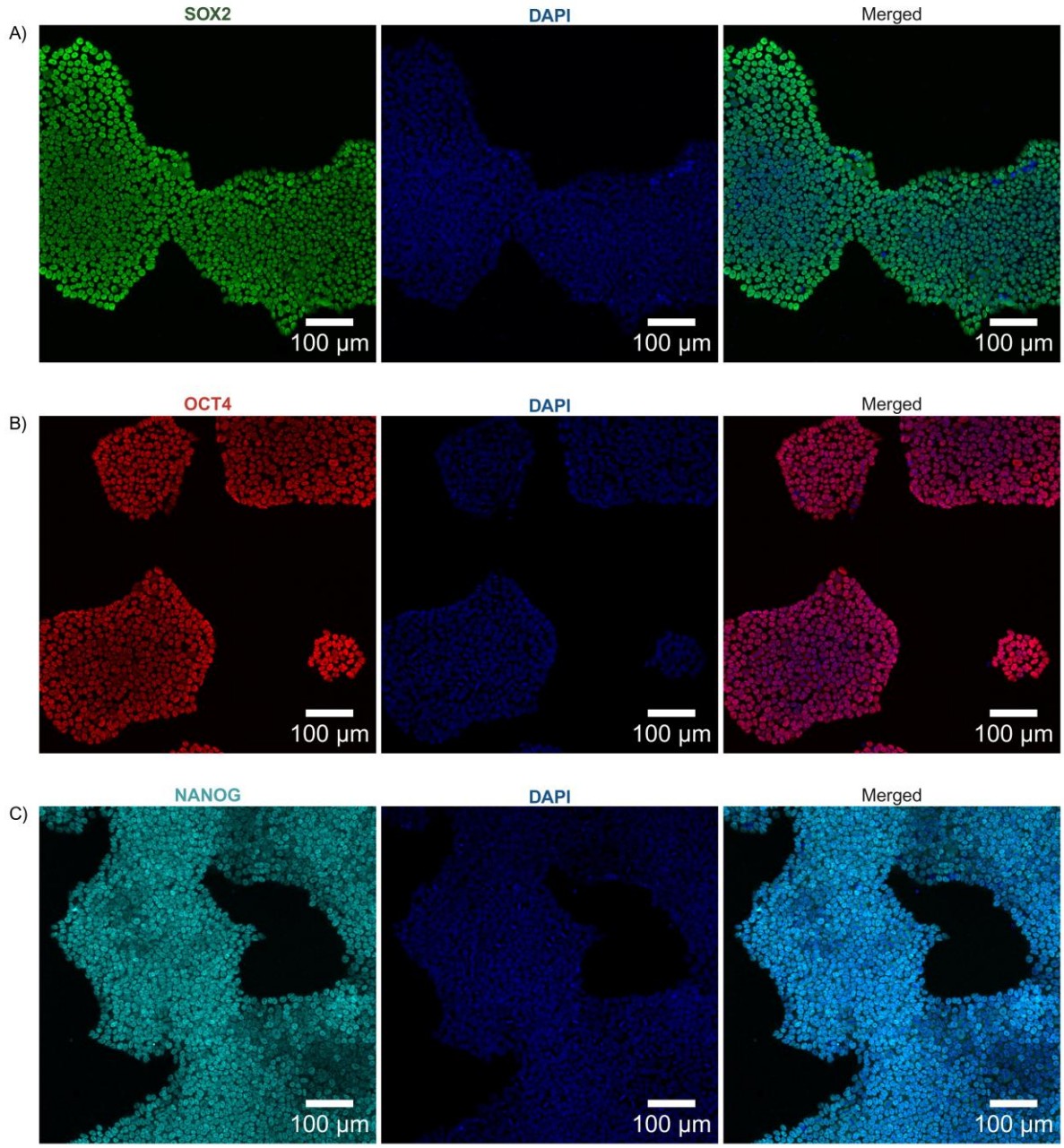
Supplementary figure 2. HDR experiment design and analysis. (A) Summary of the HDR design (gRNA #5/ssODN donor). The cut site is indicated by a red star, while the synonymous PAM-altering mutation and the therapeutic correction by a blue and green star, respectively (top). (B) ddPCR Homology Directed Repair Genome Edit Detection (HDR GED) assay for interrogating the HDR outcome at different concentration of Alt-R HDR Enhancer V2. The ratio provided is the number of HDR alleles/total number of alleles, and the percentage of total HDR efficiency can be obtained as ratio*100. Selected HDR pools are indicated by an arrow. (C) 2D amplitude view of HDR GED assay for c.718G>C, p.G240R control (left) and 0.25 µM Alt-R HDR Enhancer V2 (right). HEX amplitude on x-axis, FAM amplitude on y-axis. FAM^{+}/HEX^{+} cluster is represented in orange, FAM^{+}/HEX^{-} cluster in blue, $FAM^{-}HEX^{+}$ cluster in green.

Supplementary figure 3



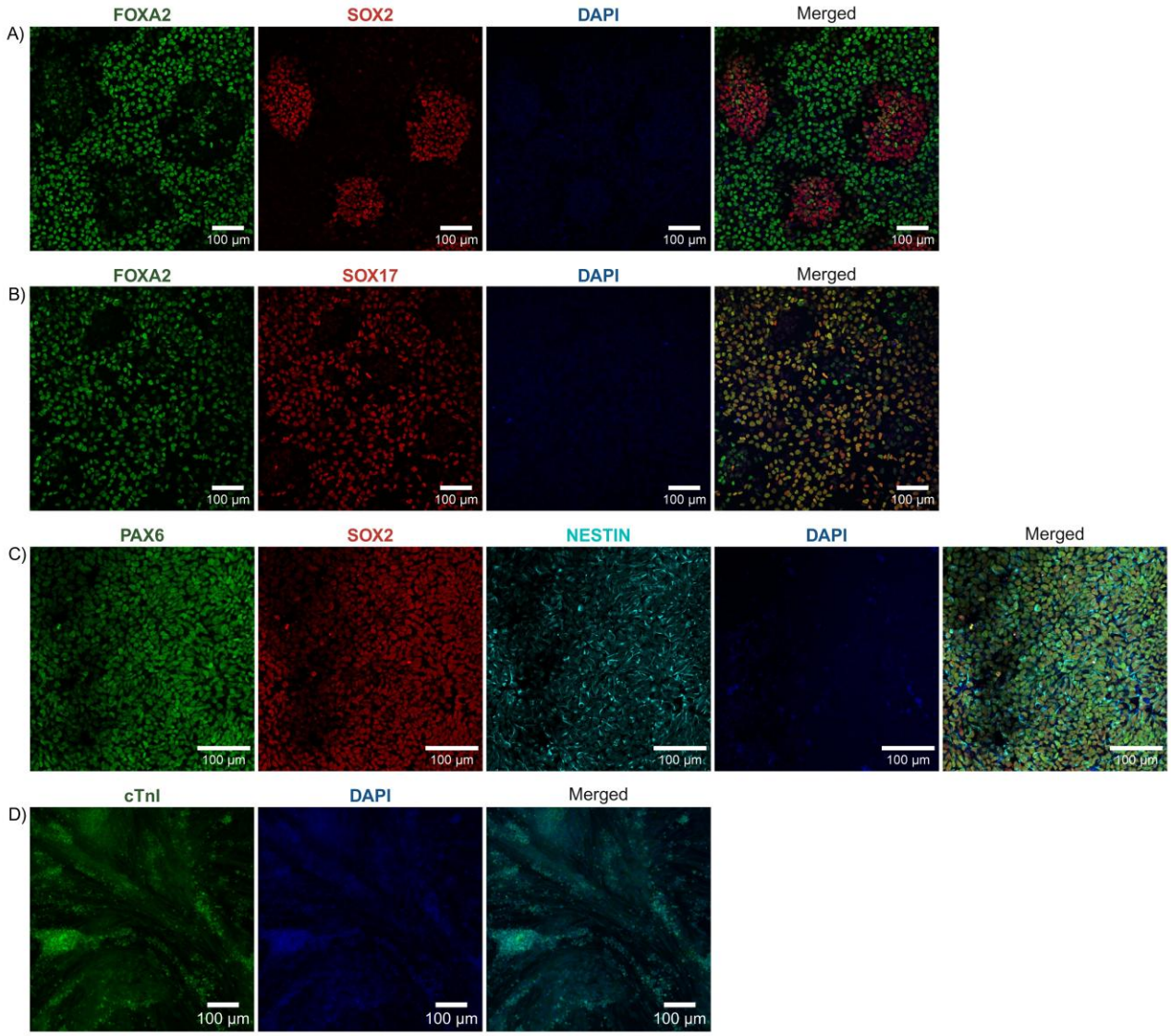
Supplementary figure 3. Single cell cloning of gene-edited iPSCs. (A) Whole view of the impedance-based monoclonality report from SEED DispenCell-S3 (left) and magnification of the single cell-induced signal record (right). Time (second) on x-axis and particle size (ohm) on y-axis. (B) Example of a growing single cell-derived, iPSC clone, after 24 hours from dispensing (left), 48 hours (middle) and 8 days (right). (100 µm and 500 µm scale bar).

Supplementary figure 4



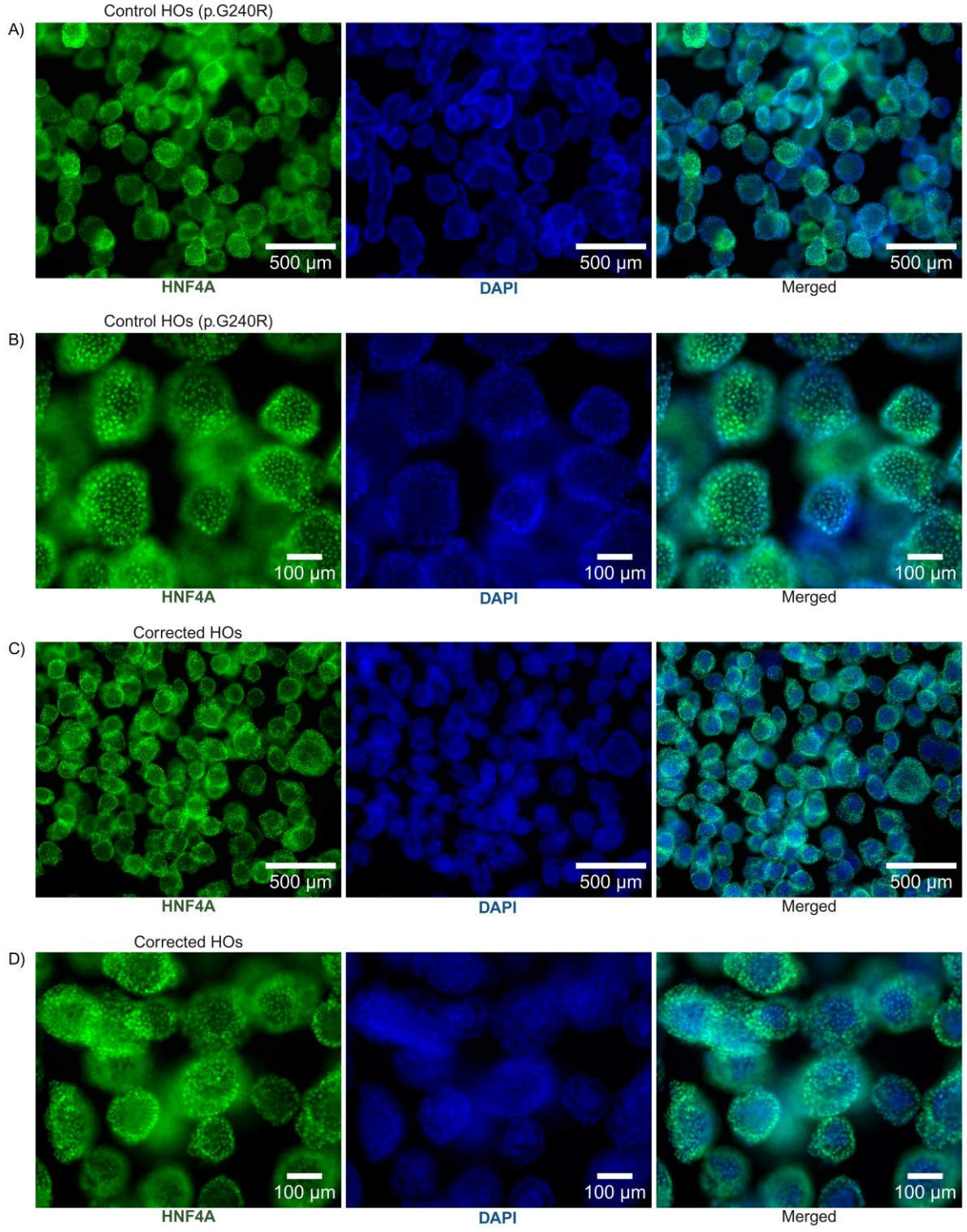
Supplementary figure 4. Single channels of the immunofluorescence analyses for the key pluripotency markers. (A) SOX2 (left, green), DAPI (middle, blue) and merged (right) (100 µm scale bar). (B) OCT4 (left, red), DAPI (middle, blue) and merged (right) (100 µm scale bar). (C) NANOG (left, cyan), DAPI (middle, blue) and merged (right) (100 µm scale bar).

Supplementary figure 5



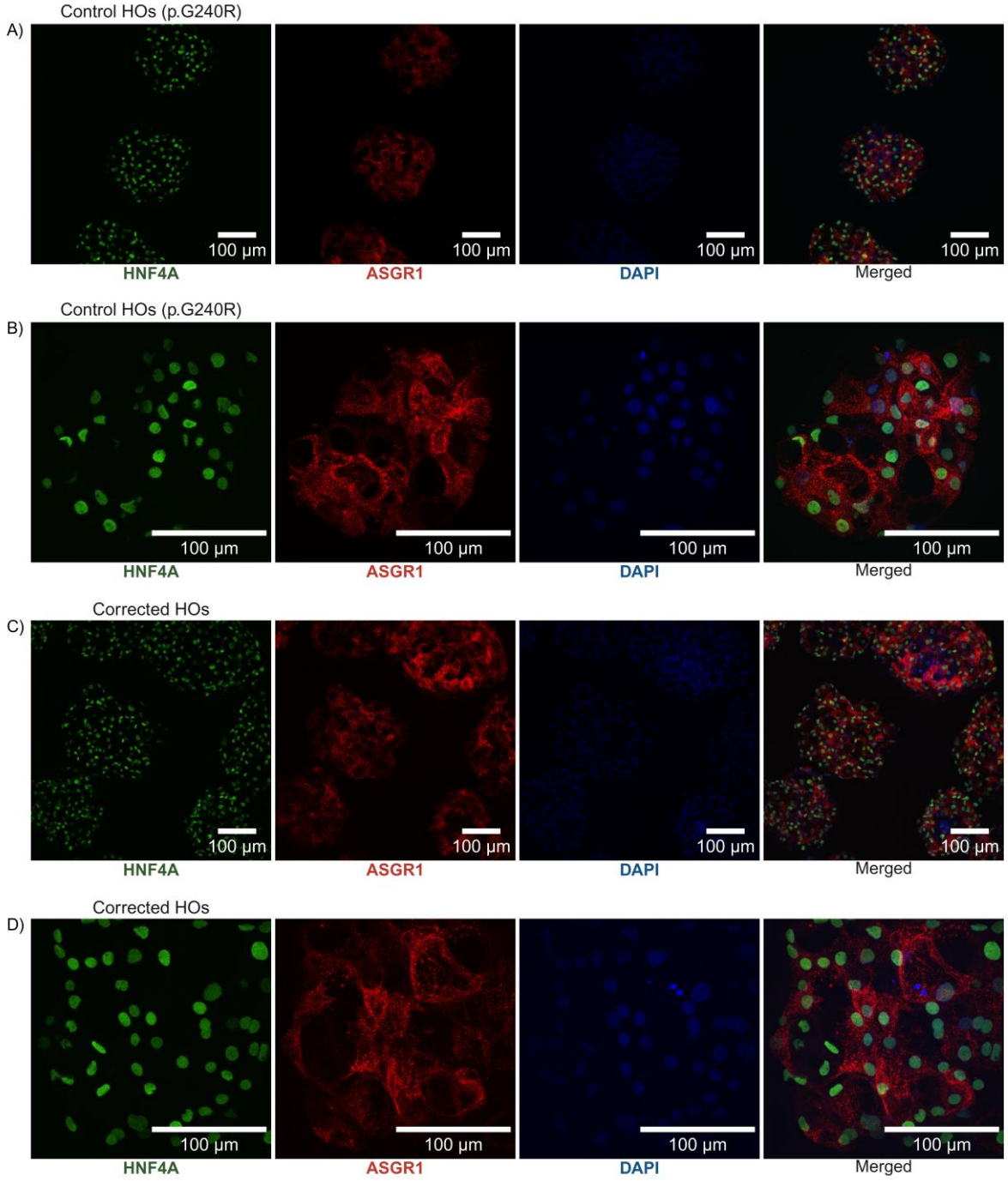
Supplementary figure 5. Single channels of the immunofluorescence analyses for the tri-lineage differentiation assay. (A) Definitive endoderm cultured with B27 (-) + 4 µM CHIR. FOXA2 (first from left, green), SOX2 (second from left, red), DAPI (third from left, blue) and merged (right) (100 µm scale bar). Zeiss LSM 880 Airyscan FAST confocal microscope. (B) Definitive endoderm cultured with B27 (-) + 3 µM CHIR. FOXA2 (first from left, green), SOX17 (second from left, red), DAPI (third from left, blue) and merged (right) (100 µm scale bar). (C) Neuroepithelium immunofluorescence analysis. PAX6 (first from left, green), SOX2 (second from left, red), NESTIN (third from left, cyan) and DAPI (fourth from left, blue) and merged (right) (100 µm scale bar). (D) Cardiomyocyte immunofluorescence analysis. cTnI (left, green), DAPI (middle, blue) and merged (right) (100 µm scale bar).

Supplementary figure 6



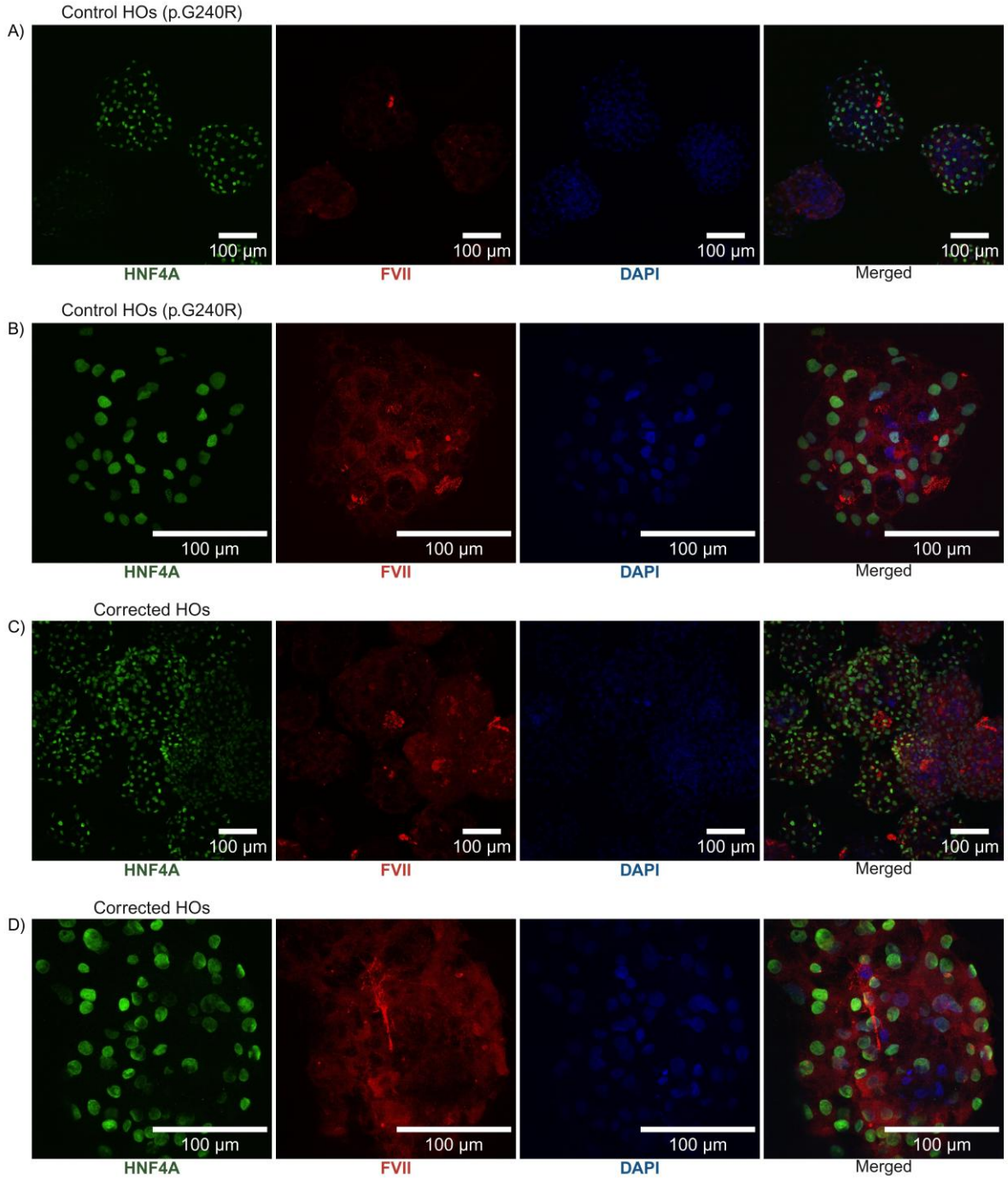
Supplementary figure 6. Single channels of the immunofluorescence analyses for HNF4A on p.G240R control and corrected HOs, at day 6. (A) p.G240R control HOs (4x magnification) (500 µm scale bar). HNF4A (left, green), DAPI (middle, blue) and merged (right). ECHO Revolve RVL-100-M. (B) p.G240R control HOs (10x magnification) (100 µm scale bar). HNF4A (left, green), DAPI (middle, blue) and merged (right). (C) Corrected HOs (4x magnification) (500 µm scale bar). HNF4A (left, green), DAPI (middle, blue) and merged (right). (D) Corrected HOs (10x magnification) (100 µm scale bar). HNF4A (left, green), DAPI (middle, blue) and merged (right).

Supplementary figure 7



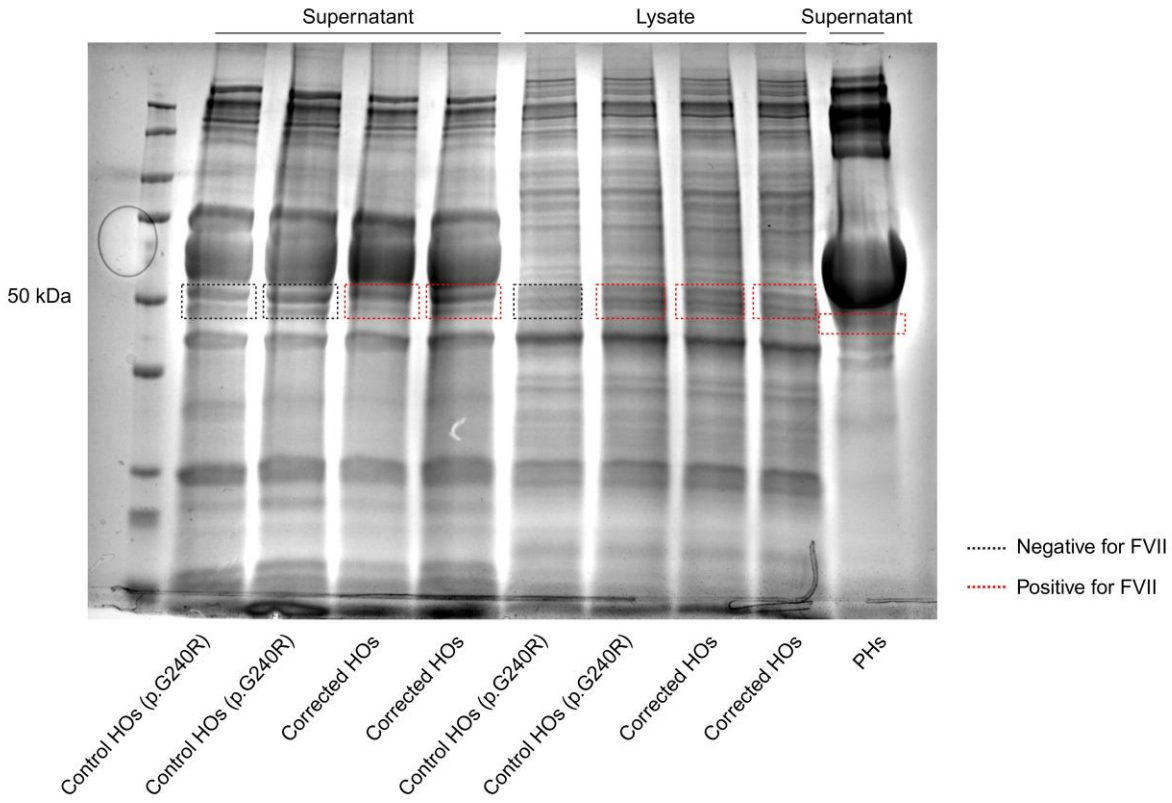
Supplementary figure 7. Single channels of the immunofluorescence analyses for ASGR1 and HNF4A markers on p.G240R control and corrected HOs, at day 19. (A) p.G240R control HOs. HNF4A (first from left, green), ASGR1 (second from left, red), DAPI (third from left, blue) and merged (right) (20x magnification) (100 µm scale bar). Andor Dragonfly Spinning Disk confocal microscope. (B) p.G240R control HOs (60x magnification) (100 µm scale bar). (C) Corrected HOs (20x magnification) (500 µm scale bar). (D) Corrected HOs (60x magnification) (100 µm scale bar).

Supplementary figure 8



Supplementary figure 8. Single channels of the immunofluorescence analyses for FVII and HNF4A markers on p.G240R control and corrected HOs, at day 19. (A) p.G240R control HOs. HNF4A (first from left, green), FVII (second from left, red), DAPI (third from left, blue) and merged (right) (20x magnification) (100 µm scale bar). Andor Dragonfly Spinning Disk Confocal Microscope. (B) p.G240R control HOs (60x magnification) (100 µm scale bar). (C) Corrected HOs (20x magnification) (500 µm scale bar). (D) Corrected HOs (60x magnification) (100 µm scale bar).

Supplementary figure 9



Supplementary figure 9. LC-MS analysis. SDS-PAGE of HO culture supernatants and cellular lysates. The gel samples of 40-60 kDa analyzed are indicated by black (negative for FVII) and red (positive for FVII) boxes.

Supplementary table file legends - See Excel File

Supplementary table file 1. Reagent and resource list. (A) List of primary and secondary antibodies used in this study. (B) Reagents and components. (C) Commercial assays and kits. (D) Oligonucleotide list that includes primers and TaqMan assays. (E) Software.

Supplementary table file 2. WES germline filtering and off-target prediction. (A) Results of the WES germline filtering. The silent, PAM-altering variant is highlighted in green. Variants were additionally verified using Integrative Genomics Viewer (IGV; igv.org) and marked with an asterisk (*) if visualized in the control iPSCs (c.718G>C, p.G240R). (B) Off-target sequences and sites predicted by IDT. (C) Off-target sequences and sites predicted by Benchling.

Supplementary video file legend

Supplementary video file 1. Contracting iPSC-derived cardiomyocytes. Bright-field microscopy recording of spontaneously contracting iPSC-derived cardiomyocytes at 2 weeks of differentiation, as part of the tri-lineage differentiation assay to assess pluripotency. ECHO Revolve RVL-100-M (40x magnification).

Supplementary materials and methods

Extended methods

The study was approved by the Norwegian regional committees for medical and health research ethics (REK 2018/777) and the Data Protection Officer at Oslo University Hospital (PVO #18/20935). The study was conducted in accordance with the Helsinki Declaration.

The reagent and resource list is present in the supplementary information (Supplementary information, table 1).

Generation and culturing of patient-derived iPSCs

Peripheral blood mononuclear cells (PBMCs) were isolated by Lymphoprep (STEMCELL; Vancouver, British Columbia, Canada) from blood samples collected from the FVII deficient patient, after written informed consent from the parents was obtained. The ethical approval was obtained from the data protection officer at Oslo University Hospital. The PBMCs were then reprogrammed to iPSCs by using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Invitrogen; Waltham, Massachusetts, USA), following the manufacturer's recommendations. When iPSC colonies appeared, they were picked and transferred to Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Gibco; Waltham, Massachusetts, USA)-treated 6 well plates (Thermo Fisher Scientific; Waltham, Massachusetts, USA) and cultured on. iPSCs were routinely expanded by clump passaging in 6 well plates, using 0.5 mM EDTA. The iPSCs were maintained at 37 °C, 5% CO_2 and in Essential 8 Medium (Gibco), supplemented with Penicillin-Streptomycin (10,000 U/mL) (Gibco) at 1% final concentration.

Tri-lineage assessment of pluripotency

Pluripotency of the iPSCs was investigated by direct differentiation to the three different germ layers. The iPSCs were directed to the definitive endoderm using the protocol developed by Mathapati et al., 2016[1] and Harrison et al., 2023[2], based on CHIR99021 treatment of iPSCs. To assess mesoderm differentiation, we used the protocol from Lian et al., 2013[3] to produce functional cardiomyocytes using various concentrations of CHIR99021 (4 µM – 10 µM) and 5 µM IWP 2 (Tocris). To assess ectoderm potential, we employed the method described by Chambers et al., 2009[4] and Maroof et al., 2013[5]. In short, neural epithelial cells were generated by using N-2 Supplement (100X) (Gibco), 10 µM SB 431542

(Tocris), 100 nM LDN-193189 (Selleckchem; Houston, Texas, USA) and 2 µM XAV 939 (Tocris).

Karyotyping

The iPSCs were karyotyped by KaryoStat+ Genetic Stability Assay Service (Thermo Fisher Scientific).

Genomic DNA and RNA isolation

Genomic DNA (gDNA) was isolated by using either the DNeasy Blood & Tissue Kit (Qiagen; Venlo, The Netherlands) or the Phire Animal Tissue Direct PCR Kit (Thermo Scientific), depending on the application. Total RNA was isolated by using the MagMAX-96 Total RNA Isolation Kit (Invitrogen).

Polymerase chain reaction (PCR) and reverse transcription quantitative PCR (RT-qPCR)

PCRs were performed using AmpliTaq Gold 360 Master Mix (Applied Biosystems; Waltham, Massachusetts, USA). The optimal annealing temperature and GC Enhancer amount was identified using gradient PCR. PCR products were confirmed by agarose gel electrophoresis, using a 1:5 dilution with gel loading dye (Purple (6X) (NEB; Ipswich, Massachusetts). When needed, the PCR products were purified using GeneJET PCR Purification Kit (Thermo Fisher Scientific). For reverse transcription quantitative polymerase chain reaction (RT–qPCR), total RNA was reversely transcribed to cDNA using qScript cDNA Synthesis Kit (QuantaBio; Beverly, Massachusetts, USA). Gene expression was assessed using TaqMan assays and analyzed with QuantStudio™ Software V1.3. All graphs were generated using GraphPad Prism v10.2.0.

CRISPR gene editing

Guide RNAs (gRNAs) and single-stranded oligodeoxynucleotides (ssODNs) donors were designed using the Alt-R CRISPR HDR Design Tool (IDT; Newark, New Jersey, USA) and Benchling software design tools (Benchling; San Francisco, California, USA). Donor templates consisted of symmetric homology arms of 40 bp, the corrective c.718C>G change and a protospacer adjacent motif (PAM)-altering modification. The PAM-altering

modifications were designed with Benchling software design tool as single-nucleotide, synonymous variants, to avoid re-cutting at the target locus, and further analyzed with MutationTaster2021, BDGP (https://www.fruitfly.org/seq_tools/splice.html), MobiDetails[6] and GeneBe (https://genebe.net/). The gene editing experiments were performed by nucleofection (Neon transfection system, Thermo Fisher Scientific) of ribonucleoprotein (RNP) complexes, assembled following IDT's recommendations[7]. In short, the CRISPR RNA (crRNA):transactivating crRNA (tracrRNA) duplexes were prepared by mixing the customized crRNAs and the Alt-R CRISPR-Cas9 tracrRNA ATTO 550, in equimolar concentrations. RNP complexes were generated by combining the Alt-R S.p. HiFi Cas9 Nuclease V3 with the crRNA:tracrRNA duplexes. The electroporation mixture was assembled by combining 1 µL RNP complex, 9 µL cell suspension and 2 µL Resuspension Buffer R, or Alt-R HDR Donor Oligo. 10 µL of this mixture was pipetted into the Neon Pipette Station and nucleofected at 1400 V, with 1 pulse at 20 ms pulse width. For screening of cleavage efficiency of each gRNA, gDNA was isolated after <72 hours.

Droplet digital PCR (ddPCR)

The ddPCR was performed by using customized Non-Homologous End Joining (NHEJ) Genome Edit Detection Assays (BioRad; Hercules, California, USA) and HDR Genome Edit Detection Assays (BioRad), following the manufacturers' instructions. The reactions were prepared by using the ddPCR Supermix for Probes (No dUTP) (BioRad) and 20 ng gDNA input template. The droplets were generated with the QX200 Droplet Generator (BioRad) and analyzed on a QX200 Droplet Reader (BioRad). Data analysis was performed using QX Manager Software, Standard Edition v2.1 (Bio-Rad).

Immunofluorescence and microscopy

Immunofluorescence analyses were performed following the methods described by Harrison et al., 2023[2] for the HOs and the Cell Signaling protocol (Danvers, Massachusetts, USA) for the iPSCs. Samples on µ-Slide 2 Well chambers were fixed with 4% paraformaldehyde (Thermo Fisher Scientific) for 15 minutes and blocked with 10% normal donkey serum (Abcam; Cambridge, United Kingdom), 0.3% triton X-100 (Sigma-Aldrich; Burlington, Massachusetts, USA) in Dulbecco's phosphate-buffered saline with no calcium or magnesium ($DPBS^{-/-}$). The antibodies were diluted in 10 mg/mL bovine serum albumin (Sigma-Aldrich) and 0.3% triton X-100 $DPBS^{-/-}$. Samples were mounted with ProLong Glass Antifade Mountant with NucBlue Stain (Invitrogen). HO suspensions were fixed for 30

minutes, then permeabilized and blocked with 10% normal donkey serum in 0.1% triton X-100 $DPBS^{-/-}$ for 30 minutes. The primary antibodies were directly diluted in the suspension and incubated for 1 hour at 37 °C. The secondary antibodies were mixed in 1% normal donkey serum and 0.1% triton $DPBS^{-/-}$, and incubated for 1 hour at 37 °C. A negative control immunostaining was performed using only secondary antibodies. The HOs were mounted with NucBlue Fixed Cell ReadyProbes Reagent (4',6-diamidino-2-phenylindole (DAPI)) (Invitrogen). Imaging was performed using the ECHO Revolve RVL-100-M, Zeiss LSM 880 Airyscan FAST confocal microscope and Andor Dragonfly Spinning Disk confocal microscope.

iPSCs single cell cloning

iPSCs were pre-incubated with E8 supplemented with 10 µM Y-27632 (Tocris), for 1 hour at 37 ºC, 5% CO_2. Then iPSCs were treated with StemPro Accutase (Gibco) supplemented with 10 µM Y-27632, for <10 minutes at 37 ºC, 5% CO_2. The iPSCs were resuspended with complete StemFlex Medium (Gibco), supplemented with 10 µM Y-27632, and filtered through a 20 µm strainer (SEED Biosciences; Epalinges, Switzerland) twice. Cells were counted with trypan blue exclusion, pelleted at 200xg for 5 minutes and resuspended at 150’000 cells/mL in StemFlex-Y-27632. 15 µL of the cell suspension was moved into the DispenMe (SEED Biosciences) vial and gently resuspended. Following the manufacturer’s instructions, the cell suspension was loaded on the Dispencell (SEED Biosciences) cell seeding device and dispensed at 1 cell/well, into Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Thermo Fisher Scientific)-coated 96 well plates, which had been pre-warmed and filled with 100 µL/well StemFlex-Y-27632. Wells in which more than one cell (multiple single cell events, doublets or aggregates) was dispensed, or where the detection signal was ambiguous or uncertain, were excluded. An optimal particle size was defined by an impedance range of ≥400 to ≤3,000 ohm. After <48 hours, fresh 150 µL StemFlex were added to dilute the Y-27632. After 48 hours, 150 µL were removed from each well and replaced with fresh StemFlex. After that, iPSCs were expanded with regular medium changes.

Sanger Sequencing and Whole Exome Sequencing (WES)

Sanger sequencing, Illumina WES analyses at 30x coverage (Clinical Research Exome - CRE V4) and 100x coverage (INVIEW Human Exome) and downstream bioinformatic analysis were performed by Eurofins Genomics (Ebersberg, Germany).

Hepatic organoid differentiation

Hepatic organoids (HOs) were generated using the protocol developed by Harrison et al.[2],. In short, iPSCs were seeded as single cells in 125 mL PC Erlenmeyer Shaker Flasks (VWR; Radnor, Pennsylvania, USA), at a concentration of $2.5 \cdot 10^5$ cells/mL in 20 mL E8, supplemented with CultureCEPT Supplement (Gibco). After 16 hours (day 0), the HOs were cultured with RPMI-B-27 Supplement (Gibco), minus insulin, supplemented with 3.8 µM CHIR99021. After 24 hours (day 1), the medium was replaced with RPMI-B-27 Supplement, minus insulin, without CHIR99021. The HOs were then cultured with SR-DMSO from day 2 until day 6, and with L-15 medium from day 7 until day 19. On day 19, the HOs were washed twice with 20 mL L-15 base (Sigma-Aldrich) and then incubated with serum-free L-15 medium, formulated as described by Harrison et al., 2023[2] but devoid of fetal bovine serum (Biowest; Bradenton, Florida, USA) and supplemented with 5 µg/mL Vitamin K_1. Both HOs and the cell supernatant were collected on day 21 for the downstream analyses, after 2 days in serum-free conditions.

Protein determination and activity assay

Supernatants were harvested from HO suspensions by centrifugation for 5 minutes at 300xg without brake and collected in 2 mL tubes. The supernatants were then centrifuged at 4 ºC, 18'118xg, for 15 minutes, and stored at -80 ºC. Protein levels (FVII:Ag) were analyzed by U-PLEX Human Factor VII Assay (Mesoscale Diagnostic; Rockville, Maryland, USA), following the manufacturer's instructions. FVII activity (FVII:C) was measured using the BIOPHEN FVII assay kit (Aniara; West Chester Township, Ohio, USA). Factor X (Abcam), albumin (Nordic Biosite; Täby, Stockholm) and α-1 Antitrypsin (A1AT) (Abcam) antigens were measured by enzyme-linked immunosorbent assay (ELISA). Thrombin generation was assessed using the calibrated automated thrombogram (CAT) (Diagnostica Stago, Asnières sur Seine, France), as described by Harrison et al.[2]. 50 µL sample was mixed with 30 µL of FVII-deficient plasma (HemosIL, Instrumentation Laboratory Company, Bedford, MA, USA) and 20 µL of either 1 pM (PPP reagent low) or 5 pM tissue factor (TF) (PPP reagent). After 10 minutes incubation at 37 °C, the thrombin generation was initiated by adding 20 µL of fluorogenic substrate in a buffer containing calcium chloride (FluCa). All the reagents were provided by Diagnostica Stago. The fluorescence was measured kinetically at 37 °C for up to 80 minutes and the results were analyzed in the thrombinoscope software. Controls included L-15 medium as negative control, PHs and pooled normal plasma (PNP) as positive controls. Cell pellets were obtained by centrifugation of 5 mL of HO suspension, the supernatant was removed and the pellet resuspended with 1 mL T-PER Tissue Protein

Extraction Reagent (Thermo Fisher Scientific), supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (100X) (Thermo Fisher Scientific), and homogenized with BD Microlance 18 G needle (Becton Dickinson; Franklin Lakes, New Jersey, USA). Sample homogenates were centrifuged at 8'000xg for 10 minutes, at 4 ºC, to obtain a clear fraction and stored at -80 ºC. The total cellular protein was quantified with Pierce BCA Protein Assay Kits (VWR). As a positive control, Human Plateable Hepatocytes, Uptake Qualified (Gibco) were cultured as a dense population of $1 \cdot 10^6$ cells/mL, in 2 mL maintenance medium (Gibco), following the manufacturer's recommendations. Samples were collected after 48 hours in maintenance medium and processed as above to generate lysates. As additional positive reference for the FVII chromogenic activity assay, HOs generated from a wild-type, healthy and unedited iPSC line (AG27) were used[2].

Liquid chromatography-mass spectrometry (LC-MS) analysis

Proteins from culture supernatants (concentrated) and cellular lysates were separated by SDS-PAGE and stained with Coomassie Blue (Thermo Fisher Scientific). Gel bands of size 40-60 kDa were excised and subjected to in-gel digestion using trypsin, after which the resulting proteolytic fragments were desalted using 10 µL STAGE-tips (Affinisep; Le Houlme, France). The samples were analyzed by LC-MS using a timsTOF Pro (Bruker Daltonik; Bremen, Germany) which was coupled online to a nanoElute nanoflow liquid chromatography system (Bruker Daltonik) via a CaptiveSpray nanoelectrospray ion source. The dried peptides were dissolved in 4 µL 0.1% formic acid and 2 µL of sample was injected. The peptides were separated on a reversed phase Aurora Elite CSI UHPLC column (C18, 15 cm x 75 µm, 1.7 µm) (IonOpticks; Fitzroy, VIC, Australia). Mobile phase A contained water with 0.1% formic acid, and acetonitrile with 0.1% formic acid was used as mobile phase B. The peptides were separated by a gradient from 0-35% mobile phase B over 30 min at a flow rate of 200 nL/min at a column temperature of 50°C. MS acquisition was performed in DDA-PASEF mode. The LC-MS data were searched against the UniProt database of Homo sapiens (20,429 entries) using Mascot version 3.0 (Matrix Science; London, UK). The following parameters were used: digestion enzyme, trypsin; maximum missed cleavage, 1; fragment ion mass error tolerance, 0.03 Da; parent ion error tolerance, 15 ppm. Oxidation of methionine, propionamidylation of cysteine, and acetylation of the protein N-terminus were specified as variable modifications. Scaffold version 5.1.2 (Proteome Software Inc.; Portland, OR, USA) was used to validate MS/MS based peptide and protein identifications. A false-discovery rate of 1% of the peptide threshold was applied to the datasets.

Statistical analyses

The statistical analyses were performed with GraphPad Prism 10.2.0. The data were generated from three independent experiments consisting of two biological replicates for the HOs and one for the PHs and are expressed as mean and standard deviation. The normal distribution of values was assessed by Shapiro-Wilk test. For the comparison among two groups the statistical significance was determined by unpaired Student's t-test. A probability value of $p<0.05$ was considered statistically significant, with * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$.

References to supplementary data

1. Mathapati S, Siller R, Impellizzeri AAR, et al. Small-molecule-directed hepatocyte-like cell differentiation of human pluripotent stem cells. Curr Protoc Stem Cell Biol 2016;2016(1):1G.6.1-1G.6.18.

2. Harrison SP, Siller R, Tanaka Y, et al. Scalable production of tissue-like vascularized liver organoids from human PSCs. Exp Mol Med 2023;55(9):2005–2024.

3. Lian X, Zhang J, Azarin SM, et al. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/β-catenin signaling under fully defined conditions. Nat Protoc 2013;8(1):162–175.

4. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol 2009;27(3):275–280.

5. Maroof AM, Keros S, Tyson JA, et al. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell 2013;12(5):559–572.

6. Baux D, Van Goethem C, Ardouin O, et al. MobiDetails: online DNA variants interpretation. Eur J Hum Genet 2021;29(2):356–360.

7. Kenny Moore C, Martin J, William S. Electroporation of human pluripotent or embryonic stem cells with CRISPR reagents Ribonucleoprotein delivery using the Alt-R ® CRISPR-Cas9 System for homology-directed repair or other genome editing applications. 2017; Coralville, IA. Integrated DNA Technologies.;1–5. www.idtdna.com/CRISPR-Cas9.