Supplemental methods

Human pluripotent cell production

HPSC lines were derived from dermal fibroblasts using the four Yamanaka's factors (OCT4, SOX2, KLF4, MYC) introduced by a variety of means (integrative retroviral vectors, episomal plasmid vectors and non-integrative Sendai vectors).

Recombinant retroviral vectors

The three transcription factors *GATA1*, *FLI1* and *TAL1* (Addgene #92416, #92415 and #92417 respectively) were individually cloned into the replication deficient 2nd generation self-inactivating pWPT lentiviral backbone (Dr. Trono, Addgene # 12255) as described (Moreau 2016) and replication deficient vector particles (LVPs) generated by transient co-transfection of HEK 293T/17 cells (ATCC CRL-11268) using the pWPT vectors and psPAX2 and pMD2.G helper plasmids (Addgene # 12260, # 12259) following local health and safety regulations. The large-scale production of lentiviral particles was carried out commercially by Vectalys (Toulouse, France) to >1.00E+09 TU/ml.
hPSC lines were transduced for a single 18-24 hr exposure to LVPs using MOI of 20 in the presence of Protamine sulphate 10µg/ml in routine basal media.

Lentiviral forward programming

Lentiviral programming followed our original published protocol with some modifications. Briefly, 24hrs prior to transduction (D-1) hPSCs were dissociated to small clumps using PBS/EDTA or single cell suspensions using TrypLE Select (Thermo Fisher Scientific) and 5.00E+04-1.00E+05 cells seeded onto vitronectin (VTN-N, Thermo Fisher Scientific) coated 12 well plates in a serum free defined media supplemented by recombinant zebrafish FGF2 15ng/ml, human Activin-A 15ng/ml (Cambridge stem cell Institute) and rock inhibitor Y27632 to 10µM (Sigma). On day of transduction (D0) the 3TFs are added at a MOI of 20 using an in-house media based on E6 (for 500ml: 500mL of DMEM/F12 (ThermoFisher Scientific), 3.6mL 7.5% $NaHCO_3$ (ThermoFisher Scientific) 10mL Insulin-Transferrin-Selenium premixed 100x solution (i.e. used as 50x here) (ThermoFisherScientific) and 5mL L-ascorbic acid 100 x (6.4mg/ml L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate)

stock solution (Sigma) supplemented with BMP4 10ng/ml, FGF2 20ng/ml and protamine sulphate 10µg/ml for 24hours followed by a PBS wash and replacing the media supplemented with BMP4 and FGF2 only (D1). On D2 the mesoderm induction media is aspirated and MK media plus TPO 20ng/ml (Biotechne) and SCF 25ng/ml (ThermoFisher Scientific) added. Cells are fed every 2-3 days by replacing half volume with 2 x media plus cytokines. At day 10 cells were collected from the supernatant and pooled with adherent cells after TrypLE treatment. FoP outcome is determined by flow cytometry of an aliquot of cells. MKs are then continued in suspension culture feeding every 2-3 days by 50% removal 2 x cytokine concentration replacement and monitoring by flow cytometry at regular intervals.

Inducible forward programming (iMK_FoP)

hPSCs are seeded for differentiation as described for viral FoP and on D0 the same mesoderm media is added (in-house E6+FGF2 20ng/ml+ BMP4 10ng/ml) excluding protamine sulphate and including doxycycline at the determined concentration for each line, for most lines 0.25µg/ml doxycycline. Mesoderm media is refreshed on D2 plus doxycycline, and on D3 media replaced with MK media plus TPO 20ng/ml (Biotechne) and SCF 25ng/ml (ThermoFisher Scientific) plus doxycycline. From this stage on the FoP is as described for viral programming plus doxycycline addition and the media is refreshed every 2 days as described for virally transduced cells. In experiments with CHIR99021 3µM (Cayman Chemical Company) or DMSO vehicle control was added for 24hrs on D1-2 of FoP and then removed.

Generation of inducible targeting constructs

We adapted a system originally developed by Matthias Pawlowski, termed the OPTi-OX system (*patent 1619876.4*) using TET-ON 3G, third generation rtTA plus the TRE component (Zhou et al. 2006) to drive exogenous gene expression**.** We target the rtTA and TRE components to two different genomic safe harbours.
ROSA26 targeting consists of CRISPR/Cas9n/guide RNA vectors (Vector maps 3, 4) plus the donor vector Rosa26-CAG-rtTA (OPTi-OX) containing a CAG promoter to drive constitutive rtTA expression. The Rosa-CAG-rtTA targeting vector was constructed by cloning the coding sequence of a third generation rtTA into the the BamHI/MluI

(NewEngland Biolabs, NEB) sites of pRosa26-CAG-EGFP thus replacing the EGFP sequence (Vector map 2).
AAVS1 targeting used ZFN vectors (pZFN_AAVS1-L-ELD and pZFN_AAVS1-R-KKR, Vector maps 5, 6): the generous gift of Dr. Kosuke Yusa (Wellcome Trust Sanger Institute, Hinxton, UK). Previously described AAVS1 left and right ZFNs amino acid sequences were modified to generate obligate heterodimer ZFNs by codon optimization for mammalian expression. The combination of these two plasmids induces a specific double strand break between exons 1 and 2 of PPR1R12C on chromosome 19 (AAVS1 locus).
To complete the AAVS1 targeting we excised the GFP open reading frame (ORF) from pAAVS1-TRE-GFP with NcoI/EcoRI (NEB) and introduced a Nco1-eGFP-GATA1-TAL1CO-FLI1CO-EcoRI polycistronic fragment from pWPT eGFP_GATA1_TAL1CO_FLI1CO.

Generation of inducible hPSC for MK-FoP

Prior to genetic modification of each hPSC a puromycin (Vector Map1: AAVS1-TRE-PC3) and a G418 survival curve (Vector map 2: Rosa-neo-CAG-rtTA) was performed. Parental lines were seeded as single cells, following TrypLE Select (Thermo Fisher Scientific) dissociation, at 5.00+04 cells per well of a 12-well plate in E8 media plus rock inhibitor Y-27632 (Sigma) 10µM overnight at 37°C and 5% CO_2. Colonies are allowed to grow for 48hrs changing the media daily then Puromycin (Sigma) 1mg/ml stock in dH20 was added at 2, 1, 0.25, 0.125, 0.0675, 0.0337 µg/ml final concentration to different wells. After monitoring and daily for a week the results are recorded generally cultures are killed by between 0.5 and 0.125 µg/ml puromycin. This is repeated for G418 (Thermo Fisher Scientific) using a working stock of 100mg/ml in dH20 and at 500 to 50µg/ml on the cells feeding and monitoring the culture as for puromycin. The range for G418 killing is 200-100µg/ml for hPSC lines tested thus far.
To create single step inducible lines low passage number parental lines were dissociated to single cells using TrypLE Select, counted using a haemocytometer and 5.00+05 to 1.00+06 cells centrifuged gently at 115g for 3minutes at room temperature. The pellet was resuspended in 100µl of nucleofector mix (82µl Human stem cell nucleofector solution 2 + 18µl of supplement 1) using Nucleofector kit VPH-5022 (Lonza) to which a mix of all six plasmids, 6µg in total, in a minimal volume was added: 1µg p Rosa26-CAG-rtTA (OPTi-OX), 1µg pRosa26-guideA_cas9n, 1µg pRosa26-guideB_cas9n, 1µg pAAVS1-TRE-GFP,

1µg pZFN_AAVS1-L-ELD, 1µg pZFN_AAVS1-R-KKR (Vector maps 1-6, Figure S4). After gentle mixing cells were transferred to a cuvette avoiding any bubbles and tapped on the bench before Nucleoporation using program A-023 in the Amaxa™ Nucleofector™ II (Lonza). Cells were recovered immediately using 500µl media and transferred to two wells of a 6 well plate coated with laminin LN-521 at 5µg/ml (BioLamina) diluted in DPBS with calcium and magnesium (ThermoFisher Scientific). Laminin plates are incubated overnight at 4°C then the coating mix aspirated and E8 media plus rock inhibitor Y-27632 (Sigma) 10µM added to receive cells. Transfected cells are incubated overnight at 37°C and 5% CO_2 then fresh media without Y-27632 added. Selection with puromycin and G418 is started 48hrs after transfection using line determined concentrations. Selection continues for 5-7 days or until all the cells on the non-transfected control well are dead. Colonies start to appear 7-10 days later and are picked into 12 well plates mechanically using a 200p pipette tip, colonies are picked relatively small to ensure maintenance of pluripotency. Colonies were expanded and transferred back to VTN coated plates by passage 3. Cells were banked as frozen vials and genomic DNA(gDNA) was prepared for genotyping.

Generation of the polycistronic cassette

The AAVS1 targeting vector was constructed using the third generation PC3 polycistronic cassette after preliminary tests indicated the best order of the rapid induxinduction of the 3TFs is slower but sustained for longers was GATA: TALCO: FLI1CO. Both FLI and TAL were codon optimised (CO) coding sequences. To ensure the translation of separate proteins from a single transcript the open reading frames of each TF was preceded by a self-cleaving 2A oligopeptides containing a conserved c-terminal motif: D(V/I) EXNPGP and the stop codon of each ORF removed during cloning except from FLI1 at the end of the cassette. When protein synthesis occurs along an ORF that contains a 2A sequence, translation is paused by the ribosome at the glycine (G) and proline (P) codons of the 2A motif. This results in the nascent protein chain, up to and including the glycine residue, being released. We considered gene order before generating the final cassette, as the resulting proteins will have either a 2A peptide attached to the C-terminus (for genes upstream of a 2A sequence), or a proline attached to the N-terminus (for genes downstream of a 2A sequence) (Hu, 2014), and cleavage efficency can be affected by the N-terminal protein following the 2A sequence. Initial tests showed that the cassette programmed more efficiently with FLI1 at the end when tested in the pWPT lentiviral system. We also used different 2A variants between each ORF

to reduce the risk of intramolecular homologous recombination (HR) E2A: Equine rhinitis A virus, P2A: Porcine teschovirus-1, T2A: *Thoseaasigna* virus. These were purchased as oligonucleotides (Table S3) and cloned in the order **GFP**-E2A-**GATA1**-P2A-**TAL1CO**-T2A-**FLI1CO.**

The cassette was generated in a series of steps first by PCR of each TF sequence from the pWPT lentiviral vectors using the Phusion Taq high-fidelity DNA polymerase according to the manufacturer's instructions (NEB). The codon optimised versions pWPT-TAL1 CO and pWPT-FLI1CO were generated by GeneArt™ gene synthesis (Thermofisher Scientific). Both have been tested for efficacy in viral programming.

The E2A sequence was generated by annealing the two oligonucleotides E2A_GATA1_Fo and E2A_GATA1_Re (Table S3) including Not I restriction sites. Each oligonucleotide at 100µM was added to 5µl NEB buffer 2.1in a final volume of 50µl and annealed in a thermocycler at 95°C 10min, 95°C 3min (-1°C/cycle) x70 cycles, then 4°C hold. A joining PCR was used to join the E2A oligonucleotide and GATA1 coding sequence. The T2A fragment was generated by annealing the two oligonucleotides T2A_FLI1_Fo and T2A_FLI1_Re (Table S3), as for the E2A sequence and joined to FLI1 coding fragment by joining PCR including a stop codon for FLI1 and Sal1 restriction sites for cloning. Similarly, the P2A-TAL1CO fragment was generated with the addition of a 5' Mlu1 site and a 3' Sal1 site. The complete cassette then assembled in sequential steps first in pWPT before Nco1/EcoR1 transfer of the complete cassette to the AAVS1 targeting vector, the final two steps involving large fragments were performed using Gibson Assembly® (NEB). Plasmid constructs were heat transformed using Stbl3 cells (ThermoFisher Scientific) and subsequent plasmid preparations were performed using endotoxin-free Nucleobond® Xtra Midi Plus EF kits (Macherey-Nagel). The final construct was sequenced using a series of primers (listed in Table S3).

Genotyping of inducible clones

Genomic DNA was extracted using the Purelink® Genomic DNA Mini Preparation kit (Invitrogen, Life Technologies) and eluted in 50µl dH20, the concentration determined using a Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies) and gDNA diluted to 10ng/µl in aliquots for genotyping and 20ng used for each reaction. All genotyping PCRs were performed using Q5®Hot Start High-Fidelity DNA polymerase (NEB) apart from the ROSA

GSH PCR which used Long Amp DNA polymerase (NEB) and the parent line checked alongside each reaction. Primers used are listed in Table S4 together with annealing temperatures used and expected amplicon size. In brief, PCR for integration at the AAVS1 GSH used 63°C for 32 cycles with 1.5minute extension giving 1692bp fragment in wildtype cells and no band when integration is homozygous. PCR for integration at the ROSA GSH used 59.3°C (determined from gradient PCR test) for 35 cycles with 3minutes extension giving a 2186bp fragment in wildtype cells and no band when integration is homozygous. Integration at the 3' and 5' AAVS1 sites used 55°C with 1.5minute extension for 35 cycles and 55°C for 1minutes extension for 35 cycles respectively. Integration at the 3' and 5' ROSA sites used 63°C, 1.5 minutes extension for 32 cycles and 65°C for 0.5 minute extension for 35 cycles respectively. For the off -target PCR there should be no band in the wildtype or the clones and pROSA_CAG_rtTa and pUC AAVS1_p-responder (eGFP_PC3) can be used as positive controls. These primers are in the donor vector sequence and outside the targeting area. This is obviously limited and we are currently in the process of whole genome sequencing for key inducible lines as well as performing karyotyping analysis.

Karyotyping and pluripotency

hPSC are cultured to 50-60% confluency and treated with 0.1µg/ml colcemid at 37°C for upto 10hrs and mitotics accumulated by collection of the supernatant plus the adherent cells, following TrypLE Select (Thermo Fisher Scientific) treatment for 5 minutes. Cells are centrifuged at 300g for 5 minutes, the supernatant aspirated and 2-3mls hypotonic solution (0.075M KCL) added. Cells are incubated at 37°C for 10 minutes to rupture the membranes, then centrifuged again as above, the supernatant aspirated carefully. Resuspend the lysed cell pellet in 5mls fresh Carnoy's solution (Glacial Acetic acid: methanol, 1:3) dropwise, whilst flicking the tube to avoid clumping and repeat the spin. Take off the supernatant and resuspend as before in 2ml Carnoy's solution and transferred to -20°C prior to transfer to the Cytogenetics laboratory, Cambridge University Hospitals, UK for karyotyping. Pluripotency was assessed as described by flow cytometry.

In-vitro derived Primary Megakaryocytes

Cord blood and adult peripheral blood, from blood donor apheresis cones, was obtained after informed consent under a protocol approved by the Cambridgeshire 4 Research Ethics

Committee (07/MRE05/44). Cord blood and peripheral blood CD34-positive haematopoietic progenitors were isolated by positive selection using CD34 microbeads (MACS Miltenyi Biotec GmbH), and cultured for 10-12 days (cord blood) or 7-10 days (peripheral blood) in Cellgro® (CellGenix GmbH) or AMK supplemented with cytokines, Thrombopoietin (TPO) 100ng/ml (Biotechne) and ILIβ 10ng/ml (Biotechne). Phenotype for CB-MKs was monitored by CD41/CD42 flow cytometry as described.

Flow cytometry analysis

Flow cytometry was carried out using a Gallios flow cytometer (Beckman Coulter). Single cell suspensions were generated from hPSCs using TrypLE Select (Thermo Fisher Scientific) dissociation followed by a wash step in basal media at 300g for 5 minutes and then stained for 20'/RT in PBS 0.2%BSA 5mM EDTA using combinations of FITC, PE and APC conjugated antibodies (Table S5). For mature MK cultures in suspension the culture was gently mixed by pipetting and an aliquot stained. We used a CD235a FITC, CD42a PE plus CD41APC mix for virally programmed cells and for inducible lines CD235aPeCy7, CD42aAPC plus CD41APCH7. Background fluorescence was set against matched isotype control antibodies and a compensation matrix defined using single-color stained cells. Flow count fluorospheres (Beckman Coulter) and 4'-6'-diamidino-2-phenylindole (DAPI)1µg/ml were used to determine viable cell count in samples.

Cell Morphology analysis

Morphology of MKs was determined using a Cytospin Cytocentrifuge (ThermoFisher Scientific) enabling slow speed depositing of MKs onto glass slides at 400g for 5 minutes. Samples are air dried briefly then fixed and stained using Rapid Romanowsky (TCS Biologicals*)*.

Immunofluorescence analysis

Megakaryocytes were cultured on human fibrinogen (20µgml^{-1}, Enzyme Research, UK) coated chamber slides (ibidi, Germany) for upto 48hrs to foster adhesion and proplatelet formation. Cells were fixed with 2% paraformaldehyde, permeabilised with 0.2%Saponin/1% BSA/0.01% Triton-X-100 and incubated for 90 minutes at RT with primary antibodies (Table

S5), washed with PBS/0.01% Triton-X-100 and incubated with fluorochrome conjugated secondary antibodies for 90 minutes at RT. Cell nuclei were stained using 1µg/ml Dapi and slides mounted suing Gelvatol or Slow Fade Diamond (Thermo Fisher Scientific). Images were acquired using a Leica DMi8 (Leica, Germany). Staining for GPIbβ (CD42c) was analysed by Image J using the class I-IV system of Aguilar et al where the degree and type of DMS were classified into classes. Class I mainly peripheral staining without cytoplasmic territories, class II some staining seen in cytoplasmic territories, class III when the cytoplasm is filled by GPIbβ staining indicating more a mature DMS and finally class IV where staining extends into proplatelets.

Intracellular flow cytometry staining

Intracellular detection for von Willebrand (VWF) was performed using the Fix and Perm cell Permeabilization Kit (Thermo Fisher Scientific). Mature MK suspension cultures were first stained with the extracellular antigens CD42a APC, CD41 APC H7 and CD9 BV510 for 15 minutes, then fixed using reagent A for a further 15 minutes, washed used in PBS 0.2%BSA 5mM EDTA and permeabilized with reagent B plus the intracellular stain VWF for 20 minutes followed by a further wash. Samples were run on Gallios flow cytometer (Beckman Coulter).

Transmission Electron Microscopy (TEM)

Samples for TEM were prepared from ≥ 10^7 viable mature MKs and at least 75% CD41/42 positive. MKs are centrifuged at 800rpm for 5 minutes, the supernatant aspirated and the pellet washed in normal saline (0.9% sodium chloride) twice x 10mls and after the second wash and centrifugation, resuspended 1ml 2% glutaraldehyde and 2% formaldehyde in 0.05M PIPES buffer pH7.4. Cells are fixed for 4-6hrs at 4°C and rinsed by centrifugation at 15,000rpm for 5minutes 5 times with 0.01M PIPES pH7.4. Excess buffer or fix is always removed immediately after spinning. After the final wash pellets were resuspended in 1ml 0.01M PIPES and stored at 4°C. Samples were transferred to Cambridge Advanced Imaging Centre (CAIC) for processing and prepared sections imaged using a Jeol JEM-1400 Transmission Electron Microscope at CAIC.

Gene expression analysis by RT-qPCR

Total RNA was prepared using the Nucleospin® RNA plus kit (Macherey-Nagel) including a DNA removal step and 500ng RNA reverse transcribed using Superscript®IV reverse transcriptase and random hexamers (Thermo Fisher Scientific). Two-step qPCR was performed with SYBR green chemistry on the ABI 7500HT (Applied Biosystems) by the relative standard curve method using HMBS endogenous control gene. Primers are listed in Table S6.

Protein expression by western blotting

Samples were harvested on D3 of differentiation for protein in RIPA (150mM NaCl,1% nonidet-40, 0.5% sodium deoxycholate, 0.1%SDS, 60mM Tris pH 8) plus protease inhibitors, 1.00E+05 in 50µl. Samples x 20µl were run on Bolt™ 10% Bis-Tris Plus 1.00mm x 12wells gels (Thermo Fisher Scientific) with 2 x sample buffer (63mM Tris pH6.8, 10%glycerol 2%SDS, 0.1% BPB) using MES running buffer and Chameleon™ Duo Pre-stained protein ladder (Licor). Wet transfer was performed using Bolt running buffer plus 10% methanol to Immobilon® -FL PVDF transfer membrane (Millipore). Primary antibody hybridisation was performed in Odyssey® Blocking Buffer (PBS) using goat polyclonal Tal1 1:1000 (Santa Cruz Biotechnology), mouse monoclonal Fli11:1000 (BD Biosciences) or goat polyclonal Gata11:1000 (biotechne) with goat polyclonal GAPDH 1: 20,000 (Origene). Donkey anti mouse IRDye 800CW or donkey anti goat IRDye 800CW (green) plus donkey anti-goat IRDye 680RD (red) 1:15,000 secondary antibodies were used (Licor). Blots were scanned using a Licor Odyssey™ Fc Scanner and analysed using Image Studio™ software (Licor).

In-vitro platelet analysis

Static platelet assays were carried out in 24 well plates. Mature MKs were centrifuged gently at 200g for 10 minutes with no brake and MK pellets resuspended in high glucose RPMI (ThermoFisher Scientific, # A1049101, ATCC modification, GMP manufacture) then plated at 5.00+04 per ml for 3 days at 37°C.
Crude supernatant containing the platelets was analysed by flow cytometry using Calcein - AM 100nM, CD41APC H7 (1:200) and CD42aAPC (1:100) (Supplementary Table S5)

for 10minutes at 37°C. Flow count fluorospheres (Beckman Coulter), were used to determine viable platelet number. Flow protocols and gating strategies were determined using human platelets from fresh whole blood (Blood donor ethics No: HBREC.2018.13) diluted in PBS/Acid-Citrate-Dextrose analysed in parallel.

Washed donor platelet preparation and bioreactor derived platelet concentration

Venipuncture of donor blood using 3.2% buffered sodium citrate vacutainers used informed consent and ethical approval (Blood donor ethics No: HBREC.2018.13). An additional 10% acid citrate dextrose (ACD, Sigma) was added to the blood before centrifugation at 100g RT for 20 mins. The platelet rich plasma (PRP) layer was collected and supplemented with 750ng/ml prostaglandin I2 (PGI2, Sigma) before centrifugation at 1400g RT for 10mins. The supernatant was aspirated and the platelet pellet resuspended in Tyrode's buffer supplemented with 10% ACD and 750ng/ml PGI2. Finally, platelets were centrifuged at 1400g at RT for 10mins and platelets carefully resuspended in Tyrode's to the indicated concentration.

Bioreactor outflows were collected in RPMI plus ACD (1:9) in 50ml LoBind conical tubes (Eppendorf), same day runs were rocked gently before pooling, taking samples for flow and adding PGI2 as combined to centrifuge at 1400g RT for 10 mins (Acc9/Dec7). The supernatant was removed and the platelet pellet resuspended gently in <100µl Tyrode's in a LoBind 2ml tube (Eppendorf) for injection. A 1µl sample for flow cytometry, as described, provided an accurate viable platelet number. Recovery on concentration is usually <50%.

Bioreactor

A second-generation bioreactor (Platelet BioGenesis) consisting of a "sandwich" design wherein a polycarbonate membrane holding the cells is pressed between two thermoplastic layers. Megakaryocytes are evenly seeded across the channels and trapped on the porous membrane through which proplatelets extend and nascent platelets are released. Three million viable megakaryocytes are seeded per run at an estimated 75% pore occupancy for the *in vitro* function studies. The set up uses a low perfusion volume of approximately 20mls and is part of a scalable design and for *in-vivo* studies an average of 1.74E+07 ± 3.04E+06 (SEM, n=6) viable MKs were seeded per run after filtering out any clumps through a 70µm filter, centrifuging at 120g for 8mins RT and resuspending in 10mls AMK.

Platelet Function Assays

Platelet function measured by flow cytometry

During bioreactor runs 200µl samples were taken and stained as described for static platelet assays. The bioreactor outflow was centrifuged at 120g for 20 min then 1.5 µM of labile PGI_2 added to the supernatant before further centrifugation at 1420g for 10 min to temporarily sequester activation. The platelet pellet was then re-suspended in Tyrode's buffer (134mM NaCl, 2.9mM KCl, 0.34mM Na2HPO4.2H20, 12mM NaHCO3, 20mM HEPES, 1mM MgCl2.6H20, 5mM D-Glucose, pH 7.3) and used for functional assays within 4 hours.

In vitro thrombus formation under flow (IVTOF)

The bioreactor outflow was treated as described, for IVTOF *in vitro* bioreactor derived or donor platelets were stained with CellTracker^{TM} Red (Thermo Fisher Scientific), and added 1:99 to whole human donor blood previously stained green with $DIOC_6$ (Sigma, 3,3'-Dihexyloxacarbocyanine Iodide) in whole blood. Samples were perfused through a collagen coated (HORM collagen, Takeda Austria) Vena8 Biochip (Cellex, Dublin) blocked with 1% BSA. Samples were run at 40 μ l/min, equal to 1000 s-1 for 1 min, as an *in vitro* approximation for clot formation in arterial shear flow, and imaged on an Axiovert 40 CFL microscope. Images were analysed using Image J software calculating the mean and standard deviation of the mean fluorescence intensity of the field of view. This figure was used as a minimum threshold allowing the measurement of the area covered by red (*in-vitro* or donor derived platelets) or green (donor) stained platelets**.** The red area (CellTracker red) was then divided by the green (DIOC6) area to give percentage incorporation.

Murine transfusion and haemostasis model

Immunodeficient NRG/J mice were platelet depleted using 0.6ug/g BW anti-CD42b antibody (Emfret Analytics, Germany) a minimum of one hour prior to a tail vein bleed both to confirm depletion and give an accurate haemoglobin level. Depletion was confirmed by full blood counts using a scil Vet abc Plus+ (Horiba Medical, Japan) with 10µl EDTA whole blood. Following confirmation of depletion, mice were injected intraperitoneally using a mixture of ketamine (100mg/kg) and xylazine (10mg/kg) and transferred to a 38°C chamber for 5minutes. Mice were then immediately intravenously (IV) injected with either Tyrode's

buffer, washed donor or *in vitro* bioreactor derived platelets. Immediately following IV injection, mouse tails were transected 2mm from the tail tip, and placed into 40ml warmed phosphate buffered saline and moved each 4 mins to 40mls of fresh saline for a total of 20mins. Following a maximum 20mins of bleeding time, mice were euthanised and blood was collected from the inferior vena cava into 100ul of 0.5M EDTA.

Flow cytometry for the haemostasis model

Collected mouse blood 5µl +45µl Tyrode's was stained using anti-mouse specific CD42d APC (eBioscience) 1:50, anti-human specific CD42 APC H7 (BD Biosciences), Calcein AM 200nM (Life Technologies) using a Gallios flow cytometer (Beckman Coulter).

Haemoglobin Assay for the haemostasis model

Haemoglobin levels were assayed using the QuantiChrom Haemoglobin Assay Kit (BioAssay Systems). Standards showed a linear response in the range of 0-200mg/dl and the assay is sensitive to 0.9mg/dl. From the raw values, a Hb concentration was obtained using the formula:

$$[Hb]_{sample} = \frac{OD_{sample\ -OD\ blank}}{OD_{calibrator-OD\ blank}} \times 100\ mg/ml$$

It is then possible to infer the volume of blood lost with the following formula based on the conservation of mass:

$$\text{Blood loss (ml)} = \frac{[Hb]_{sample} \times Volume}{[Hb]_{post\ platelet\ depletion\ level}}$$

Post platelet depletion haemoglobin levels were analysed on a haematology analyser (scil Vet abc Plus+, SCIL Animal Care Company) as a reference baseline haemoglobin level for each individual mouse.

Statistics

Results are presented as mean ± standard error of the mean (SEM) with n representing the number of biological replicates unless otherwise stated. Statistical P values were calculated by Student's t-test when comparing two samples and using ANOVA (one way Analysis Of Variance) for multiple samples. Statistical values for *in-vivo* blood loss from the haemostasis model were calculated in Prism GraphPad using ANOVA.

Supplemental Tables:

Table S1: hPSC and hESC lines used in this publication

Table S2: Components of the AMK culture media: R&D and GMP equivalent reagents

Table S3: Oligonucleotide 2A sequences and primers for sequencing the polycistronic targeting vector

Table S4: Genotyping primers for inducible hPSCs

Table S5: Antibodies/stains used for flow cytometry

Table S6: Quantitative PCR primers

Supplemental Figures:

Figure S1. AMK culture medium improves both pro-platelet and platelet formation in primary cord blood derived HSCs

Figure S2. Genetically modified inducible cell lines maintain pluripotency and show no gross genetic abnormalities.

Figure S3. Dynamics of transgene expression and differentiation of inducible cell lines

Figure S4. Vector maps 1-6 for inducible forward programming constructs.

Figure S5. The effect of CHIR99021 on the profile of programming and the enhancement in maturity of FoP-MK from clinical grade hiPSC**.**

Figure S6. Bioreactor platelet production from clinical grade hiPSCs derived MKs.

Figure S7. Platelet counts in immunodeficient NRG/J mice post anti-CD42b antibody administration and percentage ratio of human/mouse platelets in terminal bleeds.

Supplementary Tables

Table S1
hPSC and hESC lines used in this publication

Common/Lab Name	Official Name	hiPSC/HESC	Origin	DONOR SEX M/F	GMP/EUTCD grade available	REFERENCE
FFDK	HPSI0813i-ffdk_3	hiPSC	HipSCi		×	http://www.hipsci.org
QOLG	HPSI1113i-qolg_2	hiPSC	HipSCi		×	http://www.hipsci.org
LGR1.1	LIPSC-GR1.1	hiPSC	NIH/NINDS		✓	https://nindsgenetics.org/
Delta-2	DELTA- 2	hiPSC	Confidential		✓	
Delta-3	DELTA-3	hiPSC	Confidential	F	✓	
RCIB-10	CGT-RCiB-10	hiPSC	Catapult	F	✓	https://ct.catapult.org.uk
MS3	Mastershef 3	hESC	UKRMP	F	✓	https://www.ukrmp.org.uk
MS4	Mastershef 4	hESC	UKRMP	M	✓	https://www.ukrmp.org.uk
MS7	Mastershef 7	hESC	UKRMP	M	✓	https://www.ukrmp.org.uk
MS8	Mastershef 8	hESC	UKRMP	M	✓	https://www.ukrmp.org.uk
MS10**	Mastershef 10	hESC	UKRMP	M	✓	https://www.ukrmp.org.uk Merkle Nature 2017
MS11	Mastershef 11	hESC	UKRMP	M	✓	https://www.ukrmp.org.uk
KCL031	Kings College-031	hESC	UKRMP	M	✓	Jacquet Stem Cell Research 2016
KCL032	Kings College-032	hESC	UKRMP	F*	✓	Miere Stem Cell Research 2016
KCL033	Kings College-033	hESC	UKRMP	F*	✓	Devito Stem Cell Research 2016
KCL034	Kings College-034	hESC	UKRMP	M*	✓	Devito Stem Cell Research 2016
KCL037	Kings College-037	hESC	UKRMP	M	✓	Miere Stem Cell Research 2016
KCL038	Kings College-038	hESC	UKRMP	M	✓	Miere Stem Cell Research 2016
KCL039	Kings College-039	hESC	UKRMP	M	✓	Devito Stem Cell Research 2016
KCL040	Kings College-040	hESC	UKRMP	F	✓	Jacquet Stem Cell Research 2016
***siblings**	****** confirmed to have 2 TP53 mutations (R248W and G245S)** **HipSCi Human Induced Pluripotent Stem Cell Intiative** **UKRMP United Kingdom Regenerative Medicine Platform**					

Table S2
Megakaryocyte Expansion Media AMK

Component	Company	Cat. No.	GMP Compliant	ml	Concentration
Basal Media IMDM w/o phenol red	ThermoFisher Scientific (Gibco™)	21056 (500ml)	√	500	1x
Chemically defined lipid concentrate	ThermoFisher Scientific (Gibco™)	11905-031 (100ml)		5	1x
Insulin-Transferrin-Selenium (ITS -G) (100X)	ThermoFisher Scientific (Gibco™)	41400-045 (10ml)	√	5	1x
2-Mercaptoethanol	ThermoFisher Scientific (Gibco™)	21985-023 (50ml) @55mM	√	0.5	55µM
Bovine Serum Albumin (BSA) 30%	Biosera	SA-296 (1000ml)	BSE Free	8.4	0.5%

Conversion of Megakaryocyte Expansion AMK to GMP Components

Research Grade			GMP Grade	
Component	**Company**	**Cat. No.**	**Company**	**Cat. No.**
Basal Media IMDM w/o phenol red	ThermoFisher Scientific (Gibco™)	21056 (500ml)	LAB GRADE=GMP	
Chemically defined lipid concentrate	ThermoFisher Scientific (Gibco™)	11905-031 (100ml)	Sigma	L0288
Insulin-Transferrin-Selenium (ITS -G) (100X)	ThermoFisher Scientific (Gibco™)	41400-045 (10ml)	LAB GRADE=GMP	
2-Mercaptoethanol	ThermoFisher Scientific (Gibco™)	21985-023 (50ml) @55mM	LAB GRADE=GMP	
Bovine Serum Albumin (BSA) 30%	Biosera	SA-296 (1000ml)		
Human Serum Albumin			Irvine Scientific	9988
Cytokines for MK and inducible lines				
TPO	Biotechne/ R&D Systems	288-TPN	Miltenyi Biotec	170-076-134
SCF	ThermoFisher Scientific (Gibco™)	PHC2111	Miltenyi Biotec	170-076-149
Doxycycline Hyclate	Sigma	33429 (100mg)	Sigma USP Grade	1226003

Table S3
Oligonucleotide 2A sequences and primers for sequencing the polycistronic targeting vector

Oligonucleotide 2A sequences	Sequence
P2A_Fo	GCCGCACGCGTGGAAGCGGAGCTACTAACTTCAGCCTGCTGAA GCAGGCTGGAGACGTGGAGGAGAACCCT**GGACCT**ACTAGTTG
P2A_Re	**CAACTAGT AGGTCC AGGGTTCTCCTCCACGTCTCCAGCCTGCTT CAGCAGGCTGAAGTTAGTAGCTCCGCTTCCACGCGTGCGGC**
E2A_GATA1_Fo	GCGGCCGCTGGAAGCGGACAGTGTACTAATTATGCTCTCTTGA AATTGGCTGGAGATGTTGAGAGCAACCCT**GGACCT**TTCGAAAT GGAGTTCCCT
E2A_GATA1_Re	**AGGGAACTCCATTTCGAA AGGTCC AGGGTTGCTCTCAACATC TCCAGCCAATTTCAAGAGAGCATAATTAGTACACTGTCCGCTT CCAGCGGCCGC**
T2A_FLI1_Fo	TCGACTTGCGTACGGGAAGCGGAGAGGGCAGAGGAAGTCTGCTA ACATGCGGTGACGTCGAGGAGAATCC**TGGACCT**ATGGACGGCAC CATCAAAGAGGC
T2A_FLI1_Re	**TGATGGTGCCGTCCAT AGGTCC AGGATTCTCCTCGACGTCACCGCAT GTTAGCAGACTTCCTCTGCCCTCTCCGCTTCCCGTACGCAAGTCGA**
Sequencing primers	
GATA1_seq_1Re	**GTGGGAGAAAAGAAGGTACTGG**
GATA1_seq_2Re	ATTCCCGCTACCGCTG
FLI1_seq_1Re	**ATTTGCTAACGCTGCAGTCC**
FLI1_seq_1Fo	GGAGTATGACCACATGAATGG
FLI1_seq_2Re	**GCCGCATCACAATACTGG**
FLI1_seq_2Fo	GGTGAACTTTGTCCCTCC
TAL1CO_Seqi_Re	**AGCCGCAGGATCTCGTTCTT**
TAL1CO_seq_1Re	AGGCAGTTCAGCTGTCACA
TAL1CO_seq_1Fo	**CCAGACACAGAGTGCCTACC**
TAL1CO_seq_2Fo	GCAAGAACGAGATCCTGC
eGFP_Seqi_Re	**TAGCTACTAGCTAGTCGAGA**
E2A_GATA1_Fo	GCCTGCGGCCGCTGGAAGCG
E2A_GATA1_Re	**GCCGCGGCCGCCTGAGCTGAGCG**
E2A_GATA1_Fo2	CCTGGACCTTTCGAAATGGAGTTCCCTGGC

Bold **AGGTCC** Glycine /Proline cut sites for 2A sequences are indicated

Table S4
Genotyping primers for inducible hPSCs

PCR	F primer	sequence	Amplicon(bp)	Tm	Parent	HOM	HET
AAVS1 Genomic locus	F_ genome	CTGTTTCCCCTTCCCAGGCAGGTCC	1692	63	1692	no product	1692
	R_genome	TGCAGGGGAACGGGGCTCAGTCTGA					
AAVS1 5' end integration	F_ genome	CTGTTTCCCCTTCCCAGGCAGGTCC	991	65	no product	991	991
	R_(puro)	TCGTCGCGGGTGGCGAGGCGCACCG					
AAVS1 3' end integration	Fli1CO_seqi_2Fo	GCAGCAGACTGGCCAACCCT	1731	63	no product	1731	1731
	R_(genome)	TGCAGGGGAACGGGGCTCAGTCTGA					
AAVS1 off target	iOptTal1_TG_Fo	ACCGCCAGATCTCTGCATC	2748	55	no product	no product	no product
	AAVS1 off-target R	ATGCTTCCGGCTCGTATGTT					
Rosa26 genomic locus	F_ROSA	GAGAAGAGGCTGTGCTTCGG	2186	59.3	2186	no product	2186
	R_ROSA	ACAGTACAAGCCAGTAATGGAG					
3'end integration	F_ROSA_rTTA	GAAACTCGCTCAAAAGCTGGG	1896	55	no product	1896	1896
	R_ROSA	ACAGTACAAGCCAGTAATGGAG					
5'end integration	F_ROSA	GAGAAGAGGCTGTGCTTCGG	1176	55	no product	1176	1176
	R_(ROSA vector)	AAGACCGCGAAGAGTTTGTCC					
	R_(ROSA vector)neo	CCATCTTGTTCAATGGCCGAT	1228	55	no product	1228	1228
ROSA off-target	F_ROSA_rTTA	GAAACTCGCTCAAAAGCTGGG	1794	55	no product	1794	1794
	ROSA off-target R	TGACCATGATTACGCCAAGC					

Table S5
Antibodies/stains used for flow cytometry

Antibody	Antigen	Clone	Flourochrome	Assay conc/diln	Manufacturer	Catalog No
CD9	CD9	monoclonal	BV510	1:100	BD Biosciences	563640
VWF	VWF	polyclonal	FITC	1:5,000	Abcam	ab8822
IgG	Isotype	polyclonal	FITC	1:5,000	Abcam	ab127762
CD41a	ITG2AB	HIP8	APC	1:10	BD Biosciences	559777
CD41a	ITG2AB	HIP8	APC H7	1:100cells 1:200PLTs	BD Biosciences	561422
CD41a	ITG2AB	5B12	FITC	1:10	Millipore	FCMAB195F
CD42a	GP9	monoclonal	APC	1:100	Miltenyi Biotec	130-100-932
CD42a	GP9	ALMA:16	FITC	1:10	BD Biosciences	558818
CD42d	GPV	monoclonal	APC	1:50	eBioscience	17-0421-82
CD235a	GYPA	GA-R2 (HIR2)	FITC	1:100	BD Biosciences	559943
CD235a	GYPA	GA-R2 (HIR2)	PE-Cy7	1:100	BD Biosciences	563666
TRA-1-60			FITC	1:100	Millipore	FCMAB115F
SSEA4	SSEA4	REA101	PE	1:20	Miltenyi Biotec	130-098-369
Calcein-AM	Live cell stain			1:20,000 100nM	Life Technologies	17783
$DiOC_6(3)$	Live cell stain			1:1000	Sigma	318426
Cell Tracker™ Red CMPTX	Retained living cells			1:1000	ThermoFisher Scientific	C34552
DAPI	DNA stain			1µg/ml	Sigma	D9542

Table S6
Quantitative PCR primers

Primer	Sequence	Tm	Amplicon bp
HMBS_F	GGGAACCAGCTCCCTGCGAAG	60°C	145
HMBS_R	AGCTGTTGCCAGGATGATGGCAC		
Brachyury F	TATGAGCCTCGAATCCACATAGT	60°C	109
Brachyury R	CCTCGTTCTGATAAGCAGTCAC		
Hoxa9F	GGGTGACTGTCCCACGCTTGA	60°C	
Hoxa9R	AGCCAGTTGGCTGCTGGGTT		
Sall4F	AGGAATTTGTGGCGGAGAGG	60°C	205
Sall4R	GTTCACTGGAGCACCCAGC		
CDX4F	CCGATGCCAGCCTCCAATTT	60°C	175
CDX4R	CTGTGCCCATTGTACTAGACG		
VWF F	CAACACCTGCATTTGCCGAA	60°C	78
VWF R	TGACCTGTGACAAGGCACTC		
NFE2_Hsa-FO	GGGTGACTGTCCCACGCTTGA	60°C	
NFE2_Hsa-RE	TCAGACCCTGCAGCTCGGTGA		
GP1BB F	AGCTTACTGCTCCTGCTGC	60°C	300
GP1BB R	GTTGTGTCGACAGGGAAGG		
CD9 F	CCTGCTGTTCGGATTTAACTTCA	60°C	89
CD9 R	TGGTCTGAGAGTCGAATCGGA		
KDR F	GGAACCTCACTATCCGCAGAGT	60°C	132
KDR R	CCAAGTTCGTCTTTTCCTGGGC		
STAT5A F	GTTCAGTGTTGGCAGCAATGAGC	60°C	108
STAT5A R	AGCACAGTAGCCGTGGCATTGT		
DKK1 F	GGTATTCCAGAAGAACCACCTTG	60°C	128
DKK1 R	CTTGGACCAGAAGTGTCTAGCAC		
ITGA2F	CCTACAATGTTGGTCTCCCAGA	60°C	106
ITGA2R	AGTAACCAGTTGCCTTTTGGATT		

Figure S1

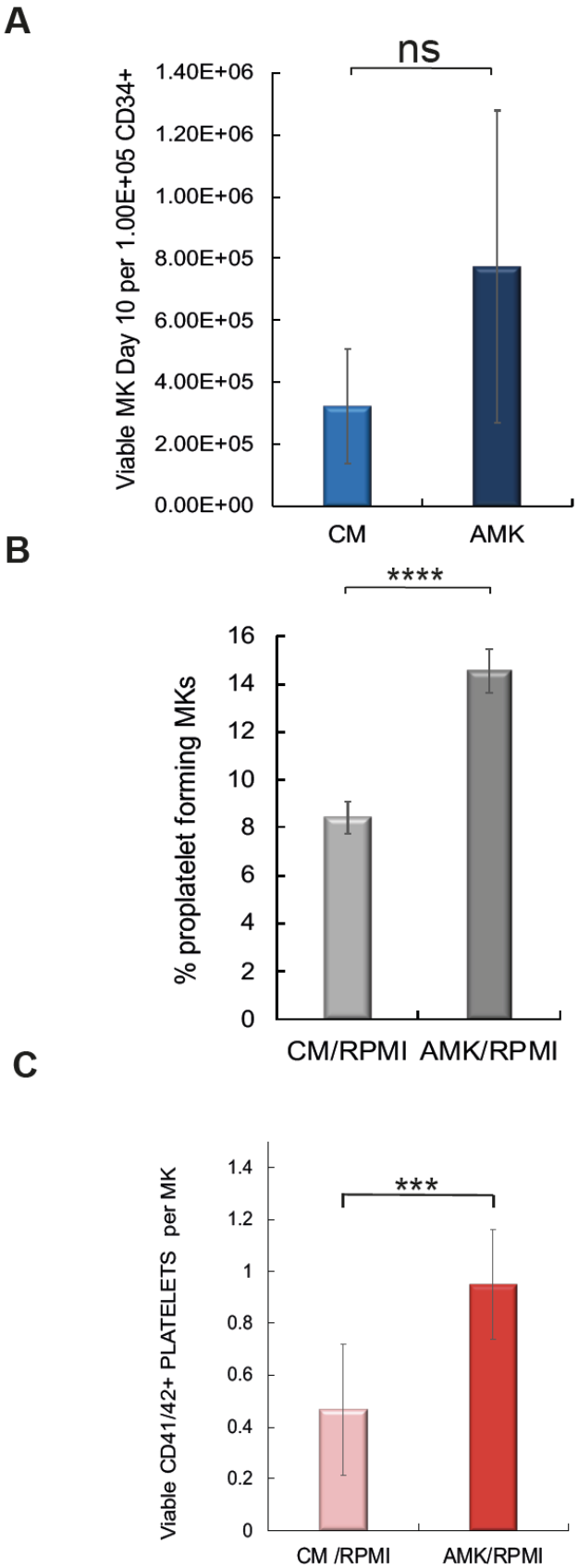

A
ns
Viable MK Day 10 per 1.00E+05 CD34+
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
CM
AMK
B
****
% proplatelet forming MKs
16
14
12
10
8
6
4
2
0
CM/RPMI
AMK/RPMI
C
***
Viable CD41/42+ PLATELETS per MK
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CM /RPMI
AMK/RPMI

Figure S1. AMK culture medium improves both pro-platelet and platelet formation in primary cord blood derived HSCs

A. Bar graph showing the number viable MKs on Day10 of culture (per 1.00E+05 CD34+ starting cells) cultured in CM or AMK. Mean ± 1 x SEM, n=4.

B. Bar graph showing the percentage of pro-platelet forming MKs cultured using CM or AMK then transferred to high glucose RPMI on fibrinogen coated chamber slides. Ttest ****p=2.9E-07, Mean ±1 x SEM, n=4.

C. Bar graph showing the number of viable Calcein-AM+ CD41+ CD42+ platelets produced per MK after culture in either CM or AMK and transfer to high glucose RPMI. Ttest ***p=0.0003, Mean ± 1 x SEM, n=4.

Figure S2

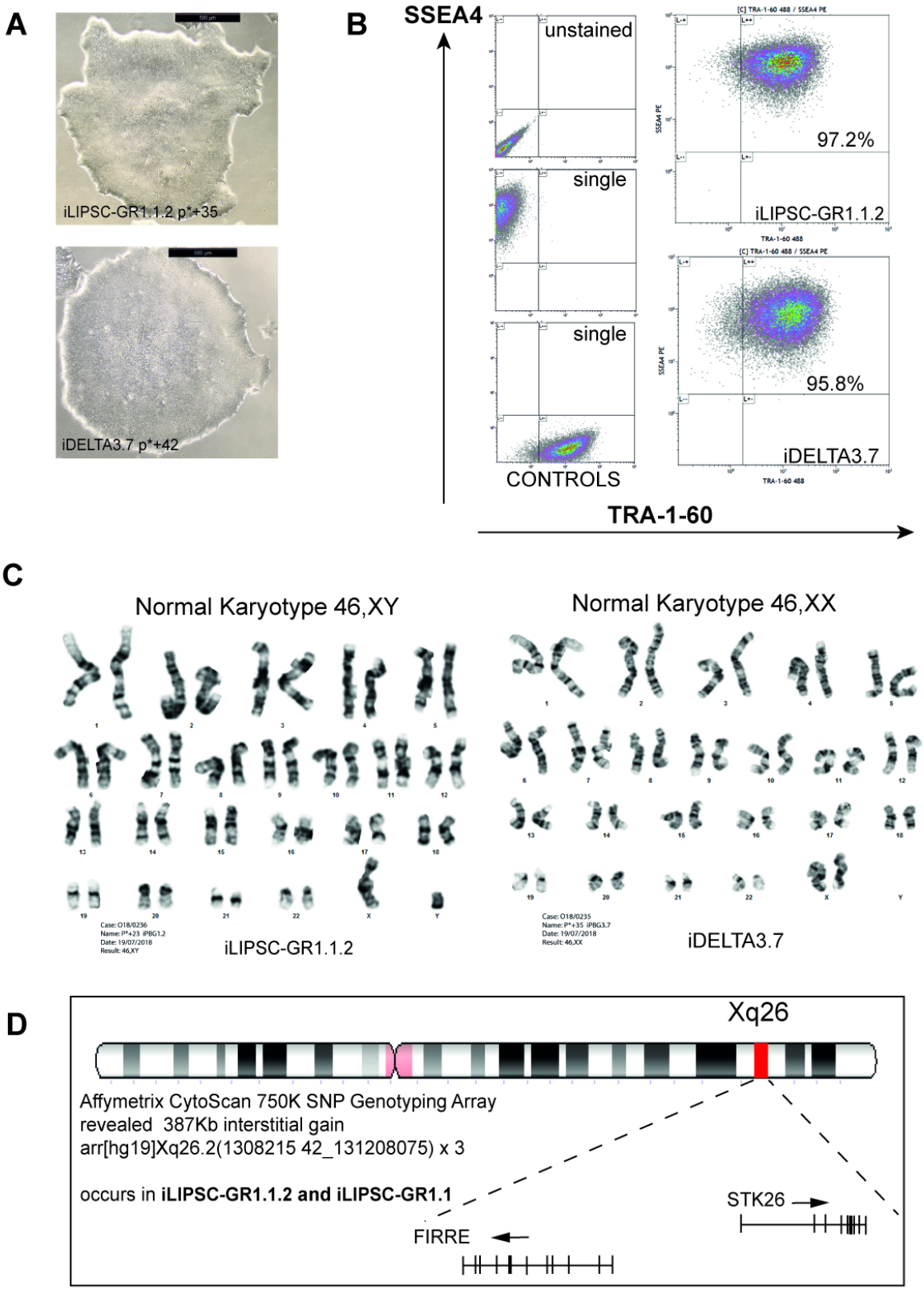

A
iLIPSC-GR1.1.2 p*+35
iDELTA3.7 p*+42
B
SSEA4
unstained
single
single
CONTROLS
97.2%
iLIPSC-GR1.1.2
95.8%
iDELTA3.7
TRA-1-60
C
Normal Karyotype 46,XY
iLIPSC-GR1.1.2
Normal Karyotype 46,XX
iDELTA3.7
D
Xq26
Affymetrix CytoScan 750K SNP Genotyping Array
revealed 387Kb interstitial gain
arr[hg19]Xq26.2(1308215 42_131208075) x 3
occurs in iLIPSC-GR1.1.2 and iLIPSC-GR1.1
STK26
FIRRE

Figure S2. Genetically modified inducible cell lines maintain pluripotency and show no gross genetic abnormalities.

A. Light microscope images of iLIPSC-GR1.1.2 p*+18 (*=23+8) (top panel) and iDELTA-3.7 p*+30 (*=13+14) (bottom panel) showing characteristic pluripotent cells growing as tightly packed colonies with defined edges. [* indicates number of passages of the parent hPSC at the time of genetic modification]

B. Left, controls for a mixed population of both lines showing unstained, single stain SSEA4, and single stain Tra-1-60. Right, flow cytometry dot plots for the same two genetically modified inducible hPSC lines showing that over 95% of cells are positive for the pluripotency markers TRA-1-60 and SSEA4.

C. Metaphase chromosome spreads for iDELTA-3.7p*+35 and iLIPSC-GR1.1.2 p*+23 showing a normal male (iDELTA-3.7) and female (iLIPSC-GR1.1.2) karyotype.

D. Affymetrix CytoScan 750K SNP genotyping arrays while normal for both iDELTA-3.7 and parent line DELTA-3 shows a gain in Xq26.2.

Figure S3

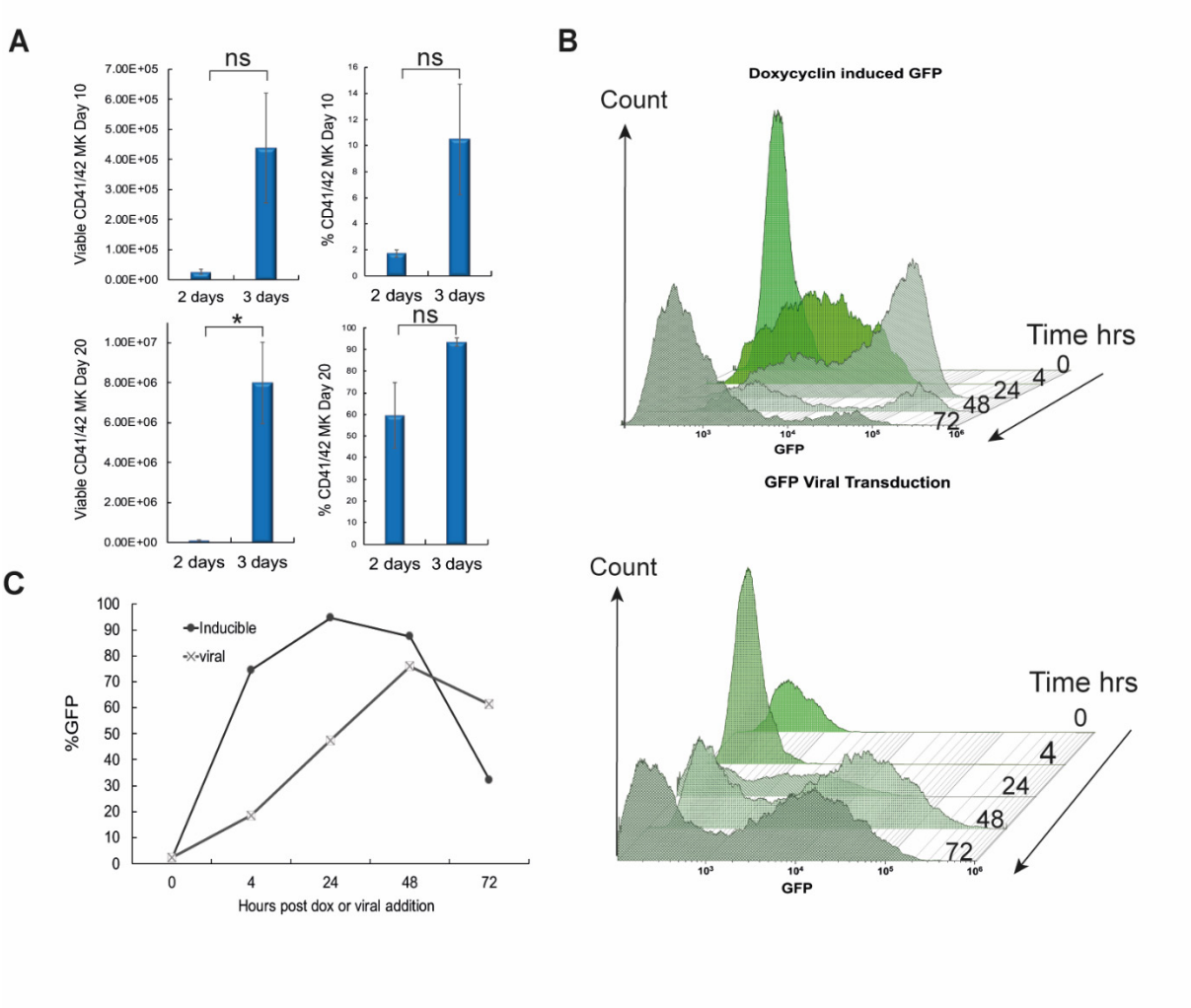

A
ns
Viable CD41/42 MK Day 10
% CD41/42 MK Day 10
2 days 3 days
*
Viable CD41/42 MK Day 20
% CD41/42 MK Day 20
B
Doxycyclin induced GFP
Count
Time hrs
0
4
24
48
72
GFP
GFP Viral Transduction
C
Inducible
viral
%GFP
Hours post dox or viral addition

Figure S3. Dynamics of transgene expression and differentiation of inducible cell lines

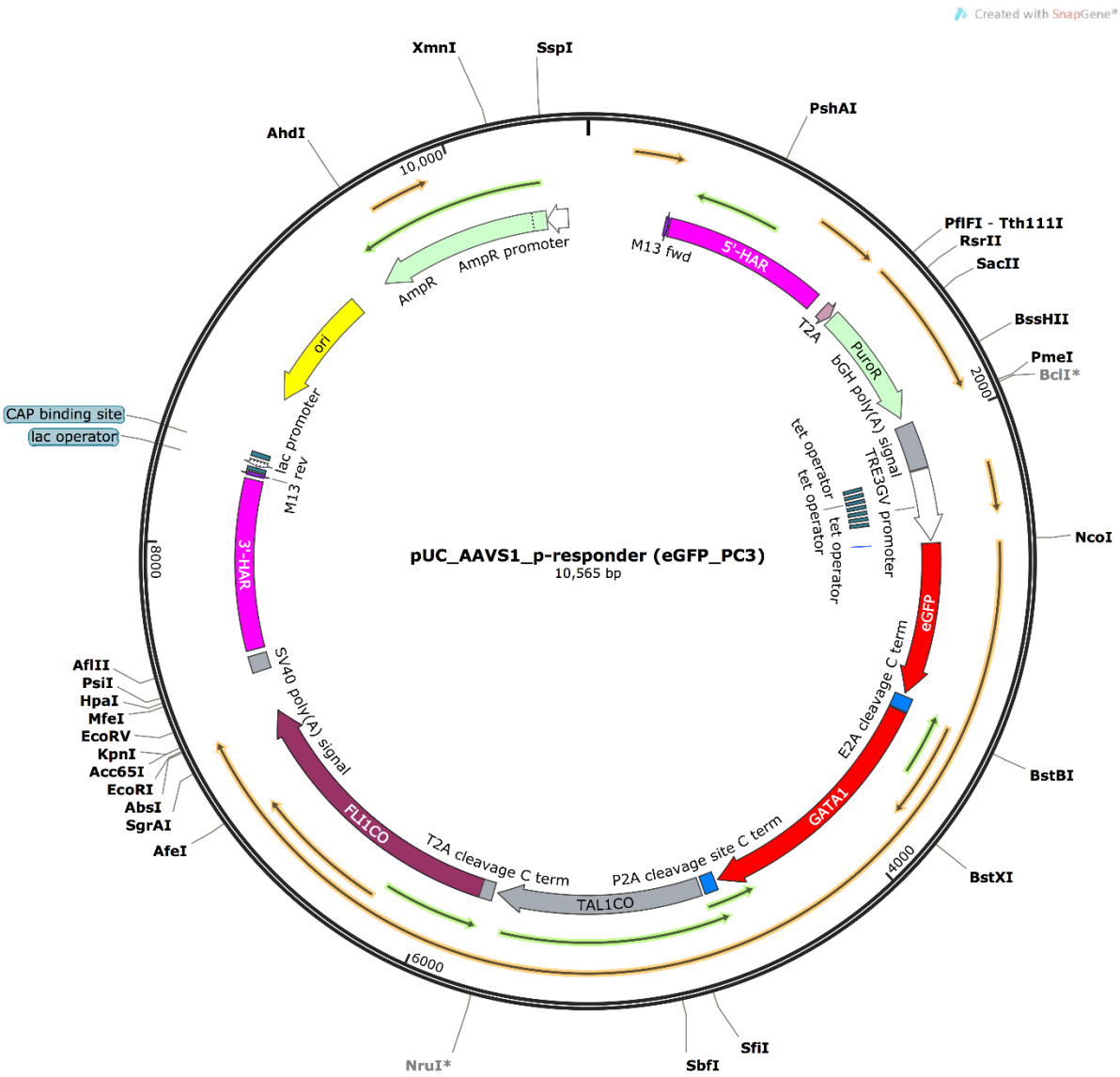
A. There is a difference in the dynamics of programming for inducible lines. We found that at day 10 of forward programming both number and the percentage of viable MKs CD41+42+ increases when mesoderm induction is extended from 2 to 3 days (Top). p = ns Mean ± 1 x SEM, n=3. By day20 (bottom) this translates to a significant increase in the number of viable MKs *p=0.019 but not in the percentage of viable MKs. Mean ± 1 x SEM, n=3. Data from iDELTA-3.7.

B. Overlay histograms obtained from parellel cultures of iLIPSC-GR1.1.2 using either 0.25µg/ml doxycycline or lentivirus of the 3TFs, GATA1,TAL1,FLI1 plus a pWPT-GFP virus at MOI 20 for each virus. GFP determined by flow cytometry as a proxy for expression of the 3TFs shows rapid induction for doxycycline induced samples (Top) plus a rapid decrease by 72hrs post induction, for virally transduced cells (bottom) the profile shows slower induction but the signal is more sustainable.

C. A plot of fluorescent intensity for the GFP profile shown in B. reveals the rapid increase and decrease of signal for the inducible system.

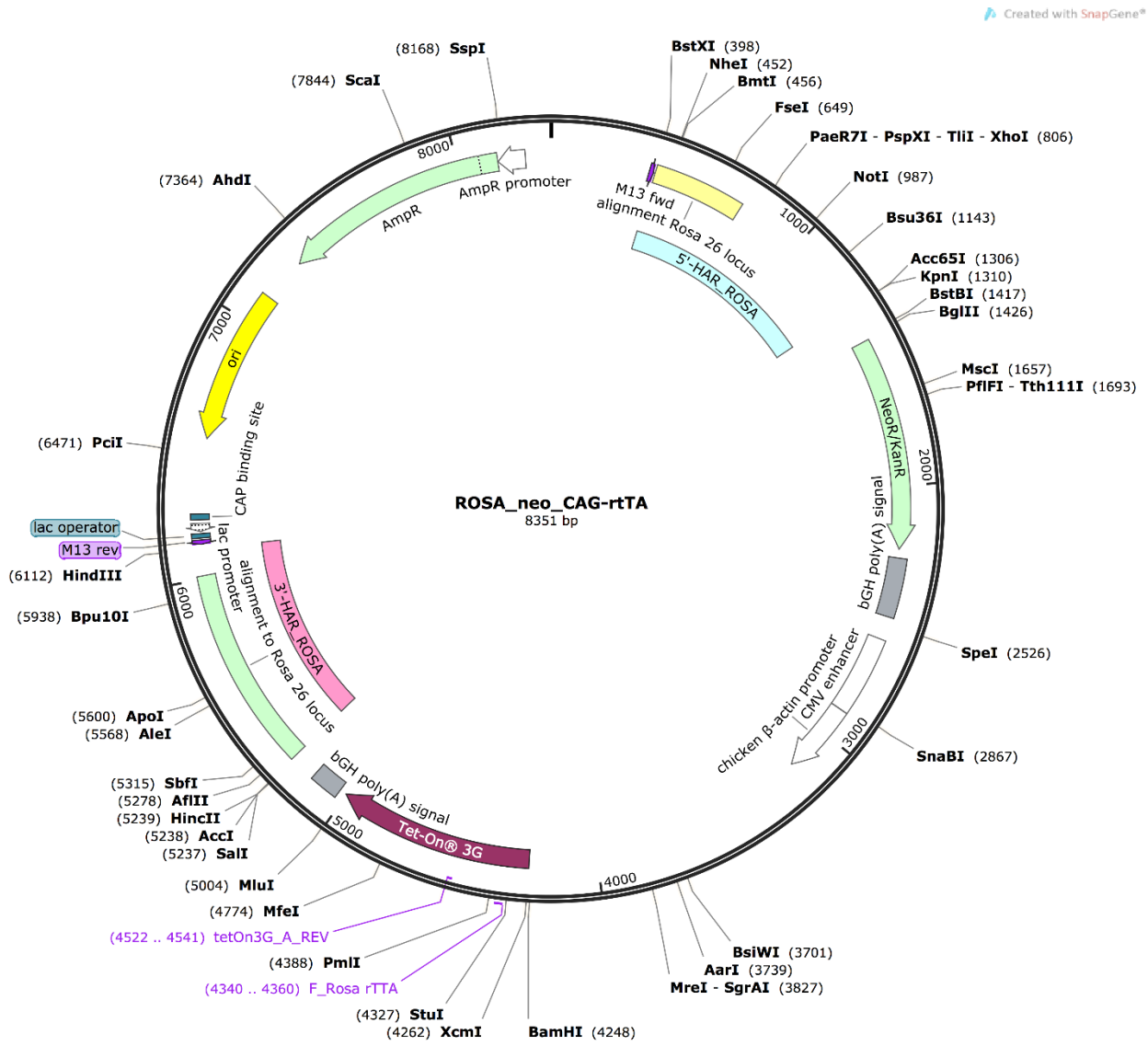
Figure S4

Vector Maps

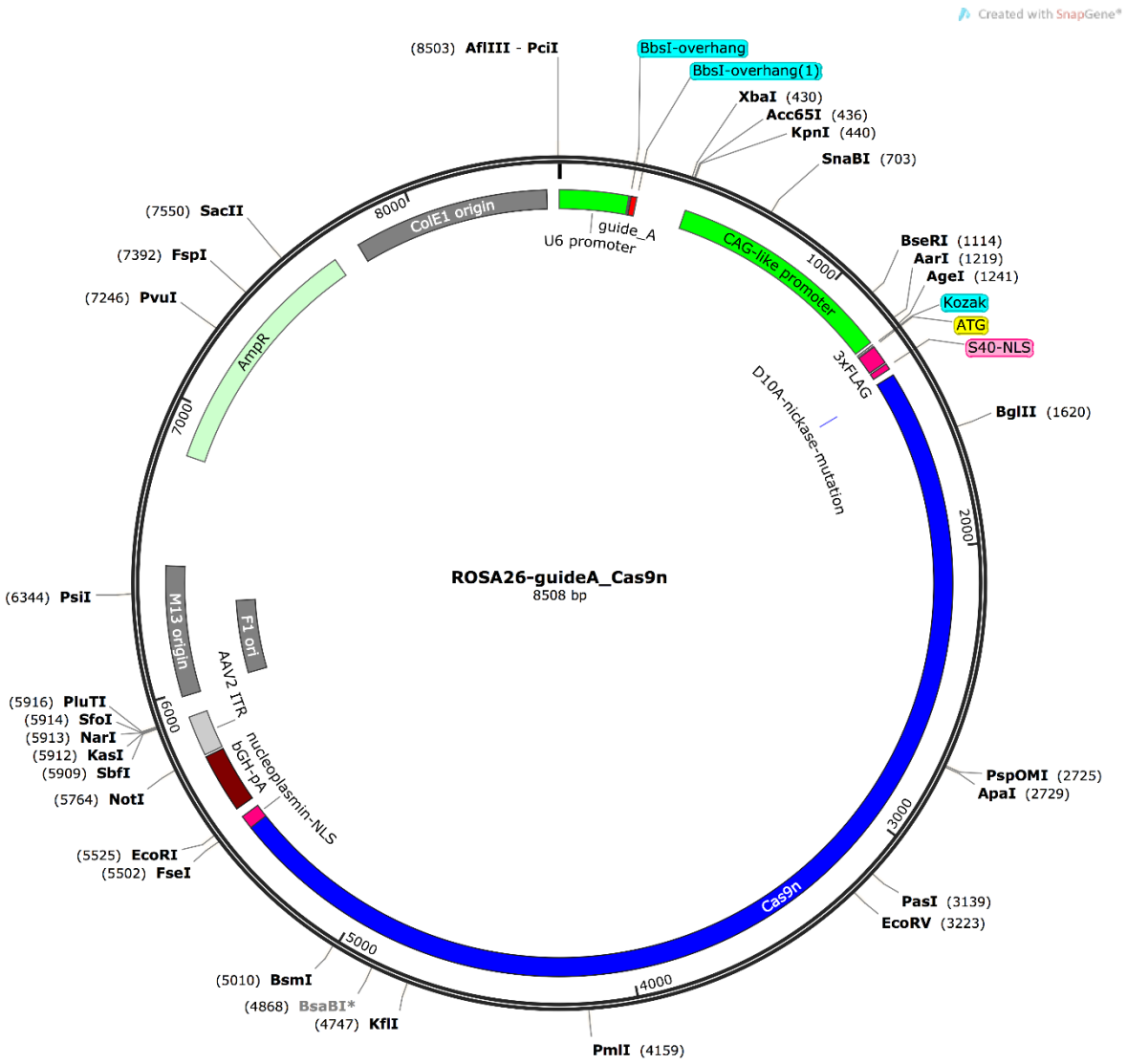


Vector map 1 AAVS1-TRE-PC3

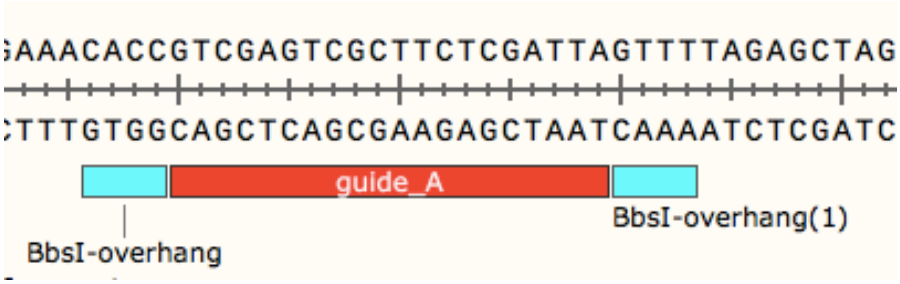
Final targeting vector containing the polycistronic cassette:
GFP-E2A-**GATA1**-P2A-**TAL1CO**-T2A-**FLI1CO**
The cassette was cloned using NcoI/EcoRI unique restriction sites.

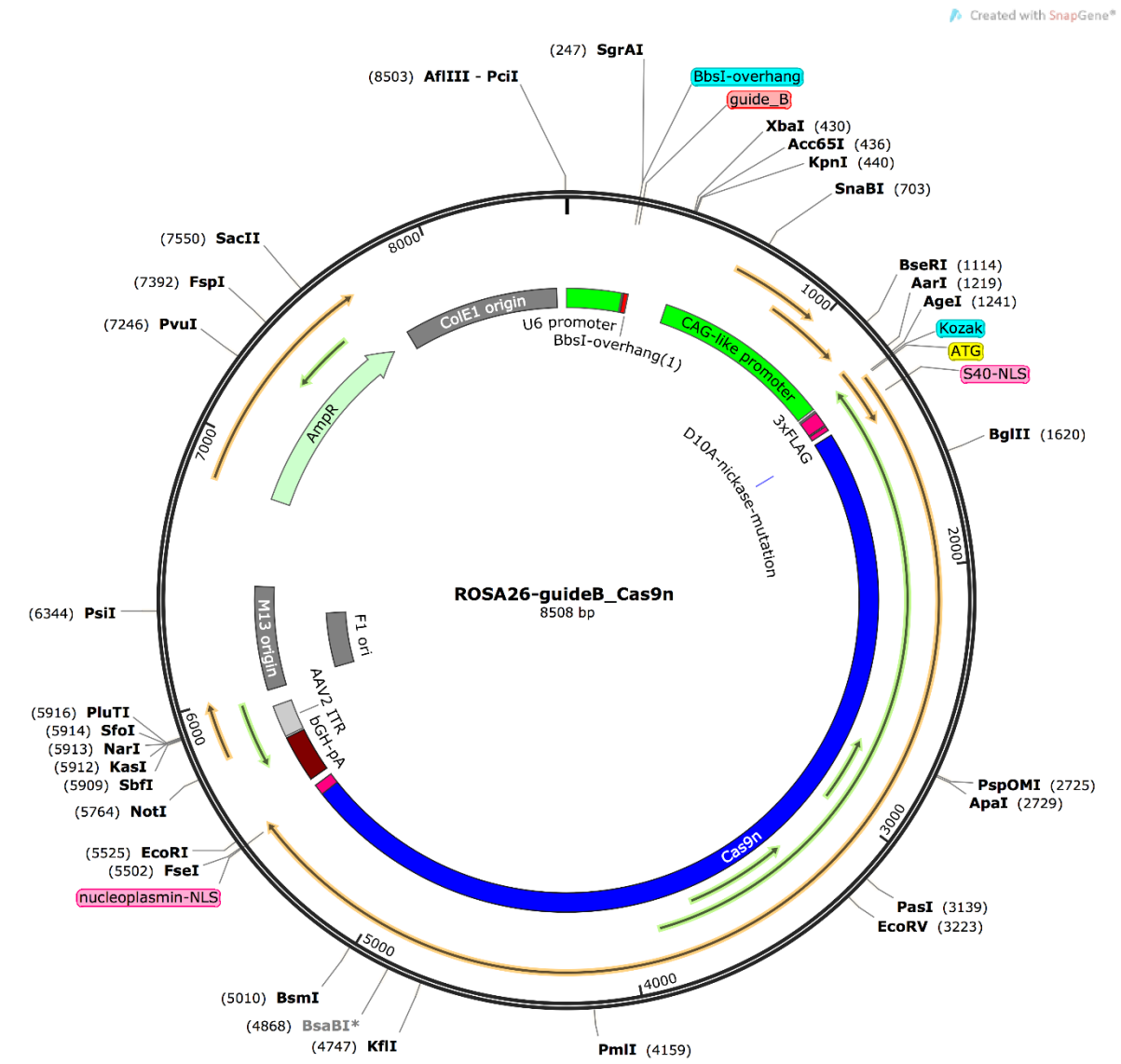


Vector map 2 Rosa 26 –neo-CAG-rtTA (OptiX system)

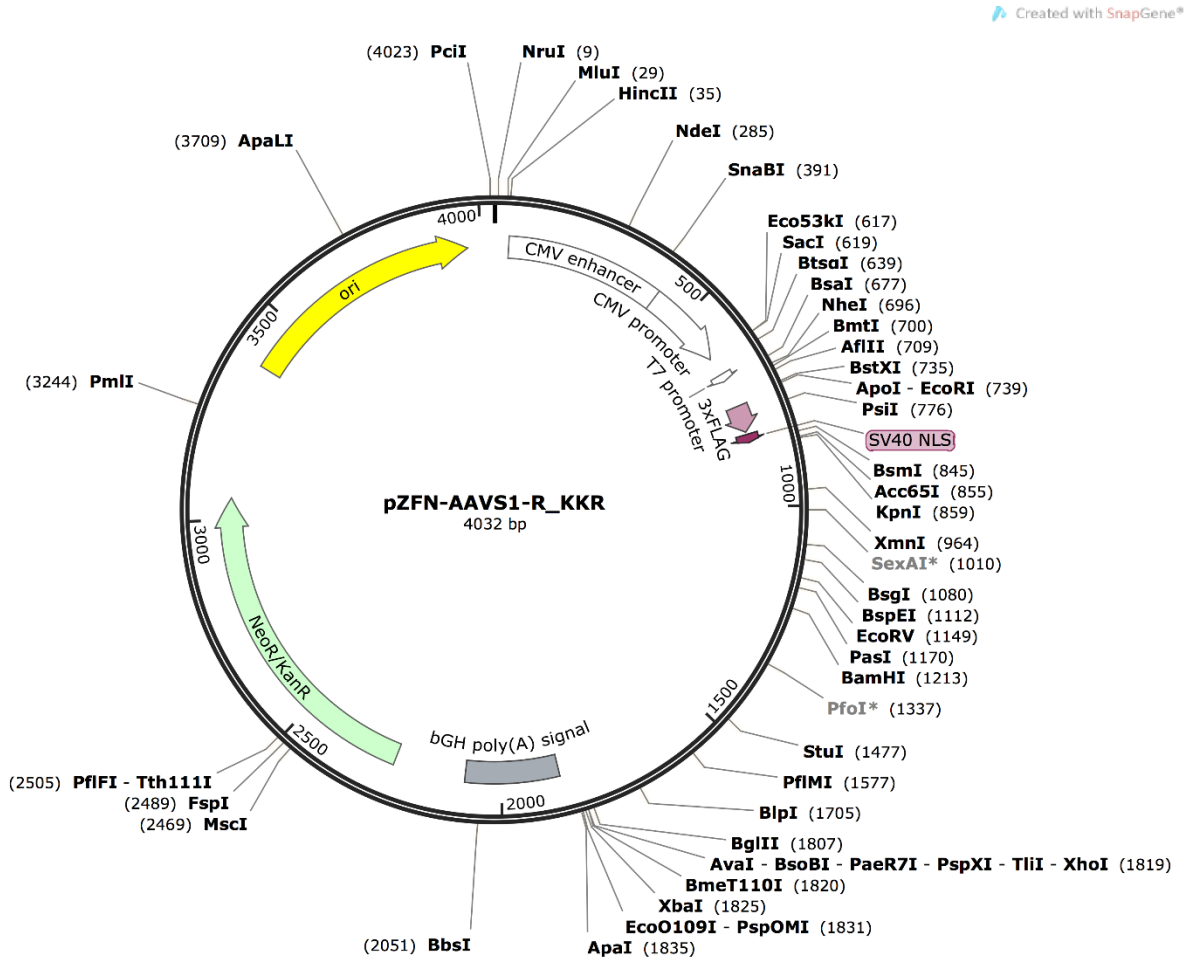


Vector map 3 Rosa26-guideA_Cas9n

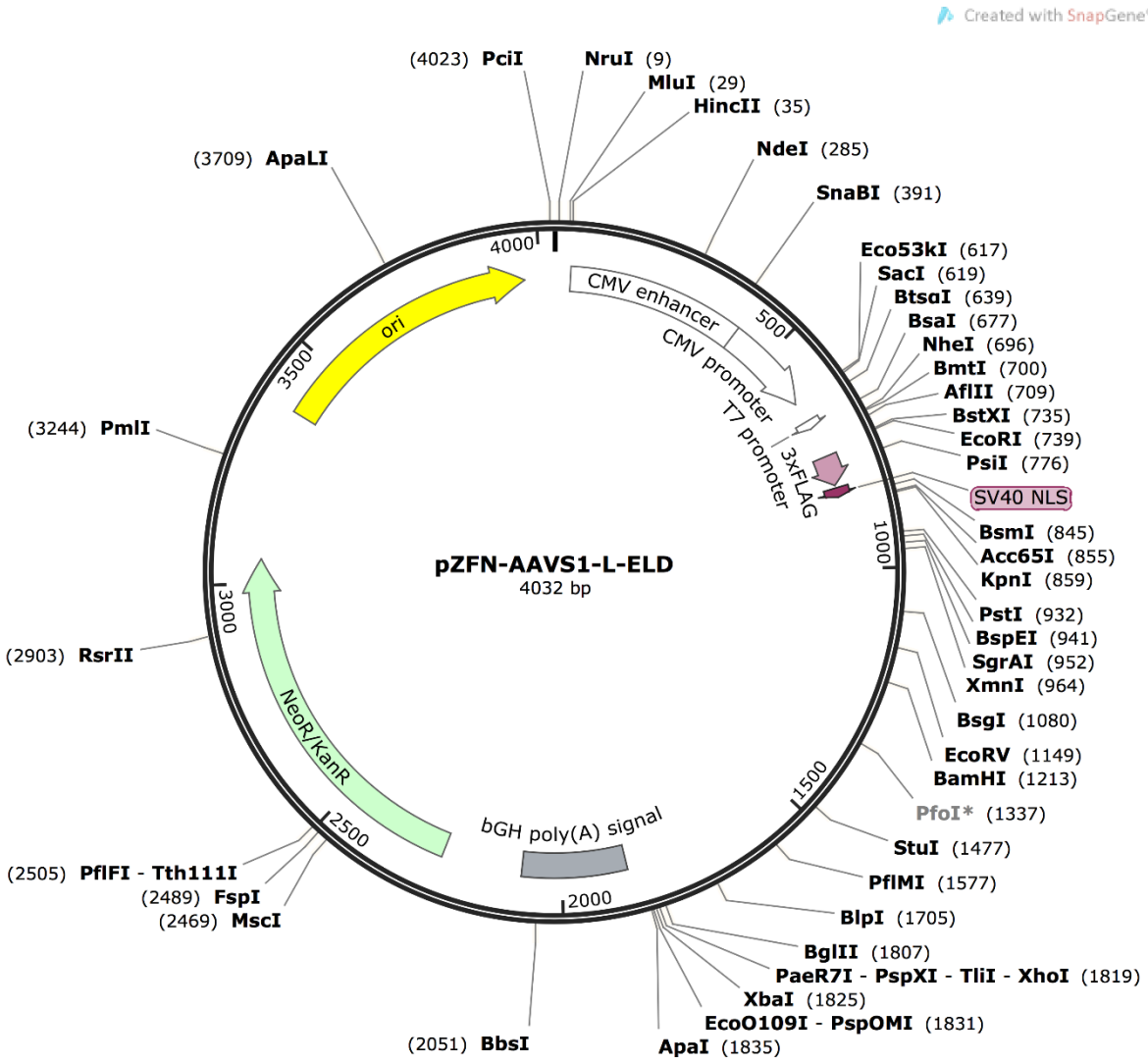




Vector map 4 Rosa26-guideB_Cas9n



Vector 5 pZFN –AAVS1-R_KRR



Vector 6 pZFN –AAVS1-L_ELD

Figure S5

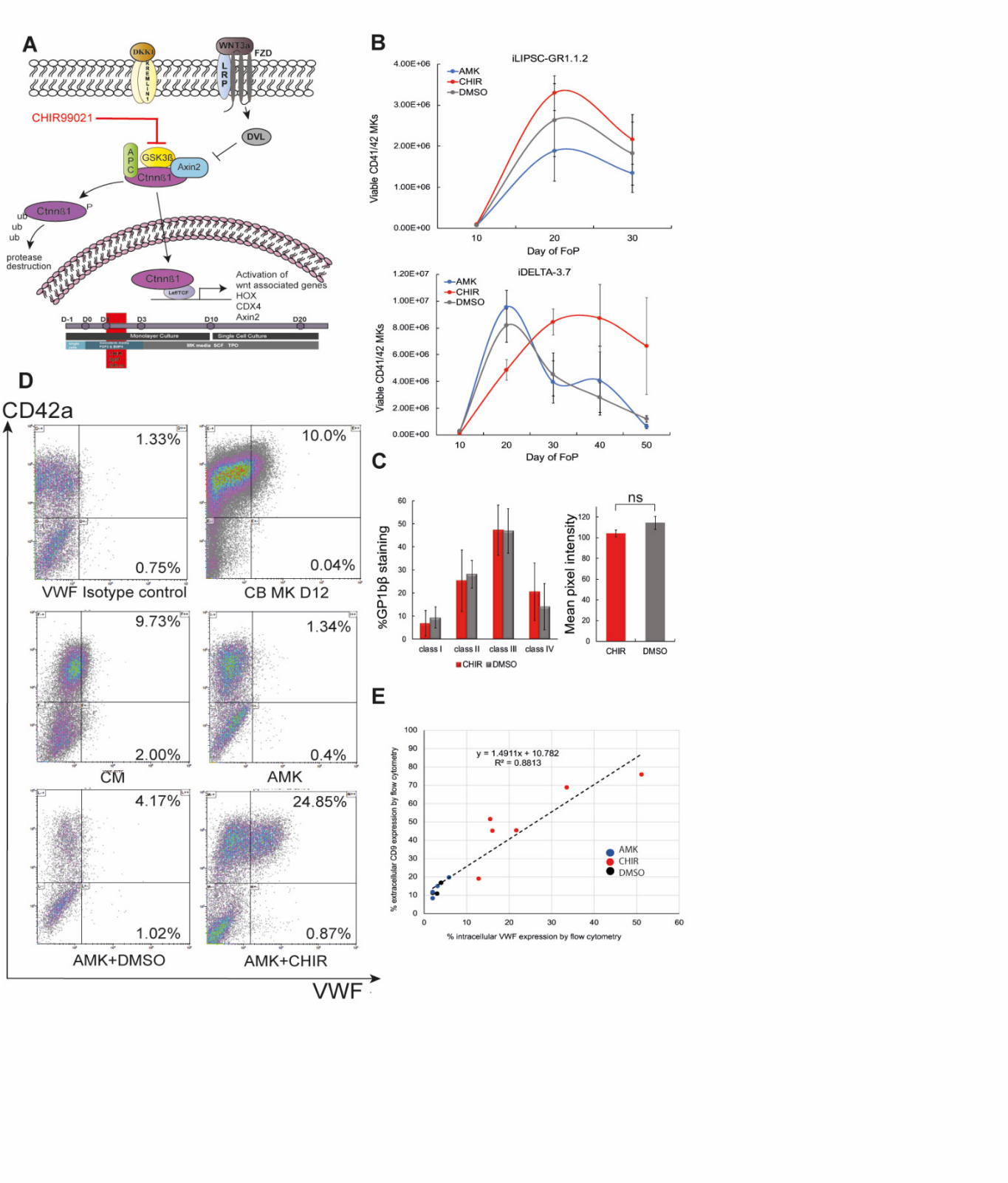

A
DKK1
WNT3a
FZD
LRP
CHIR99021
DVL
APC
GSK3ß
Axin2
Ctnnß1
P
ub
protease destruction
Activation of wnt associated genes
HOX
CDX4
Axin2
D-1
D0
D3
D10
D20
Monolayer Culture
Single Cell Culture
MK media SCF TPO
B
iLIPSC-GR1.1.2
AMK
CHIR
DMSO
Viable CD41/42 MKs
Day of FoP
iDELTA-3.7
C
%GP1bβ staining
class I
class II
class III
class IV
Mean pixel intensity
ns
D
CD42a
1.33%
0.75%
VWF Isotype control
10.0%
0.04%
CB MK D12
9.73%
2.00%
CM
1.34%
0.4%
AMK
4.17%
1.02%
AMK+DMSO
24.85%
0.87%
AMK+CHIR
VWF
E
y = 1.4911x + 10.782
R² = 0.8813
% extracellular CD9 expression by flow cytometry
% intracellular VWF expression by flow cytometry

Figure S5. The effect of CHIR99021 on the profile of programming and the enhancement in maturity of FoP-MK from clinical grade hiPSC.

A. The Wnt pathway: wnt-3a inducible signaling pathway protein 3a (wnt3a); frizzled receptor (FZD); Lipoprotein receptor-related protein (LRP) part of the receptor complex; dickkopf1 (DKK1) a negative regulator; disheveled (dvl) a negative regulator; Glycogen synthase kinase 3 Beta (GSK3β); adenomatous polyposis coli (APC); β catenin (Ctnnβ1). Arrows indicate positive interactions and bars represent negative interactions. CHIR99021 is a potent inhibitor of GSK3β which forms part of the canonical β catenin destruction complex, downstream effectors of wnt signalling. Inhibition prevents β catenin destruction resulting in transcription of wnt regulated genes and therefore acts as a wnt activator.

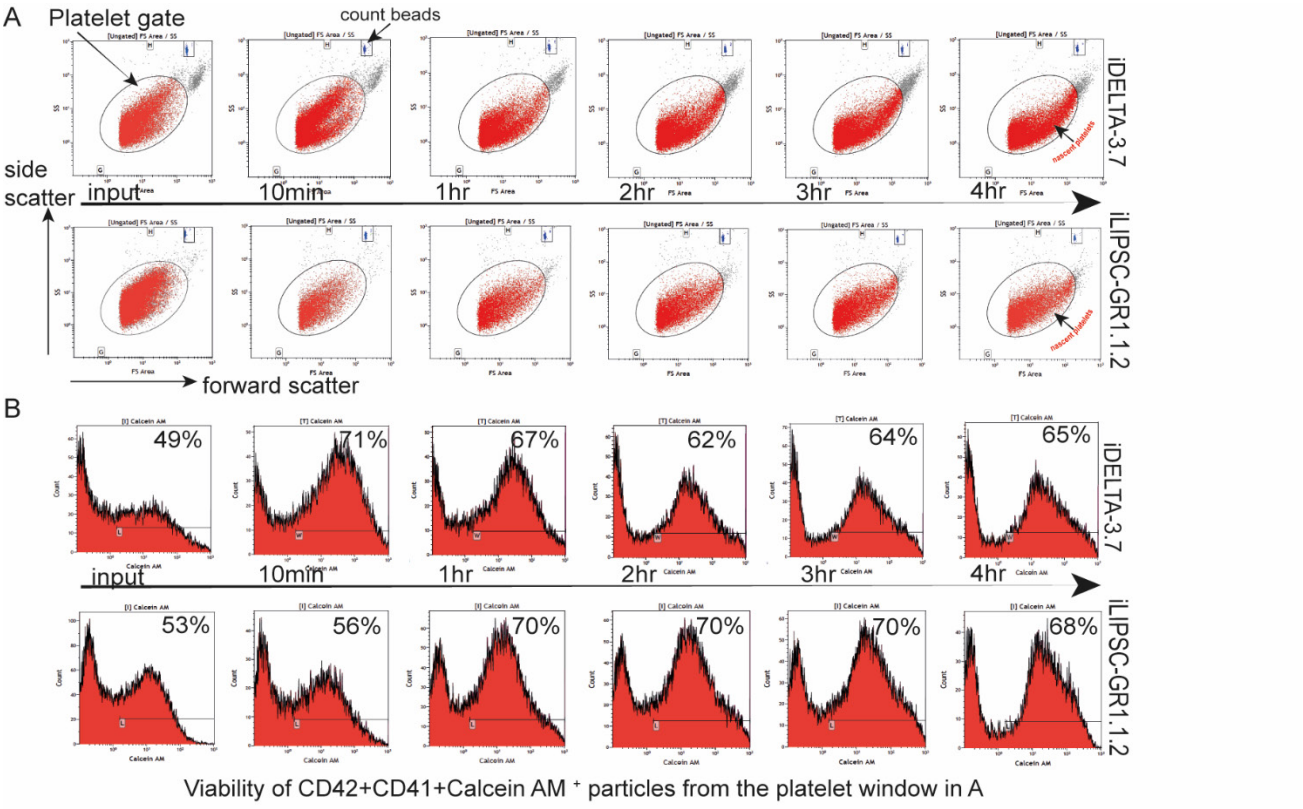
B. Line graphs for iLIPSC-GR1.1.2 (left) and iDELTA-3.7(right) for n=6 and n=10 biological replicates respectively showing the differing dynamics for the differentiation of the two clinical grade inducible lines.

C. iDELTA-3.7 MKs cultured with CHIR99021 show an additional increase in DMS development compared to DMSO in AMK. Staining of GP1Bβ in mature MKs derived after addition of CHIR99021 or DMSO showed a small further increase in class III and IV MKs above that seen in Figure 2D with fully developed DMS as per Aguilar et al.(supplemental methods) with no significant change in mean pixel intensity of the images perhaps reflecting the higher numbers of MKs forming proplatelet extensions (class IV). Data from n=3, 217 images.

D. Flow cytometry dot plots for MKs stained with CD42aAPC/VWF Fitc, gated on single fixed cells, analysed relative to isotype control. Numbers are % CD42a+VWF+ (top right quadrants) and % CD42-VWF+ (bottom right quadrants). VWF+ cells are CD42+ and CHIR99021 treatment increases intracellular VWF staining.

E. Using flow cytometry data to plot a linear regression curve of the percentage of intracellular VWF (x axis) against the percentage of extracellular CD9 (y axis), expressed from adjacent loci on chromosome 12, show a near linear relationship (R^2=0.8813), n=13 for iDELTA-3.7. AMK alone (blue circles), CHIR99021 treated (red circles), vehicle (black circles).

Figure S6



Viability of CD42+CD41+Calcein AM $^{+}$ particles from the platelet window in A

Figure S6. Bioreactor platelet production from clinical grade hiPSCs derived MKs.

A. Samples were taken throughout bioreactor runs to monitor platelet release from MKs, images show flow cytometry dot plots for the platelet window, determined using donor derived platelets, for iDELTA-3.7 (top panel, bioreactor run 2) and iLIPSC-GR1.1.2 (bottom panel, bioreactor run1). The change in platelet population from culture derived platelets already formed or forming on harvesting, to a nascent platelet population with a different forward-side scatter profile is clearly demonstrated.

B. Flow cytometry dot plots showing the viability by CalceinAM+ of the CD41+42+ platelets during the course of the bioreactor runs for iDELTA-3.7 (top panel, bioreactor run 2) and iLIPSC-GR1.1.2 (bottom panel, bioreactor run1).

Figure S7

A

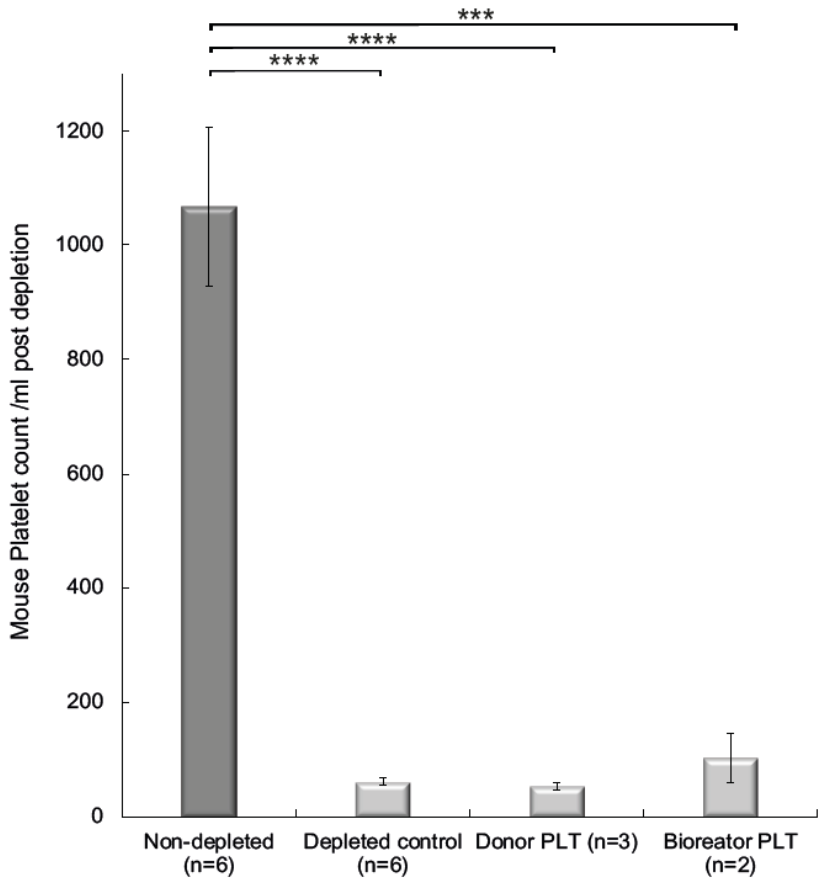

***
****
****
1200
1000
800
600
400
200
0
Mouse Platelet count /ml post depletion
Non-depleted (n=6)
Depleted control (n=6)
Donor PLT (n=3)
Bioreator PLT (n=2)

Figure S7. Platelet counts in immunodeficient NRG/J mice post anti-CD42b antibody administration and percentage ratio of human/mouse platelets in terminal bleeds.
A. Mean platelet counts for all the mice used for platelet transfusions (Figure 6A) measured from 10µl EDTA whole blood collected post anti-CD42d administration. Depleted samples analysed using ANOVA (Prism 9) plus Bonferroni multiple comparism show depleted platelet counts are significantly are significantly different from non-depleted samples. Depleted controls (n=6) v non-depleted(n=6) ****p=<0.0001, donor platelets (n=3) v non-depleted(n=6) ****p=<0.0001, bioreactor platelets (n=2) v non-depleted (n=6) ***p=<0.001.

Supplemental video

Bioreactor produced platelets 10mins to 4 hrs illustrating nascent platelet production