Figure S1: Expression of K14 and exogenous reprogramming factors in iPSCs. 
(a) K14 is homogeneously expressed in keratinocytes (left panel) and downregulated in keratinocytes derived iPSCs (right panel). (b) Relative expression of the exogenous reprogramming factors in iPSCs at days 100-125 after expansion compared to day 4 after transduction. Data represent mean and standard deviation (SD) of samples collected at day 100 and at day 125 and analysed in triplicates.
Figure S2: Flp-mediated excision of the selection cassette and lentiviral integrations.

(a) Southern blot to detect Flp-mediated excision of the PGK-neo-pA selection cassette in the targeted X-CGD iPSC clones. Genomic DNA was digested with ApaLI and hybridized with a 5’ external probe. (b) Southern blot to detect Flp-mediated excision of the lentiviral integrations in the targeted X-CGD iPSC clones. Genomic DNA was digested with BsrGI and hybridized with the PRE lentiviral probe. Note that clone Nr. 3 has excised only one of the 3 transgenes (marked by two asterisks) and clone Nr. 10 has the PGK-neo-pA cassette (blot in (a)) but none of the lentiviral transgenes. (c) The blot in (b) was hybridized with the 5’ external probe to show equal loading of the DNA.
Figure S3: Confirmation of the genetic correction by sequencing.
(a) Sequence from the surrounding genomic region of the X-CGD causing mutations (deletion: 985 del G; substitution: 962 T>G) in exon 9 of the patient CYBB gene.
(b) Sequence of the same region after gene targeting and Flp-recombination, showing the correction of the mutation and the location of the FRT site in the upstream intron.
(c) Representative SKY karyogram of the corrected (targeted and Flp-recombined) X-CGD iPSCs showing a normal male karyotype (46,XY). Chromosomes are arranged in numerical order and each chromosome is shown after staining with DAPI (left), in SKY display (middle), and classification colors. Note that areas where two chromosomes overlap lead to mixed spectra and are not rearrangements (e.g. overlap between the right chromosomes 2 and 9).
Figure S4: Expression of surface markers and gp91phox during differentiation. (a) Expression of CD10, CD13, CD31 and CD123 at day 20 and day 49 of iPSC differentiation (BAC transgenic clone PB21) (b) Expression of gp91phox of a correctly targeted clone at day 16 and at day 43 of myeloid differentiation. The third panel shows iPSCs cells carrying a BAC (clone PB13) at day 43 of differentiation.
Figure S5: ROS activity in CFUs and differentiated granulocytes assayed by NBT staining. (a) Representative pictures of a CFU from a BAC transgenic iPSC clone before and after staining with NBT. (b) Representative pictures of differentiated iPSCs stained with NBT. (c) Table showing the number of assayed clones by NBT staining. Presence of granulocytes was assayed by CD16.

| Clone Nr. | Granulocytes | NBT |
|-----------|--------------|-----|
| S22       | +            | -   |
| TG33      | +            | +   |
| TG55      | +            | +   |
| TG80      | +            | +   |
| TG84      | +            | +   |
| TG86      | +            | +   |
| TG89      | -            | -   |
| PB1       | +            | +   |
| PB5       | -            | -   |
| PB12      | +            | +   |
| PB13      | +            | +   |
| PB21      | +            | +   |
| PB23      | -            | -   |

S22 = X-CGD diff. iPSCs
TG = targeted X-CGD diff. iPSCs
PB = BAC transgenic X-CGD diff. iPSCs
| Clone | Integration site                                                                 | Chromosome | Position       | Inter- or Intragenic |
|-------|----------------------------------------------------------------------------------|------------|----------------|---------------------|
| 1     | aagagaacaggataggcaccTTAAgcaagaacattgtagagga                                     | 12         | NT_009714.17 pos. 14298583 | Intergenic          |
| 2     | ttacaagaaactgtgagcatTTAAttgcttctcagtaatgca                                     | 9          | NT_008470.19 pos. 17327134 | Intragenic          |
| 4     | gttcattattttaccattTTAAGcaggagagctgagtgaagt                                      | 8          | NT_008046 pos. 35897118  | Intergenic          |
| 5     | cttaagaaaaaaactacatTTAAnaaatatctcaagcccagcc                                    | 2          | NT_005403.17 pos. 43614805 | Intergenic          |
| 6     | ttaattattttttgagcTTAAttaatttttttaaaaa                                         | 1          | NT_004487.19 pos. 46717628 | Intragenic          |
| 12    | atgtcaacactaactctctctTTAAGcagtaaatctctctctctt                                  | 11         | NT_167190.1 pos. 3869071  | Intergenic          |
| 13    | aggccattgcccataagcacaTTAArgggaaacagaaagctgaa                                    | 14         | NT_026437.12 pos. 29528874 | Intergenic          |
| 14    | agagacaggtgtctcatTTAAtggagctgtctcaaaactcc                                     | 11         | NT_009237.18 pos. 4624398  | Intergenic          |
| 19    | acaatcttaggtaggtaggtTTAAatggaactaatattcttcag                                   | X          | NT_079573 pos. 599052       | Intergenic          |
| 21    | gccctatctctctTTAArggaggaagcagagcaga                                           | 8          | NT_008183 pos. 982031       | Intergenic          |
| 22    | tgaaacccacaagatacatTTAAtatatccacccacatagagc                                    | 20         | NT_011387.8 pos. 10410032   | Intragenic          |
| 23    | ctgagattgaaaccttgctcTTAAAtggagcatgacctctttt                                   | 2          | NT_022135 pos. 35866941     | Intergenic          |
### Table S2: List of recombineering oligos and primers

| Name              | Sequence (5’-3’)                                                                 |
|-------------------|----------------------------------------------------------------------------------|
| gp91-FRT-PGK-neo-1| tgggctacaactgagggcaattcccttgacagacacatctgtggaggaGAAGTTCC<br>TATTCTCTAGAAAAGTATAGGAACCTCAGTTTAACGC<br>GCCGCATTCTACC |
| gp91-FRT-PGK-neo-2| gataaaaactgagctaaattggcccacttagatctctgttttagggacaGAAGTTCCT<br>ATACTTTCTAGAGAATAGGAACCTTCTTTAACGGGCACGC<br>GCACACAAAAACC |
| gp91-15A-amp-1    | catatttttaggttaatattaatagatgtataatctgtctgtctGATATCGGCG<br>ACATGAGTATTCAACATTTCCGTG |
| gp91-15A-amp-2    | tatagaaaatctgaactataaatggttgagccagtaaaagaagGTCGACGAT<br>ATCTAGCGGAGGTATACGTGGCTAATATG |
| gp91-5’ttrim-amp-1| tgccacagctagctacagcagctacagcctctctgacttGGCCCTGCAGGC<br>CATCGATCAATCTAAAGTATATATGAGTAAC |
| gp91-5’ttrim-amp-2| caaggaatagttttctctactaaccactgttagaaagTTTGTTTATTTTTCTAA<br>ATAC |

lower case – homology arm; upper case bold – restriction sites, FRT sites; upper case regular – priming part

### PCR primers to detect transposition events

| Name     | Sequence (5’-3’) |
|----------|------------------|
| PB5_F    | GACGCATGCATTCTTGGAAT |
| PB5_R    | ATGCGTCATTITGACTCACG |
| PB3_F    | GAAGAAAATTTTGAGTTTTTGTITT |
| PB3_R    | CGCATGTGTTTTATCGGTCT |
| Amp_R    | ATAATACCGCCACACATAGC |
| gp91_intron10-11_R | GGTCTCCTCTCCTCCCCCTTG |

### qRT-PCR primers

| Name     | Sequence (5’-3’) |
|----------|------------------|
| Oct4-exo-F | CCGTGAAGCTGGAAAAAGAG |
| Gene   | Primer Name | Sequence            |
|--------|-------------|---------------------|
| Oct4   | exo-R       | AGATGGTGGTCTGGGCTGAAC |
| Sox2   | exo-F       | ATGCACAACAGCGAGATCAG |
| Sox2   | exo-R       | TTCTGGGCGGTTACTTTGAG |
| Klf4   | exo-F       | CCGGAGACAGGAGAAATCAA |
| Klf4   | exo-R       | TCTGATGGGGTAGGATGAGC |
| cMyc   | exo-F       | AGCGACTTGGGAGGAGGAACA |
| cMyc   | exo-R       | CTCTGACCTTTTGCCAGGAG |
| OCT4   | endo-F      | GGGTTTTTGGGATAAGTCTTTCA |
| OCT4   | endo-R      | GCCCCCCACCCTTTTGTTT |
| SOX2   | endo-F      | CAAAAATGGGCATGACAGTT |
| SOX2   | endo-R      | AGTTGGGATCGAACAAGAGCTATT |
| KLF4   | endo-F      | AGCCTAAATGGATGGGCTTGGT |
| KLF4   | endo-R      | TTGAAAACTTTGGCTTCTTTGTT |
| NANOG  | F           | ACAACTGGCGCAAGATAGCA |
| NANOG  | R           | GGTTCGCCAGTCGGGTTCAC |
| CRIPTO | F           | CGGAACGTGGACGACGATGT |
| CRIPTO | R           | GGGCAGCAGGTCAGTACAG |
| REX1   | F           | CCTGCAGGCGGAAATAGAAC |
| REX1   | R           | GACACATAGCCATCACAATAAGG |
Supplementary Material and Methods

Virus vector particle production

The pRRL.PPT.SF.hOct34.hKlf4.hSox2.hcmyc.i2dTomato.pre (LV-OKSM-Tomato) reprogramming vector containing human reprogramming factors: OCT4, KLF4, SOX2 and c-MYC was kindly donated by Axel Schambach (MHH Universität, Hannover, 1). Virus vector particle production was performed as previously described 2. Briefly, human embryonic kidney (HEK293T) cells were seeded 24 hours prior to transfection in 175 cm² flasks in DMEM (Dulbecco’s Modified Eagle Medium) supplemented with L-glutamine (Life Technologies, Darmstadt, Germany), 10% FCS (Fetal Calf Serum), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were transiently transfected by polyethylenimine with psPAX2, pMDG2 and LV-OKSM-Tomato vector. Supernatants were harvested 48 and 60 hours after transfection, filtered (0.45 µm) and concentrated using ultracentrifugation (100,000g, 4°C, 3 hours) and resuspended in Keratinocyte Growth Medium.

Culture of hES cells

H7.S6 hESCs were cultured on mouse embryonic fibroblasts (MEF) in DMEM/F12 medium supplemented with 20% Knockout Serum Replacement (Life Technologies) and 4 ng/ml basic fibroblast growth factor (Peprotech, Hamburg, Germany) and passaged using 1 mg/ml collagenase IV (Life Technologies) adding 10 µM ROCK inhibitor Y-27632 (Sigma-Aldrich, Munich, Germany). For transfections, the cells were dissociated using TrypLE (Life Technologies) and transferred to feeder-free conditions on Matrigel (BD Biosciences) in MEF-conditioned hESC medium. Permission to work with hESCs was granted by the Robert Koch Institute, Berlin, Germany (AZ 1710-79-1-4-17E1).
RNA isolation and real-time PCR

RNA isolation and reverse transcription were performed following standard procedures. Quantitative real time PCR was done using GoTaq qPCR Master Mix (Promega, Mannheim, Germany) using oligonucleotides listed in Table S2. Ct-values were normalized against GAPDH and fold differences in expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method.

Immunostaining

Cells cultured on Matrigel coated cover slips were fixed with 3.7 % formaldehyde for 15 min, incubated with 0.5% Triton X-100 in PBS (Phosphate Buffered Saline) for permeabilization for 10 min and blocked with 3% BSA (Bovine Serum Albumin)/PBS for 1 hour at room temperature. Primary antibodies, diluted in 1% BSA/0.125% Triton X-100/PBS were incubated overnight at 4°C. The following antibodies were used: OCT4 (0.5 µg/ml), NANOG (0.5 µg/ml), K14 (0.2 µg/ml; all from abcam, Cambridge, UK), SSEA1, SSEA3, SSEA4, TRA-1-60, TRA-1-18 (1:20 diluted; kindly donated from the Centre of Cell Biology, University of Sheffield). Secondary antibodies (a-rabbit Alexa 488 (Life Technologies), a-mouse-IgM-PE, a-rat-IgM-PE or a-mouse-Ig-PE (BD Pharmingen, Brussels, Belgium) were added for 30 min at 37°C diluted in 1% BSA/0.125% Triton X-100/PBS. The cells were counterstained for 5 min with Hoechst 33342 (10µg/ml; Life Technologies), mounted in Fluormount-G (SouthernBiotech, Birmingham, USA) for fluorescence microscopy (Axiophot, Zeiss, Jena, Germany) and analysed by the accompanying software (Axiovision AxiVs40 V 4.6.3.0; Zeiss).
**Teratoma formation**

1x10^6 hiPSCs were resuspended in Matrigel and injected intramuscularly into immunodeficient NSG (NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm3Wjl</sup>/SzJ) mice. 8-12 weeks after injection mice were euthanized and the isolated teratomas were fixed overnight in 4% neutrally buffered formaldehyde, dehydrated in increasing amounts of ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) according to standard procedures.

**Generation of modified BACs and targeting vectors**

Two BACs RP11-777A16 and RP11-641C23 containing the CYBB gene were purchased from Children’s Hospital Oakland Research Institute (CHORI, USA). All BAC modifications and the subsequent generation of targeting vectors were done using recombineering technology<sup>4</sup>. Oligonucleotides were purchased from Biomers (Ulm, Germany) and are listed in Table S2.

**Construction of CYBB-BAC-PB**

For transposon mediated BAC transgenesis RP11-641C23 was modified in two steps. The cassettes for recombineering were obtained by digestion of plasmids R6K-amp-PB-SB and R6K-PB-SB-UBC-BSD with PacI<sup>5</sup>. Firstly, a cassette containing 5’-PB inverted repeat (ITR), the ampicillin resistance gene (bla) and thymidine kinase from herpes simplex virus (HSV-TK) was inserted upstream of T7 sequence replacing the lox511 site. For the next step, a cassette containing 3’-PB ITR and Blasticidin-S resistance gene driven by a dual Ubiquitin-C/Em7 promoter was inserted at the other side of the BAC backbone replacing loxP site. The clones were verified by restriction digestion and sequencing.
**Generation of CYBB-FRT-PGK-neo-FRT-BAC**

To generate targeting constructs, the RP11-641C23 BAC was further modified by insertion of PGK-Em7-neo selection cassette flanked by FRT sites into intron 8 of the CYBB gene. The cassette was PCR-amplified from an R6K vector using the primers gp91-FRT-PGK-neo-1 and gp91-FRT-PGK-neo-2, which contain homology arms to the respective position in the CYBB intron.

**Generation of p15A-CYBB-FRT-PGK-neo**

To generate a targeting vector with short homology arms the fragment gp91phox with PGK-neo insertion was subcloned from the BAC into a p15A-origin vector using recombineering. The p15A-ampR cassette was PCR amplified using oligos gp91-15A-1 and gp91-15A-2.

**Trimming of CYBB-FRT-PGK-neo-FRT-BAC**

For gene targeting with large vectors, the BAC with PGK-neo insertion was trimmed by recombineering with a PCR-amplified ampicillin resistance cassette using oligos gp91-5'trim-amp-1 and gp91-5'trim-amp-2. To allow generation of targeting vectors with different length of 3’ homology arms, the restriction sites for SbfI and SfiI were additionally introduced in gp91-5'trim-amp-2. The 5’ homology arm was 8kb long for all targeting vectors allowing detection of targeting events by Southern blotting.

**Transfection and screening of clones**

**BAC lipofection of human pluripotent stem cells**

BAC was prepared using Nucleobond BAC 100 kit (Macherey-Nagel, Düren, Germany) from 400 ml of overnight bacterial culture grown at 30°C. PiggyBac transposase and BAC co-transfection was performed by lipofection as previously described\(^5\). Briefly, confluent cells grown on Matrigel coated 10 cm dish were
passaged in 1:3 dilution in the presence of 10 µM ROCK inhibitor one day before transfection. 10 µg of BAC and 10 µg of hyPBase expression vector or pUC19 control vector were dissolved overnight in 3 ml of OPTI-MEM medium and further used for transfection with Lipofectamine LTX (Life Technologies) according to the manufacturers protocol. *PiggyBac* transposase expression (hyPBase) vector was a gift from Alan Bradley, Sanger Institute, UK. Positive selection with 2 µg/ml blasticidin (Life Technologies) and negative selection with 200 nM FIAU (Fialuridine; Moravek Biochemicals, Brea, USA) started 2 and 6 days after transfection respectively. After approximately 2 weeks of selection, colonies were picked into 96 well plates, expanded and screened by PCR.

Electroporation of human pluripotent stem cells

Plasmid DNA or trimmed BAC were linearized by restriction digestion, purified by phenol-chloroform extraction and dissolved in a small volume of PBS to ~1 µg/µl. Pluripotent stem cells grown on Matrigel were dissociated using TrypLE and washed. For each transfection 3x10^6 cells were resuspended in 750 µl of culture medium and mixed with 25 µg of DNA. Electroporation was done in 4 mm cuvettes at 320 V and 250 µF using a Biorad Gene Pulser. The cell suspension was incubated 10 min at room temperature and then distributed to Matrigel coated dishes with MEF-conditioned medium supplemented with ROCK inhibitor. Selection with 100 µg/ml G418 started 2 days after transfection. After approximately 2 weeks of selection, stable resistant clones were picked to 96-well plates and expanded.

Analysis of transposition events

The presence of transposon inverted repeats and loss of the ampicillin resistance cassette in the clones that survived selection after BAC transfection was checked by
PCR analysis using primers pairs PB5_F and PB5_R; PB3_F and PB3_R for the presence of 3’PB ITR and PB5_F with Amp_R to confirm exclusion of the BAC backbone. The BAC integration sites were determined using Splinkerette PCR and sequencing as already described\(^5\),\(^7\).

**Southern blot analysis**

Genomic DNA was digested with ApaLI or BsrGI, separated by electrophoresis and transferred to nylon membranes (BNAZF810S; PALL, NY, USA). Probes were labeled with \(^{32}\)P-dCTP using High Prime (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instruction. Hybridization and washes of the membrane were done according to Church & Gilbert method. The membranes were exposed to radiosensitive screen and scanned by a FLA-3000 phosphoimager (Fuji Photo, Tokyo, Japan).

**Conventional Cytogenetic (G-banding), Spectral karyotyping (SKY) and Fluorescence In Situ Hybridization (FISH)**

Cells were treated with colcemid (0.035 µg/ml for 60 min), incubated in 0.075 M KCl for 20 min at 37°C and fixed in freshly prepared methanol/acetic acid (3:1) at room temperature and cell suspension was dropped onto glass slides. G-banding was performed using standard protocols. Spectral karyotyping (SKY) analysis was performed as described previously\(^8\). Metaphase chromosomes were hybridized for 3 days with a self-made SKY hybridization probe cocktail for human chromosomes as described\(^9\). SKY images of about 20 metaphase chromosomes per cell line stained with a mixture of 5 fluorochromes (green, orange, red, far-red, and near-infrared) were captured using an DMRXA epifluorescence microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) with an HCX PL SAPO 63x/1.30 oil objective.
(Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) with the SpectraCube® system (Applied Spectral Imaging, Migdal HaEmek, Israel) and the SKYView® imaging software (Applied Spectral Imaging, Migdal HaEmek, Israel). For FISH analysis of the integration site DNA from the CYBB BAC was directly labeled via Nick-translation in red using tetramethyl-rhodamin-5-dUTP (Roche) and hybridized on chromosome spreads together with a commercial probe targeting the centromere of chromosome X labeled in green (DXZ1; Kreatech, Amsterdam, Netherlands). FISH was carried out according to standard protocols.

In vitro differentiation

For the in vitro differentiation of hiPSCs into the haematopoietic lineage we adapted the protocol provided by Stem Cell Technologies and which was based on Ng et al. and Chadwick et al. with small modifications. Three days after passaging of hiPS cells, mTeSR medium (Stem Cell Technologies) was replaced with 3 ml per well of differentiation medium I (STEMdiff APEL medium (Stem Cell Technologies) supplemented with 1 ng/ml VEGF, 30 ng/ml BMP-4 (Miltenyi Biotec, Bergisch Gladbach, Germany), 40 ng/ml SCF (Stem Cell Factor; R&D Systems, Wiesbaden, Germany), and 25 ng/ml Activin A (R&D)). After 4-5 days of incubation, the medium was replaced with differentiation medium II (STEMdiff APEL medium supplemented with 180 ng/ml SCF, 180 ng/ml Flt-3 ligand, 10 ng/ml IL-3 (interleukin-3), 10 ng/ml TPO (thrombopoietin) (all from BD Biosciences), 10 ng/ml IL-6, 50 ng/ml G-CSF, 25 ng/ml BMP-4 (Miltenyi Biotec) and changed every 4-5 days. Between days 18-20, cells were analysed for expression of haematopoietic markers (CD34/CD45) and stem cell properties. For further differentiation we followed the protocol published by Yokoyama et al. with slight modifications. Cells were then dissociated and replated on Mitomycin-C (Sigma-Aldrich) inactivated OP9 cells in differentiation medium III.
(IMDM (Life Technologies) supplemented with 10% Fetal Bovine Serum (Thermo Fisher Scientific, Waltham, USA), 10% horse serum, 5% hybridoma medium (Biochrom, Berlin, Germany), 0.1 mM 2-mercaptoethanol (Sigma-Aldrich), 100 ng/ml SCF, 100 ng/ml Flt-3, 100 ng/ml IL-6, 10 ng/ml TPO and 10 ng/ml IL-3). From day 28-30 onwards, the cells were cultured in the presence of 50 ng/ml G-CSF (Miltenyi Biotec) in RPMI medium (PAA) supplemented with 10% FBS, 2 mM L-Glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate, 10 mM Hepes (Biochrom) and 0.1 mM 2-mercaptoethanol. Between days 43-44 cells were analysed by flow cytometry for expression of CD13 (PE labeled, BD Biosciences) and gp91phox (PE labeled anti-Flavocytochrome b558, MBL) as well as oxidase activity.

**Isolation and culture of CD34+ HSPCs**

Following informed consent and the approval of the local ethics committee (EK221102004, EK47022007) primary human haematopoietic stem and progenitor cells (HSPCs) were isolated from either mobilized peripheral blood or umbilical cord blood of healthy donors by CD34 antibody-conjugated magnetic beads according to the manufacturer’s instructions (Miltenyi Biotec). The purity of the isolated CD34+ population was confirmed by LSRII flow cytometer (BD Biosciences) and the cells were cultured for 2-5 days in X-Vivo10 medium (Lonza, Basel, Switzerland) containing 1% human serum albumin, 25 ng/ml Flt3-ligand and 10 ng/ml each TPO, IL-3 and SCF.

**Isolation of neutrophils**

Blood samples from voluntary healthy donors or X-CGD patients were used after informed consent and the approval of the local ethics committee (EK 355112012). 3%
dextran solution was added to heparin blood samples. Following sedimentation (30 min, room temperature), and centrifugation at 300g (8 min, room temperature) the cell pellet was resuspended in 500 µl PBS. To lyse the erythrocytes, 10 ml of 1x HANK’s solution (Life Technologies) was added for 22 sec. After centrifugation (300g, 8 min, room temperature) the procedure was repeated twice. Finally, blood cells were collected in PBS und used for measurement of oxidase activity or expression of surface markers.

**Antibody staining and flow cytometry**

Cells were washed with PBS and co-stained with CD34-APC-A or CD34-PerCP and CD45-V500 or CD13-PE and CD45-V500 antibodies (BD Biosciences) for 15 min at room temperature. Following a washing step with PBS, the expression of surface markers was measured by flow cytometry (LSRII; BD Biosciences). To analyze the expression of gp91phox protein the cells were fixed with Medium A (GAS001S-100, Life Technologies) for 15 min, washed with PBS and permeabilized with Medium B (GAS002S-100, Life Technologies) for additional 10 min. After washing with PBS the cells were incubated with monoclonal gp91phox PE labeled antibody (MBL International, Woburn, USA) (5 µl per 50 µl cell suspension) for 20 min at room temperature, washed with PBS and analysed by flow cytometry.

**Colony forming unit (CFU) and NBT assay**

5 x 10³ in vitro differentiated iPS cells were plated in triplicate in methylcellulose medium containing recombinant cytokines (MethoCult H4434; Stem Cell Technology, Vancouver, BC, Canada). After 2-3 weeks, colony-forming cells (CFC) were analyzed by stereomicroscope and stained after stimulation with phorbol 12-
myristate 13-acetate (PMA - 0.1 µg/ml) with Nitro Blue Tetrazolium (NBT - 50 µg/ml).

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