# Patients and methods

**Supplementary data for the manuscript: "Reconstitution of *in vivo* hematopoiesis by iPS cell-derived HLA-B4002-lacking hematopoietic stem cells of acquired aplastic anemia that escape cytotoxic T lymphocyte attack"**

**Table S1. Characteristics of the AA patient (KANA1) whose monocytes were used to generate iPS cell in this study**

| | | **KANA1 patient** |
|---|---|---|
| **Age (year)** | | 28 |
| **Gender** | | Male |
| **Disease Status** | | Very severe |
| **Months from diagnosis** | | 170 |
| **Immunosuppressive therapy (IST)** | | ATG/CsA |
| **Response to IST** | | CR |
| **Missing alleles due to 6pLOH** | **HLA-A** | *24:02* |
| | **HLA-B** | *40:02* |
| | **HLA-C** | *03:04* |
| **Retained alleles** | **HLA-A** | *02:01* |
| | **HLA-B** | *35:01* |
| | **HLA-C** | *08:01* |
| **% HLA-LLs of each lineage cells** | **Granulocytes** | 40.9% |
| | **Monocytes** | 33.3% |
| | **B cells** | 11.3% |
| | **T cells** | 11.8% |

**ATG indicates antithymocyte globulin; CsA, cyclosporine A; and CR, complete response; and HLA-lacking leukocytes, HLA-LLs.**

**Table S2. Somatic mutations identified in HLA-*B*40:02* of KANA1 patient[#]**

| **DNA changes in B4002(−)A(+) granulocytes** | **Location** | **Codon** | **Group** | **COSMIC ID** |
|---|---|---|---|---|
| **c.1A>T** | **Exon 1** | **ATG>TTG** | **Start loss** | **Novel** |
| **c.748C>T** | **Exon 4** | **CAG>TAG** | **Nonsense mutation** | **COSM4833544*** |
| **c.869T>C** | **Exon 4** | **CTG>CCG** | **Missense mutation** | **Novel** |

**#** Data are shown in a reference[1].

***** COSMIC, the Catalogue of Somatic Mutations in Cancer.

## Generation of iPSCs from non-T-cell populations in PBMCs with episomal vectors

Peripheral blood was obtained from KANA1 who had HLA-class I alleles-lacking leukocytes (HLA-LLs). Generation of iPSCs from the patient's peripheral blood mononuclear cells (PBMCs) were carried out at (Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan) using a nonintegrating method as described previously with some modifications[2]. PBMCs were separated using density gradient centrifusion with Lymphoprep, according to the manufacturer's instructions (Rodelokka, Oslo, Norway). PBMCs were transfected with episomal vectors including pCXLE-hOCT3/4-shp53, pCXLE-hSK, and pCXLE-hUL and then seeded onto mouse embryonic fibroblasts (MEF) feeder cells. These PBMCs were cultured in non-T-cell medium that mainly stimulates the proliferation of monocytes. The colonies were counted 16–35 days after plating, and the colonies similar to human embryonic stem cells were selected for further cultivation and evaluation. The chromosomal G-band analyses were performed at the Nihon Gene Research Laboratories (Sendai, Miyagi, Japan).

## Cell lines and preparation of the conditioned media

WEHI cells were purchased from Riken cell bank and were cultured in D-MEM supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine. SNL cells were purchased from Cell Biolabs, Inc. (San Diego CA, USA) and were cultured in D-MEM (high glucose) supplemented with 10% FBS, 0.1 mM MEM non-essential amino acids (NEAA) and 2 mM L-glutamine. OP9 cells were obtained from Dr. Hirao Atsushi at Cancer Research institute, Kanazawa University and were cultured in α-MEM medium supplemented with 10% FBS and 1% Pen-Strep. The OP9 cells[3] were used in iPSCs culture as a feeder cells for feeder induction culture**(Fig. S1A&B)**. We prepared the conditioned media by collection of supernatant of OP9 cells and WEHI cells culture media as described before[4].

## Protocols for the differentiation of human iPS cells (h-iPSCs) to HSCs under feeder free conditions

### StemPro®-34 SFM medium culture

iPSC colonies were collected and plated in Biocat Collagen-I 25cm$^2$ vented flasks in StemPro-34 medium (Invitrogen) supplemented with 2 mM glutamine, 30 ng/ml bone morphogenetic protein 4 (BMP4), 50 µg/mL ascorbic acid, 150 µg/mL transferrin, 0.4 mM monothioglycerol and several cytokines which were added sequentially as follows:
**Days 0-4:** Vascular endothelial growth factor (VEGF) 50 ng/mL, stem cell factor (SCF), 50 ng/mL, thrombopoietin (TPO) 50 ng/mL, FLT3-ligand (Flt3L) 50 ng/mL, bFGF 20 ng/mL.
**Days 4-8:** VEGF 50 ng/mL, SCF 50 ng/mL, TPO 50 ng/mL, Flt3L 50 ng/mL, bFGF 20 ng/mL.
**Day 8+:** SCF 50 ng/mL, TPO 50 ng/mL, IL-3, 10 ng/mL, IL-11 5 ng/mL, erythropoietin (EPO) 2 U/mL, and insulin-like growth factor (IGF) 125 ng/mL.
The iPSCs were harvested from cultures on the indicated days and processed for flow cytometry (FCM) analysis to determine the committed cell properties.

### Induction of HSC differentiation from iPSCs using conditioned media

We used the supernatant of OP9 cell and WEHI cell culture media as conditioned media. iPSC colonies were collected and plated in new fresh α-MEM medium containing 10% FBS and 10% conditioned media as supplements. iPSCs were harvested from feeder-free induction cultures on the indicated days (0, 7, 14, 21, and 28) and processed for an FCM assay to determine the committed cell properties **(Fig. S1C)**

**Figure S1**

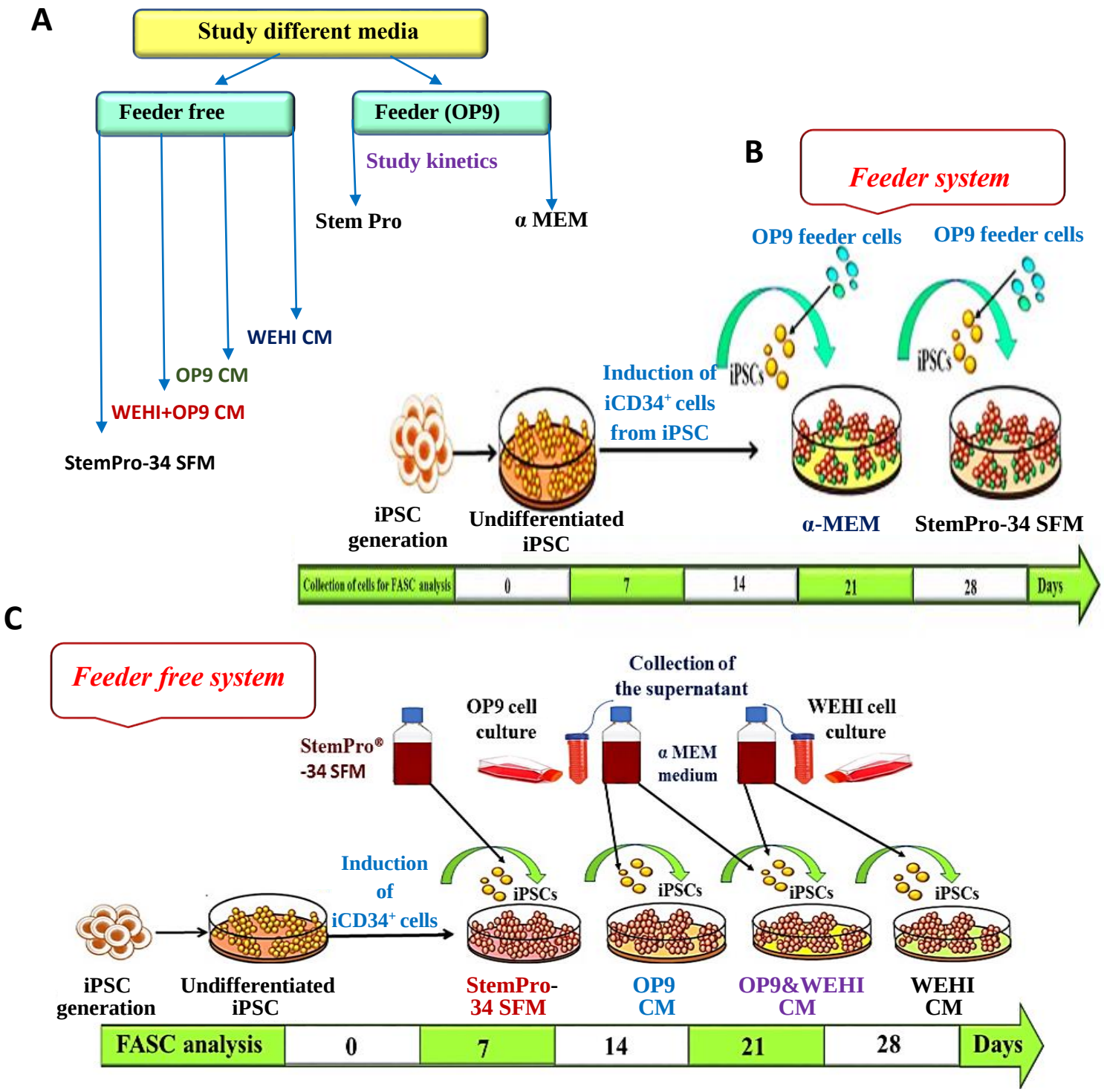


**Figure S1. Induction of iPSC-HSCs using feeder and feeder free systems.** (A) Schematic diagram and protocol for inducing CD34$^{+}$ cells from iPSCs using OP9 feeder cells in alpha MEM or StemPro-34 SFM culture media (B) and feeder free systems with Stem-Pro-34 SFM and OP9, WEHI and OP9 & WEHI conditioned media(CM) (C). The time points to study effects of different culture media on HLA.

## Detection of HLA(-) leukocytes uses flow cytometry (FMC) analysis

Heparinized PB and BM were collected from KANA1 patient. Cells were washed in phosphate-buffered saline (PBS) and resuspended in 100 μl of FACS buffer at a concentration of $10^6$ cells per ml. Cells were incubated in 1% bovine serum albumin (BSA) in PBS (blocking buffer) for 10 minutes and then stained with the appropriate dilution of the antibody and incubated for 30 minutes at 4°C in the dark. The antibodies used were monoclonal antibodies (mAbs) specific for HLA-A24, A2 and HLA-B61 as well as the lineage marker mAbs specific for CD11b in granulocytes (Gs), CD33 in monocytes (Ms), CD19 in B cells (Bs), CD3 in T cells (Ts), and NKp46 in natural killer (NK) cells. After staining, cells were washed in PBS and resuspended in FACS buffer and analyzed in a FACSCanto II instrument (Becton Dickinson, Franklin Lakes, NJ, USA) and obtained data were further analyzed with the FlowJo software package, version 10.0.7 (Treestar, Ashland, OR, USA).

## Phenotype analysis of iPSCs and differentiated iPSCs

iPSCs and differentiated iPSCs were collected using 0.05% trypsin and dissociated into a single cell suspension. Cell clumps were removed by filtering the samples through 40-mm cell strainers. The cells were then washed with PBS and resuspended in 100 μl of FACS buffer at a concentration of $10^6$ cells/ml. Cells were treated with 1% BSA in PBS and stained with various mAbs directed against iPSC and hematopoietic cell markers. The monoclonal antibodies used for this study are provided in **(Table S3)**. The appropriate isotype IgGs (BD or BioLegend) served as controls.

## Table S3. Monoclonal antibodies were used for this study

| Antigen | Isotype | Fluorescein | Source |
|---|---|---|---|
| **CD3** | IgG1 | PerCP-Cy5.5 | BD Biosciences |
| **CD11b** | IgG1 | PE | BD Biosciences |
| **CD11b** | IgG1 | APC | Beckman Coulter |
| **CD19** | IgG1 | APC-Cy7 | Beckman Coulter |
| **CD33** | IgG1 | APC | Beckman Coulter |
| **CD34** | IgG1 | FITC | BD Biosciences |
| **CD34** | IgG1 | PE | BD Biosciences |
| **CD55** | IgG2a | FITC | BD Biosciences |
| **CD55** | IgG2a | PE | BD Biosciences |
| **CD59** | IgG2a | FITC | BD Biosciences |
| **CD59** | IgG2a | PE | BD Biosciences |
| **FLAER** | - | Alex Fluor 488 | Pinewood Scientific Services |
| **HLA-ABC** | IgG1 | FITC | BD Biosciences |
| **HLA-A2** | IgG2a | FITC | One Lambda |
| **HLA-A9 (A24)** | IgG2b | FITC | One Lambda |
| **HLA-B61** | Human/mouse Chimeric IgG1 with constant Domain of mouse IgG1 | None & PE | Prepared in house[1] |
| **HLA-DR** | IgG2a | FITC | BD Biosciences |
| **HLA-E** | IgG1 | PE | eBiosciences |
| **Mouse Ig** | Goat polyclonal Ig | BV421 | BD Horizon |
| **Streptavidin** | - | PE | BD Biosciences |
| **SSEA-4** | IgG3 | PE | BioLegend |

**Abbreviations:** APC, Allophycocyanin; APC-Cy7, Allophycocyanin-Cy7 Tandem; FITC, fluorescein isothiocyanate; FLAER, Fluorescein-labeled proaerolysin; Ig, immunoglobulin; PerCP---Cy5.5, peridinin-chlorophyll proteins-Cy5.5 tandem; PE, phycoerythirin and BV421, BD Horizon Brilliant Violet 421

**HLA genotype of iPSCs**

PCR was performed in a 20 μl mixture containing 10 μM HotstarTaq Master Mix, 1 μM of HLA primer 1-1(HLA-A2-A31-A33) or primer 2-2 (HLA-A1, A11, A24) and 2 mM of DNA with the following PCR program: initial activation at 95 °C for 5 min denaturing at 94 °C for 15s, annealing at 65 °C for 15 s, extension at 72 °C for 30s, final extension at 72°C for 7 min, 35 cycles using the primer sets listed in **(Table S4)**. PCR products were analyzed by 2 % agarose gel electrophoresis, and visualized through ethidium bromide staining, the images were captured with FAS-III + imaging system (TOYOBO, Kita-ku, Osaka, Japan). HLA was genotyped using the TaqMan-Allelic discrimination method in a StepOne Plus instrument (Applied Biosystems, Foster City, CA, USA) and results were analyzed using allelic discrimination software (Applied Biosystems). The genotyping assay was conducted in 96-well PCR plates. The amplification reaction contained template DNA, TaqMan universal master mix and a specific probe for HLA to distinguish 6pLOH(-) iPSCs from 6pLOH(+) iPSCs using the primer sets listed in **(Table S5).**

**RT-PCR analysis**

Total RNA was extracted from iPSCs at day 7 of culture and iCD34$^{+}$ cells at day 21 of differentiation using the RNeasy kit (Qiagen, Hilden, Germany) and cDNA was prepared using the high capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA, USA) and was used as template in PCR amplification reactions performed with the HotstarTaq plus master mix (Qiagen). The expressions of HoxA9, CD45, CD41, and OCT3 were detected using the primer sets listed in **(Table S6)** using β-Actin as a loading control. RT-PCR was performed in a 20 μl mixture containing 10× RT buffer 2 μl, 0.8 μl of 25× dNTP mix, 2 μl of 10× RT Random, 1 μl of Multiscribe, 1 μl of RNase inhibitor and 10 μl of RNA. Four-step cycling was used for cDNA synthesis: Stage 1 for 5 min at 25 °C, one cycle of 25°C for 5 min, 37°C for 120 min (Stage 2) and 85°C at 5 min for Stage 3, Stage 4 for 4ºC. PCR amplification (initial denaturation for 4 min at 94°C, 35 cycles of 94°C for 15 s, 65°C for Oct3/4, 56 °C for hCD45 and 58°C for HoxA9 , CD41 and β-Actin, 72°C for 1 min, and then followed by 72°C for 10 min). PCR products were analyzed by 2 % agarose gel electrophoresis, and visualized through ethidium bromide staining. The expression of β-actin was used as a loading control. PCR products were analyzed by 2 % agarose gel electrophoresis and visualized with ethidium bromide staining.

**Detection of HLA allele mutations**

To identify the somatic mutations in 6pLOH(+) iPS clones and *B*40:02$^{mut}$* clone, we performed target sequencing of HLA-B using a next-generation sequencer (NGS, MiSeq; Illumina, San Diego, USA). B4002(+) granulocytes, B4002(+)T cells, or both from the KANA1 patient and his B4002(+)iPSCs were used as controls. HLA genes were enriched from genomic DNA using sequence capture (SeqCap EZ system; Roche Sequencing, Pleasanton, USA), a hybridization-based gene enrichment method. Potential mutations responsible for the missing of *HLA-B*40:02* were identified when variant reads were found only in B4002- iPSCs. All mutations were validated using deep sequencing of HLA-B locus-specific long-range PCR, as previously described[5]. HLA-B alleles carrying those mutations were determined using the nearest allele-specific single-nucleotide polymorphisms (SNPs). Reference sequences of HLA-*B*40:02* were obtained from the Immuno Polymorphism Database International ImMunoGeneTics Project HLA (IPD-IGMT/HLA database[6].

**Table S4. Sequences of primers used for PCR**

| Name | Primer | Primer sequence |
|---|---|---|
| 1-1 (A2/31/33) | FW | 5'-GCATATGACTCACCACGCTG |
| | RW | 5'-CTGCACATGGCAGGTGTATC |
| 2-2 (A11/24) | FW | 5'-AAGCCCCAGCTAGAAATGTG |
| | RW | 5'-CTGTGACTTGTGACTGCTGG |
| B1 | FW | 5'-GGAGGTTCCTCTAAGATCTCATGG |
| | RW | 5'-CAGGAGACGTGGGACAGGAG |
| B2 | FW | 5'-GGCTCTGACCAGGTCCTGTTT |
| | RW | 5'-GACCCCAAGAATCTCACCTTTTC |
| C3 | FW | 5'-GACGTTTCGAATGTGTGGTGA |
| | RW | 5'-GAACAAATTCAGGTCAGTCATGGT |
| GAPDH | FW | 5'-TGCACCACCAACTGCTTAGC |
| | RW | 5'-GGCATGGACTGTGGTCATGAG |

**Table S5. Sequences of probes used for TaqMan PCR**

| Name | Primer | Primer sequence |
|---|---|---|
| B1 | TaqMan-MGB-probe-6FAM | 5'-CTCACAGGGCATTTT |
| | TaqMan-MGB-probe | 5'-CCTCACAGGACATTT |
| B2 | TaqMan-MGB-probe-6FAM | 5'-CTCCAGGCAGCGAC |
| | TaqMan-MGB-probe | 5'-CTCCAGCCAGCGAC |
| C3 | TaqMan-MGB-probe-6FAM | 5'-TCAGAGTGTGATCACTT |
| | TaqMan-MGB-probe | 5'-TCAGAGTGTCATCACTT |
| A31/33 | TaqMan-MGB-probe-6FAM | 5'-ATGATCCAAGAAATC |
| | TaqMan-MGB-probe | 5'-CTGATGCCTGAGGTC |

**Table S6. Primers used for RT-PCR**

| Gene | Primer | Primer sequence |
|---|---|---|
| **hCD45** | FW | TTCAACTTATACCCTTCGTGTC |
| | RW | CCTGCTTTACTTTGTCCACTTC |
| **HoxA9** | FW | TGCGGGCATTTAAGTCTGTC |
| | RW | TCTACAGTAGCCCAATGGCG |
| **hGATA1** | FW | CTCCCTGTCCCCAATAGTGC |
| | RW | GTCCTTCGGCTGCTCCTGTG |
| **hCD41** | FW | GACTGTGAATGGTCTTCACCTC |
| | RW | ACACGTTGAACCATGCGTGCGA |
| **hOCT3/4-S1165** | FW | GACAGGGGGAGGGGAGGAGCTAGG |
| | RW | CTTCCCTCCAACCAGTTGCCCCAAAC |
| **hβ-actin** | FW | GTGGGGCGCCCCAGGCACCA |
| | RW | CTCCTTAATGTCACGCACGATTTC |

**Reconstitution of immunodeficient mice with AA-iCD34$^+$ cells**

The immunodeficient 57BL/6.Rag2$^{null}$Il2rg$^{null}$NOD-Sirpa (BRGS) mice model of xenotransplantation was established as described previously[7] which lacked T, B, and NK cells were used in the present study. Sublethally irradiated (150 cGy) mice of 5 and 6 weeks of age (young) and mice 4-5 months of age (adult) were used. The animals were bred and housed in pressurized ventilated cages, according to institutional regulations, in a pathogen-free room at Kanazawa University. A total of $1 \times 10^6$ iCD34$^+$ cells were injected intrafemorally into mice. BM injection was performed under full body anesthesia using 3% isoflurane. Mouse fur was removed from the knee joint and thigh to expose intact skin. A needle coupled with a 1mL syringe was inserted from the femur into the BM cavity after conformation that needle was inside BM, cells were injected in the BM. After the surgical procedure mice were placed under a heat lamp for 3-5 min to aid recovery from anesthesia and were monitored daily for any signs of discomfort and weekly weighting. At 9, 10, or 12 weeks after transplantation, samples of BM, PB, spleens, and thymuses were harvested from recipient mice and prepared for flow cytometry and immunohistochemical assays. Additional mice injected with saline solution were used as negative controls.

## FCM analysis for BRGS mice reconstituted with iCD34$^+$ cells

For analysis and sorting of human cells in the immunodeficient mice, heparinized BM, PB, spleen and thymus were collected from mice at 9, 10, or 12 weeks after transplantation and single-cell suspensions were prepared. Erythrocytes in PB and spleen were lysed using ammonium chloride solution (0.8 % $NH_4Cl$ with 0.1 mM EDTA) (Stem Cell Technologies) when human lymphoid and myeloid cells were analyzed; the remaining cells were collected and washed with PBS, resuspended and treated in 100 μl of 2% fetal bovine serum (FBS) in PBS (blocking buffer) for 10 minutes and blocked again for 2 hours with a commercial Fc-blocking antibody before staining. The cells were stained with appropriate dilution of antibodies specific for human surface markers as described in (**Table. S7**) as well as anti–mouse CD45 mAbs and incubated for 30 minutes at 4°C in the dark for flow cytometric analysis. Nonviable cells were excluded by 7-aminoactinomycin D (7-AAD) (Thermo Fisher Scientific). Human Glycophorin-A CD45(-) erythroid cells were analyzed using CD235a mAbs. To ensure specificity, control murine BM, PB, spleen and thymus cells were stained with the same cocktail of human antibodies for all the experiments reported in the study and dead cells and nonspecific signals were excluded.
For flow cytometry analysis, at least 100,000 events were collected and analyzed. The percentages of selected cell populations (based on comparison with background staining when isotype-matched antibodies are used) among the total live cells were shown.

**Table S7**. **Human monoclonal antibodies were used for analysis human cells in BRGS mice.**

| Antigen | Fluorescein | Source |
|---|---|---|
| **CD3** | APC | BD Biosciences |
| **CD4** | PE | BD Biosciences |
| **CD8** | APC-Cy7 | BD Biosciences |
| **CD15** | PE | BD Biosciences |
| **CD16** | FITC | BD Biosciences |
| **CD19** | PE-Cy7 | BD Biosciences |
| **CD33** | APC | BD Biosciences |
| **CD34** | PE | BD Biosciences |
| **CD34** | FITC | BD Biosciences |
| **CD38** | PE-Cy7 | BD Biosciences |
| **CD41a** | FITC | BD Biosciences |
| **CD45** | AmCyan | BD Biosciences |
| **CD45RA** | APC | BD Biosciences |
| **CD90** | APC | BioLegend |
| **CD108** | PE | BD Biosciences |
| **CD135** | Alexa Fluor 647 | BD Biosciences |
| **CD184** | Pacific blue | BD Biosciences |
| **CD235a** | PE | BD Biosciences |
| **IgM** | APC | BioLegend |
| **NKp46** | PE | BD Biosciences |

**Immunohistochemistry assay and H&E staining of mouse tissues**

Tissue sections were prepared from transplanted mouse femoral bone marrows and decalcified for 48 h in phosphate-buffered formalin (3.7% formalin, pH 7.4) containing 20% EDTA (Sigma), and then processed for fixed paraffin-embedding. Sections (4-μm) were mounted on slides, deparaffinized in xylene and rehydrated in grades of ethanol according to the standard methods. One set of tissue sections was stained with H&E and the other set was subjected to immunohistochemical staining using anti-human myeloperoxidase. Briefly, deparaffinized sections were incubated in Target Retrieval Solution (Dako Japan, Tokyo, Japan) at 95 °C for 20 min for pretreatment prior to immunostaining for human myeloperoxidase. Endogenous peroxidase was inactivated using methanolic-hydrogen peroxide for 20 min and incubated in normal goat serum (1:10; Vector Lab, Burlingame, CA, USA) for 20 min to block non-specific binding of the primary antibodies. The sections were then incubated at 4°C overnight with a primary mAb against human myeloperoxidase and then Dako Envision-HRP (Dako, Bunkyo-ku, Tokyo, Japan) was used. The antibodies were tested for specificity on murine BM to exclude interspecies cross-reactivity. After the benzidine reaction, the sections were counterstained with hematoxylin.

## Figure S2

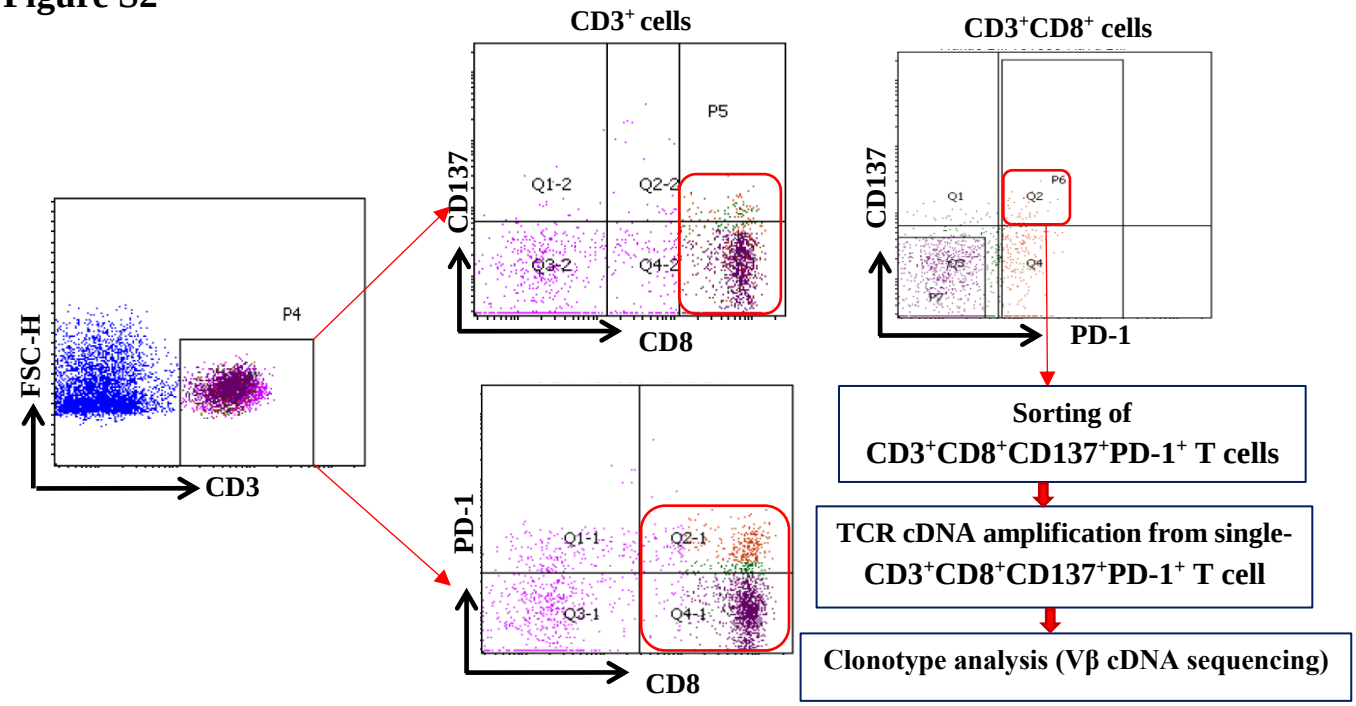


**Figure S2. TCR Vβ repertoire analysis of the bone marrow T cells from KANA1 patient**.
Putative pathogenic T cells were isolated from KANA1 patient BM using anti-CD3, CD8, CD137 and PD-1 antibodies. Thirty-seven $CD3^+CD8^+CD137_+PD\text{-}1^+$ T cells were single-cell sorted and analyzed for TCR Vβ.

# Supplemental results

**Figure S3**

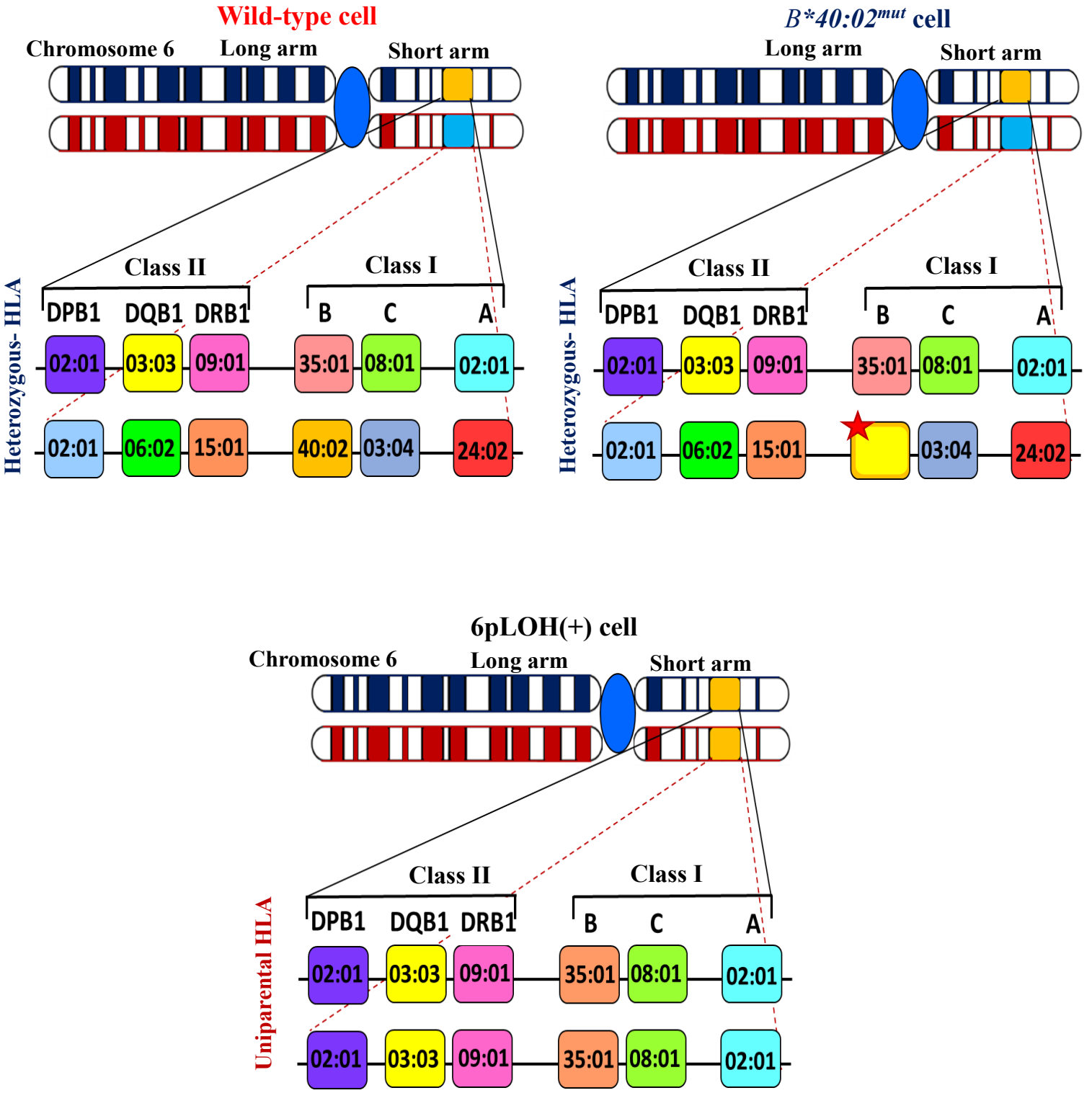


**Figure S3**. A diagram of the HLA complex of patient KANA1, showing the difference between WT cells *B*40:02*$^{mut}$ and 6pLOH(+)cells. A stop codon mutation occurred at the starting site of *B*40:02* indicated as a star.

## Figure S4

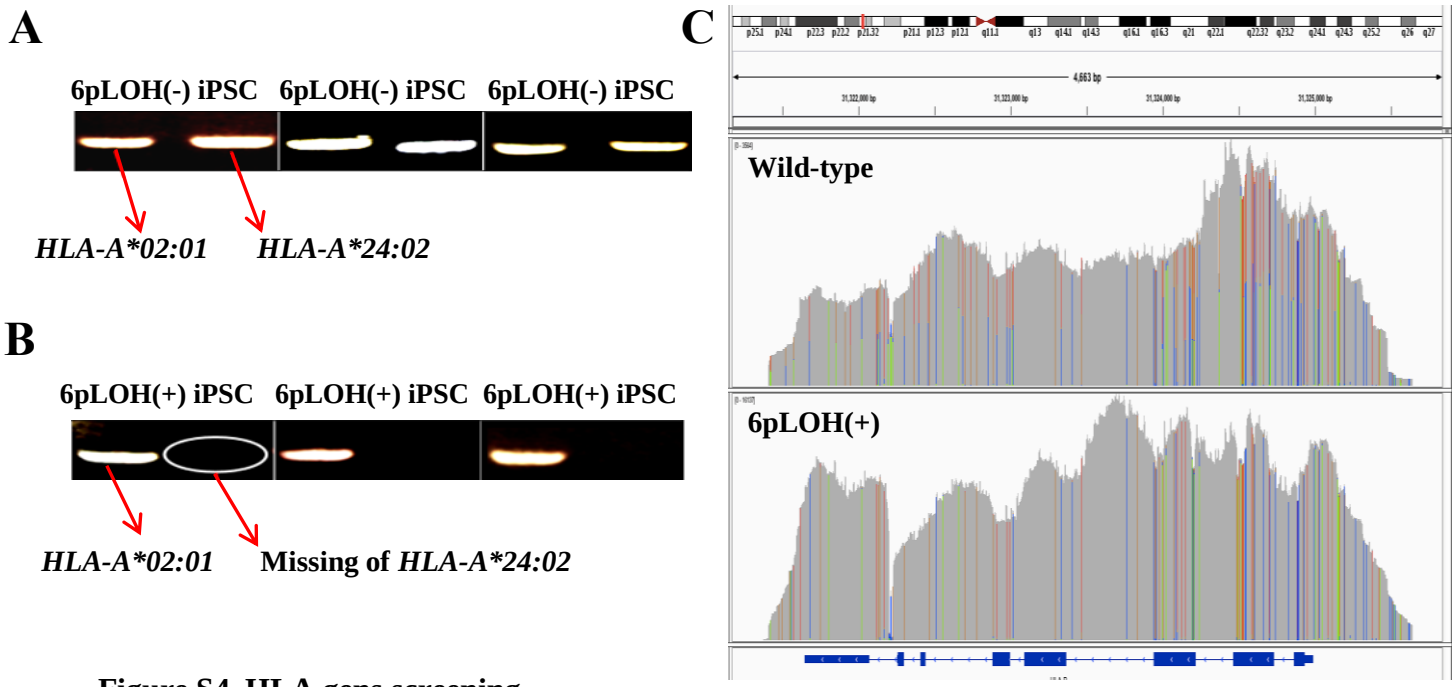


**Figure S4. HLA gens screening**

PCR analysis to detect HAL-A*02:01 and HLA-A*24:02 alleles on DNA from several iPSC lines of KANA-1. The results show the difference in the band pattern between (A) 6pLOH(-) and (B) 6pLOH(+)iPSC clones. (C) Alignment view of next-generation sequencing, the gray color denotes bases identical with the reference genome, and, green, blue, orange, and red colors denote bases different from reference genome. The column that has two colors represents heterozygous SNV that are in WT iPSC clone (E2) graph, while, one color column means homozygous SNV in 6pLOH(+) iPS clone (clone E1). The results of HLA-B allelic sequencing on a WT iPSC clone (E2) is shown as a control.

**Table S8. Characteristic of iPSC lines which were established from KANA1**

| Clone | Marker expression | | | | Clone type |
|---|---|---|---|---|---|
| | Hematopoietic | HLA-A2 | HLA-A24 | HLA-B*40:02 | |
| KANA1-A1 | + | + | - | - | 6pLOH(+) |
| KANA1-A2 | + | + | - | - | 6pLOH(+) |
| KANA1-B1 | + | + | - | - | 6pLOH(+) |
| KANA1-B2 | + | + | - | - | 6pLOH(+) |
| KANA1-C1 | + | + | - | - | 6pLOH(+) |
| KANA1-D2 | + | + | - | - | 6pLOH(+) |
| KANA1-E1 | + | + | - | - | 6pLOH(+) |
| KANA1-E2 | + | + | + | + | Wild-type |
| KANA1-E3 | + | + | + | + | Wild-type |
| KANA1-H1 | + | + | - | - | 6pLOH(+) |
| KANA1-I1 | + | + | - | - | 6pLOH(+) |
| KANA1-G1 | + | + | + | + | Wild -type |
| KANA1-G2 | + | + | + | - | $B*40:02^{mut}$ (Start loss) |
| KANA1-G3 | + | + | - | - | 6pLOH(+) |

## Figure S5

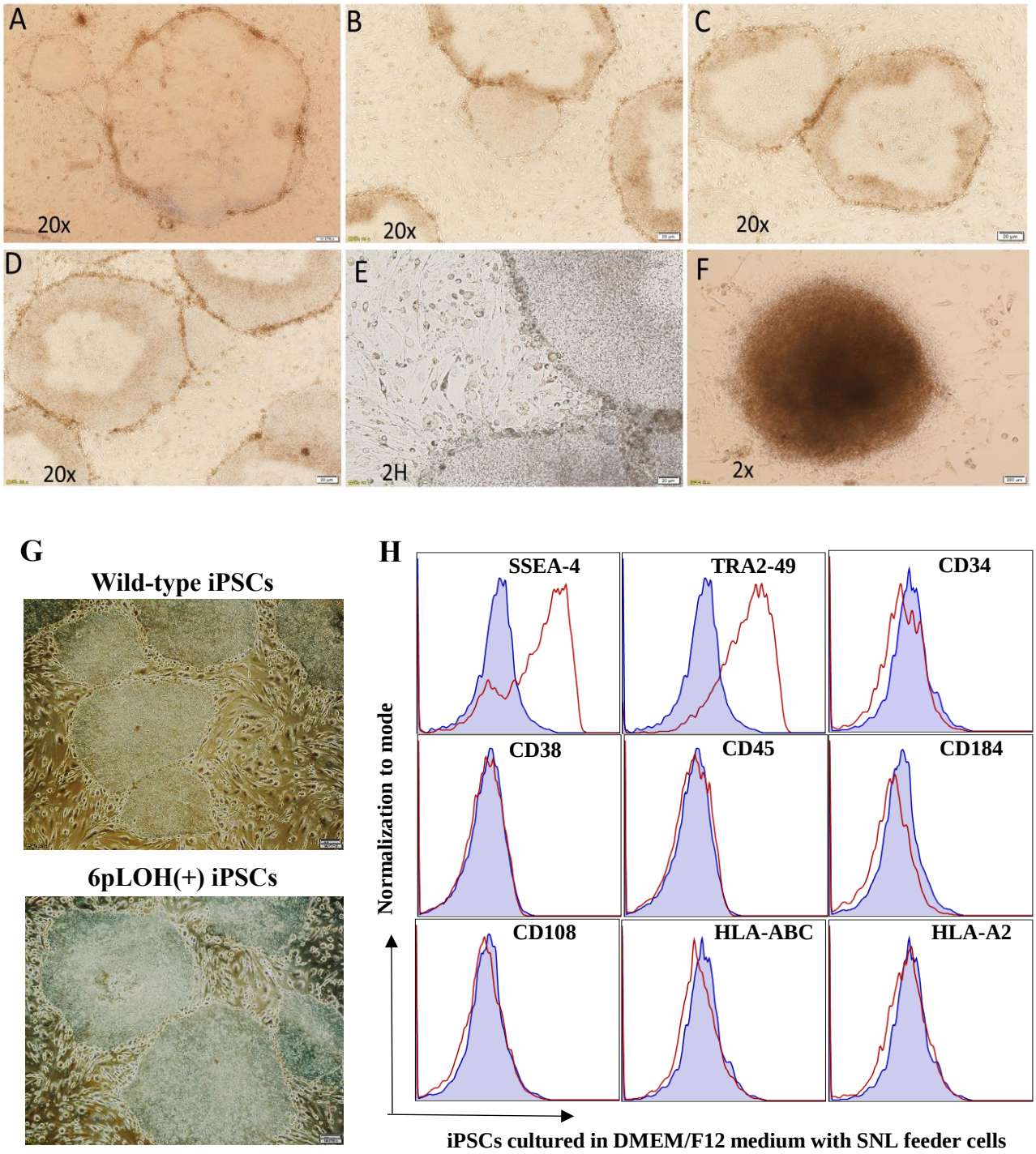


**Figure S5. Morphology of iPSCs**

Representative images of iPS cells (clone KANA1-E2 at passage 7) documented at days 0-3-5-7 of culture (A-D respectively). (E) Image of iPSCs with high magnification. (F) Image of typical iPSC colony, Bars = 20 µm (A-E), Bar= 200 (G) Comparative morphology of WT iPSCs, clone KANA1-E3, (Upper panel) and 6pLOH(+) iPSCs, clone KANA1-G3(Lower panel). Bars = 100 µm. 20X. (H) Representative histograms of iPSCs showing positive expression of iPSCs markers (SSEA-4 & TRA-49) and negative expression for hematopoietic marker and HLA(Blue histograms, isotype; Red, iPSCs).

## Figure S6

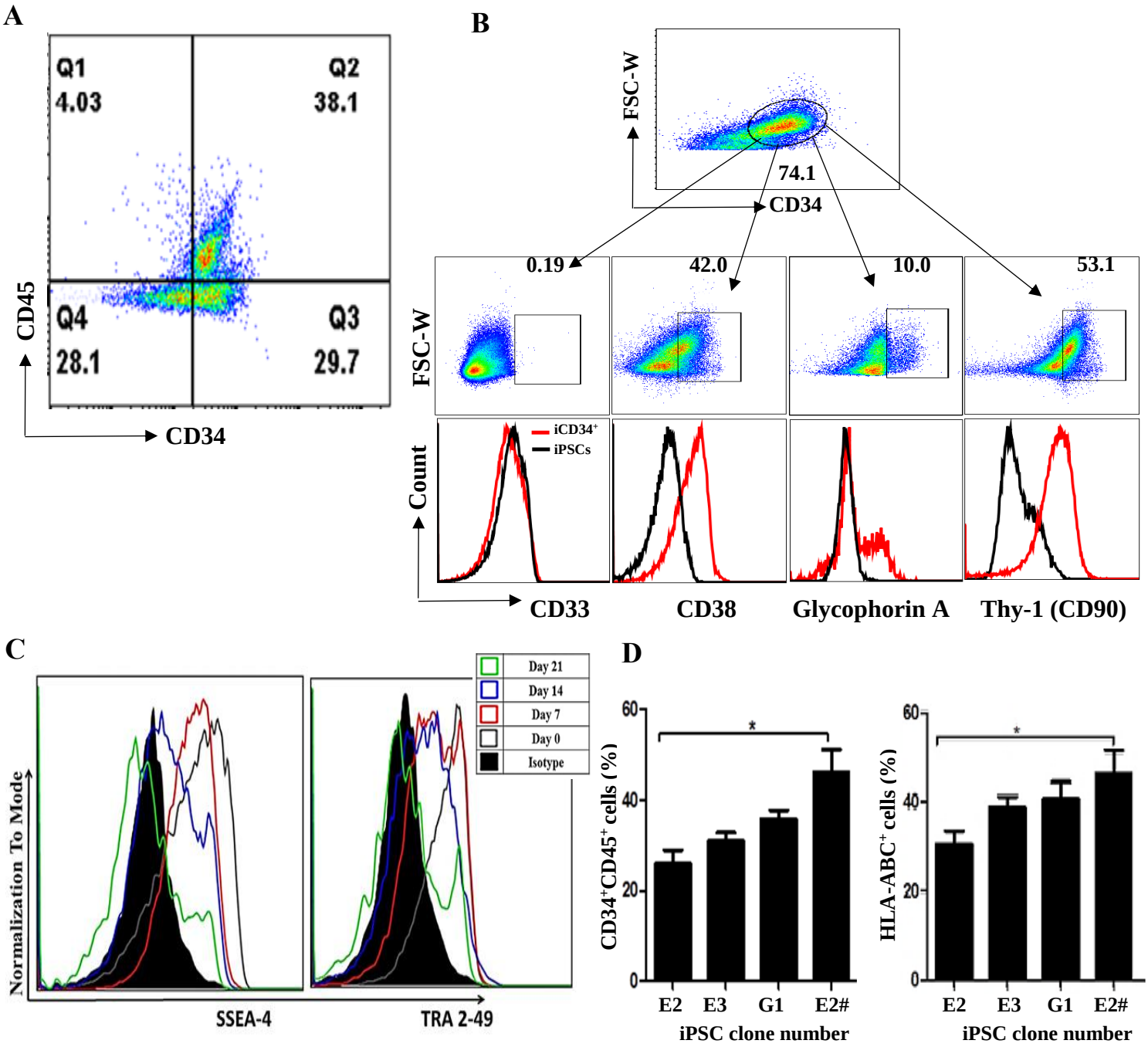


**Figure S6. Differentiation of iPSCs into hematopoietic stem cells in the presence of OP9 feeder.** (A) The percentage of $CD34^+$ & $CD34^+CD45^+$cells in iPSCs (G1 clone) cultured with OP9 feeder cells in StemPro culture medium and analyzed at day 21. (B) The $iCD34^+$ cells expressed CD38, glycohorin-A, and Thy-1 and negative for the markers of parental cells (CD33). (C) Kinetic analysis of immature markers expression on differentiating iPSCs (G1 clone). As iPSCs differentiate into $iCD34^+$ cells, they progressively lose iPSC markers (SSEA-4 & TRA-49). (D) Comparison of hematopoietic markers expression and HLA of $iCD34^+$ cells in the same or different passage number (clone E2 after 11 passages and E2# the same clone after 15 passages) showing variations iPSCs.

## Figure S7

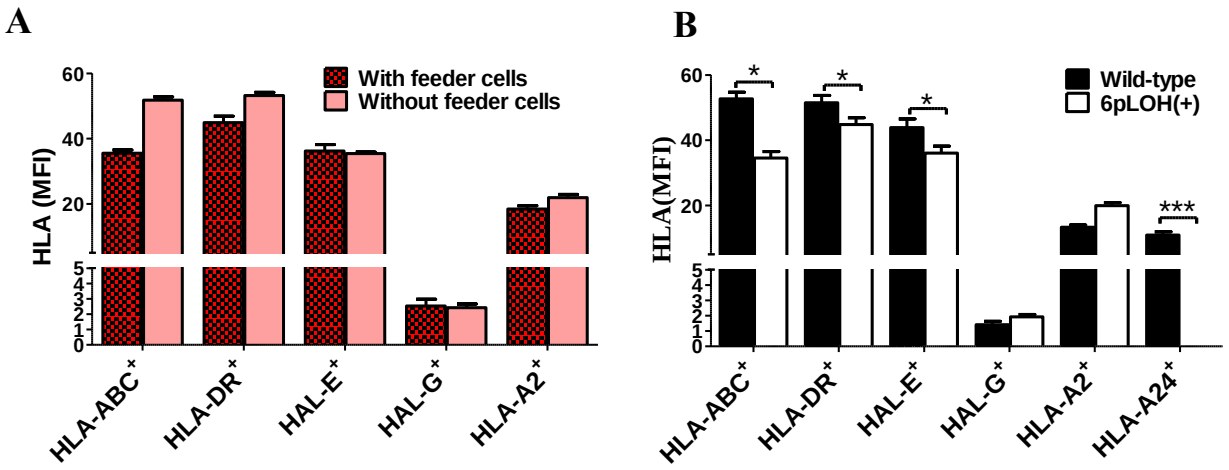


**Figure S7. HLA expression level (mean florescence intensity, MFI) in iCD34$^+$ cells.**
(A) Impact of type of culture on HLA expression profiles of 6pLOH(+)iCD34$^+$ cells cultured with feeders or without feeder cells. (B) HLA expression levels in WT and 6pLOH(+) hematopoietic cells cultured without feeder cells. Error bars represent the means ± SEM; *P <0.05, **P <0.01)

## Figure S8

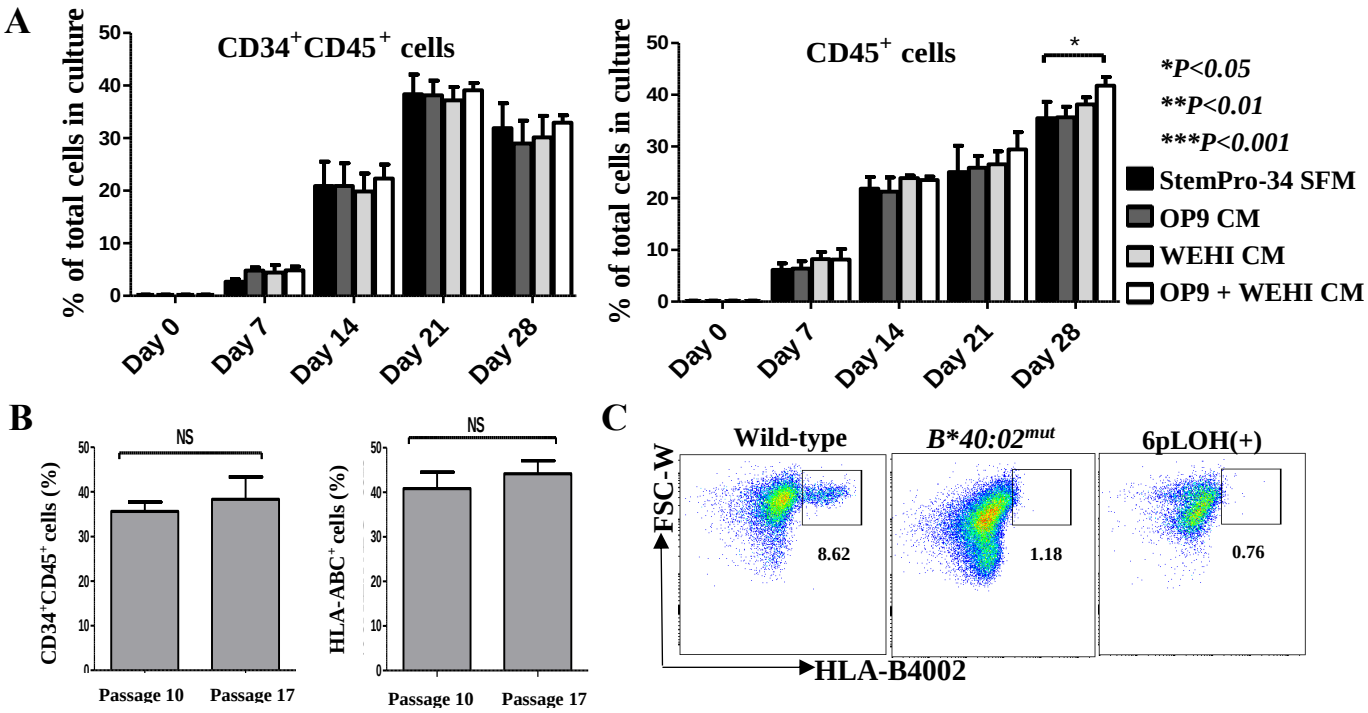


**Figure S8. Effect of culture and iPSCs passage number on derived iCD34$^+$ cells.** (A) The expression of cell surface markers on iPSCs cultured under the four differentiation methods. (B) The percentage of CD34$^+$ CD45$^+$ (left panel) and HLA expression in iCD34$^+$ cells derived from iPS cells (clone G1) used at two different passage number (passage 10 and 17). (C) The HLA-*B*40:02* expression in iCD34$^+$ cells derived from WT, *B*40:02*$^{mut}$ and 6pLOH (+) clones after their culture in MethoCult™ system.

## Figure S9

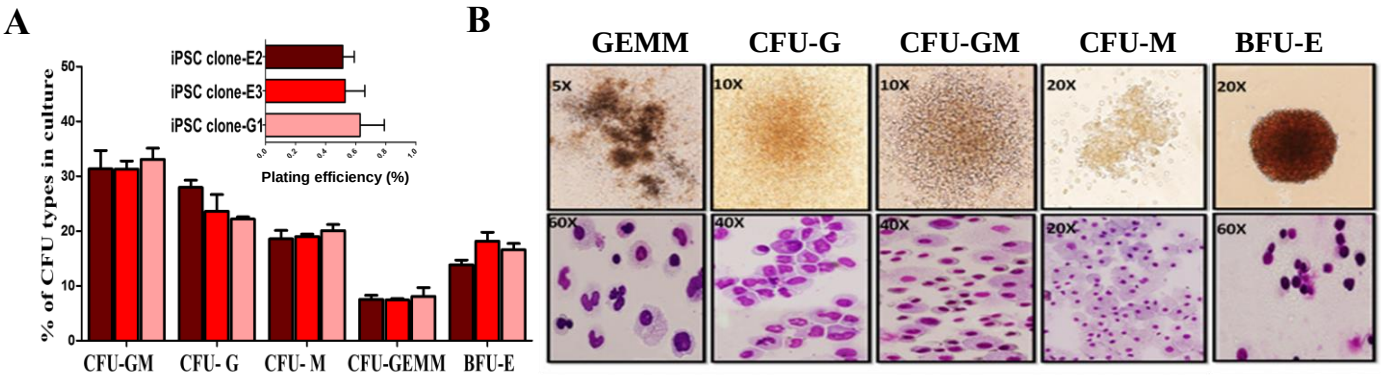


**Figure S9. Clonogenic capacity of wild-type iCD34$^+$cells.** (A) Comparison of CFU-inducing capacity from three WT iPSC clone. The data indicate the mean ± SEM of the % of CFUs obtained from 3 independent experiments. (B) The morphology of cells derived from different colonies was identified with May-Grünwald Giemsa staining.

## Figure S10

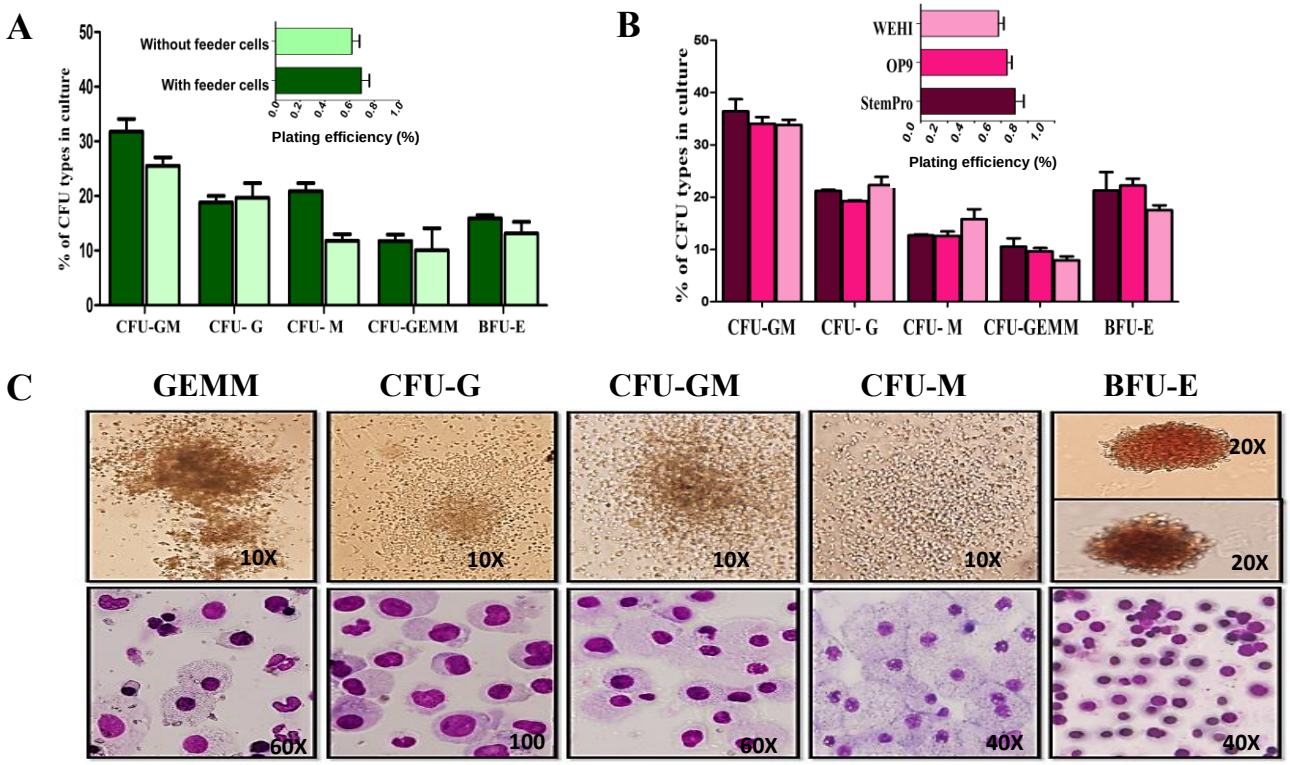


**Figure S10. Clonogenic capacity of iCD34$^+$ cells generated in feeder and feeder-free systems.** Different colony forming units (CFU) generated from iCD34$^+$ cells cultured with feeder or feeder cells (A) or with three different feeder-free systems with StemPro, conditioning media from OP9 cells or WEHI cells (B) as determined at 14 days of culture.
The percentage of colonies from 5,000 iCD34$^+$ seeded cells is shown. The data represent the mean ± SEM of duplicate cultures from three independent experiments. (C) Representative images of different CFU derived from iCD34$^+$ WT cells cultured in the presence of OP9 cells.

**Figure S11**

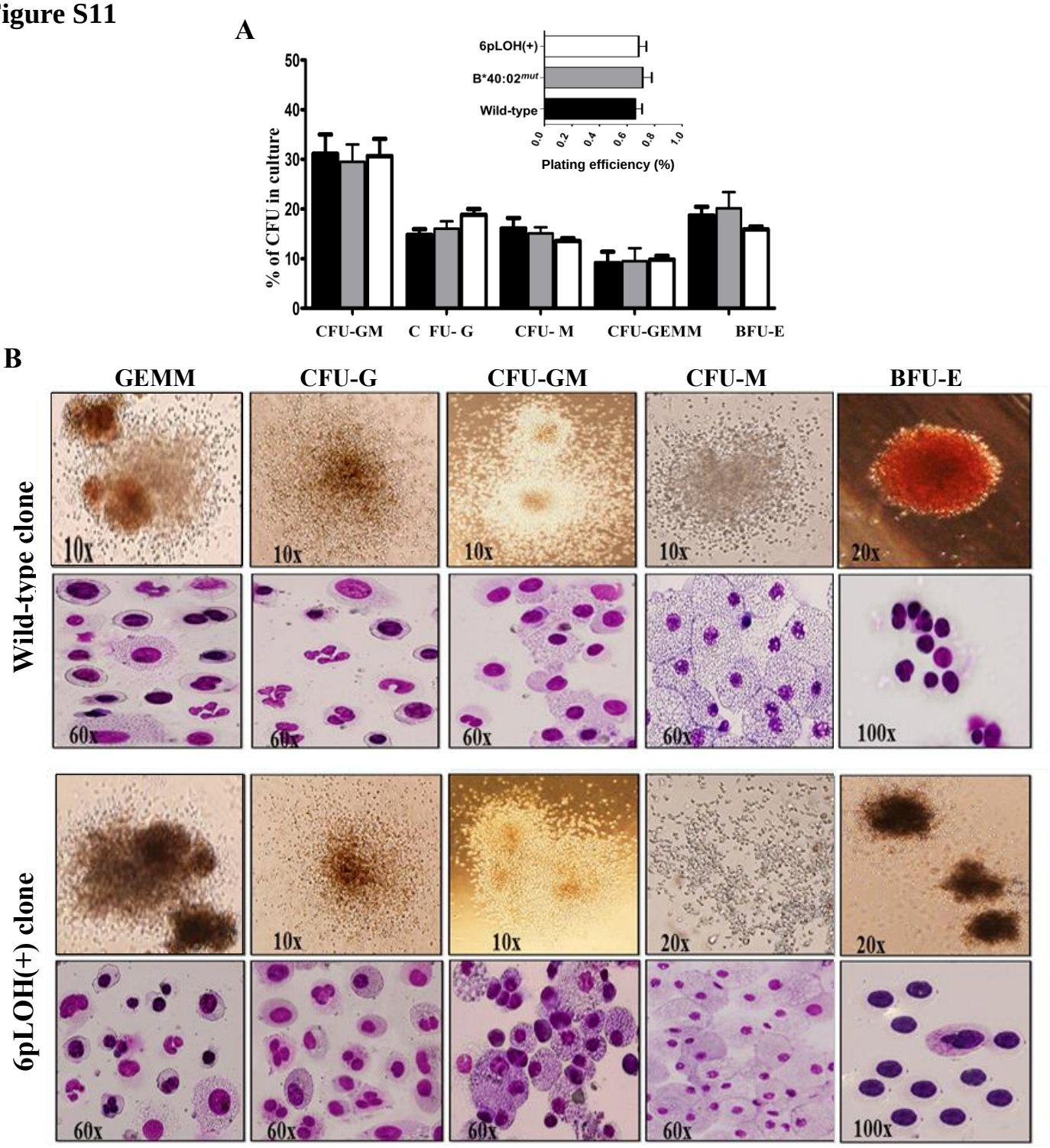


**Figure S11. Comparison of clonogenic capacities of WT and HLA-lacking iCD34$^+$ cells.**
(A) The percentage of CFUs from iCD34$^+$cells derived from iPSCs with three different HLA genotypes (WT, *B*40:02$^{mut}$* and 6pLOH (+)). The data indicate the mean ± SEM of the % of CFUs obtained from 3 independent experiments. (B) Representative CFU-GEMM, CFU-G, CFU-GM, CFU-M, and BFU-E-derived colonies from the WT-iCD34$^+$ cells (Upper panels) and 6pLOH(+) iCD34$^+$ cells (Lower panels). Below the corresponding CFU figures single cell images of cytospin preparations are shown. The depicted images were generated from May-Grünwald Giemsa staining.

## Figure S12

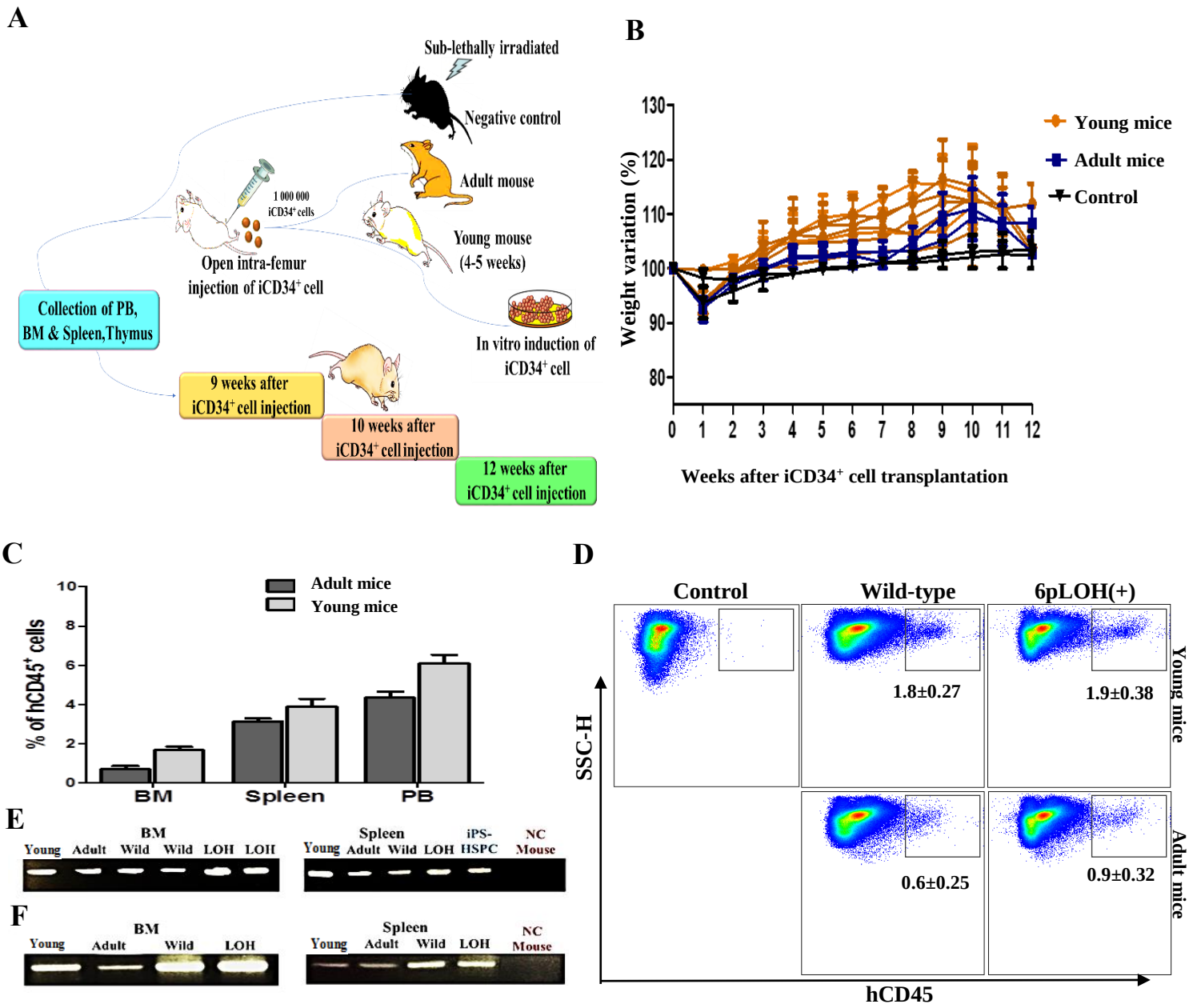


**Figure S12. Engraftment of WT and 6pLOH(+) iCD34$^+$ cells in BRGS mice.**
(A) Outline of transplantation model using iCD34$^+$ cells and stepwise of analysis of the HSPC engraftment. (B) Average weekly body weight of young and adult mice after iCD34$^+$ cell transplantation. (C) The percentages of human CD45$^+$cells in the BM, spleen, and PB of recipient young (n=2) and adult (n=2) mice, 12 weeks post-transplantation with WT or 6pLOH(+) iCD34$^+$ cells. (D) Representative FAC analysis of BM cells from transplanted mice showing the percentages of human cells (hCD45$^+$). (E and F) PCR amplification of human chromosome (E) and human CD45 gene (F) in the indicated organs derived from transplanted or control mice.

## Figure S13

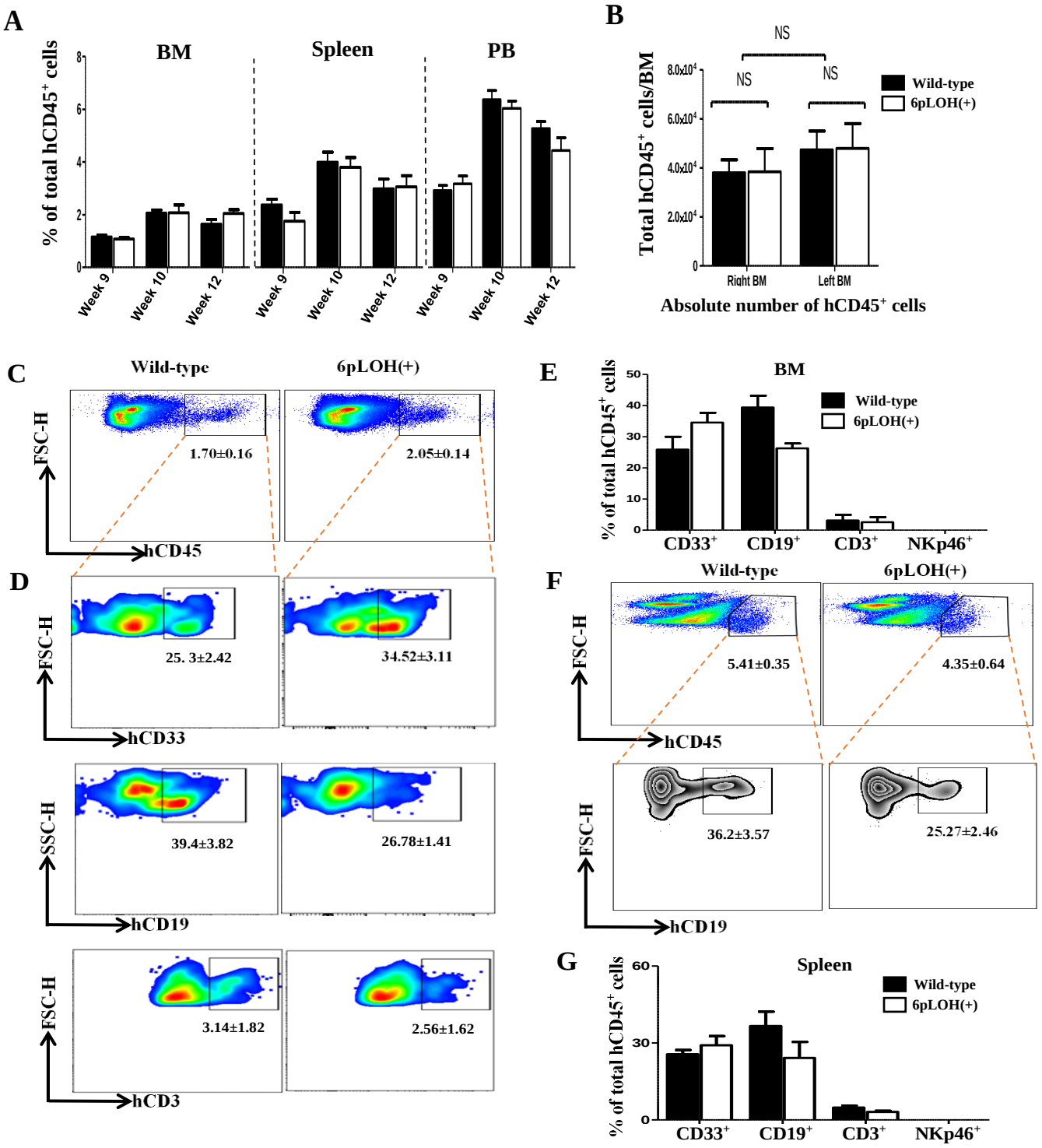


**Figure S13. Detection of human CD45$^+$ cells in the BM and spleen of young BRGS mice post-transplantation with iCD34$^+$ cells.** (A)The percentage of hCD45$^+$ cells over time in the indicated organs of the mice transplanted with WT and 6pLOH (+) iCD34$^+$ cells at 9, 10, and 12 weeks post-transplantation. Data show mean ± SEM of 3 WT and 3 6pLOH(+) cell recipients. Additional 3 mice were used as negative control. (B) The percentage of hCD45$^+$cells in bilateral femurs. (C-G) Human myeloid and lymphoid lineage cells with WT or 6pLOH(+) in the BM (C-E), and in the spleen (F and G).

## Figure S14

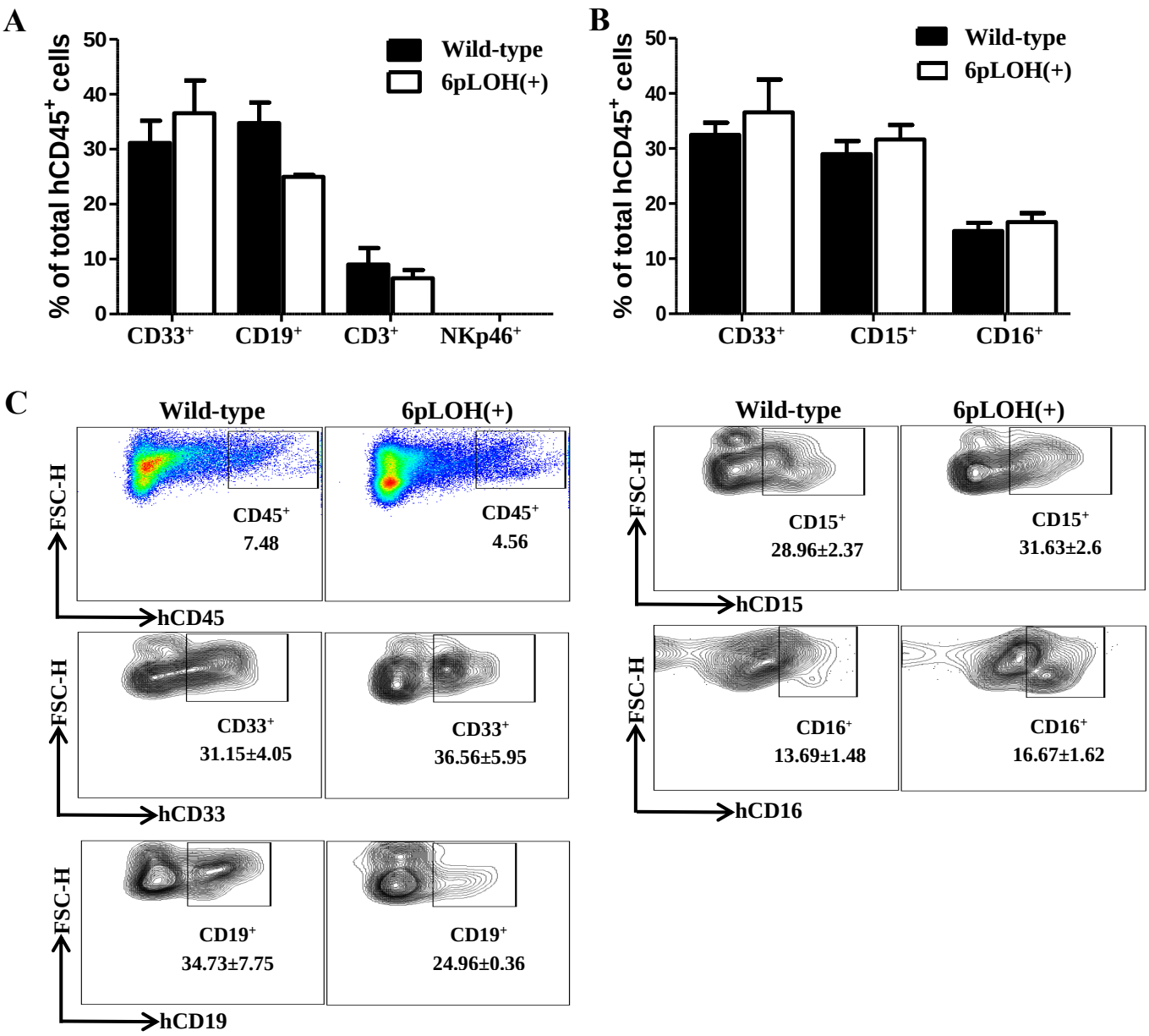


**Figure S14. Detection of human CD45$^+$ cells in the PB of young BRGS mice post-transplantation with WT and 6pLOH(+) iCD34$^+$ cells.**

(A)The percentage of human myeloid and lymphoid lineage cell in PB of the mice transplanted with WT or 6pLOH (+) iCD34$^+$ cells at 10, and 12 weeks post-transplantation. (B) The percentage of hCD33$^+$ cells, hCD15$^+$ cells, and hCD16$^+$cells among hCD45$^+$ cells in PB. The data show the mean ± SEM of 3 wild-type and 3 6pLOH(+) recipient mice. Additional 3 mice were used as a negative control. (C) Representative FCM analysis of human myeloid and lymphoid lineage cells in PB of the recipient mice.

## Figure S15

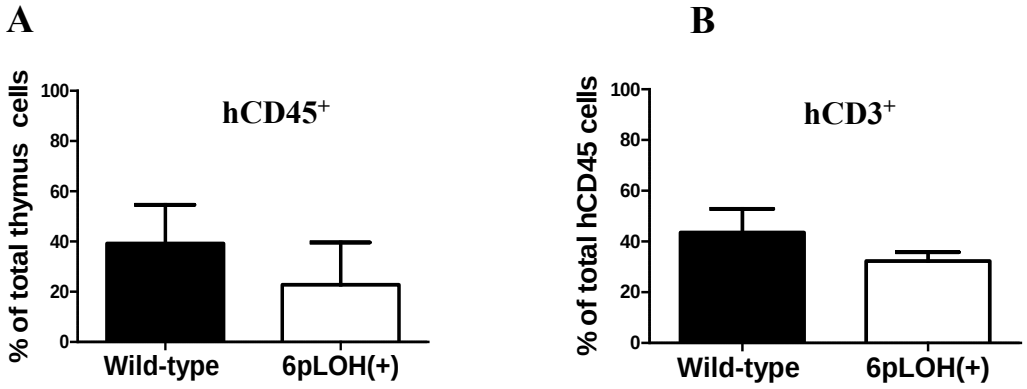


**Figure S15. Detection of human CD45$^+$ cells in the thymus of transplanted young BRGS mice.**
(A) The percentage of hCD45$^+$cells in the thymus of the mice transplanted with WT and 6pLOH(+) iCD34$^+$ cells at 10 weeks post-transplantation. (B) The percentage of hCD3$^+$cells within the hCD45$^+$ population in the thymus of the recipient mice. The data show the mean ± SEM of 3 WT and 3 6pLOH(+) recipients.

## Figure S16

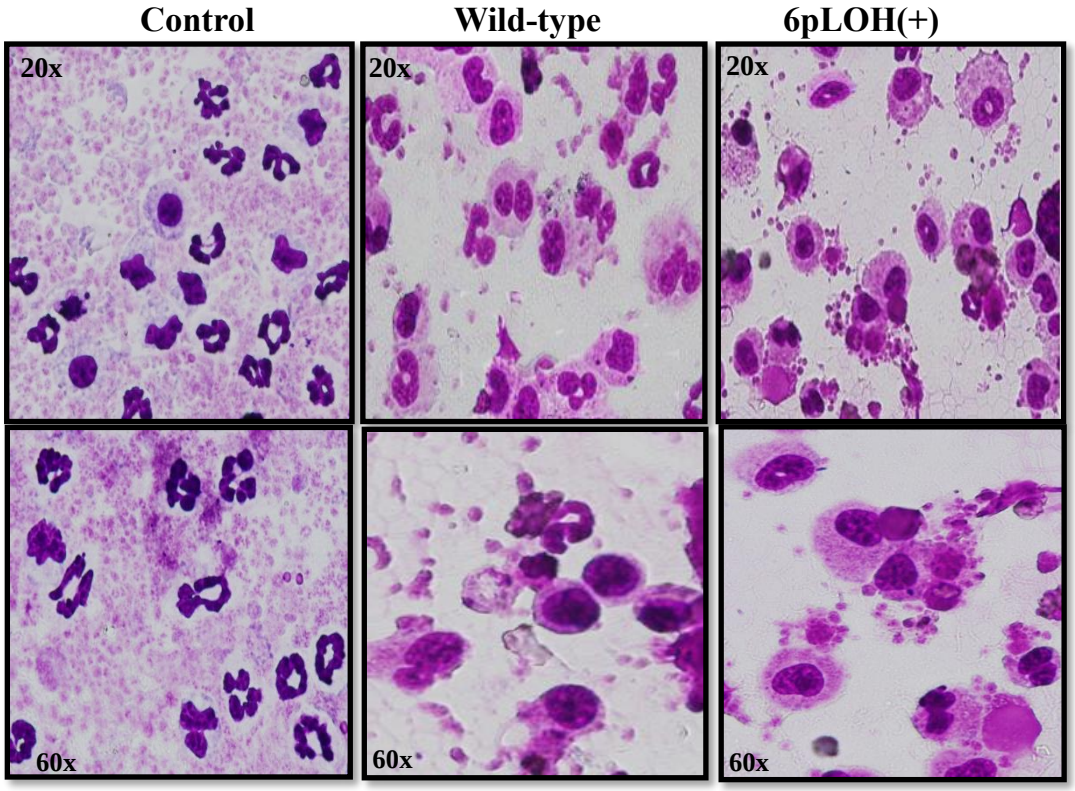


**Figure S16. Morphology of myeloid and lymphoid leukocytes in the PB of transplanted young BRGS mice**
Representative cytospin preparations from PB samples obtained from the mice at 10 week after transplantation with WT or 6pLOH(+) iCD34$^+$ cells and stained with May-Grünwald staining.

## Figure S17

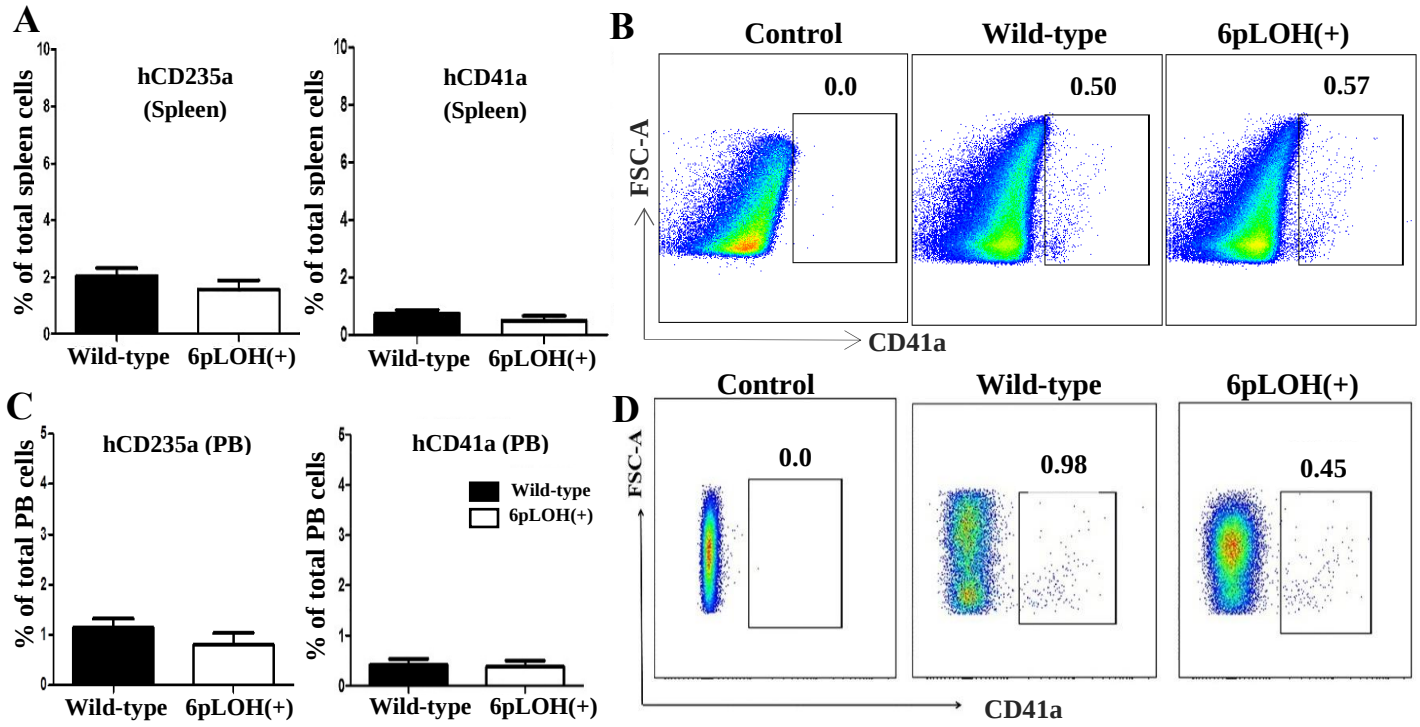


**Figure S17. Terminal differentiation of human erythropoiesis and thrombopoiesis derived from iCD34$^+$ cells in the spleen and PB of transplanted young mice.** (A) The percentages of human erythroid cells (left panel) and platelets (right panel) within the splenocytes of transplanted mice. Data indicate the mean ± SEM of 3 different experiments. (B) Representative FAC analysis showing the CD41a$^+$ cell frequency in the gates corresponding to the platelet fraction in the spleen of transplanted mice. (C) The percentages of human erythroid cells (left panel) and platelets (right panel) in the PB cells of recipient mice 10 weeks post-transplantation. (D) Representative FAC analysis showing the CD41a$^+$ population in the gate corresponding to the platelet fraction of the PB of the transplanted mice.

## Figure S18

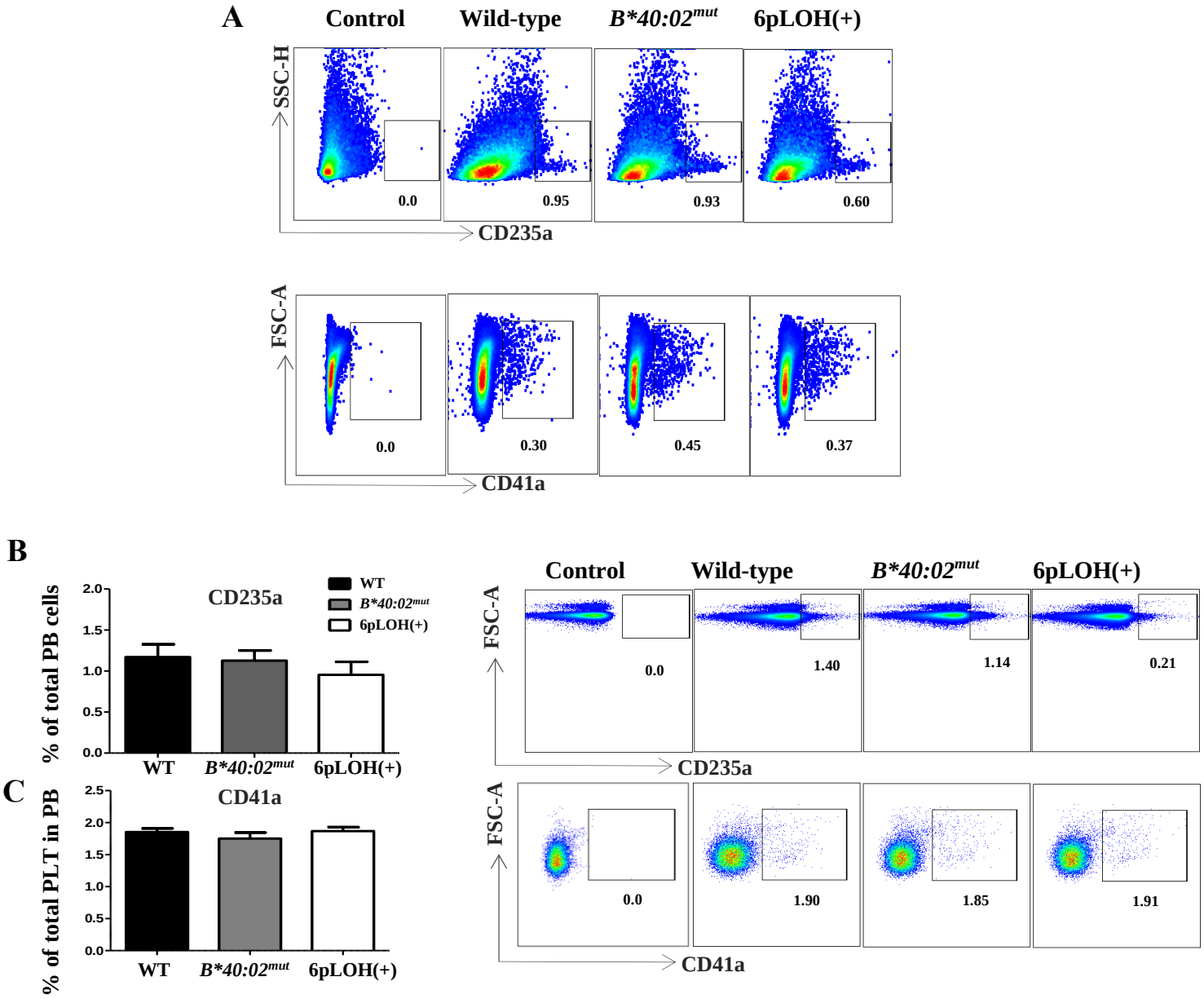


**Figure S18. Terminal differentiation of transplanted iCD34$^+$ cells into human erythroid cells and platelets in the BRGS mice.** (A) A representative set of scattergrams showing the percentage of human erythroid cells (left panel) and platelets (right panel) within the total spleen cells of the mice transplanted with different iCD34$^+$ cells. (B) The percentages of human erythroid cells in the total PB cells (left panel) and a representative set of scattergrams (right panel) showing the presence of human erythrocytes in the recipient mice 10 weeks post transplantation. (C) The percentages of human platelets (left panel) and a representative set of scattergrams (right panel) showing the presence of human platelets in the PB of transplanted mice. Data indicate the mean ± SEM of 3 WT, 2 *B*40:02$^{mut}$* and 3 6pLOH(+) recipients. Additional 4 mice were used as negative control.

## Figure S19

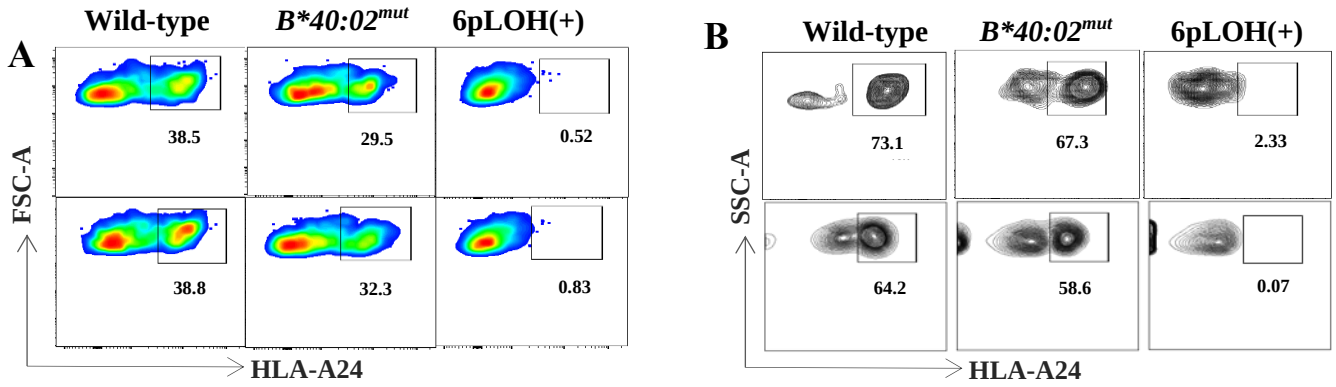


**Figure S19. HLA expression profiles of human lymphoid and myeloid cells isolated from the spleen and PB of the transplanted mice.** (A) HLA-A24:02 expression by $CD19^+$ lymphoid cells in the spleens of mice transplanted with WT, *B*40:02*$^{mut}$ or 6pLOH(+) $iCD34^+$ cells (B) Representative FACs analysis showing the HLA-A24:02 expression by $CD33^+$ cells in the PB of mice transplanted with the indicated $iCD34^+$ cells.

## Figure S20

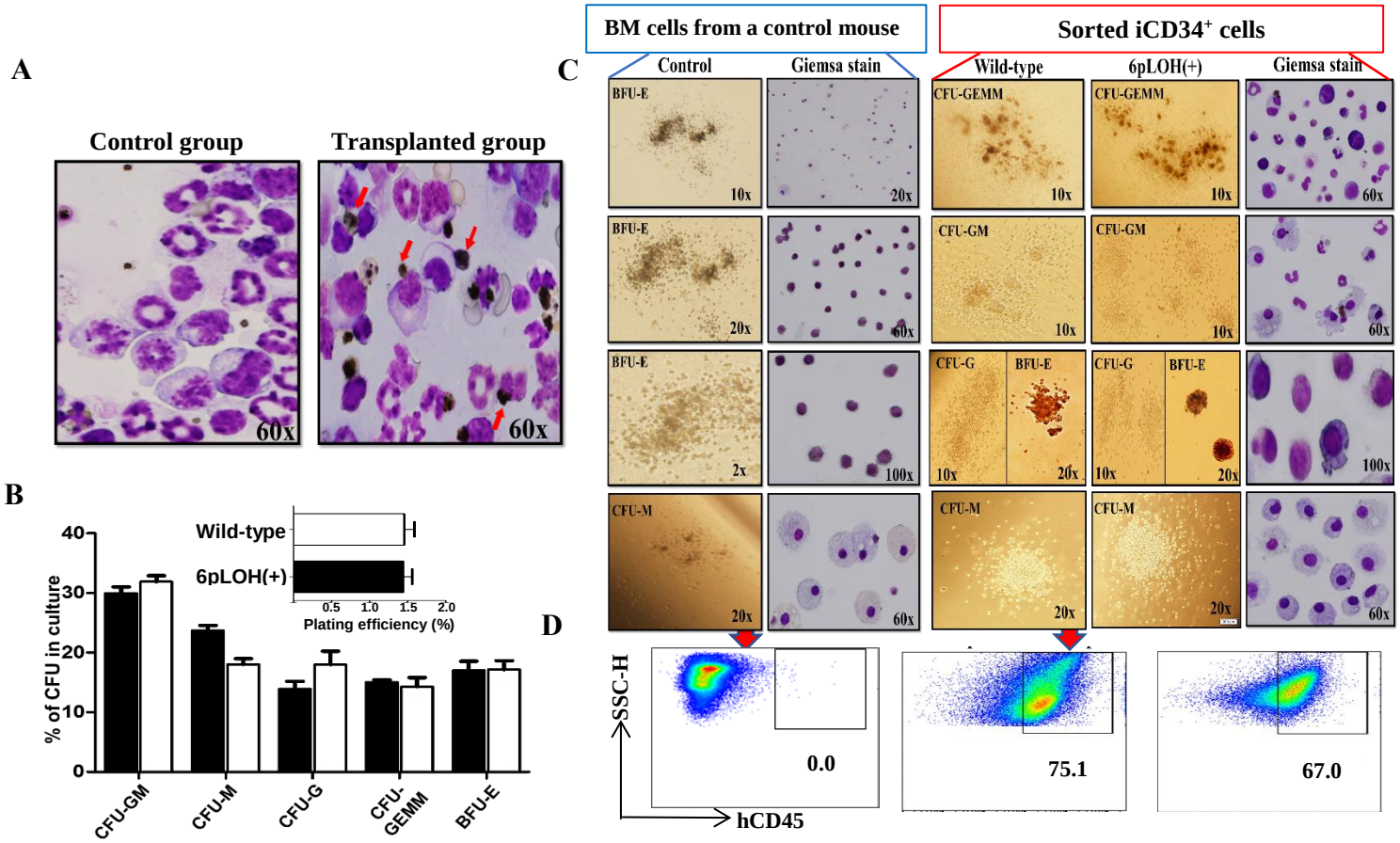


**Figure S20. Hematopoietic colony formation by human iCD34$^+$ cells collected from the BM of transplanted mice.** (A) Representative Giemsa staining of cytospin preparations from non-transplanted control and transplanted mouse BM cells incubated with anti-hCD34 mAb-coated beads. The images show attachment of the microbeads to human iCD34$^+$ cells (red arrow). (B) Clonogenic capabilities of human CD34$^+$cells derived from the BM of the mice engrafted with WT or 6pLOH(+) iCD34$^+$ cells. Data represent the mean ± SEM of percentage of colonies derived from 2x10$^3$ iCD34$^+$ cells and counted after 14 days of culture in MethoCult™ medium. Inserted graph indicate the plating efficiency of WT or 6pLOH(+) human CD34+ cells cultured in MethoCult™ medium (C) Representative images of various types of CFU-derived from the isolated CD34$^+$ cells cultured as described in B visualized directly or harvested and prepared by cytospin and followed by Giemsa staining. Mouse BM cells produced only erythroid and monocyte colonies. (D) Representative FACs analysis of cells collected from colonies formed in the methylcellulose cultures (at day 14), showing the majority of the harvested cells to be human-derived (center panel), which was similar to the cells collected from the culture of parental iCD34$^+$ cells (right panel). The left panel shows a negative control derived from the culture of the mouse BM.

## Figure S21

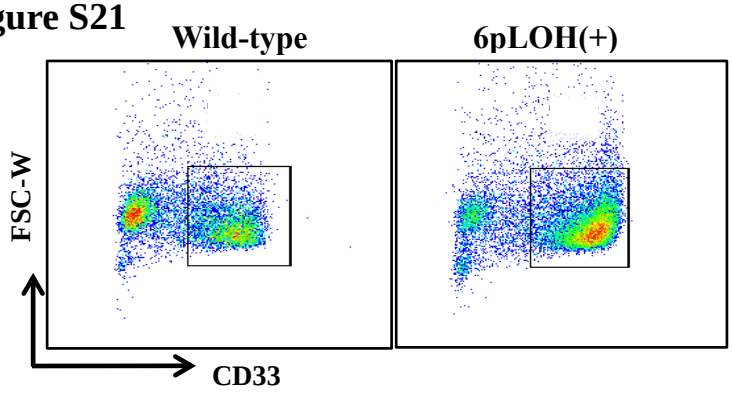


**Figure S21. Human CD33$^+$ cells in the cells collected from the methylcellulose culture of WT or 6pLOH(+) CD34$^+$ cells.** Representative FACS analysis of cells collected from the methylcellulose medium and stained with hCD33$^+$ cells.

## References


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