Escape hematopoiesis by HLA-B5401-lacking hematopoietic stem progenitor cells in men with acquired aplastic anemia

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doi:10.3324/haematol.2018.210856

Supplementary data for the manuscript: "Escape hematopoiesis by HLA-B5401-lacking hematopoietic stem progenitor cells in men with acquired aplastic anemia"

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Patients

AA patients were assessed consecutively from October 2006 to 2018 at Department of Cellular Transplantation Biology (Hematology), Kanazawa University Graduate School of Medical Science to determine the prevalence of HLA allele-lacking leukocytes (HLA-LLs) and glycosylphosphatidylinositol-anchored protein-deficient $(GPI\text{-}AP)^-$ granulocytes using methods as described previously (1). A total of 733 Japanese patients with AA were enrolled in this study. 618 patients that had been studied in our previous works (Katagiri T, et al. Blood 2011 Dec 15;118(25):6601-9, Zaimoku Y, et al. Blood 2017 May 25;129(21):2908-16) were included in this study. All patients were genotyped for HLA-A, HLA-B, HLA-C, and HLA-DRB1 alleles using the polymerase chain reaction (PCR) sequence-specific oligonucleotide method and 6pLOH was detected by GeneChip 500K arrays (Affymetrix), droplet digital polymerase chain reaction (ddPCR) using a QX200 AutoDG Droplet Digital PCR System (Bio-Rad, Hercules, USA) or a next-generation sequencer (NGS, MiSeq; Illumina, San Diego, CA) as previously described(2, 3). HLA-LLs were detected using HLA-A allele specific monoclonal antibodies and FACS Canto II (BD Biosciences, San Jose, USA) when patients' samples were available for flow cytometry. Flow cytometry results were analyzed with the FlowJo v10.1 software program (Tree star, Ashland, USA). All patients provided their informed consent to the HLA-typing and genetic analyses. The study protocols were approved by the ethical committee of Kanazawa University Institute of Medical, Pharmaceutical, and Health Sciences. Severe AA was diagnosed when at least two of the following criteria were met; the neutrophil count was $< 0.5 \times 10^9$/L, the platelet count was $< 20 \times 10^9$/L and the reticulocyte count was $< 20 \times 10^9$/L. Very severe AA was defined as a neutrophil count $< 0.2 \times 10^9$/L in addition to the criteria for severe AA. The response criteria were as previously described(4).

Patient KANA6

A 53-year old man was diagnosed as having non-severe AA. He was found to have 3.49% PNH-type granulocytes and 0.40% PNH-type erythrocytes, as well as HLA-LLs; his monocytes consisted of 21% A24(+)B5401(+) (wild-type), 61% A24(+)B5401(-) , and 16% A24(-)B5401(-) (6pLOH[+]) cells. The patient responded to cyclosporine and metenolone, and achieved complete response. The patient characteristics and the details of the patient's HLA(-) leukocytes are described on **Tables S1 & S2** and **Fig. S1 & S2**.

Table S1. Hematological profile characteristics of the AA patient (KANA6) whose monocytes were used to generate iPS cell in this study

WBC		2.9 x 10^9/L
Differential count of leukocytes	**Neutrophil**	43.0%
	Eosinophil	2.0%
	Monocyte	7.0%
	Lymphocyte	47.0%
RBCs		2.19 x 10^{12}/L
Hemoglobin		7.8 g/dL
MCV		100.9 fL
Reticulocytes		40 x 10^9/L
Platelets		21 x 10^9/L

Figure S1

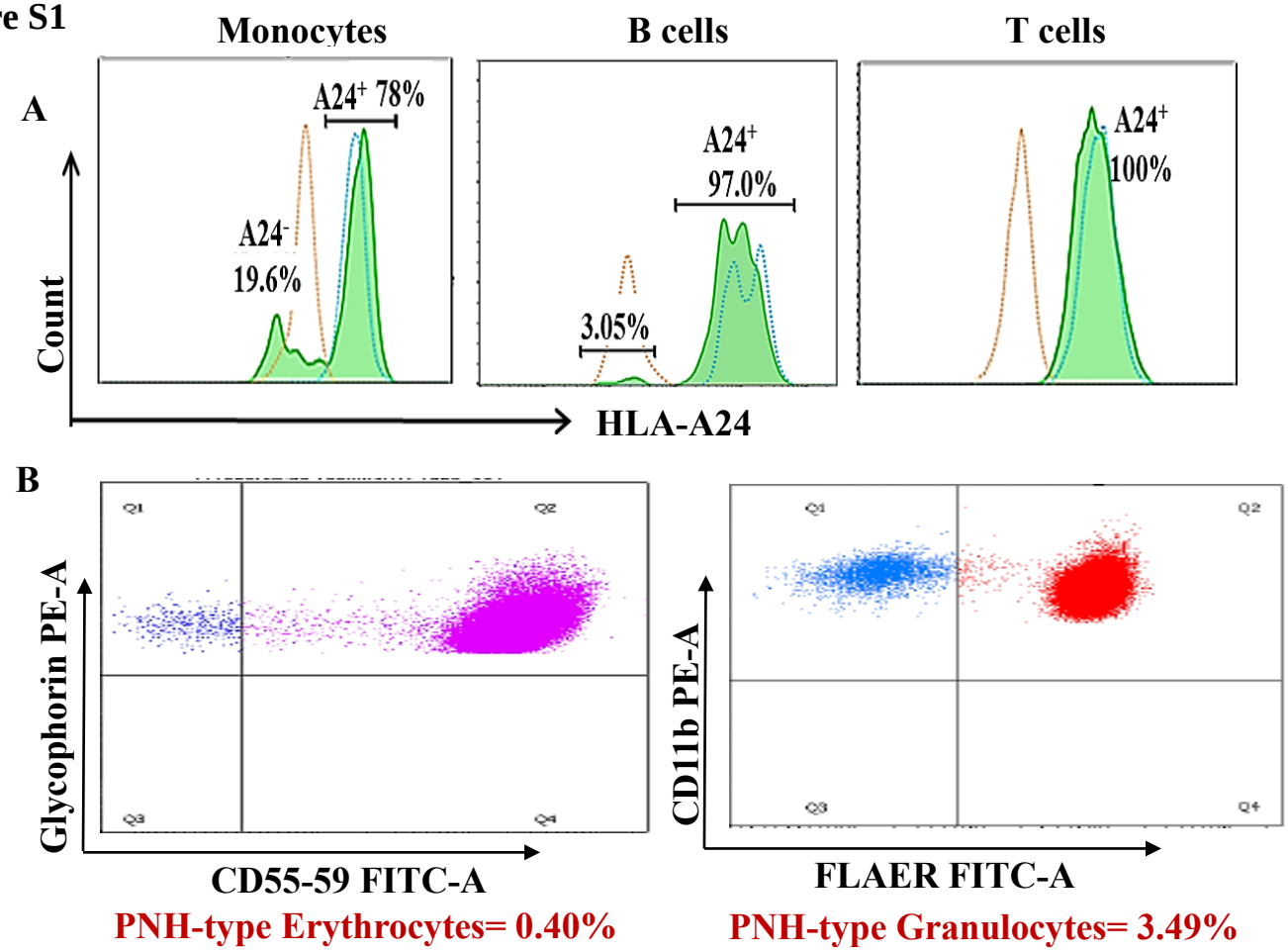

Figure S1. HLA-LLs and PNH-type cells of KANA6.
(A) HLA-A2402-lacking cells in the different lineage of leukocytes from KANA6. Histograms of HLA-A2402-expressing cells in different lineages of the patient (green lines) and a healthy individual (blue lines) are shown. (B) $CD55^-CD59$ $glycophorin^+$erythrocytes (C) Fluorescein-labeled proaerolysin $(FLAER)^-CD11b^+$ granulocytes.

Figure S2

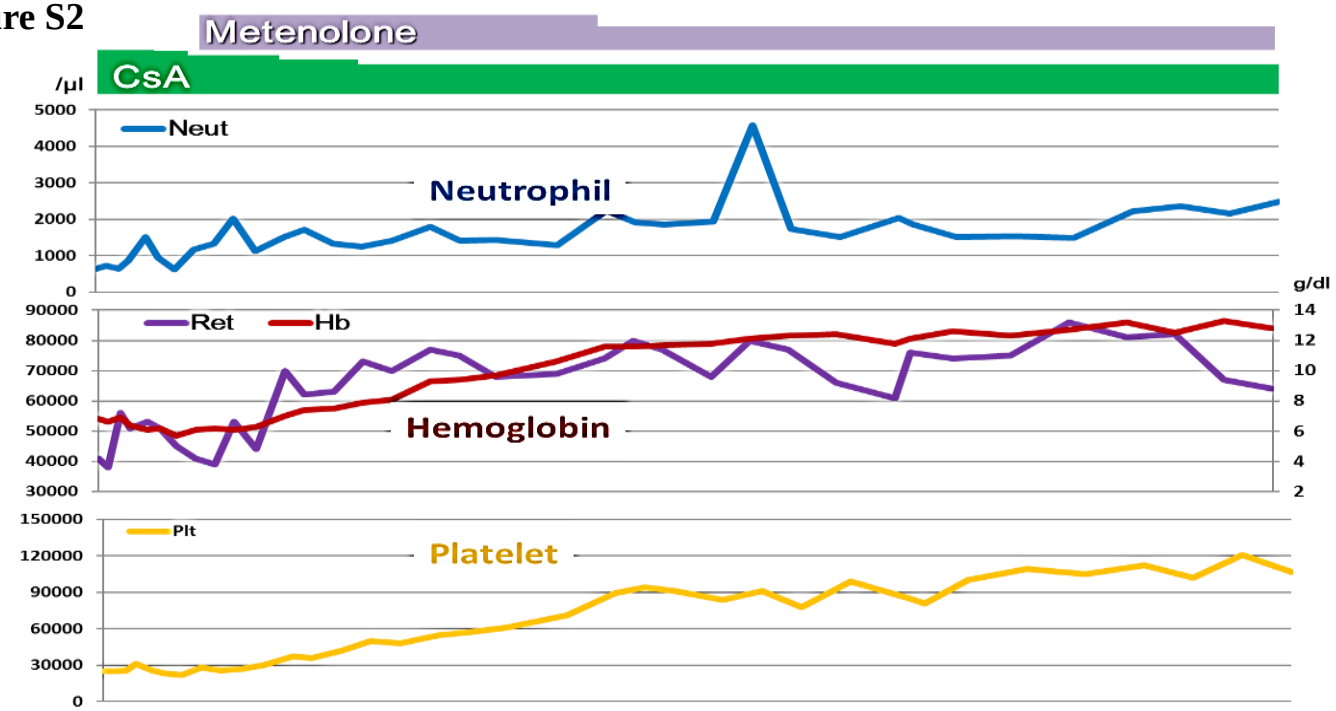

Figure S2. KANA 6's clinical course after metenolone and cyclosporine A (CsA) therapy

Table S2. Characteristics of KANA6

		KANA6
Age (year)		53
Gender		Male
Disease Status		Non-Severe
Months from diagnosis		30
Immunosuppressive therapy (IST)		CsA/anabolic steroid
Response to IST		CR
Missing alleles due to 6pLOH	**HLA-A**	*24:02*
	HLA-B	*54:01*
	HLA-C	*01:02*
	HLA-DRB1	*04:01*
Retained alleles	**HLA-A**	*01:01*
	HLA-B	*37:01*
	HLA-C	*06:02*
	HLA-DRB1	*01:01*
% of HLA-LLs in each cell lineage	**Granulocytes**	25%
	Monocytes	19.6%
	B cells	3.05%
	T cells	0%

CsA, cyclosporine A; CR, complete response; and HLA-lacking leukocytes, HLA-LLs.

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

Peripheral blood was obtained from KANA6 who had 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 modification(5). 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.

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(6) were used in iPSC culture as a feeder cells for feeder induction culture**.** We prepared the conditioned media by collection of supernatant of OP9 cells and WEHI cells culture media as described before(7).

Figure S3

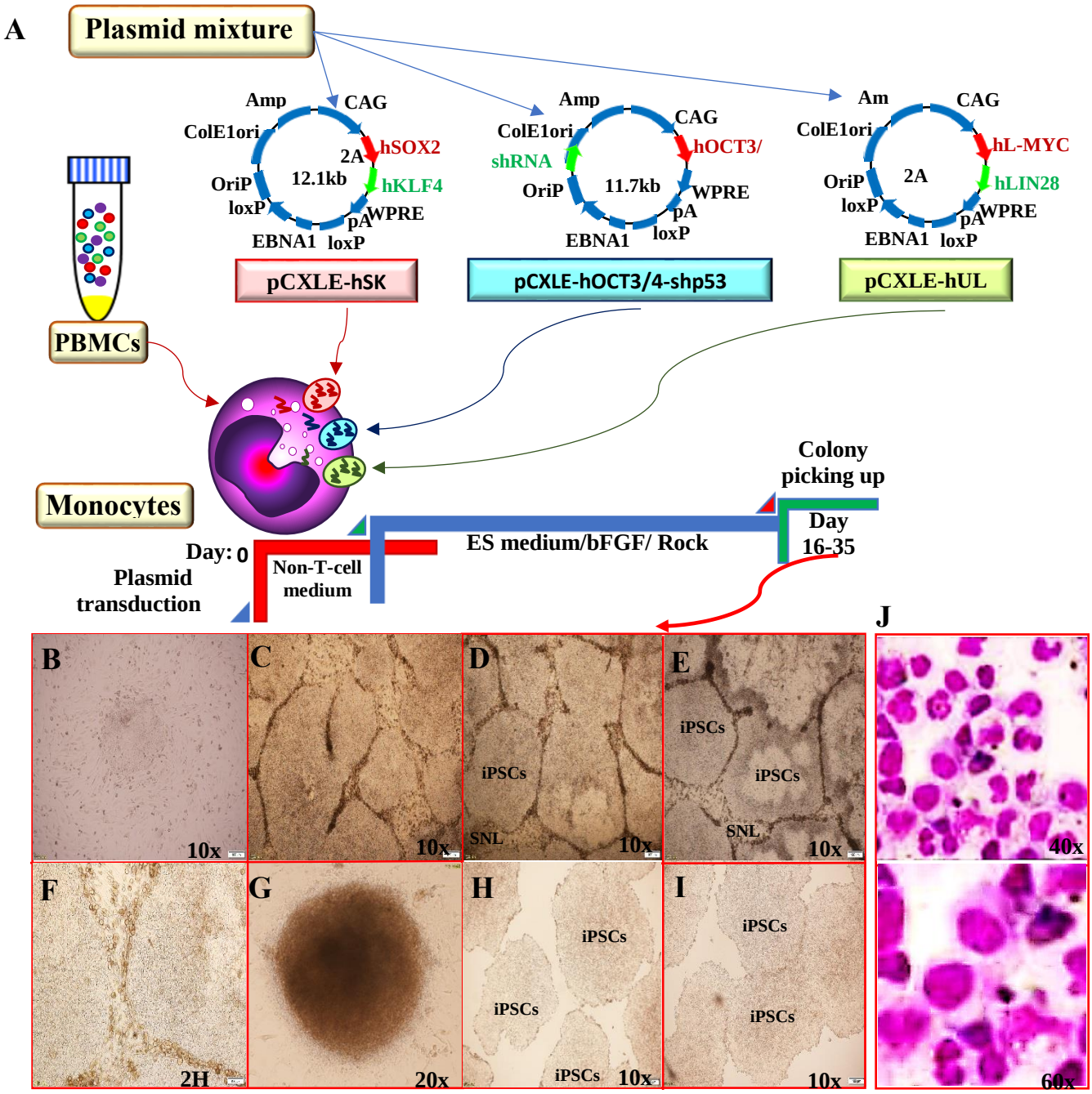

Figure S3. Generation of 6pLOH (+) and (-) iPSCs from non-T-cell populations in PBMCs with episomal vectors. (A) The iPSC induction protocol. PBMCs were transfected with episomal vectors mixture and then seeded MEF feeder cells. The colonies were counted and picked up for expansion 16–35 days after plating. (B-E) Representative images of iPS cells (clone KANA6-E1 at passage 9) documented at days 2-4-6-8 of culture (respectively). (F) Image of iPSCs with high magnification. (G) Image of typical iPSC colony, Bars = 100 µm (A-E), Bar= 200. (H&I) Image of iPSCs after feeder cells separation. (J) iPSC single cell images of cytospin preparations. The depicted images were prepared by May-Grünwald Giemsa staining.

Protocols for the differentiation of human iPS cells (h-iPSCs) to HSPCs 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 analysis to determine the committed cell properties.

Induction of HSPC 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**.**

Detection of HLA(-) leukocytes with flow cytometry (FMC)

Heparinized PB and BM were collected from KANA6 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 min and then stained with the appropriate dilution of the antibody and incubated for 30 min at 4°C in the dark. The antibodies used were monoclonal antibodies (mAbs) specific for HLA-A24, and HLA-Bw6 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 mAbs 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-Bw6	IgG2a	FITC	One Lambda
HLA-Bw4	IgG2a	PE	One Lambda
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-A1 primer or HLA-A24 primer 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 15s, 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).

Detection of HLA allele mutations

To identify the somatic mutations in 6pLOH(+) and *B*54:01*^{mut} iPSC clones, we performed targeted deep sequencing of HLA-B using a next-generation sequencer (NGS, MiSeq; Illumina, San Diego, USA). B5401(+) granulocytes, B5401 (+) T cells, or both from the KANA6 patient and his B5401 (+) 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*54:01* were identified when variant reads were found only in B5401^{-} iPSCs. All mutations were validated using targeted deep sequencing of HLA-B locus-specific long-range PCR, as previously described(8). HLA-B alleles carrying those mutations were determined using the nearest allele-specific single-nucleotide polymorphisms (SNPs). Reference sequences of HLA-*B*54:01* were obtained from the Immuno Polymorphism Database International ImMunoGeneTics Project HLA (IPD-IGMT/HLA database)(9).

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

GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase.

Colony-forming unit (CFU) assay

For methylcellulose assays, iCD34^{+} cells were isolated using human CD34 Microbeads (Miltenyi Biotec, Tokyo, Japan) and resuspended at a density of 40,000 cells/ml. A total of 400 μl of the cell resuspension was mixed with 3 ml of MethoCultTM GF H4034 (StemCell Technologies, Vancouver, Canada), and 1 ml was plated into 35-mm dishes in duplicate and incubated at 37 °C with 5% CO_2 for 14 days. Counting and characterization of CFU-derived colonies were performed using an inverted microscope. Lineage assignment was determined by a morphological analysis of Giemsa-stained individual colonies.

Reconstitution of immunodeficient mice with iCD34$^+$ cells from KANA6

Immunodeficient 57BL/6.Rag2^{null}Il2rgnull NOD-Sirpa (BRGS) mice that lack T, B, and NK cells were used for xenotransplantation as described previously(10). 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 1×10^6 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 1 mL syringe was inserted from the femur into the BM cavity, and cells were injected in the BM after confirming that the needle was inside 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 12 weeks after transplantation, samples of BM, PB, spleens, and thymuses were harvested from recipient mice and prepared for FCM 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 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 min and blocked again for 2 hours with a commercial Fc-blocking antibody before staining. The cells were stained with appropriate dilution of mAbs specific for human surface markers as described in (**Table. S5**) as well as anti–mouse CD45 mAbs and incubated for 30 min at 4°C in the dark for FCM 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 FCM 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 S5. **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

The engraftment of human cells was assessed by PCR for the alpha-satellite region of human chromosome 17 as described before(11) or by detecting human CD45 and HLA genes using genomic DNA from the BM and spleen of transplanted mice as templates. PCR was carried out with the HotstarTaq plus master mix (Qiagen, Venlo, The Netherlands) using the primer sets listed in **Table S6**. These animal studies were approved by the Institutional Animal Care and Use Committee at Kanazawa University.

Table S6. Primers used for PCR

Gene	Primer	Primer sequence
hCD45	FW	TTCAACTTATACCCTTCGTGTC
	RW	CCTGCTTTACTTTGTCCACTTC
hGATA1	FW	CTCCCTGTCCCCAATAGTGC
	RW	GTCCTTCGGCTGCTCCTGTG
hCD41	FW	GACTGTGAATGGTCTTCACCTC
	RW	ACACGTTGAACCATGCGTGCGA
hβ-actin	FW	GTGGGGCGCCCCAGGCACCA
	RW	CTCCTTAATGTCACGCACGATTTC

Table S7. HLA-class I alleles frequently lost as a results of 6pLOH in 107 6pLOH(+) patients

HLA class -I		Number (%) of 6pLOH patient
HLA-A	*A*31:01*	22 (21%)
	*A*24:02*	38 (36%)
	*A*02:06*	24 (22%)
	*A*02:01*	21 (20%)
HLA-B	*B*40:02*	50 (47%)
	*B*54:01*	11 (10%)
	*B*51:01*	5 (5%)
	*B*39:01*	6 (6%)
	*B*40:01*	3 (3%)
HLA-C	*C*03:04*	43 (40%)
	*C*01:02*	20 (19%)

Table S8. The frequencies of HLA class I alleles lost as a results of 6pLOH in 57 6pLOH(+) patients who did not carry *HLA-B*40:02*

HLA Class-1		Number (%) of 6pLOH patient
HLA-A	*A *31:01*	8 (14%)
	*A*24:02*	14 (25%)
	*A*02:06*	14 (25%)
	*A*02:01*	8 (14%)
HLA-B	*B*54:01*	11 (19%)
	*B*51:01*	4 (7%)
	*B*39:01*	5 (9%)
	*B*40:01*	4 (7%)
	*B*35:01*	2 (4%)
HLA-C	*C*03:04*	7 (13%)
	*C*01:02*	14* (25%)

*****This allele (*HLA-C*01:02*) allele was included in the lost haplotype in 8 (73%) of 11 6pLOH(+) patients who shared *HLA-B*54:01*-containin haplotype

Figure S4

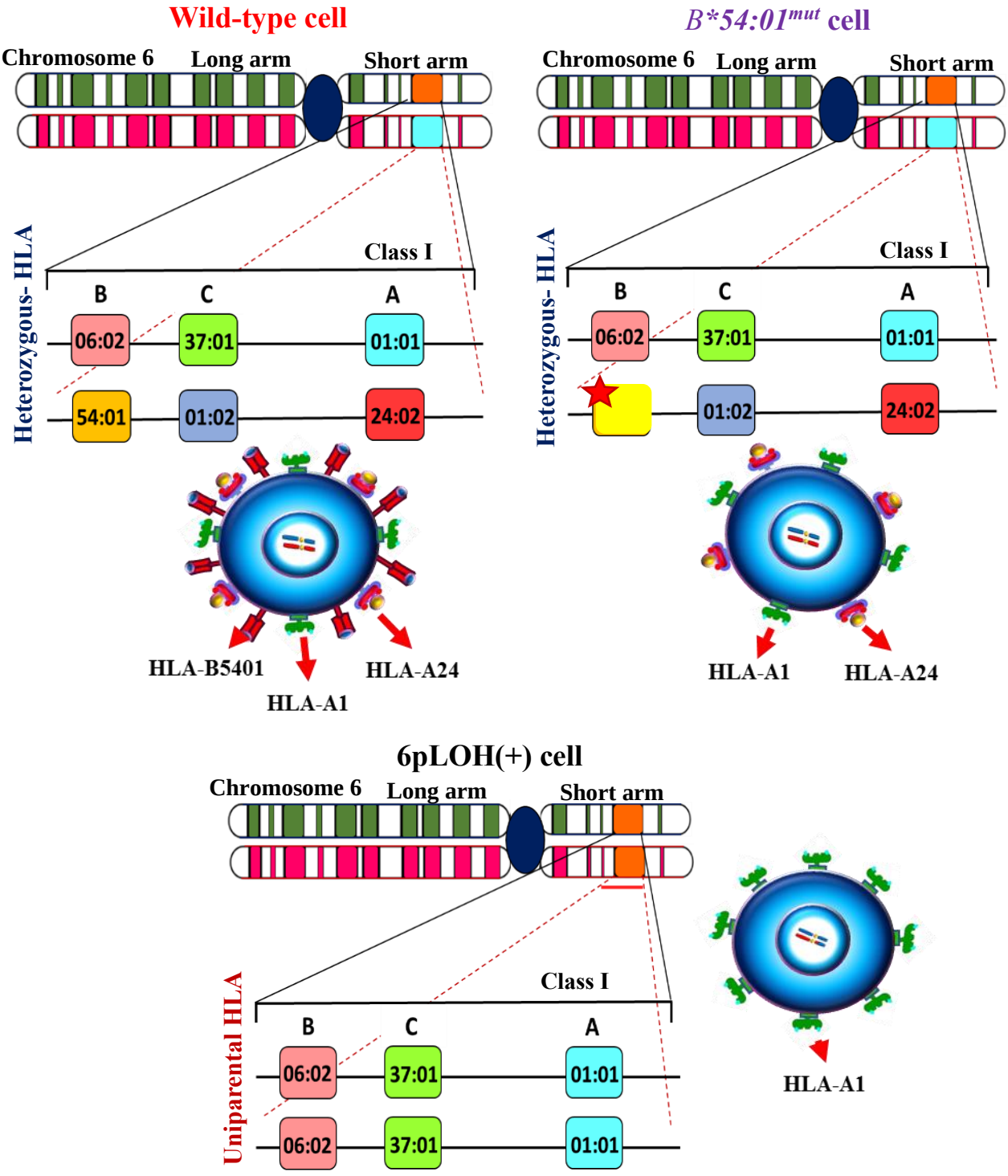

Figure S4. A diagram of the HLA complex of patient KANA6, showing the difference between WT cells *B*54:01*^{mut} and 6pLOH(+) cells. A start loss or nonsense mutation occurred at the starting site of *B*54:01* indicated as a star. Below or beside the corresponding HLA figures a proposed HLA expression patterns of single cells are shown.

Figure S5

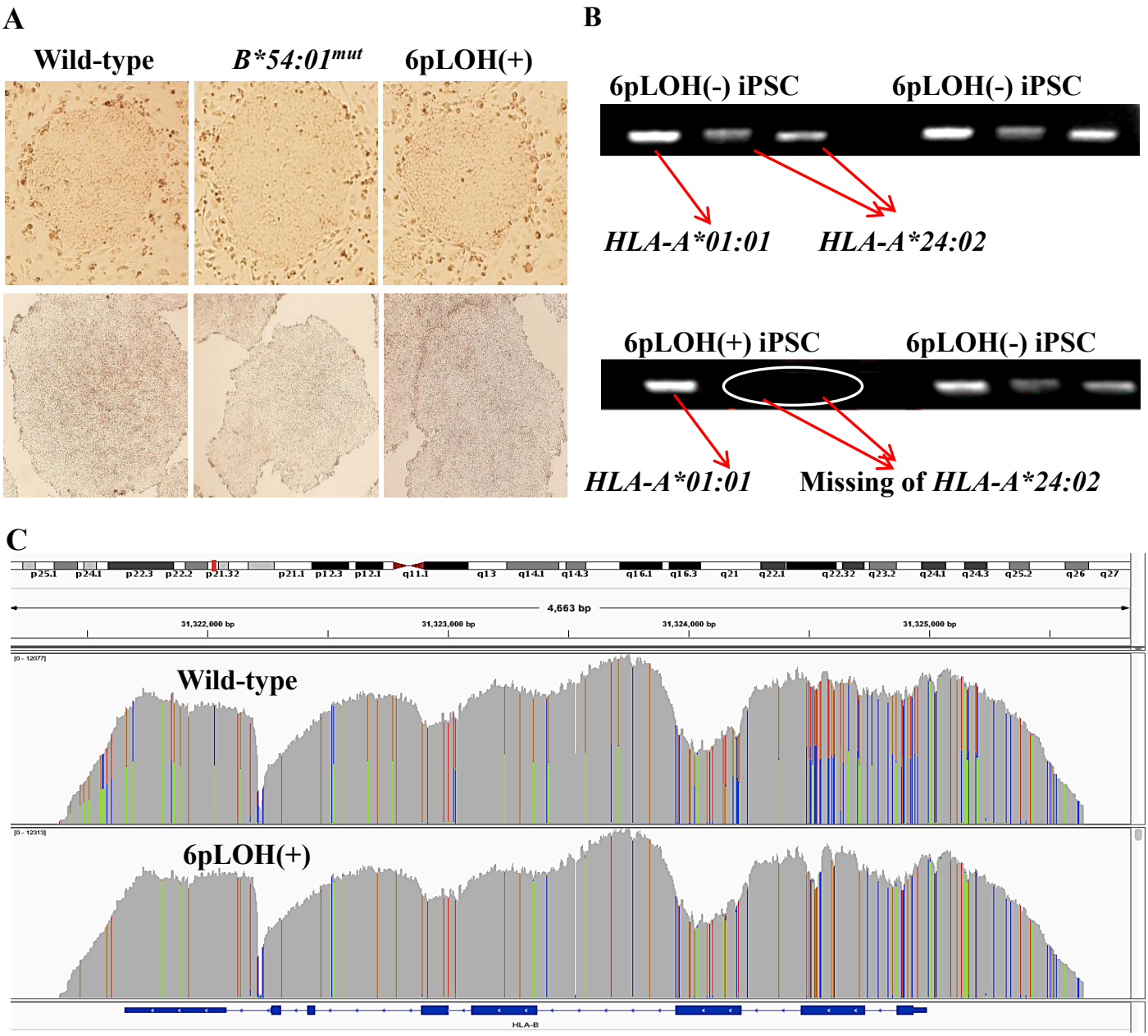

Figure S5. **Characteristic of iPSCs with different HLA genotypes establishment from the monocytes of KANA6 patient with acquired AA.** Morphology of iPSCs (A) Comparative morphology of WT (KANA6-E1), *B*54:01*^{mut} (KANA6-C1), and 6pLOH(+) (KANA6-D1) iPSCs clones, image of iPSC colonies, four days (upper panels) and 8 days after SNL cells separation (lower panels). (B) *HAL-A*01:01* and *HLA-A*24:02* alleles detected in DNA from several iPSC lines of KANA-6. The results show the difference in the band pattern between 6pLOH(-) and 6pLOH(+) iPSC clones. (C) Alignment view of next-generation sequencing. The gray color denotes bases identical with the reference genome; green, blue, orange, and red colors denote bases that were different from reference genome. The column that has two colors represents heterozygous SNV that were detected in a WT iPSC clone (E1) graph, while one color column represents homozygous SNV in a 6pLOH(+) iPSC clone (clone D1). The results of HLA-B allelic sequencing on a WT iPSC clone (E1) is shown as a control.

Figure S6

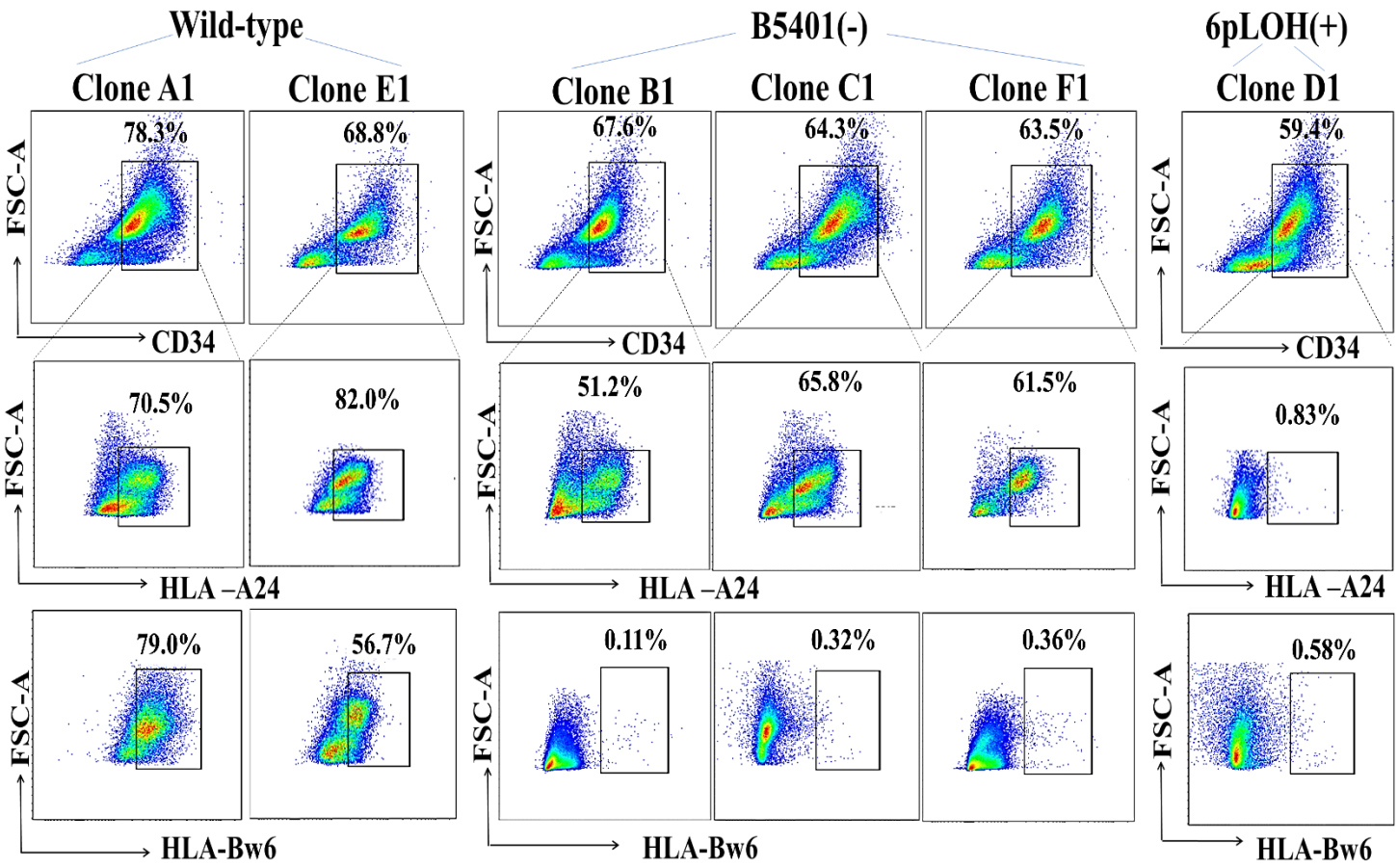

Figure S6. Characterization of HSPCs derived from KANA6's iPSCs with different HLA genotypes. (A) The expression of A2402 and Bw6 (B5401) by WT, *B*54:01*^{mut}, and 6pLOH(+) iCD34^{+} cells derived from three different iPSCs with different HLA genotypes. iPSCs cultured with a feeder-free system (StemPro) for 21 days were subjected to flow cytometry. Representative scattergrams show the WT iCD34^{+}cells to be positive for A2402 and Bw6 (left panels), the *B*54:01*^{mut} iCD34^{+}cells to be positive for A2402 and negative for Bw6 (middle panels), and the 6pLOH(+) iCD34^{+} cells to be double-negative for A2402 and Bw6 (right panels).

Figure S7

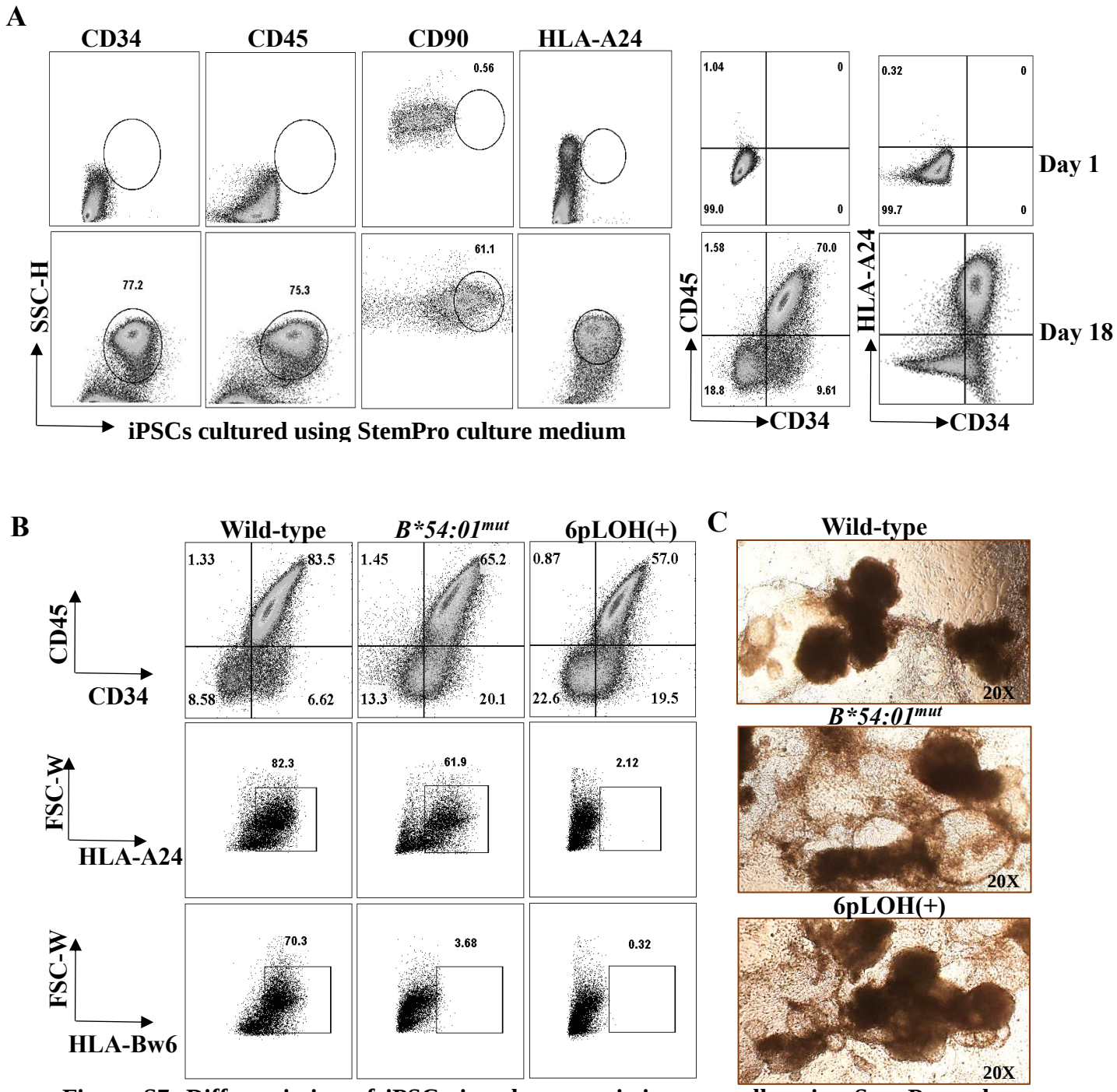

Figure S7. Differentiation of iPSCs into hematopoietic stem cells using StemPro culture medium. (A) Representative FCM results showing the percentage of $CD34^+$, $CD45^+$, CD90+, HLA-$A24^+$, $CD34^+CD45^+$ & $CD34^+$HLA-$A24^+$ cells in iPSCs (A1 clone) cultured with StemPro culture medium and analyzed at day 1 and 18. (B) The percentages of $CD34^+CD45^+$ (Upper panel), $CD34^+$HLA-$A24^+$ cells (middle panel) and $CD34^+$HLA-$Bw6^+$ cells (Lower panel) expressed by iPSCs with different HLA genotypes that were analyzed at day 18. (C) Comparative morphology of WT iPSCs, clone KANA6-E1 (Upper panel), *B*54:01*^{mut}, clone KANA6-C1 (middle panel) and 6pLOH(+) iPSCs, clone KANA6-D1 (Lower panel).

Figure S8

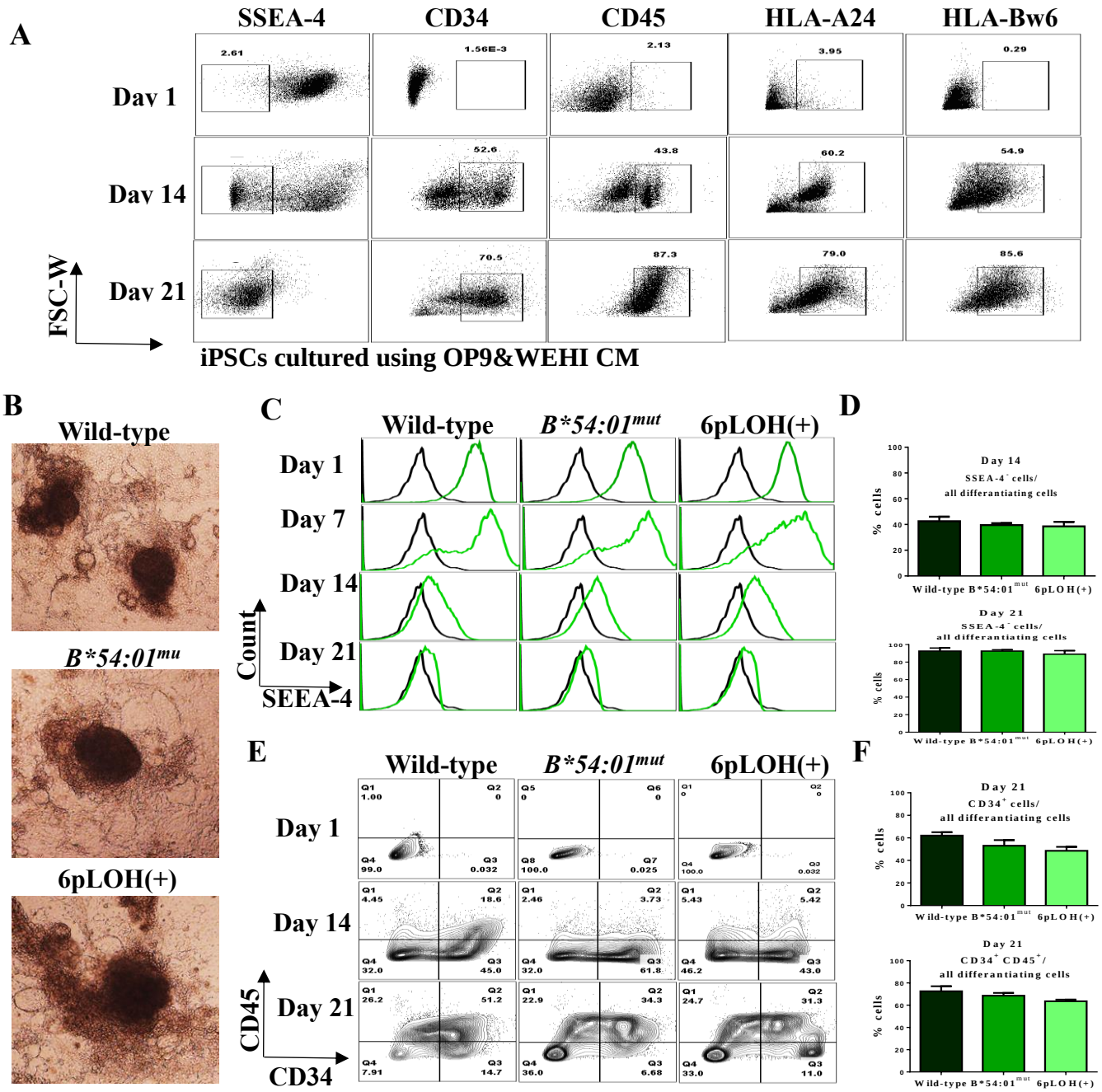

Figure S8. Differentiation of iPSCs into HSCs using OP9 & WEHI CM.
(A) Representative FCM results showing the percentage of SSEA-4^{-}, CD34^{+}, CD45^{+}, HLA-24^{+}, CD34^{+}HLA-A24^{+}& CD34^{+}HLA-Bw6^{+} cells in WT iPSCs (E1 clone) cultured with OP9 CM and analyzed at day 1, 14 and 21. (B) Comparative morphology of WT iPSCs, clone KANA6-A1 (Upper panel), *B*54:01*^{mut}, clone KANA6-B1 (middle panel) and 6pLOH(+) iPSCs, clone KANA6-D1(Lower panel) at day 14 of OP9&WEHI CM culture. (C&D) Kinetic analysis of immature marker expression on differentiating iPSCs with different HLA genotypes analyzed at day 1, 14 and 21. As iPSCs differentiate into iCD34^{+} cells, they progressively lost iPSC markers (SSEA-4). (D&E) FCM and bar graphs showing the kinetic analysis of hematopoietic markers expression percentage (CD34^{+}, CD34^{+}CD45^{+}) on differentiating iPSCs with different HLA genotypes analyzed at day 1, 14 and 21.

Figure S9

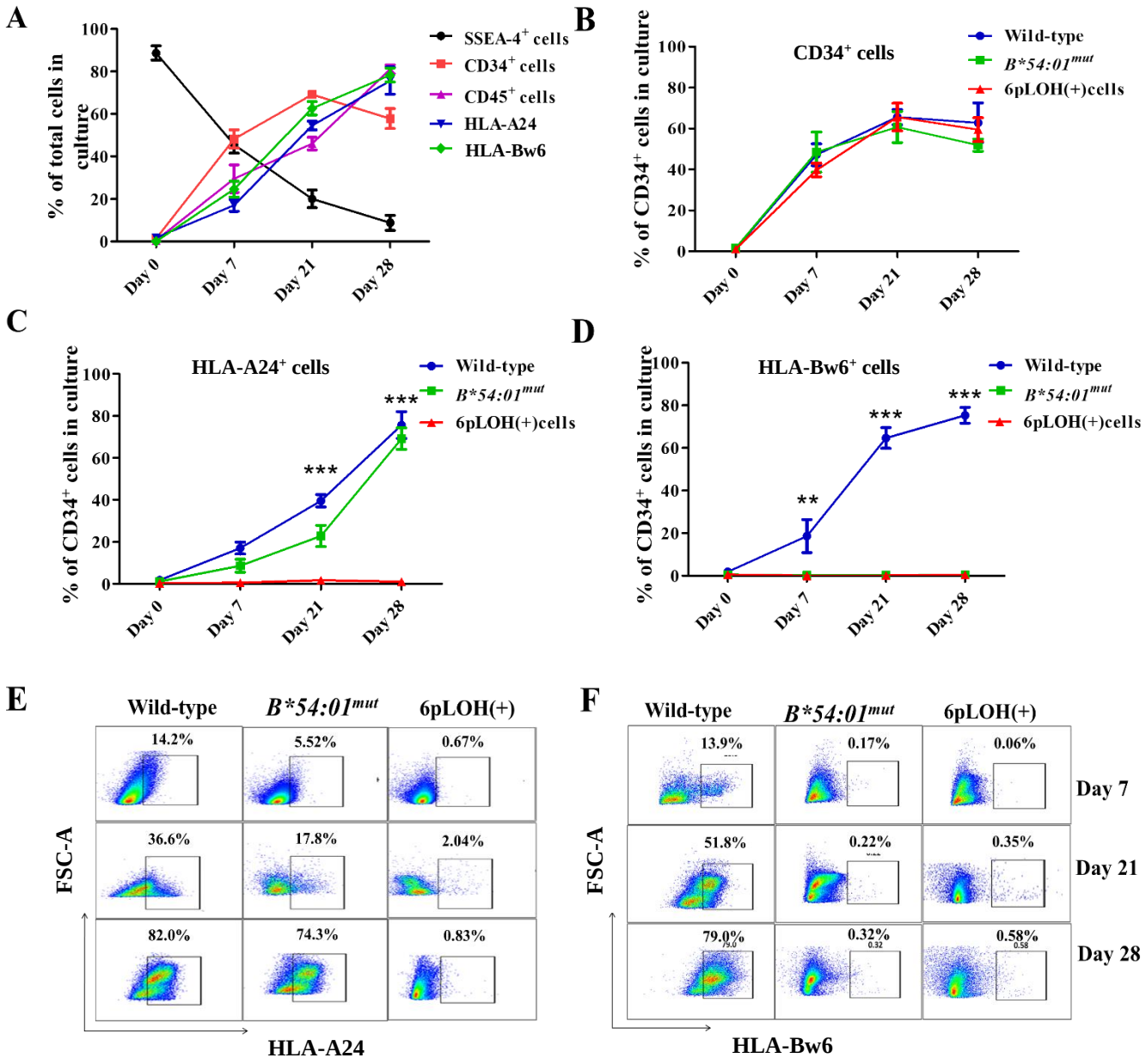

Figure S9. Kinetic expression of hematopoietic markers and HLA by HSPCs derived from KANA6-iPSCs with different HLA genotypes. (A) Cultured WT iPS clones with StemPro medium were analyzed for the percentage of SSEA-4^{-}CD34^{+} cells, CD45^{+}cells and HLA^{+} cells by the gated CD34^{+} cells. The data show the mean ± SEM from three independent experiments. (B - D) Kinetic expression of CD34, HLA-A24 and HLA-Bw6 respectively from KANA6-iPSCs with different HLA genotypes. The cells were harvested on the indicated days (0,7, 21, and 28) and examined with FCM. The data represent the mean ± SEM of the values determined by FMC. (E) Representative FCM results showing the expression of A24 and HLA-Bw6 by WT and *B*54:01*^{mut} and 6pLOH(+) iCD34^{+} cells derived from three different iPSCs with different HLA genotypes on the indicated days (0,7, 21, and 28).

Figure S10

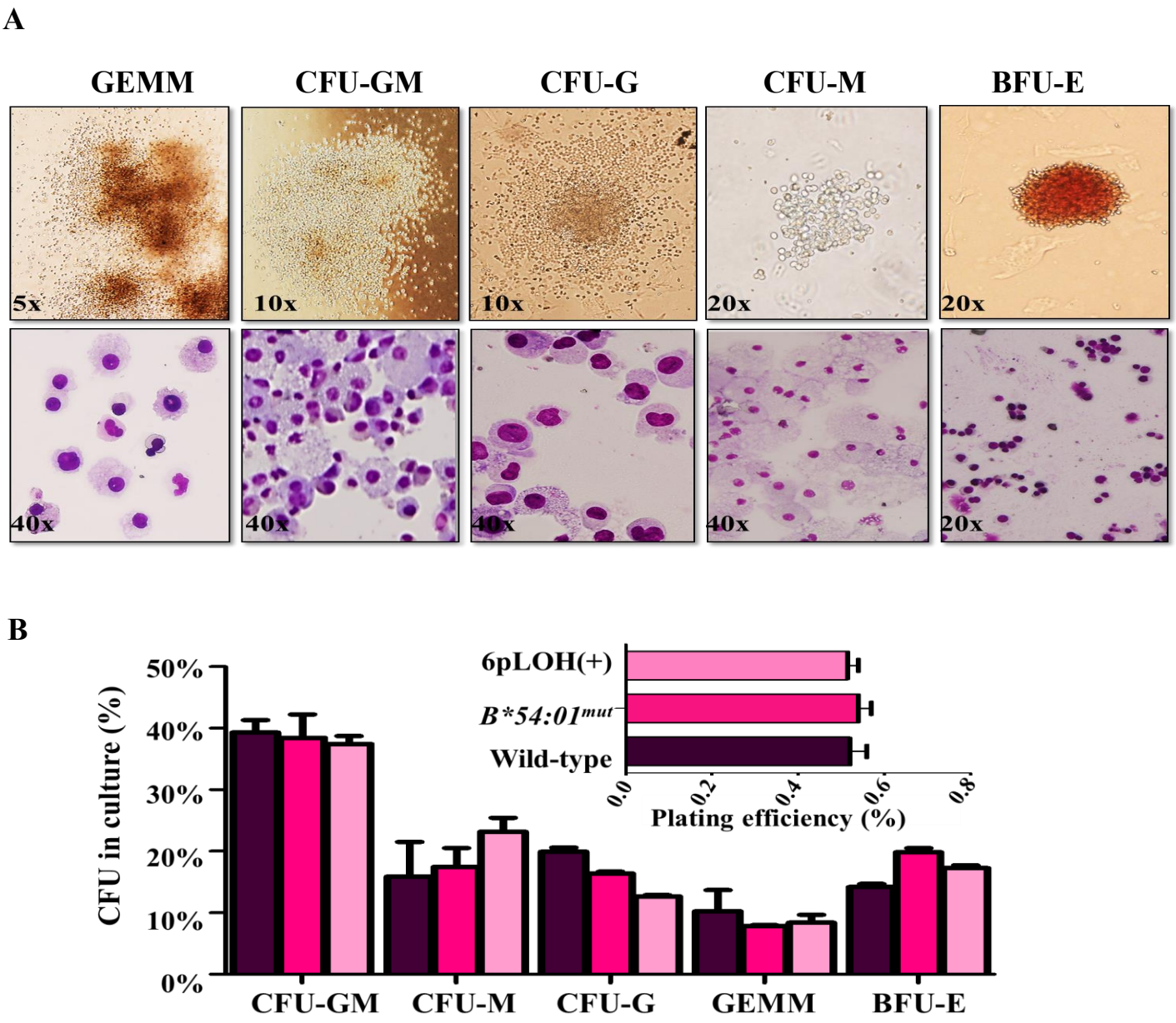

Figure S10. The hematopoietic potential of iCD34$^+$ cells derived from wild-type (WT), *B*54:01*^{mut}, and 6pLOH(+) iPSCs. (A) Representative CFU-GEMM-, CFU-G-, CFU-GM-, CFU-M-, and BFU-E-derived colonies from WT-iCD34$^+$ cells and the morphology of constituent cells stained by May-Grünwald Giemsa staining. (B) The percentage of CFUs (lower panel) and plating efficiencies (upper panel) of iCD34$^+$cells derived from iPSCs with three different HLA genotypes. The data are presented as the mean ± SEM of the percentage of CFUs obtained from three independent experiments. The plating efficiency was defined as the frequency of colonies generated from 5,000 seeded iCD34$^+$ cells (total number of colonies/5×10^3 cells seeded).

Figure S11

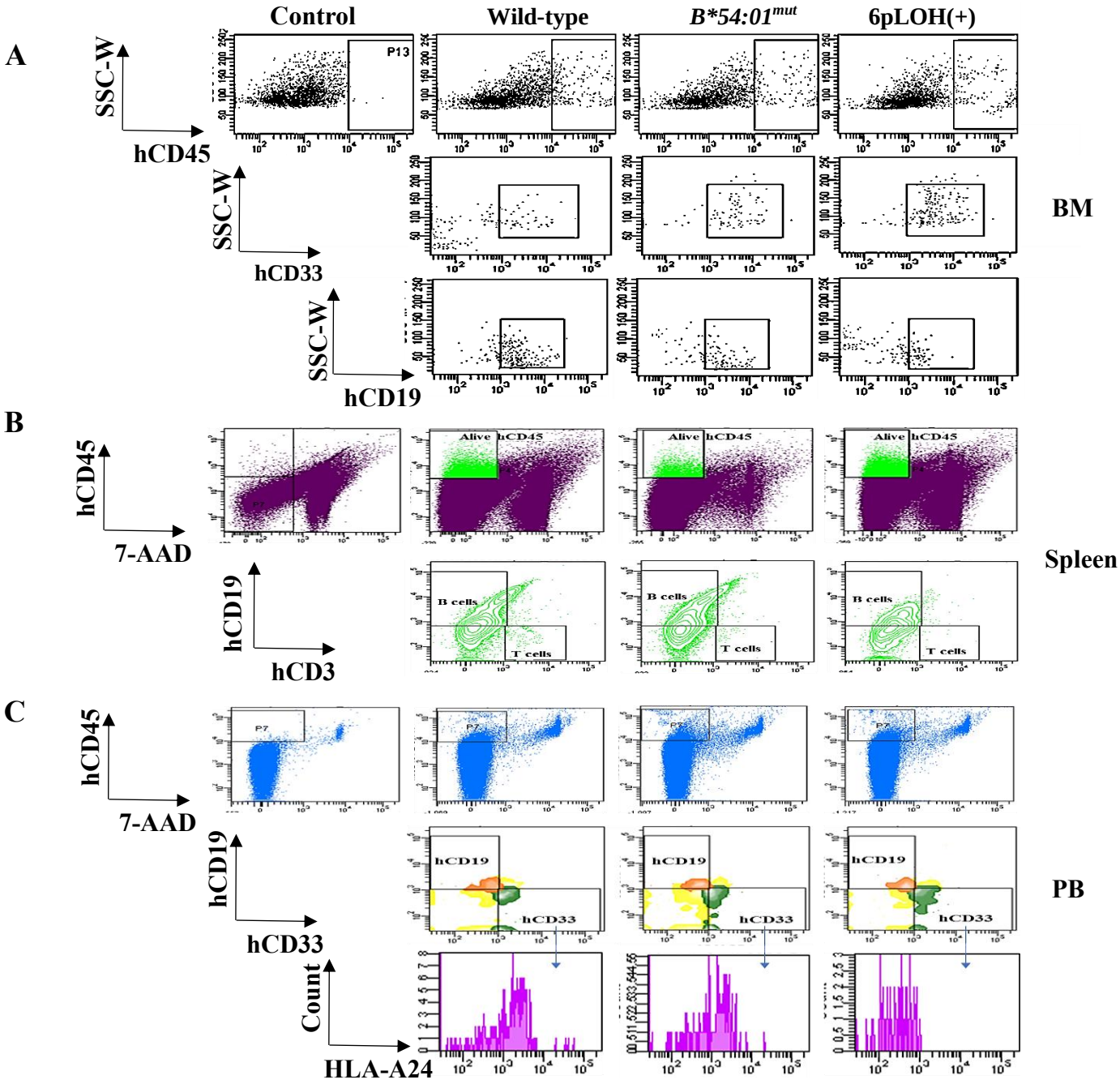

Figure S11. Engraftment of WT, *B*54:01*^{mut} and 6pLOH(+) iCD34^{+} cells in BRGS mice.
(A) Representative FCM analysis of BM cells from transplanted mice showing the percentages of human cells (hCD45^{+}) (upper panels), human myeloid cells (hCD33^{+}) (middle panels), and lymphoid cells (hCD19^{+}) (lower panels) at 12 weeks post-transplantation. (B) FCM analysis of the spleen showing the percentages of human hCD45^{+}cells (upper panels) (hCD45^{+}), and lymphoid cells (hCD19^{+}& hCD3^{+}) (lower panels). (C) Representative FCM analysis of the peripheral blood (PB) showing and human cells (upper panels), human CD33^{+} myeloid cells& lymphoid cells (hCD19^{+}) (middle panels), and HLA-A24 expression by CD33^{+} myeloid cells in the PB of mice transplanted with WT, *B*54:01*^{mut} or 6pLOH(+) iCD34^{+} cells (lower panels) at 12 weeks post-transplantation.

Figure S12

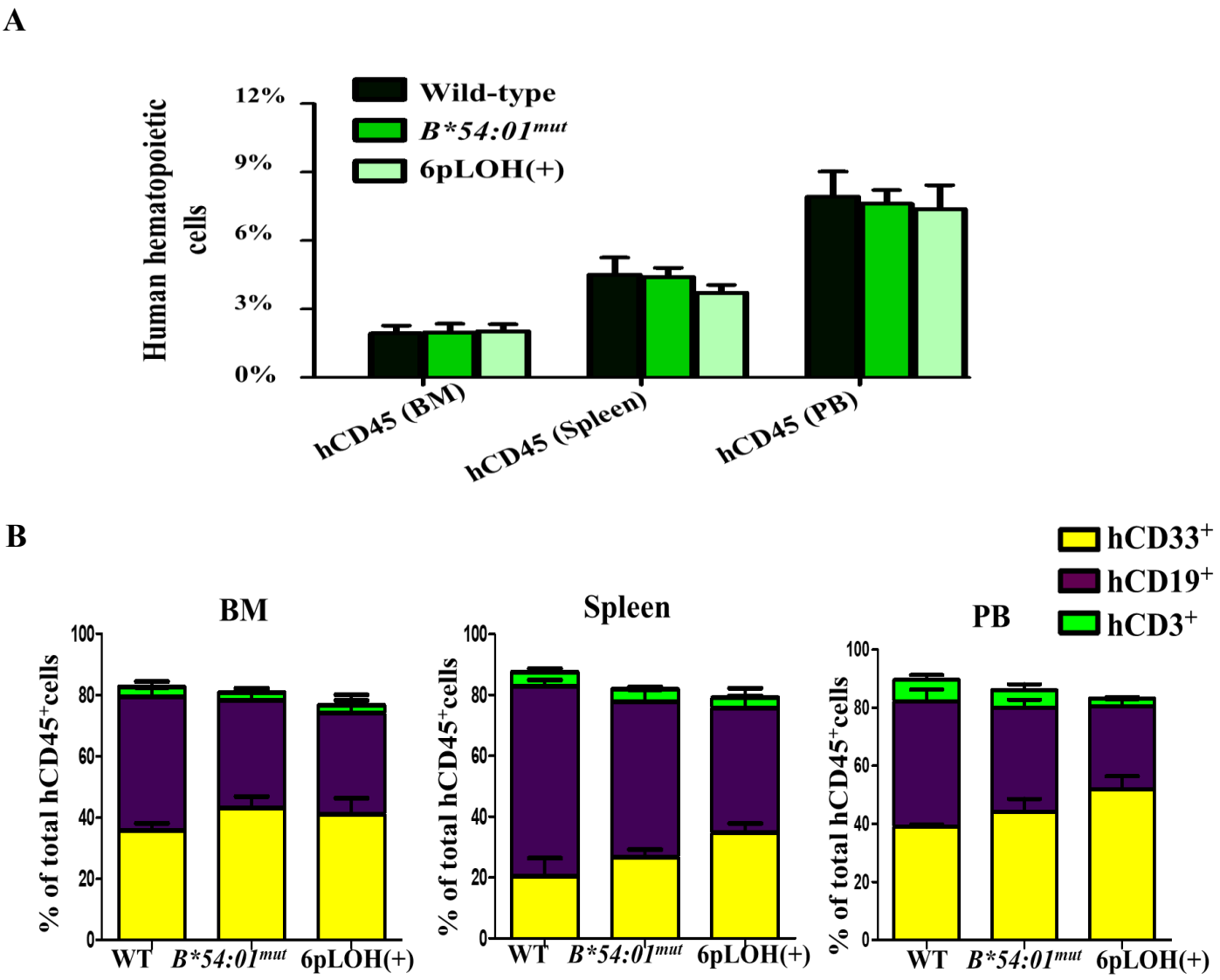

Figure S12. Engraftment of myeloid and lymphoid lineage cells derived from wild-type (WT), *B*54:01*^{mut}, and 6pLOH(+) CD34^{+} iPSCs in different organs BRGS mice. (A) Human CD45^{+} cells in different organs of BRGS mice at 12 weeks after transplantation of WT, *B**54:01^{mut}, and 6pLOH(+) CD34^{+} iPSCs. The percentages of human hematopoietic cells represent the mean ± SEM of three WT, *B**54:01^{mut}, and 6pLOH(+) cell recipients. (B) Engraftment of myeloid and lymphoid lineage cells in different organs. The percentages of each cell lineage in the BM (left panel), spleen (middle panel), and PB (right panel) are shown. The data are presented as the mean ± SEM of the percentages of each marker-positive cell obtained from three WT, *B*54:01*^{mut}, and 6pLOH(+) cell recipient mice.

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