Vitamin C–dependent lysine demethylase 6 (KDM6)-mediated demethylation promotes a chromatin state that supports the endothelial-to-hematopoietic transition

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Running title: Vc promotes a chromatin state that supports EHT

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Abstract

Hematopoietic stem cells (HSCs)/progenitor cells (HPCs) are generated from hemogenic endothelial cells (HECs) during the endothelial-to-hematopoietic transition (EHT); however, the underlying mechanism remains poorly understood. Here, using an array of approaches, including CRISPR/Cas9 gene knockouts, RNA-Seq, ChIP-Seq, ATAC-Seq, et al, we report that vitamin C (Vc) is essential in HPC generation during human pluripotent stem cell (hPSC) differentiation in defined culture conditions. Mechanistically, we found that the endothelial cells generated in the absence of Vc fail to undergo the EHT because of an apparent failure in opening up genomic loci essential for hematopoiesis. Under Vc deficiency, these loci exhibited abnormal accumulation of histone H3 trimethylation at Lys-27 (H3K27me3) a repressive histone modification that arose because of lower activities of demethylases that target H3K27me3. Consistently, deletion of the two H3K27me3 demethylases, Jumonji domain–containing 3 (JMJD3 or KDM6B) and histone demethylase UTX (UTX or KDM6A), impaired HPC generation even in the presence of Vc. Furthermore, we noted that Vc and jmjd3 are also important for HSC generation during zebrafish development. Together, our findings reveal an essential role for Vc in the EHT for hematopoiesis, and identify KDM6-mediated chromatin demethylation as an important regulatory mechanism in hematopoietic cell differentiation.
Hematopoietic stem/progenitor cells (HSPCs) hold great promise for regenerative medicine, yet remain problematic in terms of efficient generation from human pluripotent stem cells (hPSCs) such as hESCs (human embryonic stem cells) or hiPSCs (human pluripotent stem cells) (1-4). One possible reason is that the in vitro differentiation failed to fully recapitulate the developmental principles of hematopoiesis in vivo. Therefore, understanding the molecular mechanisms underlying hematopoiesis both in vitro and in vivo would be important to promote HSPC generation in vitro.

During mouse hematopoiesis, the first hematopoietic program, primitive hematopoiesis emerges in the yolk sac at embryonic (E) day 7.5 while the definitive program occurs later at around E10.5 at aorta-gonad-mesonephros (AGM) region (5). HSPCs derived at yolk sac or AGM display distinct functional characteristics in terms of engraftment in irradiated neonatal and adult mouse recipients (6). At cellular level, hematopoiesis involves sequential lineage specifications from mesoderm, hematopoietic and vascular fate to HSPCs, and then different subtypes of blood cells. Insights from different model organisms demonstrate that HSCs arise from a special type of endothelial cells, termed hemogenic endothelial cells (HECs) through endothelial-to-hematopoietic transition (EHT) (7-9). Several signaling pathways are known to regulate EHT in mouse model, such as retinoic acid (RA), Notch, TGF-β signaling (10) (11,12). HEC population is a very special subset of vascular endothelium that holds hematopoietic potential to give rise to multiple lineage HSPCs within a short developmental window. HECs isolated from mouse embryos could produce functional HSPCs in vitro (13), highlighting its essential function in HSPC generation during development. However, despite its essential role in hematopoiesis, the molecular events that specify functional HECs and the subsequent EHT remains largely unknown, particular in human background.

Here in this study, we discovered that vitamin C (Vc) is required for the generation of HPCs from hPSCs through regulating EHT. Mechanistically, Vc plays an essential role to specify a permissive chromatin state that allow endothelial cells to give rise to HPCs. Moreover, Vc is also important for HPC generation during zebrafish development. These findings reveal a previously unidentified but essential role of Vc dependent epigenetic mechanism underlying EHT during hematopoietic development.

Results: Vitamin C is required for generation of HPCs from hPSCs in defined condition

We sought to develop an efficient approach to differentiate blood cells from hPSCs in a chemical defined, serum-free and monolayer condition. At early stage of embryogenesis, blood lineages were originally developed from primitive streak (PS) and the downstream lateral mesoderm (LM) (14). Loh et al reported that Wnt inhibition and BMP activation promote LM specification in hPSC derived PS population (14). Based on this report and other literatures (2,14,15), we developed a step wise strategy to differentiate hPSCs in a defined, monolayer condition that recapitulates main stages of early hematopoiesis, including the PS, LM, HECs and then HPCs (Fig.1A). Through combined Activin/GSK3b inhibition/PI3K inhibition/FGF treatment for 1 day, nearly 100% of H1 hESCs differentiated into the Brachyury (T) positive PS mesoderm in monolayer condition (Fig.1B). Then, Wnt inhibition and BMP activation treated at the following day promoted LM specification while suppressed the paraxial mesoderm (PM) cell fate, as demonstrated by the activation of HAND1, the LM marker and suppression of MSGN1, the PM marker (Fig.1B) (15). Later at day 4, the cells were switched into the typical EHT medium containing hematopoietic cytokines to allow further hematopoiesis. Significant number of CD43+ HPCs (1) could be detected after two days of culture in EHT medium. (Fig.1C). Morphologically, typical EHT process could be observed at this stage, i.e. the endothelial cells acquired the hematopoietic morphology and became floated during culture from day4 to day8 (Fig.1D). At day 8, over 90% of floating cells were CD43+ (Fig.1E), the typical phenotype of hPSC derived HPCs (1) and they could form various blood CFUs (colony forming units) (Fig.1F and S1A). Later on at day 10, certain percentage of CD43+ HPCs(1) could be detected after two days of culture in EHT medium. (Fig.1C). Morphologically, typical EHT process could be observed at this stage, i.e. the endothelial cells acquired the hematopoietic morphology and became floated during culture from day4 to day8 (Fig.1D). At day 8, over 90% of floating cells were CD43+ (Fig.1E), the typical phenotype of hPSC derived HPCs(1) and they could form various blood CFUs (colony forming units) (Fig.1F and S1A). Later on at day 10, certain percentage of CD43+ cells became CD45+, a marker indicating mature HPCs(16,17) (Fig. 1E). Furthermore, both adult and embryonic globin HBB, HBE and HBG1 could be detected in the erythroid CFUs (CFU-E) (Fig.1H-I), demonstrating both the definitive and primitive hematopoiesis occurred during differentiation. Together, we developed a monolayer approach to
differentiate hPSCs into hematopoietic cells in a chemical defined condition.

To further characterize the role of individual factor in the basal medium in HPC generation, we surprisingly found that vitamin C was essential in HPC generation. In defined condition with no Vc, the generation of CD43+ HPCs, but not the panendothelial cells were significantly reduced (Fig.1J-K, S1B). Consistently, the endothelial cells that express both CD31 and CD43, the typical phenotype indicating EHT were significantly reduced in Vc condition without Vc (Fig.1K). Quantitatively, the percentage of CD43+ HPCs as well as their hematopoietic potential to form CFUs were significantly reduced in Vc condition (Fig.1L-N). The role of Vc in HPC generation was also verified on different hPSC lines, including multiple iPSC lines that we generated previously from different background, such as UH10, UC1, UC5, UH1 and HU-AL hiPSC (18) (Fig.S1C-F). Notably, we found that increasing the concentration of Vc showed minor further enhancement on HPC generation, suggesting the function of Vc is not dose dependent in this case (Fig.S1G). Also, we found that only Vc, but not the other antioxidants could promote the HPC generation (Fig.1N, S1H). Nevertheless, these data above suggest that Vc is an essential factor for hematopoietic differentiation in hPSCs in defined condition.

Vitamin C is required to specify functional HECs

We then examined role of Vc in more detail in the time course of blood differentiation of hPSCs. At early stage, the T positive PS population showed little difference between Vc+ and Vc- conditions (Fig.2A). To analyze the role of Vc at HEC stage, we employed the previously generated GATA2/GFP reporter hESC line (19). Consistent to our previous findings, GATA2 expression discriminated hemogenic potential endothelial cells from the non-hematopoietic endothelial cells in hESC differentiation (Fig.2A). In addition, the GATA2+ endothelial cells (G2ECs) almost do not express the non-hematopoietic endothelial cell marker – CD73 (20) (Fig.2C). At day 4 of differentiation, the generation of CD31+CD43+GATA2/eGFP+ endothelial cells (G2ECs) that contain HECs did not show much difference between Vc+ and Vc- conditions (Fig.2A-B, S2D). However, following 2 days of further differentiation, the CD43+ HPCs were significantly reduced at day 6 in Vc condition (Fig.2A). Notably, some key hematopoietic genes such as RUNX1, MYB etc. started to show differences in expression at day 4 between two conditions (Fig.2C). To precisely examine the role of Vc in HPC generation at EHT stage, we sorted the G2ECs generated in the Vc+ or Vc- condition at day 4 and re-plated them again in EHT medium either containing Vc or no Vc (Fig.2D). Interestingly, the G2ECs generated in the presence of Vc (Vc+ G2ECs) successfully produced HPCs with efficient CFU potential (Fig.S2E) in EHT condition no matter with or without Vc (Fig.2D). In contrast, the G2ECs generated in the absence of Vc (Vc- G2ECs) failed to efficiently produce HPCs even though were re-plated in EHT medium containing Vc (Fig.2D). Together, these data suggest that Vc is mainly required for the specification of endothelial cells that have hemogenic potential for HPC generation.

Endothelial Cells generated in the absence of Vc are incompetent to undergo EHT

In order to investigate the molecular mechanism underlying the functional defect in Vc- G2ECs, we generated RNA-seq data in the time course of the subsequent EHT process. Surprisingly, Vc- G2ECs and Vc+ G2ECs showed much similar profile in whole genome expression based on RNA-seq data (Fig.3A). Selected endothelial or blood related genes showed much similar expressions between two populations (Fig.3B). However, when re-plated in EHT condition, they showed distinct differentiation pathways based on principal component analysis (PCA) (Fig.3C). While the EHT of Vc+ G2ECs progressed well toward HPCs, Vc- G2ECs did not progress well toward HPCs (Fig.3C). The up or down regulated genes during EHT clearly showed distinct patterns between Vc- G2ECs and Vc+ G2ECs (Fig.3D-F). The endothelial related genes, such as CDH5, JAG1 and TEK were significantly down-regulated in Vc+ G2ECs, but remained highly expressed in Vc- G2ECs (Fig.3E and F). The hematopoietic genes, like RUNX1, MYB and IKZF1 were significantly up-regulated in Vc+ G2ECs, but repressed in Vc- G2ECs during EHT process (Fig.3E and F). Together, these data demonstrate that Vc- G2ECs are incompetent to undergo EHT, despite showed similar gene expression profiles as Vc+ G2ECs.

Vc promotes chromatin accessibility on hematopoietic genes
Ours and other reports showed that Vc regulates somatic cell reprogramming through histone and DNA demethylases (21-23). To see if similar mechanisms also regulate the generation of HECs, we examined the chromatin accessibility landscapes and epigenetic modifications in Vc- G2ECs and Vc+ G2ECs by transposase-accessible chromatin with sequencing (ATAC-seq) and chromatin immunoprecipitation followed by sequencing (CHIP-seq) on active (H3K4me3) and repressive (H3K27me3) histone modifications. Broadly, all the ATAC-seq samples exhibited the expected periodicity of insert length (Fig.S3A) and the ATAC-seq peaks were enriched in transcriptional start sites (TSS) (Fig.S3B). Despite the similar distribution of the ATAC-seq peaks in the genomic regions (Fig.4A), significant number of differential accessible chromatin regions were detected between Vc- G2ECs and Vc+ G2ECs (Fig.4B-C). Regions with increased accessibility in Vc+ G2ECs were enriched for genes associated with hematopoietic function (Fig. 4D). Conversely, chromatin regions enriched in motifs of known hematopoietic regulators were more assessable in Vc+ G2ECs than that in Vc- G2ECs (Fig.4E, FigS3C). These data indicate that Vc promotes chromatin accessibility on hematopoietic genes. Consistently, the global level of repressive histone marker, H3K27me3 was significantly reduced in Vc+ G2ECs (Fig.4F). While the active genes and the neither genes were similar, the poised bivalent genes (H3K4me3/K3K27me3)(24) showed big difference between Vc- G2ECs and Vc+ G2ECs (Fig.S4A-B, Fig.4G). Significant number of poised genes in Vc+ G2ECs became more repressed in Vc- G2ECs due to the failure to remove H3K27me3 at those loci. These genes include many known important genes in hematopoiesis, for example, MYB and IKZF1 (Fig.4H-J). Conversely, some bivalent genes in Vc+ G2ECs showed reduced H3K27me3 in Vc- G2ECs, but they are not reported to involve in hematopoiesis (Fig.4H-J). Together, these data demonstrate that Vc promotes the hematopoietic potential in endothelial cells by opening genomic loci of hematopoietic regulators.

**Vc regulates hematopoietic differentiation of hPSC through KDM6s and TET1**

The data shown above indicate that H3K27me3 might be a downstream effector regulated by Vc in HPC generation. H3K27me3 modification on chromatin could be removed by its demethylase, KDM6s that contain two members, UTX (KDM6A) and JMJD3 (KDM6B)(25). Notably, Vc has been reported to regulate the KDM6 demethylase activity in dopamine neuron (26). We then examined the global level of H3K27me3 in ECs generated with or without Vc. Consistent to the CHIP-seq data in Fig.4F, Vc+ G2ECs showed reduced level of global H3K27me3 compared with Vc- G2ECs (Fig.5A). Moreover, based a previously reported assay for KDM6 demethylase activity (26), Vc+ G2ECs also showed much higher activity of KDM6 demethylase (Fig.5B). These data indicate that KDM6s might be a critical downstream effector of Vc during hematopoietic differentiation of hPSCs. To further examine the direct role of KDM6s, we deleted the JMJC domain, the critical domain for demethylase activity in UTX and/or JMJD3 in hESCs (Fig.5C and Fig.5A-D)(27). Upon differentiation, hESCs with deletion of either UTX (UTX<sup>-/-</sup>) or JMJD3 (JMJD3<sup>-/-</sup>), or both (dKO) showed significant defects in HPC generation, but not the endothelial cells in the presence of Vc (Fig.5D-F and Fig.5E). These data indicate that JMJD3 and/or UTX are required for the normal EHT and not functionally redundant in this process. We then analyzed the differentiation of these KDM6 mutant cells in more detail. Firstly, the pluripotency marker such as OCT4, was downregulated along the differentiation in all the examined cell lines (Fig.5G), indicating the exit of pluripotency was generally not impeded in KDM6 deficient cells. The PS marker T, the LM marker HAND1 and GATA2 were all successfully up-regulated at early stage of differentiation in all KDM6 deficient hESC lines (Fig.5G). In contrast, the hematopoietic genes, such as MYB, IKZF1, LMO2 and TAL1 failed to up-regulate at later stage (Fig.5G). These data suggest that the KDM6s mainly acts at later stage of blood differentiation to ensure a normal EHT. Accordingly, H3K27me3 were more enriched on the selected hematopoietic genes in KDM6 deficient cells compared with wild type hESCs at later stage of blood differentiation (Fig.5I). In contrast, CD31<sup>+</sup>CD43<sup>-</sup> endothelial cells showed no big difference between all cell lines examined (Fig.5F). Also, deletion of JMJD3 did not affect differentiation of the GATA2<sup>+</sup> ECs (Fig.5F-G). Together, these data demonstrate that KDM6 mediated H3K27me3 demethylation is required for HPC generation at later stage of differentiation in hPSCs.
It is worthy to note that Vc was also reported to suppress leukaemogenesis through TET family enzymes, the dioxygenases that catalyze 5mC to 5hmC on DNA (28-30). To test whether if TET dependent DNA methylation is also involved in the regulation of Vc on HPC generation. We performed deletions of TET1 in human ESCs and examined their blood differentiation potential (Fig.5I-J). Indeed, HPC, but not the EC generation was reduced in TET1 deficient hESCs in the presence of Vc (Fig.5I-J). On the other hand, Vc showed a mild enhance on HPC generation in the absence of TET1. These data indicate TET mediated DNA methylation might be another downstream mechanism of Vc in HPC generation, apart from the KDM6 mediated H3K27me3 demethylation.

Vitamin C and jmjd3 are important for HSC emergence in zebrafish

To examine the role of Vc to promote hematopoiesis in vivo, we employed zebrafish as a development model as zebrafish is one of those species that cannot synthesize Vc by themselves (31). Based on previous report,(32) we fed the flk1: mCherry/cmyb:GFP(33) transgenic zebrafish with the defined diets with or without Vc (Fig.6A). After continuous feeding with Vc-diets for over two months, the total Vc concentration in the D5.5 zebrafish embryos was significantly reduced (Fig.6B). We then analyzed the blood development in embryos developed in Vc+ or Vc- condition. The zebrafish embryos developed in Vc- condition showed significantly reduced generation of flk1+cmyb+ cells (emerging HSCs(34)) in AGM region compared with Vc+ condition and controls (Fig.6C), indicating Vc plays an important role in blood development in zebrafish. Lastly, we also examined the role of jmjd3 in HSC commitment during zebrafish development with normal Vc. We injected morpholinos (MO) to knock down jmjd3 in flk1: mCherry/cmyb:GFP transgenic zebrafish(33). The flk1+ cmyb+ emerging HSCs was remarkably reduced in jmjd3 morphants (Fig.6D) in the AGM region compared with controls, suggesting jmjd3 is also important for the blood development in zebrafish.

Discussion

The hematopoietic differentiation of hPSCs provides a good model to investigate human hematopoiesis, but is largely inefficient and variable (35,36). Based on previously published literatures(2,14,15), we developed a step wise blood differentiation strategy in hPSCs, which recapitulates the main stages of early hematopoiesis, including the PS, LM, HECs and then HPCs in defined, monolayer condition. This monolayer based approach exhibits reduced batch to batch viabilities and much higher efficiencies compared previously reported protocols based on co-culture with OP9 stromal cells (37) (Fig.S6A). Even though HPCs generated in this condition still showed limited engraftment in vivo (data not shown), it provides a simple and efficient model to study human hematopoiesis in a totally defined and monolayer condition.

Based on this approach, we discovered that Vc is an essential factor in generation of HPCs, particularly at later stage of differentiation in specification of functional HECs (Fig.1). Interestingly, Vc would be no longer required for HPC generation after the stage of HEC specification. We further revealed that the endothelial cells generated in the presence or absence of Vc harbor distinct epigenetic state. Specifically, endothelial cells generated in Vc-condition exhibited high level of H3K27me3 and less DNA accessibility on hematopoietic genes. In previous reports, the methyl groups on H3K27 was shown to be removed by the JMJC domain containing protein KDM6s (KDM6A/UTX and KDM6B/JMJD3) (38). Interestingly, Vc was shown to be crucial for the optimized demethylation activity of the JMJC domain(27). Consistent to these findings, KDM6s show much reduced demethylase activity in Vc- condition. Further impairment of KDM6s in hESCs impeded EHT in the presence of Vc. More importantly, Vc and jmjd3 are also important to regulate the emergence of hematopoietic cells in zebrafish development. Therefore, our data reveal a previously unknown, but essential role of Vc dependent epigenetic mechanism in hematopoiesis, both in human model and zebrafish development.

Vc is also a well-known antioxidant, however, other antioxidants did not rescue the HPC defect in hPSCs in Vc- condition (Fig.1N, S1H). Also, Vc presence or absence do not have significant impact on the expression of Vc transporter genes – SVCT1/2 in the G2ECs (Fig.S6B). These data indicate that the major role of Vc to promote HPC generation is mainly through epigenetic regulations. The epigenetic mechanisms, such as active or repressive histone modifications, H3K4me3/H3K27me3 have been shown to be important in different type of stem cell models, including the hematopoietic cells...
Recently, EZH1, a component of PRC2 that catalyze H3K27me3 was reported to play critical roles in restricting multipotency of primitive hematopoiesis (41). However, at earlier stage of hematopoietic development, little is known how the functional HECs are specified and what molecular events drive them into HSPCs. Our findings here reveal that Vc dependent KDM6 specifies a competent epigenetic state in endothelial cells with EHT potential, further highlighting the critical role of Vc dependent epigenetics at early stage of hematopoiesis.

Recently, Agathocleous et al reported that Slc23a2 (Svct2, the key Vc transporter) knockout mice showed mild effects on embryonic HSCs frequency in the fetal liver at E17.5. However, ascorbate-depleted mice showed higher level of HSC frequency in spleen and bone marrow, indicating that depletion of Vc might result HSC expansion at later stage. Consistently, Vc was reported to suppress leukaemogenesis through TET family enzymes, the dioxygenases that catalyze 5mC to 5hmC on DNA (28-30).

Experimental procedures

Cell lines

The hPSC lines used in this study include H1 hESCs, UC1, UC5, UH1, UH-AL, UH10 hiPSCs, H1-GATA2<sup>Y/eGFP</sup> hESCs, H1-UTX<sup>Y</sup> hESCs, H1-JMJD3<sup>Y</sup> hESCs, H1-GATA2<sup>Y/eGFP</sup>-JMJD3<sup>Y</sup> hESCs, H1-GATA2<sup>Y/eGFP</sup>-JMJD3<sup>Y</sup>, HN4 hESCs and HN4-TET1<sup>Y</sup> hESCs. Among them, H1 hESCs were obtained from WiCell Research Institute; the UC1, UC5, UH1, UH-AL and UH10 hiPSCs were derived in our lab and the other cell lines were also constructed in our lab through gene editing in the H1 or HN4 hESCs.

KDM6 gene knockout in hESCs

The gene knockout of the KDM6 family including UTX and JMJD3 were mediated by the CRISPR/Cas9 system. In detail, the sgRNA were designed on the website (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design), and further cloned into pX330 plasmid. The sequence of the sgRNA are listed below: UTX sgRNA: TAAACGACAACTTACCAAGC; JMJD3 sgRNA: GCGAACACCTCGCAGTCGCC

To delete the JMJC domain of KDM6 gene, JMJD3 for instance, we further constructed a targeting vector, which contains two homology arms cloned from the genomic DNA of the H1 and H1-GATA2<sup>Y/eGFP</sup> cell line about 1 kb in the upstream and downstream of the JMJC domain respectively. A loxP-flanked PGK-puromycin cassette was further cloned into the two homology arms in the Puc57 vector. For gene targeting, we electroporated 4 µg of the targeting vector linearized by EcoRI and 2 µg CRISPR/cas9-sgRNA into H1 hESCs. Then the cells were cultured in medium with the addition of Y-27632 (10 µM, Sigma). After about 4 days, the hESC clones were selected by puromycin (0.5 µg/mL, Sigma). The positive clones were further analyzed by PCR to select the homozygous mutation. The same strategy was used to delete JMJC domain of the UTX in H1 to generate the H1-UTX<sup>Y</sup>. For H1-JMJD3<sup>Y</sup>-2# cell lines, we electroporated 400 ng of Cre recombinase into the H1-JMJD3<sup>Y</sup> cells to remove the loxp flanked PGK-puromycin cassette, followed by seeding in a single cell state in the presence of Y-27632. About 7 days later, the clones were picked and further analyzed by PCR to select the homozygous mutation. For H1-UTX<sup>Y/-/JMJD3<sup>Y</sup>-</sup> (dKO) cell lines, the same strategy was used to delete JMJC domain of the UTX in H1-JMJD3<sup>Y</sup>-2#. The homozygous mutated clones failed to express the JMJD3 and/or UTX in mRNA level (Fig.S5C,F).

Maintenance and differentiation of hPSCs

All hPSC cell lines were maintained in mTeSR1 medium (Stem Cell Technologies) on Matrigel (1:100 dilution; BD) coated plates. Prior to differentiation, the ~80% confluent hPSCs were dissociated by Accutase (Sigma) and plated on Growth Factor Reduced (GFR) Matrigel (1:100 dilution; BD) coated 12-well plates at the initial density of 0.7 to 1.5×10<sup>5</sup>/well. Particularly, thiazovivin (0.1 µM, Selleck) was added in the culture medium to inhibit hPSCs apoptosis. After overnight culture (designated as day 0), the confluence of the plated hPSCs were around 10%. Then, the hPSCs were induced for stepwise
differentiation as described in Figure 1A. Firstly, at day 0-1 of differentiation, 40 ng/ml BMP4 (Peprotech), 30 ng/ml ACTIVIN A (Sino Biological Inc.), 20 ng/ml bFGF (Sino Biological Inc.), 6 µM CHIR99021 (Selleck), 10 µM LY294002 (Selleck) were added to the basic medium (BM, mimics of the Custom mTeSR1(42)) of DMEM/F12 (GIBCO) supplemented with 1% insulin-transferrin-selenium (ITS, GIBCO), 70 mg/ml vitamin C (Vc, 2-Phospho-L-ascorbic acid trisodium salt solution, Sigma). In particular, the osmotic pressure of the BM is about 340, adjusted by 9% NaCl. Secondly, 30 ng/ml BMP, 1 µM A8301 (Selleck) and 2 µM IWR-1-endo (Selleck) were added in the BM at day 1-2 of differentiation. Then, 40 ng/ml VEGF (Sino Biological Inc.) and 50 ng/ml bFGF were in the BM at day 2-4 of differentiation. Finally, 40 ng/ml VEGF, 50 ng/ml bFGF, 10 µM SB431542 (Selleck), 10 ng/ml SCF (Peprotech), 50 ng/ml TPO (Sino Biological Inc.), 10 ng/ml IL3 (Sino Biological Inc.) and 50 ng/ml IL6 (Sino Biological Inc.) were added in the BM at day 4-6 of differentiation and further hematopoietic commitment and maturation. The hematopoietic differentiation medium in each step should be changed every day, as the cells expanded very quickly in the system. The other OP9 co-culture and embryoid body (EB) methods for hematopoietic differentiation of hPSCs was performed according to previously publication (37). The hPSCs and differentiating cells were maintained and differentiated in standard conditions (37°C, 5% CO2, over 95% humidity).

**Real-time quantitative PCR**

The total RNA were extracted by the RaPure Total RNA Micro Kit (Magen), and 2 µg RNA were reversely transcribed into cDNA according to the manufacturer’s instruction (Takara). RT-qPCR were performed with SYBR green Master Mix (Bio-Rad). GAPDH were used for normalization. All primers used in this study were listed in the Supplemental Table 1.

**Immunofluorescence**

The cell were fixed by 4% paraformaldehyde (PFA) for 30 min and incubated with the CD31 non conjugated antibody and then stained with Alexa Fluor 568. After that, the cells were further stained with CD43-FITC antibody and followed by DAPI staining. The antibodies used in this study were listed in the Supplemental Table 2.

**Western blot**

Western blot was performed as previously described (43). Briefly, the H3 (histone 3) was used as the control and the reference for quantification of the H3K27me3 modification.

**Measurement of the KDM6 demethylase activity**

The demethylase activity of KDM6s were measured by the JMJD3/UTX demethylase activity assay colorimetric kit (Epigentek). Briefly, the nuclear factions were obtained using the NE-PER nuclear and cytoplasmic extraction kit (Thermo), and 5 ug nuclear extracts were used for the demethylase activity detection.

**Flow cytometry and Fluorescence-activated cell sorting**

For cell surface staining, cells were dissociated by Accutase (Sigma) and prepared in PBS supplemented with 2% FBS and labeled with multicolor antibody combinations, incubated in 4 ºC for 15 to 30 min. For intracellular staining, cells were fixed by fixation buffer (BD) and then permeabilized using permeabilization solution (BD) before staining. For cell sorting, DAPI were used to exclude the dead cells. For flow cytometry, samples were analyzed by C6 or Fortessa (BD); and for cell sorting, cells were sorted by the Aria (BD) or Moflo (Beckman). All the antibodies used in this study were listed in the Supplemental Table 2.

**CFU assay**

The CFU assay was conducted under the manufacturer’s instruction of Methocult H4435 (Stem Cell Technologies). Briefly, single cells of indicated number were suspended in the 100 µl IMDM supplemented with 2% FBS (Biological Industries), and then mixed with 1 ml Methocult H4435. The mixture were transferred into 35 mm ultra-low attachment plates (Stem Cell Technologies). After 12 to 16 days, the CFUs were classified and counted according to the morphology. The CFU assay were performed in standard conditions (37°C, 5% CO2, over 95% humidity). In particular, the peripheral blood CD34+ cells were isolated from the mobilized peripheral blood CD34+ cells by MACS from the volunteer, which is approved by the IRB of the Third Affiliated Hospital, Sun Yat-sen University.

**RNA-Seq**
In brief, total RNA were isolated by Directzol RNA MiniPrep kit (Zymo Research), and sequencing libraries was prepared with TruSeq RNA Sample Prep Kit (Illumina) under the manufacturer’s instruction. The samples were run on MiSeq system with MiSeq Reagent Kits v2 (50 cycles) (Illumina). All RNA-Seq data were processed as previously described (44,45). In briefly, reads were aligned to an index generated from the Ensembl transcriptome version 74 (hg38), using RSEM (v1.2.19), Bowtie2 (v2.2.5), and normalized with EDASeq (v2.2.0). Particularly, gene expression is expressed as “normalized tag count”. A threshold of at least 20 normalized tags was used to filter lowly expressed transcripts. Differential expression was performed using DESeq2 (v1.8.1) and genes were considered significant if they had a Benjamini-Hochberg corrected p value < 0.05. Gene ontology was performed using clusterProfiler.

**ATAC-Seq**

ATAC-Seq and data processing were performed according to previous reports (46-48). Briefly, 50,000 cells of each sample were used to generate DNA libraries for sequencing NextSeq 500. All sequencing data were mapped on to hg38 using bowtie2. Peaks were called using MACS2 and differential accessibility was assessed using DESeq2 as previously described (48). Regions were called differentially accessible if the absolute value of the log2 (fold change) was > 0.5 at an FDR <0.1. All genome views are to the same vertical scale (0–20).

**ChIP-Seq**

ChIP-Seq and data processing were performed as previously described (49,50). In brief, 5,000,000 cells of each sample were used to generate DNA libraries for sequencing by NextSeq 500. All sequencing data were mapped on to hg38 using bowtie2, and peaks were called using SICER. To identify the H3K4me3 and H3K27me3 associated regions and map the genes, we choose the gene region containing the peaks extending ±2.5 kb from the TSS. In addition, intersectBED was used to identify the active (H3K4me3 only), repressed H3K27me3 only (K27), bivalent or poised (H3K4/27me3) and neither (H3K4/27me3 null) gene regions. Gene ontology was performed using DAVID.

**ChIP-qPCR**

ChIP-Seq and data processing were performed as previously described (51,52). DNA samples including the Input positive control and the IgG negative control were used for SYBR Green-base quantitative PCR analysis.

**Zebrafish and morpholinos (MOs)**

The transgenic zebrafish lines flk1:mCherry and cmyb:eGFP (generously provided by Dr. Wengqing Zhang) were raised and maintained at 28.5°C. The zebrafish embryos were acquired by natural spawning, and further reared in an incubator at 28.5°C. This study was approved by the Ethical Review Committee of the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences.

The zebrafish (about 6 weeks of age) were fed with conventional lab diets (comprising artemia), Vc+ and Vc- diets respectively for over 60 days to reduce Vc concentration in the zebrafish (31). The formula of the Vc- and Vc+ diets (Dysts Inc.) were formulated according to previous publication(32). The diets contains wheat gluten (150g/kg), casein (305g/kg), Egg whites (40g/kg), cellulose (30g/kg), vitamin mix (40g/kg), mineral mix (50g/kg), α-Tocopherol (0.5g/kg) and Vitamin C (10g/kg, Vc+ diets only). Among them, the vitamin mix contains the following components: vitamin A (500,000 IU/g, 0.15g/kg), vitamin D3 (400,000 United States Pharmacopeia (USP)/mg, 6.2445g/kg), vitamin K (0.025g/kg), thiamine (0.15g/kg), riboflavin (0.25g/kg), vitamin B6 (0.125g/kg), pantothenic acid (0.75g/kg), niacin (1.25g/kg), biotin (0.005g/kg), folate (0.05g/kg), vitamin B12 (0.0005g/kg), myoinositol (6.25g/kg), para-amino benzoic acid (PABA, 1g/kg), celulil (α-cellulose, 983.75g/kg); the mineral mix contains calcium carbonate (19.23g/kg), calcium phosphate dibasic (2H2O, 766.29g/kg), citric acid (5.28g/kg), cupric carbonate (0.36g/kg), ferric citrate (2.99g/kg), magnesium oxide (22.89g/kg), iron carbonate (5.65g/kg), sodium chloride (28.02 g/kg), disodium hydrogen phosphate (11.89g/kg), zinc carbonate (0.97g/kg), potassium phosphate dibasic (74.16g/kg), potassium sulfate (62.26 g/kg), potassium iodide (0.01 g/kg). After 60 days of diets consuming, the zebrafish were mating for natural spawning to acquire the embryos for analysis. At 36 hpf, the flk1’eGFP’ zebrafishes were harvested and analyzed the emerging HPCs in the AGM region by confocal microscopy. Briefly, the zebrafish embryos were scanned by a Zeiss 710 NLO confocal laser microscope and the images were generated by 3D projections. The
flk1\textsuperscript{cmyb}\textsuperscript{+} cells in the AGM were counted as emerging HPCs.

For jmjd3 knockdown in zebrafish, the zebrafish were fed with the conventional diets, and the embryos were acquired by natural spawning. The jmjd3 translation blocking MO (5' - CCCATCTCGCTGTTACTGTGTTTTC - 3') was a gift from Dr. Yu Shanhe (Liu Tingxi's lab). The control MO (5' - CCTCTTACCTCAGTTACAATTTATA - 3') was purchased from Gene Tools. All MOs were microinjected into the embryos at 1 cell stage. Also, at 36 hpf the flk1\textsuperscript{cmyb}\textsuperscript{+} zebrafishes were harvested and analyzed the emerging HPCs in the AGM region by confocal microscopy.

**Measurement of Vc in diets and zebrafish**

To measure the Vc concentration in the diets, the diets were dissolved in H\textsubscript{2}O, centrifuged and the supernatants were collected for analysis. To detect the Vc concentration in the zebrafish, the fish about 5 days post fertilization were euthanized by overdose of tricaine, weighed and homogenized by ultrasonication. The homogenates were then centrifuged, and the supernatants were collected for analysis. The Vc concentration was determined by the HPLC with electrochemical detection as previously described (31).

**Statement**

The CFU assay involved human peripheral blood were approved by IRB of the Third Affiliated Hospital, Sun Yat-sen University. Also the study abide by the Declaration of Helsinki principles.
Supporting Information: Supplementary Figures1-6 and supplemental table1-2 were shown in the Supporting Information.

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Conflict of interest: All authors declare no conflict of interest.
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Footnotes
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Abbreviations and nomenclature
Vc, Vitamin C; KDM6, lysine demethylase 6; HSCs, Hematopoietic stem cells; HPCs, Hematopoietic progenitor cells; HECs, hemogenic endothelial cells; EHT, endothelial-to-hematopoietic transition; H3K27me3, histone H3 trimethylation at Lys-27; hESCs, human embryonic stem cells; hiPSCs, human pluripotent stem cells; AGM, aorta-gonad-mesonephros; RA, retinoic acid; PS, primitive streak; LM, lateral mesoderm; PM, paraxial mesoderm; CFUs, colony forming units; G2ECs, GATA2+ endothelial cells; Vc+_G2ECs, the G2ECs generated in the presence of Vc; Vc-_G2ECs, the G2ECs generated in the absence of Vc; PCA, principal component analysis; ATAC-seq, transposase-accessible chromatin with sequencing; CHIP-seq, chromatin immunoprecipitation followed by sequencing; TSS, transcriptional start sites; EB, embryoid body; PFA, paraformaldehyde; H3, histone 3, DA, dorsal aorta; PCV, posterior cardinal vein.
Figure legends

Figure 1 Vitamin C is required in generation of HPCs from hPSCs in defined condition (A) Scheme for human hPSC based hematopoietic differentiation. (B) FACS and RT-qPCR analysis of the indicated markers at the indicated time during differentiation. Triplicate data are represented as mean + SD of a single experiment, representative of two independent experiments (C) FACS analysis of the indicated markers’ expression during differentiation. Triplicate data are represented as mean + SD of a single experiment. (D) Phase-contrast photos of the cells at the indicated time during differentiation. Scale bar: 100µm (E) FACS analysis of the CD43+ and CD45+ cell generation at the day 8 and 10 of differentiation. (F) Phase-contrast photos of the CFUs. Scale bar: 100µm (G) CFU analysis of the 5000 CD43+ cells at the indicated time during differentiation. Triplicate data are represented as mean + SD of a single experiment. (H) FACS analysis of the CD43+ and CD45+ cell generation at the day 8 and 10 of differentiation. Triplicate data are represented as mean + SD of a single experiment, representative of three independent experiments. (J) Phase-contrast photos of the cells at day 6 of differentiation with or without Vc addition. The red arrows indicate the emerged HPCs. Scale bar: 100µm. (K) Immunostaining of CD31, CD43 and DAPI of the cells at day 6 of differentiation with or without Vc addition. The white arrows indicate the emerging CD43+ cells. Scale bar: 100µm. (L) FACS analysis and the statistics of the generation of the CD43+ HPCs at day 6 of differentiation with or without Vc addition. Error bars represent mean + SD of five independent replicates. ns., no significance; **p < 0.01. (M) CFU analysis of the 10000 CD43+ cells isolated from day 6 of differentiation with or without Vc. Error bars indicate mean + SD of 8 independent replicates; *p<0.05; ***p<0.001; ns., no significance. (N) Statistics analysis of the effects of the indicated antioxidants on the CD43+ HPC generation. Error bars represent mean + SD of three independent replicates. ns., no significance; **p < 0.01; ***p < 0.001.

Figure 2 Vitamin C is required for functional HEC specification, but not thereafter (A) FACS analysis of the indicated marker expression at the indicated stage with or without the addition of Vc. (B) Statistics analysis of the CD31+CD43+GATA2+ EC generation at day 4 of differentiation with or without the addition of Vc. Error bars represent the mean+SD; n=15; ns, no significance. (C) RT-qPCR analysis of the indicated markers during the differentiation with or without the addition of Vc. Triplicate data are represented as mean + SD of a single experiment, representative of two independent experiments. The data of the HAND1 expression in the Vc plus condition was used in the Figure 1B for the comparison of MSGN1 expression. (D) Phase-contrast analysis of the indicated cells after sorting and replating in medium with or without the addition of Vc at the indicated time; FACS analysis of the HPC generation were performed at day 4 after replating. Triplicate data are represented as mean + SD of three independent experiments; ns, no significance; *** p<0.001. Scale bar: 100µm.

Figure 3 Vc+_G2ECs and Vc-_G2ECs exhibit distinct transcriptional profile (A) Paired Pearson correlation analysis of global gene expression between Vc+_G2ECs and Vc-_G2ECs cells. R, Pearson correlation coefficient. (B) Violin plot of the expression of endothelial and blood related genes. (C) PCA analysis of the samples after sorting and replating of Vc+_G2ECs and Vc-_G2ECs cells at the indicated time in the medium without Vc addition. “HPCs 60h” and “HPCs 96h” indicate the CD43+ cells isolated at 60h and 96h after replating; while “ECs 60h” represent the CD31+CD43- cells isolated at 60h after replating. (D) Heatmap of different expressed genes clustered by the pheatmap. (E) Heatmap and go analysis of the selected down-regulated cluster and all up-regulated clusters. (F) and (G) Timecourse expression of the endothelial and blood related genes after sorting and replating of Vc+_G2ECs and Vc-_G2ECs cells at the indicated time. As for the HPCs/ECs, the HPCs refers to “HPCs 60h” in Vc+_G2ECs derived cells and ECs refers to “ECs 60h” in Vc-_G2ECs derived cells.

Figure 4 Vc prevents the poised genes to become fully repressive in G2ECs (A) Distribution of the ATAC-seq peaks in the genomic regions of the Vc+_G2ECs (Vc+) and Vc-_G2ECs cells (Vc-). (B) MA plot of the Differential accessibility (log2 fold change in reads per accessible region) plotted against the mean reads per region. (C) ATAC density heatmap of the differential opened and closed regions in Vc+_G2ECs and Vc-_G2ECs cells. (D) Selected genomic views of the ATAC-seq data at the indicated
gene locus. (E) Known motif enrichment analysis of the Vc+ G2ECs and Vc- G2ECs cells by homer. Fold enrichment was calculated by Target % / Background %. (F) Pillow analysis of the H3K4me3 and H3K27me3 density at the transcriptional start site (TSS) regions in the Vc+ G2ECs and Vc- G2ECs cells. (G) Intersect of the active (H3K4me3 only), neither (H3K4/27me3 null) and bivalent (H3K4/27me3 both) modified genes in the Vc+ G2ECs and Vc- G2ECs cells. (H) Alluvial plots (left) illustrating the histone methylation changes of all bivalent genes (K4K27) in the Vc+ G2ECs and Vc- G2ECs cells. Repressed (H3K27me3 only) (I) Go analysis (middle) of the bivalent genes lost the H3K27me3 modification. Up paneal: selected GO terms (254 GO terms in all) implicating blood cell process; down panel, top 7 of the 46 GO terms in all, and no GO terms contains blood cell process. (J) Selected genomic views of the H3K4me3 and H3K27 peaks at the indicated gene locus.

**Figure 5** Deletion of KDM6 gene family (JMJD3/UTX) and TET1 severely impaired HPC generation in hematopoietic differentiation of hPSCs (A) Western blot analysis of the global H3K27me3 in the indicated cells. The upper number represents the relative grayscale value. (B) KDM6 demethylase activity of the nuclear extracts from the indicated cells. (C) Scheme of the KDM6 gene deletion and hematopoietic differentiation. (D) Phase-contrast photos of the indicated cells at day 6 of differentiation with or without Vc addition. The red arrows indicate the emerged HPCs. Scale bar: 100µm. (E) FACS analysis of the CD43+ HPC generation from indicated cells at day 6 of differentiation with or without Vc addition. Error bar represent mean + SD of at least three independent experiment. ns, no significance; ***, p<0.01. (F) Statistics of the CD31+CD43+ endothelial cells and CD43+ HPCs generation. Error bar represent mean + SD of at least three independent experiment. ns, no significance; *, p<0.05; **, p<0.01. (G) RT-qPCR analysis of the indicated markers during the differentiation with the addition of Vc. Triplicate data are represented as mean + SD of a single experiment, representative of two independent experiments. (H) ChIP-qPCR analysis of H3K27me3 density at the indicated gene locus. Triplicate data are represented as mean + SD of a single independent experiments. (I) Genotype of the TET1 mutation in the HN4 hESCs. (J) FACS analysis and statistics of the CD34+CD31+CD43+ endothelial cells and CD43+ HPCs generated at day 6 of differentiation. Error bars represent mean + SD of two independent replicates. *p<0.05;**p < 0.01; ***p<0.001.

**Figure 6** Vitamin C and jmjd3 are required for HSC generation in zebrafish (A) The formula of the Vc- and Vc+ diets. (B) Left: Vc concentration in the Vc- and Vc+ diets; Right: Vc concentration in the zebrafish fed with the indicated diets. (C) Confocal images (left) and statistics (right) using flk1:mCherry/cmyb:GFP line detected the HSC number in the zebrafish fed with lab diets (artemia, n=11), Vc- (n=25) and Vc+ (n=22) diets respectively at 36 hpf (hours post fertilization). The white arrows indicate emerging HPCs in the AGM region. ns., no significance; ***, p<0.001. (D) Confocal images (left) and statistics (right) using flk1:mCherry/cmyb:GFP line detected the HPC number in the control (n=15) and jmjd3 (n=22) morphants at 36 hpf. The white arrows indicate emerging HSCs in the AGM region. ***, p<0.001. DA, dorsal aorta; PCV, posterior cardinal vein.
Figure 1

A

| Days differentiation |
|----------------------|
| hESCs | PS | LM | HECs/ECs | pre-HPCs | HPCs |

Induction and specification

Maturation and expansion

B

C

D

E

F

G

H

I

J

K

L

M

N
Figure 2

A

Days differentiation

0 1 4 6 (day)

hESCs PS HECs/ECs HPCs

-VC

+VC

GATA2/eGFP

CD31

Percentage of CD43+CD31+GATA2+ cells(%)

B

-VC +VC

Percentage of CD43+ cells(%)

C

OCT4

SOX2

T

HAND1

KDR

RUNX1

MYB

Relative to GAPDH

D

Sort and replate (day 4)

HPCs

Vc- G2ECs

Vc+ G2ECs

Percentage of CD3+ cells(%)

*** ns.***

-VC +VC

-by guest on March 23, 2020http://www.jbc.org/Downloaded from
Figure 3

A

B

C

D

E

F

G

Timecourse expression of blood related genes

| Gene   | RUNX1 | MYB  | IKZF1 | ITGB3 | LMO2 | NFE2 |
|--------|-------|------|-------|-------|------|------|
| Log2FC |       |      |       |       |      |      |
| 0h     |       |      |       |       |      |      |
| 24h    |       |      |       |       |      |      |
| 36h    |       |      |       |       |      |      |
| 48h    |       |      |       |       |      |      |
| 60h    |       |      |       |       |      |      |

Timecourse expression of endothelial related genes

| Gene   | CDH5  | EPHB4 | ITGA9 | JAG1  | TEK   | VWF  |
|--------|-------|-------|-------|-------|-------|------|
| Log2FC |       |       |       |       |       |      |
| 0h     |       |       |       |       |       |      |
| 24h    |       |       |       |       |       |      |
| 36h    |       |       |       |       |       |      |
| 48h    |       |       |       |       |       |      |
| 60h    |       |       |       |       |       |      |

Transmembrane receptor protein serine/threonine kinase signaling pathway

Positive regulation of leukocyte migration

Positive regulation of cell motility

Regulation of leukocyte migration

Regulation of cellular response to growth factor stimulus

Collagen metabolic process

Cardiac chamber development

Cardiac chamber morphogenesis

Collagen catabolic process

Heart morphogenesis

Trabecula formation

Angiogenesis

Gas transport

Peptidyl-tyrosine autophosphorylation

Integrin-mediated signaling pathway

Positive regulation of locomotion

Positive regulation of cell migration

Mast cell activation involved in immune response

Regulation of leukocyte migration

Positive regulation of cell motility

Mast cell degranulation
Figure 4

**A**

![Percentage (%)](image)

**B**

- Vc opened regions / close to open (CO)
- Permissive Open (PO)
- Vc closed regions / close to open (OC)

**C**

- Vc opened regions / close to open (CO)
- Permissive Open (PO)
- Vc closed regions / close to open (OC)

**D**

- Vc opened regions / close to open (CO)
- Permissive Open (PO)
- Vc closed regions / close to open (OC)

**E**

| Known motif enrichment | Type | Fold enrichment | \(-\log_{10}(p\text{-value})\) |
|------------------------|------|-----------------|--------------------------------|
| ERG                    | ETS  | 2.35            | 0.015                          |
| ETV2                   | ETS  | 2.35            | 0.015                          |
| FLI1                   | ETS  | 2.35            | 0.015                          |
| ETS1                   | ETS  | 2.35            | 0.015                          |
| ETV1                   | ETS  | 2.35            | 0.015                          |
| GABPA                  | ETS  | 2.35            | 0.015                          |
| GATA1                  | ZF   | 2.35            | 0.015                          |
| GATA2                  | ZF   | 2.35            | 0.015                          |
| PLI1                   | ETS  | 2.35            | 0.015                          |
| GATA3                  | ZF   | 2.35            | 0.015                          |
| SCL                    | bHLH | 2.35            | 0.015                          |
| MYB                    | HTH  | 2.35            | 0.015                          |
| RUNX1                  | RUNT | 2.35            | 0.015                          |
| HOXA9                  | Homebox | 2.35 | 0.015                   |

**F**

- Vc+ specific bivalent genes to Vc- HECs active genes
- Vc- specific bivalent genes to Vc+ HECs active genes

**G**

- Vc+ specific bivalent genes to Vc- HECs active genes

**H**

- Vc+ specific bivalent genes to Vc- HECs active genes

**I**

- Vc+ specific bivalent genes to Vc- HECs active genes

**J**

- Vc+ specific bivalent genes to Vc- HECs active genes
Figure 5

A

H3K27me3  
H3

Vc- G2ECs  
Vc+ G2ECs

B

Relative KDM6 demethylase activity

C

UTX  
JMJD3  
E24  
E18

D

wildtype  
UTX+/-  
JMJD3-/-  
dKO

E

wildtype  
UTX+/-  
JMJD3-/-  
dKO

F

Percentage of the CD34+CD43-CD31+ cell generation (%)

G

OCT4  
T  
HAND1  
GATA2

H

IKZF1  
MYB  
LMO2  
TAL1

I

Clone  
Genotype  
Indel

WT  
TET1 +/- 1#  
TET1 +/- 2#

TET1 +/- 1#  
TET1 +/- 2#

J

Percentage of the CD34+CD43-CD31+ cell generation (%)

Percentage of CD34+ cell generation (%)

Percentage of CD31+ cell generation (%)

Percentage of CD34+CD43+ cell generation (%)

Percentage of CD34+CD43-CD31+ cell generation (%)

Percentage of CD34+CD43+ cell generation (%)

Percentage of CD34+CD43-CD31+ cell generation (%)

Percentage of CD34+CD43+ cell generation (%)

Percentage of CD34+CD43-CD31+ cell generation (%)

Percentage of CD34+CD43+ cell generation (%)
Figure 6

A

Diet ingredients (Vc+, Vc- respectively)

| Ingredient          | g/100 g |
|---------------------|---------|
| Wheat gluten        | 15      |
| Casein              | 30.5    |
| Egg whites          | 4       |
| Cellulose           | 3       |
| Vitamin mix^a       | 4       |
| Mineral mix^b       | 4       |
| Starch              | 26.5    |
| Soybean oil         | 7       |
| Soy lecithin        | 5       |
| α-Tocopherol        | 0.05    |
| Vitamin C (Vc+ diet only) | 1       |

B

Diets

C

flk1:mCherry/cmyb:GFP

D

flk1:mCherry/cmyb:GFP
Vitamin C–dependent lysine demethylase 6 (KDM6)-mediated demethylation promotes a chromatin state that supports the endothelial-to-hematopoietic transition

Tian Zhang, Ke Huang, Yanling Zhu, Tianyu Wang, Yongli Shan, Bing Long, Yuhang Li, Qianyu Chen, Pengtao Wang, Shaoyang Zhao, Dongwei Li, Chuman Wu, Baoqiang Kang, Jiaming Gu, Yuchan Mai, Qing Wang, Jinbing Li, Yanqi Zhang, Zechuan Liang, Lin Guo, Fang Wu, Shuquan Su, Junwei Wang, Minghui Gao, Xiaofen Zhong, Baojian Liao, Jiekai Chen, Xiao Zhang, Xiaodong Shu, Duanqing Pei, Jinfu Nie, and Guangjin Pan

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