Supplementary materials and methods

Antibodies

Antibodies for western blotting were used as follows: rabbit anti-human DNMT1 (cat. no. A5495, ABclonal), rabbit anti-human glyceraldehyde-3-phosphate dehydrogenase GAPDH (cat. no. AB2000, ABways), mouse anti-human caspase-3 (cat. no.66470-2-Ig, Proteintech), mouse anti-p53 (cat. no.60283-2-Ig, Proteintech), mouse anti-p21 (cat. no.60214-1-Ig, Proteintech), rabbit anti-RPL15 (cat. no.16470-1-AP, Proteintech), mouse anti-BAX (cat. no.60267-1-Ig, Proteintech), rabbit anti-human activating transcription factor 4 ATF4 (cat. no.10835-1-AP, Proteintech), rabbit anti-human protein kinase R-like endoplasmic reticulum kinase PERK (cat. no. #5683T, Cell Signaling Technology), rabbit anti-human inositol-requiring transmembrane kinase endoribonuclease-1α IRE1α (cat. no. 3294T, Cell Signaling Technology), rabbit anti-human binding immunoglobulin protein BIP (cat. no. 3177, Cell Signaling Technology), rabbit anti-human eukaryotic initiation factor 2 eIF2α (cat. no. 5324T, Cell Signaling Technology), rabbit anti-human Phospho-eIF2α (Ser51) p-eIF2α (cat. no. 3398T, Cell Signaling Technology), mouse anti-human C/EBP homologous protein CHOP (cat. no. #2895T, Cell Signaling Technology), rabbit anti-human Phospho-IRE1α (S724) p-IRE1α (cat. no. ab124945, abcam). The antibody used for dot blotting was mouse anti-human 5-Methylcytosine 5-mC (cat. no. 39649, ACTIV MOTIF). Commercial antibodies used for flow cytometry were as follows: APC-conjugated CD235a (GPA) (cat. no. 551336, BD), PE-conjugated CD235a (GPA) (cat. no. 8065789, BD), PE-conjugated CD34 (cat. no.555822, BD), PE-conjugated CD49d (α4 integrin cat. no.12-499-42, invitrogen), APC-conjugated CD49d (α4 integrin) (cat. no.130-124-229, MACS), FITC-conjugated CD36-fluoresce (cat. no.555454, BD), PE-cy7- conjugated CD123 (IL-3R) (cat. no.25-1239-42, invitrogen) APC-conjugated annexin V (cat. no.88-8103-74, eBioscience), FITC-conjugated annexin V (cat. no.640945, Biolegend) Hoechst33342 (cat. no.C0031, Solarbio), and 7AAD (cat. no.00-6993-50, eBioscience). Mouse monoclonal antibody against human band 3 was generated in our laboratory and labeled with FITC or APC as described previously [4].

Culture of CD34+cells

The cell culture details have been described previously [3,4]. The details were as follows: The day of getting CD34+ cells were recorded as day 0. The cell culture procedure was comprised of 3 phases. Composition of the base culture medium was Iscove's Modified Dulbecco's Medium, 2% human peripheral blood plasma, 3% human AB serum, 200 mg/mL human Holo-transferrin, 3 IU/mL heparin, and 10 mg/mL insulin. In the first phase (day 0 to day 7), CD34+ cells at a concentration of 105 /mL were cultured in the presence of 10 ng/mL stem cell factor, 1 ng/mL IL-3, and 3 IU/mL erythropoietin. In the second phase (day 7 to day 11), IL-3 does not need to be added into the culture medium. In the third phase that lasted until day 21, the cell concentration was adjusted to 106 /mL on day 11 and to 5×106/mL on day 15, respectively, the medium for this phase was the base medium plus 3 IU/mL erythropoietin, and the concentration of transferrin was adjusted to 1 mg/mL.

Cell cycle analysis

Edu kit was used for cell cycle measurement according to the manufacturer's protocol. In brief, 1 × 10^6 cells were incubated with 10 μM Edu for 2 h at 37 °C. After incubation, the cells were harvested and washed with 3 ml of 1% BSA in PBS. Cells were then fixed, permeabilized, and stained with Edu detection cocktail as well as 7AAD. The staining of Edu and 7AAD was analyzed by flow cytometry. Data were collected and analyzed using FlowJ, and the data are

expressed as EdU fluorescence intensity versus 7AAD [23].

RNA extraction and quantitative reverse transcription-PCR assays

RNA was extracted from cell cultures using RNA extract kits (cat.no.74104, Qiagen) according to the manufacturer's instructions. Reverse transcription was performed using a HiFi-MMLV cDNA Kit (cat.no. CW0744M, CWBIO). Quantitative reverse transcription PCR (qRT-PCR) assays were completed using SYBR™ Select Master Mix (cat.no. 4472903, Thermo Fisher Scientific). Quantitative reverse transcription-PCR was carried out on a PCR platform (LightCycler 480, Roche Life Science) using a master mix according to the manufacturer's instructions. Relative expression levels were normalized to that of GAPDH. The primer sequences are listed in Table 2. Detailed protocols for the RNA extraction, reverse transcription, primer design, and quantitative reverse transcription-PCR have been described previously [15].

Western blotting

Total cell lysates were prepared using RIPA buffer (cat.no. 89900, Thermo Fisher Scientific) in the presence of proteinase inhibitor Cocktail (cat.no. P8340, Sigma). Protein concentration was measured using a BCA protein assay kit (cat.no. P1511-5, PPLYGEN). All procedures were performed as described previously [15].

Supplementary Figure Legend

Supplemental Fig. 1 Effects of DNMT1 deficiency on early and terminal erythroid differentiation.

(A) Flow cytometric analysis of erythroid progenitor populations. Cells cultured for 6 days were stained with antibodies against IL-3R, GPA, CD34 and CD36. The IL-3R and GPA double-negative population was further separated by CD34 and CD36. The $IL\text{-}3R^{-}GPA^{-}CD34^{+}CD36^{-}$ population was identified as BFU-E and the $IL\text{-}3R^{-}GPA^{-}CD34^{-}CD36^{+}$ population was identified as CFU-E. (B) Quantification of BFUE and CFU-E at day6.

Supplemental Fig. 2 Effects of DNMT1 deficiency on early and terminal erythroid differentiation.

(A) The expression of GPA on day 7 of culture: the GPA^{+} cells were gated. (B) Quantification of GPA^{+} positive cells at indicated days. (C) Terminal erythroid differentiation was monitored on indicated days by flow cytometric analysis based on the expression of band 3 and α4 integrin. Representative plots of α4-integrin vs band 3 of GPA^{+} cells are shown and the erythroblasts are separated into 5 populations: proerythroblasts (I), early basophilic erythroblasts (II), late basophilic erythroblasts (III), polychromatic erythroblasts (IV), and orthochromatic erythroblasts (V). (D) Quantitative analyses of erythroblasts at distinct stages. (E) Representative cytospin images of erythroblasts on 7,9,11,13 and 15 days stained with May-Grünwald Giemsa. Scale bar = 10μm. The results are from 3 independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

Supplemental Fig. 3 DNMT1 regulate p21 methylation level in erythroid progenitors.

(A) DNA dot blots for 5mC at day6. (B) Quantitative analysis of 5mC levels from 3 independent experiments. (C) Prediction of p21 promoter regions CPG island.

Supplemental Fig. 4 DNMT1 regulated RPL15 promoter regions methylation level in late stage of terminal erythroblasts.

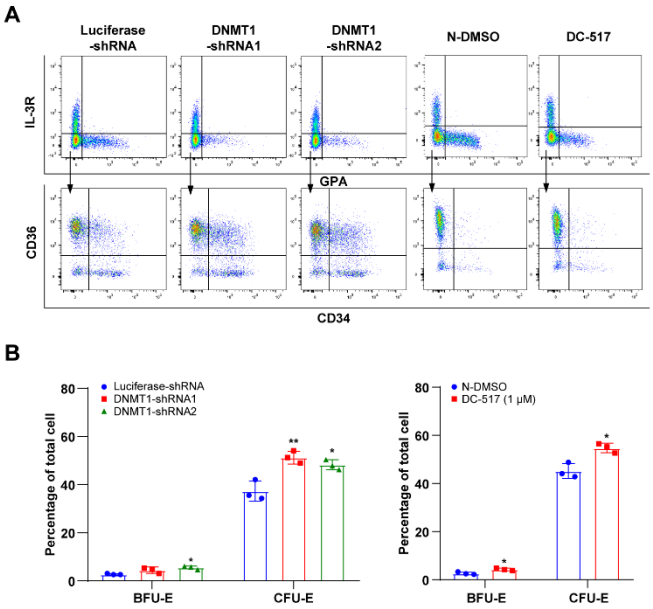
(A) DNA dot blots for 5mC at day15. (B) Quantitative analysis of 5mC levels from 3 independent experiments. (C) Prediction of RPL15 promoter regions CPG island.

Supplemental Fig. 5 DNMT1 deficiency increased apoptosis independent of p21 in late erythroblasts.

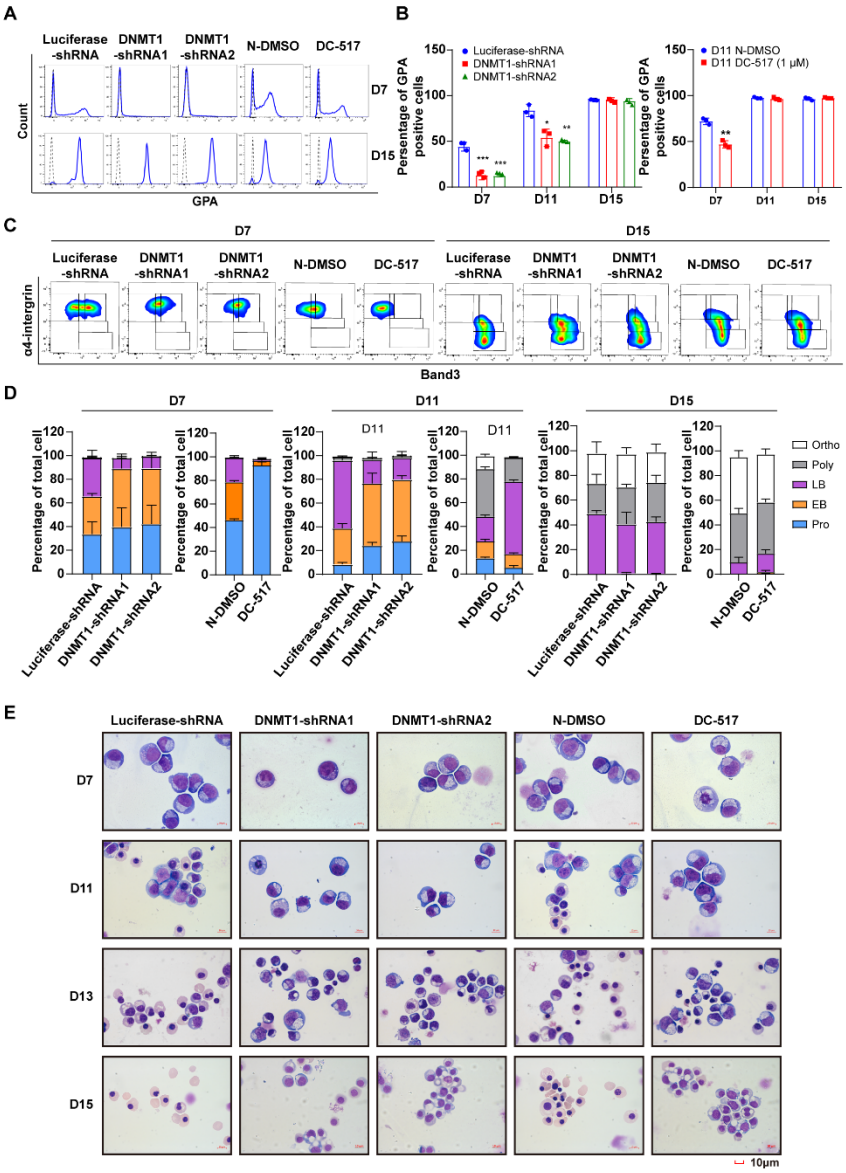
(A) Representative flow cytometry profiles of apoptosis as assessed by Annexin V and 7AAD staining of luciferase-shRNA and DNMT1-shRNA cells cultured for 15 days in the presence of DMSO or 1μM UC2288. (B) Quantitative analysis of apoptosis from three independent experiments. (C) Representative flow cytometry profiles of apoptosis as assessed by Annexin V and 7AAD staining of Normal-DMSO and DC_517 1μM cells cultured for 15 days in the presence of DMSO or 1μM UC2288. (D) Quantitative analysis of apoptosis from three independent experiments.

Supplemental Fig. 6 The mRNA express of p53 pathway genes and ER stress downstream genes

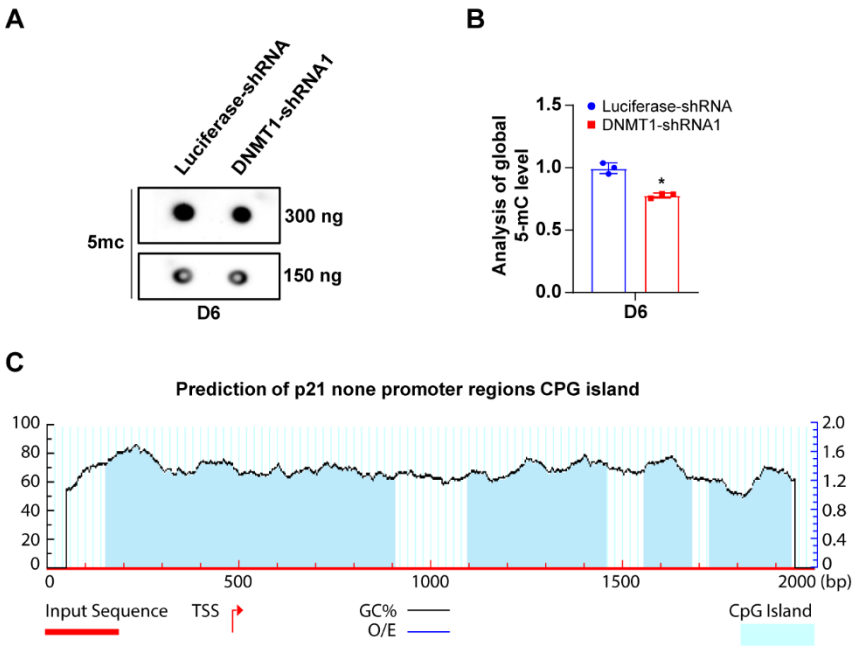
(A) The FPKM of TP53, BAX and P21. (B) qRT-PCR results showing ATF4, XBP1, DDIT3 mRNA expression levels in DNMT1 deficiency erythroblasts cultured for 15 days. GAPDH was used as internal reference.



Supplemental Fig. 1 Effects of DNMT1 deficiency on early and terminal erythroid differentiation.



Supplemental Fig. 2 Effects of DNMT1 deficiency on early and terminal erythroid differentiation.



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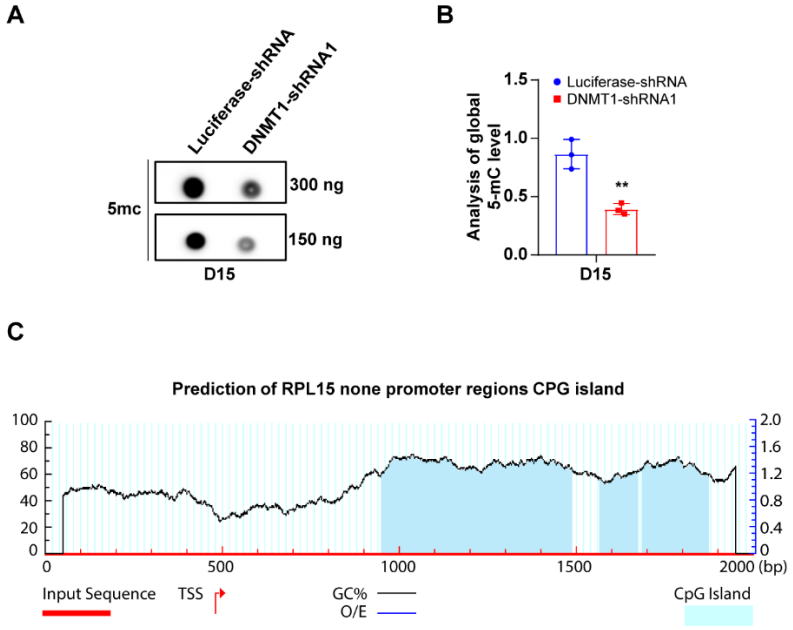
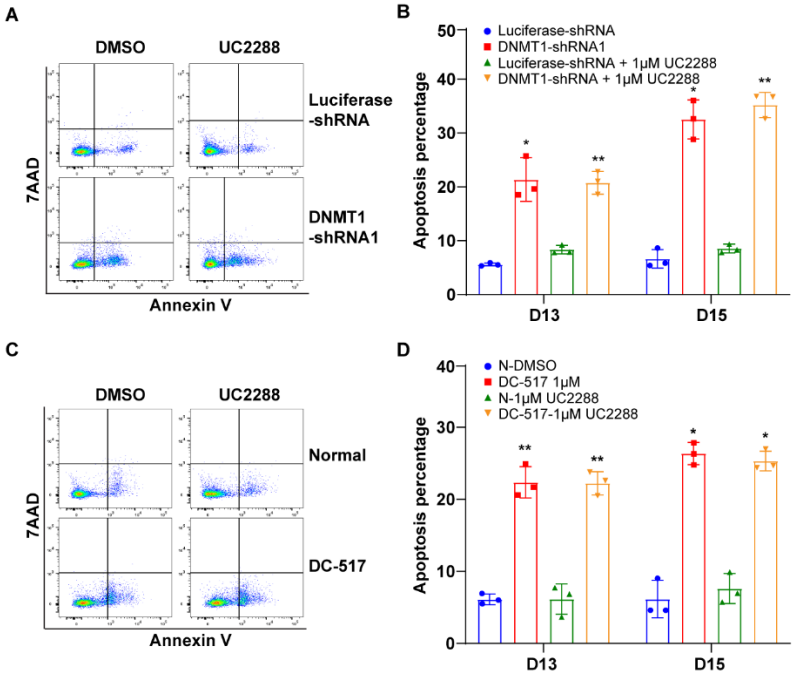


Supplemental Fig. 4 DNMT1 regulated RPL15 promoter regions methylation level in late stage of terminal erythroblasts.



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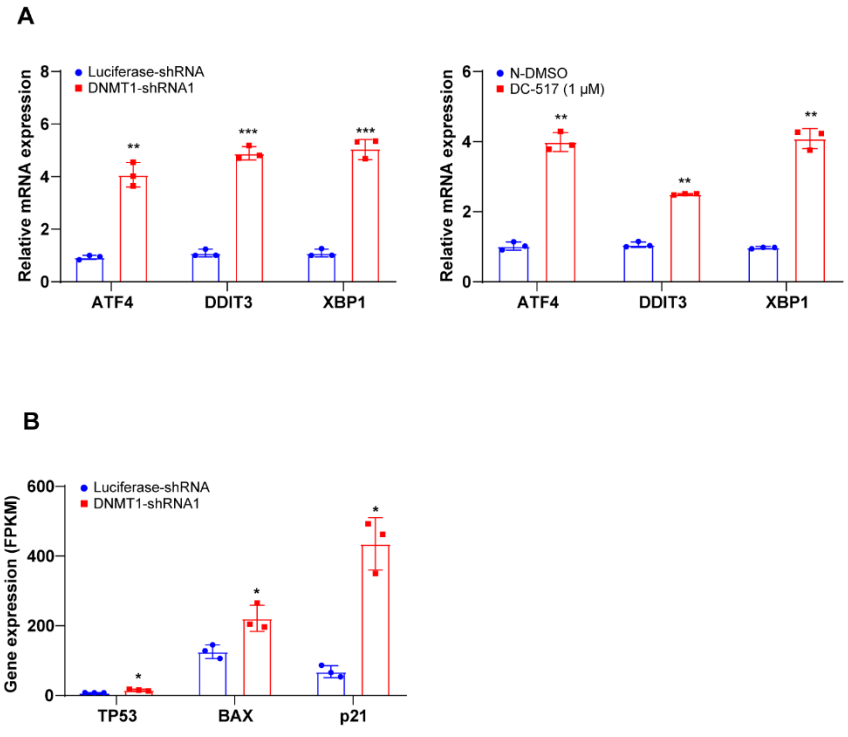

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