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FAM122A Inhibits Erythroid Differentiation through GATA1

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SUMMARY

*FAM1*22A is a highly conserved housekeeping gene, but its physiological and pathophysiological roles remain greatly elusive. Based on the fact that *FAM1*22A is highly expressed in human CD71^{+} early erythroid cells, herein we report that FAM122A is downregulated during erythroid differentiation, while its overexpression significantly inhibits erythrocytic differentiation in primary human hematopoietic progenitor cells and erythroleukemia cells. Mechanistically, FAM122A directly interacts with the C-terminal zinc finger domain of GATA1, a critical transcriptional factor for erythropoiesis, and reduces GATA1 chromatin occupancy on the promoters of its target genes, thus resulting in the decrease of GATA1 transcriptional activity. The public datasets show that *FAM1*22A is abnormally upregulated in patients with β-thalassemia. Collectively, our results demonstrate that FAM122A plays an inhibitory role in the regulation of erythroid differentiation, and it would be a potentially therapeutic target for GATA1-related dyserythropoiesis or an important regulator for amplifying erythroid cells *ex vivo*.

INTRODUCTION

Erythropoiesis, a stepwise process of differentiation by which red blood cells (RBCs) are generated from hematopoietic stem and progenitor cells (HSPCs), is finely controlled by master transcription factors that tightly regulate erythroid-specific gene expression networks (Alvarez-Dominguez et al., 2017; Li et al., 2019; Merryweather-Clarke et al., 2011; Nandakumar et al., 2016; Perreault and Venters, 2018). The core erythroid network of transcription factors is comprised of DNA-binding GATA1, TAL1, and KLF1, as well as non-DNA-binding LDB1 and LMO2 (Nandakumar et al., 2016; Xu et al., 2012). For example, it is well known that erythroid differentiation depends upon GATA-1 in a dose-dependent manner, which is also important for the survival and cell-cycle regulation of erythroid progenitors by erythropoietin (EPO) signaling (Fujiwara et al., 1996; Gutierrez et al., 2020; Xu et al., 2012). Accordingly, GATA1 deficiency arrests erythropoiesis at a proerythroblast stage and induces apoptosis (Fujiwara et al., 1996; Gutierrez et al., 2020).

As a housekeeping gene, *FAM1*22A (also known as *C9orf42*) is highly conserved among a variety of mammalian species (Eisenberg and Levanon, 2013). Previously, we reported that FAM122A inhibits the phosphatase activity of protein phosphatases of the type 2A family (PP2A) by interacting with its Aα scaffold and Bα regulatory subunits (Fan et al., 2016), a major fraction of cellular Ser/Thr phosphatase activity in any given human tissue, to play important roles in germ cell maturation, embryonic development, metabolic regulation, tumor suppression, and homeostasis of many adult organs (Reynhout and Jans-

sens, 2019). We also demonstrated that FAM122A is critical for maintaining the growth of hepatocellular carcinoma cells and acute myeloid leukemia (AML) cells in a PP2A activity-independent or -dependent manner (Liu et al., 2020; Zhou et al., 2020). However, the biological functions of FAM122A protein are poorly understood to date. Based on the fact that *FAM1*22A is highly expressed in human CD71^{+} early erythroid cells, here we report that FAM122A significantly inhibits erythrocyte differentiation in primary human erythroid cells and erythroleukemia cells through interacting with and inhibiting the transcriptional activity of GATA1.

RESULTS

Downregulation of FAM122A Expression during Erythroid Differentiation

During erythropoiesis, CD34, CD71, GATA1, and hemoglobin are expressed closely in relation to early and late erythroid progenitors, such as erythroid burst-forming unit (BFU-E) and colony-forming unit (CFU-E) and the early stages of erythroid terminal differentiation. In brief, CD34 is a marker of progenitor cells, such as early erythropoietic progenitors, including BFU-E and CFU-E but is lost with differentiation, while CD71 is expressed in late erythroid progenitors and during the early stages of terminal erythroid differentiation (Chen et al., 2009; Hu et al., 2013; Li et al., 2014). In search of the human *FAM1*22A gene expression in BioGPS datasets (http://biogps.org/dataset/GSE1133/geneatlas-u133a-gcrma/) (Su et al., 2004), we found that *FAM1*22A is highly expressed in
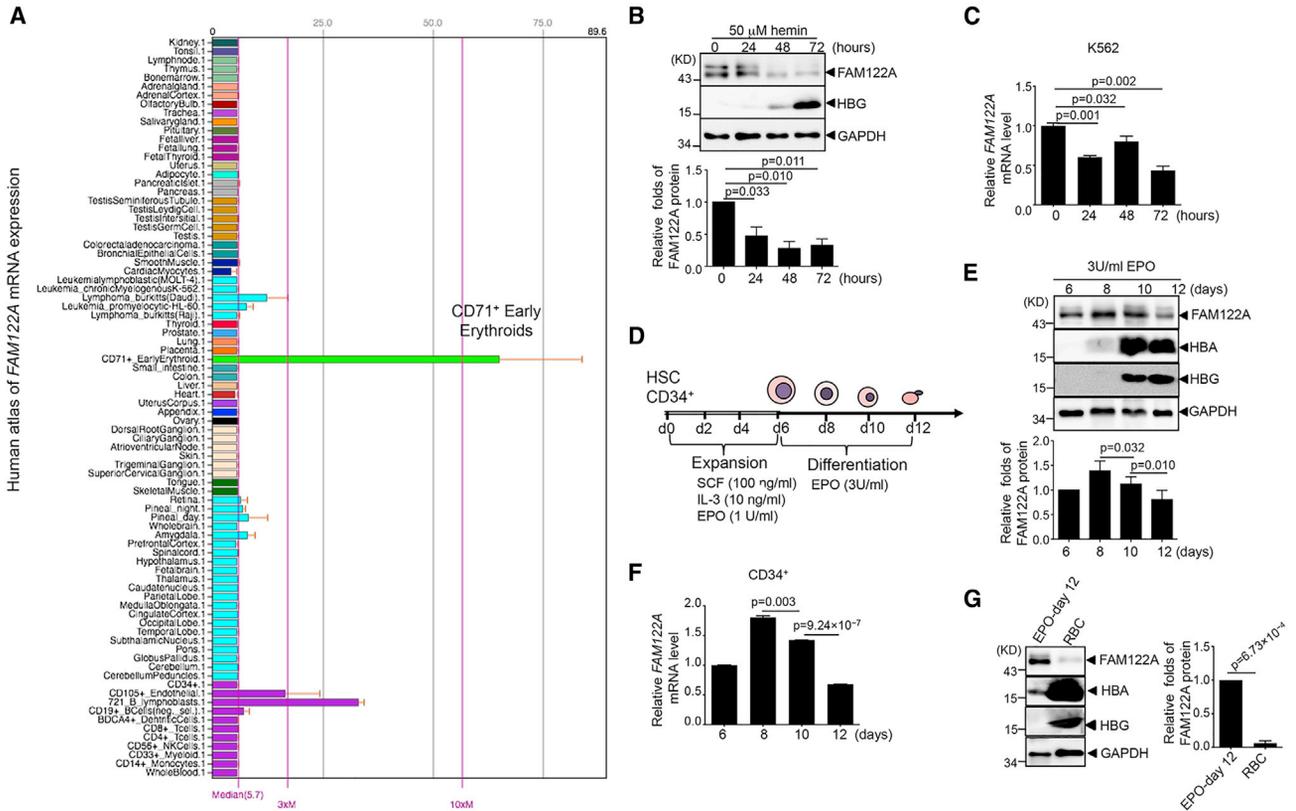
Figure 1. FAM122A Is Downregulated during Erythroid Differentiation in Human K562 and CD34+ Cells

(A) The expression of *FAM122A* gene was assessed in a variety of human tissues and cells from the gene annotation BioGPS database.

(B and C) K562 cells were treated with 50 μM hemin for the indicated times. The expression levels of FAM122A protein and mRNA were, respectively, examined by western blot (n = 3) (B) and qPCR (n = 3) (C). mRNA level data indicate the means with bar ± SD in an independent experiment (C).

(D) Schematics indicated the process of CD34+ cell expansion and differentiation induction by EPO.

(E–G) FAM122A levels were examined by western blot (E and G) (n = 3) and qPCR (F) (n = 3), in which the cells were treated as indicated in (D). GAPDH was a protein loading control, and HbG or HbA were used as indicators of erythroid differentiation. FAM122A proteins were quantified according to the densitometric value and the relative protein levels against control cells are shown as means ± SD from three independent experiments bottom panels (B and E) and right panel (G).

human CD71+ erythroid cells among all tissues and cells (Figure 1A). Thus, we asked whether FAM122A is involved in erythrocytic development, during which GATA1 is expressed in both early and late erythroid progenitors and during the early stages of terminal differentiation, but is at peak expression level in late erythroid progenitors at the same time as CD71 expression (Kobayashi and Yamamoto, 2007), and hemoglobin (Hb) is expressed during terminal differentiation. Also, erythroid cells at successive terminal stages of human erythropoiesis were also identified by using the combination of glycophorin A (GPA) and Band 3 (Auffray et al., 2001; Hu et al., 2013). Toward this end, we used hemin at 50 μM to treat human erythroleukemic K562 cells as a cellular model for erythroid differentiation induction, as indicated by benzidine staining-positive (DAB+) cells (an indicator for Hb production) and

accumulation of HbG (Rutherford et al., 1979; Wang et al., 2018). More intriguingly, our results demonstrated that FAM122A mRNA and protein levels were gradually decreased upon hemin-induced erythroid differentiation (Figures S1A, 1B, and 1C). We also *in vitro* expanded primary CD34+ HSPCs from human umbilical cord blood by 100 ng/mL stem cell factor (SCF), 10 ng/mL interleukin-3 (IL-3), and 1 U/mL EPO for 6 days, followed by 3 U/mL EPO for an additional 6 days, as depicted in Figure 1D. Consistent with the previous report (Sun et al., 2015), the treatment effectively induced CD34+ cells to undergo erythroid maturation, as assessed by morphological features and HbA/HbG expression (Figures S1B and 1E). As expected, both FAM122A protein and mRNA were reduced in terminal erythroid differentiation after EPO treatment for 12 days (Figures 1E and 1F). In line, human RBCs are
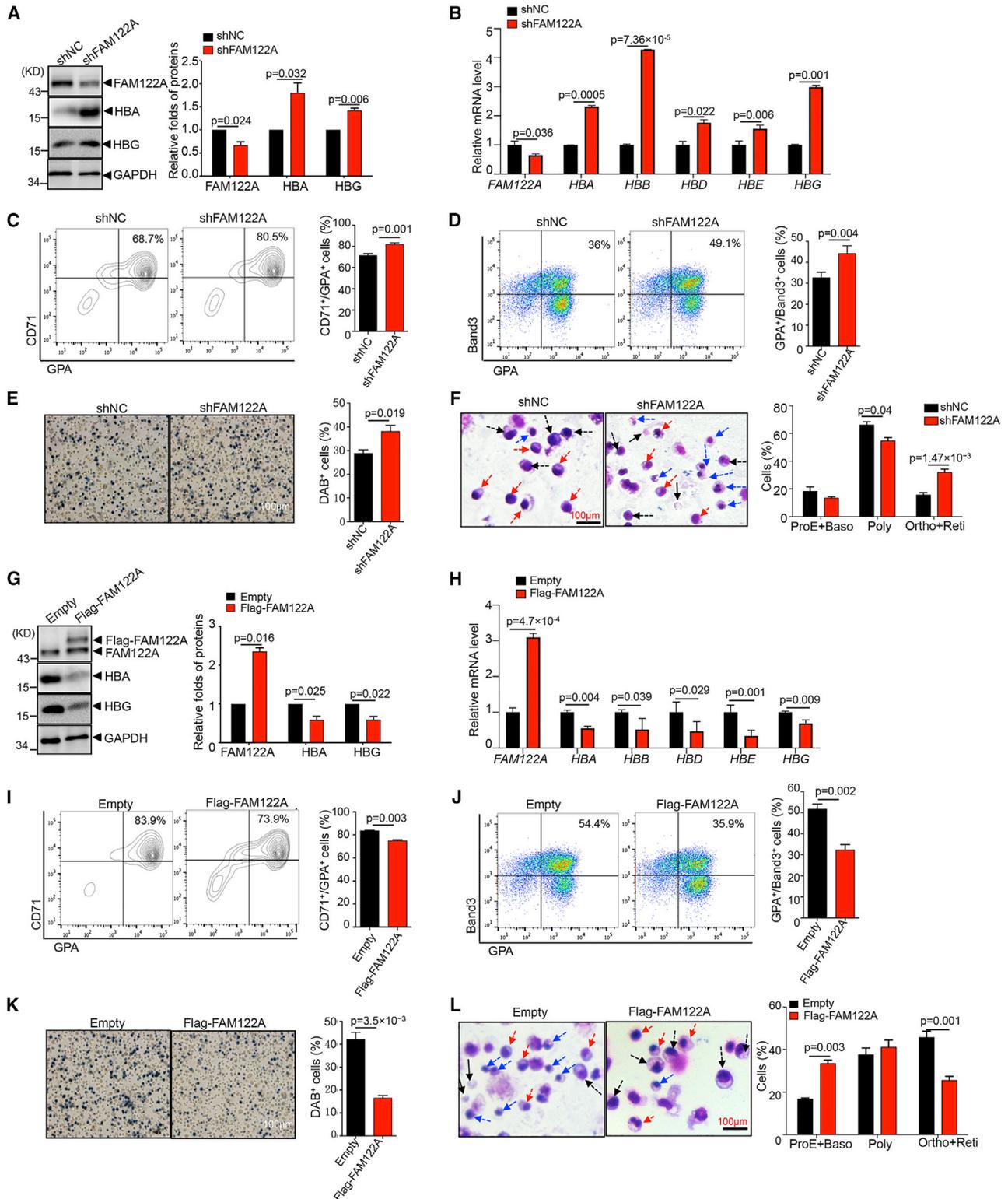
Figure 2. The Effects of FAM122A Modulation on EPO-Induced Erythroid Differentiation in CD34⁺ Cells

CD34⁺ cells were infected with lentivirus carrying shFAM122A or negative control shRNA (shNC) (A–F), as well as Flag-FAM122A or empty vectors (G–L) for 48 h, followed by induction with EPO at 3 U/mL for 4 days. Lentivirus-infected CD34⁺ cells with EPO treatment were

(legend continued on next page)

absent of FAM122A protein (Figure 1G). All these results indicate that FAM122A is downregulated during erythroid differentiation.

Inhibition of Terminal Erythroid Differentiation by FAM122A

Next, we attempted to explore the potential roles of FAM122A in erythropoiesis by small hairpin RNA (shRNA)-mediated knockdown in human CD34$^+$ HSPCs from human umbilical cord blood. For this, CD34$^+$ cells were expanded for 4 days and followed by lentivirus infection with specific shRNA against FAM122A (shFAM122A) or a negative control shRNA (shNC). Two days post-infection, a significant silencing effect was confirmed in shFAM122A-expressing CD34$^+$ cells (Figure S1C). By utilizing the flow cytometry-based strategy for isolating human BFU-E and CFU-E (Li et al., 2014), we found that FAM122A knockdown did not impact the amounts of BFU-E (IL-3R$^-$GPA$^-$CD34$^+$CD36$^-$) and CFU-E (IL-3R$^-$ GPA$^-$CD34$^-$CD36$^+$) populations (Figure S1D), and also failed to affect their colony-forming abilities (Figure S1E). Thus, we examined whether FAM122A knockdown impacts terminal erythroid differentiation in CD34$^+$ cells induced by EPO. Intriguingly, the results showed that FAM122A knockdown significantly increased various globin protein and/or gene expressions (Figures 2A and 2B), and enhanced the percentages of CD71$^+$/GPA$^+$ and GPA$^+$/Band3$^+$ cells (Figures 2C and 2D) and DAB$^+$ cells (Figure 2E). The morphological observation also showed that, under EPO induction for 4 days, the percentages of orthochromatic erythroblasts and reticulocytes were significantly increased in FAM122A knockdown CD34$^+$ cells (Figure 2F).

We also used CRISPR/Cas-9 to delete FAM122A in K562 cells, and found that FAM122A knockout (FAM122A KO) significantly enhanced hemin-induced erythroid differentiation (Figures S2A–S2C), which could be significantly rescued by re-expression of FAM122A (Figures S2D–S2F). Because the *FAM122A* gene is localized within the first intron of *PIP5K1B* (*phosphatidylinositol 4-phosphate 5-kinase*), we also found that PIP5K1B knockdown failed to influence hemin-induced erythroid differentiation (Figures S2G–S2I), excluding the role of this gene in erythroid differentiation. Notably, FAM122A knockout itself could promote the expressions of globin genes and HbG protein, and increased DAB$^+$ cells, indicating that FAM122A deletion may spontaneously trigger or be prone to erythroid differentiation.

Reciprocally, Flag-FAM122A overexpression did not influence BFU-E/CFU-E population and their colony-forming capacities *in vitro* (Figures S1F–S1H), but markedly suppressed EPO-induced erythroid differentiation (Figures 2G–2L). Also, FAM122A overexpression could significantly inhibit hemin-induced erythroid differentiation of K562 cells (Figures S2J–S2L). Cumulatively, our results indicate that FAM122A is a negative regulator for erythroid differentiation.

Contribution of GATA1 to FAM122A-Regulated Erythroid Differentiation

To identify the possible proteins interacting with FAM122A, we incubated nuclear extracts of K562 cells together with the *in-vitro*-translated GST-FAM122A with GST as a control, followed by GST pull-down. The precipitates were fractionated by SDS-PAGE and stained with Coomassie brilliant blue. The separated proteins by electrophoresis from GST-FAM122A- and GST-bound lysates were excised and further identified by liquid chromatography-tandem mass spectrometry analysis (Table S1). In total, we identified 142 FAM122A-interacting proteins, including GATA1 (Figures 3A and 3B), the latter being further confirmed by western blot (Figure S3). On the other hand, we also performed RNA sequencing to examine the global gene expression profiling of K562 cells with (FAM122A KO no. 1) and without (NC) FAM122A knockout (Figures 3C and 3D). A comparison of the transcriptomes using a statistical cutoff of p < 0.01 and a fold change >1.5 revealed that FAM122A knockout significantly altered the transcriptome of K562 cells with 133 increasing transcripts and 129 decreasing ones (Table S2). By gene ontology analysis, many upregulated genes were closely associated with the components and functions of hemoglobin for molecular functions, cellular components, or biological processes (Figures 3C and 3D; Table S2). Among these upregulated genes, some of which were confirmed by qPCR (Figure 3E), globins (*HbA1*, *HbA2*, *HbB*, *HbG1*, and *HbG2*), *ALAS2* (a critical enzyme

analyzed for the indicated protein expression with western blot (A and G) (n = 3), various globin gene levels with qPCR (B and H) (n = 3), the percentages of CD71$^+$/GPA$^+$ (C and I) (n = 3) and GPA$^+$/Band3$^+$ cells (D and J) (n = 3) with FACS, the percentages of DAB$^+$ cells (E and K) (n = 3), together with morphological observation with Giemsa staining (F and L) (n = 3). The qPCR data were analyzed by normalizing against the corresponding shNC or empty treated with EPO for 4 days (B and H). The related quantitative data are shown on the right panels (A, C, D, E, F, G, I, J, K, and L). The percentages of distinct stages during erythroid differentiation were calculated and shown, in which solid black arrows indicate reticulocytes, dotted blue arrows indicate orthochromatic erythroblasts, dotted red arrows indicate polychromatic erythroblasts, and dotted black arrows indicate proerythroblasts or basophilic erythroblasts. ProE + Baso, proerythroblasts and basophilic erythroblasts; Poly, polychromatic erythroblasts; Ortho + Reti, orthochromatic erythroblasts and reticulocytes (F and L), right panels.
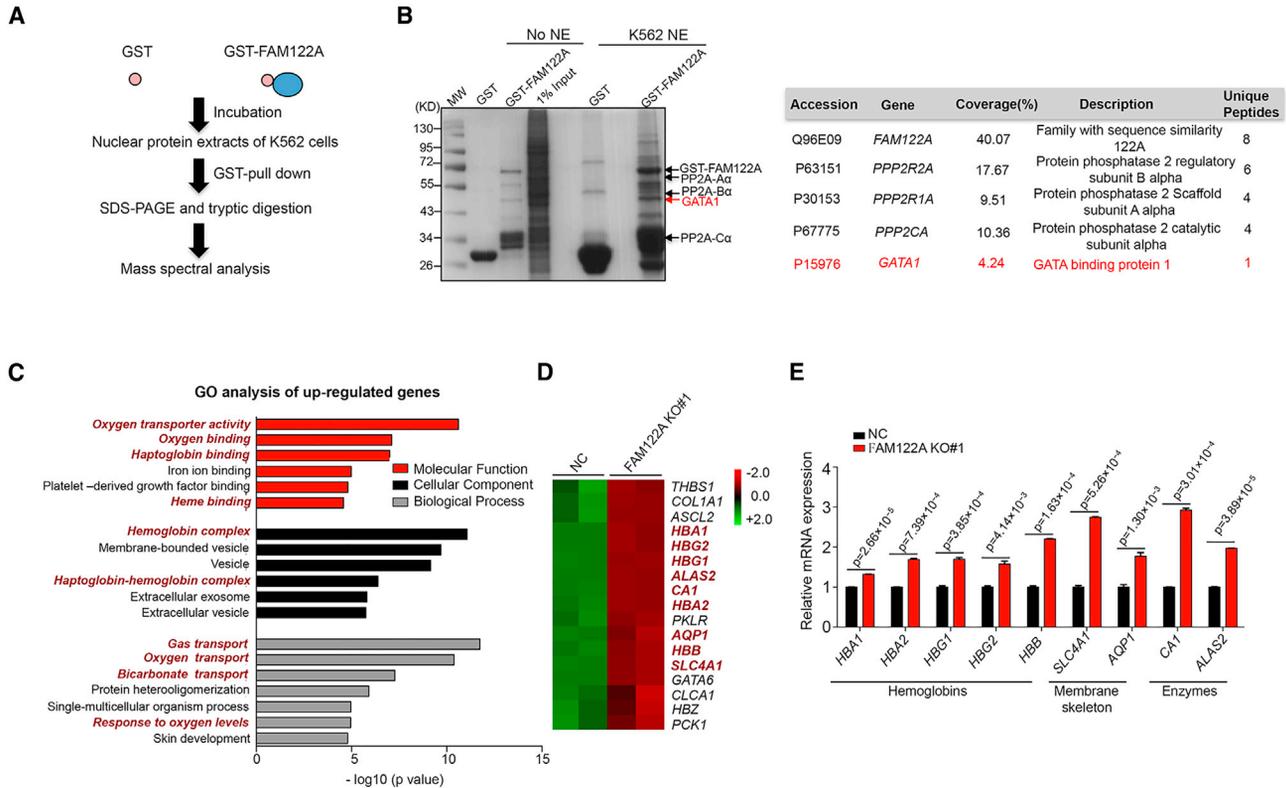
Figure 3. GATA1 Is a Potentially Interacted Protein with FAM122A and the Gene Regulation Profiling upon FAM122A Knockout

(A and B) The potential interacted proteins with FAM122A were analyzed by *in vitro* binding assay and MS analysis. Schematics showed the process of the experiments (A). Coomassie brilliant blue-stained gel of proteins bound to GST and GST-FAM122A are shown, followed by identification with tandem mass spectrometry (B). The indicated bands referred to GST-FAM122A, GATA1, and the known FAM122A interacting proteins, PP2A-Aα, -Bα, and -Cα (left panel). The indicated proteins were analyzed by scaffold 4 proteome software and their information is presented (right panel). Peptides and coverage indicate the numbers of unique peptides and the percentage of sequence coverage for identified proteins.

(C and D) FAM122A KO and NC K562 cells were subjected to RNA sequencing and bioinformatics analysis. Differentially expressed genes were analyzed by gene ontology (C), and the most upregulated genes in FAM122A KO K562 cells were enriched as indicated in molecular functions, cellular components and biological processes. Heatmap presents the enriched candidate and upregulated genes involved in erythroid maturation and function as indicated with red words (D).

(E) The representative candidate genes regulated by FAM122A were confirmed by qPCR (n = 3). Data indicate means with bar as SD in an independent experiment.

of heme synthesis) (Kaneko et al., 2014), carbonic anhydrase 1 (*CA1*) (an enzyme for catalyzing the reversible hydration of CO_2), and membrane skeleton-related genes *SLC4A1* and *AQP1* are specific target genes of GATA1 (An et al., 2014).

The above-described results led us to extrapolate that GATA1 has a role in FAM122A-modulated erythroid differentiation. To consolidate this, two pairs of GATA1 shRNAs (shGATA1 no. 1 and shGTAT1 no. 2) with shcontrol were infected into K562 cells to knockdown GATA1 in either FAM122A knockdown or shNC cells (Figure 4A). Subsequently, these cells were, respectively, treated with or without hemin at 50 μM for 48 h. As shown in Figures 4B–4D, GATA1 knockdown significantly abrogated

the effects of FAM122A silencing-increased hemoglobin and globin gene expressions either in the presence or absence of hemin induction. However, knockdown of GATA2 did not affect the effects of FAM122A silencing on differentiation (Figures S4A–S4C). These results suggest that GATA1 mediates FAM122A-regulated erythroid differentiation.

Direct Interaction of FAM122A with GATA1

To elucidate how GATA1 works in FAM122A-regulated erythroid differentiation, we found that FAM122A did not affect GATA1 expression in K562 and CD34+ cells (Figure S4D and S4E). As described above, our protein interactomic analysis showed
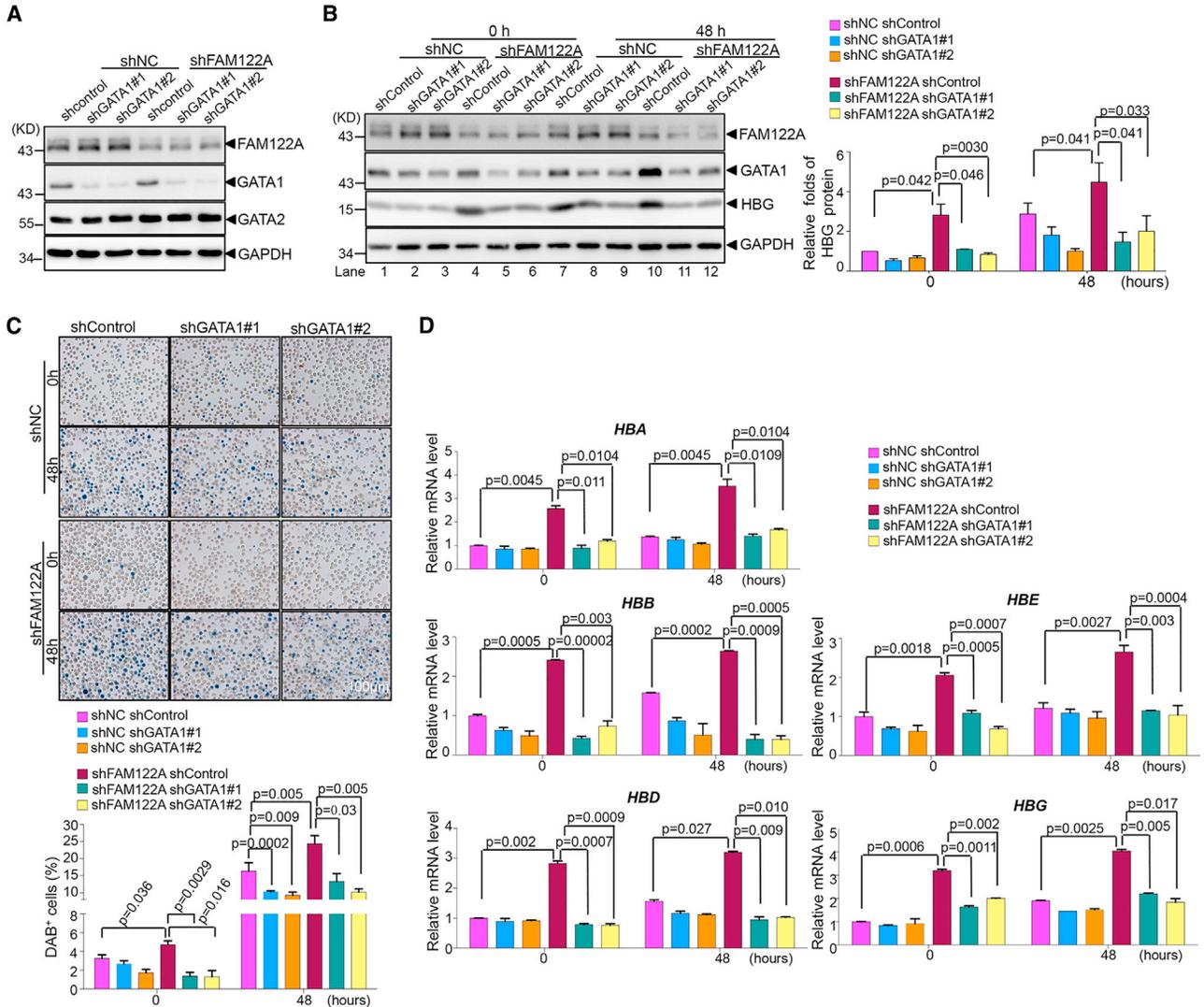
Figure 4. GATA1 Mediates FAM122A Deletion-Enhanced Erythroid Differentiation

K562 cells with stably expressing shNC and shFAM122A were further infected with lentiviruses expressing two pairs of GATA1 shRNAs, together with control shRNA as controls (shcontrol). The indicated cells were treated with or without 50 μM hemin for 48 h.

(A) The specific knockdown effect of GATA1 was confirmed by western blot (n = 3).

(B) HbG protein expression as an indicator for erythroid differentiation was examined by western blot (left panel) and its relative expression level was calculated by normalizing against shNC/shcontrol cells (right panel) (n = 3).

(C) Representative images of benzidine staining are shown in the indicated cells (upper panel) and the percentages of DAB+ cells were calculated and shown by observing five random fields (bottom panel) (n = 3).

(D) Expressions of various globin genes were examined by qPCR. Data were normalized as relative fold changes against shNC/shcontrol cells without hemin treatment and displayed as means ± SD in an independent experiment (n = 3).

that FAM122A interacts with GATA1. To confirm this, 293T cells were co-transfected with Flag-tagged FAM122A and GFP-tagged GATA1, followed by co-immunoprecipitation assays. The results showed that Flag-FAM122A could pull-down GATA1, and GFP-GATA1 could reciprocally precipitate FAM122A (Figure 5A). The physical interaction of endogenous FAM122A and

GATA1 was further confirmed in K562 cells (Figure 5B). The immunofluorescence assay also revealed intranuclear colocalization of FAM122A and GATA1 proteins in K562 and CD34+ cells (Figure 5C). The *in vitro* GST pull-down assay further showed that FAM122A interacts directly with GATA1 (Figure 5D). With a serious of GATA1-truncated mutants (Figure 5E), we showed that
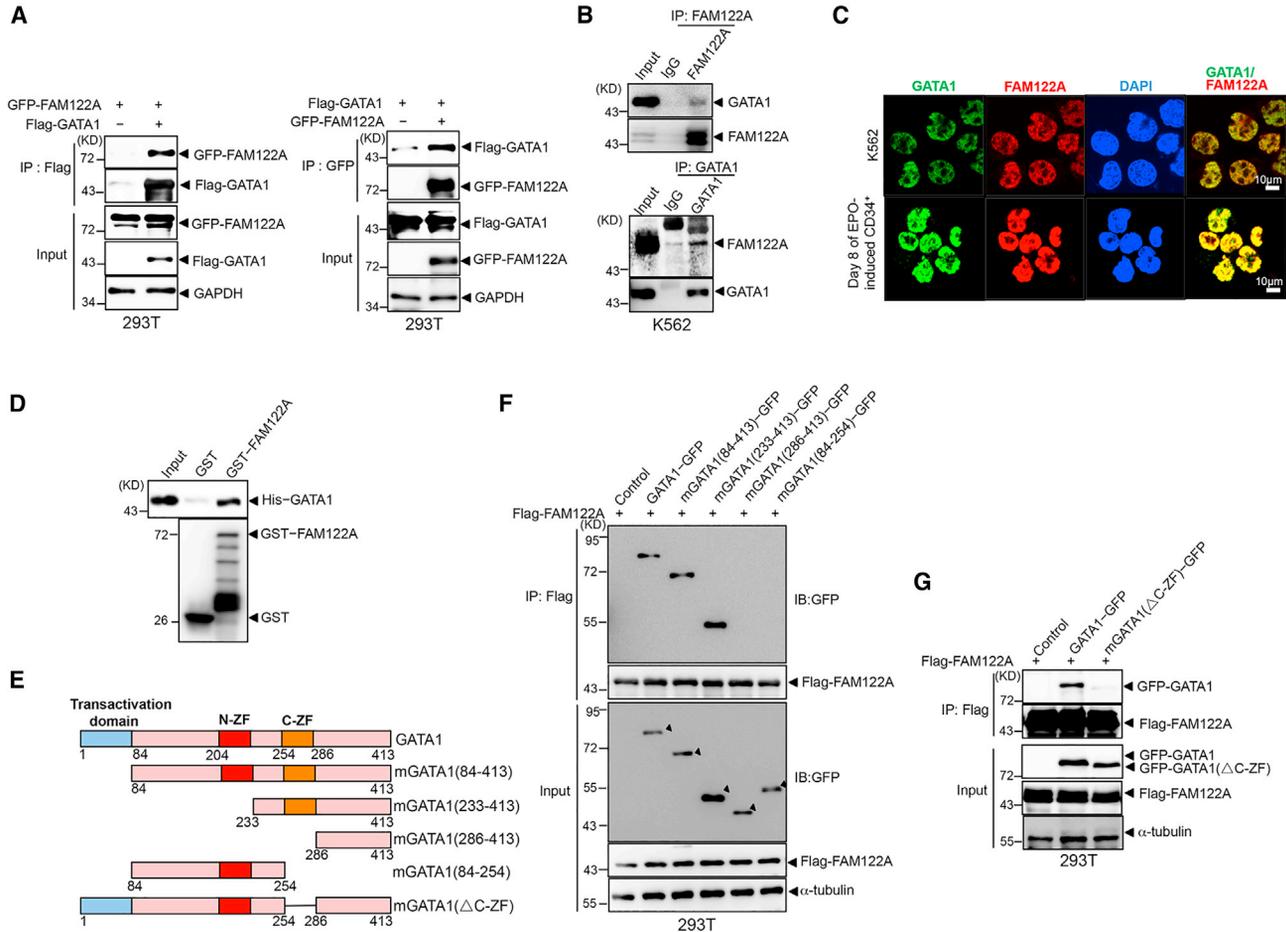
Figure 5. FAM122A Interacts Directly with GATA1 by Its C-Terminal Zinc Finger Domain

(A–D) FAM122A directly interacted with GATA1. 293T cells were transiently co-transfected with GFP-FAM122A, with or without Flag-GATA1 plasmids, and co-immunoprecipitation (coIP) was performed with anti-Flag (left panel) or anti-GFP (right panel) antibodies, respectively. Western blots were used to examine the proteins in immunoprecipitates (n = 3) (A). Interaction of endogenous FAM122A and GATA1 are shown by IP with anti-FAM122A (upper panel) or GATA1 (bottom panel) antibody in K562 cells, followed by immunoblotting with the indicated antibodies (n = 3) (B). Representative images of colocalization of endogenous FAM122A (red) and GATA-1 (green) proteins are shown in K562 (upper panels) and CD34^{+} cells treated with EPO (lower panels) (n = 3) (C). Direct interaction of FAM122A and GATA1 was analyzed by GST pull-down assay by *in vitro* expressed GST-FAM122A and His-GATA1 proteins (n = 3) (D).

(E) Structure diagram of full-length and truncated GATA1 plasmids used in (F) and (G) is shown. GATA1-expressing plasmids were sequentially truncated to produce the mGATA1 (84-413), mGATA1 (233-413), mGATA1 (286-413), mGATA1 (84-254), or mGATA1 (ΔC-ZF), which specifically deleted the C-terminal zinc finger domain for subsequent analysis of the critical domains for GATA1 and FAM122A interaction.

(F and G) 293T cells were transiently co-transfected with Flag-FAM122A and GFP-tagged different truncated/mutated GATA-1 plasmids, followed by coIP assay with anti-Flag antibody (n = 3).

the C-terminal zinc finger domain of GATA1 is crucial for the physical interaction of GATA1 and FAM122A (Figures 5F and 5G).

Inhibition of the Transcriptional Activity of GATA1 by FAM122A

Considering that the C-terminal zinc finger domain of GATA1 for its interaction with FAM122A is also a critical region for the binding of GATA1 to its target DNA (Ferreira et al., 2005; Kaneko et al., 2012), we tested whether FAM122A affects the DNA binding activity of GATA1. For this, a biotin-labeled DNA probe bearing the core canonical GATA DNA sequence (Cantor and Orkin, 2002) was incubated with purified GATA1 and/or FAM122A proteins expressed in *E. coli*. The electrophoretic mobility shift assay (EMSA) showed that a specific shift of the DNA-protein complex was observed only with the incubation of GATA1 protein (lane 3, Figure 6A) but not with the
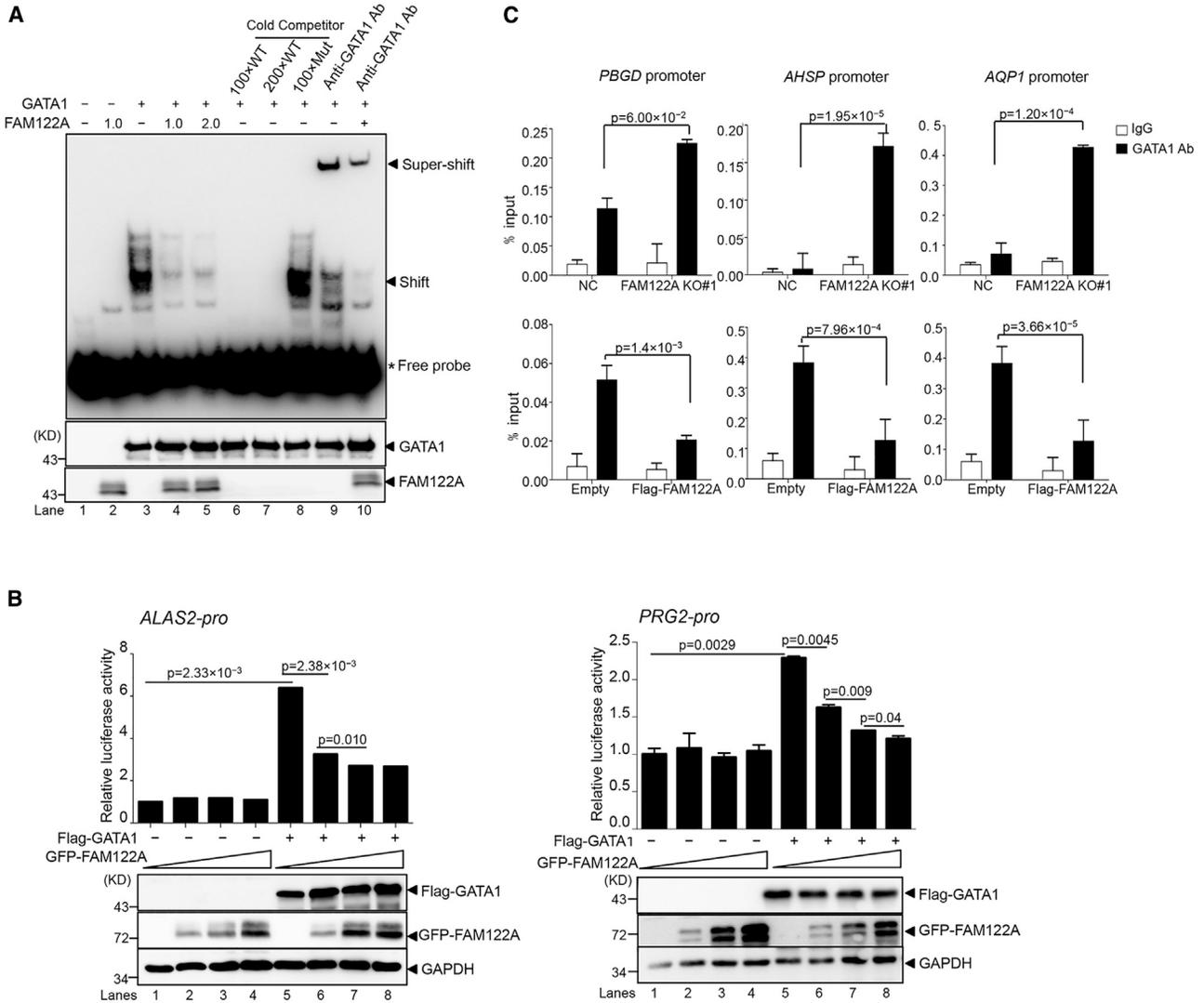
Figure 6. FAM122A Inhibits the Transcriptional Activity of GATA1 by Reducing Its Association with the Targeting DNA

(A) EMSA assay was performed to analyze the effect of FAM122A on GATA-1 binding to target DNA sequences by purified proteins expressed in *E. coli*. Specificity of the shift was confirmed by the complete competition with wild-type competitors but not the mutant one (upper panel). The protein expressions were examined by western blot (bottom panel) (n = 3).

(B) 293T cells were co-transfected with the increasing concentrations of GFP-FAM122A with or without Flag-GATA-1, together with pro-ALAS2-pGL3-basic (ALAS2-pro)/pro-PRG2-pGL3-basic (PRG2-pro) and pRL-SV40 plasmids (as an internal reference plasmid), and the luciferase activities were measured after transfection for 24 h. The efficiency of protein expression was confirmed by western blot (bottom panels) (n = 3).

(C) ChIP assay was performed to analyze GATA1 binding to the promoter and enhancer regions of *PBGD*, *AHSP*, and *AQP1* genes in FAM122A knockout (upper panels) or overexpression (bottom panels) K562 cells treated with 50 μM hemin for 48 h. Input, 1% of chromatin lysate subjected to immunoprecipitation; IgG, normal IgG as a negative control (n = 3).

FAM122A protein (lane 2, Figure 6A), while this shift band was competitively eliminated when in the presence of excessive amounts of wild-type (WT) probes without biotin labeling (lanes 6 and 7, Figure 6A), but still appeared in the presence of the excessive probe with mutant DNA binding sequence (lane 8, Figure 6A). A super-shift band could be seen when co-incubating the GATA1 antibody with the GATA1 protein (lane 9, Figure 6A), suggesting that GATA1 specifically and efficiently bound to this probe. As expected, the co-incubation of FAM122A together with GATA1 almost eliminated the shift band produced by GATA1 binding activity (lanes 4–5, Figure 6A).

Furthermore, FAM122A also reduced the super-shift band intensity (lane 10, Figure 6A). These results implied that FAM122A can significantly inhibit the DNA binding activity of GATA1.

These facts promoted us to ask whether FAM122A influences the transcriptional activity of GATA1. For this purpose, 293T cells were co-transfected with luciferase reporters containing the promoter regions of *ALAS2* or *PRG2* (Surinya et al., 1997; Wu et al., 2014) and increasing amounts of GFP-FAM122A plasmids, together with or without Flag-GATA1. The results showed that FAM122A inhibited GATA1-triggered reporter activity in a dose-dependent manner (Figure 6B).

We further assessed whether FAM122A affects the chromatin occupancy of GATA1 in FAM122A KO and NC K562 cells treated with hemin by a chromatin immunoprecipitation (ChIP) assay, and monitored GATA1 recruitment to several promoter and enhancer regions of erythroid-specific genes, including *PBGD*, *AHSP*, and *AQP1* (Hasegawa et al., 2012; Welch et al., 2004). As shown in Figure 6C, FAM122A KO significantly enhanced GATA1 chromatin occupancy at the promoter regions of these genes (upper, Figure 6C), suggesting that FAM122A deletion also increases the association of GATA1 with the promoter of its target genes *in vivo*. In addition, FAM122A overexpression reduced GATA1 chromatin occupancy on the promoters of its target genes (bottom, Figure 6C).

To further investigate the potential role of FAM122A in dyserythropoiesis, we analyzed *FAM122A* mRNA expression levels in the purified early and late erythroblasts from CD34+ cells, isolated from the peripheral blood of six transfusion-dependent patients with β-thalassemia (before transfusion) and six healthy controls (Forster et al., 2015). We found that *FAM122A* is significantly upregulated in the patient group with delayed erythroid maturation post-induction with EPO for 14 days (Figure S5A). Furthermore, we examined another dataset from a patient and her mother in one family with inherited β-thalassemia (Taghavifar et al., 2019), and found that *FAM122A* expression is also abnormally upregulated in the blood of the patient (daughter) and carrier (mother) with β-thalassemia (Figure S5B).

DISCUSSION

In this work, we showed that FAM122A is abundant in erythroid progenitor cells and downregulated during terminal differentiation, similar to the expression pattern of CD71 and/or GATA1. Moreover, FAM122A negatively regulates the terminal differentiation of erythrocytes, but does not affect the early process of erythropoiesis, as determined in either human CD34+ or K562 cells with genetically modulated *FAM122A* expression, suggesting that FAM122A specifically contributes to the process of terminal erythroid differentiation. How FAM122A expression is regulated during terminal erythroid differentiation remains to be further investigated. According to our results that both mRNA and the protein of FAM122A were downregulated during terminal differentiation, we extrapolated that the regulation of FAM122A expression during erythroid differentiation is mainly involved in its transcriptional level.

The *in vitro* protein binding assay accompanied with mass spectrometry (MS) analysis and RNA sequencing data showed that GATA1 might be involved in the effects of FAM122A on erythroid differentiation. Knockdown of GATA1, but not GATA2, can significantly rescue FAM122A silencing-enhanced erythroid gene expression and maturation potential, indicating that GATA1 mediates the effect of FAM122A-regulating erythroid differentiation. GATA1 plays a central role in the development of erythrocytes, especially in terminal erythroid differentiation (Moriguchi and Yamamoto, 2014), and abnormal regulation of GATA1 is associated with dyserythropoietic disorders (Ferreira et al., 2005; Gutierrez et al., 2020; Tremblay et al., 2018).

GATA1 activity can be regulated by transcriptional and/or translational regulation, posttranslational modification, and protein-protein interaction (Ferreira et al., 2005; Morceau et al., 2004). FAM122A modulation does not change the mRNA and protein levels of GATA1, excluding the possibility of the regulation by transcriptional or translational levels. The posttranslational modifications of GATA1, including acetylation, phosphorylation, and sumolyation, have been found to regulate its DNA binding and/or transcriptional activity (Gutierrez et al., 2020; Hernandez-Hernandez et al., 2006; Yu et al., 2010). Considering that FAM122A was previously identified as a PP2A inhibitor, we further found that FAM122A modulation did not alter the phosphorylation of GATA1 at Ser142 and Ser310 (data not shown), the latter site being correlated with the binding and transcriptional activities of GATA1 (Kadri et al., 2005; Zhao et al., 2006).

Mounting evidence shows that GATA1 exerts its function by interacting with a serious of cofactors, either co-activators or co-repressors (Ferreira et al., 2005; Gutierrez et al., 2020; Morceau et al., 2004). FAM122A interacts directly with GATA1 and inhibits its DNA binding and transcriptional activities, supporting the notion that FAM122A may act as a co-repressor of GATA1 to suppress the transcriptional activity, since their interaction reduces the association of GATA1 with the target gene promoters, thus interfering with erythroid differentiation. On the other hand, several lines of evidence showed that GATA1 is acetylated at two conserved lysine-rich motifs localized closer

to its C-terminal zinc finger domain and that this modification promotes its transcriptional activity (Lamonica et al., 2006). FAM122A can interact directly with the C-terminal zinc finger of GATA1, thus we do not exclude the possibility that this interaction may affect the acetylation state of GATA1 and/or influence its transcriptional activity, which deserves to be investigated in future.

Recently, we have demonstrated that *FAM122A* is abnormally upregulated in AML patients and its expression level is negatively correlated with the overall survival of AML patients. More importantly, FAM122A is found to be essential for the growth of AML cells *in vitro* and *in vivo* by modulating PP2A activity and sustaining c-Myc protein levels (Liu et al., 2020), showing an essential role of FAM122A in hematological malignancy. In this study, we found that FAM122A is a negative regulator of normal human erythropoiesis process possibly by acting as a corepressor to interfere with the DNA binding and transcriptional activities of GATA1, pointing to the potential and physiological role of FAM122A as a GATA1 coregulator in erythroid differentiation. The aberrant upregulation of *FAM122A* in patients with β-thalassemia further implies the important role of FAM122A in the regulation of erythropoiesis.

A deep understanding the mechanisms of erythropoiesis is extremely important and necessary for not only generating massive amounts of erythroid cells *in vitro* or *ex vivo* for transplantation and therapeutics (Chang et al., 2011; Zeuner et al., 2012), but also for providing the opportunity to govern stress or pathological dyserythropoiesis (such as blood loss, allogeneic stem cell transplantation, anemia, and β-thalassemia). Our findings propose a novel mechanism for the inhibitory effect of FAM122A on the regulation of human erythropoiesis *ex vivo* using CD34+ cells, and inhibition of FAM122A may enhance the effect of erythroid differentiation and amplify the bulk products of erythroid cells, which will potentially overcome current hurdles in the fields of bulk RBC production due to the lack of blood donor resources and high costs (Zeuner et al., 2012). During the last decade, efficient procedures or technology to produce RBC *ex vivo* using primary HSCs, embryonic stem cells, or induced pluripotent stem cells, have become an increasing concern to achieve maximal RBC quality, quantity, and maturation. Our results suggest that limitation of the inhibitory effects by negative regulatory factors, such as FAM122A, may significantly enhance the quantity of matured RBCs similar to TRAIL (Migliaccio et al., 2011; Zeuner et al., 2012).

In summary, our study demonstrates that FAM122A plays an inhibitory role in human erythropoiesis in a GATA1-dependent manner by suppressing the DNA binding and transcriptional activities of GATA1. These findings not only elucidate the new function of FAM122A in the

regulation of erythropoiesis, but also propose that FAM122A would be a potentially therapeutic target for GATA1-related dyserythropoietic disorders or an important regulator for amplifying erythroid cells *ex vivo*.

EXPERIMENTAL PROCEDURES

Cells and Culture Conditions

293T cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen). Human erythroleukemia cells K562 were cultured in RPMI 1640 medium (Invitrogen). All media were supplemented with 10% FBS (Invitrogen) and 1% penicillin/streptomycin. For erythroid differentiation, K562 cells were induced by addition of 50 μM hemin (Sigma, USA).

Purification and *In Vitro* Culture of Human CD34+ Cells

CD34+ cells were purified from human umbilical cord blood by applying CD34+ magnetic selective beads system (Miltenyi Biotec, Germany) according to the manufacturer's instructions. Cells were cultured at 10^5 cells/mL for 5–6 days in Serum-Free Expansion Medium (STEMCELL Technologies) supplemented with 10% FBS (STEMCELL Technologies), 100 ng/mL SCF, 10 ng/mL IL-3, and 1 U/mL EPO (STEMCELL Technologies) at 37°C in 5% CO_2 for cell expansion, and then cultured in the medium with the presence of 30% FBS and 3 U/mL EPO for erythroid differentiation at the indicated days (4 or 6 days). CD34+ cells were derived from human umbilical bloods obtained in the Department of Obstetrics and Gynecology of Ren-Ji hospital. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (SJTU-SM and national) and with the Helsinki Declaration of 1975, as revised in 2013. Informed consents were obtained from all patients included in the study.

Benzidine Staining, Cell Morphology, and Colony Assay

For benzidine staining, cells were washed twice with ice-cold phosphate-buffered saline. Benzidine dihydrochloride (Sigma, USA) solution was prepared with 0.5 M ethylic acid. One microliter of 30% hydrogen peroxide was prepared and added to a 50-μL benzidine solution immediately before use. Then, 1 μL of fresh whole benzidine solution containing hydrogen peroxide was added in 10 μL cells. The dark blue particles of oxidized benzidine were readily distinguished under a light microscope. Two or three hundred cells (about five fields) were examined in each assay, and the percentages of benzidine-positive cells were calculated. For cell morphology and colony assays, see Supplemental Information.

Plasmids, RNA Interference, and CRISPR/Cas9

For details, see Supplemental Information.

Flow Cytometry Analysis

To perform flow cytometry analysis of BFU-E and CFU-E cells, 2–5 × 10^5 CD34+ cells were stained with the following mouse anti-human antibodies: PE-conjugated anti-CD34 (560941, BD

Biosciences), FITC-conjugated anti-CD36 (555454, BD Biosciences), PE-Cy7-conjugated anti–IL-3R (CD123) (4318669, eBioscience), APC-conjugated anti-GPA (551336, BD Biosciences), or appropriate isotype controls (all from BD Biosciences) for 30 min in the dark. To monitor erythroblast differentiation, 2–5 × 10^5 CD34$^+$ cells were labeled with FITC-conjugated anti-CD71 (1930273, eBioscience), FITC-conjugated anti-BAND3 (130-119-780, Miltenyi Biotec), and APC-conjugated anti-GPA (551336, BD Biosciences) for 30 min. Cells were then washed twice with 1 mL phosphate-buffered saline/0.5% BSA. Labeled cells were analyzed for fluorescence emission using a FACSCaliber (BD Biosciences) and Cell Quest Pro software (BD Biosciences/Pharmingen) for acquisition and analysis.

RNA Sequencing and qPCR
See Supplemental Information. RNA sequencing data have been deposited in the Gene Expression Omnibus (GEO).

In Vitro Binding Studies and MS Analysis
GST-FAM122A (10 mg) was incubated with 2 mg of K562 nuclear extracts overnight in buffer containing 20 mM Tris-HCl (pH 7.6); 150 mM NaCl; 1 mM EDTA; 0.5% NP-40; 10% glycerol; 1 mM PMSF; protease inhibitor cocktail. Bound proteins were washed five times in the washing buffer with 50 mM Tris-HCl (pH 7.6); 300 mM NaCl; 1 mM EDTA; 0.5% NP-40; 10% glycerol, separated by SDS-PAGE and visualized by Coomassie brilliant blue staining. The separated proteins by electrophoresis from GST-FAM122A- and GST-bound lysates were excised for identification by liquid chromatography-tandem MS analysis, as described in our previous report (Shen et al., 2018, 2019).

Immunoprecipitation
For detecting the interactions between FAM122A and GATA1 and its domain mapping, 2 × 10^6 cells were harvested with immunoprecipitation lysis buffer (20 mM Tris-HCl [pH 7.6]; 150 mM NaCl; 1 mM EDTA; 0.5% NP-40; 10% glycerol; 1 mM PMSF; protease inhibitor cocktail). After brief sonication, the lysates were centrifuged (1,7000 × g, 15 min) at 4°C. After pre-clearing with normal IgG beads (A0919, Sigma, Germany) at 4°C for 2 h, supernatants were incubated with antibody against GATA-1 (sc-265, Santa Cruz, CA) or antibody against FAM122A (sc-242677, Santa Cruz), together with protein A/G Plus Agarose (sc-2003, Santa Cruz) or incubated with mouse anti-Flag M2 Agarose gel (A2220, Sigma, St. Louis, MO) or anti-GFP Agarose (D153-8, MBL, CO) overnight at 4°C. After immunoprecipitation, the beads were washed five times with washing buffer (50 mM Tris-HCl [pH 7.6]; 300 mM NaCl; 1 mM EDTA; 0.5% NP-40; 10% glycerol), then the precipitates were analyzed by western blot.

GST Pull-Down
GST alone and GST-tagged FAM122A fusion proteins were expressed in *E. coli* BL21 by induction with isopropyl β-D-1-thiogalactopyranoside at 28°C for 6 h and purified with GST Bind Resin (Novagen). GATA1 was bacterially expressed as six His-tagged protein, followed by purification using nickel-nitrilotriacetic acid resin (QIAGEN). The purified GST or GST-tagged FAM122A proteins were incubated with the purified GATA1 protein for 2 h

at room temperature. Then the precipitates were eluted by the SDS sample buffer and followed by western blot.

EMSA
EMSA was carried out using the LightShift Chemiluminescent EMSA Kit (Pierce). In brief, the following double-stranded 5′-biotin-labeled probes or unlabeled cold competitors were synthesized. Oligonucleotides probes harboring two copies of GATA1 binding sequence (sense: 5′-CACTTGATAACACAAAGT GATAACTCT-3′; antisense: 5′-AGAGTTATCACTTTGTGTTATCA AGTG-3′) and mutant competitor probes (sense: 5′-CACTTAGC GACACAAAGTAGCGACTCT-3′, antisense: 5′-AGAGTCGCTACT TTGTGTCGCTAAGTG-3′) were synthesized from Sigma. GATA1 and FAM122A protein lysates used in this assay were expressed *in vitro* by the *E. coli* BL21, the protein concentration was determined using the BCA protein assay reagent (Pierce). Binding assays were performed in a buffer containing 10 mM Tris (pH 7.6), 50 mM KCl, 1 mM EDTA, 10 mM $(NH4)_2SO_4$, 1 mM DTT, 0.2% Tween 20, and 1 μg poly(dI:dC). For the competition assay, 100-/200-fold excess of unlabeled WT competitor probes or mutant competitor probes were added to the reaction mixture. Anti-GATA1 antibody (NBP1-47492, Novus) was used for super-shift assay.

Luciferase Assay
For reporter plasmid construction, human ALAS2 gene promoter (−797∼ −617 bp) and human proteoglycan 2 gene promoter (−117 ∼ −67 bp) were PCR amplified and cloned into pGL3 basic vector (Promega). 293T cells were plated at 5 × 10^4 cells per well in 12-well plates 1 day before being transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 24 h, transfected cells were lysed with passive lysis buffer and lysates were analyzed for both firefly and Renilla luciferase activity using a Dual-Luciferase Reporter Assay Kit (Promega). Luciferase activity was normalized for transfection efficiency using Renilla activity as an internal control (10 ng).

ChIP Assay
ChIP assays were performed using Pierce Agarose ChIP Kit (26156, Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. K562 cells were treated with 50 μM hemin for 48 h, and protein-DNA complexes were crosslinked with 1% formaldehyde for 30 min at room temperature. The reaction was stopped by adding glycine to a final concentration of 125 mM and incubating for 5 min at room temperature. Chromatin solutions were precipitated overnight with rotation at 4°C using GATA1 antibody (NBP1-47492, Novus) or anti-mouse IgG (sc-3877, Santa Cruz) as a negative control. The DNA associated with immunoprecipitates was isolated and used as a template for the PCR to amplify the promoter and enhancer sequences containing the GATA1 binding element. The PCR conditions were as follows: 95°C for 3 min and 38 cycles of 30 s at 94°C, 30 s at 60°C, and 40 s at 72°C, followed by an extension time of 5 min at 72°C. The primer pairs used were as followed: PBGD gene, 5′-TCTAGTCTACTCCATGTGGC-3′ and 5′-ACCAAGGCAGTTGT CAGTGG-3′, yielding a 231-bp fragment; AHSP gene, 5′-AGGGCT CAGTAAACGTC-3′ and 5′-AGAAGGGAGAGGCTTCC-3′, yielding a 186-bp fragment; AQP1gene, 5′-AATGCAGGGCTGGGGTTAGCC

CGGCTC-3′ and 5′-TGACACCTCTTATCGCATCTGCCTCC-3′, yielding a 120-bp fragment. The precipitated DNAs were further analyzed by qPCR. Each sample was detected in triplicate, and the amount of precipitated DNA was calculated as the percentage of input sample.

Western Blots

Proteins were fractionated on 10% SDS-PAGE and transferred to the Immobilon PVDF transfer membranes (Millipore, Billerica, MA). The membrane was blocked with 5% nonfat milk or 5% BSA for 1 h and incubated with specific primary antibodies overnight at 4°C. The following primary antibodies were used: anti-GATA1 (3535, Cell Signaling Technology), anti-GATA2 (ab70366, Abcam), anti-hemoglobin α (14537-1-AP, Proteintech), anti-HBG1 (25728-1-AP, Proteintech), anti-HRP-GAPDH (60004, Proteintech), anti-phospho-GATA1 (Ser142) (11041, Signalway Antibody), and anti-phospho-GATA1 (Ser310) (11042, Signalway Antibody). Anti-FAM122A antibody was from ABclonal. The blot was washed three times with PBS-Tween and then incubated with HRP-linked secondary antibodies (7074, Cell Signaling Technology). The signals were detected by chemiluminescence phototope-HRP Kit (Millipore, WBKLS0500) according to the manufacturer's instructions. As necessary, blots were probed with anti-HRP-linked GAPDH (60004, Proteintech) antibody as loading controls.

Immunofluorescence

Cells were cytospun onto slides and fixed for 10 min at room temperature in 4% formaldehyde and permeabilized in 0.1% Triton X-100 for 15 min at room temperature. Nonspecific sites were blocked by incubation with PBS containing 2.5% BSA for 1 h at room temperature. Cells were then incubated with anti-GATA1 (NBP1-47492, Novus) and anti-FAM122A (NBP2-31646, Novus) antibodies overnight at 4°C. Cells were subsequently washed three times with 1× PBS. Secondary antibodies (Alexa Fluoro secondary 488/595) (z25402/z25407; Invitrogen, Carlsbad, CA) were applied at 1:200 dilution for 1 h at room temperature. Finally, the cells were incubated in 4′6-diamidino-2-phenylindole for 10 min at room temperature. Stained cells were visualized using a confocal laser scanning microscope Nikon Eclipse Ti (Nikon, Kanagawa, Japan).

Statistical Analyses

Data are expressed as mean ± SD and were analyzed by Student's t test, with $p < 0.05$ indicating significant difference. All experiments were repeated at least three times.

Data and Code Availability

The accession numbers for the RNA-seq data reported in this paper is GEO: GSE141735.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.stemcr.2020.07.010.

AUTHOR CONTRIBUTIONS

J.C. designed the research and performed most of the experiments. Q.Z. provided normal human umbilical cord bloods. M.H.L., Y.S.Y., and Y.Q.W. cultured the cells, analyzed the results, and carried out some of the experiments. G.Q.C. and Y.H. designed the research, analyzed and interpreted data, and prepared the manuscript.

REFERENCES

Alvarez-Dominguez, J.R., Zhang, X., and Hu, W. (2017). Widespread and dynamic translational control of red blood cell development. Blood *129*, 619–629.

An, X., Schulz, V.P., Li, J., Wu, K., Liu, J., Xue, F., Hu, J., Mohandas, N., and Gallagher, P.G. (2014). Global transcriptome analyses of human and murine terminal erythroid differentiation. Blood *123*, 3466–3477.

Auffray, I., Marfatia, S., de Jong, K., Lee, G., Huang, C.H., Paszty, C., Tanner, M.J., Mohandas, N., and Chasis, J.A. (2001). Glycophorin A dimerization and band 3 interaction during erythroid membrane biogenesis: in vivo studies in human glycophorin A transgenic mice. Blood *97*, 2872–2878.

Cantor, A.B., and Orkin, S.H. (2002). Transcriptional regulation of erythropoiesis: an affair involving multiple partners. Oncogene *21*, 3368–3376.

Chang, K.H., Bonig, H., and Papayannopoulou, T. (2011). Generation and characterization of erythroid cells from human embryonic stem cells and induced pluripotent stem cells: an overview. Stem Cells Int *2011*, 791604.

Chen, K., Liu, J., Heck, S., Chasis, J.A., An, X., and Mohandas, N. (2009). Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. Proc. Natl. Acad. Sci. U S A *106*, 17413–17418.

Eisenberg, E., and Levanon, E.Y. (2013). Human housekeeping genes, revisited. Trends Genet. *29*, 569–574.

Fan, L., Liu, M.H., Guo, M., Hu, C.X., Yan, Z.W., Chen, J., Chen, G.Q., and Huang, Y. (2016). FAM122A, a new endogenous inhibitor of protein phosphatase 2A. Oncotarget *7*, 63887–63900.

Ferreira, R., Ohneda, K., Yamamoto, M., and Philipsen, S. (2005). GATA1 function, a paradigm for transcription factors in hematopoiesis. Mol. Cell Biol *25*, 1215–1227.

Forster, L., McCooke, J., Bellgard, M., Joske, D., Finlayson, J., and Ghassemifar, R. (2015). Differential gene expression analysis in early and late erythroid progenitor cells in beta-thalassaemia. Br. J. Haematol. *170*, 257–267.

Fujiwara, Y., Browne, C.P., Cunniff, K., Goff, S.C., and Orkin, S.H. (1996). Arrested development of embryonic red cell precursors in

mouse embryos lacking transcription factor GATA-1. Proc. Natl. Acad. Sci. U S A *93*, 12355–12358.

Gutierrez, L., Caballero, N., Fernandez-Calleja, L., Karkoulia, E., and Strouboulis, J. (2020). Regulation of GATA1 levels in erythropoiesis. IUBMB Life *72*, 89–105.

Hasegawa, A., Shimizu, R., Mohandas, N., and Yamamoto, M. (2012). Mature erythrocyte membrane homeostasis is compromised by loss of the GATA1-FOG1 interaction. Blood *119*, 2615–2623.

Hernandez-Hernandez, A., Ray, P., Litos, G., Ciro, M., Ottolenghi, S., Beug, H., and Boyes, J. (2006). Acetylation and MAPK phosphorylation cooperate to regulate the degradation of active GATA-1. EMBO J. *25*, 3264–3274.

Hu, J., Liu, J., Xue, F., Halverson, G., Reid, M., Guo, A., Chen, L., Raza, A., Galili, N., Jaffray, J., et al. (2013). Isolation and functional characterization of human erythroblasts at distinct stages: implications for understanding of normal and disordered erythropoiesis in vivo. Blood *121*, 3246–3253.

Kadri, Z., Maouche-Chretien, L., Rooke, H.M., Orkin, S.H., Romeo, P.H., Mayeux, P., Leboulch, P., and Chretien, S. (2005). Phosphatidylinositol 3-kinase/Akt induced by erythropoietin renders the erythroid differentiation factor GATA-1 competent for TIMP-1 gene transactivation. Mol. Cell Biol *25*, 7412–7422.

Kaneko, H., Kobayashi, E., Yamamoto, M., and Shimizu, R. (2012). N- and C-terminal transactivation domains of GATA1 protein coordinate hematopoietic program. J. Biol. Chem. *287*, 21439–21449.

Kaneko, K., Furuyama, K., Fujiwara, T., Kobayashi, R., Ishida, H., Harigae, H., and Shibahara, S. (2014). Identification of a novel erythroid-specific enhancer for the ALAS2 gene and its loss-of-function mutation which is associated with congenital sideroblastic anemia. Haematologica *99*, 252–261.

Kobayashi, M., and Yamamoto, M. (2007). Regulation of GATA1 gene expression. J. Biochem. *142*, 1–10.

Lamonica, J.M., Vakoc, C.R., and Blobel, G.A. (2006). Acetylation of GATA-1 is required for chromatin occupancy. Blood *108*, 3736–3738.

Li, J., Hale, J., Bhagia, P., Xue, F., Chen, L., Jaffray, J., Yan, H., Lane, J., Gallagher, P.G., Mohandas, N., et al. (2014). Isolation and transcriptome analyses of human erythroid progenitors: BFU-E and CFU-E. Blood *124*, 3636–3645.

Li, W., Wang, Y., Zhao, H., Zhang, H., Xu, Y., Wang, S., Guo, X., Huang, Y., Zhang, S., Han, Y., et al. (2019). Identification and transcriptome analysis of erythroblastic island macrophages. Blood *134*, 480–491.

Liu, M.H., Chen, J., Yang, Y.S., Wang, Y.Q., Chen, G.Q., Zhang, Y., and Huang, Y. (2020). FAM122A promotes acute myeloid leukemia cell growth through inhibiting PP2A activity and sustaining MYC expression. Haematologica https://doi.org/10.3324/haematol.2020.251462.

Merryweather-Clarke, A.T., Atzberger, A., Soneji, S., Gray, N., Clark, K., Waugh, C., McGowan, S.J., Taylor, S., Nandi, A.K., Wood, W.G., et al. (2011). Global gene expression analysis of human erythroid progenitors. Blood *117*, e96–e108.

Migliaccio, G., Masiello, F., Tirelli, V., Sanchez, M., Varricchio, L., Whitsett, C., and Migliaccio, A.R. (2011). Under HEMA conditions,

self-replication of human erythroblasts is limited by autophagic death. Blood Cells Mol Dis *47*, 182–197.

Morceau, F., Schnekenburger, M., Dicato, M., and Diederich, M. (2004). GATA-1: friends, brothers, and coworkers. Ann. N. Y Acad. Sci. *1030*, 537–554.

Moriguchi, T., and Yamamoto, M. (2014). A regulatory network governing Gata1 and Gata2 gene transcription orchestrates erythroid lineage differentiation. Int. J. Hematol. *100*, 417–424.

Nandakumar, S.K., Ulirsch, J.C., and Sankaran, V.G. (2016). Advances in understanding erythropoiesis: evolving perspectives. Br. J. Haematol. *173*, 206–218.

Perreault, A.A., and Venters, B.J. (2018). Integrative view on how erythropoietin signaling controls transcription patterns in erythroid cells. Curr. Opin. Hematol. *25*, 189–195.

Reynhout, S., and Janssens, V. (2019). Physiologic functions of PP2A: lessons from genetically modified mice. Biochim. Biophys. Acta Mol. Cell Res *1866*, 31–50.

Rutherford, T.R., Clegg, J.B., and Weatherall, D.J. (1979). K562 human leukaemic cells synthesise embryonic haemoglobin in response to haemin. Nature *280*, 164–165.

Shen, S.M., Ji, Y., Zhang, C., Dong, S.S., Yang, S., Xiong, Z., Ge, M.K., Yu, Y., Xia, L., Guo, M., et al. (2018). Nuclear PTEN safeguards pre-mRNA splicing to link Golgi apparatus for its tumor suppressive role. Nat. Commun. *9*, 2392.

Shen, S.M., Zhang, C., Ge, M.K., Dong, S.S., Xia, L., He, P., Zhang, N., Ji, Y., Yang, S., Yu, Y., et al. (2019). PTENalpha and PTENbeta promote carcinogenesis through WDR5 and H3K4 trimethylation. Nat. Cell Biol *21*, 1436–1448.

Su, A.I., Wiltshire, T., Batalov, S., Lapp, H., Ching, K.A., Block, D., Zhang, J., Soden, R., Hayakawa, M., Kreiman, G., et al. (2004). A gene atlas of the mouse and human protein-encoding transcriptomes. Proc. Natl. Acad. Sci. U S A *101*, 6062–6067.

Sun, Z., Wang, Y., Han, X., Zhao, X., Peng, Y., Li, Y., Peng, M., Song, J., Wu, K., Sun, S., et al. (2015). miR-150 inhibits terminal erythroid proliferation and differentiation. Oncotarget *6*, 43033–43047.

Surinya, K.H., Cox, T.C., and May, B.K. (1997). Transcriptional regulation of the human erythroid 5-aminolevulinate synthase gene. Identification of promoter elements and role of regulatory proteins. J. Biol. Chem. *272*, 26585–26594.

Taghavifar, F., Hamid, M., and Shariati, G. (2019). Gene expression in blood from an individual with beta-thalassemia: an RNA sequence analysis. Mol. Genet. Genomic Med. *7*, e00740.

Tremblay, M., Sanchez-Ferras, O., and Bouchard, M. (2018). GATA transcription factors in development and disease. Development *145*, dev164384.

Wang, D., Si, S., Wang, Q., Luo, G., Du, Q., Liang, Q., Guo, X., Zhang, G., Feng, J., and Leng, Z. (2018). MiR-27a promotes hemin-induced erythroid differentiation of K562 cells by targeting CDC25B. Cell Physiol Biochem *46*, 365–374.

Welch, J.J., Watts, J.A., Vakoc, C.R., Yao, Y., Wang, H., Hardison, R.C., Blobel, G.A., Chodosh, L.A., and Weiss, M.J. (2004). Global regulation of erythroid gene expression by transcription factor GATA-1. Blood *104*, 3136–3147.

Wu, J., Zhou, L.Q., Yu, W., Zhao, Z.G., Xie, X.M., Wang, W.T., Xiong, J., Li, M., Xue, Z., Wang, X., et al. (2014). PML4 facilitates erythroid differentiation by enhancing the transcriptional activity of GATA-1. Blood *123*, 261–270.

Xu, J., Shao, Z., Glass, K., Bauer, D.E., Pinello, L., Van Handel, B., Hou, S., Stamatoyannopoulos, J.A., Mikkola, H.K., Yuan, G.C., et al. (2012). Combinatorial assembly of developmental stage-specific enhancers controls gene expression programs during human erythropoiesis. Dev. Cell *23*, 796–811.

Yu, L., Ji, W., Zhang, H., Renda, M.J., He, Y., Lin, S., Cheng, E.C., Chen, H., Krause, D.S., and Min, W. (2010). SENP1-mediated GATA1 deSUMOylation is critical for definitive erythropoiesis. J. Exp. Med. *207*, 1183–1195.

Zeuner, A., Martelli, F., Vaglio, S., Federici, G., Whitsett, C., and Migliaccio, A.R. (2012). Concise review: stem cell-derived erythrocytes as upcoming players in blood transfusion. Stem Cells *30*, 1587–1596.

Zhao, W., Kitidis, C., Fleming, M.D., Lodish, H.F., and Ghaffari, S. (2006). Erythropoietin stimulates phosphorylation and activation of GATA-1 via the PI3-kinase/AKT signaling pathway. Blood *107*, 907–915.

Zhou, Y., Shi, W.Y., He, W., Yan, Z.W., Liu, M.H., Chen, J., Yang, Y.S., Wang, Y.Q., Chen, G.Q., and Huang, Y. (2020). FAM122A supports the growth of hepatocellular carcinoma cells and its deletion enhances doxorubicin-induced cytotoxicity. Exp. Cell Res *387*, 111714.