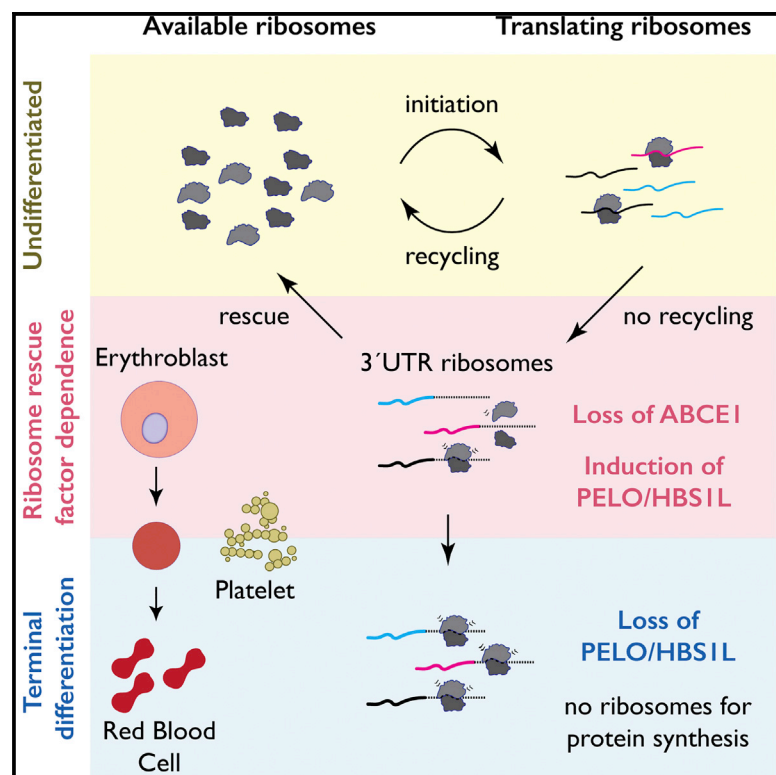


Cell Reports

Dynamic Regulation of a Ribosome Rescue Pathway in Erythroid Cells and Platelets

Graphical Abstract



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In Brief

Maturing mammalian platelets and red blood cells lack nuclei but continue protein synthesis during terminal differentiation. Using ribosome profiling, Mills et al. report abundant 3' UTR ribosomes in these lineages and show how interplay between the ribosome rescue/recycling factors PELO and ABCE1 may promote translation of critical mRNAs during differentiation.

Highlights

- Primary reticulocyte and platelet ribosome profiling reveals 3' UTR ribosome buildup
- Decreased ABCE1 during differentiation leads to ribosome recycling failure
- Regulation of the ribosome rescue factors PELO/HBS1L compensates for recycling defects

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Dynamic Regulation of a Ribosome Rescue Pathway in Erythroid Cells and Platelets

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SUMMARY

Protein synthesis continues in platelets and maturing reticulocytes, although these blood cells lack nuclei and do not make new mRNA or ribosomes. Here, we analyze translation in primary human cells from anucleate lineages by ribosome profiling and uncover a dramatic accumulation of post-termination unrecycled ribosomes in the 3' UTRs of mRNAs. We demonstrate that these ribosomes accumulate as a result of the natural loss of the ribosome recycling factor ABCE1 during terminal differentiation. Induction of the ribosome rescue factors PELO and HBS1L is required to support protein synthesis when ABCE1 levels fall and for hemoglobin production during blood cell development. Our observations suggest that this distinctive loss of ABCE1 in anucleate blood lineages could sensitize them to defects in ribosome homeostasis, perhaps explaining in part why genetic defects in the fundamental process of ribosome production (“ribosomopathies”) often affect hematopoiesis specifically.

INTRODUCTION

In mammals, immature red blood cells (reticulocytes) and platelets lack nuclei and, therefore, cannot transcribe new RNA. Despite this, retained ribosomes and pre-synthesized mRNAs permit continued protein synthesis during terminal differentiation in these cells (Heynen, 1990; Ji et al., 2011). Reticulocytes are highly translationally active and circulate for 2–3 days before completely losing ribosomes, organelles, and mRNA; these latter events define their transition to mature erythrocytes (Heynen, 1990; Wiczling and Krzyzanski, 2008). Translation by reticulocytes is prolific—as much as one-third of the final hemoglobin content of an erythrocyte is synthesized after enucleation (Heynen and Verwilghen, 1982; Heynen, 1990). Similarly, nascent platelets are released from the bone marrow containing ribosomes, organelles, and mRNA derived from the precursor megakaryocyte (Bray et al., 2013; Kissopoulou et al., 2013; Rowley et al., 2011). Platelet mRNA and ribosomes are stable for a

period of at least several days (Ault, 1993; Ault et al., 1992), and, during this time, they direct protein synthesis (Booyse and Rafelson, 1967; Weyrich et al., 1998).

Ribosome homeostasis, which refers broadly to the regulation of ribosome levels in cells, plays an essential role in determining which mRNAs are translated (Ludwig et al., 2014; Signer et al., 2014). Haploinsufficiencies of specific ribosomal proteins and defects in ribosome biogenesis perturb ribosome homeostasis and disproportionately result in hematopoietic dysfunction (Barlow et al., 2010; Buchanan et al., 1981; Draptchinskaia et al., 1999; Ebert et al., 2008; Neuwirtova et al., 2013; Ricciardi et al., 2015). Additionally, disrupted ribosome biogenesis increases free ribosomal proteins, which can activate p53 (Zhang and Lu, 2009), further modulating these phenotypes (Barlow et al., 2010). It is not well understood why these defects lead to hematopoietic tissue-specific phenotypes. Moreover, mechanisms that regulate ribosome availability in anucleate blood lineages have not been identified, although autophagy (Kundu et al., 2008), ubiquitin-dependent degradation (Etlinger and Goldberg, 1977; Wefes et al., 1995), as well as specific ribonucleases (Pisareva et al., 2015; Valentine et al., 1974) have been proposed to degrade ribosomes in reticulocytes. Delayed ribosome clearance is associated with abnormal erythroid maturation (Kundu et al., 2008; Valentine et al., 1974) and platelet dysfunction in humans (Fletcher et al., 2015; Marconi et al., 2016).

We show here that the active dissociation of the ribosomal subunits, a distinct step in the translation cycle termed ribosome recycling (Pisarev et al., 2010; Shoemaker et al., 2010), regulates ribosome homeostasis in reticulocytes and platelets. Our data suggest that loss of the ribosome recycling factor ABCE1 (Rli1p in *S. cerevisiae*), limits ribosome availability during terminal differentiation of these anucleate blood lineages, leading to the accumulation of post-termination, unrecycled ribosomes in the 3' UTRs of mRNAs. These 3' UTR ribosomes are cleared by induction of the ribosome rescue factors PELO and HBS1L (Dom34p and Hbs1p in *S. cerevisiae*), which return ribosomes to the active cellular pool. These factors act as a heteroduplex to release stalled and unrecycled ribosomes (Guydosh and Green, 2014) and compensate for *Rli1* defects in yeast (Young et al., 2015). We propose that enhanced ribosome rescue compensates for the loss of ABCE1-mediated recycling in anucleate blood lineages. Our results delineate the functional significance



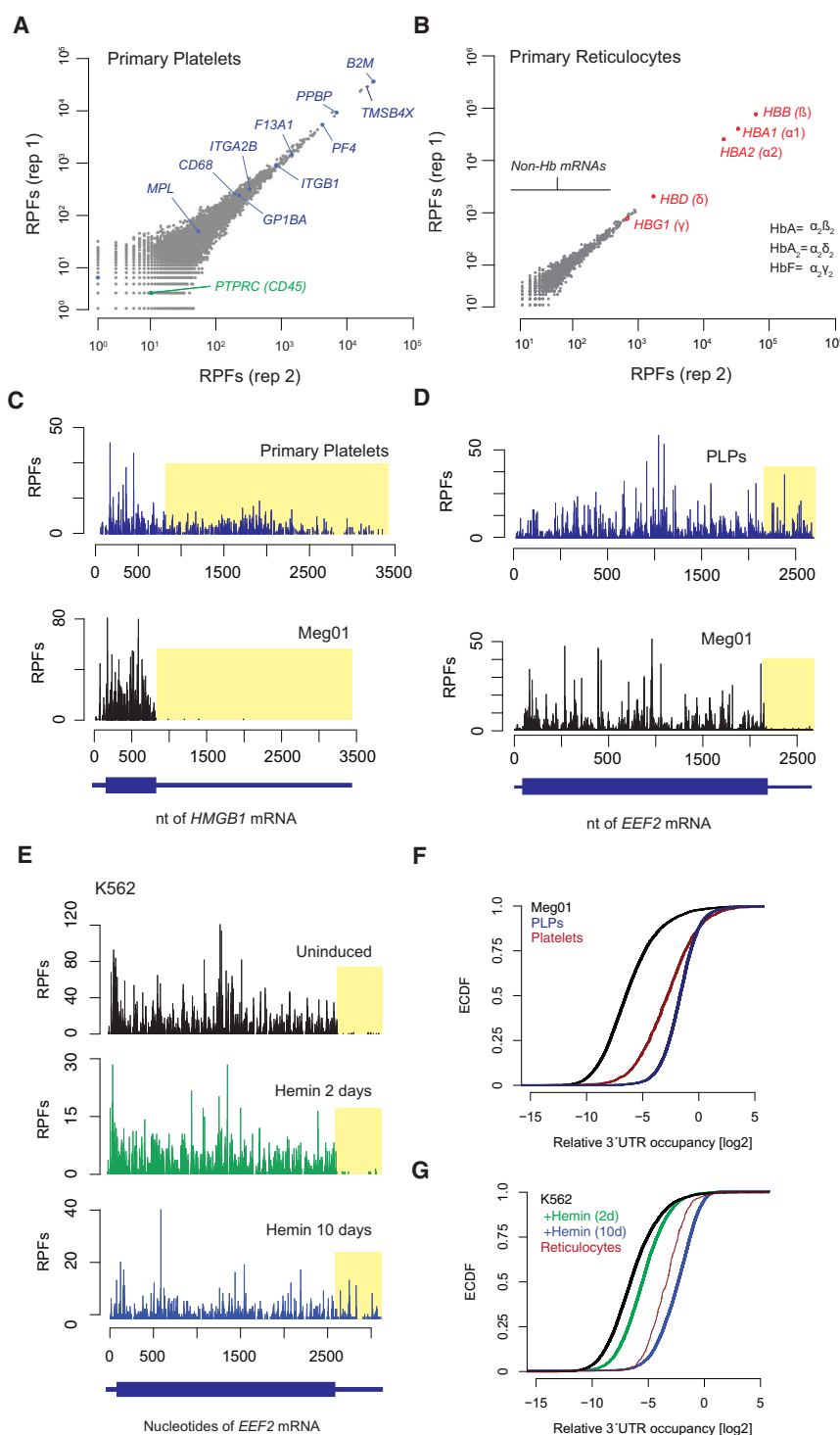


Figure 1. Platelet and Reticulocyte Ribosome Profiling Show Abundant 3' UTR RPFs

(A) Scatterplots of RPF counts per mRNA coding sequence for replicates from a single healthy human donor of primary platelets. Known platelet proteins are highlighted in blue. *PTPRC* (CD45) is a white blood cell marker (green).

(B) Replicates of RPF counts from CD71+ primary human reticulocytes.

(C) Ribosome profile of the *HMGB1* mRNA in platelets (top) and Meg01 cells (bottom).

(D) Ribosome profile of the *EEF2* mRNA from in vitro platelet-like particles (PLPs) (top) and precursor Meg01 cells (bottom).

(E) Accumulation of 3' UTR RPFs during erythroid-like differentiation.

(F and G) Cumulative histograms of relative 3' UTR occupancy for (F) primary human platelets and cell culture models and (G) primary reticulocytes and cell culture models.

RESULTS

Anucleate Cells Accumulate 3' UTR Ribosomes

We analyzed translation in primary human anucleate blood lineages by ribosome profiling, which relies on the deep sequencing of ribosome-protected fragments (RPFs) (Ingolia et al., 2009, 2011). Analysis of these data revealed ~6,700 ribosome-occupied mRNAs in platelets using a cutoff of 0.3 reads per kilobase of coding sequence per million mapped reads (Figure 1A), consistent with the high complexity of the platelet transcriptome (Bray et al., 2013; Gnatenko et al., 2003; Kissopoulou et al., 2013; Rowley et al., 2011) and proteome (Burkhart et al., 2012; Kim et al., 2014; Martens et al., 2005; Vaudel et al., 2012; Wilhelm et al., 2014). Reticulocyte ribosomes predominantly occupied adult hemoglobin (Hb) mRNAs (Figure 1B), consistent with the fact that Hb transcripts make up the majority of the reticulocyte transcriptome (Clissold et al., 1977), and mostly hemoglobin protein (Denton et al., 1975) is made at this stage of differentiation. We also detected ribosomes occupying ~400 additional mRNAs in reticulocytes, indicating that

of this regulatory switch by demonstrating that hemoglobin translation depends on PELO/HBS1L expression. This work may help rationalize the sensitivity of erythroid cell lineages to genetic diseases caused by mutations in components of the translational machinery.

many non-Hb mRNAs are also translated during this stage of development.

Surprisingly, in primary human platelets and reticulocytes, we observed substantial ribosome occupancy on the 3' UTRs (Figures 1C, 1F, and 1G). Although these regions are devoid of

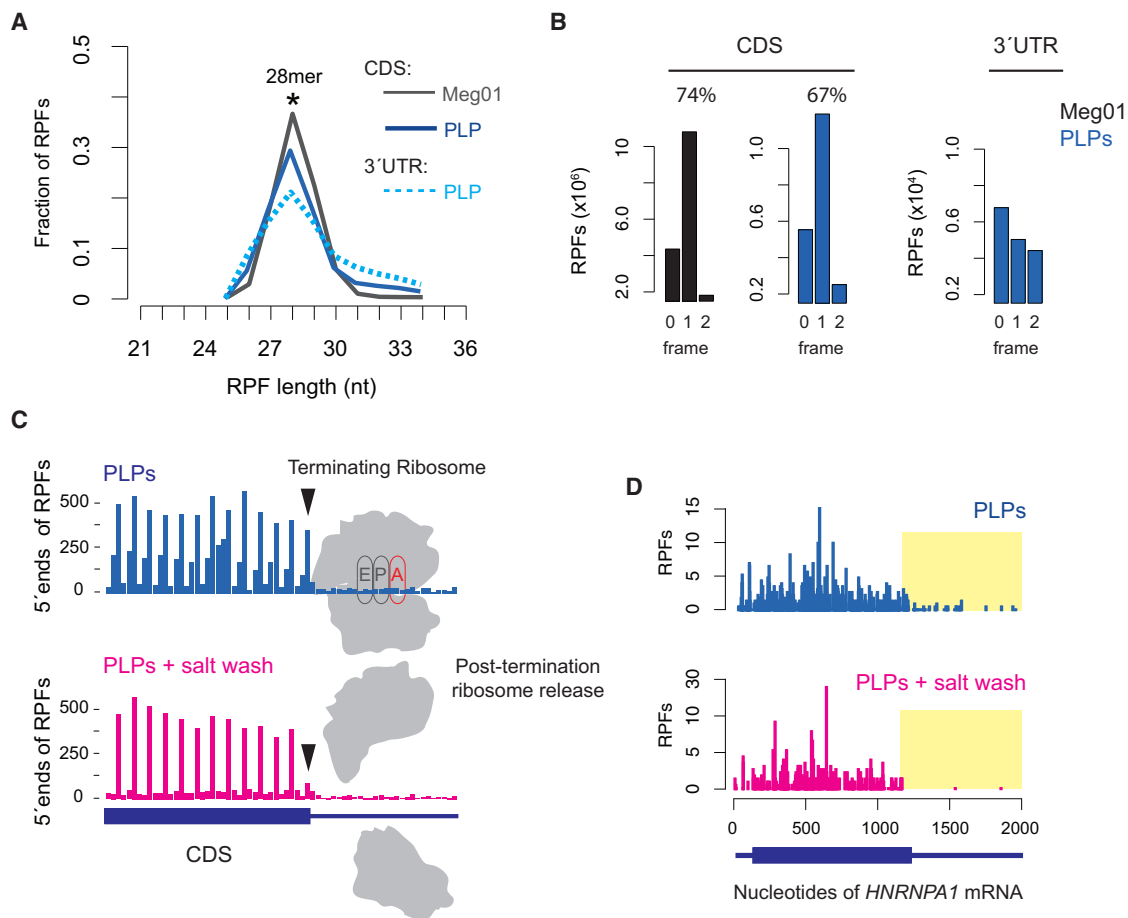


Figure 2. 3' UTR RPFs Result from Post-termination Ribosomes

(A) RPF size distributions of 3' UTR footprints relative to CDSs.

(B) Reading frame analysis of RPFs mapped to coding sequences (CDS) or 3' UTRs.

(C) 5' ends of RPFs around the stop codon. Arrows indicate ribosomes with a stop codon in the A site, which are dissociated by a high salt wash (1M KCl).

(D) Ribosome profile for the *HNRNPA1* mRNA with 3' UTRs highlighted in yellow.

ribosome footprints in other cell types, we quantified an approximately 30-fold increase in RPFs in platelets and reticulocytes compared with nucleated peripheral blood mononuclear cells (PBMCs) (Figures 1F and 1G; Figure S2A), neutrophils (Guo et al., 2010), or macrophages (Su et al., 2015). The increased density of 3' UTR RPFs is a global rather than a transcript-specific effect. In platelets, 3' UTR RPFs represented ~30% of all RPFs mapped to mRNA transcripts, whereas in reticulocytes (which have a far less complex transcriptome), 4% of all RPFs were mapped to 3' UTRs.

To explore the mechanisms underlying the 3' UTR ribosome accumulation in primary human cells, we tested whether these effects could be recapitulated by in vitro systems of blood cell differentiation. We purified in vitro platelet-like particles (PLPs) from megakaryoblast (Meg01) precursor cells that generate these particles in tissue culture (Clarke et al., 2003; Takeuchi et al., 1991, 1998) and showed that they are translationally active (Figures S1F–S1J; Supplemental Experimental Procedures). Ribosome profiling in PLPs showed that, like primary human platelets, PLPs display increased 3' UTR RPF density relative

to precursor Meg01 cells (Figures 1D and 1F). Next, we induced K562 cells toward erythroid differentiation by supplementing the growth media with hemin (Rutherford et al., 1979). These cells also exhibited a global, differentiation-dependent increase in 3' UTR RPF density (Figure 1E), consistent with observations from primary reticulocytes (Figure 1G). Together, these data establish that the striking increase in 3' UTR RPF density we observed in primary platelets and reticulocytes also occurs in cell models for these lineages.

We next set out to verify that these 3' UTR RPFs are derived from bona fide ribosomes and test whether these ribosomes are actively engaged in translation. Because previous work has shown that genetic reduction of ribosome rescue and recycling factors in yeast generates an accumulation of 3' UTR ribosomes (Young et al., 2015), we focused on the possibility that a failure of ribosome recycling may explain the abundance of 3' UTR RPFs observed in platelets and reticulocytes. The distribution of fragment sizes that mapped to 3' UTRs was nearly indistinguishable from those that mapped to the coding sequence (CDS) (Figure 2A), suggesting that they are authentic RPFs and not the

result of non-ribosomal ribonucleoprotein (RNP) complexes protecting mRNA fragments from nuclease digestion (Ingolia et al., 2014). The 3' UTR RPFs did not preferentially occupy a single open reading frame (ORF), as would be anticipated from stop codon read-through (Figure 2B; Dunn et al., 2013), however. We thus tested whether 3' UTR ribosomes are associated with a nascent polypeptide chain (indicative of active translation) by treating lysates with high salt concentrations to release vacant ribosomes lacking a nascent polypeptide (Blobel and Sabatini, 1971). This treatment released ~80% of ribosomes with a stop codon in the A site (Figure 2C, black arrows), consistent with the expectation that these ribosomes have terminated translation and released their nascent polypeptide. Critically, ribosomes in the 3' UTR were similarly released by the high-salt wash, indicating that they also lack a nascent polypeptide (Figure 2D; Figure S2B); by contrast, ribosomes within the ORF were not affected. Based on these data (and additional genetic experiments below), we conclude that a majority of ribosomes in the 3' UTR are post-termination, unrecycled ribosomes not actively translating.

Dynamic Regulation of Ribosome Recycling and Ribosome Rescue

The accumulation of vacant ribosomes downstream of the stop codon is consistent with a recycling defect. Indeed, we found that the levels of the canonical ribosome recycling factor ABCE1 (Pisarev et al., 2010) are strongly reduced in platelets compared with precursor megakaryoblast cells (Meg01) and in reticulocytes compared with undifferentiated K562 cells (Figure S2C). Moreover, declining ABCE1 levels have been observed in proteomic analyses of erythroid differentiation from primary human cells (Gautier et al., 2016; Prenni et al., 2012), although the consequences of this decline were not explored. Interestingly, ABCE1 is also depleted in PLPs (Figure 3A) and in K562 cells induced toward erythroid differentiation (Figure 3B and 3C), where RNA levels also decline (Figure S4). Natural reduction of ABCE1 in primary anucleate lineages thus occurs in our *in vitro* models, which also show 3' UTR ribosome accumulation, whereas levels of other translation factors, including the initiation factor eIF1A, the termination factor eRF1, and the core ribosomal proteins RPS6 or RPL4, do not change (Figures 3B and 3C). In reticulocytes, a likely explanation for the loss of ABCE1 is that this protein contains an obligate iron-sulfur (FeS) cluster (Barthelme et al., 2007; Figures S2F and S2G) and natural clearance of mitochondria during maturation (Kundu et al., 2008; Novak et al., 2010; Schweers et al., 2007) will inevitably impair FeS cluster biogenesis. Consistent with this model, we see clear evidence for loss of mitochondrially encoded transcripts in our RNA sequencing (RNA-seq) data (Figure S2F).

Reduced ABCE1 levels suggest a plausible mechanistic explanation for 3' UTR ribosome occupancy. However, although ABCE1 levels begin to decline immediately after K562 cells are induced to differentiate, 3' UTR ribosome accumulation was modest 2 days after induction (Figures 1E and 1G). These observations suggest that a compensatory response might allow these cells to remove post-termination ribosomes from the 3' UTRs despite reduced levels of ABCE1. To test this hypothesis, we measured the expression of the ribosome rescue factors

PELO and HBS1L, which act together to release stalled or unrecycled ribosomes that are not engaged in elongation and make them available for translation (Guydosh and Green, 2014; Shoemaker et al., 2010). In primary platelets and reticulocytes, PELO levels were strongly diminished (Figure 3E), and this down-regulation was recapitulated in PLPs (Figure 3A) and fully differentiated K562 cells (Figure 3B). Both recycling and rescue factors are attenuated in fully mature platelets and reticulocytes, consistent with the observed abundance of 3' UTR ribosomes in these lineages. Strikingly, however, PELO was initially upregulated ~5-fold in the 2 days following hemin induction in K562 cells (Figures 3B–3D) and more modestly in published quantitative mass spectrometry data from erythropoietic differentiation of primary CD34+ cells (Figure 3F; Gautier et al., 2016).

Our data reveal dynamic regulation of ribosome recycling and rescue factors in anucleate hematopoietic lineages. The delay between the decline in ABCE1 levels and the accumulation of 3' UTR ribosomes leads to a model wherein transient activation of the ribosome rescue pathway compensates for the natural decline of ribosome recycling to permit ongoing translation of critical mRNAs during terminal differentiation (Figure 3D). Subsequent combined loss of both ABCE1 and PELO late in differentiation, when less translation is required, may allow the dramatic accumulation of unrecycled ribosomes observed by ribosome profiling.

PELO Compensates for the Loss of ABCE1

We set out to directly test our model by manipulating the levels of ribosome recycling/rescue factors *in vitro*. Indeed, short hairpin RNA (shRNA) knockdown of ABCE1 in undifferentiated K562 cells (Figure 4A), which normally do not accumulate 3' UTR ribosomes, was sufficient to drive their accumulation (Figures 4B and 4C, black traces). Further, these accumulated 3' UTR ribosomes could be “rescued” by overexpression of PELO (Figures 4B and 4C, pink traces, and 4F), further validating that 3' UTR RPFs in K562 cells and PLPs represent authentic ribosomes. As anticipated, ABCE1 knockdown also led to a buildup of terminated, unrecycled ribosomes at the stop codon (Figure 4D, black trace) that was cleared by overexpression of PELO (Figure 4D, pink trace). Importantly, overexpression of PELO did not alter global translation patterns or RNA levels in the presence of normal levels of ABCE1 (Figures S3G and S3H, days 0–2). These data establish that loss of ABCE1 is sufficient to cause the accumulation of post-termination 3' UTR ribosomes in mammalian cells and that increased expression of PELO can at least in part suppress this effect.

To test whether PELO can also rescue the 3' UTR ribosomes that accumulate physiologically during differentiation, we stably overexpressed PELO (or GFP as our control) in Meg01 or K562 cells (Figures 4E and 4F) and performed ribosome profiling on PLPs and hemin-differentiated K562 cells. As anticipated, overexpression of PELO suppressed 3' UTR ribosomes strongly in PLPs (Figures 4G and 4I; Figures S3C–S3E) and more modestly in day 6 differentiated K562 cells, when endogenous levels of PELO were low (Figure 4H; Figure S3F). We conclude that the 3' UTR ribosomes that accumulate on platelet and reticulocyte mRNAs are true substrates for ribosome rescue by PELO.

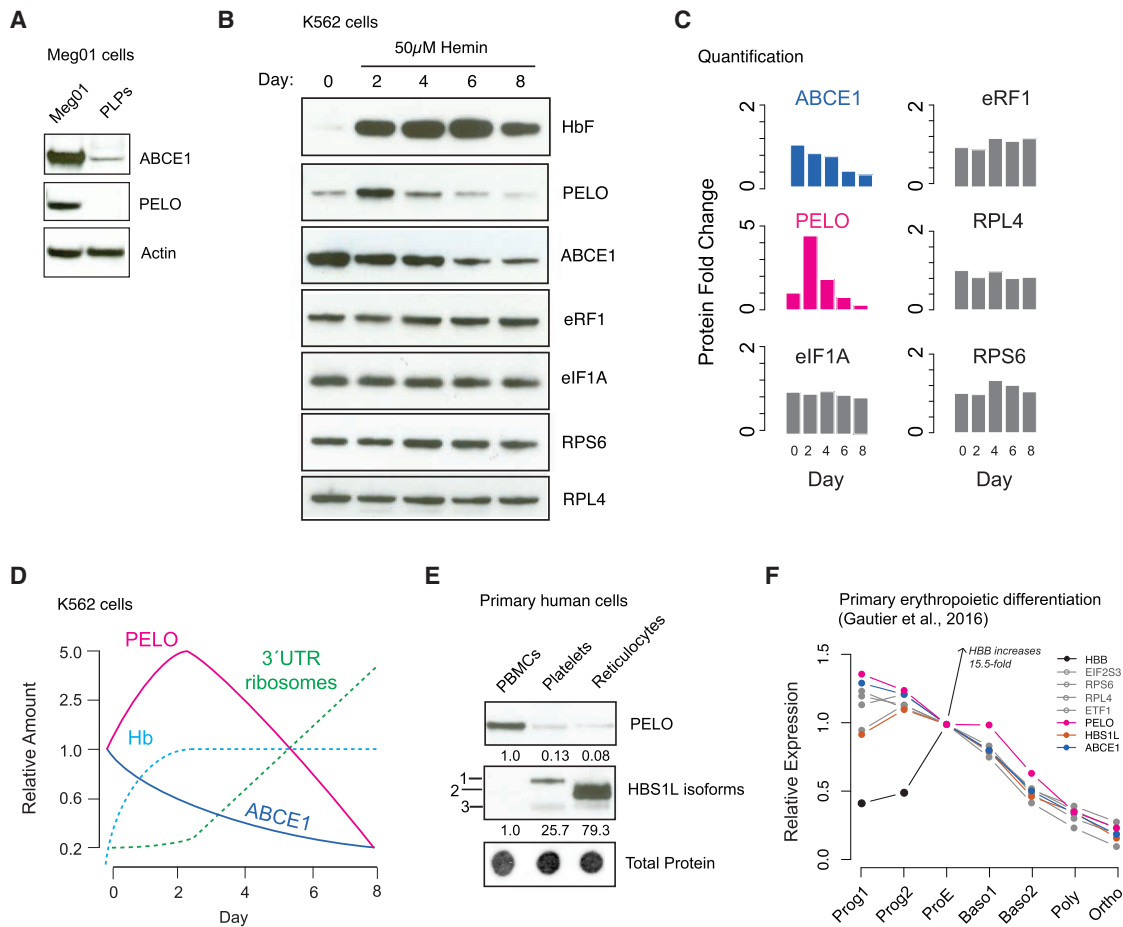


Figure 3. Dynamic Regulation of the Ribosome Recycling Machinery

(A) PELO and ABCE1 are present in Meg01 cells but absent in PLPs.

(B) Translation factor expression in differentiating K562 cells.

(C) Quantification of western blots in (B) normalized to day 0.

(D) Schematic of recycling/rescue factor changes during K562 cell differentiation in relation to hemoglobin and 3' UTR ribosome accumulation.

(E) Differential expression of PELO and HBS1L isoforms in PBMCs, platelets, and reticulocytes with band intensities normalized to total protein.

(F) Quantitative mass spectrometry data from Gautier et al. (2016) showing relative protein levels during erythropoietic differentiation of primary CD34+ cells.

PELO/HBS1L Regulate Ribosome Homeostasis

We propose that induction of the ribosome rescue factor PELO during platelet and reticulocyte maturation plays a critical role in rescuing ribosomes to maintain translational homeostasis and support the synthesis of critical proteins as ABCE1 levels decline. Reticulocytes synthesize large quantities of hemoglobin subunit proteins, as do K562 cells differentiating in response to hemin treatment (Rutherford et al., 1979), and we observed that the induction of PELO and HBS1L coincides closely with hemoglobin production (Figures 5A and 3B). We note that K562 cells primarily synthesize embryonic and fetal hemoglobin tetramers ($\alpha_2\gamma_2$ and $\alpha_2\epsilon_2$) (Rutherford et al., 1979) rather than the adult hemoglobin ($\alpha_2\beta_2$) that is made in primary reticulocytes from adults. Through analysis of our ribosome profiling and RNA-seq data, we see that hemoglobin accumulation is primarily driven by increased translation (Figure 5B). We calculated the translational efficiency (TE) as the ratio of RPFs relative to

mRNA abundance and saw that TE of the individual α and γ mRNAs encoding the HbF ($\alpha_2\gamma_2$) tetramer rises dramatically in the first 2 days of differentiation (~ 75 -fold for α -globin mRNA and ~ 8 -fold for γ -globin mRNA) and drops off rapidly thereafter (Figures 5B and 5C; Figure S4). The time course of hemoglobin production closely matches that of PELO expression (Figures 3B and 5A), suggesting that PELO/HBS1L induction may be critical in providing available ribosome subunits for its translation.

PELO/HBS1L Is Required for Hemin-Induced HbF Accumulation

In light of the tight coincidence between PELO induction and hemoglobin expression, we next asked whether PELO/HBS1L induction is required to promote hemoglobin accumulation. Defects in ribosome rescue (Figure 5E), either by knockdown of HBS1L alone (Figure 5F) or with PELO (Figure 5G), abrogated hemoglobin production. The levels of the control proteins eIF1A

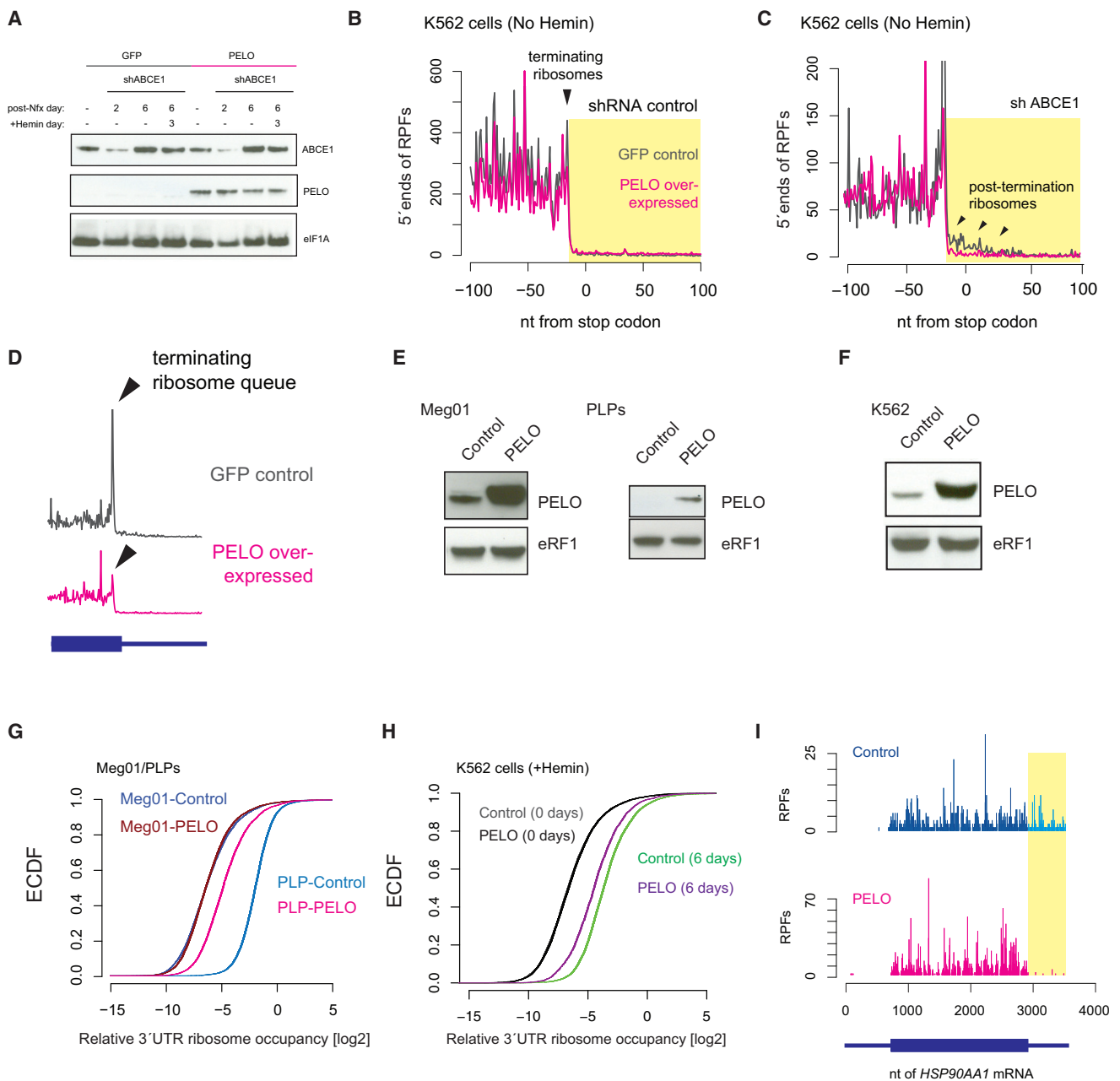


Figure 4. Ribosome Rescue by PELO/HBS1L Compensates for Declining ABCE1 Levels

(A) shRNA knockdown of ABCE1 in K562 cells overexpressing GFP or PELO. (B and C) Profile of 28- to 32-nt RPFs around stop codons. ABCE1 knockdown leads to the accumulation of post-termination ribosomes, indicated by black arrows, either at the stop codon (B) or in the 3' UTR (C). PELO overexpression suppresses this effect. (D) ABCE1 knockdown leads to a queue of terminating ribosomes on stop codons (gray trace), and overexpression of PELO prevents this effect. (E and F) Transgenic overexpression of PELO increases protein levels relative to eRF1 (control). (G and H) Restoration of PELO releases 3' UTR ribosomes in PLPs (G) and 6-day differentiated K562 cells (H). (I) Ribosome profile of the *HSP90AA1* mRNA in PLPs.

and actin remained unchanged, indicating that these changes do not reflect a loss of cell viability. These data instead argue that PELO/HBS1L induction is required for translation of a group of mRNAs that includes hemoglobin in K562 cells, where they

compensate for the natural loss of ABCE1 to maintain ribosome availability. We propose that this release of unrecycled ribosomes by PELO/HBS1L allows for the translation of critical mRNAs, such as those encoding hemoglobin (Lodish, 1974).

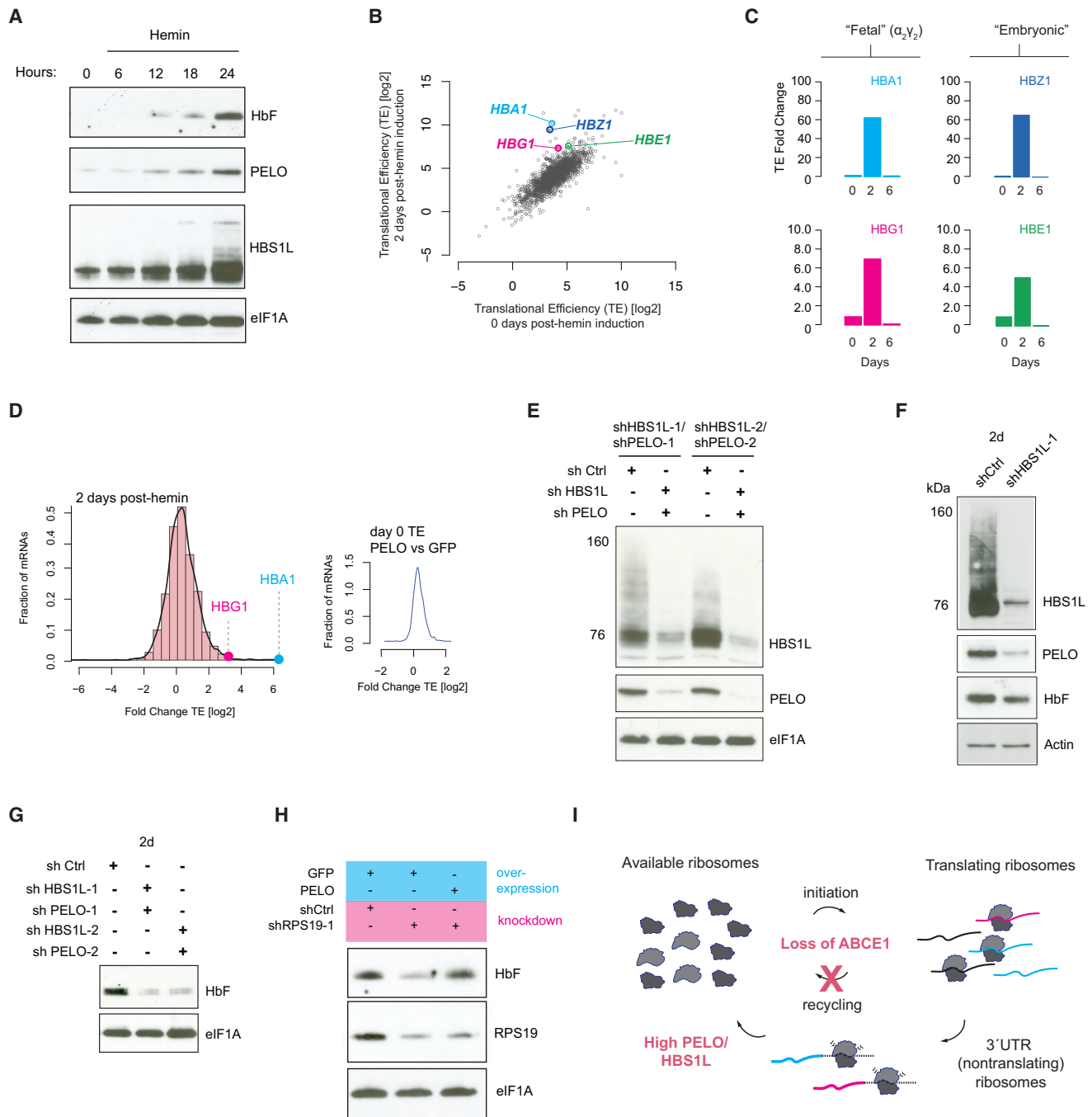


Figure 5. PELO/HBS1L Regulate Ribosome Homeostasis

(A) Hemoglobin, PELO, and HBS1L expression during the first 24 hr of hemin induction.

(B) Global translational efficiency (TE) changes after 2 days of hemin induction.

(C) TE changes for mRNAs encoding hemoglobin subunits measured 2 days post-induction.

(D) Histogram of TE changes for all mRNAs after 2 days of hemin treatment. Inset: TE ratio (PELO versus GFP) prior to Hemin induction.

(E) shRNA knockdown of PELO and HBS1L with two different shRNA hairpin sequences each.

(F and G) Hemoglobin expression after hemin induction is reduced by knockdown of HBS1L and enhanced by knockdown of PELO.

(H) RPS19 knockdown reduces hemin-induced hemoglobin accumulation suppressed by overexpression of PELO.

(I) Model showing the loss of ABCE1 in differentiating K562 cells accompanied by induction of PELO/HBS1L to maintain ribosome homeostasis.

Because hemoglobin mRNAs are well known to have relatively high initiation rates (Lodish, 1971) and should be kinetically favored in competitions with other mRNAs, an increase in ribosomes available for initiation could differentially enhance the translation of hemoglobin transcripts.

Ribosome Rescue and Disorders of Ribosome Homeostasis

Our model for the regulation of ribosome homeostasis in anucleate hematopoietic cells raised the question of how recycling and rescue factors interact with other perturbations in ribosome availability in these lineages. “Ribosomopathies” are a group of heterogeneous disorders that result from perturbations in ribosome homeostasis and often manifest in hematopoietic dysfunction (in addition to defects in other tissues) (Narla and Ebert, 2010). Heterozygous loss of several specific core ribosomal protein genes, including *RPS19*, leads to Diamond-Blackfan anemia (DBA) (Draptchinskaia et al., 1999), a disorder associated with reduced erythroid progenitor populations (Nathan et al., 1978) that causes congenital hypoplastic anemia (Diamond and Blackfan, 1938). The pathogenesis of *RPS19*-deficient DBA is complex but involves a defect in 40S ribosome biogenesis (Flygare et al., 2007) that leads to impaired survival of early erythroid progenitors (Flygare et al., 2005).

To examine the potential relevance of our observations to DBA, we depleted *RPS19* in K562 cells by shRNA knockdown (Figure 5H; Figure S5), which should reduce ribosome production by restricting ribosome proteins available for assembly. Although specific stages of erythroid development lack reliable matched counterparts in K562 differentiation, the early phase of erythropoiesis resembles an early, 24-hr time point in K562 cells because they still have nuclei and have not yet lost all ribosome recycling/ribosome rescue or arrested protein synthesis (as shown in Figure 3D). Hemoglobin accumulation was strongly abrogated by *RPS19* depletion (Figure 5H, lanes 1 and 2). Strikingly, transgenic overexpression of PELO/HBS1L restored hemoglobin levels (Figure 5H, lane 3). PELO/HBS1L can thus counteract general defects in ribosome availability resulting from defects in ribosome production as well as recycling.

DISCUSSION

Here we reveal a surprising accumulation of ribosomes on the 3' UTRs of mRNA transcripts in primary platelets and reticulocytes and argue that they are driven by cell type-specific regulation of the core ribosome recycling and rescue machinery present in all cells (Figure 5I). These distinctive features of anucleate blood cell lineages are recapitulated in cell culture models. We found that the natural loss of ABCE1-mediated ribosome recycling during terminal differentiation causes vacant, un-recycled ribosomes to enter the 3' UTR of platelet and reticulocyte mRNAs. These 3' UTR RPFs can be released by overexpression of the ribosome rescue factors PELO and HBS1L.

We further show that these ribosome rescue factors, PELO and HBS1L, along with the recycling factor ABCE1, are dynamically regulated during erythroid and platelet maturation. We propose that the upregulation of PELO/HBS1L is critical for maintaining the pool of available ribosomes for translation

when ABCE1 activity declines during the differentiation of anucleate blood cell lineages. The events that trigger ABCE1 loss are unknown, but we speculate that its obligate FeS cluster (Barthelme et al., 2007) can no longer be synthesized after mitochondrial breakdown during reticulocyte maturation.

We have identified the regulation of ribosome availability in platelets and reticulocytes as a critical role for mammalian ribosome rescue factors distinct from their known role in mRNA surveillance. Our results also point to a broader potential for rescue factors in mitigating the effects of ribosome shortage resulting from impaired ribosome biogenesis as well as physiological loss of ABCE1. Natural levels of ribosome rescue factors could modulate the sensitivity of cells to the availability of ribosomes, and PELO/HBS1L levels may increase to meet transient cellular demands for ribosomes, including those posed by declining levels of ABCE1 or ribosomal protein haploinsufficiency (Bhattacharya et al., 2010; Flygare et al., 2007). Intriguingly, genomic variants near the mammalian *HBS1L-MYB* locus are associated with multiple hematologic traits (Menzel et al., 2007). Whether these polymorphic variants affect the regulation of PELO/HBS1L we observed in platelets and erythroid cells, in addition to their well-documented effects on MYB expression (Stadhouders et al., 2014), remains an exciting area of future investigation.

EXPERIMENTAL PROCEDURES

Primary Platelets and Reticulocytes

Leukoreduced plateletpheresis samples were obtained from individual healthy donors in accordance with Institutional Review Board (IRB) #00001463 (Johns Hopkins University). Platelet samples were prepared from single-donor apheresis samples to achieve $<1 \times 10^6$ white blood cells (WBCs) per unit of $>1 \times 10^{11}$ platelets (or a WBC frequency of 1×10^{-6}).

Ribosome Profiling

Ribosome profiling was carried out as described previously (Ingolia et al., 2012). For high-salt washes, lysates were brought to 1 M KCl (final concentration) for 30 min on ice and exchanged using a desalting column (Zeba) prior to footprinting.

Meg01/PLP Cell Culture Experiments

Meg01 cells were obtained from the ATCC and cultured in RPMI 1640 (10% fetal bovine serum [FBS]). PLPs were purified from Meg01 culture supernatants by 5- μ m filtration and centrifugation at $3,000 \times g$ for 10 min (Figures S1F–S1H; Supplemental Experimental Procedures).

K562 Differentiation Experiments

K562 cells were obtained from the ATCC and cultured in RPMI 1640 (10% FBS) supplemented with 2 mM glutamine. Hemin (Sigma) was dissolved in 20 mM NaOH and sterile-filtered through a 0.22- μ m filter (Millipore) before adding to cells.

ACCESSION NUMBERS

The accession number for the ribosome profiling and mRNA sequencing data reported in this paper is NCBI GEO: GSE85864.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2016.08.088>.

AUTHOR CONTRIBUTIONS

E.W.M., R.G., and N.T.I. designed the experiments and interpreted the results. E.W.M. performed the experiments. E.W.M. and J.W. performed the initial hemin experiments. E.W.M., R.G., and N.T.I. wrote the paper with input from all authors.

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