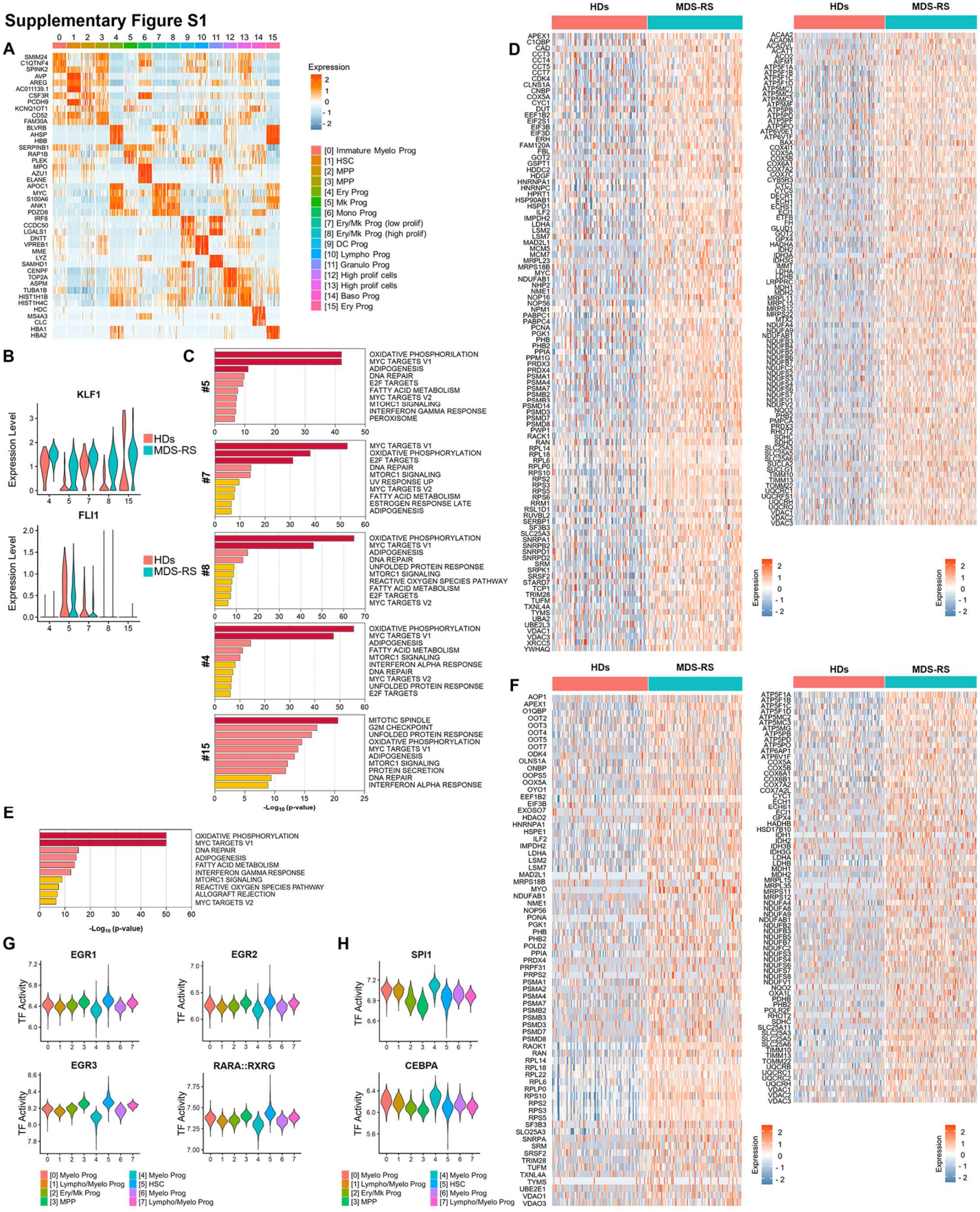
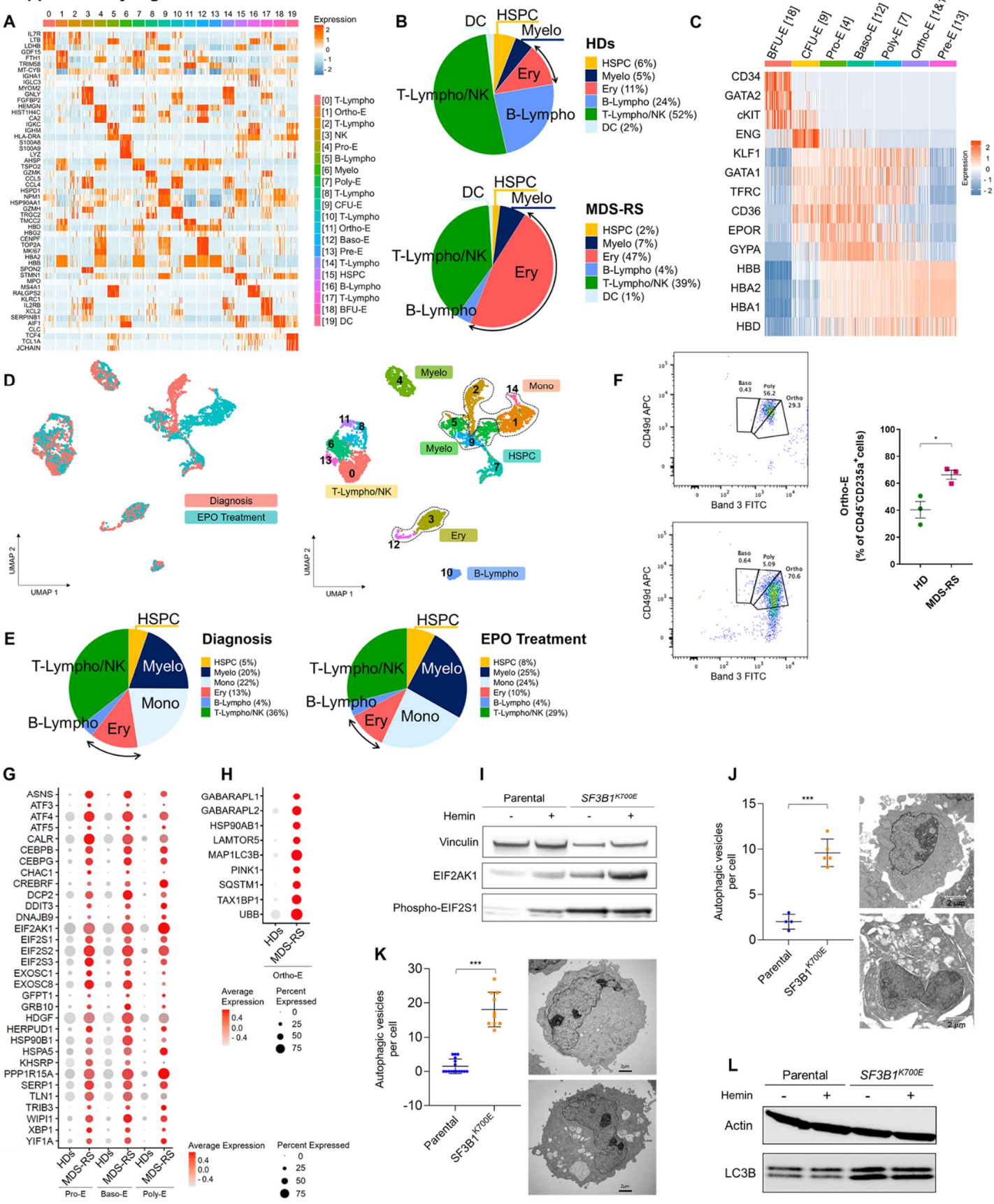

Supplementary Figure S1
A
Expression
[0] Immature Myelo Prog
[1] HSC
[2] MPP
[3] MPP
[4] Ery Prog
[5] Mk Prog
[6] Mono Prog
[7] Ery/Mk Prog (low prolif)
[8] Ery/Mk Prog (high prolif)
[9] DC Prog
[10] Lympho Prog
[11] Granulo Prog
[12] High prolif cells
[13] High prolif cells
[14] Baso Prog
[15] Ery Prog
B
KLF1
FLI1
Expression Level
HDs
MDS-RS
C
#5
OXIDATIVE PHOSPHORILATION
MYC TARGETS V1
ADIPOGENESIS
DNA REPAIR
E2F TARGETS
FATTY ACID METABOLISM
MYC TARGETS V2
MTORC1 SIGNALING
INTERFERON GAMMA RESPONSE
PEROXISOME
#7
MYC TARGETS V1
OXIDATIVE PHOSPHORYLATION
E2F TARGETS
DNA REPAIR
MTORC1 SIGNALING
UV RESPONSE UP
MYC TARGETS V2
FATTY ACID METABOLISM
ESTROGEN RESPONSE LATE
ADIPOGENESIS
#8
OXIDATIVE PHOSPHORYLATION
MYC TARGETS V1
ADIPOGENESIS
DNA REPAIR
UNFOLDED PROTEIN RESPONSE
MTORC1 SIGNALING
REACTIVE OXYGEN SPECIES PATHWAY
FATTY ACID METABOLISM
E2F TARGETS
MYC TARGETS V2
#4
OXIDATIVE PHOSPHORYLATION
MYC TARGETS V1
ADIPOGENESIS
FATTY ACID METABOLISM
MTORC1 SIGNALING
INTERFERON ALPHA RESPONSE
DNA REPAIR
MYC TARGETS V2
UNFOLDED PROTEIN RESPONSE
E2F TARGETS
#15
MITOTIC SPINDLE
G2M CHECKPOINT
UNFOLDED PROTEIN RESPONSE
OXIDATIVE PHOSPHORYLATION
MYC TARGETS V1
ADIPOGENESIS
MTORC1 SIGNALING
PROTEIN SECRETION
DNA REPAIR
INTERFERON ALPHA RESPONSE
-Log10 (p-value)
D
HDs
MDS-RS
Expression
F
HDs
MDS-RS
Expression
E
OXIDATIVE PHOSPHORYLATION
MYC TARGETS V1
DNA REPAIR
ADIPOGENESIS
FATTY ACID METABOLISM
INTERFERON GAMMA RESPONSE
MTORC1 SIGNALING
REACTIVE OXYGEN SPECIES PATHWAY
ALLOGRAFT REJECTION
MYC TARGETS V2
-Log10 (p-value)
G
EGR1
EGR2
EGR3
RARA::RXRG
TF Activity
[0] Myelo Prog
[1] Lympho/Myelo Prog
[2] Ery/Mk Prog
[3] MPP
[4] Myelo Prog
[5] HSC
[6] Myelo Prog
[7] Lympho/Myelo Prog
H
SPI1
CEBPA
TF Activity
[0] Myelo Prog
[1] Lympho/Myelo Prog
[2] Ery/Mk Prog
[3] MPP
[4] Myelo Prog
[5] HSC
[6] Myelo Prog
[7] Lympho/Myelo Prog

**Supplementary Figure S1. *SF3B1*$^{MT}$ do not affect erythropoiesis at the level of HSPCs**

**(A)** Heatmap of the expression of the top 3 genes enriched in each of the 16 clusters shown in Figure 1A. **(B)** Violin plots of *KLF1* (top) and *FLI1* (bottom) expression across the HD and MDS-RS Ery/Mk clusters shown in Figure 1A. **(C)** Pathway enrichment analysis of the genes that were significantly upregulated in MDS-RS Ery/Mk clusters 5, 7, 8, 4, and 15 as compared with the HD clusters shown in Figure 1A (adjusted $P \leq 0.05$). The top 10 Hallmark gene sets are shown. **(D)** Heatmaps of the expression of the MYC signaling (left) and oxidative phosphorylation (right) pathway genes that were significantly upregulated in the MDS-RS erythroid/megakaryocytic cell clusters as compared with the HD erythroid/megakaryocytic cell cluster shown in Figure 1A. **(E)** Pathway enrichment analysis of the genes that were significantly upregulated in the MDS-RS HSC cluster as compared with the HD HSC cluster shown in Figure 1A (adjusted $P \leq 0.05$). The top 10 Hallmark gene sets are shown. **(F)** Heatmaps of the expression of MYC signaling (left) and oxidative phosphorylation (right) pathway genes that were significantly upregulated in the MDS-RS HSC cluster as compared with the HD HSC cluster shown in Figure 1A. **(G)** Violin plots showing the activities of the TFs EGR1, EGR2, EGR3, and RARA:RXRG across the 8 clusters shown in Figure 1D. **(H)** Violin plots showing the activities of the TFs SPI1 and CEBPA across the 8 clusters shown in Figure 1D.

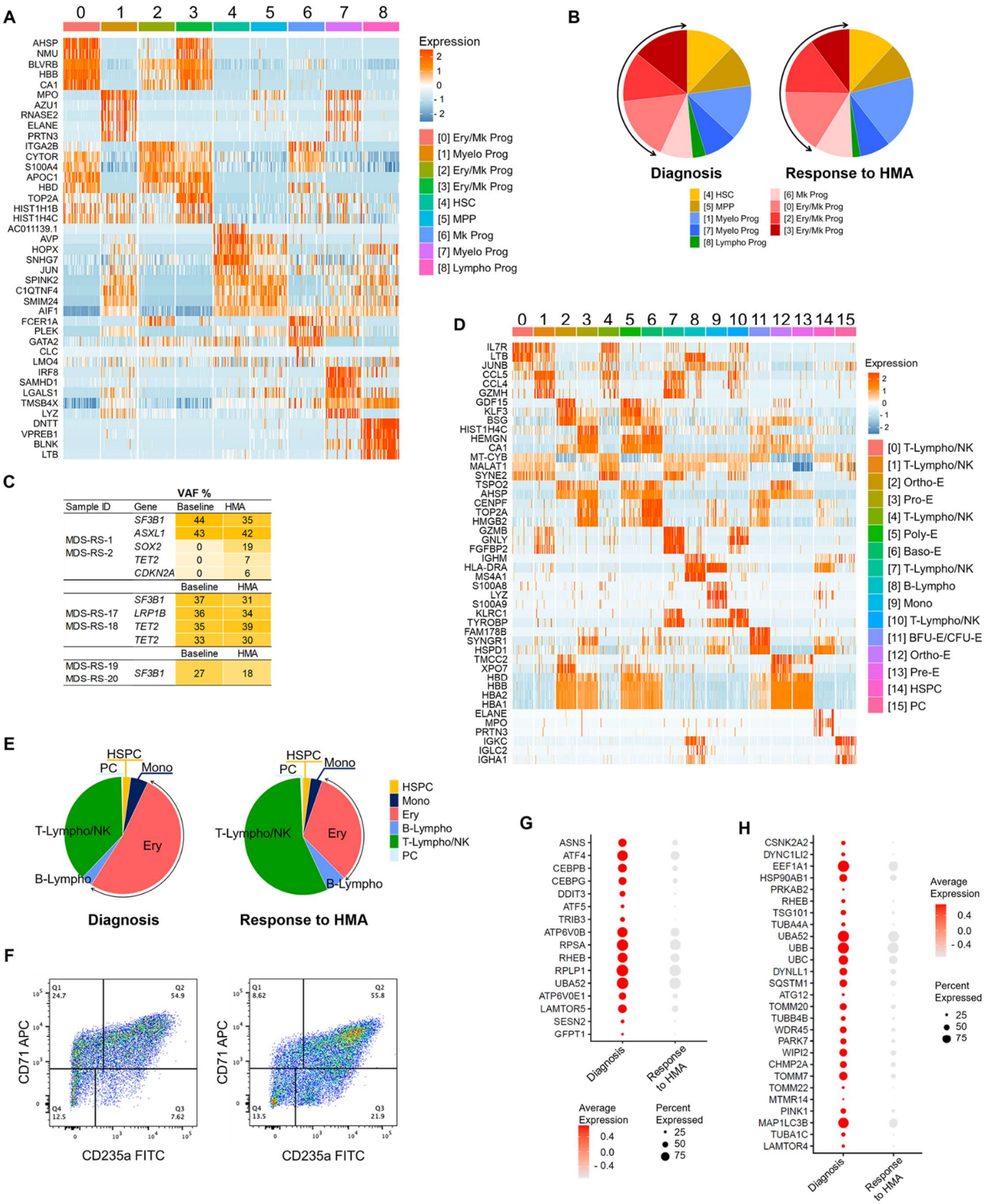
## Supplementary Figure S2



**Supplementary Figure S2. *SF3B1*$^{MT}$ arrest erythroid terminal differentiation and activate the EIF2AK1-induced response pathway to heme deficiency**

**(A)** Heatmap of the expression of the top 3 genes enriched in each of the 20 clusters shown in Figure 2A. **(B)** Distribution of HD (top) and MDS-RS (bottom) MNCs among the cluster groups shown in Figure 2A defined by distinct lineage differentiation trajectories. HSPC, hematopoietic stem and progenitor cells; Myelo, myeloid cells; Ery, erythroblasts; B-Lympho, B-lymphocytes; T-Lympho/NK, T-lymphocytes and natural killer cells; DC, dendritic cells. Arrows indicate the erythroblastic population. **(C)** Heatmap of the expression of representative surface markers (*CD34*, *cKIT*, *ENG*, *TFRC*, *CD36*, *EPOR,* and *GYPA*), transcriptional factors (*GATA1*, *GATA2*, and *KLF1*), and hemoglobin subunits (*HBB*, *HBA2*, *HBA1*, and *HBD*) across different stages of erythroid differentiation. BFU-E, phenotypic burst forming unit-erythroid cells; CFU-E, phenotypic colony formation unit-erythroid cells; Pro-E, pro-erythroblasts; Baso-E, basophilic erythroblasts; Poly-E, polychromatophilic erythroblasts; Ortho-E, orthochromatic erythroblasts; Pre-E, pre-erythrocytes. **(D)** UMAP plots of scRNA-seq data for single BM MNCs isolated from one patient with *SF3B1*-mutant MDS-RS at the time of diagnosis (n=2,608) and after EPO treatment (n=3,182). Each dot represents one cell. Different colors represent the sample origin (left) and cluster identity (right). HSPC, hematopoietic stem and progenitor cells; Myelo, myeloid cells; Mono, monocytes; Ery, erythroblasts; B-Lympho, B-lymphocytes; T-Lympho/NK, T-lymphocytes and natural killer cells. **(E)** Distribution of MNCs across the clusters shown in Figure S2D. **(F)** Left, representative flow cytometry plots showing the expression of integrin α4 (CD49d) and band 3 in CD45$^{-}$CD235a$^{+}$ erythroblasts from one HD BM sample (top) and one *SF3B1*-mutant MDS-RS BM sample (bottom). Right, frequencies of orthochromatic erythroblasts (Ortho-E) in CD45$^{-}$CD235a$^{+}$ erythroblasts isolated from HD BM samples (n=3) and *SF3B1*-mutant MDS-RS BM samples (n=3). Statistically significant differences were detected using a 2-tailed Student *t*-test. Baso-E, basophilic erythroblasts; Poly-E, polychromatophilic erythroblasts. **(G)** Dot plot of the expression of the genes belonging to the EIF2AK1-mediated pathway that were significantly upregulated in the MDS-RS Pro-E, Baso-E, and Poly-E clusters as compared with the HD Pro-E, Baso-E, and Poly-E clusters shown in Figure 2B. **(H)** Dot plot of the expression of the autophagy pathway genes that were significantly upregulated in the MDS-RS Ortho-E cluster as compared with the HD Ortho-E cluster shown in Figure 2B. **(I)** Representative Western blot analysis of EIF2AK1 and phospho-EIF2S1 in parental and *SF3B1*$^{K700E}$ K562 cells treated with vehicle (-) or hemin (+) for 3 days. Vinculin was used as a loading control. **(J)** Left, number of autophagic vesicles per cell in 4 parental and 4 *SF3B1*$^{K700E}$ K562 cells. Statistically significant differences were detected using a 2-tailed Student *t*-test. Right, representative transmission electron microscopy images of one representative parental cell (top) and one *SF3B1*$^{K700E}$ K562 cell (bottom). Scale bars represent 2 μm. **(K)** Left, number of autophagic vesicles per cell in 16 parental and 10 *SF3B1*$^{K700E}$ K562 cells after 3 days of hemin-induced differentiation. Significant differences were detected using a 2-tailed Student *t*-test. Right, representative transmission electron microscopy images of one representative parental cell (top) and one *SF3B1*$^{K700E}$ K562 cell (bottom). Scale bars represent 2 μm. **(L)** Representative Western blot analysis of LC3B in parental and *SF3B1*$^{K700E}$ K562 cells treated with vehicle (-) or hemin (+) for 3 days. Actin was used as a loading control.

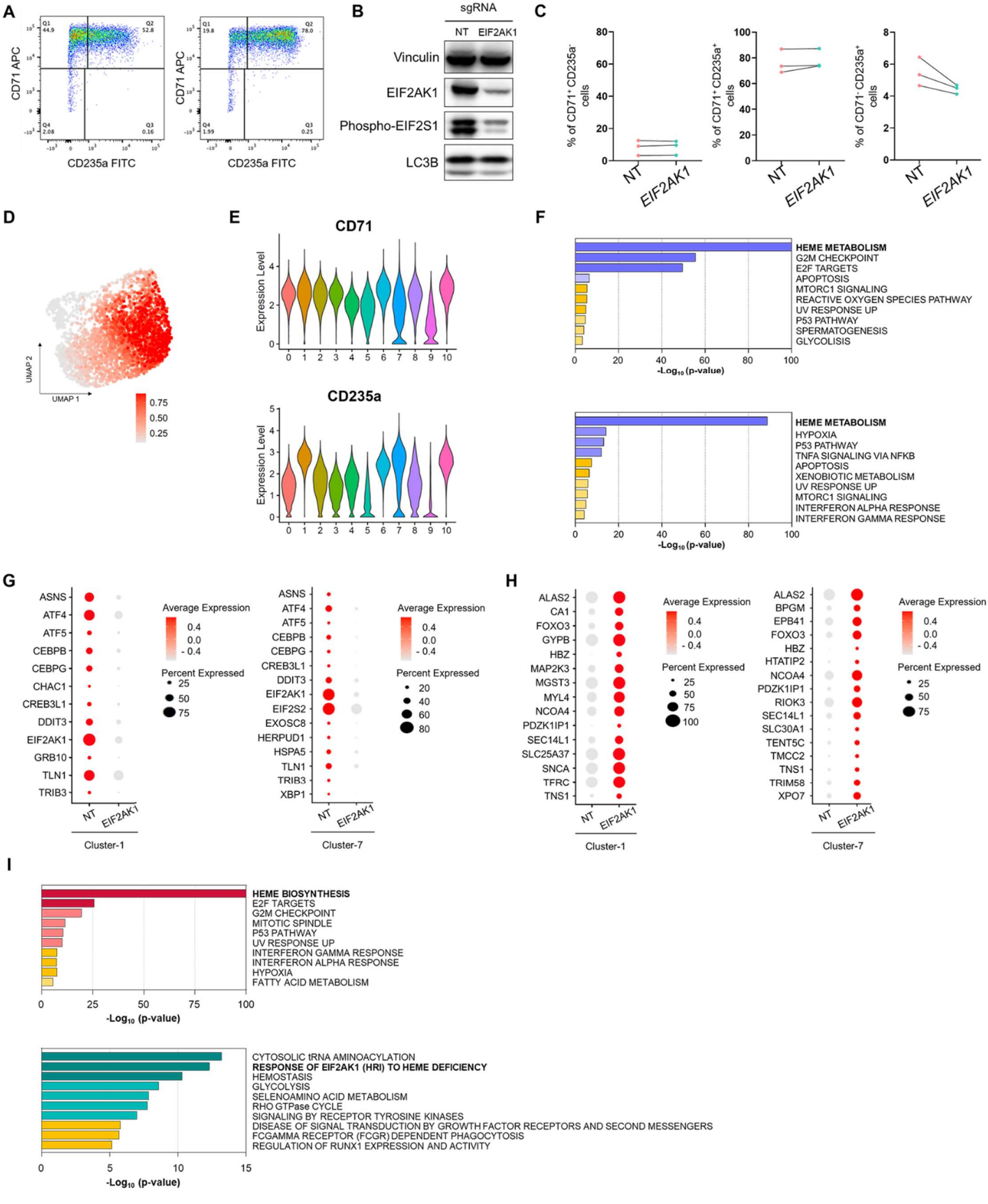
## Supplementary Figure S3



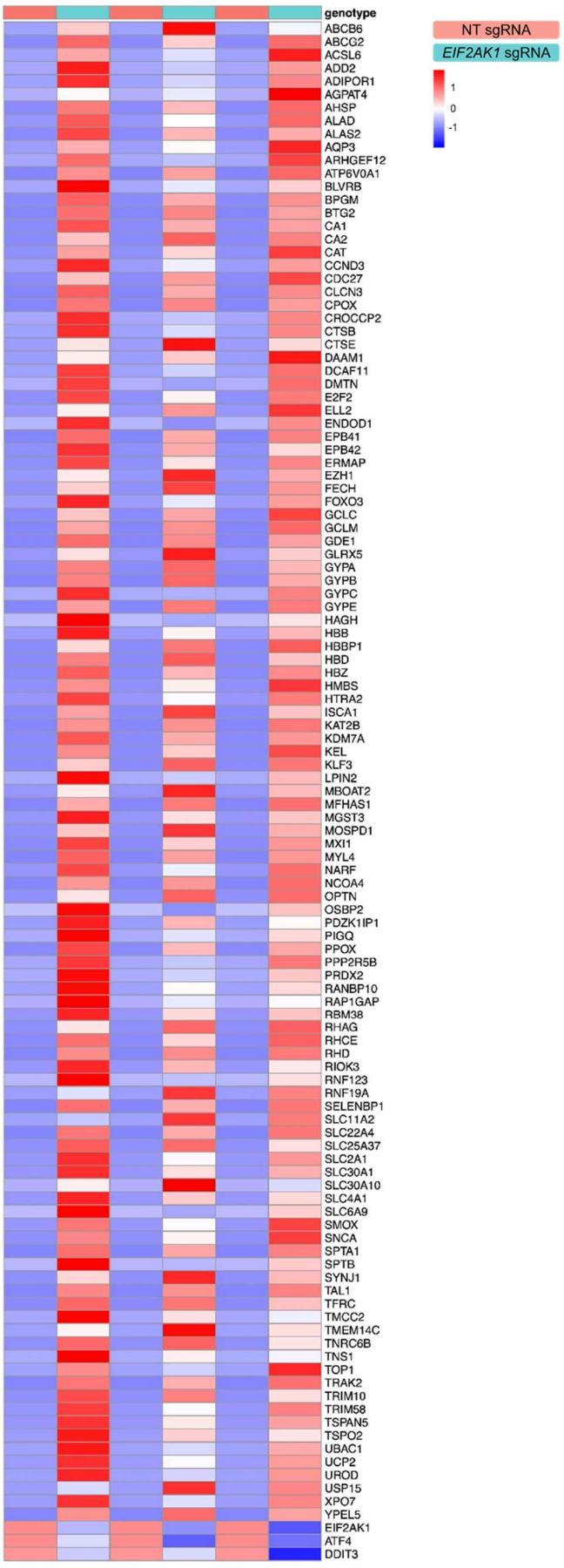
**Supplementary Figure S3. Hypomethylating agent therapy inhibits the EIF2AK1-induced response pathway to heme deficiency in terminally differentiated cells in patients who became transfusion independent.**

**(A)** Heatmap of the expression of the top 5 genes enriched in each of the 9 clusters shown in Figure 3A. HSC, hematopoietic stem cells; MPP, multipotent progenitors; Lympho, lymphoid; Ery/Mk, erythroid/megakaryocytic; Mk, megakaryocytic; Myelo, myeloid; Prog, progenitors. Arrows indicate the erythroid/megakaryocytic progenitors. **(B)** Distribution of $Lin^-CD34^+$ cells isolated from MDS-RS patients at baseline (left) and at the time of response to HMA therapy (right) across the clusters shown in Figure 3A. **(C)** Variant allelic frequencies (VAFs) of somatic mutations in relevant leukemia driver genes and/or oncogenes detected in total BM MNCs from 3 *SF3B1*-mutant MDS-RS patients at baseline or at the time of hematological response to HMA therapy. **(D)** Heatmap of the expression of the top 3 genes enriched in each of the 16 clusters shown in Figure 3B. **(E)** Distribution of MNCs isolated from MDS-RS patients at diagnosis (left) and at the time of response to HMA therapy (right) across the clusters shown in Figure 3B. HSPC, hematopoietic stem and progenitor cells; Mono, monocyte cells; Ery, erythroblasts; B-Lympho, B lymphocytes; T-Lympho/NK, T lymphocytes and natural killer cells; PC, Plasma cells. Arrows indicate the erythroblast population. **(F)** Flow cytometry plots showing the expression of CD71 and CD235a in primary $CD45^-$erythroblasts derived from *SF3B1*-mutant $CD34^+$ MDS-RS cells cultured in erythroid differentiation media for 8 days and then treated with vehicle (left) or 5-azacitidine (right) for 3 days. **(G)** Dot plot of the expression of the genes belonging to the EIF2AK1-mediated pathway that were significantly downregulated in MDS-RS Ortho-E at the time of response to HMA therapy as compared with the time of diagnosis. **(H)** Dot plot of the expression of autophagy pathway genes that were significantly downregulated in MDS-RS Ortho-E at the time of response to HMA therapy as compared with the time of diagnosis.

## Supplementary Figure S4



**J**




**K**

| | | VAF % | |
|---|---|---|---|
| Sample ID | Gene | NT | *EIF2AK1* |
| MDS-RS-3-bis | *SF3B1* | 14 | 12 |
| MDS-RS-13 | *SF3B1* | 16 | 15 |
| MDS-RS-14 | *SF3B1* | 36 | 42 |

**Supplementary Figure S4. Inhibition of EIF2AK1 overcomes the accumulation of RS and enables terminal erythroid maturation and red blood cell production**

**(A)** Flow cytometry plots showing the expression of CD71 and CD235a in representative NT (left) and *EIF2AK1* (right) sgRNA–treated *SF3B1*-mutant MDS-RS samples at day 13 of culture. **(B)** Representative Western blot analysis of EIF2AK1, phospho-EIF2S1 and LC3B in NT sgRNA– and *EIF2AK1* sgRNA–treated HD cells at day 13 of erythroid differentiation. Vinculin was used as a loading control. **(C)** Frequencies of $CD71^{+}CD235a^{-}$ (left), $CD71^{+}CD235a^{+}$ (middle), and $CD71^{-}CD235a^{+}$ (right) erythroblasts in NT sgRNA– or *EIF2AK1* sgRNA–treated HD samples (n=3) at day 13 of culture. Each symbol represents one sample; lines connect paired samples. No significant differences were detected using paired t-tests. **(D)** UMAP showing the CytoTRACE values of the cells in Figure 4C. The color indicates the degree of differentiation from low (grey) to high (red). **(E)** Violin plots of CD71 (top) and CD235a (bottom) expression across the 11 clusters shown in Figure 4C. **(F)** Pathway enrichment analysis of the marker genes of cluster 1 (top) and 7 (bottom) shown in Figure 4C (adjusted $P \leq 0.05$). **(G)** Dot plots of the expression of the genes involved in the EIF2AK1 response to heme deficiency pathway that were significantly downregulated in *EIF2AK1* sgRNA–treated cells from *SF3B1*-mutant MDS-RS in clusters 1 (left) and 7 (right) shown in Figure 4C. **(H)** Dot plots of the expression of the heme metabolism pathway genes that were significantly upregulated in *EIF2AK1* sgRNA–treated *SF3B1*-mutant MDS-RS cells from clusters 1 (left) and 7 (right) shown in Figure 4C. **(I)** Pathway enrichment analysis of the genes that were significantly upregulated (top) or downregulated (bottom) in *SF3B1*-mutant MDS-RS cells treated with *EIF2AK1* sgRNA as compared with those treated with NT sgRNAs ($P \leq 0.05$). **(J)** Heatmap of genes involved in heme metabolism that were significantly upregulated in *SF3B1*-mutant MDS-RS cells after *EIF2AK1* depletion. Expression of *EIF2AK1, ATF4,* and *DDIT3* indicate *EIF2AK1* pathway inhibition. **(K)** Variant allelic frequencies (VAFs) of $SF3B1^{MT}$ detected in non-targeting (NT) sgRNA– and *EIF2AK1* sgRNA–treated MDS-RS cells at day 13 of culture.