**Supplementary Material**

**Suppl 1.**

**Cell cultures and transfections**

K562 cells were cultured in RPMI medium (Thermo Fisher Scientific, Cat# 11875-093) containing 10% fetal bovine serum (Thermo Fisher Scientific, Cat# 16000-044) and 1× penicillin-streptomycin (Thermo Fisher Scientific, Cat# 15140-148) for 24 h before nucleofection. Cells were transfected with a GFP-tagged *SAR1A* CRISPR/Cas9 knockout plasmid (Santa Cruz Biotechnology, Cat# sc-404190-NIC) using SF Cell Line 4D-Nucleofector X Kit L (Lonza, Cat# V4XC-2024) in accordance with the manufacturer’s instructions. After 24 h of transfection, the medium was replaced with selective medium containing 2 μg/mL puromycin for 96 h. Then, the cells were treated with 100 μM hydroxyurea (Sigma-Aldrich, Cat# H8627-1G) for 72 h.

# **Flow-cytometry analysis of transfection efficiency**

Transfection efficiency was evaluated by measuring the percentage of GFP-expressing cells by flow cytometry using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA). Cells were washed with 1× PBS (Thermo Fisher Scientific, Cat# 10010-023), and then GFP was detected by using a 530/30 nm bandpass filter in the FL1 channel. Data analysis was performed with CellQuest software (BD Biosciences, San Jose, CA).

# **Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)**

# Total RNA was extracted by using the RNeasy Plus Mini kit (QIAGEN, Germantown, MD, Cat# 74134) in accordance with the manufacturer’s instructions. cDNA was synthesized with the SuperScript™ III first-strand synthesis system (Thermo Fisher Scientific), and qPCR was performed with the QuantStudio™ 6 Flex Real-Time PCR system (Thermo Fisher Scientific) using TaqMan universal master mix (Thermo Fisher Scientific, Cat# 4440047). The following primer and probe sequences were used: *SAR1A* forward primer, 5′-CAGTGTGCTCCAGTTCCTAG-3′; *SAR1A* reverse primer, 5′-TGATGTCGGATGTAGTGTTGG-3′; *SAR1A* probe, 5′-FAM-ATGTGAAGAAGAGTGGTTTTGCCTGC-BHQ1-3′; *GAPDH* forward primer, 5′-ACATCGCTCAGACACCATG-3′; *GAPDH* reverse primer, 5′- TGTAGTTGAGGTCAATGAAGGG-3′; *GAPDH* probe, 5′-HEX-AAGGTCGGAGTCAACGGATTTGGTC-BHQ1-3′.The results were analyzed with QuantStudio™ 6 Flex software, and relative expressions were determined by normalization to *GAPDH* expression.

# **Western blotting**

# Total protein lysates were extracted using RIPA buffer (Sigma-Aldrich, St. Louis, MO, Cat# R0278-50ML) supplemented with 1× protease inhibitor (Roche Life Science, Indianapolis, IN, Cat# 04-693-132-001) in accordance with the manufacturer’s instructions. The protein concentration was measured with a Pierce™ BCA protein assay kit (Thermo Fisher Scientific, Cat# 23227). Thirty micrograms of protein lysates were electrophoresed in NuPAGE™ 4–12% Bis-Tris gels (Thermo Fisher Scientific, Cat# NP0321BOX) and then transferred to polyvinylidene difluoride membranes (Thermo Fisher Scientific, Cat# IB401001) using an iBlot™ gel transfer device (Thermo Fisher Scientific). The membranes were blocked in Tris-buffered saline with 0.1% Tween-20 and 5% blotting milk (Bio-Rad, Hercules, CA) for 1 h and then incubated with primary antibody at 4 °C overnight. The primary antibodies used were anti-SAR1A (Thermo Fisher Scientific, Cat# PA5-68201), anti-hemoglobin γ (Cell Signaling Technology, Cat# 39386S), or anti-β-actin (Santa Cruz Biotechnology, Cat# sc-47778). The membranes were washed 3 times for 5 min each time and incubated with IRDye® 800CW goat anti-mouse IgG or anti-rabbit IgG secondary antibodies (LI-COR Biosciences, Lincoln, NE, Cat# 926-32210 or Cat# 926-32211) for 1 h in the dark at room temperature. After 3 washes, the fluorescence signal on the membranes was detected by using an Odyssey CLx imaging system (LI-COR Biosciences). Blots probed with anti-β-actin were used as a loading control.

**miRNA sequencing and data analysis**

Total RNA was isolated by using an miRNeasy micro kit (QIAGEN, Cat# 217084) and sequenced for miRNAs at Arraystar (Rockville, MD). The workflow pipeline for miRNA sequencing data analysis used in this study included alignment, quantification, normalization, and differential gene expression analysis. All analyses were performed in Partek Flow software (Partek Inc., St. Louis, MO). The sample FASTQ files were uploaded into Partek Flow software for processing. First, unaligned reads were trimmed adapter at the 3′ end and then trimmed bases with a minimum read length = 15 bases. Then, the trimmed reads were aligned to the whole genome of human genome assembly hg38 (GRCh38) using Bowtie aligner. The aligned reads were quantified with the human miRbase mature microRNAs version 22 annotation model and then normalized with the median ratio method. The normalized read counts were used for differential expression analysis by the DESeq2 method. A false discovery rate (FDR) was used to correct the *P*-value. FDR < 0.05 and fold-change less than −2 or greater than 2 were used to define differentially expressed miRNAs. The RNA-seq data in this publication will be deposited in NCBI’s Gene Expression Omnibus.

**qRT-PCR for miRNA expression**

Top 3 upregulated and downregulated miRNAs in each group with the largest fold-change were selected for validation of the differentially expressed miRNAs data using a TaqMan miRNA assay. Total RNA was isolated with an miRNeasy Micro Kit (QIAGEN, Cat#217084), and then 100 ng of total RNA was converted to complementary DNA (cDNA) using a TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Cat#4355596). qPCR was carried out on a QuantStudio6 instrument using a TaqMan miRNA assay and TaqMan Universal PCR Master Mix (Thermo Fisher Scientific). The relative miRNA expression was calculated by using the 2‑ΔΔCT method with wild-type control used as the calibrator and the RNU6B endogenous control for normalization.

**miRNA target prediction and KEGG pathway analysis**

Partek Flow was used to predict the targets and KEGG pathways associated with the differentially expressed miRNAs. To identify miRNA targets, TargetScan 8.0 was selected as the search mode and “Human” was selected as the organism. In addition, a *P*-value threshold of 0.01 was applied for the KEGG pathway analysis. The 11 genes (*DNMT1*, *DNMT3A*, *GATAD2B*, *HDAC1*, *IKZF1*, *KLF1*, *MYB*, *PPARGC1A*, *RUNX1*, *SOX6,* and *ZBTB7A*) were prioritized use to predict links of differentially expressed miRNAs to target genes in Fig. 2a based on prior associations with HbF regulation and consistent prediction using TargetScan.

**Statistical analysis**

The data from qRT-PCR are presented as mean ± standard deviation from 3 biological replicates. Student’s t-test was used for comparisons between 2 independent groups. A *P*-value < 0.05 was considered to be statistically significant for all analyses.