Supplemental Methods

Creation and differentiation of iPSCs from DBA patients
All iPSC lines (DBA and WT control) used in this study were derived from human primary dermal fibroblasts cultured in IMDM with 10% FBS, 1X penicillin/streptomycin (PS) (Gibco), 2 mM glutamine (Invitrogen), and 4 ng/ml bFGF (R&D). For reprogramming, fibroblasts were transduced with pHage2-CMV-RTTA-W and pHage-Tet-hSTEMCCA-loxP viruses (for RPS19+/p.Q126X) or with pHage-hSTEMMCCA-loxP virus (for RPL5+/p.R23X and all WT lines) in the presence of 5 μg/mL polybrene. At day 4, cells were split onto matrigel-coated (BD) dishes plated with irradiated mouse embryonic fibroblasts (MEF) in medium containing 0.1mM beta-mercaptoethanol (BME), 1X NEAA, 50 μg/ml ascorbic acid (AA), 4 ng/mL bFGF and for RPS19 only, 2 μg/ml doxycycline (DOX)(Sigma). At day 10, we switched to hESC media (DMEM/F12 containing 2mM of glutamine, 20% Knockout Serum Replacement (Invitrogen), 1X NEAA, 1X PS, 0.5X sodium pyruvate, 0.1mM BME, and 10 ng/ml bFGF). Four weeks later, iPSC colonies were picked and expanded for 20 passages before characterization. For cells reprogrammed with pHage-hSTEMMCCA-loxP virus (RPL5+/p.R23X and WT lines), the lentiviral cassette was subsequently removed using CRE-mediated excision. Characterization of iPSCs was based on pluripotency marker expression assessed by flow cytometry and RT-qPCR. All iPSCs analyzed in this study showed normal karyotype by G-banding. For teratoma formation, 1-2 x10^6 cells were injected into the neck of a Fox Chase SCID Beige Mice. Mice were sacrificed 6-9 weeks later.

Polysome profiling
Approximately 5 x 10^6 iPSCs were resuspended in 200 μl hypotonic buffer (1.5 mM KCl, 2.5 mM MgCl2, and 5.0 mM Tris-Cl, pH 7.4) and 200 μl hypotonic lysis buffer (same with 2% sodium deoxycholate, 2% Triton X-100, and 2.5 mM DTT) and gently disrupted using a Dounce homogenizer. The lysates were centrifuged at 8,000 g for 10 min at 4°C. The supernatant was supplemented with 4 μl RNase inhibitor (Life Technologies) and 80 μL heparin. Linear 10% to 45% sucrose gradients (80 mM NaCl, 5 mM MgCl2, 20 mM Tris-Cl, pH 7.4, and 1 mM DTT)
were formed using a Gradient Master (BioComp). Gradients were centrifuged at 38,000 rpm for 3h at 4°C and analyzed with an ISCO fractionator (Brandel).

**Pre-rRNA analysis**

Pre-rRNA analysis by Northern blotting was performed as described previously using synthetic oligonucleotide DNA probes. RNA (10 μg) was electrophoresed in 1% (wt/vol) agarose-formaldehyde gels and blotted to Hybond-N+ membranes (GE Healthcare) overnight. Oligonucleotide probes (Supplemental Table 3) were end-labeled with $[\gamma-^{32}\text{P}]$ATP and T4 polynucleotide kinase (Fisher).

**Gene targeting in human DBA iPSCs using AAVS1 zinc-finger nucleases**

Zinc-finger nuclease (ZFN) cDNAs under the control of the PGK promoter were cloned into a plasmid expression vector ($pPGK-ZFN-L(eft)$ and $pPGK-ZFN-R(ight)$). The donor construct was targeted to the $AAVS1$ locus using the AAVS1-SA-2A-puro-pA plasmid (Addgene) containing human $RPS19$, human $RPL5$ or $GFP$ cDNAs driven by the chicken β actin promoter. Approximately $1 \times 10^5$ iPSCs were plated onto puromycin-resistant MEF feeders with 10 μM Rock inhibitor (Cayman Chemical), then transfected using X-tremeGENE 9 (Roche). Puromycin (0.5 μg/mL) was added 2 days later. After 2 weeks, individual clones were picked, expanded and characterized, as described in Supplemental Methods. iPSCs clones were screened for heterozygous integration at the $AAVS1$ locus using PCR (Supplemental Figure 3 and Supplemental Table 2).

**iPSC differentiation into hematopoietic lineages via formation of embryoid bodies (EBs)**

The iPSC lines were differentiated into blood cells as described with some modifications in the cytokines added. A detailed schematic is shown in Supplemental Figure 1. Between 3-5 x 10^5 iPSCs were plated on matrigel (BD Biosciences) coated 6-well plates and cultured for 1-2 days in hESC medium (see previous section) supplemented with 10 ng/ml bFGF. Upon reaching ~80-95% confluence, cells were treated with 0.1% collagenase B for 30 min at 37°C, then 0.05% trypsin for 3 min to dissociate cells. Cells were collected and plated at 3-4 x 10^5 per well into low cluster dishes in SP34 media supplemented with 1X penicillin/streptomycin (PS, GIBCO), glutamine (2 mM), ascorbic acid (50 μg/ml, Sigma), iron saturated transferrin, (750 ng/ml,
Boehringer Mannheim), monothioglycerol (MTG, 15 mM, Sigma), BMP4 (25 ng/mL) and VEGF (50 ng/ml) for the first 2 days. The medium was replenished and supplemented until day 14 with a sequential cytokine cocktail, as shown in Supplemental Figure 1. For RPL5 experiments shown in Figure 7 of the main text, the cells in suspension were isolated on day 14 and cultured in medium supplemented with SCF (50 ng/ml), IGF-1 (25 ng/ml) and EPO (2 U/ml) for two additional weeks. For colony assays, 2 x 10³ progenitors (CD41⁺/CD34⁺/CD43⁺/CD235⁺) released from EBs were plated in methylcellulose medium (Stem Cell Technologies, Inc.) supplemented with EPO (5 U/ml), IL-3 (10 ng/ml), GM-CSF (5ng/ml) and SCF (5ng/ml). Alternatively, EBs were dissociated in 0.1% collagenase B for 1 hour at 37ºC, then 0.25% trypsin for 3 min and disrupted by gentle mechanical agitation with a 23 gauge needle and syringe. 10⁴ EB-derived cells were then plated in methylcellulose medium.

Flow cytometry and Western blotting
Antibodies used for flow cytometry are described in Supplemental Table 1. Cells were analyzed on a FACSCantoII flow cytometer (BD Biosciences) using FlowJo software (Tree Star Inc.). Western blots were performed using standard protocols. Antibodies for Western blotting were: anti-RPS19 monoclonal antibody (Abcam, 1:1000), rabbit anti-RPL5 polyclonal antibody (AssayBiotech, 1:1000), anti-GAPDH-HRP (Abcam, 1:2000) and anti-β actin-HRP (Sigma, 1:25000).

Quantitative Reverse-Transcriptase PCR (qRT-PCR)
Total RNA was isolated from cells using RNeasy (Qiagen, Inc). cDNA was produced using random hexamers with Superscript III Reverse Transcriptase (Invitrogen). As a control, dilutions of H9 ESC cDNA were used to generate a standard curve to calculate copy number of each gene relative to the housekeeping gene Cyclophilin. Quantitative PCR reactions were performed using SYBR Green qPCR Master Mix (Roche) and analyzed on a Light Cycler 480II qPCR System (Roche). Primer sequences are shown Supplemental Table 2.
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**Supplemental Table 1.** Antibodies and dilutions used for flow cytometry.
## Supplemental Table 2. Primers sequences used for RT-qPCR

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**Supplemental Table 3.** Probes used for Northern Blot analysis to analyze pre-rRNAs.
Supplemental Figure 1. Differentiation of iPSCs into hematopoietic lineages via embryoid body (EB) formation. The protocol is based on a recent report. A timeline is shown in the center of the Figure. Cytokines sequentially added are shown at the top. The flow cytometry panels at the bottom indicate the main surface phenotypes used to assess various stages of iPSC differentiation.
Supplemental Figure 2. RPS19 Q126X protein is not detected after forced expression in 293T cells or H9 ESC-derived erythroblasts. A. Schematics of lentiviral constructs encoding FLAG-tagged RPS19 WT or RPS19 Q126X. For the RPS19 Q126X construct, an in frame FLAG encoding sequence followed by a new stop codon replaced the mutant antisense codon. These cDNAs were cloned into the multiple cloning site of the HMD lentiviral vector, which contains a GFP cDNA separated from the transgene by an IRES. Viruses were produced in 293T cells according to standard protocols. B. 293T cells were infected with lentiviruses encoding FLAG-RPS19 WT, FLAG-RPS19 Q126X or empty vector. After 96 hours, cells were harvested and analyzed by Western blotting using antibodies against FLAG, RPS19 or β-actin. *, endogenous RPS19 (lower band in lanes 1 and 3); **, FLAG-RPS19 (upper band ** in lane 2). Note that enforced expression of RPS19-FLAG caused downregulation of endogenous RPS19 (lane 2),
indicating that cells autoregulate total RPS19 protein. C. H9 ESCs were induced to differentiate using the protocol described in Supplemental Figure 1 and Supplemental Methods. At day 8, hematopoietic progenitors released by EBs were infected with IRES-GFP containing lentiviruses encoding FLAG-RPS19 WT, FLAG-RPS19 Q126X or empty vector. Cells were cultured for 4 days with EPO, SCF and IGF-1 to promote proliferation of erythroid cells. After 4 days, 93% expressed erythroid markers CD71 and CD235 (not shown). Intracellular staining with anti-FLAG antibody was performed. In the GFP+ cells corresponding to the transduced fraction, FLAG-RPS19 WT protein was detected (left panel), but not FLAG-RPS19 Q126X. The absence of RPS19 mutant protein expression in panels B and C is in agreement with previous reports showing that RPS19 genes harboring premature termination codons are not expressed well, either because newly transcribed mRNA is subject to nonsense mediated decay⁵, or the nascent protein is rapidly degraded by the proteasome⁶.
**Supplemental Figure 3.** Characterization of $RPS19^{+/Q126X}$ iPSCs. 

A. Genomic DNA sequence showing heterozygosity for the c.376 C>T, p.Q126X nonsense mutation in iPSCs derived from the patient’s fibroblasts. 

B. Pluripotency markers assessed by flow cytometry of $RPS19^{+/Q126X}$ iPSCs. 

C. RT-qPCR analyzing expression of pluripotency markers in the $RPS19^{+/Q126X}$ iPSC clone, a control WT iPSC clone and the human ESC line H9. Gene expression levels are normalized to cyclophilin RNA. 

D. G-band karyotype. 

E. Teratoma formed by $RPS19^{+/Q126X}$ iPSCs showing endoderm, mesoderm and ectoderm germ layer formation.
Supplemental Figure 4. Genetic rescue of RPS19<sup>+/p.Q126X</sup> and RPL5<sup>+/p.R23X</sup> iPSCs by zinc finger mediated homologous recombination. Wild type RPS19, RPL5 or GFP cDNAs were introduced into the “safe harbor” AAVS1 locus. A. Schematics of the endogenous (upper) and targeted (lower) AAVS1 intron 1 region showing relevant PCR primer locations and SphI cleavage sites used for Southern blot analysis of gene targeting. The “integration” primers detect the modified locus with RPS19 or control GFP cDNAs inserted. Integration primer F’ represents a genomic
region just upstream of the left homology arm (HA-L) and integration primer R’ represents a region within the puromycin resistance cassette (SA-Puro). Endogenous primers F’ and R’ represent genomic regions corresponding to HA-L and HA-R respectively. The probe for Southern Blotting (panel C) is a 480-bp BamH1 fragment from the 5’ arm of homology (HA-L) of the vector plasmid AAVS1-SA-2A-puro-pA. The diagram is not drawn exactly to scale. The probe used for Southern blotting in Panel C is represented. B. PCR analysis of genomic DNA from heterozygous modified and control iPSCs identifying the recombined AAVS1 allele using the “integration” primer pair (1300 bp PCR product) and endogenous unmodified AAVS1 allele using the “endogenous” primer pair (400 bp PCR product). C. Southern Blot analysis of iPSCs with GFP, RPS19 or RPL5 targeted into the AAVS1 locus. Genomic DNA (10 μg) was digested with SphI, fractionated by agarose gel electrophoresis, transferred to a Hybond-N+ membrane and hybridized to the 32P-labeled internal probe, represented in panel A. WT and targeted alleles are represented by 6.4 and 4.2 kb bands respectively.
Supplemental Figure 5. Characterization of \( RPL5^{+/p.R23X} \) iPSCs. A. Genomic DNA sequence showing heterozygosity for the \( RPL5 \) c.67C>T, p.R23X nonsense mutation in patient-derived iPSCs. B. Pluripotency markers assessed by flow cytometry of \( RPL5^{+/p.R23X} \) iPSCs. C. RT-qPCR analyzing expression of pluripotency markers in the \( RPL5^{+/p.R23X} \) iPSC clone, a control WT iPSC clone and the human ES cell line H9. Gene expression levels are normalized to cyclophilin RNA. D. G-band karyotype. E. Teratoma formed by \( RPL5^{+/p.R23X} \) iPSCs showing endoderm, mesoderm and ectoderm germ layer formation.
References