Supplemental Materials

Supplemental Figure Legends

Figure S1. Generation of *EIF4EBP1*<sup>+/−</sup> hESCs by CRISPR/Cas9-mediated gene editing. (A) Western blot analysis of 4E-BP1 in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hESCs. β-actin was used as loading control. (B) Karyotype analysis of *EIF4EBP1*<sup>+/−</sup> hESCs. (C) The copy number variation (CNV) analysis in *EIF4EBP1*<sup>+/−</sup> and *EIF4EBP1*<sup>−/−</sup> hESCs. (D) Detection of the top 6 predicted off-target sites in *EIF4EBP1*<sup>−/−</sup> hESCs. (E) Immunofluorescence staining of pluripotency markers, including OCT4, SOX2 and NANOG in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hESCs. Scale bars, 10 μm. (F) Semi-quantitative PCR (semi-qPCR) analysis of the expression levels of pluripotency markers, including SOX2, OCT4 and NANOG in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hESCs. 18S rRNA was used as a loading control. (G) Cell cycle analysis of *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hESCs. Statistical data are presented as means ± SEM. n = 3 technical replicates. (H) CLonal expansion assay in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hESCs. Scale bars, 10 μm. Statistical data are presented as means ± SEM. n = 3 biological replicates. Two-tailed unpaired t-test.

Figure S2. Generation of *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs. (A) Flow cytometric analysis of classic markers for hMSCs, including CD73, CD90 and CD105, as well as irrelevant markers for hMSCs, including CD34, CD43 and CD45 in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs. (B) Ratio analysis of cells positive for surface antigen markers for hMSCs, including CD166, CD29, CD44, CD13 and HLA, or irrelevant markers for hMSCs, including CD14, CD19, CD164, and PDLPN, in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs. Statistical data are presented as means ± SD. n = 3 technical replicates. (C-D) Characterization of the differentiation potentials of *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs (P4) into osteoblasts (C) and adipocytes (D). Von Kossa staining was used to evaluate osteogenesis. Scale bars, 100 μm. The relative Von Kossa-positive area is quantified as fold changes of areas in *EIF4EBP1*<sup>−/−</sup> cells compared with that in *EIF4EBP1*<sup>+/+</sup> cells and presented as means ± SEM. n = 3 biological replicates. Two-tailed unpaired t-test. Oil Red O staining was used to evaluate adipogenesis. Scale bars, 100 μm. The relative absorbance of Oil Red O is quantified as fold changes of absorbance value in *EIF4EBP1*<sup>−/−</sup> cells compared with that in *EIF4EBP1*<sup>+/+</sup> cells and presented as means ± SEM. n = 3 biological replicates. Two-tailed unpaired t-test. (E) SA-β-gal staining of *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs (P4). Scale bars, 100 μm. Statistical data are presented as means ± SEM. n = 3 biological replicates. Two-tailed unpaired t-test. (F) Clonal expansion assay in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs (P4). Statistical data are presented as means ± SEM. n = 3 biological replicates. Two-tailed unpaired t-test. (G) Immunofluorescence staining of Ki67 in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs (P4). Arrows indicate Ki67-positive cells. Scale bars, 10 μm. Statistical data are presented as means ± SEM. n = 3 biological replicates. Two-tailed unpaired t-test. (H) Cell cycle analysis of *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs (P4). Statistical data are presented as means ± SEM. n = 3 biological replicates. Two-tailed unpaired t-test. (I) Heatmap showing the Euclidian distance between replicates among the indicated groups of RNA-seq data of *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs at EP (P4) and LP (P8). (J) Principal component
analysis showing high reproducibility of quantitative proteomic data between replicates in *EIF4EBP1*<sup>+/+</sup> and *EIF4EBP1*<sup>−/−</sup> hMSCs (P4). (K) A model describing the role of 4E-BP1 as a stabilizer of UQCRCC2 in *EIF4EBP1*<sup>+/+</sup> hMSCs. In *EIF4EBP1*<sup>+/+</sup> hMSCs, 4E-BP1 potentially interacts with and stabilizes UQCRCC2, thus promoting the stability of mitochondrial respiration complex III, maintaining mitochondrial homeostasis, and counteracting cellular senescence. 4E-BP1 deficiency in hMSCs results in decreased expression of UQCRCC2, destabilization of mitochondrial complex III, disrupted mitochondrial structure and function, excessive accumulation of ROS, and accelerated cellular senescence. Schematic diagram was created with BioRender.com.

**Supplemental Table Legends**

**Table S1.** Differentially expressed genes (DEGs) between *EIF4EBP1*<sup>+/−</sup> and *EIF4EBP1*<sup>+/+</sup> hMSCs.

**Table S2.** Differentially expressed proteins (DEPs) between *EIF4EBP1*<sup>+/−</sup> and *EIF4EBP1*<sup>+/+</sup> hMSCs.

**Table S3.** Sequence information of sgRNAs and primers used for gene editing, plasmid construction and qPCR analysis.
Proteomics

Figure S2

A

hMSC

B

hMSC

C

hMSC

D

hMSC

E

hMSC

F

hMSC

G

hMSC

H

hMSC

I

hMSC

J

hMSC

K

hMSC

L

hMSC

M

hMSC

N

hMSC

O

hMSC

P

hMSC

Q

hMSC

R

hMSC

S

hMSC

T

hMSC

U

hMSC

V

hMSC

W

hMSC

X

hMSC

Y

hMSC

Z

hMSC

Positive cells (%)

Surface antigen | EIF4EBP1+/+ | EIF4EBP1-/−

| CD166   | 99.93 ± 0.06 | 99.73 ± 0.06 |
| CD29    | 100.00 ± 0.00 | 98.93 ± 0.15 |
| CD44    | 95.50 ± 0.40  | 95.87 ± 1.55 |
| CD13    | 96.47 ± 0.31  | 93.53 ± 0.06 |
| HLA     | 81.67 ± 0.55  | 90.53 ± 0.15 |
| CD14    | 0.02 ± 0.02   | 0.01 ± 0.01  |
| CD19    | 0.07 ± 0.04   | 0.10 ± 0.02  |
| CD164   | 0.02 ± 0.01   | 0.04 ± 0.03  |
| PDPN    | 0.47 ± 0.12   | 0.39 ± 0.10  |

P = 0.1821

Stability of mitochondrial respiration complex III

Ki67

Relative absorbance of Oil Red O

Phase staining

Positive cells (%)

Mitochondrial ROS

RNA-seq

Phase staining

Proteomics

PC2 (24.9% explained var.)

PC1 (30.0% explained var.)