**Mice**
C57BL/6-Ly5.1 or -Ly5.2 congenic mice were used for LSK transduction and competitive repopulation assays. Animal care was in accordance with the guidelines of Keio University for animal and recombinant DNA experiments. Fbxw7 deficient HSCs were prepared as described previously.¹

**Flow cytometry**
Stained cells were analyzed and sorted by SORP FACS Aria (BD Biosciences).

**Immunocytochemistry**
Immunocytochemistry with isolated cells was performed as described previously.² Briefly, cells were attached to glass slides using Cytospin (ThermoScientific) and fixed with 4% PFA. Slides were blocked with a protein blocker (DAKO) to avoid nonspecific staining. Specimens were incubated with primary antibodies overnight in humidified chambers at 4°C, washed in PBS three times, and reacted with fluorophore-labeled secondary antibodies and nuclear stain (DAPI or TOTO-3; Molecular Probes).

**Confocal microscopy**
Fluorescence images were obtained using a confocal laser-scanning microscope (FV1000; Olympus) in the linear range to avoid fluorescence saturation. Scanning was performed in the sequential laser emission mode to avoid cross-talk between the fluorescence channels. Expression levels of c-Myc, p53, Notch1, and phosphorylated S6 protein were quantified with the integrated fluorescence intensity (IFI) of the c-Myc, p53, Notch1 or phosphorylated S6 signals normalized to the IFI of the DAPI or TOTO-3 signal in independent cells. The total number of cells and the number of Ki67 or BrdU or phosphorylated S6 positive cells (Ki67 or BrdU or phosphorylated S6-positive nuclei) were counted by laser-scanning confocal microscopy at 20× magnification, in randomly chosen fields.

**Quantitative RT-PCR**
Quantitative PCR was performed as described previously.² Total RNA was prepared with an RNeasy mini kit (Qiagen) and was reverse-transcribed with Superscript VILO (Invitrogen) to prepare cDNA. Diluted cDNA samples were analyzed with SYBR Premix Ex Taq II (TaKaRa) or TaqMan gene expression assays (Applied Biosystems) with ready-made primer sets for each gene (TaKaRa or Applied Biosystem) according to the manufacturer’s instructions. The transcripts of Fbxw7 isoforms (Fbxw7α, Fbxw7β, and Fbxw7γ) were quantified with primers described previously.³
Competitive repopulation assay
Fbxw7α or GFP control retrovirus-transduced LSK cells (3×10^3) from C57BL/6-Ly5.1 mice were cultured in SF-O3 with 100 ng/ml murine SCF (PeproTech EC Ltd) and 100 ng/ml human TPO (PeproTech EC Ltd) for 1 week to stress the HSCs. Cultivated LSK cells, together with 4×10^5 BM MNCs from C57BL/6-Ly5.2 mice, were transplanted into lethally irradiated C57BL/6-Ly5.2 congenic mice. At 4, 8, 12, and 16 weeks after transplantation, peripheral blood cells of the recipient mice were stained with PE-conjugated anti-Ly5.1 (A20) or APC-conjugated anti-Ly5.2 (104) antibodies and analyzed by a SORP FACS Aria. Secondary transplantation into lethally irradiated C57BL/6-Ly5.2 congenic mice was performed using 3,000 C57BL/6-Ly5.1^+ LSK cells from primary recipients, together with 4×10^5 BM MNCs from C57BL/6-Ly5.2 mice. Recipient mice were sacrificed for analysis 4 months after BMT.

Colony-forming cell assays
EGFP^+ transduced cells were plated (150 cells per 35 mm dish) in a semi-solid methylcellulose medium with cytokines (MethoCult M3434). Cells were incubated at 37°C in a 5% CO2 atmosphere. The EGFP^+ colony numbers were assessed at day 9. Colonies derived from high proliferative potential-CFCs (HPP-CFCs) were assessed at day 11. Four hundred GFP^+ LSK cells were transplanted into 8.5-Gy irradiated mice for the CFU-S_{12} assay. Spleens were harvested 12 days later and fixed with Bounin solution. CFU-S colonies were quantified and photographed. Each reported number of CFU-S colonies represents an average of five spleens.
Table S1. PB chimerism in secondary BMT

PB chimerism in secondary BMT of EGFP or Fbxw7α-transduced cells was analyzed 4 months after BMT in individual mice.

<table>
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<th>2Mo</th>
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<th>4Mo</th>
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<td>4/4</td>
<td>5.57 4.88 3.56 2.98</td>
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Figure S1. Confirmation of Fbxw7 expression after transduction
(A) Expression level of Fbxw7 transcripts in EGFP or Fbxw7α virus-transduced EGFP+ cells. Each value was normalized to β-actin expression and is expressed as the fold induction compared to EGFP virus-transduced control cells (mean ± SD, n = 4). (B, C) Freshly isolated CD34−Flt3−LSK cells or EGFP or Fbxw7α virus-transduced CD48− LSK cells were isolated and stained with DAPI and anti-Fbxw7 antibody. Scale bar represents 10 µm. Expression levels of Fbxw7 were quantified by IFI of the Fbxw7 signal normalized to the IFI of the DAPI signal in independent cells (*P<0.01, mean ± SEM).

Figure S2. Localization of Fbxw7 isoforms after overexpression in HSCs
EGFP+ cells were isolated from EGFP or Fbxw7α, β, or γ virus-transduced cells. Isolated cells were stained with DAPI, anti-Fbxw7 antibody or anti-nucleostemin antibody. Scale bar represents 20 µm.

Figure S3. Overexpression of Fbxw7α suppresses expression of its targets at the protein level
EGFP+ cells were isolated from EGFP or Fbxw7α virus-transduced cells. Isolated cells were stained with DAPI and either anti–c-Myc, Notch1, phosphorylated S6, p53 antibody, or isotype controls. Scale bar represents 50 µm.

Figure S4. Overexpression of Fbxw7α does not affect differentiation
Cultured EGFP or Fbxw7α virus-transduced donor-derived PB differentiation status (CD3+ or CD4/8+ T cells, B220+ B cells, or Mac-1/Gr-1+ myeloid cells) was assessed in primary BMT recipients at 4 months after BMT.

Figure S5. Hypoxia represses c-Myc through induction of Fbxw7 in the nucleus
(A) Quantitative PCR analysis of c-Myc in CD34− Flt3− LSK cells exposed to 15 h of normoxia (20% O2; open bars) or hypoxia (1% O2; closed bars) with hTPO and mSCF. Each value was normalized to β-actin expression and expressed as the fold induction compared to normoxic samples (mean ± SD, n = 4). (B) Immunocytochemical detection of Fbxw7 in CD34− Flt3− LSK cells exposed to 15 h of normoxia (20% O2; open bars) or hypoxia (1% O2; closed bars) with hTPO and mSCF. CD34− Flt3− LSK cells were stained with TOTO-3 and anti-Fbxw7 antibody. Expression levels of Fbxw7 protein were quantified by the IFI of the Fbxw7 signal normalized to the IFI of the TOTO-3 signal in independent cells (mean ± SD, scale bars represent 10 µm). (D–I) Immunocytochemical detection of Fbxw7 target proteins, c-Myc (D, E), Notch1 (F, G), and phosphorylated S6. (H, I) in wild-type or Fbxw7 deficient CD34− Flt3− LSK cells exposed to
15 h of normoxia (20% O₂; open bars) or hypoxia (1% O₂; closed bars) with hTPO and mSCF. Each group of CD34⁻ Flt3⁻ LSK cells were stained with DAPI and either anti–c-Myc, Notch1, or phosphorylated S6 antibody. Expression levels of each target protein were quantified by the IFI of each target signal normalized to the IFI of the DAPI signal in independent cells (mean ± SEM).

REFERENCES

Figure S1

A

\[ P = 3.76 \times 10^{-8} \]

B

C

\[ \text{Cellular relative IFI of Fbxw7} \]

**

n=122  n=108  n=114
Figure S2

- **Fbxw7α**
- **Fbxw7β**
- **Fbxw7γ**
Figure S3

EGFP  Fbxw7α  isotype

DAPI  DAPI  DAPI

c-Myc  c-Myc  c-Myc

Notch1  Notch1  Notch1

P-S6  P-S6  P-S6

DAPI  DAPI  DAPI

p53  p53  p53

EGFP  Fbxw7α  isotype

DAPI  DAPI  DAPI

c-Myc  c-Myc  c-Myc

Notch1  Notch1  Notch1

P-S6  P-S6  P-S6

DAPI  DAPI  DAPI

p53  p53  p53
Figure S4

Lineage differentiation in donor-derived cells (%) for Fbxw7α and EGFP.
Figure S5

A. Cellular relative IFI of c-Myc/β-actin

B. Cellular relative IFI of Fbxw7

C. 20% O₂ 1% O₂

D. Cellular relative IFI of c-Myc

E. Cellular relative IFI of c-Myc

F. Cellular relative IFI of Notch1

G. Cellular relative IFI of Notch1

H. Cellular relative IFI of P-S6

I. Cellular relative IFI of P-S6

WT

KO

n=104  n=81

n=97  n=102

n=85  n=76

n=88  n=134

P=0.003

P=1.14x10^-62

P=1.21x10^-7

P=0.04

P<0.01

P<0.01

P<0.01

P<0.01

N.S.

N.S.