Supporting Information

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SI Materials and Methods

Cell Culture. Germ-line stem (GS) cells used in the present study were derived from green mice or ROSA26 mice that were backcrossed to a DBA/2 background (1, 2). We derived *Fbxw7*$^{f/f}$ GS cells from 2- to 3-d-old *Fbxw7*$^{f/f}$ mice produced from offspring that resulted from crossing *Fbxw7*$^{f/+}$ mice in a C57BL/6 × DBA/2 background. GS cell culture conditions using StemPro-34 SFM (Invitrogen) were described previously (1). The growth factors used included 10 ng/mL human fibroblast growth factor 2 (FGF2), and 15 ng/mL rat glial cell line-derived neurotrophic factor (GDNF) (Peprotech). The cells were regularly maintained on mitomycin C (Sigma)-treated mouse embryonic fibroblasts (MEFs). *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine t-butyl ester (DAPT) (Wako Pure Chemical Industries) was added at 10 μM.

Immunostaining. Testes samples were fixed in 4% paraformaldehyde for 2 h and then frozen in Tissue-Tek OCT compound (Sakura Finetechnical). For immunostaining of cryosections, samples were treated with 0.1% Triton X-100 in PBS. After immersing them in blocking buffer (0.1% Tween 20, 3% BSA, and 10% goat serum in PBS) for >1 h, samples were incubated with primary antibodies at 4 °C overnight. Secondary antibodies were incubated for 1 h at room temperature. Samples were counterstained with Hoechst 33342 (Sigma). The images were collected using a confocal microscope (Fluoview FV1000D; Olympus). The antibodies used are listed in Table S1.

Flow Cytometry. GS cells were dissociated by incubating in cell dissociation buffer for 5 min (Invitrogen). Propidium iodide (1 μg/mL) was added to exclude dead cells. Stained cells were analyzed using a FACSCalibur (BD Biosciences). The antibodies used are listed in Table S1.

Lentivirus Infection. Full-length mouse *Fbxw7α*, human *Myc* (a gift from H. Saya, Keio University, Tokyo), human *Mycn* (Addgene), and human *Ccne1* (a gift from C. J. Sherr, St. Jude Children's Research Hospital, Memphis, TN) were cloned into the CSII-EF-IRES2-Venus (IV) vector. Human *Pin1* (Addgene) was cloned into the CSII-EF-IRES2-puro (IP) vector. Lentiviral particles were produced by transient transfection of 293T cells, and GS cells or testis cells were transfected as described previously (3). Virus titers were determined by transfecting 293T cells, and the multiplicities of infection (MOIs) were adjusted to 4.0.

Increases in the number of *Fbxw7α*-expressing GS cells were measured by plating 3×10^5 cells per 9.5 cm^2 on MEFs. The number of Venus-expressing cells was determined by FACSCalibur (BD Biosciences). For transplantation experiments, green mouse testis cells were transfected with the control or *Fbxw7α*-expressing lentiviral construct, and 6×10^4 cells were transplanted into seminiferous tubules 2 d after transfection.

For shRNA-mediated gene knockdown (KD), KD vectors were purchased from Open Biosystems. A mixture of lentiviral particles was used to transfect GS cells from ROSA mice or testis cells. pLKO1-Scramble shRNA (Addgene) was used as a control (Open Biosystems). The lentivirus titer was determined using a Lenti-X p24 rapid titer kit (Clontech). The MOIs in the KD experiment were adjusted to 4.0. All KD vectors are listed in Table S2.

Adenovirus Infection. For deletion of *Fbxw7*, dissociated testis cells were exposed to AxCANCre (RIKEN BRC) at a density of 1×10^6 cells per 9.5 cm^2, as described previously (4). After overnight incubation, the virus was removed on the next day, and cells were used for transplantation. AxCANLacZ (RIKEN BRC) was used as a control. The MOIs were adjusted to 2.0.

Apoptosis Assay. For TUNEL staining, a single-cell suspension was concentrated on glass slides by centrifugation with Cytospin 4 (Thermo Electron Corporation). After fixation in 4% paraformaldehyde for 1 h, cells were labeled using an In Situ Cell Death Detection kit (TMR red) (Roche Applied Science) according to the manufacturer's protocol. The nuclei were counterstained with Hoechst 33342 (2 μg/mL; Sigma) to determine the percentage of TUNEL-positive nuclei relative to the total number of Hoechst 33342-stained nuclei. Apoptotic cells were quantified by collecting images of stained cells using Photoshop software (Adobe Systems).

Analyses of Recipient Testes. For counting the colony number, recipient mice were killed between 6 and 8 wk after transplantation, and their testes were analyzed by observation under UV light or by staining for β-galactosidase, the *LacZ* gene product, with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) (Wako Pure Chemical Industries) (2). In experiments using green mice, testes were analyzed under UV fluorescence. A germ-cell cluster was defined as a colony when it occupied the entire basal surface of the tubule and was longer than 0.1 mm. For histological analysis, paraffin-embedded sections were stained with hematoxylin/eosin. The number of tubules with spermatogenesis, as defined by the presence of multiple layers of germ cells in the entire circumference of the tubules, was recorded for one section from each testis.

Southern Blotting. Genomic DNA was digested with StuI and transferred and hybridized with exon 4 probe, as described previously (4, 5). The PCR product was subsequently cloned into pGEMT easy vector (Promega). The plasmid was then digested with EcoRI to produce a 322-bp fragment, which was used as a hybridization probe. Band intensity was quantified using NIH image 1.62 software.

Western Blotting. Samples were separated by SDS/PAGE, transferred onto Hybond-P membranes (Amersham Biosciences), and incubated with primary antibodies. The antibodies used in the experiments are shown in Table S1. Band intensity was quantified using Multi Gauge version 3.0 software (Fuji Photo Film Co. Ltd.), and expression levels were normalized relative to those of ACTB.

Gene-Expression Analyses. Total RNA was isolated using TRIzol (Invitrogen), and first-strand cDNA was synthesized using a Verso cDNA Synthesis Kit (Thermo Fisher Scientific) for RT-PCR. For real-time PCR, the StepOnePlus Real-Time PCR system and *Power* SYBR Green PCR Master Mix were used following the manufacturer's protocol (Applied Biosystems). Transcript levels were normalized relative to those of *Hprt*. PCR conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Each PCR was run at least in triplicate. For RT-PCR, PCR conditions were 95 °C for 10 min, followed by 30 cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 1 min. The primers used for PCR are listed in Table S3.

Statistical analyses. Results are presented as means ± SEM. Significant differences between means for single comparisons were determined using the Student *t* test. Multiple comparison analyses were performed using ANOVA followed by Tukey's Honest Significant Difference (HSD) test.

1. Kanatsu-Shinohara M, et al. (2003) Long-term proliferation in culture and germline transmission of mouse male germline stem cells. *Biol Reprod* 69(2):612–616.
2. Kanatsu-Shinohara M, et al. (2011) Serum- and feeder-free culture of mouse germline stem cells. *Biol Reprod* 84(1):97–105.
3. Kanatsu-Shinohara M, et al. (2008) Long-term culture of male germline stem cells from hamster testes. *Biol Reprod* 78(4):611–617.
4. Takehashi M, et al. (2007) Adenovirus-mediated gene delivery into mouse spermatogonial stem cells. *Proc Natl Acad Sci USA* 104(8):2596–2601.
5. Onoyama I, et al. (2007) Conditional inactivation of Fbxw7 impairs cell-cycle exit during T cell differentiation and results in lymphomatogenesis. *J Exp Med* 204(12):2875–2888.

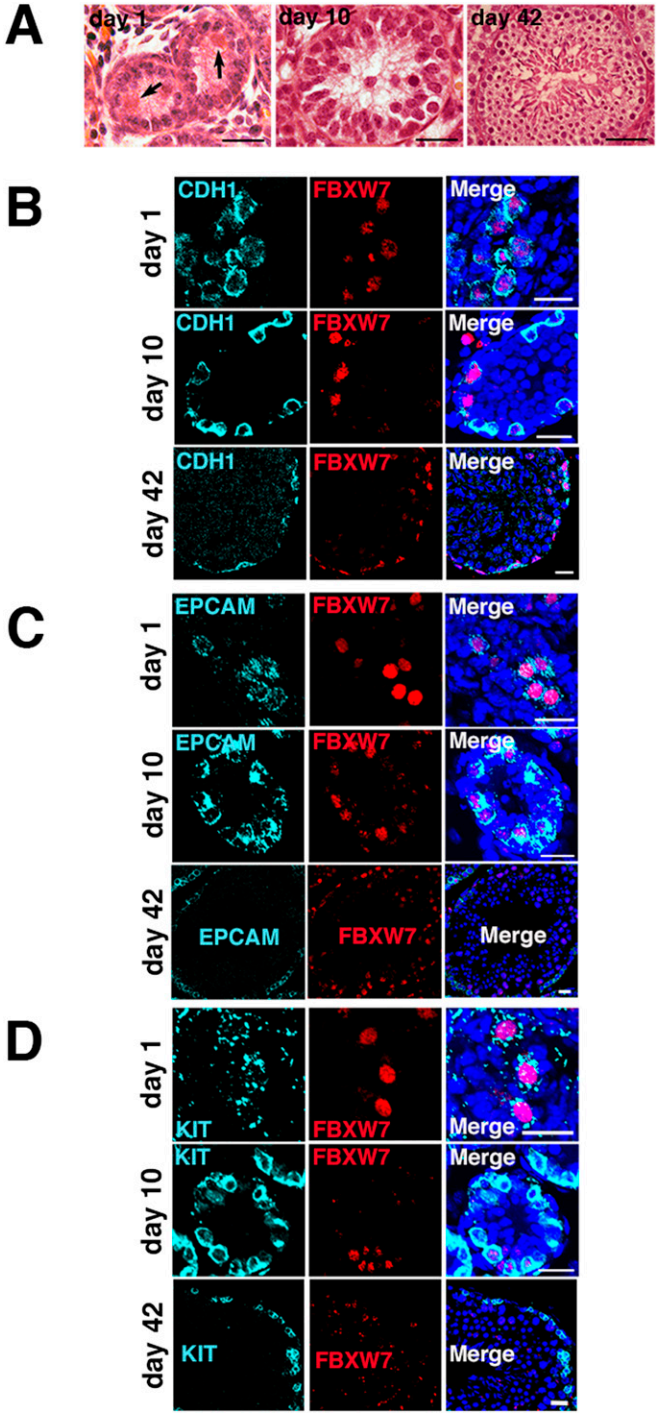

Fig. S1. Expression of F-box and WD-40 domain protein 7 (FBXW7) in testes. (*A*) Histological appearance of postnatal testes. Arrows indicate gonocytes that are not attached to the basement membrane. (*B–D*) Double immunohistochemistry of FBXW7 and cadherin 1 (CDH1) (*B*), epitherial cell adhesion molecule (EPCAM) (*C*), or kit oncogene (KIT) (*D*) during postnatal testis development. (Scale bars: *A*, 50 μm; *B–D*, 20 μm.) Stain: *A*, hematoxylin/eosin; *B–D*, Hoechst 33342.

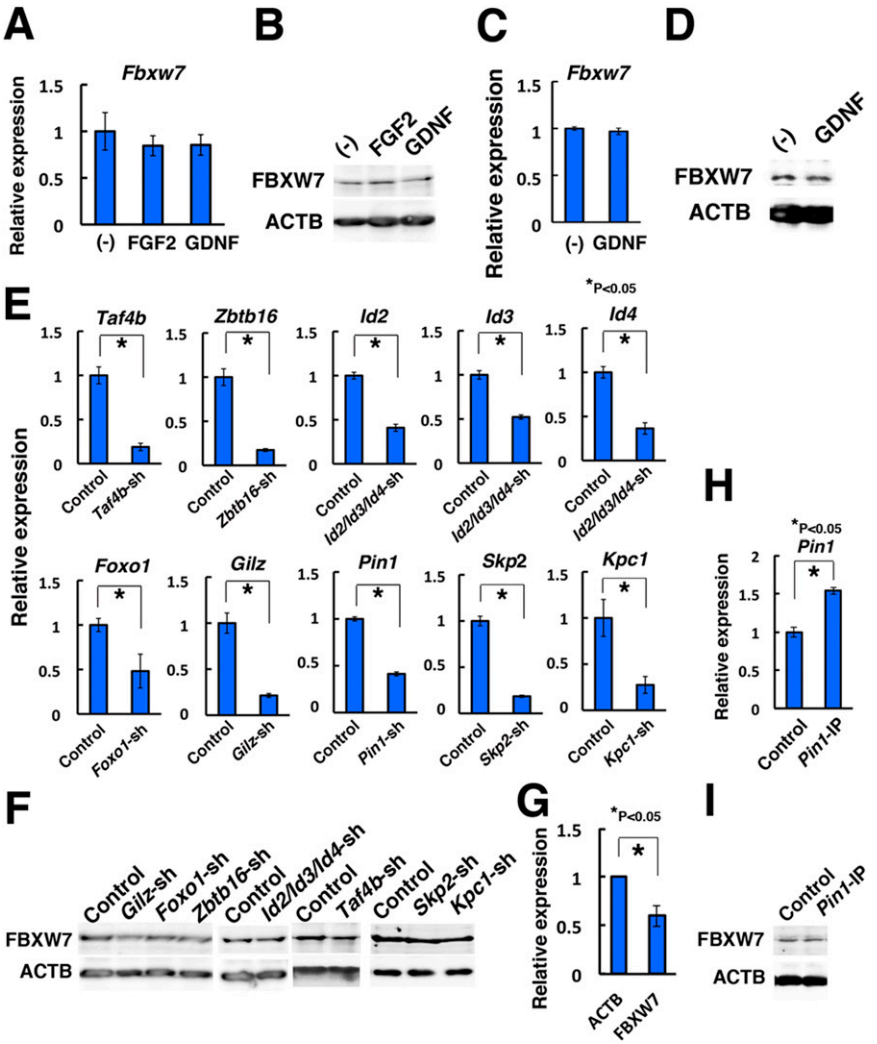

Fig. S2. Regulation of *Fbxw7* expression. (*A*) Real-time PCR analysis of *Fbxw7* expression by cytokine supplementation in GS cells. GS cells were cultured without cytokines for 3 d, and samples were collected 24 h after cytokine supplementation ($n = 9$). (*B*) Western blot analysis of FBXW7 expression by cytokine supplementation in GS cells. GS cells were cultured without cytokines for 3 d, and samples were collected 24 h after cytokine supplementation. (*C* and *D*) Expression of *Fbxw7* (*C*) and FBXW7 (*D*) in germ cells enriched from 10-d-old pup testes. Testis cells were incubated overnight on gelatin-coated plates. Germ cells were enriched by gentle pipetting and cultured on laminin-coated plates for 2 d without GDNF. Samples were collected 24 h after GDNF supplementation. Results of real-time PCR (*C*) ($n = 9$) and Western blot analysis (*D*) are shown. Increase in FBXW7 expression was 1.2 ± 0.2-fold ($n = 3$), and the difference was not significant. (*E*) Real-time PCR analysis of indicated gene expression following depletion by shRNA ($n = 6$–9). Cells were recovered 3 d after infection. (*F*). Western blot analysis of FBXW7 expression following depletion of indicated genes by shRNA. Cells were recovered 3 d after infection. (*G*) Quantification of FBXW7 expression in GS cells following *Pin1* depletion by shRNA ($n = 3$). Cells were recovered 3 d after infection. (*H*) Real-time PCR analysis of *Pin1* expression following *Pin1* overexpression in GS cells ($n = 3$). Cells were recovered 3 d after infection. (*I*) Western blot analysis of FBXW7 expression in GS cells following *Pin1* overexpression. Increase in FBXW7 expression was 1.2 ± 0.1-fold ($n = 3$), and the difference was not significant.

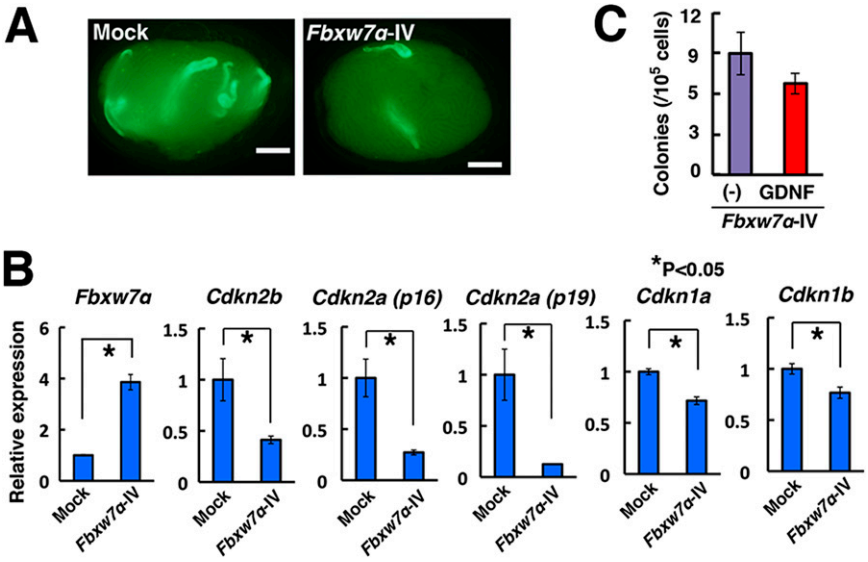

Fig. S3. Overexpression of *Fbxw7α* in GS cells. (*A*) Macroscopic appearance of a W recipient testis after transplantation of green mouse testis cells transduced with *Fbxw7α*. Cells were transplanted 2 d after infection. Colonized areas appear as green stretches of tubules under UV light. (*B*) Real-time PCR analyses of the indicated genes following *Fbxw7α* overexpression (n = 6–9). Cells were recovered 3 d after infection. (*C*) Colony counts after *Fbxw7α* overexpression and incubation with GDNF. Results of 3 experiments (n = 18). (Scale bar: *A*, 1 mm.)

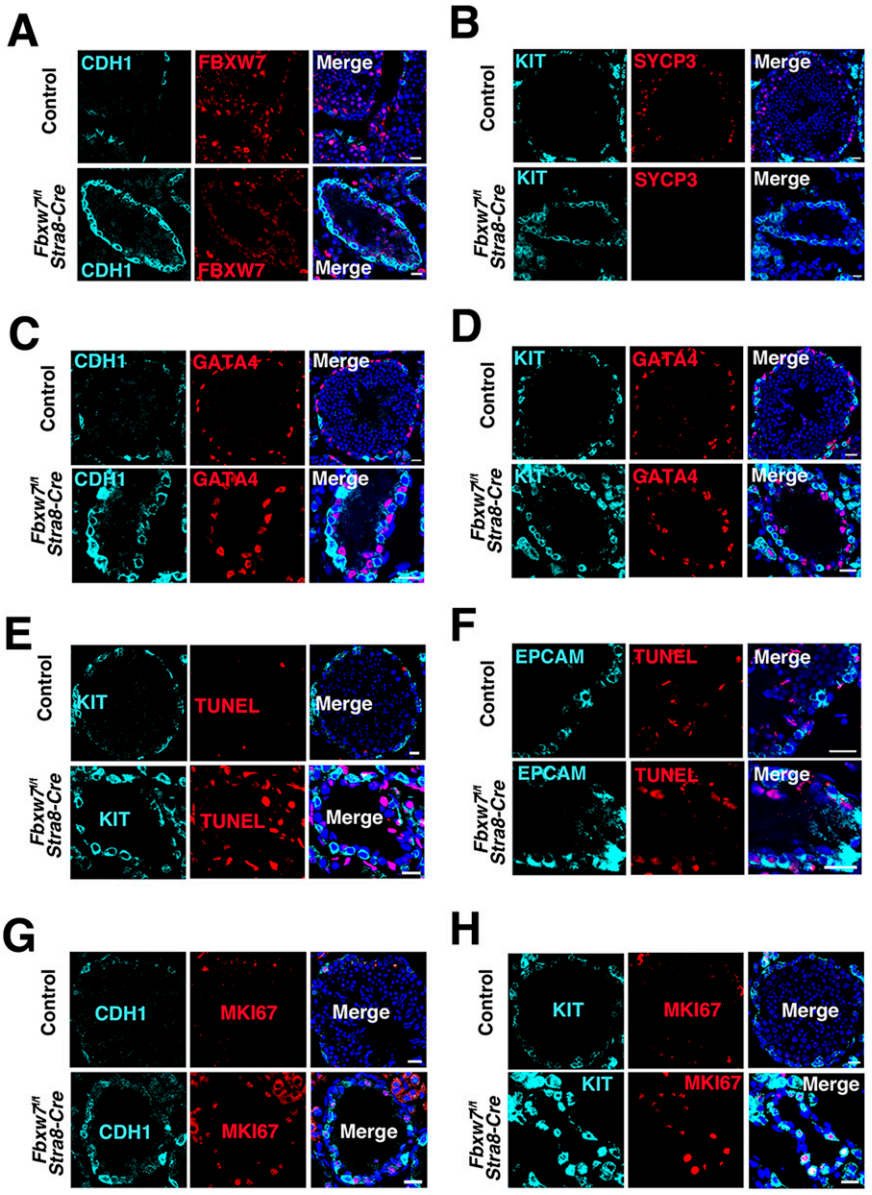

Fig. S4. Immunohistochemistry of *Fbxw7*$^{f/f}$ stimulated by retinoic acid gene 8 (*Stra8*)-*Cre* mouse testes. (*A*) Double immunohistochemistry of FBXW7 and CDH1. (*B*) Double immunohistochemistry of KIT and synaptonemal complex protein 3 (SYCP3). (*C*) Double immunohistochemisty of CDH1 and GATA binding protein 4 (GATA4). (*D*) Double immunohistochemistry KIT and GATA4. (*E*) Immunohistochemical staining of KIT and TUNEL. (*F*) Immunohistochemical staining of EPCAM and TUNEL. (*G*) Double immunohistochemistry of CDH1 and antigen identified by monoclonal antibody Ki67 (MKI67). (*H*) Double immunohistochemistry of KIT and MKI67. Counterstained with Hoechst 33342 (blue). (Scale bar: 20 μm.) Stain: *A*–*H*, Hoechst 33342.

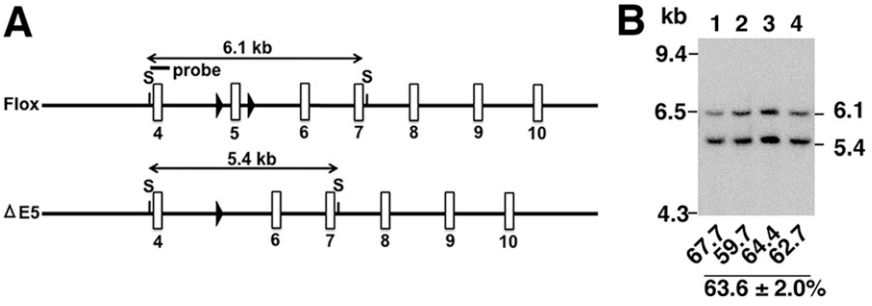

Fig. S5. Production of *Fbxw7* KO SSCs by AxCANCre transduction. (*A*) Conditional mutant mice used in the experiment. Exon 5 of the *Fbxw7* gene was deleted by Cre-mediated recombination. The indicated probe was used for Southern blot analysis. S, StuI. (*B*) Southern blot analysis to detect the deletion efficiency. Genomic DNA was digested with StuI and hybridized with the indicated probe.

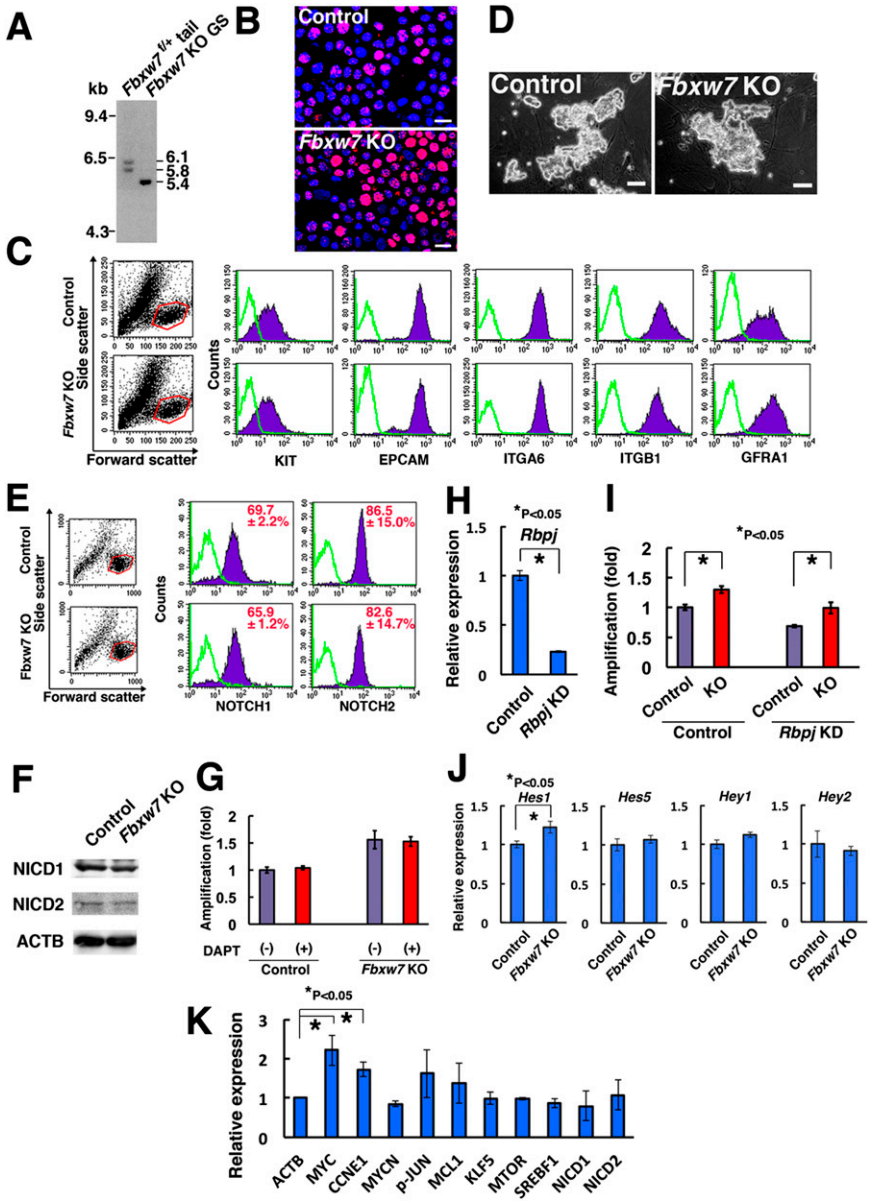

Fig. S6. Phenotype of *Fbxw7* KO GS cells. (*A*) Southern blot analysis of *Fbxw7* KO GS cells 3 wk after AxCANCre infection. (*B*) Immunocytochemistry of MKI67 in *Fbxw7* KO GS cells. Three days after infection. (*C*) Flow cytometric analysis of spermatogonia marker expression. Green lines indicate controls. (*D*) Appearance of *Fbxw7* knockout (KO) GS cells. (*E*) Flow-cytometric analysis of NOTCH1 and NOTCH2 expression ($n = 3–4$). (*F*) Western blot analysis of NICD1 and NICD2 expression. (*G*) Effect of DAPT on *Fbxw7* KO GS cell proliferation ($n = 6$). After overnight inoculation with AxCANCre, virus supernatant was removed, and cells were replated with DAPT after passage. Cell number was determined 3 d after replating. AxCANLacZ was used as a control. (*H*) Real-time PCR analysis of *Rbpj* expression following depletion by shRNA ($n = 9$). Cells were recovered 3 d after infection. (*I*) Effect of *Rbpj* depletion on *Fbxw7* KO GS cell proliferation ($n = 3$). *Fbxw7* KO GS cells were infected with shRNA against *Rbpj* and were replated after 24 h. The cells were then incubated with AxCANCre for 24 h. Virus supernatant was removed, and cells were replated in a new dish. Cell number was determined 3 d after replating. AxCANLacZ was used as a control. (*J*) Real-time PCR analysis of NOTCH target gene expression. ($n = 9$). (*K*) Quantification of Western blot band intensities for FBXW7 substrates ($n = 3–4$). (Scale bars: *B* and *D*, 20 μm.)

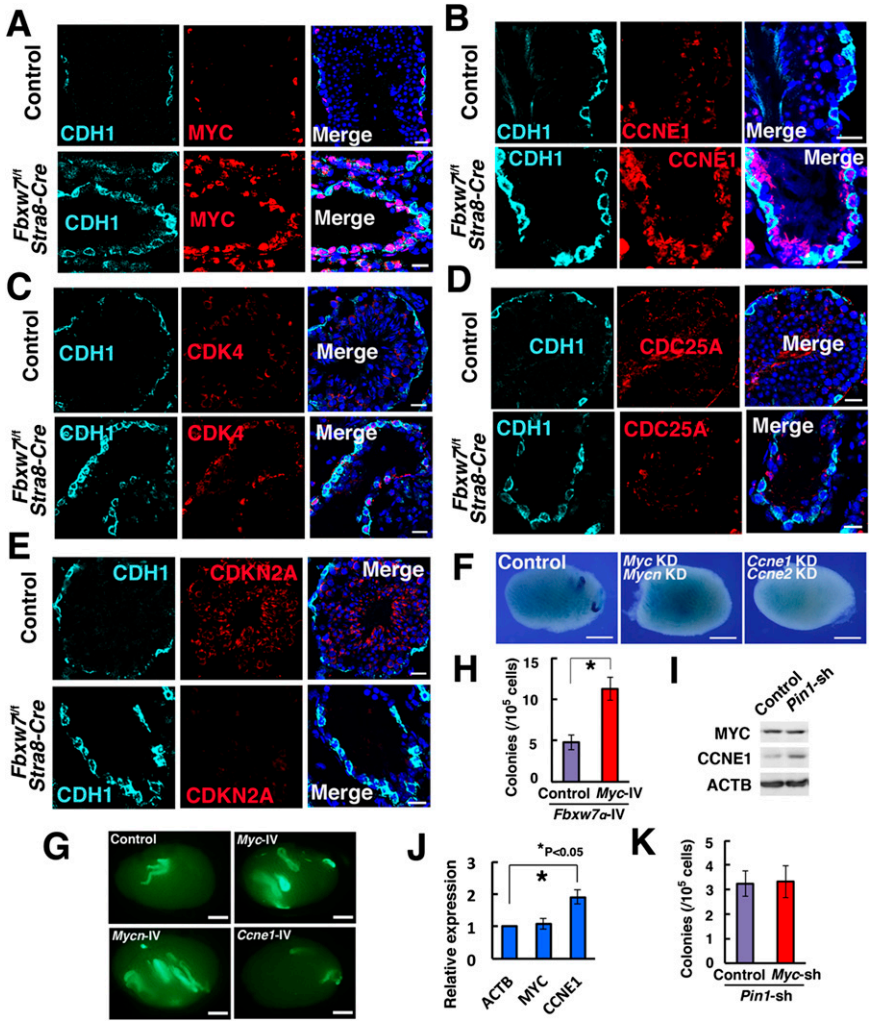

Fig. S7. Effect of Fbxw7 deficiency in myelocytomatosis oncogene (MYC) or cyclin E1 (CCNE1) expression. (*A*) Double immunohistochemistry of CDH1 and MYC in *Fbxw7*$^{f/f}$ *Stra8-Cre* testes. (*B*) Double immunohistochemistry of CDH1 and CCNE1 in *Fbxw7*$^{f/f}$ *Stra8-Cre* testes. (*C*) Double immunohistochemistry of CDH1 and CDK4 in *Fbxw7*$^{f/f}$ *Stra8-Cre* testes. (*D*) Double immunohistochemistry of CDH1 and CDC25A in *Fbxw7*$^{f/f}$ *Stra8-Cre* testes. (*E*) Double immunohistochemistry of CDH1 and cyclin-dependent kinase inhibitor (CDKN) 2A in *Fbxw7*$^{f/f}$ *Stra8-Cre* testes. (*F*) Macroscopic appearance of recipient testes transplanted with *Fbxw7* KO testis cells after transduction of shRNAs against *Myc*/*Mycn* or *Ccne1*/*Ccne2*. (*G*) Macroscopic appearance of recipient testes transplanted with green mouse testis cells transduced with a lentivirus expressing *Myc*, *Mycn*, or *Ccne1*. (*H*) Colony counts after overexpression of *Fbxw7*α and *Myc*. Results of three experiments ($n = 16$). (*I* and *J*) Effect of *Pin1* depletion by shRNA on MYC and CCNE1 expression. Western blot analysis (*I*) and quantification of band intensities (*J*) are shown ($n = 3$). Cells were recovered 3 d after infection. (*K*) Colony counts after depletion of *Pin1* and *Myc*. Results of three experiments ($n = 18$). (Scale bars: *A*–*E*, 20 μm; *F* and *G*, 1 mm.) Stain: *A*–*E*, Hoechst 33342.

Table S1. Antibodies

Antigen	Name	Company
Immunohistochemistry		
CDH1	Alexa fluor 647-conjugated rat anti-human CD324 (E-cadherin) Rat anti-human CD324 (E-cadherin)	eBioscience (clone DECMA-1)
EPCAM	Allophycocyanin (APC)-conjugated rat anti-mouse CD326 (EpCAM) Rat anti-mouse CD326 (EpCAM)	BioLegend (clone G8.8)
KIT	APC-conjugated rat anti-mouse CD117 (c-kit) Rat anti-mouse CD117 (c-kit)	eBioscience (clone ACK2)
FBXW7	Rabbit anti-human Fbxw7	Lifespan Biosciences (LS-B2909)
MKI67	Alexa fluor 488-conjugated mouse anti-human K_i-67	BD Biosciences (clone B56)
SYCP3	Rabbit anti-human Sycp3	Abcam (ab15093)
GATA4	Rabbit anti-human GATA4	Abcam (ab84593)
MYC	Rabbit anti-human c-Myc	Santa Cruz Biotechnology (sc-764)
CCNE1	Rabbit anti-rat cyclin E1	Santa Cruz Biotechnology (sc-481)
CDK4	Rabbit anti-mouse CDK4	Abcam (ab7955)
CDC25A	Rabbit anti-human CDC25A	Lifespan Biosciences (LS-B1463)
CDKN2A	Rabbit anti-mouse CDKN2A/p19 ARF	Abcam (ab80)
Secondary reagents		
Alexa fluor 488-conjugated goat anti-rabbit IgG		Invitrogen (cat. no. A11008)
Alexa fluor 568-conjugated goat anti-rabbit IgG		Invitrogen (cat. no. A11011)
Alexa fluor 647-conjugated goat anti-rabbit IgG		Invitrogen (cat. no. A21245)
Alexa fluor 647-conjugated goat anti-rat IgG		Invitrogen (cat. no. A21247)
Flow cytometry		
KIT	APC-conjugated rat-anti-mouse c-kit	eBioscience (clone ACK2)
EPCAM	Rat anti-mouse EpCAM	BioLegend (clone G8.8)
ITGA6	Rat anti-mouseCD49f (α6-integrin)	BD Biosciences (clone GoH3)
ITGB1	Biotin-conjugated hamster anti-rat CD29 (β1-integrin)	BD Biosciences (clone Ha2/5)
GFRA1	Biotin-conjugated goat anti-rat Gfrα1	R&D systems (BAF560)
NOTCH1	Biotin-conjugated goat anti-mouse Notch1	BioLegend (HMN1-12)
NOTCH2	APC-conjugated hamster anti-mouse Notch2	BioLegend (HMN2-35)
Secondary reagents		
Alexa fluor 647-conjugated hamster IgG isotype control		BioLegend (clone HTK888)
APC-conjugated Streptavidin		eBioscience (17-4317)
APC-conjugated goat anti-rat IgG+IgM		BD Bioscience (551019)
Western blotting		
FBXW7	Rabbit anti-human Fbxw7	Lifespan Biosciences (LS-B2909)
MYC	Rabbit anti-human c-Myc	Santa Cruz Biotechnology (sc-764)
MYCN	Rabbit anti-human *N*-Myc	Cell Signaling (9405)
CCNE1	Rabbit anti-rat cyclin E1	Santa Cruz Biotechnology (sc-481)
Phosphorylated JUN	Rabbit anti-human phospho-c-Jun (Ser63) II	Cell Signaling (9261)
MCL1	Rabbit anti-human Mcl-1	Abcam (ab32087)
KLF5	Rabbit anti-human Klf5	Abcam (ab137676)
MTOR	Rabbit anti-human mTOR	Cell Signaling (2972)
SREBF1	Rabbit anti-human Srebp1	Santa Cruz Biotechnology (sc-367)
ACTB	Mouse anti-β-actin antibody	Sigma (clone AC-15)
CCND1	Mouse anti-human cyclin D1	Cell Signaling (2926)
CCND2	Rabbit anti-cyclin D2	Cell Signaling (2924)
CCND3	Mouse anti-human cyclin D3	Cell Signaling (2936)
Phosphorylated MAPK14	Rabbit anti-human phospho-p38 MAPK (Thr180/Tyr182)	Cell Signaling (4511)
Phosphorylated MAP2K1	Rabbit anti-human phospho-MEK1/2 (Ser217/221)	Cell Signaling (9121)
Phosphorylated AKT	Rabbit anti-human phospho-Akt (Ser473)	Cell Signaling (9271)
CDKN2B	Rabbit anti-human CDKN2B (p15)	Cell Signaling (4822)
CDKN2A	Rat anti-mouse CDKN2D (p19)	Santa Cruz Biotechnology (sc-32748)
NICD1	Rabbit anti-activated human Notch1	Abcam (ab8925)
NICD2	Rabbit anti-human Notch2 intracellular domain	Abcam (ab52302)
Secondary reagents		
HRP (horseradish peroxidase)-conjugated horse anti-mouse IgG		Cell Signaling (cat. no. 7076)
HRP-conjugated horse anti-rabbit IgG		Cell Signaling (cat. no. 7074)
HRP-conjugated goat anti-rat IgG + IgM		Jackson Immunoresearch (112-035-044)

Table S2. KD vectors

Gene	Vector
Taf4b	TRCN0000241312
Zbtb16	TRCN0000012941
Foxo1	TRCN0000054878, TRCN0000054879, TRCN0000054880, TRCN0000054881, TRCN0000054882
Id2	TRCN0000054388, TRCN0000054389, TRCN0000054390
Id3	TRCN0000071438, TRCN0000071439, TRCN0000071440
Id4	TRCN0000071444
Gilz	TRCN0000085743, TRCN0000085744, TRCN0000085745, TRCN0000085746, TRCN0000085747
Myc	TRCN0000042513, TRCN0000042514, TRCN0000042515, TRCN0000042516
Mycn	TRCN0000042523, TRCN0000042525, TRCN0000042526, TRCN0000042527
Ccne1	TRCN0000077775, TRCN0000077776, TRCN0000077777
Ccne2	TRCN0000077779, TRCN0000077780, TRCN0000077781, TRCN0000077782
Pin1	TRCN0000012579, TRCN0000012580
Skp2	TRCN0000088758, TRCN0000088759, TRCN0000088760, TRCN0000088761, TRCN0000088762
Kpc1	TRCN0000201178, TRCN0000201651, TRCN0000192171, TRCN0000200959, TRCN0000191626
Rbpj	TRCN0000097286, TRCN0000097287, TRCN0000097288

Table S3. PCR primers and genotyping

Genotyping		
Fbxw7	Forward: TGGTATAGGCTTAACCCTATAGGG Reverse: AGCCATCTACTCTCACTCACAG WT, 650 bp; Flox, 900 bp	
R26R	IMR0315: GCGAAGAGTTTGTCCTCAACC IMR0316: GGAGCGGGAGAAATGGATATG IMR0883: AAAGTCGCTCTGAGTTGTTAT WT, ~600 bp; Flox, ~300 bp	
	Forward	Reverse
RT-PCR		
Stra8	AACGGTATCTCAACTTTTACAAGCA	ATTTCTCCTCTGGATTTTCTGAGTT
Hoxa4	TGAGCGCTCTCGAACCGCCTATACC	GATGGTGGTGTGGGCTGTGAGTTTG
Crem	GATTGAAGAAGAAAAATCAGA	CATGCTGTAATCAGTTCATAG
Piwil1	ATGATCGTGGGCATC	AGGCCACTGCTGTCATA
Clgn	ATATGCGTTTCCAGGGTGTTGGAC	GTATGCACCTCCACAATCAATACC
Sycp3	GGTGGAAGAAAGCATTCTGG	CAGCTCCAAATTTTTCCAGC
Prm	ACGAAGATGTCGCAGACGGAGGAG	CATCGGCGGTGGCATTTTTCAAGA
Hprt	GCTGGTGAAAAGGACCTCT	CACAGGACTAGAACACCTGC
qPCR		
Hprt	GCTGGTGAAAAGGACCTCT	CACAGGACTAGAACACCTGC
Fbxw7	TGCAAAGTCTCAGATTATACC	ACTTCTCTGGTCCGCTCCAGC
Fbxw7α	CTCACCAGCTCTCCTCTCCATT	GCTGAACATGGTACAAGGCCA
Fbxw7β	AGAAAATATGGGTTTCTACGG	TTGCTGAACATGGTACAAGG
Fbxw7γ	AACCATGGCTTGGTTCCTGTTG	CAGAACCATGGTCCAACTTTC
Taf4b	AGATGTTACTAAAGGCAGCC	GCAAGCTCCAACTGCTGCAA
Zbtb16	CACACTCAAGAGCCACAAGC	ATCATGGCCGAGTAGTCTCG
Foxo1	GTGAAGAGCGTGCCCTACTT	TCCTTCATTCTGCACTCGAA
Id2	ACTATCGTCAGCCTGCATCA	AGCCACAGAGTACTTTGCTA
Id3	TCGGAACGTAGCCTGGCCAT	TGGCTAAGCTGAGTGCCTCG
Id4	GTTCACGAGCATTCACCGTA	AAGGTTGGATTCACGATTGC
Gilz	CCCTAGACAACAAGATTGAGC	CTTCTCAAGCAGCTCACGAA
Pin1	AGATCACCAGGAGCAAGGAG	TGAACTGTGAGGCCAGAGAT
Skp2	GCAAAGGGAGTGACAAAGAC	TCCCAAGGAGCAGCTCATCT
Kpc1	CTCAGATGCTGAGAAGTCCA	AGTTTAGCGGTTTCCTGCTG
Cdkn2b	CAGATCCCAACGCCCTGAAC	GCAGTTGGGTTCTGCTCCGT
Cdkn2a(p16)	ACATCAAGACATCGTGCGA	TAGCTCTGCTCTTGGGATTG
Cdkn2a(p19)	GGTTCTGGTCACTGTGAGG	TGAGCAGAAGAGCTGCTACG
Cdkn1a	GCAGATCCACAGCGATATCC	CAACTGCTCACTGTCCACGG
Cdkn1b	AGGAGAGCCAGGATGTCAGC	GAATCTTCTGCAGCAGGTCG
Rbpj	AGCTGAACTTGGAAGGGAAG	CGCTGTTGCCATAGAACATC
Hes1	TATTGCCAACTGGGAGCCTG	TCTAGCCCATTCATTCCTCT
Hes5	TCCAGAGCTCCAGGCATGGC	TCTATGCTGCTGTTGATGCG
Hey1	ACGAGACCATCGAGGTGGAA	TTCCTGGCCAAAACCTGGGA
Hey2	CCTTGTGAGGAAACGACCTC	CATCACTGAGCTTGTAGCGT