

Table S1. PCR primers for RT-qPCR analysis.

Gene	Strand	Sequence (5'→3')
Mouse <i>β-TrCP2</i>	F	GAGTTCTGCACAGTCGGACA
Mouse <i>β-TrCP2</i>	R	GGTACCCAAGTCCCCTGCTA
Mouse <i>Dmrt1</i>	F	CTCATACTACGGGCCTCCCT
Mouse <i>Dmrt1</i>	R	TTCGAGCTCTCGTTGCTCAT
Mouse <i>Stra8</i>	F	TTCCTGCGTGTTCACAAGT
Mouse <i>Stra8</i>	R	ACTGGGTTGGTTGCCTTCTC
Mouse <i>Arbp</i>	F	GGACCCGAGAAGACCTCCTT
Mouse <i>Arbp</i>	R	GCACATCACTCAGAATTTCAATGG

Table S2. Commercially available antibodies used in the study.

Antigen	Provider	Catalog number
SCP3 (1/200 dilution)	Abcam	ab97672
PLZF (1/200)	Santa Cruz	sc-22839
DMC1 (1/200)	Santa Cruz	sc-22768
SNAIL1 (1/1000)	Cell Signaling	3895
EM11 (1/200)	Santa Cruz	sc-50927
STRA8 (1/500)	Abcam	ab49602
DMRT1 (1/2000)	Santa Cruz	sc-377167
Ubiquitin (1/200)	DAKO	Z0458
Hsp90 (1/2000)	BD	610418
BrdU (1/1000)	BD	555627
Myc tag (1/1000)	Santa Cruz	sc-40
FLAG tag (1/2000)	Sigma	F1804
HA tag (1/500)	Roche	11667475001
HRP-conjugated anti-rabbit IgG (1/14000)	Promega	W4011
HRP-conjugated anti-mouse IgG (1/14000)	Promega	W4021
Alexa 546-conjugated anti-rabbit IgG (1/1000)	Life Technologies	A-11035
Alexa 488-conjugated anti-mouse IgG (1/1000)	Life Technologies	A-11001

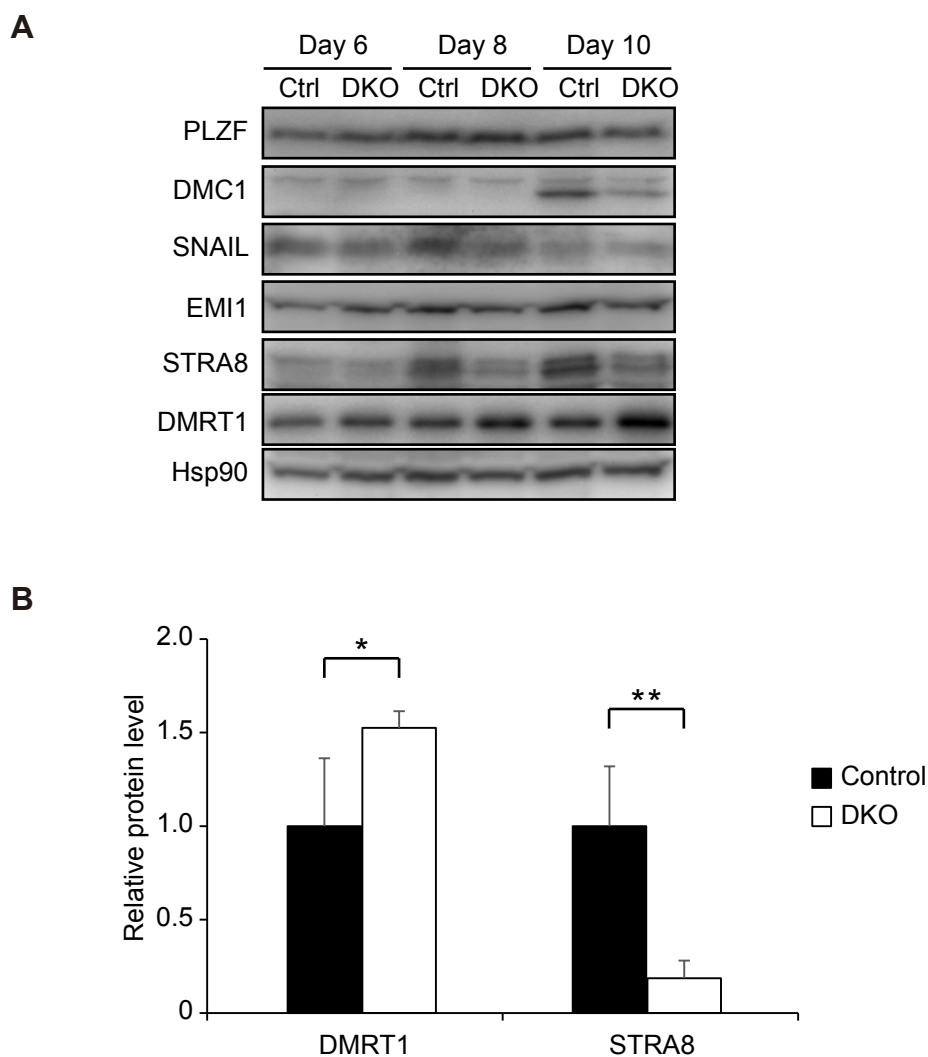
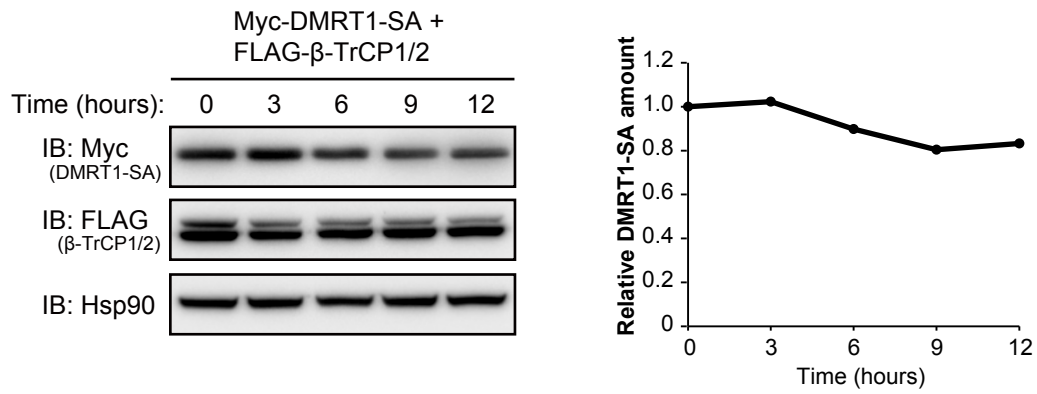


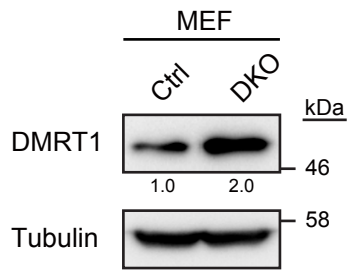
Figure S1. Accumulation of DMRT1 and reduction of STRA8 in β -*TrCP1/2* DKO testes (related to Figure 5).

(A) Immunoblot analysis of the indicated proteins in the testis of control (*Stra8-Cre*, Ctrl) and β -*TrCP1/2* DKO mice at the indicated ages. Hsp90 was examined as a loading control. (B) Quantification of band intensities of STRA8 and DMRT1 normalized by that of Hsp90 in Figure 5A measured with Image J software. * $P < 0.05$ or ** $P < 0.01$ versus age-matched control (unpaired Student's *t* test).

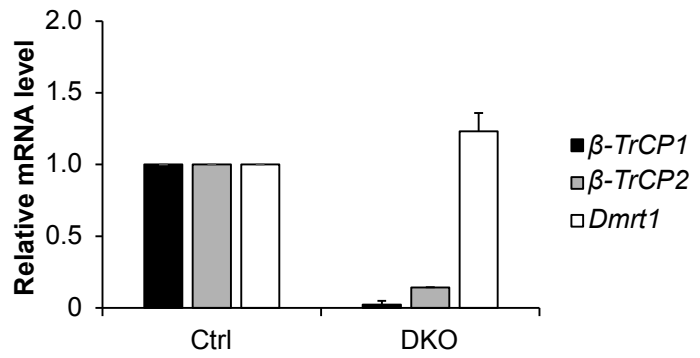
A



B



C



D

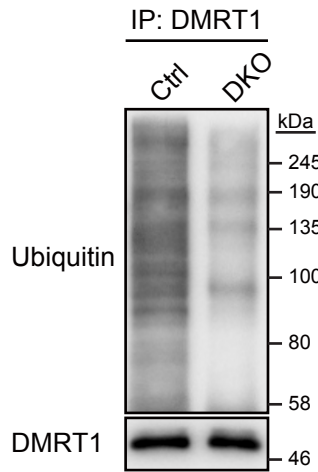


Figure S2. β -TrCP1/2 ubiquitylate DMRT1 for degradation (related to Figure 6). (A)

Cycloheximide chase analysis of the SA mutant of DMRT1 stability. 293T cells transfected with vectors for FLAG-tagged β -TrCP1 and β -TrCP2 as well as for Myc epitope-tagged SA mutant of DMRT1 were incubated with cycloheximide for the indicated times, lysed, and subjected to immunoblot analysis (IB) with the indicated antibodies. The band intensity for Myc-DMRT1 normalized by that of Hsp90 was quantified with Image J software. **(B)** Immunoblot analysis of the indicated proteins in the MEFs prepared from wild-type (Ctrl) or β -TrCP1^{-/-}; β -TrCP2^{F/F} (DKO) mice. MEFs were infected with retrovirus encoding DMRT1, selected by blasticidin, and then further infected with retrovirus encoding Cre. **(C)** RT-qPCR analysis of β -TrCP1, β -TrCP2 and *Dmrt1* mRNAs in the MEFs prepared as in **(B)**. Data are means \pm s.e.m. from two independent experiments. **(D)** In vivo ubiquitylation analysis of DMRT1. MEFs prepared as in **(B)** were treated with the proteasome inhibitor MG132 for 5 h, lysed, and subjected to immunoprecipitation (IP) with antibodies to DMRT1 under denaturing conditions followed by immunoblot analysis with antibodies to DMRT1 and to ubiquitin.

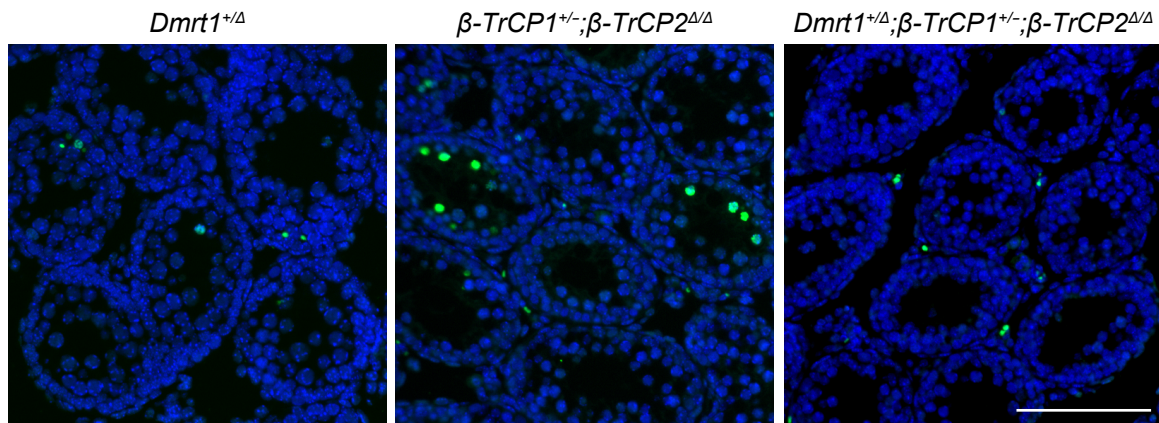


Figure S3. Amelioration of apoptosis in β -TrCP-deficient mice by heterozygous deletion of *Dmrt1* (related to Figure 8).

TUNEL staining of seminiferous tubules in mice of indicated genotypes at 14 dpp. Scale bars, 100 μ m. The percentage of TUNEL-positive tubules was presented in Figure 8D.

Class	Species	Amino acid sequence	NCBI reference
Mammals	<i>H. sapiens</i>	326 PPSSQDSGLVSLSSSS	NP_068770.2
	<i>M. musculus</i>	324 PPSSQDSGLVSLSSSS	NP_056641.2
	<i>R. norvegicus</i>	324 PPSSQDSGLVSLSSSS	NP_446158.1
Reptile	<i>A. carolinensis</i>	308 PPSSQDSGLVSLSSSS	XP_003216601.1
Birds	<i>G. gallus</i>	304 PPSSQDSGLGCLSSSE	F1P5L6 (UniProt)
	<i>C. livia</i>	238 PPSSQDSGLGCLSSSE	XP_005507217.1
	<i>Z. albicollis</i>	226 PPNSQDSGLGCLSSSS	XP_005488381.1
	<i>P. pubescens</i>	290 PASSQDSGLRCLSGSE	XP_009900057.1
	<i>E. garzetta</i>	229 PPNSQDSGLGCLSSSE	XP_009639798.1
	<i>C. canorus</i>	232 PPSSQDSGLGCLSSSE	XP_009554449.1
Amphibian	<i>X. tropicalis</i>	289 PPSSQDSGII SLSSNS	XP_002935648.2
Fish	<i>C. milli</i>	276 PSSSQDSGLI SLSSTS	XP_007890871.1
	<i>D. rerio</i>	226 SDGAQDSVSI SMIDA	NP_991191.1
	<i>β-TrCP degnon</i>	DSGxxS	

Figure S4. β -TrCP degnon sequences in vertebrate DMRT1 orthologs (related to discussion).

The amino acid sequences of DMRT1 orthologs were retrieved from the NCBI database (with the exception of that for *Gallus gallus* because of an apparent frameshift). The β -TrCP degnon is shaded, with the critical residues for association with β -TrCP indicated in red.