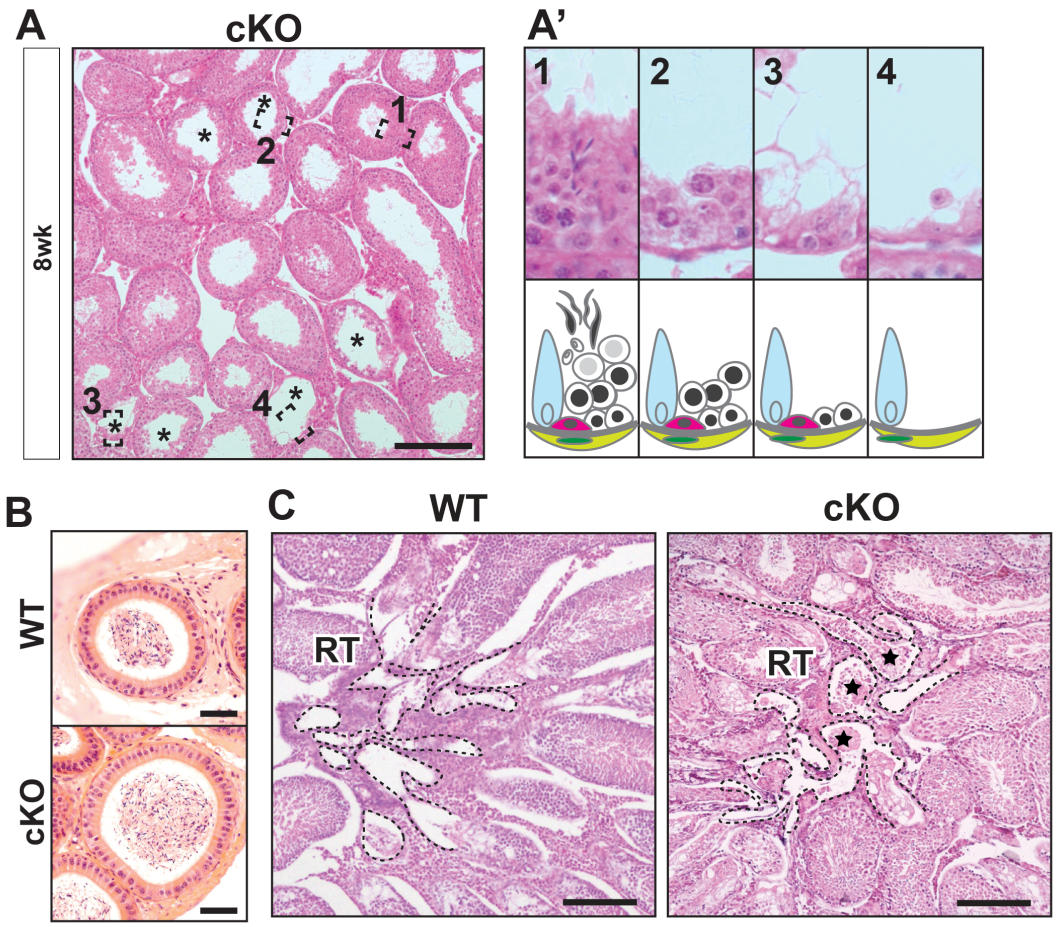


Fig. S1 Degeneration and dilation of seminiferous tubulesin cKO is independent from ER expression

(A) Histology of the testis from 8wk-old cKO mouse. Right panels A'1–4 show the regions surrounded by broken rectangles 1–4 in the left panel at higher magnifications together with schematic illustrations below. * indicates degenerative tubules in A. **(B)** Histology of the epididymis from adult WT and cKO mice. **(C)** Histology of the rete testis (RT, region outlined by broken line) in 8wk-old WT and cKO mouse. Stars represent the cell debris observed in the luminal region of RT in cKO testis. Bars, 50μm in B, 200μm in A and C.

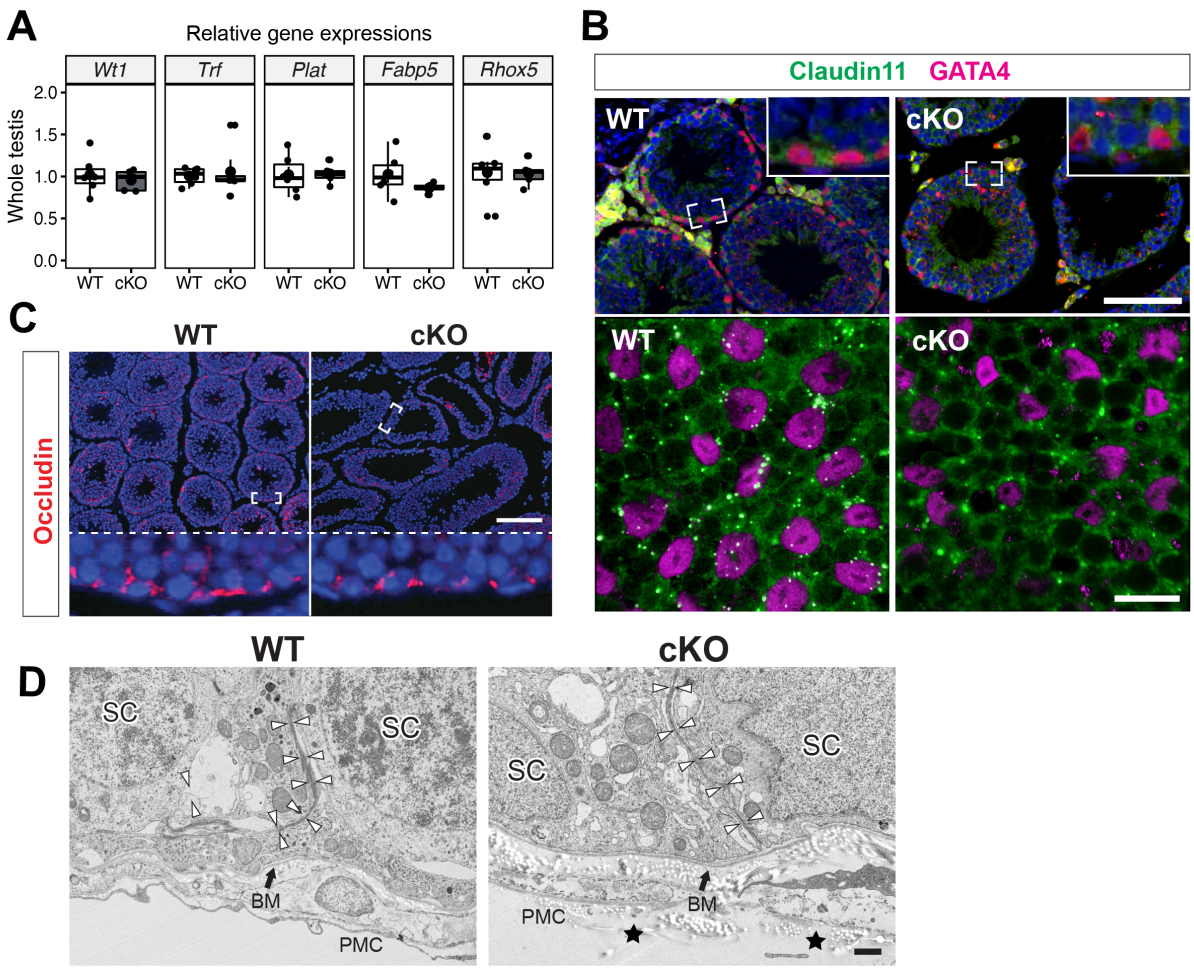


Fig. S2 Sertoli cell functionwas not affected in cKO mice

(A) Expression levels of genes related to Sertoli cell function in whole testis of 8wk-old WT and cKO mice (n=6 for WT, n=5 for cKO). **(B–D)** Blood-testis barrier (BTB) appeared to be unaffected in cKO mice. (B) Anti-Claudin 11 (green) and GATA4 (red) immunohistochemistry in testis section (upper panels) and whole-mount seminiferous tubules (lower panels) from WT and cKO mice at 8wk-old. Claudin 11 signals were observed around the GATA4-postive Sertoli cell nuclei. (C) Anti-Occludin (red) immunohistochemistry in testis section from WT and cKO mice at 8wk-old. Lower panels in C show the magnification of regions surrounded by broken rectangles. (D) Transmission electron micrographs of 8wk-old WT and cKO mouse testes, visualizing similar ultrastructure of tight junctions between Sertoli cells in WT and cKO testis. White arrowheads point to intact junctions between Sertoli cells. BM: basement membrane, SC: Sertoli cell. Stars indicate accumulated lipid-like structures around the basement membrane. Bars, 100μm in upper panels in B and C, 20μm in lower panels in B and 1μm in D.

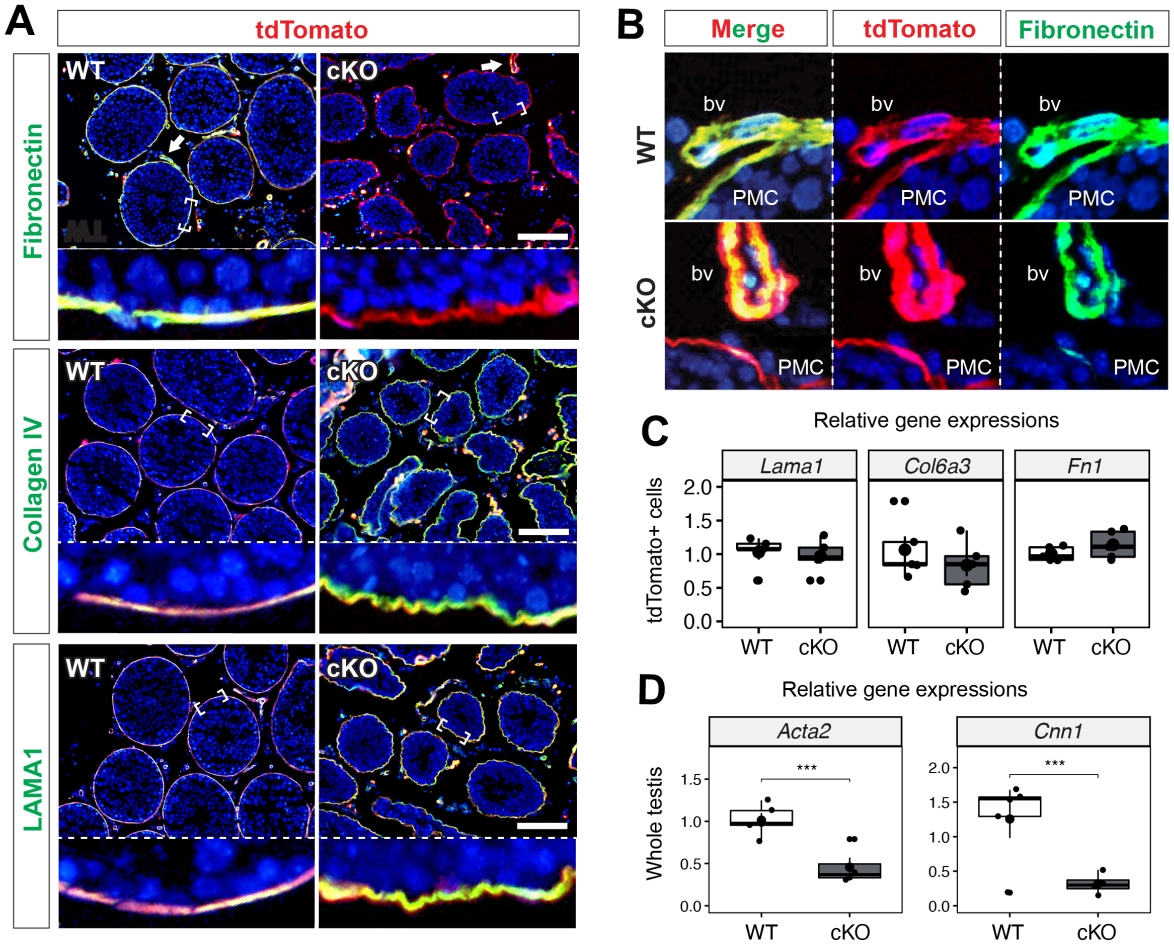


Fig. S3 Fibronectin is present in vascular smooth musclecells, but not in PMCs of cKO mice

(A, B) Cryosections of 8wk-old WT and cKO mouse testes, showing the immunoreactivity of ECM proteins (green) and tdTomato signals (red). Lower panels in A show magnification of the region surrounded by broken rectangles in upper panels. Bars, 100μm. (B) Higher magnification of the region indicated with white arrow in the top panels in A. In cKO testes, fibronectin signals were observed intensely in vascular smooth muscle cells but not in PMCs. bv: blood vessel. **(C)** Expression levels of genes encoding ECM proteins in tdTomato^{+} cells sorted from 8wk-old WT and cKO mice. (n=5 for each genotype). **(D)** Expression levels of genes encoding smooth muscle contractile proteins in whole testis from 8wk-old WT and cKO mice. (n=6 for WT, n=5 for cKO). ***p<0.005. Analysis was performed using student's t-test. Bars, 100μm.

Supplementary Tables

Table S1. List of antibodies used in this study

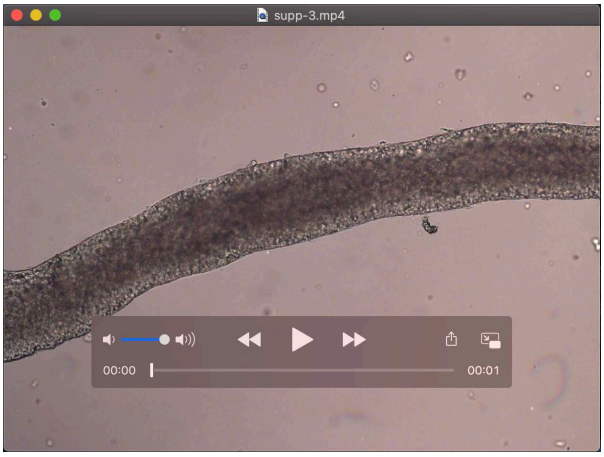
Antigen	Dilution	Description	Company	CAT#
αSMA	1/500 IHC, 1/1,000 WB	Mouse monoclonal	Sigma	A5228
c-KIT	1/200 IHC	Goat polyclonal	R&D systems	AF1356
Claudin 11	1/100 IHC	Rabbit polyclonal	Thermo Fisher	36-4500
CNN1	1/5000 WB	Rabbit monoclonal	Abcam	ab46794
CNND1	1/400 IHC	Rabbit monoclonal	Abcam	ab16663
Collagen IV	1/100 IHC	Rabbit polyclonal	Abcam	ab6586
Fibronectin	1/100 IHC	Rabbit polyclonal	Abcam	ab2413
GATA4	1/100 IHC	Goat polyclonal	Santa cruz	sc1237
GFRα1	1/100 IHC	Goat polyclonal	R&D systems	AF560
HSP70	1/1000 IHC	Rabbit polyclonal	Abcam	ab79852
HPRT1	1/20,000 WB	Rabbit polyclonal	Thermo Fisher	A305-306A
Ki67	1/100 IHC	Mouse monoclonal	Leica	NCL-L-Ki67-MM1
Laminin	1/200 IHC	Rabbit polyclonal	Abcam	ab11575
LIN28A	1/200 IHC	Rabbit monoclonal	Cell Signaling	8706
PECAM-1	1/100 IHC	Rat monoclonal	BD Pharmingen	550274
PLZF	1/100 IHC	Rabbit polyclonal	Santa cruz	sc22889
RFP	1/200 IHC	Rabbit polyclonal	MBL	PM005
SCP3	1/500 IHC	Mouse monoclonal	Santa cruz	sc74569
SOX9	1/100 IHC	Rabbit polyclonal	Milipore	AB5535
VASA	1/1000 IHC	Rabbit polyclonal	Abcam	ab13840

Table S2. List of primers used for qPCR

Target gene	Direction	Sequence (5' → 3')
Acta2	F	TGCTGTCCCTCTATGCCTCT
	R	GAAGGAATAGCCACGCTCAG
Col6a3	F	GGAACCACGGAAGAGAGCAA
	R	CAGGGAACTGACCCAAGACA
Cnn1	F	CAAGCTGGCCCAGAAATACGACC
	R	TCTTCACAGAACCCGGCTGCAG
Esr1	F	TTGAACCAGCAGGGTGGC
	R	AGGCTTTGGTGTGAAGGGTC
Esr2	F	GGCAAGCTCATCTTTGCTCC
	R	CTCATCCCTTGGGACAGCAC
Fabp5	F	CGGTCAAAACCGAGAGCACA
	R	GTGCAGACCGTCTCAGTTTTTC
Fn1	F	GCCACCATTACTGGTCTGGA
	R	ACCAGTTGGGGAAGCTCATC
Hprt	F	AGCAGTACAGCCCCAAAATGGT
	R	CCAACAAAGTCTGGCCTGTATCC
Lama1	F	TATCCTGCCCACATCAAACAG
	R	CTTGTAGGTCAAAGGCTCGG
Plat	F	AGATGAGCCAACGCAGACAA
	R	CTTGGTTCGCTGCAACTTCG
Rhox5	F	GCAGCGCACTAATTCCTTTG
	R	GCAGCCCTCCTGATCTTAAA
Trf	F	CGTAGGCGCATTCAAGTGTC
	R	ATTGGTCCCTGTCAGCCTTC
Wt1	F	CCAGTGTAAAACTTGTCAGCGAAA
	R	ATGAGTCCTGGTGTGGGTCTTC

Supplementary Movies

Movie 1. Active contraction of adult WT seminiferous tubule



Movie 2. Contraction of adult cKO seminiferous tubule

Supplementary Materials and Methods

Western blot

Testes collected from 8wk-old WT and cKO mice were rinsed with PBS and homogenized in cold RIPA buffer containing protease inhibitor cocktail (Sigma). Protein concentration was determined by a DC protein assay (Bio-Rad). Equal amounts of total protein from each sample were then mixed with SDS loading buffer and boiled at 95ºC for 10 minutes. The target proteins were separated by SDS-PAGE, then transferred onto PVDF membranes. The membranes were blocked with 5% BSA/TBST with 0.1% Tween 20, and then incubated with primary antibodies listed in Table 1 at 4°C overnight. The membranes were washed with 0.1% Tween 20/TBST and incubated with secondary antibodies for at least 1 hour at room temperature. All secondary antibodies were diluted into 5% non-fat milk in 1X TBST with 0.1% Tween 20. The membranes were washed with 0.1% Tween 20/TBST. ECL was carried out with Pierce™ ECL 2 Western Blotting Substrate (Thermo Fisher) and the signals were visualized with ChemiDoc™ XRS+ system (Bio-Rad). The expression of HPRT1 was used as an internal control in this experiment.

Efferent duct ligation

Mice were anesthetized with isoflurane, and the efferent duct of a testis was ligated under a dissecting microscope as reported previously (Smith, 1962) with 7-0 nylon suture. Contralateral testis from the same animal was kept as a control without ligation. Mice were euthanized 1 week after the ligation, and their testes were collected, weighed, dispersed into seminiferous tubule fragments and analyzed by αSMA immunohistochemistry in whole-mount preparation. Weight of the ligated testis was 1.30 ± 0.04 times heavier than the control testis at 1 week after the ligation ($p=0.034$, student's t-test, $n=3$). More than 30 whole-mount seminiferous tubules were analyzed per animal for the quantification of PMC number/half circumference of seminiferous tubules.