

Fig. S1. Localization of ACTRT1 and ACTL7A in spermatids/spermatozoa. (A) The negative control for Fig. 1B. Immunofluorescent staining of ACTRT1 and ACTL7A (red) in *Actrt1*-KO testicular spermatids. The signal of ACTRT1 was lost whereas the subacrosomal localization of ACTL7A was unaltered in *Actrt1*-KO spermatids. (B) The sperm smear from WT and *Actrt1*-KO mice was immunostained for ACTRT1 or ACTL7A (red). In mature spermatozoa, ACTRT1 and ACTL7A translocated from the subacrosomal region to the postacrosomal sheath (PAS). Sections were counterstained with the acrosome dye PNA-FITC (green) and the nuclear dye DAPI (blue). Scale bar, 5 μ m.

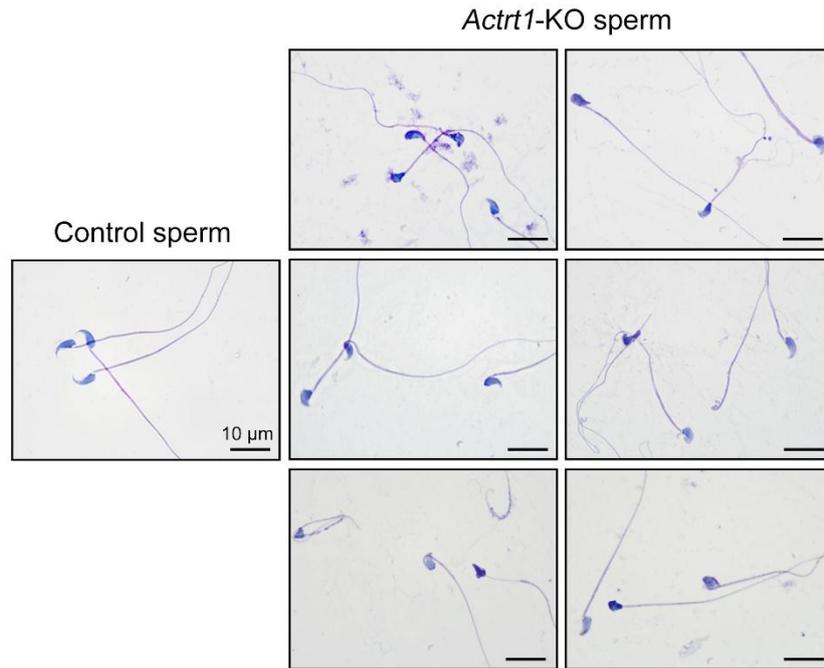


Fig. S2. Light microscopy of *Actrl1*-KO sperm. Sperm of wild-type and *Actrl1*-KO mice were stained by the Papanicolaou technique. A high ratio of sperm with deformed heads was observed in *Actrl1*-KO sperm. Scale bars, 10 μm.

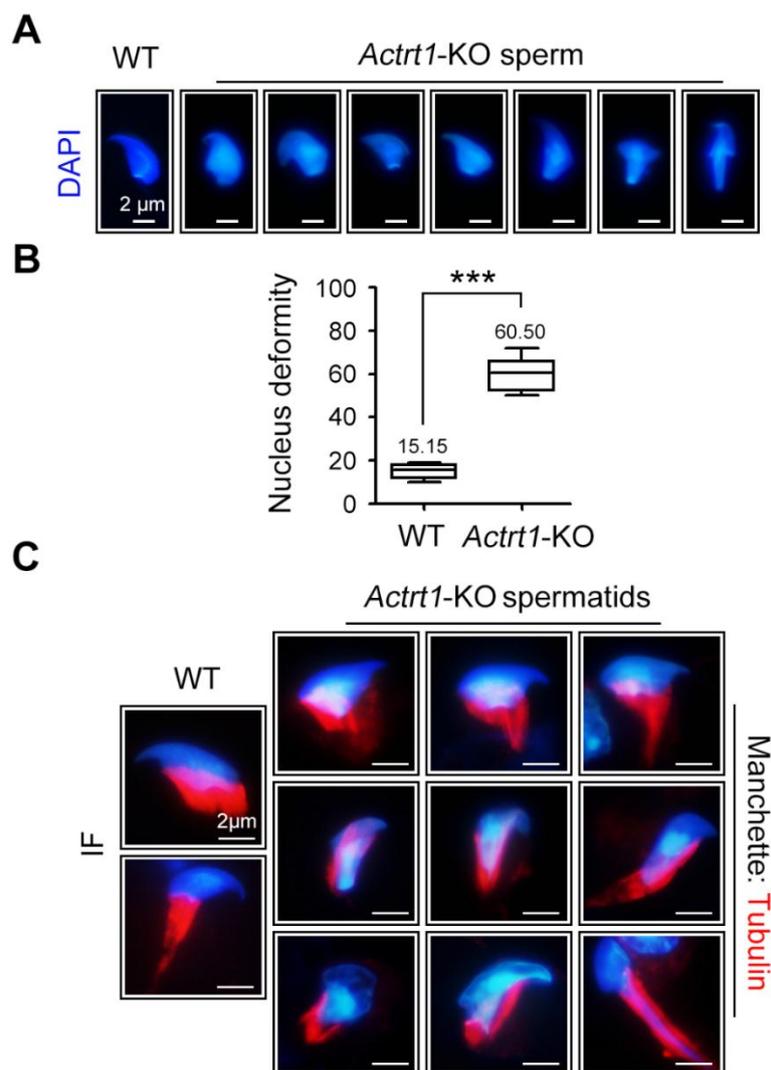


Fig. S3. Nucleus deformity and abnormal development of manchette in *Actrt1*-KO sperm. (A) Representative images of DAPI-stained nucleus of wild-type and *Actrt1*-KO sperm. Scale bars, 2 μ m. (B) The percentage of nucleus deformity in DAPI-stained wild-type and *Actrt1*-KO sperm. Data are presented as the means \pm SEM. $n=3$ mice for each group. Student's *t* test. *** $p<0.001$. At least 50 sperm were counted in each experiment. (C) The manchette of spermatids from wild-type and *Actrt1*-KO mice was observed by the staining of Tubulin-Tracker Red. Nucleus were counterstained with DAPI. Scale bars, 2 μ m.

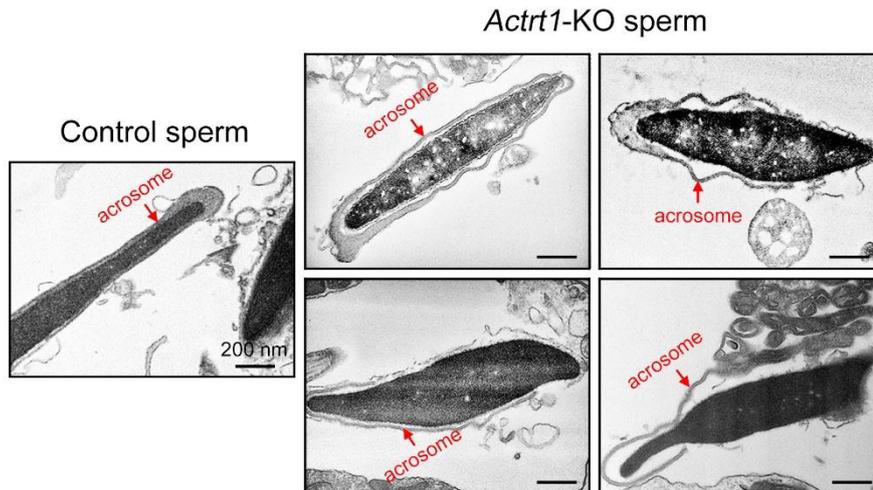


Fig. S4. TEM analysis of *Actrt1*-KO sperm. TEM analysis revealed the detachment of the acrosome from the nucleus in the *Actrt1*-KO sperm. The acrosomes were indicated by arrows in figures. Scale bars, 200 nm.

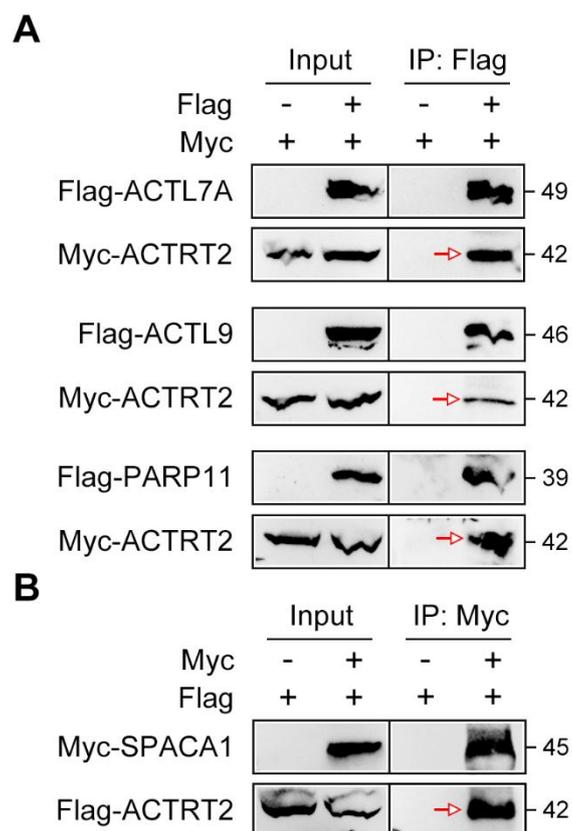


Fig. S5. ACTRT2 interacts with ACTL7A, ACTL9, PARP11, and SPACA1. (A) HEK293T cells were transfected with Flag-tagged and/or Myc-tagged recombinant plasmids. Cell lysate was subjected to immunoprecipitated Flag-tagged proteins with anti-Flag antibody. Myc-tagged ACTRT2 could be coimmunoprecipitated with Flag-tagged ACTL7A, ACTL9, and PARP11. (B) Cell lysate was subjected to immunoprecipitated Myc-tagged SPACA1 with anti-Myc antibody. Flag-tagged ACTRT2 could be coimmunoprecipitated with Myc-tagged SPACA1.

Table S1. CRISPR/Cas9 strategy to target mouse *Actrt1* gene. gRNAs are marked in blue, exons are marked in red.

[Click here to download Table S1](#)

Table S2. Primers for the genotype of *Actrt1*-KO mice.

PCR No.	Primer No.	Sequence	Band Size
<i>Actrt1</i> ^{-Y}	F1	TCATCAACACACCCGCAAC	WT: 8230 bp
	R1	CAGTTGGCTACTTGACTTGCCTAG	KO: 297 bp
	F2	GCTAGGAGTGAAACCCAATGAAC	WT: 394 bp
	R2	TTTGTAGCCCAAGGAGACAGTAC	KO: not existed

Table S3. Specific primers for amplification of *Actrt1* and *Actrt2* using RT-PCR.

mRNA	Primer No.	Sequence	Band Size
<i>Actrt1</i>	Forward	CATACCATCTGCAAGATCTAATCGG	490 bp
	Reverse	GTTGAGGATGCAGGGGAAGGT	
<i>Actrt2</i>	Forward	CCCCACGGGAGCTAGTCAGAAGAA	512 bp
	Reverse	CATCTTCTGCCCCGCCGAGACA	

Table S4. Primers for plasmid construction.

Gene	Primer No.	Sequence
<i>Actr12</i>	Forward	(KpnI) <u>GGTACCCCGACCGGGATGTTTAACCCACTGGT</u>
	Reverse	(XhoI) <u>CTCGAGCGGGAAGCACCGCCTCTGGACCAC</u>
<i>Actl7a</i>	Forward	(KpnI) <u>GGTACCCCGATGTCTCTGGATGGTGTGTGGG</u>
	Reverse	(XhoI) <u>CTCGAGCGGGAAGCACCTTCTGTAGAGGAAGAAAG</u>
<i>Actl9</i>	Forward	(KpnI) <u>GGTACCCCGATGGATGTCAATGGACACCCAAAGT</u>
	Reverse	(XhoI) <u>CTCGAGCGGAAAGTAGCATTTCGGTATAACAACCTGG</u>
<i>Spacal</i>	Forward	(KpnI) <u>GGTACCATGCGCGCCAGGGGCGC</u>
	Reverse	(XbaI) <u>TCTAGATTTCATTCCATTCACTTAAAGCGTCATCTTCATG</u>
<i>Parp11</i>	Forward	(KpnI) <u>GGTACCATGTTTCACAAGACAGAGGAGTTCTT</u>
	Reverse	(XbaI) <u>TCTAGAGTGGAAGTCTATCAGGTACTCAGGG</u>
<i>Dpy19l2</i>	Forward	(KpnI) <u>GGTACCATGGTGGGGCCGACAAG</u>
	Reverse	(ApaI) <u>GGGCCCCTTAATCTTCAGTACTCTGTACATACTATTCTG</u>
<i>Fam209</i>	Forward	(KpnI) <u>GGTACCATGCGGACGCTGCTGAGATG</u>
	Reverse	(XbaI) <u>TCTAGACTCAGAGTCCTCCTCCCCCA</u>
<i>Spata46</i>	Forward	(KpnI) <u>GGTACCATGGATAACTACTCACTCCTCAGCAC</u>
	Reverse	(XbaI) <u>TCTAGACTTGAAGGCCTGGCAGCTG</u>

Table S5. Antibodies/dyes used in this study.

Protein	Manufacturer	Cat No.	Usage	Concentration
HSP90	Proteintech	13171-1-AP	WB	1:1,000
Calnexin	Abcam	ab133615	WB	1:1,000
MLH1	Proteintech	11697-1-AP	WB	1:1,000
Vimentin	Abcam	ab92547	WB	1:1,000
Myc	Abmart	M20002	WB, IP	1:1,000; 1:100
Flag	Abmart	M20008	WB, IP	1:1,000; 1:100
PNA-FITC	Sigma-Aldrich	L7381	IF	1:500
Mito-Tracker Red	Beyotime	C1049	IF	1:5,000
Actin-Tracker Red	Beyotime	C2203	IF	1:100
Tubulin-Tracker Red	Beyotime	C1050	IF	1:100
ACTRT1	Invitrogen	PA5-31691	WB, IF	1:1,000; 1:200
ACTRT2	Proteintech	16992-1-AP	WB	1:1,000
ACTL7A	Proteintech	17355-1-AP	WB, IP, IF	1:1,000; 1:100; 1:200
SPACA1	Abcam	ab191843	WB, IP	1:1,000; 1:100
CAPZA3	Progen	GP-SH4	WB	1:1,000
CAPZB	Proteintech	25043-1-AP	WB	1:1,000
LMNA	Proteintech	10298-1-AP	WB	1:1,000
PLC ζ	Abcam	ab181816	WB	1:1,000
β -tubulin	Abmart	M30109	WB	1:1,000
Alexa Fluor 555-labeled Donkey Anti-Rabbit IgG(H+L)	Beyotime	A0453	IF	1:200
Goat anti-rabbit IgG-HRP	Abmart	M21002	WB	1:10,000
Goat anti-mouse IgG HRP	Abmart	M21001	WB	1:10,000
Rabbit Anti-guinea pig IgG HRP	Abmart	M212124	WB	1:10,000