

Supplementary Table S1. Single-guide RNAs targeting the genes' upstream (U) and downstream (D) regions used for generating knockout mice. Efficiency of embryo transplantation was presented using the number of total pups delivered by pseudopregnant mice divided by the number of total embryos used for oviduct transplantation (Total pups/embryos transplanted). Efficiency of genome editing was determined by the number of pups carrying enzymatic mutations divided by the number of pups subjected to genotyping (GM pups/pups genotyped).

Gene symbol	KO strategy	Total pups/embryos transplanted	GM pups/pups genotyped	PAM	sgRNA sequences	
<i>4921507P07Rik</i>	Zygote electroporation	14/90 (15.6%)	3/14 (21.5%)	CGG	U	GGCCGAATAGTTGTTTCGTA
				AGG	D	AACAGAATCCAGCCAAATGC
<i>Allc</i>	Zygote electroporation	15/56 (27%)	1/15 (6%)	GGG	U	GATTTGGCCTCTGAGTGCCT
				AGG	D	TAGGGAACCCCTTGAGCCGA
<i>Cabs1</i>	Zygote injection	15/77 (19%)	5/11 (45%)	TGG	U	AGTCATTTACTGAAGTAACG
<i>Fam229b</i>	Zygote electroporation	16/75 (21%)	15/16 (94%)	AGG	U	CTTCTTGAGCCCGCTAGCTA
				GGG	D	ATCATTAAAGTCTTCTGTCTGA
<i>Fscb</i>	Zygote electroporation	17/100 (23%)	10/17 (59%)	AGG	U	CCTGAAGAGCCTATATCATT
				AGG	D	TAAAGAAGTCTGAGCTATCGTC
<i>Hdgfl1</i>	Zygote electroporation	15/80 (18.8%)	9/15 (60%)	AGG	U	AGCAGCAGCGGGACCTCTCG
				GGG	D	GTTGAAGACTGGGCCCTAGT
<i>lqca</i>	Zygote electroporation	20/80 (25%)	5/20 (25%)	AGG	U	GTTCCCCCAACTTCGGCAGT
				AGG	D	TCTTCTCTCTCGGTACAC
<i>Lelp1</i>	Zygote electroporation	6/33 (18%)	3/6 (50%)	AGG	U	TGAAAGCTTCTCGAGAAAAC
				AGG	D	CCATGCCTACCCCGAAAGTG
<i>Spata24</i>	Zygote electroporation	16/40 (40%)	13/16 (81%)	GGG	U	ACAGAGCACCTGGCAACCG
				AGG	D	GACAGTGCCACTGCTCATGG
<i>Tmem97</i>	Zygote electroporation	4/46 (8.7%)	3/4 (75%)	TGG	U	CAGCTAGGCGCTGTGTCTGAG
				TGG	D	GCAGTGCTGTGGTAGCGCTT
<i>Eddm3b</i>	Zygote electroporation	10/79 (13%)	1/10 (10%)	GGG	U	TCAGGCCTTGGTCACCGTCC
<i>Lrcol1</i>	Zygote electroporation	6/81 (7.4%)	3/6 (50%)	TGG	U	GTGAGATGTCTGTCTCGGTG
				TGG	D	CACATTCCAAAAACACGGTC
<i>Tmem114</i>	Zygote electroporation	20/65 (30.8%)	11/20 (55%)	TGG	U	TGCGGGTGGGATCGCGTAAG
				TGG	D	CCAGGAGGCGCAGGGCCTTT

Supplementary Table S2. Primers and PCR conditions used for genotyping.

Gene symbol	Allele	Forward primer	Reverse primer	Band size	Annealing	Elongation
<i>4921507P07Rik</i>	WT	TAAGCCTCTGGTTTTGTGC	TACTCATGCTTTGCCTTCTCC	400 bp	65°C, 30 s	72°C, 30 s
	KO	TAAGCCTCTGGTTTTGTGC	GTCAGGACATTATTTGGTCC	346 bp	65°C, 30 s	72°C, 30 s
<i>Allc</i>	WT	GCCTTGCTGTGTTGTAAGCC	ATGGACTTGGGTTCCACCGAC	856 bp	65°C, 30 s	72°C, 1 min
	KO	CAGAGCTCTCACCGTTCTC	AGCACCATAGGGTATGTA	649 bp	65°C, 30 s	72°C, 45 s
<i>Cabs1</i>	WT	CTGTGAGAGTGCTCAGTGCC	CCCTTAGGTTTGGAGTTTGG	350 bp	65°C, 30 s	72°C, 30 s
	KO	CTGTGAGAGTGCTCAGTGCC	CCCTTAGGTTTGGAGTTTGG	345 bp	65°C, 30 s	72°C, 30 s
<i>Fam229b</i>	WT	AGGCAGGAAGCTATGTGAAGG	TACACAAGGGATAGGAAAGGTGA	422 bp	65°C, 30 s	72°C, 30 s
	KO	AGGCAGGAAGCTATGTGAAGG	GCTTAGCATGCAGAGCGCTC	347 bp	65°C, 30 s	72°C, 30 s
<i>Fscb</i>	WT	GGAAGTGTAGAAGACCTGACTGC	GAGAACTTCAGCAGAGCCC	502 bp	65°C, 30 s	72°C, 30 s
	KO	GTCCTAACAGAAACAAACCAAG	TAAGCAATCATTGTCCATGG	545 bp	65°C, 30 s	72°C, 30 s
<i>Hdgf1</i>	WT	ACCAACATTTCTTAACATGG	ACAAAGTTGATGTCAACTGG	1514 bp	60°C, 30 s	60°C, 30 s
	KO	ACCAACATTTCTTAACATGG	ACAAAGTTGATGTCAACTGG	419 bp	60°C, 30 s	60°C, 30 s
<i>lqca</i>	WT	GGGGTTGCTTGTGGAAACAC	TCAACAGCCTTCCTTCGAGT	461 bp	63°C, 30 s	72°C, 60 s
	KO	CGGCTCACATAAACAGGGCA	TCCATCAAGAACGGAGGTGAG	978 bp	63°C, 30 s	72°C, 60 s
<i>Lelp1</i>	WT	CCATGCCTACCCCGAAAGTG	CAGCCCCACAATGTTGTAGG	968 bp	65°C, 30 s	65°C, 30 s
	KO	CCATGCCTACCCCGAAAGTG	CAGCCCCACAATGTTGTAGG	509 bp	65°C, 30 s	65°C, 30 s
<i>Spata24</i>	WT	TCTGTTGCAGATGGTTCTCC	CAGGACTCTGCTCTTGACAC	542 bp	65°C, 30 s	72°C, 30 s
	KO	TTGGGGGATGAAATCCAGTC	AGCTCAGTAGAGGCGGGTGC	199 bp	55°C, 30 s	72°C, 30 s
<i>Tmem97</i>	WT	GTGACCATCTAGGCCAATGG	GGCAATATACTAACCTAAGG	591 bp	60°C, 30 s	72°C, 30 s
	KO	CTATAGCCCCGCTATATGGG	GGTGAGGTGCCAGAGTAGGG	552 bp	65°C, 30 s	72°C, 30 s
<i>Eddm3b</i>	WT	CAAAAGAGAGGGCATATAGGAGG	AGCAGAAGCAGGGCCAGGC	296 bp	65°C, 30 s	72°C, 30 s
	KO	CAAAAGAGAGGGCATATAGGAGG	GAGCAGAAGCAGGGCCTTTT	292 bp	65°C, 30 s	72°C, 30 s
<i>Lrcol1</i>	WT	CAGCCAGGGCTACACGGAAA	GATGGGGCTGCATGACGATTGGG	491 bp	65°C, 30 s	72°C, 30 s
	KO	CAGCCAGGGCTACACGGAAA	GGTCTTTGATGAGCAGAACC	489 bp	65°C, 30 s	72°C, 30 s
<i>Tmem114</i>	WT	ACTCAGTCTGATCGTGATGG	TCCGCTGCTTCTACTTGTGG	567 bp	60°C, 30 s	60°C, 30 s
	KO	GGACGCTCGCCCCGATCCGG	AAAACCTACGGGTGACTAGG	587 bp	60°C, 30 s	60°C, 30 s

Supplementary Table S3. Sanger sequencing of mutant alleles in all mouse lines. Bases in uppercase indicate exon sequences and the ones in lowercase indicate intron sequences.

Gene symbol	Upstream sequence (25 bp)	Mutation	Downstream sequence (25 bp)
<i>4921507P07Rik</i>	TACTCTAACGGACTGCACACCCCGT	-22195	GGCTGGATTCTGTTTCTGTATTAAG
<i>Allc</i>	GCAGCTGATTGATTTGGCCTCTGAG	-19639	gggaagtatttttagaattatcctc
<i>Cabs1</i>	AACTATTACCTCGGAAGGAGACCAC	-5	TTCAGTAAATGACTGCACACCGGAT
<i>Fam229b</i>	TTGGCCTATTTTGTAAGTCTATGT	-3266 +17*	TTCAGTAAATGACTGCACACCGGAT
<i>Fscb</i>	AATGTGAAGAACCTGAAGAGCCTAT	-3166	AGGTGTAACTTAAACTAATAATTT
	AATGTGAAGAACCTGAAGAGCCTAT	-3164	TCAGGTGTAACTTAAACTAATAAT
<i>Hdgf1</i>	aggaagcgttgccagcatcctcgag	-1095	GGGACAGGCCAGTTGACATCAACTT
<i>lqca</i>	CTAGGGGGAGTTCCCCAACTTCGG	-107633	AGAGGAGGAAGAGAGTTTTAAGgta
<i>Lelp1</i>	aggcccctaagaacacctgcttact	-577bp+118 [#]	AGTGAGGCACCATGGGCAACTCTCC
<i>Spata24</i>	CCTTGCGAACAGAGGAAGTCCCCGC	-5638	ACTGTCTGTCTGCCTCCTATAGCAA
<i>Tmem97</i>	GGGCCCTGGCAGCTAGGCGCTGTGT	-8286	CGTACCACAGCACTGCTAAAAGG
<i>Tmem114</i>	TGGGCCGGAGCACGTGCGGGTGGGA	-15261	CCCATCTCAGTGATGGCAACCTTT
<i>Eddm3b</i>	CCTGCTCTGATGTCCTCTCTAAAAG	-5	GCCCTGCTTCTGCTCCTGCTGTGCC
<i>Lrcol1</i>	GGTCCAACGTGAGATGTCTGTCTCG	-1196	AATGTGTGCCCTGGAGGAAGGTGAC

*+17: TTTCTTGAGCCCGCTAG

[#]+118:GAAGCTTTCAATGTGTTTCAGTCTCTGGACCATCTCATTAAAGAGCTGCTTGTCTGTGGACTTGGGGTATGTCTAGATGCCTGCACTCTGATTTCTCTTACTTTGTTTCCATTCTAG

Supplementary Table S4. Testis weight relative to body weight in wild-type and mutant males.

Gene symbol	Testis/body weight ($\times 10^3$)	No. of males	No. of testis
Wild-type	3.0 \pm 0.4	6	12
<i>Fam229b</i>	3.3 \pm 0.1	3	6
<i>Lqca</i>	3.2 \pm 0.0	2	4
<i>Lelp1</i>	4.2 \pm 0.4	2	4
<i>Spata24</i>	3.8 \pm 0.6	2	4
<i>Tmem114</i>	3.2 \pm 0.5	3	6

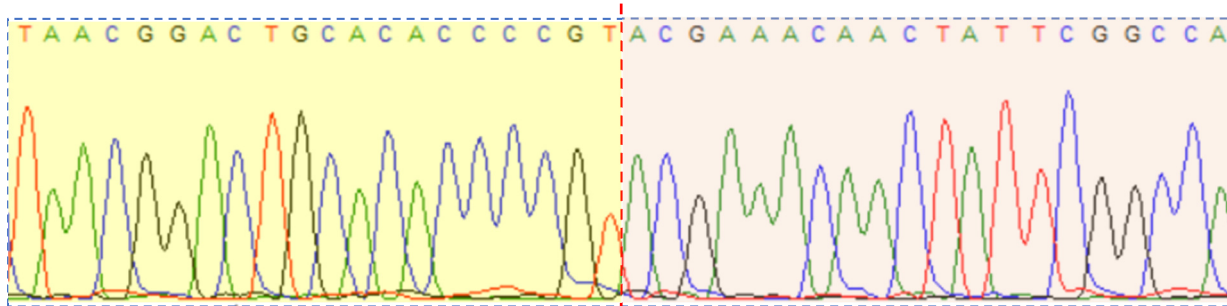
Supplementary Table S5. The 13 knockout mouse lines in this study. The RBRC No. and CARD ID are available for the mouse lines that have been deposited as frozen sperm to Riken BioResource Research Center and Center for Animal Resources and Development at Kumamoto University, respectively.

Gene symbol	Gene name	RBRC No.	CARD ID
<i>4921507P07Rik</i>	RIKEN cDNA 4921507P07 gene	11050	2957
<i>Allc</i>	Allantoicase	11054	2961
<i>Cabs1</i>	Calcium binding protein, spermatid associated 1	10333	2703
<i>Fam229b</i>	Family with sequence similarity 229, member B	11035	2942
<i>Fscb</i>	Fibrous sheath CABYR binding protein	11063	2970
<i>Hdgf1</i>	HDGF like 1	11018	2925
<i>Iqca</i>	IQ motif containing with AAA domain	TBD	TBD
<i>Lelp1</i>	Late cornified envelope-like proline-rich 1	TBD	TBD
<i>Spata24</i>	Spermatogenesis associated 24	TBD	TBD
<i>Tmem97</i>	Transmembrane protein 97	11025	2932
<i>Eddm3b</i>	Epididymal protein 3B	10345	2715
<i>Lrcol1</i>	Leucine rich colipase like 1	TBD	TBD
<i>Tmem114</i>	Transmembrane protein 114	TBD	TBD

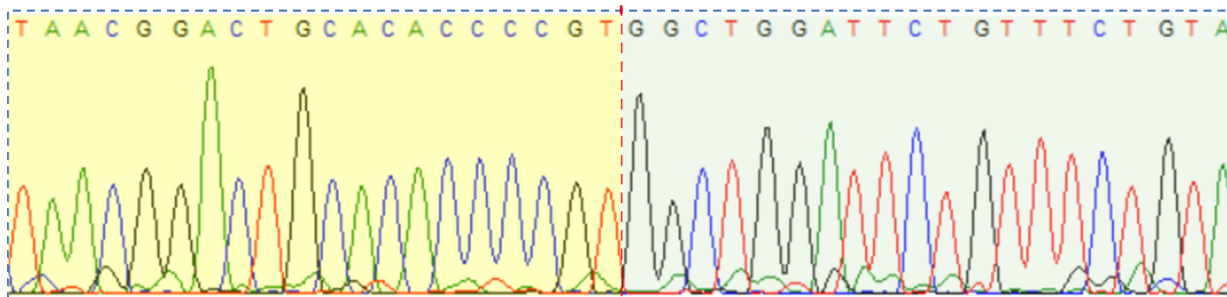
Supplementary Table S6. Additional information about the 13 mouse genes analyzed in this study. The presence of transmembrane (TM) domains and a signal peptide was predicted using TMHMM and SignalP, respectively.

Gene symbol	NCBI UID	MGI ID	Chromosome	No. of TM domain	Signal peptide	Human orthologs
<i>4921507P07Rik</i>	70821	1918071	6	0	N	<i>C7orf31</i>
<i>Allc</i>	94041	2136971	6	0	N	<i>ALLC</i>
<i>Cabs1</i>	70977	1918227	5	0	N	<i>CABS1</i>
<i>Fam229b</i>	66337	1913587	10	0	N	<i>FAM229B</i>
<i>Fscb</i>	623046	3646964	12	0	N	<i>FSCB</i>
<i>Hdgfl1</i>	15192	1194493	13	0	N	<i>HDGFL1</i>
<i>Iqca</i>	74918	1922168	1	0	N	<i>IQCA1</i>
<i>Lelp1</i>	149018	1916582	1	0	N	<i>LELP1</i>
<i>Spata24</i>	71242	1918492	18	0	N	<i>SPATA24</i>
<i>Tmem97</i>	69071	1916321	10	4	N	<i>TMEM97</i>
<i>Eddm3b</i>	219026	2684921	14	0	Y	<i>EDDM3B</i>
<i>Lrcol1</i>	381667	2686525	5	1	Y	<i>LROCL1</i>
<i>Tmem114</i>	74720	1921970	16	4	Y	<i>TMEM114</i>

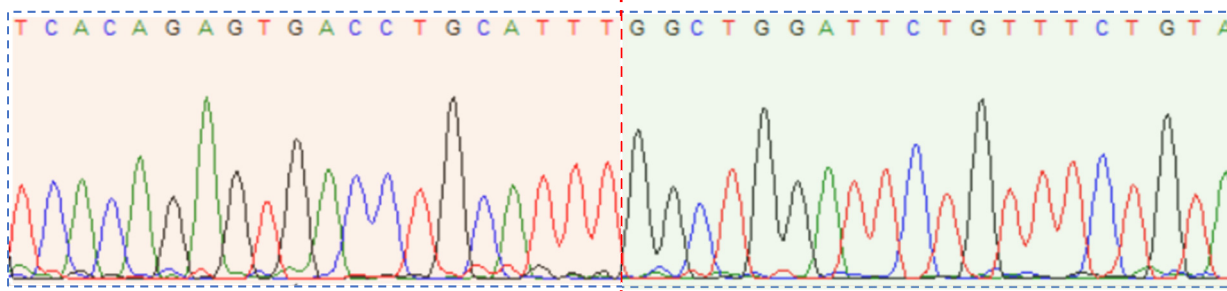
4921507P07Rik^{+/+} Upstream



4921507P07Rik^{-22195/-22195}

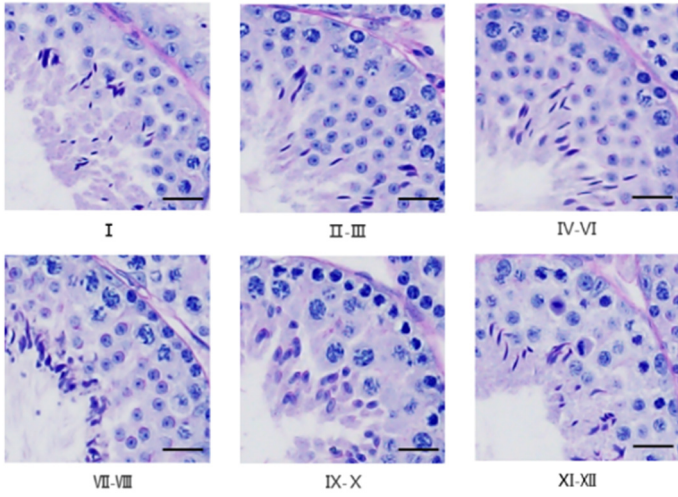


4921507P07Rik^{+/+} Downstream

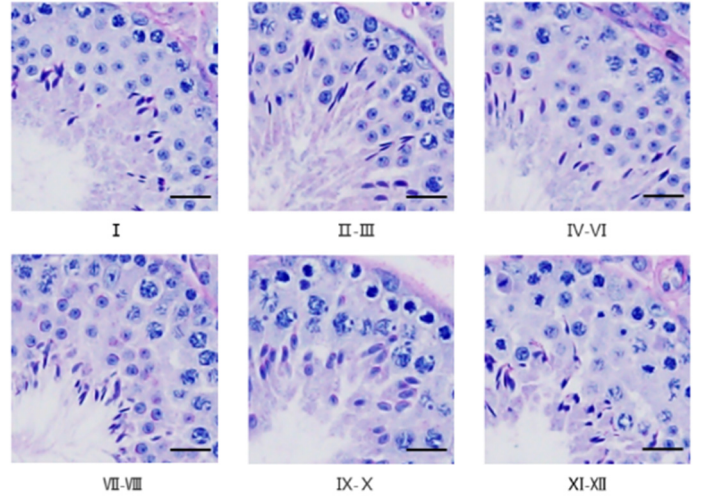


Supplementary Figure S1. Sanger sequencing of *4921507P07Rik* wild-type and knockout alleles. The upper and lower graphs indicate the upstream and downstream sequences of the wild-type allele, whereas the middle graph shows the knockout allele. The sequences deleted in the knockout mice are highlighted in red. The regions immediately upstream and downstream of the deletion are highlighted in yellow and green, respectively.

(A) Het.

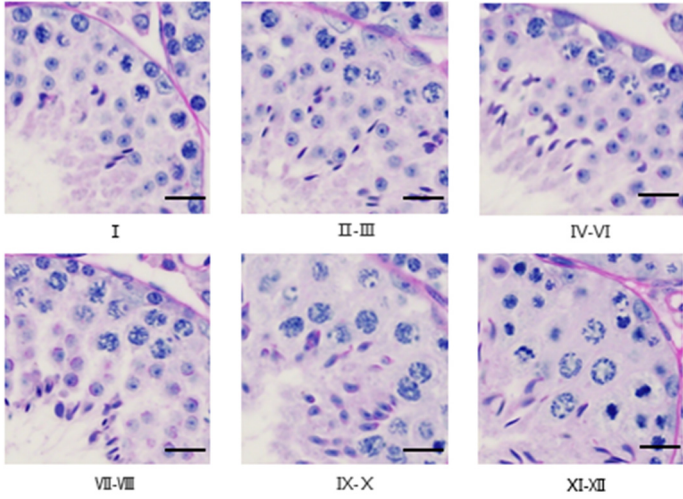


(B) Homo.

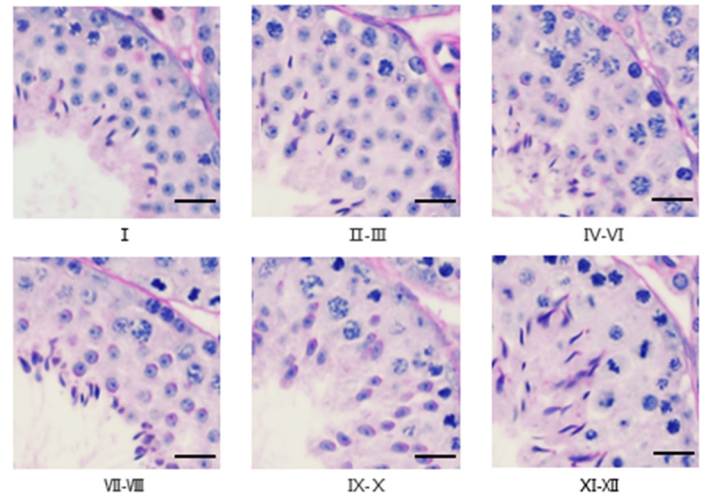


Supplementary Figure S2. Detailed analyses of spermatogenesis in **(A)** *A/lc* heterozygous and **(B)** homozygous knockout mice. Scale bars = 20 μ m.

(A) Het.

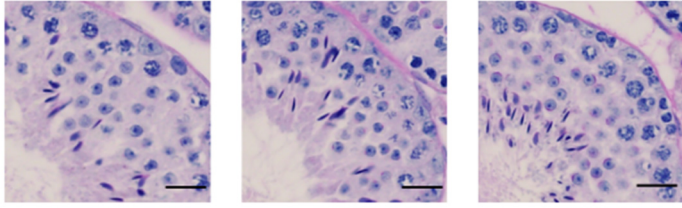


(B) Homo.



Supplementary Figure S3. Detailed analyses of spermatogenesis in **(A)** *Fscb* heterozygous and **(B)** homozygous knockout mice. Scale bars = 20 μm .

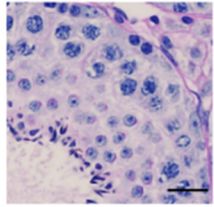
(A) Het.



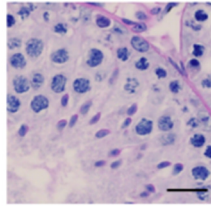
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II-III

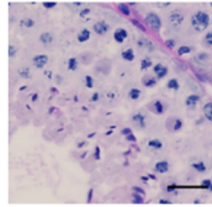
IV-VI



VII-VIII

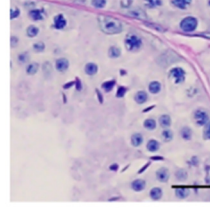


IX-X

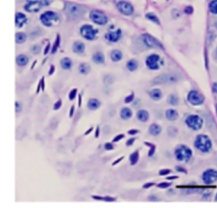


XI-XII

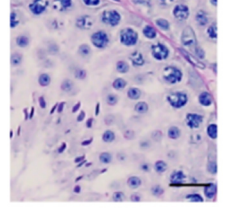
(B) Homo.



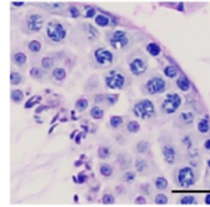
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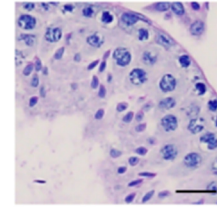
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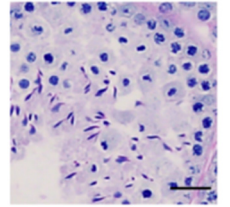
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VII-VIII



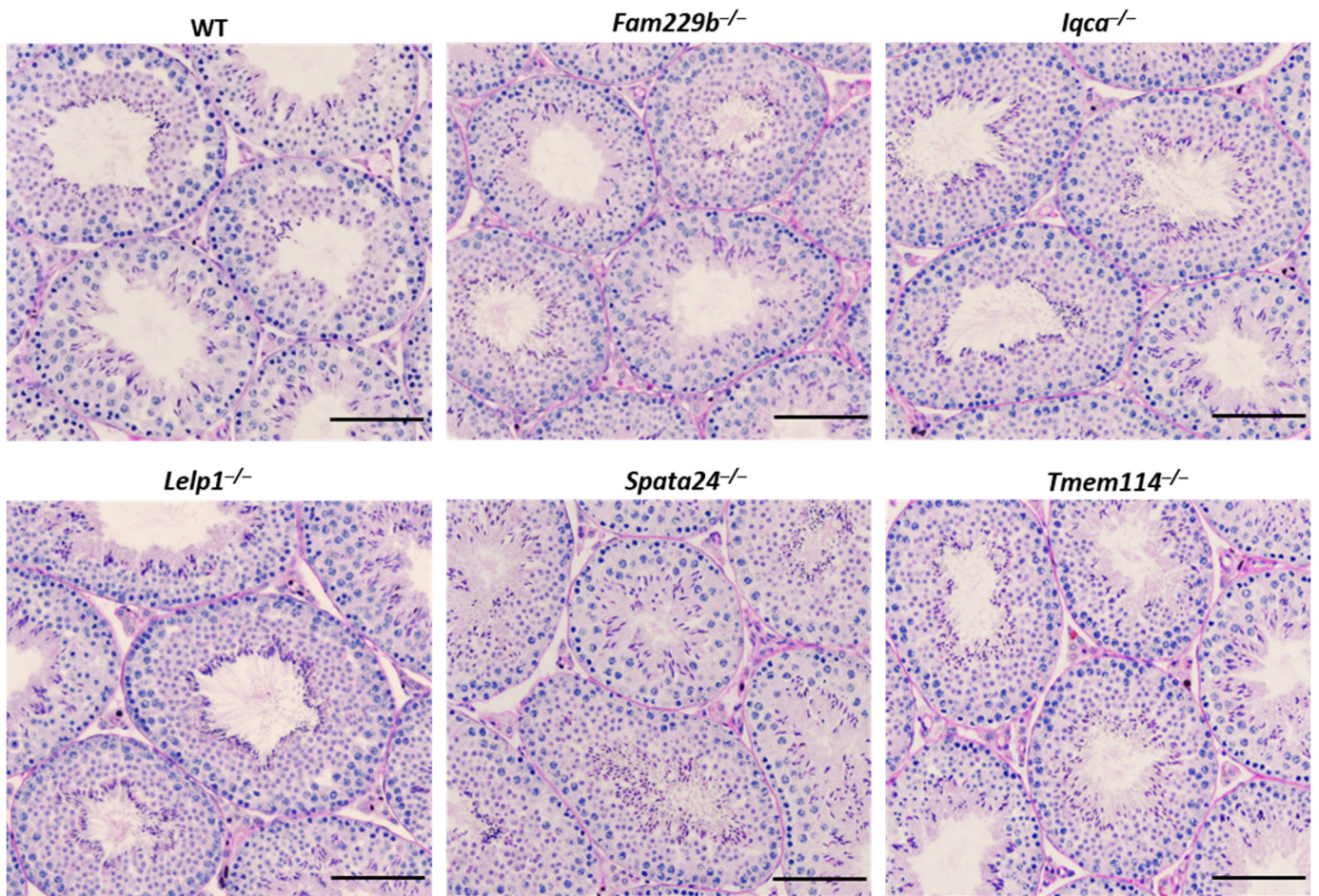
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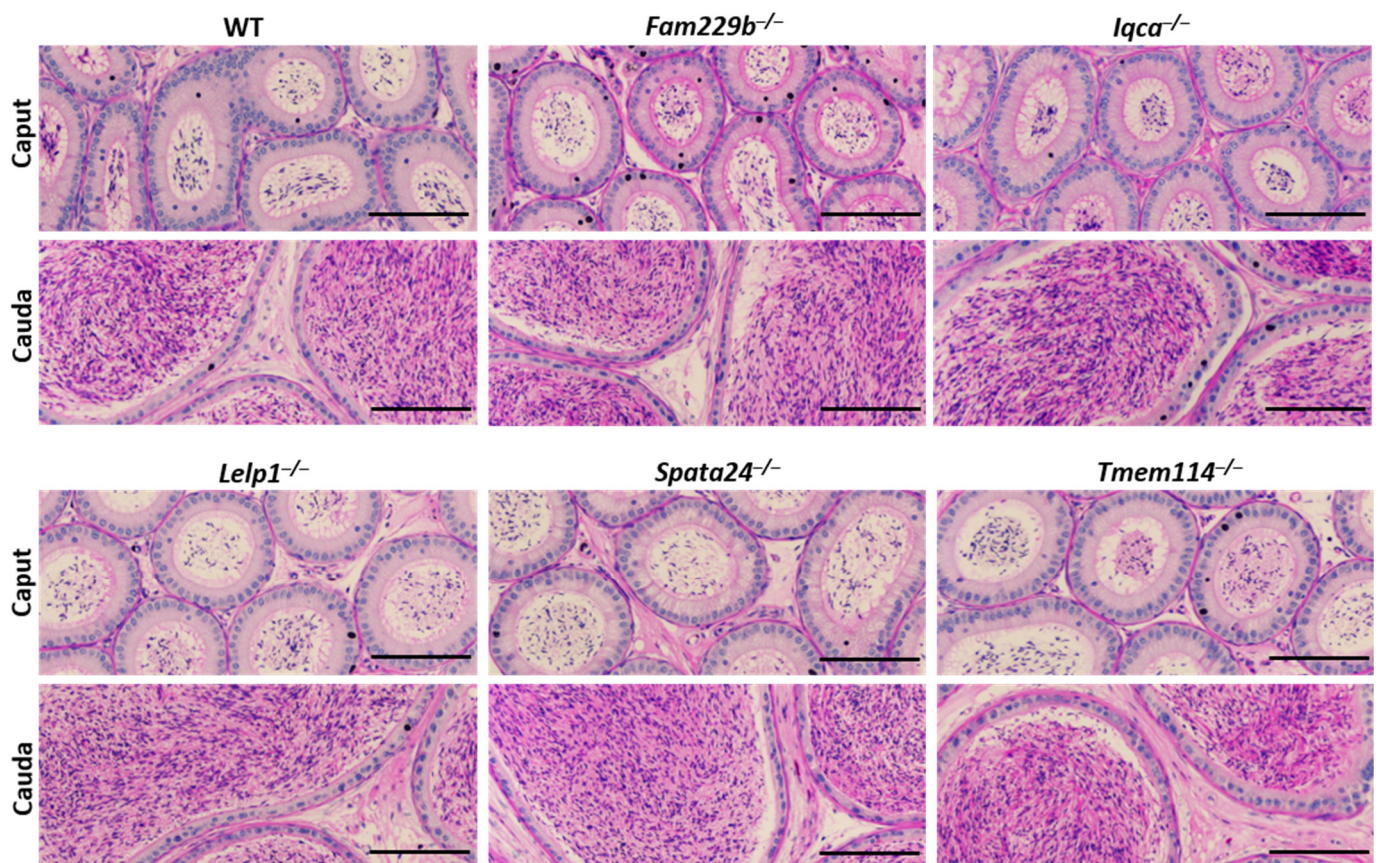
XI-XII

Supplementary Figure S4. Detailed analyses of spermatogenesis in **(A)** *4921507P07Rik* heterozygous and **(B)** homozygous knockout mice. Scale bars = 20 μ m.

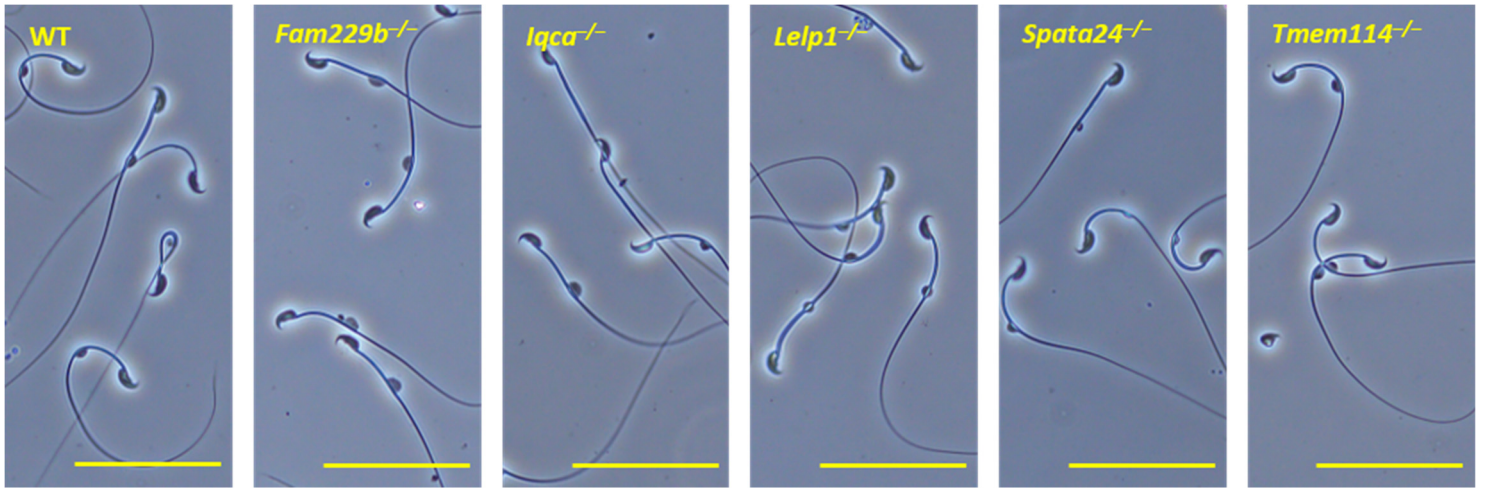
(A)



(B)



Supplementary Figure S5. Histological analyses of (A) testes and (B) epididymides in *Fam229b*, *lqca*, *Lelp1*, *Spata24* and *Tmem114* knockout males. Scale bars = 100 μ m.



Supplementary Figure S6. Morphology of spermatozoa in *Fam229b*, *lqca*, *Lelp1*, *Spata24* and *Tmem114* knockout males. Scale bars = 50 μ m.

(M) TMEM114

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Human 1 MRVHLGGLAGAAALSGALSFVLLAAAIGTDFWYIIDTERLERITGPGAQILLGGINRSQEPPLSSHSGLWRTCVRVQSECTPLMNPFRLENVTVSESSRQLLTMHGTFVILL 110
Mouse 1 MRVHLGGLAGAAALSGALSFVLLAAAIGTDFWYIIDTERLERSSQRMRDQ-EPANRSQEPPLSSHSGLWRTCVRVQSECTPLMNPFWQENVTVSESSRQLLTMHGTFVILL 109

Human 111 PLSLILMVFVGGMTGFLSFLLEKYLKLLLLLTGILFLFGAMVTLAGISVYIAYSAAAFREALCLLEEKALLDQVDHSFGWSLALGWISFIAELLTGAMFLAAARELSLRQRD 220
Mouse 110 PLSLIVMVFVGGMTGFLSFLLEKYLKLLLLLTGILFLFGAMVTLAGISVYIAYSAAAFREALCLLEERALLDQVDHSFGWSLALGWISFVSELLTGAVFLAAARELSLRQRD 219

Human 221 QAI 223
Mouse 220 QAI 222
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Supplementary Figure S7. Alignment of mouse and human peptide sequences encoded by each gene. Dark regions indicate identities between mouse and human sequences. **(A)** 4921507P07Rik. **(B)** ALLC. **(C)** CABS1 **(D)** FAM229B. **(E)** FSCB. **(F)** HDGFL1. **(G)** IQCA. **(H)** LELP1. **(I)** SPATA24. **(J)** TMEM97. **(K)** EDDM3B. **(L)** LRCOL1. **(M)** TMEM114.

Cabs1

Wild-type MAEDGSPKIYSRPPRDNSKTPTEADIFFGADNTIPKSETTITSEGDHVTS...LKEEPDDLMM*
Mutant MAEDGSPKIYSRPPRDNSKTPTEADIFFGADNTIPKSETTITSEGDHFSK*

Supplementary Figure S8. Amino acid sequences encoded by wild-type and mutant *Cabs1*.

Eddm3b

Wild-type MSLVPALMSSLKAWALLLLLLLCLQ...EDIKALEPIL*
Mutant MSLVPALMSSLKGPASAPAVPAA*

Supplementary Figure S9. Amino acid sequences encoded by wild-type and mutant *Eddm3b*.