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Original Article

Testis-enriched *Asb15* is not required for spermatogenesis and male fertility in mice

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Abstract: Background: The function of *Asb15,* which encodes an ASB protein with ankyrin (ANK) repeats and a C-terminal suppressor of cytokine signaling (SOCS) box motif, in male germ cells is poorly understood. Because expression of *Asb15* is enriched in mouse testis, it may have a role in spermatogenesis. Methods and results: We used a computer-assisted sperm analysis (CASA) system to analyze sperm from *Asb15* gene knockout (KO) mice that we generated using the clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) technique. Histological staining and immunostaining were used to evaluate spermatogenesis in *Asb15*-KO mice. *Asb15*-KO and wild-type mice showed no differences in histology or in semen quality, fertility, or sperm apoptosis. *Asb15*- and *Asb17*-double KO (dKO) mice were generated to determine whether *Asb17* compensated for the loss of *Asb15*. However, *Asb15/17*-dKO mice also showed normal fertility, except for an increase in giant cells in testicular tubules, suggesting a minor functional compensation between the two genes during spermatogenesis. Conclusions: Our study suggests that *Asb15* was individually not required for spermatogenesis or for fertility in mice. However, further investigation might be needed to reach a firm conclusion. These findings can prevent redundant research by other scientists and provides new information for further studies on the genetics of fertility in humans.

Keywords: Asb15, testis, spermatogenesis, male fertility

Introduction

There are 18 proteins in the ASB family, and all contain N-terminal ankyrin (ANK) repeats and a suppressor of cytokine signaling (SOCS) box at the C terminus [1]. They provide the substrate recognition subunit for some Elongin-Cullin-SOCS (ECS) E3 ubiquitin ligases that function as cell cycle regulators, skeletal membrane proteins, receptors, and transcription factors [2]. Several ASB family proteins, including those encoded by the *Asb3*, *Asb4*, *Asb8*, and *Asb9* genes, are expressed primarily in mouse testis and function during spermatogenesis [3-5]. *Asb1*-gene knockout (KO) mice lack spermatogenesis from some seminiferous tubules [6]. Recently, we found that *Asb17* is a key factor for spermiation [7].

Asb15, a member of the ASB protein family, helps in regulating the differentiation of muscle cells [8, 9]. However, its function in male germ cells is not known. During spermatogenesis, sequential mitosis in the spermatogonial stem cells produces spermatocytes. This is followed by the transformation of spermatocytes into spermatids via two rounds of meiosis, and the spermatids undergo morphological remodeling [10]. Spermatogenesis involves developmentally regulated, stage-specific gene expression in the germ cells [11-13]. Elucidating the genes and pathways involved in male gametogenesis may identify candidates for developing therapies to treat male infertility. Here, we characterized *Asb15*-KO mice to determine the role of the *Asb15* gene, which is highly expressed in mouse testis, in spermatogenesis.

Materials and methods

Animals

We produced CRISPR/Cas9 *Asb15*-KO mice as described previously [14, 15] using a single guide (sg) RNA that targeted exon 6 of *Asb15*. The target sequences were 5'-GGCAACGTCCACTTGAGAGACGG-3' and 5'-CCACTGTGACGTGTTAGAACATC-3'. Cas9 mRNA was made as described previously [16, 17]. We microinjected zygotes taken from a CD-1 mouse with both sgRNA and Cas9 mRNA, and we generated heterozygous mice by backcrossing the founder mice with CD-1 mice. We used the backcrossed CD-1 *Asb15*-KO and wild-type (WT) mice in the experiments as described here. To determine the genotypes, we sequenced polymerase chain reaction (PCR) products made with following forward (5'-ATACAACACCAGCCTTGAC-3') and reverse (5'-GCACGAAGATAA GACAGACT-3') primers. *Asb17*-KO mice were obtained as previously described [7]. *Asb15*-KO mice and *Asb17*-KO mice were intercrossed to produce *Asb15/17*-double KO (dKO) mice. Mice were fed under a specific pathogen-free (SPF) condition at Nanjing Medical University. Our procedures followed with the *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Ethical and Welfare Committee of Nanjing Medical University (No. 2004020).

Fertility test

Adult $WT^{(+/+)}$, $Asb15^{(-/-)}$-KO, $Asb17^{(-/-)}$-KO, and *Asb15/17*-dKO males were mated with WT females (ratio of 1:2) continuously for three months with numerical counts of all litters.

RNA extraction and reverse-transcriptase (RT)-quantitative PCR (RT-qPCR)

We used TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA) to extract RNA from testicular tissues according to the manufacturer's instructions. The RNA was reverse-transcribed into cDNA that was quantitated by quantitative real-time PCR (Applied Biosystems, Foster City, CA, USA) using 18S rRNA as the internal control. *Asb15* and 18S rRNA primers were described previously [14].

Histological analysis

We fixed the testes and epididymides from *Asb15*-KO, *Asb15/17*-dKO, and WT adults for 48 h using modified Davidson's fluid. We dehydrated the specimens in successively higher concentrations of ethanol and then embedded the samples in paraffin. The specimens were sectioned to four microns, then rehydrated, and stained with either periodic acid Schiff (PAS) or hematoxylin and eosin (H&E). Sperm collected from the cauda epididymis of 8-12-week-old WT and *Asb15*-KO mice were suspended in a culture medium to assess the semen quality using the Ceros™ II Sperm Analysis System (Hamilton Thorne, Beverly, MA, USA). We analyzed the ultrastructure of sperm from 8-12-week-old WT and *Asb15*-KO mice after they were fixed in 2% (v/v) glutaraldehyde overnight and then placed in OsO_4 (2%, w/v) for 2 h. The specimens were embedded in Araldite and sectioned to 80 nm. Specimens were examined using a JEM-1410 transmission electron microscope (JEOL, Tokyo, Japan).

Immunofluorescence

Deparaffinized, rehydrated sections were blocked using 5% (w/v) bovine serum albumin (BSA) (Sunshine, Nanjing, China) after antigen retrieval treatment and were then incubated with primary antibodies at 4°C overnight (Table S1). Slides were washed with phosphate-buffered saline (PBS) twice and incubated with Alexa-Fluor secondary antibodies (Thermo Scientific, Waltham, USA). We used a confocal laser scanning microscope (Zeiss LSM710, Carl Zeiss, Oberkochen, Germany) to collect immunofluorescent images.

TUNEL assay

Apoptotic cells were assessed using a terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay [18, 19]. We treated tissue sections with proteinase K at 20 μg/mL for 10 min at room temperature prior to incubation in an equilibration buffer for 30 min. Sections were then incubated in BrightRed Labeling Buffer for 60 min at 37°C, washed three times in PBS, and stained with 4',6-diamidino-2-phenylindole (DAPI). We used a confocal laser scanning microscope (Zeiss LSM710, Carl Zeiss, Oberkochen, Germany) to collect images.

Chromosome spread

Chromosome spreads were made as described previously [20]. We digested testicular tissue from *Asb15*-KO and WT mice with trypsin and

collagenase for 15 min at 37°C, immersed the tissue in an extraction buffer that was hypotonic, and resuspended the cell pellets in 100 mM sucrose before spreading the tissue on slides coated with a fixative. We washed the fixed specimens using PBS and immunostained them using the appropriate primary antibodies (Table S1).

Statistical analyses

Data are presented as the mean ± standard deviation. Statistical significance was determined by an unpaired Student's t-test or analysis of variance with $P<0.05$ considered to be statistically significant.

Results

Asb15 was expressed primarily in mouse testes

ASB15 is conserved among species that have proteins with the classic ANK repeats combined with the SOCS motif (Figure S1). Information from the BioGPS database (http://biogps.org) showed that ASB15 was expressed primarily in the testis (Figure S2A). Further, *Asb15* transcripts from multiple murine tissues analyzed by RT-PCR (Figure S2B) gave results similar to the data in the BioGPS database (Figure S2A).

Generation of Asb15-KO mice

We constructed the first reported *Asb15* global KO mouse strain by creating a 14-bp deletion in exon 6 using CRISPR/Cas9 genome editing (**Figure 1A**) that we confirmed by sequencing PCR products made from WT and mutant mice (**Figure 1B**). The deletion resulted in a frameshift mutation that predicted a truncated 210 amino-acid protein (**Figure 1C**). Moreover, *Asb15* transcripts decreased dramatically in *Asb15*-KO testes vs. WT (**Figure 1D**), indicating that the *Asb15* mutation led to the rapid decay of the *Asb15* mRNA.

Asb15-KO mice are fertile

Asb15-KO mice were viable with normal fertility, and they produced litter sizes similar to those of WT mice (**Figure 2A**). WT and *Asb15*-KO mice also had testes of similar size and weight (**Figure 2B** and **2C**). An analysis of sperm from *Asb15*-KO mice using CASA revealed that sperm concentration, sperm motility, and the progressive ratio of sperm were similar to the controls (**Figure 2D-F**). Sperm morphology by optical microscopy (**Figure 2G** and **2H**) or transmission electron microscopy (TEM) (**Figure 2I**) was also similar in *Asb15*-KO and WT mice. Therefore, loss of *Asb15* did not affect sperm quantity or quality and, hence, had no effect on fertility in mice.

Asb15-KO mice showed normal spermatogenesis

We examined germ cell development within the seminiferous epithelium using PAS staining. Similar to WT mice, testes from *Asb15*-KO mice exhibited complete spermatogenesis, displaying all stages of the spermatogenetic cycle (**Figure 3A**). Further, the epididymis in *Asb15*-KO mice showed no histological changes compared to WT mice (**Figure 3B** and **3C**). Thus, loss of *Asb15* had no adverse effects on spermatogenesis.

To better visualize spermatogenesis, Lin28, SOX9, PNA, HSD-3β, and TUNEL proteins were fluorescently labeled to quantify spermatogonial stem cells, Sertoli cells, acrosomes, Leydig cells, and apoptotic cells in testes, respectively. We found no difference in the number of these cell types when comparing *Asb15*-KO and WT mice (**Figure 4A-K**). To evaluate meiotic progression, we performed a chromosome spread experiment. We immunostained specimens using both SCP3 (a protein that identifies the lateral element of the synaptonemal complex) and γH2AX (a protein that identifies double-stranded DNA breaks). Using the distribution of SCP3 and γH2AX [21, 22], we identified the substages of meiotic prophase, including leptotene, zygotene, pachytene, and diplotene. These experiments revealed no morphological changes for SCP3 and γH2AX in *Asb15*-KO spermatocytes compared with the controls (**Figure 4L**). Therefore, *Asb15* was not required for mouse spermatogenesis.

Asb15/17-dKO mice showed normal spermatogenesis

We observed previously that several ASB family proteins could compensate for the loss of *Asb12* in mouse testes [14]. To determine whether other ASB protein(s) could compensate for the loss of *Asb15*, we analyzed the

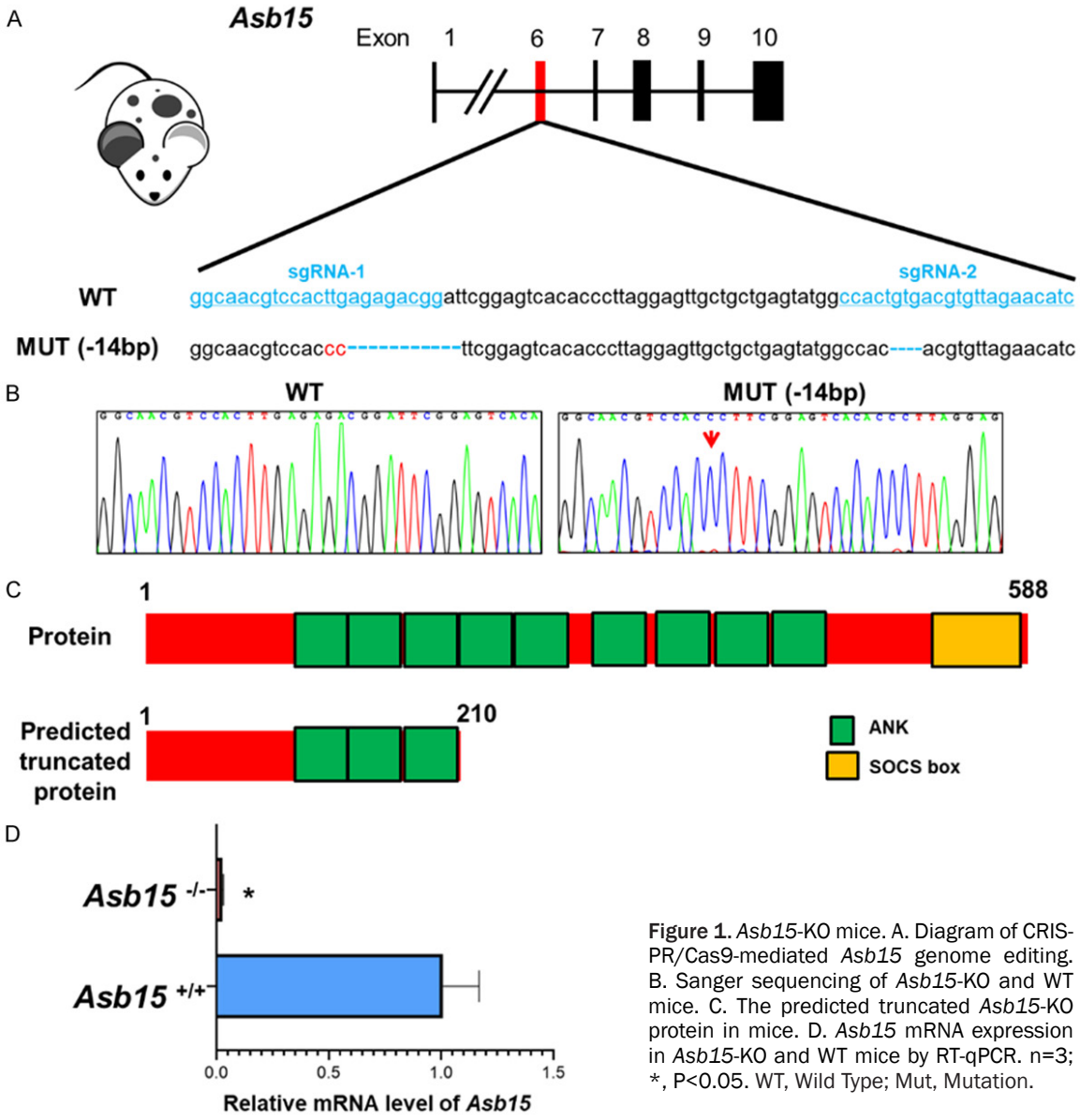


Figure 1. *Asb15*-KO mice. A. Diagram of CRISPR/Cas9-mediated *Asb15* genome editing. B. Sanger sequencing of *Asb15*-KO and WT mice. C. The predicted truncated *Asb15*-KO protein in mice. D. *Asb15* mRNA expression in *Asb15*-KO and WT mice by RT-qPCR. n=3; *, P<0.05. WT, Wild Type; Mut, Mutation.

expression pattern of ASB genes in human and mouse testes based on the single-cell RNA sequencing (scRNA-seq) data for human and mouse testes available in public databases [23-25]. In human and mouse testes, *Asb15* and *Asb17* were co-expressed in spermatids (**Figures 5** and **6**), suggesting that *Asb17* could compensate for the loss of *Asb15*. To determine the effects of *Asb15/17* deficiency on spermatogenesis, we generated *Asb15/17*-dKO mice.

Testicular morphology, assessed by PAS staining, revealed intact seminiferous tubules showing normal spermatogenesis in WT, *Asb15*-KO, *Asb17*-KO, and *Asb15/17*-dKO mice (**Figure 7A**). However, the number of multinuclear giant cells increased significantly in *Asb15/17*-dKO mice compared to the other three genotypes (**Figure 7A** and **7B**, black arrows). The fertility test showed that *Asb15/17*-dKO males produced a similar number of pups per litter compared to the WT group (**Figure 7C**), suggesting that loss of *Asb15/17* had a minor effect on mouse spermatogenesis.

Discussion

Spermatogenesis, which produces haploid sperm, is an important process in sexual repro-

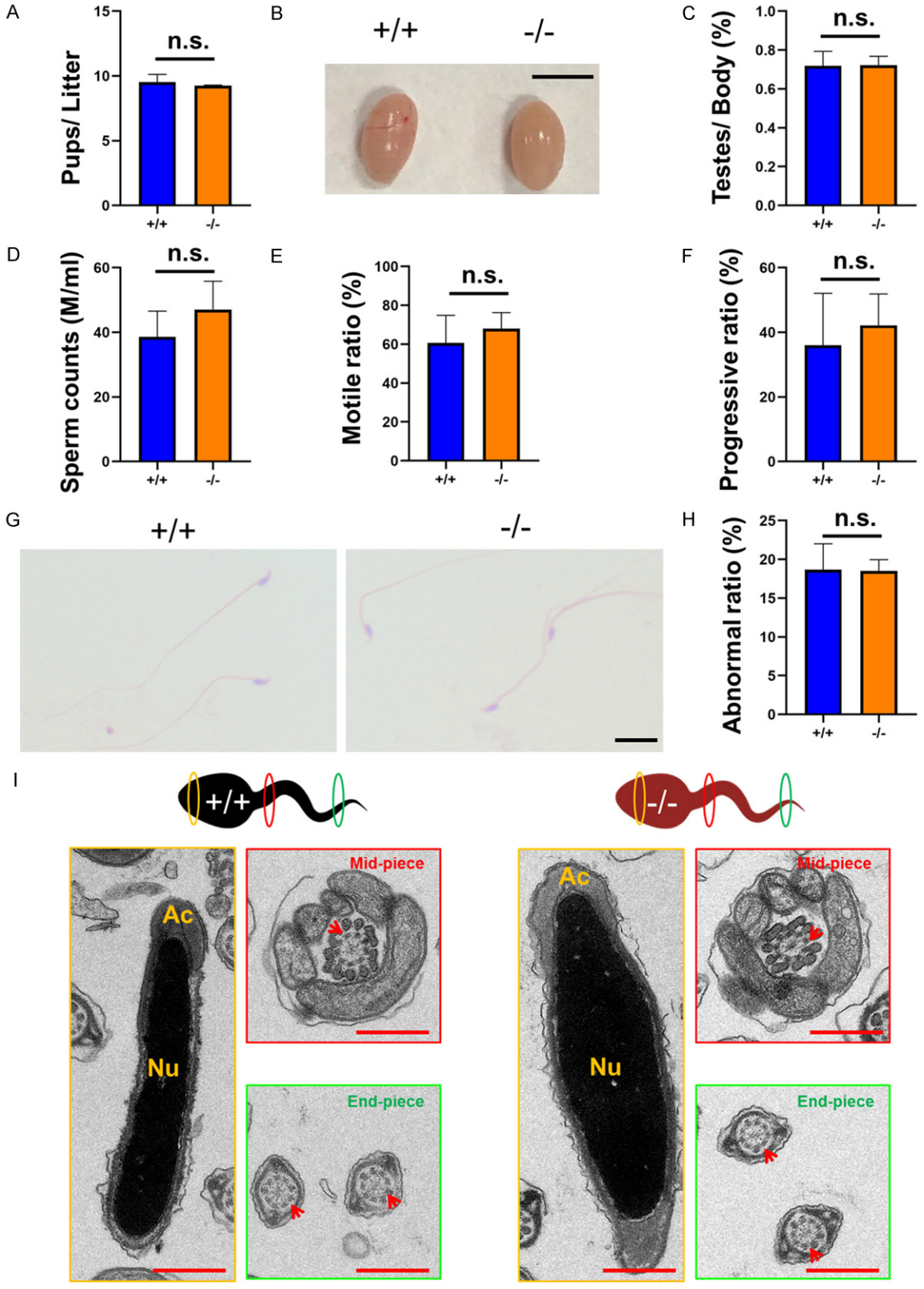


Figure 2. Fertility of *Asb15*-KO mice. (A) Fertility test for *Asb15*-KO and WT male mice. (B) Testes from *Asb15*-KO and WT mice. Scale bar =5 mm. (C) Testis/body weight ratio, (D) sperm counts, (E) sperm motility, and (F) progressive ratio for *Asb15*-KO and WT mice using CASA. M, million. (G) Sperm in the cauda epididymis and (H) quantification

of sperm in *Asb15*-KO and WT mice using H&E staining. Scale bar =20 μm. (I) Sperm in the cauda epididymis of *Asb15*-KO and WT mice shown by TEM. Arrows show the axonemes that have a 9+2 microtubule arrangement. Scale bar =500 nm. Ac, Acrosome; Nu, Nucleus; ns, not significant. For (A, C, D, E, F, H) n=3, P>0.05.

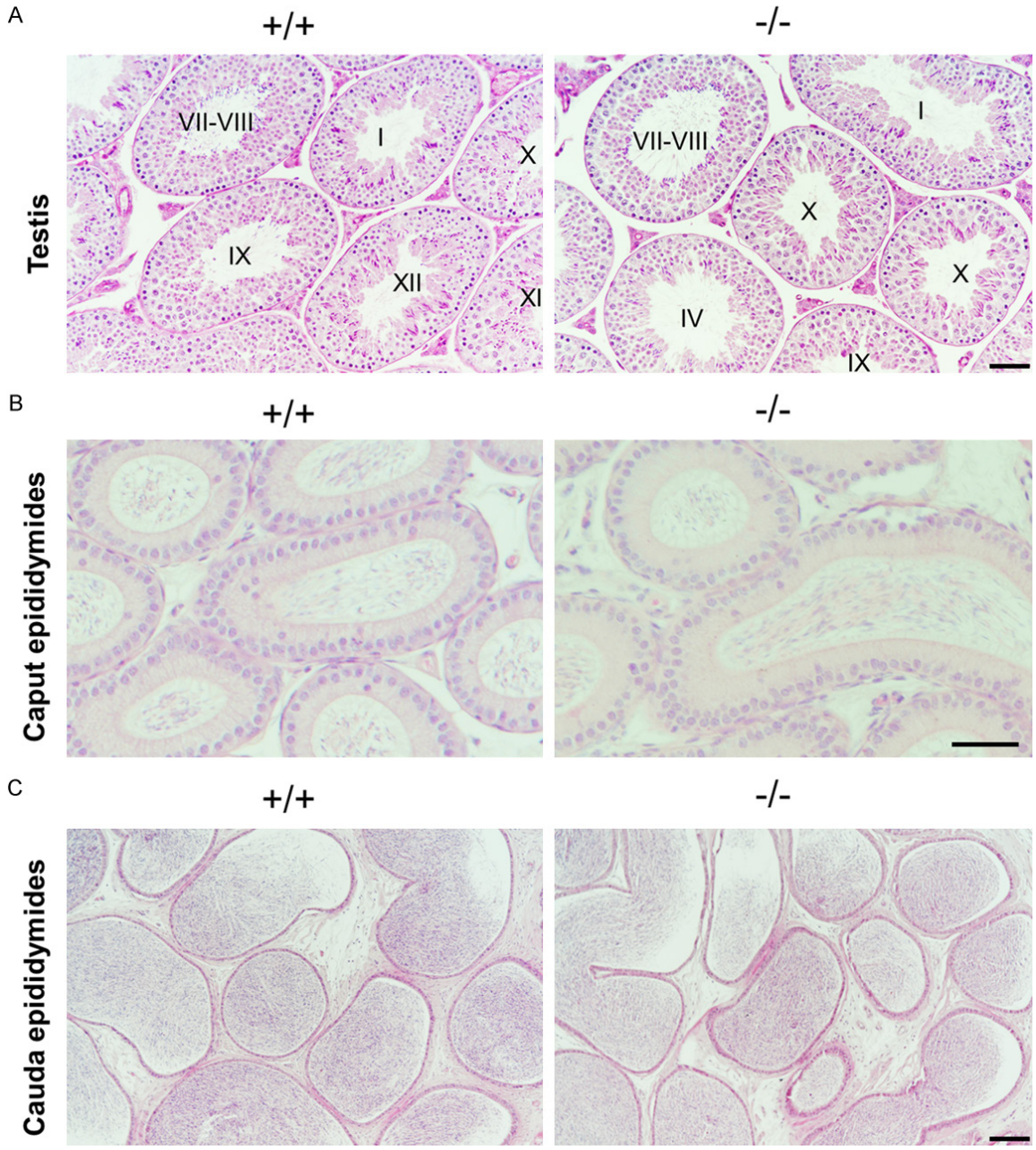


Figure 3. Histology of the testes and epididymides. A. Staining of sections of the testis from *Asb15*-KO and WT mice using PAS. B. Stained sections of the caput epididymides from *Asb15*-KO and WT mice using H&E. C. Stained sections of the cauda epididymides from *Asb15*-KO mice and WT using H&E. Scale bar =50 μm.

duction; hence, it has been conserved in evolution [26], and genes that are differentially expressed in the testes appear to be essential for spermatogenesis [27-29]. However, other studies have demonstrated that some testis-enriched genes have no effect on male fertility [30, 31].

In this study, we found that *Asb15* was expressed primarily in mouse testis, suggest-

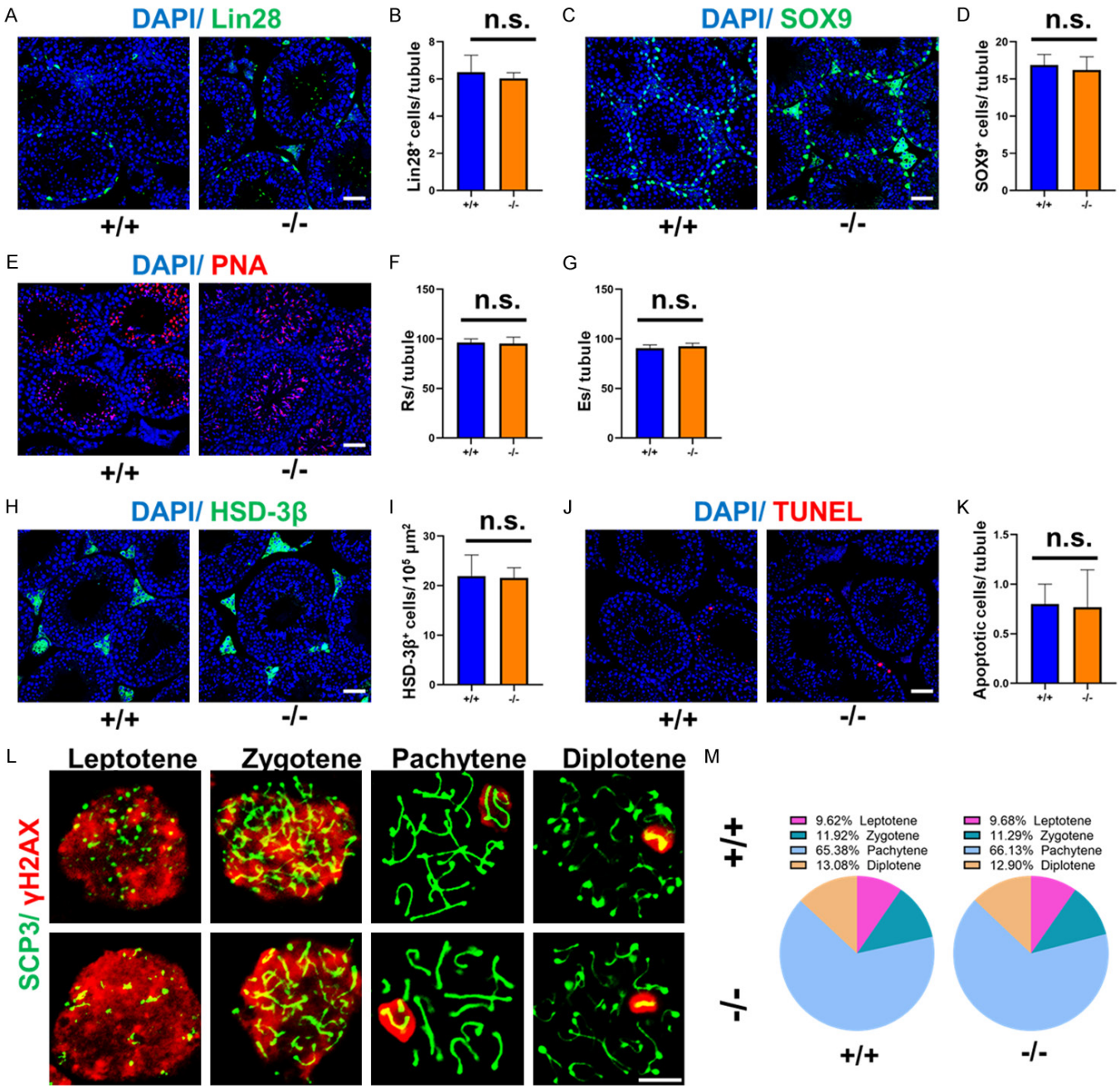
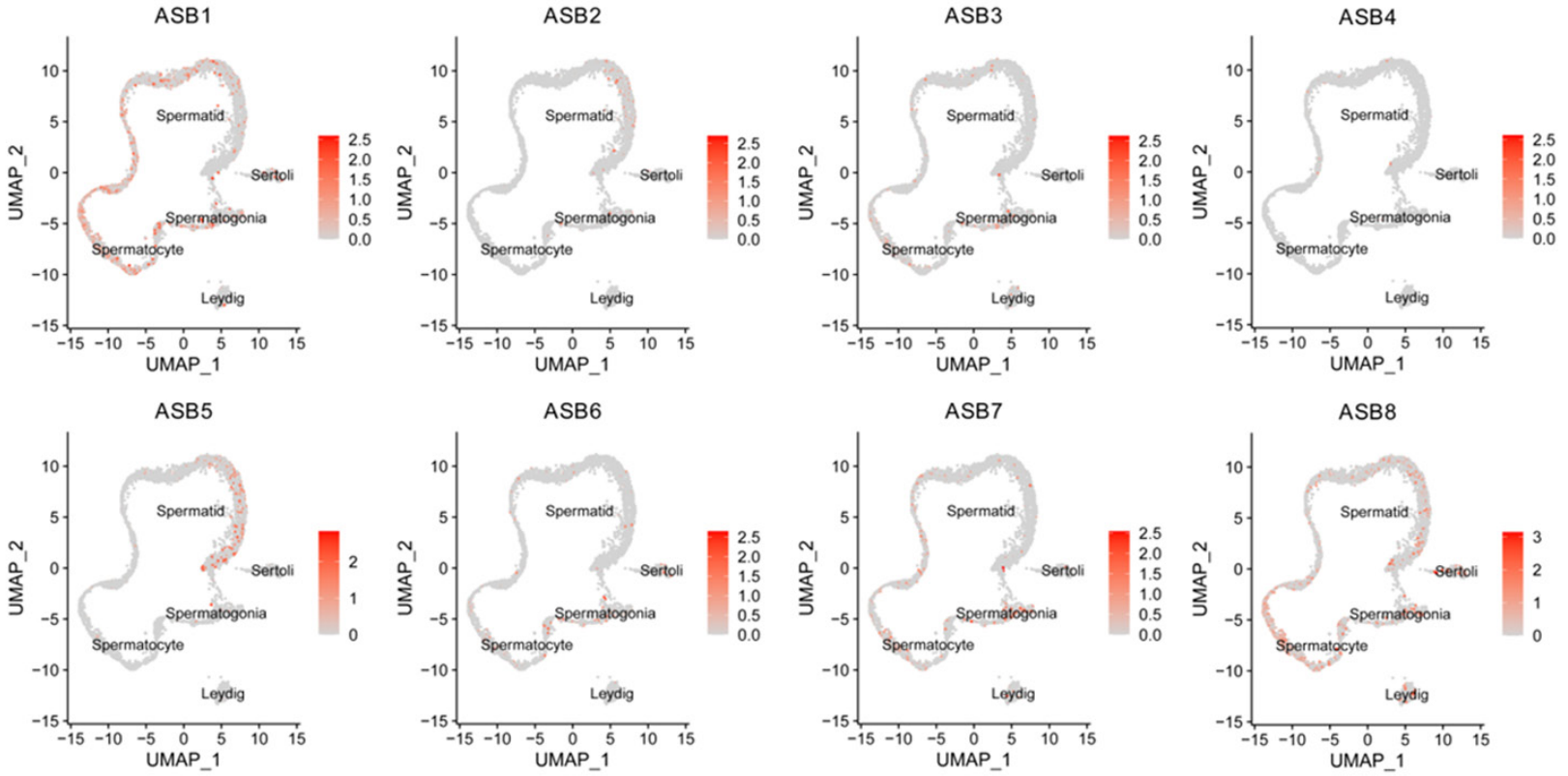

A
DAPI/ Lin28
+/+
-/-
B
n.s.
Lin28+ cells/ tubule
C
DAPI/ SOX9
D
n.s.
SOX9+ cells/ tubule
E
DAPI/ PNA
F
n.s.
Rs/ tubule
G
n.s.
Es/ tubule
H
DAPI/ HSD-3β
I
n.s.
HSD-3β+ cells/ 10⁵ μm²
J
DAPI/ TUNEL
K
n.s.
Apoptotic cells/ tubule
L
Leptotene
Zygotene
Pachytene
Diplotene
SCP3/ γH2AX
M
9.62% Leptotene
11.92% Zygotene
65.38% Pachytene
13.08% Diplotene
9.68% Leptotene
11.29% Zygotene
66.13% Pachytene
12.90% Diplotene

Figure 4. Spermatogenesis in *Asb15*-KO mice. (A) Immunostaining and (B) quantification of Lin28 in the testes from *Asb15*-KO and WT mice. (C) Immunostaining and (D) quantification of SOX9 in the testes from *Asb15*-KO and WT mice. (E) Immunostaining of PNA, (F) quantification of round spermatids (Rs), and (G) quantification of elongating/elongated (Es) spermatids in testes from *Asb15*-KO and WT mice. (H) Immunostaining and (I) quantification of HSD-3β in the testes from *Asb15*-KO and WT mice. (J) Apoptotic cells and (K) quantification of apoptotic cells in the testes from *Asb15*-KO and WT mice by TUNEL assay. (L) Co-immunostaining for γH2AX and SCP3 in the chromosome spread for spermatocytes from *Asb15*-KO and WT mice. (M) Quantification of (L). For (A, C, E, H, and J), scale bar =50 μm; For (L), scale bar =5 μm, For (B, D, F, G, I, and K), n=3, P>0.05. DAPI, 4',6-Diamidino-2-Phenylindole; γH2AX, H2A.X Variant Histone; HSD-3β, Hydroxy-Delta-5-Steroid Dehydrogenase, 3 Beta; Lin28, Lin-28 Homolog; PNA, Peanut Agglutinin; SCP3, Synaptonemal Complex Protein 3; SOX9, SRY-Box 9; TUNEL, Terminal Deoxynucleotidyl Transferase-dUTP Nick-End Labeling. ns, not significant.



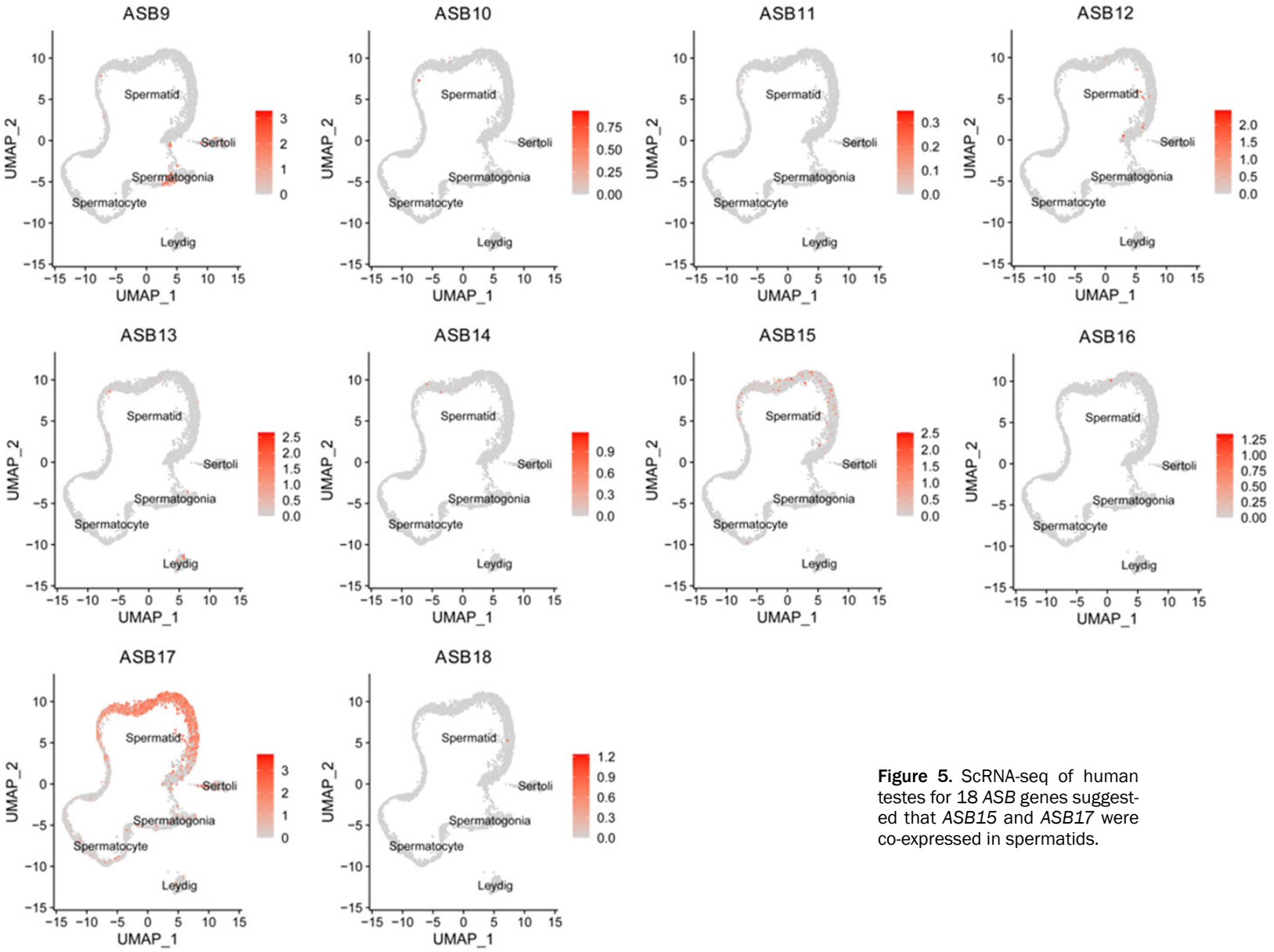


Figure 5. ScRNA-seq of human testes for 18 *ASB* genes suggested that *ASB15* and *ASB17* were co-expressed in spermatids.

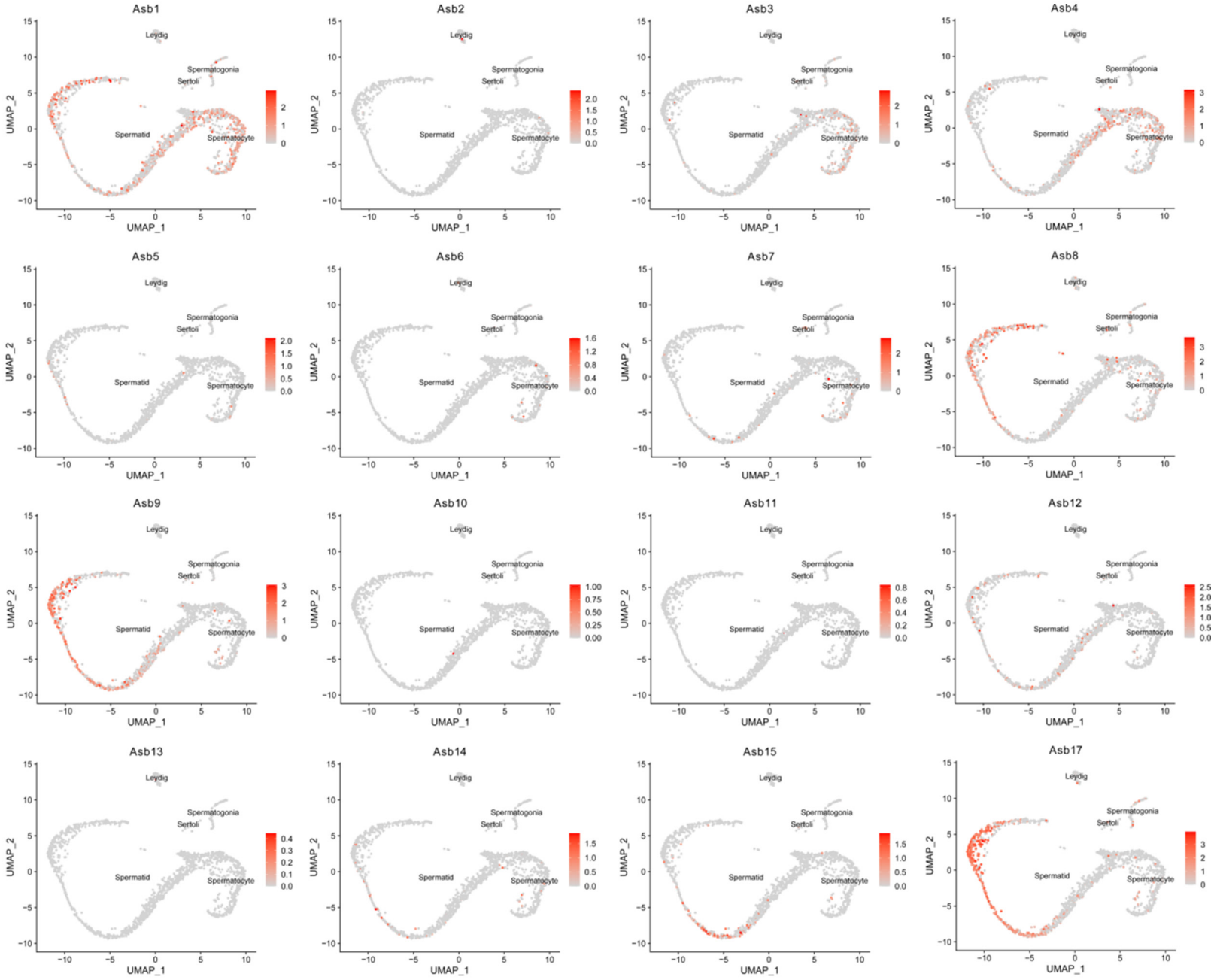

Asb1
Asb2
Asb3
Asb4
Asb5
Asb6
Asb7
Asb8
Asb9
Asb10
Asb11
Asb12
Asb13
Asb14
Asb15
Asb17
Leydig
Spermatogonia
Sertoli
Spermatid
Spermatocyte
UMAP_1
UMAP_2

Figure 6. ScRNA-seq of mouse testes for 16 *Asb* genes suggested that *Asb15* and *Asb17* were co-expressed in spermatids. *Asb16* and *Asb18* were not detected.

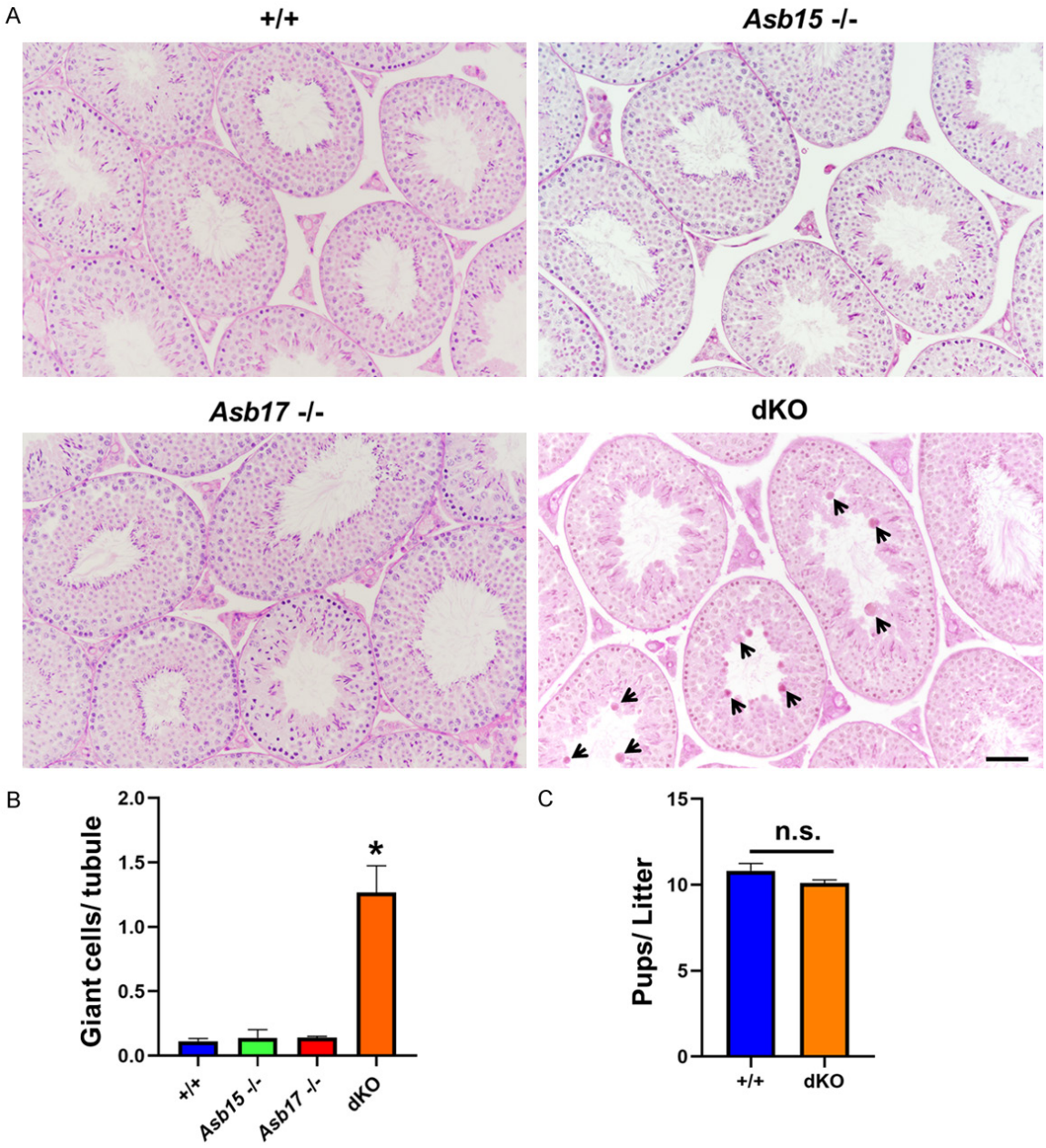


Figure 7. Fertility of *Asb15/17*-dKO mice. (A) Staining of sections of the testis from *Asb15*-KO, *Asb17*-KO, *Asb15/17*-dKO, and WT mice using PAS. Scale bar =50 μm. (B) Quantification of giant cells in (A). n=3, P<0.05 (*). (C) Fertility test for *Asb15/17*-dKO and WT mice. n=3, P>0.05. dKO, Double Knockout; ns, not significant.

ing that it plays an important role in spermatogenesis and male fertility. Therefore, we generated an *Asb15*-KO mouse line using CRISPR/Cas9 technology to determine the role of *Asb15* in mice. However, the mutant revealed no histological changes nor any effects on fertility, indicating that *Asb15* was dispensable for spermatogenesis. Data on scRNA-seq in human and mouse testes showed that *Asb15* and *Asb17* were co-expressed in spermatids, suggesting that expression of these two genes could result in compensation for the loss of the

other gene in the testes. We created a double mutant mouse lacking both genes to determine whether *Asb17* compensated for *Asb15*. However, adult *Asb15/17*-dKO mice had normal fertility, indicating that *Asb15* and *Asb17* are dispensable for male reproduction. Interestingly, the number of multinuclear giant cells was significantly greater in *Asb15/17*-dKO mice, indicating that the double KO strain might have a minor effect on spermatogenesis with no effect on male fertility. Our study provides genetic and phenotypic information that can prevent redundant research by other scientists, as well as information for further studies on the genetics of fertility in humans.

In summary, we identified *Asb15* as a gene with high levels of expression in the testis and generated *Asb15*-KO and *Asb15/17*-dKO mice. These mutants had normal spermatogenesis and normal fertility, indicating that *Asb15* and *Asb17* are not necessary for male fertility.

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Disclosure of conflict of interest

None.

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References

[1] Piessevaux J, Lavens D, Peelman F and Tavernier J. The many faces of the SOCS box. Cytokine Growth Factor Rev 2008; 19: 371-381.

[2] Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM and Hilton DJ. The SOCS box: a tale of destruction and degradation. Trends Biochem Sci 2002; 27: 235-241.

[3] Chiba T, Yao J, Higami Y, Shimokawa I, Hosokawa M and Higuchi K. Identification of differentially expressed genes in senescence-accelerated mouse testes by suppression subtractive hybridization analysis. Mamm Genome 2007; 18: 105-112.

[4] Kim SK, Rhim SY, Lee MR, Kim JS, Kim HJ, Lee DR and Kim KS. Stage-specific expression of ankyrin and SOCS box protein-4 (Asb-4) during spermatogenesis. Mol Cells 2008; 25: 317-321.

[5] Lee MR, Kim SK, Kim JS, Rhim SY and Kim KS. Expression of murine Asb-9 during mouse spermatogenesis. Mol Cells 2008; 26: 621-624.

[6] Kile BT, Metcalf D, Mifsud S, DiRago L, Nicola NA, Hilton DJ and Alexander WS. Functional analysis of Asb-1 using genetic modification in mice. Mol Cell Biol 2001; 21: 6189-6197.

[7] Shen C, Xu J, Zhou Q, Lin M, Lv J, Zhang X, Wu Y, Chen X, Yu J, Huang X and Zheng B. E3 ubiquitin ligase ASB17 is required for spermiation in mice. Transl Androl Urol 2021; 10: 4320-4332.

[8] Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcalf D and Nicola NA. Twenty proteins containing a C-terminal SOCS box form five structural classes. Proc Natl Acad Sci U S A 1998; 95: 114-119.

[9] McDaneld TG and Spurlock DM. Ankyrin repeat and suppressor of cytokine signaling (SOCS) box-containing protein (ASB) 15 alters differentiation of mouse C2C12 myoblasts and phosphorylation of mitogen-activated protein kinase and Akt. J Anim Sci 2008; 86: 2897-2902.

[10] Wu S, Yan M, Ge R and Cheng CY. Crosstalk between Sertoli and germ cells in male fertility. Trends Mol Med 2020; 26: 215-231.

[11] Zheng B, Zhao D, Zhang P, Shen C, Guo Y, Zhou T, Guo X, Zhou Z and Sha J. Quantitative proteomics reveals the essential roles of stromal interaction molecule 1 (STIM1) in the testicular cord formation in mouse testis. Mol Cell Proteomics 2015; 14: 2682-2691.

[12] Zheng B, Zhou Q, Guo Y, Shao B, Zhou T, Wang L, Zhou Z, Sha J, Guo X and Huang X. Establishment of a proteomic profile associated with gonocyte and spermatogonial stem cell matu-

ration and differentiation in neonatal mice. Proteomics 2014; 14: 274-285.
[13] Larson EL, Kopania EEK and Good JM. Spermatogenesis and the evolution of mammalian sex chromosomes. Trends Genet 2018; 34: 722-732.
[14] Zhang R, Xu J, Shen C, Zhang X, Li S, Lv J, Xu D, Huang X, Zheng B, Liu M and Wu Y. Testis-enriched Asb12 is not required for spermatogenesis and fertility in mice. Transl Androl Urol 2022; 11: 168-178.
[15] Wu Y, Wang T, Zhao Z, Liu S, Shen C, Li H, Liu M, Zheng B, Yu J and Huang X. Retinoic acid induced protein 14 (Rai14) is dispensable for mouse spermatogenesis. PeerJ 2021; 9: e10847.
[16] Zhang Y, Guo R, Cui Y, Zhu Z, Zhang Y, Wu H, Zheng B, Yue Q, Bai S, Zeng W, Guo X, Zhou Z, Shen B, Zheng K, Liu M, Ye L and Sha J. An essential role for PNLDC1 in piRNA 3' end trimming and male fertility in mice. Cell Res 2017; 27: 1392-1396.
[17] Wang X, Xie W, Yao Y, Zhu Y, Zhou J, Cui Y, Guo X, Yuan Y, Zhou Z and Liu M. The heat shock protein family gene Hspa1l in male mice is dispensable for fertility. PeerJ 2020; 8: e8702.
[18] Zheng B, Yu J, Guo Y, Gao T, Shen C, Zhang X, Li H and Huang X. Cellular nucleic acid-binding protein is vital to testis development and spermatogenesis in mice. Reproduction 2018; 156: 59-69.
[19] Shen C, Zhang K, Yu J, Guo Y, Gao T, Liu Y, Zhang X, Chen X, Yu Y, Cheng H, Zheng A, Li H, Huang X, Ding X and Zheng B. Stromal interaction molecule 1 is required for neonatal testicular development in mice. Biochem Biophys Res Commun 2018; 504: 909-915.
[20] Gao T, Lin M, Wu Y, Li K, Liu C, Zhou Q, Shen C, Zheng B and Huang X. Transferrin receptor (TFRC) is essential for meiotic progression during mouse spermatogenesis. Zygote 2021; 29: 169-175.
[21] Hua R, Wei H, Liu C, Zhang Y, Liu S, Guo Y, Cui Y, Zhang X, Guo X, Li W and Liu M. FBXO47 regulates telomere-inner nuclear envelope integration by stabilizing TRF2 during meiosis. Nucleic Acids Res 2019; 47: 11755-11770.
[22] Lin M, Lv J, Zhao D, Liu S, Xu J, Wu Y, Wang F, Zhang J, Zheng B, Shen C, Guan X, Yu J and Huang X. MRNIP is essential for meiotic progression and spermatogenesis in mice. Biochem Biophys Res Commun 2021; 550: 127-133.
[23] Zhao L, Yao C, Xing X, Jing T, Li P, Zhu Z, Yang C, Zhai J, Tian R, Chen H, Luo J, Liu N, Deng Z, Lin X, Li N, Fang J, Sun J, Wang C, Zhou Z and Li Z. Single-cell analysis of developing and azoospermia human testicles reveals central role of Sertoli cells. Nat Commun 2020; 11: 5683.
[24] Han X, Wang R, Zhou Y, Fei L, Sun H, Lai S, Saadatpour A, Zhou Z, Chen H, Ye F, Huang D, Xu Y, Huang W, Jiang M, Jiang X, Mao J, Chen Y, Lu C, Xie J, Fang Q, Wang Y, Yue R, Li T, Huang H, Orkin SH, Yuan GC, Chen M and Guo G. Mapping the mouse cell atlas by microwell-seq. Cell 2018; 172: 1091-1107 e1017.
[25] Li Y, Mi P, Wu J, Tang Y, Liu X, Cheng J, Huang Y, Qin W, Cheng CY and Sun F. High throughput scRNA-Seq provides insights into Leydig cell senescence induced by experimental autoimmune Orchitis: a prominent role of interstitial fibrosis and complement activation. Front Immunol 2021; 12: 771373.
[26] Griswold MD. Spermatogenesis: the commitment to meiosis. Physiol Rev 2016; 96: 1-17.
[27] Ikawa M, Wada I, Kominami K, Watanabe D, Toshimori K, Nishimune Y and Okabe M. The putative chaperone calmegin is required for sperm fertility. Nature 1997; 387: 607-611.
[28] Greenbaum MP, Yan W, Wu MH, Lin YN, Agno JE, Sharma M, Braun RE, Rajkovic A and Matzuk MM. TEX14 is essential for intercellular bridges and fertility in male mice. Proc Natl Acad Sci U S A 2006; 103: 4982-4987.
[29] Fujihara Y, Noda T, Kobayashi K, Oji A, Kobayashi S, Matsumura T, Larasati T, Oura S, Kojima-Kita K, Yu Z, Matzuk MM and Ikawa M. Identification of multiple male reproductive tract-specific proteins that regulate sperm migration through the oviduct in mice. Proc Natl Acad Sci U S A 2019; 116: 18498-18506.
[30] Lu Y, Oura S, Matsumura T, Oji A, Sakurai N, Fujihara Y, Shimada K, Miyata H, Tobita T, Noda T, Castaneda JM, Kiyozumi D, Zhang Q, Larasati T, Young SAM, Kodani M, Huddleston CA, Robertson MJ, Coarfa C, Isotani A, Aitken RJ, Okabe M, Matzuk MM, Garcia TX and Ikawa M. CRISPR/Cas9-mediated genome editing reveals 30 testis-enriched genes dispensable for male fertility in micedagger. Biol Reprod 2019; 101: 501-511.
[31] Park S, Shimada K, Fujihara Y, Xu Z, Shimada K, Larasati T, Pratiwi P, Matzuk RM, Devlin DJ, Yu Z, Garcia TX, Matzuk MM and Ikawa M. CRISPR/Cas9-mediated genome-edited mice reveal 10 testis-enriched genes are dispensable for male fecundity. Biol Reprod 2020; 103: 195-204.

Table S1. Antibodies used in the study

Antigen	Source	Company	Application	Dilution	RRID
Lin28	Rabbit	Abcam	IF	1:400	AB_776033
SOX9	Rabbit	Millipore	IF	1:200	AB_2239761
HSD-3β	Mouse	Santa Cruz	IF	1:500	AB_2721058
SCP3	Rabbit	Abcam	IF	1:200	AB_301639
γH2AX	Mouse	Abcam	IF	1:500	AB_470861

Lin28, Lin-28 Homolog; SOX9, SRY-Box 9; HSD-3β, Hydroxy-Delta-5-Steroid Dehydrogenase, 3 Beta; SCP3, Synaptonemal Complex Protein 3; γH2AX, H2A.X Variant Histone.

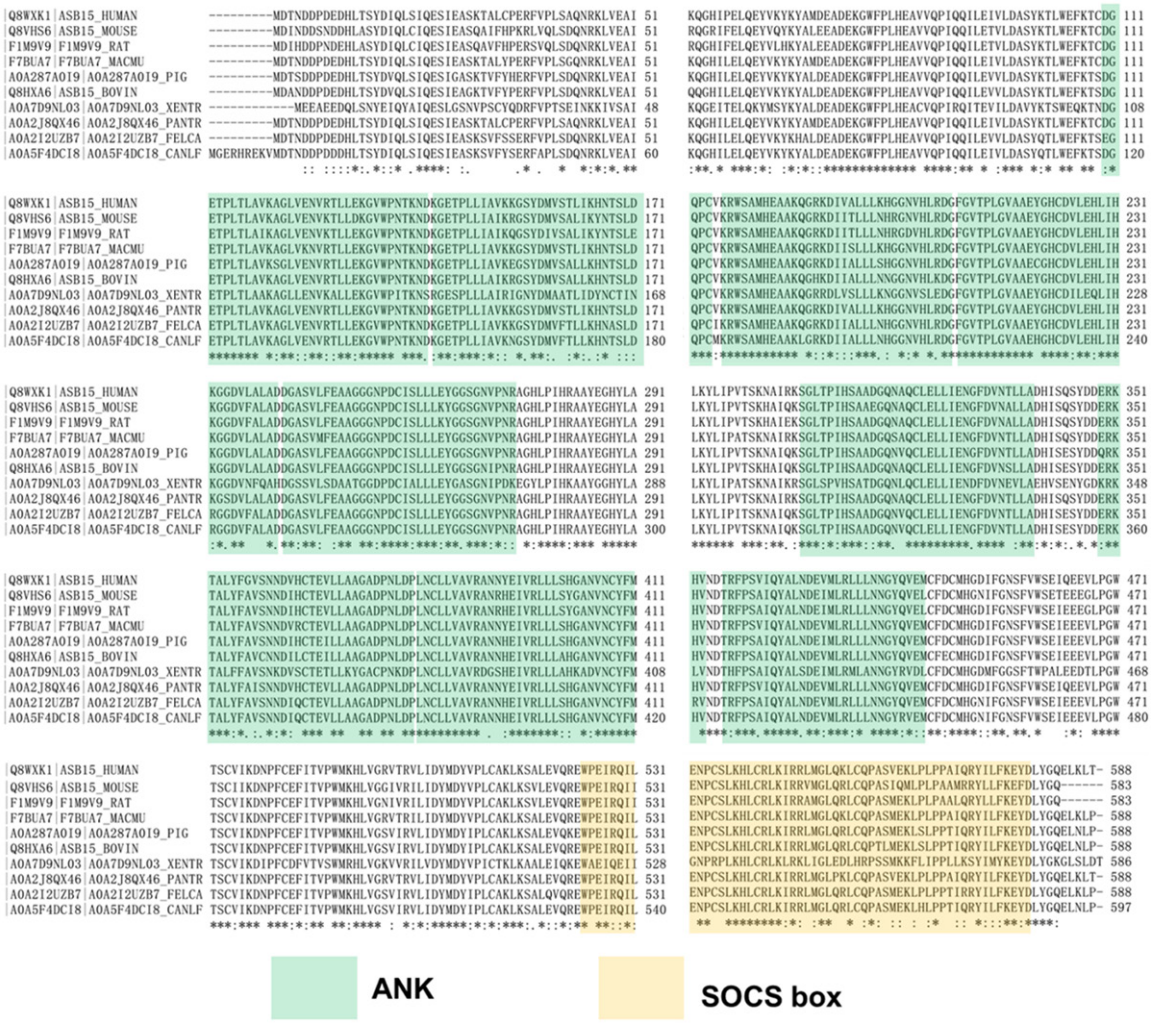

Figure S1. The result of sequence alignment of *Asb15* proteins among species suggested that *Asb15* is an evolutionarily conserved gene among species.

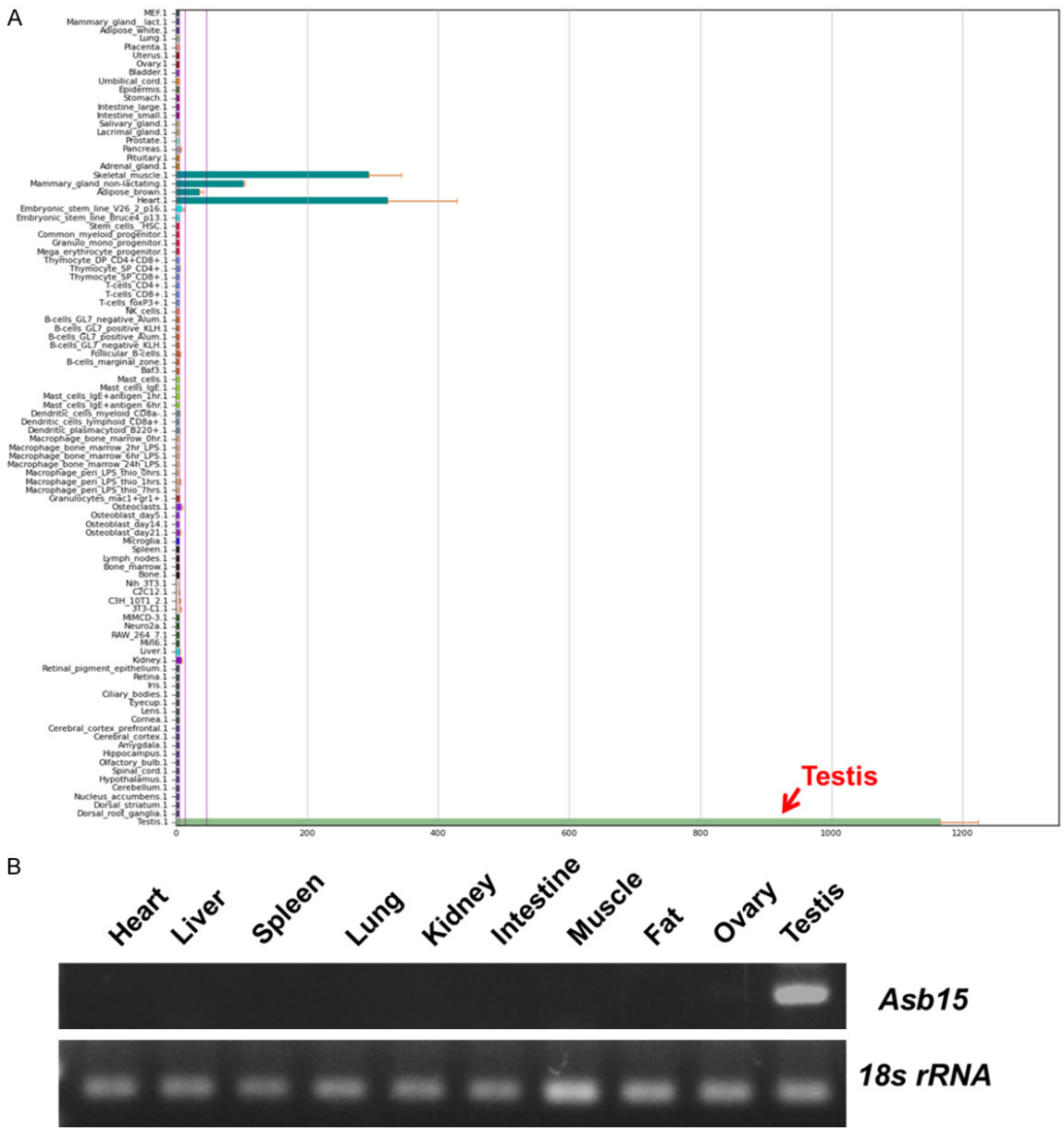

Figure S2. Expression profiling of *Asb15* in mice. A. Bioinformatic analysis of *Asb15* expression in multiple tissues and cells of mice. The data are acquired from the BioGPS database based on microarray analysis (http://biogps.org). B. Reverse transcription PCR analysis of *Asb15* in multiple murine tissues. *Asb15*, ankyrin repeat and SOCS box protein 15.