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WDFY1, a WD40 repeat protein, is not essential for spermatogenesis and male fertility in mice

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ABSTRACT

The mouse WD repeat and FYVE domain containing 1 (*Wdfy1*) gene is located in chromosome 1qC4 and spans over 73.7 kilobases. It encodes a protein of 410-amino acid protein that shares 97.8% amino acid sequence identity with the human WDFY1 protein. However, the expression pattern of WDFY1 in reproductive organs and its function in male fertility remain unknown. In this study, we generated transgenic mice expressing FLAG-Wdfy1-mCherry cDNA driven by the *Wdfy1* promoter to clarify the expression of WDFY1. The results showed that WDFY1 is highly expressed in mouse testes and located in the cytoplasm of late pachytene spermatocytes to elongated spermatids. Interestingly, the global *Wdfy1* knockout (KO) male mice displayed normal growth, development, and fertility. Further histological analysis of *Wdfy1* knockout mouse testes revealed that all spermatogenic cells are present in *Wdfy1* KO seminiferous tubules. Together, our data demonstrate that WDFY1 is dispensable for mouse spermatogenesis and male fertility.

1. Introduction

The WD40 protein family is a general term that contains a series of proteins characterized by the presence of multiple WD40 domains [1]. These WD40 domains often comprise four or more repeats of 40–60 amino acid stretches that terminate in a tryptophan-aspartic acid dipeptide [1]. The WD40 proteins are crucially involved in different cellular processes, including signal transduction, gene transcription, vesicle fusion, and spermatogenesis [2,3]. It's worth noting that a large number of WD40 proteins were highly or specifically expressed in testes and had distinct and tissue-specific functions in testicular development and spermatogenesis [2]. For example, DDB1-CUL4-associated factors (DCAFs) are a subset of WD40 family proteins, which have much more specific and complex expression patterns during testicular development and spermatogenesis [2]. It can function as the substrate receptor of Cullin4-RING-based E3 ubiquitin ligase complexes to recruit various proteins for ubiquitination, which is an essential process of spermatogenesis [4]. Moreover, WDC146, a novel WD40 repeat protein expressed preferentially in the testis in a stage-specific manner, has been reported to play a crucial role in spermatogenesis [5]. The DMX-Like 2 (DMXL2) gene encodes a protein with multiple WD40 domains, which young male knockout mice produced fewer sperm counts and had poorer sperm motility than wild-type mice at the beginning of their reproductive life [6]. In addition, several WD40 family genes, such as *β-TrCP*, *Katnb1*, *Ddb2*, *Brwd1*, *Dcaf17*, and *Lis1*, have also played critical roles during testis

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development, spermatogenesis, and fertility [7–12]. One of the WD40 family genes, *Wdfy1*, has been reported to enhance the interaction between TRIF and TLR3/4 and play a critical role in Tlr3-mediated immune responses *in vivo* [13,14]. Interestingly, in our previous study, we found that *Wdfy1*, a WD40 family gene, could bind with UHRF1 and its expression level was significantly reduced in *Uhrf1* cKO testes (*Stra8-Cre*; $Uhrf1^{flox/Del}$) [15]. Although UHRF1 had widely functioned in male germ cell development and spermatogenesis, the specific function of WDFY1 in spermatogenesis and male fertility remains unclear.

To investigate the role of WDFY1 in spermatogenesis, we first generated *Wdfy1* transgenic mice tagged by N-terminal FLAG and C-terminal mCherry for identifying its expression pattern. We then analyzed the phenotype of the *Wdfy1* gene knocked out male mice to explore the physiological function of WDFY1 in spermatogenesis and male fertility. Surprisingly, although WDFY1 was found to express in testes predominantly, the *Wdfy1* knocked out male mice were fertile without any abnormalities in spermatogenesis. As far as we know, this is the first report of testis highly-expressed *Wdfy1* gene for its *in vivo* functional analysis in mouse fertility and spermatogenesis. Collectively, our data revealed that *Wdfy1* is not essential for mouse spermatogenesis and male fertility.

2. Materials and methods

2.1. Mice

Wdfy1 heterogeneous mice with a C57BL/6N genetic background were obtained from Dr. Yu Liu's laboratory at State Key Laboratory of Virology, Frontier Science Center for Immunology and Metabolism, College of Life Sciences, Wuhan University. The heterogeneous mice were intercrossed to get homozygous mice (KO). Then the homozygous mice were verified by PCR analysis and TA clone sequencing. To test fertility, three pairs of wild-type (WT) female mice were co-caged with WT and *Wdfy1* KO male mice to determine the number and size of litters.

To construct FLAG-*Wdfy1*-mCherry plasmid, *Wdfy1* cDNA was amplified from total RNA isolated from *Wdfy1* mouse testis and added 2× FLAG tag at the 5′ end. The resultant DNA fragment was inserted into the pmCherry-N1 vector (Clontech), where the promoter was replaced by a 2.8 kb sequence upstream of the transcription start site. The success of the plasmid was confirmed by sequencing of DNA. To generate $Wdfy1^{FLAG/mCherry}$ transgenic mice, the above plasmid was injected into pronuclei of 1-cell zygotes for producing founders. The founders were examined by PCR analysis. All animal experiments were approved by the Institutional Animal Welfares and Ethics of Huazhong University of Science and Technology.

2.2. Western blotting

Proteins were extracted from various mouse tissues using RIPA buffer (CWBIO, Cat# 01408) supplemented with 1% 1 mM PMSF (Beyotime, Cat#ST505). Western blotting was performed as previously reported [15]. The primary antibodies against FLAG (Proteintech, Cat#20543-1-AP) and mCherry (Invitrogen, Cat# M11217) were used. The images were captured by using ChemiDoc XRS+ system (BIO-RAD).

2.3. RNA extraction and RT-qPCR

Total RNA was extracted from adult mouse tissues using TRIzol reagent (Invitrogen) and cDNA synthesis was performed by HiScript II 1st Strand cDNA Synthesis Kit with gDNA wiper (Vazyme) following the recommendations of the manufacturer. RT-qPCR was performed with HieffTMq PCR SYBR Green Master Mix (Yeasen) on the Light-Cycler@96 Real-Time PCR system (Roche) according to manufactures' instructions. For real-time PCR analysis, the quantification of fold change in *Wdfy1* expression was determined by the comparative C_T method. The primers designed for this study are enlisted in Supplementary Table 1.

2.4. Immunofluorescence

Testes were fixed in 4% paraformaldehyde (PFA) diluted with PBS overnight at 4 °C. Samples were then sequentially soaked in 5% and 20% sucrose in PBS at room temperature (RT) and embedded in the Tissue-Tek O.C.T compound (Sakura Finetek, 4583). 5 μm thick cryosections were cut on a Thermo Fisher Scientific CryoStar NX50 cryostat. For immunostaining, cryosections were washed with PBS and microwaved in 0.01 M sodium citrate buffer (pH = 6.0) for antigen retrieval. After cooling down to RT, sections were washed three times with PBS and blocked with buffer (containing 3% normal goat serum and 3% fetal bovine serum in 1% bovine serum albumin) for 30 min. Then, the sections were incubated with primary antibodies (anti-FLAG, Proteintech, Cat#20543-1-AP and anti-mCherry, Invitrogen, Cat#M11217) at 4 °C overnight and incubated with secondary antibody at RT for 1 h after washing with PBS three times. The sections were counterstained with DAPI, and images were obtained by fluorescence microscope.

2.5. Histology analysis

Mouse testes and epididymides were collected and fixed in Bouin's solution (Sigma Aldrich) at 4 °C overnight. Samples were washed in 70% ethanol until there was no noticeable yellow, then the samples were embedded in paraffin and sectioned at 5 μm thickness for periodic acid-Schiff (PAS) staining analyses.

2.6. Sperm migration analysis

WT females were superovulated by injecting with 8 IU pregnant mare serum gonadotropin (PMSG), followed by 8 IU human chorionic gonadotropin (hCG) after 48 h. Superovulated WT females were caged together with WT or *Wdfy1* KO males 12 h after hCG injection and checked vaginal plugs every 0.5 h. Uteri and oviducts of plugged females were excised and fixed 2 h after coitus. Samples were paraffined and sectioned to stain with hematoxylin and eosin (H&E). Sections containing utero-tubal junction (UTJ) were examined for the presence of sperm under a bright-field microscope system.

2.7. Statistical analysis

All data are presented as mean ± SEM unless otherwise noted in the figure legends. Statistical differences between datasets were assessed by one-way ANOVA or Student's *t*-test using the GraphPad Prism 9.0 software. The database support for bioinformatic analysis of expression pattern of *Wdfy1* in different types of spermatogenic cells was downloaded from NCBI's Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/gen/) data repository with the accession ID: GSE112393.

3. Results

3.1. WDFY1 is preferentially expressed in mouse testes

To investigate the expression pattern of WDFY1, we first performed western blot assays to examine the protein expression levels of WDFY1 by using commercial WDFY1 antibodies; however,

none of the commercial antibodies showed the correct band of WDFY1. We thus generated transgenic mice expressing FLAG-*Wdfy1*-mCherry cDNA driven by the *Wdfy1* promoter to identify the expression of WDFY1 (Fig. 1A and Fig. S1A and B). The western blot results in multiple tissues showed that WDFY1 is highly expressed in testes and weakly expressed in brains (Fig. 1B and C), consistent with the qPCR results (Fig. 1D). Further immunostaining revealed that WDFY1 (mCherry signals) is beginning to be expressed from the cytoplasm of pachytene spermatocytes in the seminiferous tubules of stage IX–X (Fig. 1E). With the progress of spermatogenesis, the expression of WDFY1 gradually increased and peaked in the cytoplasm of round spermatid and elongated spermatid, whereas no expression in pre-leptotene (PL), leptotene (L), zygotene (Z), and pachytene (P) of stage II–VIII (Fig. 1E). Consistent with these results, scRNA-seq analyses of the published data also showed WDFY1 highly expressed in spermatids (Fig. S1C and D), which verified the successful construction of the *Wdfy1* transgenic mice model again. Taken together, the above data show that WDFY1 is preferentially expressed in mouse testes and is mainly localized in the cytoplasm of spermatids.

3.2. *WDFY1 is not essential for spermatogenesis and male fertility*

To determine the physiological role of WDFY1 in spermatogenesis and male fertility, we obtained *Wdfy1* heterozygous mice [13] from Dr. Yu Liu's lab at Wuhan University. We then intercrossed the *Wdfy1* heterozygous mice to get *Wdfy1* homozygous mice (KO mice). The *Wdfy1* KO mice were verified by genotyping, direct sequencing analysis, and RT-qPCR assays (Fig. 2A and Fig. S2A and B). Unexpectedly, we found that the testis gross morphology and weights of *Wdfy1* KO mice were comparable with WT mice (Fig. 2B and C). Further histological analyses of testis and epididymis revealed no germ cell development and spermiogenesis defects in *Wdfy1* KO mice (Fig.2D and Fig. S2C). In addition, we found that the *Wdfy1* KO mice have normal sperm motility and the ability of sperm transportation compared with that of controls (Fig. S2D and E). The six-month fertility test results also proved that the average number of offspring per litter and the number of litters in *Wdfy1* KO males showed no discernible changes compared to WT males (Fig. 2E). Collectively, these results indicate that *Wdfy1* is not essential for spermatogenesis and male fertility in mice.

4. Discussion

In the current study, we aimed to know the expression profile of WDFY1 in testes, a WD40 protein-coding gene that belonged to WD40 family genes, and the role of WDFY1 in mouse spermatogenesis and male fertility. For this purpose, we generated a transgenic *Wdfy1*$^{FLAG/mCherry}$ mouse model using CRISPR/cas9 genome editing technology. We found that WDFY1 was highly expressed in testes, especially in the cytoplasm of round spermatids and elongated spermatids. Further, a fertility test was conducted on three different *Wdfy1* KO mice, and the results showed that *Wdfy1* KO male mice were fertile. In addition, the complete structure of seminiferous tubules and the presence of all types of germ cells were observed in the testis sections of *Wdfy1* KO mice. Thus, our

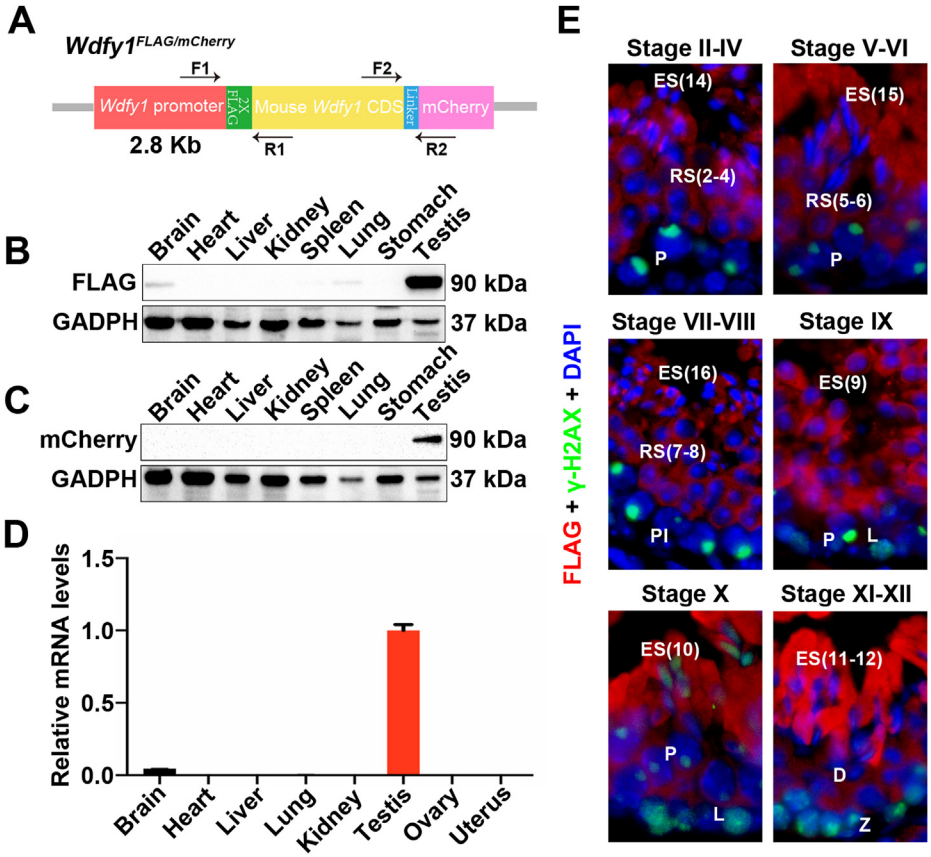

Fig. 1. The expression profile of WDFY1 in multiple tissues and different stages of spermatogenic cells. (**A**) Diagram of transgenic cassette expressing FLAG-*Wdfy1*-mCherry cDNA driven by the *Wdfy1* promoter. (**B**–**C**) Western blot analyses of WDFY1 protein levels in multiple tissues from adult male *Wdfy1*$^{FLAG/mCherry}$ mice are shown. GADPH served as a loading control. (**D**) RT-qPCR analyses of *Wdfy1* mRNA expression levels in multiple tissues from WT mice are shown. (**E**) Immunostainings of anti-mCherry and anti-γH2A.X antibodies in testis cryosections from adult male *Wdfy1*$^{FLAG/mCherry}$ mice are shown. Scale bars = 10 μm.

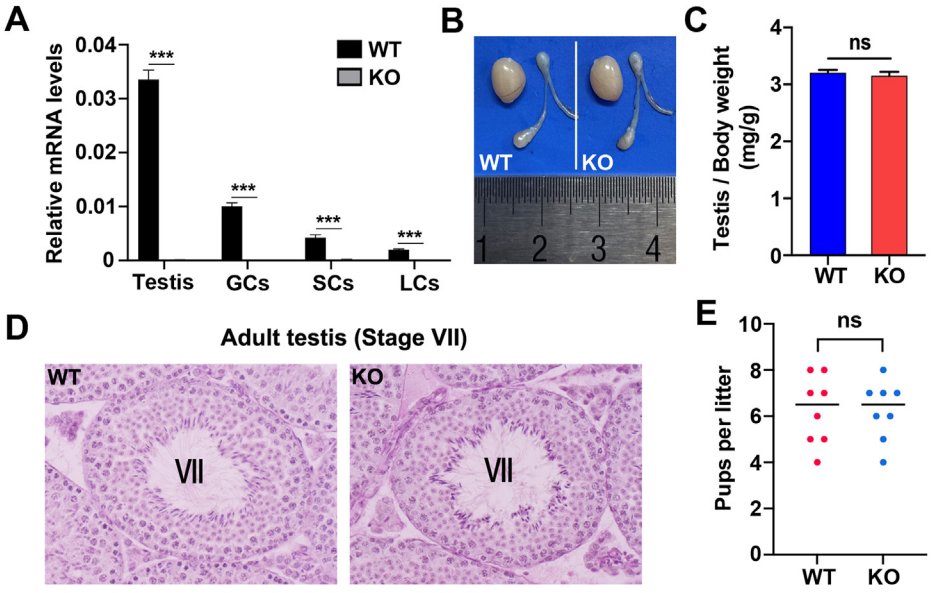

Fig. 2. WDFY1 deficiency in mice does not affect normal spermatogenesis and male fertility. (**A**) RT-qPCR analyses of *Wdfy1* mRNA levels in testes and different types of testicular cells from WT and *Wdfy1* KO mice are shown. GCs, Germ cells; SCs, Sertoli cells, and LCs, Leydig cells. Data are presented as mean ± SEM, $n = 3$. ***$P < 0.001$ by student's *t*-test. (**B**) Gross images of the testis and epididymis in adult WT and *Wdfy1* KO mice. (**C**) The histogram represents the ratio of testis to body weight in WT and *Wdfy1* KO mice. Data are presented as mean ± SEM, $n = 3$. ns, not significant. (**D**) The periodic acid-Schiff (PAS) staining of adult testes from WT and *Wdfy1* KO mice shows stage VII of testicular seminiferous tubules. Scale bars = 100 μm. (**E**) The scatter diagram shows the number of pups per litter produced from WT and *Wdfy1* KO male mice. Data are presented as mean ± SEM, $n = 3$. ns, not significant.

study suggested that *Wdfy1* is not vital for spermatogenesis and male fertility in mice. We hypothesized that the fertile phenotype of *Wdfy1* KO mice may be attributed to gene redundancy, a phenomenon that always is used to describe that inactivation of overlapping gene function could be compensated by other genes with similar functions [16]. For instance, *Ubc4*, *Kdm4d* and *Epab* are redundant in nature and highly expressed in mouse testes; their deficiency mouse model is fertile [17–20]. However, the inactivation of the *Wdfy1* gene in mice may be compensated by other unknown transcriptional factors, which need further identification. In this study, we reported that *Wdfy1* is dispensable for mouse spermatogenesis and male fertility, although it is highly expressed in testes. Till now, this is the first report regarding the *in vivo* function of *Wdfy1* in mouse testes. Therefore, this study will help researchers search for other WD40 family genes other than *Wdfy1* that play a crucial role in germ cell development and male fertility to fill the gaps in the reproductive field.

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Authors' contributions

S.Y. and W.Q. conceived and designed the study. C.L., M.X., S.G., Y.G., X.L., X.W., Y.W., S.F., J.Z. and Y, Z. performed all bench experiments. Y.L. generated the *Wdfy1* KO mouse model. C.L wrote the manuscript. S.Y. revised the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2022.01.084.

References

[1] E.J. Neer, C.J. Schmidt, R. Nambudripad, T.F. Smith, The ancient regulatory-protein family of WD-repeat proteins, Nature 371 (1994) 297–300.
[2] B.V. Mistry, M. Alanazi, H. Fitwi, O. Al-Harazi, M. Rajab, A. Altorbag, F. Almohanna, D. Colak, A.M. Assiri, Expression profiling of WD40 family genes including DDB1- and CUL4- associated factor (DCAF) genes in mice and human suggests important regulatory roles in testicular development and spermatogenesis, BMC Genom. 21 (2020) 602.
[3] T.F. Smith, C. Gaitatzes, K. Saxena, E.J. Neer, The WD repeat: a common architecture for diverse functions, Trends Biochem. Sci. 24 (1999) 181–185.
[4] X. Hou, W. Zhang, Z. Xiao, H. Gan, X. Lin, S. Liao, C. Han, Mining and characterization of ubiquitin E3 ligases expressed in the mouse testis, BMC Genom. 13 (2012) 495.
[5] S. Ito, A. Sakai, T. Nomura, Y. Miki, M. Ouchida, J. Sasaki, K. Shimizu, A novel WD40 repeat protein, WDC146, highly expressed during spermatogenesis in a stage-specific manner, Biochem. Biophys. Res. Commun. 280 (2001) 656–663.
[6] C. Gobé, M. Elzaiat, N. Meunier, M. André, E. Sellem, P. Congar, L. Jouneau, A. Allais-Bonnet, I. Naciri, B. Passet, et al., Dual role of DMXL2 in olfactory information transmission and the first wave of spermatogenesis, PLoS Genet. 15 (2019), e1007909.
[7] T. Nakagawa, T. Zhang, R. Kushi, S. Nakano, T. Endo, M. Nakagawa, N. Yanagihara, D. Zarkower, K. Nakayama, Regulation of mitosis-meiosis transition by the ubiquitin ligase beta-TrCP in male germ cells, Development 144 (2017) 4137–4147.
[8] K. Nayernia, F. Vauti, A. Meinhardt, C. Cadenas, S. Schweyer, B.I. Meyer,

I. Schwandt, K. Chowdhury, W. Engel, H.H. Arnold, Inactivation of a testis-specific Lis1 transcript in mice prevents spermatid differentiation and causes male infertility, J. Biol. Chem. 278 (2003) 48377–48385.
[9] A. Ali, B.V. Mistry, H.A. Ahmed, R. Abdulla, H.A. Amer, A. Prince, A.M. Alazami, F.S. Alkuraya, A. Assiri, Deletion of DDB1- and CUL4- associated factor-17 (Dcaf17) gene causes spermatogenesis defects and male infertility in mice, Sci. Rep. 8 (2018) 9202.
[10] L. O'Donnell, R.I. McLachlan, D.J. Merriner, M.K. O'Bryan, D. Jamsai, KATNB1 in the human testis and its genetic variants in fertile and oligoasthenoteratozoospermic infertile men, Andrology 2 (2014) 884–891.
[11] S. Pattabiraman, C. Baumann, D. Guisado, J.J. Eppig, J.C. Schimenti, R. De la Fuente, Mouse BRWD1 is critical for spermatid postmeiotic transcription and female meiotic chromosome stability, J. Cell Biol. 208 (2015) 53–69.
[12] T. Itoh, S. Iwashita, M.B. Cohen, D.K. Meyerholz, S. Linn, Ddb2 is a haploinsufficient tumor suppressor and controls spontaneous germ cell apoptosis, Hum. Mol. Genet. 16 (2007) 1578–1586.
[13] Y. Yang, Y.H. Hu, Y. Liu, Wdfy1 deficiency impairs Tlr3-mediated immune responses in vivo, Cell. Mol. Immunol. 17 (2020) 1014–1016.
[14] Y.H. Hu, Y. Zhang, L.Q. Jiang, S. Wang, C.Q. Lei, M.S. Sun, H.B. Shu, Y. Liu, WDFY1 mediates TLR3/4 signaling by recruiting TRIF, EMBO Rep. 16 (2015) 447–455.
[15] J. Dong, X. Wang, C. Cao, Y. Wen, A. Sakashita, S. Chen, J. Zhang, Y. Zhang, L. Zhou, M. Luo, et al., UHRF1 suppresses retrotransposons and cooperates with PRMT5 and PIWI proteins in male germ cells, Nat. Commun. 10 (2019) 4705.
[16] J. Láruson Á, S. Yeaman, K.E. Lotterhos, The importance of genetic redundancy in evolution, Trends Ecol. Evol. 35 (2020) 809–822.
[17] N. Iwamori, M. Zhao, M.L. Meistrich, M.M. Matzuk, The testis-enriched histone demethylase, KDM4D, regulates methylation of histone H3 lysine 9 during spermatogenesis in the mouse but is dispensable for fertility, Biol. Reprod. 84 (2011) 1225–1234.
[18] N. Bedard, P. Hingamp, Z. Pang, A. Karaplis, C. Morales, J. Trasler, D. Cyr, C. Gagnon, S.S. Wing, Mice lacking the UBC4-testis gene have a delay in postnatal testis development but normal spermatogenesis and fertility, Mol. Cell Biol. 25 (2005) 6346–6354.
[19] S. Ozturk, O. Guzeloglu-Kayisli, K.M. Lowther, M.D. Lalioti, D. Sakkas, E. Seli, Epab is dispensable for mouse spermatogenesis and male fertility, Mol. Reprod. Dev. 81 (2014) 390.
[20] Y. Lu, S. Oura, T. Matsumura, A. Oji, N. Sakurai, Y. Fujihara, K. Shimada, H. Miyata, T. Tobita, T. Noda, et al., CRISPR/Cas9-mediated genome editing reveals 30 testis-enriched genes dispensable for male fertility in mice, Biol. Reprod. 101 (2019) 501–511.