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Original Article

DCUN1D2 is insignificant for spermatogenesis and male fertility in mice

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Abstract: Objectives: Defective in cullin neddylation 1 domain containing 2 (DCUN1D2) belongs to the DCNLs family and can induce cell growth arrest and is also related to neddylation. While its potential role in spermatogenesis is hypothesized, the functional significance of DCUN1D2 in male germ cells remains undefined. Methods: To investigate this, germ cell and Sertoli cell-specific *Dcun1d2*-knockouts were generated. Sperm parameters were analyzed via computer-assisted sperm analysis (CASA), while histological and immunofluorescence staining were employed to evaluate spermatogenic progression and apoptosis. Results: Compared with the control group, *Dcun1d2* conditional knockout (cKO) mice exhibited no significant differences in histology, semen quality, sperm apoptosis or fertility tests. Conclusions: This study indicated that DCUN1D2 has no significant effect on mouse spermatogenesis or male fertility. These findings will help avoid redundant studies and provide new information for the study of human fertility genes.

Keywords: Dcun1d2, testis, spermatogenesis, male fertility

Introduction

Spermatogenesis occurs in the seminiferous tubules (STs) of the testes, maintaining continuous production of male gametes (sperm). This intricate process involves three primary phases: mitotic proliferation, meiotic division, and post-meiotic differentiation (spermiogenesis) [1]. Ubiquitination, a crucial post-translational modification (PTM) in eukaryotes, has demonstrated growing importance in regulating spermatogenesis [2-6]. Furthermore, neddylation, a ubiquitination-like modification, operates through divergent functional pathways and induces distinct biological outcomes compared to ubiquitination [7], also critically regulating meiotic recombination in spermatogenesis [8].

As the largest ubiquitin E3 enzyme family, cullin-RING ligases (CRLs) adapt interchangeable substrate receptors and require activation via cullin neddylation by NEDD8 [9, 10]. DCN-like proteins act as scaffold-like E3s to promote Cullin neddylation [11]. Notably, all DCNLs bind most Cullin subtypes, likely mediating neddylation of different Cullin subpopulations [12]. It has been reported that *Dcun1d1*$^{-/-}$ mice exhibit infertility due to spermatogenic defects, likely by promoting Cul3 neddylation to regulate spermatogenic CRL E3 activity [13].

Mammalian cells express five DCN-like (DCNL) proteins (DCUN1D1-DCUN1D5), with DCUN1D1, DCUN1D2 and DCUN1D3 exhibiting non-redundant roles in binding cullins (Culs) to regu-

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late their neddylation [12, 14]. Defective in cullin neddylation 1 domain containing 2 (DCUN1D2), a 30 kDa member of the DCNL family, contains two conserved domains: a C-terminal PONY domain essential for neddylation activation and a predicted N-terminal ubiquitin-associated (UBA) domain mediating direct ubiquitin interaction [15]. Reports demonstrate DCUN1D1 critically regulates neddylation as its E3 component, whereas DCUN1D3 acts as a tumor suppressor through DCUN1D1 neddylation antagonism [16]. Despite these advances, the functional significance of DCUN1D2 in murine spermatogenesis remains poorly characterized.

This study sought to investigate the functional contribution of *Dcun1d2* to murine spermatogenesis. Through generation and phenotypic analysis of *Dcun1d2* conditional knockout (cKO) mice, we demonstrated that *Dcun1d2* deficiency does not critically influence spermatogenic progression or male fertility parameters.

Materials and methods

Bioinformatic analysis

We obtained single-cell RNA sequencing (scRNA-seq) datasets of normal adult testicular tissues from two mammalian species through the GEO database, including mouse dataset GSM5563668 and human dataset GSE149512. The raw sequencing data were processed and analyzed using the Seurat computational framework. Following standard normalization procedures, cross-dataset integration was performed with Harmony algorithm to mitigate batch effects between species [17, 18]. For comparative analysis of conserved cellular features, gene expression patterns were systematically visualized through DotPlot representations of marker gene clusters and FeaturePlot projections of spatial distribution in reduced dimensional embeddings, as previously described [19].

Animals

Dcun1d2$^{flox/+}$ mice were purchased from Cyagen Biosciences Inc. (Suzhou, China), while *Amh/Ddx4*-Cre transgenic mice came from Nanjing Medical University's State Key Laboratory of Reproductive Medicine and Offspring Health. All mice were housed under specific pathogen-free conditions at the University's Animal Center, with experiments approved by its Animal Ethics and Welfare Committee (Approval No. 2402015). At the end of the study, mice were euthanized under carbon dioxide exposure followed by cervical dislocation.

Fertility test

In the fertility experiments, 8-week-old control and *Dcun1d2* mutant males were paired with wild-type (WT) females at a ratio of 1:2 for three months. The number of litters produced was recorded.

Polymerase chain reaction (PCR) genotyping

Dcun1d2-WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice were genotyped using PCR and agarose gel electrophoresis. The primers were: F1: 5'-TAAGTGATGAACAGCAGGGTTTTG-3'; R1: 5'-ATGTTTGCTCAGCCTGTTCTTAAC-3'.

Histology

The modified Davidson's fluid was employed to fix the testes and epididymides of adult *Dcun1d2*-WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice for 48 hours. The specimens were ethanol-dehydrated and paraffin-embedded. Sectioned into 5-μm slices, they were rehydrated and stained with periodic acid schiff (PAS) or hematoxylin and eosin (H&E) as described [20-22]. Finally, histological analysis was performed after microscopic imaging.

Computer-assisted sperm analysis (CASA)

Sperm from adult *Dcun1d2*-WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice cauda epididymides were suspended in human tubal fluid (In-VitroCare, Frederick, Maryland, USA). They were incubated at 37°C for 5 min, and assessed for quality using the Ceros™ II System (Hamilton Thorne, Beverly, Massachusetts, USA).

Immunofluorescence

Immunofluorescence staining followed established methods [23-25]. Sections underwent antigen retrieval in 10 mM citrate buffer (pH 6.0), blocking with 1% (w/v) bovine serum albumin (BSA) for 2 h, and overnight incubation at 4°C with primary antibodies: anti-3β-hydroxysteroid dehydrogenase (3β-HSD)

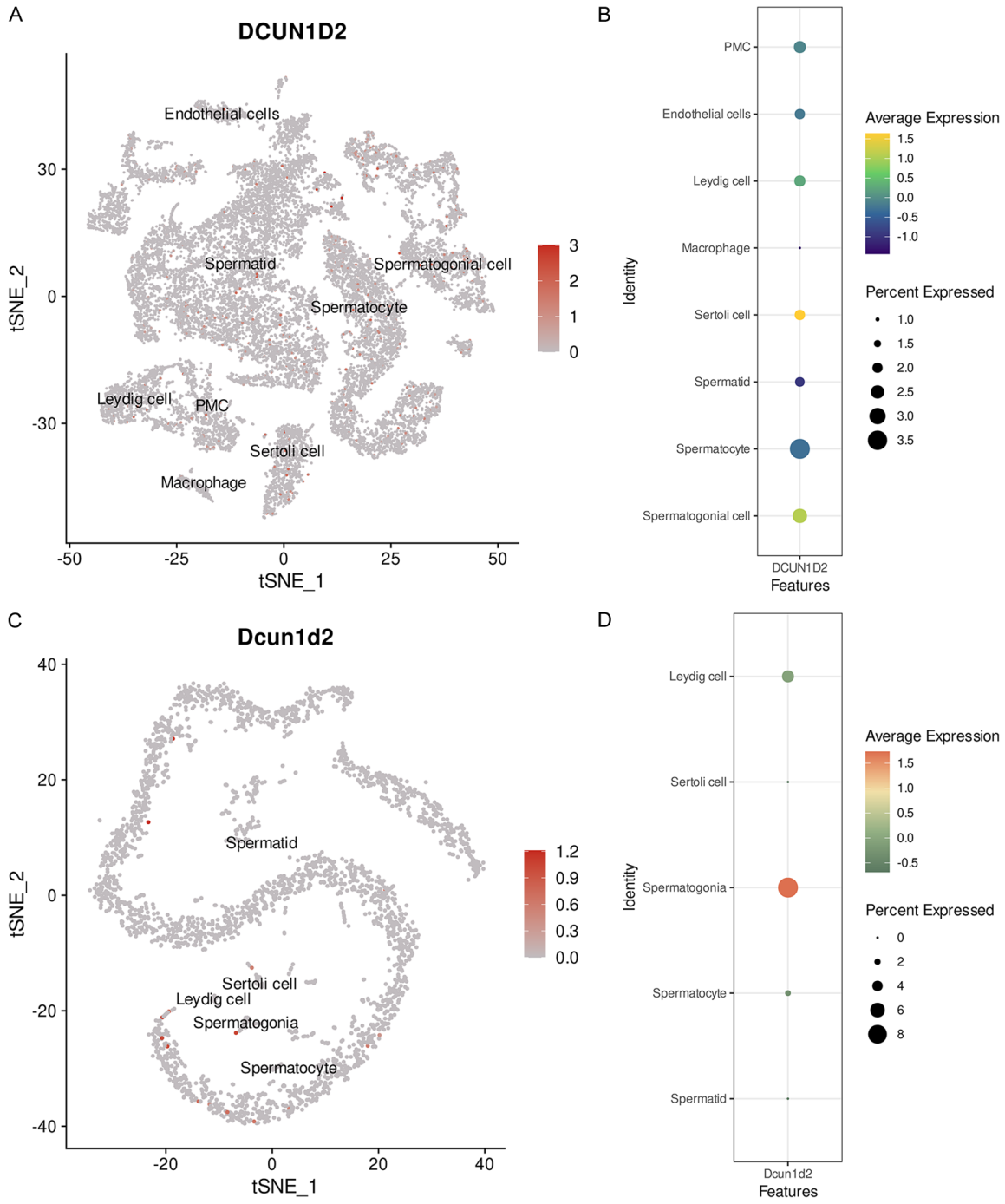

Figure 1. DCUN1D2/Dcun1d2 expression patterns in human and mouse testes. A, B. Featureplots showing the distribution of DCUN1D2/Dcun1d2 in human and mouse testes. C, D. Dotplots illustrating DCUN1D2/Dcun1d2 expression in various cell types from the human and mouse testis samples. Cell types were identified based on clustering of scRNA-seq data.

(1:500, Santa), anti-gamma H2A.X Variant Histone (γ-H2AX) (1:200, Abcam), anti-LIN28 (1:200, Abcam), anti-SRY-box transcription factor 9 (SOX9) (1:200, Millipore) and peptide nucleic acid (PNA) (1:500, Vector). Following three 10-minute phosphate-buffered saline (PBS) washes at room temperature (RT), sections were then incubated with AlexaFluor secondary antibodies (Thermo Scientific, Waltham, USA) for 1 hour at 37°C. Eventually, the sec-

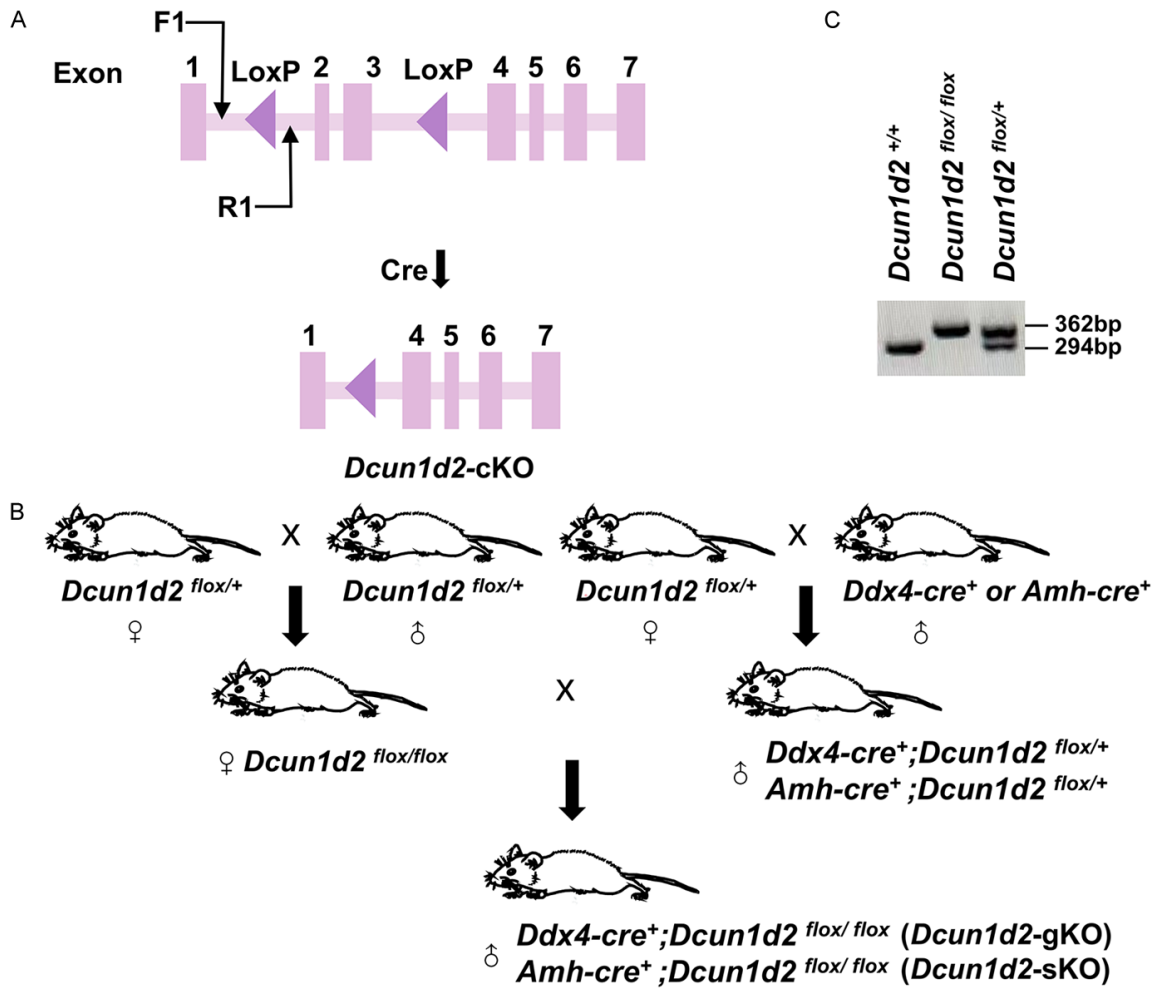

Figure 2. Generation of *Dcun1d2*-cKO mice. A. Schematic representation of the CRISPR/Cas9-mediated *Dcun1d2* conditional knockout (cKO) strategy targeting exons 2-3 flanked by loxP sites. B. Breeding strategies for producing *Dcun1d2*-cKO mice. C. Validation of mouse genotypes by PCR.

tions were stained with 4',6-diamidino-2-phenylindole (DAPI). The images were captured using a confocal microscope (LSM800, Zeiss, Oberkochen, Germany) for fluorescence analysis.

Terminal deoxynucleotidyl transferase-dUTP nick-end labeling (TUNEL) assay

Apoptotic cells were detected by published protocols [25, 26]. Paraffin-embedded testicular sections underwent proteinase K digestion (10 μg/mL, 10 min), equilibration buffer incubation (30 min), then BrightRed-labeled buffer (Vazyme, Nanjing) incubation (37°C, 1 h). After PBS washing (3×) and DAPI staining, images were analyzed using a Zeiss LSM800 confocal microscope (Oberkochen, Germany).

Statistical analysis

Data were analyzed with GraphPad Prism 9 and presented as mean ± SD. Statistical significance ($P<0.05$) was determined by Student's t-test or one-way ANOVA.

Results

DCUN1D2/Dcun1d2 expression patterns in human and mouse testes

DCUN1D2 belongs to the DCNLs family and is a neddylation-related molecule [15]. It was assessed for testicular expression and localization via public single-cell RNA sequencing (scRNA-seq) datasets. In human testes, scRNA-seq data revealed that DCUN1D2 was predomi-

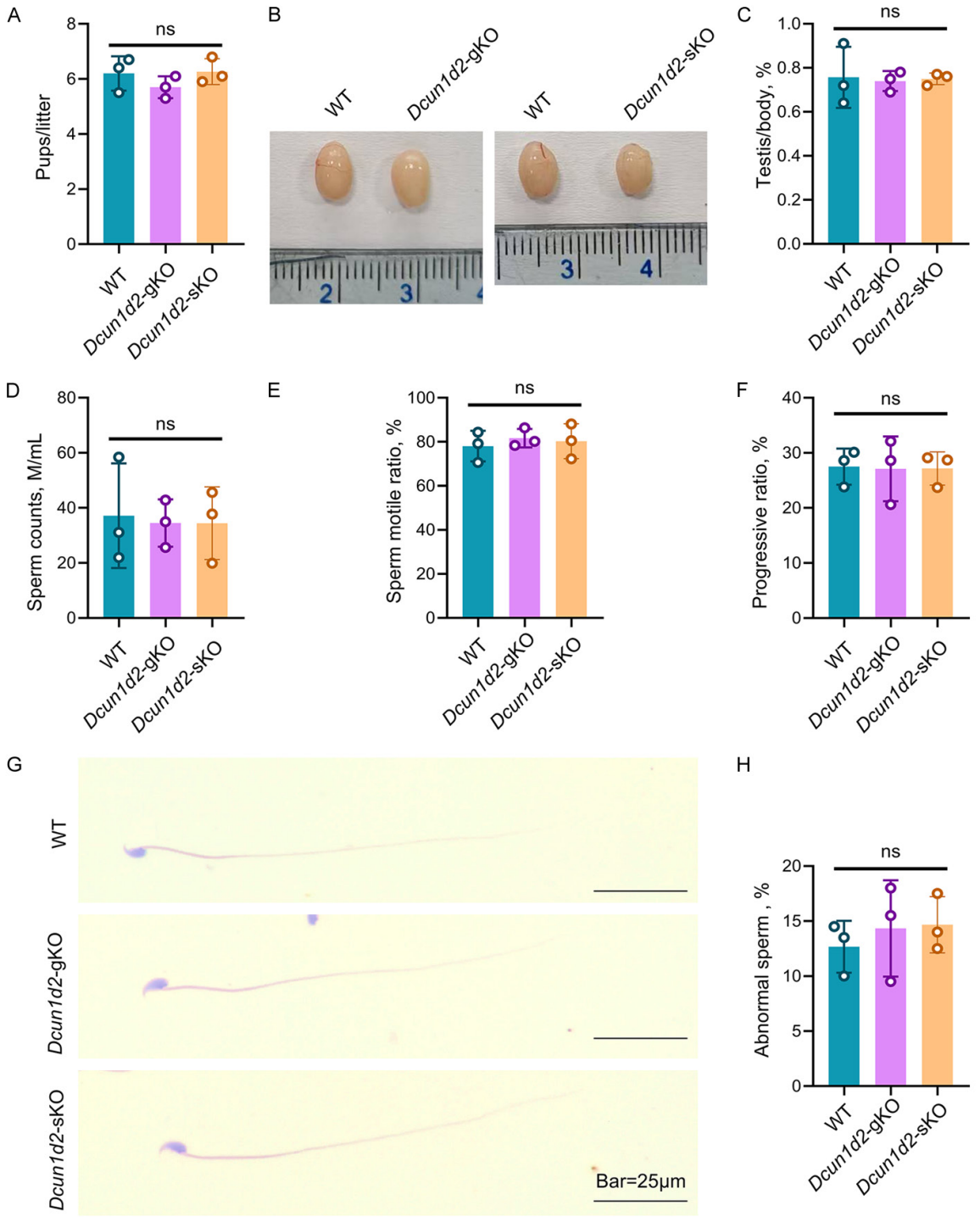

Figure 3. *Dcun1d2*-cKO mice are fertile. (A) Fertility testing of wild-type (WT) (n = 3), *Dcun1d2*-sKO (n = 3) and *Dcun1d2*-gKO (n = 3) mice. (B) Gross morphology of the testes of WT, *Dcun1d2*-sKO and *Dcun1d2*-gKO mice. (C) Testis/body weight ratios of WT (n = 3), *Dcun1d2*-sKO (n = 3) and *Dcun1d2*-gKO (n = 3) mice. (D-F) Computer-assisted sperm analysis (CASA) results for the sperm counts (D), motility (E), and progressive ratios (F) in WT, *Dcun1d2*-sKO and *Dcun1d2*-gKO mice. M, million. n = 3 for each group. (G) Hematoxylin and eosin (H&E) staining of sperm cells in the cauda epididymides of WT, *Dcun1d2*-sKO and *Dcun1d2*-gKO mice. Scale bar = 25 μm. (H) Quantitative analysis of the results shown in (G). n = 3 for each group. ns, not significant.

nantly expressed in spermatogonia and spermatocyte (**Figure 1A**, **1B**). In mouse testes, Dcun1d2 exhibited strong expression in spermatogonia (**Figure 1C**, **1D**).

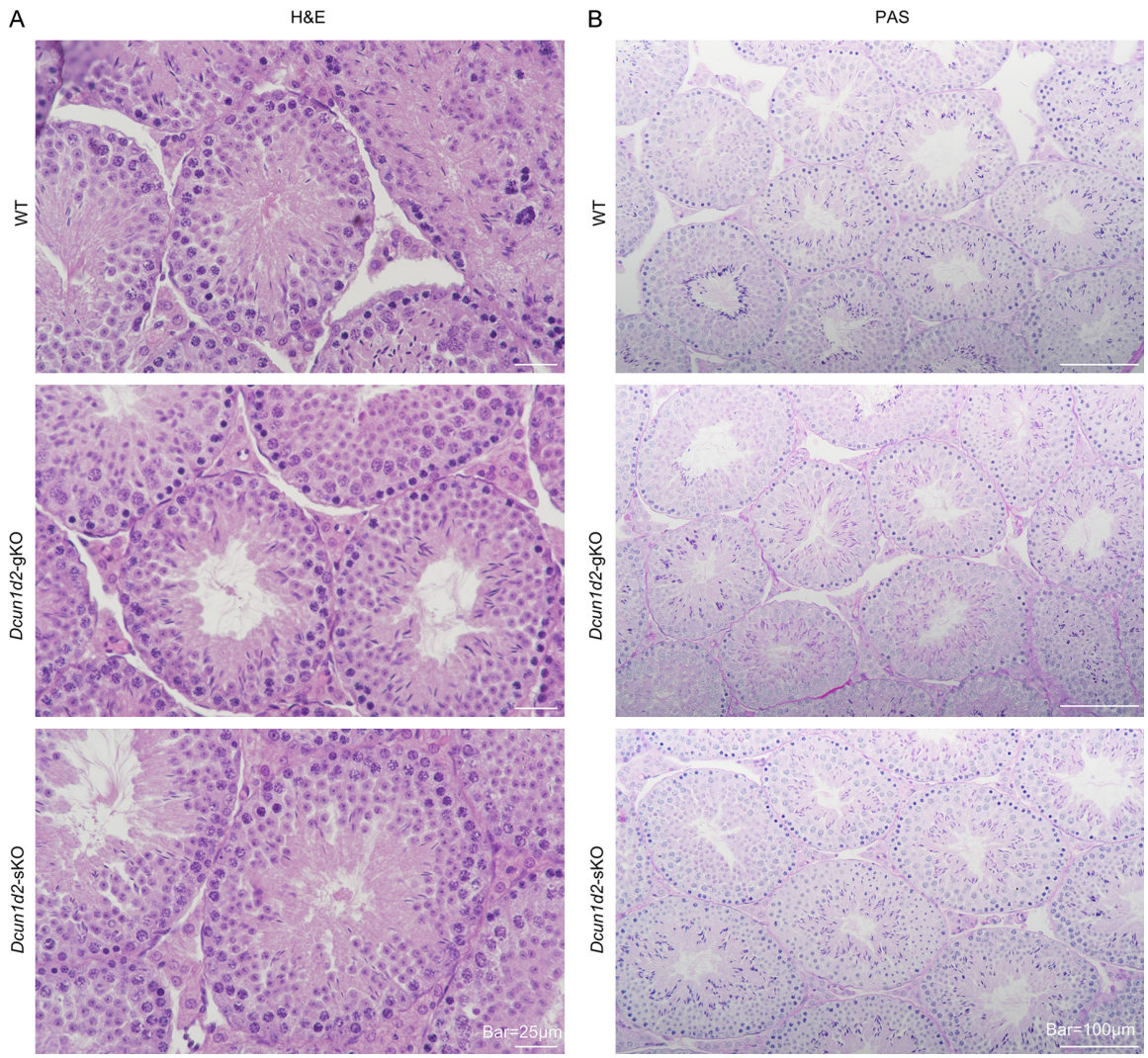

Figure 4. Testicular Histology. A. Histological assessment of spermatogenesis via H&E staining of testicular sections from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. Scale bar = 25 μm. B. Visualization of germ cell glycoprotein dynamics using periodic acid schiff (PAS) staining of testicular sections from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. Scale bar = 100 μm.

Generation of Dcun1d2-cKO mice

To generate conditional knockout (cKO) mice for the *Dcun1d2* gene, we employed CRISPR/Cas9-mediated genomic editing to flank exons 2-3 with LoxP sites (**Figure 2A**). This design enabled Cre recombinase-dependent deletion of exons 2-3, which are critical for *Dcun1d2* function. Then, by crossing *Dcun1d2*-floxed mice with *Ddx4*-Cre and *Amh*-Cre transgenic mice, respectively, we obtained germ cell- and Sertoli cell-specific *Dcun1d2*-knockout (referred to as *Dcun1d2*-gKO and *Dcun1d2*-sKO, respectively) mice (**Figure 2B**). Genotype confirmation was performed by PCR amplification, which validated the successful establishment of the *Dcun1d2*-cKO mouse strains (**Figure 2C**).

Dcun1d2-cKO mice are fertile

Fertility assessment of *Dcun1d2*-cKO mice revealed comparable litter sizes to wild-type (WT) mice (**Figure 3A**). Testicular dimensions and weight showed no significant differences between WT and *Dcun1d2*-cKO mice (**Figure 3B**, **3C**). CASA analyses revealed comparable sperm concentration, motility, and progressive velocity in *Dcun1d2*-cKO vs WT mice (**Figure 3D-F**). Additionally, H&E staining for sperm mor-

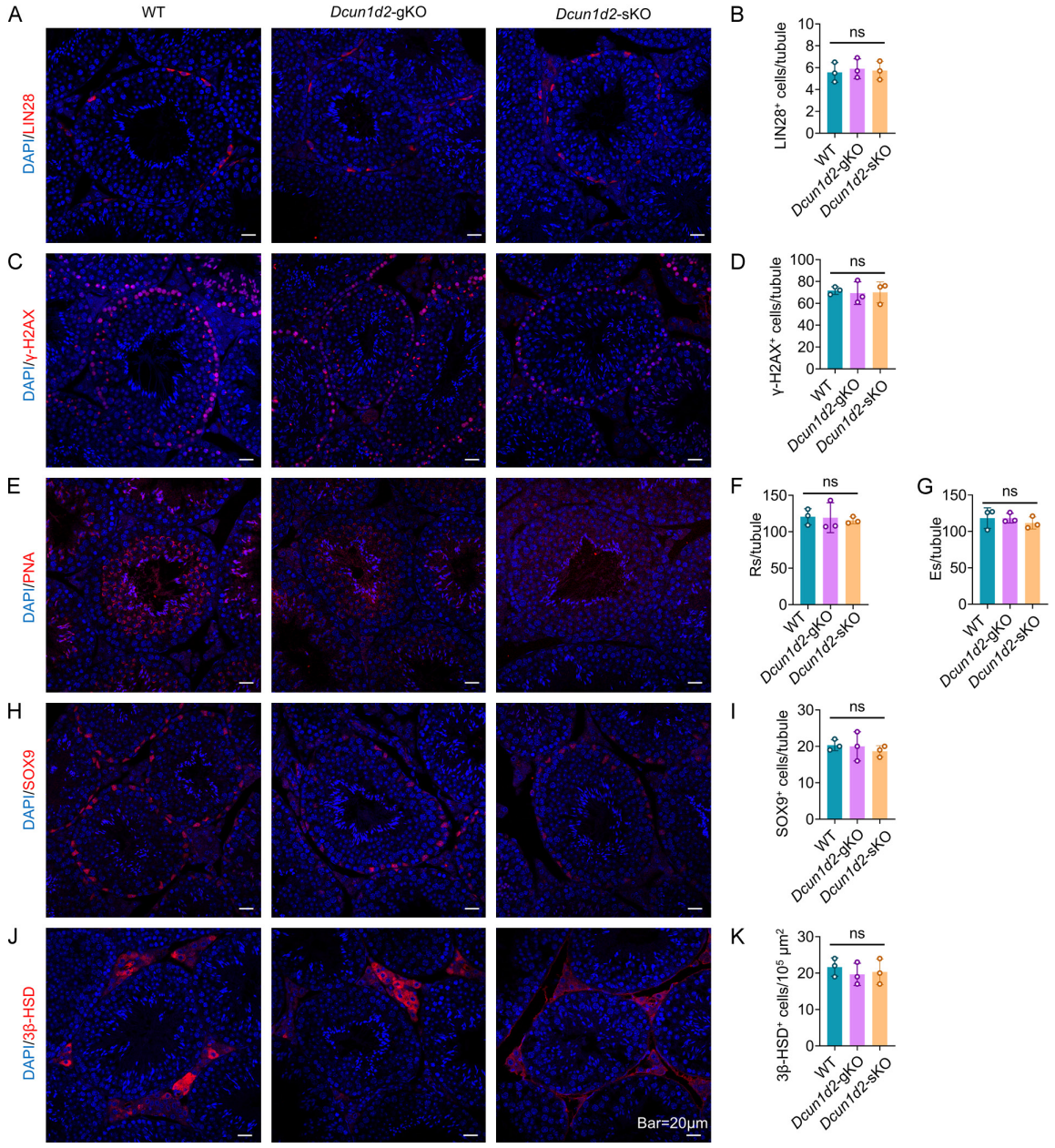

Figure 5. Immunostaining of stage-specific markers during spermatogenesis in WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. (A) Immunostaining of LIN28 in spermatogonial stem cells from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. (B) Quantification of (A). n = 3 for each group. (C) Immunostaining of γ-H2AX in spermatocytes from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. (D) Quantification of (C). n = 3 for each group. (E) Immunostaining of PNA in acrosomes from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. (F) Quantification of round spermatids (Rs) in (E). n = 3 for each group. (G) Quantification of elongating/elongated spermatids (Es) in (E). n = 3 for each group. (H) Immunostaining of SOX9 in Sertoli cells from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. (I) Quantification of (H). n = 3 for each group. (J) Immunostaining of 3β-HSD in Leydig cells from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. (K) Quantification of (J). n = 3 for each group. Scale bar = 20 μm. ns, not significant.

phology evaluation indicated no notable distinctions between WT and *Dcun1d2*-cKO mice (**Figure 3G**, **3H**). These findings collectively indicated that *Dcun1d2* deficiency did not affect murine fertility or seminal parameters.

Dcun1d2-cKO mice show normal spermatogenesis

Testicular morphology was evaluated by H&E staining, with WT and *Dcun1d2*-cKO mice

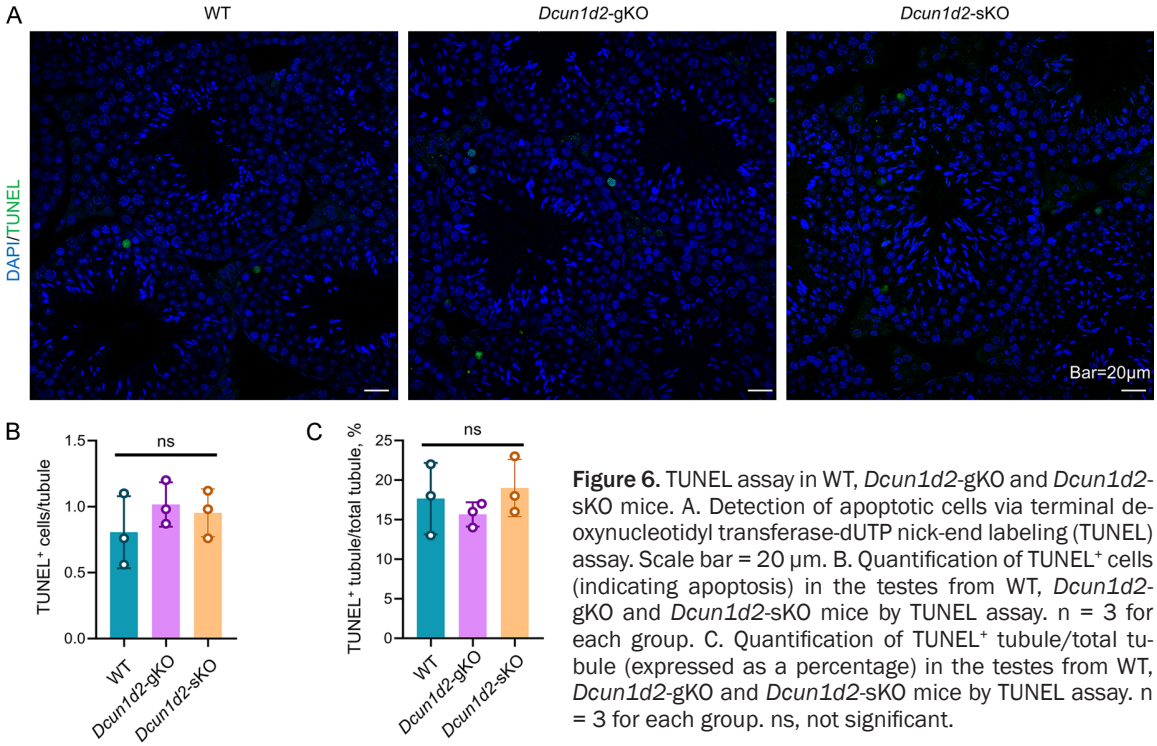

Figure 6. TUNEL assay in WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice. A. Detection of apoptotic cells via terminal deoxynucleotidyl transferase-dUTP nick-end labeling (TUNEL) assay. Scale bar = 20 μm. B. Quantification of TUNEL$^+$ cells (indicating apoptosis) in the testes from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice by TUNEL assay. n = 3 for each group. C. Quantification of TUNEL$^+$ tubule/total tubule (expressed as a percentage) in the testes from WT, *Dcun1d2*-gKO and *Dcun1d2*-sKO mice by TUNEL assay. n = 3 for each group. ns, not significant.

exhibiting intact seminiferous tubules containing spermatogenic cells at all developmental stages (**Figure 4A**). Meanwhile, the development of germ cells within the seminiferous epithelium was inspected by PAS staining. The testes of *Dcun1d2*-cKO mice, similar to those of WT mice, manifested complete spermatogenesis, revealing all stages of the spermatogenic cycle (**Figure 4B**). These observations confirmed that the deletion of *Dcun1d2* had no influence on spermatogenesis.

To comprehensively evaluate spermatogenesis, we quantified the spermatogonial stem cells, spermatocytes, acrosomes, Sertoli cells, Leydig cells, and apoptotic cells in the testes of WT and *Dcun1d2*-cKO mice by LIN28, γ-H2AX, PNA, SOX9, 3β-HSD, and TUNEL signals, respectively. Both WT and *Dcun1d2*-cKO mice exhibited comparable cell population counts across all examined cell types (**Figures 5**, **6**). These discoveries indicated that *Dcun1d2* loss did not compromise spermatogenic processes in mice.

Discussion

Mammalian spermatogenesis represents a remarkably complex differentiation cascade, transforming spermatogonial stem cells into mature spermatozoa through meticulously orchestrated mitotic, meiotic, and post-meiotic phases [27]. Ubiquitin-proteasome regulation, mediated by diverse E3 ubiquitin ligases, is indispensable throughout this process. These ligases govern critical events such as histone removal for DNA condensation and residual body degradation [1, 28]. Notably, the CRL family - constituting the largest E3 ligase group - plays multifaceted roles in germ cell development [29]. CRL components like CUL4A facilitate homologous recombination-mediated DNA repair during meiosis [30], while CUL4B maintains spermatogonial stem cell properties through somatic-germline interactions and ensures sperm motility via germ cell-autonomous functions [31].

In fact, CRLs also play a significant role in neddylation [32], a ubiquitin-like modification that involves the transfer of the ubiquitin-like protein NEDD8 to the lysine residues of target substrates. This process requires the NEDD8 cascade: E1 activating enzyme, E2 conjugating enzyme, and E3 ligase [33, 34]. DCUN1D1, a key neddylation regulator, enhances CRL function by promoting cullin neddylation, with evidence suggesting its spermatogenic role

involves CUL3 activation [13, 35]. Interestingly, while its paralog DCUN1D2 - also implicated in neddylation pathways [15] - is reported to induce cell growth arrest [36], its relevance to spermatogenesis remained unexplored.

To address this knowledge gap, we generated a germ cell-specific *Dcun1d2*-cKO mouse model using CRISPR/Cas9 technology. Contrary to expectations based on its neddylation association and in vitro phenotypes, *Dcun1d2* ablation did not impair spermatogenesis, testicular histology, or male fertility. This suggests DCUN1D2 is non-essential for murine spermatogenesis, likely due to functional redundancy within the DCNL protein family. This absence of phenotype aligns with emerging evidence that numerous testis-enriched genes - including *Socs7* [37] and *Spsb1* [38] are non-essential for murine fertility.

This functional resilience stands in sharp contrast to DCUN1D1, whose deletion disrupts spermatogenesis [13], underscoring distinct roles for DCNL paralogs despite shared neddylation pathways. While DCUN1D1 critically regulates CUL3 in germ cells, DCUN1D2 appears expendable under physiological conditions. Our findings establish DCUN1D2 as functionally redundant for murine spermatogenesis and reproduction. Given that neddylation and specific CRLs (e.g., CUL4A/B) remain demonstrably critical for fertility [30, 31], DCUN1D2's dispensability refines our understanding of regulatory plasticity within the neddylation network. Future research should prioritize genes with non-redundant, demonstrable impacts on fertility mechanisms.

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Disclosure of conflict of interest

None.

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References

[1] Xiong Y, Yu C and Zhang Q. Ubiquitin-proteasome system-regulated protein degradation in spermatogenesis. Cells 2022; 11: 1058.
[2] Ryu KY, Sinnar SA, Reinholdt LG, Vaccari S, Hall S, Garcia MA, Zaitseva TS, Bouley DM, Boekelheide K, Handel MA, Conti M and Kopito RR. The mouse polyubiquitin gene Ubb is essential for meiotic progression. Mol Cell Biol 2008; 28: 1136-1146.
[3] Xi Y, Gong C, Zhang Z, Zhu F, Zhang Y, Tang Y, Yan L, Jiang H, Qiao J and Liu Q. NAE1-mediated neddylation coordinates ubiquitination regulation of meiotic recombination during spermatogenesis. Theranostics 2025; 15: 3122-3142.
[4] Lv J, Wu T, Xue J, Shen C, Gao W, Chen X, Guo Y, Liu M, Yu J, Huang X and Zheng B. ASB1 engages with ELOB to facilitate SQOR ubiquitination and H(2)S homeostasis during spermiogenesis. Redox Biol 2025; 79: 103484.
[5] 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.
[6] Yu J, Shen C, Lin M, Chen X, Dai X, Li Z, Wu Y, Fu Y, Lv J, Huang X, Zheng B and Sun F. BMI1 promotes spermatogonial stem cell maintenance by epigenetically repressing Wnt10b/β-catenin signaling. Int J Biol Sci 2022; 18: 2807-2820.
[7] Rabut G and Peter M. Function and regulation of protein neddylation. 'Protein modifications: beyond the usual suspects' review series. EMBO Rep 2008; 9: 969-976.
[8] Jahns MT, Vezon D, Chambon A, Pereira L, Falque M, Martin OC, Chelysheva L and Grelon M. Crossover localisation is regulated by the neddylation posttranslational regulatory pathway. PLoS Biol 2014; 12: e1001930.
[9] Enchev RI, Schulman BA and Peter M. Protein neddylation: beyond cullin-RING ligases. Nat Rev Mol Cell Biol 2015; 16: 30-44.
[10] Zhang S, Yu Q, Li Z, Zhao Y and Sun Y. Protein neddylation and its role in health and diseases. Signal Transduct Target Ther 2024; 9: 85.
[11] Thomas Y, Scott DC, Kristariyanto YA, Rinehart J, Clark K, Cohen P and Kurz T. The NEDD8 E3

ligase DCNL5 is phosphorylated by IKK alpha during Toll-like receptor activation. PLoS One 2018; 13: e0199197.
[12] Keuss MJ, Thomas Y, McArthur R, Wood NT, Knebel A and Kurz T. Characterization of the mammalian family of DCN-type NEDD8 E3 ligases. J Cell Sci 2016; 129: 1441-1454.
[13] Huang G, Kaufman AJ, Ryan RJH, Romin Y, Huryn L, Bains S, Manova-Todorova K, Morris PL, Hunnicutt GR, Adelman CA, Petrini JHJ, Ramanathan Y and Singh B. Mouse DCUN1D1 (SCCRO) is required for spermatogenetic individualization. PLoS One 2019; 14: e0209995.
[14] Meyer-Schaller N, Chou YC, Sumara I, Martin DD, Kurz T, Katheder N, Hofmann K, Berthiaume LG, Sicheri F and Peter M. The human Dcn1-like protein DCNL3 promotes Cul3 neddylation at membranes. Proc Natl Acad Sci U S A 2009; 106: 12365-12370.
[15] Kajigaya H, Ishibashi T, Hayashi A, Yamaguchi Y and Baba H. Concentration of neddylation-related molecules in paranodal myelin of the peripheral nervous system. Proc Jpn Acad Ser B Phys Biol Sci 2016; 92: 56-68.
[16] Huang G, Stock C, Bommeljé CC, Weeda VB, Shah K, Bains S, Buss E, Shaha M, Rechler W, Ramanathan SY and Singh B. SCCRO3 (DCUN1D3) antagonizes the neddylation and oncogenic activity of SCCRO (DCUN1D1). J Biol Chem 2014; 289: 34728-34742.
[17] Liu C. Screen for autophagy-related biomarkers in osteoarthritis based on bioinformatic analysis. Biocell 2024; 48: 339-351.
[18] Dong Z, Zhao C, Hu S, Yang K, Yu J, Sun X and Zheng J. Bioinformatic analysis and in vivo validation of angiogenesis related genes in inflammatory bowel disease. Biocell 2023; 47: 2735-2745.
[19] Wu T, Jin X, Huang C, Yu X, Xu B, Gao W, Qiu X, Bao M, Zhao D, Feng G, Zheng B and Huang X. E3 ligase FBXO22 is not significant for spermatogenesis and male fertility in mice. Am J Transl Res 2024; 16: 1834-1844.
[20] Yang X, He L, Li X, Wang L, Bu T, Yun D, Lu X, Gao S, Huang Q, Li J, Zheng B, Yu J and Sun F. Triptolide exposure triggers testicular vacuolization injury by disrupting the Sertoli cell junction and cytoskeletal organization via the AKT/mTOR signaling pathway. Ecotoxicol Environ Saf 2024; 279: 116502.
[21] Yu J, Xu J, Li H, Wu P, Zhu S, Huang X, Shen C, Zheng B and Li W. Gold nanoparticles retrogradely penetrate through testicular barriers via Sertoli-cells mediated endocytosis/exocytosis and induce immune response in mouse. Ecotoxicol Environ Saf 2023; 255: 114827.
[22] Yu Y, Wang J, Zhou L, Li H, Zheng B and Yang S. CFAP43-mediated intra-manchette transport is required for sperm head shaping and flagella formation. Zygote 2021; 29: 75-81.
[23] Gao T, Lin M, Shao B, Zhou Q, Wang Y, Chen X, Zhao D, Dai X, Shen C, Cheng H, Yang S, Li H, Zheng B, Zhong X, Yu J, Chen L and Huang X. BMI1 promotes steroidogenesis through maintaining redox homeostasis in mouse MLTC-1 and primary Leydig cells. Cell Cycle 2020; 19: 1884-1898.
[24] 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.
[25] 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.
[26] Yu J, Yan Y, Luan X, Qiao C, Liu Y, Zhao D, Xie B, Zheng Q, Wang M, Chen W, Shen C, He Z, Hu X, Huang X, Li H, Shao Q, Chen X, Zheng B and Fang J. Srlp is crucial for the self-renewal and differentiation of germline stem cells via RpL6 signals in drosophila testes. Cell Death Dis 2019; 10: 294.
[27] Han C. Gene expression programs in mammalian spermatogenesis. Development 2024; 151: dev202033.
[28] Richburg JH, Myers JL and Bratton SB. The role of E3 ligases in the ubiquitin-dependent regulation of spermatogenesis. Semin Cell Dev Biol 2014; 30: 27-35.
[29] Zheng N and Shabek N. Ubiquitin ligases: structure, function, and regulation. Annu Rev Biochem 2017; 86: 129-157.
[30] Kopanja D, Roy N, Stoyanova T, Hess RA, Bagchi S and Raychaudhuri P. Cul4A is essential for spermatogenesis and male fertility. Dev Biol 2011; 352: 278-287.
[31] Yin Y, Liu L, Yang C, Lin C, Veith GM, Wang C, Sutovsky P, Zhou P and Ma L. Cell autonomous and nonautonomous function of CUL4B in mouse spermatogenesis. J Biol Chem 2016; 291: 6923-6935.
[32] Lin CP and Komives EA. Diversity of structure and function in Cullin E3 ligases. Curr Opin Struct Biol 2024; 88: 102879.
[33] Wang T, Jiang J, Zhang X, Ke X and Qu Y. Ubiquitin-like modification dependent proteasomal degradation and disease therapy. Trends Mol Med 2024; 30: 1061-1075.
[34] Qin X, Han X and Sun Y. Discovery of small molecule inhibitors of neddylation catalyzing enzymes for anticancer therapy. Biomed Pharmacother 2024; 179: 117356.
[35] Paccez JD, Foret CLM, de Vasconcellos JF, Donaldson L and Zerbini LF. DCUN1D1 and

neddylation: potential targets for cancer therapy. Biochim Biophys Acta Mol Basis Dis 2024; 1870: 167308.

[36] Sánchez-Abarca LI, Gutierrez-Cosio S, Santamaría C, Caballero-Velazquez T, Blanco B, Herrero-Sánchez C, García JL, Carrancio S, Hernández-Campo P, González FJ, Flores T, Ciudad L, Ballestar E, Del Cañizo C, San Miguel JF and Pérez-Simon JA. Immunomodulatory effect of 5-azacytidine (5-azaC): potential role in the transplantation setting. Blood 2010; 115: 107-121.

[37] Zhou N, Deng J, Zhou H, Hu H, Jiang B, Wu T, Shen C, Wu Y and Xie J. Testis-enriched Socs7 is not essential for spermatogenesis and male fertility in mice. Am J Transl Res 2025; 17: 1780-1791.

[38] Hu H, Zhu Y, Jiang B, Zhou N, Wu T, Wang G, Shen C, Wu Y and Xi X. Testis-enriched Spsb1 is not required for spermatogenesis and fertility in mice. Am J Transl Res 2025; 17: 1824-1833.