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Original Article

Testis-enriched *Socs7* is not essential for spermatogenesis and male fertility in mice

Nianchao Zhou[1*], Junpeng Deng[2*], Hui Zhou[1*], Haoyue Hu[1], Bing Jiang[1], Tiantian Wu[3], Cong Shen[3], Yibo Wu[1], Jianjun Xie[2]

[1]*Human Reproductive and Genetic Center, Affiliated Hospital of Jiangnan University, Wuxi 214062, Jiangsu, China;* [2]*Department of Urology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou 215002, Jiangsu, China;* [3]*State Key Laboratory of Reproductive Medicine and Offspring Health, Center for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou 215002, Jiangsu, China.* [*]*Equal contributors.*

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Abstract: Objectives: As a crucial member of the Suppressor of Cytokine Signaling (SOCS) family, SOCS7 regulates various physiological processes, including insulin resistance, inflammation, and tumor suppression. However, its role in male germ cells remains poorly understood. This study aims to investigate the function of SOCS7 in spermatogenesis and uncover its potential regulatory mechanisms. Methods: We conducted bioinformatics analyses to examine the expression profile of *Socs7* in the testes, generated *Socs7*-knockout (KO) mice using CRISPR/Cas9 genome editing, and assessed testicular morphology through histological and immunohistochemical staining. Semen quality was evaluated using computer-assisted sperm analysis (CASA), and testicular apoptosis was examined using the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Results: Bioinformatics analysis revealed high expression of *Socs7* in both human and mouse testes. However, *Socs7*-KO mice exhibited normal fertility, with no significant differences in testicular morphology, sperm quality, or spermatogenesis compared to wild-type (WT) mice. Additionally, testicular apoptosis in *Socs7*-KO mice was not significantly altered. Conclusions: Our study demonstrates that although *Socs7* is highly expressed in the testes, its deletion does not impair male fertility or spermatogenesis in mice. These findings provide valuable insights into the role of SOCS7 in male reproduction and help prevent unnecessary duplication of research efforts.

Keywords: *Socs7*, spermatogenesis, fertility, testis

Introduction

Spermatogenesis is a complex process involving a series of cell divisions and differentiation events within the seminiferous tubules of the testes, ultimately leading to the production of mature sperm [1, 2]. During this process, male germ cells undergo multiple post-translational modifications, among which ubiquitination is one of the most prominent. Ubiquitination is a key regulatory mechanism in eukaryote cells that governs protein degradation and cellular homeostasis [3]. Recent studies have demonstrated that components of the ubiquitin-proteasome system (UPS) regulate various stages of spermatogenesis at multiple levels [4, 5]. The ubiquitination process is mediated by a sequential enzymatic cascade involving E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin ligases. Among these, E3 ubiquitin ligases play a pivotal role by recognizing specific substrate proteins and facilitating their ubiquitination, making them key regulators of protein turnover [6-8].

The Suppressor of Cytokine Signaling (SOCS) protein family consists of eight members: cytokine-inducible SH2-containing protein (CIS) and SOCS1-7. All SOCS proteins share a conserved C-terminal SOCS-box, which is essential for their function [9]. The SOCS-box mediates the formation of an E3 ubiquitin ligase complex by interacting with adaptor proteins Elongin B and Elongin C, which, in turn, link the SOCS protein

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to the Cullin5 scaffold protein and the RING-box protein (Rbx2). This complex facilitates the ubiquitination and proteasomal degradation of target proteins, thereby regulating diverse cellular processes [10, 11].

As an important member of the SOCS family, SOCS7 plays a critical role in multiple physiological processes. For example, SOCS7 modulates insulin signaling by targeting insulin receptor substrate (IRS) proteins for ubiquitination and proteasomal degradation, leading to attenuation of insulin signaling and promotion of insulin resistance [12, 13]. Additionally, SOCS7 inhibits prolactin, growth hormone, and leptin signaling by interacting with STAT5 or STAT3, thereby reducing their nuclear translocation [14]. In cancer biology, SOCS7 primarily functions as a tumor suppressor. The SOCS7/HuR/FOXM1 signaling axis has been shown to inhibit the progression of high-grade serous ovarian cancer, while in hepatocellular carcinoma (HCC), SOCS7 suppresses immune evasion and tumor growth by targeting Shc1 for degradation [15, 16]. Furthermore, the SOCS7-based E3 ubiquitin ligase complex has been reported to effectively inhibit the proliferation of mutant KRAS-driven pancreatic cancer cells [17].

Although Krebs et al. previously reported that *Socs7*-knockout (KO) mice exhibited normal fertility in their study on hydrocephalus [18], the role of SOCS7 in spermatogenesis has not been systematically investigated. In this study, we employed the CRISPR/Cas9 genome editing to generate *Socs7*-KO mice and examined the effects of SOCS7 on reproductive capacity and spermatogenesis.

Materials and methods

Bioinformatic analysis

We analyzed SOCS7 expression using transcriptomic data from the BioGPS database (http://biogps.org/) for human and mouse testis. The data were processed with Seurat, integrated using Harmony, and visualized with the DotPlot and FeaturePlot functions. Cell types were identified using standard marker genes.

Mice

Following our previously described methods [19, 20], *Socs7*-KO mice were generated using CRISPR/Cas9 genome editing technology. Briefly, single-guide RNAs (sgRNAs) targeting exons 2 to 6 of the Socs7 gene were designed, with the following sequences: 5'-TGTGGAACCGTCCTGAGCACAGG-3' and 5'-GACGCTCAGGCCAACTATGCTGG-3'. Cas9 mRNA and the sgRNAs were microinjected into fertilized CD-1 mouse eggs, which were subsequently transferred into the oviducts of pseudopregnant CD-1 female mice. Genotyping of *Socs7*-KO mice was performed by polymerase chain reaction (PCR) using the following primers: forward primer 1: 5'-TGGTGTGTAGTGGAATGTGGTTTC-3'; reverse primer 1: 5'-CAGGGGATAGAGAGTCAGTTCAAATCC-3'; and reverse primer 2: 5'-AGAGAGAAAGACAGGGTCCTTACC-3'. All animals were maintained in a specific pathogen-free facility at the Animal Center of Nanjing Medical University, where experimental procedures including terminal euthanasia by cervical dislocation were conducted in accordance with the guidelines of the Animal Ethics and Welfare Committee of Nanjing Medical University (No. IACUC-2402015), which approved all animal experiments.

Fertility test

Fertility was assessed by pairing adult *Socs7*-KO and wild-type (WT) male mice (8 weeks old) with WT females (8-10 weeks old) at a 1:2 ratio for a duration of 12 weeks. The total number of litters, the number of pups per litter, and the birth dates were meticulously recorded for subsequent statistical analysis.

Computer-assisted sperm analysis (CASA)

Sperm were collected from the cauda epididymis of 8-week-old WT and *Socs7*-KO mice, and suspended in human tubal fluid (HTF) culture medium (InVitroCare, USA) containing 10% bovine serum albumin (BSA) for a 5-minute incubation. Sperm concentration, motility, and progressive motility were then assessed using the Ceros™ II sperm analysis system (Hamilton Thorne, USA).

Histological analysis

As described in our previous study [21-23], testicular tissues from 8-week-old WT and *Socs7*-KO mice were fixed in modified Davidson's fixative for 48 h, followed by dehydration in ethanol and embedding in paraffin. The tissues were

then sectioned into 5 μm thick slices, rehydrated, and stained with hematoxylin-eosin (H&E) or periodic acid-Schiff (PAS) stain for histological analysis.

Immunofluorescence

After rehydrating paraffin-embedded testicular sections, antigen retrieval was performed in citrate buffer. The sections were then blocked with 1% (w/v) bovine serum albumin (BSA) for 2 hours, as previously described [24-26]. Subsequently, the sections were incubated overnight at 4°C with the following primary antibodies: anti-lin-28 homolog (Lin28, 1:200, Abcam), anti-H2AX variant histone (γ-H2AX, 1:400, Abcam), peptide nucleic acid (PNA, 1:400, Vector), anti-SRY-box transcription factor 9 (SOX9, 1:400, Millipore), and anti-3β-hydroxysteroid dehydrogenase (3β-HSD, 1:200, Santa Cruz). The following day, the sections were washed three times with phosphate-buffered saline (PBS) and incubated with AlexaFluor secondary antibodies (Thermo Scientific, Waltham, USA) at 37°C for 1 hour. Finally, the sections were stained with 4',6-diamidino-2-phenylindole (DAPI) and analyzed by fluorescence microscopy using a confocal laser scanning microscope (LSM800, Zeiss).

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

Apoptotic cells were detected using a TUNEL assay kit (Vazyme, Nanjing, China), following a previously described protocol [27]. Briefly, paraffin-embedded testicular sections were deparaffinized, rehydrated, and incubated with proteinase K (20 μg/mL, Beyotime) at room temperature for 20 minutes. The sections were then treated with equilibration buffer for 30 minutes, followed by incubation with BrightRed Labeling Buffer at 37°C for 1 hour. After washing with PBS three times, the sections were stained with DAPI for nuclear visualization. Images were acquired using an LSM800 confocal laser scanning microscope (Zeiss, Germany). Briefly, paraffin-embedded testicular sections were deparaffinized, rehydrated, and incubated with proteinase K (20 μg/mL, Beyotime) at room temperature for 20 minutes. The sections were then treated with equilibration buffer for 30 minutes, followed by incubation with BrightRed Labeling Buffer at 37°C for 1 hour. After washing with PBS three times, the sections were stained with DAPI for nuclear visualization. Images were acquired using an LSM800 confocal laser scanning microscope (Zeiss, Germany).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software, USA). Values are presented as the mean ± standard deviation (SD) from at least three independent replicates per experiment. Differences between WT and *Socs7*-KO mice were assessed using an unpaired Student's t-test. A *P*-value of < 0.05 was considered statistically significant.

Results

Socs7 is primarily expressed in the testes of human and mice

We performed a bioinformatics analysis to investigate the expression patterns of Socs7 across various tissues and cell types using both the BioGPS database (http://biogps.org/) and publicly available single-cell RNA sequencing (scRNA-seq) datasets (GSM5563668 and GSE149512) for human and mouse testes. The analysis revealed that *Socs7* was predominantly expressed in the testes of both species (**Figures 1A**, **2A**). In human testes, *SOCS7* expression was mainly found in spermatids and spermatocytes, while in mouse testes (**Figure 1B**, **1C**), it was significantly expressed not only in spermatids and spermatocytes but also in Sertoli cells (**Figure 2B**, **2C**).

Socs7-KO mice exhibit normal fertility

To further investigate the role of SOCS7 in male reproduction, we generated *Socs7*-KO mice using CRISPR/Cas9-mediated gene editing. As shown in **Figures 3A** and S1, two sgRNAs were designed to target exons 2 to 6 of the Socs7 gene, spanning a total of 6,472 base pairs. The genotypes of the resulting mice-*Socs7*$^{+/+}$, *Socs7*$^{+/-}$ and *Socs7*$^{-/-}$- were confirmed by PCR amplification (**Figure 3B**). We performed a fertility test over a period of 3 months using both WT and *Socs7*-KO mice, with pup and litter counts recorded at regular intervals. The fertility analysis revealed no significant difference in fertility between WT and *Socs7*-KO male mice (**Figure 3C**). Additionally, no significant differences were observed in testicular morphology or the

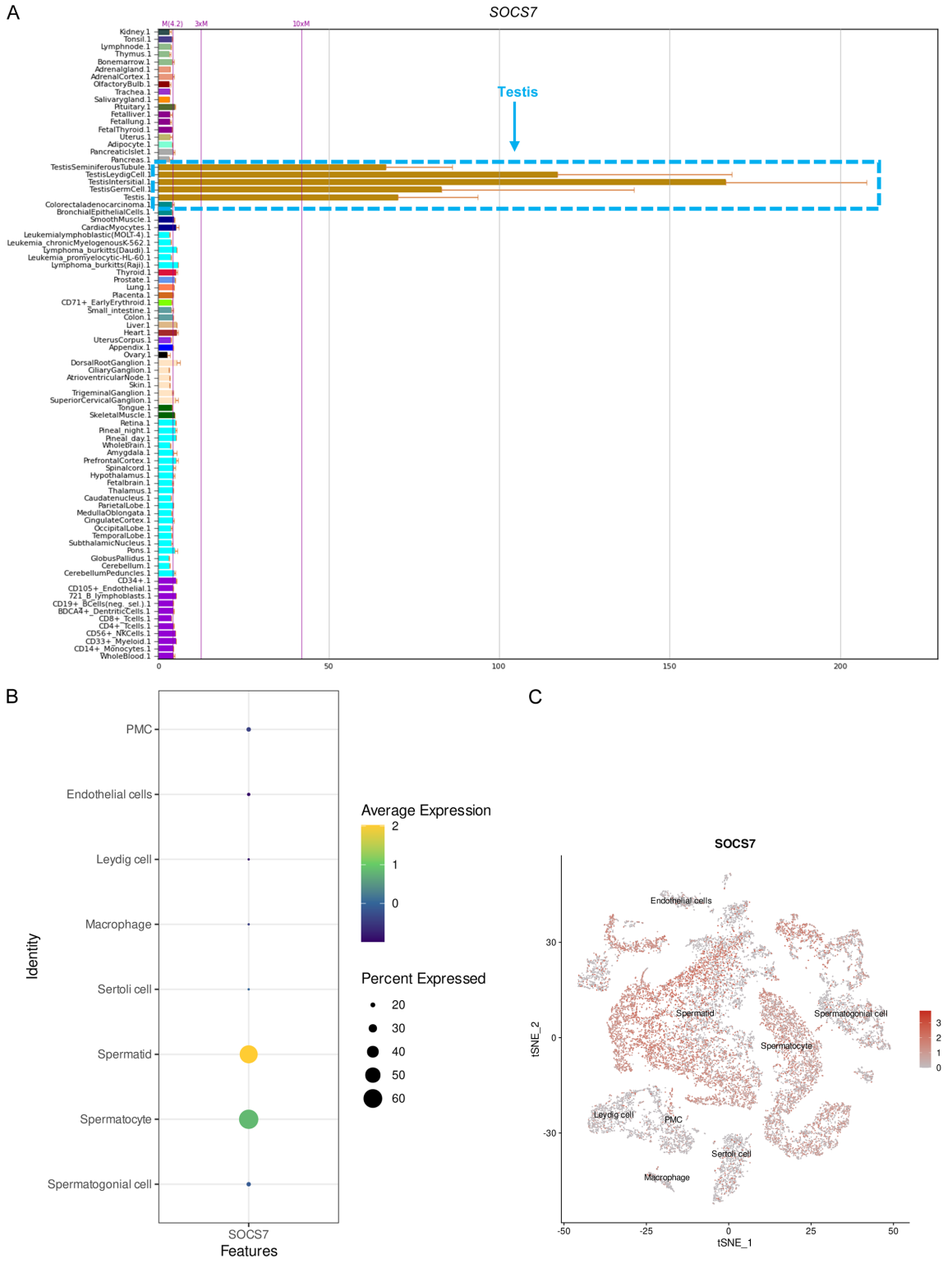

Figure 1. Expression profiling of *SOCS7* in human tissues and testes. A. Bioinformatic analysis of *SOCS7* expression across a wide range of human tissues and cell types, based on microarray data retrieved from the BioGPS database (http://biogps.org/). B. Dotplot illustrating the expression levels of *SOCS7* in distinct cell populations within human testis samples. C. t-SNE featureplots displaying the distribution of *SOCS7* expression across various cell types within human testis samples.

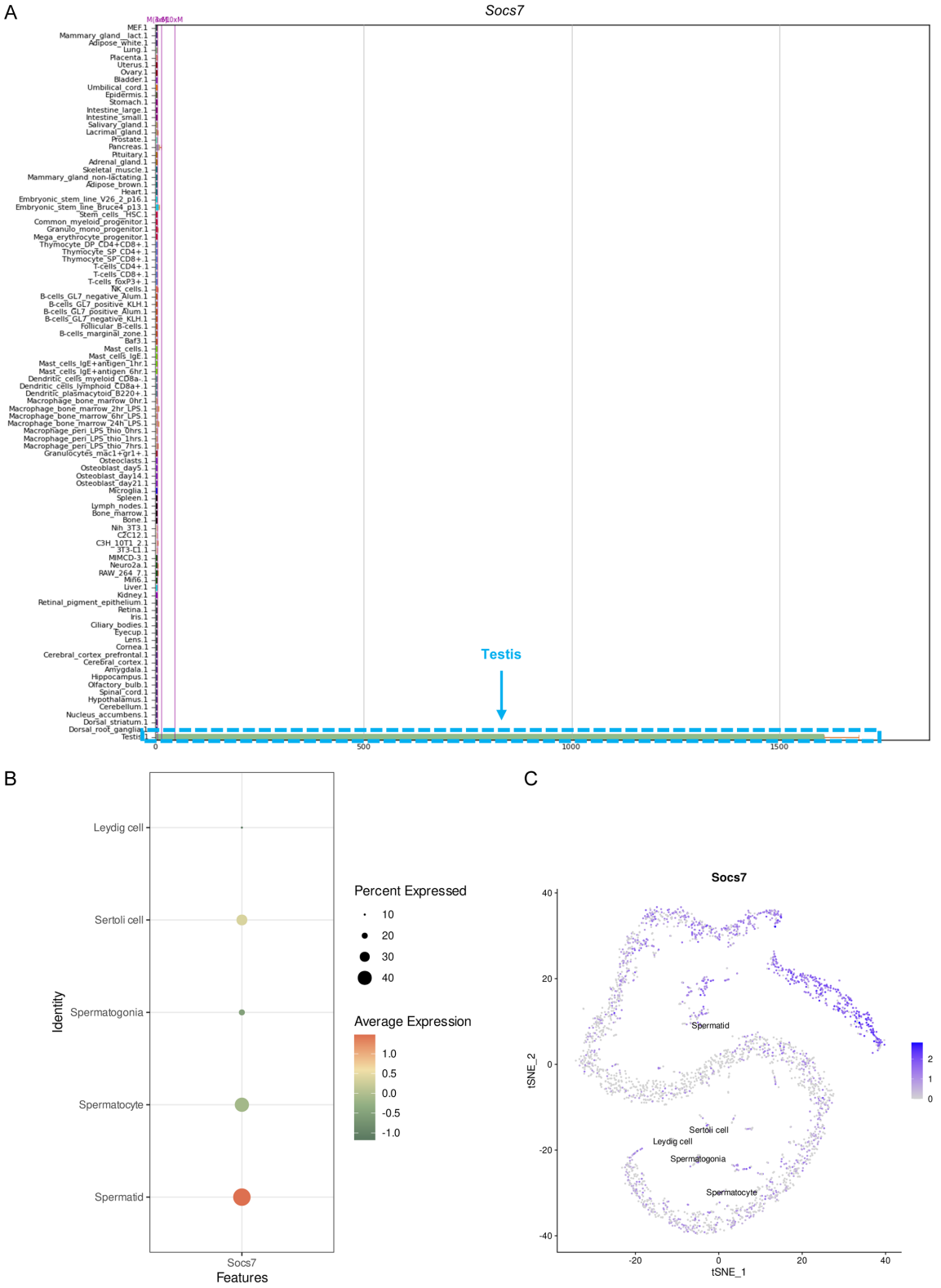

Figure 2. Expression profiling of *Socs7* in mice tissues and testis. A. Bioinformatic analysis of *Socs7* expression in multiple tissues and cells of mice, using microarray data from the BioGPS database (http://biogps.org/). B. Dotplot of *Socs7* expression in different cell types from mice testis samples. C. tSNE featureplots illustrating the distribution of *Socs7* across various cell types in mice testis samples.

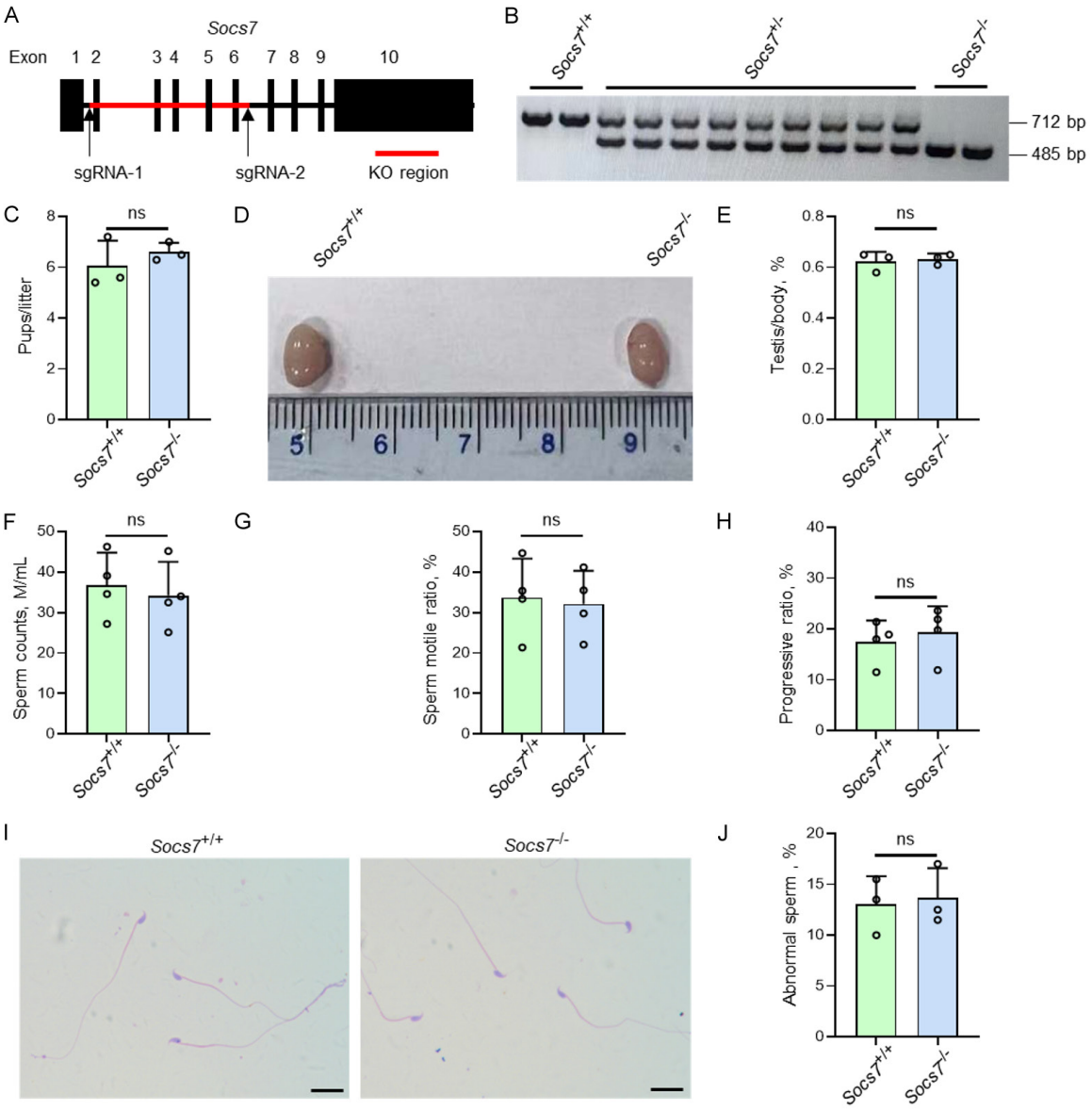

Figure 3. Generation of *Socs7*-knockout (KO) mice and assessment of fertility. (A) Schematic representation of the CRISPR/Cas9 strategy used to generate *Socs7*-KO mice. (B) PCR validation of genomic DNA from *Socs7*$^{+/+}$, *Socs7*$^{+/-}$ and *Socs7*$^{-/-}$ mice. (C) Fertility test of male wild-type (WT) and Socs7-KO mice (n = 3, P > 0.05). (D) Histological examination of testicular morphology in WT and *Socs7*-KO mice. (E) Testis/body weight ratio of WT and *Socs7*-KO mice (n = 3, P > 0.05). (F-H) CASA results of WT and *Socs7*-KO mice, including sperm count (F), motility (G) and progressive ratio (H) (n = 4, P > 0.05). (I) Representative H&E staining images of sperm from WT and *Socs7*-KO mice (Scale bar: 20 μm). (J) Quantification of abnormal sperm from H&E staining shown in (I) (n = 3, P > 0.05).

testis-to-body weight ratio between the two groups (**Figure 3D**, **3E**). Computer-Assisted Sperm Analysis (CASA) showed no statistically significant differences between WT and *Socs7*-KO mice in sperm concentration, motility, or the progressive motility ratio (**Figure 3F-H**). Moreover, H&E staining of sperm revealed normal sperm morphology in *Socs7*-KO mice (**Figure 3I**, **3J**). In summary, the knockout of SOCS7 did not noticeably affect male fertility or sperm quality.

Spermatogenesis is normal in Socs7-KO mice

The seminiferous tubules are the primary structures involved in spermatogenesis. To assess their morphology, we performed H&E staining on testicular sections from both WT and *Socs7*-

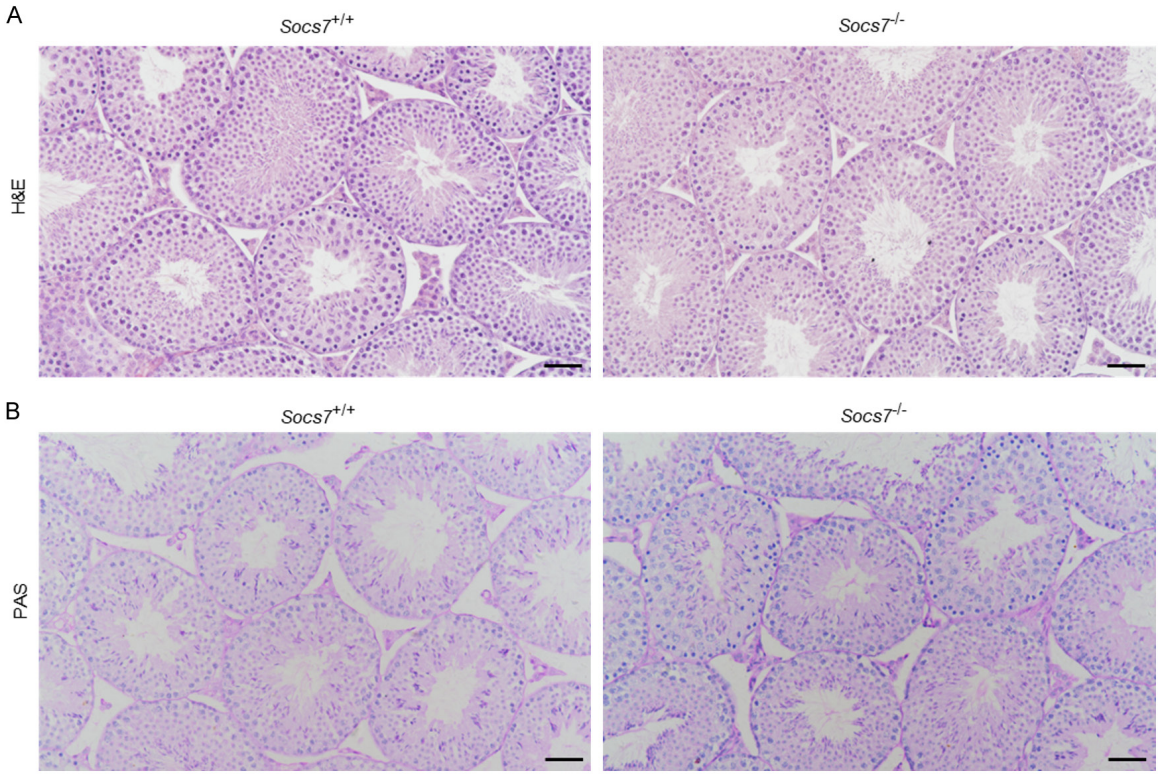

Figure 4. Histological analysis of testes from 8-week-old mice. A. H&E staining of testis sections from WT and *Socs7*-KO mice (Scale bar: 50 μm). B. PAS staining of testis sections from WT and *Socs7*-KO mice (Scale bar: 50 μm).

KO mice. The results showed no morphological abnormalities in the seminiferous tubules of *Socs7*-KO mice (**Figure 4A**). Similarly, PAS staining of testicular sections from both groups revealed intact seminiferous tubules (**Figure 4B**).

We next evaluated the expression of various germ cell markers during spermatogenesis in testicular sections from WT and *Socs7*-KO mice using immunofluorescence. The results revealed no statistically significant differences in the expression of Lin28 (spermatogonial stem cell marker) [28], γ-H2AX (spermatocyte marker) [29], PNA (acrosome marker) [30], SOX9 (Sertoli cell marker) [31], and 3β-HSD (Leydig cell marker) [32], between the two groups (**Figure 5**). These findings suggest that the knockout of *Socs7* does not significantly affect spermatogenesis in mice.

Analysis of testicular cell apoptosis in Socs7-KO mice

Although *Socs7* is significantly expressed in the testes of mice, the knockout of *Socs7* did not affect fertility or spermatogenesis. To further investigate this, we performed a TUNEL assay to assess testicular cell apoptosis in *Socs7*-KO mice (**Figure 6A**). The results showed no statistically significant differences in TUNEL-positive cells or the percentage of TUNEL-positive tubules among the total spermatogenic tubules between WT and *Socs7*-KO mice (**Figure 6B**, **6C**).

Discussion

Ubiquitination is a critical post-translational modification involved in the formation of spermatogonial stem cells and the differentiation of spermatogonia during spermatogenesis [33]. E3 ubiquitin ligases, key enzymes in the ubiquitination process, play an irreplaceable role in the sperm acrosome formation [34]. The SOCS family, including SOCS7, participate in recruiting E3 ubiquitin ligase complexes through the conserved C-terminal SOCS-box domain. SOCS7 has been shown to regulate processes such as inflammatory responses [35, 36], signal transduction (including insulin and leptin signaling) [37], and tumor proliferation (in ovar-

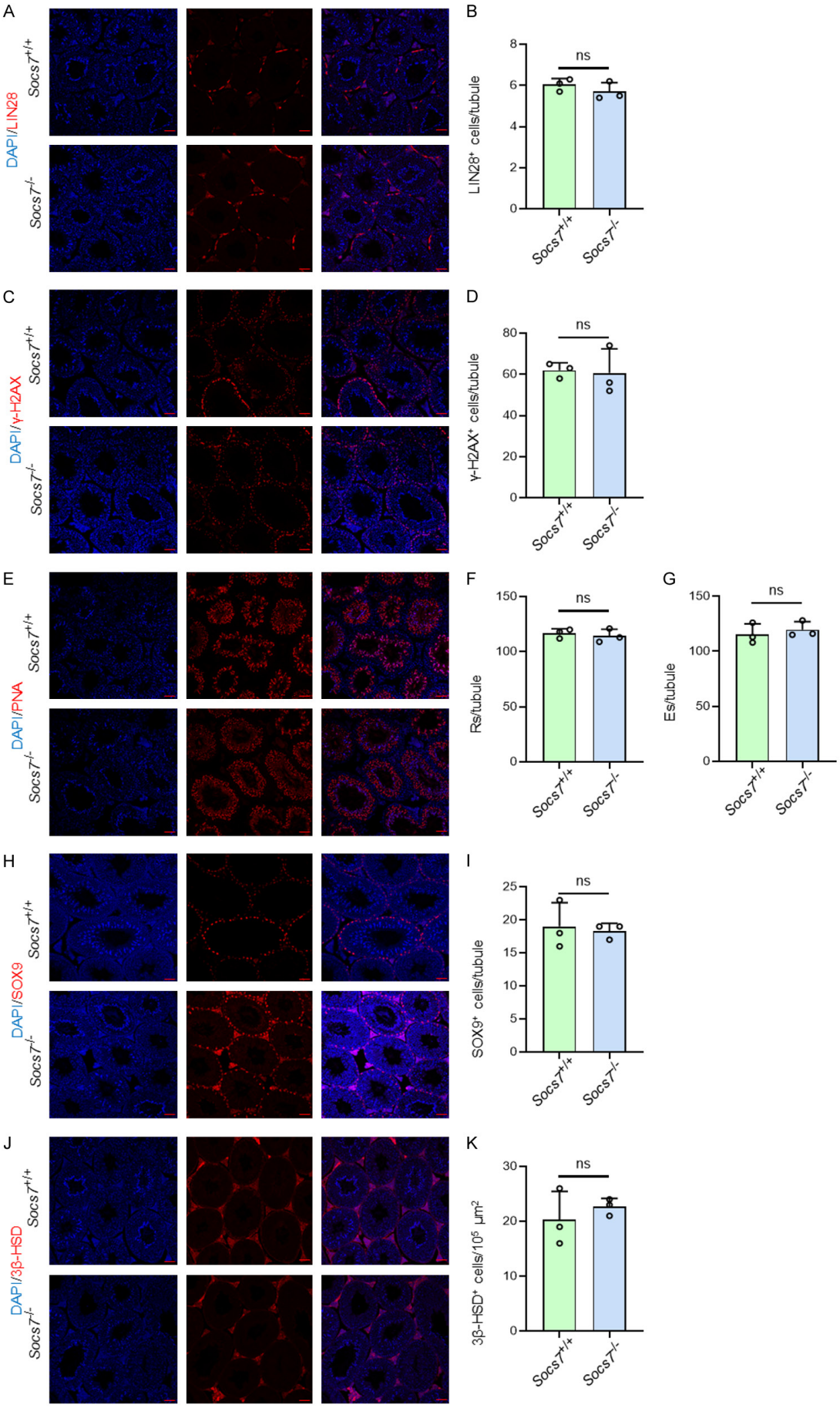
A
DAPI/LIN28
Socs7+/+
Socs7-/-
B
LIN28+ cells/tubule
ns
C
DAPI/γ-H2AX
D
γ-H2AX+ cells/tubule
E
DAPI/PNA
F
Rs/tubule
G
Es/tubule
H
DAPI/SOX9
I
SOX9+ cells/tubule
J
DAPI/3β-HSD
K
3β-HSD+ cells/10⁵ μm²

Figure 5. Immunofluorescence analysis of spermatogenesis in *Socs7*-KO mice. (A) Immunostaining of Lin28 in testis sections from WT and *Socs7*-KO mice (Scale bar: 50 μm). (B) Quantification of Lin28 expression shown in (A) (n = 3, P > 0.05). (C) Immunofluorescence of γ-H2AX in testis sections from WT and *Socs7*-KO mice (Scale bar: 50 μm). (D) Quantification of γ-H2AX levels in (C) (n = 3, P > 0.05). (E) Immunostaining of PNA in testis sections from WT and *Socs7*-KO mice (Scale bar: 50 μm). (F) Quantification of round spermatids (Rs) in (E) (n = 3, P > 0.05). (G) Quantification of elongating/elongated spermatids (Es) in (E) (n = 3, P > 0.05). (H) Immunostaining of SOX9 in testis sections from WT and *Socs7*-KO mice (Scale bar: 50 μm). (I) Quantification of SOX9 expression in (H) (n = 3, P > 0.05). (J) Immunostaining of 3β-HSD in testis sections from WT and *Socs7*-KO mice (Scale bar: 50 μm). (K) Quantification of (J) (n = 3, P > 0.05).

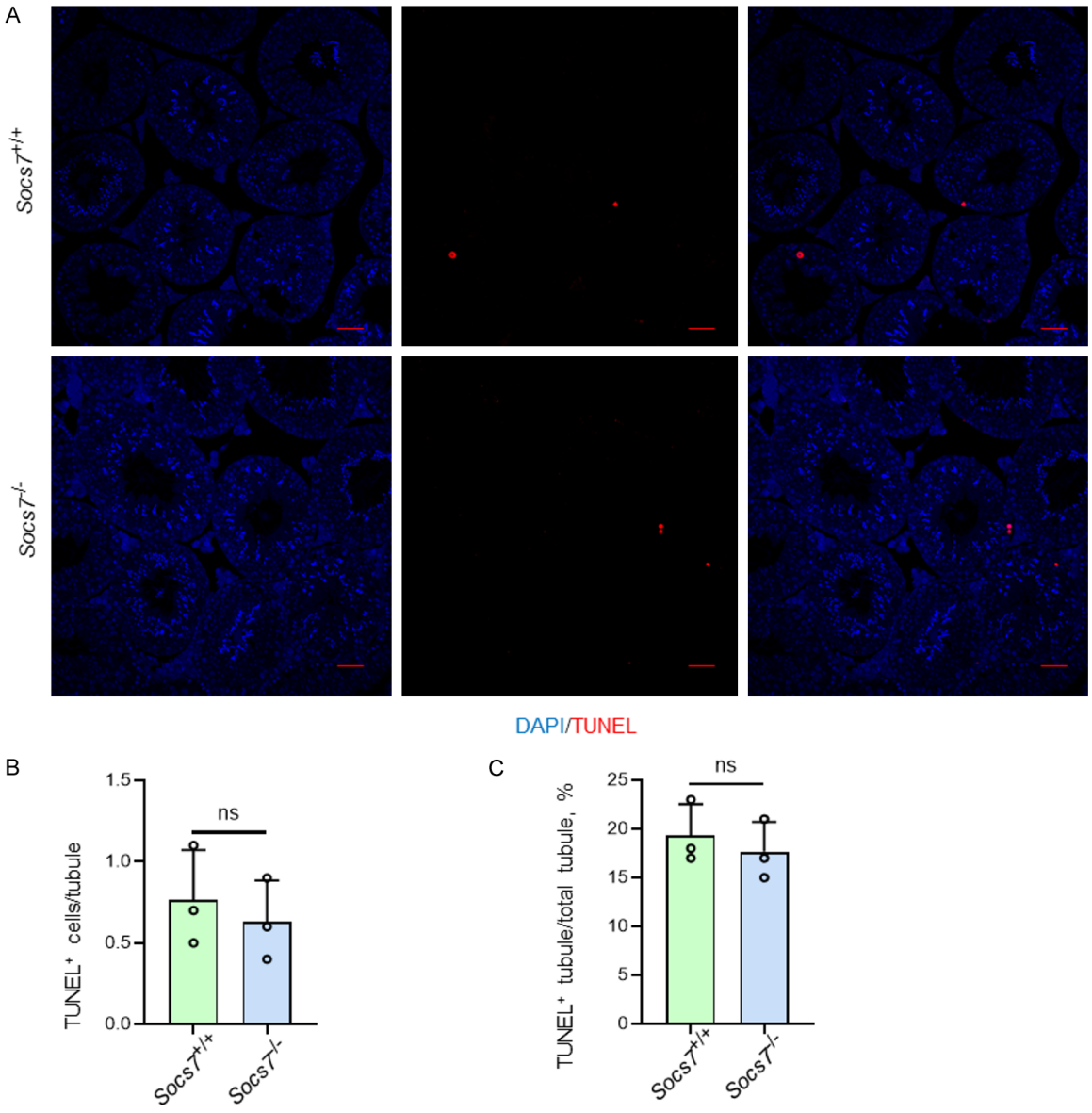

Figure 6. TUNEL assay analysis of apoptosis in *Socs7*-KO mice testes. (A) TUNEL staining of testes from WT and *Socs7*-KO mice (Scale bar: 50 μm). (B) Quantification of apoptotic cells shown in (A) (n = 3, P > 0.05). (C) Percentage of TUNEL-positive tubules among total spermatogenic tubules (n = 3, P > 0.05).

ian cancer, hepatocellular carcinoma, and breast cancer) [15, 16, 38]. However, its role in male germ cells remains unclear.

Bioinformatics analysis revealed that SOCS7 is highly expressed in both human and mouse testes, primarily in spermatogonia and sper-

matocytes, suggesting a critical regulatory role in male fertility and spermatogenesis. To investigate SOCS7's function, we generated *Socs7*-KO mice using the CRISPR/Cas9 system. Contrary to our initial hypothesis, *Socs7*-KO male mice exhibited normal fertility, with no significant differences in litter size or number of pups per litter compared to wild-type (WT) controls. Testicular morphology, seminiferous tubule structure, testis/body weight ratio, and sperm quality - including concentration, motility, and morphology - were unaffected by *Socs7* deletion. Additionally, no significant differences were found in the TUNEL assay for apoptosis or in the expression of key germ cell markers (Lin28, γ-H2AX, PNA, SOX9, and 3β-HSD) between WT and *Socs7*-KO mice. These results suggest that the loss of SOCS7 does not lead to obvious phenotypic defects in spermatogenesis, possibly due to compensatory mechanisms. For instance, other members of the SOCS family, which also contain the SOCS-box domain and interact with the E3 ubiquitin ligase complex [39], might compensate for the loss of SOCS7. Future research should explore the roles of other SOCS family members in male germ cells and identify compensatory molecules, as well as elucidate their interaction mechanisms.

In summary, our study shows that while SOCS7 is highly expressed in the testes of both humans and mice, its deletion does not significantly affect male fertility, testicular morphology, or spermatogenesis. These findings provide important insights for future research and help prevent redundant efforts in investigating similar questions in other studies.

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Disclosure of conflict of interest

None.

Address correspondence to: Cong Shen, State Key Laboratory of Reproductive Medicine and Offspring Health, Center for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou 215002, Jiangsu, China. Tel: +86-15050388584; E-mail: congshen@njmu.edu.cn; Jianjun Xie, Department of Urology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, Suzhou 215002, Jiangsu, China. Tel: +86-18012603488; E-mail: xjjmobile@njmu.edu.cn; Yibo Wu, Human Reproductive and Genetic Center, Affiliated Hospital of Jiangnan University, Wuxi 214062, Jiangsu, China. Tel: +86-13771193920; E-mail: 9862016107@jiangnan.edu.cn

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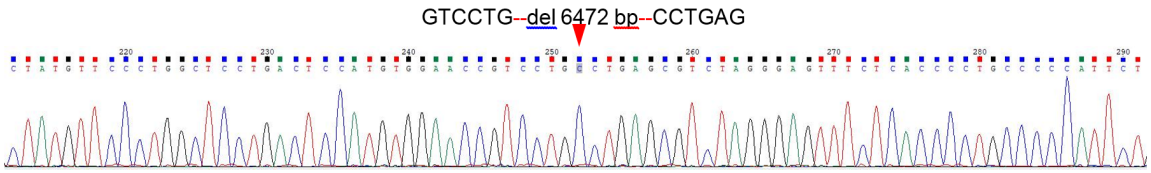

Figure S1. Sanger sequencing of PCR product in *Socs7*-KO mice.