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Loss of α -catenin in germ cells impairs spermatogenesis and flagellar function associated with teratozoospermia

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ABSTRACT

Infertility affects approximately 10%–20% of couples worldwide, with male factors accounting for roughly half of all cases. Teratozoospermia, defined by the occurrence of abnormally shaped sperm, is a significant contributor to male infertility. Here, by analyzing a publicly available RNA array dataset comparing teratozoospermia patients and normal controls, we uncovered disrupted adherens junction (AJ)-associated signaling and defective spermatogenesis within germ cells of teratozoospermia patients. In adherens junctions (AJs), α -catenin (*CTNNA1*) anchors the cadherin-catenin complex to the actin cytoskeleton and regulates actin organization, but its role in germ cell development and sperm function remains unknown. We generated a germ cell-specific *Ctnna1* conditional knockout mouse model (*Ctnna1*^{CKO}). Homozygous *Ctnna1*-deficient mice exhibited male infertility with abnormal structure and function of spermatozoa. Single-cell RNA sequencing of control and *Ctnna1*^{CKO} testes revealed an accumulation of spermatogonia with impaired differentiation and abnormal acrosome structure in *Ctnna1*-deficient mice, likely caused by disrupted splicing and meiotic processes through dysregulated vinculin. Additionally, loss of α -catenin disrupted both direct and indirect interactions between spermatogenic cells and Sertoli cells, as evidenced by N-cadherin internalization that mediates their adhesion and by reduced Cyclophilin A/CD147 paracrine signaling essential for spermatogenesis. Mechanistically, loss of α -

catenin results in disorganization of the α -catenin/vinculin/N-cadherin complex, leading to defective cell-cell interactions and impairment of spermatogenesis. Collectively, our study establishes α -catenin as a critical regulator of spermatogenesis and germ cell development and highlights its potential as a prognostic biomarker and therapeutic target for male infertility, particularly teratozoospermia.

Keywords: α -catenin; Teratozoospermia; ScRNA sequencing; Spermatogenesis; Sertoli-germ cell communication

INTRODUCTION

Infertility represents a pervasive global health burden, with an estimated 17.5% prevalence among reproductive-aged individuals (Agarwal et al., 2021; Mohammadzadeh et al., 2024). Notably, approximately 20%–30% of infertility cases are attributed solely to male factors, with their involvement extending to 50% of overall cases (Agarwal et al., 2015, 2021; Mohammadzadeh et al., 2024). While assisted reproductive technologies (ART) have improved fertility outcomes, the decline in semen quality and increasing male infertility rates pose significant challenges for patients seeking to overcome infertility (Cavalhas-Almeida et al., 2025; Minhas et al., 2021).

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Male fertility is critically dependent on spermatogenesis, a highly regulated process of dramatic shape change where spermatids differentiate into spermatozoa containing a structurally intact sperm head, a functional head-tail coupling apparatus (HTCA), and a motile flagellum with normal morphological architecture (Parker, 2020; Roldan, 2019; Wu et al., 2024). The process of spermatogenesis encompasses three sequential stages: spermatogonia mitosis, spermatocyte meiosis, and spermiogenesis (Wu et al., 2024). Defects in either mitosis or meiosis typically result in azoospermia, while failure of spermiogenesis manifests as diminished sperm concentration, compromised motility, and abnormal spermatozoa, a condition clinically termed asthenoteratozoospermia, a subtype of teratozoospermia (Cavallini, 2006; Northmore & Oh, 1998). Teratozoospermia is characterized by a percentage of morphologically normal spermatozoa below the lower reference limit, with cut-off values for normality varying greatly in recent decades from 50% (WHO, 1980) to 4% (WHO, 2010) (Coutton et al., 2015). As a heterogeneous condition, teratozoospermia encompasses a wide range of abnormal sperm phenotypes, which singularly or concurrently compromise the morphology of the sperm head, neck, midpiece, and tail.

The flagellum of spermatozoa exhibits an evolutionarily conserved “9+2” axonemal structure, consisting of nine peripheral doublet microtubules (DMTs) surrounding a central pair apparatus (CP) of two microtubules (Inaba & Mizuno, 2016). Doublet microtubules exhibit a 96 nm repeating unit architecture, within which axonemal dynein, radial spokes (RSs), and the nexin-dynein regulatory complex (N-DRC) are spatially organized (Kumar & Singh, 2021). In the midpiece of the flagellum, the “9+2” axonemal structure is encircled by nine outer dense fibers (ODFs) (Yan, 2009). These ODFs are paired with nine peripheral DMTs and enveloped by a mitochondrial sheath, collectively forming the “9+9+2” pattern (Yan, 2009). Defects in ODF1 have been demonstrated to be associated with ASS in mouse models (Yang et al., 2012). The HTCA is a complex structure composed of a proximal centriole, a distal centriole, and pericentriolar dense material (Fawcett & Phillips, 1969). This apparatus is indispensable for anchoring the sperm head to the flagellum, particularly during high-speed motility (Wu et al., 2020a). Defects in either the sperm flagellum or HTCA may impair motility or induce morphological abnormalities, clinically recognized as multiple morphological abnormalities of the sperm flagella (MMAF, which always causes severe asthenozoospermia) or acephalic spermatozoa syndrome (ASS), both of which are common forms of asthenoteratozoospermia that significantly contribute to male infertility (Jin et al., 2023a; Tu et al., 2020; Wu et al., 2020a). Previous studies have documented a co-occurrence of defects in HTCA and sperm flagellum, which phenotypically aligns with the presence of acephalic spermatozoa in teratozoospermic patients (Chemes & Rawe, 2010; Hall et al., 2013; Moretti et al., 2011; Shang et al., 2017; Yuan et al., 2015; Zhang et al., 2021b; Zhu et al., 2018).

In the testis, the cadherin/catenin complex functions as an integral unit, along with $\alpha 6 \beta 1$ integrin and the nectin/afadin complex, to finely regulate adherens junctions (AJs) dynamics at different stages of germ cell development during the seminiferous epithelial cycle (Lee et al., 2003). The ectoplasmic specialization (ES) is one of the specialized testis-specific actin-based AJs, and the apical ES is the only junction type at the Sertoli-elongating spermatid (step 8 and

beyond) interface, while the basal ES is intermixed with tight junctions, the basal tubulobulbar complex (TBC, another testis-specific AJ type), and desmosome-like junctions at the blood-testis barrier (BTB). The precise regulation of AJs and actin bundles facilitates extensive junction restructuring within the seminiferous epithelium (Cheng & Mruk, 2002; Mruk & Cheng, 2004), which in turn enables developing germ cells to migrate from the basal to the adluminal compartment for further development (Russell, 1977). Here, our unbiased analysis of a publicly available RNA array dataset (Platts et al., 2007) (GSE6872) revealed that germ cells from teratozoospermic patients exhibit pronounced defects in reproductive function alongside a notable reduction in AJ-associated signaling genes, such as *CTNNA1*. Crucially, α -catenin (encoded by *CTNNA1*) exerts a dual regulatory role in cytoskeletal dynamics and cell-cell adhesion by anchoring the cadherin-catenin complex to the actin cytoskeleton and orchestrating actin filament organization (Platts et al., 2007). However, the role of germ cell-derived AJs, especially α -catenin, in the development and function of sperm cells remains unknown.

In this study, we generated a conditional knockout (cKO) mouse model for *Ctnna1* by utilizing a male germ cell-specific Cre (*Stra8*-P2A-ZsGreen1-T2A-Cre) mouse line. Importantly, homozygous *Ctnna1*-depleted male mice were sterile with abnormal structure and function of spermatozoa. Single-cell RNA sequencing (scRNA-seq) analyses on testes from control and mutant mice revealed a remarkable accumulation of spermatogonia (SPG) with defective differentiation trajectory in the absence of *Ctnna1*, possibly due to disrupted splicing and meiosis. Additionally, we identified that loss of *Ctnna1* impairs cell-cell interactions between spermatogenic cells and Sertoli cells, with compromised Cyclophilin A/CD147 interaction that is pivotal for spermatogenesis (Bi et al., 2013). Mechanistically, α -catenin forms complexes with vinculin (VCL) and N-cadherin (*CDH2*) to maintain the link between cadherins and actin filaments. Loss of α -catenin leads to disorganization of the complex, leading to compromised spermatogenic/Sertoli cell interaction and defective spermatogenesis. Collectively, our study not only establishes the pivotal role of α -catenin in regulating spermatogenesis and germ cell development but also highlights its potential as a prognostic biomarker for male infertility, especially teratozoospermia.

MATERIAL AND METHODS

Animals

Floxed *Ctnna1* mice (*Ctnna1^{flox/flox}*) were obtained from the Jackson Laboratory (Stock number: 004604; <https://www.jax.org/strain/004604>), as previously described (Vasioukhin et al., 2001; Zhu et al., 2021). To generate germ cell-specific *Ctnna1* knockout mice, *Ctnna1^{flox/flox}* mice were first crossed with *Stra8*-P2A-ZsGreen1-T2A-Cre transgenic mice (Cyagen, Stock No. C001536; hereafter referred to as *Stra8*-Cre). Female offspring harboring the *Ctnna1^{flox/-}*; *Stra8*-Cre genotype were then bred with *Ctnna1^{flox/flox}* male mice to produce male progeny with germ cell-specific deletion of *Ctnna1* (*Ctnna1^{ckO}*). Since the *Ctnna1*-deleted allele cannot be transmitted through sperm, the control *Ctnna1^{flox/flox}* mice were generated by crossing *Ctnna1^{flox/flox}* males with *Ctnna1^{flox/flox}* females. All animals were maintained in a specific pathogen-free (SPF) facility under controlled

conditions (temperature: 23°C; 12-h light/12-h dark cycle), with ad libitum access to food and water. The primer sequences used for genotyping are provided in Supplementary Table S1. All animal experiment procedures were approved by the Animal Protection and Use Committee of Sichuan Provincial People's Hospital (Approval No. 2025-512) and conducted in accordance with the ethical standards and guidelines of the Chinese Animal Welfare Committee for the care and use of laboratory animals.

Sperm motility analysis

Spermatozoa were harvested by dissecting the cauda epididymides and incubating them in YTH buffer (M2035, Nanjing Aibei Biotechnology, China) at 37°C for 15 min. Subsequently, sperm motility parameters were analyzed using a computer-aided sperm analysis (CASA) system (ML-608JZII, Mailang, China).

Histological analysis and immunofluorescence staining

Testes and epididymides were freshly collected and fixed overnight in Bouin's solution (HT10132, Sigma, China). Following fixation, tissues were dehydrated through a graded ethanol series (70%, 80%, 95%, and 100%, each for 2 h), cleared in xylene (Mackhours, China), and infiltrated with molten paraffin at 65°C overnight. The paraffin-embedded samples were sectioned at 4 µm. Following dewaxing and rehydration, tissue sections were stained with hematoxylin and eosin (60524ES60, Yeasen, China) or Periodic acid-Schiff (C0142M, Beyotime, China) reagent according to standard histological protocols. For immunofluorescence staining, the slices were immersed in 0.01% sodium citrate buffer (pH 6.0, C1010, Solarbio, China) and boiled in water for 20 min for antigen retrieval. Primary antibodies were incubated overnight at 4°C, followed by incubation with secondary antibodies at 37°C for 1 h. The antibodies used for immunofluorescence staining are listed in Supplementary Table S2.

Transmission electron microscopy (TEM)

Tissue samples were initially prefixed in 2.5% glutaraldehyde, followed by postfixation in 1% osmium tetroxide. After fixation, samples were dehydrated through a graded acetone series (30%, 50%, 70%, 80%, 90%, 95%, and 100%), with the 100% acetone changed three times to ensure complete dehydration. The specimens were then infiltrated with increasing concentrations of Epon 812 resin in acetone at ratios of 3:1, 1:1, and 1:3, and finally embedded in pure Epon 812 embedding medium. Semithin sections were prepared for light microscopic localization, and regions enriched in spermatozoa were selected for further processing. Ultrathin sections (60–90 nm) were cut using a diamond knife, placed on copper grids, and stained with uranyl acetate for 10–15 min, followed by lead citrate staining for 1–2 min at room temperature. The sections were then examined using a JEM-1400-FLASH transmission electron microscope (JEOL, Japan).

Scanning electron microscopy (SEM)

Fresh spermatozoa samples were fixed in 2.5% phosphate-buffered glutaraldehyde. Then the specimens were dehydrated through an ascending gradient of ethanol (50%, 70%, 95%, and 100%) and air-dried. The specimens were attached to specimen holders, sputter-coated with gold particles, and then examined using a JSM-IT700HR scanning electron microscope (SEM) (JEOL, Japan).

Cell culture and *CTNNA1* knockdown

HEK293 STF cells were purchased from ATCC (American

Type Culture Collection) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 mg/mL penicillin, and 100 mg/mL streptomycin at 37°C in a 5% CO₂ atmosphere. HEK293STF were transduced with a lentivirus carrying shRNA targeting *CTNNA1* (5'-CACCTCAGAGATGGACAACTA-3', Genechem, China), according to the manufacturer's instructions.

RNA extraction and qPCR

Total RNA was extracted using TRIzol reagent (ET101-01-V2, TransGen Biotech, China) following the manufacturer's instructions. Complementary DNA (cDNA) was synthesized directly from RNA templates using the TransScript® One-Step RT-PCR SuperMix kit (AT411-02, TransGen Biotech, China). Quantitative PCR (qPCR) was performed on an ABI 7500 Real-Time PCR System using TransStart® Tip Green qPCR SuperMix (AQ142-21, TransGen Biotech, China). Gene expression was normalized to *Gapdh*/*GAPDH* as the internal control, and fold changes (FCs) were calculated using the $2^{-\Delta\Delta Ct}$ method. The sequences of primers used in this study are provided in Supplementary Table S3.

Co-immunoprecipitation and western blot analysis

Co-immunoprecipitation assays for both cellular overexpression and endogenous proteins were performed as previously described (Zhu et al., 2021). For the overexpression assay, HEK293T cell lysates overexpressing HA-tagged vinculin and FLAG-tagged α -catenin (or vector) were incubated with anti-FLAG Magnetic Beads (HY-K0201, MedChemExpress, USA). For endogenous interaction, testicular lysates from 2-month-old mice were incubated with Protein A/G magnetic beads (HY-K0202, MedChemExpress, USA) conjugated with anti- α -catenin antibody or IgG control. For western blot analysis, samples were lysed in buffer (HY-K1001, MedChemExpress, USA) supplemented with protease (HY-K0010, MedChemExpress, USA) and phosphatase (HY-K0021, MedChemExpress, USA) inhibitor cocktails, followed by ultrasonic disruption to ensure complete protein extraction. The extracted proteins were separated on 4%–20% SDS-polyacrylamide gels and subsequently transferred onto nitrocellulose membranes. Membranes were blocked with 5% skim milk in TBST for 1 h at room temperature. Subsequently, membranes were incubated overnight at 4°C with primary antibodies diluted in blocking solution. After incubation with secondary antibodies, signals were detected using the SuperSignal™ West Dura Extended Duration Substrate (34075, Thermo Fisher Scientific, USA) with a Touch Imager (e-BLOT, China) and analyzed using ImageJ software. Details of the antibodies are provided in Supplementary Table S4.

Public RNA microarray data analysis

The expression matrix of RNA microarray data of spermatozoa from control and teratozoospermic patients was obtained from GSE6872. The differential analysis was performed using the Limma R package, and genes were identified as differentially expressed if they met the criteria of a $P < 0.05$ and $|\log_2 FC| > 1$.

Preparation of single-cell suspension

Testes were isolated from three 8-week-old control and three *Ctnna1*^{cko} male mice. Tissues were dissected, and the tunica albuginea was removed in PBS. The tissue samples were washed twice with pre-chilled RPMI 1640 medium supplemented with 0.04% BSA. The tissues were then finely minced using surgical scissors into approximately 0.5 mm³

pieces and transferred to a freshly prepared digestion buffer containing 0.4 mg/mL collagenase I (Cat# 17100017, Gibco, USA) and 0.6 mg/mL collagenase II (Cat# 17101015, Gibco, USA). Enzymatic digestion was performed in a 37°C incubator for 30–60 min with gentle inversion every 5–10 min to ensure thorough mixing. Following digestion, the resulting cell suspension was filtered once or twice through a 40 µm cell strainer (BD, USA) and centrifuged at 300 ×g for 5 min at 4°C. The pellet was resuspended in an appropriate volume of culture medium and mixed with an equal volume of red blood cell lysis buffer (Cat# 130-094-183, MACS, Germany). The mixture was incubated at 4°C for 10 min. Cells were then centrifuged at 300 ×g for 5 min at 4°C, and the supernatant was discarded. The resulting pellet was washed once with culture medium, centrifuged again at 300 ×g for 5 min at 4°C, and the supernatant was removed. Finally, the cell pellet was resuspended in 100 µL of culture medium, and cell concentration and viability were determined using the Luna™ automated cell counter.

Single-cell RNA sequencing and data analysis

Cell suspensions were prepared as described above. Single-cell suspensions were processed using the 10× Genomics Chromium™ system according to the manufacturer's instructions based on the Chromium Next GEM technology. Libraries were generated using the Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (10× Genomics, USA). Briefly, individual cells were encapsulated into nanoliter-scale Gel Bead-In-Emulsions (GEMs) along with barcoded gel beads containing unique molecular identifiers (UMIs), cell barcodes, and poly (dT) primers. Following GEM generation, reverse transcription was performed to synthesize barcoded full-length cDNA from captured mRNA transcripts. After breaking the emulsion using a recovery agent, barcoded cDNA was purified and amplified via PCR. Library construction was carried out using the following steps: (1) enzymatic fragmentation, end repair, and A-tailing of the amplified cDNA; (2) size selection with SPRIselect beads; (3) adaptor ligation; (4) post-ligation cleanup with SPRIselect beads; (5) sample index PCR followed by final cleanup. The resulting indexed libraries were quantified, pooled according to the estimated number of recovered cells, and sequenced on an Illumina NovaSeq 6000 platform using paired-end 150 bp reads.

Enrichment analysis

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs) were performed using clusterProfiler. Gene Set Enrichment Analysis (GSEA) was performed using the clusterProfiler R package and gene sets from KEGG or GO databases. The signaling scores of spermatogenic cells subclusters were calculated based on the expressions of genes from KEGG or GO gene sets using the AddModuleScore function in the Seurat package.

RNA velocity analysis

To perform the RNA velocity analysis, the spliced reads and unspliced reads were recounted using the Python script velocity.py (<https://github.com/velocity-team/velocity.py>) on the Cell Ranger output folder. The calculation of RNA velocity values for each gene in each cell and the embedding RNA velocity vector to low-dimensional space were performed with the R package velocity.R. Velocity fields were projected onto the Uniform Manifold Approximation and Projection (UMAP)

embedding obtained in Seurat.

Monocle3 pseudotime analysis

Monocle3 was used to infer the cell differentiation trajectories without prior knowledge of differentiation time or direction. Under an unsupervised framework, algorithms place cells along a trajectory corresponding to a biological process by taking advantage of an individual cell's asynchronous progression.

Cell communication analysis by CellChat

The cell communication analysis was performed using the CellChat R package. First, we imported the normalized expression matrix to create the CellChat object with the createCellChat function. Secondly, the data was preprocessed using the identifyOverExpressedGenes, identifyOverExpressedInteractions and projectData functions using the default parameters. The computeCommunProb, filterCommunication (min.cells=10) and computeCommunProbPathway functions were then used to determine any potential ligand-receptor interactions. Finally, the cell communication network was aggregated using the aggregateNet function.

Collection of sperm from cauda epididymis

To clear the epididymal lumen and collect sperm from mice, tissues including the cauda epididymis and proximal vas deferens were isolated from euthanized mice. Retrograde perfusion was carried out by inserting a drawn-out PVC catheter into the vas deferens, allowing the contents to be washed out via a cut end in the proximal cauda, following previously described methods (Yeung et al., 2004).

Immunofluorescence analysis of chromosome spreads

Immunofluorescence analysis of chromosome spreads was performed using testes from postnatal day 18 *Ctnna1*^{CKO} and control mice. Testes were dissected, decapsulated, and immersed in hypotonic extraction buffer. The tissue was minced with a sterile razor blade and gently teased apart with fine forceps, followed by incubation on ice for 40 min. A 200 µL drop of 100 mM sucrose solution was placed onto a clean glass slide. Hypotonic-treated tissue fragments were transferred into the sucrose drop until the solution became visibly turbid. The remaining tissue fragments were discarded. Glass slides were pre-wetted in fixation solution containing 1% paraformaldehyde and 0.15% Triton X-100 (pH 9.2). Slides were removed from the fixative, retaining a small drop of fixative at one corner. Subsequently, 50 µL of the turbid sucrose–cell suspension was added to the retained fixative drop, mixed directly on the slide, and immediately spread evenly across the slide surface. Slides were incubated overnight at 4°C. Following incubation, slides were washed three times in 0.4% Photo-Flo solution for 10 min each, air-dried, and stored at –80°C until analysis.

Blood-testis barrier permeability assay

Two-month-old *Ctnna1*^{CKO} and control mice were intraperitoneally injected with 200 µL of 2% (w/v) Evans blue (EB) solution. Four hours post-injection, mice were anesthetized and subjected to transcardial perfusion with phosphate-buffered saline (PBS) until the effluent became clear. The testes were immediately collected and weighed. To extract the dye, testes were incubated individually in 1 mL of formamide at 37°C for 24 h. Following incubation, samples were centrifuged at 12 000 ×g for 15 min at 37°C, and 100 µL

of the supernatant was carefully transferred to a 96-well plate. A standard curve was generated using serial dilutions of EB. Absorbance was measured at 620 nm using a microplate reader (FlexA-200, Allsheng Instruments Co., Ltd, China). The EB concentration was calculated based on the standard curve, normalized to testis weight, and expressed as μg EB/mg tissue.

Multiplex immunofluorescence staining

Paraffin sections were deparaffinized and subjected to antigen retrieval, followed by blocking for 20 min. Sections were then incubated with primary antibodies overnight at 4°C. After washing three times with TBST for 3 min each, sections were incubated with the secondary antibody provided in the kit (abs50028, Absin, China) at room temperature for 10 min. Following three additional washes with TBST (3 min each), fluorescent labeling was performed using a 1 $\times$ fluorophore diluted in signal amplification buffer for one fluorescence channel (abs50028, Absin, China). Sections were incubated at room temperature in the dark for 10 min and subsequently washed three times with TBST for 5 min each. After each staining cycle, sections were subjected to antigen retrieval again, followed by blocking for 20 min, and the staining procedure was repeated as described above. This process was repeated until all target antigens were labeled. Finally, sections were counterstained with 1 $\times$ DAPI for 10 min, washed, and coverslipped with antifade mounting medium.

In vitro fertilization (IVF)

IVF was performed as previously described with minor modifications (Takeo & Nakagata, 2011). Oocytes were collected from the fallopian tubes into 200 μL HTF medium (CAT# M1130, Easy Check, China) under mineral oil. Spermatozoa were obtained from the cauda epididymis in 200 μL HTF and incubated at 34°C, 5% CO_2 for 1 h for capacitation. The supernatant containing motile sperm ($1 \times 10^6/\text{mL}$) was aspirated from the periphery of the capacitation drop and transferred into oocytes and co-incubated for 6 h, after which the medium was replaced with KSOM (CAT# M1430, Easy Check, China). Embryo development was assessed under a microscope, and blastocysts were transferred *in vivo*.

Statistical analysis

All statistical analyses were performed using GraphPad Prism v.9.0. For comparisons between two groups, an unpaired Student's *t*-test was applied when the data followed a normal distribution; otherwise, a nonparametric test with Welch's correction was used. Each experiment was independently repeated at least three times.

RESULTS

Compromised adherens junctions are associated with male teratozoospermia

To explore novel mechanisms underlying teratozoospermia, we analyzed a publicly available RNA microarray dataset (Platts et al., 2007) (GSE6872) of sperm cells from males with severe teratozoospermia and normal controls. An unbiased GO enrichment analysis of DEGs revealed a significant enrichment in biological processes (BP) and cellular components (CC) essential for male reproduction, including spermatogenesis and axonemal structure/function (Figure 1A). Notably, we observed a prominent enrichment of DEGs in the

GO molecular function (MF) term cadherin binding (Figure 1A), indicating that disturbed germ cell AJs may be associated with teratozoospermia. To determine whether the pathways mentioned above were activated or suppressed in teratozoospermia, we applied GSEA. As a result, GSEA of GO terms demonstrated that germ cells from teratozoospermia patients showed significant negative associations with reproductive functions and cadherin binding (Figure 1B–D), indicative of impaired spermatogenesis, defective axonemal structure/function, and disrupted AJs. Similarly, GSEA of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database revealed negative correlations with pathways linked to reproduction (meiosis and spliceosome) and AJs (Figure 1E). Core AJ-associated genes, including *CDH1*, *CDH5*, *CTNNA1*, and *CTNNA1* (which encode components of the cadherin/catenin complex), as well as *VCL*, *ACTB*, *RAC1*, and *CDC42* (involved in cytoskeletal regulation), were significantly downregulated in sperm from teratozoospermia patients compared with normozoospermic controls (Figure 1F). These results highlight a potential role of AJs in orchestrating the process of spermatogenesis and ciliary function. Furthermore, analysis of an independent microarray dataset (Platts et al., 2007) (GSE6967) confirmed a 2.5-fold decrease in *CTNNA1* expression levels in teratozoospermia sperm (Figure 1G). Since *CTNNA1* encodes α -catenin, which serves as a pivotal linker connecting the cadherin/catenin complex to the actin cytoskeleton network (Cheng & Mruk, 2002; Gumbiner, 2000), its reduced expression may compromise AJ integrity and thereby disrupt spermatogenesis and ciliary function. Therefore, we speculate that downregulation of *CTNNA1* may be mechanistically involved in the spermatogenic defects observed in teratozoospermia.

Loss of *Ctnna1* leads to male infertility and sperm structural defects

Given the ubiquitous expression of *CTNNA1* in both testicular germ cells and somatic cells (Supplementary Figure S1), we generated a germ cell-specific *Ctnna1* knockout mouse model to investigate the role of α -catenin in spermatogenesis. We employed a *Stra8*-P2A-ZsGreen1-T2A-Cre transgenic mouse (hereafter referred to as *Stra8*-Cre) to conditionally delete *Ctnna1* in advanced germ cells, with recombination initiated at the 1 spermatogonia (SPG) stage, preceding meiotic entry (Han et al., 2025; Lin et al., 2017) (Figure 2A). Gene deletion was initially validated by western blotting and real-time quantitative PCR (qPCR) in testes (Supplementary Figure S2A–C). However, remaining α -catenin protein was detected in whole testicular lysates from *Ctnna1*^{fllox/+}; *Stra8*-Cre (hereafter termed *Ctnna1*^{CKO}) mice, likely attributable to non-germ cell populations (Supplementary Figure S1; Supplementary Figure S2A–C) (Han et al., 2025). To verify germ cell-specific deletion, we assessed *Ctnna1* levels in purified spermatozoa and the sperm-free cauda epididymis using qPCR. The results confirmed robust depletion (> 90%) of *Ctnna1* in *Ctnna1*^{CKO} spermatozoa compared to *Ctnna1*^{fllox/fllox} (Ctrl) mice (Figure 2B), confirming efficient *Ctnna1* knockout in germ cells. Due to the presence of the *Ctnna1* null allele (*Ctnna1*^{−/−}), *Ctnna1* levels were reduced by approximately 50% in the sperm-free cauda epididymis of the *Ctnna1*^{CKO} group (Supplementary Figure S2D), confirming the absence of ectopic Cre recombinase activity in somatic tissues. Male *Ctnna1*^{CKO} mice were completely sterile and failed to produce offspring when mated with either

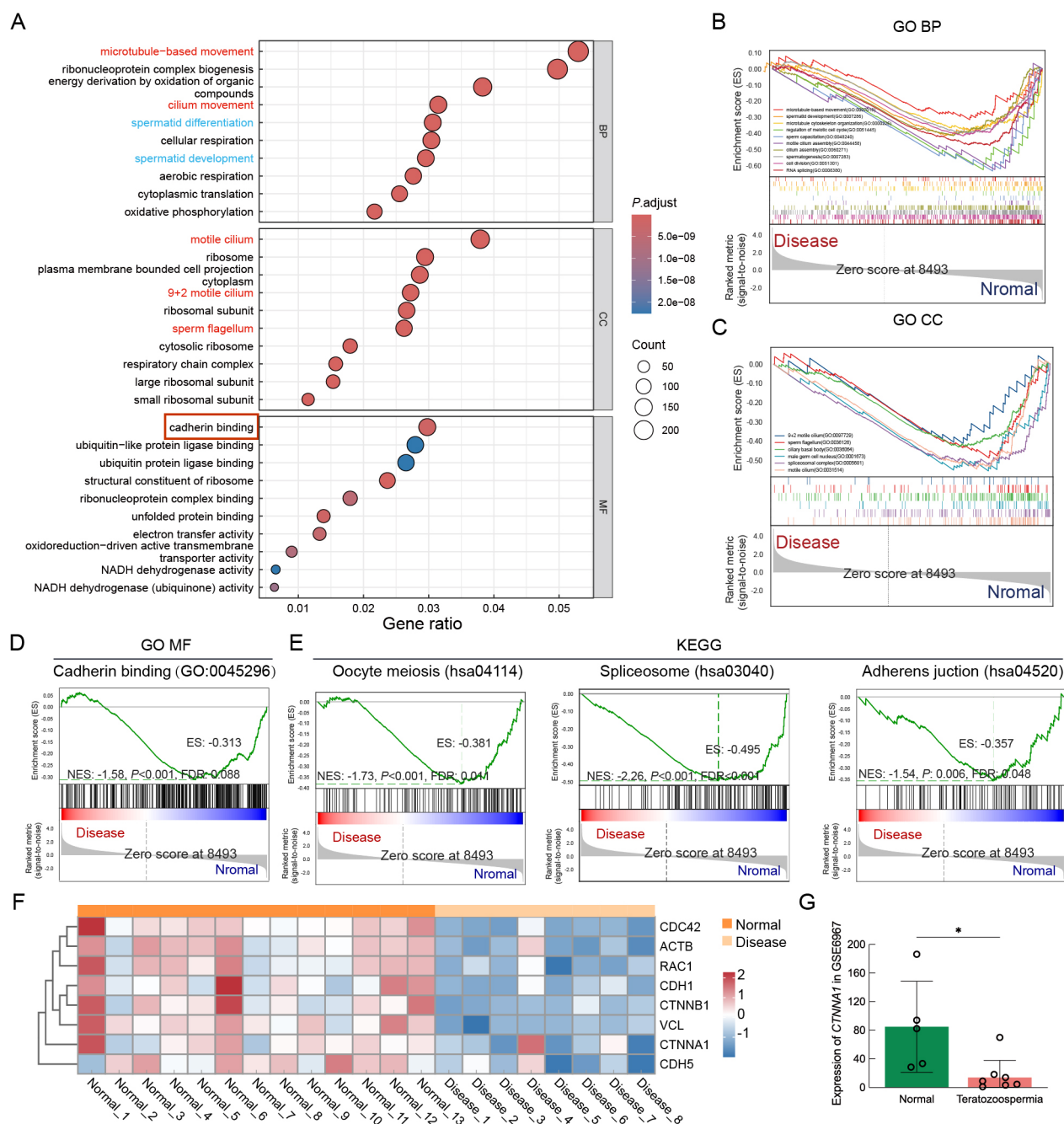


Figure 1 Compromised adherens junctions are associated with male teratozoospermia

A: Gene Ontology (GO) enrichment analysis of Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) from downregulated genes ($P < 0.05$, $|\log_2FC| > 1$) of RNA microarray dataset GSE6872. B–D: GSEA of GO terms associated with reproductive functions (B, C) and cadherin binding (D) in sperm cells from RNA microarray dataset (GSE6872), comparing teratozoospermia patients with control samples. E: GSEA of KEGG pathways associated with reproduction (meiosis and spliceosome) and AJs from RNA microarray dataset (GSE6872), comparing teratozoospermia patients with normal samples. F: Heatmap of core genes related to AJs in disease and normal groups. G: Expression levels of *CTNNA1* in normal ($n=5$) and teratozoospermia ($n=7$) groups from the GSE6872 microarray dataset. *: $P < 0.05$.

Ctnna1^{flox/flox} or wild-type (WT) females. Conversely, *Ctnna1*^{flox/flox} males successfully sired *Ctnna1*^{CKO} offspring when paired with *Ctnna1*^{CKO} females, suggesting that α -catenin is essential for male, but not female, fertility.

To uncover the cause of male infertility, the testes of adult *Ctnna1*^{CKO} and Ctrl mice were examined both grossly and histologically. Despite the observed infertility, the testes of adult *Ctnna1*^{CKO} mice exhibited no obvious morphological abnormalities compared with those of Ctrl mice (Figure 2C). Furthermore, the BTB in *Ctnna1*^{CKO} mice remained intact and

comparable to controls (Supplementary Figure S2E, F). Interestingly, although the number of spermatozoa in epididymides of *Ctnna1*^{CKO} mice appeared comparable to that of controls (Figure 2D), *Ctnna1*^{CKO} sperm displayed a markedly increased incidence of head-to-tail separation, bent flagella, and abnormal head formation, with ~38% of sperm affected compared to ~10% in controls (Figure 2E, F). Consistently, computer-assisted sperm analysis (CASA) demonstrated that *Ctnna1*^{CKO} mice exhibited reduced sperm straight-line velocity (VSL) (163 ± 31.53 vs. 217 ± 20.42 $\mu\text{m/s}$)

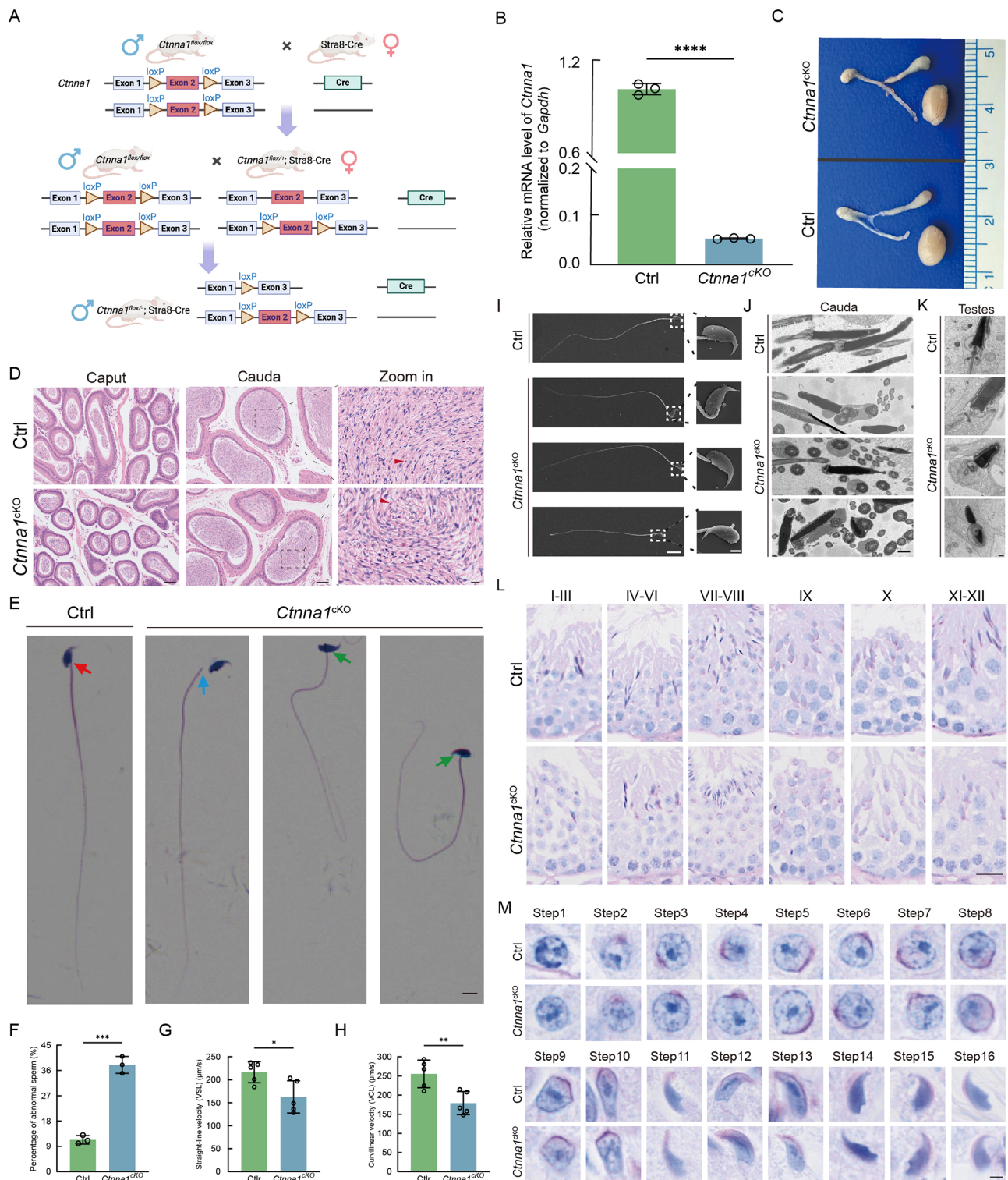


Figure 2 Loss of *Ctnna1* leads to sperm structural defects

A: Schematic diagram of the conditional *Ctnna1* knockout strategy in male mice. B: Quantification of *Ctnna1* mRNA levels in sperm from P60 Ctrl and *Ctnna1*^{KO} mice. *n*=3. C: Representative images of testes and epididymides from P60 Ctrl and *Ctnna1*^{KO} mice. D: Representative hematoxylin-eosin (HE) stained images of the epididymal caput and cauda from P60 Ctrl and *Ctnna1*^{KO} mice. Red arrows, normal sperm heads. E: Representative HE-stained epididymal sperm smears from P60 Ctrl and *Ctnna1*^{KO} mice. Red arrow, normal head-tail connection; blue arrow, head-tail dissociation; green arrows, bent neck. F: Quantification of abnormal sperm in smears from P60 Ctrl and *Ctnna1*^{KO} mice. *n*=3. G, H: Sperm straight-line velocity (VSL, $\mu\text{m/s}$) and curvilinear velocity (VCL, $\mu\text{m/s}$) in Ctrl and *Ctnna1*^{KO} mice measured using CASA. *n*=5. I: SEM images of sperm from P60 Ctrl and *Ctnna1*^{KO} mice, with high-magnification images showing sperm neck and head region. J, K: Transmission electron microscopy (TEM) images of sperm in cauda epididymis and testes from P60 Ctrl and *Ctnna1*^{KO} mice. L: Representative PAS-stained images of the 12 stages of seminiferous epithelium cycle in P60 Ctrl and *Ctnna1*^{KO} testes. M: Representative spermatid morphology at different developmental stages in P60 Ctrl and *Ctnna1*^{KO} mice. Scale bars, 100 μm and 10 μm (D); 5 μm (E); 10 μm and 2 μm (I); 1 μm (J), 3 μm (K); 20 μm (L); 2 μm (M). Data are presented as mean $\pm$ SD. Ctrl: control; SD: standard deviation. *: *P*<0.05; **: *P*<0.01; ***: *P*<0.001; ****: *P*<0.0001.

and curvilinear velocity (VCL) (191 ± 27.17 vs. 255 ± 32.19 $\mu\text{m/s}$) compared to controls (Figure 2G, H; Supplementary Video S1, 2). SEM confirmed structural defects in *Ctnna1*^{ckO} spermatozoa, characterized by head-tail dissociation and abnormal hook morphology of sperm heads (Figure 2I). Transmission electron microscopy (TEM) was then applied to examine the ultrastructure of epididymal and testicular spermatozoa collected from Ctrl and *Ctnna1*^{ckO} mice, revealing profound ultrastructural defects in *Ctnna1*^{ckO} mice, including disrupted head-to-tail junctions and bent configuration (Figure 2J, K). Periodic acid-Schiff (PAS) staining revealed the presence of all successive stages (I–XII) of the seminiferous epithelial cycle in *Ctnna1*^{ckO} mice (Figure 2L). In addition, stepwise analysis of spermatids in *Ctnna1*^{ckO} mice demonstrated the successive morphological steps of spermatid development (Figure 2M). Collectively, these findings suggest that loss of *Ctnna1* in spermatogonia impairs the structure and function of germ cells, ultimately leading to male infertility.

scRNA-seq reveals *Ctnna1* deficiency impairs spermatogenesis and axonemal structure/function

To study cellular and molecular changes associated with defects in *Ctnna1*^{ckO} sperm, we next performed scRNA-seq on the testes from Ctrl and *Ctnna1*^{ckO} mice (Figure 3A). Following the exclusion of low-quality cells and doublets, transcriptomic data were subjected to graph-based clustering and cell type annotation, with subsequent dimensionality reduction via joint uniform manifold approximation and projection (UMAP) for visualization (Figure 3B). A total of 24 234 cells were obtained after stringent quality control and subsequently annotated into distinct populations, including spermatogenic cells (*Stra8* and *Prm1*) (Zhang et al., 2024), Sertoli cells (*Aard* and *Tsx*) (Xie et al., 2025), Leydig cells (*Cyp17a1* and *Ptgds*) (Xie et al., 2025; Zhang et al., 2015), myeloid cells (*Lyz2* and *Cd74*, immune cells, mainly macrophages) (Alfano et al., 2021; Jiang et al., 2025), mural cells (*Tagln* and *Pdgfrb*, perivascular cells) (Barnett et al., 2024), fibroblasts (*Dcn* and *Igfbbp6*) (Tan et al., 2023), and endothelial cells (*Pecam1* and *Cdh5*) (Wang et al., 2024), using previously established markers (Figure 3C–Q). Pie chart analysis revealed a significant shift in cell population distribution, with a marked increase in Leydig cells (1.29% to 13.74%) and a decrease in Sertoli cells (23.74% to 2.06%), whereas the proportion of spermatogenic cells remained undisturbed following *Ctnna1* depletion (Figure 3R). Since *Ctnna1* was conditionally deleted in advanced germ cells starting from the type A1 spermatogonia stage, we performed GO enrichment analysis of all DEGs in spermatogenic cells ($|\log_2\text{FC}| > 0.58$). This analysis revealed a significant enrichment of GO terms related to sperm structure and function, including sperm flagellum assembly, sperm motility, sperm midpiece, and sperm principal piece, indicating that *Ctnna1* deficiency leads to defects in spermatogenesis and axonemal structure/function (Figure 4A), consistent with those observed in teratozoospermia patients (Figure 1A). In addition, upregulated DEGs in spermatogenic cells were only enriched in pathways related to sperm DNA condensation (Supplementary Figure S3A), whereas downregulated genes contributed mostly to spermatogenesis and axonemal structure/function (Supplementary Figure S3B). Consistently, GSEA of GO_BP and GO_CC in spermatogenic cells upon *Ctnna1* deletion revealed significant downregulation of pathways associated with axonemal structure/function

(Figure 4B–G). Core genes associated with spermatogenesis and axonemal structure/function were significantly downregulated in spermatogenic cells of *Ctnna1*^{ckO} mice (Figure 4H–S). Collectively, these results further support impaired sperm motility and flagellar development following loss of *Ctnna1*.

To assess germ cell dynamics during spermatogenesis, we analyzed both molecular signatures and changes in the number of germ cell subsets between the *Ctnna1*^{ckO} and control groups. Reclustering of spermatogenic cells identified six distinct subpopulations based on established markers (Tan et al., 2023; Zhang et al., 2023, 2024): *Stra8* and *Uchl1* for SPG, *Piwil1* and *Spag6* for spermatocytes (Scytes 1 and Scytes 2), *Spaca1* and *Spaca3* for round spermatids (Round STids), *Prm1* and *Prm2* for elongating spermatids (Elongating1 STids and Elongating2 STids) (Figure 5A, B; Supplementary Figure S4). Pie chart analysis revealed a notable shift in the distribution of these subpopulations in *Ctnna1*^{ckO} testes, characterized by an increased proportion of spermatogonia and elongating spermatids stage 2, and a decreased proportion of spermatocytes, round spermatids, and elongating spermatids stage 1 (Figure 5C), suggesting potential defects in earlier stages of spermatogenesis that may trigger abnormal progression into late elongating stages. UMAP visualization by genotype (Ctrl vs. *Ctnna1*^{ckO}) also revealed distinct cellular distributions (Figure 5D). We then mapped the activity of selected GO pathways onto the UMAP. Notably, signatures related to cilium assembly and motility were markedly reduced in spermatocytes and round spermatids of *Ctnna1*^{ckO} mice, indicating impaired structural differentiation of spermatozoa (Figure 5D–J).

Depletion of *Ctnna1* impairs the differentiation trajectory of spermatogenic cells from spermatogonia

To elucidate the developmental hierarchy of spermatogenic subpopulations, we conducted pseudotime analysis by RNA velocity (Bergen et al., 2020). RNA velocity analysis of Ctrl spermatogenic cells revealed a continuous differentiation trajectory among spermatogenic subtypes, with spermatogonia at the root and Elongating2 spermatids at the terminal end, representing early and advanced differentiation states, respectively (Figure 6A). However, *Ctnna1* depletion led to the loss of preferential orientation of the trajectory from spermatogonia to spermatocytes, and then to round spermatids (Figure 6B), suggesting perturbed differentiation from spermatogonia in the absence of *Ctnna1*. Since previous work exhibited continuous UMAP distribution between spermatogonia and other spermatogenic subpopulations (Tan et al., 2023; Zhang et al., 2023, 2024), we asked whether spermatogonia from *Ctnna1*^{ckO} mice contribute to the disconnection in our work. Therefore, we performed UMAP analyses of spermatogenic cells independently for each omics dataset, thereby preserving their unique characteristics. Consistent with prior observations (Tan et al., 2023; Zhang et al., 2023, 2024), spermatogonia and spermatocytes from Ctrl mice formed a continuous trajectory in the UMAP projection (Figure 6C). In contrast, spermatogonia from *Ctnna1*^{ckO} mice exhibited a clear disconnection from the spermatocyte cluster, indicative of disrupted germ cell differentiation (Figure 6D). Additionally, the disruption of germ cell differentiation from spermatogonia of *Ctnna1*^{ckO} mice was further proved by a disturbed differentiation trajectory using Monocle 3 (Figure 6E, F). Therefore, we conclude that

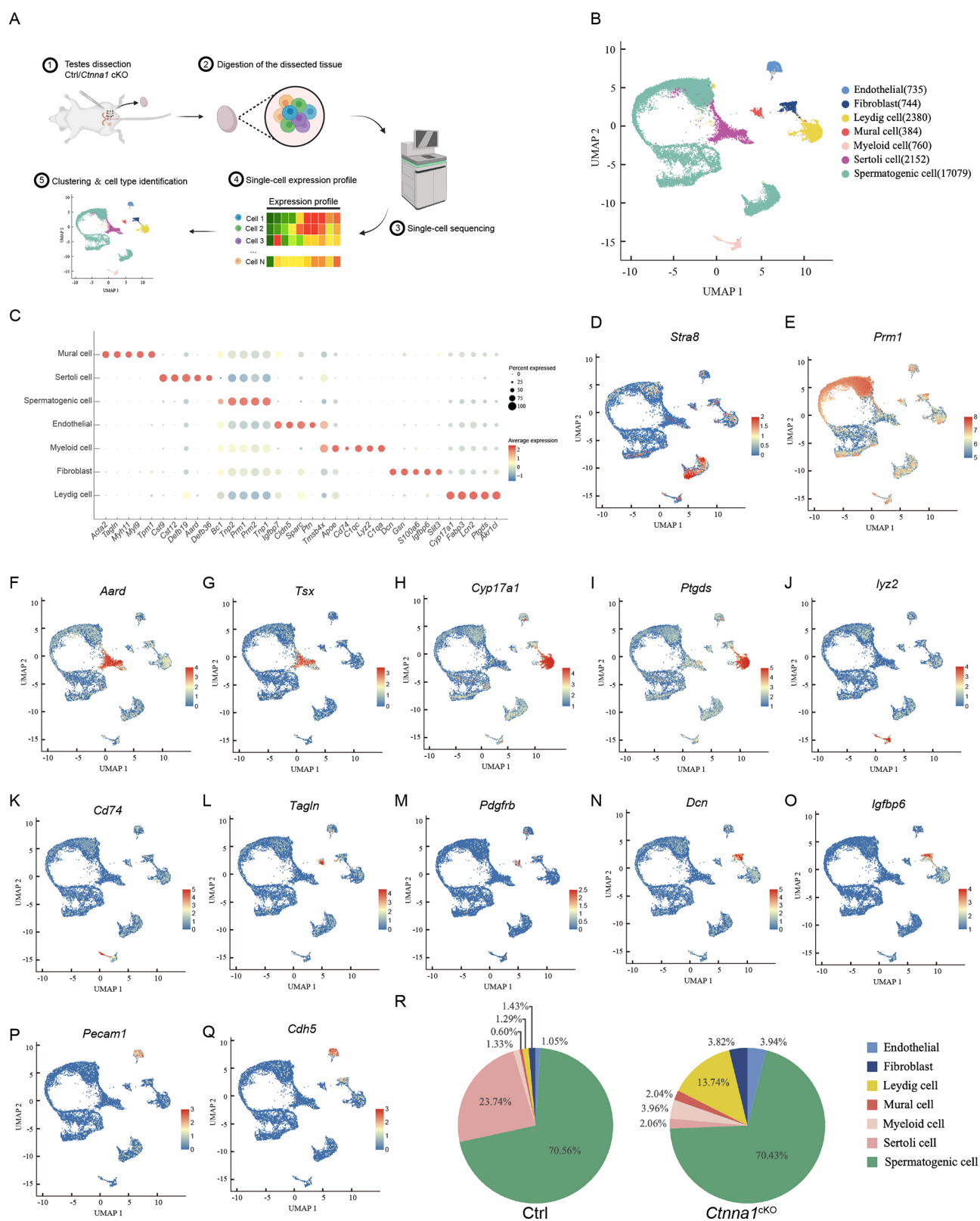


Figure 3 Single-cell RNA sequencing (scRNA-seq) of testes from P60 Ctrl and *Ctnna1*^{CKO} mice

A: Schematic diagram of scRNA-seq workflow for P60 Ctrl and *Ctnna1*^{CKO} mice testes. B: Uniform manifold approximation and projection (UMAP) plot of testicular cells, with cluster colored by identified cell types. C: Dot plot of marker genes across the annotated cell types. D–O: UMAP feature plots colored by the expression levels of marker genes expressed in the identified clusters. R: Pie charts showing the distribution of the identified cell types across samples. Ctrl: control.

depletion of *Ctnna1* in spermatogenic cells impairs the differentiation trajectory of spermatogenic cells from spermatogonia.

***Ctnna1* deficiency impairs meiosis and splicing in spermatogonia**

Given that loss of *Ctnna1* in spermatogenic cells leads to

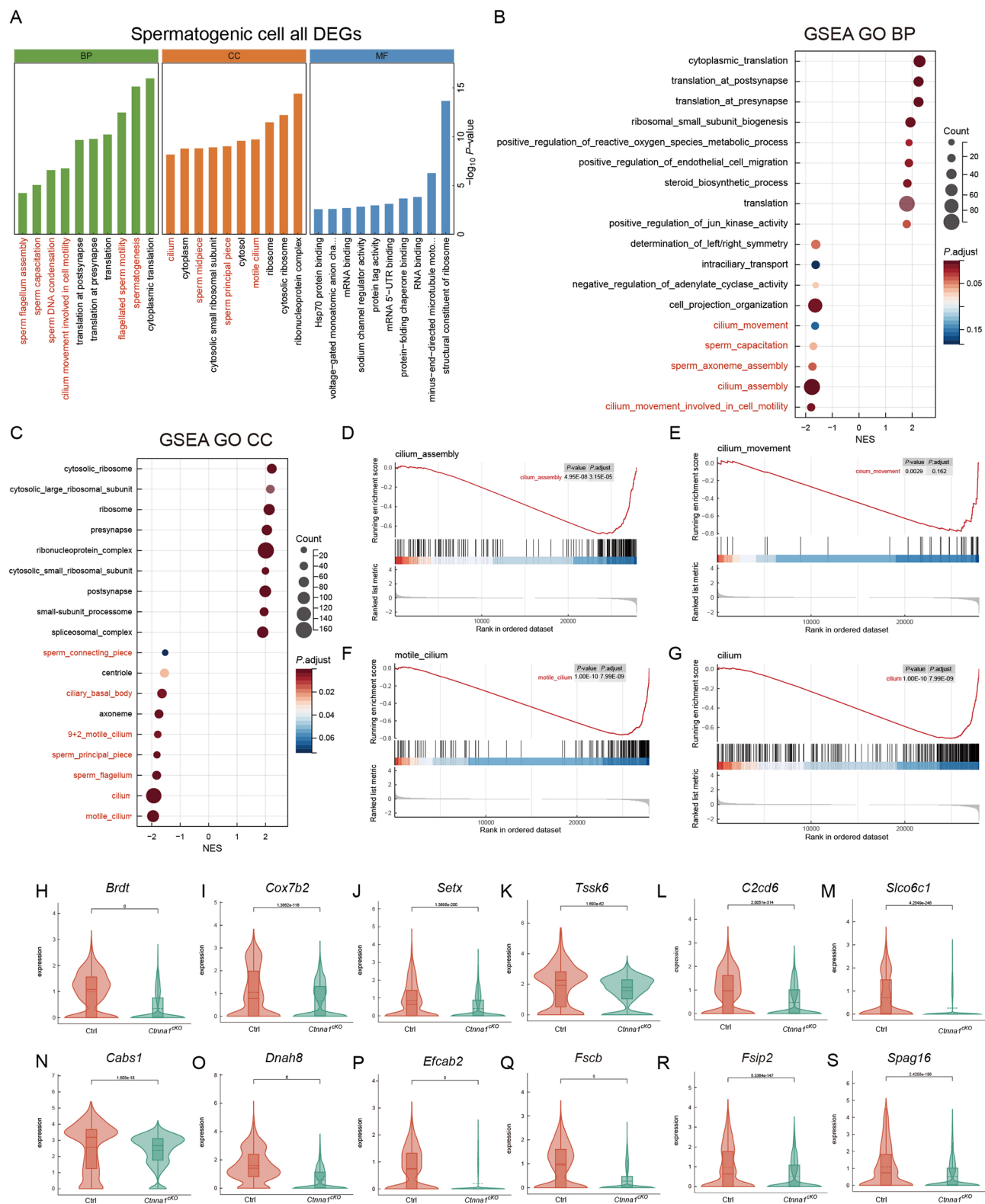


Figure 4 *Ctnna1* deficiency leads to impaired axonemal structure/function

A: Bar plots of GO enrichment analysis for DEGs in spermatogenic cells from *Ctnna1*^{CKO} versus Ctrl. B, C: GSEA dot plots of GO terms (BP and CC) in spermatogenic cells, comparing Ctrl and *Ctnna1*^{CKO} groups. D–G: GSEA enrichment plots of GO gene sets showing cilium-related pathway enrichment in spermatogenic cells of *Ctnna1*^{CKO} mice. H–S: Violin plots representing the expression of core genes related to spermatogenesis and axonemal structure/function in Ctrl and *Ctnna1*^{CKO} spermatogenic cells. Ctrl: control.

disturbed differentiation trajectory and accumulation of spermatogonia, and that *Stra8*-Cre expresses Cre recombinase preliminarily and prominently in spermatogonia, we focused our investigation on the function of α -catenin in

these cells (Endo et al., 2015; Shimada et al., 2023). We first performed immunofluorescence staining of testicular sections from P15 and P60 mice using anti-PLZF antibody to label undifferentiated spermatogonia. Consistent with the scRNA-

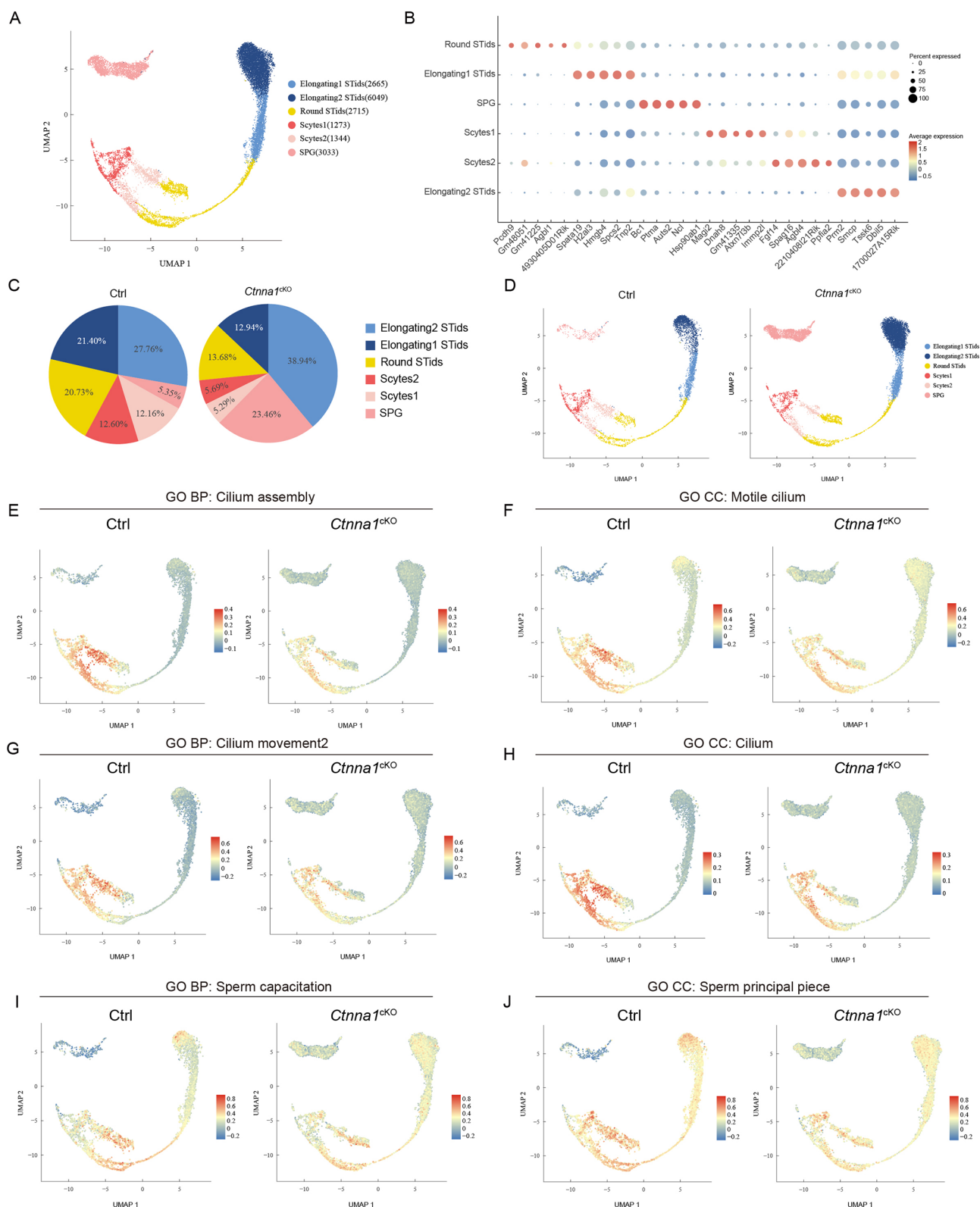


Figure 5 Identification of spermatogenic subclusters in Ctrl and *Ctnna1^{ckO}* mice

A: UMAP of integrated spermatogenic subclusters from Ctrl and *Ctnna1^{ckO}* groups. B: Dot plot for the specific top six marker genes in each spermatogenic subcluster. C: Pie charts showing the distributions of six spermatogenic subpopulations in Ctrl and *Ctnna1^{ckO}* mice. D: Split UMAP of spermatogenic subclusters in Ctrl and *Ctnna1^{ckO}* mice, respectively. E–J: UMAP plot showing activity of selected GO pathways in spermatogenic cells of Ctrl and *Ctnna1^{ckO}* groups. Ctrl: control.

seq analysis, the number of undifferentiated spermatogonia was increased in both P15 and P60 *Ctnna1^{ckO}* testes (Figure 6G–J), indicating a delayed transition from undifferentiated to differentiating spermatogonia prior to the

mitosis-to-meiosis transition. During spermiogenesis, acrosome formation drives the differentiation of round spermatids into elongating spermatids (Kavarthapu et al., 2023). We then evaluated the cap-phase acrosomes of round

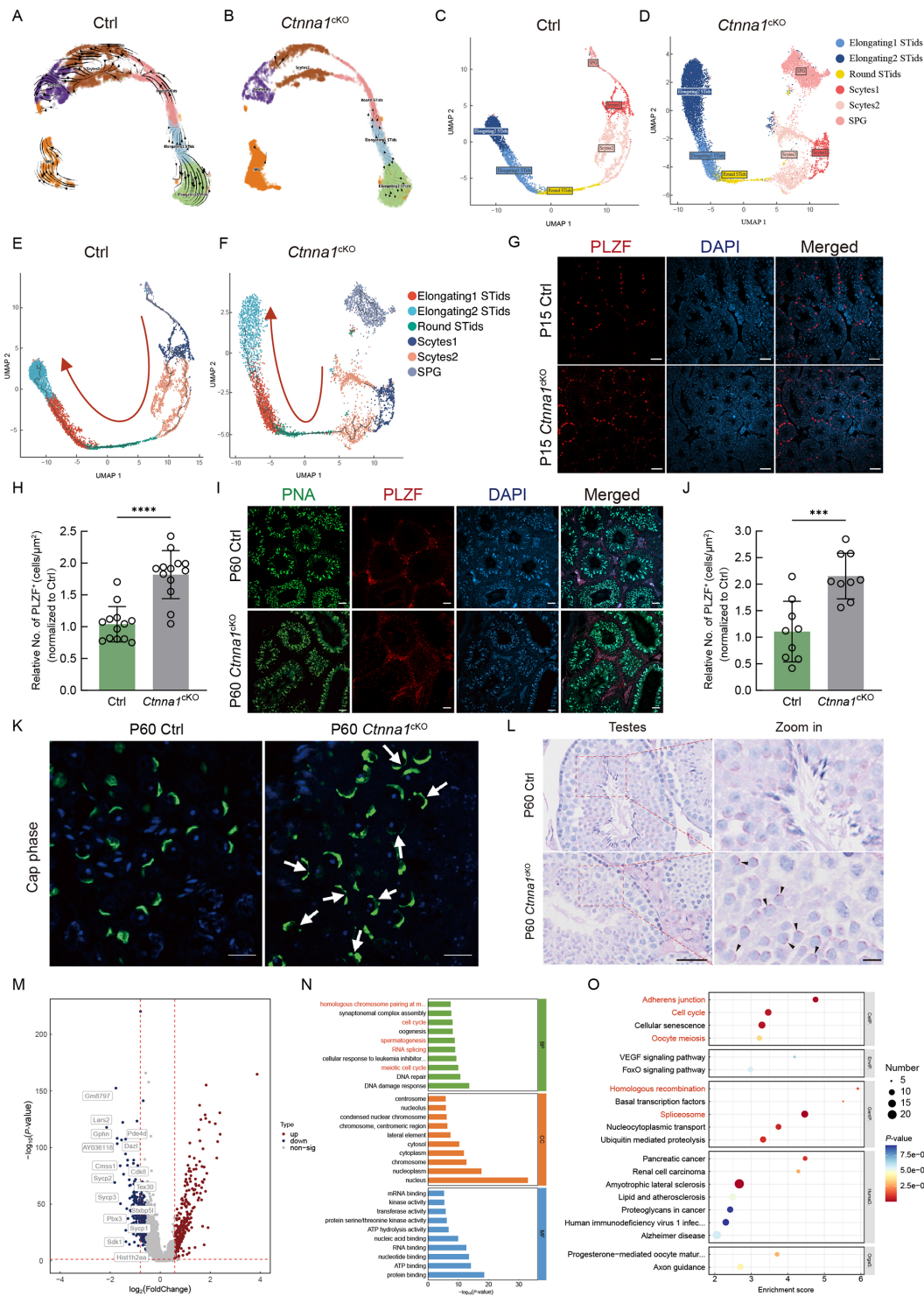


Figure 6 Loss of *Ctnna1* leads to impaired differentiation of spermatogenic cells from spermatogonia

A, B: Velocities for spermatogenic cells in a UMAP-based embedding in Ctrl (A) and *Ctnna1*^{CKO} (B) mice. C, D: UMAP of spermatogenic subclusters in Ctrl (C) and *Ctnna1*^{CKO} (D) mice. E, F: Pseudotime analysis of spermatogenic subclusters in Ctrl (E) and *Ctnna1*^{CKO} (F) mice using Monocle 3. G: Representative images of testes from P15 Ctrl and *Ctnna1*^{CKO} mice. Red, PLZF; Blue, DAPI. H: Quantification of PLZF-positive cells per μm^2 in P15 Ctrl and *Ctnna1*^{CKO} mice testes, normalized to the Ctrl group. $n=13$. I: Representative images of testes from P60 Ctrl and *Ctnna1*^{CKO} mice. Green, PNA; Red, PLZF; Blue, DAPI. J: Quantification of PLZF-positive cells per μm^2 in P60 Ctrl and *Ctnna1*^{CKO} mice testes, normalized to the Ctrl group. $n=9$. K: Representative images of cap-phase round spermatids from P60 Ctrl and *Ctnna1*^{CKO} testes. Green, PNA; Blue, DAPI. White arrows, discontinuous acrosomal structures. L: Representative PAS-stained images of cap-phase round spermatids from P60 Ctrl and *Ctnna1*^{CKO} testes. Black arrows, discontinuous acrosomal structures. M: Volcano plot of DEGs in spermatogonia from *Ctnna1*^{CKO} versus Ctrl ($\log_2\text{FC} < -0.58$, $P < 0.05$). Red and blue dots represent significantly up- and downregulated genes, respectively. N: Bar plots of GO enrichment analysis for DEGs in spermatogonia from *Ctnna1*^{CKO} versus Ctrl. O: Dot plot of significantly enriched KEGG pathways for DEGs in spermatogonia from *Ctnna1*^{CKO} versus Ctrl. Scale bars, 50 μm (G, I, and L); 10 μm (K, L). Data are presented as mean $\pm$ SD. Ctrl: control; SD: standard deviation. ****: $P < 0.001$; *****: $P < 0.0001$.

spermatids using PNA and PAS staining. We found that while Ctrl spermatids exhibited a single uniform layer of acrosomal caps, *Ctnna1*^{ckO} spermatids displayed discontinuous acrosomal structures (Figure 6K, L).

To identify transcriptional alterations in spermatogonia lacking *Ctnna1*, we conducted differential gene expression analysis, which revealed a total of 430 downregulated genes in the *Ctnna1*^{ckO} mice compared to Ctrl, including *Sycps* (*Sycp1*, *Sycp2*, and *Sycp3*) and *Tex30*, which were associated with meiosis (Figure 6M). GO analysis revealed that these downregulated genes were enriched in biological processes primarily related to spermatogenesis, RNA splicing, and the meiotic cell cycle (Figure 6N). Consistently, KEGG analysis indicated an enrichment in pathways including adherens junction, cell cycle, meiosis, and the spliceosome (Figure 6O), suggesting widespread disruptions in transcriptional programs essential for spermatogenic progression. Functional enrichment analysis showed a significant expression of RNA splicing and meiosis-related signatures mostly in spermatogonia and spermatocytes (Figure 7A–D), which were markedly reduced upon *Ctnna1* depletion in these subclusters (Figure 7E). Core genes associated with meiosis and splicing were also prominently expressed in spermatogonia and spermatocytes and downregulated in the *Ctnna1*^{ckO} group (Supplementary Figure S5). qPCR of testes from *Ctnna1*^{ckO} and Ctrl mice confirmed the downregulation of genes involved in RNA splicing and meiosis (Figure 7F–Q). Furthermore, analysis of RNA read proportions revealed distinct distributions between Ctrl and *Ctnna1*^{ckO} mice spermatogenic cells. In Ctrl spermatogenic cells, spliced reads accounted for 81%, indicative of abundant mature transcripts, whereas unspliced reads comprised 14% (Figure 7R). In contrast, *Ctnna1*^{ckO} spermatogenic cells showed a decrease in spliced reads (74%) and a marked increase in unspliced reads (21%), suggesting impaired splicing efficiency associated with *Ctnna1* deficiency (Figure 7R). Consistently, immunofluorescence analysis of chromosome spreads stained for SYCP3 and γH2AX revealed that *Ctnna1*^{ckO} testes exhibited an increased proportion of leptotene and zygotene spermatocytes, accompanied by a decrease in pachytene and diplotene populations compared with controls (Figure 7S, T). These results collectively demonstrate that loss of *Ctnna1* leads to an accumulation of spermatogonia and delayed differentiation into spermatids due to compromised meiosis and splicing.

Depletion of *Ctnna1* leads to abnormal activation of sperm DNA condensation and protein translation

Beyond the accumulation of SPG, the scRNA-seq data highlighted a marked increase in the ratio of Elongating 2 STids in *Ctnna1*^{ckO} germ cells (Figure 5C), which potentially accounts for the maintenance of the overall sperm count upon *Ctnna1* depletion. We, therefore, hypothesized that a compensatory feedback response to earlier meiotic disturbances might be triggered during post-meiotic differentiation. Intriguingly, we observed a striking molecular uncoupling between two essential spermiogenic programs: while markers required for DNA condensation, including transition proteins (*Tnp1* and *Tnp2*), protamine 1 (*Prm1*), and chromodomain helicase DNA binding protein 5 (*Chd5*) (Hermann et al., 2018; Li et al., 2014), were significantly upregulated, genes essential for the acquisition of sperm motility and flagellar integrity, such as fibrous sheath proteins (*Akap4*, *Cabyr*, and *Fscb*) and carbonic anhydrase 2 (*Car2*)

(Huang et al., 2021; Li et al., 2007; Wandernoth et al., 2015), were markedly downregulated in Elongating 2 STids from *Ctnna1*^{ckO} mice (Supplementary Figure S6A).

Heatmap analysis confirmed that the expression of these functional gene sets predominantly initiated during the post-meiotic stages, specifically from the round spermatid stage onward (Supplementary Figure S6B). Notably, within the post-meiotic population, a striking divergence in the transcriptional programs was observed between the Ctrl and *Ctnna1*^{ckO} groups. The elevated expression of DNA condensation-related genes in *Ctnna1*^{ckO} mice suggested an accelerated drive toward nuclear compaction (Supplementary Figure S6B), which potentially serves as a compensatory feedback response to earlier meiotic disturbances. Conversely, the suppression of motility-related genes indicated a fundamental failure in flagellar assembly (Supplementary Figure S6B).

Further unbiased enrichment analysis revealed that, in addition to dysregulated DNA condensation (Supplementary Figure S6C) and sperm motility (Supplementary Figure S6D) pathways, Elongating2 STids from *Ctnna1*^{ckO} exhibited a robust enrichment of upregulated genes in ribosome biogenesis and translation (Supplementary Figure S6C–E). These findings suggest that the increased proportion of late-stage elongating spermatids is likely attributed to the abnormal acceleration of DNA condensation and protein translation during post-meiotic development.

Loss of *Ctnna1* leads to the disorganization of vinculin

Beyond the transcriptional program, we also explored whether *Ctnna1* depletion affects structural proteins critical for spermatogenesis. Vinculin (encoded by *VCL*) is a cytoskeletal protein that interacts with α-catenin and plays a role in sperm capacitation, being predominantly localized to the sperm head of capacitated sperm (Roa-Espitia et al., 2016; Xie et al., 2022). Interestingly, depletion of *Vcl* in primary spermatocytes has been reported to cause decreased fertility in males through compromised meiotic progress (Petrusová et al., 2022). We, therefore, asked whether vinculin was disturbed in the absence of α-catenin. We first confirmed the robust interaction between α-catenin and vinculin by co-immunoprecipitation (Co-IP) (Figure 7U). In the cap phase, vinculin signals were largely colocalized with PNA and displayed a continuous linear distribution along the acrosomal cap, with a notable accumulation at its central region (Figure 7V), consistent with previous observations (Roa-Espitia et al., 2016; Xie et al., 2022). In contrast, *Ctnna1*^{ckO} spermatids displayed vinculin aggregation precisely at the sites of PNA signal discontinuity, indicating abnormal cap-shaped acrosome formation (Figure 7V). These findings are consistent with the abnormal hook morphology of sperm heads observed in SEM analysis (Figure 2I). Together, these data suggest that *Ctnna1* deficiency disrupts vinculin organization during acrosome biogenesis, thereby contributing to defective sperm head formation and male infertility.

Loss of *Ctnna1* in germ cells disrupts crosstalk between Sertoli cells and germ cells

In spermatogenesis, germ cells communicate with Sertoli cells and Leydig cells via cellular adhesion and paracrine signaling (Hofmann & Mcbeath, 2022). We then conducted CellChat analysis to evaluate the contribution of cell-cell communication to the impaired spermatogenesis observed in *Ctnna1*^{ckO} mice. The analysis revealed a global reduction in inferred intercellular interactions following *Ctnna1* depletion

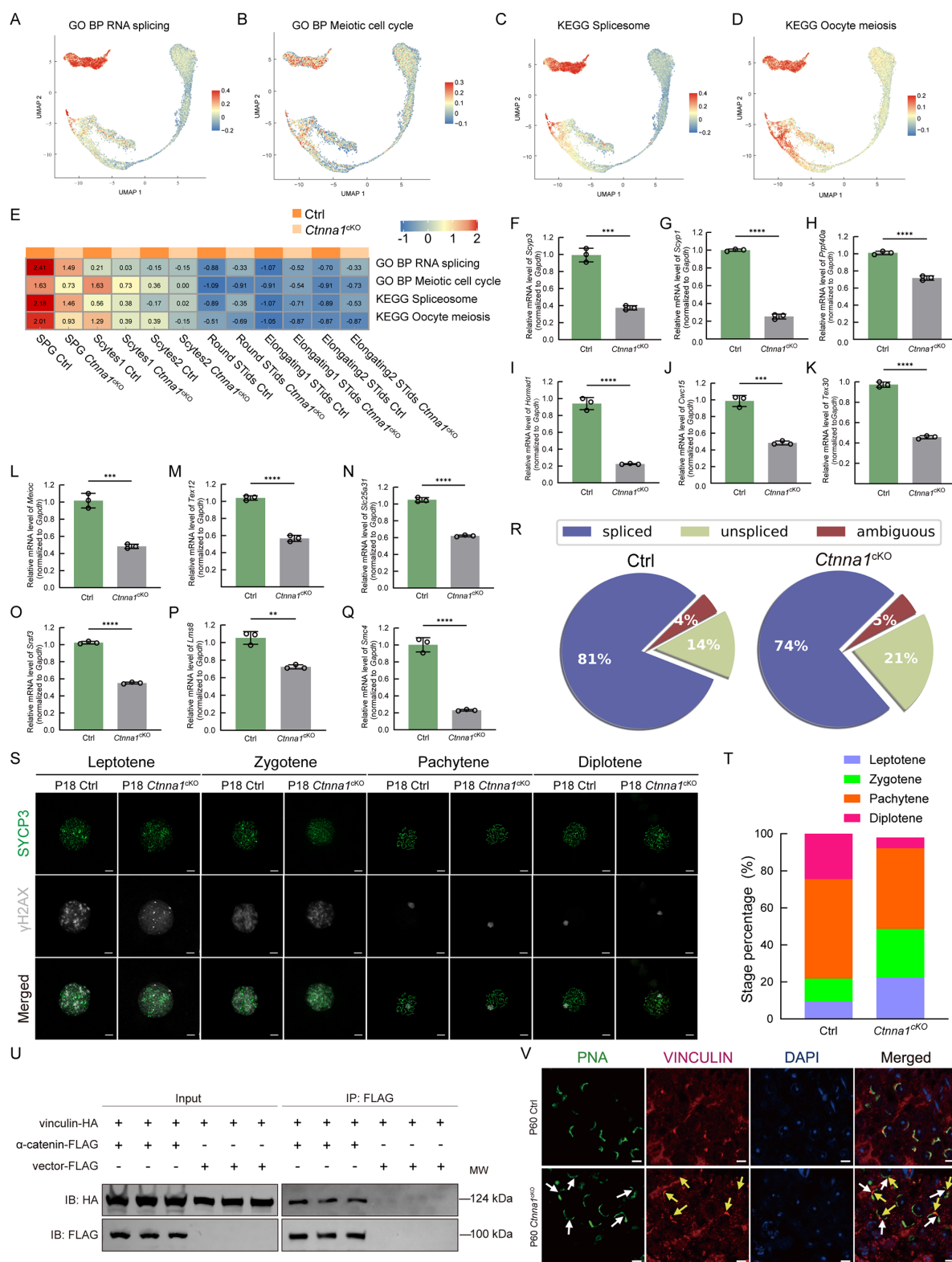


Figure 7 *Cttna1* deficiency leads to impaired meiosis/splicing in spermatogonia and disorganization of vinculin

A–D: UMAP plot showing activity of selected GO (A, B) and KEGG (C, D) pathways in spermatogonia. E: Heatmap of the expression patterns of genes associated with meiosis/splicing-related pathways. F–Q: Relative mRNA levels of meiosis- and splicing-related genes (*Sycp3*, *Sycp1*, *Prpf40a*, *Stra8*, *Hormad1*, *Cwc15*, *Tex30*, *Meioc*, *Tex12*, *Slc25a31*, *Srsf3*, *Lms8*, and *Smc4*) in Ctrl and *Cttna1*^{CKO} testes, normalized to *Gapdh*. n=3. R: Pie charts showing RNA read proportions of between Ctrl and *Cttna1*^{CKO} spermatogenic cells. S: Representative images of meiotic chromosome spreads from P18 Ctrl and *Cttna1*^{CKO} mice. Green, SYCP3; White, γH2AX; Blue, DAPI. T: Quantification of the relative proportions of spermatocytes at different meiotic stages in chromosome spreads from P18 Ctrl and *Cttna1*^{CKO} mice. U: Co-IP analysis of interaction between α-catenin-FLAG and vinculin-HA in HEK293 cells. V: Representative images of cap-phase round spermatids from P60 Ctrl and *Cttna1*^{CKO} testes. Green, PNA; Red, vinculin; Blue, DAPI. White arrows, discontinuous acrosomal structures; yellow arrows, aggregation of vinculin. Scale bars, 10 μm (S); 5 μm (V). Data are presented as mean±SD. Ctrl: control; SD: standard deviation. **: *P*<0.01; ***: *P*<0.001; ****: *P*<0.0001.

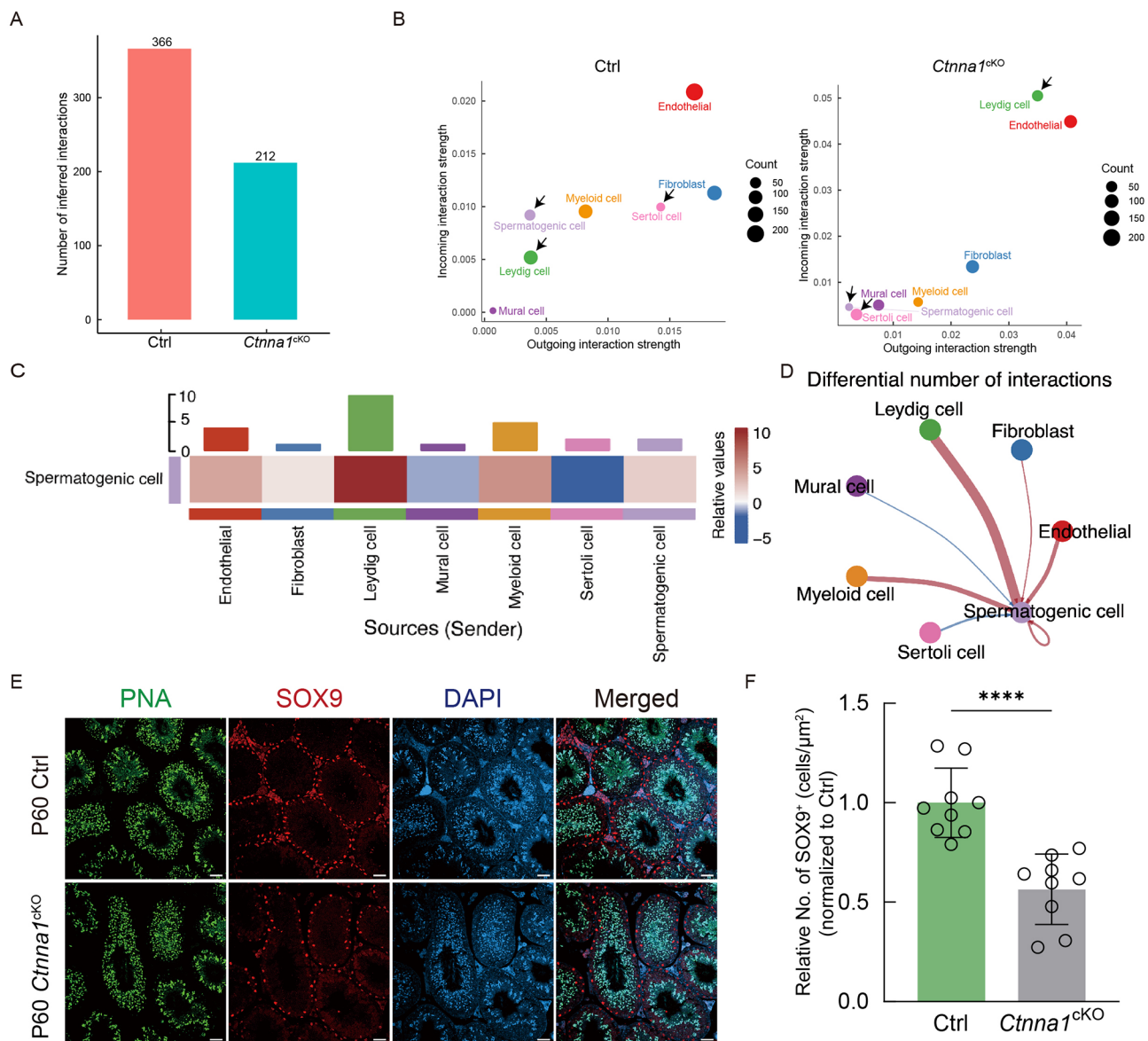


Figure 8 Loss of *Ctnna1* in germ cells leads to decreased Sertoli cells and impaired Sertoli-germ cell interactions

A: Bar plot of inferred cell-cell interactions in Ctrl and *Ctnna1*^{ckO} groups. B: The incoming and outgoing interaction strength of each cell type in Ctrl and *Ctnna1*^{ckO} groups. C: Heatmap showing the differential number of interactions between spermatogenic cells (receivers) and other cell types, comparing *Ctnna1*^{ckO} and Ctrl groups. D: Circle plot displaying the differential number of interactions between spermatogenic cells and other cell types, comparing *Ctnna1*^{ckO} and Ctrl groups. E: Representative images of P60 Ctrl and *Ctnna1*^{ckO} testes. Green, PNA; Red, SOX9; Blue, DAPI. F: Relative number of SOX9⁺ cells per μm^2 in P60 Ctrl and *Ctnna1*^{ckO} mice testes, normalized to the Ctrl group. $n=14$. Scale bars, 50 μm (E). Data are presented as mean $\pm$ SD. Ctrl: control; SD: standard deviation. ****: $P<0.0001$

(Figure 8A). A decrease in incoming interaction strength was observed in spermatogenic cells of *Ctnna1*^{ckO} mice relative to Ctrl (Figure 8B), potentially contributing to impaired spermatogenesis. Notably, the overall interaction strength of Sertoli cells and Leydig cells was drastically decreased and increased (Figure 8B), respectively. Further, focusing on the incoming signaling to spermatogenic cells, we observed a significant reduction in interactions originating from Sertoli cells and a notable increase from Leydig cells, suggesting altered paracrine regulation upon *Ctnna1* depletion (Figure 8C, D). Immunofluorescence analysis of mouse testes sections revealed a 43.5% reduction in Sertoli cell number in the *Ctnna1*^{ckO} group compared with controls (Figure 8E, F).

We next examined the alterations in interactions between Sertoli cells, Leydig cells, and subclusters of spermatogenic cells. *Ctnna1* depletion led to a decrease in the number of

inferred intercellular interactions among these cells (Figure 9A). As expected, interactions involving Sertoli cells were broadly diminished, particularly those with spermatogonia, spermatocytes, and elongating spermatids (Figure 9B, C). Quantitative assessment of overall signaling strength demonstrated that Sertoli cells were the dominant signal senders in Ctrl testes (Supplementary Figure S7A). In contrast, in *Ctnna1*^{ckO} testes, Sertoli cells exhibited markedly reduced signaling activity (Supplementary Figure S7A). Sertoli cells are essential components of the germ cell niche, supporting germ cell progression to spermatozoa through direct cell-cell contact and regulation of the extracellular microenvironment (Huang et al., 2023; O'donnell et al., 2022). Indeed, the loss of Sertoli-spermatid junctions could result in male infertility and correlate with head and midpiece malformation (Mueller et al., 2003). Therefore, we

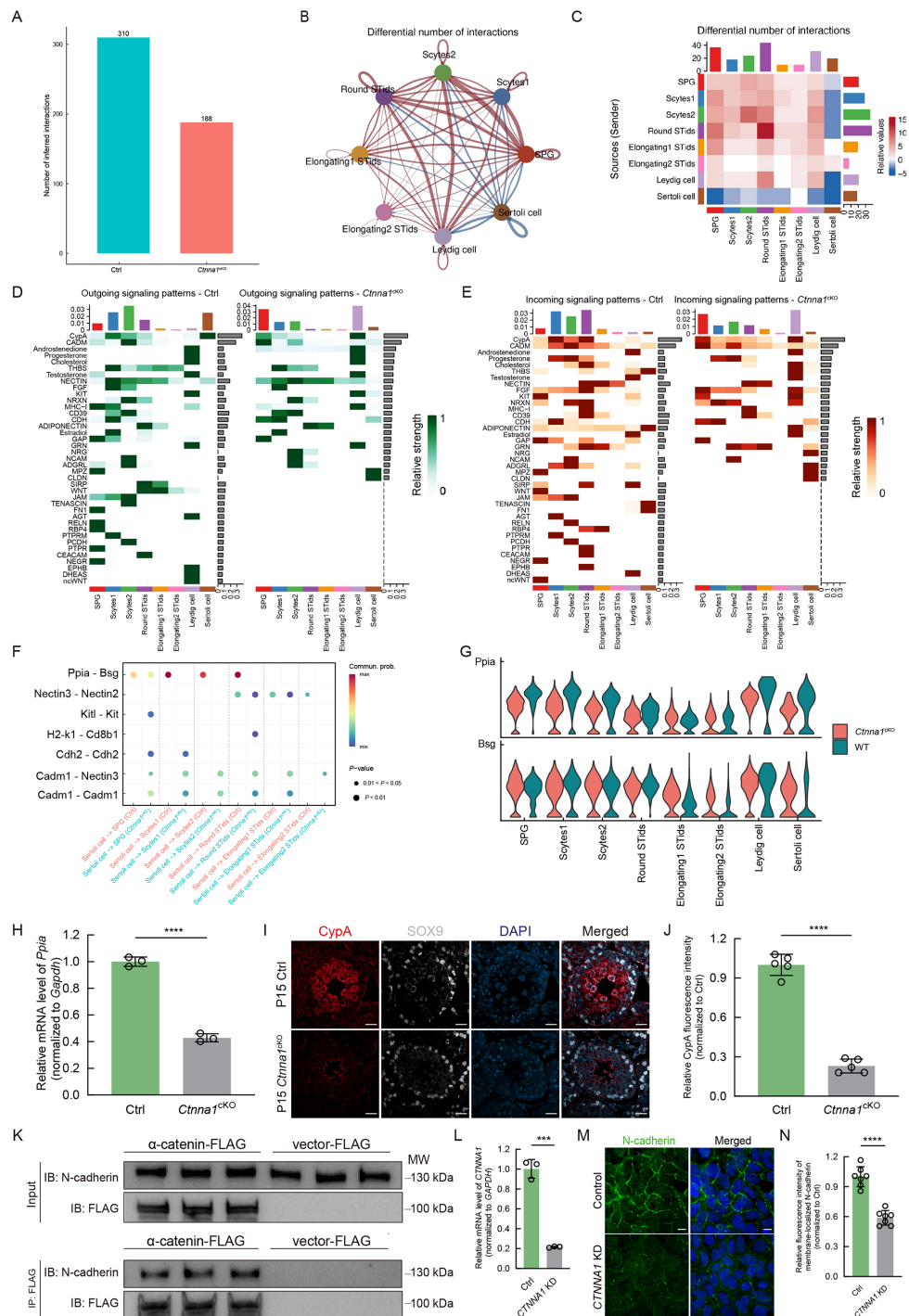


Figure 9 *Ctnna1* deficiency leads to compromised direct and indirect Sertoli-germ cell interactions

A: Bar plot of the inferred intercellular interactions among Sertoli cells, Leydig cells, and subclusters of spermatogenic cells, comparing *Ctnna1*^{CKO} and Ctrl groups. B: Circle plot displaying the differential number of interactions among subclusters of spermatogenic cells, Leydig cells, and Sertoli cells, comparing *Ctnna1*^{CKO} and Ctrl groups. C: Heatmap showing the differential number of interactions among subclusters of spermatogenic cells, Leydig cells, and Sertoli cells, comparing *Ctnna1*^{CKO} and WT groups. D: Heatmap of outgoing signaling patterns associated with Sertoli cells, Leydig cells, and spermatogenic subclusters, comparing *Ctnna1*^{CKO} and Ctrl groups. E: Heatmap of incoming signaling patterns associated with Sertoli cells, Leydig cells, and spermatogenic subclusters, comparing *Ctnna1*^{CKO} and Ctrl groups. F: The communication probability of CypA pathway ligand-receptor pairs in Ctrl and *Ctnna1*^{CKO} groups. G: The expression of *Ppia* and *Bsg* in Sertoli cells, Leydig cells, and spermatogenic subclusters in Ctrl and *Ctnna1*^{CKO} groups. H: Quantification of relative *Ppia* mRNA levels in P15 Ctrl and *Ctnna1*^{CKO} testes, normalized to *Gapdh*. *n*=3. I: Representative images of testes from P15 Ctrl and *Ctnna1*^{CKO} mice. Red, CypA; White, SOX9; Blue, DAPI. J: Quantification of relative *Ppia* mRNA levels in P15 Ctrl and *Ctnna1*^{CKO} mice, normalized to *Gapdh*. *n*=3. K: Co-IP analysis of interaction between α -catenin-FLAG and N-cadherin in HEK293 cells. L: Quantification of relative *CTNNA1* mRNA levels in control and *CTNNA1* KD HEK293 cells, normalized to *GAPDH*. *n*=3. M: Representative images of control and *CTNNA1* KD HEK293 cells. Green, N-cadherin; Blue, DAPI. N: Normalized fluorescence intensity levels for N-cadherin at the cell membrane in control and *CTNNA1* KD HEK293 cells. *n*=7. Scale bars, 20 μ m (I); 10 μ m (M). Data are presented as mean $\pm$ SD. Ctrl: control; SD: standard deviation. ***: *P*<0.001; ****: *P*<0.0001.

hypothesized that compromised interactions between Sertoli cells and spermatogenic cells may contribute to defective spermatogenesis in *Ctnna1*^{CKO} mice.

Heatmap analyses illustrated that the CypA signaling, the dominant pathway mediating cell-cell interactions, predominantly originated from Sertoli cells in controls but was markedly reduced in the *Ctnna1*^{CKO} group (Figure 9D). Consistently, the CypA pathway in the incoming signaling of spermatocytes and round spermatids was remarkably diminished in *Ctnna1*^{CKO} compared to Ctrl (Figure 9E). Furthermore, network analysis showed that the CypA pathway orchestrated robust signaling between Sertoli cells and spermatogenic cells in Ctrl mice, whereas these interactions were significantly weakened in the *Ctnna1*^{CKO} group (Supplementary Figure S7B). Dot plot of ligand (from Sertoli cells)-receptor (subclusters of spermatogenic cells) interactions revealed that the cyclophilin A (encoded by *Ppia*) and CD147 (encoded by *Bsg*) ligand receptor interaction, which belongs to the CypA pathway, was disrupted between Sertoli cells and spermatocytes/round spermatids (Figure 9F). Compared to the stable expression of the receptor *Bsg* in Sertoli and spermatogenic cells, the ligand *Ppia* was significantly downregulated in both somatic and germline compartments, including Sertoli cells, spermatogonia, spermatocytes, and round spermatids (Figure 9G). qPCR and immunofluorescence analysis of testes from P15 mice revealed a significant reduction in *Ppia* mRNA levels in the *Ctnna1*^{CKO} group compared to the controls (Figure 9H–J). Overall, the decreased cyclophilin A-CD147 interaction between Sertoli cells and germ cells may be involved in the compromised spermatogenesis in *Ctnna1*^{CKO} mice. These findings suggest a compensatory or pathological rewiring of the testicular cell communication network following *Ctnna1* loss.

Cadherins are transmembrane glycoproteins that mediate calcium-dependent adhesion between neighboring cells, thereby maintaining tissue architecture and integrity in animals (Halbleib & Nelson, 2006). Their intracellular domains recruit catenin, including β -catenin and α -catenin, to form the cadherin-catenin complex, which bridges cadherins to the actin cytoskeleton to maintain cell junction stability and tissue integrity (Maitre & Heisenberg, 2013). Given that N-cadherin was reported to be expressed on the surface of both Sertoli cells and germ cells, where it acts as a partial mediator of cell adhesion (Newton et al., 1993), we asked whether loss of *Ctnna1* affects the direct communication between Sertoli cells and germ cells through N-cadherin. The interaction between N-cadherin and α -catenin was first confirmed in HEK293T cells and testes by co-immunoprecipitation (Figure 9K; Supplementary Figure S7C). Further immunofluorescence analysis revealed that in control cells, N-cadherin primarily localized to the cell membranes, whereas its expression became diffused and internalized in cells depleted of *CTNNA1* by shRNA (Figure 9L–N), suggesting that loss of *Ctnna1* disrupts direct cell adhesion between Sertoli cells and germ cells.

Loss of *Ctnna1* in germ cells causes Sertoli cell-to-Leydig cell transdifferentiation

Sertoli and Leydig cells, the two primary somatic lineages in the testis, exhibit distinct morphologies and divergent physiological functions. Previous work has demonstrated that the loss of *Wt1* combined with the activation of Wnt/ β -catenin

signaling can drive the transdifferentiation of Sertoli cells into Leydig cells (Zhang et al., 2015). Consequently, we asked whether this transdifferentiation accounts for the marked reduction in Sertoli cells and the concurrent expansion of the Leydig cell population observed by scRNA-seq (Figure 3R). First, volcano plot of top-regulated DEGs exhibited aberrant upregulation of Leydig cell markers, including *Cyp17a1* and *Fabp3* (Xie et al., 2025; Zhang et al., 2015), within the Sertoli cells of *Ctnna1*^{CKO} mice compared to Ctrl (Supplementary Figure S8A–D). Moreover, other Leydig cell markers, *Cyp11a1*, *Star*, *Insl3*, and *Hsd3b1* (Xie et al., 2025; Zhang et al., 2015), were also upregulated in *Ctnna1*^{CKO} Sertoli cells (Supplementary Figure S8E–H), suggesting transdifferentiation from Sertoli cells to Leydig cells in the *Ctnna1*^{CKO} model. Subsequent reclustering of Sertoli and Leydig cells identified a distinct intermediate population expressing markers of both lineages (Supplementary Figure S8I, J). Notably, within this population, core Sertoli cell markers were drastically downregulated, while Leydig cell markers were significantly upregulated in *Ctnna1*^{CKO} mice compared to Ctrl (Supplementary Figure S8K, L). These results strongly substantiate the occurrence of Sertoli-to-Leydig transdifferentiation in *Ctnna1*^{CKO} mice, which could be attributable to the marked reduction in Sertoli cells and the concurrent expansion of the Leydig cell population.

Ultimately, *in vitro* fertilization (IVF) assays demonstrated that *Ctnna1*^{CKO} sperm achieved fertilization rates comparable to controls in crosses with WT females (Supplementary Figure S9). Furthermore, *Ctnna1*^{CKO} spermatozoa retained normal fertility during IVF, yielding offspring that were exclusively *Ctnna1*^{+/+}, with or without the *Stra8*-Cre transgene. These findings suggest that, despite marked structural defects, sperm lacking α -catenin retain the capacity to fertilize oocytes and support embryonic development.

DISCUSSION

Azoospermia or oligozoospermia (absence or reduced concentration of sperm), asthenozoospermia (reduced sperm motility), and teratozoospermia (increased proportion of morphologically abnormal sperm) represent the most prevalent forms of male infertility (Jin et al., 2023b; Ray et al., 2017). Although recent advances in high-throughput sequencing of infertile male cohorts have significantly expanded our understanding of the genetic underpinnings of male infertility, the etiology remains unknown in approximately 60%–70% of affected individuals (Houston et al., 2021). To explore potential molecular alterations associated with sperm morphological defects, we analyzed a publicly available microarray dataset (Platts et al., 2007) (GSE6872) profiling of sperm cells from individuals with severe teratozoospermia and normozoospermic controls. Unbiased enrichment analysis revealed that, in addition to dysregulated reproductive pathways, sperm from teratozoospermia patients exhibited reduced expression of genes involved in AJs. While previous studies have predominantly focused on the AJs between Sertoli cells, which constitute the blood-testis barrier, the role of AJs within germ cells themselves remains largely unexplored. α -catenin, a key component of AJs that serves as a central linker connecting the cadherin-catenin complex to the actin cytoskeleton (Cheng & Mruk, 2002; Gumbiner, 2000), drew our attention.

In this study, we utilized a conditional knockout mouse model to demonstrate that the loss of α -catenin function in

mouse germ cells resulted in complete sterility in male mice, characterized by abnormal sperm morphology and motility. Notably, SEM and TEM analyses of the sperm ultrastructure revealed detachment between the head and tail, bent flagella, and the abnormal hook morphology of sperm heads in *Ctnna1*^{ckO} spermatozoa. These results indicate that α -catenin is essential for male fertility and the maintenance of sperm ultrastructural integrity.

Azoospermia or oligozoospermia is typically attributed to defects in spermatogenesis, such as meiotic or spermatogenic arrest, whereas asthenozoospermia is generally associated with impaired sperm flagellar function (Jin et al., 2023b). Thus, mutations in genes responsible for flagellar assembly and the regulation of motility are widely recognized as major causes of asthenozoospermia (Tu et al., 2020). However, the defects in meiosis and spermatogenic arrest were rarely reported in asthenozoospermia-related models. Here, we observed dysregulation of meiosis and RNA splicing-related pathways not only in spermatozoa from teratozoospermic patients based on a publicly available microarray dataset (Platts et al., 2007), but also in *Ctnna1*^{ckO} spermatozoa, as revealed by our single-cell RNA-seq analyses. Benefiting from single-cell transcriptomic technology, we were able to accurately distinguish distinct subtypes of spermatogenic cells and systematically investigate the pathway alterations caused by *Ctnna1* deletion within each specific germ cell subtype. scRNA-seq revealed a dramatically increased proportion of spermatogonia and elongating spermatids stage 2, and a reduction in spermatocytes, round spermatids, and elongating spermatids stage 1. Consistent with previous findings (Wu et al., 2024), enrichment analysis revealed that meiosis- and splicing-related pathways were primarily activated in spermatogonia and secondarily in spermatocytes under normal conditions, but were markedly downregulated in both cell types upon *Ctnna1* deletion. Interestingly, pseudotime analysis revealed that following *Ctnna1* knockout, the differentiation of spermatogonia to spermatocytes was disturbed. Taken together, these observations suggest that in addition to mediating sperm ultrastructural integrity, α -catenin also participates in the meiosis and splicing processes of spermatogonia, thereby facilitating the differentiation of spermatogonia into spermatocytes.

Elucidating the temporal hierarchy between RNA splicing dysregulation and meiotic defects is critical for understanding the pathology in the *Ctnna1*^{ckO} mouse model. Our transcriptomic profiling revealed a distinct chronological pattern: pathways governing RNA splicing are predominantly active in spermatogonia and early spermatocytes, temporally preceding the full activation of the meiotic program observed in subsequent stages (Figure 7A–D). This sequential expression profile aligns with established evidence that robust RNA splicing is a prerequisite for meiotic entry, and that perturbations in splicing factors, such as BCAS2 (Liu et al., 2017), BUD31 (Qin et al., 2023), SRSF1 (Lei et al., 2023), or hnRNPH1 (Feng et al., 2022), inevitably precipitate meiotic arrest. Consequently, we believe that the splicing abnormalities in the *Ctnna1*^{ckO} mouse model arise prior to meiotic entry, and therefore are more likely to contribute to, rather than result from, meiotic failure.

Since α -catenin is a structural protein localized to adherens junctions, it is unlikely to be a direct constituent of the nuclear spliceosome. Instead, its role in mRNA processing may be mediated through its structural functions. Recent studies have

demonstrated that α -catenin/vinculin serve as a critical mechanical link between integrin-mediated adhesions and the F-actin cytoskeleton, thereby regulating extracellular matrix (ECM) mechanosensing (Kim et al., 2016; Sarpal et al., 2019), (Mukherjee et al., 2022). Furthermore, growing evidence suggests that mechanical cues, such as ECM stiffness and physical stress, are potent regulators of alternative splicing (Branco Da Cunha et al., 2016; Wang et al., 2023). Consequently, we postulate that the splicing defects observed in *Ctnna1*-deficient cells are not a result of direct α -catenin interference with the spliceosome, but rather a downstream consequence of impaired mechanotransduction and the subsequent disruption of cellular homeostasis.

Under typical circumstances, such meiotic disturbances would be expected to reduce the downstream sperm pool (Li et al., 2024; Zhang et al., 2021a). However, the overall sperm count in *Ctnna1*^{ckO} testes was undisturbed, possibly attributable to the compensatory feedback response during post-mitotic development. By hyper-activating the translational machinery and nuclear compaction program, the germline appears to “force” the progression of surviving cells toward terminal stages to offset the meiotic deficit. However, this compensatory drive comes at the cost of developmental quality. The significant downregulation of genes critical for sperm motility and flagellar assembly during post-meiosis stages suggests a functional decoupling between nuclear maturation and structural morphogenesis. Our morphological observations further corroborate the molecular findings, as the resulting sperm count, though normal in number, exhibits severe structural and functional defects. Similarly, a previous report revealed that premature translation of *Tnp2* leads to reduced sperm motility with abnormal head morphogenesis (Tseden et al., 2007). Collectively, these findings suggest that while a compensatory feedback loop may sustain sperm output volume under meiotic stress, it fails to safeguard the structural and functional integrity of the spermatozoa, ultimately leading to male infertility. Sertoli cells are indispensable for spermatogenesis, providing the essential physical framework for germ cell survival and development, as well as facilitating the release of mature spermatozoa (Wu et al., 2020b). Effective spermatogenesis critically relies on direct cell-cell contact and paracrine interactions between Sertoli cells and germ cells (Bi et al., 2013), the disruption of which invariably leads to spermatogenic failure (Akama et al., 2002). Studies in rat models have demonstrated that N-cadherin is expressed in both isolated Sertoli cells and germ cells (Chung et al., 1998). This protein is localized to the basal compartment of the seminiferous epithelium, where it contributes to inter-Sertoli and Sertoli-germ cell junctions (Johnson & Boekelheide, 2002). It is also detected in the adluminal compartment at the heads of elongating spermatids in a stage-specific manner (Johnson & Boekelheide, 2002). In our study, knockdown of *CTNNA1* resulted in internalization of N-cadherin, possibly contributing to impaired direct Sertoli-germ cell communication. Furthermore, our scRNA-seq analyses revealed a robust downregulation of the paracrine CypA pathway genes (*Ppia* and *Bsg*) within the intercellular communication network involving Sertoli cells and spermatocytes/round spermatids. The *Bsg* gene encodes basigin (CD147), a highly N-glycosylated transmembrane protein expressed in germ cells that mediates their attachment to Sertoli cells. Notably, *Bsg* deletion has been shown to cause spermatogenic arrest and disrupted interactions

between gametes and Sertoli cells (Bi et al., 2013). Therefore, these findings suggest that α -catenin deficiency in male germ cells compromises spermatogenesis, at least in part, by disrupting the direct and indirect communication with Sertoli cells.

A previous study proposed the notion that Sertoli cells possess the potential for reprogramming (Zhang et al., 2015). Our single-cell analysis unveiled a cluster of intermediate state cells that co-express Sertoli and Leydig cell markers, providing compelling evidence for cellular plasticity within the testis (Zhang et al., 2015). Notably, the transcriptomic profile of these transitioning *Ctnna1*^{CKO} mice, characterized by a profound upregulation of Leydig markers and a concurrent loss of Sertoli identity, strongly substantiates the occurrence of Sertoli-to-Leydig transdifferentiation (Zhang et al., 2015). However, while these findings suggest a phenotypic switch that is largely attributable to the marked reduction in Sertoli cells and the concurrent expansion of the Leydig cell population in *Ctnna1*^{CKO} mice, future studies are warranted to dissect the specific downstream signaling pathways by which *Ctnna1* depletion in germ cells contributes to this aberrant transdifferentiation in Sertoli cells. Another limitation of this study is the discrepancy in the estimated Sertoli cell reduction following *Ctnna1* depletion. While scRNA-seq analysis suggested a drastic decrease of over 90% (from 23.74% to 2.06%) (Figure 3R), in situ quantitative immunofluorescence staining of SOX9 revealed a more moderate reduction of approximately 43.5% (Figure 8F). We postulate that the pronounced underrepresentation of Sertoli cells in our scRNA-seq dataset is likely attributable to physical constraints, specifically their large cell size and robust adhesion to germ cells (Mehraein & Negahdar, 2011; Mruk & Cheng, 2015; Russell et al., 1990). Collectively, our findings delineate a critical role for α -catenin in orchestrating spermatogenesis and germ cell development, thereby underscoring its importance in maintaining male reproductive health. Beyond elucidating its mechanistic involvement, we also identify α -catenin as a potential diagnostic biomarker and therapeutic target for male infertility, with particular relevance to teratozoospermia.

DATA AVAILABILITY

All datasets were deposited in the Science Data Bank (DOI: 10.57760/sciencedb.37233), Genome Sequence Archive (GSA, accession No. PRJCA058019), and National Center for Biotechnology Information (NCBI BioProjectID PRJNA1420258) databases.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualization, writing-review, and writing-editing: Z.Y., M.Y., and X.Z. Investigation and writing-original draft: X.H., S.L., and R.Z. Visualization: X.H., S.L., and M.Y. Mouse breeding and genotyping: R.Z., L.P., and L.L. Statistical analysis: L.Y. and Y.H. All authors read and approved the final version of the manuscript.

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