

## *Ccdc152* is not necessary for male fertility, but contributes to maintaining sperm morphology

Ryua HARIMA<sup>1)\*</sup>, Takahiro SASAKI<sup>1)\*</sup>, Takayuki KANEKO<sup>2)\*</sup>, Fuka ASO<sup>1)</sup>, Hayato TAKASHIMA<sup>2)</sup>, Takashi TOYAMA<sup>2)</sup>, Kenshiro HARA<sup>1)</sup>, Kentaro TANEMURA<sup>1)</sup> and Yoshiro SAITO<sup>2)</sup>

<sup>1)</sup>Laboratory of Animal Reproduction and Development, Graduate School of Agricultural Science, Tohoku University, Sendai 980-8572, Japan

<sup>2)</sup>Laboratory of Molecular Biology and Metabolism, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8572, Japan

**Abstract.** Selenoprotein P (SeP) is synthesized in the liver and plays a vital role in maintaining selenium homeostasis via transport throughout the body. Previous studies have shown that SeP-deficient mice have severely reduced expression of selenoproteins essential for testicular function, leading to male infertility. We previously reported that the high expression of *Ccdc152* in hepatocytes acts as a lncRNA, suppressing SeP expression in the liver. *Ccdc152* reduces SeP translation by binding to SeP mRNA and decreasing its interaction with SECIS-binding protein 2. Although *Ccdc152* is highly expressed in testes, its function remains unclear. Therefore, this study aimed to elucidate the role of *Ccdc152* in the testes. Using the CRISPR/Cas9 system, we generated mice lacking all exons of *Ccdc152* and found that SeP expression levels in the liver and plasma, as well as overall selenium homeostasis, remained unchanged. No significant differences were observed in the expression of glutathione peroxidase 1/4 or level of selenium in the testes. Subsequent investigation of the impact on male reproductive function revealed no abnormalities in sperm motility or Mendelian ratios of the offspring. However, a slight decrease in testicular weight and an increased rate of sperm malformations in the epididymis were observed. RNA-seq and pathway analyses identified the reduced expression of multiple genes related to kinesin and reproductive pathways. Based on these findings, *Ccdc152* may not be essential for male reproductive function, but it may enhance reproductive capabilities by maintaining the expression of genes necessary for reproduction.

**Key words:** *Ccdc152*, Glutathione peroxidase, Selenoprotein P, Spermatogenesis, Sperm morphogenesis

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**S**permatogenesis is a highly complex regulatory process that can be broadly classified into three stages. The first stage involves the self-renewal and differentiation of spermatogonia, which continuously undergo mitosis and maintain testicular homeostasis [1]. The second stage is meiosis, in which spermatocytes differentiate from spermatogonia in response to retinoic acid [2, 3]. The final stage is spermiogenesis, during which spermatids undergo dramatic morphological changes and become elongated. During this process, the spermatid head elongates, chromatin condenses by replacing histones with protamine [4], and acrosomes and flagella, which are responsible for fertilization, are formed [5, 6]. The continuous production of sperm throughout an individual's lifespan is supported by active self-renewal and differentiation of spermatogonia [1, 7]. High metabolic activity during these processes leads to significant mitochondrial oxygen consumption, resulting in the production of reactive oxygen species (ROS) [8]. Moreover, mitochondria are abundant in secondary spermatocytes and the middle piece of the flagella [9, 10], which actively produces ATP, resulting in high ROS

production [11]. The accumulation of ROS leads to a reduction in sperm motility [12, 13] and an increase in cell death [14], thereby causing male infertility [11]. However, moderate amounts of ROS have positive effects on spermatogenesis and sperm function [15], including promoting spermatogonia self-renewal [16] and improving sperm function during capacitation [17–21].

The essential trace element selenium plays a pivotal role in spermatogenesis in the testes [22]. Excessive and deficient concentrations of selenium in the testicular tissue can result in defective spermatogenesis [23]. Selenoprotein P (SeP), encoded by the *SELENOP* gene, originates in the liver and serves as a selenium transporter. It is distributed to various organs through the systemic circulation and is specifically taken up by the testes via its receptor, Apolipoprotein E Receptor 2 (ApoER2) [24, 25]. Prior studies have demonstrated that selenium deficiency leads to reduced expression of enzymes such as glutathione peroxidase (GPx) 4 and GPx1 in the testes [26, 27]. This downregulation is associated with cytoskeletal abnormalities and diminished antioxidant capability, which compromise spermatogenesis.

In a previous study, we identified the *coiled-coil domain-containing protein 152* (*Ccdc152*) gene, which functions as a long non-coding RNA (lncRNA) that inhibits SeP translation in the liver. We termed this novel gene product *lncRNA inhibitor of SELENOP translation* (*L-IST*). The inhibition occurs via an interaction between mRNA and SECIS-binding protein 2 (SBP2), a protein essential for SeP translation, which in turn leads to reduced ribosomal binding to *SELENOP* mRNA and suppression of protein expression [28]. Notably, *Ccdc152/L-IST* was predominantly expressed in the testes,

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Correspondence: K Tanemura (e-mail: kentaro.tanemura.e4@tohoku.ac.jp), Y Saito (e-mail: yoshiro.saito.a8@tohoku.ac.jp)

\* R Harima, T Sasaki and T Kaneko equally contributed to this study.

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as evidenced by Human Brain Atlas data. Furthermore, the CCDC protein family, to which *Ccdc152/L-IST* belongs, has been implicated in fertility, with certain mutations resulting in infertility [29, 30]. Given these findings, we hypothesized that *Ccdc152/L-IST* plays a regulatory role in maintaining selenium homeostasis in the testes, thereby influencing male fertility. The primary objective of this study was to elucidate the effects of *Ccdc152/L-IST* on selenium homeostasis and its consequent effects on male fertility in the testicular environment.

## Materials and Methods

### Chemicals

Sodium dodecyl sulfate (SDS) and acrylamide were purchased from Nacalai Tesque (Kyoto, Japan) and FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), respectively. Penicillin and streptomycin were purchased from Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA). ISOGEN II was purchased from Nippon Gene (Tokyo, Japan). Bouin's solution and Myer's hematoxylin were purchased from Sigma-Aldrich (St. Louis, MO, USA). crRNA, tracrRNA, and Cas9 nuclease were obtained from Integrated DNA Technologies (Coralville, IA, USA). All other chemicals used were of the highest commercially available quality.

### Animals

C57BL/6N mice were purchased from SLC (Shizuoka, Japan) and maintained at  $24 \pm 1^\circ\text{C}$  and  $60 \pm 10\%$  humidity, with a 12-h light/dark cycle and free access to food (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water. All animal care and experimental procedures complied with the regulations for animal experiments and related activities of Tohoku University. The Tohoku University Institutional Animal Care and Use Committee approved this study (2019noudou-003-02).

### Generation of *Ccdc152* FLD mice

*Ccdc152* full-locus deletion (FLD) mice were generated using *i*-GONAD as previously described [31]. Briefly, two crRNAs were designed with the following sequences: 5'-GAACGGCGGACCCAGAGGTGGG-3' and 3'-CCGCCTCATGCGAAGACGGTTT-5'. crRNAs and tracrRNA were annealed at  $94^\circ\text{C}$  for 2 min and mixed with the Cas9 nuclease. This genome-editing mixture was injected into the oviduct of pregnant mice (C57BL/6N males  $\times$  females) at E0.7 and electroporated *in vivo* using a CUY21EditII Electroporator (BEX, Tokyo, Japan). Genotyping was performed using PCR with primers for *Ccdc152* wild type (WT) (Fw: 5'-GGCAGGCCTTTCAGAGTAAGCTAG-3' and Rv: 5'-GGTGTTAATCTGCGTACTGC-3') or FLD (Fw: 5'-AATTGGTGGGGCTCTAAGGTA-3' and Rv: 5'-GGTGTTAATCTGCGTACTGC-3') alleles. The PCR product using the *Ccdc152* FLD primers was Sanger sequenced and a 22,978 bp deletion was identified. To fix the mutation in the strain, the F0 founder was mated with WT C57BL/6N mice, and the resulting F1 heterozygous male and female mice were crossed to obtain an F2 homozygous mutants.

### Fertility test

A single 8-week-old male WT or *Ccdc152* FLD mouse was caged with two 8-week-old female WT mice for 2 weeks. Mating was verified by the presence of vaginal plugs. Vaginal plugs and number of pups per litter were recorded.

### Morphological and histological analysis

After measuring the body weights of both WT and *Ccdc152* FLD mice (12–14 weeks old), the mice were euthanized. The testes and epididymides were isolated and weighed. Testes and epididymides were fixed in Bouin's solution overnight at  $4^\circ\text{C}$ , gradually dehydrated by stepwise substitution with ethanol and xylene at different concentrations, and finally embedded in paraffin. Sections ( $4 \mu\text{m}$ ) were rehydrated in xylene and ethanol at different concentrations, treated with 0.5% periodic acid for 10 min, treated with Schiff's reagent (FUJIFILM Wako Pure Chemical Corporation) for 20 min, and counterstained with Myer's hematoxylin for 30 sec. Sections were examined under a microscope (BX50; Olympus, Tokyo, Japan). To measure the circumference of the tubule, periodic acid-Schiff hematoxylin (PAS-H)-stained sections were examined, and images were analyzed using the ImageJ software. In total, 150 round sections of seminiferous tubules were obtained from three mice of each genotype.

### Sperm motility, morphology, and count analysis

Spermatozoa from cauda epididymis were suspended in a human tubal fluid medium (101.6 mM NaCl, 4.7 mM KCl, 0.37 mM  $\text{K}_2\text{PO}_4$ , 0.2 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2 mM  $\text{CaCl}_2$ , 25 mM  $\text{NaHCO}_3$ , 2.78 mM glucose, 0.33 mM sodium pyruvate, 21.4 mM sodium lactate, 286 mg/l penicillin G, and 228 mg/l streptomycin) and incubated at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$  in humidified air. After incubation for 10 and 120 min, the spermatozoa were analyzed using the Sperm Motility Analysis System (SMAS; DITECT Co., Ltd., Tokyo, Japan). For morphological analysis, spermatozoa were incubated for 120 min, mounted on glass slides, air-dried, and stained with Papanicolaou stain. A total of 1200 spermatozoa were measured for normal morphology from three mice of each genotype. A hemocytometer was used for sperm counting from 1:10 dilution of spermatozoa incubated with 3% NaCl for 120 min.

### Western blotting

Tissue (50 mg) was homogenized in a buffer containing 1% SDS, and proteins were extracted. After sonication, the samples were adjusted to a uniform concentration and applied to acrylamide gels at 20  $\mu\text{g}/\text{lane}$ . Electrophoresis was performed at 300 V for 30 min, followed by transfer at 200 mA for 60 min. The gel was blocked with skim milk and incubated overnight with primary antibodies at the indicated concentrations. Secondary antibodies were added and shaken at room temperature for 1 h before detection. The antibodies used were as follows: rabbit anti-mSeP pAb [32], rabbit anti-GPx4 mAb (ab125066), and rabbit anti-GPx1 pAb (ab22604) from Abcam (Waltham, MA, USA) and mouse anti-GAPDH mAb (5A12) HRP-conjugated from FUJIFILM Wako Pure Chemical Corporation.

### Quantitative real-time PCR (qPCR)

RNA extraction was performed using ISOGEN II following the manufacturer's protocol. Briefly, tissues were homogenized in ISOGEN II solution, the aqueous layer was extracted, and total RNA was recovered via isopropanol precipitation. The concentration and quality of purified RNA were determined using a NanoDrop (Thermo Fisher Scientific). Reverse transcription was performed to obtain cDNA using a PrimeScript Reagent Kit (Takara Bio Inc., Shiga, Japan) with random 6mer and oligo dT primers, following the manufacturer's protocol. qPCR was performed using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific) and a CFX Connect thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All data were normalized to GAPDH mRNA levels. The primers used were

as follows: mouse *SelenoP*: 5'-AGCTCTGCTTGTACAAAGCC-3' and 5'-CAGGTCTTCCAATCTGGATGC-3', mouse *GPx1*: 5'-AGTCCACCGTGATGCCTTCT-3' and 5'-GAGACGCGACATTCTCAATGA-3', and mouse *Gapdh*: 5'-AACTTTGGCATTGTGGAAGG-3' and 5'-ACACATTGGGGGTAGGAACA-3'.

#### RNA sequencing (RNA-seq) analysis

Total RNA was extracted as previously described. Quality checks of the extracted RNA and RNA-seq analysis were performed using Genome-Lead (Kagawa, Japan). A total of 17 million reads were analyzed. The results were analyzed using the corresponding analysis packages in R. Differentially expressed genes were verified using padj. Differential expression was analyzed using a negative binomial distribution model.

#### Statistical analysis

Quantification of band intensity was performed using ImageJ software (version 1.53i). Statistical analyses for all experiments were performed using R (version 4.2.2). For all tests, statistical significance was set at  $P < 0.05$ .

## Results

#### Establishment of *Ccdc152*<sup>full-locus del.</sup> (*Ccdc152* FLD) mice and its effect on selenoprotein expression

Our previous research indicated that *Ccdc152* may act as a lncRNA that suppresses SeP expression in hepatocytes [28]. To investigate its physiological role *in vivo*, we generated *Ccdc152* FLD mice using the *i*-GONAD method, which involves the *in vivo* electroporation of the Cas9 protein and sgRNA complex at the one-cell stage (Fig. 1A). Full locus deletion was verified through Sanger sequencing, utilizing primers that recognize the 5' and 3' UTR (Fig. 1B). Despite the absence of *Ccdc152*, we observed no significant alterations in the SeP and GPx1 mRNA levels in the liver compared to WT controls (Fig. 1C). In addition, the protein expression levels of GPx1 and GPx4 in the liver and SeP levels in the plasma were not altered by *Ccdc152* FLD compared to WT controls (Fig. 1D–F). These observations suggest that *Ccdc152* deficiency may not influence selenoprotein expression in the liver tissue or circulating blood.

#### Effects of *Ccdc152* on selenoprotein expression in the testis

Previous research suggests high expression of *Ccdc152* in the testes [28]. Subsequent analyses were conducted on mouse testes to assess the effects of *Ccdc152* deficiency on selenoprotein expression. Testicular tissue was excised from male mice and both the mRNA and protein levels of selenoproteins were quantified. No significant changes were observed in either expression metric compared to WT controls (Fig. 2A–C). These findings implied that the absence of *Ccdc152* did not influence selenoprotein expression in the testes.

#### *Ccdc152* FLD mice have slight abnormalities in testicular morphology

It has been reported that *Ccdc152* mRNA is highly expressed in the testes [28]. We hypothesized that *Ccdc152* plays an essential role in male fertility; therefore, we evaluated the fertility of *Ccdc152* FLD mice. To assess fertility, we mated WT and *Ccdc152* FLD male mice with WT female mice and counted the number of pups per litter over 2 weeks. As shown in Fig. 3A, the number of pups from *Ccdc152* FLD male mice was comparable to that of pups from WT male mice. These results indicated that *Ccdc152* FLD male mice have normal

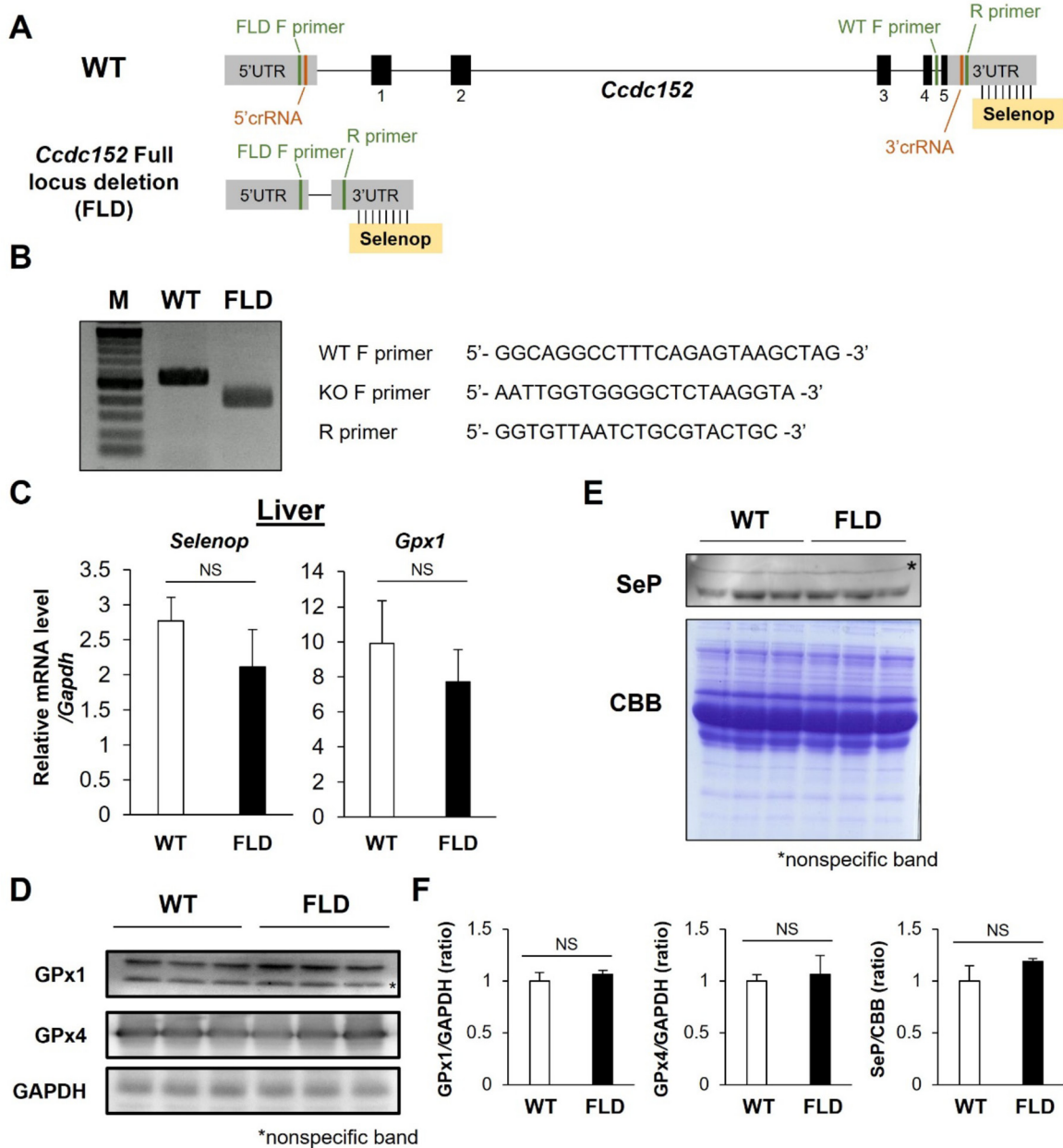
fertility. We then measured the weights of the testes and epididymis of *Ccdc152* FLD mice. The testis/body weight ratio of *Ccdc152* FLD mice was significantly lower than that of WT mice (Fig. 3B), whereas the epididymal/body weight ratio was not significantly different from the WT mice (Fig. 3C). Corresponding to the reduction in the testis/body weight ratio, the circumference of the seminiferous tubules in *Ccdc152* FLD mice was shorter than that in WT mice (Fig. 3D). To examine spermatogenesis in *Ccdc152* FLD mice, we investigated the morphology of the testes and epididymis using PAS-H staining. No significant abnormalities were detected in *Ccdc152* FLD mice compared with WT mice (Fig. 3E). Furthermore, using PAS-H staining, the seminiferous tubule stages were determined based on the shape of the acrosomes of round spermatids and the nuclei of spermatocytes and elongated spermatids. As germ cells corresponding to each stage were present in the seminiferous tubules of *Ccdc152* FLD mice, there was no disturbance in the seminiferous tubule stages, and spermatogenesis proceeded normally (Fig. 3Ea–f). In addition, no significant differences were observed between *Ccdc152* FLD and WT mice in spermatogenesis staging of the seminiferous tubules (Fig. 3Ea–f). A large number of spermatozoa were detected in the cauda epididymis of *Ccdc152* FLD, and no morphological differences were observed compared to WT mice (Fig. 3F). Testicular and epididymal histology indicated that spermatogenesis proceeded normally in *Ccdc152* FLD mice. Taken together, *Ccdc152* FLD mice did not show significantly affected fertility but there were slight morphological abnormalities, such as a decreased testis weight ratio and a shortened seminiferous tubule perimeter.

#### *Ccdc152* FLD mice have normal sperm function

We then focused on the morphology and motility of spermatozoa. Cauda spermatozoa were collected and subjected to Papanicolaou staining for morphological analysis (Fig. 4A). The percentage of spermatozoa displaying abnormal morphology was significantly increased in *Ccdc152* FLD compared to that in the WT (Figs. 4A, B). Specifically, spermatozoa head and flagella abnormalities were observed in *Ccdc152* FLD mice (Figs. 4Ac', d'). There were no changes in the number of spermatozoa in the cauda epididymis between the WT and *Ccdc152* FLD mice (Fig. 4C). Sperm motility was also measured using SMAS, and no significant changes were observed in *Ccdc152* FLD compared to the WT at 10 and 120 min of incubation (Fig. 4D). These results suggested that *Ccdc152* is involved in spermatozoa morphogenesis, but not sperm motility.

#### *Ccdc152* is involved in sperm morphogenesis

To identify the gene alterations responsible for the functional abnormalities, we conducted an RNA-seq analysis of the testes. MA plots were generated to visualize changes in gene expression, with genes showing adjusted p-values less than 0.05 highlighted in red, and selenoproteins in blue (Fig. 5A). Pathway enrichment analysis revealed an overrepresentation of pathways related to Reproduction and Kinesins, both of which are crucial for reproductive functions (Fig. 5B). Genes that exhibited particularly high levels of variability within these pathways are shown in Fig. 5C. These variable genes were further represented in volcano plots for visual inspection (Fig. 5D). Although the observed changes in gene expression were not statistically significant, consistent directional changes were observed across datasets. These findings suggest that the loss of *Ccdc152* contributes to the increased prevalence of sperm with abnormal morphology, possibly through the subtle downregulation of genes crucial for sperm morphogenesis.



**Fig. 1.** Establishment of *Ccdc152* full-locus del. (FLD) mice and its effect on selenoprotein expression. (A) Schematic representation of the *Ccdc152* genomic region. (B) Agarose gel electrophoresis of *Ccdc152* editing in *Ccdc152* FLD mice. (C–E) Expression of selenoproteins in the livers of *Ccdc152* FLD mice, assessed using (C) qPCR, (D) western blotting, and (E) plasma SeP levels. (F) Quantification of the protein expression levels in (D) and (E). Data are shown as mean  $\pm$  standard deviation (SD) (n = 3).

## Discussion

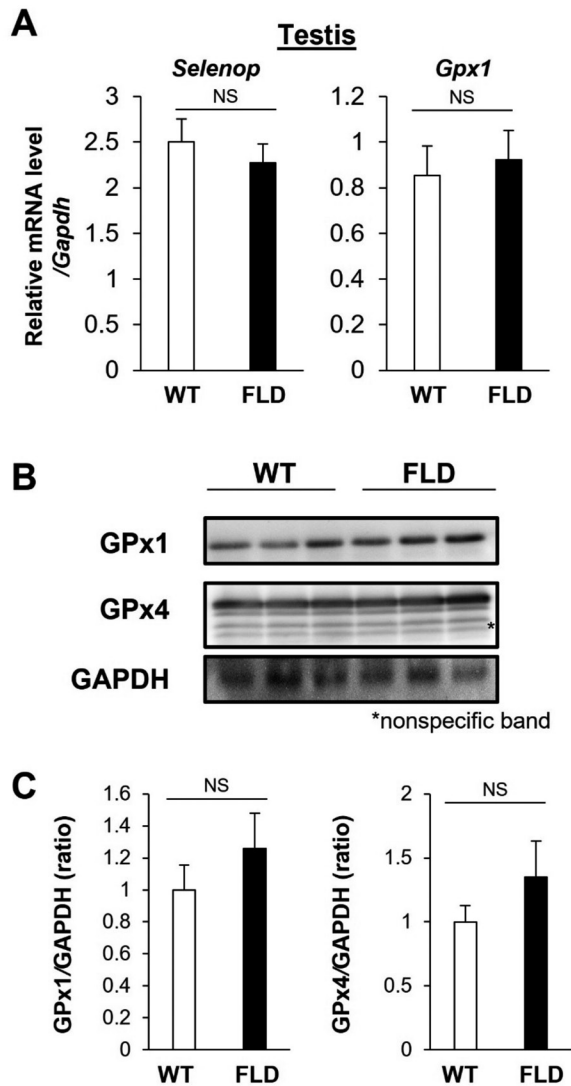
In this study, we investigated the role of *Ccdc152* in testicular function. Our results indicated that the loss of *Ccdc152* does not influence the expression of SeP or other selenoproteins in the liver or testes. However, RNA-seq data suggested that *Ccdc152* affects testicular and sperm morphogenesis by uniformly reducing the expression of kinesin family proteins and disrupting the expression of cytoskeletal-related genes, such as Formin, Ranbp10, Act111, and D7Ert443e.

It has been reported that kinesin family knockout mice show abnormal sperm morphology [33]. In the present study, *Ccdc152* FLD mice had downregulated expression of kinesins. Therefore,

downregulation of the kinesin pathway may have caused abnormal sperm morphogenesis in *Ccdc152* FLD mice. During spermiogenesis, the proteins necessary for inducing changes are transported along the microtubules and actin around the sperm head [34]. Hence, it is important for spermiogenesis that the cytoskeleton, including microtubules and actin, is formed normally. Some genes associated with cytoskeleton formation were highly differentially expressed in *Ccdc152* FLD mice. Thus, another cause of the increase in sperm morphological abnormalities could be a disturbance in cytoskeleton-related gene expression. Further studies are required to determine the role of *Ccdc152* in spermiogenesis.

In addition to kinesins, the reproductive pathway was downregulated in *Ccdc152* FLD mice, which included many genes related to





**Fig. 2.** Effects of *Ccdc152* on selenoprotein expression in the testis. (A–B) Expression of selenoproteins in the testes of *Ccdc152* FLD mice, measured using (A) qPCR and (B) western blotting. (C) Quantification of the protein expression levels in (B). Data are shown as mean  $\pm$  SD ( $n = 3$ ).

fertilization and sperm motility. In this study, *Ccdc152* deficiency resulted in malformations, including abnormal sperm morphology, and affected testicular weight during spermatogenesis. Despite these changes, fertility was not affected when measured for 2 weeks, and motile sperm were still observed. However, long-term mating experiments could not be fully evaluated to count the number of total litters and pups, which is a significant subject for future study. Furthermore, these mice may exhibit increased susceptibility to stressors such as aging, environmental toxicity, and nutritional imbalances.

We have previously reported that *Ccdc152* functions intracellularly as a lncRNA in hepatocytes, suppressing protein expression by binding to SeP mRNA during translation [28]. SeP is crucial for transporting selenium to the testes, and a lack of selenium in the testes is believed to lead to defects in spermatogenesis. Therefore, we anticipated fluctuations in selenoprotein expression in the testes of *Ccdc152* FLD mice. However, we observed no disturbances in selenoprotein levels. Mice typically consume 3–4 g of food daily. Given that the CE-2

diet provides 0.39  $\mu\text{g}$  Se/g, this equates to approximately 1.3  $\mu\text{g}$  Se intake per mouse each day [35]. In comparison, the recommended daily intake of selenium for humans is between 25–30  $\mu\text{g}$ . Therefore, when adjusted for body weight, mice ingested selenium at a rate more than 200 times greater than that of humans. Given this significant difference in selenium consumption, the effects of *Ccdc152* FLD may not have been apparent. Moreover, scRNA-seq data showed that *Ccdc152* was primarily expressed in spermatocytes, whereas SeP and its intratesticular receptor ApoER2 were expressed only in Sertoli cells and not in germ cells. Therefore, it seems unlikely that *Ccdc152* and SeP interact directly in the testis because they do not coexist in the same compartment. Given that *Ccdc152* is highly expressed in spermatocytes, it may also play a role in meiosis. Nevertheless, meiosis proceeded normally and numerous haploid cells were observed in *Ccdc152* FLD mice, indicating that *Ccdc152* is either not vital for meiosis or that a similar gene could compensate for its loss. Further analysis is required to elucidate the detailed function of *Ccdc152* in the testis.

We first hypothesized that *Ccdc152/L-IST* plays a regulatory role in maintaining selenium homeostasis in the testes thereby, we prepared *Ccdc152* FLD mice to elucidate the effects of *Ccdc152/L-IST* on selenium homeostasis. Based on the findings in the present study, *Ccdc152* may not be essential for male reproductive function, but it may enhance reproductive capabilities by maintaining the expression of genes necessary for reproduction. Further studies are necessary to understand the physiological role of *Ccdc152/L-IST* and its significance on selenium metabolism and diseases.

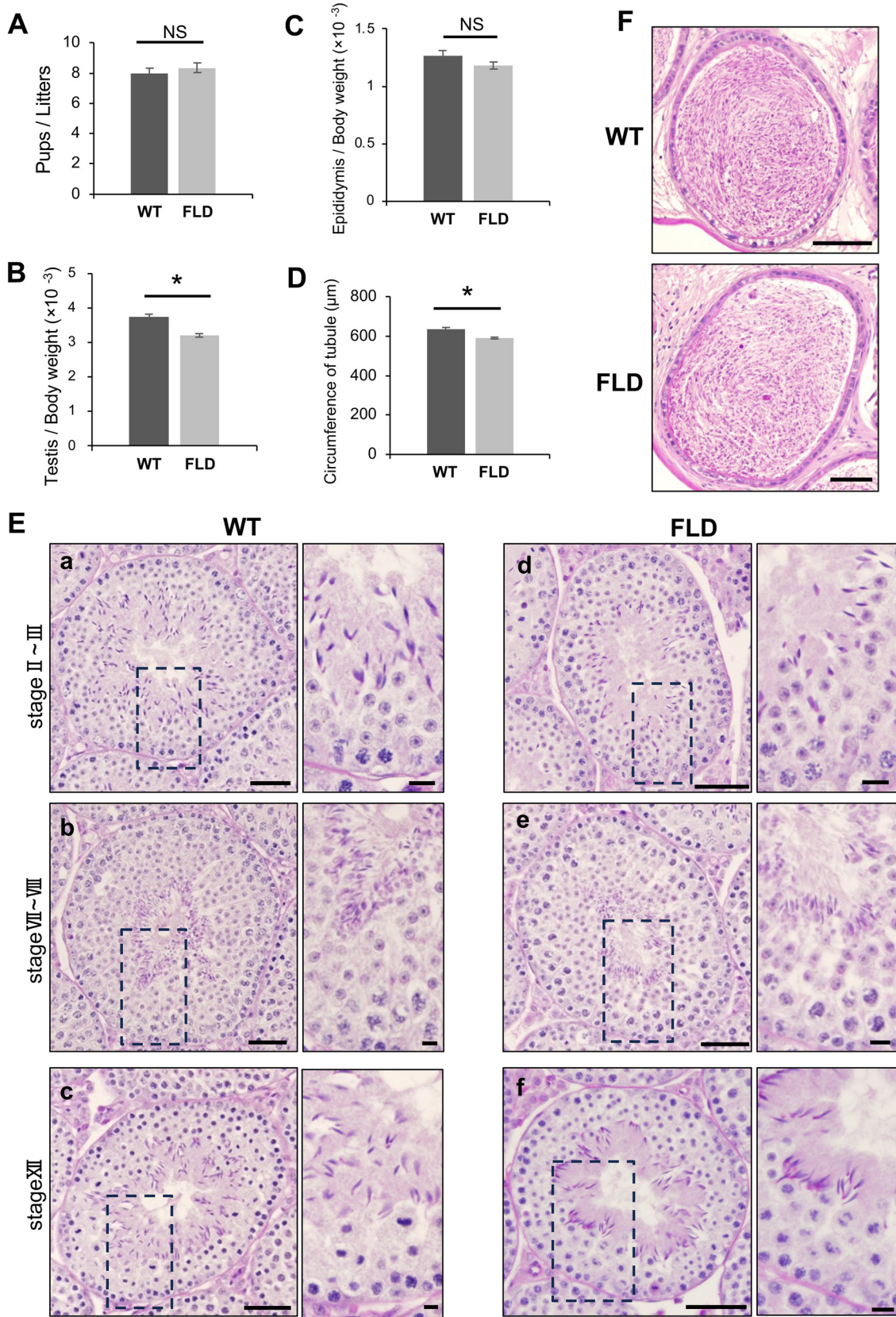
**Conflicts of interests:** The authors state no conflict of interest.

## Acknowledgements

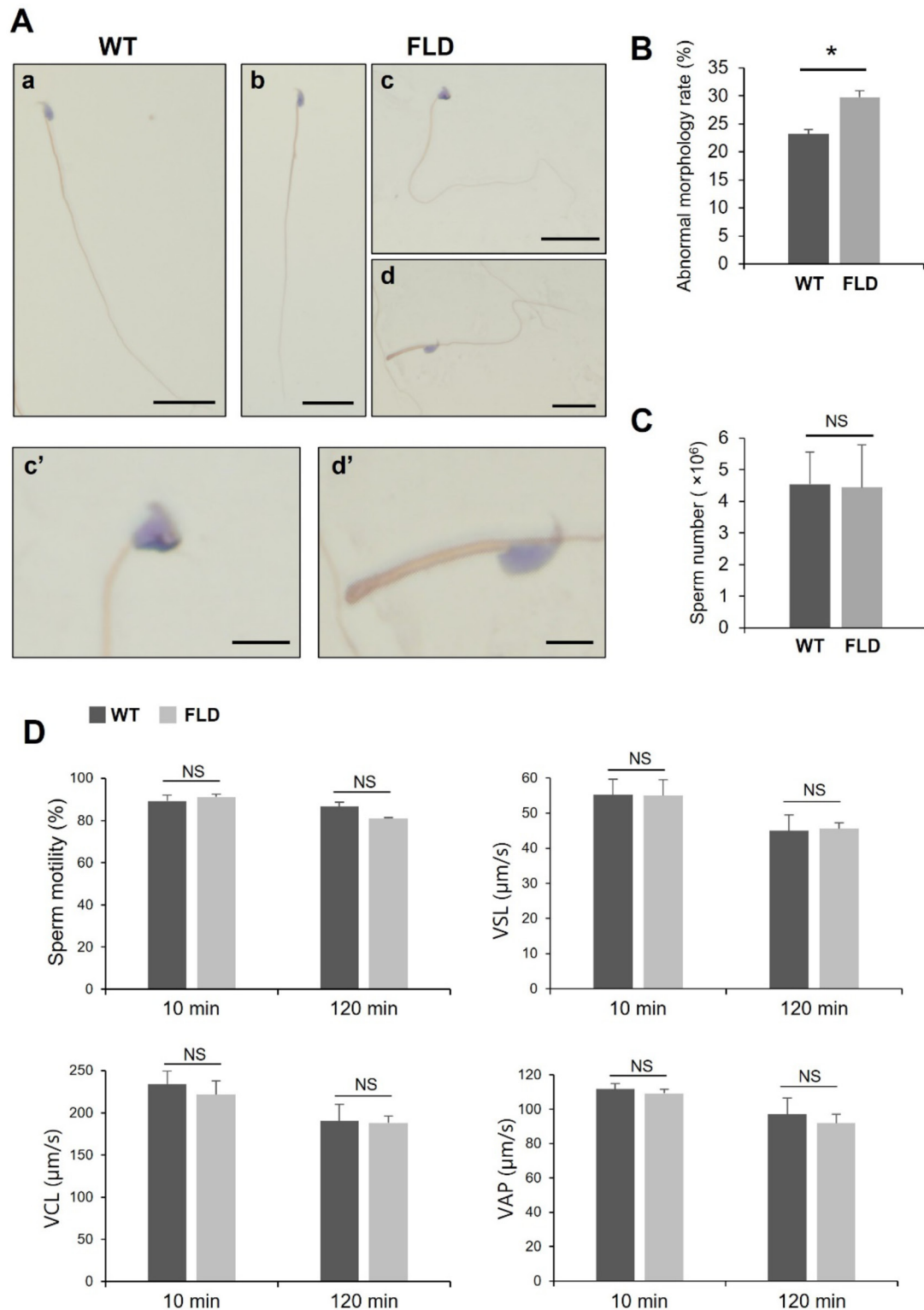
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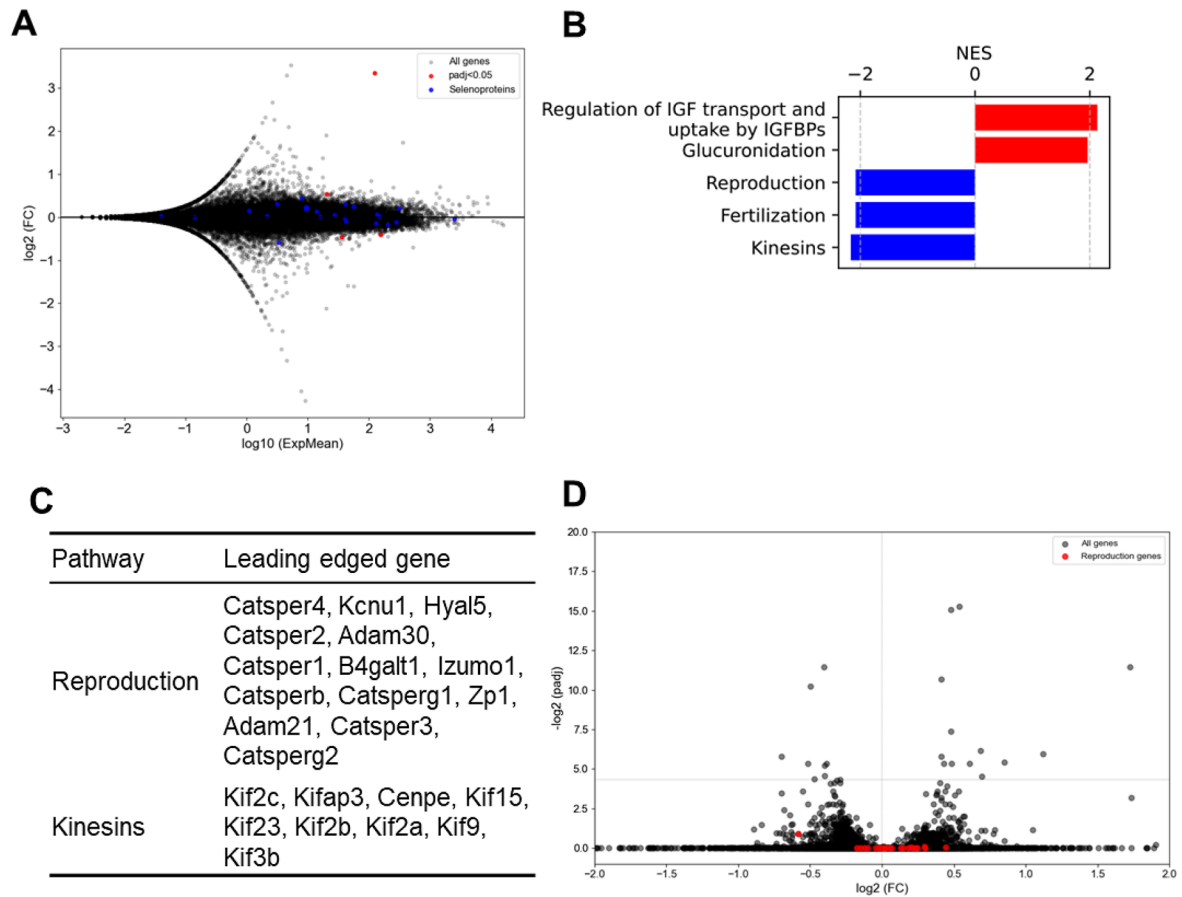


**Fig. 3.** *Ccdc152* FLD mice have slight abnormalities in testicular morphology. (A) Pups/litters in *Ccdc152* FLD mice compared with WT mice from 2 weeks of mating (Student's *t*-test, mean  $\pm$  standard error [SE],  $n = 4$ , \*  $P < 0.05$ ). Litters/plugs; WT: 8/8 ( $n = 4$ ), *Ccdc152* FLD: 12/14 ( $n = 4$ ). (B–D) Measurement of (B) testis/body weight, (C) epididymis/body weight, and (D) circumference of tubule in *Ccdc152* FLD mice compared with WT mice (Student's *t*-test, mean  $\pm$  SE,  $n = 3$ , \*  $P < 0.05$ ). (E) PAS-H staining of seminiferous tubules in (a–c) WT and (d–f) *Ccdc152* FLD mice. Seminiferous tubule stage comparison in (a, d) stage II–III, (b, e) stage VII–VIII, and (c, f) stage XII. Scale bars = 50  $\mu\text{m}$ . In each panel, a high-magnification image of a cropped site is shown (scale bars = 10  $\mu\text{m}$ ). (F) PAS-H staining of cauda epididymis in WT and *Ccdc152* FLD mice. Scale bars = 100  $\mu\text{m}$ .



**Fig. 4.** *Cdc152*<sup>full-locus del.</sup> mice have normal sperm function. (A) Papanicolaou staining of spermatozoa in (a) WT and (b–d) *Cdc152*<sup>full-locus del.</sup> mice. Scale bars = 20  $\mu\text{m}$  (a–d) and 5  $\mu\text{m}$  (c', d'). Panels show (b) normal and (c, d) abnormal morphologies. (c, d) High-power images of spermatozoa showing (c') head and (d') flagella abnormalities. (B–C) Abnormal morphology rate and sperm number in *Cdc152*<sup>full-locus del.</sup> mice compared with WT mice (Student's *t*-test, mean  $\pm$  SE,  $n = 3$ , \*  $P < 0.05$ ). (D) Sperm motility analysis in *Cdc152*<sup>full-locus del.</sup> mice compared with WT mice with incubation times of 10 and 120 min (Student's *t*-test, mean  $\pm$  SE,  $n = 3$ , \*  $P < 0.05$ ). VSL, Straight-line Velocity; VCL, Curvilinear Velocity; VAP, Average Path Velocity.





**Fig. 5.** *Ccdc152* is involved in sperm morphogenesis. RNA-seq analysis of *Ccdc152*<sup>full-locus del.</sup> mice compared with WT mice. (A) MA plot showing variations in gene expression. Genes showing adjusted p-values less than 0.05 are highlighted in red, and selenoproteins are in blue. (B) Results of the pathway analysis for genes with  $p_{adj} < 0.05$ . (C) Genes that significantly affected the pathways. (D) Volcano plot of the genes that showed variation in expression in (C). NES, normalized enrichment score.

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