Usp26 mutation in mice leads to defective spermatogenesis depending on genetic background

5

Authors and affiliations:

Kohei Sakai¹, Chizuru Ito², Mizuki Wakabayashi¹, Satoko Kanzaki¹, Toshiaki Ito¹, Shuji Takada³, Kiyotaka Toshimori^{2,4}, Yoichi Sekita¹ and Tohru Kimura^{1,*}

- ¹ Laboratory of Stem Cell Biology, Department of Biosciences, Kitasato University School of Science, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0373, Japan.
 - ² Department of Functional Anatomy and Reproductive Biology and Medicine, Graduate School of Medicine, ⁴ Future Medicine Research Center, Chiba University Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba, Chiba, 260-8670, Japan.
- ³ Department of Systems BioMedicine, National Research Institute for Child Health and Development, 2-10-1, Okura, Setagaya-ku, Tokyo, 157-8535, Japan.

Correspondence:

20 * Tohru Kimura, PhD

Laboratory of Stem Cell Biology, Department of Biosciences, Kitasato University School of Science,

1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa, 252-0373, Japan

Tel: +81-42-778-8864; Fax: +81-42-778-8864

E-mail: tkimura@ Kitasato-u.ac.jp

Figure S1

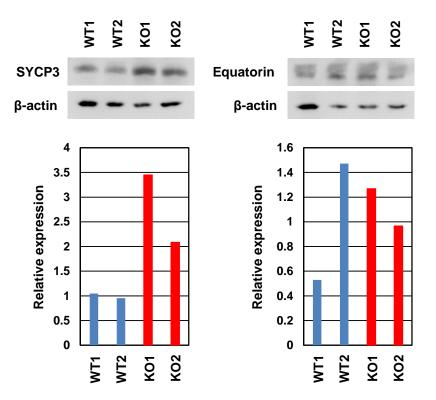


Figure S2

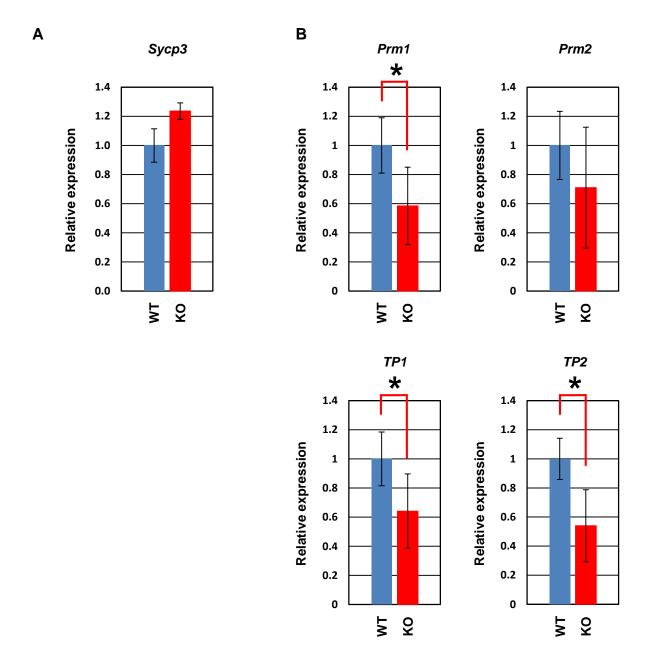
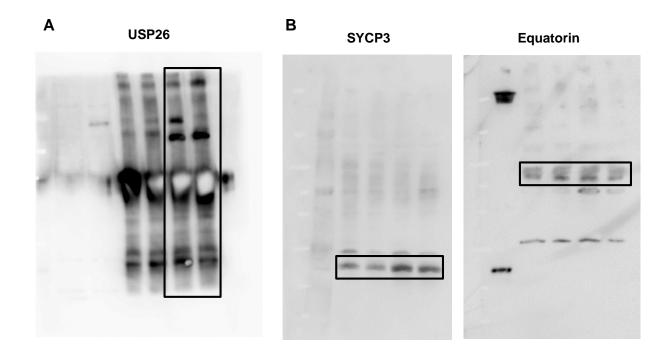
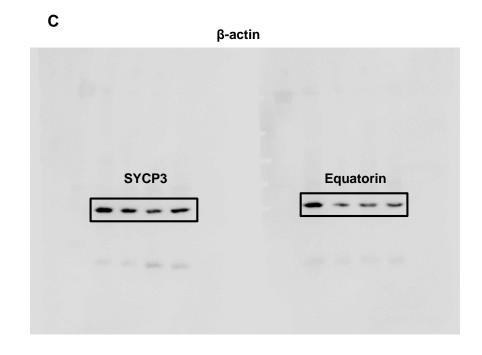


Figure S3





Supplementary Figure Legends

5

10

15

Figure S1. The protein levels of SYCP3 and Equatorin in *Usp26* mutant testis.

Testes lysates were subjected to Western blot analysis using anti–SYCP3 and anti–Equatorin antibodies. The band intensities were quantified using Image-J software and the expression levels were normalized by expression of β -actin.

Figure S2. Expression of spermatocyte and spermatid marker genes in *Usp26* mutant testis.

The mRNA levels of spermatocyte (A; Sycp3) and spermatid (B; Prm1, Prm2, TP1, TP2) marker genes in testes were quantified by RT-qPCR analysis. The expression levels were normalized by expression of the housekeeping gene Rplp0 (Arbp: $ribosomal\ protein\ large\ P0$). Expression of Prm1, TP1 and TP2 was significantly decreased in mutant mice (Student's t-test; *P < 0.05). The mean \pm standard deviation is shown.

Figure S3. Uncropped blot images for Figure 1D (A) and Figure S2 (B, C).

Supplementary Tables

20 Table S1 Primer sequences for qRT-PCR

Gene	Sequence (5' to 3')	Sequence (5' to 3')
Sycp3	ATGGAAACTCAGCAGCAAGAG	TTTACTAAAGGCATGCCTCTTAGC
Prm1	TCAAAACTCCTGCGTGAGAA	GACAGGTGGCATTGTTCCTT
Prm2	CCTGTCCATTCTGCCTGGAG	ACTCAGATCTCGTGGCTCCT
TP1	GTCTTCAAACAACACGGGGC	CGAATTTCGTCACGACTGGC
TP2	TCCTGTGTCCATTTGATCCCA	AGTCTTCCAGGGTACAAGCT
Arbp	CAAAGCTGAAGCAAAGGAAGAG	ATTAAGCAGGCTGACTTGGTTG