Expanded View Figures

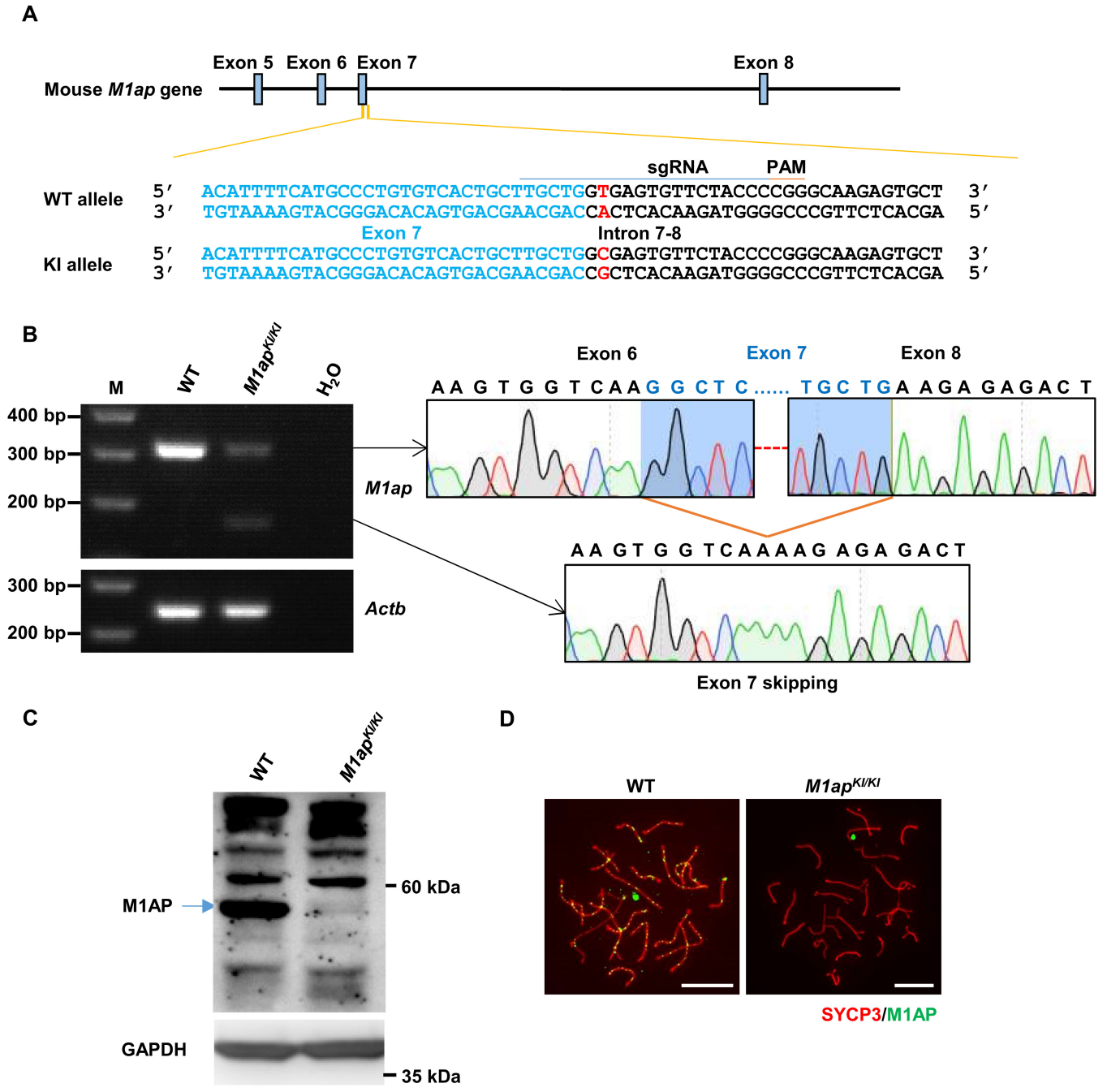

Figure EV1. Generation of *M1ap*$^{KI/KI}$ mice.

A The strategy to generate mice that carry an *M1ap* knockin (KI) mutation equivalent to that of our patients. The single guide RNA (sgRNA, underlined) was designed to target the splicing mutation site (written in red). In the KI allele, thymine was replaced by cytosine. Nucleotides from exon 7 are written in blue. WT, wild-type. PAM, protospacer adjacent motif.

B Reverse transcription-PCR analysis of WT and homozygous *M1ap*$^{KI/KI}$ mouse testes with primers spanning exons 6–8. *Actb* served as an internal control.

C Western blotting with testis lysates from WT and homozygous *M1ap*$^{KI/KI}$ mice using the anti-M1AP antibody. GAPDH was used as the loading control. The band corresponding to WT full-length M1AP protein is indicated by an arrow.

D Immunofluorescence staining of M1AP (green) and SYCP3 (red) on the spermatocyte spreads of WT and *M1ap*$^{KI/KI}$ mice. Scale bars, 10 μm.

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Figure EV2. The phenotype of *M1ap*$^{KI/KI}$ mice.

A Representative images of testes from 8-week-old control and *M1ap*$^{KI/KI}$ mice. Each grid represents 1 mm.
B The ratio of testis/body weight of 8-week-old control and *M1ap*$^{KI/KI}$ mice.
C The sperm count per epididymis of 8-week-old control and *M1ap*$^{KI/KI}$ mice.
D Representative images of hematoxylin and eosin-stained sections of cauda epididymides from 8-week-old wild-type and *M1ap*$^{KI/KI}$ mice. Scale bars, 50 μm.
E Immunofluorescence staining of SYCP3 (red) and MLH3 (green) in spermatocyte spreads from 8-week-old control and *M1ap*$^{KI/KI}$ mice. Scale bars, 10 μm.
F Number of MLH3 foci per cell in control and *M1ap*$^{KI/KI}$ spermatocytes.
G Immunofluorescence staining of SYCP3 (red) and γH2AX (green) on spermatocyte spreads from 8-week-old control and *M1ap*$^{KI/KI}$ mice. Scale bars, 10 μm.
H The percentages of spermatocytes at each stage of meiotic prophase I in 8-week-old control and *M1ap*$^{KI/KI}$ mice.

Data information: (B and C), Data are shown as the mean ± SEM of three biological replicates. (F and H) Data are shown as the mean ± SEM. *n* shows the number of cells scored from three biological replicates. $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; NS, not significant; two-tailed Student's *t*-test.

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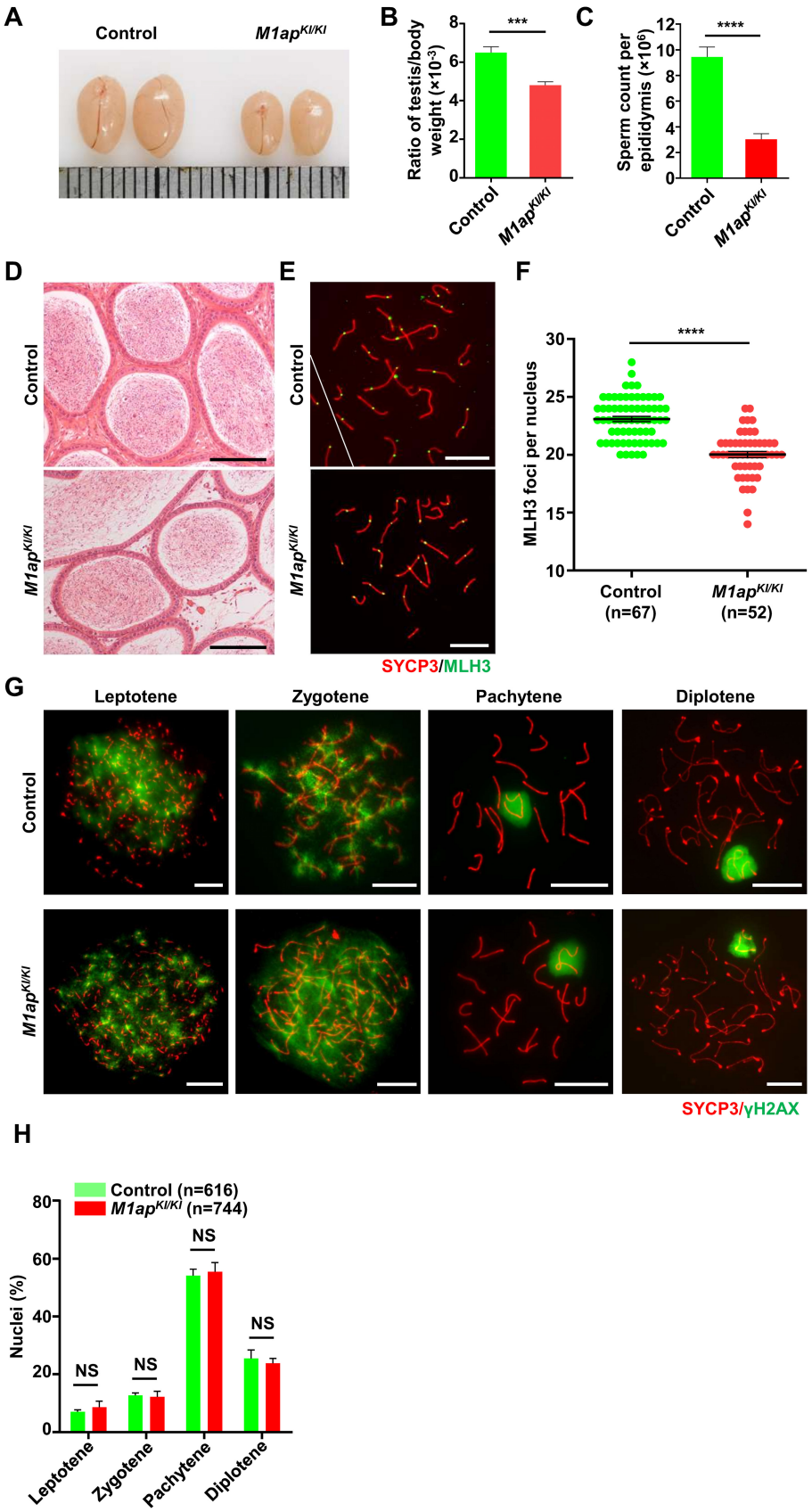

Figure EV2.

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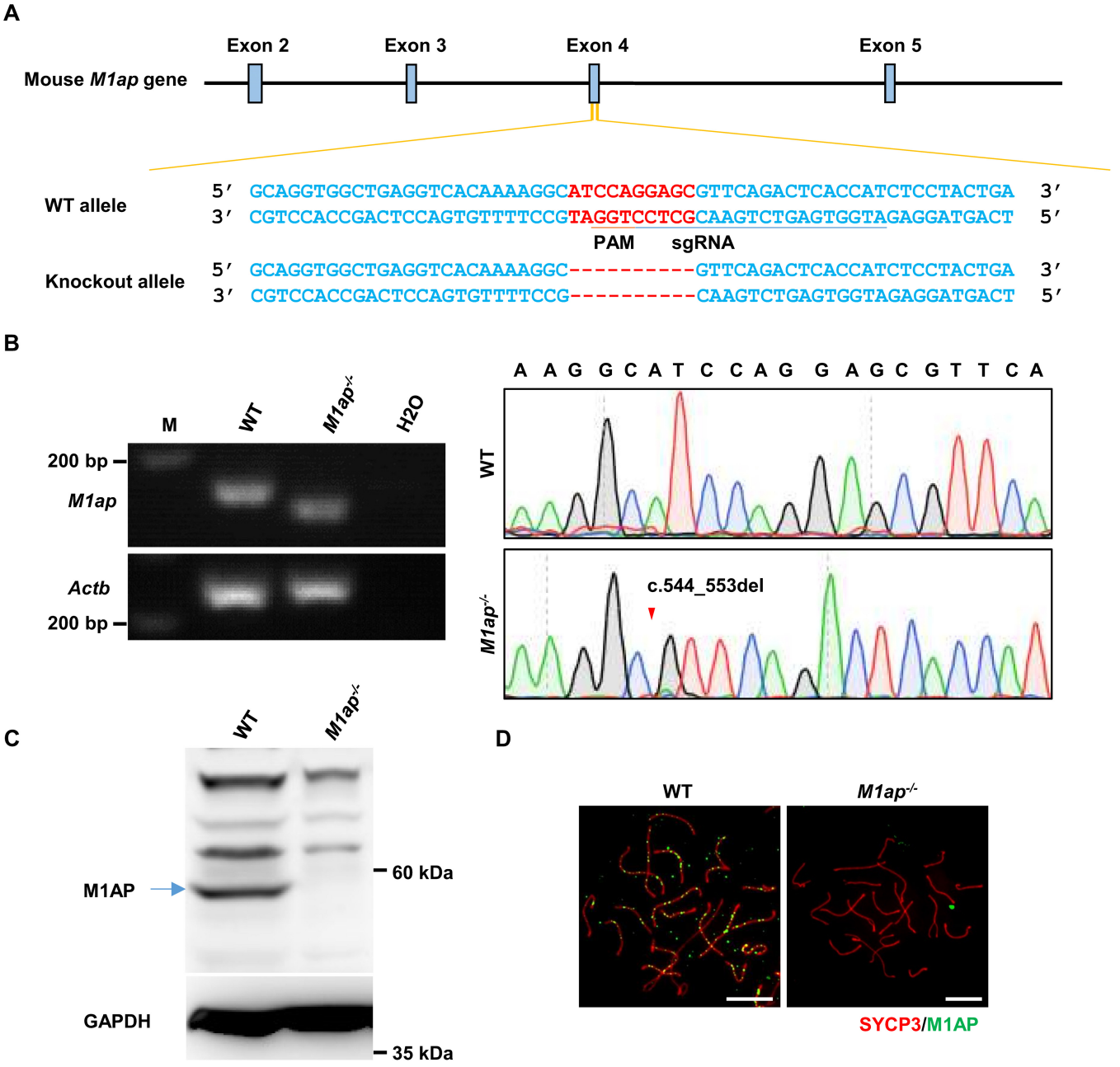

Figure EV3. Generation of *M1ap*$^{-/-}$ mice.

A The strategy to generate *M1ap*$^{-/-}$ mice. The single guide RNA (underlined) was designed to target exon 4 of the *M1ap* gene. A 10-base pair deletion was detected in the *M1ap* knockout allele. WT, wild-type. PAM, protospacer adjacent motif.

B Gel electrophoresis of the PCR products obtained from testis cDNA and subsequent Sanger sequencing showing the 10-base pair deletion in the *M1ap*$^{-/-}$ mice. *Actb* served as an internal control. The arrowhead indicates the mutation site.

C Western blotting with testis lysates from WT and *M1ap*$^{-/-}$ mice using the anti-M1AP antibody. GAPDH was used as the loading control. The band corresponding to full-length WT M1AP protein is indicated by an arrow.

D Immunofluorescence staining of M1AP (green) and SYCP3 (red) on the spermatocyte spreads of WT and *M1ap*$^{-/-}$ mice. Scale bars, 10 μm.

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Figure EV4. The phenotype of *M1ap*$^{-/-}$ mice.

A–C Representative images of testes (A), the ratio of testis/body weight (B), and the sperm count per epididymis (C) from 8-week-old control and *M1ap*$^{-/-}$ mice. Each grid represents 1 mm in (A).

D Representative images of hematoxylin and eosin-stained sections of testes and cauda epididymides from 8-week-old control and *M1ap*$^{-/-}$ mice. A magnified view of the boxed representative metaphase cell is shown in the lower left corner of the image of *M1ap*$^{-/-}$ mice. The blue arrow indicates unaligned chromosomes. Scale bars, 50 μm.

E Immunofluorescence staining of SYCP3 (red) and γH2AX (green) on spermatocyte spreads from 8-week-old control and *M1ap*$^{-/-}$ mice. Scale bars, 10 μm.

F The percentages of spermatocytes at each stage of meiotic prophase I in 8-week-old control and *M1ap*$^{-/-}$ mice.

G Quantification of nuclei with untouching XY chromosomes at pachytene.

H Immunofluorescence staining of SYCP3 (red) and MLH3 (green) in spermatocyte spreads from 8-week-old control and *M1ap*$^{-/-}$ mice. Scale bars, 10 μm.

I Number of MLH3 foci per cell.

J Meiotic metaphase I (MMI) spermatocytes stained with Giemsa. Scale bars, 10 μm.

K Number of bivalents per nucleus.

L Frequencies of nuclei with univalents.

M Frequencies of nuclei with only XY univalents, only autosome univalents, or both XY and autosome univalents.

Data information: (B and C), Data are presented as the mean ± SEM of three biological replicates. (F, G, I, and K–M) Data are presented as mean ± SEM. *n* shows the number of cells scored from at least three biological replicates. $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; NS, not significant; two-tailed Student's *t*-test.

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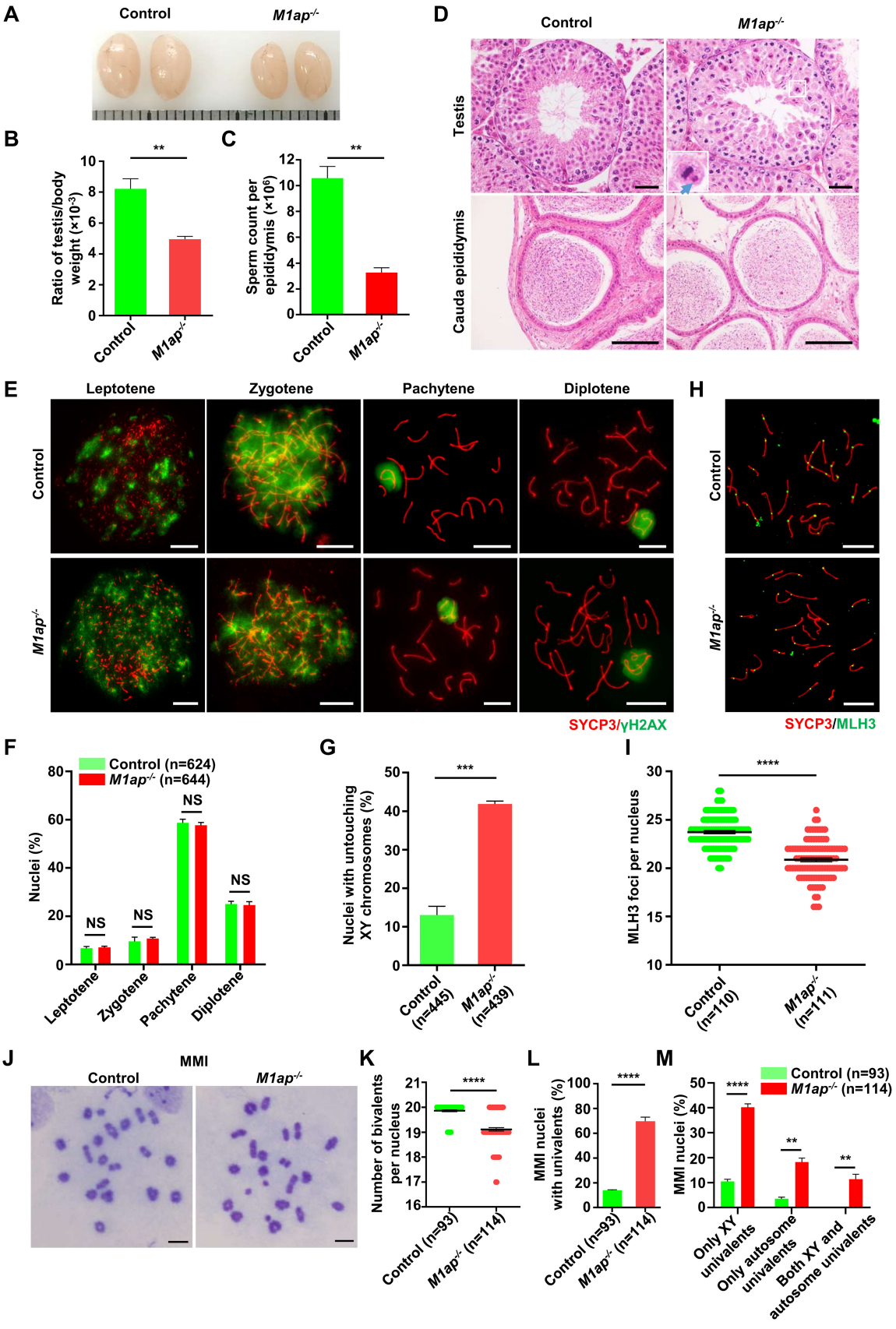
A
Control
M1ap-/-
B
Ratio of testis/body weight (×10-3)
Control
M1ap-/-
C
Sperm count per epididymis (×10^6)
Control
M1ap-/-
D
Control
M1ap-/-
Testis
Cauda epididymis
E
Leptotene
Zygotene
Pachytene
Diplotene
Control
M1ap-/-
SYCP3/γH2AX
H
Control
M1ap-/-
SYCP3/MLH3
F
Control (n=624)
M1ap-/- (n=644)
Nuclei (%)
NS
Leptotene
Zygotene
Pachytene
Diplotene
G
Nuclei with untouching XY chromosomes (%)
Control (n=445)
M1ap-/- (n=439)
I
MLH3 foci per nucleus
Control (n=110)
M1ap-/- (n=111)
J
MMI
Control
M1ap-/-
K
Number of bivalents per nucleus
Control (n=93)
M1ap-/- (n=114)
L
MMI nuclei with univalents (%)
Control (n=93)
M1ap-/- (n=114)
M
Control (n=93)
M1ap-/- (n=114)
MMI nuclei (%)
Only XY univalents
Only autosome univalents
Both XY and autosome univalents

Figure EV4.

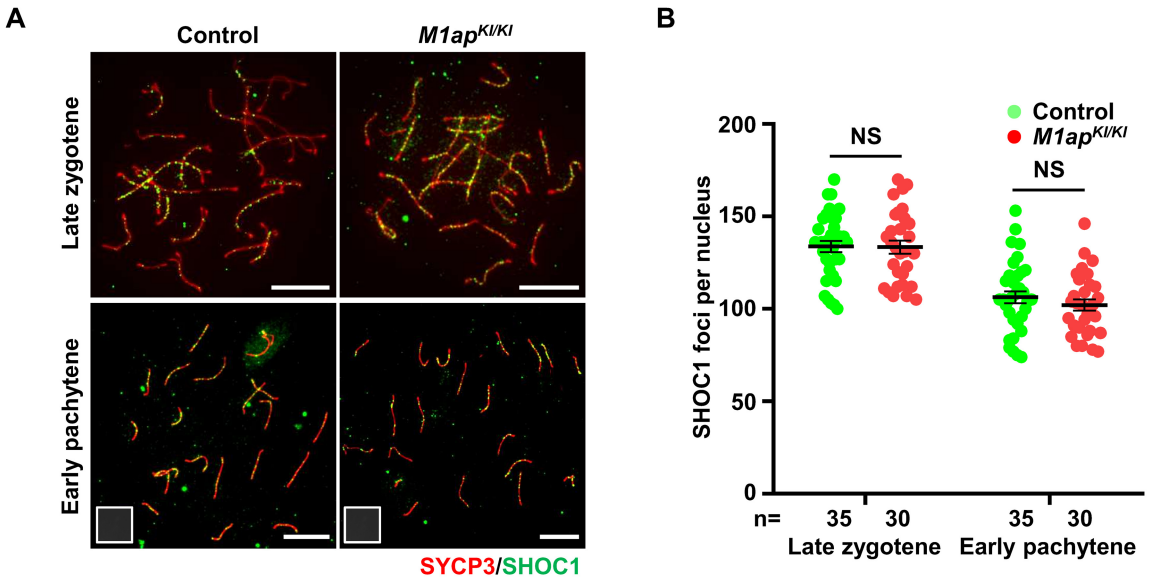

Figure EV5. No obvious differences were observed in the number of SHOC1 foci between control and *M1ap*$^{KI/KI}$ spermatocytes.

A Immunofluorescence staining with antibodies against SYCP3 (red) and SHOC1 (green) on spermatocyte spreads. Scale bars, 10 μm.

B The mean number of SHOC1 foci per cell in control and *M1ap*$^{KI/KI}$ spermatocytes at the indicated stages. Data are presented as the mean ± SEM. *n* shows the number of cells scored from three biological replicates. NS, not significant; two-tailed Student's *t*-test.

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