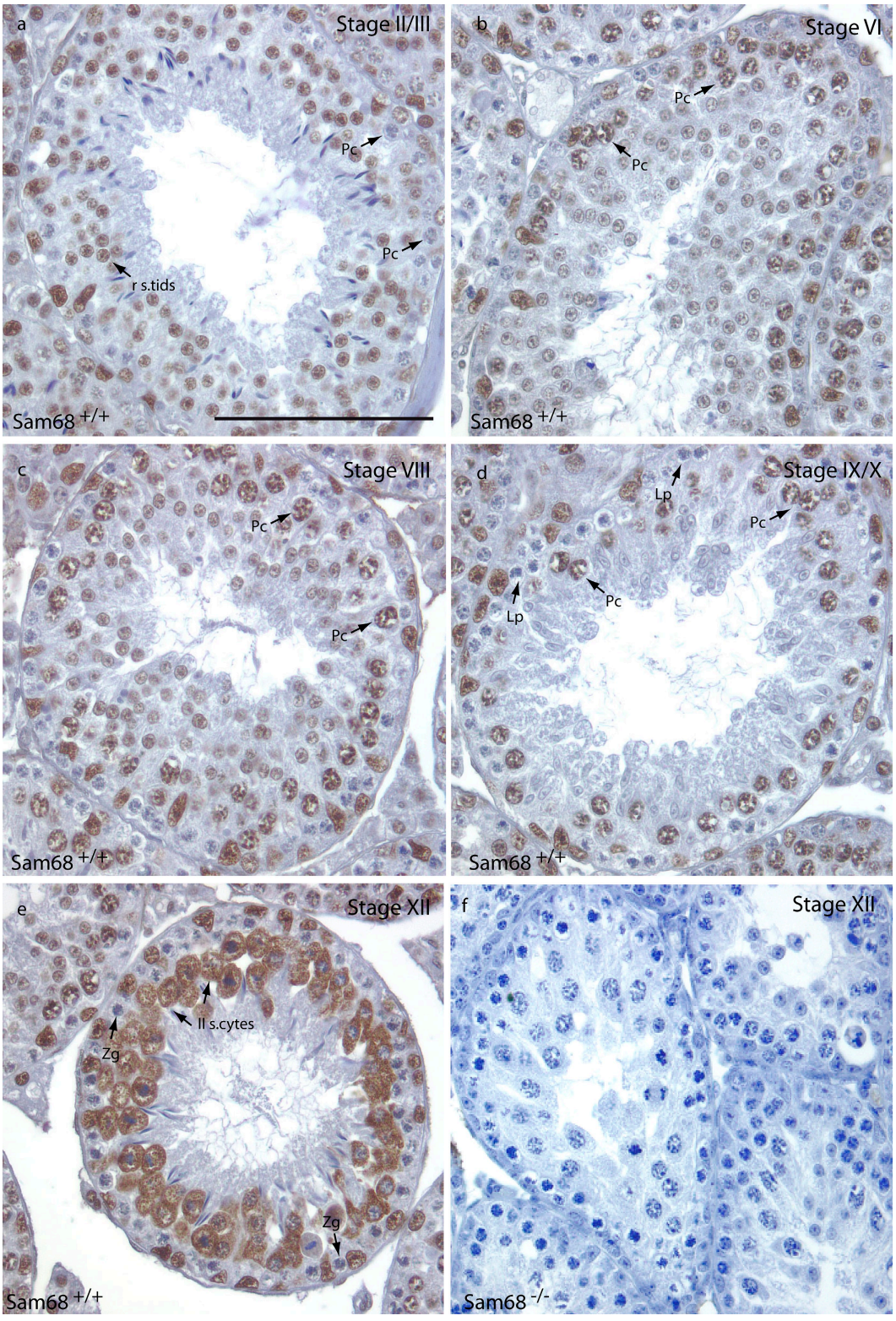



Figure S1. **Immunohistochemical analysis of Sam68 expression in adult mouse testis.** Testicular sections representing different stages of the cycle of mouse seminiferous tubules were stained with anti-Sam68 antibody and counterstained with hematoxylin to detect cell nuclei. Arrows indicate representative cells discussed in the text. The stage of the seminiferous tubule was assessed by staining an adjacent section with periodic acid-Schiff stain, and it is labeled on the top right of each panel. Sam68 protein accumulates from the mid-pachytene stage (Stage VI) to the stages approaching the meiotic divisions (stages X–XII). Stage XII tubules from *Sam68*$^{-/-}$ testis were also stained to determine the specificity of the anti-Sam68 antibody in immunohistochemistry. Lp, leptotene; Pc, pachytene; Zg, zygotene. Bar, 100 µm.

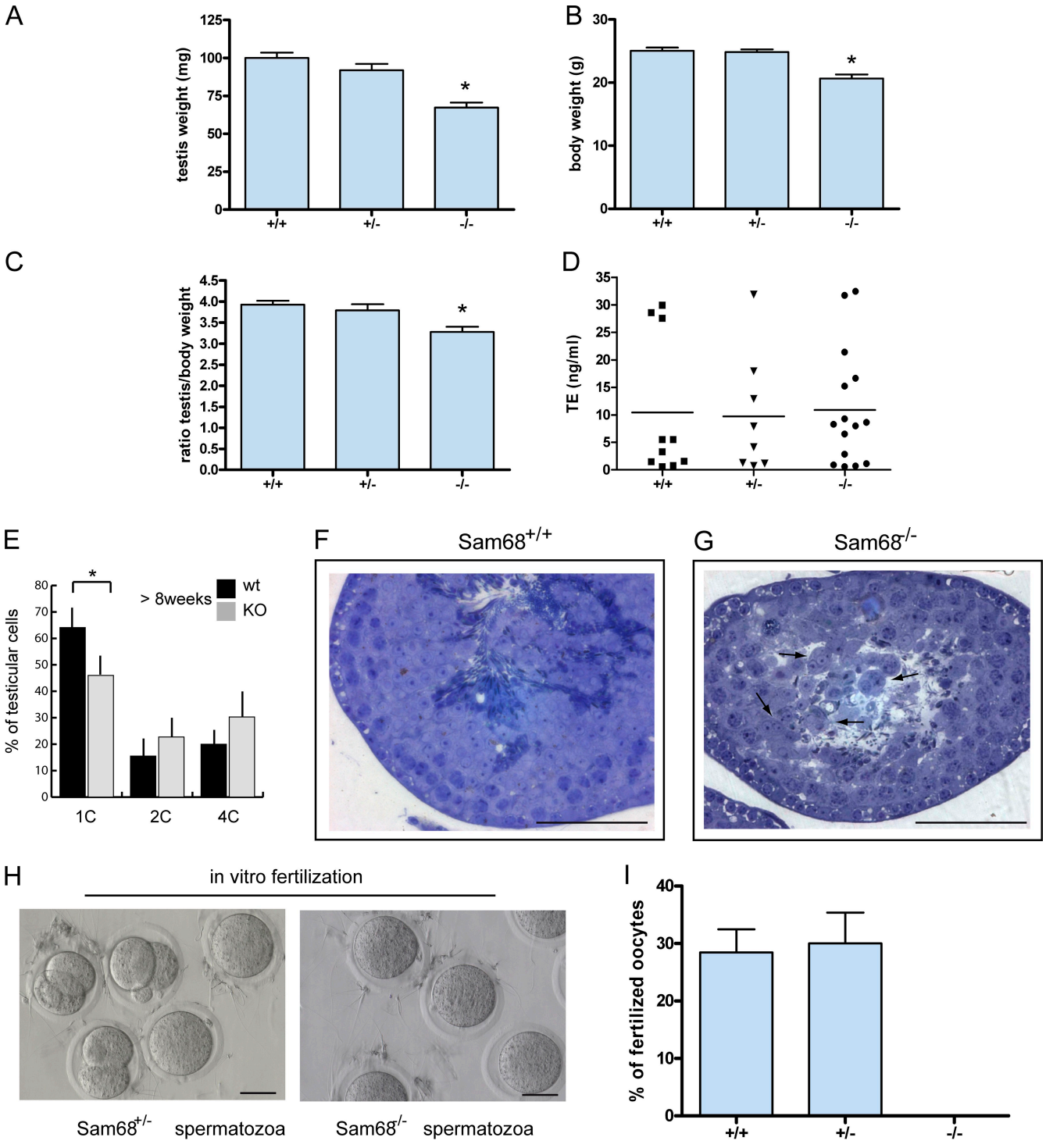



Figure S2. **Analysis of the testicular phenotype and fertility of *Sam68* knockout mice.** (A–C) Analysis of testis weight (A), whole body weight (B), and the ratio of testis/body weight (C) of *Sam68*$^{+/+}$ ($n$ = 14), *Sam68*$^{+/-}$ ($n$ = 13), and *Sam68*$^{-/-}$ ($n$ = 20) mice. *, $P < 0.01$ in $t$ test and ANOVA test. (D) Testosterone levels in serum obtained from *Sam68*$^{+/+}$ ($n$ = 10), *Sam68*$^{+/-}$ ($n$ = 8), and *Sam68*$^{-/-}$ ($n$ = 15) mice. The bar represents the mean value; each symbol represents the actual concentration in each mouse analyzed. (E) Quantitative data of the percentage of each germ cell type as determined by propidium iodine staining in three independent experiments using adult mice (≥8 wk). *, $P < 0.01$ in $t$ test. (F and G) Histological analysis on semithin sections of seminiferous tubules from wild-type (F) or knockout (G) testes. Arrows in G indicate the aberrant cells containing two or more nuclei in postmeiotic round spermatids. (H) Representative examples of in vitro fertilization experiments using wild-type oocytes and *Sam68*$^{+/+}$ or *Sam68*$^{-/-}$ spermatozoa. 24 h after in vitro fertilization, 30–40% of eggs incubated with wild-type spermatozoa were fertilized and reached the two-cell stage of embryo development. By contrast, none of the eggs incubated with knockout spermatozoa were fertilized, and they all remained at the one-cell stage. Bars, 50 µm. (I) Diagram showing the results of three independent in vitro fertilization experiments using *Sam68*$^{+/+}$, *Sam68*$^{+/-}$, and *Sam68*$^{-/-}$ spermatozoa. Data are represented as the mean + SD.

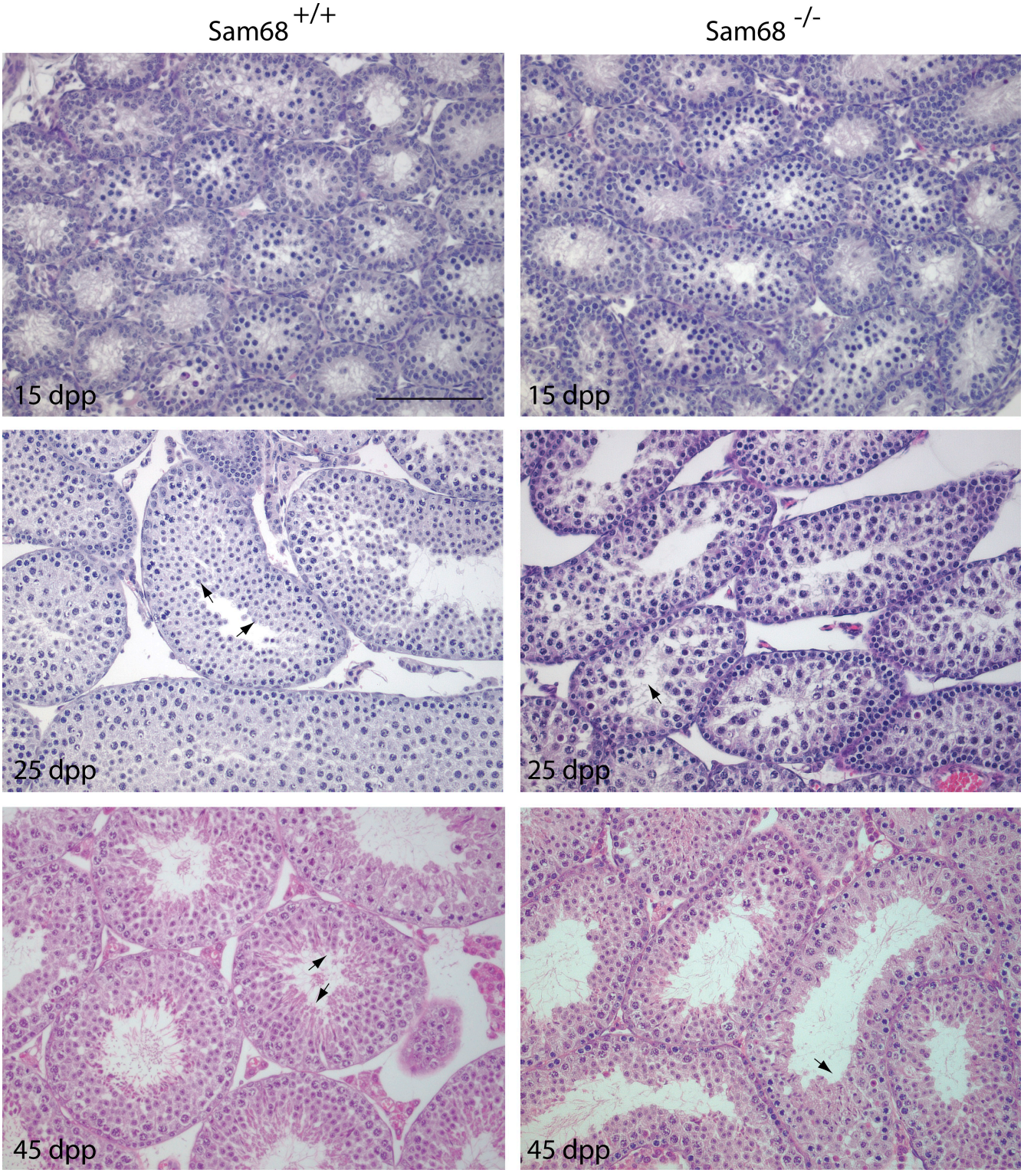



Figure S3. **Histological analysis of testes from *Sam68* knockout mice.** Histological analysis of testis from prepuberal mice (15 dpp), postpuberal mice (25 dpp), and mice that have completed the first wave of spermatogenesis (45 dpp). Arrows indicate round spermatids in the 25-dpp testes and elongated spermatids in the 45-dpp testes. Bar, 100 μm.

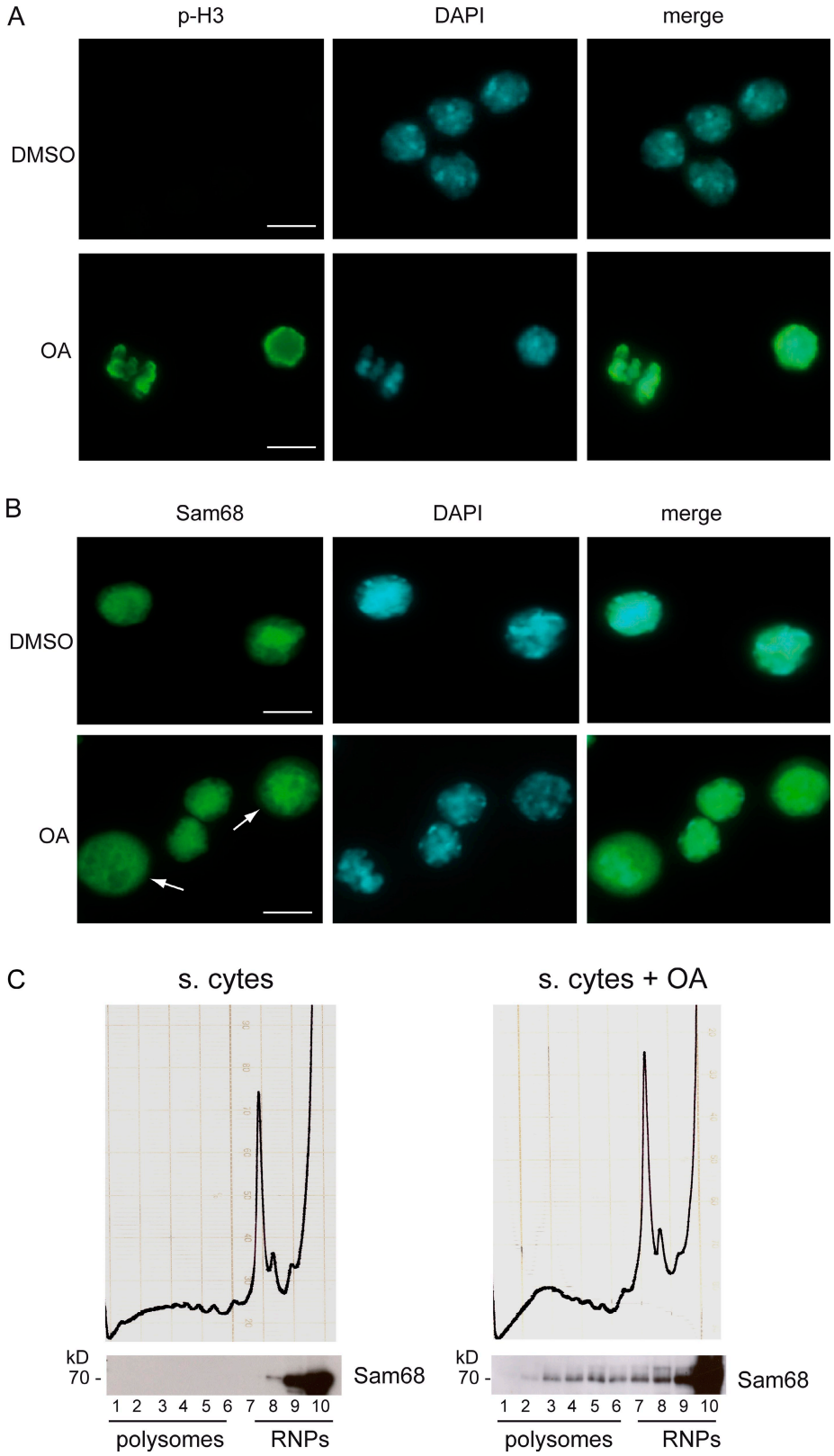
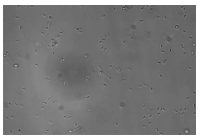
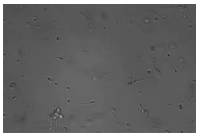


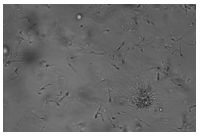

Figure S4. **Effect of stimulation with okadaic acid of pachytene spermatocytes on the subcellular distribution of Sam68.** Control (DMSO) or treated (OA) spermatocytes were analyzed by immunofluorescence with the anti-phosphoH3 (p-H3) antibody (A) or the anti-Sam68 antibody (B). Phosphorylated H3 staining indicates that spermatocytes have progressed to the M phase of the first meiotic division. Arrows in B indicate spermatocytes in which Sam68 is localized in the cytoplasm after OA treatment. Cells were also stained for DNA with Hoechst 3332. (A and B) Rightmost panels show the merged images of pH3 or Sam68 and DNA staining. (C) Absorbance profile (OD = 254 nm) of sucrose gradient sedimentation of cells extracts from control (DMSO) or treated (OA) mouse spermatocytes. The bottom panels show the Western blot analysis of Sam68 distribution in each fraction of the gradients.



Video 1. **Defects in spermiogenesis in *Sam68*$^{-/-}$ testis.** Motility is impaired in spermatozoa from *Sam68* knockout mice (~50% of the animals tested). Most of the spermatozoa produced by *Sam68*$^{-/-}$ mice were abnormally shaped and immotile. Some spermatozoa displayed motility but the majority lost their flagellum shortly after their release in fertilization medium. Images were taken from an inverted microscope (DMI6000B; Leica) using a Pan-Neofluar 40×/0.75 objective lens. Images were acquired at room temperature using an RT-slider camera (Diagnostic Instruments, Inc.) and LIF software (Leica). The display rate is 5 frames per second.



Video 2. **Defects in spermiogenesis in *Sam68*$^{-/-}$ testis.** Motility is impaired in spermatozoa from *Sam68* knockout mice (~50% of the animals tested). The few *Sam68*$^{-/-}$ spermatozoa that maintained reduced motility in culture were completely infertile even under in vitro fertilization conditions. Images were taken from an inverted microscope (DMI6000B; Leica) using a Pan-Neofluar 40×/0.75 objective lens. Images were acquired at room temperature using an RT-slider camera (Diagnostic Instruments, Inc.) and LIF software (Leica). The display rate is 5 frames per second.



Video 3. **Normal spermatozoa motility.** Movement of normal spermatozoa from a wild-type mouse contrasts with the impaired motility observed in spermatozoa from *Sam68* knockout mice. Images were taken from an inverted microscope (DMI6000B; Leica) using a Pan-Neofluar 40×/0.75 objective lens. Images were acquired at room temperature using an RT-slider camera (Diagnostic Instruments, Inc.) and LIF software (Leica). The display rate is 5 frames per second.