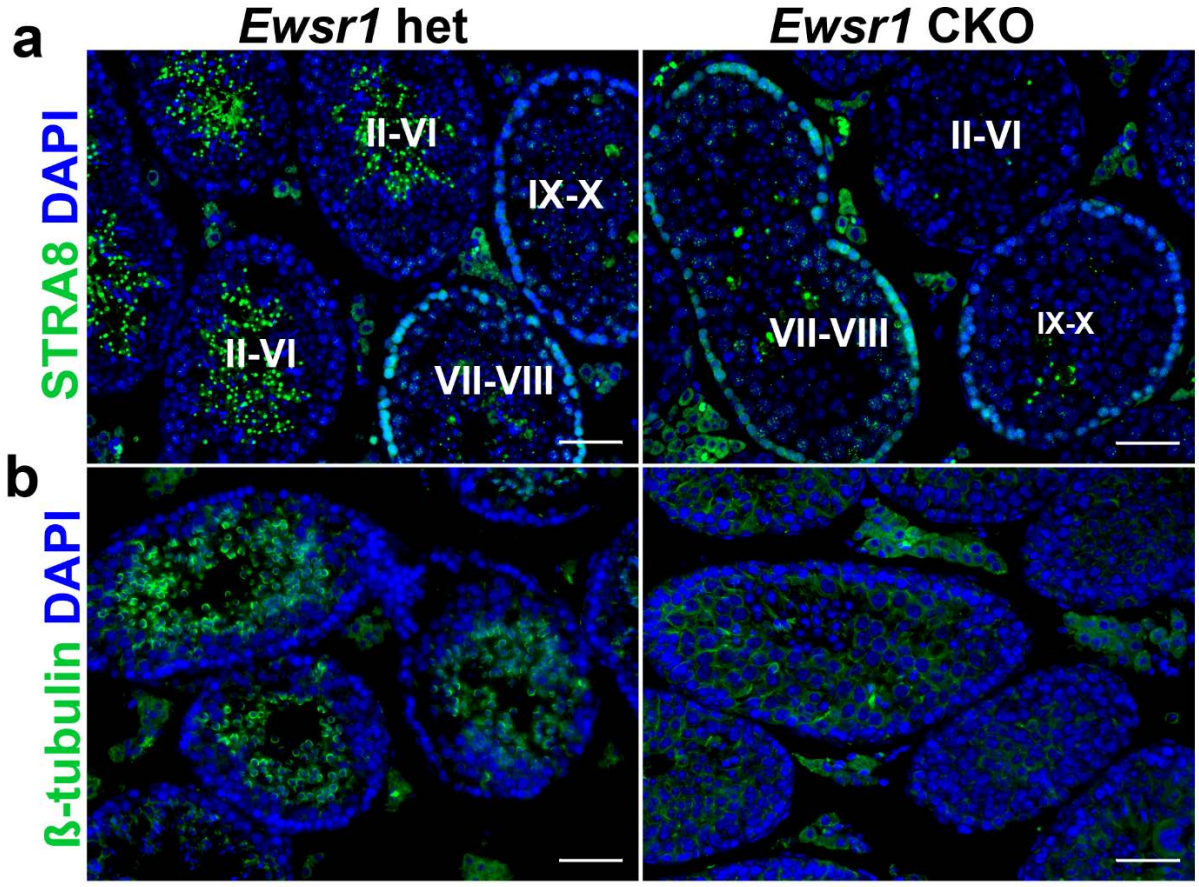

Figure S1. Loss of elongated spermatids in *Ewsr1* CKO seminiferous tubules.

(A) Immunostaining of STRA8 (green) in *Ewsr1* het (left panel) and CKO (right panel) testicular cross sections. The numbers indicate the stage of each seminiferous tubule. (B) Immunostaining of the elongated spermatid marker ß-tubulin in *Ewsr1* het (left panel) and CKO (right panel) testicular cross sections. Scale bar, 50 µm.

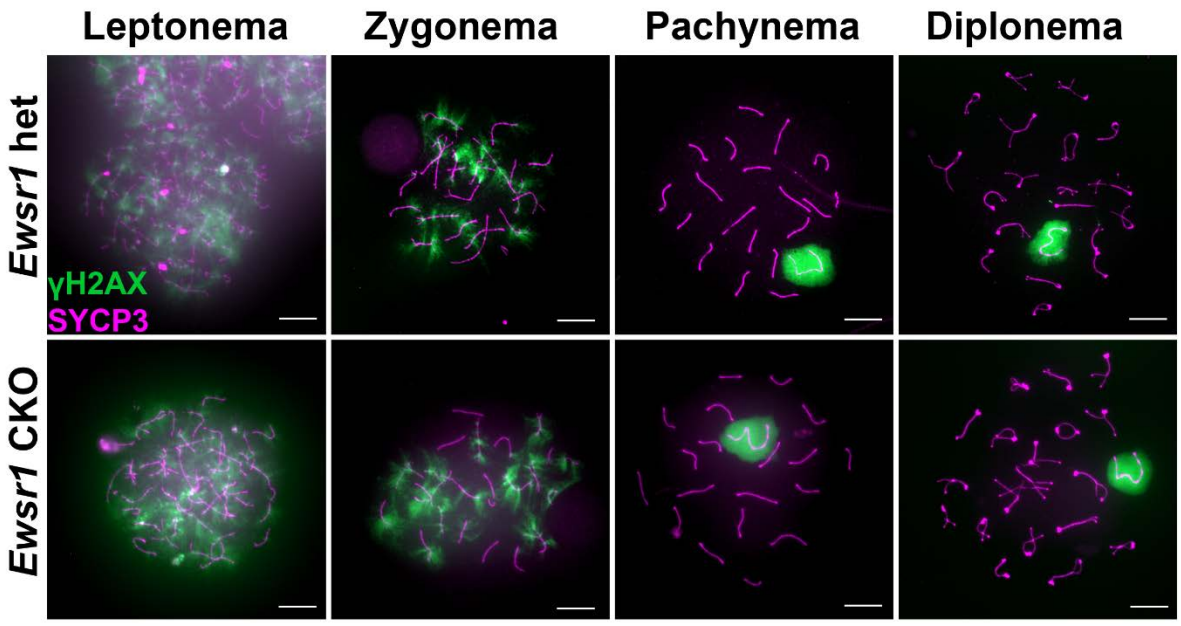

Figure S2. Meiotic recombination is not affected in the absence of EWSR1.

Immunostaining of γH2AX (green) and SYCP3 (magenta) in leptonema, zygonema, pachynema and diplonema of *Ewsr1* het (top panels) and CKO (bottom panels) spermatocytes.

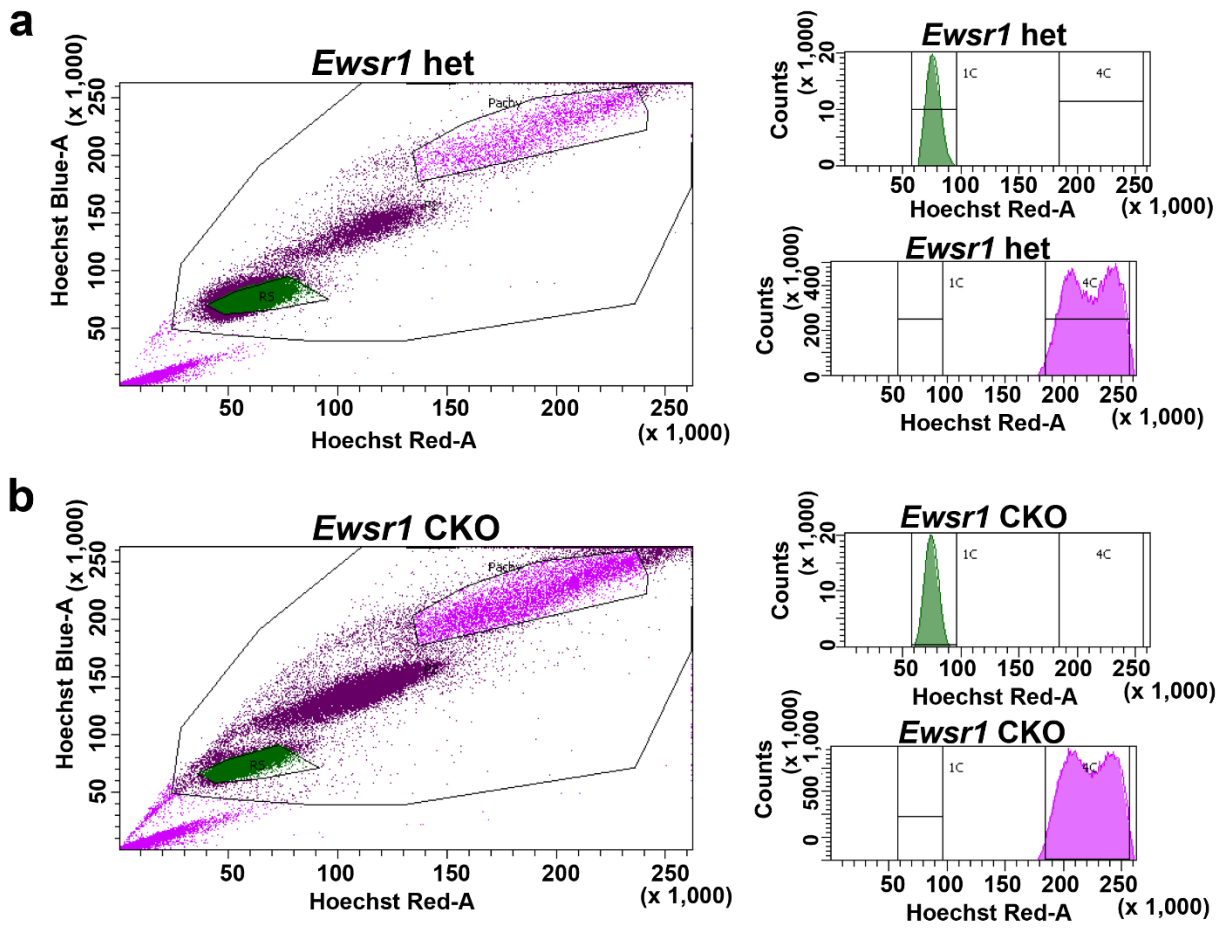

Figure S3. Flow cytometry sorting of pachynema/diplonema and round spermatids in *Ewsr1* het and CKO mice.

(A and B) 4C (pachynema/diplonema) and 1C (RS) cells were sorted based on DNA content by Hoechst 43342 staining of *Ewsr1* het (A) and CKO (B) isolated testicular germ cells.

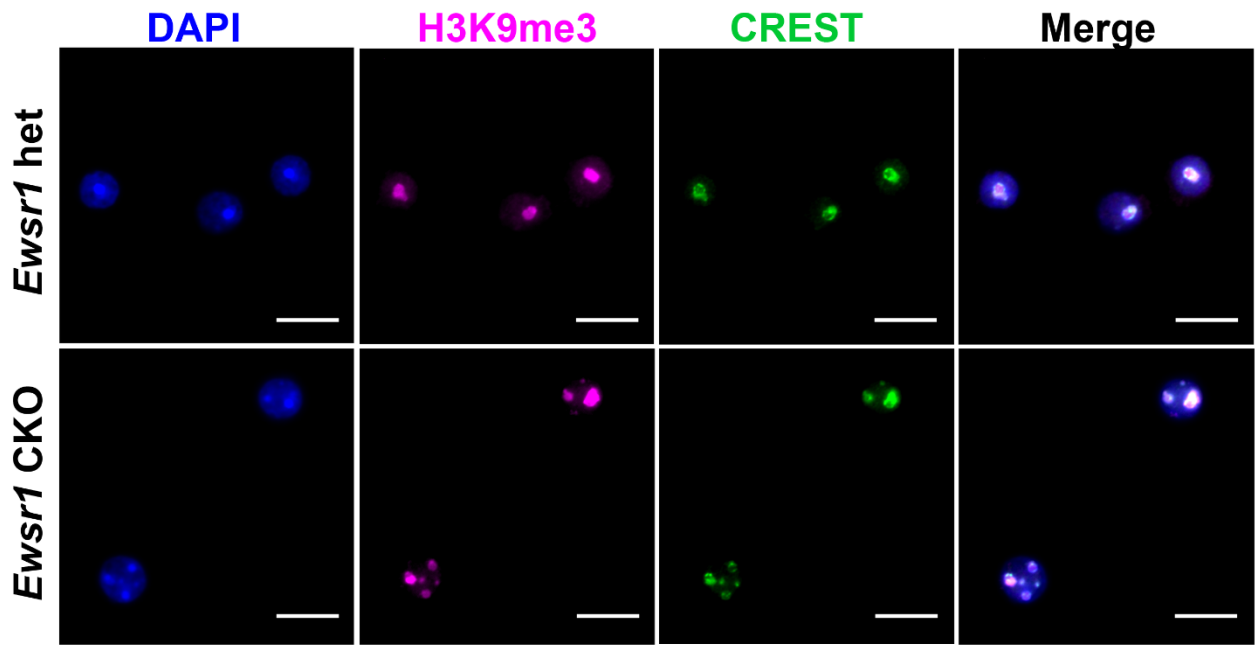

Figure S4. Increased chromocenter fragmentation in isolated round spermatids in *Ewsr1* CKO mice.

Co-immunostaining of H3K9me3 (magenta) and centromeres (CREST, green) in flow cytometry-sorted RS in *Ewsr1* het (top panels) and CKO (bottom panels) mice. Scale bar, 10 µm.

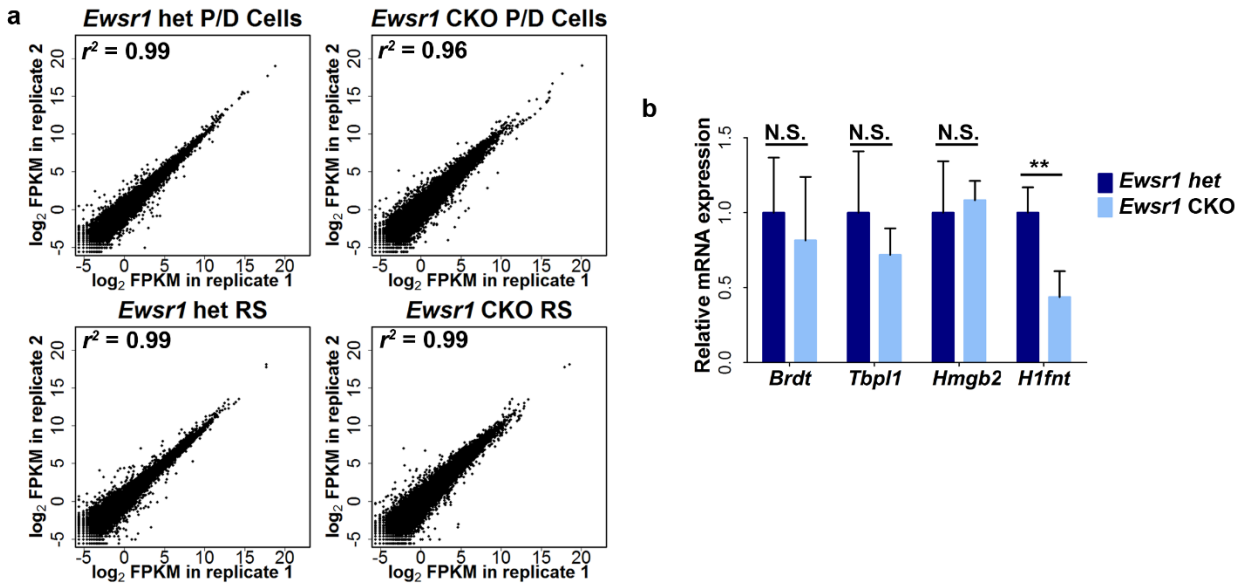

Figure S5. The transition from a meiotic to a spermiogenesis gene-expression program is affected in *Ewsr1* CKO mice.

(A) Correlation between replicates of P/D and of RS samples. R^2 values are shown in the upper left-hand corners. (B) QRT-PCR of chromocenter formation-related genes expression in 21-dpp *Ewsr1* het (dark blue) and CKO (light blue) mouse testis extracts. *β-Actin* mRNA levels were used for normalization. N.S., not significant ($p > 0.05$ by Student's *t*-test), ** $p < 0.01$ by Student's *t*-test. Data is shown as mean ± SD with 3 replicates.