

Expanded View Figures

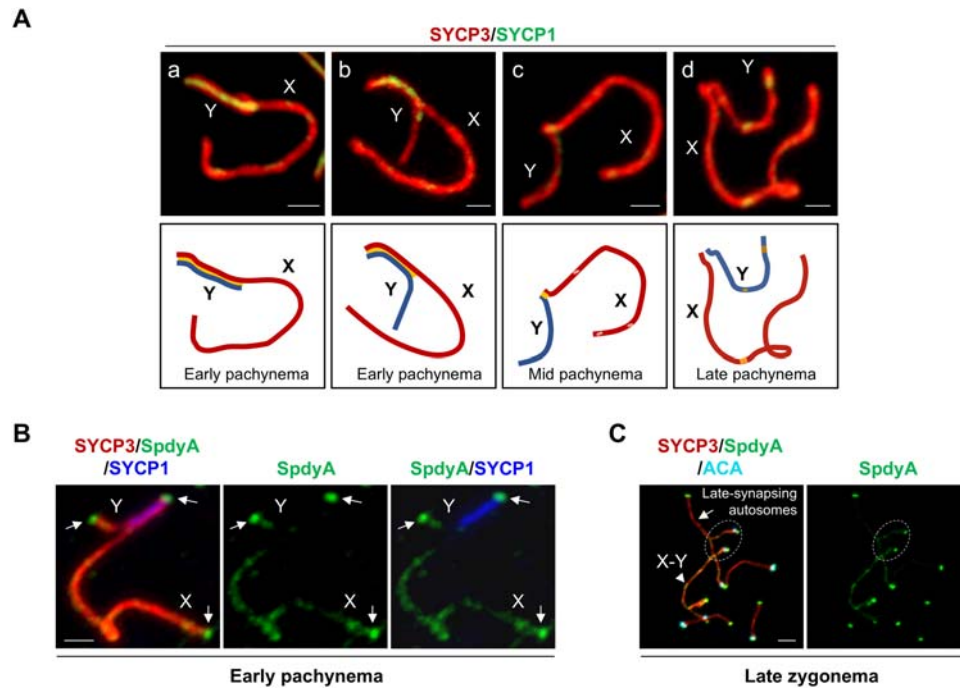


Figure EV1. Dynamic configuration changes of X and Y chromosomes and SpdyA localization at sex chromosome axes and telomeres during pachynema.

(A) Illustration of the dynamic changes in the X and Y chromosome configuration during pachynema. X and Y chromosomes were immunostained for SYCP3 (red) and SYCP1 (green). (a) At early pachynema, the X and Y chromosomes exhibit extensive side-by-side pairing, with the Y chromosomal axis fully aligned along the X chromosomal axis and with synaptonemal complex (SC) formation occurring between the aligned regions. This configuration is transient and rarely observed. (b) As pachynema progresses, the SC between the X and Y chromosomes gradually shortens. (c) By mid-pachynema, the SC becomes restricted to the PAR. (d) At late pachynema, desynapsis occurs and a chiasma forms, resulting in an "end-to-end" attachment between the X and Y chromosomes. Scale bars, 1 μ m. (B) The localization of SpdyA at telomeres (arrows) and at the unsynapsed axes of X and Y chromosomes at early pachynema. X and Y chromosomes were immunostained for SYCP3 (red), SYCP1 (blue) and SpdyA (green). Scale bar, 1 μ m. (C) SpdyA localization at the unsynapsed region (dashed circle) of late-synapsing autosomes at late zygonema. Scale bar, 2 μ m.

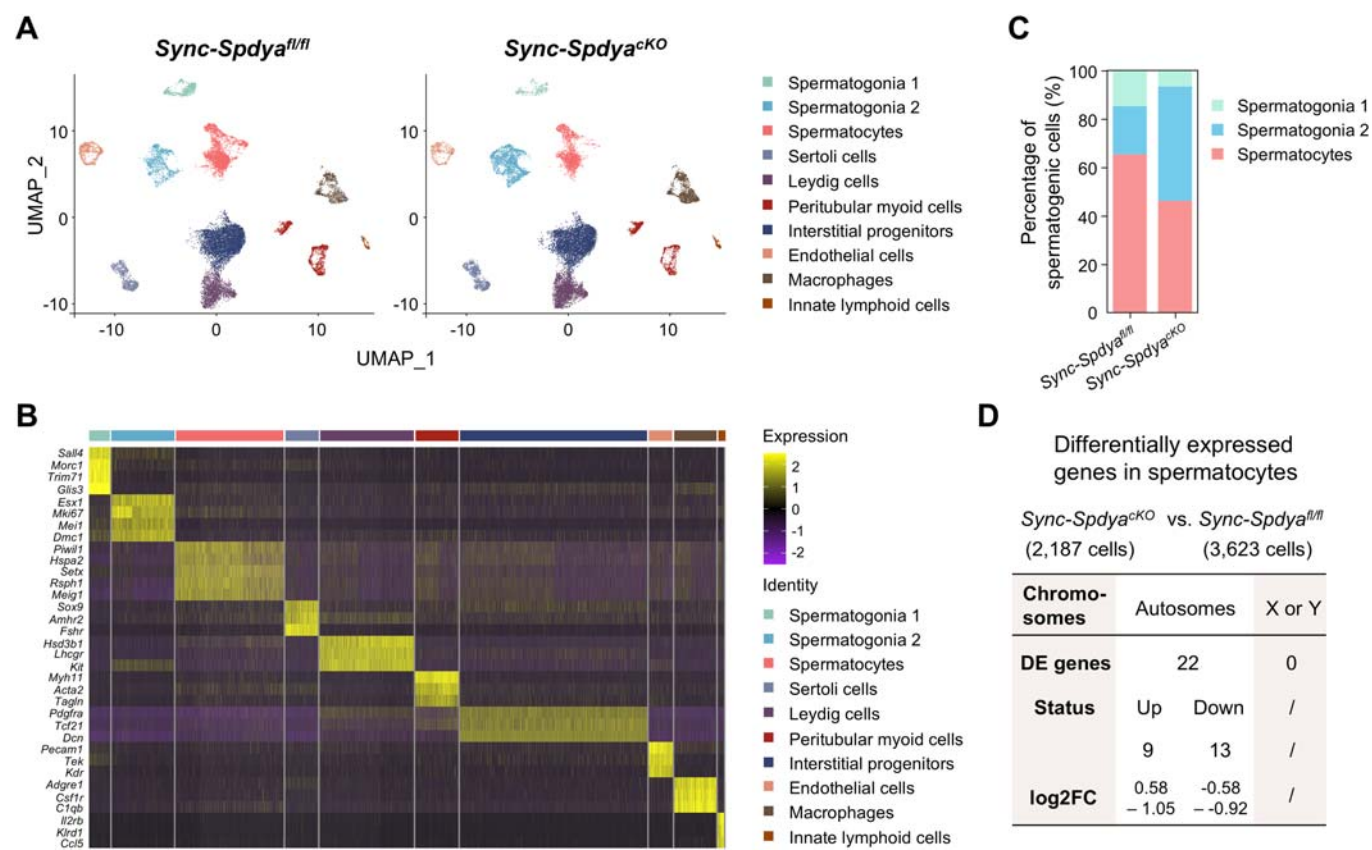


Figure EV2. Single-cell RNA-seq analysis revealed no differential expression of sex chromosome-linked genes in mid-late *Sync-SpdyacKO* pachytene spermatocytes.

(A) UMAP and clustering analysis of single-cell transcriptome data from testicular cells collected at RA11.75 from tamoxifen-treated *Spdyalfl/fl* and *Spdyalfl/fl*;Ddx4-Cre^{ERT2} mice. Each dot represents a single cell, colored according to its cluster identity. UMAP, Uniform Manifold Approximation and Projection. (B) Heatmap showing expression of marker genes for 10 identified cell types (right). The pachytene cell marker *Hspa2* is highly expressed in spermatocytes. Spermatogonia 1 and 2 likely represent undifferentiated and differentiating spermatogonia, respectively, based on their marker gene profiles. (C) Proportion of the three types of spermatogenic cell types. (D) Differential expression analysis of 2187 *Sync-SpdyacKO* and 3623 *Sync-Spdyalfl/fl* pachytene spermatocytes. DE genes, differentially expressed genes; Log2FC, log₂ fold change. Cutoff criteria: |log2FC| ≥ 0.58 and adjusted *P* value < 0.05.

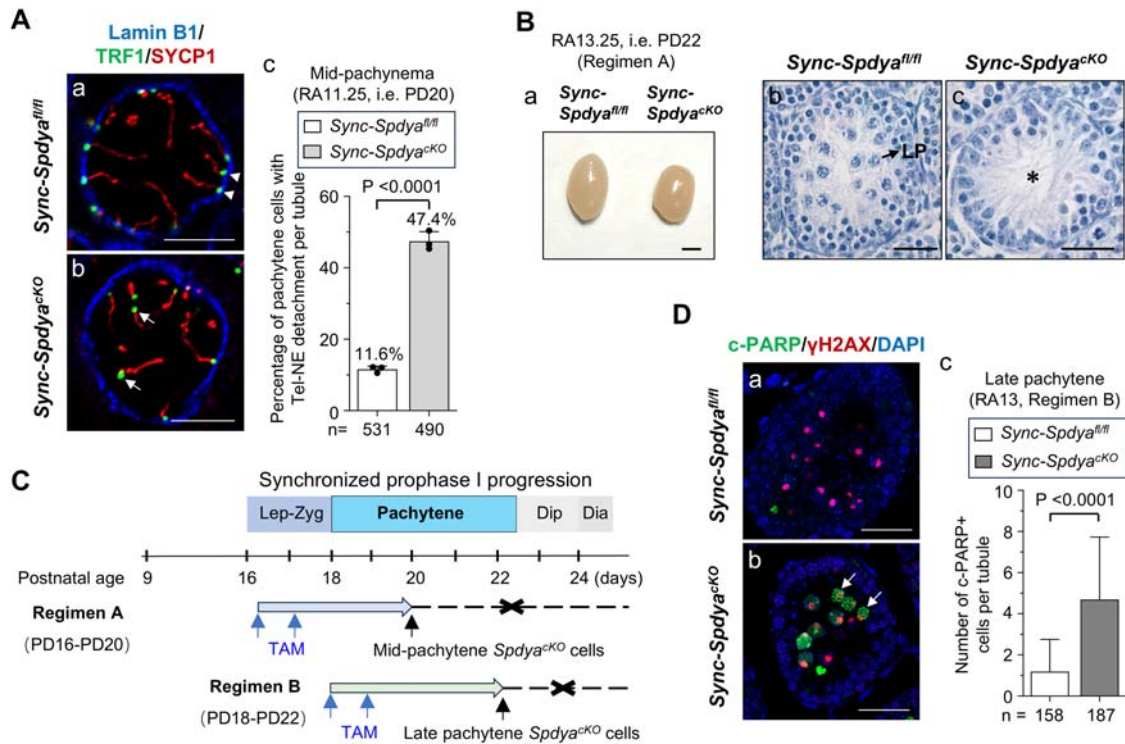


Figure EV3. Spdya is essential for spermatocyte survival throughout pachynema.

(A) Compared to *Sync-Spdya*^{fl/fl} cells (a), mid-pachytene (RA11.25) *Sync-Spdya*^{CKO} cell (b) exhibited severe Tel-NE detachment (arrows). Testis sections were immunostained for TRF1 (green), SYCP1 (red) and Lamin B1 (blue). Arrowheads indicate Tel-NE attachment. Scale bars, 5 μm. (c) Percentages of pachytene cells with Tel-NE detachment in mid-pachytene *Sync-Spdya*^{fl/fl} and *Sync-Spdya*^{CKO} testes at RA11.25. "n" represents the total number of pachytene spermatocytes scored from three mice per genotype. (B) Following tamoxifen regimen shown in Fig. 2A, the *Sync-Spdya*^{CKO} testes collected at RA13.25 were smaller in size and lacked late pachytene spermatocytes. LP, late pachytene spermatocyte; asterisk indicates loss of spermatocytes. Scale bars, 30 μm. (C) Summary of tamoxifen treatment regimens used to obtain mid- and late pachytene *Sync-Spdya*^{CKO} spermatocytes. Regimens A and B correspond to those shown in Figs. 2A and 3A, respectively. Dashed lines with crosses indicate stages at which *Sync-Spdya*^{CKO} spermatocytes could not be obtained. (D) Following Regimen B, late pachytene *Sync-Spdya*^{CKO} spermatocytes collected at RA13 exhibited apoptosis. Testis sections were immunostained for c-PARP (green) and γH2AX (red). Nuclei were counterstained with DAPI. Scale bars, 30 μm. "n" represents the total number of seminiferous tubules scored from three mice per genotype. Data information: All values are presented as the mean ± SD. Statistical analyses were performed using two-tailed Student's *t* test in (A) with $P = 0.000025$, and Mann-Whitney test in (D) with $P < 0.0000000000000001$.

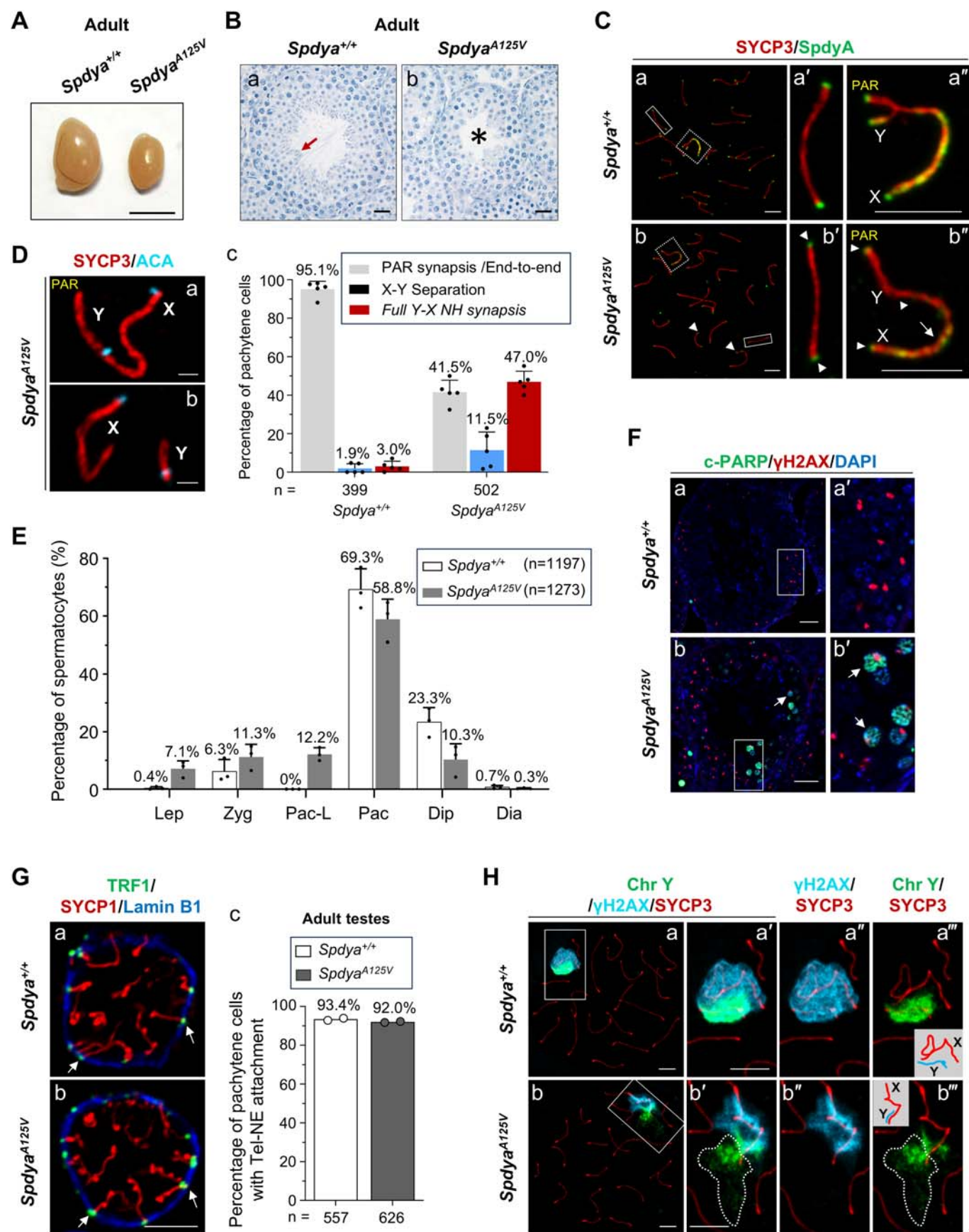


Figure EV4. Adult *SpdyA*^{A125V} mice is a hypomorphic-SpdyA model in which pachytene spermatocytes exhibited full Y-X NH synapsis and apoptosis.

(A) The size of testis was smaller in the 3-month-old (adult) *SpdyA*^{A125V} mice. Scale bar, 5 mm. (B) Histological analyses of testes from adult *SpdyA*^{+/+} (a) and *SpdyA*^{A125V} (b) mice. Red arrow indicates great amounts of elongated spermatids in *SpdyA*^{+/+} seminiferous tubule, but they were difficult to find in *SpdyA*^{A125V} seminiferous tubule (asterisk). Scale bars, 30 μ m. (C) SpdyA signal intensity was strikingly reduced at telomeres (b-b', arrowheads) and sex chromosome axes (b'', arrow) in *SpdyA*^{A125V} pachytene spermatocyte. Magnified views (a'-a'', b'-b'') are indicated by solid and dashed rectangles in (a-b). Scale bars, 5 μ m. (D) Numbers of *SpdyA*^{A125V} pachytene cells exhibited full Y-X NH synapsis (a) and X-Y separation (b). Scale bars, 1 μ m. (e) Percentages of pachytene cells with different X-Y configurations in testes from *SpdyA*^{+/+} and *SpdyA*^{A125V} adult male mice. (E) Analyses of meiotic stages in spermatocytes from adult *SpdyA*^{+/+} and *SpdyA*^{A125V} mice, based on immunostaining of chromosome spreads. Lep, leptotene; Zyg, zygotene; Pac-L, pachytene-like; Pac, pachytene; Dip, diplotene; Dia, diakinesis spermatocytes. (F) IF analyses of *SpdyA*^{+/+} (a-a') and *SpdyA*^{A125V} (b-b') testis sections immunostained for c-PARP (green) and γ H2AX (red). Solid rectangles indicate areas magnified in (a'-b'). Arrows indicate apoptotic spermatocytes. Scale bars, 30 μ m. (G) IF analyses of *SpdyA*^{+/+} and *SpdyA*^{A125V} testis sections with Lamin B1 (blue), TRF1 (green) and SYCP1 (red). Arrows indicate Tel-NE attachment. Scale bars, 5 μ m. (h) Percentages of *SpdyA*^{+/+} and *SpdyA*^{A125V} pachytene cells with intact Tel-NE attachment. (H) Sex chromosomes in late pachytene *SpdyA*^{A125V} spermatocytes with full Y-X NH synapsis displayed disrupted loop-axis organization. Chromosome spreads were subjected to immuno-FISH staining for γ H2AX (light blue), Chr Y (green) and SYCP3 (red). Magnified views (a'-a'', b'-b'') are indicated by solid rectangles in (a, b). White dashed line-enclosed area indicates Y chromatin signals located outside the γ H2AX area. Scale bars, 5 μ m. Data information: In (D, E), values are presented as the mean $\pm$ SD; In (G), data are presented as the mean with individual values. "n" represents the total number of pachytene spermatocytes scored per genotype: from five mice in (D), from three mice in (E), and from two mice in (G).