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Supplementary Materials for

SKP1 drives the prophase I to metaphase I transition during male meiosis

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This PDF file includes:

SUPPLEMENTARY MATERIALS

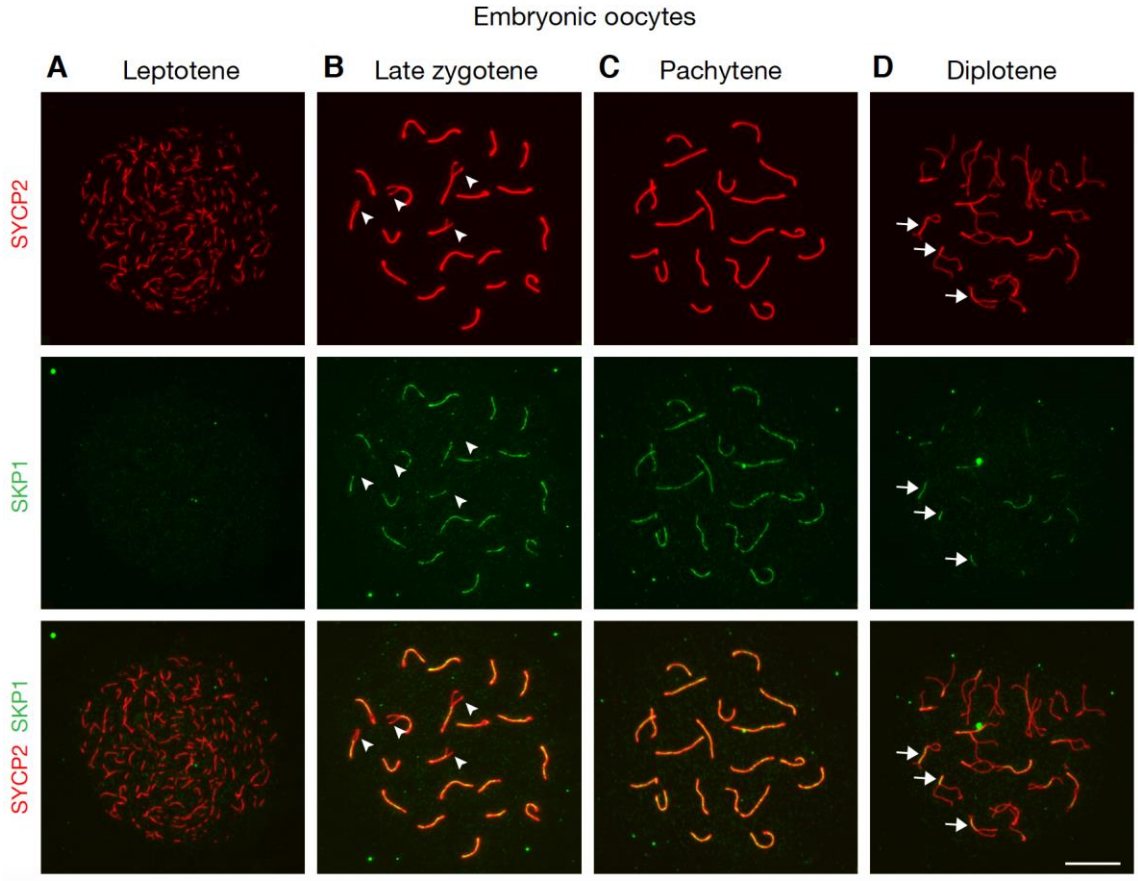

Fig. S1. Localization of SKP1 to the SC in oocytes. Surface spread nuclei of wild type E18.5 oocytes were immunostained with anti-SKP1 and SYCP2 antibodies. (**A**) Leptotene. (**B**) Late zygotene or early pachytene. (**C**) Pachytene. (**D**) Diplotene. Arrowheads (**B**) indicate the absence of SKP1 in unsynapsed regions on the synaptonemal complexes at the late zygotene /early pachytene stage. Arrows (**D**) designate the localization of SKP1 to the synapsed regions at the diplotene stage. Scale bar, 10 μm.

Fig. S2. Inactivation of the *Rec8* gene by CRISPR-Cas9–mediated genome editing. The sequences and locations of the sgRNAs are shown. In the *Rec8* knockout, exons 2- 19 are deleted.

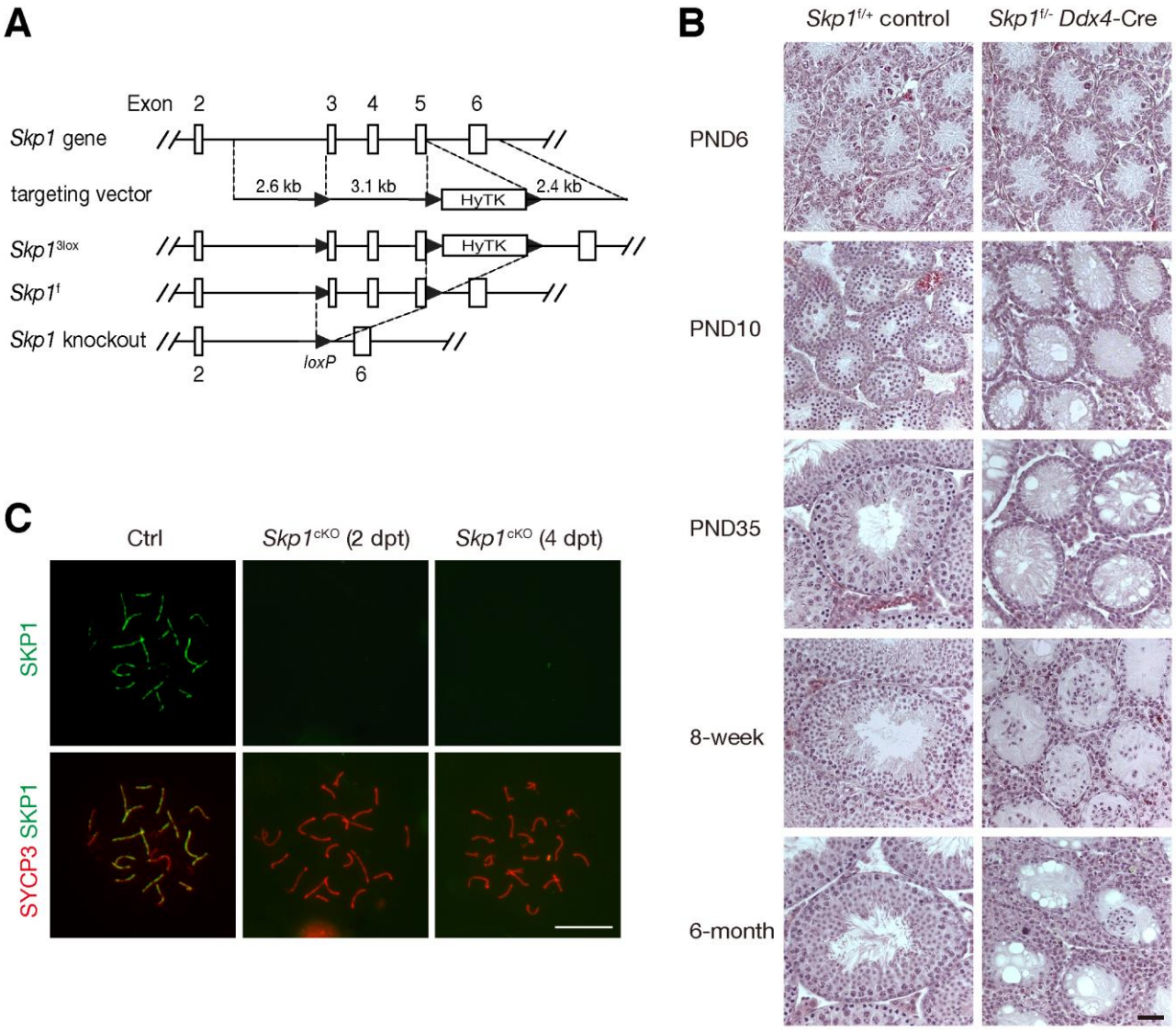

Fig. S3. Targeted inactivation of the *Skp1* gene. (**A**) Schematic diagram of the *Skp1* targeting strategy. The mouse *Skp1* gene consists of 6 exons and encodes a protein of 163 amino acids. In the conditional *Skp1*^{f} allele, exons 3-5 are floxed. Exons 3-5 encode residues 33-153. Deletion of exons 3-5 (359 nt) not only removes the majority of the protein but also results in a frameshift in the resulting *Skp1* mutant transcript. HyTK is a double selection marker and enables hygromycin-positive selection and thymidine kinase-negative selection. (**B**) Histological analysis of *Skp1*$^{f/+}$ control and *Skp1*$^{f/-}$ *Ddx4*-Cre testes at postnatal day 6 (PND6) through 6 months. *Ddx4*-Cre is germ cell-specific but constitutively active *(20)*. Note the complete loss of germ cells in *Skp1*$^{fl/-}$ *Ddx4*-Cre testes at PND10 and beyond. Scale bar, 25 μm. (**C**) Lack of SKP1 signal on the synaptonemal complexes in *Skp1*^{cKO} pachytene spermatocytes. Scale bar, 10 μm.

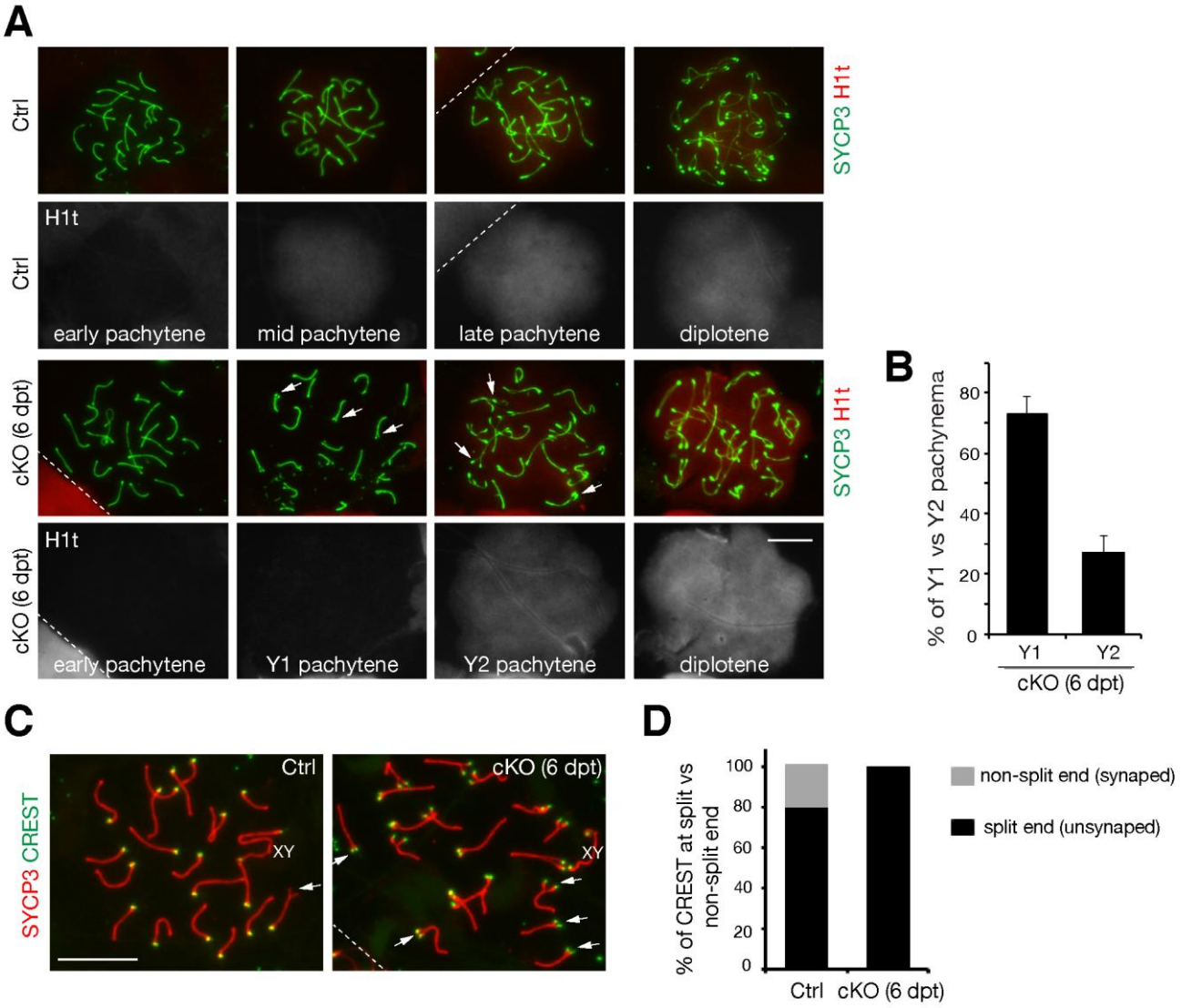

Fig. S4. H1t expression and CREST localization in WT and *Skp1*^{cKO} spermatocytes. (**A**) Staging of pachynema by H1t expression. In wild type, H1t is absent in early pachynema, appears in mid-pachynema, and increases its abundance in late pachynema and diplonema. In *Skp1*^{cKO} spermatocytes, early pachynema with no unsynapsed ends are H1t-negative, some pachynema with split ends are H1t-negative (Y1 pachynema) and thus at the early pachytene stage, and the remaining pachynema with split ends are H1t-positive (Y2 pachynema) and thus are at the mid-pachytene stage. However, Y2 pachynema could also be considered as early diplonema. (**B**) Count of H1t-negative (Y1) and H1t-positive (Y2) *Skp1*^{cKO} pachytene spermatocytes with unsynapsed split ends. (**C**) CREST marks centromeres. Pachytene spermatocytes were examined by surface nuclear spread analysis. Arrows indicate some of the desynapsed chromosome ends. (**D**) The centromeric ends are preferentially desynapsed in *Skp1*^{cKO} pachytene spermatocytes. Scale bars, 10 μm.

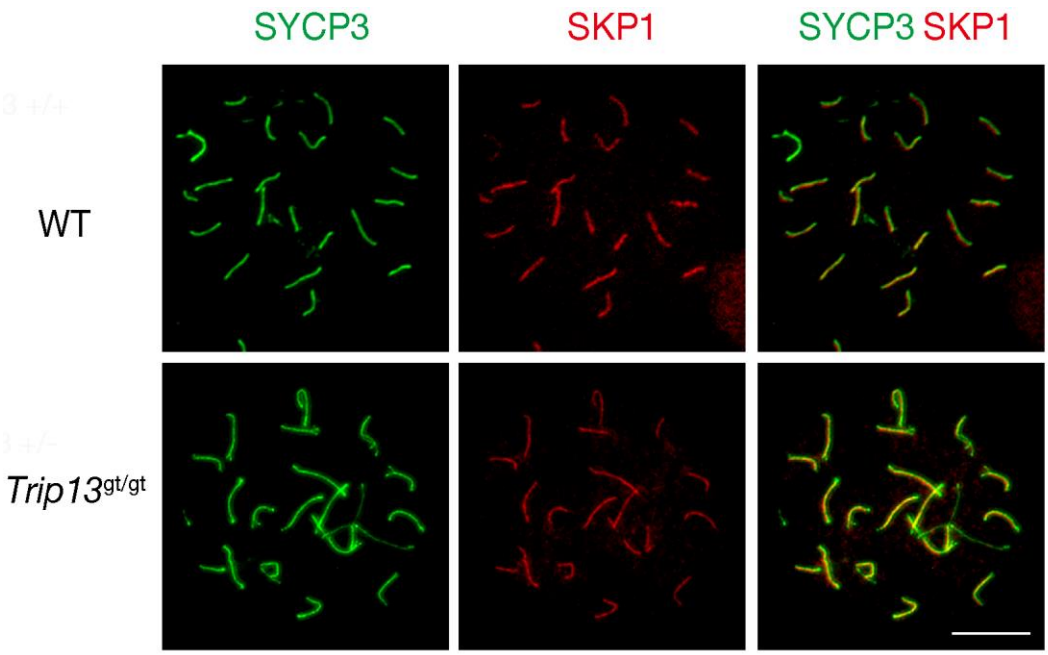

Fig. S5. Localization of SKP1 in WT and *Trip13*$^{Gt/Gt}$ pachytene spermatocytes. Surface nuclear spread analysis of wild type and *Trip13*$^{Gt/Gt}$ spermatocytes was performed by immunostaining of SKP1 and SYCP3. Scale bar, 10 μm.

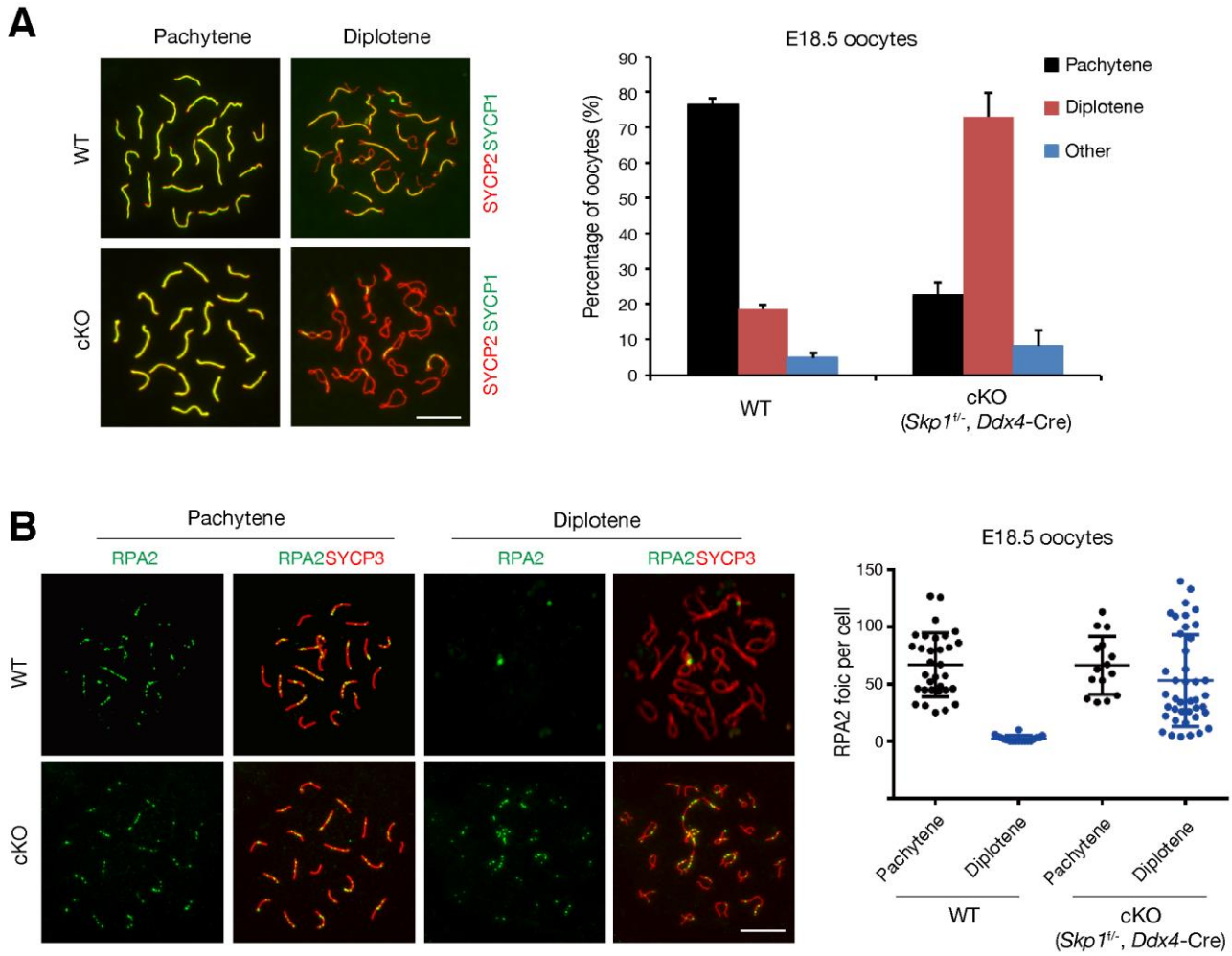

Fig. S6. Precocious chromosome desynapsis and persistence of RPA2 foci in E18.5 *Skp1*^{cKO} oocytes. *Skp1*^{cKO} oocytes were collected from E18.5 *Skp1*$^{fl/-}$ *Ddx4*-Cre embryos. (**A**) Precocious chromosome desynapsis in *Skp1*^{cKO} oocytes. Surface spread nuclei of E18.5 oocytes were immunostained with anti-SYCP1 and anti-SYCP2 antibodies. Pachytene and diplotene oocytes are shown. Graph on the right shows increased percentage of diplotene spermatocytes in E18.5 *Skp1*$^{fl/-}$ *Ddx4*-Cre embryos. Data were from three independent experiments. (**B**) Persistence of RPA2 foci in *Skp1*^{cKO} diplotene oocytes. Surface spread nuclei of E18.5 oocytes were immunostained with anti-RPA2 and anti-SYCP3 antibodies. Graph on the right shows the count of RPA2 foci. Scale bars, 10 μm.

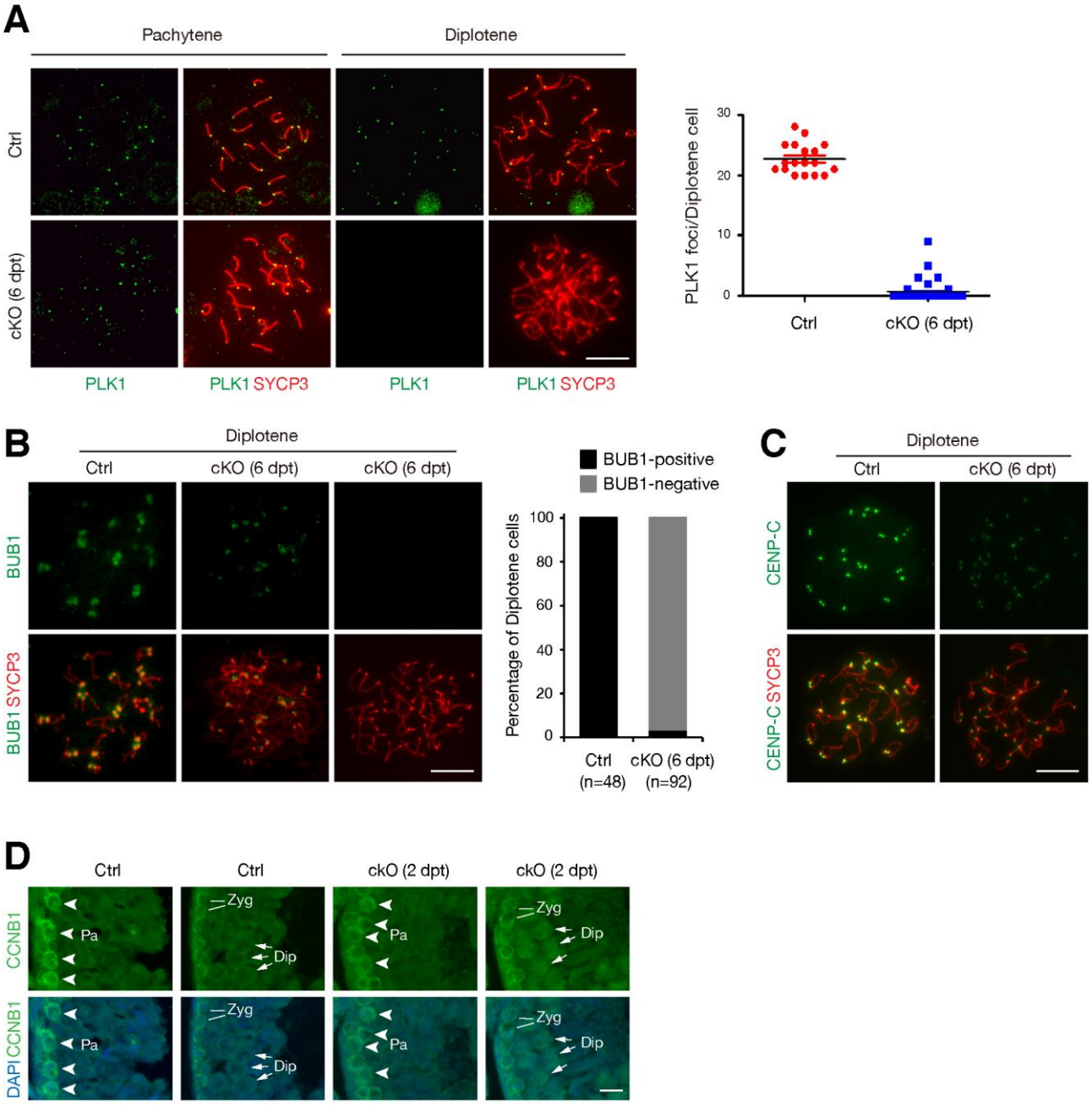

Fig. S7. Expression and localization of PLK1, BUB1, CENP-C, and CCNB1 in spermatocytes. (**A**) Loss of PLK1 from centromeres in *Skp1*^{cKO} diplotene spermatocytes. (**B**) Loss of BUB1 from centromeres in *Skp1*^{cKO} diplotene spermatocytes. (**C**) Reduced CENP-C at centromeres in *Skp1*^{cKO} diplotene spermatocytes. (**D**) Nuclear localization of CCNB1 in *Skp1*-deficient diplotene spermatocytes. Scale bars, 10 μm.