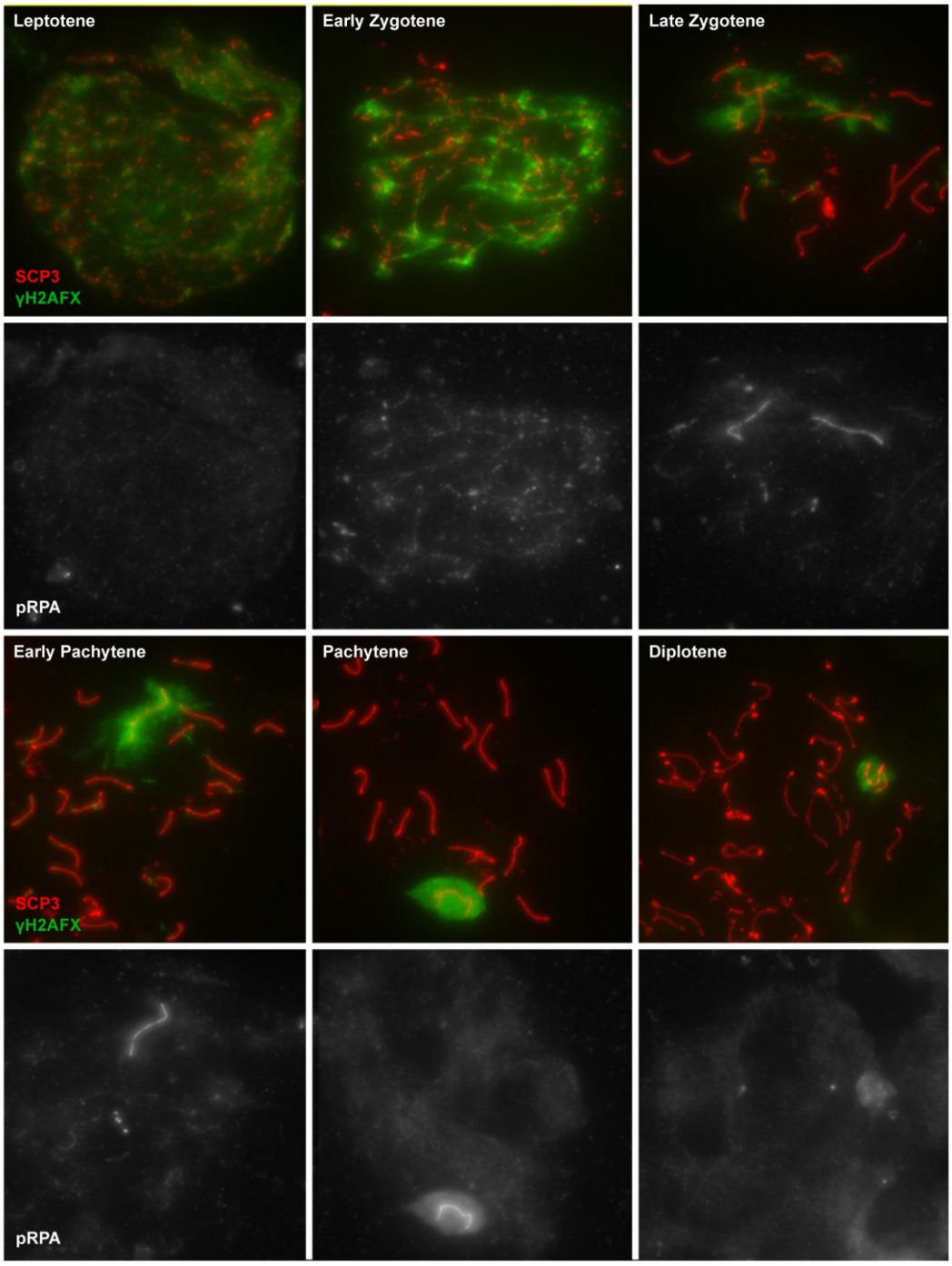

Fig. S1. Dynamics of pRPA during the first meiotic prophase. Immunofluoresence of pRPA, γH2AFX, and a component of the axial element, SCP3, in WT spermatocytes at individual stages of the first meiotic prophase. Representative immunofluorescence images from WT males.

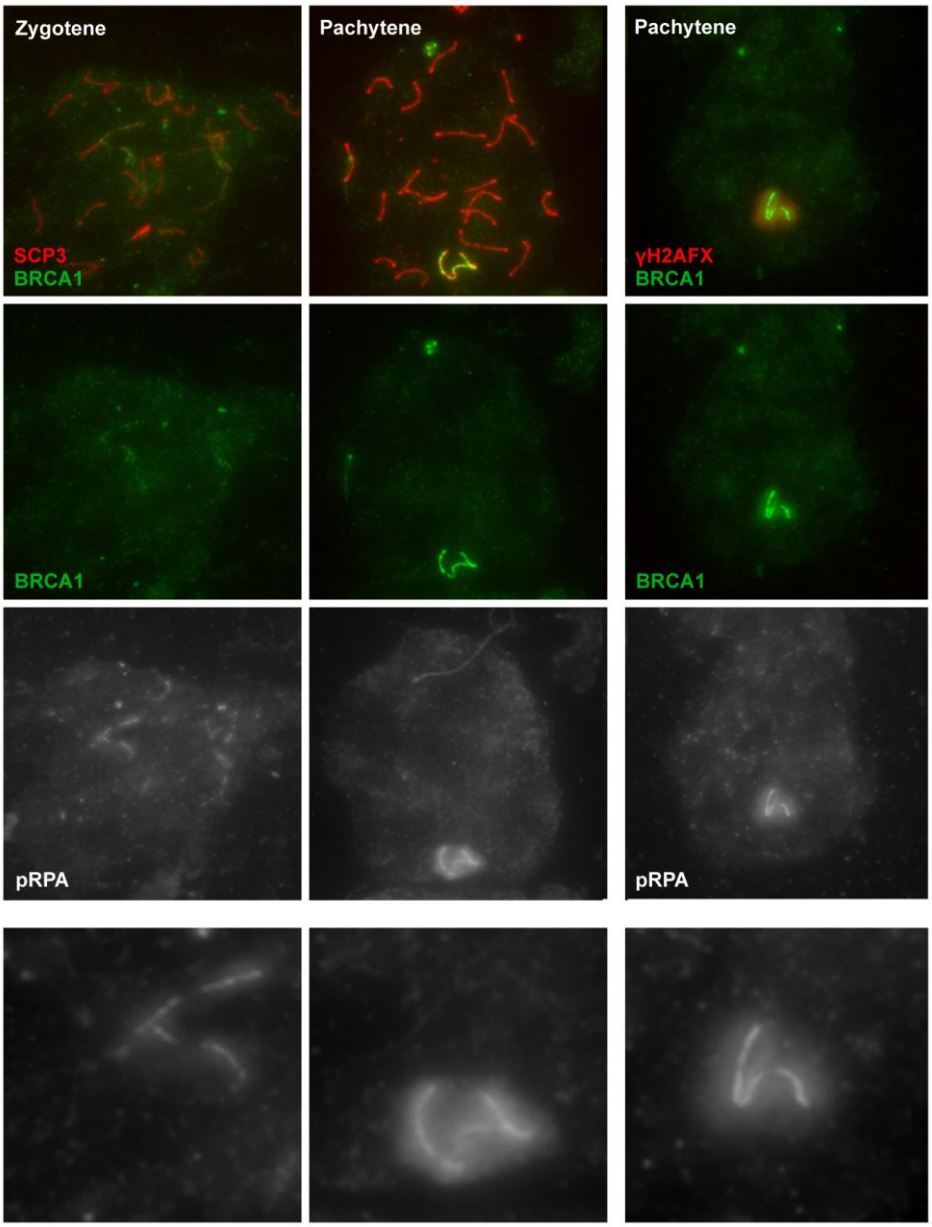

Fig. S2. pRPA enrichment is coincident with BRCA1 staining on the XY.

(**A**) pRPA in WT zygotene and pachytene spermatocytes colocalizes with BRCA1, a marker of asynapsed chromosomes. Note, pRPA staining pattern is similar regardless of co-staining with antibodies against BRCA1 or γH2AFX, suggesting signal is not a result of 'bleed through' of BRCA1 or γH2AFX immunoflourescent signal. (**B**) Higher maginification view of XY body from spermatocytes in (**A**).

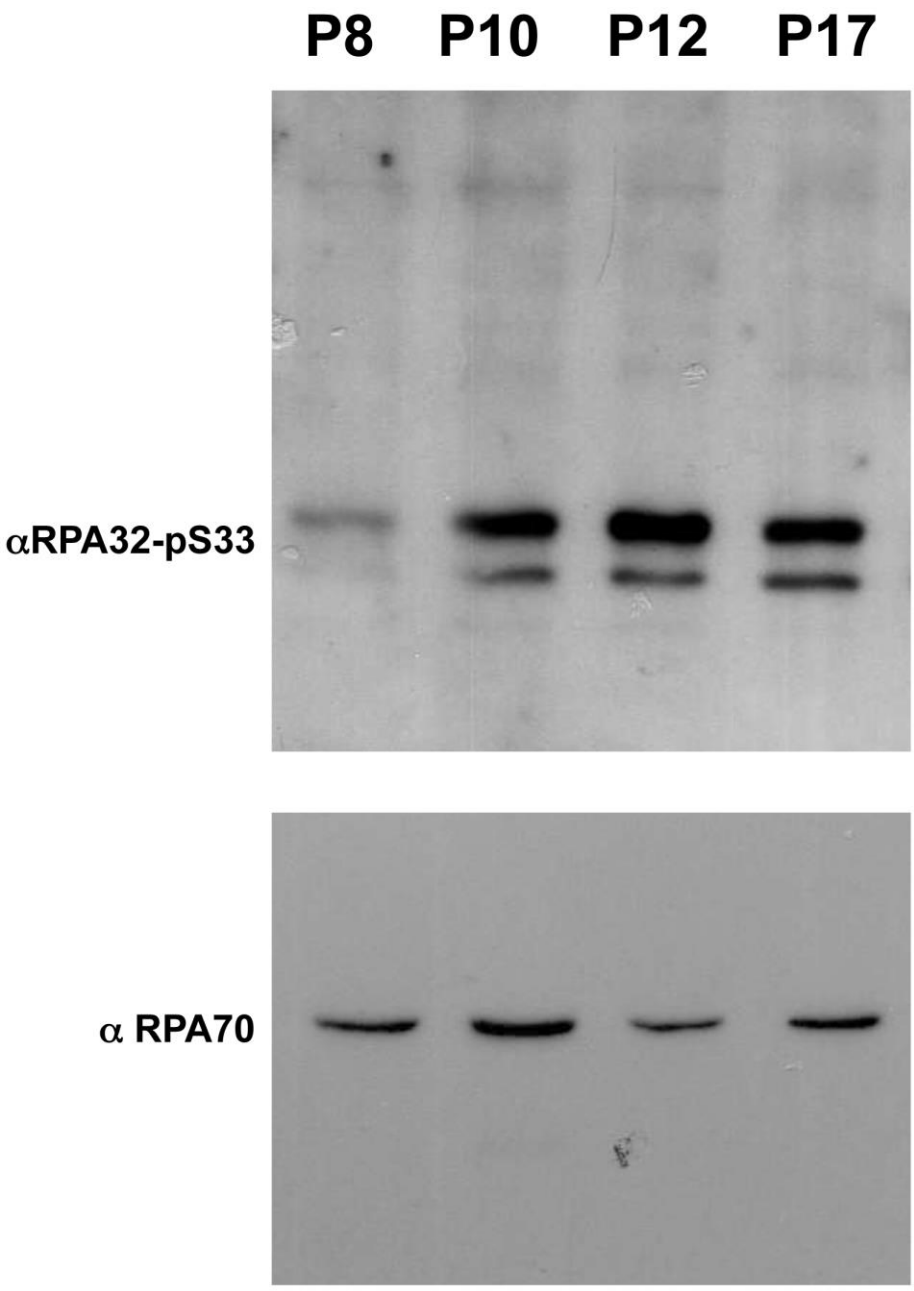

Fig. S3. Levels of phosphorylated RPA in postnatal testes. Levels of pRPA and RPA70 were assessed in whole cell extracts from testes of postnatal days 8 (P8), 10 (P10), 12 (P12), and 17 (P17). At P10, leptotene and zygotene spermatocytes are first evident.

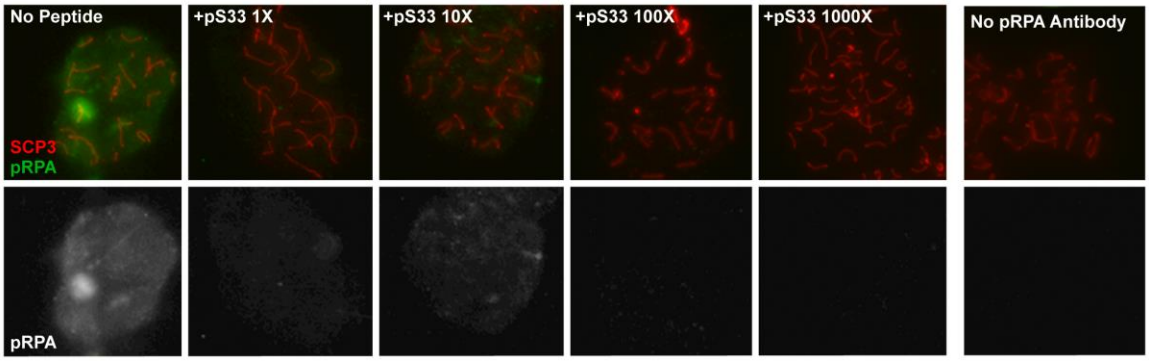

Fig. S4. Specificity of the anti-pRPA antibody determined by peptide competition. Anti-pRPA antibodies were pre-incubated with varying amounts of the immunizing peptide, ranging from an equal-molar ratio to a 1000-fold excess, then used for immunofluorescence experiments on control spermatocytes. Signal on the XY was diminished at an equal-molar excess.

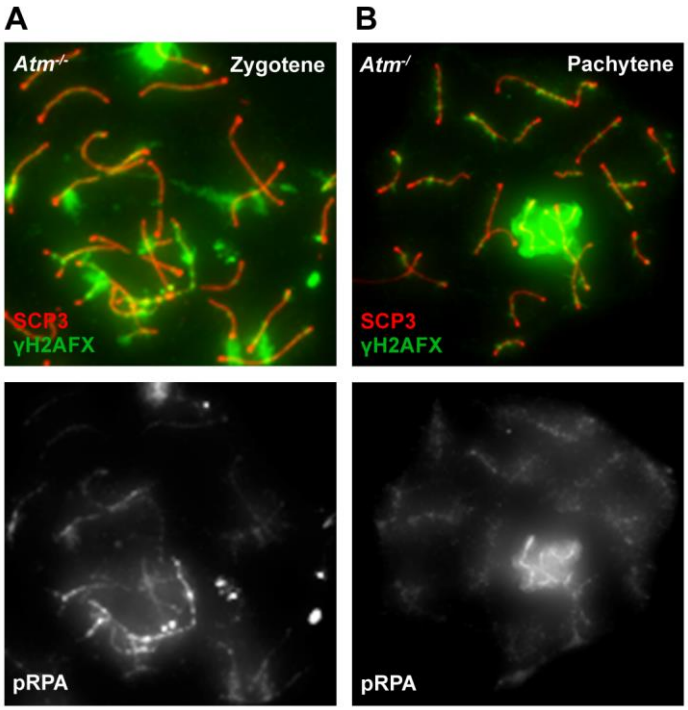

Fig. S5. RPA phosphorylation is unaffected in the absence of the ATM kinase. Zygotene (**A**) and more developmentally advanced, pachytene-like (**B**) *Atm*$^{-/-}$ spermatocytes show normal patterns of colocalization between pRPA and γH2AFX-positive domains.

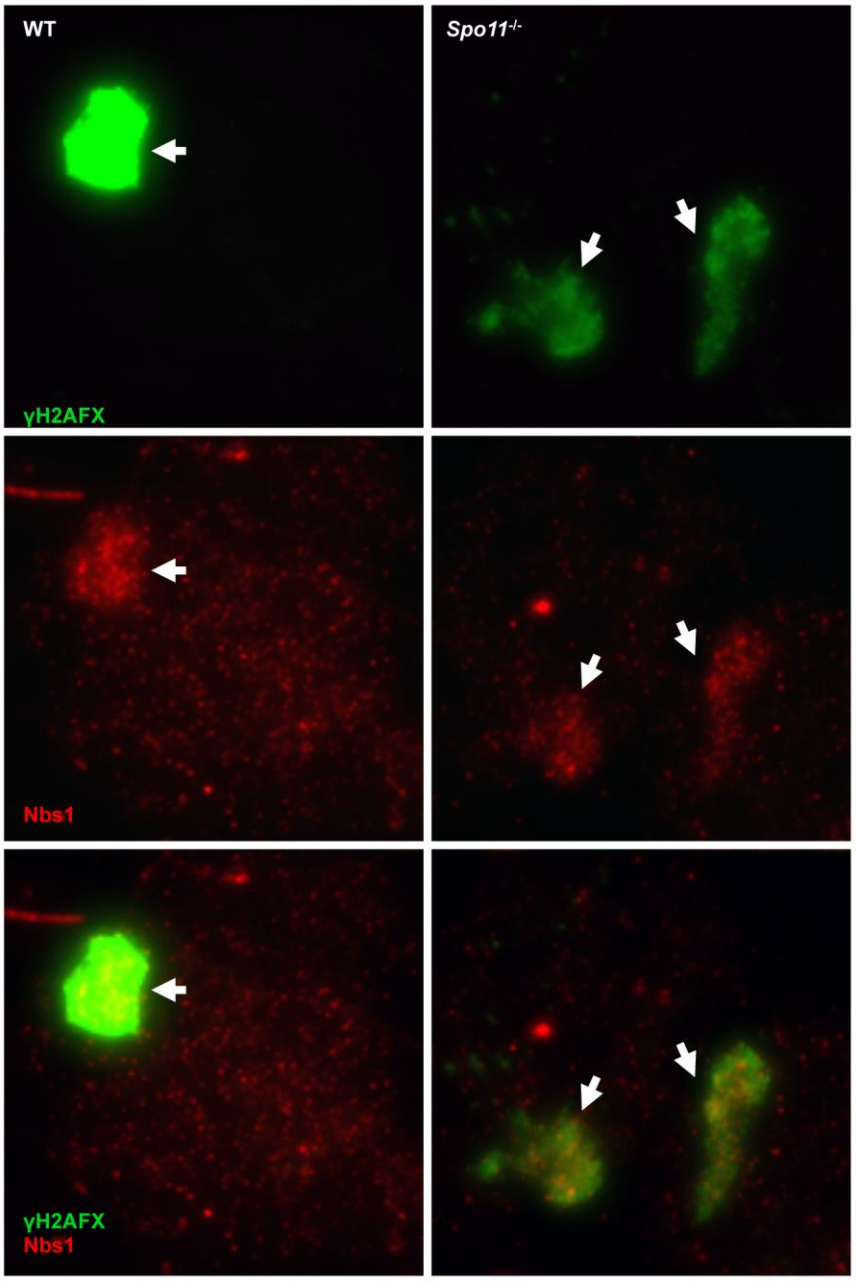

Fig. S6. Localization of Nbs1 to asynapsed chromatin in *Spo11*$^{-/-}$ spermatocytes. Indirect immunofluorescence against Nbs1 in *Spo11*$^{-/-}$ spermatocytes. Just as in WT spermatocytes, Nbs1 colocalized with γH2AFX-positive domains (white arrows) in the absence of SPO11.

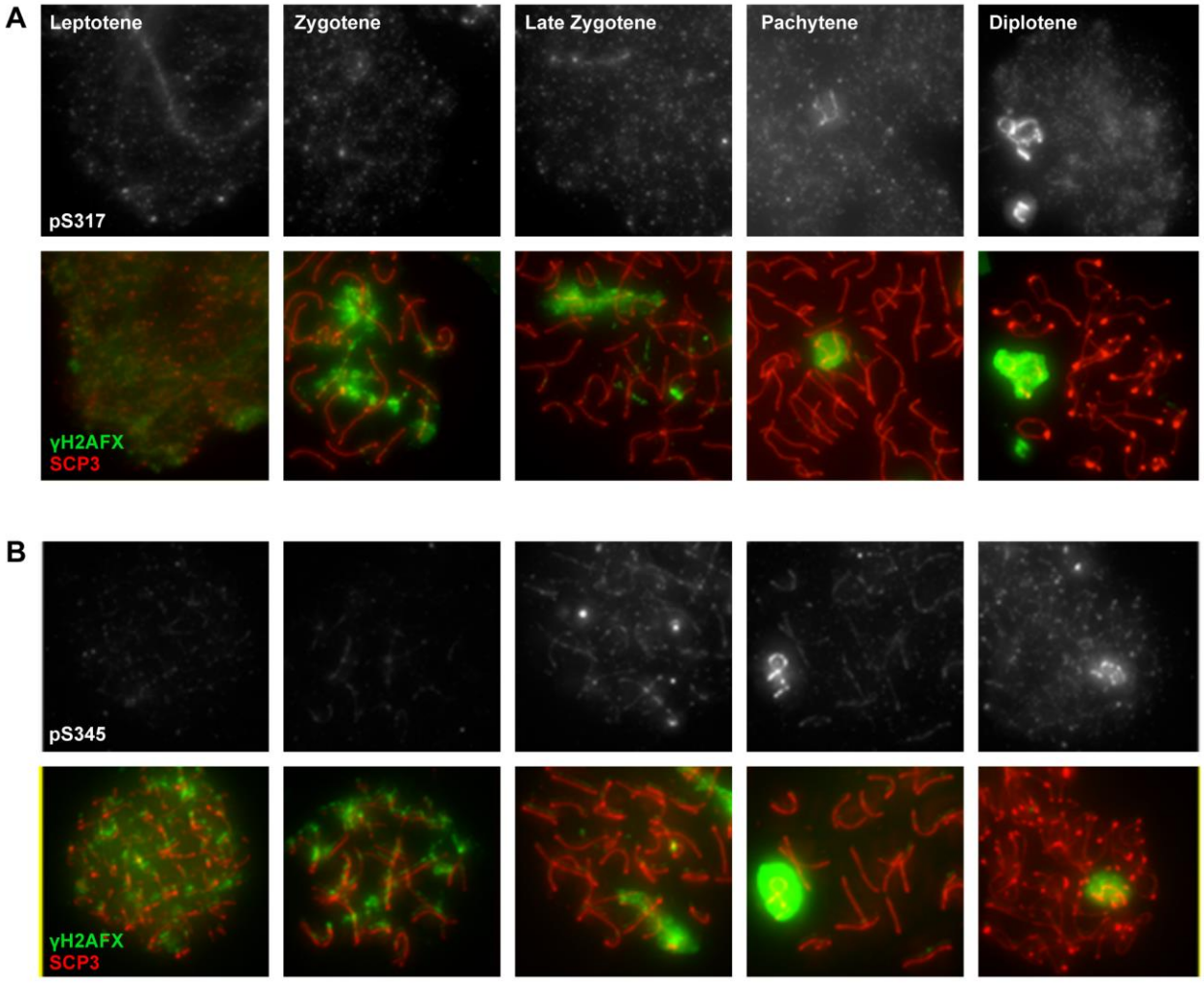

Fig. S7. Distribution of CHK1 phosphoforms in primary spermatocytes. Distribution of pCHK1 (S317) (**A**) and pCHK1 (S345) (**B**) at stages of the first meiotic prophase.

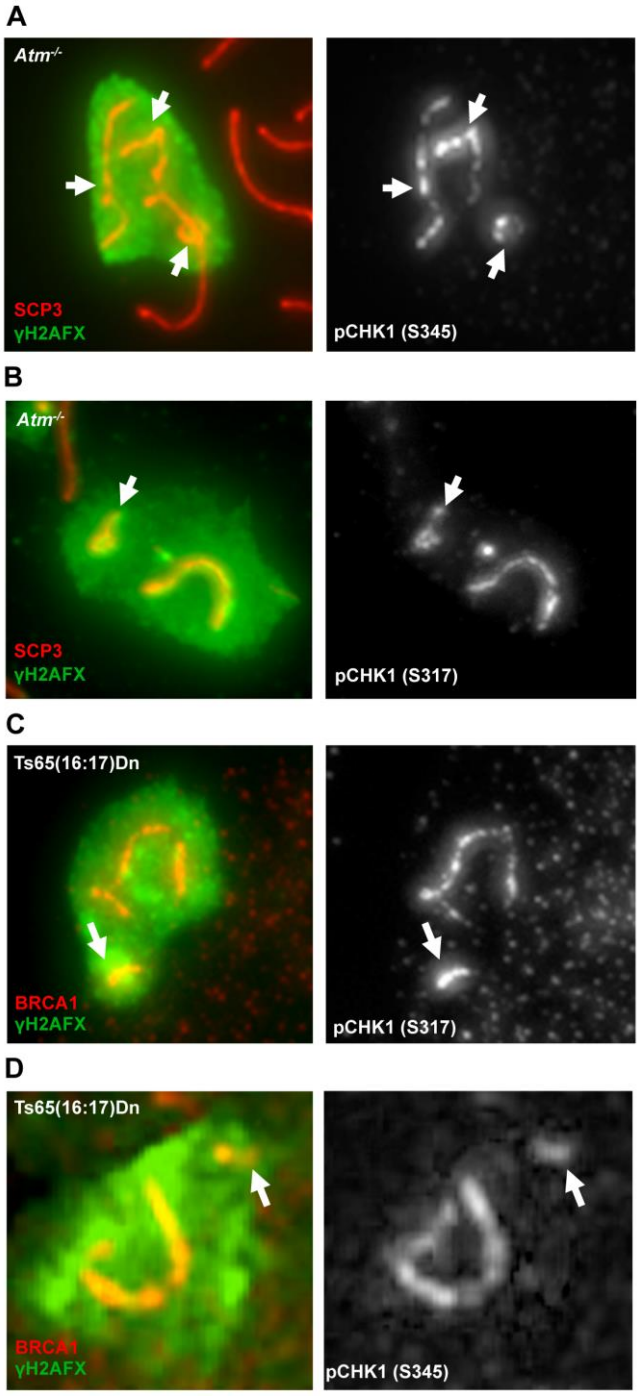

Fig. S8. Localization of CHK1 phosphoforms in *Atm*$^{-/-}$spermatocytes. Asynapsed chromosomes in *Atm*$^{-/-}$ spermatocytes are associated with pCHK1 (S317) (**A**) and pCHK1 (S345) (**B**). Similarly, both phosphorylated forms of CHK1, S317 (**C**), and S345 (**D**), are enriched on asynapsed autosomes (white arrow) in Ts65(16:17)Dn spermatocytes**.**