Lee and Hirano, http://www.jcb.org/cgi/content/full/jcb.201008005/DC1

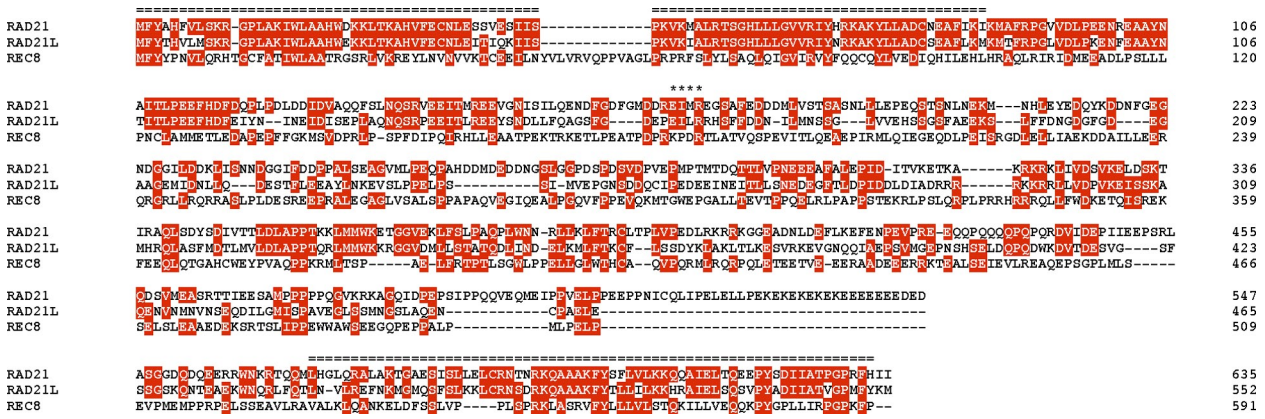

Figure S1. **Alignment of amino acid sequences between mouse RAD21, RAD21L, and REC8.** Identical amino acids between paralogs are highlighted in red. Winged-helix domains are indicated by broken double lines.

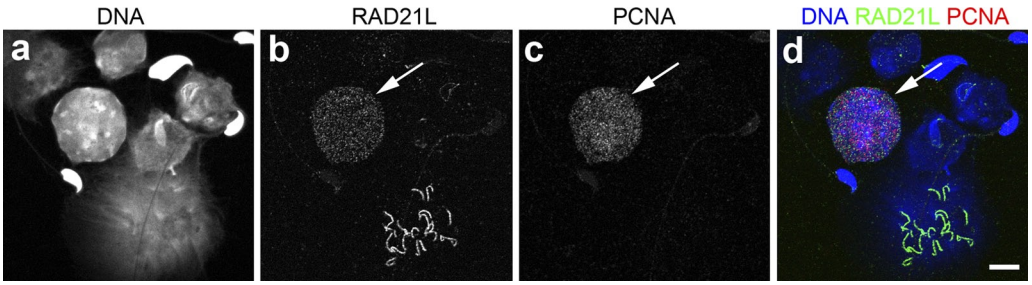

Figure S2. **RAD21L is detected in premeiotic S phase.** Nuclear spreads of testicular cells were immunofluorescently labeled with both rat polyclonal anti-RAD21L (b) and mouse monoclonal anti-PCNA (S phase marker) (c) antibodies. DNA was counterstained with Hoechst 34580 (a), and merged images are shown (d). Note that there is a cell labeled with both anti-RAD21L and anti-PCNA antibodies (indicated by arrows in b–d), revealing that RAD21L is present in premeiotic cells. Bar, 10 µm.

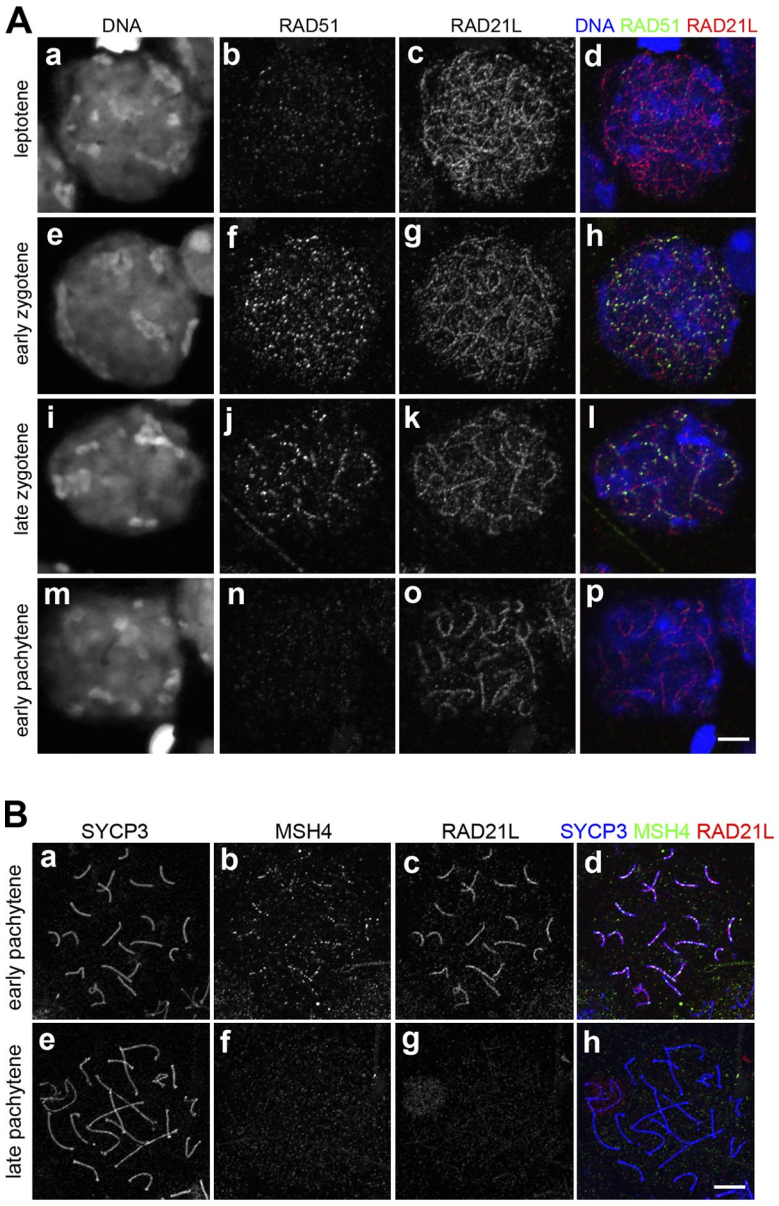

Figure S3. **The relative timing of the disappearance of RAD21L and RAD51 or MSH4 from the synaptonemal complex.** (A) Nuclear spreads from testicular cells were prepared and subjected to immunofluorescent labeling with rabbit polyclonal anti-RAD51 (b, f, j, and n) and rat polyclonal anti-RAD21L (c, g, k, and o) antibodies. DNA was counterstained with Hoechst 34580 (a, e, i, and m), and merged images are shown (d, h, l, and p). Bar, 5 µm. (B) Nuclear spreads from testicular cells were prepared and subjected to immunofluorescent labeling with mouse polyclonal anti-SYCP3 (a and e), rabbit polyclonal anti-MSH4 (b and f), and rat polyclonal anti-RAD21L (c and g) antibodies. Merged images are shown (d and h). Bar, 5 µm.

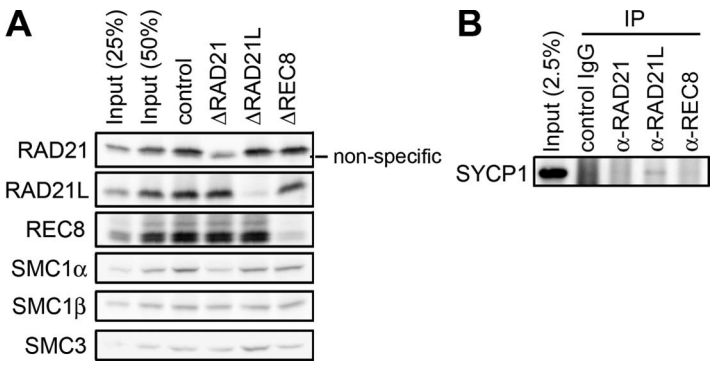

Figure S4. **Complementary analyses of cohesin complexes in testis.** (A) For immunodepletion of kleisin subunits, testis extracts were incubated with anti-RAD21, anti-RAD21L, or anti-REC8 antibody at 4°C for 1 h, and the supernatant was recovered. This operation was repeated twice more, and the final supernatant was used as RAD21-, RAD21L-, or REC8-depleted testis extracts. The depletion of RAD21 (ΔRAD21) from testis extracts reduced the remaining amount of SMC1α and SMC3. However, the depletion of REC8 (ΔREC8) also reduced the remaining amount of SMC3. However, the depletion of RAD21L (ΔRAD21L) did not affect the remaining amount of any SMC proteins, suggesting that the relative amount of RAD21L to other kleisin subunits in the testis extracts is extremely low and is consistent with the relative levels of mRNA expression described in this paper. (B) Testis extracts were immunoprecipitated with antibody against kleisin subunits or control rabbit IgG. Then the immunoprecipitates were subjected to immunoblot analysis with anti-SYCP1 antibody.

Table S1. **Probes and primers for quantitative real-time PCR**

Gene	Probes	Forward primer Reverse primer
RAD21	5′-CATGCCCCCACCACCCCCTC-3′	5′-CAGCAGAACAACCATAGAAGAATCAG-3′ 5′-TCTATTTGCCCGGCTTTCC-3′
RAD21L	5′-TCTGGACCTTGCACCCCCCACAC-3′	5′-TTGCTTCCTTTATGGACACTCTCA-3′ 5′-CCACTCCTCCCCGCTTCT-3′
REC8	5′-CTTCTCGGGTCTTCTATCTGCTCTTGGTGC-3′	5′-TCTCAGCCCCCGAAAGTTG-3′ 5′-GCCCATATGGCTTCTGTTGTTC-3′