Horn et al., http://www.jcb.org/cgi/content/full/jcb.201304004/DC1

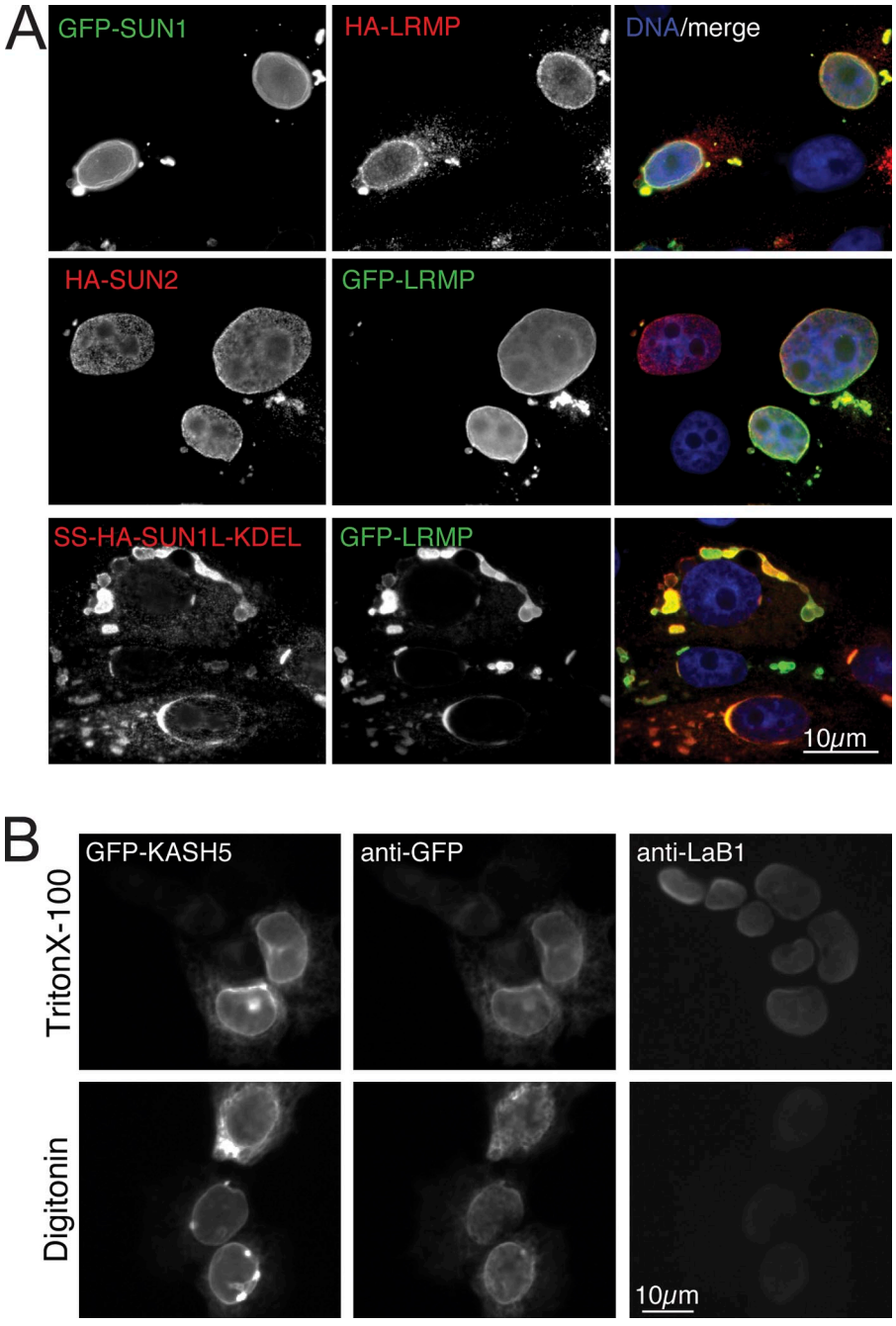

Figure S1. **LRMP behaves as an authentic KASH domain protein.** (A) HA- and GFP-tagged versions of LRMP colocalize at the NE in HeLa cells with GFP- and HA-tagged versions of SUN1 and SUN2, respectively. However, a dominant-negative form of SUN1 (SS-HA-SUN1L-KDEL) eliminates LRMP from the NE, and instead diverts the LRMP in to cytoplasmic aggregates. All samples were co-stained with DAPI to highlight nuclei. (B) KASH5 localizes to the outer nuclear membrane (ONM). HEK293 cells expressing GFP-KASH5 were fixed in formaldehyde and then differentially permeabilized with 0.002% digitonin vs. 0.2% Triton X-100. The cells were then labeled with a mouse monoclonal antibody against GFP and rabbit anti-lamin B1 (LaB1). Secondary antibodies were conjugated with Alexa Fluor 568 (goat anti–mouse IgG) and Alexa Fluor 350 (goat anti–rabbit IgG). While lamin B1, located on the nuclear face of the NE, is only detected after permeabilization with Triton X-100, the GFP moiety can be immunolabeled after permeabilization using either digitonin or Triton X-100. Because low concentrations of digitonin leave the nuclear membranes intact, these results indicate that there is a population of GFP-KASH5 that is localized to the ONM.

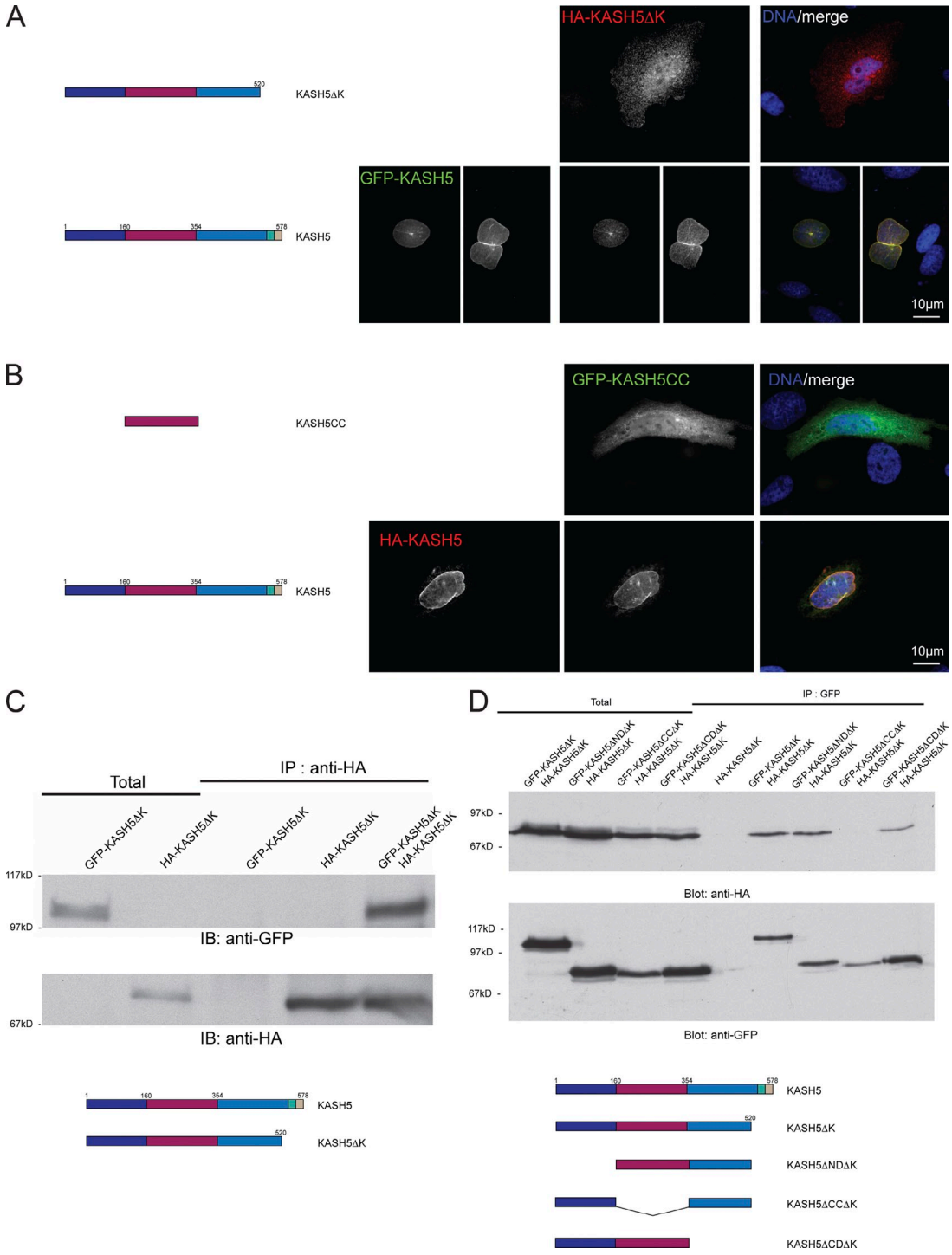

Figure S2. **KASH5 self-associates in vivo to form higher order oligomers.** (A) HeLa cells were transfected with a HA-tagged soluble form of KASH5 that lacks the KASH domain (HA-KASH5ΔK). HA-KASH5ΔK can be detected in both the nucleus and cytoplasm, but shows no NE localization (A, top panels). However, when cotransfected with a GFP-tagged version of full-length KASH5 (GFP-KASH5), HA-KASH5ΔK is recruited to the NE. A similar experiment (B) using a GFP-tagged version of the KASH5 coiled-coil domain (GFP-KASH5CC), which is also found in both the nucleus and cytoplasm (B, top panels), reveals that it is recruited to the NE by HA-tagged full-length KASH5 (HA-KASH5). These data suggest that KASH5 can self-associate and that this self-association is mediated by the coiled-coil domain. These results were confirmed in a series of coimmunoprecipitation studies. For these experiments only soluble forms of KASH5 lacking its KASH domain were used. Clearly, HA-KASH5ΔK specifically associates with GFP-KASH5ΔK (C). Using a series of deletion constructs (D) it is apparent that HA-KASH5ΔK only coimmunoprecipitates GFP-tagged forms of KASH5 that retain the coiled-coil domain.

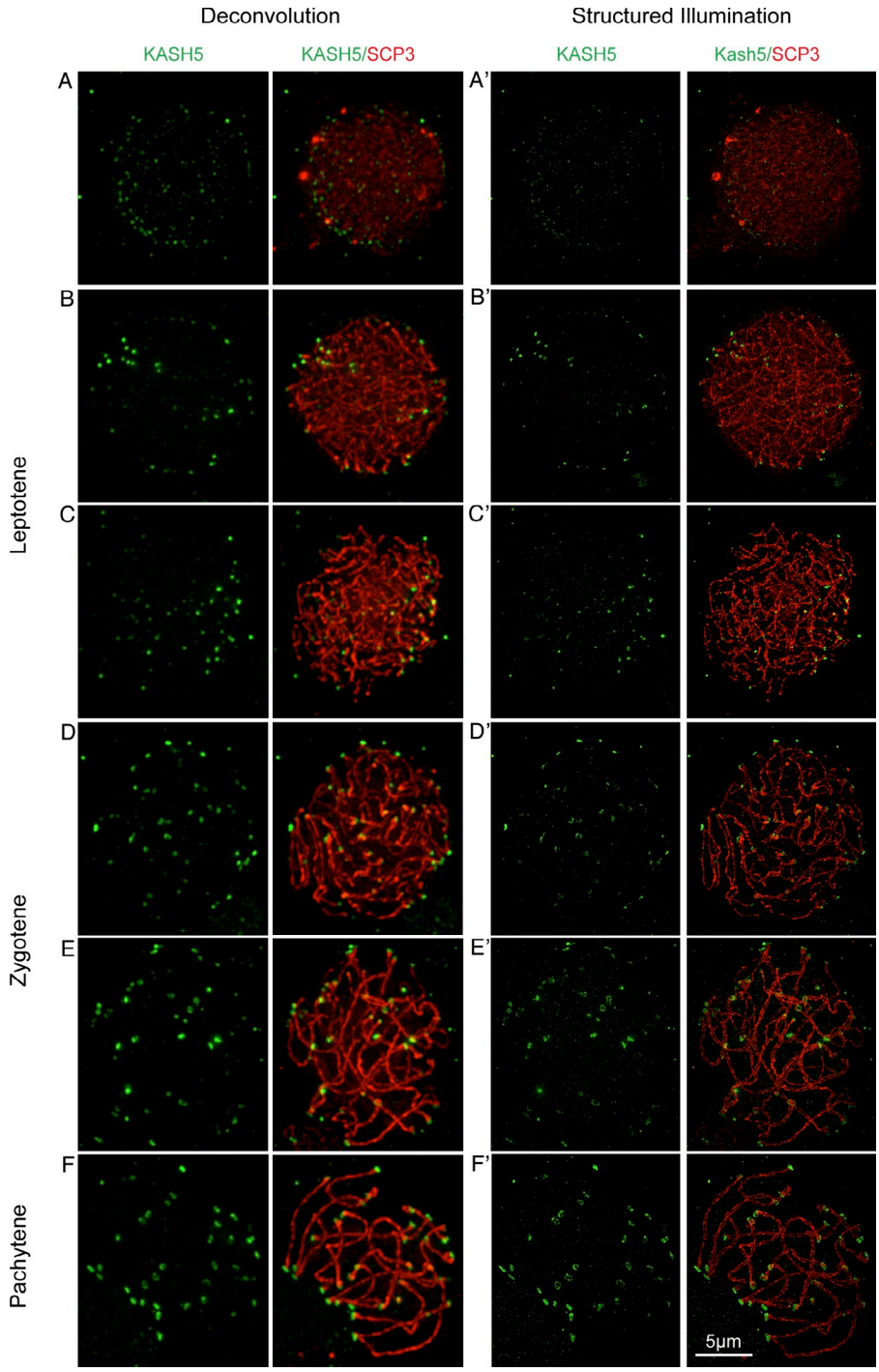

Figure S3. **Meiotic progression in wild-type spermatocytes.** Spermatocytes at successive stages of meiotic prophase 1 were immunostained with anti-KASH5 and anti-SCP3. The same cells were imaged using both conventional deconvolution microscopy (left set of images) and structured illumination microscopy (SIM, right set of images). The twofold improvement in resolution achieved with SIM reveals the alignment of SCP3-containing axial elements of synaptonemal complexes during homologous chromosome pairing. SIM also reveals the ring-like organization of KASH5 at the tips of synaptonemal complexes.

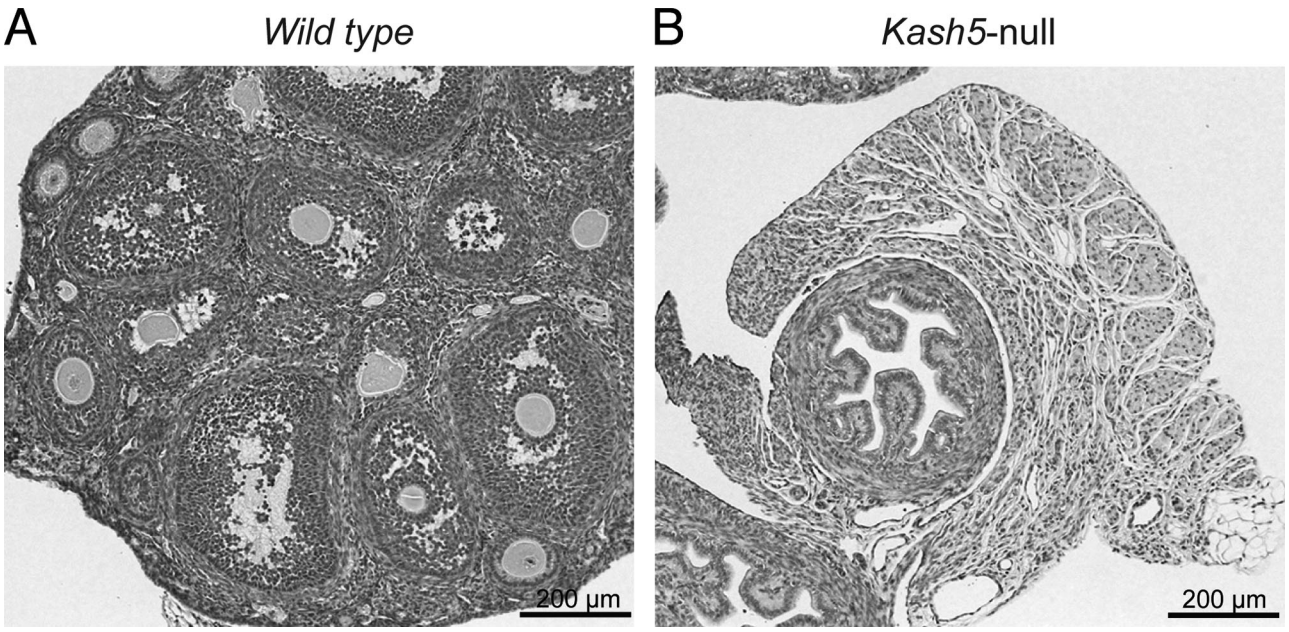

Figure S4. **Infertility in *Kash5*-null females.** Sections of ovaries from both wild-type (A) and *Kash5*-null (B) adult female littermates. Sections were stained with hematoxylin and eosin (H&E). Although wild-type ovaries display numerous follicles at various stages of development, such features are uniformly absent from the *Kash5*-null samples. Clearly, KASH5 deficiency in females is associated with a profound gametogenesis defect.

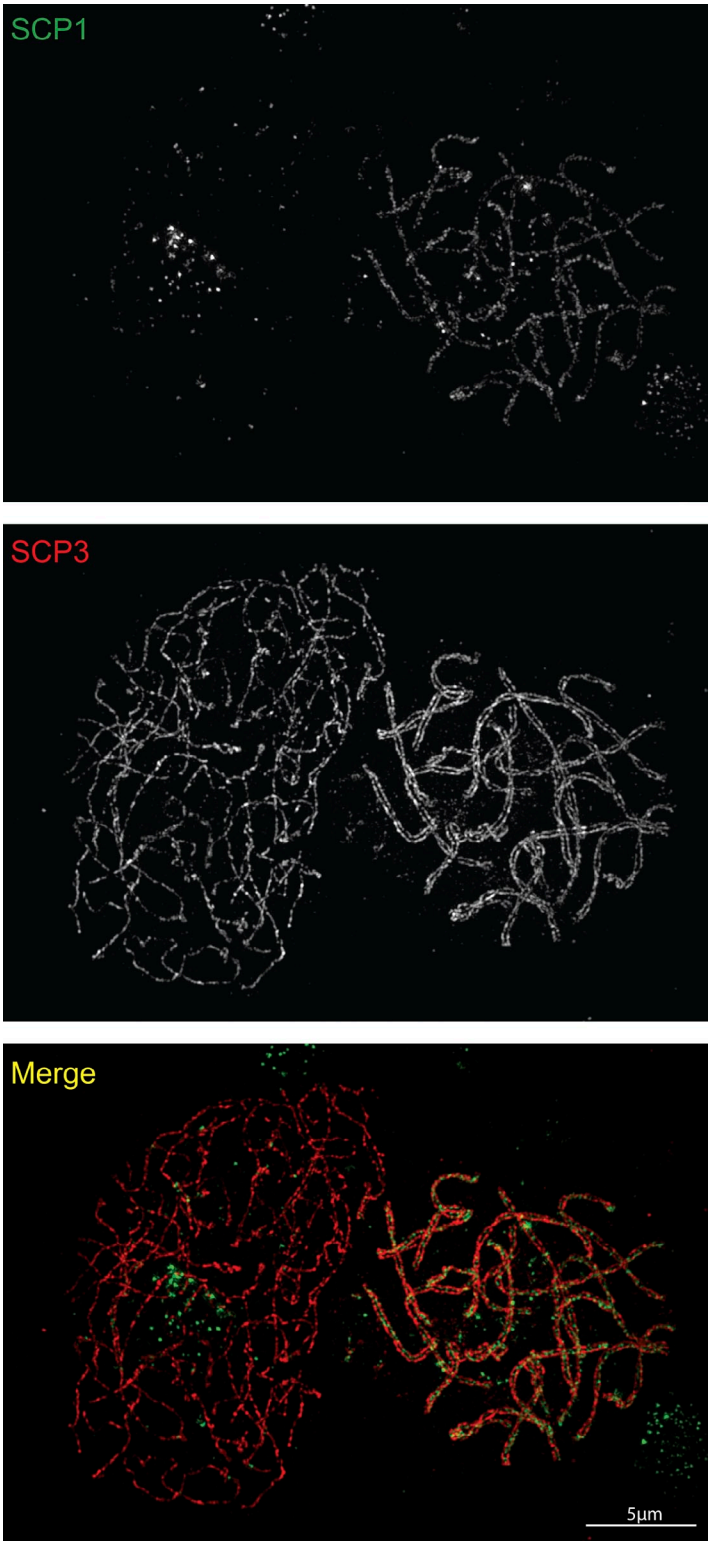

Figure S5. **Visualization of spermatocyte spreads using structured illumination microscopy (SIM).** Samples from wild-type testes were labeled with antibodies against SCP3, an axial element component of synaptonemal complexes, and SCP1, a transverse element component. A leptotene cell can be seen on the left, and a pachytene cell is on the right. It is clear that SCP1 foci are flanked by the SCP3-containing axial strands in the pachytene cell. The axial strands appear frequently to coil around each other in a loose double helix.