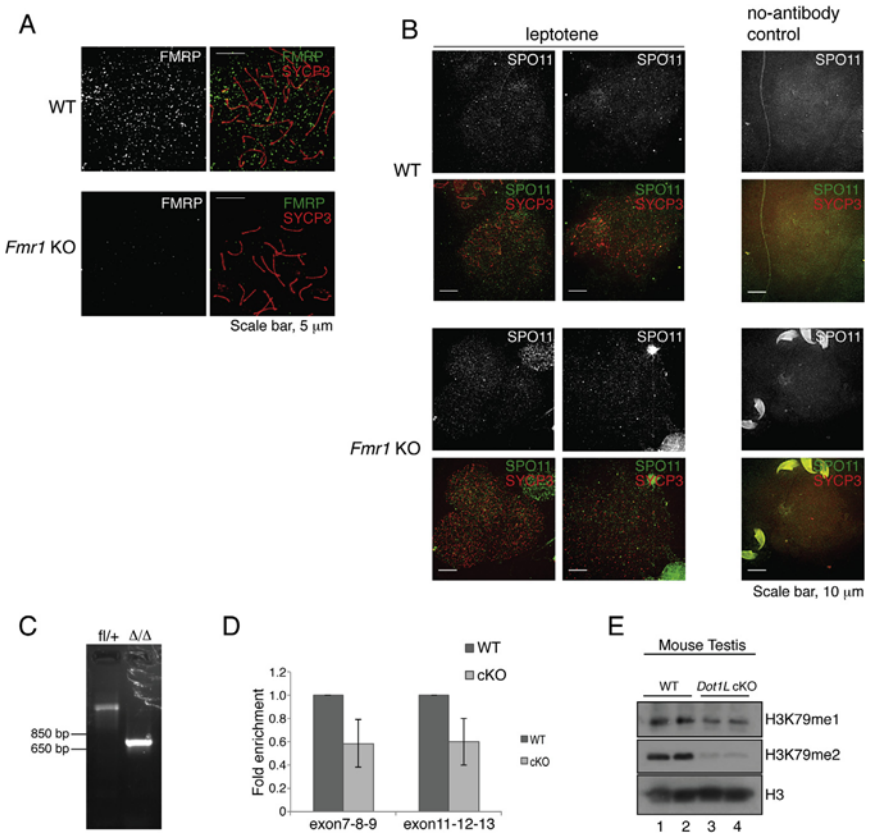

Figure S7. Control Staining of FMRP in Mouse Spermatocytes, SPO11 Staining, and Analysis of *Dot1L* cKO Testes, Related to Figures 5 and 7

(A) Immunostaining of wild-type (WT) and *Fmr1* KO meiotic spreads with anti-FMRP antibody (rabbit polyclonal anti-FMRP, Abcam) to confirm the specificity of FMRP staining. SYCP3 marks the chromosomes. Scale bar, 5 μm.

(B) SPO11 recruitment and double-strand-break formation is not perturbed in *Fmr1* KO cells. SPO11 catalyzes DSBs during the leptotene stage of meiotic prophase. Staining for SPO11 in representative WT and representative KO leptotene cells reveals no difference between WT and KO. Equivalent staining with no primary antibody is shown as a negative control. SPO11 staining alone is shown in the top row for clarity. Large green patches are sperm heads. Scale bar, 10 μm.

(C–E) *Dot1L* expression is reduced in *Dot1L* cKO testis.

(C) Genotyping for the $Dot1L^{\Delta}$ allele using genomic DNA from $Dot1L^{fl/+}$;*Mvh-Cre*- (WT, fl/+) or $Dot1L^{fl/\Delta}$;*Mvh-Cre*+ (cKO, Δ/Δ) adult testis tissue. The delta allele is predicted to be ~700 bp.

(D) qRT-PCR using two different primer sets specific for *Dot1L*, using cDNA from $Dot1L^{fl/+}$;*Mvh-Cre* (WT) and $Dot1L^{fl/\Delta}$;*Mvh-Cre*+ (cKO) adult testis. For each qPCR primer set, one primer spans an exon-exon junction in the *Dot1L* cDNA and the other primer is in a third, adjacent exon. Signal is normalized to *Actb*. Error bars signify SD for two biological replicates. Differences between means do not meet statistical significance. Note that cDNA was isolated from whole testis, which contains both somatic and germ cells, but the *Mvh-Cre* transgene is expressed only in germ cells. Therefore, some *Dot1L* expression is expected in the mixed somatic/germ cell population even in cKO testis.

(E) Western blot for H3K79me1 and H3K79me2 on mouse testis lysates showing reduction of H3K79 methylation in *Dot1L* cKO testes (lanes 3 and 4). Two samples of WT (lanes 1 and 2) and *Dot1L* cKO (lanes 3 and 4) testes are shown.