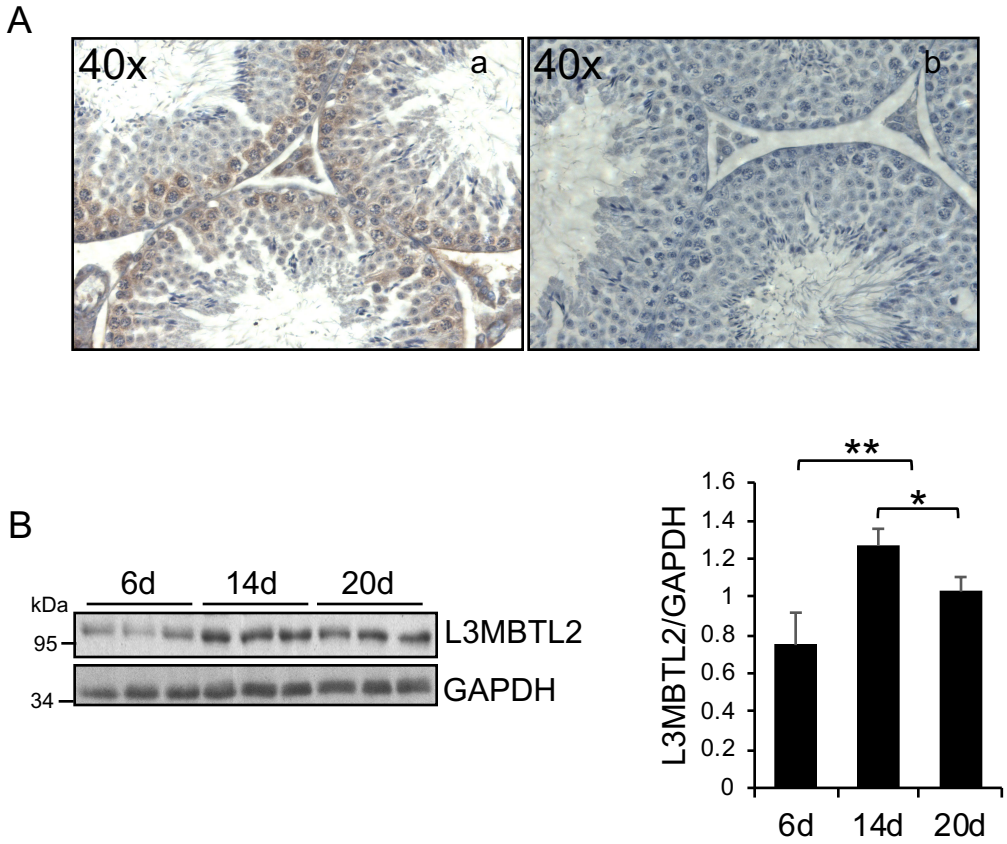
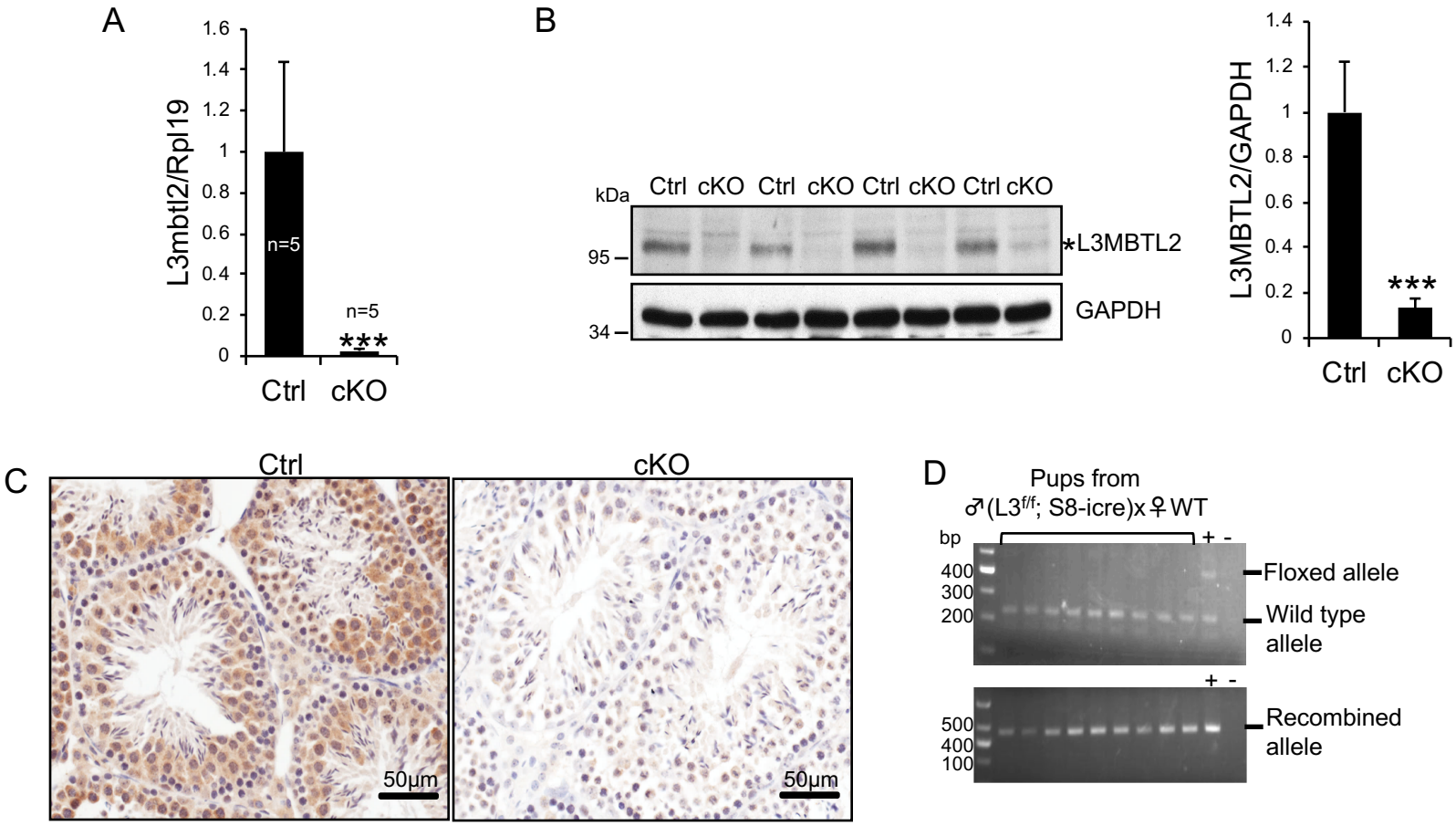
Supplemental Figure 1



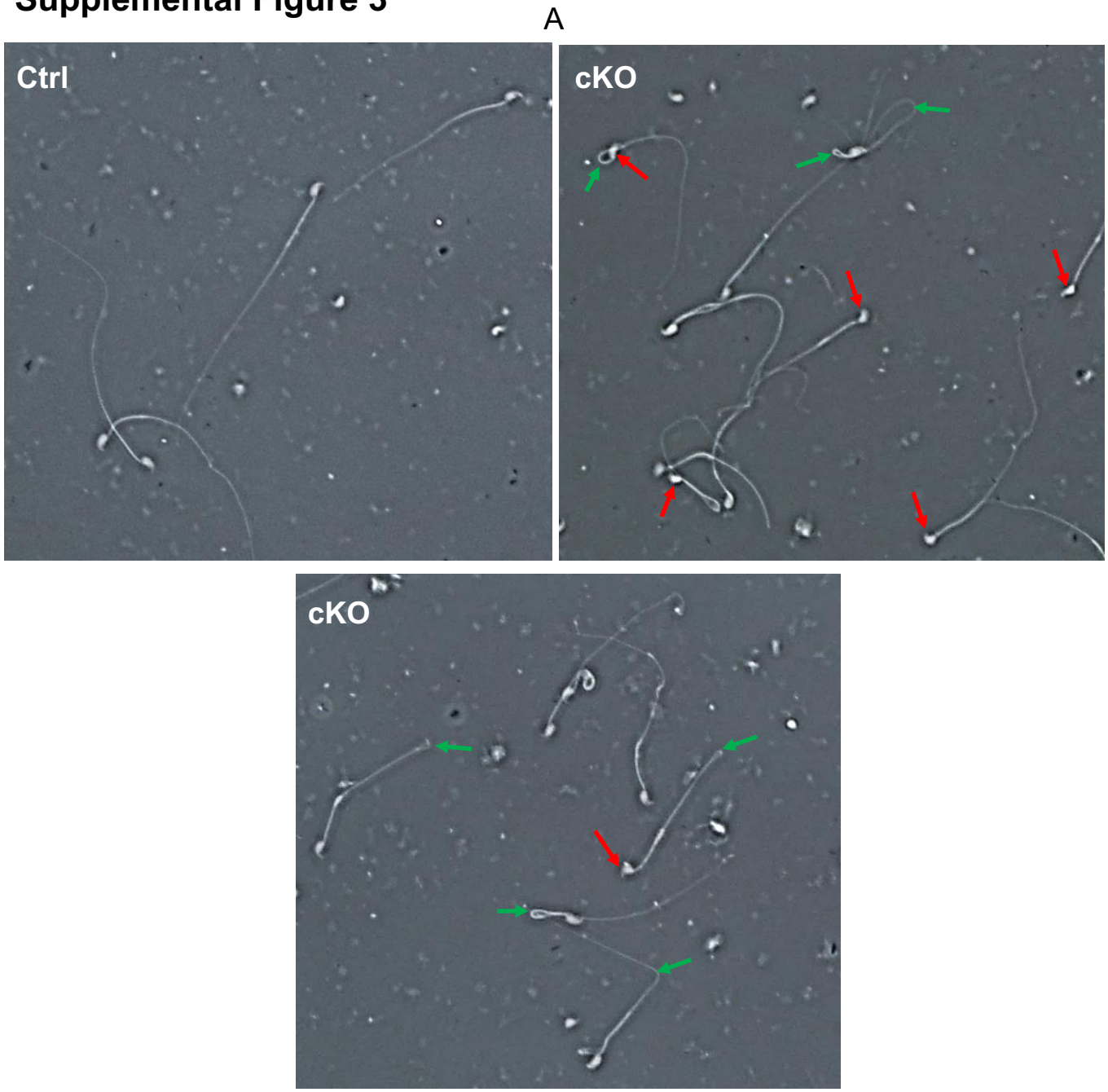
Supplemental Figure 1. Cellular localization of L3MBTL2 in WT mouse testes. (A) Immunohistochemistry for L3MBTL2 was performed on the testis of 2 months old WT mice. Brown color indicates positive staining (a). Anti-L3MBTL2 from Sigma (HPA000815) was used. Negative control (b) was performed with normal IgG instead of the primary antibody. (B) Expression of L3MBTL2 in the testis during the first wave of spermatogenesis. Immunoblots of L3MBTL2 and GAPDH in the testes of mice at different days postpartum (left panel). L3MBTL2 levels relative to GAPDH were quantified by densitometry (right panel). *$p < 0.05$; **$p < 0.01$.

Supplemental Figure 2



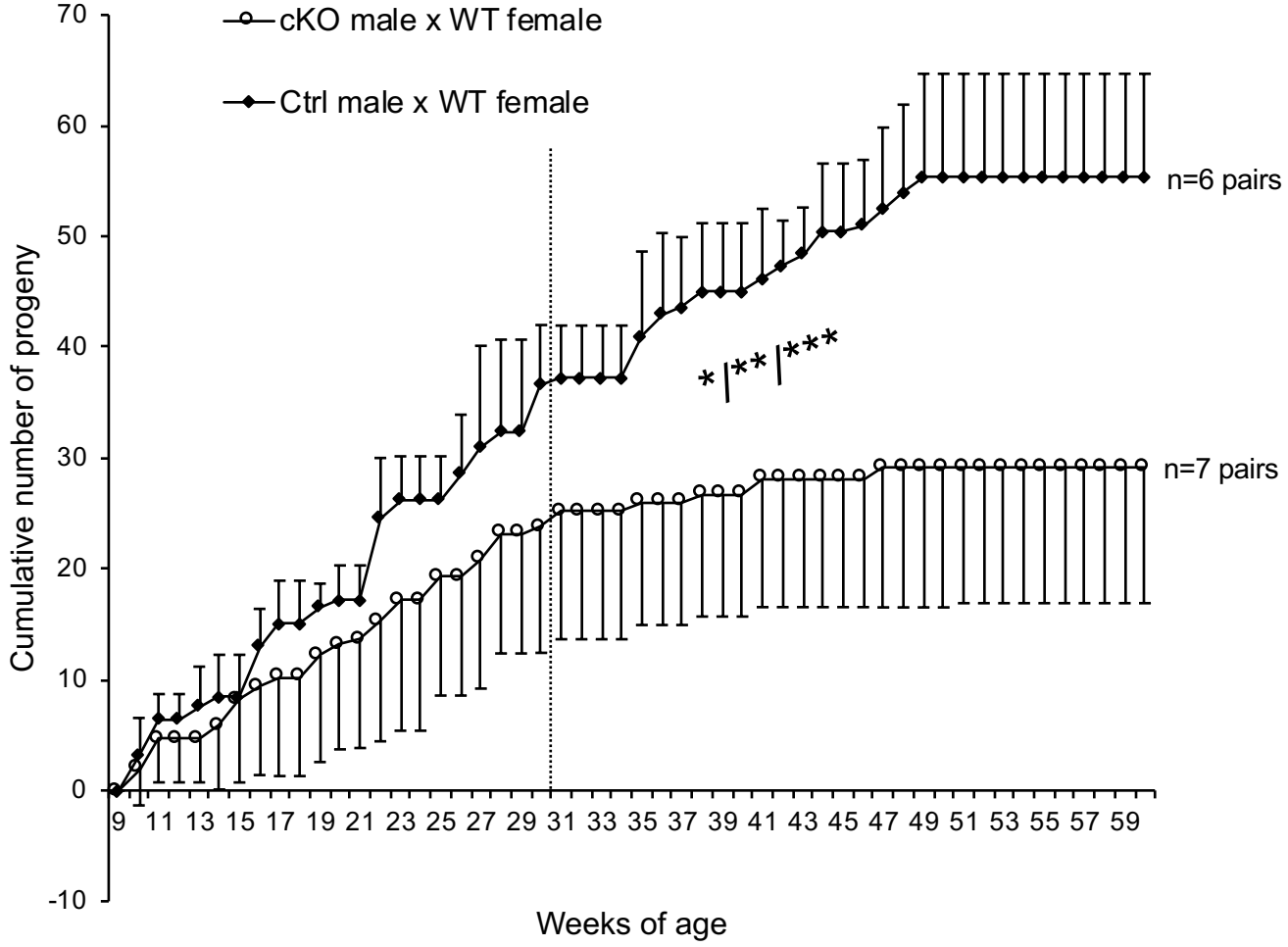
Supplemental Figure 2. Expression of L3MBTL2 in WT and L3mbtl2 cKO mouse testes. (A) Real-time PCR analysis was performed to examine L3mbtl2 mRNA expression in control (L3mbtl2$^{f/f}$) and L3mbtl2 cKO (L3mbtl2$^{f/f}$-Stra8-icre) testes collected from 2 months old mice. (B) Western blotting was performed to examine L3mbtl2 protein expression in control and cKO testes collected from 2 months old mice. GAPDH is the loading control. (C) Immunohistochemistry for L3MBTL2 was performed on the testis of 2 months old control and L3mbtl2 cKO mice. Brown color indicates positive staining. Anti-L3MBTL2 antibody from Boster (A08416) was used. (D) Genotyping PCR of pups born from a L3mbtl2 cKO male crossed with a WT female mouse at 2 months of age indicates complete cre-mediated excision of the floxed allele in the offspring. n = 5 mice for panel C. ***$p < 0.001$.

Supplemental Figure 3



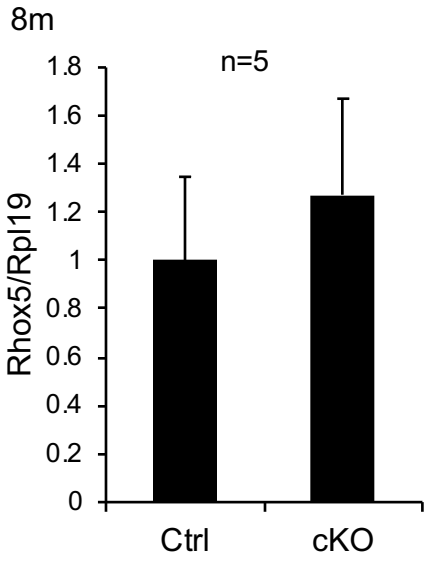
Supplemental Figure 3. (A) Normal and abnormal sperm in control (L3mbtl2$^{f/f}$) and L3mbtl2 cKO (L3mbtl2$^{f/f}$-Stra8-icre) mice at 8 months of age. (B) Quantification of normal sperm from control and L3mbtl2 cKO mice at 8 months of age. The numbers of mice are indicated. Red arrows indicate defective heads, and green arrows indicate hairpins or V-shape bending. ***$p < 0.001$.

Supplemental Figure 4



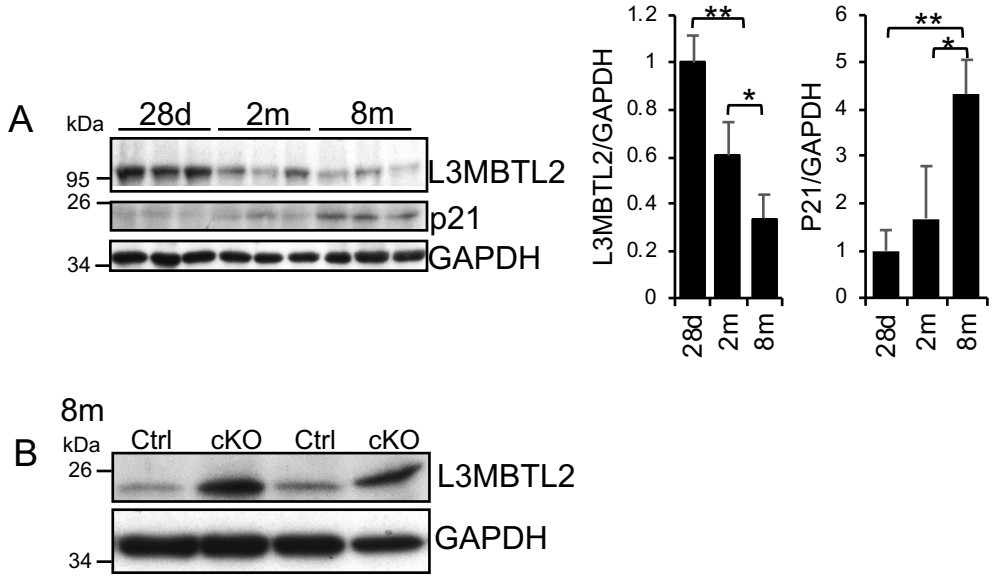
Supplemental Figure 4. L3mbtl2 cKO mice cease to produce progeny prematurely. 6-week-old control and L3mbtl2 cKO male mice were mated with 6-week-old WT females, and the cumulative numbers of pups were determined. The dotted line indicates that the cumulative numbers between control and L3mbtl2 cKO male mice become statistically different at 30 weeks of age. The numbers of mating pairs are indicated. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (cKO vs Ctrl).

Supplemental Figure 5



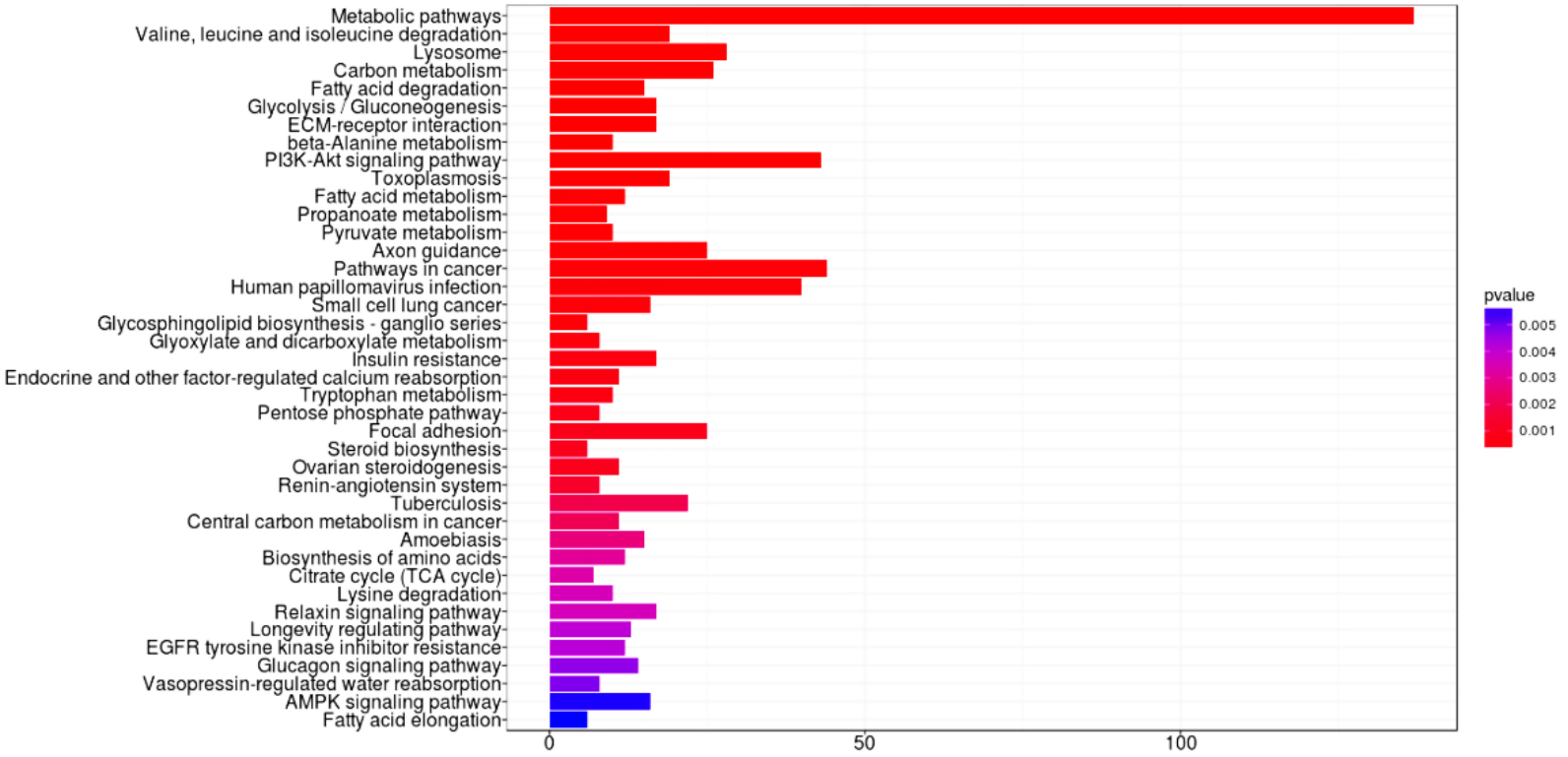
Supplemental Figure 5. mRNA expression of the testosterone responsive gene Rhox5 in the testes of control and L3mbtl2 cKO mice.

Supplemental Figure 6



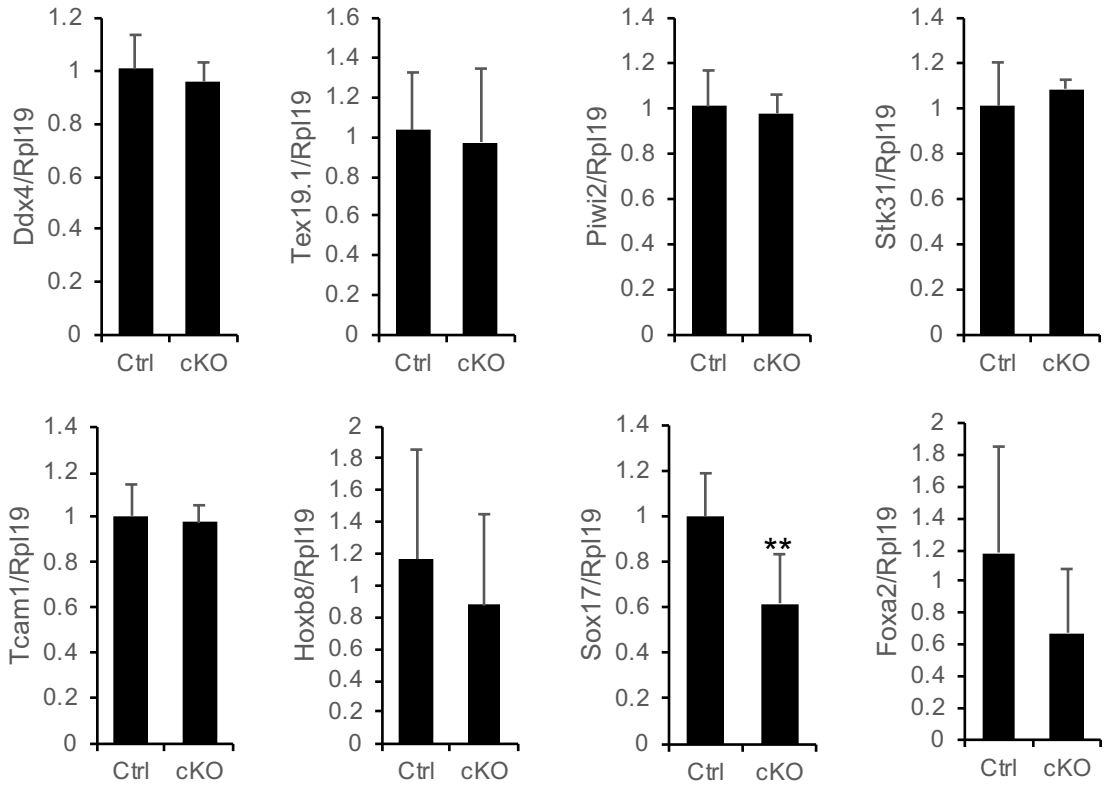
Supplemental Figure 6. L3MBTL2 and p21 protein levels in testis at different ages. (A) Expression of L3MBTL2 and p21 in the testes of WT mice at 28 days and 2 and 8 months of age was examined by Western blotting, and quantified by densitometry. (B) p21 protein levels in the testes of control and L3mbtl2 cKO mice at 8 months of age. GAPDH is the loading control. *$p < 0.05$; **$p < 0.01$.

Supplemental Figure 7



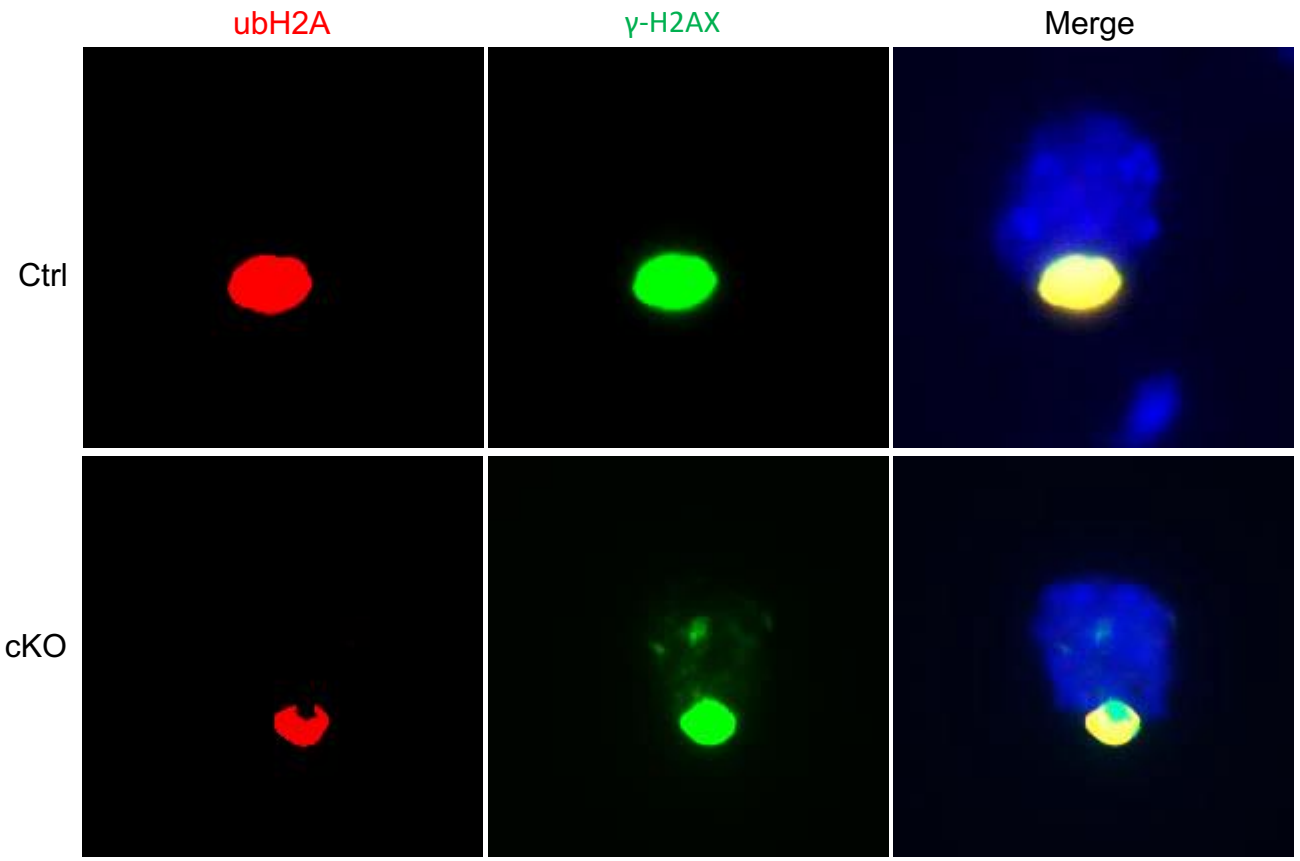
Supplemental Figure 7. KEGG pathway analysis for differentially expressed genes.

Supplemental Figure 8



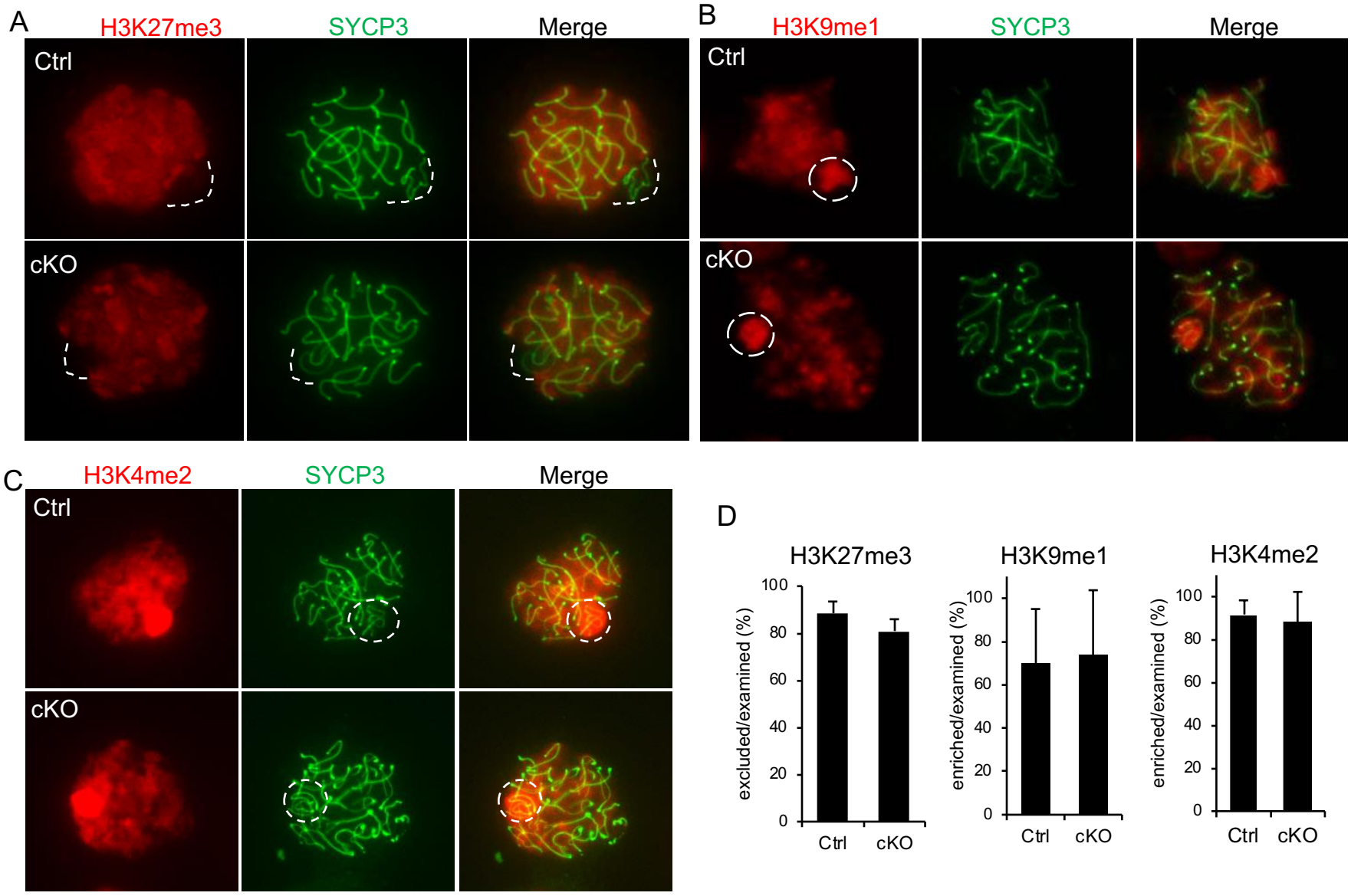
Supplemental Figure 8. Expression of mRNAs for Ddx4, Tex19.1, Piwi2, Stk31, Tcam1, Hoxb8, Sox17 and Foxa2 in the testes of control ($L3mbtl2^{f/f}$) and L3mbtl2 cKO ($L3mbtl2^{f/f}$-Stra8-icre) mice at 8 months of age. n=5. ** $P < 0.01$.

Supplemental Figure 9



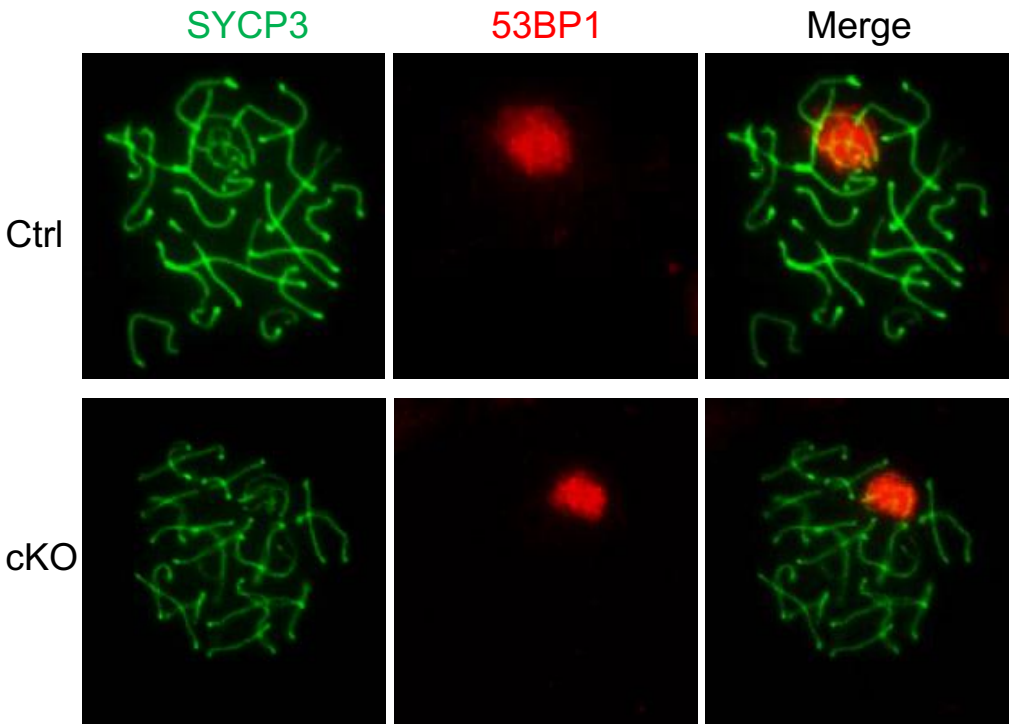
Supplemental Figure 9. Costaining of γ-H2AX and the XY body marker ubH2A in pachytene spermatocytes. Meiotic spreads were prepared from the testes of control (L3mbtl2$^{f/f}$) and L3mbtl2 cKO (L3mbtl2$^{f/f}$-Stra8-icre) mice at 8 months of age.

Supplemental Figure 10



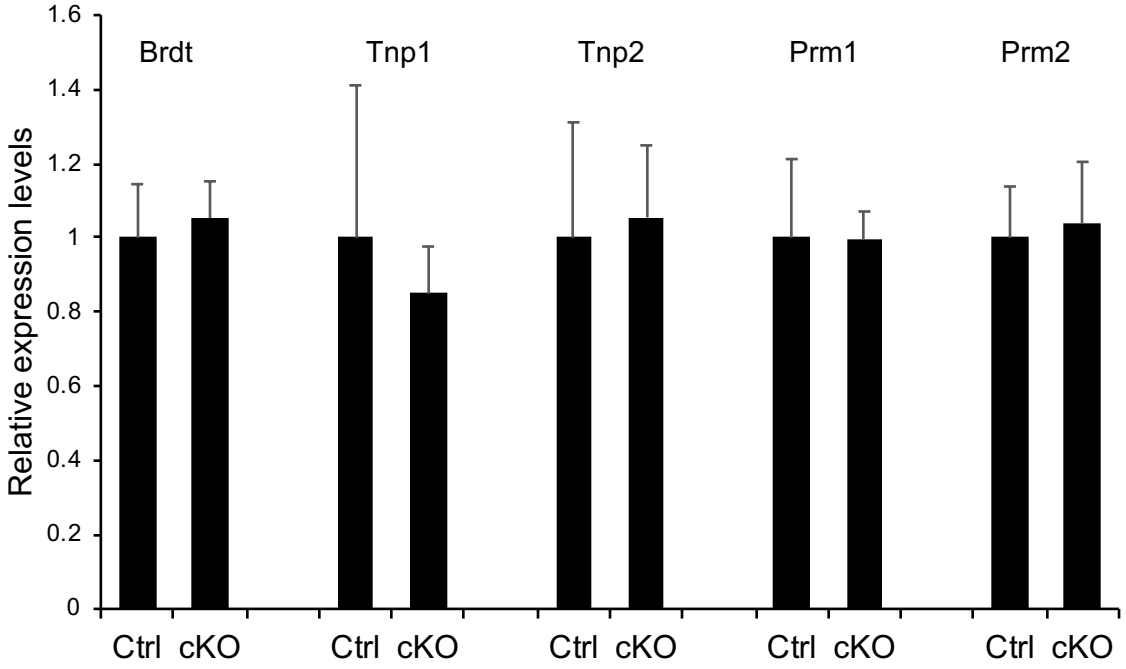
Supplemental Figure 10. Costaining of H3K27me3 (A), H3K9me1 (B), or H3K4me2 (C), and SYCP3 in meiotic spreads from the testes of control (L3mbtl2$^{f/f}$) and L3mbtl2 cKO (L3mbtl2$^{f/f}$-Stra8-icre) mice at 8 months of age. Frequency of spermatocytes, in which H3K27me3 was excluded, or H3K9me1 or H3K4me2 was enriched at the XY body was summarized (D). 3 mice in each genotype were used.

Supplemental Figure 11



Supplemental Figure 11. Costaining of 53BP1 and SYCP3 in meiotic spreads from the testes of control (L3mbtl2$^{f/f}$) and L3mbtl2 cKO (L3mbtl2$^{f/f}$-Stra8-icre) mice at 8 months of age.

Supplemental Figure 12



Supplemental Figure 12. Expression of mRNAs for the transition proteins BRDT, TNP1 and TNP2 and protamines 1 and 2 in the testes of control (L3mbtl2$^{f/f}$) and L3mbtl2 cKO (L3mbtl2$^{f/f}$-Stra8-icre) mice at 8 months of age. n=5.