Figure S1. Prss50-null mice were generated using CRISPR/CAS9 technology. A) *Prss50* mRNA sequence. Indicated in yellow is exon 2 (targeted exon). The 5' guide is indicated in purple and the 3' guide in aqua. **B)** Western blot of testis of WT and *Prss50*-null mice indicated the lack of PRSS50 in *Prss50*-null testis.

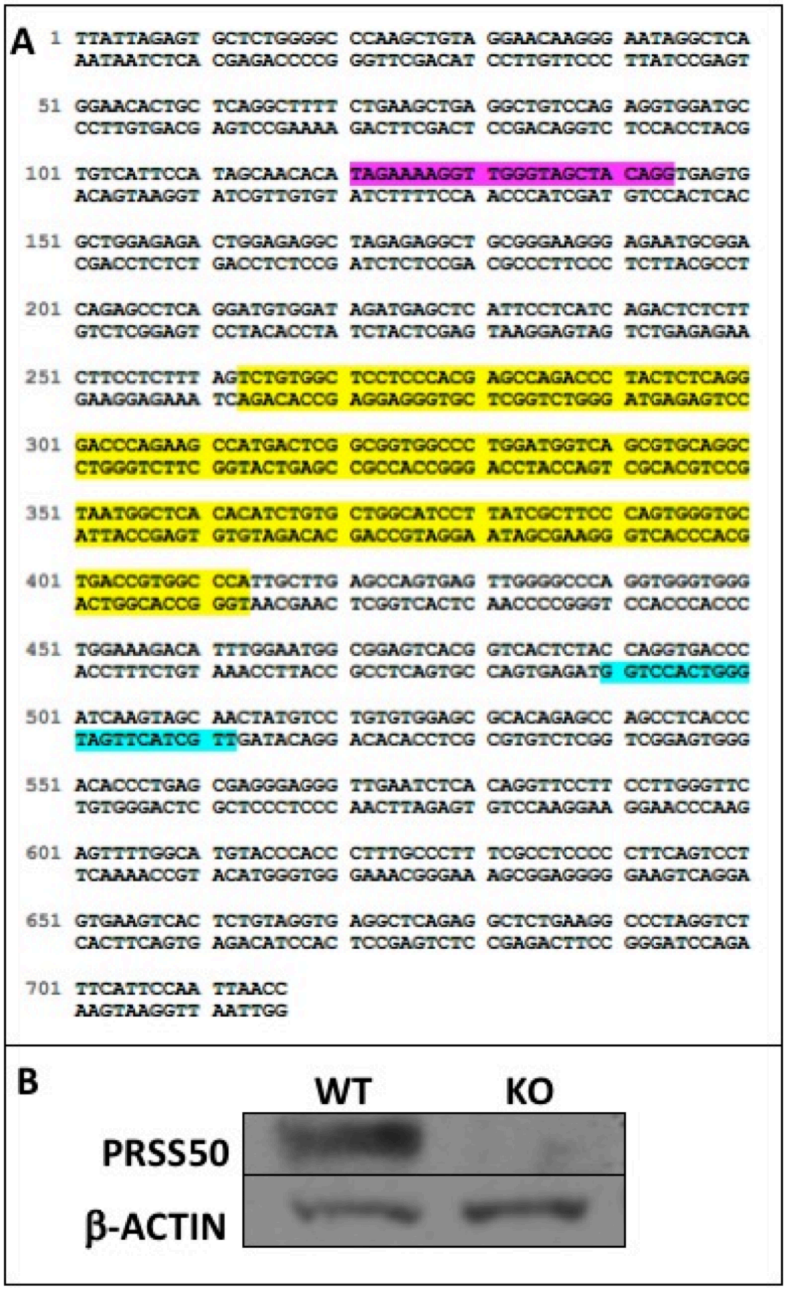


Figure S2. Staining of different markers of testicular cells and structures does not show differences between *Prss50*-null and WT mice. **A)** Testicular staining using the Leydig cell marker 3β-HSD. **B**) Testicular staining using the Sertoli and interstitial cell marker AR. **C**) Testicular staining using the spermatogonia cell marker PLZF. **D-E**) Testicular staining using the proliferation markers CCND1 and KI67. **F**) Testicular staining using the intracellular bridge marker TEX14.

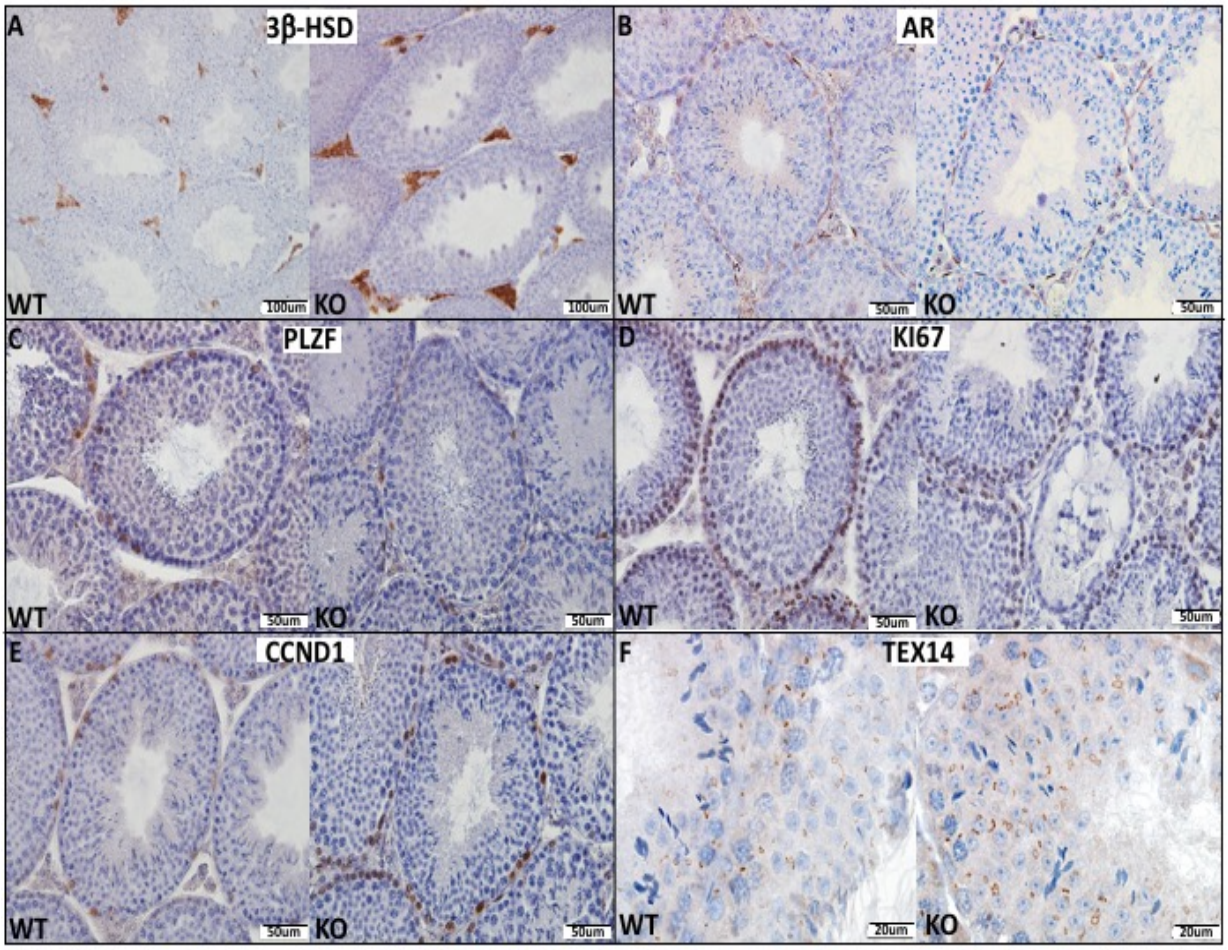


Figure S3. Level of Different testicular protein in *Prss50*-null mice. Comparison of the level of CCND1, FOX01, TEX14, and SOX9 between the testis of WT and *Prss50*-null mice. β-actin was used as an internal control. The ratio of Null vs. WT is indicated. None of the difference of ratios were statistically significant.

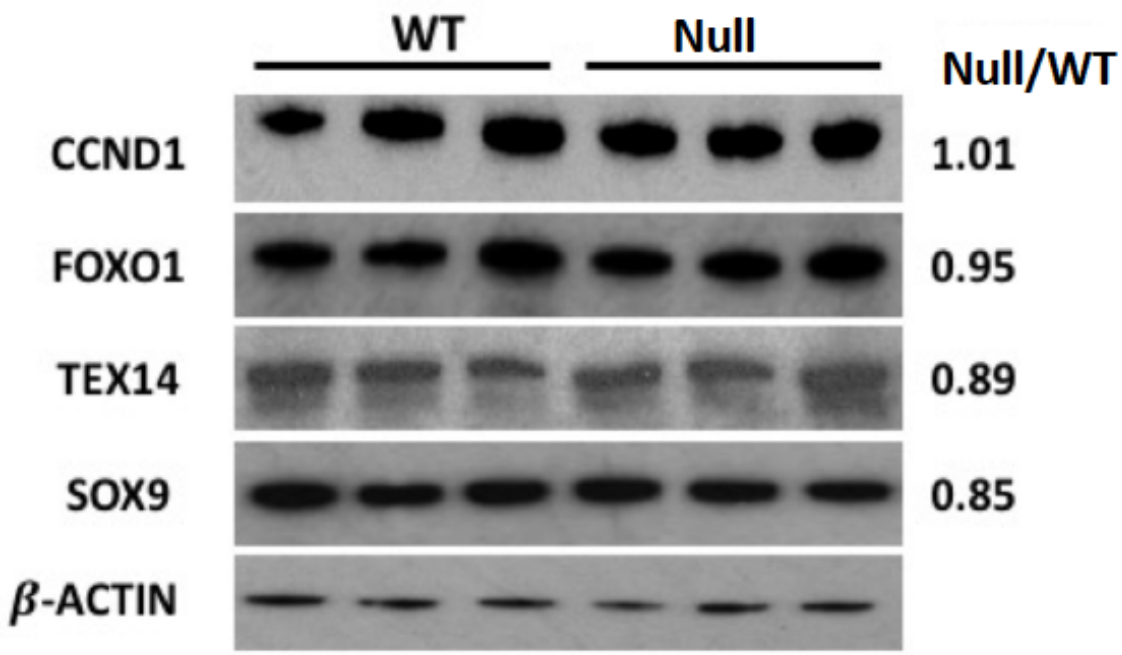


Figure 4S. Rules-based classification of sperm morphology. Sperm from WT and *Prss50*-null mice labeled with DAPI (head), MitoTracker (midpiece), and α-tubulin (tail) were subjected to HCA analysis. A total of 10,000 sperm from each sample were classified using a rules-based scheme shown. Numbers indicate the total number of sperm and percent of input.

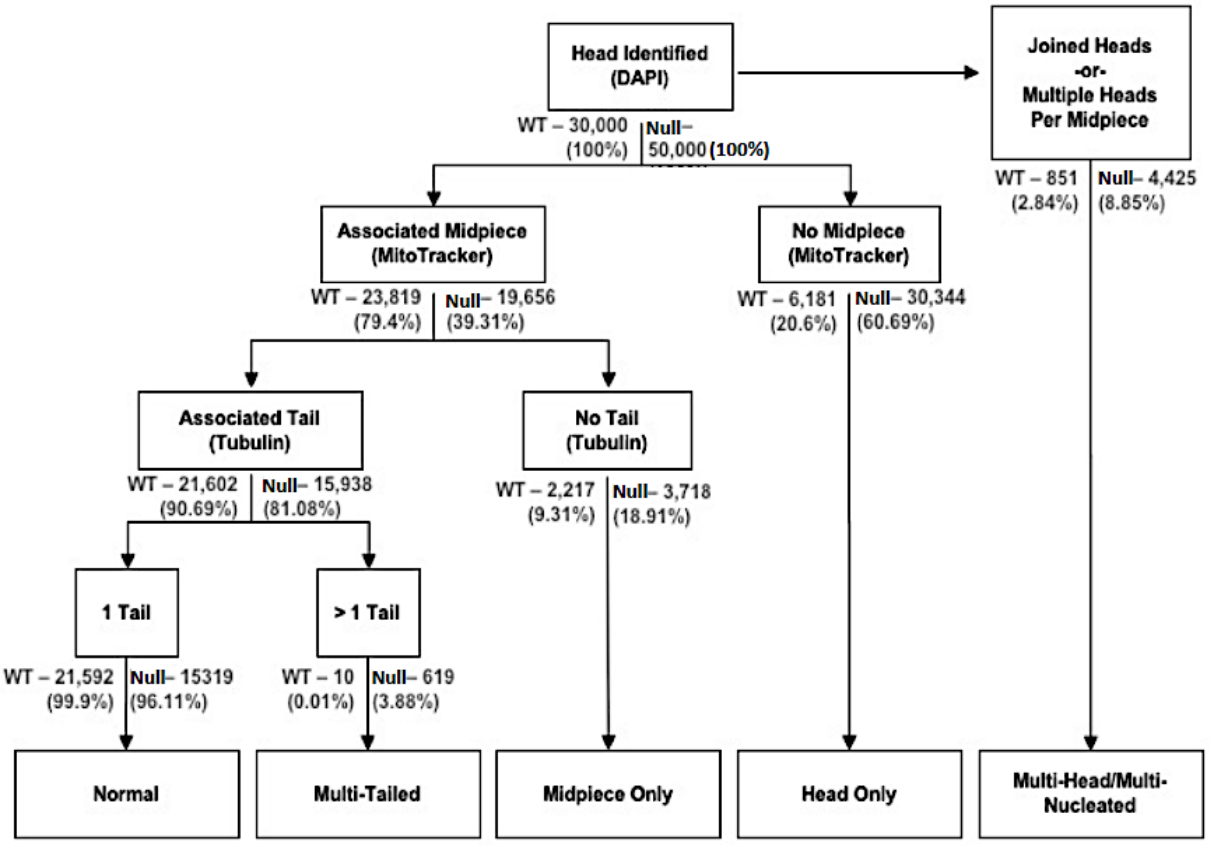


Figure S5. Sperm abnormalities in *Prss50*-null mice. *Prss50*-null sperm have multiple abnormalities including: conjoined (A-B), mitochondrial gaps in tail and bend tail (C-D), and multiple tail with single tails, or multiple heads and midpiece with a single principal piece (E-J).

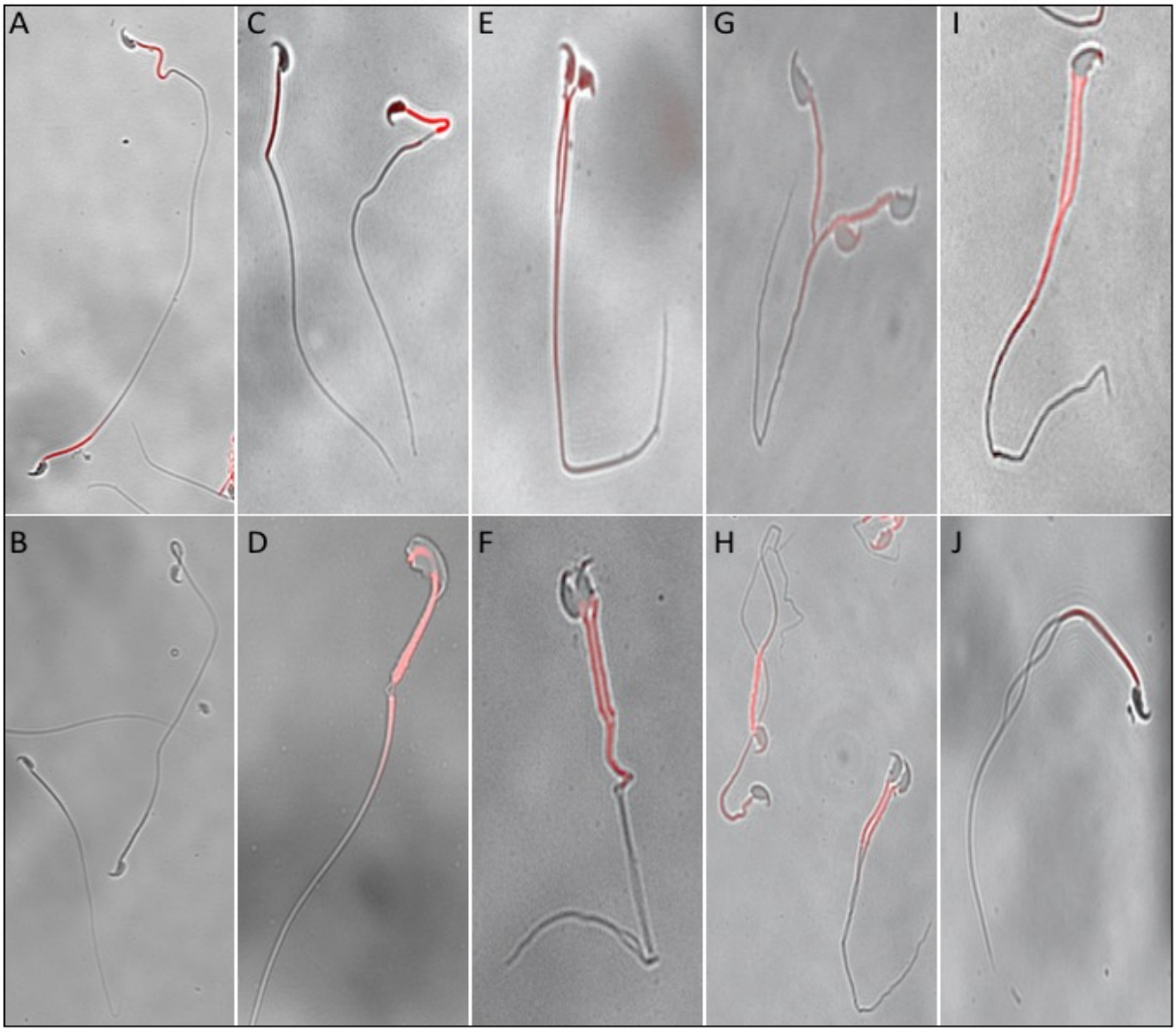


Figure S6. Sperm of *Prss50*-null mice do not have DNA damage based on Sperm chromatin structure assay (SCSA). Sperm heads from WT and *Prss50*-null mice labeled with acridine orange (AO). The SCSA simultaneously determines the % of sperm with high DNA stainability (%HDS) related to retained nuclear histones consistent with immature sperm; the % of DNA stainability versus red/red + green fluorescence (DFI); the count, area, circularity, and electricity. None significant difference between WT and null sperm was identified.

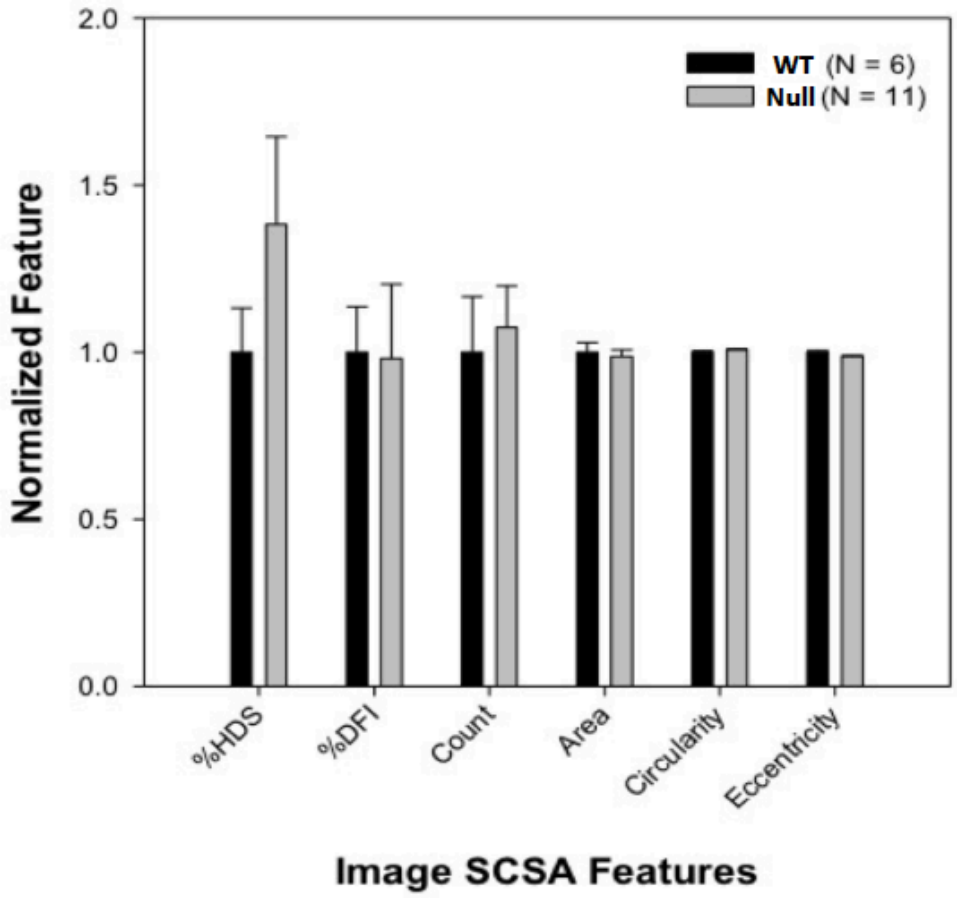
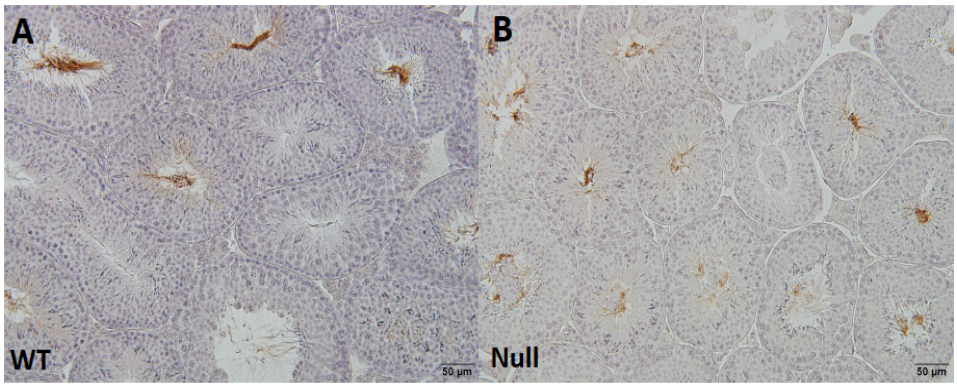
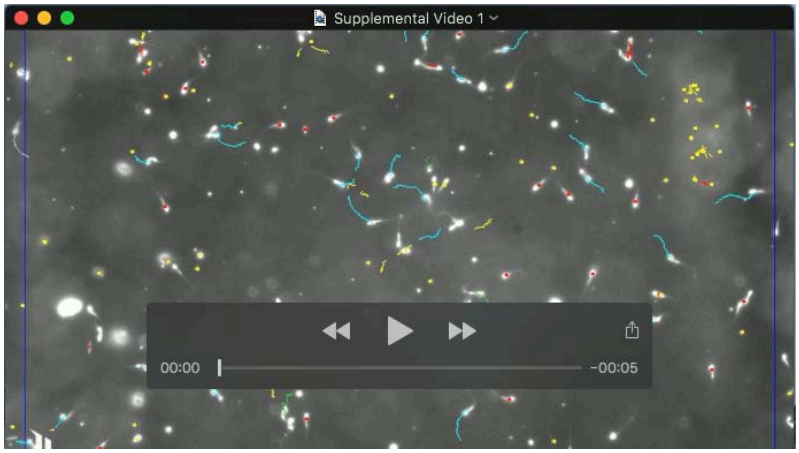
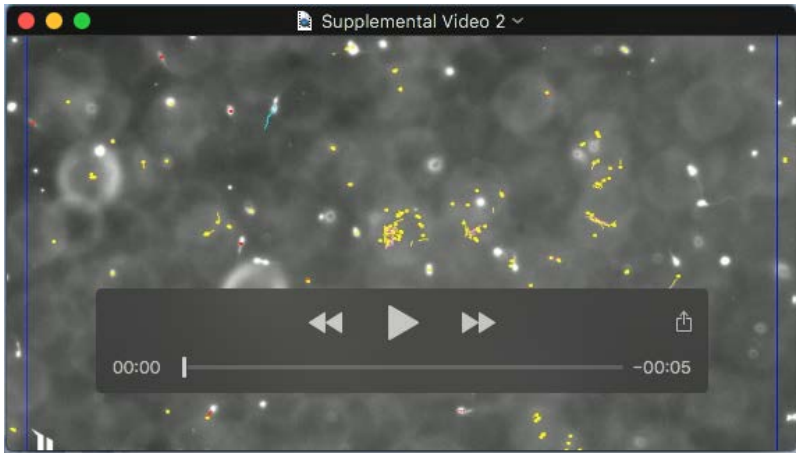


Figure S7. Testicular staining using AKAP4 does not show differences between *Prss50*-null and WT mice.

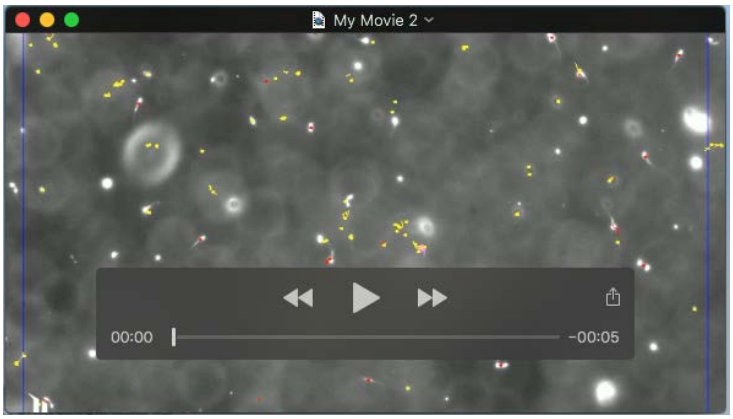




Movie 1. Frame from a microscopic video of WT sperm at 40X magnification (0.1x speed).



Movie 2. Frame from a microscopic video of *Prss50* null sperm at 40X magnification demonstrating a sperm with two heads (blue circle; 0.1x speed) as well as conjoined sperm.



Movie 3. Frame from a microscopic video of *Prss50* null sperm at 40X magnification demonstrating conjoined sperm (blue circle; 0.1x speed).

Movie 4. Frame from a microscopic video of *Prss50* null conjoined sperm at 100X magnification (0.1x speed).

Movie 5. Frame from a microscopic video of *Prss50* null two-head sperm at 100X magnification (0.1x speed).