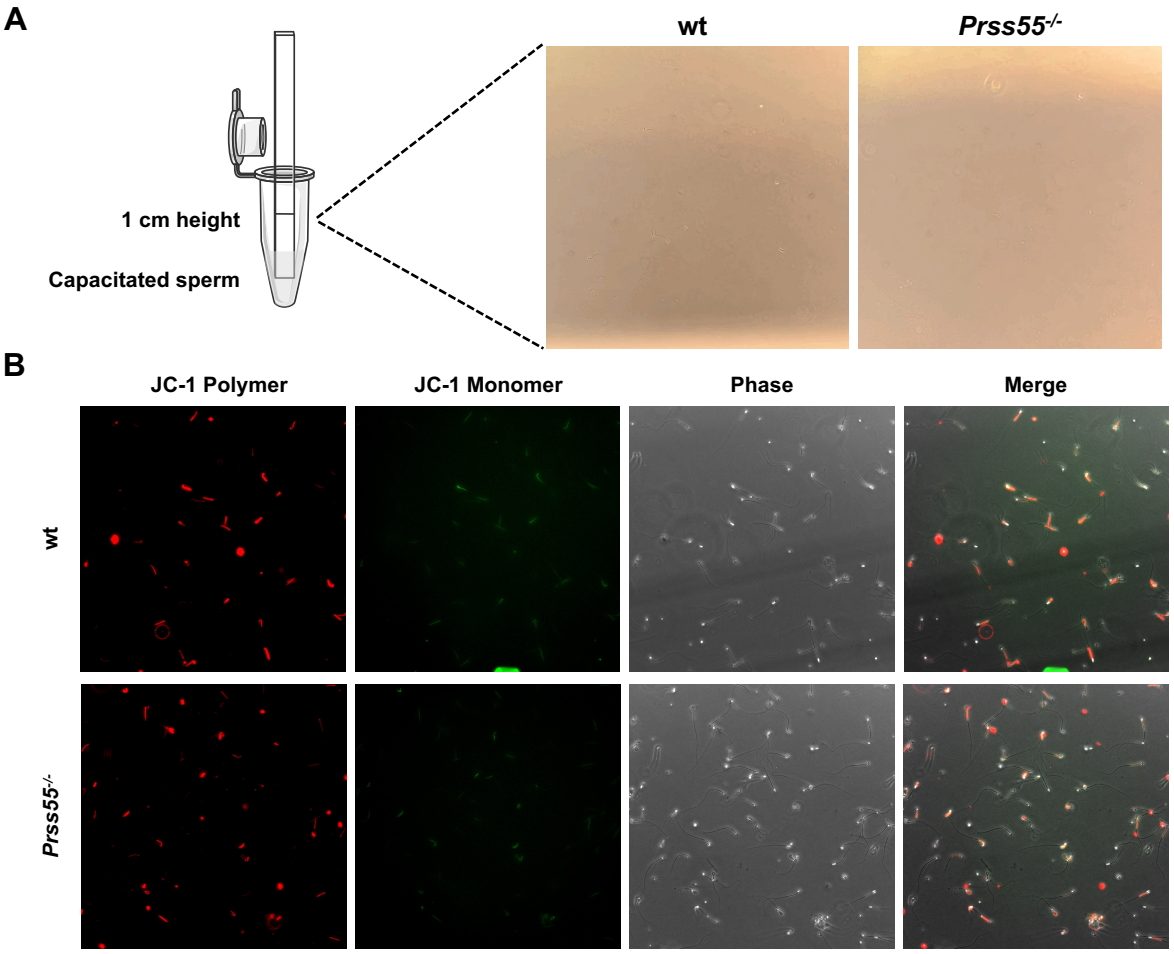
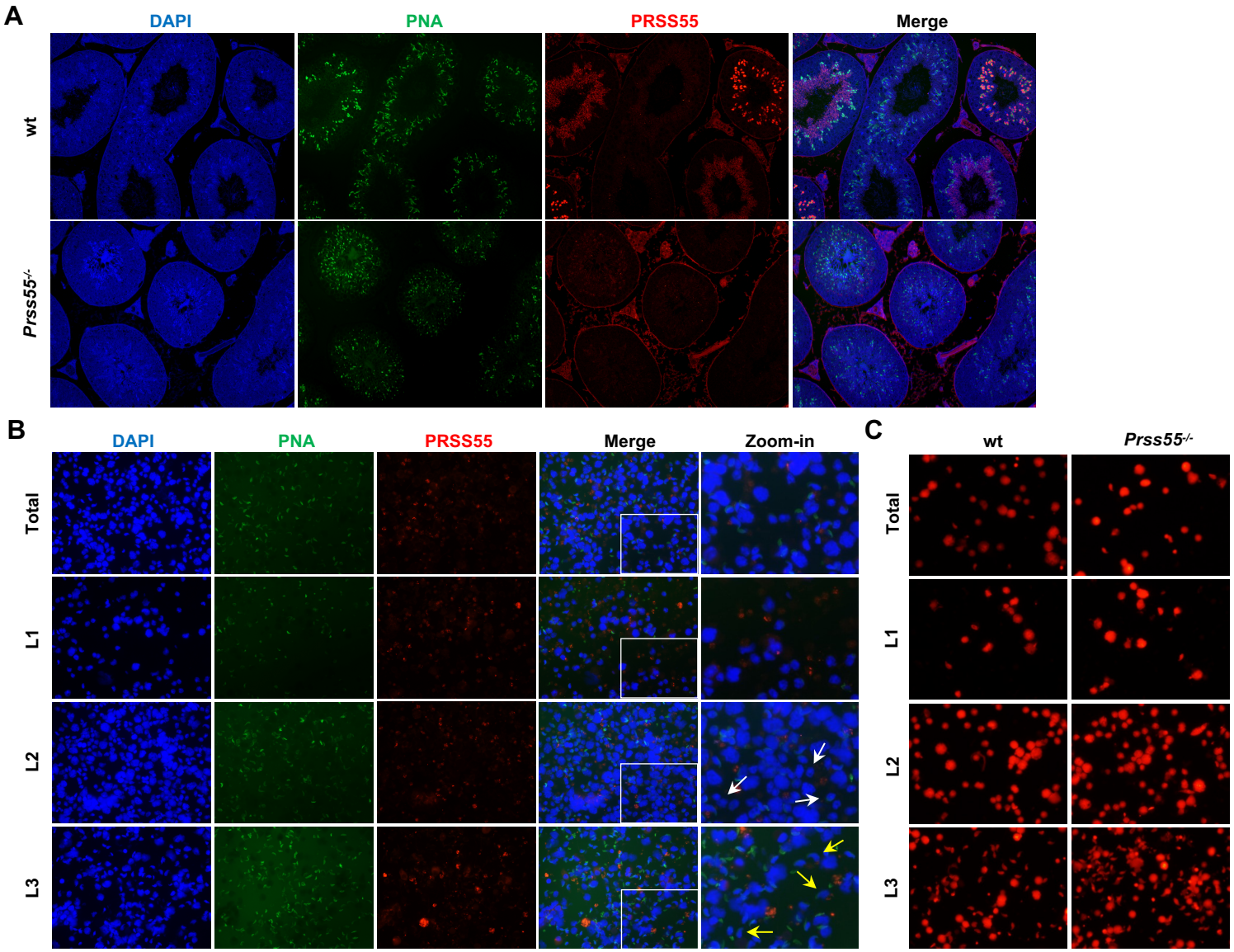
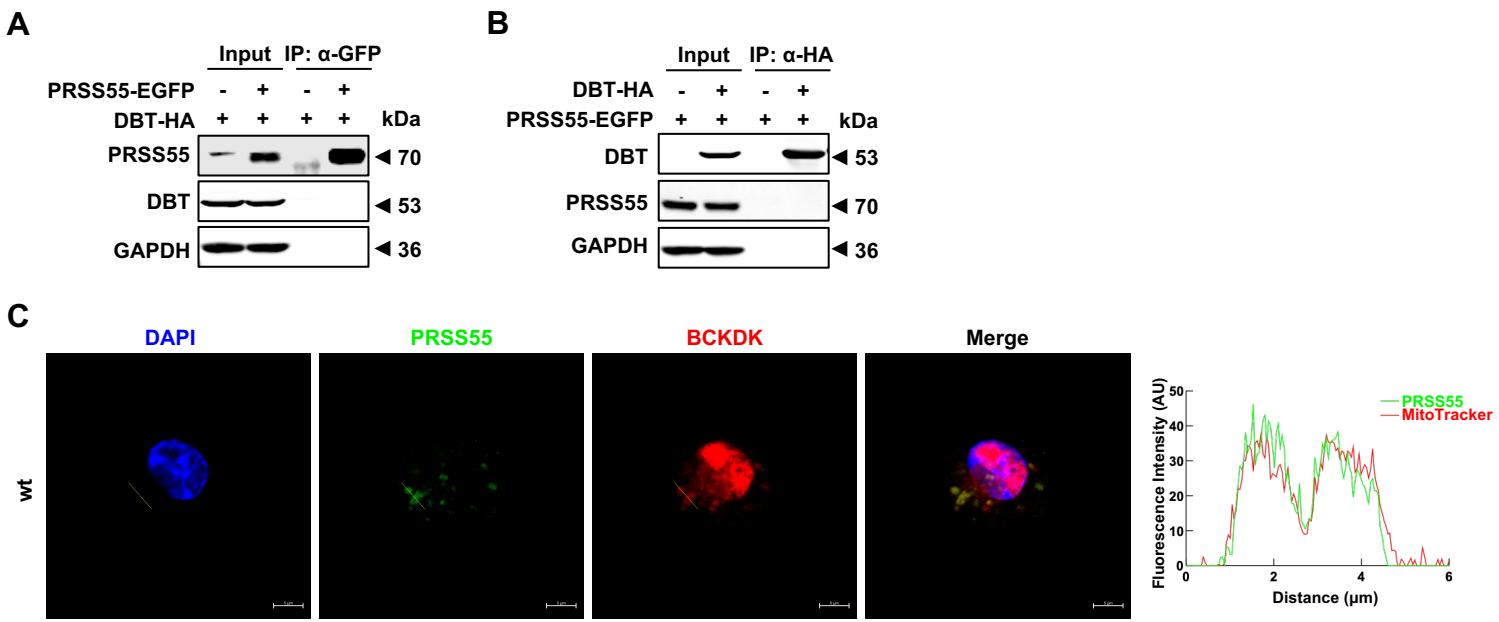


Supplementary Figure 1. *In vitro* sperm migration assay and MMP detection (A) Schematic diagram of *in vitro* sperm migration in 10% MC4000 solution (left) and representative video clips (right) captured under Nikon Eclipse Ti-S microscope, 200× magnification. (B) Representative JC-1 fluorescent images of MMP detection were obtained from a Cytation 5 cell imaging reader.



Supplementary Figure 2. Immunofluorescence staining of PRSS55. (A) PRSS55 is located at the luminal side of the seminiferous tubule. (PRSS55: red; PNA: green; DAPI: blue). (B) Immunofluorescence staining of PRSS55 of total (T), layer 1 (L1), layer 2 (L2), and layer 3 (L3) testicular cells between wt and *Prss55*$^{-/-}$ mice. Testicular L1 cells were cell debris as relatively large in size and with irregular edges; the testicular L2 cells were likely round spermatids with condensed nuclei and PNA signal appearance to resemble acrosomal vesicles (white arrowed); the testicular L3 cells were likely elongated spermatids with smaller and more compact nuclei and crescent-shaped acrosome (yellow arrowed). (C) Propidium iodide (PI) nuclear staining (red) of total, L1, L2, and L3 testicular cells between wt and *Prss55*$^{-/-}$ mice.



Supplementary Figure 3. Co-IP/MS hits validation (A, B) Co-IP of PRSS55 (A) or DBT (B) tagged as indicated with anti-tag antibodies from the cell lysates of co-transfected HEK293T cells by immunoblot. GAPDH was shown as a loading control. (C) Immunofluorescence co-localization of PRSS55 and BCKDK in wild-type mouse testicular cells (PRSS55: green; BCKDK: red; DAPI: blue). Pixel profile analysis revealed colocalization of green and red signals.