

Figure S1. Immunofluorescent staining of normal rabbit IgG (control) and PPP3R2 antibodies. (A) Immunofluorescent staining of normal rabbit IgG (control) in mouse testis. Bars: 50 μm. (B) Immunofluorescent staining of PPP3R2 in mouse sperm from either caput epididymis or cauda epididymis. Bars: 10 μm. (C) Immunofluorescent staining of normal rabbit IgG (control) in mouse mature sperm. Bars: 20 μm.

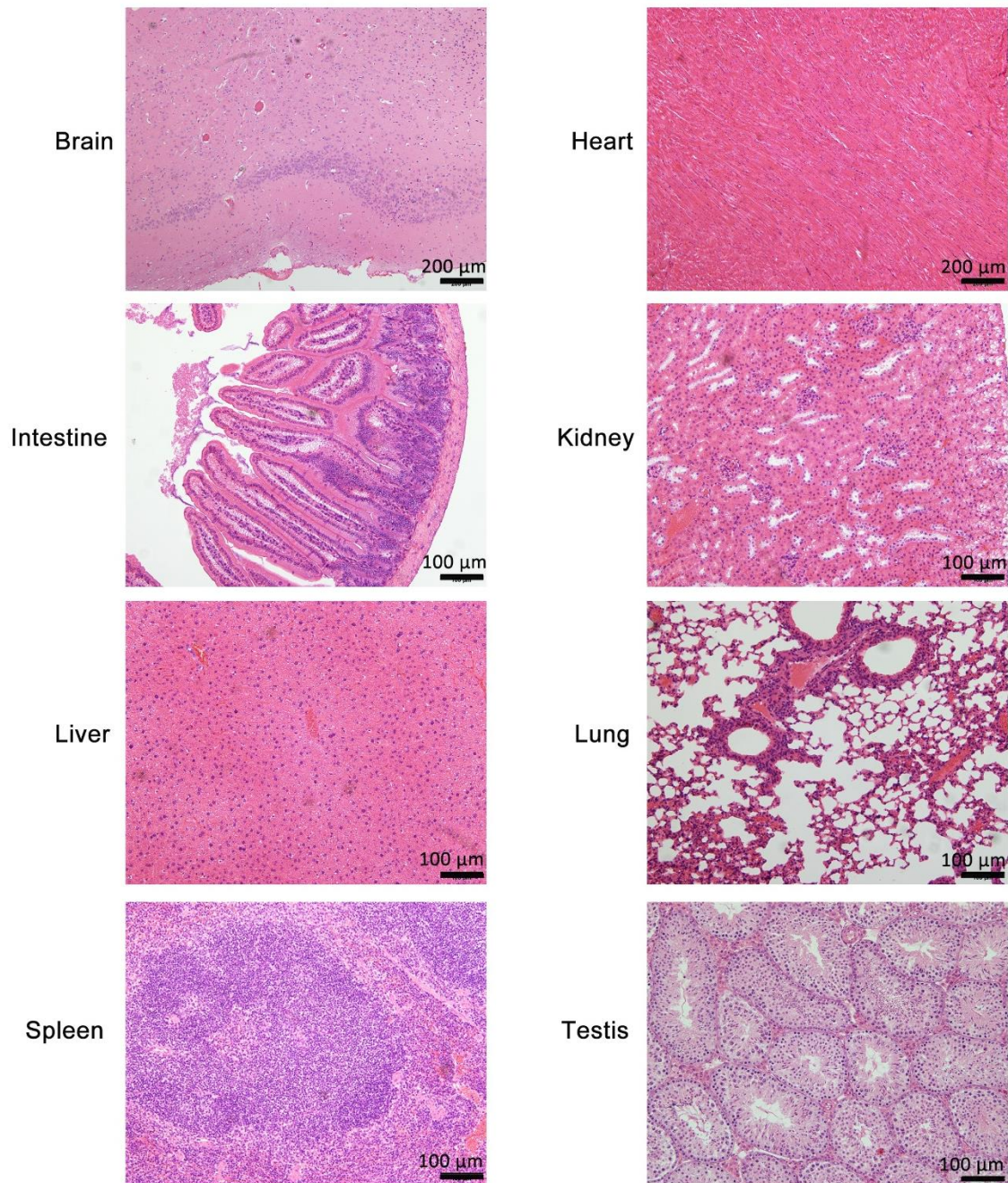


Figure S2. Histological characteristics of *Ppp3r2* KO male mice. H&E stained sections of brain, heart, intestine, kidney, liver, lung, spleen and testis indicate no significant changes in histological structures of organs in *Ppp3r2* KO mice (n = 3). Bars: 200 μm or 100 μm.

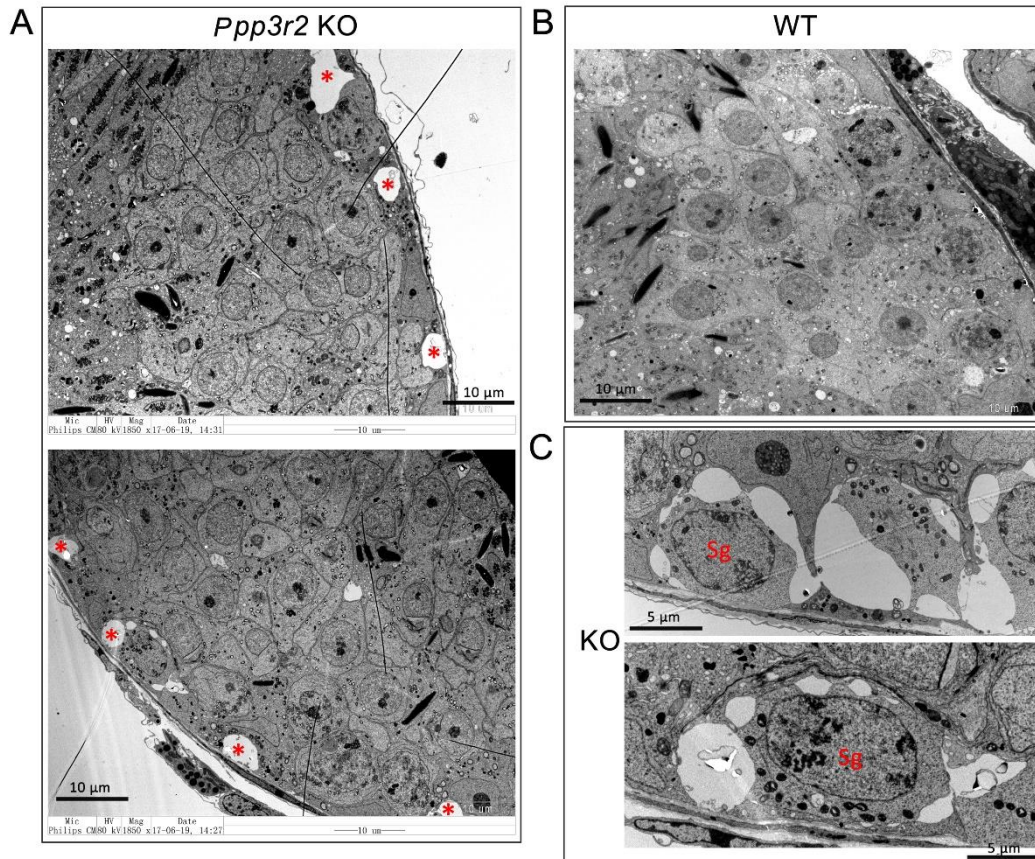


Figure S3. Testicular ultrastructure analysis. Transmission electron micrograph analysis of *Ppp3r2* KO (A) and WT testes (B) shows some asyntactic spermatogonia located along the basal region of the seminiferous epithelium in *Ppp3r2* KO testes (Asterisks). (C) Enlarged images show asyntactic spermatogonia (Sg) in *Ppp3r2* KO testes. Bar scales: 10 μm or 5 μm.

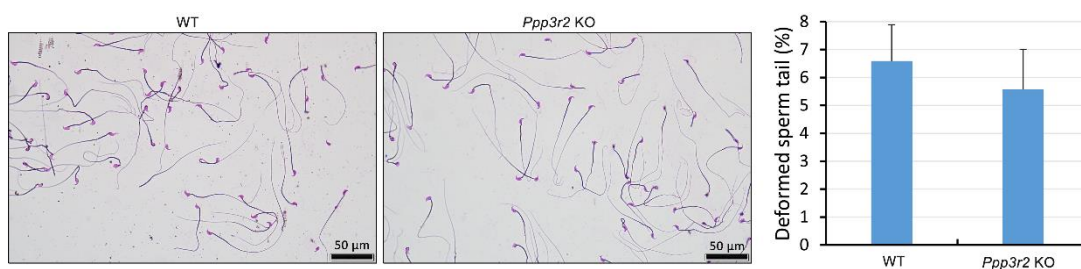


Figure S4. Morphological analysis of caput epididymis sperm in WT and *Ppp3r2* KO mice. In the sperm of *Ppp3r2* KO mice isolated from the caput epididymis, there is little sperm tail deformity ($5.57 \pm 1.43\%$) and its appearance is similar to that in WT mice ($6.59 \pm 1.30\%$) ($n=5$, $P > 0.05$).

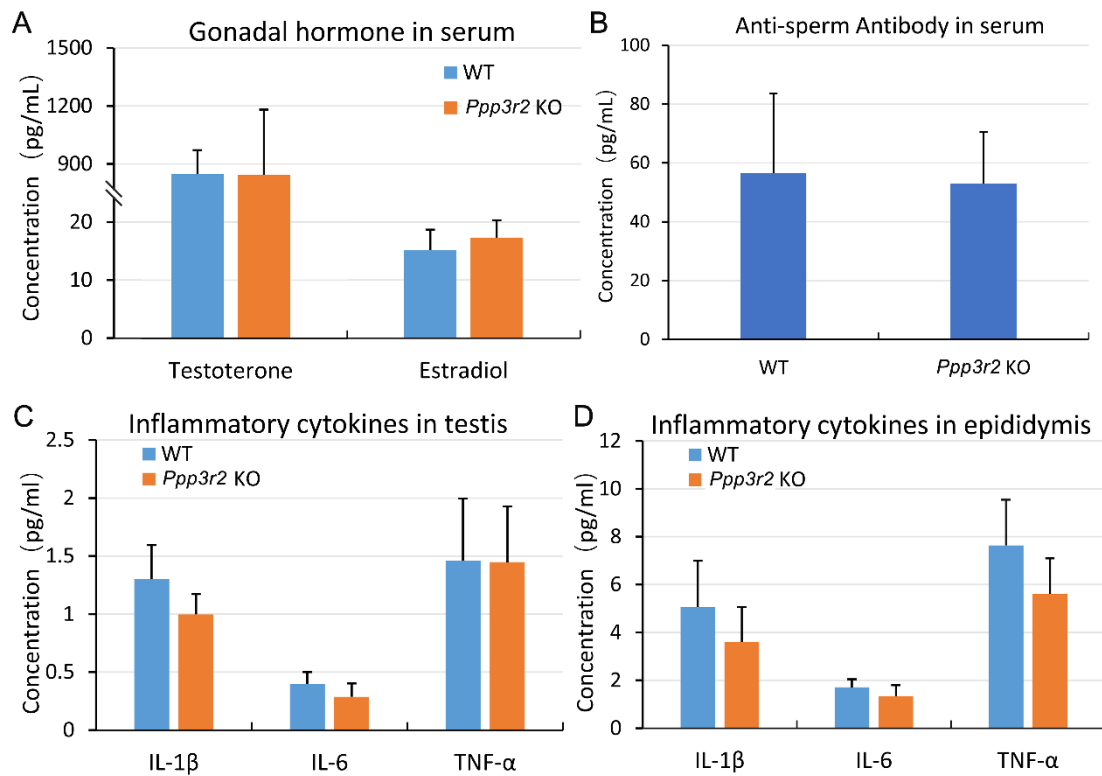


Figure S5. Gonadal hormones, anti-sperm antibody and inflammatory cytokines analyses. (A, B) Serum testosterone, estradiol and anti-sperm antibody detected by ELISA showed no significant differences between WT and *Ppp3r2* KO mice. (C, D) IL-1 β , IL-6 and TNF- α , in testis and epididymis, detected by ELISA showed no significant differences between WT and *Ppp3r2* KO mice. Error bars represent SD (n=6).

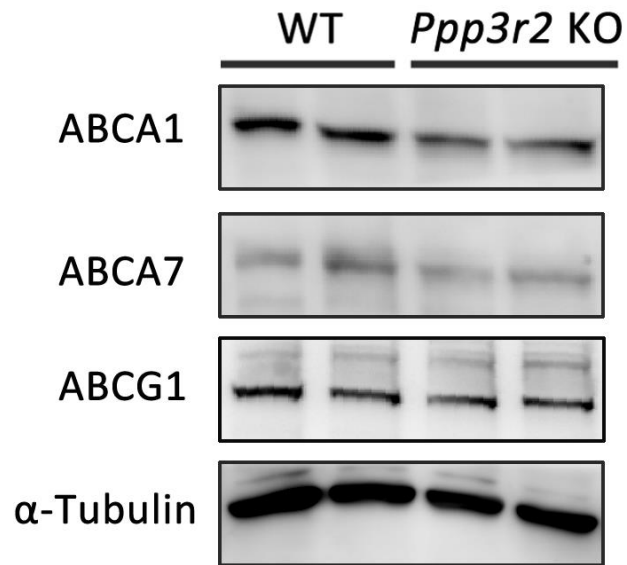


Figure S6. Western blot analysis of cholesterol transporters in sperm. ABCA1 and ABCG1 are significantly expressed in mature sperm, whereas the level of ABCA7 is relatively little. Moreover, ABCA1 expression in *Ppp3r2* KO sperm is greatly reduced compared with that in WT sperm, whereas ABCG1 and ABCA7 expression levels in WT sperm and *Ppp3r2* KO sperm are similar to one another. α -Tubulin expression validated loading control equivalence.