

Title: Multiple aspects of male germ cell development and interactions with Sertoli cells require inositol hexakisphosphate kinase-1

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S1

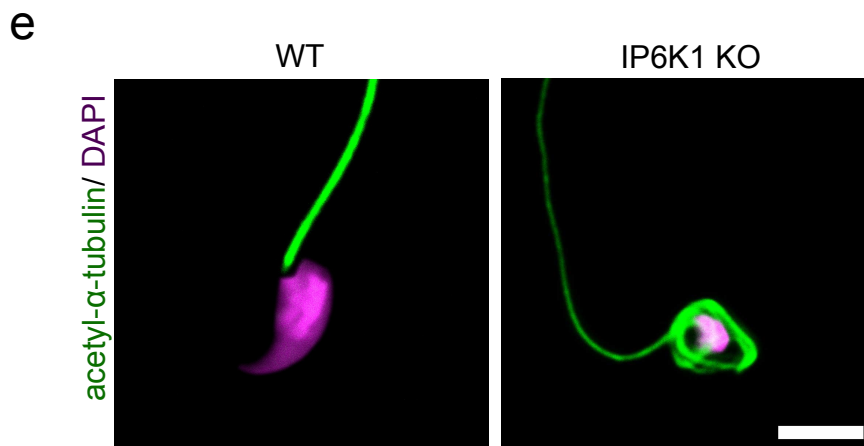
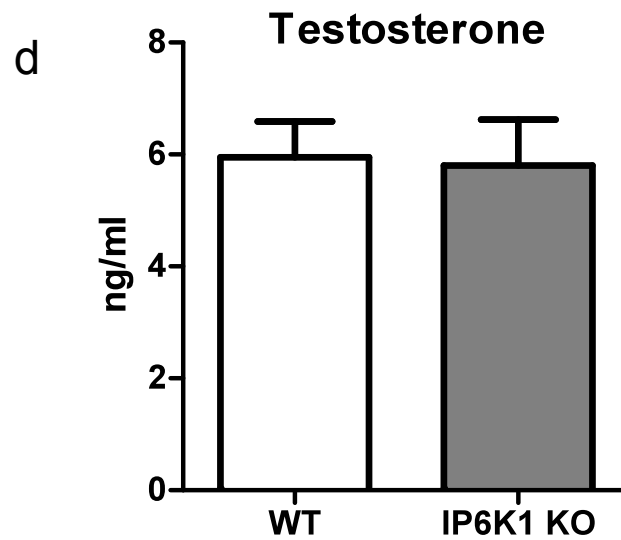
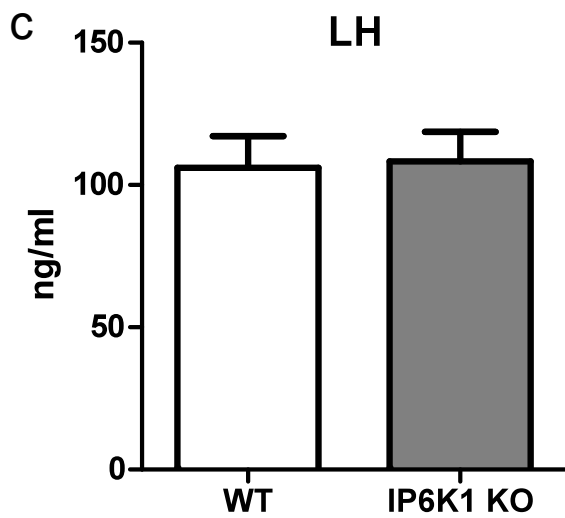
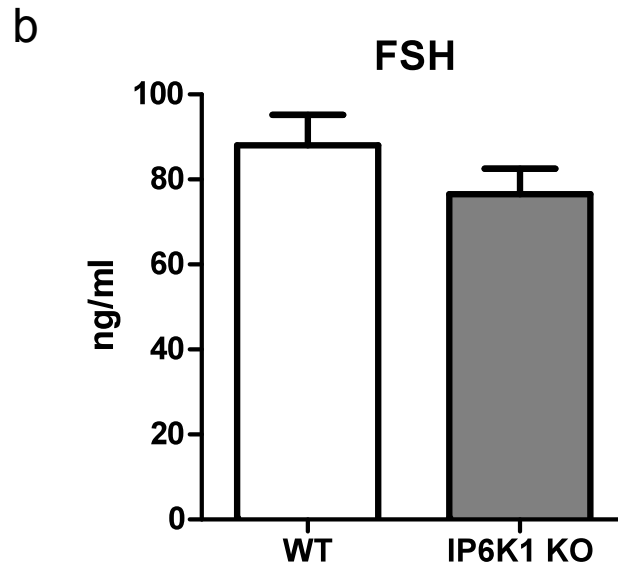
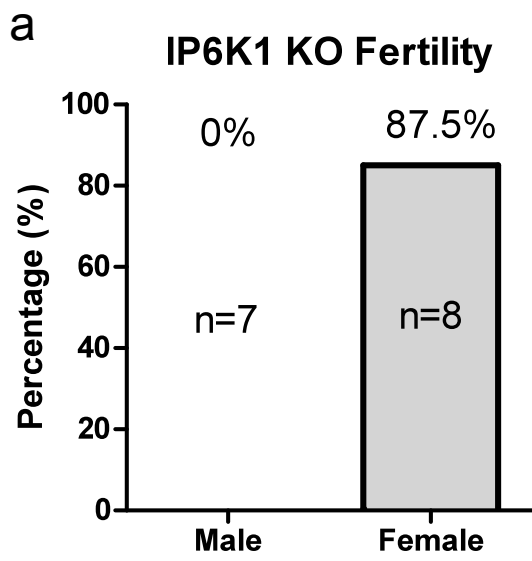


Fig. S1 *IP6K1* KO male mice were infertile. (a) *IP6K1* KO male mice (n=7) mated with wild type female mice, none of the females were pregnant; *IP6K1* KO female mice (n=8) mated with wild type male mice, Seven of 8 *IP6K1* KO females were pregnant and produced progeny. (b) Normal blood FSH levels in *IP6K1* KO male mice, determined by ELISA. (n=6, male 10 weeks old, unpaired student's t-test, mean \pm s.e.m.). (c) Normal blood LH levels in *IP6K1* KO male mice, determined by ELISA. (n=6, male 10 weeks old, unpaired student's t-test, mean \pm s.e.m.). (d) Normal blood testosterone levels in *IP6K1* KO male mice, determined by ELISA. (n=6, male 10 weeks old, unpaired student's t-test, mean \pm s.e.m.). (e) Sperm cells were isolated from epididymides. Immunostaining of acetyl- α -tubulin for sperm tails; and nucleus was stained by DAPI. Abnormal sperm heads coiled by sperm tails were seen in the *IP6K1* KOs. Scale bar 5 μ m.

S2

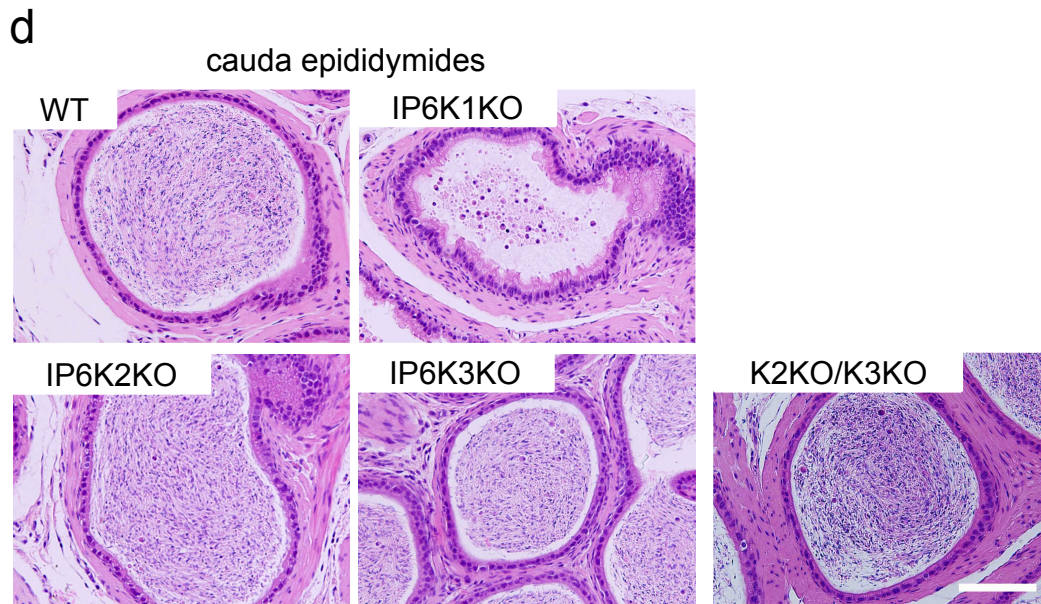
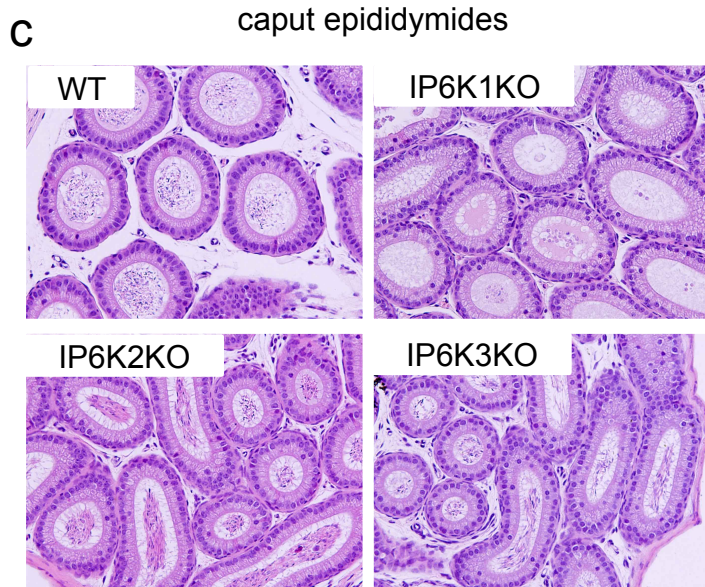
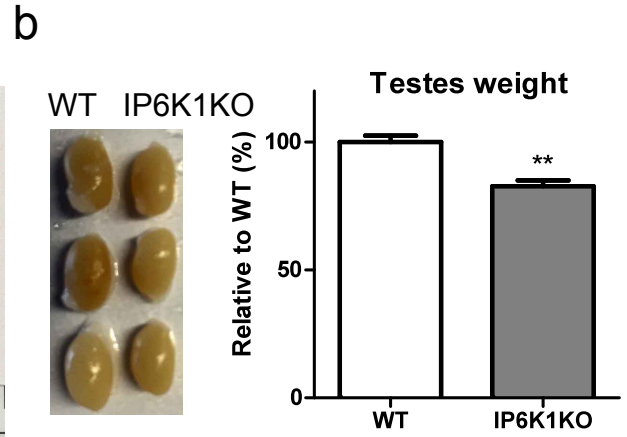
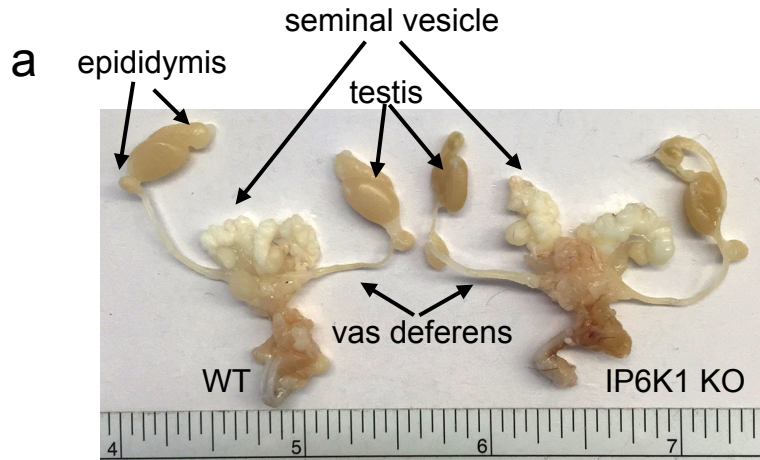


Fig. S2 *IP6K1* KO testes were smaller and devoid of sperm in epididymides. (a) Image shows the reproductive organs of *IP6K1* WT and KO male mice; The *IP6K1* KO male mice have intact reproductive organs but smaller testes. (b) Testes of *IP6K1* KO mice were smaller than those of wild type (n=6). **P<0.01 by unpaired student's t-test. (c) H&E staining of caput epididymides of wild type, *IP6K1* KO, *IP6K2* KO, *IP6K3* KO and *IP6K2/IP6K3* double knockout mice. *IP6K1* KO caput epididymides were devoid of sperms. Scale bar 100 μ m. (d) H&E staining of cauda epididymides of wild type, *IP6K1* KO, *IP6K2* KO, *IP6K3* KO and *IP6K2/IP6K3* double knockout mice. Round germ cells were seen in *IP6K1* KO cauda epididymides. Scale bar 100 μ m.

S3

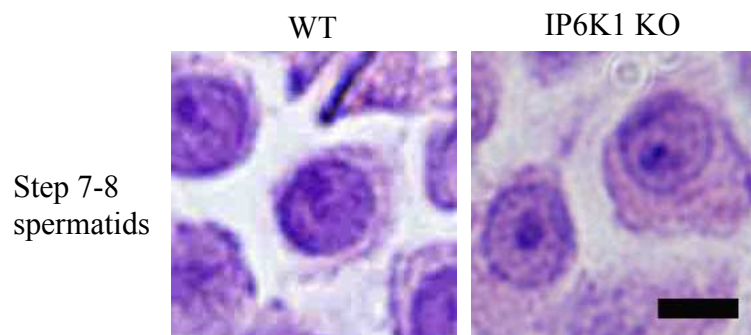
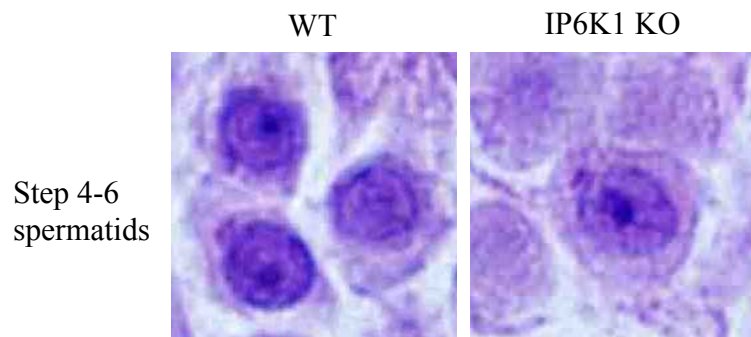
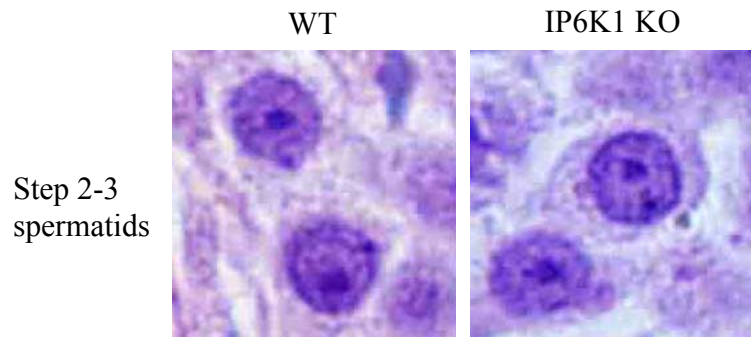
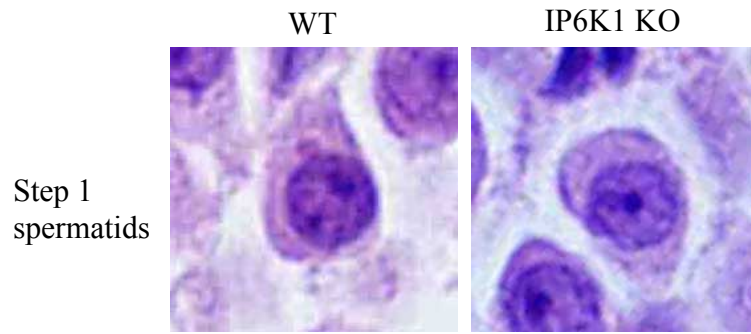


Fig. S3 H&E staining of WT and *IP6K1* KO testes revealed morphologically normal round spermatids (steps 1-8) in *IP6K1* KOs. Scale bar 5 μ m.

S4

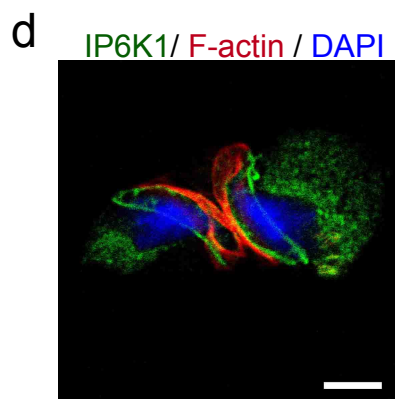
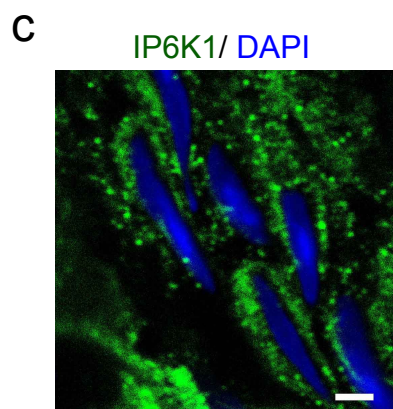
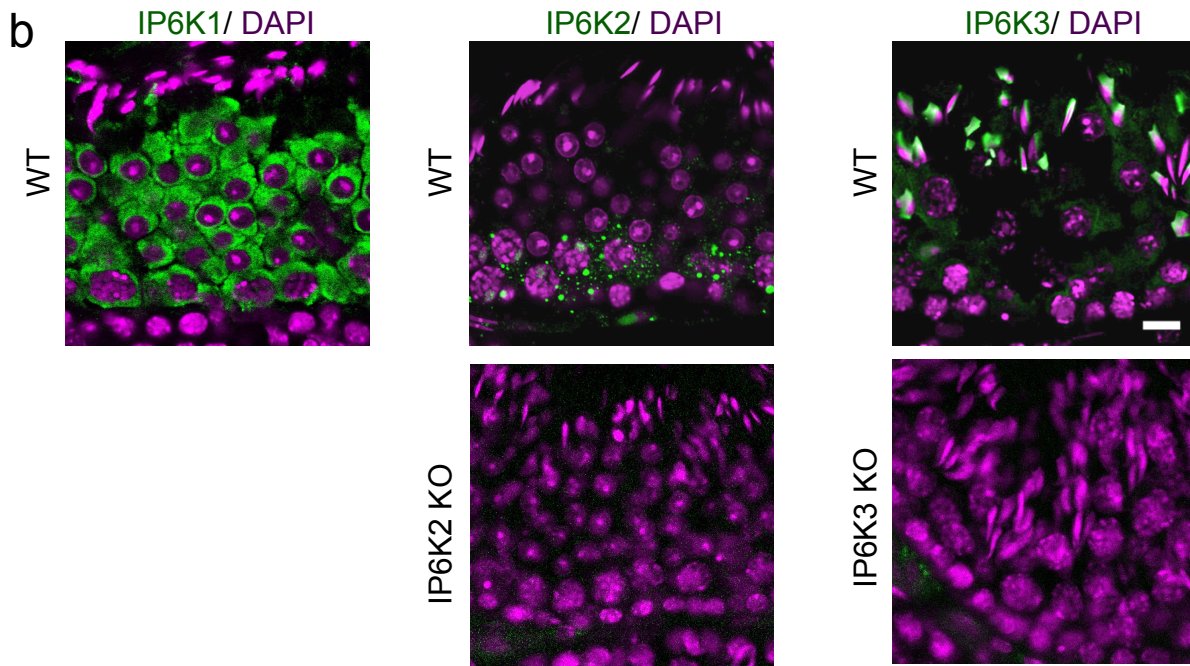
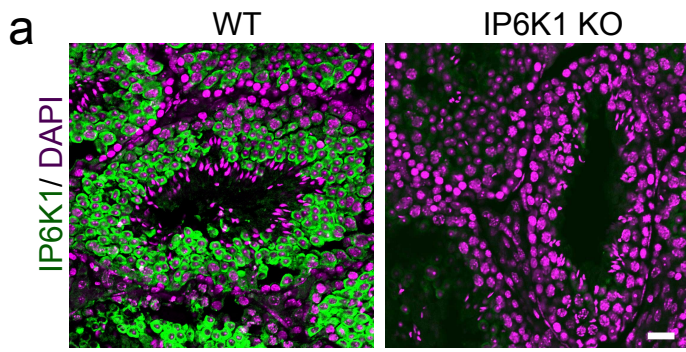
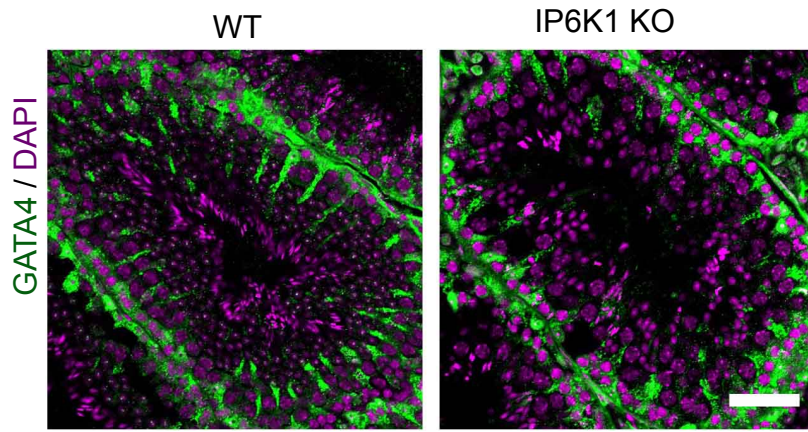


Fig. S4 IP6K1 was expressed in germ cells. (a) Immunostaining of IP6K1 in wild type and *IP6K1* KO testes, scale bar 20 μm . (b) Immunostaining of IP6K1 in wild type testes; Immunostaining of IP6K2 in wild type and *IP6K2* KO testes; Immunostaining of IP6K3 in wild type and *IP6K3* KO testes; scale bar 10 μm . (c) Immunostaining of IP6K1 in wild type testes, scale bar 2 μm . (d) Immunostaining of IP6K1 on loose spermatids released from the minced testes, scale bar 5 μm .

S5

a



b

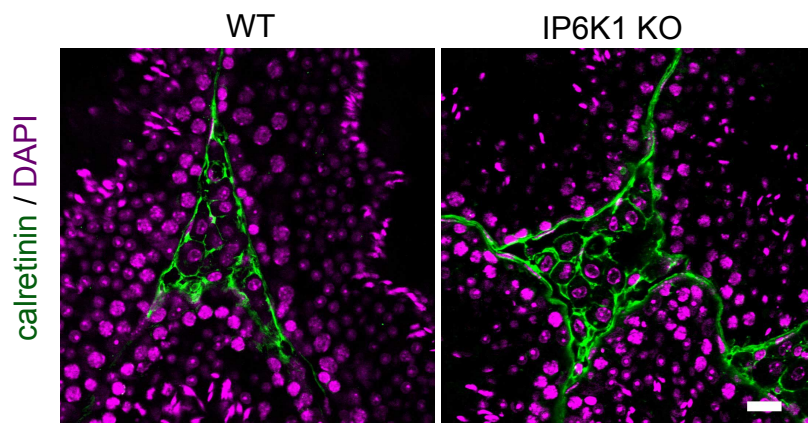


Fig. S5 Relative normal numbers of Sertoli cells and Leydig cells in *IP6K1* KOs. (a) Immunostaining of GATA4 for Sertoli cells in WT and *IP6K1* KO testes. DAPI stains nucleus. Scale bar 50 μm . (b) Immunostaining of calretinin for Leydig cells in WT and *IP6K1* KO testes. DAPI stains nucleus. Scale bar 20 μm .