Supplementary Information for

The loss-function of *KNL1* causes oligospermia and asthenospermia in mice by affecting the assembly and separation of the spindle through flow cytometry and immunofluorescence

This PDF file includes Supplementary
Figure S1–S3 and Table S1

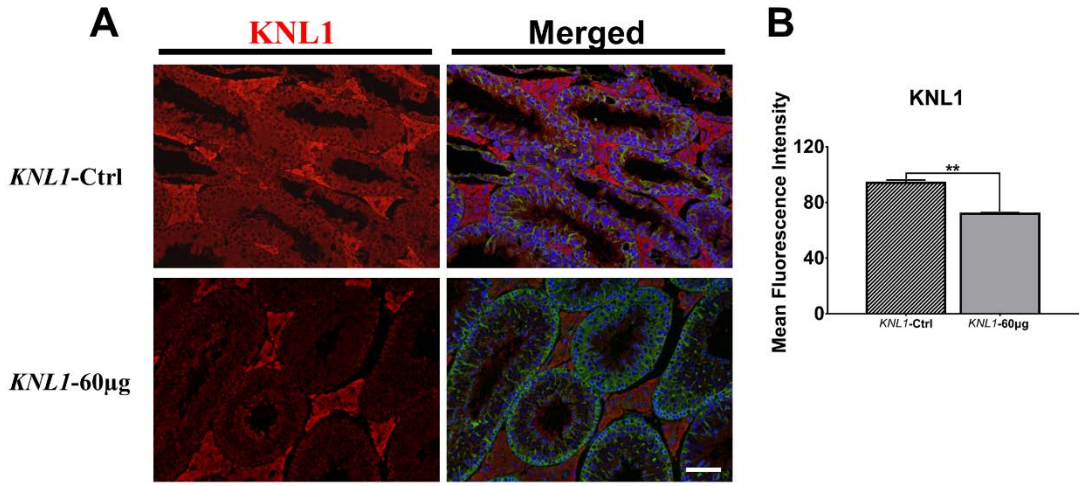

Figure S1. Immunofluorescence intensity of *KNL1* between *KNL1*-Ctrl and *KNL1*-60 μg. (**A**) *KNL1* fluorescent images of testis in *KNL1*-Ctrl and *KNL1*-60μg. Testis was immunostained with anti-*KNL1* (red). Merge was DAPI (blue), *KNL1* (red), and α-tubulin (green). (**B**) Data were presented as mean percentages (mean ± SEM) of at least three independent measurements. Asterisk denotes statistical difference level of significance (**, $p < 0.01$, ns > 0.05). Bar= 100 μm.

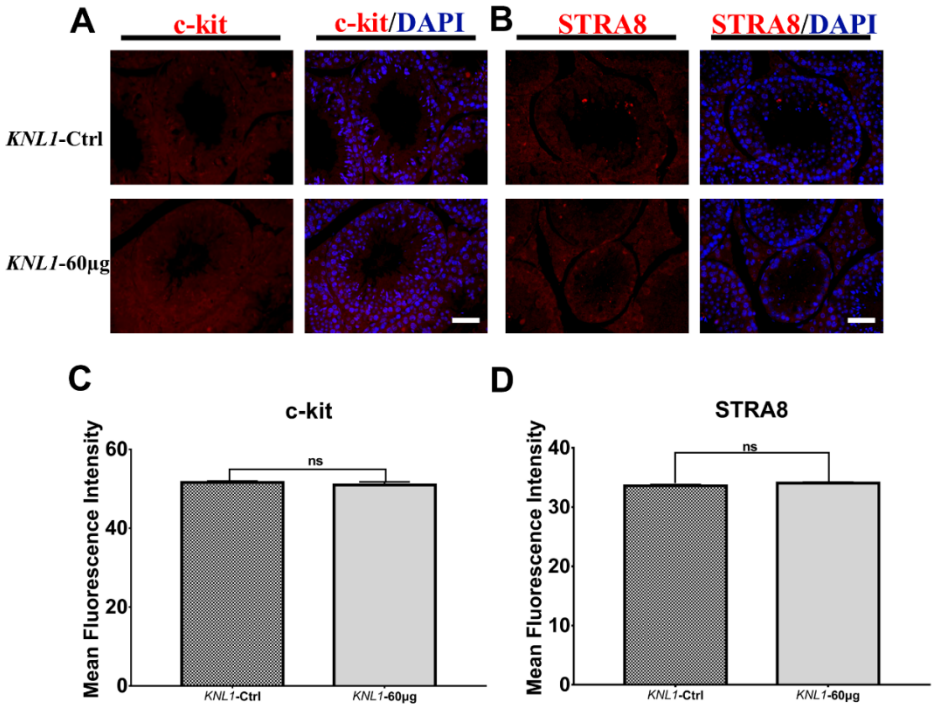

Figure S2. Immunofluorescence intensity of two markers between *KNL1*-Ctrl and *KNL1*-60 μg. (**A**)c-kit fluorescent images of testis in *KNL1*-Ctrl and *KNL1*-60 μg. Testis was immunostained with anti-c-kit(red). Merge was DAPI (blue) and c-kit (red). (**B**) STRA8 fluorescent images of testis in *KNL1*-Ctrl and *KNL1*-60 μg. Testis was immunostained with anti-STRA8 (red). Merge was DAPI (blue) and STRA8 (red). (**C,D**) Data were presented as mean percentages (mean ± SEM) of at least three independent measurements. ns denotes statistical difference at a $p > 0.05$ level of significance. Bar = 50 μm.

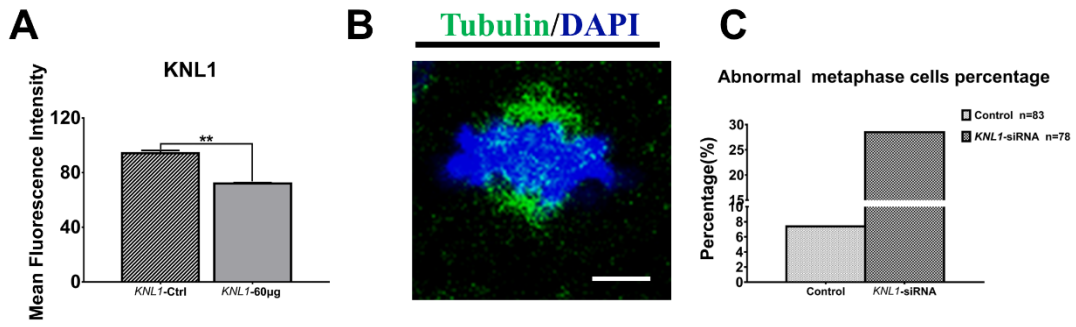

Figure S3. *KNL1* siRNA interference efficiency and immunofluorescence image of normal cell division. (**A**) *KNL1* siRNA interference efficiency in GC-2 cells. (**B**) The normal spindle and chromosome arrangement at metaphase. Bar= 5 μm. (**C**) The percentage of abnormal metaphase cells between Control-cells and *KNL1*-siRNA-cells. Asterisk denotes statistical difference level of significance (**, $p < 0.01$, ns > 0.05).

Table S1. Immunofluorescence antibody used in the experiment

Name	Brand
PLZF Rabbit Polyclonal Antibody	Beyotime
Histone *H2AX* Rabbit Polyclonal Antibody	Beyotime
STRA8 Monoclonal antibody	Proteintech
SYCP3 Polyclonal antibody	Proteintech
GFRα1 Polyclonal Antibody	Elabscience
Purified Anti-Mouse c-kit Antibody	Elabscience
Alpha Tubulin Polyclonal antibody CASC5 (E4A5L) Rabbit mAb	Proteintech CST
Goat Anti-Rabbit IgG H&L (Alexa Fluor 594)	GeneCopoeia
CoraLite594 – conjugated Goat Anti-Mouse IgG(H+L)	Proteintech
CoraLite488 – conjugated Affinipure Goat Anti-Rabbit IgG(H+L)	Proteintech