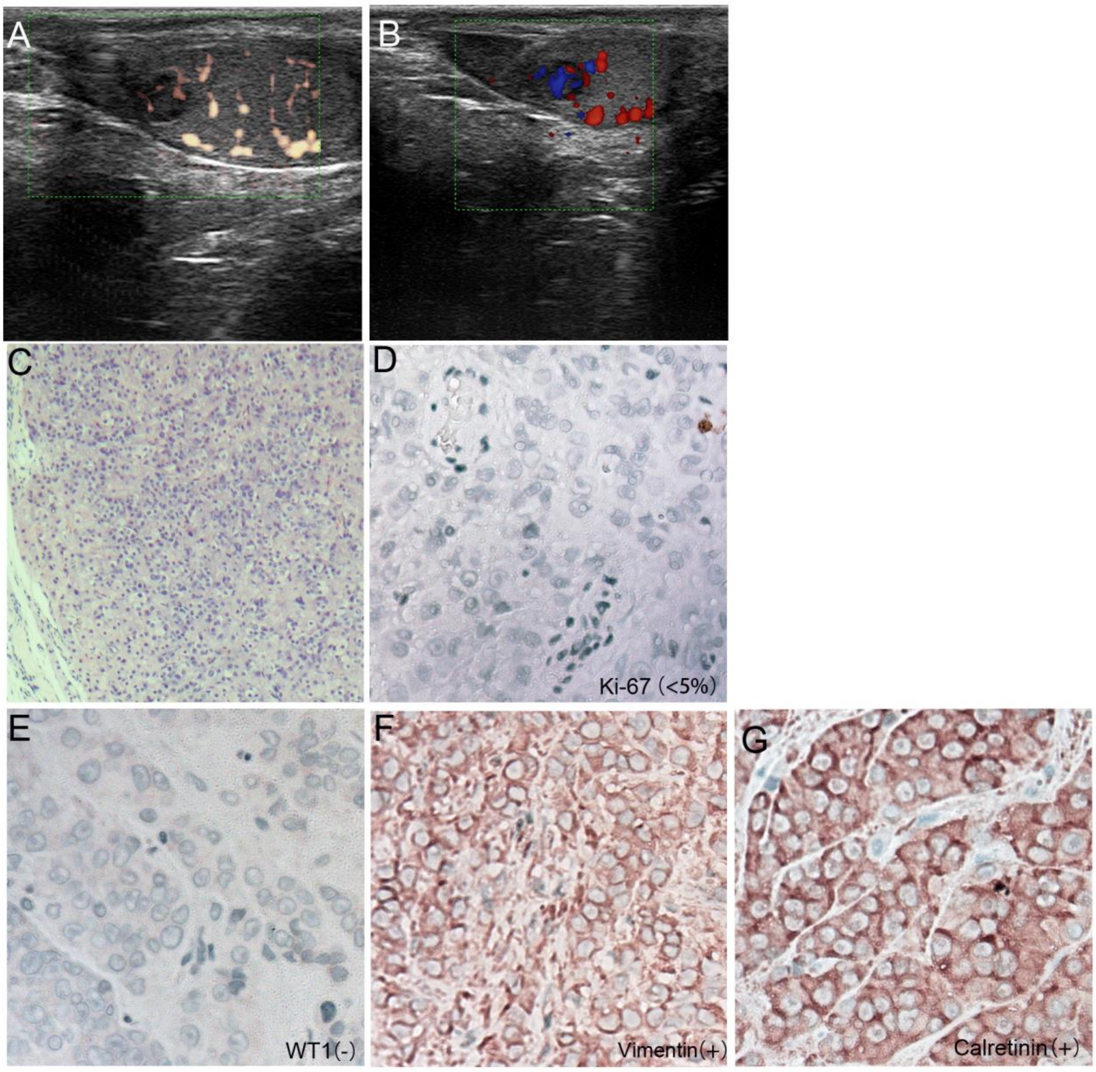
Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: X-Linked RBBP7 Mutation Causes Maturation Arrest and Testicular Tumors

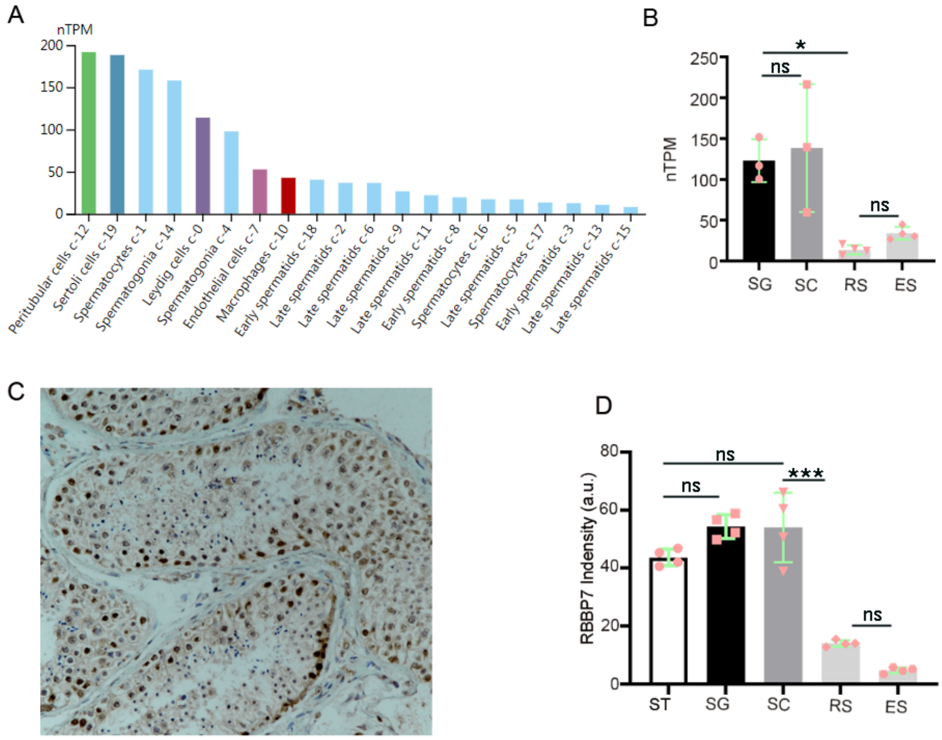
Supplementary Figures:

Supplementary Figure S1



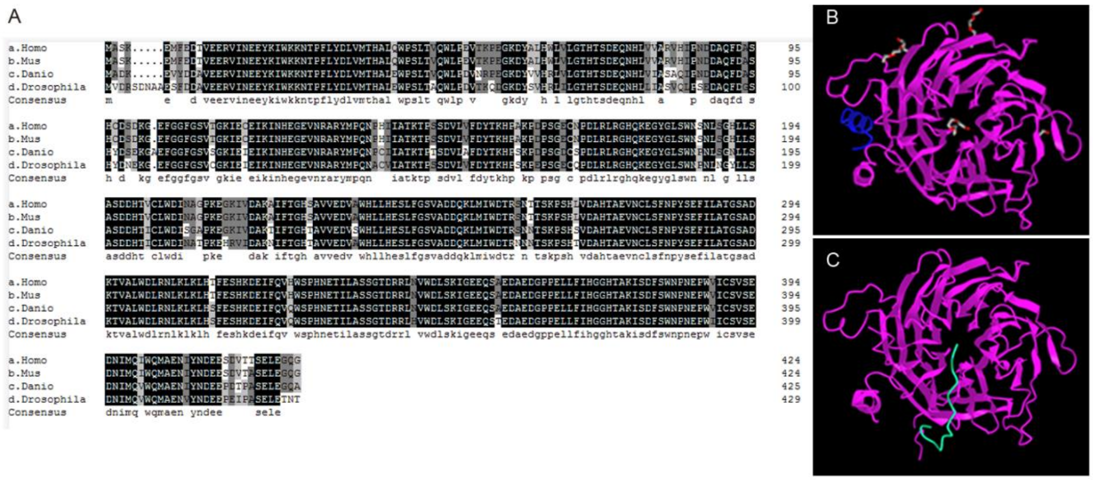
(A, B) Ultrasonography picture shows the boundary of the lesion was clear, and blood flow signal could be detected in the lesion. (C) H&E staining analysis of cross-sections of Leydig cell tumor from II:1. (D-G) Immunohistochemical staining of the Leydig cell tumor tissues with Ki-67, WT1, Vimentin, Calretinin.

Supplementary Figure S2



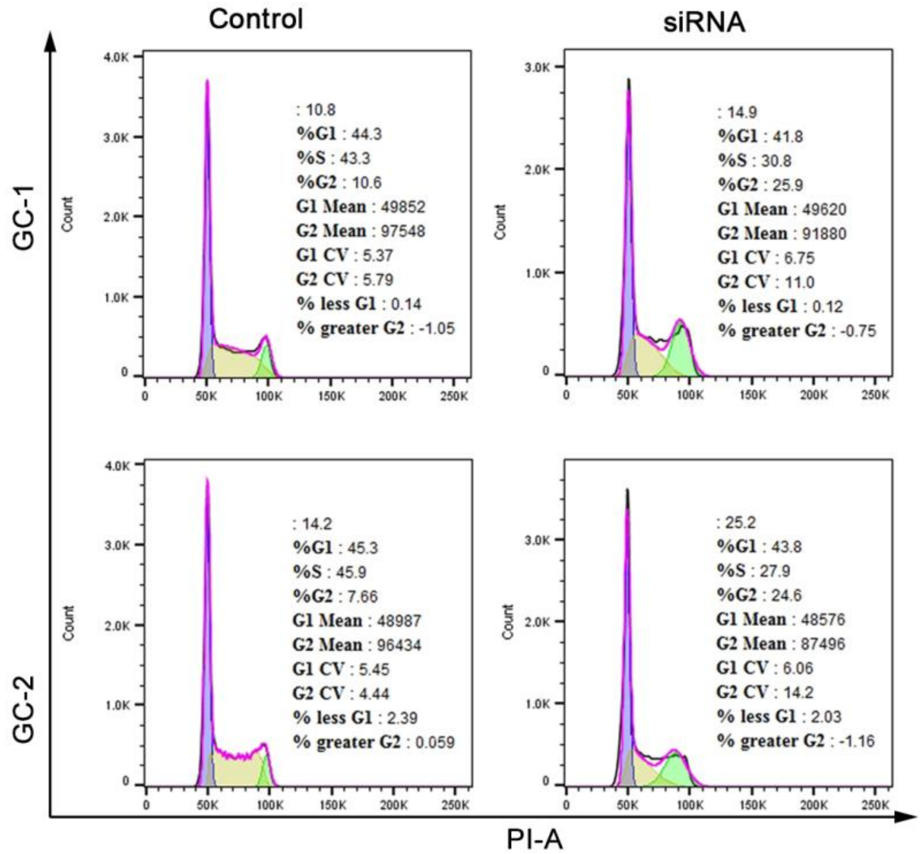
(A) Single cell analysis of human *RBBP7* in testes (data from: https://www.proteinatlas.org). (B) Graph showing the relative expression patterns of *RBBP7* during spermatogenesis stages. SG, spermatogonia; SC, spermatocyte; RS, round spermatid, ES, elongated spermatid. KIT, SOX4, and UTF1 were specifically expressed in spermatogonia cells and used as spermatogonia cells markers. SPO11, SYCP3, TOP2A were specifically expressed in spermatocyte cells. ACRV1, HEMGN, and SPACA1 were specifically expressed in round spermatids. PRM2, SPATA3, and TNP1 were specifically expressed in elongated spermatids (data from: https://www.proteinatlas.org/). (C) RBBP7 immunohistochemistry analysis of cross-section of seminiferous tubules using testicular biopsy samples from a normal control individual. (D) Quantification analyses of RBBP7 expression intensity of different cell types. ST, sertoli cell. Data are shown as mean ± SEM. *$P < 0.05$;***$P < 0.001$ by 1-way ANOVA followed by Bonferroni's post hoc test.

Supplementary Figure S3



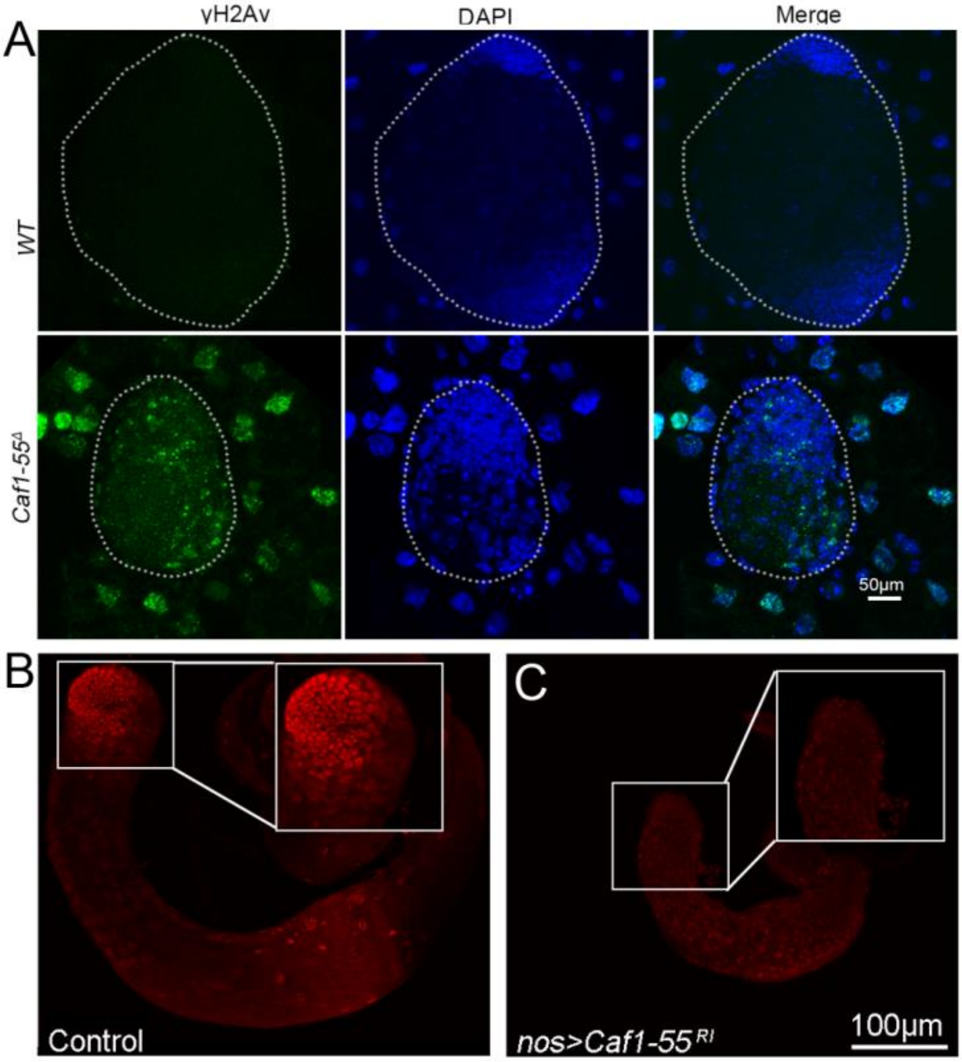
(A) Multiple sequence alignment of RBBP7 from different species, constructed using ClustalX2. Sequences from top to bottom: a. Homo, *Homo sapiens*, NP_001185648.1; b. Mus, *Mus musculus*, NP_033057.3 ; c. Danio, *Danio rerio*, NP_997775.1; d. Drosophila, *Drosophila melanogaster*, NP_524354.1; Black background indicates identical amino acid residues and similar residues are in a gray background. (B) The top view of RBBP7/Histone H4 Complex structure (PDB code: 3CFV; blue, H4; purple, RBBP7). (C) The top view of Caf1-55/Histone H3 Complex structure (PDB code: 2YBA; green, H3; purple, Caf1-55).

Supplementary Figure S4



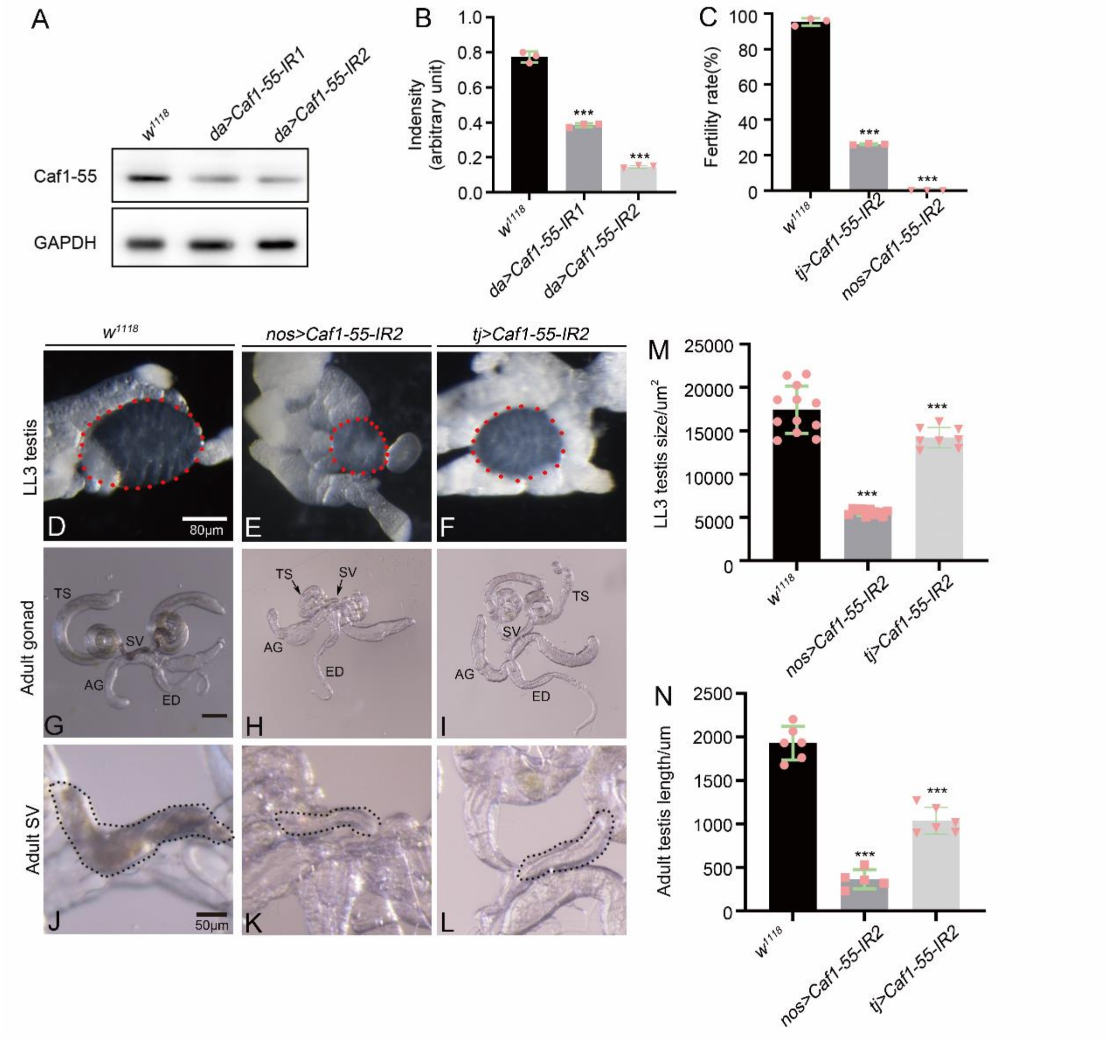
Flow cytometry analysis for cell cycle distribution of NC and siRNA-treated GC-1 and GC-2 cells.

Supplementary Figure S5



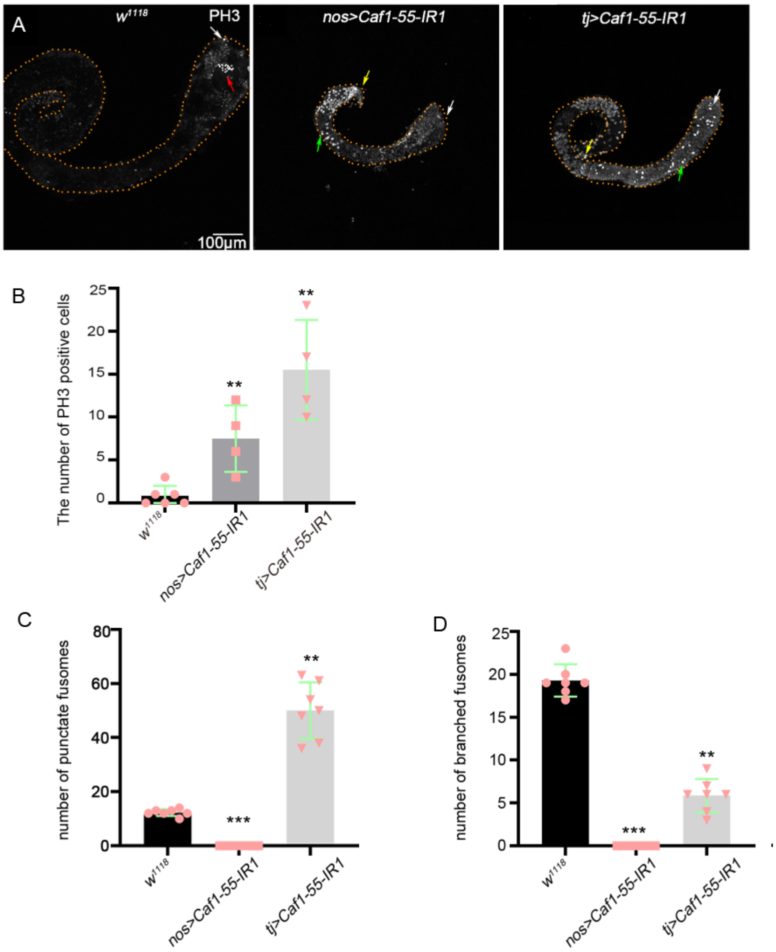
(**A**) Cafl-55 was barely detected in *Caf1-55* knockdown males. (B) Testes of the indicated genotypes. Images showing the apical testes (white dotted lines) of wild type (upper), *Caf1-55* mutant animals (lower). (C) Cafl-55 was highly enriched in the testis of adult males.

Supplementary Figure S6



(A) Western blot analysis of Caf1-55 in protein extracts from wild-type and *Nos-Gal4* driven 2 different *Caf1-55* RNAi lines to validate knockdown efficiency. (B) Quantification analyses of Caf1-55 expression levels in figure A. (C) Graph showing male fertility rate in different genotypes. (D, G, J) wild-type, in which the control images in supplemental figures 6D and 6G were the same controls shown in figure 5K (previous Figure 5H), as all the experiments were performed at the same time window. (E, H, K) *nos-Gal4* driven *Caf1-55* RNAi2 animals, (F, I, J) *tj-Gal4* driven *Caf1-55* RNAi2 animals. Graphs showing the testes of control and *Caf1-55* RNAi2 animals driven by *tj-Gal4* (F) and *nos-Gal4* (E) at the late third instar larval stage. Graphs showing the male reproductive systems of control and *Caf1-55* RNAi2 animals as driven by *nos-Gal4* (H) and *tj-Gal4* (I) from 5-day-old adults; TS, testis; SV, seminal vesicle; AG, accessory gland; ED, ejaculatory duct. (J, K, L) Magnified images of SV from different genotypes, note that the seminal vesicles of *Caf1-55* RNAi2 animals are almost empty. Quantification of the testes size from different genotypes animals at late third instar larval stage (M) and 5-day-old adult stage (N). Data are shown as mean ± SEM. ***P < 0.001 by the unpaired, two-tailed Student's t-test.

Supplementary Figure S7



(A) Immunostaining of wild-type, *nos-Gal4* driven *Caf1-55* knockdown animals, and *tj-Gal4* driven *Caf1-55* knockdown animal's testes for PH3. White arrows indicate small numbers of mitotic cells at the tip of the testes; A red arrow indicates a single PH3-positive meiotic cyst; a green arrow indicates small numbers of mitotic cells at the middle of the testes; and a yellow arrow indicates small numbers of mitotic cells at the tip of the testes. (B) Number of PH3-positive cells in the testes of different animal genotypes. (C, D) Number of punctate fusomes (C, n=7 for each genotype) and branched fusomes (D, n=7 for each genotype) in the testis of different animal genotypes. Data are shown as mean ± SEM. ***$P < 0.001$ by two-tailed unpaired Student's t-test.

Supplementary Tables

Table S1. The antibodies used for immunostaining

Antibodies	From	Dilution rate
rabbit anti-RBBP7	Abbexa，141930	1:200
guinea pig anti-Vasa	Gifted by Yu Cai, Temasek Life Sciences Laboratory, Singapore	1:15000
rat anti-E-cadherin	DSHB, DCAD2	1:50
rabbit anti-γH2Av	Rockland，33026	1:1000
rabbit anti-cleaved caspase3	Cell Signaling Technology, 9661	1:100
mouse anti-1B1	DSHB, 1B1	1:75
mouse anti-eya	DSHB,10H6	1:20
rabbit anti-PH3	Millipore,06-570	1:500
mouse anti-RBBP7	Sigbio, XG10829	1:100
rabbit anti-histone H4	Affinity, DF6950	1:200
rabbit anti-γH2AX	Abcam, ab81299	1:200
rabbit anti-BRCA1	Proteintech, 22362-1-AP	1:500
mouse Anti-WT1	Elabscience, PA6533	1:200
FITC Goat anti-Mouse IgG	Jackson Immuno, 115-095-166	1:250
Cy3 Goat anti- Mouse IgG	Jackson Immuno, 115-165-166	1:500
Cy5 Goat anti-Mouse IgG	Jackson Immuno, 115-175-166	1:250
FITC Goat anti-Rabbit IgG	Jackson Immuno, 111-085-003	1:250
Cy3 Goat anti- Rabbit IgG	Jackson Immuno, 111-165-003	1:500
Cy5 Goat anti- Rabbit IgG	Jackson Immuno, 111-175-144	1:250
FITC Goat anti- Rat IgG	Jackson Immuno, 112-095-167	1:250
Cy3 Goat anti- Rat IgG	Jackson Immuno, 112-165-167	1:500
488 Goat anti- guinea pig IgG	Jackson Immuno, 106-545-003	1:250
Cy3 Goat anti-guinea pig IgG	Jackson Immuno, 106-165-003	1:500

Table S2. The antibodies used for Western Blot

Antibodies	From	Dilution rate
rabbit anti-RBBP7	Abbexa，141930	1:500
mouse Anti-GAPDH	Proteintech, 60004-1-Ig	1:5000
mouse Anti-GAPDH	Good Here, AB-M-M001	1:2000
Goat anti-mouse HRP	Beyotime, A0216	1:5000
Goat anti-rabbit HRP	Beyotime, A0208	1:5000

Table S3. Hormone changes in the proband and his brother

Patients	Proband	Proband	Proband	Proband's brother	Proband's brother
Examination	1st test	2nd test	3rd test	1st test	2nd test
Examination time	before surgery (Aug 2019)	3-month post-surgery (Nov 2019)	20-month post-surgery (Jul 2021)	Jul 2017	Oct 2019
FSH(IU/L)	45.5	50.5	46.8	18.8	19.3
LH(IU/L)	27.3	26.1	29.4	7.0	8.6
T(nmol/L)	6.5	3.9	3.5	1.7	0.7
E_2(pmol/L)	70.0	<18.5	<18.5	-	88.4
PRL (ng/ml)	6.1	6.6	9.9	21.3	31.7