Supplemental methods and materials

Antibody preparation

Affinity-purified BNC1 polyclonal rabbit antibodies (HuaAn Biotechnology Co. Ltd., Hangzhou, China) were raised against a synthetic polypeptide (VKPERNSLGTKKGC) corresponding to the unique amino acid sequence of BNC1(amino acids 343-355), C-terminal cysteine was added to the peptide to perform consequent sulfolink coupling for affinity purification.

Hormone Assays

Blood was collected by cardiac puncture. Serum was separated by centrifugation (10 min, 7500 rpm) in microtainer brand tubes (Becton Dickinson, New York, USA), frozen and stored at −80°C. Radioimmunoassays for FSH, LH, and T were performed by using the commercially available RIA kit from Institute of Isotopes Co., Ltd.

RNA Isolation and RT–qPCR

Total RNA from eight week-old mouse testes was isolated by trizol(Takara,JP) after slight sonication homogenization. Two microgram (μg) of total RNA was reverse transcribed via a cDNA reverse-transcription kit (Takara, 4368814, JP). SYBR green reactions were performed according to the manufacturer's instructions of SYBR Green PCR Master Mix (Applied Biosystems, 4309155, USA) by an ABI 7300 real-time PCR machine (Applied Biosystems, USA) for conducting quantitative RT-PCR (RT-qPCR) analysis. Relative expression of mRNA was determined after normalization to the actin gene. All PCR primer sequences are list in Table S1. Student's t test was used to evaluate statistical significance.

Recombinant Plasmids Construction and Transfection

HEK293T cells were cultured in EMEM (Invitrogen, Massachusetts, USA) supplemented with 10% calf serum (Invitrogen, Massachusetts, USA) and incubated in 5% CO_2 at 37 °C. The pECFP-N1 vector was used for the construction of the pECFP-BNC1, pECFP-BNC1 truncation mutant plasmid. The pcDNA3.1-RFP-Flag vector was used for the construction of the RFP-TAF7L plasmid. Cloned BNC1 wild and mutant fragments and TAF7L sequence were inserted into the multiple cloning sites between HindIII and EcoRI. For transient transfection, HEK293T cells were transfected with these recombinant expression plasmids using Attractene transfection reagent (QIAGEN, 301005, Germany) according to the manufacturer's instructions. After transfection for 24 hours in a 5% CO_2 incubator at 37 °C, cells were harvested and fixed with 4% formaldehyde for 1 hour at room temperature.

Immunofluorescence and Confocal Microscopy

Mouse testis specimens were incubated with rabbit polyclonal antibody BNC1 (Huaan, Hangzhou, China) and mouse monoclonal antibody TAF7L (Abnova, H00054457-M04, Taiwan, China). After blocking non-specific binding sites in blocking buffer (0.1% Tween and 2% bovine serum albumin (BSA) in Phosphate-buffered saline (PBS)), BNC1 antibody and TAF7L antibody diluted at 1:200 were applied, and then the sections were incubated at 4 °C overnight. For negative control, antibody dilution buffer was applied instead of primary antibody. After being washed with PBS (0.01 mol/l pH 7.4), AlexaRed- labeled, goat-anti-mouse secondary antibody (Invitrogen, Alexa Fluor 488, CA, USA) and goat-anti-rabbit secondary antibody (Invitrogen, Alexa Fluor 594, CA, USA) were applied to tissue sections and incubated at room temperature for two 2 hours. Nuclei were stained using 1 μg/ml DAPI (Sigma, D9542, San Francisco, USA). Negative controls

included parallel sections treated without primary antibody. Slides were analyzed using an Olympus BX61W1 fluorescence microscope (Olympus Corporation, Tokyo, Japan).

HEK293T cells transfected with CFP–BNC1(WT) or CFP–BNC1(MT) and FLAG–TAF7L and CRL-2053 cells transfected with CFP–BNC1(WT) or CFP–BNC1(MT) were washed in PBS, fixed with 4% paraformaldehyde in PBS at room temperature for 30 minutes. After washing with PBS, nuclei were stained using 1 μg/ml DAPI (Sigma, D9542, San Francisco, USA,). Then cells were observed using an Olympus IX81 fluorescence microscope (Olympus Corporation, Tokyo, Japan) to determine the location of the protein.

***Bnc1* Knockdown with Small Interfering RNA (siRNA) of Germ Cells**

RNA knockdown of *Bnc1* in C18-4, CRL-2053 and CRL-2196 cells was achieved through transient transfection of double-stranded RNA (dsRNA) specifically targeting *Bnc1* (Sense primer 1, GGGUGUAACAUGGUGUUCAdTdT; anti-sense primer 1, UGAACACCAUGUUACACCCdTdT; sense primer 2, GCAGUCUGGUUUACCCAAdTdT; anti-sense primer 2, AUUGGGUAAACCAGACUGCdTdT. They were synthesized by Biomics Biotechnologies Co. Ltd, China). Control treatments were performed using a scrambled RNA (sense primer, UUCUCCGAACGUGUCACGUdTdT; anti-sense primer, ACGUGACACGUUCGGAGAAdTdT). *Bnc1* siRNA was dissolved in RNase-free water. C18-4 CRL-2053 and CRL-2196 (5×10^4cells) were transferred into 6-well plates, then transfected when cells reached 50% confluence. Cells were transfected with si-*Bnc1* or scramble RNA in the presence of Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc. Massachusetts, USA) according to the manufacturer's protocol. The cells that were successfully transfected exhibited GFP. The expression of several spermatogenic associative genes was detected in those cells using RT-qPCR.

Supplemental figure legends and figures

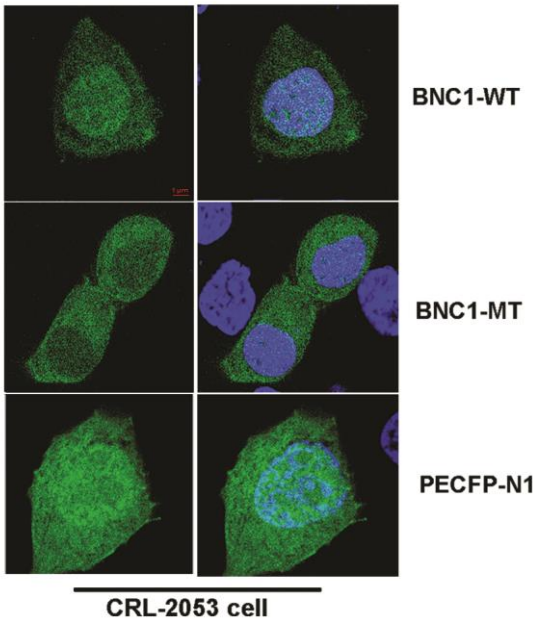

Figure S1| Nucleus localization disorder of mutated BNC1

Fluorescence microscope scanning showed the localization of wild-type or mutated BNC1 in CRL-2053 cell transfected with WT BNC1-CFP fusion plasmids or MT BNC1-CFP fusion plasmids. WT BNC1-CFP fusion protein mainly located in nuclei and MT BNC1-CFP fusion protein mainly located in plasma. Scale bar= 1μm.

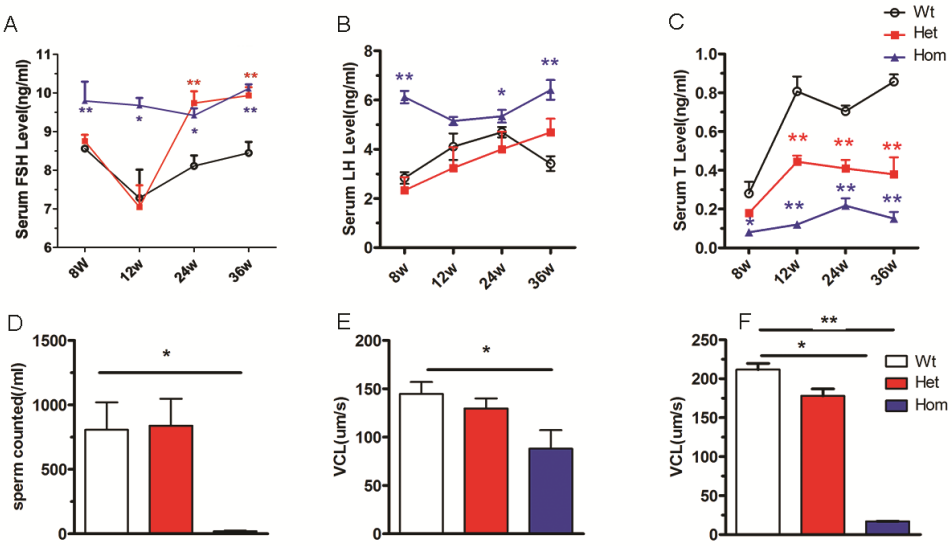

Figure S2| Hormone levels and sperm motility in mice was affected by mutated *Bnc1*

Serum levels of FSH (A), LH (B), and T (C) of Wt, Het and Hom male mice at different ages (wks); sperm counts (D) and curvilinear velocity (VCL)(E) in sperm from Wt, Het and Hom mice at the age of 8 weeks. VCL of sperm from Wt, Het and Hom mice at the age of 24 weeks (F).

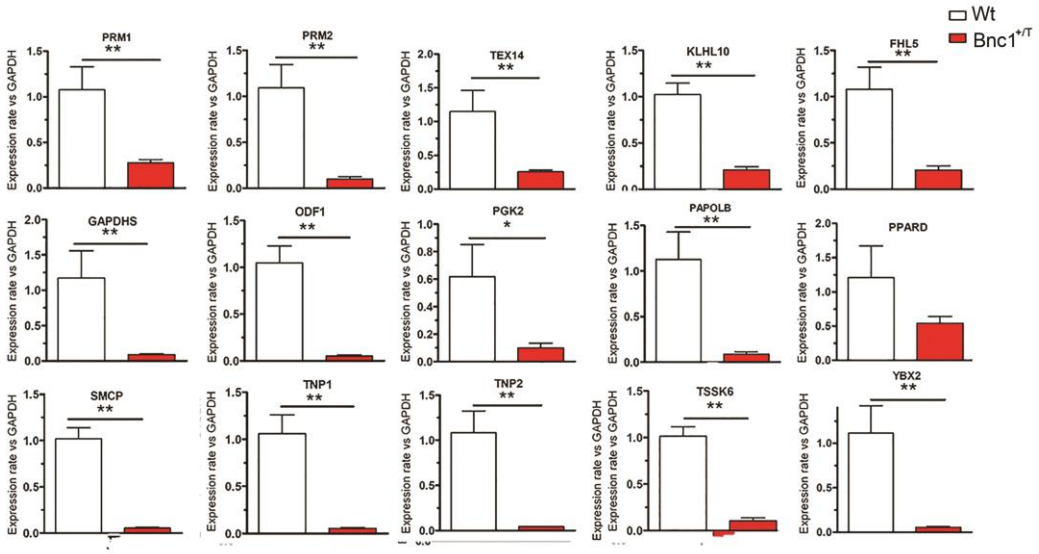

Figure S3| RT-qPCR analysis verified the results of microarray analysis.

The value of 1 was arbitrarily assigned to the expression levels of genes in Wt testis; their corresponding expression levels in *Bnc1*$^{+/T}$ testis were relative to this value. Values are expressed as mean ± SEM of three independent experiments (n=5). Asterisks denote statistically significant differences of *Bnc1*$^{+/T}$ compared with Wt. **P* < 0.05; ***P* < 0.01 (Student's t-test).

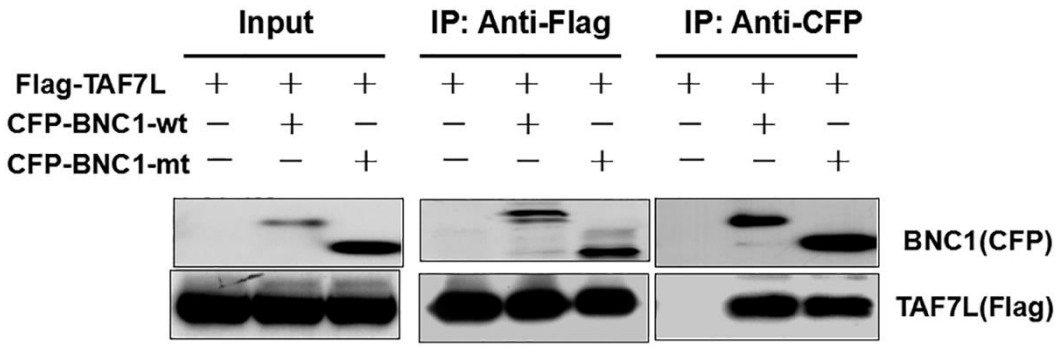

Figure S4| Co-precipitation of BNC1 and TAF7L

FLAG–TAF7L and CFP–BNC1 WT or CFP–BNC1 MT plasmids were over-expressed in HEK293T cells and IPs were performed in cell lysis using both FLAG and CFP antibodies, followed by western blotting analysis with FLAG and CFP antibodies. The results showed that TAF7L can be efficiently pulled down by both BNC1 WT and BNC1 MT proteins and vice versa.

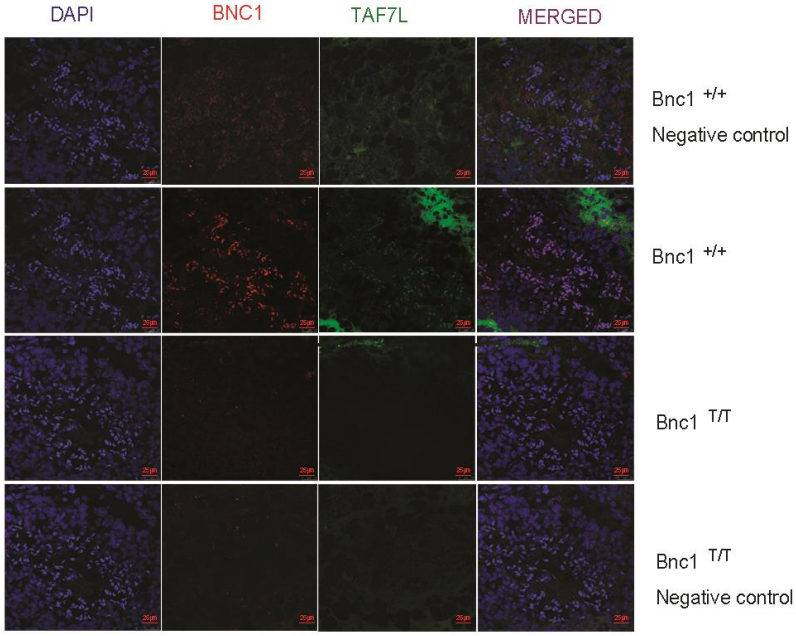

Figure S5| Co-localization of BNC1 and TAF7L in mice testis

Testis sections from *Bnc1*$^{+/+}$ or *Bnc1*$^{T/T}$ mice immunostained with DAPI (Column 1), antibodies against BNC1 (Column 2) and antibodies against TAF7L (Column 3), with negative control accordingly. Scale bar = 25μm.

Supplemental tables

Table S1 Primers for q-PCR analysis in mouse (m) or human (h)

Genes	Primers (5'-3')
m-SCP3	F-GCCAGTAACCAGAAAATTGAGC
	R- CCACTGCTGCAACACATTCATA
m-SCP1	F- TGTCCAGTCGGGAAAACATTG
	R-AGAATTAGCAACCTGTTCGAGC
m-BNC1	F- GTGTTCGATATTAGCAGCCTCAT
	R- GCCGGTCCAATAGGATTTTCAG
m-PAPOLB	F- ACACAGACCGCGAACTCAC
	R- CGGTAAGAGCCGAACGTAAAAA
m-PRM1	FCCGTCGCAGACGAAGATGTC
	R-CACCTTATGGTGTATGAGCGG
m-PRM2	F- ATGGTTCGCTACCGAATGAGG
	R-CTCCGCCTTCTGCATGACC
m-SMCP	F- GACTCACTAGACTGCTGAGGA
	R- CCTGATCTGGCAACACAGTTC
m-ODF1	F- ACTTATACATGCACCCGTACTGC
	R- GCGGGATCGCTTGGAGTAG
m-TEX14	F- TTCAGGGAAGCCCAAATAGTTC
	R- TTCAGGGAAGCCCAAATAGTTC
m-FHL5	F-TTGTGAGCAGTGTAAAGAACCAA
	R- ACCAAAGAGTGATGGCATTTGTT
m-KLHL10	F- TGAGTATGCGTACACTCGGAC
	R- CAACCCCTAACGATGCCCA
m-YBX2	F- AGCCGCTATGCCCCTAATC
	R- ACAAACCGCCTTCGATAGAAGA
m-PPARD	F- GCAGCCTCAACATGGAATGTC
	R- GAGCTTCATGCGGATTGTCC

m-PGK2	F- TTCTGCTAAGTTGACTCTGGACA
	R- AGCCTTGATTCTCTGGTTGTTTG
m-TNP1	F- ACCAGCCGCAAGCTAAAGAC
	R- GCTTCCACCTCTCTTGACGC
m-TNP2	F- TCACACCAGTAACCAGTGCAA
	R- CCCTGAGCTACGCCTCTTAG
m-GAPDHS	F- AATGGATTTGGACGCATTGGT
	R- TTTGCACTGGTACGTGTTGAT
m-TSSK6	F- TCGGACATTGCTGGCCTAC
	R- CGATCAAGGATTTGCATCGTTC
m-C-RET	F- GCACCAAGTACCGCACACT
	R- GCGGCAGTTGTAGAGAGACTTC
m-MAGEA4	F- GTCTCTGGCATTGGCATGATAG
	R-GCTTACTCTGAACATCAGTCAGC
m-KIT	F-AGGCTATCCCTGTTGTGTCTG
	R- ACATGGAGTTCACGGATGTAGA
m-OCT4	F- CGGAAGAGAAAGCGAACTAGC
	R- ATTGGCGATGTGAGTGATCTG
m-VASA	F-GAGAACACATCTACAACTGGTGG
	R- CCTCGCTTGGAAAACCCTCT
m-PLZF	F- CTGGGACTTTGTGCGATGTG
	R- CGGTGGAAGAGGATCTCAAACA
h-TAF7L	F- TGTACTGTCAGGAACCTAGCAC
	R- CAAGCGTTCTCAGGCTTTCAA
h-YBX2	F- GCTGGCAATCCAAGTCCTG
	R- TCAAATTCCACAGTCTCCCCAT
h-BNC1	F- CAGCAGTTCCTTCGTTTTGGA
	R- GGTGGTATGATGATGGATTGCTC
h-ODF1	F- GTGCGACTTGTATATGCACCC

	R- GTGATCGCTTGGAATAGCACA
h-GAPDHS	F- TGTGGGCATCAATGGATTTGG
	R- ACACCATGTATTCCGGGTCAAT

Table S2 Primers used for assaying the binding of BNC1 to target genes via ChIP-qPCR

Gene	Primers (5'-3')
GDNF	F-CGGATGGGTCTCCTGGATG
	R- CTCCAGCACGTTTCCCTCT
TEX14	F- GATGCTGGGAATGGAACCTG
	R-GATTGAGTTCCAGGCCAACC
TSSK6	F- GACCTCAAGTGCGAAAACGT
	R- GTAGGTGGTGCTCAGGTCTG
TNP2	F- AAAGCCCTGCCTCTCATCTT
	R-AAGAAGGGACACACAGAGGT
VEGFA	F- ACGTCCCCAGAGAGTGTTTT
	R- ATCCCAGGTTGCTGCTTAGG
HOOK1	F-GAGGTTCTCGGACCATGGAG
	R-CTGGCAAGCGTGGAACTAC
KLHL10	F- CACAATTTCCTGCACCCGTC
	R- AGCTGTCCTGATGCCCAT
SPATC1	F- CTGTGTTGTTCCCATTGCCA
	R- AGGGCTCAATTCTACCCACC

Table S3 Primers used for assaying the binding of TAF7L to target genes via ChIP-qPCR

Genes	Primers (5’-3’)
SPATC1	F- TGAGGATGGACACACACACA
	R- CTGACTTTCCCAGGCCATTA
TEX14	F-CATCATGTGCAGTCAGGTGTC
	R- AGGCAAGGGAGTGGAAAGAT
KLHL10	F- GGAGGCAAGTTATGGTGGTG
	R- GTCCTTCCTTGCATGATGGT
TSSK3	F-GGTGGGGAGTGCTTCTTTTG
	R-CAACTTCTGCCCACCTTGTC
PRM1	F-ATCTGTACCTGGCCATGGTG
	R-GCATCTATAACAGGCCGCAG
PRM2	F-CATGGGTCCTCTCGTAGACC
	R-CACGAGGTGTACAGGCAG