

Fig.S1. Expression profiles of MFN2 in mouse spermatogenesis. (A) RT-qPCR analyses of *Mfn2* mRNA level in 10 multiple organs in mice. Data are presented as mean \pm SEM, n = 3. (B) RT-qPCR analyses of the *Mfn2* mRNA level in developing mouse testes at postnatal day 0 (P0), P7, P14, P21, P28, P35, and in P56. Data are presented as mean \pm SEM, n = 3. (C) Expression of MFN2 protein in multiple organs. Levels of MFN2 in multiple organs were determined using western blot analyses. GAPDH was used as a loading control. (D) Western blot analyses showing MFN2 protein in developing mouse testes. GAPDH was used as a loading control. (E) Double immunostainings with MFN2 and γ -H2A.X on developing mouse testes at P0, P7, P14, P21, P28, and P35. Nuclei were stained with DAPI. Nuclei were stained with DAPI. Scale bar=10 μ m. (F) Double immunostainings with MFN2 and GCNA (a germ cell marker) on adult WT testes. Scale bar=10 μ m. Spg, spermatogonia; pL, preleptotene spermatocytes; L, leptotene spermatocytes; Z, zygotene spermatocytes; PS, pachytene spermatocytes; D, diplotene spermatocytes; M, meiotic divisions; sSc—secondary spermatocytes; RS, round spermatids; ES, elongating spermatids; SC, Sertoli cells. (G) RT-qPCR analyses of the *Mfn2* mRNA expression levels in indicated types of purified spermatogenic cells. Data are presented as mean \pm SEM, n = 3. SC, Sertoli cells; Spg, Spermatogonia; PS, Pachytene spermatocytes; RS, Round spermatids. (H) Western blot showing the protein levels of MFN2 in indicated types of purified spermatogenic cells. GAPDH served as a loading control. SC, Sertoli cells; PS, Pachytene spermatocytes; RS, Round spermatids; ES, Elongating spermatids. (I) The qualification of the protein levels of MFN2 in (H).

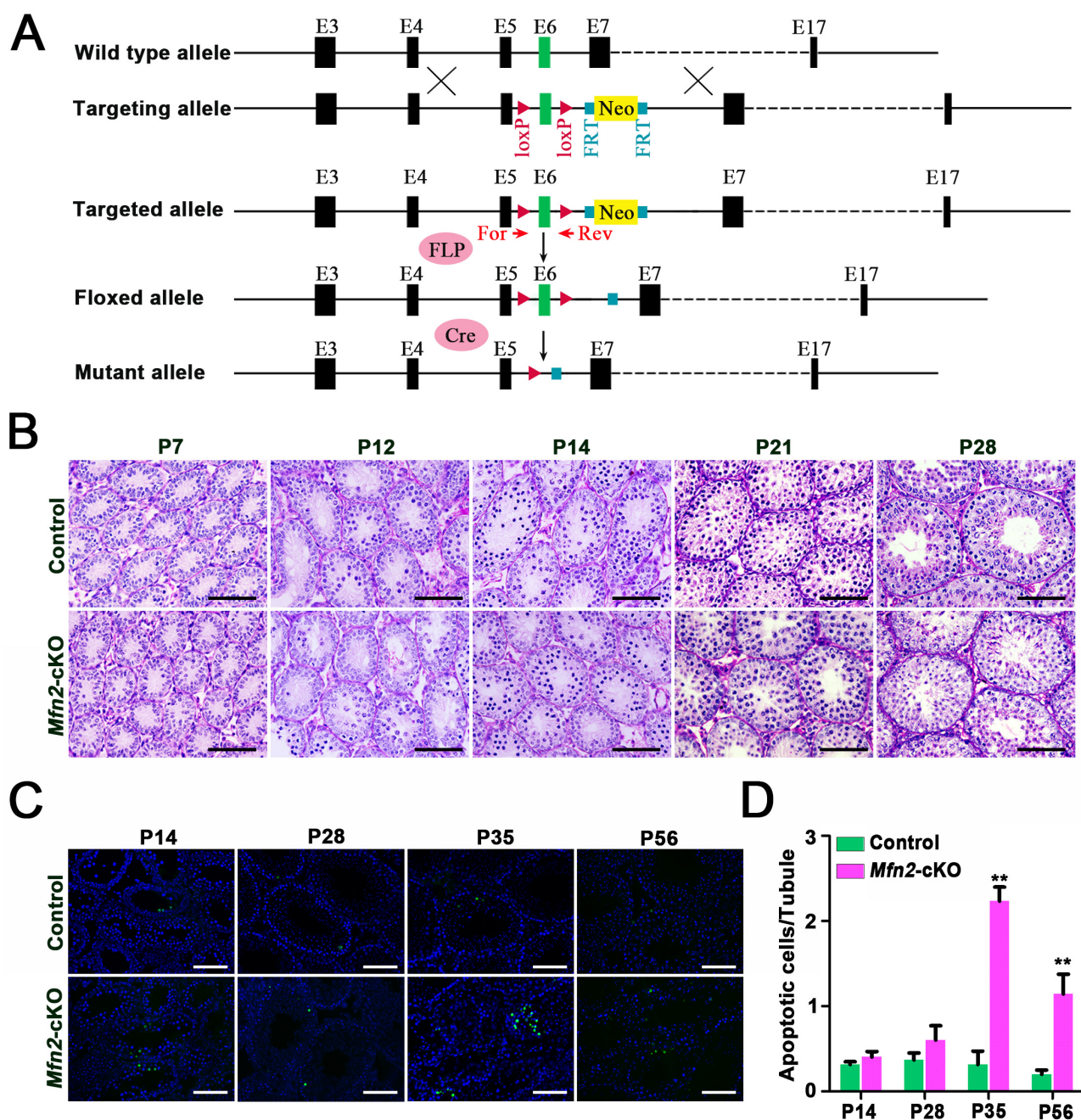


Fig.S2. Conditional inactivation of *Mfn2* causes increased apoptosis in testes. (A) Schematic representation of the targeting strategy for generating a floxed *Mfn2* allele through homologous recombination in the murine embryonic stem cells. Exons 6 will be deleted after Cre-mediated recombination. Positions of the forward (For) and reverse (Rev) primers used for genotyping are shown. (B) Periodic acid-Schiff (PAS) staining showing the histology of developing testes at P7, P12, P14, P21 and P28 from control and *Mfn2*-cKO mice. No obvious morphological defects were observed in *Mfn2*-cKO testes. Scale bars=100 μ m. (C) Representative images of TUNEL assays on testis sections from control and *Mfn2*-cKO testes at P14, P28, P35, and P56. Scale bars=100 μ m. (D) Quantification of apoptotic cells in control and *Mfn2*-cKO developing testes at P14, P28, P35, and P56. Data are presented as mean \pm SEM, n = 5. ** P <0.01.

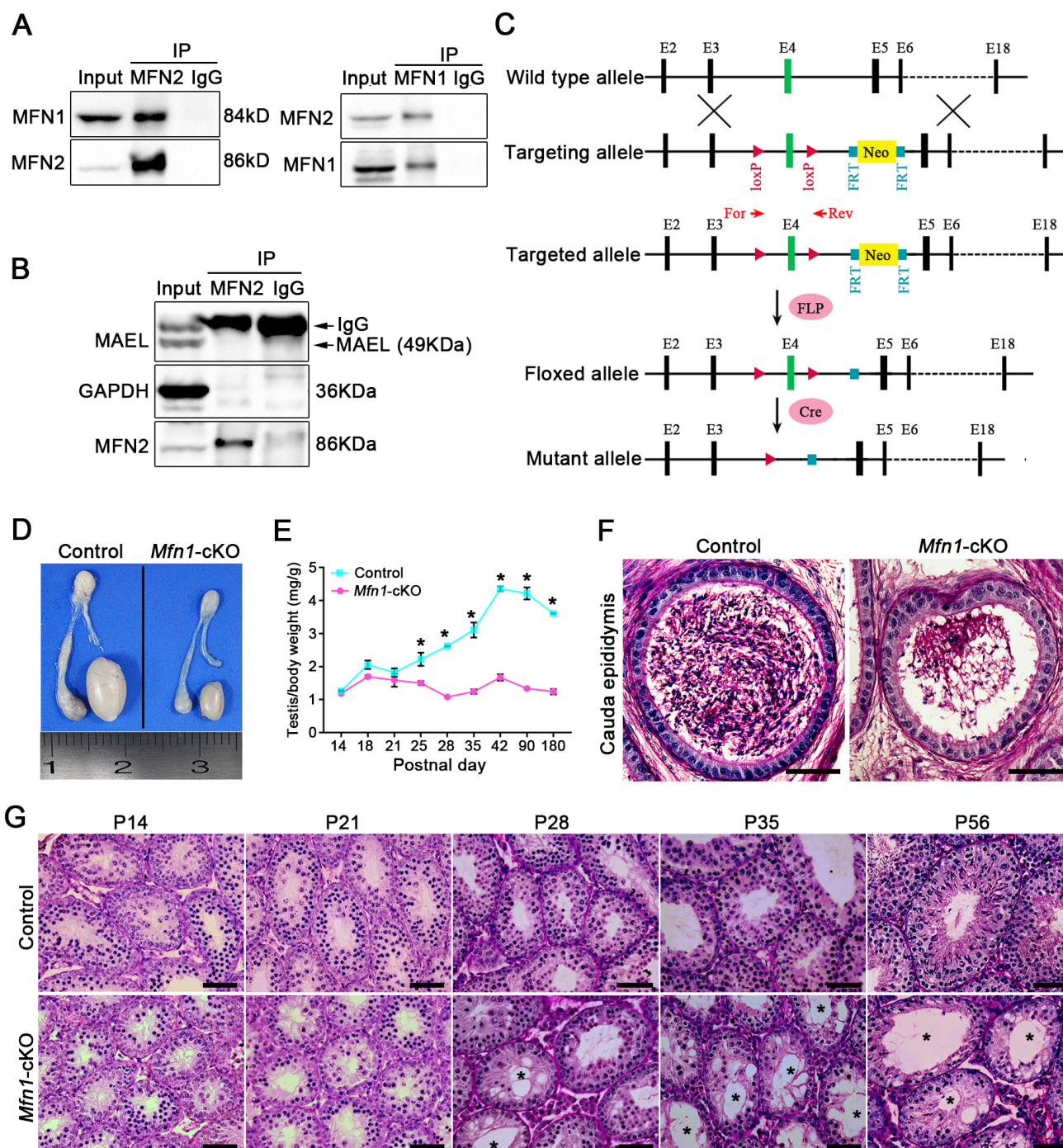


Fig.S3. MFN2 interacts with MFN1 in testis and conditional knockout of *Mfn1* leads to male infertility. (A) Immunoprecipitation showing MFN1 was detected in the immunoprecipitants from MFN2 in the mouse testes (the left panel), and reciprocal immunoprecipitation showing MFN2 was detected in the immunoprecipitants from MFN1 in the mouse testes (the right panel). (B) Immunoprecipitation showing no detection of MAEL and GAPDH in the immunoprecipitants from MFN2 in the mouse testes. (C) Schematic representation of the targeting strategy for generating a floxed *Mfn1* allele through homologous recombination in the murine embryonic stem cells. Exons 4 will be deleted after Cre-mediated recombination. Positions of the forward (For) and reverse (Rev) primers used for genotyping are shown. (D) Gross morphology of the testis and the epididymis from adult control and *Mfn1*-cKO mice. (E) Testes growth curve shows the *Mfn1*-cKO testes were significantly decreased from P25. Data are presented as mean \pm SEM, $n = 5$. * $P < 0.05$. (F)

PAS-staining showing the histology of cauda epididymis from control and *Mfn1*-cKO mice. Scale bars=100 μ m. (**G**) PAS-staining showing the histology of developing testes at P14, P21, P28, P35 and P56 from control and *Mfn1*-cKO mice. Asterisk (*) represents the vacuolated seminiferous tubules. Scale bar=50 μ m.

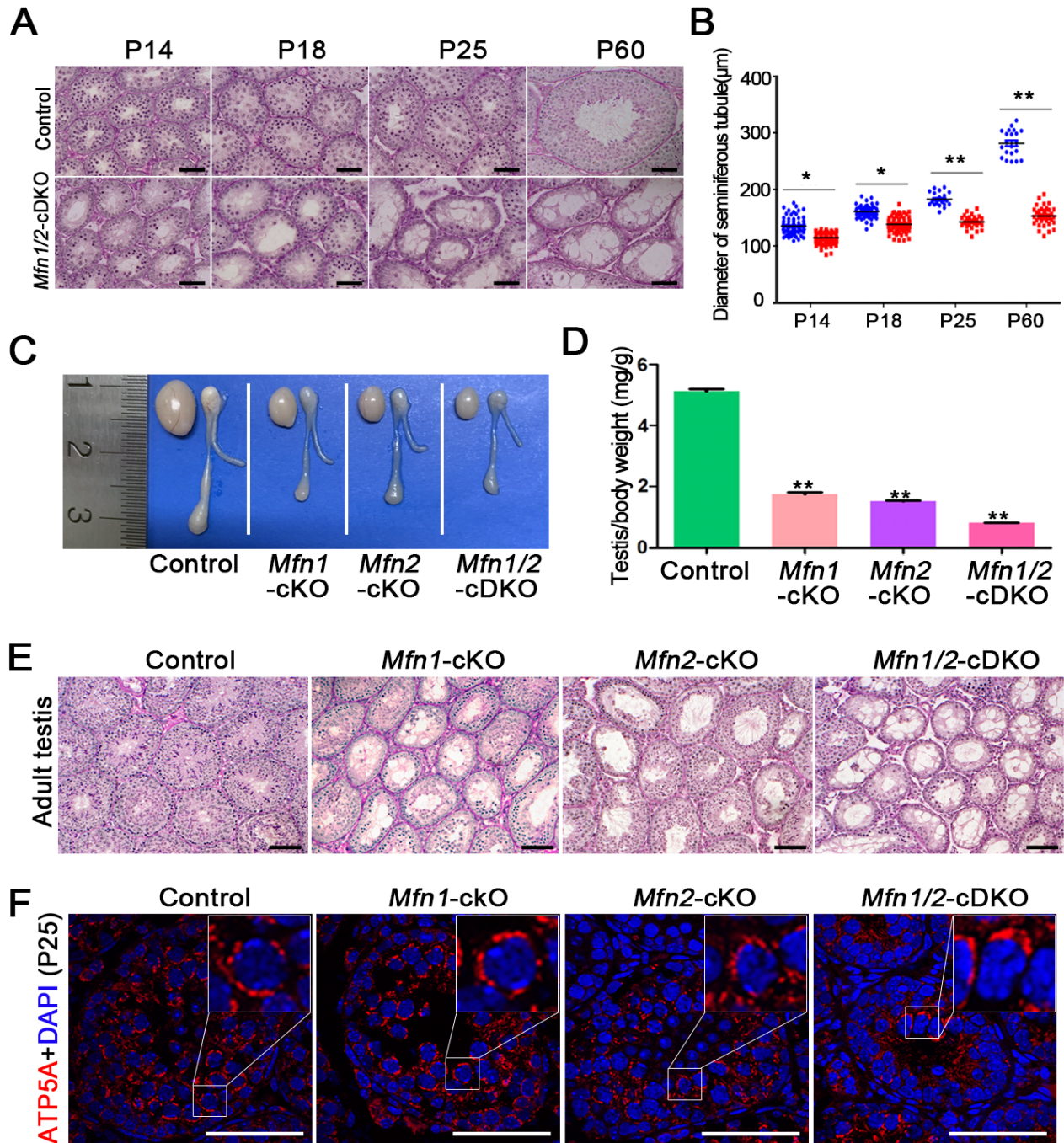


Fig.S4. Double knockout of *Mfn1* and *Mfn2* results in much more severe disrupted testicular phenotypes in mice. (A) PAS-staining showing the histology of developing testes at P14, P18, P21, and P60 from control and *Mfn1/2-cDKO* mice. Scale bar=50μm. (B) Scatter plot showing the quantification of diameters of testis seminiferous tubules at P14, P18, P21, and P60 from control and *Mfn1/2-cDKO* mice. * $P < 0.05$, ** $P < 0.01$. (C) Gross morphology of the testis and the epididymis from adult control, *Mfn1-cKO*, *Mfn2-cKO* and *Mfn1/2-cDKO* mice, respectively. (D) Histogram showing the ratio of testis weight to body weight in control, *Mfn1-cKO*, *Mfn2-cKO*, and *Mfn1/2-cDKO* mice. Data are presented as mean \pm SEM, $n = 5$. ** $P < 0.01$. (E) PAS-staining showing the histology of adult testes from control, *Mfn1-cKO*, *Mfn2-cKO* and *Mfn1/2-cDKO* mice. Scale bar=50μm. (F) Representative immunofluorescent images of ATP5A (an outer mitochondria membrane marker) showing the mitochondrial distribution in testicular sections at P25 from control, *Mfn1-cKO*, *Mfn2-cKO*, and *Mfn1/2-cDKO* testes. Scale bar=50μm.

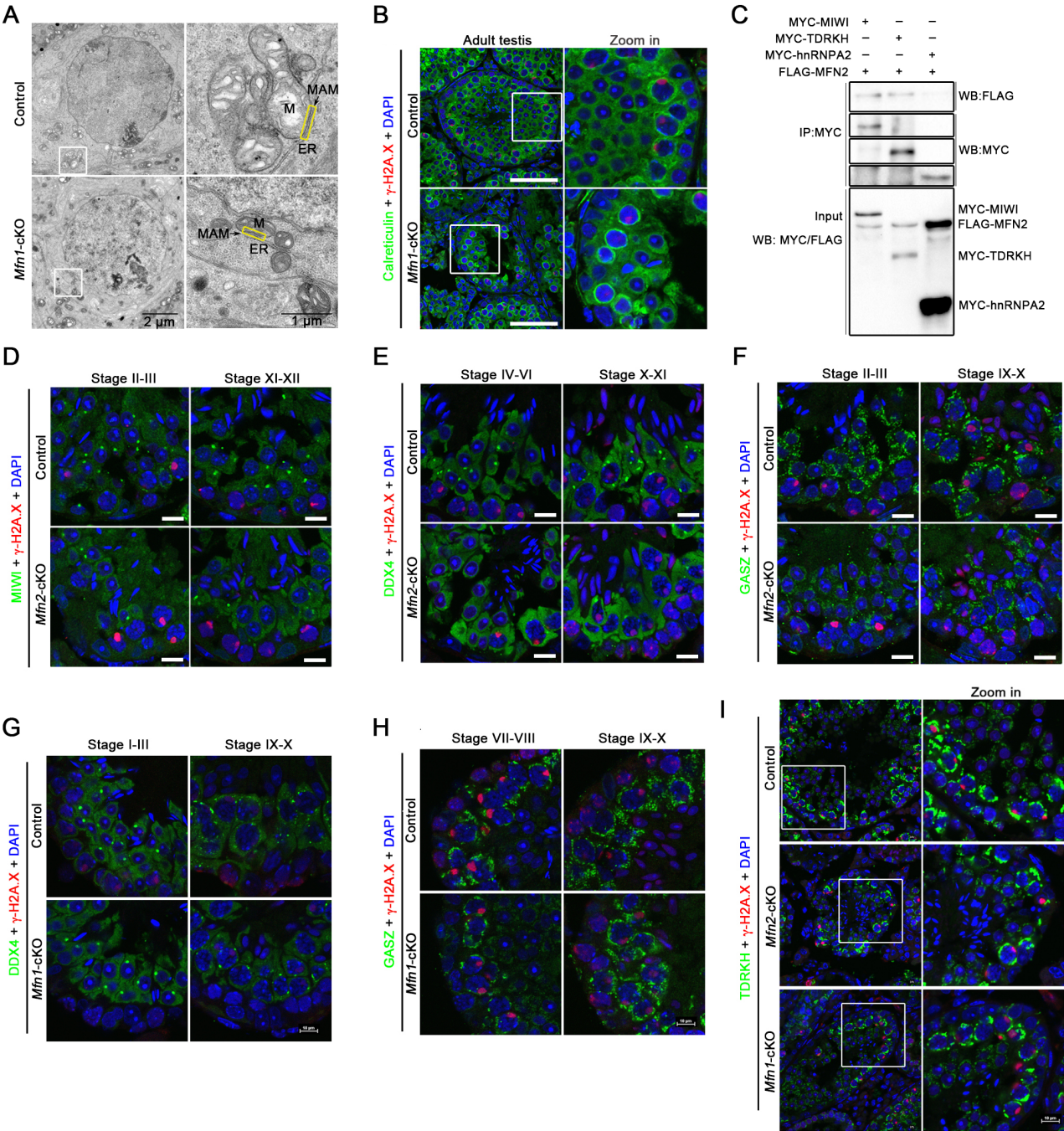


Fig.S5. Loss of MFN2, but not MFN1 in postnatal germ cells leads to ectopic expression of Nuage-associated proteins in testes. (A) TEM showing the fragmented and

swelling mitochondria (M) in pachytene spermatocytes from adult *Mfn1*-cKO mouse testes. Right panels represent the zoomed in mitochondria from the square areas in the left panels. Arrows indicate mitochondria-associated ER membranes (MAM). **(B)** Co-immunostainings of Calreticulin and γ -H2A.X in adult testes from control and *Mfn1*-cKO mice. Nuclei were stained with DAPI. Scale bar=50 μ m. **(C)** *In vitro* interaction assay showing the interaction of FLAG-MFN2 with MYC-MIWI and MYC-TDRKH in HEK293T cells, MYC-hnRNPA2 (a nuclear RNA binding protein) serves as a negative control. **(D)** Double immunostainings with MIWI and γ -H2A.X in stage II-III and XI-XII seminiferous tubules from control and *Mfn2*-cKO mice. Nuclei were stained with DAPI. Scale bar=10 μ m. **(E)** Double immunostainings with DDX4 and γ -H2A.X in stage IV-VI and X-XI seminiferous tubules from control and *Mfn2*-cKO mice. Nuclei were stained with DAPI. Scale bar=10 μ m. **(F)** Double immunostainings with GASZ and γ -H2A.X in stage II-III and IX-X seminiferous tubules from control and *Mfn2*-cKO mice. Nuclei were stained with DAPI. Scale bar=10 μ m. **(G)** Double immunostainings with DDX4 and γ -H2A.X in stage I-III and IX-X seminiferous tubules from control and *Mfn1*-cKO mice. Nuclei were stained with DAPI. Scale bar=10 μ m. **(H)** Double immunostainings with GASZ and γ -H2A.X in stage VII-VIII and IX-X seminiferous tubules from control and *Mfn1*-cKO mice. Nuclei were stained with DAPI. Scale bar=10 μ m. **(I)** Double immunostainings with TDRKH and γ -H2A.X in testicular sections from control, *Mfn1*-cKO, and *Mfn2*-cKO testes. Nuclei were stained with DAPI. Scale bar=10 μ m.

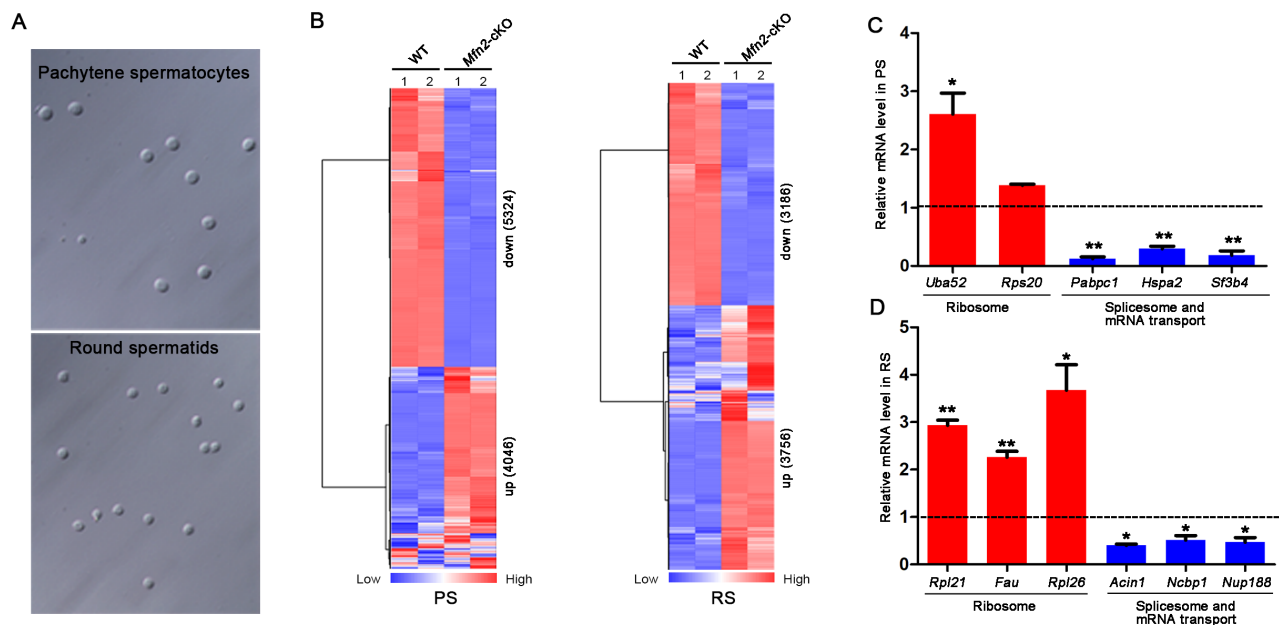


Fig.S6. RNA-seq reveals differential gene expressions in *Mfn2-cKO* pachytene spermatocytes and round spermatids. (A) Light microscopy images showing the purity and morphology of isolated pachytene spermatocytes (PS) in the upper and round spermatids (RS) in the lower, respectively. (B) Heatmaps show the differential expression genes in purified pachytene spermatocytes (left panel) and round spermatids (right panel) from adult WT and *Mfn2-cKO* testes. Significantly regulated genes have a *P*-value of <0.05 and a fold change of >2.0 . Two biological replicates indicate in the heatmap. (C-D) Validation of the up- and down-regulated genes selected from RNA-seq data in purified pachytene spermatocytes (C) and round spermatids (D) by RT-qPCR analyses. N=3.

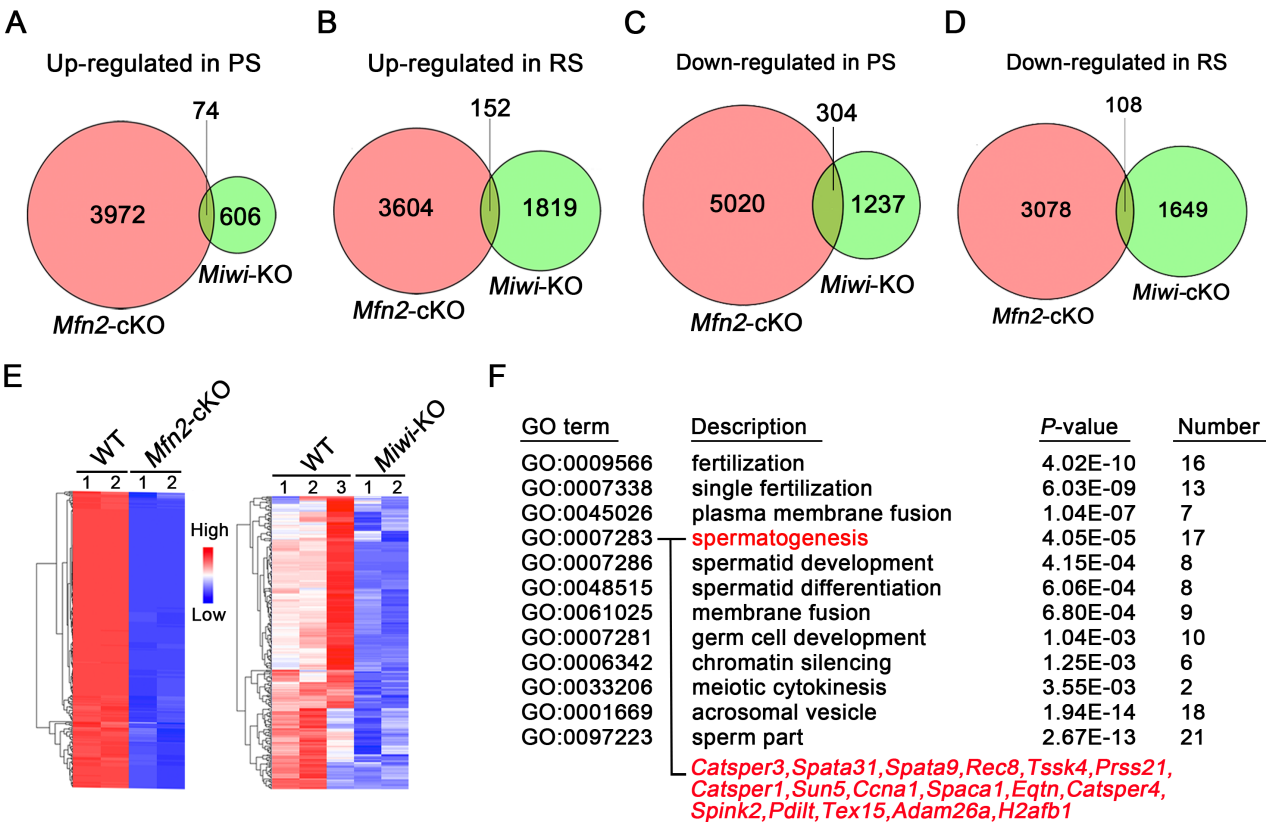


Fig.S7. The comparison of overlapped differential expressed genes in RNA-seq from *Mfn2*-cKO and *Miwi*-KO pachytene spermatocytes and round spermatids. (A-D) Venn diagrams show the intersection of significant genes differentially upregulated (A-B) and downregulated (C-D) in *Mfn2*-cKO and *Miwi*-KO pachytene spermatocytes (PS) and round spermatids (RS) respectively. **(E-F)** Heatmaps (E) and gene ontology analyses (F) show the intersection of significant genes differentially downregulated in *Mfn2*-cKO and *Miwi*-KO pachytene spermatocytes. Genes enriched in the spermatogenesis indicated in fly-out with red text according to the degree of differences.

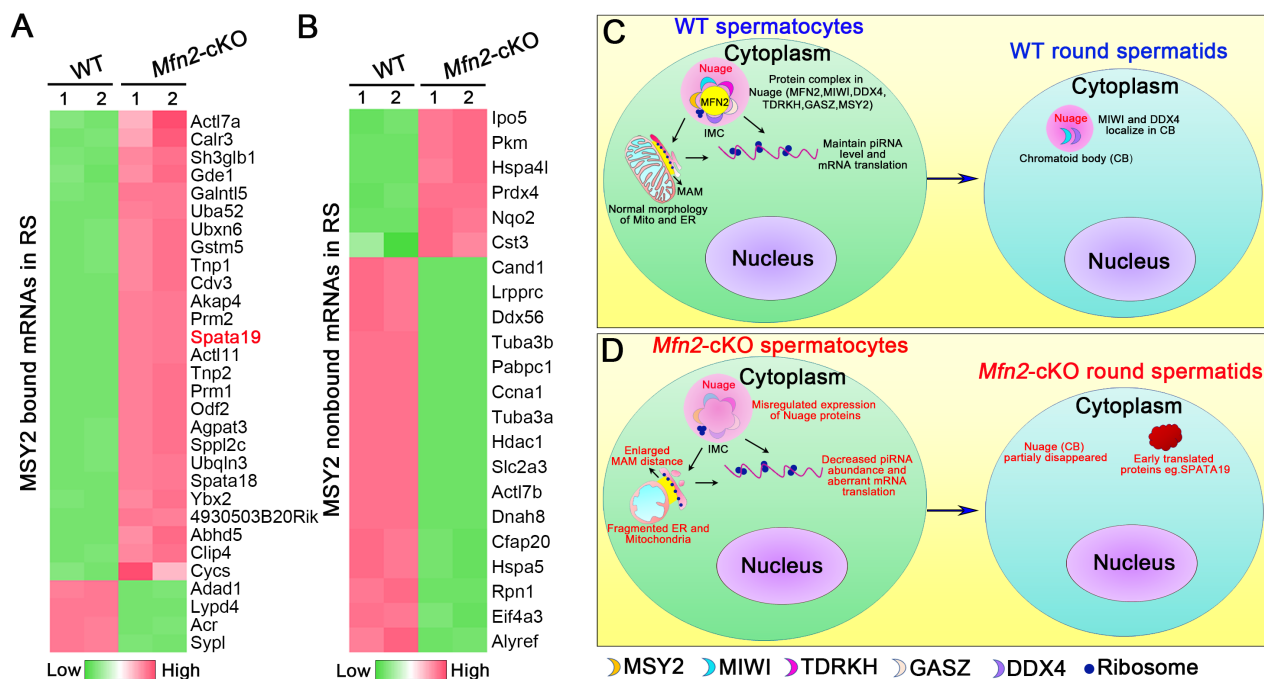


Fig.S8. Differential expressed MSY2-bound/unbound gamete-specific mRNAs analyses and working model are shown. (A-B) Heat-maps show the differential expression levels of MSY2-bound (A) and unbound (B) gamete-specific mRNAs in round spermatids (RS) from RNA-Seq data in WT and *Mfn2*-cKO mice. **(C)** In WT spermatocytes, MFN2 cooperates with Nuage associated proteins in pachytene spermatocytes and round spermatids, including MIWI, DDX4, TDRKH, GASZ, and MSY2 to form interaction complexes for maintaining normal morphology of mitochondria, MAM and ER, and mRNA translation. **(D)** In MFN2 deficient pachytene spermatocytes and round spermatids, Nuage-associated proteins are ectopically expressed, including a decrease of MIWI, DDX4, and GASZ expression, and an increase of MSY2 expression. Meanwhile, in the cytoplasm, the disassociated MFN2-Nuage protein complexes caused some MSY2-bound mRNAs are misregulated in mRNA and/or protein level, especially gamete-specific genes, such as SPATA19 is early-translated, which leads to disrupted male germ cell development and male fertility.

Table S1. Size distribution of small RNA reads after normalization with miRNA reads

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Table S2. Total 4046 up-regulated genes in *Mfn2*-cKO pachytene spermatocytes (PS) determined by RNA-Seq.

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Table S3. Total 3756 up-regulated genes in *Mfn2*-cKO round spermatids (RS) determined by RNA-Seq.

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Table S4. 34 piRNA targeting genes shown upregulated in *Mfn2*-cKO pachytene spermatocytes

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Table S5. 32 piRNA targeting genes shown upregulated in *Mfn2*-cKO round spermatids

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Table S6. The expression levels of MSY2-bound mRNA in WT and *Mfn2*-cKO round spermatids (RS) determined by RNA-Seq.

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Table S7. The expression levels of MSY2-nonbound mRNA in WT and *Mfn2*-cKO round spermatids (RS) determined by RNA-Seq.

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Table.S8. The expression levels of MSY2-bound mRNA in WT and *Mfn2*-cKO pachytene spermatocytes (PS) determined by RNA-Seq.[Click here to Download Table S8](#)**Table.S9. The expression levels of MSY2-nonbound mRNA in WT and *Mfn2*-cKO pachytene spermatocytes (PS) determined by RNA-Seq.**[Click here to Download Table S9](#)**Table S10. The antibodies used in this study.**

Name	Source	Cat number	Dilutions
Rabbit anti-Mitofusin-2 (D2D10)	Cell Signaling	9482	1:1000 for WB; 1:100 for IMF; 2~4 ug for IP
Rabbit anti-DDX4/MVH	Abcam	ab13840	1:2000 for WB; 1:200 for IMF; 2~4 ug for IP
Mouse anti-gamma-H2A.X	Abcam	ab26350	1:200 for IMF
Mouse anti-ATP5A	Abcam	ab14748	1:200 for IMF
Rabbit anti-Calreticulin	HuaBio	ET1608-60	1:100 for IMF
Rabbit anti-PIWIL1 (MIWI)	Proteintech	15659-1-AP	1:1000 for WB; 1:100 for IMF; 2~4 ug for IP
Rabbit anti-TDRKH	Proteintech	13528-1-AP	1:2000 for WB; 1:200 for IMF; 2~4 ug for IP
Rabbit anti-MSY2	Proteintech	13538-1-AP	1:1000 for WB; 1:100 for IMF; 2~4 ug for IP
Rabbit anti-GASZ	Proteintech	21550-1-AP	1:1000 for WB; 1:100 for IMF; 2~4 ug for IP
Mouse anti-MYC tag	Proteintech	60003-2-Ig	1:5000 for WB; 2~4 ug for IP
Rabbit anti-Flag tag	Proteintech	20543-1-AP	1:3000 for WB
Rabbit anti-GAPDH	Proteintech	10494-1-AP	1:5000 for WB
Rabbit anti-RPS6	Proteintech	14823-1-AP	1:1000 for WB
Mouse anti-Tubulin	Proteintech	66031-1-Ig	1:3000 for WB
Rabbit anti-SPATA19	Proteintech	16656-1-AP	1:1000 for WB; 1:100 for IMF
Rabbit anti-MFN1	ABclonal	A9880	1:1000 for WB; 1:100 for IMF; 2~4 ug for IP
Rat anti-GCNA1(TAR98)	Abcam	Ab82527	1:100 for IMF

Table S11. Primer sequences are used in this study.[Click here to Download Table S11](#)