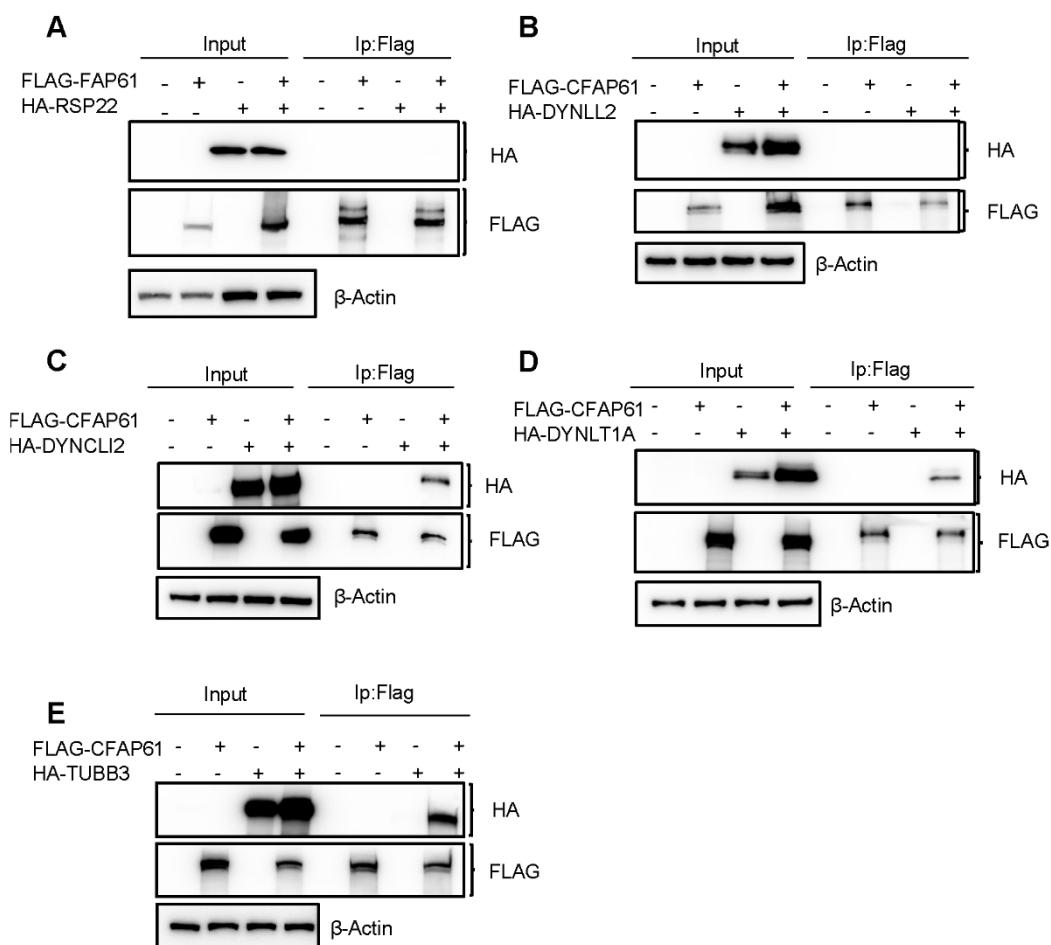
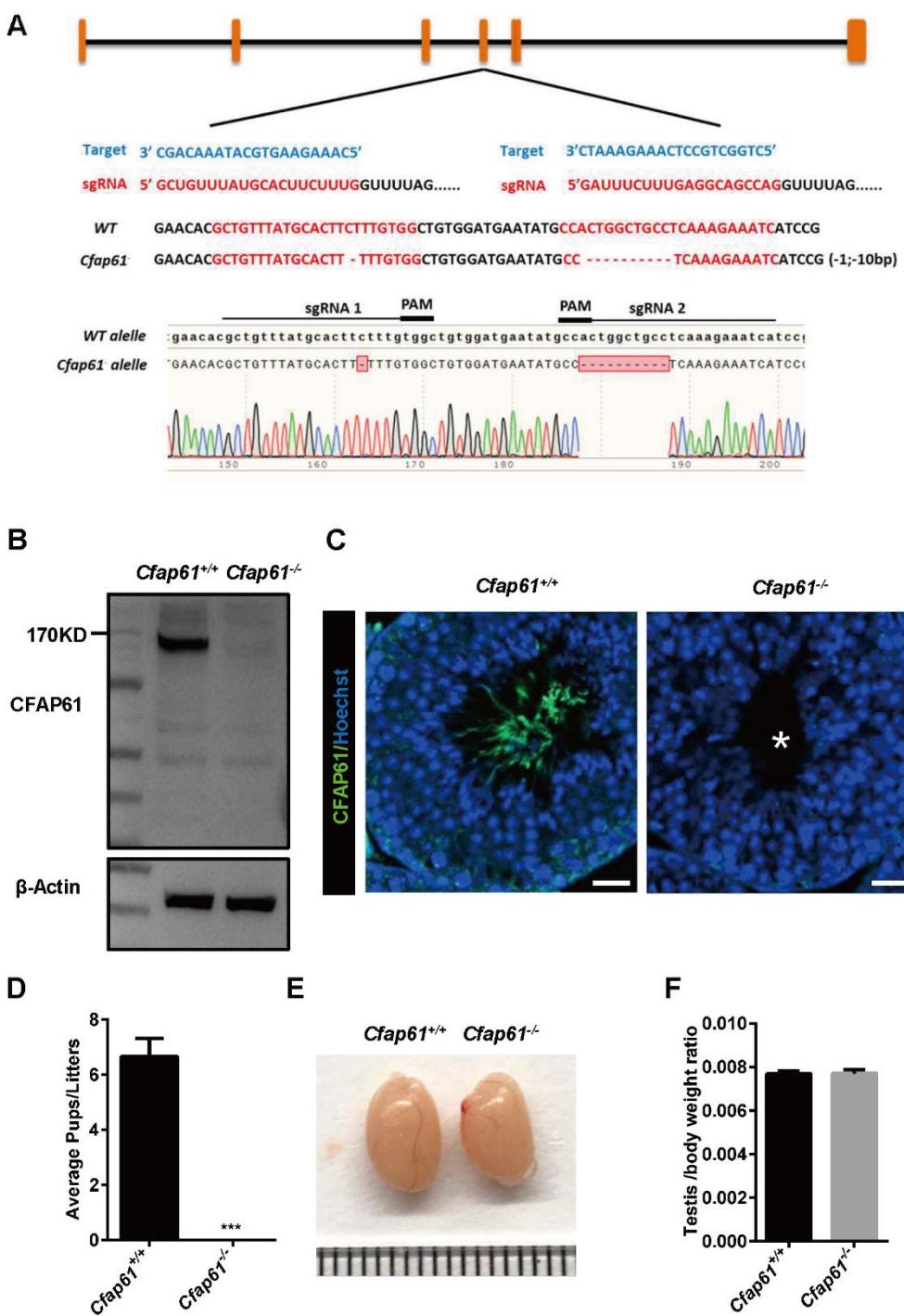
**Fig. S1. Identification of CFAP61 interacting proteins.**

(A) Heatmap representation of CFAP61 interacting proteins identified with anti-CFAP61. IP1-NC1 and IP2-NC2 represent data from two biological replicates. (B) Functional classes of CFAP61 interacting proteins. (C) Representative immunofluorescence image from proximity ligation assay (PLA) performed on epididymal sperm from wild-type and *Cfap61*^{-/-} mice. Evidence of proximity (distance < 40 nm) between CFAP61 and CSNK2A2 (upper panel)/PPP1CC (lower panel) is indicated by the appearance of red dots. Nuclei were stained with DAPI (blue). Scale bars = 10 μm

**Fig. S2. CFAP61 interacts with DYNEIN and TUBULIN.**

(A) Chlamydomonas RSP22 was expressed or co-expressed with FAP61 in HEK293T cells and FAP61 interaction with RSP22 was examined by co-immunoprecipitation.(B) Mouse DYNLL2(RSPH22) was expressed or co-expressed with CFAP61 in HEK293T cells and CFAP61 interaction with RSPH22 was examined by co-immunoprecipitation. (C-E) Mouse dynein subunits (C-D) or TUBB3 (E) were expressed or co-expressed with CFAP61 in HEK293T cells and CFAP61 interaction with DYNLL2, DYNCLI2, DYNLT1A, TUBB3 was examined by co-immunoprecipitation.

**Fig. S3.** *Cfap61^{-/-}* mice are infertile.

(A) Schematic diagram of CRISPR/Cas9 targeting strategy. The sgRNAs were designed within exon 4 of *Cfap61* and a 1-bp and 10-bp deletion was detected in *Cfap61^{-/-}* mice by Sanger sequencing. (B, C) CFAP61 was not detected in *Cfap61^{-/-}* mice by Western blotting and immunofluorescence. (D) Infertility was assessed by the number of pups per litter. (E) Kidney size was measured. (F) Testis weight was measured relative to body weight.

testis by western blot (B) and immunofluorescence (C). Scale bars =20 μ m. (D) The average litter size of WT (6.677 ± 0.667), *Cfap61^{-/-}* (0), male mice (n=3). P (*Cfap61^{-/-}* vs WT)= 0.0006. (E) The size of the testes was not altered in the WT and *Cfap61^{-/-}* adult mice. (F) The testis/body weight ratio of WT (0.007689 ± 0.0001418), *Cfap61^{-/-}* (0.007721 ± 0.000175) , male mice (n=3). P (*Cfap61^{-/-}* vs WT)= 0.8948. Data represent mean \pm s.e.m.

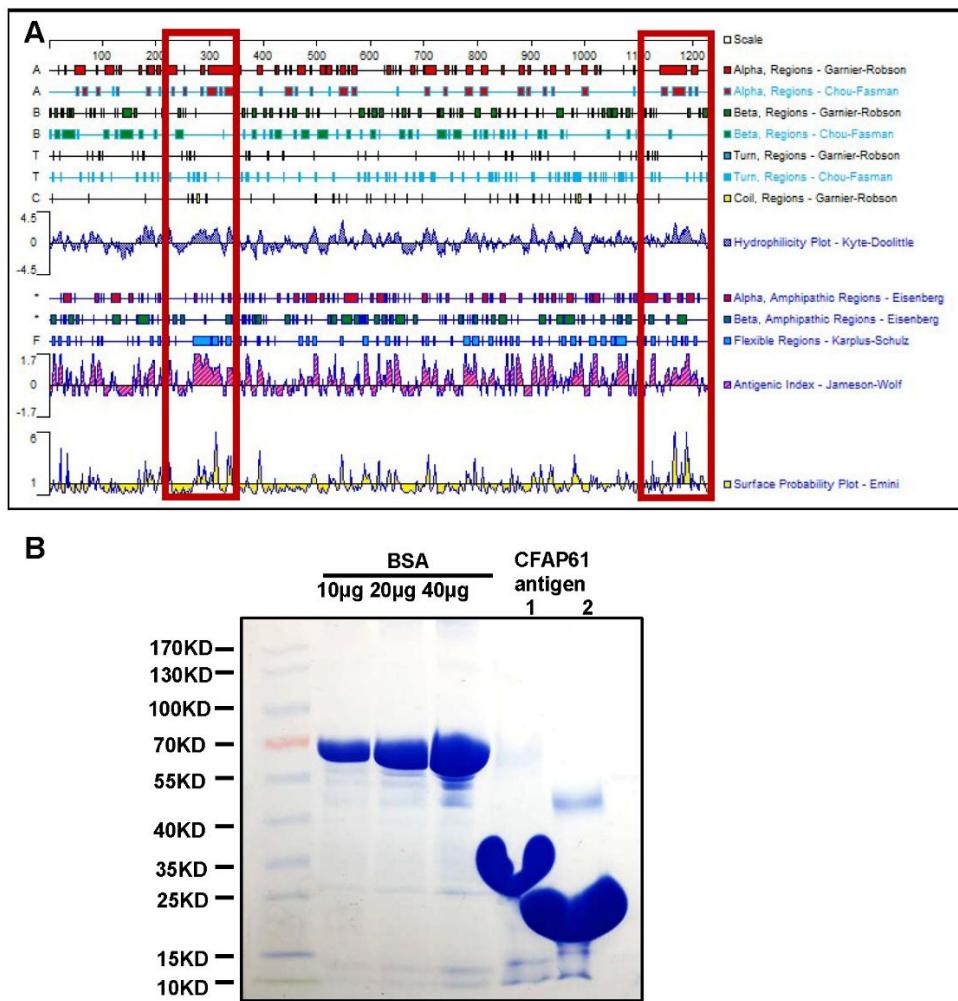


Fig. S4. Antigen selection and purification.

(A) Epitope prediction of CFAP61 by analyzing secondary structures and other indicators (flexibility, hydrophilicity, antigenicity, and surface probability). (B) Coomassie brilliant blue stained SDS-PAGE of selected antigens. Protein standards indicate molecular size in kilodaltons in leftmost lane, while next three lanes show the indicated microgram amounts of pure bovine serum albumin (BSA) and final two lanes show purified CFAP61 antigens.

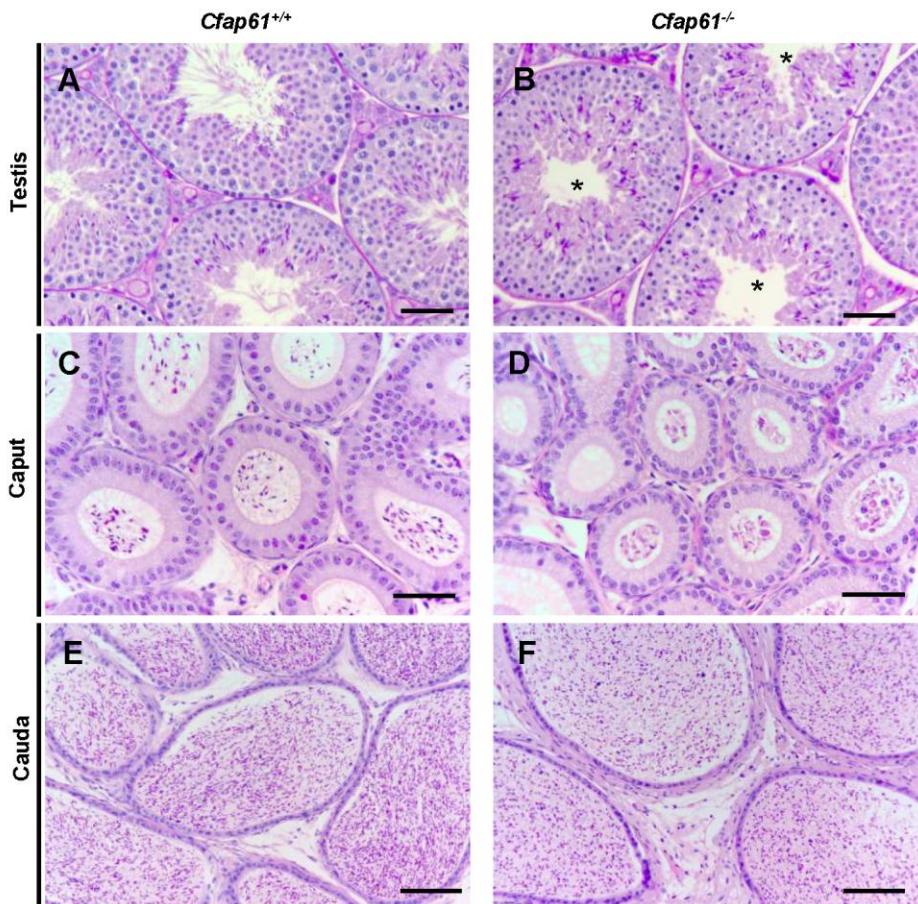


Fig. S5. Spermatogenesis is abnormal in *Cfap61*^{-/-} mice.

Sections of periodic acid Schiff-stained testis and hematoxylin and eosin-stained caput and cauda epididymis from wild-type (A, C, E) and *Cfap61*^{-/-} (B, D, F) mice. (A-D) Scale bars =50 µm. (E, F) Scale bars =100 µm.

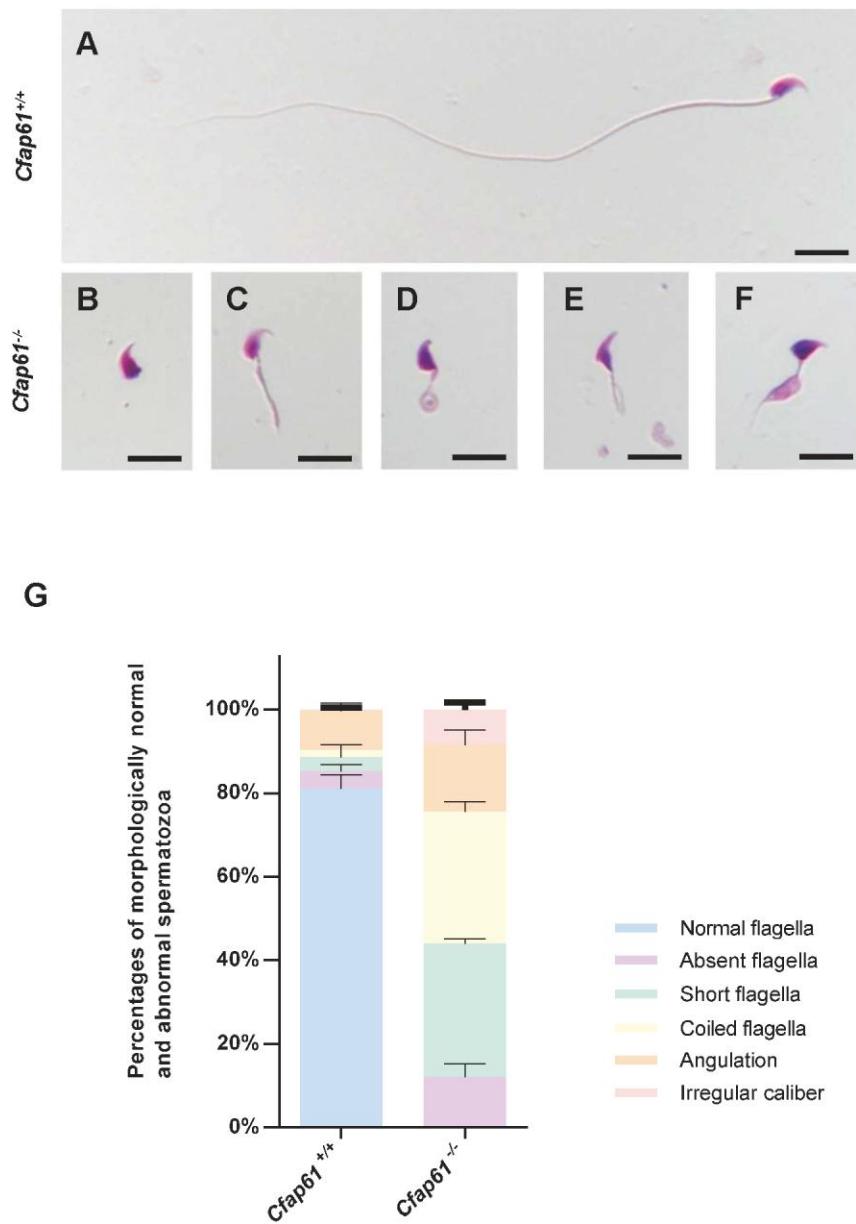


Fig. S6. Sperm morphology study in *Cfap61*^{-/-} male mice.

(A) Light microscopy showed wild type spermatozoon with normal morphology while spermatozoa from *Cfap61*^{-/-} mice manifested aberrant flagellar morphologies: absent (B), short (C), coiled (D), angulation (E) and irregular caliber (F), consistent with the MMAF phenotype. Scale bars =10 μ m. (G) Percentage of morphologically normal

and abnormal spermatozoa in WT and *Cfap61*^{-/-} male mice (n=3), evaluated following the WHO guidelines. The left column shows that the percentage of spermatozoa with normal flagella ($81.165 \pm 1.895\%$), absent flagella ($4.028 \pm 0.966\%$), short flagella ($3.414 \pm 1.709\%$), coiled flagella ($1.604 \pm 0.783\%$), angulation ($9.366 \pm 1.139\%$) and irregular caliber ($0.423 \pm 0.259\%$) in the normal group. The right column shows that the percentage of spermatozoa with normal flagella (0%), absent flagella ($12.085 \pm 1.852\%$), short flagella ($31.969 \pm 0.654\%$), coiled flagella ($31.542 \pm 1.317\%$), angulation ($15.833 \pm 2.125\%$) and irregular caliber ($8.570 \pm 0.940\%$) in the *Cfap61*^{-/-} group. $P_{\text{normal flagella}} (Cfap61^{-/-} \text{ vs WT}) = 1.77 \times 10^{-6}$, $P_{\text{absent flagella}} (Cfap61^{-/-} \text{ vs WT}) = 0.0182$, $P_{\text{short flagella}} (Cfap61^{-/-} \text{ vs WT}) = 9.84 \times 10^{-5}$, $P_{\text{coiled flagella}} (Cfap61^{-/-} \text{ vs WT}) = 4.04 \times 10^{-5}$, $P_{\text{angulation}} (Cfap61^{-/-} \text{ vs WT}) = 0.055$, $P_{\text{irregular caliber}} (Cfap61^{-/-} \text{ vs WT}) = 0.001$.

Data represent mean \pm s.e.m.

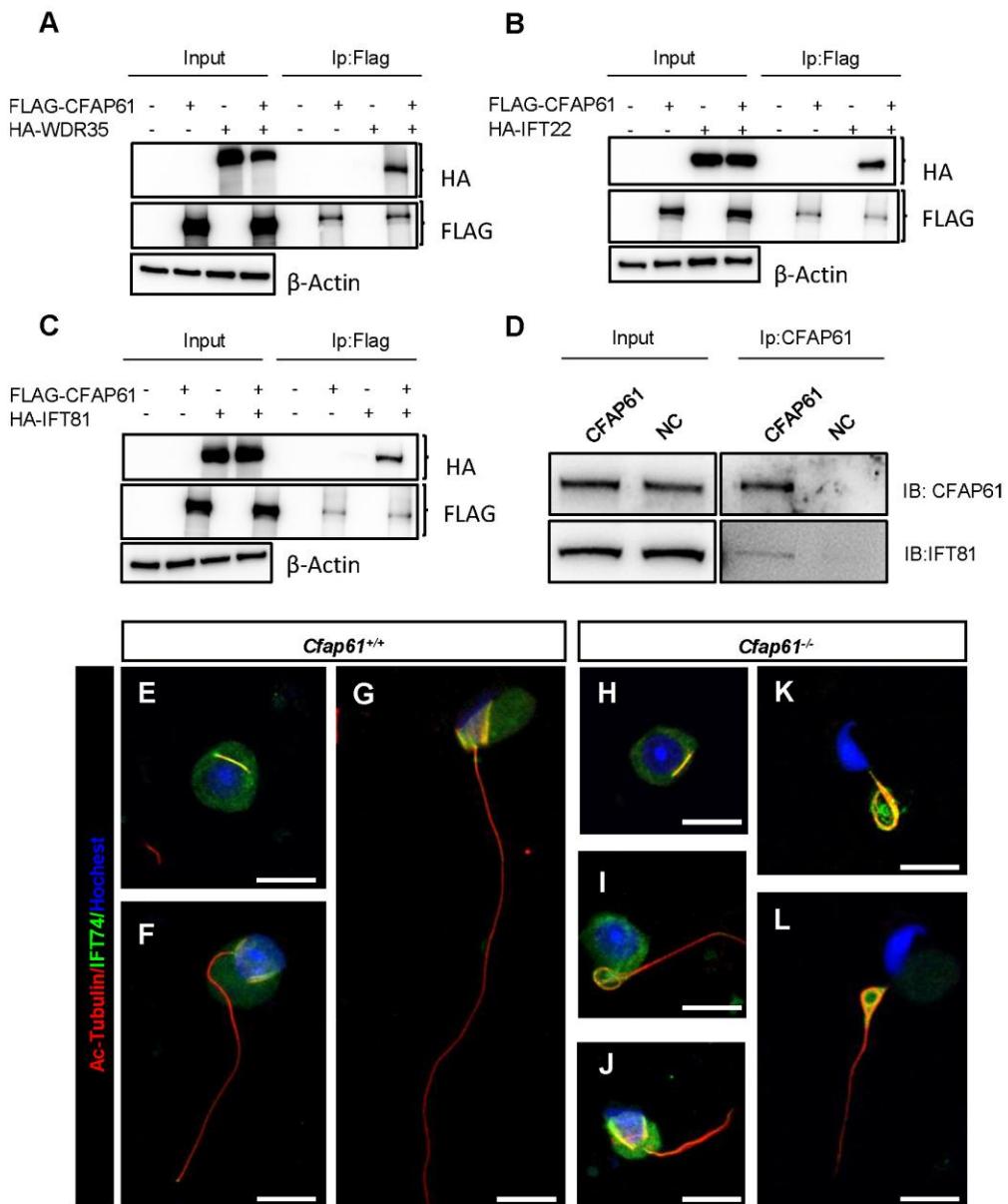


Fig. S7. Intraflagellar transport protein are retained in mature spermatozoa .

(A-C) Intraflagellar transport (IFT) proteins were expressed or co-expressed with CFAP61 in HEK293T cells, and CFAP61 interaction with IFT proteins was examined by co-immunoprecipitation. (D) Co-IP analysis of CFAP61 and IFT81 from testicular protein extracts. (E-L) Immunofluorescence analysis of acetylated-tubulin (red) and IFT74 (green) in wild-type (E-G) and *Cfap61*^{-/-} germ cells (H-L). Scale bars =10 μm.

Table S1. Primer sequences For qRT-PCR

Primer	Sequence
Cfap61-qRT-F	TCCCCATGCACACCTCTGAAC
Cfap61-qRT-R	AAGTACAGTTCTGGCACCGC
Wdr66-qRT-F	AAGCTCCTCCCTGGCTGATA
Wdr66-qRT-R	TGTCTTCAGGCTTCTGGCTG
Maats1-qRT-F	CAGCACCATGAGGCCAGACAG
Maats1-qRT-R	ACACAATAAATAACGGGTCGTACA
Rspf9-qRT-F	ATGACATCCCCAGAGGGTCT
Rspf9-qRT-R	GGAGCGTGGTAGAAGGTGAG
Ropn11-qRT-F	TCCTGCACAAGCAGTGTAGC
Ropn11-qRT-R	TCCAGCTCCAGGATCGTTCT
Rspf4a-qRT-F	TGGTCGAGACAGCTATGAGGA
Rspf4a-qRT-R	ACCAATTACAGCGACCCTGAG
18s-F	TAACGAACGAGACTCTGGCAT
18s-R	CGGACATCTAAGGGCATCACAG

Table S2. Primer sequences For plasmids construction

Primer	Sequence
Flag-Cfap61-F	AAGGATGACGATGACAAGCTTCCATCCTCACGTCCC CCAG
Flag-Cfap61-R	CCACACTGGACTAGTGGATCCCTAAATGATGCCTGGC CATGCG
HA-Armc4-F	TACGCATCAGCGAAAAGCTTGGTGTGGCGTTGACCA GGTG
HA-Armc4-R	CCACACTGGACTAGTGGATCCTCAGTTGTATCTAGCCT TTTCTGTAGCAAGAG
HA-Calm4-F	TACGCATCAGCGAAAAGCTTCTCACGGGTTACTA AGGAGGAGGTC

HA-Calm4-R	CCACACTGGACTAGTGGATCCTCAATTCAACGTGG AGGCGC
HA-Maats1-F	TACGCATCAGCGAAAAGCTTAGCCAGACAGTGACCA TCCAGGAA
HA-Maats1-R	CCACACTGGACTAGTGGATCCCTAGCCTCTCCCTCAT CCTGAGTAGG
HA-Rsph3a-F	TACGCATCAGCGAAAAGCTGCCGCCACCAACATT GGG
HA-Rsph3a-R	CCACACTGGACTAGTGGATCCTCACTCTGCCATAAGG TGTCCCC
HA-Dynll2-F	TACGCATCAGCGAAAAGCTTCTGACCGGAAGGCAG TGATCAA
HA-Dynll2-R	CCACACTGGACTAGTGGATCCCTAGCCGACTTGAAG AGGAGGATT
HA-Ropn1-F	TACGCATCAGCGAAAAGCTCCTCAGACAGACAAGC AAGTATGC
HA-Ropn1-R	CCACACTGGACTAGTGGATCCTTATTCCAGCCGAACC CTAGGGT
HA-Ropn11-F	TACGCATCAGCGAAAAGCTCCGCTGCCGACACCA CCACACTGGACTAGTGGATCCCTATATTATCTTTTTTC
HA-Ropn11-R	CAACGAAGAAGTCCGAAAGT TACGCATCAGCGAAAAGCTTGACGCCGACAGCCTCT TG
HA-Rsph9-F	CCACACTGGACTAGTGGATCCCTACAGCATGAAGGGC AAGTCCA
HA-Rsph9-R	TACGCATCAGCGAAAAGCTTTCTTCTACCTGAGCA AGAAAATTGCTGT
HA-Wdr35-F	CCACACTGGACTAGTGGATCCTTATTCTACCGAGCTGT GGCATAAAGG
HA-Wdr35-R	TACGCATCAGCGAAAAGCTTCTGAAGGCTAAGATCC TCTTCGTGGG
HA-Ift22-F	CCACACTGGACTAGTGGATCCCTAGGTGATGATGAGC ATCTCCTCGC
HA-Ift22-R	TACGCATCAGCGAAAAGCTTCTGAAGGCTAAGATCC CTTCAGCCC
HA-Ift74-F	CCACACTGGACTAGTGGATCCTCAGCTTGCTGGCAT TATGTAGAGC
HA-Ift74-R	TACGCATCAGCGAAAAGCTTAGTGACCAAATCAAAT TCATCGTGGAC
HA-Ift81-F	CCACACTGGACTAGTGGATCCTCAGAGAACCAAGCCGG TCCTCTC
HA-Ift81-R	TACGCATCAGCGAAAAGCTTCGGACAAAGTGATT TAAAAGCTGAGTT
HA-Dyncli2-F	

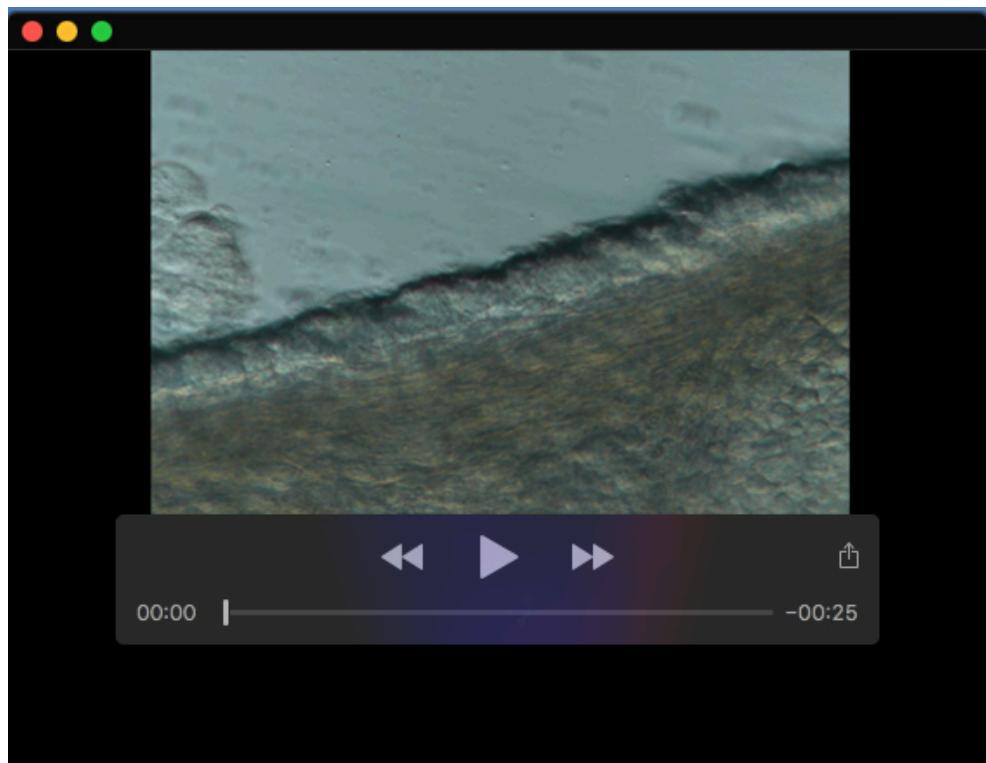
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HA-Dynll1-F	TACGCATCAGCGAAAAGCTTGCGACCGGAAGGC GG T
HA-Dynll1-R	CCACACTGGACTAGTGGATCCTAACCA CAGATTGAAC AGAAGAATGGCC
HA-Dynlrb2-F	TACGCATCAGCGAAAAGCTTACAGAAGTGGAGGAA ACCCTCAAGAG
HA-Dynlrb2-R	CCACACTGGACTAGTGGATCCCTACTCACATGGGTCT GAATGACAATCA
HA-Dynlt1a-F	TACGCATCAGCGAAAAGCTTGAAGACTTCCAGGCCT CCGAGG
HA-Dynlt1a-R	CCACACTGGACTAGTGGATCCTCAGATGGACAGTCCG AAGGTACTGA
exon1-F	TTGGCAAATCTAGAGGATCCAGGCTGCGCGTCCTCCT TG
exon1-R	AGGAGCAGAGCTTACCTCATCCGCAGCTCCAGGAC
intron1'+exon2+i	GTAAGCTCTGCTCCTGAATTATTCTATCCC
ntron2'-F	AGGTTGGCCTTTCTGCCAGGGAGGT CATGTCA
intron1'+exon2+i	AGAAAAGGCCAACCTTGCTGTTAC
ntron2'-R	GTAGTCCATGTCGACAAGCTAACGATGCCTGGCCAC GC
exon3-33-F	ATTGGGTACCTTAATAATGAGTCTCGGACCTCGC
exon3-33-R	ATCCCTCGACTTAATTACCACATTGTAGAGGTTTAC TTGCTTTAAAAAAC
firely luciferase-F	
firely luciferase-R	



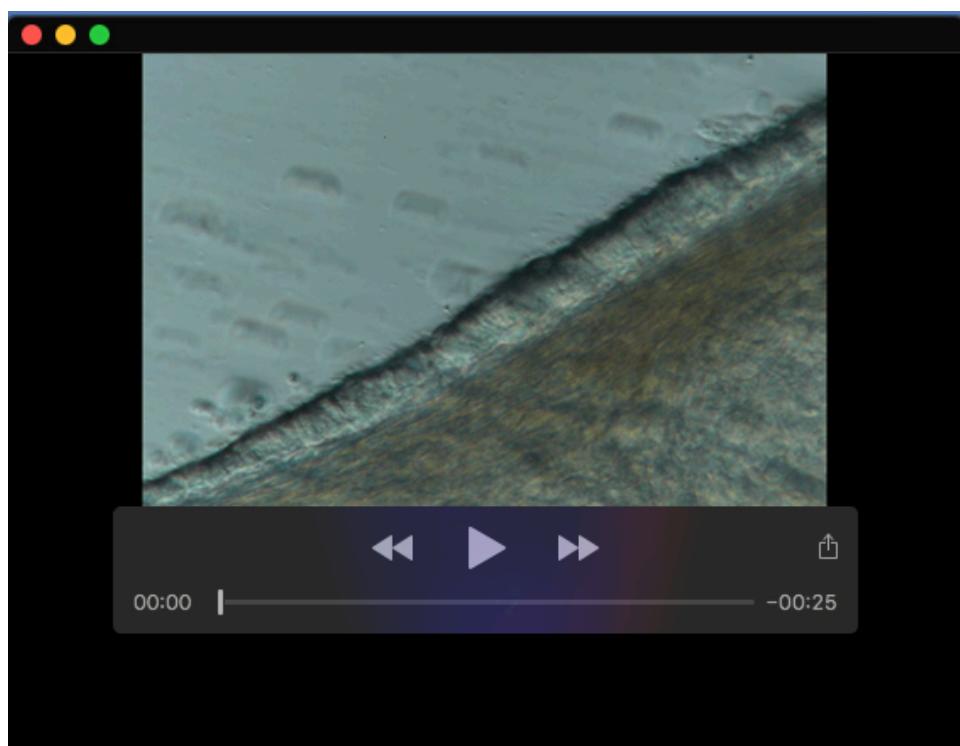
Movie 1. Motility and movement of the sperm from the caput epididymis of wild-type male mice.



Movie 2. Motility and movement of the sperm from the caput epididymis of *Cfap61*^{-/-} male mice.



Movie 3. Ciliary motility of wild type tracheal epithelial cells.



Movie 4. Ciliary motility of *Cfap61*^{-/-} tracheal epithelial cells.