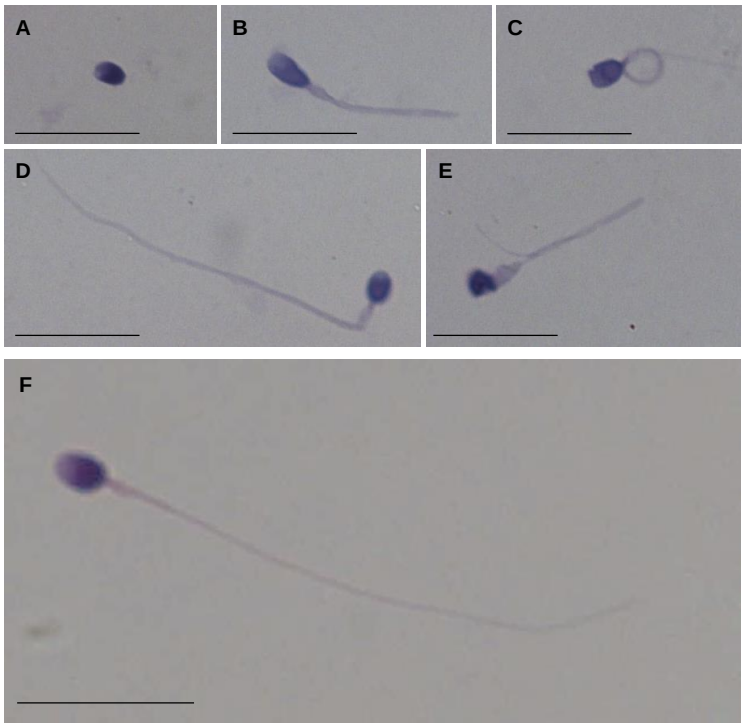
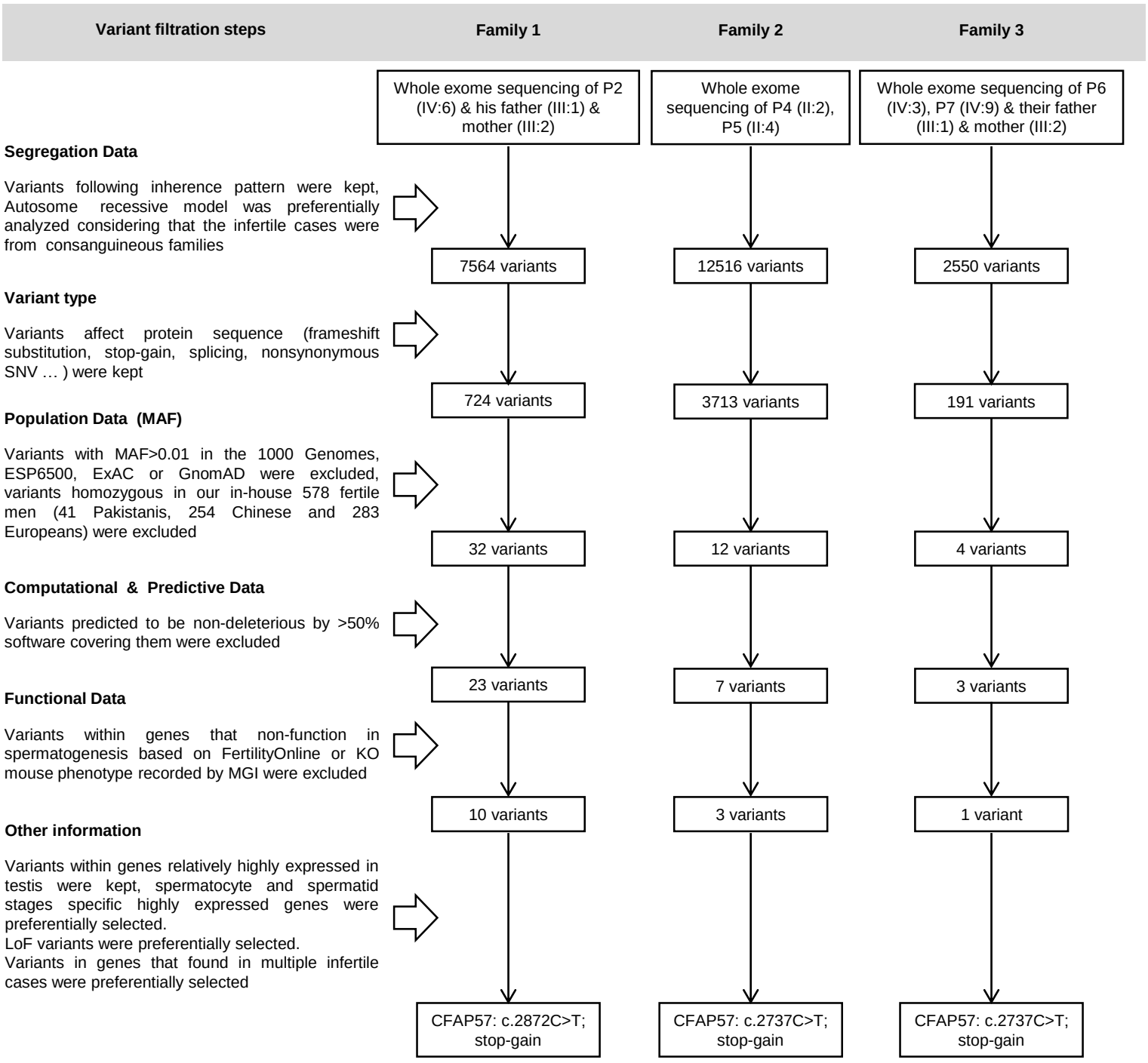
Supplementary Figure 1



Supplementary Figure 1. Sperm morphology of P7 and a fertile control. (**A-E**) Representative images showing spermatozoa with absent (A), short (B), coiled (C) and bent (D) flagella and flagella of irregular caliber (E) from P7 and spermatozoa with normal flagella (F) from a fertile control. Scale bars represent 10 µm.

Supplementary Figure 2

A



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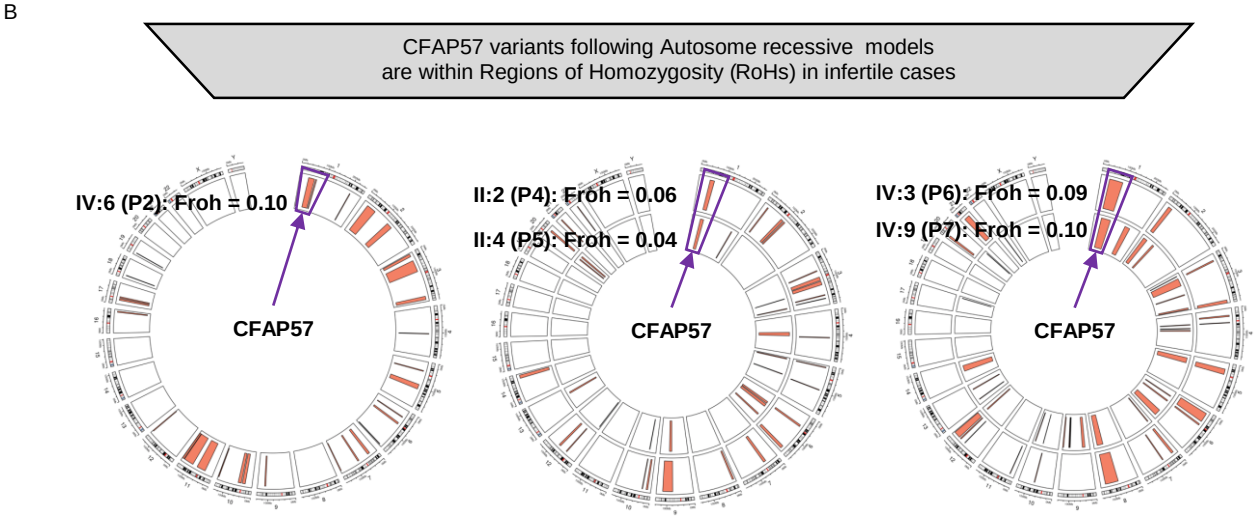
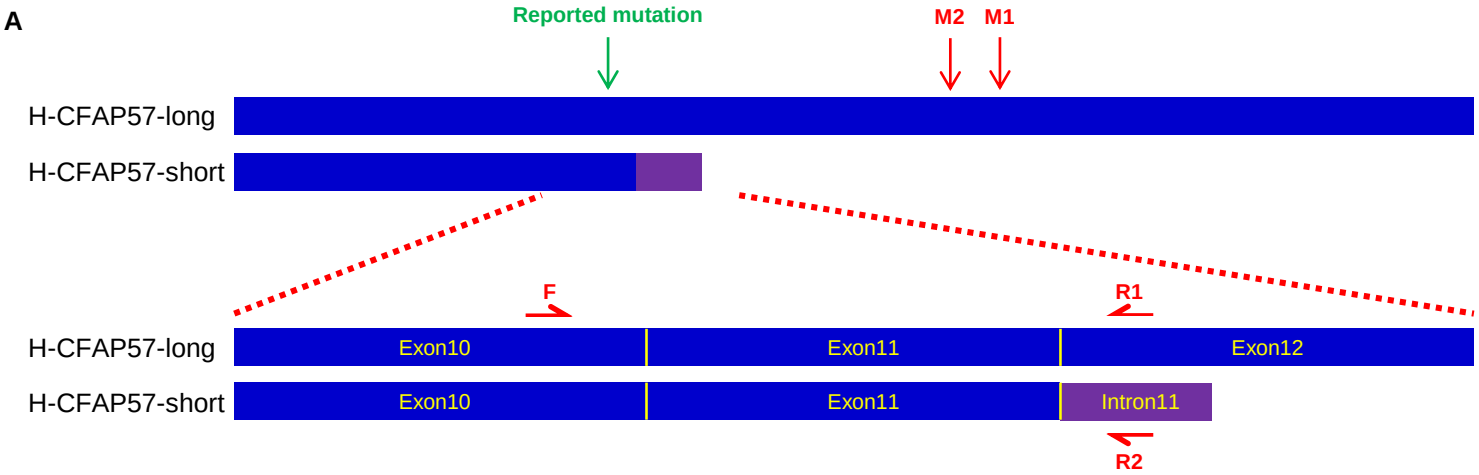
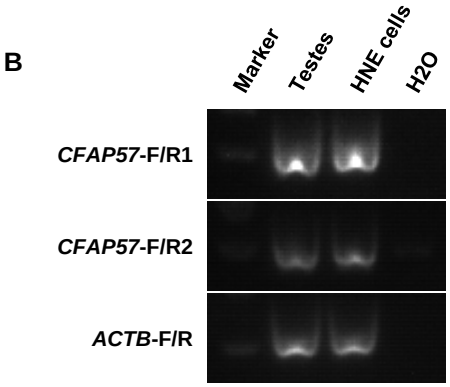


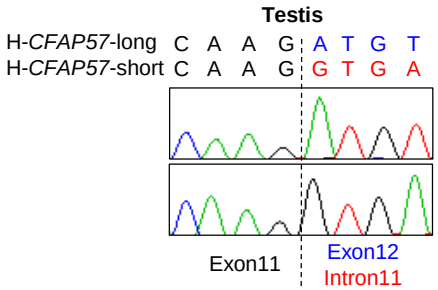
Supplementary Figure 2. Bioinformatic analysis of candidate variants. (**A**) Candidate pathogenic variants were filtered.

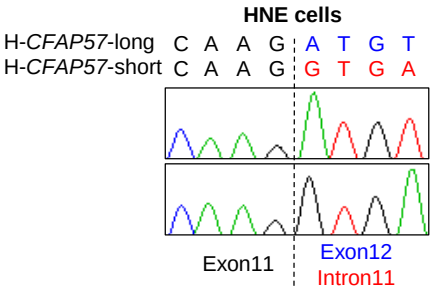
(**B**) CFAP57 variants are within RoHs in infertile cases.

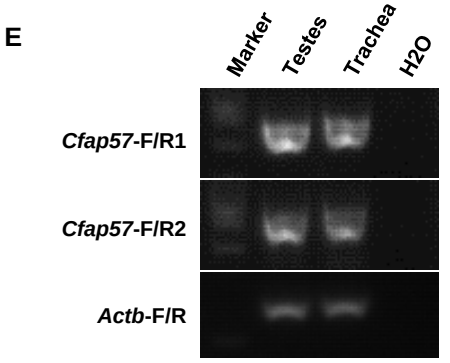
Supplementary Figure 3

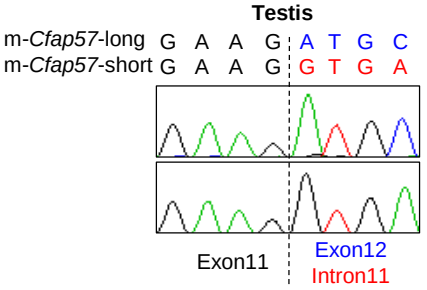


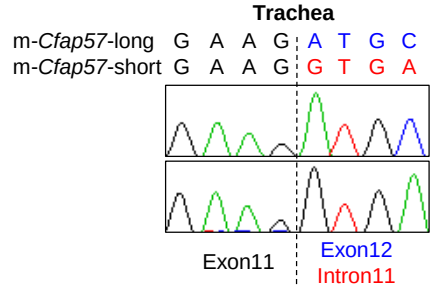












H

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Human           MSAVVAQTLHVFGLRSHVANNIFYFDEQIIIFPSGNHCVKYNVDQKWQKFIPGSEKSQGMLALSISPNRRYLAISETVQEKPAITIYELSSIPCRKRKVLNNFDFQVQKFISMAFSPDSKYLLAQTSPPE
Mouse           MSTVVAQALHVFGLRPHVTNNVFFFDEQIIIFPSGNHCVKYNIDQKWQKFIAGSDKSQGMLALAISPNRRYLAISETVQEKPAVTIYELSSIPCRKRKVLNNFDFQVQKFTSMAFSPDSKYLLTQTSPPD
Consensus       MSaVVAQaLHVFGLRpHVaNN!F%FDEQIIIFPSGNHCVKYN!DQKWQKFIaGS#KSQGMLALaISPNRRYLAISETVQEKPA!TIYELSSIPCRKRKVLNNFDFQVQKFiSMAFSPDSKYLLaQTSPP#

                131     140       150       160       170       180       190       200       210       220       230       240       250       260
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Human           SNLVYWLWEKQKVMAIVRIDTQNNPVYQVSFSPQDNTQVCVTGNGWFKLLRFAEGTLKQTSFQRGEPQNYLAHTAYADDKIVVGTDTGKLFLFESGDQRWETSIMVKEPTNGSKSLDVIQESESLIEFPP
Mouse           SNLVYWLWEKQKVMAIIKADSQNNPIYQVSISSQDNSQVCITGSGVFKLLRFAEGTLKQINFQRGESSNYLAHHAYSEDRVIVGTDTGKLFLFESGDQRWETSIMVKESTS-SRSLEVIQESESLIEFPP
Consensus       SNLVYWLWEKQKVMAI!raDsQNNP!YQVSiSpQDNsQVC!TGnGwFKLLRFAEGTLKqinFQRGEpqNYLAHaAYa#Dr!!VGTDTGKLFLFESGDQRWETSIMVKEpTn.SrSL#VIQESESLIEFPP

                261     270       280       290       300       310       320       330       340       350       360       370       380       390
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Human           VSSPLPSYEQMVRASSHSQMSMPQVFAIAAYSKGFACSAGPGRVLLFEKMEEKDFYRESREIRIPVDPQSNDPSQSDKQDVLCLCFSPSEETLVASTSKNQLYSITMSLTEISKGEPAHFEYLMYPLHSA
Mouse           LSSPVSSVERMDITTNTQQQAMPQVFAIAAYSKGFACSAGPGRVLLFEKVEEKDFYRESREIRIPTDQQSNDPSQSDKQDVLCLCFSPSEETLIASTNKNQLYSITMSLTEISKGEAAHFEYLLYPLHSA
Consensus       lSSPlpSyErMdaasnhqQqaMPQVFAIAAYSKGFACSAGPGRVLLFEKmEEKDFYRESREIRIPtDqQSNDPSQSDKQDVLCLCFSPSEETL!ASTnKNQLYSITMSLTEISKGEaAHFEYL$YPLHSA

                391     400       410       420       430       440       450       460       470       480       490       500       510       520
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Human           PITGLATCIRKPLIATCSLDRSIRLWNYETNTLELFKEYQEEAYSISLHPSGHFIVVGFADKLRLMNLLIDDIRSFKEYSVRGCGECSFSNGGHLFAAVNGNVIHVYTTTSLENISSLKGHTGKIRSIVW
Mouse           SITGLDTCIRKPLIATCSLDRSVRIWNYESNTLELYKEYQEEAYTVSLHPSGHYIVVGFADKLRLMNLLIDDIRPFKEYSVRGCKECSFSNGGHLFAAVNGNVIHIFTTTSLENINILKGHTGKIRSLVW
Consensus       pITGLaTCIRKPLIATCSLDRS!RiWNYEsNTLEL%KEYQEEAYs!SLHPSGH%IVVGFADKLRLMNLLIDDIRpFKEYSVRGCgECSFSNGGHLFAAVNGNVIH!%TTTSLENIniLKGHTGKIRSiVW

                521     530       540       550       560       570       580       590       600       610       620       630       640       650
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Human           NADDSKLISGGTDGAVYEWNLSTGKRETECVLKSCSYNCVTVSPDAKIIFAVGSDHTLKEIADSLILREISAFDVTYTAIVISHSGRMMFVGTSVGTIRAMKYPLPLQKEFNEYQAHAGPITKMLLTFDD
Mouse           NLDDSKLVSAGTDGAVYEWNLSTGKRETECVLKSCSYNSVTTSPDAKVIFAVGSDQTLKEISDSLILREIPAFDVIYTSITISHSGRMIFVGTSVGTIRAMKYPLSLQKEYNEYQAHAGPVMKMLLTFDD
Consensus       NaDDSKL!SaGTDGAVYEWNLSTGKRETECVLKSCSYNcVTvSPDAK!IFAVGSDqTLKEIaDSLILREIpAFDViYTaItISHSGRMiFVGTSVGTIRAMKYPLpLQKE%NEYQAHAGP!mKMLLTFDD

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                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Human           QFLLTAAEDGCLFTWKVFDKDGRGIKRERVGFAEEVLVTKTDMEEKAQVMLELKTRVEELKMENEYQLRLKDMNYSEKIKELTDKFIQEMESLKTKNQVLRTEKEKQDVYHMEHIEDLLDKQSRELQDM
Mouse           QFLLTVGEDGCLFTWKVFDKDGRGIKRERVGFAEEVLVTKTDMEEKAQIMLELKTRVEELKMENEYQLRLKDMNYTEKIKELTDKFIQEMESLKTKNQVLKTEKEKQDISHRERLEELVDKQTRELQDL
Consensus       QFLLTaaEDGCLFTWKVFDKDGRGIKRERVGFAEEVLVTKTDMEEKAQ!MLELKTRVEELKMENEYQLRLKDMNYsEKIKELTDKFIQEMESLKTKNQVLrTEKEKQD!sHrEriE#LlDKQsRELQD$

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Human           ECCNNQKLLLEYEKYQELQLKSQRMQEEYEKQLRDNDETKSQALEELTEFYEAKLQEKTTLLEERQEDYRQQLREFEETKKQIEEDEREIQDIKTKYEKKLRDEKESNLRLKGETGIMRKKFSSLQKEI
Mouse           ECCNNQKLLLEYEKYQELQLKSQRMQEEYEKQLRDNDETKSQALEELTEFYEAKLQEKTGLLEERQEDVRQQLREFEETKKQIEEDERDIQDIKTKYERKLRDEKESNLRLKGETGIMRKKFSSLQKEI
Consensus       ECCNNQKLLLEYEKYQELQLKSQRMQEEYEKQLRDNDETKSQALEELTEFYEAKLQEKTgLLEERQEDVRQQLREFEETKKQIEEDERDIQDIKTKYEr KLRDEKESNLRLKGETGIMRKKFSSLQKEI

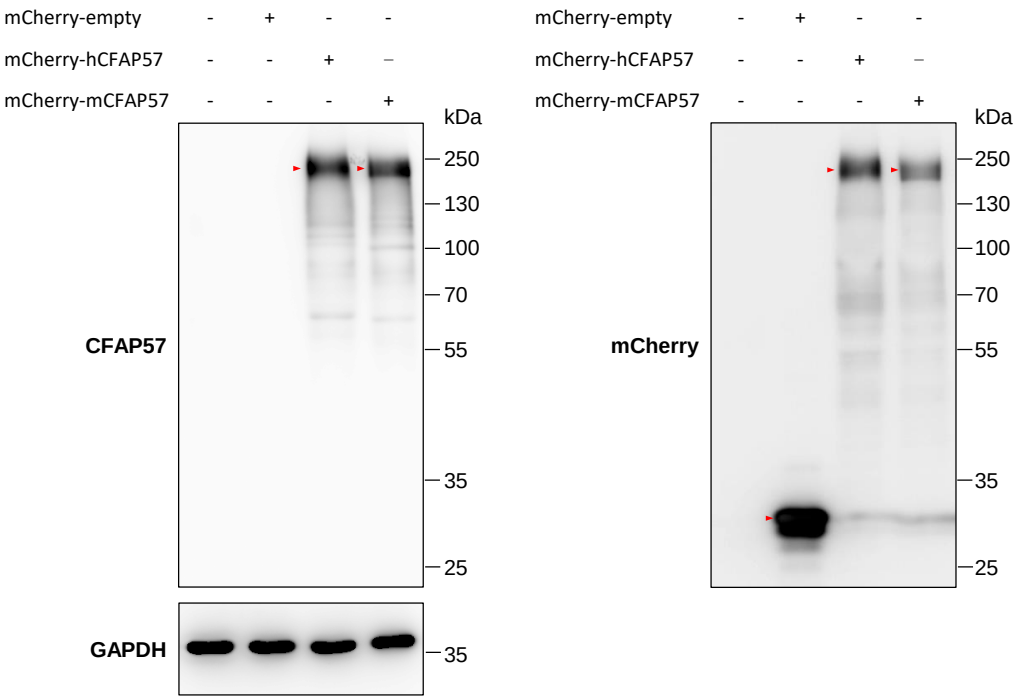
                911     920       930       940       950       960       970       980       990       1000      1010      1020      1030      1040
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Human           EERTNDIETLKGEQMKLQGVIKSLEKDIQGLKREIQERDETIQDKEKRIYDLKKKNQELGKFKFVLDYKIKELKKQIEPRENEIRVMKEQIQEMERELENFHKQNTQLELNITELMQKLRATDQEMRRER
Mouse           EERTNDIELLKSEQMKLQGIRSLEKDIQGLKREIQERDETIQDKEKRIYDLKKKNQELEKFKFVLDYKIKELKKQIEPRENEIKVMKEQIQEMERELERFHKQNTQLELNITELLQKLRATDQEMRKEQ
Consensus       EERTNDIElLKgEQMKLQG!IrSLEKDIQGLKREIQERDETIQDKEKRIYDLKKKNQELeKFKFVLDYKIKELKKQIEPRENEIrVMKEQIQEMERELEr FHKQNTQLELNITELlQKLRATDQEMRrEr

                1041    1050      1060      1070      1080      1090      1100      1110      1120      1130      1140      1150      1160      1170
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Human           QKERDLEALVKRFKTDLHNCVAYIQEPRLLKEKVRGLFEKYVQRADMVEIAGLNTDLQQEYTRQREHLERNLATLKKKVVKEGELHRTDYYRIMQENVSLIKEINELRRELKFTRSQVYDLEAALKLTKK
Mouse           QKERDLEALVRRFKTDLHNCVAYIQEPGLLKEKIRGLFEKYVQRADMVEIAGLNSDLQQEYARQREHLERNLATLKKKVIKEGELHRTDYYRIMQENVSLIKEINELRRELKLTRSQIYDLESALKVSKK
Consensus       QKERDLEALVrRFKTDLHNCVAYIQEPrLLKEK!RGLFEKYVQRADMVEIAGLNsDLQQEYaRQREHLERNLATLKKKV!KEGELHRTDYYRIMQENVSLIKEINELRRELK!TRSQ!YDLEaALKlsKK

                1171    1180      1190      1200      1210      1220      1230      1240      1250
                |--------+---------+---------+---------+---------+---------+---------+---------|
Human           VRPQEVSETEPSRDMLSTAPTARLNEQEETGRIIEMQRLEIQRLRDQIQEQEQVTGFHTILAGVRLPSLSNSEVDLEVKTN
Mouse           TRSQEVPESVISKDVVGSTSTMRLNEQEETGRIIEMQRLEIRRLRDQIQEQEQVPGFHTIAGVRLPSIVDSDVDFEVHTK
Consensus       tRpQEVpEseiSrDmlgsapTaRLNEQEETGRIIEMQRLEIrRLRDQIQEQEQVpGFHTiAGVRLPSis#S#VDlEVhTn
```

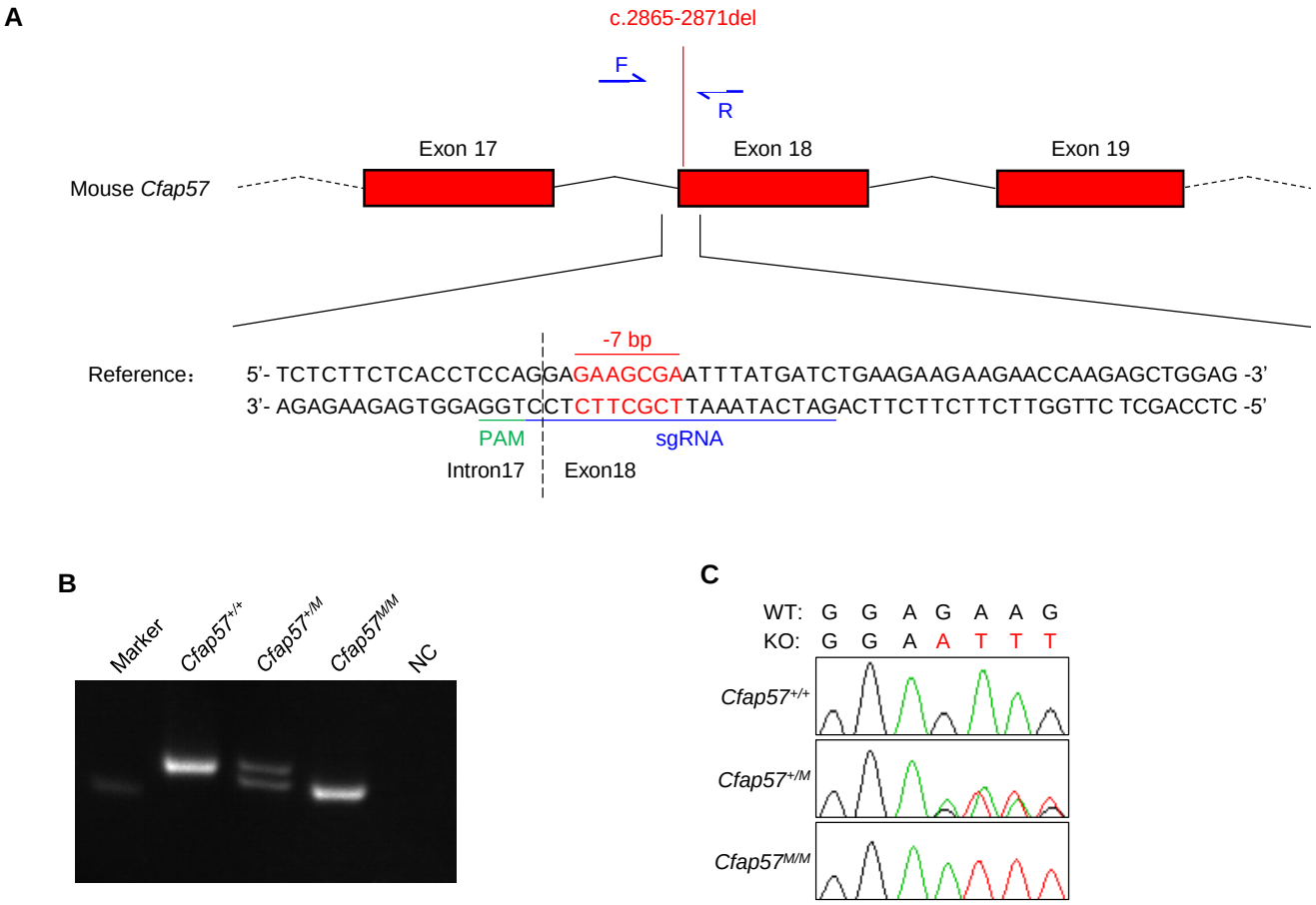
Supplementary Figure 3. Two transcripts of *CFAP57/Cfap57* are both expressed in testes and HNE cells/ mice trachea. (**A**) Schematic illustrating two transcriptions of *CFAP57*. (**B-D**) PAGE and Sanger sequencing results showing the expression of both *CFAP57* transcriptions in testes and HNE cells. (**E-G**) PAGE and Sanger sequencing results showing the expression of both *Cfap57* transcriptions in testes and trachea. (**H**) CFAP57 was conserved in human and mice. Sequence alignment shows conservation of human and mice CFAP57 protein. Red letters represent completely same sequence. The alignment was performed using the online software MultAlin (http://multalin.toulouse.inra.fr/multalin/multalin.html).

Supplementary Figure 4



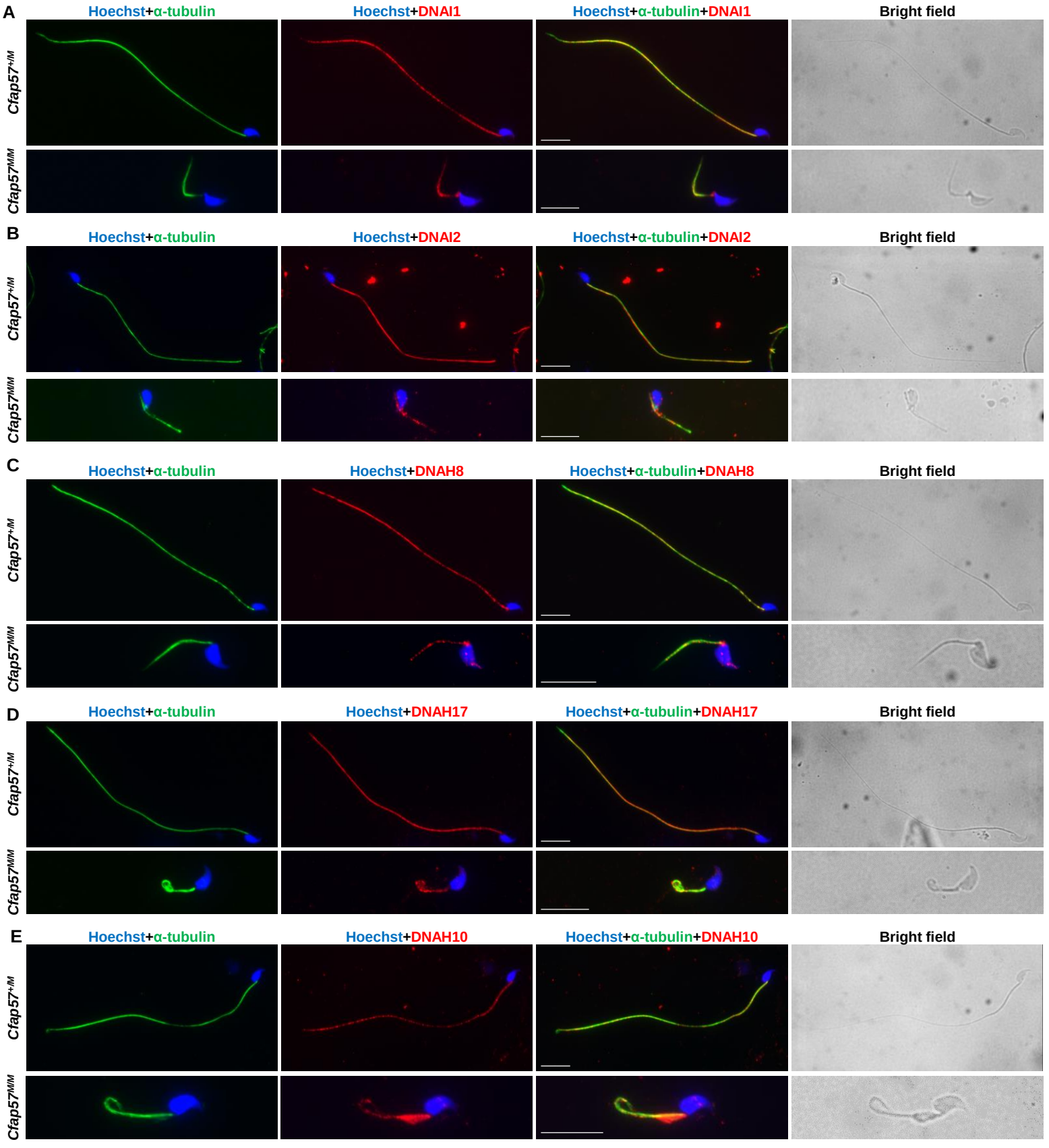
Supplementary Figure 4. Anti-CFAP57 antibody was able to recognize both human and mice CFAP57 *in vitro*. MCherry-tagged human and mice CFAP57 and mCherry were expressed in cultured HEK293T cells. Both anti-mCherry antibodies and anti-CFAP57 antibodies were used to detect the mCherry-tagged CFAP57. Arrows indicate the positions of bands corresponding to proteins of interest.

Supplementary Figure 5

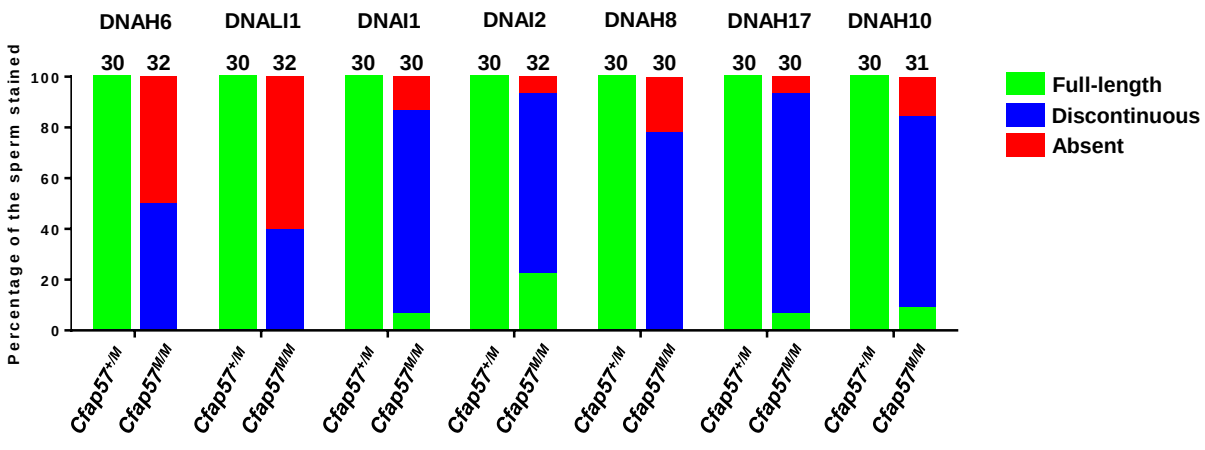


Supplementary Figure 5. *Cfap57* mutant mice were generated by CRISPR/Cas9. (**A**) Schematic illustrating construction of the mouse model (*Cfap57*$^{M/M}$). (**B**) PAGE results showing the 7 bp deletion in *Cfap57*$^{M/M}$ mice. (**C**) Sanger sequencing results of *Cfap57*$^{+/+}$, *Cfap57*$^{+/M}$ and *Cfap57*$^{M/M}$ mice.

Supplementary Figure 6

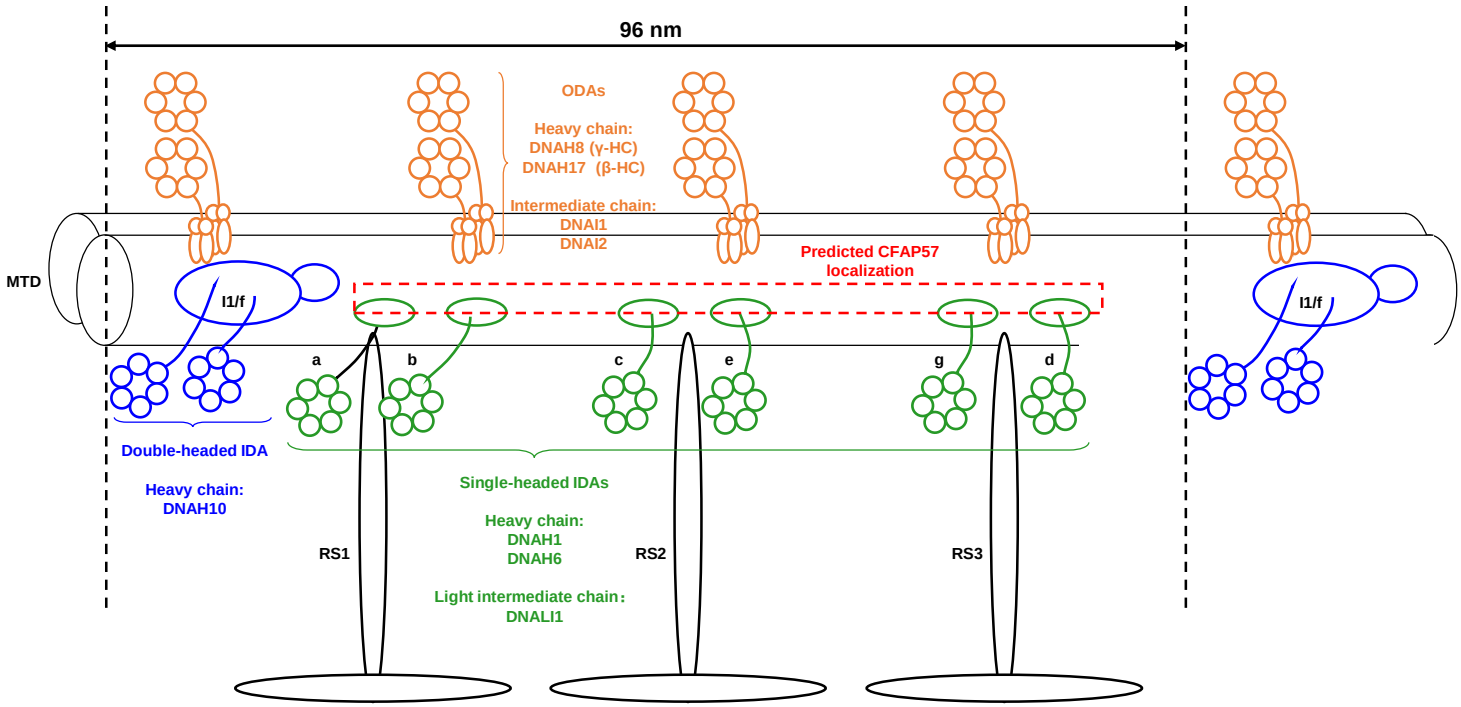


F



Supplementary Figure 6. ODA and double-headed IDA proteins still exist but show abnormal signals in sperm from *Cfap57*$^{M/M}$ mice. (**A-E**) Representative images of spermatozoa from *Cfap57*$^{+/M}$ and *Cfap57*$^{M/M}$ mice co-stained with anti-α-Tubulin and anti-DNAI1 antibodies (**A**), anti-DNAI2 antibodies (**B**), anti-DNAH8 antibodies (**C**), anti-DNAH17 antibodies (**D**), or anti-DNAH10 antibodies (**E**). Scale bars represent 10 μm. (**F**) Percentage of spermatozoa with full-length, discontinuous or absolutely absent signals of the stained antibodies. At least 30 sperm were analyzed after stained for each antibody.

Supplementary Figure 7



Supplementary Figure 7. Diagram for the localizations of proteins used as ODA/IDA markers. ODAs are drawn in orange, double-headed IDAs are drawn in blue and the single-headed IDAs are drawn in green. The red rectangle represents the predicted localization of CFAP57, which is connected to a subset of single-headed IDAs but not double-headed IDAs or ODAs.

IDA, inner dynein arm. ODA, outer dynein arm. MTD, microtubules doublet. RS, radial spoke.