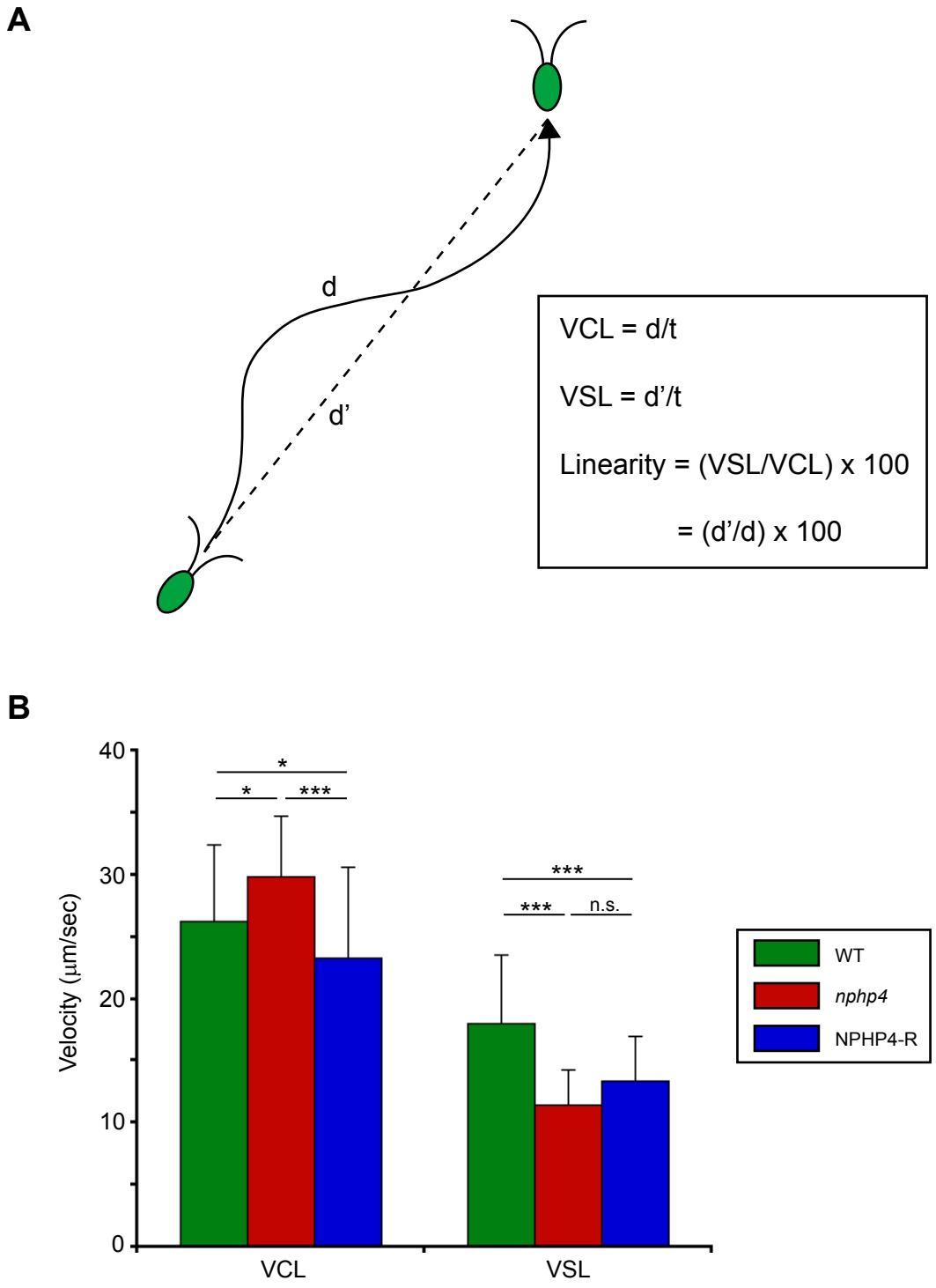

Fig. S1. Motility of *C. reinhardtii* cells analyzed by CASA. (A) Diagram indicating the two parameters used to calculate percent linearity (VSL/VCL x 100). VCL (velocity curvilinear) is "instantaneous" velocity determined point to point along the actual swimming path (d). VSL (velocity straight line) is calculated from the linear distance between the start and end points of swimming paths recorded by CASA (d'). (B) Means ± s.d. of VCL and VSL were calculated from values obtained from a total of 10 fields in each of 5 independent experiments. Statistical significance was determined by the Tukey-Kramer method: n.s., ≥0.05; *, <0.05-0.01; ***, <0.001.

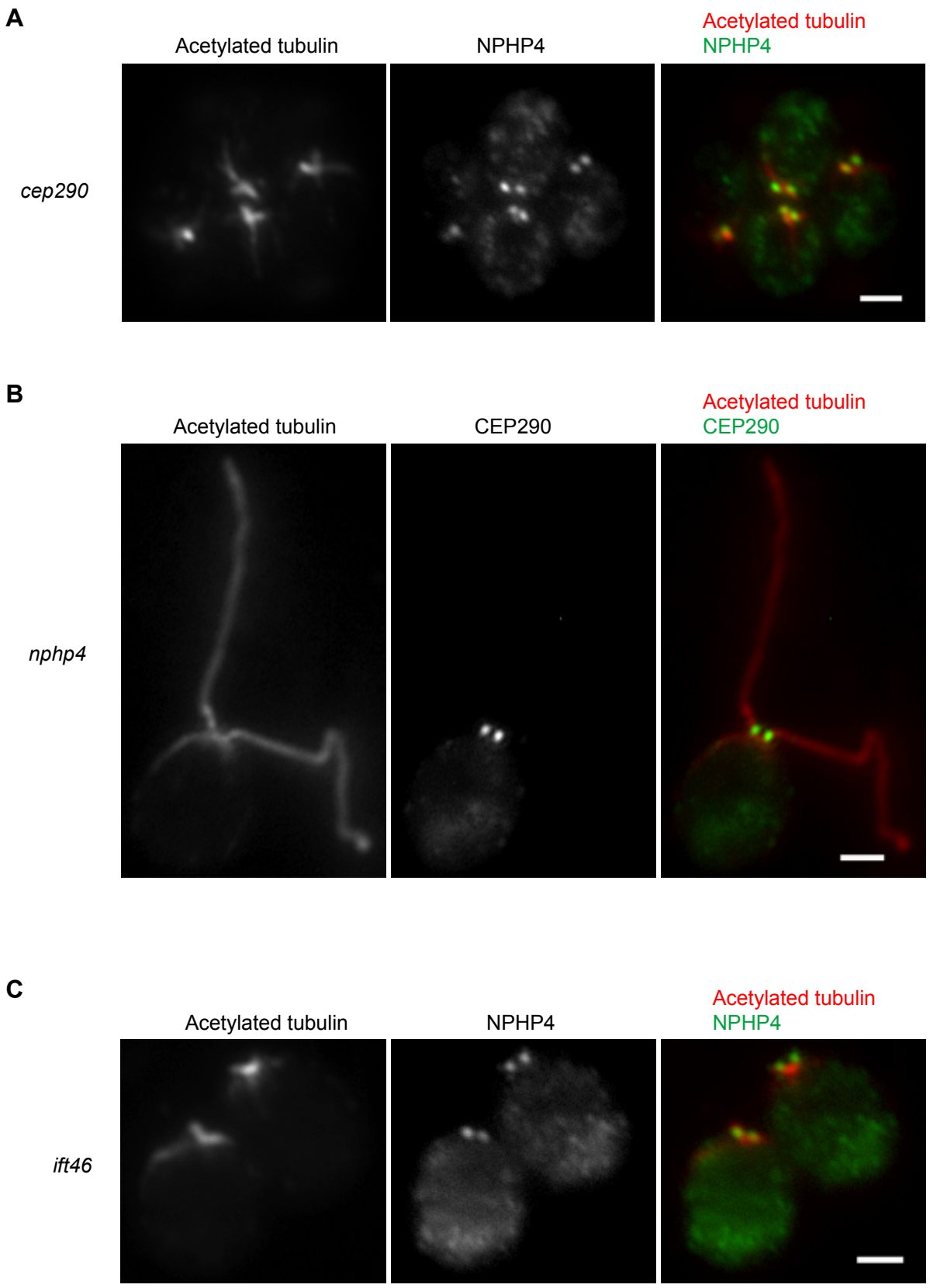

Fig. S2. NPHP4 and CEP290 localize to the TZ independently of each other. (A) Four *cep290* cells still within the mother cell wall were labeled with antibodies to acetylated tubulin and NPHP4; a merged image is on the right. (B) A *nphp4* cell was labeled with anti-acetylated tubulin and anti-CEP290; a merged image is on the right. NPHP4 is localized normally in the TZ of the *cep290* mutant, and CEP290 is localized normally in the TZ of the *nphp4* mutant. (C) Immunofluorescence microscopy of *ift46* cells labeled with antibodies against acetylated tubulin and NPHP4. The merged image is on the right. Bars are 2 μm.

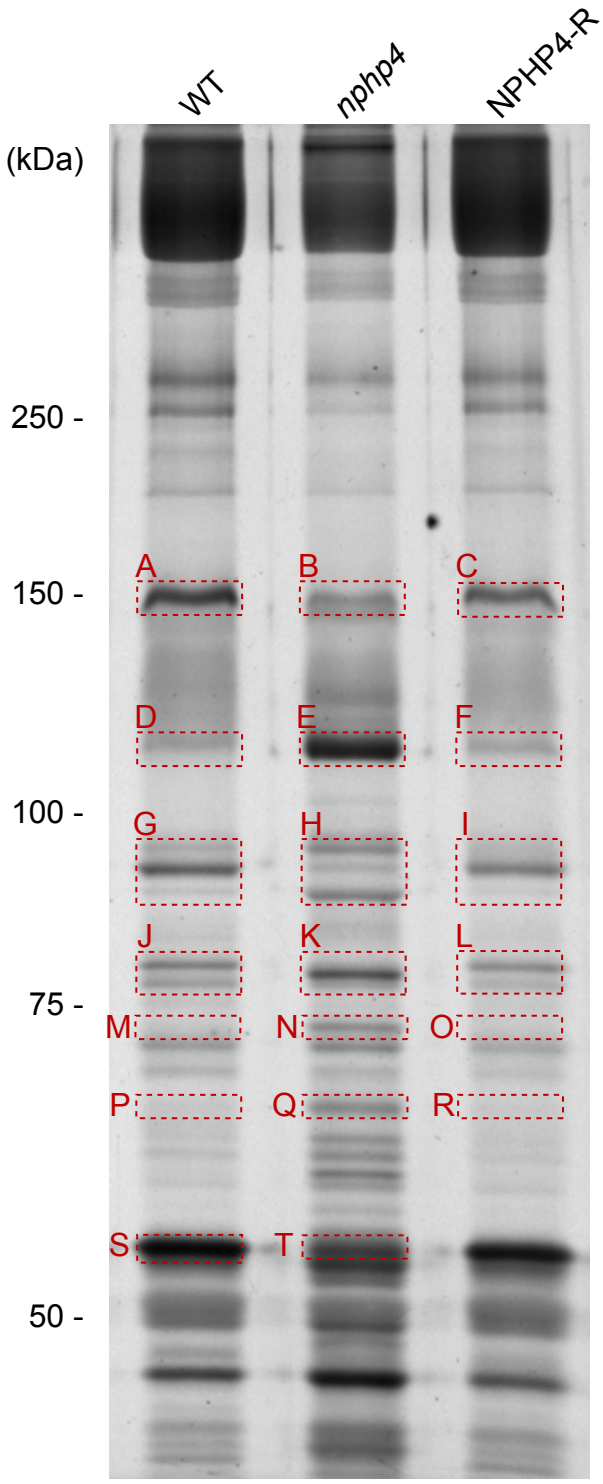

Fig. S3. *nphp4* cells have abnormal flagellar protein composition that is rescued by transformation with wild-type NPHP4. Proteins of isolated membrane-plus-matrix of wild-type (WT), *nphp4*, and NPHP4-R flagella were separated in a 6% SDS-polyacrylamide gel. Rectangles with dashed lines indicate slices excised for analysis by mass spectrometry (see text, Table I, and Table S1).

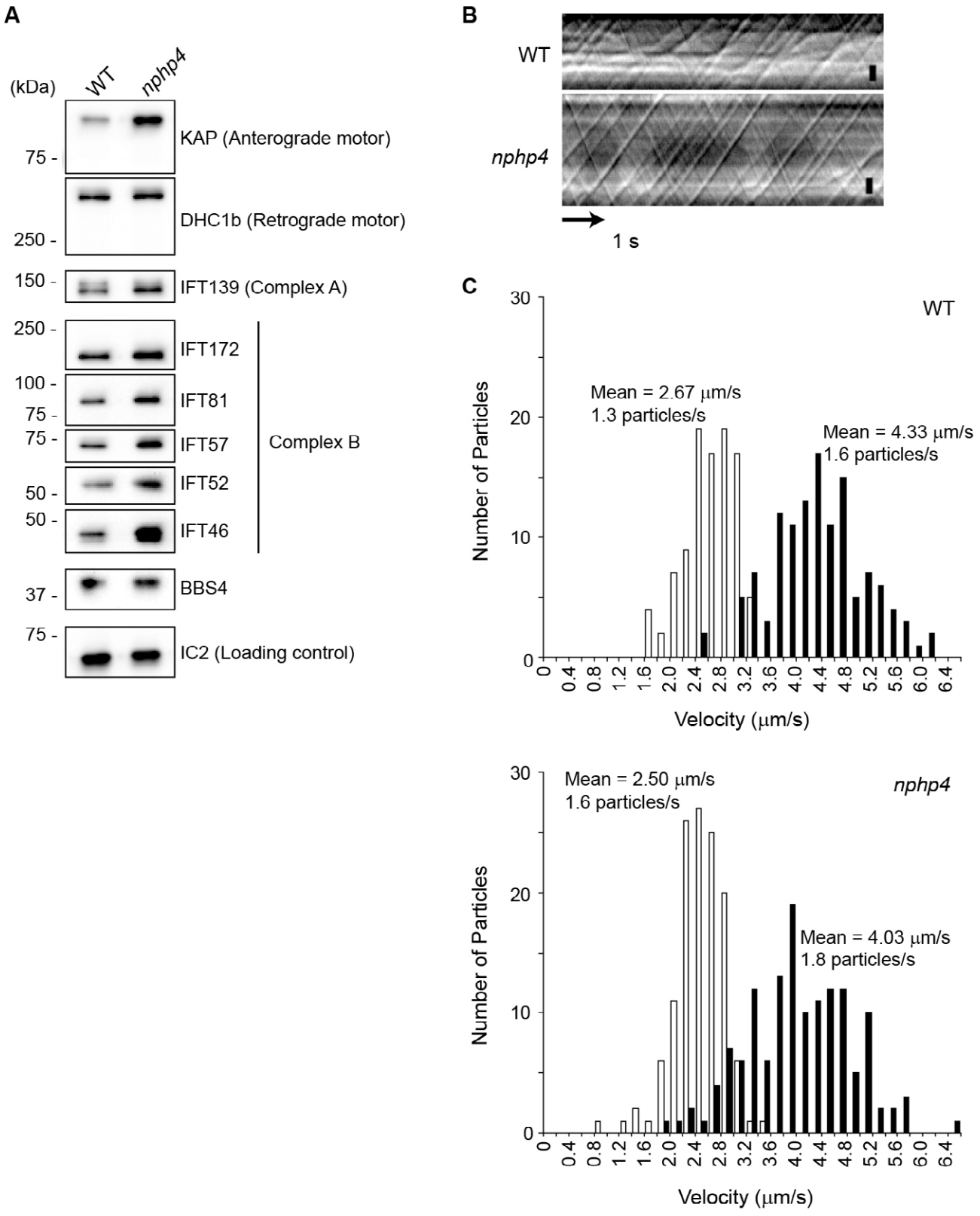

Fig. S4. The absence of NPHP4 has little or no effect on IFT. (A) Western blots of isolated wild-type and *nphp4* flagella probed with antibodies against IFT motor subunits (KAP and DHC1b); IFT complex-A protein IFT139; IFT complex-B proteins (IFT172, IFT81, IFT57, IFT52 and IFT46); and a subunit (BBS4) of the BBSome, an IFT cargo adaptor. The outer arm dynein intermediate chain IC2 was used as a loading control. (B) Kymographs of IFT in wild-type (WT) and *nphp4* flagella. IFT was recorded by DIC microscopy. Bars are 1 μm. (C) Velocity and frequency of IFT particles in wild-type and *nphp4* flagella. The values were determined from kymographs of eight movies analyzed for 10 s each. Anterograde and retrograde IFT are indicated by open and solid bars, respectively. P values between wild-type and *nphp4*: 0.0018 for anterograde velocity, 0.0016 for retrograde velocity, 0.0541 for anterograde frequency, and 0.5667 for retrograde frequency. Values were obtained by Student's t-test.

Table S1. Complete list of proteins identified by mass spectrometry analysis of selected slices of SDS-polyacrylamide gel of isolated membrane-plus-matrix fractions of wild-type, *nphp4* (Mutant), and NPHP4-R (Rescued) flagella. All proteins identified from slices A-T labeled in Figure S4 are listed with total number of peptides found for each. Protein IDs are from the Augustus 5 database. Proteins drastically decreased in *nphp4* flagella are in red and those increased are in blue (see text for criteria). Membrane-associated proteins identified by less than 5 peptides in wild-type flagella and not detected at all in the *nphp4* mutant flagella are in yellow. The protein ID for FMG-1B is not available (N/A). The gel slice from the rescued strain corresponding to slices S and T from wild type and the *nphp4* mutant respectively was lost; FAP12 was considered decreased in the mutant flagella based on the many fewer peptides found for it in the mutant flagella as compared to wild-type flagella.

Download Table S1.

Table S2. Primers used in this study

Name	Sequence
Primers used to map the deletion in the *nphp4* mutant	
NPHP4-Nf	5’-GCGCTCCACTCAATAACCAT-3’
NPHP4-Nr	5’-AGGTAAGTCCCCTCCTGGAA-3’
NPHP4-Cf	5’-AGGTAGATGGGGTGCTGGTG-3’
NPHP4-Cr	5’-TAGAATTGGGAGCCTTGACG-3’
DRC3-Nf	5’-GAATGCATCCAGGTGTGTTG-3’
DRC3-Nr	5’-ACGCCAGGTTTTTGAAGCTA-3’
DRC3-Cf	5’-ACATGGGCAAGATTGACTCC-3’
DRC3-Cr	5’-ACTGCTTGAATGTCCGCTTT-3’
531450f	5’-CAGCCAGACAACAAACGGTA-3’
531450r	5’-GCCCTGGAAGTTCTGATACG-3’
531350f	5’-TGGGCTTATTTTCTGCCAAG-3’
531350r	5’-CCTGCGACCTGTGTTACTGT-3’
531300f	5’-CTGCTATGGCTCGTGTTGTG-3’
531300r	5’-GCACATACGGTAGCGAACCT-3’
531200-Nf	5’-CATTCCTTTGAGGTGCATTG-3’
531200-Nr	5’-GCCGTTTTGTTCCCTAGTTG-3’
531200-Cf	5’-GGTATTTGCGGTCCAACACT-3’
531200-Cr	5’-ATGACATAGTCCGCCCAGTC-3’
531150-Nf	5’-GCATAAATTCGAGCGACAGC-3’
531150-Nr	5’-ACAAGTGCCGTCAACTAAGG-3’
531150-Cf	5’-TGAATGCACTGAACCGAGTG-3’

531150-Cr	5’-ATTACTCTCACTCCCACTCG-3’

Primers used to amplify the last exon of NPHP4

NPHP4Ex28-N	5’-GGATCCACGTTTGAGGTGGAGCTGCC-3’
NPHP4Ex28-C	5’-GTCGACTACTTATAAATGCGCACACGG-3’