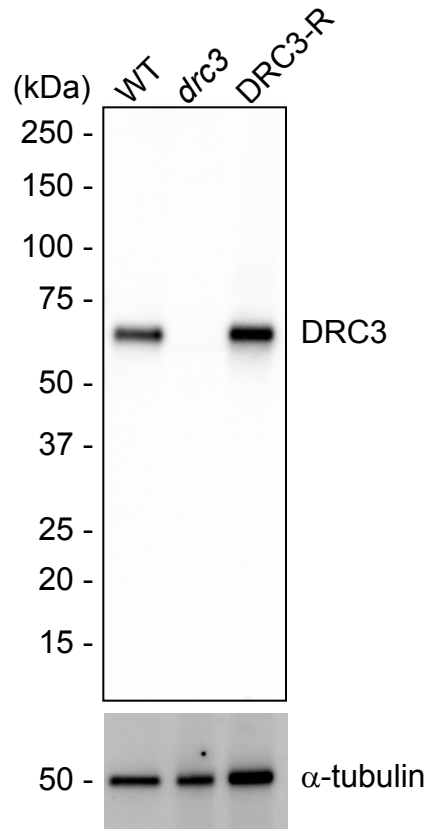


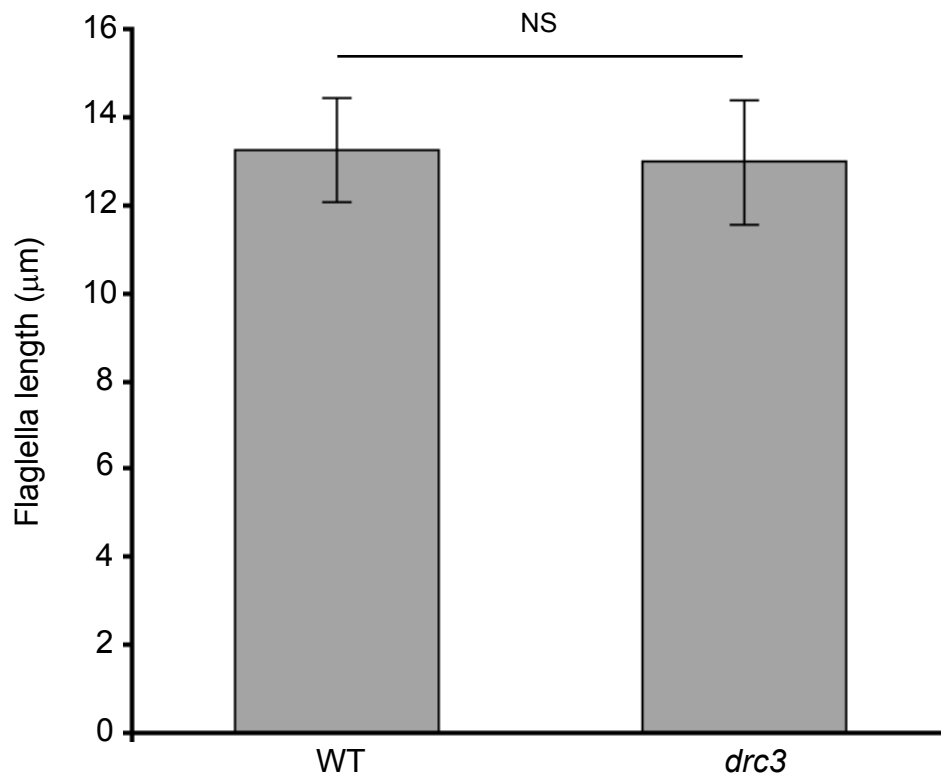
# Supplemental Materials

*Molecular Biology of the Cell*

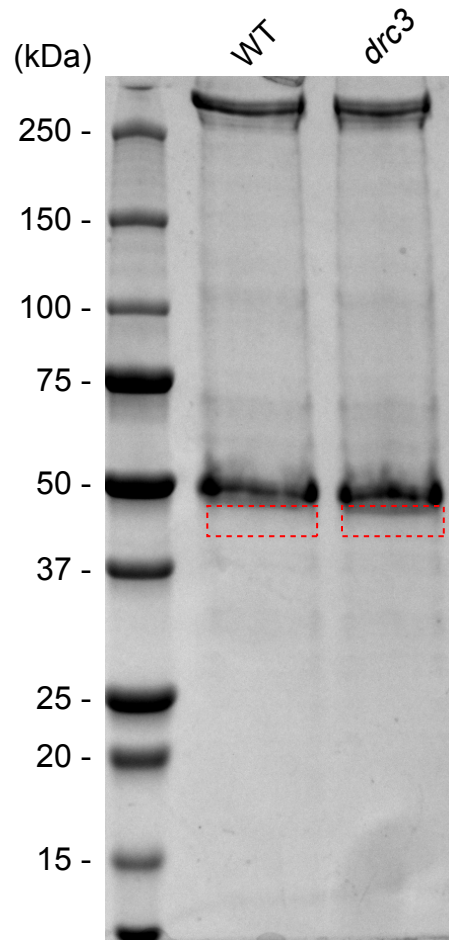
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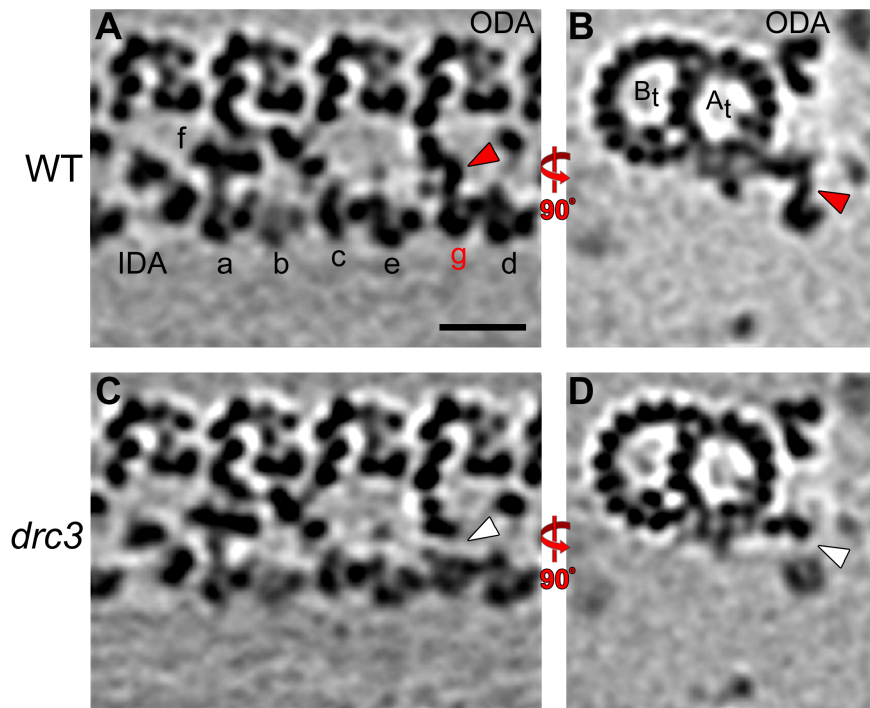
**FIGURE S1. DRC3 is not expressed in the *drc3* mutant.** Western blot of whole-cell lysates probed with anti-DRC3 antibody. The antibody recognized a band of the predicted size for DRC3 (60 kDa) in wild type (WT) and the rescued strain (DRC3-R), but not in *drc3* cells.  $\alpha$ -tubulin served as a loading control.



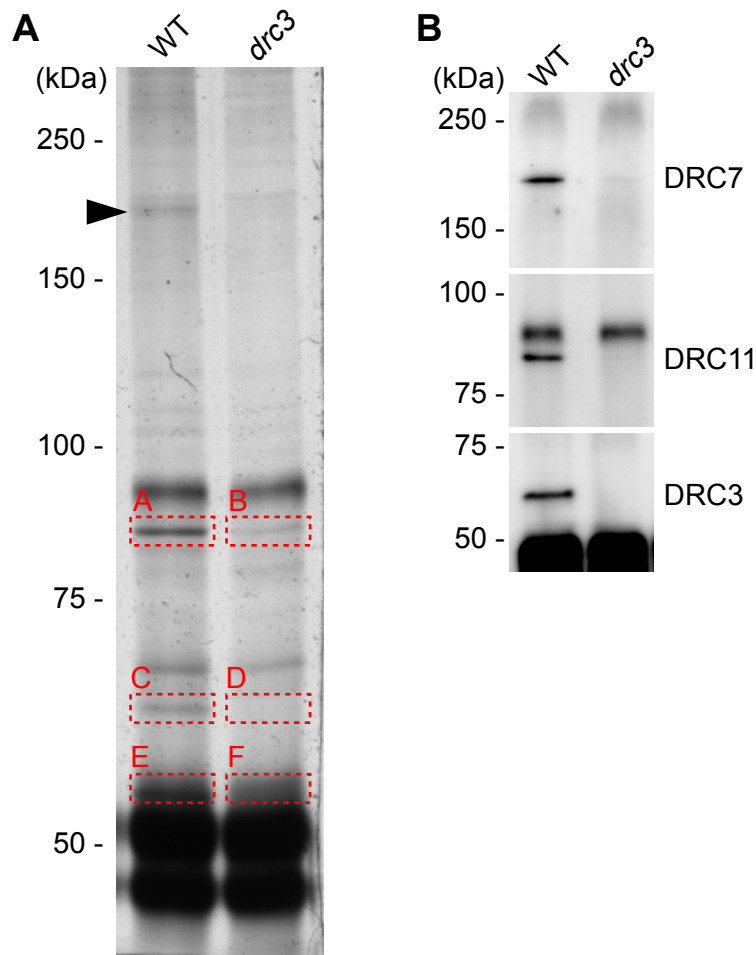
**FIGURE S2. Length of flagella in wild-type (WT) and *drc3* mutant cells.** Means  $\pm$  SDs of flagella length were determined for 30 cells of each strain. Statistical significance was determined by Student's t-test.



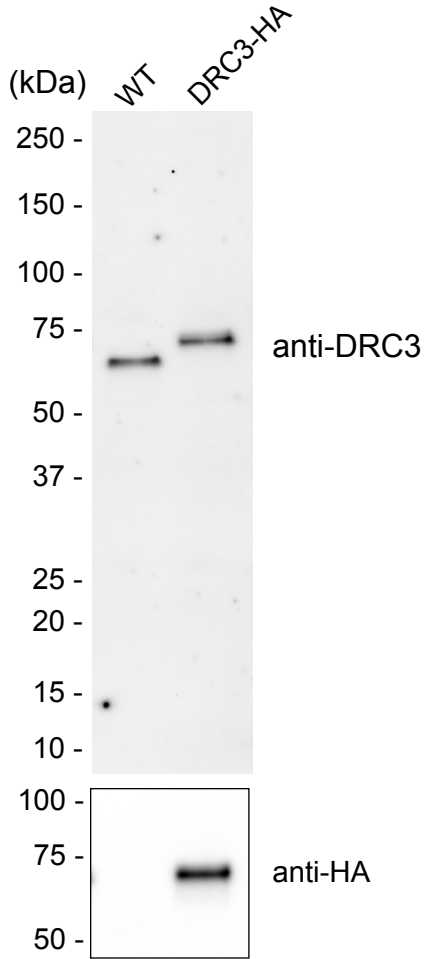
**FIGURE S3. Identification of DRC9 and DRC10 in *drc3* axonemes.** 0.4 M KI extracts of wild-type (WT) and *drc3* axonemes were separated by SDS-PAGE and then stained with Coomassie brilliant blue R-250. Rectangles with dashed lines indicate slices excised for analysis by MS.



**FIGURE S4. Identification of structural defects in *drc3* axonemes by comparison of the axonemal repeats from wild-type (WT) and *drc3* axonemes.** Longitudinal (A, C) and cross-sectional (B, D) tomographic slices of the averaged 96-nm axonemal repeats from wild type (A, B) and *drc3* (C, D) axonemes reveal the density of the L1 protrusion that directly connects to inner dynein arm g in wild type (red arrowheads), but is missing in *drc3* axonemes (white arrowheads). Note that the densities of other major axonemal structures, such as the outer dynein arms and inner arm dyneins, are not changed in *drc3*. Scale bar: 20 nm.



**FIGURE S5. Anti-DRC3 immunoprecipitates from KI extracts of isolated axonemes of wild-type (WT) and *drc3* cells.** (A) Silver-stained SDS-polyacrylamide gel of proteins pulled down with anti-DRC3. Rectangles with dashed lines indicate slices excised for analysis by MS; DRC3, 4 and 11 were identified in the gel slices (see Table S1). An arrowhead indicates a band likely to be DRC7 based on size. (B) The same samples as in (A) were analyzed in western blots probed with anti-DRC3, 7, and 11 antibodies. DRC7 and DRC11 were specifically co-precipitated with DRC3. The band present in both lanes at ~90 kDa represents anti-DRC3 antibody that was not completely denatured and was recognized by the secondary antibody.



**FIGURE S6. DRC3-HA strain used for the anti-HA immunoprecipitation experiment.**

Western blot of isolated flagella of wild type (WT) and the DRC3-HA strain, probed with antibodies against DRC3 (upper panel) and the HA peptide (lower panel). DRC3-HA is present at wild-type levels in the DRC3-HA flagella; in that sample, the band recognized by the anti-DRC3 antibody is shifted upward because of the addition of the 3xHA tag (4.4 kDa).

**Table S1**

| Slice | ProteinID | Protein description          | Number of peptides |             |
|-------|-----------|------------------------------|--------------------|-------------|
|       |           |                              | Wild type          | <i>drc3</i> |
|       |           |                              | <b>A</b>           | <b>B</b>    |
|       | 524469    | DRC11 (FAP82)                | 9                  | 5           |
|       | 523721    | RNI-like protein             | 3                  | 5           |
|       | 513888    | $\beta$ -tubulin             | 2                  | 2           |
|       | 515476    | FAP99                        | 1                  | 0           |
|       | 518501    | Dihydroorotate dehydrogenase | 1                  | 0           |
|       | 518407    | Kinesin heavy chain          | 1                  | 0           |
|       | 523861    | DNA helicase                 | 1                  | 0           |
|       | 517045    | F1-ATPase                    | 0                  | 2           |
|       | 515031    | Actin                        | 0                  | 1           |
|       |           |                              | <b>C</b>           | <b>D</b>    |
|       | 513508    | DRC3 (FAP134)                | 17                 | 0           |
|       | 518854    | Thioredoxin-like protein     | 2                  | 0           |
|       | 513514    | FAP7                         | 1                  | 1           |
|       | 510598    | IFT57                        | 1                  | 0           |
|       | 524750    | ABC transporter              | 1                  | 0           |
|       | 513771    | RSP5                         | 0                  | 1           |
|       | 516210    | TRP10                        | 0                  | 1           |
|       |           |                              | <b>E</b>           | <b>F</b>    |
|       | 512343    | DRC4                         | 19                 | 3           |
|       | 513888    | $\beta$ -tubulin             | 10                 | 4           |
|       | 516143    | Tetkin                       | 6                  | 3           |
|       | 521991    | $\alpha$ -tubulin            | 2                  | 0           |
|       | 513127    | Enolase                      | 1                  | 0           |
|       | 524918    | cAMP kinase                  | 1                  | 1           |
|       | 519178    | Phosphodiesterase            | 0                  | 1           |



**Table S1. Complete list of proteins identified by MS analysis of selected slices of SDS-polyacrylamide gel of proteins immunoprecipitated from wild-type and *drc3* axonemal extracts by anti-DRC3 antibody.** All proteins identified from slices A-F labeled in Figure S5 are listed with total number of peptides found for each. Protein IDs are from the Augustus 5 database. Peptides from DRC3, DRC4, and DRC11 (red) are much more abundant in the wild-type immunoprecipitate.

**Table S2 Primers used in this study**

| Name  | Sequence                                     |
|---|--|
| <b>Primers used to map the deletion in the <i>drc3</i> mutant</b> |  |
| DRC3Ex1F  | 5'-GAATGCATCCAGGTGTGTTG-3'                   |
| DRC3Ex2R  | 5'-ACGCCAGGTTTTTGAAGCTA-3'                   |
| DRC3Int3F   | 5'-TAGCATGAGACGTGTGTTGC-3'                   |
| DRC3Ex4R  | 5'-ACGTAGGACCTGTAATCGTG-3'                   |
| DRC3Ex6F  | 5'-TCAACACCTACACAGACGAG-3'                   |
| DRC3Ex7R  | 5'-TGTTGTGCTGCTCCAGAATG-3'                   |
| DRC3Ex7F  | 5'-TTCTGGAGCAGCACACAAG-3'                    |
| DRC3Int7R   | 5'-CACTACCGTATCACAACCTCC-3'                  |
| DRC3Int7F   | 5'-AGTGACGGTGGCATGATGG-3'                    |
| DRC3Ex8R  | 5'-GTGAAGTACCCGTTGTACTG-3'                   |
| DRC3Ex8F  | 5'-CAGTACAACGGGTACTTCAC-3'                   |
| DRC3Ex9R  | 5'-CTTCTCGAACACGGTCATGG-3'                   |
| DRC3Ex10F   | 5'-ACATGGGCAAGATTGACTCC-3'                   |
| DRC3Ex10R   | 5'-ACTGCTTGAATGTCCGCTTT-3'                   |
| <b>Primers used to screen <i>drc3/pf14</i> strains</b>            |  |
| 531300f   | 5'-CTGCTATGGCTCGTGTTGTG-3'                   |
| 531300r   | 5'-GCACATACGGTAGCGAACCT-3'                   |
| RSP3up  | 5'-ATGATGAGCGACGTTAACGC-3'                   |
| RSP3down  | 5'-ACAGGAGAACACACCGATCC-3'                   |
| DRC3Ex1F  | 5'-GAATGCATCCAGGTGTGTTG-3'                   |
| DRC3Ex2R  | 5'-ACGCCAGGTTTTTGAAGCTA-3'                   |
| NPHP4-F1  | 5'-GCGCTCCACTCAATAACCAT-3'                   |
| NPHP4-R1  | 5'-AGGTAAGTCCCCTCCTGGAA-3'                   |
| NPHP4-F2  | 5'-AGGTAGATGGGGTGCTGGTG-3'                   |
| NPHP4-R2  | 5'-TAGAATTGGGAGCCTTGACG-3'                   |
| <b>Primers used to generate pDRC3-BglII</b>                       |  |
| DRC3PFL   | 5'-GACGGTGGCATGATGGACGGTGC-3'                |
| DRC3stopSap   | 5'-AAGCTCTTCTATCTCCCATCTCGCCGCCCTCCTCCTCG-3' |

DRC3BglIISap

5'-AAGCTCTTCTGATCTGGGGGCTGAGCGGGCGTGC-3'

DRC3EcoNIDN

5'-TGTGGCTCTGGGGCTCGGGC-3'

**Primers used to amplify partial cDNA of DRC3**

DRC3cDNA-5'

5'-GGATCCCAGCATCAGGACGAGATGATTG-3'

DRC3cDNA-3'

5'-AAGCTTAGTCAAACCTCCTGCACCAGCC-3'

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