Supplementary Materials

Molecular Biology of the Cell

Saravanan *et al*.

Supplementary materials

CC number	Genotype	Reference or source
CC-5102 (wild-type, g1)	*nit1, agg1, mt*$^{+}$	(Pazour et al., 1995)
CC-5325 (wild-type)	*cw15, mt-*	(Li et al., 2016)
CC-124 (wild-type)	*nit1, nit2, agg1, mt-*	(Proschold et al., 2005)
CC-125 (wild-type)	*nit1, nit2, mt+*	(Proschold et al., 2005)
CC-3090	*ida6-1, mt*$^{+}$	(Kato et al., 1993)
CC-4483	*pf2-4, 9b11, mt*$^{-}$	(Rupp and Porter, 2003)
CC-5510	*pf2-4, DRC4-mC-TG, mt*$^{-}$	this study
CC-4495	*ida6, DRC2-GFP-TG, mt*$^{+}$	(Bower et al., 2018)
CC-5514	*ida6-1, pf2-4, DRC2-GFP-TG*	this study
CC-5515	*ida6-1, pf2-4, DRC4-mC-TG*	this study
CC-5516	*pf2-4, ida6-1, DRC2-GFP-TG, DRC4-mC-TG, mt*$^{-}$	this study
CC-5517	*pf2-4, ida6-1, DRC2-GFP-TG, DRC4-mC-TG, mt*$^{+}$	this study

Table S1) Chlamydomonas strains used in this study.

TG, transgene expressed from an ectopic locus; the TG designation and mating type were omitted in the main text and figures.

Figure S1

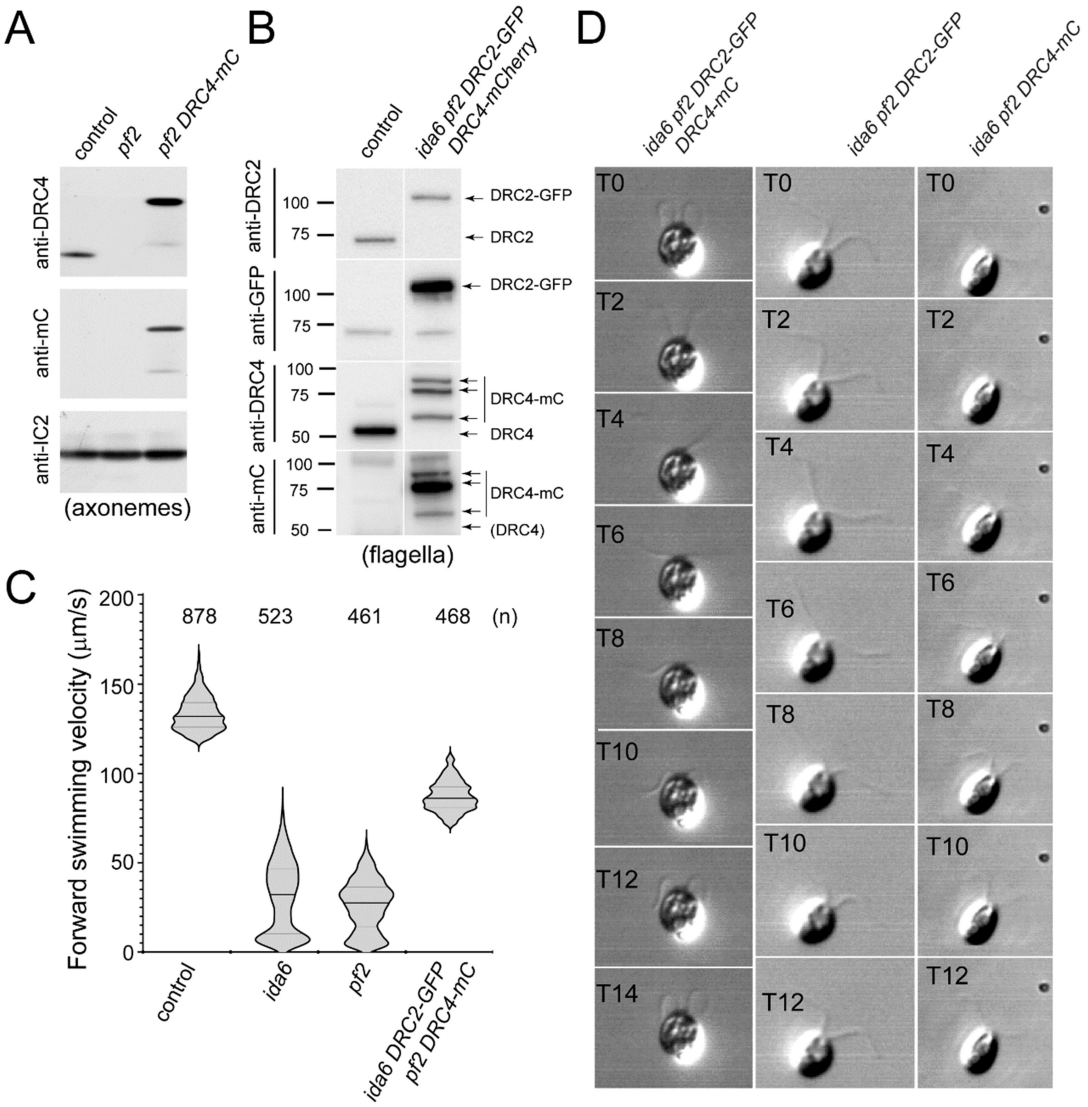

Figure S1) Loss of DRC4 severely impairs flagellar motion

A) Western Blot analyzing isolated axonemes of a wild-type control, *pf2*, and the *pf2 DRC4-mC* rescue strain with antibodies to DRC4 and mC; antibodies to IC2, an outer dynein subunit, were used to control for loading.

B) Western blot analyzing flagella isolated from wild-type (control; g1) and the *ida6 pf2 DRC2-GFP DRC4-mC* strain. Replicate membranes were stained with anti-DRC2 and anti-DRC4; anti-IC2 was used as a loading control. The positions of DRC2, DRC4, DRC2-GFP, DRC4-mC, and of a DRC4-mC fragment are indicated with arrows. Unrelated lanes were cropped. The DRC4-mC subunit often ran as three bands in flagellar samples, of which the middle one was the most prominent; this band was also the main target of anti-mC. Proteolysis of mC, which tends to fragment upon heating for SDS-PAGE, has been observed repeatedly (Gross et al., 2000). The origin of the larger immunoreactive band is unclear.

C) Violin plot showing the forward swimming velocity of wild-type, the *ida6* and *pf2* mutants, and *ida6 pf2 DRC2-GFP DRC4-mC* double-mutant-double-rescue strain. n, number of cells analyzed.

D) Still DIC images from highspeed recordings of *ida6 pf2 DRC2-GFP DRC4-mC, ida6 pf2 DRC2-GFP* and *ida6 pf2 DRC4-mC* cells. The time in ms is indicated. See related video 1.

Figure S2

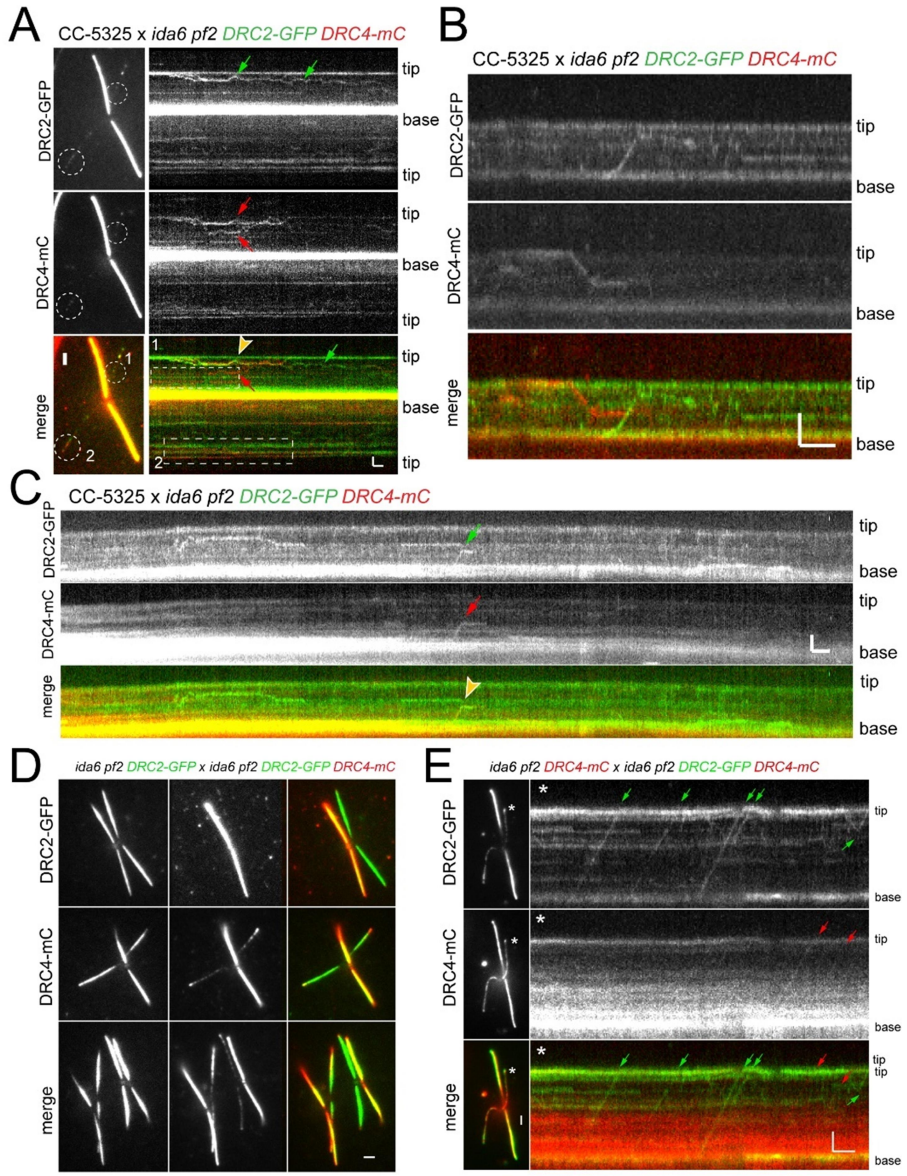

Figure S2) Analysis of DRC2-GFP and DRC4-mC transport in zygotic flagella.

A) Still images and kymograms of wild-type (CC-5325) × *ida6 pf2 DRC2-GFP DRC4-mC* zygotes. Arrows mark trajectories resulting from diffusion of the tagged proteins; co-diffusion is marked by a yellow arrowhead. Bars = 2s and 2 µm. Circles in the still images and boxed areas highlight stationary proteins indicative for association to the axoneme. Note distinct patterns of

stationary DRC2-GFP and DRC4-mC indicative for independent incorporation into the wild-type axonemes. Bars = 2s and 2 µm.

B, C) Examples of independent transport (B) and co-transport (C) of DRC2-GFP and DRC4-mC. Bars = 2s and 2 µm.

D, E) Still images (D, E) and kymograms (E) of zygotes obtained by mating *ida6 pf2 DRC2-GFP DRC4-mC* with either *ida6 pf2 DRC2-GFP* (D) or *ida6 pf2 DRC4-mC* (E). The star indicates the flagellum used for kymographic analysis. Bars = 2s and 2 µm.

Video S1) Flagellar motion analysis of DRC mutant and rescue strains

The video shows cells of the *ida6 pf2 DRC2-GFP, ida6 pf2 DRC4-mC*, and the *ida6 pf2-4 DRC2-GFP DRC4-mC* strains recorded at 1,000 fps. The timer counts seconds.milliseconds.