Online Supplemental Materials and Methods

Identification of endogenous sequence flanking the NIT1 *insertion in* oda5-2

The *oda5-2* insertional mutant did not retain vector sequence (Fig. S1A), but did retain the *NIT1* selectable marker which segregated with the Oda5- motility phenotype (Fig. S1D). Therefore, to obtain sequences flanking the *NIT1* insertion, genomic Southern blots of wild-type and *oda5-2* were screened with a 5-kb probe containing sequences to the 3' end of the *NIT1* gene (Fernandez *et al*., 1989). A 6-kb RFLP was identified in SacII-digested *oda5-2* genomic DNA (Fig. S1B). 5 to 7-kb genomic fragments from SacII-digested *oda5-2* DNA were isolated and subcloned into pSE280 (Invitrogen, Carlsbad, CA). Colony lifts were screened with the *NIT1* probe and one positive colony identified. This plasmid, p36.1, was sequenced and determined to contain both *NIT1* and unknown sequences. A small region of the unknown sequence was amplified by PCR and used to probe Southern blots of genomic DNA from wild type, *oda5-2*, and their meiotic progeny. The 36.1 probe identified a 6-kb SacII RFLP that segregated with the Oda5- phenotype (Fig. S1C and E).

Construction of an HA-Tagged ODA5 *Gene*

Plasmid p125.5 was created by ligating a 2.6-kb EcoR1-BamH1 fragment containing the terminal 2/5^{ths} of the *ODA5* gene into the pBS-SK+ vector (Stratagene). p125.5 was digested with HincII, which cuts in the SK+ vector, 5' to the EcoR1 cloning site, and at position 5901 in the *ODA5* gene. This produces a 2.3-kb fragment and a 3.1-kb fragment which includes the SK+ vector plus the 3' end of the *ODA5* gene. A 136-bp Sma1 fragment was isolated from p3xHA (Silflow *et al*., 2001) and ligated into the 3.1-kb

HincII fragment from p125.5, creating plasmid p127.3. The 2.3-kb HincII fragment from p125.5 was ligated into the Stu1 site of p127.3, creating plasmid p128.8. Lastly, plasmid p50.1 was digested with Sal1-EcoR1 and plasmid p128.2 was digested with EcoR1-BamH1. The 3.5-kb Sal1-EcoR1 fragment from p50.1 was ligated into the pUC18 vector, along with the 2.6-kb EcoR1-BamH1 fragment from p128.8. This creates construct p130.2, which contains the *ODA5* gene with the triple HA-epitope tag located 15 amino acids upstream of the *ODA5* stop codon. All constructs were sequenced and verified by restriction mapping to determine proper orientation and reading frame.

Cloning of Flagellar* AK *cDNA

GreenGenie (Li *et al*., 2003) was used to analyze the flagellar *AK* genomic structure. Primers (Integrated DNA Technologies, Coralville, IA) to predicted exons and untranslated regions were used to amplify the *AK* cDNA from a gt10 cDNA library constructed from mRNA isolated from synchronously grown cells harvested one hour after the beginning of the dark period (Pazour and Witman, unpublished results). Comparison of the cDNA sequence vs. the genomic sequence from the *Chlamydomonas* version 2.0 genome database (Joint Genome Institute, Walnut Creek, CA) verified the intron-exon boundaries and untranslated regions at the 5' and 3' ends of the cDNA.