# Supporting Information

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### SI Materials and Methods

**Strains and Special Chemicals.** *Chlamydomonas reinhardtii* strains *21gr* (mt+) (CC-1690), *6145C* (mt−) (CC-1691), *bld1* (CC-477), *bld2* (mt+) (CC-478), *shf2* (mt+) (CC-2346), are available from the *Chlamydomonas* Genetics Center, University of Minnesota, St. Paul. *lf4-3* was kindly provided by Paul Lefebvre (University of Minnesota). Sodium pyrophosphate (NaPPi), myelin basic protein, colchicine (Sigma), and alkaline phosphatase [calf intestinal phosphatase (CIP)] (NEB) were used at concentrations indicated in the main text or in *SI Materials and Methods*.

**Cell Culture, Deflagellation, Flagellar Regeneration, Flagellar Shortening, and Flagellar Length Measurements.** Growing of vegetative cells, gametogenesis, and zygote formation are described elsewhere (1). Briefly, vegetative cells grown in liquid M medium were cultured at 23 °C with aeration under 14:10 h light–dark cycle. For gametogenesis, vegetative cells were resuspended in M-N medium and grown with aeration under continuous light for 16–24 h at 23 °C. To initiate zygote formation, equal numbers of mt+ and mt− gametes were mixed and allowed to mate with air-bubbling.

Deflagellation, flagellar regeneration, induction of flagellar shortening by NaPPi, and flagellar length measurement are essentially as described previously (2–4). Briefly, the cells were deflagellated by pH shock and the deflagellated cells were resuspended in fresh medium to allow flagellar regeneration. To induce flagellar shortening, NaPPi were added to a final concentration of 20 mM (pH 7.2). For inhibition of flagellar shortening by staurosporine, 1 μM staurosporine (final concentration) was added 15 min before adding NaPPi. To determine flagellar length, cells were fixed in 1% (vol/vol) glutaraldehyde and imaged by differential interference contrast microscopy at 400× magnification on a Zeiss Axio Observer Z1 microscope (Zeiss) equipped with a QuantEM 512SC camera (Photometrics). Flagellar length was measured by using ImageJ (National Institutes of Health) and calibrated with a micrometer. For each measurement, flagella from at least 50 cells were measured.

**Nucleic Acid Manipulations, Bacterial Protein Expression, and *Chlamydomonas* Transformations.** General molecular techniques were used to generate different constructs for gene expression in bacteria or *Chlamydomonas*, which were confirmed by sequencing. Plasmid pCALK1-HA harboring triple HA-tagged full-length CALK at the C terminus was described previously (5). pCALK-K66R-HA was obtained by site-directed mutagenesis of pCALK1-HA through PCR. To prepare CALK C-terminal deletion mutant tagged with HA (CALK1-341-HA), full-length CALK DNA sequence in pCALK1-HA was replaced by the genomic DNA fragment encoding 1–341 amino acids of CALK (N-terminal region containing the kinase domain) to generate pCALK3-HA. For antibiotic selection of *Chlamydomonas* transformants, expression cassette of paromomycin-resistant gene *aphVIII* from plasmid pIS103 (6) was cloned into the previously mentioned plasmids. Glass bead method was used to transform pCALK1-HA, pCALK3-HA, and pCALK-K66R-HA into *Chlamydomonas* (7). Positive transformants were selected by immunoblotting of the transformants with anti-HA antibody.

For constructs made for expression of various forms of CALK in bacteria, general molecular techniques were used to generate truncations or point mutations of CALK cDNA including CALK1-341, CALK342-769, CALK-K66R, and CALK-T193A. These DNA fragments were cloned into pGEX4T-2 (GE Healthcare) for expression of GST-tagged fusion protein in bacteria. CALK, CALK-T193A, CALK1-341, and CALK342-769 cDNA fragments were also cloned into pET28A (Invitrogen) for expression of His-tagged fusion protein. General molecular protocols for expression and purification of bacterial expressed proteins were followed. Briefly, expression constructs were transformed into *Escherichia coli* BL21 (DE3) strain, which was grown at 37 °C with vigorous shaking. The temperature of the culture was switched to 16 °C when absorbance at 600 nm reached 0.6 and protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.5 mM under continuous shaking for 18 h. His-tagged recombinant proteins were purified by using nickel-nitrilotriacetic acid-agarose according to the instructions from the manufacturer (Invitrogen). GST-tagged proteins were purified by affinity chromatography using a glutathione-Sepharose 4B column (GE Healthcare).

**Immunoprecipitation and Pull-Down Assay.** To immunoprecipitate HA-tagged forms of CALK, $1 \times 10^8$ cells were lysed with lysis buffer [20 mM Hepes (pH 7.2), 5 mM MgCl2, 1 mM DTT, 1 mM EDTA, 20 mM beta-glycerol phosphate, 10 mM NaF, 0.1 mM Na3VO4, 150 mM NaCl, 5% glycerol, EDTA-free protease inhibitor mixture (Roche), and 25 μg/mL ALLN]. A mixture of 0.5 μg Rat anti-HA antibody (Roche) and 25 μL protein A beads was added into the lysate followed by shaking on ice for 2 h. After washing three times with lysis buffer, the beads were boiled in SDS/PAGE buffer for 5 min followed by immunoblot analysis.

**Phosphatase Treatment and in Vitro Kinase Assay.** Phosphatase treatment was essentially as described previously (5) with the following modification. The reaction contained 35 μL clear cell lysate (from $7 \times 10^6$ cells), 4 μL 10× phosphatase reaction buffer, and 2 μL CIP (NEB). After incubation at 37 °C for 30 min, the reaction was terminated by adding equal volume of 4× SDS sample buffer followed by boiling.

In vitro kinase assay was essentially as described (8). Bacterially expressed, His-tagged recombinant WT CALK or its mutants (100 ng in 20 mM Hepes, 1 mM EGTA, 1 mM DTT, 10 mM benzamidine, 0.2 mM PMSF, 10% glycerol) was assayed in 30 μL of protein kinase assay buffer [20 mM Tris, pH 8.0, 10 mM $MgCl_2$, 0.1 mM DTT, 100 μM ATP, 2 μCi ($\gamma$-$^{32}$P) ATP (6,000 mCi/mmol, Amersham Pharmacia Biotech)]. A total of 5 μg bovine myelin basic protein (Sigma) was used as substrate. The reaction was allowed to occur at 30 °C for 30 min and terminated by adding SDS sample buffer and boiling, and analyzed by SDS/PAGE and autoradiography.

For inducing bacterially expressed CALK phosphorylation, a previously published method was followed (5). Briefly, cell lysates from aflagellated cells obtained by pH shock was mixed with purified CALK variant in the presence of 1 mM ATP and incubated for 30 min at 30 °C. The reaction was stopped by adding SDS sample buffer and boiled. The samples were analyzed by SDS/PAGE and immunoblot. The molecular weight shift indicated phosphorylation.

**SDS/PAGE and Immunoblotting.** SDS/PAGE and immunoblotting were essentially as described previously (2, 5). Cell samples were lysed in lysis buffer, as described previously, and boiled in 1× SDS sample buffer for 5 min before being subjected to SDS/PAGE analysis. To analyze HA-tagged forms of CALK by immunoblotting, HA-tagged CALK variants were first immunoprecipitated by using anti-HA antibody. The antibodies used for

immunoblotting are as follows: rabbit anti-CALK (1:5,000) (5), anti-pCALK (1:150), anti-GST (1:2,500) (Abmart), mouse anti–α-tubulin (1:5,000) (Sigma), anti-Histidine (1:500) (Qiagen), and rat anti-HA (1:1,000) (Roche). Rabbit anti-pCALK antibody was made against peptide Cys-QERPVT(P)RVGT-amide (PhosphoSolutions,). This antibody was affinity-purified by using nonphosphorylated peptide. Densitometry of immunoblot was analyzed with TotalLab-Software (Nonlinear Dynamics Ltd.).

**Immunostaining.** For immunostaining of the cells, a previously published protocol was followed (9). Cells in M medium were collected by centrifugation, resuspended in M medium, fixed for 5 min at room temperature in 4% Paraformaldehyde M medium. The fixed cells were resuspended in PBS with 0.5% Nonidet P-40 for 2 min at room temperature. Then the cells were resuspended in PBS and allowed to adhere to polyethylenimine-coated microscope slides for 10 min at room temperature. The slides were immersed in 100% methanol for 10 min at −20 °C and allowed to dry. After rehydration with PBS three times, the slides were incubated for 30 min at room temperature in blocking buffer (10 mM phosphate, pH 7.2, 5% goat serum, 5% glycerol, 1% fish cold fish gelatin, 0.04% azide), followed by addition of primary antibodies and incubation at 37 °C for 1 h. After three washes of 5 min each in PBS buffer with 0.5% Tween-20, the slides were incubated for 1 h at 37 °C in blocking buffer with secondary antibodies. After three washes of 5 min each in PBS buffer with 0.5% Tween-20, the slides were washed by PBS buffer alone. The slides were mounted with Fluoromount-G (SouthernBiotech) and imaged on a Zeiss LSM780 Observer Z1 Confocal Laser Microscope (Zeiss). Images were acquired and processed by ZEN 2009 Light Edition and Adobe Photoshop software, and assembled in Adobe Illustrator (Adobe Systems). The primary antibodies used were mouse anti–α-tubulin (1:200) (Sigma), rat anti-HA (1:50) (Roche), and anti-pCALK (1:50). The secondary antibodies used were Texas Red goat anti-Rat/Rabbit IgG (1:400; Molecular Probes) and Alexa Fluor 488 goat anti-Mouse IgG (1:400; Molecular Probes).

For immunodepletion of pCALK antibody, bacterial expressed GST-CALK on glutathione beads was incubated with pCALK antibody for 30 min at 37 °C. The supernatant after brief centrifugation was used for immunostaining.


1. Pan J, Snell WJ (2005) Chlamydomonas shortens its flagella by activating axonemal disassembly, stimulating IFT particle trafficking, and blocking anterograde cargo loading. *Dev Cell* 9(3):431–438.
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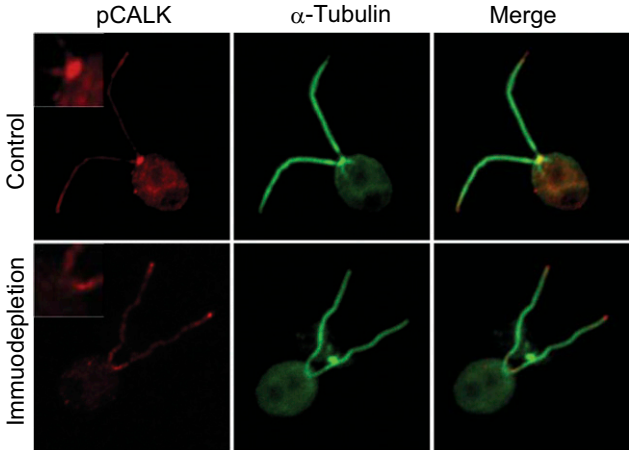


**Fig. S1.** CALK T193 phosphorylated form is specifically localized to the basal body region. *Chlamydomonas* cells were immunostained with pCALK antibody with or without immunodepletion. Anti–α-tubulin was used as control.

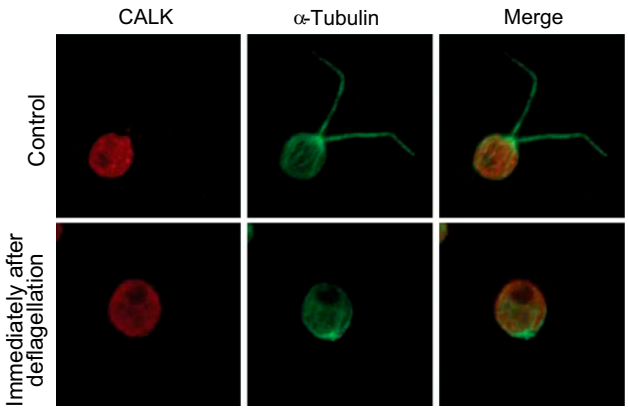


**Fig. S2.** Localization of total CALK before and after deflagellation. Cells expressing HA-CALK were deflagellated or not and subsequently immunostained with anti-HA and anti–α-tubulin antibodies.

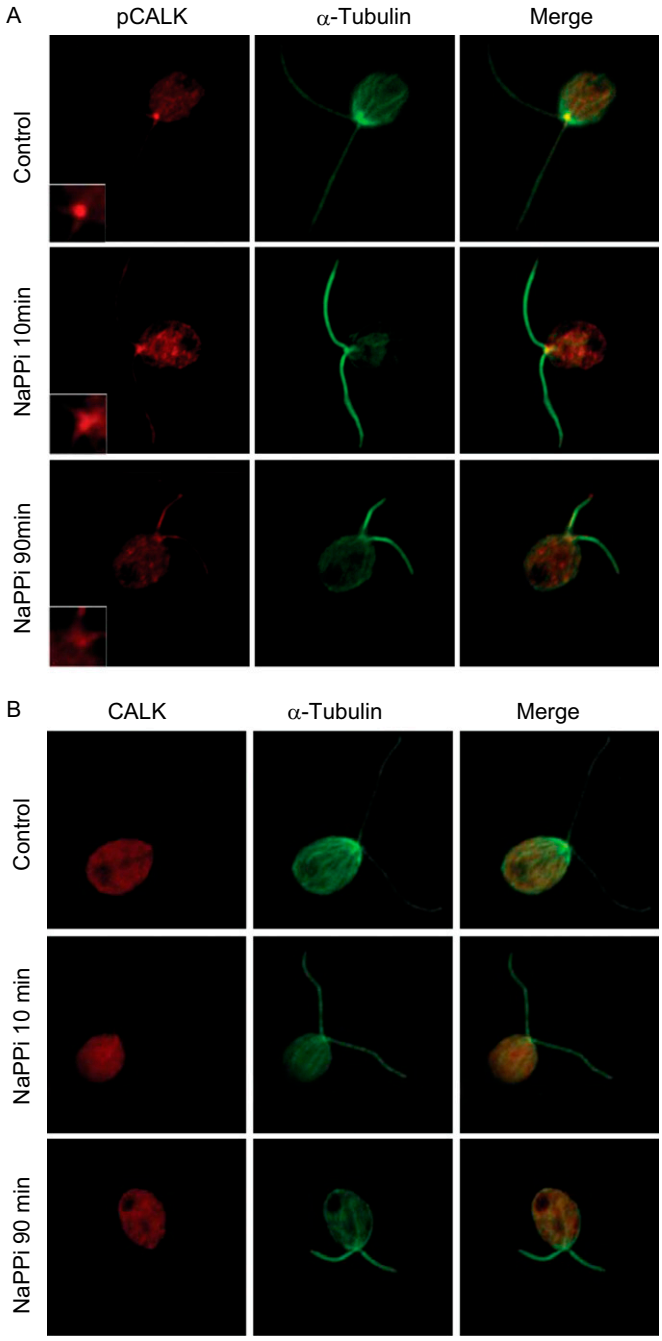


**Fig. S3.** Immunostaining assay of CALK during flagellar shortening. *Chlamydomonas* cells expressing CALK-HA were induced to shorten their flagella by 20 mM NaPPi. Cells before treatment (control) and at different times after treatment were immunostained with antibodies against pCALK and α-tubulin (*A*). (*Insets*) Higher magnification view of the basal body region. Please note the changes of pCALK at the basal body region. Cell samples were also immunostained with antibodies against HA and α-tubulin (*B*).