Supplemental Figure 1. CMF22 and basal body co-staining. Indirect immunofluorescence on detergent-extracted cytoskeletons from CMF22-HA cells. Samples were stained with anti-HA antibodies to detect CMF22-HA (green) and YL 1/2 antibodies as a marker for the basal body (red).The kDNA and nuclear DNA were stained with DAPI (blue).

Supplemental Figure 2. CMF22 knockdown does not affect axoneme ultrastructure. Transmission electron microscopy (TEM) analysis of flagella in CMF22-UKD cells grown in the absence or presence of tetracycline for 72 hours as indicated. Samples are either whole cells (upper panels) or 1% NP40-extracted samples (lower panels).

Supplemental Figure 3. Removal of tetracycline restores wild-type motility in CMF22-UKD cells. CMF22-UKD cells were induced with tetracycline for RNAi knockdown of CMF22 for 72 hours then tetracycline was removed by diluting the culture to $1x10^6$ every 24 hours for 5 days. (A) Motility traces of individual cells at day 5 after tetracycline removal. (B) Mean squared displacement (msd) of CMF22-UKD cells after 5 days tetracycline removal is plotted as a function of time interval.

Supplemental Figure 4. CMF22 Knockdown cells exhibits both reverse and forward beats. Time-lapse image series from video microscopy of CMF22-UKD cells 72 hrs post induction. Filled arrow indicates position of wave at each time point. Unfilled arrows indicate approximate position where waveform originated. “P” marks the posterior end of the cell and “A” marks the anterior end of the cell. (Top) Induction with Tet shows a reverse flagellar beat that moves toward the base of the flagellum. Frames 110 to 130 taken from Supplementary Movie 5. (Bottom) A reverse flagellar beat that moves towards the base of the flagellum, then stops and

moves toward the tip of the flagellum (bottom panels). Frames 145 to 595 taken from Supplemental Movie #8.

Supplemental Table #1: CMF22 orthologues from 115 diverse eukaryotes. The TbCMF22 protein (XP_828418.1) was used as the original query to perform reciprocal best BLAST using the NCBI BLAST portal and species specific portals. An orthologue was confirmed only if TbCMF22 was returned as the top hit upon BLAST against the *T. brucei* genome. To get diverse coverage of genomes, multiples species from the same genus were not compared. Our study found 85 orthologues for CMF22 out of 86 organisms with motile cilia, 0 out of 4 organisms with immotile cilia and 3 out of 25 organisms without cilia.

Supplemental Movie #1: 30 fps CMF22-UKD –Tet, control cells. Video microscopy shows normal motility of uninduced (-Tet) CMF22-UKD cell line. Notice that the cell rapidly travels across the field of view. Video recorded and played at 30 fps.

Supplemental Movie #2: 30 fps CMF22-UKD +Tet cells. After 72 hours of RNAi induction (+Tet), CMF22-UKD cells exhibit erratic movements in which flagellar beating is impaired and cells are not capable of propulsive motility. Notice in the knockdown (+Tet), erratic movements of the flagellar tip, bent anterior (thin end), and base-to-tip beating. Video recorded and played at 30 fps.

Supplemental Movie #3: 1000fps video of flagellar beating in control cells. Cells maintained –Tet. Videos were recorded at 1000 frames/second and played back at 50 frames/second. Note several tip-to-base beats. Figure 6 (-Tet) shows time-lapse image series taken from frames 100 to 300.

Supplemental Movie #4: 1000fps video of reverse (base-to-tip) flagellar beating in CMF22 Knockdown cells. CMF22-UKD cells were maintained +Tet for 72 hrs. Videos recorded at 1000fps and played at 50 fps. Figure 6 (+Tet) shows time-lapse image series taken from frames 150 to 350.

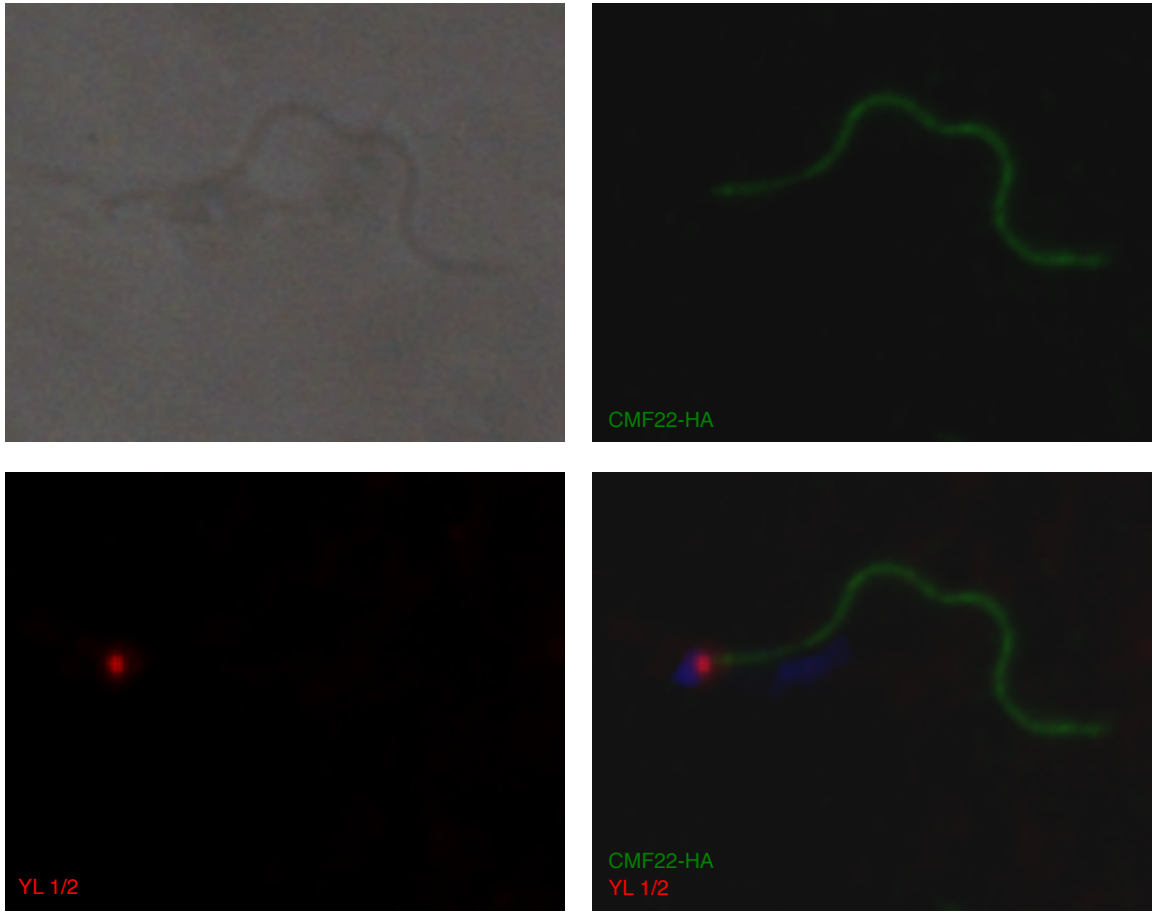
Supplemental Movie #5. 1000fps video of forward (tip-to-base) flagellar beating in CMF22 knockdown cells. CMF22-UKD cells were maintained +Tet for 72 hrs. Videos recorded at 1000fps and played at 50 fps. Video shows a flagellar beat that moves from tip toward base but then appears to stop and then reverse direction. Supplemental Figure 3 (top panels) shows time-lapse image series taken from frames 110 to 130.

Supplemental Movie #6: 30 fps video of normal cell translocation in CMF22UKD-Ri cells maintained in –Tet. Video shows essentially normal beating and translocation of CMF22-UKD-Ri cells before RNAi induction with Tet.

Supplemental Movie #7: 30 fps video of normal cell translocation in CMF22UKD-Ri cells 72 hours post-induction for RNAi against endogenous CMF22. CMF22UKD-Ri cells were maintained +Tet for 72 hrs. Video was recorded and played at 30 fps. Video shows essentially normal beating and translocation of CMF22-UKD-Ri cells after RNAi knockdown of endogenous CMF22.

Supplemental Movie #8: 1000 fps video of reverse flagellar beating followed by a forward beat in CMF22 Knockdown cells. CMF22-UKD cells were maintained +Tet for 72 hrs. Videos recorded at 1000fps and played at 50 fps. Video shows a forward beat that moves from the tip towards the base of the flagellum (frames 100 to 200) and is immediately followed by a reverse

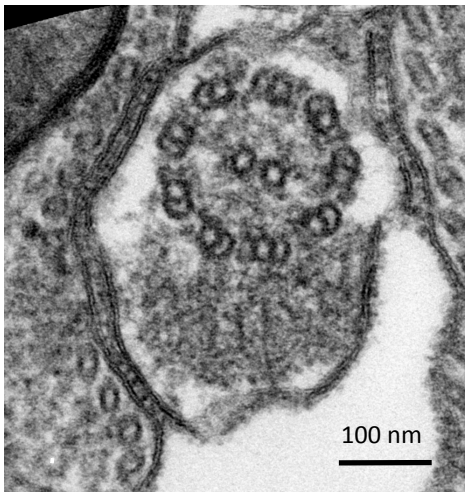
beat that moves back to the tip of the flagellum (frames 300 to 400). Supplemental Figure 3 (bottom) shows time-lapse image series taken from frames 145 to 595.


CMF22-HA
YL 1/2
CMF22-HA
YL 1/2

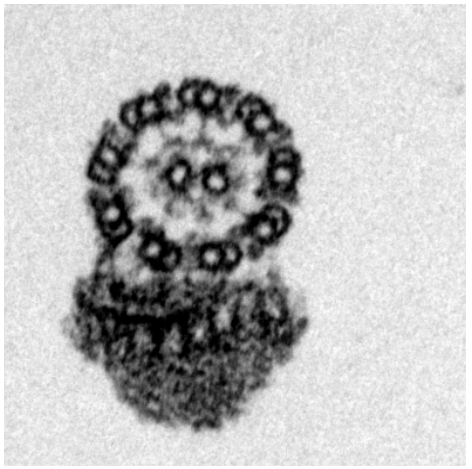
-Tet

+ Tet

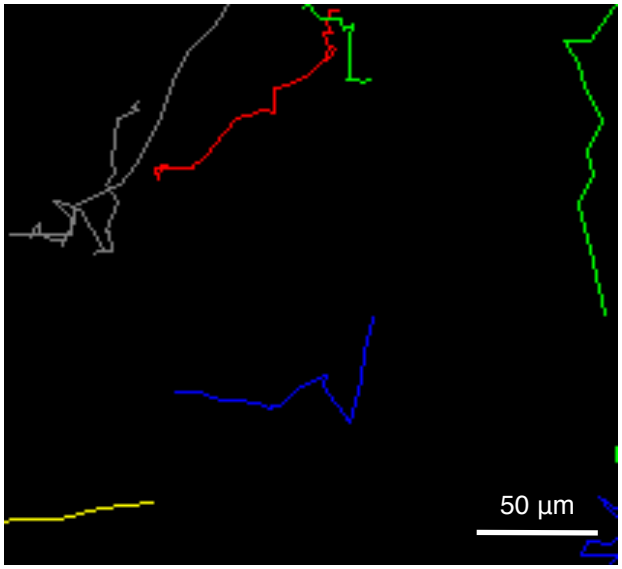
Whole Cells



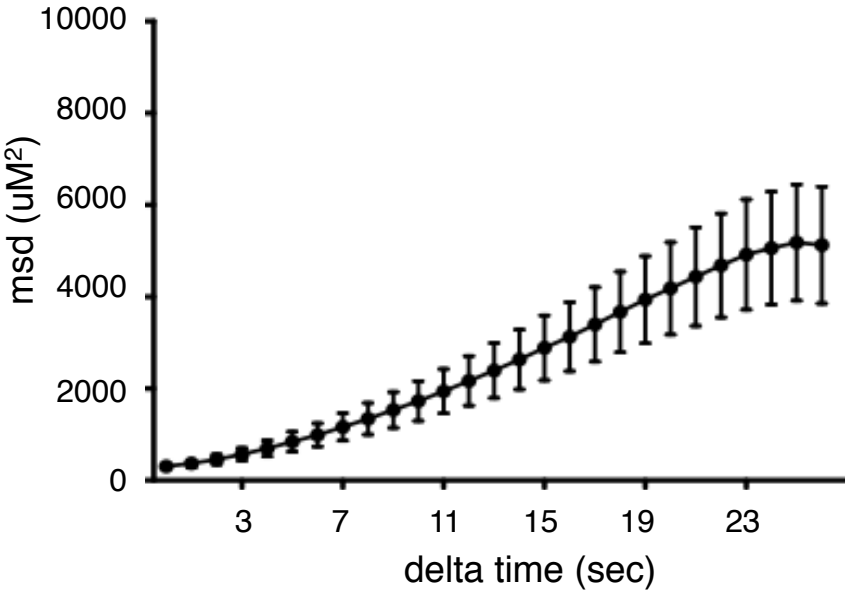
Cytoskeletons



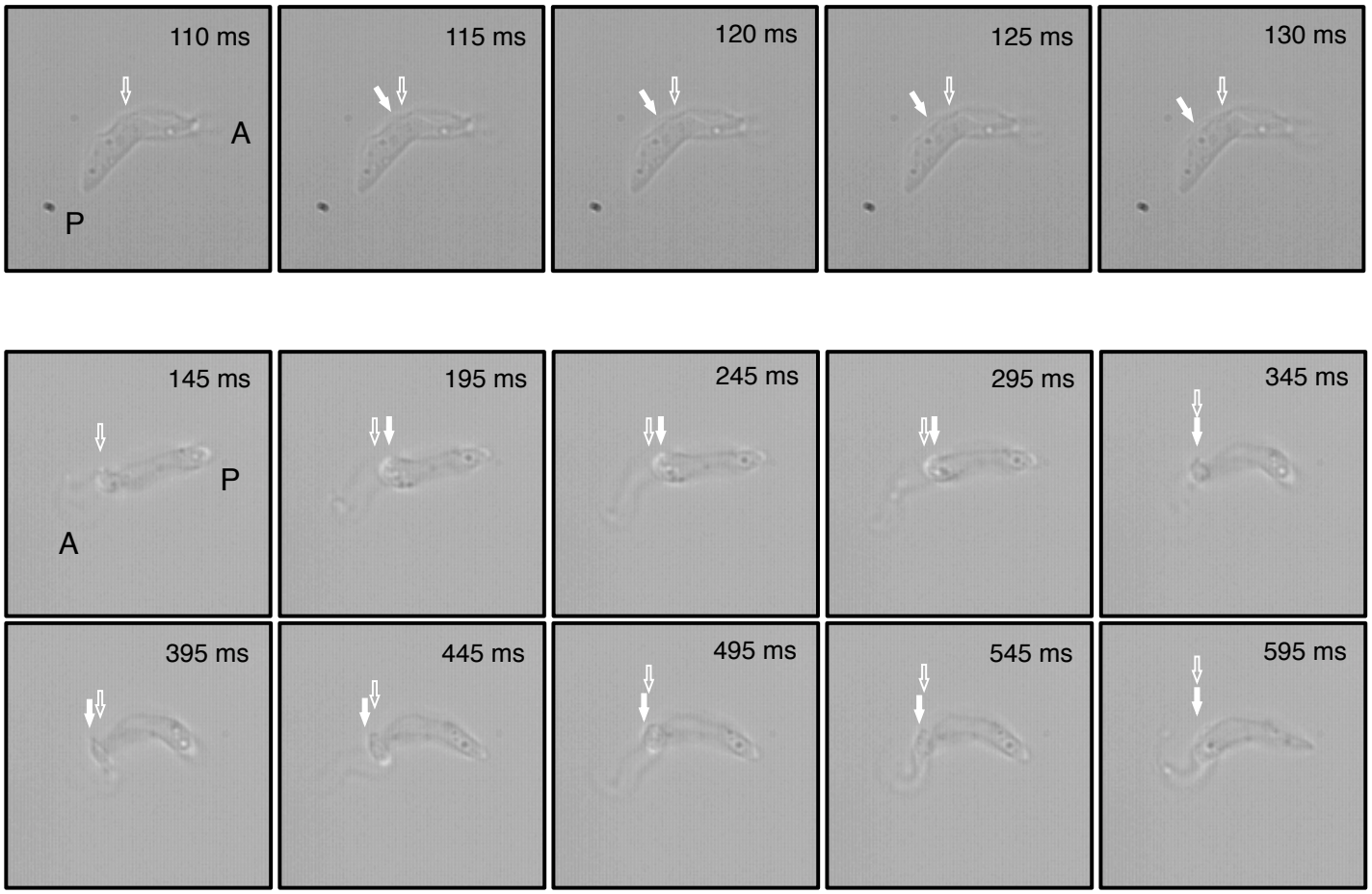
A


50 µm

B


10000
8000
6000
4000
2000
0
msd (uM²)
3
7
11
15
19
23
delta time (sec)

CMF22-UKD +Tet



Motile Cilia	CMF22 Orthologue
Acyrthosiphon pisum	XP_003242250.1
Aedes aegypti	XP_001658040.1
Ailuropoda melanoleuca	XP_002915532.1
Allomyces macrogynus	AMAG_01639.2
Amphimedon queenslandica	XP_003388958.1
Anolis carolinensis	XP_003215233.1
Anopheles gambiae	XP_320769.4
Apis melifera	XP_001122486.2
Batrachochytrium dendrobatidis	EGF82441.1
Bigelowiella natans	jgiIBigna1I142761
Bos taurus	NP_001193476.1
Branchiostoma floridae	XP_002594378.1
Canis familiaris	XP_539921.3
Capitella teleta	ELU03195.1
Chlamydomonas reinhardti	XP_001690665.1
Ciona intestinalis	XP_002129665.1
Clonorchis sinensis	GAA52427.1
Crassostrea gigas	EKC42060.1
Culex pipiens	XP_001847569.1
Cyanophora paradoxa	ConsensusfromContig6672-snap-gene-0.0
Danaus plexippus	EHJ76815.1
Danio rerio	NP_001073437.2
Drosophila melanogaster	AAM11255.1
Ectocarpus siliculosus	CBN77721.1
Emiliania huxleyi	jgiIEmihu1I235066
Equus caballus	XP_001496552.2
Felis catus	XP_003991344.1
Gallus gallus	XP_421878.2
Giardia lamblia	XP_001707075.1
Guillardia theta	EKX39704.1
Harpegnathos saltator	EFN82410.1
Helobdella robusta	jgiIHelro1I188756
Heterocephalus glaber	EHB01958.1
Homo sapiens	NP_001257513.1
Hydra magnipapillata	XP_002168984.2
Leishmania major	XP_001681896.1
Lottia gigantea	jgiILotgi1I61523I0860
Loxodonta africana	XP_003417985.1
Macaca mulatta	EHH17871.1
Meleagris gallopavo	XP_003207414.1
Micromonas pusilla	XP_003061855.1

Motile Cilia	CMF22 Orthologue
Monodelphis domestica	XP_001372750.2
Monosiga brevicollis	XP_001744417.1
Mus musculus	NP_083398.2
Myotis davidii	ELK32629.1
Naegleria gruberi	XP_002678621.1
Nasonia vitripennis	XP_003423849.1
Nematostella vectensis	XP_001637403.1
Oikopleura dioica	CBY14496.1
Oreochromis niloticus	XP_003440019.1
Ornithorhynchus anatinus	XP_001513364.2
Oryctolagus cuniculus	XP_002723285.1
Oryzias latipes	XP_004067899.1
Ovis aries	XP_004001818.1
Oxytricha trifallax	EJY71359.1
Pan troglodytes	XP_003309586.1
Paramecium tetraurelia	XP_001429179.1
Pediculus humanus	XP_002432035.1
Perkinsus marinus	XP_002776859.1
Physcomitrella patens	XP_001767278.1
Phytophthora infestans	XP_002898734.1
Plasmodium falciparum	None
Pongo abelii	XP_002818732.2
Rattus norvegicus	NP_001019488.2
Saccoglossus kowalevskii	NP_001171756.1
Saprolegnia parasitica	SPRG_02274.1
Sarcophilus harrisii	XP_003770307.1
Schistosoma mansoni	XP_002572778.1
Selaginella moellendorffii	XP_002974085.1
Spizellomyces punctatus	SPPG_02701.3
Strongylocentrotus purpuratus	XP_780770.3
Sus scrofa	XP_003133808.1
Taeniopygia guttata	XP_004175065.1
Takifugu rubripes	XP_003974316.1
Tetrahymena thermophila	XP_001015966.1
Tetraodon nigroviridis	CAG08045.1
Thalassiosira pseudonana	XP_002294237.1
Thecamonas trahens	AMSG_00296.2
Toxoplasma gondii	XP_002371250.1
Tribolium castaneum	EFA11364.1
Trichomonas vaginalis	XP_001330039.1
Trichoplax adhaerens	XP_002111442.1
Trypanosoma brucei	XP_828418.1
Trypanosoma cruzi	XP_817318.1
Volvox carteri	XP_002951335.1
Xenopus tropicalis	XP_002932048.1

Immotile Cilia	CMF22 Orthologue
Trichinella spiralis	None
Caenorhabditis elegans	None
Brugia malayi	None
Ascaris suum	None
No Cilia	**CMF22 Orthologue**
Arabidopsis thaliana	None
Aureococcus anophagefferens	EGB03618.1
Chlorella variabilis	EFN52029.1
Coprinopsis cinerea	None
Cryptococcus neoformans	None
Cryptosporidium parvum	None
Cyanidioschyzon merolae	None
Dictyostelium discoideum	None
Encephalitozoon cuniculi	None
Entamoeba histolytica	None
Glycine max	None
Neurospora crassa	None
Oryza sativa	None
Ostreococcus tauri	XP_003078557.1
Phaeodactylum tricornutum	None
Populus trichocarpa	None
Rhizopus oryzae	None
Saccharomyces cerevisiae	None
Schizosaccharomyces pombe	None
Solanum tuberosum	None
Theileria annulata	None
Ustilago maydis	None
Vitis vinifera	None
Zea mays	None