## **SUPPLEMENTAL FIGURES**

**Figure S1. Alignment of the four** *Paramecium BUG22* gene DNA sequences and the human cDNA. The four *Paramecium BUG22* genes, which encode the same protein, as well as the human cDNA, are aligned to show up their differences and provide divergent sequences to design specific RNAi probes. Lines in red and green represent short RNAi probes respectively specific for inactivation of the *Paramecium* and human genes. Lines in purple and blue represent long RNAi probes to respectively inactivate the *Paramecium BUG22* paralogs *a/b* and *c/d*. The *Paramecium* introns are highlighted in pale blue.

BUG22a BUG22b BUG22c BUG22d	1 1 1 1	ATGTTCAAGAACTCA ATGTTCAAGAACTCA ATGTTCAAGAACTCA ATGTTCAAGAACTCA	TTCTAAAGTGGATTT TTCTAAAGTGGATTT TTCC <mark>A</mark> AAGTGGATTT TTCTAAAGTGGATTT	CTTTCAATATTA CTTTCCATATTA CTTTCAATTTTA CTTTCAAT <mark>T</mark> TTA	ATATTCAATAG ATATTCAATAG ATATTCAATAG ATATTCAATAG	GATCAAAGCC GATCAAAGCC GATCAAAGCC GATCAAAGCC GATCAAAGCC	ATTGTAAATTI ATTGTAAATTI CTTGTAGATTI CTTGTAGATTI	rgggataagc/ rgggataagc/ rgggataaac/ rgggataaac/	NAAgtaattta NAAgtaattta NAAgtatttt NAAgtatttta	itgattggatt itaattgcatt tagttcgaat itaggtccaat	atttaag <mark>T</mark> atttaagT at <mark>ga</mark> aagT att <mark>a</mark> aagT	CAAGAATGG CAAGAATGG TAAGAATGG TAAGAATGG
Hs-BUG22	1	ATGTTCAAAAAC <mark>A</mark> CC	TTC <mark>C</mark> AGAG <mark>C</mark> GG <mark>C</mark> TTC	CTCTCCATCCTC	TACAGCATCG	G <mark>CAGC</mark> AAGCC	fc <mark>tg</mark> caaat <mark>c</mark> i	rggga <mark>c</mark> aaa− <i>i</i>	AAG <mark>GTA</mark> C			GGAATGG
BUG22a BUG22b BUG22c BUG22d	121 121 121 121	ACATATTAAACGTAT ACATATTAAACGTAT ACATATCAACGAAT ACATATCAACGAAT	CACAGATCAAGACAT CACAGATCAAGACAT TACAGA <mark>CT</mark> AAGATAT TACAGAT <mark>T</mark> AAGATAT	'TTAATCATCAGT 'TTAATCATCAGT 'T <mark>C</mark> AATCATCAGT 'TTAATCATCAGT	ACT <mark>TGAAAT</mark> A ACT <mark>T</mark> GAAATA CCTAGAAATC TCTAGAAATC	ATGGGTAC <mark>C</mark> A ATGGG <mark>A</mark> ACCA ATGGGTACAA ATGGGTACAA	ATGTGAGTACI ATGTGAGTACI ATGTAAGTACC ATGTGAGTACC	AATTTCATA AATTTCATA CAATTTCATT CAATTTCATT	AC-TGCTCCTO AC-TGCTCCTO AC-TGCTCCTO AC-TGCTCCTO	SC <mark>C</mark> GATCCCAA SCTGA <mark>C</mark> CCTAA SCTGATCCAAA SCTGATCCAAA	AGAAACAT AGAAACAT AGAAACTT AGAAAC <mark>T</mark> T	TGGGAATTA TAGGAATTA TGGGAAT <mark>C</mark> A TGGGAAT <mark>C</mark> A
Hs-BUG22	96	C <mark>CA</mark> CATCAAA <mark>A</mark> GAAT	cac <mark>tgataa</mark> tgacat	CC <mark>AGTC</mark> CCTG <b>G</b> T	GCTAGAGATT	C <mark>AAGG</mark> ACAA	atgta <mark>ag</mark> aco	CA <mark>CA</mark> TAT <mark>AT</mark> C	ACATGC-CCT	gc <mark>agac</mark> cc <mark>c</mark> aa	ga <mark>a</mark> gacg <mark>c</mark>	TGGGAATTA 
BUG22a BUG22b BUG22c BUG22d Hs-BUG22	240 240 240 240 215	AATTGCCATTCTTAG AATTGCCTTTCTTAG AATTACCTTTCTTAG AATTACCTTTCTTAG AATTACCTTTCTTAG	TCATGAT <mark>C</mark> ATTAAGA TCATGATTATTAAGA TAATGATTATTAAGA TAATGATTATTAAGA TCATGATTATCAAAA	ATgtaat-ttat ATgtaatattgt ATgtaaaatcgt ATgtaaattcat AC	tggtatta-t tggcattaat catatttaat catatttaat	gattagCTTA gattagCTTA atctagCTTA atctagCTTA atctagCTTA	AAAAATACTTO AAAAATATTTO AAAAATACTTT AAAAATACTTT AGAAGTA <mark>T</mark> TTT	ACATTTGAG ACTTTTGAAC TACATTTGAAC TACCTTTGAAC TACCTTT <mark>C</mark> GAAC	TTGTAGGTAT TTGTAGGTTTT TTGCAAGTCC TTGCAAGTCC TTGCAAGTCC	'GGATGATAAG 'GGATGATAAG 'GGATGACAAA 'GGATGACAAA <b>'AGATGACAAG</b>	AATGTCAG. AATGTCAG. AATGTCAG. AATGT <mark>T</mark> AG. AATGT <mark>GC</mark> G	AAGGAGA AAGAAGA AAGAAGA TT AAGAAGA TTCGTCGCTT
BUG22a BUG22b BUG22c BUG22d Hs-BUG22	358 360 360 360 309	TAGAGCATC <mark>G</mark> AATTA TAGAGCATC <mark>G</mark> AATTA CAGAGCATCTAATTA CAGAGCCTCTAATTA CAGAGCCTCTAATTA	TTAAgtaattag TTAAgtaattag TCAAgtacatettaa TCAAgtacatettaa CCAG	tataaagaatgt tataaagaatgt aacacattat-t aacacattat t	acTCAACTAC acTCAACTAC acTCCACAAC acTCCACAAC acTCAACAAC ACCACCAC	GAGAGT <mark>G</mark> AAA( GAGAGT <mark>G</mark> AAA) CAGAGTCAAA( CAGAGTCAAA( CC <mark>GGGGTCAAA)</mark>	CCTTTCATCTO CCTTTCATTTO CCATTTATCTO CCATTTATCTO CCATTTATCTO	STAC <mark>A</mark> ATGCC STACAATGCC STACTATGCC STACTATGCC STACTATGCC STACCATGCC	PATGAGA <mark>T</mark> TGG PATGAGA <mark>T</mark> TGG PATGAGACTGG PATGAGACTGG CATG <mark>C</mark> GGCTGG	SATGAGGGATG SATGAGGGATG SATGAAGGATG SATGAAGGATG SATGA <mark>C</mark> GG <mark>C</mark> TG	GAATCAAA GAATCAAA GAAT <mark>T</mark> AAA GAATCAAA GAA <mark>C</mark> CAGA	T <mark>CT</mark> AATTCA T <mark>CT</mark> AATTCA TTCAGTTCA TTCAGTTCA TTCAGTTCA
BUG22a BUG22b BUG22c BUG22d Hs-BUG22	415 417 419 419 344	AACCTTTCATCTGTA AACCTTTCATTGTA AACCATTTATCTGTA AACCATTTATCTGTA AACCGTTCATCTGC	CAATGCCTATGAGAT CAATGCCTATGAGAT CTATGCCTATGAGAG CTATGCCTATGAGAG CCATGCCCATGCGGG	TGGATGAGGGAT TGGATGAGGGAT TGGATGAAGGAT TGGATGAAGGAT TGGATGAAGGGT	'GGAATCAAAT 'GGAATCAAAT 'GGAAT <mark>T</mark> AAAT 'GGAATCAAAT' 'GGAA <mark>C</mark> CAGAT	CTAATTCAAC CT <mark>A</mark> ATTCAAC TCAGTTCAA <mark>T</mark> TCAGTTCAA <mark>T</mark> TCAGTTCAAC	ITCTCTGATTT ITATCTGATTT ITATCTGATTT ITATCTGATTT ITC <mark>CTA</mark> GACTT	TCAO <mark>C</mark> AGAAG/ TCAOCAGAAG/ TCAOTAGAAG/ TCAOTAGAAG/ TCAO <mark>ACGCC</mark> G/	AGCATATGGTA AGCATATGGTA AGCATATGGTA AGCATATGGTA AGCATACGGCA	ACAAACTATAT ACAAACTATAT ACTAACTACAT ACTAACTA	AGAAACAC AGAAACAC AGAAACAT AGAGACAT CGAGACCC	TCCGgtatc TCCGgtatc TACGgta TACGgta TC
BUG22a BUG22b BUG22c BUG22d	535 537 537 537	catat <mark>caatctc</mark> att cat <mark>t</mark> tcaatctcatt catatagatgcaaat catatagatgcaat	tt <mark>c-tag</mark> AGTTCAAA ttg-tagAGTTCAAA ataatagAGTTCAAA ataatagAGTTCAAA	.ТТСАТGССААТТ .ТТСАТGССААТТ .ТАСАТGССААТТ .ТАСАТGССААТТ .ТАСАТGССААТТ	GCAGAATCAG GCAGAATCAG GCAGAAT <mark>T</mark> AG GCAGAAT <mark>T</mark> AG	AAGAATCTAT AAGAATCTAT AAGAATCTAT AAGAATCTAT	IT <mark>T</mark> AGTGATCO IT <mark>T</mark> AGTGATCO ITCAGTGATCO ITCAG <mark>C</mark> GATCO	STCTTTACAG STCTTTACAG STTTATATAG STTTATATAG STTTATATAG	"GAAGAAGAG "GAAGAAGAAT "GAAGACGAAT "GAAGACGAAT	'TACC <mark>C</mark> CCTGA 'TACCACCTGA 'TGCCACCTGA 'TGCC <mark>C</mark> CCTGA	ATTCAAGT ATTCAACT ATT <mark>T</mark> AAAT ATT <mark>T</mark> AAAT	TATTCTTAC TATTCTTAC TATTCTTAC TATTCTTGC
Hs-BUG22	457		<mark>AGAGT</mark> GCAGA	T <mark>C</mark> CATGC <mark>A</mark> AATT	GTCGCATCCG	A <mark>CGGGT</mark> TAC	ITC <mark>TCA</mark> GACAC	GA <mark>CT</mark> CTACTCA	AGAAGA <mark>T</mark> GAGC	TGCCCGCAGA	GTTCAAA <mark>C</mark>	TGTATCT-C
BUG22a BUG22b BUG22c BUG22d	654 656 657 657	ССАТСТАААААСААС ССАТСТАААААСААС ССАТСТАААААТААС ССАТСТАААААТААС	GATGA GATGA GATGA GATGA									
Hs-BUG22	556	CCAGTTCACAACAAC	GCAAAGCAATAA									

Figure S2. Centriolar localization of a human Bug22p in HeLa cells. A. HeLa cells expressing the GFP-HsBug22p protein were immuno-labeled with the 20H5 monoclonal antibody that recognizes centrin, which is a cytological marker of centrioles. Note the colocalization of Bug22p with centrin. B: Untransformed HeLa cells double labeled with GTL3 and the 20H5 anti-centrin showing co-localization of the markers. C. HeLa cells expressing the GFP-HsBug22p protein were immuno-labeled with the anti-GTL3 antibody that recognizes Bug22p. The immuno-labeling perfectly coincides with the GFP fluorescence. Bar = 10  $\mu$ m.



Figure S3. Effect of BUG22 RNAi on basal body and ciliary rootlet organization at two days of RNAi. A and D. Control Nd7p-depleted cells. B, C, E and F. Bug22p-depleted cells. A-C. ID5 anti-tubulin labeling basal bodies. D-F. Anti-ciliary rootlet labeling. Note the disorganization of basal body arrangement revealed by the observation of erratic ciliary rootlet orientations in Bug22p-depleted cells (highlighted in insets). Bar =  $10 \mu m$ .



## Figure S4. Comparison of ciliatures of Bug22p-depleted and Ift172p-depleted cells at two days of RNAi. A. Control cell depleted in its Nd7p protein with normal cilia as viewed in immunofluorescence using an anti-Paramecium tubulin. B. Bug22p-dpleted cell harboring normal cilia. C. In contrast, Ift172p-depleted cells have lost all their cilia and display only some remnants on their anterior ventral field (arrow). Bar = $10 \mu m$ .



## Video 1. High-speed video microscopy of ciliary beating in Nd7p-depleted cells. By

viewing the sequence at low speed or frame by frame using Quicktime, the decomposition of the ciliary beat is shown, with a clear appearance of the power stroke, while the recovery stroke, less visible, occurs out of focus, parallel to the cell surface.

## Video 2. High-speed video microscopy of ciliary beating in Bug22p-depleted cells.

Comparing this sequence with the recording of Nd7p-depleted control cells of Video 1 shows that the distinction between power and recovery stroke has been lost, that the frequency of beating is much greater and that the rigidity of many cilia is impaired, prominently in their distal half.