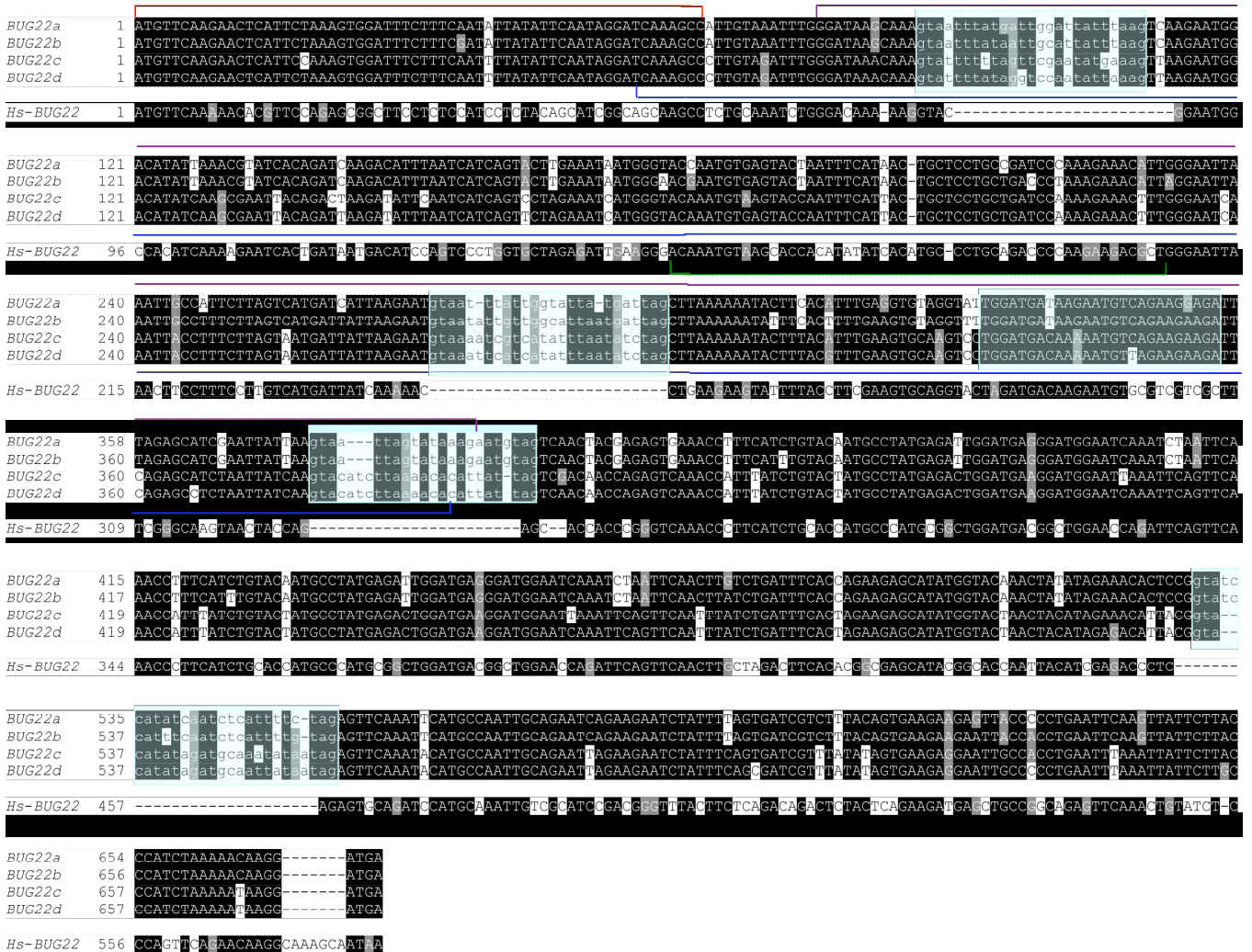
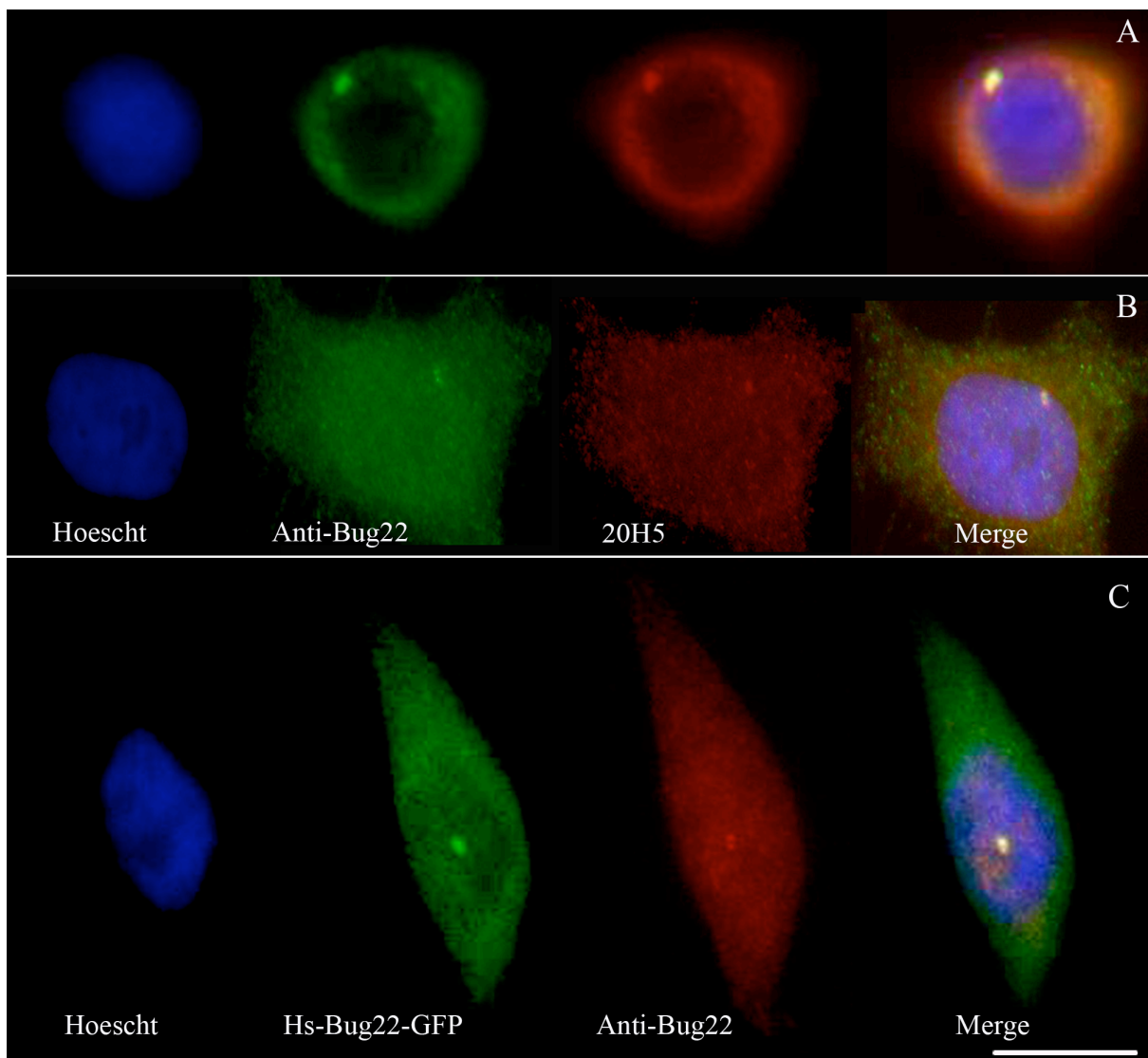


## SUPPLEMENTAL FIGURES

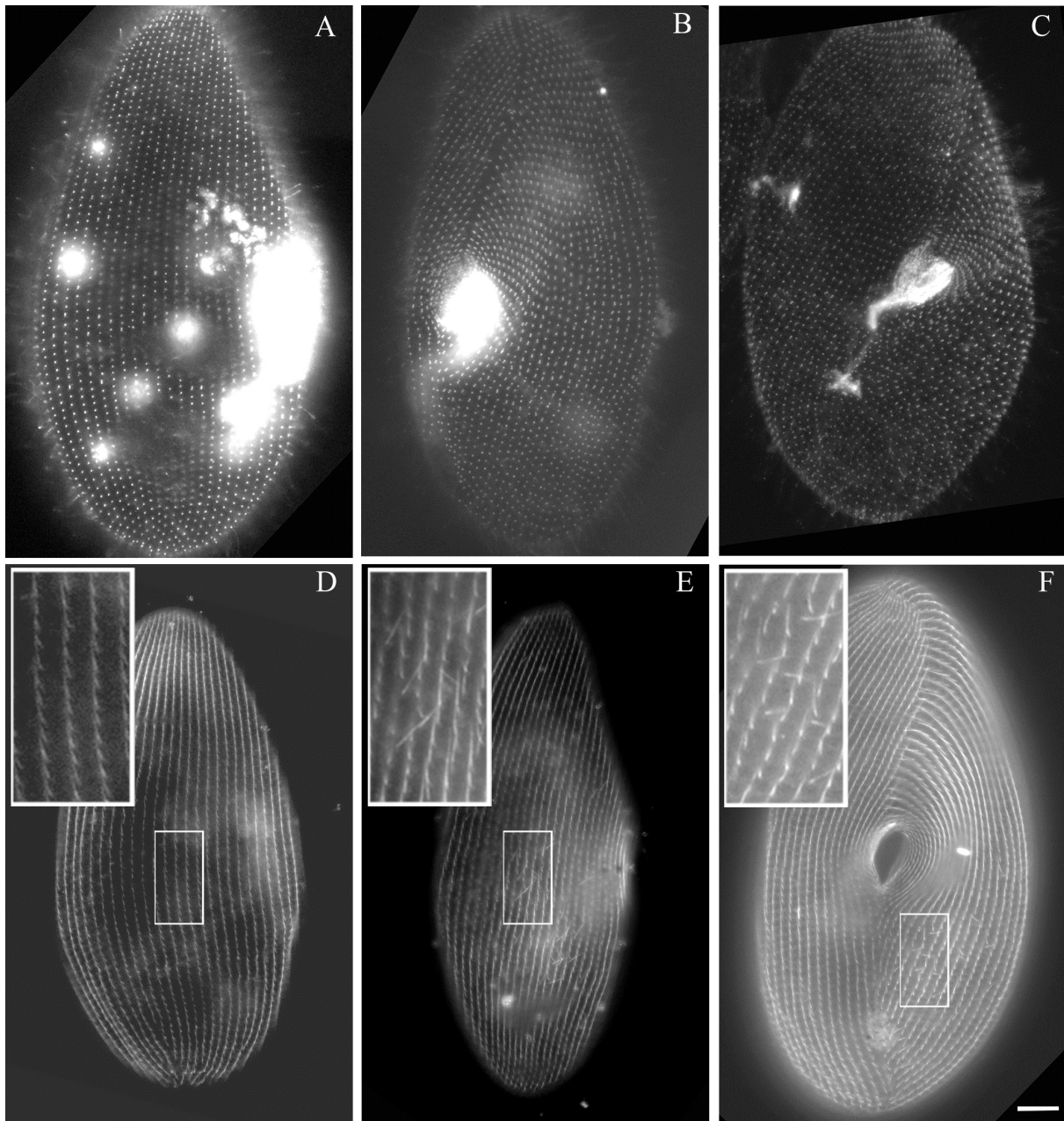
**Figure S1. Alignment of the four *Paramecium* *BUG22* gene DNA sequences and the human cDNA.** The four *Paramecium* *BUG22* genes, which encode the same protein, as well as the human cDNA, are aligned to show up their differences and provide divergent sequences to design specific RNAi probes. Lines 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.



**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 co-localization 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.

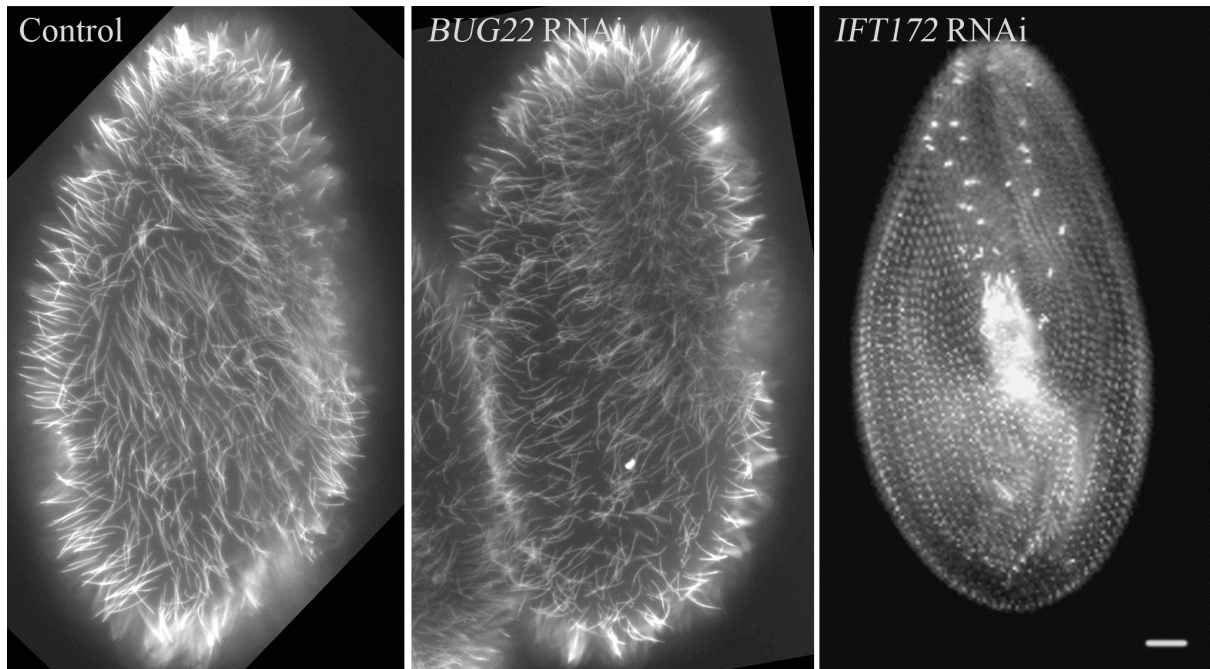


**Figure S3. Effect of BUG22 RNAi on basal body and ciliary rootlet organization at two days of RNAi.** A and D. Control Nd7p-depleted cells. B, C, E and F. Bug22p-depleted cells. A-C. ID5 anti-tubulin labeling basal bodies. D-F. Anti-ciliary rootlet labeling. Note the disorganization of basal body arrangement 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.