

Supplementary Materials for
Giant proteins in a giant cell: Molecular basis of ultrafast Ca²⁺-dependent cell contraction

Jing Zhang *et al.*

Corresponding author: Wei Miao, miaowei@ihb.ac.cn; Jie Xiong, xiongjie@ihb.ac.cn

Sci. Adv. **9**, eadd6550 (2023)
DOI: 10.1126/sciadv.add6550

The PDF file includes:

Figs. S1 to S11
Tables S1 and S2
Legends for movies S1 and S2

Other Supplementary Material for this manuscript includes the following:

Movies S1 and S2

Supplementary Table 1. Statistical data on the genomes of *Spirostomum minus* and other ciliates.

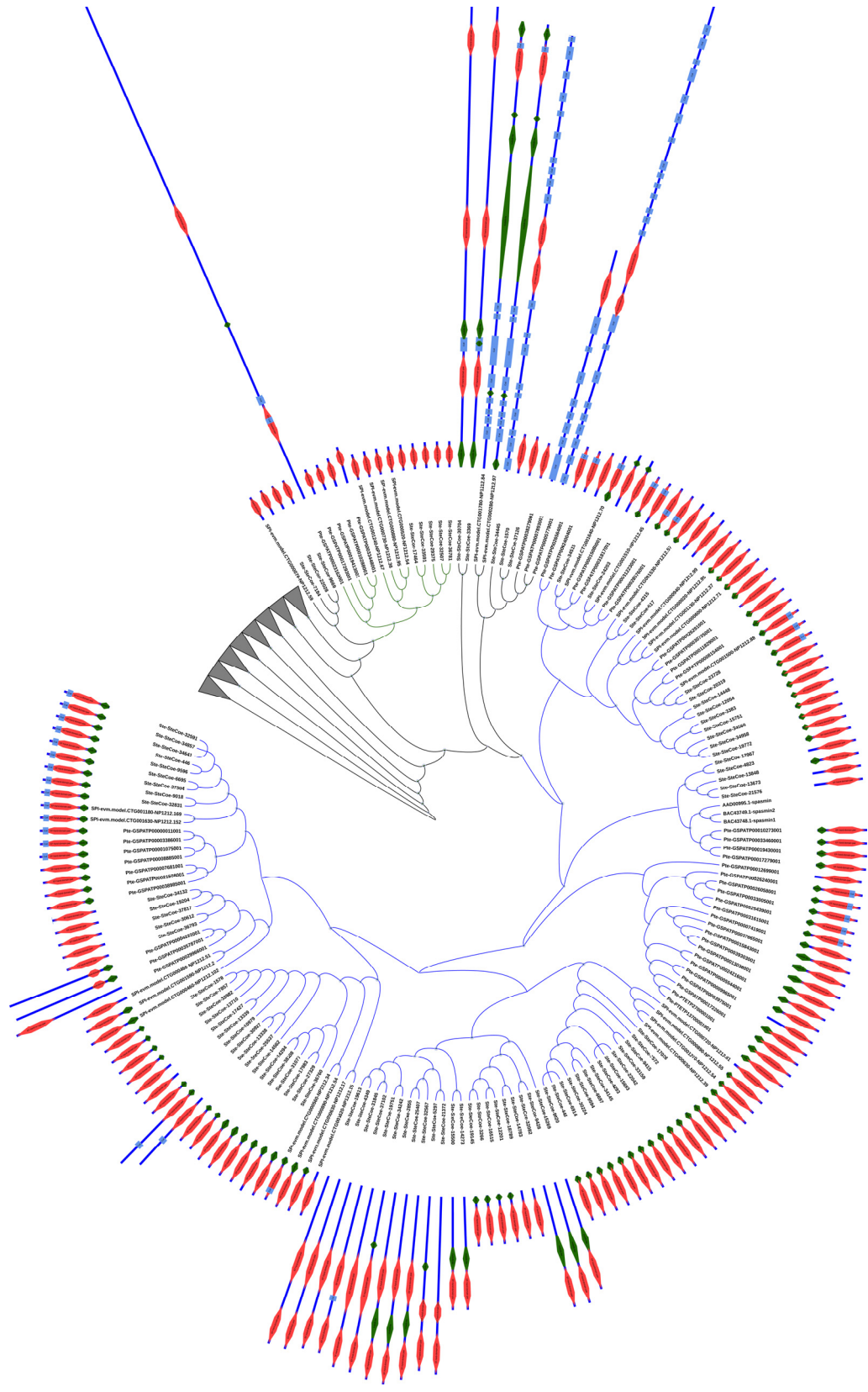
Species	<i>Spirostomum minus</i>	<i>Stentor coeruleus</i>	<i>Paramecium tetraurelia</i>	<i>Tetrahymena thermophila</i>
Genome size (Mb)	66.6	77.8	72.1	103.0
No. of scaffolds	194	2628	697	181
N50 (Kb)	277	55	413	520
GC content	55.6%	30%	28%	22%
No. of genes	38,210	34,494	39,642	26,996
Gene density (per Mb)	573	443	549	262

Supplementary Table 2. The percentage of abnormal cells in RNAi experiments.

	RNAi experiment	Start cell number	A	B	C	D	E
HT115	R1	50	2.0%	4.0%	4.0%	0.0%	0.0%
	R2	50	2.0%	4.0%	0.0%	2.0%	0.0%
	R3	50	6.0%	8.0%	0.0%	0.0%	0.0%
	R4	200	8.0%	0.5%	0.5%	1.5%	0.0%
	R5	200	3.0%	0.5%	0.0%	0.0%	0.0%
L4440	R1	50	0.0%	0.0%	0.0%	0.0%	0.0%
	R2	50	0.0%	0.0%	0.0%	0.0%	0.0%
	R3	50	10.0%	6.0%	0.0%	2.0%	0.0%
	R4	200	2.0%	1.5%	0.5%	0.5%	0.0%
	R5	200	1.5%	2.5%	0.5%	1.0%	0.0%
GSBP1	R1	50	6.0%	2.0%	0.0%	0.0%	2.0%
	R2	50	4.0%	0.0%	0.0%	0.0%	2.0%
	R3	50	10.0%	6.0%	6.0%	10.0%	4.0%
	R4	200	2.0%	1.5%	0.5%	0.5%	0.5%
	R5	200	2.0%	1.0%	0.0%	0.0%	0.0%
GSBP2	R1	50	2.0%	6.0%	2.0%	0.0%	0.0%
	R2	50	4.0%	0.0%	2.0%	2.0%	2.0%
	R3	50	10.0%	10.0%	2.0%	6.0%	2.0%
	R4	200	4.5%	2.0%	0.0%	1.5%	0.0%
	R5	200	4.0%	2.0%	0.0%	0.0%	1.0%

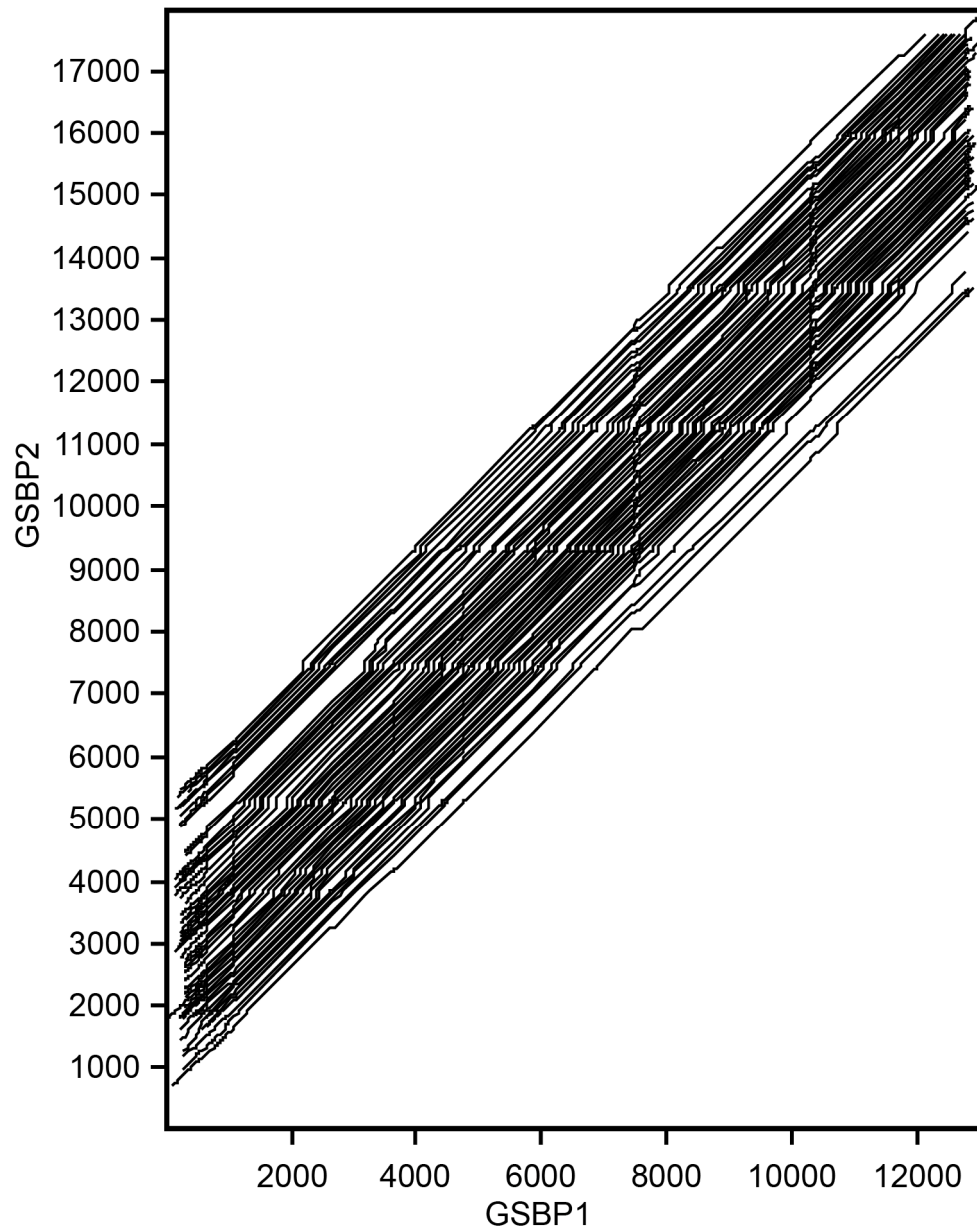
R1-R5: five replicates of RNAi experiments

Start cell number: the number of cells that were picked up and used for the RNAi experiments.

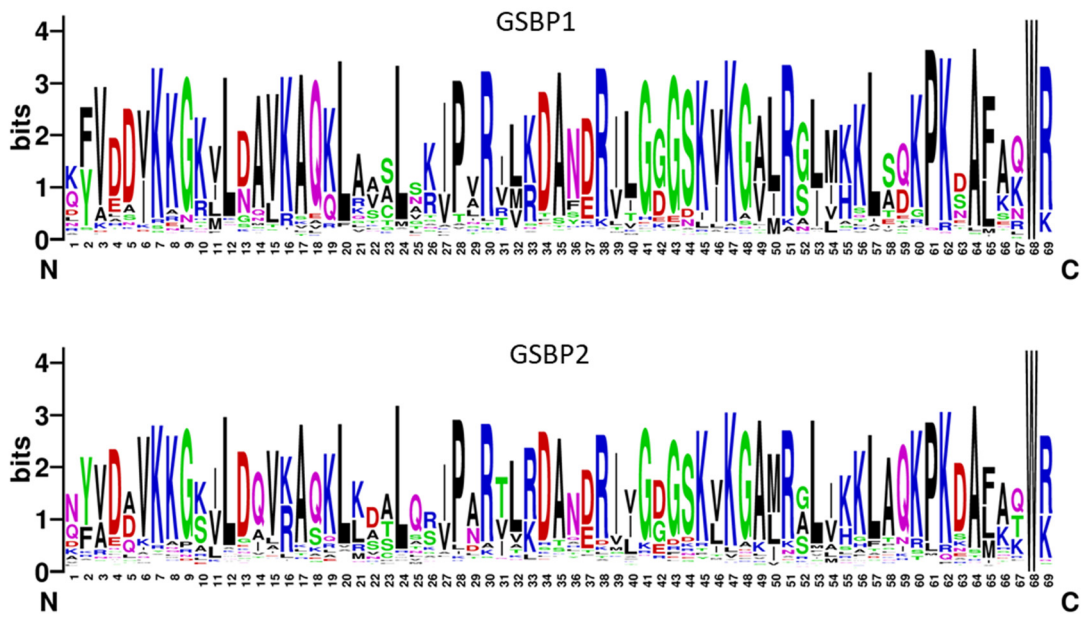


Supplementary Fig. 1. Phylogenetic tree showing all EF-hand proteins in *Spirostomum minus*, *Stentor coeruleus*, and *Paramecium tetraurelia*. Spasmin genes as well as their closest cluster are shown, whereas other EF-hand proteins are collapsed. The clade highlighted in blue is shown in Fig.1. A clade of

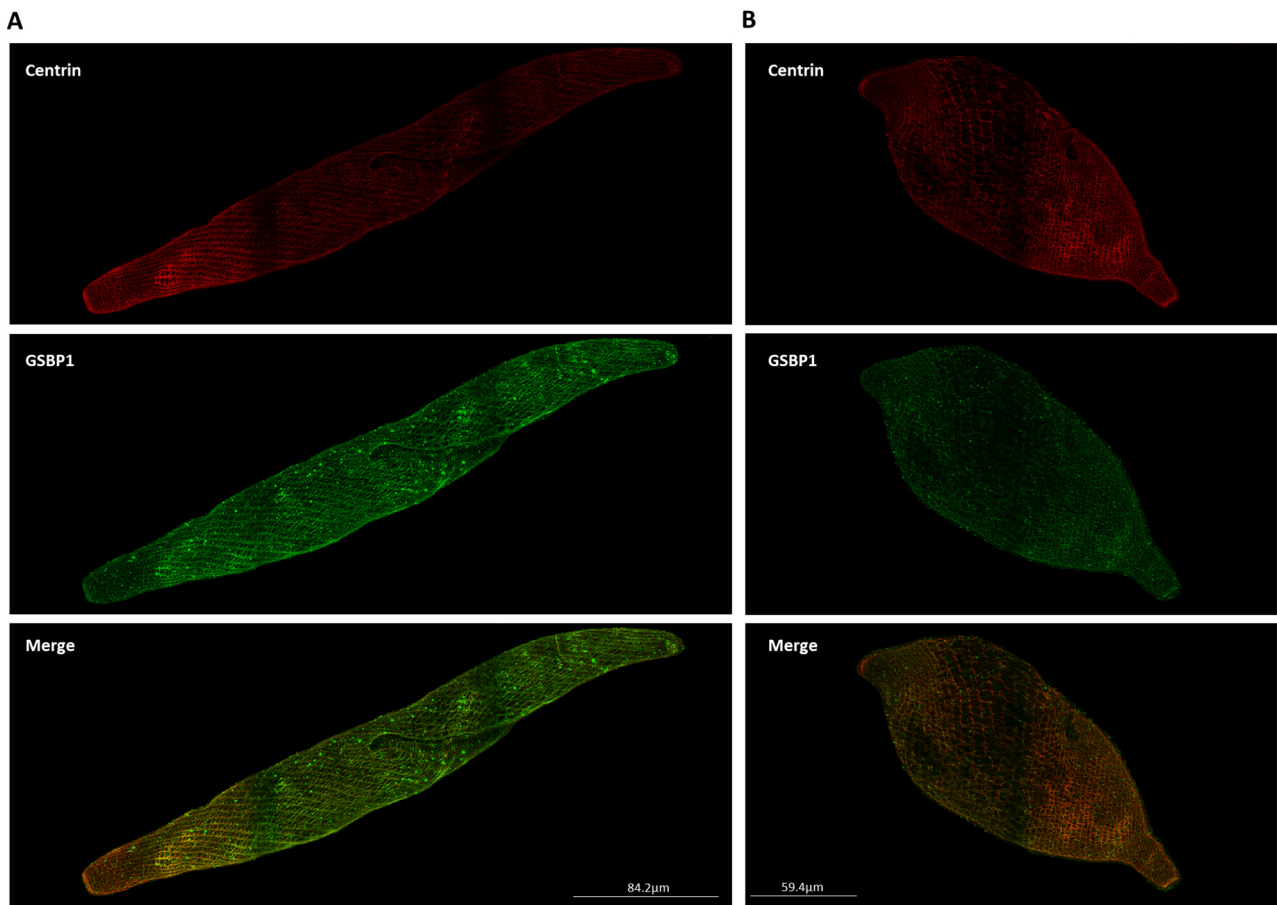
EF-hand genes with short length (green branches) closely cluster with spasmin genes (blue branches). Predicted domain organization was assigned to each spasmin. Taxon names prefixed with “SPI”, “Ste” and “Pte” are genes from *S. minus*, *S. coeruleus*, and *P. tetraurelia*, respectively.



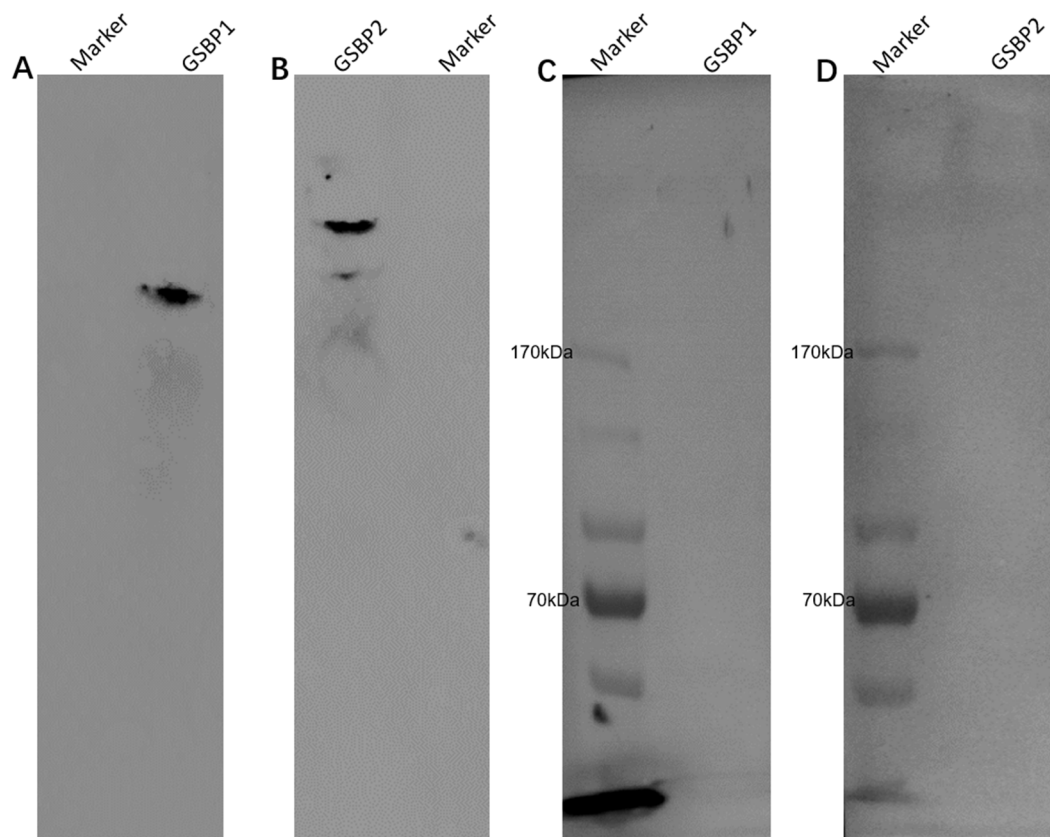
Supplementary Fig. 2. Pairwise alignment between GSBP1 and GSBP2 proteins. Two proteins show 54% identity at protein sequence level.



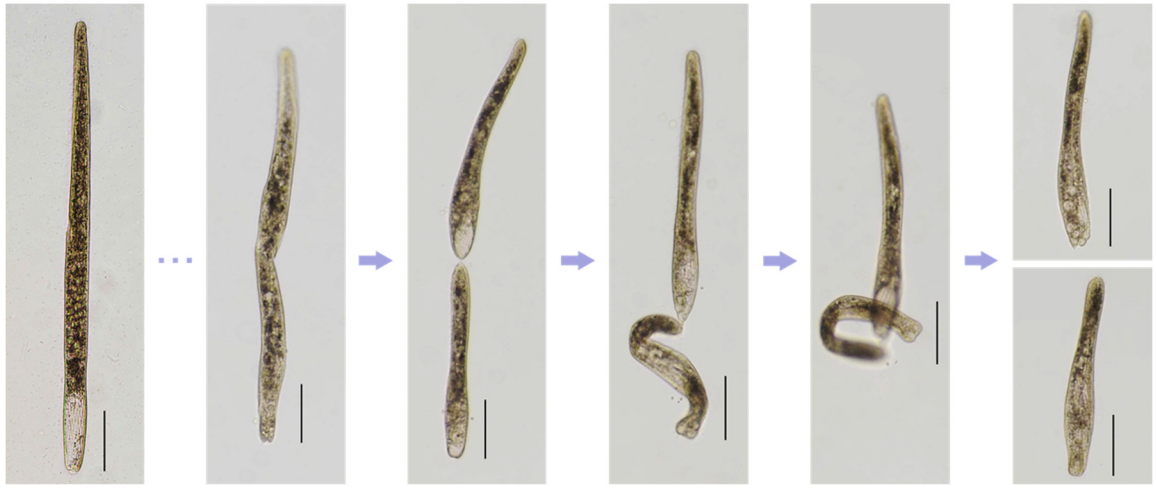
Supplementary Fig. 3. Sequence logo of 69 AAs interval between two neighboring tryptophan sites.



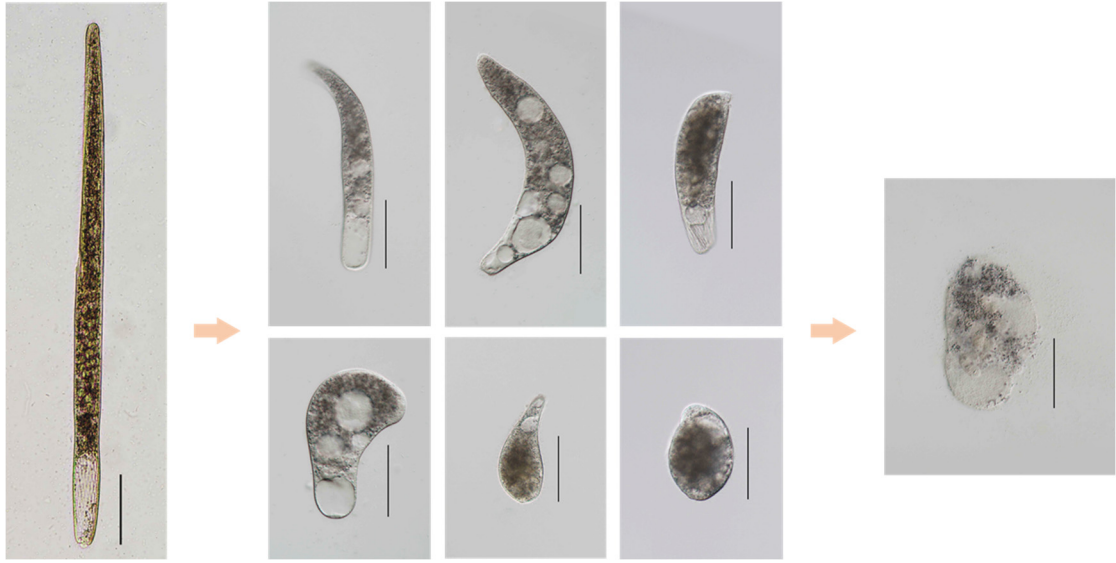
Supplementary Fig. 4. Co-localization of GSBP1 and spasmin in *Spirostomum minus*. Red, IF using anti-centrin antibody (mouse) to show location of spasmin; Green, IF using anti-GSBP1 antibody (rabbit). A, a cell in a somewhat elongated state; B, a cell in an almost fully contracted state.



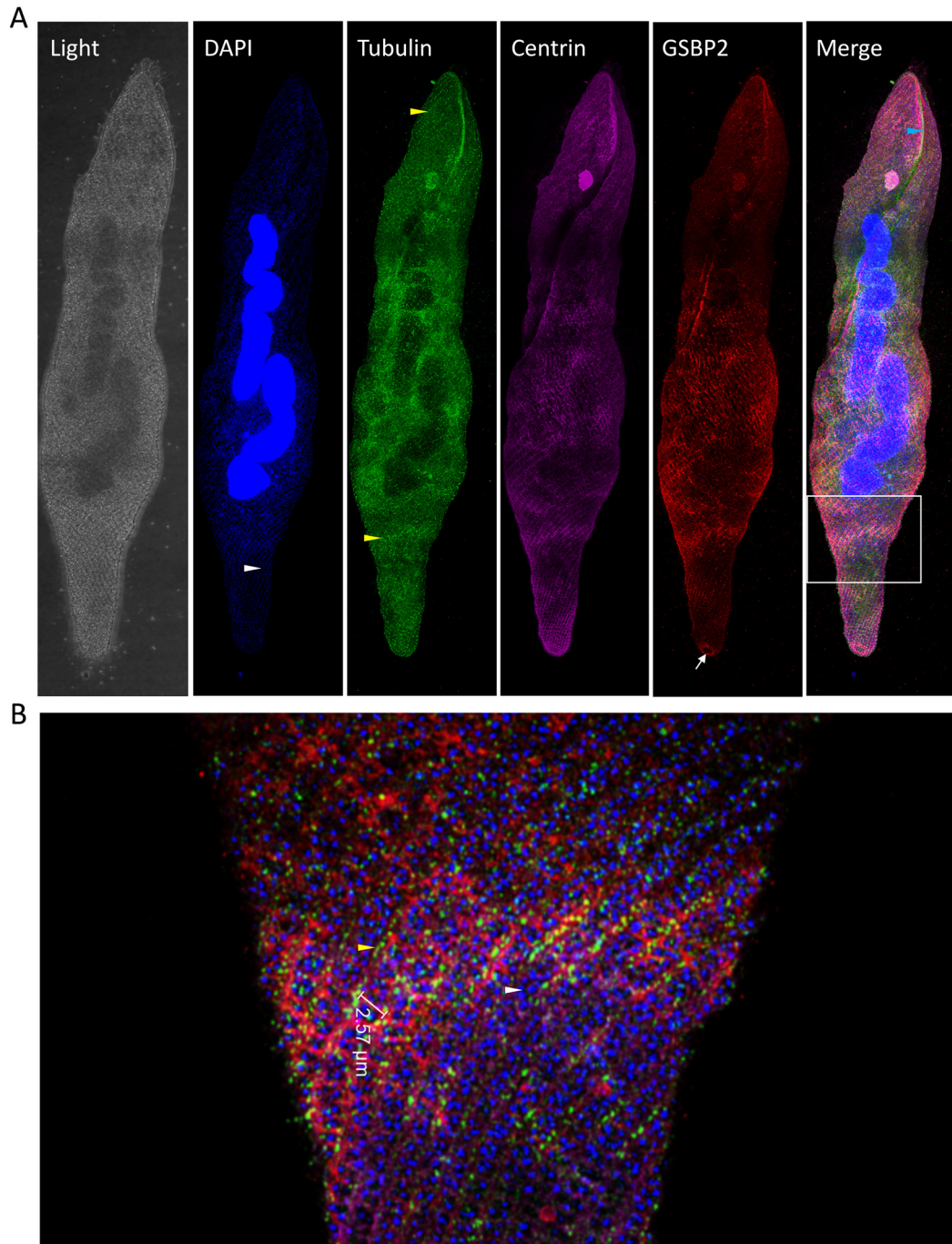
Supplementary Fig. 5. Western blot confirmation of the GSBP1 and 2 antibodies. A & C, GSBP1; B & D, GSBP2. In A and B, a 25-40kDa marker (Cat No. DM2005, Coolaber, Beijing, China) was loaded in the western blot experiments, but it seems ran out of the gel. C & D were used to check whether the GSBP antibodies could unspecifically bind to small proteins.



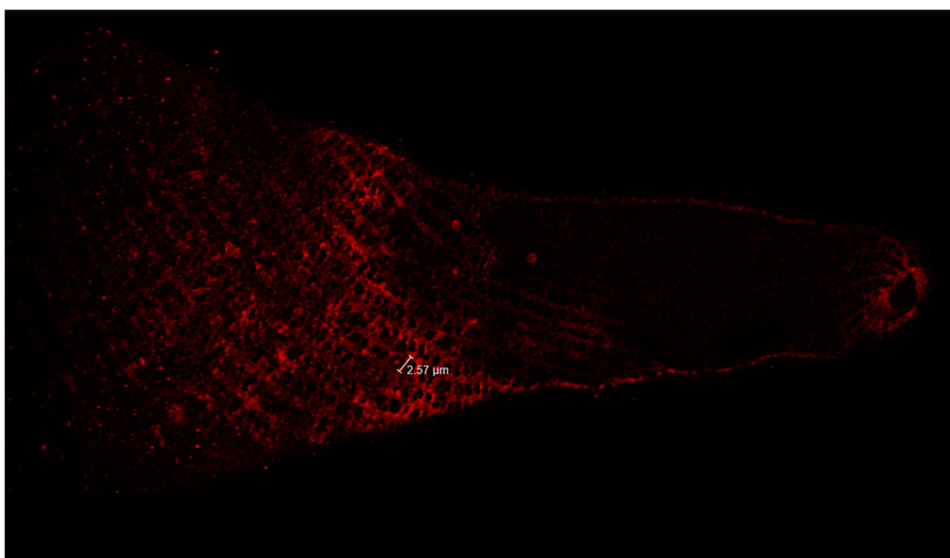
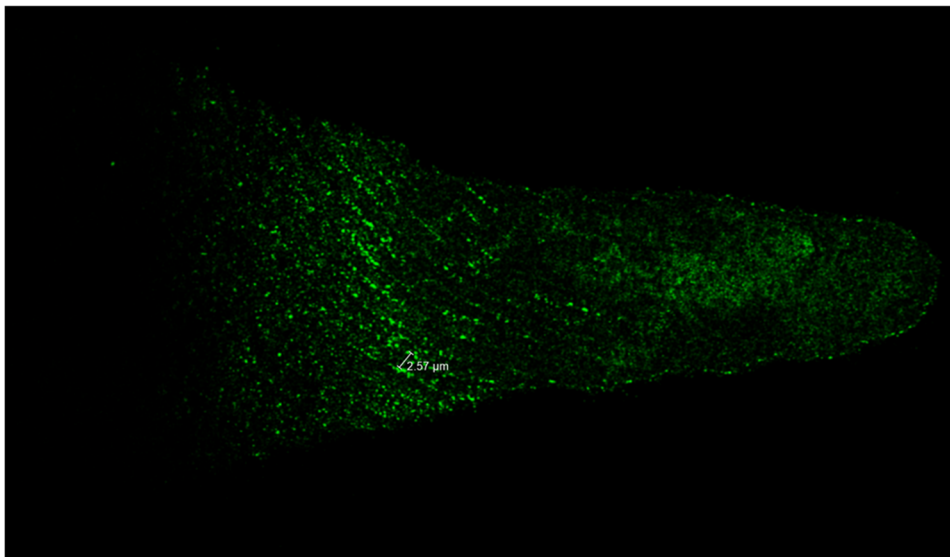
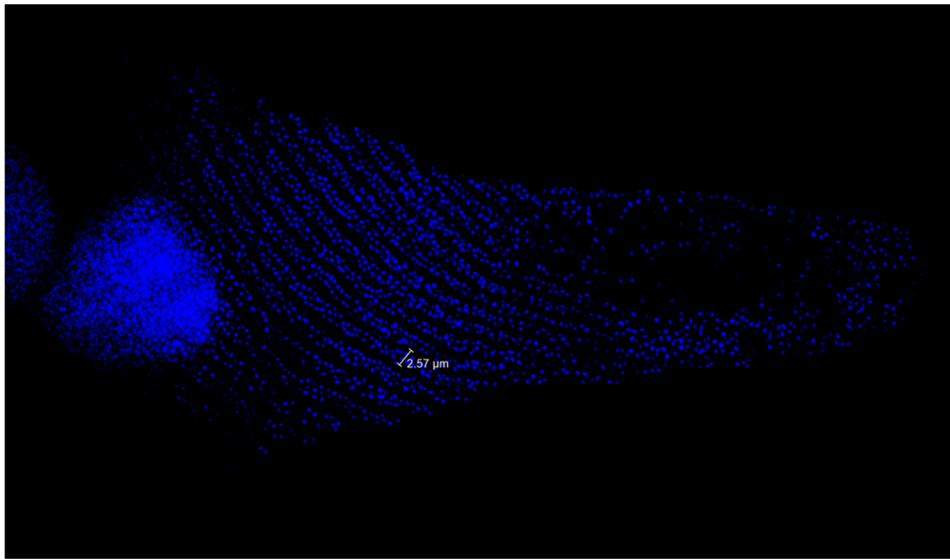
Supplementary Fig. 6. Cell division process of *Spirostomum minus*. Scale bar, 100 μm .



Supplementary Fig. 7. Cell death process of *Spirostomum minus*. Scale bar, 100 μm .



Supplementary Fig. 8. Distribution patterns of subcellular structures in *Spirostomum minus*. A, super resolution IF image showing the localization of tubulin, spasmin and GSBP2. Blue, DAPI staining of DNA, note that the foci (arrowhead) indicate the mitochondria; Green, IF using anti-beta-tubulin antibody (rat), represents the microtubule-based fibrillar bundles. Purple, IF using anti-centrin antibody (mouse). Red, IF using anti-GSBP2 antibody (rabbit). B, distribution patterns of the subcellular structures. Approximate distance between two neighboring grooves is indicated by scale bar (2.57 μm).

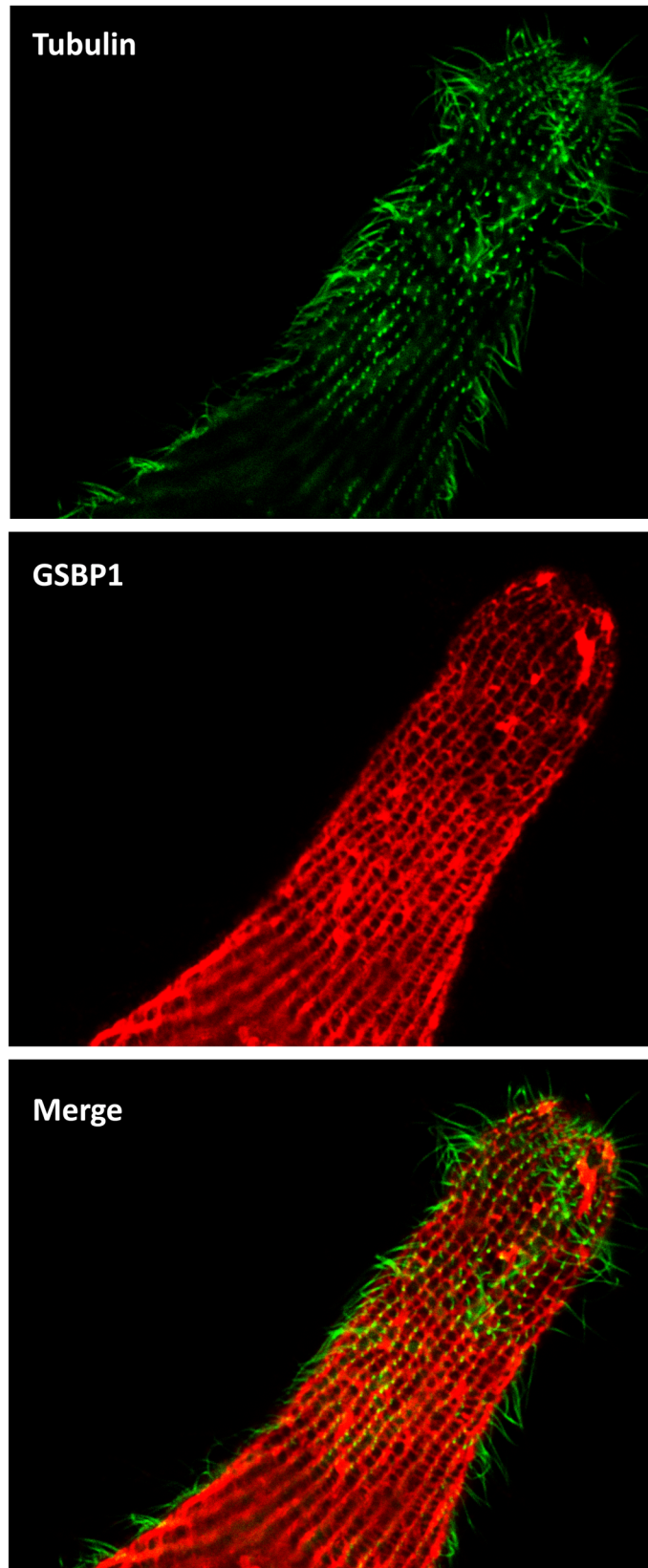


Supplementary Fig. 9. Localization of mitochondria, tubulin and GSBP2 in *Spirostomum minus*. Blue, DAPI staining showing the mitochondria; Green, IF using anti-beta-tubulin antibody (rat); Red, IF

using anti-GSBP2 antibody (rabbit).



Supplementary Fig. 10. A *Spirostomum minus* cell ejecting undigested food at the posterior.



Supplementary Fig. 11. Basal bodies of cilia are located near cross points in the mesh-like contractile fibrillar structure. Green, tubulin stained using the Tubulin-Atto 488 as in Fig. 1; Red, IF using anti-GSBP1 antibody (rabbit).

Movie S1. *Spirostomum minus* cells contracting frequently in a solution of 10^{-3} mol/L Ca^{2+} .

Movie S2. A GSBP1 RNAi *Spirostomum minus* cell that has lost its ability to contract in a solution of 10^{-3} mol/L Ca^{2+} .