The nuclear transport factor CSE1 drives macronuclear volume increase and macronuclear node condensation in *Stentor coeruleus*

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Summary

The giant ciliate, *Stentor coeruleus*, provides a unique opportunity to study nuclear shape because its macronucleus undergoes a rapid, dramatic, and developmentally regulated shape change. During a 2 hour time period within cell division and regeneration, the 400 um long moniliform macronucleus condenses into a single mass, elongates into a vermiform shape, and then renodulates, returning to its original beads-on-a-string morphology.[1] Previous work from the 1960's - 1980's demonstrated that the macronuclear shape change is a highly regulated part of cell division and regeneration, but there were no molecular studies into this process.[2,3] With the recent availability of a sequenced *Stentor* genome, a transcriptome during regeneration, and molecular tools like RNAi, it is now possible to investigate the molecular mechanisms that drive macronuclear shape change.[4–6] We found that the volume of the macronucleus increases during condensation, suggesting an inflation-like mechanism. When the nuclear transport factor, CSE1, is knocked down by RNAi, this volume increase is reduced, and the nodes are unable to fuse. This leads to a long-lasting change in nuclear morphology. We found that CSE1 is mainly cytoplasmic during interphase and in early regeneration, and then accumulates inside the macronucleus during condensation. At the end of regeneration CSE1 is degraded while the macronucleus returns to its pre-condensation volume. We propose a model in which nuclear transport via CSE1 increases the volume of the macronucleus, driving the condensation of the many nodes into a single mass.

Introduction/Results

Nuclear size and shape are among the most visible and important aspects of cell geometry. In most mammalian cell types, the nucleus is spheroidal in shape and the nucleus to cytoplasm ratio is tightly controlled. Breakdown in control of nuclear size and shape is an indicator of major problems within the cell. For example, the main criteria used to diagnose and stage cancerous cells since the 1800's are defects in the size and shape of the nucleus.[7] Yet our knowledge of the causes and consequences of these shape changes is extremely limited. In the example of cancerous cells, many components of the cell are misregulated, making it difficult to determine which changes specifically affect nuclear structure. An alternative approach for learning how cells regulate the shapes and sizes of their nuclei is by studying cells that naturally undergo developmentally regulated and dramatic nuclear shape changes as part of their normal physiology. A system that has a developmentally regulated shape change will allow us to more readily link changes in gene expression to alterations in nuclear shape. The more extreme the shape change, the better, because a dramatic shape change makes it easier to quantify the change and to detect subtle effects of perturbations that might be missed if the

normal nuclear changes are less dramatic. While some specific cell types in metazoans such as neutrophils or *Xenopus* epidermal tail fin cells develop lobed and branched nuclear shapes, most metazoan model systems maintain spheroid nuclei.[8,9] There is, however, a classical model organism whose extreme and developmentally regulated shape change creates an opportunity to dissect the mechanisms of nuclear shape control: *Stentor coeruleus*.

Structure and dynamics of *Stentor's* macronucleus

Stentor coeruleus is a giant ciliate that can extend up to 1 mm long. *Stentor* is a cone-shaped cell with a ciliated oral apparatus (OA) at the wide anterior end (Figure 1A). Cortical rows of microtubules and cilia run down the length of the cell to the holdfast at the posterior end. This large and complex cell has a correspondingly large macronucleus that is about 400 um in length and contains approximately 60,000 copies of its genome.[5] The macronucleus is visible without any staining due to the difference in refractive index between the cytoplasm and the macronucleus. The macronucleus appears as a string of clear beads that are about 30 um in diameter (Figure 1A). The many nodes of the macronucleus are continuous with each other and are connected by thin regions that are about 1-2 um wide. The nodes are contained by a single nuclear envelope, and both the nodes and the connections between them contain DNA (Figure 1B). Transmission electron microscopy of a node shows a double-membrane nuclear envelope surrounding chromatin, as well as multiple dark-staining areas that are consistent with prior descriptions of *Stentor* nucleoli (Figure 1C).[10] Thus, while having an unusual shape, the *Stentor* macronucleus shares the same ultrastructural organization of other eukaryotic nuclei.

In addition to its remarkable shape, *Stentor's* macronucleus also undergoes a dramatic, regulated, and reversible shape change. During vegetative cell division, a new OA forms in the middle of the cell and the two daughter cells split after OA development is complete. This process happens in 8 stages that each take approximately 1 hour, the last 5 of which are illustrated in Figure 1D. During this process, the macronuclear nodes condense into a single mass, elongate into a sausage-like shape, and then renodulate. The macronucleus does not undergo mitosis; instead it is split by the cleavage furrow between the two daughter cells as they separate.[11] This process, called the macronuclear shape-change cycle, also occurs when *Stentor* regenerates its OA after removal by cutting or by sucrose shock.[1] This allows us to experimentally induce this nuclear shape change synchronously in a population of cells. This inducibility, coupled with the developing set of tools available in *Stentor* such as RNAi make *Stentor* an interesting system in which to study the regulation of nuclear shape change.[4]

Previous work from the 1960's - 1980's has investigated the macronuclear shape change cycle through micro-transplantation experiments and by electron microscopy. Early studies showed that a change in the cytoplasm is sufficient to cause macronuclear condensation, as evidenced by the fact that a nucleus transplanted from a non-regenerating cell into a regenerating cell will undergo the shape change.[1,12] Macronuclei transplanted into stage 3 regenerating *Stentor* condensed more efficiently than macronuclei transplanted into stage 4 hosts - suggesting that the cytoplasmic conditions needed for condensation are present about three hours after sucrose shock.[2] These experiments showed that the interactions between the macronucleus and cytoplasm are crucial for condensation of the macronucleus. Condensation itself involves alterations to the chromatin: electron microscopy studies show that the chromatin

appears completely homogeneous by the time the macronucleus begins elongation.[10] Elongation of the macronucleus involves the microtubule cortex in addition to the cytoplasm. Electron microscopy studies have shown that a sheath of microtubules, as well as nuclear envelope-bound channels of microtubules piercing the macronucleus, are formed during elongation.[10,13] Grafting experiments showed that elongation of the macronucleus is aligned with the cortical rows.[3] These previous studies have left us with a picture of an extremely complex and regulated process - however virtually nothing is known about the physical or molecular nature of the macronuclear shape change process.

Macronuclear volume change during regeneration

At a physical level, converting from a string of small spheres to a single large compact shape will entail either an increase in volume or a decrease in surface area. One simple hypothesis is that the more compact shape is achieved by increasing the nuclear volume, much like inflating a balloon. To test this idea, we asked how the volume and node count change during the macronuclear shape change cycle. The large size and constant motion of *Stentor* cells poses a challenge for live cell imaging. We found that a Shaeffer rotocompressor allows the entire macronucleus to stay in focus while slowing the stentor's movement, yet still allows the cell to live and regenerate. We imaged the later half of regeneration using a Zeiss Axiozoom (Figure 1E). Based on these images, we then calculated the approximate volume of the macronucleus as described in Methods (see also Supplemental Figure S1A). We found that the volume of the macronucleus dramatically increases at exactly the same time that the node count decreases during macronuclear condensation (Figure 1F). This trend occurred for 6 of the 9 macronuclear cycles that were imaged (Supplemental Figure S1B). The time that the macronucleus spent in its condensed state varied, but was followed by elongation into a vermiform shape. We observed nodulation occurring simultaneously across the entire length of the macronucleus (Figure 1E).

Identifying CSE1 as a regulator of the macronuclear shape change cycle

What molecular players are driving these dramatic physical changes? De Terra previously showed that some factor is present in the cytoplasm of regenerating cells approximately 3 hours after induction of regeneration that stimulates nuclear condensation.[2] Given the fact that regeneration in *Stentor* is accompanied by a specific gene expression program, we hypothesized that the cytoplasmic alteration might involve induction of a gene product involved in nuclear transport. To investigate this idea, we first asked whether the macronuclear shape-change cycle is in fact dependent on gene expression during regeneration. We treated stentors with cycloheximide just after sucrose shock to prevent new protein synthesis. None of the cycloheximide treated stentors condensed their macronuclei to be fewer than 3 nodes, while 83% of DMSO treated stentors condensed their macronuclei (Figure 2A), indicating that synthesis of one or more protein products during regeneration plays a role in condensation.

Next, we looked for potential candidates in the list of top differentially expressed genes during regeneration.[6] Because nuclear transport has been shown to affect overall nuclear size, we focused on genes encoding potential nuclear transport factors.[14,15] Among the differentially expressed genes predicted to encode nuclear transport-related proteins, the gene CSE1, stood

out as a candidate because it is highly expressed early in regeneration before the macronuclear shape change cycle takes place (Figure 2B). The peak of CSE1 expression occurs at 120 minutes post-sucrose shock, shortly before the window in which the cytoplasm is able to set up the macronucleus for its later condensation.[2] Thus, while CSE1 is expressed well before the macronuclear shape change occurs, it is expressed at exactly the expected time if it were important for macronuclear condensation. In other model systems, CSE1 is an export factor that is necessary to export importin alpha, thus making it available for the nuclear import of other proteins.[16,17] In that sense, although technically an exportin, CSE1 is a factor whose overall function is to promote nuclear import. This candidate therefore fulfilled the requirements for our hypothetical mediator of increased macronuclear volume during *Stentor* regeneration. The predicted domain structure of *Stentor* CSE1 is similar to *S. cerevisiae* CSE1 (Figure 2C). *Stentor* CSE1 is homologous to CSE1 in other organisms, including CSE1 in other ciliates (Figure 2D, Supplemental Figure S8). There are two *Stentor* genes homologous to CSE1; these two *Stentor* genes are 92% identical to each other at the amino acid level (Supplemental Figure S7A). One of the *Stentor* CSE1 homologs was split in two during genome assembly, so it is represented by both gene identification numbers associated with this gene (SteCoe_28919 + SteCoe_28918). Both SteCoe_28374 and SteCoe_28918 are up-regulated during regeneration.[6] In the following experiments we used the Stentor gene SteCoe_28374 to create RNAi constructs because the complete gene was represented in the RNA-seq data (Supplemental Figure S6 and S7B-C).

Is CSE1 necessary for volume increase and/or shape change during the macronuclear shape-change cycle? To test this, we fed *Stentor* bacteria expressing RNAi constructs directed against CSE1 for 7 days, and measured the macronuclear shape-change cycle in these cells, induced by sucrose shock. We imaged these cells for 1 hour longer than control *Stentor* to account for *CSE1(RNAi) Stentor's* slightly slowed regeneration (Supplemental Figure S2B). Four out of six cells showed no clear peak in volume (Figure 3A,B and Figure S2). CSE1 RNAi cells generally had smaller increases in volume than wild-type stentors, and reached their maximum volumes with a slower rate of growth than wild-type stentors (Figure 3C, D). CSE1 RNAi cells were unable to condense their nucleus into 3 nodes or less. We observed that the nodes often bunched together, but many nodes failed to fuse (Figure 3E). This data shows that CSE1 is necessary for both macronuclear condensation and volume increase during the macronuclear shape-change cycle.

The physiological impact of differences in nuclear shape remains poorly understood. Does the cell care what shape its nucleus is? Given the shape phenotype we observed with RNAi knockdown of CSE1, we can now ask: what are the consequences of the macronucleus failing to undergo its normal shape-change cycle due to a lack of CSE1? We imaged macronuclear structure 24 hours post-sucrose shock. Control RNAi cells had mostly circular macronuclear nodes, with an average circularity of 0.74 (Figure 3F). We knocked down CSE1 with two RNAi constructs: CSE1 RNAi A and CSE1 RNAi B. Both RNAi constructs resulted in a wide variety of macronuclear shapes, with some macronuclei being elongated or having jagged edges (Figure 3F). We quantified the average node circularity for each stentor, and found that the average node circularity of *CSE1 RNAi Stentor* is significantly decreased (Figure 3G). This indicates that the shape change cycle may be required to maintain the normal shape of the nuclear nodes. We further observed morphological changes to the overall cell shape. Wild type

Stentors typically have elongated tails while they are undisturbed and freely swimming - we observed this in control RNAi cells. In CSE1 RNAi cells, we observed many free-swimming cells with shortened tails (Figure 3H). These cells still had a functional holdfast and were able to contract, albeit less so due to their shorter lengths. This suggests that the components of the tail were still present. In that sense, the phenotype resembles a "gap" mutant of *Drosophila*.

CSE1 localization during the macronuclear shape change cycle

In order to track CSE1 localization throughout regeneration, we raised a custom antibody against *Stentor* CSE1 (Supplemental Figure S3). In non sucrose shocked cells, CSE1 is present in cytoplasmic puncta in both PFA- and methanol-fixed cells (Figure 4 and Supplemental Figure S4). In three cases we observed CSE1 staining become concentrated around the periphery of the macronucleus; this occurred in cells 6 hours post sucrose shock, which corresponds to the time at which macronuclei are in the process of condensing. (Supplemental Figure S4). When the macronucleus is condensed, CSE1 is present in the interior of the macronucleus (Figure 4). This occurs in both PFA-fixed stentors that have been permeabilized with Triton-X100, as well as methanol-fixed stentors, showing that the intranuclear punctate signal is not an artifact of fixation conditions (Supplemental Figure S4). Both fixation conditions show that CSE1 dynamically re-localizes from being mainly cytoplasmic, to mainly intranuclear as the macronucleus condenses. We have also observed this relocalization in stentors undergoing cell division, suggesting that this localization change is a part of the macronuclear shape-change cycle, and not a stress response to sucrose shock (Supplemental Figure S5). In the course of these observations, we noted that by 7 hours into regeneration, CSE1 signal is dramatically reduced (Figures 4 and S4). This occurs at roughly the time at which the macronucleus begins to elongate and decrease in volume.

Discussion

Early work on *Stentor* showed that there is a complex interplay between the cytoplasm, the microtubule cortex, and the macronucleus during its shape change cycle. Macronuclei are dependent on unidentified components in the cytoplasm present at particular stages in order to progress into the next stage of the cycle, and the structural components of the macronucleus itself change dramatically throughout the cycle. The macronuclear shape change cycle consists of many different processes occurring over three phases: 1. the fusion of the nodes coupled with their migration towards the center of the cell and a near-doubling of the nuclear volume; 2. the condensed macronucleus reducing its volume to baseline and elongating along microtubule structures; and 3. the elongated macronucleus rapidly re-nodulating along its entire length. In this paper we have identified the first molecular component involved in the regulation of macronuclear shape change: CSE1.

CSE1 is necessary for the fusion of nodes and the volume increase that occurs during phase 1 of the macronuclear shape change cycle. We have shown that when stentors are treated with CSE1 RNAi, the macronuclear condensation is defective - the nodes do not fuse to the same extent as wild type, the volume increases less, and the rate at which the macronucleus increases in volume is reduced. We also observed that, in CSE1 RNAi cells, the nodes still appear to migrate towards each other, suggesting that some other factor is driving the positioning of the nodes.

How might CSE1 be driving the volume increase of the macronucleus? Alterations in nucleocytoplasmic transport of proteins have been shown to affect the volume of nuclei in metazoan cells. While some studies suggest overall flux of proteins into the nucleus can increase volume, the import of lamins is especially effective at facilitating nuclear volume increase.[14,15,18] *Stentor* has no genes that are homologous to lamins. Further investigation into the structure and composition of *Stentor's* nuclear envelope are needed to determine if there are some proteins that play a similar role as lamins do in metazoans.

While it is unclear exactly which proteins are trafficked into the macronucleus during condensation, CSE1 likely plays a role in their import. In other systems, CSE1 keeps the cycle of nuclear import running by exporting importin alpha out of the nucleus, thus ensuring importin alpha is available in the cytoplasm to import more proteins into the nucleus. When CSE1 levels are depleted in *S. cerevisiae*, importin alpha is sequestered inside the nucleus.[16] In *Drosophila*, the CSE1 homolog dcas switches from a predominantly cytoplasmic to a nuclear localization at different stages of oogenesis.[19] This redistribution of CSE1 in *Drosophila* is thought to reflect changes in nuclear transport, given that stages with high amounts of nuclear dcas correspond to stages in which the overall protein levels of the nucleus are increased.[19] In *Stentor*, the re-localization of CSE1 from the cytoplasm to the macronucleus during condensation suggests that a similar shift in the overall direction of nuclear transport towards the macronucleus has taken place (Figure 4A). Degradation of CSE1 protein after condensation could be causing a decrease in nuclear import, allowing the macronucleus to decrease in volume. Overall, the picture emerges of *Stentor* transiently increasing its levels of CSE1 in order to drive more import of material into the macronucleus during the condensation phase of the nuclear shape change (Figure 4C).

Besides an increase in volume, the other notable aspect of the macronuclear shape change cycle is the fusion of the nodes. The fusion may simply be a direct physical result of this volume increase. Node fusion does not require any membrane fusion, as the nodes are, from the beginning, linked by thin regions and are contained within a single nuclear envelope. Nodes that have been severed from each other cannot fuse.[20] If the volume increase outpaces an increase in nuclear surface area, then the beads on a chain shape cannot be maintained. The macronucleus would more and more begin to resemble the shape with the maximum volume:surface area ratio: a sphere. If CSE1 cells are unable to increase their volume as much as wild type, then the most energetically favorable path would be to remain in a moniliform shape, or to have incomplete node fusion. Altering the nuclear shape may be a common way to accommodate alterations in the surface area:volume ratio of nuclei. For example, some *sec* mutant *S. cerevisiae* cells have their nuclear envelope growth outpace the growth of their nuclear volume, and develop bilobed nuclei as a result.[21]

How might *Stentor* benefit from undergoing the macronuclear shape change cycle? One feature that separates *Stentor's* macronuclear shape change cycle from other models in metazoans is the reversibility of the change - *Stentor's* macronucleus completes the process looking similar to how it began, albeit with a slightly increased number of nodes. Because of this, the purpose of the macronuclear shape change cycle has been unclear. A variety of competing hypotheses have been proposed for why *Stentor* would compact its nucleus during division, ranging from the idea that it makes it easier to split during cytokinesis, to the hypothesis that it allows *Stentor* to rapidly increase its number of nodes.[1] No consensus has

been reached on this matter. It was interesting to note, then, that *CSE1 RNAi Stentor*, in addition to failing to compact fully, were unable to maintain a normal moniliform shape after regeneration was complete. This suggests that condensing the macronucleus is important for maintaining its normal shape. Biophysical measurements of membrane tension and nucleoplasmic density before, during, and after macronuclear condensation would help us understand how the macronuclear shape change cycle helps maintain the moniliform shape of *Stentor* during interphase.

We also observed that some *CSE1 RNAi Stentor* appeared to have shortened posterior halves after 7 days of RNAi feeding (Figure 3H). It is plausible that the misshapen macronucleus of CSE1 RNAi *Stentor* is unable to extend the length of the cell to properly distribute mRNA throughout the entire cytoplasm, and the posterior half begins to shrink as a result. In other species of *Stentor*, the macronuclear shape usually corresponds to the size of the cell. The smallest species, *Stentor multiformis*, have spherical macronuclei. Intermediate species like *Stentor roseli* have vermiform macronuclei. *Stentor coeruleus* is one of the largest *Stentor species*, and like other giant heterotrich ciliates like *Spirostomum*, it has a moniliform macronucleus.[1] This shape of nucleus could be useful for stretching the macronucleus across long distances in giant ciliates, thus providing a local source of message for different regions of the cell. The misshapen macronuclei of CSE1 RNAi cells may be unable to efficiently reach across the length of *Stentor* needed to support the maintenance of all of its cellular structures.

Giant and syncytial cells in fungi and animals often have many nuclei that are distributed throughout the cell, and these distributions are important for the cells to function properly. In muscle fibers, the nuclei are located at the periphery of the fiber, and spaced such that the distance between them is maximized.[22] In various muscle diseases, and also during muscle repair, the nuclei are often clustered in the center of the muscle fiber. [23,24] The hyphae of fungi like *Ashbya gossypii* also have multiple nuclei distributed along their lengths.[25,26] In mutant strains of *Ashbya* where nuclei are randomly spaced, nuclei that are clustered together undergo mitosis at similar times - disrupting the cell cycle independence of each nucleus within the hyphae.[27] When cells reach large size scales, regulating the distribution of nuclear material appears to be important for maintaining the overall cellular architecture.

The macronucleus of *Stentor coeruleus* undergoes a rapid and dramatic nuclear shape change that has long fascinated cell biologists. We have now identified the first molecular player in this shape change: CSE1. This nuclear transport factor is necessary for node condensation and volume increase to occur, and its re-localization and degradation correspond to the morphological changes of the macronucleus. The macronuclear shape change cycle is a complex process, and further understanding it will require studying more genes and investigating the physical changes that happen to the macronucleus throughout this cycle.

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Materials and Methods

Stentor Strains and culturing

All RNAi, immunofluorescence, and live imaging experiments were carried out with *Stentor coeruleus* originally obtained from Carolina Biological Supply Company (Burlington, NC) and cultured in the lab. Images in figures 1A and 1C were taken of wild *Stentor coeruleus* that were obtained from North Lake in Golden Gate Park, San Francisco, CA and then cultured in the lab. *Stentor* were cultured using the same protocol detailed in Lin, 2018.[28]

Live Imaging of Macronuclear Shape Change Cycle

Stentor were sucrose shocked in a 15% sucrose in Carolina Spring Water (CSW) solution (Carolina Biological Supply) for two minutes. The shock was halted by rapidly diluting 2 mL of shocked stentors into 50 mL of CSW. Cells were incubated at room temperature for 4-5 hours. The stentors were then loaded into a Schaeffer rotocompressor (Biological Institute of Philadelpiha, Philadelphia PA) and compressed until their movement was just halted. Although not used for the imaging reported here, we have also found that the Janetopolous rotocompressor (Invivo-Imaging.com) also works well to compress *Stentor.*[29] The rotocompressed stentors were then imaged using a Zeiss Axiozoom V16 equipped with a Nikon Rebel T3i SLR Camera. Timelapse images were taken every 5 minutes either manually or automatically using DSLR Remote Pro (Breeze Systems Ltd., Camberly, Surrey, UK).

Volume Calculations

The edges of the macronuclear nodes were manually traced on a transparent layer above each *Stentor* image using the pen tool in Affinity Designer 1.9.3 (Affinity.serif.com, Serif (Europe) Ltd., Nottingham, UK). Each outline was saved as a PNG with transparent background. In FIJI each outline was filled in and converted to a binary image using a custom FIJI macro.[30] The binary images were loaded into Affinity Designer 1.9.3, and, using the lasso tool, the nodes were manually arranged so that the midline of the macronucleus was horizontal. The horizontal images were opened in FIJI and each image was cropped and converted to binary to be prepped for further analysis with Python. The python-ready images were then analyzed in a Jupyter notebook to calculate the volume of the macronucleus at each timepoint by assuming rotational symmetry around the horizontal axis of each node.[313233,343536] Details of the calculation are provided in Supplemental Figure S1A. The number of nodes for each timepoint was visually counted from the outline images.

Phylogenetic Analysis

CSE1 homologs were identified using BLASTP.[37] CSE1 amino acid sequences were uploaded into MEGAX and aligned using MUSCLE.[38–40] Phylogenetic trees were generated using the Maximum Likelihood Method.[41] Domains of *Stentor* CSE1 were identified using InterPro.[42] The multiple alignments were displayed using boxshade (https://sourceforge.net/projects/boxshade/)

Cloning

Genes were amplified with PCR from genomic DNA extracted from *Stentor* using a DNeasy Blood and Tissue Kit: Animal Blood Spin-Column Protocol (QIAGEN, Germantown, MD). Genes

were inserted into a pPR-T4P plasmid using ligation independent cloning.[43] The resulting vectors were then transformed into HT115 *E. coli.* The two CSE1 RNAi constructs target non-overlapping regions of the CSE1 gene. CSE1 RNAi A encompasses DNA bases 1247-2084, while CSE1 RNAi B targets 2089-2841. Primer sequences are listed in Supplemental Figure S6.

Cycloheximide Treatment

Cells were either treated with 0.01% DMSO or 5 mg/mL cycloheximide in CSW immediately after sucrose shock. Single cells were then placed into individual wells in a 96-well plate and observed every 15 minutes using a Zeiss Stemi Stereomicroscope. The time at which each macronucleus condensed to at least 3 nodes was recorded.

RNAi

HT115 bacteria containing the RNAi constructs were grown to OD600 0.4-0.6, and then induced with 1 mM IPTG. Induction took place for either 4 hours at 37°C or overnight at room temperature. *Stentor* were added to fresh CSW and fed pellets of bacteria expressing RNAi daily for 7 days.

Hoechst Staining

Stentors were incubated in 10 ug/mL Hoechst 33342 diluted in CSW for 30 minutes. Cells were then transferred to CSW and incubated for another 30 minutes. Stentors were compressed in a rotocompressor to image the macronucleus.

Macronuclear Shape Analysis

Images of macronuclear nodes were thresholded in FIJI, and the circularity of each node was measured.[30] For each stentor with multiple nodes, the circularity of the nodes were averaged together. Thus the circularity reported in each datapoint in Figure 3 is the average node circularity per *Stentor*. This ensures that cells with many nodes do not overpower cells with fewer nodes when determining the overall node circularity of each population of *Stentor.*

CSE1 Antibody Generation

Pre-immune bleeds from rabbits were first screened in order to avoid using any rabbits that already produce antibodies that react with *Stentor* proteins in immunofluorescence and in western blots. This was done by incubating either fixed stentors or western blot membranes with pre-immune rabbit serum diluted at a 1:500 ratio, then staining with secondary antibodies. A custom anti-CSE1 antibody was generated using the peptide MVDFTSIFTKC (Bethyl Laboratories, Montgomery, TX). A column for affinity purification was prepared by binding the peptide to a SulfoLink resin column using the SulfoLink Immobilization Kit (Thermo Fisher). Antibodies were affinity-purified from serum using this column.

Immunofluorescence with PFA Fixation

Cells were fixed in 2% paraformaldehyde in 0.5x PBS at 4°C overnight. Cells were then rinsed in TBS and then permeabilized in 0.5% Triton X-100 in TBS. Cells were then rinsed in 0.1% Triton X-100 in TBS and blocked in 2% BSA, 0.1% Sodium Azide in TBS for 1 hour at room

temperature. Primary and secondary antibodies were incubated with fixed stentors for 1 hour each at room temperature. Primary antibodies used in this study are Anti-Nup98 (mab21A10, Abcam, Waltham, MA) and a custom-generated CSE1 antibody. Secondary antibodies used in this study are Alexa-488 goat-anti-rabbit (AB_2576217, Thermo Fisher Scientific, Waltham, MA) and Alexa-488 goat–anti-mouse (Life Technologies, Grand Island, NY). Cells were washed three times with 0.1% Triton-X100 in TBS after both antibody incubations. Cells were then placed into mounting medium (80% glycerol, 1% DMSO in 50 mM Tris pH 8.0). Slides were prepared using 0.25 mm silicone spacers in between the coverglass and slide.

Peptide Block

Stentor anti-CSE1 antibody and the peptide used to generate this antibody (MVDFTSIFTKC) were mixed at a 1:10 antibody:peptide molar ratio and incubated for 2 hours at room temperature.

Immunofluorescence with Methanol Fixation

Cells were fixed in -20°C methanol for either 1 hour or overnight. Cells were rinsed with 1:1 PBS:Methanol, and then again with PBS. Cells were blocked with 2% BSA in PBS for 1 hour at room temperature. Primary and secondary antibodies were incubated with stentors for 1 hour at room temperature each. Cells were rinsed with PBS and then mounted in Vectashield mounting medium. Slides were prepared using 0.25 mm silicone spacers in between the coverglass and slide.

Fluorescence Imaging

Stentors were imaged with a DeltaVision deconvolution microscope with a 20x air objective and a CoolSnap HQ camera. Z-stacks were taken with 2 um step sizes. This microscope was controlled using SoftWorx (Applied Precision). Stentors were also imaged on a Nikon Ti microscope equipped with an Andor Borealis CSU-W1 spinning disk confocal, an Andor Zyla 4.2 sCMOS camera, and a Plan Fluor 40x/1.3 oil immersion objective. This microscope was controlled using Micro-manager software.[44] Nuclear envelope immunofluorescence images were taken on a Zeiss LSM980 Airyscan confocal microscope equipped with a C-Apochromat 40x/1.20 water immersion Korr objective.

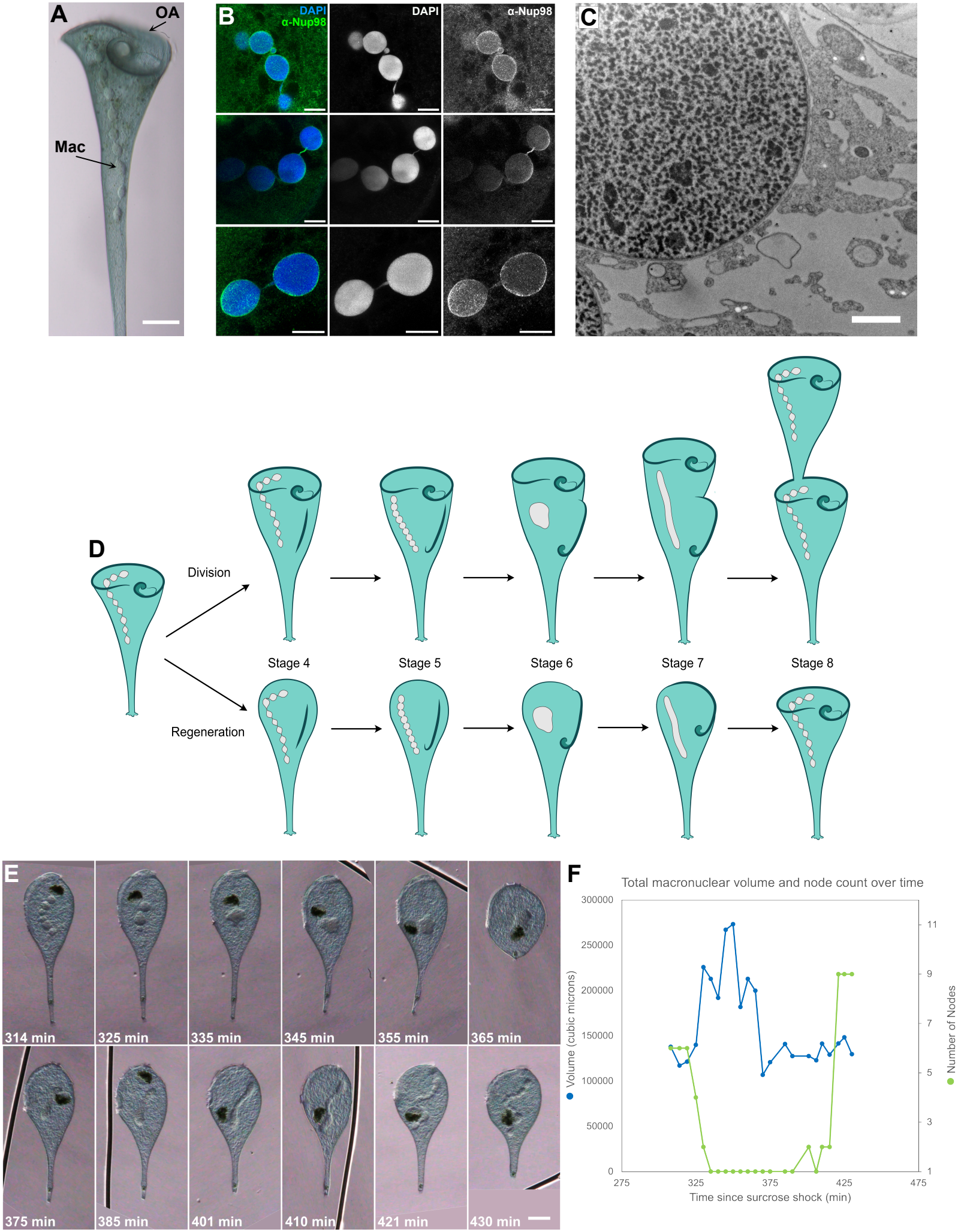
CSE1 Average Intensity Measurements

Image stacks of *Stentor* were converted into max projections. The autofluorescence of *Stentor's* pigment stripes was used to define the boundary of the cell: this channel was thresholded and converted to a binary image in FIJI. The boundary of this binary image was transferred to the CSE1 channel image, and the average CSE1 fluorescence intensity within this boundary was measured.

Transmission Electron Microscopy

Stentors were starved such that the cytoplasm was free of visible food vacuoles and the macronucleus was clearly visible under a dissecting microscope. The fixation protocol is derived from Wloga, 2008.[45] Fifty stentors were washed with 10 mM Tris-HCl pH 7.4. Immediately after the rinse, stentors were fixed for 1 hour in ice-cold 1% osmic acid, 1.5% glutaraldehyde, 25 mM

cacodylate buffer pH 7.4. After fixation, the stentors were rinsed overnight at 4C in 50 mM cacodylate buffer pH 7.4. The stentors were then embedded in agar blocks, dehydrated in ethanol series, and embedded in Epoxy resin. Using the PowerTomeXL ultramicrotome, sections of 70nm were collected on single slot, formvar, carbon coated, copper grids and post stained with 1% uranyl acetate and Reynolds lead citrate. Imaging was done using JEOL transmission electron microscope 200CX.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Total macronuclear volume and node count over time
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Number of Nodes
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300000
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150000
100000
50000
0
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3
1

Figure 1: Macronuclear shape change in *Stentor* involves reversible increase in nuclear volume (A) Brightfield image of *Stentor coeruleus* (scale bar = 100 um). The oral apparatus (OA, arrow) curls around the anterior of the cell. The macronucleus (Mac, arrow) is visible as a moniliform series of connected nodes within the cell, resembling a string of glass beads. The macronucleus extends from the membranellar band to the beginning of the thin contractile tail in the posterior half of the cell.

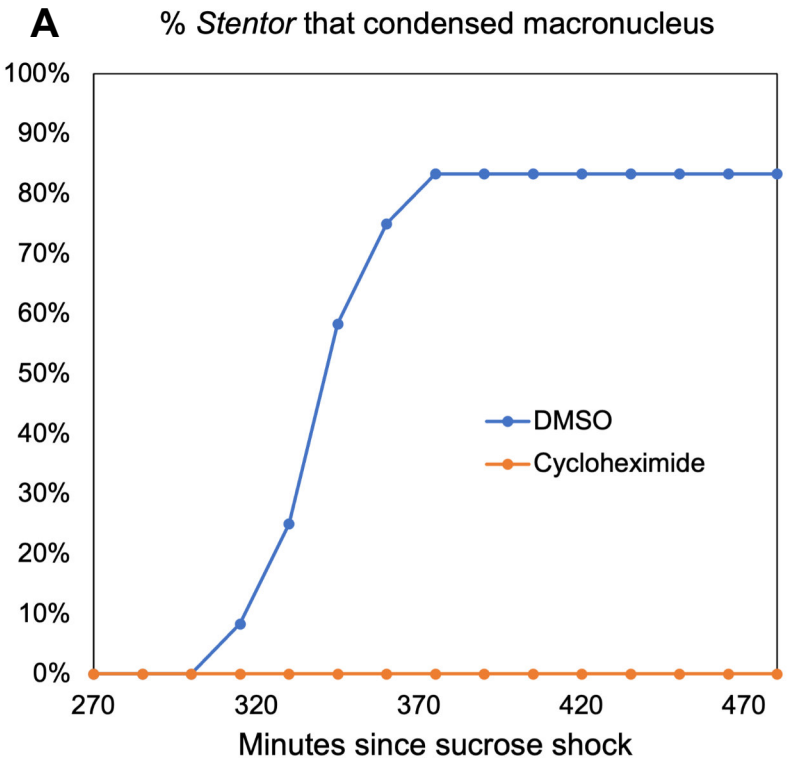
(B) Immunofluorescence images of the macronucleus in methanol-fixed cells stained with DAPI and anti-Nup98, an antibody that detects nuclear pores (scale bars = 20 um). DNA is present in both the nodes and the thin connecting regions between the nodes. The nuclear envelope surrounds both the nodes and the connecting regions. Images were taken with the same objective, but cropped to highlight regions of interest.

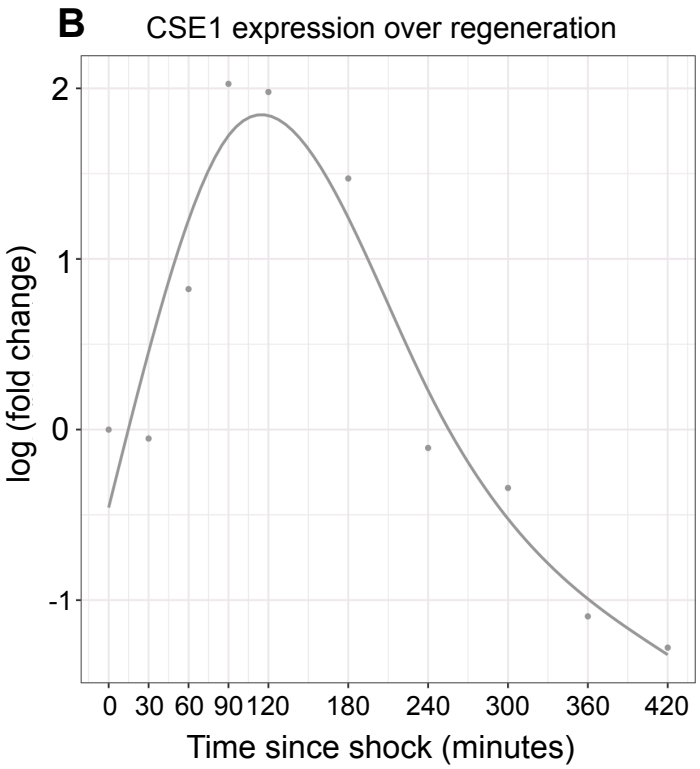
(C) Transmission electron microscopy of the macronucleus (scale bar = 2 um). A double membrane nuclear envelope surrounds the macronuclear node. The node contains multiple large features whose staining is consistent with them being nucleoli as described in Pelvat, 1982.[10] Chromatin density appears to vary throughout the node, with no region having overall denser or lighter staining than another.

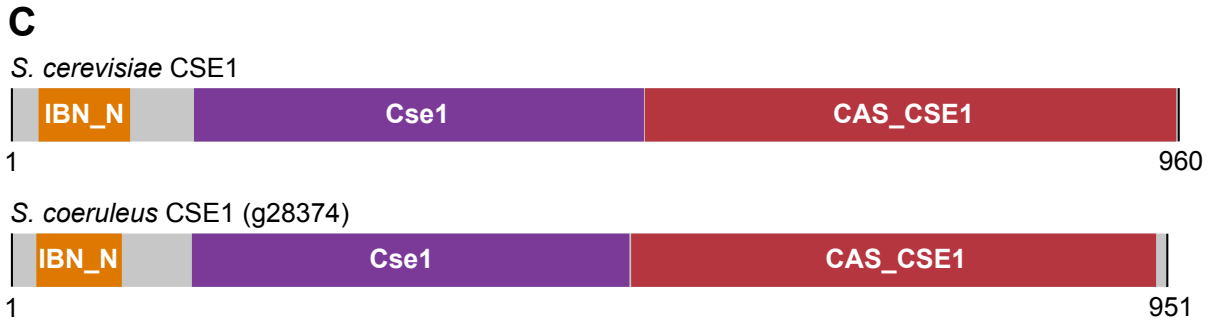
(D) Diagram of the macronuclear shape-change cycle in both cell division and regeneration. *Stentor* division and regeneration has 8 morphological stages that take place over the course of 8 hours. During cell division, a new oral apparatus forms in the middle of the cell. This oral primordium elongates, curls at the posterior end to form a new oral pouch, then inserts into the cleavage furrow to form the new oral apparatus of the posterior daughter cell. During this process the thin connecting regions shorten, the macronuclear nodes come into direct contact with each other, and eventually they fuse together to produce a single compact shape. The macronucleus then elongates into a vermiform shape before renodulating back into a moniliform shape. The macronucleus is cut in two by the constriction formed between the anterior daughter cell's tail and the posterior daughter cell's frontal field. During regeneration, the oral apparatus and the macronucleus undergo the same morphological changes with the same timing as in the stages of cell division.

(E) The macronuclear shape-change cycle observed during regeneration in a living *Stentor coeruleus* cell. The cell was immobilized and compressed in a Schaeffer rotocompressor. Scale bars = 75 um, time since sucrose shock is in the lower right corner of each image. The macronucleus condenses into a single node (t = 335 min), elongates (t = 375-401 min), and then renodulates (t = 421 min). The dark object seen in this time series is a food vacuole.

(F) The total macronuclear volume was calculated by estimating the volume of a stack of cylinders along the midline of the macronucleus (Supplemental Fig S1). The number of macronuclear nodes were counted for each frame. The macronuclear volume peaks as the number of nodes decreases to one. The macronuclear volume returns to baseline as the macronucleus elongates into a vermiform shape. The macronucleus re-nodulates all at once, bringing the node count up rapidly.


A
% Stentor that condensed macronucleus
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
270
320
370
420
470
Minutes since sucrose shock
DMSO
Cycloheximide


B
CSE1 expression over regeneration
log (fold change)
2
1
0
-1
0 30 60 90 120 180 240 300 360 420
Time since shock (minutes)


C
S. cerevisiae CSE1
IBN_N
Cse1
CAS_CSE1
1
960
S. coeruleus CSE1 (g28374)
IBN_N
Cse1
CAS_CSE1
1
951

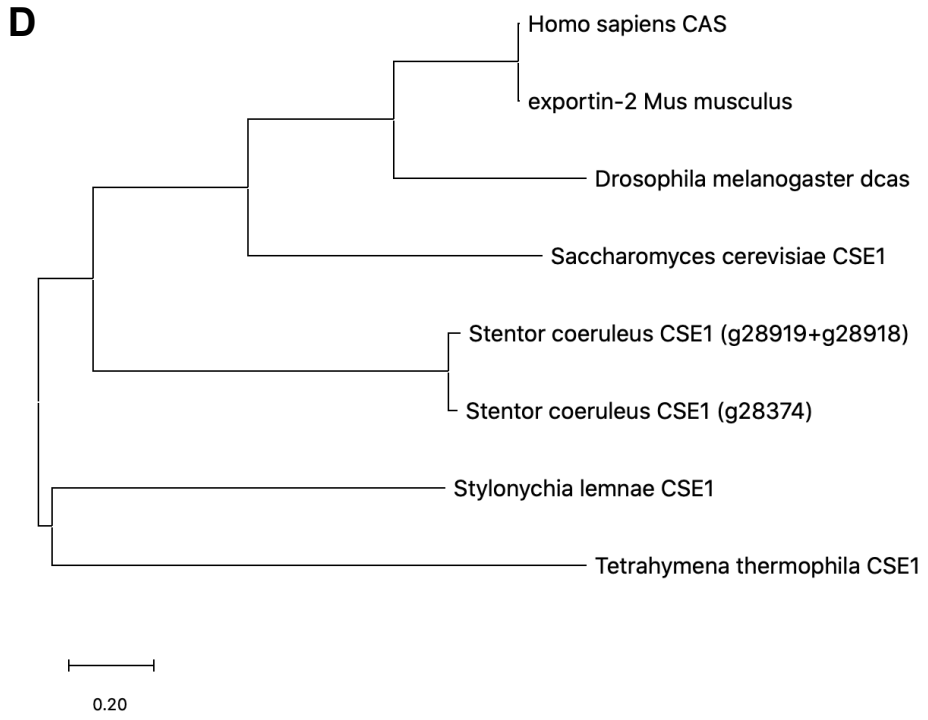

D
Homo sapiens CAS
exportin-2 Mus musculus
Drosophila melanogaster dcas
Saccharomyces cerevisiae CSE1
Stentor coeruleus CSE1 (g28919+g28918)
Stentor coeruleus CSE1 (g28374)
Stylonychia lemnae CSE1
Tetrahymena thermophila CSE1
0.20

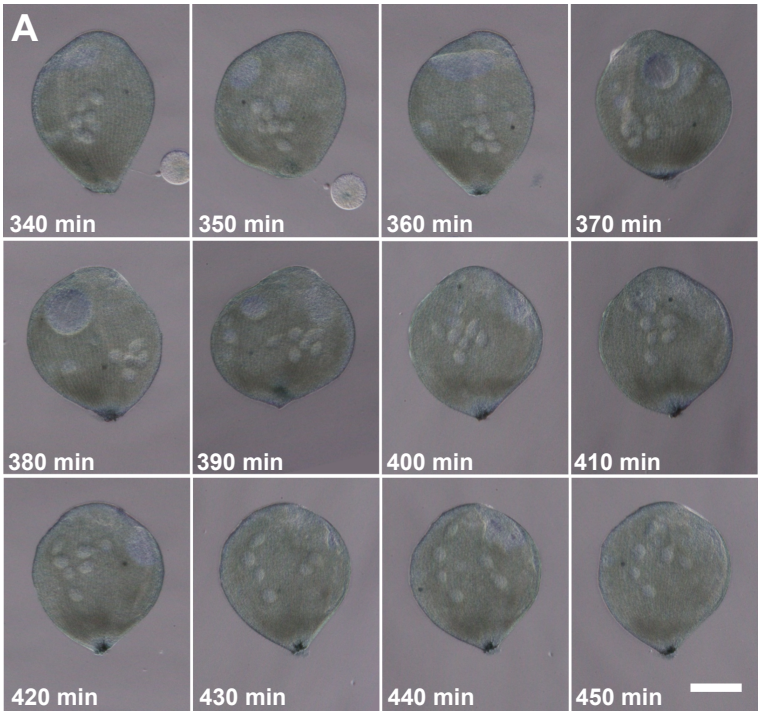
Figure 2: Expression of a CSE1 ortholog during macronuclear shape change

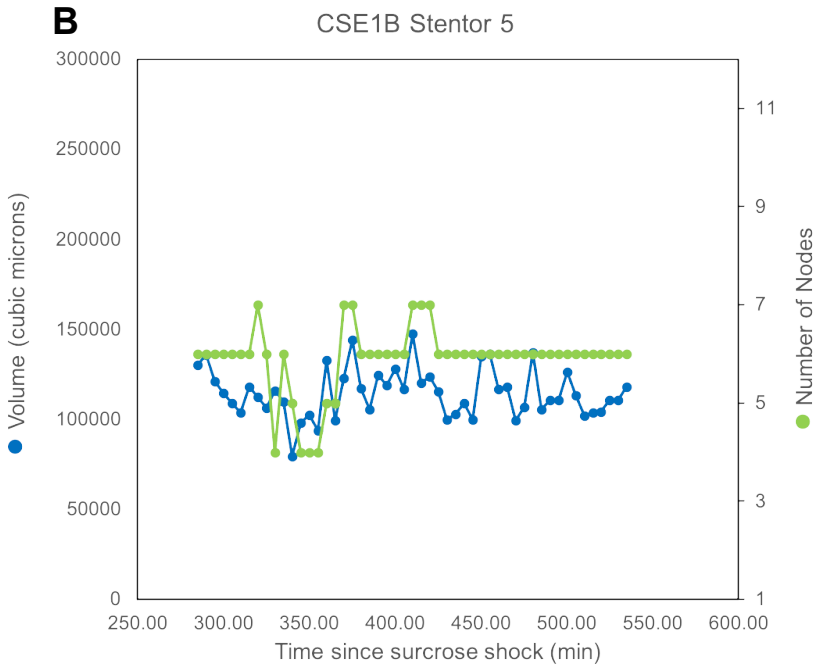
(A) Macronuclear condensation requires protein synthesis. Graph plots the percent of *Stentor* that condensed their macronucleus (defined as having 3 nodes or less) as a function of time during regeneration induced by sucrose shock. Cells were either treated with 0.01% DMSO or 5 mg/mL cycloheximide in CSW immediately after sucrose shock. 83% of cells treated with DMSO had condensed their macronucleus by 375 minutes after sucrose shock (n = 12). None of the cells treated with cycloheximide condensed their macronucleus (n = 12).

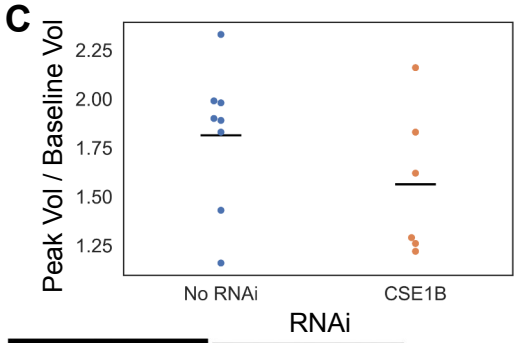
(B) CSE1 expression peaks about 120 minutes after sucrose shock, with mRNA levels increasing 4-fold relative to non-regenerating *Stentor*.

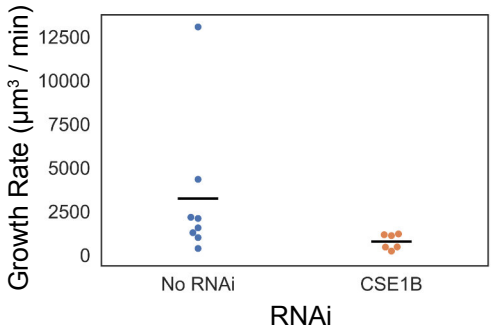
(C) Diagram of domain structures of *S. cerevisiae* CSE1 protein and *S. coeruleus* CSE1 protein. Pfam domains were determined using InterPro.

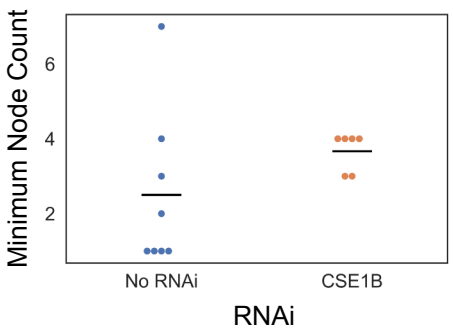
(D) Phylogenetic tree showing the two *Stentor coeruleus* CSE1 homologs and their relationship to CSE1 homologs in other organisms. One of the *Stentor* CSE1 genes was split in two during genome assembly, so the combined sequence was used to construct the tree (g28919+g28918).

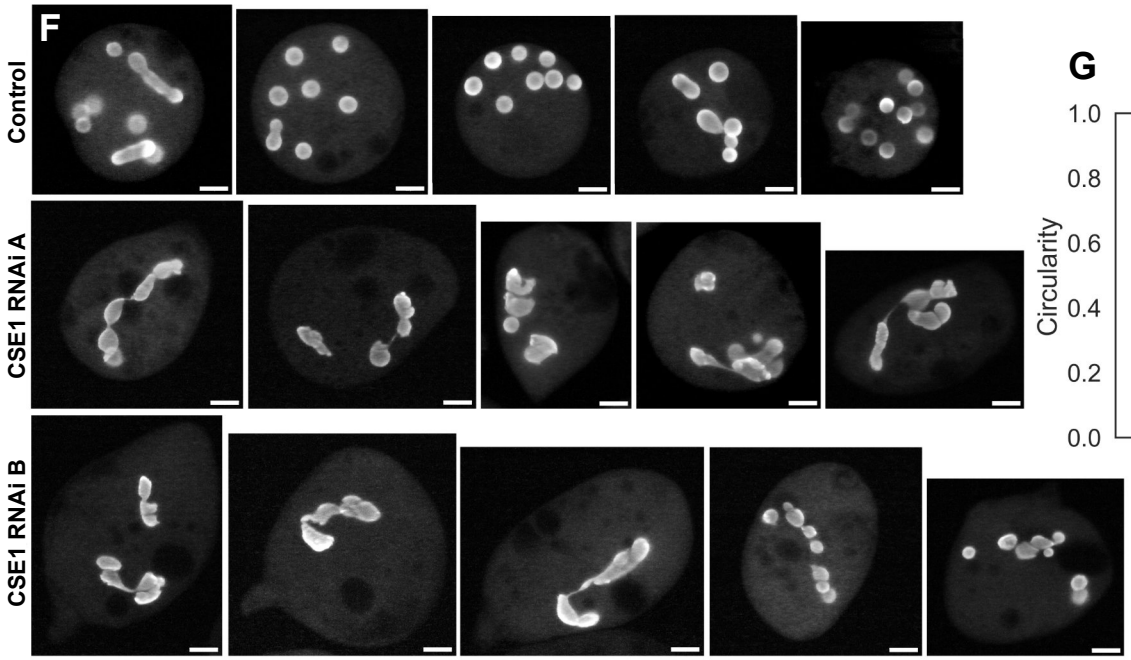

A
340 min
350 min
360 min
370 min
380 min
390 min
400 min
410 min
420 min
430 min
440 min
450 min

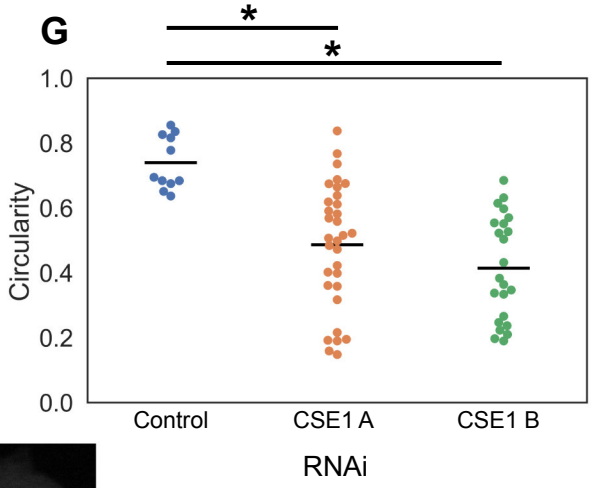

B
CSE1B Stentor 5
Volume (cubic microns)
Number of Nodes
Time since surcrose shock (min)
300000
250000
200000
150000
100000
50000
0
11
9
7
5
3
1
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00


C
Peak Vol / Baseline Vol
2.25
2.00
1.75
1.50
1.25
No RNAi
CSE1B
RNAi


D
Growth Rate (μm³ / min)
12500
10000
7500
5000
2500
0
No RNAi
CSE1B
RNAi


E
Minimum Node Count
6
4
2
No RNAi
CSE1B
RNAi


F
Control
CSE1 RNAi A
CSE1 RNAi B


G
*
*
Circularity
1.0
0.8
0.6
0.4
0.2
0.0
Control
CSE1 A
CSE1 B
RNAi

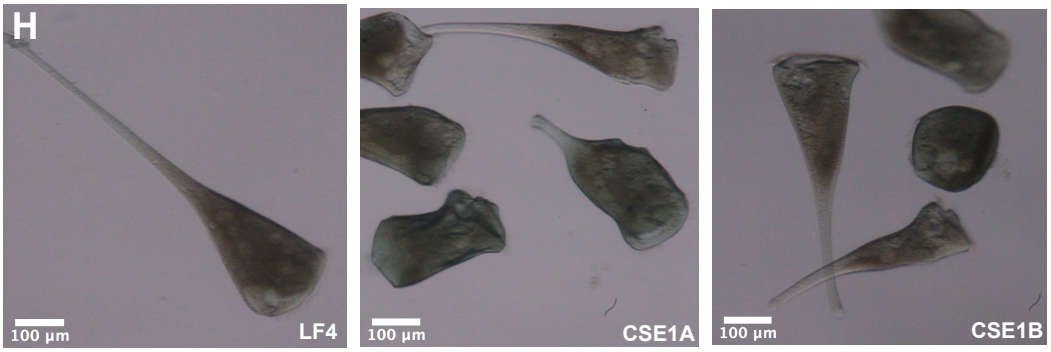

H
100 μm
LF4
100 μm
CSE1A
100 μm
CSE1B

Figure 3: CSE1 is required for normal macronuclear shape change

(A) Brightfield images of a rotocompressed *CSE1(RNAi) B Stentor* during regeneration (Scale bar = 100 um). The macronuclear nodes clump together and then spread out. The nodes never condensed into a single mass in this cell.

(B) Plot showing volume and number of macronuclear nodes over time after sucrose shock. This *CSE1(RNAi) B Stentor* did not show a peak in macronuclear volume during regeneration. The node count starts at 6 nodes, decreases slightly to 4 nodes, and then returns to 6 nodes.

(C) Plot showing the ratio between the peak volume and the baseline volume for macronuclei in regenerating *Stentor*, comparing control to CSE1 RNAi. The baseline volume was determined by visually inspecting a plot of volume versus time and estimating the average volume before condensation began. The peak volume is the maximum volume measured. For control (non-RNAi) *Stentor*, the average peak/base value is 1.81. For *CSE1 (RNAi) B Stentor*, the average peak/base value is 1.56. 1/8 non-RNAi *Stentor* did not show a clear peak in volume, while 4/6 *CSE1(RNAi) B Stentor* did not show a clear peak in volume (Supplemental Fig. S3)

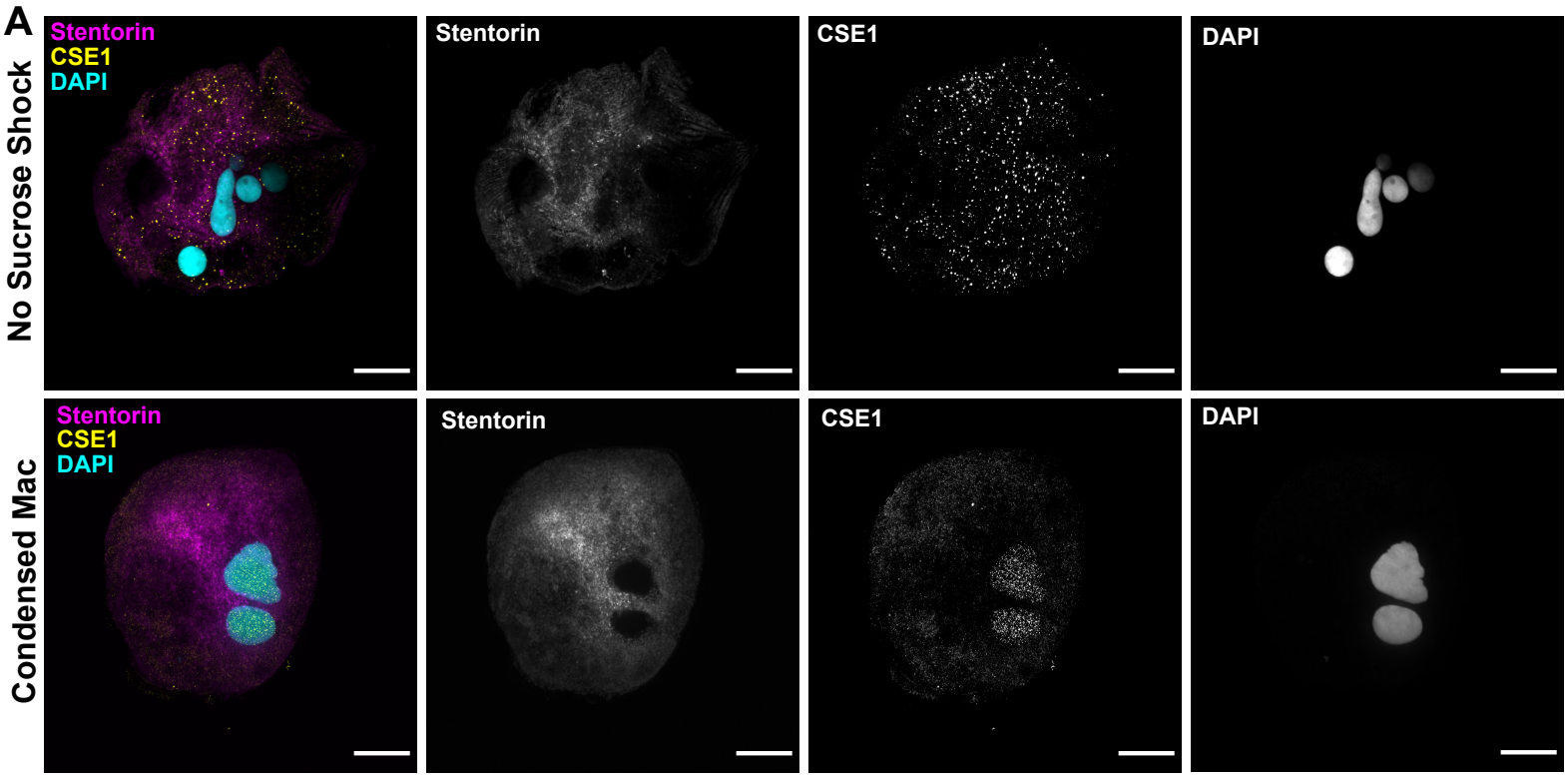
(D) Plot showing the volume growth rate of the macronucleus during condensation. Growth rate was measured as the macronuclear volume change from baseline to peak, divided by the time over which the volume change took place. Nuclear volume in non-RNAi *Stentor* grew with an average growth rate of 3,200 um^3/min, with one stentor reaching a rate of 13,000 um^3/min. In contrast, *CSE1(RNAi) B Stentor* averaged 780 um^3/min.

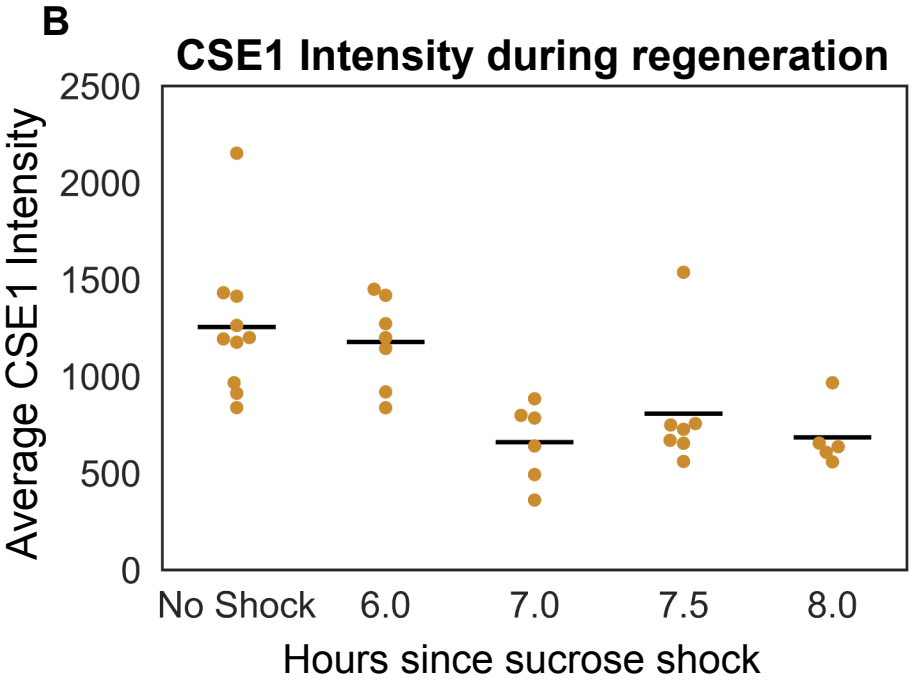
(E) Plot of the minimum number of nodes reached by each stentor at the point of maximal nuclear condensation. The average minimum number of nodes for Non-RNAi treated *Stentor* is 2.5, for *CSE1(RNAi) B Stentor* the average minimum node number is 3.7. 75% of Non-RNAi *Stentor* condensed their macronucleus to 3 nodes or less, while only 33% of *CSE1(RNAi) B Stentor* reached 3 nodes. None of the *CSE1(RNAi) B Stentor* were able to condense their macronuclei into a single mass.

(F) Images of macronuclei 24 hours after sucrose shock (Scale bars = 50 um). *Stentor* were stained with Hoechst 33342 and rotocompressed. While some nodes in control *(LF4 RNAi) Stentor* are elongated, most are circular. Both *CSE1(RNAi) A* and *CSE1(RNAi) B Stentor* have irregularly shaped macronuclei, as well as a few round nodes.

(G) Plot showing the average node circularity for each stentor cell. The average circularity is 0.74 for control *(LF4 RNAi) Stentor* (n = 11), 0.49 for *CSE1(RNAi) A Stentor* (n = 32), and 0.41 for *CSE1(RNAi) B Stentor* (n = 23). * $P < 0.01$, Kolmogorov-Smirnov Test.

(H) Brightfield images of *Stentor* after 7 days of RNAi feeding, without performing any sucrose shock (Scale bars = 100 um). *Stentor* with short tails are present in populations of both *CSE1(RNAi) A* and *CSE1(RNAi) B Stentor.*


A
No Sucrose Shock
Condensed Mac
Stentorin
CSE1
DAPI


B
CSE1 Intensity during regeneration
Average CSE1 Intensity
2500
2000
1500
1000
500
0
No Shock
6.0
7.0
7.5
8.0
Hours since sucrose shock

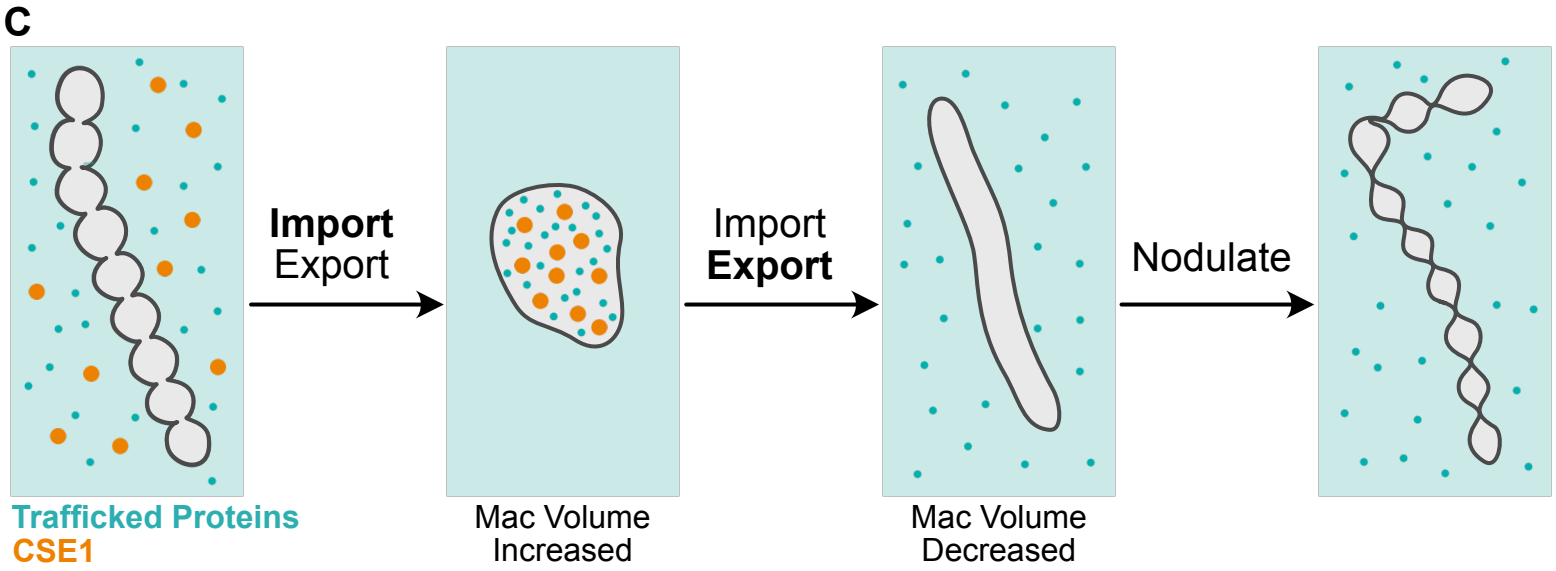

C
Import
Export
Import
Export
Nodulate
Trafficked Proteins
CSE1
Mac Volume
Increased
Mac Volume
Decreased

Figure 4: Dynamic relocalization of CSE1 during nuclear shape change

(A) Immunofluorescence images of *Stentor* showing the localization of CSE1 (scale bars = 50 um). *Stentor* were fixed with paraformaldehyde, stained with antibodies against CSE1 as well as DAPI to detect DNA, and imaged on a spinning disk confocal microscope. The cytoplasm was visualized by imaging autofluorescence generated by the blue pigment stentorin. In cells that have not been sucrose shocked, CSE1 is present in cytoplasmic puncta, with little staining present inside the macronucleus. In cells with condensed macronuclei during regeneration, CSE1 is present in intranuclear puncta, while cytoplasmic staining is decreased.

(B) Average CSE1 intensity per cell. *Stentor* were fixed in methanol and CSE1 immunofluorescence was performed. All cells were imaged with the same light intensity and exposure settings. The average intensity of CSE1 staining, expressed in arbitrary units, was measured for each cell imaged. The signal from stentorin was used to define the area of the cell – the average CSE1 intensity over this area was measured. Non-shocked cells had an average CSE1 intensity of 1255, and cells 6 hours after sucrose shock had a similar CSE1 intensity of 1177. At 7 hours the average intensity drops by approximately half to 660 and remains low until the end of regeneration. The average intensity at 7.5 hours is 807, and at 8 hours it averages 685.

(C) Model for how CSE1 may be promoting macronuclear condensation and volume increase. Nuclear proteins imported and exported from the macronucleus are represented by small blue dots – the exact identities of these proteins are currently unknown. CSE1 is represented by larger orange dots. The cytoplasm is light blue while the nucleoplasm is light gray. Before condensation, CSE1 and many proteins are cytoplasmic. We hypothesize that during compaction, nuclear import increases. This increased transport increases the amount of proteins inside the macronucleus, which in turn leads to the volume increases and causes the macronucleus to condense into a single mass to accommodate this change in surface to volume ratio. CSE1 is localized mainly to the nucleoplasm at the stage of high compaction. Shortly after condensation, CSE1 protein levels begin to drop, causing nuclear transport to shift towards nuclear export, such that the macronuclear volume decreases, and the macronucleus can achieve an elongated shape. At the end of the macronuclear cycle the macronucleus nodulates and CSE1 degradation is complete.

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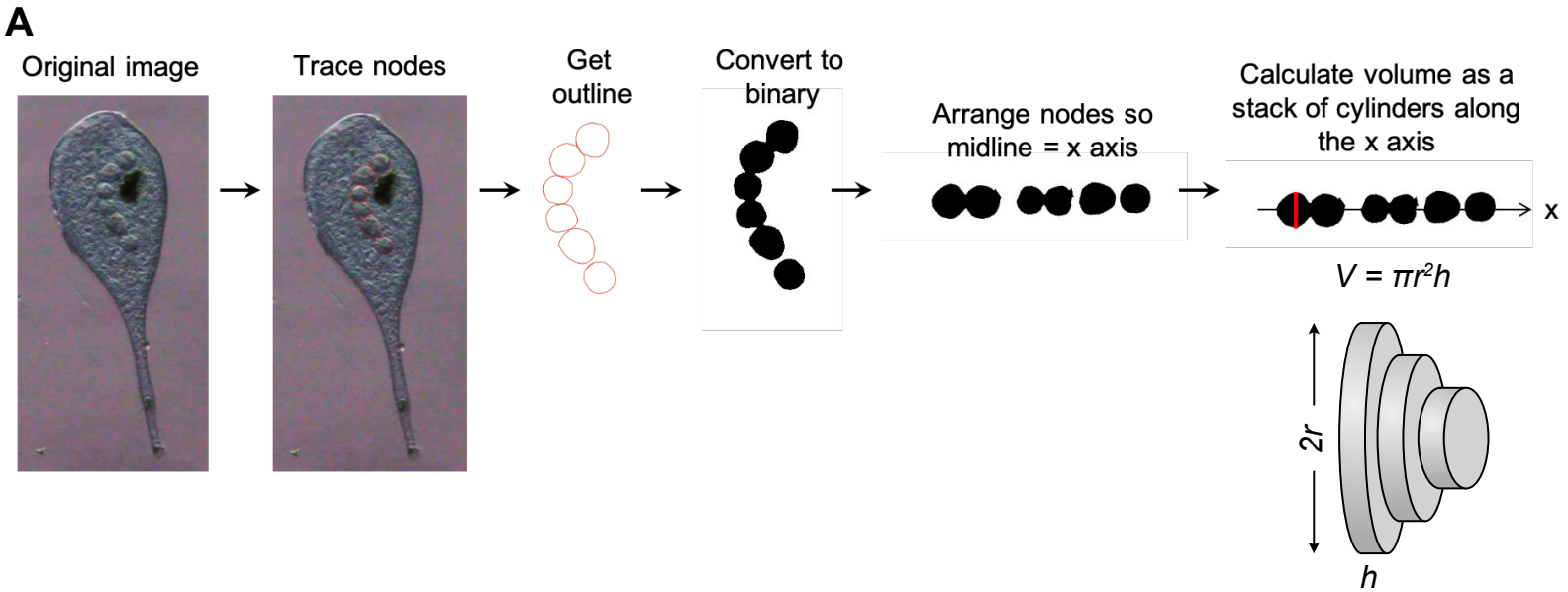
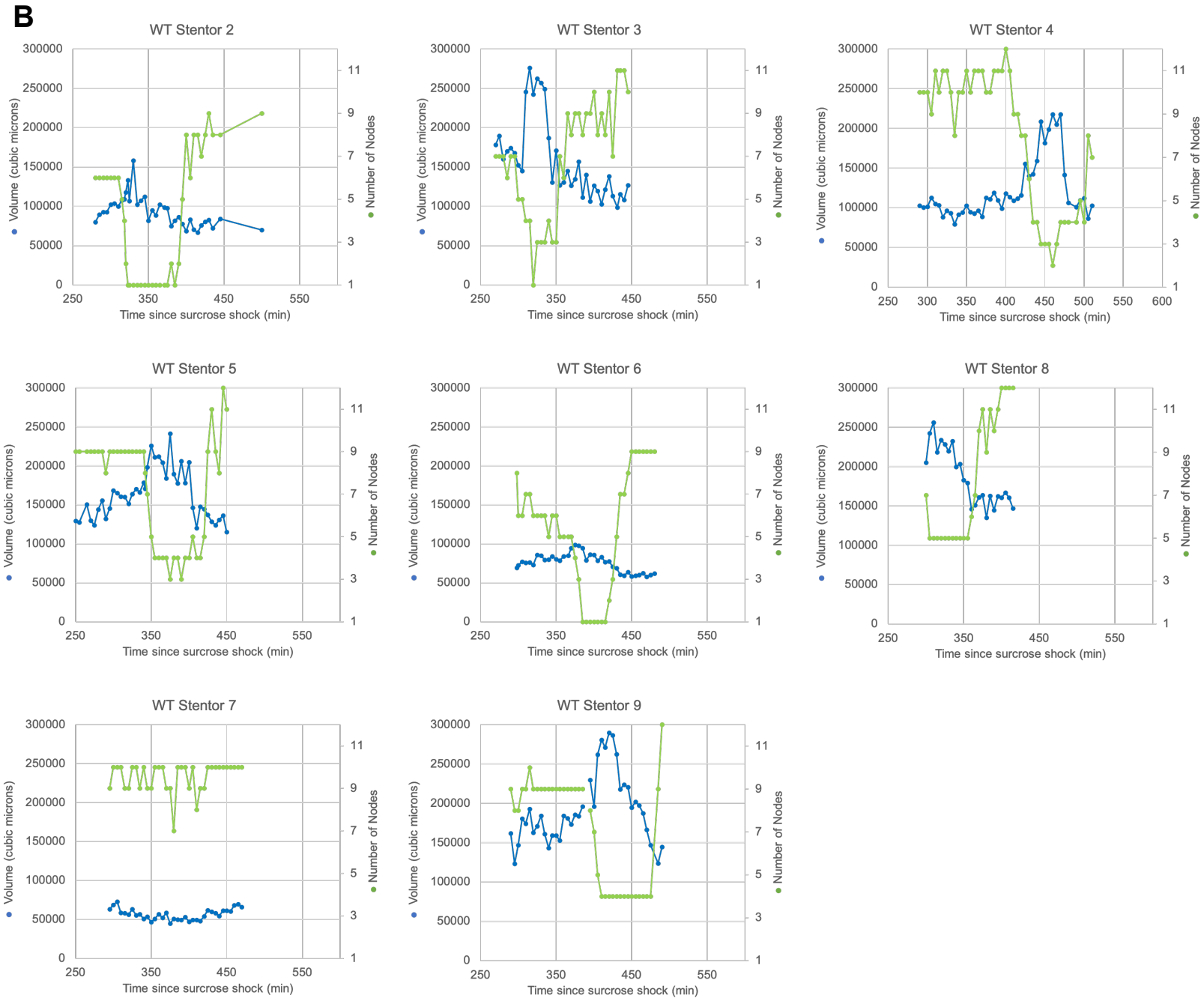

A
Original image
Trace nodes
Get outline
Convert to binary
Arrange nodes so midline = x axis
Calculate volume as a stack of cylinders along the x axis
x
V = πr²h
2r
h

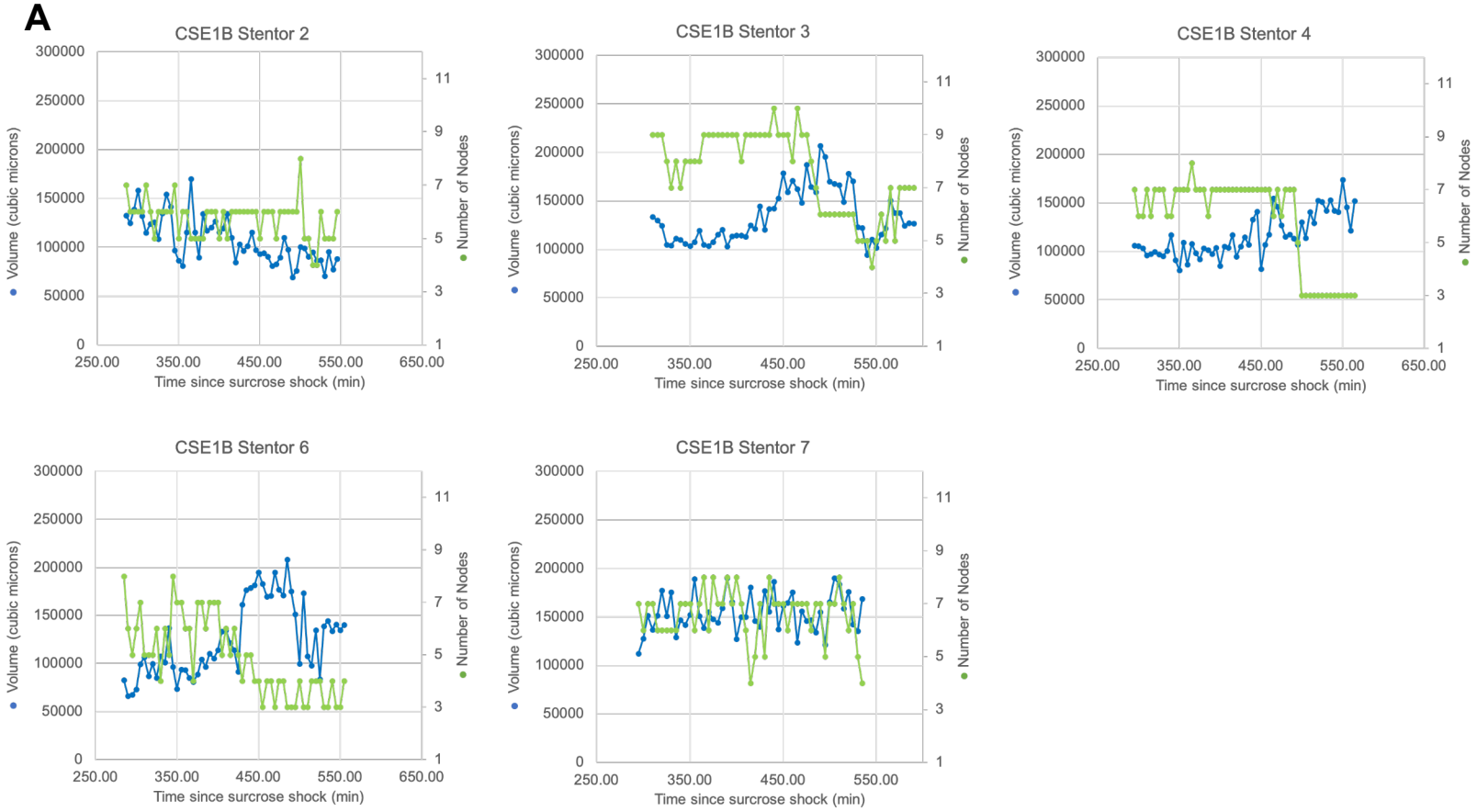

B
WT Stentor 2
WT Stentor 3
WT Stentor 4
WT Stentor 5
WT Stentor 6
WT Stentor 8
WT Stentor 7
WT Stentor 9
Volume (cubic microns)
Number of Nodes
Time since surcrose shock (min)

Supplemental Figure S1: Macronuclear volumes and node number during regeneration of non-RNAi *Stentor*

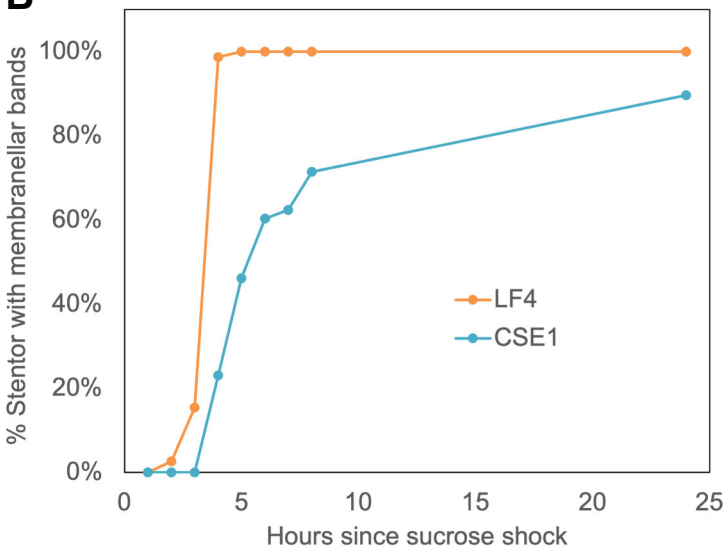
(A) Diagram of macronuclear volume calculation workflow. The macronuclear nodes are traced by hand. The traced outline is then separated from the image and filled in to create a binary image of the macronucleus. Then, the macronucleus image is split up and individual node images rotated so that the midline of the macronucleus is now a horizontal line along the x axis. The volume of the macronucleus is calculated by assuming rotational symmetry around the x axis to generate a series of cylinders of radius equal to that of the macronucleus relative to the axis at that position, and then adding up the volumes of cylinders along the macronucleus. The height of each cylinder is 1 pixel length along the x axis, and the diameter of each cylinder is the thickness of the macronucleus at that point. The red line illustrates a side view of one of these cylinders. The volume for each cylinder is calculated and added together to get the total macronuclear volume.

(B) Plots of WT *Stentor* volume and node number changes during regeneration that were not included in Fig 1. *Stentor* 8 was omitted from later data analysis because a pre-condensation baseline could not be determined. In general, the macronuclear volume peaks as the node number drops, indicating that macronuclear condensation is linked to an increase in macronuclear volume.

A


CSE1B Stentor 2
CSE1B Stentor 3
CSE1B Stentor 4
CSE1B Stentor 6
CSE1B Stentor 7
Volume (cubic microns)
Number of Nodes
Time since surcrose shock (min)
300000
250000
200000
150000
100000
50000
0
11
9
7
5
3
1
250.00
350.00
450.00
550.00
650.00

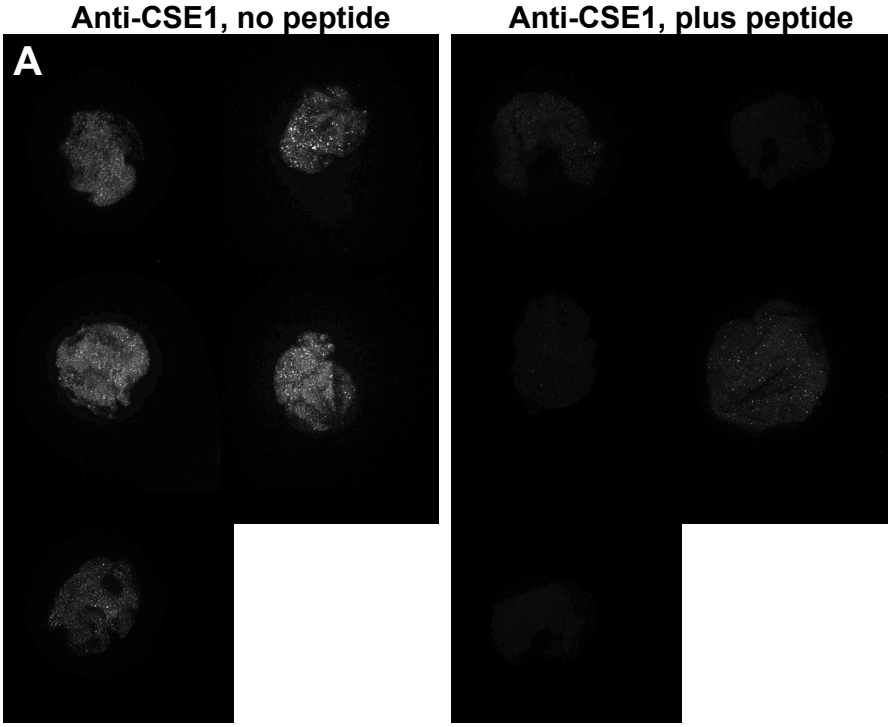
B

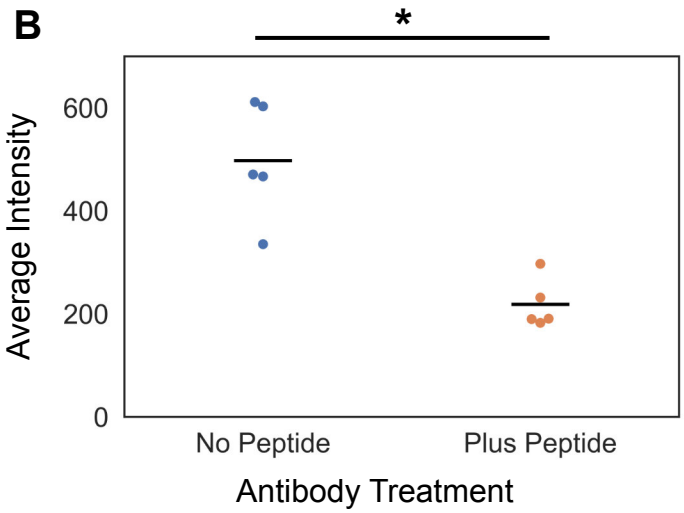

% Stentor with membranellar bands
100%
80%
60%
40%
20%
0%
0
5
10
15
20
25
Hours since sucrose shock
LF4
CSE1

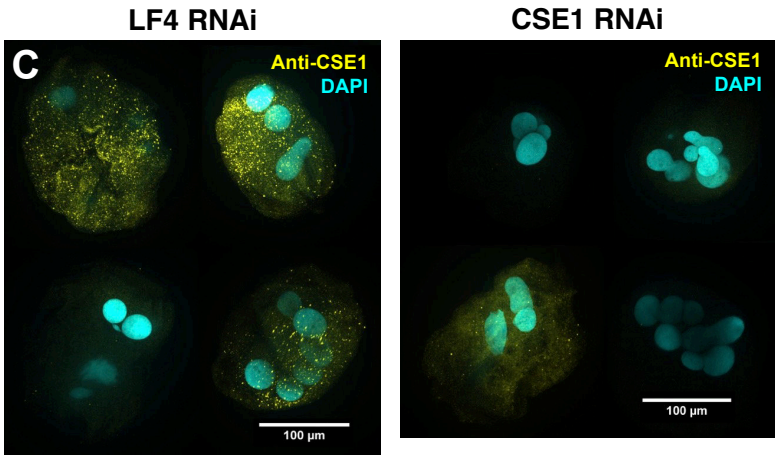
Supplemental Figure S2: Regeneration of *CSE1(RNAi) Stentor*

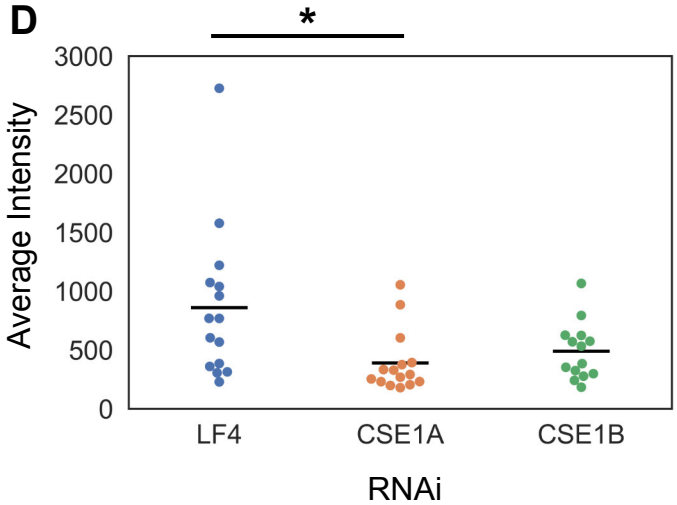
(A) Plots of *CSE1(RNAi) B Stentor* volume and node number changes during regeneration from additional examples that were not shown in Fig 3. Most *Stentor* do not have any clear peak in volume. While node count slightly decreases in some cells, a node count below 3 was never observed.

(B) Plot showing the percentage of *Stentor* that re-grew membranellar bands at various times after sucrose shock. *LF4 (control RNAi) Stentor* show a pronounced jump to 100% re-grown membranellar bands (stage 4 of regeneration) at about 4 hours (n = 77) consistent with the normal timing of regeneration. *CSE1(RNAi) A Stentor* show a lag in membranellar band regeneration, requiring more than 5 hours for half the cells to show a membranellar band. By 24 hours, 90% of cells grew a membranellar band (n = 77).


Anti-CSE1, no peptide
Anti-CSE1, plus peptide
A


B
*
Average Intensity
600
400
200
0
No Peptide
Plus Peptide
Antibody Treatment


LF4 RNAi
CSE1 RNAi
C
Anti-CSE1
DAPI
100 µm
Anti-CSE1
DAPI
100 µm


D
*
Average Intensity
3000
2500
2000
1500
1000
500
0
LF4
CSE1A
CSE1B
RNAi

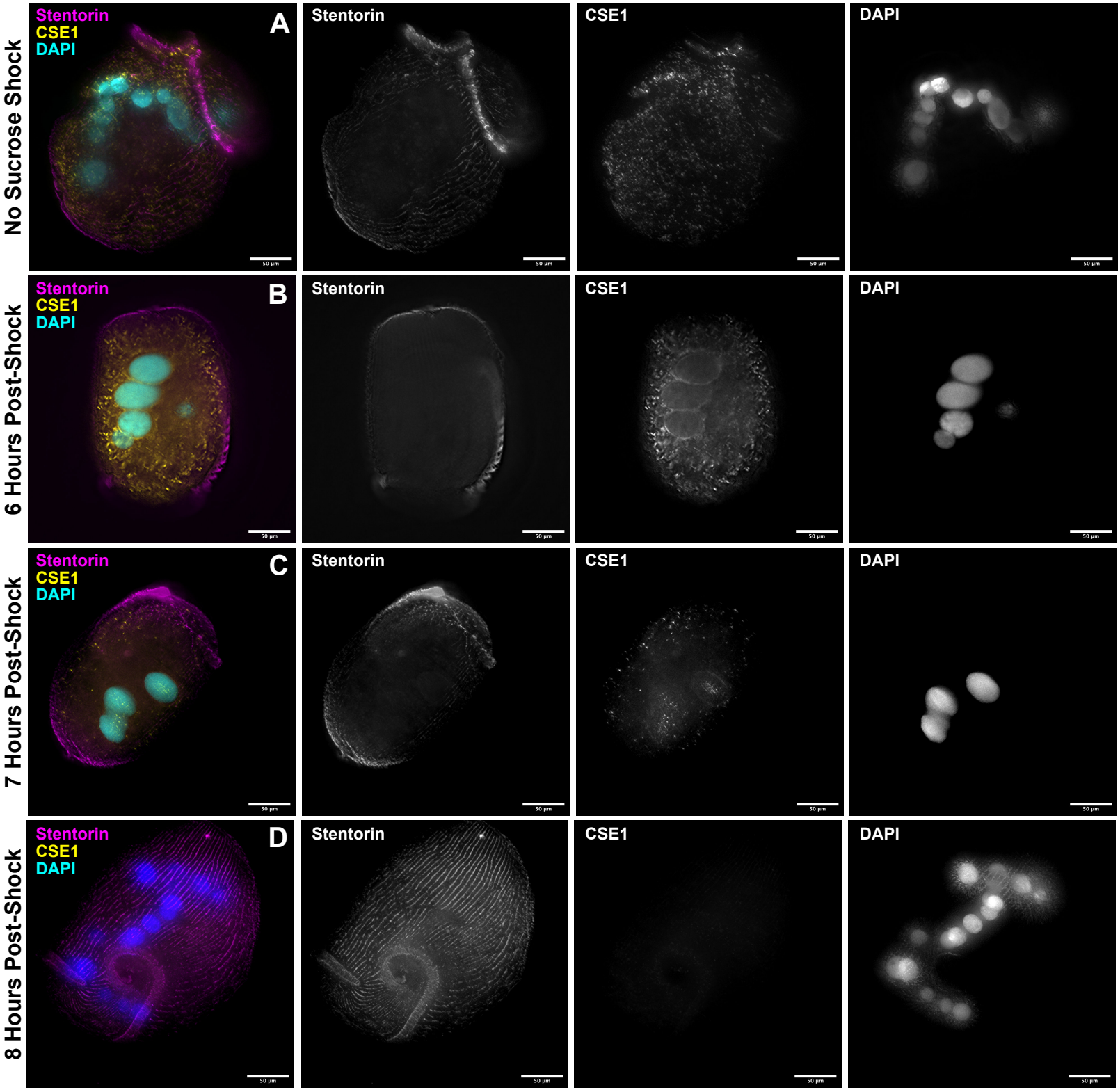
Supplemental Figure S3: Controls for anti-CSE1 antibody specificity

(A) Peptide blocking control. Images of PFA fixed *Stentor* stained with anti-CSE1 either without or with pre-incubation with CSE1 peptide. Cells were imaged with a W1 spinning disk confocal.

(B) Plot showing the average CSE1 staining intensity in PFA fixed *Stentor* when the CSE1 antibody is either on its own or pre-incubated with CSE1 peptide. Without the peptide block, the average CSE1 intensity is 497 (n = 5). With peptide blocking the average CSE1 intensity drops in half to 218. *P < 0.01, Two-tailed T-Test.

(C) RNAi control for antibody specificity. Images of PFA fixed *LF4(RNAi)* or *CSE1(RNAi) Stentor* stained with anti-CSE1. Cells were imaged with a W1 spinning disk confocal.

(D) Plot showing the average CSE1 staining intensity in PFA fixed *Stentor* treated with RNAi. In *LF4(RNAi) Stentor*, the average intensity is 860. In *CSE1(RNAi) A Stentor*, the average intensity is 388. The average intensity of *CSE1(RNAi) B Stentor* is 489. *P < 0.05, Two-tailed T Test.


No Sucrose Shock
6 Hours Post-Shock
7 Hours Post-Shock
8 Hours Post-Shock
Stentorin
CSE1
DAPI
A
B
C
D
50 µm

Supplemental Figure S4: Immunofluorescence of CSE1 in methanol fixed *Stentor* at different stages of regeneration

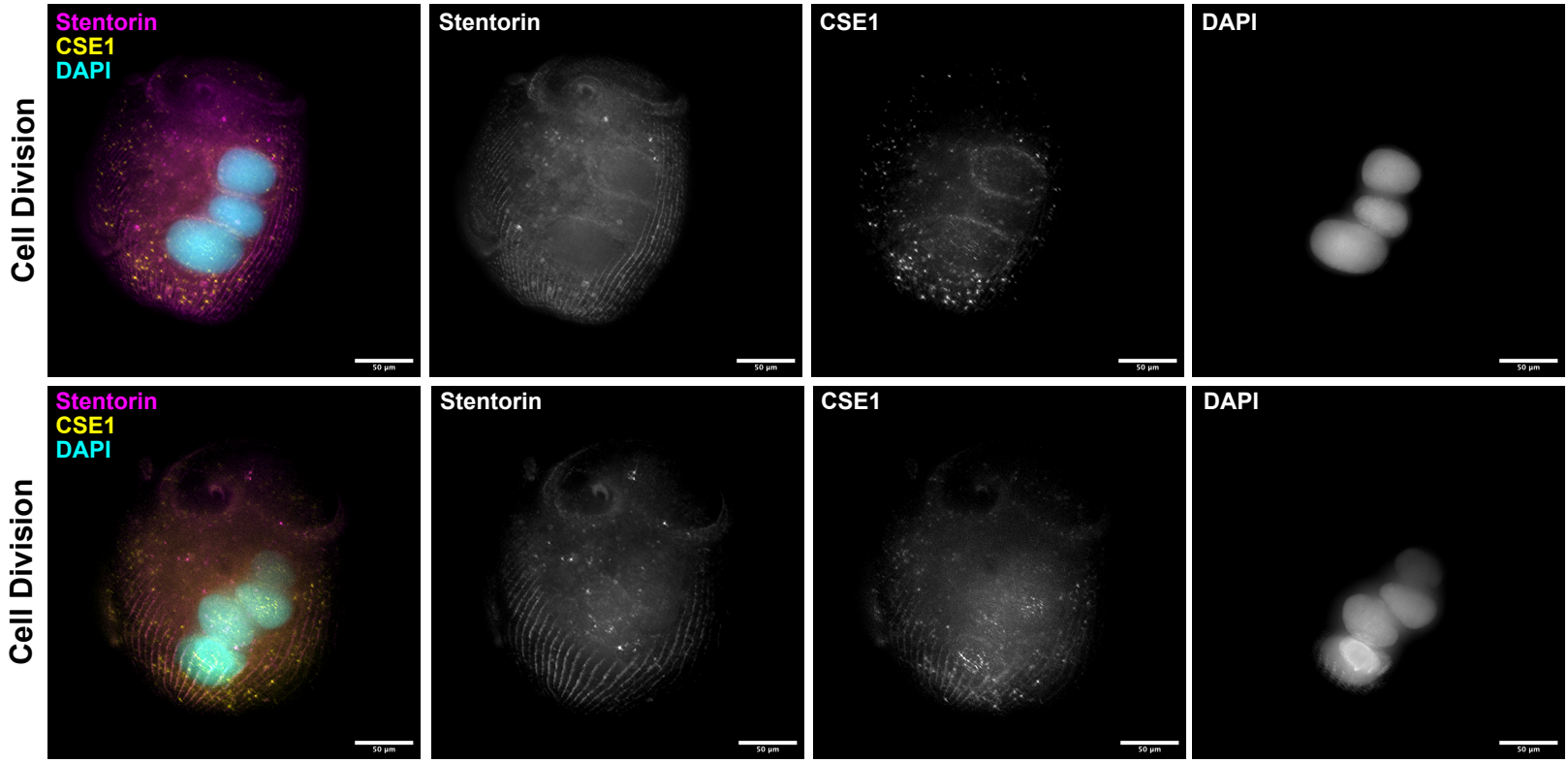
All cells were imaged with a 20x air objective on a DeltaVision deconvolution microscope. Scale bars = 50 um.

(A) *Stentor* that have not undergone sucrose shock have cytoplasmic puncta of CSE1.

(B) Six hours post sucrose shock is generally the time at which the macronucleus begins condensation. At this time we observed CSE1 staining around the periphery of the macronucleus.

(C) At 7 hours post sucrose shock the macronucleus in this cell is condensed. At this time we observe CSE1 puncta inside of the macronucleus.

(D) At 8 hours post sucrose shock, the total CSE1 signal is greatly decreased relative to pre-sucrose shock stentors. The intensity range displayed in the CSE1 image for 8 hours post sucrose shock is the same as the intensity range displayed in the non-sucrose shock CSE1 image (A).


Cell Division
Stentorin
CSE1
DAPI
Stentorin
CSE1
DAPI
Cell Division
Stentorin
CSE1
DAPI
Stentorin
CSE1
DAPI

Supplemental Figure S5: Immunofluorescence of CSE1 in dividing *Stentor* fixed with methanol.

Stentor late in cell division are readily identifiable in culture because they have a visible membranellar band developing on the side of the cell, as well as the original membranellar band at the anterior end. Cells were imaged with a 20x air objective on a DeltaVision deconvolution microscope. Scale bars = 50 um. In cells with condensed macronuclei we observe CSE1 around the periphery of the macronucleus, as well as punctate CSE1 staining in the interior of the macronucleus.

RNAi Name	Target Gene	Position within coding region	Forward Primer	Reverse Primer
CSE1A	CSE1 (g28374)	1247 - 2084	AACTGGAAGCAAATGGATGC	AGCCATAGATGAGGCATTGG
CSE1B	CSE1 (g28374)	2089-2841	TGCCTCATCTATGGCTCCTC	TTGAAGAGTTGGGCGTATCG
LF4	LF4	8-884	CAGAATACCGTTTGATATCAAAAAAAGGTGAAGG	CTACTTGCCTCATCAACAAAATCTTTGAAATAAGG
Mob1	Mob1	40-672	AAGAAGCGAATTGAAAAAGGCCAGC	GTTACCAGAAGCTTCTCTTTCCATTCTTTCC

Supplemental Figure S6: RNAi constructs

List of RNAi constructs and primers used in this study.

A

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                                        20                      40
SteCoe_28919_28918 MVDFTSIFTKCFSAQFAVRVEGEKELKLLEDQSEFAGSLITYFSSPSTPPPLSQMAAV 58
      SteCoe_28374 MVDFTSIFTKCFSAQFTVRVEGEKELKLLEDQSEFAGSLITYFSSPSTPPPLSQMAAV 58
                    60                      80                      100
SteCoe_28919_28918 SFKNYIIKRWAPEEGPTIPPETKILIKRNIYSVMVNAVPSVSSQLXPPETKILIKRNI 116
      SteCoe_28374 NFKNYIIKRWAPEEGPTIPPEGKILIKRNIYTVMVNAIPSVSSQL------------- 103
                       120                     140                     160
SteCoe_28919_28918 YSVMVNAVPSVSSQLRESIEWIAKHDFPQDWPDLIQSLYQGLSIGYXRKHMAVINTLM 174
      SteCoe_28374 ---------------RESIEWIAKHDFPQNWTDLIQSLYQGLSIGYTNNPMAVINTLM 146
                          180                     200                     220
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      SteCoe_28374 TCHKLFKRYRYSFRSDELWSEIKLVVDSLFHVYFATATQVYSCLQNAQTEDEVIRHIS 204
                             240                     260                     280
SteCoe_28919_28918 SFIPLLKVFISLNGQDIPQQFDDTLKEWMTLLNYLLNYLSPLLHDDFKLFLLKSKVMK 290
      SteCoe_28374 TFIPLLKVFISLNGQDIPQQFDDTLKEWMTLLNYLLNYLSPLLQDDLKLFLLKSKVMK 262
                                300                     320                     340
SteCoe_28919_28918 CLTLYAQKYDEDFEPYVKDFCTSVWDLLARASGFSQYDRFVSSCLEYFRVVTFKPQIA 348
      SteCoe_28374 CLTLYAQKYDEDFEPYVKDFCTSVWDLLSRASGFSQYDRFVSACLEYFRVVTFKPQIA 320
                                   360                     380                     400
SteCoe_28919_28918 ELIHGNLNIMFTNLILPNMIISLDEEDLAETAPMEFVKMFLEDANEDTRRCACGQLMK 406
      SteCoe_28374 ELIHGNLNIMFTNLILPNMIISLDEEDLADTAPMEFVKMFLEDANEDTRRCACGQLMK 378
                                      420                     440                     460
SteCoe_28919_28918 VLIKQFPDDINRLVLEQQNTVIQGFRSNPNNNWKQMDALVLMLSGVFPTLYTPRNGAS 464
      SteCoe_28374 VLIKQFPDDINKLVLEQQNTVIQGFRSNPNNNWKQMDALILMLSGMFPTLYTPRNGAS 436
                                         480                     500                     520
SteCoe_28919_28918 SVATSQAHILELYNNLVCPQLANQNFPILITSCLKFIYVYRNQFLKEMLLDIMGKVIG 522
      SteCoe_28374 SVATSQAHILELYNNLVCPQLANHSFPILITSCLKFIYVYRNQFLKEMLLDIMGKVIG 494
                                            540                     560                     580
SteCoe_28919_28918 FLDSNNVLLASYAAATLERLLMIKNDKDLIFTKDFLSSCLNQLLQNIAMALQKHPKNT 580
      SteCoe_28374 FLDSNNVLLASYAAATLERLLMIKNDRDLIFTKEFLSSCLNQLLQSIAMALQKHPKNT 552
                                               600                     620
SteCoe_28919_28918 YIMNAFFRVIWISQDLFSSFAVPACDIFINYIKQVLSEPQSSEPHFNWLLFECIALAM 638
      SteCoe_28374 YIMNAFFRVIWISQDLFSSFAVPACDIFISYIKQVLSEPQSSEPHFNWLLFECIALAM 610
                    640                     660                     680
SteCoe_28919_28918 KWSGPGISDIQKKIEPYMALIIQKSNADLLPYAFQIQAFFIRLLINLSQTNQNLISSI 696
      SteCoe_28374 KWSGPGISDIQKKLEPYMALIIQKSNADLLPYAFQIQAFFIRLLSSISQTNQNLISSI 668
                       700                     720                     740
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      SteCoe_28374 LPIDNWESGSRYYLPTLVIFLENLLMTNASSMAPQISALCNIAHKLFTLGLDGQAFSL 726
                          760                     780                     800
SteCoe_28919_28918 LTTLIETYAFENLHPYMHPIYLIIFTKLHNSKSQNIRLSPRFHKGSILFVSSVILKYG 812
      SteCoe_28374 LTTLIETYSFENLHPYMHPIYMIIFTKLHNSKSQNIRLSPRFHRGSIMFVSSFILKYG 784
                             820                     840                     860
SteCoe_28919_28918 WKVLSDSMNSVQPGIFFMLVKGQILQNLRSIETIVERRAVILAMSVLMQNIEVTNELW 870
      SteCoe_28374 WNVLSDSMNSVQPGIFFMLVKGQILQNLRSIETIVERRAVILAMSALMQNTEVTNELW 842
                                880                     900                     920
SteCoe_28919_28918 INIVISVSKILDSTTNILSGTVYSNGLVDLPEENTIQMTRDSFQKVYSTEMPLTDKYA 928
      SteCoe_28374 INIVISVSKILDSTTNILSGTVYSNGLVDLPEENTIQMTRDSFQKVYSAEIPLTDKYP 900
                                   940                     960
SteCoe_28919_28918 SLPNEKMFFINTICSQQYPTGSFWSFAGQHLDQQVPTILNRYAQLFNQQIR 979
      SteCoe_28374 HLPNEKMFFINTICNQQYPTGSFWSFAGQHLDQQIPTILNRYAQLFNQQIR 951
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B

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                                  20                        40                        60
                                   |                         |                         |
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CSE1_A_28374 AACTGGAAGCAAATGGATGCTTTAATACTTATGCTTTCTGGGATGTTTCCAACTTTATACACT 63
                              80                        100                       120
                               |                         |                         |
CSE1_A_28918 CCAAGAAATGGTGCATCATCAGTAGCGACATCACAAGCGCATATACTTGAACTTTATAACAAT 126
CSE1_A_28374 CCAAGAAATGGGGCTTCATCAGTAGCGACATCACAAGCACATATTCTTGAACTTTATAACAAT 126
                          140                       160                       180
                           |                         |                         |
CSE1_A_28918 TTAGTCTGTCCTCAATTAGCTAATCAGAATTTCCCTATACTTATTACAAGTTGTCTTAAGTTT 189
CSE1_A_28374 TTGGTCTGTCCTCAACTTGCTAATCATAGTTTCCCTATACTAATAACAAGTTGTCTTAAGTTT 189
                      200                       220                       240
                       |                         |                         |
CSE1_A_28918 ATTTACGTCTACAGAAACCAGTTTTTAAAAGAAATGCTTTTGGACATAATGGGAAAAGTTATA 252
CSE1_A_28374 ATTTATGTCTACAGAAATCAGTTTTTAAAAGAAATGCTATTAGATATCATGGGAAAAGTTATT 252
                  260                       280                       300
                   |                         |                         |
CSE1_A_28918 GGGTTTTTAGACAGTAACAATGTTTTATTAGCAAGTTATGCTGCGGCTACACTTGAAAGGCTG 315
CSE1_A_28374 GGGTTCTTAGATAGTAACAACGTATTATTAGCCAGTTATGCTGCAGCTACTCTTGAAAGACTT 315
                320                       340                       360
                 |                         |                         |
CSE1_A_28918 CTTATGATAAAAAATGATAAGGATTTGATTTTCACTAAAGACTTCCTGTCTTCATGTTTGAAC 378
CSE1_A_28374 CTTATGATAAAAAACGACCGGGATCTTATTTTCACTAAAGAGTTCCTATCATCATGTTTAAAT 378
             380                       400                       420                       440
              |                         |                         |                         |
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CSE1_A_28374 CAATTATTACAAAGTATCGCTATGGCTCTTCAGAAACACCCAAAAAACACTTATATTATGAAT 441
                                  460                       480                       500
                                   |                         |                         |
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CSE1_A_28374 GCATTTTTTAGGGTTATTTGGATATCCCAAGATTTGTTTTCAAGTTTTGCAGTCCCAGCTTGT 504
                              520                       540                       560
                               |                         |                         |
CSE1_A_28918 GACATTTTTATCAATTACATCAAACAAGTTCTTTCTGAGCCTCAGAGTTCAGAACCTCATTTC 567
CSE1_A_28374 GACATTTTTATCAGTTATATCAAGCAAGTACTTTCTGAGCCTCAAAGCTCAGAACCTCATTTC 567
                          580                       600                       620
                           |                         |                         |
CSE1_A_28918 AATTGGTTGCTCTTTGAGTGTATCGCCCTAGCTATGAAATGGTCAGGGCCTGGGATTTCTGAT 630
CSE1_A_28374 AACTGGTTACTTTTTGAATGTATTGCTTTAGCTATGAAATGGTCTGGGCCTGGGATTTCTGAT 630
                      640                       660                       680
                       |                         |                         |
CSE1_A_28918 ATACAGAAAAAAATCGAACCTTACATGGCTTTAATAATCCAAAAATCCAACGCAGATCTTTTA 693
CSE1_A_28374 ATCCAGAAAAAACTCGAACCTTATATGGCTCTGATAATCCAAAAGTCCAACGCAGACCTTTTG 693
                  700                       720                       740
                   |                         |                         |
CSE1_A_28918 CCATATGCTTTTCAGATCCAAGCTTTTTTCATAAGACTCCTGATTAATCTTTCACAAACCAAT 756
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                 |                         |                         |
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              |                         |                         |
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C

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                                  20                  40                  60
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                320                 340                 360
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CSE1_B_28374 CATGTTGGTTAAAGGGCAGATATTGCAAAATTTAAGGTCTATTGAAACAATTGTTGAAAGAAG 378
             380                 400                 420                 440
CSE1_B_28918 AGCTGTGATTTTAGCTATGAGTGTGCTTATGCAAAATATTGAAGTTACTAATGAATTGTGGAT 441
CSE1_B_28374 AGCTGTGATTTTAGCTATGAGTGCGCTTATGCAGAATACTGAGGTTACTAATGAACTATGGAT 441
                                460                 480                 500
CSE1_B_28918 TAATATTGTTATTAGTGTTAGTAAGATACTTGATAGTACAACGAATATACTTAGTGGGACGGT 504
CSE1_B_28374 TAATATTGTTATTAGTGTTAGTAAAATACTTGATAGTACAACAAATATACTTAGTGGGACAGT 504
                            520                 540                 560
CSE1_B_28918 GTATTCTAATGGACTTGTTGACTTACCTGAAGAAAACACTATACAAATGACACGAGACTCCTT 567
CSE1_B_28374 GTATTCTAATGGACTTGTTGACTTGCCTGAAGAAAACACGATACAAATGACTAGAGATTCCTT 567
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CSE1_B_28374 CCAAAAAGTATACAGCGCTGAAATCCCTTTAACAGACAAATACCCACACCTTCCTAACGAAAA 630
                    640                 660                 680
CSE1_B_28918 AATGTTCTTCATCAACACAATCTGTAGCCAGCAATACCCAACTGGATCCTTCTGGAGCTTTGC 693
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                700                 720                 740
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```

Supplemental Figure S7: Alignment of *Stentor's* CSE1 genes

(A) Pairwise alignment of protein sequences of *Stentor's* two CSE1 orthologs. Gene 28374 is the CSE1 gene that RNAi constructs were made against. Gene 28919+28918 is a slightly longer ortholog that encodes a protein 92% identical to that encoded by gene 28374. Identical residues are colored in blue. The green box shows the peptide sequence that the CSE1 antibody was raised against.

(B) Pairwise alignment of the DNA sequence targeted by CSE1 RNAi Construct A (SteCoe_28374) and the corresponding DNA sequence of SteCoe_28918. These sequences are 89% identical (780/878 nucleotides). Identical nucleotides are colored in blue.

(C) Pairwise alignment of the DNA sequence targeted by CSE1 RNAi Construct B (SteCoe_28374) and the corresponding DNA sequence of SteCoe_28918. These sequences are 90% identical (678/754 nucleotides). Identical nucleotides are colored in blue.

```
SteCoe_g28919+8     1 ----MVDFTSI---FTKCFSAQFAVRVEGEKELKLLEDQSEFAGSLITYFSSPSTPPPLS
SteCoe_g28374       1 ----MVDFTSI---FTKCFSAQFTVRVEGEKELKLLEDQSEFAGSLITYFSSPSTPPPLS
SacCer_CSE1         1 ----MSDLETVAKFLAESVIASTA--KTSERNLRQLETQDGFGLTLLHVIASTNLPLSTR
DroMel_CSE1         1 MEVTEANLQLLAGYLQQTLSADPNVRRPAEKLLESTELQQNYPILLLNLIDKAQMDMTTR
HomSap_CAS          1 MELSDANLQTLTEYLKKTLDPDPAIRRPAEKFLESVEGNQNYPLLLLTLLEKSQ-DNVIK
MusMus_XPO2         1 MELSDANLQTLTEYLKKTLDPDPAIRRPAEKFLESVEGNQNYPLLLLTLLEKSQ-DNVIK

SteCoe_g28919+8    54 QMAAVSFKNYIIKRW---APEEGPTIPPETKILIKRNIYSVMVNAVPSVSSQLXPPETKI
SteCoe_g28374      54 QMAAVNFKNYIIKRW---APEEGPT----------------------------IPPEGKI
SacCer_CSE1        55 LAGALFFKNFIKRKW---VDENGNH---------------------------LLPANNVE
DroMel_CSE1        61 VAGAIAFKNYIKRNWAAHLDSDGPD---------------------------RIHESDRN
HomSap_CAS         60 VCASVTFKNYIKRNW--RIVEDEPN---------------------------KICEADRV
MusMus_XPO2        60 VCASVTFKNYIKRNW--RIVEDEPN---------------------------KICEADRV

SteCoe_g28919+8   111 LIKRNIYSVMVNAVPSVSSQLRESIEWIAKHDFPQDWPDLIQSLYQGLSIGYXRKHMAVI
SteCoe_g28374      83 LIKRNIYTVMVNAIPSVSSQLRESIEWIAKHDFPQNWTDLIQSLYQGLSIGYTNNPMAVI
SacCer_CSE1        85 LIKKEIVPLMISLPNNLQVQIGEAISSIADSDFPDRWPTLLSDLASRLSND----DMVTN
DroMel_CSE1        94 TIKTLIVTLMLHSPVALQKQLSDAVSIIGKYDFPKKWPQLIDEMVERFASG----DFNVI
HomSap_CAS         91 AIKANIVHLMLSSPEQIQKQLSDAISIIGREDFPQKWPDLLTEMVNRFQSG----DFHVI
MusMus_XPO2        91 AIKANIVHLMLSSPEQIQKQLSDAISIIGREDFPQKWPDLLTEMVNRFQSG----DFHVI

SteCoe_g28919+8   171 N-TLMTCHKLFKRYRYSFRSDELWSEIKLVVDSL---FNVYFATATQVYSCLQNAQTEDE
SteCoe_g28374     143 N-TLMTCHKLFKRYRYSFRSDELWSEIKLVVDSL---FHVYFATATQVYSCLQNAQTEDE
SacCer_CSE1       141 KGVLTVAHSIFKRWRPLFRSDELFLEIKLVLDVFTAPFLNLLKTVDEQITANENNKASLN
DroMel_CSE1       150 NGVLQTAHSLFKRYRYEFKSQALWEEIKFVLDRMAKPLTDLLQATMQLTKVHENNAGALK
HomSap_CAS        147 NGVLRTAHSLFKRYRHEFKSNELWTEIKLVLDAFALPLTNLFKATIELCSTHANDASALR
MusMus_XPO2       147 NGVLRTAHSLFKRYRHEFKSNELWTEIKLVLDAFALPLTNLFKATIELCSTHANDASALR

SteCoe_g28919+8   227 VIRHISSFIPLLKVFISLNGQDIPQQFDDTLKEWMTLLNYLLNYLSPLLHDDFK------
SteCoe_g28374     199 VIRHISTFIPLLKVFISLNGQDIPQQFDDTLKEWMTLLNYLLNYLSPLLQDDLK------
SacCer_CSE1       201 IL--FDVLLVLIKLYYDFNCQDIPEFFEDNIQVGMGIFHKYLSYSNPLLEDPDETEHASV
DroMel_CSE1       210 VI--YGSLVLVNKVFFSLNSQDLPEFFEDNINTWMGAFIQQLAADVPSLRTADD-EDAGV
HomSap_CAS        207 IL--FSSLILISKLFYSLNFQDLPEFFEDNMETWMNNFHTLLTLDNKLLQTDDE-EEAGL
MusMus_XPO2       207 IL--FSSLILISKLFYSLNFQDLPEFFEDNMETWMNNFHTLLTLDNKLLQTDDE-EEAGL

SteCoe_g28919+8   281 LFLLKSKVMKCLTLYAQKYDEDFEPYVKDFCTSVWDLLARASGFSQYDRFVSSCLEYFRV
SteCoe_g28374     253 LFLLKSKVMKCLTLYAQKYDEDFEPYVKDFCTSVWDLLSRASGFSQYDRFVSACLEYFRV
SacCer_CSE1       259 LIKVKSSIQELVQLYTTRYEDVFGPMINEFIQITWNLLTSISNQPKYDILVSKSLSFLTA
DroMel_CSE1       267 LEHLRAQVCENICLYAKKYDEEFKPFMEQFVTAVWELLVKTSLHTKYDSLVSHALQFLSV
HomSap_CAS        264 LELLKSQICDNAALYAQKYDEEFQRYLPRFVTAIWNLLVTTGQEVKYDLLVSNAIQFLAS
MusMus_XPO2       264 LELLKSQICDNAALYAQKYDEEFQRYLPRFVTAIWNLLVTTGREVKYDLLVSNAIQFLAS

SteCoe_g28919+8   341 VTFKPQIAELIHGN--LNIMFTNLILPNMIISLDEEDLAETAPMEFVKMFLEDANEDTRR
SteCoe_g28374     313 VTFKPQIAELIHGN--LNIMFTNLILPNMIISLDEEDLADTAPMEFVKMFLEDANEDTRR
SacCer_CSE1       319 VTRIPKYFEIFNNESAMNNITEQIILPNVTLREEDVELFEDDPIEYIRRDLEGSDTDTRR
DroMel_CSE1       327 VADRQHYQSIFENPEILAQICDKVVIPNLDIRPSDEEIFEDSPEEYIRRDIEGSDIDTRR
HomSap_CAS        324 VCERPHYKNLFEDQNTLTSICEKVIVPNMEFRAADEEAFEDNSEEYIRRDLEGSDIDTRR
MusMus_XPO2       324 VCERPHYKNLFEDQNTLTSICEKVIVPNMEFRAADEEAFEDNSEEYIRRDLEGSDIDTRR

SteCoe_g28919+8   399 CACGQLMKVLIKQFPDDINRLVLEQQNTVIQGFRSNPNNNWKQMDALVLMLSGVFPTLYT
SteCoe_g28374     371 CACGQLMKVLIKQFPDDINKLVLEQQNTVIQGFRSNPNNNWKQMDALILMLSGMFPTLYT
SacCer_CSE1       379 RACTDFLKELKEKNEVLVTNIFLAHMKGFVDQYMSDPSKNWKFKDLYIYLFTALAINGNI
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DroMel_CSE1       387 RAACDLVKTLSINFEQKIFGIFGQYLERLLTKYKENPATNWRSKDTAIYLVTSWASRGGT
HomSap_CAS        384 RAACDLVRGLCKFFEGPVTGIFSGYVNSMLQEYAKNPSVNWKHKDAAIYLVTSLASKAQT
MusMus_XPO2       384 RAACDLVRGLCKFFEGPVTGIFSGYVNSMLQEYAKNPSVNWKHKDAAIYLVTSLASKAQT

SteCoe_g28919+8   459 PRNGASSVATSQAHILELYNNLVCPQLANQN---FPILITSCLKFIYVYRNQFLKEMLLD
SteCoe_g28374     431 PRNGASSVATSQAHILELYNNLVCPQLANHS---FPILITSCLKFIYVYRNQFLKEMLLD
SacCer_CSE1       439 TNAGVSS-TNNLLNVVDFFTKEIAPDLTSNNIPHI-ILRVDAIKYIYTFRNQLTKAQLIE
DroMel_CSE1       447 QKHGITQ-TSELVPLPEFCAQQIIPELERPNINEFPVLKAAAIKYVMVFRSILGPQVLAS
HomSap_CAS        444 QKHGITQ-ANELVNLTEFFVNHILPDLKSANVNEFPVLKADGIKYIMIFRNQVPKEHLLV
MusMus_XPO2       444 QKHGITQ-ANELVNLTEFFVNHILPDLKSNNVNEFPVLKADGIKYIMIFRNQVPKEHLLV

SteCoe_g28919+8   516 IMGKVIGFLDSNNVLLASYAAATLERLLMIKNDKD---LIFTKDFLSSCLNQLLQNIAMA
SteCoe_g28374     488 IMGKVIGFLDSNNVLLASYAAATLERLLMIKNDRD---LIFTKEFLSSCLNQLLQSIAMA
SacCer_CSE1       497 LMPILATFLQTDEYVVYTYAAITIEKILTIRESNTSPAFIFHKEDISNSTEILLKNLIAL
DroMel_CSE1       506 CLPQLIRHLPAESSVVHSYAACSVEKILSMRDASN--AIVFGPQILAPYTTELISGLFAT
HomSap_CAS        503 SIPLLINHLQAESIVVHTYAAHALERLFTMRGPNN--ATLFTAAEIAPFVEILLTNLFKA
MusMus_XPO2       503 SIPLLISHLEAESIVVHTYAAHALERLFTMRGSNN--TTLFTAAEIAPFVEILLTNLFKA

SteCoe_g28919+8   573 LQKHP-------KNTYIMNAFFRVIWISQDLFSSFAVPACDIFINYIKQVLSEPQSSEPH
SteCoe_g28374     545 LQKHP-------KNTYIMNAFFRVIWISQDLFSSFAVPACDIFISYIKQVLSEPQSSEPH
SacCer_CSE1       557 ILKHGSSPEKLAENEFLMRSIFRVLQTSEDSIQPLFPQLLAQFIEIVTIMAKNP--SNPR
DroMel_CSE1       564 LSLPGSG-----ENEYVMKAIMRSFSVLQSAAMPFMGVALPRLTEILTQVAKNP--SRPQ
HomSap_CAS        561 LTLPGSS-----ENEYIMKAIMRSFSLLQEAIIPYIPTLITQLTQKLLAVSKNP--SKPH
MusMus_XPO2       561 LTLPGSS-----ENEYIMKAIMRSFSLLQEAIIPYIPTLITQLTQKLLAVSKNP--SKPH

SteCoe_g28919+8   626 FNWLLFECIALAMKWSG----PGISDIQKKIEPYMALIIQKSNADLLPYAFQIQAFFIRL
SteCoe_g28374     598 FNWLLFECIALAMKWSG----PGISDIQKKLEPYMALIIQKSNADLLPYAFQIQAFFIRL
SacCer_CSE1       615 FTHYTFESIGAILNYTQRQNLPLLVD---SMMPTFLTVFSEDIQEFIPYVFQIIAFVVEQ
DroMel_CSE1       617 FNHYLFETLALCIKIVCHADSSAVSSFEEALFPVFQGILQQDIVEFMPYVFQMLSVLLEM
HomSap_CAS        614 FNHYMFEAICLSIRITCKANPAAVVNFEEALFLVFTEILQNDVQEFIPYVFQVMSLLLET
MusMus_XPO2       614 FNHYMFEAICLSIRITCKANPAAVVNFEEALFLVFTEILQNDVQEFIPYVFQVMSLLLET

SteCoe_g28919+8   682 L---INLSQTNQNLISSILPIDNWESGSRYYLPTLVIFLENLLATNASSM----APQISA
SteCoe_g28374     654 L---SSISQTNQNLISSILPIDNWESGSRYYLPTLVIFLENLLMTNASSM----APQISA
SacCer_CSE1       672 S---ATIPESIKPLAQPLLAPNVWELKGN--IPAVTRLLKSFIKTDSSIF-----PDLVP
DroMel_CSE1       677 REGTGTIPEPYWALFPCLLSPALWDRTGN--VTPLIRLISAFIKQGSAQI--QALGKLSG
HomSap_CAS        674 HK--NDIPSSYMALFPHLLQPVLWERTGN--IPALVRLLQAFLERGSNTIASAAADKIPG
MusMus_XPO2       674 HK--NDIPSSYMALFPHLLQPVLWERTGN--IPALVRLLQAFLERGSSTIATAAADKIPG

SteCoe_g28919+8   735 LCNIAHKLF-NLGLDGQAFSLLTTLIETYAFENLHPYMHPIYLIIFTKLHNSKSQNIRLS
SteCoe_g28374     707 LCNIAHKLF-TLGLDGQAFSLLTTLIETYSFENLHPYMHPIYMIIFTKLHNSKSQNIRLS
SacCer_CSE1       722 VLGIFQRLIASKAYEVHGFDLLEHIMLLIDMNRLRPYIKQIAVLLLQRLQNSK------T
DroMel_CSE1       733 ILGIFQKMIASKANDHEGFYLLQNLLSYYPPAEIQTNLRQIFGLLFQRLSLSK------T
HomSap_CAS        730 LLGVFQKLIASKANDHQGFYLLNSIIEHMPPESVDQYRKQIFILLFQRLQNSK------T
MusMus_XPO2       730 LLGVFQKLIASKANDHQGFYLLNSIIEHMPPESVDQYRKQIFILLFQRLQNSK------T

SteCoe_g28919+8   794 PRFHKGSILFVSSVILKYGWKVLSDSMNSVQPGIFFMLVKGQILQNLRSIETIVERRAVI
SteCoe_g28374     766 PRFHRGSIMFVSSFILKYGWNVLSDSMNSVQPGIFFMLVKGQILQNLRSIETIVERRAVI
SacCer_CSE1       776 ERYVKKLTVFFGLISNKLGSDFLIHFIDEVQDGLFQQIWGNFIITTLPTIGNLLDRKIAL
DroMel_CSE1       787 PKYLSGIIIFFSFYVIKFSGSQMAQLIDEIQPNLFGMLLDRVFITEMGKIPKEQDRKMVA
HomSap_CAS        784 TKFIKSFLVFINLYCIKYGALALQEIFDGIQPKMFGMVLEKIIIPEIQKVSGNVEKKICA
MusMus_XPO2       784 TKFIKSFLVFINLYCIKYGALALQEIFDGIQPKMFGMVLEKIIIPEIQKVSGNVEKKICA
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SteCoe_g28919+8  854 LAMSVLMQNI-----EVTNELWINIVIS-VSKILDSTTNILSGTVYSNGLVDLPEENTIQ
SteCoe_g28374    826 LAMSALMQNT-----EVTNELWINIVIS-VSKILDSTTNILSGTVYSNGLVDLPEENTIQ
SacCer_CSE1      836 IGVLNMVING-----QFFQSKYPTLISSTMNSIIETASSQSIA-NLKNDYVDLDNLEEIS
DroMel_CSE1      847 VGVTKLLTETPEILQQQYATFWPRLLHS-LIDLFERPPEKLMG--LEIGETAGVAEDPDA
HomSap_CAS       844 VGITKLLTECPPMMDTEYTKLWTPLLQS-LIGLFELPEDDTIP-----DEEHFIDIEDTP
MusMus_XPO2      844 VGITKLLTECPPMMDTEYTKLWTPLLQS-LIGLFELPEDDSIP-----DEEHFIDIEDTP

SteCoe_g28919+8  908 MTRDSFQKVYSTEMPLTDKYASL---PNEKMFFINTICS-QQYPTGSFWSFAGQHLDQQV
SteCoe_g28374    880 MTRDSFQKVYSAEIPLTDKYPHL---PNEKMFFINTICN-QQYPTGSFWSFAGQHLDQQI
SacCer_CSE1      890 TFGSHFSKLVSISEKPFDPLPEIDVNNGVRLYVAEALNKYNAISGNTFLNTILPQLTQEN
DroMel_CSE1      904 GYQVAFAQLTHAQPNQQDHLAEI---KDARQFLATSLSKFAQARAGEFSTLLSP-LEPEY
HomSap_CAS       898 GYQTAFSQLAFAGKKEHDPVGQM--VNNPKIHLAQSLHKLSTACPGRVPSMVSTSLNAEA
MusMus_XPO2      898 GYQTAFSQLAFAGKKEHDPVGQM--VNNPKIHLAQSLHKLSTACPGRVPSMVSTSLNAEA

SteCoe_g28919+8  964 PTILNRYAQLFNQQIR
SteCoe_g28374    936 PTILNRYAQLFNQQIR
SacCer_CSE1      950 QVKLNQLLVGN-----
DroMel_CSE1      960 KQVLQKYCDQAGVRIA
HomSap_CAS       956 LQYLQGYLQAASVTLL
MusMus_XPO2      956 LQYLQGYLQAASVTLL
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Supplemental Figure S8: Multiple alignment of CSE1 genes

Multiple alignment of both *Stentor coeruleus* CSE1 orthologs and CSE1 homologs from *S. cerevisiae, D. melanogaster, H. sapiens, and M. musculus.* Mulitiple alignment performed in MEGAX using MUSCLE, displayed using Boxshade. The N-terminal half of CSE1 has multiple contact sites with importin alpha and is generally more conserved.[46,47] In *S. cerevisiae* CSE1, the region from Asn346 to Asn379 is necessary for forming a complex with Ran-GTP and importin-alpha, and acts as a hinge between the N and C terminal halves of CSE1 (Green box).[47] The tryptophan at position 419 in S. cerevisiae CSE1 is conserved in other CSE1 homologs as well as related proteins like importin betas.[47] This tryptophan is also conserved in *Stentor* CSE1 (magenta box). Asp220 in *S. cerevisiae* CSE1 has been shown to be necessary for CSE1 to form a complex with importin alpha and RanGTP – this residue is conserved in *Stentor* CSE1 (blue box).[48]