The architecture and operating mechanism of a cnidarian stinging organelle

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The stinging organelles of jellyfish, sea anemones, and other cnidarians, known as nematocysts, are remarkable cellular weapons used for both predation and defense. Nematocysts consist of a pressurized capsule containing a coiled harpoon-like thread. These structures are in turn built within specialized cells known as nematocytes. When triggered, the capsule explosively discharges, ejecting the coiled thread which punctures the target and rapidly elongates by turning inside out in a process called eversion. Due to the structural complexity of the thread and the extreme speed of discharge, the precise mechanics of nematocyst firing have remained elusive. Here, using a combination of live and super-resolution imaging, 3D electron microscopy, and genetic perturbations, we define the step-by-step sequence of nematocyst operation in the model sea anemone Nematostella vectensis. This analysis reveals the complex biomechanical transformations underpinning the operating mechanism of nematocysts, one of nature’s most exquisite biological micro-machines. Further, this study will provide insight into the form and function of related cnidarian organelles and serve as a template for the design of bioinspired microdevices.
nidarian nematocysts are complex subcellular weapons with highly specialized forms and functions. Nemato-
cysts are Golgi-derived intracellular organelles comprised of venomous threads enclosed within a pressurized capsule. When triggered, the capsule discharges, ejecting its thread as a harpoon that penetrates targets, delivering a cocktail of neurotoxins.

At the cellular level, nematocyst discharge is among the fastest mechanical processes in nature, known to be completed within 3 milliseconds in Hydra nematocysts. Measurements performed on high-speed video of Hydra stenoteles reveal that the initial phase of pressure-driven capsule explosion and subsequent thread ejection occurs in as fast as 700 nanoseconds. This initial stage of explosive discharge is comparable to other ultra-fast projectile systems found in nature such as fungal spore discharge, pollen ejection, and discharge of the ballistic organelles of dinoflagellates.

Previous studies indicate that the high-speed of nematocyst discharge is driven by the accumulation of osmotic pressure inside the capsule by a matrix of cation binding poly-γ-glutamate polymers (PGs) and the elastically stretched capsule wall releasing energy by a powerful spring-like mechanochemical force during discharge. Upon triggering, but prior to discharge, the capsule approximately doubles in volume due to the rapid influx of water. This causes the matrix to swell osmotically and stretches the capsule wall. Energy is subsequently utilized to eject the thread with high velocity, which imparts and penetrates target tissue. The later phases of nematocyst discharge involve the elongation of the thread, which proceeds on a slower timescale and is completed in milliseconds. During this phase, the nematocyst thread undergoes a shape transformation, turning inside-out through a process called eversion which is caused by the release of both osmotically generated pressure and elastic energy stored in the thread. Thus, the nematocyst operates in distinct phases that involve an initial phase of piercing the target and later phases of eversion to form a lumen.

Nematocyst characteristics vary significantly among different cnidarian species, exhibiting diversity in capsule size and thread morphology, but all retain a similar mechanism of operation involving an evertible tubule driven by explosive ejection.

To explore nematocyst biology in a genetically tractable system, here we interrogate the operation of the nematocyst thread in the sea anemone Nematostella vectensis. Nematostella harbors two types of nematocysts: microbasic p-mastigophores and basi-
trichous isorhizas, the latter having short and long varieties. In sea anemones, nematocyst capsules are sealed by three apical flaps connected to the stinging thread. This thread is composed of two distinct sub-structures: a short, rigid, and fibrous shaft and a long thin tubule decorated with barbs. The shaft is composed of three helically coiled filaments, and is initially ejected as a compressed projectile, piercing the target, and later everts to form a lumen through which the remainder of the thread, the tubule, is released. While it is known that shaft eversion entails a geometric transformation from a tightly compressed coil to a hollow syringe, the mechanisms driving this process are poorly understood. Further, tubule eversion significantly differs from that of the triple helical shaft, as the tubule everts by turning inside-out in the absence of helical filaments. The release of pressure and elastic energy stored in the capsule is theoretically sufficient to drive the initial ejection and penetration of the shaft, however, additional energy sources are likely to be required for further elongation of the thread. Due to the speed and complexity of these events, the precise stages of discharge and eversion have thus far remained elusive.

Here, we demonstrate the structural composition and mechanical transformations of both the shaft and the tubule during distinct phases of nematocyst discharge in Nematostella, and further report the operating mechanism of the nematocyst thread sub-structures. Our analysis reveals the complex structure and the sophisticated biomechanical transformations underpinning the operational mechanism of nematocysts.

Results

Visualizing nematocysts in Nematostella. To understand the distribution of stinging cells (nematocytes) and their nematocysts in Nematostella, we first created a transgenic line expressing EGFP in nematocytes under the control of the nematogalectin promoter region (nematogalectin > EGFP, Fig. 1a). Nematogalectin is a major component of the nematocyst, and it is incor-
porated into the thread structure during its morphogenesis. This protein is thought to act as a substrate for the assembly of other structural proteins into the thread, thus its temporal expression defines a useful window for visualizing nematocysts. Live imaging of transgenic primary polyps showed that the ten-
tacles were heavily populated with EGFP+ nematocytes bearing the long form basitrichs (Fig. 1a). The body column was popu-
lated with the shorter variety along with a few p-mastigophores. Intriguingly, we found that nematocytes were connected through neurite-like processes which formed local networks (Fig. 1a, arrow). Nematocytes are known to form synapses and act as afferents or effectors but can also operate cell-autonomously.

Thus, the observed networks might function in regulating collective behavior and coordinated activity of nematocyte populations. In EGFP+ nematocytes, fluorescence was detected throughout the cytoplasm and the sensory apparatus but was excluded from the capsule (Fig. 1b). The capsule wall and thread are built, in part, of minicollagens which allow the construction of a variety of structural fibers by cross-linking. We exploited this to visualize the capsule content by treating live animals with fluorescent TRITC which was incorporated into the nematocyst thread during its maturation, presumably through a reaction with minicollagens.

In basitrich type nematocytes, TRITC incorporation was seen only after thread invagination (Supplementary Fig. 1a, arrows). In contrast, nematocytes harboring maturing threads were devoid of TRITC (Supplementary Fig. 1a, dashed arrows). This suggests that the dye specifically accumulates in invaginated parts of the thread inside the capsule. We found that shRNA-mediated knockdown of the nematogalectin-like gene Nemve1_232014 resulted in abnormal capsules and prevented thread formation (Supplementary Fig. 1b). This suggests that lectins play a critical role in thread and capsule morphogenesis (Supplementary Fig. 1b, dashed arrows). We further confirmed that knockdown resulted in a two-fold reduction of Nemve1_232014 mRNA expression by qPCR, suggesting that this protein must be present in abundance for proper assembly of the thread and capsule ( Supplementary Fig. 1c). Utilizing a combination of nematogalectin–EGFP and TRITC dye labeling, we next analyzed the architecture of the thread from its development to its final morphology after firing (Fig. 1b, c; Supplementary Movie 1). In contrast to the dense shaft of p-mastigophores (Fig. 1c, arrows), in which the dye intensity was very high compared to the tubule, fluorescent TRITC incorporated with similar intensity in both the shaft and tubule of basitrichs (Fig. 1c, dashed arrows). The more uniform labeling of basitrichs and their prevalence in primary polyps led us to investigate thread operation in this nematocyst type.

The architecture of undischarged nematocysts. To analyze the structure of the shaft and tubule and thereby determine their functionality, we next performed 3D-reconstruction of undis-
charged basitrich capsules from serial sections using scanning electron microscopy (SEM; Fig. 1d; Supplemental Movie 2). We
Fig. 1 The architecture of undischarged nematocysts.  

a Nematocytes (green) of a transgenic *N. vectensis* primary polyp expressing EGFP under the control of the nematogalectin promoter. aI EGFP expression at the tentacle tip. aII EGFP expression on the body column. The arrow points to the network of cellular processes connecting nematocytes. The images are representative of primary polyps tentacles and body columns from 6 spawns.  

b Close-up view of a single nematocyte on the body column of a primary polyp expressing EGFP in nematocytes (green). The cnidocil (sensor) apparatus, cell body, and neurite-like processes are shown. TRITC (magenta) labels the capsule, the centrally positioned shaft, and the folds of the tubule (n = 10 primary polyp body columns, five experiments).  

c Nematocyst morphologies in *N. vectensis* based on the fluorescence signal of incorporated TRITC. Basitrichous isorhizas capsules (n = 19 short, n = 20 long) with densely labeled shaft (arrow) and coiled tubule (dashed arrow) continuous with the compressed shaft (left and middle panels). Microbasic p-mastigophores capsules (n = 10) shaft (arrow) with its distinctive V-shaped notch (right panel, dashed arrow) Images representative of purified nematocysts from ~300 primary polyps). Scale bars 1 μm.  

d Longitudinal section of a nematocyst showing densely coiled shaft filaments (blue), a portion of the coiled tubule (magenta), and the two connector regions, the capsule-shaft connector and shaft-tubule connector (yellow). The apical flaps are seen in a partially open conformation (dashed box). Corresponding 3D reconstruction of the longitudinal section shows the capsule, central shaft (blue), a portion of the attached tube (magenta), connector regions (yellow), and apical flaps (dashed box).  

e Transverse section of a nematocyst showing the capsule wall, dense lamellar shaft, and the propeller shaped tubule. (n = 2 complete volume capsules images from ~350 capsules visible in 2 primary polyp samples.)
found that the compressed shaft consisted of tightly coiled filaments vertically aligned to the capsule aperture formed by the apical flaps (Fig. 1d, box). The filaments were composite structures with stacks of lamellae built from electron-dense and electron-lucent layers. These results confirm a similar triplet lamellar structure to that observed in *Anemonia sulcata* by Godknecht and Tardent (1988), who noted the tip of the shaft was formed by staggered lamellae converging at a small area pointing towards the capsule aperture.

Close inspection of our SEM data further revealed that the thread wall encasing the shaft filaments was connected to the apical flaps with a loose capsule-to-shaft connector. A similar shaft-to-tubule connector was located between the basal end of the shaft and the apical end of the tubule. The tubule was twisted, forming pleats in regular lengthwise segments (Supplemental Movies 2, 3). In capsule cross-sections, the shaft lamellae were observed to be tightly coiled and compressed, while the tubule cross-section exhibited a propeller-shaped structure (Fig. 1e; Supplementary Movie 4).

### Nematocyst discharge and eversion of the stinging thread

To determine the distinct phases of thread operation, we next recorded fluorescent high-speed movies of discharge events in TRITC-labeled animals (Supplementary Movies 5–7). Following in situ nematocyte stimulation, the compressed shaft was first ejected as a dense projectile which then rapidly expanded to form an elongated cylinder through which the tubule emerged (Fig. 2aII–aIV, arrows; Supplementary Movie 5). Based on these observations, we defined three principal phases of nematocyst operation: shaft discharge (Phase I), shaft eversion (Phase II), and tube eversion (Phase III; Fig. 2, box). Using SEM, we visualized the ultrastructure of the discharging shaft. In the undischarged state, we observed sparse lamellae decorating the region where the shaft tapered to the capsule-shaft connector (Fig. 2b, dashed arrow). During the early stages of discharge, the everted capsule-shaft connector formed a skirt around the traversing shaft, creating a double-walled structure (Fig. 2c, arrow) with the unevorted shaft moving forward inside the connector (Fig. 2c, blue). The everted capsule-shaft connector was externally covered with sparse filaments resembling irregular spines originating from the evasion of the lamellae observed in the undischarged state (Fig. 2c, dashed arrow). Serial SEM sections also captured an everted connector (Fig. 2d, arrow) in which the tubule could be observed departing the capsule (Fig. 2d, dashed arrow; Supplementary Movie 8). Finally, in SEM sections of a partially discharged nematocyst thread, we observed the unevorted tubule traversing inside of its everted fraction, which was decorated externally with hollow barbs (Fig. 2e, arrow; Supplementary Movie 9).

To understand the structural changes described above, we next analyzed super-resolution images of TRITC-labeled threads undergoing eversion. This approach allowed us to demonstrate the existence of a triple helical geometry of the uncoiled shaft filaments together with the traversing unevorted tubule which could be traced by the labeling of the barbs (Fig. 2f). Interestingly, the thread wall did not incorporate TRITC and was invisible in fluorescent images but could be seen in corresponding SEM cross-sections as an electron-lucent layer that enclosed the unevorted tubule in its compacted state (Fig. 2f, fII). The highly ordered arrangement of barbs within the unevorted tubule indicates that these structures are stacked as a column, which appeared as a single filament in fluorescent images (Fig. 2f, fI). Further, SEM cross-sections through the shaft showed that its filaments consisted of lamellae that encased the traversing unevorted tubule, as seen in fluorescent images (Fig. 2f, fIII, arrow).

To visualize the thread wall that was otherwise invisible in optical images, we next focused our attention on other components of the thread. Together with minicollagens, the nematocysts of *Hydra* and *Nematostella* contain glycans and show similarities to the extracellular matrix in composition. The nematocyst-specific lectin, Nematogalectin, acts as a scaffold linking the minicollagens to glycans, mainly consisting of a non-sulfated chondroitin sheath. Considering the presence of lectins in the structure, we hypothesized that the presence of GAGs could be detected with fluorescently labeled sugar-binding lectins. Thus, we stained discharged nematocysts with fluorescent dye-conjugated Wheat Germ Agglutinin (WGA), which is selective for GlcNAc chains, and found that WGA strongly bound to the electron-lucent thread wall (Fig. 2g). Co-staining with WGA and TRITC showed that the WGA-stained material did not co-localize with TRITC, but rather formed a laminate with the TRITC-labeled structures. Finally, images of threads in an early everted state showed that the TRITC-labeled shaft surrounded the WGA-labeled tubule wall. The connector regions lacking filaments or bars which were poorly labeled with TRITC were more strongly labeled with fluorescent WGA (Fig. 2gI, gIII).

Importantly, we observed that TRITC labeling overlapped with the shaft filaments in the transillumination channel, suggesting that the shaft fibers harbor TRITC labeled material (Supplementary Fig. 2a, arrows). In contrast, fluorescent WGA labeling was enriched in the interior, surrounding the wall structure. The WGA layer formed repetitive lamellae with the TRITC labeled regions but did not overlap with TRITC labeled material (Supplementary Fig. 2b, c, TRITC; arrows; WGA, dashed arrows). By combining TRITC and WGA labeling with antibody staining of the minicollagen Nco4, we determined that TRITC signal was present in the threads of the mature capsules only where the thread is invaginated. TRITC did not label maturing capsules, which can be stained with *Nematostella* minicollagen Nco4 as reported by Zenkert et al. (2010)24,25. In tentacle tips, developing capsules deep inside the ectoderm stained with Nco4 antibody while fully matured capsules lining the surface of the tentacle epithelium were not stained (Supplementary Figs. 3, 4, arrows). In conclusion, these results suggest that the thread consists of two layers: a TRITC-labeled layer forming the TRITC-detectable shaft filaments and bars, and a WGA-labeled layer forming the overall cylindrical thread wall, including the connector regions, shaft wall, and tubule wall.

### The mechanism of shaft eversion

Structural studies of nematocyst threads penetrating gel substrates indicate that shaft eversion is initiated at the shaft’s apex. To determine how the shaft transforms from its compressed state to a loose triple helical structure, we captured the early stages of discharge by treating *Nematostella* primary polyps with a solution that simultaneously triggers discharge and rapidly fixes the samples. The reconstructed sequence of events from still images revealed the complex geometric transformation of the shaft as it exited the capsule in a coiled configuration (Fig. 2h and Supplementary Fig. 5a, arrows). We found that during shaft discharge (Phase I), the ejected shaft continued to move forward as a dense projectile inside the capsule-shaft connector until the connector extended to its maximal length. During shaft eversion (Phase II), the filaments started to uncoil from the apex of the shaft, turning inside out, thereby everting, while the basal end of the shaft moved forward inside the uncoiling filaments (Supplementary Fig. 5b). The evertor tip of the shaft exhibited a spearhead-like structure (Supplementary Fig. 5c, arrow). The tubule was attached to the basal end of the shaft through the shaft-tubule connector and was thus pulled through the newly formed lumen inside the uncoiled shaft filaments. This movement resulted in
complete eversion of the three filaments where the shaft’s former apical end became its basal end. Finally, the everted shaft lumen opened, permitting the movement of the shaft-tubule connector, initiating Phase III. The emergence of barbs on the everted tubule exterior demarcated a boundary between the shaft-tubule connector and the tubule itself, and thus marked the beginning of tubule eversion (Fig. 2g; Fig. 2h, last panel. Supplementary Fig. 5a, last panel). Altogether, these data indicate that shaft eversion executes a reproducible series of physical transformations that involve the uncoiling and forward motion of its filaments.
The mechanism of tubule eversion. SEM and fluorescent images revealed differences in composition and structure between the triple-helical fibrous shaft and the smooth cylindrical tubule (Fig. 3a, b). While shaft eversion can be explained by the motion of three filaments, the tubule lacks such symmetry and likely everts by a mechanism that involves the unfolding and untwisting of the tubule wall19,29. Live imaging revealed that during tubule elongation the forward-moving tubule untwisted and relaxed to a cylindrical state (Fig. 2a–IV; Fig. 3b, Stages 1, 2; Supplementary Movie 5). At the evert tip, the helical twists of the evverting tubule segment could be seen due to the concentration of WGA staining along the barb pockets (Fig. 3c). In contrast, in a live image captured shortly after the initiation of tubule eversion, the tubule was seen as a double-walled cylindrical structure with a lumen between the uneverted and everted walls, suggesting that it likely relaxed and untwisted rapidly (Fig. 3d, arrows). These results indicate that tubule eversion likely occurs in stages involving untwisting of its propeller-like shape to a cylindrical conformation, and that the action of the remnant segment untwisting, and relaxing feeds forward the remaining uneverted tubule to the distal tip.

*Hydra* spinalin is a glycine- and histidine-rich protein present in the spine structures on the surface of the shaft in *Hydra* nematocysts51,52. To test the role of the centrally stacked barbs in tubule eversion in *Nematostella*, we used shRNA53,54 to knock down vlg243188, previously reported to be a nematocyte-specific gene encoding a spinalin-like product55. However, further analysis suggests that vlg243188 encodes a fibroin-like factor quite distant in sequence composition to *Hydra* spinalin51,52, and direct orthologs of *Hydra* spinalin were not identified in *Nematostella*59. Nevertheless, we found that vlg243188 knockdown resulted in weakly labeled, thinned shaft filaments and in some cases visibly disrupted the structure of the barbs. The loss of TRITC intensity indicated that a fraction of the dye was also incorporated into the shaft structure, either directly or indirectly due to the presence of vlg243188. While the knockdown disrupted the structure of the barbs and their arrangement (Fig. 3e, arrows), this did not appear to affect thread operation. However, loss of vlg243188 resulted in increased bending of the tubule compared to controls (Fig. 3e, dashed arrow). This observation suggests that vlg243188 is a component of the thread that plays a role in its structural integrity but not its operation. Further, we noted that the stereotypical helical arrangement of the barbs and their stacked configurations was disrupted in samples exhibiting the strongest phenotypes (Fig. 3f, arrow, Supplementary Fig. 6a, boxes). Measurement of the TRITC intensity of the shaft structures in discharged threads indicated that TRITC incorporation was reduced following vlg243188 knockdown, with an approximate 65% reduction in mRNA levels (Supplementary Fig. 6d, e). In discharged nematocysts, the fully everted thread appeared to be an isodiometric tube composed of a WGA-labeled wall equipped with TRITC-labeled barbs and shaft filaments, excluding the connector regions (Fig. 3g, dashed boxes). The barbs decreased in density from the proximal to the distal end of the tubule, and sparsely decorated the distal region (Fig. 3g–IV). We hypothesize that the barbs, internally stacked before eversion and externally helically distributed after eversion, might function as a skeleton that prevents further bending and kinking for the elongating tubule. Indeed, as the barbs lessened distally, the tubule appeared to become more prone to kinking compared to the proximal regions which could bend in smooth curves (Fig. 3g, arrow). Interestingly, in live imaging of an elongating thread, we observed that the tubule performed smooth 180° turns in its barb dense proximal region (Supplementary Movie 10). Altogether, our data indicate that tubule eversion involves the unfolding of the tubule wall in which barbs likely provide structural support for the elongating thread.

**Model of the geometric transformation of the shaft and tubule.** These findings allowed us to build a model describing the key aspects of the observed geometric eversion in three phases. Our results suggest that the shaft filaments are attached apically to the capsule flaps, and basally to the tubule via connector regions (Fig. 4a). Phase I: Upon discharge, the shaft is ejected along with the capsule-shaft connector covering the ejecting shaft. A double-walled structure is formed (Fig. 4b, c). Based on still images and movies, shaft eversion occurs after complete ejection of the shaft. Thus, we postulate that the connector accumulates maximal elastic stress when the ejected shaft reaches its maximal distance from the capsule (Fig. 4c). Phase II: Elastic stress on the capsule-shaft connector creates outward forces applied to the apex of the compressed shaft filaments resulting in detachment of the filaments and initiation of the eversion process due to the release of elastic stress within the shaft (Fig. 4d–g, initiation). Upon completion of the sequence, a lumen is formed within the shaft that is protected by the thick filaments (Fig. 4h, end of Phase II). Phase III: The final phase commences with the release of the shaft-tubule connector which everts by folding on itself forming a double-walled structure (Fig. 4i). The unaevorted segment of the
tubule then progressively exits the capsule and moves through the everted portion of the elongating thread (Fig. 4j).

Discussion
In this article, we described the 3D organization of the nematocyst and the sequence of geometric transformations that occur upon its activation. We also suggest a model explaining the specific mechanisms of thread eversion. Based on our results, we conclude that nematocyst operation occurs in three stages involving a complex transformation of the shaft and the elongation of the tubule, during which energy stored in the overall structure is transformed to kinetic energy. The shaft performs two critical functions: first as a compressed syringe to penetrate the target cuticle; second as a protective tunnel for passage of the thin tubule.

The structure of the shaft of the anthozoan nematocysts exhibits a staggered lamellar structure that differs from the specialized Hydra stenoteles which exhibit an arrowhead-like coiled styllet3,17,36,37. Godknecht and Tardent have previously
suggested that the staggered arrangement of the lamellae as seen
in the shafts of *Anemonia Sulcata* nematocysts results in a
“hammer-drill-like” impact on a small area of the target during
eversion\(^*\)\(^{17}\). In *Hydra* stenoteles, the tip of the stylet impacts and
pierces the target at a single point\(^*\(^{2,3,11}\). Thus, the kinetics of
discharge in the *Hydra* stenoteles is orders of magnitude faster
than the slower process of shaft eversion we observed in *Nema-
tostella* nematocysts\(^*\(^{1,12}\).

The shaft eversion process resembles the mechanics of a
Y-shaped slingshot wherein elastic energy is stored in two bands
attached to a pad containing a projectile. Upon release of the pad,
the stretched bands experience a geometric eversion. The elastic
buffer zone for the transition of the three-connector rapidly forms a cylindrical tube which might act as a cylindrical state. The double-walled structure seen upon energy by acting as a spring that is released by relaxation to a tubule without undergoing eversion, suggesting that the twisted twisted tubule. Relaxation (Fig. 3d, second panel, arrow) likely allows the flow of PG matrix from the capsule into the lumen, recharging the forces that push the tubule forward. The process is repeated until the tubule is fully elongated or reaches an obstacle (Supplementary Movies 10, 11). In summary, this study demonstrates the operational capability of the nematocyst as a complex and self-assembling biological micromachine. We propose that these ancient and sophisticated organelles represent an ideal model for biologically inspired microscale devices that could be utilized in diverse applications ranging from medical technology to materials science.

**Methods**

**Animal husbandry.** Animals were raised at 23 °C in 12 parts per thousand (ppt) artificial seawater (ASW; Sea Salt, Instant Ocean). Spawning induction and dejellying were carried out as previously described. Embryos and polyps were cultured at either room temperature 23 °C or 25 °C.

**Generation of the nematogalctin > EGFP transgenic reporter line.** The transgenic reporter line was generated by meganuclease-mediated insertion of a plasmid containing EGFP under the control of a nematocyte-specific N. vectensis nematogalctin promoter. This construct was generated as part of a dual reporter system that harbors a mScarlet-I neuronal reporter that was not analyzed here and will be described in a forthcoming publication.

**In vivo labeling of Nematostella with (5,6)-tetrathymethyleneadamine isothiocyanate (TRITC).** Live Nematostella vectensis planulae (2 dpf) were allowed to react with the amine reactive rhodamine derivative 5,6-tetrathymethylene rhodamine-6-isothiocyanate. TRITC (Cayman Chemical, No. 19593) for a short duration (30 min–1 h). The animals were incubated for 1 h at a final concentration of 1 μM for live imaging of discharge. For fixed specimens, TRITC at a final of 25 μM is incubated for 1 h with 2dpf larvae. The fluorescent dye stained the animals without any apparent toxicity up to 25 μM concentration tested in this study. Upon incubation, the reactive dye was removed by multiple washes. Animals were transferred to clean dishes in dye-free medium for 3–5 days until mature nematocytes emerged and the non-specific background fluorescence disappeared substantially. Stock solutions of 25 mM TRITC was prepared and stored at −20 °C and used without an observable reduction in the chemical reactivity.

**Electron microscopy.** For scanning electron microscopy (SEM) of topography (Fig. 3a), specimens were processed according to previous reports. Briefly, samples were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M NaCacodylate buffer and stained with aqueous tannic acid, osmium tetroxide, thiocarbo- dymide, or 0.1% aqueous uranyl acetate. Samples were dehydrated in a graded series of ethanol and critical point dried in a Tousimis Samdry 795 critical point dryer, mounted on stubs, and imaged in a Hitachi TM-4000 tabletop SEM at 15 kV with BSE detector. For electron microscopy (EM) of internal ultrastructure, samples were fixed as above, with secondary fixation in 1% buffered osmium tetroxide for 1 h and en bloc staining in 0.5% aqueous uranyl acetate carried out overnight at 4 °C. A graded series of ethanol was used for dehydration with acetone as a transition solvent and infiltration in Hard Plus resin (Electron Microscopy Sciences). Samples were cured for 48 h at 60 °C and serial sections were cut at 50 nm using a Diatome 45-degree ultra-diamond knife or an AT-4 35-degree diamond knife on a Leica UC7 ultramicrotome. Sections were collected on slot grids for STEM imaging and a flat substrate (coverlip or silicon chip) for SEM imaging, and post stained using 4% uranyl acetate in 70% methanol for 4 min and Sato’s triple lead stain for 5 min. Sections on flat substrate were mounted on stubs, the underside of the coverlip painted with silver paint to mitigate charging, and all were coated with 4 nm carbon in a Leica AC6000 coater. Sections were imaged in a Zeiss Merlin SEM using the aSTEM or QBSR detector, SmartSEM (6.0.0, Zeiss), and Atlas 5.2.15 software ( Fibics, Inc.). Serial images were aligned and traced for 3D modeling in IMOD (4.9.10) and 3D models rendered in Blender 2.92 (Blender Foundation). Straightening of fully discharged nematocyte (Fig 2e) was done in Fiji (Image.sc) and false coloring were done in Photoshop 2021 (Adobe, Inc.).

**Live imaging of nematocyst discharge.** The nematocysts of the TRITC treated animals were discharged by decreasing the pH of the medium using acetic acid. The nematocysts discharge in vivo when the medium becomes acidic. Primary polyps were immobilized in glass bottom dishes by sandwiching between a glass slide and the bottom of a glass bottom dish using silicone sealant. The images were captured after dropwise addition of glacial acetic acid (17%) to the ASW, which triggers capsule discharge when the pH sufficiently decreases in the medium below a certain threshold. Live imaging of the nematocyst maturation and discharge events were recorded with Yokogawa CSU-w1 spinning disc system on a Nikon Ti2 platform with 100× objective.

**Immunofluorescence.** TRITC treated primary polyps were fixed in Ladvosky’s fixative (ethanol:formaldehyde:acetic acid = 1:2:1, 30:10:4.36) overnight. The fixed polyps were washed with 3 times the volume of PBS to remove the fixative. Samples were permeabilized with 0.1% Triton-X100 in PBS, pH 7.4 for 15 min. After several additional washes in PBST (0.1% Tween 20 in PBS, pH 7.4), the polyps were first blocked for 1 h and incubated over night at 4 °C with NvNcol-4 (1:500) in PBST supplemented with 10% goat serum. Minicollagen and Ncol43 Anti-NvNcol-4 antibody raised against Nematostella vectensis minicolla- gen NvNcol-4 protein (Rabbit, dilution 1:500) was a kind gift from Suet Ozbek, Heidelberg University). The polyps were washed three times in PBST supplemented with 10% goat serum and incubated with Alexa Fluor 647 coupled anti-rabbit secondary antibody (Goat anti-rabbit IgG (H + L), 1:500, Thermo Fisher, Cat. #: A21245, Lot: 1981173) and WGA-OregonGreen (dilution 1:500, Invitrogen Cat. #: W7024B, Lot: 2290804) overnight in PBST supplemented with 10% goat serum. Thereafter, the polyps were washed several times in PBS and incubated in 90% PBS/Glycerol overnight. The polyps were transferred to a glass slide and mounted on a glass slide with ProLong Glass antifade mounting medium (Thermo Fisher, Cat. #: P36960). Fluorescence images were acquired using Yokogawa CSU-w1 on a Nikon Ti2 platform. Super-resolution fluorescence confocal images were acquired using the Zeiss LSM 880 in Airyscan mode.
Super-resolution fluorescence confocal imaging. In Fig. 2f, TRITC treated primary polyps were fixed in Ladovsky's fixative (ethanol:formaldehyde:acetic acid:DDH2O: 20:10:1:36) overnight24–26. After several washes, the polyps were transferred to PBS/Glycerol (PBS) and incubated overnight. The polyps were transferred to a glass slide and mounted with ProLong Glass Antifade Mountant (ThermoFisher, Cat. #: P36982). The polyps were spread onto the glass slides. The labeled tentacles with partially discharged nematocysts were imaged either intact or crushed with the cover slide to detach the capsules from the tissue. The fluorescence imaging was performed using the Zeiss LSM780 in Airyscan mode.

Purification, discharge, and staining of TRITC labeled nematocysts. TRITC treated primary polyps were frozen in liquid nitrogen, thawed, and macerated manually with a plastic pestle. The samples were suspended in 1 ml Percoll (50%, v/v; Sigma Cat. #: P1644) in 300 mM sucrose supplemented with 0.01% Tween20 to prevent adhesion to the microcentrifuge tubes. The tube is further disrupted by pipetting up and down. The mixture is allowed to settle on ice for 30 min and centrifuged for 15 min at 950 g. The pellet is washed twice with PBS with 0.01% Tween-20 resuspended in nematocyst discharge buffer (10 mM Tris, pH 7.5, 10 mM CaCl2). The discharge was initiated by the addition of 1 mM DTT and incubated for 30 min. Upon incubation for 30 min, 1 μg/ml Wheat germ agglutinin conjugated with OregonGreen (dilution 1:500, Invitrogen, Cat. # W7024B, Lot: 2298084) was added to the tube and incubated for 1 h. The stained samples were washed twice with PBST and centrifuged at 1000 x g for 5 min. A loose pellet is seen and suspended in PBST 5 μl aliquots were spread onto glass slides and mounted with ProLong Glass Antifade Mountant. The images were acquired using Yokogawa CSU-W1 spinning disc system on a Nikon T2 platform with 100X objective.

shRNA knockdown. The short hairpin RNA targeting the putative Nematostella genes vlg243188 and Nenv1_232041 were synthesized by T7 polymerase reaction, purified using Direct-zol RNA miniprep Plus kit (Zymo Research, Cat. #: R2072). Purified shRNA was microinjected into unfertilized eggs at a concentration of 1 μg/μl or electroporated at a concentration of ~600 ng/μl according to the methods described previously25,26. The eggs were fertilized with sperm from wild-type or transgenic males. Following fertilization, the embryos were incubated for 48 h until the tentacle stage. The embryos were transferred to a glass slide and mounted with ProLong Glass Antifade Mountant. The images were acquired using Zeiss LSM780 in Airyscan mode.

Statistics and reproducibility. For the EM micrographs in Figs. 1d, e and 2b–e, serial sections from two different animals were acquired. From 350 capsules observed in the volumes for both animals, we acquired high-resolution images of three complete volumes of undischarged capsules and two partial volumes of discharged capsules; one complete volume of a discharged capsule in Phase 1, two complete volumes of discharged capsules in Phase 2, and three partial volumes and one complete volume of discharged capsules in Phase 3.

For qRT-PCR verification of target gene knockdown, three independent knockdown experiments were performed with ~200 polyps per sample. Each sample was analyzed in triplicate. Graphs were generated and statistical analyses were performed using GraphPad Prism (9.3.1). Statistical significance was determined using a two-tailed, unpaired students t-test, p-values are indicated in the figure legends. Cohen’s d was used to assess the effect size for all t-test analyses (mean difference between groups divided by pooled standard deviation).

Representative fluorescence images of partially discharged nematocysts were acquired from the tentacles of TRITC-treated animals fixed with Ladovsky’s reagent with identical results in 10 independent labeling and discharge experiments. Figure 1a is a representative image of transgene expression observed in animals from six independent spawnings. In Fig. 1b, the nematocyte and its capsule was representative of body column nematocytes (n = 10 primary polyp body column, 5 experiments). In Fig. 1c, capsules were representative high-magnification fluorescent images of small (n = 19) and large (n = 20) basitrichs and p-mastigophores (n = 10) purified from ~200 primary polyps. The image sequence in Fig. 2a and the Supplementary Videos 5–7, 10, and 11 were recorded from six independent live discharge experiments.

Figure 2f and Supplementary Fig. 2 were representative super-resolution images of the shaft and tube of partially discharged threads from four independent experiments. In Fig. 2g and h, the discharge sequence was reconstructed from the representative images of partially discharged threads (n = 67 partially discharged threads from primary polyp tentacles, three experiments). Figure 3a is a representative SEM image of partially discharged threads (n = 6). Figure 3b is a representative image of WGA- and TRITC-stained primary polyp tentacles fixed with Ladovsky’s reagent (n = 10 primary polyp tentacles, three experiments). Figures 3c, d were representatives of Stage 1 threads (Fig. 3c, n = 20) and double walled Stage 2 threads (Fig. 3d, n = 8) among the visible polyp threads on a primary polyp tentacle. Figure 3e, f are representative fluorescence images acquired from partially discharged primary polyp tentacles of scramble control shRNA (n = 0/25 threads from 1/27 tentacles) and vlg243188 shRNA (n = 25/25 threads from 1/25 tentacles) from 5 knockdown experiments. Figure 3g was a representative image of purified and fully discharged threads from ~300 primary polyps (n = 15 threads).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Source data are provided with this paper. Original data underlying this manuscript can be accessed from the Stowers Original Data Repository (ODR) at: http://www.stowers.org/research/publications/lipbp-1684

The correspondence and materials requests should be addressed to MG2@stowers.org. Source data are provided with this paper.

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Author contributions
A.K. and M.C.G. concepted this study. A.K., B.R., and M.C.G. wrote the manuscript. A.K., S.M.C., M.M. K.Z., and B.R. performed the experiments and analyzed the data.

Competing interests
The authors declare no competing interests.

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