A peer-reviewed version of this preprint was published in PeerJ on 26 August 2019.

Fujita S, Kuranaga E, Nakajima Y. 2019. Cell proliferation controls body size growth, tentacle morphogenesis, and regeneration in hydrozoan jellyfish *Cladonema pacificum*. PeerJ 7:e7579
https://doi.org/10.7717/peerj.7579

Cell proliferation controls body size growth, tentacle morphogenesis, and regeneration in hydrozoan jellyfish *Cladonema pacificum*

Sosuke Fujita [1], **Erina Kuranaga** [1], **Yuichiro Nakajima** [Corresp. 1, 2]

[1] Graduate School of Life Sciences, Tohoku University, Sendai, Japan

[2] Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Sendai, Japan

Corresponding Author: Yuichiro Nakajima
Email address: yuichiro.nakajima.d2@tohoku.ac.jp

Jellyfish have existed on the earth for around six hundred million years and have evolved in response to environmental changes. Hydrozoan jellyfish, members of phylum Cnidaria, exist in multiple life stages, including planula larvae, vegetatively-propagating polyps, and sexually-reproducing medusae. Although free-swimming medusae display complex morphology and exhibit increase in body size and regenerative ability, their underlying cellar mechanisms are poorly understood. Here, we investigate the roles of cell proliferation in body-size growth, appendage morphogenesis, and regeneration using *Cladonema pacificum* as a hydrozoan jellyfish model. By examining the distribution of S phase cells and mitotic cells, we revealed spatially distinct proliferating cell populations in medusae, uniform cell proliferation in the umbrella, and local cell proliferation in tentacle bulbs. Blocking cell proliferation by hydroxyurea caused inhibition of body size growth and defects in tentacle branching, nematocyte differentiation, and regeneration. Local cell proliferation in tentacle bulbs is observed in medusae of two other hydrozoan species, *Cytaeis uchidae* and *Rathkea octopunctata*, indicating that it may be a conserved feature among hydrozoan jellyfish. Altogether, our results suggest that hydrozoan medusae possess actively proliferating cells and provide experimental evidence regarding the role of cell proliferation in body-size control, tentacle morphogenesis, and regeneration.

Cell proliferation controls body size growth, tentacle morphogenesis, and regeneration in hydrozoan jellyfish *Cladonema pacifica*

[1]Sosuke Fujita, [1]Erina Kuranaga and [1,2]Yu-ichiro Nakajima

[1]Graduate School of Life Sciences, Tohoku University, Sendai, Japan

[2]Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Sendai, Japan

Corresponding Author: Yu-ichiro Nakajima

Address: 6-3 Aoba, Aramaki-Aza, Aoba-ku, Sendai, 980-8578,

Email address: yuichiro.nakajima.d2@tohoku.ac.jp

Abstract

Jellyfish have existed on the earth for around six hundred million years and have evolved in response to environmental changes. Hydrozoan jellyfish, members of phylum Cnidaria, exist in multiple life stages, including planula larvae, vegetatively-propagating polyps, and sexually-reproducing medusae. Although free-swimming medusae display complex morphology and exhibit increase in body size and regenerative ability, their underlying cellar mechanisms are poorly understood. Here, we investigate the roles of cell proliferation in body-size growth, appendage morphogenesis, and regeneration using *Cladonema pacificum* as a hydrozoan jellyfish model. By examining the distribution of S phase cells and mitotic cells, we revealed spatially distinct proliferating cell populations in medusae, uniform cell proliferation in the umbrella, and local cell proliferation in tentacle bulbs. Blocking cell proliferation by hydroxyurea caused inhibition of body size growth and defects in tentacle branching, nematocyte differentiation, and regeneration. Local cell proliferation in tentacle bulbs is observed in medusae of two other hydrozoan species, *Cytaeis uchidae* and *Rathkea octopunctata*, indicating that it may be a conserved feature among hydrozoan jellyfish. Altogether, our

results suggest that hydrozoan medusae possess actively proliferating cells and provide experimental evidence regarding the role of cell proliferation in body-size control, tentacle morphogenesis, and regeneration.

Introduction

Cell proliferation lies at the core of controlling cell number in Metazoa and thus contributes to the growth and the maintenance of animal body and organs (*Leevers & McNeill 2005; Penzo-Mendez & Stanger 2015*). During development, cell proliferation plays a critical role in body-size increase by adding cells into tissue layers, and it further generates cellular resources for different cell types by multiplying progenitors (*Gillies & Cabernard 2011; Hardwick et al. 2015*). Later in adults, proliferating cells are required for physiological cell turnover and for the replacement of damaged cells after tissue injury (*King & Newmark 2012; Pellettieri & Sanchez Alvarado 2007*). These roles of cell proliferation in multicellularity must be conserved throughout evolution: indeed, sponges, one of the earliest metazoan organisms, have acquired mechanisms to allow cell turnover by controlling proliferative capacities (*Alexander et al. 2014; Kahn & Leys 2016*).

As the sister group of bilaterians and early-branching metazoans, cnidarians have been studied as a model to understand evolutionary development (*Genikhovich & Technau 2017*). Cnidarians are diploblastic and radially symmetric animals that include diverse species such as corals, sea anemones, hydroids, and jellyfish (*Technau & Steele 2011*). During the embryonic development of the sea anemone *Nematostella vectensis*, cell proliferation is coordinated with epithelial organization and is involved in tentacle development (*Fritz et al. 2013; Ragkousi et al. 2017*). Cnidarians are also known for their regenerative abilities: for instance, *Hydra* polyps have been used for a century to investigate mechanisms of metazoan regeneration (*Fujisawa 2003; Galliot & Schmid 2002*). The basal head regeneration of *Hydra* relies on cell proliferation triggered by dying cells (*Chera et al. 2009b; Galliot & Chera 2010*). *Hydractinia* polyps regenerate through cell proliferation and the migration of stem-like cells (*Bradshaw et al. 2015; Gahan et al. 2016*). Although much has been learned about mechanisms controlling embryogenesis and growth during regeneration, it is unclear how cnidarians

integrate cell proliferation to control their body size and maintain tissue homeostasis under normal physiological conditions.

Among cnidarians, hydrozoan jellyfish have a complex life cycle including planula larvae, sessile polyps, and free-swimming medusae. While polyps undergo asexual reproduction to grow vegetatively, medusae generate gametes to perform sexual reproduction. Despite the limited life span compared to the long-lived or possibly immortal polyps, the size of medusae increases dramatically (*Hansson 1997; Miyake et al. 1997*). Furthermore, medusae maintain their regenerative capacity for missing body parts by integrating dedifferentiation and transdifferentiation (*Schmid & Alder 1984; Schmid et al. 1988; Schmid et al. 1982*). Recent studies using the hydrozoan jellyfish *Clytia hemisphaerica* have provided mechanistic insights into embryogenesis, nematogenesis, and egg maturation (*Denker et al. 2008; Momose et al. 2008; Quiroga Artigas et al. 2018*). However, little is known about the mechanism that controls body size growth in medusae. It is also unclear whether cell proliferation is required for tentacle morphogenesis and regeneration of hydrozoan jellyfish.

The hydrozoan jellyfish *Cladonema* is an emerging model, with easy lab maintenance and a high spawning rate, that is suitable for studying diverse aspects of biology including development, regeneration, and physiology (*Fujiki et al. 2019; Graziussi et al. 2012; Suga et al. 2010; Takeda et al. 2018b; Weber 1981*). *Cladonema* is characterized by small-sized medusae with branched tentacles. Using specialized adhesive tentacles, *Cladonema* can adhere to different substrata, such as seaweed, in the field. The species *Cladonema pacificum,* originally found along coastal areas in Japan, have nine main tentacles with a stereotyped branching pattern (Fig. 1A). During the *Cladonema medusa's* maturation, body size increases, and each main tentacle grows and exhibits branching morphology (*Fujiki et al. 2019*), providing an ideal system to dissect the cellular mechanisms associated with jellyfish growth and morphogenesis.

In this study, we investigate the role of cell proliferation in medusa growth and morphogenesis, using *Cladonema pacificum* as a model of hydrozoan jellyfish. We show that cell proliferation occurs evenly across the medusa body, including the umbrella and manubrium, with the exception of the tentacles, where cell proliferation is spatially localized to the bulb area. Blocking cell-cycle progression with a

pharmacological assay inhibits the increase of body size, tentacle branching, and nematocyte differentiation, which suggests that cell proliferation is necessary for growth and tentacle morphogenesis. We further show that cell proliferation is required for tentacle regeneration in *Cladonema* medusae. Our findings reveal cell proliferation's critical roles in the development and maintenance of the *Cladonema* body and appendages and provide a basis for understanding growth-control mechanisms in hydrozoan jellyfish.

Materials & Methods

Animal cultures

We used *Cladonema pacificum* (strains 6W and UN2), *Cytaeis uchidae* (strain ♀17) and *Rathkea octopunctata* (strain MF-1) medusae for this research. The medusae were cultured in plastic cups (V-type container, V-7 and V-8, AS ONE) at 20°C (*Cladonema* and *Cytaeis*) or 4 °C (*Rathkea*), and their polyps were maintained in the cups (V-7) at 20 °C or 4 °C in darkness. Vietnamese brine shrimp (A&A Marine LLC) were fed to medusae and polyps. Artificial sea water (ASW) was prepared by SEA LIFE (Marin Tech, Tokyo). Pictures of medusae were taken through a LEICA S8APO microscope with a Nikon digital camera (D5600).

Immunofluorescence

The medusae were anesthetized with 7% $MgCl_2$ in ASW for 10 min and fixed 4% paraformaldehyde (PFA) in ASW for 1 hr. After fixation, the samples were rinsed in 1x PBS and washed 3 times (10 min each) in PBS containing 0.1% Triton X-100 (0.1% PBT). The samples were incubated in primary antibodies in 0.1% PBT overnight at 4 °C. The antibodies used were rabbit anti-Phospho-Histone H3 (Ser10) (1:500; Upstate, 06-570) and mouse anti-α-Tubulin (1:500; SIGMA, T6199). After the primary antibody incubation, the samples were washed 3 times (10 min each) in 0.1% PBT and incubated in secondary antibodies (1:500; ALEXA FLUOR Goat anti-mouse IgG, ALEXA FLUOR Goat anti-rabbit IgG, Life Technologies) and Hoechst 33342 (1:250; Thermo Scientific) in 0.1% PBT for 1hr in dark. After 4 washes (10 min each) in 0.1% PBT, the samples

were mounted on slides with 70% glycerol. Confocal images were collected through Leica SP8 or SP5 confocal microscopes. Z-stack images were performed using ImageJ/Fiji software.

EdU labeling

The medusae were incubated with 20 μM EdU (EdU kit; Invitrogen, 1836341) in ASW for 24 hr or 150 μM for 1hr. After EdU treatment, the medusae were anesthetized with 7% $MgCl_2$ in ASW for 10 min and fixed 4% paraformaldehyde (PFA) in ASW for 1 hr. After fixation, the samples were rinsed in 1x PBS and washed 3 times (10 min each) in 0.1% PBT. The samples were incubated with a EdU reaction cocktail (1x reaction buffer, $CuSO_4$, Alexa Fluor azide, and 1x reaction buffer additive; all included in EdU kit; Invitrogen, 1836341) for 30 min in the dark. After the EdU reaction, the samples were washed 3 times (10 min each) in 0.1% PBT and Hoechst 33342 (1:250; Thermo Scientific) in 0.1% PBT for 1hr in dark. The samples were washed 4 times (10 min each) in 0.1% PBT and were mounted on slides with 70% glycerol.

Hydroxyurea treatment

The live medusae were incubated with 10mM hydroxyurea (HU) (Wako, LKP3349) in ASW (ASW only for control) (Fig. 2-4). HU incubation was continued for 9 days and HU solution or ASW was changed every other day.

Measurement of umbrella size and tentacle length

Pictures of medusae were taken with a Nikon D5600, and umbrella size was measured using polygon selections with ImageJ software (Fig. 2B). We measured the length and width of medusae under the microscope using an ocular micrometer and multiplied the length and width to generate a value for umbrella size (Fig. 2D). Tentacle length was measured daily under the microscope with an ocular micrometer (Fig. 4D).

DAPI poly-γ-glutamate staining

This protocol was adapted from (*Szczepanek et al. 2002*): The medusae were anesthetized with 7% $MgCl_2$ in ASW for 10 min and fixed with 4% PFA in ASW for 1 hr.

After fixation, the samples were rinsed in 1x PBS and washed 3 times (10 min each) in 0.1% PBT. The samples were incubated in DAPI (1:500; Polysciences, Inc.) in PBT for 60 min. After the DAPI incubation, samples were washed 4 times (10 min each) in PBT and mounted on slides with 70% glycerol in DW. Samples were scanned with a combination of 488nm excitation and 555nm emission filter using either Leica SP8 or SP5 confocal microscopes. Using ImageJ, we performed Z-stacks and counted nematocysts. Empty nematocysts were counted manually.

Dissection of tentacles for regeneration

Tentacles' basal sides were dissected with small scissors, leaving the tentacle bulbs intact. Amputated medusae were fed every other day.

Results

Cell proliferation patterns in the medusa *Cladonema pacificum*

To understand the spatial pattern of cell proliferation in *Cladonema* medusa, we performed 5–ethynyl–2'–deoxyuridine (EdU) staining (*Salic & Mitchison 2008*). EdU-positive cells, which indicate S-phase or the former S-phase cells, were broadly detected in the whole medusa body including the umbrella, the manubrium (a supporting organ of the oral in medusae), and the tentacles (Fig. 1B and 1C). In the tentacles, large numbers of EdU positive cells were located at their base, called tentacle bulbs, suggesting that tentacle bulbs might behave as a proliferation zone (Fig. 1D). We confirmed that these EdU-positive cells were proliferating cells using the mitotic marker, anti-Phospho-Histone 3 (PH3) antibody. PH3-positive cells were detected in both the umbrella and the tentacle bulbs (Fig. 1E and 1F). We further observed mitotic spindles, detected with an anti-α Tubulin antibody in PH3-positive cells (Fig. 1E). These results suggest that cell proliferation may occur uniformly in the medusa body, while a subset of cell proliferation could occur locally in the tentacle bulbs. Based on these observations, we hypothesized that uniform cell proliferation may control body size growth while local cell proliferation in the tentacle bulbs may contribute to tentacle morphogenesis.

188

189 **Cell proliferation is necessary for the control of body size**

190 Animal body size increases upon intake of nutrition because nutrition influences cell
191 proliferation and cell growth (*Bohnsack & Hirschi 2004*). We first monitored the body
192 size of juvenile medusae by focusing on the size of their umbrella because the umbrella
193 grows in direct proportion with whole body size. Under normal feeding conditions, the
194 medusa umbrella size increased dramatically by 54.8%, from 0.62±0.02 mm^2 to
195 0.96±0.02 mm^2 during the first 24 hours, with a subsequent minor increase observed
196 over the following 5 days (0.98±0.03mm^2) (Fig. 2A and 2C). By contrast, under starved
197 conditions, the size of medusa umbrella did not increase, compared to controls, and
198 rather gradually decreased over the following 5 days. Moreover, fewer EdU positive
199 cells were detected in the starved medusae than in fed controls (Fig. 2B), suggesting
200 that, at the cellular level, nutrition affects cell proliferation in medusae. These results
201 indicate that body-size growth in juvenile medusa depends on available nutrition.
202 To test the hypothesis that uniform cell proliferation in medusa contributes to
203 body-size increase, we performed a pharmacological assay to block cell-cycle
204 progression using hydroxyurea, a cell-cycle inhibitor that causes G1 arrest (*Koc et al.*
205 *2004*). Under hydroxyurea treatment, S phase cells detected by EdU staining
206 disappeared from the medusa body (Fig. 2D). By tracking the size of umbrella, we found
207 that hydroxyurea-treated medusae did not exhibit the size increase that was observed in
208 controls (Fig. 2E). Together, these results suggest that cell-cycle progression affects
209 body size in *Cladonema* medusae.

210

211 **Cell proliferation is necessary for tentacle morphogenesis**

212 In *Clytia hemisphaerica*, another hydrozoan jellyfish, stem-like cells or progenitors are
213 proposed to exist in tentacle bulbs (*Denker et al. 2008*). The local cell proliferation
214 observed in the tentacle bulbs of *Cladonema* medusa may reflect such stem or
215 progenitor cell populations (Fig. 1D and 1F). To test the hypothesis that local cell
216 proliferation in tentacle bulbs contributes to tentacle morphogenesis, we first focused on
217 tentacle branching. Although the initial tentacles have one branch in juvenile medusa,
218 the number of branches gradually increases during medusae maturation (*Fujiki et al.*

2019). In our normal feeding condition, the branching number reached approximately three (2.98±0.05 per tentacle) by day 9 (Fig. 3A and 3C). By contrast, when cell proliferation was blocked with hydroxyurea, none of the medusae exhibited the typical increase in branched tentacles; rather, all maintained only one branch (Fig. 3B and 3C). This result points to cell proliferation in tentacle bulbs as a necessary component for normal tentacle branching.

Cnidarian tentacles have nematocysts, organelles specific to the cnidarian phylum that are utilized for food capture and defense against predators (*Kass-Simon & Scappaticci 2002*). In *Clytia hemisphaerica*, stem-like cells or progenitors in tentacle bulbs seem to supply nematocysts at the tips of tentacles via cell proliferation, migration to the tip, and differentiation (*Denker et al. 2008*). This evidence raises the possibility that cell proliferation also controls nematocyte development or nematogenesis in hydrozoan jellyfish. To monitor nematocytes in *Cladonema* tentacles, we utilized DAPI, a nuclear staining dye that can label poly-γ-glutamate synthesized in the nematocyst wall (*Szczepanek et al. 2002*). Using poly-γ-glutamate staining, we discovered nematocyte size variations ranging from $2\mu m^2$-$110\mu m^2$ (Fig. 3D). Because nematocytes increase in size during maturation, small nematocysts tend to be immature nematocysts. We also found that some of the nematocysts were empty, suggesting that such nematocytes had been depleted (Fig. 3D). In order to investigate whether cell proliferation in tentacle bulbs also contributes to nematocyte maturation, we examined the size distribution and emptiness of nematocytes after cell-cycle blocking with hydroxyurea. Compared to controls, the rate of small nematocysts significantly reduced in the medusae under the treatment of hydroxyurea (HU+: 65.1±3.6%; HU-: 85.3±2.3%, Fig. 3D and 3E). We further detected that the rate of the empty nematocysts was higher in the medusae with hydroxyurea treatment than in controls (HU+: 25.7±3.1%; HU-: 14.1±3.1%, Fig. 3D and 3F). These results indicate that even after discharge, nematocytes are still actively supplied by progenitor cell proliferation and that this refill is prevented when cell proliferation is blocked. Taken together, our data suggest that cell proliferation in tentacle bulbs plays an important role in both tentacle branching and nematogenesis.

249

250 **Cell proliferation is necessary for tentacle regeneration**

251 Cnidrians are known to have a high regenerative capacity (*Galliot & Schmid 2002;*
252 *Holstein et al. 2003*), and the hydrozoan jellyfish *Cladonema* species exemplifies this
253 typical regenerative ability (*Weber 1981*). Given the localization of proliferative cells in
254 the *Cladonema* tentacle bulb, we decided to investigate the nature of tentacle
255 regeneration. After dissecting tentacles at their base, we monitored the process of
256 tentacle regeneration (Fig. 4A). During the first 24 hours, wound healing occurred at the
257 dissected area. Subsequently, the tip of tentacle became elongated and started
258 branching on day 2 (Fig. 4A). At day 4, fully branched tentacles were observed (Fig.
259 4A), suggesting that tentacle regeneration may follow normal tentacle morphogenesis
260 after elongation.

261 To examine the initial stage of tentacle regeneration, we examined the
262 distribution of proliferating cells using PH3 staining to visualize mitotic cells. While
263 dividing cells were frequently observed near the amputated area, mitotic cells were
264 dispersed in uncut control tentacle bulbs (Fig. 4B). We quantified the number of PH3-
265 positive cells present in the tentacle bulbs and found a significant increase in PH3-
266 positive cells in the tentacle bulbs of amputee medusae, compared to controls (Fig. 4C).
267 These observations indicate that initial regenerative responses accompany the active
268 increase of cell proliferation in tentacle bulbs. In order to test the role of cell proliferation
269 in tentacle regeneration, we blocked cell-cycle progression using hydroxyurea after
270 dissection and monitored the length of regenerating tentacles. While the tentacles
271 continued to elongate from the bulb structure after dissection in controls, tentacles in
272 animals treated with hydroxyurea were not able to elongate despite displaying normal
273 wound healing (Fig. 4D). These results demonstrate that cell proliferation in tentacle
274 bulbs is required for proper tentacle regeneration.

275

276 **Cell proliferation patterns across different hydrozoan jellyfish**

277 Hydrozoan jellyfish constitute the most broadly varied class of cnidarian jellyfish with
278 approximately 2,700 species worldwide featuring highly diverse morphological and
279 physiological characteristics (*Cartwright & Nawrocki 2010; Schuchert 2019*). For

instance, *Cytaeis uchidae* has four tentacles, and their polyps live exclusively on one type of shell: *Niotha livescens* (*Takeda et al. 2018a; Takeda et al. 2013*). Another species, *Rathkea octopunctata,* has eight grouped-tentacles, and their juvenile medusae asexually produce medusae that grow out of the manubrium (*Berrill 1952; Schuchert 2007*). To gain insight into the conserved and diversified nature of cell proliferation in hydrozoan jellyfish, we investigated the spatial pattern of cell proliferation in *Cytaeis* and *Rathkea* medusae. In *Cytaeis* medusa, EdU-positive cells were observed in manubrium, tentacle bulbs, and at the top of the umbrella (Fig. 5A and 5B). PH3-positive cells were also detected in the same regions, suggesting that proliferating cells in *Cytaeis* are distributed in a pattern similar to that observed in *Cladonema*. (Fig. 5C and 5D). By contrast, in *Rathkea octopunctata,* EdU-positive cells and PH3-positive cells were mostly restricted to the manubrium and tentacle bulbs (Fig. 5E-G). Of note, proliferating cells were frequently detected in the medusa buds that grew out of the manubrium (Fig. 5E and 5F), which may reflect asexual reproduction in *Rathkea* medusae. These results suggest that cell proliferation may occur in tentacle bulbs across hydrozoan medusae commonly, while cell proliferation patterns may vary in a species-specific manner with physiology.

Discussion

In this study, we show that the body size of *Cladonema* medusae is influenced by cell proliferation following uptake of nutrition. Without nutrition and under the blocking of cell-cycle progression, body-size increase is inhibited (Fig.2). Intriguingly**,** despite the significant differences between fed and starved animals and between hydroxyurea-treated and -untreated animals, the body size of *Cladonema* medusae increases during the first 24 hours regardless of condition (Fig 2). These results can be explained by cell growth via protein synthesis (*Schiaffino et al. 2013*) or accretionary growth, in which cells secrete extracellular matrix to increase extracellular regions, as has been suggested in the growth of cartilage and bone (*Karsenty et al. 2009; Wang et al. 2014*). Given the large amount of collagen that jellyfish contain (*Khong et al. 2016; Miura & Kimura 1985*), extracellular matrix may increase their size during the initial growth of juvenile medusae. Another interesting feature we observed is that the body size of the

starved medusae gradually decreases after 24 hours (Fig. 2B). Similarly, upon starvation, *Hydra* polyps cease asexual budding and decrease their size (*Buzgariu et al. 2008; Chera et al. 2009a*), suggesting that cnidarian animals are sensitive to nutrition availability and adapt to metabolic changes. At the organ and tissue level, such size reduction can occur via autophagy or cell death during starvation in diverse phyla (*Jeschke et al. 2000; O'Brien et al. 2011; Thongrod et al. 2018; Tracy & Baehrecke 2013*). Cnidarians thus may utilize similar mechanisms to reduce cell size and/or cell number to adjust their body size in response to environmental changes. Molecularly, TOR and Hippo signaling are conserved machinery that control organ size, and, as such, these molecules may also play an important role in cnidarian growth control (*Coste et al. 2016; Ikmi et al. 2014; Loewith & Hall 2011; van Dam et al. 2011*).

Hydrozoan animals are known to possess interstitial stem cell populations, called i-cells. In *Hydra* and *Hydractinia* polyps, i-cells are localized to the body column and have the potential to differentiate into several cell types including nematocytes, nerve cells, and gametes (*Gold & Jacobs 2013; Hemmrich et al. 2012; Hobmayer et al. 2012; Kunzel et al. 2010; Muller et al. 2004*). By contrast, the current understanding of the localization and roles of stem-like cells or i-cells in hydrozoan jellyfish are limited (*Leclere et al. 2012*). In *Cladonema* medusae, proliferative cells are distributed in tentacle bulbs (Fig. 1), which have been similarly observed in the tentacle bulbs of the *Clytia* medusa (*Denker et al. 2008*). Our pharmacological experiments confirmed that cell proliferation contributes to tentacle branching, nematogenesis, and tentacle regeneration in *Cladonema* (Fig. 3 and Fig. 4), suggesting that these proliferative cells may behave as progenitors or stem-like cells. We further found similar distribution of proliferative cells in tentacle bulbs of *Cytaeis uchidae* and *Rathkea octopunctata* (Fig. 5). Together, these results suggest that the distribution of proliferative cells in tentacle bulbs are widely conserved in hydrozoan jellyfish, while such cells might exist in other tissue to allow body-size increase and species-specific life styles.

Acknowledgements

We thank R. Deguchi (Miyagi Education Univ. Japan) for sharing jellyfish species and helpful discussion. We thank H. Takashima for technical assistance. We thank Kuranaga lab members for discussion.

References

Alexander BE, Liebrand K, Osinga R, van der Geest HG, Admiraal W, Cleutjens JP, Schutte B, Verheyen F, Ribes M, van Loon E, and de Goeij JM. 2014. Cell turnover and detritus production in marine sponges from tropical and temperate benthic ecosystems. *PLoS One* 9:e109486. 10.1371/journal.pone.0109486

Berrill NJ. 1952. Growth and Form in Gymnoblastic Hydroids .2. Sexual and Asexual Reproduction in Rathkea .3. Hydranth and Gonophore Development in Pennaria and Acaulis .4. Relative Growth in Eudendrium. *Journal of Morphology* 90:1-32. DOI 10.1002/jmor.1050900102

Bohnsack BL, and Hirschi KK. 2004. Nutrient regulation of cell cycle progression. *Annu Rev Nutr* 24:433-453. 10.1146/annurev.nutr.23.011702.073203

Bradshaw B, Thompson K, and Frank U. 2015. Distinct mechanisms underlie oral vs aboral regeneration in the cnidarian Hydractinia echinata. *Elife* 4:e05506. 10.7554/eLife.05506

Buzgariu W, Chera S, and Galliot B. 2008. Methods to investigate autophagy during starvation and regeneration in hydra. *Methods Enzymol* 451:409-437. 10.1016/S0076-6879(08)03226-6

Cartwright P, and Nawrocki AM. 2010. Character evolution in Hydrozoa (phylum Cnidaria). *Integr Comp Biol* 50:456-472. 10.1093/icb/icq089

Chera S, Buzgariu W, Ghila L, and Galliot B. 2009a. Autophagy in Hydra: a response to starvation and stress in early animal evolution. *Biochim Biophys Acta* 1793:1432-1443. 10.1016/j.bbamcr.2009.03.010

Chera S, Ghila L, Dobretz K, Wenger Y, Bauer C, Buzgariu W, Martinou JC, and Galliot B. 2009b. Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. *Dev Cell* 17:279-289. 10.1016/j.devcel.2009.07.014

Coste A, Jager M, Chambon JP, and Manuel M. 2016. Comparative study of Hippo pathway genes in cellular conveyor belts of a ctenophore and a cnidarian. *Evodevo* 7:4. 10.1186/s13227-016-0041-y

Denker E, Manuel M, Leclere L, Le Guyader H, and Rabet N. 2008. Ordered progression of nematogenesis from stem cells through differentiation stages in the tentacle bulb of Clytia hemisphaerica (Hydrozoa, Cnidaria). *Dev Biol* 315:99-113. 10.1016/j.ydbio.2007.12.023

Fritz AE, Ikmi A, Seidel C, Paulson A, and Gibson MC. 2013. Mechanisms of tentacle morphogenesis in the sea anemone Nematostella vectensis. *Development* 140:2212-2223. 10.1242/dev.088260

Fujiki A, Hou S, Nakamoto A, and Kumano G. 2019. Branching pattern and morphogenesis of medusa tentacles in the jellyfish Cladonema pacificum (Hydrozoa, Cnidaria). *Zoological Letters* 5. ARTN 12 10.1186/s40851-019-0124-4

Fujisawa T. 2003. Hydra regeneration and epitheliopeptides. *Dev Dyn* 226:182-189. 10.1002/dvdy.10221

Gahan JM, Bradshaw B, Flici H, and Frank U. 2016. The interstitial stem cells in Hydractinia and their role in regeneration. *Curr Opin Genet Dev* 40:65-73. 10.1016/j.gde.2016.06.006

Galliot B, and Chera S. 2010. The Hydra model: disclosing an apoptosis-driven generator of Wnt-based regeneration. *Trends Cell Biol* 20:514-523. 10.1016/j.tcb.2010.05.006

Galliot B, and Schmid V. 2002. Cnidarians as a model system for understanding evolution and regeneration. *Int J Dev Biol* 46:39-48.

Genikhovich G, and Technau U. 2017. On the evolution of bilaterality. *Development* 144:3392-3404. 10.1242/dev.141507

Gillies TE, and Cabernard C. 2011. Cell division orientation in animals. *Curr Biol* 21:R599-609. 10.1016/j.cub.2011.06.055

Gold DA, and Jacobs DK. 2013. Stem cell dynamics in Cnidaria: are there unifying principles? *Dev Genes Evol* 223:53-66. 10.1007/s00427-012-0429-1

Graziussi DF, Suga H, Schmid V, and Gehring WJ. 2012. The "eyes absent" (eya) gene in the eye-bearing hydrozoan jellyfish Cladonema radiatum: conservation of the retinal determination network. *J Exp Zool B Mol Dev Evol* 318:257-267. 10.1002/jez.b.22442

Hansson LJ. 1997. Effect of temperature on growth rate of Aurelia aurita (Cnidaria, Scyphozoa) from Gullmarsfjorden, Sweden. *Marine Ecology Progress Series* 161:145-153. DOI 10.3354/meps161145

Hardwick LJ, Ali FR, Azzarelli R, and Philpott A. 2015. Cell cycle regulation of proliferation versus differentiation in the central nervous system. *Cell Tissue Res* 359:187-200. 10.1007/s00441-014-1895-8

Hemmrich G, Khalturin K, Boehm AM, Puchert M, Anton-Erxleben F, Wittlieb J, Klostermeier UC, Rosenstiel P, Oberg HH, Domazet-Loso T, Sugimoto T, Niwa H, and Bosch TC. 2012. Molecular signatures of the three stem cell lineages in hydra and the emergence of stem cell function at the base of multicellularity. *Molecular Biology and Evolution* 29:3267-3280. 10.1093/molbev/mss134

Hobmayer B, Jenewein M, Eder D, Eder MK, Glasauer S, Gufler S, Hartl M, and Salvenmoser W. 2012. Stemness in Hydra - a current perspective. *Int J Dev Biol* 56:509-517. 10.1387/ijdb.113426bh

Holstein TW, Hobmayer E, and Technau U. 2003. Cnidarians: an evolutionarily conserved model system for regeneration? *Dev Dyn* 226:257-267. 10.1002/dvdy.10227

Ikmi A, Gaertner B, Seidel C, Srivastava M, Zeitlinger J, and Gibson MC. 2014. Molecular evolution of the Yap/Yorkie proto-oncogene and elucidation of its core transcriptional program. *Molecular Biology and Evolution* 31:1375-1390. 10.1093/molbev/msu071

Jeschke MG, Debroy MA, Wolf SE, Rajaraman S, and Thompson JC. 2000. Burn and starvation increase programmed cell death in small bowel epithelial cells. *Dig Dis Sci* 45:415-420.

Kahn AS, and Leys SP. 2016. The role of cell replacement in benthic-pelagic coupling by suspension feeders. *R Soc Open Sci* 3:160484. 10.1098/rsos.160484

Karsenty G, Kronenberg HM, and Settembre C. 2009. Genetic control of bone formation. *Annu Rev Cell Dev Biol* 25:629-648. 10.1146/annurev.cellbio.042308.113308

Kass-Simon G, and Scappaticci AA. 2002. The behavioral and developmental physiology of nematocysts. *Canadian Journal of Zoology* 80:1772-1794. 10.1139/Z02-135

Khong NM, Yusoff FM, Jamilah B, Basri M, Maznah I, Chan KW, and Nishikawa J. 2016. Nutritional composition and total collagen content of three commercially important edible jellyfish. *Food Chem* 196:953-960. 10.1016/j.foodchem.2015.09.094

King RS, and Newmark PA. 2012. The cell biology of regeneration. *J Cell Biol* 196:553-562. 10.1083/jcb.201105099

Koc A, Wheeler LJ, Mathews CK, and Merrill GF. 2004. Hydroxyurea arrests DNA replication by a mechanism that preserves basal dNTP pools. *J Biol Chem* 279:223-230. 10.1074/jbc.M303952200

Kunzel T, Heiermann R, Frank U, Muller W, Tilmann W, Bause M, Nonn A, Helling M, Schwarz RS, and Plickert G. 2010. Migration and differentiation potential of stem cells in the cnidarian Hydractinia analysed in eGFP-transgenic animals and chimeras. *Dev Biol* 348:120-129. 10.1016/j.ydbio.2010.08.017

Leclere L, Jager M, Barreau C, Chang P, Le Guyader H, Manuel M, and Houliston E. 2012. Maternally localized germ plasm mRNAs and germ cell/stem cell formation in the cnidarian Clytia. *Dev Biol* 364:236-248. 10.1016/j.ydbio.2012.01.018

Leevers SJ, and McNeill H. 2005. Controlling the size of organs and organisms. *Curr Opin Cell Biol* 17:604-609. 10.1016/j.ceb.2005.09.008

Loewith R, and Hall MN. 2011. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* 189:1177-1201. 10.1534/genetics.111.133363

Miura S, and Kimura S. 1985. Jellyfish mesogloea collagen. Characterization of molecules as alpha 1 alpha 2 alpha 3 heterotrimers. *J Biol Chem* 260:15352-15356.

Miyake H, Iwao K, and Kakinuma Y. 1997. Life history and environment of Aurelia aurita. *South Pacific Study* 17:273-285.

Momose T, Derelle R, and Houliston E. 2008. A maternally localised Wnt ligand required for axial patterning in the cnidarian Clytia hemisphaerica. *Development* 135:2105-2113. 10.1242/dev.021543

Muller WA, Teo R, and Frank U. 2004. Totipotent migratory stem cells in a hydroid. *Dev Biol* 275:215-224. 10.1016/j.ydbio.2004.08.006

O'Brien LE, Soliman SS, Li X, and Bilder D. 2011. Altered modes of stem cell division drive adaptive intestinal growth. *Cell* 147:603-614. 10.1016/j.cell.2011.08.048

Pellettieri J, and Sanchez Alvarado A. 2007. Cell turnover and adult tissue homeostasis: from humans to planarians. *Annu Rev Genet* 41:83-105. 10.1146/annurev.genet.41.110306.130244

Penzo-Mendez AI, and Stanger BZ. 2015. Organ-Size Regulation in Mammals. *Cold Spring Harb Perspect Biol* 7:a019240. 10.1101/cshperspect.a019240

Quiroga Artigas G, Lapebie P, Leclere L, Takeda N, Deguchi R, Jekely G, Momose T, and Houliston E. 2018. A gonad-expressed opsin mediates light-induced spawning in the jellyfish Clytia. *Elife* 7. 10.7554/eLife.29555

Ragkousi K, Marr K, McKinney S, Ellington L, and Gibson MC. 2017. Cell-Cycle-Coupled Oscillations in Apical Polarity and Intercellular Contact Maintain Order in Embryonic Epithelia. *Curr Biol* 27:1381-1386. 10.1016/j.cub.2017.03.064

Salic A, and Mitchison TJ. 2008. A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc Natl Acad Sci U S A* 105:2415-2420. 10.1073/pnas.0712168105

Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, and Sandri M. 2013. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS J* 280:4294-4314. 10.1111/febs.12253

Schmid V, and Alder H. 1984. Isolated, mononucleated, striated muscle can undergo pluripotent transdifferentiation and form a complex regenerate. *Cell* 38:801-809.

Schmid V, Alder H, Plickert G, and Weber C. 1988. Transdifferentiation from striated muscle of medusae in vitro. *Cell Differ Dev* 25 Suppl:137-146.

Schmid V, Wydler M, and Alder H. 1982. Transdifferentiation and regeneration in vitro. *Dev Biol* 92:476-488.

Schuchert P. 2007. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 2. *Revue Suisse De Zoologie* 114:195-396. DOI 10.5962/bhl.part.80395

Schuchert P. 2019. World Hydrozoa Database. . Accessed at http://wwwmarinespeciesorg/hydrozoa.

Suga H, Tschopp P, Graziussi DF, Stierwald M, Schmid V, and Gehring WJ. 2010. Flexibly deployed Pax genes in eye development at the early evolution of animals demonstrated by studies on a hydrozoan jellyfish. *Proc Natl Acad Sci U S A* 107:14263-14268. 10.1073/pnas.1008389107

Szczepanek S, Cikala M, and David CN. 2002. Poly-gamma-glutamate synthesis during formation of nematocyst capsules in Hydra. *J Cell Sci* 115:745-751.

Takeda N, Deguchi R, and Itabashi T. 2018a. Reproductive Strategies in Marine Hydrozoan Jellyfish: Sexual Medusae and Asexual Polyps. *Reproductive and Developmental Strategies Diversity and Commonality in Animals (Springer, Tokyo)*:157-174.

Takeda N, Kon Y, Quiroga Artigas G, Lapebie P, Barreau C, Koizumi O, Kishimoto T, Tachibana K, Houliston E, and Deguchi R. 2018b. Identification of jellyfish neuropeptides that act directly as oocyte maturation-inducing hormones. *Development* 145. 10.1242/dev.156786

Takeda N, Nakajima Y, Koizumi O, Fujisawa T, Takahashi T, Matsumoto M, and Deguchi R. 2013. Neuropeptides trigger oocyte maturation and subsequent spawning in the hydrozoan jellyfish Cytaeis uchidae. *Mol Reprod Dev* 80:223-232. 10.1002/mrd.22154

Technau U, and Steele RE. 2011. Evolutionary crossroads in developmental biology: Cnidaria. *Development* 138:1447-1458. 10.1242/dev.048959

Thongrod S, Wanichanon C, Kankuan W, Siangcham T, Phadngam S, Morani F, Isidoro C, and Sobhon P. 2018. Autophagy-Associated Shrinkage of the Hepatopancreas in Fasting Male Macrobrachium rosenbergii Is Rescued by Neuropeptide F. *Front Physiol* 9:613. 10.3389/fphys.2018.00613

Tracy K, and Baehrecke EH. 2013. The role of autophagy in Drosophila metamorphosis. *Curr Top Dev Biol* 103:101-125. 10.1016/B978-0-12-385979-2.00004-6

van Dam TJ, Zwartkruis FJ, Bos JL, and Snel B. 2011. Evolution of the TOR pathway. *J Mol Evol* 73:209-220. 10.1007/s00239-011-9469-9

Wang W, Rigueur D, and Lyons KM. 2014. TGFbeta signaling in cartilage development and maintenance. *Birth Defects Res C Embryo Today* 102:37-51. 10.1002/bdrc.21058

Weber C. 1981. Structure, histochemistry, ontogenetic development, and regeneration of the ocellus of Cladonema radiatum dujardin (cnidaria, hydrozoa, anthomedusae). *J Morphol* 167:313-331. 10.1002/jmor.1051670306

Figure legends

Figure 1. Cell proliferation patterns in *Cladonema* medusa

(A) Adult medusa of *Cladonema pacificum*. (B) Distribution of S-phase cells in the *Cladonema pacificum* medusa (1 day old) revealed by EdU staining (20μM, 24hr incubation). (C) Uniform distribution of S-phase cells (EdU+) in a medusa umbrella (1 day old). (D) Local distribution of S-phase cells (EdU+) in medusa tentacle bulbs (1 day old). (E) Mitotic cells detected by anti-PH3 in a medusa umbrella (8 day old). (F) Mitotic cells (PH3+) in medusa tentacle bulbs (1 day old). Arrows indicate EdU-positive (C, D) and PH3-positive (E, F) cells, respectively. Scale bar: (A) 1mm, (B-D) 100μm, (E, F) 50μm.

Figure 2. Cell proliferation is necessary for body-size growth

(A) *Cladonema pacificum* newborn medusa (0 day old) and juvenile medusa (8 day old). (B) Distribution of S-phase cells in control medusa and starved medusa with EdU staining (150μM, 1hr). (C) Quantification of umbrella size in control and starved medusae. Control medusae were fed every other day. Error bar: SD, ***$p < 0.0005$. (D) Distribution of S-phase cells in medusa of control (HU-) and hydroxyurea (HU) treatment detected by EdU staining (20μM, 24hr). No S-phase cells were detected in HU+ medusa. (E) Quantification of body size in control and in HU conditions. HU suppresses body-size growth. HU-: control medusae incubated in ASW, HU+: medusae incubated in HU 10mM ASW. Both HU+ and HU- were fed every other day. Error bar: SD, ***$p < 0.0005$. Scale bar: (A) 1mm, (B and D) 100μm.

Figure 3. Cell proliferation is necessary for tentacle morphogenesis

(A) Control (HU-) medusa incubated in ASW for 9 days. The picture shows the representative image of medusae with three branched tentacles. (B) The medusa incubated in10mM HU (HU+) ASW for 9 days. The picture shows the representative image of medusae with one branched tentacle. (C) Quantification of branching numbers per tentacle at Day 0 and Day 9. HU+: *n*=313, HU- condition: *n*=199. Error bars: SD, ***$p < 0.001$. (D) Nematocytes in tentacles labeled by DAPI (poly-γ-gultamate) in the 8 day old medusa incubated in ASW (HU-) or 10mM HU ASW (HU+). Arrows indicate small nematocysts, and arrowheads indicate empty nematocysts. (D) The rate of small nematocysts (size: 2-40 μm^2) in HU- and HU+ medusa. HU+: *n*=19, HU-: *n*=18. (E) The rate of empty nematocysts in HU- and HU+ medusa. HU+: *n*=19, HU-: *n*=18.

Figure 4. Cell proliferation is necessary for tentacle regeneration

(A) Tentacle regenerative processes after amputation in an adult medusa. Series of pictures show the growing tentacle over 4 days. (B) Mitotic cells (PH3+) in tentacle bulbs of the unremoved control and the dissected medusa. Arrowheads indicate PH3-positive cells. (C) Quantification of proliferative cells in tentacle bulbs for control and after amputation. Control: *n*=26, Amputation: *n*=11. error bar: SD, ***$p < 0.0005$. (D) Quantification of tentacle length after amputation in control (HU-) and 10mM HU treatment (HU+). Scale bar: (A) 1mm, (B) 100μm.

Figure 5. Cell proliferation patterns across different hydrozoan jellyfish

(A) Distribution of S-phase cells in the *Cytaeis uchidae* medusa (30 day old) revealed by EdU staining (EdU: 20μM, 24hr). (B) Distribution of S-phase cells (EdU+) in *Cytaeis* medusa (11 day old). (C) Mitotic cells (PH3+) in the umbrella of *Cytaeis* medusa (30 day old). (D) Mitotic cells in *Cytaeis* medusa tentacle bulbs (30 day old). (E) Distribution of S-phase cells (EdU+) in the *Rathkea octpunctata* juvenile medusa (EdU: 20μM, 24hr). (F) Mitotic cells (PH3+) in a manubrium of *Rathkea* juvenile medusa. (G) Mitotic cells (PH3+) in *Rathkea* juvenile medusa tentacles. Arrows indicate PH3-positive mitotic cells. Scale bars: 100μm.

Figure 1(on next page)

Cell proliferation patterns in *Cladonema* medusa

(A) Adult medusa of *Cladonema pacificum*. (B) Distribution of S-phase cells in the *Cladonema pacificum* medusa (1 day old) revealed by EdU staining (20μM, 24hr incubation). (C) Uniform distribution of S-phase cells (EdU+) in a medusa umbrella (1 day old). (D) Local distribution of S-phase cells (EdU+) in medusa tentacle bulbs (1 day old). (E) Mitotic cells detected by anti-PH3 in a medusa umbrella (8 day old). (F) Mitotic cells (PH3+) in medusa tentacle bulbs (1 day old). Arrows indicate EdU-positive (C, D) and PH3-positive (E, F) cells, respectively. Scale bar: (A) 1mm, (B-D)100μm, (E, F) 50μm.

Figure 1

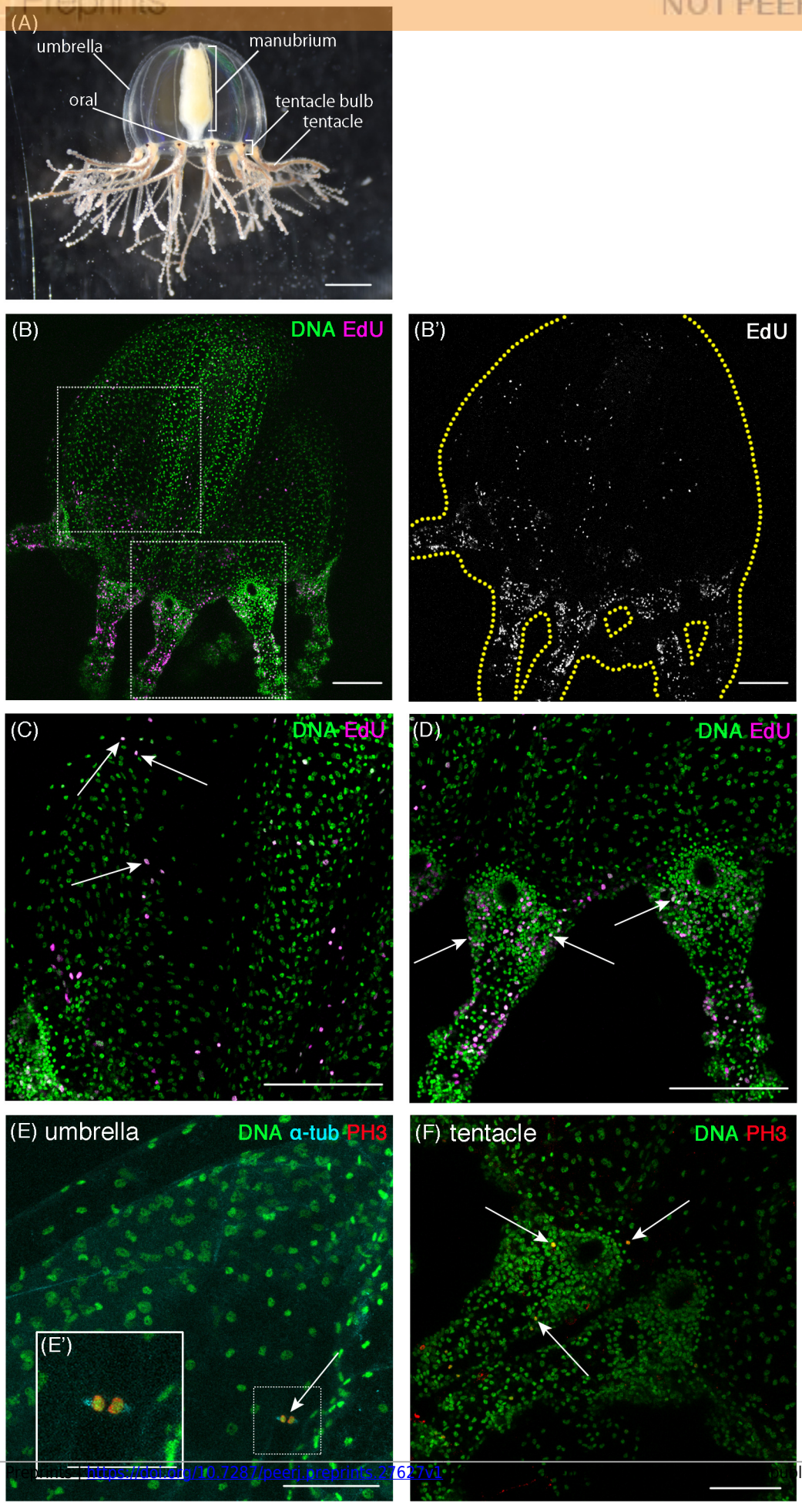
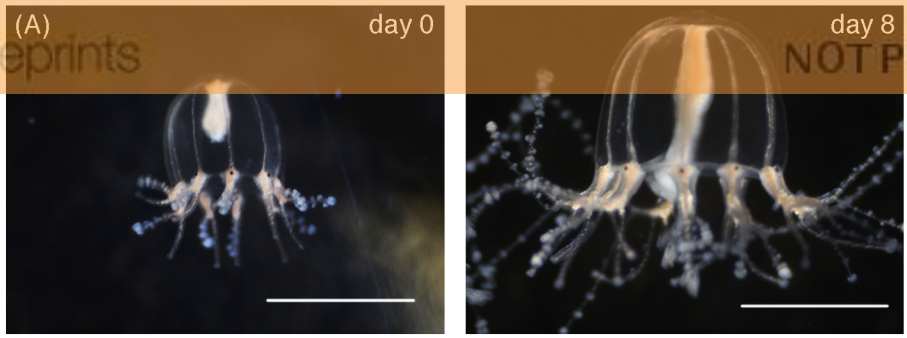


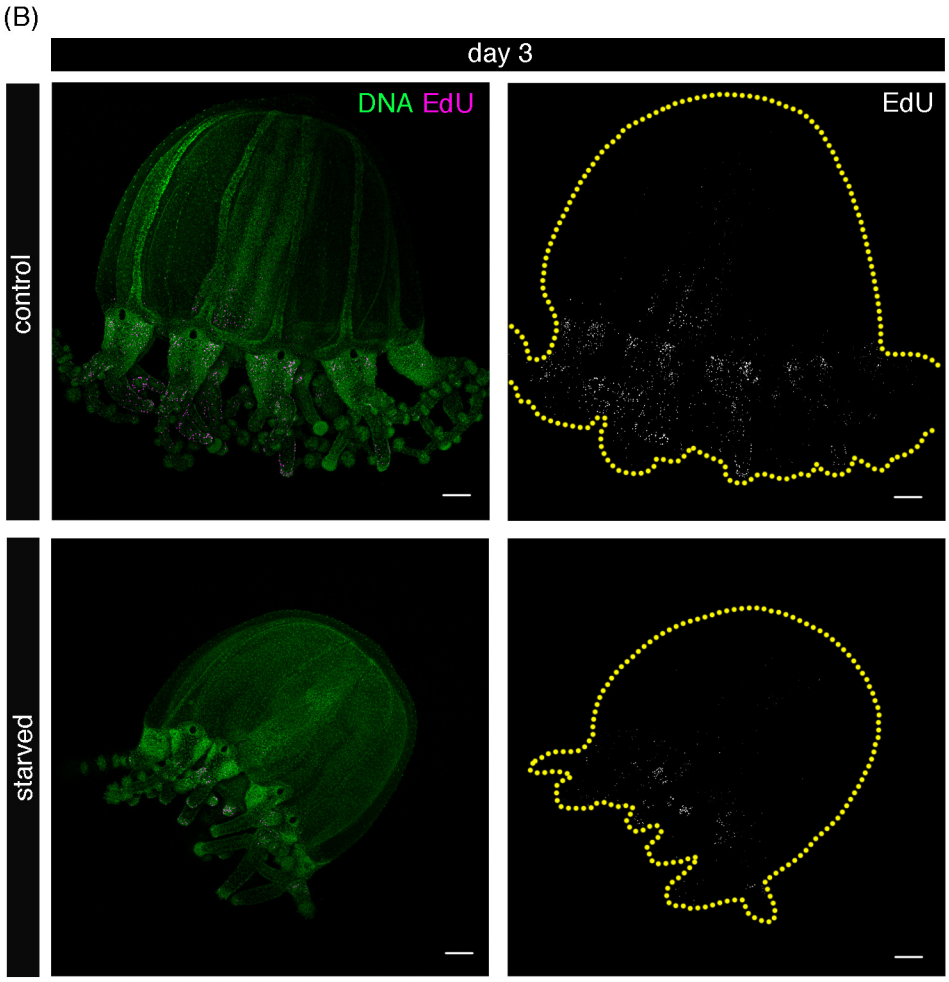
Figure 2(on next page)

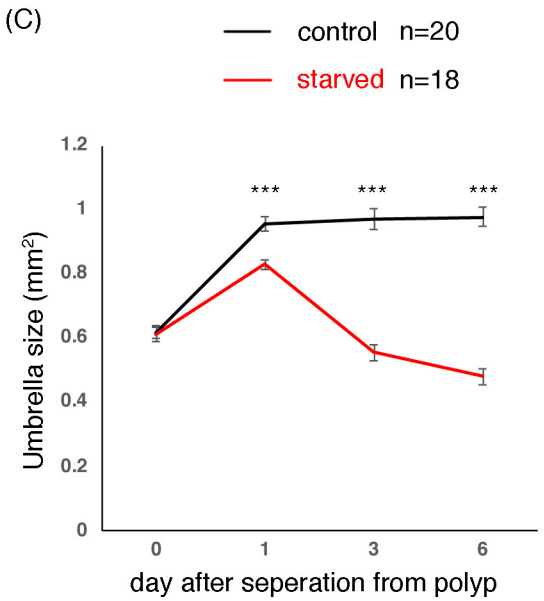
Cell proliferation is necessary for body-size growth

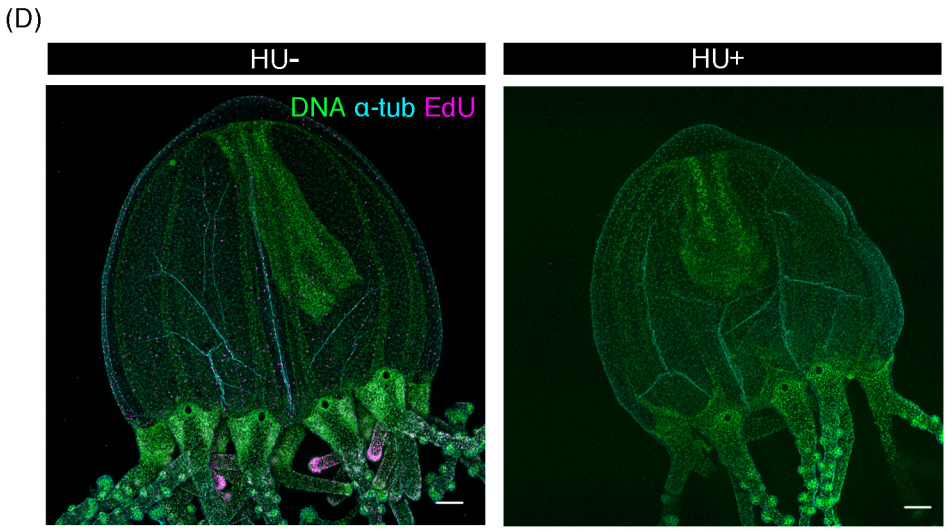
(A) *Cladonema pacificum* newborn medusa (0 day old) and juvenile medusa (8 day old). (B) Distribution of S-phase cells in control medusa and starved medusa with EdU staining (150μM, 1hr). (C) Quantification of umbrella size in control and starved medusae. Control medusae were fed every other day. Error bar: SD, ***$p < 0.0005$. (D) Distribution of S-phase cells in medusa of control (HU-) and hydroxyurea (HU) treatment detected by EdU staining (20μM, 24hr). No S-phase cells were detected in HU+ medusa. (E) Quantification of body size in control and in HU conditions. HU suppresses body-size growth. HU-: control medusae incubated in ASW, HU+: medusae incubated in HU 10mM ASW. Both HU+ and HU- were fed every other day. Error bar: SD, ***$p < 0.0005$. Scale bar: (A) 1mm, (B and D) 100μm.

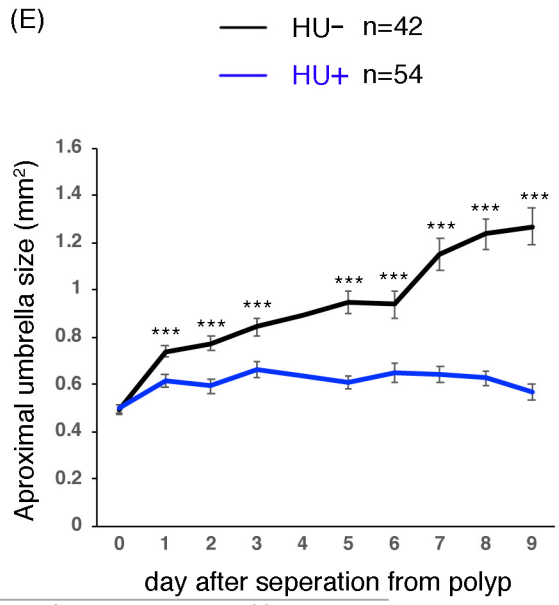
Figure 2











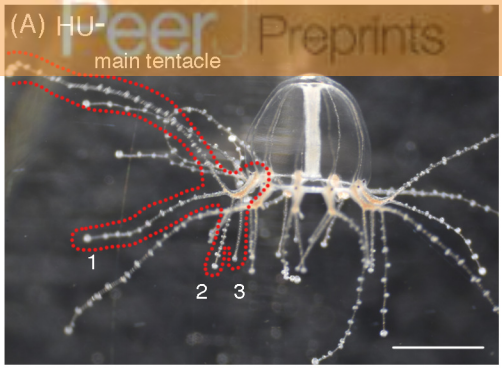
PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27627v1 | CC BY 4.0 Open Access | rec: 2 Apr 2019, publ: 2 Apr 2019

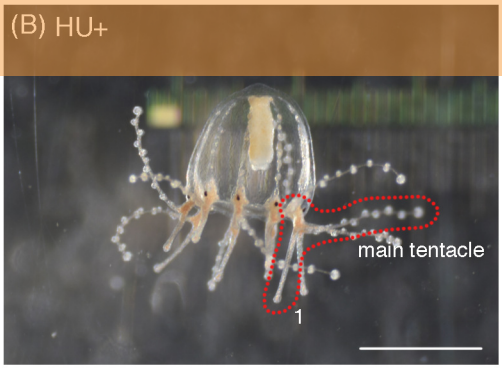
Figure 3(on next page)

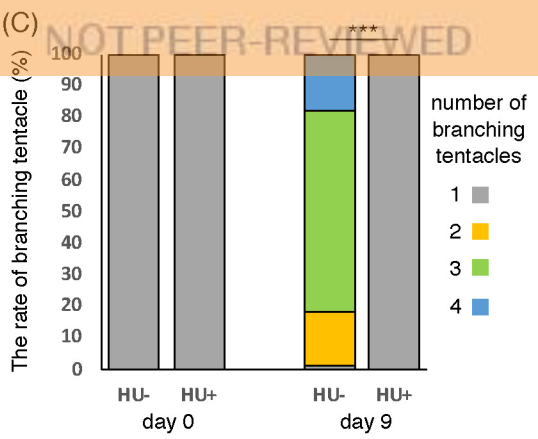
Cell proliferation is necessary for tentacle morphogenesis

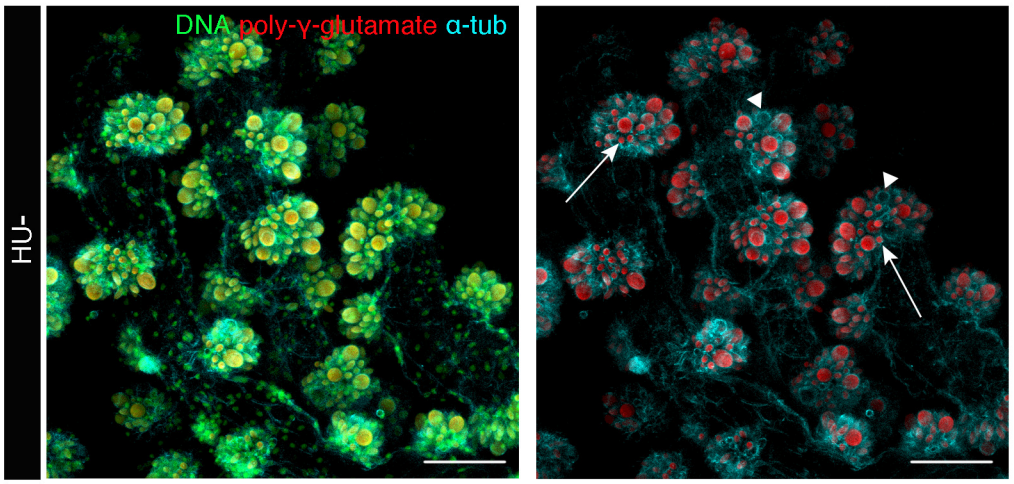
(A) Control (HU-) medusa incubated in ASW for 9 days. The picture shows the representative image of medusae with three branched tentacles. (B) The medusa incubated in10mM HU (HU+) ASW for 9 days. The picture shows the representative image of medusae with one branched tentacle. (C) Quantification of branching numbers per tentacle at Day 0 and Day 9. HU+: n=313, HU- condition: n=199. Error bars: SD, ***$p < 0.001$. (D) Nematocytes in tentacles labeled by DAPI (poly-γ-gultamate) in the 8 day old medusa incubated in ASW (HU-) or 10mM HU ASW (HU+). Arrows indicate small nematocysts, and arrowheads indicate empty nematocysts. (D) The rate of small nematocysts (size: 2-40 μm^2) in HU- and HU+ medusa. HU+: n=19, HU-: n=18. (E) The rate of empty nematocysts in HU- and HU+ medusa. HU+: n=19, HU-: n=18.

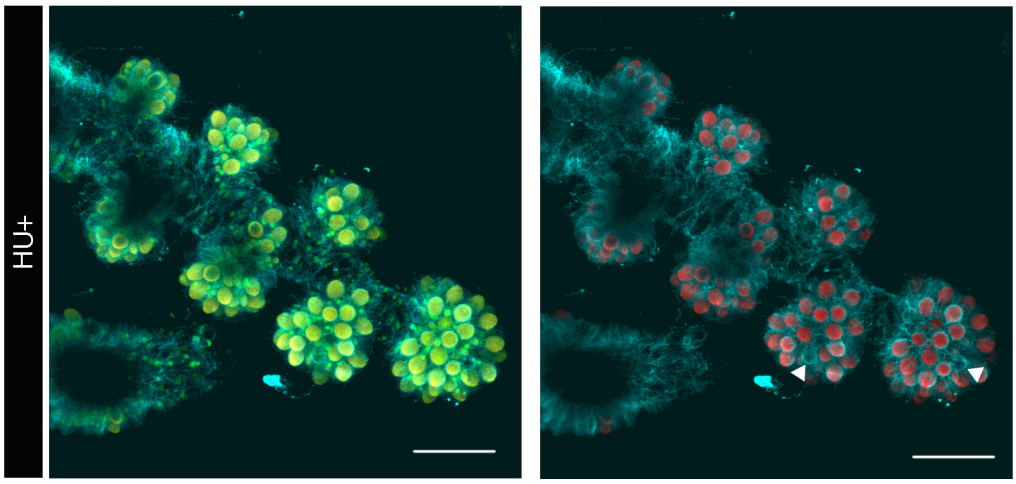
Figure 3

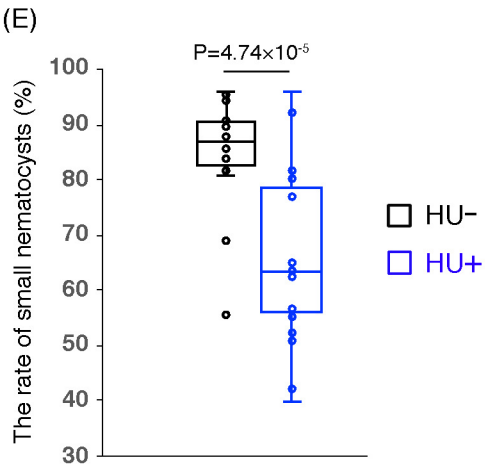












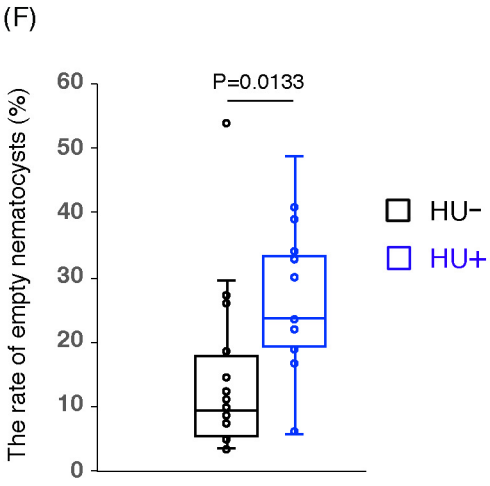


Figure 4(on next page)

Cell proliferation is necessary for tentacle regeneration

(A) Tentacle regenerative processes after amputation in an adult medusa. Series of pictures show the growing tentacle over 4 days. (B) Mitotic cells (PH3+) in tentacle bulbs of the unremoved control and the dissected medusa. Arrowheads indicate PH3-positive cells. (C) Quantification of proliferative cells in tentacle bulbs for control and after amputation. Control: n=26, Amputation: n=11. error bar: SD, ***$p < 0.0005$. (D) Quantification of tentacle length after amputation in control (HU-) and 10mM HU treatment (HU+). Scale bar: (A) 1mm, (B) 100µm.

Figure 4

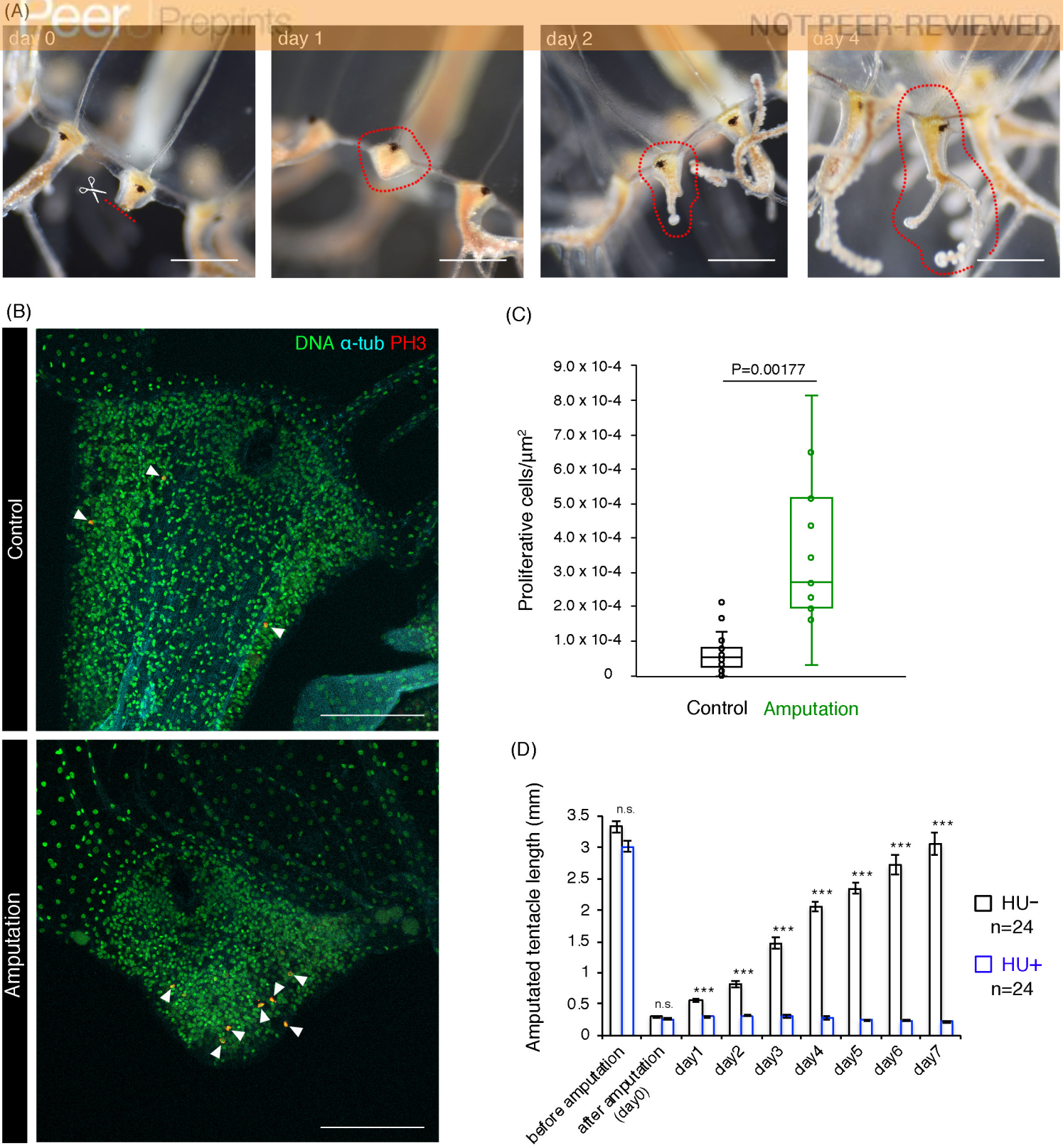


Figure 5(on next page)

Cell proliferation patterns across different hydrozoan jellyfish

(A) Distribution of S-phase cells in the *Cytaeis uchidae* medusa (30 day old) revealed by EdU staining (EdU: 20μM, 24hr). (B) Distribution of S-phase cells (EdU+) in *Cytaeis* medusa (11 day old). (C) Mitotic cells (PH3+) in the umbrella of *Cytaeis* medusa (30 day old). (D) Mitotic cells in *Cytaeis* medusa tentacle bulbs (30 day old). (E) Distribution of S-phase cells (EdU+) in the *Rathkea octpunctata* juvenile medusa (EdU: 20μM, 24hr). (F) Mitotic cells (PH3+) in a manubrium of *Rathkea* juvenile medusa. (G) Mitotic cells (PH3+) in *Rathkea* juvenile medusa tentacles. Arrows indicate PH3-positive mitotic cells. Scale bars: 100μm.

Figure 5