Dinoflagellate symbionts escape vomocytosis by host cell immune suppression

Marie R. Jacobovitz, Sebastian Rupp, Philipp A. Voss, Ira Maegele and Annika Guse

Alveolata comprises diverse taxa of single-celled eukaryotes, many of which are renowned for their ability to live inside animal cells. Notable examples are apicomplexan parasites and dinoflagellate symbionts, the latter of which power coral reef ecosystems. Although functionally distinct, they evolved from a common, free-living ancestor and must evade their host’s immune response for persistence. Both the initial cellular events that gave rise to this intracellular lifestyle and the role of host immune modulation in coral–dinoflagellate endosymbiosis are poorly understood. Here, we use a comparative approach in the cnidarian endosymbiosis model Aiptasia, which re-establishes endosymbiosis with free-living dinoflagellates every generation. We find that uptake of microalgae is largely indiscriminate, but non-symbiotic microalgae are expelled by vomocytosis, while symbionts induce host cell innate immune suppression and form a lysosomal-associated membrane protein 1-positive niche. We demonstrate that exogenous immune stimulation results in symbiont expulsion and, conversely, inhibition of canonical Toll-like receptor signalling enhances infection of host animals. Our findings indicate that symbiosis establishment is dictated by local innate immune suppression, to circumvent expulsion and promote niche formation. This work provides insight into the evolution of the cellular immune response and key steps involved in mediating endosymbiotic interactions.
maturation process\textsuperscript{12,23–27}. Apoptosis has been suggested as an alternative, post-phagocytotic sorting mechanism for incompatible symbionts\textsuperscript{23,24}. However, neither escaping apoptosis nor digestion has been experimentally shown to play a role in the clearance of incompatible microalgae during symbiosis establishment. In fact, no cellular mechanism or molecular pathway of how hosts select symbionts and distinguish between different microbes has been confirmed experimentally and the role of innate immunity suppression in symbiosis establishment remains unknown.

This limited understanding is largely a consequence of the lack of an experimental system that allows investigation of these questions with cellular resolution. To overcome this, we established larvae of the sea anemone \textit{Exaiptasia diaphana} (previously \textit{E. pallida}; commonly, Aiptasia) as a tractable model\textsuperscript{29–31}. We exploit the ability of naturally aposymbiotic (symbiont-free) Aiptasia larvae to phagocytose symbionts from the environment with similar specificities as coral larvae in order to dissect symbiosis establishment under controlled conditions. Importantly, due to their small size and transparency, they are amenable to high-resolution microscopy and cell biological analyses\textsuperscript{32–35}. Here, we utilize Aiptasia larvae as an experimental system to address the fundamental question of how suppression of host immunity is involved in symbiont selection. We describe a mechanism for the selective maintenance of intracellular symbionts involving cell-specific suppression of the host innate immune system to avoid expulsion from the host cell (the common fate of non-symbiotic microalgae), and not phagolysosomal digestion as commonly thought.

**Results**

**Phagocytosis of microalgae is indiscriminate, but intracellular maintenance is specific.** To gain insight into the mechanisms that support intracellular maintenance of symbionts and removal of non-symbiotic microorganisms, we developed a comparative system exploiting the ability of naturally aposymbiotic (symbiont-free) Aiptasia larvae to phagocytose symbionts and other particles from the environment\textsuperscript{36–38}. For comparison with the dinoflagellate symbiont \textit{Breviolum minutum} (previously \textit{Symbiodinium minutum}, strain SSB01)\textsuperscript{34,35}, we first chose \textit{Chromera velia} (previously, \textit{C. parkeae}), \textit{Nannochloropsis oculata} and \textit{Microchloropsis gaditana} (Fig. 1a) are phagocytosed by the endodermal cells of Aiptasia larvae (Fig. 1b and Extended Data Fig. 1). For further comparative analyses, we chose \textit{N. oculata} and \textit{M. gaditana}, neither of which had any previously reported connection to corals and Aiptasia\textsuperscript{39,40}. Moreover, we screened six distinct apicomplexan-unrelated microalgae (Fig. 1a) and found that \textit{Isochrysis} species, \textit{Chlorella} species, \textit{Dunaliella salina}, \textit{Chlamydomonas parkeae}, \textit{Nannochloropsis oculata} and \textit{Microchloropsis gaditana} (Fig. 1a) are phagocytosed by the endodermal cells of Aiptasia larvae (Fig. 1b and Extended Data Fig. 1). For further comparative analyses, we chose \textit{N. oculata} and \textit{M. gaditana}, neither of which had any previously reported connection to corals or anemones; however, both are known for high lipid contents\textsuperscript{41,42} and thus may be used as food by Aiptasia larvae. We confirmed that all four microalgae are fully internalized using confocal microscopy (Fig. 1c).

To compare the uptake efficiency and long-term host tolerance between symbiotic microalgae and non-symbiotic microalgae, we quantified the proportion of infected larvae and the number of intracellular microalgae over time. Naturally, infection efficiencies vary between batches, but we found that after 24 h the proportion of larvae infected with \textit{M. gaditana} (85.2%) was significantly higher compared with the proportion infected with \textit{N. oculata} (35.7%), while symbionts (\textit{B. minutum} and \textit{C. velia}) showed intermediate results (60.5 and 40.5%, respectively). The proportion of larvae containing symbionts remained relatively constant, even after removal of microalgae from the environment at 24 h post-infection (Fig. 1d). In contrast, the proportion of larvae infected with the other microalgae decreased over time. The most rapid reduction occurred with \textit{N. oculata}, followed by \textit{M. gaditana}, with a more gradual decrease with \textit{C. velia}. While the number of symbionts increased due to proliferation within the host (Fig. 1e)\textsuperscript{43}, the mean microalga cell count per larva for all of the other microalgae decreased (Fig. 1e). Taken together, our results indicate that an array of microalgae are taken up effectively by Aiptasia larvae, suggesting that decisive symbiont selection mechanisms occur after uptake, and that the host clearance response varies between the distinct microalgal types.

**After phagocytosis, non-symbiotic microalgae are cleared by expulsion and not digestion.** Microalgae, including symbionts, are intracellularly phagocytosed, an ancient process by which cells internalize large particles from the environment. Originally utilized by single-celled organisms such as amoeba to acquire food, phagocytosis has evolved to become an important part of immunity for killing invading microbes in macrophages of higher metazoa\textsuperscript{44,45}. Accordingly, we speculated that non-symbiotic microalgae are cleared by intracellular digestion, characterized by consecutive fusion of the nascent phagosome with endocytic vesicles to ultimately mature into the digestive phagolysosome. Interestingly, \textit{N. oculata}- and \textit{M. gaditana}-containing phagosomes appeared to be devoid of lysosomal-associated membrane protein 1 (LAMP1), a marker protein associated with late endosomes and lysosomes\textsuperscript{46}, and was found to be only weakly associated with \textit{C. velia}-containing phagosomes. In contrast, the symbiosome (the organelle in which the symbiont resides) was already heavily decorated with LAMP1 shortly after symbiont uptake (Fig. 2a and Supplementary Videos 1–3, validation of antibody in Extended Data Fig. 2). Thus, it appears that symbionts, and not the non-symbiotic invaders, rapidly establish a LAMP1-positive niche, allowing intracellular persistence. This then raises the question of how non-symbiotic microalgae, destined for removal (Fig. 1d), are cleared by the host cell after phagocytosis.

To monitor the elimination of non-symbiotic microalgae, we established live imaging to observe their fate over time. After 24 h of infection, larvae were embedded in low-gelling agarose (LGA) and imaged every 15 min for 48 h. We selected larvae that appeared to have intracellular microalgae, as indicated by continuous and
synchronous movement of both the microalga and the rotating larva (Supplementary Video 4); this was clearly distinct from the non-synchronous movement of microalgae located in the gastric cavity (asterisk in Supplementary Video 4). Nearly all intracellular symbionts were maintained inside Aiptasia larvae for the entire duration of the observation period (Fig. 2b,c, Supplementary Video 5 and Supplementary Table 1), with one exception where at one time point a pair of symbionts moved asynchronously in the gastric cavity of a larva (Supplementary Video 6). Furthermore, symbionts frequently replicated (11 replication events in 13 larvae), suggesting
that immobilization in LGA does not affect larval or symbiont physiology or symbiosis stability.

Interestingly, most larvae that had phagocytosed *M. gaditana* cells (four out of seven), *N. oculata* cells (seven out of eight) and *C. velia* cells (six out of eight) expelled the microalgae (42.1, 92.3 and 58.3% of total microalgae, respectively), while digestion was not observed (Fig. 2b,c, Supplementary Videos 7–9 and Supplementary Table 1). After expulsion, the microalgae appeared intact and healthy and re-acquisition of non-symbiotic microalgae by larvae occurred frequently (Supplementary Table 1). To test whether expulsion was a common response to inert particles in addition to living microalgae, we imaged larvae containing intracellular polystyrene beads of comparable size to the microalgae. We saw that beads were also expelled from (11 out of 11; 46.4% of total microalgae) and re-acquired by all larvae (Fig. 2b,c, Supplementary Table 1 and Supplementary Video 10). It is also worth noting that, in one event, a *C. velia* cell replicated during imaging (Supplementary Video 9).

Together, this indicates that expulsion, and not phagolysosomal digestion, is the prevalent response to the uptake of non-symbiotic microalgae, we imaged larvae containing intracellular polystyrene beads of comparable size to the microalgae. We saw that beads were also expelled from (11 out of 11; 46.4% of total microalgae) and re-acquired by all larvae (Fig. 2b,c, Supplementary Table 1 and Supplementary Video 10). It is also worth noting that, in one event, a *C. velia* cell replicated during imaging (Supplementary Video 9).

To test whether differential expulsion dynamics contribute to the differences in uptake efficiency and/or rates of loss between the distinct microalgae (Fig. 1d), we monitored symbionts, *M. gaditana*, *N. oculata* and *C. velia* by live imaging during the first 12 h after uptake (after infection for 1 h). We found that while symbionts and *C. velia* were expelled relatively rarely (12.5 and 27.6%, respectively), *M. gaditana* and *N. oculata* were expelled with high frequency (73.9 and 73.5%, respectively) (Fig. 2d). The average time until the first expulsion was significantly shorter for *M. gaditana* (5.6 h) and *N. oculata* (5.7 h) compared with *C. velia* (9.7 h) and symbionts (10.8 h); however, the expulsion dynamics were highly variable, suggesting that it is a stochastic process (Fig. 2e). Microalgae were frequently re-acquired and the average number of re-acquisition events per microalga was particularly high for *N. oculata* (9.7 h) compared with *C. velia* (5.6 h) and *M. gaditana* (5.7 h) (Fig. 2f). This suggests that differences in infection between distinct microalgae (Fig. 1d), we monitored symbionts, *M. gaditana*, *N. oculata* and *C. velia* by live imaging during the first 12 h after uptake (after infection for 1 h). We found that while symbionts and *C. velia* were expelled relatively rarely (12.5 and 27.6%, respectively), *M. gaditana* and *N. oculata* were expelled with high frequency (73.9 and 73.5%, respectively) (Fig. 2d).

**Expulsion of incompatible microalgae is actin independent and is regulated by extracellular signal-regulated kinase 5 (ERK5).** Interestingly, expulsion of microalgae by Aiptasia larvae resembles the non-lytic expulsion of a living organism from phagocytic cells, known as vomocytosis; both the phagocyte and the expelled microbe remain undamaged during this process (reviewed in ref. 46). Because the host phagocyte remains intact during this process, immune stimulation may be prevented, facilitating the dissemination of pathogens within the infected organism. To date, vomocytosis has been observed in an array of animal macrophages and amoeba in response to fungal pathogens (reviewed in ref. 48). The dynamics and mechanism of vomocytosis are distinct from constitutive exocytosis, the default expulsion mechanism to release phagosomal content in amoeba. While constitutive exocytosis occurs consistently ~80 min after phagocytosis and depends on actin and the actin-regulating protein WASH (Wiskott–Aldrich syndrome protein and SCAR homologue), vomocytosis is stochastic, occurring between 2 and 12 h after phagocytosis, and is actin independent (46–48).

Indeed, expulsion of microalgae by Aiptasia larvae is a highly stochastic process (Fig. 2e). Moreover, we found that expulsion of *M. gaditana* was unaffected by inhibition of actin polymerization via latrunculin B (LatB), suggesting that vomocytosis, rather than constitutive exocytosis, is at play (Fig. 3a and Extended Data Fig. 3).

In vertebrate macrophages, vomocytosis is negatively regulated by ERK5 (also known as mitogen-activated protein kinase 7 (MAPK7)), as well as the upstream mitogen-activated protein kinase 5 (MAP2K5). ERK5 inhibition by XMD17-109 significantly increased rates of vomocytosis (46). Aiptasia contains several MAPKs and one ERK5 homologue, with a conserved ATP-binding site (amino acids 61–69 in *Homo sapiens* ERK5—the target of XMD17-109), as well as a clear MAP2K5 homologue (Extended Data Fig. 4). To examine whether the function of ERK5 as a negative regulator of vomocytosis is conserved in Aiptasia, we incubated Aiptasia larvae with the ERK5 inhibitor shortly before as well as during a 24-h infection period. We found that the number of larvae infected with symbionts was massively reduced upon ERK5 inhibition (Fig. 3b), due to increased symbiont expulsion (Fig. 3c). Moreover, upon inhibition of ERK5, LAMP1 accumulation did not occur, resembling non-symbiotic microalgae-containing phagosomes (Fig. 3d,e). This further supports that Aiptasia larvae use vomocytosis for the removal of incompatible microalgae—a process that symbionts escape to initiate the stable partnership between symbiont and host cell and to promote niche formation.

**Modulation of host immunity prevents symbiont vomocytosis.** How do symbionts circumvent vomocytosis? Animals have evolved an array of defence mechanisms to protect themselves from invaders, and modulation of host immunity occurs in both parasitic and mutualistic symbioses, including those involving apicomplexan parasites and dinoflagellate symbionts (reviewed in refs. 12,46–48). To specifically ask whether host immune modulation is involved in

---

**Fig. 2 | Non-symbiotic microalgae are removed by expulsion.** a Only the symbiont was maintained in a LAMP1-positive symbiosome, as early as 6 h post-infection (h.p.i.), while the other microalgae showed no (or only slight) LAMP1 staining. Magenta, cyan and white indicate LAMP1, nuclei and microalgae, respectively (n = 4, with 30 larvae each time). Scale bar, 10 μm. b During live imaging of larvae, symbionts were rarely expelled and replicated within the host endoderm. *M. gaditana*, *N. oculata*, *C. velia*, *velia* and beads were all expelled at some point during 48 h of imaging. For the symbiont, 13 larvae were imaged with a total of 27 algae (7 larvae and 19 microalgae for *M. gaditana*; 7 larvae and 13 microalgae for *N. oculata*; 8 larvae and 12 microalgae for *C. velia*; 11 larvae and 28 beads for beads). Spontaneous expulsion was significantly higher in *N. oculata*-containing larvae (P = 0.00077) than in symbiont-containing larvae. Eggs bars represent means ± 95% confidence intervals. c Exemplary images during live imaging. The stills show DIC and red autofluorescence of microalgae photosynthetic pigments. Scale bar, 30 μm. Insects: threefold magnification of the areas indicated by a white box in the main images. For the corresponding videos, see Supplementary Videos 6–10. d Live imaging 12 h after 1-h infection revealed low levels of symbiont expulsion but significantly higher levels of expulsion of *M. gaditana* (P = 0.0000058) and *N. oculata* (P = 0.000021) when compared to the symbiont, and also when compared with *C. velia* (P = 0.0014 for *M. gaditana* and P = 0.0035 for *N. oculata*). Microalgae that were not expelled during the time of observation were set to 12 for the quantification. Error bars represent means ± 95% confidence intervals. e The time to the first expulsion was highly stochastic during the 12-h observation period, with significant differences between the symbiont and *M. gaditana* (P = 0.0025) and *N. oculata* (P = 0.0052), as well as between *M. gaditana* and *C. velia* (P = 0.036). f *M. gaditana* in particular (P = 0.00010), but also *N. oculata* (P = 0.0049), was frequently re-acquired during the observed period compared with the symbiont. In d–f, n = 4 with around ten larvae each. The statistics are based on a two-sided generalized linear mixed model of the data with pairwise P values adjusted using the Tukey method. *P* < 0.05, **P < 0.01, ***P < 0.001.

---
escaping vomocytosis, we first asked how intracellular symbionts or non-symbiotic microalgae affect host immunity at the cellular level by comparing the expression levels of known immune-related genes (based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations for Aiptasia) in cells containing either symbionts or M. gaditana, which are lost rapidly, yet remain intracellular long enough for analysis (Fig. 1d). We infected Aiptasia larvae with symbionts or M. gaditana for 24–48 h, dissociated infected larvae and sequenced groups of 8–12 endodermal cells. Specifically, we compared symbiont-containing cells with cells containing...
**M. gaditana**, as well as aposymbiotic neighbouring cells from symbiont- or *M. gaditana*-containing endoderms and aposymbiotic cells from naive endoderms (Fig. 4a and Extended Data Fig. 5).

We found that the expression of an array of immune genes was significantly lower in symbiont-containing cells compared with all other samples (Fig. 4a). This involved ten KEGG pathways related to metazoan innate immunity, with some genes present in multiple pathways and multiple transcripts annotated as the same gene (for example, *TRAF3*) (Fig. 4a). This suggests that suppression of host innate immunity upon intracellular establishment of dinoflagellate symbionts may occur at a much broader scale at the cellular level than was previously reported for whole organisms\(^\text{12}\). Specifically, the expression

---

**Fig. 3 | Symbiosis establishment relies on vomocytosis inhibition.**

- **a.** Inhibition of actin polymerization with LatB did not affect the expulsion of *M. gaditana* from infected larvae during 12 h of live imaging, as would be expected from constitutive exocytosis (*n* = 3).
- **b, c.** ERK5 inhibition with XMD17-109 significantly (*P* = 0.000013) reduced the fraction of symbiotic larvae when pre-treated for 1 h and treated during a 24-h infection (*n* = 6) (**b**), and increased (*P* = 0.013) the percentage of symbionts expelled during 12 h of live imaging (*n* = 5) (**c**). **d.** Larvae with normal ERK5 activity were able to form a LAMP1-positive symbiosome, while larvae treated with the ERK5 inhibitor XMD17-109 showed massively reduced accumulation of LAMP1 when pre-treated for 1 h and treated during a 5-h infection. Magenta, cyan and white indicate LAMP1, nuclei and symbiont, respectively. Scale bar, 10 µm. **e.** ERK5 inhibition significantly (*P* = 0.0000027) reduced the fraction of symbionts with LAMP1 accumulation (*n* = 3). Bars above graphs represent duration of infection (black dots), treatment (grey fill) and live imaging (where applicable). In all graphs, error bars represent means ± 95% confidence intervals. The statistics are based on a two-sided generalized linear mixed model accounting for repeated measurements. *P* < 0.05, **P < 0.01, ***P < 0.001.
Fig. 4 | Local suppression of host innate immunity is a prerequisite for symbiosis establishment. **a**, Several genes in immunity-related pathways were differentially downregulated in symbiont-containing cells but not in *M. gaditana*-containing cells or aposymbiotic (Apo) cells from infected larvae (the colours indicate the centred log[fold change] according to DESeq2; with downregulation in red and upregulation in blue). The heatmap shows all differentially regulated genes for symbiont-containing versus aposymbiotic cells, symbiont-containing versus aposymbiotic cells from symbiotic larvae (*M. gaditana*-containing cells) and symbiont-containing versus *M. gaditana*-containing cells within the following KEGG pathways: C-type lectin receptor signalling (ko04620), toll-like receptor (TLR) (ko04622), retinoic acid-inducible gene I (RIG-I)-like receptor (ko04621), Janus-kinase (JAK)-signal transducer and activator of transcription protein (STAT) (ko04064), MAPK (ko04010), nF-κB (ko04064), nucleotide-binding oligomerization domain (NOD)-like receptor (ko04621), complement and coagulation cascades (ko04610), and tumour necrosis factor (TNF; ko04668). Significantly differentially expressed genes compared between populations of single cells are indicated with blue (upregulated) or red (downregulated) dots. Gene names in red indicate special interest, as mentioned throughout the text. KEGG annotation was automatically based on homology. NCBI RefSeq numbers are given next to the gene name. **b**. Simplified TLR pathway according to KEGG annotations (genes in white could not be identified in Aiptasia), representing gene expression in symbiotic cells (centred log[fold change] as in a). Statistically significant changes between symbiotic and aposymbiotic cells are highlighted with asterisks, changes between symbiotic and aposymbiotic cells from symbiotic larvae are indicated with squares and changes between symbiont- and *M. gaditana*-containing cells are indicated with triangles. IkBα, nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha; IKKa/β, inhibitor of nuclear factor kappa-B kinase subunit; IRAK, interleukin-1 receptor associated kinase; NEMO, NF-κB essential modulator; TAB1, transforming growth factor-β-activated kinase 1; TAK1, TGF-β-activated kinase 1; TRAF6, TNF receptor-associated factor 6.
TLR signalling plays a fundamental role in animals’ innate immunity to recognize and eliminate invasive microbes, including the apicomplexan parasites from the genera Plasmodium and Toxoplasma\(^{49,50}\). TLRs function as pattern recognition receptors and consist of an extracellular recognition domain constructed from tandem copies of a leucine-rich repeat (LRR) motif, a transmembrane domain and a cytoplasmic signalling domain (Toll/interleukin-1 receptor (TIR) domain)\(^{51}\). To date, canonical TLRs that have both extracellular LRR domains and intracellular TIR domains have not been identified in Aiptasia; however, two proteins containing only LRR domains were identified in Hydra, along with two proteins containing only LRR domains\(^{52}\), and it is known that the functional TLR of Hydra is similarly split into two proteins (HyLRR + HyTIR)\(^{53}\). To test whether modulation of TLR signalling affects initiation of the interaction between the host and microalgae, we challenged apomictic Aiptasia larvae with lipopolysaccharide (LPS)—a ligand for mammalian TLR that is commonly used to elicit an immune response—for 1 h before and during infection with microalgae (Fig. 5a). LPS treatment massively decreased the proportion of symbiont-infected larvae to 25% of the untreated control (Fig. 5a). Surprisingly, once symbionts were intracellularly integrated for more than 24 h, LPS treatment did not affect symbiosis stability (Fig. 5b). Together, this suggests that for both the dinoflagellate symbiont and the apicomplexan-related invader, activation of host immunity pathways conflicts with initiation of the symbiotic interaction; however, the apicomplexan parasites from the genera Plasmodium and Toxoplasma.

**Fig. 5 | Immune stimulation enhances the expulsion of symbionts during initial interaction.** a, The percentage of symbiont-infected Aiptasia larvae was significantly (\(P = 0.00000000024\)) reduced when larvae were pre-treated for 1 h with LPS, followed by a 24-h exposure to microalgae and LPS. The percentage of C. velia-infected larvae was also reduced (\(P = 0.000000000024\)), but to a lesser degree. There was no effect observed for M. gaditana- or N. oculata-infected larvae (\(n = 5\)). b, LPS exposure for 24 h after established symbiosis (24 h infection + 24 h incubation without symbionts) did not influence symbiont maintenance (\(n = 5\)). c, LPS treatment induced expulsion. LPS exposure for 1 h before infection and during 1 h of infection significantly (\(P = 0.00038\)) enhanced the number of larvae with expulsion events, as observed during 1.5 h of live imaging (\(n = 3\) with a total of 42 microalgae (LPS treatment); \(n = 4\) with a total of 50 microalgae (control)). d, MyD88 inhibition enhanced the maintenance of symbionts in larvae (\(P = 0.00000053\)), while the maintenance of other microalgae was unaffected (\(n = 10\) (symbionts); \(n = 3\) (M. gaditana and C. velia); \(n = 13\) (N. oculata)). e, Model of microalgae uptake in Aiptasia endodermal cells. While the uptake of symbionts (red) leads to downregulation of immunity genes until a functional LAMP1-positive symbiosome is formed, non-symbiotic microalgae (yellow) do not elicit a strong transcriptomic response in the host cells and are subsequently expelled by vomocytosis. Bars above graphs represent duration of infection (black dots), treatment (grey fill) and live imaging (where applicable). All graphs show ±95% confidence intervals, with statistics based on a two-sided generalized linear mixed model accounting for repeated measurements. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\).
once symbionts are stably integrated into the host cell, symbiosis stability is not compromised by immune activation. This is probably due to the fact that a large proportion of host immune signalling is transcriptionally repressed by that time (Fig. 4a).

To directly test whether immune activation via LPS treatment induces symbiont expulsion, we used live imaging to monitor the fate of phagocytosed symbiont cells following LPS exposure. We found that symbionts were expelled more frequently in the presence of LPS (Fig. 5c). Thus, immune stimulation by LPS counteracts the robustness of symbiosis establishment due to an increase in expulsion events of newly phagocytosed symbionts. In contrast, LPS treatment did not significantly reduce the infection efficiency for _N. oculata_ and _M. gaditana_ microalgae, probably because the removal via vomocytosis is stochastic and occurs naturally so quickly within the first 12–24 h that artificial immune stimulation by LPS may not have an additional effect (Figs. 1d and 5a). Taken together, this suggests that vomocytosis is an immune response in Aiptasia larvae that is used for protection against invaders. However, compatible symbionts suppress vomocytosis—a key step to begin an endosymbiotic lifestyle with their hosts.

**TLR signalling is modulated via myeloid differentiation primary-response gene 88 (MyD88) during intracellular establishment of symbionts.** In mammals, most TLR-mediated activation of the immune system depends on the critical adapter molecule MyD88. During _Plasmodium_ and _Toxoplasma_ infection, MyD88 plays a key role in mounting an immune response in the host. For example, MyD88 is involved in innate sensing of _Toxoplasma gondii_ infection, and MyD88 knockout mice are more susceptible to infection (reviewed in ref. 5). Interestingly, expression levels of Aiptasia MyD88 were significantly lower in cells that phagocytosed symbionts compared with neighbouring apysymbiotic cells, _M. gaditana_-containing cells or apysymbiotic cells from naive larvae (Fig. 4a,b). To directly test whether decreased MyD88 activity affects the retention of symbionts or non-symbiotic microalgae, we infected Aiptasia larvae for 24 h, then inhibited the activity of MyD88 using an inhibitory peptide that specifically interferes with human MyD88 homodimer formation 5. The peptide mimics the solvent-exposed BB loop within the MyD88 TIR domain, a stretch of seven amino acids that is highly conserved between human and Aiptasia MyD88 (Extended Data Fig. 6). Indeed, we found that addition of the inhibitory peptide significantly increased the number of larvae that contained symbionts compared with the control; however, no such effect was observed for _M. gaditana_, _N. oculata_ or _C. velia_ (Fig. 5d). This suggests that inhibition of MyD88 facilitates symbiont persistence within the first 24–48 h of infection but is not sufficient on its own to allow the persistence of non-symbiotic microalgae.

**Discussion**

**A model to explain selective maintenance of intracellular dinoflagellate symbionts.** The selection of compatible symbionts from the environment is a key step during the establishment of cnidian–dinoflagellate endosymbiosis. Here, we observe the fate of phagocytosed compatible symbionts and incompatible microalgae in Aiptasia larvae in real time and further investigate these observations with gene expression analysis, chemical perturbations and cell biology approaches. Our findings led us to propose a model in which apysymbiotic Aiptasia larvae constitutively acquire and release diverse microalgae from and to the environment. By default, phagocytosed, non-symbiotic microalgae are removed by vomocytosis. The acquisition of suitable symbionts leads to the suppression of innate immunity pathways in the host cell, probably by suppressing the highly conserved TLR signalling pathway by targeting MyD88. This suggests that local modulation of innate immunity in the host cell is crucial for symbionts to escape expulsion by vomocytosis and promote intracellular LAMP1 niche formation (Fig. 5e). Thus, we define escaping vomocytosis as a critical step during symbiosis establishment that requires suppression of host immunity, allowing us to directly link host immune modulation with a biological function.

**Towards a molecular understanding of symbiont selection and niche formation.** Experimental evidence suggests that one symbiont selection criterion is cell size: smaller microalgae are taken up more efficiently than larger ones. Thus, the physical properties of the symbiont play a role in symbiosis establishment 6,28. Moreover, specific recognition mechanisms between symbiont and host cells have been implied to promote the initial interaction 3,25,59, but cnidian hosts also non-specifically take up inert beads and incompatible microalgae. Both fail to establish a long-term partnership, probably due to their inability to manipulate the host immune system to promote a stable symbiotic interaction 3,22,50,54. At the mechanistic level, it is commonly thought that symbionts employ similar tactics as pathogenic microorganisms to accommodate long-term intracellular residency 4,22,56. Some pathogens, for example, modulate the TGF-β pathway during infection to promote their own tolerance 53,54. Similarly, the TGF-β pathway has been proposed to be involved in cnidian–dinoflagellate endosymbiosis; specifically, the addition of an anti-human TGF-β antibody impairs symbiont uptake in Aiptasia anemones and coral larvae 54,66. However, it remains unclear whether modulation of the TGF-β pathway occurs in vivo during symbiosis establishment, and if so whether this is specific to symbiont-containing cells or rather an organism-wide effect. Here, we uncover a different means for manipulating the immune response to regulate symbiosis. Our data suggest that transcriptional repression of MyD88, a conserved cytoplasmic adapter that integrates virtually all TLRs in mammals 4, may promote symbiosis establishment in Aiptasia larvae. While MyD88 activity has been shown to impair infection with the apicomplexan parasites _Plasmodium_ and _Toxoplasma_ in mammals 49,50, its role in endosymbiosis establishment was unclear until now. Analogous to counteracting apicomplexan infection in mammals, MyD88 activity may impair the initiation of cnidian–dinoflagellate symbiosis, a principle that appears to be common for immune challenge in mutualistic and parasitic intracellular algae.

Along these lines, it is also worth noting that various pathogens, including _Listeria monocytogenes_, _Salmonella enterica_ and _Cryptococcus neoformans_, have been shown to reside in pathogen-containing vacuoles that are decorated with LAMP1 (refs. 12,32), similar to what we observe here for the intracellular symbionts in Aiptasia larvae, revealing another commonality between endosymbionts and certain intracellular pathogens (Fig. 2a). These types of pathogen-containing vacuoles are thought to resemble modified lysosomes that provide an intracellular niche for replication without being acidic or digestive (two common features of classical lysosomes) 11. Currently, the intracellular niche of dinoflagellates in the cnidian host is not well described; however, in this context, we made three interesting discoveries: (1) LAMP1 niche formation is impaired upon inhibition of ERK5, a negative regulator of vomocytosis (Fig. 3d,e); (2) non-specific immune stimulation via LPS increases symbiont expulsion within the first 24 h of symbiosis establishment (Fig. 5a,c); and (3) LPS treatment has no effect once symbionts have been integrated into host cells for 24 h (Fig. 5b). This suggests that activation of host immunity pathways conflicts with initiation of the symbiotic interaction; however, once symbionts are stably integrated within the LAMP1 niche, symbiosis stability is not compromised by immune activation, probably due to the fact that a large proportion of host immune signalling is transcriptionally repressed by this time (Fig. 4a).

In contrast with previous organism-wide analyses 12,15,21, we found that expression of the NF-κB transcription factor itself was
not downregulated in symbiotic cells. In fact, compared with apo-
symbiotic cells from symbiotic larvae, NF-κB was even upregulated
(Fig. 4a). This is in accordance with a host cell-specific response
directly linked to symbiont phagocytosis and suggests that tran-
scriptional downregulation of NF-κB itself is not required for sym-
biosis establishment at the individual host cell level but may play a
role at the organism level or once the symbiotic association becomes
more mature. However, we observed significant transcriptional
repression of multiple effectors of the second branch of TLR signal-
ing, which was mediated by the transcription factor AP-1, includ-
ing one transcript identified as AP-1, in symbiont-containing cells
(Fig. 4b). This showed that host immune suppression occurs at a
much broader scale at the cellular level than was previously reported
for whole organisms43, a distinct response that probably reflects the
immediate impact of symbiont phagocytosis by host cells.

Establishing endosymbiosis in cnidarian cells relies on escap-
ing expulsion, not digestion. We found that symbiont uptake
induces immune suppression in order to bypass clearance by
vomocytosis (the fate of non-symbiotic phagocytosed particles) and
not to escape phagolysosomal digestion. Phagocytosis is an
ancient process used by unicellular organisms to acquire and
digest food65. Moreover, microbial killing by professional macro-
phages in higher metazoans is thought to derive from phagotro-
phy66–68. Thus, being a primitive and highly conserved mechanism,
it seemed likely that intracellular digestion would be used by
cnidarians to eliminate incompatible invaders. In fact, symbi-
ont digestion has been implicated in the maintenance of stable
dinoflagellate numbers in cnidian–dinoflagellate symbiosis and
coral bleaching65–67. Accordingly, symbionts halting phagosomal
maturation and thus avoiding intracellular digestion have been
implied to play a role in symbiosis establishment43,68–70. In con-
trast, symbiont expulsion has been described as a key mechanism
by which intact symbiotic microalgae are lost from host tissue
during heat-induced bleaching71. Additionally, it was suggested
that symbiont expulsion is involved in regulating symbiont den-
sity in the host72. However, the proportions of healthy symbiont
cells, debris and other microorganisms, as well as the life stage of
the symbiont, differ between the expelled materials of different
species examined73. Here, we uncover a vital role for expulsion
in defining partner specificity during larval stages in the endo-
symbiotic relationship between cnidarians and their dinoflagel-
late symbionts. While non-pathogenic microalgae are removed
by expulsion, selective intracellular persistence of symbionts
requires avoidance of expulsion by vomocytosis, a phenomenon
preceded by host immune suppression. This raises the interesting
question of whether the same mechanisms are at play in bleach-
ing, the regulation of symbiont density and symbiosis establish-
ment. For example, while our data suggest that Aiptasia larvae
probe their environment using a trial-and-error mechanism, it is
possible that the constitutive acquisition and release of incom-
patible microalgae that we observe in aposymbiotic larvae only
occurs until a compatible symbiont strain has colonized the host.
The immune suppression associated with the uptake may slow
or halt this indiscriminate process to favour symbiont replica-
tion and dispersal rather than continuous de novo acquisition.
Accordingly, even in partially bleached hosts, the remaining
symbionts may suppress the uptake of new microalgae from the
environment to re-colonize the host by distributing descendants
of the original symbiont strain. This could explain why anemo-
nes, and even entire coral colonies, are often stably inhabited
by only a single Symbiodiniaceae type, or a few different ones at
most74–81. Alternatively, differences may exist between larval
and adult life stages, with larvae being more promiscuous and
effective in phagocytosing microalgae compared with their adult
counterparts82.

Vomocytosis as an ancient mechanism of innate immunity.
Currently, vomocytosis is thought to function as an escape route
for pathogens from specialized immune cells, possibly in an
effort to promote dissemination44. This concept is largely based
on experimental evidence for fungal pathogens from the genus
Cryptococcus, members of which are vomocytosed from cultured
macrophages and zebrafish44,62,63. Specifically, it was thought that
fungal pathogens had evolved the capacity for vomocytosis in
response to selective pressure exerted during the interaction with
predatory amoeba64. It is unclear whether vomocytosis is initi-
ated by the host or the invader, but the inability of heat-killer
C. neoformans cells to undergo vomocytosis and its dependency
on the fungal polysaccharide capsule suggest that vomocytosis is
triggered by the pathogen85,86. Interestingly, sporadic reports about
other pathogens that appear to use vomocytosis-like mechanisms
are increasing87–92. Here, we extended the observation of vomocy-
tosis to a non-pathogenic interaction occurring between Cnidaria
and diverse microalgae and inert beads. We found that Aiptasia lar-
vae indiscriminately phagocytose microalgae from the environment
and, only after uptake, select for suitable symbionts by eliminat-
ing the incompatible microalgae or indigestible particles via vomocy-
tosis, suggesting that the process is probably triggered by the host.
This is distinct from amoeba, which release only live fungal cells
via vomocytosis but rely on WASH-mediated constitutive exo-
cytosis for expulsion of inert beads and heat-killed fungal cells83.
Mechanistically, vomocytosis involves the prevention of LAMP1
accumulation in cnidarian larvae, which is in line with observations
for vomocytosed C. neoformans cells in animal macrophages (this
study and ref. 86).

To date, constitutive exocytosis has not been assessed in cni-
darian larvae; however, we found that inhibition of actin polym-
erization, which halts constitutive exocytosis, does not alter the
frequency or dynamics of the expulsion of M. gaudíta cells (Fig. 3a),
suggesting that—at least for large particles (≥3 µm)—vomocy-
tosis is the predominant mechanism at play in these organisms.
Vomocytosis of indigestible material, non-symbiotic microalgae or
even pathogenic microbes may be an effective clearing mechanism
for these small, motile organisms, which are in constant contact with
the environment. Similarly, constitutive exocytosis and vomocytosis
are efficient clearance mechanisms for free-living, unicellular amoe-
bae, while the phagocytic cells of multicellular organisms rely on the
retention and digestion of the phagocytosed material to avoid
infecting neighbouring cells or tissue. Aiptasia larvae represent an
interesting intermediate between these two. They are multicellular
organisms with structured tissues; however, both the ectoderm and
endoderm are in constant contact with the environment, thus the
tight regulation of vomocytosis that occurs in macrophages is not
only unnecessary in Aiptasia larvae but detrimental for successful
symbiosis establishment. Moreover, our data suggest that symbionts
circumvent this default clearing by inducing localized host immune
suppression. Therefore, vomocytosis may not be specific to certain
pathogens but rather an evolutionarily ancient defence mechanism
to fight invaders that was co-opted by symbiotic cnidarians to
selectively promote maintenance of intracellular symbionts. More
broadly, we propose that vomocytosis is an ancient clearing mecha-
nism of animal cells and that the ability to escape vomocytosis was
key for the evolution of an intracellular lifestyle for alveolates.

Conclusion
Cnidarians, such as corals and anemones, are evolutionarily ancient
animals with simple body plans, yet possess cells with complex
immune capacities, providing a powerful experimental system
to dissect the basis for the evolution of intracellular lifestyles and
partner specificity. Uncovering the cell biology of symbiosis estab-
lishment using comparative, experimental approaches in a dinofla-
gellate–cnidarian model will be key to understanding the origin of
mutualistic and parasitic intracellular lifestyles of alveolates within animal cells.

Methods

Live organism culture and maintenance. Microalgal cultures. For the infection experiments of Aiptasia larvae, we used B. minutum clade B (SSB01; symbiont)35, M. gaditana CCMF526 (National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences), N. scutula and C. velia (Norwegian Culture Collection of Algae K-1276; Norwegian Institute for Water Research). All cultures were grown in cell culture flasks in 0.22 μm filtered sterilized 1X Diagio’s IMK medium (Wako Pure Chemical Corporation) on a 12 h light/12 h dark cycle under 20–25 μmol m−2 s−1 of photosynthetically active radiation, as measured with an LIC-1800 PAR Quantum Sensor (LI-COR; Lincoln, NE), and grown on high density photobioreactor (200; Apose) or low density photobioreactor cultures of B. minutum (SSB01; symbiont), N. scutula and C. velia were grown at 26 °C, and M. gaditana was grown at 18 °C. All microalgae (including M. gaditana) cultures to be used for infections were kept at 26 °C for 1–2 weeks post-splitting before infection.

Aiptasia spawning and larval culture conditions. Aiptasia clonal lines F003 and CC7 (Carolina Biological Supply Company; 162865) were induced to spawn following the previously described protocol36. Aiptasia larvae were maintained in glass beakers in filter-sterilized artificial seawater (FASW) at 26 °C and exposed to a 12 h light/12 h dark cycle.

Infection assay. At least three biological replicates (that is, distinct spawning events) of naturally apsymbiotic Aiptasia larvae were collected and diluted to a concentration of 300–500 larval per ml of FASW in glass beakers. Between 4 and 8 d post-fertilization, the larvae were infected with 1×10 µg/ml of the respective microalgal stock cultures. Beakers were kept at 26 °C and exposed to a 12 h light/12 h dark cycle. After a 24-h infection, the larvae were washed to remove the microalgae, then fresh FASW was added.

Quantification of infection efficiency. Infected larvae were fixed at 1, 2, 3, 6 and 10 d post-infection using a 4% formaldehyde solution (F1635; Sigma–Aldrich) for 30 min at room temperature, followed by two washes in 0.1% Triton X-100 in phosphate-buffered saline (PBS–Triton) (3051; Carl Roth), and mounted in 87% glycerol (G5516; Sigma–Aldrich) in PBS with the addition of 2.5 mg/ml 1,4-diazabicyclo[2.2.2]octane (also known as DABCO) (D27802; Sigma–Aldrich). At least 50 larvae per replicate per microalgal type were counted (Nikon Plan Fluor 20x objective). Data recording was performed in Microsoft Excel version 16.16.16. Representative differential interference contrast (DIC) and epifluorescence images of the microalgal autofluorescence were taken. Microscopic analysis was conducted with a Nikon Eclipse Ti inverted microscope using a Nikon Plan Fluor 40x air objective. Images were processed with Fiji software1.

Imaging and staining procedures in Aiptasia larvae. Fluorescence staining for F-actin. Infected larvae were fixed 24 h post-infection using 4% formaldehyde solution for 30 min at room temperature, followed by one wash in 0.05% Tween 20 (P7994; Sigma–Aldrich) in PBS–Triton (PBS–T) for 5 min. For permeabilization, larvae were rotated in 1.5 ml Eppendorf tubes at 0.25 rpm in a solution of 1% PBS–Triton and 0.1% 3% glutaraldehyde (Electron Microscopy Sciences). For blocking, the permeabilization solution was exchanged with 5% normal goat serum (005-000-121; Jackson ImmunoResearch) in 0.05% PBS–Triton. Larvae remained in the blocking buffer for 30 min at room temperature while rotating. After two washes in 0.05% PBS–Triton, larvae were incubated in Phalloidin-Atto 565 (A12379; Sigma–Aldrich) diluted 1:200 in 0.05% PBS–Triton on a 12 h light/12 h dark cycle. After a 45-min wash, the larvae were washed to remove the phalloidin, then fresh FASW was added.

Larae were fixed for 45 min in 4% formaldehyde at room temperature, followed by three washes in 0.2% PBS–Triton and one wash in PBS. Larvae were then permeobilized in 0.2% PBS–Triton for 1 h at room temperature, followed by blocking in 5% normal goat serum and 1% bovine serum albumin in 0.2% PBS–Triton (blocking buffer) for 1 h. Primary antibody (rabbit-anti-LAMP1) was pre-adsorbed with 1% PBS–Triton, Bovine serum albumin 4% milk in 0.1% PBS–Triton and then added to the blocking buffer. Unretreated and treated extracts were diluted 1:1 in 1× loading dye and heated to 100 or 60 °C, respectively, for 5 min. Samples were loaded into a 4–20% precast gel (Bio-Rad; 4561065), which was run at 90 V for 15 min then at 200 V for 1 h at room temperature in 1x sodium dodecyl sulfate running buffer. The proteins were transferred onto a nitrocellulose membrane at 0.37 A for 1 h and 15 min at room temperature in 1X transfer buffer (200 ml methanol, 100 ml 25% tris buffer, and 800 ml water). The membrane was blocked for 1h at room temperature in 4% milk in 0.1% PBS–Triton. The blot was divided in two and incubated in either LAMP1 antibody diluted 1:2000 in blocking buffer or pre-adsorbed LAMP1 antibody overnight at 4 °C. The following day, the blots were washed in 0.1% PBS–Triton 3X for 15 min at room temperature. The secondary antibody, goat-anti-rabbit Alexa Fluor 488 (Jackson ImmunoResearch), was diluted 1:5000 in 1× PBS–Triton and one final wash in 1X PBS. The blot was developed using 1:1 enhanced chemiluminescent solution (GEPN2232; Sigma–Aldrich) and was imaged using a Kodak X-OMAT Image Station 4400 system (Eastman Kodak).

Immunofluorescence staining (LAMP1). Larvae were fixed for 45 min in 4% formaldehyde at room temperature, followed by three washes in 0.2% PBS–Triton and one wash in PBS. Larvae were then permeobilized in 0.2% PBS–Triton for 1 h at room temperature, followed by blocking in 5% normal goat serum and 1% bovine serum albumin in 0.2% PBS–Triton (blocking buffer) for 1h. Primary antibody (rabbit-anti-LAMP1) was pre-adsorbed with 1% PBS–Triton, Bovine serum albumin 4% milk in 0.1% PBS–Triton and then added to the blocking buffer. Unretreated and treated extracts were diluted 1:1 in 1× loading dye and heated to 100 or 60 °C, respectively, for 5 min. Samples were loaded into a 4–20% precast gel (Bio-Rad; 4561065), which was run at 90 V for 15 min then at 200 V for 1 h at room temperature in 1x sodium dodecyl sulfate running buffer. The proteins were transferred onto a nitrocellulose membrane at 0.37 A for 1 h and 15 min at room temperature in 1X transfer buffer (200 ml methanol, 100 ml 25% tris buffer, and 800 ml water). The membrane was blocked for 1h at room temperature in 4% milk in 0.1% PBS–Triton. The blot was divided in two and incubated in either LAMP1 antibody diluted 1:2000 in blocking buffer or pre-adsorbed LAMP1 antibody overnight at 4 °C. The following day, the blots were washed in 0.1% PBS–Triton 3X for 15 min at room temperature. The secondary antibody, goat-anti-rabbit Alexa Fluor 488 (ab130089; Abcam) was diluted 1:500 in blocking buffer and incubated for 1 h at room temperature. Larvae were washed twice in 0.2% PBS–Triton, following by a 15-min incubation with 10 μg/ml Hoechst protected from light at room temperature, then two final washes in 0.2% PBS–Triton and one in PBS before mounting in 87% glycerol. Larvae were imaged on a Leica TCS SP8 confocal laser scanning microscope using a 63x glycerol immersion lens (numerical aperture = 1.30) and Leica LAS X software. Hoechst, Alexa 488 and symbiont autofluorescence were excited with 405-, 496- and 633-nm laser lines, respectively. Fluorescence emission was detected at 410–510 nm for Hoechst, 501–541 nm for goat-anti-rabbit Alexa Fluor 488 and 645–741 nm for symbiont autofluorescence.

Transcriptome. Sample collection and sequencing. Larvae (300 ml−1) were infected with either 105 ml−1 symbionts or 105 ml−1 M. gaditana or left uninfected at 6–7 d post-fertilization for 24–48 h. Per replicate, three to five larvae were transferred to 5 ml calcium- and magnesium-free artificial seawater (CMF-ASW; https://doi.org/10.1101/pdb.rc12053) and incubated for 5 min. They were then incubated in 70 μl Pronase (0.5% in CMF-ASW; 10165921001; Sigma–Aldrich) and sodium thioglycolate (1% in CMF-ASW; T0632; Sigma–Aldrich) for approximately 2 min after pipetting up and down three to five times to remove the ectodermal cells from the larva. The endoderm was transferred to 70 μl FAWS and residual ectodermal cells were mechanically removed before transfer to fresh FAWS. Cells were separated using tweezers, with the whole procedure not taking longer than 30 min. Pools of 7–20 cells (either symbiotic cells, apsymbiotic cells from symbiotic arena, symbiotic cells from apsymbiotic arena, M. gaditana containing cells or microalgae-free cells from M. gaditana-containing larvae) were picked using a micropipette needle (Science Products; GB100T-8P) pulled to an opening diameter of 8–12 μm (Micropipette Puller P-97; Sutter Instrument). Capillaries contained 4.3 ml lysis buffer (0.2% Triton X-100, 1 μl ml−1 Protein RNase Inhibitor (333539901; Sigma–Aldrich), 1.25 μl M-oligo-dT30VN and 2.5 μM dNTP mix) and cells were flushed out of the capillary with lysis buffer, then flash
frozen. Sequencing libraries were prepared by RNA reverse transcription and pre-amplification of complementary DNA of 21 PCR cycles, as described in Picelli et al. Complementary DNA libraries were prepared for Illumina sequencing and sequenced on a NextSeq 500 (Illumina) with 75-base pair-end reads. Reads were deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA) (see ‘Data availability’ statement).

**Computational methods.** Paired reads were mapped to the Aiptasia genome version GCF_001417965.1 using HISAT2 version 2.1.0 with default settings (except --X 2000 --no-discardant --no-unal --no-mixed). Transcripts were quantified in Trinity version 2.5.1 using salmon version 0.10.2 with default settings. Principal component analysis was conducted using a Perl script supplied with Trinity for Trinity version 2.5.1 using salmon version 3.5.2 (ref. 32). Graphing of the results was restricted to pathways involved in infection, using the R package ComplexHeatmap in KNIME. Mean-corrected log [fold expressions] from DESeq2 were used in pathview in R as a basis for the shown pathway (Fig. 4b). The custom KNIME workflow used for processing and analysing the data is available at https://doi.org/10.24433/CO.0872345.v1.

**Exogenous immune perturbations.** LPS/ERK5 inhibitor treatment. Aiptasia larvae were washed and diluted to a concentration of 300–500 larvae per ml and then incubated with either 20 µg/ml LPS (from Escherichia coli O127:B8; Sigma–Aldrich), 1 µM XMD17-109 (0.1% DMSO; Carl Roth) or 0.1% DMSO for 1 h. Larvae infected with B. minutum were then added to 105 microalgae per ml of the respective microalgal types, incubated at 26 °C and exposed to a 12 h light/12 h dark cycle. For a 24-h infection, the larvae were fixed in 4% formaldehyde for 30 min, washed in PBS and mounted in 100% glycerol for counting. The infection status was quantified in at least 100 larvae per infection using a Nikon Eclipse Ti epifluorescence microscope using a Nikon Plan Fluor 20× objective, utilizing microalgal autofluorescence.

**LPS post-infection treatment.** Washed and diluted Aiptasia larvae (300–500) were mixed with B. minutum (1.0×105 microalgae per ml) and left for infection at 26 °C with a 12 h light/12 h dark cycle for 24 h. They were then washed/filtered to remove excess microalgae and incubated further for 24 h. Half of the infected larvae were exposed to 20 µg/ml LPS for 24 h before fixation with 4% formaldehyde. They were then washed in PBS and mounted in glycerol. Infection status was assessed in a 100 larvae per infection using a Nikon Eclipse Ti epifluorescence microscope with a Nikon Plan Fluor 20× objective, utilizing microalgal autofluorescence. Data recording was performed in Microsoft Excel version 16.16.

**Live imaging of early infection with LPS/ERK5 inhibitor treatment.** Aiptasia larvae (300–500) were incubated with either 25 µg/ml LPS, 1 µM XMD17-109 or 0.1% DMSO for 1 h before infection with B. minutum (1.0×105 microalgae per ml) for 1 h. They were then processed with Fiji software for LAMP1 as described above. Symbionts with strong LAMP1 staining were stained for LAMP1 as described above. Images were processed with Fiji software.

**MyD88 inhibitor peptide treatment.** At 6 d post-fertilization, larvae were infected with 1.0×106 cells per ml of B. minutum (SSB01; symbiont), M. gaditana, N. oculata or C. velia for 24 h. Infected larvae were washed with FASW to remove microalgae from the water, and 10 U ml−1 penicillin and 10 µg ml−1 streptomycin (P4333; Sigma–Aldrich) were added. Infected larvae were incubated for 24 h at 26 °C under a 12 h light/12 h dark cycle with either 50 µM MyD88 inhibitor peptide or 50 µM control peptide (NBFP-29328; Novus Biologicals). The larvae were fixed in 4% formaldehyde and the infection efficiency was quantified as previously described.

**LAMP1 assessment after ERK5 inhibitor treatment.** Aiptasia larvae were exposed to LPS (100-fold dilution of LPS) for 1 h and then incubated with 1.0×105 cells per ml of B. minutum (SSB01; symbiont) for 5 h. The larvae were then stained for LAMP1 as described above. Symbionts with strong LAMP1 staining were assessed on a Leica TCS SP8 confocal laser scanning microscope using a 63× glycerol immersion lens (numerical aperture = 1.30) and Leica LAS X software. For the control, the LAMP1 staining was evaluated in 20 symbiotic larvae; however, due to low infection numbers after XMD17-109 treatment, only eight, four and seven larvae could be evaluated in the respective biological replicates.

**Live imaging of early infection with LatB treatment.** To determine the optimal concentration of LatB, Aiptasia larvae were incubated with 0.01, 0.05, 0.10 or 0.25 µM LatB for 6 h, fixed and then stained with phalloidin to visualize F-actin as described above. Actin levels were reduced at 0.05 µM LatB compared with the control, but the larvae still appeared healthy and intact, in contrast with what was observed at higher concentrations (Extended Data Fig. 3a). Therefore, we used 0.05 µM LatB for the live imaging. Briefly, Aiptasia larvae (300–500) were infected with M. gaditana (1.0×105 microalgae per ml) for 1 h followed by a 15-min incubation with either 0.05 µM LatB in DMSO or DMSO alone (control). Larvae were mounted in µ-Dishes in both LGA and FASW (as described above), supplemented with LatB or DMSO and imaged on a Nikon Eclipse Ti inverted microscope using a Nikon Plan Fluor 20× air objective. Images were taken every 5 min in DIC and TexasRed channels. Images were processed with Fiji software. For each independent experiment (n=3), between eight and ten larvae with a total of eight to 16 microalgae were observed.

**ERK5/MAP2Ks phylogeny.** To resolve the evolutionary history and classification of Aiptasia MAPKs and MAP2Ks, we first used well-defined bait sequences (human MAPK7 and MAP2K5) and reciprocal BLAST searches to identify and collate the MAPK and MAP2K repertoire in Aiptasia. Aiptasia sequences were then used to retrieve other MAPK and MAP2K sequences of animals, including key cnidarian species from public databases, using BLASTP. Hydrolacia ehinita sequence was downloaded from https://research.nhgt.nh.gov/hydrolacia/download/ and manually curated and translated. The resulting protein sequences were dereplicated and then aligned using ClustalW (GONNET; gap opening cost = 3; gap extension cost = 1.0). Then, automated trimming was performed using trimAl (using standard parameters; http://trimal.cgenomics.org). Best-fitting amino acid substitution models were determined using ModelFinder (-m MF -msub nucleotide AUTO) with the maximum likelihood trees generated. Trees were visualized using FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/), Adobe Illustrator CC 2018 and Affinity Designer version 1.9.1. Original tree files with accession numbers are provided (Supplementary Data 1 and 2).

**Statistical notes.** Complete statistical analysis, including raw data, R scripts, results and repeat numbers, can be found at https://doi.org/10.24433/CO.0872345.v1. For analysis, a two-sided generalized linear mixed model was used in R version 4.0.3 (ref. 32) using the gam function from the mgcv package. Treatment of larvae within one well in a repeat was taken into account with the random intercept (‘Well’ ID, bset=’re’). For all of the experiments that were performed side by side, the random intercept ‘(Repeat,Date, bset=’re’) was used to account for repeat measurements. Additionally, for all of the graphs in Figs. 1 and 2, multiple comparisons were performed with the emmeans package, using the default Tukey correction methods for P-values.

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

**Data availability.** Raw reads of the RNA sequencing data can be accessed at the National Center for Biotechnology Information SRA with the following accession numbers: SRX7119772–7119776 (cells fromaposymbiotic larvae), SRX7119778 (symbiotic cells) and SRX7119779–7119781 (aposymbiotic cells from symbiotic larvae; combined in the SRA project SRP229372); and SRX7229076–7229080 (M. gaditana-containing cells) and SRX7229075–7229077 (microalgae-free cells from M. gaditana-containing larvae) (combined in the SRA project SRP233508). Source data are provided with this paper.

**Code availability.** The KNIME® workflow used for analysis, as well as other raw data and R scripts for analysis, can be found at https://doi.org/10.24433/CO.0872345.v1.

Received: 4 December 2019; Accepted: 25 March 2021; Published online: 29 April 2021

**References.**
1. Saldariaga, J. F., Taylor, F. J. R., Cavalier-Smith, T., Menden-Deuer, S. & Keeling, P. J. Molecular data and the evolutionary history of dinoflagellates. *Eur. J. Protistol.* 40, 85–111 (2004).
2. Seeger, F. & Steinfelder, S. Recent advances in understanding apicomplexan parasites. *F1000Res.* 5, 10 (2016).
3. Kwong, W. K., del Campo, J., Mathur, V., Vermeij, M. J. A. & Keeling, P. J. A widespread coral-infecting apicomplexan with chlorophyll biosynthesis genes. *Nature* 568, 103–107 (2019).
4. De Vargas, C. et al. Eukaryotic plankton diversity in the sunlit ocean. *Science* 348, 1261605 (2015).
5. Baker, A. C. Flexibility and specificity in coral–algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annu. Rev. Ecol. Evol. Syst.* 34, 661–688 (2003).
6. Yellowlees, D., Rees, T. A. V. & Leggat, W. Metabolic interactions between algal symbionts and invertebrate hosts. *Plant Cell Environ.* 31, 679–694 (2008).
LaJeunesse, T. C. et al. Systematic revision of Symbiodiniaceae highlights
Bucher, M., Wolfowicz, I., Voss, P. A., Hambleton, E. A. & Guse, A.
Grawunder, D. et al. Induction of gametogenesis in the cnidarian
Dunn, S. R. & Weis, V. M. Apoptosis as a post-phagocytic winnowing
Mohamed, A. R. et al. The transcriptomic response of the coral
Berthelier, J. et al. Implication of the host TGF
Matthews, J. L. et al. Optimal nutrient exchange and immune responses
Mansfield, K. M. et al. Transcription factor NF-
Janouškovec, J., Horák, A., Oborník, M., Lukeš, J. & Keeling, P. J. A
Janouškovec, J. et al. Apicomplexan-like parasites are polyphyletic and widely
Isolation of clonal axenic strains of the symbiotic dinoflagellate

Buchner, M., Wolfowicz, I., Voss, P. A., Hambleton, E. A. & Guse, A.
Development and symbiosis establishment in the cnidarian endosymbiosis model
Hamblyton, E. A. et al. Sterol transfer by atypical cholesterol-binding NPC2 proteins in coral–algal symbiosis. eLife 6, e43923 (2019).
Bucher, M., Wolflowicz, I., Voss, P. A., Hamblyton, E. A. & Guse, A.
Lafrance, T. C. et al. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr. Biol. 28, 2570–2580 (2018).
Xiang, T., Hambleton, E. A., DeNofrio, J. C., Pringle, J. R. & Grossman, A. R. Isolation of clonal axenic strains of the symbiotic dinoflagellate Symbiodinium and their growth and host specificity. J. Phycol. 49, 447–458 (2013).
88. Picelli, S. et al. Full-length RNA-seq from single cells using Smart-Seq2.

89. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change in data from RNA-seq experiments.

90. R Core Development Team. R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, 2018).

91. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32, 2847–2849 (2016).

92. Berthold, M. R. et al. in Data Analysis, Machine Learning and Applications. Studies in Classification, Data Analysis, and Knowledge Organization (eds Preissach, C. et al.) 319–326 (Springer, 2008).

93. Luo, W. & Brouwer, C. Pathway: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics 29, 1830–1831 (2013).

94. Capelli-Gutierrez, S., Silla-Martines, J. M. & Gabaldon, T. trime: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973 (2009).

95. Wood, S. N. Generalized Additive Models: An Introduction with R (Chapman and Hall/CRC, 2017).

96. Keeling, P. J. & Burki, F. Progress towards the tree of eukaryotes. Curr. Biol. 17, R808–R817 (2019).

97. Hardiman, G., Rock, F. L., Balasubramanian, S., Kastelein, R. A. & Bazan, J. F. Molecular characterization and modular analysis of human CD140C. Oncogene 13, 2467–2495 (1996).

98. Vyne, L. et al. Reconstructing the TIR side of the MydDosome: a paradigm for TIR–TIR interactions. Structure 24, 437–447 (2016).

99. Loiarro, M. et al. Peptide-mediated interference of TLR/IL-1R signalling in Plasmodium falciparum. PLoS ONE 11, e0152693 (2016).

100. Van Treuren, W. et al. Live imaging of Aiptasia larva, a model system for coral and anemone anemone, using a simple microfluidic device. Sci. Rep. 9, 9275 (2019).

101. Winchester, B. G. Lysosomal membrane proteins. J. Biol. Chem. 284, 15809–15814 (2009).

102. Berthold, M. R. et al. in Data Analysis, Machine Learning and Applications. Studies in Classification, Data Analysis, and Knowledge Organization (eds Preissach, C. et al.) 319–326 (Springer, 2008).

103. Supplementary information is available at www.nature.com/reprints.

104. We thank D. Pavlinic and V. Benes (GeneCore Facility, EMBL Heidelberg) for assistance with the Smart-Seq2 protocol and sequencing library preparation; D. Ibberson (Deepseqlab, Heidelberg University) for assistance with the Smart-Seq2 protocol; C. Rippe for access to the bioanalyzer; L. Hambleton for help with antibody purification; B. G. Bergherm for initiating live imaging of Aiptasia larva; M. Mercker (Bionum) for advice on statistical analysis; F. Frischknecht, T. Gilmore, T. Holstein and S. Lemke for advice; and R. May for advice and comments on the manuscript. Funding was provided by the Deutsche Forschungsgemeinschaft (DFG) (Emmy Noether Program Grant PI 1128/3-1 and H2001), European Research Council (ERC Consolidator Grant 734715) to A.G., a scholarship from the CellNetworks Excellence Cluster (Heidelberg University) Postdoctoral Program to S.R. and a PhD scholarship within the graduate school Evolutionary Novelty and Adaptation by the Baden-Württemberg Landesgraduiertenförderung Program to P.A.V.

105. Author contributions M.R.J., S.R. and A.G. conceived of the study. M.R.J., S.R., P.A.V., I.M. and A.G. developed the project. M.R.J., S.R. and I.M. performed the investigation. M.R.J., S.R. and A.G. interpreted the data. A.G. provided the resources. P.A.V. and I.M. contributed reagents and materials. A.G. supervised the project. M.R.J., S.R. and A.G. wrote the original draft of the manuscript. P.A.V., I.M. and A.G. reviewed and edited the manuscript. M.R.J. and S.R. contributed equally to this work. A.G. supervised the project. M.R.J., S.R. and A.G. administered the project. A.G. acquired the funding.

106. Competing interests The authors declare no competing interests.

107. Additional information Extended data is available for this paper at https://doi.org/10.1038/s41564-021-00897-w.

108. Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41564-021-00897-w.

109. Correspondence and requests for materials should be addressed to A.G.

110. Peer review information Nature Microbiology thanks Alejandro Sánchez-Alvarado, Simon Day and Christian Voolstra for their contribution to the peer review of this work.

111. Reprints and permissions information is available at www.nature.com/reprints.

112. Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2021
Extended Data Fig. 1 | Uptake of microalgae is indiscriminate. a, Additional microalgae screened: Isochrysis sp., Chlorella sp., D. salina, and C. parkeae. Images are DIC and red autofluorescence of microalgae photosynthetic pigments. Scale bar indicates 25 μm. b, Aiptasia larvae were infected at 4-6 days post fertilization (dpf) for 24 hours and were washed into fresh FASW. Error bars represent SEM. n=3 for all except C. parkeae with n=1.
Extended Data Fig. 2 | N-deglycosylation of LAMP1 in Aiptasia homogenate. Verification of α-LAMP1 antibody used in Figs. 2a, 3d,e by Western blot. LAMP1 has been observed to run at a higher than predicted weight (38 kDa) because it is heavily glycosylated\(^{10}\). Deglycosylation of homogenates of symbiotic and aposymbiotic adult Aiptasia CC7 using PNGase F resulted in a shift to a lower molecular weight. As control, extracts were detected with LAMP1 antibody pre-adsorbed with the peptide used for raising the antibody.
Extended Data Fig. 3 | Inhibition of actin polymerization does not affect expulsion of non-symbiotic microalgae. **a.** Analysis of the effects of different concentrations of LatrunculinB (LatB) on Aiptasia larvae to determine a suitable concentration for live imaging analysis. Larvae were incubated for 6 hours in LatB, washed, fixed and the f-actin levels were assessed by fluorescence microscopy. 0.01µM does not affect the overall levels or distribution of actin. In contrast, LatB concentrations >0.1µM substantially decreased actin levels and impacted the integrity of Aiptasia larvae (see arrowheads pointing to ‘holes’ within the organisms). Accordingly, an intermediate concentration of 0.05µM LatB which substantially reduced f-actin levels without compromising larval integrity was used for live imaging in Fig. 3a). **b.** Inhibition of actin polymerization with Latrunculin B did not affect the time to expulsion of *M. gaditana* from infected Aiptasia larvae.
Extended Data Fig. 4 | ERK5 and MAP2K5 homologues in Aiptasia. Phylogenetic analysis of ERK5 and MAP2K5 from Aiptasia. a + b are collapsed trees of Aiptasia MAPK (A) or MAP2K (B) in comparison to several other cnidarian and vertebrate species. Red arrowheads or writing indicate presence of an Aiptasia homolog. Both Aiptasia ERK5 and MEKS cluster within ERK5 (MAPK7) or MAP2K5, respectively. Full tree can be accessed through Supplementary Files 1 and 2.
Extended Data Fig. 5 | Cell-specific characterization for transcriptomic analysis. **a**, Schematic of Aiptasia larvae used for cell-specific sequencing. Ectodermal cells were removed resulting in only endodermal cells that were dissociated and selected for based on contents: aposymbiotic cells from symbiotic larvae (Symbiont-Apo), symbiotic cells from symbiotic larvae (Symbiont (red)), aposymbiotic cells from aposymbiotic larvae (Apo), cells containing *M. gaditana* from larvae infected with *M. gaditana* (*M. gaditana* (yellow)), and aposymbiotic cells from larvae infected with *M. gaditana* (*M. gaditana-Apo*). **b**, Principal Component Analysis (PCA) plot of host gene expression in different conditions.
Extended Data Fig. 6 | Amino acid sequence similarity between human and Aiptasia MyD88. Human MyD88 homo-dimerizes to trigger a downstream signaling cascade leading to immune activation. It consists of three domains, the death domain (DD), the interdomain (ID) and the C-terminal TIR domain. The human TIR domain is key for homo-dimerization with other TIR domains from MyD88 or other TIR domain containing proteins. Three distinct regions contributing to homo-dimerization have been identified by crystallography, NMR and mammalian two-hybrid analysis. However, the so-called BB-loop within the TIR domain, a solvent-exposed stretch of 7 residues (RDLVPIT) is particularly critical for homodimerization in human MyD88. Accordingly, cell-permeable peptides mimicking the 7 residues of the BB-loop of human MyD88 interfere with homo-dimerization. The TIR domains (black box/upper alignment) of mammals and Aiptasia are well conserved (~50% sequence identity). Moreover, the BB-loop (red box) is almost identical and key residues (*) are conserved. Identical amino acids have black background, similar aa have gray background and aa with white background are not similar according to blosum62 scoring.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  *Give P values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

*Our web collection on statistics for biologists contains articles on many of the points above.*

Software and code

Policy information about availability of computer code

Data collection

For wet-lab data, Microsoft Excel v16.16.6 was used. For microscopic image acquisition, either NIS Elements v4.51.01 or Leica LAS X v3.5.5.19976 were used.

Data analysis

For RNAseq analysis, HISAT2 version 2.1.0, Trinity v2.5.1 using salmon v0.10.2, R v3.5.2 - v4.0.3, DEseq2(v 1.30.1), ComplexHeatmap (v 2.6.2), and KNIME v4.3.1 were used. For wet-lab data, including statistics, R was used with following packages: readxl (v 1.3.1), lubridata (v 1.7.1), mgcv (v 1.8-34), ggsignif (v 0.6.1), readr (v 1.4.0), emmeans (v 1.5.4), tidyr (v 1.1.3), plyr (v 1.8.6), dplyr (v 1.0.4), edgeR (v 3.32.1), DEseq2 (v 1.30.1), cluster (v 2.1.1), Biobase (v 2.50.0), qvalue (2.22.0), ComplexHeatmap (v 2.6.2), knitr (v 1.31), circlize (v 0.4.12), gRDevices (v 0.4.3), colorRamps (v 2.3)

For image processing, Fiji (ImageJ v 2.10/1.53c) or Leica LAS X was used.

For phylogenetic trees, BLAST, BLASTP, ClustalW(GONNET, goc: 3, gec: 1.8), trimAI, ModelFinder within FigTree v1.4.4 were used.

Final graphic arrangements were done in Adobe Illustrator CC2018, or in Affinity Designer v 1.9.1.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

*Reads from transcriptome analysis have been deposited on NCBI sequence read archive (SRA) with following accession numbers: cells from aposymbiotic larvae (SRX7119772-7119776), symbiotic cells (SRX7119782-7119787), aposymbiotic cells from symbiotic larvae (SRX7119777-7119781), combined in the SRA project.*
SRP229372, as well as M. gaditana-containing cells (SRX7229078-7229080), and microalgae-free cells from M. gaditana-containing larvae (SRX7229075-7229077), combined in the SRA project SRP233508. Raw data for other figures can be found at https://doi.org/10.24433/CO.0872345.v1 as well es in SourceData Files.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- Sample size: For infection experiments, 1 replicate included a sample size of around 50-100 individual larvae, which we believe to be a good representation of the whole replicate (10-30 % of the initial 300-500 larvae used). Due to technical constraints, sample sizes were smaller for live imaging (around 10 per replicate), as stated in material and methods section.

- Data exclusions: No data were excluded.

- Replication: Three to five independent replicates were usually performed, as is common in the field. Despite some variation within the groups (especially due to infection efficiency), differences between treatments were consistent.

- Randomization: Exposure and control larvae were taken from same spawning in batch.

- Blinding: Blinding was not deemed necessary, as distinct algae were counted within the larvae, and no subjective grading was applied.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology         |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

Antibodies used: rabbit-anti-LAMP1 (BioScience GmbH, produced from peptide IGRKRSQRGYEKV), goat-anti-rabbit AlexaFluor 488 (abcam, Cat# Ab150089, Lot# GR119971-1), Peroxidase AffiniPure Goat Anti-Rabbit IgG (Jackson ImmunoResearch, Cat# 111-035-144, Lot# 127629)

Validation: Antibody was validated by competition with the peptide against which it was produced (Supplementary Fig 6), additionally N-deglycosylation was used, as LAMP1 is known to be highly glycosylated. Goat-anti-rabbit AlexaFluor488 was validated by seller: https://www.abcam.com/goat-rabbit-igg-fc-alexa-fluor-488-ab150089-references.html#top-0 Peroxidase Goat Anti-Rabbit was validated by seller: https://www.jacksonimmuno.com/catalog/products/111-035-144

Animals and other organisms

Policy information about: studies involving animals, ARRIVE guidelines recommended for reporting animal research

Laboratory animals: Exaiptasia pallida (Aiptasia) strains F003 (female) and CC7 (male) used for spawning when > 6 month old, and their offspring.

Wild animals: No wild animals were used.
| Field-collected samples | No field-collected samples were used. |
|-------------------------|--------------------------------------|
| Ethics oversight        | No ethical approval was necessary, as work on Aiptasia does not need approval. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.