The scavenger receptor repertoire in six cnidarian species and its putative role in cnidarian-dinoflagellate symbiois

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Many cnidarians engage in a mutualism with endosymbiotic photosynthetic dinoflagellates that forms the basis of the coral reef ecosystem. Interpartner interaction and regulation includes involvement of the host innate immune system. Basal metazoans, including cnidarians have diverse and complex innate immune repertoires that are just beginning to be described. Scavenger receptors (SR) are a diverse superfamily of innate immunity genes that recognize a broad array of microbial ligands and participate in phagocytosis of invading microbes. The superfamily includes subclades named SR-A through SR-I that are categorized based on the arrangement of sequence domains including the scavenger receptor cysteine rich (SRCR), the C-type lectin (CTLD) and the CD36 domains. Previous functional and gene expression studies on cnidarian-dinoflagellate symbiosis have implicated SR-like proteins in interpartner communication and regulation. In this study, we characterized the SR repertoire from a combination of genomic and transcriptomic resources from six cnidarian species in the Class Anthozoa. We combined these bioinformatic analyses with functional experiments using the SR inhibitor fucoidan to explore a role for SRs in cnidarian symbiosis and immunity. Bioinformatic searches revealed a large diversity of SR-like genes that resembled SR-As, SR-Bs, SR-Es and SR-Is. SRCRs, CTLDs and CD36 domains were identified in multiple sequences in combinations that were highly homologous to vertebrate SRs as well as in proteins with novel domain combinations. Phylogenetic analyses of CD36 domains of the SR-B-like sequences from a diversity of metazoans grouped cnidarian with bilaterian sequences separate from other basal metazoans. All cnidarian sequences grouped together in a subclade separately from bilaterian sequences with moderate support. Functional experiments were carried out on the sea anemone *Aiptasia pallida* that engages in a symbiosis with *Symbiodinium minutum* (clade B1). Experimental blocking of the SR ligand binding site with the inhibitor fucoidan reduced the ability of *S. minutum* to colonize *Aiptasia* suggesting that host SRs play a role

in host-symbiont recognition. In addition, incubation of symbiotic anemones with fucoidan elicited an immune response, indicating that host SRs function in immune modulation that results in host tolerance of the symbionts.

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Abstract

Many cnidarians engage in a mutualism with endosymbiotic photosynthetic dinoflagellates that forms the basis of the coral reef ecosystem. Interpartner interaction and regulation includes involvement of the host innate immune system. Basal metazoans, including cnidarians have diverse and complex innate immune repertoires that are just beginning to be described. Scavenger receptors (SR) are a diverse superfamily of innate immunity genes that recognize a broad array of microbial ligands and participate in phagocytosis of invading microbes. The superfamily includes subclades named SR-A through SR-I that are categorized based on the arrangement of sequence domains including the scavenger receptor cysteine rich (SRCR), the C-type lectin (CTLD) and the CD36 domains. Previous functional and gene expression studies on cnidarian-dinoflagellate symbiosis have implicated SR-like proteins in interpartner communication and regulation. In this study, we characterized the SR repertoire from a combination of genomic and transcriptomic resources from six cnidarian species in the Class Anthozoa. We combined these bioinformatic analyses with functional experiments using the SR inhibitor fucoidan to explore a role for SRs in cnidarian symbiosis and immunity. Bioinformatic searches revealed a large diversity of SR-like genes that resembled SR-As, SR-Bs, SR-Es and SR-Is. SRCRs, CTLDs and CD36 domains were identified in multiple sequences in combinations that were highly homologous to vertebrate SRs as well as in proteins with novel domain combinations. Phylogenetic analyses of CD36 domains of the SR-B-like sequences from a diversity of metazoans grouped cnidarian with bilaterian sequences separate from other basal metazoans. All cnidarian sequences grouped together with moderate support in a subclade separately from bilaterian sequences. Functional experiments were carried out on the sea anemone *Aiptasia pallida* that engages in a symbiosis with *Symbiodinium minutum* (clade B1). Experimental blocking of the SR ligand binding site with the inhibitor fucoidan reduced the ability of *S. minutum* to colonize *A. pallida* suggesting that host SRs play a role in host-symbiont recognition. In addition, incubation of symbiotic anemones with fucoidan elicited an immune response, indicating that host SRs function in immune modulation that results in host tolerance of the symbionts.

Introduction

Cnidarians such as reef-building corals engage in an intimate mutualistic symbiosis with photosynthetic dinoflagellates in the genus *Symbiodinium* that together form the trophic and structural foundation of coral reef ecosystems. *Symbiodinium* spp. provide large amounts of reduced organic carbon to the host in exchange for inorganic nutrients, a high light environment and refuge from herbivory (Yellowlees et al. 2008). In the majority of cnidarian-*Symbiodinium* interactions, the symbionts are taken up by host cells *via* phagocytosis. Instead of being digested as food, the symbionts resist host destruction and persist in host cells by residing in vacuoles known as symbiosomes (Davy et al. 2012). The molecular interplay between host cnidarian and resident symbionts during both the establishment and ongoing maintenance of the symbiosis is critical for a healthy holobiont (Weis & Allemand 2009).

Animal innate immune systems are central to managing microbes by both tolerating and promoting the survival of beneficial symbionts and resisting and destroying negative invaders (Bordenstein & Theis 2015; McFall-Ngai et al. 2013; Schneider & Ayres 2008). With the increased availability of sequence resources, there is now ample evidence that innate immune pathways are ancestral and that basal metazoans including cnidarians possess many of these pathways originally described in mammals and flies (Fuess et al. 2016; Miller et al. 2007; Yuen et al. 2014). Furthermore there are numerous examples of expansions of some innate immune gene families in invertebrates that are larger than those in vertebrate genomic repertoires, including NOD-like receptors, scavenger receptors, TIR-domain-containing proteins and ficolins (Baumgarten et al. 2015; Buckley & Rast 2015; Hamada et al. 2013; Pancer 2000; Poole & Weis 2014; Shinzato et al. 2011). A class of well-described host-microbe molecular interactions mediated by innate immunity are the PRR-MAMP interactions where microbe-associated molecular patterns (MAMPs) on the surface of microbes, such as lipopolysaccharide or glycans, are recognized by pattern recognition receptors (PRRs) on the surface of host cells (Janeway & Medzhitov 2002). These steric interactions launch a series of downstream signalling cascades in the host that serve to resist and destroy negative invaders or tolerate and nurture positive microbes. Genomic and transcriptomic studies of cnidarians are revealing the presence of many classical PRRs that have been extensively characterized in higher metazoans (Fuess et al. 2016; Miller et al. 2007).

One group of PRRs in the Metazoa are the scavenger receptors (SRs), so-named for their role in the scavenging and clearing of microbial invaders, modified host molecules, and apoptotic cell debris (Areschoug & Gordon 2009; Canton et al. 2013). SRs have a high affinity for a wide range of ligands and this flexibility of ligand binding has led them to be described as ‘molecular fly paper’ (Krieger 1992). A key role of SRs in innate immune function is their action as PRRs on phagocytic cells where they mediate direct non-opsonic phagocytosis of pathogenic microbes (Areschoug & Gordon 2009). SRs are thought to engage in heteromultimeric signalling

complexes, known as signalosomes, involving multiple PRRs and other molecules that together effect signal transduction in cells, thereby alerting them to microbes or modified host molecules (Canton et al. 2013). The SR superfamily is a large group of structurally diverse transmembrane cell surface glycoproteins, divided into nine classes SR-A through SR-I (Canton et al. 2013; Krieger 2001). The classes have overlapping specificities that result in an enormous breadth of MAMP recognition (Krieger 1992). Members within a given class share some sequence homology, with little-to-no homology occurring between classes. The classes are grouped by their multiple domains with no single domain common to all (Gordon 2002; Gough & Gordon 2000). SR domains occur on the extracellular portion of the protein; the proteins are anchored in the cell membrane with transmembrane domain(s) and contain short cytoplasmic tail(s). Figure 1 depicts the four SR classes that are relevant to this study. SRs are a potential target for manipulation by invading parasites, pathogens and potentially mutualists. Several pathogens have evolved mechanisms to evade SR-mediated recognition (Areschoug & Waldemarsson 2008; Faure & Rabourdin-Combe 2011). Indeed, certain human pathogens exploit specific SRs for their own benefit. For example, the Hepatitis C virus (HCV) (Catanese et al. 2007) and the malaria parasite *Plasmodium falciparum* (Ndungu et al. 2005; Rodrigues et al. 2008) have surface ligands that are recognized by SR-B1, and both use this recognition to gain entry to host cells.

SR-As and SR-Is contain the scavenger receptor cysteine rich (SRCR) domain, which consists of a 110 aa motif with conserved spacing of six to eight cysteines (Hohenester et al. 1999). The SRCR domain is found in a wide range of membrane and soluble proteins and often occurs in multiple repeats arrayed on the protein (Hohenester et al. 1999; Martinez et al. 2011; Sarrias et al. 2004). Some SR-As and SR-Es contain C-type lectin domains (CTLDs), a common domain in many proteins, that are often involved in lectin-glycan interactions (Cambi et al. 2005). SR-Bs contain the CD36 domain and have two cytoplasmic tails rooted in the membrane with two transmembrane regions, forming an extracellular loop (Silverstein & Febbraio 2009). SR genes encoding SRCR, CTLD and CD36 domains have been described in invertebrates (Hibino et al. 2006; Lehnert et al. 2014; Pancer et al. 1997; Schwarz et al. 2007; Wood-Charlson & Weis 2009). However, a detailed bioinformatic characterization of cnidarian SR genes homologous to vertebrate SR-As, SR-Bs, SR-Es and SR-Is is lacking, as are any functional studies exploring the function of these proteins.

SRs are of interest in studies of cnidarian immunity and symbiosis. First, interactions between SR-E-like host lectin-like proteins and symbiont surface glycans play an important role in host-symbiont recognition during onset of symbiosis (reviewed in Davy et al. 2012). In addition, SR-B homologues in two species of sea anemone, *Anthopleura elegantissima* (Rodriguez-Lanetty et al. 2006) and *Aiptasia pallida* (Lehnert et al. 2014), were found to be highly expressed in symbiotic compared to aposymbiotic individuals. For *A. pallida* this was a dramatic difference in expression where symbiotic anemones had 28-fold greater expression than aposymbiotic

139 animals. These studies suggest that SR-E and SR-B homologues are playing a role in host-
140 symbiont communication.
141
142 There were two aims for this study. The first was to identify SRs in six cnidarian species, all in
143 Class Anthozoa (corals, sea anemones and others), using a variety of genomic and transcriptomic
144 resources, and compare the repertoire to vertebrate SRs of known function. This provides a
145 platform for identifying potential roles of cnidarian SR proteins in immunity and symbiosis. The
146 second aim was to perform simple functional experiments to examine the role of SRs in
147 symbiont recognition and uptake by the sea anemone *A. pallida,* a well-studied model system for
148 the study of coral-dinoflagellate symbiosis. We hypothesized that if a symbiont is co-opting host
149 SRs to initiate tolerogenic pathways that dampen or prevent an immune response, blocking SR-
150 ligand-binding capabilities would induce an immune response.
151

Materials and Methods

Anthozoan genomic and transcriptomic resources

To characterize the SR protein repertoire in cnidarians, six species with publically available resources were searched. These included three anemone species: *A. elegantissima* (Kitchen et al. 2015), *Aiptasia pallida* (Baumgarten et al. 2015; Lehnert et al. 2012), and *Nematostella vectensis* (Putnam et al. 2007), and three coral species: *Acropora digitifera* (Shinzato et al. 2011), *A. millepora* (Moya et al. 2012) and *Fungia scutaria* (Kitchen et al. 2015). These species were selected based on the availability of transcriptomic and genomic resources, and to include a diversity of organisms within Class Anthozoa. These resources were derived from various developmental stages and symbiotic states (Table 1). All resources were used as provided, with the exception of the *A. pallida* transcriptome, for which raw Illumina sequence reads for accession SRR696721 were downloaded from the sequence read archive entry (http://www.ncbi.nlm.nih.gov/sra/SRX231866) and reassembled using Trinity (Grabherr et al. 2011) resulting in a better assembly than the original one performed.

SR sequence searching

Twenty-four non-cnidarian sequences were obtained, primarily from GenBank and other publically available databases (Table S1), for use in creating multiple sequence alignments and phylogenetic trees. Eleven human SR genes were chosen for production of reference protein domain architecture diagrams, to compare predicted cnidarian proteins with human SR proteins of known function (Figure 2).

To search for cnidarian SR proteins, databases were queried using several search strategies to ensure all sequences were recovered. BLASTp or tBLASTn searches with mouse and human SR protein sequences (SR-A1, MARCO, SRCL, CD36, SRB1, LMP2, and LOX1) (Supplementary file 1) and consensus sequences (pfam01130: CD36, pfam00530: SRCR) from the conserved domain database (http://www.ncbi.nlm.nih.gov/cdd) as queries were performed for each resource. Keyword searches were used with the terms SR, CD36, LMP2, SRCR, and scavenger where GO or KEGG annotations were available. Lastly, representative *N. vectensis* sequences of each protein type (SRCR-domain-containing, CD36, SRB1, and LOX1) were also used as queries for tBLASTn searches of the other five cnidarian resources. A high e-value cutoff ($1x10^{-1}$) was used in the BLAST searches to recover divergent sequences. All BLAST searches were performed using the default settings in Geneious pro version 7.1.8 with the exception of *N. vectensis*, for which searches were performed through the Joint Genome Institute online portal using the default settings (Kearse et al. 2012). A list of metazoan resources searched are listed in Table S1. Blast query sequences and cnidarian sequences identified are tabulated in Supplementary File 1.

To confirm that the sequences obtained contained SR domains, nucleotide sequences were translated using Geneious and then annotated using the InterProScan plugin (Quevillon et al. 2005). Only sequences in which two or more databases within InterProScan found either SRCR, CD36, or CTLD domains with an e-value of $<1x10^{-4}$ were used. Where the InterProScan plugin was unable to resolve protein domains, (this occurred for approx. 1 in 10 sequences) the sequences were analysed using the online protein domain database PfamA (http://pfam.sanger.ac.uk) using the default program settings. Sequences for each species were aligned and those that were identical or almost identical (<5 aa difference in the conserved domains) were omitted from the analysis as they likely represented artifacts of assembly or different isoforms of the same protein. Sequences missing a start or stop codon were removed from the analysis.

Only proteins that showed significant PfamA matches to a CTLD, SRCR and/or CD36 domains were included in the study. Diagrammatic representations of the protein domain configurations were produced using this information. Protein domain architectures were grouped together according to common domains and compared to known human SR proteins (Figure 2).

Phylogenetic analysis of SR-B homologues

A multiple sequence alignment of a subset of CD36-domain-containing sequences was performed with the MAFFT v 7.017 plug-in (Katoh et al. 2002) through Geneious (Kearse et al. 2012), using the default settings. The program ProtTest v2.4 (Abascal et al. 2005) was used to apply AIC1, AIC2 and BIC2 model selection criteria to a variety of possible substitution matrices and rate assumptions to obtain the best-fit model of protein evolution. The results from the overall comparison of these metrics indicated that the best-fit model for the full-length alignment was WAG+G+F. A maximum likelihood tree was produced using FastTree v2.1.5 (Price et al. 2010). Bootstrap support values were generated using the online program SEQBOOT (Felsenstein 2005) and values above 0.6 support were displayed at the nodes. A PhyML (Guindon et al. 2005) alternate tree produced identical topology (data not shown).

Maintenance and preparation of anemone and dinoflagellate cultures

Symbiotic *A. pallida* cultures were maintained in saltwater aquaria at 26°C with a 12/12 h light/dark photoperiod, and were fed twice weekly with live brine shrimp nauplii. Animals were rendered aposymbiotic by incubation for 8 h at 4 °C twice weekly for six weeks, followed by maintenance in the dark for approximately one month. Anemones were fed twice weekly with brine shrimp, and cleaned of expelled symbionts and food debris regularly.

Cultured dinoflagellates, *Symbiodinium minutum*, clade B1 (culture ID: CCMP830) were maintained in 50 ml flasks in sterile Guillard's f/2 enriched seawater culture medium (Sigma, St. Louis, MO, USA). Dinoflagellate cultures were maintained at 26°C on a 12/12 h light/dark photoperiod.

In preparation for experimental manipulations, individual anemones were placed in 24-well plates in 2.5 ml of 1-µm filtered seawater (FSW) and acclimated to the well-plate for 3-4 days, with the FSW replaced daily. Well plates containing aposymbiotic anemones were kept in the dark and symbiotic anemones were maintained in an incubator at 26°C with a 12/12 h light/dark photoperiod. Animals were not fed during the experimental time period.

Addition of fucoidan to block SR binding function

To explore a role for SRs in the onset of symbiosis, fucoidan, a known SR ligand, was added to anemones to block SR binding sites. Fucoidan is a protein derived from the brown alga *Fucus vesiculosus*; this polyanionic ligand is known to bind SRCR and CD36 domains in SR-As and SR-Bs, respectively (Dinguirard & Yoshino 2006; Hsu et al. 2001; Thelen et al. 2010).

To examine the effect of blocking SR ligand binding capabilities on symbiont colonization success, aposymbiotic anemones (n = 3 per treatment per time point) were pre-incubated in fucoidan (Sigma, St. Louis, MO, USA), at a concentration of 0 (FSW control), 100, 200 and 400 µg/ml for 18 h, according to Bowdish Lab protocols (online at McMaster University; www.bowdish.ca/lab/protocols). Fucoidan-treated aposymbiotic anemones were subsequently re-inoculated with *S. minutum* CCMP830. CCMP830 cells were pelleted from the culture medium, re-suspended in FSW, and then added to anemones in well-plates to a final concentration of $2x10^5$ symbionts per ml. After incubation for 12 h at 26°C in the light, anemones were rinsed twice with FSW and fucoidan treatments were refreshed. To test the effect of fucoidan exposure on host health, a second control treatment (fucoidan-washed control) was prepared where aposymbiotic anemones were pre-incubated in 200 µg/ml fucoidan for 18 h, and then washed with FSW prior to being inoculated with symbionts as described above. Anemones for all treatments were sampled at 48 and 96 h post-infection (three tentacles per anemone, for n = 3 anemones per treatment per time point).

A second experiment was designed to explore a role for SR binding in host immune tolerance during symbiosis. We hypothesized that if a symbiont is co-opting host SRs to initiate tolerogenic pathways (such as the TGFβ pathway) that dampen or prevent an immune response, blocking SR-ligand-binding capabilities could induce an immune response upon the addition of lipopolysaccharide (LPS). LPS is a MAMP that has been shown to induce an anemone immune response measured as increased nitric oxide (NO) production (Detournay et al. 2012; Perez & Weis 2006). Symbiotic anemones were incubated at increasing concentrations of fucoidan: 0 (FSW control), 100, 200, 400 and 800 µg/ml, for 4 h, prior to the addition of 1 µg/ml of LPS (Sigma, St. Louis, MO, USA) (dissolved in 0.1% v/v DMSO) for a further 12 h. The FSW control was also exposed to 1 µg/ml LPS for 12 hours. NO production by hosts was quantified as described below.

Quantifying colonization success and host NO production using confocal microscopy

Colonization success was assessed fluorometrically by confocal microscopy, following methods described in detail by Detournay et al (2012). Briefly, following experimental manipulation, solutions in wells containing anemones were replaced with 1 ml of relaxing solution (1:1 0.37 M $MgCl_2$: FSW). Samples were observed under a Zeiss LSM 510 Meta microscope with a 40x/0.8 water objective lens and a working distance of 0.8–3.2 mm. Before image scanning, the focal plane of the optical section was adjusted to include the gastrodermal cells within the anemone tentacle. For each experiment, all images were obtained with the same software scanning settings, including detector gain and laser intensity. *S. minutum* cells present were visualized by detecting chlorophyll autofluorescence with excitation and emission wavelengths of 543 and 600-700 nm, respectively. Fluorescence was quantified by first defining the gastrodermal tissue area within the anemone tentacles as a region of interest and then measuring the mean fluorescence intensity (MFI) for that region with the LSM 5 software. Intensity of chlorophyll autofluorescence for each pixel was measured and a threshold value corresponding to the background was defined by measuring the MFI at 600 nm of a gastrodermal region without symbionts (threshold MFI =20). Colonization success was expressed as percent of pixels with autofluorescence intensity above the threshold. In colonization experiments, each treatment represents a sample size of three anemones per treatment and time-point, with percent colonization taken as the mean of six tentacles per anemone. Three untreated symbiotic anemones (six tentacles per anemone) were examined to determine a baseline colonization level for symbiotic anemones.

The statistical significance of colonization success under the treatments described above was assessed using a mixed-effects model. As measures on multiple samples (i.e., tentacles) per anemone violate independence assumptions, a mixed effect was used, treating anemone as a random effect to account for correlation among samples with anemones. Main effects included time and treatment, and their interaction was estimated to account for differences between treatments at each time point. The full model can be written as:

$$y_{i,j} = \beta X_i + \mu_j + \epsilon_{i,j}$$

Here, $y_{i,j}$ is the logarithm of percent colonization (plus a small constant) of tentacle i within anemone j, β is a vector of effects to be estimated, X is a design matrix encoding the treatment and time point, as well as interaction term contrasts, μ_j is a normally distributed random effect for anemone j, and $\epsilon_{i,j}$ are normally distributed residuals. The model was estimated using the LME4 packages (Bates et al. 2015), for the statistical computing software R (R-Core-Team 2012) (www.R-project.org), the script and data used for statistical analyses are given in Supplementary Files 2, 3 and 4.

To measure and visualize production of NO by confocal microscopy, animals were treated as described in detail previously (Detournay et al. 2012; Detournay & Weis 2011). Animals were transferred to a microfuge tube containing 500 µl of relaxing solution and 15 µM 4-amino-5-methylamino-2,7 difluorofluorescein diacetate (DAF-FM DA, Molecular Probes, Eugene, OR, USA) with excitation and emission wavelengths of 488 and 510– 530 nm, respectively. Samples were incubated for 30 min in the dark and then rinsed twice with relaxing solution. Fluorescence of the DAF-FM DA probe was quantified as described above for chlorophyll autofluorescence quantification.

The statistical analysis of this experiment used a similar model as above, but treating the fucoidan concentration as a continuous variable and fitting a linear slope to the fluorescence intensity. We also added a random effect for tentacle within anemone to account for non-independence of readings within each tentacle. Using the notation above, the model can be written as

$$y_{k,i,j} = \alpha + \beta F_k + \mu_j + \gamma_{i(j)} + \epsilon_{k,i,j}$$

Here, $y_{k,i,j}$ is the fluorescence reading k within tentacle i of anemone j, α is the intercept and β is the slope of the regression line relating fluorescence to fucoidan concentration F_k, μ_j is a normally distributed random effect for anemone j, $\gamma_{i(j)}$ is a normally distributed random effect for tentacle i within anemone j, and $\epsilon_{k,i,j}$ are normally distributed residuals.

334 **Results**
335
336 Annotated predicted cnidarian SR proteins are illustrated according to their domain architecture
337 and compared with known human SR protein domain organization (Figure 2). Overall, cnidarian
338 SR-like proteins fall into four groups: SR-As, SR-Es, SR-Is and SR-Bs. The SRCR domain is
339 present in all groups except the SR-Bs.
340
341 *Cnidarian SRCR-containing proteins*
342 Vertebrate SR-As are defined by a collagen domain coupled with most proteins containing either
343 an SRCR domain or a CTLD at the C terminus (Bowdish & Gordon 2009). Only two sequences
344 meeting these criteria were identified in the cnidarian resources searched. Both are in *A.*
345 *digitifera* and contain a CUB domain in addition to two collagen domains and one SRCR.
346 Human SR-Es are defined by the presence of only CTLDs (Zani et al. 2015). The human lectin-
347 like oxidized low-density lipoprotein receptor 1 (LOX1) has an N-terminal cytoplasmic tail, a
348 transmembrane domain and a single C-terminal CTLD (Canton et al. 2013). Numerous LOX1-
349 like sequences were identified in all of the cnidarian resources searched. SR-Is in humans are
350 defined by containing only SRCR domains in various numbers of repeats and are grouped into
351 three classes: CD5, CD6 and CD163. SR-I-like sequences are abundant in all cnidarian
352 resources, in the same configurations as human SR-Is. SRCR repeat numbers range from one to
353 twenty-three.
354
355 A variety of SRCR-domain-containing proteins were also identified in cnidarian sequence
356 resources that could not be classified into any of the vertebrate classes of scavenger receptors.
357 Several cnidarians genes with SRCRs and CUB domains were identified that resemble 'human
358 deleted in malignant brain tumor' (DMBT) protein that contains eight SRCR repeats, a single
359 CUB domain and a zona pelucida domain at the C-terminal end. Predicted cnidarian proteins that
360 resemble DMBT contain one to three CUB domains combined with a range of other protein
361 domains, including MAM, fibronectin UBOX and multiple SRCRs. Five of the six cnidarian
362 resources contain sequences with a potentially novel domain configuration of multiple SRCRs,
363 several other domains, including multiple immunoglobulin domains, and a C-terminal trypsin
364 domain.
365
366 *Cnidarian SR-B-like proteins*
367 Searches identified eighteen full-length putative cnidarian SR-B sequences, all containing a
368 CD36 domain. Full-length proteins were defined as those containing both transmembrane
369 regions that form the SR-B extracellular loop configuration. Humans have four distinct SR-Bs -
370 CD36, SRB1 & 2, and LMP2 - while the six cnidarian species searched contained between two
371 and four full-length proteins.
372
373 *Phylogenetic analysis of SR-B-like proteins*

Phylogenetic analysis was carried out on the CD36 domains from SR-B-like sequences identified (Figure S1 and Figure 3). Protein sequence alignments of the predicted SR-B-like proteins from cnidarians, combined with a subset of vertebrate and invertebrate sequences, revealed that the CD36 domain is highly conserved across metazoans. Cnidarian sequences showed moderate homology to human SR-Bs, with 26-32%, 28-37% and 28-33% identity to human CD36, LMP2, and SR-B1, respectively. Identities within the cnidarian group were substantially higher, ranging from 39 to 95%, with the two *Acropora* species showing the highest homology to each other. Cnidarian sequences showed between 21 and 27% identity to the predicted SR-B-like protein sequence from the sponge, *Suberites domuncula.* Predicted cnidarian proteins lacked one of the three pairs of cysteine residues known to form three disulphide bridges in the human CD36 protein (Figure S1) (Silverstein and Febbraio, 2009). However, a pair of cysteine residues was found in all cnidarian study species at positions C107 and C117. Predicted cnidarian proteins had 8-10 N-linked glycosylation sites compared with eleven and eight sites in human SR-B1 and CD36, respectively.

Putative cnidarian SR-B proteins grouped with high support in a large clade with the bilaterians and separately from other basal metazoans. Within this large clade, cnidarians grouped together, forming a separate clade from the bilaterians. Within the cnidarian clade, there were three well-supported sub-clades, two containing both coral and anemone species and a third, containing only anemone sequences (Figure 3). Corals and sea anemones sequences formed distinct groupings within each of these clades. In contrast, bilaterian invertebrate sequences grouped with mammalian sequences in several different sub-clades of SR-Bs: LMP2, CD36, CD36-like, SR-B1, and SR-B1-like proteins.

Experimental blocking of SR proteins with fucoidan reduces colonization success and elicits an immune response in A. pallida

Fucoidan-treated anemones showed significantly lower levels of colonization (0-3%) than either the FSW control or anemones pre-incubated in fucoidan and then rinsed 48 h prior to time zero Colonization success decreased significantly in a dose-dependent manner (Figure 4, Bayesian $P < 0.0001$).

A second fucoidan experiment investigated the possible immune-regulation role of an SR in symbiosis maintenance. Symbiotic anemones were treated with increasing concentrations of fucoidan and were subsequently immune-challenged by incubation with LPS. The FSW control-treated anemones had low levels of NO production, a proxy for an immune response, measured as MFI of the NO-specific probe DAF-FM DA in tentacles, in response to incubation in LPS. In contrast, fucoidan-treated anemones showed a significant (Bayesian $P < 0.0001$) dose-dependent response of increasing NO production with increasing concentrations of fucoidan (Figure 5).

Discussion

An expanded SRCR-domain-containing protein repertoire in cnidarians

The SRCR-domain-containing protein repertoire in cnidarians, is expanded compared to that in humans, with the *A. pallida* genome containing the highest number at 36 genes (Figure 2). This finding is consistent with numerous other studies describing expansions of innate immune gene families in invertebrates (see Introduction). Other examples of SRCR-domain-containing protein repertoire expansion have been described in invertebrates, specifically in the sea urchin, *Strongylocentrotus purpuratus* and the cephalochordate *Branchiostoma floridae*, which have 218 and 270 SRCR-containing sequences respectively (Huang et al. 2008; Pancer 2000; Pancer et al. 1999; Rast & Messier-Solek 2008). These numbers are high compared to the 16 genes present in humans. In addition, cnidarian SRCR-domain-containing proteins include a variety of genes with novel domain combinations that have not been found in other organisms (Figure 2). Identification of these novel domain combinations in cnidarian immune gene repertoires is consistent with other studies of basal metazoan immune genes (Hamada et al. 2013; Poole & Weis 2014; Ryu et al. 2016). The searches for SR genes in the three transcriptomes (Table 1) likely revealed underestimates of the total SR repertoire, given that transcriptomes represent snapshots of the whole genome.

CTLD-domain-containing SRs in cnidarians

In contrast to the human genome, which contains a single LOX1 gene, all six cnidarian resources searched contained multiple LOX1-like SR-Es (Figure 2). These searches add to previous characterizations of lectin-like proteins in cnidarians, including in corals and sea anemones (Jimbo et al. 2005; Jimbo et al. 2000; Kvennefors et al. 2010; Kvennefors et al. 2008; Meyer & Weis 2012; Vidal-Dupiol et al. 2009; Wood-Charlson & Weis 2009). Human LOX1 has a diversity of signalling functions, including in recognition of microbes *via* host CTLD-microbe glycan binding: a PRR-MAMP interaction (Canton et al. 2013). In cnidarians, previous studies have detailed a role for lectin-glycan interactions in the establishment of cnidarian-dinoflagellate symbioses (reviewed in Davy et al. 2012). The identification of multiple LOX1-like proteins and several other CTLD-containing proteins with novel domain combinations across the six species examined further strengthens the hypothesis that host CTLD-symbiont glycan binding plays an important role in host innate immunity and host-symbiont recognition. Cnidarian CTLD-domain-containing proteins described here provide potential target proteins for future experimental investigation of the lectin-glycan interactions.

CD36-domain-containing SRs in cnidarians

Phylogenetic analysis of metazoan CD36 domains from SR-B homologues showed a well-supported clade of cnidarian sequences (Figure 3). A large analysis including additional sequences from basal metazoans is required to more definitively reveal deep branching patterns of this gene. The observed differing location of cysteine pairs within the CD36 domain in cnidarian sequences compared to vertebrate ones also occurred in other invertebrates (Figure

S1). As with the cnidarians searched, *C. elegans* contained one differing pair and the three sponges, *Oscarella carmella*, *S. domuncula*, and *Amphimedon queenslandica*, and the ctenophore *Mnemiopsis leidyi* had no sequence pairs in common with vertebrates. These differences may explain why antibodies to human and mouse SR-B1 and CD36 failed to label proteins in *A. pallida* in immunoblot experiments (E.F. Neubauer, unpublished data).

Functional experiments suggest that blocking SRs decreases colonization success and increases the stress response to immune challenge in A. pallida

Colonization success in aposymbiotic *A. pallida* challenged with *S. minutum* CCMP830 displayed a dose-dependent response to incubation in the SR inhibitor fucoidan, exhibiting decreasing colonization success with increasing concentrations of fucoidan (Figure 4). In vertebrates, fucoidan blocks the positively-charged ligand binding sites on SR-As and SR-Bs, and can thereby block phagocytic activity in macrophages (Dinguirard & Yoshino 2006; Hsu et al. 2001; Li et al. 2008). The observed inhibition of colonization in cnidarians suggests that phagocytosis of symbionts is likewise inhibited and provides evidence that one or multiple SRs with SRCR and/or CD36 domains function in host-symbiont recognition during onset of symbiosis.

Previous transcriptomic studies in *A. elegantissima* and *A. pallida* have found SR-B homologues to be upregulated in symbiotic compared to aposymbiotic anemones, suggesting that they play a role in the symbiosis. Our experiments showing that incubation in fucoidan causes a dose-dependent immune response in symbiotic *A. pallida* (Figure 5), further implicates a role for SRs in immune tolerance and regulation of symbiosis. In previous work on *A. pallida,* we showed that symbiotic anemones produced significantly less NO in response to an immune challenge with LPS than did aposymbiotic animals, suggesting that symbionts are modulating the host immune response (Detournay et al., 2012). The increase in this response in symbiotic anemones incubated in fucoidan suggests that this immune modulation involves an SR ligand-binding domain. Such a response is reminiscent of immune modulation by a variety of invading microbes (Janeway & Medzhitov 2002).

In summary, this study provides the first description of the diversity of SRs in cnidarians. Members include proteins with domain combinations that are highly similar to those in vertebrates as well as those that possess novel combinations. Initial functional experiments using the SR inhibitor fucoidan suggest that SRs play a role in the regulation of cnidarian-dinoflagellate symbioses. Future functional studies on candidate SRs identified in this study can further explore their role in cnidarian immunity and symbiosis.

Acknowledgements

We thank Eli Meyer for the reassembly of the *A. pallida* transcriptome and Philipp Neubauer for assistance with statistical analysis. We wish to acknowledge the Confocal Microscopy Facility at the Center for Genome Research and Biocomputing at Oregon State University.

498 **References**

499

500 Abascal F, Zardoya R, and Posada D. 2005. ProtTest: selection of best-fit models of protein
501 evolution. *Bioinformatics* 21:2104-2105. DOI:
502 Areschoug T, and Gordon S. 2009. Scavenger receptors: role in innate immunity and microbial
503 pathogenesis. *Cellular Microbiology* 11:1160-1169. DOI: 10.1111/j.1462-
504 5822.2009.01326.x
505 Areschoug T, and Waldemarsson J. 2008. Evasion of macrophage scavenger receptor A-
506 mediated recognition by pathogenic Streptococci. *EMBO Journal* 38:3068-3079. DOI:
507 10.1002/eji.200838457
508 Bates D, Maechler M, Ben B, and Walker S. 2015. Fitting linear mixed-effects models using
509 lme4. *Journal of Statistical Software* 67:1-48. DOI: 10.18637/jss.v067.i0
510 Baumgarten S, Simakov O, Esherick LY, Liew YJ, Lehnert EM, Michell CT, Li Y, Hambleton
511 EA, Guse A, Oates ME, Gough J, Weis VM, Aranda M, Pringle JR, and Voolstra CR.
512 2015. The genome of *Aiptasia*, a sea anemone model for coral symbiosis. *Proceedings of*
513 *the National Academy of Sciences* 112:11893-11898. DOI: 10.1073/pnas.1513318112
514 Bordenstein SR, and Theis KR. 2015. Host Biology in Light of the Microbiome: Ten Principles
515 of Holobionts and Hologenomes. *PLoS Biol* 13:e1002226. DOI:
516 10.1371/journal.pbio.1002226
517 Bowdish DME, and Gordon S. 2009. Conserved domains of the class A scavenger receptors:
518 evolution and function. *Immunological Reviews* 227:19-31. DOI: 10.1111/j.1600-
519 065X.2008.00728.x
520 Buckley KM, and Rast JP. 2015. Diversity of animal immune receptors and the origins of
521 recognition complexity in the deuterostomes. *Developmental and Comparative*
522 *Immunology* 49:179-189. DOI: 10.1016/j.dci.2014.10.013
523 Cambi A, Koopman M, and Figdor CG. 2005. How C-type lectins detect pathogens. *Cellular*
524 *Microbiology* 7:481-488. DOI: 10.1111/j.1462-5822.2005.00506.x
525 Canton J, Neculai D, and Grinstein S. 2013. Scavenger receptors in homeostasis and immunity.
526 *Nature Reviews Immmunology* 13:621-634. DOI: 10.1038/nri3515
527 Catanese MT, Graziani R, von Hahn T, Moreau M, Huby T, Paonessa G, Santini C, Luzzago A,
528 Rice CM, Cortese R, Vitelli A, and Nicosia A. 2007. High-avidity monoclonal antibodies
529 against the human scavenger class B type I receptor efficiently block hepatitis C virus
530 infection in the presence of high-density lipoprotein. *Journal of Virology* 81:8063-8071.
531 DOI: 10.1128/JVI.00193-07
532 Davy SK, Allemand D, and Weis VM. 2012. Cell biology of cnidarian-dinoflagellate symbiosis.
533 *Microbiology and Molecular Biology Reviews* 76:229-261. DOI: 10.1128/mmbr.05014-
534 11
535 Detournay O, Schnitzler CE, Poole A, and Weis VM. 2012. Regulation of cnidarian–
536 dinoflagellate mutualisms: Evidence that activation of a host TGFβ innate immune
537 pathway promotes tolerance of the symbiont. *Developmental and Comparative*
538 *Immunology* 38:525-537. DOI: 10.1016/j.dci.2012.08.008
539 Detournay O, and Weis VM. 2011. Role of the sphingosine rheostat in the regulation of
540 cnidarian-dinoflagellate symbioses. *Biological Bulletin* 221:261-269. DOI:
541 Dinguirard N, and Yoshino TP. 2006. Potential role of a CD36-like class B scavenger receptor in
542 the binding of modified low-density lipoprotein (acLDL) to the tegumental surface of

Schistosoma mansoni sporocysts. *Molecular Biochemical Parasitology* 146:219-230. DOI: 10.1016/j.molbiopara.2005.12.010

Faure M, and Rabourdin-Combe C. 2011. Innate immunity modulation in virus entry. *Current Opinion in Virology* 1:6-12. DOI: 10.1016/j.coviro.2011.05.013

Felsenstein J. 2005. SEQBOOT—bootstrap, jackknife or permutation resampling of molecular sequence, restriction site, gene frequency or character data. University of Washington, Seattle.

Fuess LE, Pinzón C JH, Weil E, and Mydlarz LD. 2016. Associations between transcriptional changes and protein phenotypes provide insights into immune regulation in corals. *Developmental & Comparative Immunology* 62:17-28. DOI: 10.1016/j.dci.2016.04.017

Gordon S. 2002. Pattern recognition receptors doubling up for the innate immune response. *Cell* 111:927-930. DOI: 10.1016/S0092-8674(02)01201-1

Gough PJ, and Gordon S. 2000. The role of scavenger receptors in the innate immune system. *Microbes and Infection* 2:305-311. DOI: 10.1016/S1286-4579(00)00297-5

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, and Regev A. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 29:644-652. DOI: 10.1038/nbt.1883

Guindon S, Lethiec F, Duroux P, and Gascuel O. 2005. PHYML Online—a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* 33:W557-W559. DOI: 10.1093/nar/gki352

Hamada M, Shoguchi E, Shinzato C, Kawashima T, Miller DJ, and Satoh N. 2013. The complex NOD-like receptor repertoire of the coral *Acropora digitifera* includes novel domain combinations. *Molecular Biology and Evolution* 30:167-176. DOI: 10.1093/molbev/mss213

Hibino T, Loza-Coll M, Messier C, Majeske AJ, Cohen AH, Terwilliger DP, Buckley KM, Brockton V, Nair SV, Berney K, Fugmann SD, Anderson MK, Pancer Z, Cameron RA, Smith LC, and Rast JP. 2006. The immune gene repertoire encoded in the purple sea urchin genome. *Developmental Biology* 300:349-365. DOI: 10.1016/j.ydbio.2006.08.065

Hohenester E, Sasaki T, and Timpl R. 1999. Crystal structure of a scavenger receptor cysteine-rich domain sheds light on an ancient superfamily. *Nature Structural Biology* 6:228-232. DOI: 10.1038/6669

Hsu HY, Chiu SL, Wen MH, Chen KY, and Hua KF. 2001. Ligands of macrophage scavenger receptor induce cytokine expression via differential modulation of protein kinase signaling pathways. *Journal of Biological Chemistry* 276:28719-28730. DOI: 10.1074/jbc.M011117200

Huang S, Yuan S, Guo L, Yu Y, Li J, and Wu T. 2008. Genomic analysis of the immune gene repertoire of amphioxus reveals extraordinary innate complexity and diversity. *Genome Research* 18:1112-1126. DOI: 10.1101/gr.069674.107

Janeway CA, and Medzhitov R. 2002. Innate immune recognition. *Annual Review of Immunology* 20:197-216. DOI:

Jimbo M, Koike K, Sakai R, Muramoto K, and Kamiya H. 2005. Cloning and characterization of a lectin from the octocoral *Sinularia lochmodes*. *Biochemical and Biophysical Research Communications* 330:157-162. DOI:

588 Jimbo M, Yanohara T, Koike K, Koike K, Sakai R, Muramoto K, and Kamiya H. 2000. The D-
589 galactose-binding lectin of the octocoral *Sinularia lochmodes*: characterization and
590 possible relationship to the symbiotic dinoflagellates. *Comparative Biochemistry and*
591 *Physiology Part B: Biochemistry and Molecular Biology* 125:227-236. DOI:
592 Katoh K, Misawa K, Kuma K-i, and Miyata T. 2002. MAFFT: a novel method for rapid multiple
593 sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30:3059-
594 3066. DOI:
595 Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A,
596 Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, and Drummond A. 2012.
597 Geneious Basic: an integrated and extendable desktop software platform for the
598 organization and analysis of sequence data. *Bioinformatics* 28:1647-1649. DOI:
599 Kitchen SA, Crowder CM, Poole AZ, Weis VM, and Meyer E. 2015. *De novo* assembly and
600 characterization of four anthozoan (Phylum Cnidaria) transcriptomes. *G3:*
601 *Genes|Genomes|Genetics* 5:2441-2452. DOI: 10.1534/g3.115.020164
602 Krieger M. 1992. Molecular flypaper and atherosclerosis: structure of the macrophage scavenger
603 receptor. *Trends in Biochemical Sciences* 17:141-146. DOI: 10.1016/0968-
604 0004(92)90322-Z
605 Krieger M. 2001. Scavenger receptor class B type I is a multiligand HDL receptor that influences
606 diverse physiologic systems. *Journal of Clinical Investigation* 108:793-797. DOI:
607 10.1172/JCI14011
608 Kvennefors EC, Leggat W, Kerr CC, Ainsworth TD, Hoegh-Guldberg O, and Barnes AC. 2010.
609 Analysis of evolutionarily conserved innate immune components in coral links immunity
610 and symbiosis. *Developmental and Comparative Immunology* 34:1219-1229. DOI:
611 10.1016/j.dci.2010.06.016
612 Kvennefors ECE, Leggat W, Hoegh-Guldberg O, Degnan BM, and Barnes AC. 2008. An ancient
613 and variable mannose-binding lectin from the coral *Acropora millepora* binds both
614 pathogens and symbionts. *Developmental and Comparative Immunology* 32:1582-1592.
615 DOI: 10.1016/j.dci.2008.05.010
616 Lehnert E, Burriesci M, and Pringle J. 2012. Developing the anemone *Aiptasia* as a tractable
617 model for cnidarian-dinoflagellate symbiosis: the transcriptome of aposymbiotic *A.*
618 *pallida*. *BMC Genomics* 13:271. DOI: 10.1186/1471-2164-13-271
619 Lehnert EM, Mouchka ME, Burriesci MS, Gallo ND, Schwarz JA, and Pringle JR. 2014.
620 Extensive differences in gene expression between symbiotic and aposymbiotic
621 Cnidarians. *G3: Genes|Genomes|Genetics* 4:277-295. DOI: 10.1534/g3.113.009084
622 Li B, Lu F, Wei X, and Zhao R. 2008. Fucoidan: structure and bioactivity. *Molecules* 13:1671-
623 1695. DOI: 10.3390/molecules13081671
624 Martinez VG, Moestrup SK, Holmskov U, Mollenhauer J, and Lozano F. 2011. The conserved
625 scavenger receptor cysteine-rich superfamily in therapy and diagnosis. *Pharmacological*
626 *Reviews* 63:967-1000. DOI: 10.1124/pr.111.004523
627 McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier
628 N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer
629 N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG,
630 Rumpho M, Sanders JG, Tautz D, and Wernegreen JJ. 2013. Animals in a bacterial
631 world, a new imperative for the life sciences. *Proceedings of the National Academy of*
632 *Sciences* 110:3229-3236. DOI: 10.1073/pnas.1218525110

633 Meyer E, and Weis VM. 2012. Study of cnidarian-algal symbiosis in the 'omics' age. *Biological*
634 *Bulletin* 223:44-65. DOI:
635 Miller D, Hemmrich G, Ball E, Hayward D, Khalturin K, Funayama N, Agata K, and Bosch T.
636 2007. The innate immune repertoire in Cnidaria - ancestral complexity and stochastic
637 gene loss. *Genome Biology* 8:R59. DOI:
638 Moya A, Huisman L, Ball E, Hayward D, Grasso L, Chua C, Woo H, GATTUSO JP, Forêt S,
639 and Miller D. 2012. Whole transcriptome analysis of the coral *Acropora millepora*
640 reveals complex responses to CO_2-driven acidification during the initiation of
641 calcification. *Molecular Ecology* 21:2440-2454. DOI: 10.1111/j.1365-
642 294X.2012.05554.x
643 Ndungu FM, Urban BC, Marsh K, and Langhorne J. 2005. Regulation of immune response by
644 *Plasmodium*-infected red blood cells. *Parasite Immunololgy* 27:373-384. DOI:
645 10.1073/pnas.230096397
646 Pancer Z. 2000. Dynamic expression of multiple scavenger receptor cysteine-rich genes in
647 coelomocytes of the purple sea urchin. *Proceedings of the National Academy of Sciences*
648 97:13156-13161. DOI: 10.1073/pnas.230096397
649 Pancer Z, Munkner J, Muller I, and Muller W. 1997. A novel member of an ancient superfamily:
650 sponge (*Geodia cydonium*, Porifera) putative protein that features scavenger receptor
651 cysteine-rich repeats. *Gene* 193:211-218. DOI: 10.1016/S0378-1119(97)00135-2
652 Pancer Z, Rast JP, and Davidson EH. 1999. Origins of immunity: transcription factors and
653 homologues of effector genes of the vertebrate immune system expressed in sea urchin
654 coelomocytes. *Immunogenetics* 49:773-786. DOI: doi:10.1007/s002510050551
655 Perez S, and Weis VM. 2006. Nitric oxide and cnidarian bleaching: An eviction notice mediates
656 the breakdown of symbiosis. *Journal of Experimental Biology* 209:2804-2810. DOI:
657 Poole AZ, and Weis VM. 2014. TIR-domain-containing protein repertoire of nine anthozoan
658 species reveals coral–specific expansions and uncharacterized proteins. *Developmental*
659 *and Comparative Immunology* 46:480-488. DOI: 10.1016/j.dci.2014.06.002
660 Price MN, Dehal PS, and Arkin AP. 2010. FastTree 2 – Approximately Maximum-Likelihood
661 Trees for Large Alignments. *PLoS One* 5:e9490. DOI: 10.1371/journal.pone.0009490
662 Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H,
663 Lindquist E, Kapitonov VV, Jurka J, Genikhovich G, Grigoriev IV, Lucas SM, Steele
664 RE, Finnerty JR, Technau U, Martindale MQ, and Rokhsar DS. 2007. Sea anemone
665 genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science*
666 317:86-94. DOI: 10.1126/science.1139158
667 Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, and Lopez R. 2005.
668 InterProScan: protein domains identifier. *Nucleic Acids Research* 33:W116-W120. DOI:
669 10.1093/nar/gki442
670 R-Core-Team. 2012. R: A language and environment for statistical computing. R Foundation for
671 Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www. R-
672 project. org.
673 Rast JP, and Messier-Solek C. 2008. Marine invertebrate genome sequences and our evolving
674 understanding of animal immunity. *Biological Bulletin* 214:274-283. DOI:
675 Rodrigues CD, Hannus M, Prudêncio M, Martin C, Gonçalves LA, Portugal S, Epiphanio S,
676 Akinc A, Hadwiger P, Jahn-Hofmann K, Röhl I, van Gemert G-J, Franetich J-F, Luty
677 AJF, Sauerwein R, Mazier D, Koteliansky V, Vornlocher H-P, Echeverri CJ, and Mota
678 MM. 2008. Host scavenger receptor SR-BI plays a dual role in the establishment of

679 malaria parasite liver infection. *Cell Host & Microbe* 4:271-282. DOI:
680 10.1016/j.chom.2008.07.012
681 Rodriguez-Lanetty M, Phillips W, and Weis V. 2006. Transcriptome analysis of a cnidarian -
682 dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC*
683 *Genomics* 7:23. DOI: 10.1186/1471-2164-7-23
684 Ryu T, Seridi L, Moitinho-Silva L, Oates M, Liew YJ, Mavromatis C, Wang X, Haywood A,
685 Lafi FF, Kupresanin M, Sougrat R, Alzahrani MA, Giles E, Ghosheh Y, Schunter C,
686 Baumgarten S, Berumen ML, Gao X, Aranda M, Foret S, Gough J, Voolstra CR,
687 Hentschel U, and Ravasi T. 2016. Hologenome analysis of two marine sponges with
688 different microbiomes. *BMC Genomics* 17:1-11. DOI: 10.1186/s12864-016-2501-0
689 Sarrias M, Grønlund J, and Padilla O. 2004. The scavenger receptor cysteine-rich (SRCR)
690 domain: an ancient and highly conserved protein module of the innate immune system.
691 *Critical Reviews in Immunology* 24:1-37. DOI: 10.1615/CritRevImmunol.v24.i1.10
692 Schneider DS, and Ayres JS. 2008. Two ways to survive infection: what resistance and tolerance
693 can teach us about treating infectious diseases. *Nature Reviews Immunology* 8:889-895.
694 DOI: 10.1038/nri2432
695 Schwarz RS, Hodes-Villamar L, Fitzpatrick KA, Fain MG, Hughes AL, and Cadavid LF. 2007.
696 A gene family of putative immune recognition molecules in the hydroid *Hydractinia*.
697 *Immunogenetics* 59:233-246. DOI: 10.1007/s00251-006-0179-1
698 Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M,
699 Koyanagi R, Ikuta T, Fujiyama A, Miller DJ, and Satoh N. 2011. Using the *Acropora*
700 *digitifera* genome to understand coral responses to environmental change. *Nature*
701 476:320-323. DOI: 10.1038/nature10249
702 Silverstein RL, and Febbraio M. 2009. CD36, a scavenger receptor involved in immunity,
703 metabolism, angiogenesis, and behavior. *Science Signaling* 2:re3. DOI:
704 10.1126/scisignal.272re3
705 Thelen T, Hao Y, Medeiros AI, Curtis JL, Serezani CH, Kobzik L, Harris LH, and Aronoff DM.
706 2010. The class A scavenger receptor, macrophage receptor with collagenous structure is
707 the major phagocytic receptor for *Clostridium sordellii* expressed by human decidual
708 macrophages. *Journal of Immunology* 185:4328-4335. DOI: 10.4049/jimmunol.1000989
709 Vidal-Dupiol J, Adjeroud M, Roger E, Foure L, Duval D, Mone Y, Ferrier-Pages C, Tambutte E,
710 Tambutte S, Zoccola D, Allemand D, and Mitta G. 2009. Coral bleaching under thermal
711 stress: putative involvement of host/symbiont recognition mechanisms. *BMC*
712 *Physiolology* 9:14. DOI: 10.1186/1472-6793-9-14
713 Weis VM, and Allemand D. 2009. What determines coral health? *Science* 324:1153-1155. DOI:
714 10.1126/science.1172540
715 Wood-Charlson EM, and Weis VM. 2009. The diversity of C-type lectins in the genome of a
716 basal metazoan, *Nematostella vectensis*. *Developmental and Comparative Immunology*
717 33:881-889. DOI: 10.1016/j.dci.2009.01.008
718 Yellowlees D, Rees TAV, and Leggat W. 2008. Metabolic interactions between algal symbionts
719 and invertebrate hosts. *Plant Cell & Environment* 31:679-694. DOI: doi:10.1111/j.1365-
720 3040.2008.01802.x
721 Yuen B, Bayes JM, and Degnan SM. 2014. The Characterization of Sponge NLRs Provides
722 Insight into the Origin and Evolution of This Innate Immune Gene Family in Animals.
723 *Molecular Biology and Evolution* 31:106-120. DOI: 10.1093/molbev/mst174

724 Zani IA, Stephen SL, Mughal NA, Russell D, Homer-Vanniasinkam S, Wheatcroft SB, and
725 Ponnambalam S. 2015. Scavenger receptor structure and function in health and disease.
726 *Cells* 4:178-201. DOI: 10.3390/cells4020178
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729 **Table 1.** Information on the cnidarian sequence resources used in this study. Non-symbiotic
730 refers to species that do not form symbioses with dinoflagellates. Aposymbiotic refers to species
731 that do form symbioses but the material from which the sequencing was performed did not
732 contain symbionts.
733

Organism	Developmental stage	Symbiotic state	Data type	Reference
Nematostella vectensis	Larvae	Non-symbiotic	Genome	(Putnam et al. 2007)
Anthopleura elegantissima	Adult	Aposymbiotic	Transcriptome	(Kitchen et al. 2015)
Aiptasia pallida	Adult	Aposymbiotic	Transcriptome	(Lehnert et al. 2012)
Aiptasia pallida	Adult	Aposymbiotic	Genome	(Baumgarten et al. 2015)
Acropora digitifera	Sperm	Aposymbiotic	Genome	(Shinzato et al. 2011)
Acropora millepora	Adult and larvae	Symbiotic	Transcriptome	(Moya et al. 2012)
Fungia scutaria	Larvae	Aposymbiotic	Transcriptome	(Kitchen et al. 2015)

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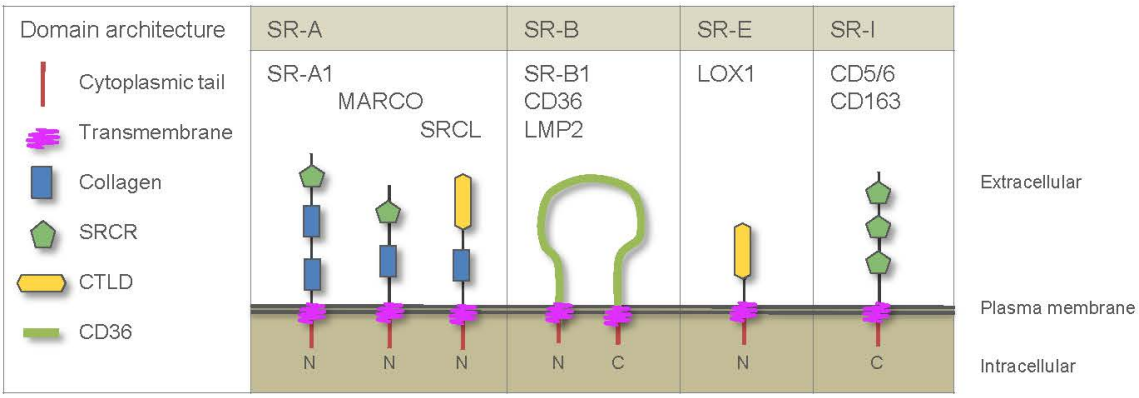
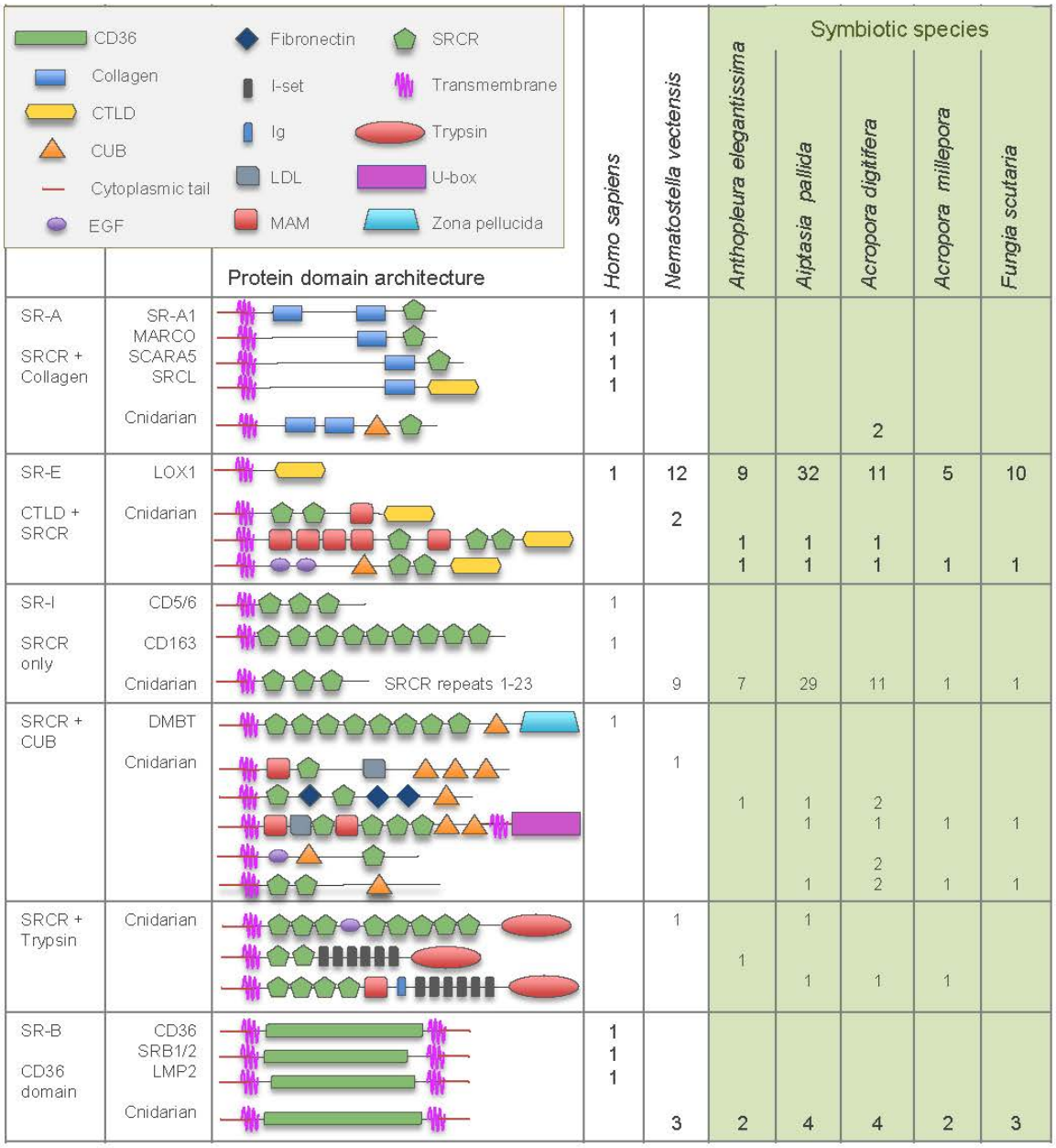
737 **Figure Legends**
738
739 **Figure 1.** Domain architecture of vertebrate SRs relevant to this study. All SR sequences are
740 anchored in the membrane with one or two transmembrane domains. All have very short
741 cytoplasmic tails and extensive extracellular ligand-binding domains. SR-As contain a collagen
742 domain(s) and can include an SRCR or a CTLD. SR-Bs have two cytoplasmic tails on either side
743 of a CD36 domain that forms an extracellular loop. SR-Es are defined by the presence of a
744 CTLD. SR-Is have multiple SRCR repeats and no other identifiable extracellular domains. C,
745 carboxy terminus; CTLD, C type lectin domain; LOX1, lectin-like oxidized low density
746 lipoprotein receptor 1; MARCO, macrophage receptor with collagenous structure; N, amino
747 terminus; SRCL, scavenger receptor with C-type lectin; SRCR, scavenger receptor cysteine-rich
748 domain.
749
750 **Figure 2.** Domain architecture of cnidarian SR domains in the six resources searched compared
751 to human SRs. Identified cnidarian SR-A-like and SR-E-like sequences display diverse domain
752 architecture and include novel domain combinations not found in vertebrates. SR-I-like
753 sequences had a varying number of SRCR repeats. A variety of SRCR-domain-containing
754 cnidarian sequences identified did not fit the criteria of any vertebrate SR classes and are
755 presented as SRCR + CUB domains or SRCR + trypsin domains. SR-B-like domain
756 combinations closely resembled vertebrate SR-Bs with two transmembrane domains, two
757 cytoplasmic tails and a CD36 domain. CTLD, C type lectin domain; CUB, complement C1r/C1s,
758 Uegf, BMP1; DMBT, deleted in malignant brain tumor protein; Ig, immunoglobulin; I-Set,
759 intermediate set of immunoglobulin domain; LDL, low density lipoprotein; LOX1, lectin-like
760 oxidized low density lipoprotein receptor 1; EGF, epidermal growth factor; MAM meprin/A5-
761 protein/PTPmu; MARCO, macrophage receptor with collagenous structure; SCARA5, scavenger
762 receptor class A member 5; SRCL, scavenger receptor with C-type lectin; SRCR, scavenger
763 receptor cysteine-rich domain; U-box, ubiquitin box. Human SR data taken from Canton et al
764 (2013). (See Supplementary File 1 for sequence information.)
765
766 **Figure 3.** Maximum-likelihood tree of SR-Bs from across the Metazoa. The tree was constructed
767 with the CD36 domain of each protein using FastTree v 2.1.5. Bootstrap support values were
768 generated using SEQBOOT, values above 0.6 are displayed at nodes. The alignment, including
769 organism names, is displayed in Figure S1.
770
771 **Figure 4.** Experimental colonization by *S. minutum* CCMP830 of aposymbiotic *A. pallida*
772 treated with increasing levels of the SR inhibitor, fucoidan. Graph shows percent colonization
773 success as measured by surface area of host gastrodermis occupied by symbionts (see Methods
774 for details) as a function of time after inoculation. Two controls were included: FSW alone and
775 an 18 h incubation in 200 µg/ml fucoidan in FSW followed by a 48 h recovery in FSW to test for
776 fucoidan toxicity to the animals. Anemones in experimental fucoidan treatments exhibited a

777 dose-dependent response with decreased colonization success with increasing fucoidan
778 concentrations. Bars represent means ± SD, n = 3 anemones per treatment. Asterisks indicate
779 high ($p > 0.999$) posterior probability of treatment effects being different from controls under the
780 Bayesian ANOVA model.
781
782 **Figure 5.** Effect of SR inhibition by fucoidan on immune stimulation in symbiotic *A. pallida*.
783 Immune stimulation of animals was elicited by incubation in 1 µg/ml LPS overnight prior to the
784 experiment. Immune stimulation was measured by quantifying DAF-FM DA, a probe for the
785 presence NO, itself a marker for immune stress. Graph shows MFI of DAF-FM DA in tentacles
786 in response to incubation in increasing concentrations of fucoidan. Animals exhibited a
787 significant dose-dependent response to fucoidan (Bayesian generalized linear mixed model, $P <$
788 0.0001), with increasing NO production with increasing SR inhibition by fucoidan. Bars
789 represent means ± SD; n = 3 anemones. Inset: representative confocal images of tentacles
790 incubated in FSW only and 800 µg/ml fucoidan. DAF-FM DA (green) symbiont
791 autofluorescence (red).
792
793

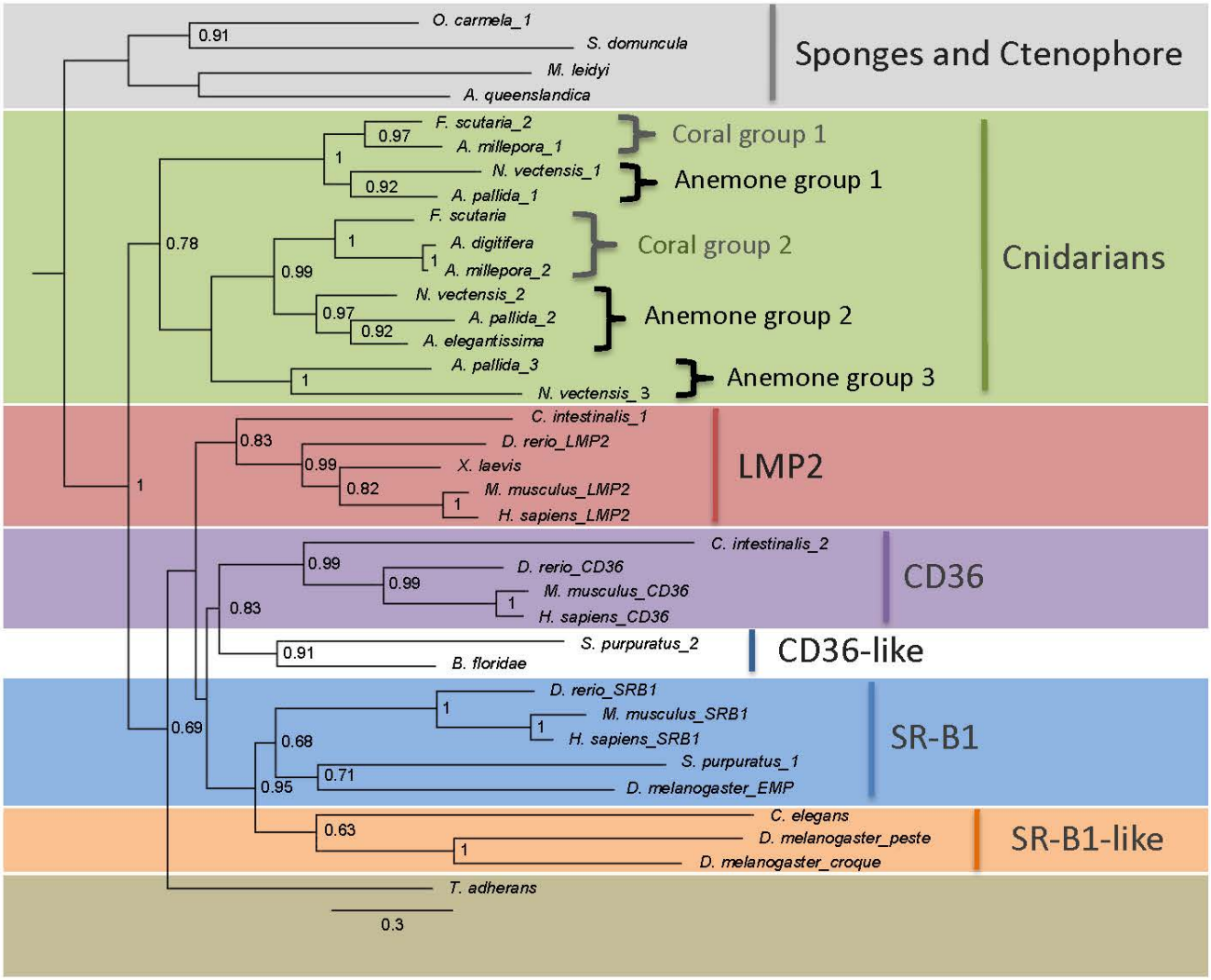


794
795 Figure 1
796

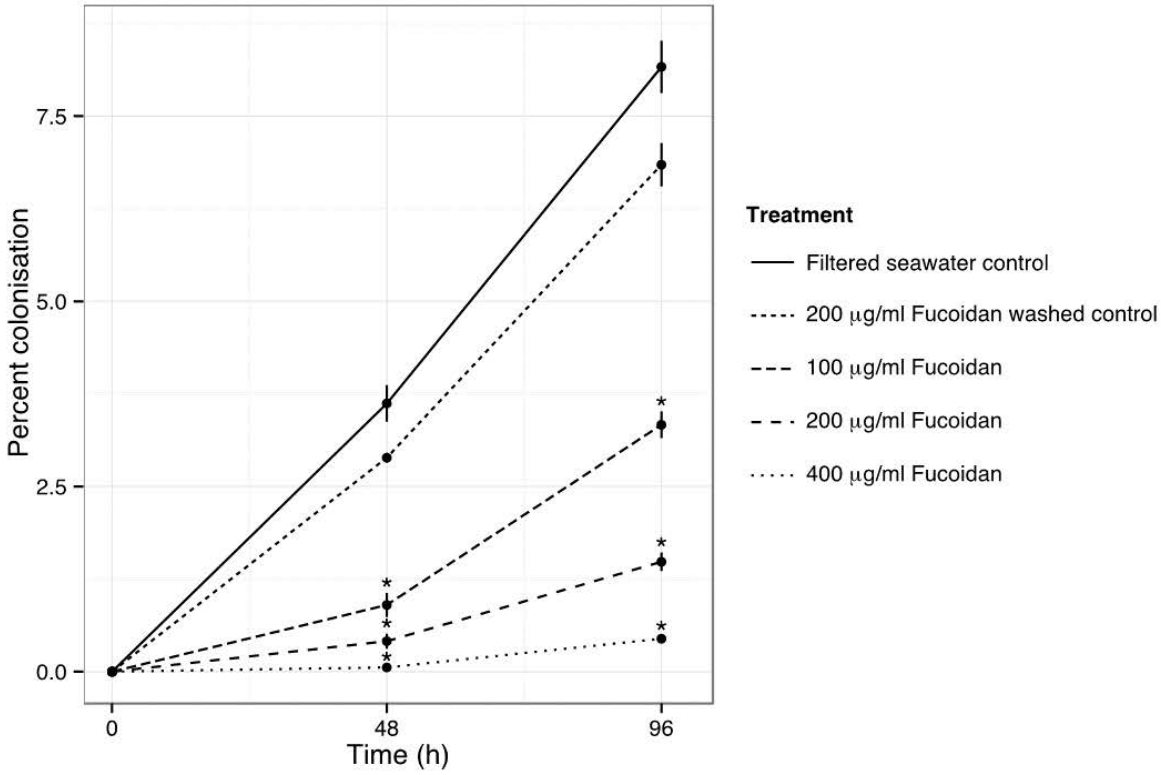


		Protein domain architecture	*Homo sapiens*	*Nematostella vectensis*	Symbiotic species: *Anthopleura elegantissima*	*Aiptasia pallida*	*Acropora digitifera*	*Acropora millepora*	*Fungia scutaria*
SR-A SRCR + Collagen	SR-A1 MARCO SCARA5 SRCL Cnidarian		1 1 1 1				2		
SR-E CTLD + SRCR	LOX1 Cnidarian		1	12 2	9 1 1	32 1 1	11 1 1	5 1	10 1
SR-I SRCR only	CD5/6 CD163 Cnidarian	SRCR repeats 1-23	1 1	9	7	29	11	1	1
SRCR + CUB	DMBT Cnidarian		1	1	1	1 1 1	2 1 2 2	1 1	1 1
SRCR + Trypsin	Cnidarian			1	1	1 1	1	1	
SR-B CD36 domain	CD36 SRB1/2 LMP2 Cnidarian		1 1 1	3	2	4	4	2	3

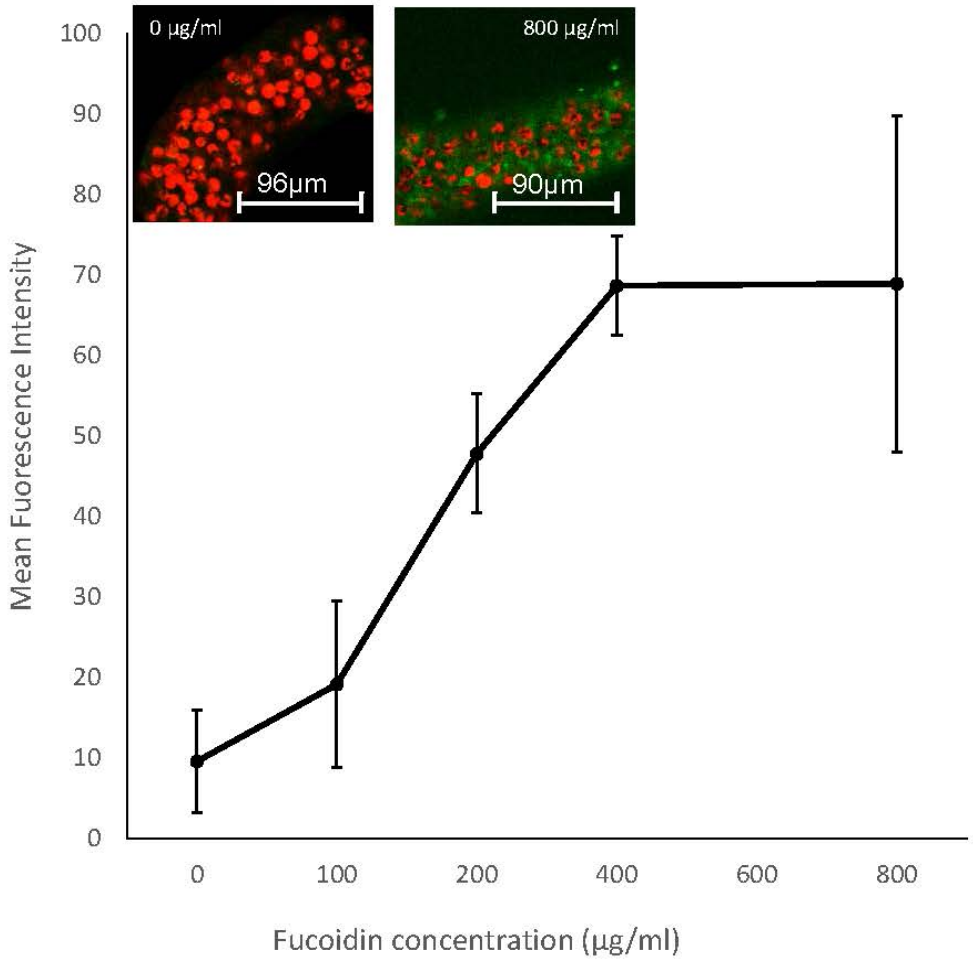
Figure 2



800
801 Figure 3
802



803
804 Figure 4
805



806
807 Figure 5