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2 **Single cell lineage dynamics of the endosymbiotic cell type in a soft coral *Xenia* species**

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1 **Abstract:**
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3 **Many hard and soft corals harbor algae for photosynthesis. The algae live inside coral cells**
4 **in a specialized membrane compartment called symbosome, which shares the**
5 **photosynthetically fixed carbon with coral host cells, while host cells provide inorganic**
6 **carbon for photosynthesis¹. This endosymbiotic relationship is critical for corals, but**
7 **increased environmental stresses are causing corals to expel their endosymbiotic algae, i.e.**
8 **coral bleaching, leading to coral death and degradation of marine ecosystem². To date, the**
9 **molecular pathways that orchestrate algal recognition, uptake, and maintenance in coral**
10 **cells remain poorly understood. We report chromosome-level genome assembly of a fast-**
11 **growing soft coral, *Xenia* species (sp.)³, and its use as a model to decipher the coral-algae**
12 **endosymbiosis. Single cell RNA-sequencing (scRNA-seq) identified 13 cell types, including**
13 **gastrodermis and cnidocytes, in *Xenia* sp.. Importantly, we identified the endosymbiotic cell**
14 **type that expresses a unique set of genes implicated in the recognition,**
15 **phagocytosis/endocytosis, maintenance of algae, and host coral cell immune modulation. By**
16 **applying scRNA-seq to investigate algal uptake in our new *Xenia* sp. regeneration model,**
17 **we uncovered a dynamic lineage progression from endosymbiotic progenitor state to**
18 **mature endosymbiotic and post-endosymbiotic cell states. The evolutionarily conserved**
19 **genes associated with the endosymbiotic process reported herein open the door to decipher**
20 **common principles by which different corals uptake and expel their endosymbionts. Our**
21 **study demonstrates the potential of single cell analyses to examine the similarities and**
22 **differences of the endosymbiotic lifestyle among different coral species.**

23
24 Similar to other endosymbiotic cnidarian, corals take up the *Symbiodiniaceae* family of
25 dinoflagellate algae into their gastrodermis through feeding. Some cells in the gastrodermis,
26 lining the digestive tract, appear to have the ability to recognize certain types of algae. Through
27 phagocytosis and by modulating host immune responses, the correct alga type is preserved and
28 enclosed by endomembranes to form symbiosomes in coral cells¹. The symbosome membrane is
29 believed to contain transporters that mediate nutrient exchange between the algae and host cells⁴.
30 In recent years, many comparative transcriptome analyses were performed on whole organisms
31 to identify gene expression changes before and after algae colonization or bleaching using

1 different cnidarian species. This led to the identification of genes whose up- or down-regulation
2 could contribute to endosymbiosis. Comparative genomic and transcriptomic information in
3 endosymbiotic and non-endosymbiotic cnidaria species were also used to search for genes that
4 may have evolved to mediate recognition or endocytosis of *Symbiodiniaceae*⁵⁻⁸. These
5 approaches, however, do not differentiate whether the altered genes are expressed in the host
6 endosymbiotic cells or other cell types without additional criteria. Protein inhibition or activation
7 studies were also used to suggest the host proteins containing C-type lectin domains, scavenger
8 receptor domains, or thrombospondin type 1 repeats to be involved in algal uptake and
9 immunosuppression⁹⁻¹¹. The broad expression and function of these proteins coupled with
10 potential off-target effects of inhibitors greatly limit data interpretation. Therefore, a systematic
11 description of genes and pathways selectively expressed in the host endosymbiotic cells is much
12 needed to begin to understand the potential regulatory mechanisms underlying the entry,
13 establishment, and possibly expulsion of *Symbiodiniaceae*.

14 To enable systematic study of coral biology, we reason that it is important to first define
15 gene expression characteristics of each cell type in corals because it would offer an opportunity
16 to identify the molecular signature of the cell type that performs endosymbiosis. We focused on a
17 soft coral pulsing *Xenia* sp. (Fig. 1a, b, Supplementary Video 1) that grows in a laboratory
18 aquarium rapidly. Using Illumina short-read and Nanopore long-read sequencing (Extended data
19 Tables 1, 2), we assembled the *Xenia* genome into 556 high quality contigs. Applying
20 chromosome conformation capture (Hi-C)¹², we further assembled these contigs into 168
21 scaffolds with the longest 15 contain 92.5% of the assembled genome of 222,699,500 bp, which
22 is similar to GenomeScope estimated genome size (Fig. 1c, Extended Data Fig. 1). The *Xenia*
23 genome has by far the longest scaffold length and thus most contiguous assembly compared to
24 the published cnidarian genomes (Fig. 1d). Genome annotation using several bulk RNA-
25 sequencing (RNA-seq) data showed that *Xenia* sp. has similar number of genes as other cnidaria
26 (Extended Data Tables 3, 4). Consistent with previous phylogenetic analysis¹³, soft corals *Xenia*
27 sp. and *Dendronephthya gigantean* grouped together, they branched later than the freshwater
28 *Hydra* from the stony corals, and they are more distantly related to the stony corals than
29 *Nematostella* and *Aiptasia* (Fig. 1e).

30 We next performed single cell RNA-seq (scRNA-seq)¹⁴ of whole polyps, stalks, or
31 tentacles using the 10xGenomics platform. By t-distributed stochastic neighbor embedding (t-

1 SNE)¹⁵, we grouped the high-quality single cell transcriptomes covering 19,976 genes into 13
2 cell clusters with distinct gene expression patterns (Fig. 2a, b, Extended Data Fig. 2a,
3 Supplementary Table 1). For validation, we attempted to identify two previously characterized
4 cnidarian cell types, cnidocytes for prey capture/defense and gasterodermis cells. We found that
5 cluster 12 cells express cnidocyte marker genes, *Minicollagens* and *Nematogalecins*¹⁶⁻¹⁸ (Fig.
6 2c). Further analysis of cluster 12 single-cell transcriptomes revealed two sub-clusters (Fig. 2d,
7 Extend Data Fig. 2b). *Minicollagens* are expressed in both sub-clusters, whereas
8 *Nematogalecins* are preferentially expressed in one sub-cluster (Fig. 2e and Extended Data Fig.
9 2c). RNA *in situ* hybridization (ISH) confirmed the expression of a *Nemetogalectin* to be more
10 restricted than the *Minicollagen* in *Xenia* pinnules (Fig. 2f, g). Interestingly, *Xenia* cells in
11 clusters 2, 8, and 13 express genes encoding collagens and proteases (Fig. 2h) shown to be
12 enriched in gastrodermis of *Nematostella*¹⁶. RNA ISH for *Collagen 6* (expressed by clusters 2
13 and 8), *Astacin-like metalloendopeptidase2* and another gene Xe_003623 (both expressed by all
14 3 clusters) showed their presence in the gastrodermis (Fig. 2h-j, Extended Data Fig. 2d-h). Thus,
15 our clustering analyses has identified different types of cnidocytes and gastrodermis cells in
16 *Xenia*.

17 To identify the cell type for endosymbiosis, we took advantage of the auto-fluorescence
18 of *Symbiodinaceae*. Using fluorescence-activated cell sorting (FACS), we separate the algae-
19 containing and algae-free *Xenia* cells (Fig. 3a, b, Extended Data Fig. 3a-d) and performed bulk
20 RNA-seq of these cell populations (Supplementary Table 2). By comparing these bulk cell
21 transcriptomes with genes expressed in each cluster, we found that cluster 13 cells exhibited the
22 highest similarity with the algae-containing cells (Fig. 3c, d, Supplementary Table 3, 4).
23 RNAscope ISH for two cluster 13 marker genes, one encoding a protein with Lectin and Kazal
24 Protease inhibitor domains (LePin) and the other encoding Granulin 1, showed expression of
25 both genes in algae-containing gastrodermis cells (Fig. 3e, Extended Data Fig. 3e, f). Thus, of
26 the 3 gastrodermis cell types, cluster 13 cells play a unique role in endosymbiosis.

27 Among the top 43 marker genes highly enriched in cluster 13 endosymbiotic cells, 30
28 encode proteins with domains of known or predicted functions, including receptors, extracellular
29 matrix proteins, immune response regulators, phagocytosis/endocytosis, or nutrient transports
30 (Fig. 3f, Extended data Fig. 4, Supplementary Table 3). Three proteins, encoded by *CD36*,
31 *DMTB1*, and *CUZD1*, contain CD36 or scavenger receptor (SR) domains known to recognize a

1 wide range of microbial surface ligands and mediate their phagocytosis, while modulating host
2 innate immune response (Extended Data Fig. 4a)^{10,19,20}. Among them, CUZD1 is least
3 understood, but it is similar to DMBT1 in domain organization. Like CD36, DMBT1 functions
4 in pattern-recognition of microbes. DMBT1 is expressed on the surface of mammalian gastro-
5 intestinal tracts where it recognizes poly-sulfated and poly-phosphorylated ligands on microbes,
6 represses inflammatory response, and regulates gastro-intestinal cell differentiation²¹. *LePin* and
7 *Granulin1*, used in ISH, have homologs in *Aiptasia*, stony and soft corals. Since *LePin* has an N-
8 terminal signal peptide followed by multiple domains including H- and C-type lectins and Kazal
9 type serine protease inhibitor (Extended Data Fig. 4b), it may confer selectivity for
10 *Symbiodiniaceae*. *Granulin1* is one of three *Granulin*-related genes expressed selectively in
11 endosymbiotic cells and in mammals Granulins were shown to modulate immune response²².

12 Phagocytosis of *Symbiodiniaceae* by the similar sized gastrodermis cells requires
13 substantial host cell expansion, but genes regulating the expansion have remained unknown.
14 Among the endosymbiotic marker genes, we found *Plekhg5*, which encodes a highly conserved
15 RhoGEF (Extended Data Fig. 4c). The *Xenopus* *Plekhg5* localizes to the apical membrane of
16 epithelial cells and recruits actomyosin to induce cell elongation and apical constriction²³. Thus,
17 *Plekhg5* is a prime candidate in regulating the apical membrane extension of endosymbiotic cells
18 to engulf *Symbiodiniaceae* during early stages of phagocytosis. Upon phagocytosis,
19 *Symbiodiniaceae* are enclosed by the host membrane to form symbiosomes²⁴. Although the
20 symbiosome compartment is acidified similarly to lysosomes²⁵, it is unclear whether specific
21 genes are involved in symbiosome formed. *Xenia* sp. has two genes encoding lysosome-
22 associated membrane glycoproteins (Lamp) that are more similar to the characterized Lamp1
23 than Lamp2. The *Xenia* *Lamp1-L* encodes a larger protein and is an endosymbiotic marker gene,
24 whereas the *Lamp1-S* encodes a smaller Lamp1 and is uniformly expressed across cell clusters
25 (Extended Data Fig. 4d, e). Since Lamps are known to regulate phagocytosis, endocytosis, lipid
26 transport, and autophagy²⁶, Lamp1-L may regulate symbiosome formation and/or function.
27 Several endosymbiotic marker genes encode enzymes that may promote the establishment of
28 endosymbiosis or facilitating nutrient exchanges between algae and the host. For example, α -CA
29 is a predicted transmembrane carbonic anhydrase that may concentrate CO₂ for photosynthesis
30 by the algae. There are also nine genes potentially participate in nutrient exchanges as they

1 encode highly conserved transporters for sugar, amino acid, ammonium, water, cholesterol, or
2 choline (Fig. 3f).

3 To better understand the lineage sequence and temporal dynamics of the *Xenia*
4 endosymbiotic cells, we developed a *Xenia* regeneration model. We surgically cut away all
5 tentacles from *Xenia* polyps and found the stalks regenerated all tentacles in several days (Fig.
6 4a). Individual tentacles also regenerated into full polyps but requiring a longer time (not
7 shown). BrdU labeling showed that the proliferated BrdU⁺ cells in the gastrodermis began to
8 take up algae at day 4 of regeneration (Extended Data Fig. 5a, b). We performed scRNA-seq of
9 the regenerating stalks and pooled the data with the scRNA-seq samples described above,
10 followed by clustering them into 13 cell types. Nearly all cell clusters from the non-regeneration
11 samples matched the clusters in the pooled samples and 100% of cells in the endosymbiotic cell
12 cluster 13 in non-regeneration samples are found in the endosymbiotic cluster 13 in the pooled
13 samples (Extended Data Fig. 5c). This allowed us to define endosymbiotic cells in the
14 regenerative sample.

15 We performed pseudotemporal ordering of the endosymbiotic *Xenia* cells by Monocle 2¹⁴
16 (Fig. 4b), which uses reversed graph embedding to construct a principal curve that passes
17 through the middle of the cells on the tSNE space. Because this trajectory analysis does not
18 provide a direction of cell state progression, we used velocity²⁷ to determine the directionality of
19 lineage progression of endosymbiotic cells in the regenerating sample. Velocityo calculates RNA
20 velocity by comparing the number of un-spliced and spliced reads, which measure the expected
21 change of gene expression in the near future, thereby providing the directionality of cell state
22 change. This allowed the identification of early and late stages of endosymbiotic cells in the
23 regenerating sample (Extended data Fig. 5d). The cell trajectory showed that the early (green)
24 and late (red) stage cells are mapped to the early and late pseudotime, respectively (Fig. 4c),
25 revealing the pseudotime represents actual lineage progression. Gene expression modeling
26 revealed significant changes along the pseudotime. Further hierarchical clustering revealed
27 distinct gene expression patterns, which helped us to define four endosymbiotic cell states (Fig.
28 4d, Supplementary Table 5).

29 To further understand the cells in the four states, we compared single cell transcriptomes
30 against transcriptomes from the bulk RNA-seq of FACS-sorted algae-containing or algae-free
31 cells and plotted the expression correlation along the pseudotime. State3 cells showed the

1 strongest correlation with the algae-containing cells followed by state1 and 2, whereas state4
2 cells showed the least correlation (Fig. 4e). Thus, state3 represents the mature agal-containing
3 cells. Moreover, whereas state3 cells showed least correlation with algae-free cells, state1 and 4
4 cells showed similar correlations with algae-containing and algae-free cells (Fig. 4e). These
5 suggest that state1 cells are pre-endosymbiotic progenitors that can transition through state2 to
6 become state3 mature algae-containing cells, while state4 represents post-endosymbiotic cells.
7 Consistent with this, the endosymbiotic marker genes we identified showed a gradual up-
8 regulation along the psuedotime from state1 through state2 to peak at state3, followed by a down
9 regulation in state4 (Fig. 4f).

10 Analyzing the differentially expressed genes in each state suggests their roles in
11 regulating endosymbiotic cell lineage development. For example, the state1 cells express two
12 genes encoding a Secreted Frizzled-Related Protein (SFRP) and Elk1. SFRP modulates Wnt
13 signaling during development and differentiation, whereas Elk1 is known to regulate cell
14 proliferation and differentiation^{28,29}. State1 cells also express a G-protein coupled receptor Mth
15 with an N-terminal extracellular domain, which should allow the creation of specific antibodies
16 that recognize these cells for live-sorting by FACS to aid future characterization of cells at this
17 state. State1 and 2 cells express genes encoding thrombospondin type 1 repeats (TSR). Since
18 TSR binds to CD36, which can convert TGF β to active form to regulate immune tolerance³⁰, the
19 TSR-containing genes expressed in state1/2 cells may directly suppress immune response of host
20 endosymbiotic cells during *Symbiodiniaceae* recognition and uptake. Our analyses suggest state4
21 cells as post-endosymbiotic. Further studies of the genes defining state4 cells should clarify if
22 these cells lose algae due to aging or stress, and if they share features of bleached endosymbiotic
23 cells.

24 Here we demonstrate the power of advanced genomic and bioinformatic tools in studying
25 coral biology. Although we focused on studying the endosymbiotic cell lineage, the regenerative
26 processes for the other cell types can be similarly comprehended as more information becomes
27 available. We reveal 4 distinct *Xenia* endosymbiotic cell states and propose a lineage progression
28 from state1 progenitors through state2 cells engaging in algae recognition to state3 mature
29 endosymbiotic cells followed by state4 post-endosymbiotic status. Since state4 transcriptome has
30 lowest expression of endosymbiotic marker genes among all four states, we speculate that these
31 post-endosymbiotic cells represent a terminal state, which is either no-longer competent for algae

1 re-uptake or can only regain endosymbiotic competence after a long recovery. The presence of
2 state4 could offer new insights into why coral bleaching is devastating because bleached
3 endosymbiotic cells may be similar to the state4 cells that cannot re-uptake or require a long time
4 to recover and re-uptake algae. It will be interesting to test if efficient recovery from bleaching
5 relies on state1 progenitors to make new endosymbiosis-competent cells. It is also feasible to
6 further test whether forced regeneration by fragmenting bleached corals can stimulate state1
7 progenitor expansion and endosymbiosis restoration.

8

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14

15 **Author contributions:** CMF and YZ conceived and supervised the project. MH, CMF, and YZ
16 designed experiments. MH performed the experiments. MH and XZ analyzed the data. MH, XZ,
17 CMF, and XZ interpreted the data and wrote the manuscript.

18

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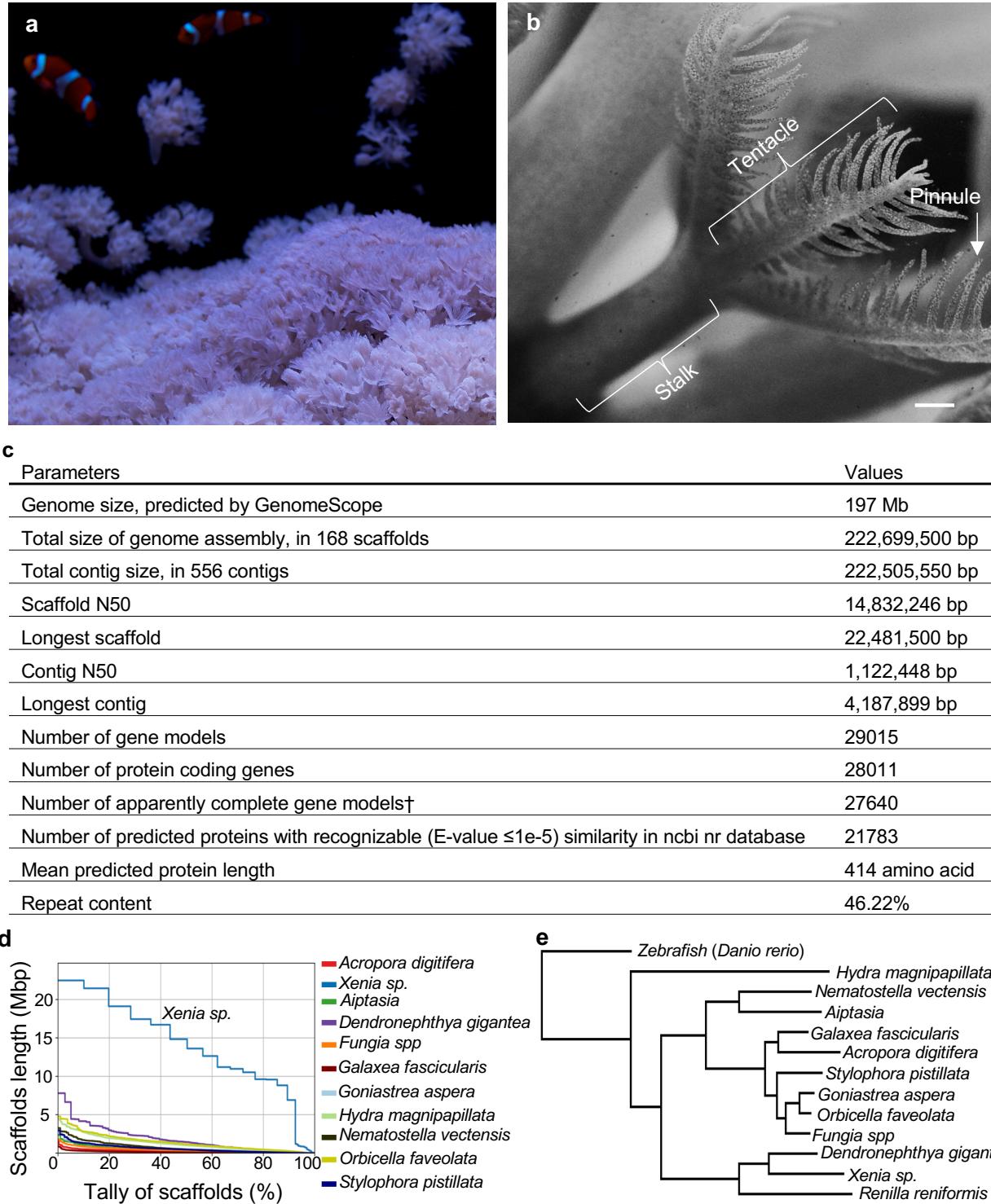


Fig. 1. High quality genome assembly for *Xenia* sp. **a**, *Xenia* sp. grown in a laboratory aquarium. **b**, An enlarged view of a *Xenia* sp. polyp with its main sub-structures indicated. Scale bar, 1 mm. **c**, A summary of *Xenia* sp. genome assembly and gene annotation. †, genes encoding protein sequences with apparent in frame start and stop codons. **d**, Comparisons of the assembled scaffold lengths (y-axis) and tallies (x-axis) of 11 sequenced cnidarians including *Xenia* sp. **e**, Evolutionary comparisons of *Xenia* sp. with other cnidarians as indicated. Zebrafish was used as an outgroup. The phylogenetic branch points were assigned with 100% confidence.

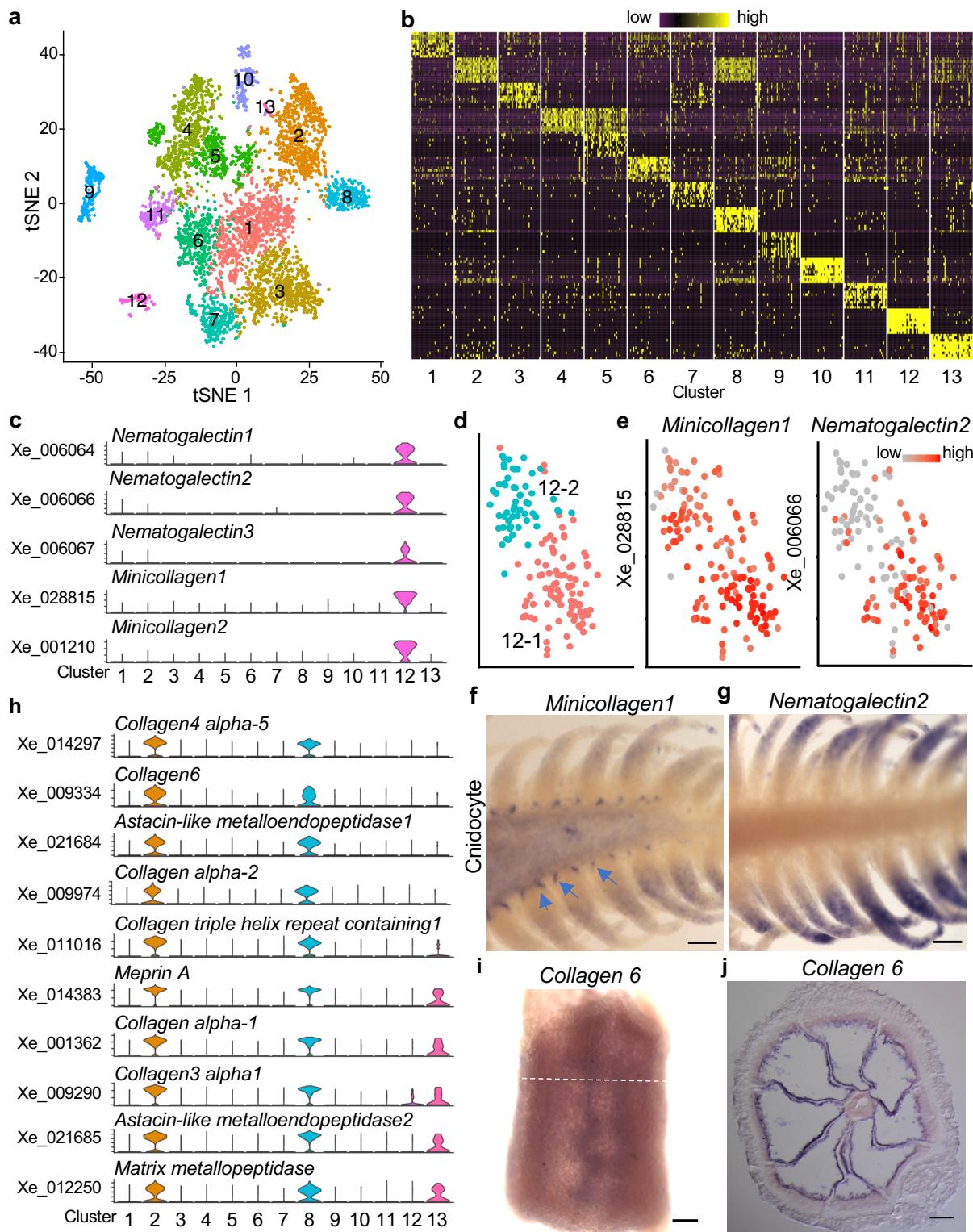


Fig. 2. scRNA-seq transcriptomes delineate 13 cell types in *Xenia* sp. **a**, Transcriptomes of individual *Xenia* sp. cells obtained by scRNA-seq were grouped into 13 clusters (color coded) and presented in the tSNE space. Each colored dot represents one cell. **b**, Gene expression heatmap (scale on the top) for the top 10 gene markers defining each cluster. Each column and row represent one cell cluster and gene, respectively. 40 cells were randomly selected from each of the 13 cell clusters for plotting. **c**, Expression profiles of the indicated marker genes defining cluster 12. **d**, Cluster 12 cells is sub-divided into two populations (12-1 and 12-2, color-coded) and displayed in a tSNE space. Each colored dot represents a cell. **e**, Expression levels (scale to top right) of two cluster 12 markers, *Minicollagen1* and *Nematogalectin2*, are shown in the tSNE plot. **f**, **g**, Whole mount RNA *in situ* hybridization of *Minicollagen1* (**f**) and *Nematogalectin2* (**g**) showing their expression in tentacles. Arrows indicate the expression of *Minicollagen1* at the base of pinnules. **h**, Expression profiles of marker genes enriched in clusters 2, 8, and 13. **i**, **j**, RNA *in situ* hybridization of *Collagen 6*. Whole mount view of the stalk in **i** and cross section image in **j**. The white dashed line in **i** indicates the cross section level in **j**. Scale bars, 100 μ m (**f**, **g**, **j**), 150 μ m (**i**).

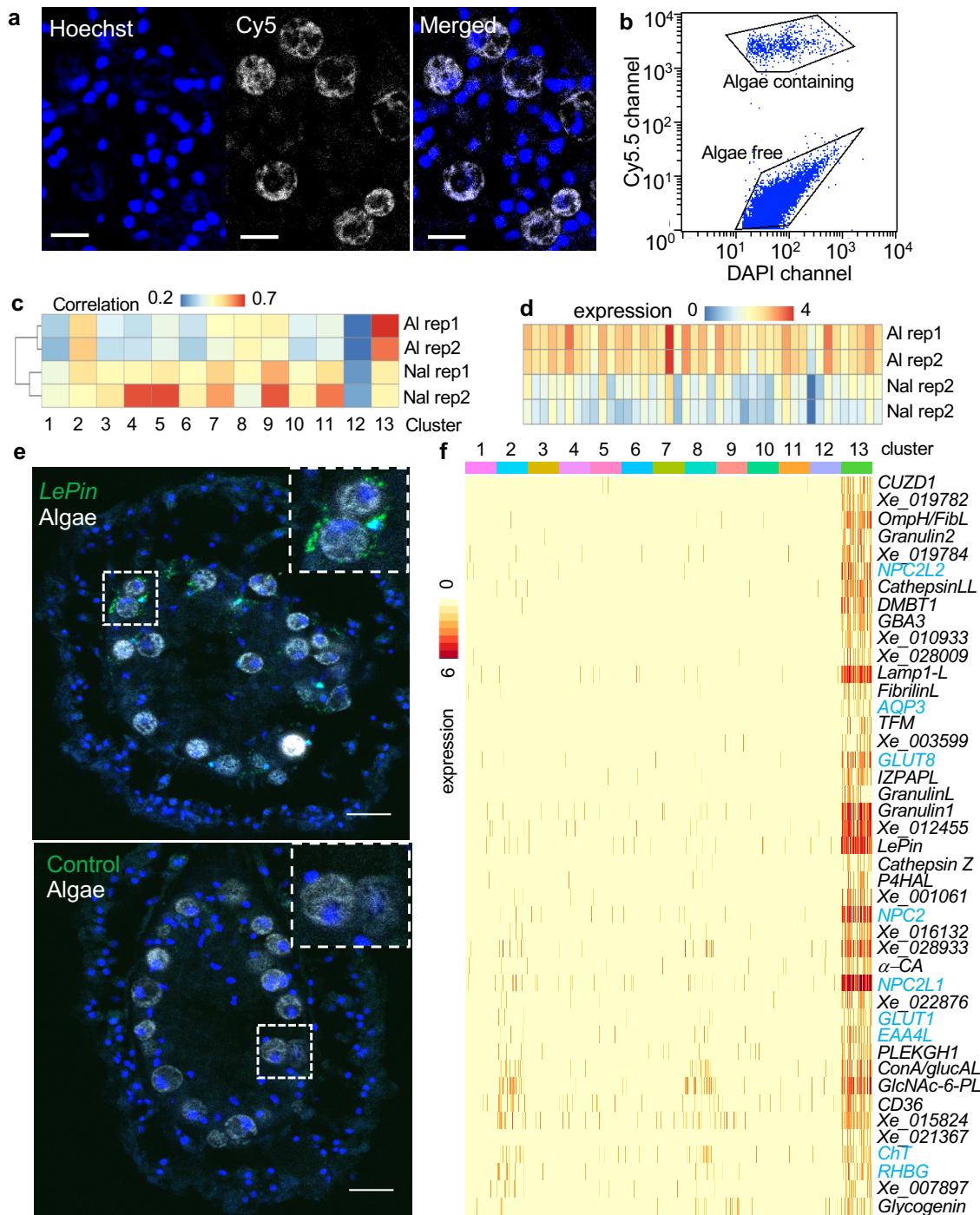


Fig. 3. Identification of genes specifically expressed in *Xenia* sp. endosymbiotic cells. **a**, The endosymbiotic algae in *Xenia* display autofluorescence in the far-red channel. A cross section of *Xenia* with *Xenia* and algal nuclei stained by Hoechst in blue and algae autofluorescence in white. **b**, A FACS profile of dissociated live *Xenia* cells using Cy5.5 and DAPI channels. **c**, Pearson correlation of gene expression between the scRNA-seq data of 13 cell clusters and the bulk RNA-seq data of two biological replicates of FACS-isolated algae-containing (Al rep1 and Al rep2) and algae-free (Nal rep1 and Nal rep2) cells. **d**, Heatmap showing the expression levels of the top 43 markers genes for cluster 13 cells in FACS-sorted algae-containing and algae-free cells. **e**, Ultra-sensitive fluorescence RNA *in situ* hybridization by RNAscope probing for *LePin* (green, top) and control (bottom). A *Xenia* cell with algae and *LePin* expression is enlarged to the top right. Algae autofluorescence is in white. Hoechst staining of all nuclei are in blue. Scale bars, 10 μ m. **f**, Heatmap showing the enrichment levels of the cluster 13's top 43 marker genes in all 13 cell clusters. Transporters are highlighted in blue.

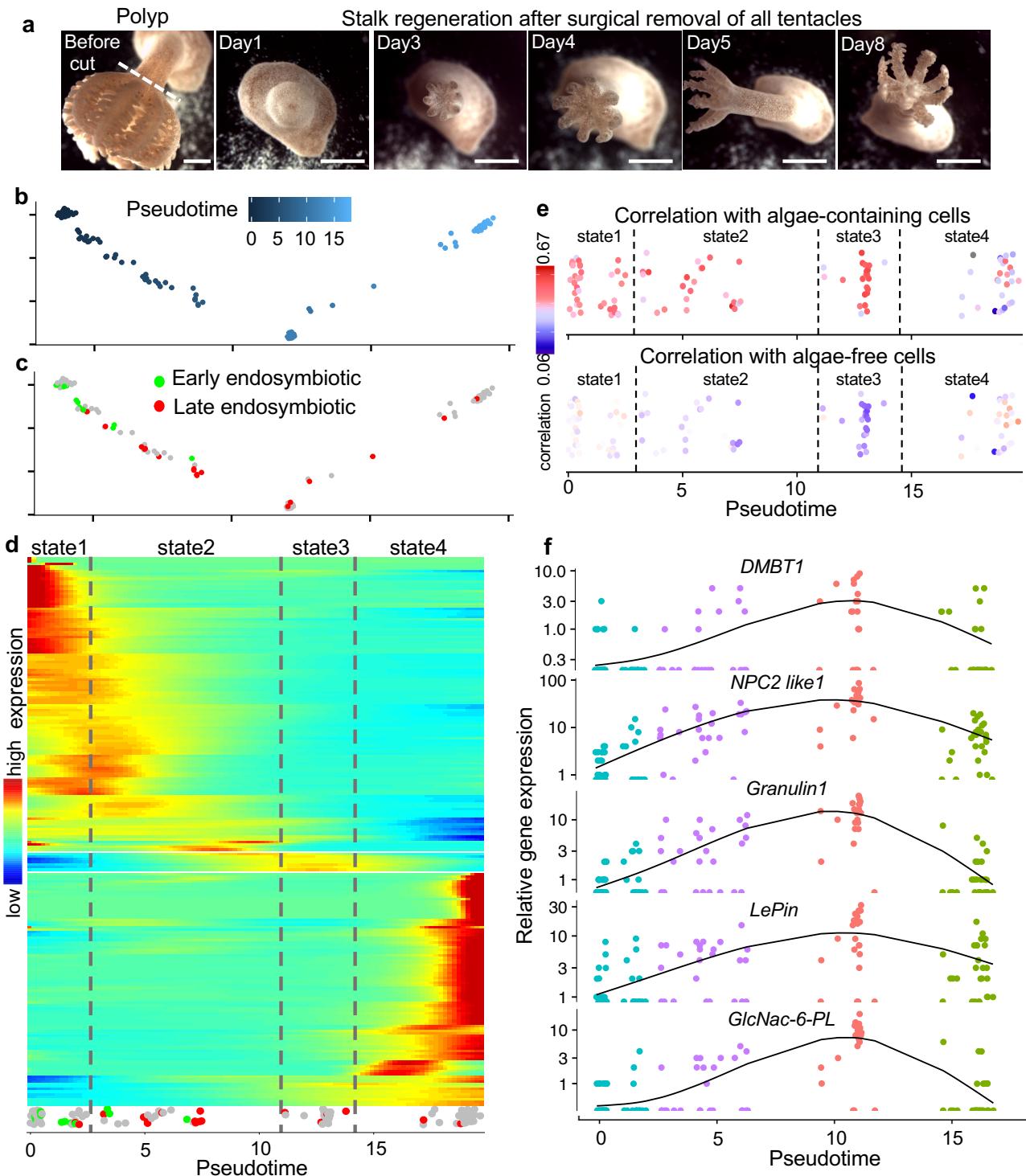
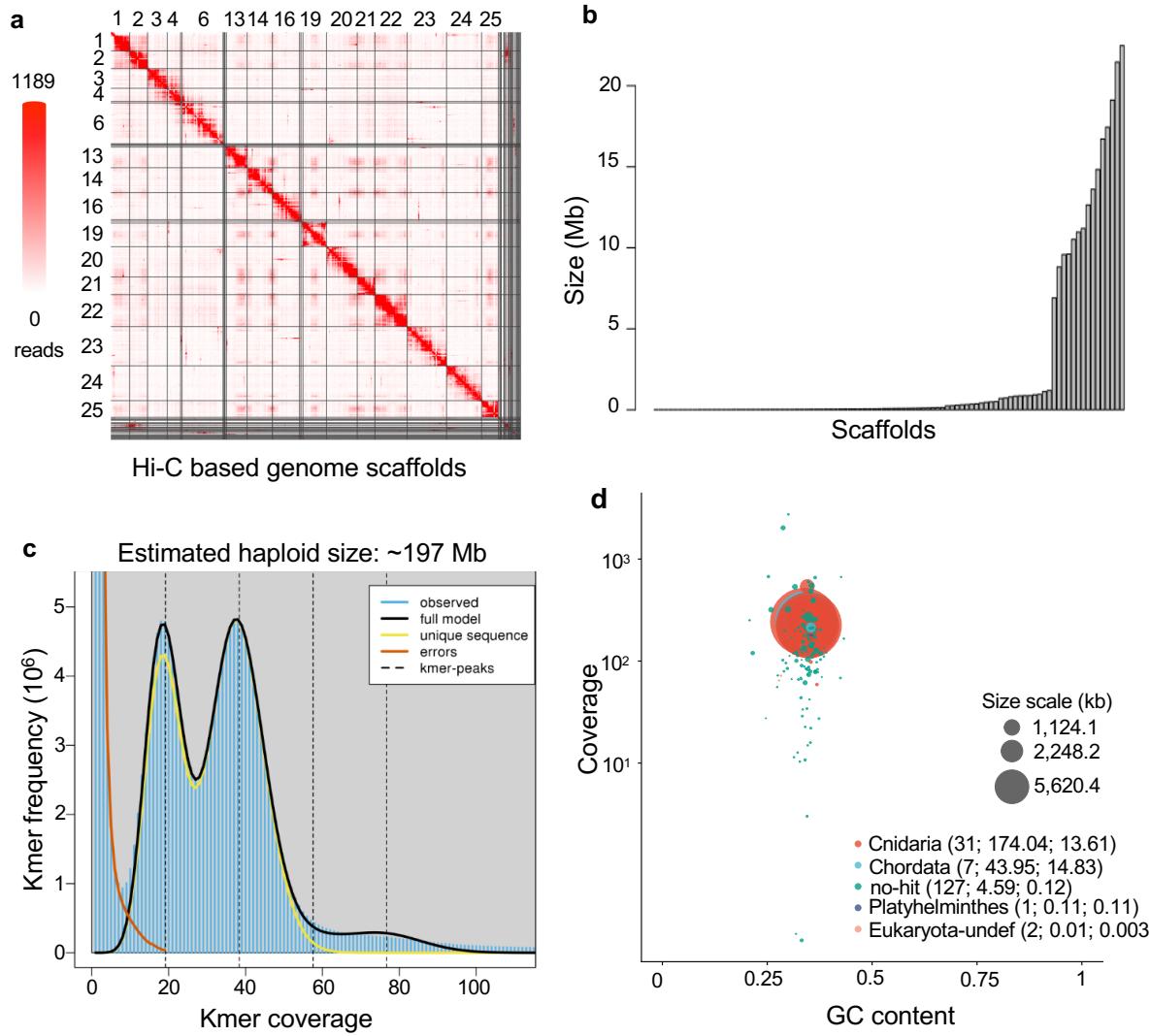
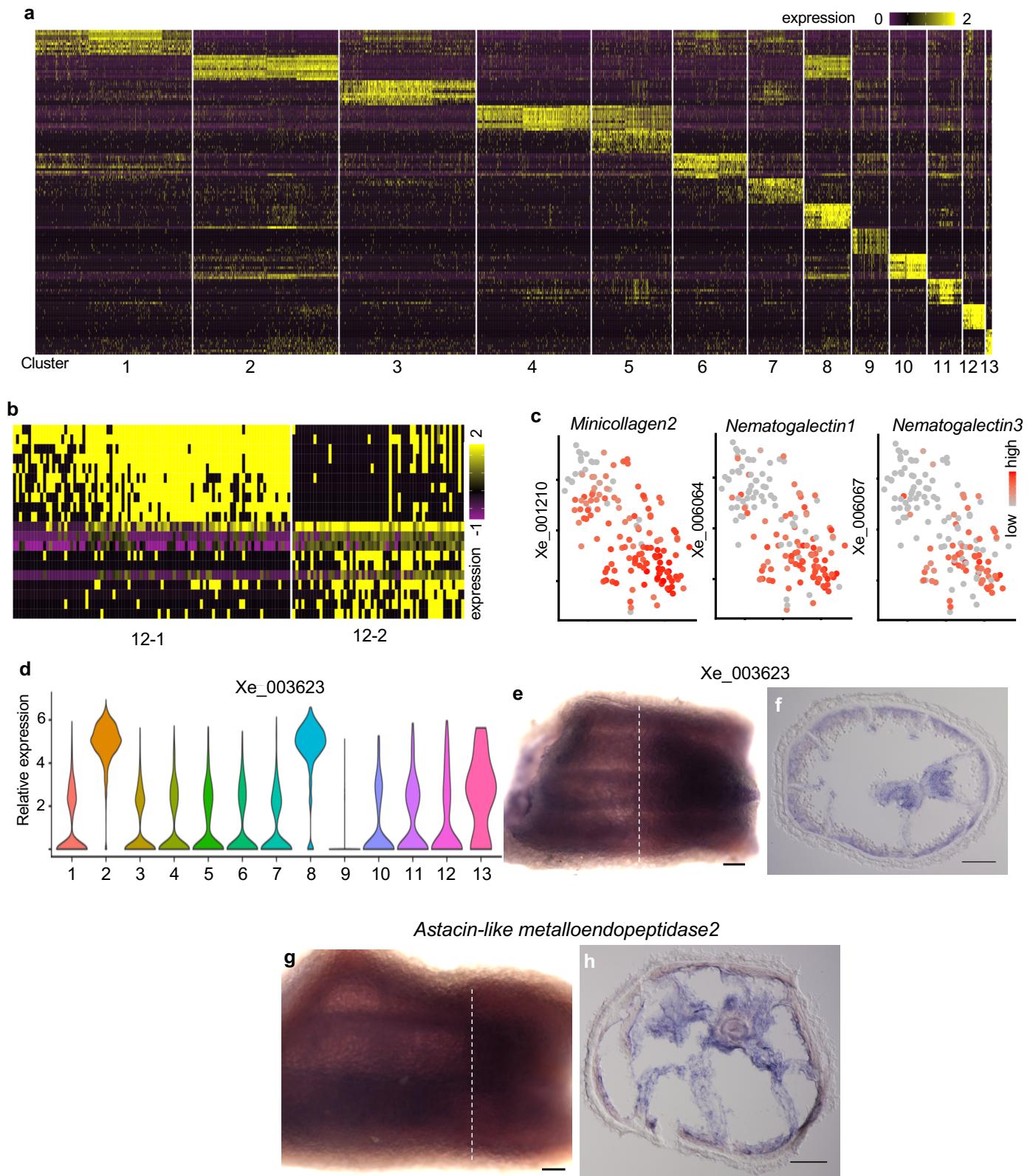


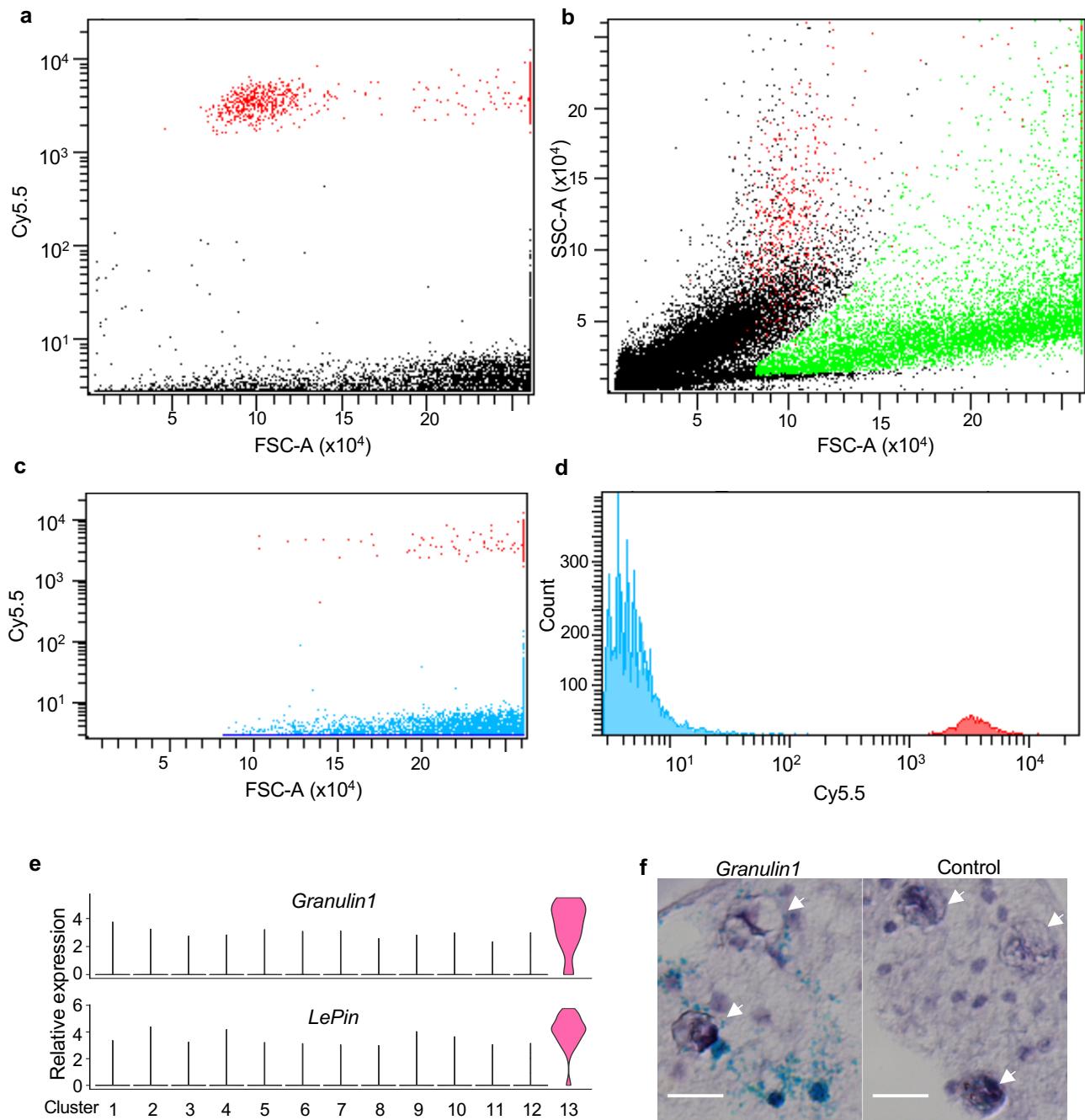
Fig. 4. Dynamic lineage progression of endosymbiotic cells. **a**, An example *Xenia* sp. polyp shown in the first image is surgically cut at the white dashed line to remove all the tentacles. A surgically cut stalk is shown to regenerate in successive days as indicated. Scale bars, 1 mm. **b**, Pseudotime trajectory of all endosymbiotic cells (a dot represents a cell) identified in regeneration and non-regeneration scRNA-seq datasets. The pseudotime indicator is shown at the top. **c**, Distribution of early (green) and late (red) lineage cells predicted by velocyto based on scRNA-seq of the regenerating sample is highlighted on the same pseudotime plot shown in **(b)**. **d**, Heatmap for gene expression levels along pseudotime. The endosymbiotic cells (as in **c**) used to model gene expression along the pseudotime line are shown at the bottom for reference; color key for gene expression level is shown to the left. Four cell states (state1-4, separated by dashed lines and indicated at the top) are defined by differential gene expression. **e**, Gene expression correlation between individual cells (shown as dots) in 4 cell states and the transcriptomes of the FACS-isolated algae-containing (top) or algae-free (bottom) cells; The heatmap key at the left shows Pearson correlation coefficient. **f**, Expression dynamics of endosymbiotic marker genes in the pseudotime through 4 cell states: cyan, purple, flesh, and green, represent state1, 2, 3, 4 cells (each dot represents a cell), respectively.



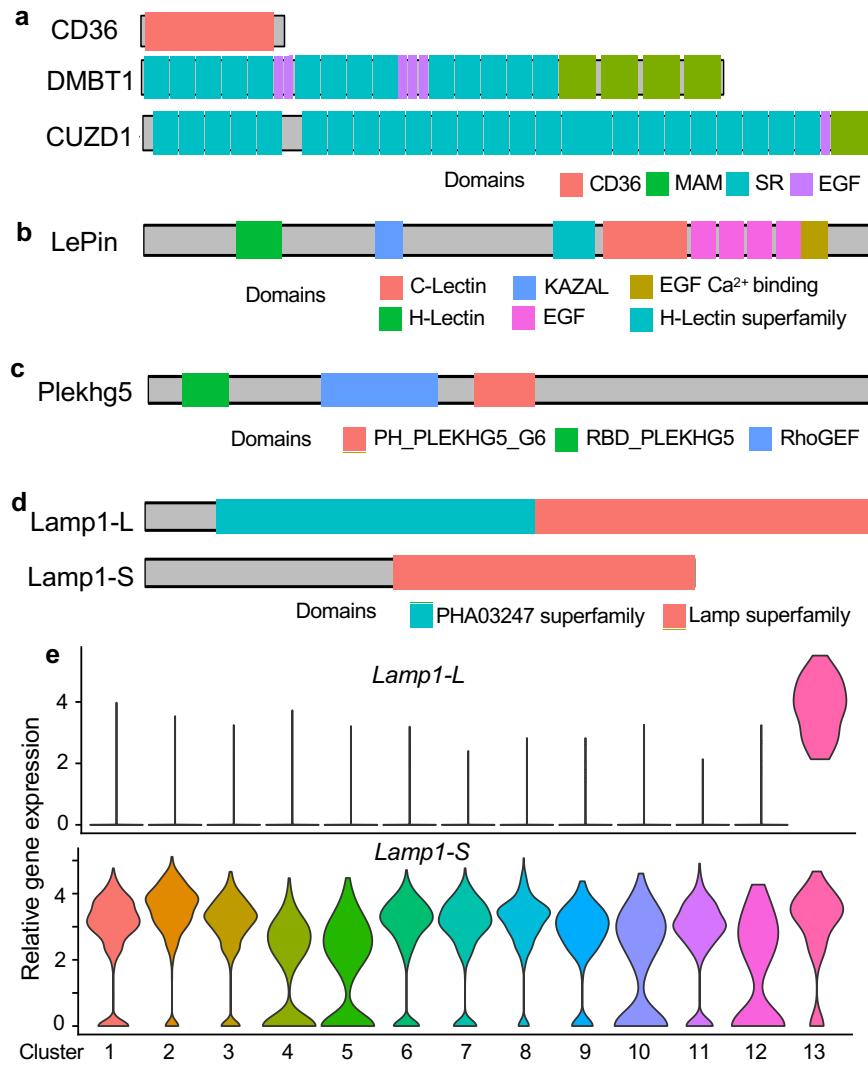
Extended Data Fig. 1. Additional genome assembly data. **a**, Hi-C based *Xenia* sp. genome assembly. The scaffolds are separated by grids demarcated by black lines. The numbers for the 15 longest scaffolds out of the total 168 are shown. **b**, Size distribution of *Xenia* sp. genome scaffolds with each bar on the x-axis representing a scaffold. **c**, *Xenia* sp. genome is predicted to be diploid, as expected, with a haploid genome size of ~197 Mb based on GenomeScope analysis of Illumina short reads. **d**, Contamination analysis by BlobTools revealed a similar GC content and genomic coverage across most scaffolds. Each colored circle in the graph represents a scaffold. Larger circles have longer scaffold lengths (see the three grey circles for length scale used in the plot). The color codes represent the closest species group that have the highest sequence similarities to the *Xenia* sp. scaffolds (the first number in each parenthesis shows the *Xenia* scaffold number followed by the combined length of the scaffolds and scaffold N50 in Mb).



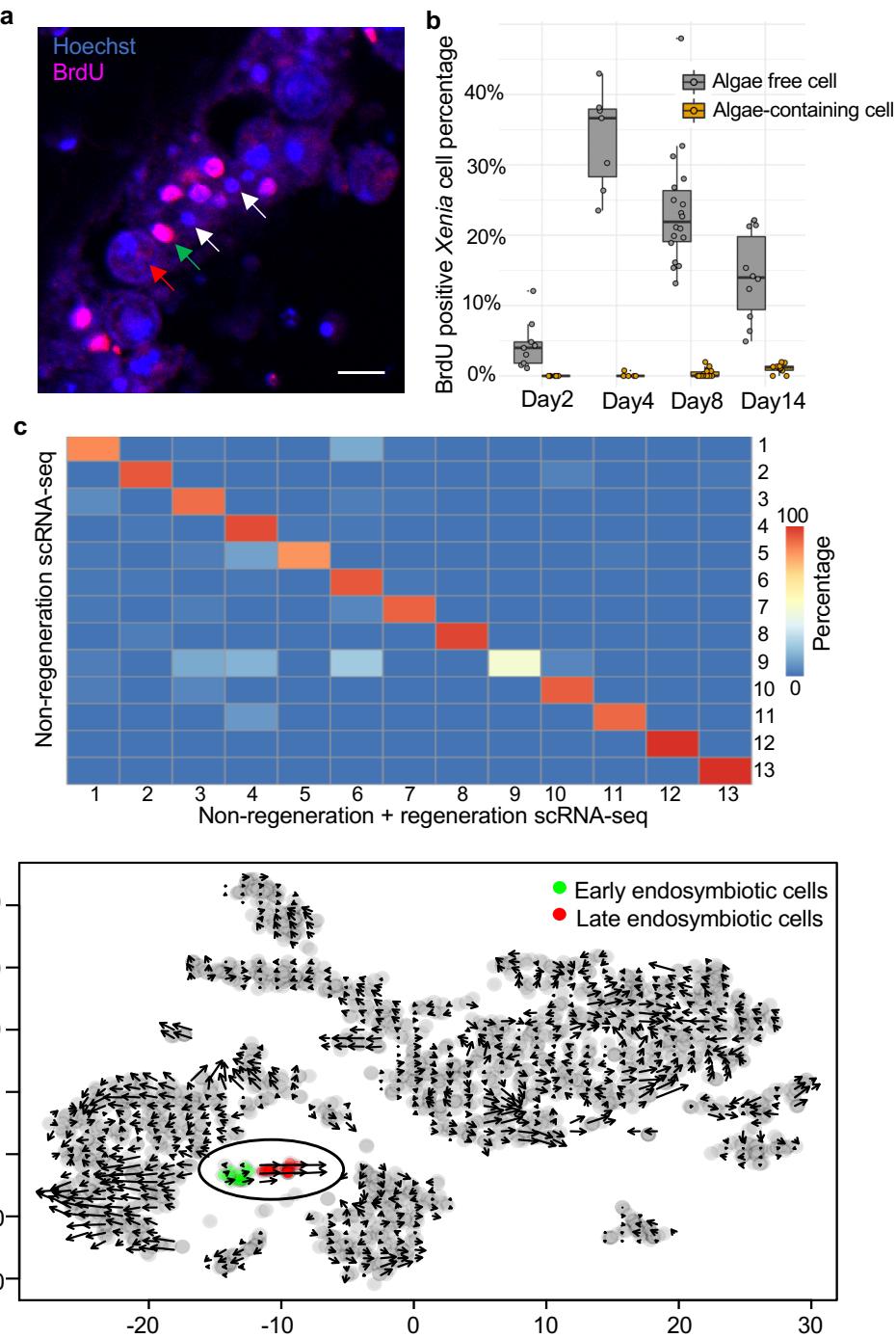
Extended Data Fig. 2. Additional scRNA-seq analyses. **a**, Heatmap showing differential gene expression patterns of all cells in the assigned 13 cell clusters (indicated at the bottom). Each column is one cell cluster and each row represents one gene. **b**, Heatmap showing differential gene expression patterns of two sub-clusters in cluster 12, 12-1 and 12-2. **c**, Expression levels (see colored expression scale) of 3 cluster 12 markers, *Minicollagen2*, *Nematogaelectin1*, and *Nematogaelectin3* are shown in the tSNE plots. **d**, Expression levels of Xe_003623, a non-conserved and uncharacterized cluster 13 marker genes, in each of the 13 cell types defined by scRNA-seq and Seurat. **e-h**, RNA *in situ* hybridization of Xe_003623 (**e**, **f**) and *Astacin-like metalloendopeptidase2* (**g**, **h**). The white dashed lines in the whole mount views (**e**, **g**) indicate the cross section cut (**f**, **h**). Scale bars, 150 μ m (**e**, **g**), 100 μ m (**f**, **h**)



Extended Data Fig. 3. Additional FACS and scRNA-seq analyses. **a**, Dissociated *Xenia* cells were first sorted by Cy5.5 and FSC-A to identify the potential algae-containing cells (Red dots). **b**, Mapping the potential algae-containing population (red dots) onto the FSC-A and SSC-A space suggests that these are real cells. To gate for algae-free cells, we first used DAPI to stain the nuclei of dissociated, fixed, and permeabilized *Xenia* cells and identified the DAPI positive sub-population (green dots), which represents cells. **c**, Further removing of Cy5.5 strong algae-containing cells among the DAPI positive population led to the sorting of algae-free coral cells (blue dots). **d**, The counts distribution of algae-containing (red) and algae-free (blue) cells in our sorting. **e**, Piano plots of the expression profiles of *Granulin1* and *LePin* in the 13 clusters defined by scRNA-seq and Seurat. **f**, Ultra-sensitive chromogenic RNA *in situ* hybridization by RNAscope probing for *Granulin1* (left) and control (right). Positive signals are blue. Samples were counterstained with Hematoxylin. White arrows indicate *Symbiodiniaceae*. Scale bars, 10 μ m.



Extended Data Fig. 4. Selected endosymbiotic marker genes with known domains. **a**, Scavenger receptors: CD36, DMBT1, and CUZD1. **b**, LePin. **c**, Plekhg5. **d**, Lamp1-L and Lamp1-S. **e**, Piano plots of expression profiles of Lamp1-L and Lamp1-S in 13 clusters defined by scRNA-seq and Seurat.



Extended Data Fig. 5. Additional analyses of endosymbiotic cell lineage. **a**, A representative image of BrdU labeling (red), overlaid with Hoechst (blue DNA stain) in a cross section of a regenerating *Xenia* sp. stalk. White, red, and green arrows indicate BrdU-negative (BrdU⁻) *Xenia* nuclei, an alga, and a BrdU-positive (BrdU⁺) *Xenia* nucleus juxtaposed to the alga, respectively. **b**, Boxplot. Percentages (y-axis) of BrdU⁺ *Xenia* cells at the indicated regeneration time points (x-axis). Each dot represents data from one section. Three independent samples were pooled and plotted for each time point. The algae-containing proliferated *Xenia* cells were estimated as those whose BrdU⁺ nuclei were juxtaposed to algae. The medians are indicated as lines in the box. The upper and lower edge of the box represents upper and lower quartiles, respectively. **c**, Comparisons of cell clusters between the non-regeneration samples (y-axis) and all samples (i.e. regeneration and non-regeneration samples combined, x-axis). The heatmap shows the percentage of cells in each of the 13 cluster defined by the non-regeneration samples that are found in the corresponding 13 types defined by all samples. **d**, Velocity analysis of the scRNA-seq data from day 4 regenerating *Xenia* sp. stalks. Each dot represents a cell and arrows indicate the directions of RNA-velocity. The endosymbiotic cell cluster is outlined and the predicted early and late cell states are colored green and red, respectively.

library	read number (M*)	read length	pair-end	data (G**)
1	27.2	150	No	4.09
2	19.7	150	No	2.91
3	73.9	75	No	5.54
4	127.4	150	Yes	38.2

* M, million; ** G, giga base

Extended Data Table 1. Summary of Illumina sequencing for genome assembly. The table shows sequence information from 4 library preparations for Illumina sequencing. Read number indicates total number of reads obtained. Read length indicates individual read length, by paired-end sequencing. Data indicate total sequence data in giga bases.

library	read number	max (bp*)	mean (bp)	median (bp)	> 5 kb**	>10 kb	>20 kb	data (G***)
run1	819,205	62,624	4,488	4,341	38.4%	3.5%	0.19%	3.68
run2	328,045	266,931	11,716	4,343	46.1%	29.9%	17.3%	3.84
run3	210,985	310,074	12,677	5,400	52.4%	32.7%	18.3%	2.67
run4	1,912,621	143,219	5,228	4,959	49.5%	7.27%	0.37%	10

* bp, base pair; ** kb, kilobase; *** G, giga base

Extended Data Table 2. Summary of Nanopore sequencing for genome assembly. Statistics of all sequence information from 4 different runs of Nanopore sequencing, including max, mean, and median reads length statistics, and the percentages of reads that have bigger sizes than the indicated number, > 5 kb, > 10 kb, and > 20 kb.

samples	library	read number (M*)	data (G**)
whole polyp	pair-end	34.2	5.12
Stalk	pair-end	36.0	5.40
Tentacle	pair-end	39.8	5.97
Regeneration 4d stalk	pair-end	38.9	5.84
Opti-Prep lv1	pair-end	29.6	4.43
Opti-Prep lv2	pair-end	31.8	4.76
Opti-Prep lv3	pair-end	33.6	5.03
Opti-Prep lv4	pair-end	31.1	4.68

* M, million; ** G, giga base

Extended Data Table 3. Transcriptomes for gene annotation. A summary of all the transcriptome data used for gene annotation. RNA isolated from different samples as indicated were used for Illumina sequencing in order to cover as many expressed gene as possible. Opti-Prep, density-based separation of dissociated *Xenia* cells into 4 different layers (see Method). Lv1, Lv2, Lv3, and Lv4 indicate layer 1, layer 2, layer 3, and layer 4 cells, respectively, used to make the RNA-seq libraries.

Parameter	species	<i>Xenia</i> <i>sp.</i>	<i>Aiptasia</i>	<i>Nematostella</i> <i>vectensis</i>	<i>Acropora</i> <i>digitifera</i>
Predicted genome size (Mb)		197	260	329	420
Assembly size (Mb)		222.7	258	356	419
Total contig size (Mb)		222.5	213	297	365
Total contig size as % of assembly size		99.9	82.5	83.4	87
Contig N50 (Kb)		1,122	14.9	19.8	10.9
Scaffold N50 (Kb)		148,322	440	472	191
Number of gene models		29015	29269	27273	23668
Number of complete gene models		27640	26658	13343	16434
Mean exon length (bp)		204	354	208	230
Mean intron length (bp)		448	638	800	952
Mean protein length (number of amino acids)		414	517	331	424

Extended Data Table 4. Comparisons of *Xenia* sp. genome assembly with the indicated published cnidarian genomes.
Number of gene models indicate predicted gene model number, whereas number of complete gene models represent number of genes with clearly predicted in-frame start and stop codons.

1 **Coral aquarium**

2 The coral aquarium is established in a tank (Reefer 450 system, Red Sea). The artificial
3 sea water made from Coral Pro Salt (Red Sea) was first incubated with live rocks for two months
4 before introducing *Xenia sp.*, other corals, fish, snails, and hermit crabs. The aquarium is
5 maintained at ~80°F with ~25% change of sea water every 1-2 weeks. The light is provided by
6 Hydra 26™ HD LED (Aqua Illumination) with 60% power on during 10:00 AM to 7:30PM. The
7 fish were fed with fish pellets (New Life Spectrum Marine Fish Formula) and Green Marine
8 Algae (Ocean Nutrition).

9

10 **Genomic DNA isolation from *Xenia sp.***

11 To enable Nanopore DNA sequencing, we modified a protocol³¹ that allowed the
12 isolation of long DNA fragments. For each DNA preparation, one or two *Xenia sp.* colonies
13 containing ~30 polyps were collected from the aquatic tank and washed 3 times for 5 min each
14 with Ca²⁺ and Mg²⁺ free artificial sea water (449 mM NaCl, 9 mM KCl, 33 mM Na₂SO₄, 2.15
15 mM NaHCO₃, 10 mM Tris-HCl, 2.5 mM EGTA, pH8.0). Tentacles were cut away as the mucus
16 surrounding them affected the quality of the isolated DNA. The remaining stalks and the bases of
17 individual *Xenia* colonies were placed in 100 µl DNAzol (Invitrogen) in a 1.5 ml microfuge tube.
18 The tissues were cut into small pieces by a scissor to make the fragment sizes of ~1/10th of the
19 original size. These fragments were further minced by a small pestle made for 1.5 ml microfuge
20 tubes (Fisher Scientific, 12-141-364). Then 900 µl DNAzol were added followed by vortexing
21 the sample and then transferred to a 15 ml conical tube. 4 ml of DNAzol and 50 µl of 10 mg/ml
22 RNase A were then added to the tube and mixed followed by incubation at 37°C for 10 min. 25
23 µl of 20 mg/ml proteinase K were then added, mixed, and the tube was incubated at 37°C for
24 another 10 min. The sample was centrifuged at 5000xg for 10 min. The supernatant was
25 transferred to another 15 ml tube. After adding 2.5 ml ethanol, the tube was gently mixed by
26 inverting several times. The tube was let to stand at room temperature for 3 min followed by
27 centrifugation at 1000xg for 10 min to pellet the DNA. The supernatant was discarded and the
28 DNA pellet was resuspended in with 500 µl TE (10 mM Tris-HCl, 1 mM disodium EDTA, pH
29 8.0) buffer. After the DNA had dissolved, 500 µl of phenol: chloroform: isoamyl alcohol
30 (25:24:1) was added, and the tube was placed on the Intelli-Mixer RM-2S for mixing using
31 program C1 at 35 rpm for 10 min. The mixture was then transferred to a 2 ml phase-lock gel

1 (QuantaBio, Cat. 2302820) and centrifuged at 4500 rpm for 10 min. The aqueous phase was
2 transferred into a new 2 ml tube, 200 μ l 5 M ammonium acetate and 1.5 ml ice-cold ethanol were
3 added followed by centrifugation at 10,000xg for 10 min to pellet DNA. The pellet was washed
4 twice with 1 ml 80% ethanol. After removing as much ethanol as possible, the DNA pellet was
5 left to dry at 42°C for 1 min, and then resuspended in 50 μ l TE buffer.

6

7 **Illumina sequencing**

8 Genomic DNA prepared as above was fragmented into ~400 bp and libraries were made
9 with ThruPLEX DNA-Seq kit (TaKaRa) according to the manufacturer's manual. These libraries
10 were sequenced using the NEXseq500 platform with NextSeq® 500/550 High Output Reagent
11 Cartridge v2 (Illumina).

12

13 **Nanopore sequencing**

14 Genomic DNA was used to build Nanopore sequencing libraries with Ligation
15 Sequencing Kit (SQK-LSK108, Oxford Nanopore Technologies), following the manufacturer's
16 manual. For the first 3 runs, genomic DNA was not fragmented in order to generate long reads.
17 To obtain more reads, for the 4th run of Nanopore sequencing, genomic DNA was sheared to 8-
18 10 kilobases by g-TUBE™ (Covaris, 520079). The libraries were sequenced in R9.4.1 flow cells
19 on MinION device (Oxford Nanopore Technologies) and the data were combined for genome
20 assembly.

21

22 **Chromosome conformation capture (Hi-C)**

23 To perform Hi-C on *Xenia sp.* tissue, we modified our previously published protocol for
24 nuclear in situ ligation³² as described in detail below.

25 1. Fix and dissociate tissues

26 Fix 8 polyps (~10⁸ cell) with 4% paraformaldehyde (PFA) overnight. After washing
27 twice with 3.3x PBS³³ and dissociating the tissue in 2 ml 3.3x PBS using a 7 ml glass Dounce
28 tissue grinder (Wheaton), another 3 ml 3.3x PBS was added. The mixture was then transfer to a
29 15 ml conical tube and centrifuged at 1000g for 3 min (Sorvall Lynx 6000 centrifuge,
30 ThermoFisher Scientific). The pellet was washed once with 5 ml 3.3x PBS.

31 2. Nuclear permeabilization and chromatin digestion

1 The pellet from step 1 was resuspended in 10 ml ice-cold Hi-C lysis buffer (10 mM Tris,
2 pH8.0, 10 mM NaCl, 0.2% NP-40, 1x protease inhibitors cocktail (Roche, 04693132001)) and
3 rotated for 30 min at 4°C followed by centrifugation at 1,000g for 5 min at 4°C. The pellet was
4 washed with 1 ml ice-cold 1.2x NEB3.1 (120µl NEB3.1 to 880µl ddH₂O) buffer and transferred
5 to 1.5 ml microfuge tube followed by centrifugation at 1,000g for 5 min at 4°C. The pellet was
6 washed again with 1 ml ice-cold 1.2x NEB3.1 followed by centrifugation. After removing the
7 supernatant, 400 µl 1.2x NEB3.1 buffer and 12 µl of 10% SDS were added to the pellet. P200
8 pipet tip was used to thoroughly resuspend and dissociate the pellet. The mixture was then
9 incubated at 65°C for 10 min at 950 rpm in a Thermomixer (Eppendorf). After cooling the mix
10 on ice for 5 min, 40 µl 20% Triton X-100 was added to the mixture to neutralize the SDS. After
11 carefully mixing by pipetting with a P200 pipet tip and inverting the tube several times, the
12 mixture was then incubated at 37°C for 60 min with rotation (950 rpm) in a Thermomixer. To
13 digest the crosslinked genomic DNA, 30 µl of 50 U/µl BglII (NEB R0144M) was added to the
14 mixture and incubated overnight at 37°C with rotation at 950 rpm in a Thermomixer.

15 3. Fill in 5'overhang generated by BglII digestion with biotin

16 A nucleotide mix containing dATP, dGTP and dTTP was made by adding 1µl each of
17 100 mM dATP, dGTP and dTTP into 27µl ddH₂O. To the 480.0µl BglII digested nuclear
18 preparation from the above step 2, 4.5 µl of the nucleotide mix, 15 µl 1 mM biotin-16-dCTP
19 (Axxora, JBS-NU-809-BIO16) and 10 µl 5U/µl Klenow (NEB, M0210L) were added followed
20 by incubation at 37°C for 90 min with intermittent gentle shaking at 700 rpm for 10s after every
21 20s using Thermomixer. The tube was also taken out and inverted every 15-20 min. After this
22 incubation, the mixture was kept on ice.

23 4. Proximity ligation

24 The mixture from step 3 was transferred to a 50 ml conical tube followed by adding 750
25 µl 10x T4 ligase buffer (NEB B0202S, no PEG), 75 µl 100x BSA (NEB), 6140.5 µl water, 25 µl
26 30 U/µl T4 DNA ligase (Thermo Scientific, EL0013), and incubating at 16°C overnight.

27 5. Reverse crosslink and DNA isolation

28 25 µl 20 mg/ml proteinase K (Invitrogen, 25530-049) was added into the reaction
29 mixture from step 4 and the mixture was divided equally into 8x1.5 ml microfuge tubes (~950 µl
30 per tube). The tubes were then incubated overnight at 65°C with rotation at 950 rpm in a

1 Thermomixer. Next day, 3 μ l 20 mg/ml proteinase K were added to each tube followed by
2 incubation at 65°C for 2 hours with mixing in Thermomixer. The mixtures were combined into
3 one 50 ml conical tube. After cooling down to room temperature, 10 ml phenol (pH 8.0) (Sigma)
4 was added and mixed by vortex for 2 min. The mixture was then centrifuged for 10 min at
5 3,000g (Sorvall Lynx 6000 centrifuge). The supernatant containing the DNA was mixed with 10
6 ml phenol:chloroform (1:1) (pre-warmed to room temperature) and vortexed for 2 min. The
7 whole mixture was then transferred to a 50 ml MaXtract High Density tube (Qiagen, 129073)
8 and centrifuged at 1,500g for 5 min (Sorvall Lynx 6000 centrifuge). The top phase containing the
9 Hi-C DNA was transferred to a 50 ml conical tube and the volume (usually ~10 ml) was adjusted
10 to 10 ml with 1xTE as needed. To pellet the DNA, 1 ml 3M Na-acetate, 5 μ l 15 mg/ml
11 GlycoBlue (Invitrogen AM9515) and 10 ml isopropanol were added to the mixture and
12 incubated at -80°C for >1 hour. The DNA was then pelleted by centrifugation at 17,000g for 45
13 min at 4°C (Sorvall Lynx 6000 centrifuge). The Hi-C DNA pellet was resuspended in 450 μ l
14 1xTE and transferred to an 1.5 ml microfuge tube followed by adding 500 μ l phenol:chloroform
15 (1:1). After mixing by vortex, the mix was centrifuged at 18,000g for 5 min at room temperature.
16 The top aqueous layer was collected into another tube followed by adding 40 μ l 3M Na-acetate,
17 1 μ l 15 mg/ml GlycoBlue (Invitrogen AM9515, 300 μ l) and 1 ml ice-cold 100% ethanol. After
18 incubating at -80°C for >30 min, the DNA was centrifuged at 21000g for 30 min at 4°C. The
19 DNA pellet was washed with freshly prepared 70% ethanol and air dry followed by dissolving in
20 45 μ l EB (10mM Tris, pH8.0). The contaminated RNA in the DNA preparation was digested by
21 adding 0.5 μ l 10 mg/ml RNaseA and incubated at 37°C for 30 min.

22 6. Remove biotin from the free DNA (un-ligated DNA) ends

23 To remove the biotin at the free DNA ends, 1.0 μ l 10 mg/ml BSA (NEB, 100x), 10.0 μ l
24 10x NEB 2.1 buffer, 1 μ l 10 mM dATP, 1 μ l 10 mM dGTP and 5 μ l T4 DNA polymerase (NEB
25 M0203S), and 42 μ l water were added to 40 μ l (~3 μ g) Hi-C DNA preparation from step 7. The
26 mixture was divided into two equal aliquots in 2 PCR tubes and incubate at 20°C for 4 hours. 2
27 μ l 0.5 M EDTA was added to each of the two tubes to stop the reaction. The Hi-C DNA was then
28 purified using the Clean and Concentrator Kit (ZYMO, D4013) followed by elution with 50 μ l
29 EB.

30 7. Biotin pull down of DNA and second DNA digestion

1 60 µl Dynabeads MyOne Streptavidin C1 (Invitrogen) was washed in 1.5 ml non-sticking
2 microfuge tubes (Ambion) with 200 µl 2x binding buffer (BB, 10 mM Tris, pH8, 0.1 mM
3 EDTA, 2 M NaCl) twice followed by resuspension in 50 µl 2x BB. The 50 µl Hi-C DNA from
4 step 6 was added followed by rotating for 30 min using Intelli-Mixer (ELMI) at room
5 temperature. The beads were collected using a magnetic stand and washed with 100 µl 1x BB
6 followed by washing with 100 µl 1x NEB4 buffer twice and resuspending in 50 µl 1x NEB4
7 buffer. The DNA on beads was digested using 1 µl 10U/µl AluI (NEB, R0137S) at 37°C for 60
8 min. The beads were collected on a magnetic stand followed by washing with 100 µl 1x BB and
9 then 100 µl EB. The beads were resuspended in 30 µl EB.

10 8. A-tailing

11 The 30 µl beads from step 7 were mixed with 5 µl NEB Buffer 2, 10 µl 1 mM dATP, 2 µl
12 H₂O, 3 µl Klenow (3'-5' exo-) (NEB M0212L) and incubated at 37°C for 45 min. After the
13 reaction, the beads were collected by a magnetic stand followed by washing with 100 µl 1x BB
14 and then 100 µl EB. The beads were resuspended in 50 µl EB.

15 9. Sequencing adaptor ligation

16 The 50 µl beads from step 8 was mixed with 3.75 µl sequencing adapter (TruSeq RNA
17 Sample Prep Kit v2), 10 µl 1x T4 DNA ligase buffer, 3 µl T4 DNA Ligase (30U/µl) (Thermo
18 Scientific, EL0013) and incubate at room temperature for 2 hours. The beads were collected by a
19 magnetic stand followed by washing twice with 400 µl 1x BB + 0.05% Tween, 200 µl 1x BB,
20 and then 100 µl EB. The beads were resuspended in 40 µl EB. To release the DNA from the
21 beads, the mixture was incubated at 98°C for 10 min and then centrifuged at 500 rpm to pellet
22 the streptavidin beads.

23 10. TruSeq RNA Library Prep Kit was used to make DNA sequencing library (8 PCR cycle was
24 used) and the DNA was sequenced by NextSeq 500.

25

26 **Single Cell RNA-seq (scRNA-seq)**

27 1 polyp, 8 tentacles, two stalks, or 2 regenerating stalks of *Xenia sp.* were dissociated into
28 single cells in 1 ml digestion buffer, containing 3.6 mg/ml dispase II (Sigma, D4693), 0.25
29 mg/ml Liberase (Sigma, 5401119001), 4% L-cysteine in Ca²⁺ free sea water (393.1 mM NaCl,
30 10.2 mM KCl, 15.7 mM MgSO₄·7H₂O, 51.4 mM MgCl₂·6H₂O, 21.1 mM Na₂SO₄, and 3 mM

1 NaHCO₃, pH 8.5) and incubated for 1 hour at room temperature. After digestion, fetal bovine
2 serum was added to a final concentration of 8% to stop enzymatic digestion. Cell suspension was
3 filtered through a 40 µM cell strainer (FALCON). Cells were counted by hemocytometer and
4 17000 cells per tissue sample were subjected to single cell library preparation using the 10x
5 Genomics platform with Chromium Single Cell 3' Library and Gel Bead Kit v2 (PN-120267),
6 Single Cell 3' A Chip Kit (PN- 1000009), and i7 Multiplex Kit (PN-120262). Libraries were
7 sequenced using Illumina NextSeq 500 for paired-end reads. Read1 is 26 bp while Read2 is 98
8 bp.

9

10 **Bulk RNA-seq**

11 Total RNA was isolated from 3 polyps, 32 tentacles, or 6 stalks by RNeasy Plus Mini Kit
12 (QIAGEN). To obtain additional transcriptome from different cell types, we dissociated coral
13 tissue into individual cells according to a previously published method³⁴ and subjected the
14 dissociated cells to OptiPrep based cell separation³⁵. Cells with different density were separated
15 into four different layers and RNA was isolated from each layer with the same kit described
16 above. For transcriptome of FACS sorted algae-containing and algae-free cells, 3 polyps were
17 dissociated with the same protocol as used in the scRNA-seq and the dissociated cells were
18 subjected to FACS. Cy5.5 positive and negative cells were collected as algae-containing and
19 algae-free cells, respectively, and used for total RNA extraction as above. cDNA libraries were
20 built according to TruSeq Stranded mRNA Library Prep Kit (Illumina) and subjected to Illumina
21 NextSeq 500 for sequencing. For gene annotation, paired-end sequencing of 75 bp for each end
22 was used. For FACS sorted bulk cell transcriptomes, single end sequencing of 75 bp was used.

23

24 **Xenia regeneration and BrdU tracing**

25 Individual *Xenia* sp. polyps were placed into a well of 12-well cell culture plate
26 (Corning) containing 2 ml artificial sea water from the aquatic tank. The polyps were allowed to
27 settle in the well for 5~7 days before cutting away the tentacles. For BrdU tracing, 0.5 mg/ml
28 BrdU was added into the well 2 days prior to sample harvest. The BrdU labeled stalks were fixed
29 by 4% PFA overnight followed by washing with PBST (PBS+0.1% Tween 20) twice for 10 min
30 each. The stalk was then balanced with 30% sucrose overnight followed by embedding in OCT,
31 frozen in dry ice/ethonal and subjected to cryo-sectioning. The slides were washed with PBS 3

1 times for 5 min each time followed by treating with 2 M HCl containing 0.5% Triton X-100 for
2 30 min at room temperature. The slides were then incubated with PBST (0.2% Triton X-100 in
3 PBS) 5 min for 3 times each followed by blocking with 10% goat serum and then incubating
4 with mouse anti-BrdU antibody (ZYMED, 18-0103, 1:200 dilution in 10% goat serum) at 4°C
5 overnight. Slides were washed with PBST 3 times for 10 min each followed by incubation with
6 the secondary antibody (Invitrogen) for 1 hour at room temperature and washing with PBST 3
7 times for 10 min each. The nuclei were counterstained with Hoechst 33342 and the signal was
8 visualized using a confocal microscope (Leica). Clear BrdU signal in the nucleus labeled by
9 Hoechst was counted as a BrdU⁺ cell. If the *Xenia* BrdU⁺ nucleus was juxtaposed to an alga, it
10 was counted as an alga containing BrdU⁺ *Xenia* cell.

11

12 **Whole mount RNA *in situ* hybridization**

13 To perform RNA *in situ* hybridization on *Xenia*, we modified the whole mount RNA *in*
14 *situ* hybridization protocol for zebrafish³⁶ as described below.

15 For making gene specific probes, we design primers (Supplementary table 6) to genes of
16 interest for polymerase chain reaction (PCR) to amplify gene fragments from *Xenia* sp. cDNA.
17 T3 promoter sequence was added to the 5' of the reverse primers so that the PCR products could
18 be directly used for synthesizing anti-sense RNA probes by T3 RNA polymerase (Promega,
19 P2083) using DIG RNA Labeling Mix (Roche, 11277073910). DIG-labeled RNA probes were
20 purified by RNA Clean and Concentrator-5 (ZYMO), heated to 80°C for 10 min, immediately
21 transferred on ice for 1 min, and then diluted in Prehyb⁺ buffer (50% Formamide, 5XSSC, 50
22 µg/ml Heparin, 2.5% Tween 20, 50 µg/ml SSDNA (Sigma, D1626)) to a final concentration of
23 0.5 µg/ml, and stored in -20°C until use.

24 *Xenia* polyps were relaxed in Ca²⁺ free sea water (described above) for 30 min and fixed
25 in 4% PFA in Ca²⁺ free sea water overnight at 4°C. Fixed polyps were washed with PBST (0.1%
26 Tween 20 in PBS) twice for 10 min each, and then incubated in 100% methanol at -20°C
27 overnight. Next day, the tissues were washed sequentially in 75%, 50%, and 25% methanol for 5
28 min each and then washed in PBST for 10 min. They were then treated with 50 µg/ml proteinase
29 K in PBST for 20 min followed by washing in PBST briefly. The tissues were post-fixed in 4%
30 PFA at room temperature for 20 min and then washed with PBST 2 time for 10 min each. Pre-
31 hybridization was performed in Prehyb⁺ at 68°C for 2 h, followed by incubation with probes in

1 Prehyb⁺ overnight at 68°C. After probes were removed, samples were washed sequentially in 2X
2 SSC (0.3 M NaCl and 0.03 M sodium citrate) containing 50% formamide for 20 min twice, 2X
3 SSC containing 25% formamide for 20 min, 2X SSC for 20 min twice, and 0.2X SSC for 30 min
4 3 times each, all at 68°C. Then, samples were washed in PBST at room temperature for 10 min
5 and incubated in DIG blocking buffer (1% ISH blocking reagent (Roche, 11096176001) in
6 maleic acid buffer (0.1M maleic acid, 0.15 M NaCl, pH 7.5) for 1 hour at room temperature,
7 followed by incubation in anti-DIG antibody (Anti-Digoxigenin-AP (Roche, 11093274910)) at
8 1:5000 dilution in DIG blocking buffer overnight at 4°C. Next day, the samples were wash in
9 PBST for 10 min 3 times each at room temperature, then in 9.5T buffer (100 mM Tris-HCl
10 pH9.5, 50 mM MgCl₂, 100 mM NaCl, 0.1% Tween 20) for 10 min 3 times each at room
11 temperature. Hybridization signals were revealed by incubation in BCIP/NBT buffer (1
12 SIGMAFAST™ BCIP®/NBT tablet (Sigma, B5655) in 10 ml H₂O)) at 4°C until brown-purplish
13 colors were sufficiently dark. For this study, the color development step took 48-60 hours. The
14 samples were then wash in PBST twice for 10 min each. The samples were post-fixed in 4%
15 PFA overnight at 4°C followed by washing in PBST twice for 10 min each and then washing in
16 methanol for 3 h at room temperature. The tissues were kept in PBS and imaged using SMZ1500
17 microscope (Nikon) under Ring Light System (Fiber-Lite). For cross section of stalks, the whole
18 mount sample was processed for frozen cryosection as described above.

19

20 **RNAscope *in situ* hybridization assay for *LePin* and *Granulin1* expression**

21 To visualize RNA expression in endosymbiotic cells, we used the ultrasensitive
22 RNAscope ISH approach (Advanced Cell Diagnostics Inc, ACD). *LePin*- or *Granulin1*-specific
23 oligo probes were ordered from ACD (see Supplementary Table S6 for further information). The
24 fluorescent RNAscope assay was carried out by RNAscope® Multiplex Fluorescent Reagent Kit
25 v2 (ACD) according to manufacturer's protocol. The Chromogenic assay was carried by
26 RNAscope® 2.5 HD Duplex Detection Kit (ACD) according to manufacturer's protocol. Both
27 assays used the cryo-section of the fixed *Xenia* polyp prepared according to the manufacturer's
28 protocol.

29

30 **Genome assembly**

1 Sequencing data from Nanopore were used to initiate the genome assemble by Canu
2 (v1.7)³⁷. The assembled genome was further polished with Illumina short reads by Nanopolish
3 (v0.9.2, <https://github.com/jts/nanopolish>) with 5 cycles, which resulted in 1482 high quality
4 contigs for the diploid genome. The diploid genome assembly was separated into haploid by
5 HaploMerger2³⁸. The haploid genome assembly was further subject to Hi-C assisted scaffolds by
6 3D *de novo* assembly pipeline³⁹.

7

8 **Gene annotation**

9 Funannotate genome annotation pipeline (v1.3.3,
10 <https://github.com/nextgenusfs/funannotate>) was used to annotate the *Xenia sp.* genome. In brief,
11 transcriptome data were assembled by Trinity (v2.6.6)⁴⁰ and used to generate the gene models
12 based on the presence of mRNA by PASApipeline (v2.3.2)⁴¹. These gene models were used as
13 training sets to perform *de novo* gene prediction by AUGUSTUS (v3.2.3)⁴² and GeneMark-ES
14 Suite (v4.32)⁴³. All gene models predicted by PASApipeline, AUGUSTUS, and GeneMark were
15 combined and subject to EVidenceModeler to generate combined gene models⁴⁴. The predicted
16 genes were filtered out if more than 90% of the sequence overlapped with repeat elements as
17 identified by RepeatMasker and RepeatModeler (<http://www.repeatmasker.org>). PASA was
18 further used to add 3' and 5' untranslated region (UTR) sequences to the remaining predicted
19 genes. Pfam (v31.0), Interpro (v 67.0), Uniprot (v2018_03), BUSCO (v1.0)⁴⁵ databases and
20 eggno-g-mapper (v1.3)⁴⁶ were used to annotate the function of these gene models.

21

22 **Phylogeny tree analysis**

23 OrthoFinder (v2.2.7)⁴⁷ was used to find orthologs from different species and build the
24 phylogenetic tree. Diamond (v0.9.21) is used to align the orthologs. MSA and FastTree methods
25 were used to infer the gene trees and phylogenetic tree.

26

27 **Single cell clustering and marker gene identification**

28 The raw single cell sequencing data was de-multiplexed and converted to FASTAQ
29 format by Illumina bcl2fastaq software. Cell Ranger (v2.1.1,
30 <https://support.10xgenomics.com/single-cell-vdj/software/pipelines/latest/what-is-cell-ranger>)
31 was used to de-multiplex samples, process barcodes and count gene expression. The sequence

1 was aligned to the annotated *Xenia sp.* genome and only the confidently mapped and non-PCR
2 duplicated reads were used to generate gene expression matrix. Cells with UMI numbers less
3 than 400 or mitochondria gene expression > 0.2% of total genes were filtered out. To further
4 remove outliers, we calculated the UMI number distribution detected per cell and removed cells
5 in the top 1% quantile. To remove batch effect, we applied Seurat canonical correlation analysis
6 (CCA) alignment method for data integration⁴⁸. For each dataset, we identified top 1000 genes
7 with the highest dispersion. We combined the top 1000 genes from each dataset and ran a
8 canonical correlation analysis to generate the CCA space. The first 16 canonical correlation
9 vectors were used to generate the dimensional reduction which was used for further clustering
10 analysis. Clustering and marker gene identification was performed with Seurat by setting the
11 resolution to 0.6, which led to the identification of 13 cell clusters.

12

13 **Identification of *Xenia sp.* cells performing endosymbiosis with *Symbiodiniaceae***

14 The bulk transcriptome data of FACS isolated algae-containing or algae-free cells were
15 aligned to *Xenia sp.* genome by STAR (v2.5.3a)⁴⁹. Individual gene expression (RPKM) for each
16 sample were calculated by RSEM (v1.3.0)⁵⁰. The gene expression levels of each bulk RNA-seq
17 of FACS-sorted cells were compared with the gene expression levels calculated using average
18 UMI (unique molecular index) number for each gene in each cell cluster identified by scRNA-
19 seq. Pearson correlation coefficient was calculated for each comparison.

20

21 **Pseudotime analysis**

22 To infer the trajectory of endosymbiotic *Xenia* cells, we combined scRNA-seq data of
23 regenerating and non-regenerating samples by Seurat CCA alignment. All cells belonging to the
24 endosymbiotic cell cluster (Cluster 13) were subjected to Monocle (v2.10.1)⁵¹ analyses. These
25 cells were further divided into two sub-clusters and the top 1000 differentially expressed genes
26 between these two groups were used as ordering genes to construct the trajectory by DDRTree
27 algorithm. The differentially expressed genes along pseudotime is detected by
28 differentialGeneTest function in Monocle.

29

30 **RNA velocity**

1 RNA velocity estimation was carried out by *velocyto.R* program (<http://velocyto.org>,
2 v0.6) according to the instruction⁵². Briefly, *velocyto* used raw data of regeneration sample to
3 count the spliced (mRNA) and un-spliced intron reads for each gene to generate a loom file. This
4 loom file was loaded into R (v3.5) by *read.loom.matrices* function and used to generate RNA
5 velocity map. The RNA velocity map was projected into the same t-SNE space identified by
6 Seurat.

7

8 **Data availability**

9 Raw sequence data for this study is available in NCBI BioProject under accession
10 PRJNA548325. Assembled genome and annotation files are available at
11 https://drive.google.com/open?id=1qmqYMepjIsYnOrBuUAe_1-GB-y8oqy0C.

12

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