Science Advances
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Supplementary Materials for

Single-cell RNA-seq reveals distinct metabolic "microniches" and close host-symbiont interactions in deep-sea chemosynthetic tubeworm

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Sci. Adv. **10**, eadn3053 (2024)
DOI: 10.1126/sciadv.adn3053

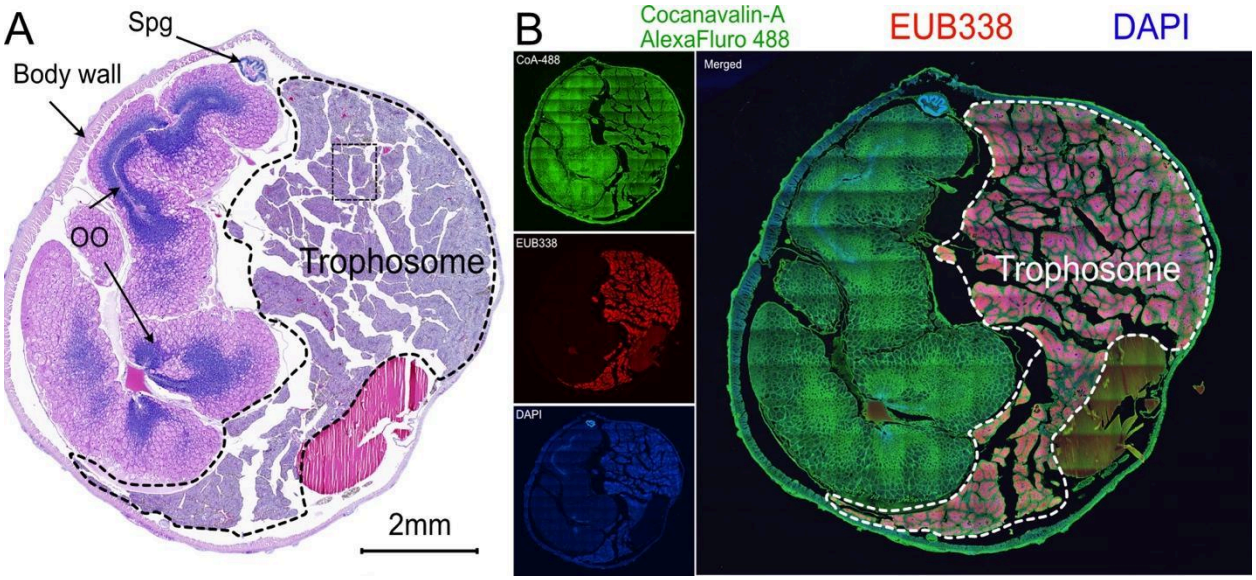
The PDF file includes:

Figs. S1 to S20
Legends for tables S1 to S6

Other Supplementary Material for this manuscript includes the following:

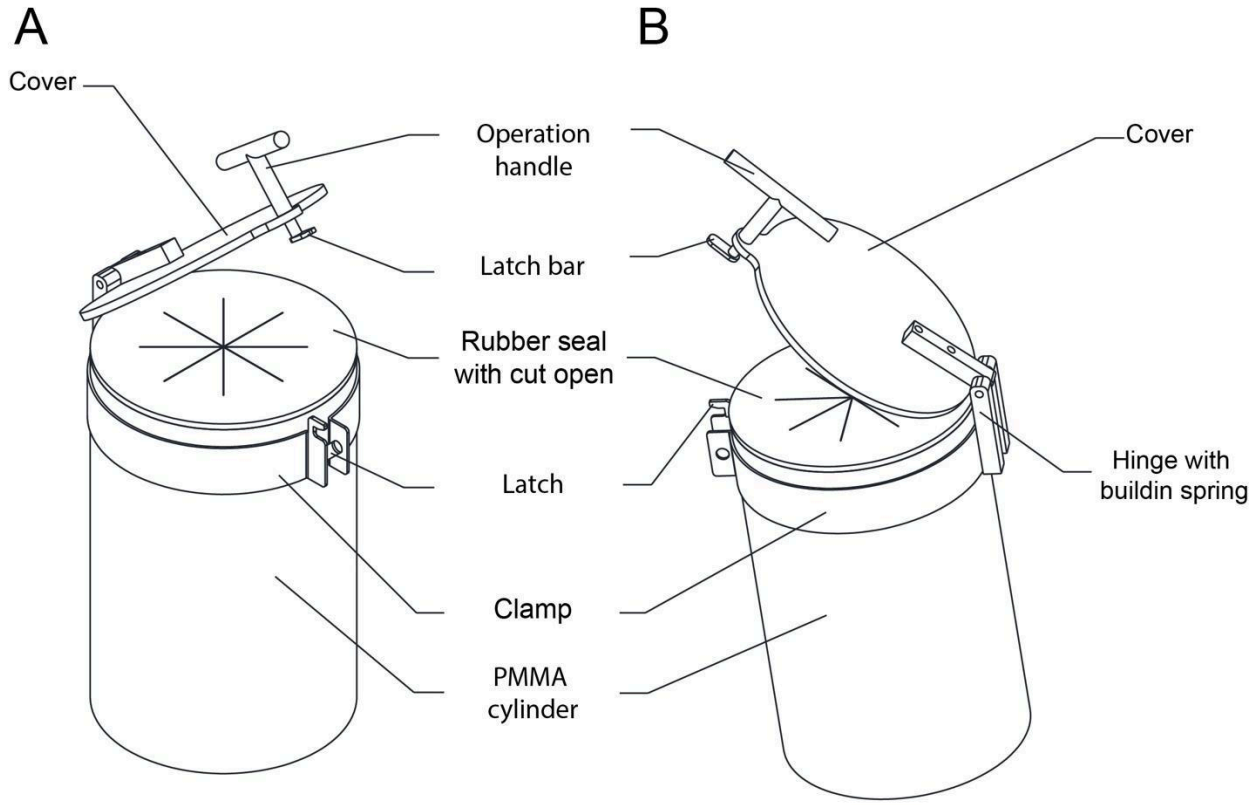
Tables S1 to S6

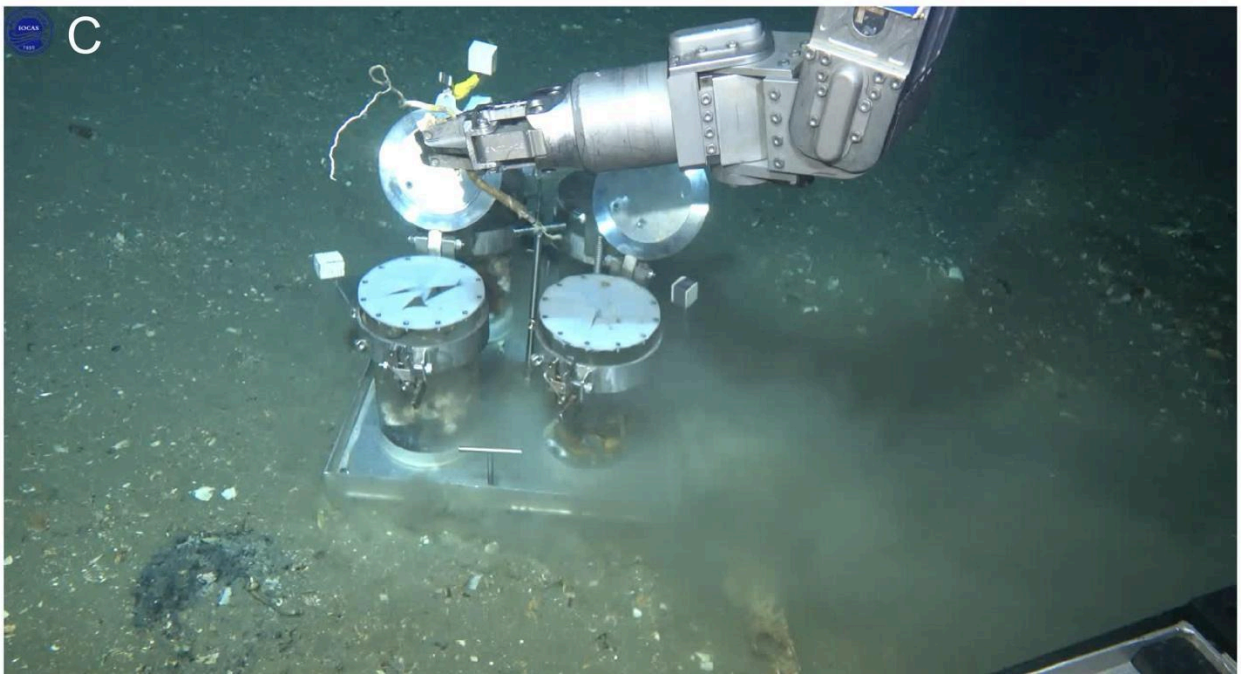
Fig. S1.



Supplementary Figure S1: The trophosome of a *Paraescarpia echinospica* tubeworm. The trophosome was circled in a long dashed line. (A) Hematoxylin and Eosin (H&E) staining of a cross-section of an adult *P. echinospia* tubeworm, the trophosome was circled in a long dashed line. The fine dash-lined square is shown in Figure 1D. (B) Fluorescent *in situ* hybridization (FISH) analysis of a cross-section of an adult *P. echinospica* tubeworm. The intracellular endosymbionts could be detected in the trophosome of the tubeworm. The trophosome was circled in a long dashed line. OO, oocyte; Spg: Spermatogonia.

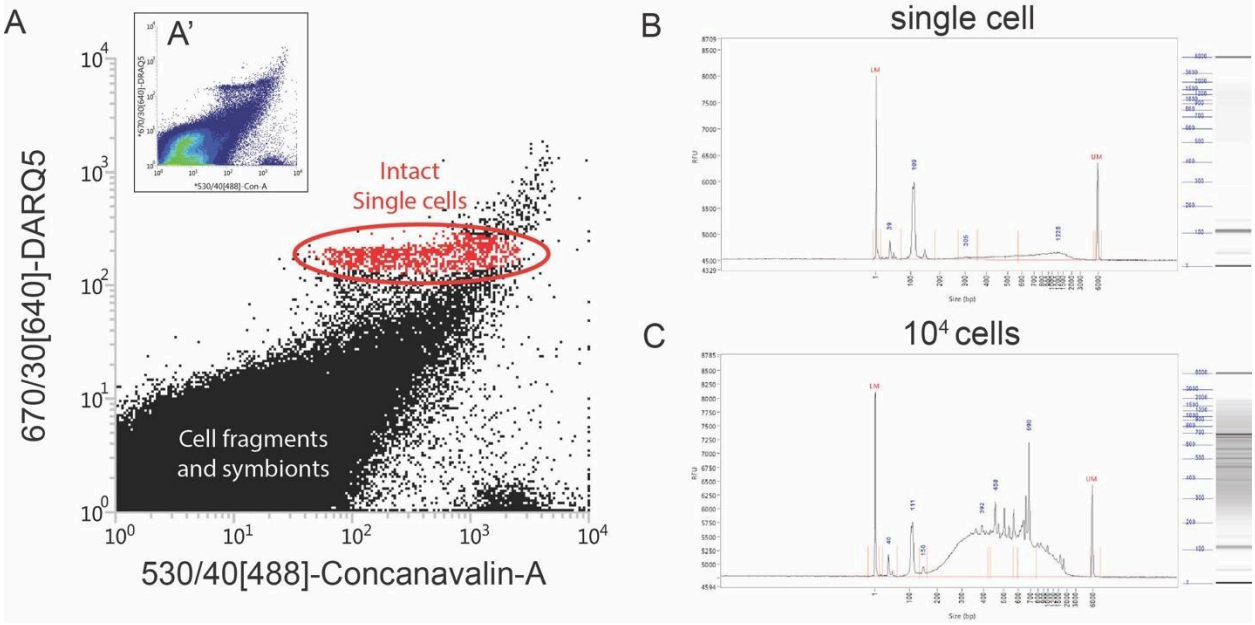
Fig. S2.


A
Cover
Operation handle
Latch bar
Rubber seal with cut open
Latch
Clamp
PMMA cylinder
B
Cover
Hinge with buildin spring


C

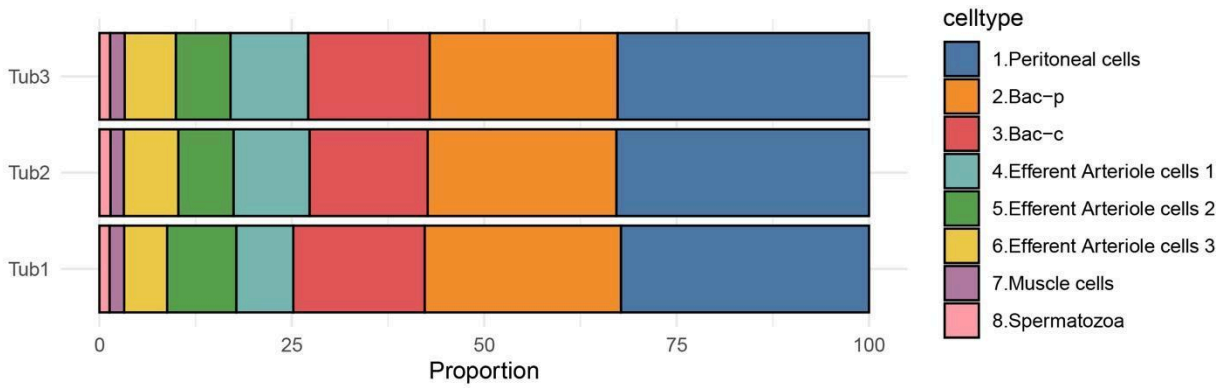
Supplementary Figure S2: The deep-sea *in situ* animal fix apparatus. (A) and (B) front and back view of the deep-sea *in situ* animal fix apparatus. (C) Photographic image of *ROV* "Faxian" collecting a *Paraescarpia echinospica* tubeworm during 2020 cruise at "F-site" cold-seep.

Fig. S3.



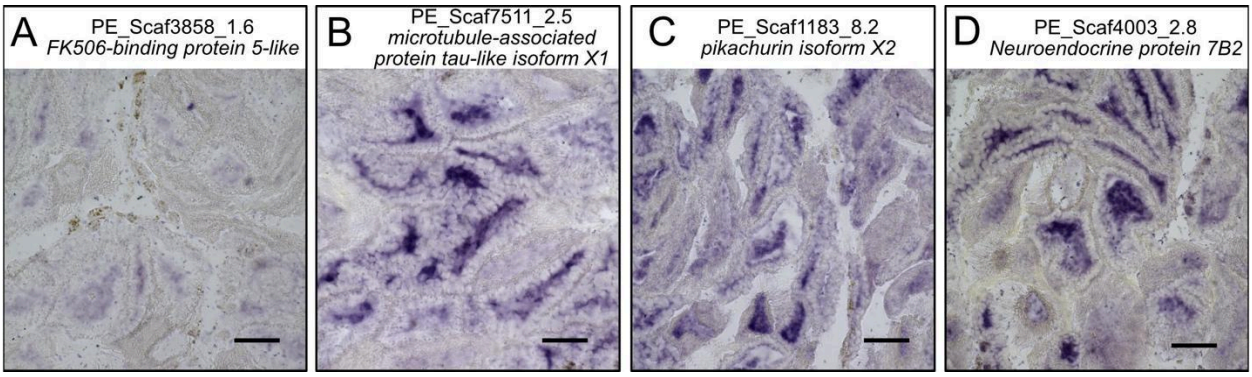
Supplementary Figure S3: Fluorescent active cell sorting (FACS) and cDNA quality check of the *in situ* fixed *Paraescarpia echinospica* trophosome cells. (A) Flow cytometry profiles of *Paraescarpia echinospica* trophosome cells ACME-dissociated cells stained with DRAQ5 (nucleus) and Concanavalin-A 488 (cytoplasm). The gated singlets are labelled in red. (B) and (C) examining the cDNA quality of single-gated cells and 10^4 gated cells.

Fig. S4.



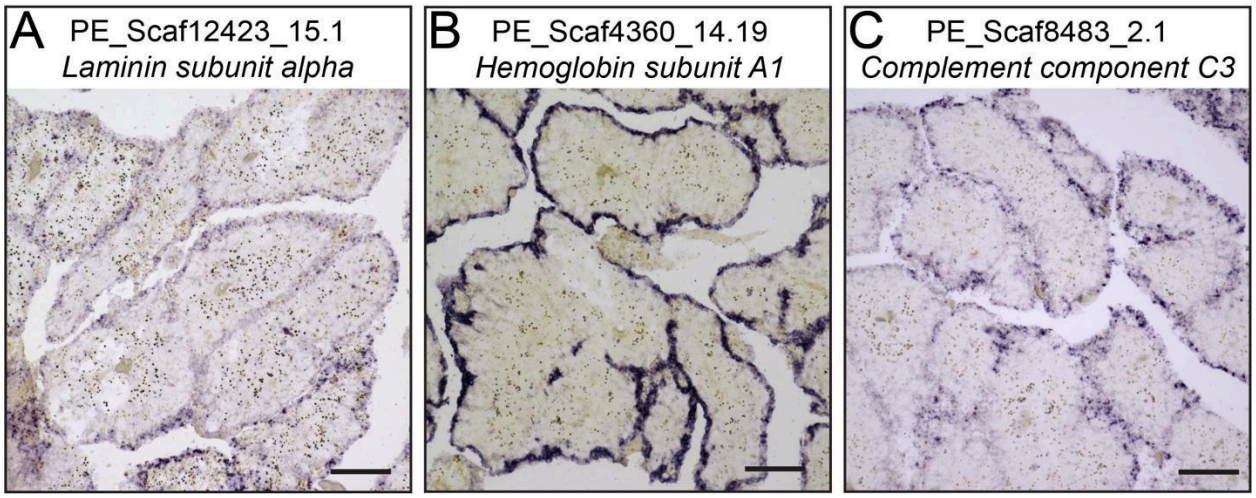
Supplementary Figure S4: Proportion of different cell types in three scRNA-seq runs.

Fig. S5.



Supplementary Figure S5: *in situ* hybridization analyses of the gene markers of the spermatozoa cell cluster. (A) gene encoding FK506-binding protein 5-like (PE_Scaf3858_1.6), (B) gene encoding microtubule-associated protein tau-like isoform X1 (PE_Scaf7511_2.5), (C) gene encoding pikachurin isoform X2 (PE_Scaf1183_8.2), and (D) gene encoding Neuroendocrine protein 7B2 (PE_Scaf4003_2.8). Scale bar = 50 μm

Fig. S6.



Supplementary Figure S6: *in situ* hybridizations of additional peritoneal cell markers. (A) gene encoding Lamin subunit alpha (PE_Scaf12423_15.1), (B) gene encoding Hemoglobin subunit A1 (PE_Scaf4360_14.19), and (C) gene encoding Complement component C3 (PE_Scaf8483_2.1). Scale bar = 100 μm

Fig. S7.

>PE_Scaf4360_14.19 hemoglobin subunit A1
*MKVPCILLVLLGAVVVATA*EKCNDLERIKVKMQWAKAYSFGANRAKFGDALWANVFNYAPDARSIFESVKSENMKSPEFQAHIAR
VLGGLDRVISMLDSKPTLDADLAHLKSQHDPRGLDPATFVVFRQALIATVAGTFGVCFDVPAWQRCFNVIAKGITGSDTFA

>PE_Scaf4360_15.1 hemoglobin subunit A1
*MKTLIILLGFVACAFAT*DCGMLQRIKVKQQWATVYSSGIAREDFGEAIWKAVFAQAPQARALFKRVGVDDIHSPAFKAHIARVNGG
LDMAISLLDNEPTLKAELAHLNGQHKERGIPSNYYDVFIRALHAVVPAALGRCFDHPAWDACSDVIIAGIRQ

>PE_Scaf4360_14.5 hemoglobin subunit A2
*MKSLIAFVCLVAAVNYCCA*DHVCGPLQRLKVKRQWAEAYGSGNRREDFGHYIWSHVFQHSPAARDMFKRVRGDNIHTSAFRAH
ATRVLGGLDMCVALLDVEPVLDSQLAHLSQQHATRGVEAAHYETFEHAVMMGIENVIGAEVFDQDAWKPCLKVITGGIQG

>PE_Scaf7478_3.3 hemoglobin subunit B1
*MNSLILALMLCGATVALVKA*EASDHCSYEDAEIVMKEWQHILGNGQSAPILLVAANVFFTGLFEKVPTSGALFKRVNVADMHSGE
FQAHTMRVMTGLDELINKLHSPAVLDSMLAHLAEQHAVRDGVTHELFHVFRDVMYDSLGQLLDEYNPDAWRNCMFHILYGIAGA
LP

>PE_Scaf8283_1.7 hemoglobin subunit B1
*MNSLILALVLCGATVALA*EGETSSHCSFEEADIVMKEWQHILSHGDSATILMRAANVVFSGMFEKEPSSAALFNRVNVADMHSGE
FHAHTMRVMTGLDELINKLHSPAVLDSMLAHLAEQHALRDGVTHELFHVFRDILYSSLGQLLDEYNPDAWKSCMFHILYGIAGALP

>PE_Scaf8283_3.3 hemoglobin subunit B1
*MNSLILALVLCGATVALA*SEYCSYEDADIVMNEWQHILGSGNSAPILMRAANVIFSAMFEKDPSSRDLFNRVNVADMHSGEFHAH
TLRVMNGLSELINKLHSPAVLDSMLAHLAEQHAVRDGVTHEQFHVFRDILYGSLGQLLDEYNPDAWKNCMFGILYGIAGSLP

>PE_Scaf350_4.9 hemoglobin subunit B1
*MHRLAPFLLVVVCGATATLG*RTFCSRDDADIAIAQWTQGFGIGGNINPKAVITGSTGFFVRMMQMDPSIKPLLSKVNVDDVNSAE
FGAHALRVMIGLDLCVNALNDIPLLEEITSHLAKQHAARIGVKREHLFLLFESTLKAFPKLIDNFNGDAWYNCLEPVFKALTVDLP

>PE_Scaf7230_5.8 hemoglobin subunit B1
*MNPLSTFLLVVCASSAVLG*EYCSEADATIVIDQWTSIYNAGVSSASRATLGNQIFSTLFKLAPDSESLFARVGVDDMSSGAFRAHA
SRVLSGLDMGINSLKQTATLNSLTEHLAAQHIARPGVKAVYFRVMGKVLMTALPTLIEDFNPDAWRNCLLPLKQAISKGLPVMRLLL
GCLSLFLSCAMAWTGYNFCVKCDAEVVKAQWNVFYAAPNSGTSKYMLASHIFDRLFASSPDAKDLFKRVNVHDQSSPEFQAHVI
RVINGLDLCINSLGNRPLLESVTDHMATQHFVREGVTQTHFDPSTTSTTTRGRAASPQ

>PE_Scaf1853_2.14 hemoglobin subunit B1
*MKTVFLLVALSCVTMCLA*NEHNKVCTHTDADAVTEMWESVWSAQNSEQVRVELAEEIFEYIFQKNPAAKELFTRVNVADVNSPE
FQAHVVRVINGLDILINFLDDLPSLEAAAGHLADQHAVRAGVTKAAFQLMDDAFVELLPQIVDNFNPDTWQRCWHSAVDIITEVLP

>PE_Scaf1853_3.4 hemoglobin subunit B1
*MKTVFLLLALSCMTSCMA*HSSQCTHDDAAQVIKMWESVWGAQNSDKIRIVLAEEAFAHIFQRDPGAKQLFSRVNVADIHSPEFE
AHVVRVLNGLDILINFLDDRPSLEAAAKHLADQHVARPGVSVGYFQVMDDAFVEILPQVVDHFDPDTWQNCWHSAIDIITSKLP

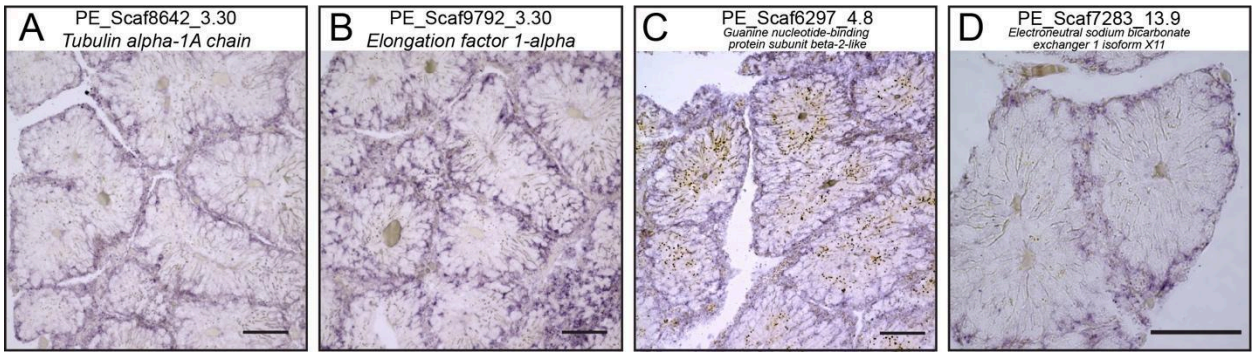
>PE_Scaf10099_2.6 L1 linker protein of V1 giant hemoglobin
*MKGLTAVVVLAVLLGVSLEG*CVGSGMGYGFASSKLQIQQRRVDRLKNSIAGLRAKVEQAQSNGDKVALDHLNTRLLLLDAPNCP
ENSVRCGHSEQCMSELEFCDGVQDCNNNFDEEACFSAPPSGSFWAGDLYHAGCGFIENSNLRLFITGHDHKYYFGAHVGISAS
MVIRHPSNGGITTHNYNLEGCYSFGKRELDLDAPGNAFELRCNTIRSEEWLSCDLVQMTGGNKCGHAHLVKQPYSFGCMTGTIA
CIFITLRPPEGFKNAILVVTESAVWRHCDREEDHCNTFNLFDAVVVSKIRTNTRYSRPGLLSAGQAPRG

>PE_Scaf7855_5.11 L4 linker protein of V1 giant hemoglobin
*MRRLLALLAVVAAVRG*FSTHLNADHVHDQDYPNDVPSKGVKPADRETAISSIELEVSADANDLRMNELQGELDDLAAEVEDMPS
EPANTKSQLYYMRLLKGNGCGDRQFQCLQASDEVPQCIPNIAVCDGVQDCKNGNDETSSICKNHTPVGSSWGGDLEWVGCAA
HRSKRVYVVITRAWQDDYLPSMQKVAATLVFSWVEDGKTMTNTEQMEGHYCYGGHALKLRASNGHVAADIVCNFIDPNHCHGKI
FKASTGTVCSRVILTRQ

>PE_Scaf6206_2.11 L4 linker protein of V1 giant hemoglobin
*MQSLMVLLLAVAAVRG*FSSHLDADHVHDQDYPYGMGLKAPFAWTSPGTAISSIELEVSADANDLRMDDLQAQLDELQTEADKISI
EAPTVWPWVSFIYLRFGNSCDKGRFQCSISSDEVKQCVSNLAVCDGITDCKNGNDEDKLICRNHMPLGSSWAGDLDWVGCEAH
RSKRLYVVVTRVWKDFFMPSVQKIAVNLIFTWVEVGRTMTNMVQAEGRYYHATHSFELNADPNSHLSADITCSFTDDNHCHGVIF
QATTGTLCGRIILIRSSPERITLSVSRQHE

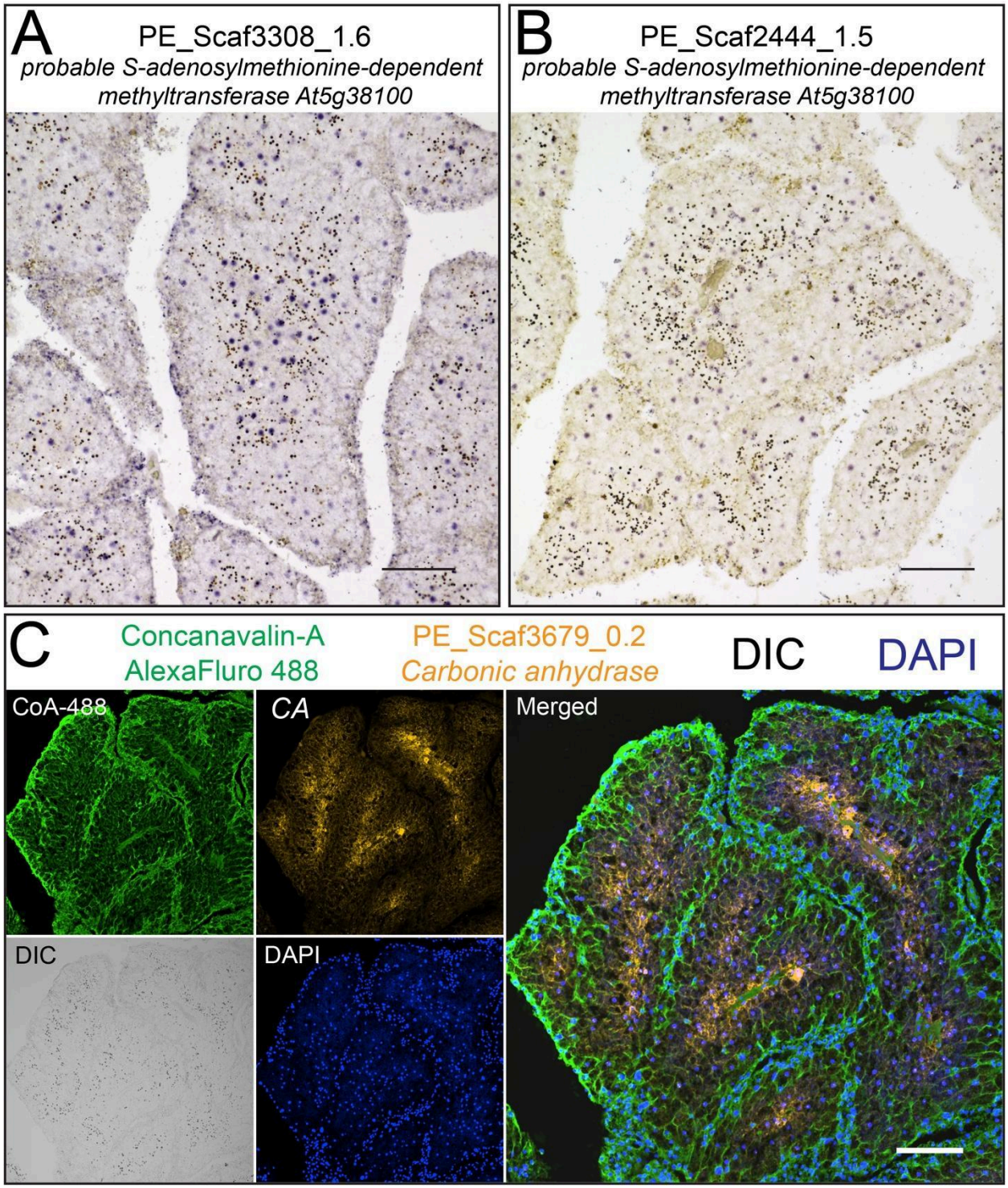
Supplementary Figure S7: Signal peptides of extracellular hemoglobin components.

Fig. S8.



Supplementary Figure S8: *in situ* hybridization analyses of additional EA cell gene markers. (A) gene encoding Tubulin alpha-1A chain (PE_Scaf8642_3.30), (B) gene encoding Elongation factor 1-alpha (PE_Scaf9792_3.30), (C) gene encoding Guanine nucleotide-binding protein subunit beta-2-like (PE_Scaf6297_4.8), and (D) gene encoding Electroneutral sodium bicarbonate exchanger 1 (PE_Scaf7283_13.9). Scale bar = 100 μm.

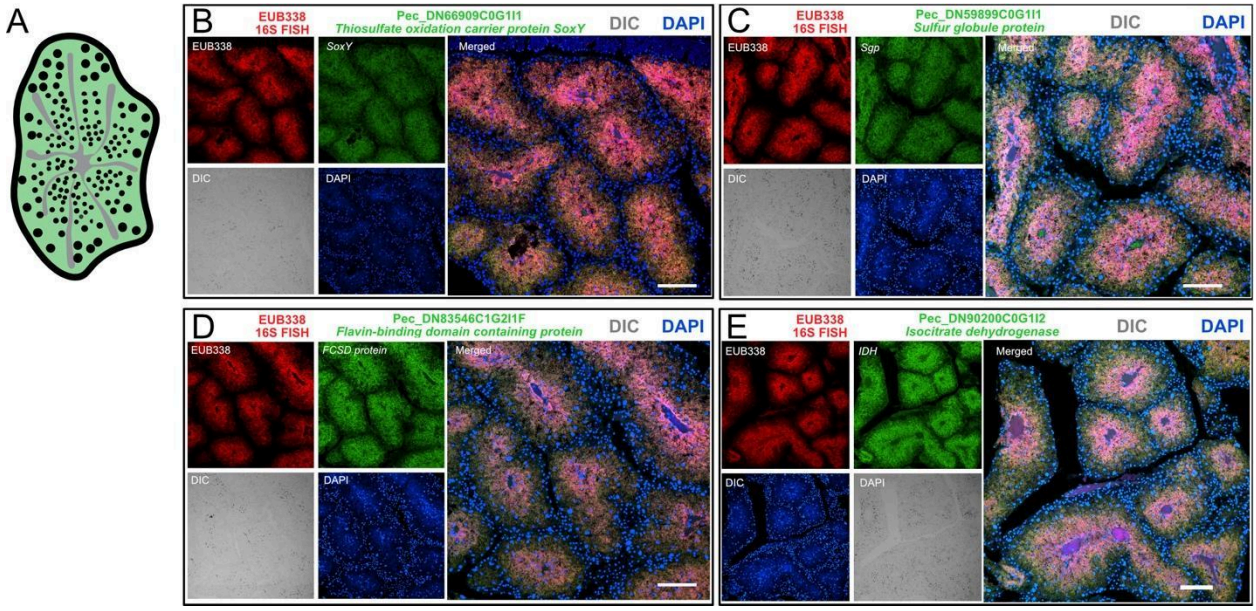
Fig. S9.



Supplementary Figure S9: *in situ* hybridization and FISH analyses of additional Bacteriocytes markers. (A) and (B): genes encoding probable SAM-dependent

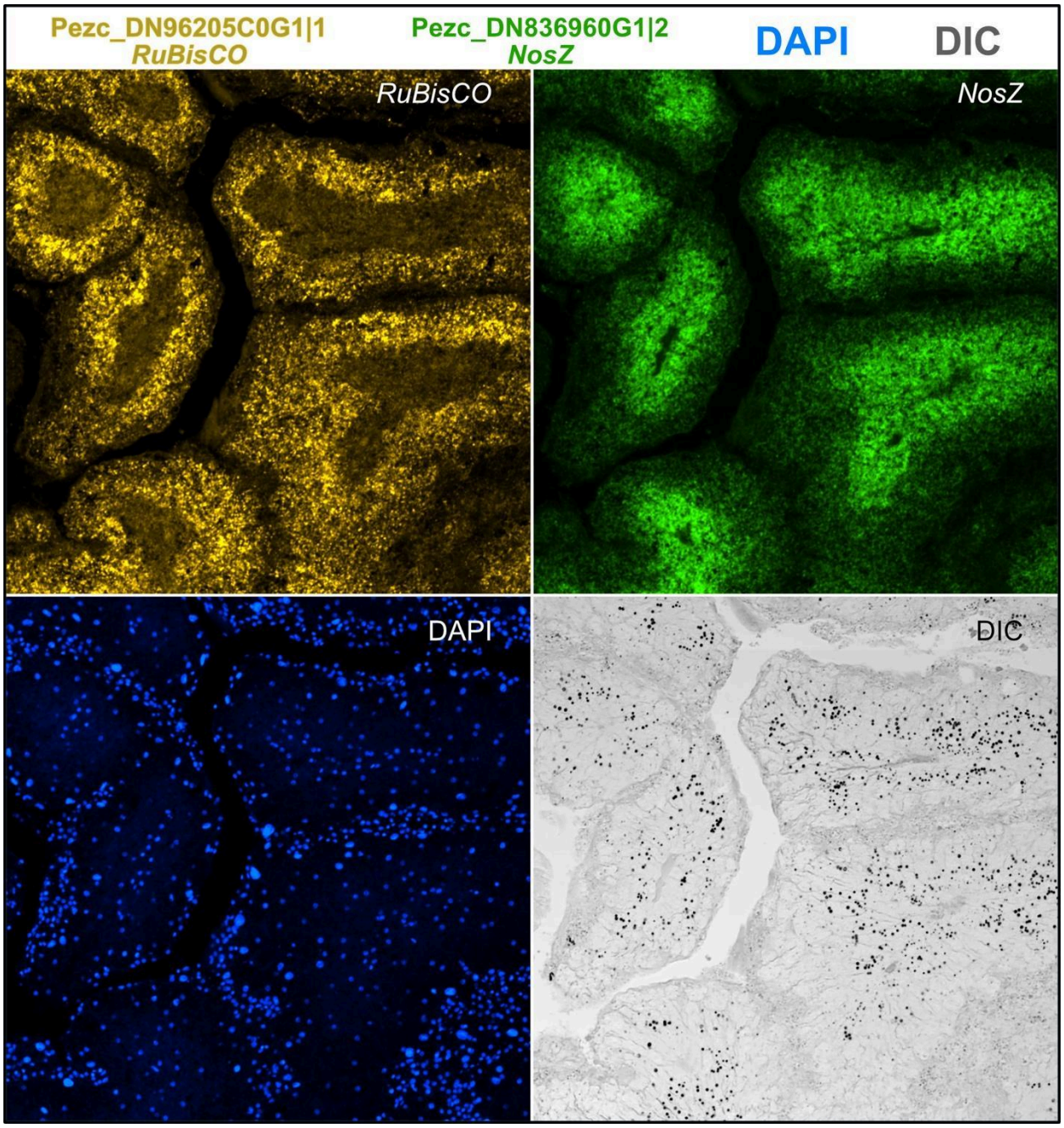
methyltransferase (PE_Scaf3308_1.6 and PE_Scaf2444_1.5), showing both genes are expressed in the center bacteriocytes. (C) FISH analysis of gene encoding Carbonic anhydrase (PE_Scaf3679_0.2). Scale bar = 100 μm

Fig. S10.



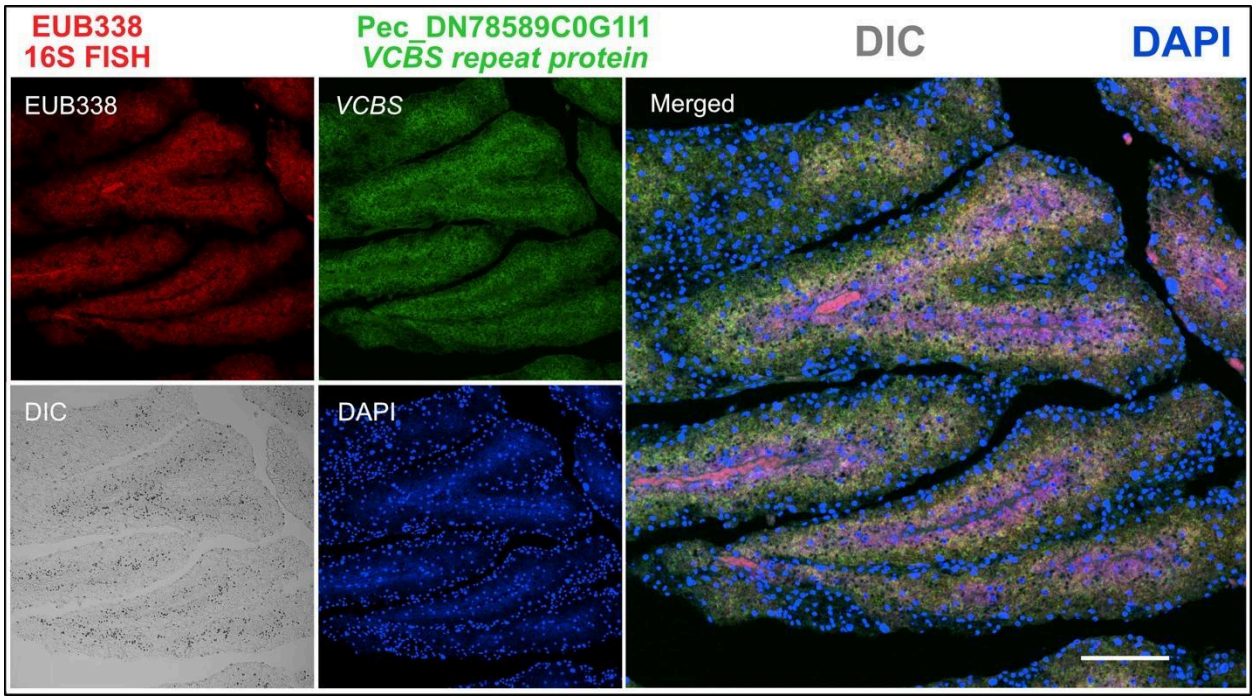
Supplementary Figure S10: Analysis of expression of key symbiont metabolic genes. (A) diagram showing the expression patterns of these genes, with no difference between the periphery and center region. (B)-(E) examined key metabolic genes that showed no difference between periphery and center symbionts. Scale bar = 100 μm

Fig. S11.



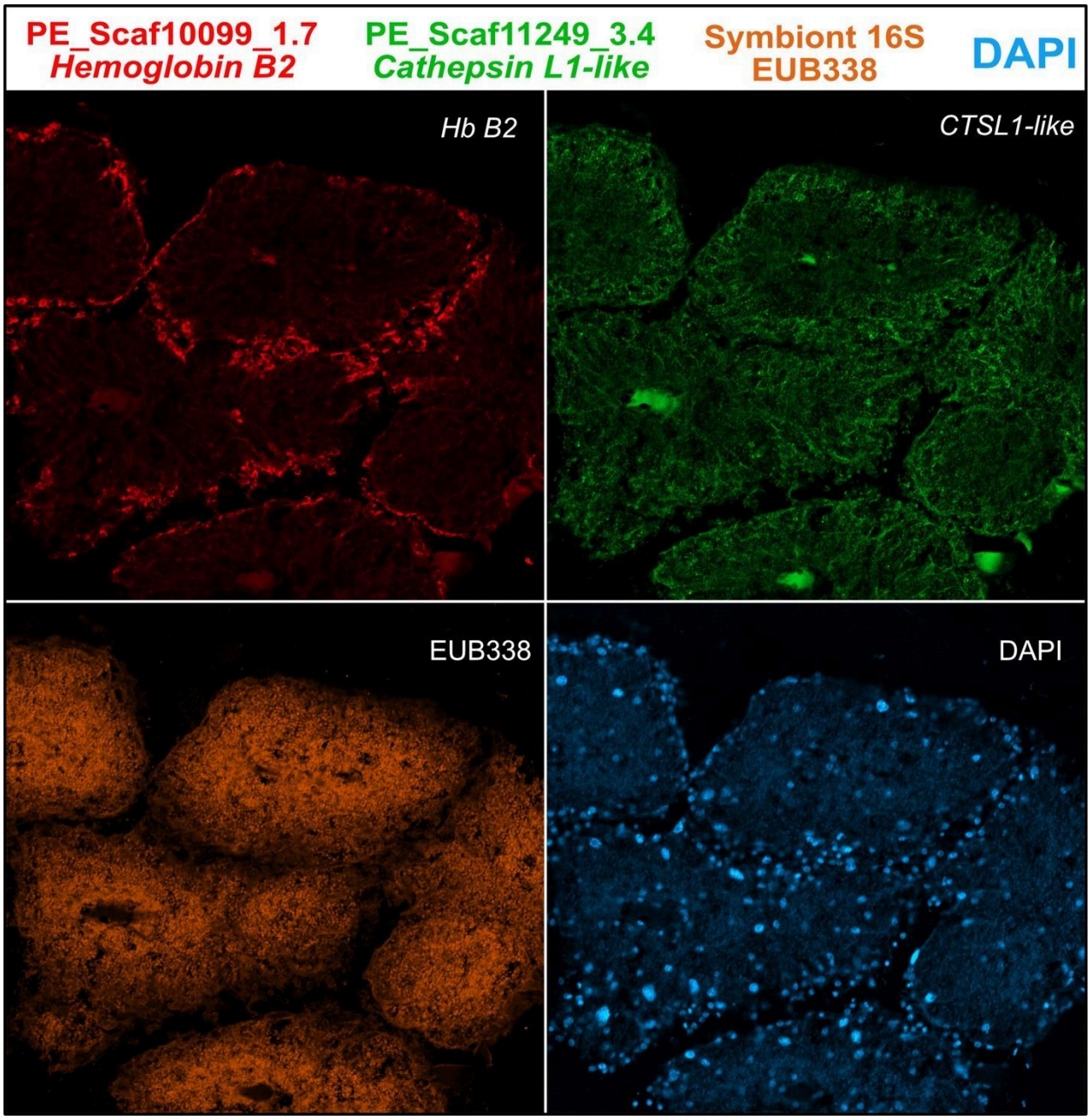
Supplementary Figure S11: Double fluorescent *in situ* hybridization analysis of symbiont *RuBisCO* and *NosZ*. Double fluorescent *in situ* hybridization analysis of genes encoding symbiont RuBisCO (Pezc_DN96205C0G1I1) and symbiont NosZ (Pec_DN83696C0G1I2). The merged image is shown in Figure 6B.

Fig. S12.



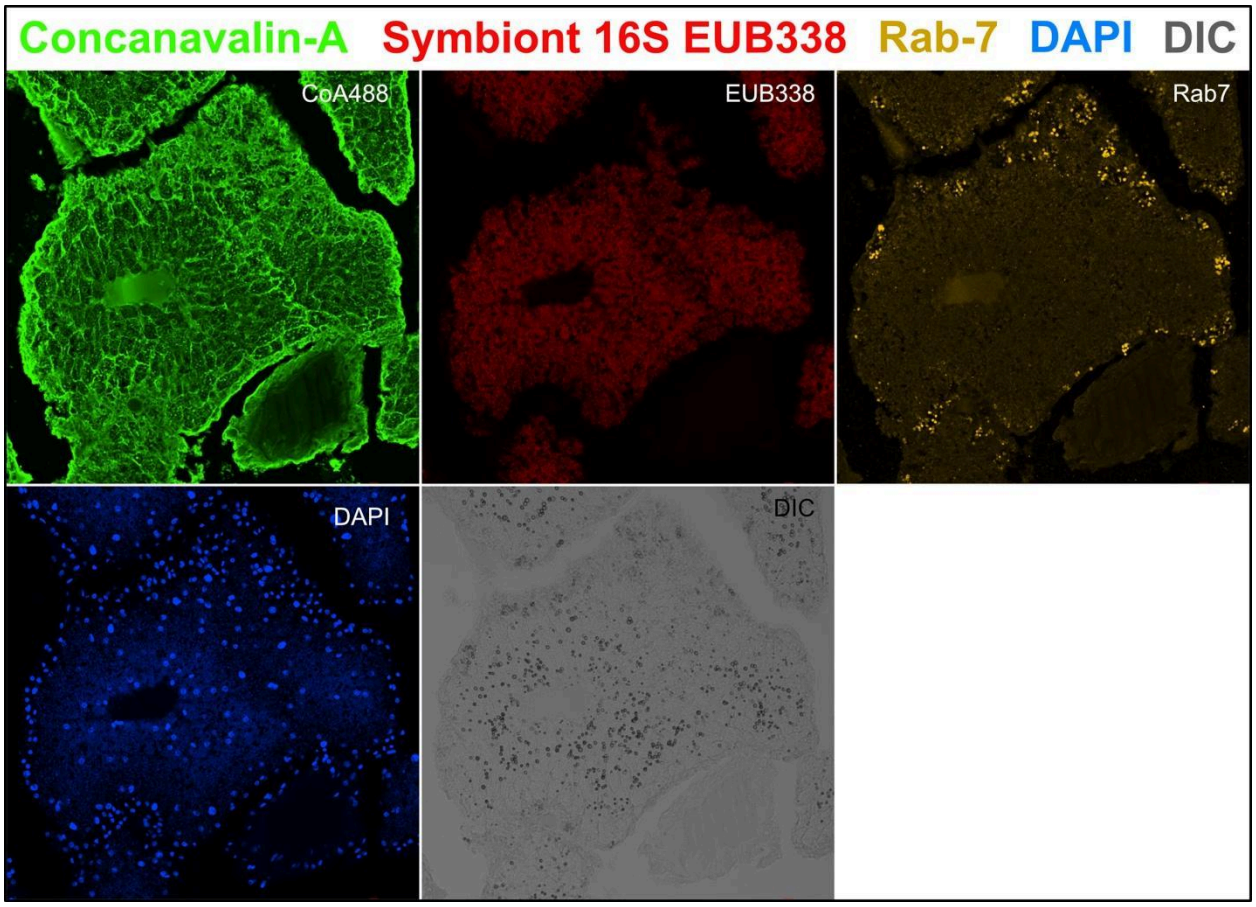
Supplementary Figure S12: Double fluorescent *in situ* hybridization of symbiont *VCBS repeat protein* and symbiont 16S rRNA. Double fluorescent *in situ* hybridization of symbiont gene encoding VCBS repeat protein (Pec_DN78589C0G1I1) and symbiont 16S rRNA, showing the gene encoding VCBS repeat protein is expressed in the symbionts located in the periphery of trophosome lobules. Scale bar = 100 μm

Fig. S13.



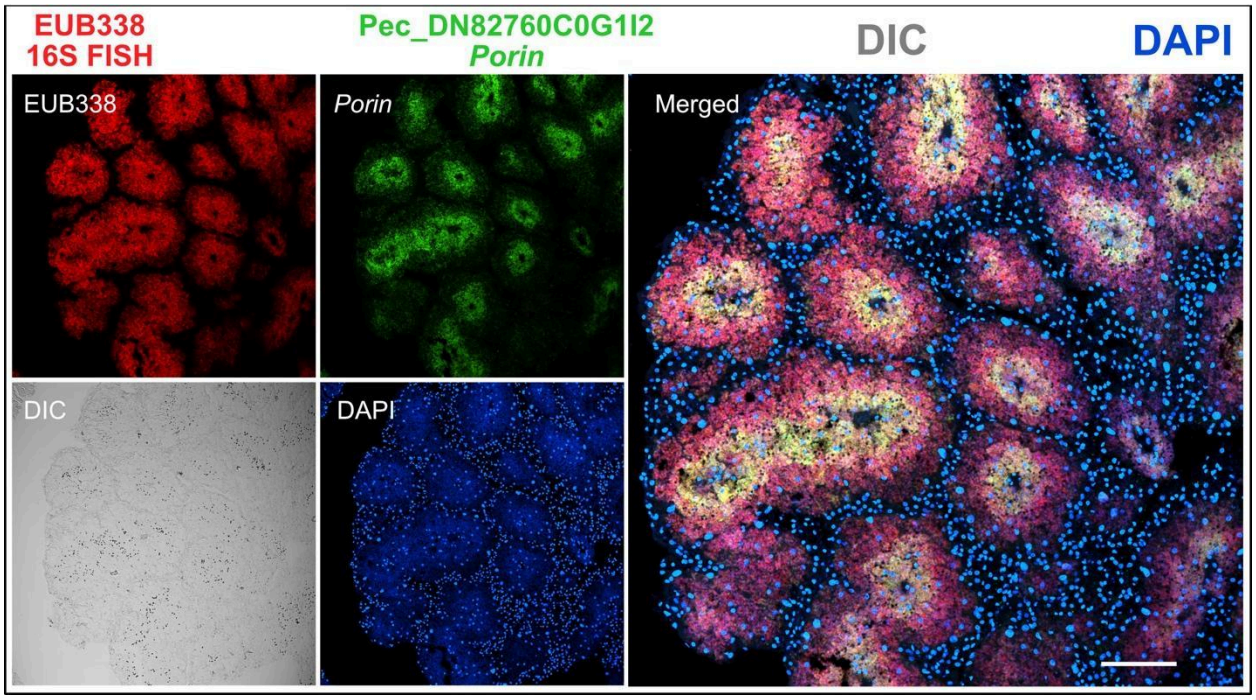
Supplementary Figure S13: Multicolor fluorescent *in situ* hybridization analysis of host *Hemoglobin B2*, host *Cathepsin L1-like*, and symbiont 16S rRNA. Multicolor fluorescent *in situ* hybridization analysis the genes encoding tubeworm host Cathepsin L1-like (PE_Scaf11249_3.4), host Hemoglobin B2 (PE_Scaf10099_1.7), and symbiont 16S rRNA. The merged image is shown in Figure 6D.

Fig. S14.



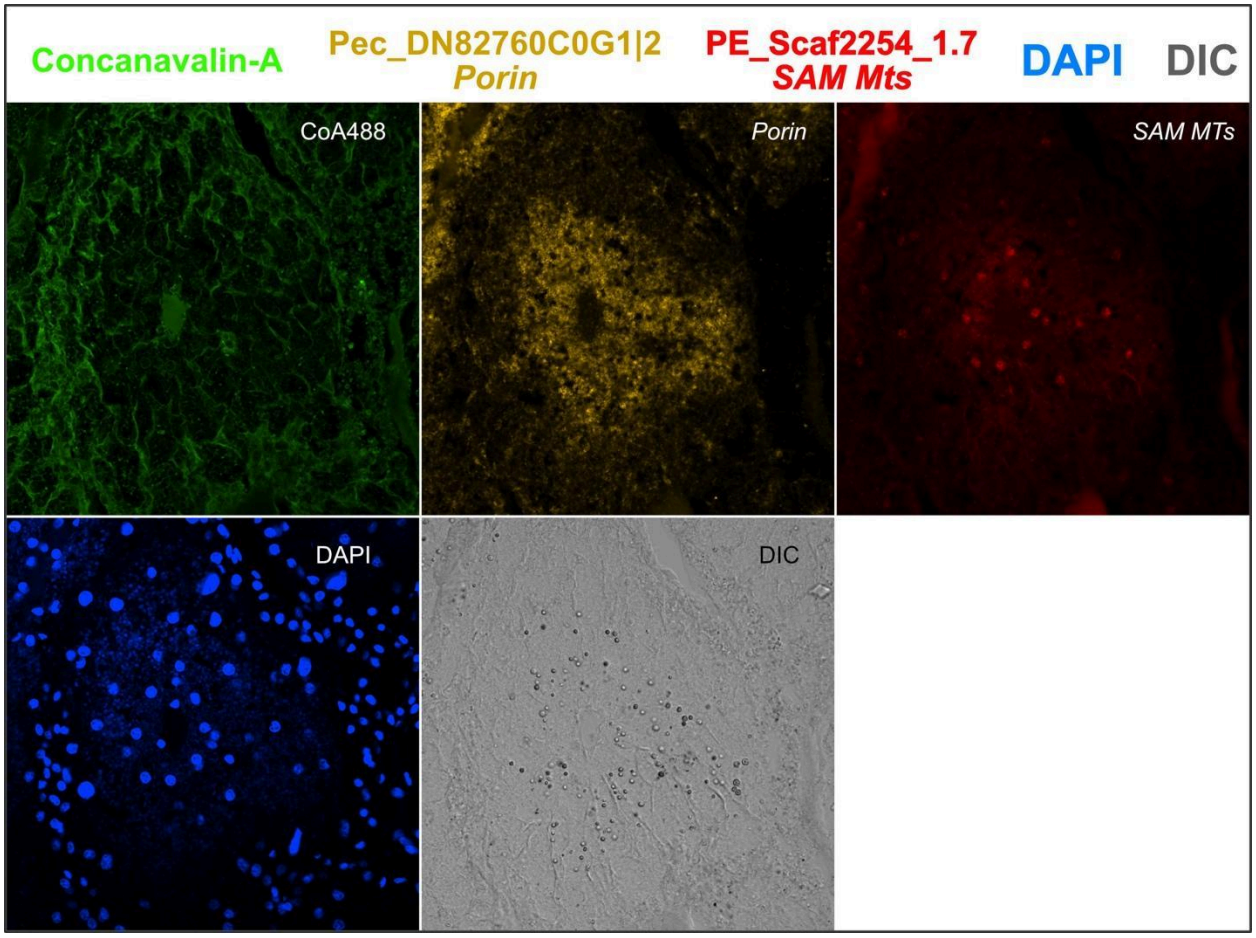
Supplementary Figure S14: FISH-IHC analysis of the symbiont 16S rRNA and late endosome marker Rab-7. The merged image is shown in Figure 6E.

Fig. S15.



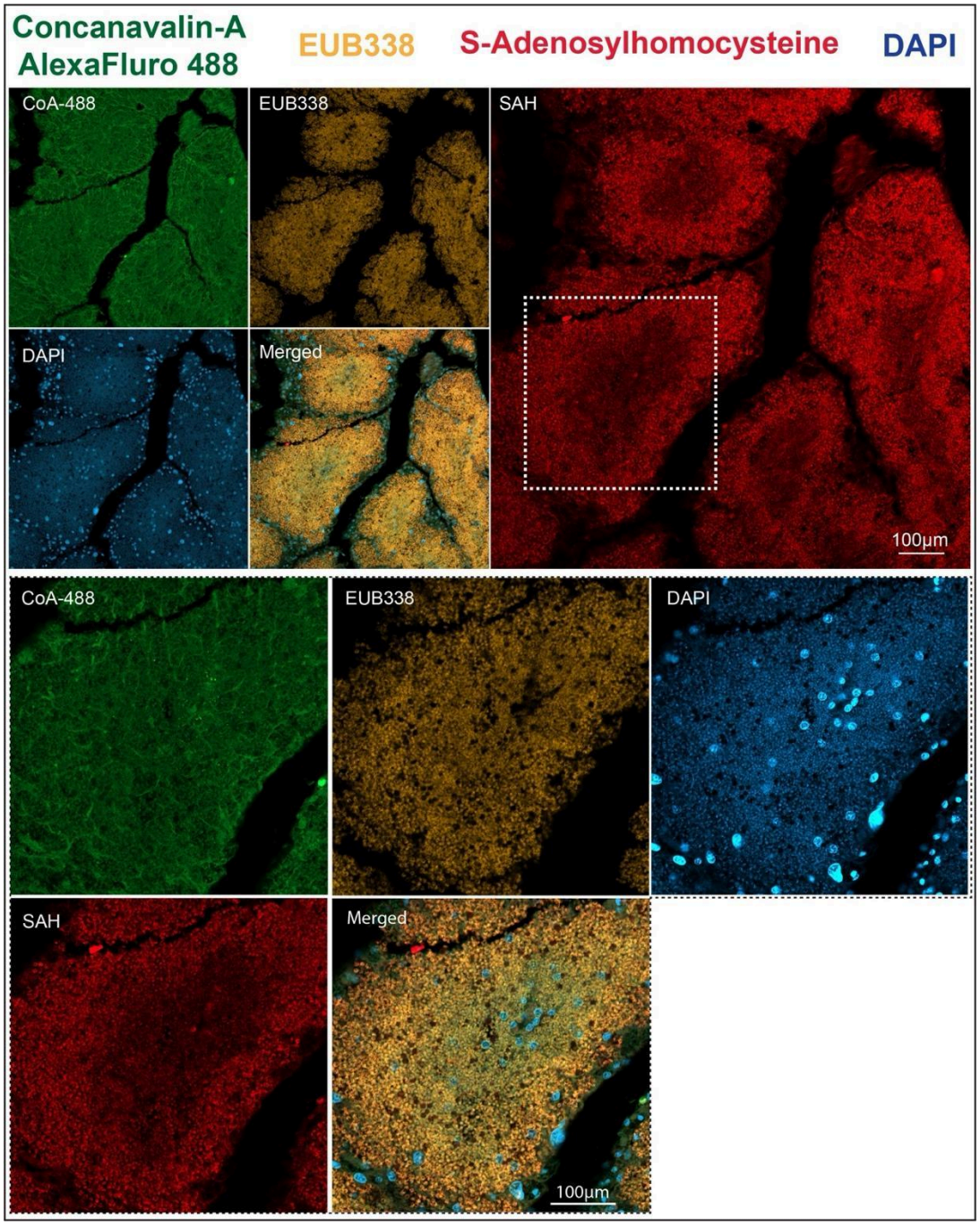
Supplementary Figure S15: Double fluorescent *in situ* hybridization analysis of symbiont *Porin* and 16S rRNA. Double fluorescent *in situ* hybridization analysis of symbiont gene encoding porin (Pec_DN82760C0G1I2), showing gene encoding porin is highly expressed in the symbionts located in the lobule center. Scale bar = 100 μm.

Fig. S16.



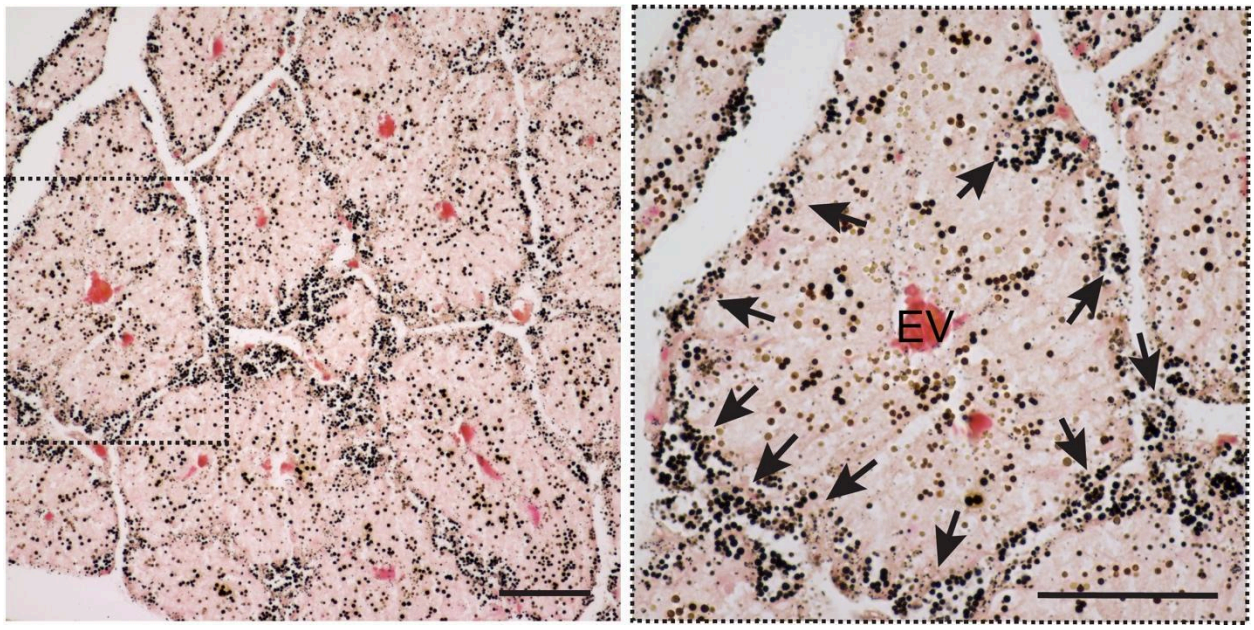
Supplementary Figure S16: Double *in situ* hybridization analysis of symbiont *Porin* and host *SAM Mts*. Double *in situ* hybridization of genes encoding symbiont Porin (Pec_DN82760C0G1I2) and host SAM-dependent methyl transferase (PE_Scaf2254_1.7). The merged image is shown in Figure 6I.

Fig. S17.



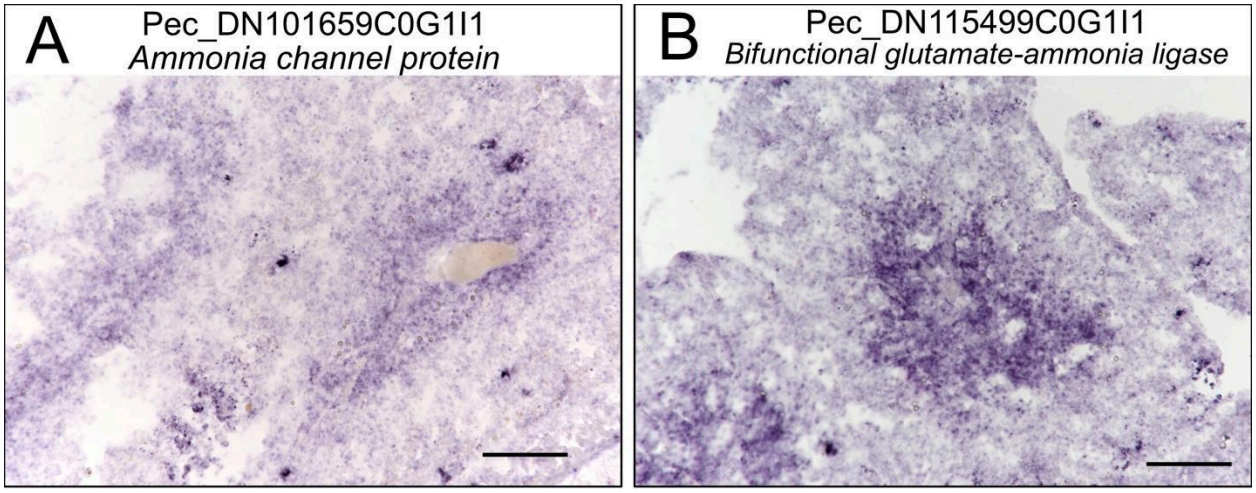
Supplementary Figure S17: IHC-FISH analysis of S-adenosylhomocysteine (SAH) distribution in trophosome lobules. The SAH staining in dash lined area is shown in Figure 6J. Scale bar = 100 µm.

Fig. S18.



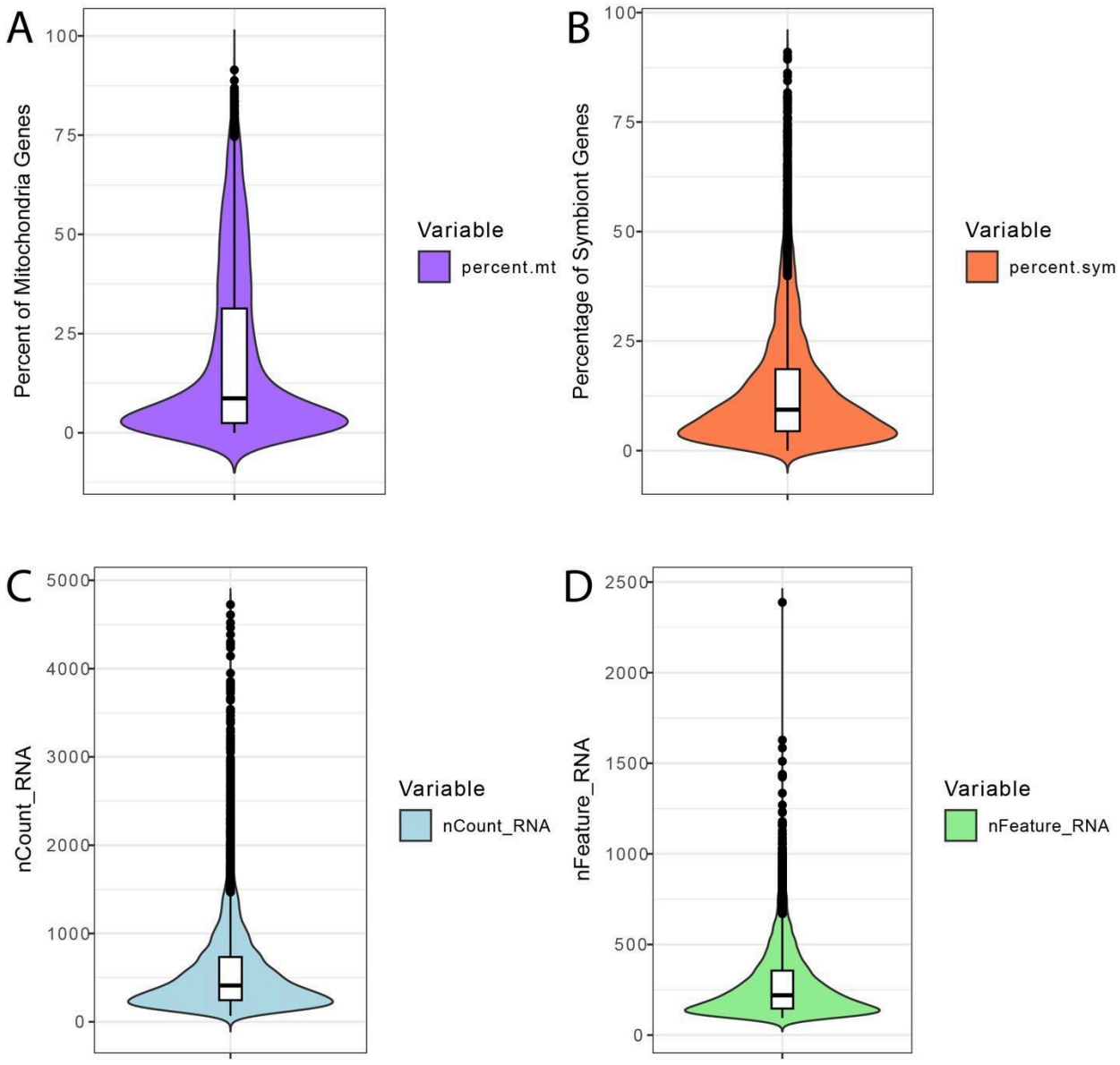
Supplementary Figure S18: Gomori methenamine silver staining analysis. Gomori methenamine silver staining analysis of the trophosome urate/uric acid deposits, showing the black urate/uric acid deposit around the periphery of each trophosome lobule (indicated by black arrows). Scale bar = 100 μm

Fig. S19.



Supplementary Figure S19: *in situ* hybridization analyses of symbiont ammonia-related genes. *in situ* hybridization analyses of genes encoding (A) symbiont Ammonia channel protein (Pec_DN101659C0G1I1) and (B) Bifunctional glutamate-ammonia ligase (Pec_DN115499C0G1I1), showing both genes are expressed in the symbiont in lobule center. Scale bar = 50 μm

Fig. S20.



Supplementary Figure S20: Violin plots showing scRNA-seq data quality metrics. (A) Percentage of mitochondria genes; (B) symbiont gene counts; (C) nCount_RNA (number of UMI); and (D) nFeature_RNA (number of genes) across all cells were analyzed by Seurat.

Captains of Supplementary Tables:

Supplementary Table S1: Identified cell markers of the muscle cell, Spermatozoa cell, and an Ambiguous cell cluster.

Supplementary Table S2: Identified cell markers of the Peritoneal cells.

Supplementary Table S3: Identified cell markers of the three efferent arteriole cell clusters.

Supplementary Table S4: Identified cell markers of the two bacteriocyte clusters.

Supplementary Table S5: Primers used to generate in situ hybridisation probes.

Supplementary Table S6: HCR Primers.