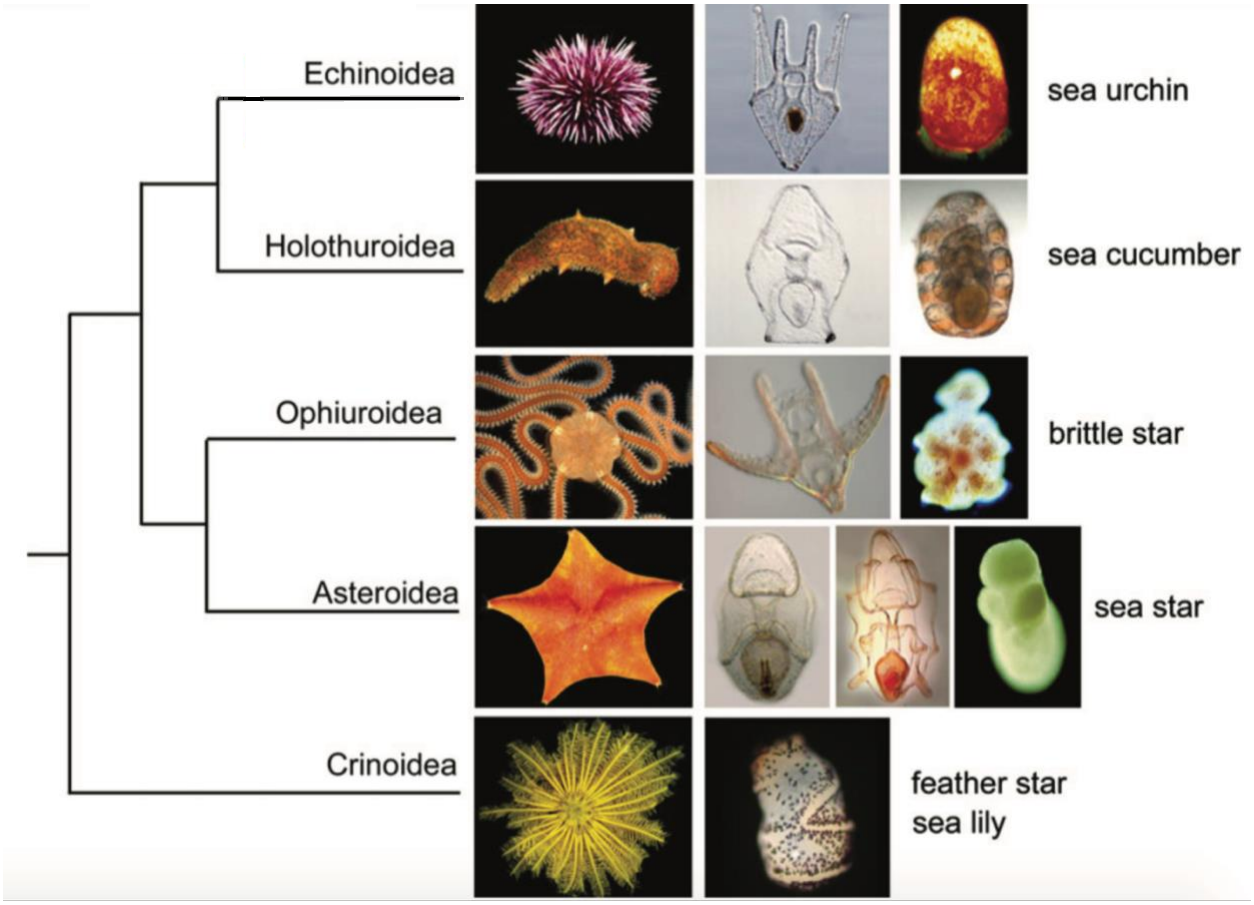
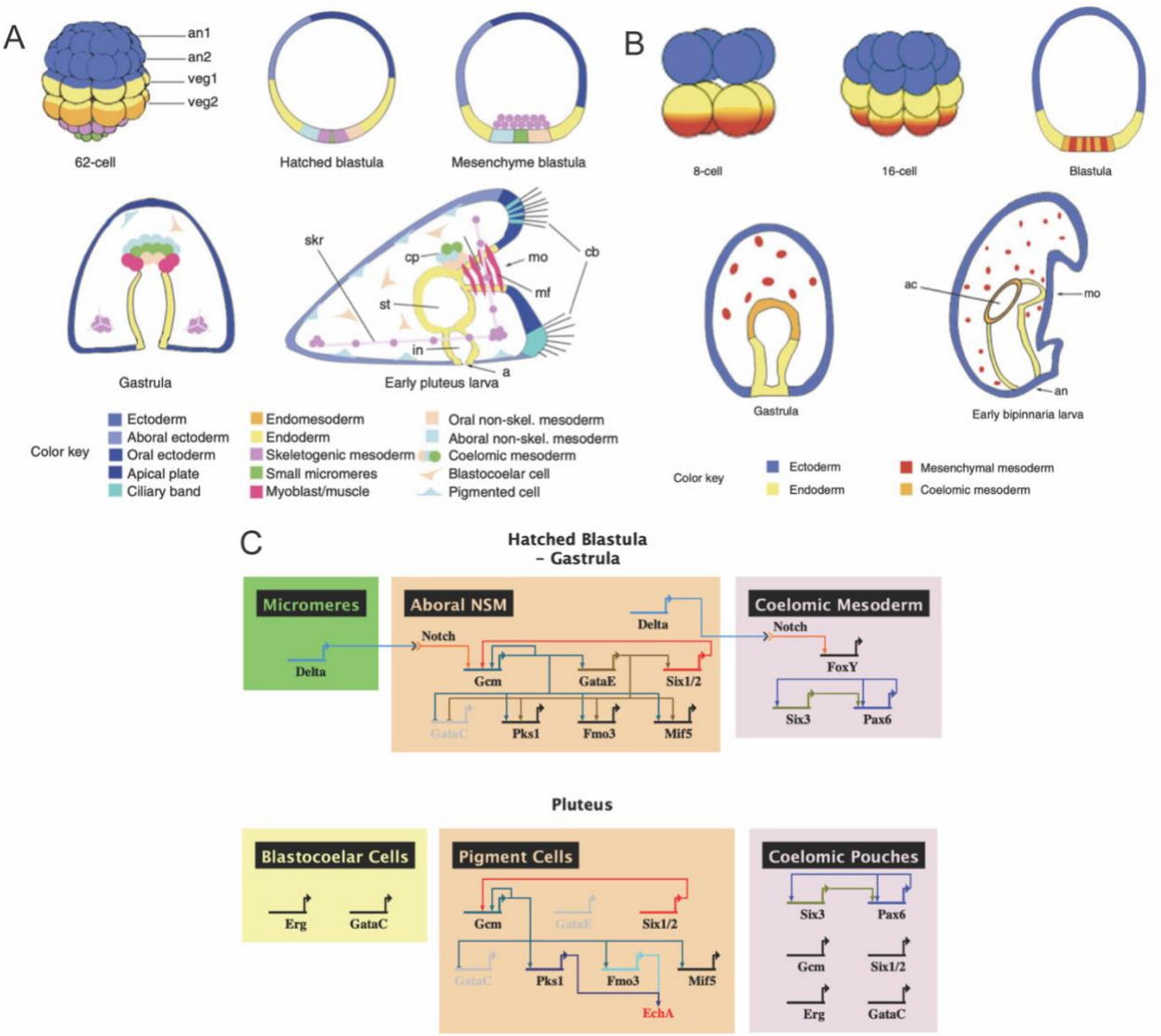
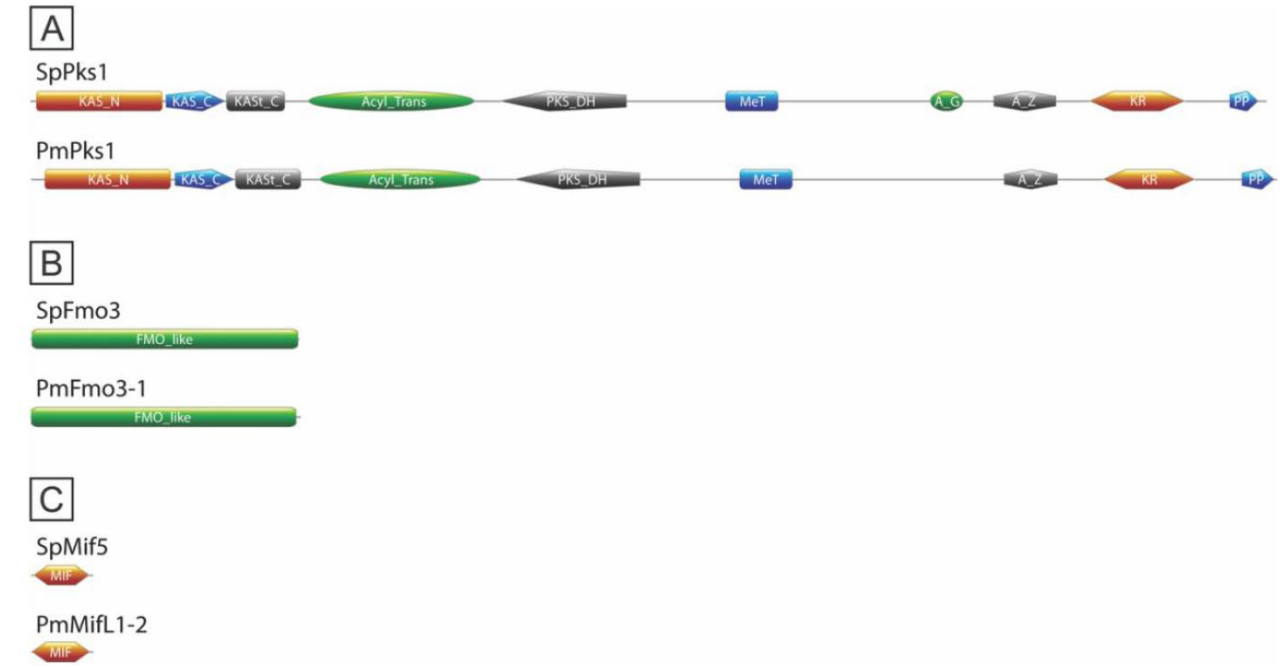
Supplementary Information

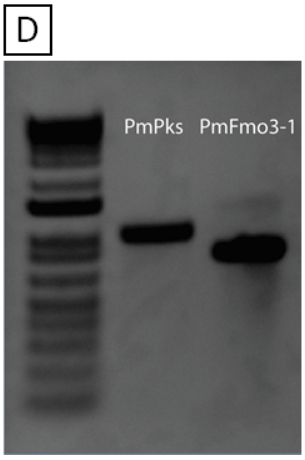


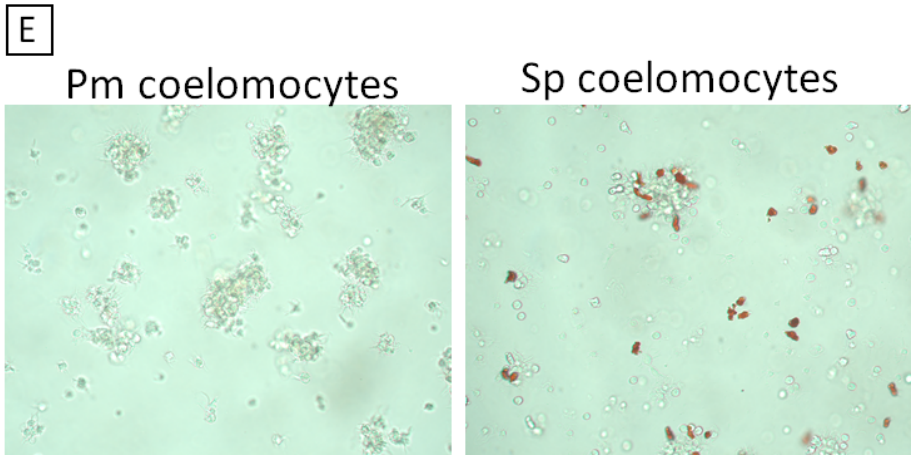
Supplementary Figure S1. Phylogeny of echinoderms with larval and adults forms. From left to right, images show adult, feeding larval and non-feeding larval forms (except for holothuroids, where only feeding larval forms are shown). Crinoids lack a feeding larval form. Early asymmetric cell division is found only in the echinoid lineage. This figure is adapted from (Arnone *et al.,* 2015)[1].



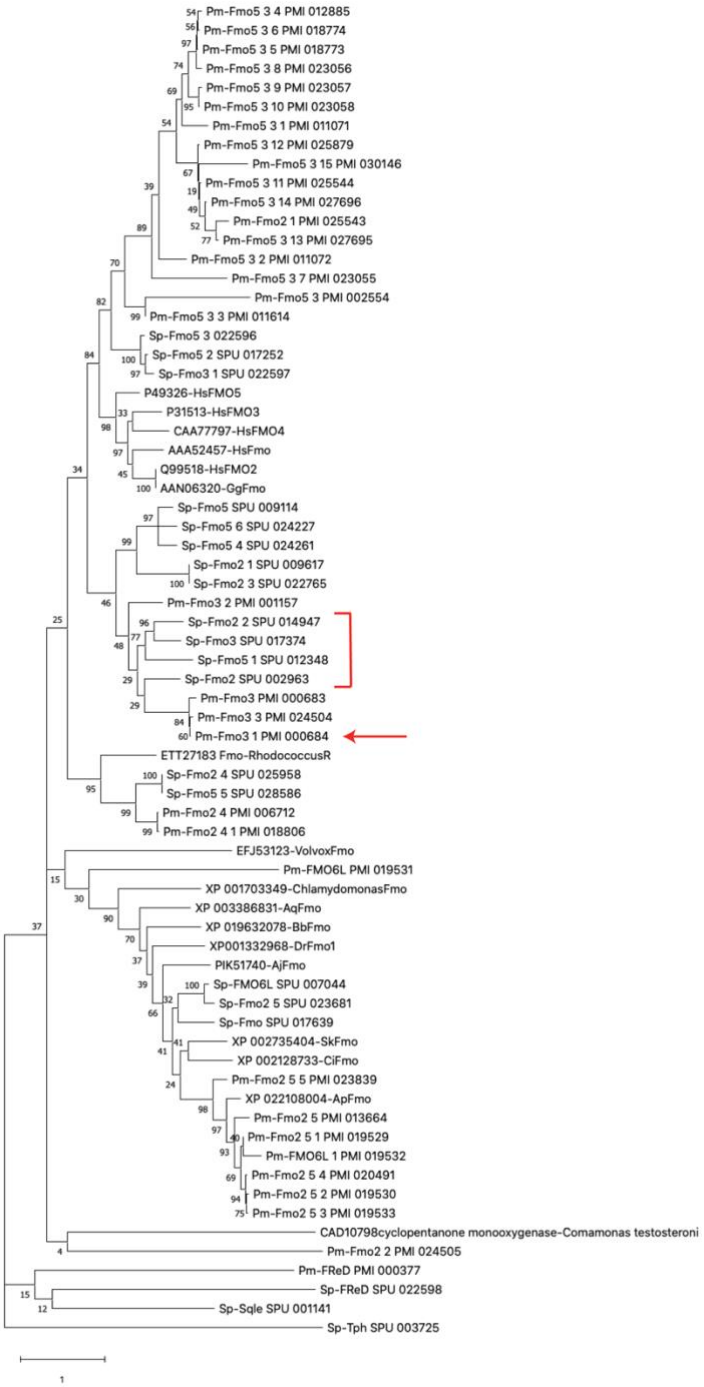
Supplementary Figure S2. Sea urchin and sea star embryogenesis. A. Sea urchin mesodermal lineages are spatially separated in the vegetal plate by the hatched blastula stage. During gastrulation, coelomic pouch precursors are specified, and pigment cells (aboral mesoderm) and blastocoelar cells (oral mesoderm) migrate into the blastocoel. Abbreviations: *a* anus, *an* animal, *cb* ciliary band, *cp* coelomic pouch, *in* intestine, *mf* muscle fiber, *mo* mouth, *skr* skeletal rod, *st* stomach, *veg* vegetal. **B.** Sea star blastula possess interwoven blastocoelar cell and coelomic pouch precursors in the mesodermal vegetal plate. Blastocoelar cells ingress into the blastocoel during gastrulation. Abbreviations: *ac* anterior coelom, *an* anus, *mo* mouth. These figure illustrations **(A&B)** are adapted from (Arnone *et al.,* 2015)[1]. **C.** The simplified mesodermal gene regulatory network of sea urchins during embryogenesis and larval stages constructed in BioTapestry[2-9].





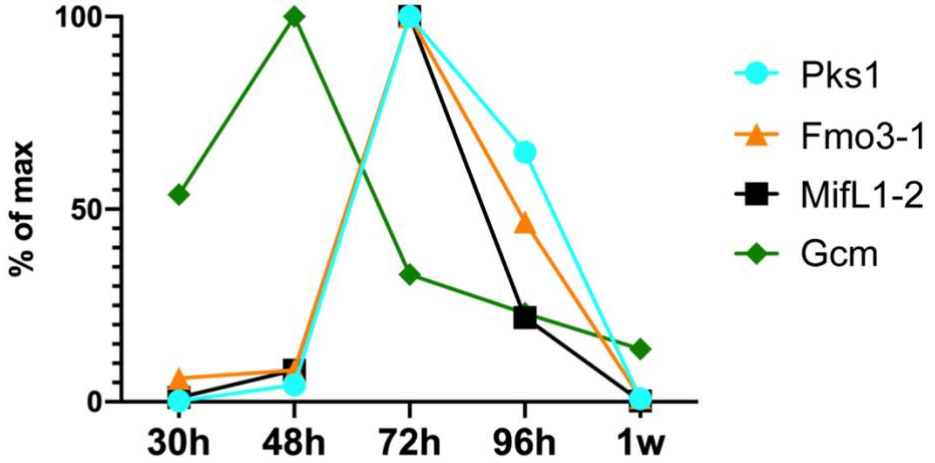


Supplementary Figure S3. Sea urchin and sea star pigment cell-specific genes.
Protein domains of A. SpPks1 and PmPks1; B. SpFmo3 and PmFmo3-1; C. SpMif5 and PmMifL1-2. All sequences have identical protein domains except SpPks1 and PmPks1, where SpPks1 has a second alcohol dehydrogenase domain absent from PmPks1. Likely protein domains were obtained using the protein database search feature of Pfam10. Images were produced using the PROSITE MyDomains web tool11. Abbreviations correspond to the following protein domains. KAS_N: Beta-ketoacyl synthase, N-terminal domain; KAS_C: Beta-ketoacyl synthase, C-terminal domain; KASt_C: Ketoacyl-synthetase C-terminal extension; Acyl_Trans: Acyl transferase domain; PKS_DH: Polyketide synthase dehydratase; MeT: Methyltransferase domain; A_G: Alcohol dehydrogenase GroES-like domain; A_Z: Zinc-binding alcohol dehydrogenase; KR: Ketoreductase domain; PP: Phosphopantetheine attachment site; FMO-like: Flavin-binding monooxygenase-like; MIF: Macrophage migration inhibitory factor.
Coelomocytes. D. PCR showing the expression of PmPks1 and PmFmo3-1 in the Pm coelomocytes. E. Representative brightfield images of c of Pm and Sp. No pigmented cells were observed in Pm.

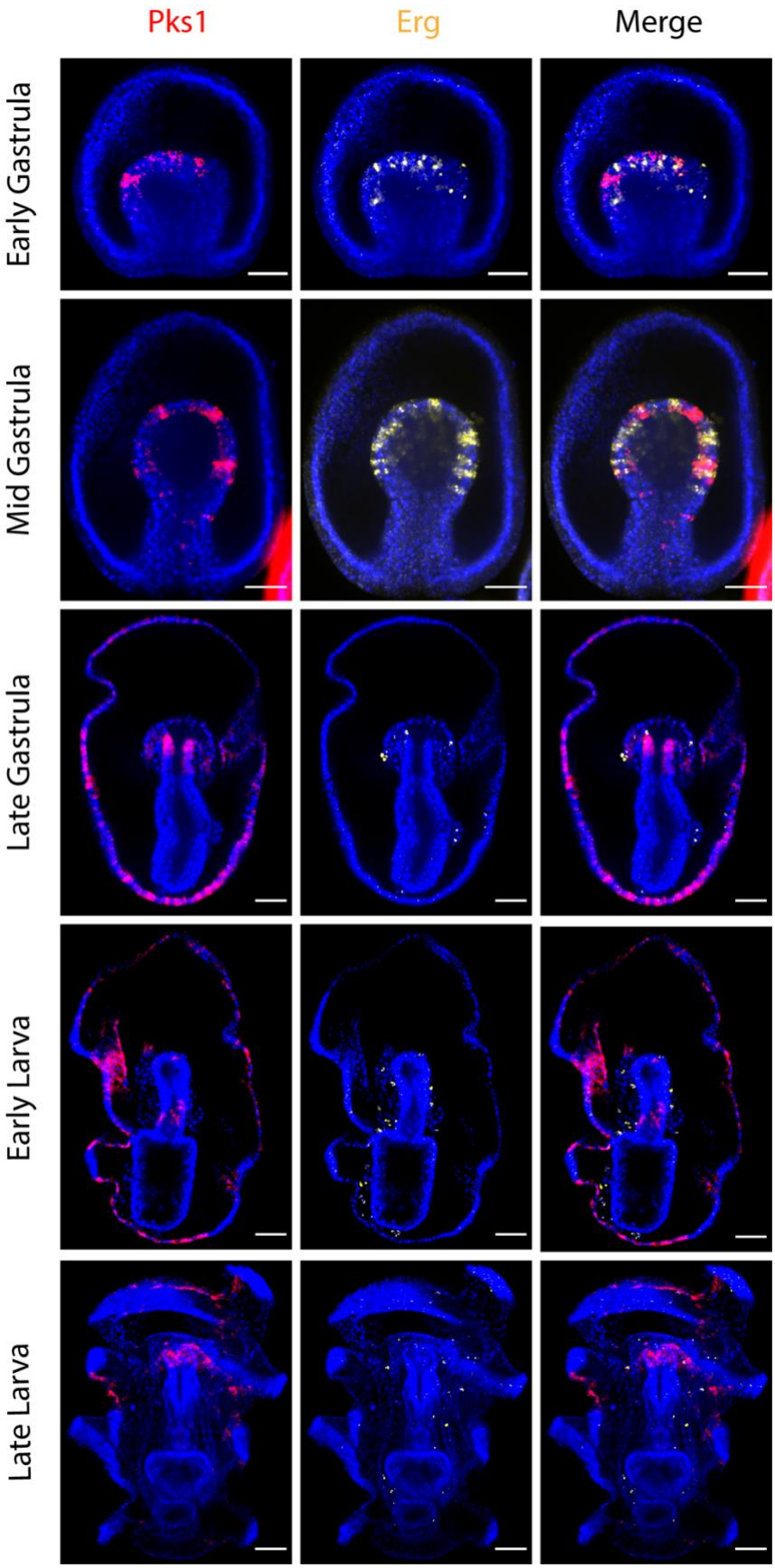


Supplementary Figure S4. *PmFmo3-1* is the ortholog of *SpFmo3*. A maximum likelihood tree showing the phylogeny of all annotated *Strongylocentrotus purpuratus* and *Patiria miniata* flavin-dependent monooxygenases (Fmo's). Non-echinoderm Fmo protein sequences were also included. 500 bootstrap replicates were used to construct the tree. *SpFmo3, SpFmo5_1, SpFmo2_2* and *SpFmo2* are the pigment cell-specific Fmo's in sea urchin larvae, shown within the red bracket[12]. *PmFmo3-1* (PMI_000684), *PmFmo3* (PMI_000683) and *PmFmo3_3* (PMI_024504) are most closely related to this cluster.

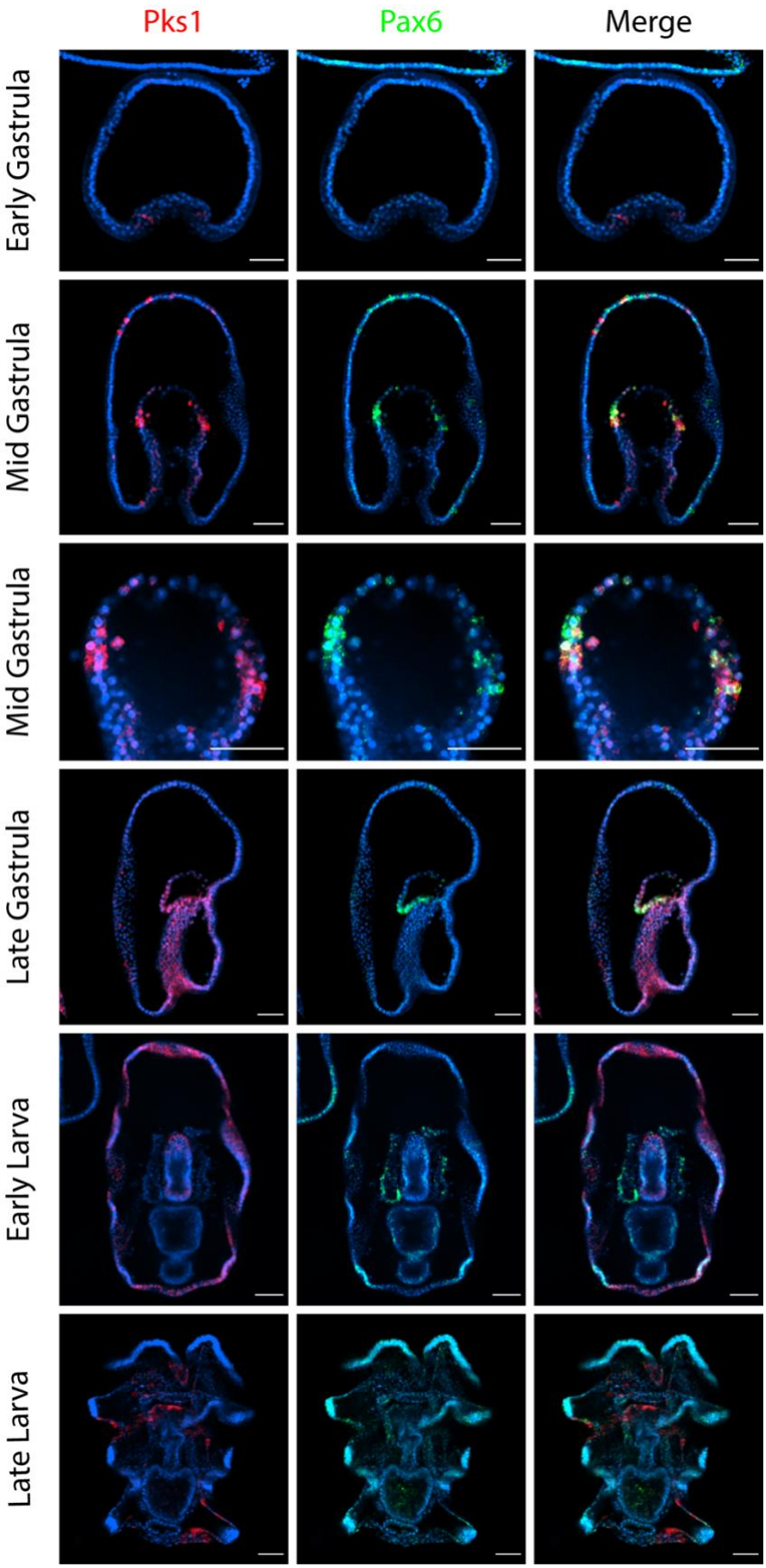
PmFmo3-1 (red arrow) displayed the highest sequence similarity to *SpFmo3*, so the two genes were determined to be orthologous.



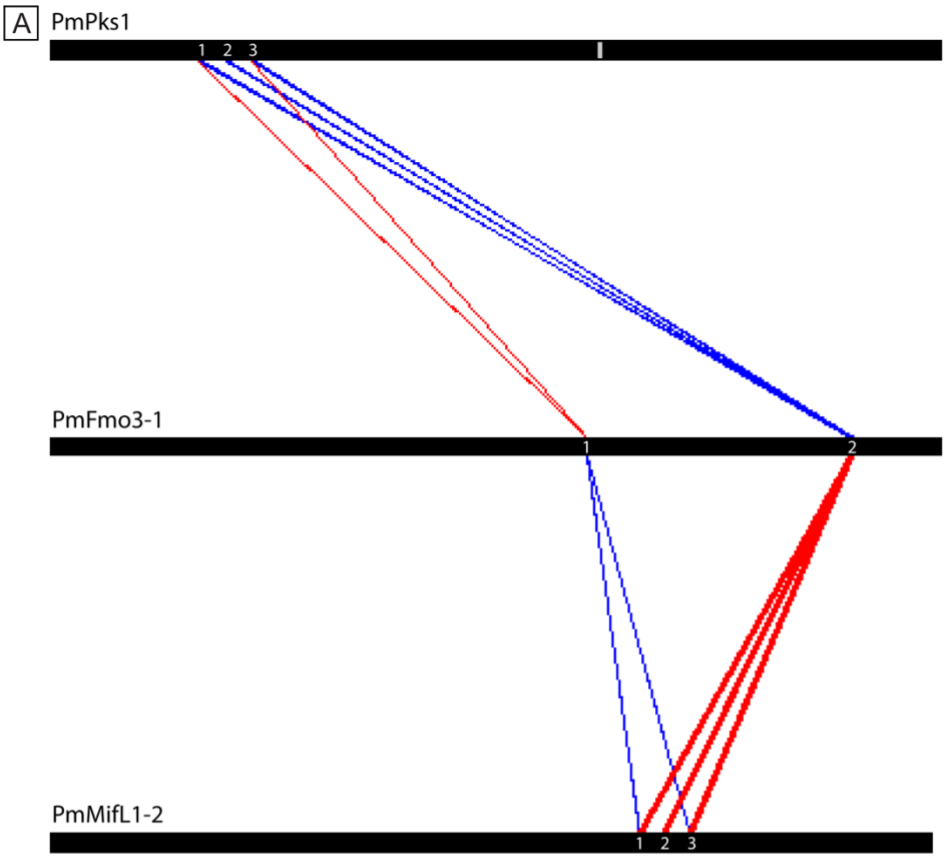
Supplementary Figure S5. Time course qRT-PCR during sea star development. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) shows the expression pattern of orthologs to sea urchin pigment cell-specific genes. Values are plotted as percent of the maximum expression value. *PmPks1, PmFmo3-1, PmMifL1-2 and PmGcm* transcripts are present from the early gastrula stage (30h post fertilization) through the late larval stage (1w). *PmGcm* expression peaks during gastrulation and decreases during the larval stages. *PmPks1, PmFmo3-1 and PmMifL1-2* have similar expression profiles, showing robust expression during the late gastrula stage (72h). At all other stages except the early larval stage (96h), transcript levels of *PmPks1, PmFmo3-1* and *PmMifL1-2* are only a fraction of this value.


Pks1
Erg
Merge
Early Gastrula
Mid Gastrula
Late Gastrula
Early Larva
Late Larva

Supplementary Figure S6. *PmPks1* is not expressed in blastocoelar cells nor their precursors. Confocal images show the expression pattern of *PmPks1* and *PmErg* using double FISH. *PmErg* expression marks the blastocoelar cell lineage. Blastocoelar cells have a mesodermal origin, undergoing an epithelial to mesenchymal transition away from the archenteron after the mid gastrula stage. *PmPks1* is also expressed in cells in the archenteron, however *PmErg* and *PmPks1* are expressed in distinct cells. After the mid gastrula stage, *PmErg* is expressed in migratory blastocoelar cells while *PmPks1* is expressed primarily in the ectoderm. Nuclei are shown in blue with DAPI. Scale bars are 50 μm.


Pks1
Pax6
Merge
Early Gastrula
Mid Gastrula
Mid Gastrula
Late Gastrula
Early Larva
Late Larva

Supplementary Figure S7. *PmPax6* and *PmPks1* transcripts colocalize during but not after gastrulation. Confocal images show the expression pattern of *PmPks1* and *PmPax6* using double FISH. *PmPks1* expression first appears in the invaginating vegetal plate at the early gastrula stage, while *PmPax6* expression is first detected at the mid gastrula stage. By the mid gastrula stage, though, *PmPks1* and *PmPax6* are expressed in the same cells in the archenteron. Following gastrulation, during the larval stages, *PmPax6* transcripts are found in the coelomic pouches and the ciliary bands while *PmPks1* is mainly expressed in the ectoderm. Nuclei are shown in blue with DAPI. Scale bars are 50 μm.



B

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PmPks1_1        GGGAAGAGGGGCCAGGAATTGCCTCAGATTGCACCACAGAGCATCTAGAATCCAAAATTT   60
PmPks1_2        --------------------CCTCAGAATACACCACAGAGCATCTAGAATTCTAAATTT   39
PmPks1_3        ----AAAGGAGCCAGGAATCGCCTCAGATTGCACCACAGAGCATCTAAAATCCAAAATGT   56
PmFmo3-1_1      ----------------------------TCGCAACCAAGAGCATCTAGAACCCAAAAA--   30
PmFmo3-1_2      GGTTAAAGTGGTCAGAAATTGCCTCAGATTGCGCTACAGAGCGTCTAGAATCAATTTTTT   60
PmMifL1-2_1     GTCGAAAGGAGCCAGGAATCGCCTCAGATTGCACCACAGAGCATCTAGAATCCAAAATTT   60
PmMifL1-2_2     GGAAAAAGGGGCCAGGAGTCCCCTCAGAATACACCACACAGCATCTGAAATTCCAATTTT   60
PmMifL1-2_3     GGGAAGAGGAGCCAGGAATCGCCTCAGATTGCACCACAGAGCATGTAGAATCCAAAATTT   60
                                          *     * *** * *  **

PmPks1_1        CCCGGGGCCCTTAAGCGGGCCCGG       84
PmPks1_2        TACGGGGCCCTTAAGCGGGCCCGG       63
PmPks1_3        TTCGGGCCCTTAAGCGGGCCCCG-       79
PmFmo3-1_1      ------------------------       30
PmFmo3-1_2      TCCGGGCCCTTAAGCGAGCCCCG-       83
PmMifL1-2_1     CCCGGGGCCCTTAAGCGGGCCCCG       84
PmMifL1-2_2     CTGGGGCCCTTAAGCGGGCCCCG-       83
PmMifL1-2_3     CCCGGGGCCCTTAAGCGGGCCCCG       84
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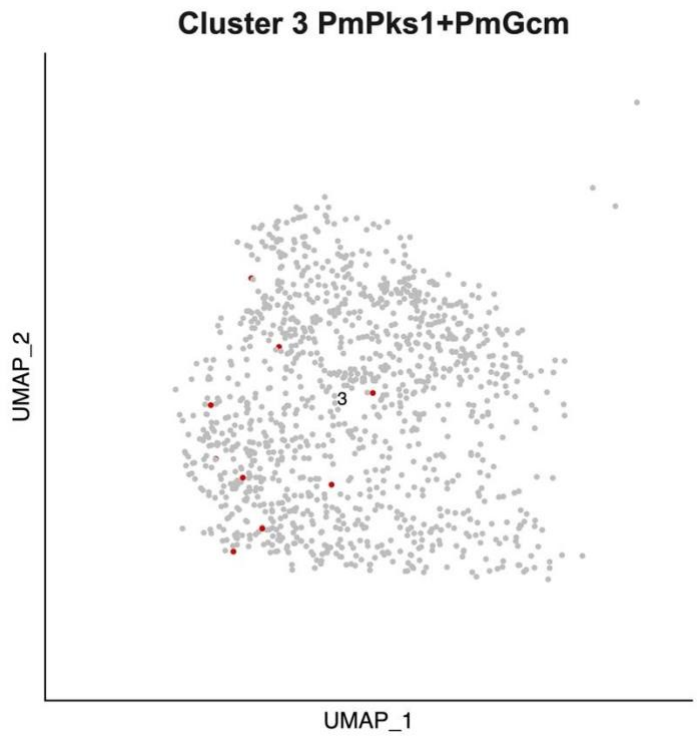
Supplementary Figure S8. Multi-sequence alignment of 10kb upstream of *PmPks1, PmFmo3-1* and *PmMifL1-2*. A. MussaGL software (http://woldlab.caltech.edu/~king/mussagl_manual/) was used for pairwise alignment of 10kb upstream of the transcription start sites of *PmPks1, PmFmo3-1* and *PmMifL1-2* (black bars). The minimum length of aligned sequences was 30 nucleotides, sharing at least 80% sequence identity. Lines between black bars demonstrate a shared sequence, with red lines indicating parallel alignment and blue lines indicating antiparallel alignment. Red/blue line thickness shows the relative length of the aligned sequences. Since the red and blue lines interconnect, a shared sequence is present in at least 2 locations 10kb upstream each of *PmPks1, PmFmo3-1* and *PmMifL1-2*. **B.** Alignment of shared sequences using Clustal Omega[13,14]. TFBind identified the sequence bracketed in red, shared by 5 of 8 aligned sequences, as a possible GATA protein binding site[15].

Cluster 0	Ectoderm
Cluster 1	Ectoderm
Cluster 2	Ectoderm: Apical
Cluster 3	Mesoderm
Cluster 4	Ectoderm: Lateral
Cluster 5	Undetermined
Cluster 6	Ectoderm: Ventral
Cluster 7	Ectoderm: Oral
Cluster 8	Endoderm: Midgut/hindgut
Cluster 9	Gcm enriched
Cluster 10	MifL1-2 enriched

Supplementary Table S1. Cluster annotations for sea star scRNA-seq data[16].

Cluster 0	Ectoderm/uncharacterized
Cluster 1	Apical Ectoderm
Cluster 2	Differentiated Pigment Cells
Cluster 3	Ciliary Band Neurons (1)
Cluster 4	Apical Plate/uncharacterized
Cluster 5	Oral Ectoderm/Mouth
Cluster 6	Lateral Ectoderm (right)
Cluster 7	Ciliary Band Neurons (2)
Cluster 8	Aboral Ectoderm (1)
Cluster 9	Aboral Ectoderm (2)
Cluster 10	Apical Plate, Proneural (2)
Cluster 11	Skeleton
Cluster 12	Mesodermal Cells
Cluster 13	Mitotic Pigment Cells
Cluster 14	Mid-gut
Cluster 15	Serotoninergic Neurons (apical plate) (1)
Cluster 16	Ciliary Band Neurons (3)
Cluster 17	Serotoninergic Neurons (apical plate) (2)

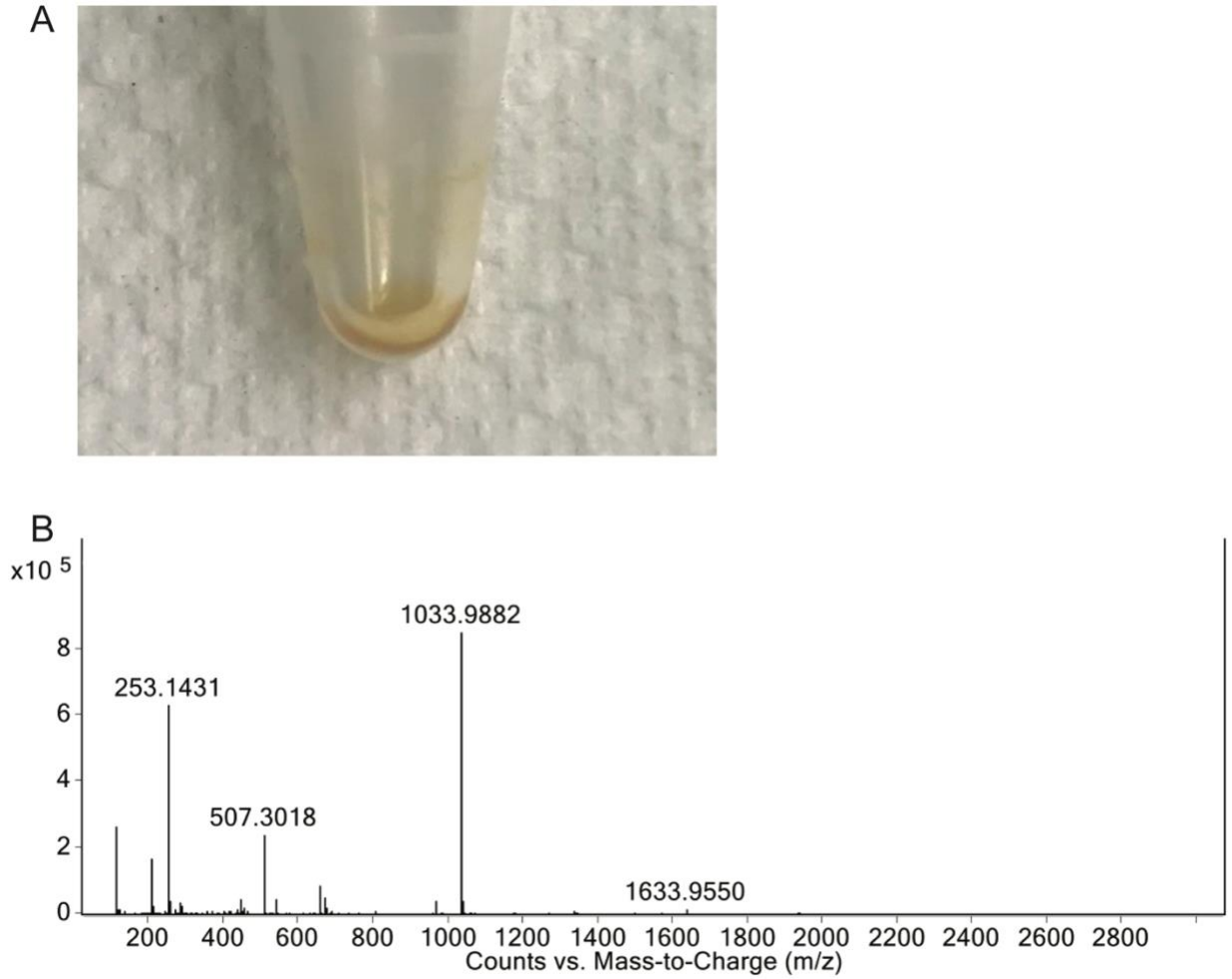
Supplementary Table S2. Cluster annotations for sea urchin scRNA-seq data[12].



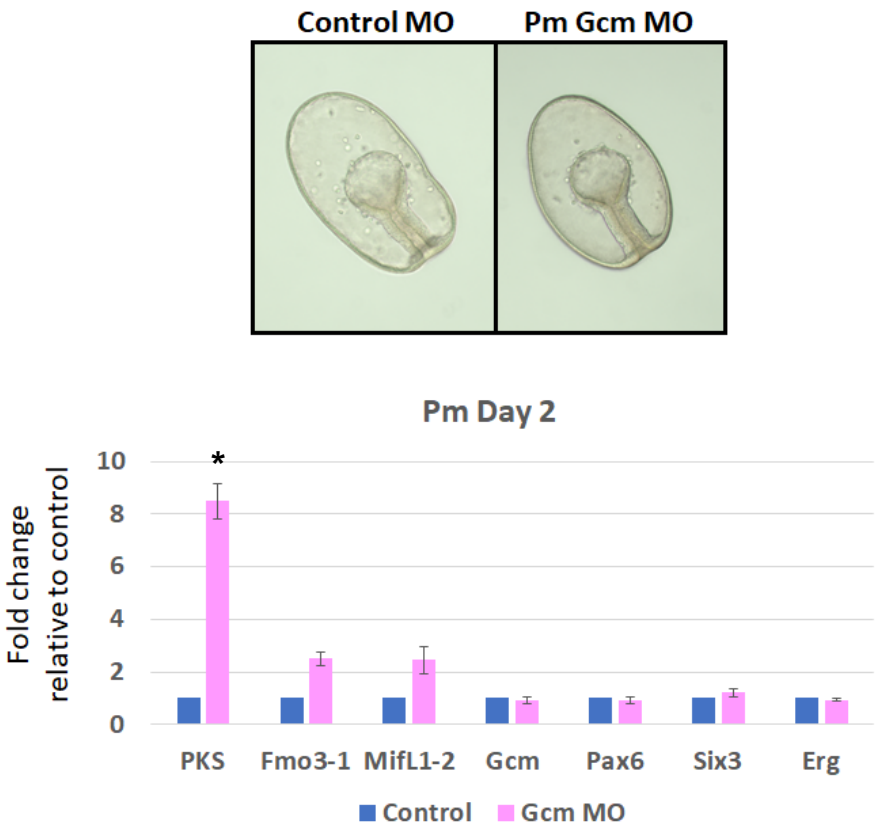
Supplemental Figure S9. Enhanced view of coexpression analysis of *PmPks1* and *PmGcm* in sea star gastrula cluster 3[16].

Compound Label	RT	Mass	Abundance	Target Mass
Spinochrome E: C10 H6 O8	4.664	254.0076	41241	254.0063
Spinochrome B: C10 H6 O6	5.65	222.0176	4731	222.0164
Spinochrome 282: C12 H10 O8	5.666	282.0374	4354	282.0376
Spinochrome C: C12 H8 O8	6.786	280.0222	67732	280.0219
Echinochrome A: C12 H10 O7	7.17	266.0434	1325583	266.0427

Supplementary Table S3. Pigment composition of sea urchin larvae. Following pigment extraction, pigment composition of *S. purpuratus* larvae was determined by LC-MS. Larvae were found to contain the naphthoquinone pigments Spinochrome E ($C_{10}H_6O_8$), Spinochrome B ($C_{10}H_6O_6$), Spinochrome 282 ($C_{12}H_{10}O_8$), Spinochrome C ($C_{12}H_8O_8$) and Echinochrome A ($C_{12}H_{10}O_7$). Echinochrome A was the most abundant molecule, almost 20-fold more abundant than Spinochrome C, the second most abundant molecule.



Supplementary Figure S10. Sea star larvae contain pigment though not any known naphthoquinone molecules. *P. miniata* larvae underwent pigment extraction in parallel with *S. purpuratus* larvae. **A.** Chemical extraction of sea star larvae produced a light orange pigment as opposed to the bright red of sea urchin larval pigments. **B.** The chemical extract was analyzed by LC-MS. The most abundant molecular peak appears at 253.1431 m/z (1033.9882 m/z is a standard). The molecular formula for this peak could not be determined.



Supplementary Figure S11. Gcm perturbation in the Sea star embryos. Morpholino against Pm Gcm was injected and the resulting embryos were either imaged or analyzed by qPCR at 48 hpf. (* p value < 0.005)

Gene Name	Gene ID	Forward Primer	Reverse Primer
PmPks1	PMI_000680	AGGGTTCCAGGGACAGAACT	GCCTGATGAAGATGGCTCTC
PmFmo3_1	PMI_000684	ATCATTGGTGGAGGGATCAG	CTGGTCGGAGATTCTCATCG
PmMifL1_2	PMI_012936	TGTGGAGAAATTGCTTGTCG	AAGCTGCGAAAGTTCCTTGA
PmGcm	PMI_000568	AAGCAGGCGGACAAGAAAT	CCCTTTGGACTGGAAAATGA
PmSix3	PMI_030378	ATATGGGACGGTGAGCAAAA	TCGGTTCTTGAACCAATTCC
PmPax6	PMI_028207	CATCAGCGTGTACCAGCCTA	CCATCATGCTCCGGTAACTT
Pm Erg	PMI_028116	AAGATGACCGACCCGGACGA	TGCCGTGGACCTTGGTCATA

Supplementary Table S4. qPCR primers are oriented in the 5'-3' direction.

Gene Name	Gene ID	Forward Primer	Reverse Primer
PmPks1	PMI_000680	AAGCAGCTTGGCATTGAGAT	GTCTCTGCCTTGTCCTCCAG
PmFmo3-1	PMI_000684	ATGGCGTTCAGTGACTACCC	ACGTGCATGTACAGGTCCAA
PmMifL1-2	PMI_012936	CGCTTTCCCAAAATTTCAAG	AATTCAAGCGTCTGCTCCAC
PmGcm	PMI_000568	CACACACCAATGAGGCAAAC	GTCGAATTGTCCGGCTATGT
PmErg	PMI_028116	ATCGGAGTGGTCAGATCCAG	ATATCCGTTGCCAGAAGTCG
PmSix3	PMI_030378	TCACATCCTGGAAAACCACA	CCCGATGGCGTAAACTCTAA
PmPax6	PMI_028207	AGACTCAAGCGGAAACTCCA	GCATCAACCTCGTTTCCATT
PmGataC	PMI_017988	ACCATCCAGTGGTTCGAGAG	CCAGTCTTCTCTTGGGCTTG

Supplementary Table S5. Primers for FISH probe generation are oriented in the 5'-3' direction.

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