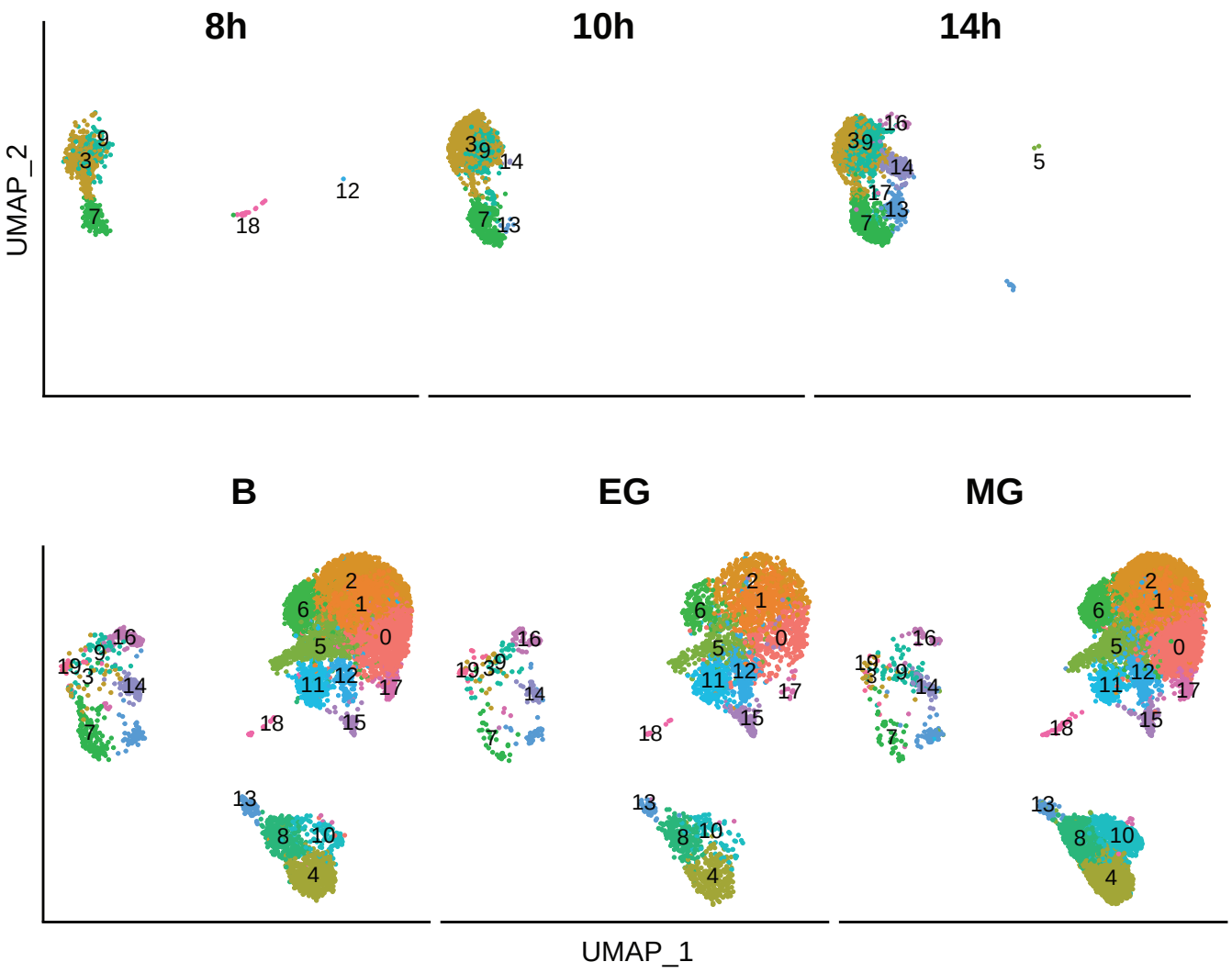

Fig. S1. Identification of cell states across early sea star development. UMAP visualization of six integrated datasets, separated by embryonic stage: 8-hpf (868 cells), 10-hpf (1,318 cells), 14-hpf (2,448 cells), blastula (7,272 cells), early gastrula (3,349 cells), and mid-gastrula (10,448 cells).

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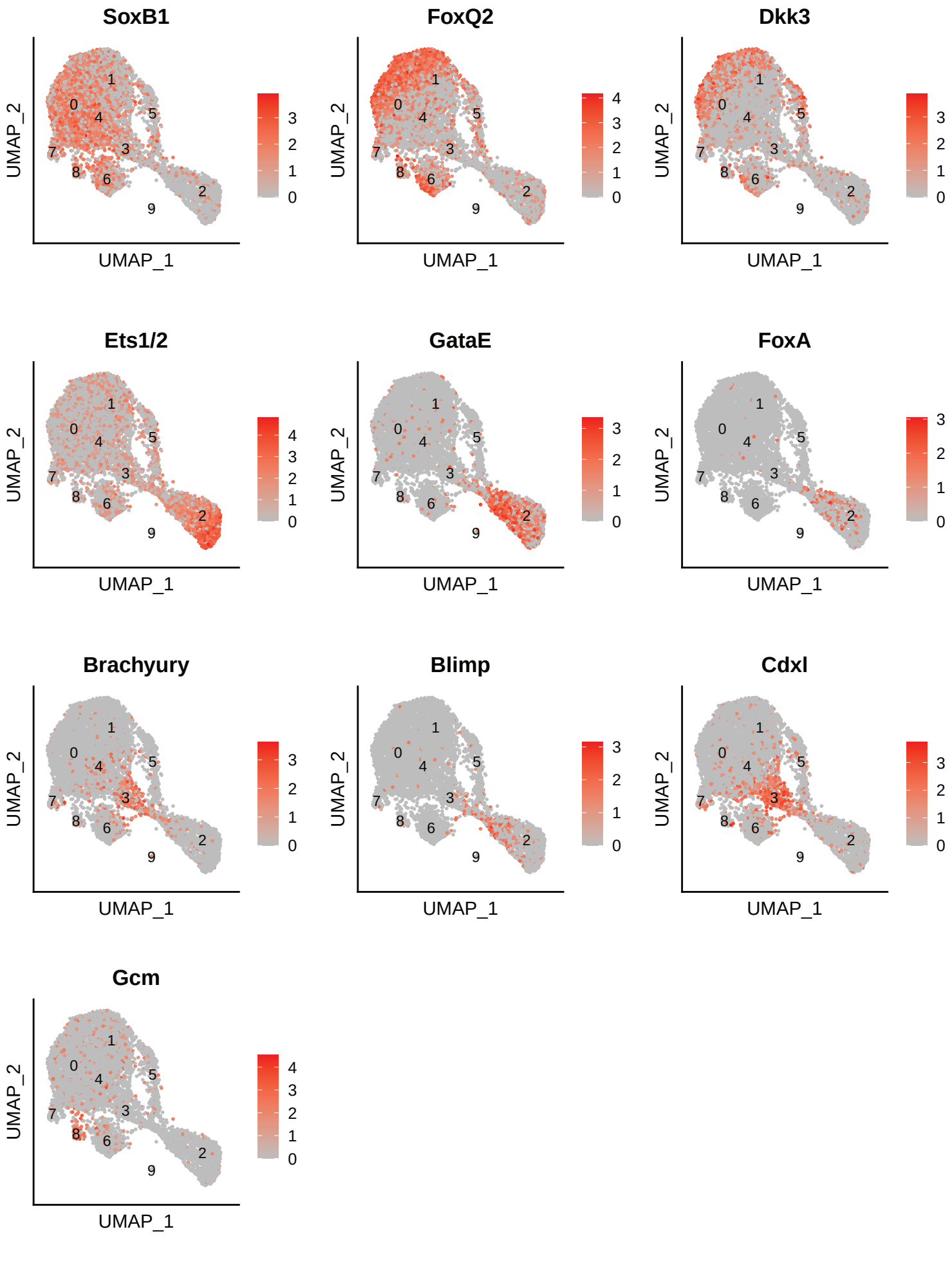

Fig. S2. Marker gene expression at blastula stage. Feature plotsshowing expression of ectodermal, mesodermal, and endodermal marker genes at blastula stage. Average gene expression level displayed by color intensity.

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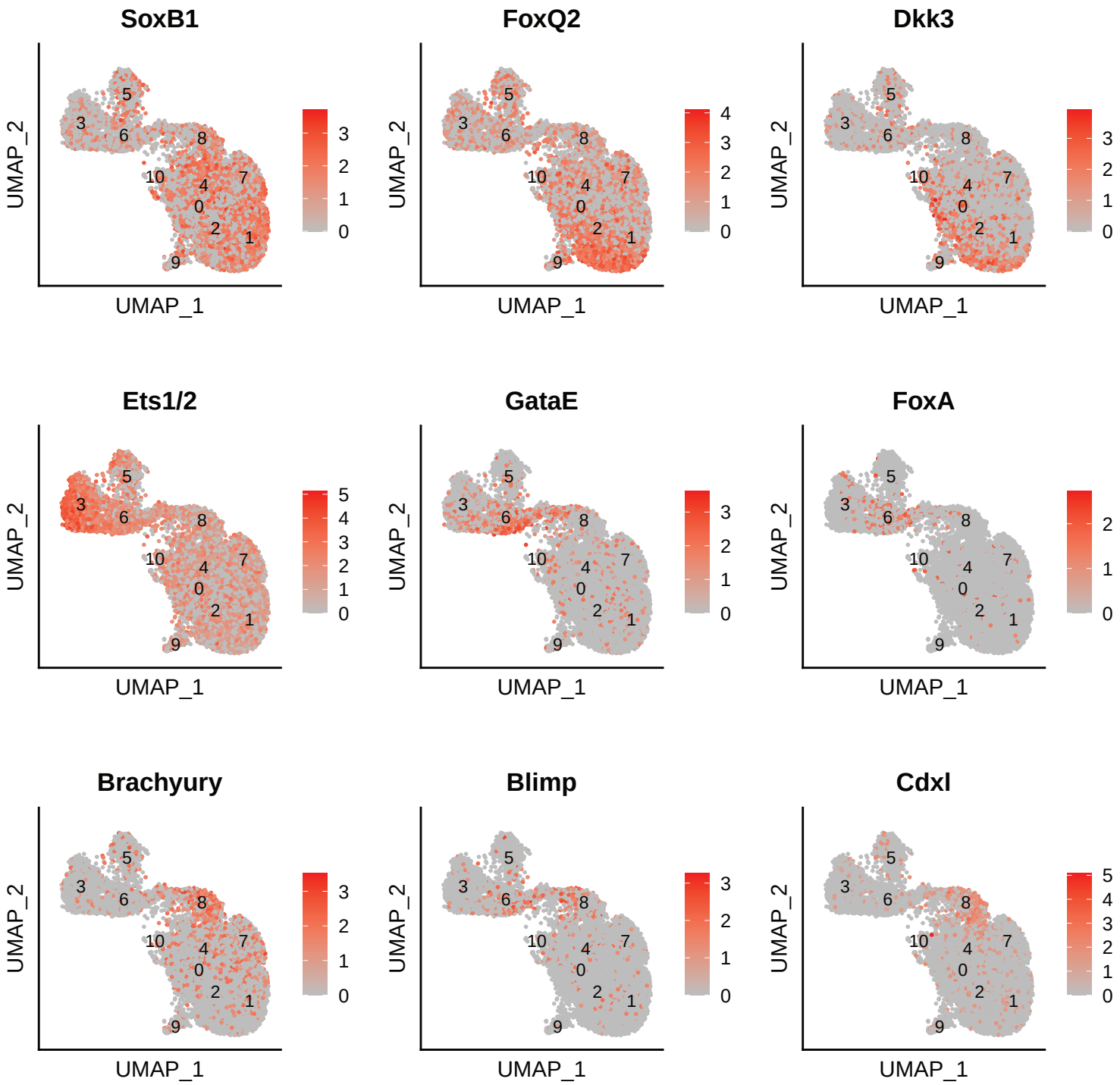

Fig. S3. Marker gene expression at mid-gastrula stage. Featureplots showing expression of ectodermal, mesodermal, and endodermal marker genes atmid-gastrula stage. Average gene expression level displayed by color intensity.

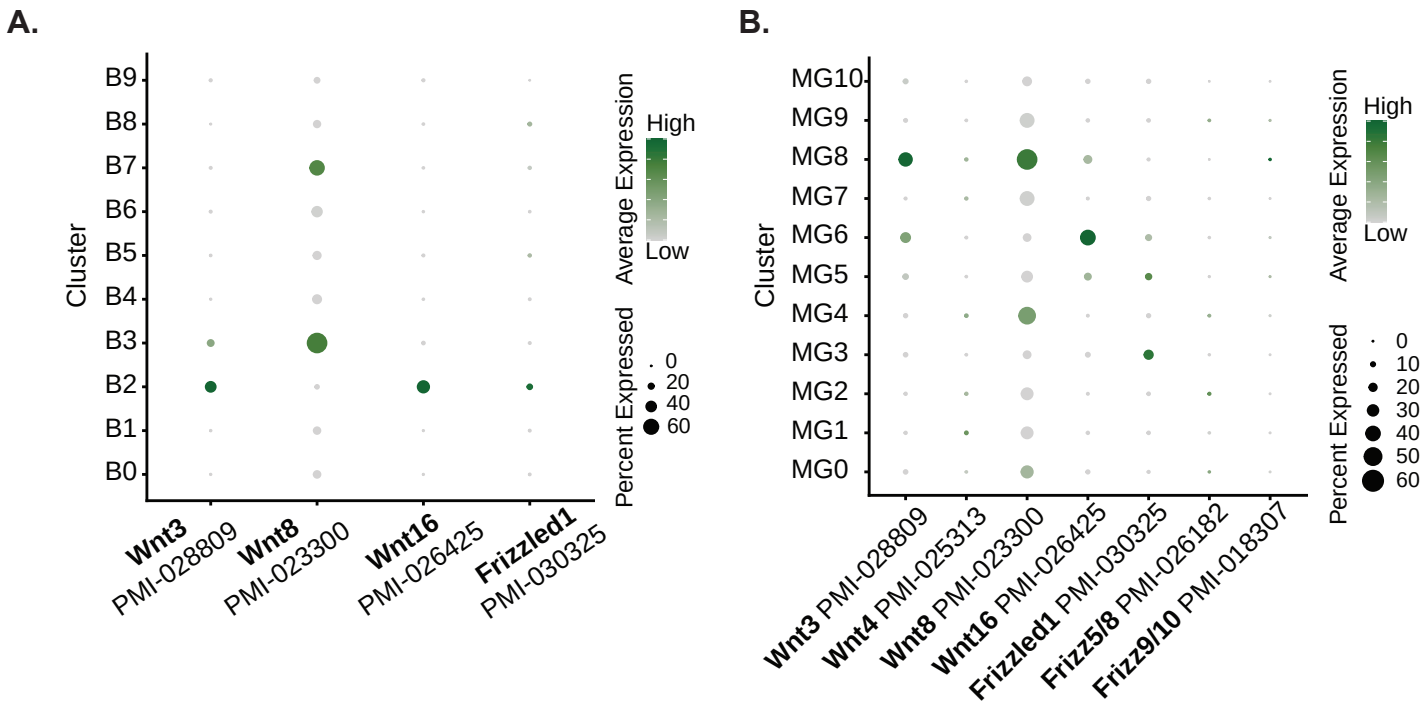

Fig. S4. Wnt signaling component expression. (A) Dot plot of Wntsignaling pathway component expression at blastula stage. Co-expression of Wnt3, Wnt16, and Frizzled1 receptor (Annotated as Frizz in the dataset) seen in cluster B2. (B) Dot plot of Wnt signaling pathway component expression at mid-gastrula stage.

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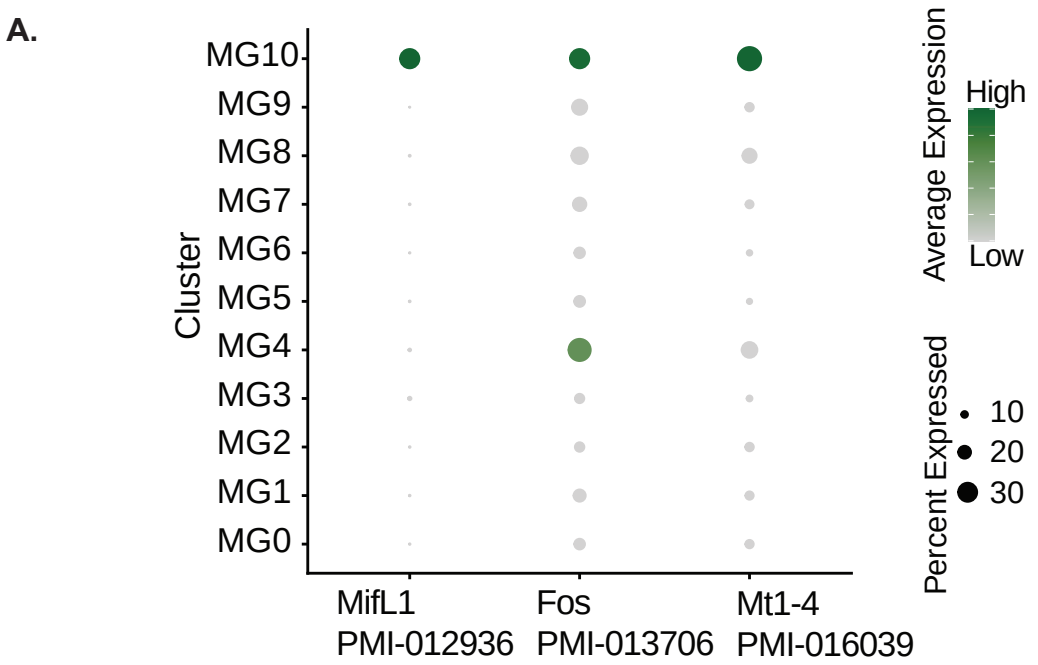

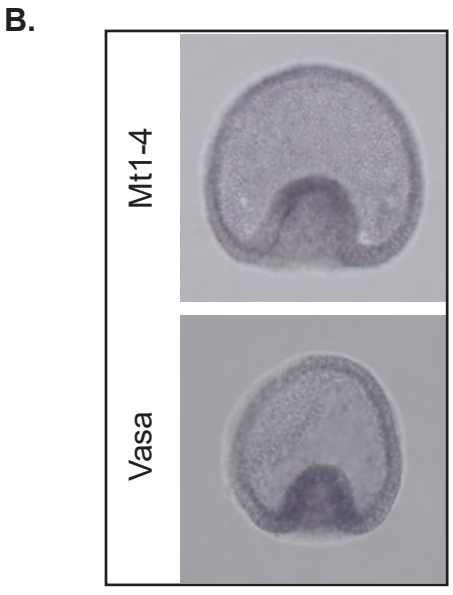

Fig. S5. MifL1-enriched cluster expression. (A) Dot plot showingexpression of sea urchin PMC marker genes Mt1-4 and Fos in cluster 10, MifL1- enriched cluster, at mid-gastrula stage. (B) Whole mount in situ hybridizations for Mt1-4 and Vasa in sea star gastrulae.

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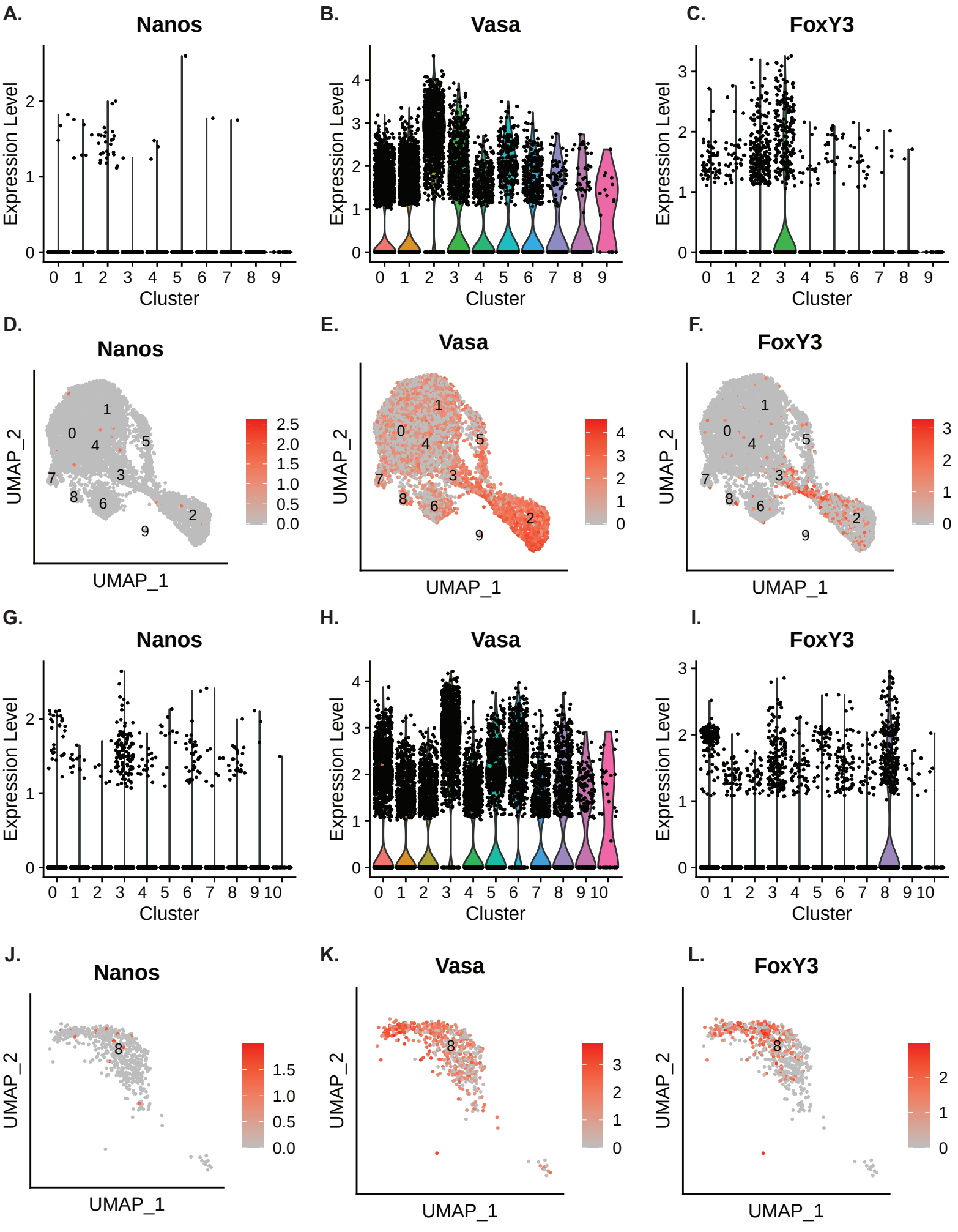
A.
Nanos
Expression Level
Cluster
B.
Vasa
Expression Level
Cluster
C.
FoxY3
Expression Level
Cluster
D.
Nanos
UMAP_2
UMAP_1
E.
Vasa
UMAP_2
UMAP_1
F.
FoxY3
UMAP_2
UMAP_1
G.
Nanos
Expression Level
Cluster
H.
Vasa
Expression Level
Cluster
I.
FoxY3
Expression Level
Cluster
J.
Nanos
UMAP_2
UMAP_1
K.
Vasa
UMAP_2
UMAP_1
L.
FoxY3
UMAP_2
UMAP_1

Fig. S6. Vasa, Nanos, and FoxY3 expression. Violin plots showing Nanos, Vasa, and FoxY3 expression per cluster in blastula stage (Hibino et al.) and mid-gastrula stage (G-I). Feature plots showing expression of Nanos, Vasa, and FoxY3in blastula stage (D-F) and in cluster 8 of mid-gastrula stage (J-L).

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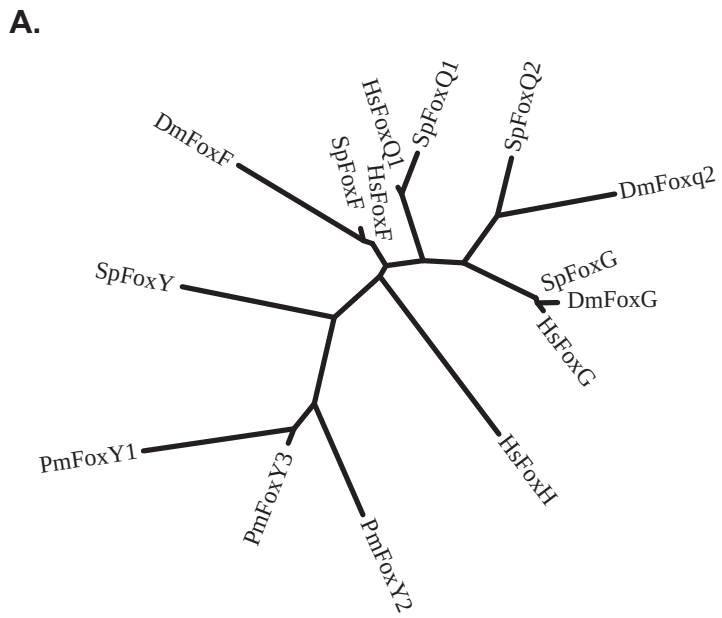
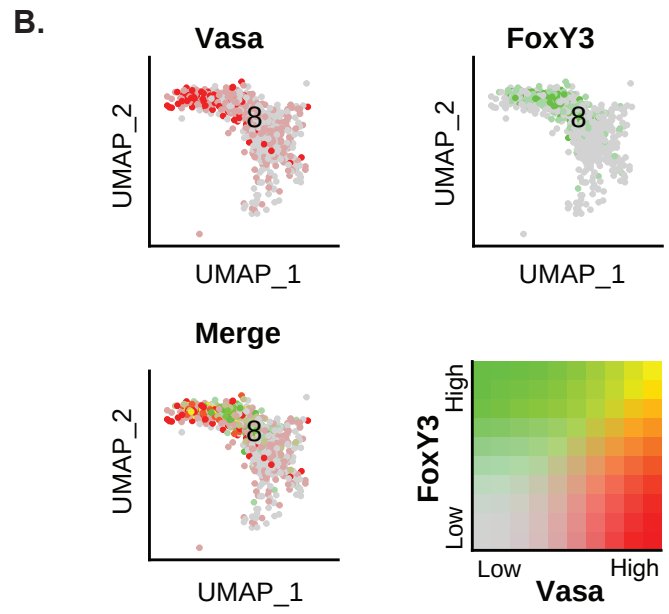
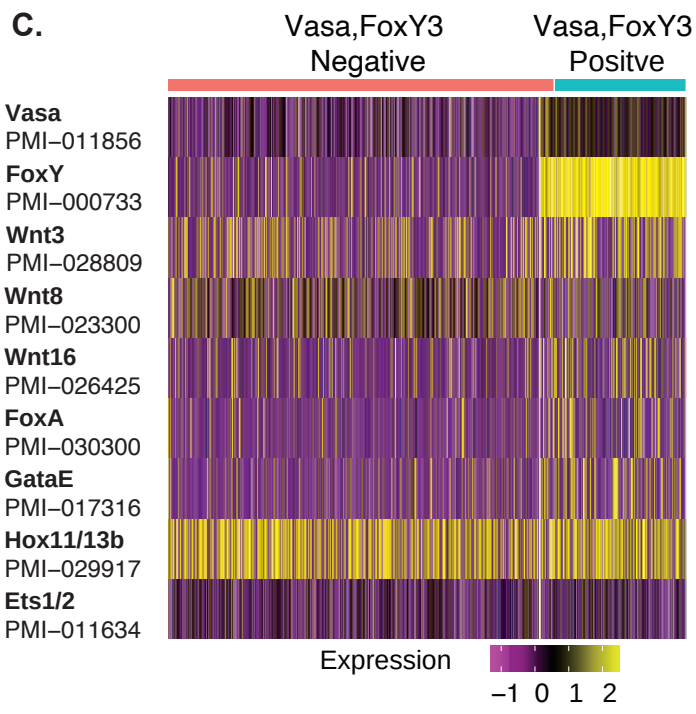

A.
DmFoxF
HsFoxQ1
SpFoxQ1
SpFoxQ2
SpFoxF
HsFoxF
DmFoxq2
SpFoxY
SpFoxG
DmFoxG
HsFoxG
HsFoxH
PmFoxY1
PmFoxY3
PmFoxY2


B.
Vasa
FoxY3
Merge
UMAP_2
UMAP_1
8
FoxY3
High
Low
Low
High
Vasa


C.
Vasa,FoxY3 Negative
Vasa,FoxY3 Positve
Vasa
PMI–011856
FoxY
PMI–000733
Wnt3
PMI–028809
Wnt8
PMI–023300
Wnt16
PMI–026425
FoxA
PMI–030300
GataE
PMI–017316
Hox11/13b
PMI–029917
Ets1/2
PMI–011634
Expression
–1 0 1 2

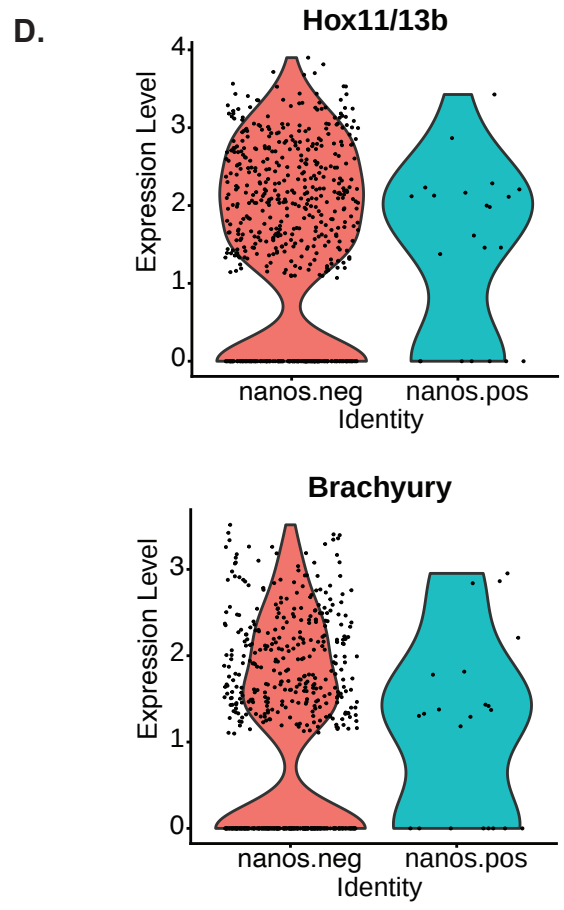

D.
Hox11/13b
Expression Level
4
3
2
1
0
nanos.neg
nanos.pos
Identity
Brachyury
Expression Level
3
2
1
0
nanos.neg
nanos.pos
Identity

Fig. S7. Vasa and FoxY3 expression. (A) Phylogenetic tree of FoxY transcription factors. Pm (*Patiria miniata*), Sp (*Strongylocentrotus purpuratus*), Dm (*Drosophila melanogaster*), Hs (*Homo sapiens*). FoxY3 (PMI_000733), previously annotated as FoxQ1, was found to be most similar to SpFoxY based on multiple protein sequence alignment. FoxY1 (PMI_028394). FoxY2 (PMI_008472). (B) Vasa expression in 395 cells of mid-gastrula cluster 8 (hindgut) shown in red (top left). FoxY3 expression in 221 cellsof cluster 8 shown in green (top right). Merge showing co-expression of Vasa and FoxY3 in 167 cells (bottom left). Color scale for expression level (bottom right). (C) Heatmap comparing expression in cells co-expressing Vasa and FoxY3 to those that donot co-express Vasa and FoxY3 in cells of cluster 8 of mid-gastrula stage (hindgut). (D) Violin plot of Hox11/13b and Brachyury expression in Nanos negative and Nanos positive cells of mid-gastrula cluster 8.

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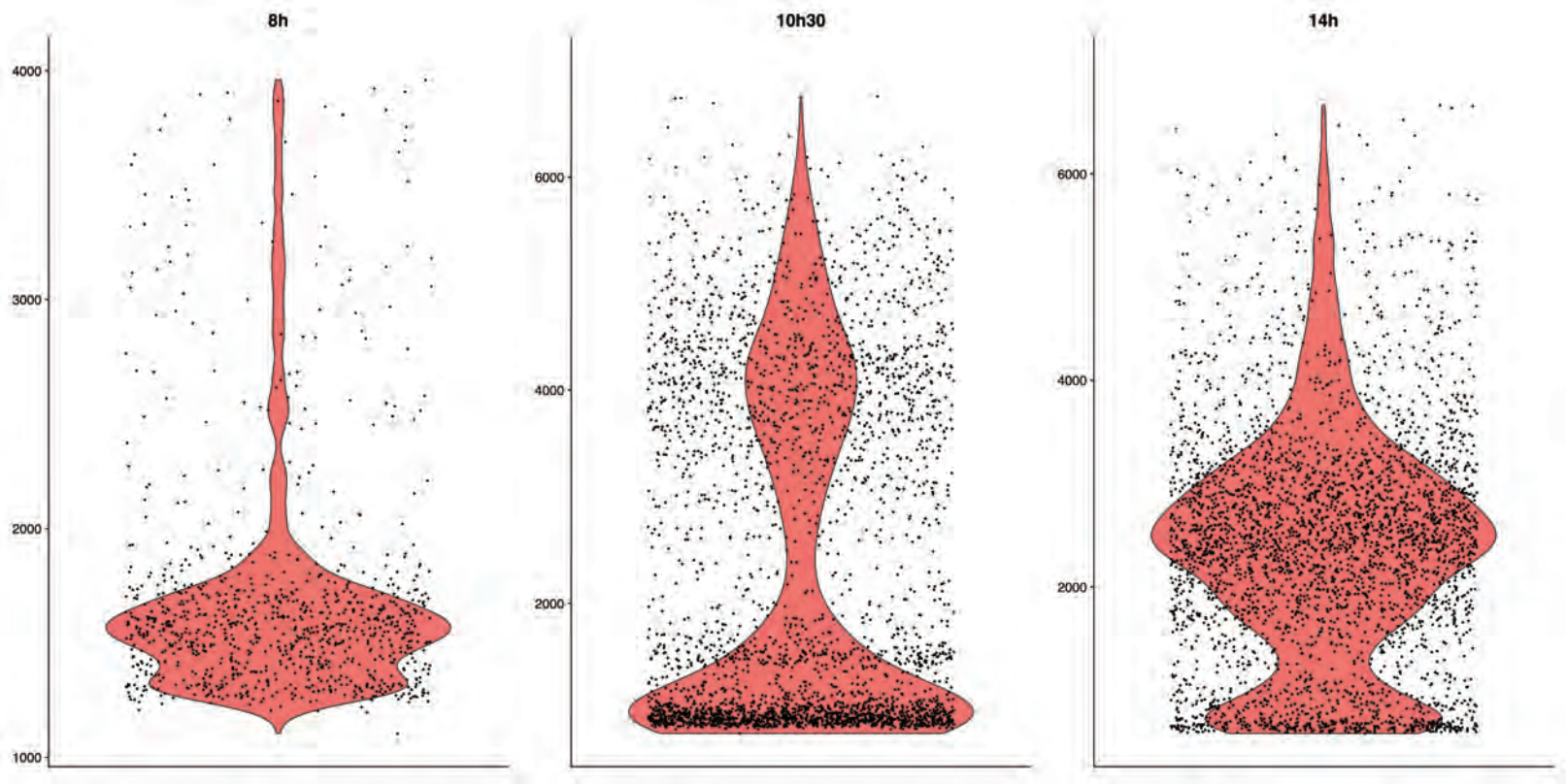

Fig. S8. Total genes detected per cell across developmental time points.

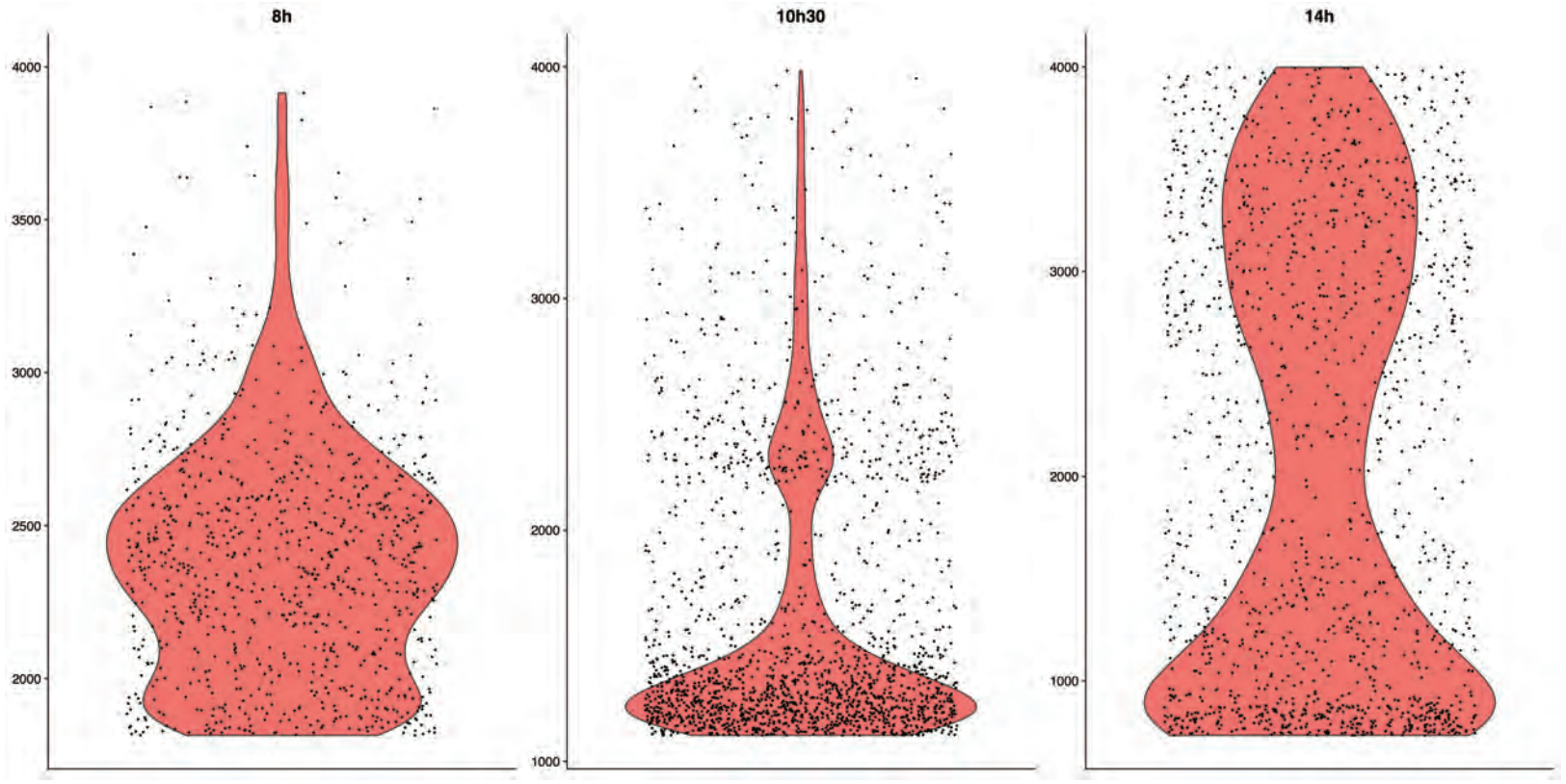

Fig. S9. Total UMIs detected per cell across developmental time points.

Table S1. Blastula Stage Cell State Identification and proportion of cells per cluster: 7,272 total cells

		Marker gene	**Number of cells**	**Percent of dataset**
Cluster 0 ●	Ectoderm:	SoxB1, Dkk3	2349	32.3
Cluster 1 ●	Ectoderm: Apical, Neuronal	FoxQ2, DKK3, Syt14 (synaptotagmin)	1528	21.0
Cluster 2 ●	Presumptive mesoderm	Ets1/2, GataE, Tbr, Wnt16,	1006	13.8
Cluster 3 ●	Presumptive endoderm	Cdxl, Bra, Nk1, Wnt8, FoxY3 (annotated as Foxq1)	695	9.5
Cluster 4 ●	Ectoderm: Oral	Nodal, Lefty, Chordin, Bmp2/4	521	7.2
Cluster 5 ●	Ectoderm: Apical pole	Dkk3, Hyalin	489	6.7
Cluster 6 ●	Ectoderm: Lateral	Wnt16, Nodal, Lefty	455	6.2
Cluster 7 ●	Ectoderm: Vegetal	Fos1, Mt1-4, Wnt8, Jun	124	1.7
Cluster 8 ●	Gcm enriched	Gcm, Delta	87	1.2
Cluster 9 ●	MifL1 enriched	MifL1, Mt1-4, Ets4	18	0.2

Mid-Gastrula Stage Cell State Identification and proportion of cells per cluster: 10,448 total cells

		Marker gene	**Number of cells**	**Percent of dataset**
Cluster 0 ●	Ectoderm	SoxB1	2407	23.0
Cluster 1 ●	Ectoderm	SoxB1, FoxQ2 (PMI-009167 Nkx3.2)	1437	13.7
Cluster 2 ●	Ectoderm: Apical	Dkk3, FoxQ2	1193	11.4
Cluster 3 ●	Mesoderm	Ets1/2, Frizz, Vasa	1143	10.9
Cluster 4 ●	Ectoderm: Lateral	SoxB1, Wnt8	1118	10.7
Cluster 5 ●	Undetermined	Fic9, Fbn3	924	8.8
Cluster 6 ●	Ectoderm: Ventral	GataE, Wnt16, Blimp1, Vasa, FoxA,	720	6.9
Cluster 7 ●	Ectoderm: Oral	Nodal, Lefty, Bmp2/4, Nkx2.1	672	6.4
Cluster 8 ●	Endoderm: Midgut/hindgut	Cdxl, Blimp1, Wnt3, Wnt8, Brachyury, FoxY3 (annotated as Foxq1)	595	5.7
Cluster 9 ●	Gcm enriched	Gcm	193	1.8
Cluster 10 ●	MifL1 enriched	MifL1, Fos, ribosomal proteins	46	0.4

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Table S2. Number of Nanos, Vasa, and FoxY3 expressing cells in cluster 8 (hindgut) and cluster 3 (archenteron) regions.

	Nanos	Vasa	FoxY3	Nanos,Vasa	Vasa,FoxY3	Nanos,Vasa,FoxY3	Total
Cluster 8	22	395	221	21	167	9	595
Cluster 3	117	1099	177	112	167	19	1143

Table S3. Blastula Cluster Markers

Click here to download Table S3

Table S4. MidGastrula Cluster Markers

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Table S5. MG Cluster 8 Vasa Positive

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Table S6. Harmony Cluster Integration Markers

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Supplementary Data 1. PmAnalysis.

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