Molecular evidence for early deuterostome origins of ovarian cell types and neuroendocrine control of reproduction

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Supplementary Figures

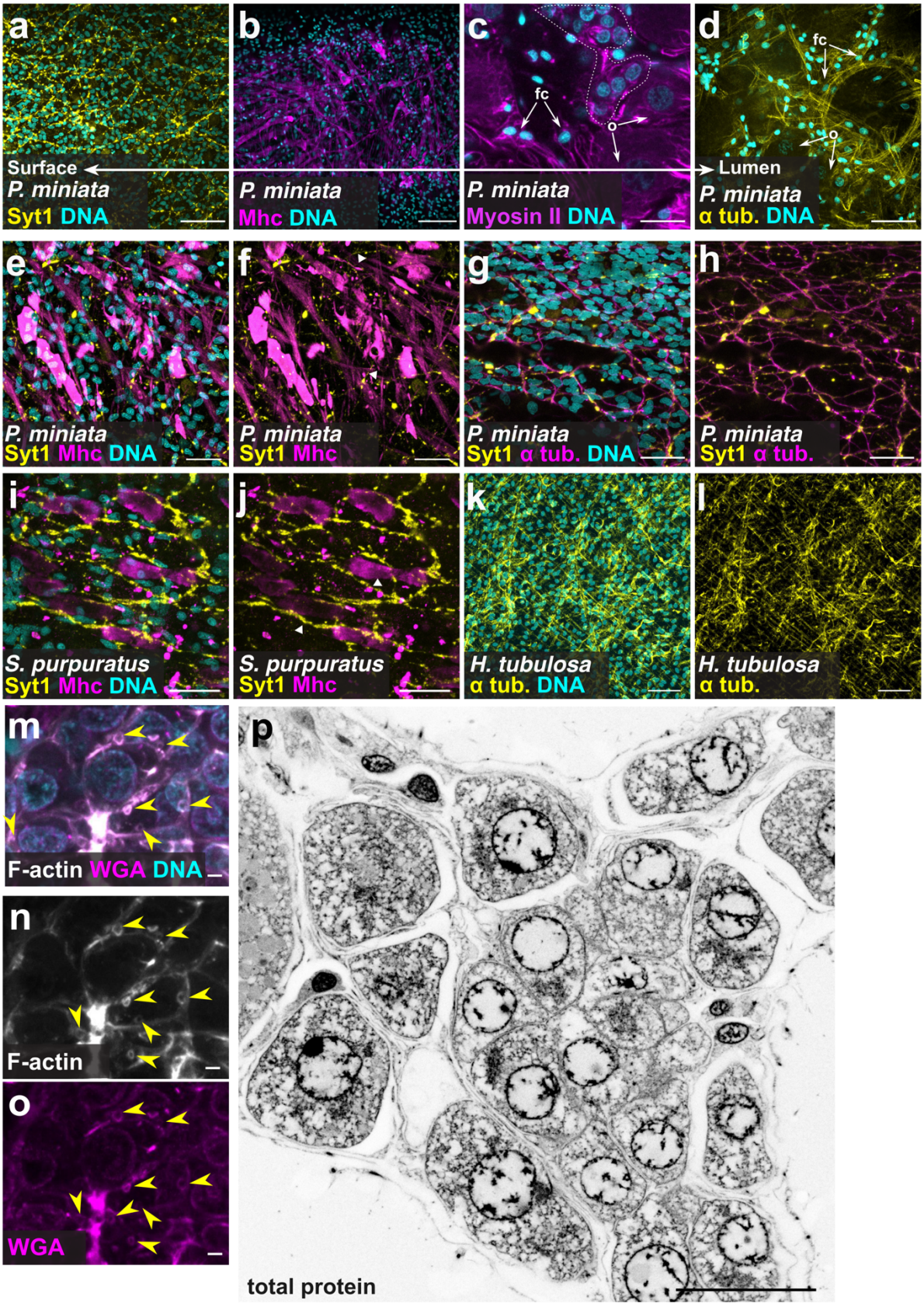

a
Surface
P. miniata
Syt1 DNA
b
P. miniata
Mhc DNA
c
fc
o
P. miniata
Myosin II DNA
d
fc
o
Lumen
P. miniata
α tub. DNA
e
P. miniata
Syt1 Mhc DNA
f
P. miniata
Syt1 Mhc
g
P. miniata
Syt1 α tub. DNA
h
P. miniata
Syt1 α tub.
i
S. purpuratus
Syt1 Mhc DNA
j
S. purpuratus
Syt1 Mhc
k
H. tubulosa
α tub. DNA
l
H. tubulosa
α tub.
m
F-actin WGA DNA
n
F-actin
o
WGA
p
total protein

Figure S1. Immunohistochemical identification of echinoderm ovarian cell types. (a-d) IHC for synaptotagmin 1 (Syt1) labeling neurons (a), for myosin heavy chain (Mhc) labeling muscles (b) and for myosin (c) and α-tubulin (DM1α) (d) labeling follicle cells. Scale bars for panels a and d are 40 μm; for panel b 60 μm; for c 20 μm. **(e-f)** IHC for Syt1 and Mhc to visualize the interconnection of ovarian neurons and muscles. Scale bars for panels are 20 μm. **(g-h)** IHC for Syt1 and α-tubulin co-labels the ovarian neurons. Scale bars for panels are 20 μm. **(i-j)** IHC for Syt1 and Mhc confirms the presence of the neurons and muscles respectively in the sea urchin *S. purpuratus* ovary. Scale bars are 25 μm. **(k-l)** IHC for α-tubulin labels the ovarian neurons in the sea cucumber *H. tubulosa* ovary. Scale bars are 50 μm. Nuclei were labelled with DAPI (cyan). fc, follicle cells; o, oocytes. White arrowheads in panels f and j are used to indicate axonal projections. (**m-o**) Phalloidin and wheat germ agglutinin (WGA) lectin co-staining labeling the cell boundaries and membranes. Yellow arrowheads indicate intercellular bridge-like structures. Scale bar is 1 μm. (**p**) Full-sized image of total protein in nest using pan-expansion microscopy from Fig. 1h. Scale bar is 20 μm.

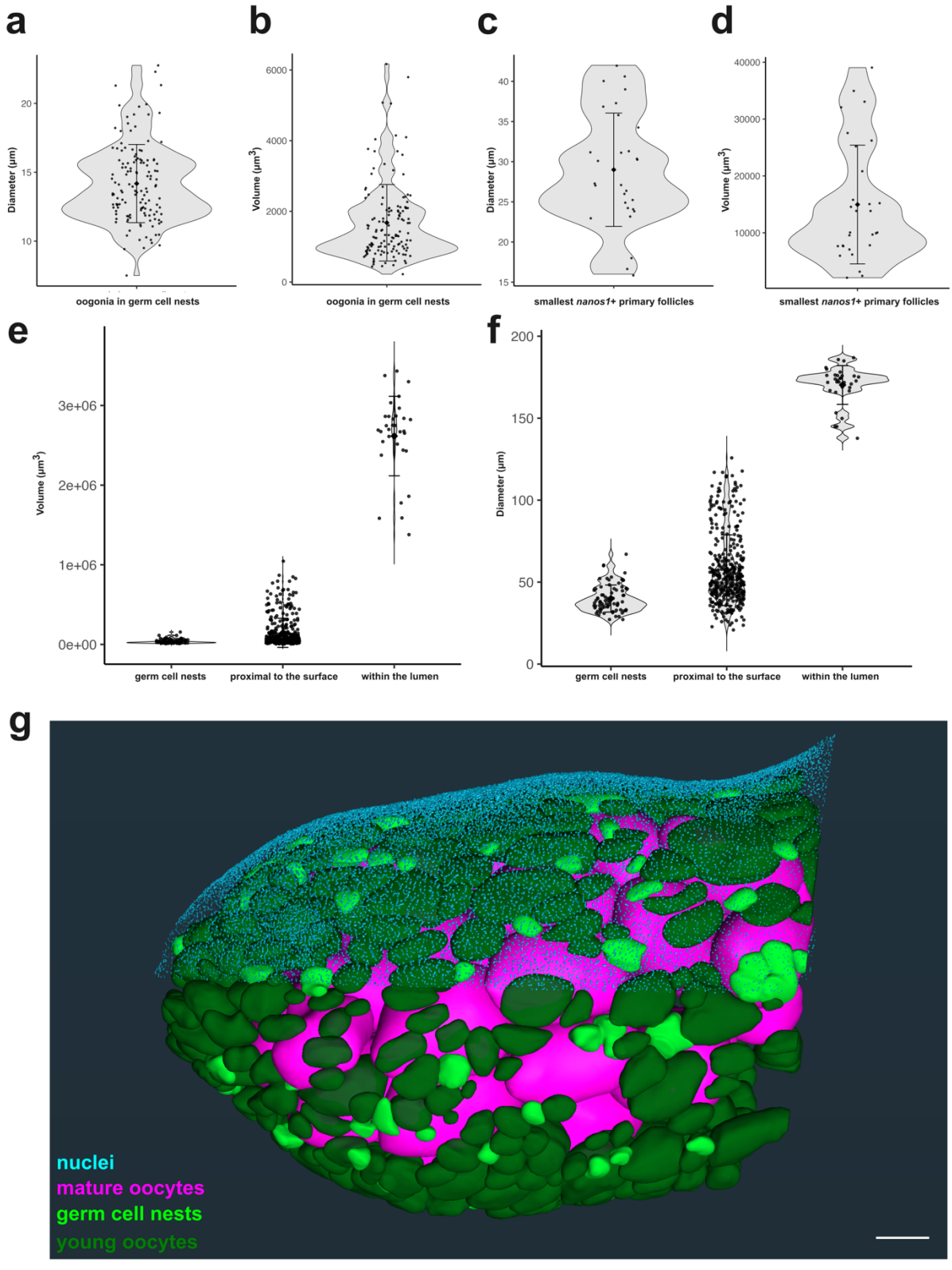

a
Diameter (µm)
oogonia in germ cell nests
b
Volume (µm³)
oogonia in germ cell nests
c
Diameter (µm)
smallest nanos1+ primary follicles
d
Volume (µm³)
smallest nanos1+ primary follicles
e
Volume (µm³)
germ cell nests
proximal to the surface
within the lumen
f
Diameter (µm)
germ cell nests
proximal to the surface
within the lumen
g
nuclei
mature oocytes
germ cell nests
young oocytes

Figure S2. Size and spatial distributions of oogonia and oocytes in the sea star ovary. (a-b) Violin plot showing the experimentally measured diameter (a) and estimated volume (b) of each oogonium within germ cell nests. **(c-d)** Violin plot showing the measured diameter (c) and estimated volume (d) of the smallest *nanos1* positive oocyte in close proximity to the germ cell nests. **(e-f)** Violin plot of the segmentation-based calculated volumes (e) and estimated diameter (f) of germ cell nests, oocytes close to the ovarian surface, and those within the lumen. In panels a-f the mean ± SD values are also depicted. **(g)** 3D rendering of segmented germ cell nests and oocytes. Scale bar is 100 μm. Ovary seen from a side view. Estimated volumes or diameters were calculated based on experimentally measured diameters or volumes respectively, assuming the shape of the oogonia and oocytes is spherical. Source data are provided as a Source Data file.

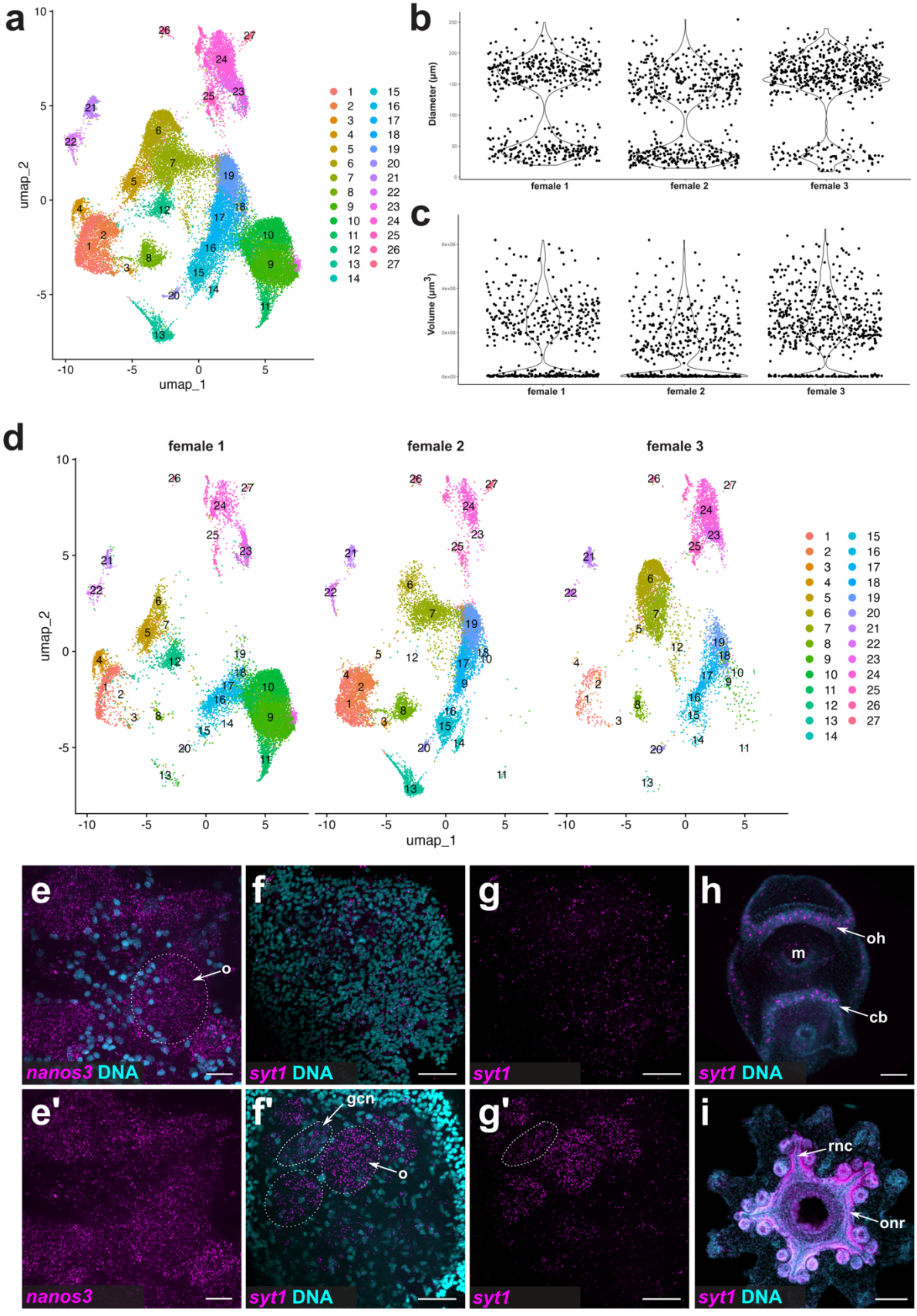

a
b
c
d
e
e'
f
f'
g
g'
h
i
umap_1
umap_2
Diameter (µm)
Volume (µm³)
female 1
female 2
female 3
nanos3 DNA
syt1 DNA
syt1
nanos3
o
gcn
m
oh
cb
rnc
onr

Figure S3. *P. miniata* ovary cell atlas and cell contribution of the different libraries. **(a)** Integrated UMAP of the three different scRNA-seq libraries, in which color code is indicative of different clusters. **(b-c)** Violin plot showing the experimentally measured diameter (b) and estimated volume (c) of the oogonia and oocytes within the ovaries used for the reconstruction of the single cell atlas. Due to the capturing system limitations only the oocytes with a size ≤ 40µm in diameter are included in the single cell atlas. **(d)** Integrated UMAP of the three different scRNA-seq libraries, split by the cell contribution of each library. The color code is indicative of different clusters. **(e-e')** HCR for the *nanos3* gene with (e) and without (e') the nuclei channel (DAPI) confirmed its localization in the germ lineage. Dotted circles in panel e are indicative of oocytes. Scale bars are 20 µm. **(f-g')** HCR for the *syt1* gene with (f-f') and without (g-g') the nuclei channel (DAPI) shows expression in the neurons (f-g) as well as the germ cell nests and oocytes (f'-g'). Dotted circles in F' are indicative of oocytes. Germ cell nests in panels f' and g' are outlined with dotted lines. Scale bars are 20 µm. **(h-i)** HCR for the *syt1* gene shows expression in the nervous system of the *P. miniata* larva (h) and post-metamorphic juvenile (i), confirming the specificity of the probe. Scale bars for panels h and i are 50 and 100 µm respectively. cb, ciliary band; gcn, germ cell nest; m, mouth; o, oocytes; oh, oral hood; onr, oral nerve ring; rnc, radial nerve cord. Source data are provided as a Source Data file.

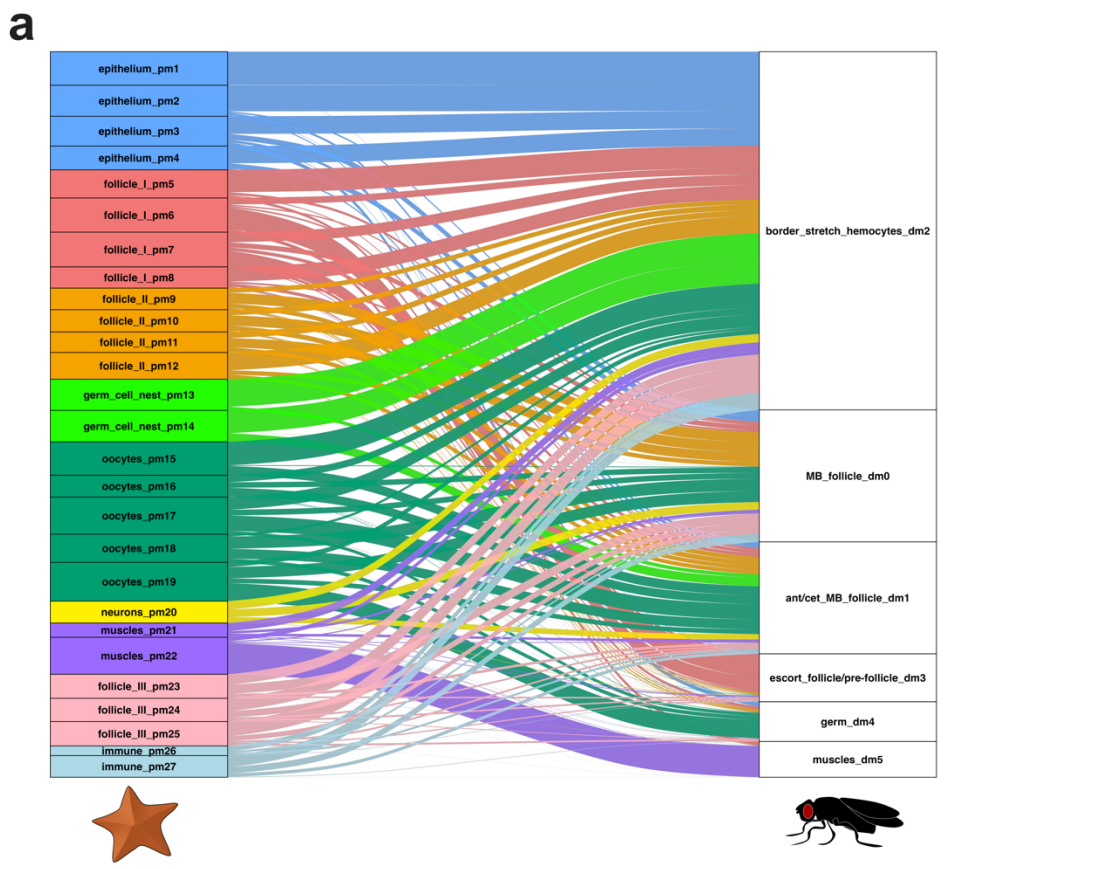

a
epithelium_pm1
epithelium_pm2
epithelium_pm3
epithelium_pm4
follicle_I_pm5
follicle_I_pm6
follicle_I_pm7
follicle_I_pm8
follicle_II_pm9
follicle_II_pm10
follicle_II_pm11
follicle_II_pm12
germ_cell_nest_pm13
germ_cell_nest_pm14
oocytes_pm15
oocytes_pm16
oocytes_pm17
oocytes_pm18
oocytes_pm19
neurons_pm20
muscles_pm21
muscles_pm22
follicle_III_pm23
follicle_III_pm24
follicle_III_pm25
immune_pm26
immune_pm27
border_stretch_hemocytes_dm2
MB_follicle_dm0
ant/cet_MB_follicle_dm1
escort_follicle/pre-follicle_dm3
germ_dm4
muscles_dm5
b
epithelium_pm1
epithelium_pm2
epithelium_pm3
epithelium_pm4
follicle_I_pm5
follicle_I_pm6
follicle_I_pm7
follicle_I_pm8
follicle_II_pm9
follicle_II_pm10
follicle_II_pm11
follicle_II_pm12
germ_cell_nest_pm13
germ_cell_nest_pm14
oocytes_pm15
oocytes_pm16
oocytes_pm17
oocytes_pm18
oocytes_pm19
neurons_pm20
muscles_pm21
muscles_pm22
follicle_III_pm23
follicle_III_pm24
follicle_III_pm25
immune_pm26
immune_pm27
stromal_mm0
granulosa_mm1
epithelium_mm10
oocytes_mm11
luteal_mm12
B-lymphocytes__mm13
epithelium_mm15
stromal_mm2
theca_mm3
endothelium_mm4
phagocytes_mm5
T-lymphocytes_mm6
granulosa_mm7
stromal_mm8
phagocytes_mm9
c
epithelium_pm1
epithelium_pm2
epithelium_pm3
epithelium_pm4
follicle_I_pm5
follicle_I_pm6
follicle_I_pm7
follicle_I_pm8
follicle_II_pm9
follicle_II_pm10
follicle_II_pm11
follicle_II_pm12
germ_cell_nest_pm13
germ_cell_nest_pm14
oocytes_pm15
oocytes_pm16
oocytes_pm17
oocytes_pm18
oocytes_pm19
neurons_pm20
muscles_pm21
muscles_pm22
follicle_III_pm23
follicle_III_pm24
follicle_III_pm25
immune_pm26
immune_pm27
undefined
upper oral ectoderm
intestine
acinar-like cells
cardiac sphincter
apical plate
stomach 3
blastocoelar cells
neurons
coelomic pouches
esophageal muscles
lower oral ectoderm
stomach 1
skeleton
immune cells
ciliary band
aboral ectoderm
stomach 2
pyloric sphincter
anus
esophagus

Figure S4. Cross-species comparison of sea star ovarian cell types with protostome and deuterostome representatives with SAMap. Sankey plot depicting the alignment scores between adult sea star ovarian cell atlas with the **(a)** adult fruit fly ovary (26), **(b)** adult mouse ovary (23) and **(c)** the sea urchin larva (18). The width of the alluvial link corresponds to the alignment score between the compared clusters. The greater the width the higher the alignment score. The alignment scores can be found in supplementary material (Supplementary Data 8-10). Source data are provided as a Source Data file.

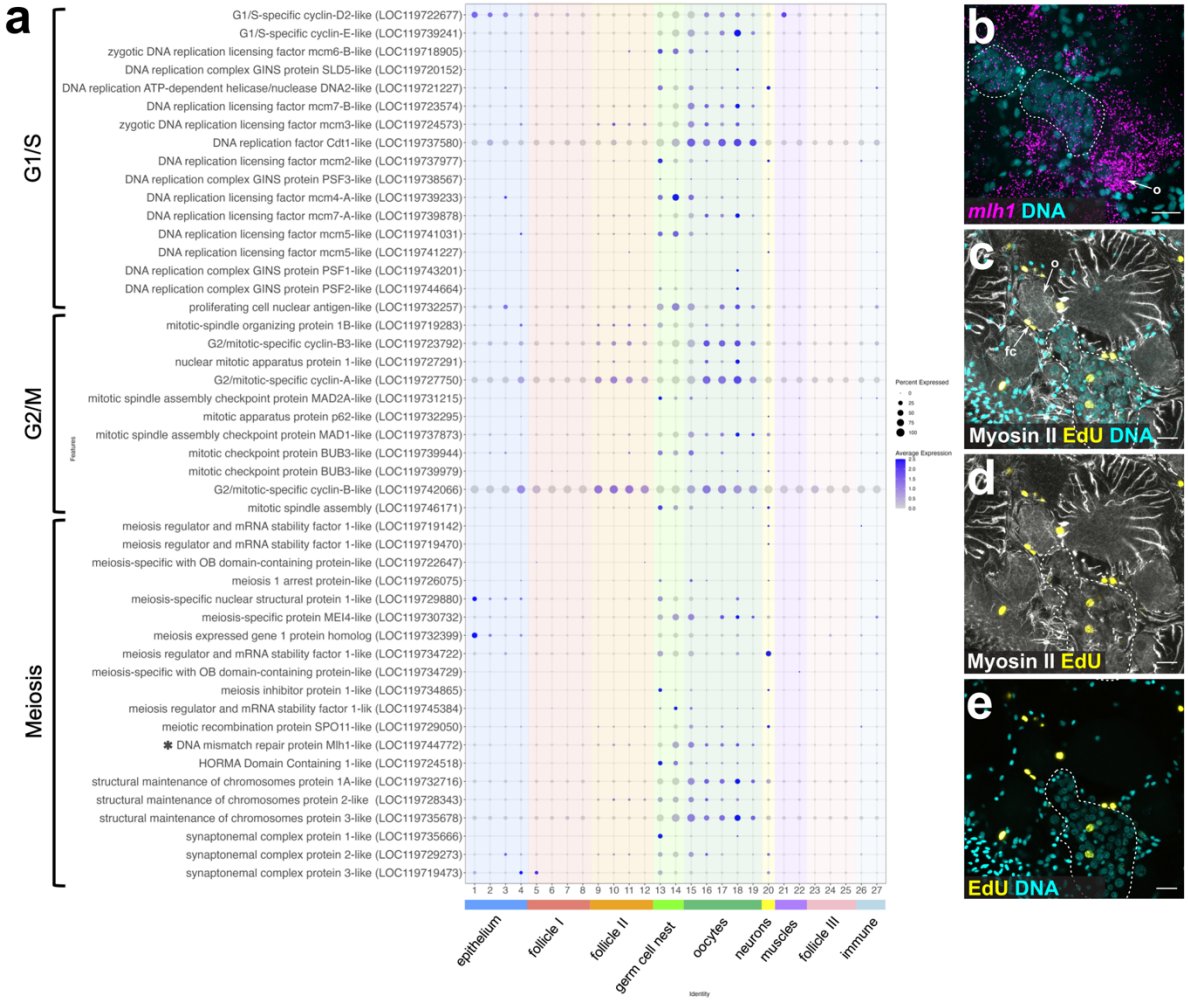
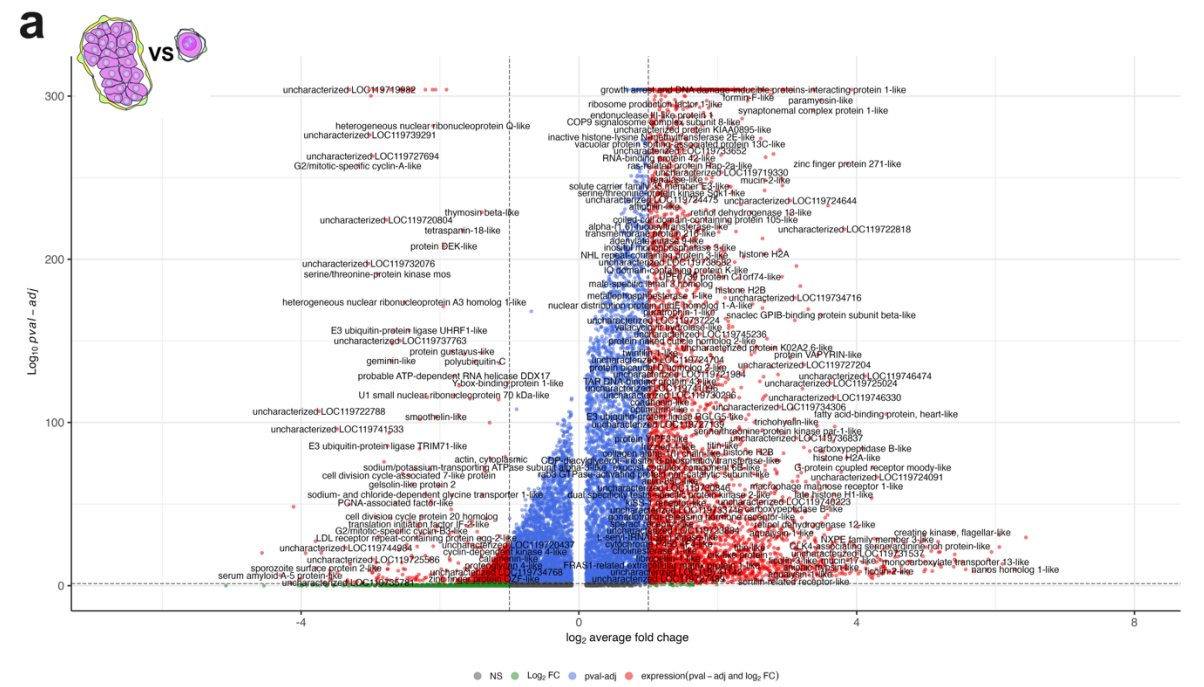
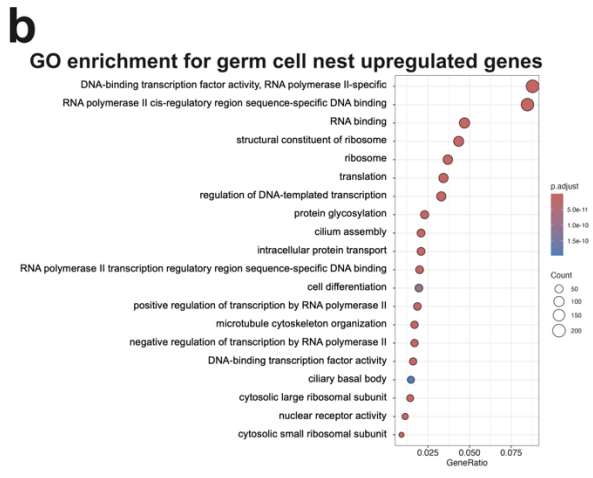
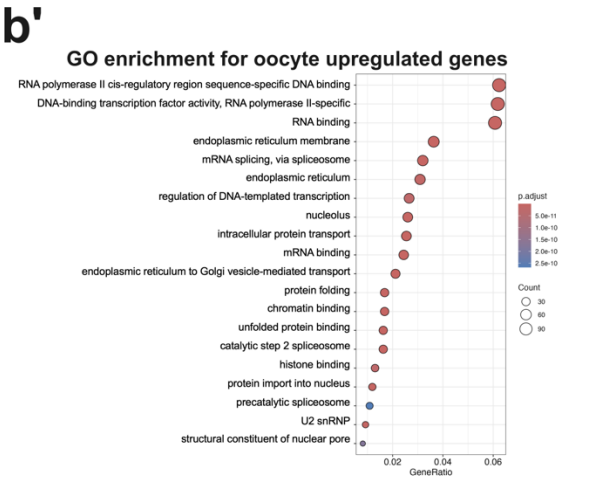


Figure S5. Identification of mitotic and meiotic molecular signatures in the sea star ovary. (a) Dotplot showing the average expression of the genes used for the scoring analysis shown in Fig. 4a. **(b)** HCR for the meiotic marker *mlh1* labelling the germ cell nests and oocytes of various sizes. **(c-e)** IHC for Myosin II labeling cell peripheries with the EdU proliferation assay confirms that cells from both the somatic and germ lineages are actively proliferating in the adult sea star ovary. Nuclei are labelled with DAPI (cyan). Dotted lines outline the germ cell nest in panels c-e. Scale bars are 20 μm. fc, follicle cells; o, oocytes. Asterisk is used to indicate the gene whose predicted expression pattern has been confirmed with HCR. Source data are provided as a Source Data file.


a
vs
Log10 pval – adj
log2 average fold chage
NS
Log2 FC
pval-adj
expression(pval – adj and log2 FC)


b
GO enrichment for germ cell nest upregulated genes
DNA-binding transcription factor activity, RNA polymerase II-specific
RNA polymerase II cis-regulatory region sequence-specific DNA binding
RNA binding
structural constituent of ribosome
ribosome
translation
regulation of DNA-templated transcription
protein glycosylation
cilium assembly
intracellular protein transport
RNA polymerase II transcription regulatory region sequence-specific DNA binding
cell differentiation
positive regulation of transcription by RNA polymerase II
microtubule cytoskeleton organization
negative regulation of transcription by RNA polymerase II
DNA-binding transcription factor activity
ciliary basal body
cytosolic large ribosomal subunit
nuclear receptor activity
cytosolic small ribosomal subunit
GeneRatio
p.adjust
Count


b'
GO enrichment for oocyte upregulated genes
RNA polymerase II cis-regulatory region sequence-specific DNA binding
DNA-binding transcription factor activity, RNA polymerase II-specific
RNA binding
endoplasmic reticulum membrane
mRNA splicing, via spliceosome
endoplasmic reticulum
regulation of DNA-templated transcription
nucleolus
intracellular protein transport
mRNA binding
endoplasmic reticulum to Golgi vesicle-mediated transport
protein folding
chromatin binding
unfolded protein binding
catalytic step 2 spliceosome
histone binding
protein import into nucleus
precatalytic spliceosome
U2 snRNP
structural constituent of nuclear pore
GeneRatio
p.adjust
Count

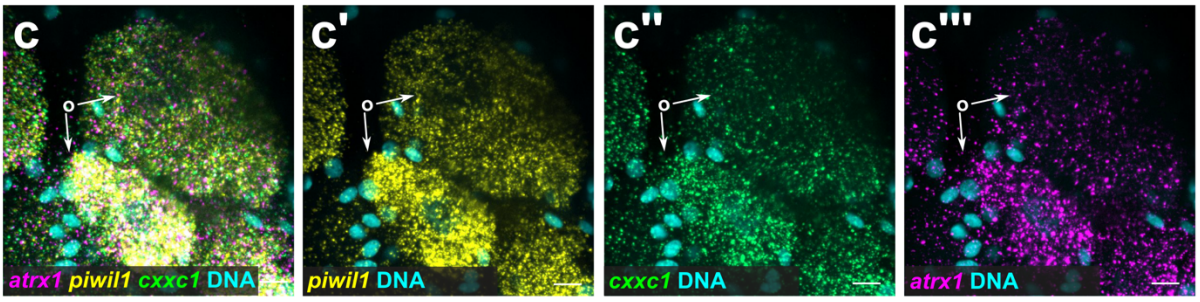
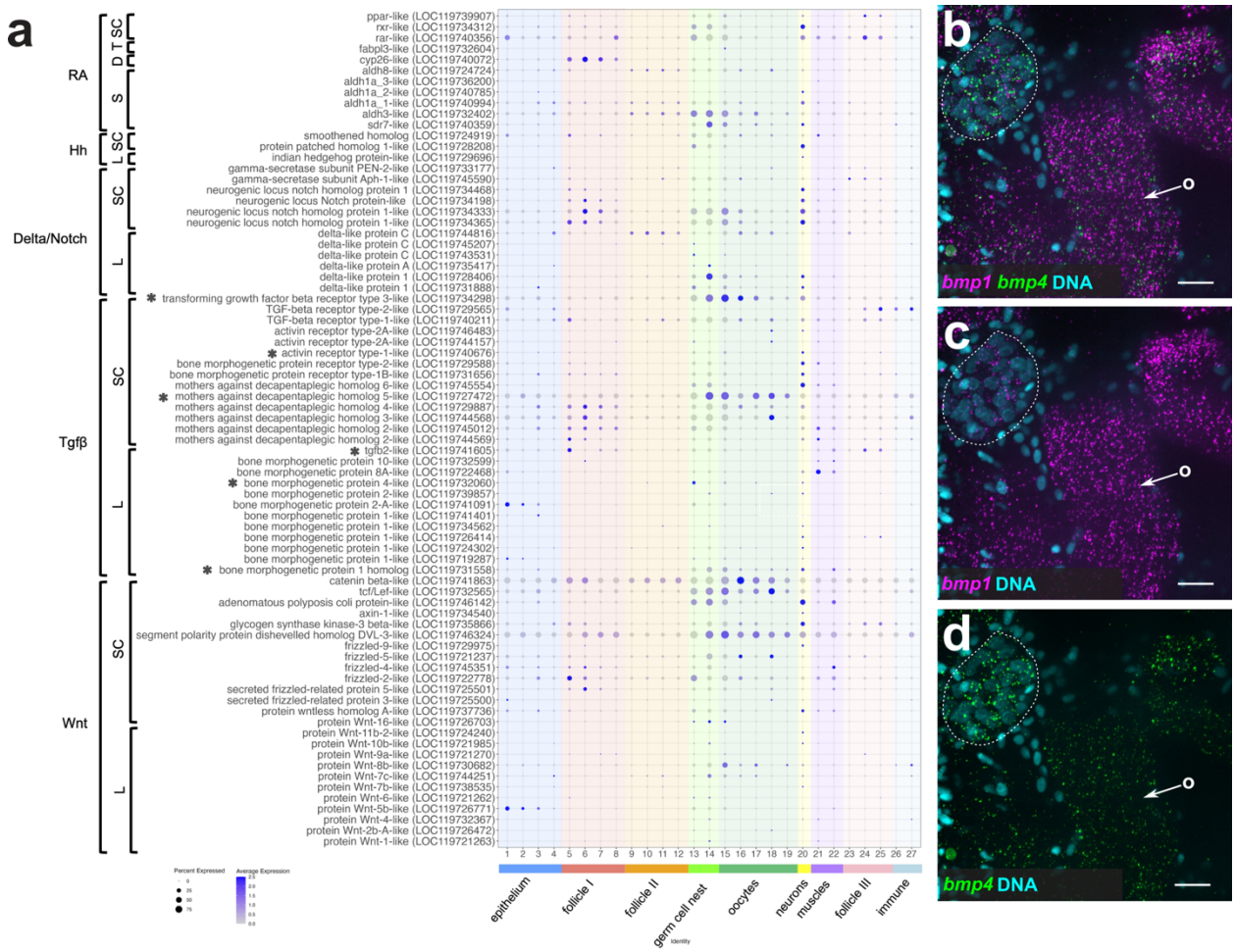

c
atrx1 piwil1 cxxc1 DNA
c'
piwil1 DNA
c''
cxxc1 DNA
c'''
atrx1 DNA

Figure S6. Differential expression patterns along the sea star oogenesis developmental trajectory. (a) Volcano plot showing the differential expression of the germ cell nest (clusters 13 and 14) genes versus the oocyte reference group (clusters 15-19). **(b-b')** Dotplot showing the top 20 enriched gene ontology terms associated with the germ cell nest (b) and the oocyte (b') upregulated genes. **(c-c''')** HCR for the transcriptional regulators *atrx1* and *cxxc1* paired with *piwil1* shows expression in oocytes. Scale bars are 10 μm. Source data are provided as a Source Data file.



Figure S7. Distinct transcription factor expression profiles during early oogenesis. Heatmap of Top 50 transcription factors defining the germline clusters. Each column represents a cell from the integrated dataset and each column is grouped by cluster.


a
RA
Hh
Delta/Notch
Tgfβ
Wnt
SC
L
D T SC
S
ppar-like (LOC119739907)
rxr-like (LOC119734312)
rar-like (LOC119740356)
fabp9-like (LOC119732604)
cyp26-like (LOC119740072)
aldh8-like (LOC119724724)
aldh1a_3-like (LOC119736200)
aldh1a_2-like (LOC119740785)
aldh1a_1-like (LOC119740994)
aldh3-like (LOC119732402)
sdr7-like (LOC119740359)
smoothened homolog (LOC119724919)
protein patched homolog 1-like (LOC119728208)
indian hedgehog protein-like (LOC119729696)
gamma-secretase subunit PEN-2-like (LOC119733177)
gamma-secretase subunit Aph-1-like (LOC119745590)
neurogenic locus notch homolog protein 1 (LOC119734468)
neurogenic locus Notch protein-like (LOC119734198)
neurogenic locus notch homolog protein 1-like (LOC119734333)
neurogenic locus notch homolog protein 1-like (LOC119734365)
delta-like protein C (LOC119744816)
delta-like protein C (LOC119745207)
delta-like protein C (LOC119743531)
delta-like protein A (LOC119735417)
delta-like protein 1 (LOC119728406)
delta-like protein 1 (LOC119731888)
transforming growth factor beta receptor type 3-like (LOC119734298)
TGF-beta receptor type-2-like (LOC119729565)
TGF-beta receptor type-1-like (LOC119740211)
activin receptor type-2A-like (LOC119746483)
activin receptor type-2A-like (LOC119744157)
activin receptor type-1-like (LOC119740676)
bone morphogenetic protein receptor type-2-like (LOC119729588)
bone morphogenetic protein receptor type-1B-like (LOC119731656)
mothers against decapentaplegic homolog 6-like (LOC119745554)
mothers against decapentaplegic homolog 5-like (LOC119727472)
mothers against decapentaplegic homolog 4-like (LOC119729887)
mothers against decapentaplegic homolog 3-like (LOC119744568)
mothers against decapentaplegic homolog 2-like (LOC119745012)
mothers against decapentaplegic homolog 2-like (LOC119744569)
tgfb2-like (LOC119741605)
bone morphogenetic protein 10-like (LOC119732599)
bone morphogenetic protein 8A-like (LOC119722468)
bone morphogenetic protein 4-like (LOC119732060)
bone morphogenetic protein 2-like (LOC119739857)
bone morphogenetic protein 2-A-like (LOC119741091)
bone morphogenetic protein 1-like (LOC119741401)
bone morphogenetic protein 1-like (LOC119734562)
bone morphogenetic protein 1-like (LOC119726414)
bone morphogenetic protein 1-like (LOC119724302)
bone morphogenetic protein 1-like (LOC119719287)
bone morphogenetic protein 1 homolog (LOC119731558)
catenin beta-like (LOC119741863)
tcf/Lef-like (LOC119732565)
adenomatous polyposis coli protein-like (LOC119746142)
axin-1-like (LOC119734540)
glycogen synthase kinase-3 beta-like (LOC119735866)
segment polarity protein dishevelled homolog DVL-3-like (LOC119746324)
frizzled-9-like (LOC119729975)
frizzled-5-like (LOC119721237)
frizzled-4-like (LOC119745351)
frizzled-2-like (LOC119722778)
secreted frizzled-related protein 5-like (LOC119725501)
secreted frizzled-related protein 3-like (LOC119725500)
protein wntless homolog A-like (LOC119737736)
protein Wnt-16-like (LOC119726703)
protein Wnt-11b-2-like (LOC119724240)
protein Wnt-10b-like (LOC119721985)
protein Wnt-9a-like (LOC119721270)
protein Wnt-8b-like (LOC119730682)
protein Wnt-7c-like (LOC119744251)
protein Wnt-7b-like (LOC119738535)
protein Wnt-6-like (LOC119721262)
protein Wnt-5b-like (LOC119726771)
protein Wnt-4-like (LOC119732367)
protein Wnt-2b-A-like (LOC119726472)
protein Wnt-1-like (LOC119721263)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
epithelium
follicle I
follicle II
germ cell nest
oocytes
neurons
muscles
follicle III
immune
Percent Expressed
Average Expression
Identity
b
bmp1 bmp4 DNA
c
bmp1 DNA
d
bmp4 DNA
o

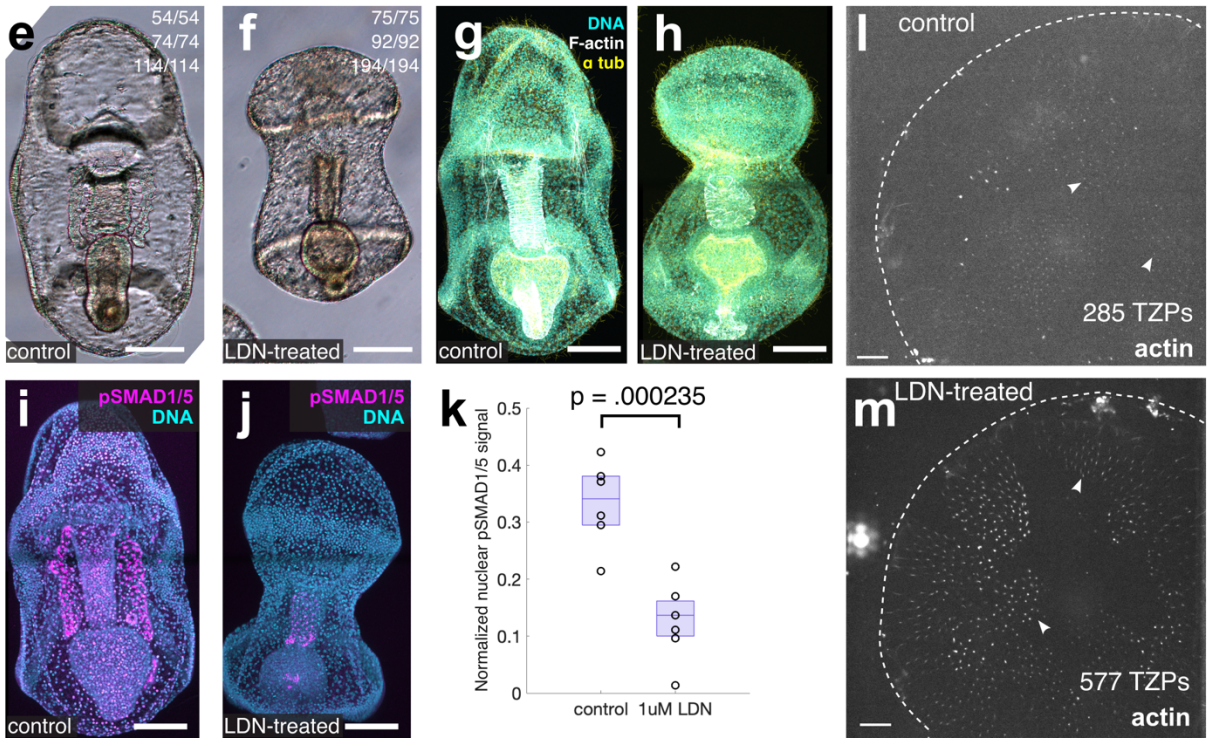

e
54/54
74/74
114/114
control
f
75/75
92/92
194/194
LDN-treated
g
DNA
F-actin
α tub
control
h
LDN-treated
i
pSMAD1/5
DNA
control
j
pSMAD1/5
DNA
LDN-treated
k
p = .000235
Normalized nuclear pSMAD1/5 signal
0
0.1
0.2
0.3
0.4
0.5
control
1uM LDN
l
control
285 TZPs
actin
m
LDN-treated
577 TZPs
actin

Figure S8. Expression patterns of evolutionarily conserved ovarian signaling pathways and validation of LDN-193189 inhibitor. (a) Dotplot showing the average expression of the genes used for the scoring analysis shown in Fig. 6a. D, degradation; L, ligand; S, synthesis; SC, signaling cascade; T, transport. **(b-d)** HCR for the *bmp1* and *bmp4* ligands confirms their spatial expression in the germ cell nests and oocytes. Nuclei are labelled with DAPI (cyan). Dotted lines outline the germ cell nest. o, oocytes. Asterisks indicate genes whose predicted expression pattern has been confirmed with HCR or IHC. The expression patterns can be found within the same figure and Fig. 6. (**e-j**) Larvae exposed to either FSW (e,g,i) or FWS + 1 μM LDN-193189 (f,h,j) from 24 hours post-fertilization until 72 hours post-fertilization, when they were imaged. (e,f) are brightfield images, and the numbers show the number of larvae showing the normal (for control) or radialized (for LDN-treated larvae) phenotype in each of three experiments. (g,h) are fixed and stained with DRAQ5 (DNA), phalloidin (actin), and immunofluorescence for DM1α (α-tubulin). (i-j) are fixed and stained for pSMAD1/5 and DRAQ5 (DNA). The pSMAD1/5 signal is lost in the LDN-treated larvae. Scale bar for e-j is 100 μm. Radialization phenotype is seen in (f,h,j). (**k**) Quantification of dorsal pSMAD1/5 signal between untreated larvae and those treated with 1 μM LDN-193189 from 24-72 hours post fertilization. Each dot represents a larva (n=6 in each condition), p-value from 2-sided t-test = .000235, box indicates the median and upper and lower quartile. (**l-m**) Follicles extracted from ovary explants cultured in either FSW or FSW + 1 μM LDN-193189 for four days. Follicle cell actin is stained with SPY555-FastAct. Dotted lines outline the follicles. Arrowheads point to TZPs. Shown are two follicles from the dataset in Fig. 6g. Scale bars are 20 μm. Source data are provided as a Source Data file.

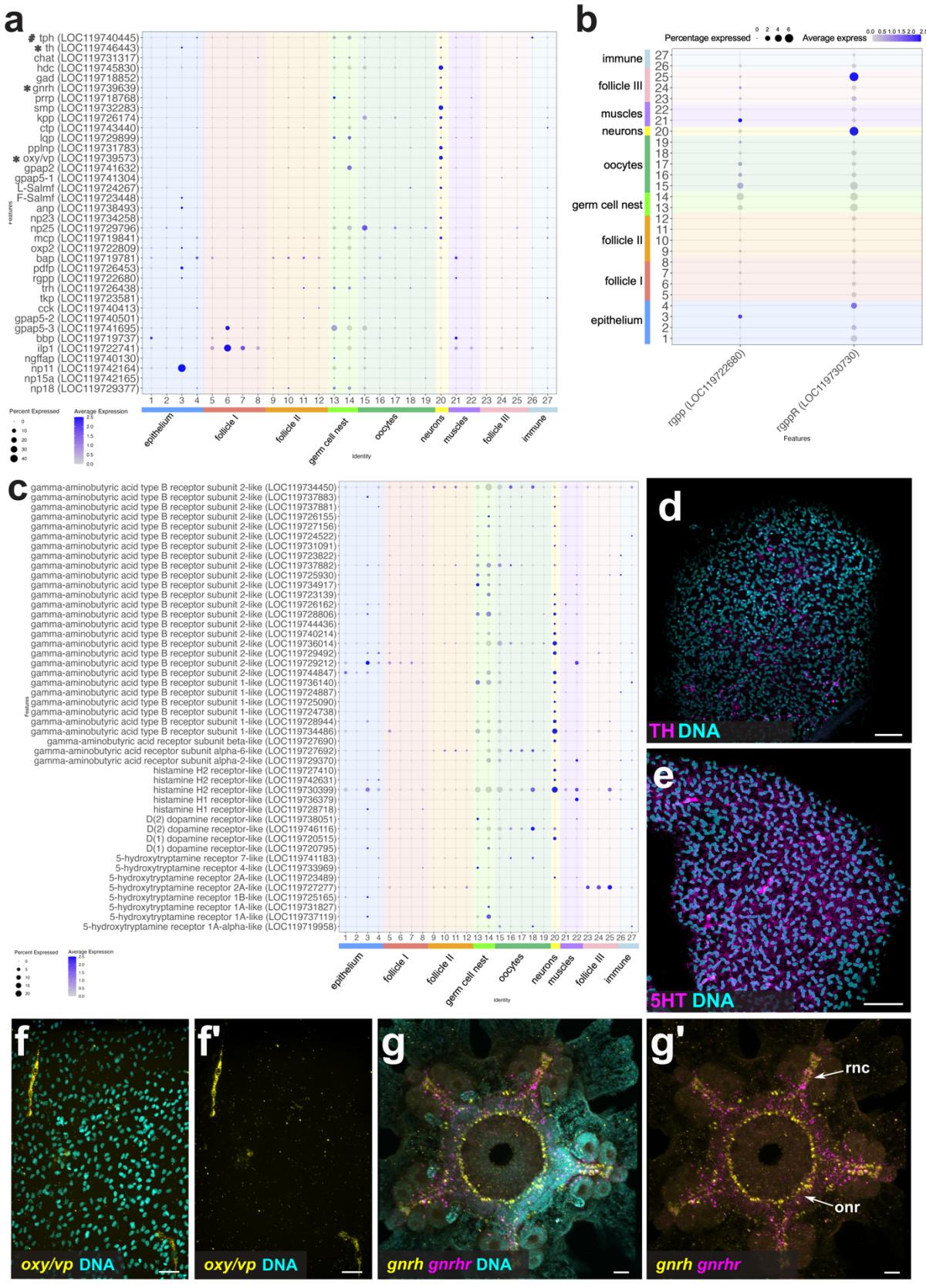

a
tph (LOC119740445)
th (LOC119746443)
chat (LOC119731317)
hdc (LOC119745830)
gad (LOC119718852)
gnrh (LOC119739639)
prrp (LOC119718768)
smp (LOC119732283)
kpp (LOC119726174)
ctp (LOC119743440)
lqp (LOC119729899)
pplnp (LOC119731783)
oxy/vp (LOC119739573)
gpap2 (LOC119741632)
gpap5-1 (LOC119741304)
L-Salmf (LOC119724267)
F-Salmf (LOC119723448)
anp (LOC119738493)
np23 (LOC119734258)
np25 (LOC119729796)
mcp (LOC119719841)
oxp2 (LOC119722809)
bap (LOC119719781)
pdfp (LOC119726453)
rgpp (LOC119722680)
trh (LOC119726438)
tkp (LOC119723581)
cck (LOC119740413)
gpap5-2 (LOC119740501)
gpap5-3 (LOC119741695)
bbp (LOC119719737)
ilp1 (LOC119722741)
ngffap (LOC119740130)
np11 (LOC119742164)
np15a (LOC119742165)
np18 (LOC119729377)
Features
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
epithelium
follicle I
follicle II
germ cell nest
oocytes
neurons
muscles
follicle III
immune
Identity
Percent Expressed
Average Expression
b
Percentage expressed
Average express
immune
follicle III
muscles
neurons
oocytes
germ cell nest
follicle II
follicle I
epithelium
rgpp (LOC119722680)
rgppR (LOC119730730)
Features
c
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119734450)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119737883)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119737881)
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gamma-aminobutyric acid type B receptor subunit 2-like (LOC119734917)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119723139)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119726162)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119728806)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119744436)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119740214)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119736014)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119729492)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119729212)
gamma-aminobutyric acid type B receptor subunit 2-like (LOC119744847)
gamma-aminobutyric acid type B receptor subunit 1-like (LOC119736140)
gamma-aminobutyric acid type B receptor subunit 1-like (LOC119724887)
gamma-aminobutyric acid type B receptor subunit 1-like (LOC119725090)
gamma-aminobutyric acid type B receptor subunit 1-like (LOC119724738)
gamma-aminobutyric acid type B receptor subunit 1-like (LOC119728944)
gamma-aminobutyric acid type B receptor subunit 1-like (LOC119734486)
gamma-aminobutyric acid receptor subunit beta-like (LOC119727690)
gamma-aminobutyric acid receptor subunit alpha-6-like (LOC119727692)
gamma-aminobutyric acid receptor subunit alpha-2-like (LOC119729370)
histamine H2 receptor-like (LOC119727410)
histamine H2 receptor-like (LOC119742631)
histamine H2 receptor-like (LOC119730399)
histamine H1 receptor-like (LOC119736379)
histamine H1 receptor-like (LOC119728718)
D(2) dopamine receptor-like (LOC119738051)
D(2) dopamine receptor-like (LOC119746116)
D(1) dopamine receptor-like (LOC119720515)
D(1) dopamine receptor-like (LOC119720795)
5-hydroxytryptamine receptor 7-like (LOC119741183)
5-hydroxytryptamine receptor 4-like (LOC119733969)
5-hydroxytryptamine receptor 2A-like (LOC119723489)
5-hydroxytryptamine receptor 2A-like (LOC119727277)
5-hydroxytryptamine receptor 1B-like (LOC119725165)
5-hydroxytryptamine receptor 1A-like (LOC119731827)
5-hydroxytryptamine receptor 1A-like (LOC119737119)
5-hydroxytryptamine receptor 1A-alpha-like (LOC119719958)
Features
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
epithelium
follicle I
follicle II
germ cell nest
oocytes
neurons
muscles
follicle III
immune
Identity
Percent Expressed
Average Expression
d
TH DNA
e
5HT DNA
f
oxy/vp DNA
f'
oxy/vp DNA
g
gnrh gnrhr DNA
g'
rnc
onr
gnrh gnrhr

Figure S9. Neuropeptides and neurotransmitters expressed in the intrinsic ovarian nervous system. (a) Dotplot showing the average expression of known echinoderm neurotransmitter synthesizing enzymes and neuropeptides. **(b)** Dotplot showing the average expression of the relaxin-like gonad-stimulating peptide (*rgpp*) and its receptor *rgppR*. **(c)** Dotplot showing the average expression of sea star neurotransmitter receptors. **(d)** Immunofluorescence of TH shows the presence of subepidermal ovarian dopaminergic neurons. Scale bar is 40 μm. **(e)** Immunofluorescence of 5HT confirms the presence of subepidermal ovarian serotonergic neurons. Scale bar is 40 μm. **(f-f')** HCR for *oxy/vp* neuropeptide marks two *oxy/vp* positive neurons per ovarian lobe. Scale bars are 20 μm. **(g-g')** HCR for *gnrh* and *gnrhr* shows expression in the post-metamorphic *P. miniata* nervous system confirming the specificity of the probes. Scale bars are 50 μm. onr, oral nerve ring; rnc, radial nerve cord. Asterisks are used to indicate genes whose predicted expression pattern has been confirmed with HCR or IHC. The expression patterns can be found within the same figure and Fig.7. Hash symbol is used to indicate the indirect validation of the predicted tph expression profile through 5HT immunostaining. Source data are provided as a Source Data file.