

Supplemental Figures and Tables

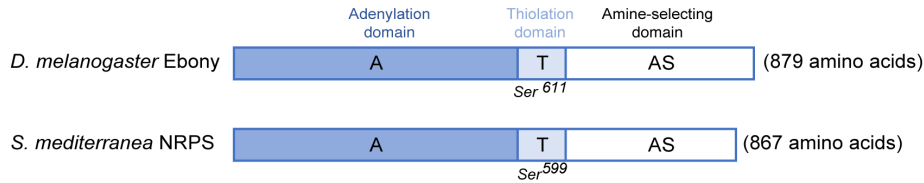
Fig. S1



Fig. S1. Single-cell RNA sequencing of cells from sexual *S. mediterranea*. (A) tSNE plot of 39 clusters. (B) t-SNE plots of representative genes for nine major planarian tissue classes previously characterized in asexual *S. mediterranea* (14). All somatic tissue classes are present except for pharyngeal cells, which are not detected in this sexual sc-seq dataset since we enriched for reproductive tissues lacking this organ. (C) t-SNE plots showing expression of somatic gonadal gene markers *dmd1*, *ophis*, *LamA*, *aadc*, and *nrps*.

Fig. S2

A



B

<i>Dmel-Ebony</i>	1	-----MGSPLQLSTVKGIQDDFYVPRALHRIFFEEQQLRHADKVALIYQPSITGGMAPSQ-----	56
<i>Smed-NRPS</i>	1	---MKVMNQLSNTSHCLYGENDSYEHQNNLATYFEMNCLNDCRDKDNPHI AAIHNDSF-----	57
<i>Sm-NRPS</i>	1	1MPQSTAQLKSPLLHLLLENLTQSSICTSTA IWHVNPVNFVFCVHNHNSFDNKNNSVTTITITDVTSTNNHKNNTYDYEQQEQWNS E E I NSNQESNEI Y TMT	100
<i>Dmel-Ebony</i>	57	YRQMRNERANRAARLLVAETHGRF-----LQPNSDGDFIVAVCMQPSSEGLVTTLLAIWRKAGAYLFI D P S F P A N R T H H I L L E A K	134
<i>Smed-NRPS</i>	58	Y S M L N T K A N I V A K N L I R S C N L E I -----P M N E C L V G W L D E G F E R L Y S I M A C L K L G L V F V P L A K N R N S D L L K R I D K C N	131
<i>Sm-NRPS</i>	101	F L K L N N A A N R V A M N L A N Y L E R K W S S I T N K I N R T Q L N Q H S L S D E P I E L R N Q S E T V I A L F M P P G I D R I I V V G I A C M K L H A Y M P L D R N V P A G R I T O L H K L K	200
<i>Dmel-Ebony</i>	135	P T L V I R D D D -----I D A G R F Q G -----T P T L S T T E L Y A K S L Q L A G S N L L S E M L -----R G	180
<i>Smed-NRPS</i>	132	L S I I I H K K C -----S D I D L L S N -----D N S I Q I T V L M D T L D E S K I T D S F N L P R E Y N P -----P O	181
<i>Sm-NRPS</i>	201	P I L I L I D K D Y Y D F I Y D D D H N D N D K M S D L S S S I D N N N K S L S R K L S S N D F I I G N L N Q L K L T F Q L F D V K V Y E Y I K L M K L S K Y Y S R S D I Y T A S I P I R V C L F P F	300
<i>Dmel-Ebony</i>	181	G N D H I A I V L Y T S G S T G V P K G V R L P H E S I L N R L Q W W -----A T F P Y T A N E A V S V F -----K T A L T F V D S I A E L W P G M C G L A I L V V P K A V T K -----	262
<i>Smed-NRPS</i>	182	S R E K T V V L H T S G S S G T P K T I K I T S A O L F N R L F W Q W -----R N L P F K S N E I V G H -----R G F M F V D N L V E C L S C I L S T V T M V I V S P T E A G -----	262
<i>Sm-NRPS</i>	301	E S D P I V L V L F T S G S T S S G P K V K L R T T Q L F N R L E W Q W S T S D M D L P N F E A T N S N T S V K R I G L A K T A W G F V D A F T E L F S C L L A G I P V V Y R G G S A G P S E	399
<i>Dmel-Ebony</i>	263	-----D P O R I V A L L E R Y K I R R L V L V P T L R S L M Y L K M E G G A A Q K L Y N I Q I W V C S G E P L S V S L A S S F D O Y F D E G V H R L Y N F Y G S T E V L G D V T Y F A C E S K	358
<i>Smed-NRPS</i>	263	-----D I V K L A E V I Q K Y S V S W I L T V P S L L Q K W L K O L -----D N Y S D I L A L S S L S T V V S S G E M L F P S L A K K C L A T F N R N S C K L V N L Y G S T E V C G D V C C Q T L Y S I	356
<i>Sm-NRPS</i>	400	K S I T V G Q L I N L T K H F K I S H I T T V P Q M N L W L K Q L R K P E I V T S H L S S L R T V I V S G D I V H R K M A C E F Q L E K N P E M R L I N L Y G T T E V A G D V T G L V F R G E	499
<i>Dmel-Ebony</i>	359	K O L S L Y D N V -----P I G I P L S N T V Y Y L L D A D -----P I G I P L S N T V Y Y L L D A D -----Y R P V K N G E I G E I F A S G L N	402
<i>Smed-NRPS</i>	357	N D V R M N S K N -----K F L S V G T P I S N N Q V F E S N S D N E -----K F L S V G T P I S N N Q V F E S N S D N E -----B E V I V I G K N	397
<i>Sm-NRPS</i>	500	I D V K K H T K V V P C G L E R E N N K S G K P V L S V G T V I Q G T A I E I V Q D D D H H E H E K D N E N Q P D K W S N P S L S I I G S V D R K P N W D K P F K I L P K G H I G H V C I G Q Q	599
<i>Dmel-Ebony</i>	403	L A A G Y V N G R D P E R F L E N F L A V E -----K K Y A R L Y R T G D V S L S -----K N G S I M Y E B R T D S Q V K I R G H R V D L S E V K N V A E L P -----	474
<i>Smed-NRPS</i>	398	V S -----E T G -----E T G -----G L A F O T G D V G F I I -----A D K K L F I G R I I D D M V K V G K K I F T K B I S T A M V H S -----D V D N C	454
<i>Sm-NRPS</i>	600	V S D S A S R C Q R I E S L P E D L N C V D T N K C K S D V E S C E N N S S K E I R V F M P Q D L G F I D P Q T N H L Y C G R T N E L I K I N G I R F H A N D I D N L F I E L K N W K A K M N T N C	699
<i>Dmel-Ebony</i>	475	-----L V D K A I -----V L C Y H A G Q V D Q A I L A F V K L R D D A P M V T E M Q M E ---A R L K D K L A D Y M T P Q V V I L E H V P L L V N G K V D R Q A L L	547
<i>Smed-NRPS</i>	455	Y T I Q L I I S G R P Q -----L V S F F T T K T E I G N K T K -----T K M E I N -----N I L M N H S L N V C L P R L E Y I K S I P I Q P O S M K P D K K M L	523
<i>Sm-NRPS</i>	700	T R E E L L V N K V S E T V T L T I Q T V H G R D L K L V C F Y V L H M N E N Q N T M N I E P K E N Y D K L E D L P K Q D D F I A V F S H Y L P P Y L S R T F I N I D H I P L M R T S G K V K E Y R	799
<i>Dmel-Ebony</i>	548	K T Y E T A N N E -----G D S S I V L D F D Y S Q -----V P E D L K L T -----A R D L F E T V G G V I G R S T R A T L A F -----H S N F Y E L G	608
<i>Smed-NRPS</i>	524	-----C K I A K -----K I L S E N R M K T L T H G K H L L S S Q T L D L T D G L I D D I N S Q K H Y I Y E I L A K -----H L S L L D E I D D S M K F Y D I G	596
<i>Sm-NRPS</i>	800	Q Y Y S K H H C E I S E I T K V L Q P G W N D P V K H M T E N N S T S D Q S F K -----N S R D F K L S R G R -----E R A R K V L A E V L G I ---R G P N G D V I G R P K D D E D F Y L L G	889
<i>Dmel-Ebony</i>	609	G N S I N S I F T V T L R E K G Y N I G I S E F I A A K N G E I I E K M A A N H -----D A V O L E E E S L N A -----C P H L K M E	669
<i>Smed-NRPS</i>	597	G S L T I V I C I A D L N R K G F S C T T E V F H N S I G E L V E C I L E G S N S K I R N N -----S D Y L F K Y D M K N -----D F T	660
<i>Sm-NRPS</i>	890	G S L L T V L T E Q L R Q L G N V N L D V F E T K T G I G S I L T L S Q N T E S D F L K T Q E P F T S S W T V K E I S M N K V L K K S H T O N L I N R I P L M E D E C Y L S P T I C P Q G S Y E	989
<i>Dmel-Ebony</i>	670	A V P L R L E H -----R O E V D I I V A S F Y N K A D E Q W L K P G V L R T D Y S D I N D I W N V L V E R D L S F V V -----Y D T N D T R I G E T A L -----N F D A R N	747
<i>Smed-NRPS</i>	661	V E Q I O Y E N -----K T E I I E F L V E N F Y T K E L V V R Y L N -----T P Y K V F E V V S E I F O L S L R S G C S F C I -----R N S I S K S I V G L Q L S E D S S Y E I P N	740
<i>Sm-NRPS</i>	990	I F I E W N D G N F S V T E R H E I V D V L V N A F I E K O R L S H A L L -----L D R T O L T E A I -----E V E L N A H K N N P G I V L T A R Y Y Y E N P Y E H T F V N K L V G V I I -----S L P A K H	1082
<i>Dmel-Ebony</i>	748	E P E V D I K S K L L I V F E L F E C E G P I R B N Y L P K G L N Q I H S F M M G T A E K L N -----P R E N I A C M H F M E H E V L R V R E K Q F A G I F T N T S P L Q	833
<i>Smed-NRPS</i>	741	T S Q L Y K L D E E N S L D C F L Q N K P R I A E F L K T P K L N -----V S V V A L S G T L N -----K S I V I Q L Y E I E K H T I D L Q C K N K T I E I I N T S E A I K	822
<i>Sm-NRPS</i>	1083	V S L H L T P K L A L V Q R E F D E C S N K ---Q Q F D I S M D N L A T O M V A I T S Q S P Y S K S Y Q Y M L S N W K K I S L K L L T R L E R D L R I A K A G Y S E V I F F N T S E A T K	1180
<i>Dmel-Ebony</i>	834	Q L A D V Y H Y K T L L N F Q V N E Y V H S D G S R P F G D A P D E Q R A I V H W K E V G K -----	879
<i>Smed-NRPS</i>	823	K I C S E L N V R L I K S T S M S H F L N E S Q C Q Y L R S L S M S D V Y G H Y M V L D L -----	867
<i>Sm-NRPS</i>	1181	E V C S Q L G Y K V I Q T I M L K S F M N K E N -----L L L P Q Y E R I R C S X M I K E L I N P S S	1227

Fig. S2. *Smed-nrps* encodes a non-ribosomal peptide synthetase. (A) Schematic showing adenylation (A), thiolation (T), and amine-selecting (AS) domains in *D. melanogaster* Ebony and the *Schmidtea mediterranea* homolog NRPS. *Drosophila* and *S. mediterranea* share a conserved serine residue in their thiolation domains. NRPS proteins like Ebony can conjugate β -alanine to various biogenic amines (e.g., dopamine, histamine, etc.). This enzymatic process involves three steps: adenylation of β -alanine catalyzed by the adenylation (A) domain; covalent attachment of β -alanine to a phosphopantetheinyl group on a conserved serine within the thiolation (T) domain; and binding of an amine in the amine-selecting (AS) domain, which facilitates nucleophilic attack of the NRPS-bound β -alanine resulting in a β -alanyl-amine dipeptide product. **(B)** Protein alignment of *Drosophila* Ebony, *S. mediterranea* NRPS, and *S. mansoni* NRPS. The serine thiolation site (marked by an asterisk) in the T domain (delineated by red lines) is conserved.

Fig. S3

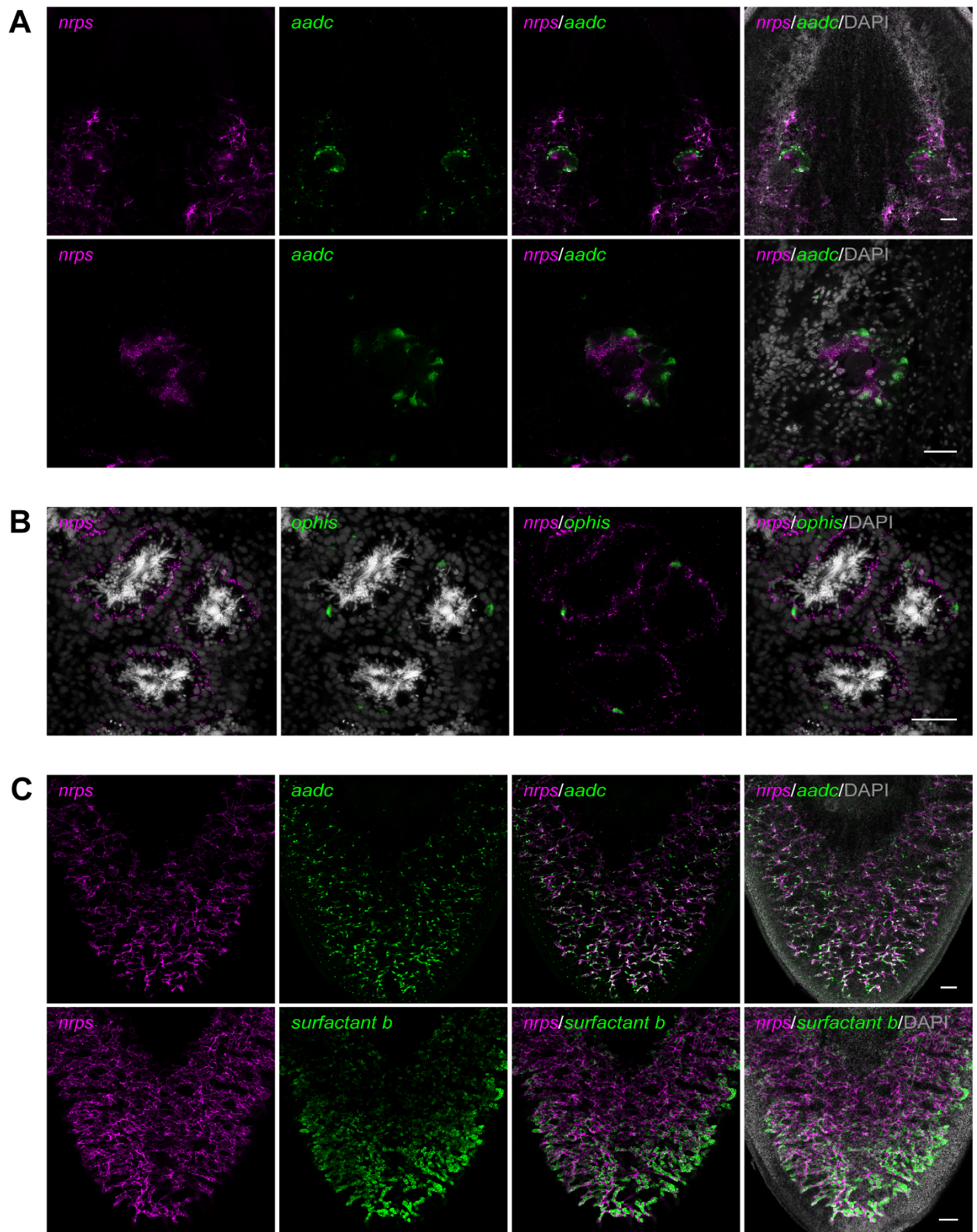
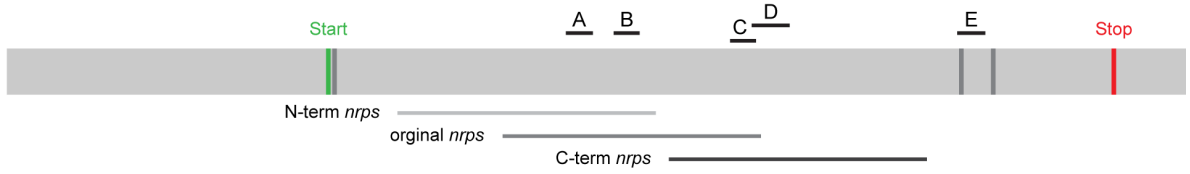


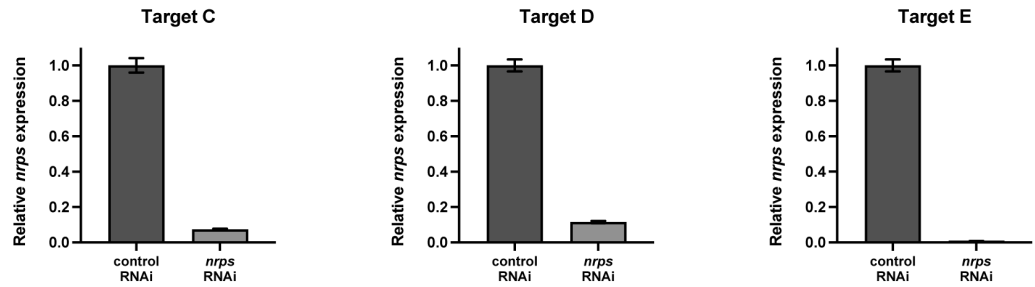
Fig. S3. *nrps* is expressed in somatic gonadal niche cells. (A) Projection of ventral head region (top) and confocal section of ovary (bottom) showing dFISH of *nrps* (magenta) and *aadc* (green). (B) Confocal section of testes with *nrps* (magenta; cytoplasmic localization) and *ophis* (green; nuclear) co-expressing cells. *ophis* RNA localizes mainly to the nucleus of somatic gonadal cells, which extend long *nrps*⁺ cytoplasmic projections that encyst developing germ cells. (C) Projections of confocal sections showing dFISH of *nrps* (magenta) with *aadc* (green; top), or yolk cell marker *surfactant b* (green; bottom) in the ventral posterior region of sexually mature planarians. Nuclei are counterstained with DAPI (gray; A-C). Scale bars, 100 μ m (A, top; C), 50 μ m (A, bottom; B).

Fig. S4

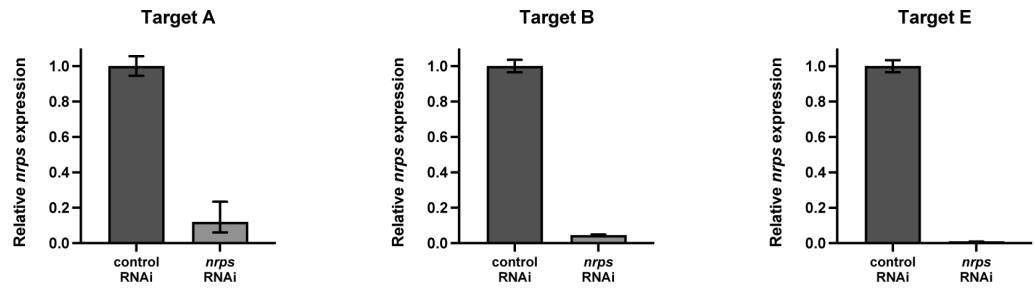
A *nrps* (SMEST023215002.1):



B *nrps* RNAi (N-term):



nrps RNAi (C-term):



nrps RNAi (original):

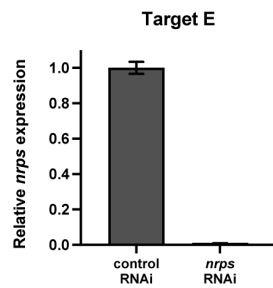


Fig. S4. Testing *nrps* RNAi specificity and quantifying *nrps* expression in knockdown animals. (A) To test for *nrps* RNAi specificity and exclude the possibility of off-target effects, RNAi was performed with dsRNA targeting three ~1 kb regions of *nrps*: the original region used throughout this study, and 2 non-overlapping regions (N-terminus vs C-terminus). *nrps* gene (gray bar) is shown with positions for start (green) and stop (red) codons, exon-exon boundaries (dark gray), cloned regions (bottom), and qPCR amplicons (top: A-E). (B) qPCR analysis of *nrps* mRNA expression normalized to β -*tubulin* in control and *nrps* RNAi animals depicting efficient knockdown of *nrps* after RNAi. Top: dsRNA targeting the N-terminus of *nrps* was used for RNAi-mediated knockdown of *nrps*, and qPCR primers targeting regions C, D, and E were used to quantify *nrps* expression levels. Middle: dsRNA targeting the C-terminus of *nrps* was used for RNAi and qPCR primers targeting regions A, B, and E were used to quantify *nrps* expression levels. Bottom: dsRNA targeting the original cloned amplicon of *nrps* and qPCR primers targeting region E were used to quantify *nrps* expression levels. N = 4 biological replicates (3 technical replicates each). Bar graphs depict relative quantification ($2^{-\Delta\Delta Ct}$) values normalized to control RNAi with 95% confidence intervals.

Fig. S5

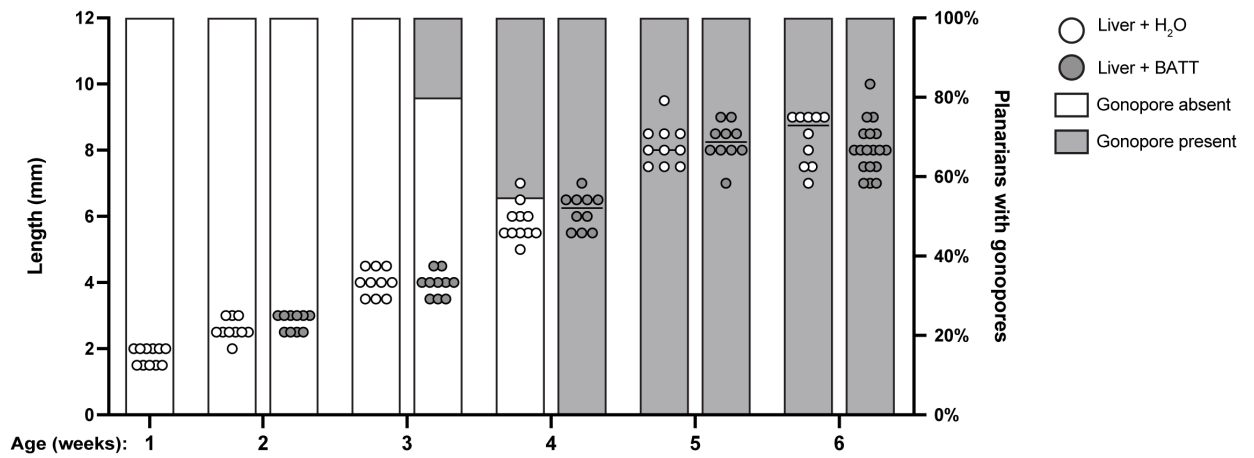


Fig. S5. BATT triggers sexual maturation. Quantification of sexual planarian length (mm; left Y axis; horizontal line represents median) and gonopore presence (right Y axis) during development. One-week old hatchlings were fed liver +/-BATT for 6 weeks. Supplementation with BATT did not affect growth but triggered precocious sexual maturation (evidenced by the presence of a gonopore) in +BATT individuals. n=10-18 planarians per time point.

Table S1. Information for transcripts mentioned in this paper.

Gene	Reference	Sequence information
<i>nrps</i>	This paper	Planmine SMEST.1: SMEST023215002.1
<i>nrps (gut)</i>	This paper	Planmine SMEST.1: SMESG000017098.1
<i>klf4l</i>	Issigonis et al., 2022	Planmine SMEST.1: SMEST031008001.1
<i>Laminin A</i>	Issigonis et al., 2022	Planmine SMEST.1: SMEST056013009.1
<i>delta3</i>	Khan et al., 2022	Genbank accession: OL957299
<i>nanos</i>	Wang et al., 2007	Genbank accession: EF035555.1
<i>dmd1</i>	Chong et al., 2013	Genbank accession: KC736555.1
<i>ophis</i>	Saberi et al., 2016	Genbank accession: KX018822.1
<i>surfactant B</i>	Rouhana et al., 2017	Genbank accession: KY847536.1
<i>tph</i>	Curie & Pearson, 2013	Genbank accession: KF134114.1
<i>aadc</i>	Curie & Pearson, 2013	Planmine SMEST.1: SMEST022173002.1

Table S2. Primer sequences.

Cloning into pJC53.2	Forward	Reverse
<i>nrps</i>	TCGTTTGCCACAGAACAGGA	CGATTAGGCCGTCGGTTAGG
<i>nrps (gut)</i>	CCGATTTTGGCAGCTTCTGG	CTCAGCCACCCATTTTCGTCT
<i>nrps (N-term)</i>	TGGATGAAGGCTTTGAGAGG	GATTCGGCCGCAAATAAATA
<i>nrps (C-term)</i>	ACAGCCGTTATGGTCCACTC	ACCGACACGTTTAGCTTTGG
qPCR	Forward	Reverse
<i>β-tubulin</i>	TGGCTGCTTGTGATCCAAGA	AAATTGCCGCAACAGTCAAATA
Target A	TCGTAAGCAGCGGTGAAAT	CTTCCGTTGAGCCGTAAAGA
Target B	GCACACCCATTTGGAACAATC	CAGTCCACAAGTCGGTGATAC
Target C	TGCTCTGTAAGATTGCGAAGA	GTCTAGAGTGGTTTGGGATGAG
Target D	CCCAAACCACTCTAGACCTAAC	GAGTCACCACCAATATCGTAGAA
Target E	ACGTCTGAAGCAACGAAGAA	GTGAGCGCAAATATTGACATTGA