Supplemental Figures and Tables

Fig. S1

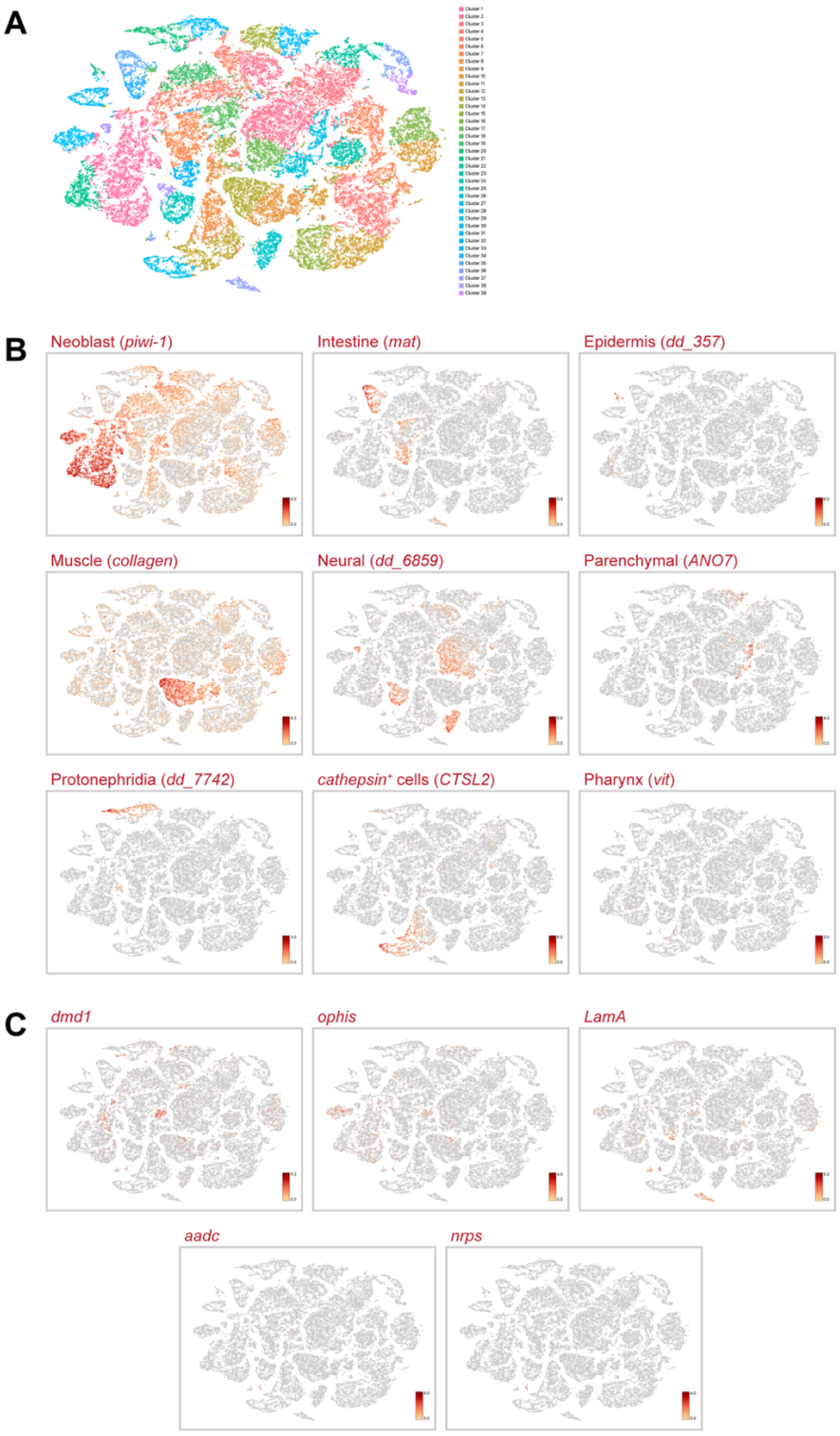


Fig. S1. Single-cell RNA sequencing of cells from sexual *S. mediterranea*. (**A**) tSNE plot of 39 clusters. (**B**) t-SNE plots of representative genes for nine major planarian tissue classes previously characterized in asexual *S. mediterranea* (14). All somatic tissue classes are present except for 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

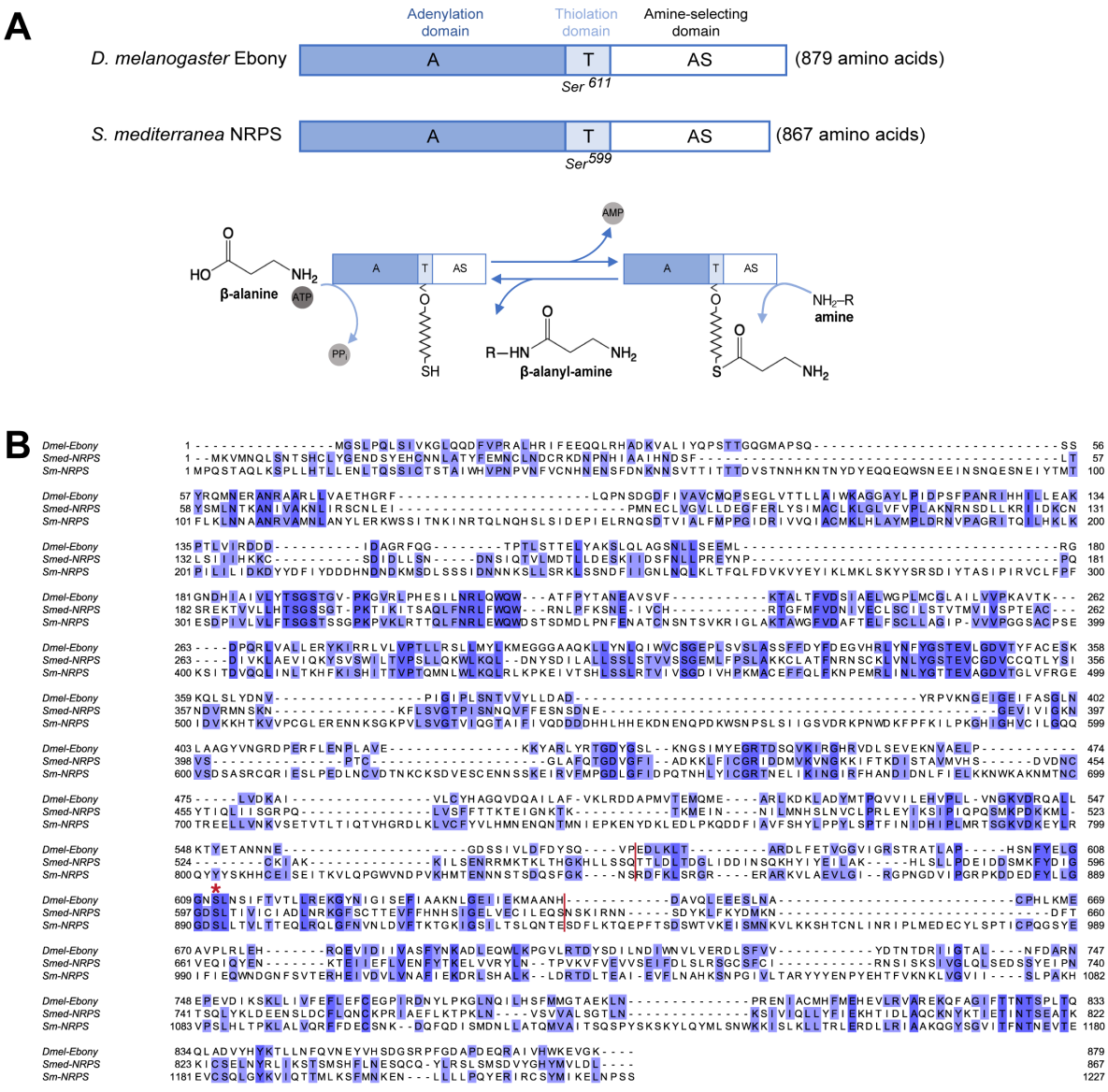


Fig. S2. *Smed-nrps* encodes a non-ribosomal peptide synthetase. (**A**) Schematic showing adenylation (A), thiolation (T), and amine-selecting (AS) domains in *D. melanogaster* Ebony and the *Schmidtea mediterranea* homolog NRPS. *Drosophila* and *S. mediterranea* share a conserved serine residue in 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

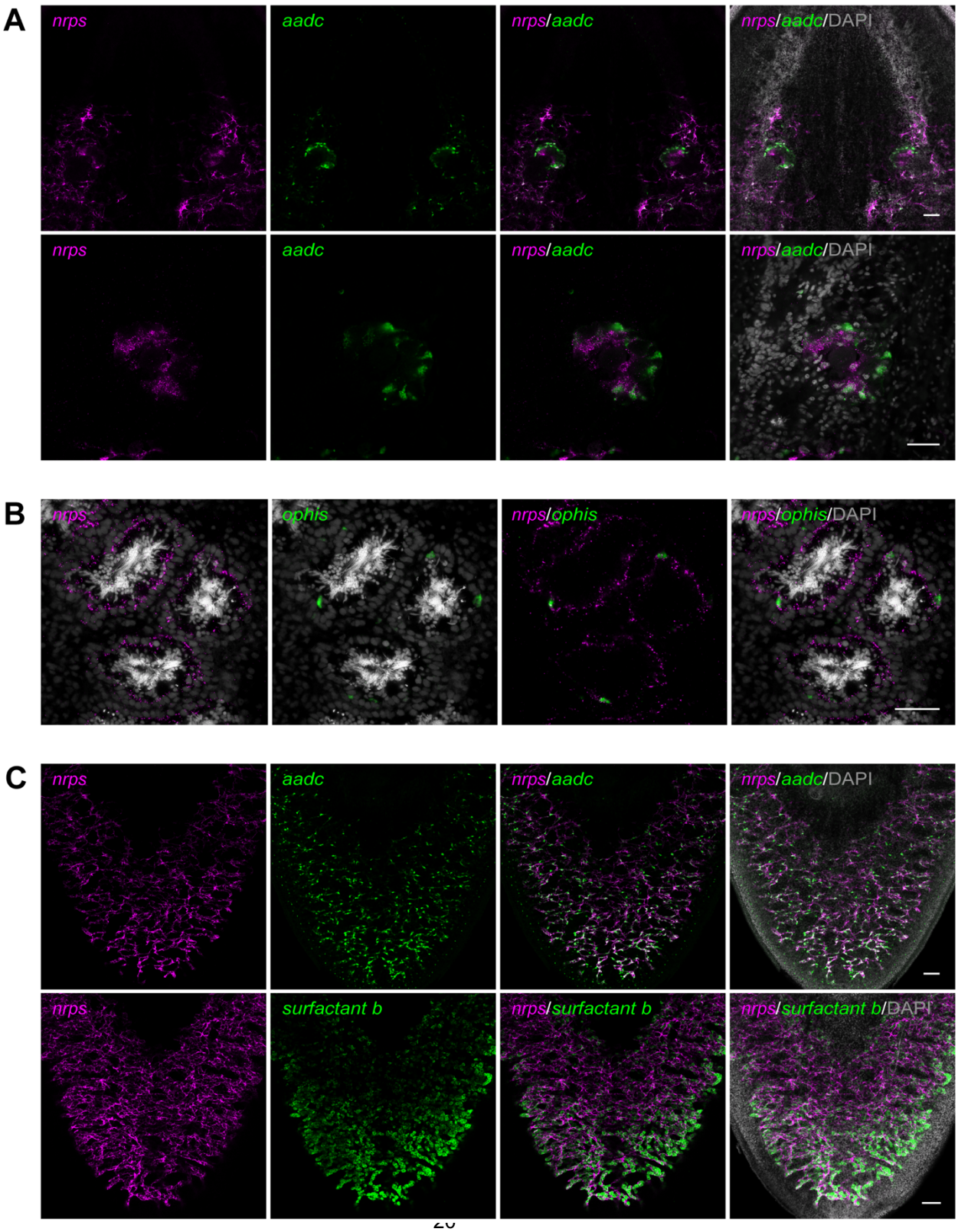
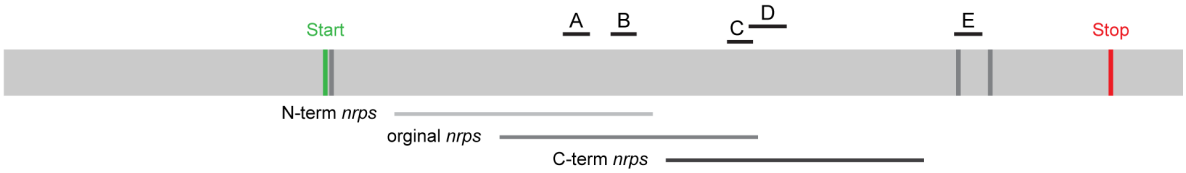

A
nrps
aadc
nrps/aadc
nrps/aadc/DAPI
nrps
aadc
nrps/aadc
nrps/aadc/DAPI
B
nrps
ophis
nrps/ophis
nrps/ophis/DAPI
C
nrps
aadc
nrps/aadc
nrps/aadc/DAPI
nrps
surfactant b
nrps/surfactant b
nrps/surfactant b/DAPI

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 μm (A, top; C), 50 μm (A, bottom; B).

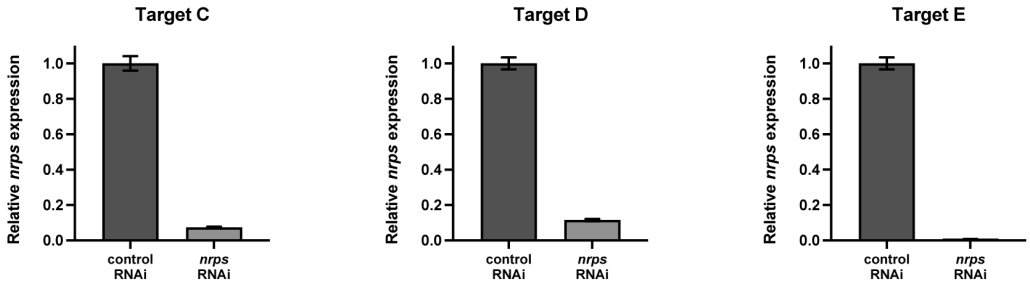
Fig. S4

A *nrps* (SMEST023215002.1):

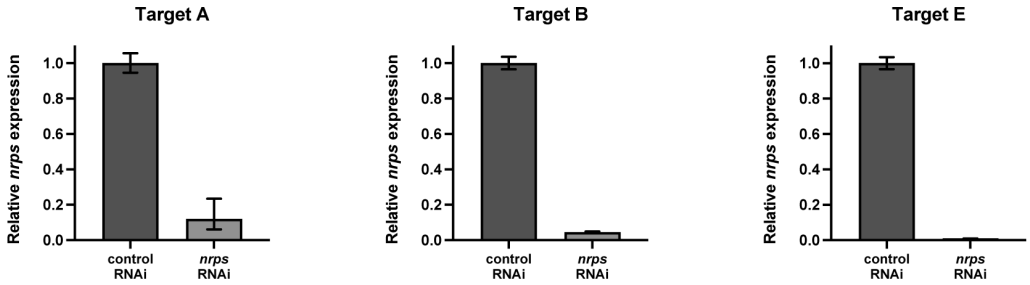


B

***nrps* RNAi (N-term):**



***nrps* RNAi (C-term):**



***nrps* RNAi (original):**

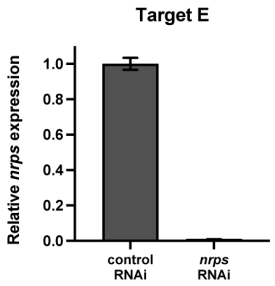


Fig. S4. Testing *nrps* RNAi specificity and quantifying *nrps* expression in knockdown animals. (**A**) To test for *nrps* RNAi specificity and exclude the possibility of off-target effects, RNAi was performed with dsRNA targeting three ~1 kb regions of *nrps*: the original region used throughout this 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

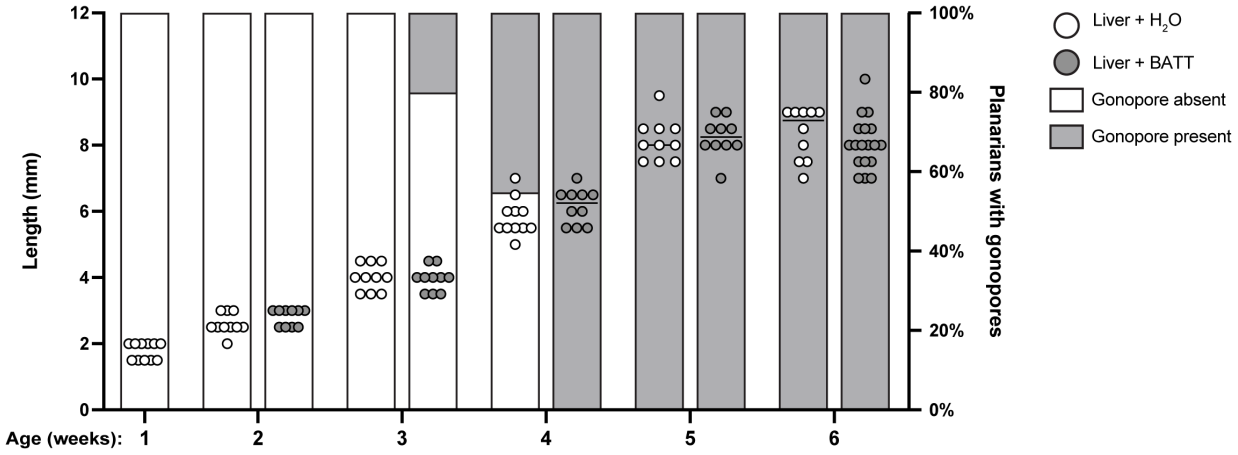


Fig. S5. BATT triggers sexual maturation. Quantification of sexual planarian length (mm; left Y axis; horizontal line represents median) and gonopore presence (right Y axis) during development. One-week old hatchlings were fed liver +/–BATT for 6 weeks. Supplementation with BATT did not affect growth 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
nrps	This paper	Planmine SMEST.1: SMEST023215002.1
***nrps* (gut)**	This paper	Planmine SMEST.1: SMESG000017098.1
klf4l	Issigonis et al., 2022	Planmine SMEST.1: SMEST031008001.1
Laminin A	Issigonis et al., 2022	Planmine SMEST.1: SMEST056013009.1
delta3	Khan et al., 2022	Genbank accession: OL957299
nanos	Wang et al., 2007	Genbank accession: EF035555.1
dmd1	Chong et al., 2013	Genbank accession: KC736555.1
ophis	Saberi et al., 2016	Genbank accession: KX018822.1
surfactant B	Rouhana et al., 2017	Genbank accession: KY847536.1
tph	Curie & Pearson, 2013	Genbank accession: KF134114.1
aadc	Curie & Pearson, 2013	Planmine SMEST.1: SMEST022173002.1

Table S2. Primer sequences.

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