SUPPLEMENTARY MATERIAL

corresponding to:

Diverse miRNA spatial expression patterns suggest important roles in homeostasis and regeneration in planarians
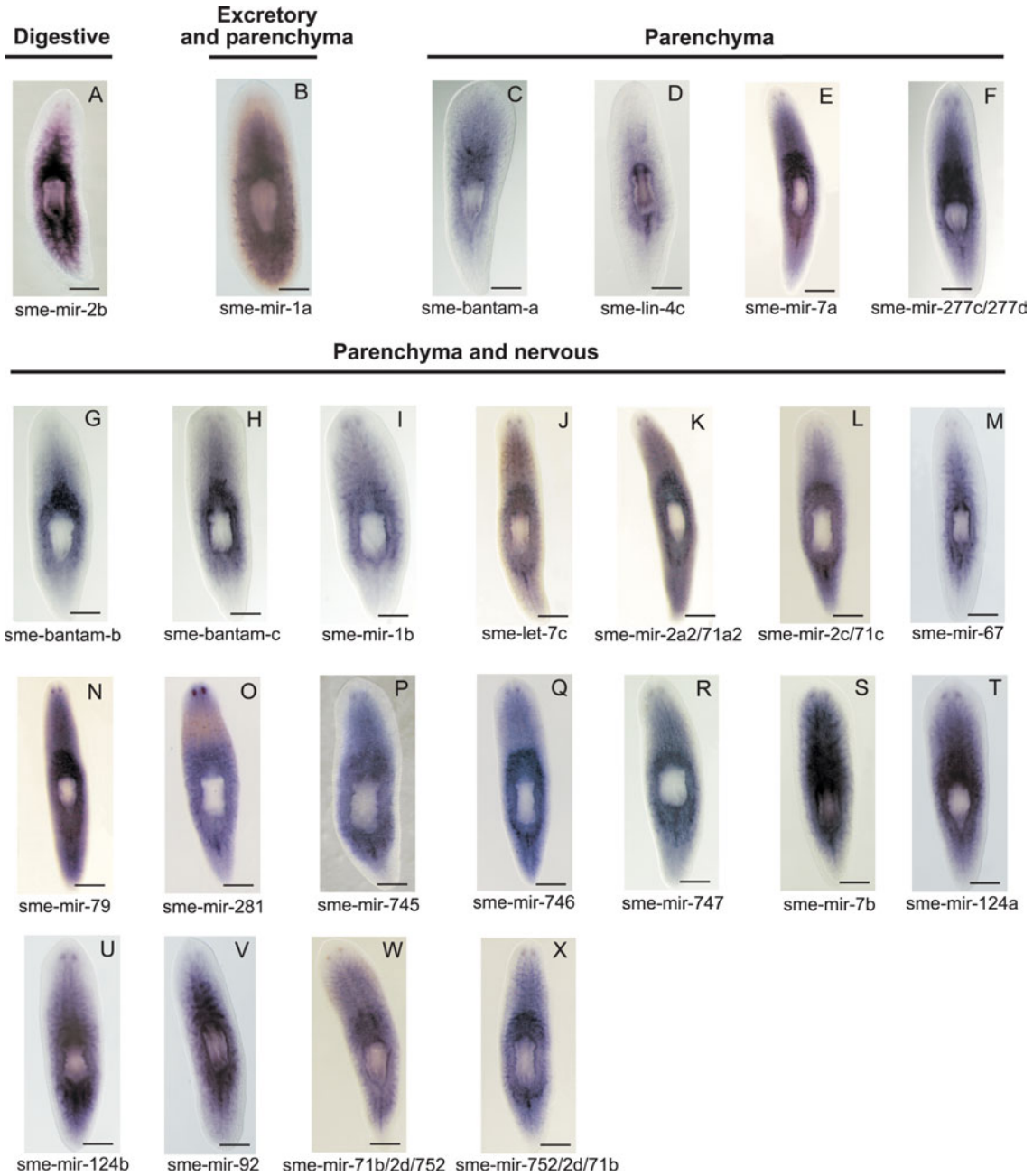
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and A. AZIZ ABOOBAKER*

SUPPLEMENTARY TABLE

OLIGOS USED FOR AMPLIFYING PROBE TEMPLATES FROM GENOMIC DNA

| miRNA	Forward Oligo (5'-3')	Reverse Oligo (5'-3')	Product Length (bp)
sme-bantam-a	CCAATGACACCAAACACCAATCGAT	TGTTGCCCACTGTCATTTTACCAGC	423
sme-bantam-b	TGTGTGCAATGAGTCATTCAATGCG	TTGACGTAAGGATCGCCCTGATTTT	523
sme-bantam-c	CAGACAGTACAATATGATTGTG	GTACGACACAATTCACATTCAG	503
sme-let-7a	TTTTCTAGCCATTGTGAACAGTT	ATCTCCAAAATTAACCAATTCTGAA	541
sme-let-7b	TATAACGAGACAAAGAAATGCACTC	CCTCAAATATCAAAGTAGAGCAATT	501
sme-let-7c	CAAAACATGCAACGTGGAATCTA	AACAACATTTGCAGATAGCTTAAA	622
sme-lin-4a	TTGAATGCAATCCAATAAACATG	TGAAAACACCACTAACAACCAAT	457
sme-lin-4b	ACTAGGTCAAGTTAGTGAACTGCCTCAG	GGAAATTCGCTGATAAAGTCAACACA	503
sme-lin-4c	CCAATCAAATCGACTGTTAGCCAG	CCTTGCACTTGAAGGAAATTTGCC	487
sme-mir-1a	TCAGTATGCCAGTTTGTGATTCG	ATAAATTGCTATTCGGAACGTTG	405
sme-mir-1b	TGTGCACAATAACTATTATAACC	CTTATGATTAACTTGCTAAATAA	458
sme-mir-1c	CTGTGCCTGGATGATGAGTGCTA	CATCTATCTATCCAACGAGATCCC	444
sme-mir-2a-1	AAGTGCTATTAGTGAAATAAATT	TAAATTAAAATACTTTAAACTGATA	451
sme-mir-2a-2	TGGTCATTTCTTTGCCTAGATTT	AAAATGCCTTGCTCAAATTGAAT	454
sme-mir-2b	GGCAATTTAATTTCAAAAAATTTG	TGGCATAAACATTGAAATGATAAA	476
sme-mir-2c	GCCTTAGTAATTATTTCATTTCAAGG	AATTTCCACATATTCCGAAGATTT	470
sme-mir-2d	TATTCTTTTTCGAAATTTTCGCTG	TATATAATTATCATATCACAGCCGCC	492
sme-mir-7a	TAGTAATATTACCATACAGTTTATTTC	ATAACTTACTGCTTTAAGAAGAAA	510
sme-mir-7b	GGTCGATTGGTCGGCAAATGAGCT	CAAGGGGCAGCAAGGAACAGTAAA	502
sme-mir-7c	GTTGTTGTTGTTGCTGCTGTTGTTGTT	GCAGGGAAGTTTCGATTCAGTTTTTCA	613
sme-mir-8	ACCCTCAATTCTTCAGTTACGTGGCC	AAGCCCACAAATGTTGTCGTTTCAC	538
sme-mir-10	AGAGAGATCCAGGCGGCATGCGTA	GCCTGAGAAGAAGCTCCATTGATCC	671
sme-mir-12	ATAATAATTGTGGTAGTTAAACGGG	GGCAATTACATCGTGTTTATTCTA	464
sme-mir-13			
sme-mir-31a	CTGAAACTTTTTCTTATCGCCAGTG	GCTTTCTTTTCGATGTGGATGA	645
sme-mir-31b	TTATCAGATGCTGAGCTCACAGA	GTAATTTGTGTGTTGCCCCTAT	550
sme-mir-36	TCATTGTCATTGCAGAAGTGAG	CAGAAACAGTATGGCGTTTTACC	599
sme-mir-61	GCAATTTAAATTGGGCACAAACT	AGTCCAATTATTGCAATGAACTTT	579
sme-mir-67	TCTCATGGACTGCATACGAGA	CCCATTCGATGGAGATTCTAAA	525
sme-mir-71a-1			
sme-mir-71a-2			
sme-mir-71b	ACCACCAATGCTGACTGTGA	CACAGCATCATCACCTCAATAA	684
sme-mir-71c			
sme-mir-79	TAGACAAAGCCGCAGTTTAAATGC	GTTTTCACGGACGATTCATGTA	699
sme-mir-87a	CTCCCTTTAAATTCTATGCTGGTC	CCATTGAAAACAACCGAAGA	673
sme-mir-87b	TGAATTTGCATCTCTTGAATCGGC	GCACACAAACACGAGATGAATGAA	692
sme-mir-92	GCACTTGTCTCAATATCTGAAACC	GAGATTGATTCGCCTTTATGC	556
sme-mir-124a	TGAAGTAAGATATTTATATTCCCTTC	ACCAGGAGACTACTCTGATTATTAT	600
sme-mir-124b	TAAACCATTTAATTCAGCATTCACCGC	TGCTTTCACCATACAAATACCGCATTT	515
sme-mir-124c	ATTTAGCACACATCTTGACAGAGC	TTATAGTTCTGAAAATAGCCTTCG	500
sme-mir-133	TTATTTCAAATTTCAATTCTACGTA	TTTAATACAATTTCAAGAACCAATA	500
sme-mir-184	ATCATAATTAGAAATTATTATTTTT	TATCAATAATAATAATAACAATAAT	550
sme-mir-190a	GCCACCTATTAATGCCGATTAGAGCG	TCCTAAATATAAATTTAAATATAAA	500
sme-mir-190b	CAAATGTGCATGTATTTATAATGGTGA	TATTTTTAAAACAAGCACCGCATTT	519
sme-mir-219	CCTGCTTTTATATTATTATCAACAC	CTATGCTTTTATATGTTTACGGTTA	500
sme-mir-277a	AATCACTGTGAAAATGCATTATCTG	TTTCCATTAATATGATAAGACGATT	609
sme-mir-277b	ACTTGTATTATTGTTGTATTGTTATTG	TATCCGATAGTGCATTTATACATTT	489
sme-mir-277c	ACAGTGCATAATTAATATGATAAAC	TAATTAAAATATATCATCTGATAAA	520
sme-mir-277d			
sme-mir-278	TTTCCTCGCATTAACCGTCTGTTGA	CATTCCGCGTTTCCAGTCATTTGTA	659
sme-mir-281	TTCGGACAAGCTGGCTAAATGAACA	TTGCGCATTTCATAATGTTTTTGCC	561
sme-mir-745	TGGTTTAGTATCGAATCTTACTGTG	CCTAAAAACAACCAGTTCTTGG	537
sme-mir-746	AATTCTATAGACGCATTTGGGAA	AAGGTGACGCTTCTCAAATTAT	444
sme-mir-747	GAATTATTGACCAAGTTTGCCT	TCTCAATTCCAAGTATTCTTCG	542
sme-mir-748	AACAAAATGAAATTAGTTACTC	TAGATTTCAAGATTATTTATAA	298
sme-mir-749	GGCATTATTGCCGTAGGTAGGAACG	AAATTGGCCCCCCACTAGTCAGAA	484
sme-mir-750	TCTGAGAATGAATTCGACGCAAACC	CACATCGCATTTGAAAACATGTTGA	469
sme-mir-751	TTGTATGATATTTTGTCGTGGC	GTGAGGTCATCAAAGACTATCAAAA	536
sme-mir-752	GACATCATTATTTGACTGTCGA	TTTCAGAATGCTCATTGAGTTC	505
sme-mir-753	GGTGGAATTCCCTGATTCAGTTC	ACCAGGAGCAGTGGTACCAGAACCT	611
sme-mir-754			
sme-mir-755			
sme-mir-756	TAATGAATGTTGTTCTCCACTATGC	AAAGAGCGGCCATGTTTATTTT	417
sme-mir-A	GTGGATGTATTCCAAACACATGGCG	AATCAAGGGAAGGGCGACCTCA	456
sme-mir-B	AAAAATGTTATAAATCCCCGGAACC	CGAACCCGGAGTTTTGAAATCA	486
sme-mir-C	TTCCTATCATTCTCAGTTGTCT	CCTAGTGGAAAATCTTAATGTG	485
sme-mir-D			
sme-mir-Ea	CGAACACACAACACCATGAAAACTT	AGCCTTGAACCATCGCTCATTTCA	668
sme-mir-Eb			
sme-mir-F	GATTAAAATTTTGATCTACTGG	ATGGAGTATTGAGTGAACATTG	491
sme-mir-G	ACCGTAGCACACCGCACTATCAAAA	GATGGAAATTTTTGAACGGCTTC	568

The different colours indicate different miRNA clusters with the exception of black colouring which indicates miRNAs excluded from the analyses.

Digestive

sme-mir-2b

Excretory and parenchyma

sme-mir-1a

Parenchyma

sme-bantam-a

sme-lin-4c

sme-mir-7a

sme-mir-277c/277d

Parenchyma and nervous

sme-bantam-b

sme-bantam-c

sme-mir-1b

sme-let-7c

sme-mir-2a2/71a2

sme-mir-2c/71c

sme-mir-67

sme-mir-79

sme-mir-281

sme-mir-745

sme-mir-746

sme-mir-747

sme-mir-7b

sme-mir-124a

sme-mir-124b

sme-mir-92

sme-mir-71b/2d/752
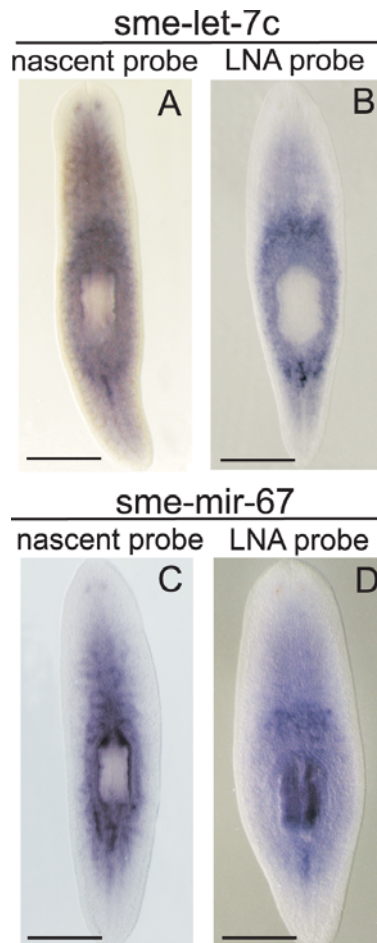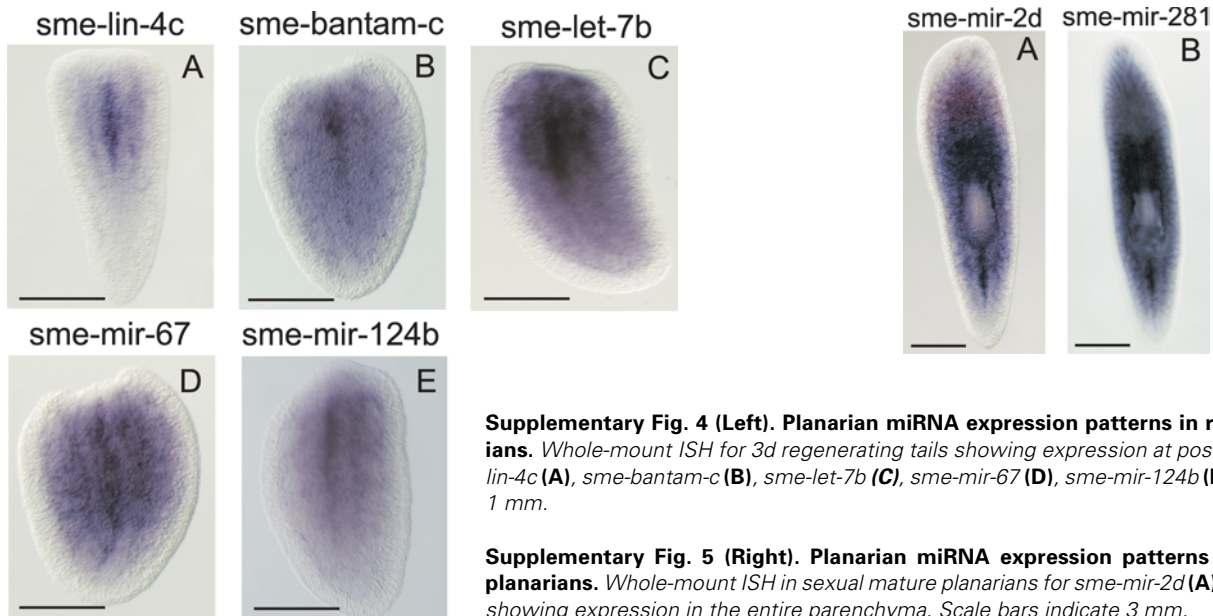

sme-mir-752/2d/71b

Supplementary Fig. 1. Planarian miRNA expression patterns in asexual *S. mediterranea. This figure shows whole-mount ISH for miRNAs that do not appear in Fig. 1. Scale bars indicate 0.7 mm.*
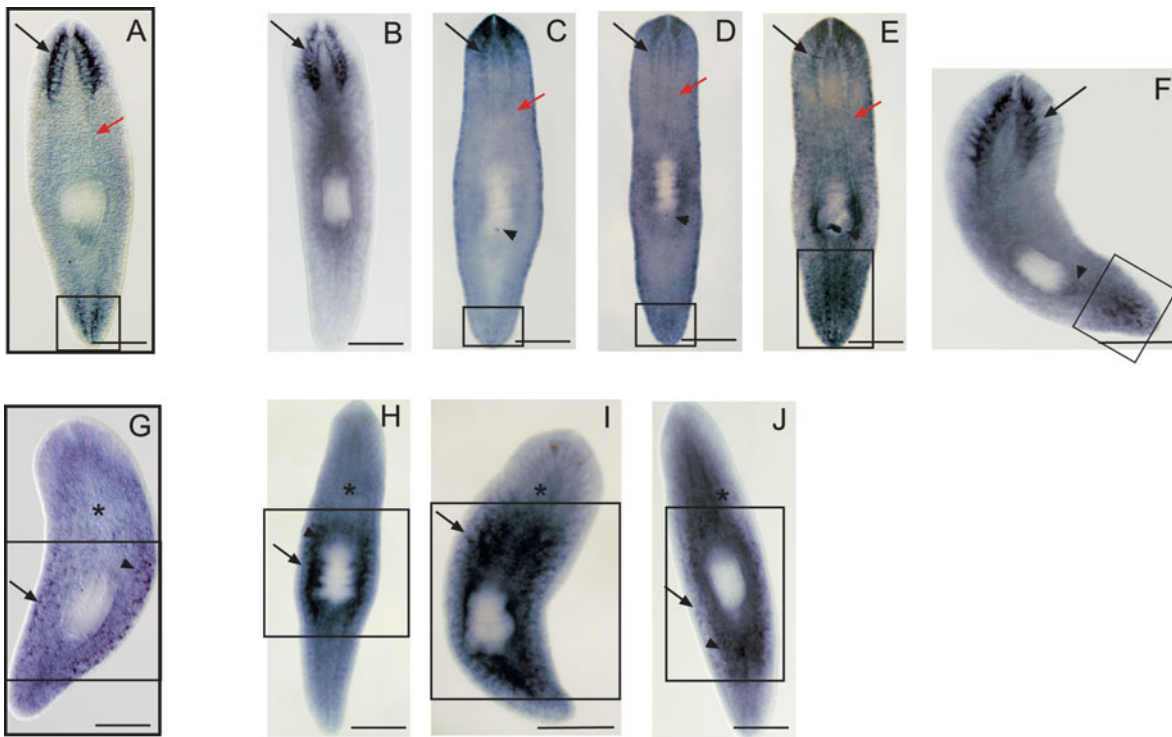
Supplementary Fig. 2. Maximum variation in miRNA expression patterns in asexual *S. mediterranea*. *This figure shows whole-mount ISH for sme-mir-31b and sme-mir-C and how the expression patterns can look somewhat different for a non-planarian expert, however always show a subset of specific features. **(A-F)** sme-mir-31 is classified as expressed in the CNS (around the brain ganglia and nerve cords) and shown in A and Fig.1U. In* in situ *experiments for this miRNA, A is representative 59% of animals while B represents 3% of the cases, C, D and E collectively represent around 20% and F represents around 20%; A shows expression around the brain ganglia (black arrow), nerve cords (red arrow) and posterior enhanced expression (square); B shows only expression around the brain ganglia; C, D and E show expression around the brain ganglia, nerve cords, posterior enhanced expression and in the innervation of the mouth (arrowhead) The differences among these three are based on the intensity of the staining in relation to the background, for instance E has a much more intense staining for all the features compared to C and D; another clear difference is that posterior staining is broader in E respect C and D. This could be explained in terms of physiological/homeostatic stage, for instance E could be a 20 days regenerating planarian or an adult planarian close to fission, both stages are not possible to be distinguish visually from a standard adult. F shows expression around the brain ganglia, very weakly in the nerve cords, posterior enhanced expression and mouth. **(G-J)** sme-mir-C is classified as expressed in the excretory system and parenchyma and shown in G and Fig. 1I. In* in situ *experiments for this miRNA, G represents 60% of animals while H and I represent 20% of animals respectively and J represents 20% of animals. G is expressed in the mid-posterior part of the excretory system (arrowhead), in sub-epidermal marginal adhesive gland cells (arrow) and in the entire parenchyma (asterisk); H to J are expressed in the same regions however the high intensity of the expression in the mid-posterior part of the excretory system (arrowhead) decreases its resolution. Scale bars indicate 1 mm.*
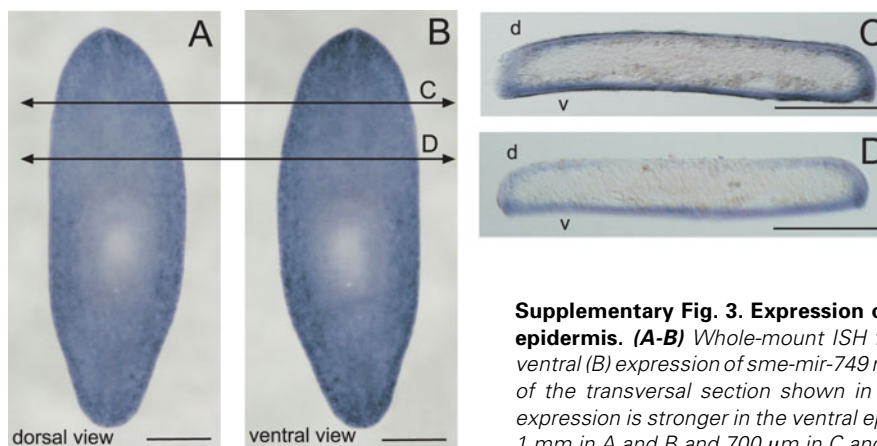
Supplementary Fig. 3. Expression of sme-mir-749 is restricted to an area near surface/ epidermis. *(A-B)* *Whole-mount ISH for adult asexual planarians showing the dorsal (A) and ventral (B) expression of sme-mir-749 near the epidermis; the arrows in A and B indicate the level of the transversal section shown in C and D.* *(C-D)* *Transversal sections indicate that the expression is stronger in the ventral epidermis (v) than in the dorsal one (d). Scale bars indicate 1 mm in A and B and 700 μm in C and D.*

sme-lin-4c

A

sme-bantam-c

B

sme-let-7b

C

sme-mir-67

D

sme-mir-124b

E

sme-mir-2d

A

sme-mir-281

B

Supplementary Fig. 4 (Left). Planarian miRNA expression patterns in regenerating planarians. *Whole-mount ISH for 3d regenerating tails showing expression at posterior midline of sme-lin-4c* **(A)**, *sme-bantam-c* **(B)**, *sme-let-7b* **(C)**, *sme-mir-67* **(D)**, *sme-mir-124b* **(E)**. *Scale bars indicate 1 mm.*

Supplementary Fig. 5 (Right). Planarian miRNA expression patterns in sexually mature planarians. *Whole-mount ISH in sexual mature planarians for sme-mir-2d* **(A)** *and sme-mir-281* **(B)** *showing expression in the entire parenchyma. Scale bars indicate 3 mm.*

sme-let-7c

nascent probe

A

LNA probe

B

sme-mir-67

nascent probe

C

LNA probe

D

Supplementary Fig. 6. miRNA expression patterns with nascent probes versus LNA probes. *The figure corroborates the patterns obtained for two miRNAs using nascent probes, by using LNA probes. Expression pattern of sme-let-7c using the nascent probe in adult asexual planarians* **(A)** *is the same as using a LNA probe* **(B)**. *Expression of sme-mir-67 using the nascent probe in adult asexual planarians* **(C)** *is the same as using a LNA probe* **(D)**. *Scale bars indicate 1 mm.*
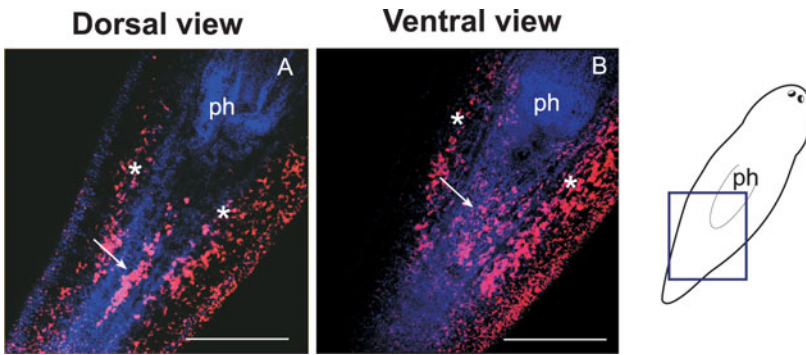
Supplementary Fig. 7. Dorso-ventral distribution of sme-let-7c mature miRNA transcript detected by a LNA probe. *A and B show confocal projections from different focal planes of the same sample.* **(A)** *Confocal projection of some slices from the dorsal part of the animal. sme-let-7c is stained in red and nuclei in blue. Note that there is staining in the dorsal midline (arrow) more diffuse staining in the dorso-lateral part of the parenchyma (asterisks).* **(B)** *Confocal projection of some slices from the ventral part of the animal. sme-let-7c is stained in red and nuclei in blue. Note that there is staining in the ventral midline (arrow) and also in the dorso-lateral part of the parenchyma (asterisks). The scheme on the right indicates by a blue square the area shown in A and B. ph indicates pharynx. Scale bars indicate 500 μm.*
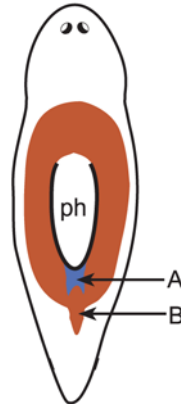
Supplementary Fig. 8. Scheme showing two different cell populations in the planarian parenchyma. *miRNA expression patterns in irradiated and non-irradiated animals show that population A is insensitive to irradiation whereas population B is sensitive to irradiation. ph, pharynx.*