SUPPLEMENTARY MATERIAL

Supplementary Tables

Table S1. Analysis of piRNA distribution amongst histone loci in the *Schmidtea mediterranea* genome.

Human Histone H2A, H2B, H3, H4 and *C. elegans* Histone H1-like (HIL-1) protein homologes (GenBank I.D. provided) were found in the *S. mediterranea* Genome Database (SmedGD; Robb et al., 2008) via TBLASTN searches (E-values provided). The top 20 homologs according to E-values, where further analyzed. Genomic reads corresponding to cDNA clones utilized in this study were found by BLASTN and are indicated in the fifth column. The presence of piRNA islands and repetitive elements mapping within 1 kilobase of histone sequence genomic regions (according to SmedGD; smedgd.neuro.utah.edu) are indicated as absent (-), present (*). Codes for piRNA islands according to SmedGD within homology regions are included in the ninth column.

Table S2. Additional primer sequences.

Forward (F) and reverse (R) primers used for measuring changes in histone and neoblast markers gene expression by RT-qPCR (top), and primers used for partial *gH4* riboprobe template synthesis, are listed. The combination of primers gH4-F_37 with gH4-R_474, and gH4-F_474 with gH4-R_861, were used for riboprobe synthesis corresponding to the first and second halves of *gH4* cDNA, respectively. For smaller sections of *gH4* sequence we used the following combination of primers (5' to 3') gH4-F_37 and gH4-R_283; gH4-F_258 and gH4-R_474; gH4-F_474 and gH4-R_671; gH4-F_647 and gH4-R_861. T3 promoter sequence was included in the reverse primer where indicated by capital letters.

Supplementary Figure Legends

Figure S1. *Germinal Histone H4* transcripts localize to chromatoid bodies, whereas transcripts for other neoblast marker genes do not.

Representative analysis of FISH signal followed by Y12 immunofluorescence of chromatoid bodies from confocal sections of neoblasts using Zen 2011 software (Carl Zeiss, Germany). **(A)** *gH4* transcripts (magenta) specifically localize to chromatoid bodies (green). Signal intensity (Y-axis; graph) for *gH4* signals (magenta arrows) aligns with peaks from Y12 signals (green arrows). **(B)** *Smed-bruli* FISH signal (magenta) peaks (magenta arrows) do not align with intensity peaks of Y12 (green arrows). Distance within line of analysis (red arrow on image) corresponds to X-axis in graph. **(C)** *gH4* localization to chromatoid bodies is not affected by non-specific dsRNA feedings. Percentage of chromatoid bodies containing *gH4* mRNA foci are shown for animals 4 days after eating liver without dsRNA (control), or 1, 4, or 10 days (D1, D4, D10) after eating liver containing dsRNA of *gH4* or transposable element *TE10* sequence. Columns represent average from analyses of three of more biological replicates. Error bars represent standard deviation. Colocalization of *gH4*/Y12 signals was only affected by *gH4* RNAi.

Figure S2. *gH4* transcripts in neoblasts and chromatoid bodies are canonical histone H4 mRNAs.

(A) Representation of the original cDNA clone used as *gH4* riboprobe template (top) and predicted canonical Histone H4 mRNA (bottom). Histone mRNA 3'-end processing is mediated by a conserved stem-loop structure shortly downstream

of the ORF; they are not polyadenylated. **(B)** FISH analyses suggest that *gH4* transcripts present in neoblasts and chromatoid bodies contain a short 3'UTR. Seven riboprobes (gray bars) corresponding to different portions of the original *gH4* cDNA (Fig. S2A; top) were used for detection of chromatoid body material as in Fig. 1A. Only probes containing sequence corresponding to the ORF region detected transcripts in neoblasts and chromatoid bodies (green framed insets), whereas probes corresponding to 3'UTR sequence showed only background-level signals in neoblasts (unframed insets). **(C-E)** Northern blot analyses verify that transcripts detected by *gH4* riboprobes in planarian neoblasts are canonical histone mRNAs. (C) Northern blot analysis using full-length *gH4* riboprobe reveals a single band of approximately 350 nucleotides in total asexual planarian RNA. (D) Northern blot analysis of total RNA, RNA retained in oligo-(dT) sepharose (p(A)+) and non-polyadenylated RNA (p(A)-) confirmed that the material recognized by the *gH4* riboprobe lacks a poly(A) tail. *Smedwi-1* mRNA is detected in poly(A)+ fraction and served as control for polyadenylated mRNA recovery. For this assay, oligo-(dT) retention performed at room temperature and requires a stretch of at least 12 adenosines, therefore mRNAs detected in the p(A)+ fraction are believed to carry a *bona fide* poly(A) tail. The slight difference in migration of *Smedwi-1* mRNA in p(A)+ and total RNA fractions is believed to be an artifact due to the oligo-(dT) fractionation process, which has been observed with other mRNAs. Ribosomal RNA (rRNA) was used as a non-polyadenylated control. (E) Northern blot analysis of RNA from planarians untreated (control) and four days post-irradiation (irradiated) confirm that *gH4*

transcripts are stem cell specific. rRNA levels are shown as loading controls.

Figure S3. Planarian histone mRNAs localize to chromatoid bodies.

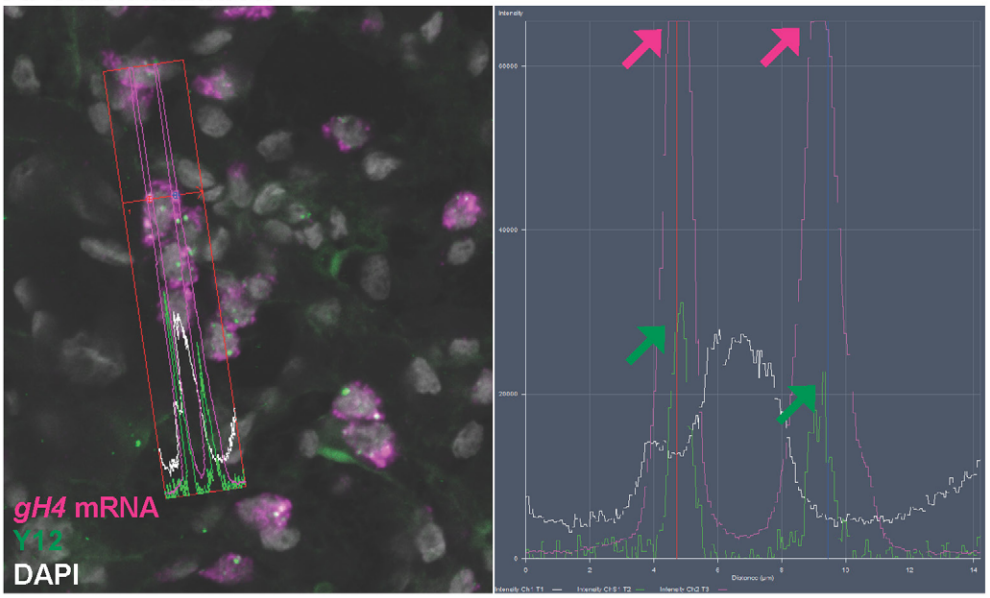
(A) Stem loop sequence structures representative of metazoan, parasitic flatworm *Schistosoma mansoni*, and analyzed *Schmidtea mediterranea* histone mRNAs. *S. mansoni* structure adapted from (Anderson et al., 2012). R = A or G (purine); Y = C or U (pyrimidine); B = C, G, or U; N = A, C, G, or U. **(B-F)** Single FISH analysis of *histone H1*, *H2A*, *H2B*, *H3* and *gH4* mRNA (magenta; B-F), followed by Y12 immunofluorescence (green; B'-F') shows that mRNAs representative of each histone are present in chromatoid bodies. Merged images (B''-F'') and visualization of cell nuclei by DAPI staining (gray; B'''-F''') are shown. Yellow arrows denote chromatoid bodies containing the mRNAs of various tested histones.

Figure S4. Planarian histone mRNA localization to chromatoid appears to be independent of SLBP.

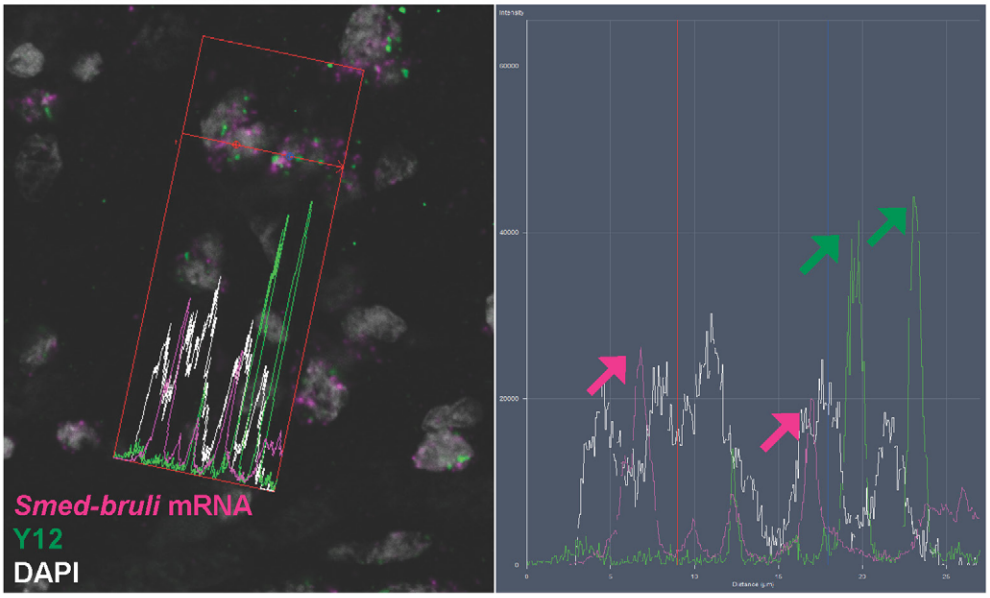
(A and B) *gH4* FISH (magenta; A and B) followed by Y12 immunofluorescence (green; A' and B') performed on control planarians (A-A''') and *SLBP(RNAi)* (B-B'''). Merged images (A'' and B'') reveal that *gH4* mRNA still localizes to chromatoid bodies in the lingering neoblasts after two weeks of *SLBP* knockdown. DAPI staining of nuclei is shown in blue (A''' and B'''). Solid yellow arrows indicate *gH4* FISH and Y12 immunofluorescence colocalized signals. Scale bar = 10 μm. **(C)** Predicted RNA stem-loop structures from different *histone H3* loci and respective cDNA clones determined using mfold (mfold.rna.albany.edu). **(D-F)** FISH analyses performed using probes

corresponding to *gH4* mRNA (magenta) and three different *histone H3* loci with varying degrees of stem-loop sequence conservation (gold; see Table S1 for more information) followed by Y12 immunofluorescence (green). All tested varieties of *histone H3* transcripts were detected in chromatoid bodies, regardless of carrying a perfectly conserved stem-loop sequence (D), single nucleotide changes in the stem sequence (imperfect; E), or several changes in stem and loop structure sequence (aberrant; F). FISH signals from probes corresponding to transcripts with variations in stem loop structures (E-F) are believed to be specific, since their closest paralogs with normal loop structures present in the *S. mediterranea* genome are below 84% identical, which is below the threshold for cross-hybridization observed in our and previous studies (Lecuyer et al., 2007). DAPI was used to visualize nuclei (blue). Scale bar = 10 μm.

A. Colocalization



B. No colocaliation



C. RNAi does not have non-specific effects in chromatoid body structure

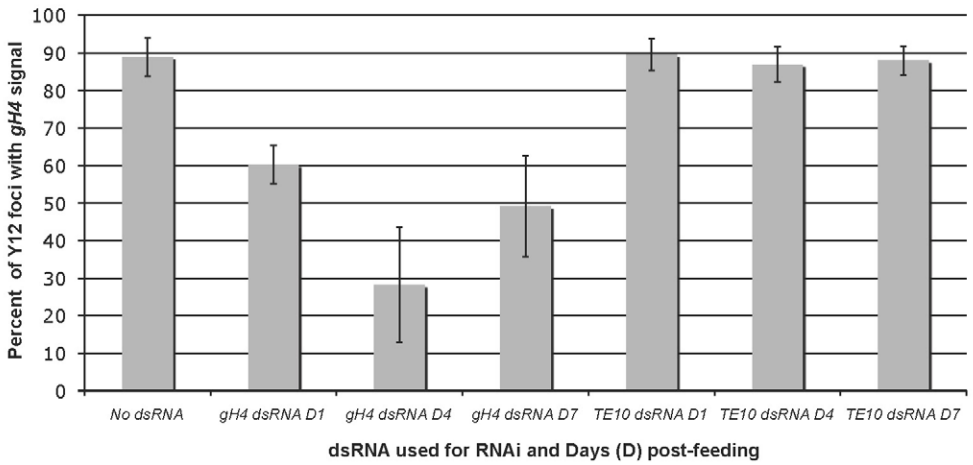
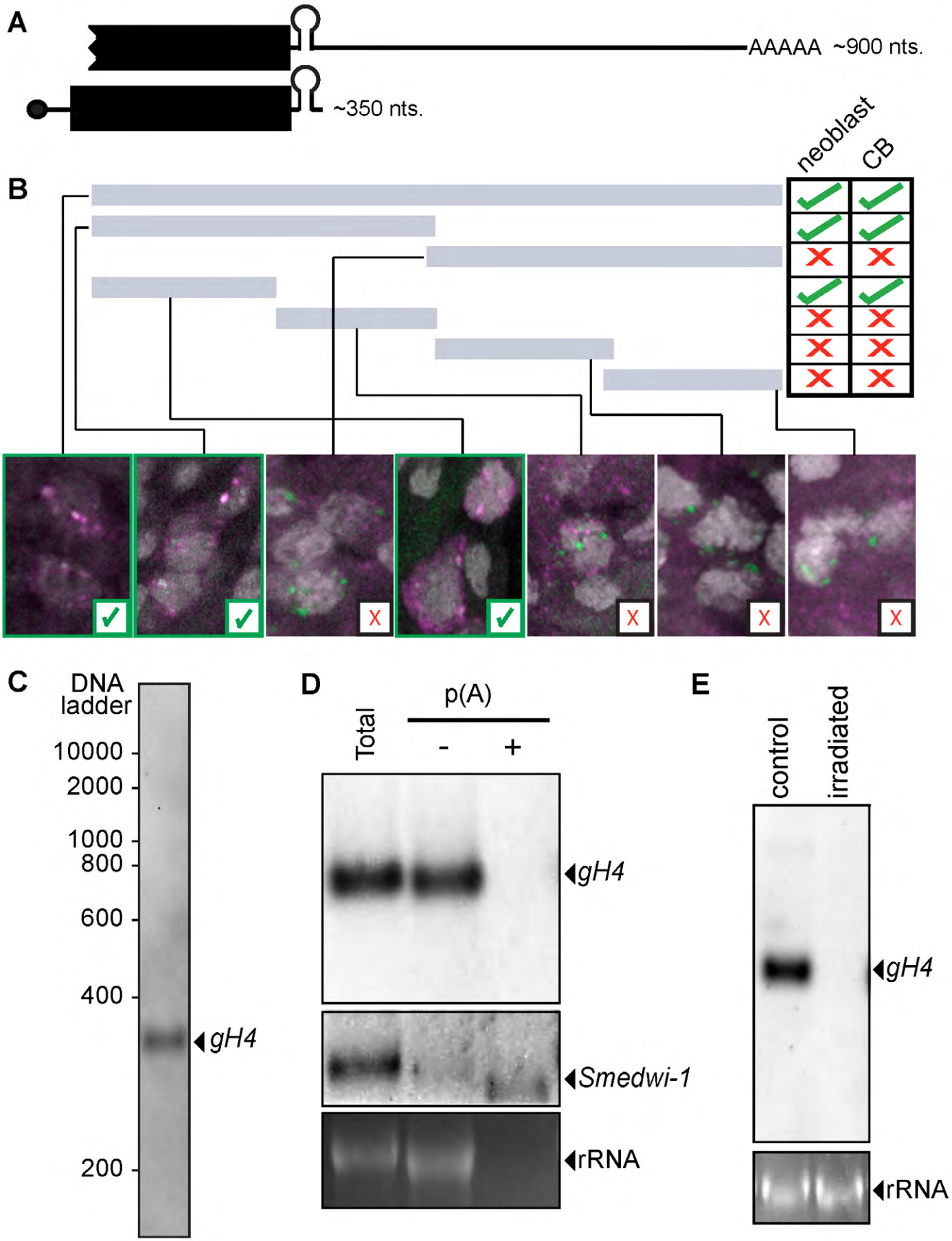


Figure S2


A
AAAAA ~900 nts.
~350 nts.
B
neoblast
CB
C
DNA ladder
10000
2000
1000
800
600
400
200
gH4
D
Total
p(A)
-
+
gH4
Smedwi-1
rRNA
E
control
irradiated
gH4
rRNA

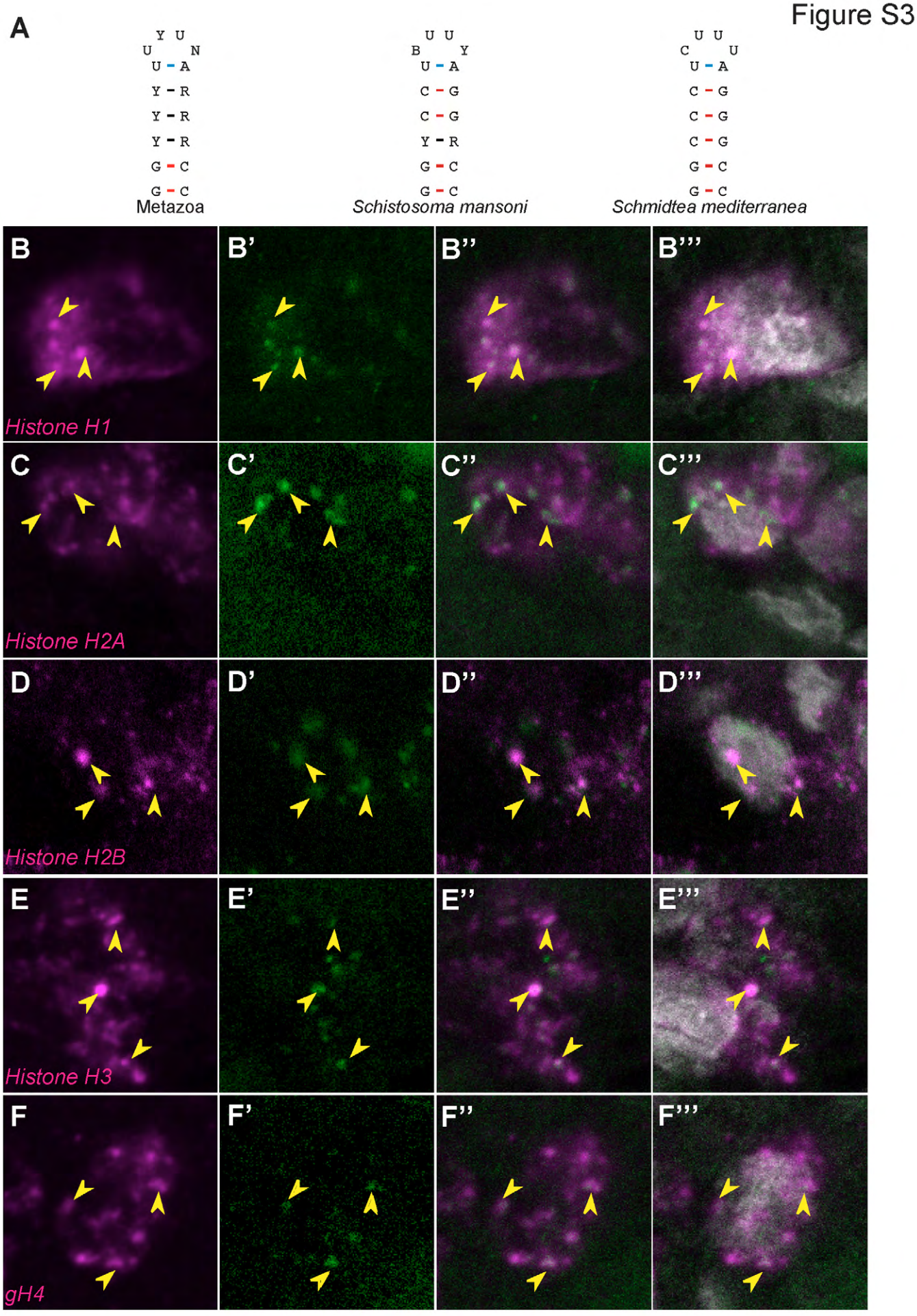

A
Y U
U N
U - A
Y - R
Y - R
Y - R
G - C
G - C
Metazoa
U U
B Y
U - A
C - G
C - G
Y - R
G - C
G - C
Schistosoma mansoni
U U
C U
U - A
C - G
C - G
C - G
G - C
G - C
Schmidtea mediterranea
B
B'
B''
B'''
Histone H1
C
C'
C''
C'''
Histone H2A
D
D'
D''
D'''
Histone H2B
E
E'
E''
E'''
Histone H3
F
F'
F''
F'''
gH4

Table S1. Analysis of piRNA distribution among histone loci in the *Schmidtea mediterranea* genome.

Histone Type	GenBank I.D.	Genomic Locus	TBLASTN E-value	Corresponding cDNA	sense piRNA	antisense piRNA	Repetitive Elements	piRNA Islands	Comments
H1-like	CAB01632.1 (Ce)	v31.004990	1.00E-07	*Histone H1*	***	-	*	175415, 175416, 175417	
		v31.018508	1.00E-04		-	-	*		
		v31.017587	1.00E-04		-	-	*		
		v31.007255	2.00E-04		-	-	*	194602, 194603, 194604	inverted duplication w/intergenic piRNAs
H2A	AAN59974.1 (Hs)	v31.003169	3.00E-48		-	-	*		
		v31.000031	1.00E-46	*Histone H2A*	***	-	*	5797-5817; 5784-5791	tandem duplication w/intergenic and intragenic piRNAs
		v31.000124	9.00E-46		***	-	*	18297-18299 (>100 more)	tandem duplication w/intergenic and intragenic piRNAs
		v31.001314	5.00E-45		-	-	*		
		v31.002596	7.00E-45		-	-	-		
		v31.000610	1.00E-44		**	-	-	62893, 62894	
		v31.002922	7.00E-44		-	-	*		
		v31.000688	1.00E-43		-	-	*		
		v31.001438	2.00E-41		-	-	*		
		v31.008399	2.00E-39		-	-	-		
		v31.002385	8.00E-38		-	-	*		
		v31.045984	8.00E-38		-	-	*		
		v31.002800	3.00E-35		*	-	*	144912	
		v31.003116	2.00E-30		-	-	*		
		v31.009608	1.00E-28		-	-	*		
		v31.019624	1.00E-27		*	-	*	235041	
		v31.001713	5.00E-27		-	-	*		
		v31.049150	5.00E-27		-	-	*		
		v31.018961	8.00E-27		-	-	*		
		v31.004866	1.00E-26		-	-	*		
		v31.003804	1.00E-26		-	-	*		
		> 400 more	3.00E-26 to 8.1						
H2B	CAA41051.1 (Hs)	v31.014939	8.00E-43		-	-	*		
		v31.005020	1.00E-42		-	-	-		
		v31.000688	4.00E-41		-	****	*	68320-68331; 68337-68342	tandem duplication w/intergenic and intragenic piRNAs
		v31.000124	4.00E-41	*Histone H2B*	*	*	*	18296; 18348	
		v31.000572	2.00E-40		-	-	*	60148; 60159-60162	tandem duplication w/intergenic piRNAs
		v31.002212	2.00E-40		-	-	*		
		v31.001736	5.00E-40		-	-	*		
		v31.008285	9.00E-40		-	-	*		
		v31.045597	9.00E-40		-	-	*		
		v31.000756	2.00E-37		**	*	*	72002-72003; 71973	
		v31.006479	1.00E-29		-	-	-		
		v31.023146	1.00E-28		-	-	-		
		v31.021105	2.00E-28		-	-	*		
		v31.026089	8.00E-28		-	-	*		
		v31.000063	1.00E-26		*****	-	-	10419-10423	
		v31.011360	8.00E-24		-	-	*		
		v31.015823	5.00E-23		-	-	*		
		v31.018852	1.00E-22		-	-	-		
		v31.013070	7.00E-22		-	-	*		
		v31.012806	6.00E-16		-	-	*		
		v31.010181	4.00E-15		-	-	*		
		... 44 more	1.00E-14 to 8.4						
H3	AAN39284.1 (Hs)	v31.000688	2.00E-70		*	-	*	68334-68341; 68331-68325	inverted duplication w/intergenic and intragenic piRNAs
		v31.002008	2.00E-70	*H3 imperfect stem*	-	-	*		
		v31.005767	2.00E-70		*	-	*	183508	
		v31.003741	2.00E-70		*	-	*	160380	
		v31.005509	2.00E-70	*H3 perfect stem-loop*	*	-	*	180581	
		v31.003583	2.00E-69	*H3 aberrant stem-loop*	*	-	*	158060	
		v31.001027	2.00E-64		-	-	*		
		v31.002106	6.00E-60		*	-	-	127184	
		v31.001251	3.00E-52		-	-	*		
		v31.009630	2.00E-48		-	-	-		
		v31.000503	2.00E-46		-	-	-		
		v31.003160	1.00E-36		*	-	-	151719	
		v31.000192	2.00E-32		-	-	*		
		v31.006511	1.00E-30		-	-	*		
		v31.012775	2.00E-23		-	-	*		
		v31.002039	3.00E-23		-	-	*		
		v31.005153	2.00E-22		-	*	*	177006	possible pseudogene
		v31.000876	1.00E-21		-	-	*		
		v31.020820	2.00E-21		**	*	*	237202; 237217-237230	inverted duplication w/intergenic and intragenic piRNAs
		v31.014377	5.00E-20		-	-	*		
		v31.009200	7.00E-20		-	-	*		
		... 12 more	8.00E-20 to 6.5						
H4	AAA52652.1 (Hs)	v31.006602	5.00E-40		-	-	*		
		v31.013216	5.00E-40		-	-	*		
		v31.000809	5.00E-40		**	-	-	74551-74552	
		v31.003127	5.00E-40		-	-	-		
		v31.017320	5.00E-40		*	*	*	230639; 230640	
		v31.001066	6.00E-40	*germinal histone H4*	*	-	*	89245-89247	inverted duplication w/intergenic and intragenic piRNAs
		v31.003066	7.00E-40		-	-	*		
		v31.002433	2.00E-28		-	-	*		
		v31.004713	6.00E-26		-	-	-		
		v31.001345	4.00E-24		****	-	-	101368-101371	
		v31.006244	4.00E-16		*	-	-	187328	
		v31.000411	1.00E-14		-	-	-		
		v31.000702	2.00E-12		-	-	*		
		v31.005770	2.00E-12		*	*	-	183514 ;183517	
		v31.005158	3.00E-12		-	-	-		
		v31.000858	2.00E-11		-	-	-		
		v31.021260	3.00E-10		-	-	-		
		v31.020257	6.00E-09		-	-	-		
		v31.023734	6.00E-09		-	-	-		
		v31.003761	9.00E-09		-	-	*		
		v31.000148	3.00E-07		-	-	-		
		... 31 more	2.00E-06 to 7.3						

(Hs) Homo sapiens
(Ce) Caenorhabditis elegans
* sense and antisense piRNAs within 1 kb locus

Table S2. Additional primer sequences

Primers used in RT-qPCR	
H1-F	tggcaacaaaggaaaaggtg
H1-R	ttggtgctggcttctttgag
H2A-F	ttcccagtgggtcgtattca
H2A-R	aacggcagccaaatataccg
H2B-F	tggaatttctggcaaagcaa
H2B-R	tagcctccgaagcgattctt
H3-F	tggtggaaaagctccacgta
H3-R	gcgacagttccaggacgata
gH4-F	aggaaaaggtggagcaaagc
gH4-R	tctgattgctggctttgtga
Smedwi2-F	gagccacgtgaaagattgga
Smedwi2-R	ccagttgccgcatcactatt
PCNA-F	tgaaagccgctgattcaagt
PCNA-R	aagtgatctccatccaagtcca
Primers used in partial *gH4* riboprobe synthesis	
gH4-F_37	ataacattcaagggtatccacaaag
gH4-R_283	TATAATTAACCCTCACTAAAGGGAGAttatttaacctccaaaaccgtacaa
gH4-F_258	tttgtacggttttggaggttaaata
gH4-R_474	TATAATTAACCCTCACTAAAGGGAGAtaatgcgagacaaatcacgttacta
gH4-F_474	tagtaacgtgatttgtctcgcat
gH4-R_671	TATAATTAACCCTCACTAAAGGGAGAtacatcgcttgattaaaatggatct
gH4-F_647	agatccattttaatcaagcgatgta
gH4-R_861	TATATTTAACCCTCACTAAAGGGAGAatgtcacagaaaaatgcaaatacaa