Supplemental Material to:

Hen1 is required for oocyte development and piRNA stability in zebrafish

Leonie M. Kamminga[1,3], Maartje J. Luteijn[1,3], Marjo J. den Broeder[1], Stefan Redl[2], Lucas J.T. Kaaij[1], Elke F. Roovers[1], Peter Ladurner[2], Eugene Berezikov[1] and René F. Ketting[1,4]

[1]Hubrecht Institute-KNAW & University Medical Centre Utrecht, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [2]Institute of Zoology, Technikerstrasse 25, A-6020 Innsbruck, Austria

[3]Contributed equally to the work

[4]Correspondence: r.ketting@hubrecht.eu

Running title: Hen1 function in zebrafish germ cells

Key words: piRNA/Piwi/Hen1/zebrafish/germ line

Oligonucleotide sequences used in this work:

Genotyping

Hen1_exon5_F	AGGAAACAGCTATGACCATGAAAG AGGCAATGAAAGTACATC
Hen1_intron5/6	TGTAAAACGACGGCCAGTGAT TTAGGGAATGCAATTT ACC

Hen1 RT-PCR

RT_exon5_6_F1	ACAGACTGCTATACAGGGTGGTG
RT_exon6_R	TAAGCGCACTTCCATCAGC
RT_exon1_2_F2	CCGACCTAGAAAGGTCATCG
RT_exon 3_4_R2	TGTTCGATCAGCTCAACACAT
GAPDH_F	acagttgtaagcaatgcctcct
GAPDH_R	tatcccagaattcctttcatgg

Contructs for RNA injection, using MultiSite Gateway® cloning technology (Invitrogen)

GFP:Hen1FL

FL_3'_Fw	GGGGACAGCTTTCTTGTACAAAGTGG ccacc Acc gcc aca ccg ttc tc
FL/nos__F	GGGGACAGCTTTCTTGTACAAAGTGGccaccCATCTGAGGCAGCAATGG
NosUTR_3'_R	GGGGACAACTTTGTATAATAAAGTTGGAAAATGTTTATATTTTCCTCAC

GFP: Hen1MTD

MTD_3'_Fw	GGGGACAGCTTTCTTGTACAAAGTGG ccacc Acc gcc aca ccg ttc tc
MTD/nos _F	cagattgcagtgttccagagaGAGCGGACATTGATGCTC
NosUTR_3'_R	GGGGACAACTTTGTATAATAAAGTTG GAAAATGTTTATATTTTCCTCAC

GFP: Hen1CTD

Hen1_CTD_F	ggtagctatggagacgaa GAGCGGACATTGATGCTC
CTD/nos_3'_F	GGGGACAGCTTTCTTGTACAAAGTGGccaccCATCTGAGGCAGCAATGG
NosUTR_3'_R	GGGGACAACTTTGTATAATAAAGTTGGAAAATGTTTATATTTTCCTCAC

Hen1FL:GFP

FL_5'_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCATGACCGCCACACCGTTCTC
FL_5'_R	GGGGACCACTTTGTACAAGAAAGCTGGGTGTTCGTCTCCATAGCTACCAAG

qRT-PCR

EnSpm_F	GATTGGCCATTGTGTTCAC ATGC
EnSpm_R	GCTGTGACTGTCATAGGTTTACC
I1_F	GAGTTGA GGATTGGAAGATCATT
I-1_R	CTTGAGTTTTAGCAATAGAAAATCTAAA TC
GypsyDR2_F	GAAATCACCTGTGCATTTAC
GypsyDR2_R	ATGCAG ACATTGGGTAAAGC
Ngaro1_F	GGGAGCGATCGAGACCTACC
Ngaro1_R	CAATCATATCACGTGCTCCTCTCG
Polinton-1_F	CCTGACAATGTT GTCAGCCTG
Polinton-1_R	CATGAAAGCTAAGGGTATAACTCTG
Ef1a_F	GGCCACGTCGACTCCGGAAAGTCC
Ef1a_R	CTCAAAACGAGCCT GGCTGTAAGG
GAPDH_F	GTGGAGTCTACTGGTGTCTTC
GAPDH_R	GTG CAGGAGGCATTGCTTACA
Tubulin_F	CCTGCTGGGAACTGTATTGT
Tubulin_R	TCAATGAGTTCCTTGCCAAT

Probes

piR28	5'-AGCCTCTGGAGCTGGTTGGCAT

piR30	5'-TGGACGAAAAGATTCGCTGGCA
piR31	5'-GCATGTGGCTCTGTGTT
piR80	5'-TCGTCGCGGAGCAGCCCAT
let-7	5'-AACTATACAACCTACTACCTCA

Primers *in situ* probes

F_vasa probe	GGTAGCTCATGGAAAATGACTGGTGATTCGTTCAG
R_vasa probe	CGGTAATACGACTCACTATAGGGCTCAGTGAGTCACAAAGTC

TUT1_Exon5/6 _Fw	AACAACAAGGTGGCGATGA
TUT1_Exon7/8_T7_Rev	CAGGtaatacgactcactatagggTGCAACAGTTTTTCTAACGTG
TUT2_RT_exon7_8_Fw	GGACAGAATCAGTGGAGTGGA
TUT2_RT_exon11_12_T7_Rev	CAGG taatacgactcactataggg GCTTGTCCCATTTGAAAACA
TUT4_exon5/6_Fw	cccgtcttacaagagatttttga
TUT4_exon8/9_T7_Rev	CAGGtaatacgactcactataggggaagttggtcattttgcgaga
TUT7_Exon24_25_Fw	TTTCTCTAAAGTGTGTGATATTGGAGA
TUT7_27_28_T7_Rev	CAGGtaatacgactcactatagggCTCAAGTCGAACGGATCCTC

Primers mRNA expression of *dr tutases* in multiple tissues

TUT1_Exon5/6 _Fw	AACAACAAGGTGGCGATGA
TUT1_Exon7/8_T7_Rev	CAGGtaatacgactcactatagggTGCAACAGTTTTTCTAACGTGTC
TUT2_exon7_8_Fw	GGACAGAATCAGTGGAGTGGA
TUT2_exon11_12_T7_Rev	CAGGtaatacgactcactatagggGCTTGTCCCATTTGAAAACAG
TUT3_Exon56_Fw	CAAGCAGCAATATCCTGTGTT
TUT3_Exon8/9_T7_Rev	CAGGtaatacgactcactatagggGCATTGCCCCATATGAACTT
TUT4_Fw	aacagtcgtgtgcagcatct
TUT4_Rev	cagcatccttgtgttgtgct
TUT5_Exon6/7 _Fw	CAGTTTCTTACAGTTACACCCAAGA
TUT5_Exon8/9_T7_Rev	CAGG taatacgactcactataggg CCC AAA GTG CTG TCA CTG
TUT6_Fw	TCGTCTGTGAACACCTTTGGGCTT
TUT6_T7Rev	CAGG taatacgactcactataggg GTCGATACCTGAACACAACTGCAG
TUT7_Exon24_25_Fw	TTTCTCTAAAGTGTGTGATATTGGAGA
TUT7_27_28_T7_Rev	CAGG taatacgactcactataggg CTCAAGTCGAACGGATCCTC

Supplemental Figure legends

Figure S1. Wild-type and *hen1* mutant gonads.
Gonads were dissected from *hen1* heterozygous or homozygous mutants, and stained for *vasa* mRNA to visualize germ cells. All pictures were taken at a 2.5x maginification.

Figure S2. Beta-elimination analysis
(A) The left panel shows β-elimination on synthetic RNA with or without a 2' OMethyl group. RNA without modification is sensitive to β-elimination which results in a shift on gel, whereas RNAs with a 2')-methyl modification is no longer sensitive. The right panel shows the same treatments on piRNAs isolated from immunoprecipitation with Ziwi antibodies.
(B) Northern blots on total RNA isolated from the indicated genetic backgrounds, probed for the indicated piRNAs. Blots were done using both testis RNA and ovary derived RNA. Hen1 mutant ovary RNA was isolated from the one female individual we retreived.

Figure S3. Cloned RNA species.
Bar diagram displaying the different types of RNA that were sequenced. "Ligation" and "polyA" refer to the method used to tag the 3' end of the small RNAs for cloning purposes. "Other piRNA" refers to a class of piRNAs that map to regions of the genome for which there is no annotation.

Figure S4. Ping-pong signatures.
Ping-pong signature of piRNAs cloned from wild-type and *hen1* mutant animals. First we asked for piRNAs that map to opposite strands of the same locus, then we asked how many bases their 5' ands were overlapping. Results are plotted as a percentage relative to the total number of overlapping piRNAs.

Figure S5. Deep sequencing analysis.
(A) Length of A and U tails observed on piRNAs.

(B) Scatter-plot of transposon read counts observed in wild-type and *hen1* mutant libraries. Each dot represents an individual transposon type.
(C) Percentage uridylated piRNAs versus cloning frequency of piRNAs derived from retro-elements or DNA elements in hen1 mutants. Retro-element-derived piRNAs are more frequently uridylated than piRNAs coming from DNA elements (10.0 ± 1.7% versus 8.2 ± 1.9%; p = 3*10-9).
(D) Similar plot as in S5B, but now focused only on transposons that have at least 3.000 reads in both libraries. The scale is now linear.

Figure S6. *dr tutase4* cDNA
This partially hypothetical open reading frame is based on XM_002666528.1 and XM_001335483.3. These two gene predictions fuse together as we determined through RT-PCR and sequencing. The fusion point between the two predictions is given in bold and the region we verified by sequencing is underlined.

Figure S7. Expression analysis of *dr tutases*
(A) RT-PCR analysis on total RNA derived from various tissues using primers to amplify fragments from the transcripts of the indicated genes. For each gene a schematic drawing indicates the domain structure of the encoded proteins.
(B) *In situ* hybridisations for *dr tutases1*, *2, 4* and *7* on adult ovaries. Purple staining indicates mRNA expression.

genotype	#♂	# ♀
+/+	108	136
+/-	295	346
-/-	296	1

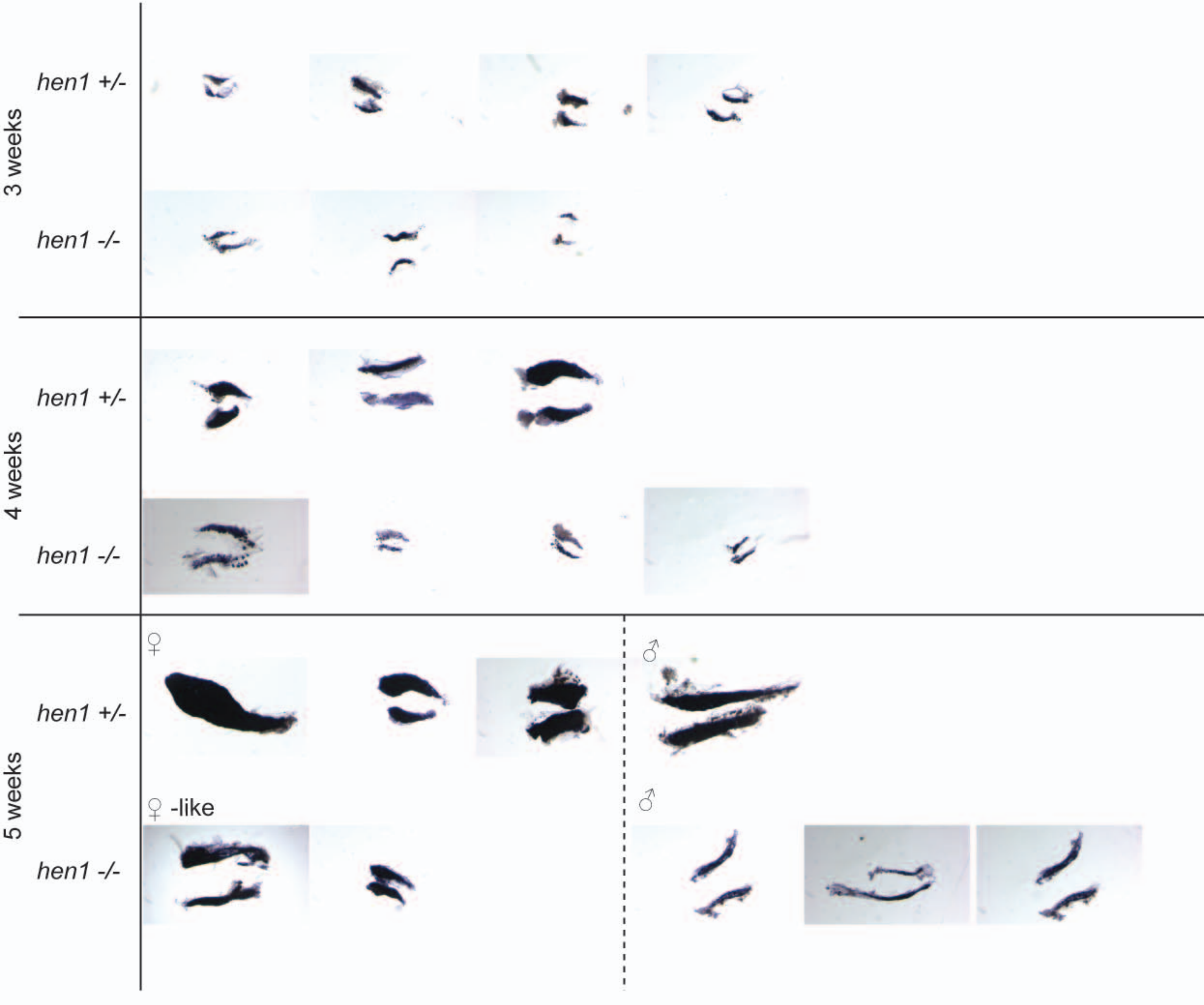
Table S1
Sex ratios in offspring of *hen1* heterozygous animals

Category	Wild-type	hen1
miRNA	217847	416433
Transposon piRNA	2590397	3122019
genic piRNA	430583	410056
other piRNA	3899033	4133676
rRNA	6801	14087
snRNA	11091	18146
snoRNA	389	708
tRNA	29	36

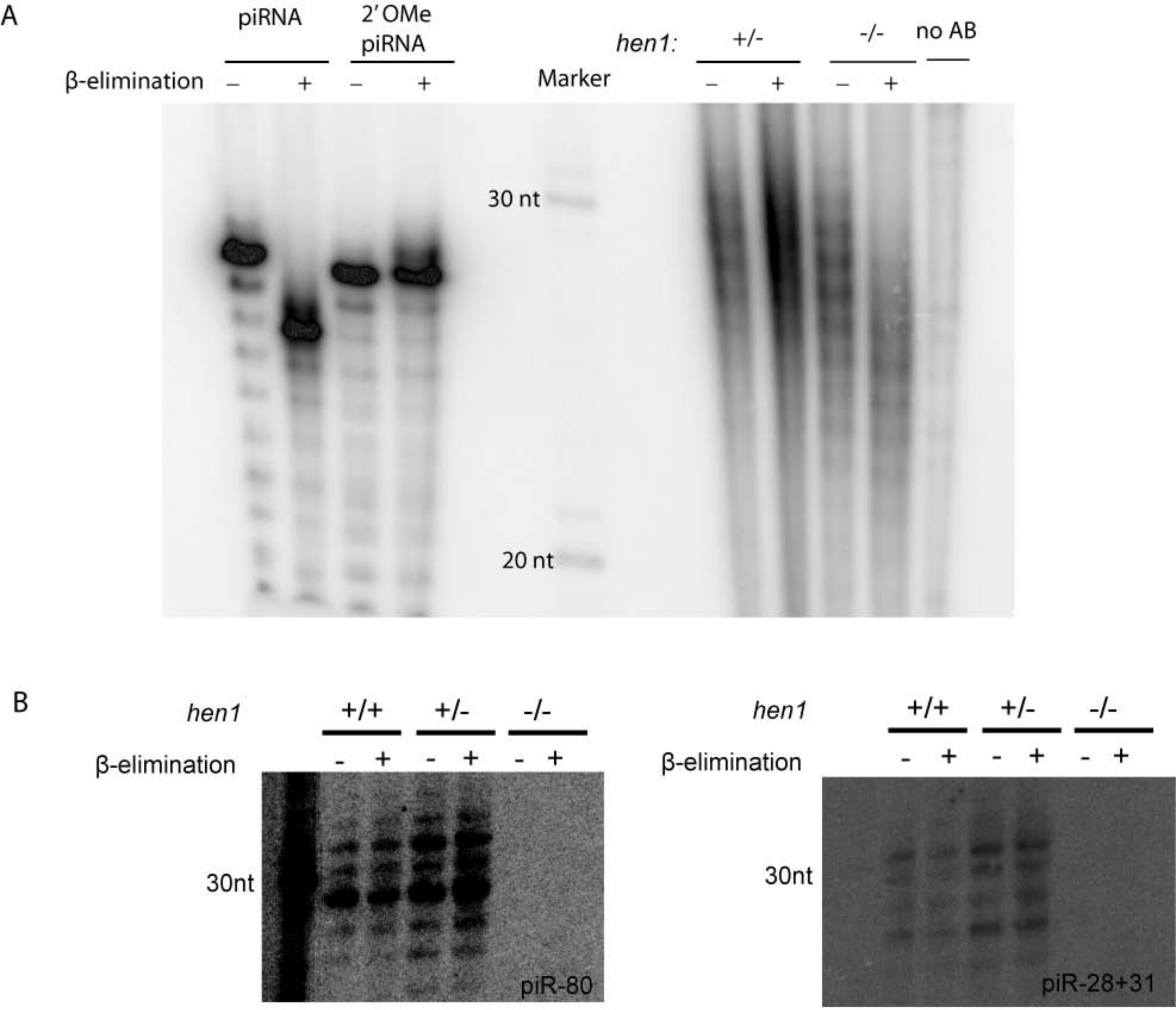
TableS2. Deep-sequencing numbers

TUTase	Accession number	# amino acids	Chromosomal position (Zv8)	Predicted or cDNA
drTUTase1	XM_687164.3	580	Chr 12: 27,703,665-27,713,295	pred.
drTUTase2	NM_001020600.1	489	Chr5:53,398,137-53,412,679	cDNA
drTUTase3	XM_692023.4	653	Chr7:38,268,984-38,302,884	pred.
drTUTase4	XM_002666528.1 XM_001335483.3	1445	Chr23: 38936010-38950932 (inverted segment of the genome)	pred.
drTUTase5	XM_680973.3	762	Chr19:26,790,880-26,801,515	pred.
U6 drTUTase	BC098614.1	797	Chr12:11,013,190-11,027,473	cDNA
drTUTase7	XM_685750.4	1199	Chr5: 71874529 - 71919997	pred.

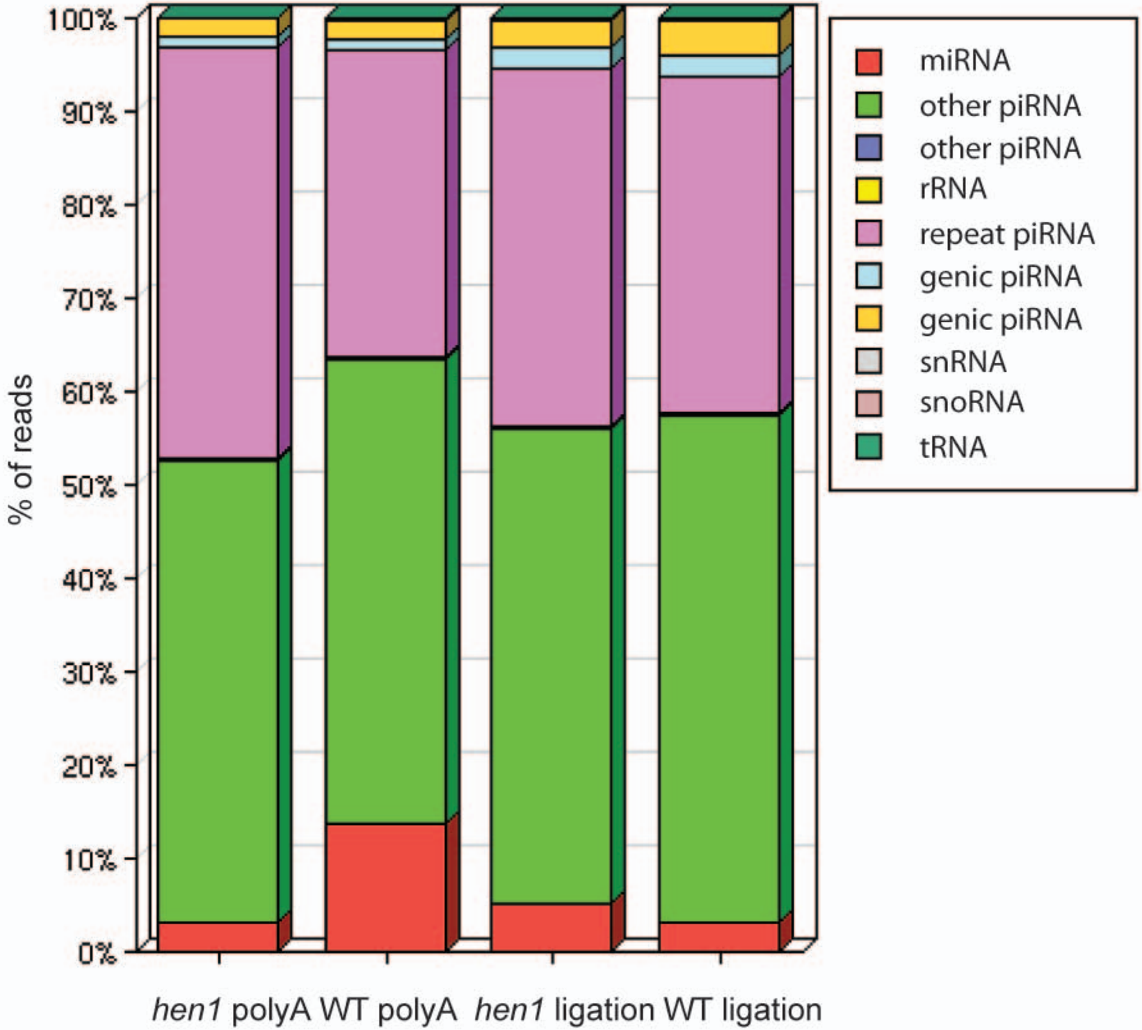
Table S4. TUTases in the zebrafish.
Predicted indicates computationally identified transcripts based on homolgies with ESTS from both zebrafish itself and other species. cDNA indicates a complete cDNA clone has been identified and sequenced.



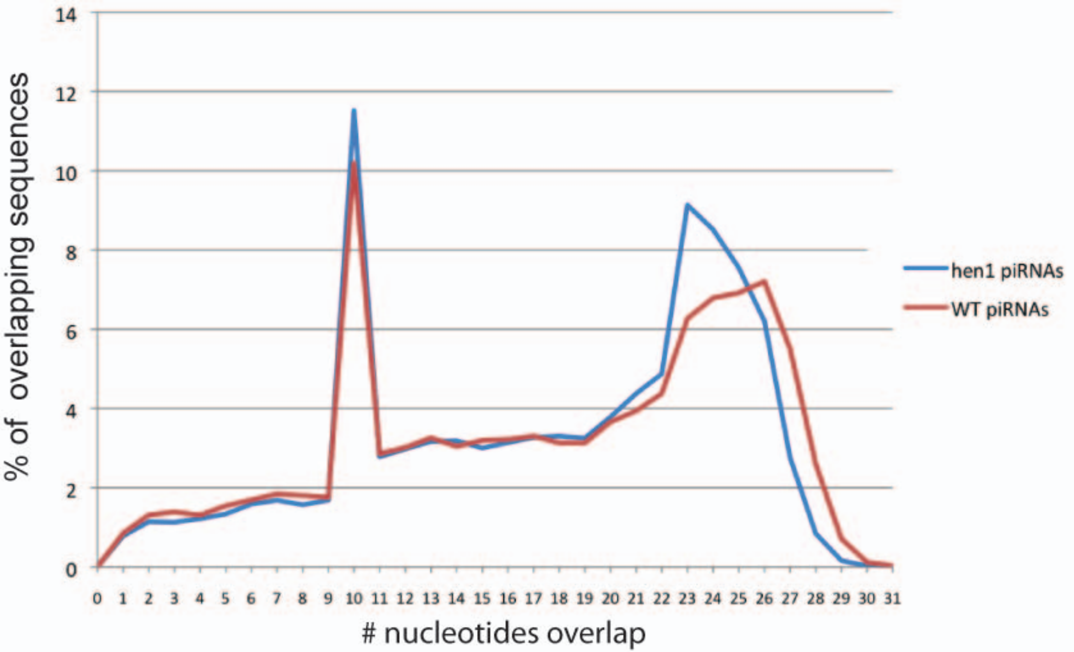
Kamminga_Figure S1



Kamminga_Figure S2

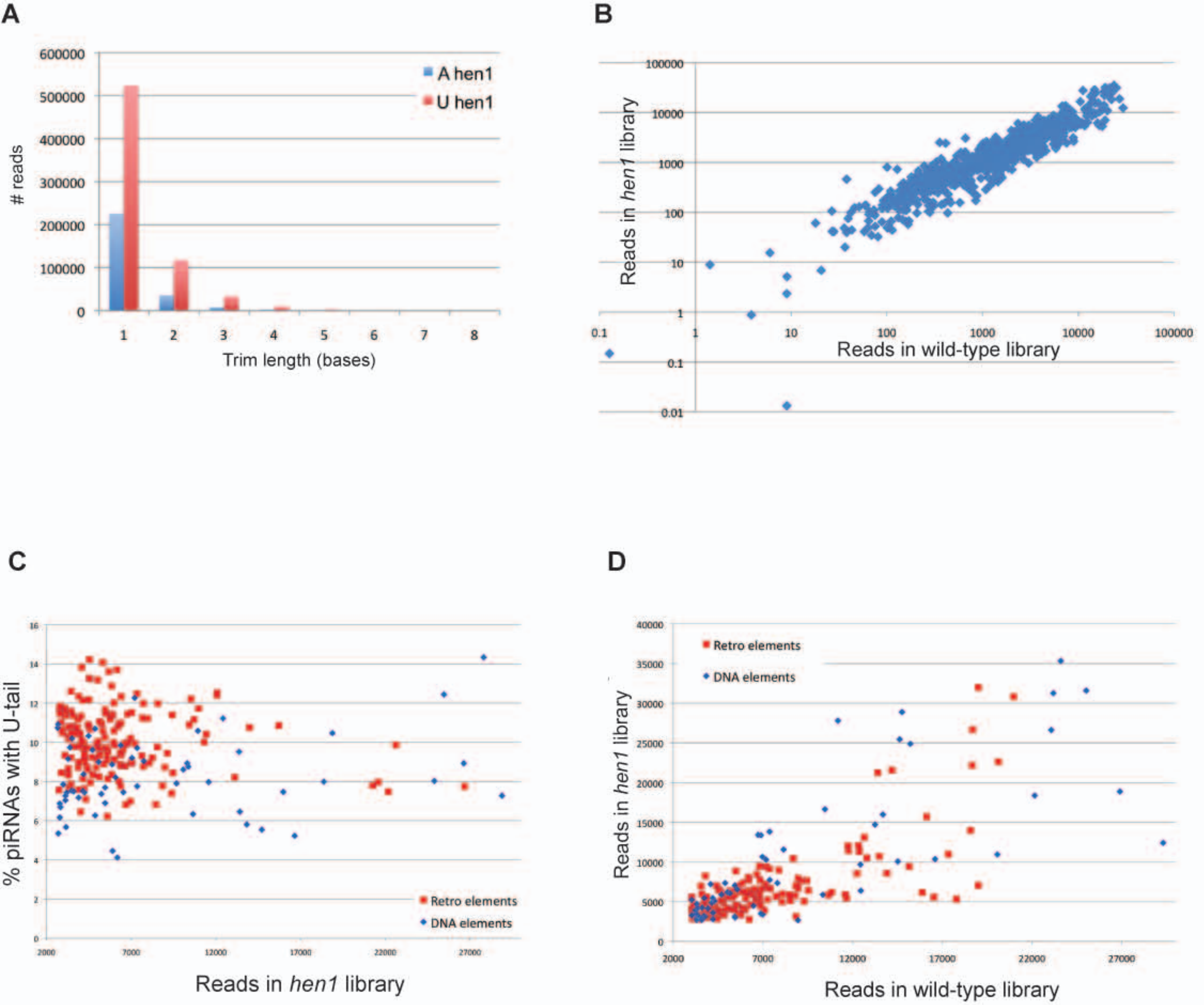


Kamminga_Figure S3



	# of sequences showing overlap
hen1 piRNAs	317695.5
WT piRNAs	215079.7

Kamminga_Figure S4



Kamminga_Figure S5

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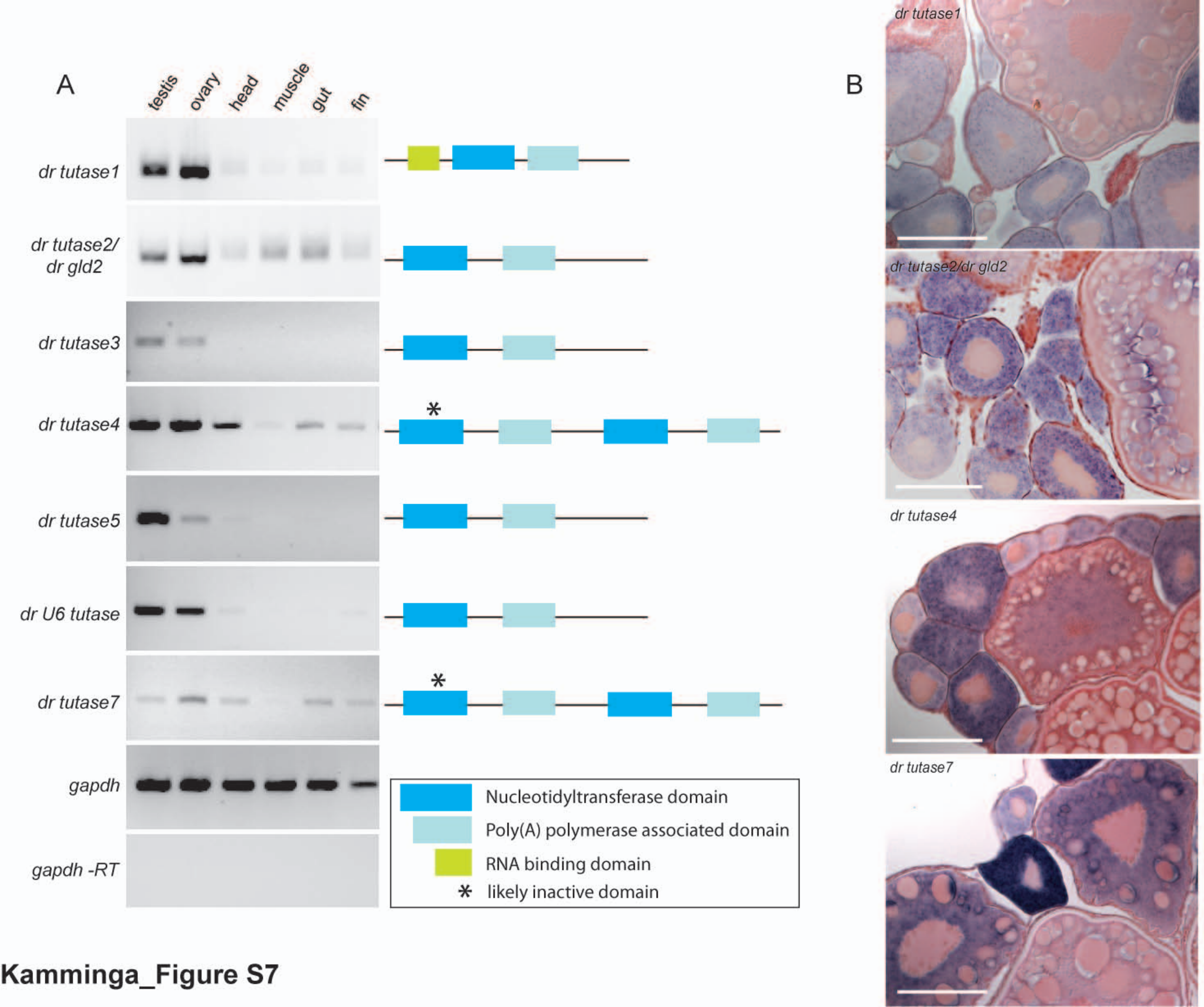
Kamminga_FigureS6



Kamminga_Figure S7