

Clues of *in vivo* nuclear gene regulation by mitochondrial short non-coding RNAs

Marco Passamonti

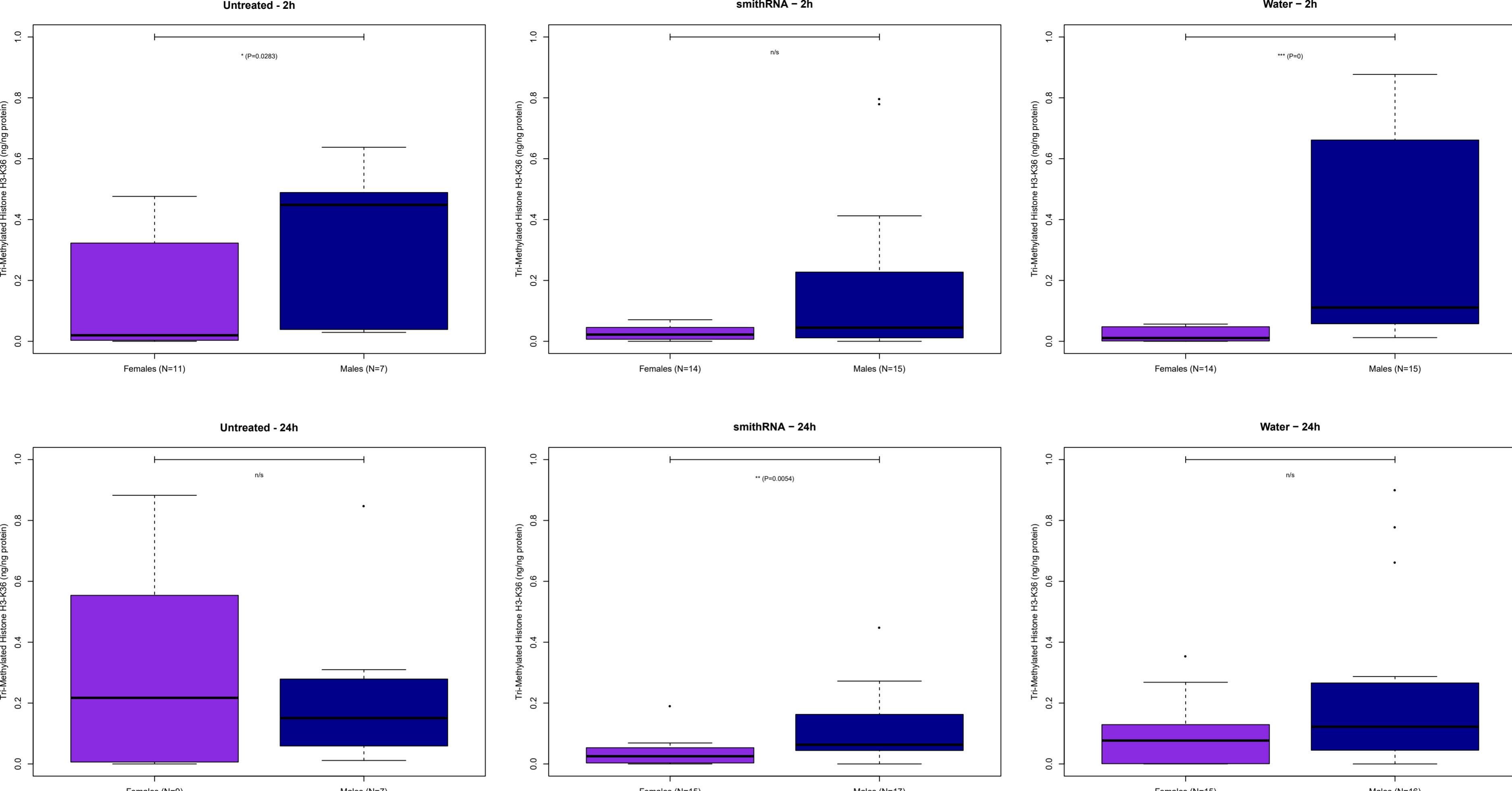
Marco Calderone

Manuel Delpero

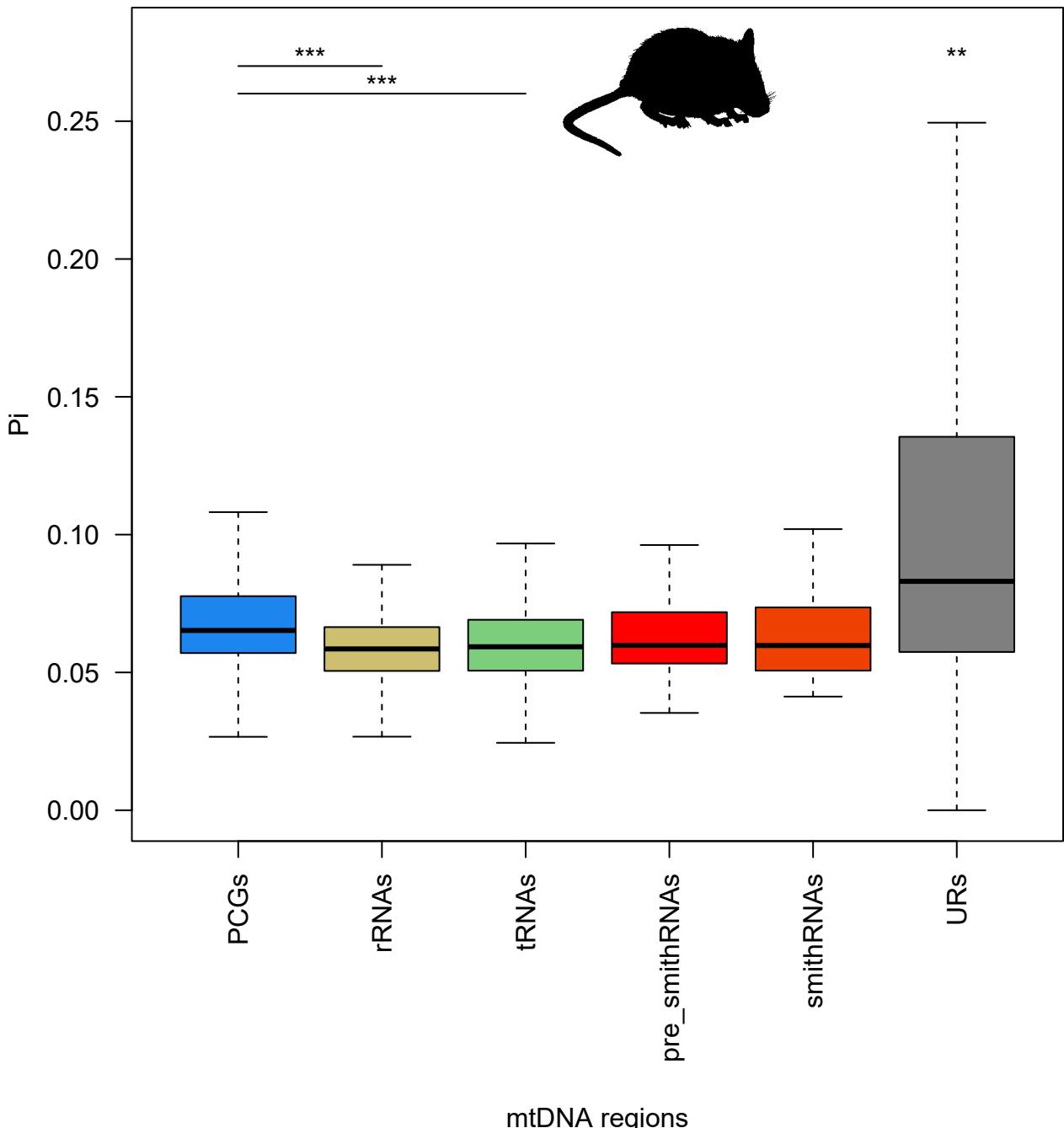
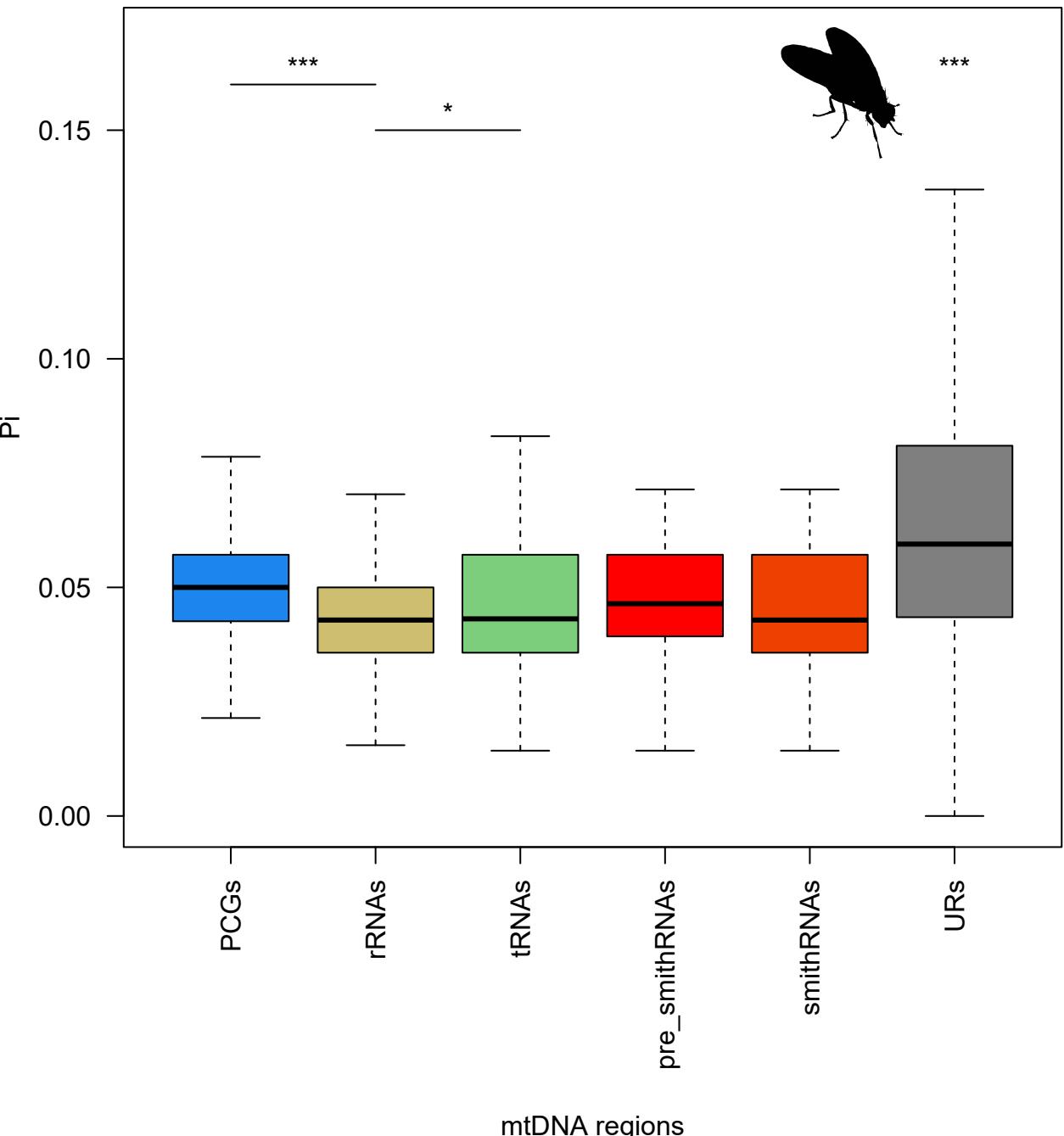
Federico Plazzi

Supplementary Dataset S1. Newly annotated smithRNAs in *Danio rerio*, *Drosophila melanogaster*, and *Mus musculus*. For naming conventions, see Methods.

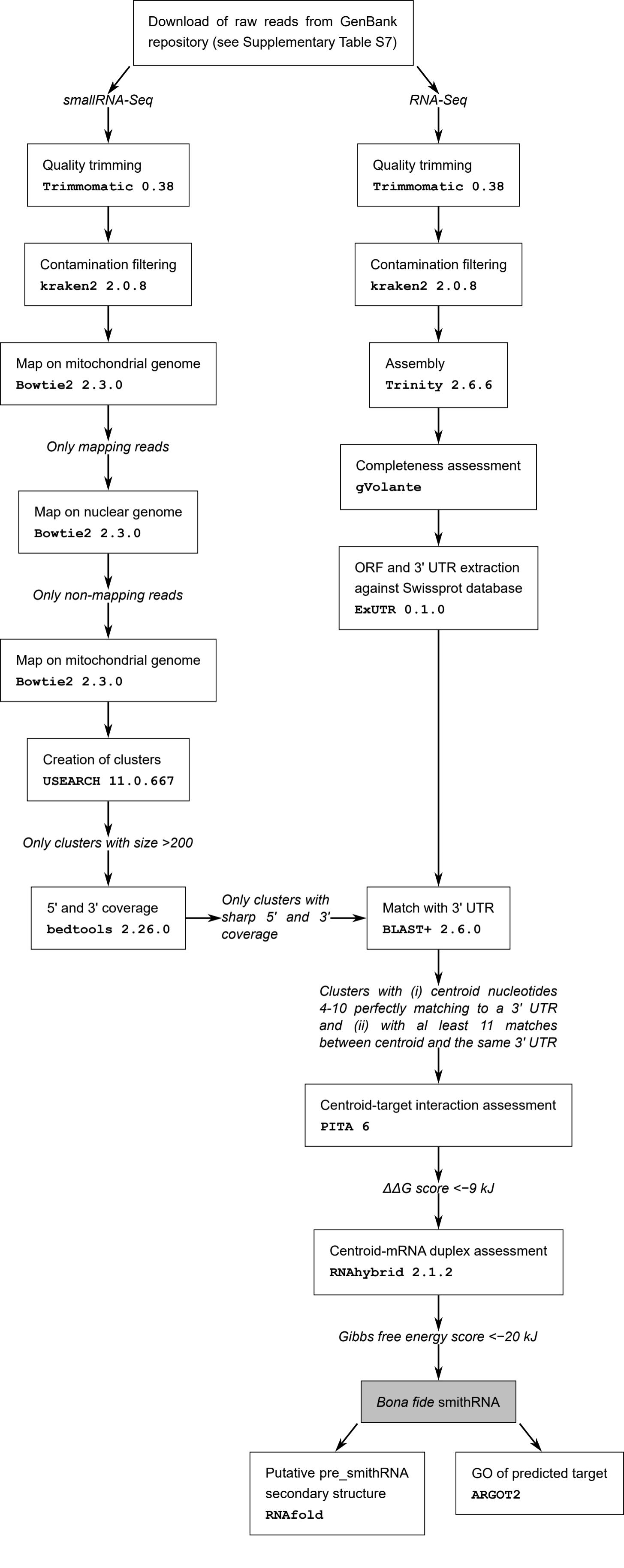
**Supplementary Dataset S2. Secondary structure of genomic context.** The secondary structure of the genomic context of each smithRNA provided in Supplementary Dataset S1 online is shown in the dot-bracket (Vienna) format; the Gibbs free energy is provided within parentheses, after the secondary structure. See Methods for naming conventions.



**Supplementary Figure S1. Effect of the injection of M\_smithRNA106t on Manila clams subdivided by time after treatment and sex.** Untreated, uninjected individuals; smithRNA, individuals injected with M\_smithRNA106t; water, individuals injected with ddH<sub>2</sub>O. The black line is the median; the two hinges of the box approximate the first and the third quartile; whiskers extend to a roughly 95% confidence interval.



**Supplementary Figure S2.** Nucleotide diversity ( $P_i$ ) computed along a sliding window over an alignment of (a) 43 *Drosophila melanogaster* and (b) 162 *Mus musculus* complete mitochondrial genomes. All significant two-tailed pairwise Dunn's test comparisons have an associated  $P < 0.0133$  for *Dr. melanogaster* and  $P < 0.0026$  for *M. musculus*. The black line is the median; the two hinges of the box approximate the first and the third quartile; whiskers extend to a roughly 95% confidence interval.



Supplementary Figure S3. The bioinformatic pipeline used for

the detection of putative smithRNAs in model species.

Supplementary Table S1. Synonymous and nonsynonymous substitutions in PCGs flanking pre-smithRNA loci.

Gene	Length of amplified region	Substitutions		Total
		synonymous	nonsynonymous	
F-nad5 (N=15)	249 bp	1	0	1
F-nad6 (N=15)	492 bp	7	8	15
F-cox2-2 (N=13)	132 bp	0	1	1
M-cox1 (N=25)	229 bp	6	1	7
M-nad1 (N=27)	281 bp	6	1	7
M-nad5 (N=27)	286 bp	12	17	29
M-nad6 (N=27)	492 bp	11	4	15

Supplementary Table S2. Pairwise Dunn's test levels of significance for treatment with M\_smithRNA145t.

Hours after treatment	Sex	Comparison <sup>1</sup>	Dunn's test level of significance
2	F	Untreated–M_smithRNA145t	0.145
		Untreated–F_smithRNA107t	0.002
		M_smithRNA145t–F_smithRNA107t	0.001***
	M	Untreated–M_smithRNA145t	0.794
		Untreated–F_smithRNA107t	1.000
		M_smithRNA145t–F_smithRNA107t	0.662
24	F	Untreated–M_smithRNA145t	1.000
		Untreated–F_smithRNA107t	0.852
		M_smithRNA145t–F_smithRNA107t	0.576
	M	Untreated–M_smithRNA145t	0.657
		Untreated–F_smithRNA107t	1.000
		M_smithRNA145t–F_smithRNA107t	0.605

<sup>1</sup> Untreated, individuals not injected; M\_smithRNA145t, individuals injected with 100 µg of M\_smithRNA145t (involved in histone acetylation regulation); F\_smithRNA107t, individuals injected with 100 µg of F\_smithRNA107t (not involved in histone acetylation regulation).

Supplementary Table S3. GO terms associated with predicted targets of newly annotated smithRNAs provided in Supplementary Dataset S1 online.

**Supplementary Table S4.** Hits of putative smithRNAs detected in *Danio rerio*, *Drosophila melanogaster*, and *Mus musculus*. Sequences were queried against miRbase (<http://www.mirbase.org/>), piRBase (<http://www.regulatoryrna.org/database/piRNA/index.html>), and fRNADB ([http://togodb.biosciencedbc.jp/togodb/view/frnadb\\_summary#en](http://togodb.biosciencedbc.jp/togodb/view/frnadb_summary#en)). When a complete identity was found, the e-value is not provided.

smithRNA	Hit <sup>1</sup>	Organism	Locus <sup>2</sup>	e-value	Database
DaRe_F_smithRNA32r	bdi-miR162	<i>Brachypodium distachyon</i>	3: 49592002-49592134 [+]	1.6	miRBase
DaRe_F_smithRNA32r	pab-miR162a	<i>Picea abies</i>	MA_230619: 2413-2531 [-]	1.6	miRBase
DaRe_M_smithRNA10t	hsa-miR-1286	<i>Homo sapiens</i>	chr22: 20249134-20249211 [-]	5.9	miRBase
DaRe_M_smithRNA10t	ptr-miR-1286	<i>Pan troglodytes</i>	chr22: 6839054-6839130 [-]	5.9	miRBase
DaRe_M_smithRNA10t	ppy-miR-1286	<i>Pongo pygmaeus</i>	chr22: 16146328-16146402 [-]	5.9	miRBase
DaRe_M_smithRNA10t	ggo-miR-1286	<i>Gorilla gorilla</i>	chr22: 3520215-3520325 [-]	5.9	miRBase
DaRe_M_smithRNA49t	piR-mmu-3852	<i>Mus musculus</i>	chrM:3848-3879:+	0.098	fRNADB
DrMe_F_smithRNA127r	FR508480	<i>Drosophila</i>		0.002	fRNADB
DrMe_F_smithRNA127r	FR486950	<i>Drosophila</i>		0.002	fRNADB
DrMe_F_smithRNA127r	FR476767	<i>Drosophila</i>		0.002	fRNADB
DrMe_F_smithRNA127r	FR454233	<i>Drosophila</i>		0.002	fRNADB
DrMe_F_smithRNA127r	FR430424	<i>Drosophila</i>		0.002	fRNADB
DrMe_F_smithRNA127r	FR428652	<i>Drosophila</i>		0.002	fRNADB
DrMe_F_smithRNA127r	FR426856	<i>Drosophila</i>		0.002	fRNADB
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DrMe_F_smithRNA127r	FR505461	<i>Drosophila</i>		0.006	fRNADB
DrMe_F_smithRNA127r	FR497675	<i>Drosophila</i>		0.006	fRNADB
DrMe_F_smithRNA127r	FR476766	<i>Drosophila</i>		0.006	fRNADB
DrMe_F_smithRNA127r	FR414623	<i>Drosophila</i>		0.006	fRNADB
DrMe_F_smithRNA127r	FR497134	<i>Drosophila</i>		0.025	fRNADB
DrMe_F_smithRNA134r	FR408244	<i>Drosophila</i>		4e-04	fRNADB
DrMe_M_smithRNA128r	piR-dme-2563361	<i>D. melanogaster</i>	chrM:12877-12905:-		piRBase
DrMe_M_smithRNA128r	FR465521	<i>Drosophila</i>		7e-08	fRNADB
DrMe_M_smithRNA128r	FR472905	<i>Drosophila</i>		4e-06	fRNADB
DrMe_M_smithRNA128r	FR491761	<i>Drosophila</i>		2e-05	fRNADB
DrMe_M_smithRNA128r	FR461400	<i>Drosophila</i>		2e-05	fRNADB
DrMe_M_smithRNA128r	FR457376	<i>Drosophila</i>		2e-05	fRNADB
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DrMe_M_smithRNA128r	FR491760	<i>Drosophila</i>		7e-05	fRNADB
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DrMe_M_smithRNA128r	FR439102	<i>Drosophila</i>		7e-05	fRNADB
DrMe_M_smithRNA128r	FR439101	<i>Drosophila</i>		7e-05	fRNADB

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DrMe_M_smithRNA141r	FR416077	<i>Drosophila</i>	0.018	fRNAdb
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DrMe_M_smithRNA141r	FR416076	<i>Drosophila</i>	0.070	fRNAdb
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DrMe_M_smithRNA141r	FR432800	<i>Drosophila</i>	0.27	fRNAdb
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DrMe_M_smithRNA144r	FR497449	<i>Drosophila</i>	0.18	fRNAdb
DrMe_M_smithRNA144r	FR467084	<i>Drosophila</i>	0.18	fRNAdb
DrMe_M_smithRNA144r	FR425386	<i>Drosophila</i>	0.18	fRNAdb
MuMu_F_smithRNA11r	piR-mmu-55370144	<i>Mus musculus</i>	chrM:1093-1118:+	piRBase
MuMu_F_smithRNA156nc	piR-mmu-4681867	<i>Mus musculus</i>	chrM:15615-15642:-	piRBase
MuMu_F_smithRNA24r	piR-mmu-51313768	<i>Mus musculus</i>		piRBase
MuMu_F_smithRNA37t	piR-mmu-10841588	<i>Mus musculus</i>		piRBase
MuMu_F_smithRNA7r	piR-mmu-31083152	<i>Mus musculus</i>	chrM:779-809:+	piRBase
MuMu_M_smithRNA8r	piR-mmu-1076637	<i>Mus musculus</i>	chrM:838-866:+	piRBase

<sup>1</sup> Name of retrieved piRNA/miRNA, or fRNAdb Accession.

<sup>2</sup> When available.

Supplementary Table S5. GenBank Accession Numbers of complete mitochondrial genomes of *Drosophila melanogaster* and *Mus musculus*.

Species	GenBank Accession Number
<i>Drosophila melanogaster</i>	AF200828
	CM008274
	CM010505
	CM010512
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	CM010526
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HQ877407  
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JF286601  
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NC\_010339  
NC\_012387

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Supplementary Table S6. PCR primers.

Primer name	Sequence	Reference
MLUR_3604	5'-AGTTTCAAAGCTCTATATTGGGTTA-3'	[50]
MLUR_4442	5'-TTTTAAAACCTCATGGATTCATTACAA-3'	[50]
RuPhM_p1_156F	5'-GCTCCTACTAGTAATATTCTTT-3'	This study
RuPhM_p1_1543R	5'-TTTTATGATTAAACTACCAAAACC-3'	This study
RuPhM_p3_12612F	5'-TTATGTTAACGCGAGATATAAGTA-3'	This study
RuPhM_p3_13106R	5'-AAAGAAAATTATTACCAATCCATG-3'	This study
RuPhM_p4_14438F	5'-GATAGGATTGAAAGATAAAACGTT-3'	This study
RuPhM_p4_14943R	5'-AAAATAACAATAACCCCTAACCTAA-3'	This study
RuPhF_p1_169F	5'-GTCTTCCTATTAATTAGTGTTG-3'	This study
RuPhF_p1_1526R	5'-TTCTTATTAAACTACCAAAACAC-3'	This study
RuPhF_p3_1285F	5'-TGAAATGAGGTCTTCTAATATAAG-3'	This study
RuPhF_p3_1921R	5'-CTTTAAAACCTAAGCCATTAAATA-3'	This study

Supplementary Table S7. SRA Accession Numbers of raw reads used in this study.

Dataset	SRA Accession Number
Ovary Replicate 1 sRNASeq; <i>Danio rerio</i> ; miRNA-Seq	SRR1265755
Ovary Replicate 1 sRNASeq; <i>Danio rerio</i> ; miRNA-Seq	SRR1265756
Ovary Replicate 1 sRNASeq; <i>Danio rerio</i> ; miRNA-Seq	SRR1265757
Testis Replicate 1 sRNASeq; <i>Danio rerio</i> ; miRNA-Seq	SRR1265758
Testis Replicate 1 sRNASeq; <i>Danio rerio</i> ; miRNA-Seq	SRR1265759
Testis Replicate 1 sRNASeq; <i>Danio rerio</i> ; miRNA-Seq	SRR1265760
ovary_rep1; <i>Danio rerio</i> ; RNA-Seq	SRR6841457
ovary_rep1; <i>Danio rerio</i> ; RNA-Seq	SRR6841458
ovary_rep1; <i>Danio rerio</i> ; RNA-Seq	SRR6841459
ovary_rep2; <i>Danio rerio</i> ; RNA-Seq	SRR6841460
ovary_rep2; <i>Danio rerio</i> ; RNA-Seq	SRR6841461
ovary_rep2; <i>Danio rerio</i> ; RNA-Seq	SRR6841462
testis_rep1; <i>Danio rerio</i> ; RNA-Seq	SRR6841469
testis_rep1; <i>Danio rerio</i> ; RNA-Seq	SRR6841470
testis_rep1; <i>Danio rerio</i> ; RNA-Seq	SRR6841471
testis_rep2; <i>Danio rerio</i> ; RNA-Seq	SRR6841472
testis_rep2; <i>Danio rerio</i> ; RNA-Seq	SRR6841473
testis_rep2; <i>Danio rerio</i> ; RNA-Seq	SRR6841474
smalllRNA X^X (control) ovary Replica 1; <i>Drosophila melanogaster</i> ; ncRNA-Seq	SRR5181429
smalllRNA X^X (control) ovary Replica 2; <i>Drosophila melanogaster</i> ; ncRNA-Seq	SRR5181430
smalllRNA X^Y (control) testis Replica 1; <i>Drosophila melanogaster</i> ; ncRNA-Seq	SRR5181435
smalllRNA X^Y (control) testis Replica 2; <i>Drosophila melanogaster</i> ; ncRNA-Seq	SRR5181436
mRNA X^X (control) ovary Replica 1; <i>Drosophila melanogaster</i> ; RNA-Seq	SRR5181417
mRNA X^X (control) ovary Replica 2; <i>Drosophila melanogaster</i> ; RNA-Seq	SRR5181418
mRNA X^Y (control) testis Replica 1; <i>Drosophila melanogaster</i> ; RNA-Seq	SRR5181423
mRNA X^Y (control) testis Replica 2; <i>Drosophila melanogaster</i> ; RNA-Seq	SRR5181424
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR083999
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR084000
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR084001
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR084005
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR084006
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR084007
Illumina HiSeq 1500 paired end sequencing of SAMD00048521	DRR084021
Illumina HiSeq 1500 paired end sequencing of SAMD00048521	DRR084022
Illumina HiSeq 1500 paired end sequencing of SAMD00048521	DRR084024
Illumina HiSeq 1500 paired end sequencing of SAMD00048521	DRR084031
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR084032
Illumina HiSeq 1500 sequencing of SAMD00048521	DRR084033
P14_Con_s1; <i>Mus musculus</i> ; ncRNA-Seq	SRR3521024

P14_Con_s2; <i>Mus musculus</i> ; ncRNA-Seq	SRR3521025
P14_Con_1; <i>Mus musculus</i> ; RNA-Seq	SRR3520994
P14_Con_2; <i>Mus musculus</i> ; RNA-Seq	SRR3520995

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