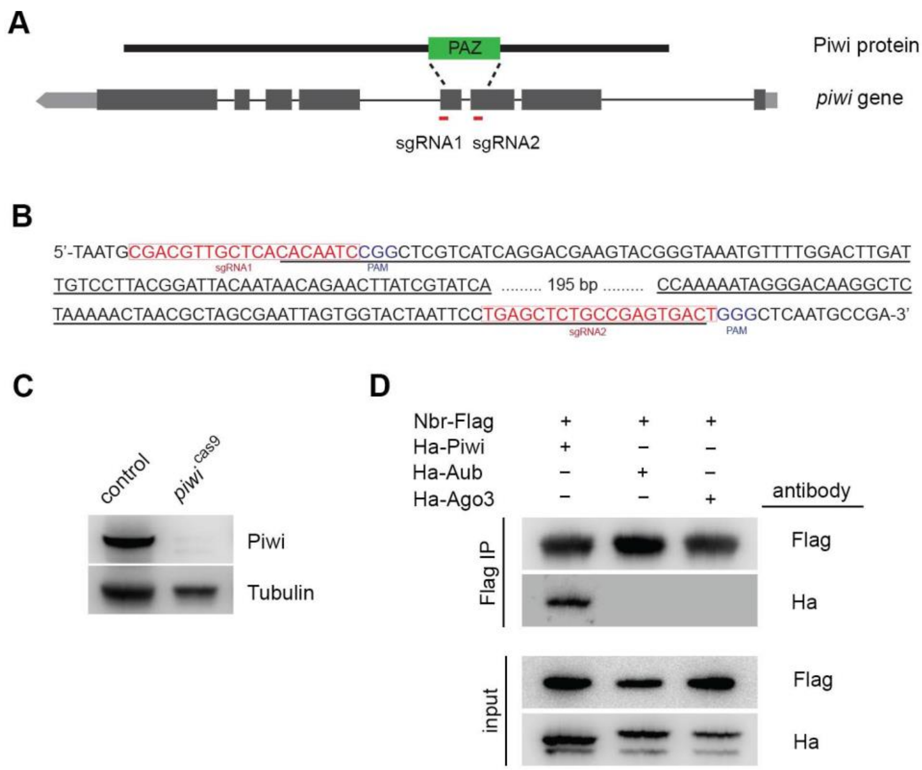


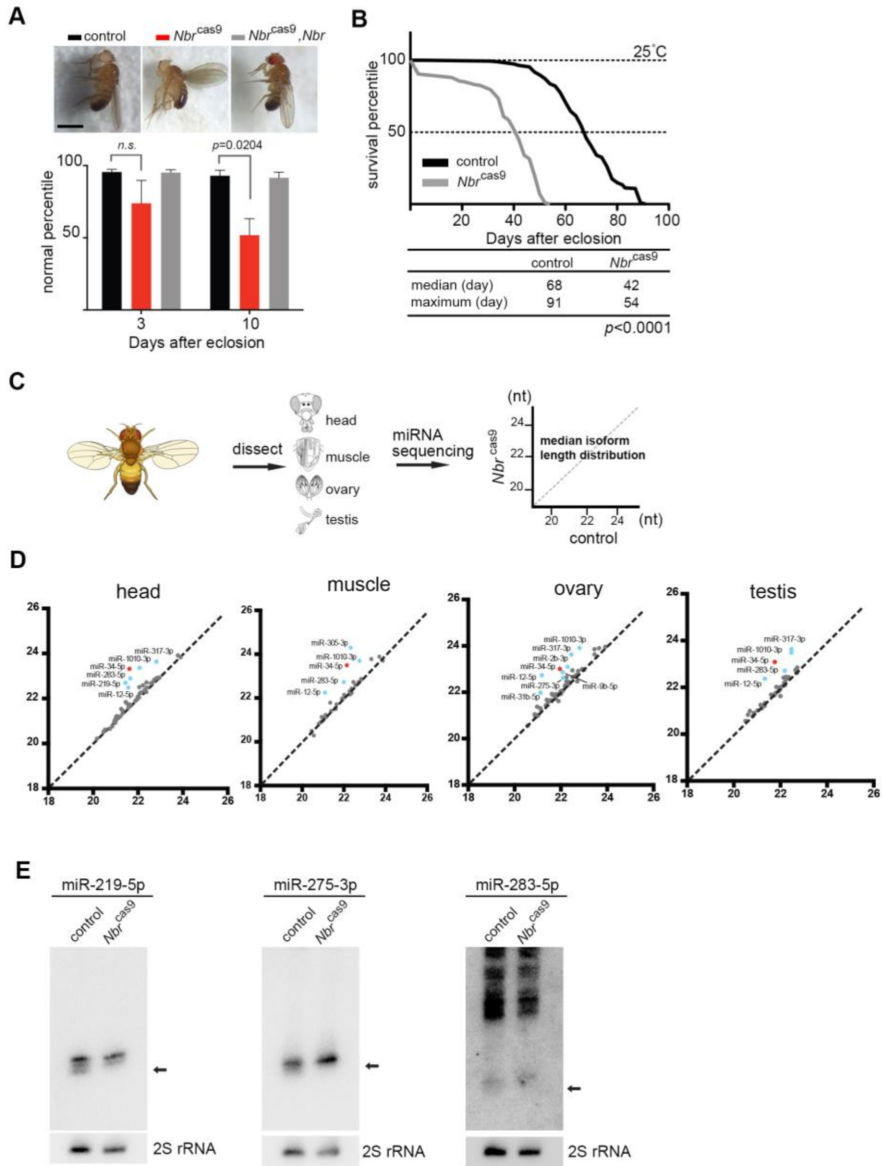
Supplementary Figure 1. Nbr interacts with Piwi at the protein level.

A. Map of the *piwi* locus and guiding RNAs used to make *piwi*^{cas9}. To make *piwi* loss-of-function allele based on the CRISPR/Cas9 method, sgRNA 1 and sgRNA 2, as illustrated in red lines, were used to induce site-specific deletion. The deleted region corresponds to the PAZ domain of Piwi.

B. *piwi*^{cas9} carries a genomic deletion on the *piwi* gene. Part of *piwi* genomic sequence was shown to illustrate design features of CRISPR/Cas9-mediated *piwi* loss-of-function allele. sgRNAs and PAMs were highlighted at a nucleotide resolution. Nucleotides removed were underlined. Resulted *piwi* mutant flies were backcrossed to the control homogeneous background for five generations, to ensure background clearance.

C. Western blot confirms the specificity of Piwi antibody. Flies of indicated genotypes were assayed by western analysis using the polyclonal antibody for Piwi. Proteins were from ovaries. Genotypes: control (5905). *piwi*^{cas9} (*piwi*$^{cas9/cas9}$).

D. Nbr interacts with Piwi. In *Drosophila* S2 cells, co-IP experiments were conducted to determine if Nbr could interact with Piwi, Aub, and Ago3. Proteins were from S2 cells.



Supplementary Figure 2. Loss of *Nbr* induces adult-onset, age-associated deficits, and miRNA trimming defects.

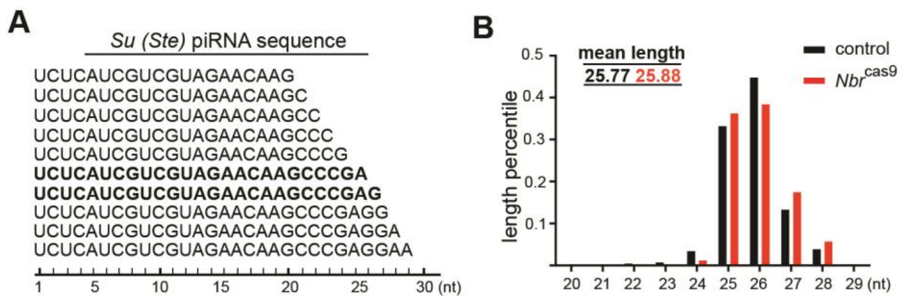
A. Lack of *Nbr* triggers an adult-onset, progressive deficit. *Nbr*^{cas9} flies exhibited expected adult emergence and gross morphology, suggesting normal development. Examination of adult animals revealed that, whereas controls had flat wing posture (top left panel), some *Nbr*^{cas9} flies showed wing held-up (Top middle panel), a phenotype commonly linked with stress conditions. Upon slight age, the defect of wing postures became significantly enhanced, suggesting progressive deterioration. Flies were raised at 25°C. Wing held up phenotype: Mean ± SD, n ≥ 40 flies (student *t*-test). Genotypes: control (5905), *Nbr*^{cas9} (*Nbr*$^{cas9/cas9}$), *Nbr*^{cas9} *Nbr* (pUAST-*Nbr*, *Nbr*$^{cas9/cas9}$; GeneSwitch-*tubulin*-GAL4).

B. *Nbr*^{cas9} flies (grey) had a sharp decline in adult viability compared to controls (black). Flies were raised at 25°C. Mean ± SE, n ≥ 200 flies for curve (p<0.0001, log-rank test). Genotypes: 5905 and *Nbr*$^{cas9/cas9}$.

C. A scheme to reveal miRNAs trimmed by Nbr. Using dissected adult tissues, we compared length for all miRNA reads between control and mutant. Our rationale lies at the fact that *Nbr* loss would accumulate higher isoforms due to defective trimming, thus tweaking the ratio among isoforms.

D. miRNAs with altered length in *Nbr*^{cas9}. Scatterplots for indicated adult tissues were made based on miRNA length alterations between control and *Nbr*^{cas9}. To highlight significantly lengthened miRNAs, we chose a cutoff of 0.5 nt, a mean length difference for particular miRNAs between control and mutant. In the graph, miR-34-5p was highlighted in red, while other miRNAs with a significantly lengthened size in *Nbr*^{cas9} were shown in blue. Genotypes: 5905 (x-axis) and *Nbr*$^{cas9/cas9}$ (y-axis).

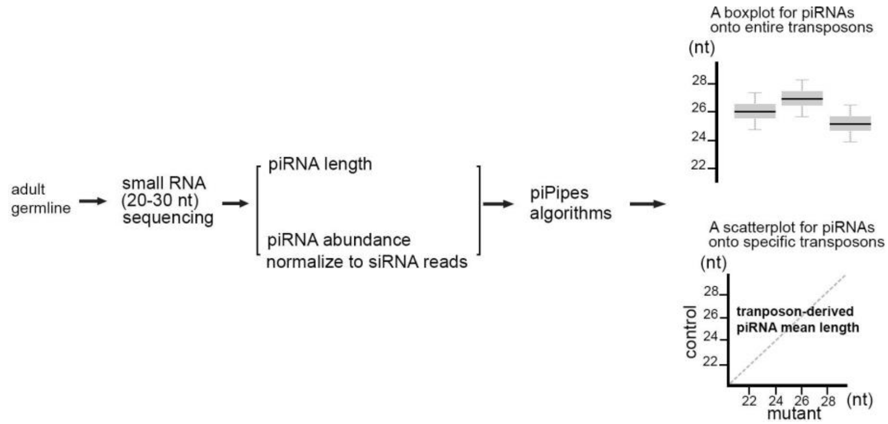
E. Nbr-substrate miRNAs show abolished lower isoforms in *Nbr*^{cas9}. Small RNA northern was using RNA from heads (miR-219-5p, miR-283-5p) and ovaries (miR-275-3p). Arrow indicated trimmed isoforms in control, which became disappeared upon *Nbr* loss. 2S rRNA was used as a loading control. Genotypes as in B.



Supplementary Figure 3. *Nbr* loss impacts sequence-specific piRNA

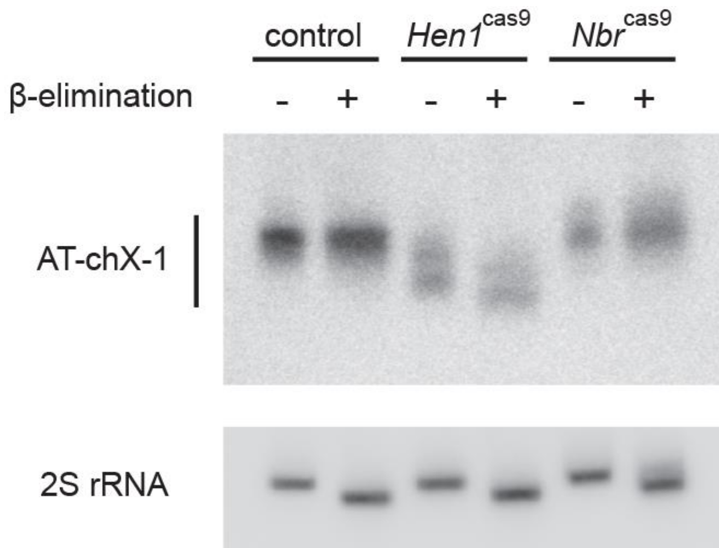
A. In the deep sequencing result, a testicular piRNA, *Su(Ste),* has a defined sequence, starting with a 5’ uridine, with heterogeneous 3’ ends of nested series. Main forms of 25 and 26 nt were shown in bold.

B. Length distribution graph indicates that *Su(Ste)* piRNAs accumulate more long forms in *Nbr*^{cas9} than in control. RNAs were from testis. Genotypes: 5905 and *Nbr*$^{cas9/cas9}$.

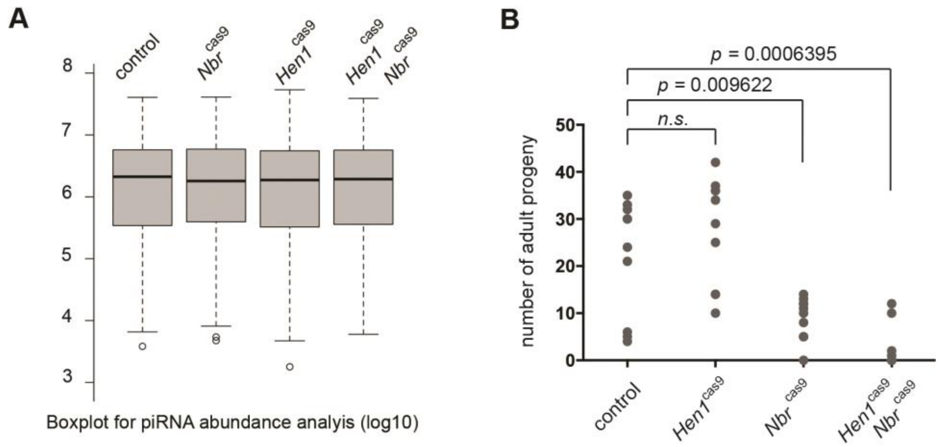


Supplementary Figure 4. Computational pipeline to analyze piRNAs

A dedicated algorithm called piPipes was used for piRNA analysis. Through piPipes, piRNAs mapped to 127 major *Drosophila* transposons were used. A mean value was calculated based on the length of piRNAs mapped to specific transposons. In boxplots, the horizontal bar (black) inside the box represented the median length of all 127 transposons, while the area of box (grey) represented the range of 25%-75% in the mean length of all 127 transposons. The whiskers indicated variability outside the upper and lower quartiles. Outliers beyond the whiskers were plotted as points. The use of boxplot allowed side-by-side comparison across multiple genotypes/samples. In scatterplots, individual transposons were shown as single dots (black), allowing comparison for individual transposons between two genotypes/samples.



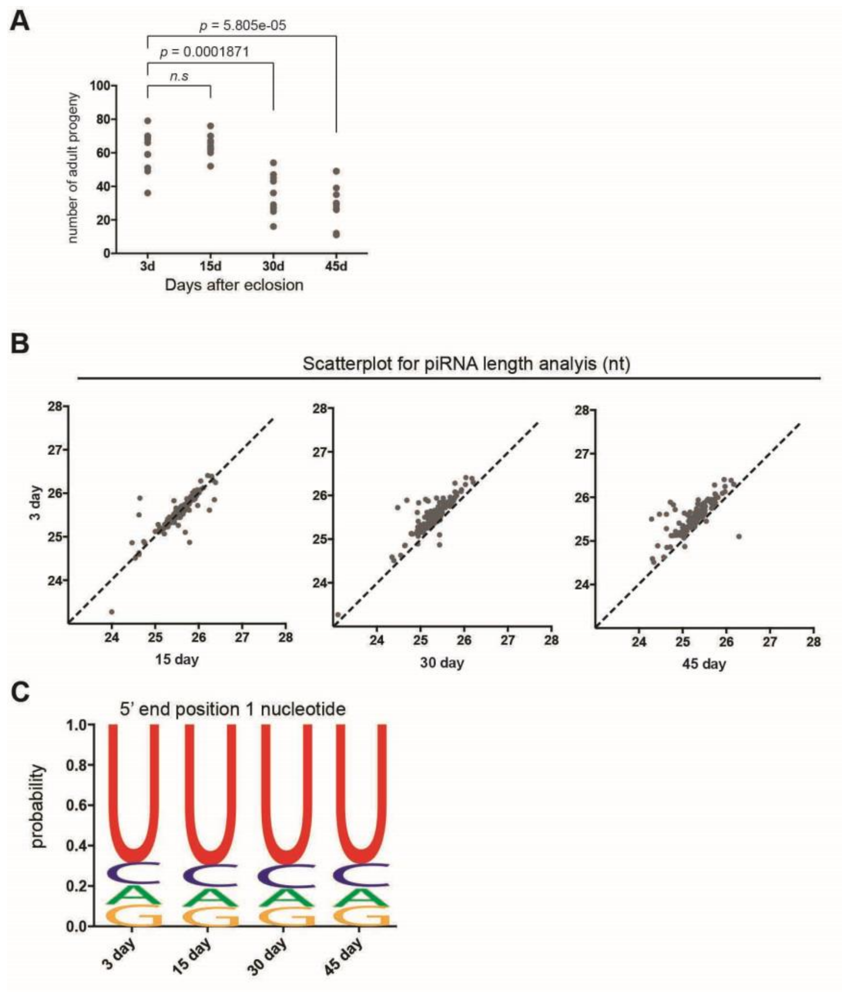
Supplementary Figure 5. piRNAs remain 3' methylated in *Nbr* mutants.
Small RNA northern blot revealed that, AT-chX-1 piRNAs were resistant to beta-elimination, in control and *Nbr*^{cas9} mutants, but not in *Hen1*^{cas9} mutants, suggesting that these piRNAs were 2'-*O*-methylated at 3' ends. Note that AT-chX-1 became shorter in *Hen1*^{cas9} mutants compared to controls, reflecting enhanced trimming upon loss of *Hen1*. RNAs were from testis. Genotypes: control (5905), *Hen1*^{cas9} (*Hen1*$^{cas9/cas9}$), *Nbr*^{cas9} (*Nbr*$^{cas9/cas9}$).



Supplementary Figure 6. Characteristics of mutants.

A. Analysis of piRNA abundance. RNAs were from ovaries of 3d old animals with indicated genotypes. Boxplots for normalized read counts were shown as log10 value. (*Nbr*^{cas9} vs. 5905: $p = 0.05525$; *Hen1*^{cas9} vs. 5905: $p = 0.0003845$; *Nbr*^{cas9}, *Hen1*^{cas9} vs. 5905: $p = 0.233$; Wilcoxon signed-rank test). Genotypes: control (5905), *Hen1*^{cas9} (*Hen1*$^{cas9/cas9}$), *Nbr*^{cas9} (*Nbr*$^{cas9/cas9}$) and double mutant.

B. Flies without *Nbr* show reduced female fecundity. Fertility test revealed that whereas *Hen1*^{cas9} mutants had normal fertility, *Nbr*^{cas9} mutants and *Hen1*^{cas9} *Nbr*^{cas9} double mutants demonstrated a significant decrease in female fecundity. n=10 independent tests, (student ***t***-test with Welch correction). Genotypes: control (5905), *Hen1*^{cas9} (*Hen1*$^{cas9/cas9}$), *Nbr*^{cas9} (*Nbr*$^{cas9/cas9}$) and double mutant.

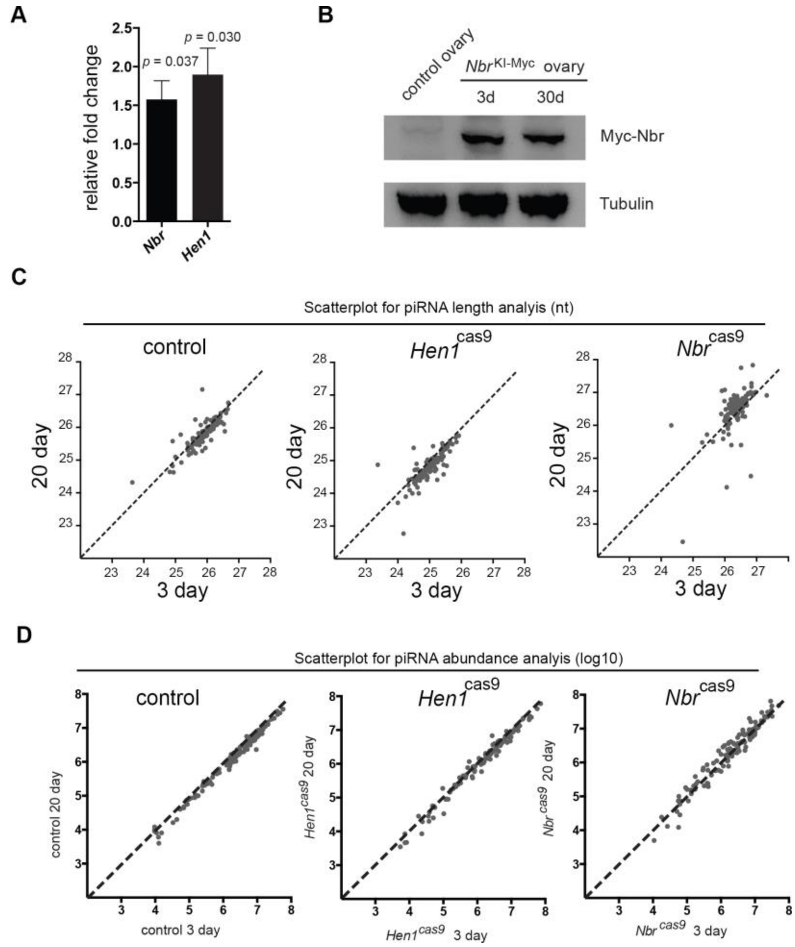


Supplementary Figure 7. With age, piRNAs become progressively shortened from 3' ends.

A. Female fecundity shows age-associated decline. n=10 independent tests, (student ***t***-test with Welch correction). Genotypes: control (5905).

B. piRNAs become progressively shortened with age. Scatterplots for piRNA length analysis revealed age-dependent length changes. (15d vs. 3d: $p = 0.001371$; 30d vs. 3d: $p < 2.2\times10^{-16}$; 45d vs. 3d: $p < 2.2\times10^{-16}$; Wilcoxon signed-rank test). RNAs were from ovaries of indicated ages. Genotype as in A.

C. piRNAs in adult flies with indicated ages show the same bias for 5' uridine (1U). Genotype as in A.



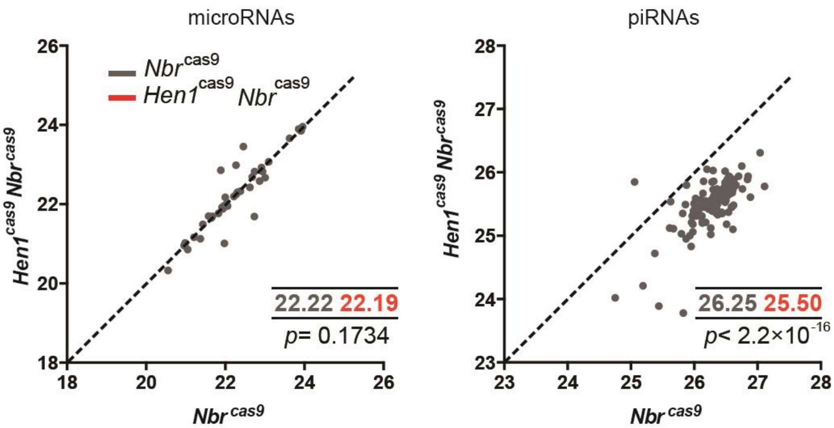
Supplementary Figure 8. *Nbr* contributes to the age-modulated piRNA profile.

A. *Nbr* and *Hen1* show increased expression level with age. qRT-PCR was used. RNAs were from ovaries at 3d or 30d. Mean ± SD, n = 3 independent experiments (student *t*-test). Genotype (5905).

B. Nbr proteins show unchanged levels with age. Proteins were from ovaries of indicated age. Genotypes: control (5905). *Nbr*$^{\text{KI-Myc}}$ (*Nbr*$^{\text{KI-Myc/KI-Myc}}$).

C. *Nbr* contributes to age-modulation of piRNAs. Scatterplots showed that, with slight aging, piRNAs became shortened in control (left panel) and *Hen1*$^{\text{cas9}}$ (middle panel), but not in *Nbr*$^{\text{cas9}}$ (right panel). RNAs were from ovaries of indicated genotypes. (5905: 20d vs. 3d: $p = 2.948\times10^{-10}$; *Hen1*$^{\text{cas9}}$: 20d vs. 3d: $p = 2.634\times10^{-15}$; *Nbr*$^{\text{cas9}}$: 20d vs. 3d: $p = 7.098\times10^{-8}$; Wilcoxon signed rank test). Genotypes: control (5905), *Hen1*$^{\text{cas9}}$ (*Hen1*$^{\text{cas9/cas9}}$), *Nbr*$^{\text{cas9}}$ (*Nbr*$^{\text{cas9/cas9}}$).

D. *Nbr* modulates piRNA abundance with age. Scatterplots showed a trend in that, with slight aging, piRNA levels became decreased in control (left panel) and *Hen1*^{cas9} (middle panel), but not in *Nbr*^{cas9} (right panel). RNAs were from ovaries of indicated genotypes. (5905: 20d vs. 3d: $p < 2.2\times10^{-16}$; *Hen1*^{cas9}: 20d vs. 3d: $p = 8.454\times10^{-11}$; *Nbr*^{cas9}: 20d vs. 3d: $p = 0.1271$;Wilcoxon signed-rank test).



Supplementary Figure 9. piRNAs may be trimmed by an unknown "trimmer". Sequence analysis and comparison revealed piRNAs, but not miRNAs, became further shortened in *Hen1*^{cas9} *Nbr*^{cas9} double mutants compared to *Nbr*^{cas9} single mutants. This data may implicate the effect of a second "unknown" trimmer that modulates piRNA sequence. RNAs were from ovaries of indicated genotypes. Wilcoxon rank sum test.

Supplementary Table 1. miRNAs trimmed by Nbr

This table summarizes a list of miRNAs that are trimmed by Nbr at a tissue resolution. For miRNAs with relatively high expression, small RNA northern was used for validation, together with small RNA sequencing data.

	tissue type	validation
miR-2b-3p	ovary	sequencing
miR-3-3p	whole body	sequencing; small RNA northern
miR-7-5p	whole body	sequencing; small RNA northern
miR-9b-5p	ovary	sequencing
miR-10-3p	whole body	sequencing; small RNA northern
miR-11-3p	whole body	sequencing
miR-12-3p	head;muscle;testis;ovary	sequencing; small RNA northern
miR-31b-5p	ovary	sequencing
miR-34-5p	head;muscle;testis;ovary	sequencing; small RNA northern
miR-100-5p	whole body	sequencing
miR-190-5p	whole body	sequencing; small RNA northern
miR-219-5p	head	sequencing; small RNA northern
miR-263a-5p	whole body	sequencing
miR-263b-5p	whole body	sequencing
miR-275-3p	ovary	sequencing; small RNA northern
miR-281-1/2-5p	whole body	sequencing
miR-283-5p	head;muscle;testis;ovary	sequencing; small RNA northern
miR-305-5p	muscle	sequencing; small RNA northern
miR-317-3p	head;ovary;testis	sequencing; small RNA northern
miR-1010-3p	head;muscle;ovary;testis	sequencing

Supplementary Table 2. Mean length for piRNA lengths in different mutants

Summary for major transposons in the fly genome was listed. Transposons further analyzed by qRT-PCR were in red.

	control	*Nbr*[cas9]	*Hen1* [cas9]	*Hen1* [cas9] *Nbr* [cas9]
frogger	25.34	27.11	25.48	25.78
aurora_element	25.33	26.54	23.96	25.37
R1_2	25.38	26.5	25.35	25.56
jockey2	24.75	25.86	24.38	25.49
X_element	25.41	26.49	24.44	25.53
jockey	24.89	25.93	24.23	25
G6	25.31	26.3	24.6	25.54
GATE	25.38	26.37	24.73	25.66
Tc1_2	25.91	26.84	24.92	25.89
transib1	25.45	26.31	24.87	25.86
R1A1-element	25.68	26.54	24.41	25.65
Rt1a	25.81	26.66	24.7	25.8
baggins	25.86	26.6	25.04	25.86
Max-element	25.87	26.6	24.67	25.68
stellateHet	25.76	26.49	24.98	25.61
G2	26.03	26.75	24.92	26.1
BS3	25.9	26.61	24.92	25.1
Rt1b	25.65	26.35	24.46	25.59
17	25.64	26.33	25.07	25.71
stellate	25.95	26.62	24.91	25.48
HeT-A	25.49	26.13	24.34	25.22
gypsy8	25.92	26.55	25.67	25.94
gypsy7	25.97	26.59	24.58	25.45
suffix	24.79	25.38	24.67	24.72
Rt1c	25.78	26.37	24.97	25.5

opus	25.88	26.47	24.99	25.75
Doc	25.38	25.96	24.36	25.21
Fw3	25.41	25.99	24.59	25.34
suste	25.44	26.01	24.59	25.46
mdg3	25.5	26.07	24.53	25.39
Osvaldo	26.02	26.59	24.36	25.45
diver2	25.56	26.12	24.84	25.31
G3	25.67	26.23	25.06	25.42
roo	25.94	26.5	25.18	25.69
looper1	25.49	26.02	25.06	25.33
INE_1	25.58	26.11	24.82	25.3
S_element	25.75	26.28	24.94	25.85
1731	25.77	26.3	24.81	25.45
Fw2	25.46	25.98	25.07	25.06
Doc3_eleme	25.56	26.08	24.75	25.43
G_element	25.84	26.36	24.65	25.74
accord	25.47	25.98	24.71	25.18
TAHRE	25.69	26.2	24.74	26.05
BS	25.77	26.27	24.97	25.57
invader3	25.79	26.29	24.97	25.5
G4	25.79	26.29	25.01	25.43
R2_element	25.89	26.39	25.25	25.62
1360	25.93	26.43	24.89	25.6
Porto1	25.67	26.16	25.21	25.45
pogo	25.7	26.18	25.12	25.21
F_element	25.51	25.99	24.49	25.39
Ivk	25.83	26.3	24.97	25.73
Tirant	25.96	26.43	25.29	25.37

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TART_C_TAR	25.72	26.18	24.65	25.55
accord2	25.93	26.39	25.18	25.62
transib2	26.24	26.7	25.45	25.94
I-element	25.78	26.24	24.72	25.47
Doc2_eleme	25.72	26.17	24.92	25.48
copia	26.1	26.55	24.57	25.71
HMS_Beagle	26.01	26.44	25.11	25.59
mst40	25.73	26.15	25.13	25.86
Tc1	25.54	25.95	25.43	24.83
3S18	25.69	26.1	24.6	25.57
gypsy12	25.94	26.35	25.03	25.58
rooA	26.03	26.44	25.16	25.67
invader2	26.12	26.53	25.12	25.81
diver	25.63	26.02	24.76	25.59
McClintock	25.91	26.3	25.3	26.03
gypsy3	25.95	26.34	24.85	25.36
HMS_Beagle	26.13	26.51	24.74	25.18
Stalker	26.23	26.61	25.38	25.73
invader4	26.53	26.89	24.54	25.61
G5A	25.78	26.13	24.37	25.2
Cr1a	26.17	26.52	25.05	25.89
Stalker4	26.21	26.56	25.35	25.66
NOF	25.77	26.11	25.08	25.48
hobo	26.14	26.48	25.28	25.77
gypsy4	26.15	26.49	25.14	25.67
Stalker2	26.32	26.66	25.48	25.8
invader1	25.96	26.29	24.77	25.51
Dm88	26.3	26.63	25.16	25.89

Q_element	26.71	27.04	25.45	26.31
springer	25.98	26.3	24.96	25.39
gtwin	26.55	26.85	25.85	25.94
blood	26.06	26.35	25.05	25.43
Helena	26.21	26.5	25.37	25.67
297	26.29	26.58	25.31	25.75
TARTA	25.86	26.14	25.32	25.67
gypsy2	26.14	26.42	25.51	25.72
Idefix	26.15	26.43	25.29	25.57
mdg1	26.16	26.44	25.17	25.35
Circe	26.03	26.31	25.13	25.63
gypsy10	26.26	26.53	25.57	25.64
gypsy9	26	26.26	24.09	25.12
ZAM	26.03	26.28	25.21	25.39
Tabor	26.19	26.44	25.44	25.58
micropia	26.52	26.76	25.19	25.74
412	26.08	26.31	25.42	25.58
gypsy	26.08	26.3	25.39	25.58
gypsy6	24.99	25.19	24.17	24.21
rover	25.61	25.8	24.74	25.03
flea	25.84	26.03	24.9	25.46
G5	25.86	26.05	24.7	25.37
Transpac	26.18	26.37	24.88	25.33
hopper2	25.26	25.44	23.53	23.89
Stalker3	26.38	26.56	25.59	25.93
S2	25.86	26.03	24.86	25.31
Burdock	25.84	26	24.6	25.51
Quasimodo	26.1	26.23	25.11	25.47

gypsy11	26.37	26.49	25.28	25.84
mariner2	25.54	25.63	24.55	25.54
hopper	25.75	25.82	24.46	25.35
gypsy5	26.42	26.49	25.55	25.71
Bari1	25.55	25.61	24.68	25.12
Doc4_element	25.82	25.87	24.55	24.95
transib3	26.23	26.26	24.53	25.02
FB	24.74	24.75	24.24	24.02
HB	26	25.97	25.5	25.31
Juan	26.53	26.49	25.7	26.02
G7	26.02	25.88	24.66	25.8
invader5	25.96	25.67	25.14	25.11
Tom1	25.92	25.06	25.69	25.85
transib4	26.14	22.75	24.72	25.4

Supplementary Table 3. piRNA abundance in different mutants

Transposons further analyzed by qRT-PCR were in red.

	control	*Nbr* ^{cas9}	*Hen1* ^{cas9}	*Hen1* ^{cas9} *Nbr* ^{cas9}
R1A1-element	40555792	40918873	53738491	38956250
roo	34546733	35677729	28894838	35213717
Rt1b	29893067	25404192	24329941	23540594
GATE	29765871	35781378	37764822	36524184
Max-element	23878570	25915874	24995514	25651025
gtwin	18375221	18757781	15419291	14571126
F_element	15829868	19596311	13607558	21286131
412	13198049	15899266	15254488	17610607
Doc	12467788	14848681	9749282	12410250
X_element	12327023	10212972	10113076	12670588
Stalker	11054786	13311987	10426252	11011733
Rt1a	10994816	16169664	11452185	14926210
Stalker4	10673738	13019675	10454206	11022726
Doc3_element	9375582	10887232	6993792	7804955
invader4	9235976	5773847	7579986	6632277
HeT-A	9183285	12781578	9122531	8472331
gypsy12	9083661	10678551	11400864	10570090
297	8993479	28827042	27198270	34197061
baggins	8694890	7710230	6498647	6779597
G_element	8311368	8700602	6584400	6133818
mdg1	8243544	9424532	9163660	9354462
opus	7499437	5915539	9016755	9486698
gypsy4	7489508	7631847	5406112	6786272
blood	7362489	6970538	6238376	7016394
gypsy6	7139684	7690015	6041633	6593523
G2	7063689	5572263	5550461	5677810
Stalker2	6868471	8201728	6108261	6372059

rooA	6690092	9158656	5677760	8500340
Cr1a	6662661	8449936	8062565	8541705
Circe	6311811	6415745	6729623	5672003
TART_C_TAR	5917997	5977014	6808865	6027270
gypsy	5749546	5892751	4519596	4736007
springer	5716495	5417446	4028415	4104576
TAHRE	5466748	7620330	5754409	4856857
gypsy2	5430234	5767966	5318034	5257971
flea	5287676	1147693	7972798	1225206
1731	5230114	3279110	2961506	3748954
gypsy10	5122992	5046472	3982439	4931326
Tabor	4764209	6048619	4705033	5737882
HMS_Beagle	4541105	3387774	2720143	2966920
Quasimodo	4414961	4078264	3124211	3514222
BS	4390388	4191312	2563274	4923770
Idefix	4228648	4258130	3982744	4772878
HMS_Beagle	4224771	2270861	2103363	2171112
3S18	4188719	5760739	3948456	5686902
aurora_element	4021801	3685493	3357721	3857174
rover	3913934	4439335	3740341	4413802
17	3757103	4108886	5071916	5560644
gypsy3	3682778	3554386	2830184	2884272
gypsy8	3655179	3728606	2722929	3411123
micropia	3559118	2417278	2013311	2119921
jockey	3376139	3297674	3546945	4146091
mdg3	3296156	1235716	2524741	1345156
1360	3285512	5156759	5335734	6568159
jockey2	2981839	1342190	1868498	1301785
NOF	2918775	2971627	3143341	4836216
Doc2_element	2688086	2651555	2132027	2253330

invader3	2584627	2078870	1284719	2739027
G6	2576079	627074	945758	379915
invader1	2458468	1693207	1281924	1931929
diver	2372031	1987955	1590040	1618401
R2_element	2260035	2483339	1794447	1517019
gypsy5	2111953	2479300	2062569	2389746
accord	2106928	1801875	3248478	2882497
hobo	1954811	1809029	1713479	2094122
Osvaldo	1877233	1627265	932764	1151843
Ivk	1845080	1550842	1819617	1687646
I-element	1718477	1794646	2068711	1382814
diver2	1550859	1815087	1296286	1787576
mst40	1530674	1362018	1409514	1731263
accord2	1527798	2096587	1512048	2010345
invader2	1523485	1374037	1423788	1649748
suste	1482810	1785831	1558074	1835264
ZAM	1476973	1666397	1097342	1276684
S_element	1444778	1610151	2033213	2184220
transib2	1366037	1032032	2351520	1815126
G5	1315973	1064191	274110	232997
Bari1	1314823	1157956	874114	1132476
BS3	1295669	1514826	1189674	1432758
Juan	1283974	1530649	1264812	1231537
Dm88	1261245	1345772	696032	1065214
TARTA	1230226	1386330	2538630	1668275
Burdock	1182124	1236358	1616024	1696223
copia	1168058	1237437	919496	2176140
McClintock	1130508	1836263	1800464	2155603
Q_element	1007549	1362920	876787	1298171
Rt1c	921877	979467	516378	460132

Transpac	664480	842077	1398779	919364
G4	655365	668500	515109	503493
R1_2	599844	565842	538270	427914
pogo	550207	20837	64731	89295
INE_1	536650	537523	411091	342295
Porto1	444522	490967	325868	381115
G5A	343242	364904	118977	103700
Fw2	322497	326305	540390	437726
gypsy7	317098	433764	258502	359194
gypsy11	308232	318691	322509	308570
Helena	269447	393630	418463	418829
Stalker3	245588	282495	217954	282695
Tc1_2	230163	308591	156323	336822
suffix	225365	382428	109094	282544
looper1	204936	131436	157977	113107
hopper	199395	217477	232908	283920
Doc4_element	185309	169848	95216	85550
hopper2	183781	255913	464215	500264
G3	149500	157859	85833	113329
stellateHet	127860	156267	178952	145553
Tirant	115183	106368	173196	90752
stellate	93590	118223	122122	85177
transib3	90213	95001	14480	30864
FB	82216	96167	90481	108577
gypsy9	74008	50655	29568	58405
Fw3	64433	72945	29862	48315
Tc1	57170	88076	27440	44741
transib1	50297	54878	48608	35706
HB	44986	43360	26373	29927
frogger	37533	48780	35868	19516

S2	30233	65358	31936	38142
mariner2	17963	13494	7214	10729
Tc3	8331	8130	4704	5988
Tom1	8029	21705	20794	27279
G7	7580	9506	5654	6052
Bari2	7498	4743	1789	5988
transib4	6560	5420	12446	16633
invader5	3827	8130	7513	7386

Supplementary Table 4. Mean length for piRNA lengths in adult life cycle

Transposons further analyzed by qRT-PCR were in red.

	3 day	15 day	30 day	45 day	30 day compared to 3 day
HB	25.72	25.98	24.48	24.83	-1.24
transib4	25.89	24.65	24.69	24.72	-1.2
gypsy9	25.83	25.43	24.96	24.79	-0.87
S2	25.91	25.91	25.12	25.67	-0.79
invader5	25.88	25.92	25.16	25.75	-0.72
Tc1_2	25.87	25.73	25.18	25.49	-0.69
mariner2	25.61	26.25	24.93	24.47	-0.68
Tc1	25.94	25.95	25.37	25.36	-0.57
invader4	26.28	26.05	25.79	25.73	-0.49
I_element	25.41	25.29	24.95	24.92	-0.46
G3	25.61	25.59	25.15	24.63	-0.46
gypsy7	25.94	25.87	25.52	25.62	-0.42
transib1	25.64	25.75	25.24	25.04	-0.4
Tirant	25.84	25.76	25.44	25.72	-0.4
Bari2	25.5	24.63	25.1	24.29	-0.4
NOF	25.51	25.42	25.14	25.21	-0.37
Q_element	26.41	26.21	26.04	25.95	-0.37
1731	25.62	25.49	25.26	25.3	-0.36
mdg3	25.13	25.07	24.79	24.85	-0.34
Porto1	25.28	25.07	24.94	24.63	-0.34
frogger	25.85	26.36	25.51	25.47	-0.34
accord2	25.66	25.48	25.33	25.17	-0.33
R2_element	25.55	25.5	25.22	25.16	-0.33
Osvaldo	25.74	25.67	25.41	25.32	-0.33
X_element	25.12	25	24.8	24.78	-0.32
Quasimodo	25.85	25.77	25.54	25.35	-0.31
Bari1	25.32	25.34	25.01	25	-0.31
aurora_element	25.12	25.1	24.82	24.74	-0.3
gypsy11	26.08	25.97	25.79	25.74	-0.29
gypsy5	25.96	25.88	25.68	25.58	-0.28
ZAM	25.83	25.74	25.55	25.41	-0.28
rover	25.35	25.27	25.08	25.01	-0.27
Circe	25.71	25.63	25.44	25.33	-0.27
Doc3_element	25.45	25.33	25.18	25.14	-0.27
BS3	25.71	25.68	25.44	25.47	-0.27
stellate	25.65	25.59	25.38	25.29	-0.27
invader3	25.67	25.58	25.41	25.36	-0.26
copia	25.6	25.5	25.34	25.11	-0.26
F_element	25.19	25.2	24.93	24.96	-0.26
Ivk	25.51	25.46	25.25	25.19	-0.26
Rt1c	25.6	25.65	25.34	25.39	-0.26
pogo	25.33	25.44	25.07	25.12	-0.26

G_element	25.65	25.55	25.4	25.35	-0.25
GATE	25.19	25.12	24.94	24.94	-0.25
rooA	25.79	25.74	25.54	25.52	-0.25
G5	25.54	25.47	25.29	25.25	-0.25
G2	25.83	25.76	25.58	25.5	-0.25
Fw2	25.28	25.22	25.04	25.04	-0.24
opus	25.63	25.62	25.39	25.36	-0.24
Dm88	26.06	25.91	25.82	25.76	-0.24
gypsy8	25.7	25.65	25.46	25.42	-0.24
suffix	24.59	24.63	24.35	24.31	-0.24
Idefix	25.92	25.82	25.69	25.55	-0.23
Rt1a	25.59	25.56	25.36	25.39	-0.23
Helena	25.85	25.89	25.62	25.35	-0.23
transib2	25.72	25.66	25.49	25.48	-0.23
gypsy3	25.7	25.6	25.47	25.42	-0.23
Doc2_element	25.46	25.44	25.23	25.22	-0.23
R1_2	25.23	25.29	25	25.1	-0.23
suste	25.22	25.1	24.99	24.84	-0.23
Doc	25.13	25.1	24.91	24.94	-0.22
HMS_Beagle	25.75	25.75	25.53	25.45	-0.22
gypsy4	25.93	25.85	25.71	25.58	-0.22
baggins	25.59	25.52	25.37	25.31	-0.22
Stalker3	26.25	26.39	26.03	25.97	-0.22
gypsy12	25.61	25.57	25.39	25.29	-0.22
gtwin	26.39	26.3	26.18	26.11	-0.21
gypsy6	24.85	24.76	24.64	24.64	-0.21
BS	25.59	25.57	25.38	25.4	-0.21
hopper2	24.86	24.47	24.65	24.72	-0.21
HMS_Beagle	25.93	25.88	25.72	25.64	-0.21
1360	25.63	25.62	25.42	25.51	-0.21
TART_C_TAR	25.35	25.27	25.15	25.2	-0.2
Cr1a	25.79	25.74	25.59	25.5	-0.2
TAHRE	25.43	25.36	25.23	25.16	-0.2
412	25.91	25.81	25.72	25.63	-0.19
gypsy10	26.01	25.86	25.82	25.76	-0.19
roo	25.71	25.62	25.52	25.51	-0.19
hobo	26.01	26.01	25.82	25.78	-0.19
Transpac	25.59	25.67	25.4	25.39	-0.19
Juan	26.13	26.11	25.94	25.97	-0.19
R1A1_element	25.39	25.37	25.21	25.17	-0.18
3S18	25.49	25.51	25.31	25.27	-0.18
McClintock	25.57	25.47	25.39	25.26	-0.18
Stalker	26	25.98	25.83	25.81	-0.17

Stalker4	25.96	25.94	25.79	25.74	-0.17
accord	25.23	25.24	25.07	25.07	-0.16
gypsy	25.87	25.84	25.71	25.65	-0.16
invader1	25.52	25.64	25.36	25.37	-0.16
17	25.31	25.3	25.15	25.2	-0.16
FB	23.27	24	23.11	22.9	-0.16
S_element	25.51	25.52	25.35	25.36	-0.16
Tabor	25.95	25.94	25.79	25.66	-0.16
Stalker2	26.09	26.06	25.93	25.91	-0.16
G6	25.15	25.11	24.99	24.94	-0.16
TARTA	25.35	25.3	25.19	25.28	-0.16
blood	25.72	25.73	25.57	25.5	-0.15
diver2	25.31	25.32	25.16	25.19	-0.15
G4	25.59	25.66	25.44	25.37	-0.15
diver	25.41	25.33	25.27	25.25	-0.14
Max_element	25.55	25.57	25.42	25.46	-0.13
hopper	25.07	25.21	24.95	25.03	-0.12
jockey2	24.51	24.54	24.39	24.34	-0.12
gypsy2	25.85	25.82	25.73	25.68	-0.12
HeT_A	25.24	25.24	25.12	25.05	-0.12
mdg1	25.88	25.85	25.76	25.65	-0.12
invader2	25.61	25.78	25.5	25.4	-0.11
mst40	25.37	25.38	25.26	25.23	-0.11
G5A	25.26	25.3	25.16	25.01	-0.1
springer	25.81	25.81	25.71	25.7	-0.1
micropia	26.3	26.33	26.21	26.17	-0.09
transib3	25.45	25.58	25.36	25.54	-0.09
jockey	24.63	24.63	24.55	24.57	-0.08
Burdock	25.33	25.39	25.25	25.28	-0.08
Rt1b	25.43	25.48	25.35	25.33	-0.08
Doc4_element	25.15	25.14	25.07	25.05	-0.08
297	25.75	25.78	25.68	25.66	-0.07
flea	25.53	25.64	25.47	25.37	-0.06
Fw3	25.26	25.53	25.2	25.09	-0.06
INE_1	25.37	25.4	25.33	25.26	-0.04
stellateHet	25.44	25.57	25.45	25.3	0.01
looper1	24.89	24.74	24.94	24.43	0.05
Tom1	25.1	25.69	25.44	26.29	0.34
G7	24.87	25.79	25.44	25.05	0.57

Supplementary Table 5. piRNA abundance in adult life cycle

Transposons further analyzed by qRT-PCR were in red.

	3 day	15 day	30 day	45 day
R1A1_element	40149494	36291700	25497365	26969138
GATE	36200017	31304570	27711696	26748727
roo	35675764	30643595	19848730	19264499
Rt1b	34953281	26327271	22733196	22880241
Max_element	27013660	19962756	15565090	15456889
gtwin	20638963	24749484	17198903	18627402
X_element	18882137	16030127	12404895	12860296
F_element	17841155	13882554	10670632	10532304
412	14199186	14886229	10912423	10447799
Rt1a	12342207	9037441	7017629	7211254
Stalker	11815536	11149429	8209559	8095500
297	11733421	9899430	6068894	6503649
Stalker4	11359090	10775434	7849227	7737890
Doc	11155458	8622955	6514117	6600559
HeT_A	10866145	8044428	5371141	5636340
gypsy12	9858453	7255424	6741561	6355131
baggins	9479222	9181496	6010960	5739030
Doc3_eleme	9233522	6726210	4901132	4895609
G_element	9038177	8347814	6189835	7051197
G2	8977548	8157545	6108594	7401294
invader4	8688627	3194886	1776162	1926899
mdg1	8629514	8354851	5872604	5587464
opus	8509769	6055708	4776948	4665800
blood	8216045	7447303	5678949	5567485
gypsy4	8079424	7515554	5153740	5221108
Stalker2	7968694	7702292	5928923	5765623
gypsy6	7833386	7263663	5403867	5837567
Cr1a	7643300	5730179	4289993	3950680
rooA	6664184	4620345	3477339	3000546
TART_C_TAR	6565761	4234011	3270942	3897139
TAHRE	6547458	5278724	3796539	3643353
springer	6034446	5960137	4562510	4531777
gypsy	5926067	5853801	4275651	4720695
gypsy2	5804611	6312498	4713480	5169306
flea	5804114	5333677	3398308	4003042
1731	5335138	4799662	4028331	4385128
Circe	5285349	4785171	4338762	4258726
jockey2	5137398	4772287	4844968	5538225
jockey	5116156	2888586	2899912	2867890
aurora_element	5039573	4201405	3254655	3301386
gypsy10	4919490	5155103	3379989	3502320
17	4842873	3911056	3204167	2638982

Tabor	4818395	4853131	3187811	3236161
3S18	4810801	4261124	2765247	2995868
rover	4688795	4510215	3111821	3063232
BS	4509666	4416808	3736840	3688545
1360	4482970	4077240	2744281	3432990
mdg3	4268598	4140842	2536220	2827198
Idefix	4184704	3741405	2450034	2175237
HMS_Beagle	4047172	3546749	2837921	2808631
gypsy8	3985241	3041061	2038114	2048662
Quasimodo	3761814	3697928	2655263	2786049
gypsy3	3704236	3328920	2459980	2531901
Doc2_element	3276300	2463778	1897414	2188494
HMS_Beagle	3088080	2841300	2384560	2617595
G6	3050094	3265730	2347513	3420069
NOF	3011242	2451218	2009031	1914479
diver	2665323	2183802	1675724	2014422
Ivk	2641988	2253030	1864675	1844343
accord	2620613	2343948	2083276	1670584
micropia	2519827	2668472	2125307	2413575
hobo	2492375	2353232	1447496	1641215
I_element	2344597	1798880	1418673	1480244
R2_element	2312717	2154713	1865020	1678777
invader3	2118410	2351199	1768962	2523771
gypsy5	2117436	2076401	1508718	1309772
BS3	2076707	1500980	1145342	1227964
invader1	1996632	1510904	1419024	1642850
S_element	1983889	1749371	1314173	1384391
accord2	1837048	1339040	1018886	1049342
Osvaldo	1800284	1566766	956962	1081023
suste	1769987	1427051	1164533	1274612
diver2	1654800	1222300	1046871	975509
Bari1	1617589	1696346	1393285	1651368
ZAM	1497284	1476513	913261	1016746
invader2	1494532	1013052	752028	703943
G5	1488462	1549040	1310582	1323043
TARTA	1448778	819524	624228	741940
transib2	1446683	1409786	962421	1171414
Juan	1412748	869102	699604	761760
McClintock	1382263	1186945	840793	920253
mst40	1312908	1090813	828324	901922
Burdock	1170489	854586	610722	749044
Dm88	1054366	781726	546047	589883
Rt1c	1042622	936303	707642	689381

Q_element	1022428	849717	591619	533779
copia	862532	817383	582655	695158
G4	697355	734439	529479	499899
INE_1	652425	520421	372078	335836
Transpac	636517	549715	332734	388516
R1_2	603864	547275	414498	483696
Porto1	584686	496188	398384	381970
pogo	576576	583754	451750	496305
G5A	399482	401396	284512	335612
gypsy7	380036	239860	176674	180249
Helena	374873	353301	292051	296274
Fw2	359787	222923	181493	185351
hopper2	317303	221750	160005	177218
gypsy11	285920	300027	231100	197246
Tc1_2	267403	223833	146688	154295
suffix	244809	186652	163388	131056
looper1	237418	318165	197189	290165
hopper	229359	177266	124378	122331
Stalker3	227080	234181	170115	184483
Doc4_eleme	210917	170387	158771	160762
stellateHet	183820	126164	80324	77346
stellate	126277	91664	66487	64054
G3	125407	94663	99224	62350
Fw3	106822	92368	67660	91478
Tirant	100437	114981	78011	87151
Tc1	90072	93740	76416	77920
gypsy9	86561	64218	50454	54897
FB	72769	61616	51875	43906
transib3	67171	54944	41652	55638
frogger	60667	58249	23945	39532
transib1	60025	43633	40111	63124
HB	45792	24157	19818	17715
S2	39590	32801	26868	22301
mariner2	15780	11236	3130	7380
G7	14206	6097	4821	4739
Tom1	12933	8989	3969	2138
invader5	12239	6742	7095	4887
transib4	11757	9551	14054	10996
Bari2	9901	5337	4392	4276

Supplementary Materials and Methods

Fly genetics

To make *Nbr*^{cas9} flies, we co-injected two sgRNAs (100ng/ul; target sequences: 5'-GGCGCGGGAGAGCTTACGCT-3' and 5'- GGAGTTAAAAAGCAGCGCTT-3') with Cas9 mRNA (1µg/µl) into fly embryo. To make *Hen1*^{cas9} flies, we co-injected two U6a-sgRNA-short plasmids (250ng/ul; targets sequence: 5'-CTTAACCAGCGTTAATCCAC-3'; 5'-GGATATGCTGGAGATGCAGT-3') into fly embryo expressing *nano*-Cas9 (TH00788. N). To make *piwi*^{cas9} flies, we co-injected two U6a-sgRNA-short plasmids (250ng/ul; targets sequence: 5'- CGACGTTGCTCACACAATC-3'; 5'- TGAGCTCTGCCGAGTGACT-3') into fly embryo expressing *nano*-Cas9 (TH00788. N). To make *Nbr*$^{KI\text{-}Myc}$ flies, we injected one sgRNA (250ng/ul; target sequences: 5'-GGCTCTTGCGTGCCATTTCTCA-3') and an oligo template with Myc tag sequence (5'-GGTTCTCCATGTTCTCCTCGTCCGACTCAAAGCCGGCGGGTATTGCGTTGTACATGTGGCTCTTGCGTGCCAGATCCTCTTCAGAGATGAGTTTCTGTTCCATTTCTCACGGGTTTCCCGTGCCACTGCAGAGCGAAGCAAGTGCAATTGCCAATTGCAAATGCAATTGACGGTAA-3'). To screen flies carrying mutant alleles, we used single fly PCR assay. Single fly was homogenized in 50µl squashing buffer (10 mM Tris buffer [pH 8.0], 25 mM NaCl, 1 mM EDTA, 200 µg/ml Proteinase K), then incubated at 37℃ for 30 min followed by inactivation at 85℃ for 10 min. For PCR amplification, 1 µl of DNA extract was used as template. PCR primers were as follows: *Nbr*^{cas9} (Forward: 5'-CCTGGATTCTGAATGGATGC-3', Reverse: 5'-TCACTTAACATGGGCACCCCG-3'), *Hen1*^{cas9} (Forward: 5'-GTGGACATCGACAAGTCCTTGCTC-3', Reverse: 5'-GCAATAGGCATATTACAAGTGTCC-3'), *piwi*^{cas9} (Forward: 5'-CGTTGGATTCATATCGTGTGCTGAG-3', Reverse: 5'-CAGGTCAAGAATCGGACGGACTAGC-3'), and *Nbr*$^{KI\text{-}Myc}$ (Forward: 5'-GAACAGAAACTCATCTCTGAAGAGG-3', Reverse: 5'-TAAAGGCTATCATGCTGGTG-3'). To generate *Nbr* pUAST constructs, RT-PCR amplification was conducted using RNA from whole flies, with primers (Forward: 5'- ATG TATCCAGTTGGACAACAG-3', Reverse: 5'-TCACTTATCGTCGTCATCCTTGTAATCCTTAACATGGGCACCCCG-3').

Small RNA northern

Oligo probes were used to make probes. Oligos used for miR-34-5p (5'-GATAATACGACTCACTATAGGGAGA-3'/5'-AAAAAATGGCAGTGTGGTTAGCTGGTTGTGTCTCCCTATAGTGAGTCGTATTATC-3'), miR-219-5p (5'-GATAATACGACTCACTATAGGGAGA-3'/5'-AAAAAATGATTGTCCAAACGCAATTCTTCTCCCTATAGTGAGTCGTATTATC-3'), miR-275-3p (5'-GATAATACGACTCACTATAGGGAGA-3'/5'-AAAAAATCAGGTACCTGAAGTAGCGCGCGTCTCCCTATAGTGAGTCGTATTATC-3'), miR-283-5p (5'-GATAATACGACTCACTATAGGGAGA-3'/5'-AAAAAAAAATATCAGCTGGTAATTCTGGTCTCCCTATAGTGAGTCGTATTATC-3'), AT-chX-1 (5'-GATAATACGACTCACTATAGGGAGA-3'/5'-AAAAAATGTTTCATCGTTAGACGGCTCGGGCGTCTCCCTATAGTGAGTCGTATTATC-3'), and 2S rRNA (5'- GATAATACGACTCACTATAGGGAGA-3'/5'-TGCTTGGACTACATATGGTTGAGGGTTGTATCTCCCTATAGTGAGTCGTATTATC-3').

Development • Supplementary information

Molecular biology

To clone full-length cDNA, RT-PCR primers were as follows: *Nbr*-Flag (Forward: 5'-ATGGCACGCAAGAGCCACATG-3', Reverse: 5'-TCACTTATCGTCGTCATCCTTGTAATCCTTAACATGGGCACCCCG-3'), Ha-*piwi* (Forward: 5'-ATGTACCCATACGATGTTCCAGATTACGCTGCTGATGATCAGGGACGT-3', Reverse: 5'-TTATAGATAATAAAACTTCTTTTC-3'), Ha-*aub* (Forward: 5'-ATGTACCCATACGATGTTCCAGATTACGCTAATTTACCACCAAACCCTG-3', Reverse: 5'-TTACAAAAAGTACAATTGATTC-3'), Ha-*ago3* (Forward: 5'-ATGTACCCATACGATGTTCCAGATTACGCTTCTGGAAGAGGAAATTTG-3', Reverse: 5'-TTAAAGATAAAATAGTTTTTCAG-3'). qRT-PCR primers were as follows: *rp49* (5'-CCGCTTCAAGGGACAGTATCTG-3'/5'-ATCTCGCCGCAGTAAACGC-3'), *piwi* (5'-CGTCCACTTAACGAAGATGATTC-3'/5'-CCTCGAGAGCTCTTCTCTCTCTT-3'), *aub* (5'-GTCCCTCGATAGAGAAATCCAGT-3'/5'-CGTGAATACACCAAATCCGTTAT-3'), *Ago3* (5'-AAATTTGATCGCAAGCAGTTTGA-3'/5'-AAACGTCGCAGCATAGCATTAAA-3'), *Nbr* (5'-GAAGACGTGCAGCTACTTGCATT-3'/5'-CTTCAGAATGAGCTCCAGCAGAG-3'), *Hen1* (5'-TAATCGAGCACGTTTACGACGAT-3'/5'-GTGACCACTCGAACTTGTGATCC-3'), *412* (5'-CAACAGCTCATCACCACCAGACA-3'/5'-AGATTGGGTCGTGTTGAAGCAGT-3'), *accord* (5'-ACAATCCACCAACAGCAACA-3'/5'-AAAAGCCAAAATGTCGGTTG-3'), *accord2* (5'-GAGGTCGTTGAATAGACTGCCCG-3'/5'-GGTCGCCGATGCCCTATTACAAT-3'), *blood* (5'-AGAGGGGAGGTGTAGTATGTGCA-3'/5'-GCTTATGCGCATATATGGCCAGC-3'), *diver* (5'-GGCACCACATAGACACATCG-3'/5'-GTGGTTTGCATAGCCAGGAT-3'), *diver2* (5'-TGCAGTTGATTCCTGGCAGAGAC-3'/5'-GGCTTCCAACAGAGTCCTCAAGG-3'), *gtwin* (5'-TTCGCACAAGCGATGATAAG-3'/5'-GATTGTTGTACGGCGACCTT-3'), *gypsy* (5'-GGGTACAGAGTTTGCAGGTGGAA-3'/5'-TCTCCAGGCCACATACTCGTCTT-3'), *gypsy10* (5'-GCATACCCAGATTCCACTCAGCC-3'/5'-CTGGGTGACGATTGGTGTTTTGC-3'), *gypsy5* (5'-TGCGAGCTTATCAGACGTTAGGG-3'/5'-GGATAGGCAAATGTCCTGAGGGG-3'), *gypsy6* (5'-GACAAGGGCATAACCGATACTGTGGA-3'/5'-AATGATTCTGTTCCGGACTTCCGTCT-3'), *het-A* (5'-CGCGCGGAACCCATCTTCAGA-3'/5'-CGCCGCAGTCGTTTGGTGAGT-3'), *hopper* (5'-TAAGTATGGCTGCAAGATCCCGC-3'/5'-ATTTGAGTGGCCCAAGACAGCAA-3'), *idefix* (5'-ATTCACCGCGTTTCATACCG-3'/5'-TCTGACTCTCGCGTTGTCTT-3'), *I-element* (5'-GTCGTGCCTCTCAGTCTAAAGCC-3'/5'-GAGCCCGGATTAGCGGTATTGTT-3'), *invader1* (5'-GTACCGTTTTTGAGCCCGTA-3'/5'-AACTACGTTGCCCATTCTGG-3'), *invader3* (5'-CAGCTATCGTTGGTGGATGCAGA-3'/5'-GGTTTCCGTTGCTGTTGTTCTCG-3'), *Max* (5'-TCTAGCCAGTCGAGGCGTAT-3'/5'-TGGAAGAGTGTCGCTTTGTG-3'), *mdg1* (5'-AACAGAAACGCCAGCAACAGC-3'/5'-CGTTCCCATGTCCGTTGTGAT-3'), *NOF* (5'-GTGAAACCCGATGAACAATGCGG-3'/5'-CTTCCTTCCTCCACAATGCAGCT-3'), *R1A1* (5'-AATTCCCGAGCTGTGCTAGA-3'/5'-GTCTCAAGGCACCTTTCAGC-3'), *rt1a* (5'-CCACACAGACTGAGGCAGAA-3'/5'-ACGCATAACTTTCCGGTTTG-3'), *ZAM* (5'-ACTTGACCTGGATACACTCACAAC-3'/ 5'-GAGTATTACGGCGACTAGGGATAC-3').