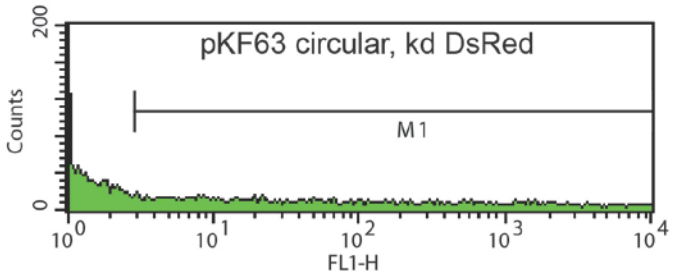
Supplementary material

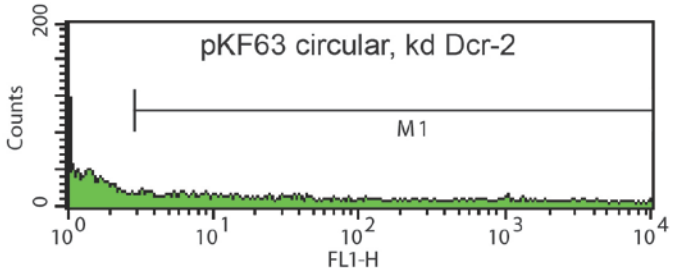
Michalik et al., “A small RNA response at DNA ends in Drosophila”

Figure S1: Flow cytometry analysis of transfected, circular and linearized GFP expression plasmid

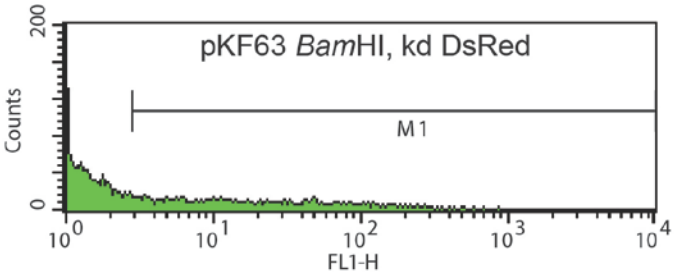
The panels on the left show the fluorescence distribution as intensity histograms of the measured cells. The marked region (M1) is a window that was defined to exclude the majority of non-transfected cells (see bottom panel of control transfected cells). The mean fluorescence intensity and the proportion of GFP-positive cells are indicated in the table to the right of the histograms. Linearization of the plasmid with *Bam*HI leads to a roughly 20-fold reduction in mean GFP fluorescence intensity (note the logarithmic scale in the histograms) but only a twofold reduction in the number of GFP positive cells. This indicates that the transfection efficiency is most likely comparable for linear and circular plasmids. While for the circular plasmid the mean fluorescence intensity is not affected by knock-down of Dcr-2, the *Bam*HI-linearized plasmid produces a twofold stronger GFP expression upon depletion of Dcr-2 in this experiment. These measurements are examples of the raw data for Fig. 1 of the manuscript.



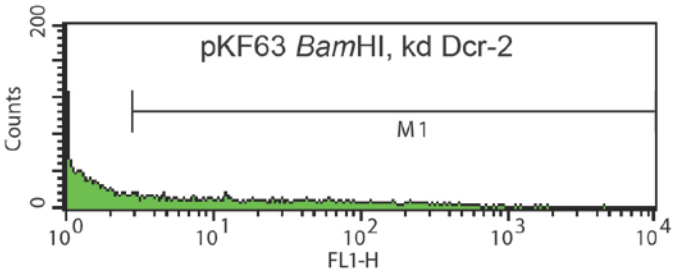
Marker	Left, Right	Events	% Total	Mean
All	1, 9910	10000	100.00	271.94
M1	3, 9910	4213	42.13	643.74



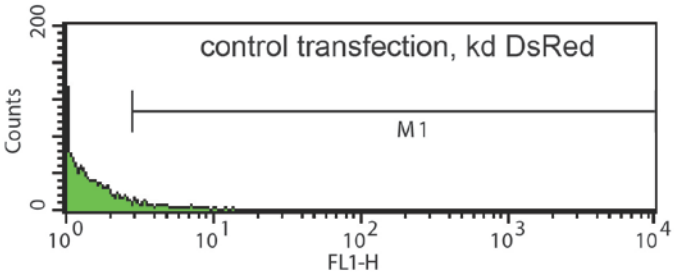
Marker	Left, Right	Events	% Total	Mean
All	1, 9910	10000	100.00	284.19
M1	3, 9910	4213	42.13	672.82



Marker	Left, Right	Events	% Total	Mean
All	1, 9910	10000	100.00	8.71
M1	3, 9910	2196	21.96	35.36



Marker	Left, Right	Events	% Total	Mean
All	1, 9910	10000	100.00	15.11
M1	3, 9910	2402	24.02	59.20



Marker	Left, Right	Events	% Total	Mean
All	1, 9910	10000	100.00	1.21
M1	3, 9910	223	2.23	4.41

Figure S2: Mean GFP fluorescence intensities without normalization

A) The mean fluorescence intensity of cells transfected with linearized plasmids was calculated and is depicted here. The clear reduction, as also seen in the histograms of Fig. S1, is visible. This is the same data as shown in Fig. 1A of the manuscript but before normalization to the control treatment.
B) Recognition sequences of the nicking-endonucleases employed in our study.
C) The mean intensity was calculated as in A) but for the experimental data with the nicking endonucleases. This is the same data as shown in Fig. 1C but before normalization. Treatment with the nicking endonucleases results in clearly decreased GFP expression, indicating that the DNA was indeed nicked and that repair of these nicks is not immediate.

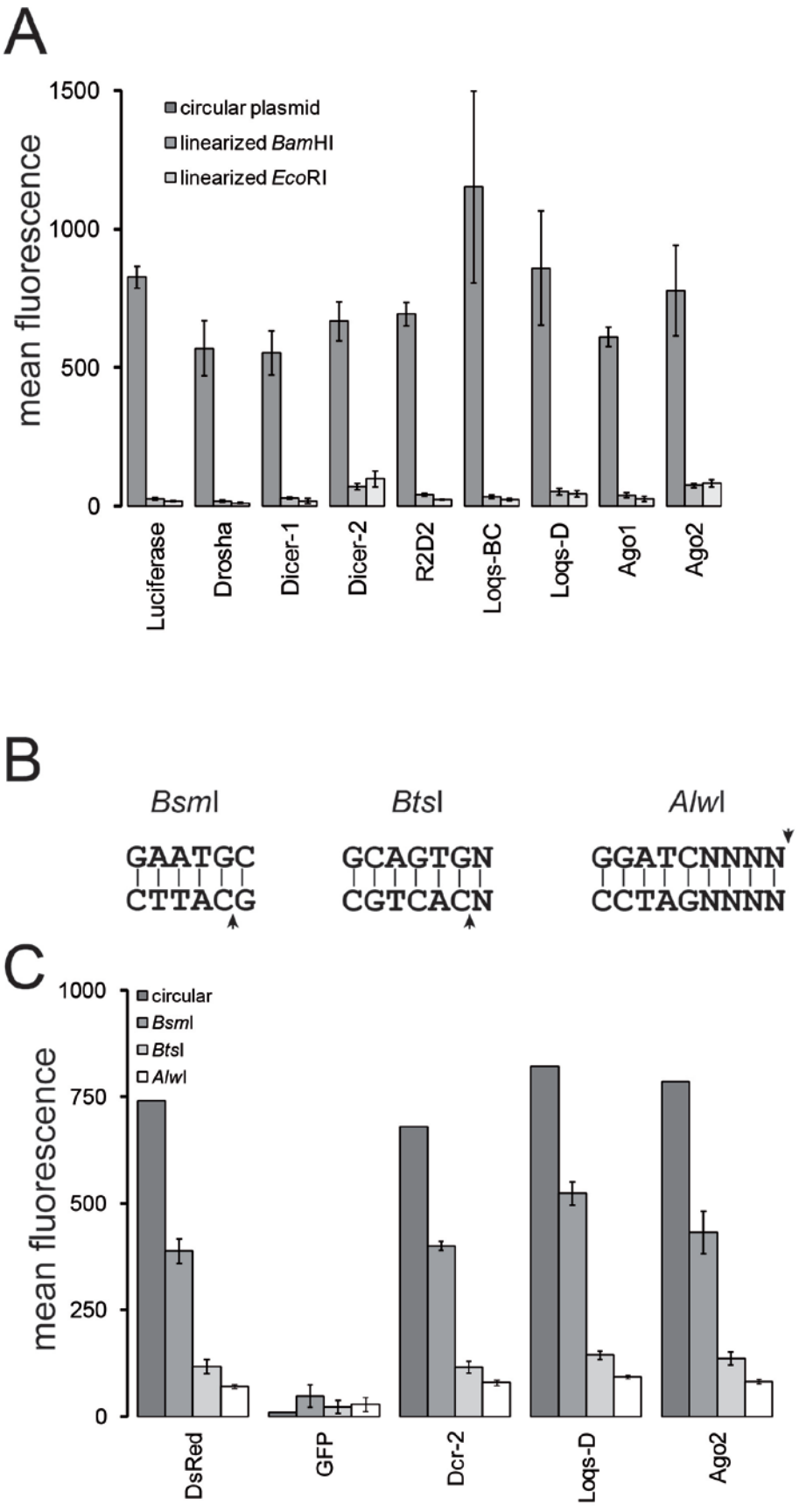
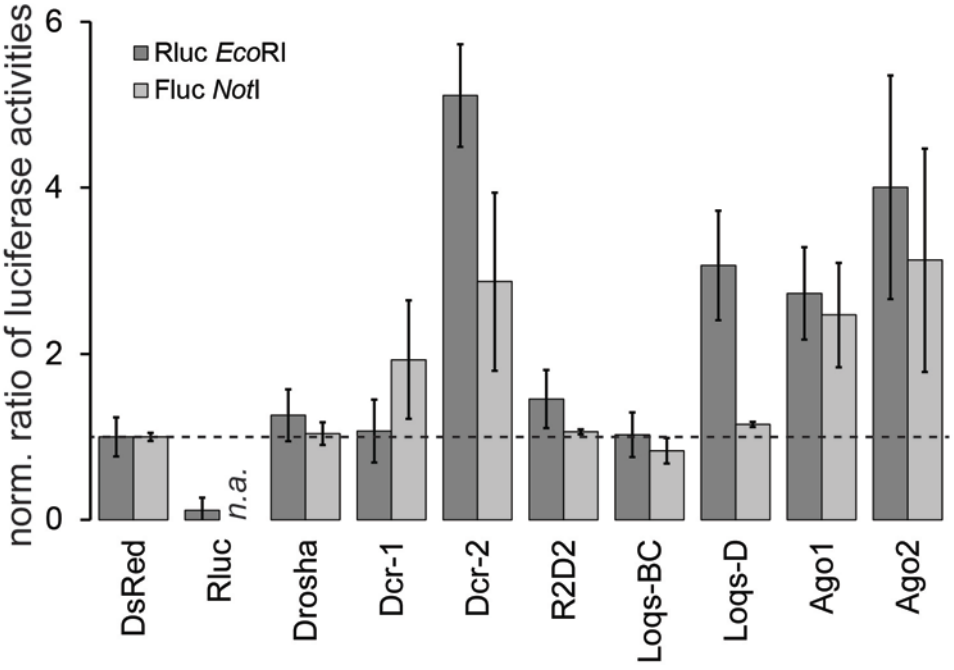

A
1500
1000
500
0
mean fluorescence
circular plasmid
linearized BamHI
linearized EcoRI
Luciferase
Drosha
Dicer-1
Dicer-2
R2D2
Loqs-BC
Loqs-D
Ago1
Ago2
B
BsmI
BtsI
AlwI
GAATGC
CTTACG
GCAGTGN
CGTCACN
GGATCNNNN
CCTAGNNNN
C
1000
750
500
250
0
mean fluorescence
circular
BsmI
BtsI
AlwI
DsRed
GFP
Dcr-2
Loqs-D
Ago2

Figure S3: The small RNA response is independent of the GFP reporter system

We created a *Renilla*-Luciferase expression vector by replacing the GFP coding sequence in our plasmid. In addition, a separate plasmid with the tubulin promotor and 3'-UTR driving the expression of Firefly luciferase was generated. With the dual luciferase assay system we can normalize for transfection efficiency by calculating the ratio of the two reporters. Either plasmid can be linearized (e.g. *Renilla* with *Eco*RI, Firefly with *Not*I) and combined with the other luciferase vector in circular form. Upon prior depletion of small RNA biogenesis factors, de-repression is observed for both linearized vectors. The small RNA response is therefore not specific to the GFP expression construct we used in Fig. 1 of the manuscript. One potential difference between the two linearized plasmids is that the *Not*I-cut Firefly expression plasmid has a less pronounced requirement for the Dicer-2 co-factor Loqs-PD.



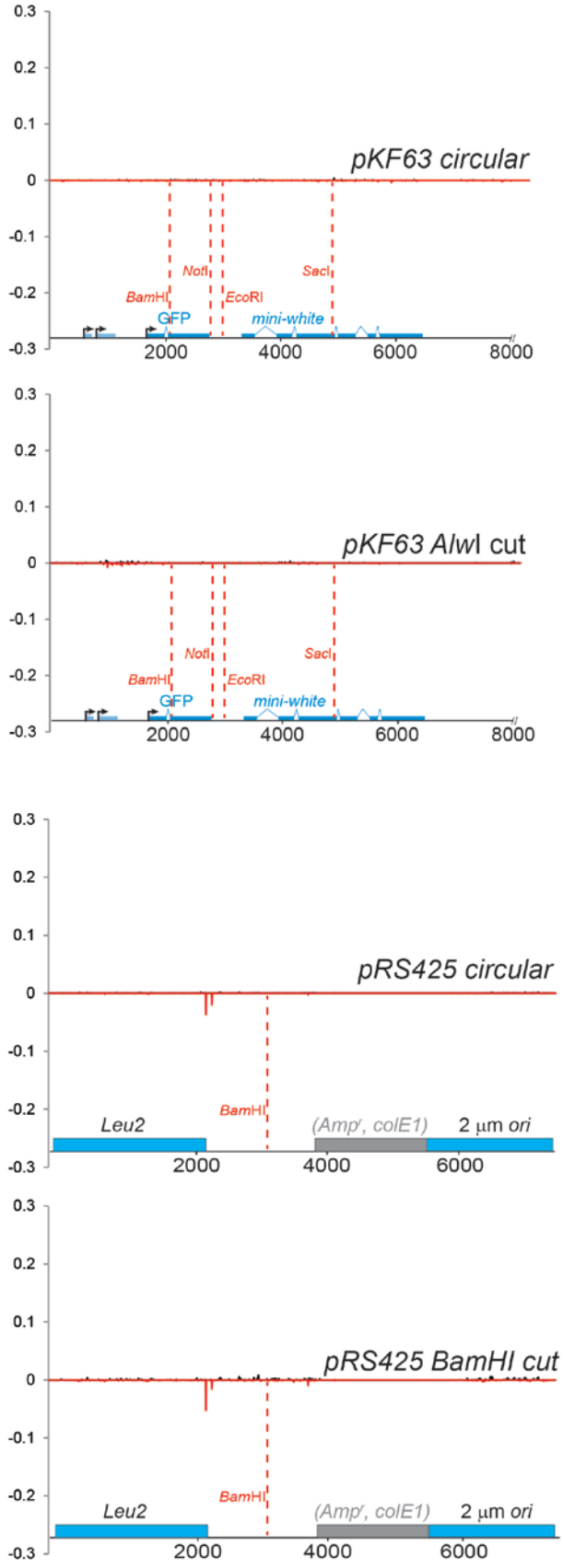


Figure S4: Profiling of break-induced small RNAs by deep sequencing

This figure is analogous to Fig.4 of the manuscript, the graphs depict the sense (black) and antisense (red) matching reads as ‰ of genome matching 21-23 nt reads in the respective library. The top two panels show reads mapping to the circular (upper) or nicked (lower) GFP expression plasmid. The two bottom panels show the distribution of reads mapping to the circular (upper) or *Bam*HI-linearized (lower) yeast plasmid. The region shaded in gray corresponds to sequences shared between the two plasmids (they are beyond pos. 8000 in the pKF63 diagrams); reads mapping to this region were not considered in our analysis as they cannot be uniquely attributed to their origin.

Figure S5: Size distribution of plasmid-matching reads

The small RNA libraries were mapped to the constructs as in Table S1 and Fig. 3 and S4 but a larger range of input sizes was taken. The size distribution of the matching reads is depicted as ‰ of the total reads in the library. In all cases, a sharp peak at 21 nt can be seen, consistent with the notion that the small RNAs are siRNAs. Sense and antisense-matching reads are represented with roughly equal abundance, arguing that double-stranded RNA was the precursor.