Contrasting epigenetic control of transgenes and endogenous genes promotes post-transcriptional transgene silencing in *Arabidopsis*

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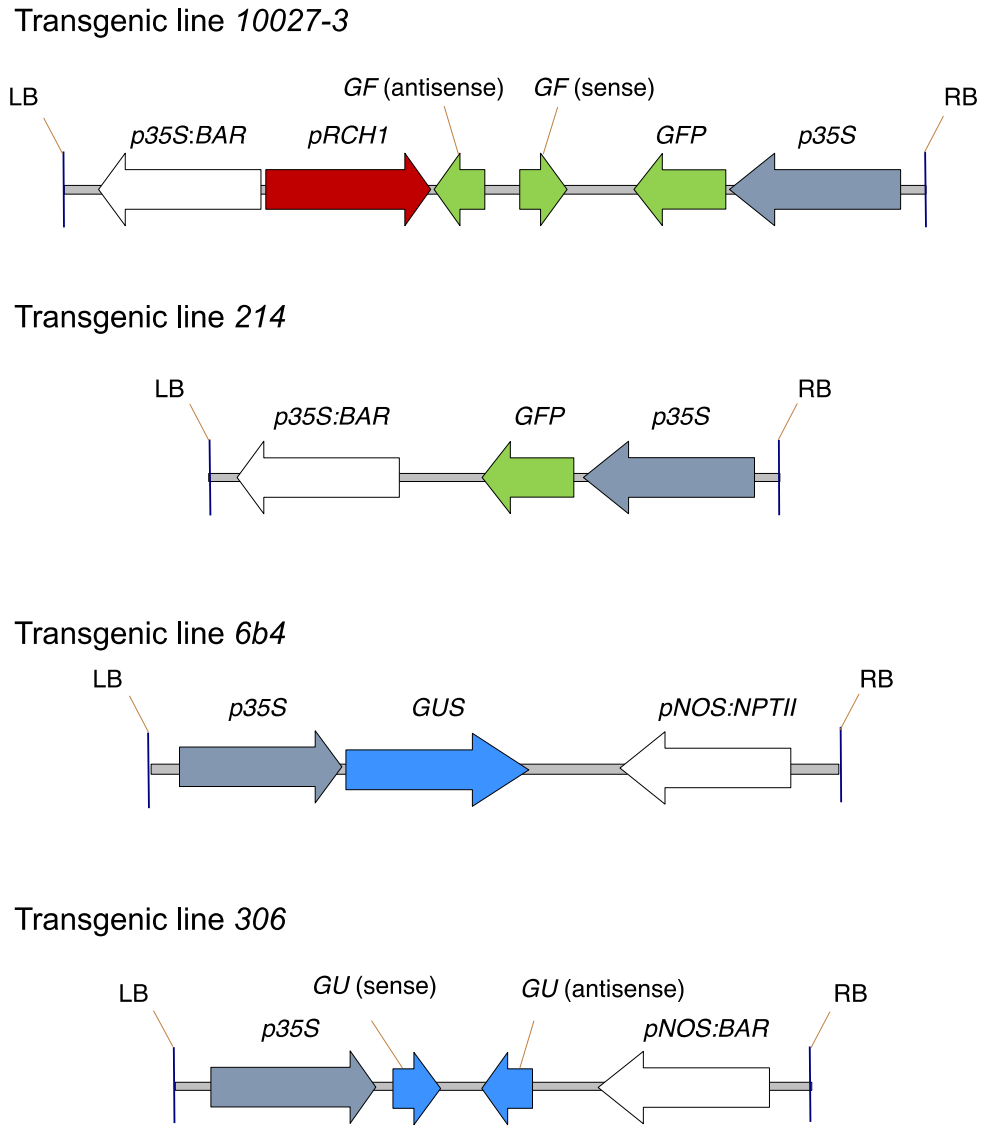
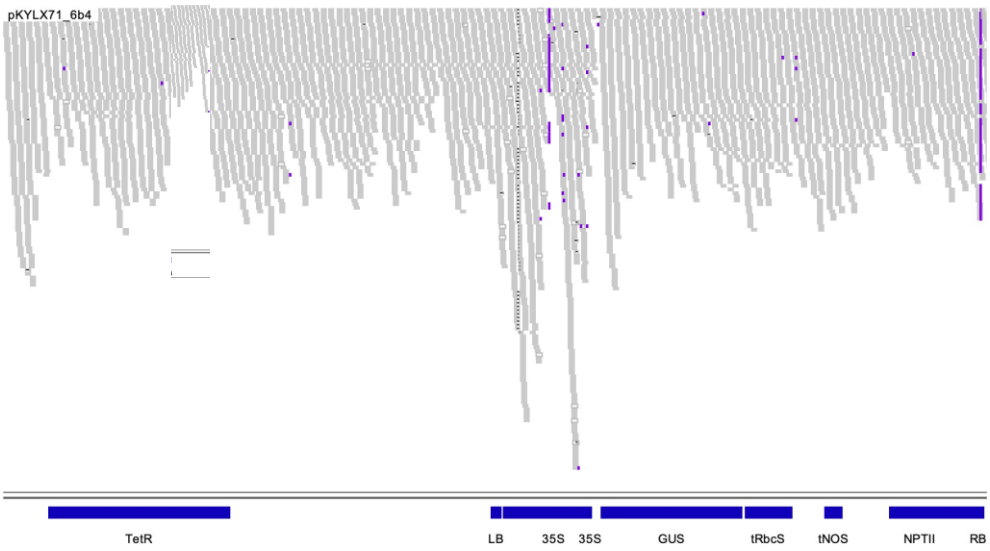
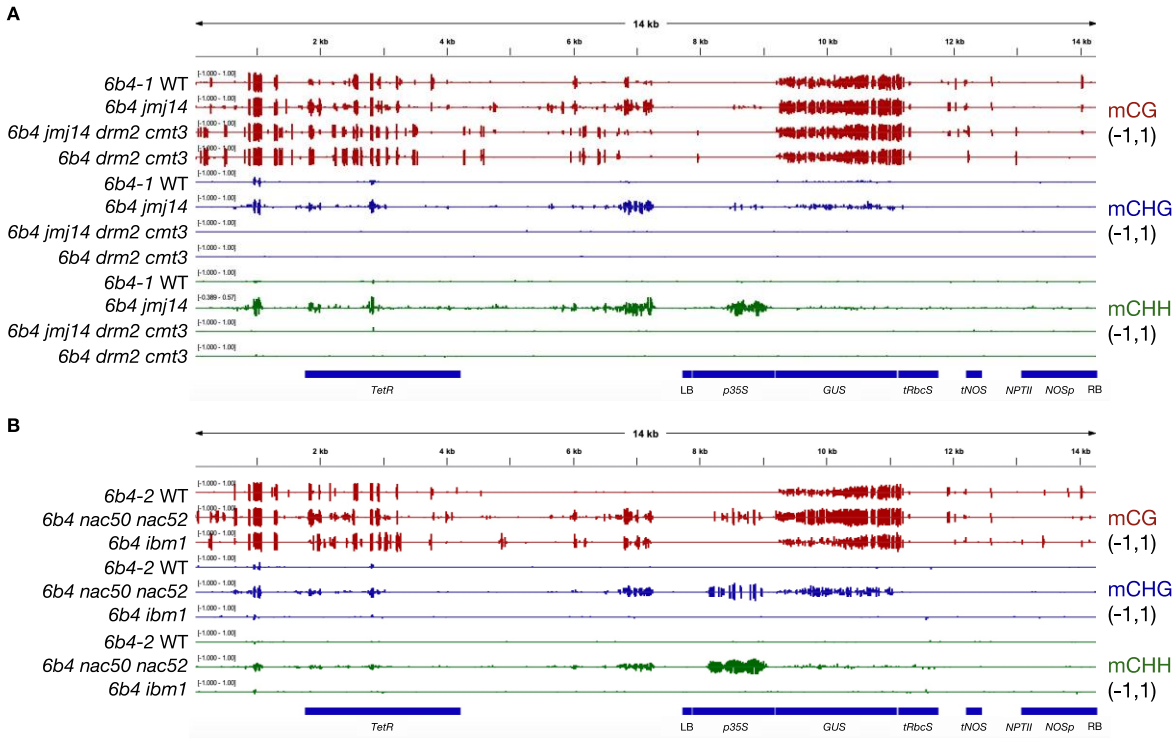


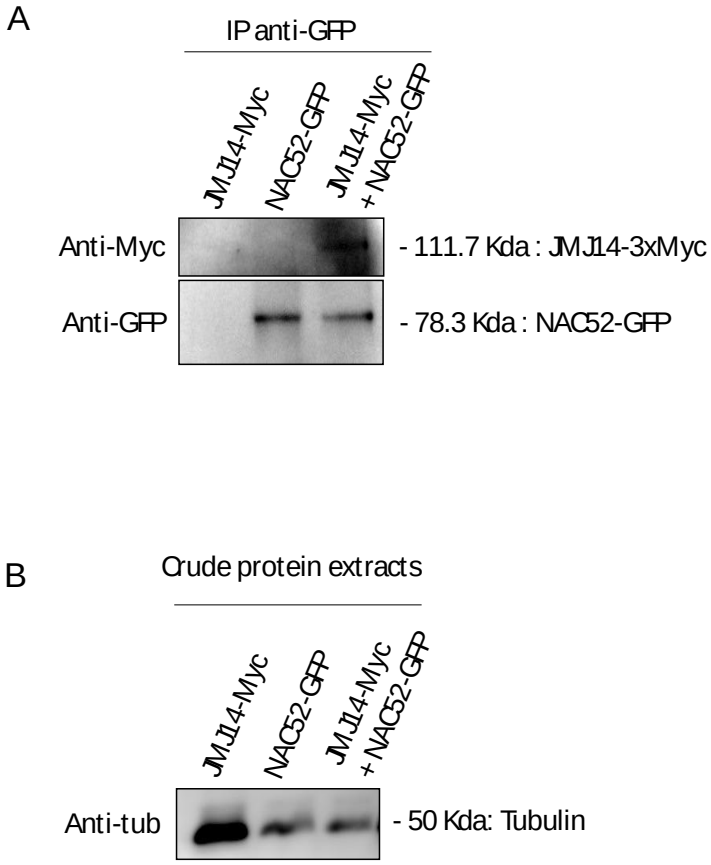
Supplementary Figure 1. T-DNA maps for transgenic reporter lines of *Arabidopsis*. A *BAR* transgene was used as a selectable transformation marker for transgenic lines *10027-3* [1], *214* [2], and *306* [3], whereas a *NPTII* transgene was used as a selectable transformation marker for transgenic line *6b4* [3]. The *pRCH1* promoter drives root tip-specific expression of the *GF* hairpin RNA in transgenic line 10027-3. LB and RB, left- and right-borders of the T-DNA.



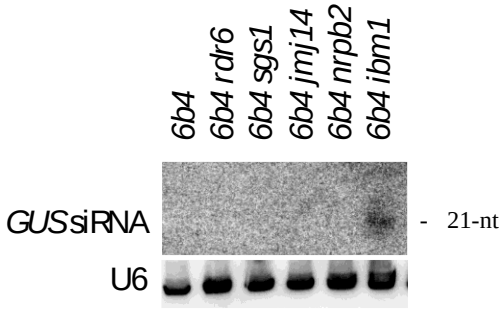
Supplementary Figure 2. Read coverage reveals integration of the entire plasmid at the *6b4* locus.
Browser track showing bisulfite paired-end reads mapping to the *6b4* locus. Purple lines represent single nucleotide errors in the reads or reference sequence. The *6b4* locus includes the tetracycline resistance gene (*TetR*), the left- and right-border sequences (LB and RB) flanking the *35S* promoter (*p35S*), *GUS* coding sequence (*GUS*) and *Rubisco* terminator (tRbcS), and the *NOS* promoter (*NOSp*) and terminator (*tNOS*) driving the kanamycin resistance gene (*NPTII*).



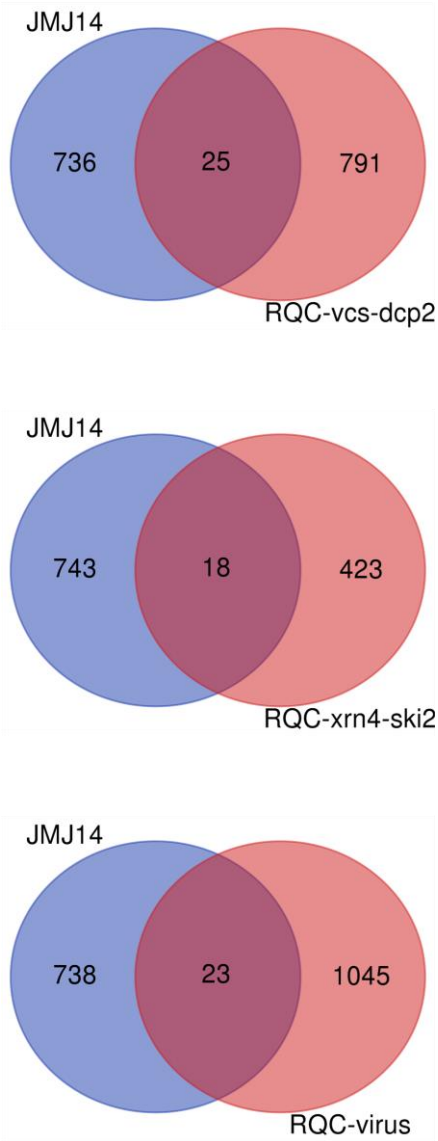
Supplementary Figure 3. JMJ14 and NAC50/NAC52 prevents CHG and CHH methylation over the majority of the *6b4* locus.
Browser tracks showing CG, CHG and CHH methylation levels for the entire *6b4* locus in leaf tissue from (A) 5-week- and (B) 8-week-old plants. The *6b4* locus includes the tetracycline resistance gene (*TetR*), the left- and right-border sequences (LB and RB) flanking the *35S* promoter (*p35S*), *GUS* coding sequence (*GUS*) and *Rubisco* terminator (tRbcS), and the *NOS* promoter (*NOSp*) and terminator (*tNOS*) driving the kanamycin resistance gene (*NPTII*). Bisulfite data were analyzed using R version 4.0.2.



Supplementary Figure 4. JMJ14 and NAC52 interact.
Proteins were extracted from plants carrying *pJMJ14:JMJ14-Myc, pUBQ10:NAC52-GFP* or both constructs and submitted to GFP immunoprecipitation. A. Immunoprecipitated proteins were separated on SGS-page gel and analyzed after transfert using anti-Myc and anti-GFP antibodies. B. A distinct gel run with crude extracts was analyzed using anti-tubulin antibodies to ensure that similar amounts of proteins were used for immunoprecipitation. Uncropped and unprocessed scans of images are provided as a Source Data file.



Supplementary Figure 5. IBM1 impairment provokes spontaneous PTGS of *GUS* at the *6b4* locus.
LMW RNAs were extracted from bulks of 50 plants of the indicated genotypes grown in vitro for 18 days. The Northen blot was hybridized with a *GUS* probe, revealing the presence of *GUS* siRNAs in *6b4 ibm1* plants, indicating that the *p35S:GUS* transgene is silenced by PTGS in this mutant background. Uncropped and unprocessed scans of images are provided as a Source Data file.



Supplementary Figure 6. JMJ14 binding is not required for the production of siRNAs from endogenous genes when RQC is impaired.
Venn diagrams showing the overlap between JMJ14-binding genes and endogenous genes producing siRNAs when RQC is impaired by *dcp2* or *vcs* mutations, *ski2* and *xrn4* mutations or infection by viruses. In each case, the overlap is similar to that expected by chance.

Supplementary Table 1. List of the primers used in this study.

Primer name	Sequence	Used for
Promoter 35S-F	CTACAAATGCCATCATTGCG	ChIP
Promoter 35S-R	AAGGATAGTGGGATTGTGCG	ChIP
GUS 5'-F	TCCTGTAGAAACCCCAACCC	ChIP
GUS 5'-R	TGGCCTGCCCAACCTTT	ChIP
GUS 3'-F	GTATCAGTGTGCATGGCTGG	ChIP
GUS 3'-R	AGTTCATGCCAGTCCAGCG	ChIP
GAPDH-F	GGTACGACAACGAATGGGGT	ChIP
GAPDH-R	TGACTGCGCATGGAATCAGT	ChIP
JMJ14 Q183*-F	CCTCTGAAGGAGAAAAAAATATGGGAGAA	*jmj14-5* genotyping
JMJ14 Q183*-R	GCATTCCTTAAAGTATTCATCATACTCCT	*jmj14-5* genotyping
JMJ14 Intron521-F	TGTGGAATTGTGTTTATTTCCTGCA	*jmj14-6* genotyping
JMJ14 Intron521-R	TGATAAGCAAAAGCCACGAACTTAA	*jmj14-6* genotyping
JMJ14 G331E-F	TGTGCTTTCTAGGAGCTATGTACTAACC	*jmj14-7* genotyping
JMJ14 G331E-R	AGACTCAGCATGGTTTCCAGGGGTC	*jmj14-7* genotyping

Supplementary Table 2. Summary of whole-genome bisulfite sequencing.

Sample	Median coverage	mCG (%)	mCHG (%)	mCHH (%)	mCHH (chloroplast)
6b4-1	21.3	24.6	8.3	2.3	0.5
6b4-2	20.1	26.2	9.6	3.3	0.8
6b4 nac50 nac52	19.1	28.0	8.7	3.1	0.7
6b4 jmj14	19.8	25.5	8.1	2.2	0.5
6b4 jmj14 cmt3 drm2	19.3	25.6	0.7	1.2	0.5
6b4 cmt3 drm2	21.3	24.9	0.8	1.4	0.6
6b4 ibm1	20.8	26.8	19.1	3.1	0.7

Note: Mean genomic coverage and methylation percentages for CG, CHG and CHH context. Cytosines covered by 3 or more reads were used to calculate genome-wide methylation percentages.

Supplementary references

1. Taochy, C. *et al.* A genetic screen for impaired systemic RNAi highlights the crucial role of DICER-LIKE 2. *Plant Physiol* **175**, 1424-1437 (2017).
2. Brosnan, C.A. *et al.* Nuclear gene silencing directs reception of long-distance mRNA silencing in Arabidopsis. *Proc Natl Acad Sci U S A* **104**, 14741-6 (2007).
3. Beclin, C., Boutet, S., Waterhouse, P. & Vaucheret, H. A branched pathway for transgene-induced RNA silencing in plants. *Curr Biol* **12**, 684-8 (2002).