Supplementary Information (Methods and 3 Supplementary Figures + legends)

A SNF2-like protein faciliates dynamic control of DNA methylation

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Supplementary Methods

Primers used for genotyping:

The α' promoter silencing system and the *drd1* mutant alleles have been described previously (Kanno et al., 2004). The work reported here was carried out using plants carrying the *drd1-6* allele. The *drd1-6* mutation produces a G to A substitution (TGGTCA to TGATCA), producing a new *Nde*II restriction enzyme site (/GATC) that can be used for genotyping after PCR amplification with the following primer set:

5' AGC TAA GGG ATG GAA ACT AGG 3'

5' CGA GAT GCT CCA ACA AGC GCG 3'

α' promoter system: primers for genotyping

Target insertion site (wild type sequence)

Tar5: 5' TTG GGA TTT CCA GAC GTC AC 3'

Tar3: 5' TCA TTT GAC CGT CGC TCA TC 3'

Target insert

GFP5GT: 5' GTG GAG AGG GTG AAG GTG ATG C 3'

GFP5GT3: 5' GCC ATG TGT AAT CCC AGC AGC 3'

Silencer insertion site (wild type sequence)

5gsil5: 5' GGG AAG TAA AAA TCT CAC GAG 3'

5gsil3: 5' GGT TGC ATC TCG ATT TAT GAG 3'

Silencer insert

Nosp: 5' ATG AGC GGA GAA TTA AGG G 3'

a'pro5': 5' CAA CAC GTA CTC ACA AAG G 3'

The NOS promoter silencing system has been described previously (Aufsatz et al., 2002a,b; 2004). For genotyping the target K and silencer H complexes in the α' promoter and NOS promoter systems, the following primer sets were used.

Target insertion site (wild type sequence)

KKWT-F 5' ATG GAC ATC CCC GGC AAA TG 3'

KKWT3-2 5' CAT TGT ACT GCT CTG CTT GAT ACT GCT TGA 3'

Target insert

TargF 5' ATG CCA TCT CCA TCA ACG TC 3'

TargR 5' TTT CTG ACG TAT GTG CTT AG 3'

Silencer insertion site

Sil5 5' GAG ATA GTG GAG CAA TCT CTG AGA TG 3'

Sil3 5' TTC ATA CGA GAC CCT CTG TTT TGG C 3'

Silencer insert

5'Hyg 5' GTC CTG CGG GTA AAT AGC TGC 3'

3'Hyg 5' CGT CTG CTG CTC CAT ACA AGC 3'

Legends to Supplementary Figures

Supplementary Figure 1. Transgene constructs and analysis of DNA methylation by using methylation-sensitive restriction enzymes.

A) The Target construct in the α' promoter system contains an α'pro-*GFP* (green fluorescent protein) reporter gene flanked by an intact nopaline synthase (*NOS*) gene on the right and a NOSpro-*NPTII* (neomycinphsophotransferase) gene, which provides resistance to kanamycin, on the left. The Silencer construct contains an inverted repeat of α' promoter sequences (black arrowheads) that is transcribed by the 35S promoter. A 19S promoter drives expression of a hygromycin phosphotransferase (*HPT*) gene, which provides resistance to hygromycin. The pUC119 and pUC18 sequences in the Target and Silencer constructs, respectively, allow cloning of the transgene inserts by plasmid rescue (Kanno et al., 2004).
B) The Target construct in the NOSpro system contains an intact *NOS* gene and a NOSpro-*NPTII* gene (Np-NPTII), which confers resistance to kanamycin. The

Silencer construct contains an inverted repeat of NOS promoter sequences (black arrowheads) that is transcribed by the 35S promoter and a 19Spro-*HPT* gene conferring resistance to hygromycin (Aufsatz et al., 2002a).

Abbreviations: LB, RB: left and right T-DNA borders. Arrows indicate direction of transcription. T, transcription termination signal.

C) The α' promoter covered by α' promoter double stranded RNA (white bar) is 257 bp in length; +1 indicates the transcription start site. Sequences outside this region of RNA-DNA sequence identity are shown in black. The approximate positions of the recognition sequences for methylation sensitive restriction enzymes used to analyze cytosine methylation are indicated. The exact positions of these sites are shown in Supplementary Fig. 3. A standard double digest using *Kpn*I and *Nde*I was performed, after which one of the methylation-sensitive restriction enzymes was added. Part of the *GFP* coding region (hatched) was used to probe the Southern blots.

D) The NOSpro covered by NOSpro double stranded RNA (white bar) is 259 bp in length; +1 indicates the transcription start site. Sequences outside this region of RNA-DNA sequence identity are shown in black. The approximate positions of the recognition sequences for methylation sensitive restriction enzymes used to analyze cytosine methylation are indicated. The exact positions of these sites are shown in Supplementary Fig. 2. A standard double digest using *Eco*RI and *Pst*I was performed, after which one of the methylation-sensitive restriction enzymes was added. Part of the *NPTII* coding region (hatched) was used to probe the Southern blots

Supplementary Figure 2. Bisulfite sequence analysis of NOS promoter methylation in wild type plants (genotype *K*/-;*H*/-;*D*/*D*) from the '*de novo*' set-up (Fig. 2D). DNA was prepared from rosette leaves of an F1 plant. Following bisulfite sequence and PCR amplification using the primer set shown, seven clones were sequenced. Three of these revealed methylation at cytosines in all sequence contexts within the region of RNA-DNA sequence homology (underlined), which is the pattern expected for RNA-directed DNA methylation. Four clones lacked methylation, consistent with the gradual acquisition of methylation of the NOS promoter in response to RNA signals. The bisulfite sequence analysis was also carried out on a *drd1-6* mutant (genotype *K*/-;*H*/-;*d*/*d*) from the '*de novo*' set-up (Fig. 2E). The sequence of ten clones showed complete conversion of cytosines to thymines (not shown), consistent with no methylation induced in the mutant. The positions of restriction enzyme sites used for the Southern blot analyses are indicated. Circles indicate CGs, boxes CNGs and triangles CNNs.

Filled figures signify methylation. The NOSpro has an overall GC content of 45%. It contains 19 CGs, 12 CNGs and 44/32 CNNs (top/bottom DNA strand).

Supplementary Figure 3. Bisulfite sequence analysis of α' promoter methylation in:

(A) 'maintenance' set-up, wild type (Fig. 2G) (genotype *K/K*;-/-;*D/D*). Corresponding Southern data using methylation-sensitive restriction enzymes shown in Fig. 4G, left.

(B) 'maintenance' set-up, *drd1-6* mutant (Fig. 4H) (genotype *K*/*K*;-/-;*d*/*d*). Corresponding Southern data using methylation-sensitive restriction enzymes shown in Fig. 4H, left.

(C) *drd1-1* mutant, third generation (genotype *K*/*K*;*H*/*H*;*d*/*d*).

(D) wild type plants homozygous for target and silencer complexes (genotype *K*/*K*;*H*/*H*;*D*/*D*).

The data shown in 'C' and 'D' have been published previously in graph form (Kanno et al., 2004) and are included here for making comparisons to 'A' and 'B'. DNA was prepared from rosette leaves (Kanno et al., 2004). The underline indicates the region of RNA-DNA sequence identity. The primer set used for PCR amplification is shown and the positions of restriction enzyme sites used for the Southern blot analyses are indicated. Sequences of 16 cloned PCR amplification products are shown. Circles indicate CGs, boxes CNGs and triangles CNNs. Filled figures signify methylation. The α' promoter has an overall GC content of 46%. It contains 9 CGs, 9 CNGs and 71/24 CNNs (top/bottom DNA strand). Overall, the bisulfite data are consistent with the Southern blot data. However, maintenance of CG methylation in the *drd1* mutant (B) at the *Hpy*CH4IV site appears low in the bisulfite experiment (CG methylation in 1/16 cloned sequences) compared to the Southern analysis (50% or more of the signal is in the methylated fragment; Fig. 4H, I, left, H lane). Moreover, considerable methylation is observed in the bisulfite sequence analysis at the *Scr*FI site at –260, which is somewhat higher than expected from the faint methylated fragment observed in the Southern blot analysis (Fig. 4H, I, left, Sc lane). In this case though, note that there is an additional, completely unmethylated *Scr*FI site at -10 that does not contain a CG dinucleotide. These differences underscores the need to use both approaches to analyze methylation. Analysis with methylation-sensitive restriction enzymes is limited by the availability of suitable restriction enzyme sites in a given sequence, but has the advantage that methylation in the entire DNA preparation is analyzed. Bisulfite sequencing measures

methylation at all Cs in a given DNA sequence, but is potentially biased because a PCR amplification step is required following the bisulfite treatment.

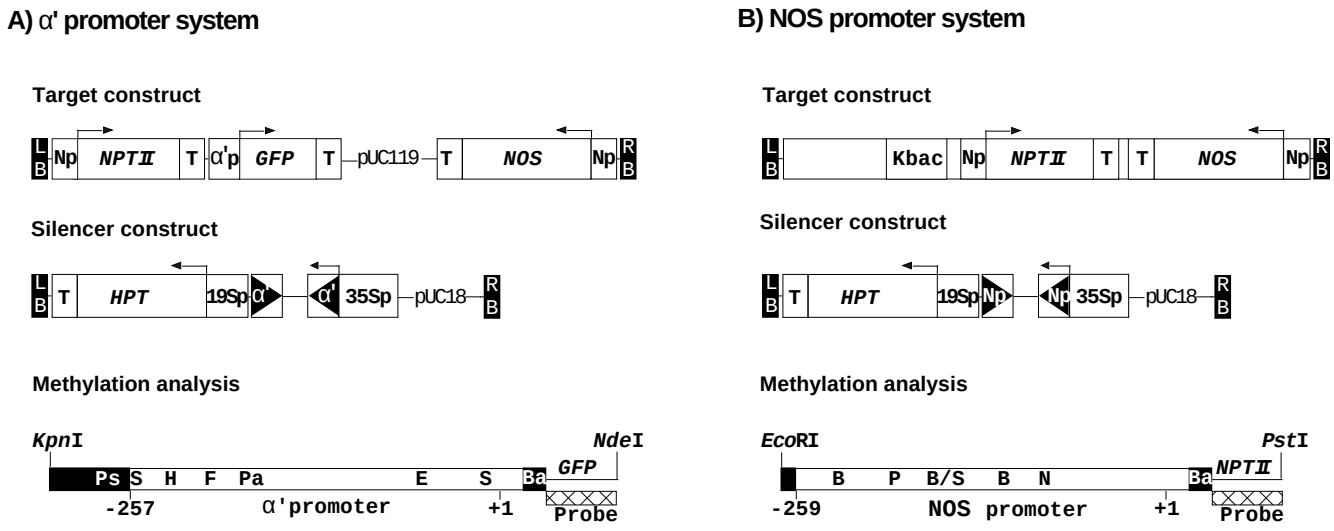
References for Supplementary Information and Figures

Aufsatz W, Mette MF, Matzke AJM, Matzke M (2004) The role of MET1 in RNA-directed *de novo* and maintenance methylation of CG dinucleotides. *Plant Mol Biol* **54**: 793-804.

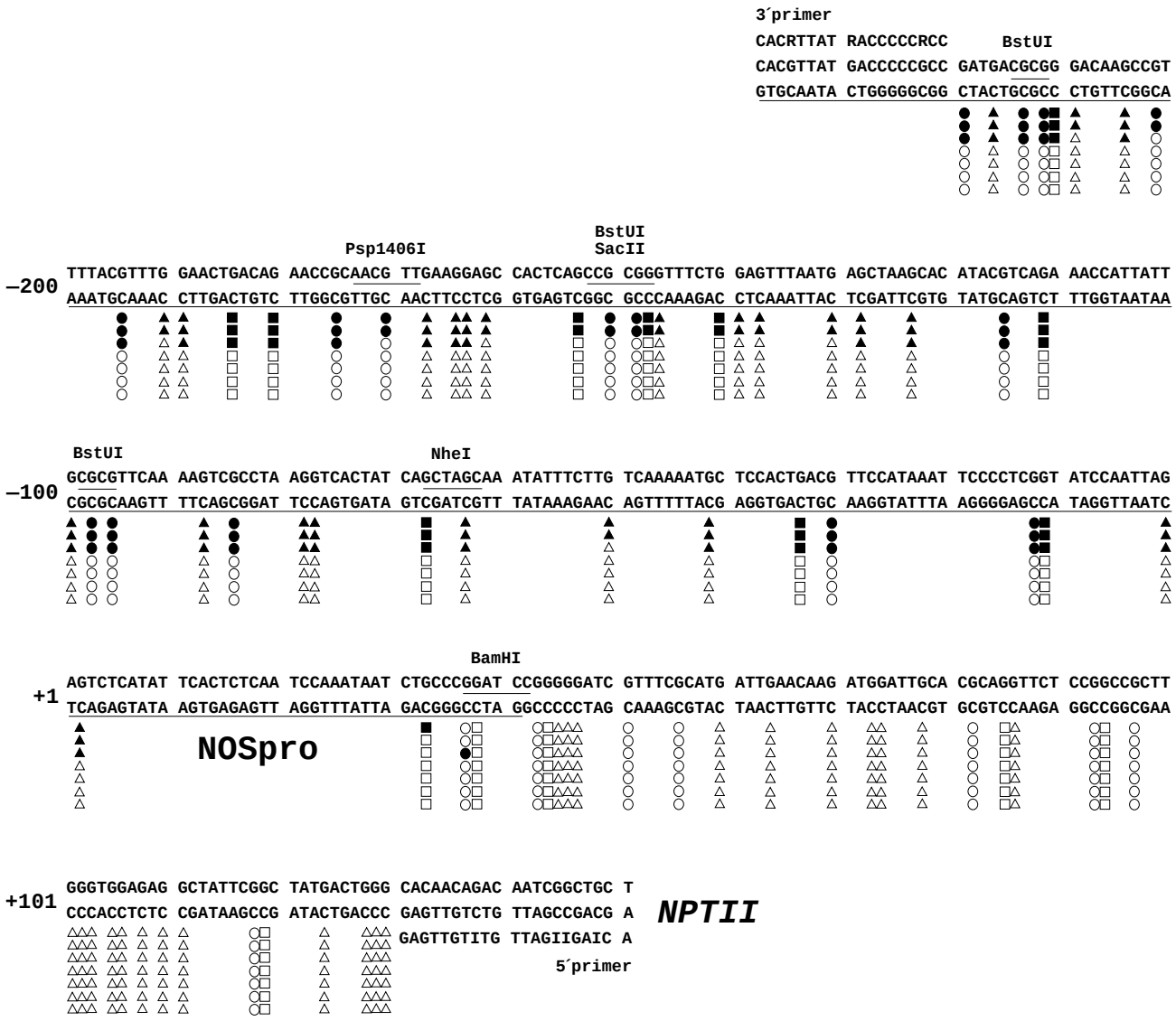
Aufsatz W, Mette MF, van der Winden J, Matzke AJM, Matzke M (2002) RNA-directed DNA methylation in *Arabidopsis*. *Proc Natl Acad Sci USA* **99**: 16499-16506.

Aufsatz W, Mette MF, van der Winden J, Matzke M, Matzke AJM (2002) HDA6, a putative histone deacetylase needed to enhance DNA methylation induced by double stranded RNA. *EMBO J* **21**: 6832-6841.

Kanno T, Mette MF, Kreil DP, Aufsatz W, Matzke M, Matzke AJM (2004) Involvement of putative SNF2 chromatin remodeling protein DRD1 in RNA-directed DNA methylation. *Curr. Biol*. **14**: 801-805.

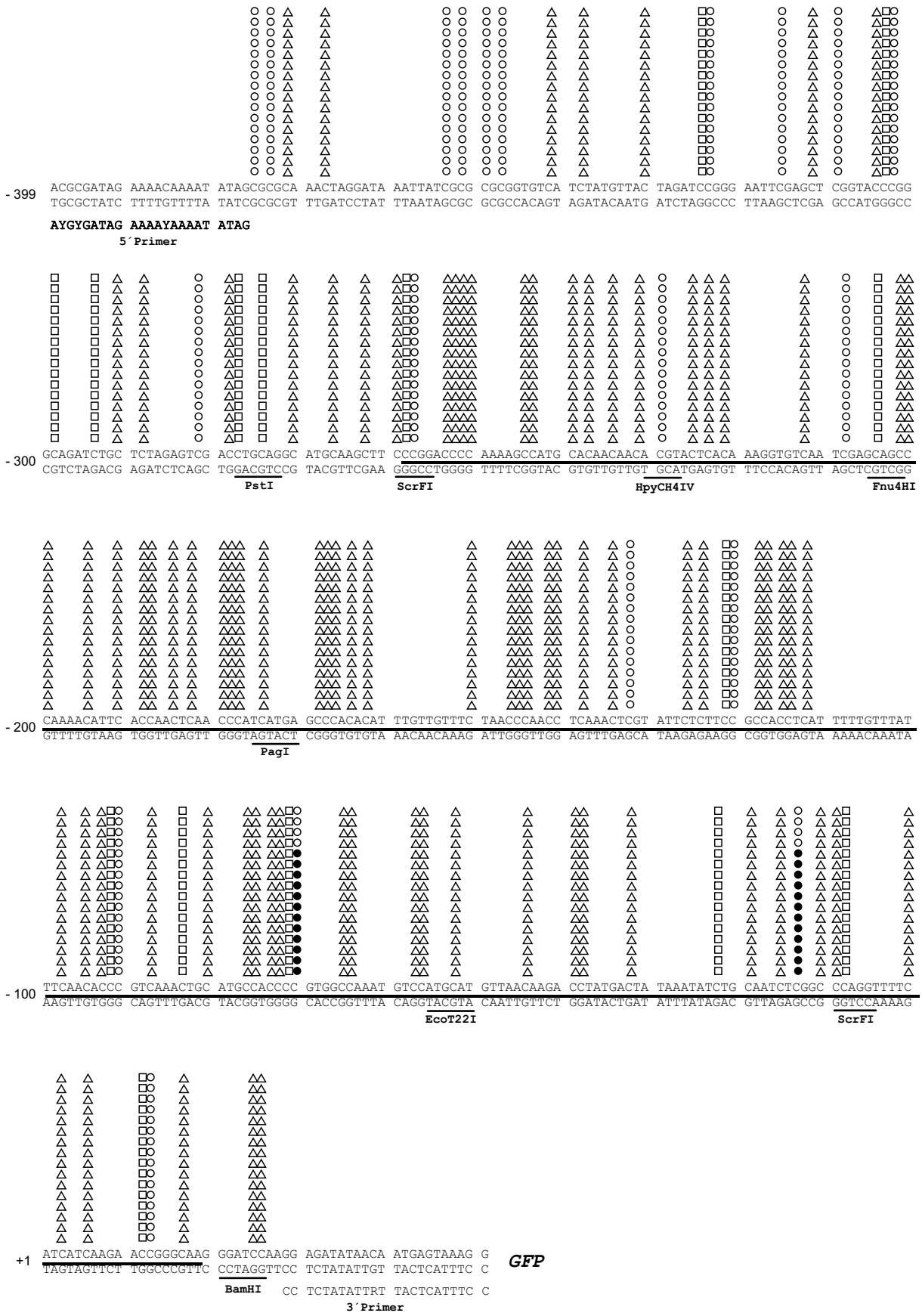


Supplementary Fig. 1 Kanno *et al.*



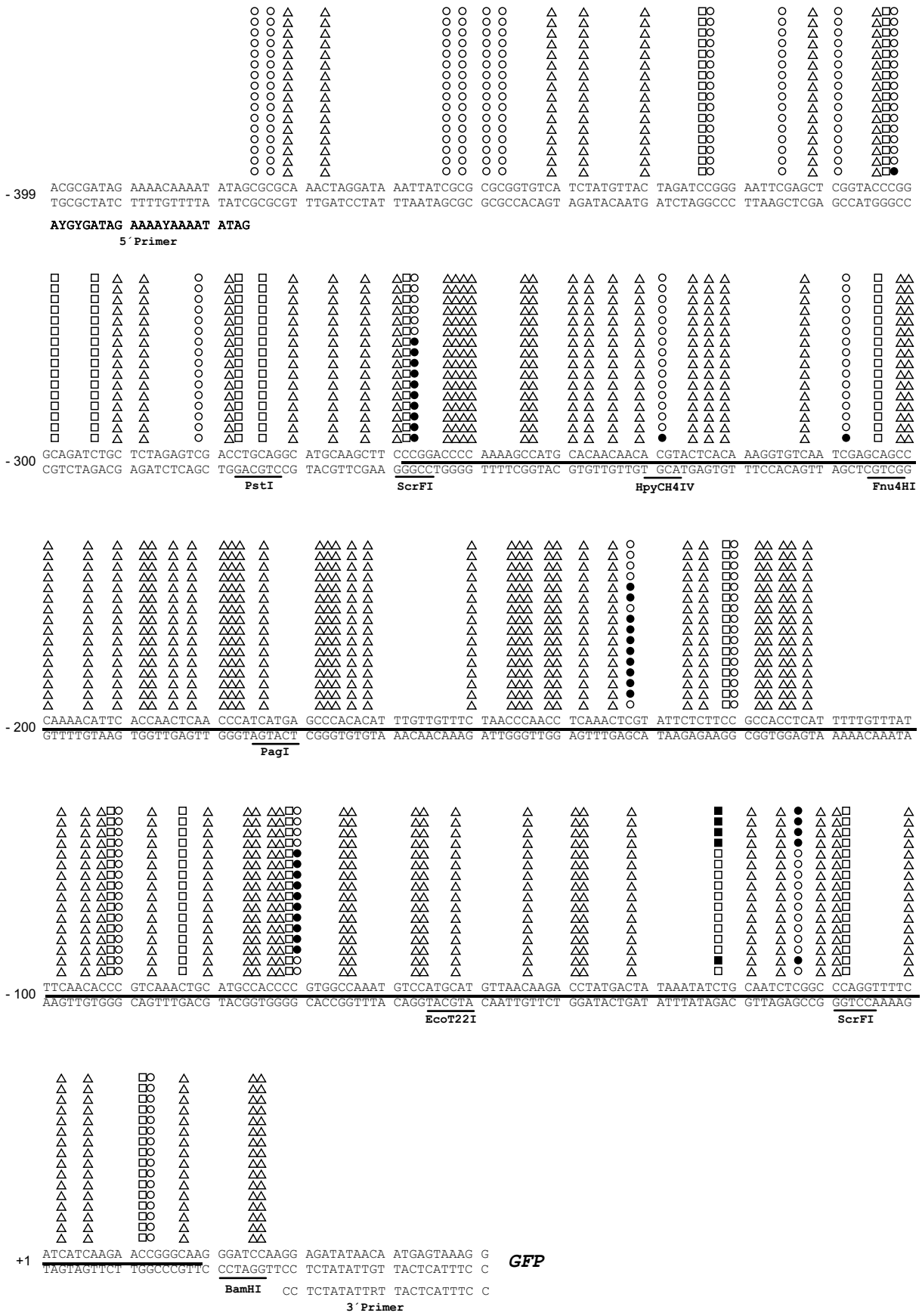
Supplementary Fig. 2 Kanno *et al.*

Bisulfite sequencing NOSpro-NPTII bottom strand of "*de novo* genotype" *K/-; H/-; D/D*
○CG □CNG △CNN; open: unmethylated; filled:methylated;



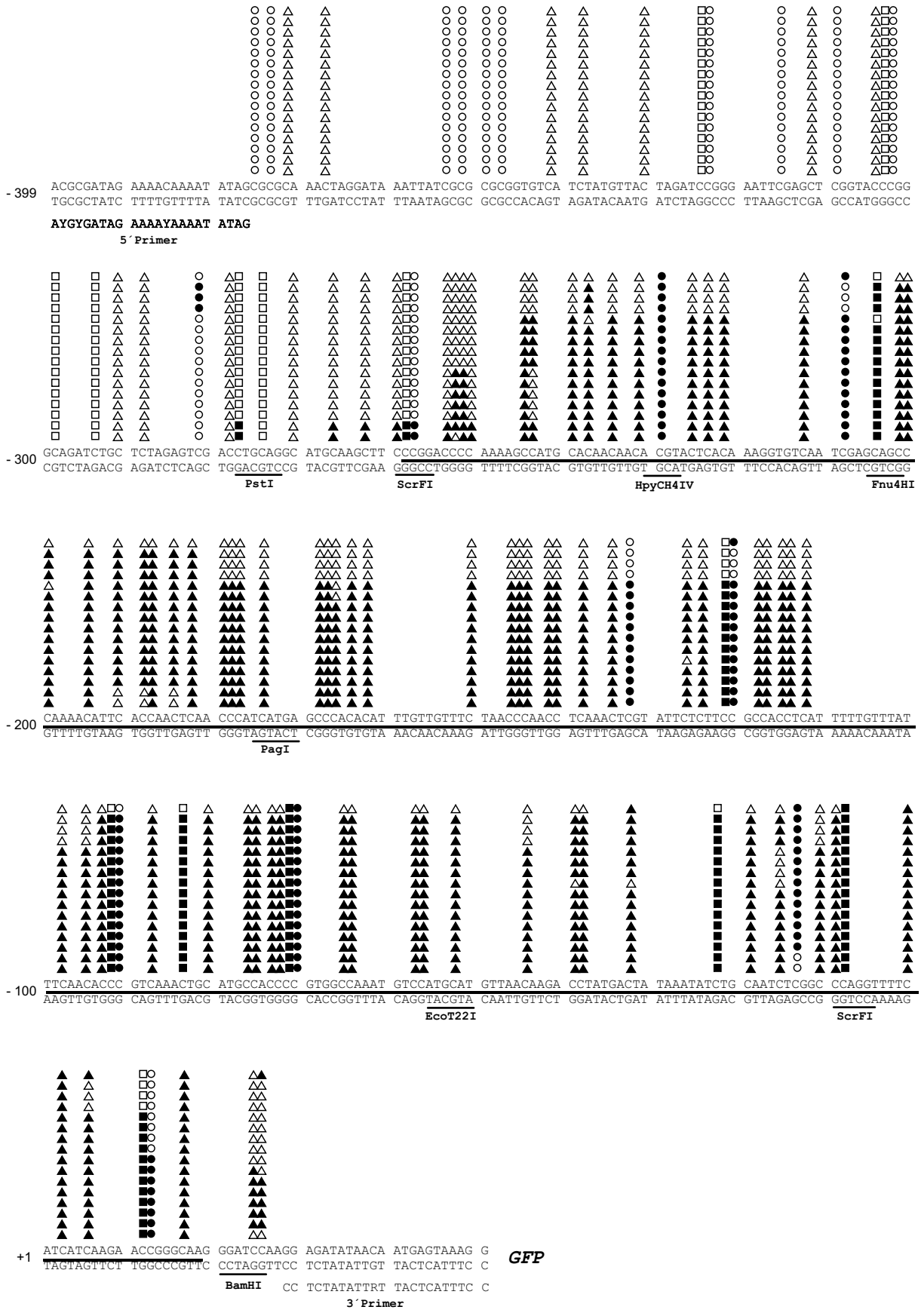
Supplementary Fig. 3A Kanno *et al.*

Bisulfite sequencing ´pro-GFP top strand of "maintenance genotype" *K/K; -/-; D/D*
○CG □CNG △CNN; open: unmethylated; filled: methylated



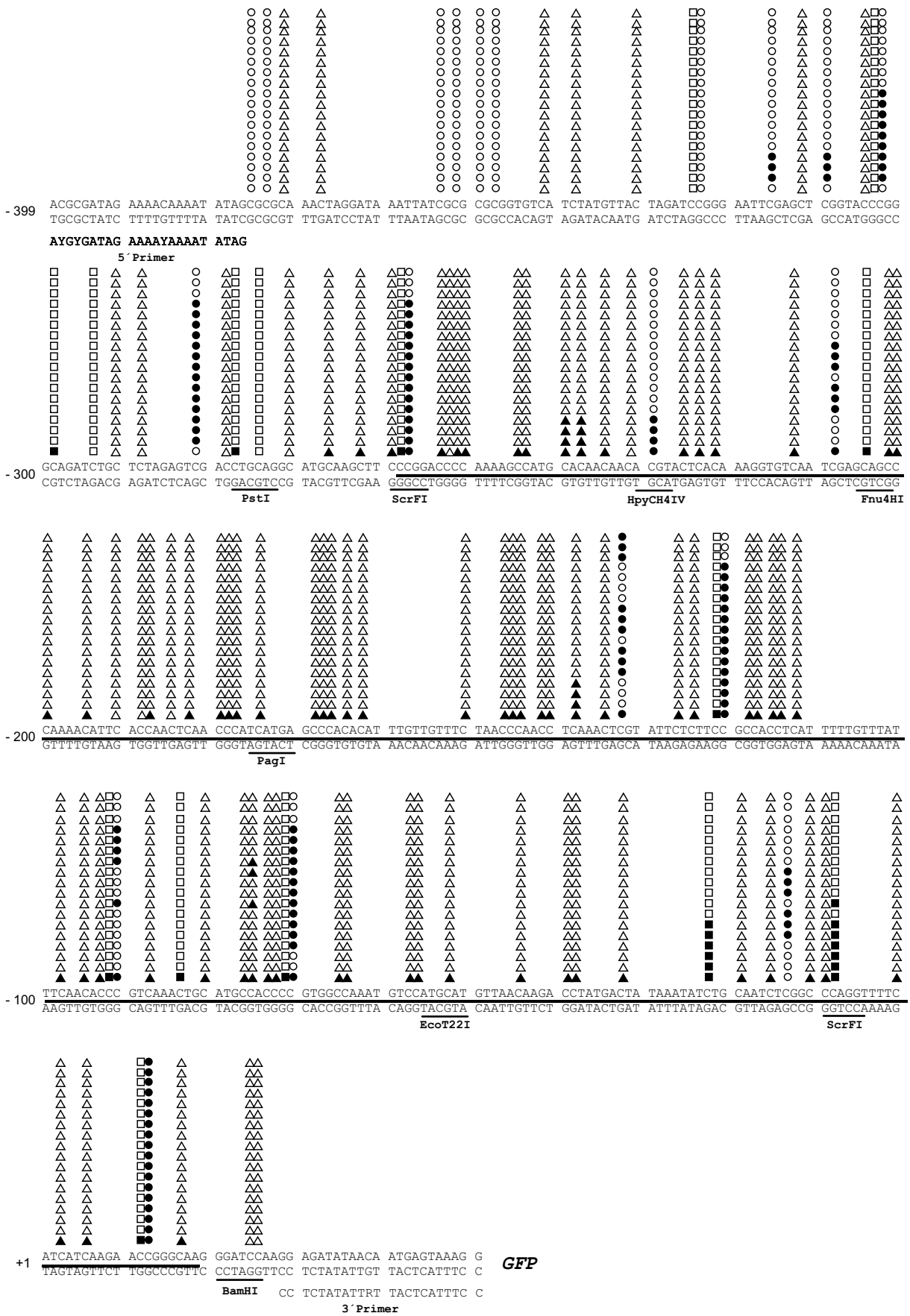
Supplementary Fig. 3B Kanno *et al.*

Bisulfite sequencing ´pro-GFP top strand of "maintenance genotype" *K/K; -/-; d/d*
○CG □CNG △CNN; open: unmethylated; filled: methylated



Supplementary Fig. 3C Kanno *et al.*

Bisulfite sequencing ´pro-GFP top strand of *K/K; H/H; D/D*
○CG □CNG △CNN; open: unmethylated; filled: methylated



Supplementary Fig. 3D Kanno *et al.*

Bisulfite sequencing ´pro-GFP top strand of *K/K; H/H; d/d*
○CG □CNG △CNN; open: unmethylated; filled: methylated