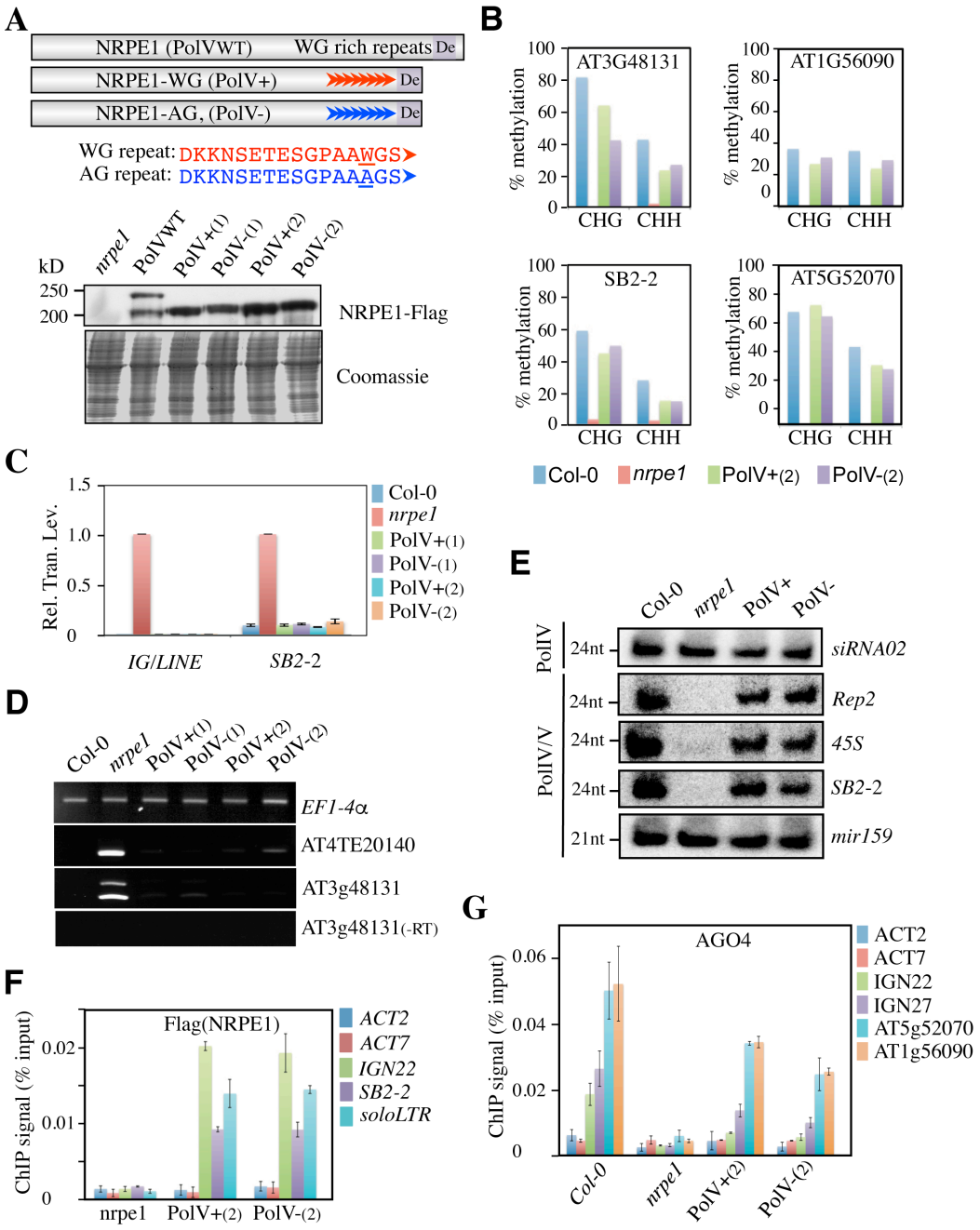
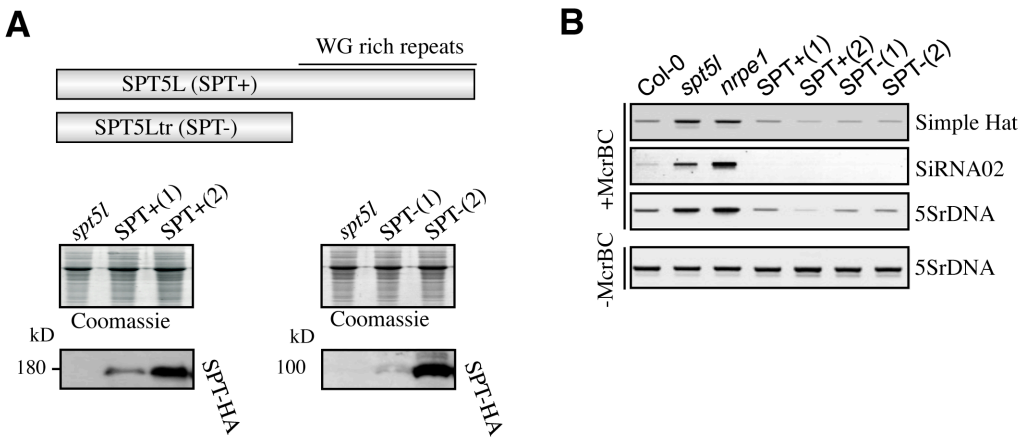

A
NRPE1 (PolVWT)
WG rich repeats
De
NRPE1-WG (PolV+)
NRPE1-AG, (PolV-)
WG repeat: DKKNSETESGPAAWGS
AG repeat: DKKNSETESGPAAAGS
kD
250
200
nrpe1
PolVWT
PolV+(1)
PolV-(1)
PolV+(2)
PolV-(2)
NRPE1-Flag
Coomassie
B
AT3G48131
AT1G56090
SB2-2
AT5G52070
% methylation
CHG
CHH
Col-0
nrpe1
PolV+(2)
PolV-(2)
C
Rel. Tran. Lev.
IG/LINE
SB2-2
Col-0
nrpe1
PolV+(1)
PolV-(1)
PolV+(2)
PolV-(2)
D
EF1-4α
AT4TE20140
AT3g48131
AT3g48131(-RT)
E
PolIV
PolIV/V
24nt
21nt
siRNA02
Rep2
45S
SB2-2
mir159
F
Flag(NRPE1)
ChIP signal (% input)
ACT2
ACT7
IGN22
SB2-2
soloLTR
nrpe1
PolV+(2)
PolV-(2)
G
AGO4
ChIP signal (% input)
ACT2
ACT7
IGN22
IGN27
AT5g52070
AT1g56090

Supplemental Fig. S1: NRPE1 AGO hooks are not mandatory for RdDM

A) Schematic representation and western blot analysis of four independent Flag-tagged NRPE1-WG/PolV+(1/2) and NRPE1-AG/PolV-(1/2) variant lines. Coomassie blue is used as loading control. B) Bisulfite analysis of DNA methylation in CHG and CHH contexts at several RdDM targets in Col-0, *nrpe1*, PolV+(2) and PolV-(2) lines. C) Quantitative RT-PCR analyses of IG/LINE and SB2-2 loci in Col-0, *nrpe1*, PolV+(1,2) and PolV-(1,2) lines. qRT-PCR values were normalized to *ACTIN7* and to *nrpe1* values. Rel. Tran. Lev. stands for Relative Transcript Level. Data represent the means of 3 independent experiments and error bars the corresponding standard deviation values. D) Semiquantitative RT-PCR analyses of AT4TE20140 and AT3g48131 loci in Col-0, *nrpe1*, PolV+(1,2) and PolV-(1,2) lines. EF1α was used as loading control. Minus RT (-RT) reactions are controls for genomic DNA contamination. E) Analysis of siRNA levels by Northern blot in Col-0, *nrpe1*, PolV+ (pool of variant lines PolV+(1,2)) and PolV- (pool of variant lines PolV-(1,2)) lines. Mir159 is used as a loading control. F) ChIP analysis of Pol V variants using the anti-Flag antibody in *nrpe1*, PolV+(2), and PolV-(2) lines. Actin 2 and actin 7 are used as negative controls. Values are means +/-SD from three independent amplifications. G) ChIP analysis of AGO4 binding in *nrpe1*, PolV+(2) and PolV-(2) lines. Actin 2 and actin 7 are used as negative controls. Values are means +/-SD from three independent amplifications.

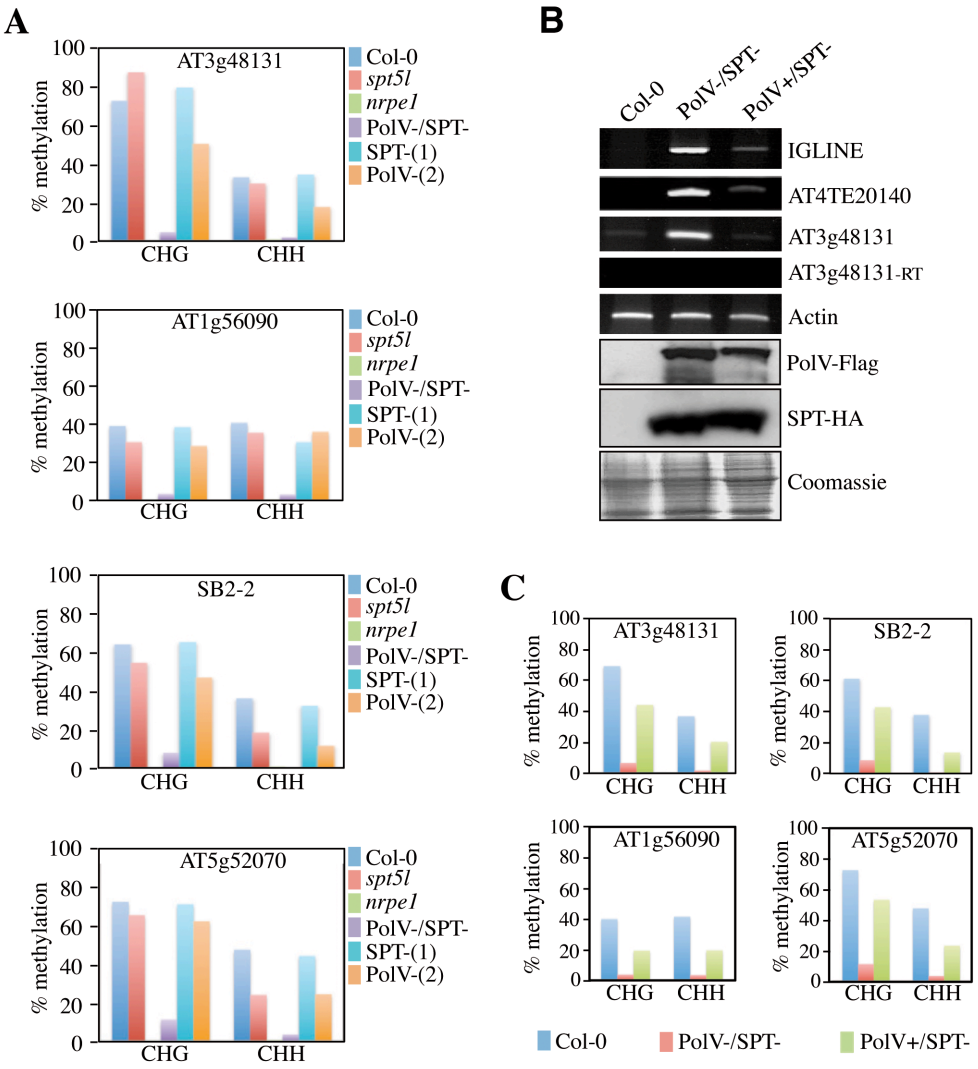
Supplemental_Fig_S2



Supplemental Fig. S2: SPT5L AGO hooks are not mandatory for RdDM

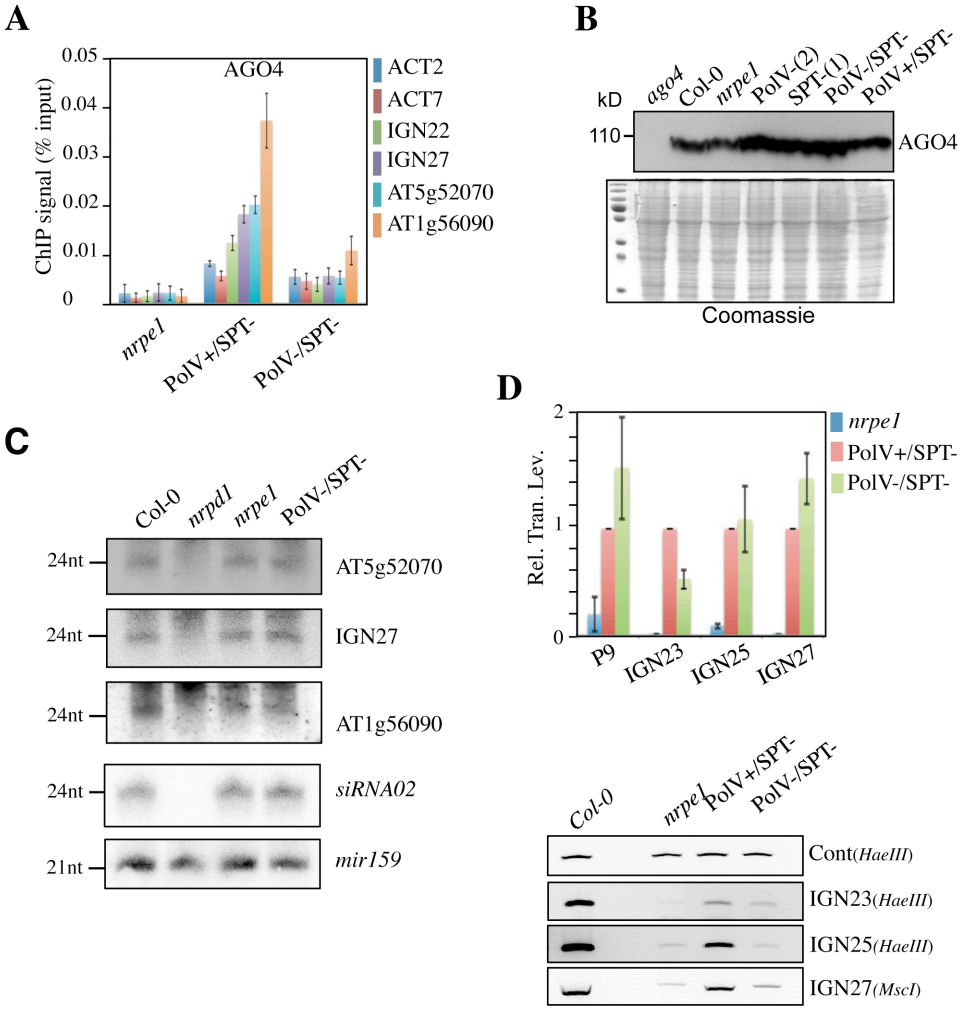
A) Schematic representation and western blot analysis of four independent HA-tagged SPT5L/SPT+(1/2) and truncated SPT5Ltr/SPT-(1/2) variant lines. Coomassie staining is used as a loading control. B) Analysis of DNA methylation at Simple Hat, siRNA02, 5SrDNA loci into *nrpe1*, *spt5l*, Col-0, SPT+(1,2) and SPT-(1,2) lines. Genomic DNA was digested with the McrBC methylation sensitive enzyme and used as a template for PCR. Undigested DNA at the 5SrDNA was used as a loading control.

Supplemental_Fig_S3



Supplemental Fig.S3: Methylation analysis of RdDM targets.

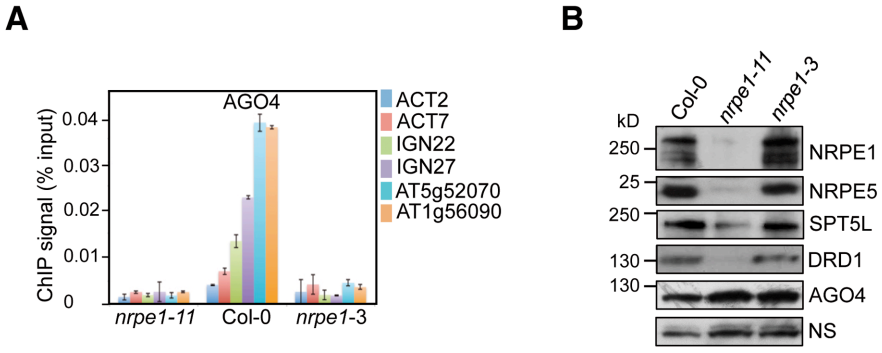
A) Analysis of DNA methylation by bisulfite sequencing in CHG and CHH contexts at four RdDM targets (namely AT3g48131, SB2-2, AT1g56090 and AT5g52070) in wild-type (Col-0), *nrpe1*, *spt5l*, PolV-/SPT- cross line and two SPT-(1) and PolV-(2) complemented lines. B) *Top panel*: Transcript level analysis by RT PCR at RdDM targets in Col-0, PolV-/SPT- and PolV+/SPT- cross lines. *ACTIN2* is used as a loading control and –RT reactions are used to assess genomic DNA contamination. *Bottom panel*: Detection of NRPE1 and SPT5L variants by western blots in the crosses lines. NRPE1 and SPT5L variants were detected respectively with anti-Flag and anti-HA antibodies. Coomassie blue staining indicates equal loading. C) Analysis of DNA methylation by bisulfite sequencing in CHG and CHH contexts at 4 RdDM targets in Col-0, and the PolV-/SPT- and PolV+/SPT- cross lines.


A
AGO4
ChIP signal (% input)
0.05
0.04
0.03
0.02
0.01
0
ACT2
ACT7
IGN22
IGN27
AT5g52070
AT1g56090
nrpe1
PolV+/SPT-
PolV-/SPT-
B
kD
ago4
Col-0
nrpe1
PolV-(2)
SPT-(1)
PolV-/SPT-
PolV+/SPT-
110
AGO4
Coomassie
C
Col-0
nrpd1
nrpe1
PolV-/SPT-
24nt
AT5g52070
IGN27
AT1g56090
siRNA02
21nt
mir159
D
Rel. Tran. Lev.
2
1
0
nrpe1
PolV+/SPT-
PolV-/SPT-
P9
IGN23
IGN25
IGN27
Col-0
nrpe1
PolV+/SPT-
PolV-/SPT-
Cont(HaeIII)
IGN23(HaeIII)
IGN25(HaeIII)
IGN27(MscI)

Supplemental Fig. S4: AGO hooks and not P5RNAs are the major determinants of AGO4 recruitment to RdDM loci

A) Chromatin immunoprecipitation analysis of AGO4 binding using anti-AGO4 antibodies in *nrpe1*, PolV+/SPT- and PolV-/SPT- lines. The tested targets are indicated on the right. Actin2 and Actin7 are used as negative controls. Values are means +/-SD from two independent amplifications. B) Analysis of the AGO4 protein accumulation by western blots in *ago4*, Col-0, *nrpe1*, the PolV-(2) and SPT-(1) complemented lines and in PolV-/SPT- and PolV+/SPT- cross lines. Coomassie blue staining is used as a loading control. C) Analysis of siRNA levels by Northern blot in Col-0, *nrpd1*, *nrpe1*, and PolV-/SPT- lines. Mir159 is used as a loading control. D) *Top panel* P9, IGN23, IGN23, IGN27 transcript accumulation was tested in *nrpe1*, PolV+/SPT- and PolV-/SPT- lines. Rel. Tran. Lev. stands for Relative Transcript Level normalized to Actin and PolV+/SPT- using the ΔΔCt method. *Bottom panel*: Analysis of DNA methylation by Chop-PCR at IGN23, IGN25 and IGN27 loci. Genomic DNA was digested with *HaeIII* or *MscI* methylation sensitive enzymes and used as template for PCR. The *RDRP* gene has no *HaeIII* site and was used as control (cont). DNA methylation was assessed in PolV+/SPT- and PolV-/SPT- cross lines in the right panel. Col-0 and *nrpe1* mutant were used as controls.

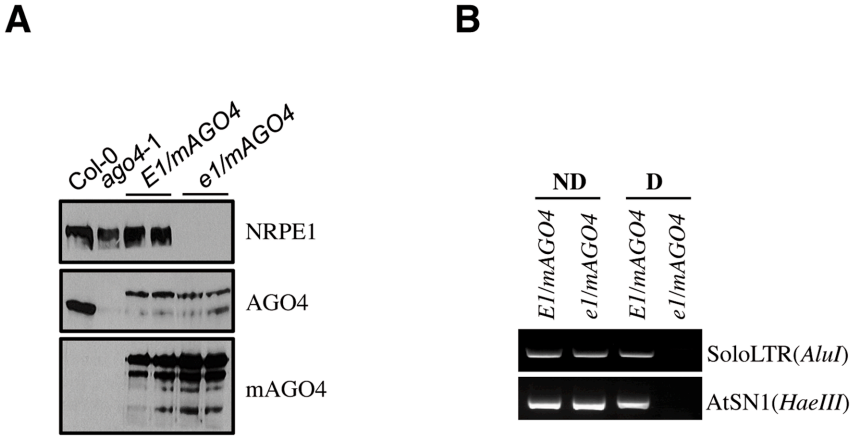
Supplemental_Fig_S5



Supplemental Fig. S5: Molecular analysis of the *nrpe1-3* catalytic mutant.

A) Chromatin immunoprecipitation analysis of AGO4 binding in *nrpe1-11* and *nrpe1-3* Pol V mutants at different RdDM targets, as indicated on the right. Actin2 and Actin7 were used as controls. Values are means +/-SD of two independent amplifications. B) Analysis by western blot of the accumulation levels of RdDM actors in Col-0, *nrpe1-11* and *nrpe1-3* PolV mutants. Antibodies against NRPE1, NRPE5, DRD1, SPT5L and AGO4 were used as indicated on the right. NS indicates a non-specific band that is used as a loading control.

Supplemental_Fig_S6



Supplemental Fig. S6: Molecular analysis of cmycAGO4 lines used in LChIP.

A) Analysis by western blot of the accumulation levels of NRPE1 and AGO4 proteins in Col-0, *ago4*-1, *E1/mAGO4 and e1/mAGO4* lines. Antibodies against NRPE1, AGO4 and Cmyc tag were used as indicated on the right. B) Analysis of DNA methylation by Chop PCR at SB2-2 and soloLTR loci in *E1/mAGO4 and e1/mAGO4* lines. Genomic DNA digested with *HaeIII and AluI* methylation sensitive enzymes (D) was used as template for PCR. Undigested DNA (ND) was used as a control.