# Supporting Information

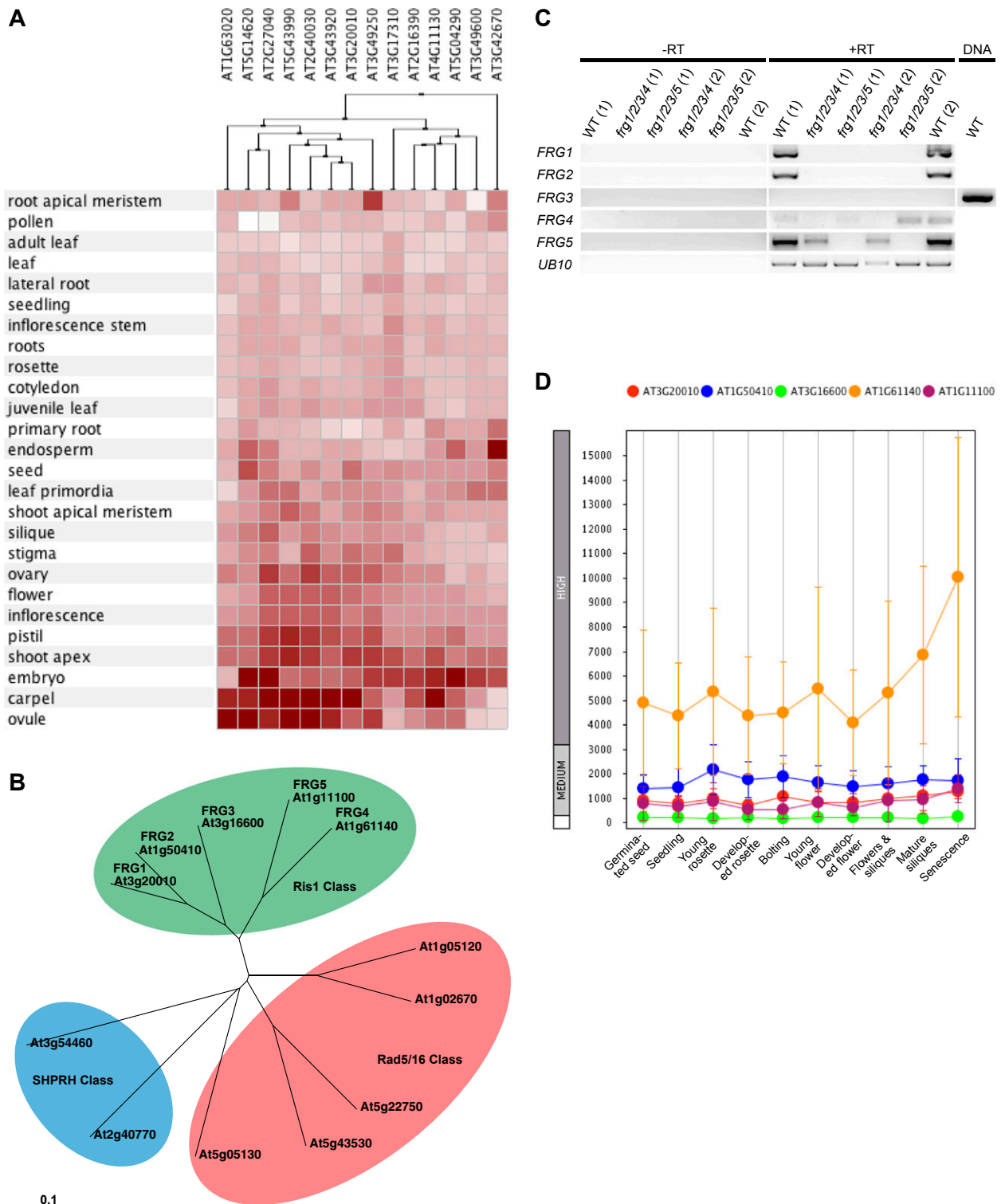## Groth et al. 10.1073/pnas.1420515111



**Fig. S1.** Expression patterns of RdDM genes and the *FRG* gene family. (*A*) Hierarchical cluster analysis of expression levels of 14 RdDM genes, including *FRG1*, in 26 different anatomical parts from microarray data (ATH1). (*B*) Phylogenetic tree (neighbor joining) of FRG1 to FRG5 proteins. (*C*) Confirmation of T-DNA insertion mutants by RT-PCR on PCR template without reverse transcription (−RT), cDNA (+RT), and DNA template. Ubiquitin (UB10) was used as control. (*D*) Mean expression levels (±SD) of *FRG* family members from microarray data (ATH1) by developmental stage/tissue.
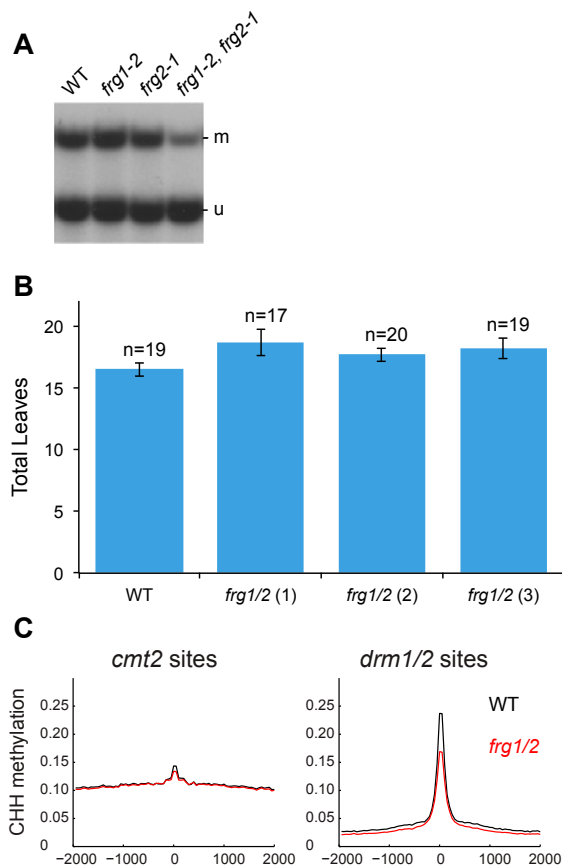
**Fig. S2.** Analysis of DNA methylation in *frg1/2* double-mutants. (*A*) DNA blot analysis of CHG methylation at the MEA-ISR in wild-type, as well as *frg1-2* and *frg2-1* single- and double-mutants. Genomic DNA has been digested with the methylation sensitive enzyme MspI; the upper and lower bands correspond to methylated (m) and unmethylated (u) fractions, respectively. (*B*) Flowering time (mean ± SE) of T1 offspring of wild-type and *frg1/2* double-mutants transformed with an unmethylated FWA transgene. Numbers in parentheses indicate individual lines. (*C*) Average distribution of CHH methylation levels over *cmt2* and *drm1/2* CHH hypo DMRs. The *x* axis indicates distance from the DMR midpoints in base pairs. The *frg1-1* mutant allele has been used for all experiments, unless indicated differently.
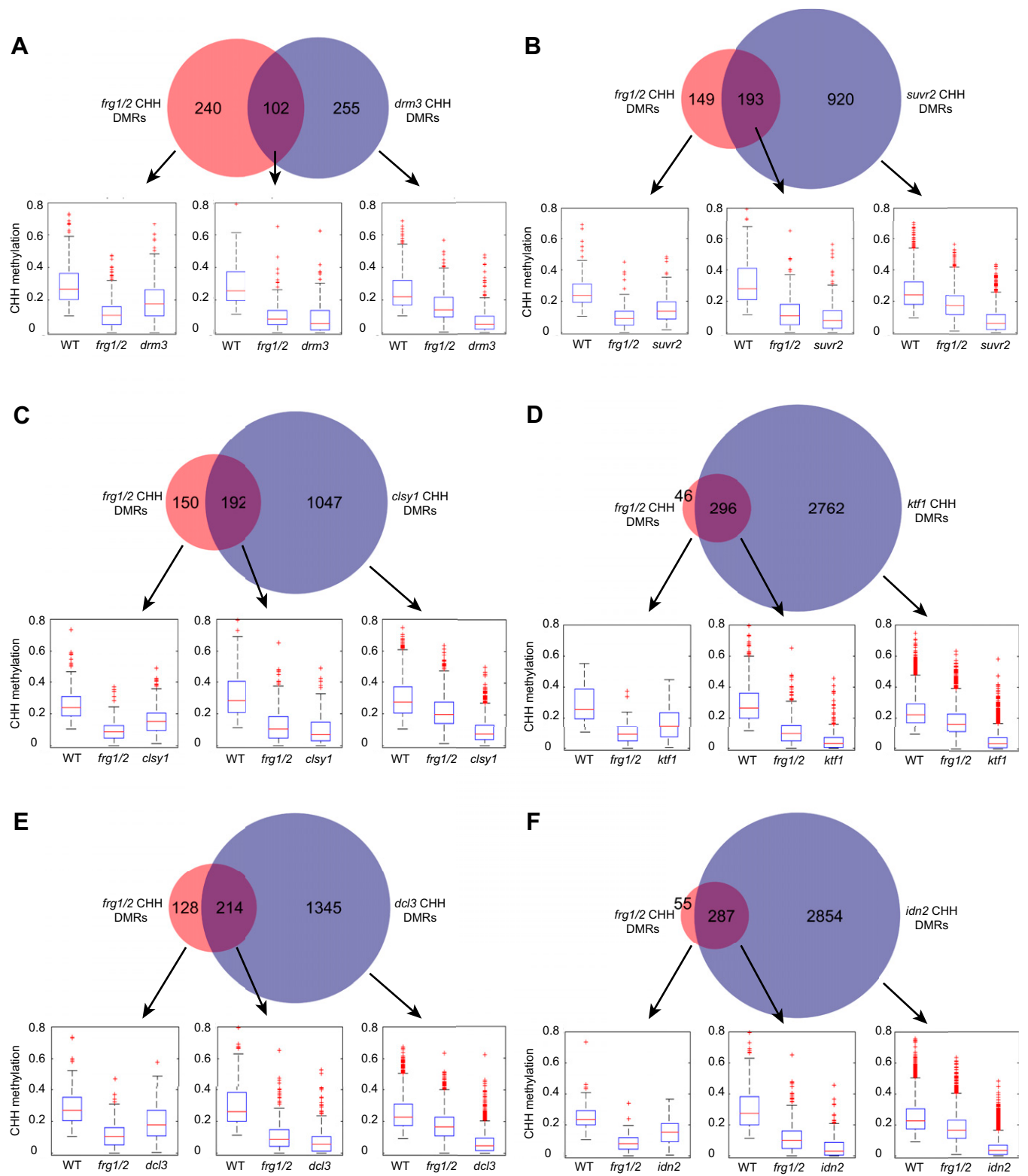
**Fig. S3.** *frg1/2* double mutants show general reduction in CHH methylation. Overlap between *frg1/2* and *drm3* (*A*), *suvr2* (*B*), *clsy1* (*C*), *ktf1* (*D*), *dcl3* (*E*), or *idn2* (*F*) CHH hypo-DMRs. Box plots show CHH methylation levels in the respective DMR fractions.
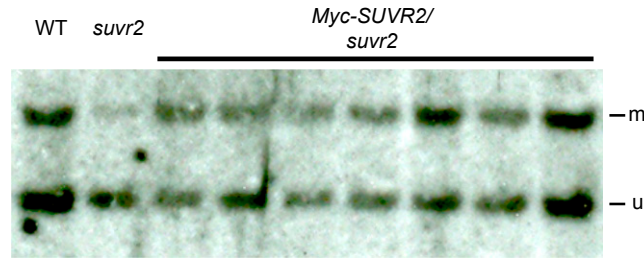
**Fig. S4.** DNA blot showing that Myc-tagged SUVR2 complements the DNA methylation defect of *suvr2* at the *MEA-ISR* locus. Seven individual T1 lines are shown.

**Table S1. List of genes that coexpress with RdDM genes and corresponding coexpression coefficient**

| DCL3 | DRM2 | RDR2 | AGO4 | NRPE2 | NRPD1 | NRPE1 | DRD1 | IDN2 | DRM3 | DMS3 | RDM1 | KTF1 | DMS4 | IDP2 | SUVR2 | Count | Gene* |
|------|------|------|------|-------|-------|-------|------|------|------|------|------|------|------|------|-------|-------|-------|
| 0.751 | 0.646 | | 0.766 | 0.677 | | | | | | 0.698 | | 0.619 | 0.657 | 0.574 | 0.661 | 9 | **FRG1** |
| 0.749 | | 0.752 | 0.675 | | | | | | 0.647 | 0.706 | | 0.635 | 0.681 | | | 7 | U.K.L1 |
| 0.739 | | 0.905 | | 0.704 | 0.704 | | | | | | | 0.671 | 0.765 | | | 6 | AT5G25475 |
| | 0.74 | | 0.591 | | 0.674 | | | 0.718 | 0.619 | | | 0.634 | | | | 6 | ROS3 |
| 0.708 | 0.641 | | | | | | | | 0.627 | | | 0.626 | 0.657 | 0.679 | | 6 | AT3G15120 |
| 0.74 | | 0.845 | | | | | | 0.782 | | | | 0.672 | 0.810 | | | 5 | **IDP2** |
| 0.75 | | 0.845 | | | | | | 0.781 | | | | 0.671 | 0.795 | | | 5 | **IDP1** |
| 0.728 | | | | | | | | 0.892 | | | | 0.62 | 0.812 | 0.658 | | 5 | **RDR2** |
| 0.738 | | 0.759 | | 0.654 | | | | | | 0.698 | | 0.663 | | | | 5 | **SUVR2** |
| | | 0.817 | | | | | | 0.84 | | | | 0.583 | 0.647 | 0.6 | | 5 | AT1G18950 |
| 0.675 | | 0.756 | 0.669 | | | | | | 0.69 | | | 0.616 | | | | 5 | AT5G14610 |
| | 0.644 | 0.785 | | | | | | | 0.619 | | | 0.591 | 0.724 | | | 5 | CHR11 |
| | 0.74 | | | | | 0.596 | 0.665 | 0.71 | | | | 0.603 | | | | 5 | AT5G58120 |
| 0.683 | | 0.782 | | | | | | 0.81 | | | | 0.592 | 0.687 | | | 5 | AT1G65370 |
| | | 0.811 | | | | | | 0.858 | | | | | 0.666 | 0.881 | | 4 | AT1G10250 |
| | | 0.794 | | | | | | 0.798 | | | | 0.584 | 0.651 | | | 4 | SPT16 |
| | | | | | | | 0.77 | 0.699 | 0.694 | | | 0.624 | | | | 4 | **NRPD2** |
| | | 0.765 | | | | | | 0.652 | | | | 0.606 | 0.721 | | | 4 | AT5G43820 |
| 0.701 | | | | | 0.683 | | | | 0.693 | 0.662 | | | | | | 4 | GIP1 |
| 0.682 | | | | | | | | 0.722 | | | | 0.637 | 0.674 | | | 4 | AT1G03830 |
| | 0.659 | | | | 0.661 | | | | | 0.637 | 0.677 | | | | | 4 | SUS2 |
| | | | | | 0.666 | | | | | 0.677 | | 0.599 | | 0.55 | | 4 | TPR9 |
| | | | | | 0.747 | | 0.713 | | 0.714 | | | | | | | 3 | OSD1 |
| | | | | | 0.663 | | 0.772 | 0.66 | | | | | | | | 3 | BET10 |
| | | | | | 0.687 | | 0.678 | | 0.704 | | | | | | | 3 | AT4G22860 |
| | | | | | 0.657 | | | | | | | 0.631 | 0.74 | | | 3 | **DCL3** |
| | | | | | 0.678 | | 0.675 | 0.669 | | | | | | | | 3 | AT2G25740 |
| | | | | | 0.694 | | | | | | | 0.595 | | 0.701 | | 3 | **DRM3** |
| | | | | | 0.659 | | | | | 0.659 | | 0.601 | | | | 3 | HMT2 |

*Bold font indicates that gene is known component of RdDM; underlining indicates that that gene has a closely related gene in the *Arbidopsis* genome.

**Table S2. Quantification of FRG1 and SUVR2 peptides identified by coimmunoprecipitation and mass spectrometry of cell extract from FRG1-FLAG expressing *Arabidopsis***

| Replicate | | Spectra[†] | | | | Unique peptides | | | | NSAfe5[‡] | | | | % FRG1[§] | | | |
|-----------|------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|
| Name: TAIR ID | M.W.* (kDa) | First | Second | Third | Fourth | First | Second | Third | Fourth | First | Second | Third | Fourth | First | Second | Third | Fourth |
| FRG1: At3g20010 | 115.5 | 338 | 292 | 41 | 78 | 31 | 53 | 29 | 26 | 2,136 | 490 | 199 | 697 | 100 | 100 | 100 | 100 |
| SUVR2: At5g43990 | 82.0 | 13 | 36 | 6 | 3 | 7 | 26 | 6 | 3 | 118 | 86 | 42 | 38 | 5.5 | 17.6 | 21.2 | 5.5 |

*Molecular weight in kilodalton.
[†]Total number of peptides per gene.
[‡]Spectral counts normalized to protein length and sample complexity.
[§]NSAfe5 values relative to FRG1 in percent.

**Table S3.   Read statistics for bisulfite, mRNA, and smRNA sequencing**

| Bisulfite sequencing | Raw reads | False positive rate (chloroplast) |
|---|---|---|
| *frg1 frg2* | 295,072,635 | mCG = 0.76% |
| | | mCHG = 0.73% |
| | | mCHH = 0.48% |
| mRNA-seq | | Uniquely mapped reads |
| WT, replicate 1 | 23,862,024 | 21,836,316 |
| WT, replicate 2 | 24,362,091 | 22,156,201 |
| WT, replicate 3 | 22,556,627 | 20,503,528 |
| *frg1 frg2*, replicate 1 | 24,431,834 | 22,231,394 |
| *frg1 frg2*, replicate 2 | 22,800,646 | 20,723,455 |
| *frg1 frg2*, replicate 3 | 22,673,935 | 20,630,999 |
| smRNA-seq | | Uniquely mapped reads in size classes 18–34 nt |
| WT, replicate 1 | 15,125,244 | 6,413,067 |
| WT, replicate 2 | 20,330,633 | 8,396,833 |
| WT, replicate 3 | 16,638,823 | 7,733,505 |
| *frg1 frg2*, replicate 1 | 16,253,897 | 7,132,876 |
| *frg1 frg2*, replicate 2 | 14,696,182 | 6,501,880 |
| *frg1 frg2*, replicate 3 | 16,442,801 | 6,762,502 |
| *frg1to5*, replicate 1 | 16,443,065 | 6,834,495 |
| *frg1to5*, replicate 2 | 17,912,561 | 7,563,709 |
| *frg1to5*, replicate 3 | 15,282,482 | 6,428,431 |
| *suvr2*, replicate 1 | 17,288,074 | 7,422,332 |
| *suvr2*, replicate 2 | 17,435,948 | 5,943,106 |
| *suvr2*, replicate 3 | 23,194,034 | 9,676,255 |
| *dcl3*, replicate 1 | 24,860,052 | 8,185,932 |
| *dcl3*, replicate 2 | 20,365,508 | 6,511,050 |
| *dcl3*, replicate 3 | 22,444,088 | 5,654,602 |
| *drm1 drm2*, replicate 1 | 23,940,572 | 9,274,756 |
| *drm1 drm2*, replicate 2 | 23,071,493 | 8,502,891 |
| *drm1 drm2*, replicate 3 | 22,453,773 | 6,884,763 |

# Other Supporting Information Files

Dataset S1 (XLSX)