

Supporting Information

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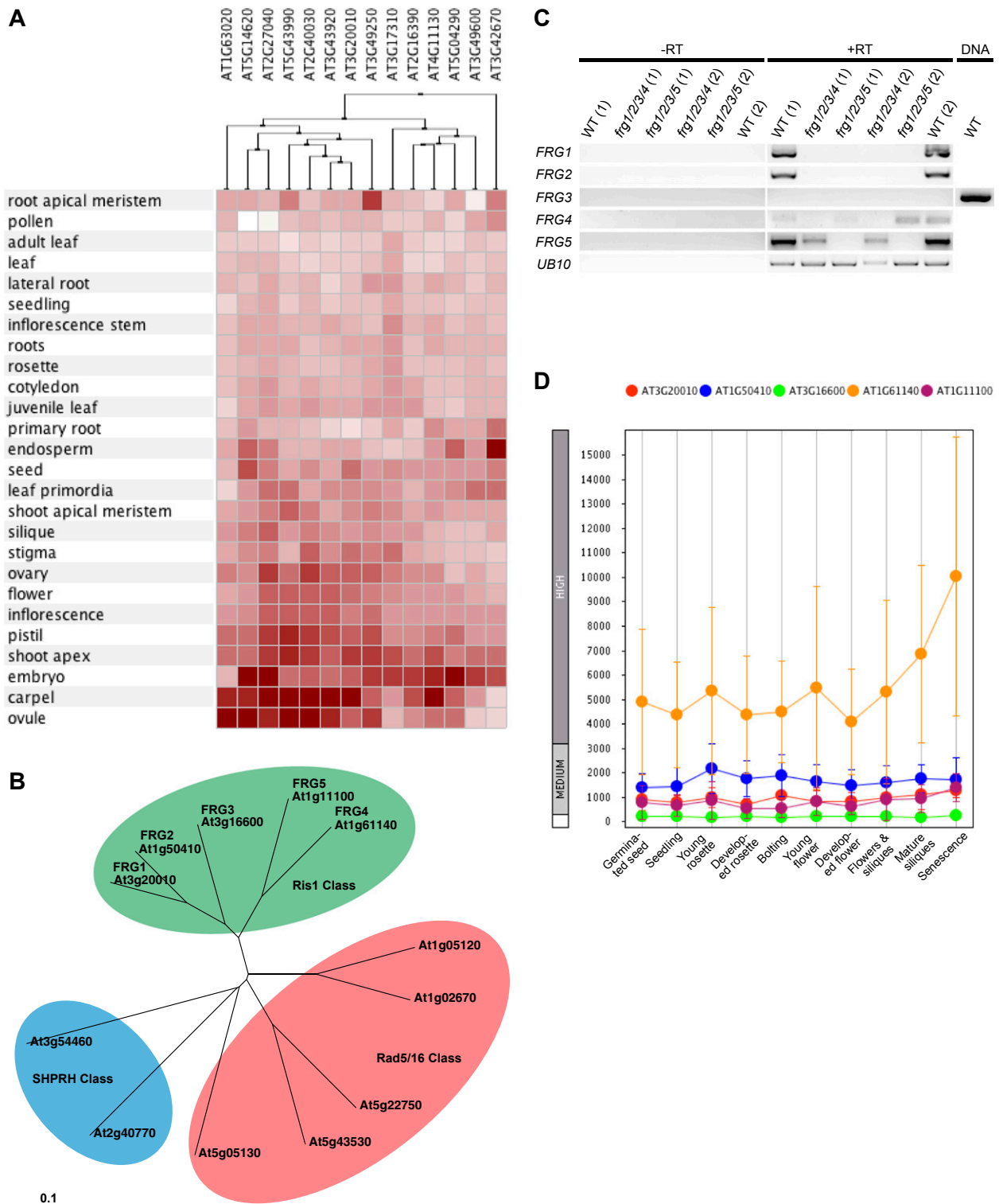


Fig. S1. Expression patterns of RdDM genes and the *FRG* gene family. (A) Hierarchical cluster analysis of expression levels of 14 RdDM genes, including *FRG1*, in 26 different anatomical parts from microarray data (ATH1). (B) Phylogenetic tree (neighbor joining) of *FRG1* to *FRG5* proteins. (C) Confirmation 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 (\pm SD) of *FRG* family members from microarray data (ATH1) by developmental stage/tissue.

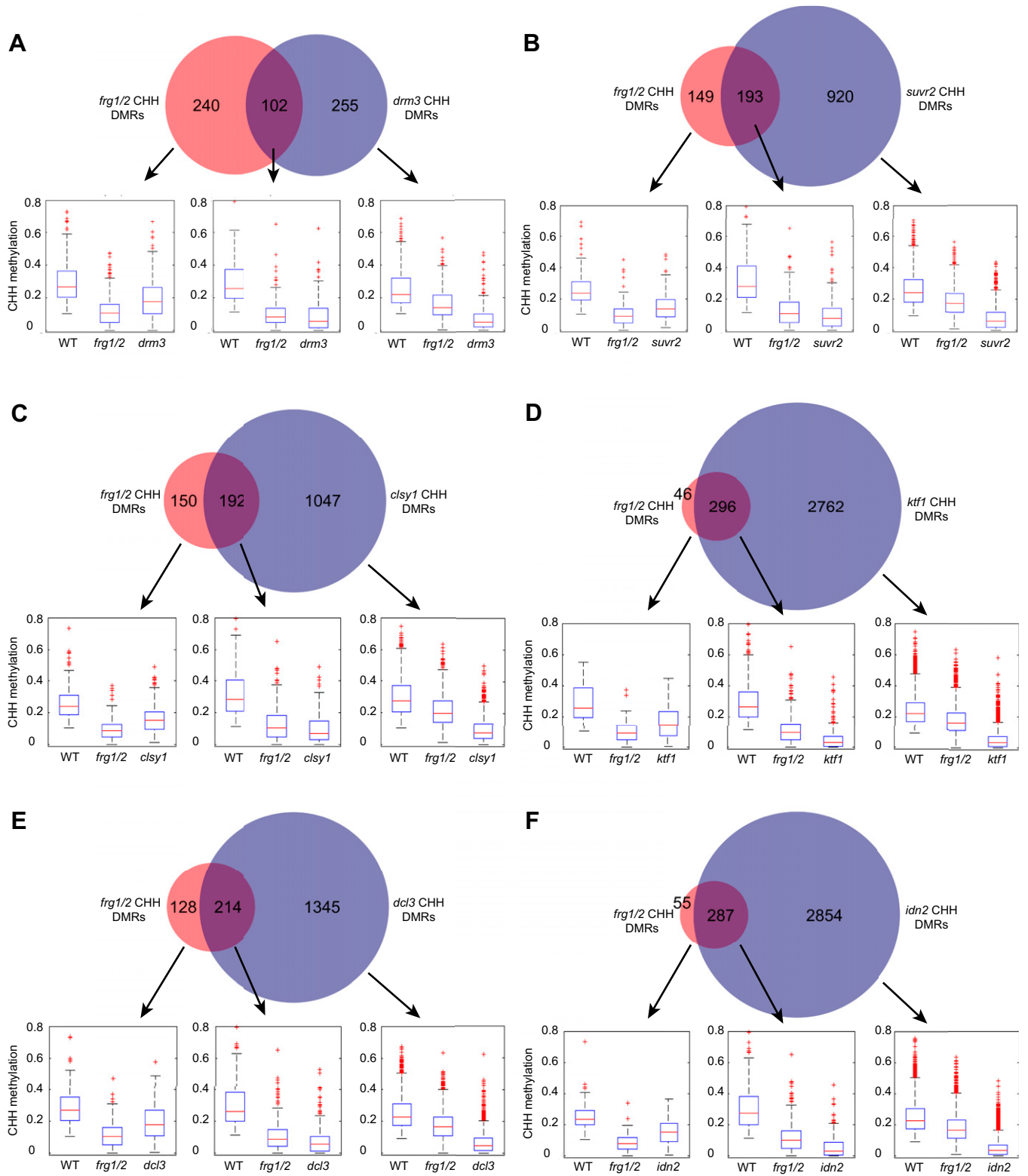


Fig. S3. *frg1/2* double mutants show general reduction in CHH methylation. Overlap between *frg1/2* and *drm3* (A), *svr2* (B), *clsy1* (C), *ktf1* (D), *dcl3* (E), or *idn2* (F) CHH hypo-DMRs. Box plots show CHH methylation levels in the respective DMR fractions.

MEA-ISR

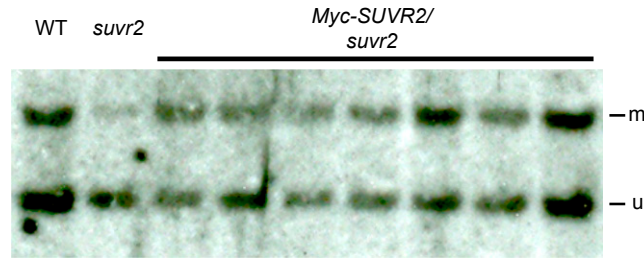


Fig. S4. DNA blot showing that Myc-tagged SUVR2 complements the DNA methylation defect of *suvr2* at the *MEA-ISR* locus. Seven individual T1 lines are shown.

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

<i>DCL3</i>	<i>DRM2</i>	<i>RDR2</i>	<i>AGO4</i>	<i>NRPE2</i>	<i>NRPD1</i>	<i>NRPE1</i>	<i>DRD1</i>	<i>IDN2</i>	<i>DRM3</i>	<i>DMS3</i>	<i>RDM1</i>	<i>KTF1</i>	<i>DMS4</i>	<i>IDP2</i>	<i>SUVR2</i>	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>U.K.L1</u>
0.739		0.905		0.704	0.704							0.671	0.765			6	<u>AT5G25475</u>
	0.74		0.591		0.674			0.718	0.619			0.634				6	<u>ROS3</u>
0.708	0.641								0.627			0.626	0.657	0.679		6	<u>AT3G15120</u>
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	<u>AT1G18950</u>
0.675			0.756	0.669						0.69		0.616				5	<u>AT5G14610</u>
	0.644	0.785							0.619			0.591	0.724			5	<u>CHR11</u>
	0.74					0.596	0.665	0.71				0.603				5	<u>AT5G58120</u>
0.683		0.782						0.81				0.592	0.687			5	<u>AT1G65370</u>
		0.811						0.858					0.666	0.881		4	<u>AT1G10250</u>
		0.794						0.798				0.584	0.651			4	<u>SPT16</u>
							0.77		0.699	0.694		0.624				4	NRPD2
			0.765						0.652			0.606	0.721			4	<u>AT5G43820</u>
0.701					0.683				0.693	0.662						4	<u>GIP1</u>
0.682								0.722				0.637	0.674			4	<u>AT1G03830</u>
	0.659								0.637		0.677					4	<u>SUS2</u>
									0.666			0.599		0.55		4	<u>TPR9</u>
								0.747	0.713		0.714					3	<u>OSD1</u>
								0.663	0.772	0.66						3	<u>BET10</u>
								0.687	0.678		0.704					3	<u>AT4G22860</u>
								0.657				0.631	0.74			3	DCL3
								0.678	0.675	0.669						3	<u>AT2G25740</u>
								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 *Arabidopsis* genome.

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

Replicate	M.W.* (kDa)	Spectra [†]				Unique peptides				NSAfe5 [‡]				% FRG1 [§]			
		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)
<i>frg1 frg2</i>	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
<i>frg1 frg2</i> , replicate 1	24,431,834	22,231,394
<i>frg1 frg2</i> , replicate 2	22,800,646	20,723,455
<i>frg1 frg2</i> , 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
<i>frg1 frg2</i> , replicate 1	16,253,897	7,132,876
<i>frg1 frg2</i> , replicate 2	14,696,182	6,501,880
<i>frg1 frg2</i> , replicate 3	16,442,801	6,762,502
<i>frg1to5</i> , replicate 1	16,443,065	6,834,495
<i>frg1to5</i> , replicate 2	17,912,561	7,563,709
<i>frg1to5</i> , replicate 3	15,282,482	6,428,431
<i>suvr2</i> , replicate 1	17,288,074	7,422,332
<i>suvr2</i> , replicate 2	17,435,948	5,943,106
<i>suvr2</i> , replicate 3	23,194,034	9,676,255
<i>dcl3</i> , replicate 1	24,860,052	8,185,932
<i>dcl3</i> , replicate 2	20,365,508	6,511,050
<i>dcl3</i> , replicate 3	22,444,088	5,654,602
<i>drm1 drm2</i> , replicate 1	23,940,572	9,274,756
<i>drm1 drm2</i> , replicate 2	23,071,493	8,502,891
<i>drm1 drm2</i> , replicate 3	22,453,773	6,884,763

Other Supporting Information Files

[Dataset S1 \(XLSX\)](#)