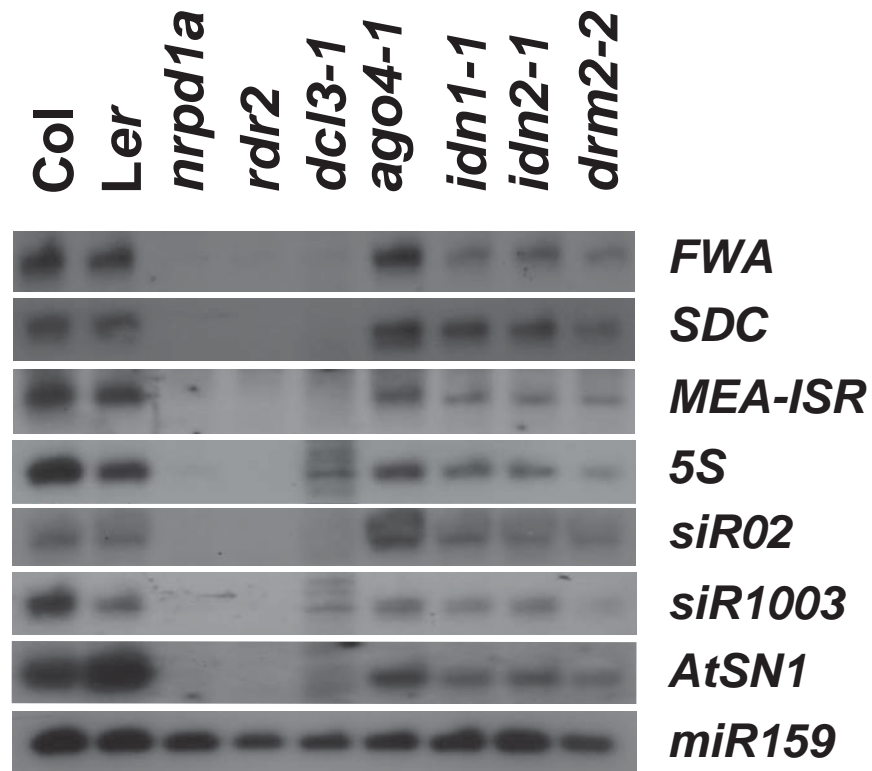
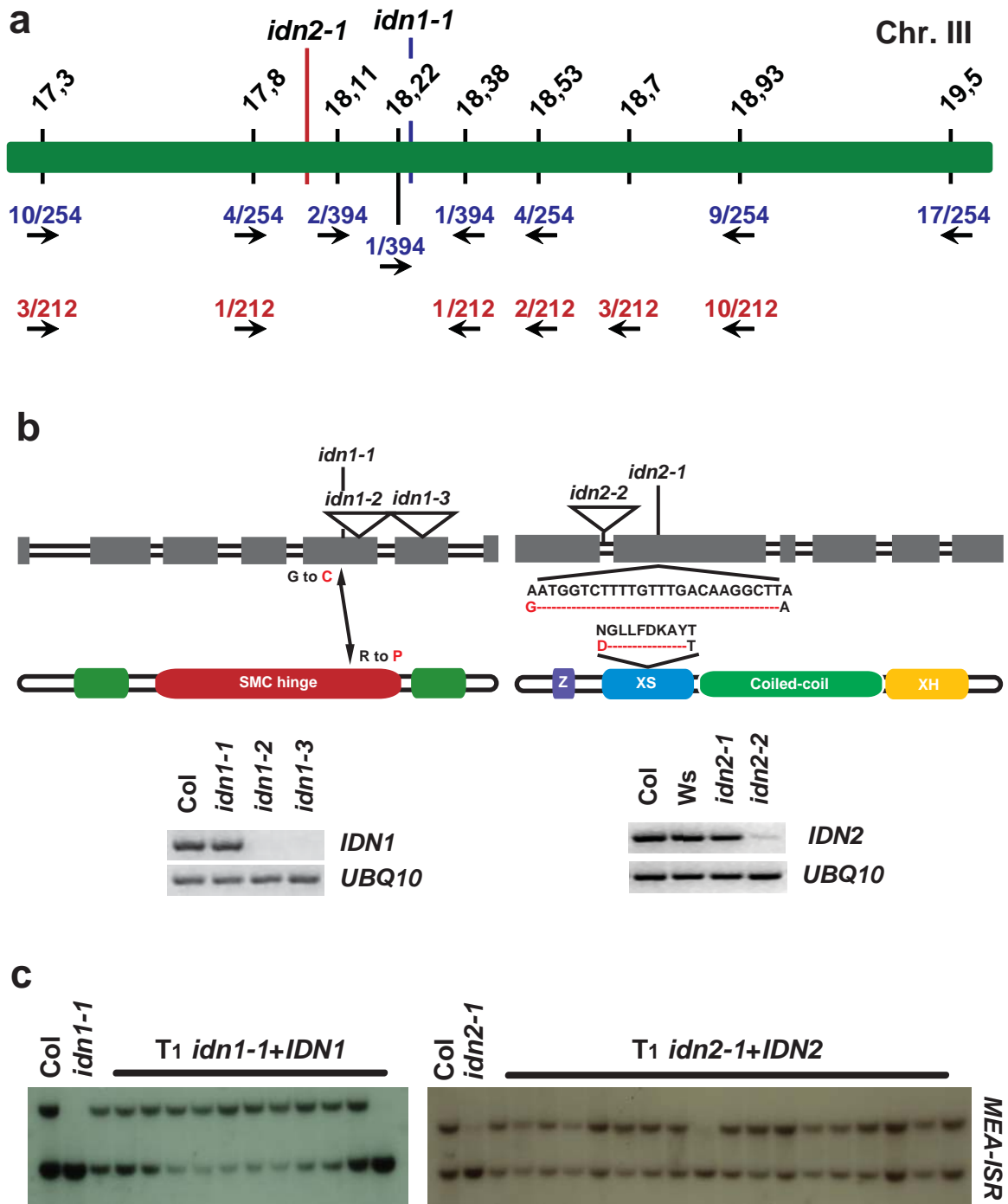


**IDN1 and IDN2: two proteins required for *de novo* DNA methylation in *Arabidopsis thaliana*.**

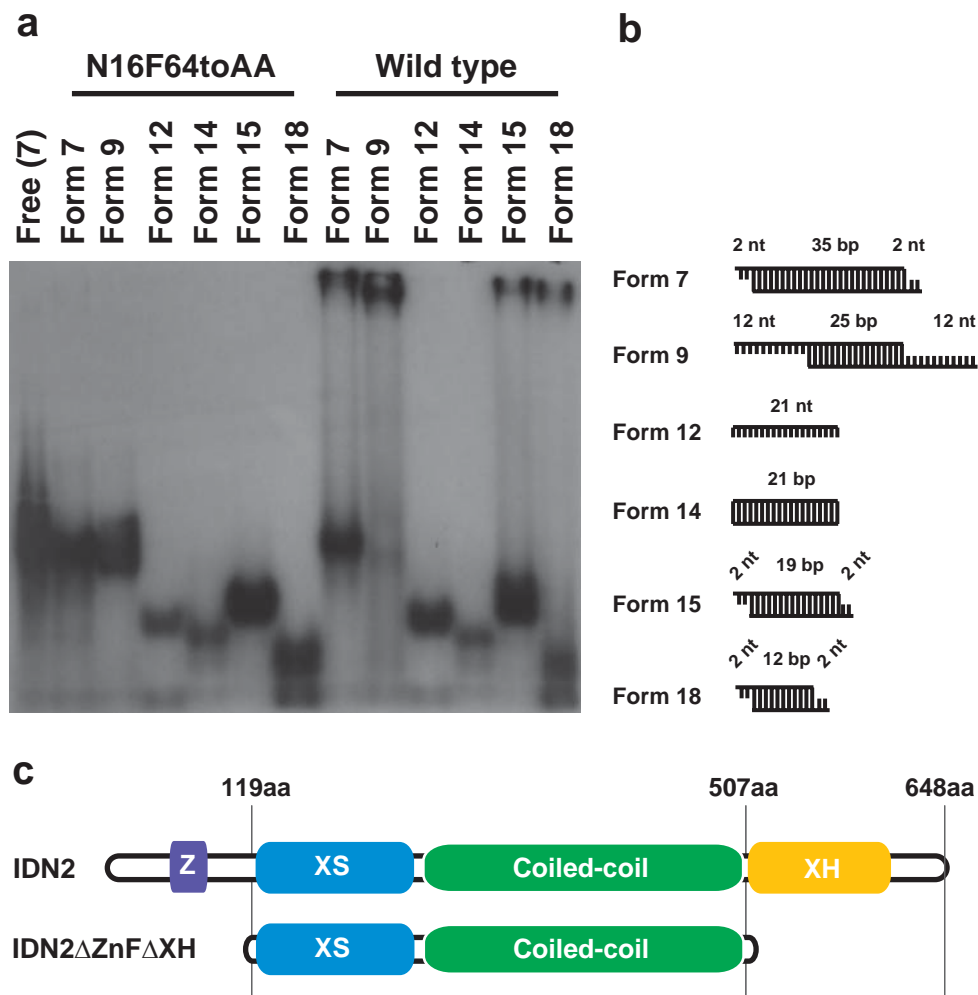
**Israel Ausin, Todd C. Mockler, Joanne Chory, and Steven E. Jacobsen**



**Supplementary Figure 1.** Northern blot analysis of siRNAs abundance in wild type, *idn* mutants, and other RdDM mutants at several loci. Hybridization with *miR159* probe is shown as loading control.



**Supplementary Figure 2.** Map based cloning of *IDN* genes. **a)** Genetic and physical map in the *IDN1* and *IDN2* genomic region. Numbers on the upper side of the green bar indicate the physical position in mega base pairs of markers used for mapping. Genetic distances to *idn1-1* (blue) and *idn2-1* (red) are indicated below every marker as the ratio of recombination events/ total  $F_2$  plants examined. Blue and red vertical bars depict *idn1-1* and *idn2-1* physical position, respectively. **b)** Structure of *IDN* genes and proteins. Exons are shown as grey boxes and introns as white bars. *idn* allele locations are shown in the upper part, capital letters correspond to the wild type/mutated nucleotides and amino acids. *IDN* gene expression levels in wild type and different *idn* mutant alleles are showed in the bottom panel. **c)** *idn1-1* and *idn2-1* genetic complementation. The figure shows the *MEAs-ISR* phenotype of wild type, *idn* mutants and several  $T_1$  *IDN* transformed plants.



**Supplementary Figure 3.** IDN2 XS domain RNA binding assay. **a)** Electrophoretic mobility shift assay showing binding of IDN2 $\Delta$ ZnF $\Delta$ XH to different RNA species. **b)** Schematic view of the different RNA species used in this assay<sup>14</sup>. **c)** Schematic view of IDN2 whole protein and the portion used in this assay. We could not successfully express the XS domain alone, but the XS domain plus coiled coil domain was expressed and soluble.

**Supplementary Table1**

## List of T-DNAs used in this work

TDNA_ID	Locus
SALK_000033	At4g10070
SALK_000645	At1g29940
SALK_000684	At5g56140
SALK_002477	At5g47620
SALK_003066	At3g53460
SALK_003772	At5g57140
SALK_005045	At3g45630
SALK_007447	At3g08620
SALK_007855	At5g56930
SALK_011416	At3g12640
SALK_012103	At3g52260
SALK_012148	At5g59950
SALK_013918	At5g53060
SALK_015201	At3g23900
SALK_016153	At2g25910
SALK_017222	At3g09160
SALK_017606	At3g15010
SALK_019314	At2g38025
SALK_019552	At3g54770
SALK_019773	At3g54230
SALK_020712	At4g28990
SALK_022160	At1g49760
SALK_022644	At3g26932
SALK_023061	At1g74230
SALK_023307	At3g48830
SALK_024210	At5g19960
SALK_024223	At4g12640
SALK_024784	At5g51730
SALK_025329	At3g63450
SALK_025341	At1g14790
SALK_025437	At4g14660
SALK_025521	At5g37030
SALK_026462	At3g10845
SALK_032699	At2g24590
SALK_034824	At5g37370
SALK_035330	At1g03360
SALK_036272	At1g66370
SALK_036327	At3g09160
SALK_036489	At1g58350
SALK_036777	At1g79100
SALK_036865	At3g26420
SALK_037042	At2g38025
SALK_037336	At2g02150
SALK_038117	At5g16840
SALK_038853	At3g57420
SALK_039135	At2g48010
SALK_039333	At3g52980
SALK_040864	At1g55310
SALK_041205	At4g03110

SALK_041457	At5g54900
SALK_041849	At3g13570
SALK_043248	At5g65260
SALK_043415	At1g01080
SALK_044432	At3g53870
SALK_047804	At5g40490
SALK_048634	At2g22600
SALK_048681	At4g37900
SALK_051144	At5g43090
SALK_052810	At3g57420
SALK_053222	At1g51520
SALK_053886	At1g60830
SALK_055376	At4g20920
SALK_058098	At3g49500
SALK_059236	At2g33440
SALK_059877	At2g16940
SALK_062177	At5g51300
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SALK_065234	At5g51120
SALK_066102	At4g10400
SALK_066841	At4g34950
SALK_067855	At3g20420
SALK_068296	At4g36960
SALK_068614	At1g09230
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SALK_069968	At5g05470
SALK_070953	At3g05760
SALK_074281	At4g03120
SALK_076082	At2g29140
SALK_076911	At5g03480
SALK_080562	At4g14610
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SALK_083356	At1g67400
SALK_087049	At2g44940
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SALK_088139	At2g43370
SALK_089931	At2g32600
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SALK_093123	At2g28530
SALK_093140	At5g64490
SALK_094414	At3g47120
SALK_094502	At1g31600
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SALK_095380	At2g22100
SALK_095639	At1g67950
SALK_095723	At5g07060
SALK_096775	At5g46840
SALK_097166	At2g25850
SALK_097962	At4g34950
SALK_098086	At1g09660

SALK_098485	At5g06210
SALK_106689	At2g28450
SALK_108458	At4g14090
SALK_110434	At5g03330
SALK_112300	At1g14790
SALK_114729	At1g73490
SALK_116982	At5g59860
SALK_117149	At2g22090
SALK_117672	At5g10800
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SALK_120729	At4g30100
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SALK_124187	At2g48010
SALK_124568	At1g33470
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SALK_132014	At1g20880
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SALK_006049	At2g16940
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SALK_136270	At1g69250
SALK_090357	At1g13190
SALK_130322	At1g27750
SALK_128379	At4g26650
SALK_007833	At1g48920
SALK_144707	At2g39140
WiscDsLox256E06	At4g32850
WiscDsLox287G04	At1g49760
WiscDsLox289_292D17	At1g80070
WiscDsLox289_292M16	At3g45630
WiscDsLox289_292N13	At2g43970
WiscDsLox293-296invN19	At3g52380
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WiscDsLox323G06	At1g71500
WiscDsLox328D12	At3g48830
WiscDsLox335D11	At3g26420
WiscDsLox373D06	At2g20490

WiscDsLox376C09	At3g25470
WiscDsLox377-380G20	At1g76050
WiscDsLox384B10	At5g18810
WiscDsLox390G04	At3g21500
WiscDsLox413-416N7	At3g04610
WiscDsLox425H12	At1g74230
WiscDsLox446E01	At4g19610
WiscDsLox449D08	At3g50100
WiscDsLox453-456F8	At4g32720
WiscDsLox457-460H17	At2g31610
WiscDsLox461-464D10	At3g53870
WiscDsLox461-464L2	At5g16840
WiscDsLox467D11	At3g54230
WiscDsLox7F05	At5g61030
WiscDsLox238E04	At2g28380
WiscDsLox246B03	At5g64200
WiscDsLox247E04	At4g02430
WiscDsLox335D09	At3g25470
WiscDsLox340C12	At1g71800
WiscDsLox382G12	At4g25500
WiscDsLox384G9	At4g13070
WiscDsLox461-464N10	At5g02530
WiscDsLox461-464N18	At1g27750
SALK_013483	At1g11420
SALK_071707	At1g11420
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SALK_106147	At5g13020
SALK_032667	At5g23800
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SALK_017750	At4g11560
SALK_094920	At4g12620
SALK_140010	At4g14700
SALK_042536	At4g12620

SALK_074694	At5g04240
SALK_025269	At2g38950
SALK_049697	At1g62310
GABI_085H03	At1g09060
SALK_135712	At4g20400
SALK_142477	At1g62830
GABI_438B06	At1g62830
SALK_073422	At1g30810
SALK_135831	At3g13682
SALK_003313	At3g45880
SALK_122006	At3g48430
GABI_454C10	At3g20810
SAIL_680_G02	At5g06550
WiscDsLox263E02	At4g21430
SAIL_811_H12	At3g20810
WiscDsLox376H12	At2g38950

**Supplementary Table2** List of primers used in this work

<b>Primer</b>	<b>Sequence 5'-3'</b>
JP2004	GGTTTTATATTAATATTAAGAGTTATGGGTYGAAGTTT
JP4423	AACCAAATCATTCTCTAAACAAAATATAAAAAATC
JP1026	AAAGTGGTTGTAGTTTATGAAAGGTTTTAT
JP1027	CTTAAAAAATTTTCAACTCATTTTTAAAAA
JP6349	GAAAAAGTTGGAATGGGTTTGGAGAGTTTAA
JP6350	CAACAAACCCTAATATATTTTTATATTTAAAC
JP5353	GGAGAGCCCAACAACCCTATT
JP5354	TTGGGATTTGATGGTGTTTGAG
JP5359	TGGGACTCGCTCAATGTT
JP5502	TCATCTGGGTGTGTTTCATTGGC
JP6509	TGTCCATCATCTTGCAGCTCTAA
JP6510	AAGACCAAAGGAGGCAAAAGG
JP3483	GATCTTTGCCGAAAACAATTGGAGG
JP3484	CGACTTGTCATTAGAAAGAAAGAGAT

## SUPPLEMENTARY METHODS

**Plant materials.** We used the following Arabidopsis strains: the wild type *Ler*, *Ws*, and *Col*; the recessive *ago4-1* allele in *Ler* background; recessive alleles in *Col* background, *idn1-1=dms3-4* from our screen; *idn1-2=dms3-5* (SALK\_068723); *idn1-3=dms3-6* (SALK\_125019); *idn2-1* from our screen; *cmt3-11*, *nrdp1a*, *rdr2-1*, *dcl3-1*, *drm2-2*; and mutated alleles in *Ws* background, *idn2-2* (FLAG\_550B05).

**Bisulfite analysis.** We performed sodium bisulfite sequencing using EZ DNA Methylation Gold (Zymo Research) by following the manufacturer's instructions. The primers used for *FWA* were JP2004 and JP4423, for *MEA-ISR* were JP1026 and JP1027 and for *SDC* were JP6349 and JP6350. We cloned the resulting PCR fragments into pCR2.1-TOPO (Invitrogen) and analysed 15 to 22 clones per sample. All primers are listed in Table S2.

**McrBC-PCR assay.** We digested approximately 500ng of DNA using 20 units of McrBC (New England Biolabs) overnight in a total volume of 25  $\mu$ L. We used 1-2  $\mu$ L for the PCR. PCR was stopped after 21-30 cycles for gel-based analysis. The primers used for *SDC* were JP5353 and JP5354 and for *UBQ10* were JP3483 and JP3484. All primers are listed in Table S2.

**SDC/FWA transformation and flowering time analysis.** We transformed plants with *SDC* as reported in<sup>8</sup>. We performed *FWA* transformation using an AGL0 *Agrobacterium tumefaciens* strain carrying a pCAMBIA3300 vector with an engineered version of *FWA* in which an *EcoRI* site was converted into a *BglII* site. For selection, we sprayed the resultant T<sub>1</sub> population with a 1:1000 dilution of Finale<sup>TM</sup>. We measured flowering time of resistant plants as the total number of leaves (rosette and cauline leaves) developed by a plant.

**Expression analysis.** We analyzed *SDC* expression as reported in<sup>8</sup>. We analysed *IDN1* (=DMS3) and *IDN2* expression by RT-PCR. We isolated total RNA using TRIzol Reagent (Invitrogen) and then synthesized cDNA first strand using SuperScriptII (Invitrogen) by following manufacturer's procedures. The primers used for *IDN1* (=DMS3) were JP5359 and JP5502 and for *IDN2* were JP6509 and JP6510. As a control

we also amplified *UBQ10* using the primers JP3483 and JP3484. PCR was stopped after 21-25 cycles for gel-based analysis. We performed small RNA analysis as described in<sup>8</sup>. All primers are listed in Table S1.

**Southern blotting.** We performed *MEA-ISR* and 5S Southern blotting, hybridization and analysis as described in S1.

***AtSN1 HaeIII* cutting assay.** We performed *AtSN1 HaeIII* cutting assay described in S2

**RNA binding assays** were performed as described in<sup>14</sup>

## **SUPPLEMENTARY REFERENCES**

S1. Lianna M. Johnson, et al. *PLoS Genetics* **4**, e1000280. (2008)

S2. Onodera, Y. et al. *Cell* **120**, 613-22.(2005)