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

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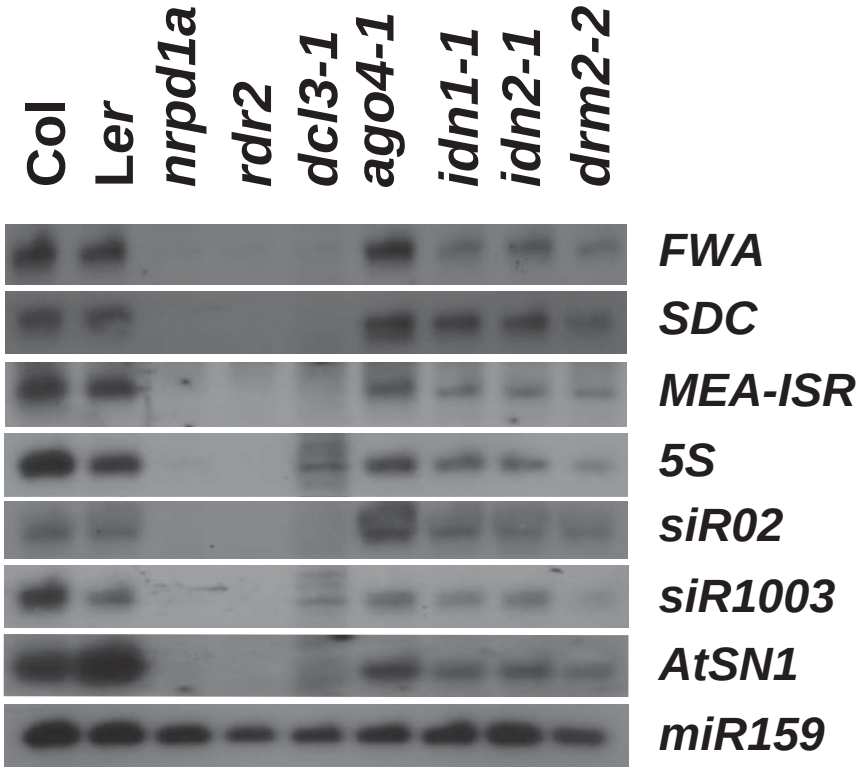
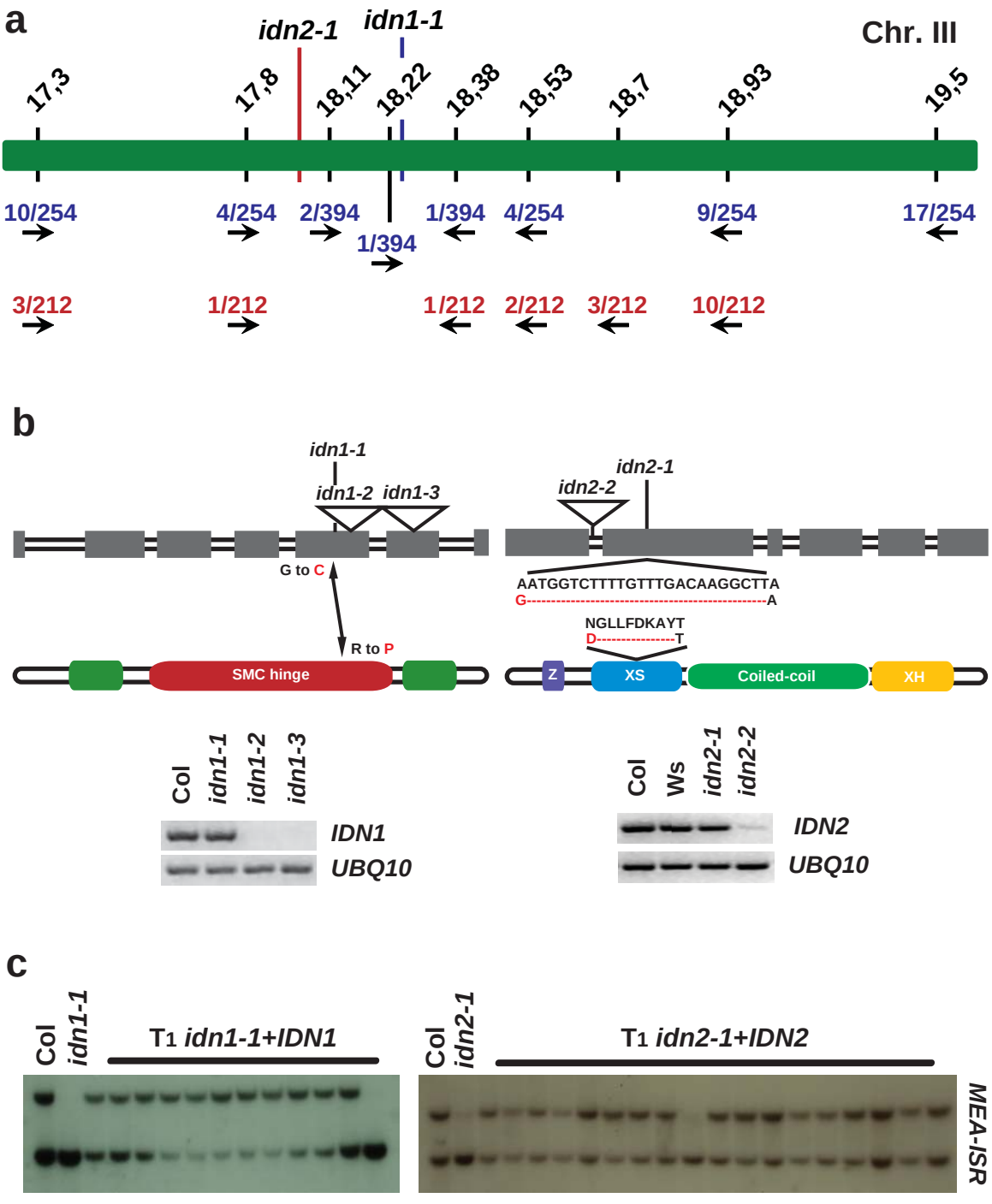


Supplementary Figure 1. Northern blot analysis of siRNAs abundance in wild type, *idn* mutants, and other RdDM mutants at several loci. Hybridization with *miR159* probe is shown as loading control.



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 *MEA-ISR* phenotype of wild type, *idn* mutants and several T_1 IDN transformed plants.

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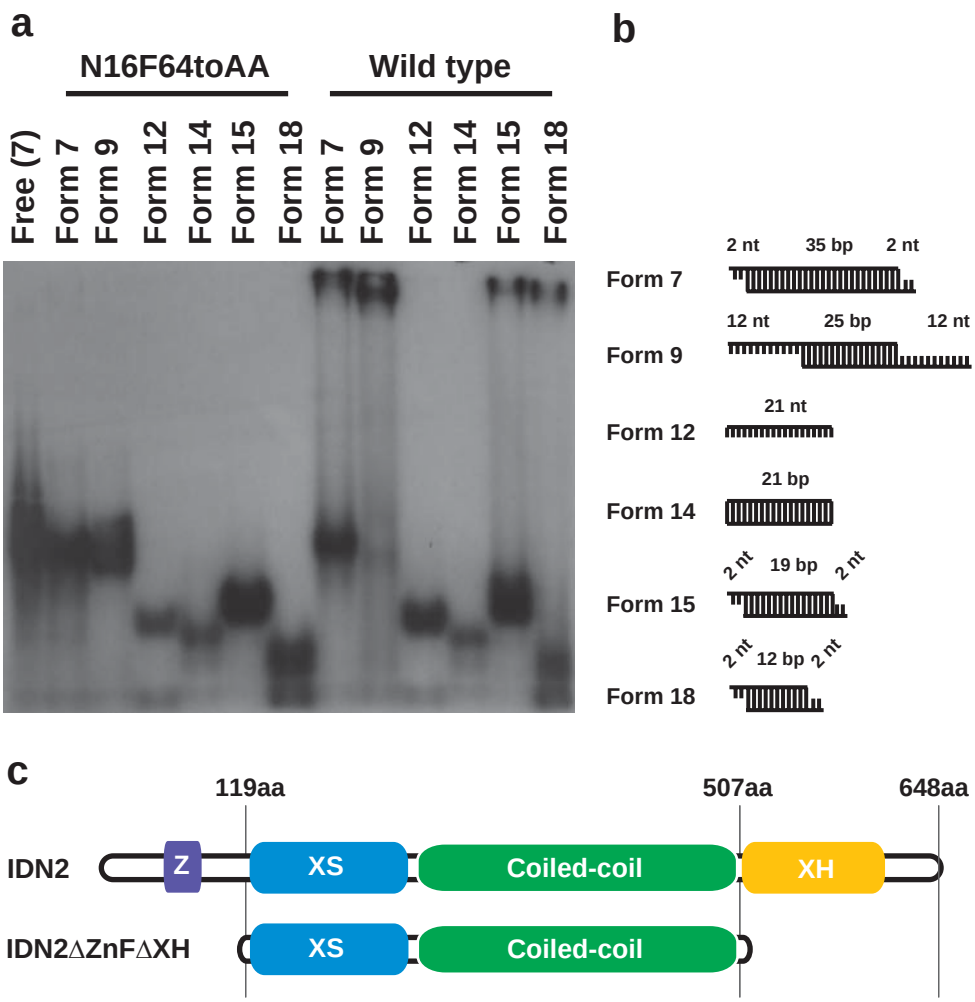

Supplementary Figure 3. IDN2 XS domain RNA binding assay. **a)** Electrophoretic mobility shift assay showing binding of IDN2ΔZnFΔXH to different RNA species. **b)** Schematic view of the different RNA species used in this assay[14]. **c)** Schematic view of IDN2 whole protein and the portion used in 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
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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
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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
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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

Primer	Sequence 5'-3'
JP2004	GGTTTTATATTAATATTAAAGAGTTATGGGTYGAAGTTT
JP4423	AACCAAAATCATTCTCTAAACAAAATATAAAAAAATC
JP1026	AAAGTGGTTGTAGTTTATGAAAGGTTTTAT
JP1027	CTTAAAAAATTTTCAACTCATTTTTAAAAAA
JP6349	GAAAAAGTTGGAATGGGTTTGGAGAGTTTAA
JP6350	CAACAAACCCTAATATATTTTATATTAAAAC
JP5353	GGAGAGCCCAACAACCCTATT
JP5354	TTGGGATTTGATGGTGTTTGAG
JP5359	TGGGGACTCGCTCAATGTT
JP5502	TCATCTGGGTGTGTTCATTGGC
JP6509	TGTCCATCATCTTGCAGCTCTAA
JP6510	AAGACCAAAGGAGGCAAAAGG
JP3483	GATCTTTGCCGGAAAACAATTGGAGG
JP3484	CGACTTGTCATTAGAAAGAAAGAGAT

SUPPLEMENTARY METHODS

Plant materials. We used the following Arabidopsis strains: the wild type L*er* , Ws, and Col; the recessive *ago4-1* allele in L*er* 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*, *nrpd1a*, *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 µL. We used 1-2 µL 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[8]. 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_1 population with a 1:1000 dilution of Finale™. 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[8]. 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[8]. 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 [14]

SUPPLEMENTARY REFERENCES

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

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