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## The impact of post-translational modifications on the activity of ARGONAUTE1 in *Arabidopsis thaliana*

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ARGONAUTE1 (AGO1) is the main effector of post-transcriptional gene silencing in plants, and plays roles in plant development and plant resistance against pathogens, so, its activity needs to be tightly regulated (Mallory and Vaucheret, 2010; Baumberger and Baulcombe, 2005). It is known that AGO1 is regulated post-transcriptionally by a feedback loop involving miR168, but less is known about its regulation at post-translational level (Rhoades *et al.*, 2002; Vaucheret *et al.*, 2004). Among the post-translational regulation mechanisms, post-translational modifications (PTMs) allow to quickly modulate protein activity in response to environmental stimuli. In animals, the members of AGO/PIWI family expressed in germline cells have been shown to be methylated on Arginine (R-methylated) (Kirino *et al.*, 2009; Nishida *et al.*, 2009). This modification impacts protein stability and/or small RNAs loading, leading to defects in germ cell maintenance or specification (Kirino *et al.*, 2009; Nishida *et al.*, 2009). In this context, we decide to see if this regulation mechanism is also observed in AGO1 protein involved in meristem formation in *A.thaliana*.

### 1. R-methylation pathways in *A.thaliana*

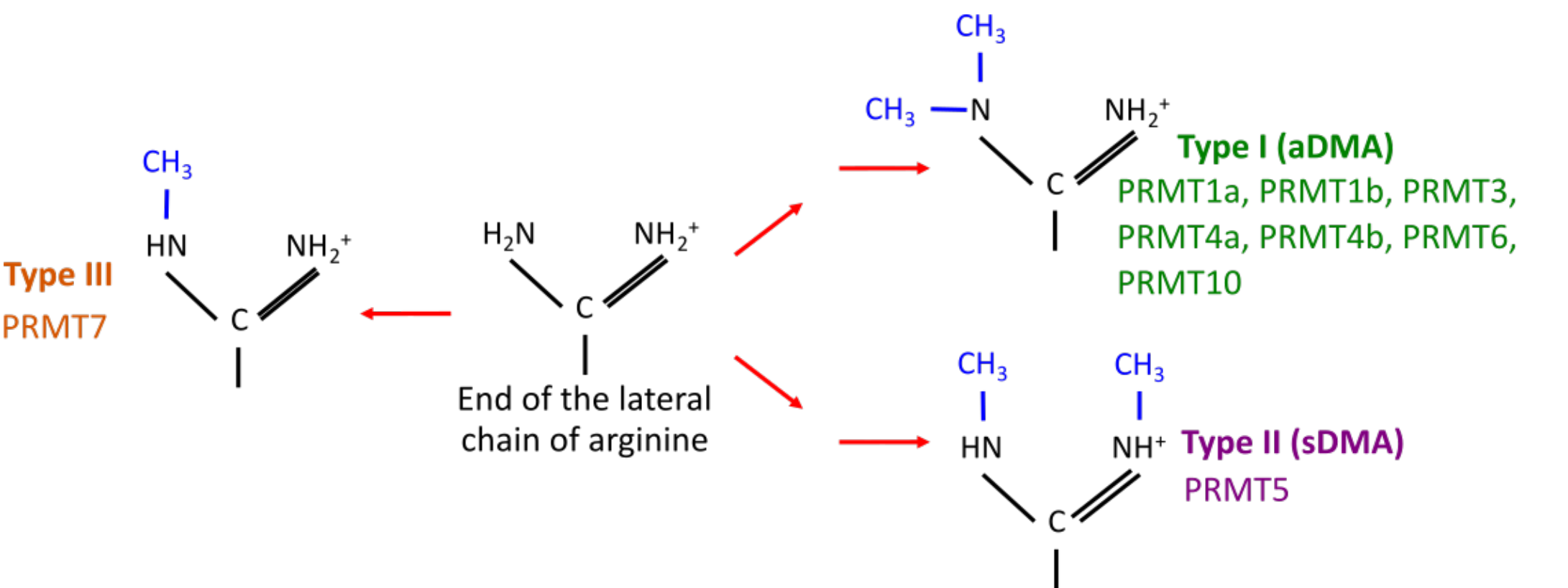


Figure 1. R-methylation deposition by PRMT proteins in *A.thaliana*.

aDMA = asymmetric dimethylation, sDMA = symmetric dimethylation

### 2. R-methylation predicted sites localization on AtAGO1 protein and their conservation in plants

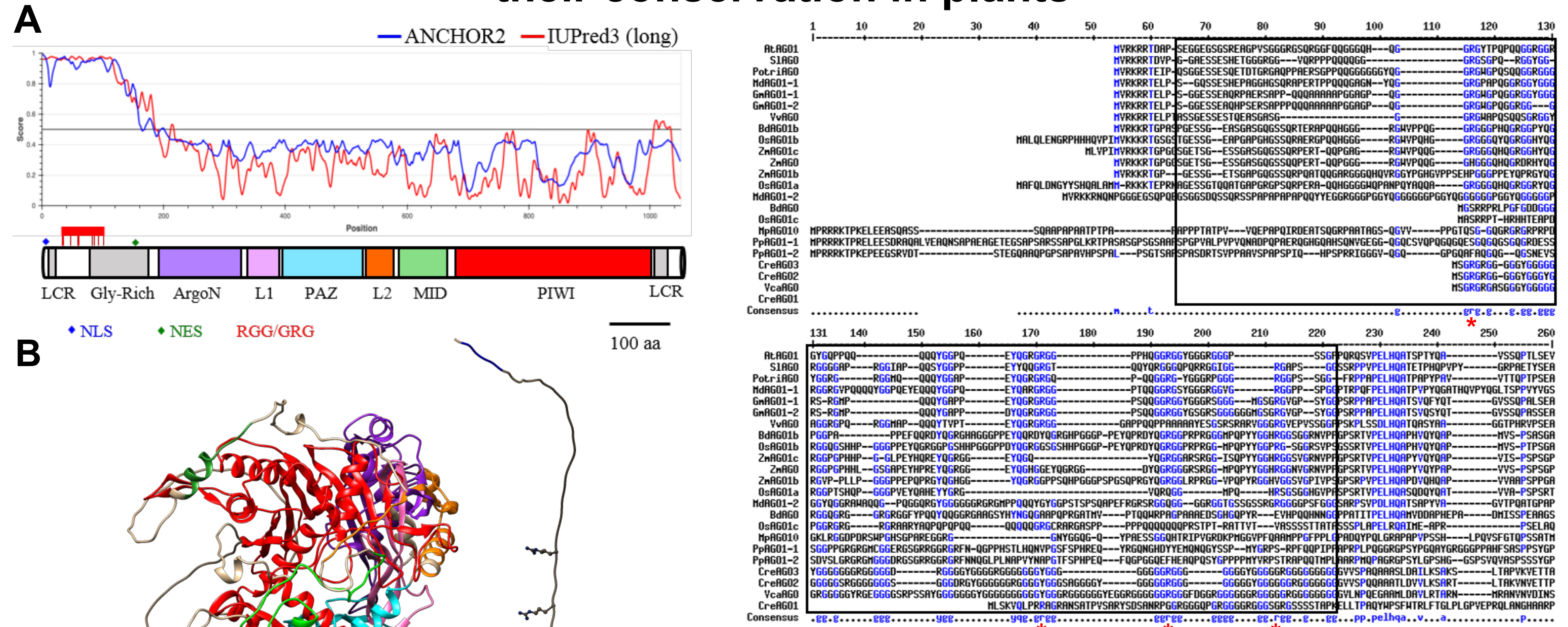


Figure 2. Predicted methylation sites in AGO1 are located in a disordered N-ter region.

(A) Prediction of long intrinsically disordered unstructured regions and protein binding motifs in disordered protein regions in AtAGO1 using ANCHOR2 in IUPRED3 (<https://iupred.elte.hu/>). (B) Prediction of the 3D structure of AGO1 using AlphaFold protein structure database (<https://alphafold.ebi.ac.uk/prediction>; Jumper *et al.*, 2021; Varad *et al.*, 2021).

Figure 3. Conservation of R-methylation predicted sites among plant homologs of AtAGO1.

Alignment of GRG/RGG containing regions of homologs of AtAGO1 from ten different plant species (At: *Arabidopsis thaliana*, Cre: *Chlamydomonas reinhardtii*, Vca: *Volvox carteri*, Mp: *Marchantia polymorpha*, Pp: *Physcomitrium patens*, Bd: *Brachypodium distachyon*, Os: *Oryza sativa*, Zm: *Zea mays*, Sl: *Solanum lycopersicum*, Vv: *Vitis vinifera*, Potri: *Populus trichocarpa*, Md: *Malus domestica*, Gm: *Glycine max*). Highlighted arginines by red asterisks correspond to conserved arginines in RGG/GRG motifs (conserved in 40% of the sequences).

### 4. The impacts of the *prmt5* mutation on mRNA and miRNA levels

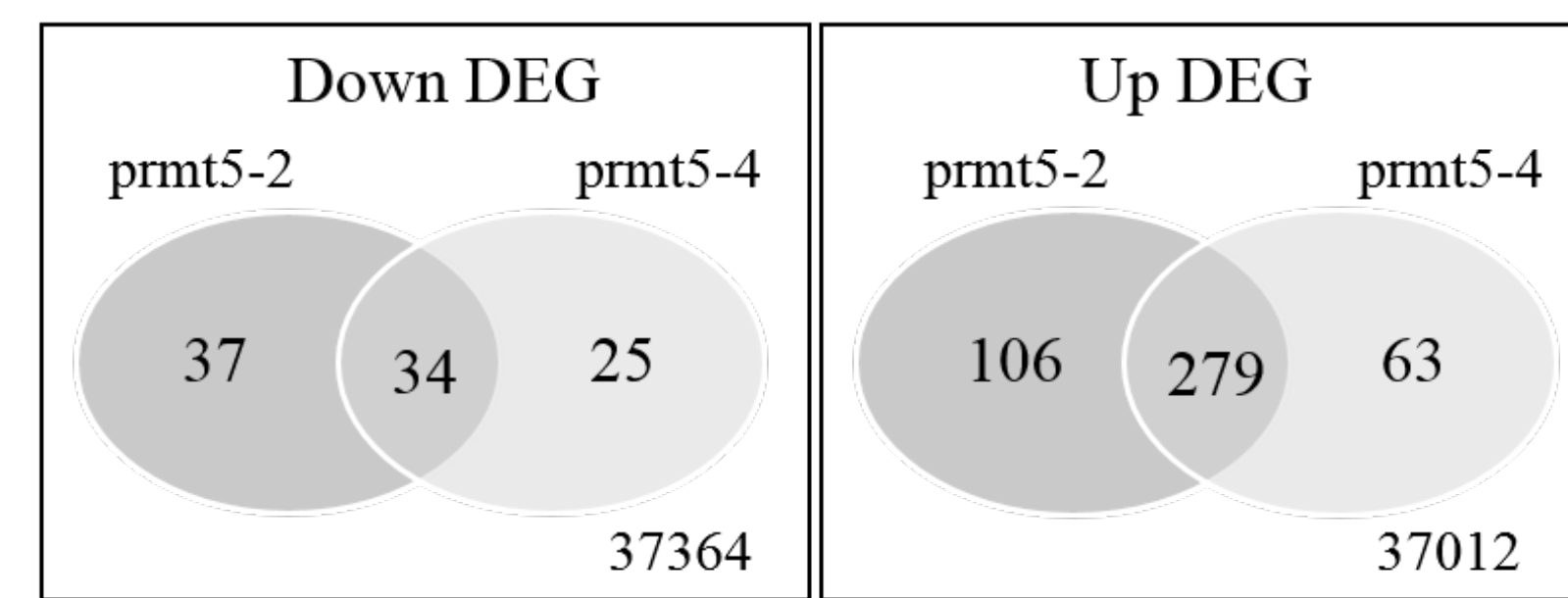


Figure 7. Differentially detected transcripts are mainly upregulated in *prmt5* mutants.

Venn diagrams obtained from polyA-RNAseq analyses, performed from Col0, *prmt5-2* and *prmt5-4* closed buds total RNA. Downregulated (left) and up-regulated genes (right) in *prmt5* mutants buds compared to Col0. The number of genes expressed in both *prmt5* mutants and that do not differ from Col0 are indicated at the bottom right of each panel.

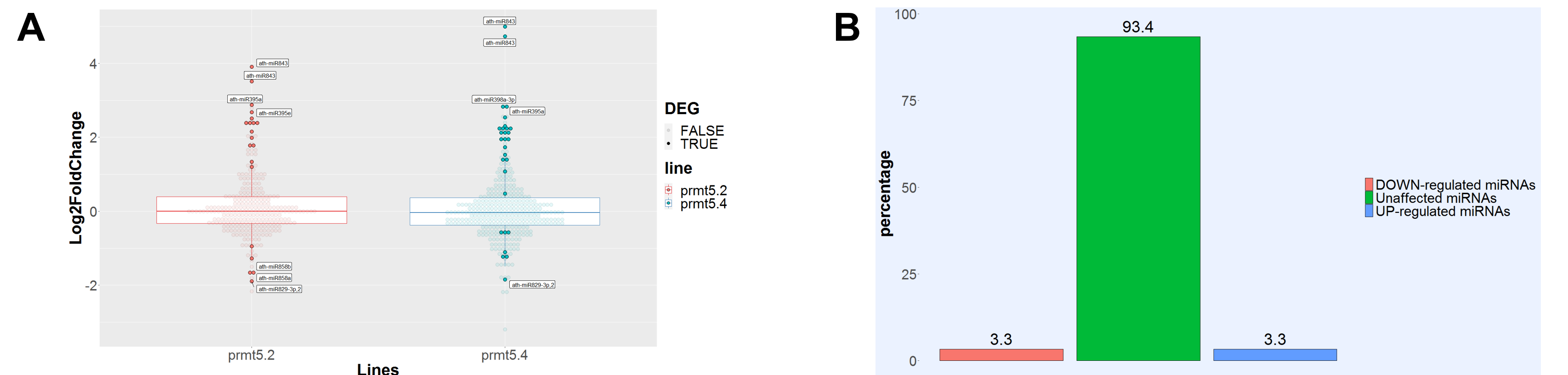


Figure 8. Only few miRNAs are differentially loaded into AGO1 in *prmt5* mutants..

(A) Mean expression levels of individual miRNAs in closed buds and open flowers of *prmt5-2* and *prmt5-4* plants relative to Col-0 plants. Horizontal segments indicate the median of the expression levels. Each dot corresponds to a single miRNA. Dark and light dots show differentially (false discovery rate adjusted P-value, 0.05) and not differentially accumulated miRNAs, respectively. (B) Bar plot representing the variations of miRNAs loading into AGO1 (down-regulated, unaffected and up-regulated) in *prmt5-2* and *prmt5-4* compared to Col0. AGO1-IP were performed on closed buds extracts.

### Conclusion

AGO1 protein is symmetrically and asymmetrically dimethylated in closed buds in *A.thaliana*, and the sDMA deposition is PRMT5-dependent. Methylation sites are conservationally organised as a cluster in N-ter region of AGO1 homologs among plants. The absence of sDMA on AGO1 in *prmt5* may be compensated by the presence of aDMA on the same sites. So it will be crucial to get rid of methylation marks to study methylation effects on AGO1. We will perform further investigations using AGO1 non methylable forms.

### 3. AGO1 R-methylation status and AGO1 stability in *prmt5* mutant

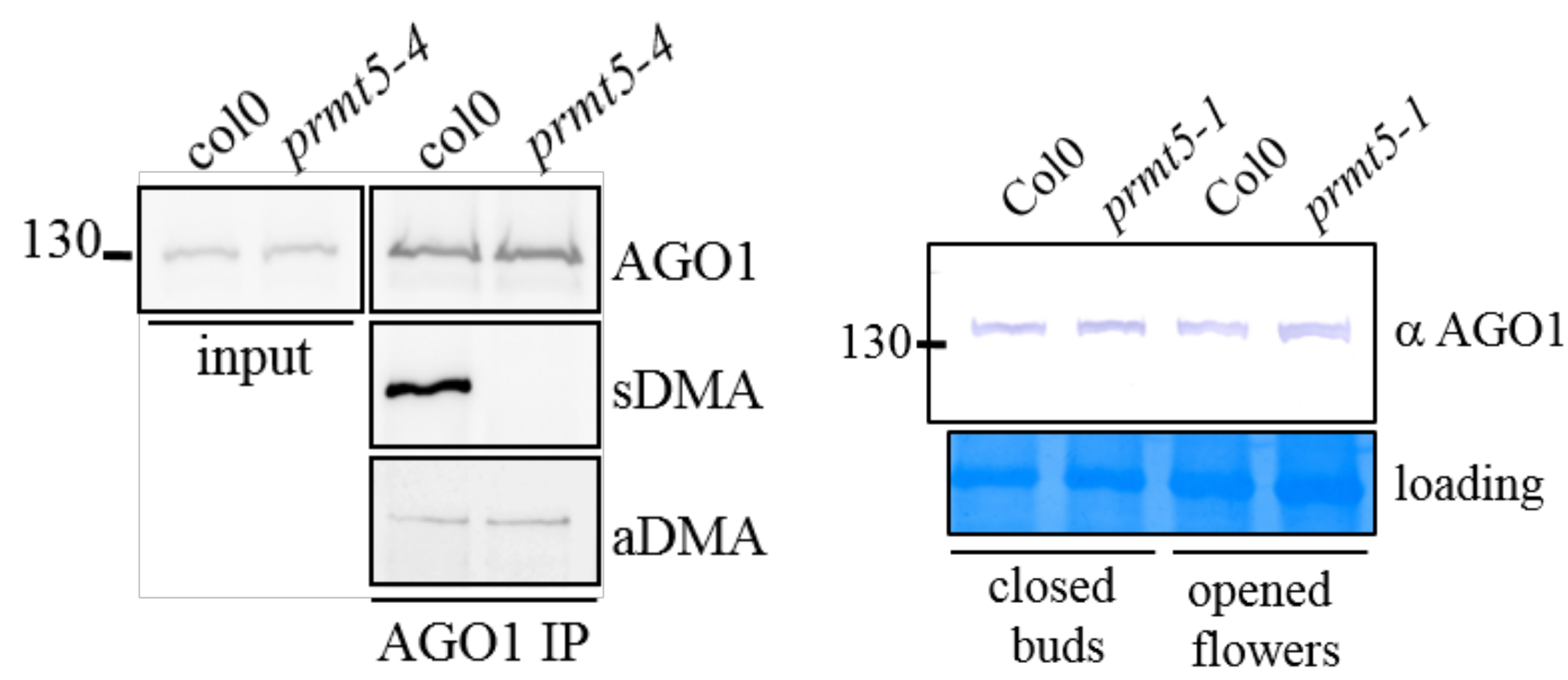


Figure 4. AGO1 is symmetrically and asymmetrically dimethylated and the sDMA deposition on AGO1 is PRMT5-dependent.

AGO1 IPs performed on total protein extracts from Col0 and *prmt5-4* buds. The immunoprecipitated AGO1 protein, sDMA and aDMA modifications are detected using specific antibodies.

Figure 6. AGO1 accumulation is not significantly impacted by *prmt5* mutation.

Immunodetection of AGO1 on total protein extracts of closed buds and opened flowers from Col0 and *prmt5-1*. The membrane is stained with colloidal coomassie as a loading control.

	col	<i>prmt5-2</i>	<i>prmt5-1</i>
Normalization factor	1.30	1.38	1.00
Spectra/AGO1	11757	1660	2287
SC% AGO1	66%	68%	68%
Mascot threshold (p>0.05)	32	32	33

	score max	#spc	score max	#spc	score max	#spc
R30(diMe)	39	9	41	10	37	5
R34(diMe)	23	2	61	11	53	22
R59(diMe)			29	3	26	8
R62(diMe)			97	13	93	20
R83(diMe)			28	2	21	2
R85(diMe)			28	2	27	4
R94(diMe)			18	1	24	2
R101(diMe)	34	10	46	11	45	15

Figure 5. sDMA sites are converted in aDMA sites in *prmt5* mutants.

Identification of dimethyl-R sites by MS/MS analysis on AGO1-IPs obtained from Col0, *prmt5-2* & *prmt5-1* closed buds extracts.

### 5. The localization of AGO1 in *prmt5*

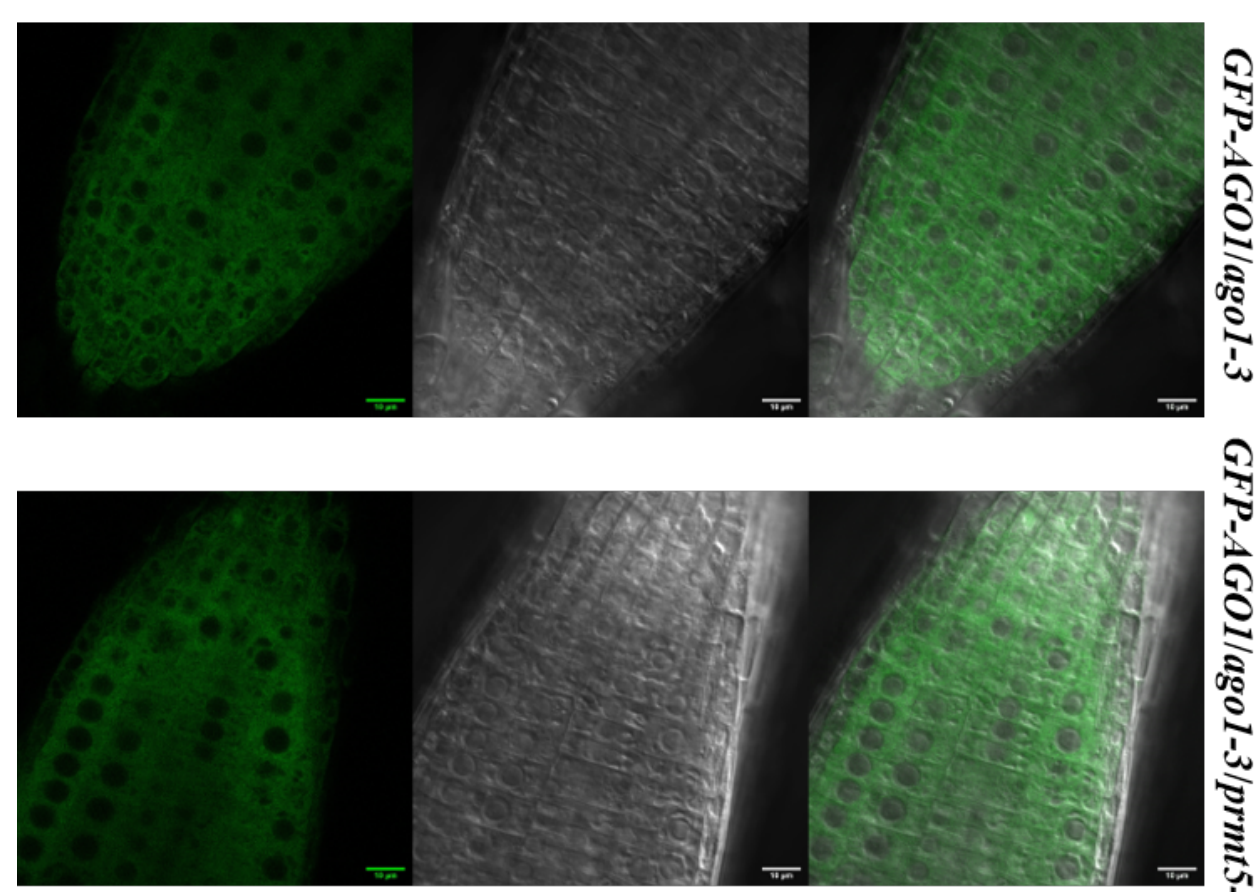


Figure 9. GFP-AGO1 subcellular localization is not affected by *prmt5* mutation.

Confocal images obtained from root tips of 5-days-old *GFP-AGO1/ago1-3*; *GFP-AGO1/ago1-3/prmt5-1*, transgenic seedlings. From left to right: GFP signal, transmission acquisition and merged image.