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Supplementary Materials for

Active DNA demethylation regulates tracheary element differentiation in *Arabidopsis*

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/26/eaaz2963/DC1)

Tables S1 to S5

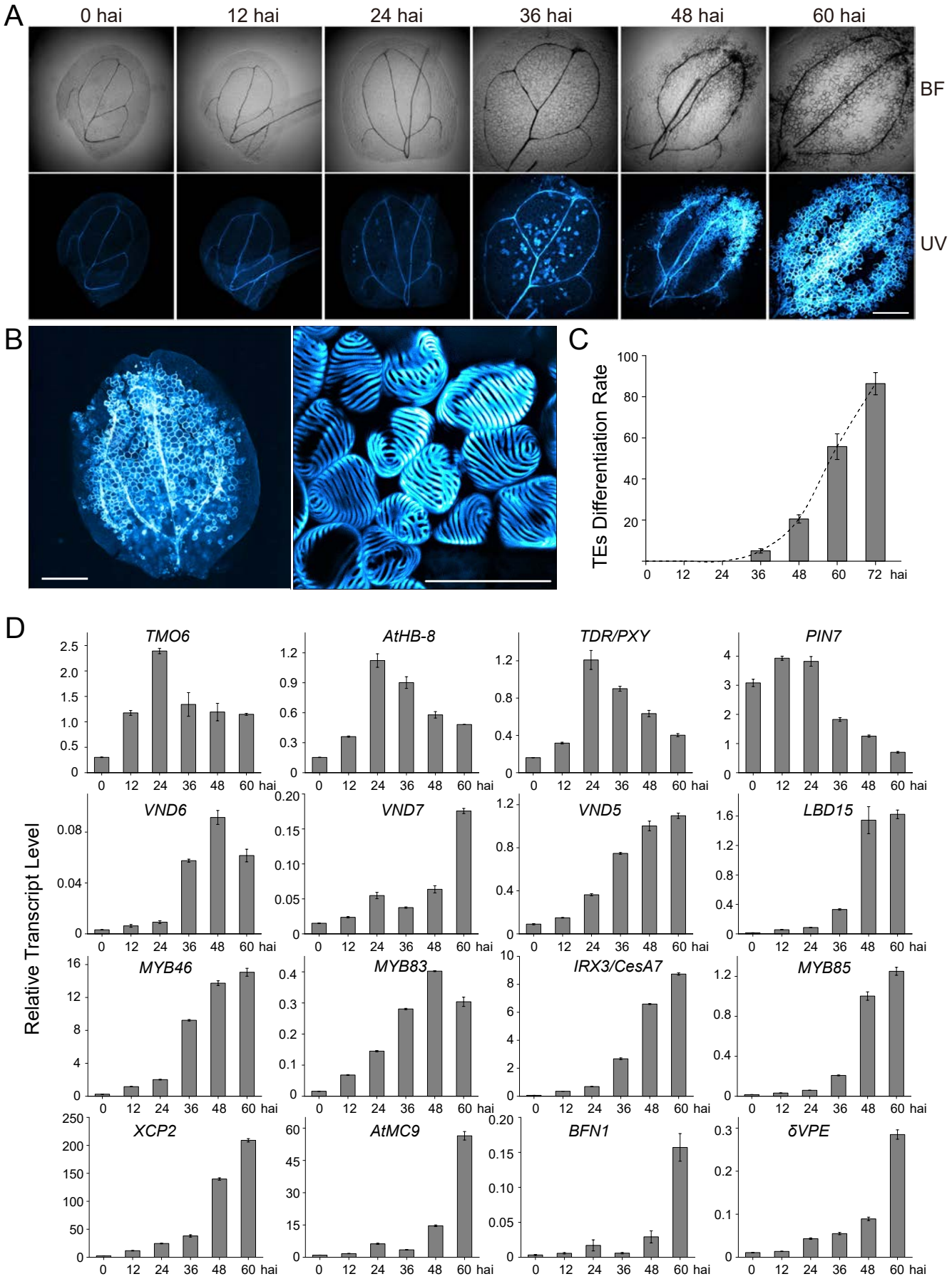

A
0 hai
12 hai
24 hai
36 hai
48 hai
60 hai
BF
UV
B
C
TEs Differentiation Rate
100
80
60
40
20
0
12
24
36
48
60
72
hai
D
Relative Transcript Level
TMO6
AtHB-8
TDR/PXY
PIN7
VND6
VND7
VND5
LBD15
MYB46
MYB83
IRX3/CesA7
MYB85
XCP2
AtMC9
BFN1
δVPE

Fig. S1. Validation of the VISUAL system.

(**A**) Images showing tracheary element (TE) differentiation in Col-0 cotyledons at six time points (0, 12, 24, 36, 48 and 60 hai). Both differential interference contrast (DIC) and UV images show secondary cell wall thickening as TE differentiation progresses. Scale bar: 750 μm. (**B**) UV images showing TE differentiation in a Col-0 cotyledon at 72 hai. Left: ectopic TEs spread throughout the cotyledon at 72 hai, Scale bar: 500 μm. Right: high-resolution three-dimensional reconstruction of a differentiated TE. Scale bar: 100 μm. (**C**) The rate of TE differentiation at six time points in Col-0 cotyledons. Error bars indicate SD, n = 50. (**D**) Relative expression levels of marker genes at different time points during TE differentiation. *TIP41* served as an internal control gene. Data are presented as the mean ± SD of three biological replicates.

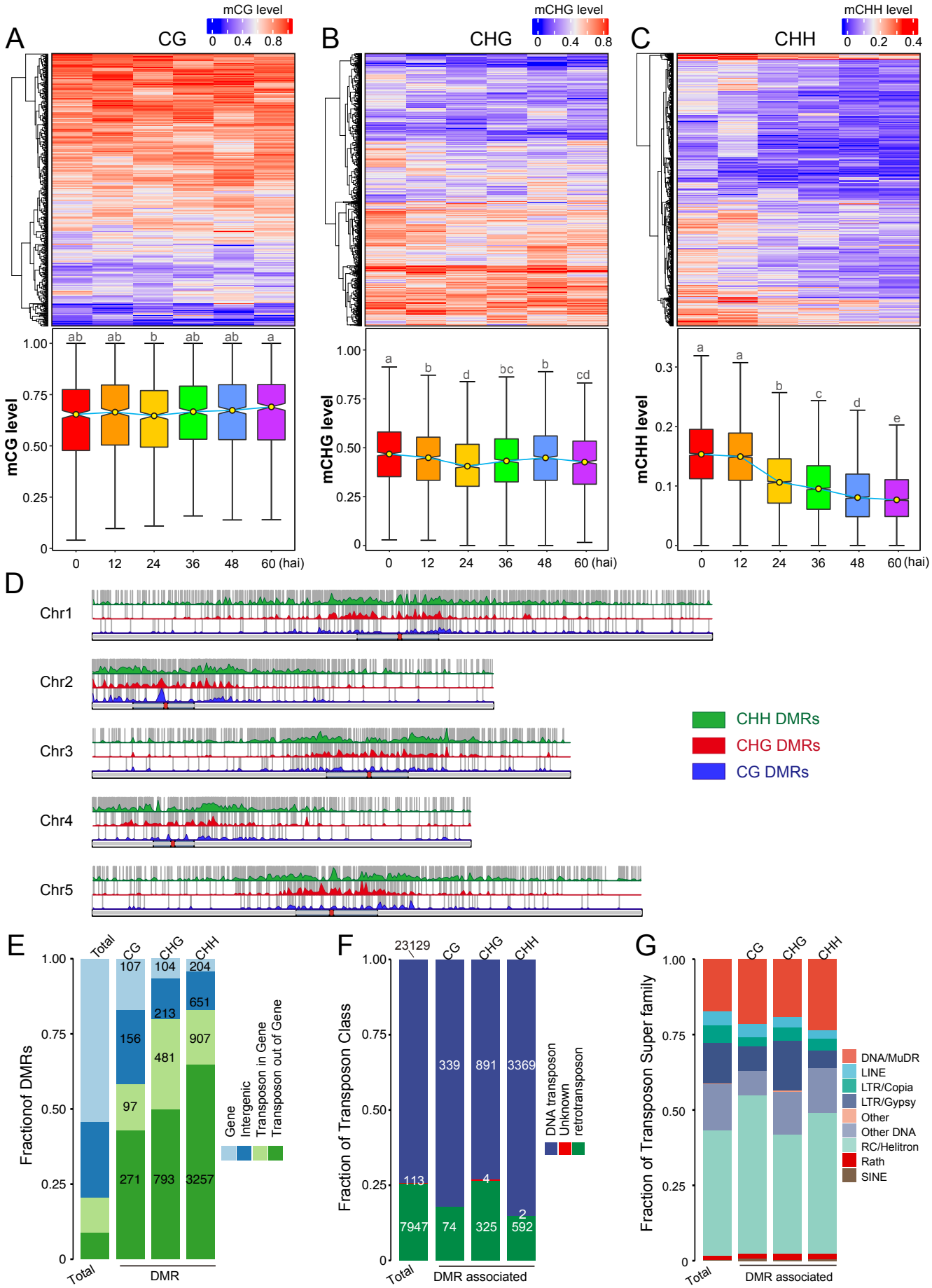

A
CG
mCG level
0 0.4 0.8
mCG level
1.00
0.75
0.50
0.25
0
ab ab b ab ab a
0 12 24 36 48 60 (hai)
B
CHG
mCHG level
0 0.4 0.8
mCHG level
1.00
0.75
0.50
0.25
0
a b d bc b cd
0 12 24 36 48 60 (hai)
C
CHH
mCHH level
0 0.2 0.4
mCHH level
0.3
0.2
0.1
0
a a b c d e
0 12 24 36 48 60 (hai)
D
Chr1
Chr2
Chr3
Chr4
Chr5
CHH DMRs
CHG DMRs
CG DMRs
E
Fractionof DMRs
1.00
0.75
0.50
0.25
0
Total CG CHG CHH
107 156 97 271
104 213 481 793
204 651 907 3257
Gene
Intergenic
Transposon in Gene
Transposon out of Gene
Total
DMR
F
Fraction of Transposon Class
1.00
0.75
0.50
0.25
0
23129 CG CHG CHH
113 7947
339 74
891 4 325
3369 2 592
DNA transposon
Unknown
retrotransposon
Total
DMR associated
G
Fraction of Transposon Super family
1.00
0.75
0.50
0.25
0
CG CHG CHH
DNA/MuDR
LINE
LTR/Copia
LTR/Gypsy
Other
Other DNA
RC/Helitron
Rath
SINE
Total
DMR associated

Fig. S2. Differential DNA methylation analysis during ectopic TE differentiation.
(**A-C**) DNA methylation levels of identified CG-DMRs (**A**), CHG-DMRs (**B**), and CHH-DMRs (**C**). Upper panel: heatmaps showing the DNA methylation levels of CG-DMRs (**A**), CHG-DMRs (**B**) and CHH-DMRs (**C**) at different time points during ectopic TE differentiation. Lower panel: box plots showing the CG, CHG and CHH methylation levels of the corresponding type of DMR at different time points during ectopic TE differentiation. Significant differences between two groups are marked with different letters ($p < 0.001$, Mann-Whitney *U* test). (**D**) Distribution of differentially methylated regions (DMRs) on five chromosomes. (**E**) Proportion of CG-DMRs, CHG-DMRs and CHH-DMRs in genic regions, intergenic regions and transposons. Total bar showing the proportions of lengths in different regions. (**F**) Proportions of DNA transposons and retrotransposons in DMR-associated transposons. (**G**) Proportions of different transposon superfamilies in DMR-associated transposons.

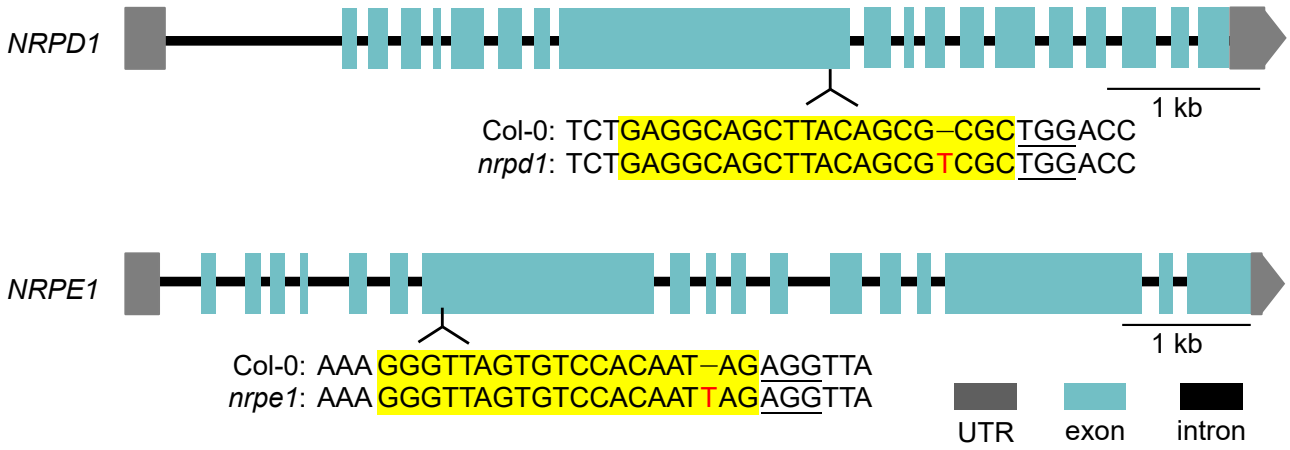
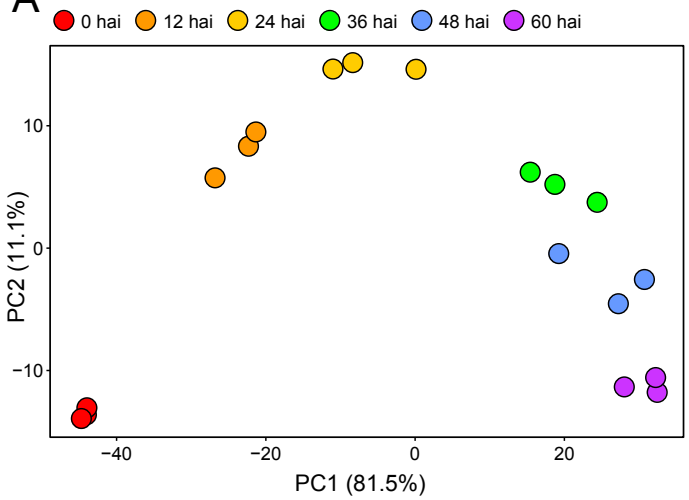
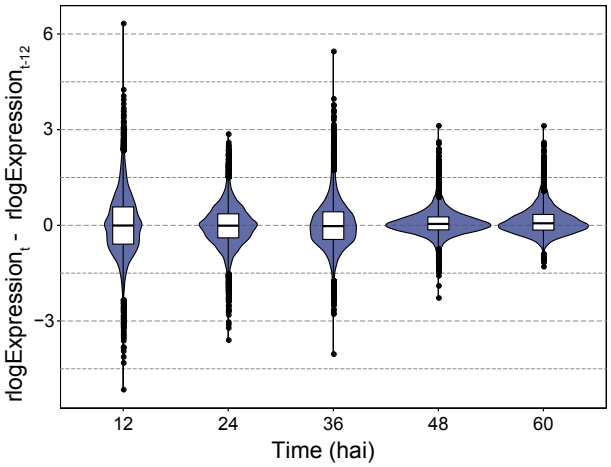


Fig. S3. Generation of *NRPD1* and *NRPE1* mutants using the CRISPR/Cas9 system.
CRISPR/Cas9-induced insertions in *nrpd1* and *nrpe1*. The sgRNA targeting sequence is highlighted in yellow. The PAM site is underlined.

A

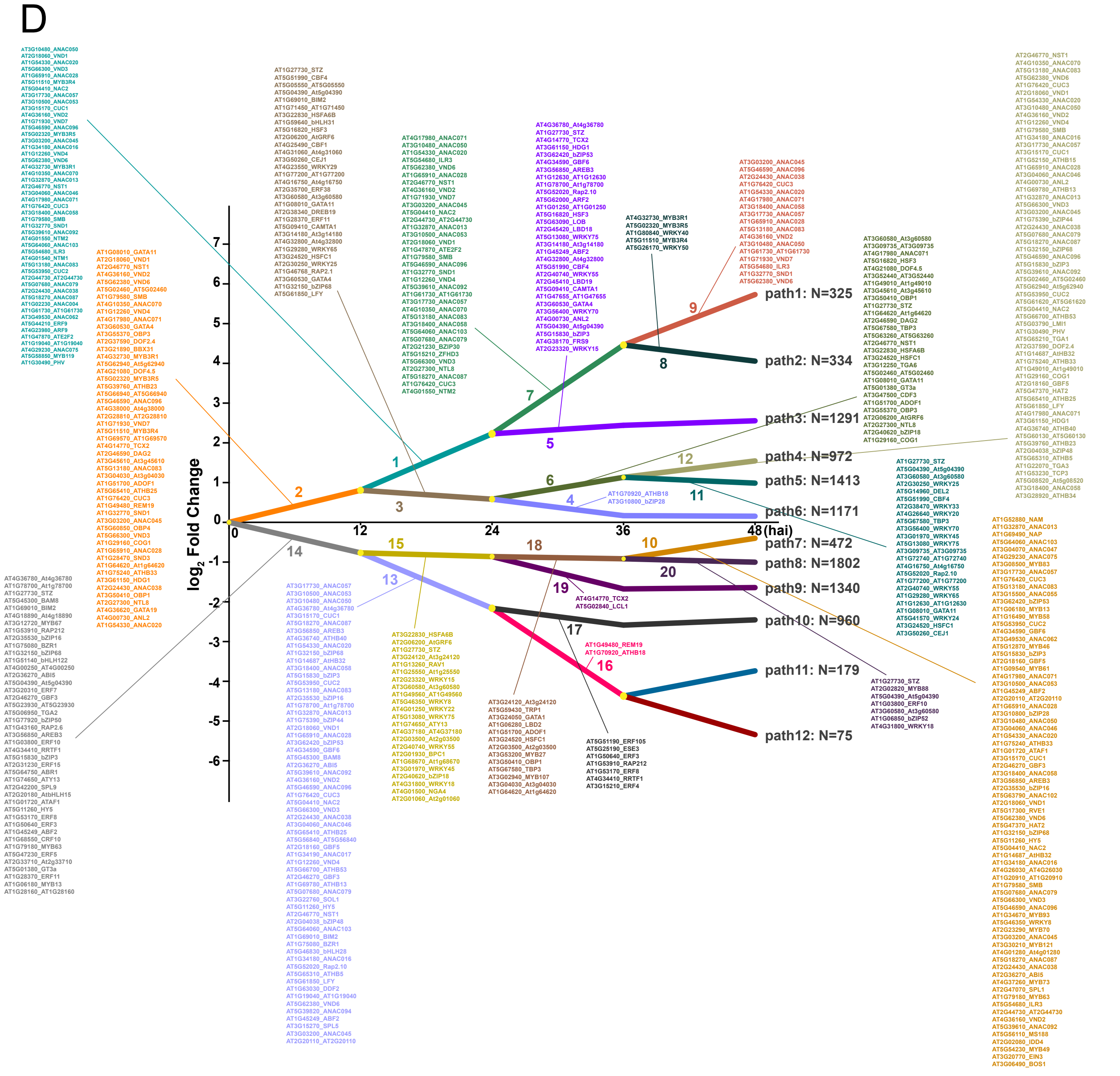


B



C

Time points	0 hai	12 hai	24 hai	36 hai	48 hai
12 hai	1,911				
	1,560				
24 hai	2,733	830			
	2,605	703			
36 hai	3,547	2,043	1,255		
	3,609	2,060	926		
48 hai	3,707	2,361	1,727	339	
	3,527	2,135	879	65	
60 hai	4,212	3,083	2,889	1,178	501
	3,572	2,160	1,380	119	22


D
log2 Fold Change
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
0
12
24
36
48 (hai)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
path1: N=325
path2: N=334
path3: N=1291
path4: N=972
path5: N=1413
path6: N=1171
path7: N=472
path8: N=1802
path9: N=1340
path10: N=960
path11: N=179
path12: N=75
AT3G10480_ANAC050
AT2G18060_VND1
AT1G54330_ANAC020
AT5G66300_VND3
AT1G65910_ANAC028
AT5G11510_MYB3R4
AT5G04410_NAC2
AT3G17730_ANAC057
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AT3G15170_CUC1
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AT1G71930_VND7
AT5G46590_ANAC096
AT5G02320_MYB3R5
AT3G03200_ANAC045
AT1G34180_ANAC016
AT1G12260_VND4
AT5G62380_VND6
AT4G32730_MYB3R1
AT4G10350_ANAC070
AT1G32870_ANAC013
AT2G46770_NST1
AT3G04060_ANAC046
AT4G17980_ANAC071
AT1G76420_CUC3
AT3G18400_ANAC058
AT1G79580_SMB
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AT5G54680_ILR3
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AT5G53950_CUC2
AT2G44730_AT2G44730
AT5G07680_ANAC079
AT2G24430_ANAC038
AT5G18270_ANAC087
AT1G02230_ANAC004
AT1G61730_AT1G61730
AT3G49530_ANAC062
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AT4G23980_ARF9
AT1G47870_ATE2F2
AT1G19040_AT1G19040
AT4G29230_ANAC075
AT5G58850_MYB119
AT1G30490_PHV
AT1G08010_GATA11
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AT2G46770_NST1
AT4G36160_VND2
AT5G62380_VND6
AT5G02460_AT5G02460
AT1G79580_SMB
AT4G10350_ANAC070
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AT4G17980_ANAC071
AT3G60530_GATA4
AT3G55370_OBP3
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AT3G21890_BBX31
AT4G32730_MYB3R1
AT5G62940_At5g62940
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AT5G02320_MYB3R5
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AT5G11510_MYB3R4
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AT2G46590_DAG2
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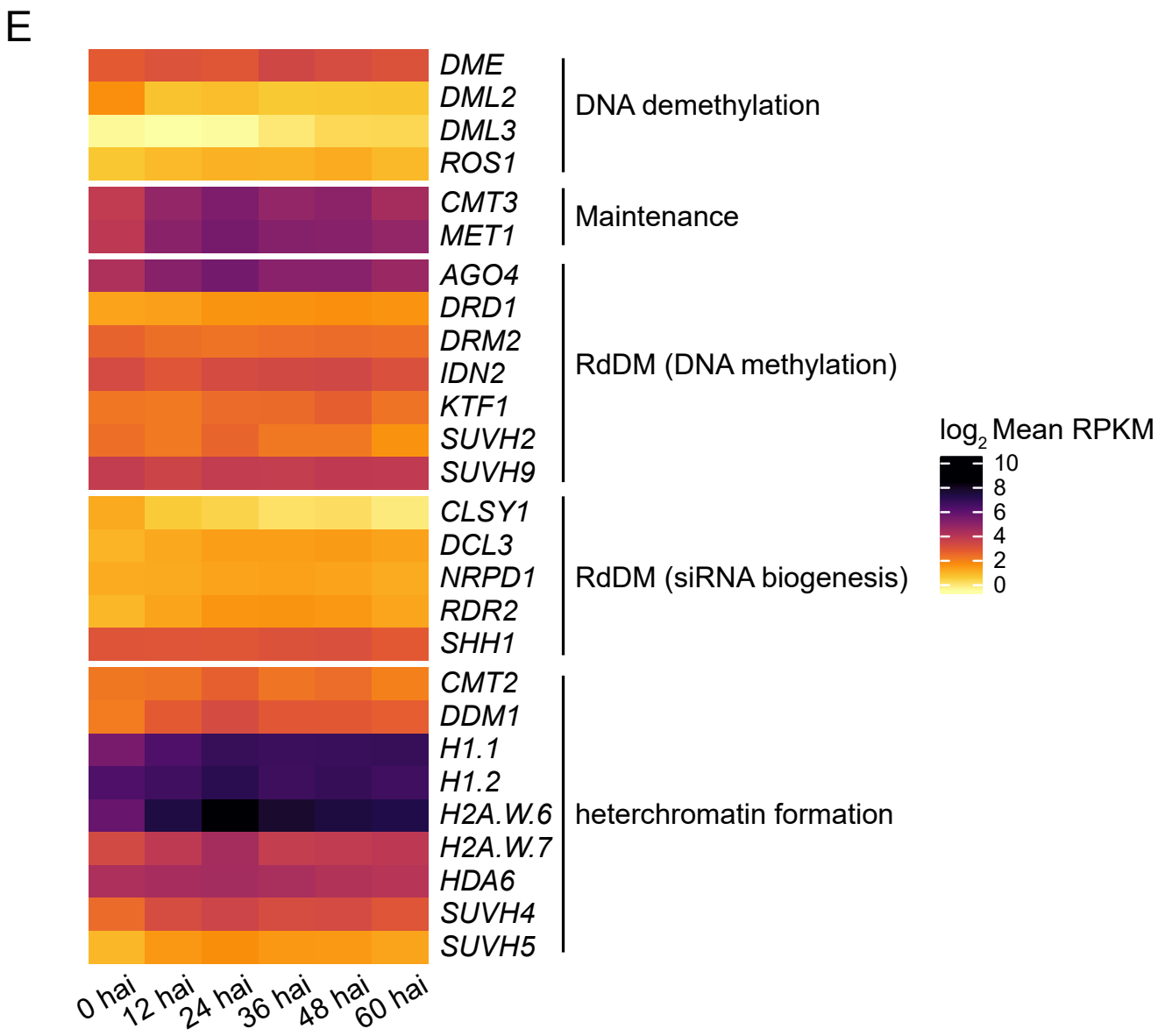
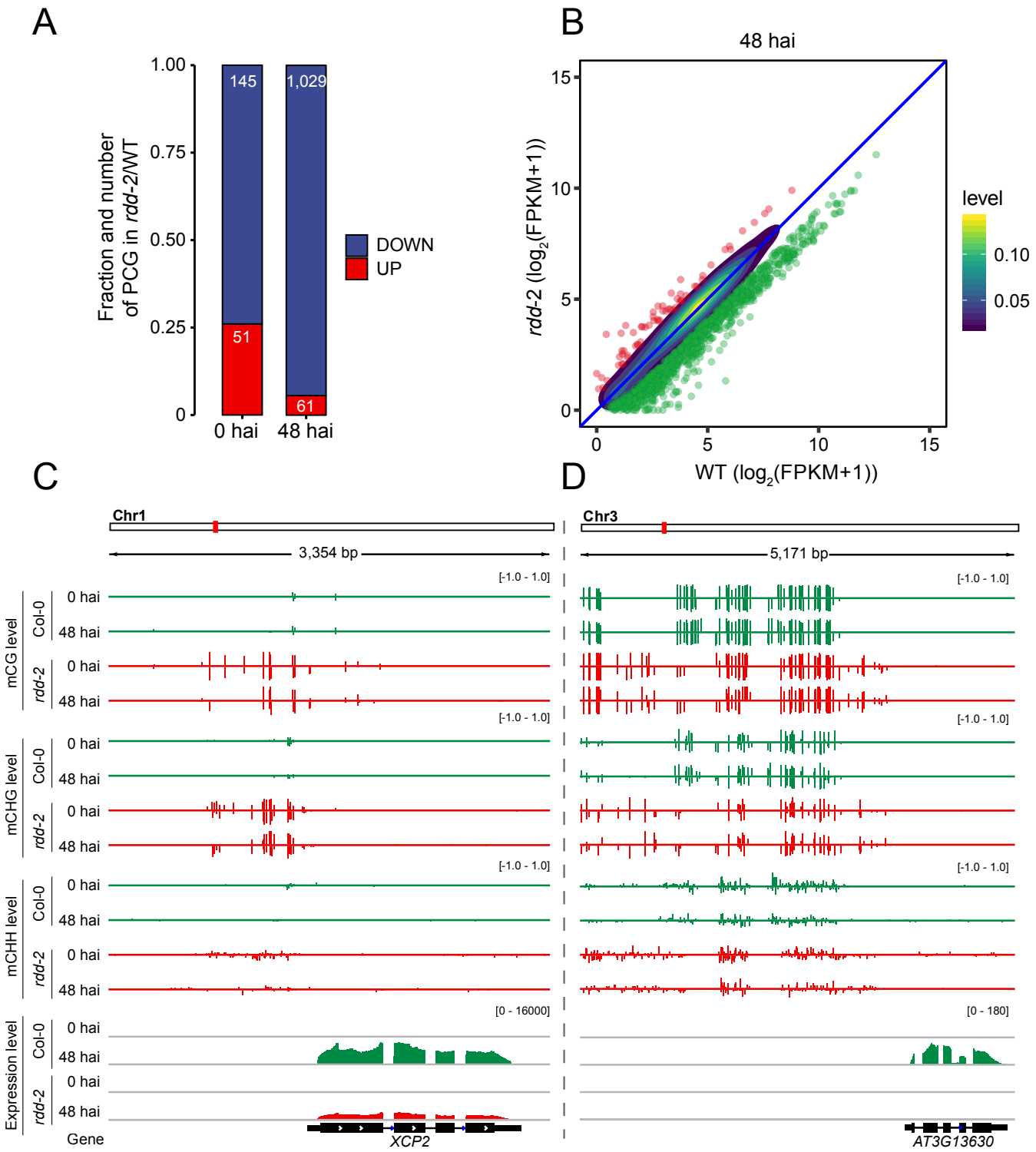


Fig. S4. Transcriptome analysis during ectopic TE differentiation.
(**A**) Principal component analysis (PCA) of the transcriptome at different time points during TE differentiation. Samples from the same time point are indicated by the same color. (**B**) Violin plot showing the extent and distribution of gene expression changes at adjacent time points. (**C**) Numbers of differentially expressed genes between any two time points. The numbers of up-regulated and down-regulated genes are colored red and blue, respectively. **(D)** The DREM model showing all transcription factors assigned to each path (ranked from high to low) is shown. The *y*-axis indicates the $\log_2$ (fold-change of expression level) at different time points during TE differentiation relative to 0 hai. The *x*-axis indicates the induction time in hours (hai). **(E)** Heatmaps showing the relative expression levels of genes in DNA methylation/demethylation pathways during ectopic TE differentiation. Values are expressed as the average $\log_2$ (RPKM) for 3 biological replicates. RPKM: reads per kilobase of transcript per million mapped reads.


A
Fraction and number of PCG in rdd-2/WT
1.00
0.75
0.50
0.25
0
145
51
1,029
61
0 hai
48 hai
DOWN
UP
B
48 hai
rdd-2 (log2(FPKM+1))
WT (log2(FPKM+1))
level
0.10
0.05
C
D
Chr1
3,354 bp
Chr3
5,171 bp
[-1.0 - 1.0]
mCG level
mCHG level
mCHH level
Expression level
Col-0
rdd-2
0 hai
48 hai
[0 - 16000]
[0 - 180]
Gene
XCP2
AT3G13630

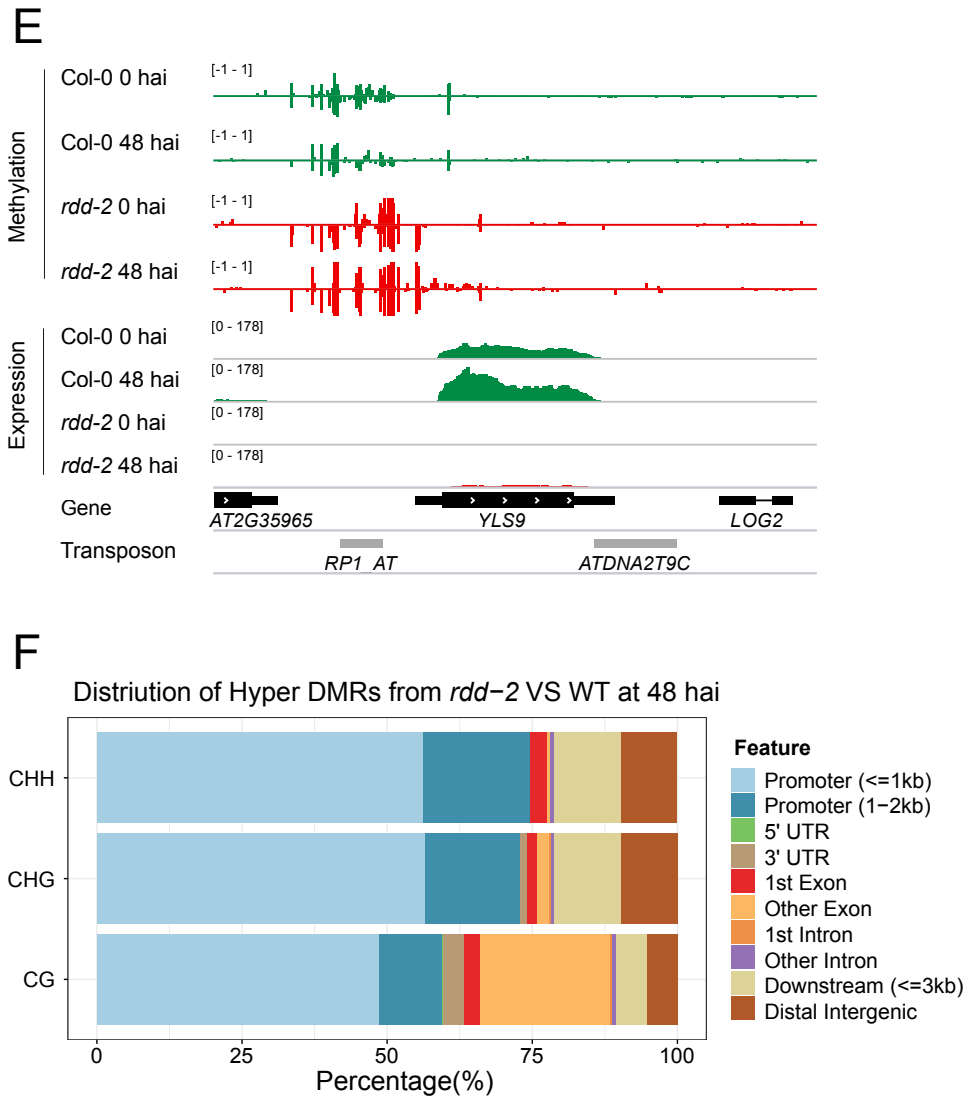


Fig. S5. DEG and DMR analysis of *rdd-2*-versus-Col-0 at 0 hai and 48 hai.
(**A**) Fraction and number of up-regulated and down-regulated genes in *rdd-2* relative to Col-0 at 0 hai and 48 hai. (**B**) Scatterplot of gene expression levels in *rdd-2* and Col-0 at 48 hai. Values are expressed as the average $\log_2$ (FPKM+1) for 2 biological replicates. FPKM: fragments per kilobase of transcript per million mapped reads. **(C,D)** DNA methylation in CG/CHG/CHG contexts and gene expression of *XCP2* **(C)** and *AT3G13630* **(D)** in Col-0 and *rdd-2* at 0 hai and 48 hai during TE differentiation. **(E)** DNA methylation and gene expression of *YLS9* in Col-0 and *rdd-2* at 0 hai and 48 hai during TE differentiation. **(F)** Distribution of hyper DMRs of *rdd-2*-versus-WT at 48 hai over genome features.