SI Appendix

SI Materials and methods

Tomato transformation

Tomato transformation

For tomato transformation, a 223bp fragment (Fig. S2A) corresponding to part of the highly conserved HhH-GPD domain of DNA glycosylase Lyase was amplified from the *SlDML1* cDNA and cloned in sense and antisense orientation in pK7GWIWG2 (I) plasmid to form a hairpin structure necessary for to RNA silencing. The recombinant plasmid named pK7GWSlDML was introduced in the *A. tumefaciens* strain GV3101.
Subsequently, tomato cotyledon transformation was done as described in Gonzalez *et al.* (S1). Twenty five regenerated shoots were selected from independent calli and cultured as described in How Kit *et al.* (S2). Twenty five kanamycin resistant T0 plants were transferred to the greenhouse and grown to obtain T1 seeds.
Among the 25 independent T0 transgenic plants obtained, 8 including plants 2 and 8, presented delayed and limited ripening phenotypes. Plants 2 and 8 were selected for further studies and self-pollinated and backcrossed, respectively, to generate lines 2 and 8. Backcrossing of the T0 plant 8 was necessary due to flower abnormality. Twenty five T1 plants were grown in each case and were classified based on the level of *SlDML* expression as determined by semi quantitative RT–PCR on 20 dpa fruits and used for preliminary phenotype characterization. No flower abnormality was observed on T1 plants that were therefore self-pollinated. Complete phenotypic and molecular analyses were performed on T2 plant population obtained after selfing of a single homozygote (line 2) or hemizygote (line 8) T1 plant.

VIGS experiments

For VIGS experiments a 480 bp PCR amplified fragment corresponding to the 5' coding sequence of *SlDML2* was inserted into the PVX vector (21). This part of the gene has no significant homology with any of the other tomato *SlDML* genes. VIGS and analysis of VIGS experiments were as described (21), using 80 independent fruits injected at 12 days post anthesis.

Molecular Analysis

Gene expression analysis

Absolute quantification of transcript was performed as described (S3). For each gene, PCR fragments were cloned and controlled by sequencing and a calibration curve was done. For comparative RT-QPCR, experiments were performed as described (S2). Normalization of data was done according to Pfaffl *et al.* (S4) using EF1 alpha as a reference gene and a reference sample. An ANOVA two ways was performed and difference in gene expression levels between Wild Type and transgenic plants were

assessed using a student t test (n=3; *: p<0.05; **: p<0.01; ***p<0.001). All primers used for RT PCR analysis are listed in Table S2.

For microarrays analysis, total RNA was isolated from *rin*, *nor Cnr* and wild type Ailsa Craig pericarp samples according to methods as described in (21). The concentration of RNA was determined using an Agilent Bioanalyser 2100 (Agilent Technologies). Total RNA was treated with DNA-free (Ambion) as per the manufacturer's instructions. RNA was then hybridized to the Syngenta Tomato Affymetrix GeneChip.™ The microarrays were normalized using the Robust Multichip Average (RMA) method with the Bioconductor Affymetrix package (S5), which both accounts for the background correction using the perfect match (PM) features for quantile normalization of all the arrays (S6) and for condensing probes into probe regions (hereafter referred to as genes) (S7). The expression values are arbitrary units of fluorescence intensity.

A linear mixed model was fitted to the logarithm of the DML data with genotype, developmental stage and gene considered as fixed effects and plant and fruit-within-plant as random terms in the model. The significance of the fixed effects and their interactions were tested using the Variance ratio F- tests output by the mixed model fitting routines within the Genstat 17 statistical package

McrBC-PCR Analysis

For methylation analysis, genomic DNA were purified from fruit pericarp using the illustra DNA extraction kit Phytopure (GE Healthcare, UK), quantified at 260nm and quality control was performed after electrophoresis on a 1% agarose gel. For McrBC-PCR methylation analysis, 1µg of genomic DNA was digested with McrBC (NEB) for 5h according to manufacturer instructions with or without GTP as a negative control. PCR amplification was performed with 50 ng of genomic DNA with the relevant primers shown in Table S2.

Bisulfite sequencing

Gene specific BS sequencing was performed essentially as described in (28). Briefly, PCR primers for bisulfite treated DNA amplification were designed with Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) using the unconverted genomic DNA sequence as input sequence. As every C nucleotide can be potentially methylated in plants and in order to avoid any sequence selection bias during PCR amplification, "C" and "G" nucleotides were replaced by "Y" and "R" nucleotides in forward and reverse primers respectively. All primers are listed in Table S2.

One microgram of genomic DNA was used for bisulfite treatment conversion using the EpiTect 96 Bisulfite Kit (Qiagen, Courtaboeuf/France) according to manufacturer's

instructions. The absence of unconverted genomic DNA was assessed processing a whole-genome amplified sample (Whole Genome Amplification (WGA) kit, Sigma-Aldrich) simultaneously with the bisulfite conversion of all samples, which presented a DNA methylation value of 0% for every C position of each amplicon after pyrosequencing. PCR amplification of the selected promoter fragments, purification of PCR products and pyrosequencing experiments were performed as described (S8). DNA methylation patterns were analyzed with the PyroMArk CpG software (Qiagen) and by an in-house developed Microsoft Excel Visual Basic Application for Cytosines outside CpG sites. For each sample, average DNA methylation value of each cytosine or cytosine group (CC, CCC) obtained by pyrosequencing of the three regions of interest (CNR, NOR and PSY1) were pooled to generate a matrix. Heatmap representation of the data was then performed using "heatmap2" function of "gplots" package of the R software. Hierarchical unsupervised clustering between columns and rows were computed using Euclidean distance and complete linkage method as agglomerative method.

Metabolite analysis

Ethylene production analysis

Ethylene production was assayed on individual fruit after 2 h by withdrawing 1-ml gas samples from sealed jars. Gas samples were analyzed via gas chromatography (7820A GC system Agilent Technologies, Santa Clara, CA, USA, http:// www.chem.agilent.com/en-US/products-services/Instruments-Systems/Gas-Chromatography/7820A-GC/Pages/default.aspx). Ethylene was identified via co-migration with an ethylene standard and quantified with reference to a standard curve for ethylene concentration.

Carotenoid analysis

Carotenoid analysis was done as described (S3). Samples from transgenic plants DML2A, B and DML8A, B were compared to those of WT1 and WT2 respectively. Differences between samples were evaluated using an ANOVA and Tukey's test (n=3, *: $p<0.05$; **: $p<0.01$; ***$p<0.001$).

^{1}H-NMR Metabolite analysis

For ^{1}H-NMR analysis, polar metabolites were extracted from ground *Solanum lycopersicum L.*, cv WVA106 pericarp fruit. Briefly, the frozen powdered samples were lyophilised and polar metabolites were extracted from 15 to 30 mg of lyophilised

powder samples with an ethanol–water series at 80°C. The supernatants were combined, dried under vacuum and lyophilized. Each lyophilized extract was solubilized in 500 µL of 300 mM potassium phosphate buffer pH 6.0, in D_2O. Ethylene diamine tetraacetic acid disodium salt (EDTA) was added at a final concentration of 3 mM. Each extract was titrated with KOD solution to pH 6 and lyophilized again. The lyophilized titrated extracts were stored in darkness under vacuum at room temperature, before ^{1}H-NMR analysis was completed within one week.

^{1}H-NMR analysis was performed using 500 µL of D_2O with sodium trimethylsilyl [2,2,3,3-$^2H_4$] propionate (TSP, 0.01% final concentration for chemical shift calibration) added to the lyophilized titrated extracts. The mixture was centrifuged at 17,700 *g* for 5 min at room temperature. The supernatant was then transferred into a 5 mm NMR tube for acquisition. Quantitative ^{1}H-NMR spectra were recorded at 500.162 MHz and 300 K on a Bruker Avance III spectrometer (Wissembourg, FR) using a 5-mm broadband inverse probe, a 90° pulse angle and an electronic reference for quantification The assignments of metabolites in the NMR spectra were made by comparing the proton chemical shifts with literature or database values (S9), by comparison with spectra of authentic compounds and by spiking the samples. For assignment purposes, ^{1}H-^{1}H COSY, spectra were acquired for selected samples. For absolute quantification three calibration curves (glucose and fructose: 2.5 to 100 mM, glutamate and glutamine: 0 to 30 mM) were prepared and analysed under the same conditions. The glucose calibration was used for the quantification of all amino-acids, as a function of the number of protons of selected resonances except fructose, glutamate and glutamine that were quantified using their own calibration curve. The metabolite concentrations were calculated using AMIX (version 3.9.10, Bruker) and Excel (Microsoft, Redmond, WA, USA) softwares.

To explore the metabolite multidimensional data set, one unsupervised multivariate statistical method was used on mean-centered data scaled to unit variance: Principal Component Analysis (PCA). PCA was used to visualize the grouping of the different samples without any knowledge of their group. PCA was performed of absolute concentration of 31 metabolites issued from ^{1}H-NMR analysis of transgenic and corresponding WT controls tomato pericarp fruit harvested at 20, 35, 40, 55, 70, 85 and 110 days post pollination (dpa), using SAS software version 8.01 (SAS Institute 1990).

For individual metabolites, means ± standard deviations (sd) were calculated from three biological replicates. For all biochemical analyses two extractions were completed to measure the concentration of each biological replicate, then the mean of three biological replicates was calculated. Mean comparisons were conducted using an ANOVA followed by Tukey's t –test.

SI Supplementary Figures

Fig. S1

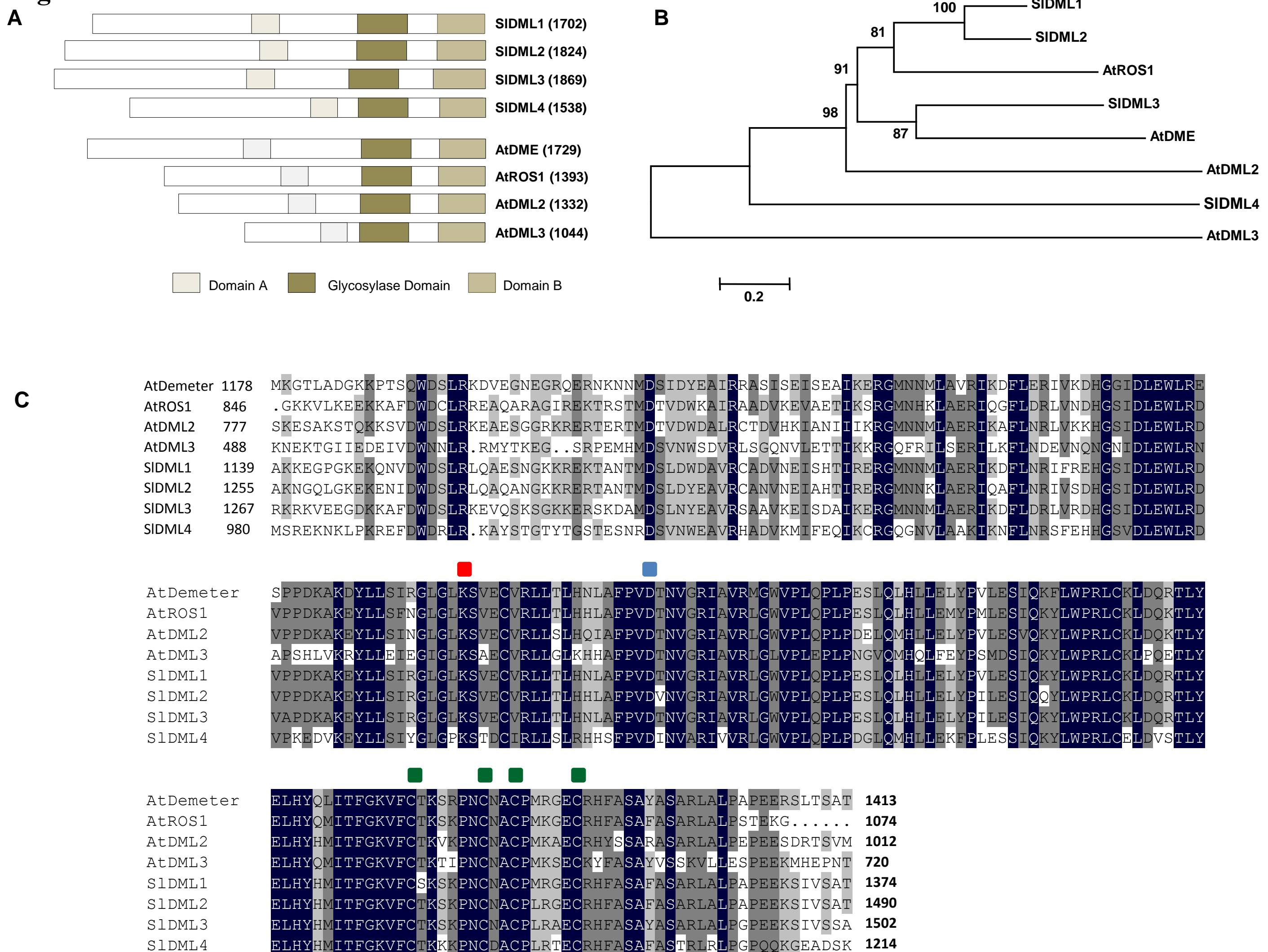

Figure S1: Comparison of DNA Glycosylase Lyase sequences from tomato and from *Arabidopsis thaliana*. (A) Schematic diagram of domain structures of tomato and *Arabidopsis thaliana* DNA Glycosylase Lyase proteins. Numbers on the right indicate the number of amino acids in each protein. The positions of the domains are indicated in Table S1. (B) Phylogenetic analysis. An unrooted dendogram was generated using the Maximum Likelihood method in MEGA v.6 based on the JTT model (S 10). Sequences of DNA glycosylase proteins were aligned using Muscle. The numbers at the branching points indicate the percentage of times that each branch topology was found during bootstrap analysis (n=1000). (C) The Helix-hairpin-helix -Gly/Pro rich domain (HhH-GPD) domain of tomato DNA Demethylase was aligned with those of *Arabidopsis*. Red dot indicates the highly conserved Lysine residue necessary for catalytic activity, blue dot shows the conserved aspartic acid residue present in the active site and green dots show the cysteine residues that constitute a [4Fe–4S] cluster (23). Accession numbers are indicated in Table S1.

Fig.S2

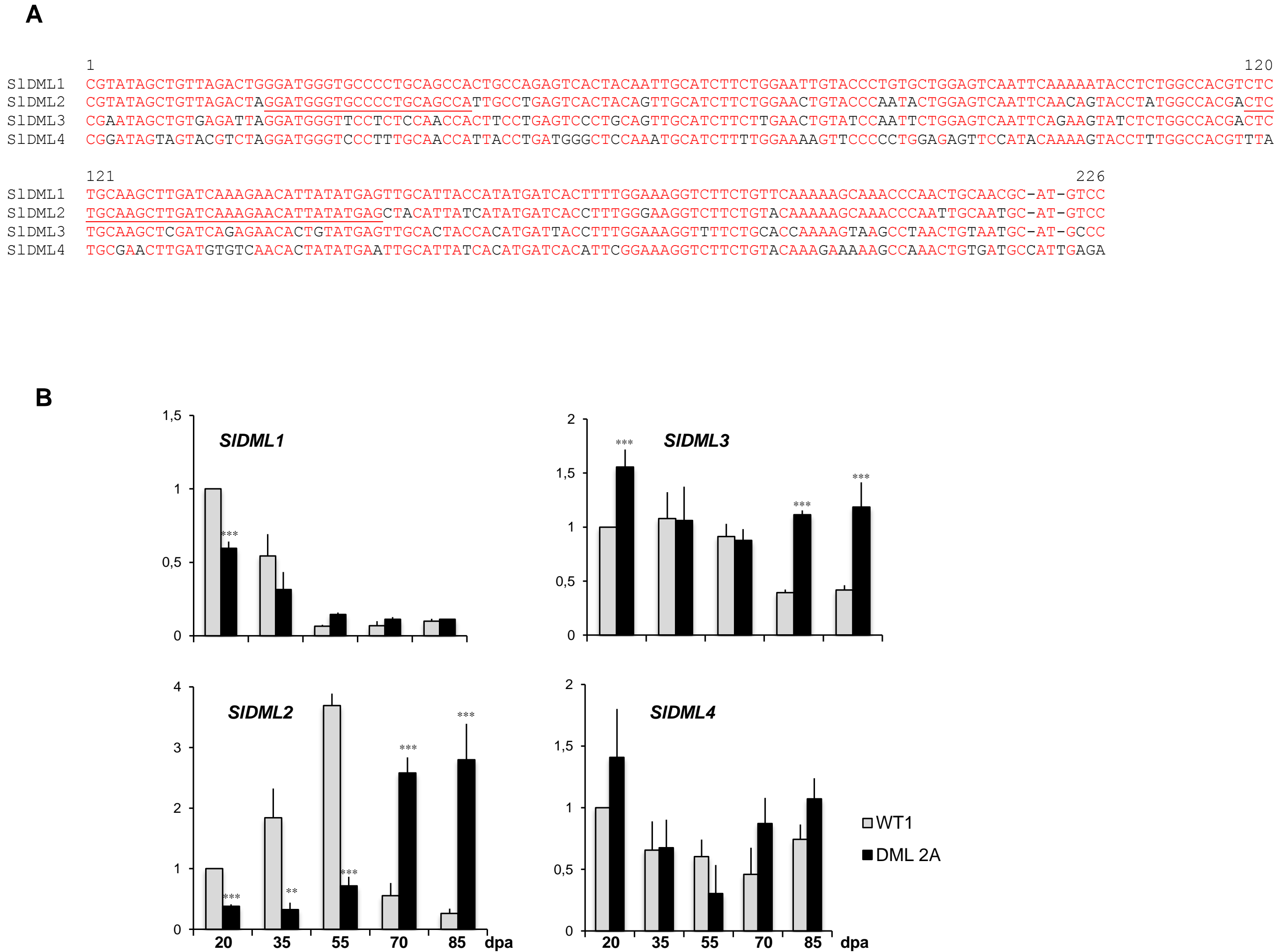

Figure S2:

(A) RNAi strategy: the sequence encoding part of the Helix-hairpin-helix -Gly/Pro rich domain (HhH-GPD) of *SlDML1* (+3897; +4123) was used to generate an RNAi construct in the vector pK7GWIWG2 (I). Alignment with corresponding sequences of *SlDML2* (+4252; +4378), *SlDML3* (+4191; +4470) and *SlDML4* (+3328; +3551) are shown. Sequence homology of *SlDML1* with the corresponding domain of *SlDML2, SlDML3, and SlDML4* is 90%, 83% and 75% respectively. Nucleotides shown in black correspond to differences between *SlDML 2, 3*, and *4* and *SlDML*1. Alignment was performed using the multalin software (http://multalin.toulouse.inra.fr/multalin/multalin.html).

(B) Residual *SlDML* gene expression: *SlDML* gene expression was measured by Real time RT-PCR analysis in WT1 and DML2A (line 2) fruits at 20, 35, 55 (Br+16), 70 (Br+31) and 85 (Br+46) dpa. An ANOVA was performed and differences with WT1 fruits of the same age were analyzed using a student t test (n=3). Stars indicate difference between WT and transgenic fruits of the same age (*: p<0.05; **: p<0.01; ***p<0.001). The Br stage (39 dpa) was not analyzed due to a limited number of fruits produced by the transgenic plants of line 2 as explained in the methods.

Fig. S3

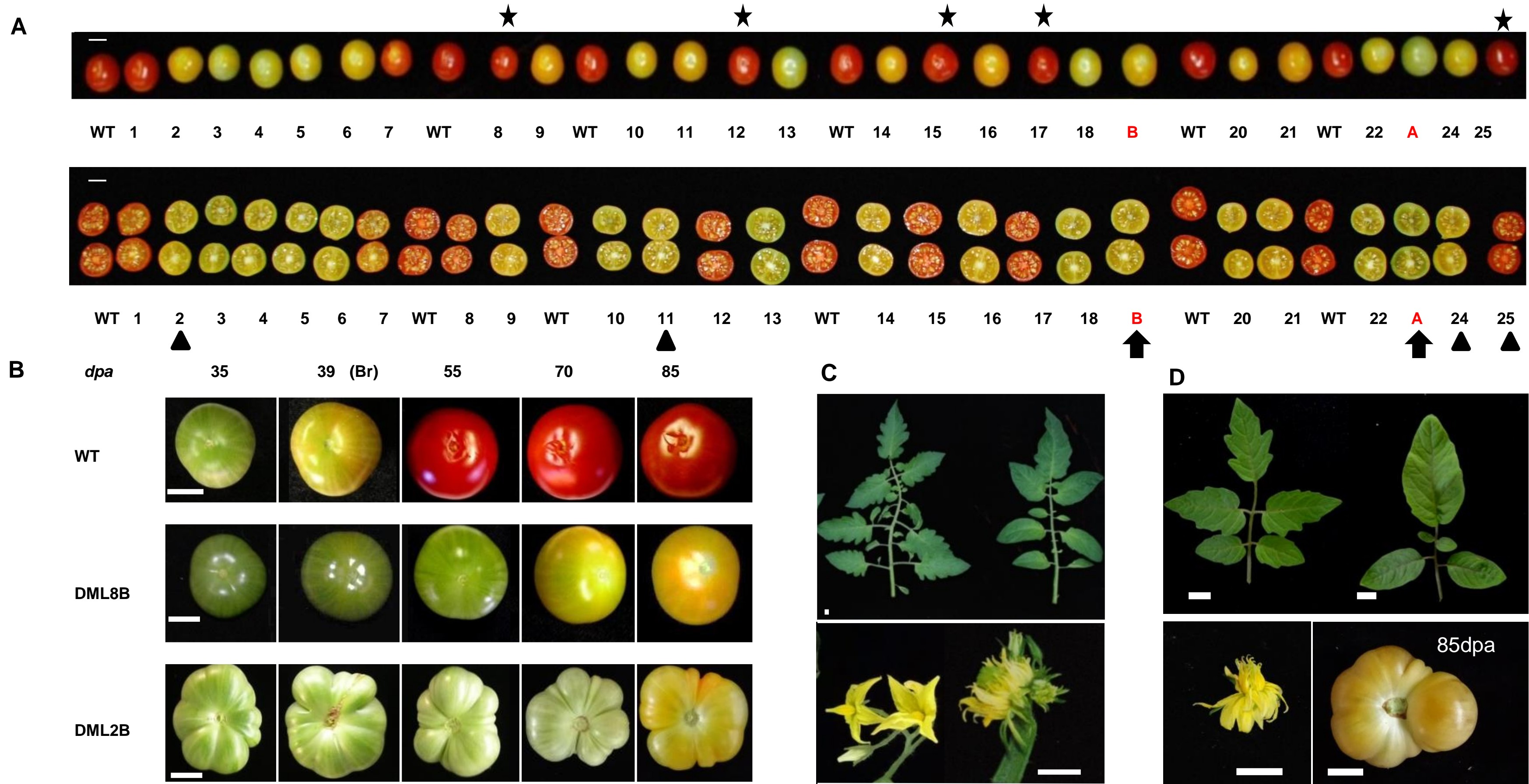

Figure S3: Fruit, flower and leaf phenotypes of transgenic RNAi plants. (A) Phenotype of fruits formed on T2 plants (line 8) obtained after self-pollination of a single T1 parent. Fruits were harvested at 85 dpa. Number refers to individual plants from which fruits were harvested. Fruits representative of each plant are shown although on a single plant, fruit phenotype intensity may vary depending on plant age and position of the fruit. ★ indicates azygous plants that have lost the transgene after segregation. In this situation fruit ripening reversed to WT. 🡅 design plants DMLA and DMLB that were selected for metabolic, gene expression and methylation analysis. ▲ indicate additional plants used as control for carotenoid measurement and or gene expression analysis and or methylation analysis. White bar: 1 cm. Similar ripening phenotypes were obtained in T2 plants of line 2. (B) Typical fruits of plants DML8B, and DML2B are shown along with WT fruits of the same age. Developmental stages are indicated. Plants of line 8 were self-pollinated whereas flowers of plant 2B required to be back crossed with WT pollen to allow fruit development. Fruits of plant DML2B are characterized, in addition to the delayed ripening phenotype by an increased number of locules. (C) Leaf (upper panel) and flowers (lower panel) of WT (left) and transgenic plants of line 2 (right). Leaf lacks indentation and flowers are fasciated. Such flowers need to be hand pollinated and will give fruits with multiple carpels (up to 18 in a few cases). (D) Representative leaf (upper panel) of WT (left) and transgenic T2 plants of line 1 (right). Representative flower (left) and fruit (right) of transgenic T3 plants of line 1 (lower panel).

Fig. S4

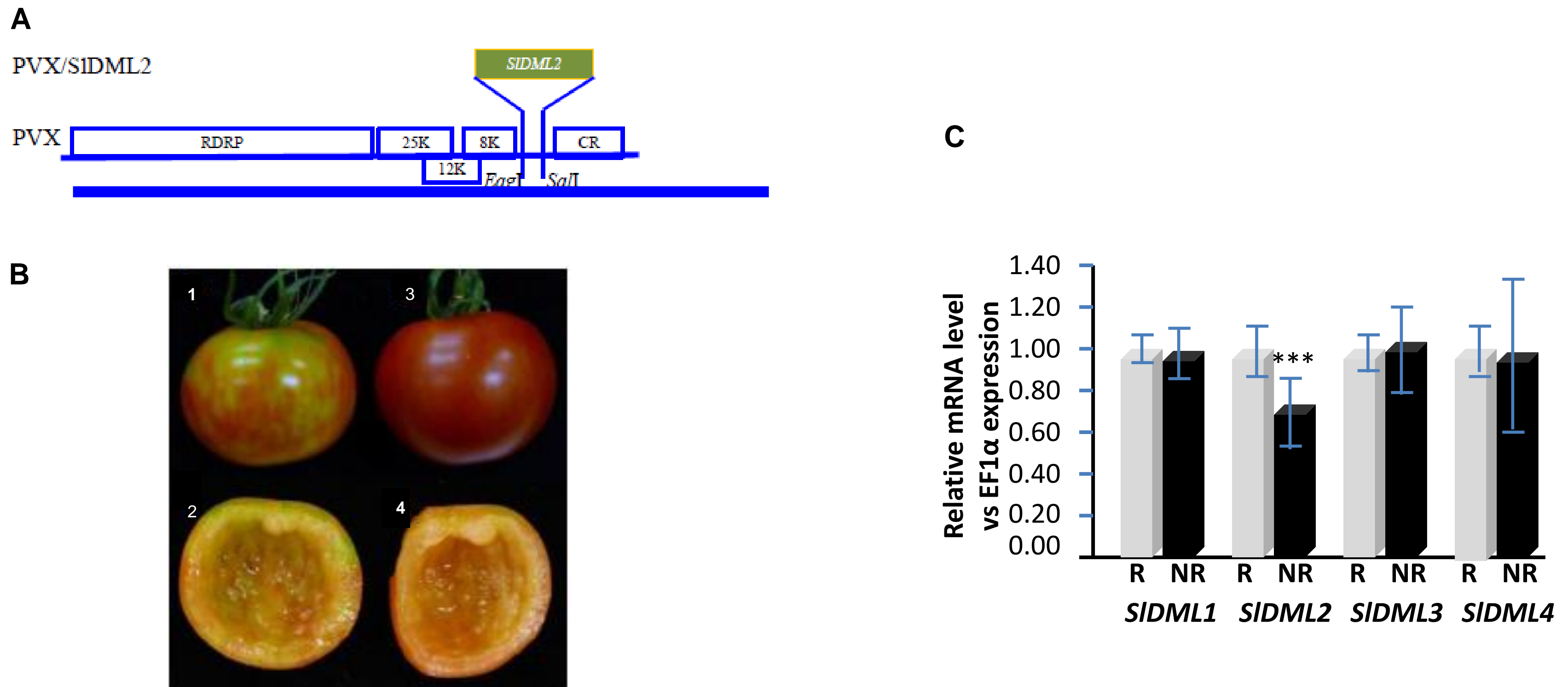

Figure S4: VIGS analysis of SlDML2 function in tomato fruit ripening. (A) Construction of PVX/SlDML2. The specific 5' coding region (1- 480) of *SlDML2* mRNA was PCR amplified and cloned into the PVX vector to generate PVX/SlDML2. (B) Fruits from Ailsa Craig plants were injected with PVX/SlDML2 **(1, 2)** or PVX **(3, 4)** at 14 dpa. Fruits were photographed 2 days after the breaker stage (Br + 2, 43dpa); **(2, 4)** Inside of fruits 1-3, respectively. Ripening-inhibited sectors in fruits injected with PVX/SlDML2 remain green.(C) *SlDML* gene expression analysis in ripening (R) and non ripening (NR) sectors of fruits treated with PVX/SlDML2. Values are normalized to EF1α and to the expression of the corresponding gene in the ripening sectors which represent the 100% of expression level. *** indicate significant difference ($p<0.005$) between R and NR sectors as determined with a student t test (n=3).

Fig. S5

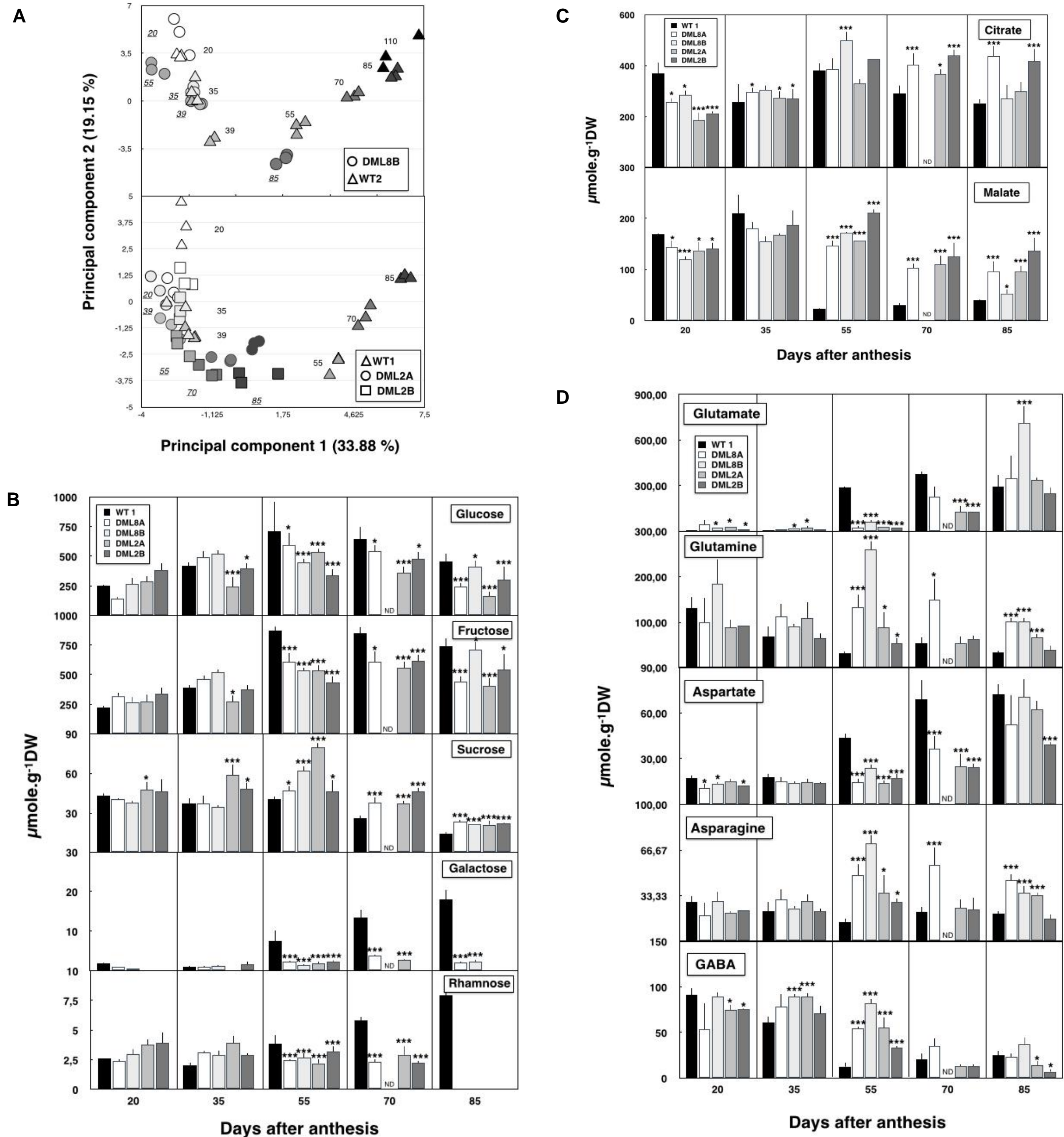

Figure S5: characterization of the metabolites content of the transgenic RNAi fruits. (**A**) PCA was performed with MatLab Software (version 7.4.0) to evaluate the grouping of samples without any knowledge of their group, using the absolute concentration of 31 metabolites determined from ^{1}H-NMR analysis of WT2 (▵) and DML8B (o) [upper panel] and WT1 (▵) and DML2A (o) and DML2B (□) [lower panel] tomato pericarp fruit harvested at 20, 35, 39, 55, 70, 85 and 110 dpa. PC1, (33.88% of the global variability) separates WT fruits at 55, 70, 85 [upper and lower panels] and 110 dpa, [upper panel] from all other samples. PC2 (19.15% of the total variability) separates fruit samples of WT1, WT2 and transgenic (DML8B and DML2A, 2B) at 20 dpa from 35dpa and 39dpa, irrespective to their genotype. (**B**) Sugar (sucrose, glucose, fructose, galactose and rhamnose), (**C**) organic acids (malic and citric acids) and (**D**) amino acids (aspartate, asparagine, glutamate, glutamine and GABA) content was determined by quantitative ^{1}H NMR spectroscopy as previously described (Sup ref S11) using for each stage and plant a minimum of 6 fruits in 3 biological replicates. An ANOVA one way was performed and difference between samples were evaluated using a t student test (n=3). Stars indicate difference between WT and transgenic fruits of the same age (*: p<0.05; **: p<0.01; ***: p<0.001).

Fig. S6

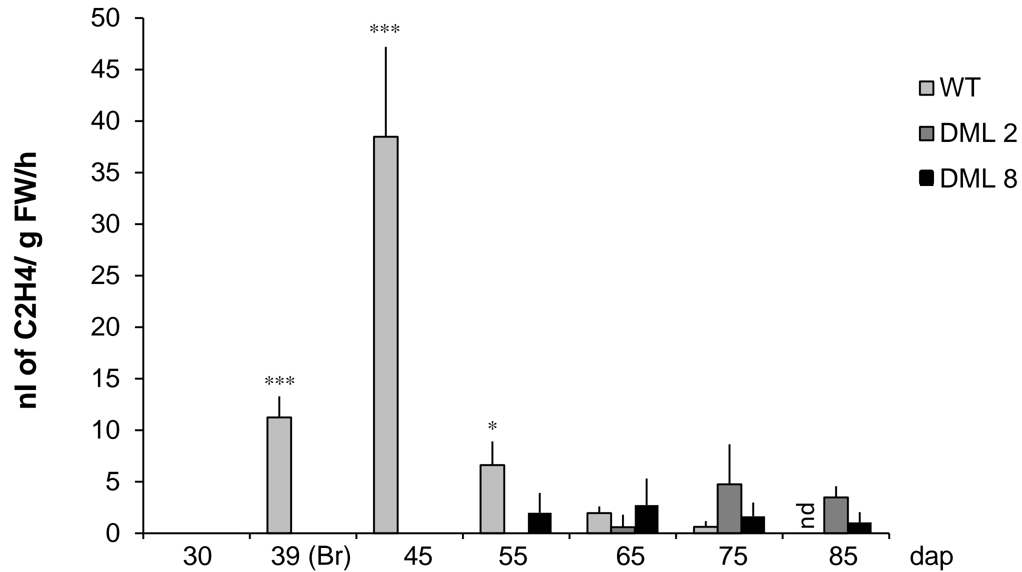

Figure S6: Ethylene production in WT and transgenic fruits

Fruits were harvested from 2 (line 2) or 4 (line 8) independent T2 plants at the developmental stages indicated and analyzed individually for ethylene production**.** Values represent the average of a minimum of 4 to 10 independent fruits for each line and time point. An Anova was performed and differences between samples were evaluated using a turkey's test. Stars indicate difference between WT and transgenic fruits of the same age (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). The Breaker stage (Br) for WT fruits corresponds to 39 +/-1 dpa.

Fig S7

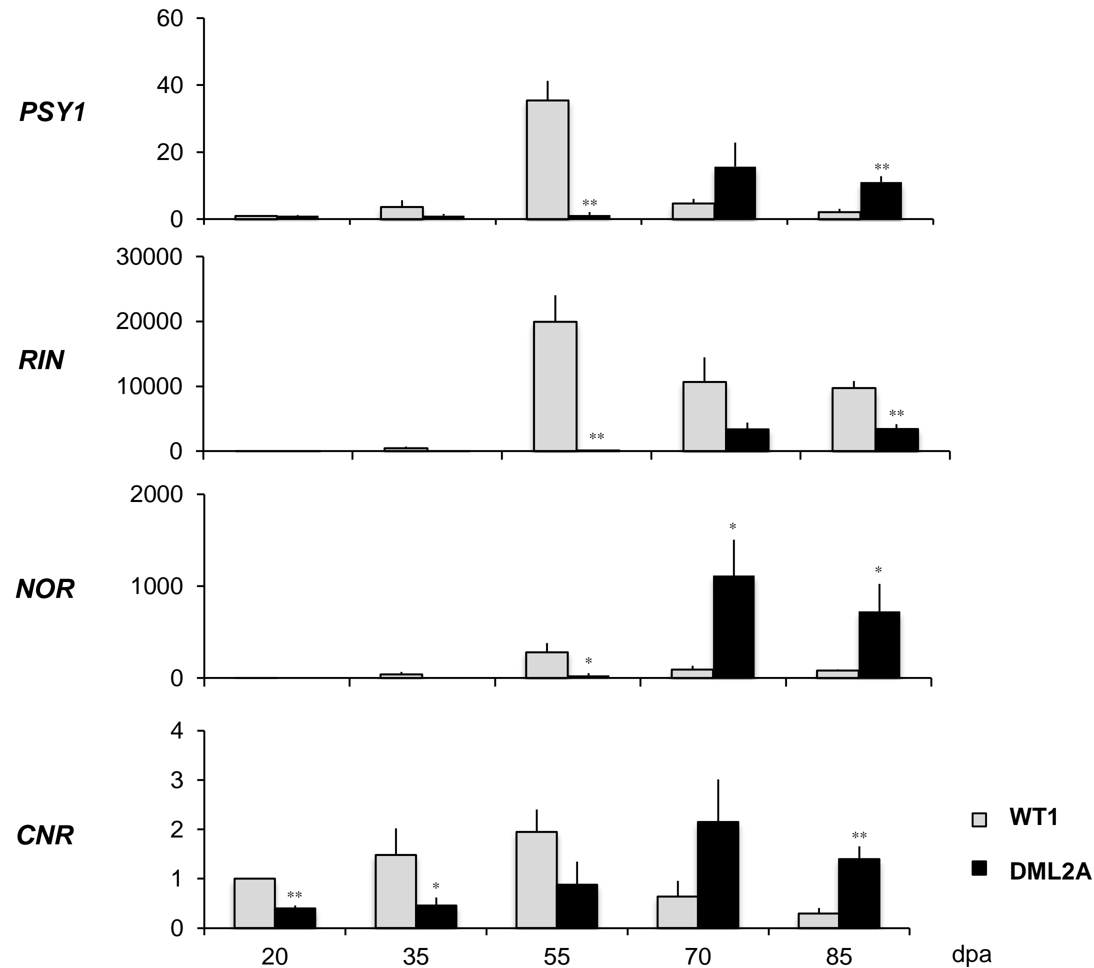

Figure S7: Candidate gene expression during the ripening of RNAi transgenic fruits of line 2 plants

RIN, NOR, CNR and PSY1 gene expression was analyzed in the transgenic plant DML2A during fruit ripening using real time RT-PCR. Primers are listed in Table S2. Values are normalized to EF1α and to the WT fruits at 20dpa. Stars (*: $p<0.05$; **: $p<0.01$; ***: :$p<0.001$) indicate significant difference between WT and DML2A plants at a given stage using a student t test (n=3).

Figure S8

***RIN* (Solyc05g012020).**

-2132

ATCTGGTACATAAACTATTGTGCTTATGTAGAATTTGGGGAAGAAACGTCAA*GGAATATA*
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GATGCACTAATGTTTGCCACTAATTCTTAAGATAGATAACAAACACATCTAAACATTATTA
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***NOR* (Solyc10g006880).**

-2568

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*GA**C**[2]A**C3**TAGG**CC**[4]AAATTTGT**CC**[5]AAAGAGTAG**CC**[6]TAGAAATG**CTTTC**[7]TTAT**C**[8]TTATTAT*
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PSY1 (Solyc03g031860).

-2345

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GTTAGAGGGTAAGTTACGCATTTAGTCGTAACTTAGTCAAACTTCGTAATAATTT
AGTAAGTTAAAATATATTAGAAATTTTCAGAATTCATAAACTTTAAATTTTAAATTTTG
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GGGTGGAATTTGTGTAAGTTTTGTTTCCTTTC[1]AGTTC[2]TTGATATATAAAGTTGC[3]AATC[4]T
TTAAC[5]ATTC[6]TTTGTTCAC[7]TTTC[8]TATAGGTTTGC[9]TAGGTTC[10]GGTTAAATTC[11]AGTAGC[1]

[2]TTTAGTTTAAACCC[13]TATGC[14]GGAATAGAGAATGTGTAAAC[15]TTTAAACTTC[16]AAATTTT
GGCTCC[17]GC[18]ATACG[19]AC[20]TAGC[21]GAC[22]TATATAATAATAGGAATTGAGCACTTGGCTT
TTGTATATAGCTTCTATGTGTACCAAAATTAGAAAATCAGGCGATTATTATAATCTTGTTG
ACTAAATATAGAATGCATCCATTACCCCCAAAAAGTGTGATTCCACTGTCATAGGAGGTT
TTTTTTATTTCATTTTATTTGTGCTTTCAATAATGTAGAGTAGTTTTACAAAGATCCTTTCT
TTGTGACACATGGTAGGTAATATTGCTGATTTTGTTGTAGTTTTGGGGTTATAAAGTTTCA
AATTATTTATACTGGAGGGTAGGGGTGGGGGTTGTCTATAATGCAGGTTATGGTTTTAC[1]
GTGAACTC[2]AATAATTATTGTAGATAC[3]TAAGAAATCC[4]ACTC[5]AGTGTTC[6]TTGC[7]GGTGTC[8]
TTGC[9]TTTTGATTTC[10]AGC[11]ATC[12]AC[13]TTGTAGTTGATTGTGTTTAGATTATC[14]AC[15]ATTAT
TC[16]TGTGGC[17]TGTAAC[18]TGTATCC[19]TTGTTAGTTGC[20]TTTGTTTC[21]TAC[22]AC[23]TGTTGTTT
TCCCTC[24]TTTTATACC[25]TATTTTGATATGTTGTACTCGAACGAGGGTCATCGGGGAACA
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CCTCTTGTAGGATTTCACTATATTGTAATATTAACTTGAGGTCACTATAGGAGCTCAAA
AACTTCTAATTTTGAATCAATGTCTGGTTATACTTTTTTTGTCATAACTGTATCTCA
AATGTGGTGTTTGGTTTATCTCATTTTGCAGAAGTCAAGAAACAGGTTACTCCTG
TTTGAGTGAGGAAAAGTTGGTTTGCCTGTCTGTGGTCTTTTTATAATCTTTTTCTA
CAGAAGAGAAAGTGGGTAATTTTGTTTGAGAGTGGAAATATTCTCTAGTGGGAAT
CTACTAGGAGTAATTTATTTTCTATAAACTAAGTAAAGTTTGGAAGGTGACAAAAA
GAAAGACAAAAATCTTGGAATTGTTTTAGACAACCAAGGTTTTCTTGCTCAGAAT
G

CNR (Solyc02g077920).
-3526
TCACAATCACAAGCAGGCAGTGAAACAATTACATCAAGCTCGTTTCATAGATTCTTCATT
TTGGAATAATAGCTTGTCACAGAGTCTGTTCCTTGTTTCAAATTAGCAATTTCTGCGCAC
AAATAATAAATCCTCGTCAAATTCGATCTATCAAAACGCTCCTTGTATTCATCCCATACCT
TCTTCGCAATTGAAGCATAAACTATGCTTGGCATCAAATCAGCTGTAACAGTACTTCCTA
TCCATGATAACACAATTGCATTAAATTTTTCCCATTACGCTTCTAGGTCTCCTTTAAACTT
ACTCTTTGTGCAGTTTCCATCCACAAATCCAAGTTTTACTTTGCCTCGCAGTGCTAGTTT
CATCGATTTGCTCCATAGAGAGTAGTTCTCTGGTCCTGTGAGTTTGATCGGAGTTATTAC
TAAGTCTGGAGAATCAGAAGCTTAAAGATAGAGAGGATGATGATGATTAATTTTTGTTG
C[1]AGC[2]TGGAACTTCC[3]ATC[4]TGTAGC[5]AC[6]ATTGTTCTCC[7]TGTC[8]ATTTCC[9]TAAAC[10]TAATT
GATTGC[11]GATTAACTC[12]AAATTCC[13]TAAGC[T]C[14]AGC[15]TGAAGGTGAGATC[16]GCC[17]GCTC[18]
TGATACC[19]ATGTTAATTTGTAGTTATGTATGTAGAATTTATGGTGAATAAGTTC[20]ACC[21]A
TTGATGAATTTTC[22]TAAGCTGCTACAGAGATATTGGAAGAGAAAAAGAGGATCACTATT
TCATTGAATCTAAATTGAATTATCTTTTTTTAATCATAATTGATGGCTAGTACTGTTATAGG
TCCAGCTAACCTACTTCTAGAAAGTTCCATTTTAACTGACCTCATAACAAATTGTAACTAA
TTTTGTTAGCTACATCACAAATGACACTTACAAGAATAACAGTAATAAGAAACAAGTTATT
TCAACAGCTATCATTTATTATGTTACCTCATCTTGTATCGTGTTAATCCGTACAGACATAA
TTAAAATACAAAATAAGAAAATTAGAACTAGAGGCTCTAAACAGGAAATTTCAGGAAGTT
CCACCTCTGCCTAGCTATATTACATGATTTAAAAGGTATAATACAAGATGAACTCCTTAAA
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GATACATATTATTATGAGATGACATATTTATGTGGTTAACTTAATTATGCGATAGACAAAT
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GACAAATTAAGGGACTTATCATGTATTATGCCCTTCAAAAAGATTACTTCTTTATTAATGA
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ATTGTCAATTGTCATTCCTTCGACAACAAGGGAATATATAGTTGGAATACTTCTGTCCCA
TCCATCCTGCCTACACACAAGTTATTCATTCTAACCTGTCTTTGCCTACTAAGATACGTCT

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TCATTCATATGGTTAATAGCAACAGAGTTGTCTTTGTATTAGTGCACTATCAATTT*AATAC*
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CAAGAAGAAGAAAAAAAGGCAAATATGATATAAAACCATTTAACAAAGTCCATATCACAAA
AATTAGACGGCAAAATCATACACGACTAATTTATAGATTCACTGAACCATGCAATTCTATA
CCGTTCACTTCCAATAAATAAAACATAATACACTATGTTTAGAGTACAAGACTCTCCTTGC
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TCATTTGTTAATTCATTTTGAGTAATGTAACTGTGCAAGATAATGATGTTTTTTTTCCTTTT
TGGTTAACTAAGATAATGATGGATTTAGGTAGATGAATTAGAC[1]ATC[2]TAGTGATAATGG
AGAGCC[3]AGTGC[4]AAC[5]AATTGAGC[6]ACTC[7]TAC[8]TGGACC[9]GAC[10]ATGGAC[11]AAC[12]TGAGA
GACC[13]AAC[14]TTGC[15]AGTATTATAATAGTGC[16]AAATTATAGTTTAGTC[17]GACTCCCTTC[18]G
GAATC[19]TAC[20]TAC[21]ATAAAGAAC[22]TACC[23]ATAAAC[24]TATGTTAGATGGC[25]TATTAC[26]GGA
GTTTAAATTAAACTC[27]GAAAATATC[28]AGAAAAAGAAGTAACTTC[29]AACC[30]AATTAC[31]AATG
C[32]ATACCC[33]TTATC[34]AC[35]AAGTGAAAAAGAGTAAAC[36]GTGCC[37]AAACTC[38]TTTTGATCCC
TCC[39]AAAGCTAGAGGAAAAGAGTGAGCAATTCACTACAAACCACTGGCTTTGGTCTAT
GGTTGACACAACTCCTCGGCTAATTGGTCAAAATATCTTGTGACCACCAACCAGCAAGC
ACTAAATTGGATGTTCTATCAGCTTCTTTACATCATAAAACAGTGAATACTGAACGCTGA
*GAGGCTAACT*GACTGCCCAAAAAAACCTTGACAAAAAGTTAGTGGAGTAACTACCTAGG
AGTAAATTCAATAGTAGACCTTGAAAAGAACTTTAGCAAAGTCATCATAAATGCTCTTCAC
GTCTCATGTACTATGTTAAGGAATGGTCACATTTCTCTCTGCATTAAAGCTAGTTCATGTT
AAAAGTTGAGGCCGGTAGTAGTTTCAACTTTCAATTTAATTCCACCTTTCCTGGCCCACT
TCTGTACGGAACACCAATCAGAATCTTTAGTTCATCTTAACACCAAAGCATCTCCACTTA
GACACTTACTAGACTTCACATAGGAGGAAAAATATGGAACTGGTGGTCCTCACACGTAC
TTACCTTTCTTTTTTTACCTTTGTTCAAGTTTCATACTCTTTTATCTGGCTTCCTCACTCTA
TTTTGGCCCAATAGGTTCTCCTCACAGGGATG

Figure S8: Promoter sequences analyzed using MCR-BC PCR and bisulfite Pyrosequencing

Promoter sequences of *RIN, NOR, PSY1* and *CNR* genes. Bases are numbered from the ATG. Fragment analyzed using McrBC-PCR (Fig. 5) are shown in italic. For the *NOR*, *CNR* and *PSY1* promoter fragments, sequences analyzed by Bisulfite-pyro-sequencing are underlined and limited either by red primers (PCR fragment 1) or by blue primers (PCR fragment 2). For each fragment analyzed by BS pyro-sequencing cytosines or group of cytosines for which the methylation level has been determined, have been numbered starting from the 5' part of the amplified sequence. ATG is shown.

Fig S9

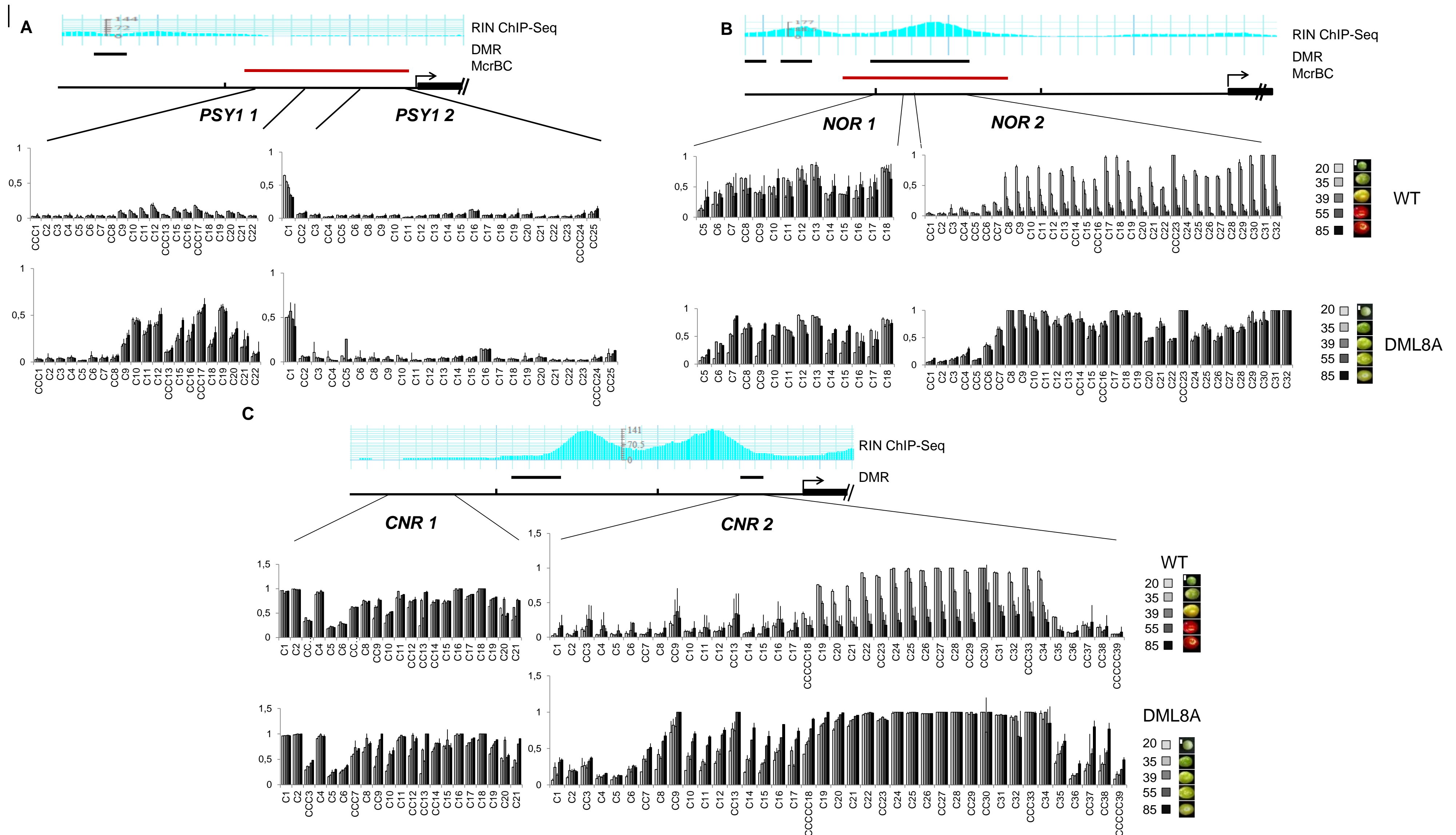

Figure S9: Gene targeted Bisulfite Pyrosequencing Analysis: Methylation levels of the *PSY1* (A), *NOR* (B), and *CNR* (C) promoters at the two PCR fragments shown in Fig. S8. Methylation level at 20, 35, 39 (Br), 55 and 85 dpa is shown in WT fruits (upper panels) and in the transgenic RNAi line DML8A (lower panels) at the same stages. Fruit phenotypes are indicated on the right. DNA sequence is depicted as a black line on which are shown the differentially methylated regions (DMR) as determined using McrBC-PCR (red lines, this study), DMRs identified in (20) (black lines) and the position of the RIN BS identified by RIN-ChIP sequencing (20). Note the higher methylation level of *PSY1* fragment 1 (A; CC8 to C22) at all stages observed in plant DML8A as compared to WT as well as the reduction in methylation at the *NOR* (B) and *CNR* (C) fragment 2 during WT fruit ripening but not in fruits of the DML8A plant .

SI Supplementary Tables

Supplementary Table S1:

Database and Accession numbers		Name	Domain A		Glycosylase domain		Domain B		Total size (aa)
			Position	size(aa)	Position	size(aa)	Position	size(aa)	
SGN	Solyc09g009080.2.1	SlDML1	691-811	121	1151-1366	216	1498-1702	205	1702
	Solyc10g083630.1.1	SlDML2	846-966	121	1267-1482	216	1615-1824	210	1824
	Solyc11g007580.1.1	SlDML3	836-955	120	1279-1494	216	1645-1869	225	1869
	Solyc03g123440.2.1	SlDML4	786-900	115	992-1206	215	1346-1538	193	1538
NCBI	NP_196076.2	AtDME	678-796	119	1190-1405	216	1530-1729	200	1729
	NP_181190.3	AtROS1	508-626	119	857-1072	216	1191-1393	203	1393
	NP_187612.5	AtDML2	477-595	119	789-1004	216	1129-1332	204	1332
	NP_195132.3	AtDML3	331-445	115	500-712	213	841-1044	204	1044

Supplementary Table 1: Tomato and Arabidopsis *DML* genes Accession number of the tomato and Arabidopsis Demeter like cDNA is indicated together with the size of the corresponding proteins, and the position of the three conserved domains characteristic of the DNA Glysosylase-Lyase[23].

Supplementary Table S2: list of primers

Supplementary Table 2		
Primers for qRT-PCR		
NCBI Accession		
AK326269.1	EF1alpha F	GCTGTCGGTGTTGTCAAGAAT
	EF1 alpha R	CATCACACTGCACAGTTCACT
XP_006341256.1	SlDML1 F	GGGCTGAACAAGCTAACAACA
	SlDML1 R	TGACCACCCTAAGTATCAGCTACA
XP_004249459.1	SlDML2 F	AGTACTCATGCCAAAGCCAAA
	SlDML2 R	CCTATCTTCTTTTTACCGACTGGA
XP_004250000.1	SlDML3 F	GCAGAATTGAAGTCACCCTTG
	SlDML3 R	GATGGCTCAGTTTGTGAGCA
XP_004236376.1	SlDML4 F	GAGCGAGTGTGGGAACAAC
	SlDML4 R	ATGCGGGCAATGAATGAGTC
NM_001247741.1	Rin F	AACATCATGGCATTGTGGTG
	Rin R	GTGTTGATGGTGCTGCATTT
	Psy1 F	ATCTTTGGTCTTGTACCGCAAA
KC767847.1	Psy1 R	GGCAGTTTTTGTAGGAGGCACA
NM_001247249.1	NOR F	AGAGAACGATGCATGGAGGTTTGT
	NOR R	ACTGGCTCAGGAAATTGGCAATGG
XM_004232854.1	CNR F	GCCAAATCAAGCAATGATGA
	CNR R	TCGCAACCATACAGACCATT
Primers for RNAi construction		
	DMLENTRS	CACCGTATAGCTGTTAGAC
	DMLENTRAS	GAACATGCGTTGCAGTTG
Primers for McrBC analysis of promoter fragments		

Solgene accession		
Solyc02g077920	CNR F	TGAGCATCAACCACTCCTAATA
	CNR R	CAGACTTAGTAATAACTCCGAT
Solyc03g123630.2.1	PM F	AAACTAGACCATGAGTGTTGAGA
	PM R	TTTTAGAGTGAATTACAGAGAAGC
Solyc03g031860	PSY1 F	TTGTTATATACAGGGGGTGGAGTT
	PSY1 R	TAGAGTGGACACAGACCATAGCTC
Solyc10g006880	NOR F	CAAAAATTGTCCAAGTTAGGGCTAC
	NOR R	GTGGAGGTCGTCATTAGCATAAAT
Solyc05g012020	RIN F	GTAGAATTTGGGGAAGAAACGTC
	RIN R	TATCAATAGTCACATCCCCTTGTG
Primers for bisulfite sequencing analysis		
PCR primers	Sl_NOR_F1	GATTGGTYATGTGAAGGATATGTG
	Sl_NOR_R1	Biotin-CTCRRATARARACAACAAATACRAC
	Sl_NOR_F2	ATYGATAGAGAYAAGTTGTTGTAAA
	Sl_NOR_R2	Biotin-ACRCARAACTACTTTATCCTRCACA
	Sl_PSY1_F1	ATTGTTGAAAGAGAGGGTGGAA
	Sl_PSY1_R1	Biotin-CAAAARCCAARTRCTCAATTCCTA
	Sl_PSY1_F2	GGGTTGTYTATAATGYAGGTTATGG
	Sl_PSY1_R2	Biotin-CCTCRTTCRARTACAACATATCAAA
	Sl_CNR_F1	AAGATAGAGAGGATGATGATGATTAA
	Sl_CNR_R1	Biotin-TCTTCCAATATCTCTRTARCARCTT
	Sl_CNR_F2	TGATGGATTTAGGTAGATGAATTAG
	Sl_CNR_R2	Biotin-TTRCTCACTCTTTTCCTCTARCTT
	Sl_NOR_pyro1F1	TGAAGGATATGTGTTGAAA
Pyrosequencing primers	Sl_NOR_pyro1F3	YAATGAAAGAATATTATAAA
	Sl_NOR_pyro2F1	GAGAYAAGTTGTTGTAAAAA
	Sl_NOR_pyro2F2	TYAAAYTTYTGTTGTAAAAT
	Sl_NOR_pyro2F3	GTTTYYTYTTTAATTAAATG
	Sl_PSY1_pyro1F1	AATTTGTGTAAGTTTTGTTT
	Sl_PSY1_pyro1F2	GCGGAATAGAGAATGTG
	Sl_PSY1_pyro2F1	AATGYAGGTTATGGTTTT
	Sl_PSY1_pyro2F2	GTTGATTGTGTTTAGATTAT
	Sl_CNR_pyro1F1	GATGATTAATTTTTGTTG
	Sl_CNR_pyro1F2	AGYTGAAGGTGAGAT
	Sl_CNR_pyro2F1	ATTTAGGTAGATGAATTAGA
	Sl_CNR_pyro2F3	AGTGYAAATTATAGTTTAGT
	Sl_CNR_pyro2F4	ATYAYAAGTGAAAAAGAGTA

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