



Supplementary Information for
CHROMOMETHYLTRANSFERASE3/KRYPTONITE maintain the
sulfurea paramutation in *Solanum lycopersicum*

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SI References

Other supplementary materials for this manuscript include the following:

Supplementary files S1 to S3

Material and Methods

Plant methods. Plants were germinated and grown in *F2* compost and transferred to *John Innes 2* compost three weeks after germination. Plants were grown in 16h light (22°C) and 8h dark (18°C) cycles with 70% humidity and light intensity 300 $\mu\text{mol l}^{-1} \times \text{s}^{-1}$ PAR. Emasculation and pollination were carried out following the Tomato Genetics Resource Centre guidelines - https://tgrc.ucdavis.edu/Guidelines_Emasculating_and_Pollinating_Tomatoes.pdf. The plant lines used for this study are summarised in **Table S2**. For molecular analyses leaves were excised from 4-week-old plants using sharp tweezers. In the case of *sulf* plants, leaves with higher degree of chlorosis were selected for each individual. Tissue was flash frozen in liquid nitrogen.

Genotyping. Genomic DNA was isolated from 100mg of leaf tissue from 4-week-old plants using the Dneasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. For all genotyping reactions genomic DNA was diluted to a final concentration of 10ng/ μL in nuclease free water. *cmt3* and *kyp* mutants were genotyped using oligonucleotides as described in (1) and also listed in **Table S3**. The mutant *cmt3* allele was amplified using Phusion High-Fidelity DNA Polymerase (Thermo Scientific). The WT CMT3 alleles and KYP WT and mutant alleles were amplified with DreamTaq DNA Polymerase (Thermo Scientific). *nrpe1* mutants were genotyped with the oligonucleotides listed in **Table S3** followed by T7 endonuclease digestion. Two digestion reactions were setup per sample: one with 10 μL of sample PCR reaction alone and the other with 5 μL of sample PCR reaction mixed together with 5 μL of a WT cv. M82 PCR reaction to allow identification of *nrpe1* homozygotes upon T7 digestion. 1.5 μL Buffer 2 (New England Biolabs) and 1.5 μL of nuclease free water were added to each reaction and denatured for 5 min at 95°C, followed by annealing with a 95°C – 85°C ramp with 2°C/sec increments and a ramp 85°C – 25°C with 0.1°C/sec increments. 2 μL of T7 endonuclease (New England Biolabs) diluted to 2U/ μL in 1X Buffer 2 (New England Biolabs) were added to each reaction and incubated at 37°C for 1 hour. The fragments were separated in a 1.5% agarose gel immediately after digestion. All genotyping PCR conditions are detailed in **Table S4**.

Tomato CHG subcontext analysis. Whole-genome bisulphite sequencing data for duplicates of *TAB2⁺* and *TAB2^{sulf}* epigenotypes were retrieved from BioProject SRP066362 (2). Paired reads were trimmed with Trim Galore! v0.4.4 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and aligned to *Solanum lycopersicum* assembly Heinz 1706 version 3.00 with Bismark v0.19.1 (3). Following deduplication and methylation report generation with Bismark, differentially methylated regions were defined using HOME v1.1 (4), filtering for at least 20% difference in absolute methylation levels between the *TAB2⁺* and *TAB2^{sulf}* plants, and at least 5 cytosines in the DMR. Extracted data was used for subcontext analysis using an in-house R script (**Supplemental file S1**). The output was parsed and plotted using the scripts available at <https://github.com/clauidiamartinho/Martinhoetal2021>.

McrBC-qPCR. Genomic DNA was isolated from 100mg of leaf tissue using Dneasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The DNA samples used for McrBC digestion were the same as the samples using for genotyping. To determine the proportion of DNA methylation at DMR1 McrBC digestion were carried out followed by quantitative real time PCR (qPCR). McrBC digestion was performed as described in (5). 10 μL qPCR reactions were assembled using 4 μg of digested or undigested genomic DNA and 1X Luna® Universal qPCR Master Mix (New England Biolabs). qPCR was carried out with one denaturation step at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 10 sec annealing at 60°C for 20 sec, and primer extension at 72°C for 30 sec. Upon completion of the cycling steps, a final extension at 72°C for 5 min on a CFX384 system (Bio-Rad) was performed. The sequences of oligonucleotides used for McrBC-qPCR are listed in **Table S3**. DNA methylation proportion was calculated using the formula $100 \times \{1 - 2^{(\text{Ct}_{\text{undigested}} - \text{Ct}_{\text{digested}})}\}$. Negative methylation values were converted to 0% DNA methylation as they represent no loss of amplification and therefore no methylation.

Expression analysis. Total RNA isolation was performed with the Direct-zol RNA Miniprep (Zymo Research) and Trizol reagent (Invitrogen) according to the manufacturer's instructions. For quantitative RT-PCR analyses 1 μg of total RNA was reverse transcribed using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions with random hexamer

primers. Quantitative PCR was performed in reactions containing 1X Luna® Universal qPCR Master Mix (New England Biolabs) on a CFX384 system (Bio-Rad). qPCR was carried out denaturation step at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 10 sec, annealing/extension at 60°C for 30 sec. Relative expression was calculated using the $\Delta\Delta Ct$ method ($2^{-\Delta\Delta Ct}$) using the geometric mean of two reference genes (**Table S3**). Oligonucleotides used for expression analyses are listed in **Table S3**.

sRNA-seq. Total RNA isolation was performed with the Direct-zol RNA Miniprep (Zymo Research) and Trizol reagent (Invitrogen) according to the manufacturer's instructions in leaf tissue of 4-week-old plants. For M82/*sulf* and F2 CMT3 sets, sRNA libraries were prepared using the NEBNext multiplex small RNA library prep kit (New England Biolabs) according to the manufacturer's instructions. Libraries were indexed during the PCR step with 12 cycles and size-selected using BluePippin. Pooled libraries were sequenced on a NextSeq500 (Illumina). NRPE1 library preparation and sequencing were outsourced to Novogene. Raw data (already demultiplexed and available in fastq format) was processed using the Snakemake pipeline available at https://github.com/seb-mueller/snakemake_sRNAseq. Briefly, raw data was quality controlled using FastQC (v0.11.7) followed by 3' adaptor removal (trimming) using cutadapt removing Illumina universal adapters. All sequences <15 nt and >40 nt in length were discarded, and the remaining sequences mapped to the reference genome (Heinz 1706 genome version 3.0). Mapping was performed using Bowtie version 1.2 with uniquely mapping with "bowtie --wrapper basic-0 -v 0 -k 1 -m 1 --best -q" which only reports sRNAs mapping to unique locations. 0 mismatches were employed using bowtie version 1.2. DMR1 sRNA quantity was normalized as count per million (CPM) basing it on the total number of reads mapped at DMR1 (please see coordinates in Table S1) using deeptools version 3.3.1. The config.yaml file used for this analysis is supplied as **Supplemental file S2**.

Whole genome bisulphite sequencing. Genomic DNA was isolated from 100 mg leaf tissue 4-week-old plants using Dneasy Plant Mini Kit (Qiagen). Library preparation and sequencing were carried out by Novogene. In brief, DNA samples were fragmented into 200-400bp using Covaris S220. Terminal repairing, A-ligation, methylation sequencing adapters ligation were performed to the DNA fragments. Bisulphite treatment was carried out with Accel-NGS Methyl-Seq DNA Library Kit (Illumina Cat No. 30096) followed by size selection and PCR amplification steps. Whole genome bisulphite processing raw data (paired-end; already demultiplexed and available in fastq format) was processed using the bisulfite Snakemake pipeline available at <https://github.com/seb-mueller/snakemake-bisulfite>. Briefly, raw data was quality controlled using FastQC (v0.11.7) followed by 3' adaptor removal (trimming) using trim_galore (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) removing Illumina universal adapters. Mapping and cytosine methylation calling was done using Bismark (3) on the reference genome (Heinz 1706 genome version 3.00) in CpG, CHG and CHH contexts. The config.yaml file used for this analysis is supplied as **Supplemental file S3**.

ChIP-qPCR. Chromatin extraction was carried out as described in (1) using 1g of leaf tissue of 4 week-old plants. Chromatin was fragmented between 200-600bp in a Covaris E220 evolution (duty cycle: 20%, peak intensity: 140, cycles of burst: 200, time: 3 min). Immunoprecipitation was carried out as described in (1) with Anti-Histone H3 (di methyl K9) antibody - ChIP Grade (abcam, ab12220) and Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (abcam, ab8580). The material was reverse cross-linked by adding NaCl to a final concentration of 200mM and incubating at 65°C O/N. This was followed by a 30 min treatment with 1µL RNase A (Thermo Scientific) at 37°C and a 90 min treatment with 1.5 µl Proteinase K (Thermo Scientific) at 65°C. DNA was purified using the MinElute Kit (Qiagen) according to manufacturer's instructions and eluted in 35µl EB buffer. The eluted DNA was used to quantify enriched DNA fragments by standard qPCR methods using 1X Luna® Universal qPCR Master Mix (New England Biolabs) on a CFX384 system (Bio-Rad). Enrichment of DNA fragments for H3K9me2 and H3K4me3 analysis were calculated as % input ($2^{(Ct \text{ input adjusted} - Ct \text{ IP})} \times 100$) and normalised using the enrichment found for an unrelated reference locus *CAC3*. Oligonucleotides employed in this analysis are listed in **Table S3**.

Maize CHG subcontext analysis. For bisulphite sequencing, genomic maize DNA was extracted (6) from pools of 30-100 embryos or half of an ear. Bisulphite treatment was performed using 400ng of

DNA and the EZ DNA Methylation-Gold kit (Zymo Research, D5006). The DNA regions of interest were PCR amplified (10 min 95°C, followed by 40 PCR cycles (30 sec 95°C, 30 sec appropriate annealing temp, 30 sec 72°C), and 5 min at 72°C). The PCR was performed using MethylTaq DNA polymerase (Diagenode, C09010010). For primer sequences, amplicon sizes and annealing temperatures see **Table S5**. In order to monitor for complete bisulphite conversion, a conversion control (Fie2 fragment) was amplified and analysed in each experiment (**Fig S13**) (similar to the -302 to -91 fragment described in (7)). PCR fragments were ligated into the pJET 1.2 vector (CloneJet PCR Cloning Kit, Thermo Scientific) according to manufacturer's instructions. Positive Clones were identified by colony-PCR, Plasmid DNA was isolated from positive clones (GeneJet Plasmid Miniprep Kit, Thermo scientific) and subjected to Sanger sequencing. For the conversion control 14-16 clones were analysed and for each other fragment 22-31 clones were analysed. Frequency of DNA methylation at individual CHG motifs was inferred using Kismeth (8).

Hi-C. 0.5g leaf tissue of 4-week-old plants (per replicate) was fixed in 1% formaldehyde (v/v) and 0.01% Triton-X (v/v) for 20 min using vacuum infiltration. The reaction was quenched by adding glycine to a final concentration of 125 mM for 10 min using vacuum infiltration. The leaf tissue was rinsed 3 times with MilliQ water, pat dried and flash frozen in liquid Nitrogen. The *in situ* Hi-C library preparation was performed essentially as described in (9). For individual samples in each replicate was homogenized for preparing libraries. The libraries were sequenced on an Illumina NextSeq500 instrument with 2 x 75 bp reads. The Hi-C raw reads were mapped to *Solanum lycopersicum* genome Heinz 1706 assembly SL3.00 with an iterative mapping pipeline (9). The removal of PCR duplicates and reads filtering were performed as described in (9). Hi-C reads of each sample are summarised in **Table S6**. Hi-C map normalization with bin size 100kb was done using the “HiTC” package in R (10).

Data visualisation and statistical analysis

Plots for ChIP-seq, sRNA-seq and subcontext analysis were carried out using ggplot2 (11) and computationally reproducible scripts are available at <https://github.com/clauidiamartinho/Martinhoetal2021>. Plots depicting DNA methylation levels determined by McRBC and expression levels were generated by employing the webtool PlotsOfData (12). Statistical tests were carried out in R using the function *wilcox.test* and *p.adjust.method = "BH"*. Genome browser images were generated in IGV v2.7.2 (13).

Data availability

All sequencing datasets, including sRNA, bisulphite and Hi-C were deposited at ArrayExpress (EMBL-EBI) under the accession numbers: E-MTAB-10556, E-MTAB-10557, E-MTAB-10565, E-MTAB-10568 and E-MTAB-10574.

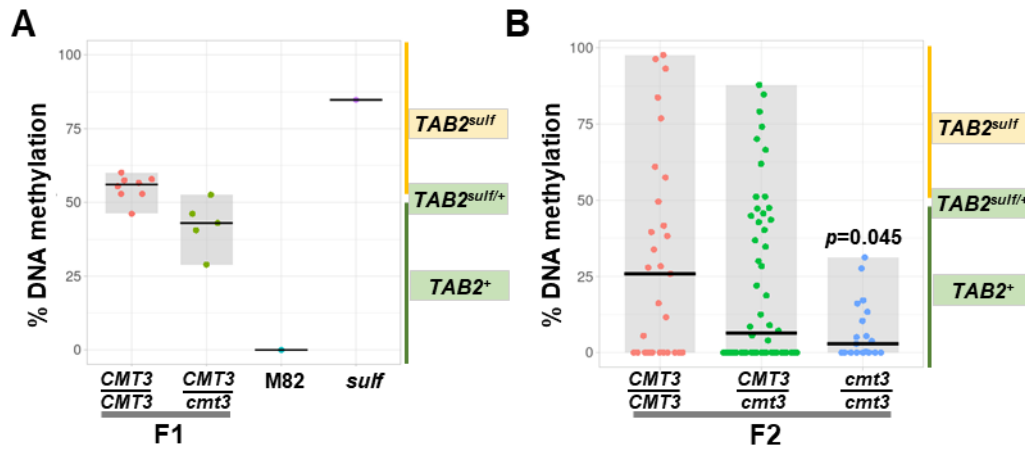


Fig. S1. – DMR1 DNA methylation is reduced in *cmt3* mutants. Jittered dots depict DNA Methylation percentage at DMR1 in individual plants determined by McrBC-qPCR. The summary of the data is shown as horizontal line indicating the median. Grey boxes illustrate the data range. Plants result from the cross between *CMT3/CMT3 TAB2^{sulf}* and *CMT3/cmt3 TAB2⁺* (Fig 1C). **A–** F1 sibling plants: *CMT3/CMT3* $n=8$; *CMT3/cmt3* $n=5$; controls: M82- (*S. lycopersicum* cv. M82) *CMT3/CMT3 TAB2⁺* $n=1$; *sulf* - (*S. lycopersicum* cv. Lukullus) *CMT3/CMT3 TAB2^{sulf}* $n=1$. **B–** F2 sibling plants: *CMT3/CMT3* $n=29$; *CMT3/cmt3* $n=52$; *cmt3/cmt3* $n=19$. p -value *cmt3/cmt3* versus *CMT3/CMT3* was calculated employing a Mann-Whitney-Wilcoxon test. Yellow boxes refer to plants displaying *sulf* chlorosis and green boxes refer to green plants.

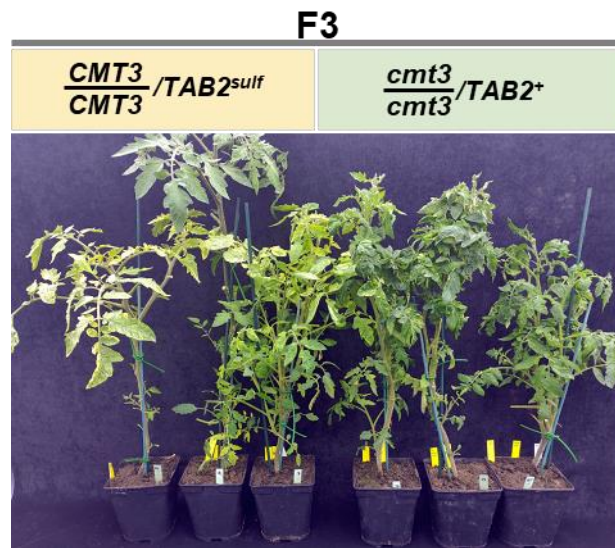


Fig. S2. CMT3 suppresses *sulf* chlorosis. 2-month-old F3 sibling plants $CMT3/CMT3\ TAB2^{sulf}$ and $cmt3/cmt3\ TAB2^{+}$. Yellow boxes refer to plants displaying *sulf* chlorosis and green boxes refer to green plants.

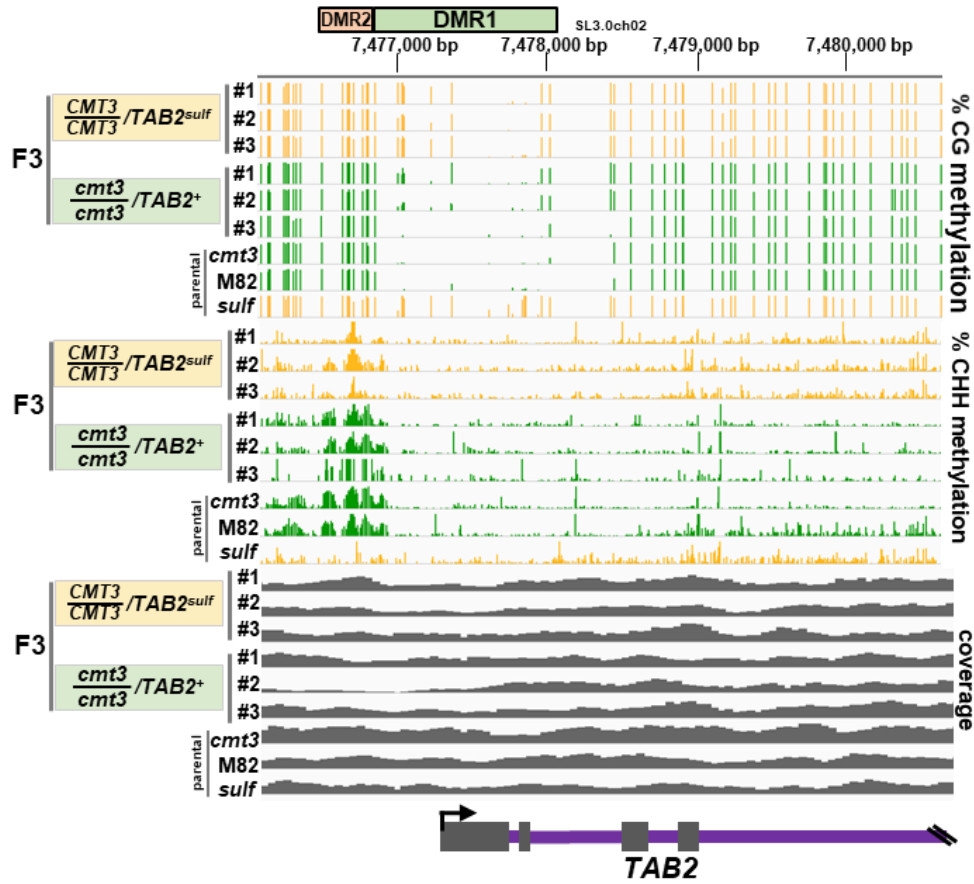


Fig. S3. – CG and CHH DNA methylation levels at DMR1 in F3 plants (F3 Fig. 1). *TAB2* IVG screenshot bisulphite sequencing data, % CG DNA methylation, range [0-100]. % CHH DNA methylation, range [0-100]. Green tracks refer to green leaf phenotype and yellow tracks refer to plants which display chlorosis. Sequencing coverage range [0-65]. Yellow tracks and boxes refer to plants displaying *sulf* chlorosis and green tracks and boxes refer to green plants.

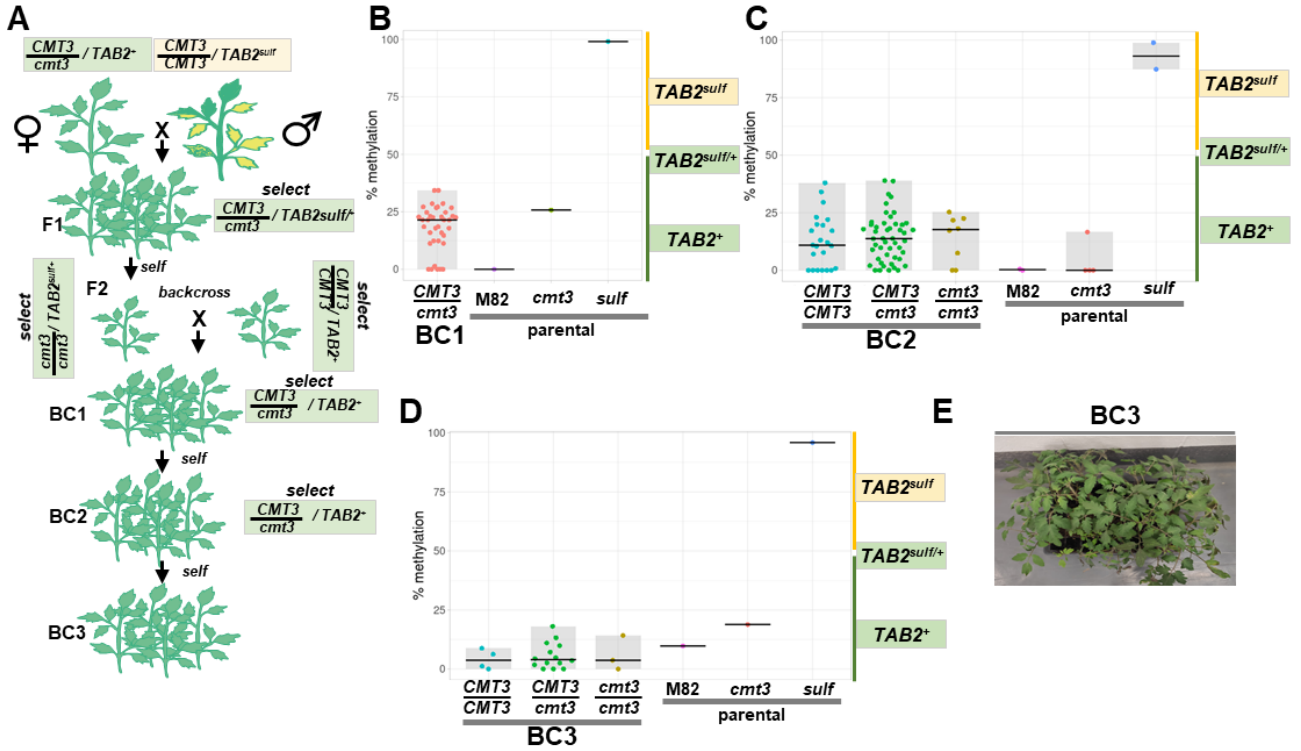


Fig. S4. CMT3 maintains *sulf* memory. **A**– Diagram illustrates crossing scheme used to obtain backcrossed populations. **B–D** Jittered dots depict % of DNA methylation at DMR1 in individual plants determined by MspI digestion followed by qPCR. The summary of the data is shown as horizontal line indicating the median. Grey boxes illustrate the data range. **B**– Plants denote first backcross generation (BC1 - Fig. S4A) $n=21$; Control plants: M82- (*S. lycopersicum* cv. M82) $CMT3/CMT3 TAB2^+ n=1$; *sulf*- (*S. lycopersicum* cv. Lukullus) $CMT3/CMT3 TAB2^{sulf} n=1$; F2 parent - $cmt3/cmt3 TAB2^+ n=1$. **C**– Second backcross generation sampling (BC2 – Fig. S4A). BC2 plants: $CMT3/CMT3 TAB2^+ n=23$; $CMT3/cmt3 TAB2^+ n=48$; $cmt3/cmt3 TAB2^+ n=8$. Control plants (these samples were processed in different batches and one control plant was added per batch). M82- (*S. lycopersicum* cv. M82) $CMT3/CMT3 TAB2^+ n=2$; *sulf*- (*S. lycopersicum* cv. Lukullus) $CMT3/CMT3 TAB2^{sulf} n=2$; $cmt3-cmt3/cmt3 TAB2^+ n=4$. **D**–Third backcross generation sampling (BC3 – Fig. S4A). BC3 plants: $CMT3/CMT3 TAB2^+ n=3$; $CMT3/cmt3 TAB2^+ n=14$; $cmt3/cmt3 TAB2^+ n=3$. Control plants: M82- (*S. lycopersicum* cv. M82) $CMT3/CMT3 TAB2^+ n=1$; *sulf*- (*S. lycopersicum* cv. Lukullus) $CMT3/CMT3 TAB2^{sulf} n=1$; $cmt3-cmt3/cmt3 TAB2^+ n=1$. **F**– BC3 population consists of 100% green plants – 4-week-old plants. Yellow boxes refer to plants displaying *sulf* chlorosis and green boxes refer to green plants.

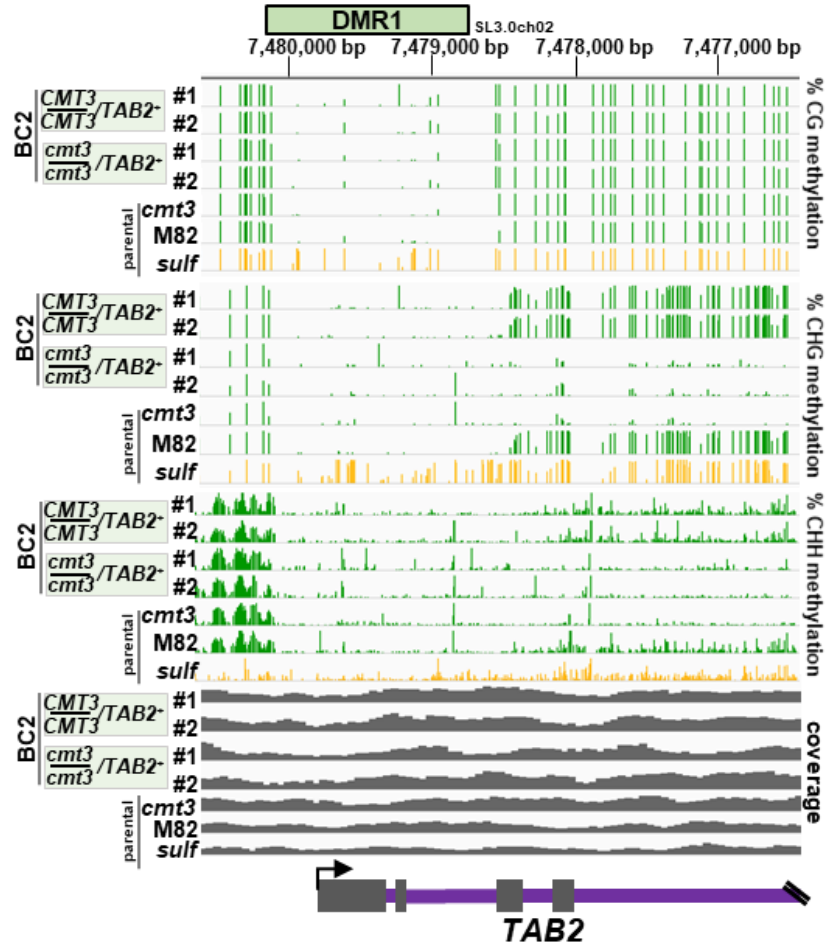


Fig. S5. Backcrossed populations don't recover DMR1 DNA methylation. CG and CHH DNA methylation levels at DMR1 in backcrossed plants – generation 2 (BC2) plants (Fig. S4A). *TAB2* IGV screenshot bisulphite sequencing data, % CG DNA methylation, range [0-100]. % CHH DNA methylation, range [0-100]. Green tracks refer to green leaf phenotype and yellow tracks refer to plants which display chlorosis. Sequencing coverage range [0-72]. Yellow tracks refer to plants displaying *sulf* chlorosis and green boxes and tracks refer to green plants.

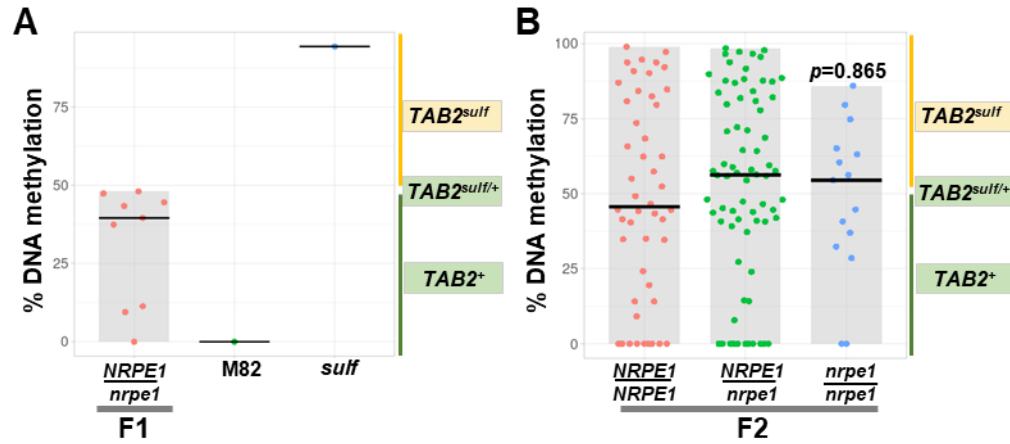


Fig. S6. DMR1 DNA methylation is unaffected in *nrpe1* mutants. Jittered dots depict DNA methylation percentage at DMR1 in individual plants determined by M_{cr}BC-qPCR. The summary of the data is shown as horizontal line indicating the median. Grey boxes illustrate the overall data range. Plants are derived from crosses between *NRPE1/NRPE1 TAB2^{sulf}* and *nrpe1/nrpe1 TAB2⁺* (Fig. 2A). **A**— F1 sibling plants: *NRPE1/nrpe1* *n*=9; controls: M82- (*S. lycopersicum* cv. M82) *CMT3/CMT3 TAB2⁺* *n*=1; *sulf* - (*S. lycopersicum* cv. Lukullus) *CMT3/CMT3 TAB2^{sulf}* *n*=1. **B**— F2 sibling plants: *NRPE1/NRPE1* *n*= 48; *NRPE1/nrpe1* *n*=72; *nrpe1/nrpe1* *n*=15. *p*-value *nrpe1/nrpe1* versus *NRPE1/NRPE1* was calculated by employing a Mann-Whitney-Wilcoxon test. Yellow boxes refer to plants displaying *sulf* chlorosis and green boxes refer to green plants.

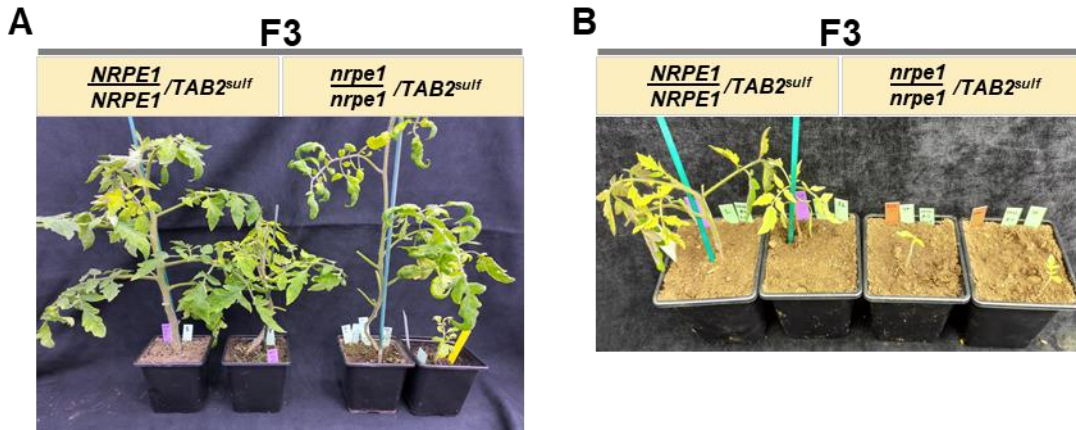


Fig. S7. *Sulf* phenotype is maintained in the *nrpe1* background. F3 plants derived from crossing *NRPE1/NRPE1 TAB2^{sulf}* and *nrpe1/nrpe1 TAB2⁺* (Fig. 2A). **A**– 6-week-old. **B**– 4-week-old. Yellow boxes refer to plants displaying *sulf* chlorosis.

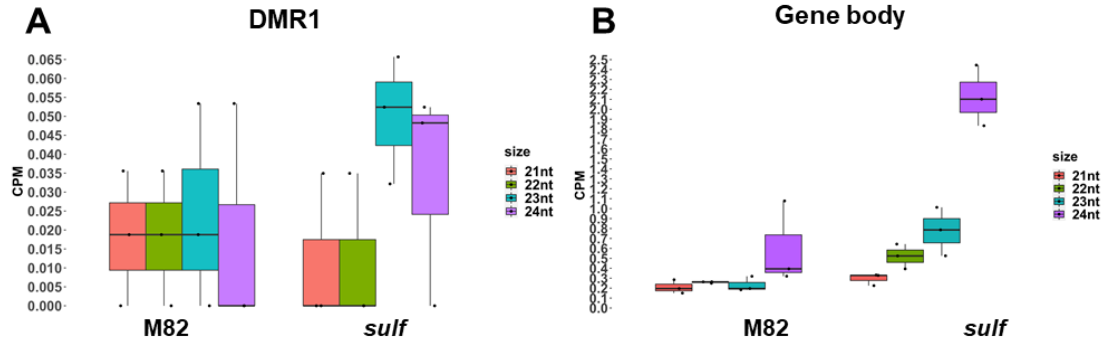


Fig. S8. sRNA accumulation in *sulf* at TAB2. sRNA accumulation in count per million (CPM). Jitter dots represent biological replicates $n=3$. The summary of the data is shown as horizontal line indicating the median. Error bars represent standard deviation. **M82–** (*S. lycopersicum* cv. *M82*) $TAB2^+$ $n=3$; ***sulf*–** (*S. lycopersicum* cv. *Lukullus*) $TAB2^{sulf}$ $n=3$. **A–** DMR1. **B–** Gene body. Coordinates are provided in Table S1.

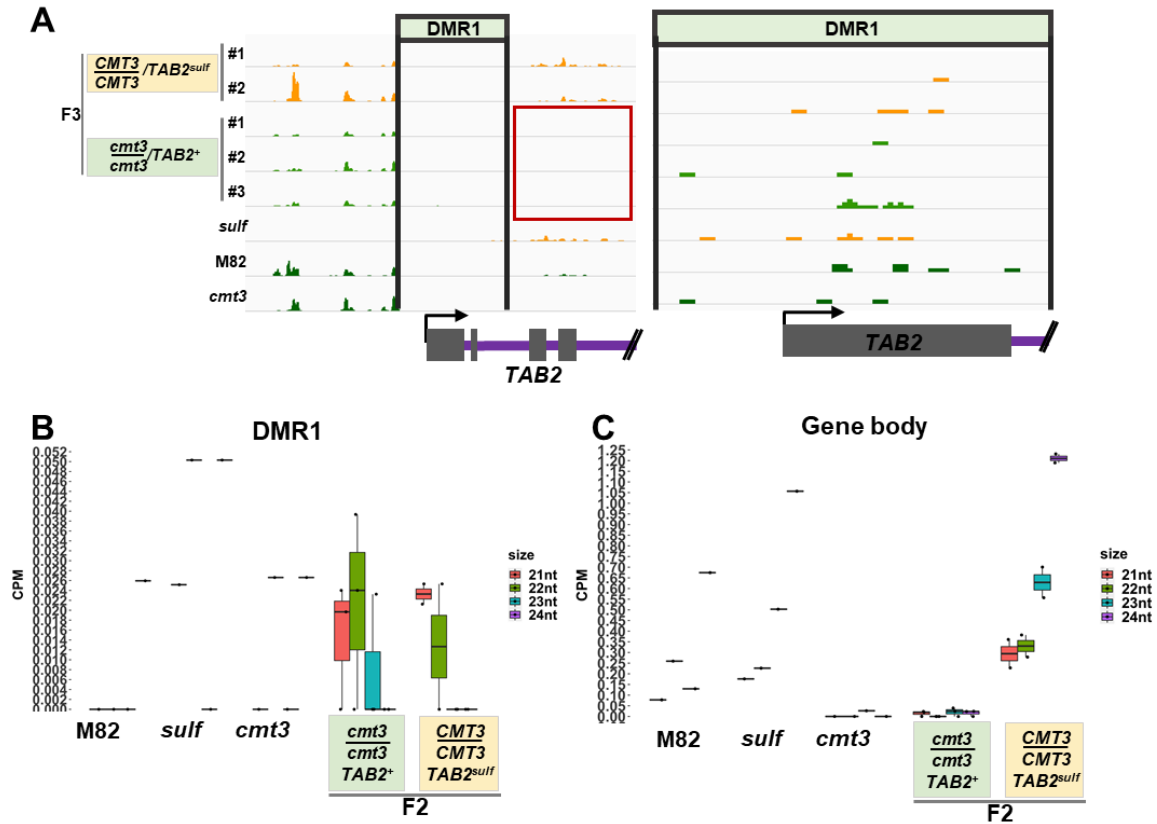


Fig. S9. sRNA accumulation at *TAB2* in *cmt3* and *CMT3* F2 plants (Fig. 1C and Fig. S1 B). **A**– *TAB2* IGV screenshot small-RNA sequencing data. Left panel – sRNA accumulation in DMR1 and *TAB2* gene body, range [0-8.53]; Right panel – zoom in DMR1 region, range [0-8.53]. Red box highlights absence of sRNA accumulation in the gene body region in the *cmt3* background. **B** and **C**– Boxplots depict siRNA accumulation in counts per million (CPM). Jitter dots represent biological replicates $n=3$. The summary of the data is shown as horizontal line indicating the median. Error bars represent standard deviation. **B**– DMR1. **C**– Gene body. **A-C**: controls: **M82**– (*S. lycopersicum* cv. *M82*) *TAB2*⁺ $n=3$; **sulf**– (*S. lycopersicum* cv. *Lukullus*) *TAB2*^{sulf} $n=3$; **cmt3**– control *cmt3/cmt3* *TAB2*⁺ (*S. lycopersicum* cv. *M82*) F3 *CMT3/CMT3* *TAB2*⁺ $n=2$; F2 plants: *cmt3/cmt3* *TAB2*⁺ $n=3$, *CMT3/CMT3* *TAB2*^{sulf} $n=2$. Yellow boxes refer to plants displaying *sulf* chlorosis and green boxes refer to green plants.

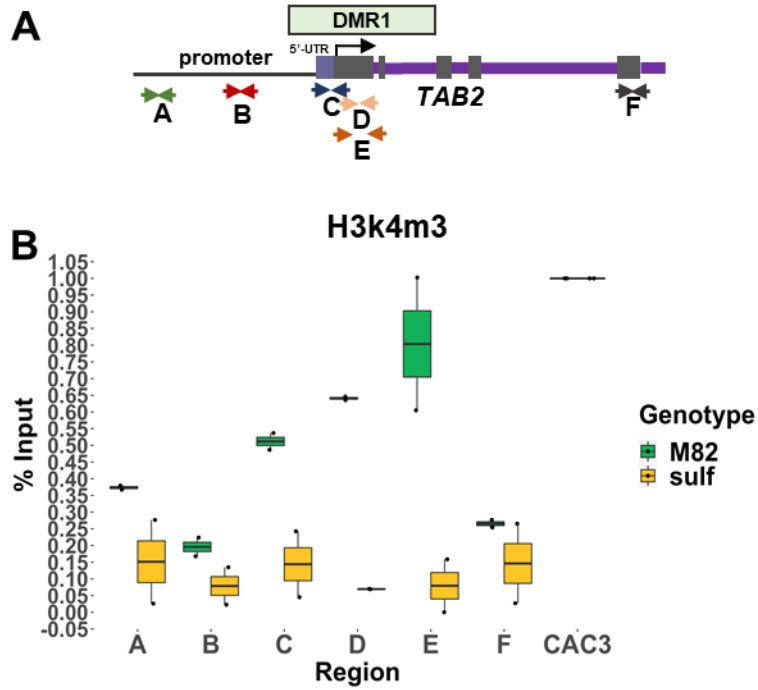


Fig. S10. H3K4me3 levels at *TAB2*. **A**– Diagram represents the relative oligonucleotide position spanning the *TAB2* locus used for ChIP-qPCR experiment in Fig. S10B (sequences are listed in Table S3). **B**– Box plot depicts H3K4me3 enrichment per % input normalised to *CAC3* reference locus determined by ChIP-qPCR. Jittered dots represent different biological replicates. **M82**– *S. lycopersicum* cv. M82 *TAB2*⁺ (green) n= 2; **sulf**– *S. lycopersicum* cv. *Lukullus* *TAB2*^{sulf} (yellow) n=2. The summary of the data is shown as horizontal line indicating the median of biological replicates. Error bars represent the standard deviation.

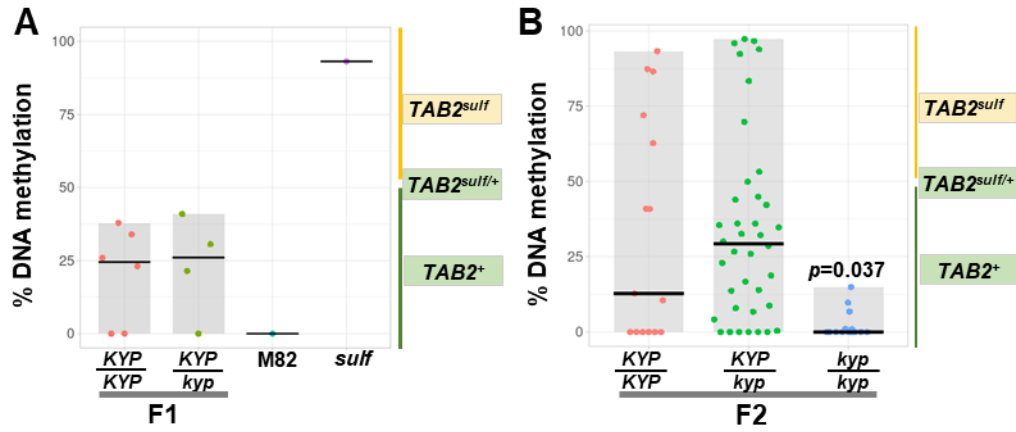


Fig. S11. DMR1 DNA methylation is reduced in *kyp* mutants. Jittered dots depict DMR1 DNA methylation percentage in individual plants determined by McrBC-qPCR. The summary of the data is shown as horizontal line indicating the median. Grey boxes illustrate the data range. Plants result from the cross between *KYP/KYP TAB2^{sulf}* and *KYP/kyp TAB2⁺* (Fig. 3C). **A**— F1 sibling plants: *KYP/KYP* n=8; *KYP/kyp* n=5; controls: **M82**— (*S. lycopersicum* cv. *M82*) *TAB2⁺* n=3; **sulf**— (*S. lycopersicum* cv. *Lukullus*) *TAB2^{sulf}* n=3. **B**— F2 sibling plants: *KYP/KYP* n=15; *KYP/kyp TAB2^{sulf}* n=38; *kyp/kyp TAB2⁺* n=15. p-value *kyp/kyp* versus *KYP/KYP* was calculated employing a Mann-Whitney-Wilcoxon test. Yellow boxes refer to plants displaying *sulf* chlorosis and green boxes refer to green plants.

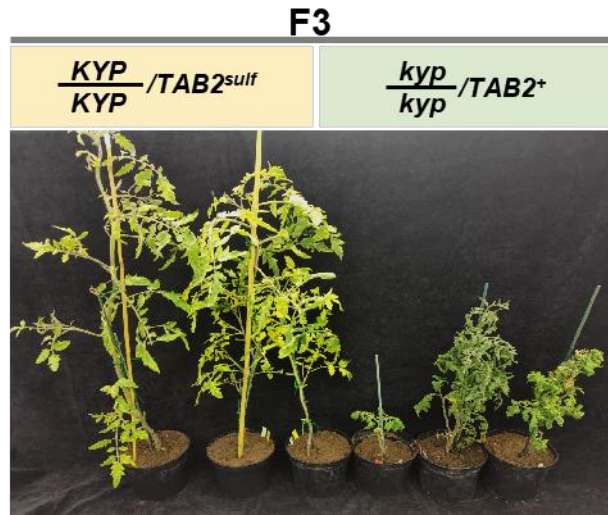


Fig. S12. KYP suppresses *sulf* chlorosis. 2-month-old F3 sibling plants $KYP/KYP\ TAB2^{sulf}$ and $kyp/kyp\ TAB2^{+}$. Yellow boxes refer to plants displaying *sulf* chlorosis and green boxes refer to green plants.

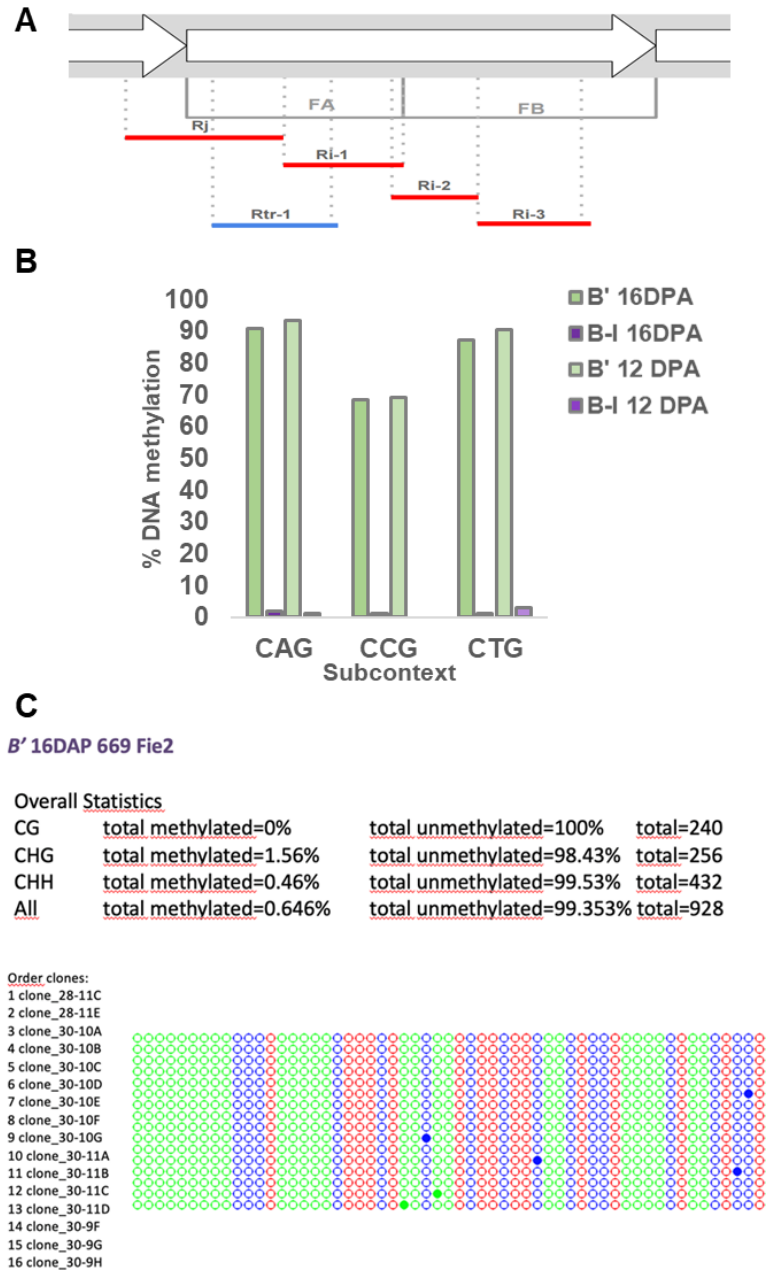


Fig. S13. CHG subcontext analysis at the *b1* locus in maize. **A** – Schematic depiction of one repeat of the *b1* hepta-repeat. One repeat is 853 bp long. For Bisulfite-sequencing fragment Rj (320 bp) was analysed. **B**– Mean percentage of DNA methylation at the different CHG subcontexts at Rj region. n CAG = 7; n CCG = 4; n CTG = 7; **C**– Average and clone-distribution of DNA methylation levels at the unmethylated *Fie2* locus in B' samples – low methylation levels confirm bisulphite conversion was complete.

Name	Chromosome	start	end
Control 1	SL3.0ch02	3606037	3607183
Control 2	SL3.0ch02	32797030	32798176
Control 3	SL3.0ch02	35314142	35315288
Control 4	SL3.0ch02	35727400	35728546
Control 5	SL3.0ch02	44030115	44031261
DMR1	SL3.0ch02	7478826	7480096
Gene body	SL3.0ch02	7477602	7478748

Table S1. DMR1 and Gene body coordinates used for the analyses in this manuscript. Random control regions in SL3.00ch02 were used for subcontext analysis Fig. 1B.

Plant line	Epigenotype	Genotype				Described in
	<i>TAB2</i> (Solyc05g005200)	<i>CMT3</i> (Solyc01g006100)	<i>KYP</i> (Solyc02g094520)	<i>NRPE1</i> (Solyc01g096390)	cultivar	
<i>sulfurea</i> (<i>sulf</i>)	<i>TAB2sulf</i>	WT	WT	WT	Lukullus	Hagemann and Gouil
M82	<i>TAB2+</i>	WT	WT	WT	M82	TGRC - acession LA3475
<i>cmt3</i>	<i>TAB2+</i>	CRISPR deletion	WT	WT	M82	Wang et al
<i>kyp</i>	<i>TAB2+</i>	WT	CRISPR deletion	WT	M82	Wang et al
<i>nrpe1</i>	<i>TAB2+</i>	WT	WT	CRISPR deletion	M82	Gouil et al

Table S2. Plant lines employed in this study.

Number	Name	Gene ID	Sequence 5'-3'	orientation	Description	Purpose
204	204_CMT3_WT	Solyc01g006100	TAGGGTGATGTTGATGTTGTATG	Forward	Genotyping <i>cmt3</i> mutant	Genotyping
205	205_CMT3_MU		TAGGGTGATGTTGATGCGGT	Forward		
206	206_CMT3_R		ATCGCCCAAGGAAACCATTG	Reverse		
201	201_KYP_WT	Solyc02g094520	AAGATTTAGCAGAAACCTGCA	Forward	Genotyping <i>kyp</i> mutant	
202	202_KYP_MU		AAGATTTAGCAGAAACAGAGG	Forward		
203	203_KYP_R		TTGGCTGTTGCAGTTTTCCCTGAG	Reverse		
275	nrpe1 Fw	Solyc01g096390	AGATGAAGACCTTGTTCATCAG	Forward	Genotyping <i>nrpe1</i> mutant	
276	nrpe RV		TGTGATAACATCATTGAGAATTG	Reverse		
23	23_Small_fw	Solyc05g005200	TTTGATGGCTTGTCTAAGCTGC	Forward	DMR1 McrBC	McrBC- qPCR
24	24_Small_rv		AGCTCTCAGGATCAATTTTCAGGG	Reverse		
35	CAC3 fw qPCR	Solyc08g006960	TGTCGTCAAAAGGTAGCGTCCTCCGT	Forward	Reference genes	Expression analysis
36	CAC3 rv qPCR		AGCCCAACTTCAGATCAGGCATTCCA	Reverse		
37	SKP1 FW qPCR	Solyc11g042930	TCAGGGCACCTCTTCGATCTGATCCT	Forward		
38	SKP1 RV qPCR		TGTCTGCCACGGTCTGGCAAGTGA	Reverse		
43	SLTAB2_qPCR_CM1	Solyc05g005200	AAGGAGCCAAGCCACTTCTT	Forward	TAB2 expression	
44	SLTAB2_qPCR_CM1		GCAACTGGACAAAAGCCAC	Reverse		
73	TAB2_Chip-qPCR3_fw	upstream Solyc05g005200	GCCTCACACTCTTCTTTGCC	Forward	Region A	ChIP-qPCR
74	TAB2_Chip-qPCR4_rev		GTTGCCAGCTAGCTCACAAA	Reverse		
232	232TAB2_mnase_fw11	upstream Solyc05g005200	AAAGTGAGAGATGCGAAAGAGAGA	Forward	Region B	
233	233TAB2_mnase_rev12		TTCGTAGCAAACAATTGTCATTCA	Reverse		
238	238TAB2_mnase_fw17	upstream Solyc05g005200	CGATATTTAGGCCGAAGAAAGAG	Forward	Region C	
239	239TAB2_mnase_rev18		ACAAGCCATCAAACCCAAATT	Reverse		
236	236TAB2_mnase_fw15	upstream Solyc05g005200	AATTTGGGTTTGATGGCTTGT	Forward	Region D	
237	237TAB2_mnase_rev16		TTGCAACTGATTTAAAGGGGAAT	Reverse		
23	23_Small_fw		TTTGATGGCTTGTCTAAGCTGC	Forward	Region E	
24	24_Small_rv		AGCTCTCAGGATCAATTTTCAGGG	Reverse		
77	TAB2_Chip-qPCR7_fw		GGAAGCAGCAAAGAAAGCCT	Forward	Region F	
78	TAB2_Chip-qPCR8_rev		AGGAGGTGGCAGGTTTAAACA	Reverse		
79	CAC3_Chip-qPCR1_fw	Solyc08g006960	GATGAGTGACGGAGCCAGTA	Forward	CAC3 used for normalisation	
80	CAC3_Chip-qPCR2_rev		CACAAAAGAGGCCTGCAGAG	Reverse		
207	207_T135_K9control_F	-	CCAGCCATAACAACCAACTTC	Forward	Amplifies T135 transposon elements	
208	208_T135_K9control_R	-	GCAGACCACCAAATCCAATC	Reverse		

Table S3. Oligonucleotides used for tomato experiments – all oligonucleotides were designed using the *Solanum lycopersicum* assembly Heinz 1706 version SL3.00.

WT *CMT3* allele

Component	volume (μL)
Green buffer (10X)	2
dNTPs (10mM each)	0.5
Oligos 204+206 mix (10μM each)	1
DreamTAQ (5U/μL)	0.2
template (10ng/μL)	2
Nuclease free water	14.3
total	20

Cycling conditions
95°C 3min 95°C 30sec, 58°C 20sec, 72°C 30sec (26 cycles) 72°C 5min

mutant *cmt3* allele

Component	volume (μL)
HF buffer (5X)	4
dNTPs (10mM each)	0.5
Oligos 205+206 mix (10μM each)	1
Phusion (2U/μL)	0.2
template (10ng/μL)	2
Nuclease free water	12.3
total	20

Cycling conditions
98°C 30sec 98°C 10sec, 65°C 30sec, 72°C 1min (26 cycles) 72°C 5min

WT *KYP* allele

Component	volume (μL)
Green buffer (10X)	2
dNTPs (10mM each)	0.5
Oligos 201+203 mix (10μM each)	1
DreamTAQ (5U/μL)	0.2
template (10ng/μL)	2
Nuclease free water	14.3
total	20

Cycling conditions
95°C 3min 95°C 30sec, 58°C 20sec, 72°C 30sec (25 cycles) 72°C 5min

mutant *kyp* allele

Component	volume (μL)
Green buffer (10X)	2
dNTPs (10mM each)	0.5
Oligos 202+203 mix (10μM each)	1
DreamTAQ (5U/μL)	0.2
template (10ng/μL)	2
Nuclease free water	14.3
total	20

Cycling conditions
95°C 3min 95°C 30sec, 58°C 20sec, 72°C 30sec (25 cycles) 72°C 5min

***NRPE1* allele**

Component	volume (μL)
HF buffer (5X)	4
dNTPs (10mM each)	0.5
Oligos 275+276 mix (10μM each)	1
Phusion (2U/μL)	0.2
template (10ng/μL)	2
Nuclease free water	12.3
total	20

Cycling conditions
98°C 30sec 98°C 10sec, 60°C 20sec, 72°C 30sec (35 cycles) 72°C 5min

Table S4. PCR conditions used for all genotyping carried out in this study.

Fragment	Name	Primer Sequence ^a	Size (bp) ^b	Tm °C
Rj	KL1310	TGGTGTTTAAAAATTYATGTTTTGTG	320	50
	KL1844	TCCACRARTCATCRTCTCAAACA		
Fie2	KL1426	AAGATTTGAGATTYGATTGAAGTGTG	225	52
	KL1427	CTTCCCCTCCRCCTAATTCTCCTTA		

Table S5. Oligonucleotides used for maize experiments.

Sample	Total reads sequenced	Total mapped reads	Total mapped reads after removing PCR duplicates	Total Hi-C reads after filtering
Replicate 1				
M82	256,921,384	149,308,782	121,182,766	47,391,805
sulf	197,852,756	116,087,535	96,693,269	34,996,574
Replicate 2				
M82	56,174,862	32,892,122	32,181,207	13,128,114
sulf	55,212,666	31,349,383	30,419,079	12,043,402
Replicate 3				
M82	64,995,720	39,275,083	38,441,719	14,651,575
sulf	37,915,680	23,337,027	22,656,587	9,378,235

Table S6. Number of Hi-C reads (statistics on mapping, PCR duplicates, and finally retained true Hi-C reads).

Dataset S1 (separate file). Script used to extract percentage of tomato DNA methylation at specific CHG subcontexts.

Dataset S2 (separate file). Config.yaml file used for sRNA-seq analysis.

Dataset S3 (separate file). Config.yaml file used for bisulphite-seq analysis.

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