New Phytologist Supporting Information

Article title: **Role of H1 and DNA methylation in selective regulation of transposable elements during heat stress**
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The following Supporting Information is available for this article:

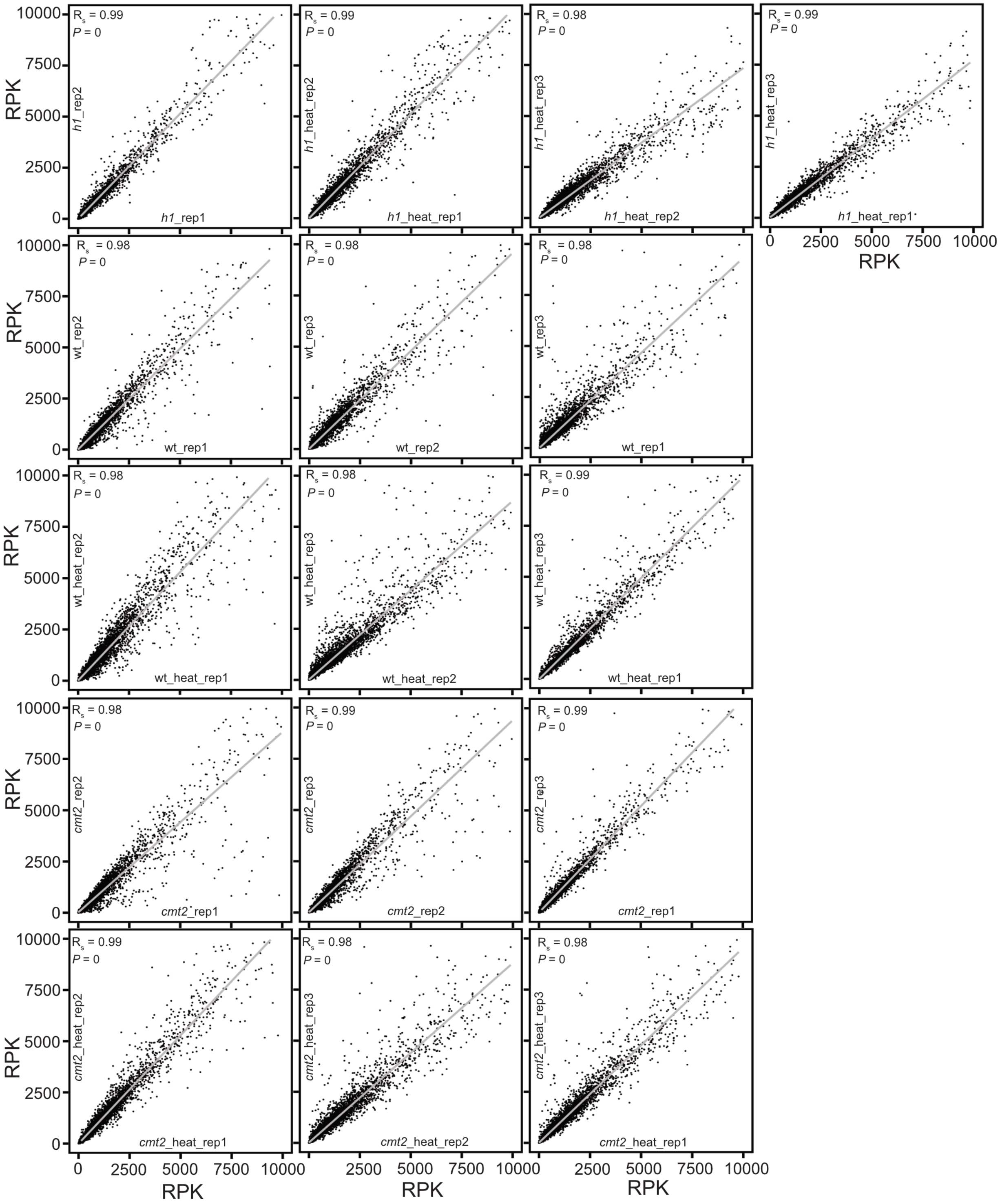

Fig. S1 Correlation analysis of RNA-seq samples. Correlation plots of RPK (reads per kilobase) for genes between replicates (rep1, rep2 and rep3) of RNA-seq samples. R_s represents Spearman correlation coefficient.

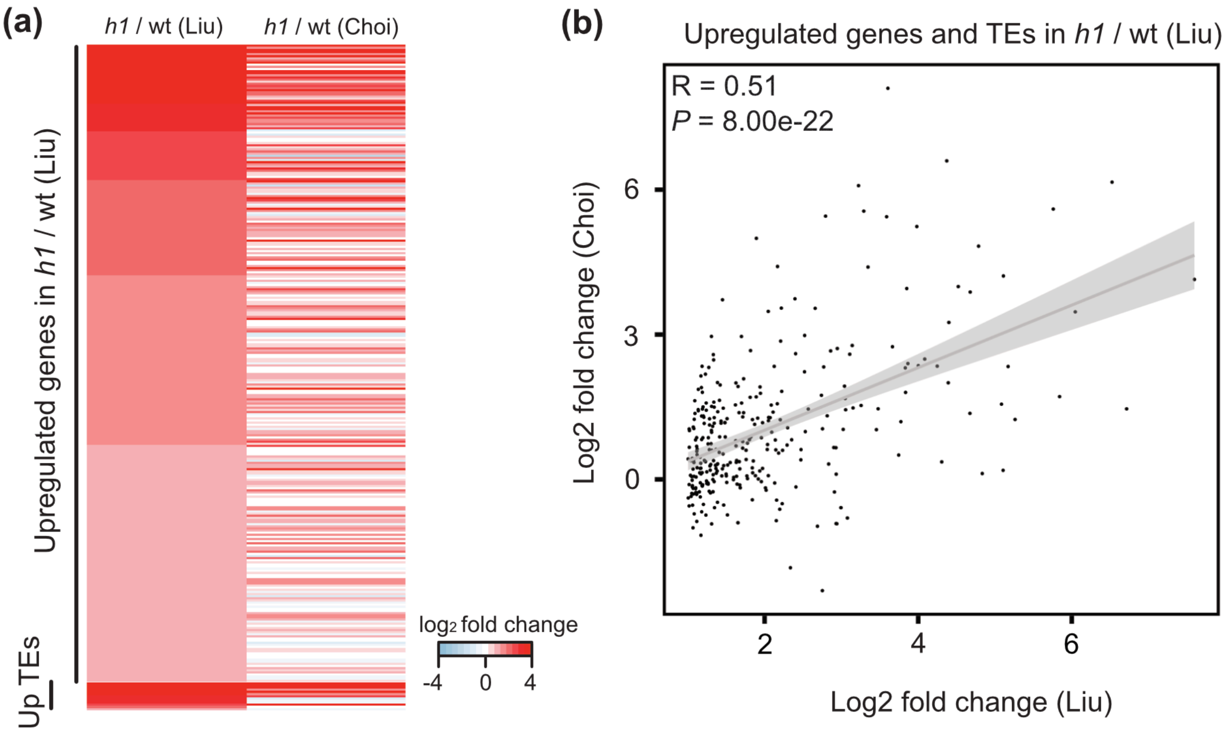

Fig. S2 Comparison of data in this study with previously published data (Choi *et al*., 2020). Heatmap (a) showing the expression changes (log2 fold change) of upregulated genes and TEs (Up TEs) in *h1* versus wt (*h1 / wt*) between our data (Liu) and Choi *et al*., 2020 (Choi). Correlation plot (b) of upregulated genes and TEs in *h1* versus wt between our data (Liu) and Choi *et al*., 2020 (Choi). R represents Pearson correlation coefficient.

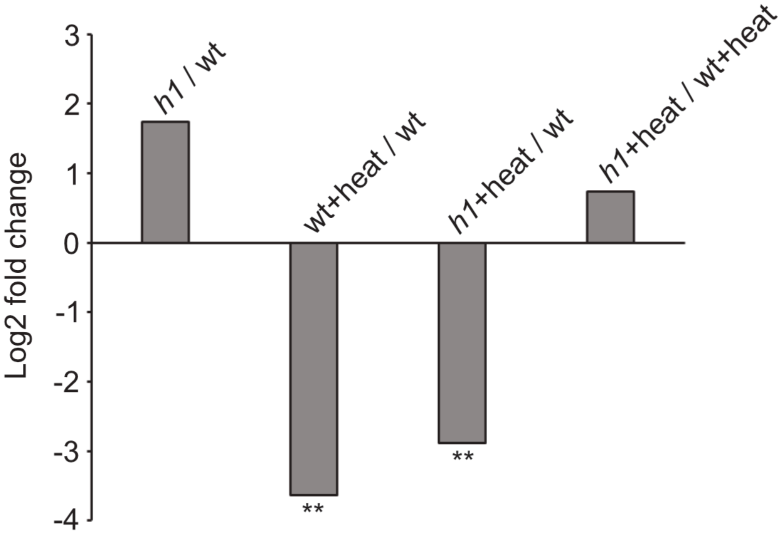

Fig. S3 *H1.3* expression does not change in heat-treated *h1* mutants compared to heat-treated wild type. RNA-seq data showing *H1.3* expression ($\log_2$ fold change) under different conditions, including *h1* versus wild type (*h1* / wt), wt plus heat versus wt (wt+heat / wt), *h1* plus heat versus wt (*h1*+heat / wt) and *h1* plus heat versus wt plus heat (*h1*+heat / wt+heat). **, adjusted *P*-value (padj) < 0.05.

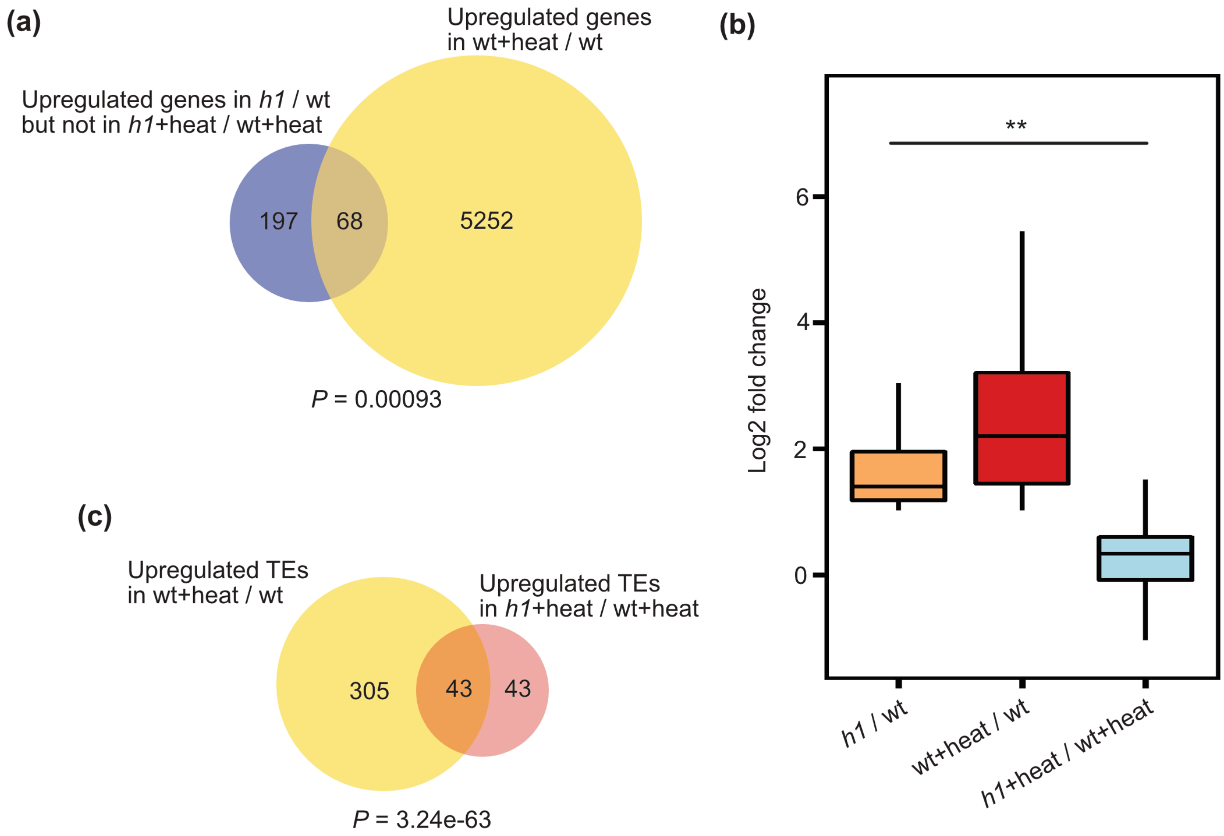

Fig. S4 A substantial proportion of upregulated genes in *h1* mutants and TEs in *h1* upon heat are heat responsive in wild type. Venn diagram (a) shows upregulated genes in *h1* versus wild type (*h1* / wt) and wt plus heat versus wt (wt+heat / wt). Boxplot (b) shows the 68 heat responsive genes (overlapped genes shown in panel (a)) which were upregulated in *h1* versus wt but did not change in *h1* plus heat versus wt plus heat (*h1*+heat / wt+heat). **, $P < 0.01$ (Wilcoxon test). The lower and upper hinges of the boxplots correspond to the first and third quartiles of the data, the black lines within the boxes mark the median. Venn diagram (c) shows upregulated TEs in wt plus heat versus wt and *h1* plus heat versus wt plus heat.

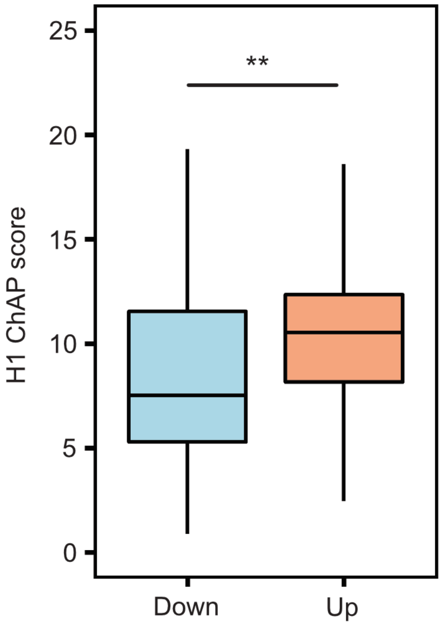

Fig. S5 H1 represses gene expression after heat stress. H1 enrichment (H1 ChAP score) in wild type on downregulated (Down) and upregulated (Up) genes in *h1* plus heat versus wild type plus heat. ChAP, chromatin affinity purification. **, $P < 0.01$ (Wilcoxon test). The lower and upper hinges of the boxplots correspond to the first and third quartiles of the data, the black lines within the boxes mark the median.

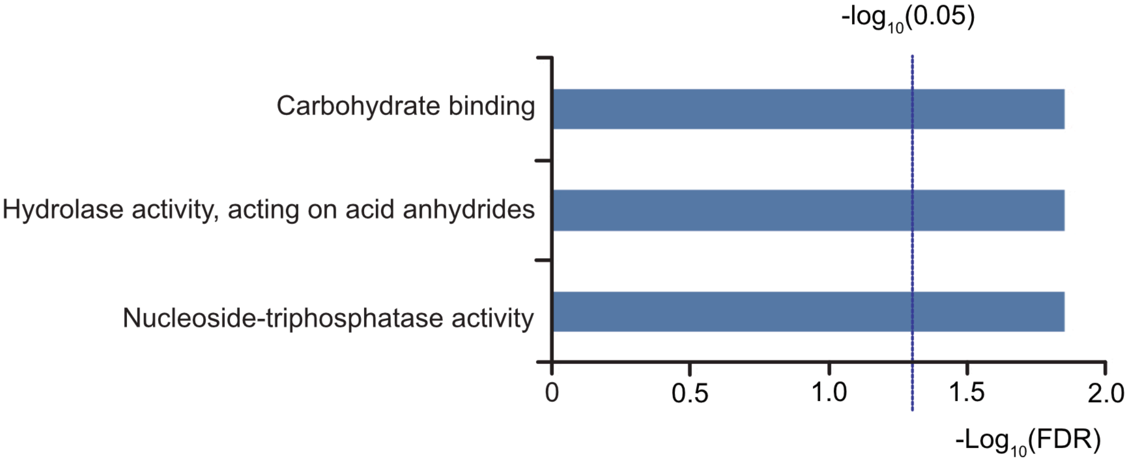

Fig. S6 Gene ontology (GO) terms of upregulated genes in *h1* plus heat versus wild type plus heat.

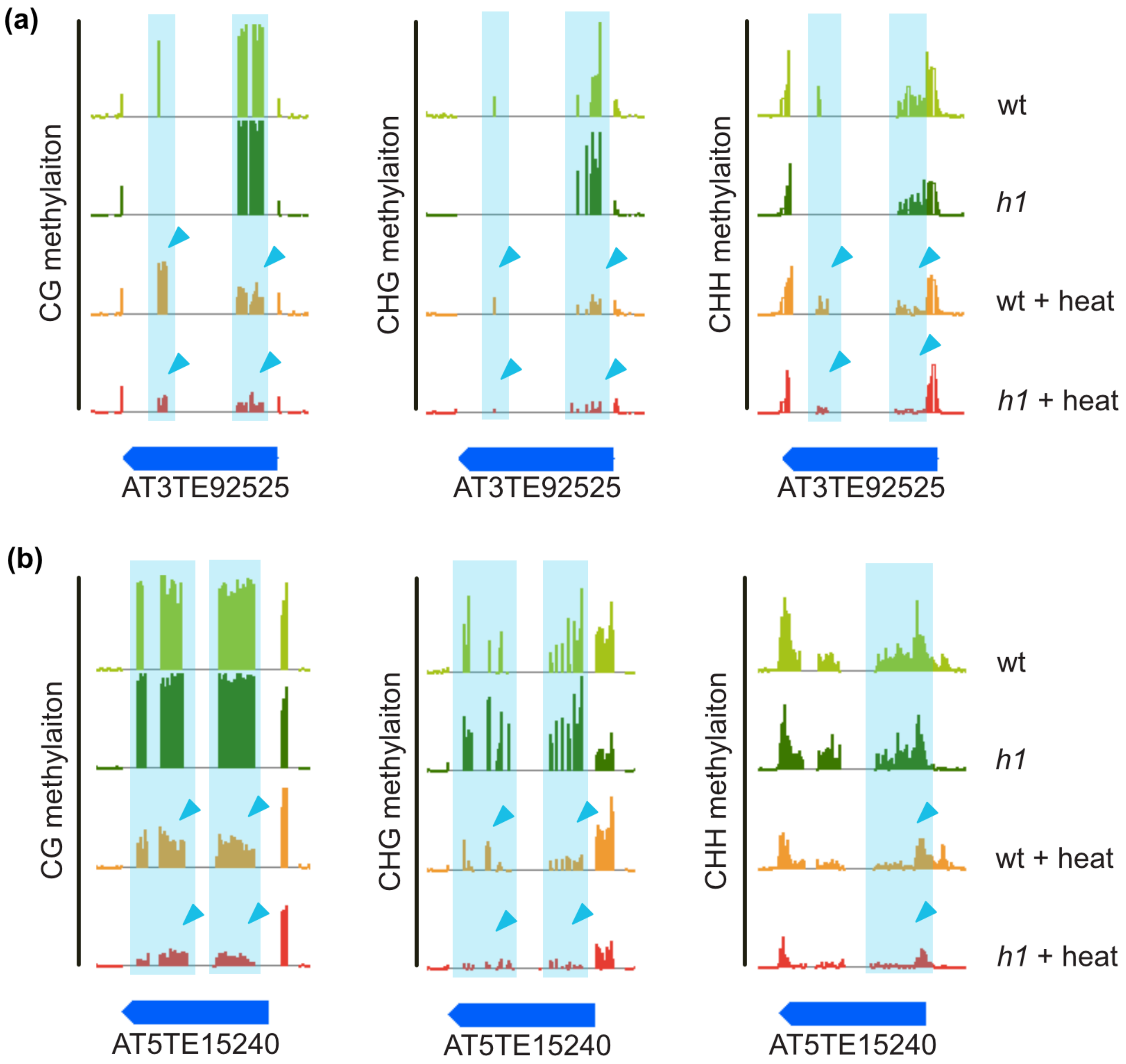

Fig. S7 Decreased CG, CHG and CHH methylation on upregulated *COPIA78/ONSEN* elements, including *ONSEN 2* (*AT3TE92525*, (a)) and *ONSEN 3* (*AT5TE15240*, (b)) in *h1* plus heat (*h1* + heat) versus wild type plus heat (wt + heat). Triangle points to the decreased DNA methylation region.

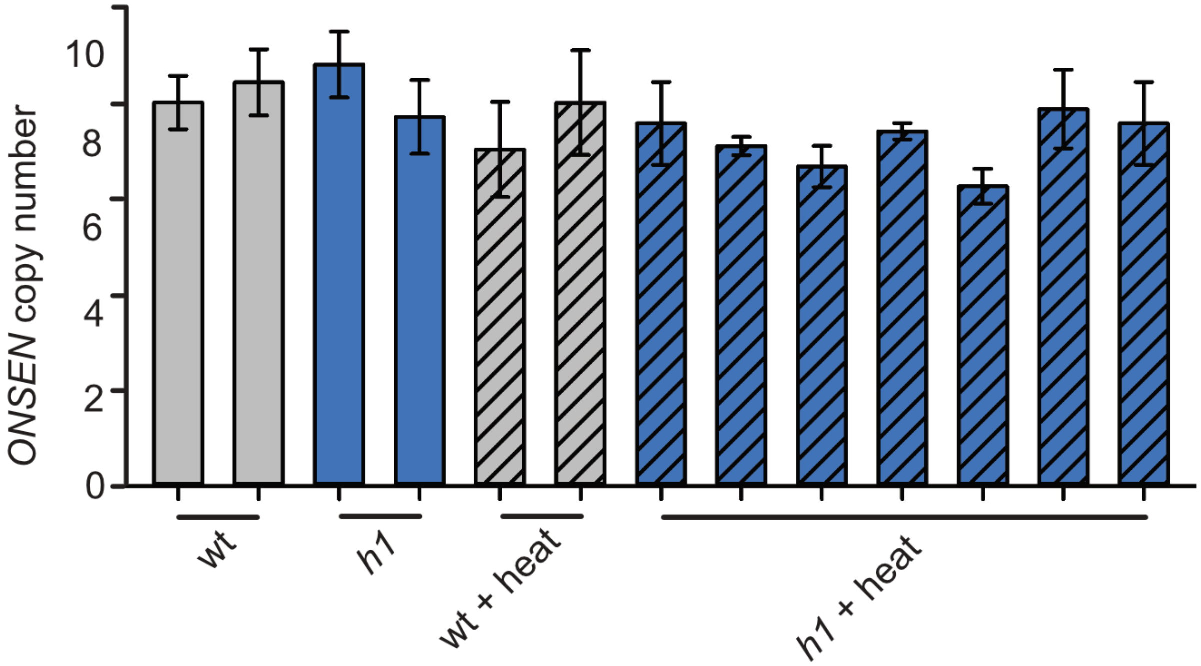

Fig. S8 H1 does not affect *ONSEN* remobilization upon heat. *ONSEN* copy number in the second generation of wild type (wt), *h1* mutants, wt subjected to heat stress (wt + heat) and *h1* subjected to heat stress (*h1* + heat). Each column represents single seedling progeny from specified plant pools. Each bar represents 1SD.

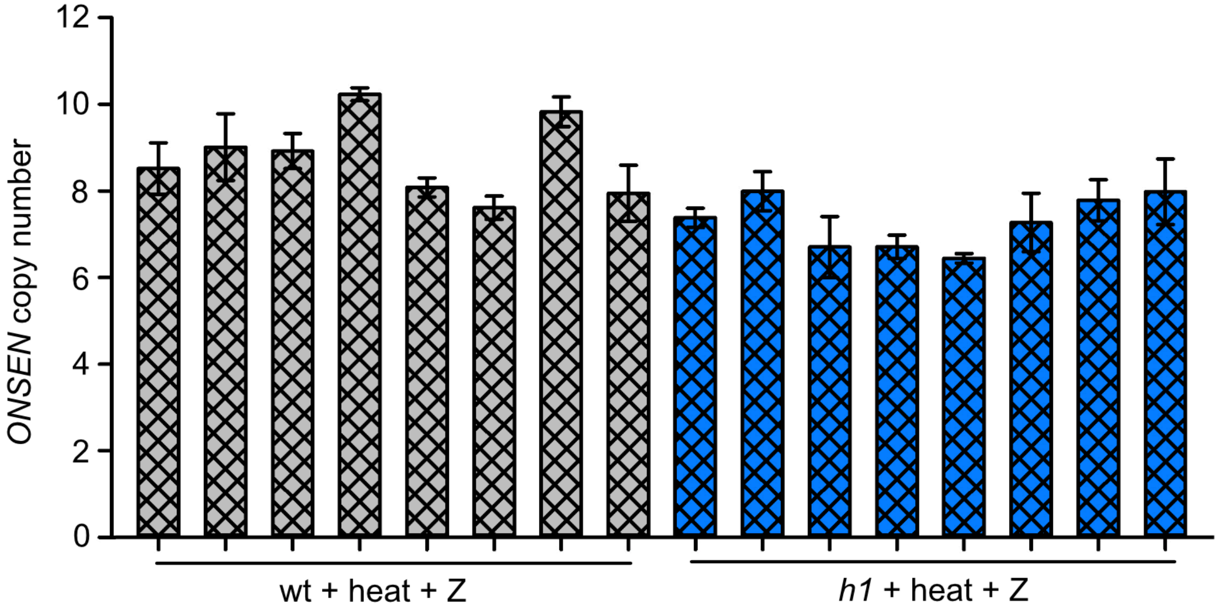

Fig. S9 *ONSEN* does not amplify in the progeny of heat and zebularine treated wild type and *h1* mutants. *ONSEN* copy number in the second generation of wild type (wt) and *h1* mutants subjected to heat stress and zebularine treatment (wt + heat + Z and *h1* + heat + Z). Each column represents single seedling progeny from specified plant pools. Each bar represents 1SD.

Table S1 Primers used in the manuscript

AT number	Gene ID	Sequence	Reference
multiple	*COPIA78*	CGGTGCTCACAAAGAGCAACTATG	*COPIA*78qF2 (Pecinka *et al.*, 2010)
	COPIA78	ATCCTTGATAGATTAGACAGAGAGCT	*COPIA*78qR3 (Pecinka *et al.*, 2010)
AT4TE15820	*GYPSY*	AACGAATCCGGAAGCGTCAC	
	GYPSY	TCTTCTACAGTCGGGCCTGG	
AT3G26650	*GAPDH*	CCTGAGATGTTGGCATGGT	
	GAPDH	AATGCGAAGCCTGCTTGA	
AT3G18780	*ACTIN2*	TTCCGCTCTTTCTTTCCAAG	
	ACTIN2	CCATTGTCACACACGATTGG	

Table S2 Mapping statistics of RNA sequencing data (see separate file).

Table S3 Lists of deregulated genes in *h1* mutants before and after heat stress (see separate file).

Table S4 Lists of upregulated TEs in *h1* mutants and *cmt2* mutants before and after heat stress (see separate file).

References

Choi J, Lyons DB, Kim MY, Moore JD, Zilberman D. **2020**. DNA methylation and histone H1 jointly repress transposable elements and aberrant intragenic transcripts. *Molecular Cell* **77**: 310-323.e7.

Pecinka A, Dinh HQ, Baubec T, Rosa M, Lettner N, Scheid OM. **2010**. Epigenetic regulation of repetitive elements is attenuated by prolonged heat stress in *Arabidopsis*. *The Plant Cell* **22**: 3118–3129.