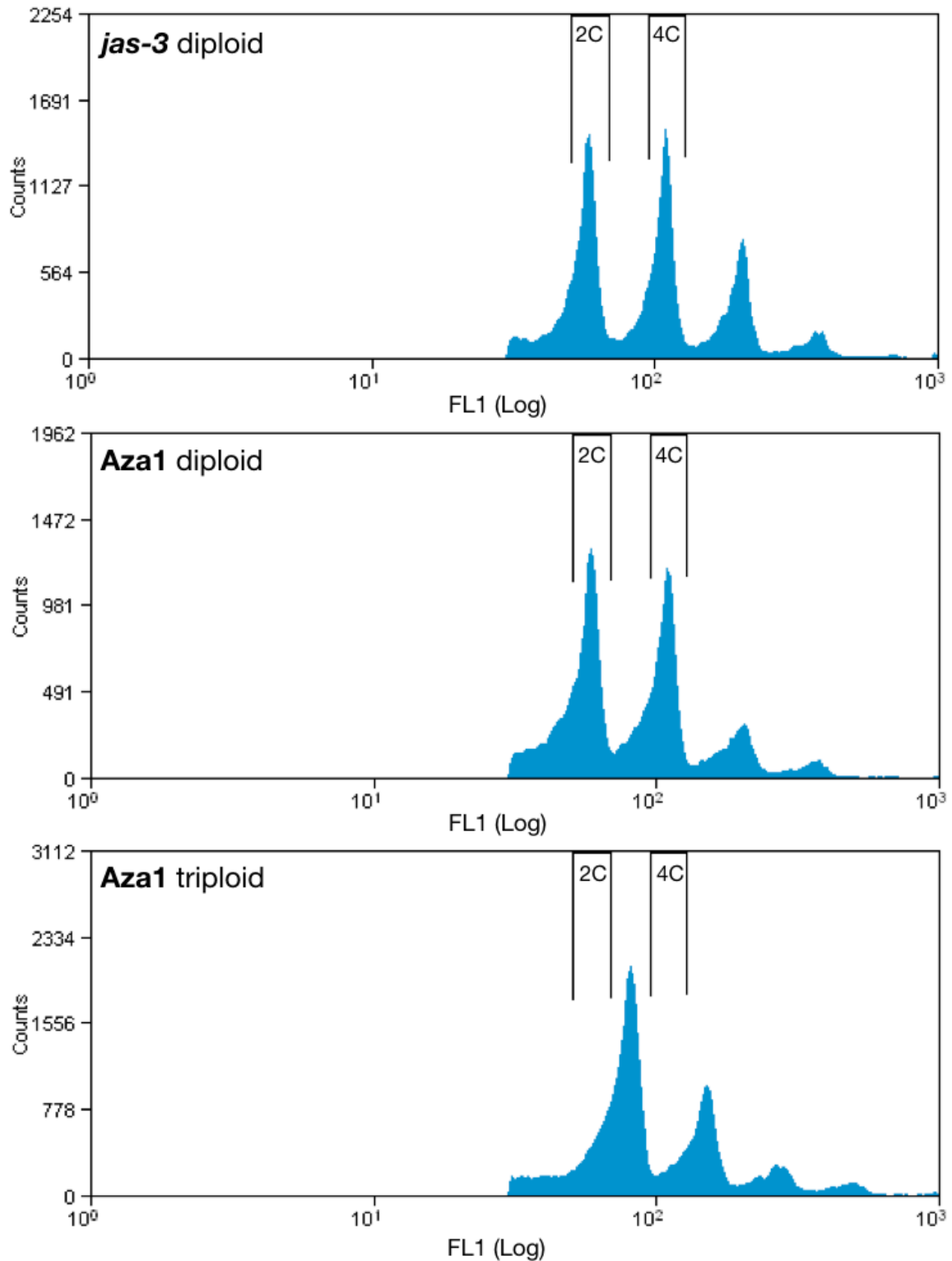


Supplemental Figure 1. Quantification of diploid pollen in *jas-3* plants. (Supports Figure 1)

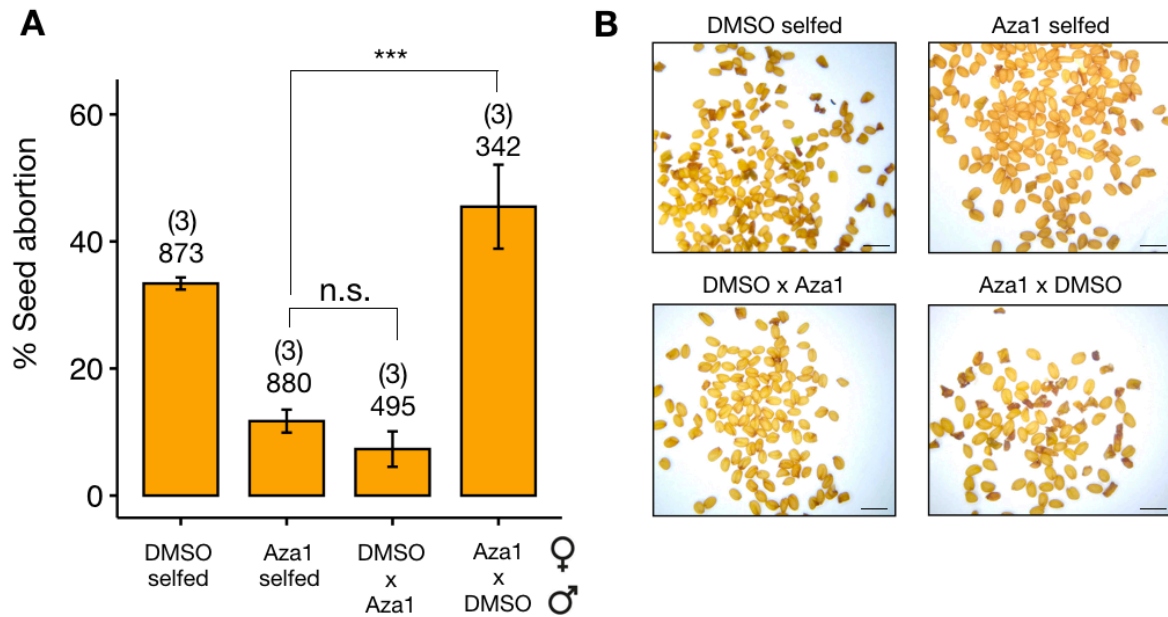
(A) The proportion of haploid (1n) and diploid (2n) pollen produced in *jas-3* mutants was quantified by flow cytometry, as pollen populations are characterized by an elevated high angle scatter (SSC) and autofluorescence (FL1).

(B) Boxplot was used to show the distribution of diploid (2n) pollen among individual DMSO and Aza1 plants. Number on top of the boxes represent the number of plants used. A Wilcoxon test was used to compare the mean values between the suppressor Aza1 and the DMSO control. n.s. is not significant ($p=0.937$).



Supplemental Figure 2. Ploidy analysis by flow cytometry. (Supports Figure 1)

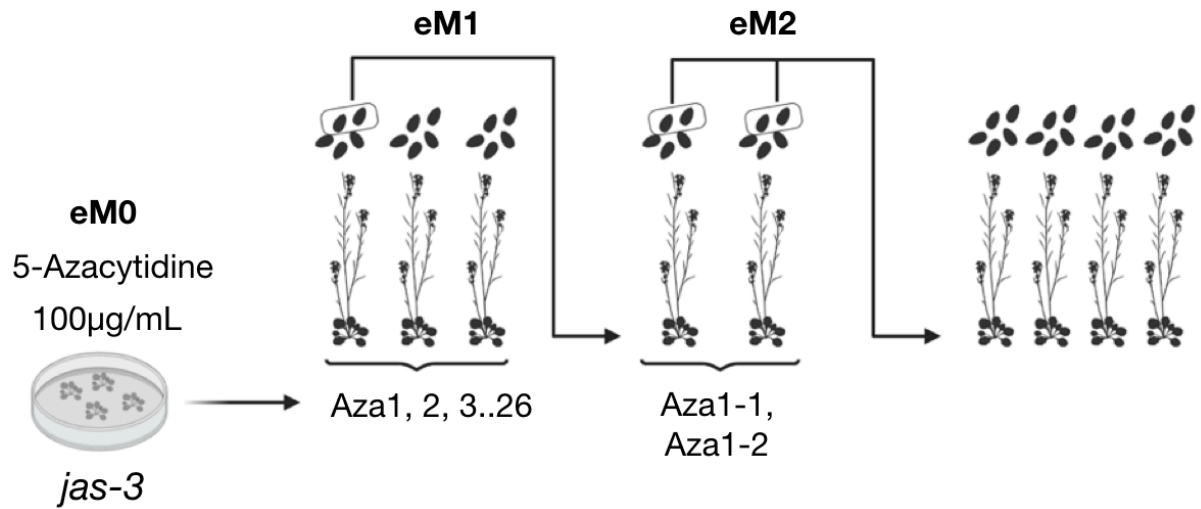
Leaf tissue was chopped in Galbraith buffer with a razor blade, stained with SYBR Green dye (Lonza), and analyzed on a CyFlow Space cytometer (Sysmex). Nuclei of young diploid leaves in Arabidopsis is characterized by two prominent 2C and 4C peaks. Triploid individuals show a proportional increase in signal intensity.



Supplemental Figure 3. Suppression of the triploid block in plants treated with 5-Azacytidine is a paternal effect. (Supports Figure 1)

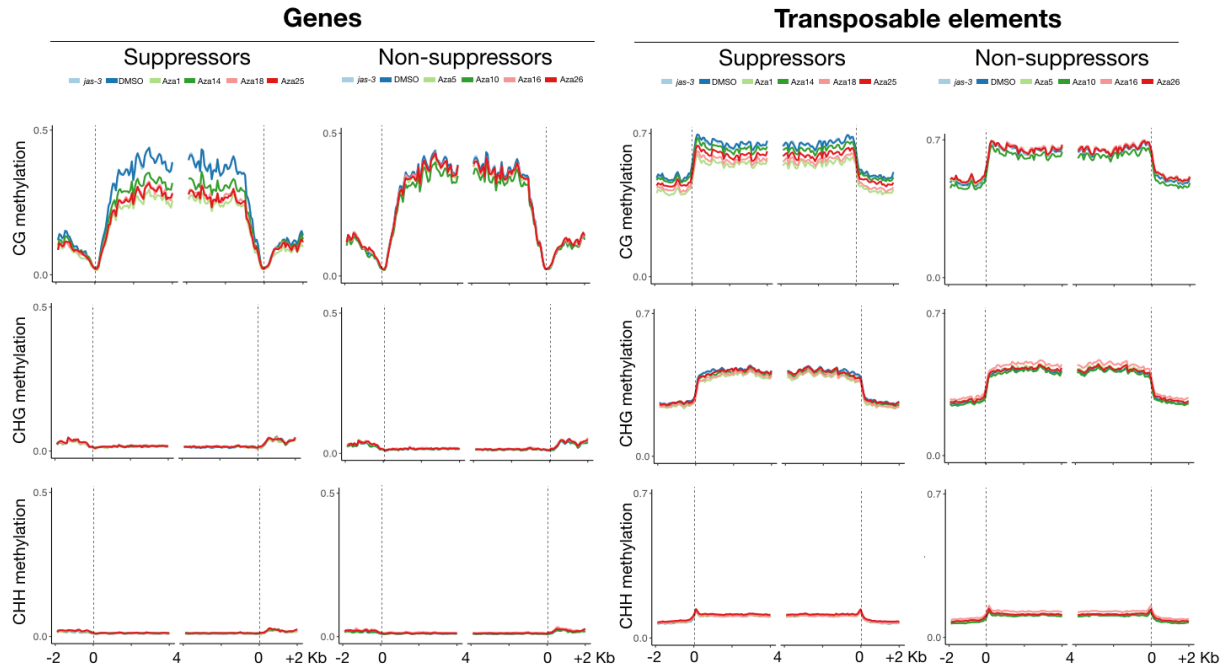
(A) A parental effect in triploid block suppression after epimutagenesis was investigated by performing reciprocal crosses between the strong suppressor Aza1 and DMSO control plants. Numbers on top of each bars represent the number of plants used (top) and total amount of seeds counted (bottom). Statistically significant difference in the percentage of collapsed seeds was calculated by ANOVA with a post hoc Dunnett test, using Aza1 as the reference group (n.s. is not significant, and *** is $p \leq 0.001$).

(B) Representative seeds images are shown for the controls (selfed plants), and reciprocal crosses. Scale bars represent 1mm.



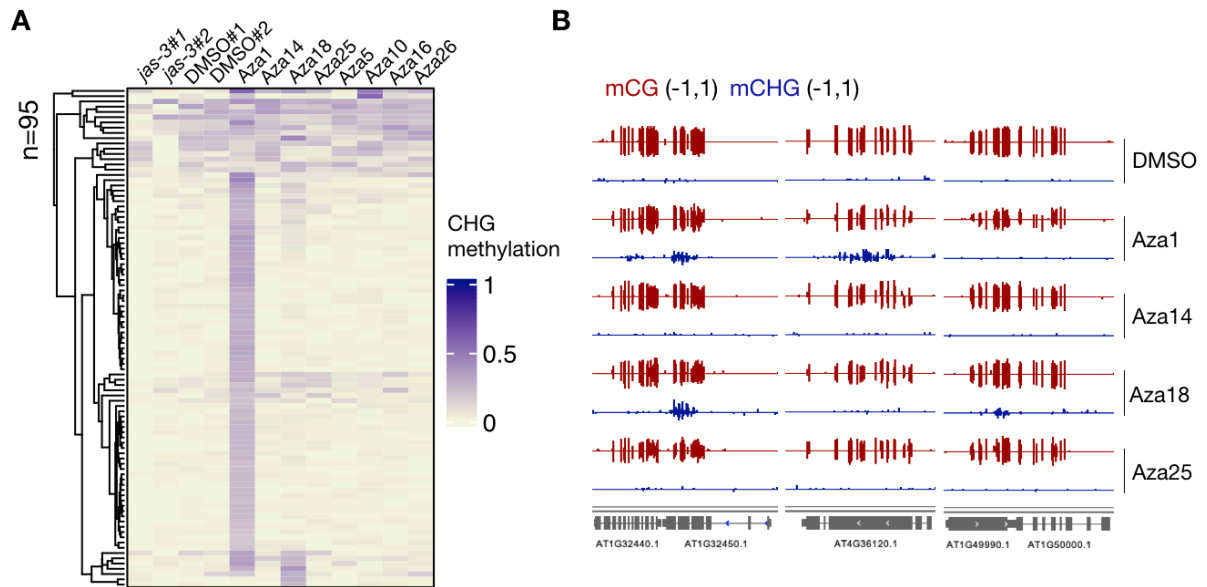
Supplemental Figure 4. Schematic depicting *jas-3* epimutagenesis and transgenerational analysis of the triploid block in suppressor lines. (Supports Figures 1, 2 and 4)

Diploid seeds from *jas-3* were treated with 100 µg/mL of 5-Azacytidine during germination and early growth. Treated eM0 plants were then transferred to soil to recover, and allowed to self-fertilize. Selfed *jas-3* mutants produce diploid seeds that are viable, and triploid seeds that abort. Diploid plants were selected after each generation and allowed to self-fertilize, and the triploid block was quantified by counting the number of aborted seeds in individual siblings.



Supplemental Figure 5. CG, CHG and CHH methylation profiles at protein-coding genes and transposable elements. (Supports Figure 2)

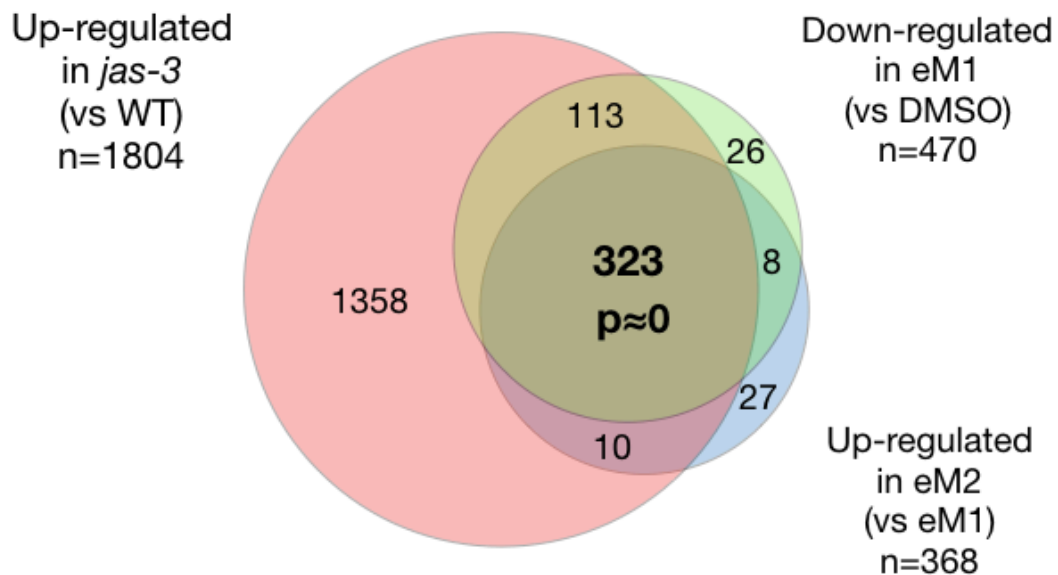
Protein-coding genes (left panels) and transposable elements (TEs, right panels) annotated according to the TAIR10 reference genome were aligned at the 5' and 3' ends (dashed lines), and average CG, CHG and CHH methylation levels for 100-bp intervals were plotted for untreated *jas-3* and DMSO controls, suppressor and non-suppressor lines. Loss of DNA methylation at genes and TEs occurs mainly in the CG context and is observed only in suppressor lines.



Supplemental Figure 6. Ectopic CHG methylation in suppressor lines. (Supports Figure 2)

(A) Heatmap representation of CHG methylation levels at ectopic CHG DMRs detected in the suppressor line *Aza1*, as compared to the untreated control *jas-3*. Average CHG methylation mapping to these DMRs is presented as heatmap for two independent replicates of untreated *jas-3* and DMSO controls, suppressor and non-suppressor lines.

(B) Genome browser tracks show DNA methylation at CG and CHG context for selected loci showing ectopic CHG methylation.

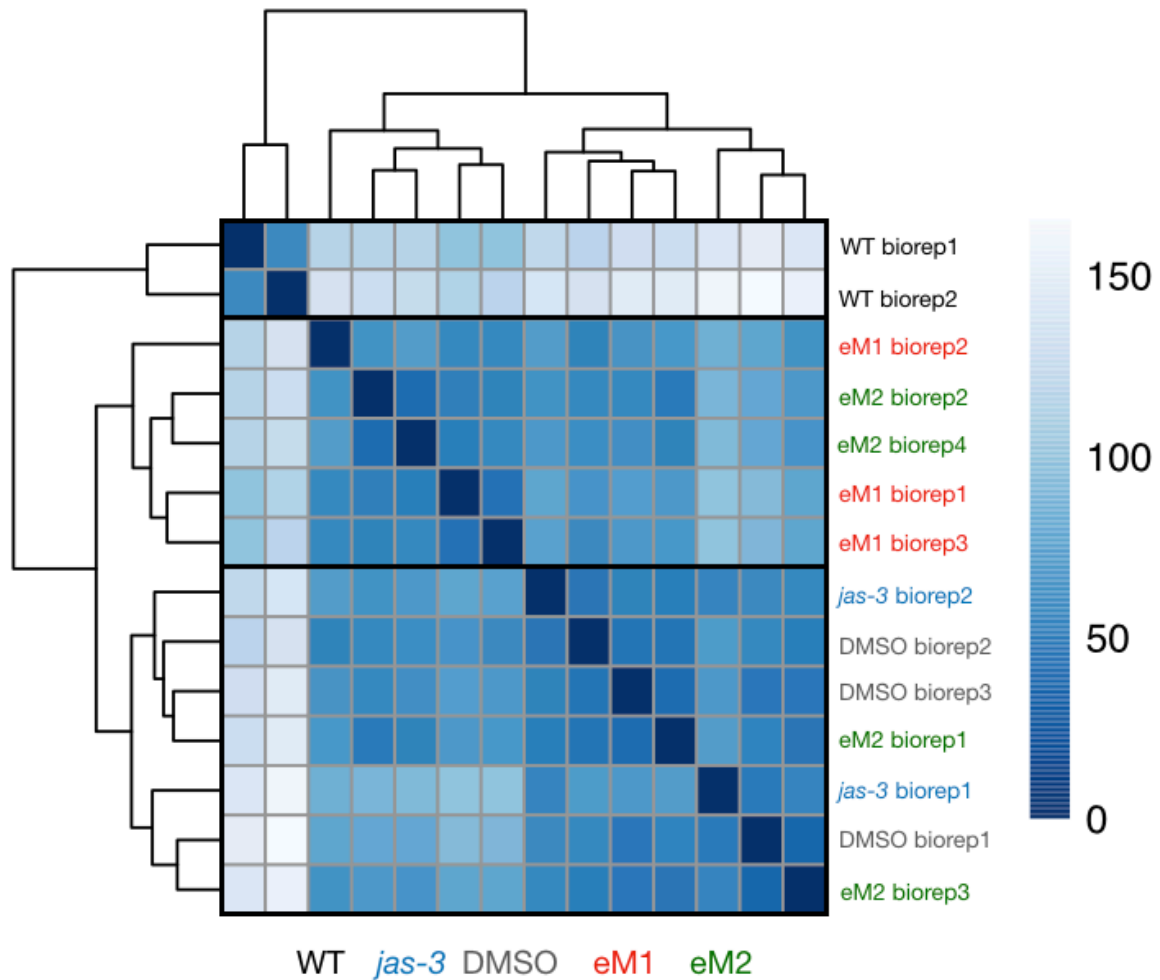
A**B**

Supplemental Figure 7. Differentially expressed genes in suppressor lines. (Supports Figure 4)

(A) Venn diagram shows overlap between genes up-regulated in untreated *jas-3* (vs WT), and genes up-regulated in DMSO controls (vs WT).

(B) Venn diagram shows the overlap of genes up-regulated in *jas-3* (vs WT) with genes transiently down-regulated in eM1 (vs DMSO), and up-regulated in eM2 (vs eM1). The majority of genes down-regulated in eM1 are genes that are up-regulated in *jas-3* mutant siliques, and expression of these genes is restored in eM2.

The statistical significance of the observed overlaps between differentially expressed genes was calculated using the R package SuperExactTest (Wang et al., 2015).



Supplemental Figure 8. Clustering of RNA-seq datasets. (Supports Figure 4)

Transformed data was used for sample clustering to visualize sample-to-sample distances (see Methods). The heatmap of this distance matrix shows an overview over similarities and dissimilarities between all RNA-seq datasets produced in this study.