

Figure 1. The triploid block is suppressed in *jas-3* plants treated with 5-Azacytidine.

(A) The triploid block response was quantified by counting the number of aborted seeds in five siliques of untreated *jas-3* plants and DMSO controls, and plants exposed to three different concentrations of 5-Azacytidine (25, 50 and 100 µg/mL). Numbers on top of each box represent the number of individual plants used. Statistically significant difference in the percentage of collapsed seeds was calculated by ANOVA with a post hoc Dunnett test, using *jas-3* as the reference group (n.s. is not significant, * is $p \leq 0.05$, and *** is $p \leq 0.001$).

(B) Representative images of seeds from DMSO controls and the strong suppressor Aza1, showing a decrease in the level of seed abortion in the suppressor line.

(C) Seed abortion and germination assays were performed in the first generation after treatment with 5-Azacytidine (eM1) to evaluate the transgenerational stability of the suppressive effect. Numbers on top of each box indicate the number of siblings (top) and total number of seeds (bottom) counted. Statistically significant difference in the percentage of non-germinated seeds was calculated by ANOVA with a post hoc Dunnett test, using DMSO as the reference group (n.s. is not significant, * is $p \leq 0.05$, and *** is $p \leq 0.001$).

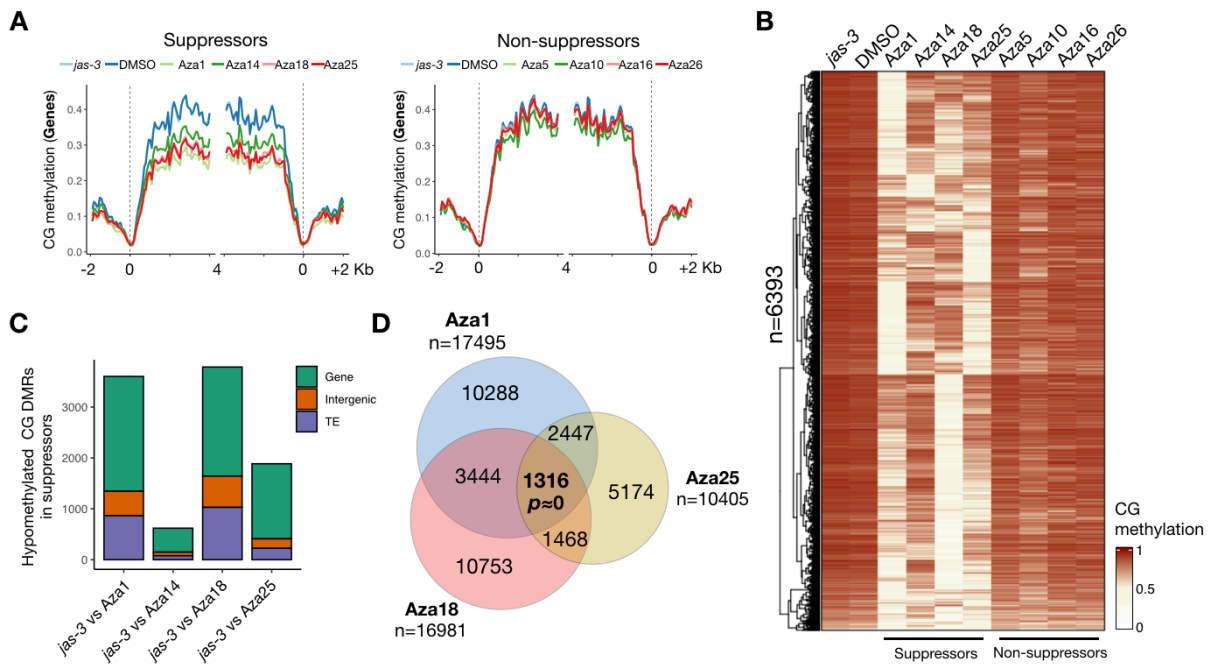


Figure 2. Transgenerational inheritance of CG hypomethylation occurs specifically in suppressor lines.

(A) Metaplots show CG methylation levels at protein-coding genes annotated according to the TAIR10 reference genome, and aligned at the 5' and 3' ends (dashed lines). Average CG, methylation was calculated for 100-bp intervals and plotted for untreated *jas-3* and DMSO controls, suppressor and non-suppressor lines, showing that loss of CG methylation occurs specifically in the suppressor lines.

(B) Heatmap representation of CG methylation levels at hypomethylated CG DMRs detected in the suppressor lines (Aza1, Aza14, Aza18 and Aza25) as compared to the untreated control *jas-3*. Average CG methylation mapping to these DMRs is presented as heatmap for untreated *jas-3* (2 replicates) and DMSO controls (2 replicates), suppressors and non-suppressors.

(C) Hypomethylated CG DMRs detected in each suppressor line were mapped to the genomic features annotated in the TAIR10 reference genome, showing that the majority of DMRs overlap with protein-coding genes and transposable elements (TEs).

(D) Venn diagram shows a significant overlap between differentially methylated 100bp bins detected in the three strongest suppressor lines (Aza1, Aza18 and Aza25). The statistical significance of the observed regions was calculated using the R package SuperExactTest (Wang et al., 2015).

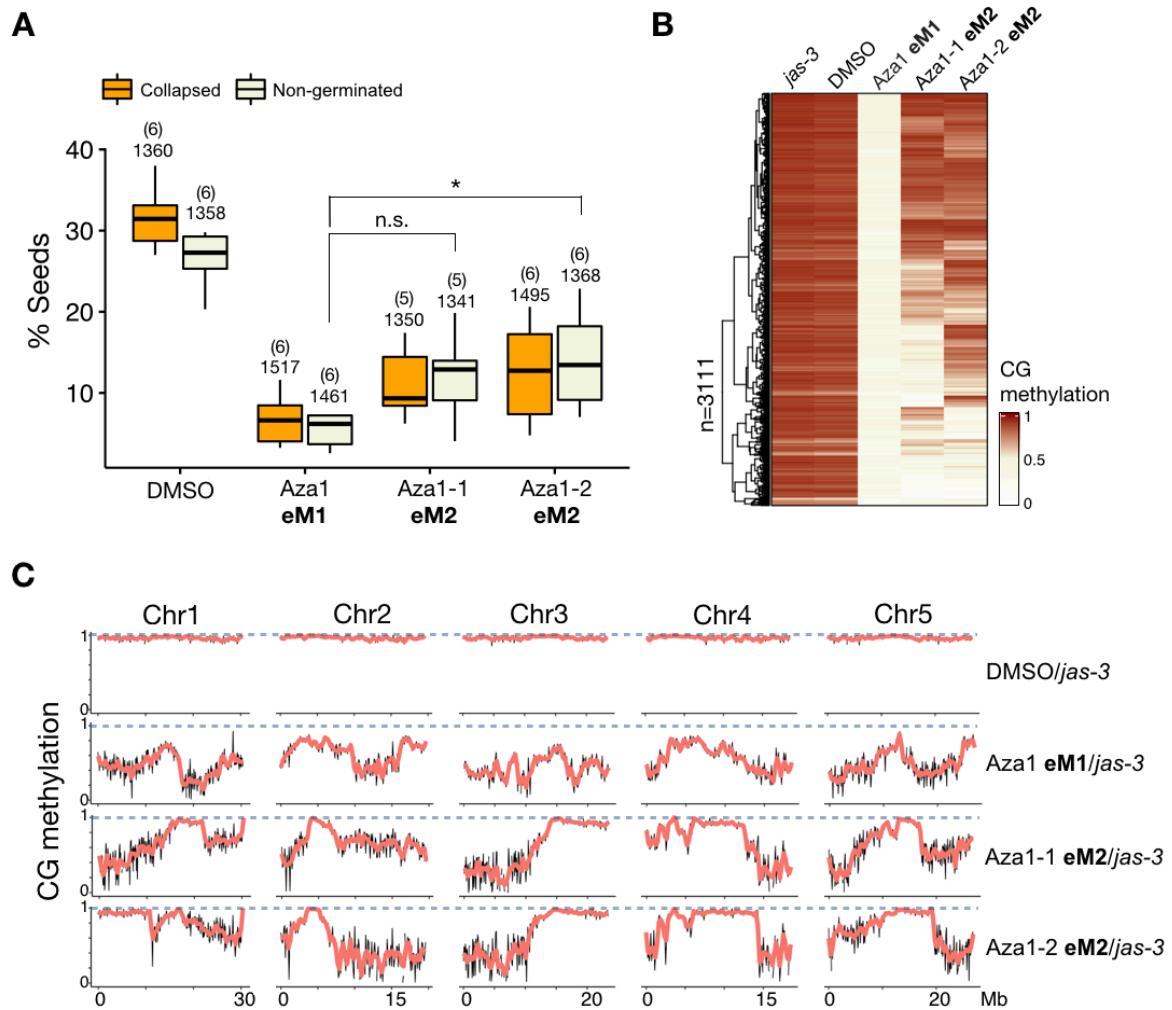


Figure 3. The triploid block response and CG methylation are partially restored in the second generation after treatment with 5-Azacytidine.

(A) Seed abortion and germination assays were performed for two consecutive generations after treatment with 5-Azacytidine (eM1 and eM2), showing that the triploid block response is only partially restored in two independent lines in the second generation after epimutagenesis (eM2). Numbers on top of each box indicate the number of siblings (top) and total number of seeds (bottom) counted. Statistical significance was calculated by ANOVA with a post hoc Dunnett test, using Aza1 as the reference group (n.s. is not significant, and * is $p \leq 0.05$).

(B) Average CG methylation mapping to hypomethylated CG DMRs detected between the suppressor line Aza1 and the untreated control *jas-3* is presented as heatmap for untreated *jas-3* (2 replicates) and DMSO (2 replicates), Aza1 eM1, and two eM2 lines (Aza1-1 and Aza1-2), to show that CG methylation is also partially restored in eM2 lines.

(C) CG methylation of untreated *jas-3*, DMSO, eM1 and eM2 lines was mapped to 100kb bins across the Arabidopsis genome. CG methylation levels in DMSO, eM1 and eM2 datasets were then divided by the levels in *jas-3*, and plotted separately for all five chromosomes to show

patterns of hypomethylation. This shows that for most of the genome, the distribution of CG hypomethylation is identical between the two eM2 lines, although there are particular loci where CG methylation is segregating.

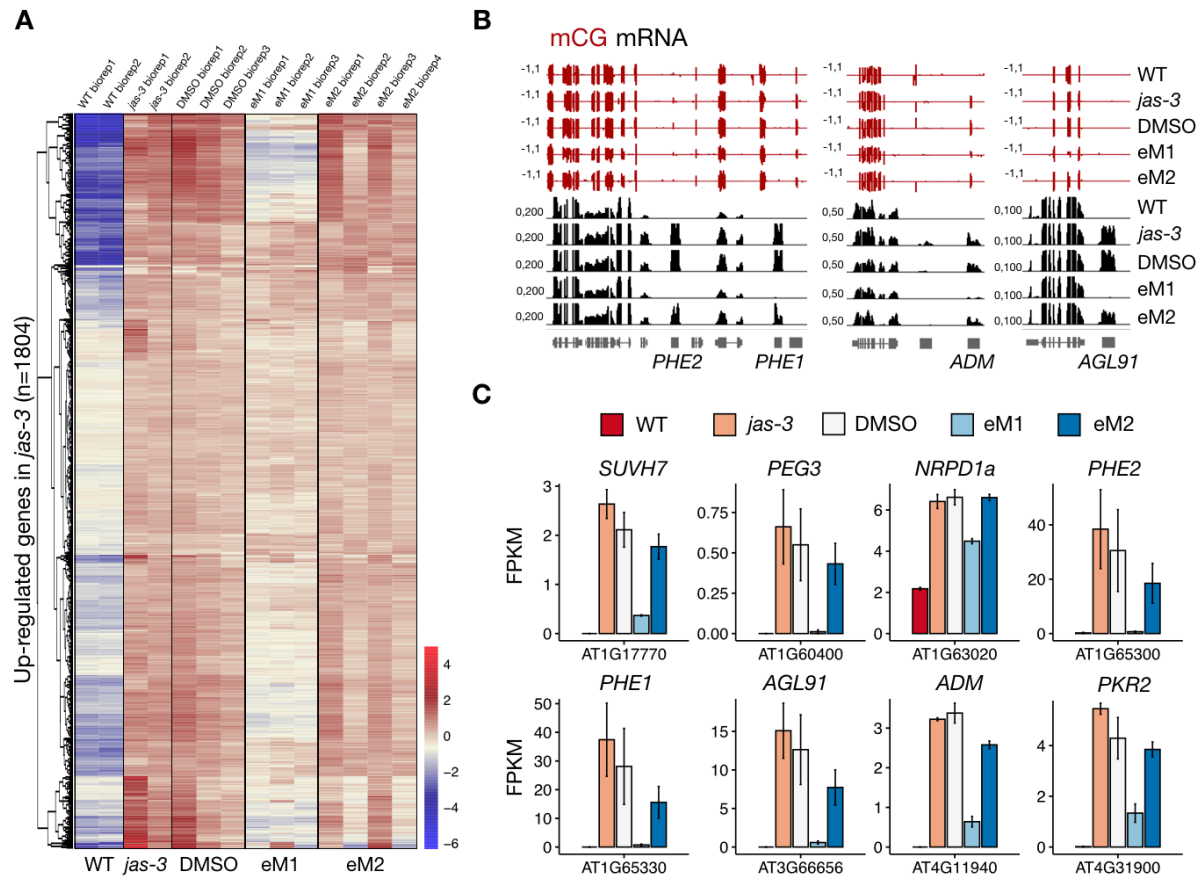


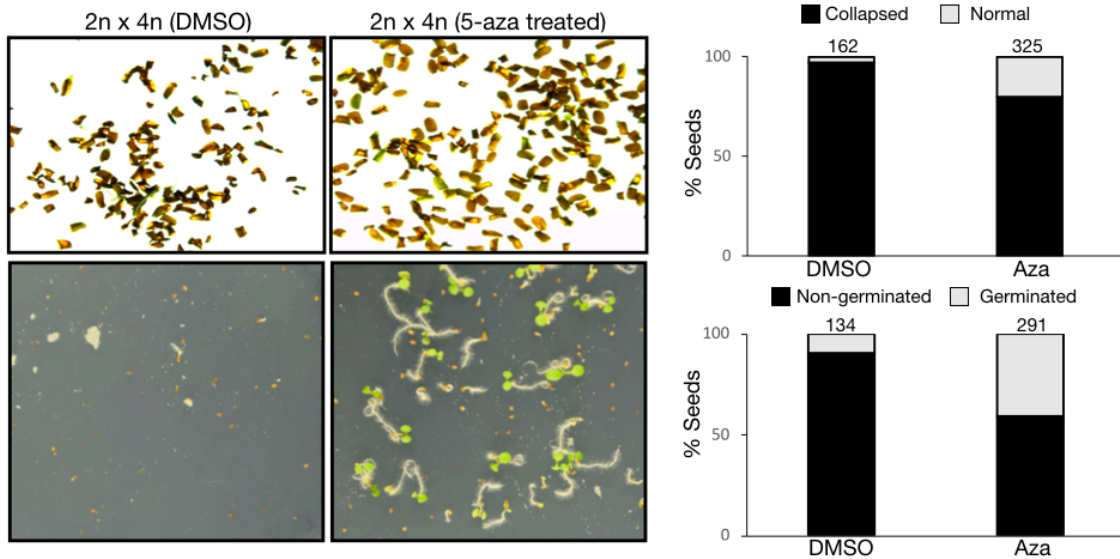
Figure 4. Paternally expressed genes are down-regulated in suppressor lines.

(A) Differential expression analysis was performed between wild-type Col-0 (WT) and *jas-3* mutant siliques, and 1804 genes were found up-regulated in *jas-3* (fold change ≥ 2 , adjusted *p*-value < 0.01). Transformed raw counts for these genes are presented as an heatmap for WT, *jas-3* and DMSO, Aza1 eM1 and eM2 biological replicates (see Methods), to show that a proportion of genes up-regulated in untreated *jas-3* and DMSO controls are transiently down-regulated in the first generation after treatment with 5-Azacytidine.

(B) Genome browser tracks display CG methylation (pooled seedlings) and mRNA levels (siliques) in *jas-3*, DMSO, Aza1 eM1 and Aza1 eM2 plants.

(C) Raw counts were normalized as fragments per kilobase per million (FPKM) and plotted as barplots with mean values and standard error.

A *Arabidopsis thaliana* 2x x 4x (Col-0)



B *Capsella rubella* x *Capsella grandiflora*

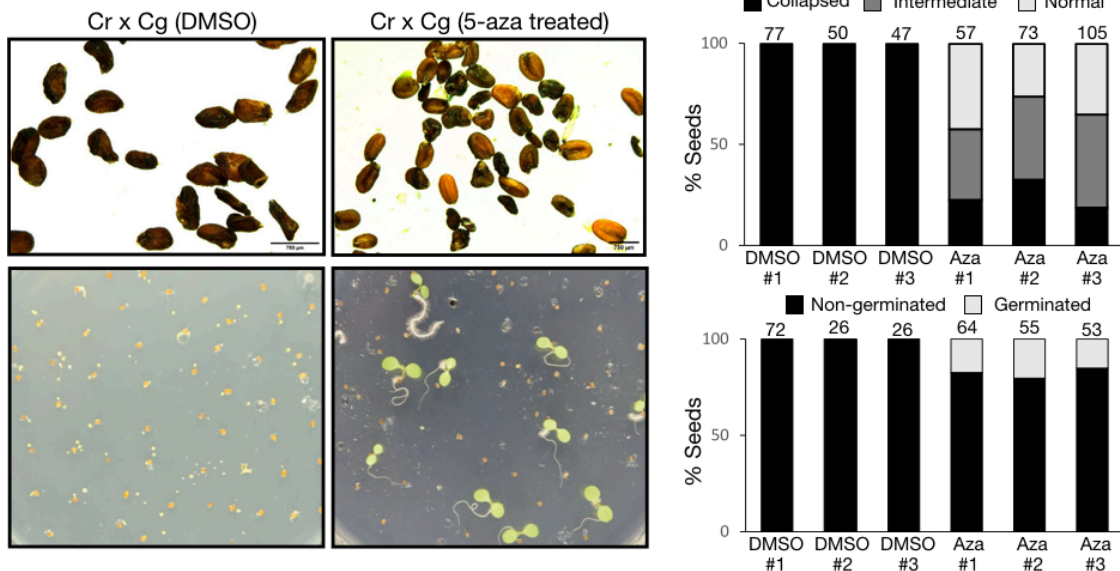


Figure 5. Chemically-induced epimutagenesis allows bypassing interploidy and interspecific hybridization barriers.

(A) Pollen from tetraploid *Arabidopsis* plants in Col-0 background was used to pollinate diploid Col-0 plants. Triploid seeds resulting from this cross abort at high frequencies when the paternal parent is treated with DMSO, while 40% of viable triploid seeds were detected when pollen was derived from plants that have been treated with 5-Azacytidine.

(B) In interspecific crosses between diploid *Capsella rubella* (Cr) and *Capsella grandiflora* (Cg), hybrid seeds abort, resembling interploidy hybridizations with paternal excess. When *C. grandiflora* plants are treated with 5-Azacytidine, approximately 20% of hybrid seeds germinated.