Targeted suppression of siRNA biogenesis in Arabidopsis pollen reveals distinct Pol IV activities in the sperm and vegetative cell lineages

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Abstract

In plants, small-interfering RNAs (siRNAs) are mainly produced from transposable elements (TEs) within the RNA-directed DNA methylation pathway (RdDM), which is particularly important during reproductive development in many plant species. However, there is limited understanding of the origins, dynamics and function of TE-derived siRNAs acting in different cellular and developmental contexts. Here, we used the RNaseIII-like protein RTL1 to suppress siRNA biogenesis specifically in Arabidopsis pollen, and found distinct siRNA subsets produced in the sperm and vegetative cell lineages via RNA polymerase IV (Pol IV). We show that gametophytic siRNAs produced in the vegetative cell reinforce RdDM in pollen and interploidy hybridization barriers in the seed, while siRNAs produced specifically in sperm cells do not impact the epigenome and are dispensable for the "triploid block". These results demonstrate that active siRNA biogenesis in the two pollen cell types has contrasting roles in transposon silencing and transgenerational epigenetic inheritance.

Full Text

Small RNA pathways have been extensively characterized in plants, and were shown to play important roles in development and reproduction[1,2]. Plant small RNAs are 20- to 24-nucleotides (nt) in length and may be further divided into microRNAs (miRNA) and small-interfering RNAs (siRNAs) depending on their precursor transcript and processing machinery, although they all share the ultimate goal to guide silencing effectors to complementary RNA targets[3,4]. A massive expansion of plant siRNA pathways came with the appearance of RNA polymerases solely dedicated to the production of siRNAs and their targets[3,4], such as Pol IV and Pol V that mediate transcriptional silencing of genes and transposable elements (TEs) via the RNA-directed DNA methylation (RdDM) pathway[5]. This mechanism is essential for normal development and fertility in many plant species[6–8], but has limited phenotypic impact in the model plant *Arabidopsis thaliana*, which allows studying siRNA biogenesis and activity in different developmental contexts. However, most studies have used genetic mutations that disrupt siRNA biogenesis throughout plant development, in a way that is difficult to investigate the origins and dynamics of siRNA subsets in specific tissues and cell types. For example, in the Arabidopsis male gametophyte, three haploid cells differentiate after meiosis to form a mature pollen grain[9]. After the first pollen mitosis, the vegetative cell nucleus exits cell cycle and undergoes epigenetic reprogramming leading to transcriptional activation of TEs that are rapidly targeted by multiple small RNA-mediated pathways[10–14]. In contrast, the generative cell undergoes a second mitosis and gives rise to twin sperm cells that slowly progress through S-phase prior to double fertilization, when RdDM pathways are transiently switched off[10,15]. For this reason, epigenetic reprogramming in the male germline has been largely associated to the activity of siRNAs originated from the vegetative cell[12,16,17] and anther tissues during meiosis[18], but the origins, dynamics and functions of siRNAs accumulated in mature pollen are still poorly understood.

We then developed a strategy to suppress siRNA biogenesis specifically in Arabidopsis pollen using the endogenous antiviral protein RTL1, which was previously shown to suppress siRNA biogenesis when expressed by strong constitutive promoters[19]. Ectopic expression of *RTL1* in pollen of Col-0 background was achieved by using the well-known *LAT52* and *MGH3* promoters that are specifically expressed in the vegetative and sperm cell lineages, respectively[20] (Extended Data Fig. 1), and two independent transgenic lines of each construct were then used for small RNA sequencing (*pLAT52::RTL1* is hereafter designated as VC-RTL1, and *pMGH3::RTL1* is SC-RTL1) (Fig. 1a, Extended Data Fig. 2). Differential expression analysis using wild-type pollen (WT Col-0) as control revealed 817 loci with significantly reduced small RNA levels in VC-RTL1 and 247 in SC-RTL1 pollen, which were mostly non-overlapping (Fig. 1b, Extended Data Fig. 2a and Supplementary Data 1). A few hundred loci with higher small RNA levels were also detected in both VC-RTL1 and SC-RTL1 lines (Extended Data Fig. 2b), but are likely indirect normalization effects resulting from the strong loss of siRNAs in these lines. We then focused on down-regulated siRNA loci and confirmed the specificity of our experiment by inspecting siRNA levels at protein-coding genes targeted by post-transcriptional gene silencing (PTGS) pathways, but expressed in either the vegetative or sperm cells (Fig. 1c). In agreement with the transcriptomes of purified pollen nuclei[21], we observe that 21/22-nt siRNAs mapping to vegetative cell-expressed genes are depleted in VC-RTL1 pollen, while 21/22-nt siRNAs mapping to sperm cell-expressed genes are specifically lost in SC-RTL1 pollen (Fig. 1d). These results demonstrate that ectopic expression of *RTL1* is able to suppress siRNA biogenesis in the two pollen cell types.

As expected, siRNA depletion in VC-RTL1 and SC-RTL1 lines occurred primarily at TEs from the LTR/Gypsy superfamily (Fig. 2a), which are the main producers of 21- to 24-nt siRNAs in pollen via Pol IV activity[13,22,23]. However, the number of TE loci that lost siRNAs in VC-RTL1 pollen is substantially higher than in SC-RTL1 (Fig. 2a), and strikingly, different TE families were differentially targeted in the two cell types (Fig. 2b, c and Extended Data Fig. 3a). This includes *ATGP1*, *VANDAL3* and *ATLANTYS2* families that lost siRNAs only in VC-RTL1 pollen, while siRNAsfrom *ATGP2*, *ATGP2N*, *VANDAL6* and *ATCOPIA36* were specifically depleted in SC-RTL1 pollen (Fig. 2b, c and Extended Data Fig. 3a). Notably, siRNA loss in VC-RTL1 pollen is particularly pronounced at the 21/22-nt size range, while all siRNA types are strongly affected in SC-RTL1 pollen (Fig. 2c, d).

Biogenesis of 24-nt siRNAs in sperm cells was particularly surprising, given that downstream RdDM components are not expressed in this cell type[10,24]. Therefore, in order to confirm that TE-derived siRNAs depleted in VC-RTL1 and SC-RTL1 pollen are indeed produced by Pol IV, we took advantage of a Fluorescence Tagged Line (FTL1230) carrying a transgene genetically linked to the *NRPD1a* gene that encodes the largest subunit of Pol IV (*AT1G63020*) (Extended Data Fig. 4a). This transgenic line contains a pollen-specific DsRed marker gene[25], which was then crossed with the *nrpd1a-3* mutant to obtain double heterozygous FTL1230/+;*nrpd1a-3/+* plants that allowed purification of wild-type (DsRed positive, WT^{FTL}) and mutant (DsRed negative, $nrpd1a\text{-}3^{FTL}$) pollen by fluorescence-activated cell sorting (FACS) (Extended Data Fig. 4b, c). This experiment confirmed a strong depletion of TE-derived siRNAs in $nrpd1a\text{-}3^{FTL}$ pollen, including TEs that lost siRNA in VC-RTL1 and SC-RTL1 pollen such as *ATGP1* and *ATGP2N*

(Fig. 2d and Extended Data Fig. 4d). Taken together, these results demonstrate that the two pollen cell types produce different siRNA subsets that are dependent on gametophytic activity of Pol IV. However, siRNAs from most TE families are not completely lost in *nrpd1a-3*^{FTL}, VC-RTL1 and SC-RTL1 pollen (Fig. 2d and Extended Data Fig. 4d), suggesting that some pollen siRNAs originate from earlier stages of pollen development or before meiosis.

Targeted suppression of siRNA biogenesis in the sperm and vegetative cell lineages was an opportunity to investigate their potentially different roles in modulating the pollen epigenome. We then profiled DNA methylation levels in VC-RTL1 and SC-RTL1 pollen using whole-genome bisulfite sequencing (WGBS), which was again compared to WT Col-0 controls and WT^{FTL} and *nrpd1a-3*^{FTL} pollen purified by FACS (Extended Data Fig. 4b, c). Analysis of differentially methylated regions (DMRs) detected 560 hypomethylated CHH DMRs in *nrpd1a-3*^{FTL} pollen that largely overlapped with that in VC-RTL1 (Fig. 3a, Extended Data Fig. 5 and Supplementary Data 2). This clearly demonstrates that ectopic RTL1 expression in the vegetative cell is able to mimic the gametophytic loss of Pol IV activity, while the levels of CHH methylation were practically unchanged in SC-RTL1 pollen (Fig. 3a and Extended Data Fig. 5). Importantly, these results also support the idea that Pol IV activity during pollen development is limited, as the loss of CHH methylation was not as strong as what is normally seen in sporophytic tissues of *nrpd1a* mutants. This is illustrated with methylome analysis of homozygous *nrpd1a* and *p35S::RTL1* seedlings (Extended Data Fig. 6), where the loss of CHH methylation is much stronger than in *nrpd1a-3*^{FTL} and VC-RTL1 pollen. This shows that active siRNA biogenesis during pollen mitosis is required to reinforce RdDM at certain TEs, but is not sufficient to fully restore DNA methylation in the vegetative cell.

We then performed RNA sequencing to investigate the impact of siRNA loss and DNA hypomethylation in the transcriptomes of SC-RTL1 and VC-RTL1 pollen. Differential expression analysis revealed that gene expression was strongly affected in VC-RTL1 pollen with 718 loci up-regulated and 855 loci down-regulated (Extended Data Fig. 7 and Supplementary Data 4). This includes TEs that are equally up-regulated in *nrpd1a-3*^{FTL} mutant pollen, such as retrotransposons from the *ATHILA4A*, *ATHILA4* and *ATGP1* families that lost siRNA in VC-RTL1 pollen (Fig. 2b), although CHH methylation is only mildly affected (Fig. 3b). Intriguingly, siRNA loss in SC-RTL1 pollen did not impact gene expression, as only six genes (including *RTL1*) were significantly deregulated (Fig. 3c, Extended Data Fig. 7 and Supplementary Data 4). Combined, our results show that epigenetic changes induced by ectopic *RTL1* expression in pollen are at least partially associated with targeted suppression of Pol IV-dependent siRNA biogenesis in the vegetative cell, while siRNA biogenesis in sperm cells appears to be mostly uncoupled from downstream effectors of gene silencing.

The role of gametophytic small RNAs in germline reprogramming and epigenetic inheritance remains poorly understood, but previous studies in Arabidopsis have shown that paternal RdDM pathways establish strong interploidy hybridization barriers in the seed, which is also called the “triploid block”[13,22,26,27]. In order to test if gametophytic siRNAs produced during pollen development are indeed required for the triploid block response, we introduced VC-RTL1 and SC-RTL1 transgenes into the *jas-*

3 mutant (Col-0 background) that produces approximately 35% of unreduced diploid pollen and triploid seeds that collapse at high frequencies[28,29]. A *jas-3*/*nrpd1a-3* double mutant was also generated to confirm a significant reduction in triploid seed collapse resulting from the loss of Pol IV-dependent siRNAs in pollen (Fig. 4a and b), as previously demonstrated with tetraploids and *osd1* mutants[22,26,27]. Strikingly, suppression of triploid seed collapse was equally observed in three independent *jas-3*;VC-RTL1 lines over five consecutive generations, while there was no significant effect in *jas-3*;SC-RTL1 plants (Fig. 4a, b). The increased number of viable triploid seeds in *jas-3*;VC-RTL1 lines is directly observed by inspecting the presence of larger seeds in their progeny (Fig. 4b), but was also confirmed based on the fertility defects of triploid plants (Extended Data Fig. 8). Importantly, increased viability of triploid seeds in *jas-3*;VC-RTL1 lines strongly correlates with down-regulation of the paternally expressed imprinted gene *PHERES1* (*PHE1*) that is an essential regulator of genomic imprinting and interploidy hybridization barriers with paternal excess[30] (Extended Data Fig. 8). Similar observations have been reported in several epigenetic suppressors of the triploid block[22,31,32], thus indicating that siRNA biogenesis in the pollen vegetative cell impacts imprinted gene expression in hybrid seeds.

In summary, we provide evidence that Pol IV-dependent siRNA biogenesis is reinforced during pollen development in the two differentiated cell types, which was unexpected given that RdDM is not active in the male germline[10,11,24]. More importantly, we show that Pol IV produces siRNA from different TE loci in the sperm and vegetative cell lineages that are only separated by two mitotic divisions. We can speculate that this reflects their distinct chromatin organizations, as the vegetative cell nucleus undergoes genome-wide changes in DNA methylation and loss of heterochromatin after the first pollen mitosis[10,11,33], while sperm nuclei have highly condensed chromatin[34] and undergo genome-wide loss of H3K27me3[35]. These differences also highlight the importance of having mobile siRNAs produced in germline companion cells in pollen[12,16,17,36], in anthers[18] and in the female gametophyte[37], as it could compensate the fact that Pol IV-dependent siRNA biogenesis is limited to only certain TEs in the germline. Our results provide further support to these ideas, as Pol IV-dependent siRNAs produced in the vegetative cell are required to trigger the triploid block in the next generation. This mechanism potentially requires mobile siRNAs that must first accumulate in sperm cells in order to reach the early endosperm after fertilization, as well as some of the many Argonaute (AGO) proteins that are highly abundant in sperm cells[38]. AGO1 and AGO5 are good candidates in this model, as they have been both independently implicated in the triploid block response[14]. This hypothesis would also explain why RTL1 activity in sperm cells is not able to target siRNAs originated from the vegetative cell, as mobile siRNAs might get rapidly loaded into AGO proteins that protect them from RTL1 activity. Alternatively, mobile siRNAs accumulated in sperm cells could also be modified in a way that prevents targeting by RTL1, but these interesting ideas await further investigation.

Finally, it remains unclear the biological significance of active siRNA biogenesis in sperm cells, given that hundreds of genes and TEs produce siRNAs that are not immediately engaged in PTGS or RdDM. This is particularly puzzling for *ATGP2* and *ATGP2N* siRNAs, which combined represent a large proportion within the most abundant TE-derived siRNAs produced in Arabidopsis pollen (Extended Data Fig. 3b). The

targeting specificity of Pol IV to most RdDM loci has been first demonstrated by its interaction with the SHH1 protein[39], which could be a missing component in sperm cells. Instead, the CLASSY family of chromatin remodeling factors or the Pol IV-interacting protein ZMP might provide such specificity, as they seem to control siRNA biogenesis and DNA methylation in a locus- and tissue-specific manner[40–42]. As downstream components of RdDM are not expressed in sperm cells[10], one possibility is that Pol IV-dependent siRNAs are sequestered only to participate in RdDM later in sperm development during pollen tube growth, or maybe after fertilization in the early zygote and endosperm. However, a recent analysis of parental siRNA contributions in the Arabidopsis endosperm does not support this idea, as there were no major effects in gene expression and DNA methylation caused by the loss of paternal siRNAs[43]. This raises yet another possibility that sperm small RNAs mediate PTGS mainly by translational repression, as previously shown with artificial miRNAs[36]. Independently of their mode of action, it is tempting to speculate that sperm-borne miRNAs and siRNAs are part of a surveillance mechanism that is primed to act only in situations of epigenetic instability caused by stress or hybridizations leading to bursts of TE expression and activity. These interesting questions may now be tested by using RTL1-mediated suppression of sperm siRNAs in different plant species and developmental contexts.

Methods

Plant materials, plasmid cloning and plant transformation

The Arabidopsis mutant lines *jas-3* (SAIL_813_H03) and *nrpd1a-3* (SALK_128428), as well as the fluorescence-tagged line FTL1230[25] were used in this study, and are all in Col-0 background. Plants were grown in greenhouse long-day conditions (16h light and 8h dark). The *pLAT52::RTL1-Myc* and *pMGH3::RTL1-Myc* constructs were generated by PCR amplification of the respective promoters from genomic DNA (primers listed in Extended Data Table 1), and cloned into the *p35S::RTL1-Myc* binary plasmid[19]. The 35S promoter was replaced using *Hind*III and *Xba*I restriction sites, and each plasmid was transformed into Arabidopsis Col-0 and *jas-3* mutants by floral dipping[44]. Transgenic seeds were surface sterilized with 50% bleach followed by 70% ethanol for 2 minutes, washed with sterile deionized water and sowed on agar plates (0.5X MS medium, 1% sucrose, pH 5.7) supplemented with cefotaxime (250mg/L, Duchefa) and hygromycin (25mg/L, Duchefa). Plates were placed in a growth chamber at 23°C, 70% humidity, 120 mE m^{-2} light with a 16-h light/8-h dark (long days) photoperiod for two weeks, and seedlings were then transferred to soil and grown in greenhouse long-day conditions to complete the life cycle (16h light and 8h dark).

RT-qPCR analysis

Pollen was purified by collecting open flowers into a 50mL falcon tube, vortexing for 3 minutes in 20mL of 100mM phosphate buffer, and filtering through a 50µm mesh. Pollen was then concentrated by centrifugation (3 min, 5000 RPM), and disrupted by shacking in the presence of glass beads (Sigma) with a Retsch homogenizer. Total RNA was then extracted using the Direct-zol RNA Microprep kit (Zymo)

following the manufacturer's instructions. Developing siliques were collected 7 days after pollination, and total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) following the manufacturer's recommendations for seed tissues (RLC buffer). 1µg of total RNA was then subjected to DNase (Invitrogen) treatment and converted into cDNA using Superscript II RT and random primers (Invitrogen). RT-qPCR was performed on a CFX Connect Real-Time PCR machine (BioRad) using SsoAdvanced Universal SYBR Green Supermix (BioRad). Primers used for RT-qPCR reaction are listed in Extended Data Table 1. *MGH3* and *ACT2* were used as internal controls.

Fluorescence-activated cell sorting

The FTL1230 line contains a pollen-expressed transgene genetically linked to *NRPD1a* (Extended Data Fig. 6), and is composed of a DsRed marker gene driven by the *LAT52* promoter[25]. This line was crossed with the homozygous mutant *nrpd1a-3*, and theresulting heterozygous *nrpd1a-3/+* F1 plants allowed purification of wild-type (DsRed positive) and mutant *nrpd1a-3* (DsRed negative) pollen by Fluorescence-Activated Cell Sorting (FACS) (Extended Data Fig. 6). Pollen was purified by collecting open flowers into Eppendorf tubes, vortexing in 2 mL of 100-mM sodium phosphate buffer (pH 7) for 3 min, and filtering through a 50µm nylon mesh (CellTrics, Sysmex). Pollen populations are characterized by an elevated high angle scatter (SSC) and autofluorescence, which were then separated based on the differences in DsRed signal intensity.

Small RNA sequencing and analysis

Library construction and sequencing was performed at BGI Genomics (Hong Kong). Single-end 50-nt reads were pre-processed by filtering collapsed reads according to length and quality. Filtered reads were mapped to the Arabidopsis TAIR10 genome with bowtie[45], reporting all multi-mappers. Only perfect-match reads were used for all downstream analysis. Reads were normalized by dividing non-redundant read counts by the number of genomic hits, and subsequently calculating the number of reads per million of filtered (18-30 nt) and perfectly mapped reads. Differential expression analysis was performed with the R package DESeq2[46] using an FDR adjusted p-value of 0.05. Additional downstream analyses and plots were done with custom R scripts. Graphical outputs were produced using the R package “ggplot2”. A summary of all small-RNA sequencing data generated in this study is presented in Supplementary Data 5, and is publicly available in the NCBI’s Gene Expression Omnibus under the accession number GSE231668.

Whole-genome bisulfite sequencing and DNA methylation analysis

Genomic DNA was purified from total pollen, and library preparation and sequencing were performed by BGI Genomics (Hong Kong) as paired-end 100bp reads using DNBSEQ technology. Pre-processed and high-quality reads were mapped to the TAIR10 genome using bismark with default settings for paired-end libraries[47], and all figures and downstream analysis were performed using custom R scripts. Graphical outputs were produced using the R package “ggplot2” and “ComplexHeatmap”. Methylome data from

nrpd1a-3 seedlings was already available[48]. DMRs were defined as 100-bp bins containing at least four, five or six differentially methylated CGs, CHGs or CHH and with an absolute methylation difference of at least 0.4, 0.35 or 0.25, respectively. Bins localizing within 200 bp of each other were merged and considered as DMRs. A summary of all WGBS sequencing data generated in this study is presented in Supplementary Data 5, and is publicly available in the NCBI's Gene Expression Omnibus under the accession number GSE231667.

RNA sequencing and analysis

Sequencing of messenger RNA was performed by BGI Genomics (Hong Kong) as paired-end 100bp reads using DNBSEQ technology. High-quality raw reads were aligned to the TAIR10 genome using STAR[49]. Normalization and analysis of differential gene expression was performed using the R package DESeq2[46], with an FDR adjusted p-value of 0.05. Graphical outputs were produced using the R packages "ggplot2", "pheatmap", and "ComplexHeatmap". A summary of all RNA sequencing data generated in this study is presented in Supplementary Data 5, and is publicly available in the NCBI's Gene Expression Omnibus under the accession number GSE231669.

Triploid block quantification and statistical analysis

The triploid block response in *jas-3* lines was quantified by imaging dry seeds from six siliques under a stereoscopic microscope (Nikon), and counting the number of normal and collapsed seeds. Statistically significant differences in the percentage of collapsed seeds were calculated by one-way analysis of variance (ANOVA) with a post hoc Dunnett test, using the R packages "ggpubr" and "multcomp".

Declarations

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Author contributions

F.B. designed the study; K.P. and M.S. performed the experiments; F.B. analyzed the data and wrote the manuscript with contributions from K.P. and M.S.

Competing interests

The authors declare no competing financial interests.

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Figures

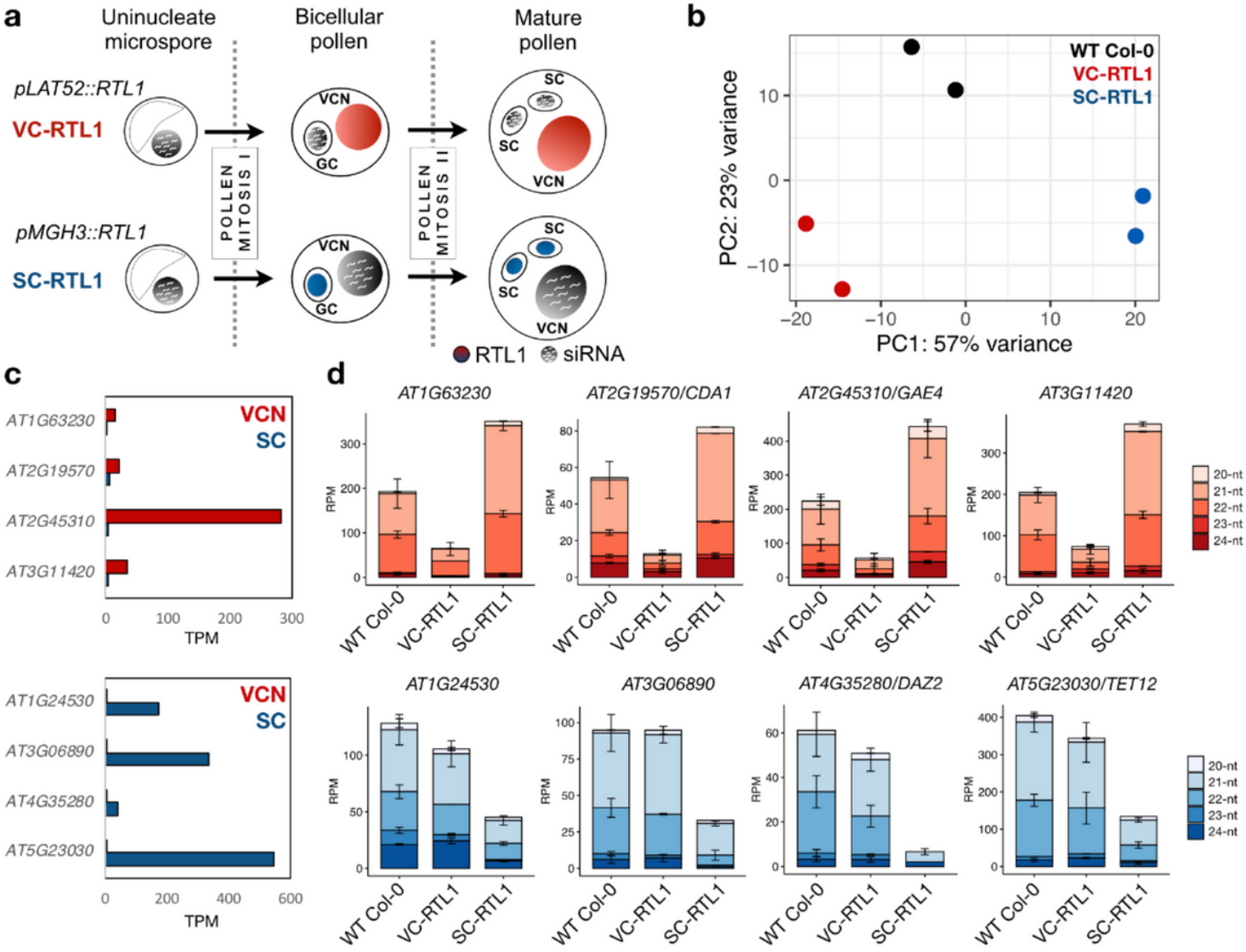

Figure 1

Ectopic expression of *RTL1* suppresses siRNA biogenesis in Arabidopsis pollen.

a, Schematic representation of *RTL1* expression in pollen driven by the *pLAT52* and *pMGH3* promoters that are specifically expressed in the vegetative cell and sperm cells, respectively. *pLAT52::RTL1*and *pMGH3::RTL1* transgenic lines are designated as VC-RTL1 and SC-RTL1, respectively. VCN is vegetative cell nucleus, GC is generative cell and SC is sperm cell. **b**, Principal component analysis shows reproducibility of small RNA sequencing experiments for two independent transgenic lines of each construct and wild-type Col-0, and highlights variance along PC1 and PC2 between all sample types. **c**, Protein-coding genes expressed in either the vegetative cell or sperm cells were selected based on their ability to produce 21/22-nt siRNAs in wild-type Col-0 pollen. TPM is transcripts per million. **d**, Genes expressed in the pollen vegetative cell show reduced levels of 21/22-nt siRNA in VC-RTL1, while genes expressed in sperm cells showed reduced levels of 21/22-nt siRNA only in SC-RTL1 pollen. Error bars represent the standard error, and RPM is reads per million.

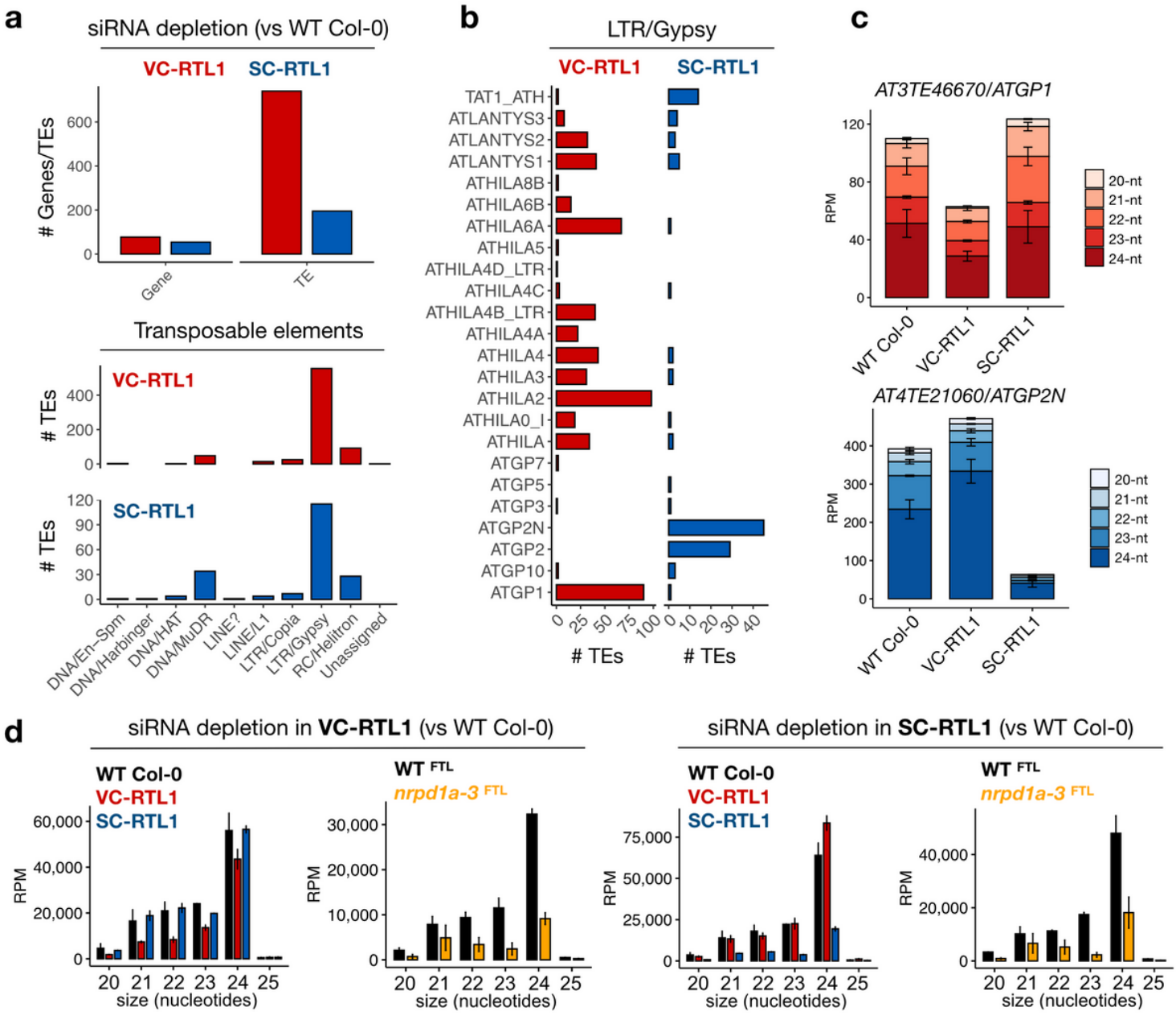

Figure 2

TE-derived siRNAs are differentially expressed in the sperm and vegetative cell lineages via Pol IV.

a, Comparative siRNA analysis between transgenic and WT Col-0 pollen shows that siRNA depletion in VC-RTL1/SC-RTL1 pollen occurs primarily at TEs. Among all TEs annotated in the Arabidopsis TAIR10 genome, the LTR/Gypsy superfamily was found over-represented in the lists of differentially expressed siRNA loci. **b**, Different members of the LTR/Gypsy superfamily showed significantly reduced siRNA levels in VC-RTL1 or SC-RTL1 pollen. **c**, Many retrotransposons from the *ATGP1*family lost siRNAs specifically in VC-RTL1 pollen, while *ATGP2* and *ATGP2N* lost siRNAs specifically in SC-RTL1 pollen. **c**, siRNAs depleted in VC-RTL1 or SC-RTL1 pollen are dependent on Pol IV activity. Error bars represent the standard error, and RPM is reads per million.

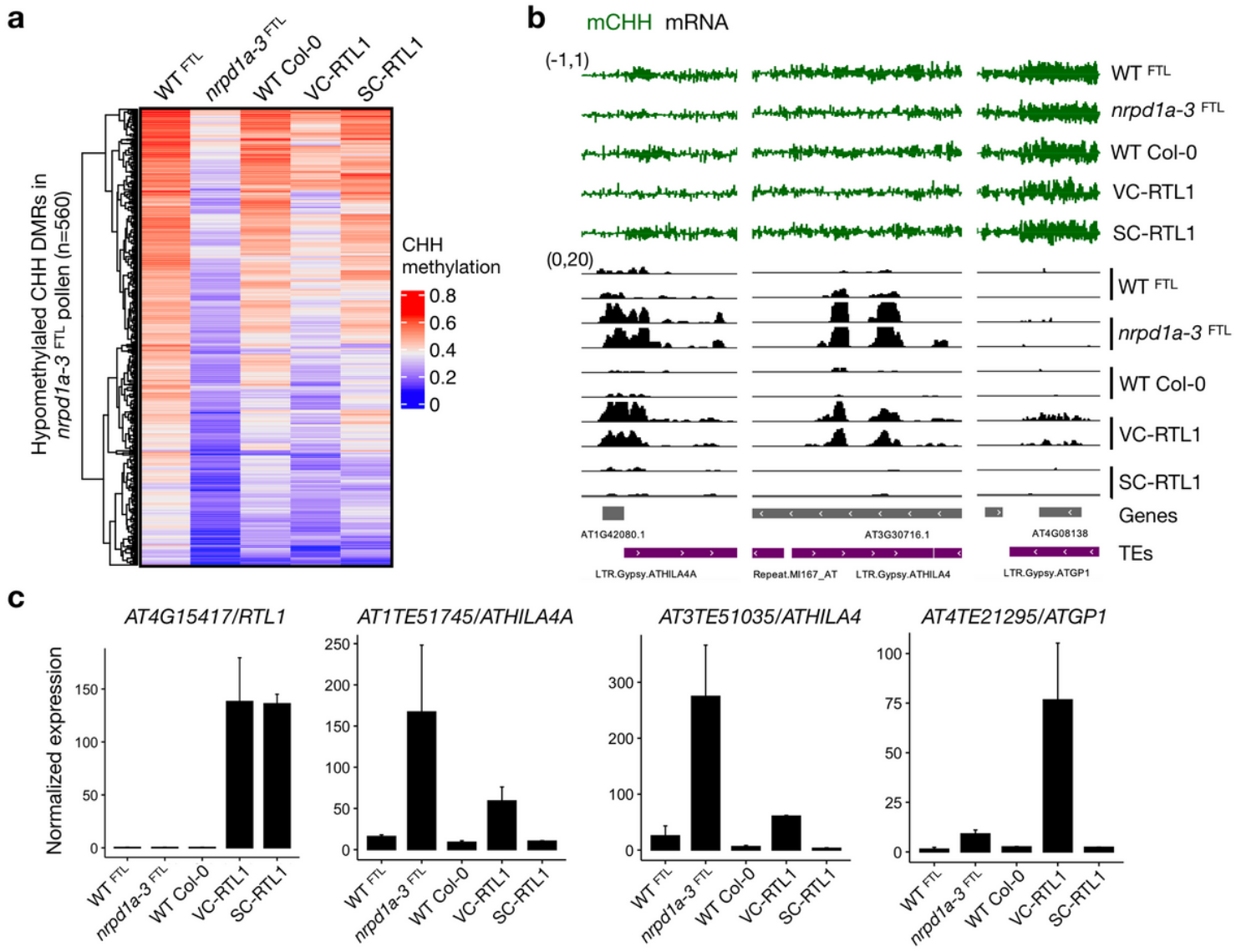

Figure 3

Targeted suppression of siRNA biogenesis in the vegetative cell impacts the pollen epigenome.

a, Heatmap representation of CHH methylation levels at 560 hypomethylated DMRs in *nrpd1a-3*^{FTL} pollen (as compared to WT^{FTL}) shows strong overlap with reduced CHH levels in VC-RTL1 pollen, while SC-RTL1 remains similar to wild-type Col-0. **b**, Genome browser tracks show partial CHH hypomethylation in *nrpd1a-3*^{FTL} and VC-RTL1 pollen, which is sufficient to increase expression of retrotransposons. **c**, RNA sequencing confirmed *RTL1* expression in both VC-RTL1 and SC-RTL1 pollen, but up-regulation of LTR/Gypsy retroelements from the *ATHILA4A*, *ATHILA4* and *ATGP1* families occurs only in VC-RTL1 and *nrpd1a-3*^{FTL} pollen. Error bars represent the standard error, and normalized expression values were calculated by DESeq2.

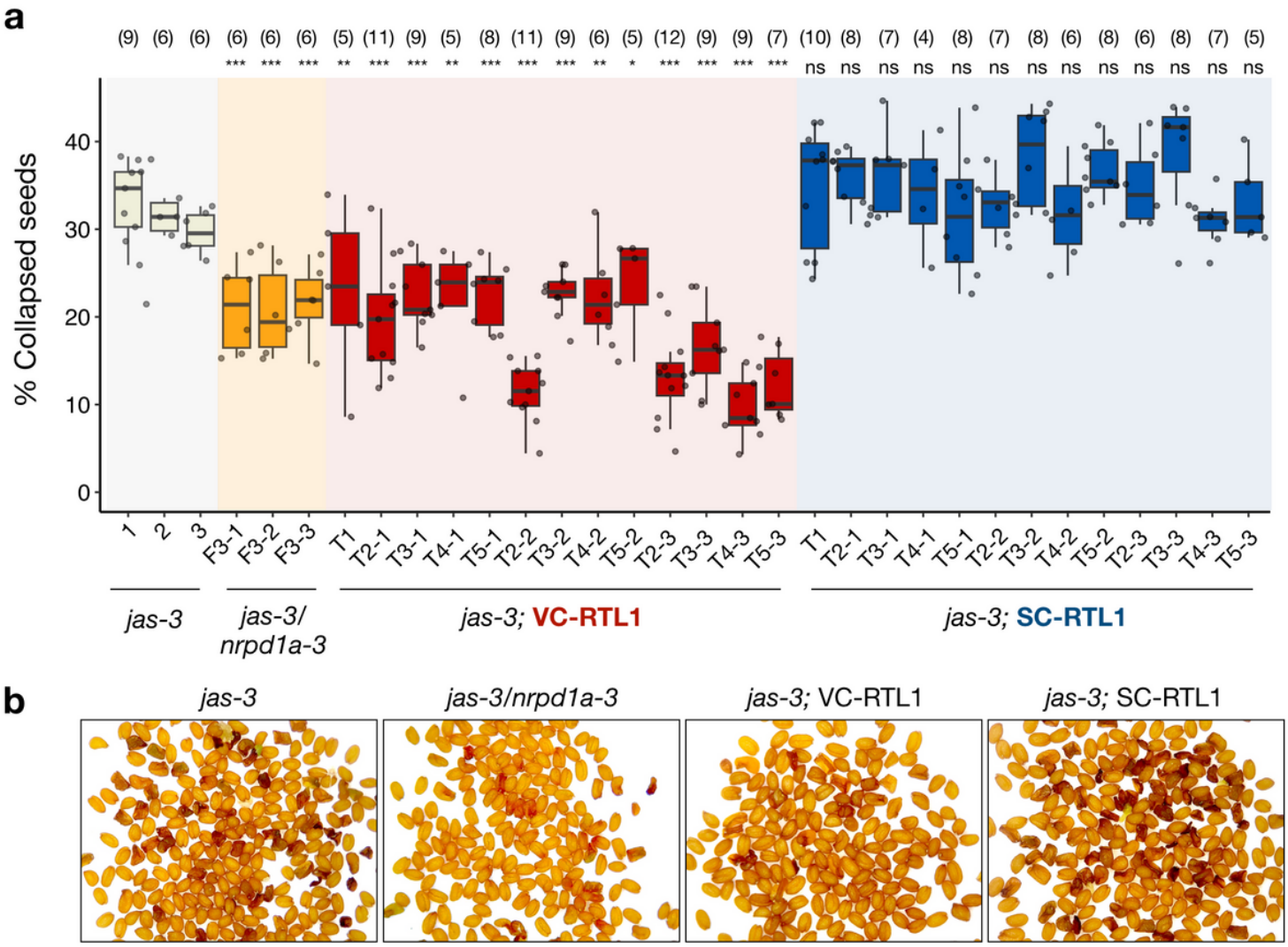

Figure 4

TE-derived siRNAs involved in the triploid block are produced in the pollen vegetative cell.

a, The triploid block response in *jas-3* mutants was quantified by counting the number of collapsed seeds in six siliques of selfed plants that reflect the amount of diploid pollen produced in this mutant (30 to 40%). Seed abortion is significantly reduced in *jas-3/nrpd1a-3* double mutant plants in the F_3 generation, and also across five consecutive generations of three independent *jas-3*;VC-RTL1 lines, while *jas-3*;SC-RTL1 remains similar to *jas-3*. Numbers above each box represent the number of individual plants used. Statistically significant differences in the percentage of collapsed seeds were calculated by ANOVA with a post hoc Dunnett test, using *jas-3* as the reference group (ns is not significant, * is $P < 0.05$, ** is $P < 0.01$, *** is $P < 0.001$). Boxes represent the interquartile range (IQR) showing the lower (Q1) and upper (Q3) quartiles surrounding the median (central line), and whiskers represent the minimum (Q1 −1.5*IQR) and maximum (Q3 + 1.5*IQR) values. **b**, Representative images of seeds show lower levels of seed abortion in suppressor lines *jas-3/nrpd1a-3* and *jas-3*;VC-RTL1, in comparison with a non-suppressor line *jas-3*;SC-RTL1 and *jas-3* controls.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryData1.xlsx
- SupplementaryData2.xlsx
- SupplementaryData3.xlsx
- SupplementaryData4.xlsx
- SupplementaryData5.xlsx
- ExtendedData.docx