PNAS

Supplementary Information for

DNA polymerase epsilon interacts with SUVH2/9 to repress the expression of genes associated with meiotic DSB hotspot in *Arabidopsis*

Cong Wang[1], Jiyue Huang[2], Jun Zhang[1], Yue Yu[1], Gregory P. Copenhaver[3, 4], Chenjiang You[1]* and Yingxiang Wang[1,2]*

Correspondence: Y.W.: yx_wang@fudan.edu.cn; C.Y.: cjyou@fudan.edu.cn

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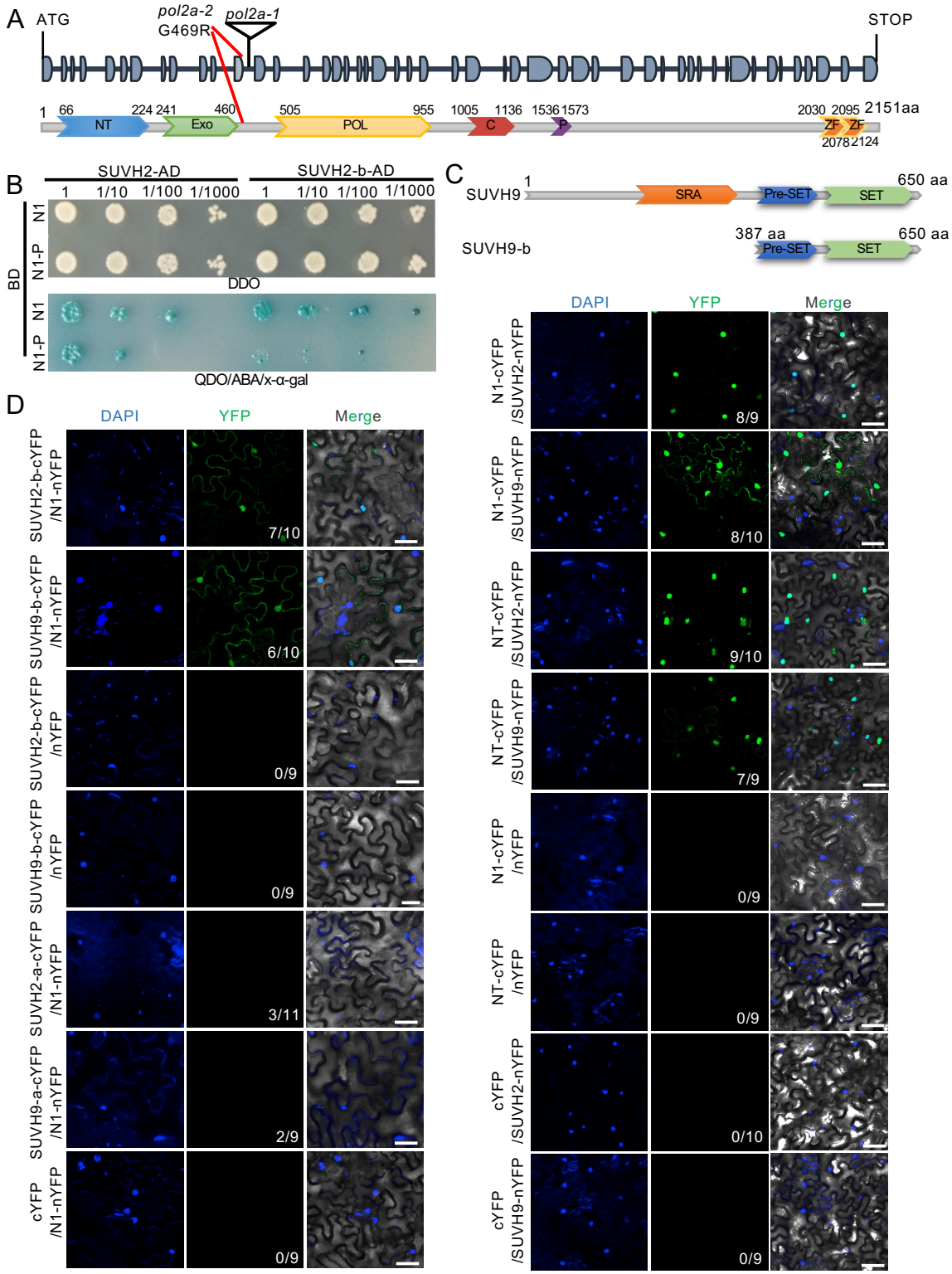


Fig. S1. The interaction assay between POL2A and SUVH2 or SUVH9

(A) Diagrams showing the full-length genomic and domain structures of POL2A with the two *pol2a* mutants. *Pol2a-1* with T-DNA insertion in N-terminal 12th intron dramatically decrease the expression of POL2A and *pol2a-2* harboring a point mutation near EXO (exonuclease) domain. (B) Diagrams showing the full-length and truncated forms of SUVH9. (C) Point mutation (*pol2a-2*) of N1 attenuates the interaction with SUVH2, detected by yeast two-hybrid assay using gradient dilution. N1 showing interaction with SUVH2/SUVH2-b at low concentrations “1/100”or “1/1000”, but N1-P not. The initial concentration of “1” means “$OD_{600} \approx 0.2$”. (D) The interaction between N1/NT of POL2A and SUVH2/SUVH9 or SUVH2-b/SUVH9-b as verified by BiFC assay. At least 3 individual plants are used for infiltration. Numbers in the bottom right corners indicate the number of positive interactions / all leaves for infiltration.

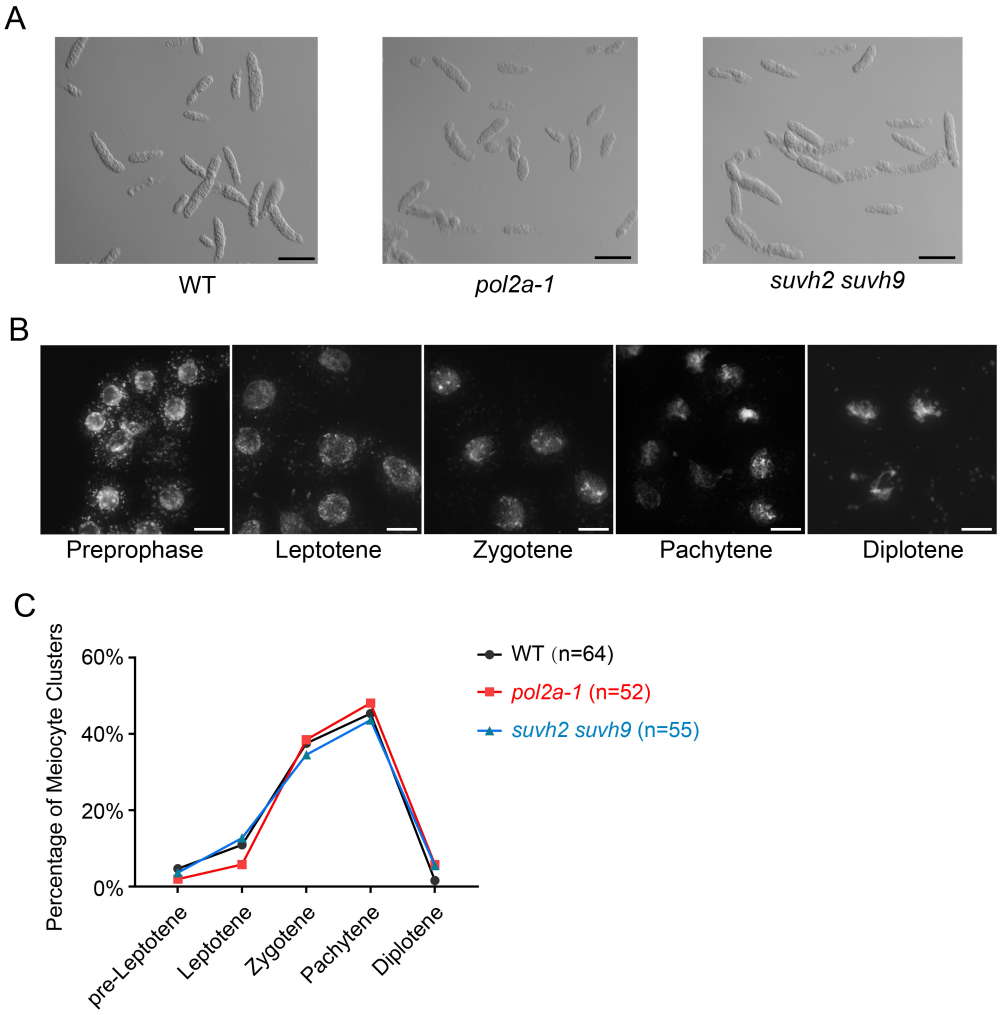


Fig. S2. Meiotic stage assay of isolated meiocytes from WT, *pol2a-1,* and *suvh2 suvh9*
(A) Male meiocyte clusters isolated from stage 9-10 flower buds from WT, *pol2a-1* and *suvh2 suvh9*. (B) Meiotic chromosome morphology of meiocyte clusters. (C) Percentages of preprophase, leptotene, zygotene, pachytene, and diplotene meiocyte clusters in WT, *pol2a-1*, and *suvh2 suvh9*. Scale bars: a, 100 μm, b, 10 μm.

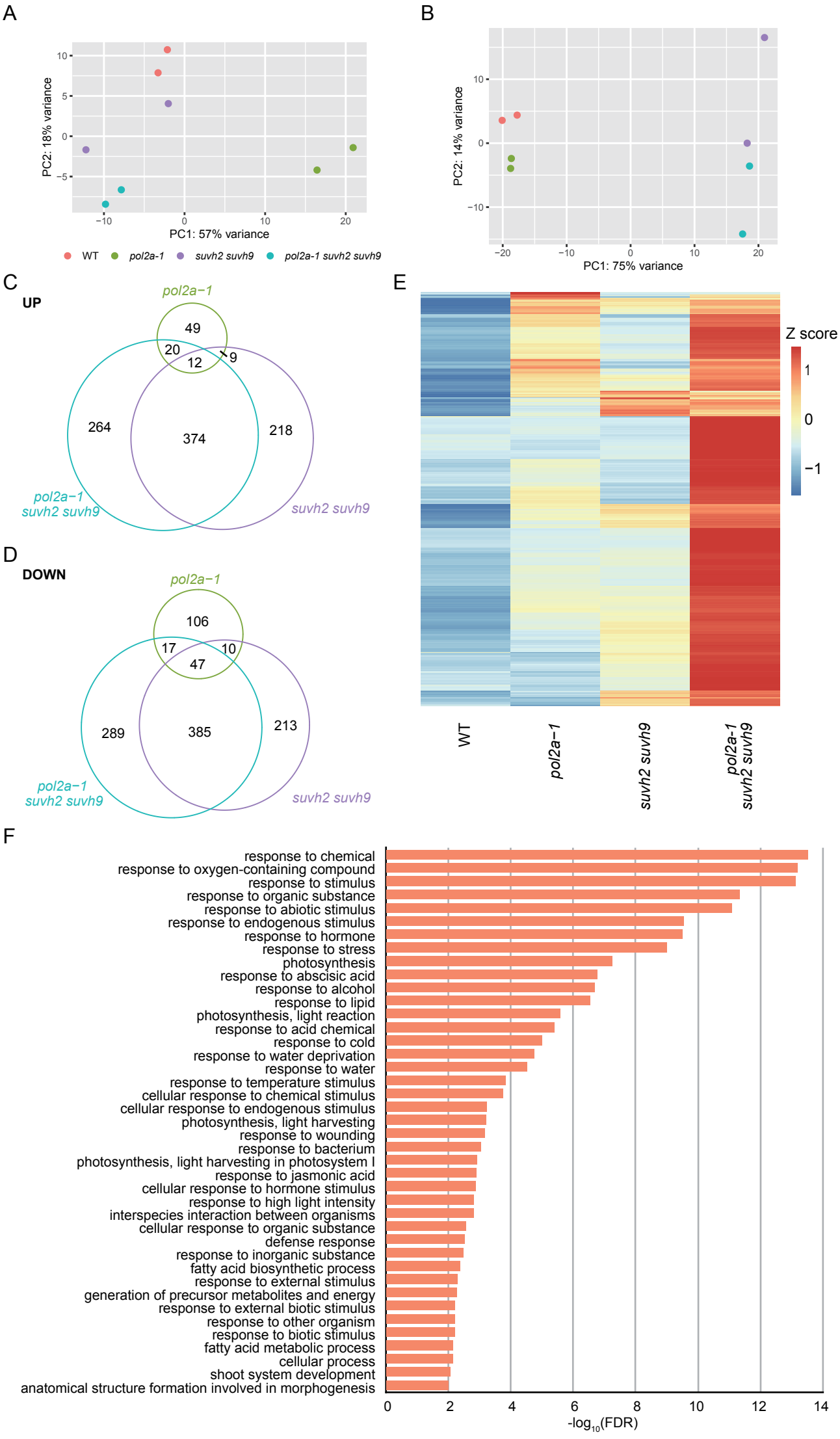

A
PC2: 18% variance
PC1: 57% variance
WT
pol2a-1
suvh2 suvh9
pol2a-1 suvh2 suvh9
B
PC2: 14% variance
PC1: 75% variance
C
UP
pol2a−1
49
20
12
9
264
374
218
pol2a−1
suvh2 suvh9
suvh2 suvh9
D
DOWN
pol2a−1
106
17
10
47
289
385
213
pol2a−1
suvh2 suvh9
suvh2 suvh9
E
Z score
1
0
−1
WT
pol2a−1
suvh2 suvh9
pol2a-1
suvh2 suvh9
F
response to chemical
response to oxygen-containing compound
response to stimulus
response to organic substance
response to abiotic stimulus
response to endogenous stimulus
response to hormone
response to stress
photosynthesis
response to abscisic acid
response to alcohol
response to lipid
photosynthesis, light reaction
response to acid chemical
response to cold
response to water deprivation
response to water
response to temperature stimulus
cellular response to chemical stimulus
cellular response to endogenous stimulus
photosynthesis, light harvesting
response to wounding
response to bacterium
photosynthesis, light harvesting in photosystem I
response to jasmonic acid
cellular response to hormone stimulus
response to high light intensity
interspecies interaction between organisms
cellular response to organic substance
defense response
response to inorganic substance
fatty acid biosynthetic process
response to external stimulus
generation of precursor metabolites and energy
response to external biotic stimulus
response to other organism
response to biotic stimulus
fatty acid metabolic process
cellular process
shoot system development
anatomical structure formation involved in morphogenesis
-log10(FDR)

Fig. S3. Transcription analysis of meiocytes and seedlings in *pol2a-1*, *suvh2 suvh9* and *pol2a-1 suvh2 suvh9* mutants.

(A) PCA results showing the reproducibility of mRNA sequencing data in meiocytes of WT, *pol2a-1*, *suvh2 suvh9* and *pol2a-1 suvh2 suvh9*. (B) PCA results showing the reproducibility of mRNA sequencing data of seedlings in WT, *pol2a-1*, *suvh2 suvh9* and *pol2a-1 suvh2 suvh9*. (C) Venn diagram showing up-regulated genes in seedlings of each mutant compared to WT. (D) Venn diagram for down-regulated genes in seedlings of each mutant compared to WT. (E) Heatmap for the expression level in FPKM of 865 up-regulated genes of WT and in each mutant. (F) Gene ontology enrichment analysis of 865 up-regulated gene in meiocytes of *pol2a-1 suvh2 suvh9*.

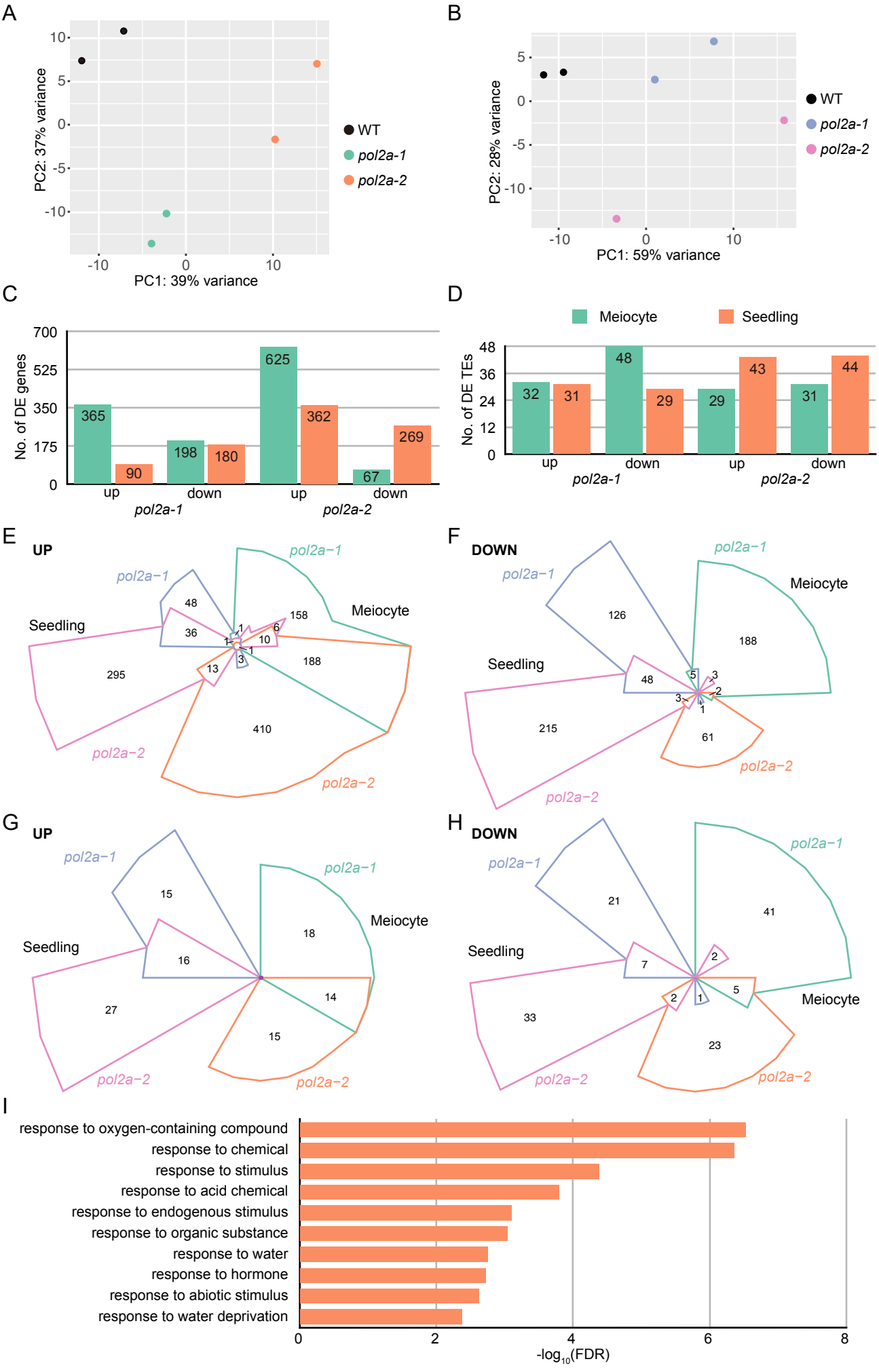

A
PC2: 37% variance
PC1: 39% variance
WT
pol2a-1
pol2a-2
B
PC2: 28% variance
PC1: 59% variance
C
No. of DE genes
365
90
198
180
625
362
67
269
up
down
pol2a-1
pol2a-2
D
Meiocyte
Seedling
No. of DE TEs
32
31
48
29
29
43
31
44
E
UP
Seedling
Meiocyte
48
36
158
10
188
295
13
410
F
DOWN
126
188
48
215
61
G
UP
15
18
16
27
14
15
H
DOWN
21
41
7
2
33
23
I
response to oxygen-containing compound
response to chemical
response to stimulus
response to acid chemical
response to endogenous stimulus
response to organic substance
response to water
response to hormone
response to abiotic stimulus
response to water deprivation
-log10(FDR)

Fig. S4. Transcription analysis of meiocytes and seedlings in *pol2a* mutants.
(A) PCA results showing the reproducibility of mRNA sequencing data in meiocytes of WT, *pol2a-1* and *pol2a-2*. (B) PCA results showing the reproducibility of mRNA sequencing data of seedlings in WT, *pol2a-1* and *pol2a-2*. (C) Bar plot showing DEGs in meiocytes and seedlings of *pol2a-1* and *pol2a-2* compared to WT. (D) Diagram showing DE TEs in meiocytes and seedlings of *pol2a-1* and *pol2a-2* compared to WT. (E) Venn diagram for up-regulated genes in meiocytes and seedlings of *pol2a-1* and *pol2a-2* compared to WT. (F) Venn diagram for down-regulated genes in meiocytes and seedlings of *pol2a-1* and *pol2a-2* compared to WT. (G) Venn diagram for up-regulated TEs in meiocytes and seedlings of *pol2a-1* and *pol2a-2* compared to WT. (H) Venn diagram for down-regulated TEs in meiocytes and seedlings of *pol2a-1* and *pol2a-2* compared to WT. (I) Gene ontology enrichment analysis of all up-regulated gene in meiocytes of *pol2a-1 and pol2a-2*.

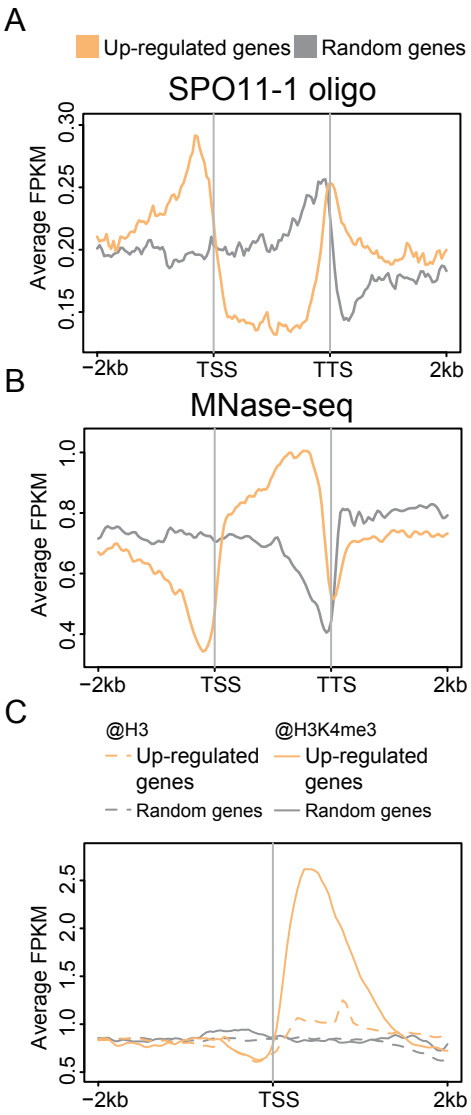


Fig. S5. The 865 up-regulated genes by POL2A-SUVH2/9 have features of meiotic DSB hotspots
(A-B) Density of SPO11-1 oligos (A) and nucleosome occupancy (B) around the 865 genes upregulated in WT. (C) Distribution of H3K4me3 along the 865 upregulated genes and random genes in WT.

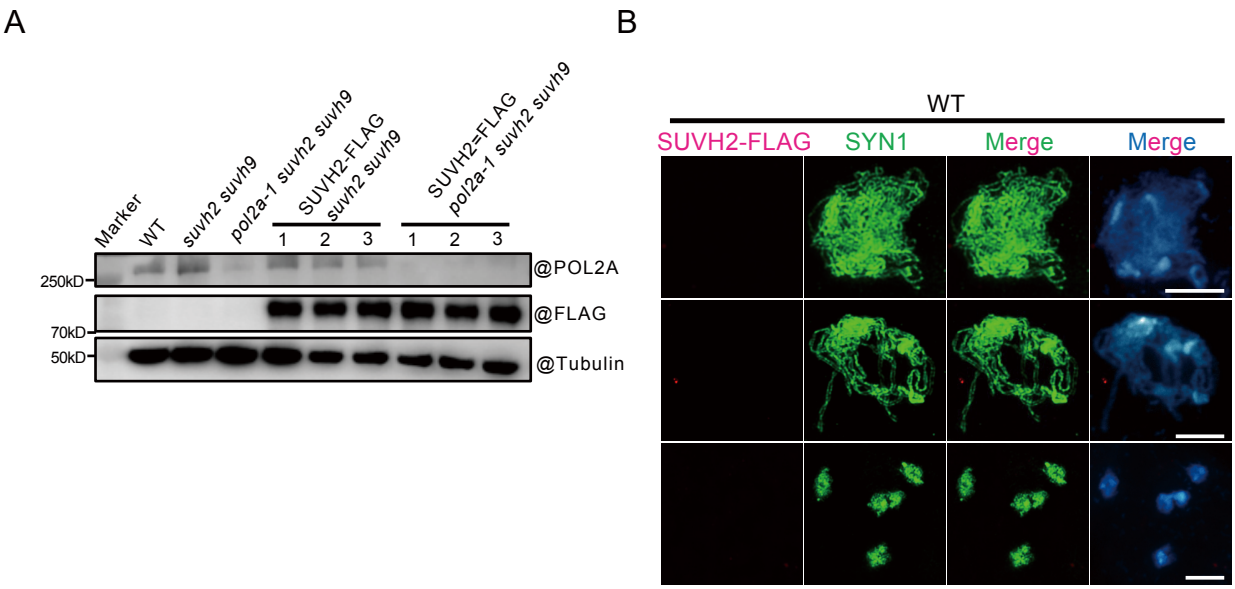


Fig. S6. The endogenous expression of SUVH2-FLAG in transgenic plants.
(A) Immunoblotting to measure SUVH2-FLAG expression of inflorescences in *suvh2 suvh9* and *pol2a-1 suvh2 suvh9* backgrounds using the FLAG antibody. Each replicate is the proteins extracted from inflorescences of one individual plant. Tubulin is used as a loading control and POL2A indicates its endogenous expression in each plant. (B) Immunostaining of FLAG and SYN1 in WT is used as a negative control. Scale bars: 5 μm.

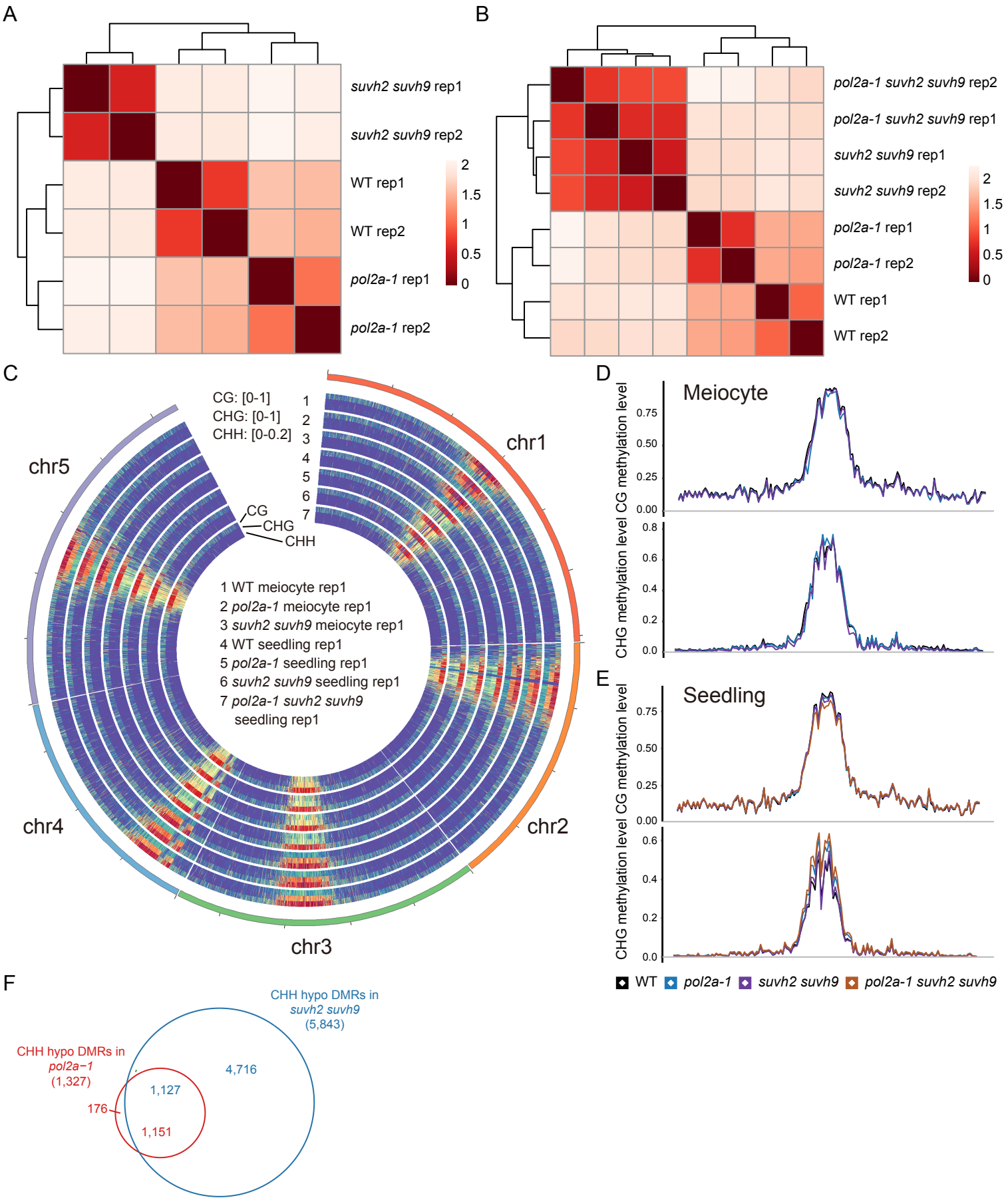


Fig. S7. DNA methylation profiles in meiocytes and seedlings

(A) Distance matrix of global DNA methylation levels showing the repeatablity of in two biological replicates in meiocytes of WT, *pol2a-1*, and *suvh2 suvh9*. (B) Distance matrix of global DNA methylation levels showing the repeatablity of in two biological replicates in seedlings of WT, *pol2a-1*, *suvh2 suvh9* and *pol2a-1 suvh2 suvh9*. (C) A circos plot showing the DNA methyaltion landscape for all three contexts in both meiocytes and seedlings of WT, *pol2a-1*, and *suvh2 suvh9*, and also in seedlings of *pol2a-1 suvh2 suvh9*. (D-E) The DNA methylation profiles of chromosome 1 in the CG and CHG contexts in meiocytes (D) and seedlings (E) of WT (black), *pol2a-1* (blue), *suvh2 suvh9* (purple) and *pol2a-1 suvh2 suvh9* (brown). (F) Venn diagram showing the CHH hypo DMRs in *pol2a-1*, and *suvh2 suvh9* compared to WT. Most POL2A-regulated hypomethylated regions are also hypomethylated in *suvh2 suvh9*.

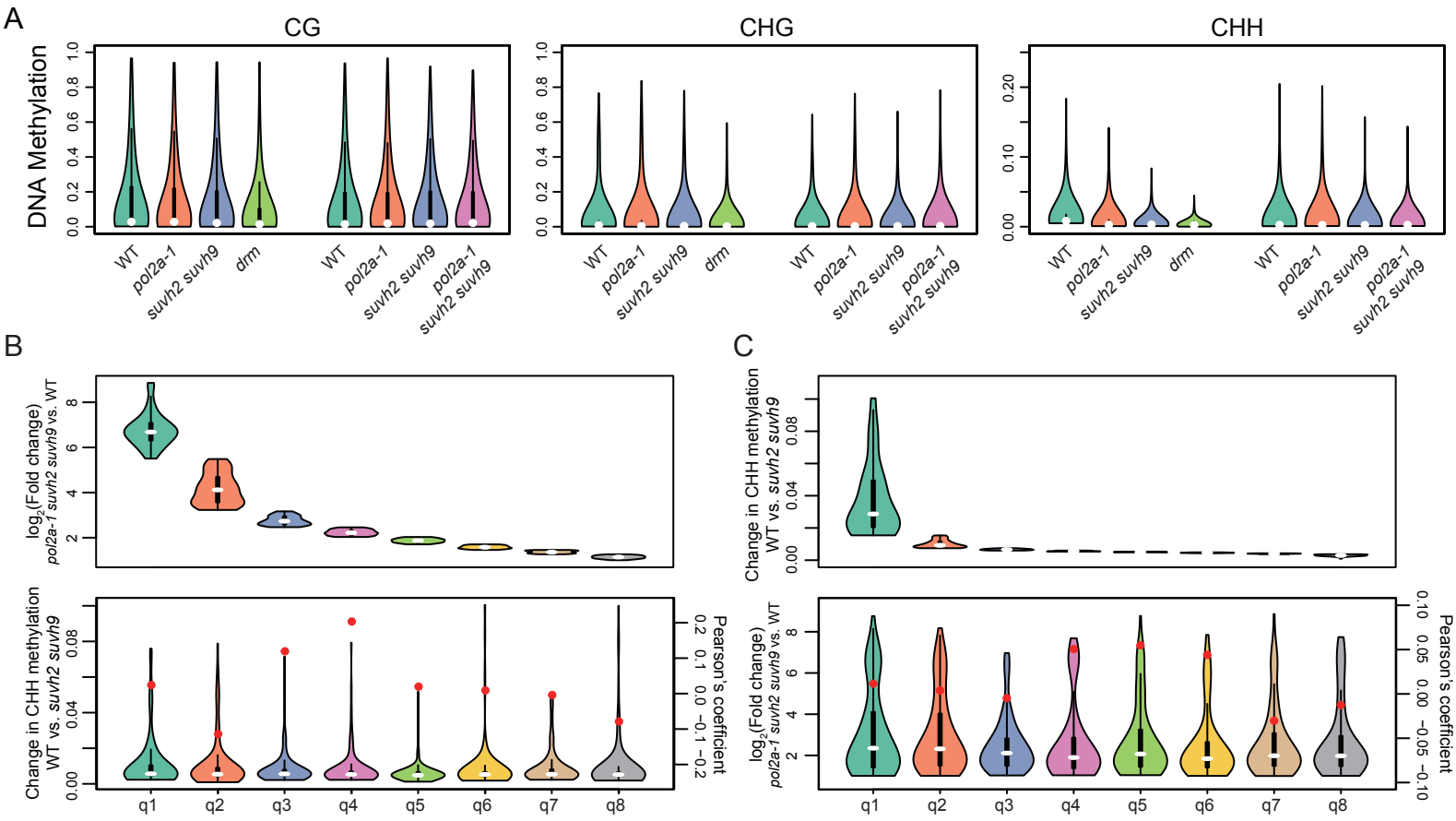


Fig. S8. CHH methylation on 865 up-regulated genes is not positively correlated with their expression.
(A) Violin plots for the CG, CHG and CHH methylation on promoters of 865 up-regulated genes in *pol2a-1 suvh2 suvh9* compared to WT. (B-C) Correlation analysis of CHH methylation in promoters and expression of 865 up-regulated genes in *pol2a-1 suvh2 suvh9* compared to WT. Genes were divided into 8 quantiles, each of which contains around 108 genes, either according to their fold changes of expression between *pol2a-1 suvh2 suvh9* and WT (D), or according to the methylation changes between WT and *suvh2 suvh9* (E). In both assays, the Pearson's correlation coefficient between methylation changes and expression changes in each quantile is low, ranging from -0.11 to 0.20. Coefficients are represented by red dots designated to the secondary axis.

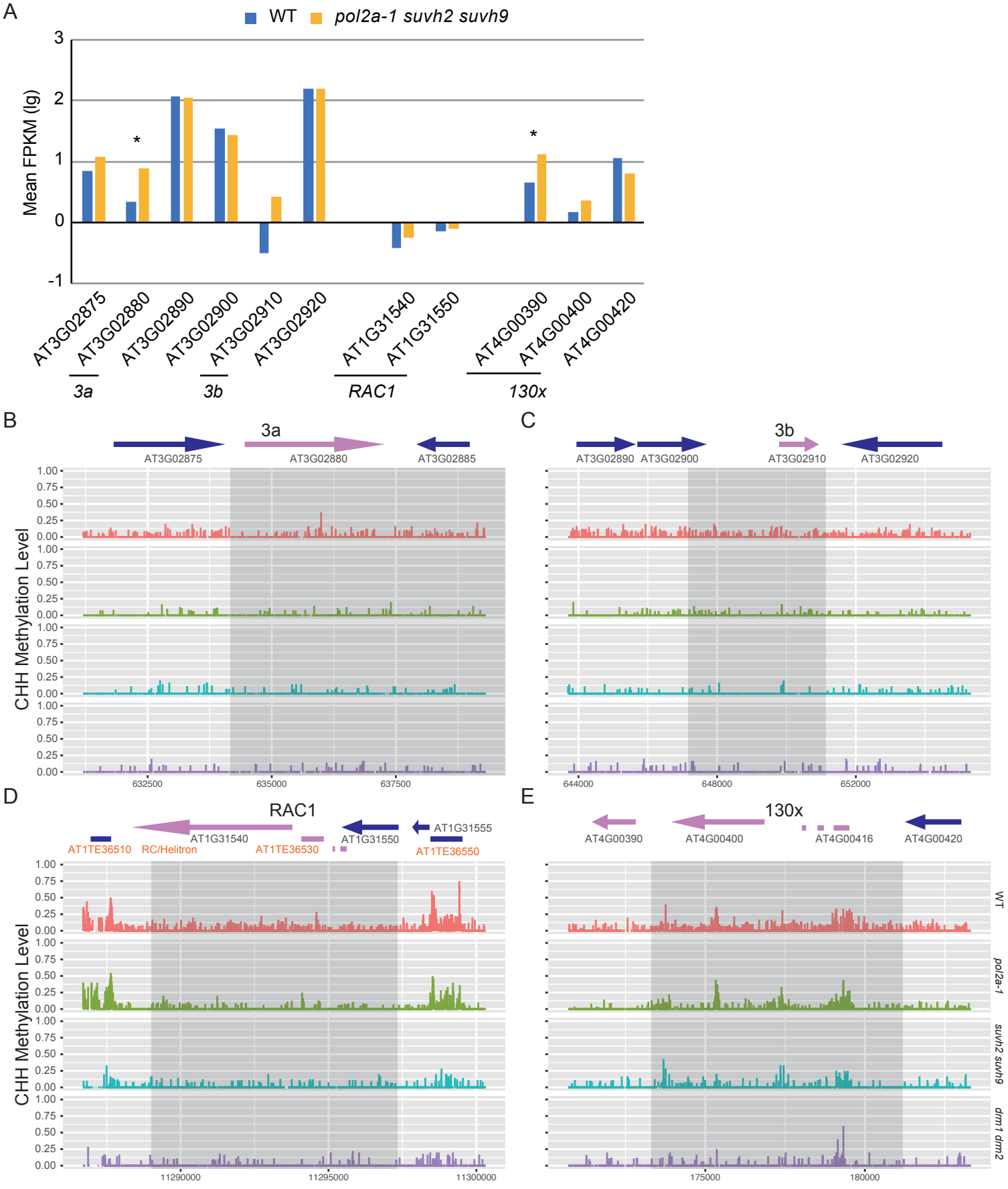
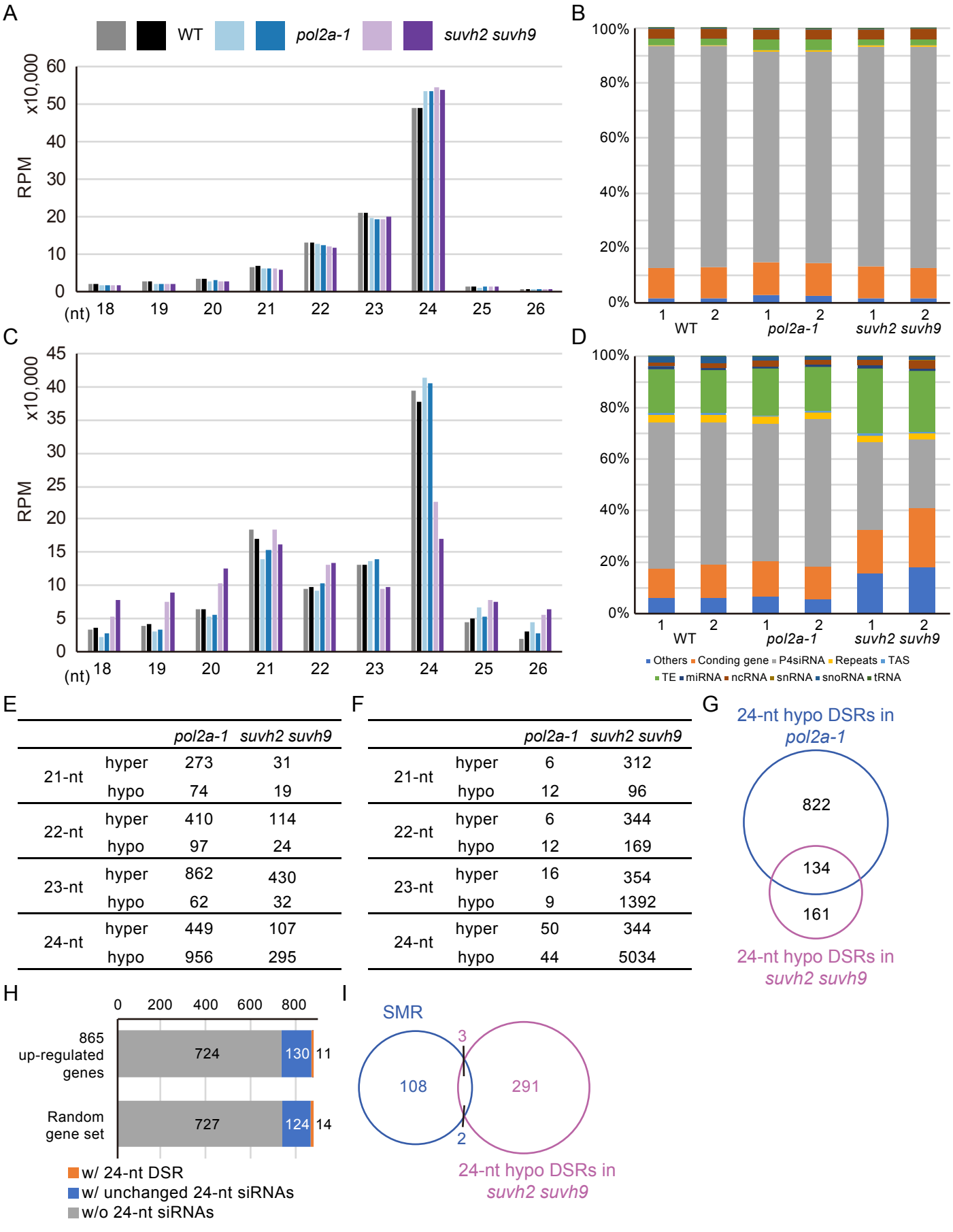


Fig. S9. The transcript and DNA methylation level of the known recombination hotspots 3a, 3b, RAC1, and 130x in meiocytes of each mutant.

(A) The RNA transcript levels of the genes within or near the 3a, 3b, RAC1, and 130x regions in meiocytes from *pol2a-1 suvh2 suvh9*, which is known as DSB hotspots. *: P <0.01. AT3G02880 is a gene encoding an NBS-LRR protein, which is commonly associated with DSBs. Genes whose promoters located in hotspots are underlined. (B-E) Snapshots showing CHH methylation at the 3a (B), 3b (C), RAC1 (D), and 130x (E) regions in meiocytes of WT, *pol2a-1*, *suvh2 suvh9* and *drm1 drm2*. Shadow regions refer to the reported DSB hotspots. Gene and TE positions are labelled above plots.



E

		pol2a-1	*suvh2 suvh9*
21-nt	hyper	273	31
	hypo	74	19
22-nt	hyper	410	114
	hypo	97	24
23-nt	hyper	862	430
	hypo	62	32
24-nt	hyper	449	107
	hypo	956	295

F

		pol2a-1	*suvh2 suvh9*
21-nt	hyper	6	312
	hypo	12	96
22-nt	hyper	6	344
	hypo	12	169
23-nt	hyper	16	354
	hypo	9	1392
24-nt	hyper	50	344
	hypo	44	5034

Fig. S10. Comparison of sRNAs in meiocytes and seedlings of WT, *pol2a-1* and *suvh2 suvh9*

(A) Size distribution of small RNAs (sRNAs) in meiocytes of WT, *pol2a-1* and *suvh2 suvh9*. (B) Assigned genomic features of 24-nt siRNAs in meiocytes of WT, *pol2a-1* and *suvh2 suvh9*. (C) Size distribution of sRNAs in seedlings in WT, *pol2a-1* and *suvh2 suvh9*. (D) Assigned genomic features of 24-nt siRNAs in seedlings of WT, *pol2a-1* and *suvh2 suvh9*. (E-F) Number of 21-24 nt differential siRNA regions (DSRs) in meiocytes (E) and seedlings (F) of *pol2a-1* and *suvh2 suvh9* compared to WT. (G) Venn diagram showing the overlap between 24-nt hypo DSRs in *pol2a-1* and *suvh2 suvh9* compared to WT, respectively. (H) Number of promoters of 865 up-regulated genes and random gene sets.without 24-nt siRNAs, with unchanged 24-nt siRNAs, and with 24-nt hyper/hypo DSRs in *pol2a-1* vs. WT. (I) Venn diagram showing the overlap between SMRs and 24-nt hypo DSRs in *suvh2 suvh9* vs. WT.

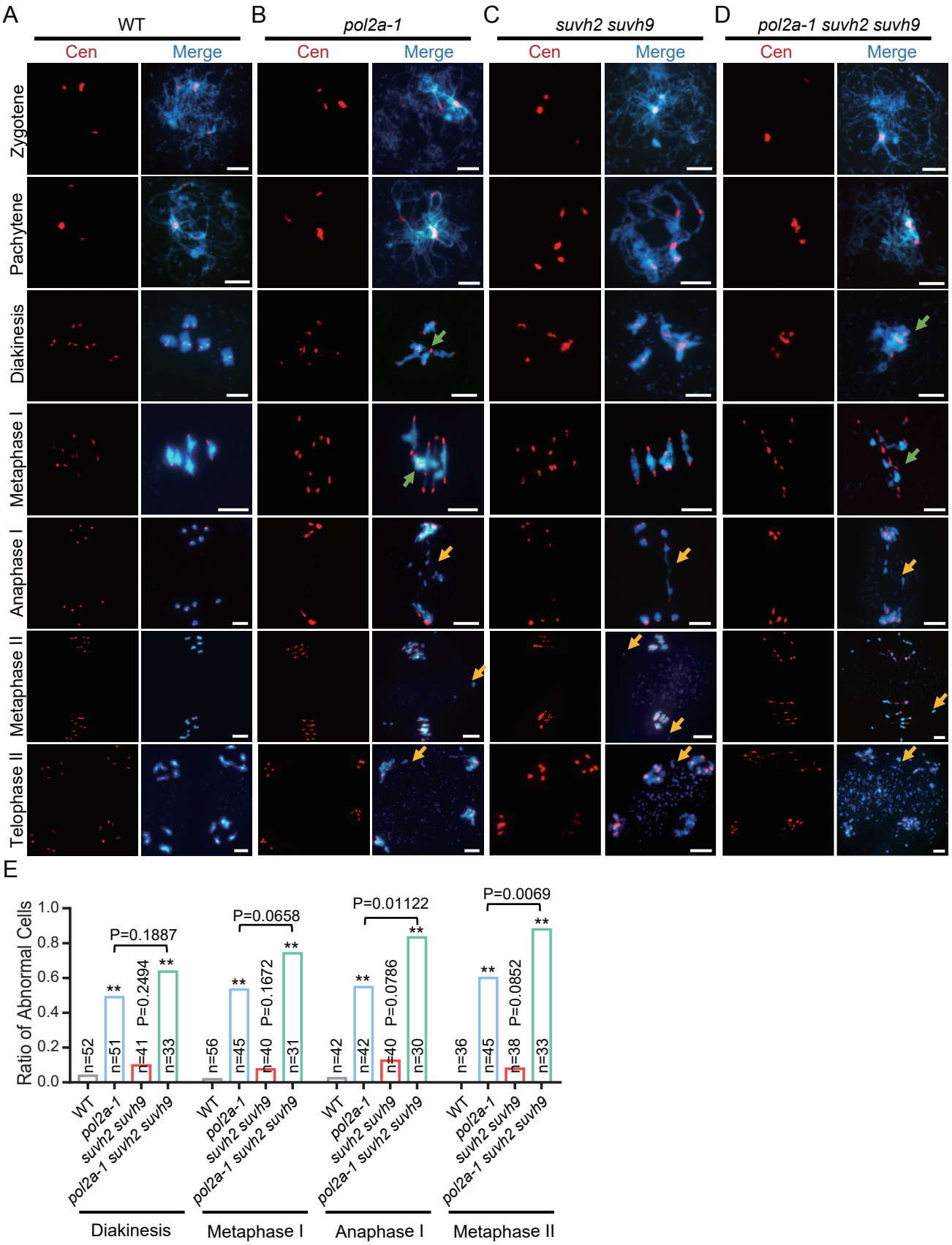


Fig. S11. Meiotic chromosome morphology in *pol2a-1, suvh2 suvh9*, and *pol2a-1 suvh2 suvh9*

(A-D) Meiotic chromosome morphology at zygotene, pachytene, diakinesis, metaphase I, anaphase I, metaphase II and telophase II of WT (A), *pol2a-1* (B), *suvh2 suvh9* (C) and *pol2a-1 suvh2 suvh9* (D). Green arrows indicate the association of non-homologs, and yellow arrows indicate the chromosomal fragmentations or bridge. Scale bars: 5 μm. (E) Bar plots showing ratios of abnormal cells in diakinesis, metaphase I, anaphase I, and metaphase II in WT and mutants. P values refer the comparison between WT and mutants unless specified. **: P < 0.01, χ2 test.

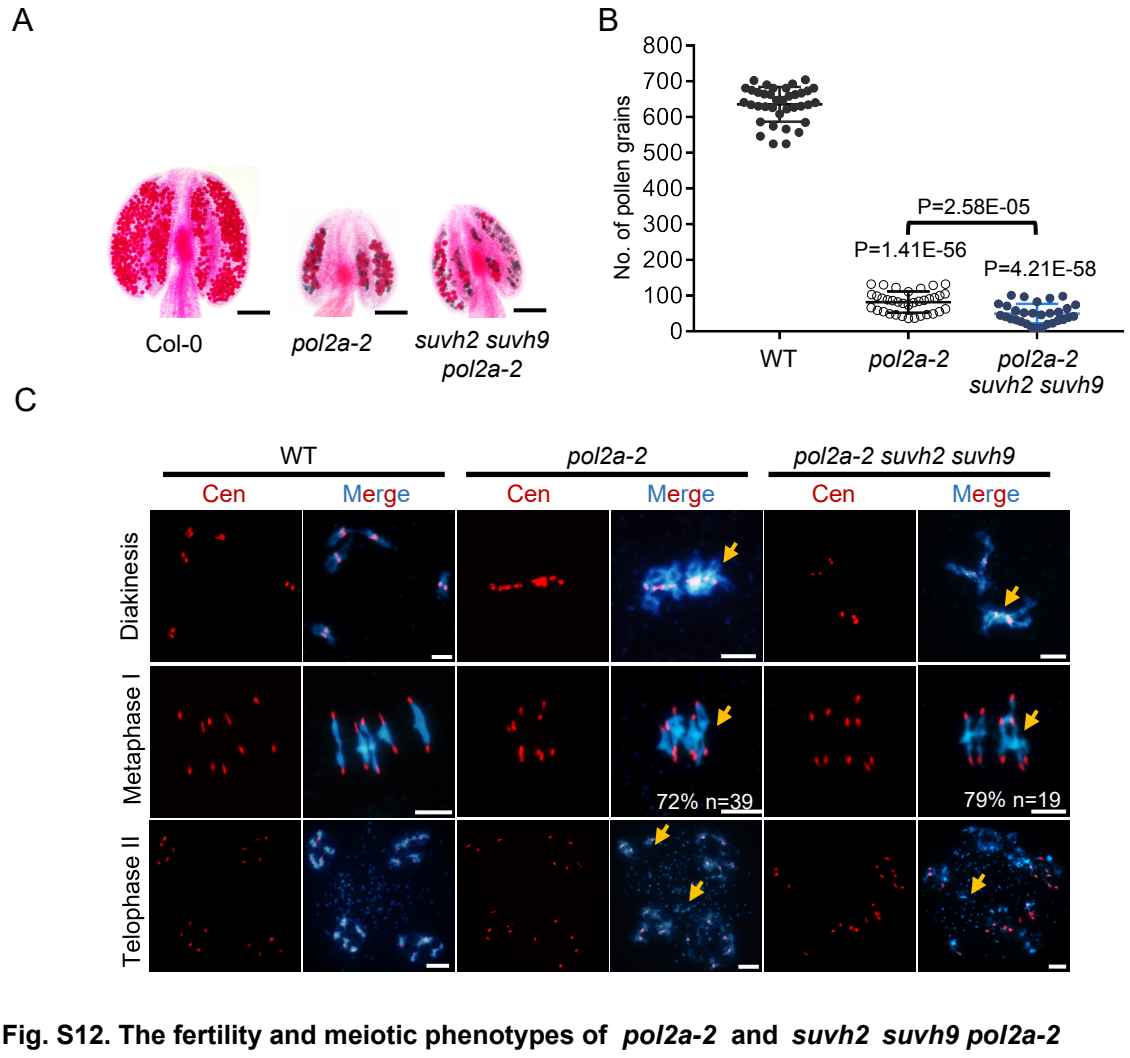


Fig. S12. The fertility and meiotic phenotypes of *pol2a-2* and *suvh2 suvh9 pol2a-2*
(A) Alexander red stained anthers of WT, *pol2a-2*, *and pol2a-2 suvh2 suvh9*. (B) The number of viable pollen grains of WT, *pol2a-2* and *pol2a-2 suvh2 suvh9*. Two-tailed Student's t-test. (C) Meiotic chromosome morphology at diakinesis, metaphase I and telophase II of WT, *pol2a-2*, *suvh2 suvh9* and *pol2a-2 suvh2 suvh9*. Yellow arrows indicate the nonhomology interaction and chromosome fragmentation. Numbers in the bottom right corners indicate the ratio of abnormal cells out of all cells counted. Scale bars: a, 1 mm; c, 5 μm. P values refer the comparison between WT and mutants unless specified.

Table S1. Oligonucleotides, related to Experimental Procedures

Oligonucleotide	Sequence (5'-3')
Primers for Transgenic Lines	
Suvh2pro-LR-F	CTATGACATGATTACGAATTCGAGCCTGGTTTTGAGAGAG
Suvh2pro-LR-R	GGATCCCCGGGTACCGAGCTCCCTTCCCAATGAACCGAAG
SUVH2-Kpn1-F	TCCGGTACCATGAGTACATTGTTACCATTTCCTG
SUVH2-SalI-R2	ATTGGTCGACGTTGCAGATGGCGAGCTTGC
Suvh9pro-LR-F	CTATGACATGATTACGAATTCCTATGAGACGCTTACAGGGGGTTG
Suvh9pro-LR-R	GGATCCCCGGGTACCGAGCTCTTTTTGCAACGAGAAGCG
SUVH9-BamHI-F	TCGGGATCCATGGGTTCTTCTCACATTCCTCTTG
SUVH9- SalI-R	GCGTCGACATTACAAATGGCAAGCTTGGCG
Primers for Yeast Two Hybird	
POL2A-N1-bait-EcoR1-F	TAAGAATTCAGCGGAGATAATCGAAGACGGGATC
POL2A-N1-bait-Sal1-R	CGCGTCGACTTTGTTGGGACATACAACATTTGCCTTG
N1-NT -BamHI-R1	CGAGGATCCTCAACGGACTTCTGCGCGTTGAAG
N1-EXO-EcoRI-F2	TCCGAATTCGTTTGTGCCTTCGATATAGAGACAAC
POL2A-N2-EcoR1-F	TAAGAATTCGCGATGGAAAAGCCCCAGACAAT
POL2A-N2-Sal1-R	CGCGTCGACTTTAATGAGTTTCAGCTCCCCTCTACGC
N1-G469R_F1	TGAGGTTTTACGTAAAAGGAGTGGCACC
N1-G469R_R1	TTTTACGTAAAACCTCATCAGGGACCAT
SUVH2-SmaI-F	TCCCCCGGGGATGAGTACATTGTTACCATTTCCTG
SUVH2-BamHI-R	CGGGATCCGTTGCAGATGGCGAGCTTGCC
SUVH2-a-BamHI-R	CGGGATCCTAGAAACACGGGTACATTCTCCTTC
SUVH2-b-SmaI-F	TCCCCCGGGGGACCTTTCTAACAAGAAGGAGA
SUVH2-c- BamHI -R	CGGGATCCACCGGTTTCCTTCGATCTGAA
SUVH9-NdeI-F	GTCCATATGATGGGTTCTTCTCACATTCCTCTTG
SUVH9-BamHI-R	TGAGGATCCTTAATTACAAATGGCAAGCTTGGCG
SUVH9-b-NdeI-F	GTCCATATGTCGAATGGGAAGGAGAATGTTCCTG
Primers for BIFC	
N1-BamHI-F	TAAGGATCCAGCGGAGATAATCGAAGACGGGAT
N1-SalI-105-R	GCGTCGACTCATTTGTTGGGACATACAACATTTGCCT
N1-SalI-103-R	GCGTCGACTTTGTTGGGACATACAACATTTGCCT
N1-NT-SalI-R	GCGTCGACCTAACGGACTTCTGCGCGTTGAAG
N1-EXO-BamHI-F	TAAGGATCCGTTTGTGCCTTCGATATAGAGACAAC
SUVH2-BamHI-F	CGCGGATCCATGAGTACATTGTTACCATTTCCTG
SUVH2-b-BamHI-F2	CGCGGATCCGACCTTTCTAACAAGAAGGAGA
SUVH2-SalI-R	ATTGGTCGACGTTGCAGATGGCGAGCTTGC
SUVH2-a-103-SalI–R2	ATCGGTCGACTAGAAACACGGGTACATTCTCCTTC
SUVH2-SalI-105-R2	ATTGGTCGACCTAGTTGCAGATGGCGAGCTTGC
SUVH9-BamHI-103-F	TCGGGATCCATGGGTTCTTCTCACATTCCTCTTG
SUVH9-b-BamHI-103-F	CGGGATCCTCGAATGGGAAGGAGAATGTTCCTG
SUVH9-a- 103-SalI -R	ATTGGTCGACCTCAGCCTGTCCTTCAATCCTCT
SUVH9-103-SalI-R	GCGTCGACATTACAAATGGCAAGCTTGGCG
SUVH9-105-SalI-R	GCGTCGACCTAATTACAAATGGCAAGCTTGGCG

Primers for Pull-down	
N1-BamHI-103-F3	TAAGGATCCAGCGGAGATAATCGAAGACGGGAT
N1-SalI-105-R3	GCGTCGACTCATTTGTTGGGACATACAACATTTGCCT
SUVH2-BamHI-103-F2	CGCGGATCCATGAGTACATTGTTACCATTTCCTG
SUVH2-SalI-R2	ATTGGTCGACCTAGTTGCAGATGGCGAGCTTGC
Primers for genotyping mutant alleles	
pol2a-1-genomic band-F	AAGGTGAATGTCGAGCTAAATTCGCT
pol2a-1-genomic band-R	AAGGTGAATGTCGAGCTAAATTCGCT
pol2a-1-T-DNA band-F	CTATGGCTCTTTATGGGTTGC
LBb1.3	ATTTTGCCGATTTCGGAAC
pol2a-2-seq-F	GGTGCATTAATTAGCTATGATACAATCA
pol2a-2-seq-R	CTCGCAGACAATGGCCTCCTACT
hygromycin-F	CTACACAGCCATCGGTCCAGAC
hygromycin-R	GGGAGTTTAGCGAGAGCCTGAC
suvh9 genomic band-F	GTCCTTCAATCCTCTCCAACC
suvh9 T-DNA band-R	CAAACAAAACCCATTTCTTCG
suvh2 genomic band-F	CAACTAGCCGAAGAAATGAGG
suvh2 T-DNA band-R	TACTTCAACCCCTGTGACTGG

Dataset S1. Expression and function annotation of 865 up-regulated genes in *pol2a-1 suvh2 suvh9* vs. WT in WT and mutant backgrounds

Dataset S2. Expression and function annotation of known genes involved in meiosis in WT and mutant backgrounds