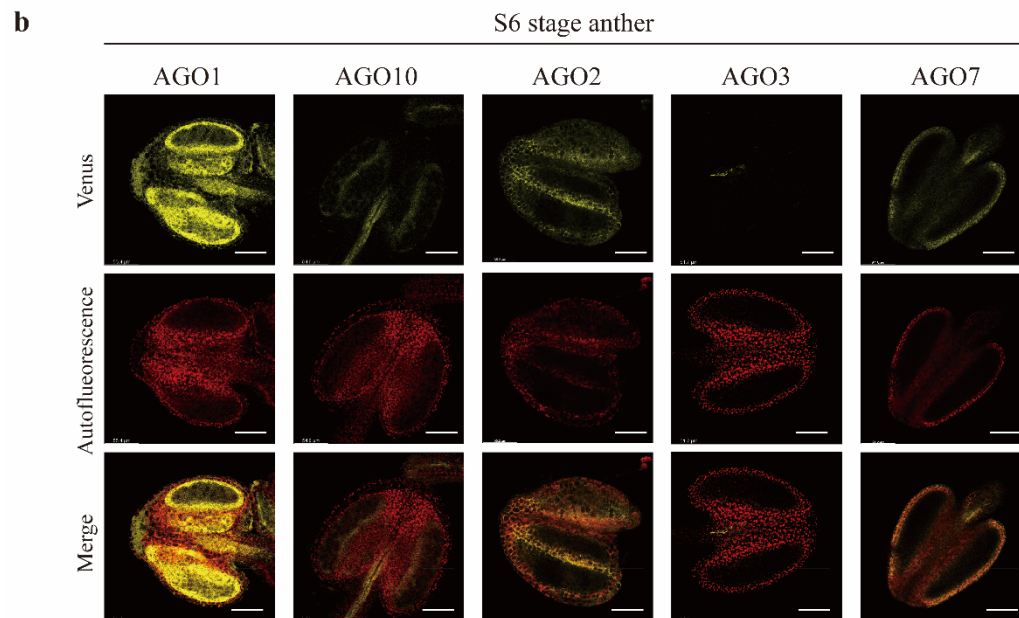
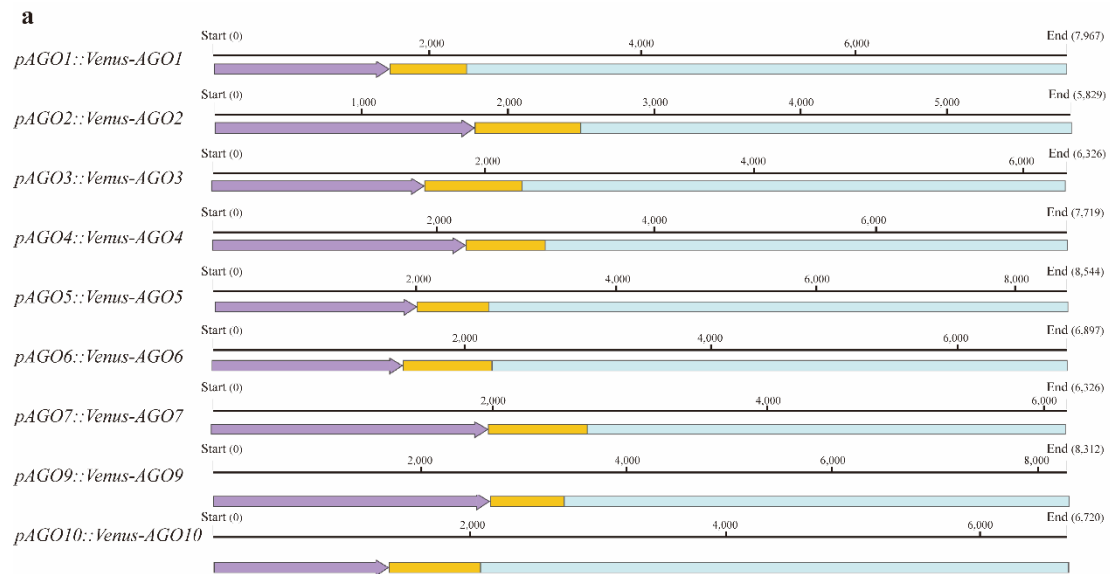




1

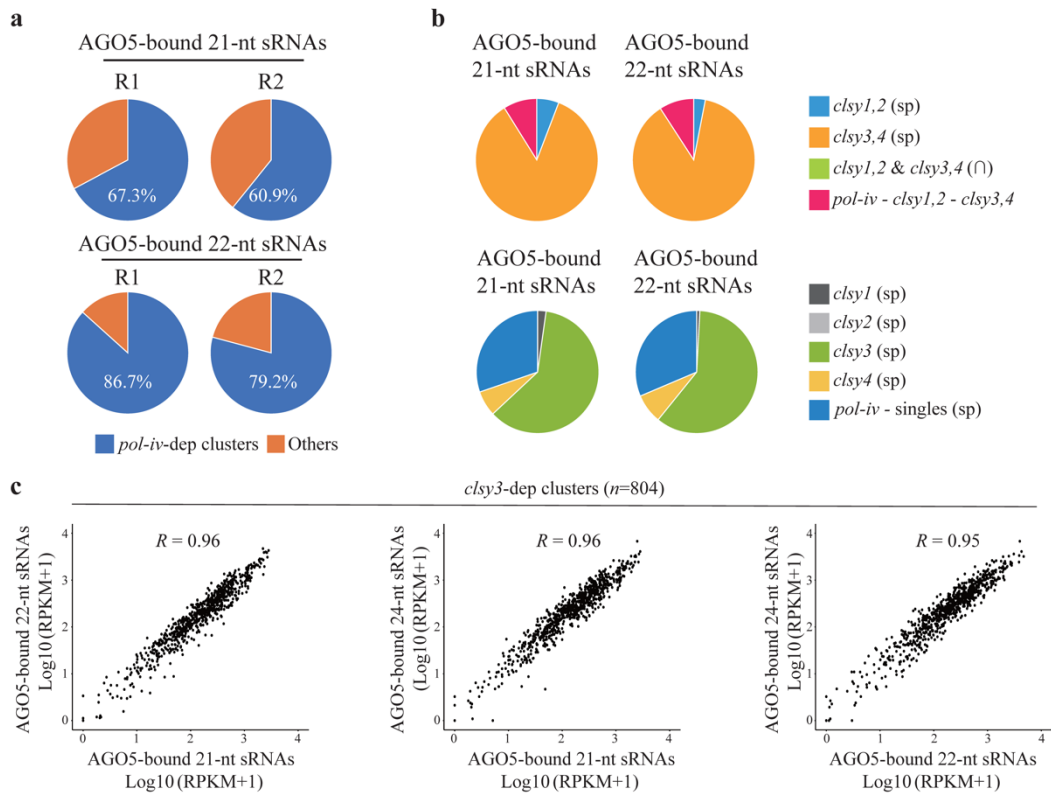
2 **Supplementary Fig. 1 | AGO1/10/2/3/7 are barely detectable in meiocytes. a,** DNA sequence

3 **used for making transgenic Venus-tagged AGO (AGO1-AGO10 excluding AGO8) reporter lines. b,**

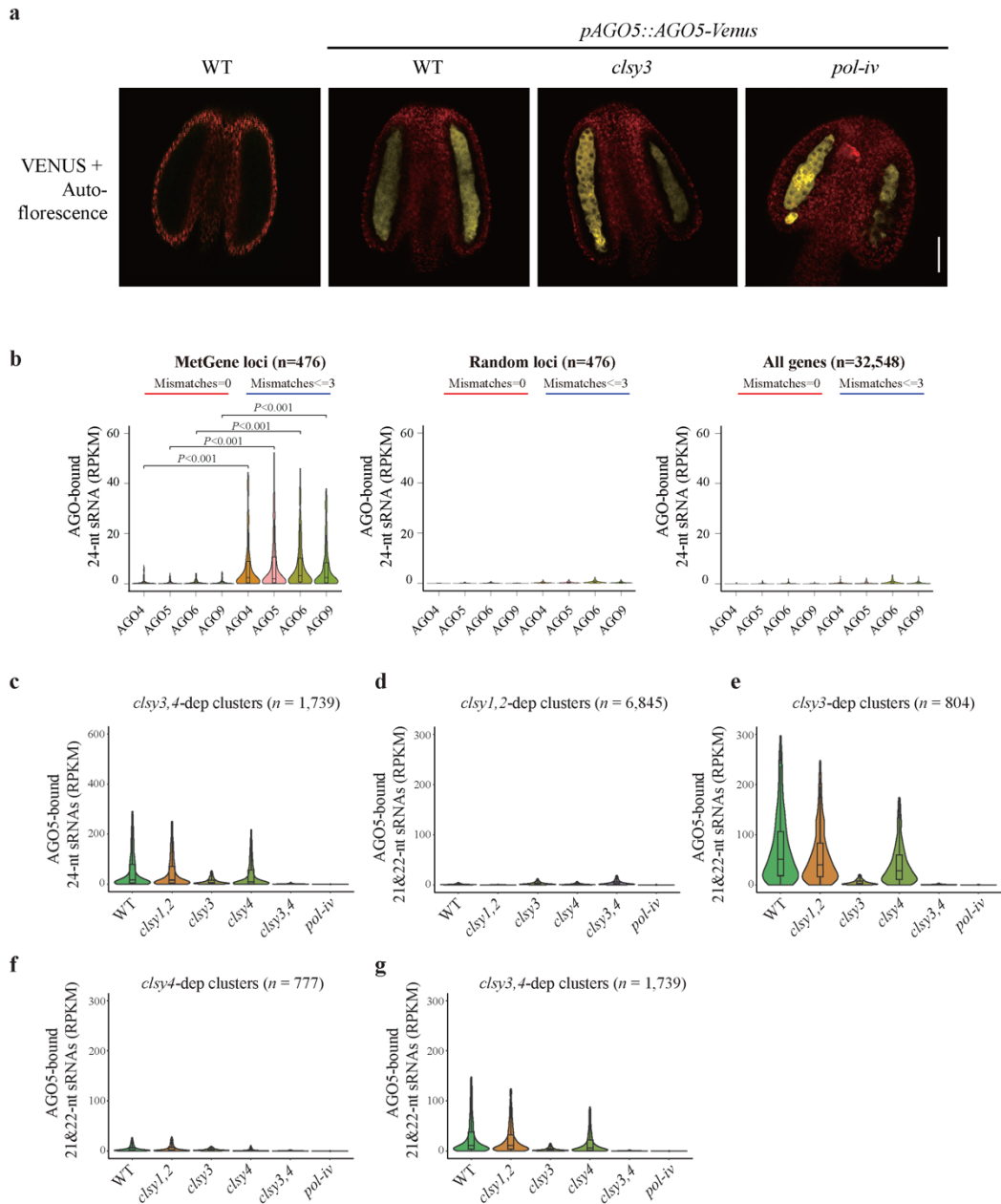
4 **Confocal images capturing Venus-AGO1/10/2/3/7 signals at Stage 6 anther of their corresponding**

5 **reporter lines, scale bars=25μm.**

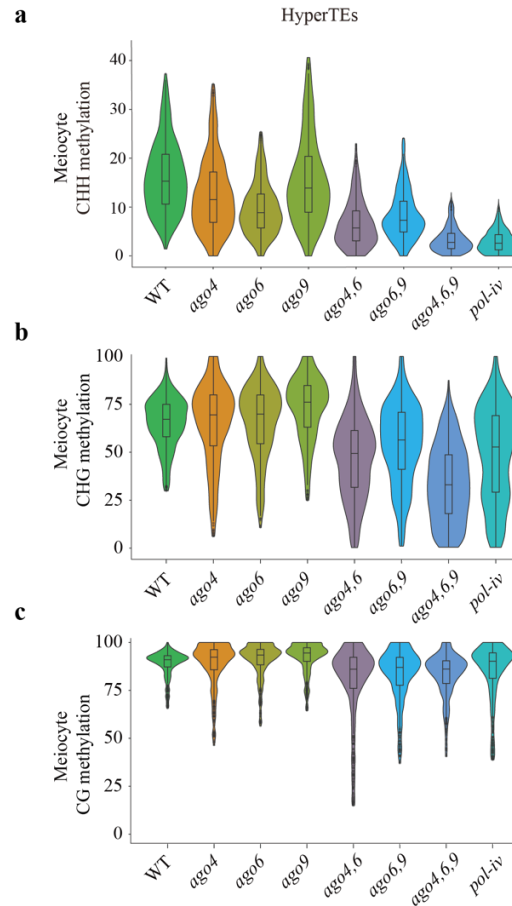
6



Supplementary Fig. 2 | The majority of AGO5-bound 21- and 22-nt sRNAs are derived from *pol-iv*-dependent sRNA clusters. **a**, Pie chart presenting the percentage of AGO5-bound 21- or 22-nt sRNAs that can be perfectly aligned to *pol-iv*-dep sRNA clusters accounting to total mapped AGO5-bound 21- or 22-nt sRNAs. The data of two biological replicates (R1 and R2) are shown. **b**, Pie chart showing the fraction of AGO5-bound 21- or 22-nt sRNAs that mapped to different classes of *clsy*-dep clusters accounting for those mapped to *pol-iv*-dep clusters. **c**, Scatter plots depicting correlations between abundance levels of AGO5-bound 21-, 22-, and 24-nt sRNAs abundance at *clsy3*-dep sRNA clusters. Pearson's R values are displayed.



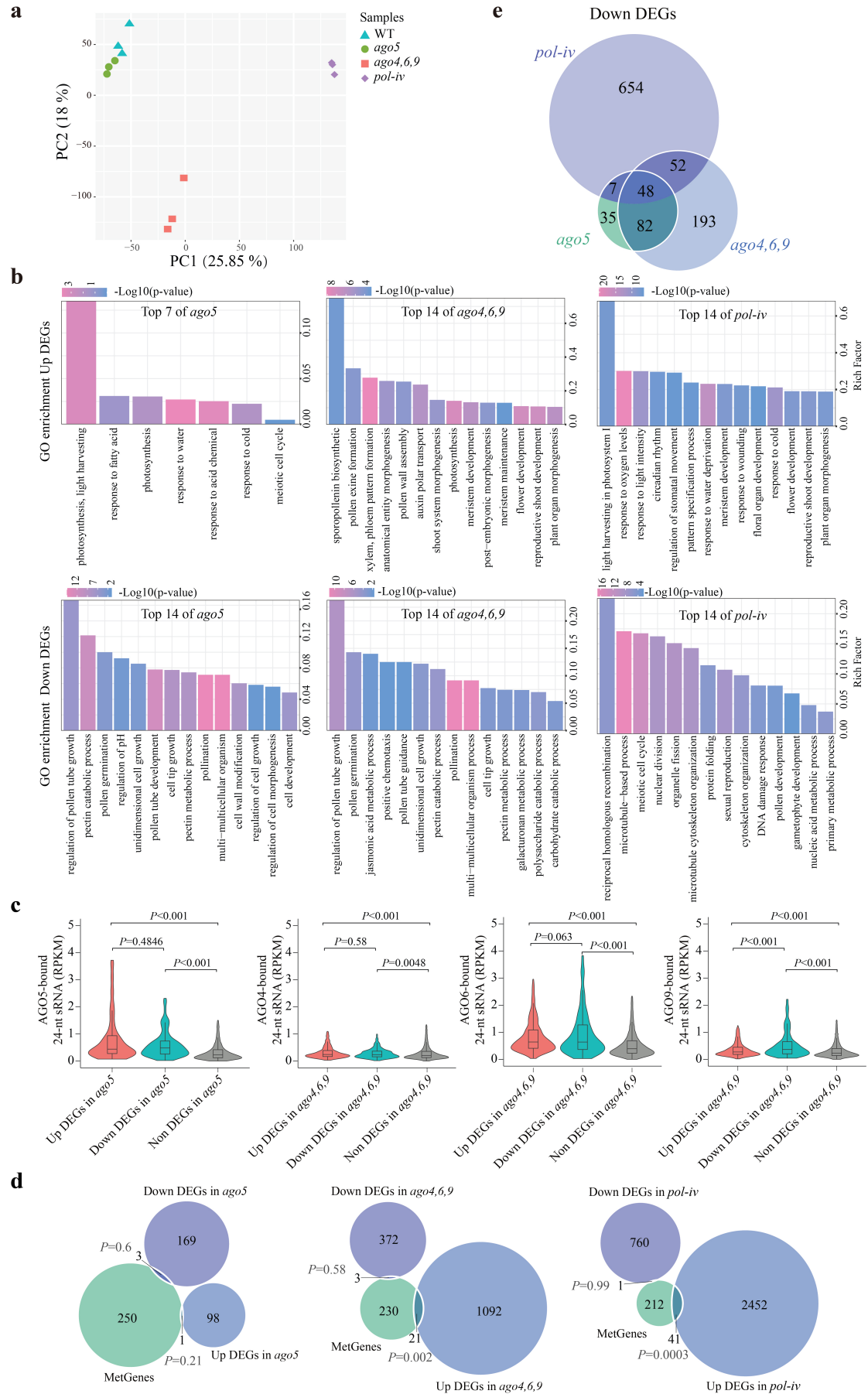
Supplementary Fig. 3 | Biogenesis of AGO5-bound siRNAs requires Pol IV and CLSY3. **a**, Confocal images of Stage 6 anthers in the indicated genotypes: WT, *AGO5-VENUS*, *AGO5-VENUS clsy3* and *AGO5-VENUS pol-iv* (from left to right). Scale bar, 25 μ m. **b**, Boxplots showing the abundance of AGO-bound 24-nt sRNAs at MetGenes (left), a random set of loci matched for size and genomic features (middle), and all genes (right), with or without mismatches as indicated. **c**, Boxplots showing AGO5-bound 24-nt sRNA abundance at *clsy3,4*-dependent clusters in indicated genotypes. **d-g**, Boxplots showing the abundance of AGO5-bound 21&22-nt sRNAs at *clsy1,2*-dependent, *clsy3*-dependent, *clsy4*-dependent, and *clsy3,4*-dependent clusters. Box plots show the median (center line), interquartile range (box), and $1.5 \times$ IQR whiskers. *P* values were calculated using a two-sided Kolmogorov–Smirnov test.



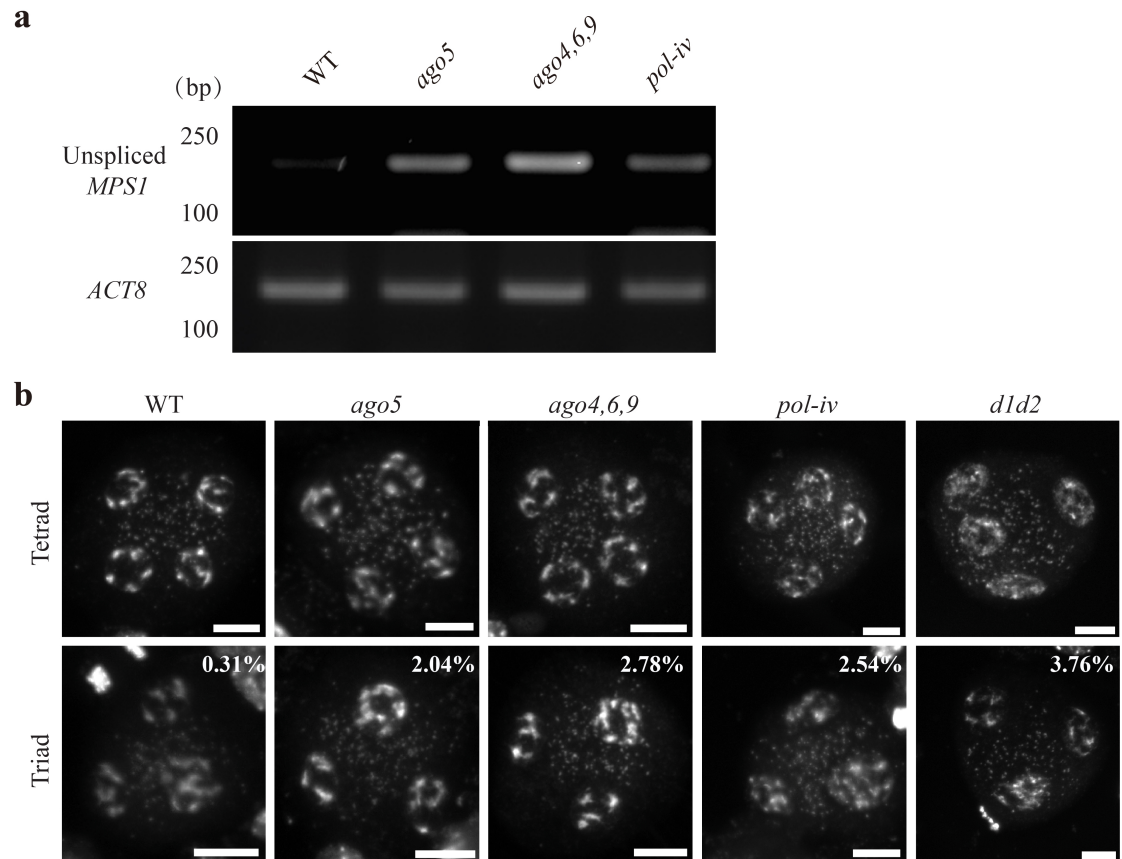
29

30 **Supplementary Fig. 4 | AGO4/6/9 but not AGO5 are involved in RdDM in the meiocyte. a-c,**
 31 Boxplots showing CHH (a), CHG (b) and CG (c) methylation levels at HyperTEs (n= 797) in male
 32 meiocytes from plants of indicated genotypes. Box plots show the median (center line), interquartile
 33 range (box), and $1.5 \times \text{IQR}$ whiskers.

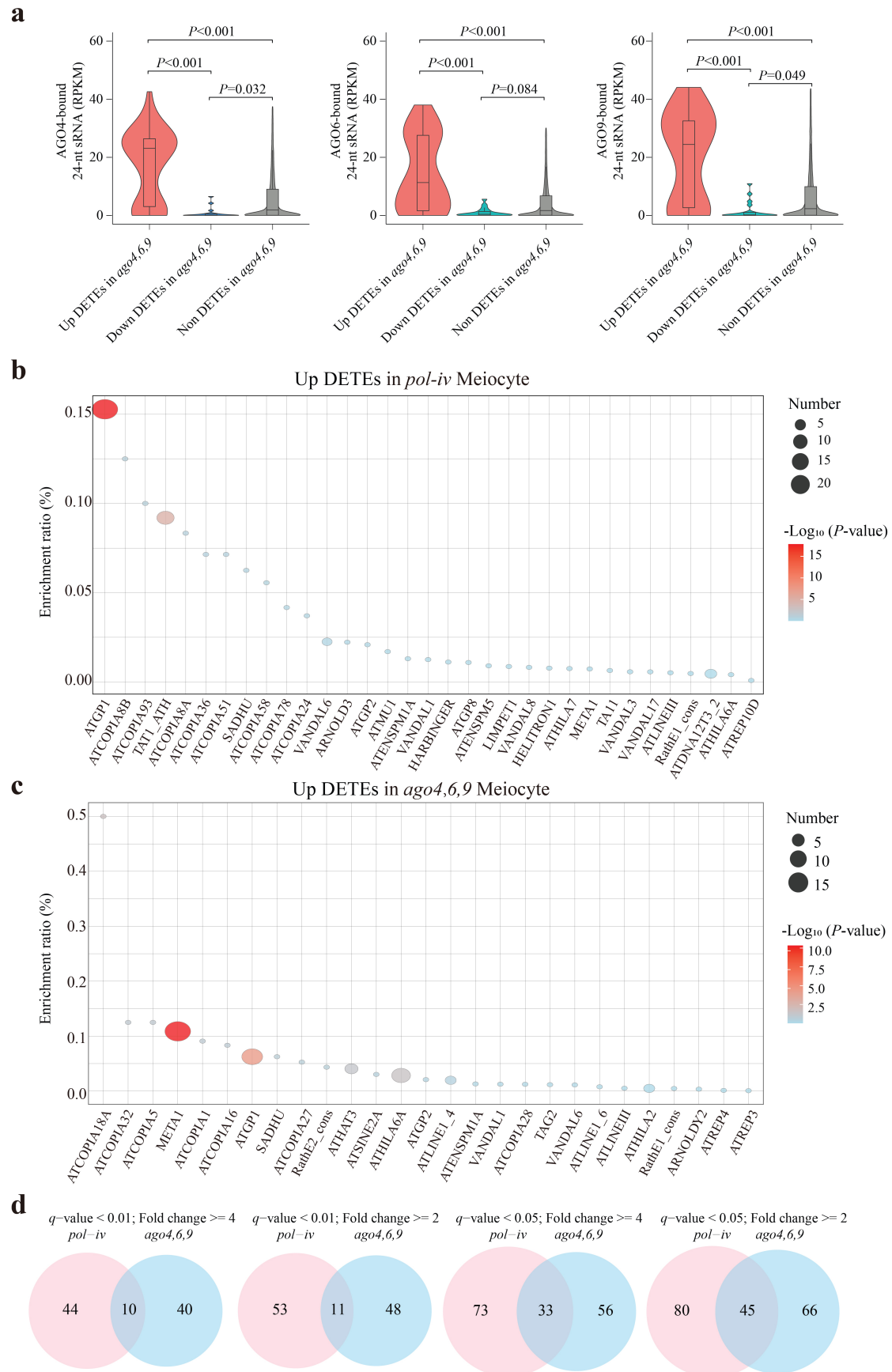
34



Supplementary Fig. 5 | Differential gene expression analysis in WT, *ago5*, *ago4,6,9* and *pol-iv* meiocytes. **a**, PCA analysis of RNA-seq biological replicates from WT, *ago5*, *ago4,6,9* and *pol-iv* meiocytes. **b**, GO term analysis for upregulated or downregulated differentially expressed genes (DEGs) in *ago5*, *ago4,6,9* and *pol-iv* meiocytes compared to WT. *P* values were calculated using the hypergeometric test. **c**, Abundance of AGO-bound 24-nt sRNAs at DEGs that are upregulated in corresponding *ago* mutants compared to WT (Up DEGs), in comparison to genes that are downregulated (Down DEGs), or genes that are not differentially expressed (non-DEGs). Box plots show the median (center line), interquartile range (box), and $1.5 \times \text{IQR}$ whiskers. *P* values were calculated using a two-sided Kolmogorov–Smirnov test. **d**, Venn diagram showing the overlap of MetGenes (a total of 254 genes overlap with MetGenes within their gene bodies) with DEGs identified in *ago5*, *ago4,6,9* and *pol-iv* meiocytes. *P* values were calculated using a hypergeometric test. **e**, Venn diagram showing the overlap of down-regulated genes identified in *ago5*, *ago4,6,9* and *pol-iv* meiocytes, compared to WT meiocytes.



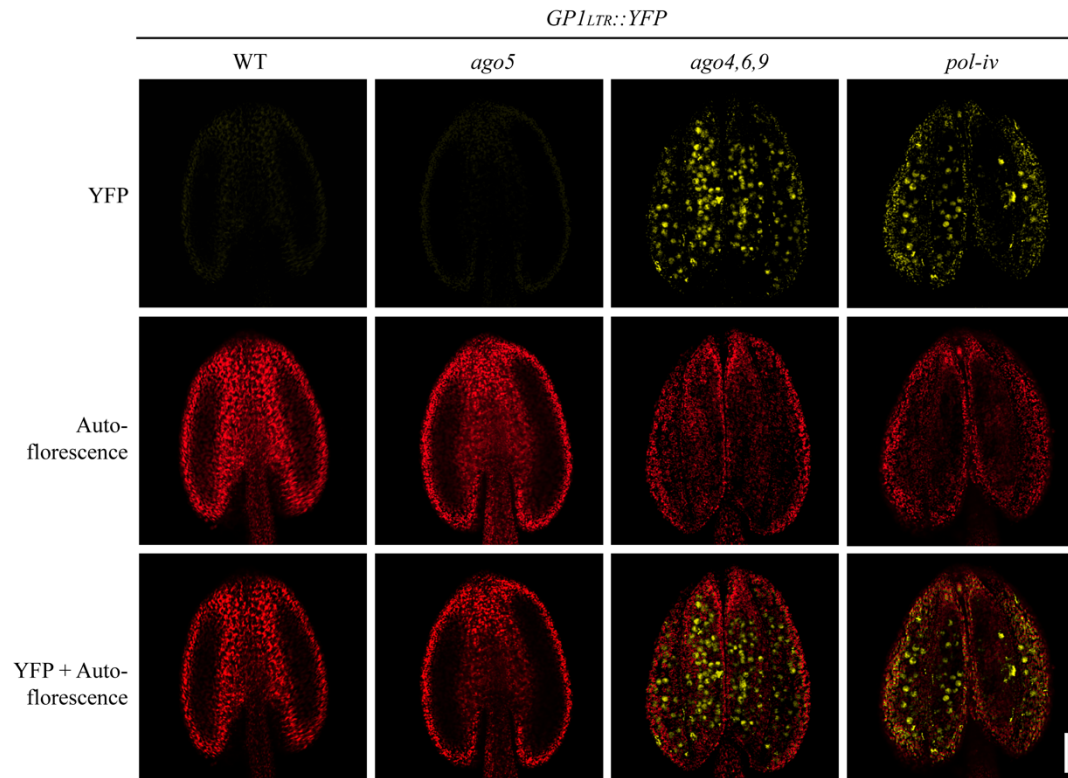
Supplementary Fig. 6 | *Ago5*, *ago4,6,9* and *pol-iv* mutant meiocytes exhibit increased *MPS1* mis-splicing and meiotic triad formation. a, RT-PCR of *MPS1* transcript with unspliced last intron in WT, *ago5*, *ago4,6,9* and *pol-iv* meiocytes. *ACTIN8* was used as a control. **b**, Representative DAPI-stained chromosome spreads from male meiocytes of indicated genotypes at telophase II. Percentages indicate the fraction of nuclei displaying triad phenotype. *dld2*, *drm1 drm2* double mutant. Scale bars, 10 μ m.



Supplementary Fig. 7 | TEs are up-regulated in *pol-iv* and *ago4,6,9* meiocytes. a, AGO4, 6, 9-bound siRNAs were significantly enriched at TEs that are upregulated in *ago4,6,9* triple mutant

62 compared to WT (Up DETEs), in comparison to TEs that are downregulated (Down DETEs), or
63 TEs that are not differentially expressed (non-DETEs). Box plots show the median (center line),
64 interquartile range (box), and $1.5 \times \text{IQR}$ whiskers. *P* values were calculated using a two-sided
65 Kolmogorov–Smirnov test. **b,c**, Enrichment analysis of TEs up-regulated in *pol-iv* (b) or *ago4,6,9*
66 (c) meiocytes. *P* values of were calculated using the hypergeometric test. **d**, Corresponding figure
67 for Fig. 6d, in which ~20% of upregulated TEs overlap between *ago4,6,9* and *pol-iv* meiocytes
68 under stringent cutoffs (*q*-value < 0.01 and Fold change ≥ 4). This is likely an underestimate of
69 overlap, as the overlap between upregulated TEs in *ago4,6,9* and *pol-iv* increases substantially when
70 less stringent cutoffs are applied, indicating that many TEs are transcriptionally affected in both
71 mutants but fall below the strict threshold in one condition.

72



Supplementary Fig. 8 | AtGP1 AT2TE23670 is activated in *ago4,6,9* or *pol-iv* mutant germlines.
GPI_{LTR}::YFP anthers in indicated genetic backgrounds. Scale bars, 25 μ m.

77 **Supplementary Table 1. Summary statistics for RIP-seq libraries.** Rep, biological
78 replicate.

Library Name	Raw reads	Clean reads (18-28 bp)	Mapped reads (18-28 bp)	Mapping rate (%)
AGO4 RIP-seq in WT floral buds rep1	59,011,278	45,586,859	34,287,410	75.21%
AGO4 RIP-seq in WT floral buds rep2	41,503,321	33,518,837	27,033,281	80.65%
AGO5 RIP-seq in WT floral buds rep1	58,246,070	38,619,813	29,440,213	76.23%
AGO5 RIP-seq in WT floral buds rep2	63,118,823	37,707,966	29,136,806	77.27%
AGO6 RIP-seq in WT floral buds rep1	46,944,277	28,498,665	21,694,408	76.12%
AGO6 RIP-seq in WT floral buds rep2	100,820,542	50,277,153	39,086,108	77.74%
AGO9 RIP-seq in WT floral buds rep1	29,718,099	20,937,370	15,384,248	73.48%
AGO9 RIP-seq in WT floral buds rep2	32,813,249	23,997,113	18,018,179	75.08%
AGO5 RIP-seq in <i>clsy1,2</i> floral buds rep1	43,685,890	35,806,912	29,784,714	83.18%
AGO5 RIP-seq in <i>clsy1,2</i> floral buds rep2	36,469,968	14,877,343	10,221,449	68.70%
AGO5 RIP-seq in <i>clsy3</i> floral buds rep1	62,983,412	31,682,639	23,413,625	73.90%
AGO5 RIP-seq in <i>clsy3</i> floral buds rep2	55,240,730	37,025,411	31,476,199	85.01%
AGO5 RIP-seq in <i>clsy4</i> floral buds rep1	75,534,086	22,626,112	18,510,716	81.81%
AGO5 RIP-seq in <i>clsy4</i> floral buds rep2	72,304,817	27,467,470	21,552,151	78.46%
AGO5 RIP-seq in <i>clsy3,4</i> floral buds rep1	59,736,771	30,252,468	24,739,091	81.78%
AGO5 RIP-seq in <i>clsy3,4</i> floral buds rep2	57,031,825	29,979,911	25,090,623	83.69%
AGO5 RIP-seq in <i>pol-iv</i> floral buds rep1	53,268,034	18,643,132	13,420,919	71.99%
AGO5 RIP-seq in <i>pol-iv</i> floral buds rep2	61,804,087	18,369,545	12,697,589	69.12%
GFP RIP-seq in WT floral buds (35S::GFP) rep1	52,782,626	15,971,201	11,919,855	74.63%

GFP RIP-seq in WT floral buds (<i>35S::GFP</i>) rep2	43,389,964	14,164,358	11,386,174	80.39%
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Supplementary Table 2. Summary statistics for BS-seq libraries. Rep, biological replicate.

Library Name	Raw reads	Clean reads	Mapped reads	Mapping rate	Bisulfite conversion rate
WT meiocytes BS-seq rep1	57,931,024	57,701,823	31,221,375	54.11%	99.58%
WT meiocytes BS-seq rep2	58,867,287	58,615,828	31,325,499	53.44%	99.73%
<i>ago5</i> meiocytes BS-seq rep1	41,397,999	40,649,658	18,059,970	44.43%	99.64%
<i>ago5</i> meiocytes BS-seq rep2	50,194,997	49,475,306	23,154,657	46.80%	99.60%
<i>ago4</i> meiocytes BS-seq rep1	42,534,773	42,101,195	25,636,899	60.89%	99.35%
<i>ago4</i> meiocytes BS-seq rep2	38,147,737	37,852,922	23,502,782	62.09%	99.45%
<i>ago6</i> meiocytes BS-seq rep1	48,928,251	48,427,864	30,097,448	62.15%	99.37%
<i>ago6</i> meiocytes BS-seq rep2	40,751,443	40,483,622	26,365,337	65.13%	99.46%
<i>ago9</i> meiocytes BS-seq rep1	43,812,367	43,566,306	27,658,867	63.49%	99.46%
<i>ago9</i> meiocytes BS-seq rep2	44,254,353	43,941,929	28,099,158	63.95%	99.31%
<i>ago6,9</i> meiocytes BS-seq rep1	42,923,669	42,106,321	22,299,149	52.96%	99.61%
<i>ago6,9</i> meiocytes BS-seq rep2	49,835,290	48,831,190	26,185,972	53.63%	99.62%
<i>ago4,6</i> meiocytes BS-seq rep1	39,062,413	38,084,225	19,626,031	51.53%	99.64%
<i>ago4,6</i> meiocytes BS-seq rep2	30,827,994	30,313,739	15,346,540	50.63%	99.64%
<i>ago4,6,9</i> meiocytes BS-seq rep1	66,399,208	64,955,444	28,024,827	43.14%	99.64%

<i>ago4,6,9</i> meiocytes BS-seq rep2	77,457,290	75,799,732	41,477,773	54.72%	99.64%
<i>pol-iv</i> meiocytes BS-seq rep1	44,045,004	43,819,143	25,033,362	57.13%	99.37%
<i>pol-iv</i> meiocytes BS-seq rep2	45,033,341	44,579,463	25,245,943	56.63%	99.60%

Supplementary Table 3. Summary statistics for RNA-seq libraries. Rep, biological replicate.

Library Name	Raw reads	Clean Reads	Mapped Reads	Mapping Rate
WT meiocytes RNA-seq rep1	29,755,163	29,598,139	28,605,948	96.65%
WT meiocytes RNA-seq rep2	24,844,194	24,696,089	24,005,137	97.20%
WT meiocytes RNA-seq rep3	21,954,776	21,864,576	21,102,823	96.52%
<i>ago5</i> meiocytes RNA-seq rep1	23,106,089	23,024,285	22,368,946	97.15%
<i>ago5</i> meiocytes RNA-seq rep2	22,333,369	22,274,726	21,626,094	97.09%
<i>ago5</i> meiocytes RNA-seq rep3	23,501,503	23,359,258	22,531,606	96.46%
<i>ago4,6,9</i> meiocytes RNA-seq rep1	33,702,304	32,683,946	31,688,993	96.96%
<i>ago4,6,9</i> meiocytes RNA-seq rep2	29,225,006	28,546,665	27,644,339	96.84%
<i>ago4,6,9</i> meiocytes RNA-seq rep3	17,113,990	17,089,362	16,609,479	97.19%
<i>pol-iv</i> meiocytes RNA-seq rep1	21,828,197	21,651,317	19,906,936	91.94%
<i>pol-iv</i> meiocytes RNA-seq rep2	21,092,719	21,016,104	19,202,379	91.37%
<i>pol-iv</i> meiocytes RNA-seq rep3	18,900,343	18,829,446	17,115,616	90.90%

Supplementary Table 4. Frequency of meiotic triads in indicated genotypes. *P* value, Fischer's exact test. Significant *P* values (*P* < 0.05) are indicated by asterisks.

	Triads (%)	n= total observations	<i>P</i> value vs WT	<i>P</i> value vs <i>drm1drm2</i>
<i>ago5</i>	2.04%	393	0.046*	0.225
<i>ago4,6,9</i>	2.78%	396	0.015*	0.504
<i>pol-iv</i>	2.54%	276	0.027*	0.467
<i>drm1drm2</i>	3.76%	266	0.003*	
WT	0.31%	324		0.003*