

Adaptive reduction of male gamete number in the selfing plant *Arabidopsis thaliana*

Tsuchimatsu *et al.*

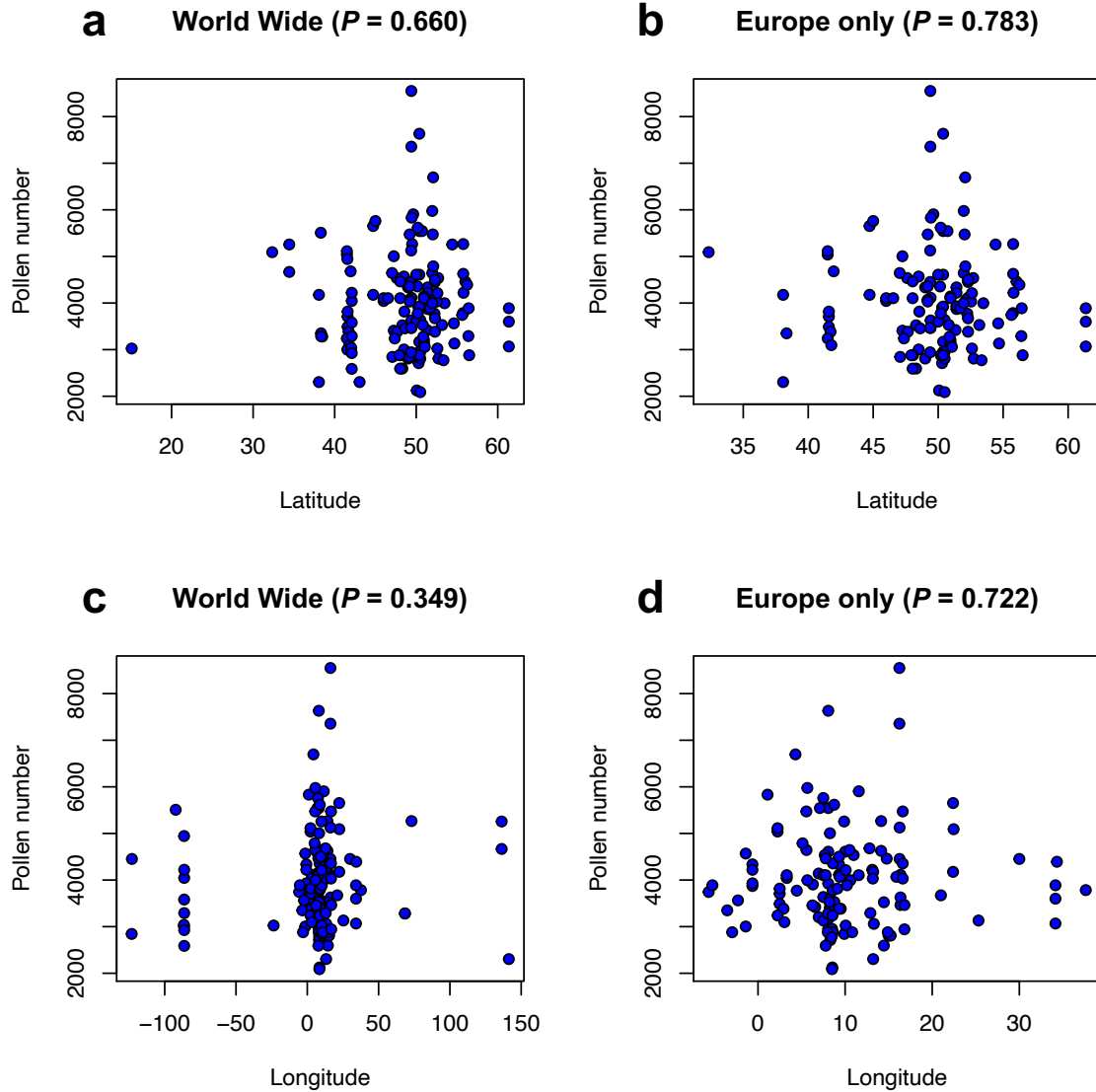
Supplementary Note 1. Correlations of pollen number with published traits and genotypes.

We analyzed the correlation of our data for pollen number per flower with the dataset of 107 published phenotypes, which includes diverse phenotypes of flowering and defense-related ionomics and developmental phenotypes, but not the numbers of ovules, flowers, or seeds. We found no significant correlations after performing Bonferroni correction for multiple testing (see main text). When multiple testing was not considered, seven traits showed a correlation ($P < 0.05$). Among these, fresh and dry weight of plants (75_FW and 76_DW, respectively, in Supplementary Table 3) were positively correlated with pollen number per flower. This suggests that larger plants produce more pollen per flower, which is consistent with empirical observations. Because plant size was measured mostly before flowering and the generation of pollen grains, these data do not suggest that larger plant size is a consequence of a larger number of pollen grains.

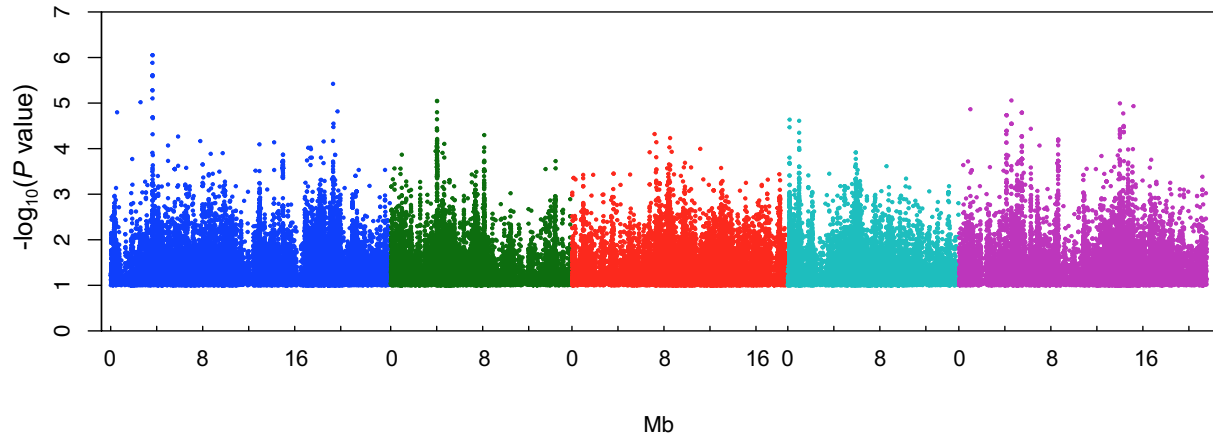
We also examined whether pollen number was associated with *S*-haplotypes (haplogroups A, B, or C)¹. Because self-compatibility has been shown to have evolved independently in each haplogroup, it may be possible that different alleles are responsible for reduced pollen numbers. However, no significant correlation was found ($P = 0.682$; ANOVA; Supplementary Table 4).

Supplementary Note 2. Selection scan and enrichment analysis in various datasets and cut-offs.

To deal with possible confounding factors, we performed selection scans and iHS enrichment analyses using various datasets and cut-offs. First, we performed the same enrichment analysis with $MAF > 0.15$ (Supplementary Fig. 4), in addition to $MAF > 0.1$. When we considered $MAF > 0.15$, we found the significant enrichment for pollen grain number associated loci under the thresholds of top 1% and 2.5% tails (but not under 10% and 5% thresholds). Second, to deal with the geographically biased sampling, we also performed the selection scan with 144 accessions that were used for GWAS. We still found the significant iHS enrichment for pollen grain number associated loci (Supplementary Fig. 5). Third, to verify the effect of setting window, we performed the iHS enrichment analysis per SNP basis, still finding the significant iHS enrichment for pollen grain number associated loci (Supplementary Fig. 6).

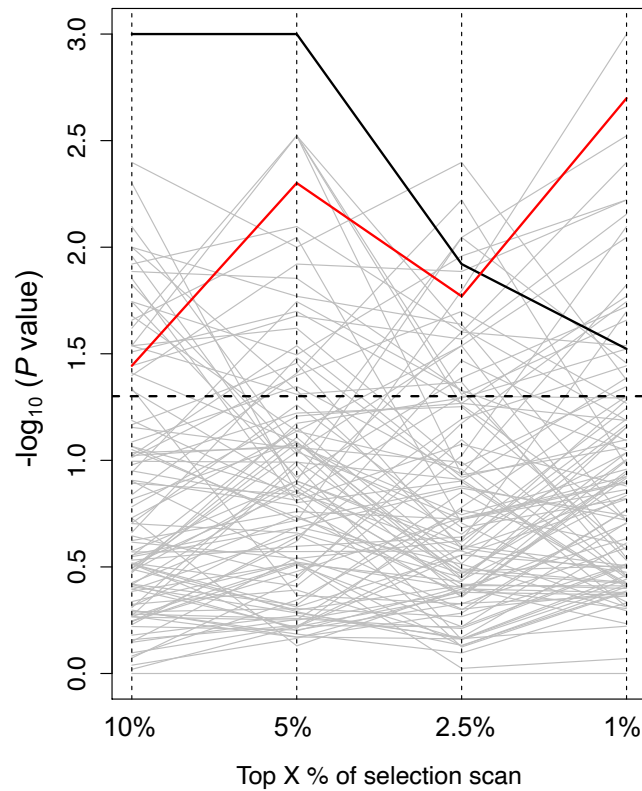


Supplementary Figure 1. Relationships between pollen number and geographic locations. (a) and (b) show the correlations between pollen number and latitude, (c) and (d) show the correlations between pollen number and longitude. (b) and (d) focus only on the European accessions. None of them showed statistically significant correlations. P values were calculated by two-sided tests without adjustments for multiple comparisons. Source data are provided as a Source Data file.



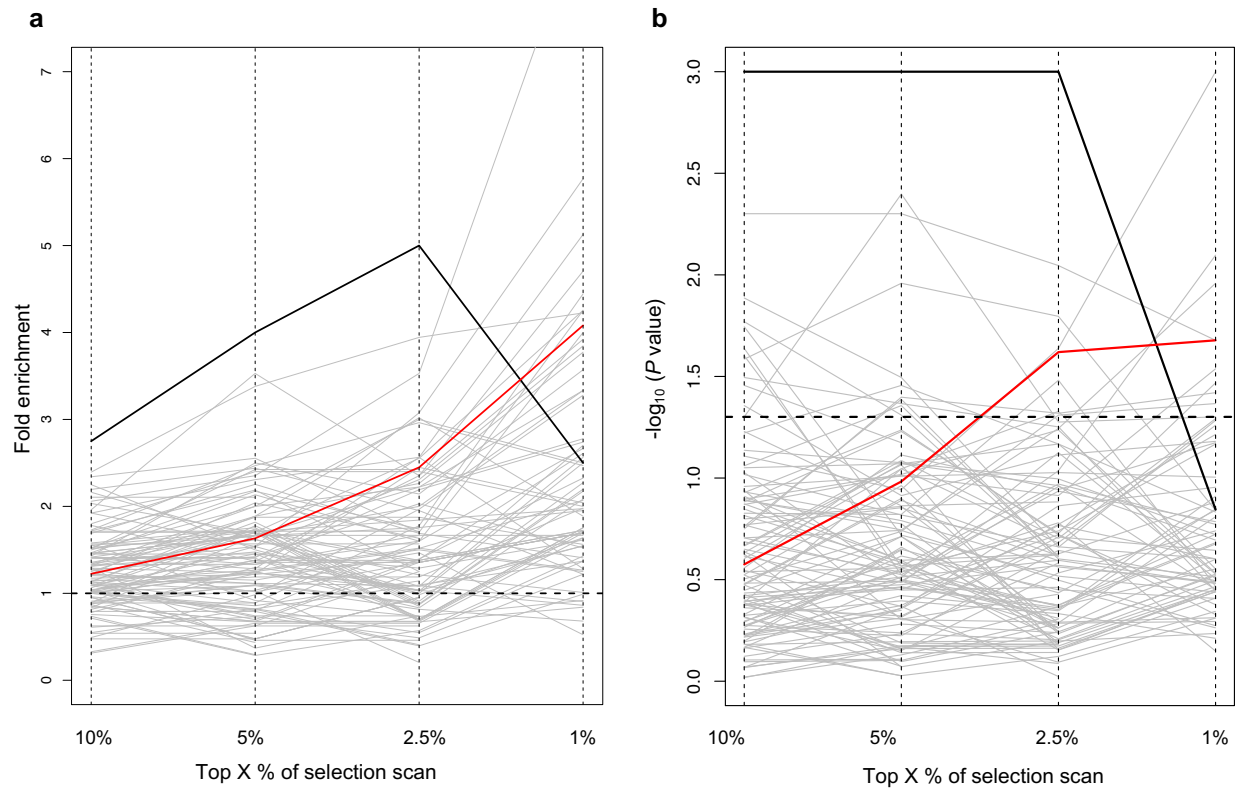
Supplementary Figure 2. Genome-wide association study of ovule number using 151 worldwide accessions.

The five chromosomes are depicted in different colors. SNPs with a minor allele frequency > 0.15 are shown. Data of ovule number is shown in Supplementary Table 2.



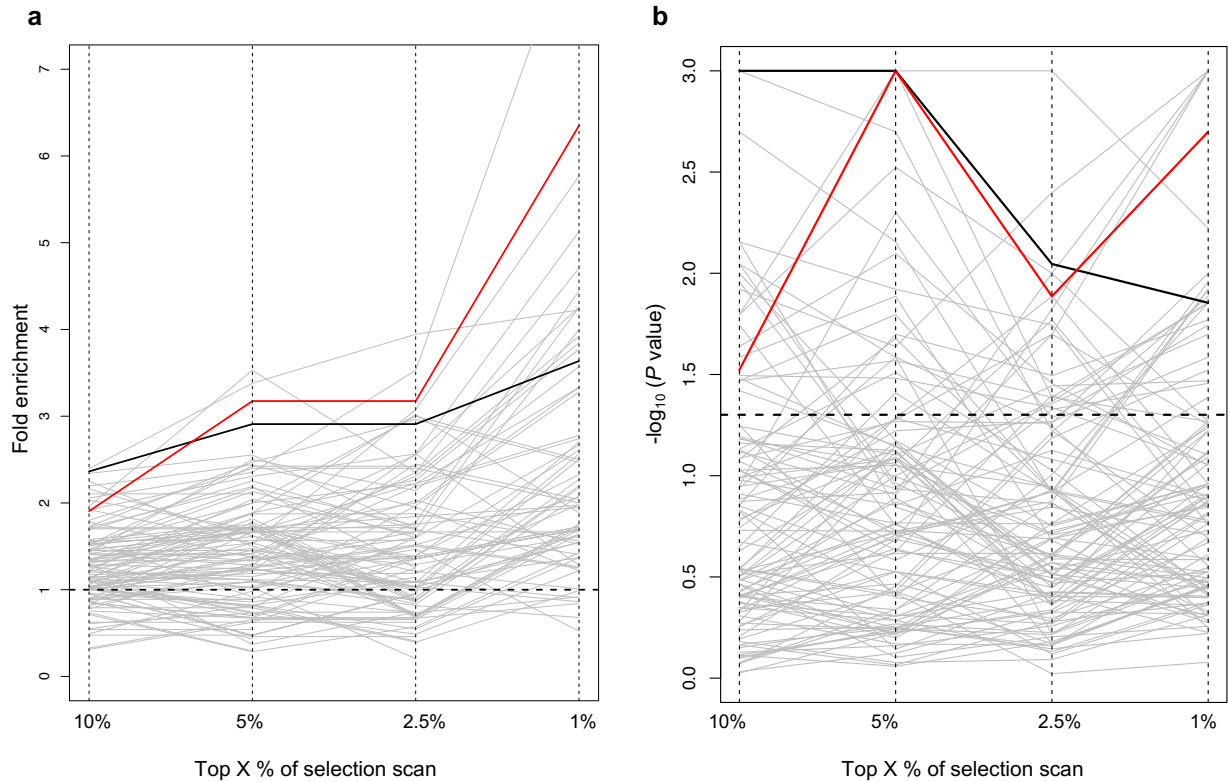
Supplementary Figure 3. *P* values of the iHS enrichment analysis based on permutation tests.

P values of the iHS enrichment analysis (Fig. 1j) are shown. Each line indicates a phenotype (red: pollen number, black: ovule number, gray: 107 phenotypes²). The X-axis quantifies the extreme tails of the iHS statistic. A horizontal dashed line indicates the threshold of $P = 0.05$. Note that the minimum *P* value is 0.001 because permutation was performed 1,000 times (see Methods). Source data are provided as a Source Data file.

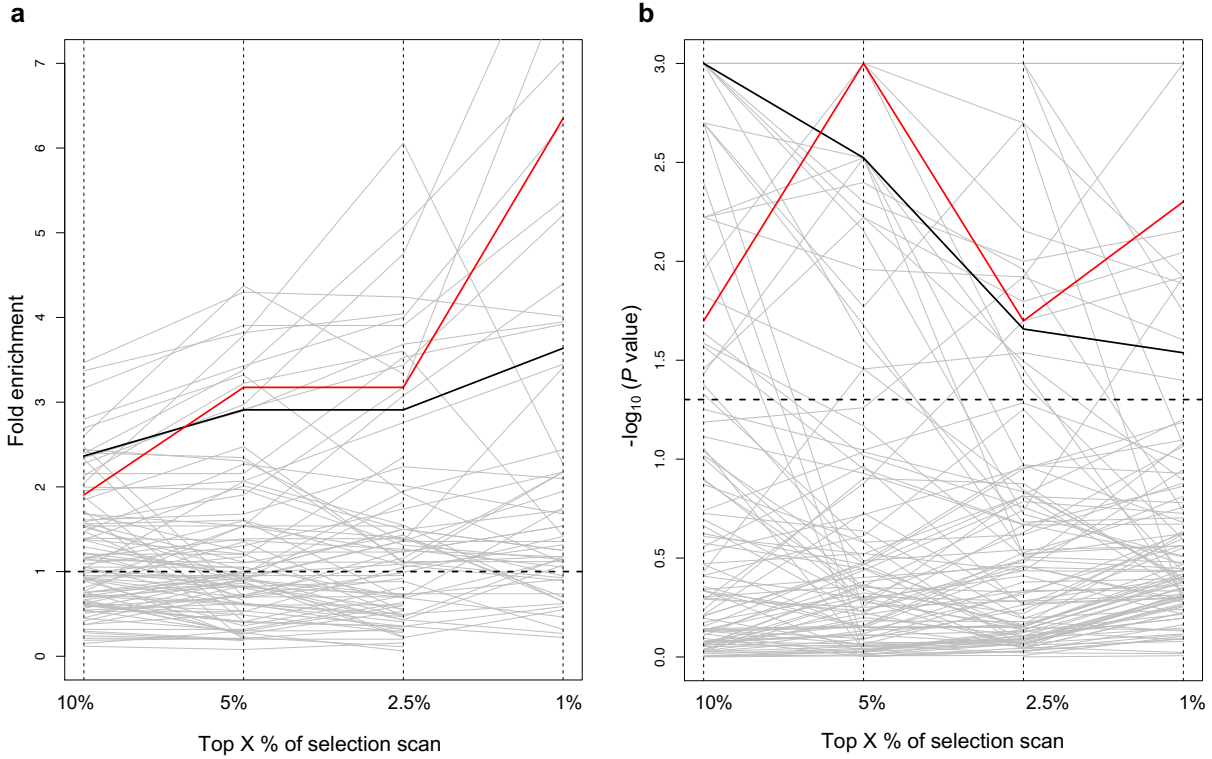


Supplementary Figure 4. Selection scan with the minor allele frequency cut-off of 0.15.

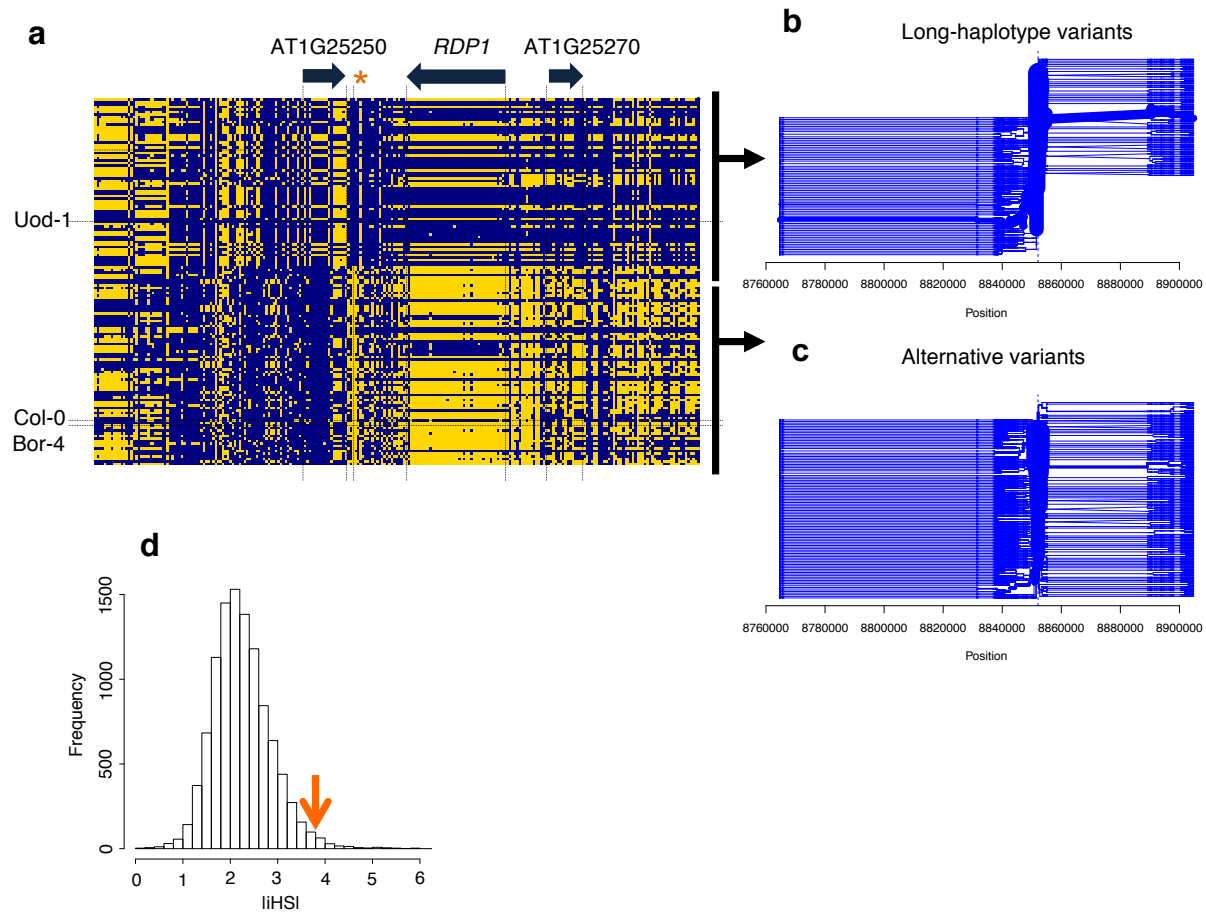
Each line indicates a phenotype (red: pollen number, black: ovule number, gray: 107 phenotypes²). **a** The x-axis quantifies the extreme tails of the iHS statistic. **b** P values of the iHS enrichment analysis based on permutation tests. A horizontal dashed line indicates the threshold of $P = 0.05$. Note that the minimum P value is 0.001 because permutation was performed 1,000 times (see Methods). Source data are provided as a Source Data file.



Supplementary Figure 5. Selection scan using 144 accessions that were also used for GWAS. Each line indicates a phenotype (red: pollen number, black: ovule number, gray: 107 phenotypes²). **a** The x-axis quantifies the extreme tails of the iHS statistic. **b** P values of the iHS enrichment analysis based on permutation tests. A horizontal dashed line indicates the threshold of $P = 0.05$. Note that the minimum P value is 0.001 because permutation was performed 1,000 times (see Methods). Source data are provided as a Source Data file.

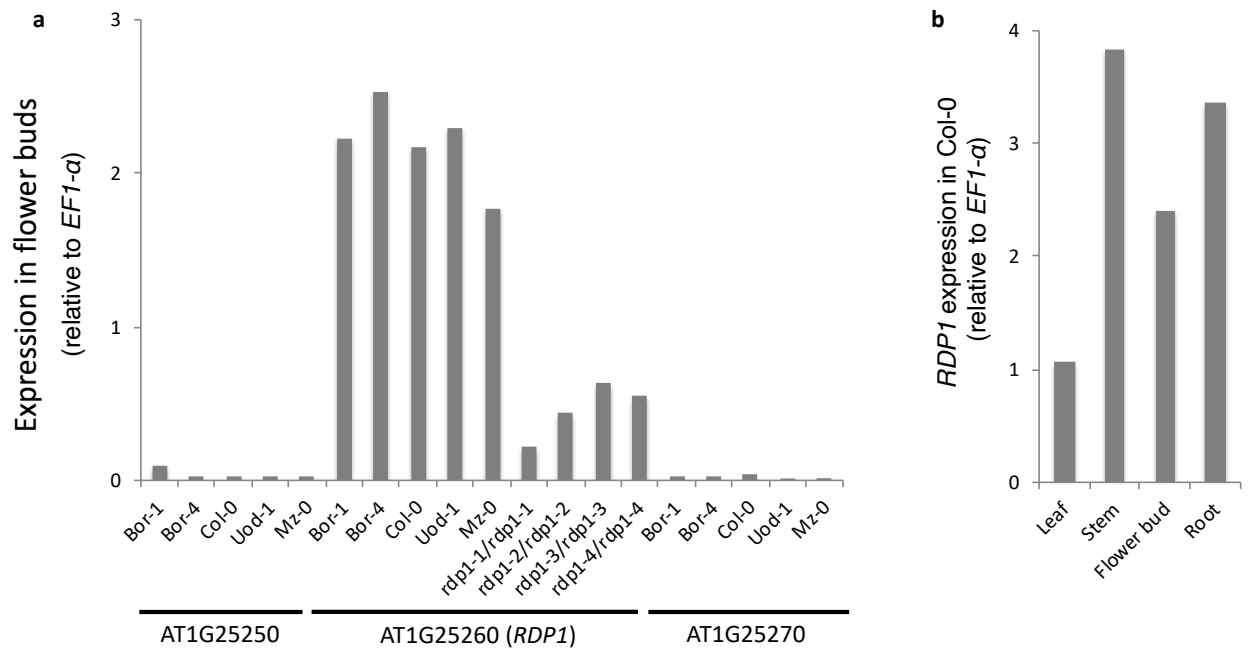


Supplementary Figure 6. Selection scan analyzed per SNP basis. Each line indicates a phenotype (red: pollen number, black: ovule number, gray: 107 phenotypes²). **a** The x-axis quantifies the extreme tails of the iHS statistic. **b** P values of the iHS enrichment analysis based on permutation tests. A horizontal dashed line indicates the threshold of $P = 0.05$. Note that the minimum P value is 0.001 because permutation was performed 1,000 times (see Methods). Source data are provided as a Source Data file.



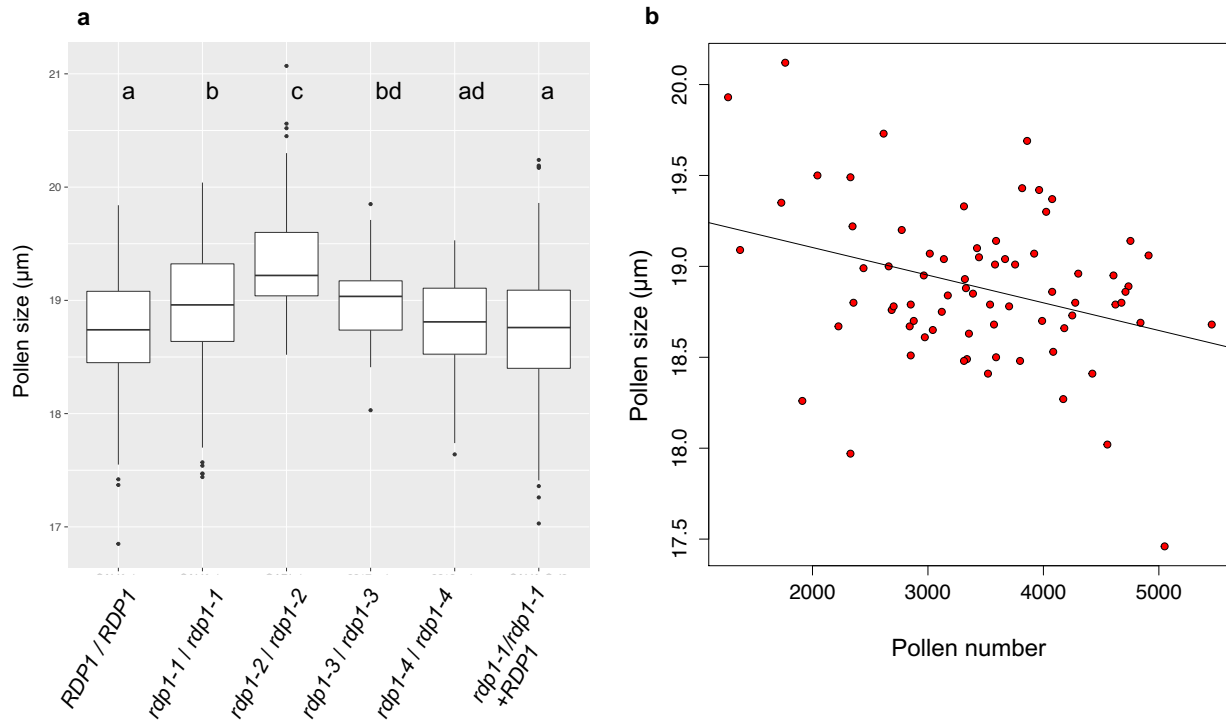
Supplementary Figure 7. A signature of selection at the *RDP1* region.

a A haplotype map of 144 accessions at the *RDP1* region. Accessions were sorted by SNP 1-8852112 (asterisk) that is significant both in the iHS scan and GWAS. Yellow and blue dots indicate polymorphisms. **b, c** Haplotype bifurcation diagrams for the long haplotype (**b**) and the other variants (**c**) in the *RDP1* region. Line width corresponds to the number of individuals with the indicated haplotype. **d** Genome-wide distribution of the absolute values of the iHS statistics. The *RDP1* region is in the upper tail of the distribution (arrow, empirical $P = 0.0149$, one-sided test). Source data underlying Supplementary Fig. 7a and 7d are provided as a Source Data file.



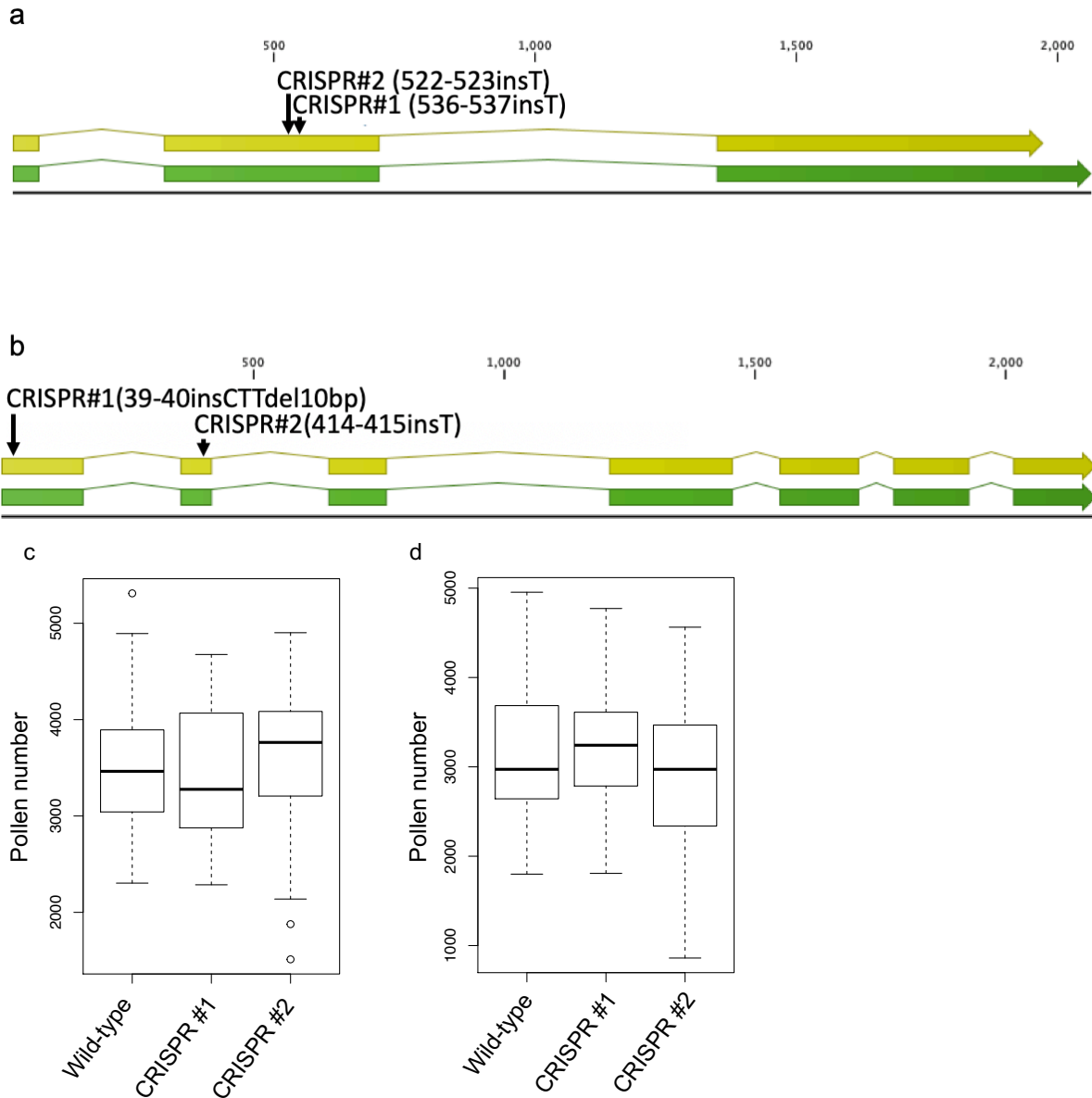
Supplementary Figure 8. Expression levels of *RDP1* and neighboring genes.

a Expression of *RDP1* (AT1G25260) and neighboring genes (AT1G25250 and AT1G25270) in flower buds of natural accessions and mutants. **b** Expression levels of *RDP1* in leaf, stem, flower bud, and root of the Col-0 accession. Gene expression levels were normalized to *EF1-α* (AT5G60390). Source data are provided as a Source Data file.



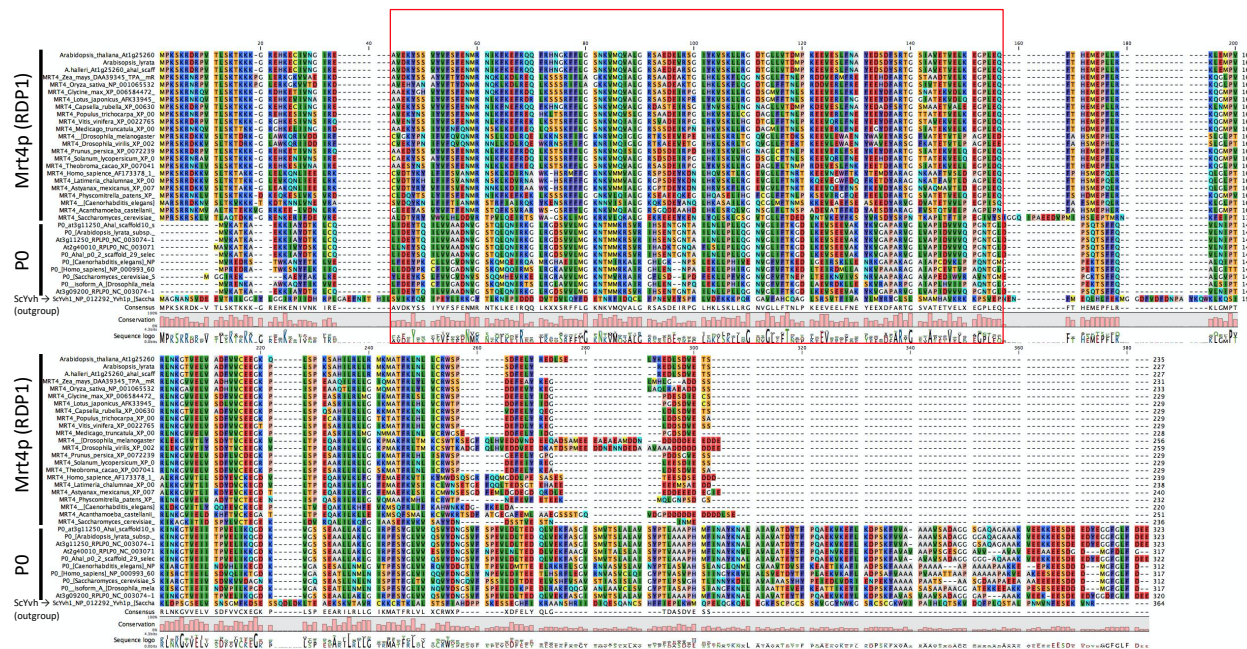
Supplementary Figure 9. Pollen size in *RDP1* mutants and its correlation with pollen number.

a Pollen size differences between four types of homozygous mutants (wild type (*RDP1/RDP1*), *rdp1-1/rdp1-1*, *rdp1-2/rdp1-2*, *rdp1-3/rdp1-3*, and *rdp1-4/rdp1-4*), and the lines complemented with the Col-0 allele (*rdp1-1/rdp1-1+RDP1*). Letters (a–d) indicate significant differences in pollen number or size between lines, determined by a nested analysis of variance and *post hoc* Tukey test ($P < 0.05$; See Supplementary Table 9 for P values of each comparison). Numbers of flowers measured (left to right, as plotted): $N = 135, 132, 45, 40, 60, 411$. Boxplots show center line: median; box limits: upper and lower quartiles; whiskers: not greater than 1.5 times the interquartile range; point: outlier. **b** Pollen size is negatively correlated with pollen number in the Col-0 accession ($N = 73, P = 0.00501$). This negative correlation may be attributed to developmental constraints³. Source data are provided as a Source Data file.



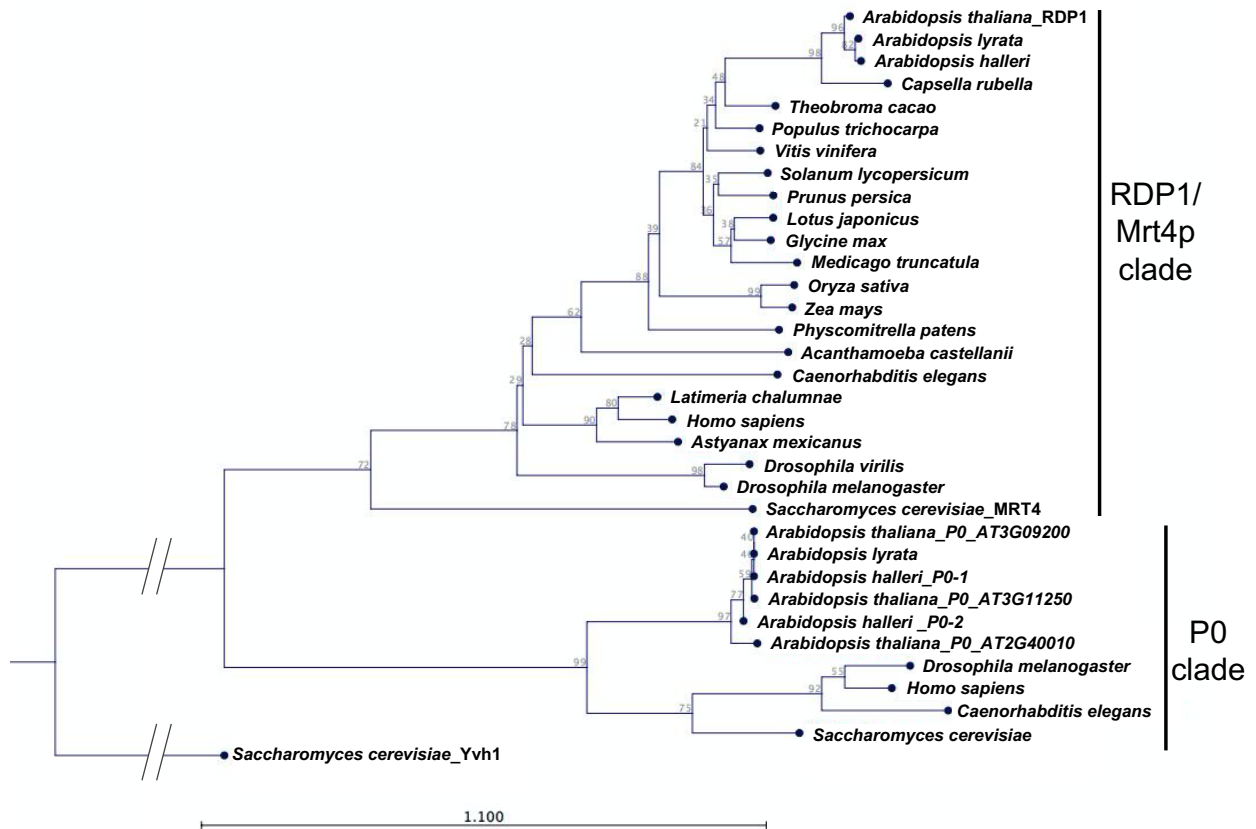
Supplementary Figure 10. Function of neighboring genes of *RDPI*.

a, b Gene structures of AT1G25250 (**a**) and AT1G25270 (**b**). Yellow and green boxes indicate coding region and exon respectively. Numbers indicate base pair from start codon. Each mutation points are shown by arrows. **c, d** Pollen number did not differ significantly between wild type and the null mutants by CRISPR-Cas9 (**c**; AT1G25250, **d**; AT1G25270). Two-sided *t*-test against wild type: CRISPR #1 of AT1G25250, $P = 0.5757$; CRISPR#2 of AT1G25250, $P = 0.4115$; CRISPR#1 of AT1G25270, $P = 0.4224$; CRISPR#2 of AT1G25270, $P = 0.2275$. Boxplots show center line: median; box limits: upper and lower quartiles; whiskers: not greater than 1.5 times the interquartile range; point: outlier. Number of flowers pollen-counted (left to right, as plotted): $N = 46, 18$ and 54 for (**c**); $N = 54, 127$ and 57 for (**d**). Source data underlying Supplementary Fig. 10c and 10d are provided as a Source Data file.



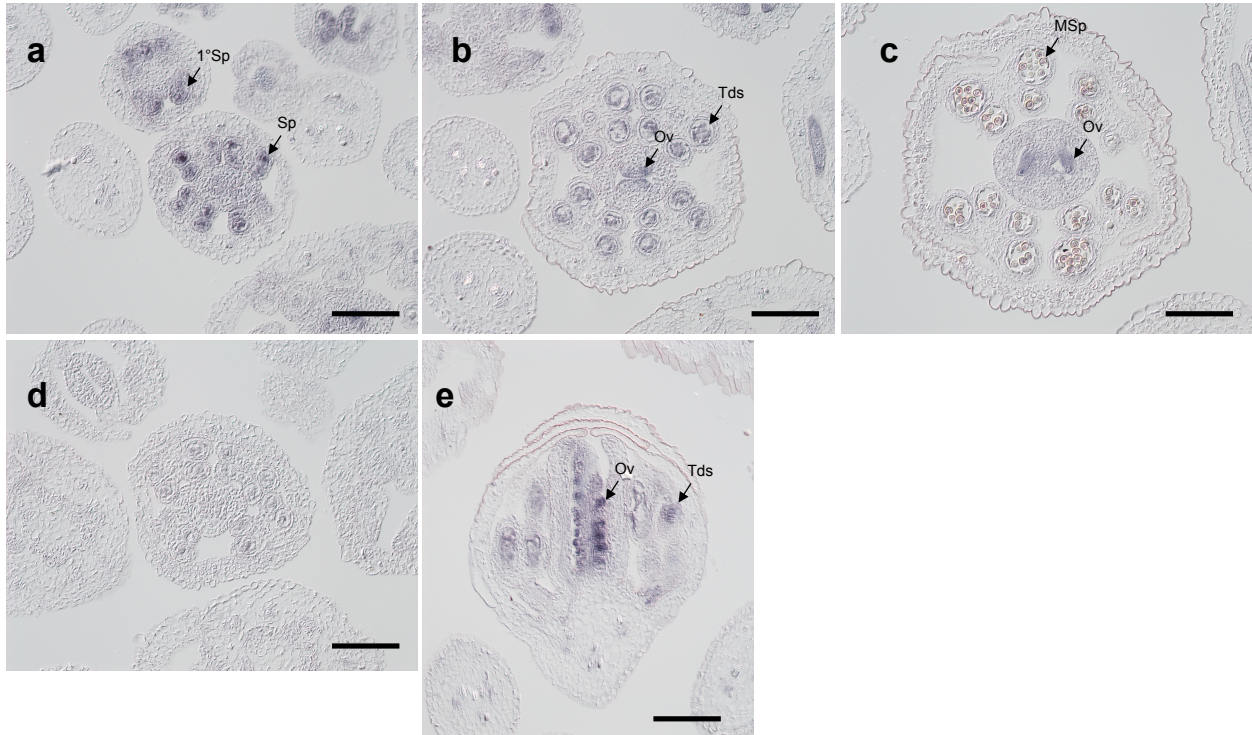
Supplementary Figure 11. Alignment of RDP1/Mrt4p and P0 protein sequences generated by ClustalW.

Accession numbers of used sequences are listed in the Supplementary Table 11. The phylogenetic tree (Supplementary Fig. 12) was generated using the aligned region indicated by the red box (44–153 aa).



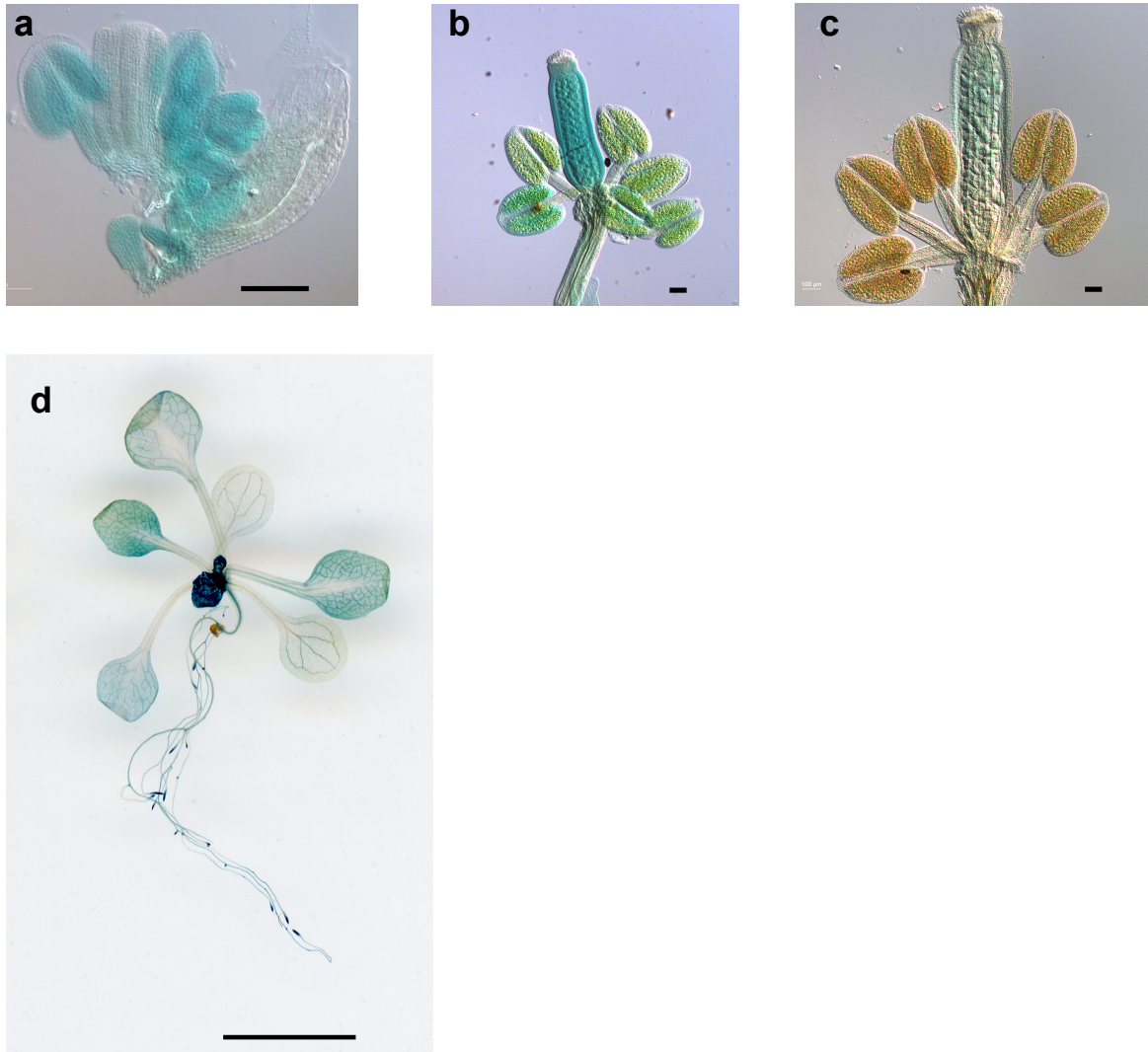
Supplementary Figure 12. Phylogenetic tree of RDP1/Mrt4p and P0.

Phylogenetic tree was constructed by using the neighbor-joining method⁴. The scale bar indicates the genetic distance based on the Kimura model⁵. Values on the branches indicate the percentage of 1,000 bootstrap replicates. See Supplementary Fig. 11 for the alignment used for this tree. ScYvh1 was used as an outgroup.

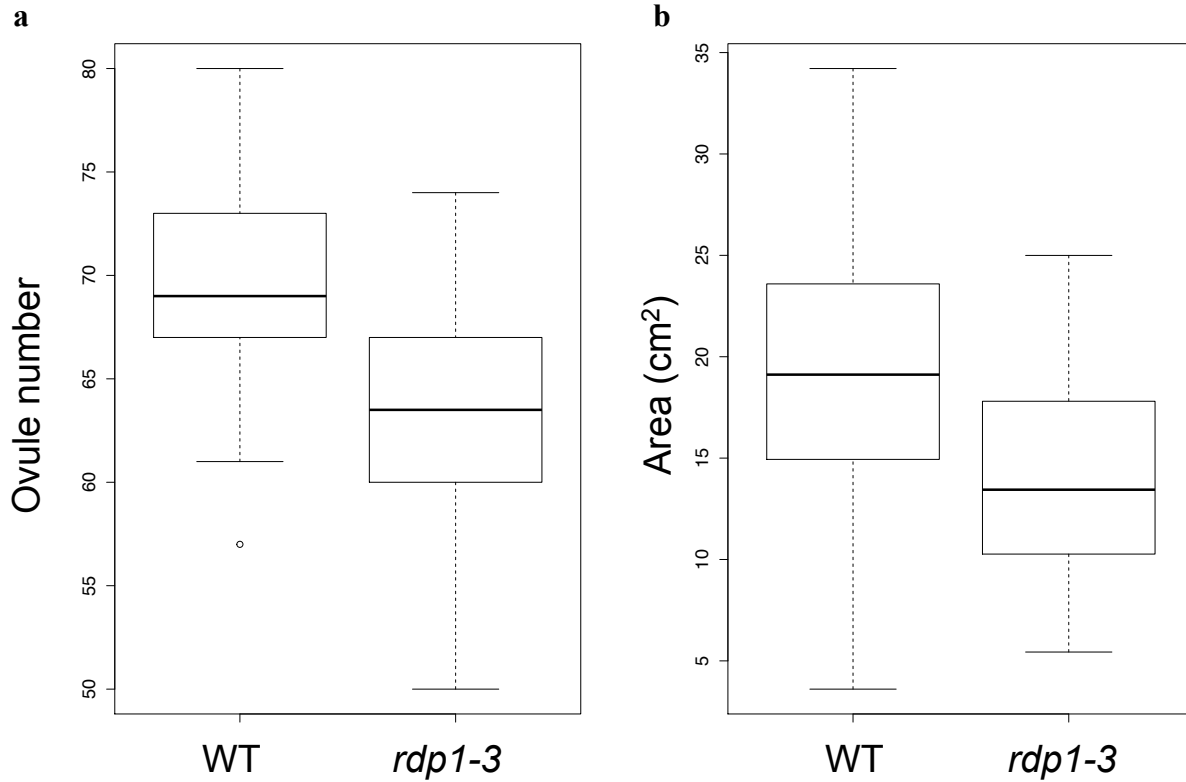


Supplementary Figure 13. *In situ* hybridization analysis of *RDPI*.

RDPI expression was analyzed in Col-0 with *RDPI* antisense (**a**, **b**, **c**, and **e**) or sense (**d**) probes. Transverse (**a–d**) and longitudinal (**e**) cross sections of the flower buds. *RDPI* expression was detected at the early stages of pollen lineage such as primary sporogenous layer (1°Sp in stage 7 flowers in (**a**)), sporogenous cell (Sp in stage 8 flowers in (**b**)), and tetrad (Tds in stage 10 flowers (**b**, **e**)), but not in the microspore (MSp in stage 11 flowers in (**c**)). *RDPI* expression was also detected in stage 10 and 11 ovules (Ov in (**b**, **c**, **e**)). Scale bars; 100 μ m. A similar expression pattern was observed at least two individuals (**a–e**).



Supplementary Figure 14. GUS staining patterns of plants with *RDP1pro::GUS* construct. GUS-stained samples were observed at flower stage 9 (**a**), 11 (**b**), 13 (**c**), and seedling (**d**). Scale bars: 100 μm for **a–c**, 5 mm for **d**. *RDP1* expression was preferentially detected in young stamens (**a**), in pistils (**b**), and in shoot and root apical meristems and young leaves (**d**). A similar GUS staining pattern was observed at least four individuals (**a–d**).



Supplementary Figure 15. Pleiotropy of *rdp1-3*.

a Ovule number per flower was reduced in *rdp1-3* (N = 42, wild type; N = 53, *rdp1-3/rdp1-3*; *t* test, $P = 2.34 \times 10^{-8}$). **b** Rosette area was smaller in *rdp1-3* (N = 49, wild type; N = 27, *rdp1-3/rdp1-3*; two-sided *t* test, $P = 0.000986$). Boxplots show center line: median; box limits: upper and lower quartiles; whiskers: not greater than 1.5 times the interquartile range; point: outlier. Source data are provided as a Source Data file.

a

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Col-0 GCCATATAGC ACGACATATA TATAGCCAC ATACAATCAA TATTGCTCT AGTAAAAATA AAAATAATA TATTGTTCA AGTACACTTC AATTGAAAA 100
Uod-1 .....G.....C.....G..... 100
Bar-4 .....C..... 100
Col-0 CAATAATATA GTGTATGCTT ATAAATATTA TCAAATCTTA ATAAATAAAT TGTCTTTTAC CTTCCCAAAT AAACCTCTCG TCCAAGAACA AAACACAAGC 200
Uod-1 ..... 200
Bar-4 ..... 200
Col-0 CACGGCGACT ACTACGAGCA TGAGAAGGGT TGAGGACTTC TACAAATGAG TGGACCCATC CCCTTAGTCT CGAACAAATT GCCAGTAGTG AGATCACTAC 300
Uod-1 ..A.....A.....G..... 300
Bar-4 .....A..... 300
Col-0 TCACTACTAA TGGTTGTTCC TATTGTTCC TATACGTTA CATTGTTTAA TTTCCGAGCA TTCATATAAA TAATTTATAA TTCATTATTA AGAGGTTTAA 400
Uod-1 .....C.....C.....T..... 400
Bar-4 .....C..... 400
Col-0 TAATATGATT TTGAAAGTAC CACTTTTTAA TTAATTTTTA TAACATTAAT TTTAAACTT TAATTTCTTA ACTTAAAAAC TTTTGCACAA CAAAACAAAA 500
Uod-1 ..... 500
Bar-4 ..... 500
Col-0 AAAACTAAAA AACTATAAT CCATTTAAAT TTAACATAGT ACACCCCTCA ATGACAAAAA AAAATCGGCC CAGATTTCTG ATAGCTAAAA GGCCTGAAAC 600
Uod-1 ..... 600
Bar-4 .....T..... 600
Col-0 GGGCCGGATC CGGATCGGTC TGAATTAGGG GTCCAGATTA AAAAGTGCBA ATTAAGCAAA ACAAGGGAAC AAGGGTTTCA GTTCCCTGA AGAAAATTGA 700
Uod-1 .....G..... 700
Bar-4 .....G..... 700
Col-0 AAGGAGCTTA ATAAACAGAA GCCGTTCTCC TTCACTTTCG TTCCTCTCAC TTCCATACAA CATTGAAAC CTCAGTATG CCTAAATCAA AGCGAGATAG 800
Uod-1 ..... 800
Bar-4 ..... 800
Col-0 ACCAGGTTAG AACTCTTCTT CCTTCACTCT TTCTGCTCTC TATTGCTGTA ACCATTGTAT CTCTGTCAC TGGTTTGGAA GCTGTAACCT TTGTAGCCGC 900
Uod-1 ..... 900
Bar-4 .....G.....G..... 900
Col-0 CGTCAAGGTT TTAGGTTT TGTAGGTTT TTAGAGGAAC CCTTTGAAA AAAACTTTAA CTGTTGGAG TTAGTAATGT TTTTTCATAG CTCTATGTT 1000
Uod-1 .....G..... 1000
Bar-4 ..... 1000
Col-0 TGAATCGATT GATGAATGGT TTATCTAAGG GTTTTGTCTT CTGATTTTTT GCTGCAAGTA CTTTGTCTAA GACGAAGAAG AAAGGAAGAG AGCATAAGGA 1100
Uod-1 ..... 1100
Bar-4 .....T..... 1100
Col-0 GTGTATTGTG AATGGAATTA GGGAGGCTGT TGAATAATAT TCCTCTGTCT ATGTTTTCTC TTTTGAAAAC ATGAGAAATA TCAAATTCOA GGAGTTTAGG 1200
Uod-1 ..... 1200
Bar-4 ..... 1200
Col-0 CAGCAGTTTA GGCACAATGG AAAGTTAGTT TCTTTGCTC TCTTCTGGTT GATGTTAGAA TTACTTGAAT GTTATGACTG ACTCGGTTCT TATTGTCTA 1300
Uod-1 ..... 1300
Bar-4 .....T..... 1300
Col-0 GGTCTTCTCT AGGTTCGAAC AAAGTATGTC AGTTGCTCT TGGTCTTCT GCAGAAGATG AACTCCGTTT TGGTATCTAC AAAGTCTCCA AGTTTGTGT 1400
Uod-1 ..... 1400
Bar-4 ..... 1400
Col-0 ATTCTGCTTC TTACAATGGT TCTTTATGT TAAATGGTCA TTTTGTGCA GTTAGATTTA CATATGTTGT GGAATGTTGT TTCAGCTGCT TCGTGGTGT 1500
Uod-1 ..... 1500
Bar-4 ..C.....C..G..T..C.....T.....C..... 1500
Col-0 ACTGGACTTC TTGTTACTGA TATGCCAAAG GAAGAATGCG AAAGGTATCT ATCTATGAT CTCTTGACGT CCTCTTTTTT CCTCTTTTGC TTTGGTGAA 1600
Uod-1 ..... 1600
Bar-4 .....A.....T.....CT.....AA.....CTC.....A..G..GG.....A..... 1589
Col-0 CAACAGTGAC GGGTTTGAAT TGTATACATG TCTAGTTGTT GTAACGTTC ACAATACTTC TTCTGGTTGT GTATTGCAGC TTATTTAACG CTTATGAGGA 1700
Uod-1 ..... 1700
Bar-4 .....A..G..T.....C..A.....CT...G.....C.....GA.....C..... 1687
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Col-0 TTCGACTTT TCAAGGACCG GAAGCATTGC AGTAGAAACG GTTTGTGAAC AGAACTTGTG CCATCTCTAT ATACTGTTGT TTCTAGATAA ATAAATAAGC 1800
 Uod-1 1800
 Bor-4 T G C 1787

Col-0 TTGAACATGA TCAGGCTCTG CT..TCTCTT TGTATTATTA AAAACTTTGT TTCGTTACTT TTATCTTAAAT TACTATATTG GTACCACCTA AATGATTAGG 1898
 Uod-1 CT A 1900
 Bor-4 G CC C G G T 1885

Col-0 TTGAACGAA GGAAGGTCT CTAGAACAGT TCACACATGA GATGGAACCA CTTCTTCGGA AGCTTGAAAT GCCTGTACGA CTAACAACAAAG GTCTGTCTGC 1998
 Uod-1 2000
 Bor-4 G G T T C 1985

Col-0 TGACCATGTT AATATTCTCT TTTCTCATAC TCACCTTTGC TATCTATATT AATCCAAAAA CTTATTTCCT CTTCTTATTT TAATATGTAG GTACTGTGGA 2098
 Uod-1 G 2100
 Bor-4 A T A 2077

Col-0 GTTGGTCTCT GACTTTGTAG TTTGTGAAGA AGGAAAACAA CTTTCGCCAA AATCAGCCCA CATTTCGTA ATAAGACTTC TGTATACCAA AACCTTTCC 2198
 Uod-1 2200
 Bor-4 2177

Col-0 TTTTAATTGG TATGTTTAT TCTTATACT TGTTTGTTA CTTTGCAGCG TCTATTGCGA ATGAAGATGG CTACTTTCAA GCTGAACCTG CTCTGCAGAT 2298
 Uod-1 2300
 Bor-4 2277

Col-0 GGAGCCCTAG TGACTTTGAG CTTTACAGAG AAGATCTGTC AGAGCTTTAC CGAGAAGATC TCTCAGACGT TGAACCTCT TAGGAAGCCA AGATTTTGAC 2398
 Uod-1 2400
 Bor-4 T 2377

Col-0 TCCACCCCTT AAATTTTCT TAATATCATC ACTATCACAT GGATTTTATA TTGTGGCACA TGGTTTGCAT TCAAATGTTT TAAGTCAAAG TTTTGTCTGC 2498
 Uod-1 G 2500
 Bor-4 G 2477

Col-0 TTTACATGTT TTGTAGAATA TCAACTATC TTCAAGAACC CCCTATCCTA ATATTGGCAA ATCAATCTTG AAAACCTTGT TTTTCTTATT CAGCCACATT 2598
 Uod-1 2600
 Bor-4 2577

Col-0 ATGGAATTC TTAICTCAATT TGCTTACTTT TTACAGATT ATAGATGGTG TTGGGACCGA ATTAATTGGC ATGCGTATGA TAATTTAAG CAAGTTTTTC 2698
 Uod-1 2700
 Bor-4 2677

Col-0 TTCTTGCTTT TAAATATTATT GTGGTCCATG GGCTTGAGAG CAGTCCAACA ACGTTCACAC GTCGTCATC TCTTTTGATA ATTTCTTGT T-TTTGTTTT 2797
 Uod-1 G T 2800
 Bor-4 C G T 2777

Col-0 TTTGTTGTT TTTTAGCAAT CGAAACTAT TCGGTTTGAC TAGGTGAGCT TAGAGTTGTG ATGTTAGGGA TAGAAGCAGT TTTCTACGAA AGGGGTTGAA 2897
 Uod-1 T C 2899
 Bor-4 T C 2877

Col-0 GATACTATGA GCGAATGCG GTTGAACAAA ATAGAACCCA CCGGAGATT TGGTAAAAGA TTAACCGGAA AGGCGATACT TCCAACCTCG ACGTAACAAA 2997
 Uod-1 C 2999
 Bor-4 2977

Col-0 TGTTTAGTTG TAGATTTTCA ACATTTT 3024
 Uod-1 G 3026
 Bor-4 G 3004

b

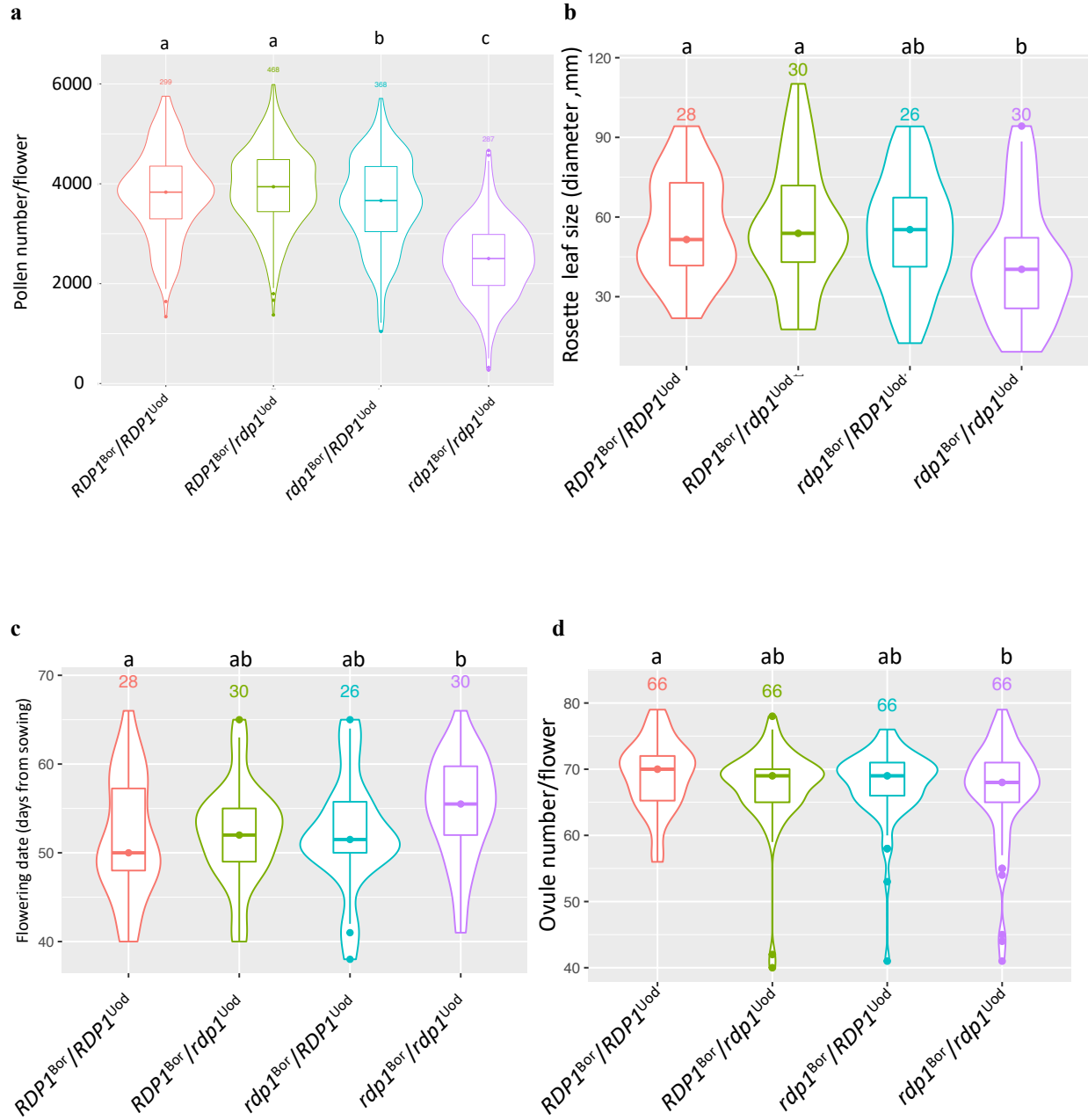
Col-0 MPKSKRDRPV TLSKTKKKGK EHKECIVNGI REAVEKYSSV YVFSFENMRN IKFKEFRQOF RHNGKFFLGS NKVMQVALGR SAEDELRSGL YKVKSKLLRGD 100
 Uod-1 100
 Bor-4 100

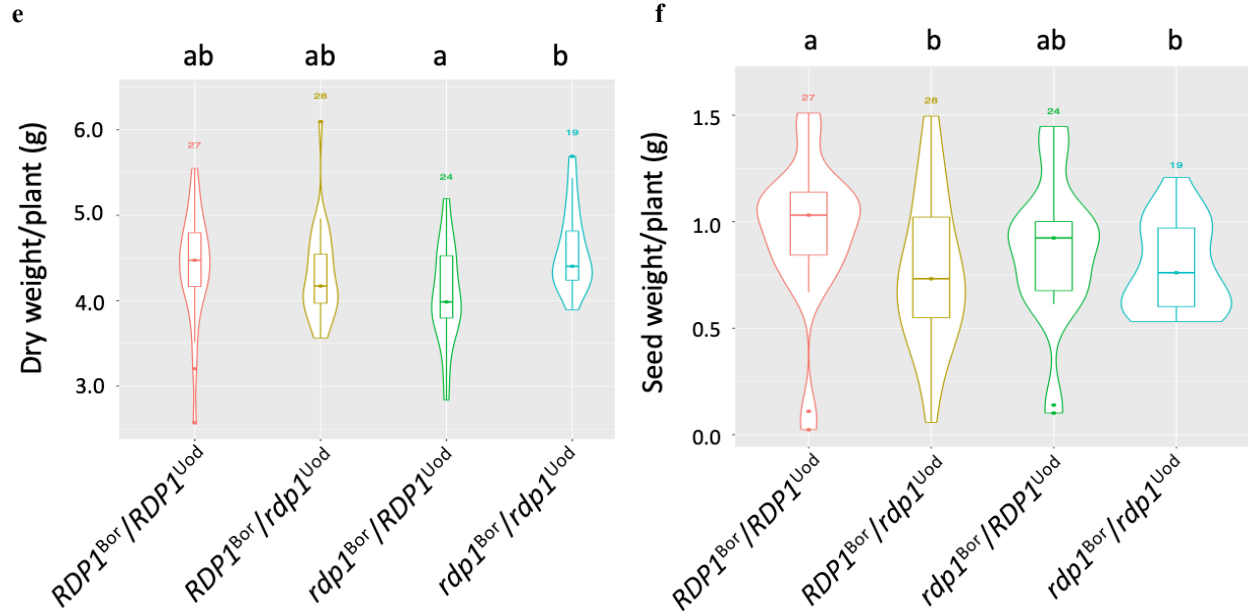
Col-0 TGLLVTDMPK EEVESLFNAY EDSDFSRTGS IAVETVELKE GPLEQFTHM EPLLRKLEMP VRLNKGTVEL VADFVVC EEG KQLSPKSAHI LRLLRMKMAT 200
 Uod-1 200
 Bor-4 200

Col-0 FKLNLLCRWS PSDFELYRED LSELYREDLS DVETS* 236
 Uod-1 236
 Bor-4 L 236

Supplementary Figure 16. Sequences of the *RDPI* gene.

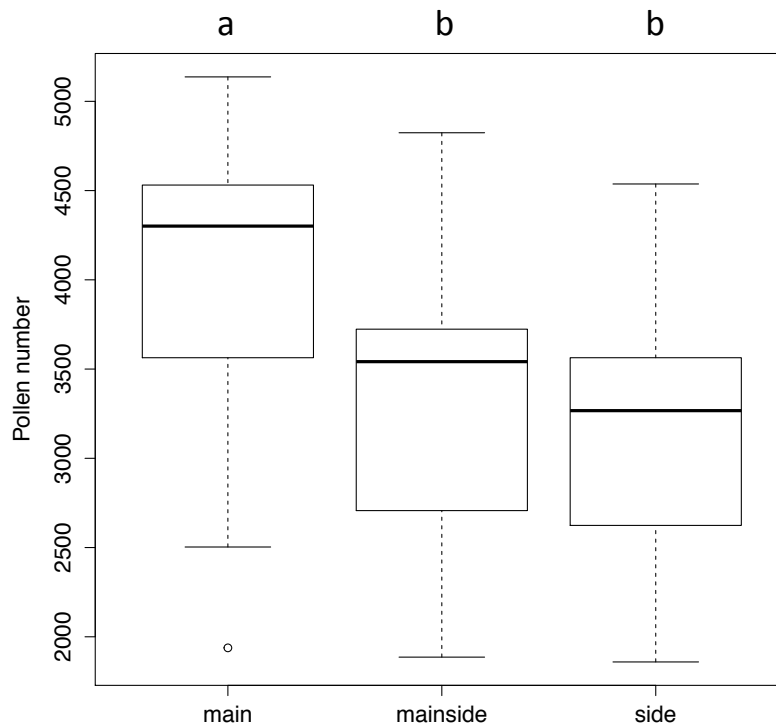
Nucleic acid (a) and amino acid (b) sequences of Col-0, Uod-1, and Bor-4 are shown. **a** Nucleic acid sequences containing *RDPI* with 777 bp upstream and 643 bp downstream sequences. **b** Amino acid sequences of *RDPI*. Dots represent identical sequence with Col-0. Dashes indicate deletion sequence.





Supplementary Figure 17. Phenotypes of F₁ plants obtained from the cross of *RDP1^{Bor}/rdp1^{Bor}* and *RDP1^{Uod}/rdp1^{Uod}*.

Pollen number (a), rosette leaf size (b), flowering date (days from sowing) (c), ovule number (d), dry weight (e) and seed weight (f) in *RDP1^{Bor}/RDP1^{Uod}*, *RDP1^{Bor}/rdp1^{Uod}*, *rdp1^{Bor}/RDP1^{Uod}*, *rdp1^{Bor}/rdp1^{Uod}* plants. Sample numbers are shown above the plots. *P* values were listed in Supplementary Table 12. Letters (a, b, c) indicate significant differences determined by *t*-test; *P* < 0.05. For pollen number phenotype, the same data set was partially used with Fig. 3c. Boxplots show center line: median; box limits: upper and lower quartiles; whiskers: not greater than 1.5 times the interquartile range; point: outlier. Violin shape corresponds to the density of data. Source data are provided as a Source Data file.



Supplementary Figure 18. Pollen number in different floral stems.

Main stems (main, N = 19), side stems branched from main stems (mainside, N = 20), and side stems (side, N = 19). Col-0 plants were used. Letters (a and b) indicate significant differences in pollen number between positions determined by analysis of variance and *post hoc* Tukey test ($P < 0.05$; main vs. mainside: $P = 0.0160$; main vs. side: $P = 0.00255$; mainside vs. side: $P = 0.769$). Boxplots show center line: median; box limits: upper and lower quartiles; whiskers: not greater than 1.5 times the interquartile range; point: outlier. Source data are provided as a Source Data file.

Supplementary Table 1. Pollen number phenotype of 144 accessions used for GWAS.

ecotype_id	Pollen number	ecotype_id	Pollen number	ecotype_id	Pollen number
5837	7355.416	7201	3420.000	7337	3276.000
6041	4459.114	7205	5904.000	7340	4068.000
6108	4219.922	7210	2808.000	7343	4032.000
6189	5265.087	7223	3168.000	7344	3384.000
6193	4626.375	7224	7632.000	7351	3888.000
6903	8549.642	7227	2952.000	7353	6696.000
6907	3871.534	7228	4608.000	7354	2880.000
6909	5508.000	7229	3924.000	7355	4680.000
6911	3024.000	7231	5544.000	7372	2844.000
6915	3904.466	7238	3708.000	7373	5256.000
6929	3280.487	7239	3816.000	7376	5760.000
6931	5265.087	7242	4536.000	7378	4104.000
6933	3488.480	7244	3600.000	7379	4464.000
6936	4043.128	7246	3528.000	7381	2592.000
6937	4336.052	7252	3564.000	7382	4788.000
6938	3784.004	7255	4104.000	7384	5472.000
6939	5090.893	7256	3132.000	7386	5616.000
6940	2710.240	7258	2952.000	7390	5976.000
6943	4336.052	7260	2088.000	7392	3996.000
6945	3771.871	7262	2808.000	7394	3672.000
6951	3465.081	7265	4608.000	7403	5004.000
6956	3627.142	7268	4536.000	7404	3024.000
6957	4112.459	7275	3060.000	7405	4212.000
6958	4103.793	7276	3780.000	7406	3384.000
6959	3003.163	7277	2880.000	7408	4500.000
6960	4568.310	7280	3528.000	7411	2880.000
6961	3349.818	7282	2880.000	7413	3132.000
6963	3284.820	7283	5652.000	7418	3240.000
6966	4219.922	7284	4176.000	7423	4032.000
6967	3929.599	7287	2772.000	7424	5472.000
6970	3384.484	7291	2304.000	7425	4356.000
6972	4667.107	7292	4176.000	7430	3456.000
6975	2594.110	7297	3816.000	7438	3067.294
6976	3523.146	7299	4644.000	7446	3600.000
6977	4452.181	7300	3240.000	7449	3888.000
6979	3407.016	7301	5040.000	7457	4392.000
6980	4452.181	7303	5112.000	7514	4944.431
6982	4112.459	7305	3996.000	7515	3000.563
6984	5126.425	7307	2880.000	7520	3465.081
6985	4452.181	7309	5544.000	7521	2942.499
7169	5256.000	7310	4356.000	7523	3290.887
7172	4068.000	7316	4644.000	7525	3037.829
7176	3636.000	7317	2844.000	8258	3290.887
7178	2808.000	7320	5832.000	8608	3581.210
7188	2124.000	7328	3096.000	8730	2926.033
7192	3744.000	7330	2304.000	8770	2587.177
7195	4140.000	7331	3888.000	8787	4045.728
7199	3204.000	7333	4104.000	8791	4219.922

Supplementary Table 2. Ovule number phenotype of 151 accessions used for GWAS.

ecotype_id	Ovule number	ecotype_id	Ovule number	ecotype_id	Ovule number
5837	64.000	7192	78.667	7333	86.667
6041	87.556	7195	77.200	7337	79.600
6108	78.667	7199	66.250	7340	84.667
6189	74.000	7201	69.500	7343	71.500
6193	76.500	7205	66.000	7344	74.667
6903	85.333	7210	80.000	7352	50.000
6907	65.500	7223	74.000	7353	58.500
6908	64.800	7224	75.250	7354	56.500
6909	66.500	7227	60.750	7355	57.500
6911	71.000	7228	70.667	7372	69.500
6915	76.000	7229	61.000	7373	75.500
6928	54.000	7231	74.800	7376	60.000
6929	72.500	7238	56.500	7378	69.000
6931	79.000	7239	69.500	7379	63.500
6933	64.800	7242	62.000	7381	65.750
6936	81.067	7244	76.000	7382	70.750
6937	73.250	7246	66.000	7384	51.500
6938	65.000	7252	47.333	7386	65.800
6939	76.000	7255	73.750	7390	50.250
6940	59.429	7256	62.500	7391	47.000
6943	79.000	7258	66.000	7392	72.000
6944	84.000	7260	69.250	7394	70.000
6945	58.933	7262	72.000	7403	75.500
6951	68.286	7265	61.200	7404	64.000
6956	61.429	7268	78.667	7405	71.500
6957	64.000	7275	85.000	7406	68.000
6958	77.333	7277	55.500	7408	72.000
6959	73.750	7280	63.500	7411	65.500
6960	75.500	7282	71.000	7413	68.000
6961	70.286	7283	56.500	7418	61.600
6963	76.000	7284	55.000	7423	72.000
6966	81.538	7287	60.000	7424	66.500
6967	69.000	7291	52.500	7425	71.500
6970	65.500	7292	70.000	7430	76.750
6972	73.667	7296	74.000	7438	67.000
6975	71.000	7297	70.667	7446	50.000
6976	61.667	7299	75.000	7449	66.000
6977	68.667	7300	57.000	7457	71.333
6978	66.667	7301	41.000	7514	70.000
6979	73.500	7303	60.667	7515	47.000
6980	73.500	7305	84.000	7520	84.727
6982	64.000	7307	68.000	7521	71.429
6984	86.000	7309	55.500	7523	56.500
6985	65.000	7310	83.600	7525	67.200
7169	44.333	7316	58.667	8258	75.500
7172	77.000	7317	65.800	8534	59.333
7176	69.500	7320	60.000	8608	60.000
7178	75.500	7328	49.000	8730	62.667
7181	52.000	7330	66.000	8770	65.333
7188	65.500	7331	62.750	8787	71.500
				8791	68.000

Supplementary Table 3. Association between pollen and ovule numbers and the diverse set of 107 phenotypes.

Category	Phenotype name	r (pollen)	P value (pollen)	r (ovule)	P value (ovule)	Category	Phenotype name	r (pollen)	P value (pollen)	r (ovule)	P value (ovule)
FL	1 LD	-0.652	0.374	-1.534	0.136	FL	40 2W	-1.246	0.216	-3.355	<0.001
FL	2 LDV	-1.013	0.169	-1.827	0.061	FL	41 4W	-1.365	0.165	-3.282	<0.001
FL	3 SD	-0.967	0.303	-2.047	0.046	FL	42 8W	-1.565	0.128	-4.481	0.001
FL	4 SDV	-0.964	0.942	-1.595	0.118	FL	43 FLC	-1.367	0.179	-4.743	0.057
FL	5 FT10	-0.613	0.543	-0.483	0.630	FL	44 FRI	-0.520	0.605	-1.825	0.074
FL	6 FT16	-1.075	0.289	-1.726	0.091	FL	45 8W,GH,FT	-0.918	0.363	-4.724	<0.001
FL	7 FT22	-1.731	0.089	-3.354	0.015	FL	46 8W,GH,LN	-0.372	0.742	-1.106	0.263
DEV	8 Seed Dormancy	-0.939	0.341	-0.218	0.827	FL	47 0W,GH,FT	-1.505	0.145	-3.817	0.004
DEF	9 Emc5	-1.075	0.324	-1.238	0.226	FL	48 0W,GH,LN	-1.152	0.254	-3.469	0.012
DEF	10 Emw1	1.598	0.193	-0.072	0.949	FL	57 FT,Field	0.667	0.073	0.974	0.326
DEF	11 Emv1	1.927	0.067	-0.061	0.951	FL	58 FT,Diameter,Field	-1.439	0.152	-0.412	0.682
DEF	12 Hkx1	1.310	0.195	-0.505	0.613	FL	59 FT,GH	0.101	0.811	-3.173	0.002
DEF	13 Noco2	1.242	0.220	1.527	0.137	DEV	60 FT,Duration,GH	-1.516	0.137	-1.623	0.110
ION	14 L17	2.136	0.039	3.028	0.004	DEV	61 LC,Duration,GH	-1.973	0.052	-3.069	0.004
ION	15 B11	-1.023	0.125	0.039	0.971	DEV	62 LFS,GH	-1.845	0.070	-3.123	0.003
ION	16 N123	0.306	0.763	-0.306	0.415	DEV	63 MT,GH	0.732	0.464	2.074	0.035
ION	17 Mj25	1.671	0.101	-0.361	0.750	DEV	64 RP,GH	1.195	0.237	3.340	0.001
ION	18 P31	-1.694	0.169	-0.148	0.909	DEF	65 AL1	-0.291	0.767	-1.201	0.230
ION	19 S34	0.591	0.590	0.164	0.776	DEF	66 AL1,CFU2	-0.420	0.672	0.926	0.356
ION	20 K39	0.950	0.532	-2.185	0.034	DEF	67 As	-2.974	0.009	-4.940	0.000
ION	21 Cx43	0.714	0.473	-0.655	0.517	DEF	68 As,CFU2	-0.142	0.887	-0.355	0.731
ION	22 Mm55	-0.865	0.320	1.153	0.254	DEF	69 Bs	1.587	0.120	-0.862	0.391
ION	23 F56	0.268	0.775	-1.843	0.071	DEF	70 Bs,CFU2	0.202	0.835	-0.102	0.919
ION	24 Cx59	-0.743	0.463	-0.890	0.559	DEF	71 AL2	-1.251	0.218	-1.634	0.094
ION	25 N160	0.452	0.650	-0.088	0.926	DEF	72 AL2,CFU2	-0.648	0.510	-0.601	0.503
ION	26 C165	0.071	0.943	0.630	0.520	DEF	73 As2	-0.242	0.804	-0.782	0.435
ION	27 Z166	0.607	0.509	-1.076	0.285	DEF	74 As2,CFU2	0.963	0.332	1.379	0.170
ION	28 As75	0.267	0.789	1.820	0.075	DEV	75 FW	2.634	0.010	0.874	0.385
ION	29 Sx2	0.902	0.371	0.629	0.537	DEV	76 DW	2.160	0.037	1.045	0.303
ION	30 Mx98	-0.115	0.906	0.143	0.828	DEV	77 LES	-1.077	0.287	0.397	0.629
ION	31 Cx114	0.912	0.369	0.092	0.927	DEV	78 YEL	-0.201	0.846	0.332	0.741
DEF	32 LavPnB.L	0.747	0.463	1.303	0.261	DEV	79 LY	-0.968	0.345	0.547	0.584
DEF	33 LavPnB.L.I	0.978	0.335	1.248	0.215	FL	80 LN10	-0.384	0.708	0.581	0.514
DEF	34 LavPnB.L.II	0.651	0.637	0.457	0.659	FL	81 LN16	1.276	0.207	2.153	0.038
DEF	35 LavB.L	1.072	0.157	1.365	0.171	FL	82 LN22	1.727	0.092	3.126	0.001
FL	39 0W	-1.728	0.036	-4.574	<0.001	DEV	158 Silique.16	-0.324	0.749	-0.463	0.151

FL: flowering phenotypes; DEV: developmental phenotypes; DEF: defense-related phenotypes; ION: ionomites phenotypes. For the details of phenotypes, see Atwell et al (ref. 2).
r: Pearson's correlation coefficient. P values were calculated by two-sided tests without adjustment for multiple comparison. For pollen number, log-transformed phenotype values were used.

Category	Phenotype name	r (pollen)	P value (pollen)	r (ovule)	P value (ovule)
DEV	159 Silique.22	-1.867	0.064	-0.657	0.514
DEV	161 Germ.10	1.237	0.231	-1.290	0.203
DEV	162 Germ.16	1.393	0.187	-0.017	0.982
DEV	163 Germ.22	-1.055	0.325	-0.697	0.517
DEV	164 Width.10	-0.951	0.371	1.140	0.254
DEV	165 Width.16	0.501	0.563	1.815	0.072
DEV	166 Width.22	0.312	0.723	1.021	0.312
DEV	167 Chloros.10	-0.745	0.460	-1.004	0.318
DEV	168 Chloros.16	-0.634	0.526	-1.256	0.215
DEV	169 Chloros.22	1.543	0.130	1.848	0.071
DEV	170 Anthocyanin.10	-0.974	0.357	0.999	0.322
DEV	171 Anthocyanin.16	1.426	0.164	0.979	0.342
DEV	172 Anthocyanin.22	-0.917	0.462	1.704	0.095
DEV	173 Leaf serr.10	0.407	0.608	1.139	0.259
DEV	174 Leaf serr.16	1.279	0.203	0.642	0.514
DEV	175 Leaf serr.22	-0.147	0.837	-0.693	0.548
DEV	176 Leaf roll.10	0.189	0.851	0.075	0.928
DEV	177 Leaf roll.16	0.367	0.627	-1.191	0.252
DEV	178 Leaf roll.22	-0.186	0.852	0.816	0.394
DEV	179 Rosette Erect.22	-0.244	0.951	-0.186	0.868
DEF	183 Trichome.length	-0.765	0.424	-0.201	0.843
DEF	184 Trichome.avg.JA	-1.566	0.123	-0.494	0.622
DEF	185 Aphid.number	-0.184	0.854	-0.521	0.577
DEF	186 Bacterial.liter	0.517	0.607	0.443	0.659
DEV	272 Seedling.Growth	0.126	0.918	0.873	0.387
DEV	273 Vern.Growth	-0.166	0.866	-0.017	0.991
DEV	274 After.Vern.Growth	0.620	0.368	0.803	0.423
DEV	277 Secondary.Dormancy	-2.262	0.030	1.605	0.104
DEV	278 Germ.in.dark	-2.180	0.030	-2.280	0.020
DEV	279 DSDS50	0.237	0.811	1.101	0.270
DEV	280 Seed.bank.133.91	0.151	0.804	0.640	0.397
DEV	281 Storage.7.days	-0.610	0.542	1.578	0.131
DEV	282 Storage.28.days	0.341	0.745	0.643	0.547
DEV	283 Storage.36.days	0.490	0.624	-0.672	0.429

Supplementary Table 4. S-haplogroups of the accessions used for pollen number GWAS.

ecotype_id	Accession name	S-haplogroup	ecotype_id	Accession name	S-haplogroup	ecotype_id	Accession name	S-haplogroup
5837	Bor-1	A	7201	Kr-0	C	7337	Si-0	A
6041	Lis-3	NA	7205	Krot-2	NA	7340	Sav-0	A
6108	T480	NA	7210	La-1	A	7343	Sp-0	A
6189	TDr-2	NA	7223	Li-2:1	A	7344	Sg-1	A
6193	TDr-7	NA	7224	Li-3	A	7351	Ty-0	NA
6903	Bor-4	A	7227	Li-5:2	NA	7353	Tha-1	NA
6907	CIBC-17	A	7228	Li-5:3	NA	7354	Ting-1	NA
6909	Col-0	A	7229	Li-6	A	7355	Tiv-1	NA
6911	Cvi-0	B	7231	Li-7	A	7372	Tscha-1	NA
6915	Ei-2	A	7238	LI-1	A	7373	Tsu-0	NA
6929	Kondara	A	7239	LI-2	C	7376	Tu-1	A
6931	Kz-9	A	7242	Lo-2	A	7378	Uk-1	A
6933	LL-0	A	7244	Mnz-0	NA	7379	Uk-2	A
6936	Lz-0	C	7246	Ma-2	A	7381	Uk-4	A
6937	Mrk-0	A	7252	Mc-0	NA	7382	Utrecht	NA
6938	Ms-0	A	7255	Mh-0	NA	7384	Ven-1	NA
6939	Mt-0	A	7256	Mh-1	A	7386	Vi-0	A
6940	Mz-0	A	7258	Nw-0	A	7390	Wag-3	NA
6943	NFA-10	A	7260	Nw-2	A	7392	Wag-5	NA
6945	Nok-3	A	7262	Nw-4	A	7394	Va-1	A
6951	Pu2-23	A	7265	Nd-0	A	7403	Wei-1	A
6956	Pu2-7	A	7268	Np-0	NA	7404	Wc-1	A
6957	Pu2-8	NA	7275	No-0	A	7405	Wc-2	A
6958	Ra-0	C	7276	Ob-0	A	7406	Wt-1	A
6959	Ren-1	A	7277	Ob-1	NA	7408	Wt-3	NA
6960	Ren-11	A	7280	Old-1	A	7411	Wl-0	NA
6961	Se-0	A	7282	Or-0	A	7413	Wl-2	A
6963	Sorbo	A	7283	Ors-1	NA	7418	Zu-1	A
6966	Sq-1	A	7284	Ors-2	NA	7423	Jl-2	A
6967	Sq-3	A	7287	Ove-0	A	7424	Jl-3	A
6970	Ts-1	A	7291	Pa-2	A	7425	Jl-4	NA
6972	Tsu-1	A	7292	Pa-3	A	7430	Nc-1	A
6975	Uod-1	A	7297	Pf-0	A	7438	N13	NA
6976	Uod-7	A	7299	Pi-2	A	7446	N4	NA
6977	Van-0	A	7300	Pla-0	A	7449	N7	NA
6979	Wei-0	A	7301	Pla-1	A	7457	Rld-2	A
6980	Ws-0	A	7303	Pla-3	A	7514	RRS-7	NA
6982	Wt-5	C	7305	Pt-0	A	7515	RRS-10	NA
6984	Zdr-1	A	7307	Pn-0	A	7520	Lp2-2	A
6985	Zdr-6	A	7309	Po-1	A	7521	Lp2-6	A
7169	Hh-0	NA	7310	Pr-0	A	7523	Pna-17	NA
7172	Hl-3	A	7316	Rhen-1	NA	7525	Rmx-A180	NA
7176	Is-1	A	7317	Rl-0	NA	8258	Ba4-1	NA
7178	Jm-1	A	7320	Rou-0	A	8608	11ME1.23	NA
7188	Kelsterbach-2	A	7328	Sf-2	A	8730	11PNA1.9	NA
7192	Kil-0	A	7330	Sapporo-0	NA	8770	11PNA3.65	NA
7195	Kl-1	A	7331	Sh-0	A	8787	11PNA3.86	NA
7199	Kl-5	A	7333	Sei-0	A	8791	11PNA3.90	NA

The S-haplogroup assignment was based on Shimizu et al (ref. 1).

NA: not known.

Supplementary Table 5. Association between pollen and ovule numbers and climate variables.

	<i>r</i> (pollen)	<i>P</i> value (pollen)	<i>r</i> (ovule)	<i>P</i> value (ovule)
LATITUDE	0.0252	0.9799	0.4996	0.6182
LONGITUDE	0.4021	0.6883	1.3283	0.1865
bio4.temperature.seasonality	-0.0660	0.9475	1.3853	0.1684
bio5.maximum.temperature.in.the.warmest.month	-0.2095	0.8344	-0.9161	0.3613
bio6.minimum.temperature.in.the.coldest.month	-0.0695	0.9447	-2.2712	0.0248
bio13.precipitation.in.the.wettest.month	-1.2478	0.2145	-1.0581	0.2920
bio14.precipitation.in.the.driest.month	-0.3502	0.7268	-0.5938	0.5537
bio15.precipitation.cv	0.0513	0.9592	1.0617	0.2904
PAR_SPRING	0.3633	0.7170	-1.5635	0.1204
length.of.the.growing.season	0.1641	0.8699	-1.2036	0.2310
number.of.consecutive.cold.days	-0.2329	0.8162	0.8765	0.3824
number.of.consecutive.frost.free.days	-0.0637	0.9493	-2.9642	0.0036
relative.humidity.spring	-0.5172	0.6060	0.3048	0.7610
daylength.spring	-0.2057	0.8374	-0.0372	0.9704
aridity.index	-0.5043	0.6150	-1.7755	0.0782

For the details of climate variables, see Hancock et al (ref. 6).

r: Pearson's correlation coefficient (two-sided test without adjustment for multiple comparison).

For pollen number, log-transformed phenotype values were used.

Supplementary Table 6. Detailed SNP information of top five GWAS peaks with the 10-kb window-based iHS scores.

Chromosome	Position	GWAS score (-log ₁₀ (<i>P</i> value))	MAF	iHS score	<i>P</i> value of iHS*
1	8858371	2.53E-08	0.167	-3.792	0.015
1	23683182	5.89E-08	0.160	2.398	0.359
2	10391465	4.58E-07	0.264	-3.561	0.026
3	15598975	1.32E-06	0.486	-1.608	0.873
5	101835	4.38E-06	0.424	1.695	0.829

**P* values of strongest iHS values in 10-kb windows that encompass the GWAS-SNP (one-sided test).

Supplementary Table 7. Top rank traits showing fold enrichments of more than 3 in the selection scan.

Phenotype name	Phenotype category	Short description	Fold enrichment	P value
Pollen number	This study	Pollen number per flower	6.35	0.002
Manganese (Mn55)	Ionomics	<i>In planta</i> ion concentration of Manganese	5.88	0.006
Bs CFU2	Defense-related	<i>In planta</i> bacterial growth (number of CFU / leaf area) of the <i>Pseudomonas viridiflava</i> strain RMX3.1b	5.77	0.003
Leaf roll 22	Developmental	Presence or absence of rolled leaves at 8 weeks post germination for plants grown at 10° C	5.13	0.001
LN10	Flowering	Leaf number at flowering time, 10° C	4.71	0.004
LN16	Flowering	Leaf number at flowering time, 16° C	4.44	0.006
Aphid number	Defense-related	Aphid offspring	4.44	0.008
RP GH	Developmental	Number of days between the appearance of the first flower and the plant complete senescence	4.26	0.009
LN22	Flowering	Leaf number at flowering time, 22° C	4.17	0.007
Storage 28 days	Developmental	Primary dormancy	4.00	0.015
As	Defense-related	Disease presence or absence following inoculation with <i>Pseudomonas viridiflava</i> strain RMX23.1b	3.92	0.022
Nickel (Ni60)	Ionomics	<i>In planta</i> ion concentration of Nickel	3.85	0.019
Noco2	Defense-related	Disease presence or absence following inoculation with <i>Perenospora parasitica</i> isolate Noco2	3.77	0.017
Ovule number	This study	Ovule number per flower	3.64	0.03
Molybdenum (Mo98)	Ionomics	<i>In planta</i> ion concentration of Molybdenum	3.57	0.03
Germ 10	Developmental	Days from removal from stratification until emergence of first cotyledon at 10° C	3.27	0.018

Pollen and ovule numbers, and phenotypes of Atwell et al. (ref. 2) are shown.

Phenotype categories are based on Atwell et al (ref. 2). Fold enrichment and P values of the top 1% iHS tail are shown (one-sided test).

Supplementary Table 8. Pairwise *P* values of Fig. 2a.

Pairwise genotype combinations		<i>P</i> value
<i>RDP1/RDP1</i>	<i>rdp1-1/rdp1-1</i>	<2.2e-16
<i>rdp1-1/rdp1-1+RDP1</i>	<i>rdp1-1/rdp1-1</i>	<2.2e-16
<i>rdp1-3/rdp1-3</i>	<i>rdp1-1/rdp1-1</i>	0.183
<i>rdp1-4/rdp1-4</i>	<i>rdp1-1/rdp1-1</i>	<2.2e-16
<i>rdp1-2/rdp1-2</i>	<i>rdp1-1/rdp1-1</i>	0.173
<i>rdp1-1/rdp1-1+RDP1</i>	<i>RDP1/RDP1</i>	0.894
<i>rdp1-3/rdp1-3</i>	<i>RDP1/RDP1</i>	<2.2e-16
<i>rdp1-4/rdp1-4</i>	<i>RDP1/RDP1</i>	<2.2e-16
<i>rdp1-2/rdp1-2</i>	<i>RDP1/RDP1</i>	<2.2e-16
<i>rdp1-3/rdp1-3</i>	<i>rdp1-1/rdp1-1+RDP1</i>	<2.2e-16
<i>rdp1-4/rdp1-4</i>	<i>rdp1-1/rdp1-1+RDP1</i>	<2.2e-16
<i>rdp1-2/rdp1-2</i>	<i>rdp1-1/rdp1-1+RDP1</i>	<2.2e-16
<i>rdp1-4/rdp1-4</i>	<i>rdp1-3/rdp1-3</i>	0.982
<i>rdp1-2/rdp1-2</i>	<i>rdp1-3/rdp1-3</i>	0.043
<i>rdp1-2/rdp1-2</i>	<i>rdp1-4/rdp1-4</i>	0.0013

P values were calculated by two-sided tests with adjustments for multiple comparisons (post hoc Tukey test).

Supplementary Table 9. Pairwise *P* values of Supplementary Fig. 9.

Pairwise genotype combinations		<i>P</i> value
<i>RDP1/RDP1</i>	<i>rdp1-1/rdp1-1</i>	2.88E-04
<i>rdp1-1/rdp1-1+RDP1</i>	<i>rdp1-1/rdp1-1</i>	2.06E-05
<i>rdp1-3/rdp1-3</i>	<i>rdp1-1/rdp1-1</i>	0.999
<i>rdp1-4/rdp1-4</i>	<i>rdp1-1/rdp1-1</i>	2.86E-02
<i>rdp1-2/rdp1-2</i>	<i>rdp1-1/rdp1-1</i>	5.28E-05
<i>rdp1-1/rdp1-1+RDP1</i>	<i>RDP1/RDP1</i>	0.998
<i>rdp1-3/rdp1-3</i>	<i>RDP1/RDP1</i>	0.0266
<i>rdp1-4/rdp1-4</i>	<i>RDP1/RDP1</i>	0.999
<i>rdp1-2/rdp1-2</i>	<i>RDP1/RDP1</i>	<2.2e-16
<i>rdp1-3/rdp1-3</i>	<i>rdp1-1/rdp1-1+RDP1</i>	0.024
<i>rdp1-4/rdp1-4</i>	<i>rdp1-1/rdp1-1+RDP1</i>	0.999
<i>rdp1-2/rdp1-2</i>	<i>rdp1-1/rdp1-1+RDP1</i>	<2.2e-16
<i>rdp1-4/rdp1-4</i>	<i>rdp1-3/rdp1-3</i>	0.137
<i>rdp1-2/rdp1-2</i>	<i>rdp1-3/rdp1-3</i>	4.90E-03
<i>rdp1-2/rdp1-2</i>	<i>rdp1-4/rdp1-4</i>	<2.2e-16

P values were calculated by two-sided tests with adjustments for multiple comparisons (post hoc Tukey test).

Supplementary Table 10. Primer list.

Target	Purpose	Forward primer		Reverse primer	
		Primer name	sequence (5'-3')	Primer name	sequence (5'-3')
AT1G25250	quantitative reverse transcription PCR	3931_A1lg25250RtFl	ACACCAGCTAACAGATCCAGATG	3932_A1g25250RtFl	AGATCTCACACACGTACCGATCTG
AT1G25260 (<i>RDPI</i>)	quantitative reverse transcription PCR	3933_A1lg25260RtFl	AGCGAGATAGACCAGTGCATTTGTC	3934_A1g25260RtFl	CAACAGCCTCCCTAATTCATTCAC
AT1G25270	quantitative reverse transcription PCR	4075_A1g25270RtFl	TTCGGATCATGACTGGGAGCAATG	4076_A1g25270RtFl	CGAAACCACGATTCGCCGAATAAAGC
AT5G60390 (<i>EF1-α</i>)	quantitative reverse transcription PCR	3529_AhEF1aF	TGAGCAGCTCTTCTTGCTTCA	3530_AhEF1aR	GGTGGTGGCATCCATCTTGTTACA
AT1G25250	genotyping for GT_5_108298 (wild-type)	3100_CT_5_107916_LP	AGACATGTTGGCTCAGGACAC	3101_GT_5_107916_RP	ATGTAAGGTGCGAGGTCAATG
	genotyping for GT_5_108298 (mutant)	3100_CT_5_107916_LP	AGACATGTTGGCTCAGGACAC	3264_Ds3-1	ACCCGACCGGATCGTATCGGT
AT1G25260 (<i>RDPI</i>)	genotyping for <i>rdp1-1</i> (wild-type)	3619_A1lg25260F04	TATGATACCCCAATTGAATG	3195_SA_064854_RP	TTTCCATTGTGCCTAAAACCTGC
	genotyping for <i>rdp1-1</i> (mutant)	2939_SALK_T_DNA_LB	ATTTTGCCGATTTTCGGAAC	3195_SA_064854_RP	TTTCCATTGTGCCTAAAACCTGC
	genotyping for <i>rdp1-2</i> (wild-type)	3622_A1lg25260F07	TTGTAGTTTGTGAAGAAGG	3551_A1lg25260R01	GTTTAAAATGAGAGAACC
	genotyping for <i>rdp1-2</i> (mutant)	3622_A1lg25260F07	TTGTAGTTTGTGAAGAAGG	2946_GABI_T_DNA_LB	ATATTGACCATCATACTCAITGC

Supplementary Table 11. Accession numbers for Supplementary Figs 11 and 12.

Gene family	Species	Accession number or AGI code
RDP1/Mrt4p	<i>Arabidopsis thaliana</i> (Col-0 accession)	AT1G25260
	<i>Arabidopsis thaliana</i> (Mz-0 accession)	LC164158
	<i>Arabidopsis thaliana</i> (Bor-4 accession)	LC164159
	<i>Arabidopsis thaliana</i> (Uod-1 accession)	LC504218
	<i>Arabidopsis lyrata</i>	LC164160
	<i>Arabidopsis halleri</i>	LC164161
	<i>Capsella rubella</i>	XP006304175
	<i>Theobroma cacao</i>	XP007041559
	<i>Populus trichocarpa</i>	XP002299012
	<i>Vitis vinifera</i>	XP002276595
	<i>Solanum lycopersicum</i>	XP004230519
	<i>Prunus persica</i>	XP007223904
	<i>Lotus japonicus</i>	AFK33945
	<i>Glycine max</i>	NP001235726
	<i>Medicago truncatula</i>	XP003593096
	<i>Oryza sativa</i>	XP015618389
	<i>Zea mays</i>	NP001148655
	<i>Physcomitrella patens</i>	XP024389099
	<i>Acanthamoeba castellanii</i>	XP004351551
	<i>Caenorhabditis elegans</i>	NP495470
	<i>Latimeria chalumnae</i>	XP005986121
	<i>Homo sapiens</i>	AF173378_1
	<i>Astyanax mexicanus</i>	XP007251523
	<i>Drosophila virilis</i>	XP002050865
	<i>Drosophila melanogaster</i>	NP610554
	<i>Saccharomyces cerevisiae</i>	AJS35570
	P0 protein	<i>Arabidopsis thaliana</i>
<i>Arabidopsis thaliana</i>		AT3G11250
<i>Arabidopsis thaliana</i>		AT2G40010
<i>Arabidopsis lyrata</i>		EFH58973
<i>Arabidopsis halleri</i>		LC164162
<i>Arabidopsis halleri</i>		LC164163
<i>Drosophila melanogaster</i>		NP524211
<i>Homo sapiens</i>		NP000993
<i>Caenorhabditis elegans</i>		NP492766
<i>Saccharomyces cerevisiae</i>	NP013444	
Yvh1	<i>Saccharomyces cerevisiae</i>	NP012292

Supplementary Table 12. *P* values for Supplementary Fig. 17.

Pollen number shown in Supplementary Fig. 17a.

	$RDP1^{Bor}/RDP1^{Uod}$	$RDP1^{Bor}/rdpl^{Uod}$	$rdpl^{Bor}/RDP1^{Uod}$	$rdpl^{Bor}/rdpl^{Uod}$
$RDP1^{Bor}/RDP1^{Uod}$	-	-	-	-
$RDP1^{Bor}/rdpl^{Uod}$	0.06361	-	-	-
$rdpl^{Bor}/RDP1^{Uod}$	0.01248	1.23E-06	-	-
$rdpl^{Bor}/rdpl^{Uod}$	< 2.2e-16	< 2.2e-16	< 2.2e-16	-

Rosette leaf size shown in Supplementary Fig. 17b.

	$RDP1^{Bor}/RDP1^{Uod}$	$RDP1^{Bor}/rdpl^{Uod}$	$rdpl^{Bor}/RDP1^{Uod}$	$rdpl^{Bor}/rdpl^{Uod}$
$RDP1^{Bor}/RDP1^{Uod}$	-	-	-	-
$RDP1^{Bor}/rdpl^{Uod}$	0.9843	-	-	-
$rdpl^{Bor}/RDP1^{Uod}$	0.7395	0.7479	-	-
$rdpl^{Bor}/rdpl^{Uod}$	0.0239	0.03439	0.06804	-

Flowering date shown in Supplementary Fig. 17c.

	$RDP1^{Bor}/RDP1^{Uod}$	$RDP1^{Bor}/rdpl^{Uod}$	$rdpl^{Bor}/RDP1^{Uod}$	$rdpl^{Bor}/rdpl^{Uod}$
$RDP1^{Bor}/RDP1^{Uod}$	-	-	-	-
$RDP1^{Bor}/rdpl^{Uod}$	0.7504	-	-	-
$rdpl^{Bor}/RDP1^{Uod}$	0.8215	0.9439	-	-
$rdpl^{Bor}/rdpl^{Uod}$	0.04641	0.05926	0.07581	-

Ovule number shown in Supplementary Fig. 17d.

	$RDP1^{Bor}/RDP1^{Uod}$	$RDP1^{Bor}/rdpl^{Uod}$	$rdpl^{Bor}/RDP1^{Uod}$	$rdpl^{Bor}/rdpl^{Uod}$
$RDP1^{Bor}/RDP1^{Uod}$	-	-	-	-
$RDP1^{Bor}/rdpl^{Uod}$	0.1237	-	-	-
$rdpl^{Bor}/RDP1^{Uod}$	0.1591	0.8317	-	-
$rdpl^{Bor}/rdpl^{Uod}$	0.04947	0.549	0.4183	-

Dry weight shown in Supplementary Fig. 17e.

	$RDP1^{Bor}/RDP1^{Uod}$	$RDP1^{Bor}/rdpl^{Uod}$	$rdpl^{Bor}/RDP1^{Uod}$	$rdpl^{Bor}/rdpl^{Uod}$
$RDP1^{Bor}/RDP1^{Uod}$	-	-	-	-
$RDP1^{Bor}/rdpl^{Uod}$	0.5022	-	-	-
$rdpl^{Bor}/RDP1^{Uod}$	0.0608	0.154	-	-
$rdpl^{Bor}/rdpl^{Uod}$	0.2847	0.0571	0.002835	-

Seed weight shown in Supplementary Fig. 17f.

	$RDP1^{Bor}/RDP1^{Uod}$	$RDP1^{Bor}/rdpl^{Uod}$	$rdpl^{Bor}/RDP1^{Uod}$	$rdpl^{Bor}/rdpl^{Uod}$
$RDP1^{Bor}/RDP1^{Uod}$	-	-	-	-
$RDP1^{Bor}/rdpl^{Uod}$	0.04554	-	-	-
$rdpl^{Bor}/RDP1^{Uod}$	0.355	0.291	-	-
$rdpl^{Bor}/rdpl^{Uod}$	0.04923	0.877	0.3233	-

Bold *P* values are significant at $P < 0.05$ (two-sided test, post hoc Tukey test).

Supplementary References

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