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SUPPLEMENTARY INFORMATION

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Sperm cells are passive cargo of the pollen tube in plant fertilization

Jun Zhang[1†], Qingpei Huang[1†], Sheng Zhong[1†], Andrea Bleckmann[2], Jiaying Huang[1], Xinyang Guo[1], Qing Lin[1], Hongya Gu[1,3], Juan Dong[4], Thomas Dresselhaus[2] and Li-Jia Qu[1,3]*

[1]State Key Laboratory for Protein and Plant Gene Research, Peking-Tsinghua Center for Life Sciences at College of Life Sciences, Peking University, Beijing 100871, China. [2]Cell Biology and Plant Biochemistry, Biochemie-Zentrum Regensburg, University of Regensburg, 93053 Regensburg, Germany. [3]The National Plant Gene Research Center (Beijing), Beijing 100101, China. [4]The Waksman Institute of Microbiology, Rutgers the State University of New Jersey, Piscataway, New Jersey 08854, USA. [†]These authors contributed equally to this work. *e-mail: qulj@pku.edu.cn

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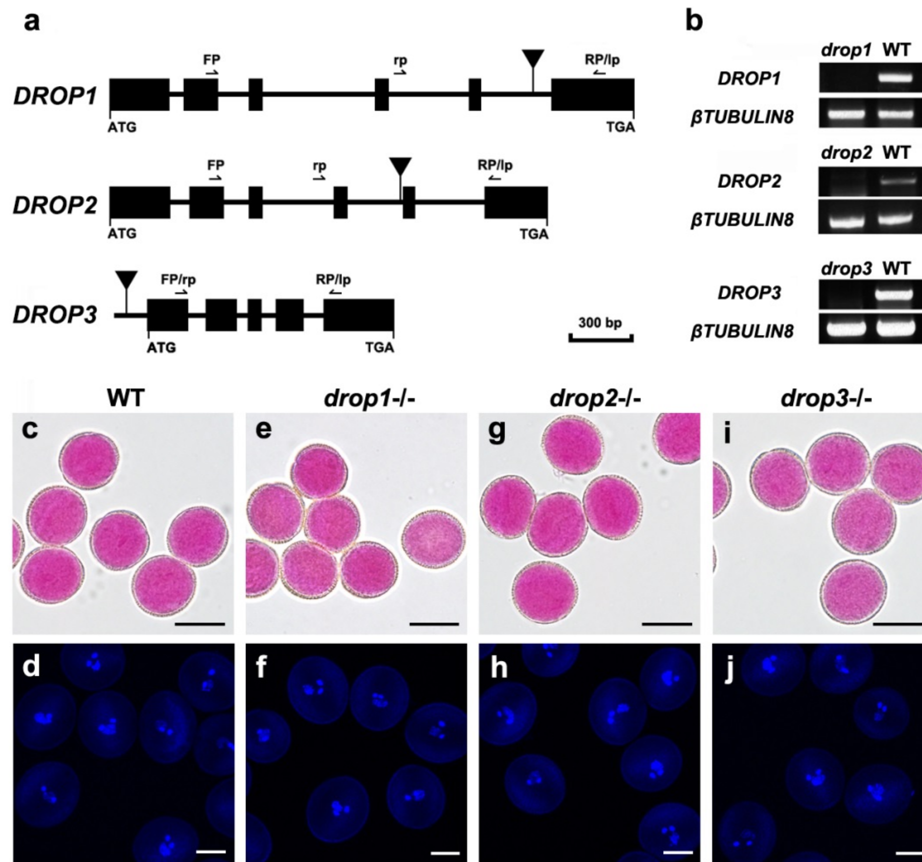

Supplementary Fig 1 | *drop1*, *drop2* and *drop3* single mutants do not have defects in pollen development. a, Schematics to show gene structure and T-DNA insertion sites in *DROP1*, *DROP2* and *DROP3* genes. Black box, exons; black line, introns; black triangle, T-DNA insertion sites. The rp/fp primers were used to amplify the insertional alleles, whereas FP/RP primers were used to confirm reverse transcription. **b,** RT-PCR to evaluate the expression level of *DROP* genes in their respective mutants. mRNAs were extracted from opening flowers and RT-PCR was performed for 35 cycles. **c-j,** Histochemical staining to evaluate mature pollen activity and development in wild-type and *drop* mutants. **c, e, g, i,** Alexander's staining for pollen viability. **d, f, h, j,** DAPI staining to visualize DNA. Scale bars, 20 μm (**c, e, g, i**); 10 μm (**d, f, h and j**).

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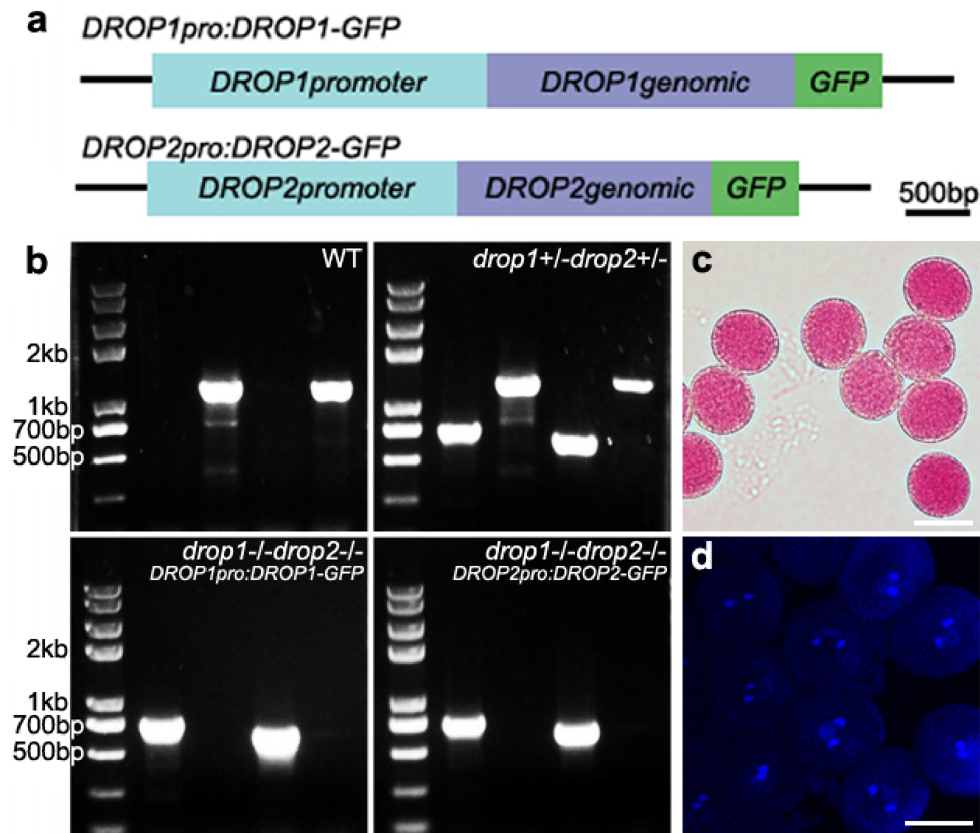

Supplementary Fig 2 | *drop1*-/- *drop2*-/- double mutants can be fully rescued by *DROP1pro:DROP1-GFP* and *DROP2pro:DROP2-GFP*. a, Schematics of complementation constructs. **b,** Genotyping PCR to show the presence of *DROP1pro:DROP1-GFP* or *DROP2pro:DROP2-GFP* constructs in complemented *drop1*-/- *drop2*-/- mutant plants. *DROP1pro:DROP1-GFP* or *DROP2pro:DROP2-GFP* were transformed into *drop1*+/- *drop2*+/-. Multiple independent homozygous *drop1-/- drop2-/-* mutations were identified (bottom two panels) from T3 transgenic plants that were complemented by the GFP-fusion constructs. **c,** Alexander's staining of pollen from rescued *drop1*-/- *drop2*-/- plants. **d,** DAPI staining of pollen grains from the rescued *drop1*-/- *drop2*-/- plants. Scale bars, 20 µm (**c, d**).

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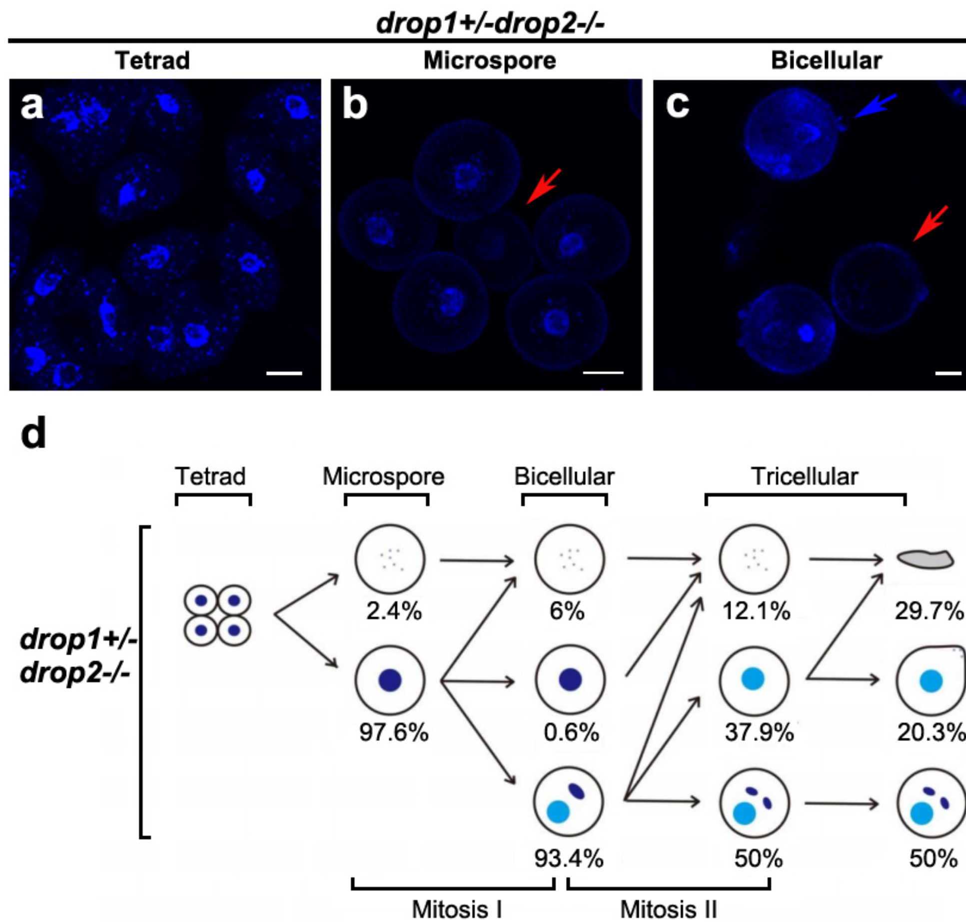

Supplementary Fig 3 | *drop1- drop2-* pollen start to exhibit nuclear division defects from the microspore stage. a-c, DAPI staining of *drop1*+/- *drop2*-/- pollen at the designated stages. Red arrow indicates abnormal pollen grains with faint nucleus-like signals, which would eventually disappear (**b, c**); blue arrow indicates the WT-looking pollen but contains only one nucleus. **d,** Schematic diagram and statistical analysis of developmental defects in *drop1*+/- *drop2*-/- mutants. Scale bars, 10 μm (**a-c**).

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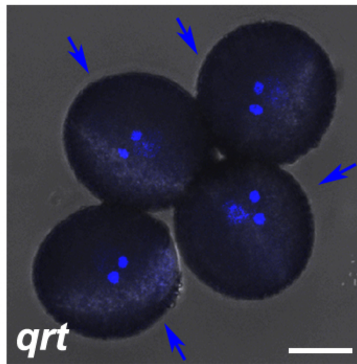

Supplementary Fig 4 | Mature pollen grains of the *qrt1* mutant. This picture is merged from a bright-field and a DAPI-staining UV image. Blue arrows indicate tri-cellular pollen grains. Scale bars, 10 µm.

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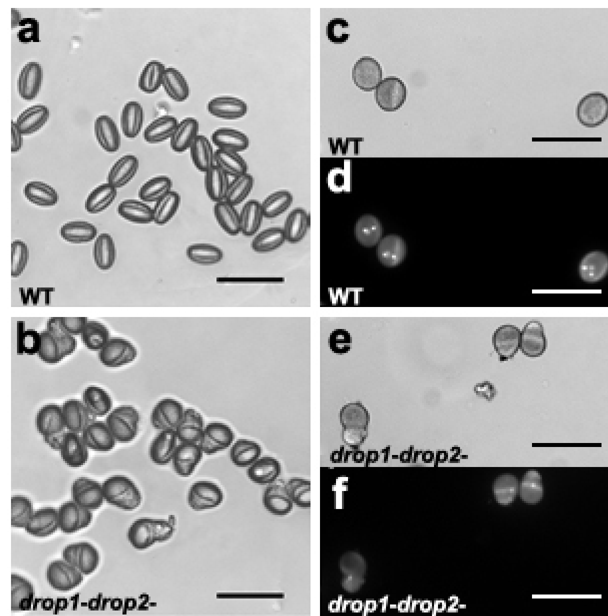

Supplementary Fig 5 | DAPI-staining of manually-picked *drop1- drop2-* pollen grains. a, b, Manually-picked wild-type (**a**) and *drop1- drop2-* pollen grains (**b**). **c, e,** Bright field images of manually picked wild-type (**c**) and *drop1- drop2-* pollen grains (**e**). **d, f,** DAPI staining of (**c**) and (**e**), respectively. Scale bars, 5 µm.

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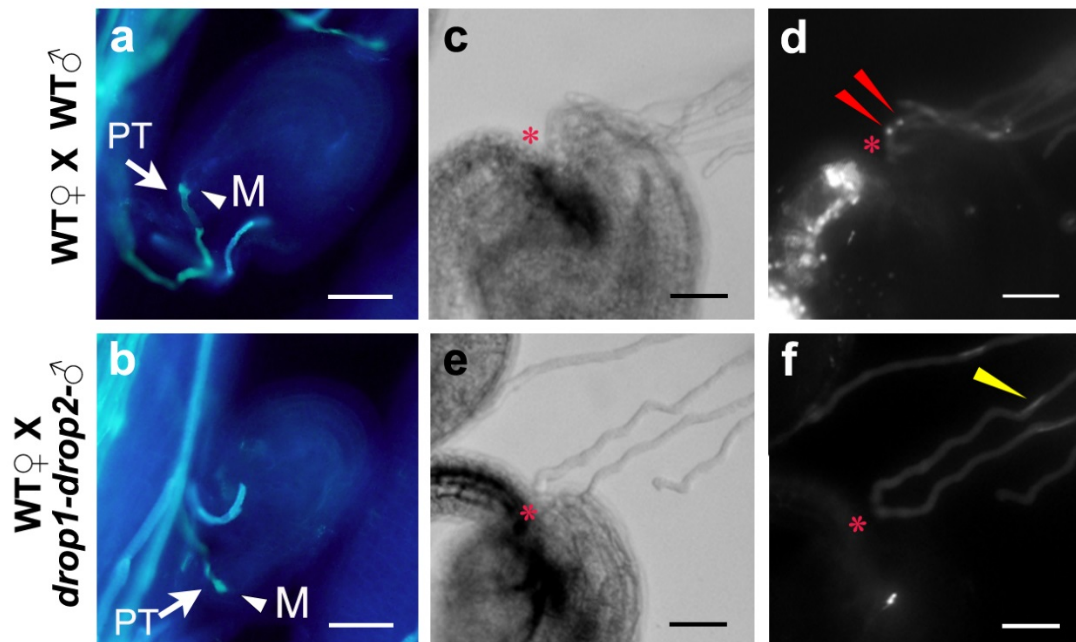

Supplementary Fig 6 | *drop1- drop2-* pollen show normal growth and guidance *in vivo* and *in vitro*. a-d, Aniline blue staining to show *in vivo* pollen tube growth of wild-type (**a**) and *drop1- drop2-* mutant (**d**). Wild-type and *drop1- drop2-* pollen were deposited on emasculated wild-type pistils and after 12 hours, the pistiles were stained to visualize tube growth. White arrow indicates a pollen tube entering the micropyle (arrowhead). **b, e,** Bright field images show targeted growth of WT (**b**) and *drop1- drop2-* (**e**) pollen tubes towards detached ovules in semi-*in vivo* pollen tube guidance assays. **c, f,** DAPI staining of plant materials used in (**b, e**), respectively, reveals the identity of sperm cells in WT pollen tubes (red arrowheads in **c**), but not in the mutants (**f**). Asterisk indicates the micropyle; yellow arrowhead indicates the vegetative nucleus. Scale bars, 20 µm (**a-f**).

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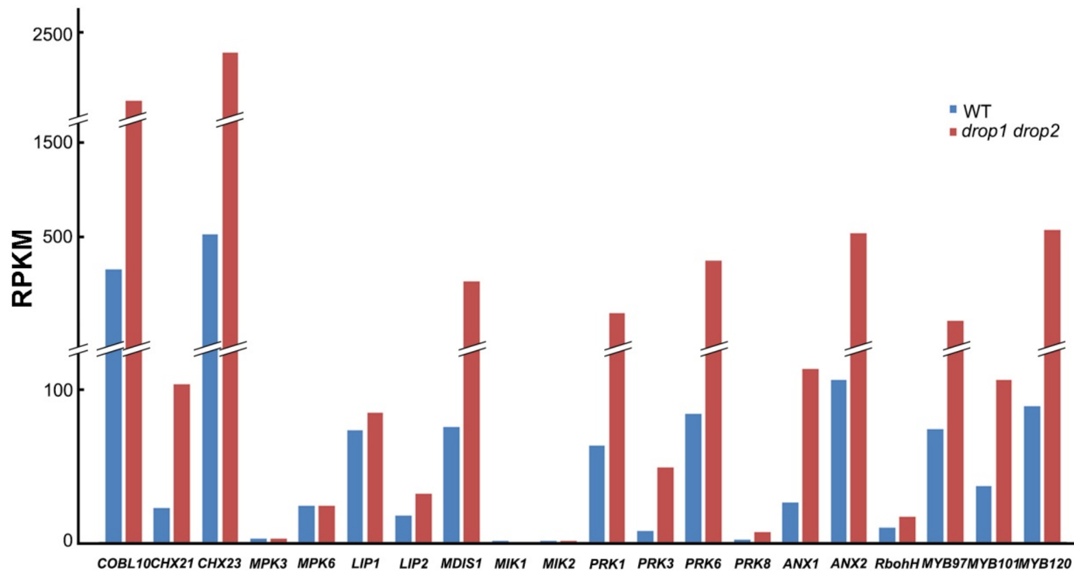

Supplementary Fig 7 | Genes involved in pollen tube growth and guidance are up-regulated in *drop1- drop2-* semi-*in vivo* pollen tubes. Relative expression levels of genes in the wild-type were detected by high-throughput RNA-sequencing using semi-*in vivo* pollen tubes. Relative expression levels of genes in *drop1- drop2-* mutant were detected by single-cell RNA-sequencing in semi-*in vivo* pollen tubes. RPKM, Reads Per Kilobases per Millionreads.

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Supplementary Table 1 | Genetic analysis of *drop* mutants

Table 1. Genetic analysis of *drop* mutants

a. Self-fertilization (Parent)	Progeny							Ratio (AA:Aa:aa)	Theoretical Ratio
	DROP1+/+	*drop1+/-*	*drop1-/-*	*DROP2+/+*	*drop2+/-*	*drop2-/-*	Total		
drop1+/-	76	152	73	301	-	-	301	1:2:0.96	1:2:1
drop2+/-	305	-	-	76	155	74	305	1:2:03:0.97	1:2:1
drop1+/-drop2-/-	186	178	0	-	-	364	364	1:0.96:0	1:2:1
drop1-/-drop2+/-	-	-	314	162	152	0	314	1:0.94:0	1:2:1
b. Reciprocal cross (Parent)	**Progeny**							**TE^{M}**	**TE^{F}**
	DROP1+/+	*drop1+/-*	*drop1-/-*	*DROP2+/+*	*drop2+/-*	*drop2-/-*	Total		
drop1+/-drop2-/-♂ × WT♀	270	0	-	-	270	-	270	0%	NA
WT♂ × *drop1+/-drop2-/-*♀	80	76	-	-	156	-	156	NA	95.0%
drop1-/-drop2+/-♂ × WT♀	-	180	-	180	0	-	180	0%	NA
WT♂ × *drop1-/-drop2+/-*♀	-	196	-	104	92	-	196	NA	88.5%

Because of the loss of the kanamycin selection marker for the SALK mutants, we used PCR to determine the transmission efficiencies (TE). TE is calculated according to the following: TE=number of progenies with T-DNA insertion/number of progenies without T-DNA insertion X 100%. 1:1 for the reciprocal crosses is the expected value for the normal gamete transmission. TEF, female transmission efficiency; TEM, male transmission efficiency; NA, not applicable.

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Supplementary Table 2 | List of primers. Names and sequences are listed in pairs as they have been used.

DROP1-CDS-F	ATGATGAACTCTTCTCTTCTAACTC
DROP1-CDS-RSC	TCACGCTTTCGAAACGGATA
DROP1-CDS-RNSC	CGCTTTCGAAACGGATACGG
DROP2-CDS-F	CACCATGAACTCCTCGTCTCTTCT
DROP2-CDS-R SC	TCACGGCTTGGAAACGGAGGGA
DROP2-CDS-R NSC	TCACGGCTTGGAAACGGAGGGA
DROP1-lp	GAAGACGACCTCTCGGTCAC
DROP1-rp	GGGGTAATTCTTTTACAGAG
DROP1-lp2	TGGCTTCGTTCAGAGAACAC
DROP2-lp	TTGGTGCTCCGTCATCTTCG
DROP2-rp	AACAGATGCAGAATTGCTAACAAGG
DROP2-lp2	CCAAAGTGTACGCAAATACG
DROP1-FP	GGACAAACGCAAACGCAAAC
DROP1-RP	GAAGACGACCTCTCGGTCAC
DROP2-FP	AGTATCGCCGAACGGTTACG
DROP2-RP	TTGGTGCTCCGTCATCTTCG
DROP3-FP	ACCAGTTCCATCATCCTCAG
DROP3-RP	GCATTGAGCCGTCCTCCTGC
DROP1-progene-BP-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAGTGAACAC GCCAACACAAG
DROP1-progene-BP-R-NSC	GGGGACCACTTTGTACAAGAAAGCTGGGTCCGCTTTCGAA ACGGATACGG
DROP2-progene-BP-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAGAACGCGG CGGAGGAAGAA
DROP2-progene-BP-R-NSC	GGGGACCACTTTGTACAAGAAAGCTGGGTCCGGCTTGGAA ACGGAGGGAG

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SUPPLEMENTARY METHODS

Total RNA isolation and RT-PCR. Total RNAs were extracted from inflorescence tissue of 10-days-bolting plants using Plant Total RNA Isolation Kit (GeneMark, Beijing). RNA reverse-transcription, cDNA synthesis and RT-PCR were conducted according to the protocols previously described[1]. The primers DROP1-FP/RP and DROP2-FP/RP were used to amplify the fragment spanning the T-DNA insertion site in *drop1* and *drop2* mutants, respectively.

Plasmid construction and plant transformation. To generate the DROP1pro:DROP1-GFP and DROP2pro:DROP2-GFP construct, native promoters and full length genomic regions of *DROP1* and *DROP2* were amplified by primers DROP1-progene-BP-F / DROP1-progene-BP-R-NSC and DROP2-progene-BP-F / DROP2-progene-BP-R-NSC, respectively (Supplementary Table 2), using total genomic DNA from Arabidopsis as template and then cloned into the pDONR221-D (Invitrogen) vector to generate pDONR221-DROP1pro:DROP1 and pDONR221-DROP2pro:DROP2 by BP reaction. For complementation, pDONR221-DROP1pro:DROP1 and pDONR221-DROP2pro:DROP2 plasmids were cloned into a GATEWAY-compatible destination vector PK7FWG0, which was modified from PK7FWG2 (Department of Plant Systems Biology, VIB-Ghent University, Ghent, Belgium) through LR reaction (Invitrogen). Constructs were then transformed into *Agrobacterium tumefaciens* GV3101, using a freeze-thaw procedure. Arabidopsis transformation and transgenic plant screening were conducted as reported[2].

DAPI staining. DAPI staining was performed as the protocol described previously[1].

Reference

1. Liu, J.J. et al., Targeted degradation of the cyclin-dependent kinase inhibitor ICK4/KRP6 by RING-type E3 ligases is essential for mitotic cell cycle progression during Arabidopsis gametogenesis. *Plant Cell* **20,** 1538-1554 (2008).
2. Qin, G.J. et al., An indole-3-acetic acid carboxyl methyltransferase regulates Arabidopsis leaf development. *Plant Cell* **17,** 2693-2704 (2005).

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