Supplementary Information for

Autophagy modulates Arabidopsis male gametophyte fertility and controls actin organization

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[6]These authors contributed equally to the article.

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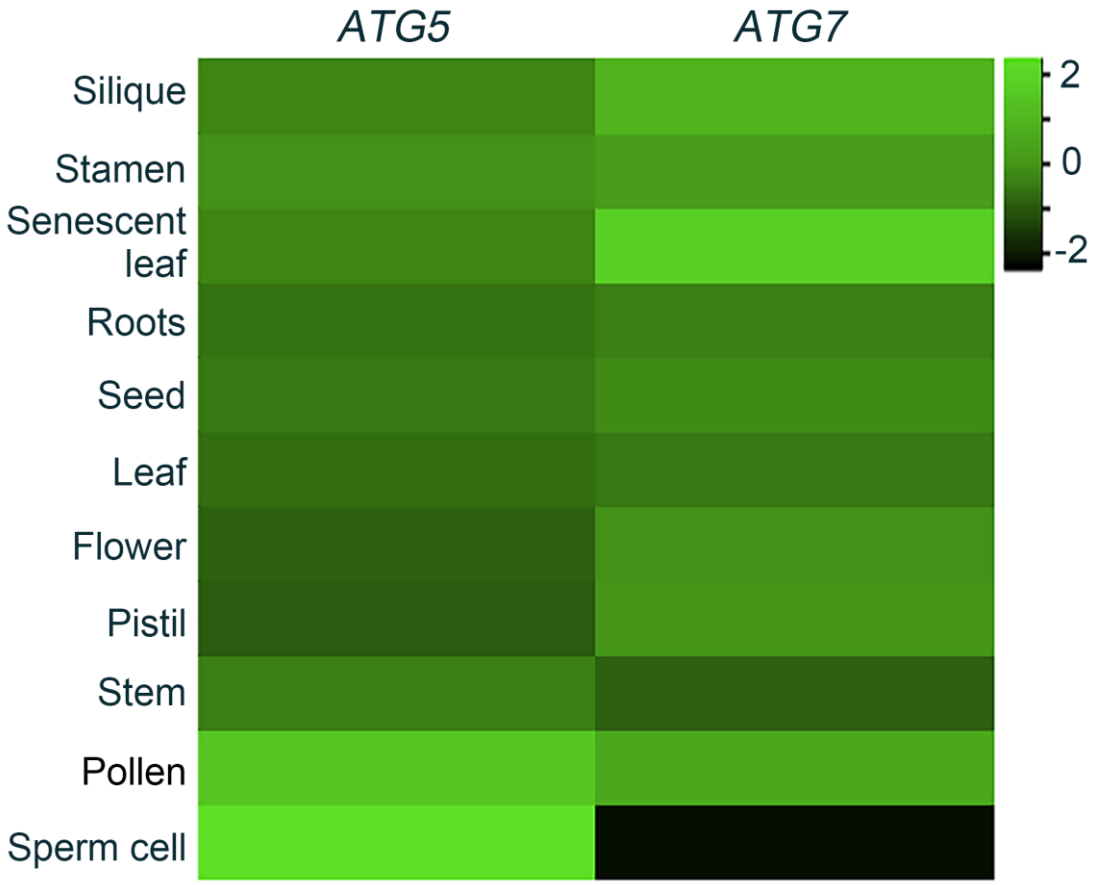
Supplementary Figures: 13

Supplementary Tables: 4

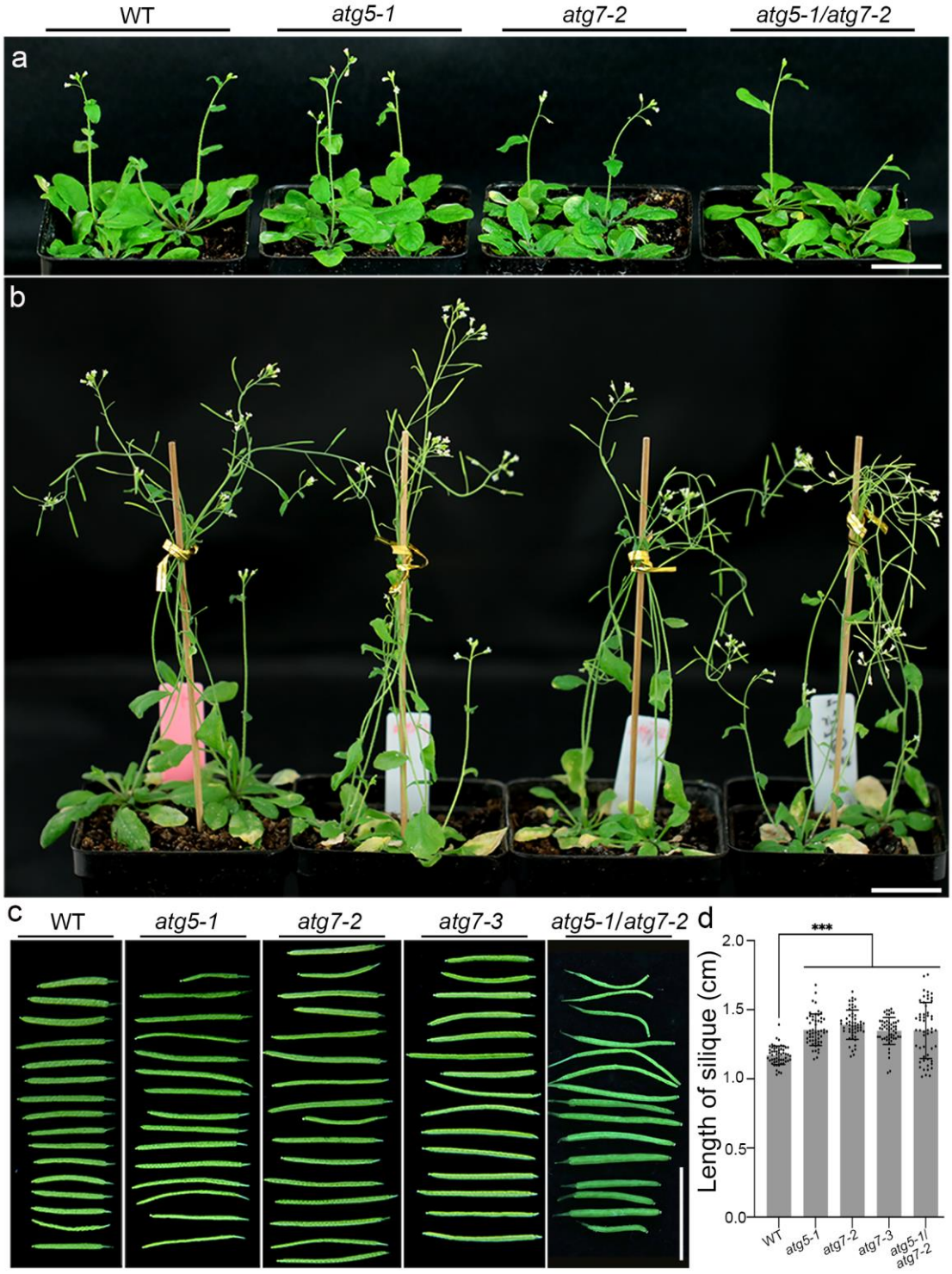
Supplementary Figure 1



Supplementary Figure 1. Gene expression profile analysis of *ATG5* and *ATG7* genes in different tissues of Arabidopsis.

The data of expression profile for *ATG5* and *ATG7* were obtained from the microarray database (https://genevestigator.com/). The heatmap image was created by Origin software. The green box represents higher gene transcriptional level, whereas the black box represents lower gene expression level. The scale bar represents row.

Supplementary Figure 2



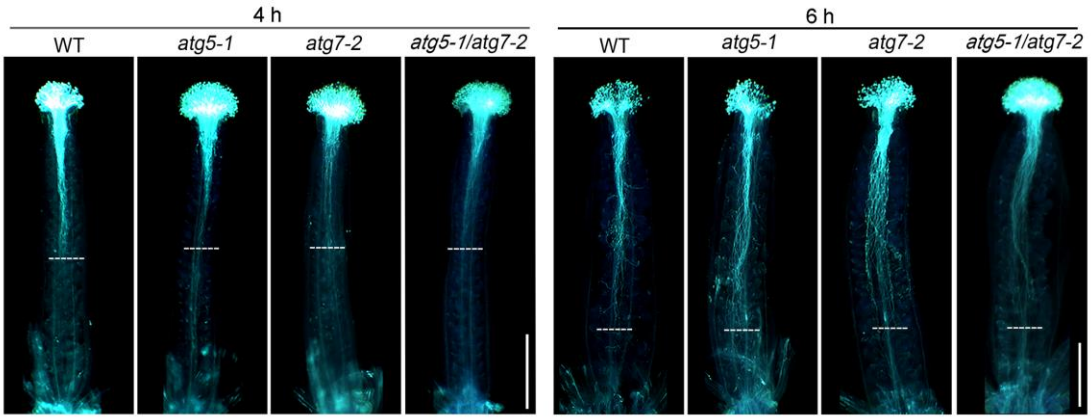
Supplementary Figure 2. Phenotypic overview of *atg5* and atg7 mutants during flowering and silique formation.

a, b Overview images of Arabidopsis plants of WT, *atg5-1*, *atg7-2* and *atg5-1*/*atg7-2* that were cultivated for 35 days and 45 days respectively under the standard Arabidopsis growth condition. Scale bars = 2.5 cm.

c Representative images of siliques harvested from one branch of inflorescence of WT, *atg5-1*, *atg7-2*, *atg7-3* and *atg5-1*/*atg7-2*.

d Statistical calculation of silique length in WT, *atg5-1*, *atg7-2*, *atg7-3* and *atg5-1*/*atg7-2* was performed using two-tailed paired Student's *t*-test, with n=53 independent replicates for each sample (error bars ± SD, $***p < 0.001$). Source data are provided in Source data file. Scale bars = 1.5 cm.

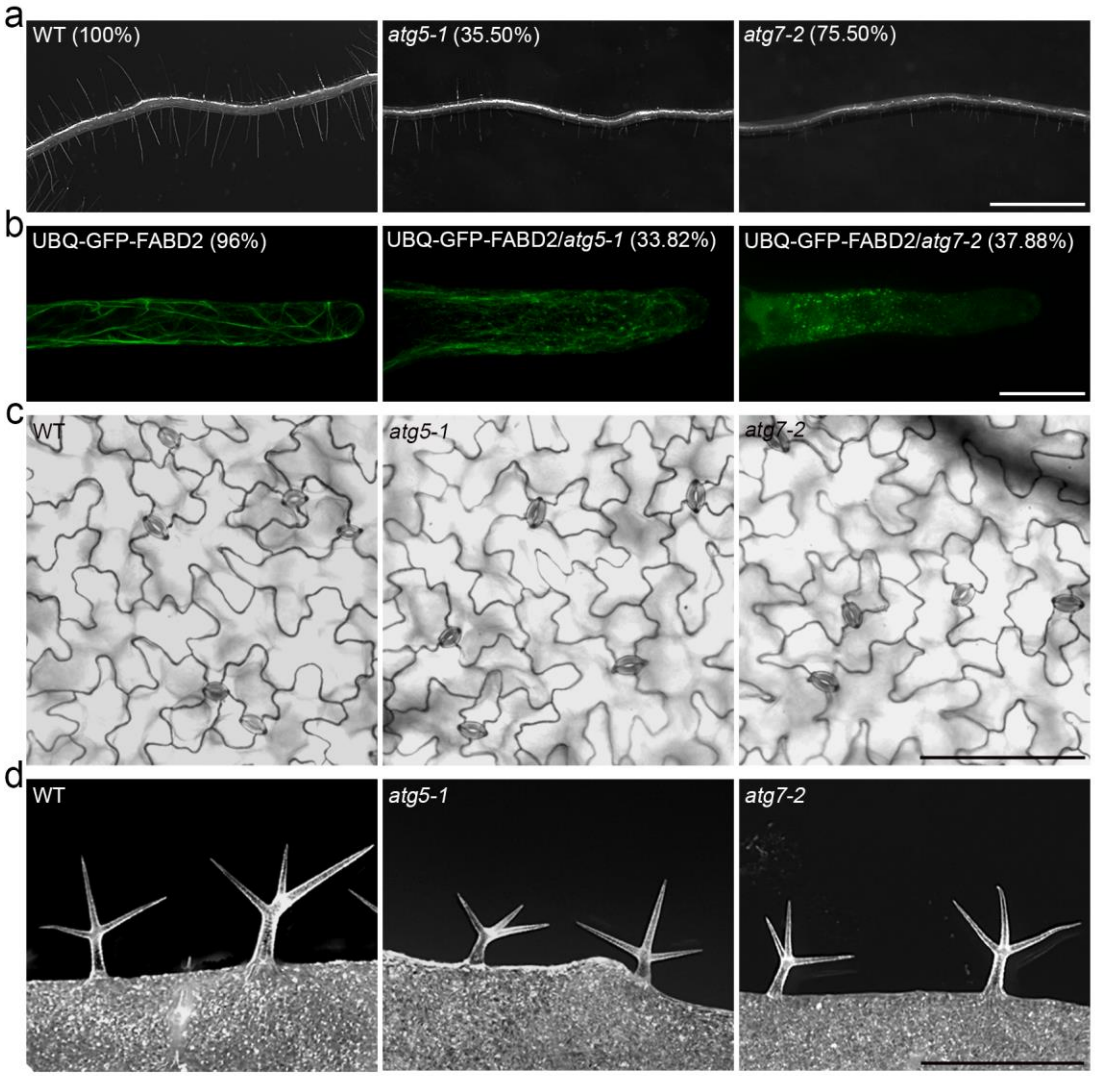
Supplementary Figure 3



Supplementary Figure 3. *In vivo* pollen tube germination assay of the WT, *atg5-1*, *atg7-2* and *atg5-1*/*atg7-2* after pollination for 4 and 6 h.

Representative images of *in vivo* pollen tube germination and growth of WT, *atg5-1*, *atg7-2* and *atg5-1*/*atg7-2* after pollination for 4 and 6 h respectively. A total of 50 independent replicates of the individual samples were observed, yielding consistent results. Dashed lines indicate the farthest position that pollen tubes could reach into the styles. Scale bars = 400 μm.

Supplementary Figure 4



Supplementary Figure 4. Phenotypic analysis of typical polarized cell types in *atg5-1* and *atg7-2*.

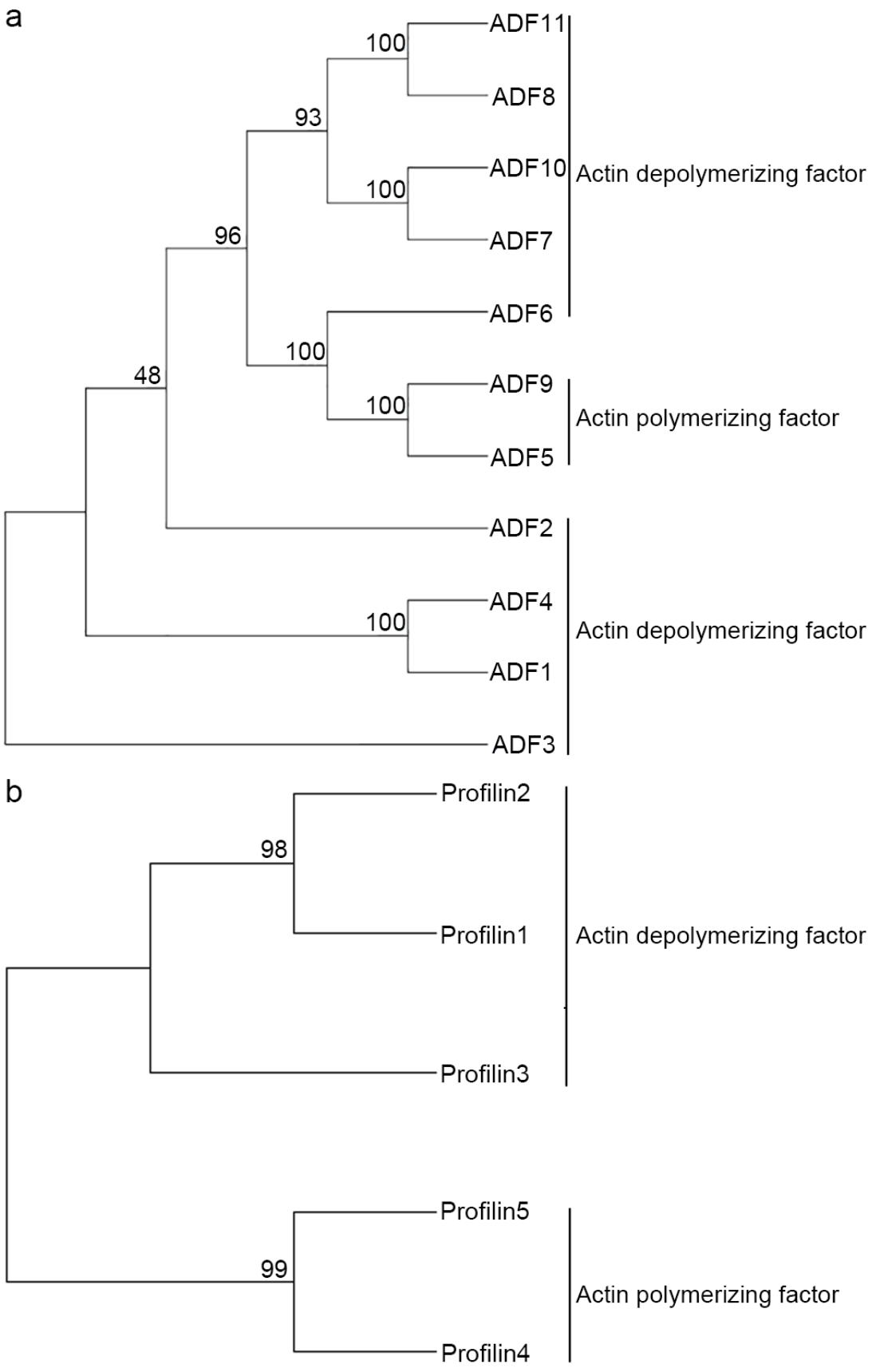
a Representative images of Arabidopsis root hairs of WT, *atg5-1* and *atg7-2*. A total of n=47 (WT), n=48 (*atg5-1*) and n=45 (*atg7-2*) independent experimental replicates were conducted for each sample, yielding consistent results. Source data are provided in Source data file. Scale bar = 100 μm.

b Representative images of the organization of actin filaments in the root hair of *pUBQ::GFP-FABD2*, *pUBQ::GFP-FABD2/atg5-1* and *pUBQ::GFP-FABD2/atg7-2*. A total of n=50 (*UBQ-GFP-FABD2*), n=68 (*UBQ-GFP-FABD2/atg5-1*) and n=66 (*UBQ-GFP-FABD2/atg7-2*) independent

experimental replicates were conducted for each sample, yielding consistent results. Source data are provided in Source data file. Scale bars = 25 μm.

c and d Representative images of Arabidopsis leaf pavement cells and trichomes of WT, *atg5-1* and *atg7-2*. A total of three independent experimental replicates were conducted for each sample, and consistent results were obtained. Scale bars: 100 μm (**c**) and 500 μm (**d**).

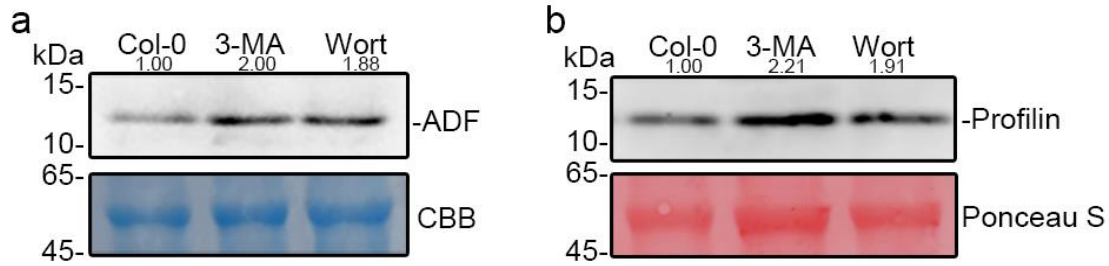
Supplementary Figure 5



Supplementary Figure 5. Phylogenetic and functional analysis of ADF and Profilin families.

a, b Phylogenetic tree of ADF and Profilin family members were built by adopting amnio acid sequences from *Arabidopsis thaliana.* The unrooted tree was constructed by using MEGA11 based on the multiple sequence alignment of the ADF and Profilin family protein sequences *via* the neighbor-joining (NJ) method. The number at each node represents the bootstrap value from 1000 replicates.

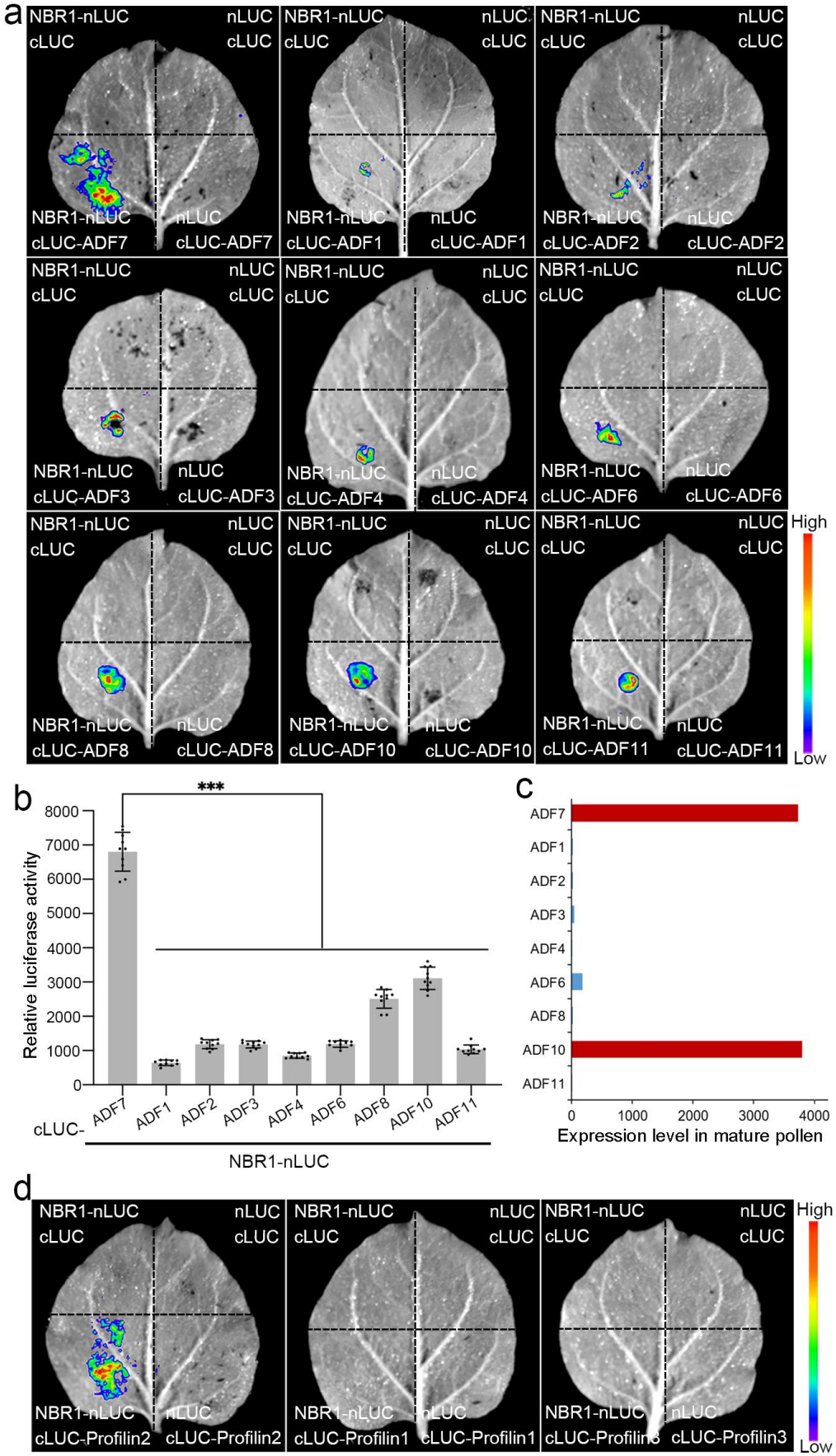
Supplementary Figure 6



Supplementary Figure 6. Effects of pharmaceutical inhibition on autophagic degradation of ADF and Profilin in Arabidopsis pollen.

a, b Western blotting analysis of ADF and Profilin levels in WT Arabidopsis mature pollens following treatment with 2.5 mM 3-Methyladenine (3-MA) and 8.25 μM wortmannin (Wort) treatment for 30 min. Detection was performed using anti-ADF and anti-Profilin antibodies. Western blotting was performed in three independent experimental replicates for each sample, and protein levels were quantified using ImageJ software. Source data of blotting are provided in Source data file.

Supplementary Figure 7



Supplementary Figure 7. Luciferase complementation assay to probe protein interactions between NBR1 and members of the ADF and Profilin families.

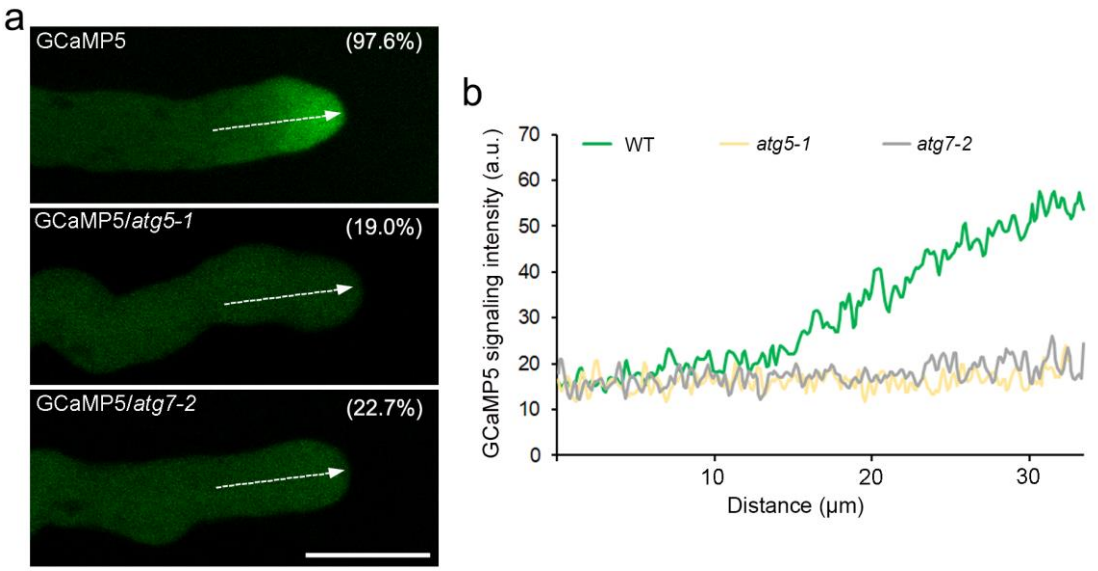
a Luciferase complementation assay demonstrates the interaction between NBR1 and members of the ADF family. Positive interactions were determined by the detection of fluorescence.

b Quantitative analysis of relative luciferase activity of the interaction strength between NBR1 and ADF family members. Statistical analysis was performed using two-tailed paired Student's *t*-test with n=10 independent experimental replicates (error bars ± SD, $***p < 0.001$). Source data are provided in Source data file.

c Expression levels of *ADF* family members in mature pollens of *Arabidopsis thaliana*. The data of expression level were obtained from the TAIR database (https://www.arabidopsis.org/).

d Luciferase complementation assay for assessing the interactions between NBR1 and the Profilin family proteins. Ten independent experimental replicates were conducted for each sample, yielding consistent results. Positive interactions were determined by the detection of fluorescence.

Supplementary Figure 8

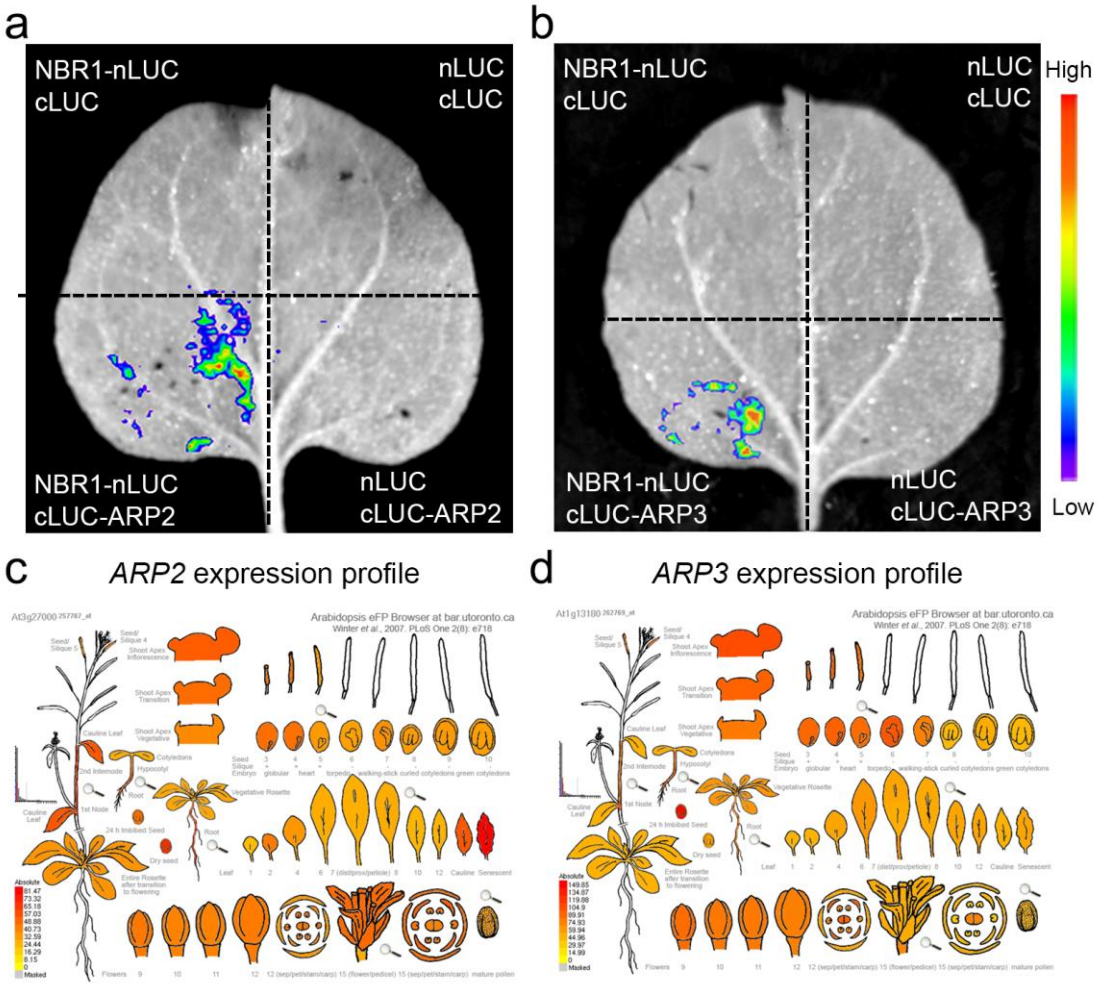


Supplementary Figure 8. Disruption of tip-focused calcium gradient in *atg5-1* and *atg7-2*.

a Representative images of growing Arabidopsis pollen tubes expressing GCaMP5 in WT, GCaMP5/*atg5-1* and GCaMP5/*atg7-2* respectively. A total of n=125 (WT), n=105 (*atg5-1*) and n=110 (*atg7-2*) independent experimental replicates were conducted for each sample, yielding consistent results. Source data are provided in Source data file. Scale bar = 25 μm.

b Analysis of fluorescent signal intensity of GCaMP5 along the white arrows depicted in the pollen tubes of WT, GCaMP5/*atg5-1* and GCaMP5/*atg7-2* pollen tubes as shown in (**a**) using ImageJ software.

Supplementary Figure 9

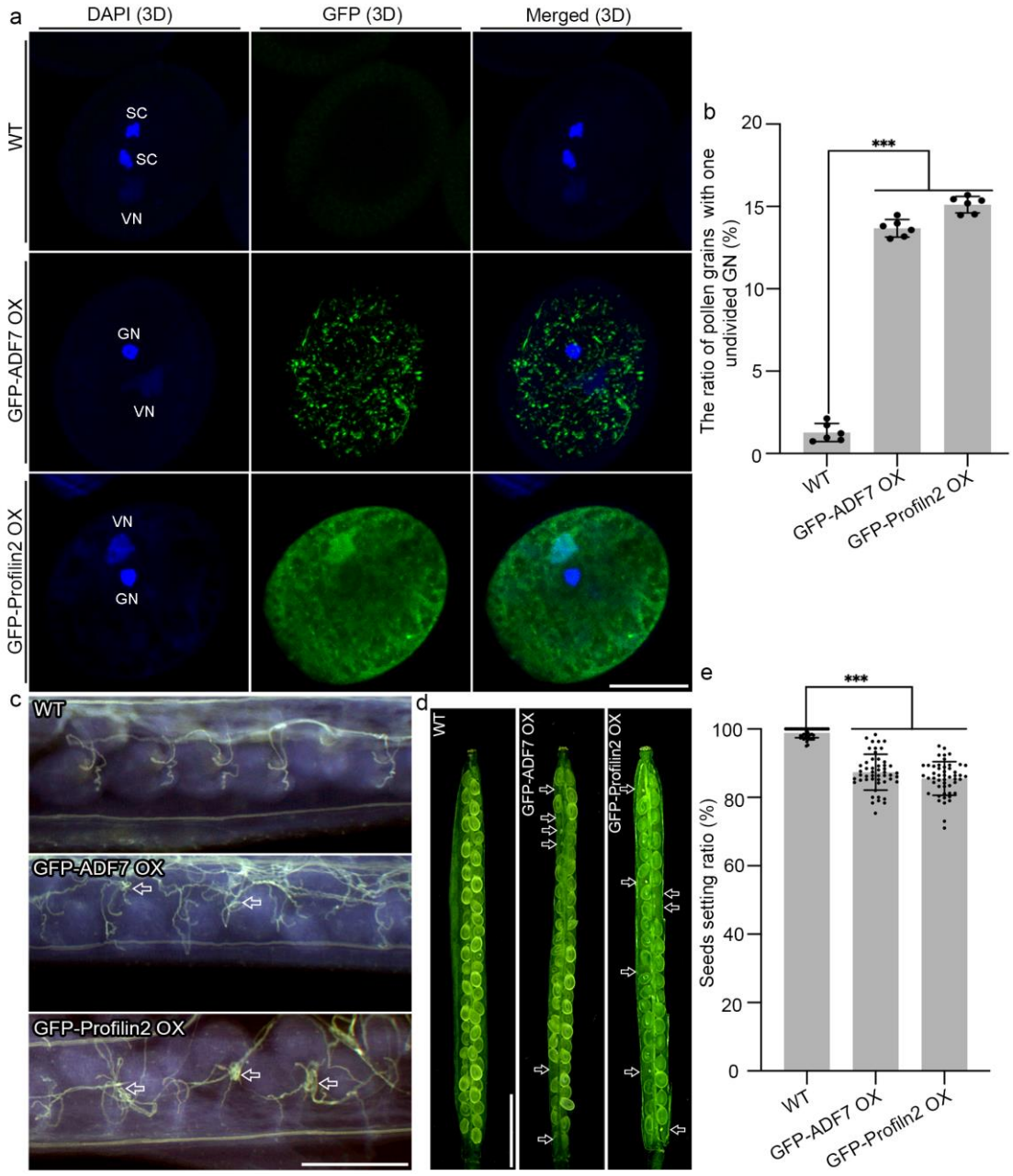


Supplementary Figure 9. Interaction of ARP2 and ARP3 with NBR1 and their expression levels in pollen

a, b Luciferase complementation assay to evaluate the interactions between NBR1 and ARP2 (**a**) and NBR1 and ARP3 (**b**). Three independent experimental replicates were conducted for each sample, and consistent results were obtained. Positive interactions were indicated by the detection of fluorescent colors.

c, d Gene expression profile of *ARP2* (**c**) and *ARP3* (**d**) in Arabidopsis pollen, obtained from the BAR database (https://bar.utoronto.ca/). Red boxes represent higher transcriptional levels, whereas white boxes indicate lower expression levels.

Supplementary Figure 10



Supplementary Figure 10. Defects in sperm cell biogenesis and pollen tube growth during fertilization in transgenic Arabidopsis overexpressing GFP-ADF7 and GFP-Profilin2.

a 3-D images of mature pollen grains from WT and OX plants of GFP-ADF7 and GFP-Profilin2 stained with DAPI. Scale bars = 10 μm.

b Statistical calculation of abnormal pollens lacking of two sperm cells from the WT, GFP-ADF7 OX and GFP-Profiln2 OX were performed using two-tailed

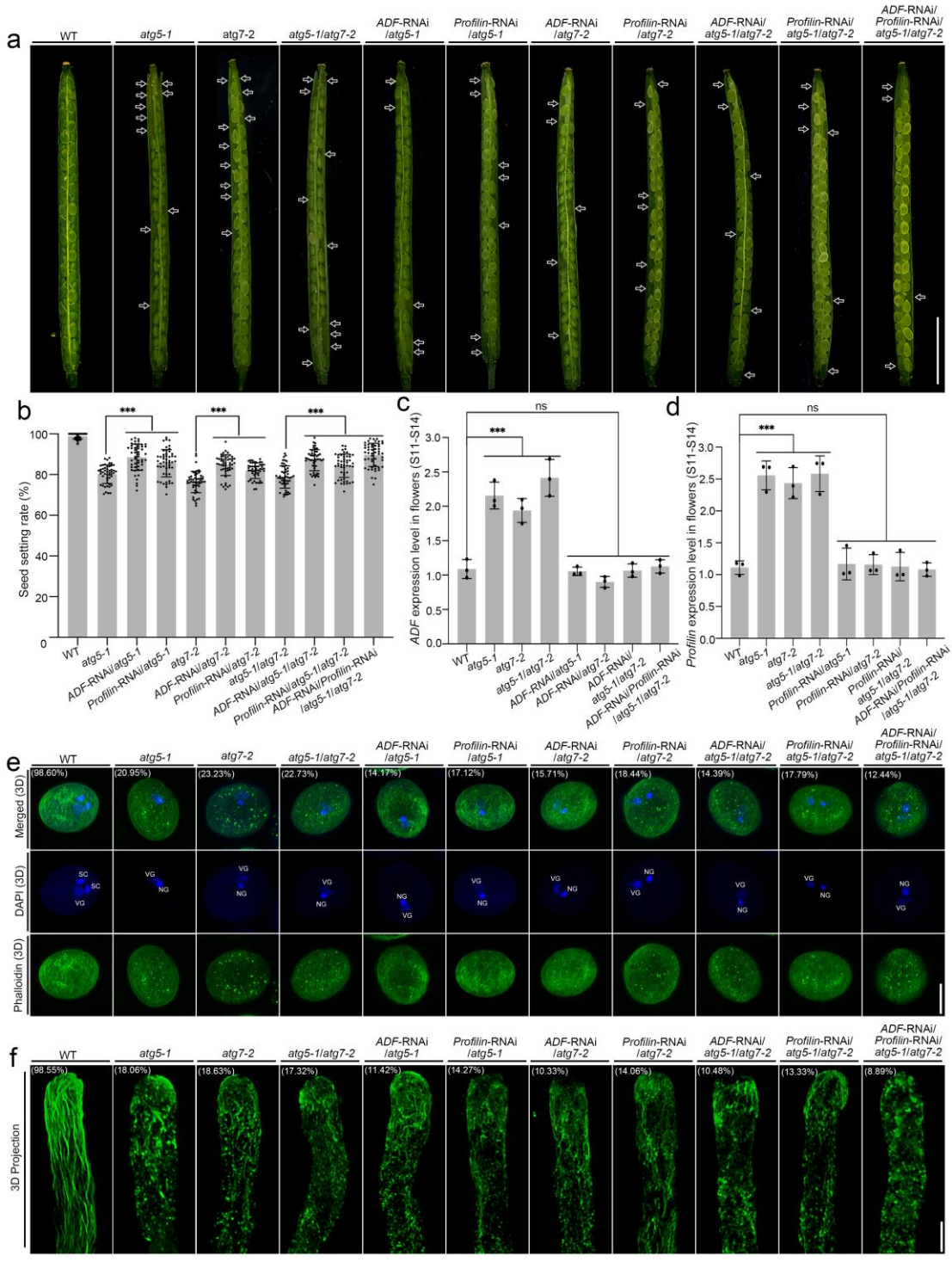
paired Student's *t*-test respectively. The number of cells examined was n=1486 (WT), n=1418 (GFP-ADF7 OX) and n=1731 (GFP-Profiln2 OX) across three independent experiments for each sample (error bars ± SD, ∗∗∗p < 0.001). Source data are provided in Source data file.

c *In vivo* pollen tube growth and targeting to ovules during fertilization in the WT, GFP-ADF7 and GFP-Profiln2 Arabidopsis respectively. Twisted and abnormal accumulation of pollen tubes were indicated by the arrows. Three independent experimental replicates were conducted for each sample, and consistent results were obtained. Scale bars = 100 μm.

d Representative images of seed settings within siliques harvested from the WT, GFP-ADF7 and GFP-Profiln2 Arabidopsis. Scale bars = 2 mm.

e Statistical calculation of seed setting in the WT, GFP-ADF7 and GFP-Profiln2 were performed using two-tailed paired Student's *t*-test with n=50 independent experimental replicates (error bars ± SD, ∗∗∗p < 0.001).

Supplementary Figure 11



Supplementary Figure 11. Partial restoration of seed setting rate, sperm cell biogenesis and actin organization in *atg5-1, atg7-2* and *atg5-1/atg7-2* mutants through RNAi of *ADFs* and *Profilins* in Arabidopsis.

a Representative silique images from WT, *atg5-1*, *atg7-2*, *atg5-1*/*atg7-2*, and transgenic lines expressing *ADF*-RNAi or *Profilin*-RNAi in the *atg5-1* (*ADF*-RNAi/*atg5-1*, *Profilin*-RNAi/*atg5-1*), *atg7-2* (*ADF*-RNAi/*atg7-2*, *Profilin*-RNAi/*atg7-2*) and *atg5-1*/*atg7-2* (*ADF*-RNAi/*atg5-1*/*atg7-2*, *Profilin*-RNAi/*atg5-1*/*atg7-2* and *ADF*-RNAi/*Profilin*-RNAi/*atg5-1*/*atg7-2*) backgrounds. Scale bars = 2 mm.

b Statistical calculation of seed setting rate in WT, *atg5-1*, *atg7-2*, *atg5-1*/*atg7-2*, and the transgenic RNAi lines were performed using two-tailed paired Student's *t*-test with n=50 independent experimental replicates (error bars ± SD, ∗∗∗p < 0.001, ns, no significant). Source data are provided in Source data file.

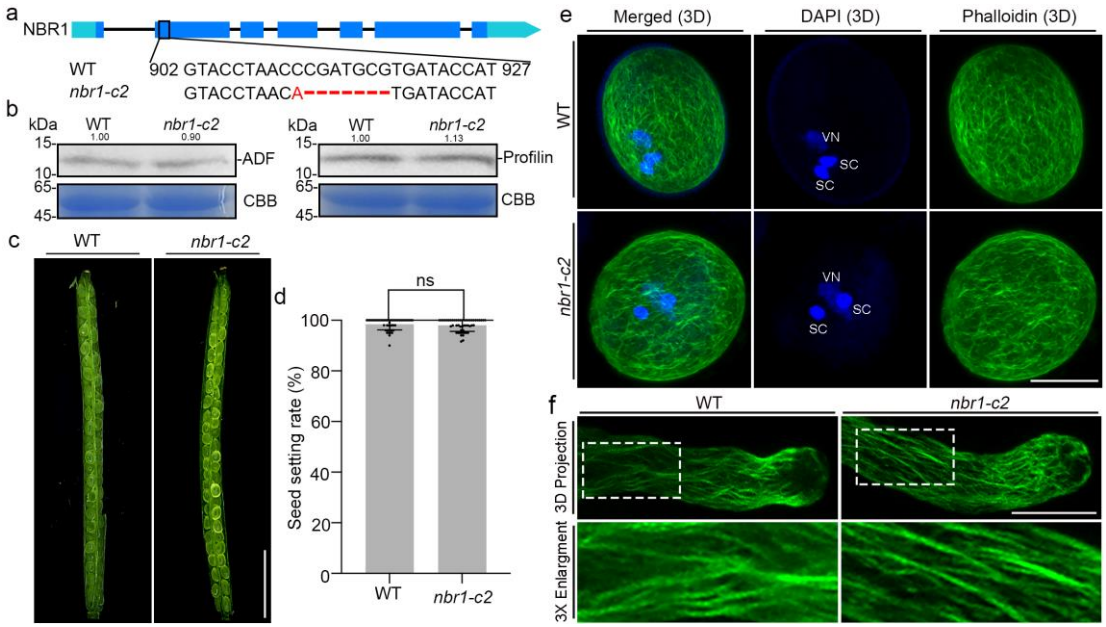
c, d RT-qPCR analysis of the expression levels of *ADF* and *Profilin* genes in flowers (stage 11-14) from the WT, *atg5-1*, *atg7-2*, *atg5-1*/*atg7-2*, and the transgenic RNAi lines were performed using two-tailed paired Student's *t*-test with n=3 independent experimental replicates (error bars ± SD, ∗∗∗p < 0.001, ns, no significant). *Actin2* was used as an internal control. Source data are provided in Source data file.

e 3-D confocal microscopy images were obtained to visualize sperm cells and spatial actin organization in mature pollen grains of WT, *atg5-1*, *atg7-2*, *atg5-1*/*atg7-2*, and transgenic *ADF*- and *Profilin*-RNAi lines in the *atg5-1*, *atg7-2*, *atg5-1*/*atg7-2* background. Samples were stained with Alexa-488 phalloidin and DAPI. Three independent experimental replicates were conducted for each sample, and consistent results were obtained. Source data are provided in Source data file. Scale bars = 10 μm.

f 3-D confocal microscopy images of spatial actin organization in 6-h germinated Arabidopsis pollen tubes of WT, *atg5-1*, *atg7-2*, *atg5-1*/*atg7-2*, and transgenic *ADF*- and *Profilin*-RNAi lines in the *atg5-1*, *atg7-2*, *atg5-1*/*atg7-2* background. Pollen tubes were stained with Alexa-488 phalloidin. Three independent experimental replicates were conducted for each sample, and

consistent results were obtained. Source data are provided in Source data file. Scale bars = 10 μm.

Supplementary Figure 12



Supplementary Figure 12. Phenotypic analysis of seed setting, sperm cell biogenesis and actin organization in *nbr1-c2* mutant.

a Schematic diagram of the *NBR1* gene sequence in *nbr1-c2* mutant generated by CRISPR/Cas9. The exons of the *NBR1* are highlighted in dark blue.

b Western blotting analysis of ADF and Profilin protein levels in pollen grains of Arabidopsis WT, and *nbr1-c2* using anti-ADF and anti-Profilin antibodies. Western blotting was performed in three independent experimental replicates, yielding consistent results. Source data of blotting are provided in Source data file.

c Representative silique images from WT and *nbr1-c2*. Scale bars = 2 mm.

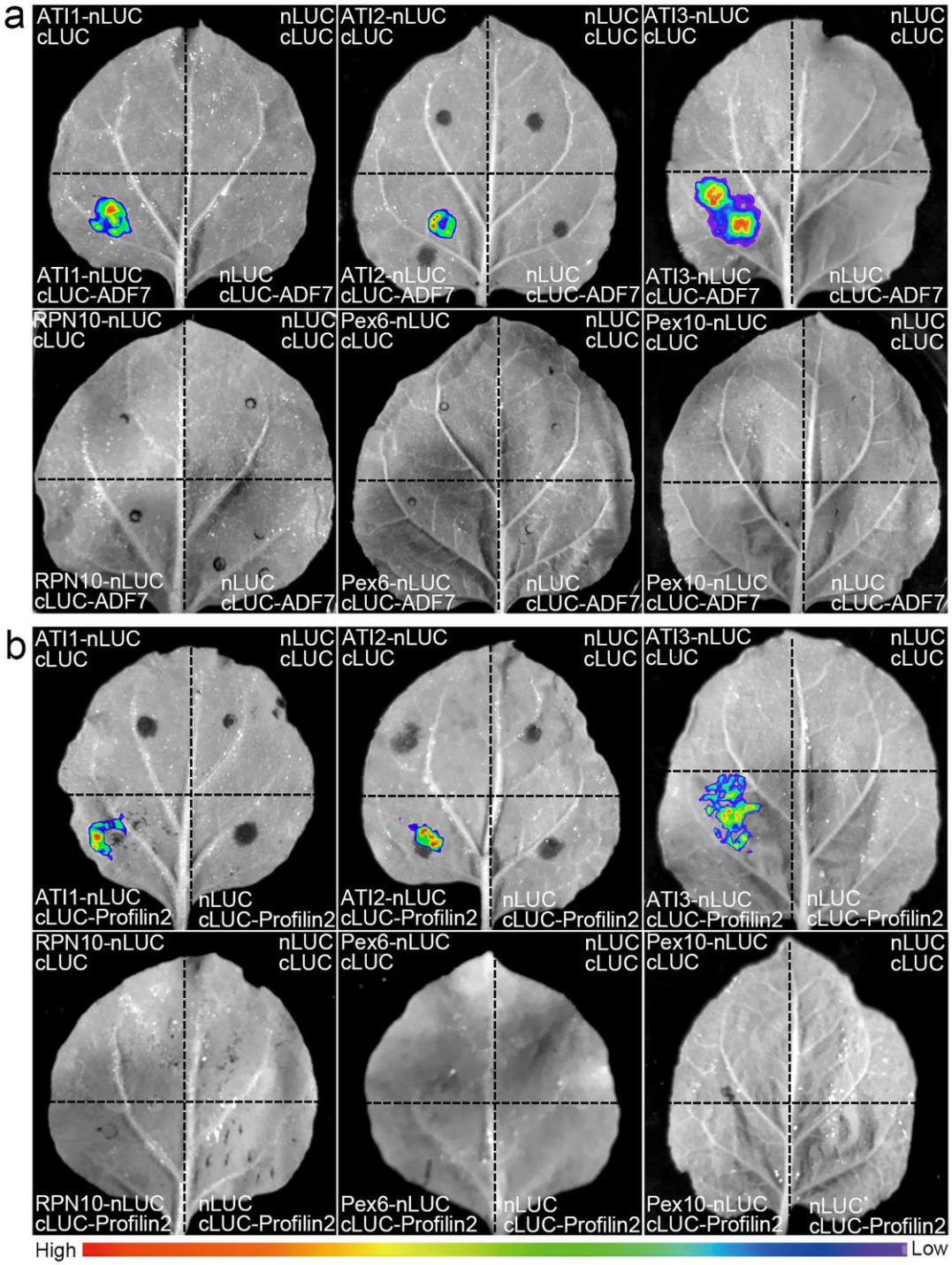
d Statistical calculation of seed setting rate in WT and *nbr1-c2* were performed using two-tailed paired Student's *t*-test with n=50 independent experimental replicates (error bars ± SD, ns, no significant). Source data of blotting are provided in Source data file.

e 3-D confocal microscopy images sperm cell formation and actin organization in mature pollen grains of WT and *nbr1-c2*, stained with Alexa-488 phalloidin

and DAPI. A total of three independent experimental replicates were conducted for each sample, yielding consistent results. Scale bars = 10 μm.

f 3-D confocal microscopy images of actin organization in 6-h germinated Arabidopsis WT and *nbr1-c2* pollen tubes that were stained with Alexa-488 phalloidin. A total of three independent experimental replicates were performed for each sample, yielding consistent results. Scale bars = 10 μm.

Supplementary Figure 13



Supplementary Figure 13. Screening of alternative autophagic receptors for interactions with ADF7 and Profilin2.

a, b Cytoplasmic autophagic receptors including ATI1, ATI2, ATI3, RPN10, Pex6, and Pex10 were screened for the interactions with ADF7 and Profilin2 by luciferase complementation assays. Three independent experimental

replicates were conducted for each sample, and consistent results were obtained. Positive interactions were determined by the detection of fluorescent colors.

Supplementary Table 1. Primers used in construction of plasmids.

Primer name	Primer sequence (5'-3')
ADF7-F	GGGGGTACCATGGCGAACGCGGC
ADF7-R	GGGGTCGACCTAGAGAGCTCGGCT
Profilin2-R	GGGGGTACCATGTCGTGGCAATCAT
Profilin2-R	GGGGTCGACTTAGAGACCAGACTCGA
ADF-RNAi-F	GGGCTCGAGAAACTTACGAGGAGTTTGC GGGAAGCTTCATCTCTGTTGGATCAG
ADF-RNAi-R	GGGGGTACCCATCTCTGTTGGATCAG GGGTCTAGAAAACTTACGAGGAGTTTGC
Profilin-RNAi-F	GGGCTCGAGTCGGCCAAGACGGC GGGAAGCTTGGTTCATCATAGATACCAAAGAC
Profilin-RNAi-R	GGGGGTACCGGTTCATCATAGATACCAAAG GGGTCTAGATCGGCCAAGACGG
GCaMP5-F	GGGGGATCCTCTAGAATGGGTTCTCATCATCATCATC
GCaMP5-R	GGGGGTACCCCCGGGTTACTTCGCTGTCATCATTTG

Supplementary Table 2. Primers used for genotyping and RT-qPCR.

Primer name	**Primer sequence (5‘-3’)**
ATG5-F	ATTTGCTATTTGTTTGGCACG
ATG5-F	TAGCATCTGAATTTCATAACCAATCTCGATACAC
ATG5-R	TACCGTTCATGACAGAGGTCC
ATG7-F	ATAATAACGCTGCGGACATCTACATTTT
ATG7-F	CGTGTAACAGTGCATTGTTGG
ATG7-R	GGAGCTTAACAAAGGGAAACG
ATG5-Q-F	ACTGATACCATGTGAAGGAG
ATG5-Q--F	GTATAGGCATCAAGATCACC
ATG7-Q-F	GAAGATTGTCTAGGTCGTGG
ATG7-Q-R	CCTGCTTTCTCTTGTATCGG

Supplementary Table 3. Primers used in Y2H assay.

Primer name	Primer sequence (5'-3')
ADF7-F	GGGGAATTCATGGCGAACGCGGCGTCG
ADF7-R	GGGGGATCCCTAGAGAGCTCGGCTTTTG
Profilin2-F	GGGGAATTCATGTCGTGGCAATCATAC
Profilin2-R	GGGGGATCCTTAGAGACCAGACTCGATAAG
ATG8d-F	GGGGAATTCATGGCGATTAGCTCCTTC
ATG8d-R	GGGGGATCCTTAGAAGAAGATCCCGAAC
NBR1-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTC TACTGCTAACG
NBR1-R	TACGATTCATCTGCAGCTCGAGTCAAGCCTCC TTCTCCCC
NBR1ΔUBA1ΔUBA2 -F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCT ACTGCTAACG
NBR1ΔUBA1ΔUBA2-R	TACGATTCATCTGCAGCTCGAGGTCATTCTTTT CTATATC
NBR1ΔPB1ΔZZΔFW-F	TGGCCATGGAGGCCAGTGAATTCGTGGAGATA ACCATGCTC
NBR1ΔPB1ΔZZΔFW-R	TACGATTCATCTGCAGCTCGAGTCAAGCCTCC TTCTCCCC
NBR1ΔPB1ΔZZΔFW-F	TGGCCATGGAGGCCAGTGAATTCGTGGAGATAACCATGCT C
NBR1ΔPB1ΔZZΔFW-R	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC
NBR1ΔZZΔFWΔUBA-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA CG
NBR1ΔZZΔFWΔUBA-R	GGGCTGCAGTCAGCCAGCGTTCACATTG
NBR1ΔZZ-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA CG
	CTTCATTGGGTGTAGTTCAACATCTGCATCC
NBR1ΔZZ-R	TGCAGATGTTGAACTACACCCAATGAAGTCC
	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC
NBR1ΔFW-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA C
	GCACCTCTCAGGTGTTGCATCTCTGAAGAATTCTGTTG
NBR1ΔFW-R	ATTCTTCAGAGATGCACACCTGAGAGGTGC
	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC
NBR1ΔZZΔFW-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA C
	GCACCTCTCAGGTGTTGCATCTCTGAAGAATTCTGTTG

	GACTTCATTGGGTGTAGCATCTCTGAAGAATTCTGTTG
NBR1ΔZZΔFW-R	AATTCTTCAGAGATGCTACACCCAATGAAGTCC
	ATTCTTCAGAGATGCACACCTGAGAGGTGC
	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC

Supplementary Table 4. Primers used in luciferase complementation assay.

Primer name	Primer sequence (5'-3')
ADF1-F	GGGGGTACCATGGTAAGCTCAATTTTCC
ADF1-R	GGGGTCGACTTAGTTGGCACGGCTC
ADF2-F	GGGGGTACCATGTTGTGCTTCTTGC
ADF2-R	GGGGTCGACTTAGTTGGTGCGGC
ADF3-F	GGGGGTACCATGGCTAATGCAGCATC
ADF3-R	GGGGTCGACTCAATTGGCTCGGCT
ADF4-F	GGGGGTACCATGGCTAATGCTGCG
ADF4-R	GGGGTCGACTTAGTTGACGCGGCT
ADF6-F	GGGGGTACCATGTCTTTCAGAGGAC
ADF6-R	GGGGTCGACTCAGTTCGCTCGTTC
ADF7-F	GGGGGTACCATGGCGAACGCGGC
ADF7-R	GGGGTCGACCTAGAGAGCTCGGCT
ADF8-F	GGGGGTACCATGACTACGTGTGTG
ADF8-R	GGGGTCGACTCAGAGATTGAGTCG
ADF10-F	GGGGGTACCATGGCGAACGCGGC
ADF10-R	GGGGTCGACCTAGAGAGCTCGACT
ADF11-F	GGGGGTACCATGTGTTTGACTGTGC
ADF11-R	GGGGTCGACTCAGAGATTGACTCG
Profilin1-F	GGGGGTACCATGTCTTGGCAATCATAC
Profilin1-R	GGGGTCGACTTAGAGTTCAGACTCGATAAG
Profilin2-F	GGGGGTACCATGTCGTGGCAATCAT
Profilin2-R	GGGGTCGACTTAGAGACCAGACTCGA
Profilin3-F	GGGGGTACCATGTCGTGGCAGAC
Profilin3-R	GGGCTGCAGTCAGAGCCCCGATTC
ATG8d-F	GGGGGTACCATGGCGATTAGCTCC
ATG8d-R	GGGGTCGACTTAGAAGAAGATCCC
NBR1-F	AACACGGGGGACGAGCTCGGTACCATGGA GTCTACTGCTAACGC
NBR1-R	CCATTTGTTGGATCCCGGGTACCAGCCTCC TTCTCCCCTG
*NBR1*Δ*UBA1*Δ*UBA2*-F	AACACGGGGGACGAGCTCGGTACCATGGA GTCTACTGCTAACGC
*NBR1*Δ*UBA1*Δ*UBA2*-R	GTACGAGATCTGGTCGACGTCATTCTTTTC TATATC
*NBR1*Δ*PB1*Δ*ZZ*Δ*FW*-F	GGGGGTACCGTGGAGATAACCATGCTC

NBR1ΔPB1ΔZZΔFW-R	GTACGAGATCTGGTCGACAGCCTCCTTCT CCCCTG
ARP2-F	GGGGGTACCATGGACAACAAAAACGTC
ARP2-R	GGGGTCGACTTAAGCTTGGCTCATTTTATTC
ARP3-F	GGGGGTACCATGGATCCGACTTCTCG
ARP3-R	GGGGTCGACTCAATACATTCCCTTGAACAC
ATI1-F	GGGGGTACCATGGCTAACAATGAGGAGC
ATI1-R	GGGGTCGACGACCTCACTGGAGGAG
ATI2-F	GGGGGTACCATGGCTGACAAAGATGAAG
ATI2-R	GGGGTCGACACGGTCGTTTTGGGC
ATI3-F	GGGGGTACCATGAATAGAAATTCCAAAGGAAG
ATI3-R	GGGGTCGACCAAAATCTCCCACTCAGAATC
RPN10-F	GGGGGTACCATGGTTCTCGAGGCGAC
RPN10-R	GGGGTCGACTCACTTCTTCTCATCCTCGC
Pex6-F	GGGGGATCCATGGTGGAGAGACGGAATC
Pex6-R	GGGGTCGACGCTCGAACGGCCTTG
Pex10-F	GGGGGTACCATGAGGCTTAATGGGGATTC
Pex10-R	GGGGTCGACAAAATCAGAATGATACAAACAAACC
NBR1ΔPB1ΔZZΔFW-F	TGGCCATGGAGGCCAGTGAATTCGTGGAGATA ACCATGCTC
NBR1ΔPB1ΔZZΔFW-R	TACGATTCATCTGCAGCTCGAGTCAAGCCTCC TTCTCCCC
NBR1ΔPB1ΔZZΔFW-F	TGGCCATGGAGGCCAGTGAATTCGTGGAGATAACCATGCT C
NBR1ΔPB1ΔZZΔFW-R	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC
NBR1ΔZZΔFWΔUBA-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA CG
NBR1ΔZZΔFWΔUBA-R	GGGCTGCAGTCAGCCAGCGTTCACATTG
NBR1ΔZZ-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA CG
	CTTCATTGGGTGTAGTTCAACATCTGCATCC
NBR1ΔZZ-R	TGCAGATGTTGAACTACACCCAATGAAGTCC
	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC
NBR1ΔFW-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA C
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NBR1ΔFW-R	ATTCTTCAGAGATGCACACCTGAGAGGTGC
	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC
NBR1ΔZZΔFW-F	TGGCCATGGAGGCCAGTGAATTCATGGAGTCTACTGCTAA

	C
	GCACCTCTCAGGTGTTGCATCTCTGAAGAATTCTGTTG
	GACTTCATTGGGTGTAGCATCTCTGAAGAATTCTGTTG
NBR1ΔZZΔFW-R	AATTCTTCAGAGATGCTACACCCAATGAAGTCC
	ATTCTTCAGAGATGCACACCTGAGAGGTGC
	TACGATTCATCTGCAGCTCGAGTCAAGCCTCCTTCTCCCC