

Article title: ATBS1-INTERACTING FACTOR 2 negatively modulates pollen production and seed formation in *Arabidopsis*

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The following Supporting Information is available for this article:

Figure S1. Expression level-dependent aerial and silique phenotypes of AIF2-overexpressing independent transgenic lines (*p35S::AIF2-EGFP/Col-0*, AIF2ox).

Figure S2. Expression of the native promoter-driven and CaMV35S promoter-driven AIF2 protein in floral organs.

Figure S3. Reduced pollen production in AIF2ox plants.

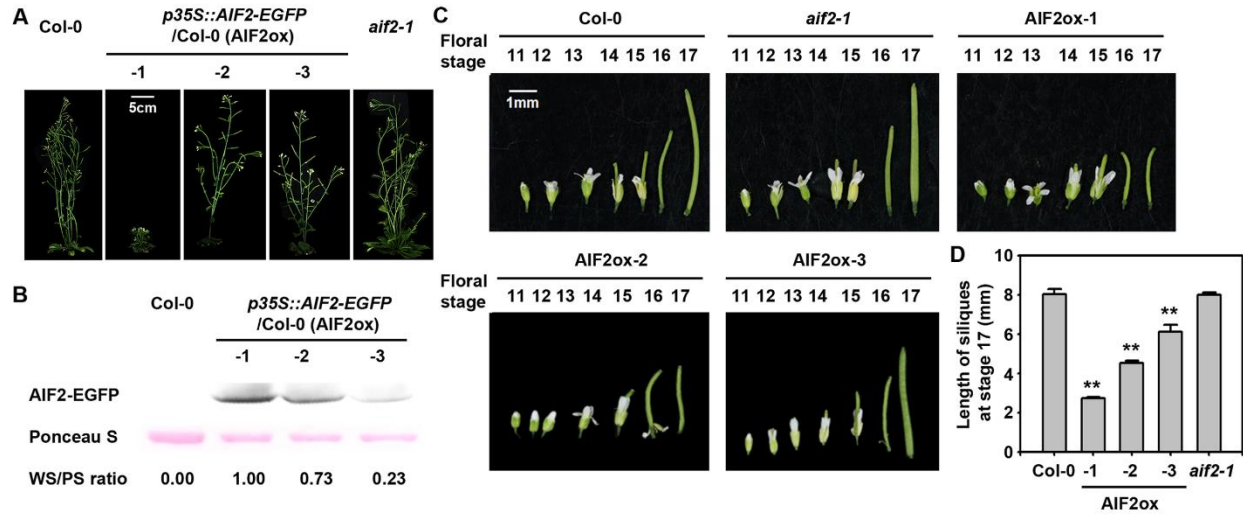
Figure S4. Efficacy of *in vitro*-germinated pollen tube growth.

Figure S5. Relative expression of *AIF2* (At3g06590) in developing seeds.

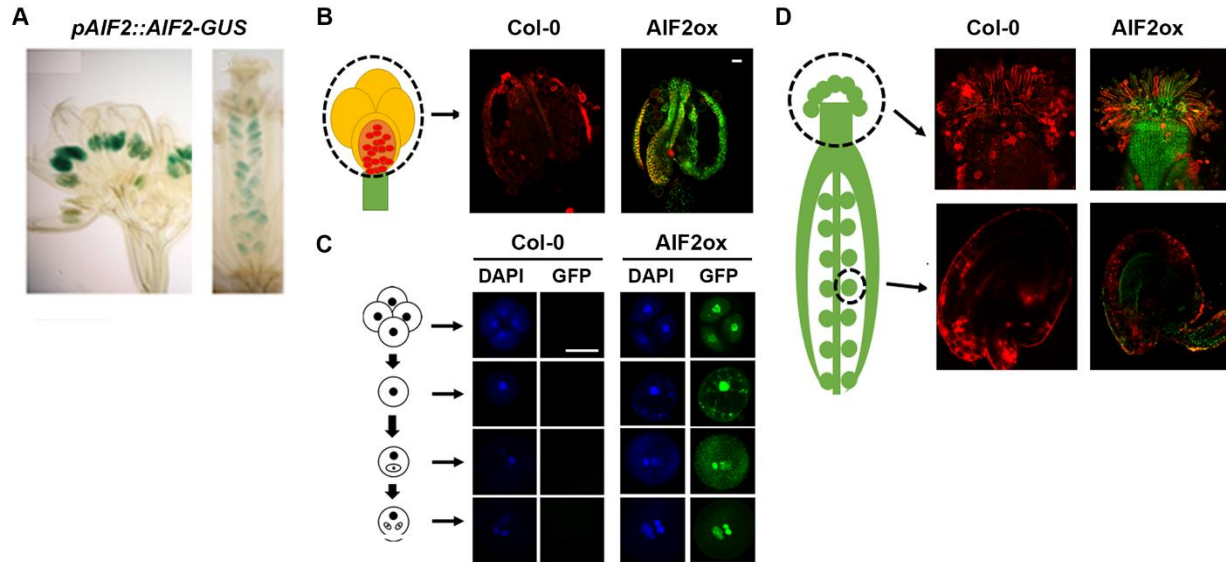
Figure S6. Morphology of seeds stained with mPS-PI, followed by confocal microscope observation.

Table S1. Primers used in cDNA amplification of *AIF2*

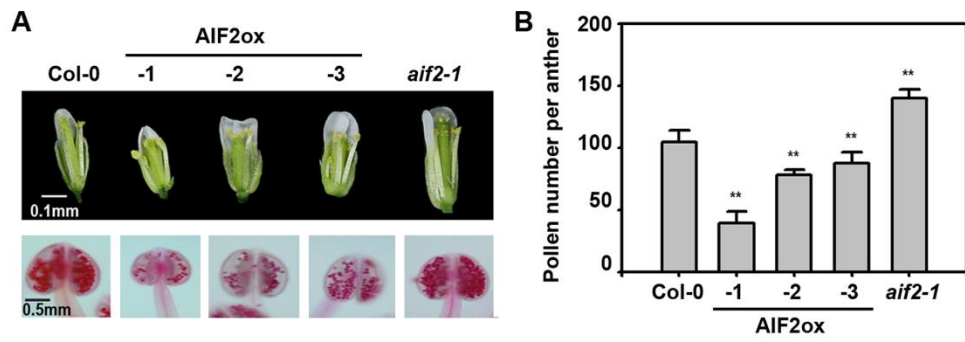
Table S2. Primers used in quantitative real time RT-PCR analysis



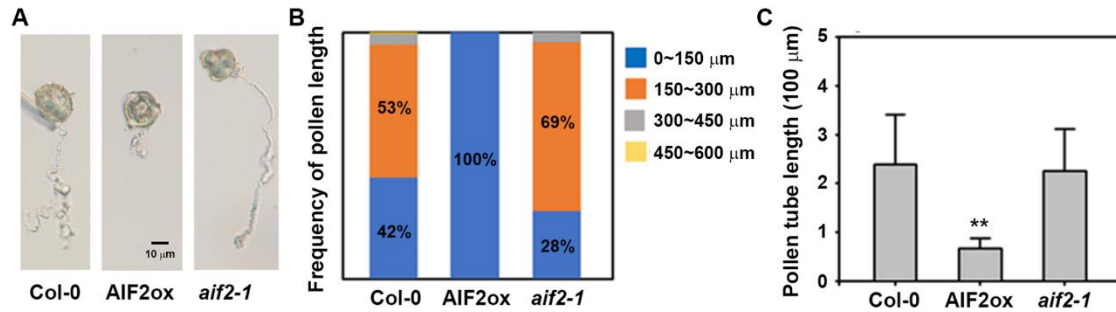
Supplementary Figure S1. Expression level-dependent aerial and silique phenotypes of AIF2-overexpressing independent transgenic lines (*p35S::AIF2-EGFP/Col-0*, AIF2ox). **(A)** Aerial phenotypes of 8-week-old plants. **(B)** Western blot analysis showing ectopic expression of AIF2-EGFP, ranging from high to low levels compared with the Columbia wild-type (Col-0) control. Total protein extracts were isolated from leaves of plants described in (A), size-fractionated by SDS-PAGE, and probed against anti-GFP antibodies. Ponceau S staining was used to normalize AIF2 expression level (WS/PS ratio). **(C, D)** Pictures showing floral stage-dependent growth of siliques (C) and measurement of silique length at stage 17 (D). Number of siliques examined > 50.



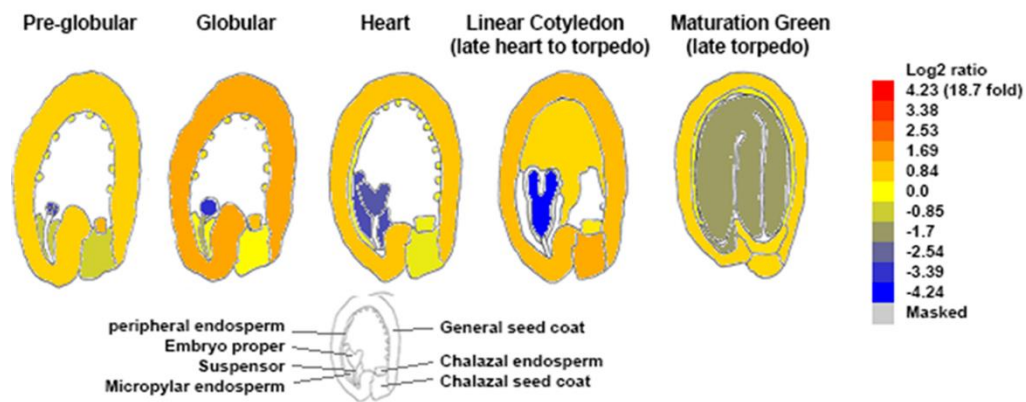
Supplementary Figure S2. Expression of the native promoter-driven and CaMV35S promoter-driven AIF2 protein in floral organs. **(A)** accumulation of the native promoter-driven AIF2-GUS protein in pollen grains and ovules **(B-D)** accumulation of ectopically-expressed AIF2-EGFP protein in anthers **(B)**, developing pollens **(C)**, and stigma/style and ovules **(D)**. Bar represents 10 μ m in length. Red: PI-stained fluorescence, Blue: DAPI-stained fluorescence, Green: GFP fluorescence.



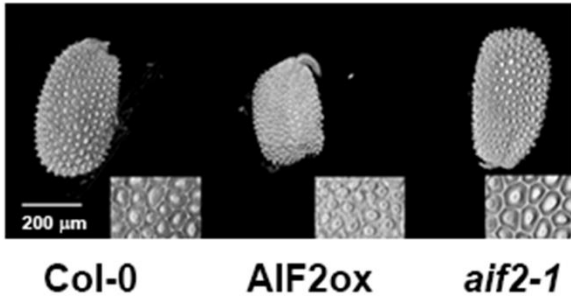
Supplementary Figure S3. Reduced pollen production in AIF2ox plants. **(A)** Anthers of different transgenic lines were stained with Alexander's solution and **(B)** the resulting dye-stained pollens were counted. Number of anthers examined > 50.



Supplementary Figure S4. Efficacy of *in vitro*-germinated pollen tube growth. Pollen grains of Col-0, AIF2ox, *aif2-1* plants were grown on a solid germination medium for 6 hrs in dark at room temperature. Data show (A) pictures of germinated pollen grains, (B) frequency of pollens that show tube growth in a range of the indicated length, and (C) the average tube length of all germinated pollens. Number of pollens examined > 3,000 taken from 20-25 open flowers.



Supplementary Figure S5. Relative expression of *AIF2* (At3g06590) in developing seeds. Tissue-specific expression was analyzed using a plant eFP viewer tool provided by ePlant web site (<http://bar.utoronto.ca/eplant/>, University of Toronto).



Supplementary Figure S6. Morphology of seeds stained with mPS-PI, followed by confocal microscope observation. The insert in picture is the magnified image of seed surface.

Supplementary Table S1. Primers used in cDNA amplification of <i>AIF2</i>		
Amplification	Gene fragment cloned	Primer sets (5' to 3')
AIF2FL	Full-length cDNA of <i>AIF2</i> coding region	CACCATGGCGTCTCTGATCTCAGATAT
		AATCGGTGGAGGAGCTGAGCCG
AIF2dC	cDNA encoding N-terminus and bHLH domain of <i>AIF2</i>	CACCATGGCGTCTCTGATCTCAGATAT
		CTCCAGAGCCTGAATATAATCAGTTGC

Supplementary Table S2. Primers used in quantitative real time RT-PCR analysis		
Gene	Locus	Primer set (5' to 3')
<i>IND</i>	At4g00120	CAGCCCCAAAAGAAGCATGATGG
		TTAGGGTTTCGGGAGGTGGATCTAA
<i>ALC</i>	At5g67110	CTCTCGAGCTTTCTCCGAACGATTC
		TGTCTTGGCCAGTTTCAGAGACTCC
<i>MSI</i>	At5g22260	TGGTGGGTGGTCAAATAGAG
		TCATCATTCCTACGTTCCCT
<i>SPL</i>	At4g27330	TCGCTAGAGCAGCTTCAGTT
		CCTCCATTGGTCCCGTAT
<i>TDF1</i>	At3g28470	CGGTCCTCAAGTAGTGGG
		ATGTATTCGGCTTCGATGTT
<i>TCPI</i>	At1G67260	GGTACGGTGAAGAAGAAGTGG
		TCCTCTAGCTTTGGCTCCTAG
<i>TCP4</i>	At3G15030	AGGAGCAGAGCATCCGAGTA
		ATTGACGGCGGGAGAAAAC
<i>YUCCA1</i>	At4g32540	CTCGTCCGACATAACGCATCTCCT
		CCCAACCGGTGTATTTCCAAAC
<i>YUCCA2</i>	At4g13260	TCCCTAAAGATTTCTGTGAG
		CCACCGTGATACATACTCC
<i>YUCCA6</i>	At5g25620	GGGAGAAGAAGGCACGAC
		GAAGGCTGAGCACCGAAG
<i>ARF6</i>	At1g30330	GCATGCTGATGTGGAGACTGATGAA
		GGCTGCTGCGAGTAATCCAAGG
<i>ARF8</i>	At5g37020	TGGGTCAACAGGGTCATGAAGGAGA
		TGTGGTGGTAGGCTTGGGTAATTGG
<i>DWF4</i>	At1g78540	ACAGCAAACAACGGAGCG
		TCTGAACCAGCACATAGCCT
<i>SHB1</i>	At1g78540	CAGGTTCAAGCACTGAGGAGT
		TGCTTCCTCGGTTTAGAGTA
<i>IKU1</i>	At2g35230	TTTGGGTGTGAATAGGATTGG
		CGCTGCAACCGTGTATTCT
<i>MINI3</i>	At1g55600	TTTGATGATATTGCAACGGAA
		GATCCTTTGTGTCTTGCTTGT
<i>AP2</i>	At4g36920	ATTCGGCTAATTCGAAGCATAA
		AGAGGAGGTTGGAAGCCATT

Supplementary Table S2. Primers used in quantitative real time RT-PCR analysis		
Gene	Locus	Primer set (5' to 3')
<i>ARF2</i>	At5g62000	TTTACCAGCAAGCGGACTTT
		CTGGCTGAGGACATCCAGTT
<i>SWEET11</i>	At3g48740	GGAGCCACACGTGCTAAGATTATCG
		ACCAAGCACATTCGGGAAAGCAA
<i>SWEET12</i>	At5g23660	GCGCCGCTTAGCATTATCAGAAC
		TGCACCTAGAACAAACCCAATCACG
<i>SWEET15</i>	At5g50800	CGTGGCTCGTGTGATAAAGACAAAG
		CCACCACGTTTGGAATCGCTATG
<i>FAD2</i>	At3g12120	TAACGTTATCGCCCCTACGTCAGC
		AATTGGTGGCGACGTAGTAGAAGCA
<i>FAD3</i>	At2g29980	CACTCGCGGTTCTTAAAGTCTACGG
		TGGCGTCGACCAAGTGATAGTGA
<i>LPCAT1</i>	At1g12640	GCAACAAGCGATCAGTCCGAAAATG
		AAGCGCGGGAAACTTACTTACCG
<i>LPCAT2</i>	At1g63050	TGCGGTTTCAGATTCCGCTTTTCT
		TGTTGCCACCGGTAAATAGCTTTTCG
<i>PDAT1</i>	At5g13640	TGCGAGCTTCCCTCAGTATGTAACG
		GCTGGATCCAACCCAGTTTCATTGT
<i>UBC1</i>	At1g14400	TCATCGCACGGTGATATTGAGAATC
		TCGACATCCTCCTTTCTTTCGTGGA