

A rapid alkalization factor-like peptide EaF82 impairs tapetum degeneration during pollen development through induced ATP deficiency

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This Supplementary Data file includes:

1. Supplementary Table 1-3, 7-9

Table S1. List of primer sequences used in current study.

Table S2. The Hybrigenics' references of seven selected clones for 1-by-1 assays.

Table S3. The summary of read counts of RNAseq analysis in current study.

Table S7. A subset of FPKM related to tapetum genes.

Table S8. The EaF82 interacting proteins.

Table S9. Summary of interaction matrix and results.

2. Supplementary Figures S1-12

Figure S1. Genetic cassettes used in this study.

Figure S2 Alkalization assays of inactive EaF82-S as well as a representative of collected pollen grains and tobacco suspension cells.

Figure S3. Tissue specific expression of *EaF82* promoter in Arabidopsis transgenic *EaF82p::EaF82-sGFP* (TA) lines.

Figure S4. Arabidopsis transgenic *EaF82p::EaF82-sGFP* lines (TA-1, -3, -4, and -5).

Figure S5. Immunoblots of Arabidopsis transgenic *EaF82p::EaF82-sGFP* (TA-4) flower proteins against anti-EaF82 and anti-GFP antibodies.

Figure S6. Arabidopsis transgenic *35Sp::EaF82-sGFP* (TB) lines.

Figure S7. RT-qPCR of *GUS* expression levels.

Figure S8. Male gametophyte development of Arabidopsis transgenic *35Sp::EaF82-sGFP* (TB) line.

Figure S9. The numbers of seeds per silique with different length (cm) in Arabidopsis transgenic *35Sp::EaF82* (TC) line.

Figure S10. RNAseq analysis of early developmental flower buds of two independent *35Sp::EaF82* (TC1 and TC2) and vector control (C) transgenic lines.

Figure S11. Solid growth tests on +/- Histidine and +/- 3-AT plates.

Figure S12. A “DomSight” of AKIN10 (AT3G01090) displays the information of bait and prey structural, functional and interaction domains.

Supplementary Tables 4-6 in Excel spreadsheets

Table S4. Common DEGs that both TC-1 and TC-2 increase two-fold compared to the vector control.

Table S5. Common DEGs that both TC-1 and TC-2 decrease two-fold compared to the vector control.

Table S6. A subset of upregulated and downregulated DEGs with FDR<0.05.

Supplementary Data Sets 1-3 in Excel spreadsheets

Data Set S1. The FPKM values for normalized read counts of RNAseq analysis of Arabidopsis transgenic *35Sp::EaF82* (TC) and vector control (C) lines.

Data Set S2. Gene Ontology enrichment analysis of upregulated DEGs.

Data Set S3. Gene Ontology enrichment analysis of downregulated DEGs.

Table S1. List of primer sequences used in current study

AGI ID	gene name	Forward sequence (5'-3')	Reverse sequence (5'-3')
At1G19890	<i>MGH3</i>	AGGCAGCCGAAGCATACTTG	ACCCTTTTGGCGTGAATCG
At1G19960	<i>AT1G19960</i>	CGGCGGCTCCTTTGG	CCACACTTGCTTCCTCACCTTT
At1G21000	<i>AT1G21000</i>	ACGGTCCCAATGGTGATCA	GGAGAGAACTAAAACCACCTTCATC
At1G24520	<i>BCP1</i>	CGATGACGATGCAGCTCCTA	TAGAGGACCAGCCACTGCAA
At1G28270	<i>RALFL4</i>	CAATGCAACCTACCCTTTAACCA	GTCTTCCCCGATGCAACCT
At1G29140	<i>AT1G29140</i>	TGAGTGCAAGGGTCGTGAGA	TTGTCGGTCACGGCTTCTTT
At1G35490	<i>AT1G35490</i>	CATCGAGCGCGTTTGAGA	TTGTAGCACTGGATGGTCCTTT
At1G61563	<i>RALFL8</i>	CCCACCCTAACACCTGCAA	CTTTGGACCTGTTTCACGATGG
At1G61566	<i>RALFL9</i>	CCCACCCTAACACCTGCAA	ATCTTCTCGCATCTCTCTGGTA
At1G80660	<i>HA9</i>	TGGCACCGTGTGAGCAAAA	CTTGCACTCTCCCGAAGGT
At2G07040	<i>PRK2A</i>	GCGCGCAAGAGCTCATG	TTGGTCACACGGCTTTGTTTT
At2G07560	<i>HA6</i>	GGGTTGCACGTCAGAGAGTT	TTGGAGGGTCAAAGAGTGGC
At2G18470	<i>PERK4</i>	GCAAGAAATGGCTCGAATGG	CGCCCCGAATGACGAAT
At2G21480	<i>AT2G21480</i>	TGAACGGTGTGAGGTCTTG	ATCCCTGCTTCCCCATACT
At2G22055	<i>RALFL15</i>	CAAAGCCCACCCTAACACCT	ACCTGTATCACGACGGCAAC
At2G33775	<i>RALFL19</i>	TGTTGATCCTTGGCCTCTGA	GGGTCCATGTGGCATTGG
At2G36080	<i>ABS2</i>	GGAGAGCCAAAGAGGCAACT	CCGAATCTAGCTGGCACTCC
At2G45660	<i>AGL20</i>	ACTCTTGGGAGAAGGCATAGGAA	TCAAGCTGTTGCTCAATCTGTTG
At3G08560	<i>VHA-E2</i>	AAGCCAAAGTCGGGTCTCCTA	AGGAGGTGGAGGGAGGAAGA
At3G25165	<i>VHA-E2</i>	GGACGAGTTGAGGCTAACGATAA	GGAGCATTAGGACGCAAGCA
At3G42640	<i>HA8</i>	GGGGAGCAAGAGGCTTCAAT	CCTTCGAGGAGACGAGCATC
At4G10603	<i>AT4G10603</i>	CCGTTGTTTCGGCTAGGTGTAAG	CATTGCCTTGCCCCCTTTA
At4G14020	<i>AT4G14020</i>	ACATTTACCGAAAACGACGAAGAA	TTCTTTCACCCACGTGTTATTCA
At4G24540	<i>AGL24</i>	ACGTTGGAAAGGGCAAACTG	TTTGTGGTCACCGACTCTGTCT
At4G35900	<i>FD</i>	GCAAGACTCAAGAGACAACAAG	CAAAATGGAGCTGTGGAAGAC
At4G39110	<i>AT4G39110</i>	AGGGCAAAGCAGAAGAGACC	TTCGTTGGTGGTGACTGAGG
At5G17480	<i>PC1</i>	ATGGCTGATGCAACGGAGAA	GAGCTTCTTCGAGTTCGGCT
At5G28680	<i>ANX2</i>	AGCAGTTTTTGTCTTGTGGGG	CTGGAGGCTGTTTTCTGCAA
At5G45880	<i>AT5G45880</i>	CTTGCCGTGTCCAATTCGTT	TTCTGCTCCTGCACTCCAAC
At5G57350	<i>HA3</i>	TTCTGCTGATGCACGTCTT	TTCCCTGGACCTTTCGTTG
At5G62165	<i>AGL42</i>	CCAGCAATCACGACTCACAAAT	TTGTATCATGTGGCTTGCTTCTT
	<i>EaF82</i>	GCATGTGGTGTCACTCCTCTTAT	ATCTTCTTCCGAGTCCCAAA
	<i>NPTII</i>	AAGATGGATTGCACGCAGGTTC	ACGGGTAGCCAACGCTATGTC
	<i>GUS2</i>	TGGCCTGGCAGGAGAACT	ACGTATCCACGCCGTATTCG

Table S2. The Hybrigenics' references of seven selected clones for 1-by-1 assays

ATMB_RP1_hgx4998v1_pB27_A-237 ABCF4 [402-557]*
ATMB_RP1_hgx4998v1_pB27_A-240 ALATS [36-282]
ATMB_RP1_hgx4998v1_pB27_A-66 FKBP-like peptidyl-prolyl cis-trans isomerase family protein [43-190]
ATMB_RP1_hgx4998v1_pB27_A-262 PAPP2C [177-287]
ATMB_RP1_hgx4998v1_pB27_A-94 TCH4 [24-178]

ATMB_RP1_hgx4998v1_pB27_A-125 AKIN10 [422-535]
ATMB_RP1_hgx4998v1_pB27_A-157 SYTA [309-536]

*The amino acid residues of proteins been identified in prey fragments are indicated in brackets. The prey fragments that do not stop with the natural stop codon of the protein have a short additional peptide at the C-terminal end which comes from the SfiI restriction side, being used for cloning of the library fragments.

Table S3. The summary of read counts of RNAseq analysis in current study

Samples ID	Read	Read length bp	Index	Number of Reads
C1	SE	76	CTGAAGCT+AGGCTATA	19,858,927
C2	SE	76	TAATGCGC+AGGCTATA	18,609,048
C3	SE	76	CGGCTATG+AGGCTATA	18,331,258
TC1-1	SE	76	CTGAAGCT+GCCTCTAT	20,035,018
TC1-2	SE	76	TAATGCGC+GCCTCTAT	19,216,782
TC1-3	SE	76	CGGCTATG+GCCTCTAT	20,693,114
TC2-1	SE	76	CTGAAGCT+AGGATAGG	23,037,461
TC2-2	SE	76	TAATGCGC+AGGATAGG	21,102,277
TC2-3	SE	76	CGGCTATG+AGGATAGG	21,339,999

Table S7. A subset of FPKM related to tapetum genes.

Gene id	Gene short name	locus	length	FPKM_ C1	FPKM_ C2	FPKM_ C3	FPKM_ TC1-1	FPKM_ TC1-2	FPKM_ TC1-3	FPKM_ TC2-1	FPKM_ TC2-2	FPKM_ TC2-3
Tapetum-related												
AT5G07280	EMS1/EXS	5:2284829 -2288855	4.03E+0 3	12.94	13.09	15.33	16.05	12.87	13.77	15.54	14.20	14.92
AT4G24972	TPD1	4:1283735 2- 12840230	1.34E+0 3	6.83	4.42	6.49	3.74	5.79	5.30	5.00	4.87	4.98
AT1G34210	SERK2	1:1245856 3- 12462905	2554	9.38	10.01	9.56	10.24	10.99	7.75	10.13	6.65	9.18
AT1G34210	SERK2	1:1245858 9- 12462752	2267	6.15	5.44	8.14	5.18	5.28	4.95	5.58	10.70	6.98
AT1G71830	SERK1	1:2701815 7- 27022117	2570	12.46	13.51	14.42	14.26	14.75	14.41	15.81	14.42	15.47
AT3G28470	TDF1/MYB35	3:1067450 7- 10675724	1025	1.37	2.02	1.91	2.62	0.97	1.34	2.08	1.48	2.71
AT2G16910	AMS	2:7331720 -7334254	1893	33.39	33.40	45.57	48.79	29.50	33.23	47.66	35.97	44.85
AT5G56110	MYB188/MYB103/MYB80	5:2271919 0- 22720664	963	3.39	2.56	4.60	2.55	1.20	2.07	2.87	1.67	3.67
AT5G22260	MS1	5:7367634 -7370295	2.19E+0 3	2.59	2.50	3.25	3.28	2.23	1.66	3.51	2.43	3.18

AT4G21330	DYT1	4:1134992 1- 11350694	624	0.13	0.68	0.28	1.92	0.27	0.87	1.58	1.09	1.32
AT4G20900	MS5	4:1118410 2- 11185844	1353	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AT4G20900	MS5	4:1118410 2- 11185844	1305	1.17	0.73	1.80	1.81	0.57	1.00	1.29	1.38	1.47
AT3G51280	MS5-like	3:1903713 9- 19039048	1649	20.20	27.64	26.55	38.31	41.52	32.95	50.05	41.90	41.58
AT3G11980	MS2/FAR2	3:3814230 -3817117	2117	51.40	33.34	54.30	60.38	45.99	35.70	64.57	52.92	69.76
AT4G33790	MS2-like/FAR3	4:1620409 5- 16207956	1956	0.73	0.49	1.02	1.31	0.54	1.30	0.22	1.51	0.75
AT4G33790	MS2-like/FAR3	4:1620409 5- 16207956	1776	39.84	34.90	39.31	37.02	40.67	27.61	28.08	28.10	32.53
AT4G27330	SPL/NZZ	4:1368207 7- 13683594	1329	6.96	6.80	9.52	5.98	3.94	6.38	5.31	5.33	5.67
AT5G50260	CEP1*	5:2045531 6- 20457068	1580	6.55	2.78	5.29	0.27	0.37	0.53	0.28	0.15	0.22
AT2G01570	RGA	2:255246- 257568	2.32E+0 3	33.43	39.86	40.28	38.23	40.33	38.79	37.86	40.58	43.17
AT4G02780	GA1	4:1237670 -1244822	2675	0.41	0.62	0.57	0.59	0.64	0.38	0.56	0.62	0.70
AT1G14920	GAI	1:5148981 -5151415	2434	19.30	23.02	21.23	25.44	28.62	24.79	25.76	24.24	29.28
AT5G06100	MYB33	5:1837834 -1840706	2266	2.96	2.58	4.12	3.55	3.76	6.08	6.83	5.50	2.60
AT5G06100	MYB33	5:1837834 -1840727	2206	8.33	7.79	6.27	8.20	6.43	4.91	4.10	5.46	8.46
AT5G06100	MYB33	5:1837895 -1840701	2537	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.13
AT5G06100	MYB33	5:1837913 -1840159	1741	0.00	0.00	0.00	0.00	0.21	0.00	0.23	0.39	0.73
AT5G06100	MYB33	5:1837913 -1840706	2443	0.20	0.00	1.79	0.62	0.72	1.02	0.36	0.40	0.04
AT5G06110	MYB33	5:1840754 -1843872	2554	28.83	19.05	32.29	36.99	33.59	28.20	35.01	26.77	31.72
AT3G11440	MYB65	3:3602035 -3605172	2.23E+0 3	0.00	0.25	0.26	0.00	0.00	0.00	0.00	0.00	0.00
AT3G11440	MYB65	3:3602035 -3605172	2140	0.00	1.94	0.63	0.42	1.31	0.00	0.37	0.00	0.78
AT3G11440	MYB65	3:3602348 -3605110	2089	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AT3G11440	MYB65	3:3602883 -3605104	2009	3.24	0.53	2.97	2.36	1.68	3.34	3.44	3.07	2.28

*: The gene is in the downregulated DEG list.

Table S8. The EaF82 interacting proteins

	name	ATG	# clones	score
	Arabidopsis thaliana - BIP1	AT5G28540	16	A
p	Arabidopsis thaliana - ALATS	AT1G50200	10	A
p	Arabidopsis thaliana - SYTA	AT2G20990	10	A

	Arabidopsis thaliana - F17H15.1	AT2G25970	7	A
	Arabidopsis thaliana - MNJ7.18	AT5G47590	7	A
	Arabidopsis thaliana - SOX	AT3G01910	7	B
	Arabidopsis thaliana - F14O23.22	AT1G71840	5	B
	Arabidopsis thaliana - HSC70-1	AT5G02500	5	B
	Arabidopsis thaliana - F5F19.6	AT1G52000	4	B
	Arabidopsis thaliana - FP3	AT5G63530	4	B
p	Arabidopsis thaliana - PAPP2C	AT1G22280	4	B
p	Arabidopsis thaliana - ABCF4	AT3G54540	3	C
	Arabidopsis thaliana - LOS2	AT2G36530	3	C
	Arabidopsis thaliana - SPDS2	AT1G70310	3	C
	Arabidopsis thaliana - emb1507	AT1G20960	3	C
	Arabidopsis thaliana - heat shock protein 70-3	AT3G09440	3	C
	Arabidopsis thaliana - MFB16.14	AT5G50740	5	D
	Arabidopsis thaliana - PATL1	AT1G72150	5	D
	Arabidopsis thaliana - ACT2	AT3G18780	2	D
	Arabidopsis thaliana - AMY3	AT1G69830	2	D
	Arabidopsis thaliana - Carbohydratebinding-like fold	AT3G62360	2	D
	Arabidopsis thaliana - F13M22.2	AT2G37520	2	D
p	Arabidopsis thaliana - FKBP-like	AT3G55520	2	D
	Arabidopsis thaliana Translation elongation factor EFG/EF2 protein (SCO1)	AT1G62750	2	D
	Arabidopsis thaliana - HIP1	AT4G22670	2	D
	Arabidopsis thaliana - IDM2	AT1G54840	2	D
	Arabidopsis thaliana - MYC3	AT5G46760	2	D
	Arabidopsis thaliana - SK5	AT3G60020	2	D
	Arabidopsis thaliana - SRC2	AT1G09070	2	D
p	Arabidopsis thaliana - TCH4	AT5G57560	2	D
	Arabidopsis thaliana - clathrin	AT5G05010	2	D
p	Arabidopsis thaliana - AKIN10	AT3G01090	1	D
	Arabidopsis thaliana - DRH1	AT3G01540	1	D
	Arabidopsis thaliana - F18O14.16	AT1G19400	1	D
	Arabidopsis thaliana - HSP91	AT1G79930	1	D
	Arabidopsis thaliana - MBP1	AT1G52040	1	D
	Arabidopsis thaliana - MDJ22.18	AT5G22760	1	D
	Arabidopsis thaliana - MJJ3.25	AT5G05830	1	D
	Arabidopsis thaliana - MPK17.2	AT5G36740	1	D
	Arabidopsis thaliana - PBB2	AT5G40580	1	D
	Arabidopsis thaliana - Phox3	AT5G20360	1	D
	Arabidopsis thaliana - SGT1A	AT4G23570	1	D
	Arabidopsis thaliana - T19D16.19	AT1G10890	1	D
	Arabidopsis thaliana - T1K7.11	AT1G26520	1	D
	Arabidopsis thaliana - cpHsc70-1	AT4G24280	1	D
	Arabidopsis thaliana - hypothetical protein	AT3G59430	1	D

Table S9. Summary of interaction matrix and results

Interaction Matrix					Selection Medium					
N# Interaction*	Type	Bait	Prey	Prey clone reference	DO-2	DO-3	DO-3 +	DO-3 +	DO-3 +	DO-3 +

							1mM 3-AT	5mM 3-AT	10mM 3-AT	50mM 3-AT
1	Hybrigenics' positive control	SMAD	SMURF	/	+	+	+	+	+	+
2	Negative control	F82	pP7ø	/	+	-	-	-	-	-
3	Negative control	pB27ø	ABCF4	pB27_A-237	+	-	-	-	-	-
4	Interaction	F82	ABCF4	pB27_A-237	+	+	+	+	+/-	-
5	Negative control	pB27ø	ALATS	pB27_A-240	+	-	-	-	-	-
6	Interaction	F82	ALATS	pB27_A-240	+	+	+	-	-	-
7	Negative control	pB27ø	FKBP_like peptidyl-prolyl cis-trans isomerase family protein	pB27_A-66	+	-	-	-	-	-
8	Interaction	F82	FKBP_like peptidyl-prolyl cis-trans isomerase family protein	pB27_A-66	+	+	+	+/-	-	-
9	Negative control	pB27ø	PAPP2C	pB27_A-262	+	-	-	-	-	-
10	Interaction	F82	PAPP2C	pB27_A-262	+	+	+	+	+	
11	Negative control	pB27ø	TCH4	pB27_A-94	+	-	-	-	-	-
12	Interaction	F82	TCH4	pB27_A-94	+	+	+	-	-	-
13	Negative control	pB27ø	AKIN10	pB27_A-125	+	-	-	-	-	-
14	Interaction	F82	AKIN10	pB27_A-125	+	+	+	+	+/-	-
15	Negative control	pB27ø	SYTA	pB27_A-157	+	-	-	-	-	-
16	Interaction	F82	SYTA	pB27_A-157	+	+	+	-	-	-

* A single clone of each negative control is spotted in duplicates. pB27: LexA DNA Binding Domain (DBD) vector (LexA-bait); pB27ø: empty pB27 vector; pP7/pP6: Gal4 Activation Domain (AD) vector (AD-prey); pP7ø: empty pP7 vector; DO-2: selective medium without tryptophan and leucine; DO-3: selective medium without tryptophan, leucine and histidine; 3-AT: 3-aminotriazole.

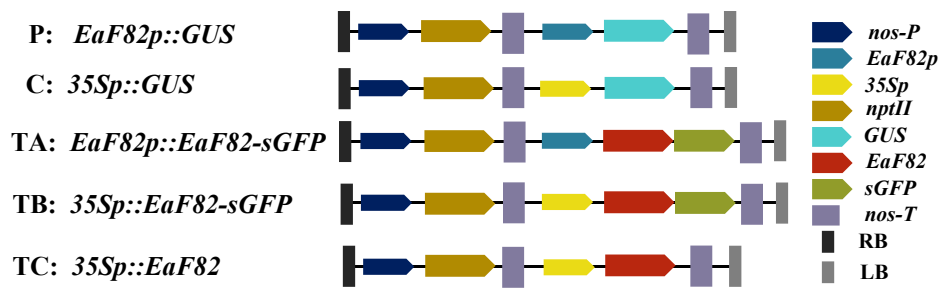


Figure S1 Genetic cassettes used in this study. *EaF82p*: *EaF82* promoter; *35Sp*: CaMV 35S promoter; *sGFP*: *Green Fluorescent Protein* (S65T); *GUS*: *uidA*; *nptII*: *neomycin phosphotransferase II*; *nos-P*: *nopaline synthase* promoter; *nos-T*: *nos* terminator; RB: right border; LB, left border.

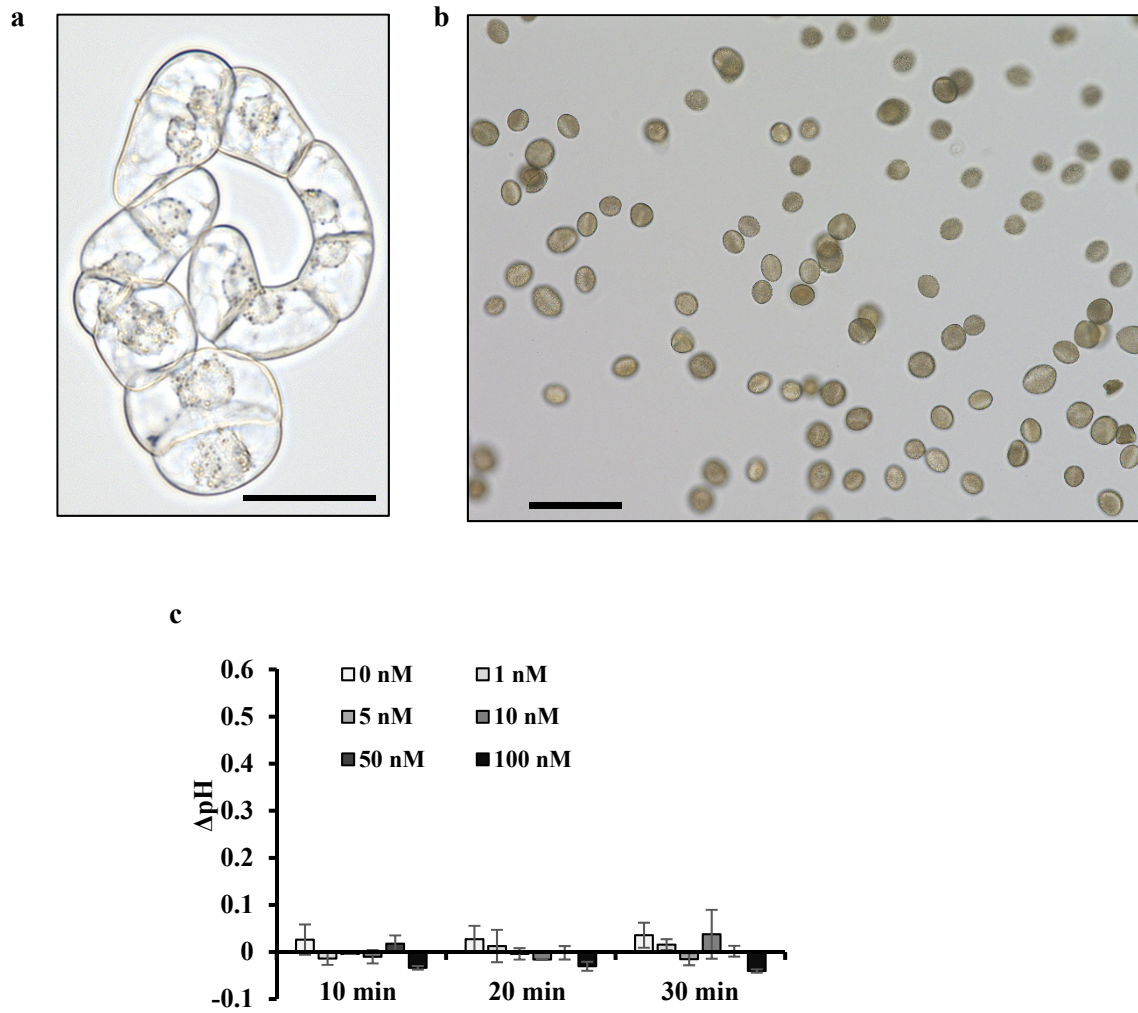


Figure S2 Alkalinization assays of inactive EaF82-S as well as a representative of collected pollen grains and tobacco suspension cells. **a** A representative of tobacco suspension cells used for alkalinization assay. Bar=50 μ m. **b** A representative of collected pollen in HEPES potassium salt buffer for protein isolation. Bar=100 μ m. **c** Alkalinization assays of inactive EaF82-S. Data plotted is the concentration curve. The pH changes (ΔpH) were calculated as measured pH value after applying inactive EaF82-S for 10, 20 and 30 min minus the pH value at 0 min at each concentration from 0 to 100nM. The data plotted were the average of three independent experiments \pm SD.

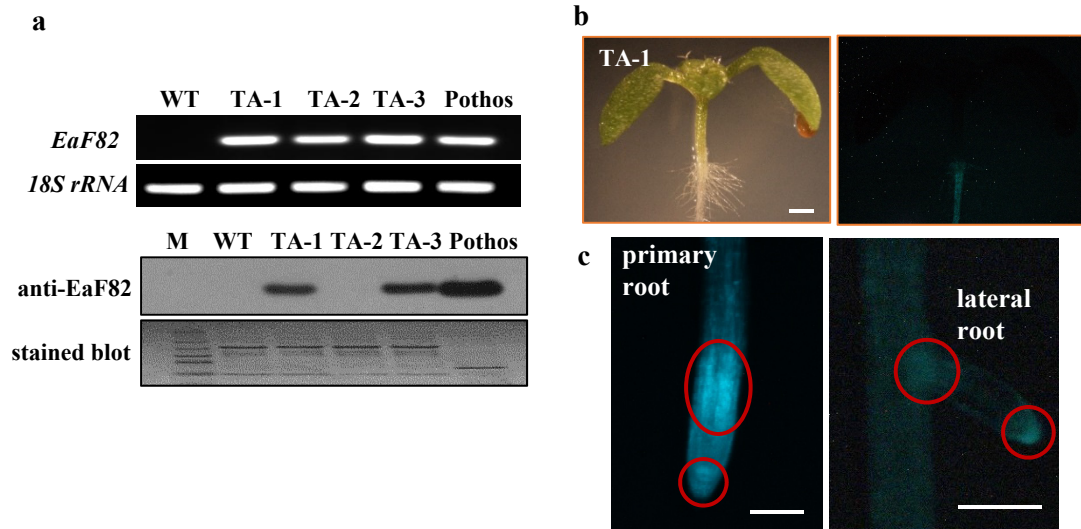


Figure S3 Tissue specific expression of *EaF82* promoter in Arabidopsis transgenic *EaF82p::EaF82-sGFP* (TA) lines. **a** RT-PCR (upper panel) and immunoblot (lower panel) of three 7-day old subline seedlings (TA-1, 2, and 3). PCR was performed using primer pairs specific to *EaF82* and *18S rRNA* gene (as internal control). Stained blot shows protein loading. *EaF82* originated from ‘Golden Pothos’ was used as a positive control. **b** 10-day old light grown seedlings (left) and the detected GFP signals in roots (right). Bar = 1mm. **c** GFP signals detected in primary and lateral roots are circled in red. Bar = 500 μ m.

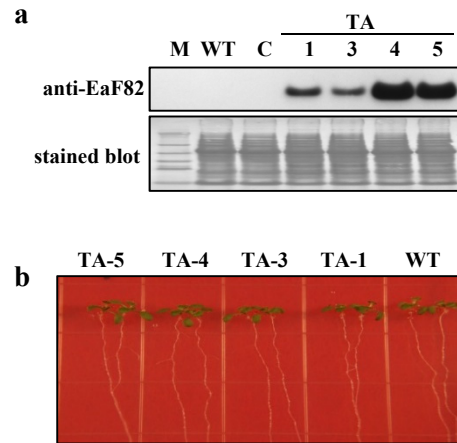


Figure S4 Arabidopsis transgenic *EaF82p::EaF82-sGFP* lines (TA-1, -3, -4, and -5). **a** Immunoblot of seedling proteins against anti-EaF82. C: vector control. WT: wild-type. M: protein size marker. **b** 10-day old seedlings germinated on MS medium show normal growth as WT.

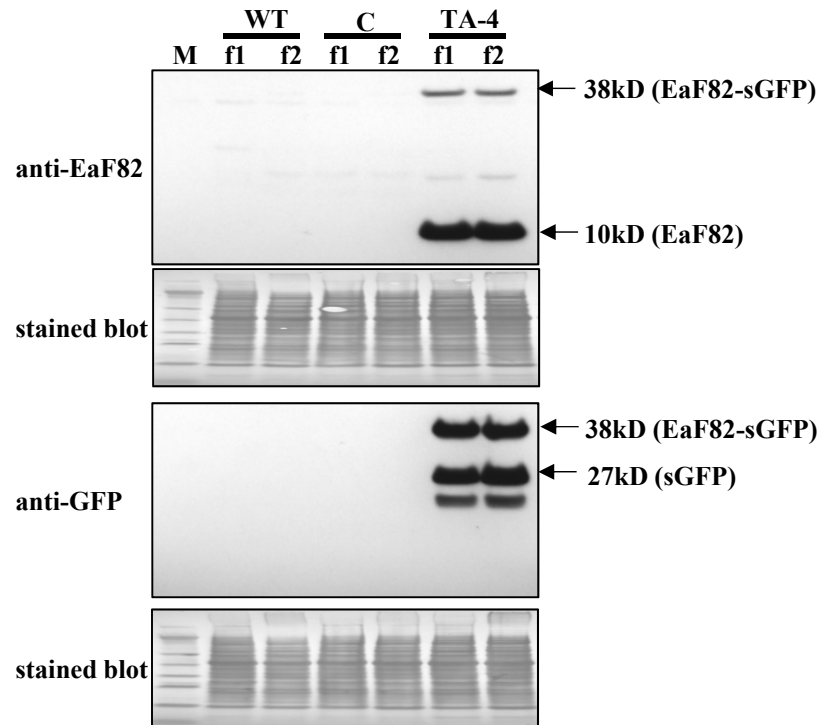


Figure S5 Immunoblots of Arabidopsis transgenic *EaF82p::EaF82-sGFP* (TA-4) flower proteins against anti-EaF82 and anti-GFP antibodies. Stained blots show protein loading. C: vector control. WT: wild-type. M: protein size marker. f1 and f2: two independent pools of flower tissues.

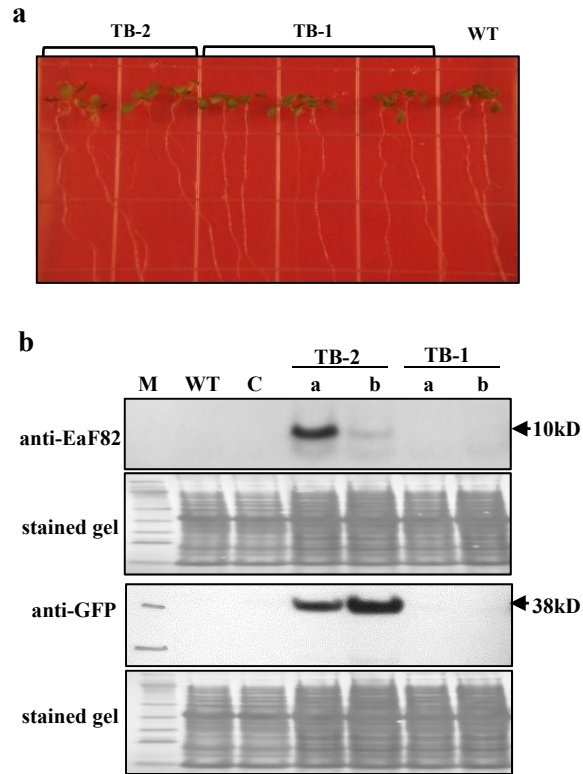


Figure S6 Arabidopsis transgenic *35Sp::EaF82-sGFP* (TB) lines. **a** 10-day old seedlings germinated on MS medium show normal growth as wild-type (WT). **b** Immunoblot of seedling proteins against anti-EaF82 and anti-GFP. TB-1 and -2 are two independent lines, while a, and b are two sublines.

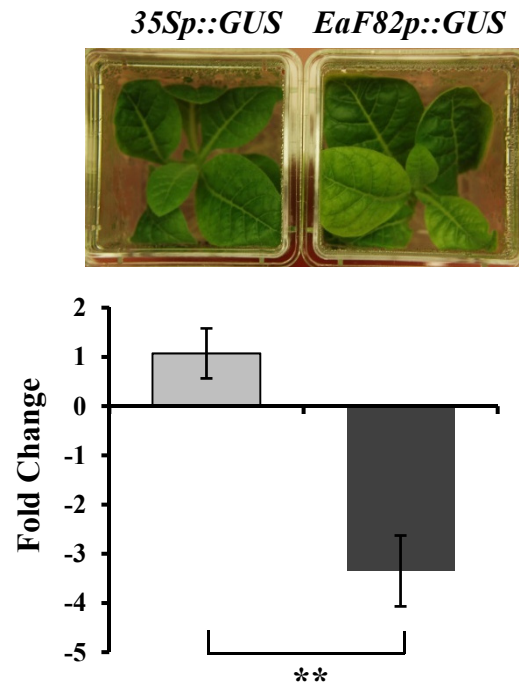


Figure S7 RT-qPCR of *GUS* expression levels. Transgenic tobacco plants carrying *GUS* driven by CaMV 35S promoter (*35Sp::GUS*) and *EaF82* promoter (*EaF82p::GUS*), respectively (upper panel). *GUS* expression levels of each transgenic line were first normalized with their internal *18S rRNA* expression level. The fold changes were then calculated as *EaF82p::GUS* comparing to *35Sp::GUS* (as 1). Data plotted are the average of fold changes in three biological replicates \pm SD. ** $P < 0.01$.

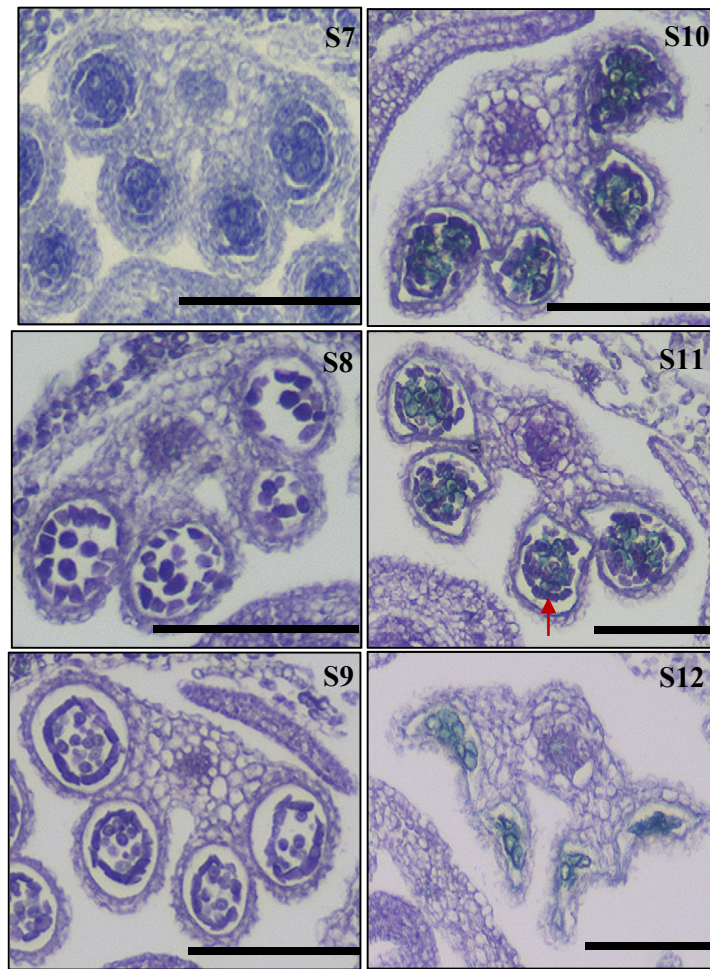


Figure S8 Male gametophyte development of *Arabidopsis* transgenic *35Sp::EaF82-sGFP* (TB) line. The stages S7 to S12 of a second independent TB flower cluster. The delayed degeneration of tapetum at stage S11 is indicated in red arrow. Underdeveloped pollen at stage S12 are stained in light green. Bar = 100 μm.

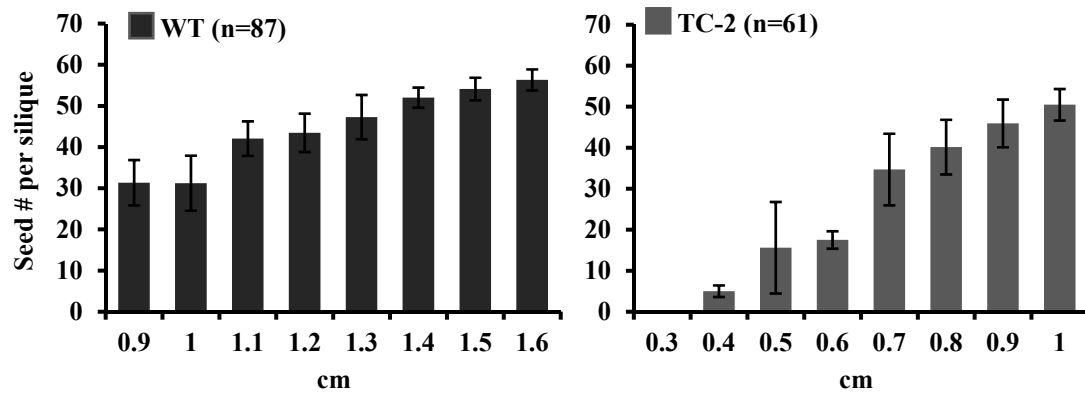


Figure S9 The numbers of seeds per silique with different length (cm) in *Arabidopsis* transgenic 35Sp::EaF82 (TC) line. Data plotted are the average \pm SD. WT: wild-type; n: number of siliques.

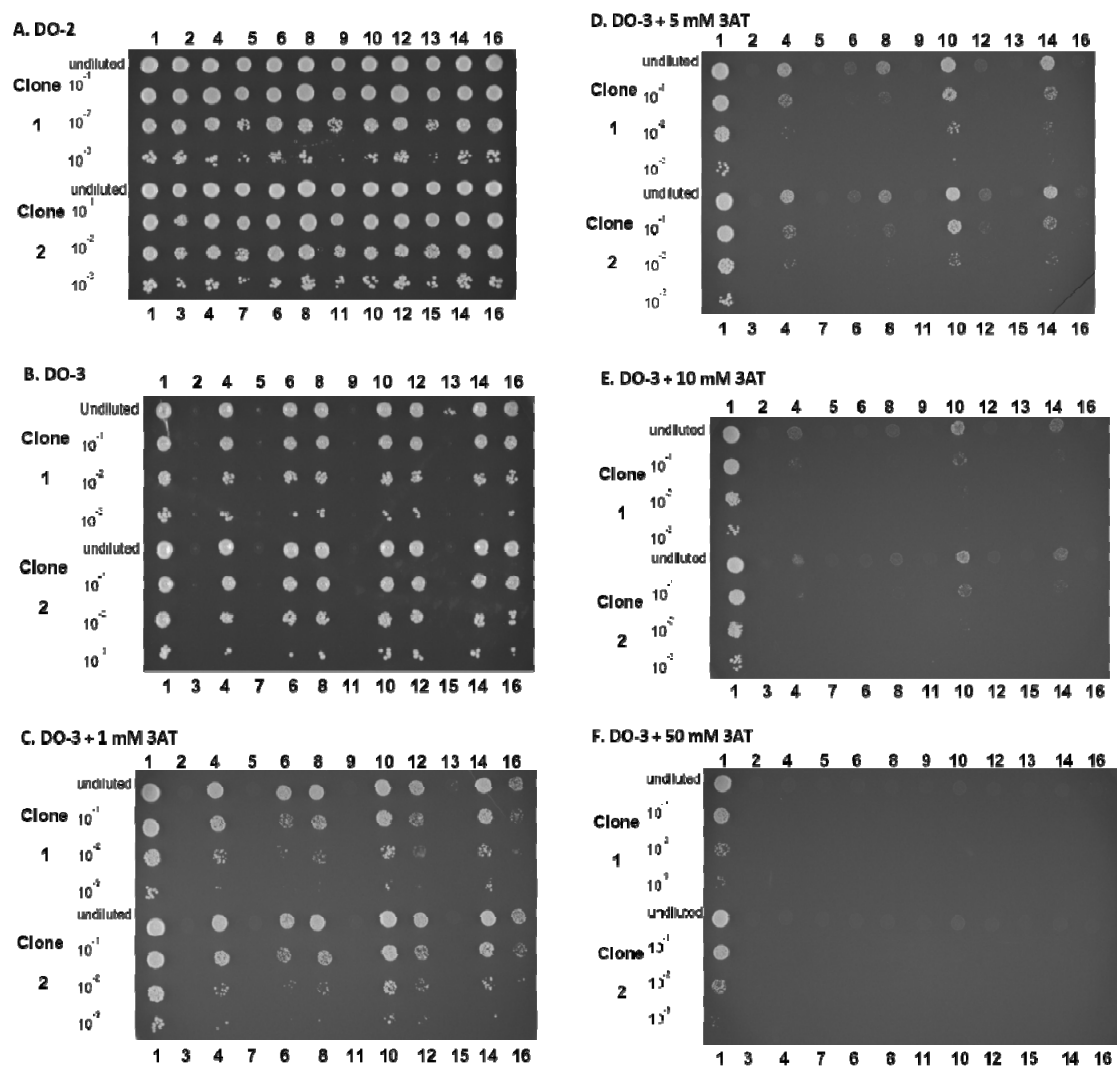


Figure S11 Solid growth tests on +/- Histidine and +/- 3-AT plates. **A** DO-2, **B** DO-3, **C** DO-3 + 1 mM AT, **D** DO-3 + 5 mM AT, **E** DO-3 + 10 mM AT, and **F** DO-3 + 50 mM AT. Detailed information of the interacting #1-16 are listed in Supplementary Table 8.

DomSight index

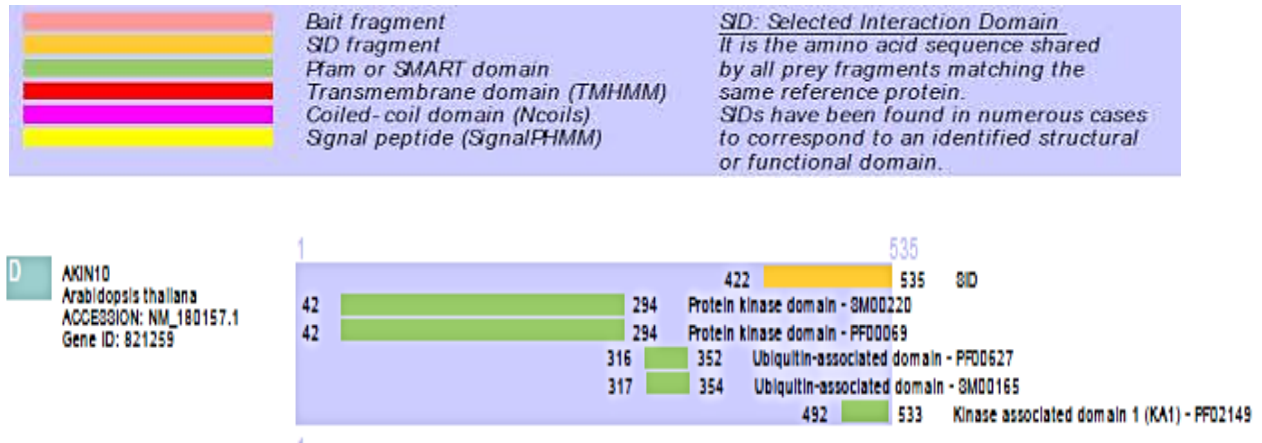


Figure S12 A “DomSight” of AKIN10 (AT3G01090) displays the information of bait and prey structural, functional and interaction domains. The SID domain (amino acid residues 422-535) contains kinase associated domain 1 (KA1).