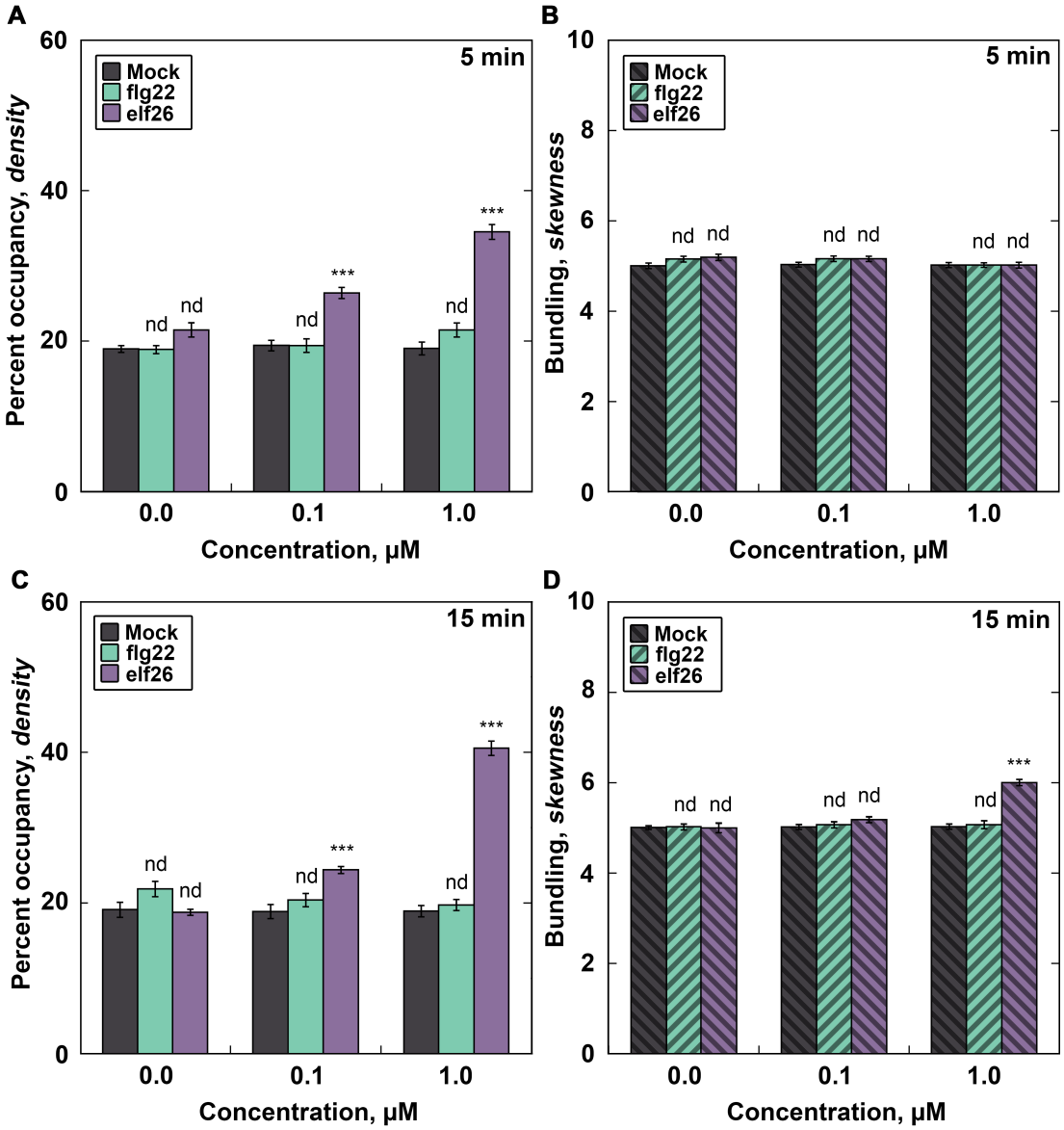
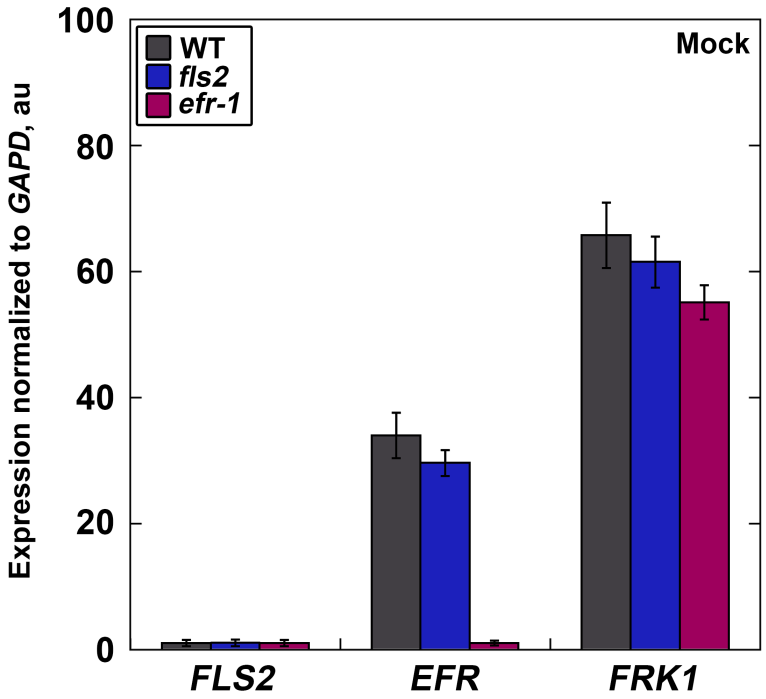


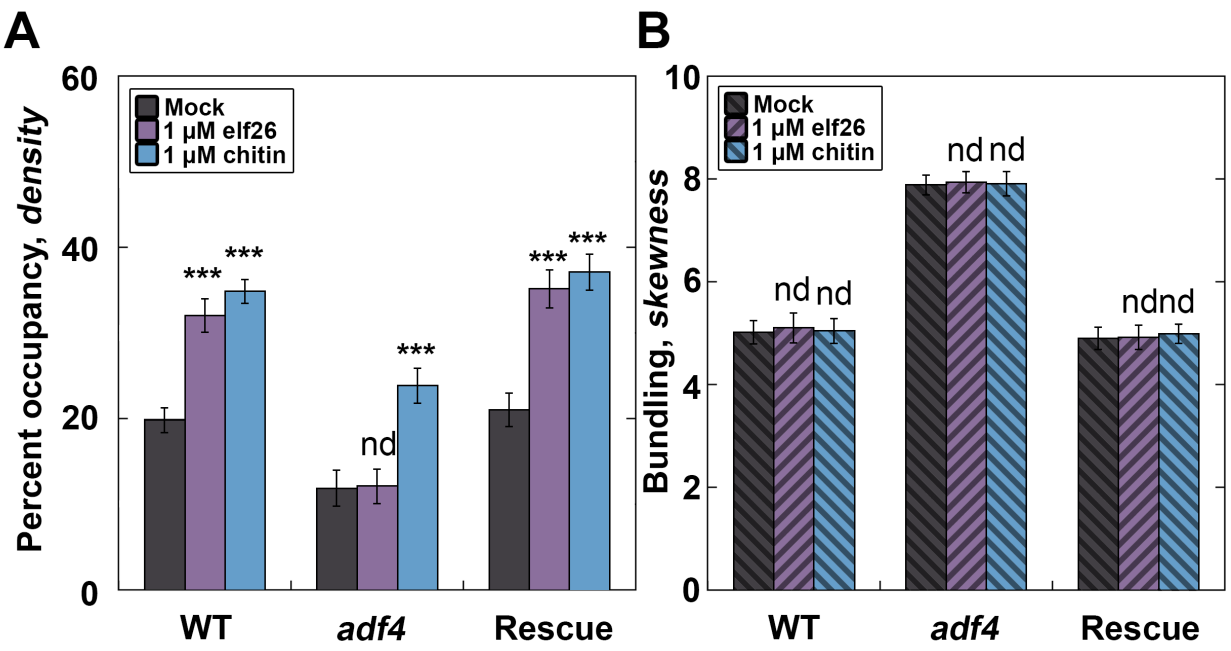
Supplemental Figure 1. Dose-dependent changes in cortical actin arrays are elicited by elf26 treatment.

(A) Actin filament density was enhanced in a dose-dependent fashion. Hypocotyls were treated with elf26 peptide at various concentrations for 5 min, and actin filament abundance was measured in epidermal cells from the base of the hypocotyl. Cells treated with 0.1 or 1 μM elf26 had significantly increased actin filament density. **(B)** The extent of actin filament bundling was not significantly different from mock for any treatment investigated. The same images analyzed in **(A)** were measured for actin filament bundling. **(C)** Actin filament density in epidermal cells from the base of the hypocotyl, treated with 0.1 or 1 μM elf26, was significantly increased 15 min after treatment compared to mock control. **(D)** The extent of actin filament bundling was only elevated after 15 min of treatment with 1 μM elf26 peptide, compared to mock. Treatment with flg22 peptide did not elicit any measurable changes to either actin architecture parameter at any concentration tested. Values given are means ± SEM (*n* = 300 cells per concentration and treatment from at least 30 hypocotyls). Asterisks represent significant differences by ANOVA, with Tukey HSD post-hoc analysis (nd = not significantly different from mock; *** = $P < 0.001$).

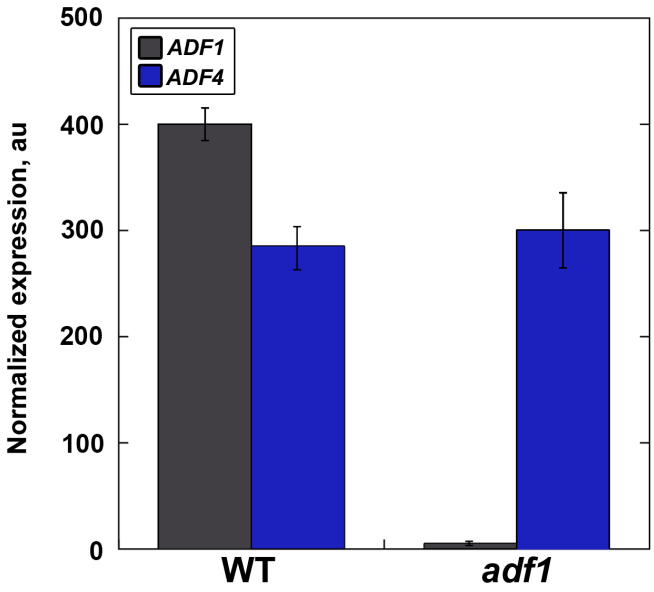


Supplemental Figure 2. *FLAGELLIN SENSING2* is not expressed in dark-grown *Arabidopsis* seedlings.

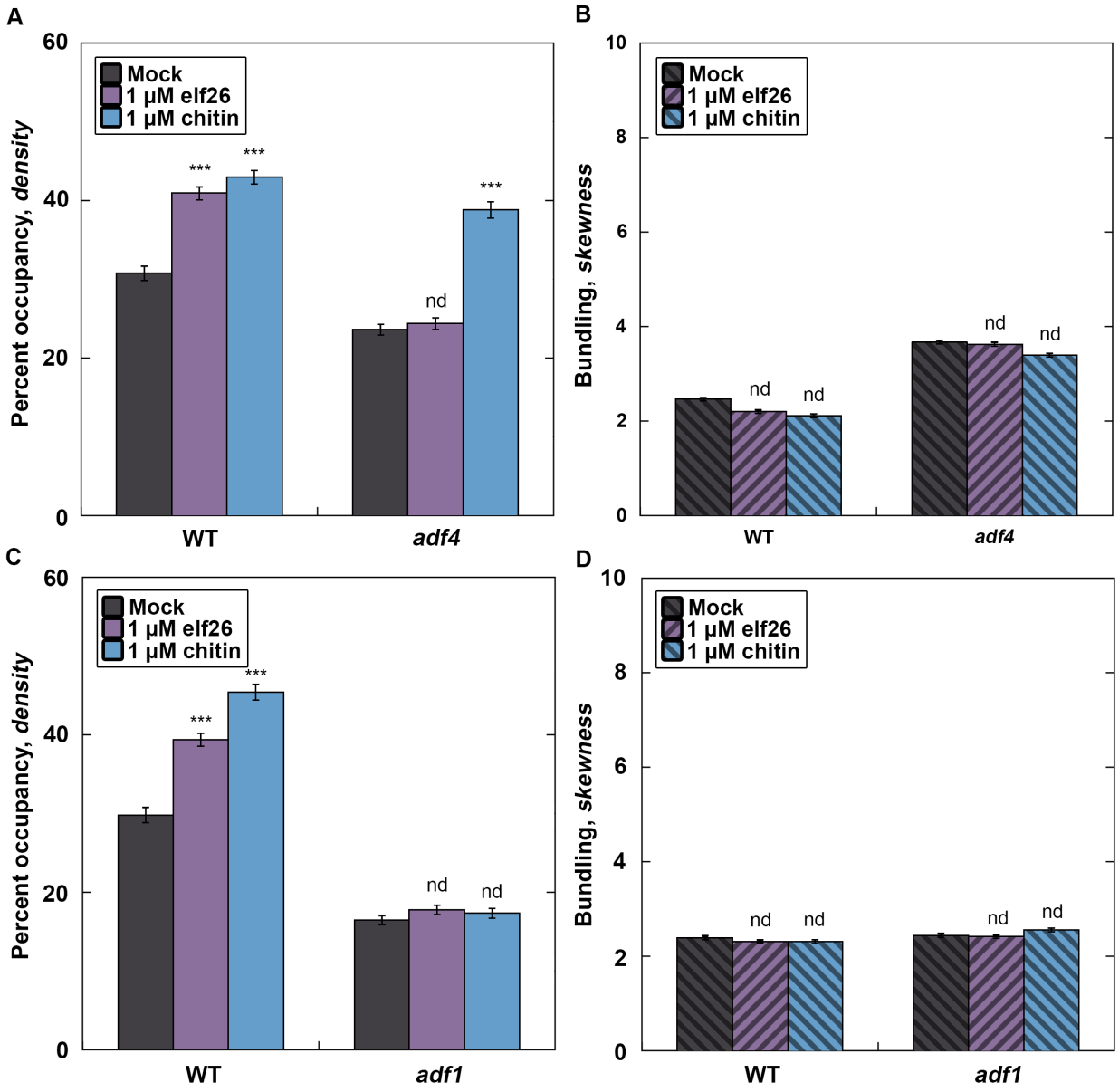
Real-time quantitative PCR (RT-qPCR) analyses of gene transcripts in wild-type (WT) and several *Arabidopsis* knockout mutants, *fls2 efr-1,* and *adf4*. Expression was confirmed by RT-qPCR on 5-days-after-germination, dark-grown seedlings. *FLS2* expression was absent in the *fls2* knockout mutant, as well as all other genotypes grown in the dark. Additionally, *EFR* transcripts were absent in the *efr-1* knockout mutant. Expression of *FRK1* and the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPD*), was absent from controls lacking reverse transcriptase (not shown). Mean values from triplicate biological samples and technical replications are plotted ± SD, normalized to GAPD expression. (au, arbitrary units)



Supplemental Figure 3. A 35S:*ADF4*;*adf4* rescue line responds to elf26 treatment.
(A) Actin filament abundance, or percent occupancy, was measured at the base of 5 days-after-germination hypocotyls. Epidermal cells from WT and the rescue line responded to treatment with 1 µM elf26 or 1 µM chitin, whereas the *adf4* mutant did not respond to elf26 treatment but did respond to chitin. **(B)** The extent of actin filament bundling was measured from the same images used in **(A).** There was no significant difference between treatments with flg22, elf26, or mock. Values given are means ± SEM (n = 100 cells per treatment, from at least 25 hypocotyls). Asterisks represent significant differences by ANOVA, with Tukey HSD post-hoc analysis ($P < 0.001$; nd = not significantly different from mock).



Supplemental Figure 4. The homozygous *adf1* mutant is a knockout line.
Quantitative real-time PCR (qRT-PCR) analysis of *ADF* transcripts in wild-type, and *adf1* homozygous mutant lines. Expression of *ADF1* and *ADF4* transcripts was confirmed by qRT-PCR on 5 DAG etiolated WT seedlings. *ADF1* was absent from the *adf1* knockout mutant, whereas *ADF4* is expressed at WT levels. Expression was normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) and transcripts were not detected in controls lacking reverse-transcriptase (not shown). Quadruple biological and triplicate technical replications are plotted ± SEM.



Supplemental Figure 5. The *adf1* mutant fails to respond to elf26 or chitin treatment.
(A, C) Quantitative values for actin filament density were measured following treatment with mock, 1 µM elf26 or 1 µM chitin. Epidermal cells from WT hypocotyls responded to elf26 or chitin treatment with an increase in actin filament abundance **(A, C)**. An increase in actin filament density occurred following chitin treatment of the *adf4* mutant; however, there was no change to filament abundance following treatment with elf26 **(A)**. In contrast, the *adf1* mutant did not respond with an increase in filament abundance following treatment with either MAMP **(C)**. **(B, D)** The extent of actin filament bundling was measured in the *adf4* **(B)** and *adf1* mutants **(D)**; however, there were no significant differences among treatments with mock, elf26 or chitin. Images of epidermal cells located at the base of 5 DAG hypocotyls were obtained from either *adf4* or *adf1* homozygous mutants or their WT siblings. Values given are means ± SEM (n = 200 cells from 10 hypocotyls per treatment and genotype). Asterisks represent significant differences by ANOVA, with Tukey HSD post-hoc analysis ($P < 0.001$; nd = not significantly different from mock).

Supplemental Table 1. Actin-dynamics parameters from mock and elf26-treated *adf4* hypocotyl epidermal cells

Stochastic Dynamics Parameters		WT + Mock	WT + 1 µM elf26	*adf4* + Mock	*adf4* + 1 µM elf26
Elongation rate; µm s^{-1}		1.7 ± 0.1‡	1.8 ± 0.1[nd]	1.7 ± 0.1[nd]	1.8 ± 0.1[nd,nd]
Severing frequency; breaks $\mu m^{-1}s^{-1}$		0.015 ± 0.002	0.009 ± 0.001[c]	0.007 ± 0.001[c]	0.005 ± 0.001[c,nd]
Max. filament length; µm		13.6 ± 0.6	18.4 ± 0.6[c]	17.4 ± 0.7[c]	19.1 ± 0.6[c,nd]
Max. filament lifetime; s		20 ± 1	26 ± 1[c]	25 ± 1[b]	26 ± 2[c,nd]
Re-growth of severed ends; %		2.5 ± 0.4	2.5 ± 0.5[nd]	2.6 ± 0.5[nd]	2.6 ± 0.5[nd,nd]
Annealing of severed ends; %		2.2 ± 0.5	8.9 ± 0.9[c]	5.3 ± 0.9[a]	8.8 ± 0.9[c,d]
Filament origin; % per cell:	*de novo*	34.3 ± 0.4	28.7 ± 0.4[b]	32.0 ± 0.4[nd]	23.0 ± 0.4[c,e]
	ends	21.0 ± 0.3	20.3 ± 0.4[nd]	21.7 ± 0.4[nd]	19.7 ± 0.3[nd,nd]
	side	44.7 ± 0.4	50.7 ± 0.4[a]	46.3 ± 0.5[nd]	57.3 ± 0.6[c,e]

‡ Values given are means ± SEM, with n > 50 filaments from n > 30 epidermal cells and at least 10 hypocotyls per line.
[nd] Not significantly different from mock control value by Student's *t* test; *P*-value > 0.05.
[a] Significantly different from WT + mock control value by Student's *t*-test; *P*-value ≤ 0.05.
[b] Significantly different from WT + mock control value by Student's *t*-test; *P*-value ≤ 0.01.
[c] Significantly different from WT + mock control value by Student's *t*-test; *P*-value ≤ 0.001.
[d] Significantly different from *adf4* + mock control value by Student's *t*-test; *P*-value ≤ 0.05.
[e] Significantly different from *adf4* + mock control value by Student's *t*-test; *P*-value ≤ 0.001.

Supplemental Table 2. Gene-specific primers used for real-time quantitative PCR

Gene	Forward Primer	Reverse primer
GAPD	5'-CACTTGAAGGGTGGTGCCAAG-3'	5'-CCTGTTGTCGCCAACGAAGTC-3'
FLS2	5'-TTGTCCACGTAAGATGTTCCAG-3'	5'-TTGCAGCGAAGTCACATATTG-3'
EFR	5'-TGGAAATAACTCGTCCAGTGG-3'	5'-CAGATGGGTTACCATCACTGG-3'
FRK1	5'-GGGTCAGATTTCAACAGTTGTC-3'	5'-AATAGCAGGTTGGCCTGTAATC-3'
WRKY33	5'-GTGATATTGACATTCTTGACGA-3'	5'-GATGGTTGTGCACTTGTAGTA-3'
PHI1	5'-TTGGTTTAGACGGGATGGTG-3'	5'-ACTCCAGTACAAGCCGATCC-3'
NHL10	5'-TTCCTGTCCGTAACCCAAAC-3'	5'-CCCTCGTAGTAGGCATGAGC-3'
CYP81F2	5'-AAATGGAGAGAGCAACACAATG-3'	5'-ATCGCCCATTCCAATGTTAC-3'