Abscisic acid-induced degradation of *Arabidopsis* guanine nucleotide exchange factor requires calcium-dependent protein kinases

Zixing Li, Yohei Takahashi, Alexander Scavo, Benjamin Brandt[#], Desiree Nguyen,

Philippe Rieu, Julian I. Schroeder[*]

Division of Biological Sciences
Cell and Developmental Biology Section
University of California, San Diego
La Jolla, CA 92093-0116

Corresponding author: Julian I. Schroeder

Email: jischroeder(at)ucsd.edu

www.pnas.org/cgi/doi/10.1073/pnas.1719659115

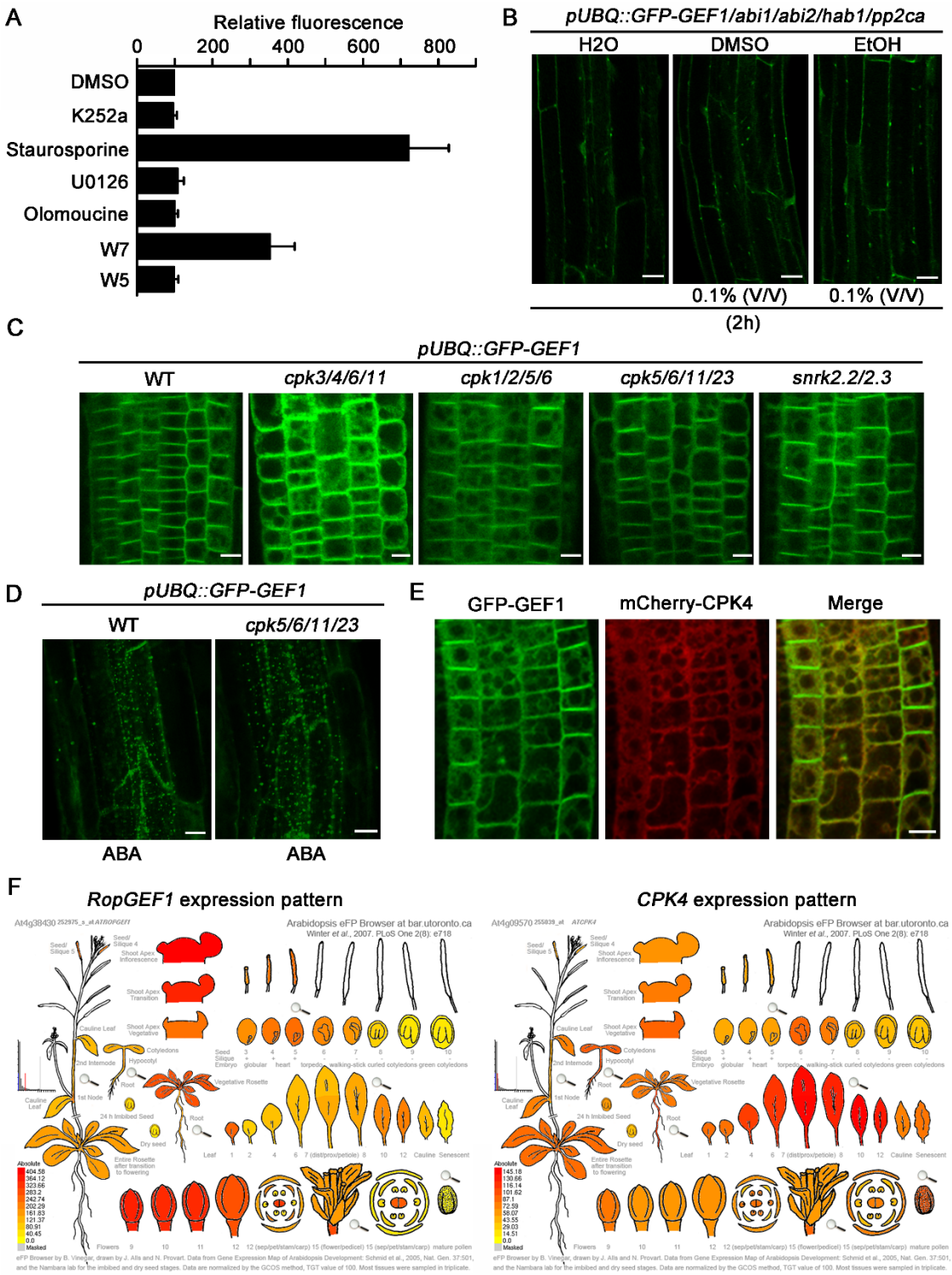
A
Relative fluorescence
0
200
400
600
800
DMSO
K252a
Staurosporine
U0126
Olomoucine
W7
W5
B
pUBQ::GFP-GEF1/abi1/abi2/hab1/pp2ca
H2O
DMSO
EtOH
0.1% (V/V)
0.1% (V/V)
(2h)
C
pUBQ::GFP-GEF1
WT
cpk3/4/6/11
cpk1/2/5/6
cpk5/6/11/23
snrk2.2/2.3
D
pUBQ::GFP-GEF1
WT
cpk5/6/11/23
ABA
ABA
E
GFP-GEF1
mCherry-CPK4
Merge
F
RopGEF1 expression pattern
CPK4 expression pattern
Arabidopsis eFP Browser at bar.utoronto.ca
Winter et al., 2007. PLoS One 2(8): e718

Fig. S1. Subcellular localization of GFP-GEF1. (*A*) Quantification of relative fluorescence intensities in Fig 1A (relative to that of DMSO treatment). Confocal parameters: Zeiss LSM 710 (objective: 20x; laser: 488; pinhole: 90 μm; digital gain: 1; channel: 8 bit; average: line 4; zoom: 1; master gain: 980). Data represent mean ±SD of three replicates. 5 cells in root mature region were analyzed in each replicate for each treatment. Average fluorescence intensity of each cell was calculated with Image J software. (*B*) GFP fluorescence in root epidermal cells of 5-day-old plants expressing *GFP-GEF1* in the *abi1/abi2/hab1/pp2ca* quadruple mutant background, treated with the indicated solvents for two hours. (*C*) Expression of GFP-GEF1 in the indicated mutant backgrounds. Scale bars =10 μm. (*D*) Subcellular localization of GFP-GEF1 after 1 hour ABA exposure in the indicated mutant backgrounds. Scale bar = 10 μm. (*E*) Co-localization assay of CPK4 and GEF1 in *Arabidopsis* root tissue. Scale bar = 10 μm. (*F*) Expression patterns of CPK4 and GEF1 analyzed through eFP browser data sets at bar.utoronto.ca (1, 2).

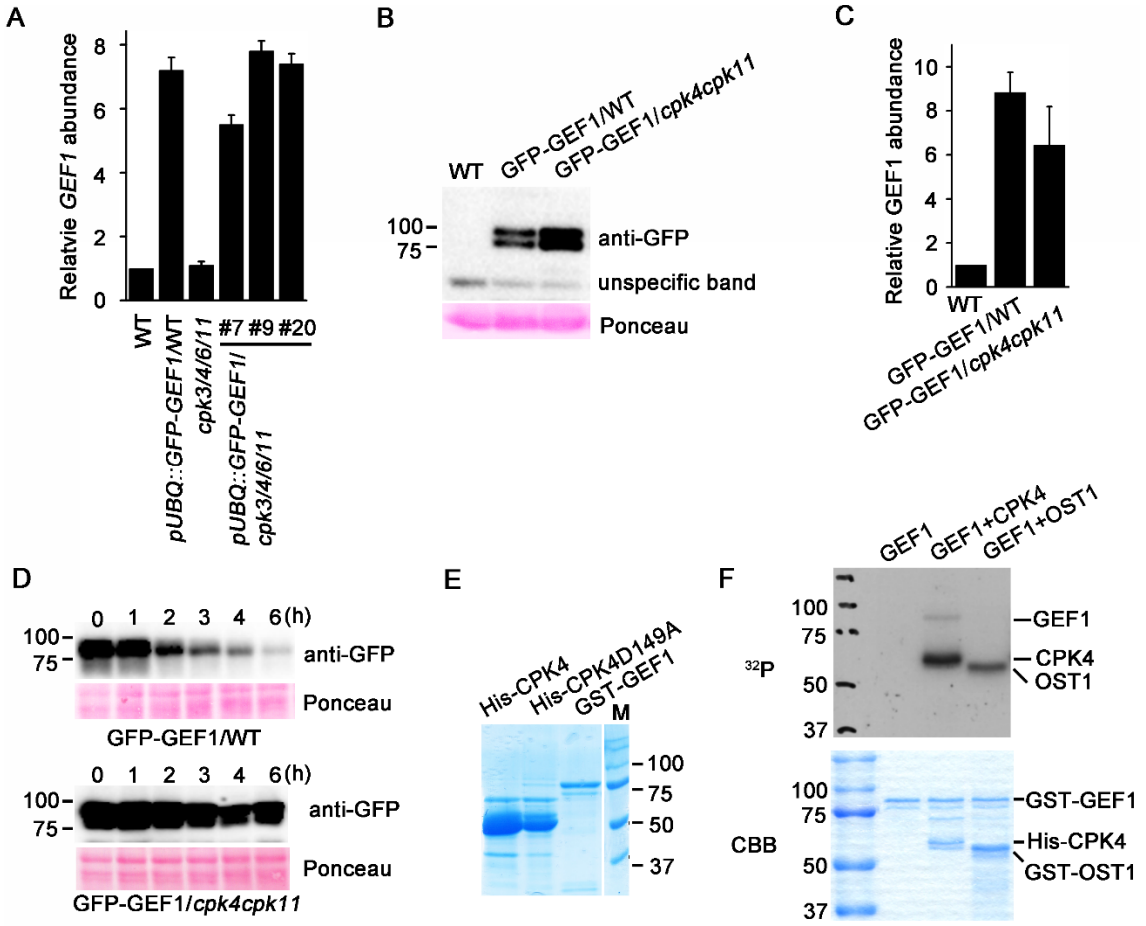

Fig. S2. (*A*) RT-qPCR analysis of transcript levels of GEF1 in the indicated genotypes. The values represent the mean ±SD (n=3). (*B*) Immunoblot analysis of GFP-GEF1 protein in 10-day-old *Arabidopsis* seedlings expressing *GFP-GEF1* in the indicated genotype backgrounds. Total protein extracts (30 μg) were subjected to immunoblot analysis with GFP antibody. Ponceau staining of PVDF membrane was used as a loading control. (n=2). (*C*) RT-qPCR analysis of transcript levels of *GEF1* in the indicated genotypes. The values represent the mean ±SD (n=3). (*D*) Immunoblot analyses of GFP-GEF1 protein abundance in response to ABA treatment for the indicated times. 10-day-old *Arabidopsis* seedlings were immersed in 1/2 MS liquid medium for 1h then transferred into 1/2MS medium supplemented with 50 μM ABA and treated for the indicated times. (n=2). (*E)* SDS-PAGE gel showing the purified His-CPK4, His-CPK4-D149A and GST-GEF1 protein. M: Molecular weight markers. (*F)* Phosphorylation assay of GEF1 by CPK4 and OST1. Approximately 2 μg of His-CPK4, GST-OST1 and GEF1 respectively were mixed, kinase reactions were carried out at room temperature for 1h

(n=4).

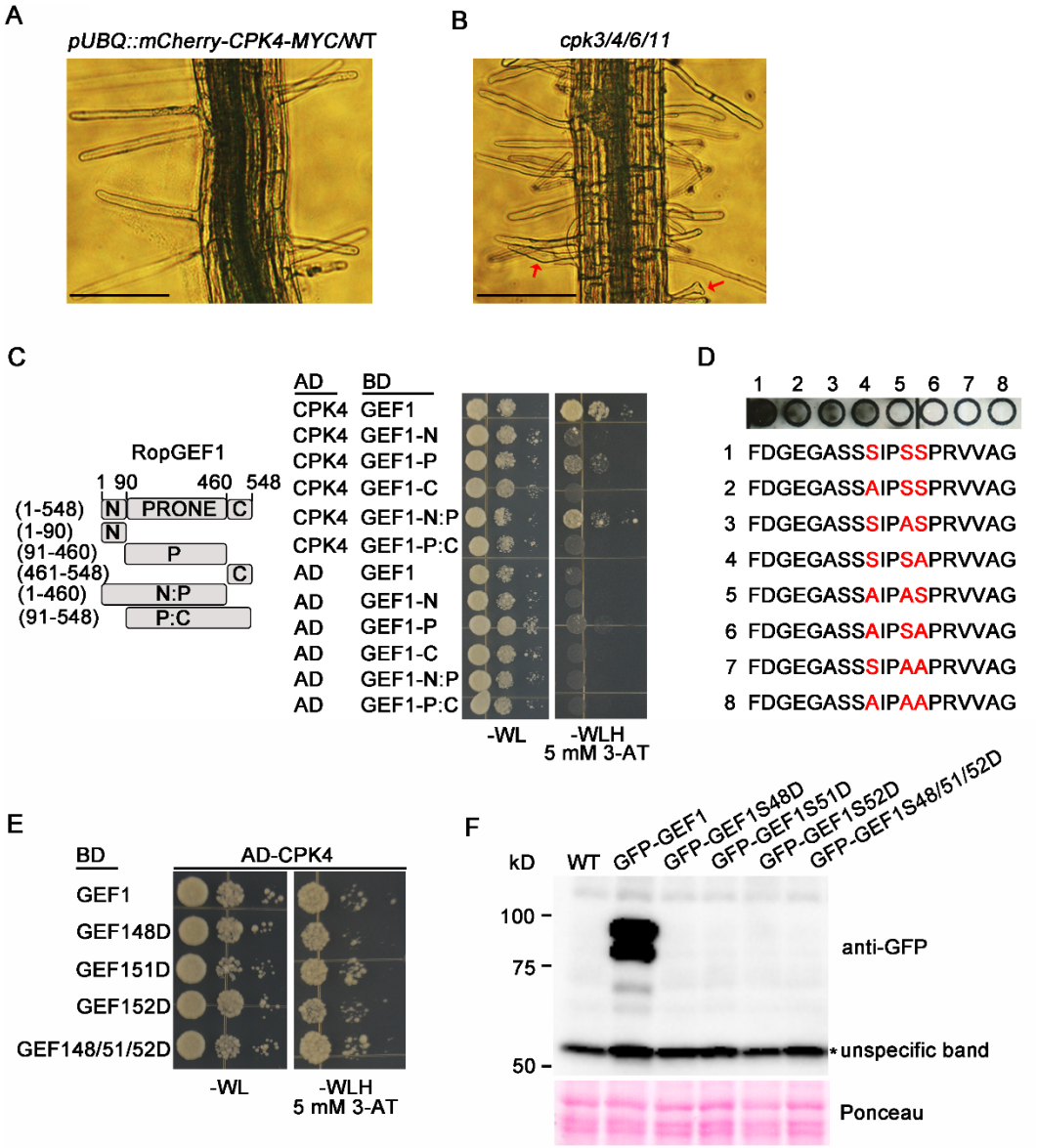

Fig. S3. (*A*) Root hair phenotype in CPK4 overexpression plants in wild type (Col-0) background. *(B)* Root hair phenotype in *cpk3/4/6/11* quadruple mutant plants. Red arrows point to root hairs showing defective development. Scale bar = 100 µm. (*C*) Yeast-2-hybrid interaction assays of the indicated domains of GEF1 with CPK4. (*D*) *In vitro* kinase assay using His-CPK4 and synthetic peptides. After 30 minutes kinase treatment, blots were stripped with 8M urea to remove residual stickiness and phosphorylated peptides were detected with the phospho-Ser/Thr antibody. (*E*) Yeast-2-hybrid assays of the interaction between CPK4 and GEF1 or GEF1 phosphomimic variants. (*F*) Immunoblot analysis of GFP-GEF1 protein in 10-day-old Arabidopsis seedlings

expressing GFP-GEF1 or GFP-GEF1 phosphomimic variants in wild type plants. (n=2).

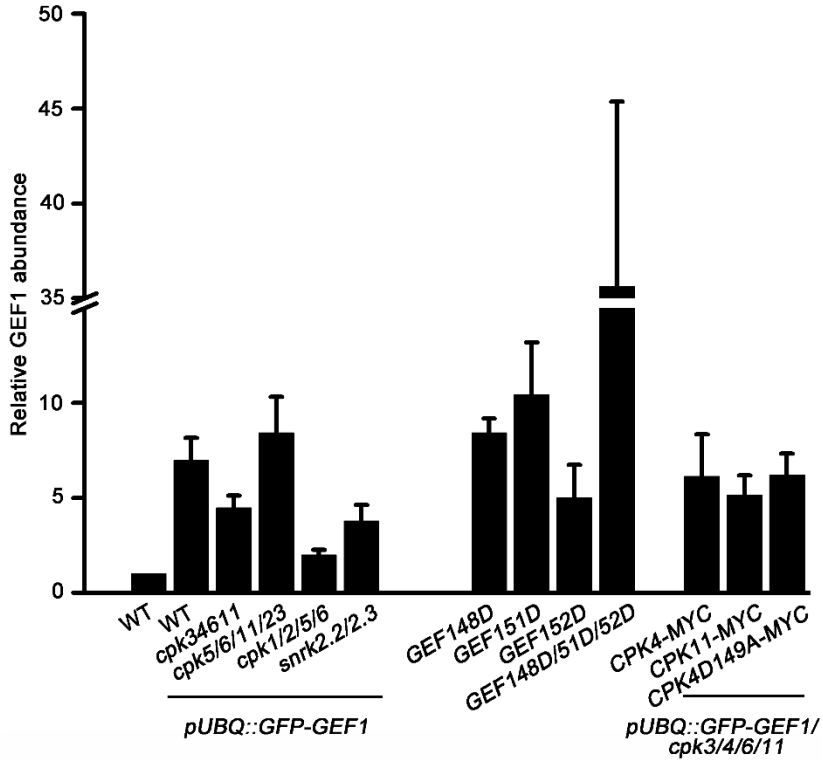

Fig. S4. RT-qPCR analysis of transcript levels of *GEF1* in the indicated genotypes. The values represent the mean relative to the wild type *GEF1* transcript ±s.d. (n=3).

References

1. Schmid M, *et al.* (2005) A gene expression map of Arabidopsis thaliana development. *Nat Genet* 37(5):501-506.
2. Winter D, *et al.* (2007) An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. *PLoS One* 2(8):e718.