Supplemental data

Material and Methods

Plant growth conditions

Surface-sterilized seeds were plated on growth medium (GM) containing Murashige and Skoog basal salts, 1.0% (w/v) sucrose, 0.05% (w/v) Mes (pH 5.7) and 0.8% (w/v) Bact Agar (BECTON, DICKINSON). Seeds were then transferred to a growth room at 22°C under continuous white light (20-50 μ mol/m2/second).

Plasmid construction and histochemical staining for GUS

For construction of each *pACAP::GUS* plasmid, the 2 kb upstream sequence of each *ACAP* transcriptional start site was amplified from *Arabidopsis* ecotype Columbia genomic DNA by PCR with gene-specific primer sets. They were subcloned into pENTR/D/TOPO vector (Invitrogen), and then integrated into the pBGGUS binary vectors (Kubo et al., 2005). The resulting plasmids were transformed into *Arabidopsis* ecotype Columbia by the floral dip method. Histochemical GUS staining was performed with seedlings of the F2 generation or embryos of the F3 generation. Samples were fixed in 90% (v/v) acetone for longer than 45 minutes at -20°C.After washing in 100

mM sodium phosphate buffer pH 7.2 at least 3 times, they were immersed in a reaction mixture containing 1 mM 5-bromo-4-chloro-3-indolyl glucuronide, 0.5 mM potassium ferricyanide, and 0.5 mM potassium ferrocyanide in 100 mM sodium phosphate buffer pH 7.2 and incubated at 37°C for 2 hours (*pVAN3::GUS*) or 24 hours (*pVAL1*,*2*,*3::GUS*) in the dark. After the reaction, samples were mounted with a mixture of chloral hydrate, glycerol, and water and observed under a light microscope equipped with a Nomarski optics.

Plasmid construction and transient expression

cDNAs for *Arabidopsis* ACAPs were isolated by RT-PCR using total RNA prepared from seedlings of *Arabidopsis* Columbia ecotype with gene-specific primer sets. Open reading frames (ORFs) of *ACAPs* were fused to the ORF of *sGFP* (provided by Yasuo Niwa of Schizuoka University) or *VENUS* (provided by Atsushi Miyawaki of RIKEN Brain Science Institute) in the directions of 5'-*ACAP*-*XFP*-3' and subcloned into the expression vector which is derived from pUC18 and contains the *CaMV 35S* promoter and the *Nos* terminator. *35S::ARA7-mRFP* (Ueda et al., 2004), 35S:: *mRFP-ARA6* (Ueda et al., 2004), *35S::mRFP-SYP41*(Uemura et al., 2004), and *35S:: ST-mRFP* (provided by Keiko Shoda of RIKEN Discovery Research Institute) were used as intracellular markers. Double transient expression of *35S::ACAP-XFP* and of intracellular markers in the protoplasts of *Arabidopsis* cultured suspension cells were analyzed as described by Ueda et al. (Ueda et al., 2001).

For double labeling with sGFP and mRFP, fluorescence was observed with Zeiss confocal laser scanning microscope, LSM510 (Carl Zeiss Co., Ltd, Tokyo, Japan). Cells expressing only sGFP or VENUS tagged ACAP was observed with a confocal laser microscope system: Olympus BX52 fluorescence microscope equipped with CSU10 and a EM CCD camera, iXon (ANDOR).