Supplementary Methods

Plant growth conditions. Surface-sterilized seeds were plated on growth medium containing Murashige and Skoog basal salts, 1% (w/v) sucrose, 0.05% (w/v) MES (pH 5.7) and 1.5% (w/v) agar. Seeds were then transferred to a growth room at 22°C under continuous white light (20-50 mmol m⁻²s⁻¹).

Enhancer screening. clen1 was isolated from 16,000 ethylmethane-sulfonate (EMS)-mutagenized clv2-101 mutant seeds. From these seeds, 48 candidates showing enlarged SAMs and strong resistance to CLV3 and CLE25 treatment, which strongly inhibits root growth, were isolated.

Identification of the causal gene in the clen1 mutant. A combined approach using next-generation sequencing and map-based cloning was applied as described previously [20]. clv2 clen1 genomic DNA was subjected to analysis using the SOLiD system to search for difference from the Col-0 reference genome. After detecting DNA substitutions, we extracted mutations in
protein-coding genes located around the mapped position. Mapping populations were generated by crossing *clen1* mutants in the Col-0 background with Landsberg *erecta* (La-er). In the F2 generation, homozygous mutants were selected for segregation analyses.

**Complementation for the *clv2 clen1* mutant.** Full-length *AGB1* cDNAs was amplified by RT-PCR using the primers *AGB1*-F1 (GGGGACAAGTTTGTACAAAAAAGCAGGCTATGTCTGTCTCCGAGCTCA AAGAACGCCA) and *AGB1*-R1 (GGGGACCACTTTGTACAAAAAGCTGGGTTCAAATCACTCTCCTGTGT CCTCCAAAC). The *AGB1* cDNA was cloned into donor vector using Gateway BP clonase (Life Technologies). Genomic *AGB1* fragment was PCR amplified from Col-0 genomic DNA by PCR using the primers attB1-AGB1-F (AAAAAGCAGGCTGCTGTTCGTAAGGAGAATCAATGGGCT) and attB2-AB1-R (AGAAAGCTGGGTGGGAAAGCATGGATGAAGAAGATGAGCG), then the
PCR fragment were subjected for the 2nd PCR as manufacturer’s instructions. The PCR product was cloned into donor vector using Gateway BP clonase (Life Technologies). The resulting entry clone was then integrated into binary vectors pGWB402 and pGWB501, respectively, using LR clonase (Life Technologies) [35]. And the resultant plasmids were used for Agrobacterium-mediated transformation of clv2 clen1.

**AGB1 overexpression analyses.**

The entry clone harboring AGB1 cDNA was integrated into the binary vector pGWB406 using LR clonase (Life Technologies) [35]. The pGWB402-AGB1 and pGWB406-AGB1 vectors were transformed into wild-type plants. After antibiotic selection, the phenotypes and GFP-signals of the plants were observed using an MZ16F dissecting microscope (Leica Microsystems) and an FV1000 multi-photon confocal microscope (Olympus), respectively.

**mRNA in situ hybridization.** *In situ* hybridization was performed according
to the method described in a previous report [36]. The \textit{GPA1, AGB1, AGG1} and \textit{AGG2} antisense probes were generated from the 1100-, 1020-, 610- and 1000-bp fragments that were amplified by PCR using the following primers:

\begin{align*}
\text{GPA1} & \quad (\text{CACCAGGCTGCTGAAATCGAAAGAC}) \\
& \quad \text{and} \quad (\text{TAAAAGGCCAGCCTCCAGTAA}) \quad \text{AGB1} \\
& \quad (\text{CACCTCCGTGACCAGCTTAGACAGA}) \\
& \quad \text{and} \quad (\text{CCTGTACACAAGGGACTTCCA}) \quad \text{AGG1} \\
& \quad (\text{CACCAGAGACTTCGACGACAATTCAA}) \\
& \quad \text{and} \quad (\text{AAGCTGTAAGCCTGAAAATCCTCA}) \quad \text{AGG2} \\
& \quad (\text{CACCTTTTTTCAGTCTCCCCCAACTCA}) \\
& \quad \text{and} \quad (\text{AGCCTCTCTCAGAGCTCACC}) \\
\end{align*}

The probes were hybridized at 45°C for \textit{AGB1} and at 42°C for \textit{GPA1, AGG1} and \textit{AGG2}.

\textbf{Bimolecular fluorescence complementation.} Entry clones containing cDNA inserts for AGB1, CLV1, CLV2 and RPK2 [37] were integrated into BiFC
vectors [38] using LR clonase (Life Technologies) and purified using the Purelink Plasmid Midiprep Kit (Life Technologies). For BiFC experiments, 5 μg of each Venus-N-terminal- and Venus-C-terminal-tagged protein expression vector was co-transformed. Five micrograms of the mCherry expression plasmid was simultaneously introduced as a marker for transformation efficiency [39]. To quantify protein-protein interactions, the fluorescence intensities of Venus (BiFC) and mCherry (transformation marker) from approximately 20 cells were measured using the same microscope settings. A reliable BiFC signal was recognized in cells showing Venus/mCherry ratios of >0.33 for CLV1-CLV2 and RPK2-AGB1 interactions. The number of cells showing ratios exceeding these values was scored [40]. The BiFC signals were observed using a Leica SP5 confocal laser scanning microscope (Leica microsystems).

**Co-immunoprecipitation.** To test the interaction between RPK2 and AGB1 *in vivo*, the entire cytoplasmic domain of RPK2, designated RPK2c, was used
for co-IP because transmembrane proteins are difficult to isolate, and this region of the receptor is expected to serve as an interaction domain for cytoplasmic signal transducers. The RPK2c fragment was PCR amplified from wild-type cDNA using the primers RPK2c-F (CACCATGGATATCGGAGTTCCAATAAC) and RPK2-R2 (ACACGACGGAGGTGTTAGCTG). The mCherry fragment was PCR amplified from the vector described before using the primers mCherry-TOPO-F (CACCATGGTGAGCAAGGGCGAGGAG) and mCherry-dSTOP-R (CTTGTACAGCTCGTCCATGC). The PCR product was then cloned using the pENTR/D-TOPO Cloning Kit (Life Technologies). Entry clones containing AGB1, RPK2c or mCherry were integrated into transient expression vectors [41] using LR clonase and purified using the Purelink Plasmid Maxiprep Kit (Life Technologies). For transient expression analysis, 60 μg of the expression vectors was introduced into 1,000 μl of a protoplast suspension at a concentration of \(2 \times 10^7\) protoplasts ml\(^{-1}\) using the PEG-mediated transformation method, as described above. Protein
extraction and immunoprecipitation using the anti-GFP antibody and the μMACS GFP isolation kit (Miltenyi Biotec) was performed as described elsewhere [41]. Frozen protoplasts were homogenized and diluted in extraction buffer (10 mM HEPES [pH 7.5], 100 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% Triton X-100, and a protease inhibitor cocktail (Roche)). After being vortexed and centrifuged, the protein extracts were mixed with 40 μl of Anti-GFP MicroBeads, then incubated for 45 min at 4°C. The Venus-bound proteins were precipitated in columns according to the manufacturer’s instructions.

**Western blotting.** Western blotting analysis was performed as described previously, with minor modifications [42]. Extracted samples were resuspended in LDS loading buffer (Life Technologies) and Sample Reducing Agent (Life Technologies) and then incubated at 98°C for 2 min. Proteins were fractionated in NuPAGE Novex 4-12% Bis-Tris gels (Life Technologies) and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad).
Precision Plus Protein All Blue Standards (Bio-Rad) were used as molecular weight markers. The membranes were blocked in TBST (10 mM Tris-Cl, pH 8.0, 150 mM NaCl and 0.05% Tween 20) containing 5% nonfat dried milk at room temperature for 1 h, followed by incubation with anti-FLAG M2-HRP (Sigma-Aldrich A8592; 1:1,000) or anti-GFP-HRP (Miltenyi Biotec 130-091-833; 1:2,500) in TBST containing 2.5% nonfat dried milk at room temperature for 1 h and three washes with TBST. The membrane was finally treated with Western BLoT Hyper HRP Substrate (TaKaRa), and luminescence was detected using the ChemiDoc XRS system (Bio-Rad).
References


