Supporting Information

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SI Materials and Methods

Plant Materials and Reporter Constructs. *clv1–11*, *clv3–2*, and *clv2–1* alleles in L-*er* background have been previously described (36, 37). The *pWOL*::*GFP* line in Columbia (Col-0) background has been previously described (38) and it recapitulates expression patterns observed in the shoot and root via in situ hybridization (30). The *pARR5*::*GFP* line in WS ecotype has been previously described (13). The *pWUS*::*GFP-ER* and *pCLV3*::*GFP-ER* lines have also been previously described (10, 34) (for details of other lines see below). The *pWUS*::*dsRED-N7* construct in the T-DNA vector pMLBART (39) conferring Basta resistance in plants is composed of 3.33 kb of upstream regulatory sequence from the *WUS* gene fused to dsRED followed by the N7 nuclear localization sequence (40) with 1.31 kb of *WUS* 3′-untranslated sequence. For double transgenic plants with various reporters, *pWUS*::*DsRed-N7* was transformed into respective backgrounds as previously described (29). Reporter lines were subsequently crossed into mutant backgrounds. Cytokinin receptor mutants in Col-0 background were genotyped as previously reported (41).

Quantitative Real-Time PCR (qRT-PCR). Quantitative real-time PCR (qRT-PCR) was performed with Roche Universal Probe Library hydrolysis probes. Each sample represents tissue harvested from 50 two-week-old seedlings just transitioned to flowering. Meristem tissue from 50 plants was harvested and pooled from seedlings after removing leaves, cotyledons and root tissue followed by liquid nitrogen flash freezing and homogenization. Relative expression by qRT-PCR was normalized to *NM_128399.2* which has been shown to be a superior reference gene for qRT-PCR analysis, constant against various treatments, including cytokinin treatment (42). Transcript abundance of this gene is 1/10 of *UBQ10* transcript levels and therefore more similar to *WUS* transcript levels. Similar trends were also observed using *UBIQUITIN 10 (42)*. All samples were run in at least triplicate. Total RNA was isolated using the RNeasy mini kit (Qiagen). RNA concentration and quality was assayed using nanodrop spectrometer (Agilent). First-strand cDNA synthesis was performed with 2 μg total RNA using SuperScript II RNase H- reverse transcriptase (Invitrogen) and 20-mer oligo dT primers according to the manufacturer's instructions. Real-time PCR amplifications were performed in triplicate in 96-well plates in a 20-μL reaction volume on a Roche LightCycler 480 system. Unlabeled gene-specific primers in combination with a gene-specific hydrolysis probe from the Roche Universal Probe Library Set were used to detect gene-specific amplification products. For *WUS* quantification, primers spanned two intron sequences which eliminated products from potential genomic DNA contamination under our PCR settings. Gene specific primers 5′-ggattttcagctactcttcaagcta-3′ and 5′- ctgccttgactaagttgacacg-3′with UPL probe 157 were used to amplify and detect *NM_128399.2*, the primers 5′-tcagagaacatcttgcctcgt-3′ and 5′-atttcacaggcttcaataagaaatc-3′ with UPL probe 17 were used to amplify *ARR5*, 5′-ggatacatcgccccagagt-3′ and 5′-tccaaattcaccaacaggttt-3′ with UPL probe 33 were used for *CLV1*, the primers 5′-aaccaagaccatcatctctatcatc-3′ and 5′- ccatcctccacctacgttgt-3′ with probe 33 were used to amplify *WUS*, and the primers 5′-gaagttcaatgtttcgtttcatgt-3′ and 5′-ggattatacaaggccccaaaa-3′ with UPL probe 119 were used to amplify and detect *UBQ10*. Error bars of real-time qRT-PCR experiments in Fig. 1 are derived from three independent biological experiments each run in triplicate, the cytokinin serial dilution curve in Fig. 2*A* which is derived from two independent biological experiments. We show the mean and SEM between respective biological replicates. Analysis and plots in Fig. 5 are derived from pooled tissue run in triplicate.

Plant Growth and Cytokinin Treatment Conditions. Plants were grown as previously described (29). Cytokinin treatments with N6-benzylaminopurine (BAP; Sigma–Aldrich Co.) were performed as described (23) except that shoots were sprayed with the respective solutions. For analysis of carpel number, plants were treated three times at 1-week intervals. *clv3–2* mutants and wild-type plants in Fig. 3*F* and Fig. S1*F*, *I*, and *K* were treated once every second day. Phenotypic analysis was performed on soil. Flowers at positions 3–20 of at least 10 plants were counted for carpel numbers of cytokinin and mock treated samples. At least two independent biological experiments were performed for each genotype. Imaging was performed as previously described (29). Membranes were stained with FM4–64 dye unless otherwise noted (29). Computational modeling is described in the Computational Modeling in *SI Appendix*.

Cycloheximide Treatments. Plants were pretreated with 10 μM cycloheximide (Sigma–Aldrich Co.) for 30 min. then treated with respective BAP or mock solutions also containing 10 μM cycloheximide and harvested 4 h after treatment.

39. Gleave AP (2002) A versatile binary vector system with a T-DNA organizational structure conducive to efficient integration of cloned DNA into the plant genome. *Plant Mol Biol* 20:1203–1207.
40. Cutler SR, Ehrhardt DW, Griffitts JS, Somerville CR (2000) Random GFP::cDNA fusions enable visualization of subcellular structures in cells of *Arabidopsis* at a high frequency. *Proc Natl Acad Sci USA* 97:3718–3723.
41. Higuchi M, et al. (2004) In planta functions of the *Arabidopsis* cytokinin receptor family. *Proc Natl Acad Sci USA* 101:8821–8826.
42. Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible W (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiol* 139:5–17.

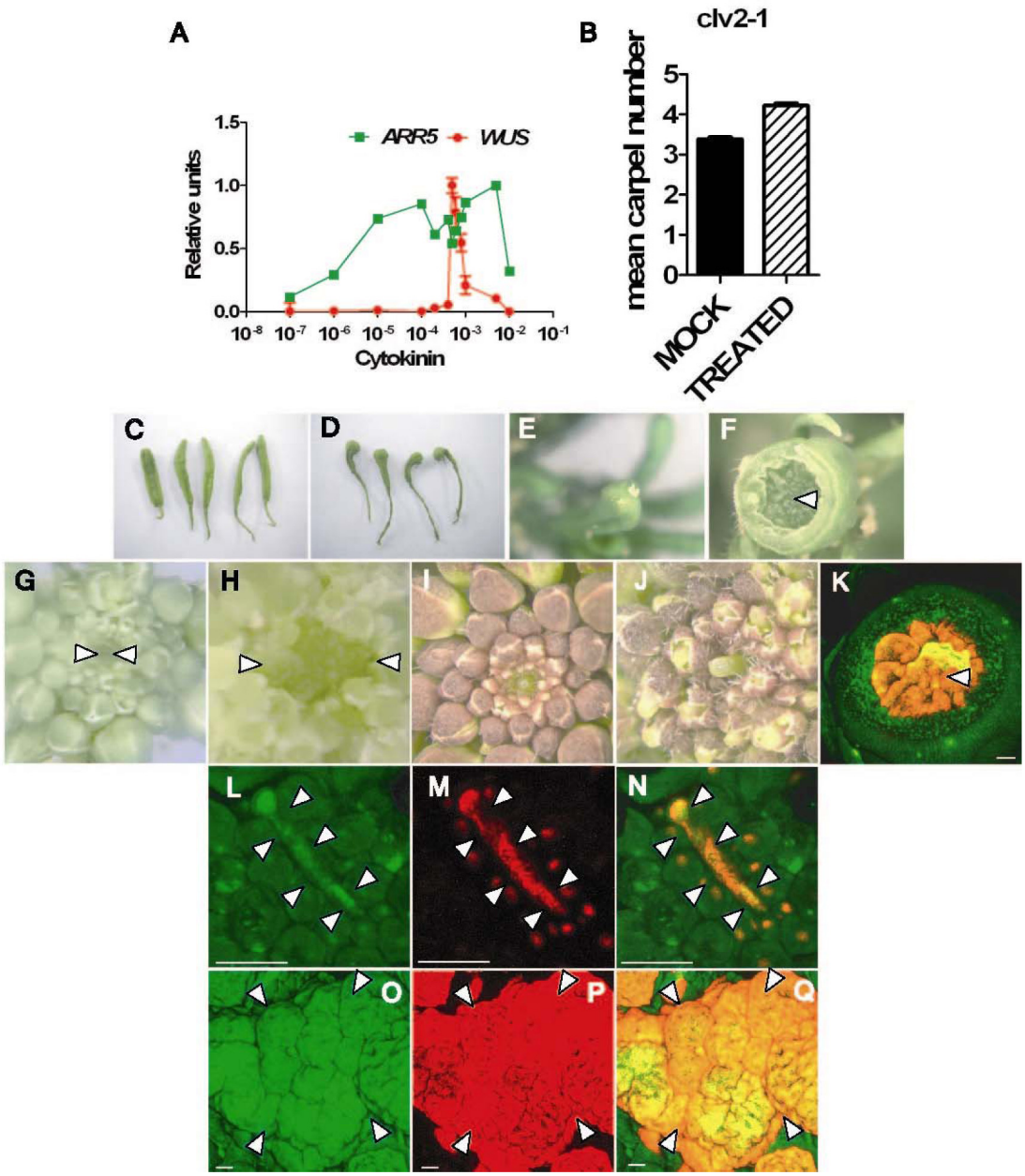

Fig. S1. (*A*) Finer serial dilutions reveal a steep rise in *WUS* transcript starting around 400 μM and peaking near 600 μM, overlapping with a dip of *ARR5* transcript. (*B*) Mean carpel numbers from either mock or cytokinin treated *clv2–1* flowers. Error bars, SEM. (C and D) Phenotypes of mock (*C*) or treated (*D*) *clv1–11* carpels. Cytokinin treatment enhanced the club shaped carpel phenotype. (*E* and *F*) *clv3–2* mock (*E*) or cytokinin-treated (*F*) flowers. Treated flowers were observed with 14 carpels with a central mass of undifferentiated tissue (arrowhead) which was never observed in mock treated flowers. (*G* and *H*) Mock (*G*) or cytokinin-treated (*H*) *clv3–2* meristems showing massive enlargement of the *clv3–2* SAM (bounded by arrows) after cytokinin treatment. (*I* and *J*) Mock (*I*) or treated (*J*) *clv1–11* meristems. Treated meristems were slightly wider (50 vs. $\approx$100 μm) and qualitatively taller than mock treated meristems. (*K*) undifferentiated tissue within the center of the gynoecium (arrowheads, Fig. S1*F*) formed after repeated treatment of *clv3–2* flowers was marked by *WUS* (red) and *AHK4* (green) reporter expression. (*L–N*) *AHK4* (green) and *WUS* (red) reporter expression in the untreated linear fasciated *clv3–2* SAM (arrows) and floral meristems or (*O–Q*) in the grossly enlarged cytokinin treated *clv3–2* SAM (arrows) and floral meristems. (Scale bars, 100 μm.)

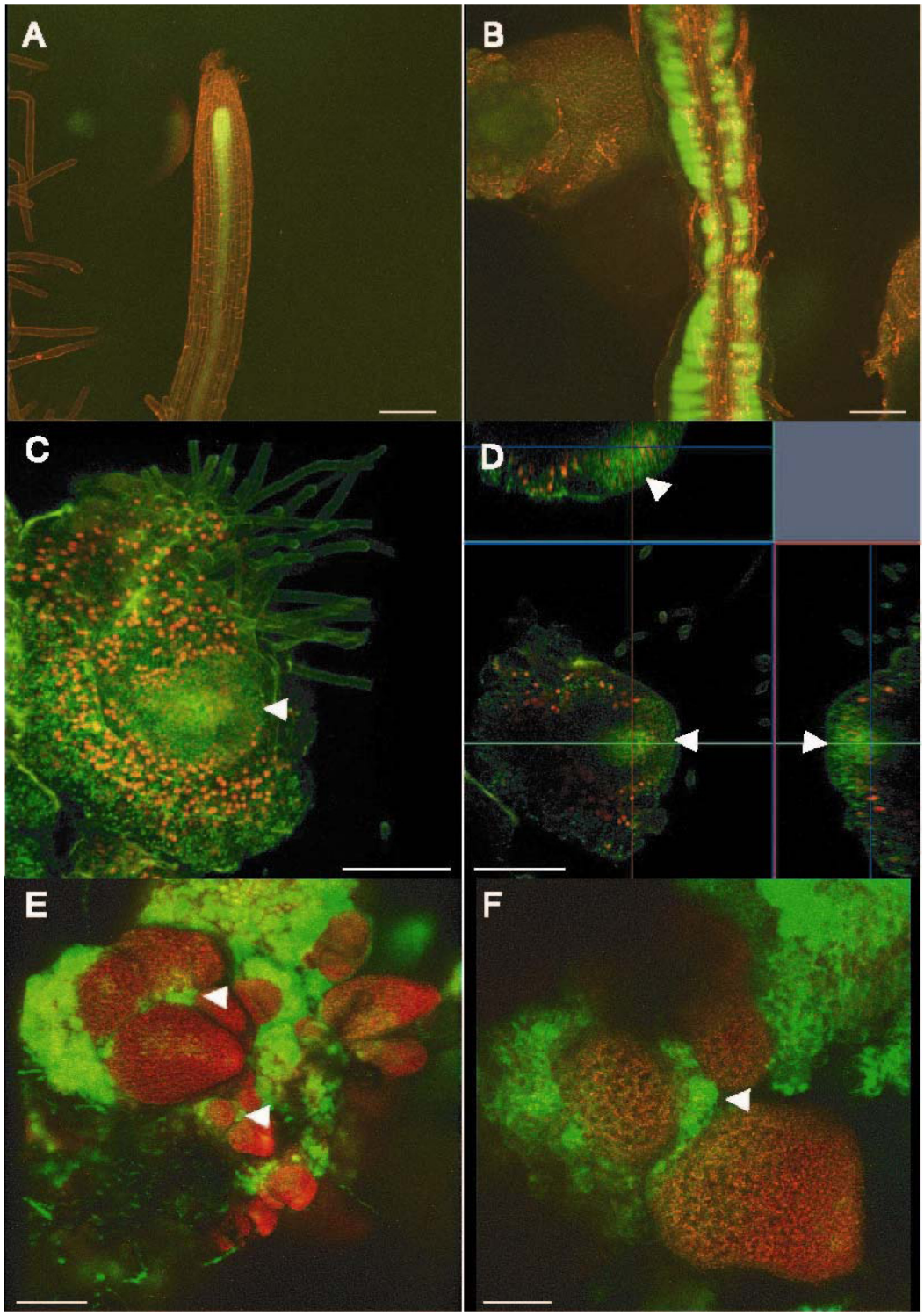

Fig. S2. (*A* and *B*) *AHK4* reporter expression in the untreated root (*A*) and proliferating cells after culture on auxin-rich medium (*B*). Receptor expression is both stronger and broader in auxin treated samples. (*C* and *D*) *AHK4* and *WUS* reporter overlap in the developing rib zone of new shoot meristems forming from callus. (*E* and *F*) *pTCS*::*GFP* report is also active in the developing RM of regenerating SAMs in culture and peripheral callus cells. Error bars, 100 μm in (*A* and *B*) 200 μm in (*E*), and 50 μm in (*C*, *D* and *F*). Arrowheads mark regenerating SAMs.

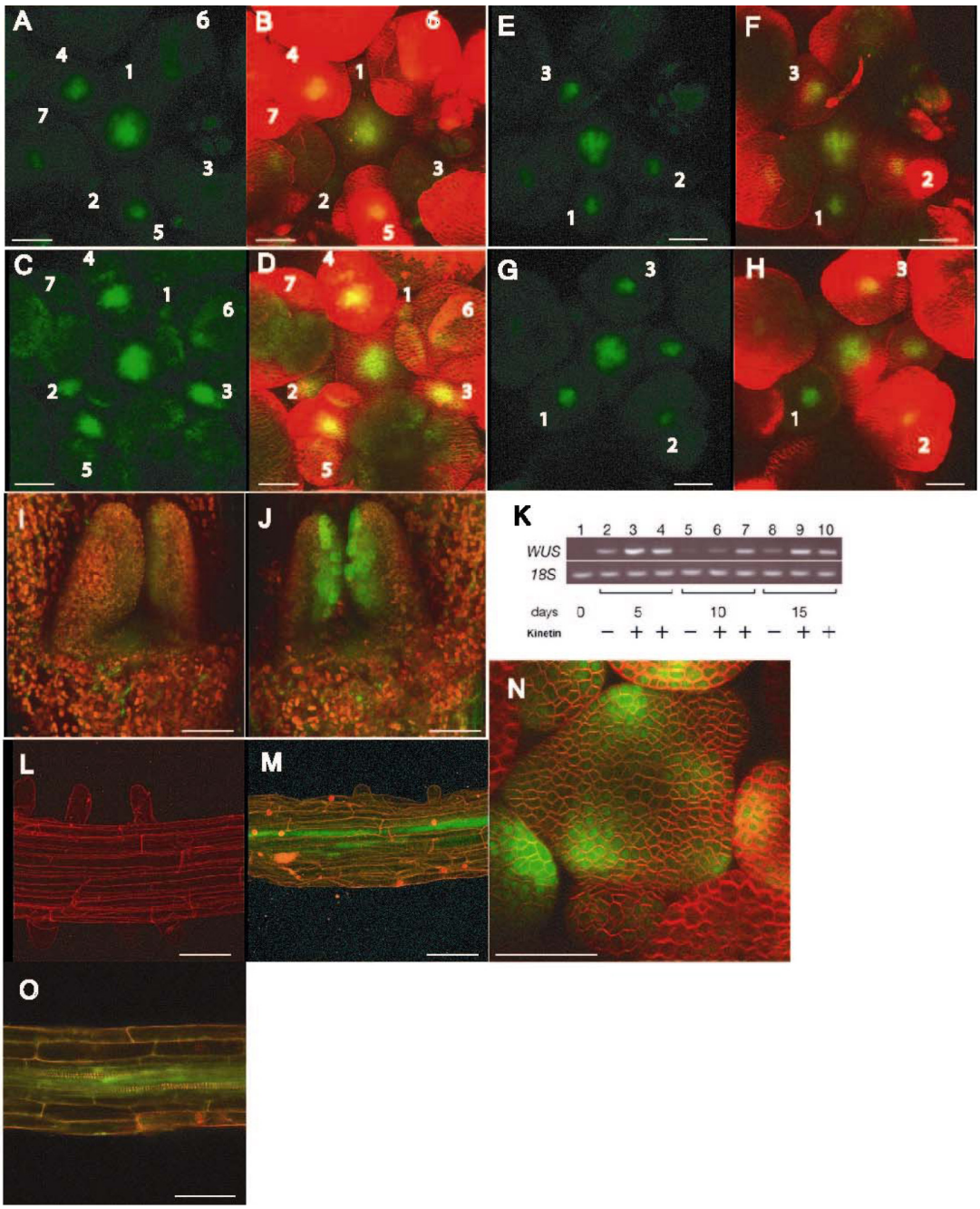

Fig. S3. (*A–D*) are representative results of live imaging experiments of *WUS* reporter (green) before (*A* and *B*) ($n = 10$) and after 24 h of cytokinin treatment (*C* and *D*) ($n = 10$). (*E–H*) are representative results of live imaging experiments of *WUS* reporter (green) before (*E* and *F*) ($n = 10$) and after 24 h of mock treatment (*G* and *H*) ($n = 10$). *WUS* expression is induced in the adaxial sides of cytokinin treated leaves (*J*) but not mock treated samples (*I*). (*K*) Cytokinin treatment induces *WUS* expression in root explants. Semiquantitative RT-PCR on root explants after 0, 5, 10, and 15 days of culture in the absence (−) or presence (+) of the cytokinin (50 μg/L kinetin). *WUS* is not expressed in root plants at 0 days of culture (lane 1) but becomes expressed after prolonged culture on MS media (lanes 2–10) and this expression is enhanced by the presence of cytokinin (lanes 3, 4, 6, 7, 9, and 10). (*L* and *M*) *WUS* reporter expression (green) in the stele of cytokinin treated root explants (*M*) but not untreated roots (*L*). (*N*) AHP6 reporter, *pAHP6*::*AHP6-GFP* (green), a component known to repress cytokinin signaling[28] is expressed in organ primordia where Type-A ARRs are down-regulated. (*O*) AHK4/CRE1 is expressed in the root stele, where ectopic *WUS* is induced after cytokinin treatment. (Scale bars, 50 μm.)

Other Supporting Information Files

SI Appendix