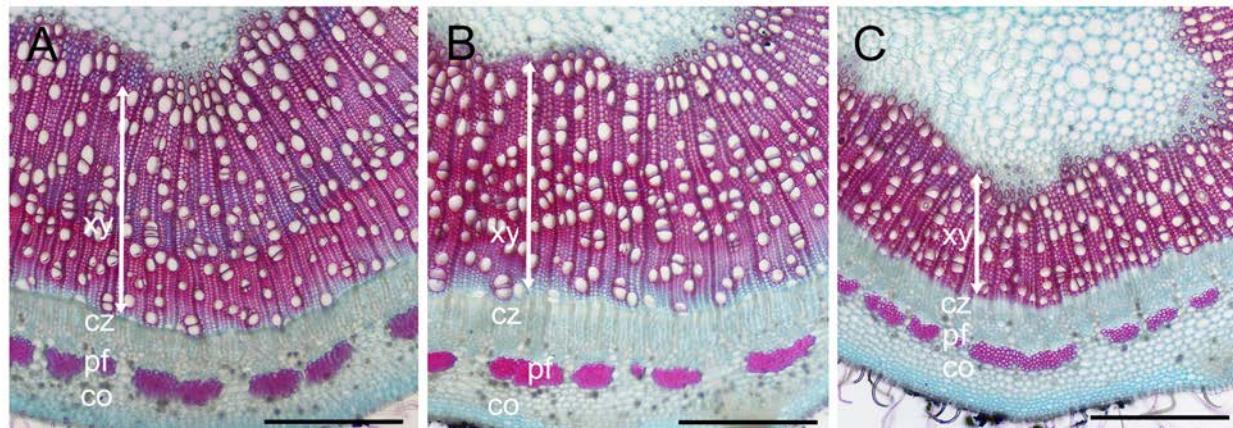
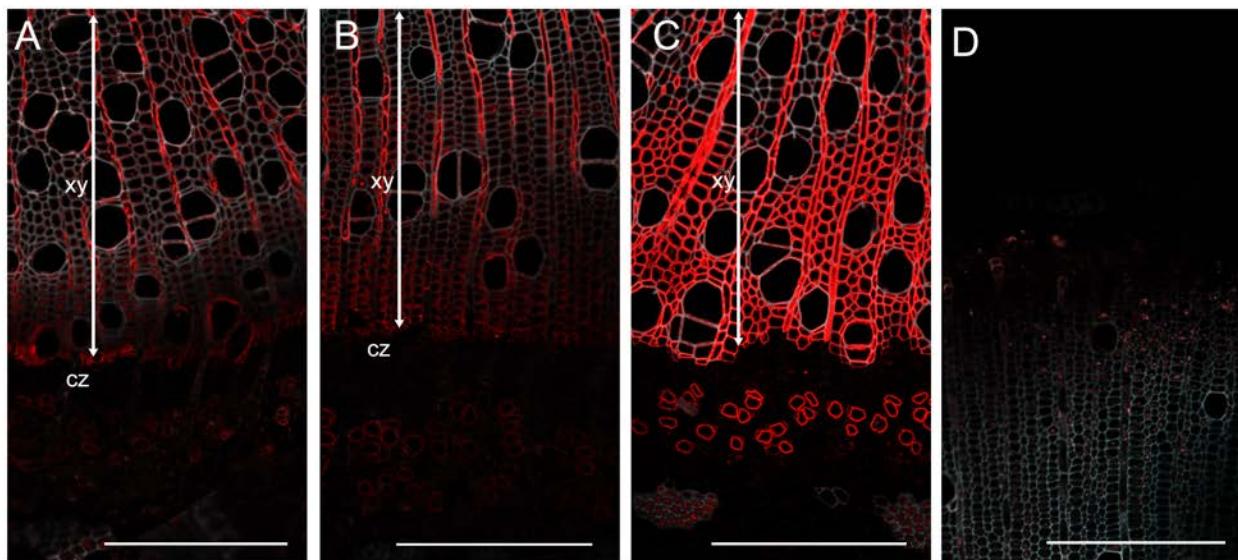


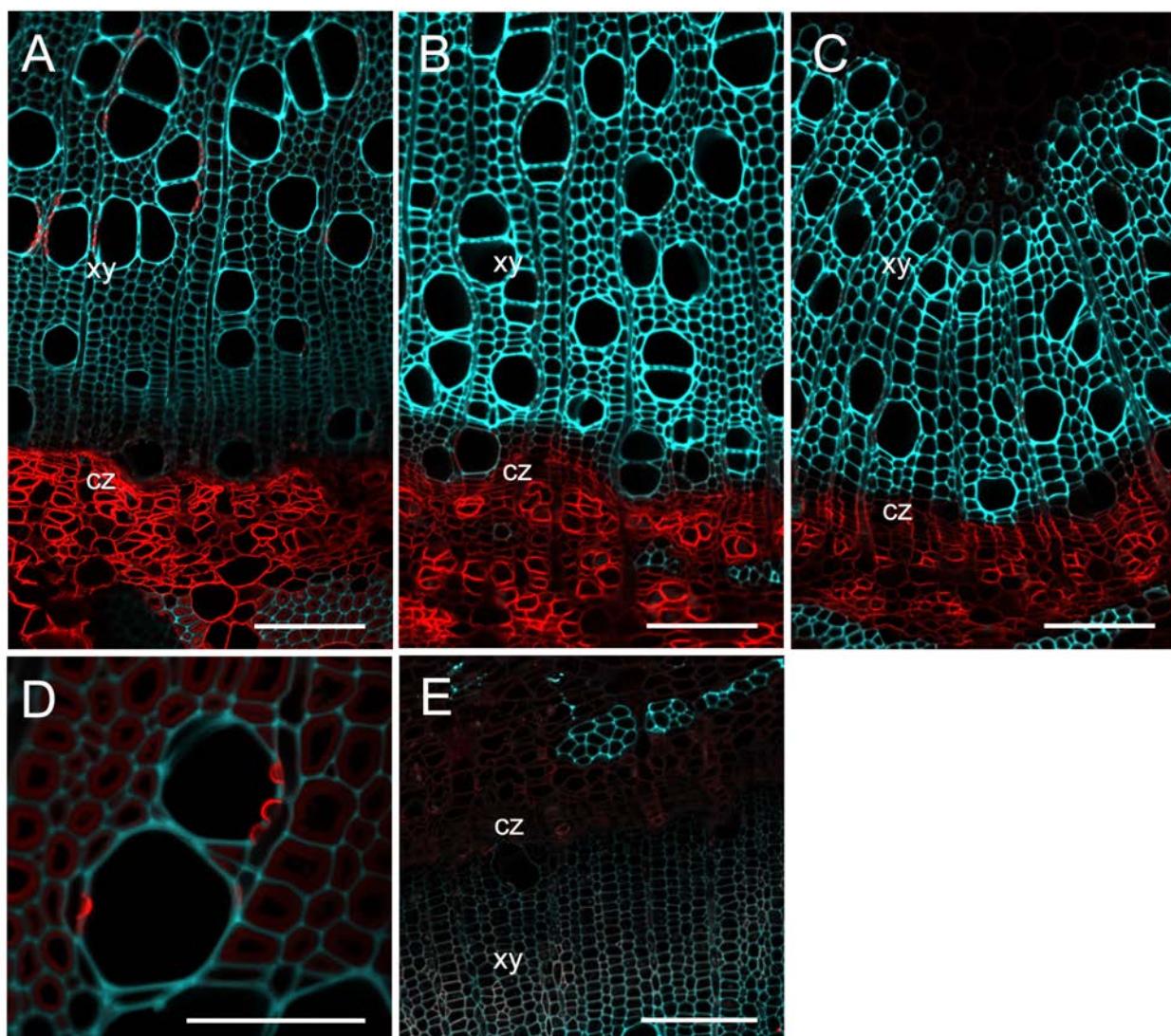
Supplemental Figure 1. Quantification of gravitropic bending and longitudinal growth in ARK2 genotypes during a two week gravistimulus trial. A-C, Time lapse movies (three biological replicates per genotype) were digitized in Image J by tracing each stem image from the base of the stem to the point where primary bending defines an apex of curvature. (A), Lift of the stem at apex of curvature, normalized for total stem height over time. (B), Rate of stem bending over time, normalized for stem length. (C), Stem curvature over time. (D), Quantification of elongative growth for gravistimulated or upright control trees after two week growth.



Supplemental Figure 2. Opposite wood histology. Histological staining with phloroglucinol and astra blue of stem transverse sections from trees subjected to two week gravistimulation. (A), miRNA-ARK2, (B) wild-type, (C), OE-ARK2. Scale bars, A-C 500 μ m. (co, cortex; cz, cambial zone; pf, phloem fiber; xy, xylem)



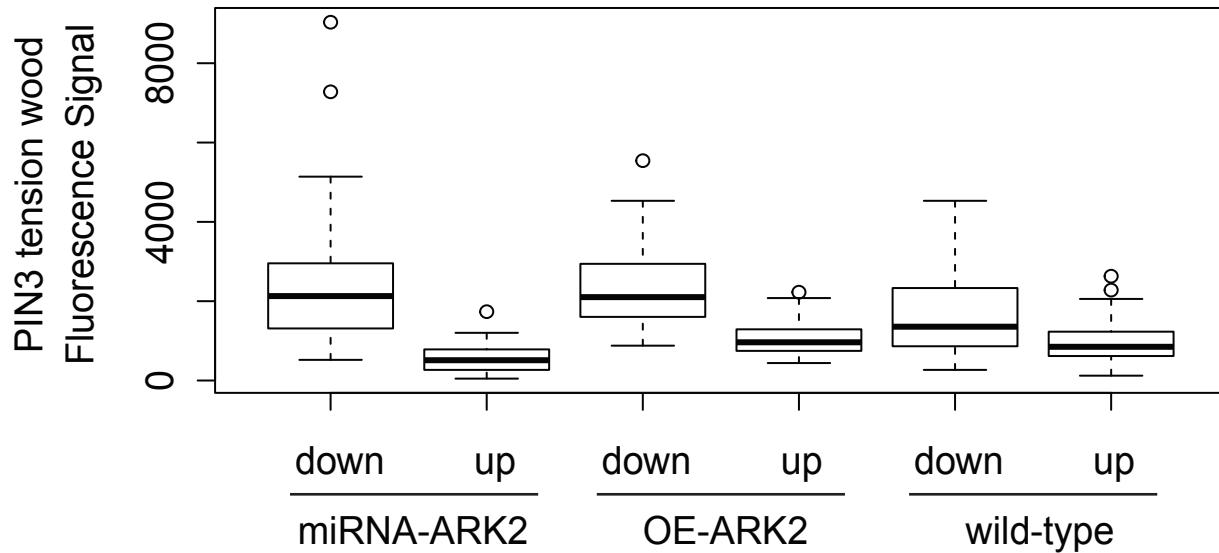
Supplemental Figure 3. JIM14 immunolocalization of arabinogalactan protein epitopes in opposite wood of *ARK2* genotypes, and negative control showing background labeling. A-C, Immunolocalization with JIM14 to reveal arabinogalactan protein epitopes in opposite wood for miRNA-ARK2 (A), wild type (B), and OE-ARK2 (C). Panel (D) shows background in a section for which the primary JIM14 antibody was omitted and probed with the secondary antibody alone. In each panel, red signal is from JIM14 labelling while blue signal is ultraviolet autofluorescence from lignified cell walls. Scale bars, A-C 500 μm ; D-F, 200 μm . (co, cortex; cz, cambial zone; pf, phloem fiber; xy, xylem)



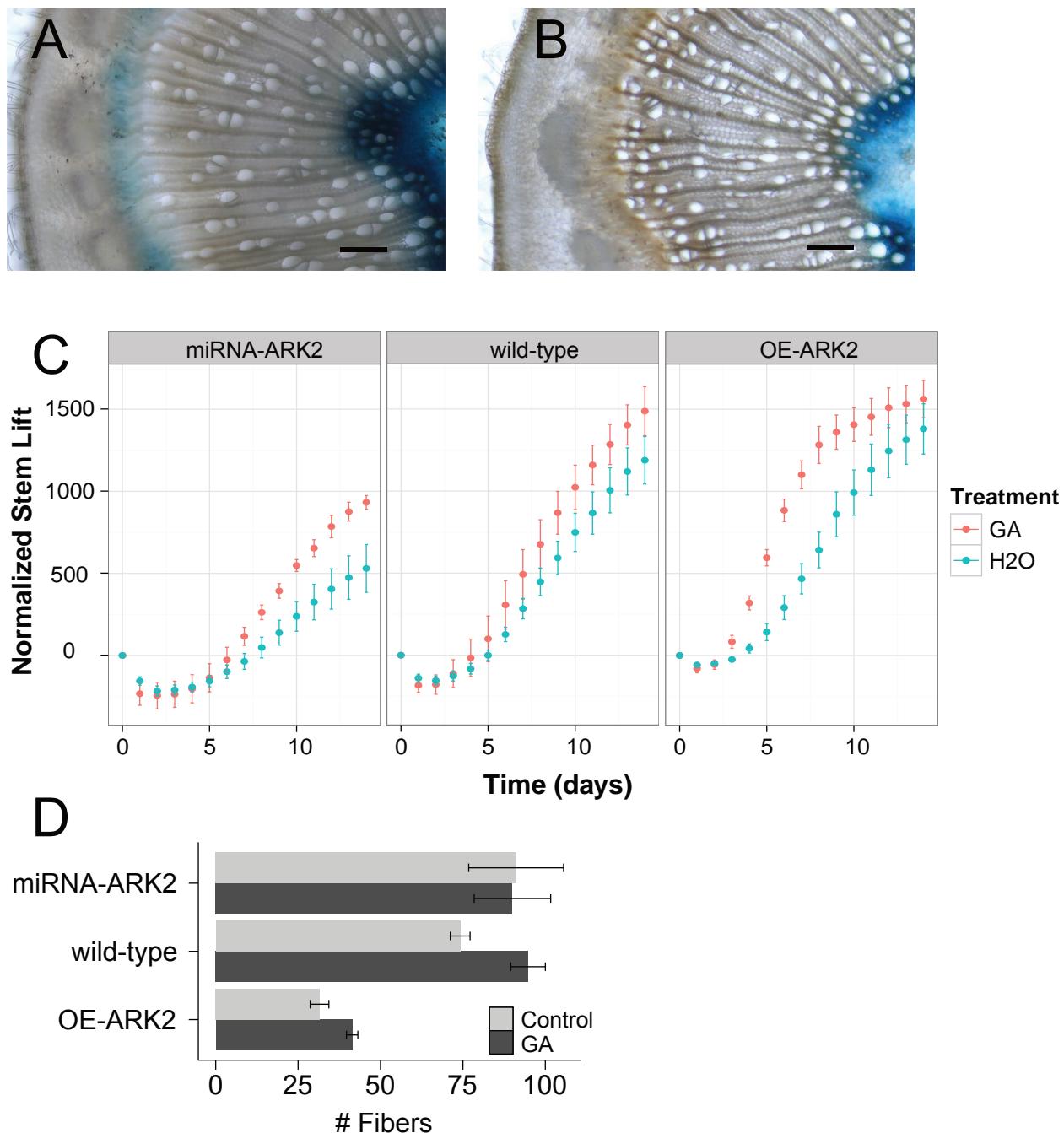
Supplemental Figure 4. Imaging of XET activity in opposite wood of ARK2 genotypes. XET labeling in opposite wood, which lacks G-layers and does not show XET labeling of fibers. XET activity was imaged using a XXXG-SR fluorescent substrate. **A-C**, XET labeling in opposite wood of miRNA-ARK2 (**A**), wild type (**B**), and OE-ARK2 (**C**) was found in the phloem and cortex. (**D**), XET labelling between neighboring vessels and fibers. (**E**), Control section was boiled for 10 minutes prior to XET labelling as a negative control. Scale bars, **A-C, E** 100 μm ; **D**, 50 μm . (cz, cambial zone; xy, xylem)



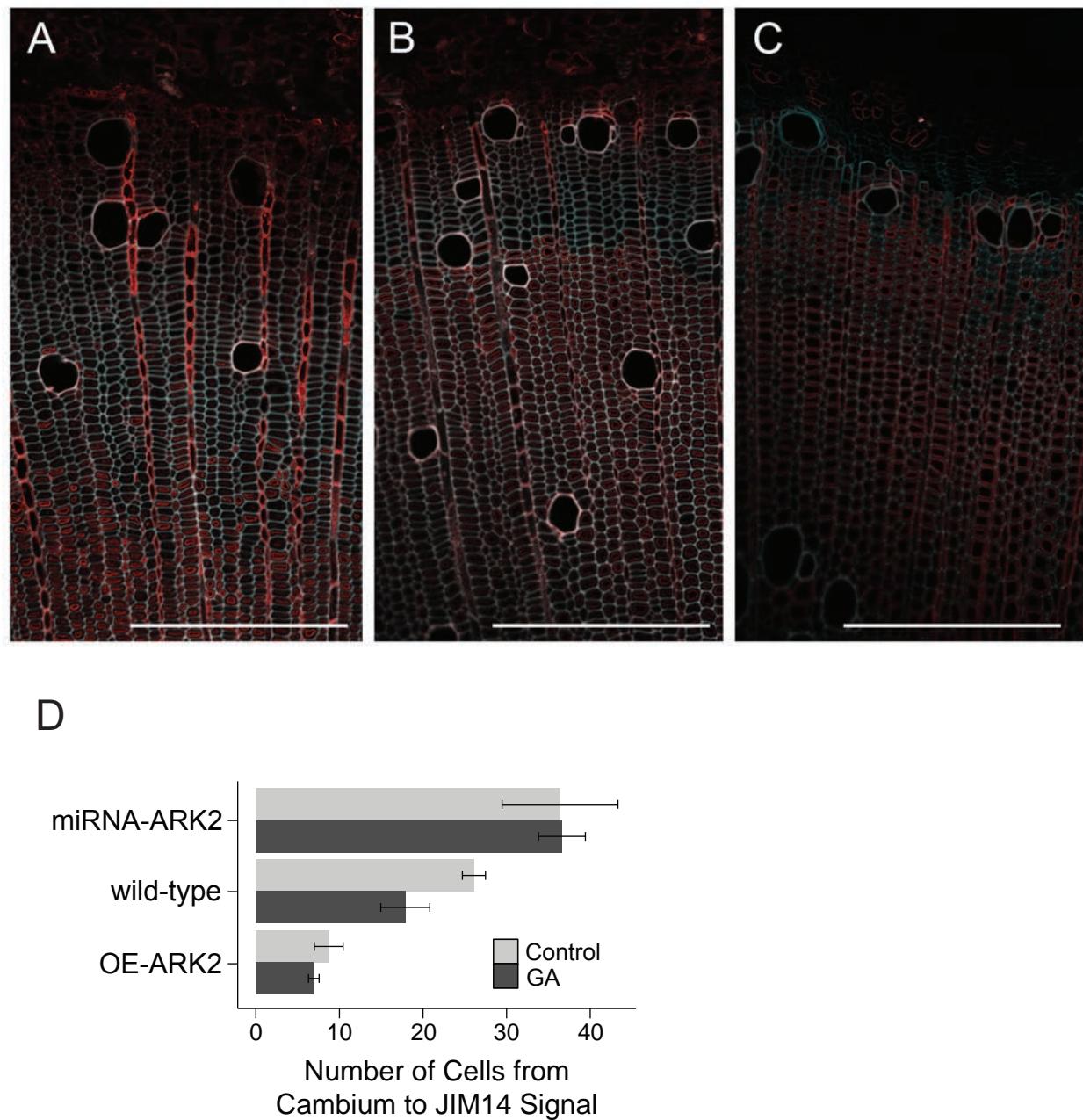
Supplemental Figure 5. PIN3 and control immunolocalization. (A), Immunoblot of membrane proteins extracted from *Populus* leaf and stem tissues probed with PIN3 antibody. Expected size for PIN3 proteins is approximately 70kDa. Because of challenges in extracting membrane proteins from woody tissues, PIN3 was detectable only in the leaf but not stem extracts. Lanes on right from blot processed without PIN3 primary antibody to show negligible background signal. (B), Immunofluorescence signal alone and (C), immunofluorescence signal merged with brightfield image for section in which immunolocalization was performed omitting the primary PIN3 antibody, showing negligible background signal and thus good specificity.



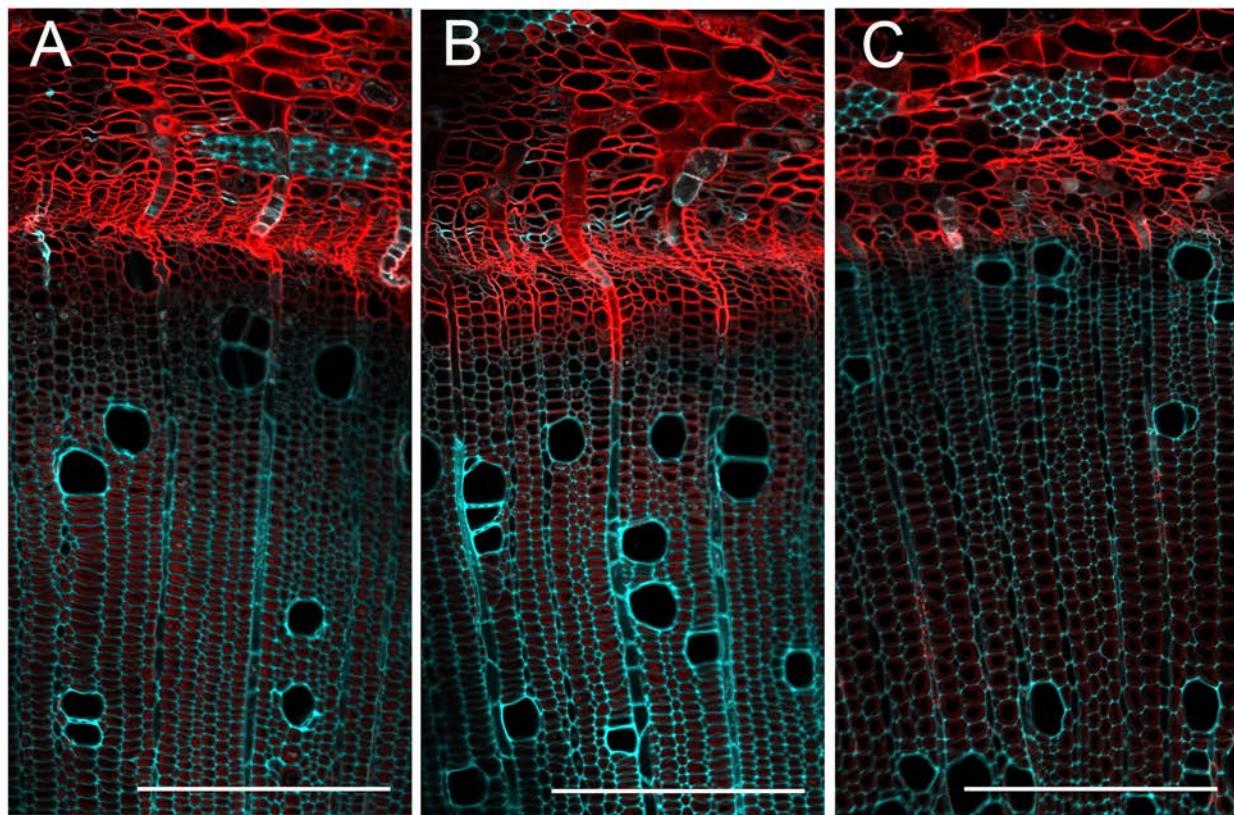
Supplemental Figure 6. PIN3 quantification in tension wood and localization in opposite wood. Immunolocalizations with PIN3 antibody were used to quantify the fluorescent signal in confocal images for individual cells from 4-day gravistimulated trees of each *ARK2* genotype. Signal was quantified in the apical (“up” towards the sky) or basal (“down” towards the ground) membranes within PIN3-expressing cells, relative to the gravitational vector.



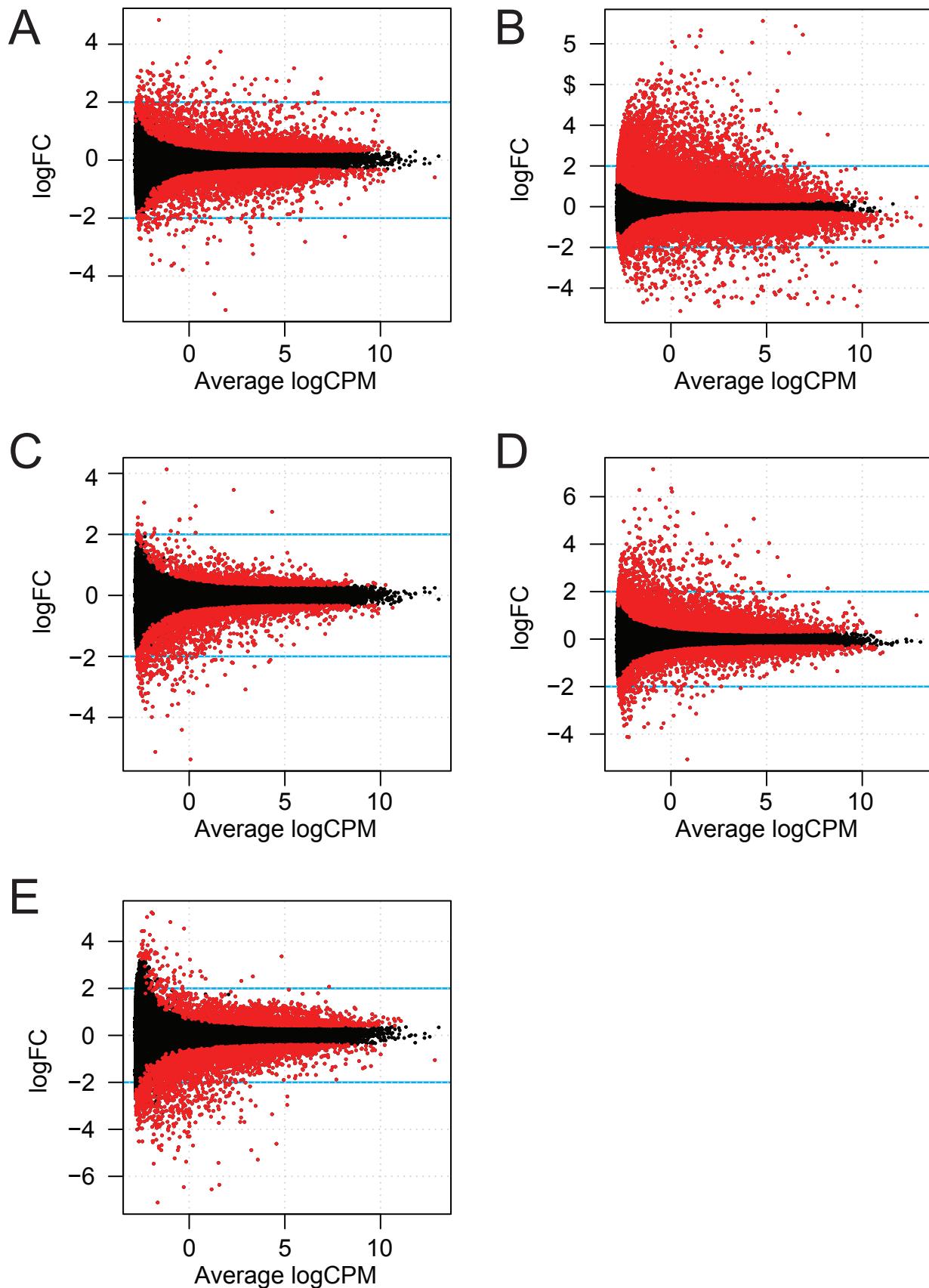
Supplemental Figure 7. GA effects on DR5:GUS expression in upright stems, and on gravibending in ARK2 genotypes. DR5:GUS expression in upright stems of GA-treated (A) and untreated (B) trees. (C) Lift of the stem at apex of curvature, normalized for total stem height over time, for ARK2 genotypes treated with GA or water control. Time lapse movies (three biological replicates per genotype) were digitized in Image J by tracing each stem image from the base of the stem to the point where primary bending defines an apex of curvature. (D) Quantification of number of tension wood fibers produced during a two week gravistimulus trial for each ARK2 genotype. Welch two sample t-test analysis of GA effects: miRNA p=0.95, wild type p=0.02, OE-ARK2 p=0.02.



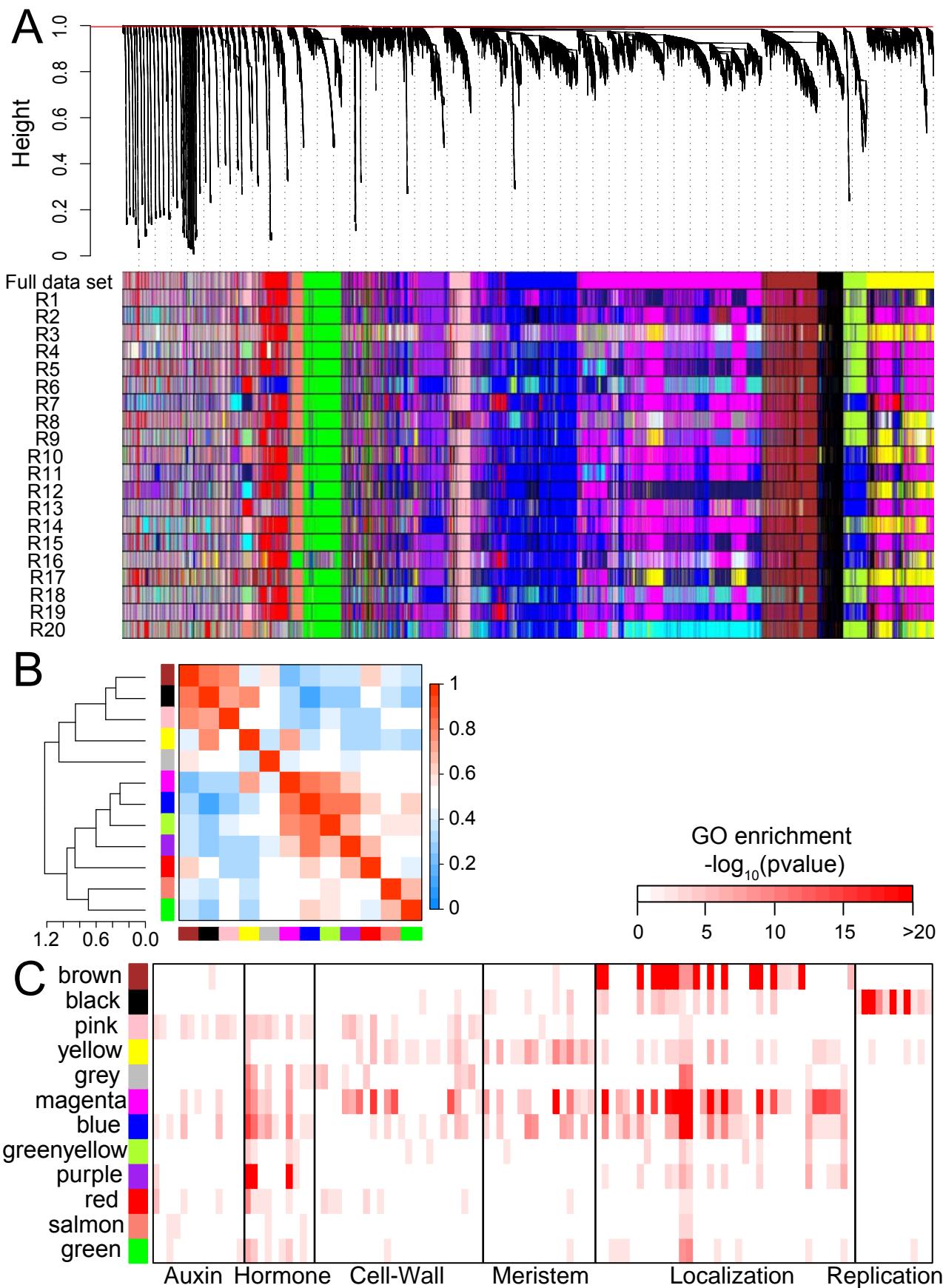
Supplemental Figure 8. JIM14 immunolocalization for stem sections of trees treated with GA. JIM14 immunolocalization for stem sections of GA-treated trees of (A) miRNA-ARK2, (B) wild-type, and (C) OE-ARK2. In each panel, red signal is from JIM14 labelling while blue signal is ultraviolet autofluorescence from lignified cell walls. Scale bars 200 μ m. (D) Quantification of the number of cells between the cambium and the first fiber cell with JIM14 immunofluorescence for GA treated and control trees of each ARK2 genotype.



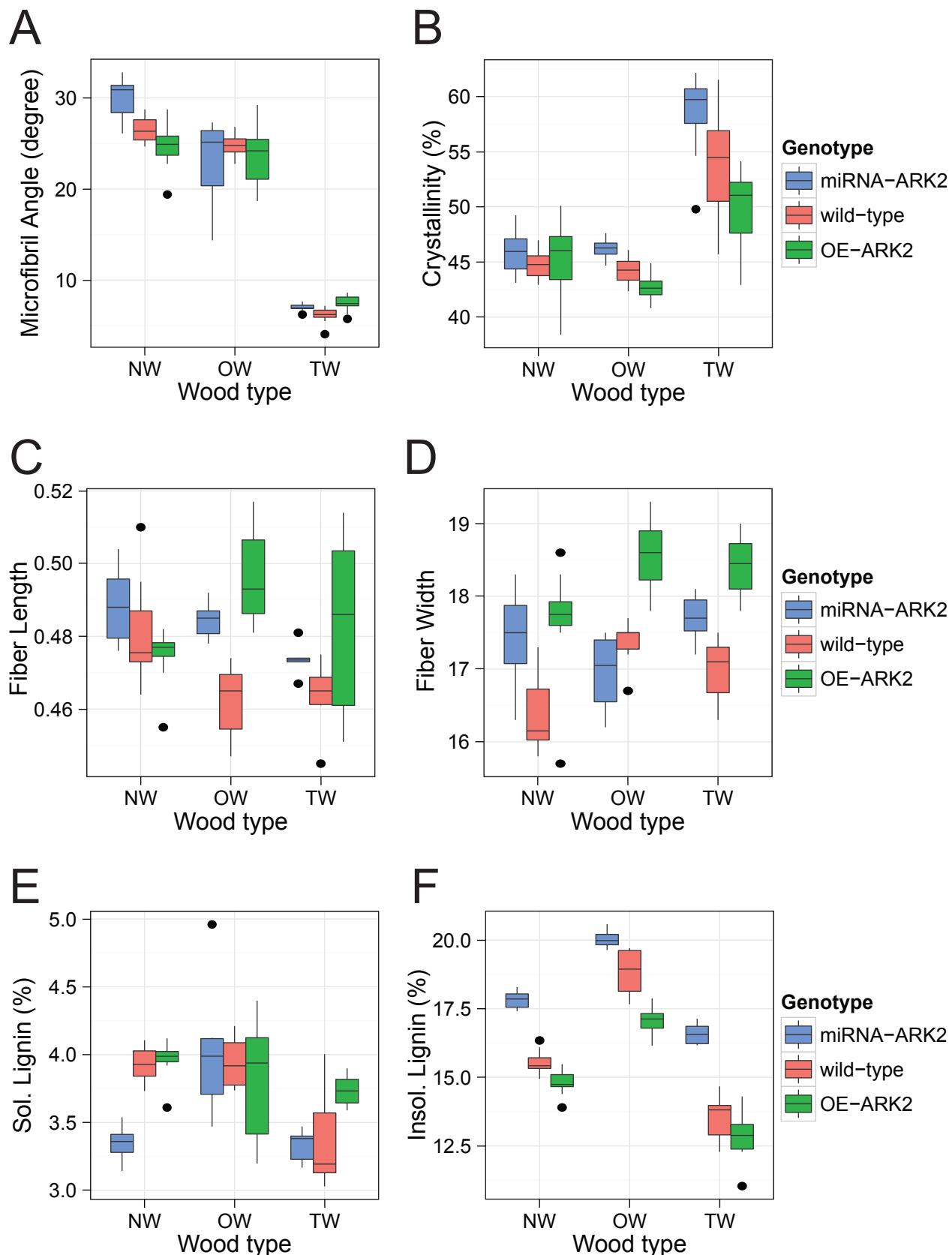
Supplemental Figure 9. In situ imaging of XET activity in stems of trees treated with GA. Incorporation of fluorescent XET substrate XXXG-SR imaged for (A) miRNA-ARK2, (B) wild-type, and (C) OE-ARK2 stem sections from trees treated with GA. In each panel, red signal is from fluorescent XET substrate XXXG-SR incorporation, while blue signal is ultraviolet autofluorescence from cell walls. Inset boxes show magnified view of position where XET labeling first occurs in G-layers for each ARK2 genotype. Scale bars 100 μ m.

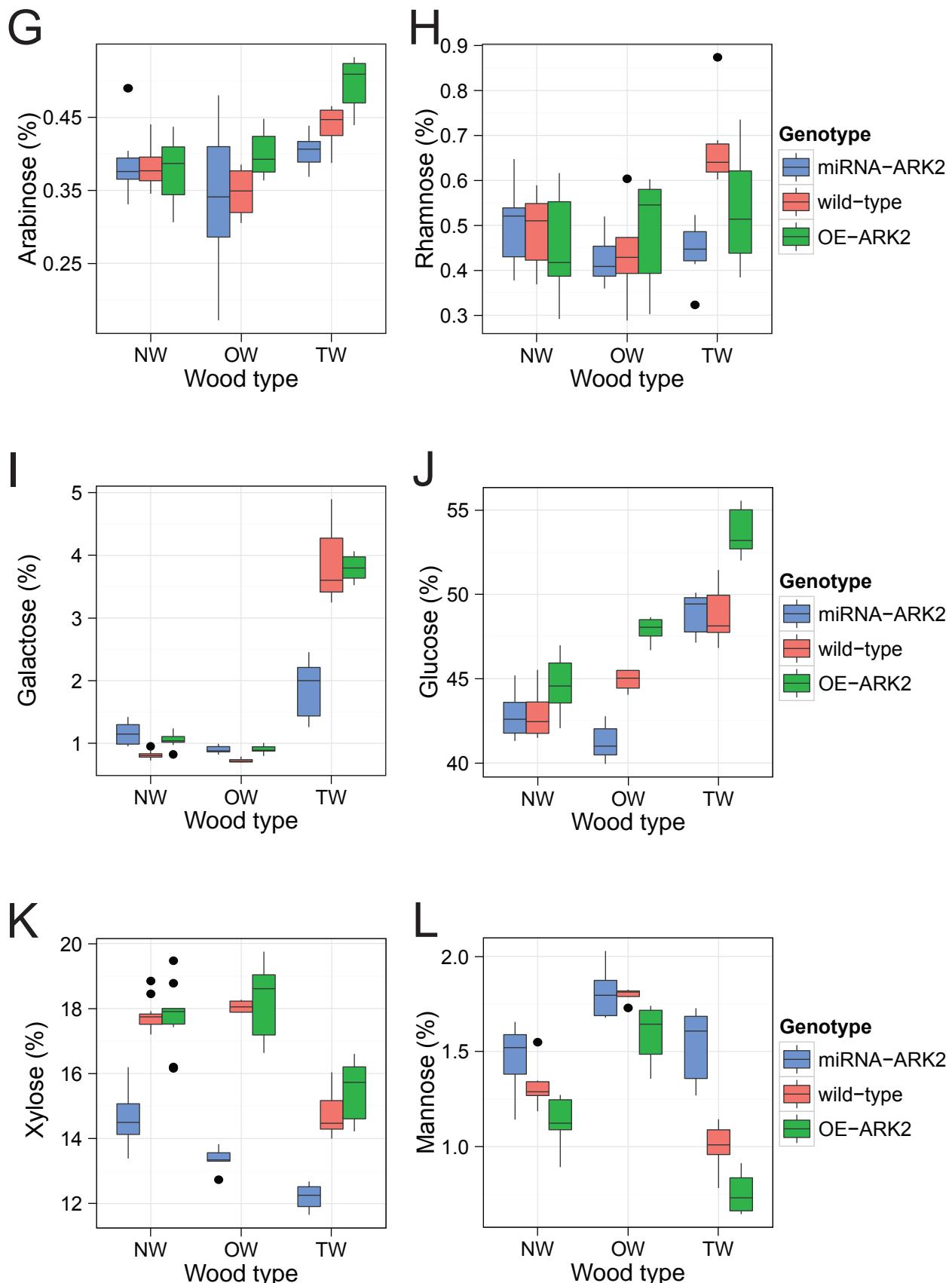


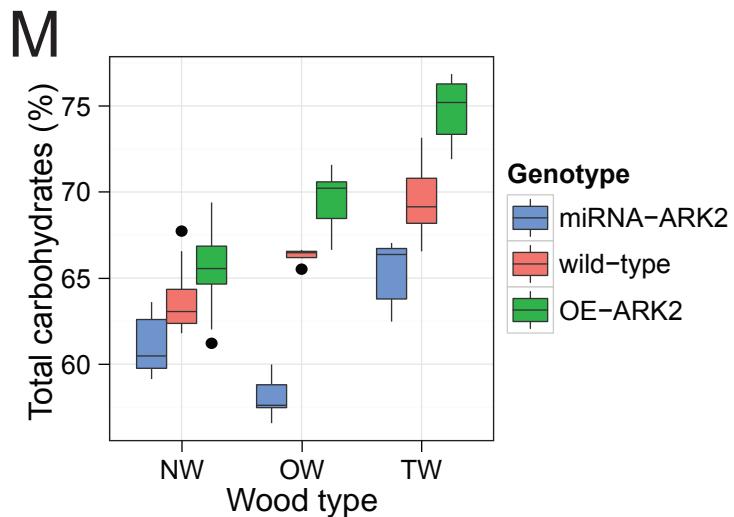
Supplemental Figure 10. Differential expression of genes in opposite wood versus tension wood and normal wood. In all plots, data points in red show statistically significant differential expression. Blue lines correspond to a two-fold difference in expression. **(A)** Genes differentially expressed in tension wood when compared to normal and opposite wood. **(B)** Genes differentially expressed in opposite wood when compared to normal and tension wood. **(C)** Genes differentially expressed in miRNA-ARK2 compared to wild type. **(D)** Genes differentially expressed in OE-ARK2 compared to wild type. **(E)** Genes differentially expressed in response to GA compared to water controls.



Supplemental Figure 11. Characterization of gene co-expression network modules. **(A)** Stability analysis of gene modules. Results of modules calculated using all full RNA-seq libraries (top row) or with a random sample of 75% of the RNA-seq datasets. **(B)** Heatmap showing strength of correlations among all possible combinations of co-expression gene modules. **(C)** Heatmap showing enrichment for modules for GO terms from hormone, cell wall, meristem, localization and replication related ontologies.







Supplemental Figure 12. Quantification of wood property and chemistry traits for wood types within each *ARK2* genotype. (A) Cellulose microfibril angle. (B) Cellulose crystallinity. (C) Fiber length. (D) Fiber width. (E) Soluble lignin. (F) Insoluble lignin. (G) Arabinose. (H) Rhamnose. (I) Galactose. (J) Glucose. (K) Xylose. (L) Mannose. (M) Total carbohydrate.