Supplemental Information for:

Engineering Plant Cell Walls: Tuning Lignin Monomer Composition for Deconstructable Biofuels Feedstocks or Resilient Biomaterials

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Calculation of Intra-Cell Wall Void Space

To quantify the differences in porosity and delamination within cell walls, TEM micrographs were processed to threshold intra-wall void spaces into regions from which size and shape descriptors could be measured. Six regions of interest (ROI) from each of four different images from each of the six samples were analyzed to determine void space. To select a threshold that would distinguish cell wall material from void space, the mean and standard deviation of pixel values within a ROI known to contain only void space (Figure S1a *), such as the cell lumen, was measured. The threshold was then determined as the pixel value two standard deviations above the mean pixel value of the designated void region, calculated as

$$T = \bar{x}_v + 2\sigma_v$$

where T is the threshold value, and $\overline{x}_v$ and σ_v are the mean and standard deviation, respectively, of the pixel values from a known void region. Examples of measured ROIs and the binary images determined using this thresholding method are shown in Figure S1.

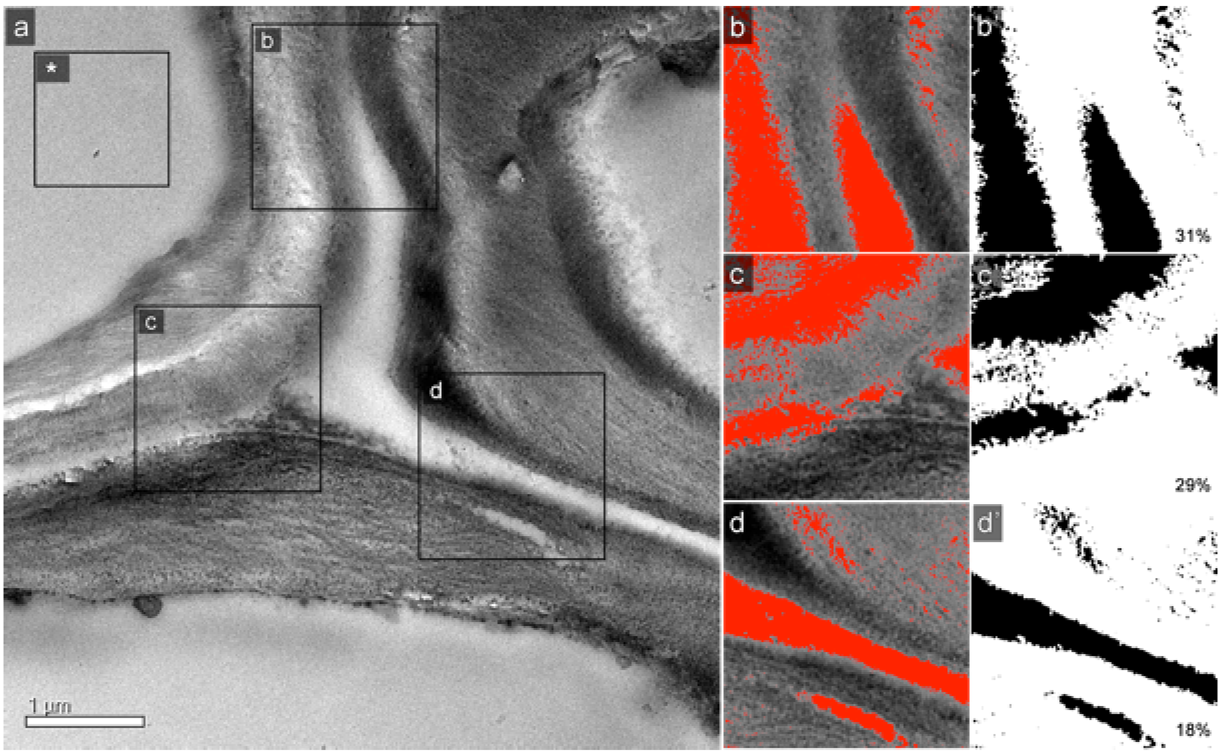

Figure S1. Explanation of void space calculation. (a) Entire original TEM image of maleic acid treated cell walls. The region of interest denoted with the asterisk designates the known void region from which the threshold value was calculated. (b-d) Examples of intra-cell wall ROIs sub-selected from the original image. The left panels show the effect of applying the calculated threshold to the regions of interest and the resulting void space value.

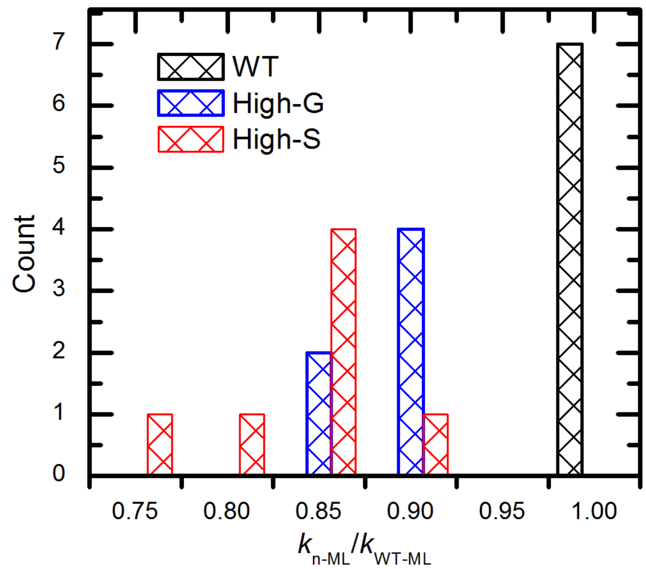

Figure S2. Statistical analysis of CR-FM stiffness in the middle lamella region of cell walls. Histogram of all middle lamella data obtained from the 20 CR-FM images. The *x*-axis in the histograms is equivalent to the *z*-axis in the CR-FM images in Fig. 5 in the main text and represents the contact stiffness k normalized to the contact stiffness $k_{WT\text{-}ML}$ of the wild-type middle lamella. Student *t*-tests (2 tail test, equal variance) comparing the wild type and two mutants reveals ~95 % confidence that the high-S type (*fah* 1 C4H:F5H) and high-G type (*fah* 1) middle lamellas differ in stiffness from each other and >99.9 % confidence that they differ from the wild type. Mean values of normalized contact stiffness for the C4H:F5H and fah 1-2 types are 0.83 ± 0.05 and 0.89 ± 0.03, respectively.