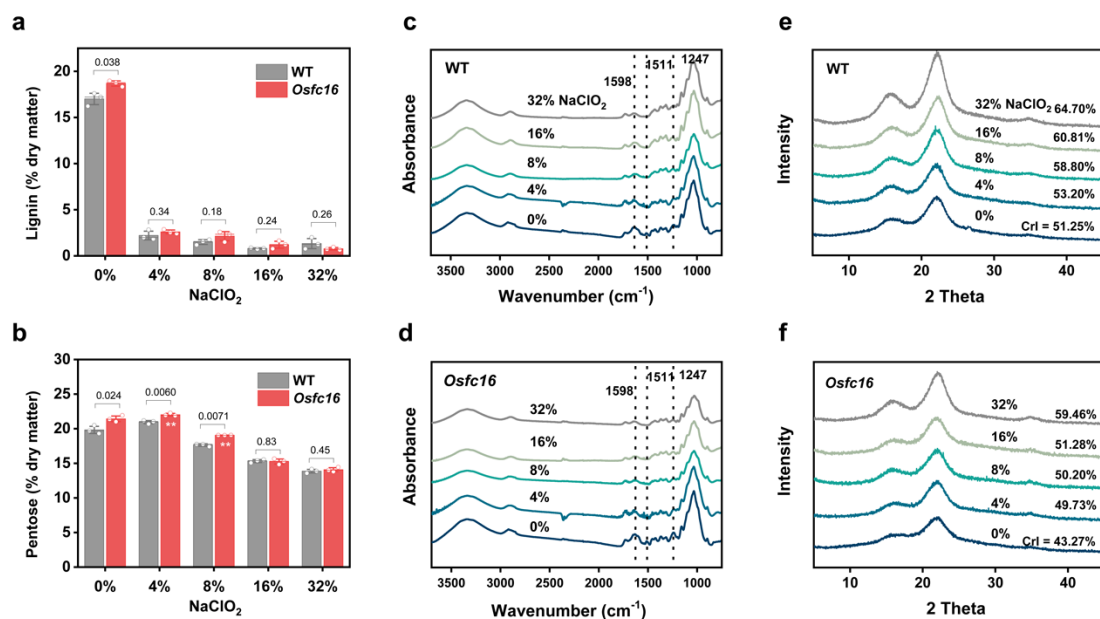


**Single-molecular insights into the breakpoint of cellulose nanofibers
assembly during saccharification**

Zhang and Hu *et al.*

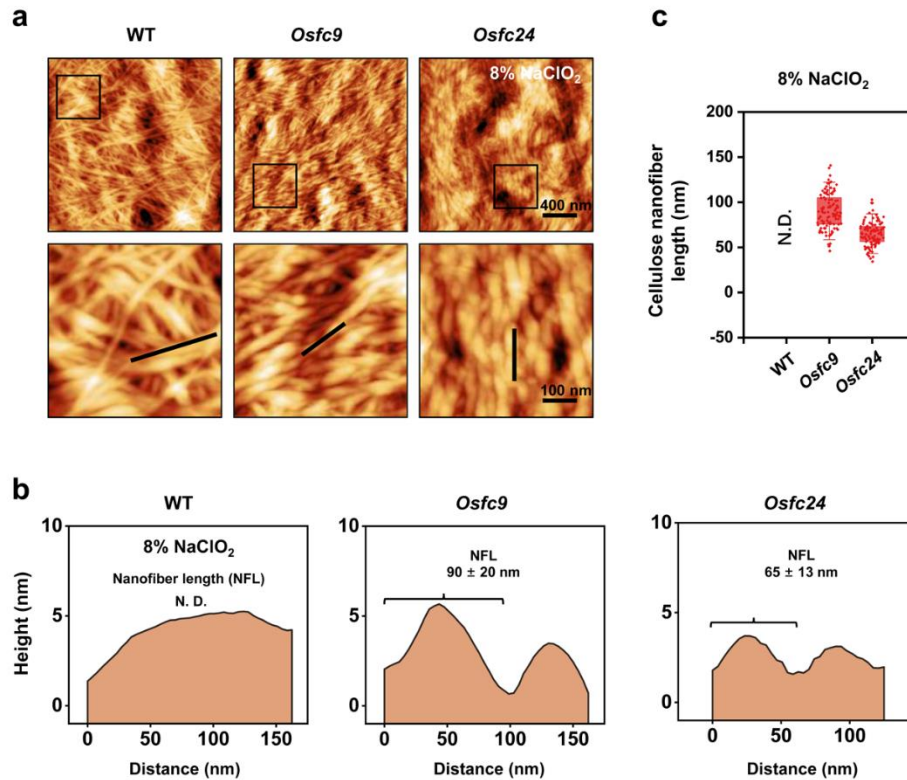
Supplementary Table 1. Characteristic bands of the FT-IR spectra.

Reported wavenumber (cm ⁻¹)	Observed wavenumber (cm ⁻¹)	Functional group	Assignment
829	831	C—H breathing	H-lignin
898	898	C—H vibration	Cellulose
1051	1051	C—O—C ring skeletal vibration	Hemicelluloses
1163	1164	C—O—C asymmetric stretching	Cellulose
1247	1247	C—O—C stretching of aryl-alkyl ether	Lignin
1373	1371	C—H ₂ scissoring	Cellulose
1430	1430	C—H ₂ bending	Cellulose
1460	1460	C—H ₃ asymmetric bending	Lignin
1515	1511	C=C stretching of the aromatic ring	Lignin
1590	1598	C=C stretching	Lignin
1603	1603	C=C stretching	Lignin
1735	1735	C=O stretching of acetyl or carboxylic acid	Hemicelluloses & Lignin
2900	2900	C—H stretching	Cellulose
3350	3378	O—H stretching	Cellulose



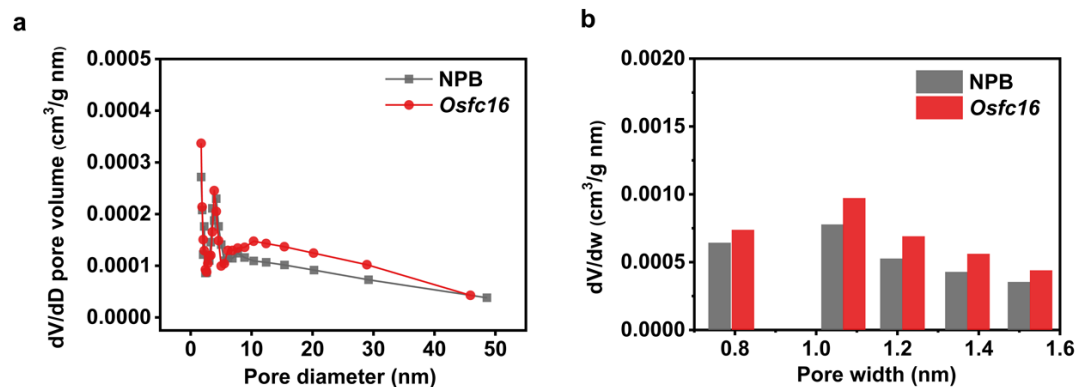
Supplementary Fig. 1. Wall polymers levels and properties in stem tissue residues of *Osf16* mutant and WT after NaClO_2 treatments.

a Lignin content. **b** Pentoses content crudely as hemicellulose. Bars as means $\pm$ SD ($n = 3$ biologically independent samples). **c**, **d** Fourier transform infrared spectroscopic profiling. Information of chemical bonds for all major peaks listed in Supplementary Table 1. **e**, **f** XRD spectroscopic profiling. Significant differences between the WT and mutant were determined using two-tailed Student's t -test: $**P < 0.01$, $*P < 0.05$. Source data are provided as a Source Data file.



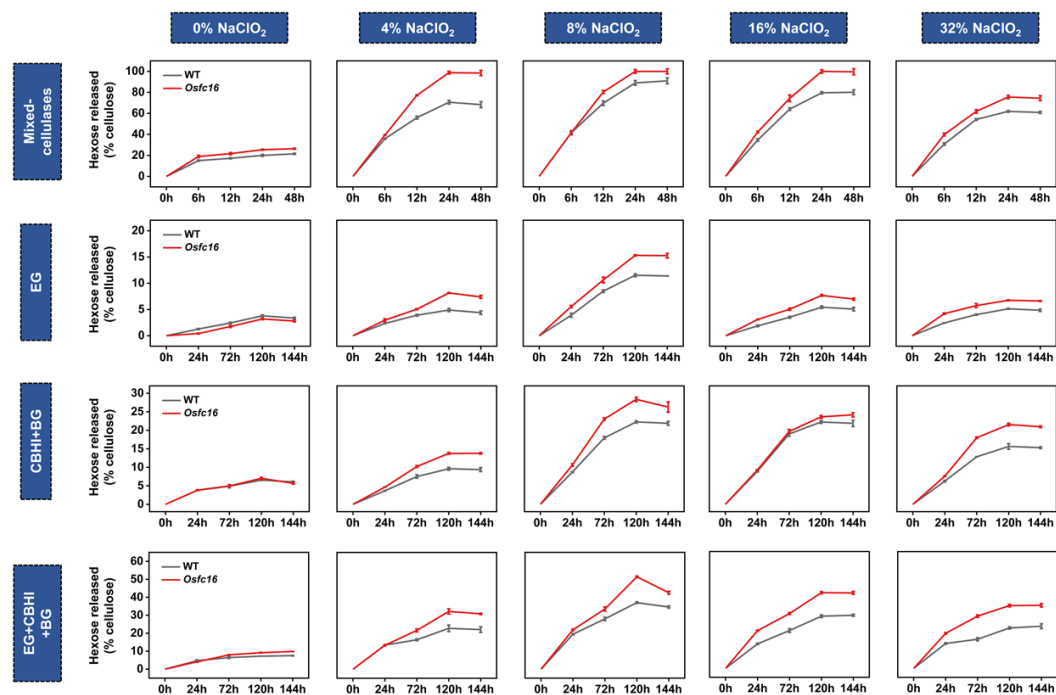
Supplementary Fig. 2. *In situ* measurements of cellulose defects in *Osf9* and *Osf24* mutants.

a Classic AFM topography in plant cell walls after 8% NaClO₂ treatment. **b** Cross profiles corresponding for black lines in **(a)** to illustrate alternate breakpoints for nanofibers lengths. **c** Average nanofibers lengths distribution in **(b)**. NFL, nanofibers length, data as means ± SD (n = 100 nanofibers counted from 3 biologically independent samples). Data in **(c)** are displayed as box and whisker plots with individual data points. The error bars represent 95th and 5th percentiles. Center line, average; box limits, 25th and 75th percentiles. AFM experiments were repeated at least 3 times independently with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 3. Raised nanopore volumes in *Osf16* mutant.

a BJH adsorption micropore and **b** Horvath-Kawazoe nanopore distribution of crude cellulose substrates after 8% NaClO_2 extraction with mature straws. Source data are provided as a Source Data file.



Supplementary Fig. 4. One-step enzymatic hydrolyses with stem powders samples of *Osf16* mutant and WT after NaClO_2 treatments with time course. Bars as means $\pm$ SD (n = 3 biologically independent samples). Source data are provided as a Source Data file.