

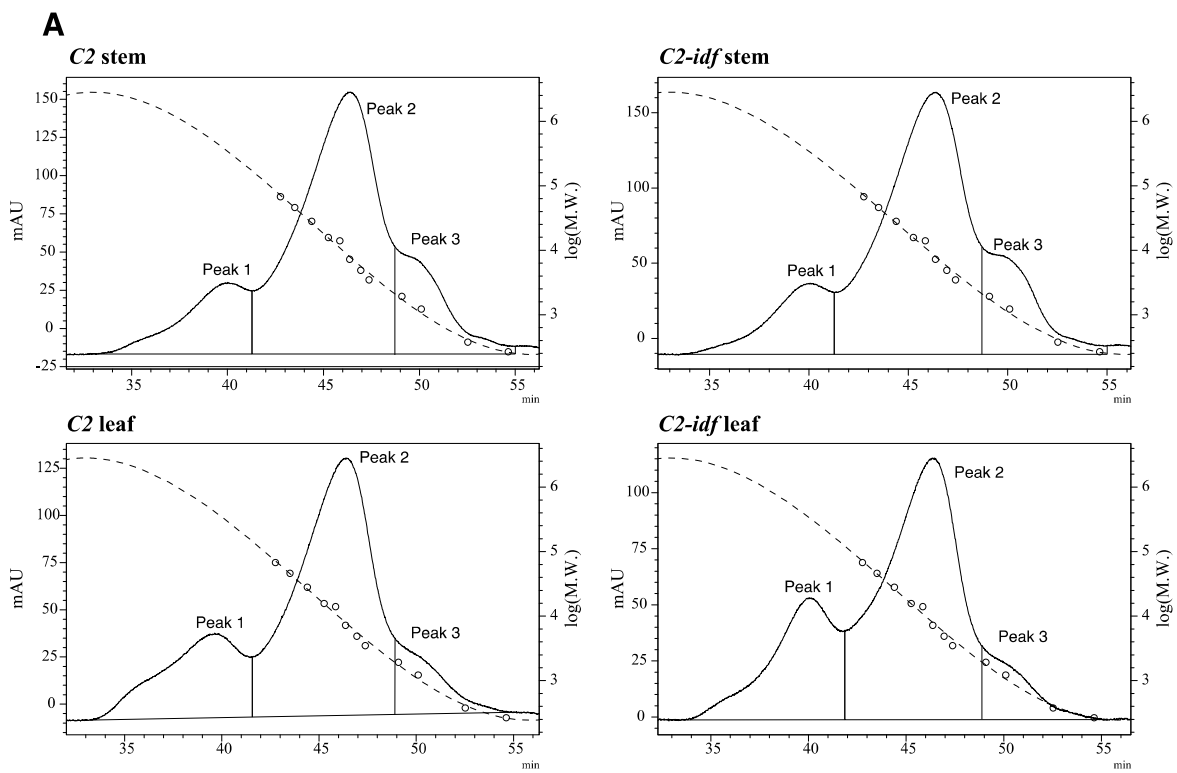
1 **SUPPLEMENTAL FILES**

2 **Supplemental File S1. MS/MS spectra of the characterized metabolites**

3 See separate file, Supplemental File S1.pdf

4

5 **Supplemental Figure S1. GPC chromatographic comparison of lignin from *C2-Idf* mutant and *C2***  
6 **control stem and leaf. (A)** The dashed line shows the standard curve ( $R^2=0.9962$ ) of polystyrene with  
7 12 data points. The molecular weight distribution curves were each divided into three peaks and the  
8 Mn, Mw, and polydispersity index (Mw/Mn) of each peak were analyzed and shown in the table (B).  
9 Peak 1 represent an abnormal large molecular weight for lignin samples and was therefore regarded  
10 as an unknown contaminant, the peak 2 represented the major lignin polymer and peak 3 originated  
11 from lignin oligolignols with molecular weight at around 800-1000. Statistics were performed on  
12 peak 2. The Mn, Mw and Mw/Mn values of stem and the Mn value of leaf were not significantly  
13 different between the *C2-Idf* mutant and *C2* control (Student t-test, p-value>0.05). The Mw and  
14 Mw/Mn values of leaf were significantly different between the *C2-Idf* mutant and *C2* control (8%  
15 increase in *C2-Idf* as compared to *C2*, Student t-test, p-value=0.03 for Mw, and 4% increase, Student  
16 t-test, p-value=0.04 for Mn/Mw). Average  $\pm$  standard deviation are given for 4 to 6 biological  
17 replicates.



**B**

<i>C2</i> stem				<i>C2-Idf</i> stem			
	Mn	Mw	Mw/Mn		Mn	Mw	Mw/Mn
Peak 1	421200 $\pm$ 37000	720000 $\pm$ 66500	1.71 $\pm$ 0.03	Peak 1	477100 $\pm$ 55300	812300 $\pm$ 112500	1.70 $\pm$ 0.08
Peak 2	8500 $\pm$ 300	25000 $\pm$ 1400	2.95 $\pm$ 0.06	Peak 2	8300 $\pm$ 300	24200 $\pm$ 1400	2.92 $\pm$ 0.06
Peak 3	810 $\pm$ 50	1100 $\pm$ 30	1.34 $\pm$ 0.04	Peak 3	900 $\pm$ 80	1100 $\pm$ 50	1.29 $\pm$ 0.06
<i>C2</i> leaf				<i>C2-Idf</i> leaf			
	Mn	Mw	Mw/Mn		Mn	Mw	Mw/Mn
Peak 1	507100 $\pm$ 23700	891700 $\pm$ 42200	1.76 $\pm$ 0.05	Peak 1	405300 $\pm$ 76700	710200 $\pm$ 144600	1.75 $\pm$ 0.05
Peak 2	8000 $\pm$ 300	21100 $\pm$ 1000	2.62 $\pm$ 0.05	Peak 2	8400 $\pm$ 300	22900 $\pm$ 1100	2.71 $\pm$ 0.07
Peak 3	800 $\pm$ 20	1100 $\pm$ 10	1.33 $\pm$ 0.02	Peak 3	800 $\pm$ 80	1000 $\pm$ 60	1.38 $\pm$ 0.06

19 **Supplemental Table S1. Growth parameters of *C2-ldf* mutant and *C2* control plants.** Plant height and dry biomass measurements were performed on  
 20 senesced greenhouse-grown plants. DW: dry weight; SEM: standard error of the mean with n=12; n.s.: not significantly different; underlined values:  
 21 significantly different compared to the control; \*:0.05≥p>0.01, \*\*: p<0.01.

22

Growth parameter	<i>C2</i> (mean ± SEM)	<i>C2-ldf</i> (mean ± SEM)	% difference	t-test
Plant height with tassel (cm)	296.33 ± 17.78	292.01 ± 19.01	-1.4	n.s.
Leaf DW (g)	36.11 ± 6.71	<u>45.13</u> ± <u>6.20</u>	25	*
Stem DW (g)	90.79 ± 31.45	<u>121.80</u> ± <u>28.08</u>	34.16	**
Total DW (g) (leaves + stems)	126.90 ± 37.76	<u>166.94</u> ± <u>33.94</u>	31.55	**

23

24 **Supplemental Table S2. Targeted search for oligolignols isolated from stem and leaf of *C2-ldf* and *C2* control plants.** b.d.: below detection limit; SD:  
 25 standard deviation, p-values are calculated via Student t-tests statistics. Nomenclature of the oligolignols is explained in Morreel et al., (2004; 2010a;  
 26 2010b), the prime (') signifies an aldehyde moiety.

27

nr	Name	[M-H] <sup>+</sup>	RT (min)	<i>C2</i> internode (Mean ± SD)	<i>C2-ldf</i> internode (Mean ± SD)	p-value	Ratio <i>C2-ldf</i> / <i>C2</i>	<i>C2</i> leaf (Mean ± SD)	<i>C2-ldf</i> leaf (Mean ± SD)	p- value	Ratio <i>C2-ldf</i> / <i>C2</i>
1	<b>G(8-O-4)S(8-5)G 1</b>	583.2180	14.81	29243 ± 7109	39767 ± 20579	0.267	1.36	2539 ± 934	2162 ± 896	0.442	0.85
2	<b>G(8-O-4)S(8-5)G 2</b>	583.2180	15.61	13912 ± 3862	14941 ± 9379	0.903	1.07	1411 ± 648	887 ± 640	0.125	0.63
3	<b>G(8-O-4)S(8-8)G</b>	583.2194	17.04	2494 ± 653	4590 ± 3247	0.110	1.84	b.d.	b.d.	-	-
4	<b>G(8-O-4)S(8-5)G'</b>	581.2034	16.93	2172 ± 4397	1062 ± 826	0.611	0.49	2011 ± 546	1889 ± 1202	0.372	0.94
5	<b>S(8-O-4)S(8-8)S</b>	643.2390	16.54	713 ± 398	984 ± 727	0.465	1.38	3233 ± 2903	2791 ± 742	0.645	0.86
6	<b>G(8-O-4)G(8-O-4)S(8-8)S</b>	809.3000	18.09	2153 ± 1182	4997 ± 3693	0.083	2.32	1110 ± 327	1415 ± 646	0.439	1.27

28

29

30 **Supplemental Table S3. Relative quantification of lignin units and linkages based on volume integrals from the HSQC spectra of stem and leaf of C2-Idf**  
 31 **mutant and C2 control plants.** The lignin units were expressed as a percentage of the sum of G (guaiacyl), S' (benzylic-oxidized S units, or syringaldehyde- or  
 32 sinapaldehyde-derived), and S (syringyl) units. The linkage types were expressed as percentage of the sum of interunit linkage types A-C (including C'). Units  
 33 relatively quantified (as volume integrals only) include: cinnamaldehyde end-groups X2; benzaldehyde end-groups X3, phenylcoumaran ( $\beta$ -5) units B,  $\beta$ -aryl  
 34 ether ( $\beta$ -O-4) units A; resinol ( $\beta$ - $\beta$ ) units C; and  $\beta$ - $\beta'$  tetrahydrofuran units C'. n= 6 biological replicates. **Bold or underlined values indicate significantly**  
 35 **increased or decreased values, respectively, as compared to those of the control plants.**, pCA: p-coumarate, H: p-hydroxyphenyl unit, FA: ferulate, T: triclin  
 36 unit. SD: standard deviation, inf: infinite, n.d.: not detected, -: not applicable, n.s.: not significant, \*:0.05<p≤0.01, \*\*: 0.01<p≤0.001, \*\*\*: p<0.001.

feature	C2 stem (mean ± SD)	C2-Idf stem (mean ± SD)	% difference	t-test	C2 leaf (mean ± SD)	C2-Idf leaf (mean ± SD)	% difference	t- test
pCA%	98.2 ± 3.7	91.2 ± 4.8	-9.1	n.s	56.5 ± 2.7	<u>52.8 ± 2.3</u>	<u>-6.5</u>	*
H%	1.0 ± 0.1	<u>0.6 ± 0.2</u>	<u>-43.3</u>	*	10.1 ± 1.8	8.5 ± 1.0	-16.4	n.s.
FA%	1.8 ± 0.9	1.6 ± 0.8	-9.5	n.s.	2.4 ± 1.1	2.2 ± 0.2	-9.3	n.s.
G%	25.6 ± 2.3	<b>30.4 ± 0.5</b>	<b>18.7</b>	**	55.6 ± 1.4	55.3 ± 1.7	-0.6	n.s
S'%	0.8 ± 0.3	0.8 ± 0.2	4.0	n.s.	n.d.	n.d.	-	-
S%	73.6 ± 2.2	<u>68.8 ± 0.6</u>	<u>-6.6</u>	**	44.4 ± 1.4	44.7 ± 1.7	0.7	n.s
T%	1.9 ± 0.3	n.d.	<u>inf down</u>	***	27.6 ± 1.4	<u>n.d.</u>	<u>inf down</u>	***
Total (G+S+S')	100	100			100	100		
S/G	3.0 ± 0.5	<u>2.3 ± 0.1</u>	<u>-20.7</u>	**	0.8 ± 0.1	0.8 ± 0.1	1.3	n.s.
X2 (cinnamaldehyde end-group)%	0.9 ± 0.4	1.0 ± 0.3	13.5	n.s.	n.d.	<b>0.4 ± 0.3</b>	<b>inf up</b>	**
X3 (benzaldehyde end- group)%	- ± -	0.1 ± 0.1	66.6	n.s.	n.d.	n.d.	-	-
B ( $\beta$ -5)%	0.6 ± 0.3	0.9 ± 0.6	57.9	n.s.	2.0 ± 0.5	<b>4.2 ± 0.9</b>	<b>109.6</b>	***
A ( $\beta$ -O-4)%	94.4 ± 0.9	<u>92.6 ± 1.2</u>	<u>-2.0</u>	*	97.7 ± 0.7	<u>93.9 ± 1.5</u>	<u>-3.9</u>	***
C ( $\beta$ - $\beta$ , resinol)%	0.3 ± 0.3	0.6 ± 0.6	67.6	n.s.	0.3 ± 0.3	<b>1.2 ± 0.5</b>	<b>310.3</b>	**
C' ( $\beta$ - $\beta$ , tetrahydrofuran)%	4.7 ± 0.7	<b>6.0 ± 0.6</b>	<b>27.9</b>	*	n.d.	<b>0.7 ± 0.5</b>	<b>inf up</b>	***
Total (A+B+C+C')	100	100			100	100		

37

38

39 **Supplemental Table S4. Saccharification efficiency of senesced stem and leaf biomass from *C2-Idf* mutant and *C2* control plants.** The numbers listed in this  
40 table are visualized in the graphs of Figure 4.

41 See separate file, Supplemental Table S4.xlsx

42