

SUPPORTING FIGURES AND TABLES :

Enhanced characteristics of genetically modified switchgrass (*Panicum virgatum* L.) for high biofuel production[†]

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Fig.S1. Genomic DNA PCR and qRT-PCR for PvMYB4-OX lines in the Alamo ST1 background.

- (a) The *pANIC2B-PvMYB4* construct used for switchgrass transformation. *OsAct1*, promoter of rice *Actin1* gene; *rubi3*, promoter of *rubisco3* gene; *ZmUbi1*, promoter of maize *ubiquitin 1* gene. *hph*, hygromycin resistance gene for transformation selection marker. LB and RB, left and right borders of the T-DNA sequences. The double arrows show the primer pairs used for genomic DNA PCR testing. HPH, hygromycin-specific gene primer pairs; Gene specific, primer pairs covering the ZmUBI1 promoter region and PvMYB4 gene specific region.
- (b) Genomic DNA PCR. L7, L9, L10 and ST1 are transgenic and non-transgenic control lines.
- (c,d) qRT-PCR analysis showing (c) *PvMYB4* transgene transcripts in control and *PvMYB4*-OX transgenic switchgrass and (d) endogenous *PvMYB4* transcripts; the endogenous gene is down-regulated by overexpression of the *PvMYB4* transgene in switchgrass. Primer pairs used were as been reported before¹². Data are means \pm SE (n=3). L1 (high overexpression), L6 (intermediate overexpression), L9 and L10 (transgenic controls) were chosen for further analysis.
- (e) Visible phenotypes of control (Line 2A) and *PvMYB4*-OX switchgrass (Line 1C).

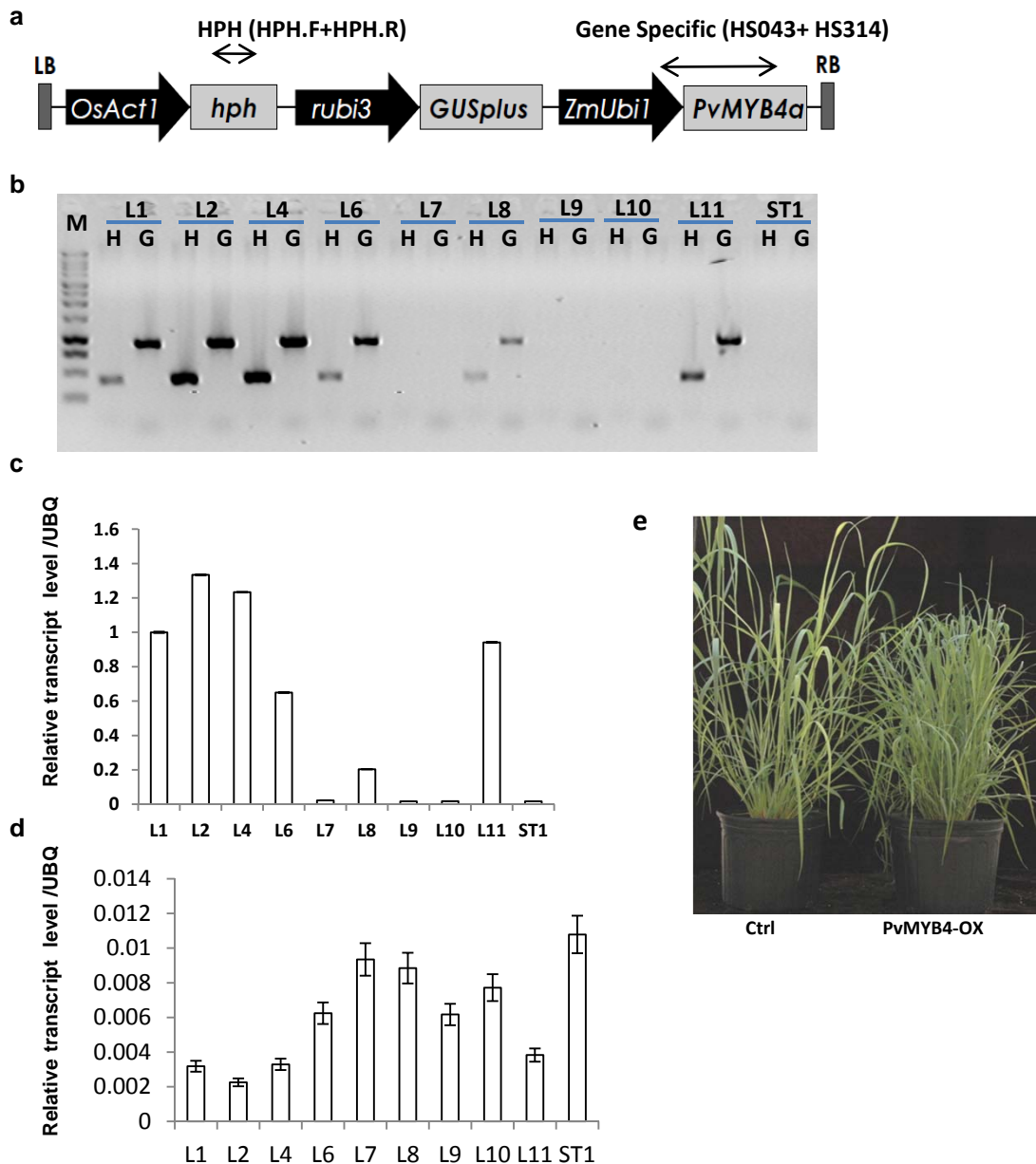


Fig. S2. PvMYB4-OX transgenic switchgrass lines have reduced levels of soluble phenolic compounds and reduced ester- and ether-linked *p*-coumaric/ferulic acid ratios.

(a) Levels of total soluble phenolic compounds as determined by reaction with Folin Ciocalteau reagent. (b) Levels of ester- and ether-linked wall-bound *p*-coumaric/ferulic acid (*p*-CA/FA) ratios. All data are means \pm SE (n=3). Different letters on the bars of figures indicate significant differences at the $p < 0.05$ level. Mean comparisons, based on mean separation test results, cannot be compared across variables in panel **b**. Lines L9, L10, L1 and L6 are in the Alamo ST1 genetic background, lines 2A, 2B, 1A, 1C and 1E in the Alamo ST2 genetic background.

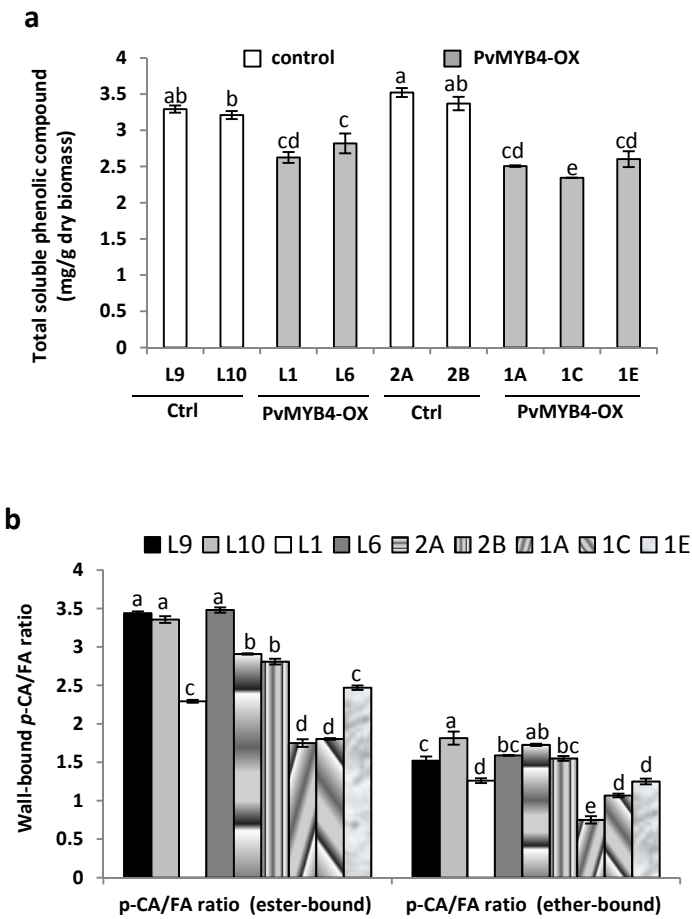


Fig. S3. Comparisons of methanol- (blue), 0.1 M NaOH- (red) and 2.0 M NaOH- (green) extracted cell wall residues determined by solid-state ^{13}C CP/MAS NMR spectroscopy. Panels (a) and (b) show the NMR spectra for control line 2A and PvMYB4-OX line 1C, respectively.

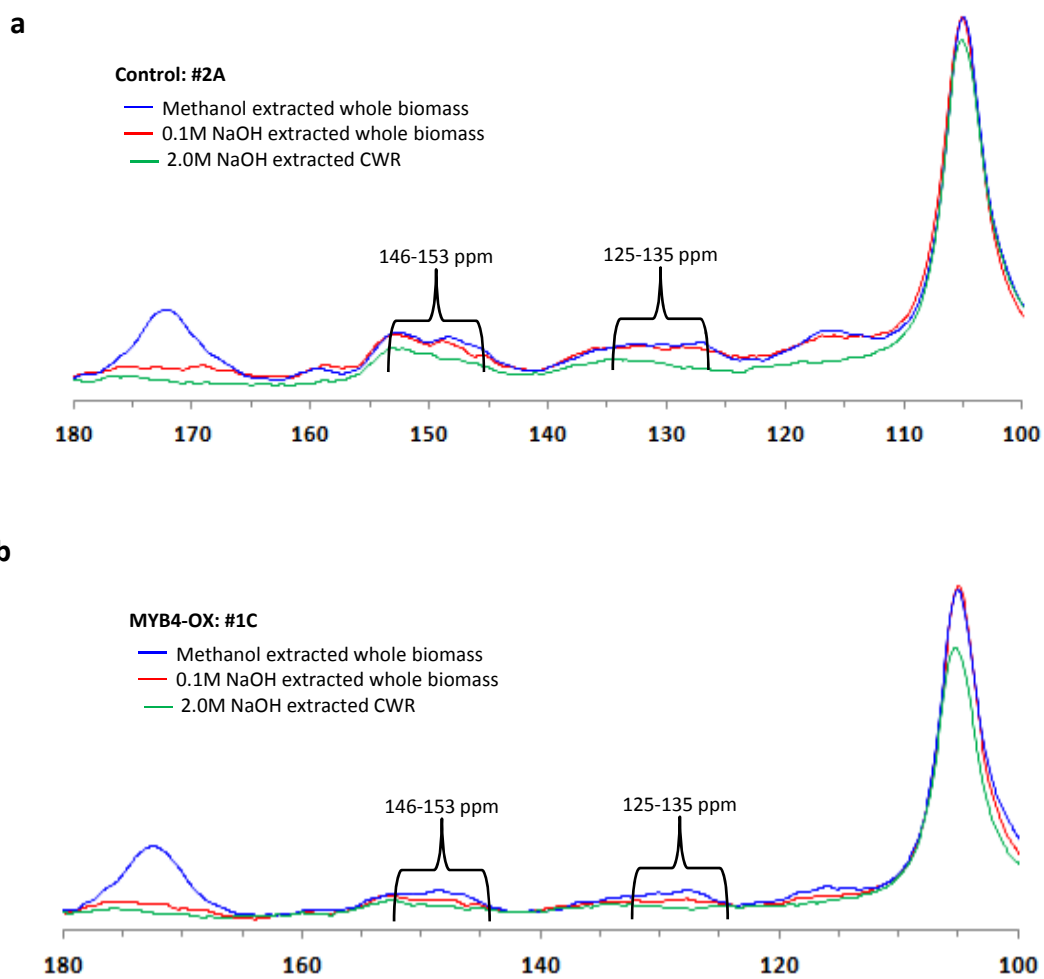


Fig. S4. Gel permeation chromatography analysis of isolated lignins from PvMYB-OX and control switchgrass.
Control lines: 2A and 2B. MYb4-OX lines: 1C and 1D.

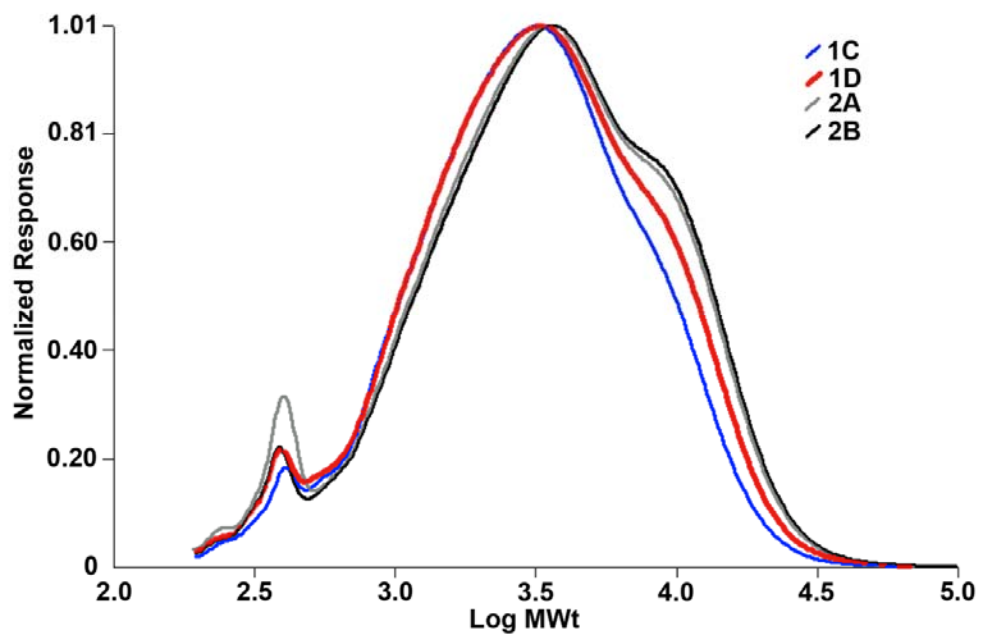
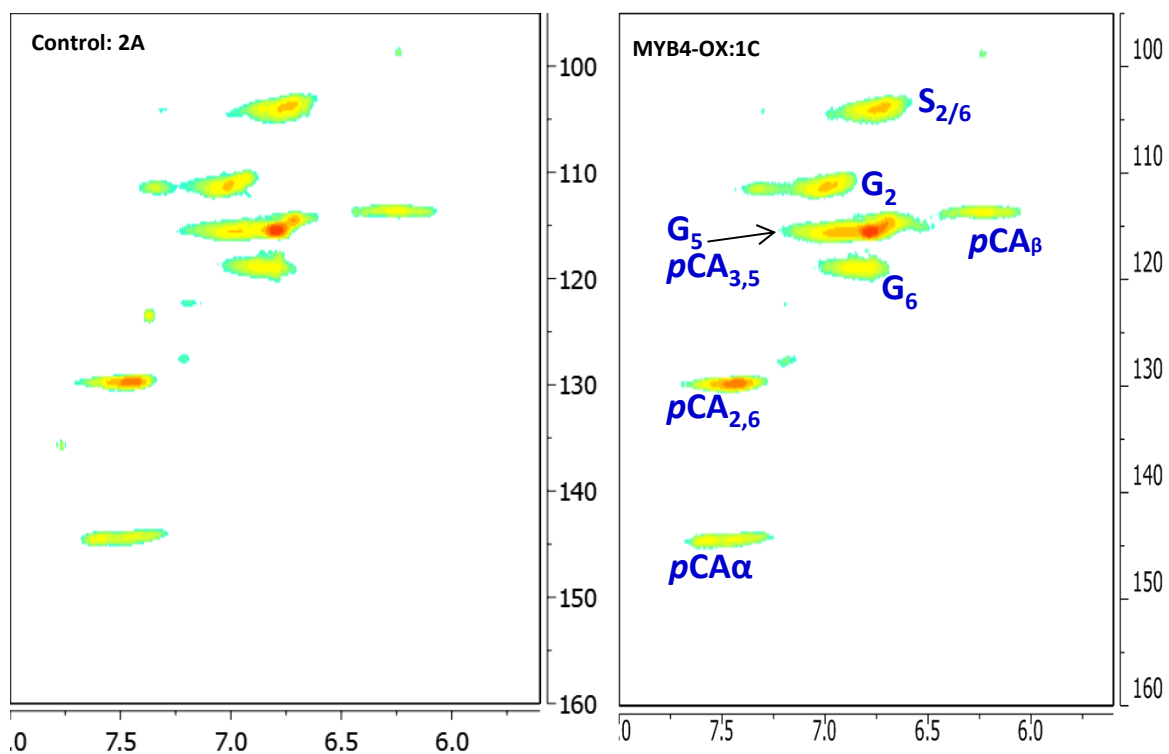


Fig. S5: Partial ^{13}C - ^1H HSQC spectra of milled lignins showing the C–H correlations from aromatic rings of the structural units.
pCA, *p*-coumaryl unit. G, coniferyl unit. S, sinapyl unit.



Aromatic regions of a ^{13}C - ^1H HSQC spectrum switchgrass lignin.

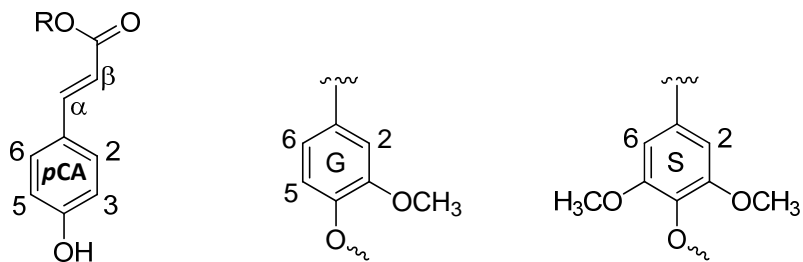


Fig. S6. Glycome profiling of extracts from alcohol insoluble cell wall residues from switchgrass.

Sequential extraction of alcohol insoluble cell wall materials from control and PvMYB4-OX transgenic lines was performed using a set of solvents with increasing strength, as indicated). The bars on the top panels of the figure indicates the amount of carbohydrates released by sequential extraction of the wall residues. The highlighted boxed areas show regions of difference between control and transgenics, and are featured in **Fig. 5** along with details of the antibodies.

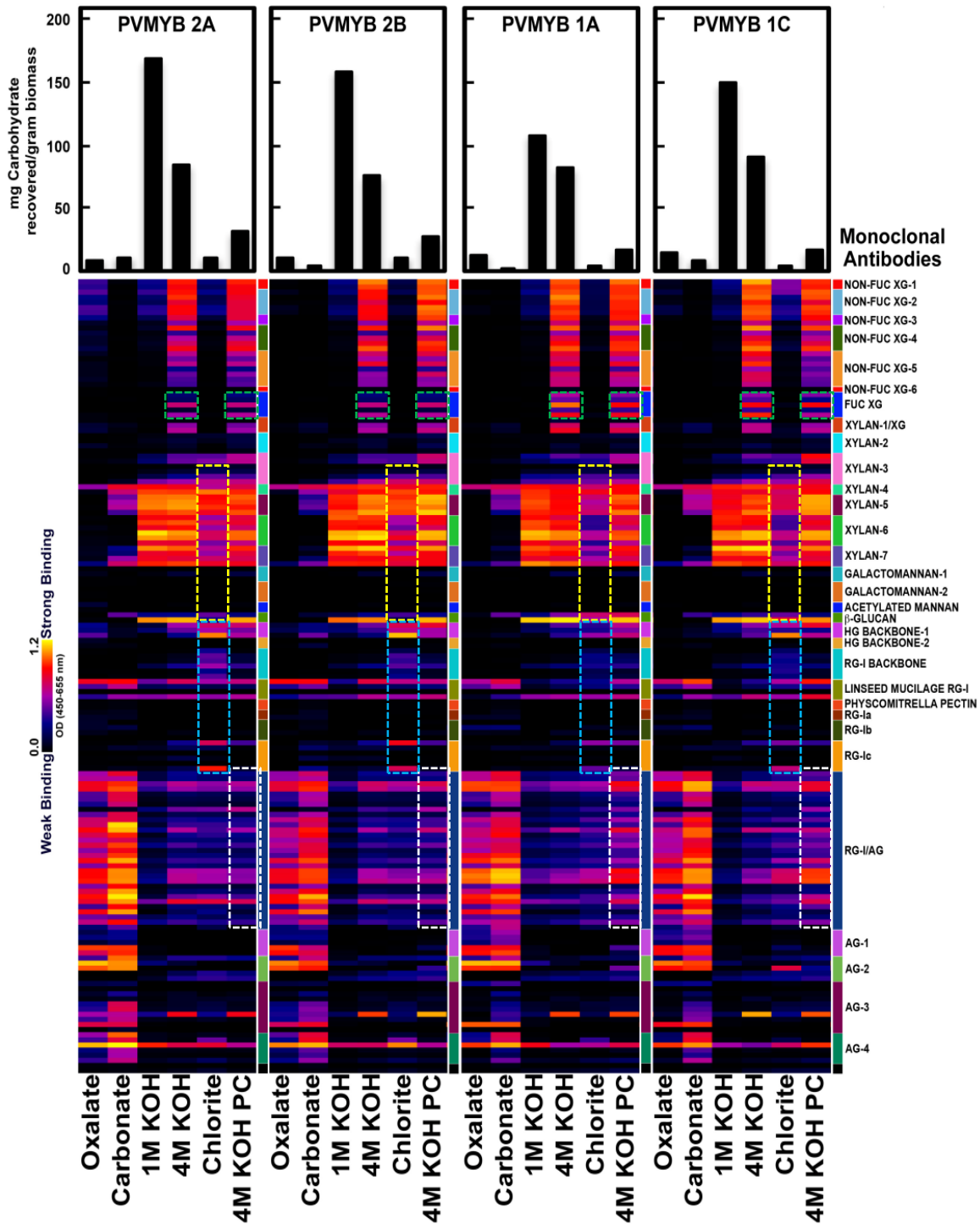


Table S1: Sugar composition (mg/g dry biomass) of whole tiller biomass determined by quantitative saccharification assay.

Lines	Glucose	Xylose	Galactose	Arabinose	Total sugars	Glucose (% of control)
Control (ST1)	339.2±7.1	158.0±10.1	20.8±0.5	29.4±0.4	549.3±17.1	100.0
L1	383.9±1.8*	161.8±1.1	28.2±0.5*	36.1±0.2*	613.1±3.2*	113.2
L6	319.7±12.2	129.0±13.5	16.9±0.7*	30.2±1.7	497.8±27.8	94.3
L8	351.3±1.4	157.8±4.2	16.6±0.1*	29.3±0.7	557.5±6.3	103.6
Control (ST2)	343.1±2.6	190.0±4.2	17.7±0.3	30.2±0.3	583.2±3.9	100.0
1A	320.2±25.4	142.6±24.7	25.0±1.6*	34.2±4.4	524.9±56.0	93.3
1B	359.0±1.4*	178.5±1.7*	24.7±0.1*	35.3±0.1*	601.1±3.0*	104.6
1C	353.6±3.3	169.1±0.9*	25.1±0.5*	34.5±0.2*	585.5±4.5	103.1
1D	344.1±3.3	179.5±2.4*	24.0±0.5*	34.1±0.2*	584.5±6.3	100.3
1E	371.8±3.7*	190.8±2.2	21.7±0.1*	34.0±0.4*	620.8±6.0*	108.4

Control for ST1 lines is the average of control lines L9 and L10. Control for ST2 lines is the average of control lines 2A and 2B. All data are means \pm SE (n=6 for control lines and n=3 for transgenic lines). Asterisks indicate values that were determined by the Student *t*-test to be significantly different from their equivalent control ($p<0.05$).

Table S2: Levels of total sugars (mg/g dry cell wall residues) from control and PvMYB4-OX switchgrass after removal of soluble sugars and starch.

Lines	Glucose	Xylose	Galactose	Arabinose	Total sugars	Glucose(% of control)
Control (ST1)	468.2±8.6	254.0±2.2	12.8±0.6	24.1±0.7	759.0±9.6	100.0
L1	474.0±7.9	261.4±0.5	14.7±0.1	29.7±0.2	779.8±8.8	101.2
L6	477.1±2.1	261.2±1.3	14.5±0.6	28.7±1.9	781.5±3.3	101.9
L8	472.3±5.1	259.2±0.2	13.0±0.7	25.8±2.3	770.3±2.3	100.9
Control (ST2)	426.1±5.7	270.5±3.2	12.1±0.3	25.0±0.9	733.7±8.0	100.0
1A	438.1±1.8	257.9±0.2	16.2±0.3	33.2±1.3	745.3±0.4	102.8
1B	443.9±1.8*	270.1±0.3	12.6±0.1	30.9±0.5	757.5±0.9	104.2
1C	448.2±0.0*	264.5±2.2	13.5±0.3	31.8±0.5	758.0±1.4	105.2
1D	444.8±7.2	274.5±4.3	13.1±1.1	31.3±3.9	763.7±6.5	104.4
1E	423.8±3.5	271.5±4.1	13.3±0.1	33.5±0.4	742.0±7.3	99.5

No significant differences among lines were detected. Control for ST1 lines is the average of control lines L9 and L10. Control for ST2 lines is the average of control lines 2A and 2B. All data are means \pm SE (n=4 for control lines and n=2 for transgenic lines). Asterisks indicate values that were determined by the Student *t*-test to be significantly different from their equivalent control ($p < 0.05$).

Table S3: Levels of pectin (mg/g alcohol insoluble cell wall residue) extracted from control and PvMYB4-OX switchgrass.

Lines	Water extracted	Na-AC/EDTA extracted	HCl extracted	Total pectin
2A (control)	0.73 ± 0.05	0.33 ± 0.14	5.93 ± 0.08	6.98 ± 0.18
1C	0.93 ± 0.04 *	0.52 ± 0.13 *	6.97 ± 0.17 *	8.42 ± 0.22 *
1D	0.77 ± 0.07	0.50 ± 0.13	7.10 ± 0.27 *	8.37 ± 0.34 *
L10 (control)	0.96 ± 0.04	0.40 ± 0.01	4.82 ± 0.12	6.14 ± 0.10
L1	1.04 ± 0.06	0.49 ± 0.23	7.13 ± 0.08 *	8.66 ± 0.32 *

All data are means ± SE (n=3). Asterisks indicate values that were determined by the Student *t*-test to be significantly different from their equivalent control ($p < 0.05$).

Table S4: Plant cell wall glycan-directed monoclonal antibodies (mAbs) used for glycome profiling analyses.

The groupings of antibodies are based on a hierarchical clustering of ELISA data generated from a screen of all mAbs against a panel of plant polysaccharide preparations^{1,2} that groups the mAbs according to the predominant polysaccharides that they recognize. The majority of listings link to the WallMabDB plant cell wall monoclonal antibody database (<http://www.wallmabdb.net>) that provides detailed descriptions of each mAb, including immunogen, antibody isotype, epitope structure (to the extent known), supplier information, and related literature citations.

1. Pattathil S, Avci U, Baldwin D et al. A comprehensive toolkit of plant cell wall glycan-directed monoclonal antibodies. *Plant Physiol* 2010; **153**:514-525.
2. Pattathil S, Avci U, Hahn MG Immunological approaches to plant cell wall and biomass characterization: glycome profiling. *Methods Mol Biol* 2012, **908**: 61-72.

Glycan Group	mAb Names	Glycan Group	mAb Names	Glycan Group	mAb Names	Glycan Group	mAb Names		
Non-Fucosylated Xyloglucan-1	CCRC-M95	Xylan-5	CCRC-M144	RG-Ia	CCRC-M5	Arabinogalactan-2	JIM14		
	CCRC-M101		CCRC-M146		CCRC-M2		JIM19		
Non-Fucosylated Xyloglucan-2	CCRC-M104		CCRC-M145	CCRC-M155	RG-Ib		JIM137	JIM12	CCRC-M133
	CCRC-M89		Xylan-6	CCRC-M153			JIM101	CCRC-M133	CCRC-M107
	CCRC-M93	CCRC-M151		CCRC-M61		Arabinogalactan-3	JIM4		
	CCRC-M87	CCRC-M148		CCRC-M30			CCRC-M31		
CCRC-M88	CCRC-M140	RG-Ic		CCRC-M23	JIM17		CCRC-M26		
Non-Fucosylated Xyloglucan-3	CCRC-M100		CCRC-M139	CCRC-M17	CCRC-M19		JIM15	JIM8	
	CCRC-M103		CCRC-M138	CCRC-M18	CCRC-M56	CCRC-M85	CCRC-M81		
Non-Fucosylated Xyloglucan-4	CCRC-M58		Xylan-7	CCRC-M160	Arabinogalactan-3	CCRC-M18	MAC266	PN 16.4B4	
	CCRC-M86	CCRC-M137		CCRC-M56		CCRC-M85			
	CCRC-M86	CCRC-M152		CCRC-M16		CCRC-M81			
	CCRC-M55	CCRC-M149		Arabinogalactan-4		MAC266			
CCRC-M52	Galactomannan-1	CCRC-M60	PN 16.4B4						
CCRC-M99		CCRC-M75	CCRC-M41		MAC207				
Non-Fucosylated Xyloglucan-5		CCRC-M54	CCRC-M70		CCRC-M80	JIM133			
		CCRC-M48	CCRC-M74	CCRC-M79	JIM13				
	CCRC-M49	Galactomannan-2	CCRC-M44	CCRC-M92					
	CCRC-M96		CCRC-M166	CCRC-M33	CCRC-M91				
	CCRC-M50		CCRC-M168	CCRC-M32	CCRC-M78				
CCRC-M51	CCRC-M174		CCRC-M13	Unidentified					
CCRC-M53	CCRC-M175	CCRC-M42	MAC265						
Non-Fucosylated Xyloglucan-6	CCRC-M57	Acetylated Mannan	CCRC-M24		CCRC-M97				
Fucosylated Xyloglucan	Xylan-1/XG		CCRC-M169		CCRC-M12	CCRC-M7			
			CCRC-M102	CCRC-M77	CCRC-M7				
			CCRC-M39	CCRC-M77	CCRC-M7				
		CCRC-M106	CCRC-M77	CCRC-M7					
Xylan-2	CCRC-M111	CCRC-M170	CCRC-M77	CCRC-M77					
	CCRC-M108	β-Glucan	LAMP	CCRC-M25					
	CCRC-M109		BG1	CCRC-M9					
	Xylan-3	HG Backbone-1	HG Backbone-2	CCRC-M131	CCRC-M128				
CCRC-M38				CCRC-M126					
JIM5				CCRC-M134					
CCRC-M117				CCRC-M125					
Xylan-4	CCRC-M113	JIM136	CCRC-M123	CCRC-M122					
	CCRC-M120	JIM7	CCRC-M122	CCRC-M121					
	CCRC-M118	RG-I Backbone	CCRC-M121	CCRC-M112					
	CCRC-M116		CCRC-M69	CCRC-M21					
CCRC-M114	CCRC-M35		JIM131						
CCRC-M154	CCRC-M36		CCRC-M22						
Xyloglucan-6	CCRC-M119	Linseed Mucilage RG-I	CCRC-M14	JIM1					
	CCRC-M115		CCRC-M129	CCRC-M15					
	CCRC-M110		CCRC-M72	CCRC-M8					
	CCRC-M105		JIM3	JIM16					
Xyloglucan-6	CCRC-M117	Physcomitrella Pectin	CCRC-M40	JIM93					
	CCRC-M113		CCRC-M98	JIM94					
	CCRC-M120		CCRC-M94	JIM11					
	CCRC-M118		CCRC-M94	MAC204					
Xyloglucan-6	CCRC-M116	CCRC-M94	CCRC-M161	JIM20					
	CCRC-M114	CCRC-M94	CCRC-M164						
	CCRC-M154								
	CCRC-M150								