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Supplementary Information for

Mechanistic Insights from Plant Heteromannan Synthesis in Yeast

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Materials and Methods

Cloning of Plant Genes and *Pichia* Transformation

Plant genes were cloned using the EasySelect *Pichia* Expression Kit (Invitrogen), according to the manufacturer's manual. Full-length cDNA sequences, including their native stop codons, were inserted into the multiple cloning site of *pPICZ B*-based vectors (Invitrogen) and transformed in *E. coli TOP10F'* cells for propagation. The *pPICZ B* vector contains a methanol-inducible *pAOX1* promoter for recombinant protein expression and a constitutively expressed marker for Zeocin selection in bacteria and yeast (Fig. S4*A*). The following transcripts were amplified using the primers listed in Supplemental Table S3 from their native tissues: *AkCSLA3* (GenBank: ADW77641.1) from the developing konjac corm (1), *AtCSLA2*, *AtMSR1* and *AtCSLA7* from developing Arabidopsis siliques (2). For *AtCSLA7*, "GCT" was added following the native start codon to ensure efficient translation initiation in yeast. The coffee *CcMANS1* and *CcMSR1* were synthesized in a codon-optimized form for *Pichia* expression (GeneArt), and without the restriction sites present in the *pPICZ B* vector. Multimeric gene constructs were assembled *in vitro* using an enhanced version of the method described in the Multi-Copy Pichia Expression Kit manual (Invitrogen). The *Pme*I site in *pAOX1* was domesticated (removed) using the Phusion Site-Directed Mutagenesis Kit (Thermo Fisher Scientific) to yield a *pPICZ X* vector (Fig. S4*B*), which was used to clone *MSR* genes. Additional site-directed mutagenesis experiments (including domestication of *BamH*I site in *AtCSLA7*) were performed using the same kit, and the primers listed in Table S3. Each *MSR* transcriptional unit, flanked by unique *Bgl*II and *BamH*I sites, was cut from *pPICZ X* and inserted into the *BamH*I site of a *pPICZ B* + *CSLA* vector (Fig. S4*B*).

Constructs were verified by colony PCR and Sanger sequencing using the vector- and gene-specific primers listed in Table S3. Transgenes were integrated into the *pAOX1* region of the *Pichia pastoris* X-33 genome via homologous recombination. *Pichia* cells were transformed via the condensed protocol (3), using 150 ng of linearized plasmid DNA. At least three independent *Pichia* colonies containing the desired transgene(s) were identified by genotyping using *pPICZ* vector and insert-specific primer combinations that would produce unique amplicons (Table S3).

Pichia Growth and Cell Wall Isolation

For each construct, cells from at least three independent *Pichia* transformants were grown in buffered glycerol-complex medium (BMGY) to accumulate biomass, and were then induced to express recombinant proteins for 24 h using buffered methanol-complex medium (BMMY), as described in the EasySelect *Pichia* Expression Kit (Invitrogen). Cells were grown at 30°C and 225 rpm, in 14 mL culture tubes or in 24-well plates (Fig. 5, Fig. S1*B*, Fig. S2, Fig. S4 and Fig. S5). In culture tubes, cells were grown overnight in 2 to 3 mL of BMGY, collected by centrifugation (2000 *g* for 2 min), and re-suspended in an equal volume of BMMY. To compare more strains in parallel, *Pichia* was cultured in multi-well plates as previously described (4), with minor modifications. Cells were grown in 600 µL of BMGY for around 60 h, which leads to glycerol depletion (4), and then the *pAOX1* promoter was induced by the addition of 600 µL of BMMY2 (4), containing 1% methanol (twice as much as in the regular BMMY medium).

After methanol induction, *Pichia* cells were transferred to 2 mL tubes and collected by centrifugation. For wall AIR isolation, cells were mechanically lysed with 1 mL of ethanol and glass beads (Sigma, Catalog number G8772) for 2:30 min at 30 Hz using a ball mill (Retsch Mill MM400). After centrifugation for 2 min at 10000 *g*, the supernatant was discarded and the pellet was washed further with 1 mL of chloroform:methanol (1:1 v/v), and finally with 1 mL of acetone. The resulting AIR material was dried, and directly used for carbohydrate analysis or for enrichment of HM polysaccharides. For Fig. S1*B*, glycans in 600 µL of the BMMY supernatant

(after pelleting cells) were precipitated with 1.4 mL of ethanol, and washed as described for the wall AIR material.

Enrichment of HM Polysaccharides

Mannosylated proteins were extracted from *Pichia* wall material with 1 M NaOH for 60 min at 75°C and 1400 rpm in a thermomixer (Eppendorf), as previously described (5). After neutralizing the solution with acetic acid, AKI polymers were pelleted at 20000 *g* for 2 min and washed with water to remove the alkaline-soluble carbohydrates. The resulting AKI material was resuspended in 600 µL of water by mixing at 30 Hz for 2:30 min using a ball mill, and used immediately for enzymatic digestion or stored at 4°C.

Yeast β-1,3-glucans were enzymatically removed from *Pichia* AKI material as previously described (5). Samples were mixed for 2 days at 37°C with at least 1 mg of Zymolyase 20T from *Arthrobactor luteus* (USBiological), in 100 mM potassium phosphate buffer (pH 7.0), containing sodium azide as a preservative. The resulting EM polysaccharides, which remain insoluble, were then washed twice with water.

Structural Analysis of Polysaccharides

Glycosidic linkages in *Pichia* material were analyzed by gas chromatography–mass spectrometry following derivatization of partially methylated alditol acetates as described (6), with modifications by (7). Carbohydrates were separated using a 5977A Series GC-MS system (Agilent) equipped with a SP-2380 capillary column (Supelco). Carbohydrate peaks were annotated based on their retention time and ion spectra relative to standards.

Monosaccharide composition was analyzed via high-performance anion-exchange chromatography coupled with pulsed electrochemical detection (HPAEC-PAD), as previously described (2, 8), on a 940 Professional IC Vario ONE/ChS/PP/LPG instrument (Metrohm) equipped with CarboPac PA20 guard and analytical columns.

For enzymatic characterization, AKI or EM polymers were incubated with 1 U of endo-1,4-β-Mannanase (Megazyme, E-BMABC) or Cellulase (Megazyme, E-CELBA) for 30 min at 40°C and 1000 rpm in a thermomixer (Eppendorf). Afterwards, the samples were centrifuged for 2 min at 20000 *g*, and 100 uL of the supernatant was used to quantify for the amount of sugar release. Total hexose sugars were quantified using the anthrone assay (9), and the monosaccharide composition of the solubilized material was determined by HPAEC-PAD.

For ^{1}H-NMR analysis, enriched HM polysaccharides were dissolved in 0.3 mL of 10% NaOD / D_2O solution (99.9% atom D), containing 0.05% (w/w) of 3-(trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salt. The ivory nut mannan, konjac glucomannan, yeast β-glucans, curdlan (all from Megazyme), cellopentaose, and cellulose (both from Sigma–Aldrich) standards were dissolved as described for the *Pichia* samples. The ^{1}H-NMR spectra were recorded on a Varian 400 MHz NMR spectrometer equipped with a ^{1}H/^{13}C probe at 298 K. All chemical shifts were referenced relative to 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid (0.00 ppm for ^{1}H). The NMR data was processed and analyzed using Bruker's Topspin 3.5 software.

Prior to SEC analysis, EM polymers were incubated for 16 h in 10% NaOH at 60°C and 1400 rpm in a thermomixer (Eppendorf). The material was then mixed for 10 min at 30 Hz using a ball mill, neutralized with acetic acid, and sonicated for a total of 120 min at 80°C (vortex mixing every 30 min). After centrifugation for 10 min at 10000 *g* the amount of polysaccharides in the supernatant was quantified with the anthrone assay. Solubilized EM polymers (180 to 300 µg) were injected into an NGC Quest 10 Plus Chromatography System (Bio-Rad) equipped with a Tricorn Superose 6 column (GE Healthcare) and a refractive index detector (RID 20A, Shimadzu). Water was used as a mobile phase, and an equal amount of each sample was also injected after digestion with 10 U of endo-1,4-β-mannanase (Megazyme, E-BMABC) for 30 min

at 40°C and 1000 rpm. Dextran standards of known molecular size were subjected to SEC similar to the *Pichia* samples.

Fluorescent Imaging of *Pichia* Cells

Polysaccharides in *Pichia* cell walls were labelled using a protocol previously used for plant seeds (10, 11) with modifications. After 24 h of methanol induction, 100 µL of *Pichia* cells were pelleted by centrifugation at 400 *g* for 2 min. Cells were resuspended in 100 µL of phosphate-buffered saline (PBS), pH 7.0 solution. After adding 0.6 µL of 2-mercaptoethanol and 1 µL of Zymolyase 20T (125 µg), cells were incubated for 20 min at 30°C, 1000 rpm in a thermomixer (Eppendorf) to partially remove native yeast polymers. The cells were pelleted and washed with PBS, before sequentially incubating them in antibody solutions as previously described (10). The LM21 and LM22 primary antibodies (PlantProbes, www.plantprobes.net), which are directed against HM polysaccharides (12), were diluted 1:5, while Goat anti-Rat IgG Alexa Fluor 488 (Invitrogen) secondary antibody was diluted 1:50. Native polysaccharides in the yeast wall were counterstained with 1% (w/v) calcofluor white for 60 min, and then rinsed well with PBS.

The plasma membrane of cells expressing sfGFP or sfGFP-AkCSLA3 proteins was stained with the FM4-64 dye (Invitrogen) immediately before imaging, as previously described (13).

Fluorescent signals were acquired with a Leica DM2000 microscope equipped with pE-300 white (CoolLED) illumination and an L5 filter cube (Leica), or with a Leica TCS SP8 STED 3X confocal system. On the Leica SP8 system, the following parameters were used to detect the different fluorophores: calcofluor (405 nm excitation, 405 to 450 nm emission); sfGFP and Alexafluor 488 (488 nm excitation, 500 to 530 nm emission); FM4-64 (552 nm excitation, 590 to 700 nm emission). For each experiment, images were processed uniformly using the Fiji software (14).

Quantitative reverse transcription PCR (RT-qPCR)

Pichia cells were grown in the 24-well plate format described above. After 24 h of methanol induction, 150 uL of cells were transferred to new tubes for RNA extraction, and the rest of the cell culture was used for wall carbohydrate analysis. Total RNA was isolated using the YeaStar RNA Kit (Zymo, R1002), according to the manufacturer's instructions, including on-column digestion with DNase I Set (Zymo, E1010). The eluted RNA was used immediately or stored at -80ºC. To remove any remaining DNA contamination, at least 1 µg of eluted RNA was further treated with DNase I in a new tube and purified using the RNA Clean & Concentrator™-5. The final concentration and purity of nucleic acids was measured using the Eppendorf BioSpectrometer, and Qubit dsDNA and RNA high sensitive kits. First-strand cDNA was synthesized from 200 ng of RNA using the iScript kit (BioRad, 170-8891).

RT-qPCR was performed on a MyiQ system using iQ SYBR Green Supermix (BioRad), according to manufacturer's protocol. Each 20 µL reaction contained 300 nM of F and R primers and 0.2 µL of first-strand cDNA. Negative controls with only RNA were also tested for each sample. The primers used for the RT-qPCR experiment are listed in Table S4. *Pichia ARG4*, argininosuccinate lyase (15) and *TAF10*, subunit of Transcription initiation factor TFIID (16), were previously validated as reference genes. Fold change gene expression, normalized to the geometric mean of *Pichia ARG4* and *TAF10*, was calculated using the Pfaffl method (17, 18).

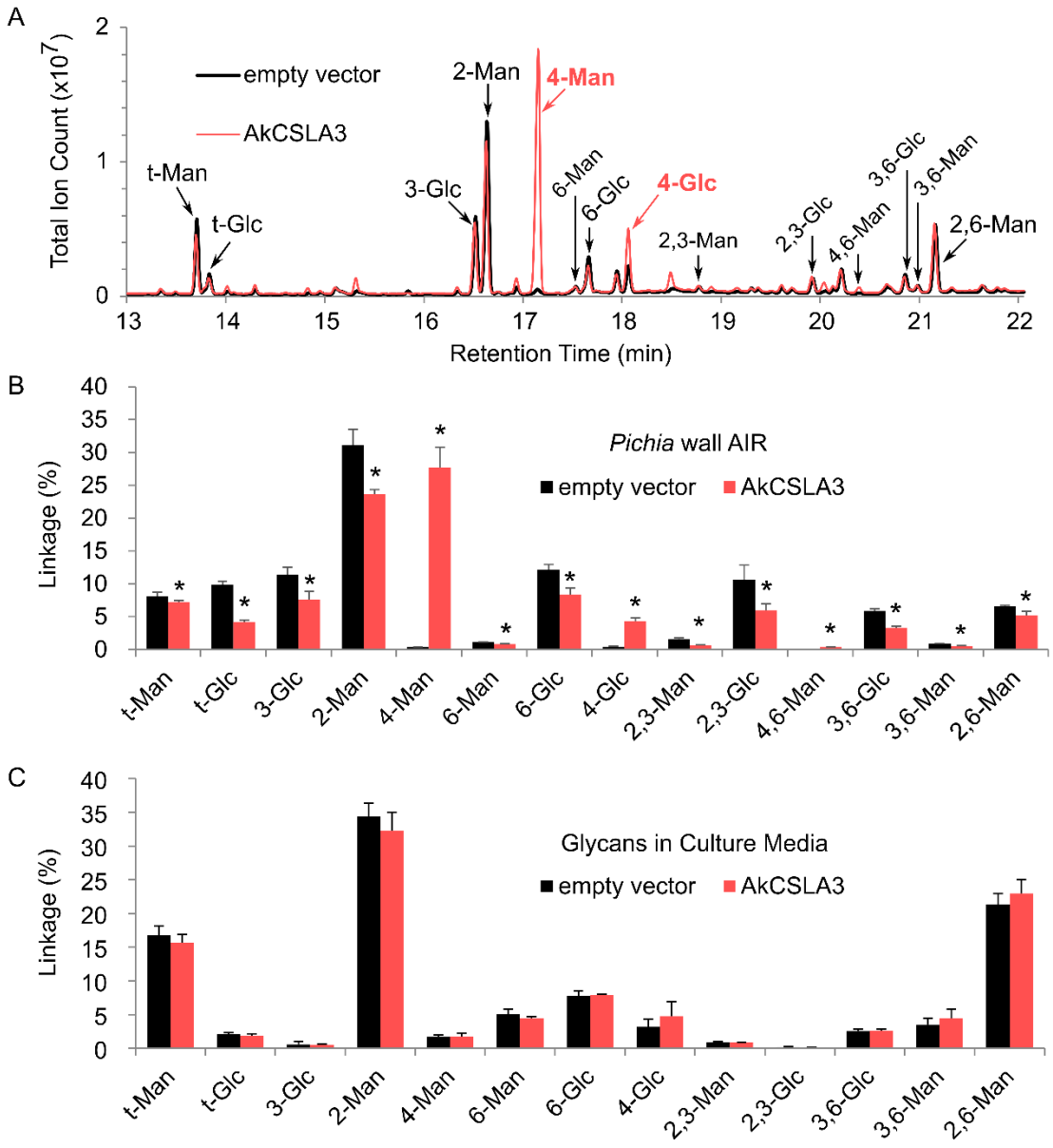

Fig. S1. Analysis of polysaccharides in *Pichia* cells and growth media. (*A*) GC-MS chromatogram of the cell wall glycosidic linkages (quantified in Fig. 1) produced by the empty vector control and the konjac AkCSLA3. Non-labelled peaks could not be assigned to carbohydrate derivatives. (*B*) Linkage composition of the cell wall alcohol-insoluble residue (AIR). Data show mean + SD of 6 independent transformants for each genotype. (*C*) Linkage composition of the ethanol-precipitable glycans secreted into the culture media by *Pichia* cells. Data show mean + SD of 3 biological replicates. In (*B*) and (*C*), asterisks mark significant changes between the empty vector control and AkCSLA3 strain (two-tailed *t*-test, $P < 0.05$).

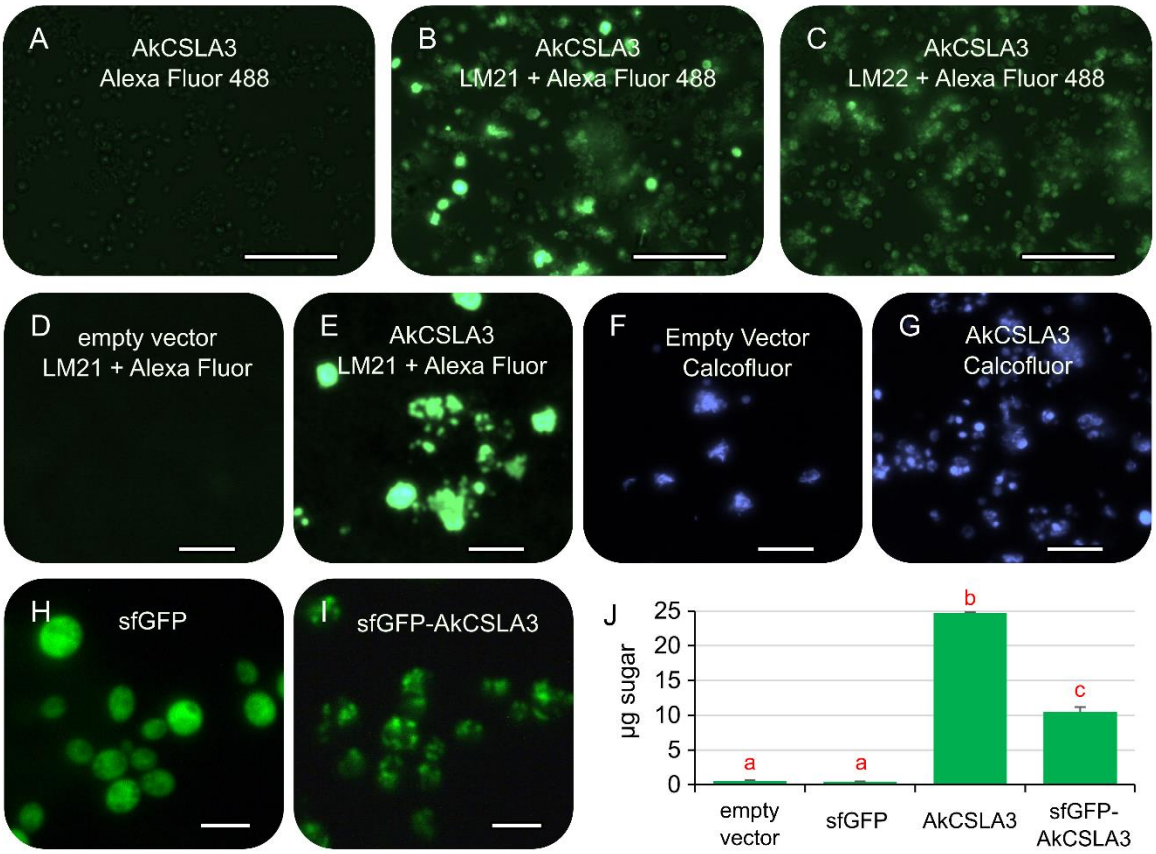

Fig. S2. Imaging of polysaccharides in the *Pichia* cells. (*A*) Yeast cells expressing AkCSLA3 immunolabeled without a primary antibody. (*B*) LM21 and (*C*) LM22 immunolabeling of the AkCSLA3 cells. (*D*) and (*E*) LM21 labelling on empty vector strain and AkCSLA3 expressing strain. (*F*) and (*G*) Calcofluor labelling of both *Pichia* strains. (*H*) and (*I*) Imaging of Pichia cells expressing super folder green fluorescent protein (sfGFP) or sfGFP-tagged AkCSLA3, respectively. (*J*) Amount of sugar released by β-1,4-mannanase from enriched HM polysaccharides extracted from *Pichia* cells (see Fig. S3 for details) imaged in panels (*H*) and (*I*). Data show mean + SD of 3 technical replicates. Different letters indicate significant changes based on one-way ANOVA with post-hoc Tukey HSD Test ($P<0.01$). (Scale bars: *A* to *C*, 50 µm; *D* to *I*, 10 µm).

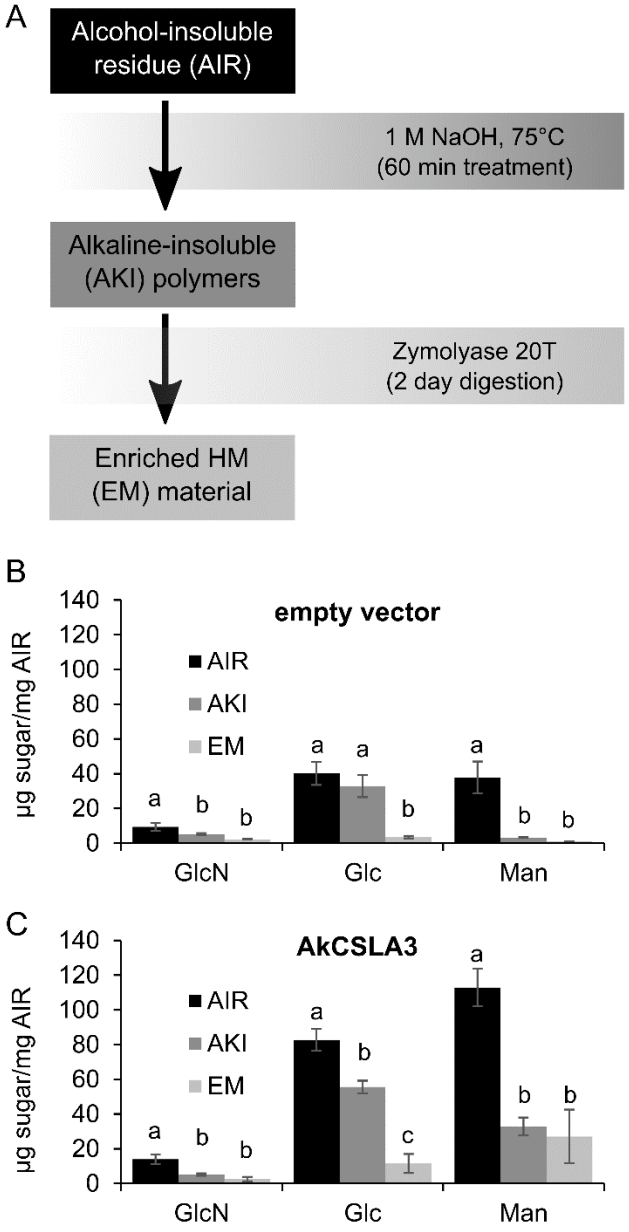

Fig. S3. Chemical and enzymatic enrichment of HM polymers from the *Pichia* cell wall. (*A*) Strategy for enriching HM polysaccharides from *Pichia* wall AIR. (*B*) and (*C*) Monosaccharide composition of AIR material, and sequentially isolated AKI and EM fractions from empty vector and AkCSLA3 strains, respectively. Data show the mean ± SD of 3 biological replicates. Glucosamine (GlcN) is derived from yeast chitin. For each sugar in panels (*B*) and (*C*), distinct letters indicate significant changes between different wall fractions based on one-way ANOVA with post-hoc Tukey HSD Test ($P < 0.01$).

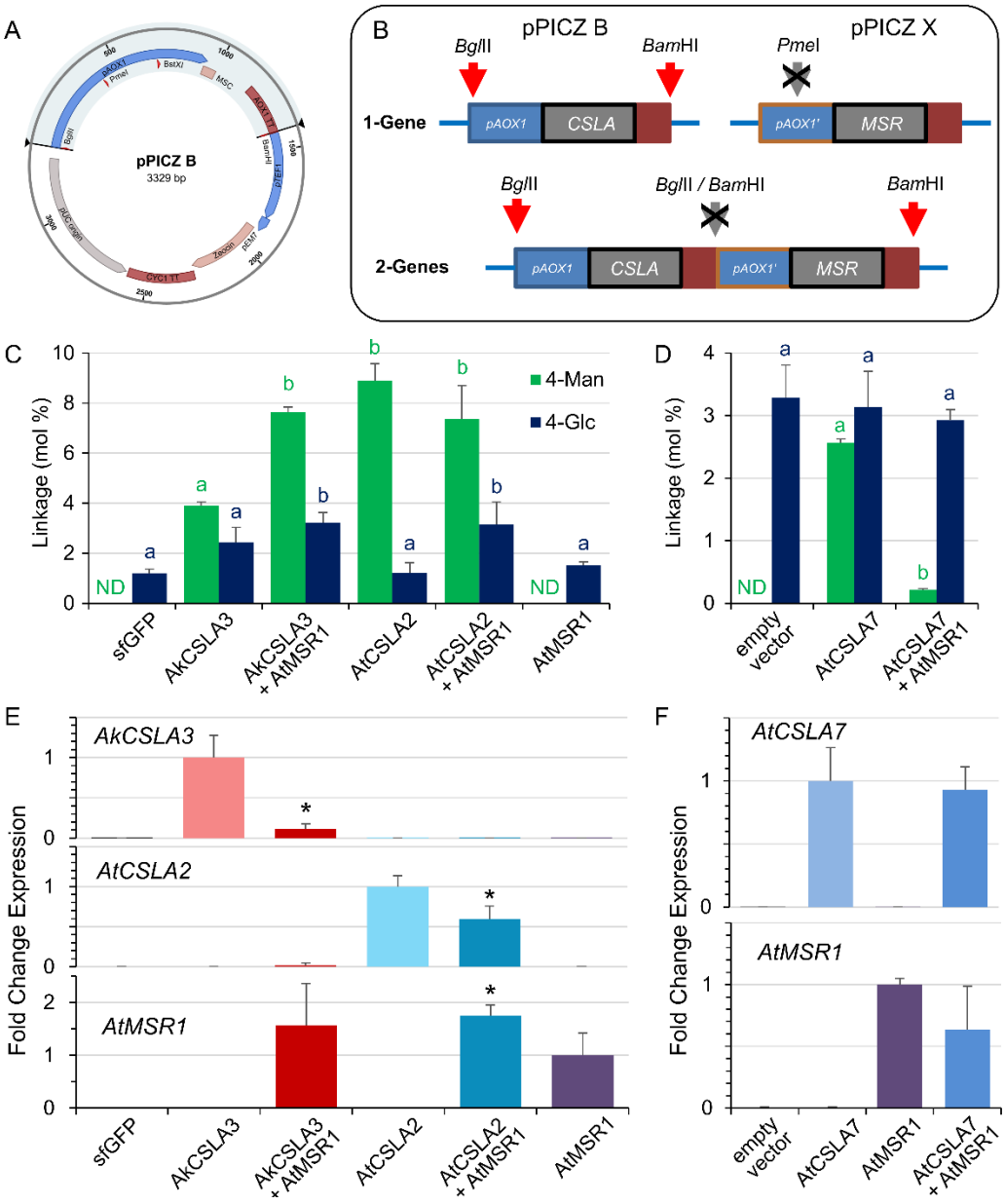

Fig. S4. *Pichia* multimeric gene constructs. (*A*) Graphic map of the pPICZ B vector. The *Bgl*II-*Bam*HI cassette contains a full transcriptional unit. (*B*) The *Pme*I site in the pPICZ B vector was removed to produce the pPICZ X entry vector. Two distinct transcriptional units cloned in the pPICZ B and pPICZ X vectors can be joined using *Bgl*II / *Bam*HI complementary ends. (*C*) Glycosyl linkage analysis of *Pichia* wall AIR. (*D*) Glycosyl linkage analysis of AKI polymers. Values represent the molar percentage of total carbohydrate linkages detected, and show mean + SD of 3 biological replicates (only 2 for AtCSLA7 and AtCSLA7 + AtMSR1). For each linkage, distinct letters indicate significant changes in one-way ANOVA with post-hoc Tukey HSD Test ($P < 0.05$). (*E*) and (*F*) Transcript levels quantified with RT-qPCR. The expression of each gene of interest was normalized to *Pichia ARG4* and *TAF10* reference genes and set as 1.0 in the respective single gene strain. Data show mean + SD of three biological replicates. Asterisks mark a significant change relative to the respective single gene strain (two-tailed *t*-test, $P < 0.05$).

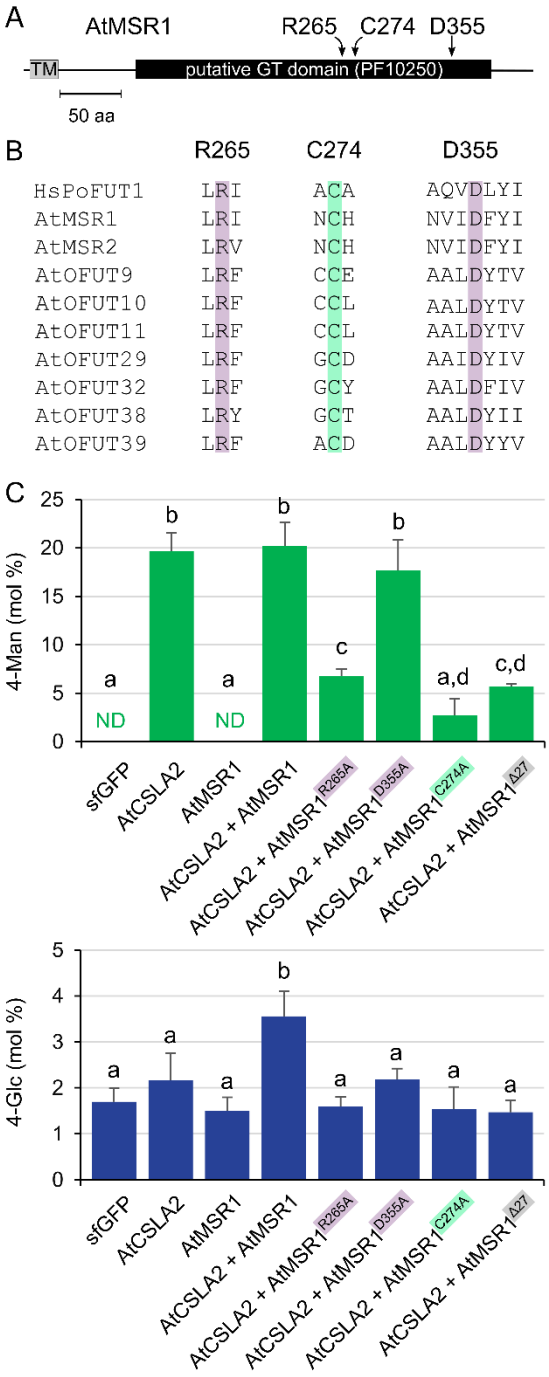

Fig. S5. Characterization of conserved AtMSR1 amino acids. (*A*) Features of the AtMSR1 protein, including conserved residues. TM – transmembrane domain. (*B*) Alignment of AtMSR1 and related Arabidopsis proteins to the human protein o-fucosyltransferase1 (HsPoFUT1), showing three conserved amino acids. (*C*) Effect of missense mutations and TM deletion (Δ27) on AtMSR1 function. Abundance of 4-Man and 4-Glc linkages in AKI polymers, as a molar percentage of total carbohydrates detected. Data show mean + SD of 3 biological replicates. ND, not detected. Distinct letters indicate significant changes in one-way ANOVA with post-hoc Tukey HSD Test ($P < 0.05$).

Table S1. Glycosidic linkage composition of HM produced in *Pichia*. Table shows the full dataset for the enriched mannan (EM) material analyzed in Fig. 3*C*. Values represent molar percentage of total carbohydrates detected. "Others" include trace amounts of 3,6-Man and 2,6-Man linkages. Data show mean ± SD of 4 technical replicates.

	empty vector	**AkCSLA3**	**AkCSLA3 + AtMSR1**	**AtCSLA2**	**AtCSLA2 + AtMSR1**
t-Man	1.7 ± 0.2	3.0 ± 0.6	1.6 ± 0.2	2.6 ± 0.2	1.3 ± 0.1
t-Glc	10.6 ± 0.4	2.9 ± 0.3	2.0 ± 0.3	2.3 ± 0.0	2.6 ± 0.0
3-Glc	4.6 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.8 ± 0.0	0.7 ± 0.0
2-Man	3.9 ± 0.2	1.5 ± 0.2	0.8 ± 0.1	2.2 ± 0.1	1.0 ± 0.1
4-Man	0.0 ± 0.0	66.0 ± 1.0	69.4 ± 0.3	74.2 ± 0.4	64.9 ± 0.3
6-Glc	51.4 ± 0.4	8.2 ± 1.3	4.6 ± 0.2	10.8 ± 0.1	9.4 ± 0.5
4-Glc	9.6 ± 0.2	14.5 ± 1.8	19.7 ± 0.2	2.5 ± 0.1	17.0 ± 0.6
3,6-Glc	14.8 ± 0.4	1.7 ± 0.4	0.6 ± 0.1	2.8 ± 0.0	2.1 ± 0.1
Others	3.4 ± 0.4	1.4 ± 0.2	0.8 ± 0.0	1.9 ± 0.1	1.1 ± 0.1

Table S2. Peaks in the ^{1}H-NMR spectra of HM produced in *Pichia*.
Standard polysaccharides and enriched mannan (EM) material from *Pichia* were analyzed using similar conditions. The data represent chemical shifts (ppm) of each peak. EM material from the empty vector control did not show any carbohydrate peaks. *Pichia* strains expressing *Arabidopsis thaliana* CSLA2 (AtCSLA2) or *Amorphophallus konjac* CSLA3 (AkCSLA3) were examined in two independent experiments (Exp 1 and Exp 2).

Polymer / *Pichia* Strain	H1	H2	H3	H4	H5	H6a	H6b
Mannan	4.67	4.04	3.70	3.80	3.42	3.70	3.85
Glucomannan							
β-1,4-mannan	4.68	4.04	3.70	3.80	3.42	3.70	3.85
β-1,4-glucan	4.46	3.29	3.44-3.62	3.44-3.62	3.44-3.62	3.73-3.91	3.73-3.91
Cellopentose	4.46	3.29	3.53	3.53	3.53	3.91	3.76
Yeast β-glucans							
β-1,3-glucan	4.70	3.46	3.61	3.39	3.39	3.92	3.61
β-1,6-glucan	4.42					4.29	
Curdlan	4.70	3.46	3.61	3.39	3.39	3.92	3.61
Exp 1 – AtCSLA2 (mannan**)**	4.68	4.04	3.70	3.80	3.42	3.70	3.85
Exp 1 – AkCSLA3							
β-1,4-mannan	4.67	4.04	3.70	3.80	3.42	3.70	3.85
β-1,4-glucan	4.46	3.30	3.44-3.62	3.44-3.62	3.44-3.62	3.73-3.91	3.73-3.91
Exp 2 – AkCSLA3							
β-1,4-mannan	4.67	4.04	3.70	3.80	3.42	3.70	3.85
β-1,4-glucan	4.46	3.29	3.44-3.62	3.44-3.62	3.44-3.62	3.73-3.91	3.73-3.91
Exp 2 – AkCSLA3 + AtMSR1							
β-1,4-mannan	4.67	4.04	3.70	3.80	3.42	3.70	3.85
β-1,4-glucan	4.46	3.29	3.44-3.62	3.44-3.62	3.44-3.62	3.85	3.85
Exp 2 – AtCSLA2 (mannan)	4.67	4.04	3.70	3.80	3.42	3.73-3.91	3.73-3.91
Exp 2 – AtCSLA2 + AtMSR1							
β-1,4-mannan	4.67	4.04	3.70	3.80	3.42	3.70	3.85
β-1,4-glucan	4.46	3.29	3.44-3.62	3.44-3.62	3.44-3.62	3.73-3.91	3.73-3.91

Table S3. Sequences of primers used for cloning, genotyping and sequencing. Primers are sorted based on their primary purpose. F or R denote the forward or reverse orientation. Introduced restriction sites are marked in bold, and modified bases are in red.

	Primer Target and Direction	5' to 3' Sequence
cDNA cloning	AkCSLA3 (ATG to TAG) F	atc**TTCGAA**ATGGCCATCGACTGG
	AkCSLA3 (ATG to TAG) R	tga**CACGTG**CTACTTTTCACTAGGAACAAAGG
	AtCSLA2 (ATG to TAG + 8 bp of 3' UTR) F	gta**TTCGAA**ATGGACGGTGTATCACCAAAG
	AtCSLA2 (ATG to TAG + 8 bp of 3' UTR) R	aat**CACGTG**CACAACTACTAACTCGGGACATAAG
	AtMSR1 (ATG to TGA) F	ga**TTCGAA**ATGGGTGTTGATTTGAGGCA
	AtMSR1 (ATG to TGA) R	gtt**CACGTG**TCAGCAAAAGCATGAATAAGCC
	AtCSLA7 (ATG, + gtc, to TAG) F	ta**CACGTG**atATGgctTCTCCTCTCCCAATCTTCC
	AtCSLA7 (ATG, + gtc, to TAG) R	gcctgttt**CACGTG**CTATGCTAAGTAAGAGGAAGCAGG
Sequencing + Genotyping	pPICZ primer F	GACTGGTTCCAATTGACAAGC
	pPICZ primer R	GCAAATGGCATTCTGACATCC
	AkCSLA3 R	TATTGCCATCTTCCTCACCAG
	AkCSLA3 F	GAGCAGGAGTCAGGCTCATC
	AtCSLA2 F	CGCCGGAATATGGAGAATAG
	pPICZ 3' end of pUC origin F	CTTTTCTACGGGGTCTGACG
	pPICZ B cloning site R	CACGTGAATTCCTCGTTTCG
	AtMSR1 R	ACAGGATCAGTTTCGCCATC
	AtMSR1 F	GGTGGCTAAGCATCTTGGAG
	AtCSLA7 R	GAACCCAAAGAAGGCAAATG
	AtCSLA7 F	TCCAGCCAGTAAGGAGTTGG
	CcMANS1 R	CGACGATTTTACGGACGAAG
	CcMANS1 F	AGAGTTGGCTTTGGTTCAGG
	CcMSR1 R	AGGATTCGTGCCATTCAGTC
	CcMSR1 F	TTCCTTGGACGGTGTTATCC
Site-Directed Mutagenesis	pPICZ B (no PmeI, 2 bp del) F	aaacgctgtcttggaacctaatatg
	pPICZ B (no PmeI, 2 bp del) R	actgtcagttttgggccatttg
	AtCSLA7 (no BamHI) F	ATGATTCCACGGAcCCAGCCAGTAA
	AtCSLA7 (no BamHI) R	CAAGCACTTGAATTATTATACGATTCGATGGC
	AtMSR1 (R265A) F	CATAGCAGTAGACCTTgcAATCGACATACTTG
	AtMSR1 (R265A) R	AAACGGCCACCGGATTTACGG
	AtMSR1 (D355A) F	GCTTATGTAGAAagCGATAACATTTTCGTACTCGG
	AtMSR1 (D355A) R	TCAAGAAGCGATGTCTTTGTTCCAGC
	AtMSR1 (C274A) F	GAGAAGAAAAATgctCATACAACTGGTGTAGTAGGG
	AtMSR1 (C274A) R	AAGTATGTCGATTCTAAGGTCTACTGCTATGAAACGG
	AtMSR1 (Δ27) F	gccTTCGAAATGGATTACTTTGATTCTCTTCAGGAG
	AtMSR1 (Δ27) R	gttCACGTGTCAGCAAAAGCATGAATAAGCC
Genotyping	pPICZ X mutated PmeI region F	TGACAGAAACGCTGTCTTGG
	AtCSLA2 multimer F	CAATGTCTTTCTTCATCAGTGG
	start of AtMSR1 R	AGCATCTGTCCAAGCATCAC
	binds start of CcMSR1 R	TCAGGATAGCAGCAACAACC
	binds end of CcMANS1 F	AGGCTGGTGCCTTCTTCAC

Table S4. Sequences of primers used for RT-qPCR.
For each primer, F or R indicates its orientation and the third column lists the sequence source.

Primer Target and Direction	5' to 3' Sequence	Design Tool / Reference
Pichia ARG4 F	TCCTCCGGTGGCAGTTCTT	Primer-BLAST (19)
Pichia ARG4 R	TCCATTGACTCCCGTTTTGAG	published sequence (15)
Pichia TAF10 F	ATTGAGGAAGAGCCCGAACC	published sequence (15)
Pichia TAF10 R	GCGTGAATTCTTCGTCCTCC	Primer-BLAST (19)
AkCSLA3 F	TGCATCTCCCGACAACCATC	Primer-BLAST (19)
AkCSLA3 R	TCCCGATCTCCAACAAACCG	Primer-BLAST (19)
AtCSLA2 F	TTGCGGCTTTCTTGTTCGTG	Primer-BLAST (19)
AtCSLA2 R	CATAAGTCCCGATCCAGCCC	Primer-BLAST (19)
AtMSR1 F	ATGACGCTGTATCATCGAAAGGG	Primer-BLAST (19)
AtMSR1 R	GCCACCATTACAGCATCAGTG	Primer-BLAST (19)
AtCSLA7 F	AAAGGCTCTGGTCATGGGCTTG	QuantPrime (20)
AtCSLA7 R	TAGCTCCAGCAAATGCACCCTCTC	QuantPrime (20)

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