Expression of a Trichoderma reesei β-1,4 endo-xylanase in tall fescue modifies cell wall structure and digestibility and elicits pathogen defence responses

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Expression of a *Trichoderma reesei* ß-1,4 endo-xylanase in tall fescue modifies cell wall structure and digestibility and elicits pathogen defence responses

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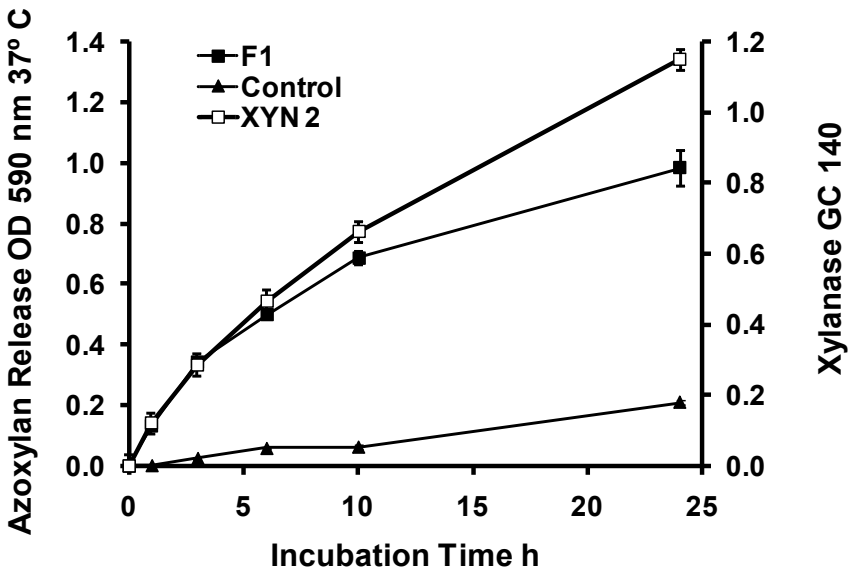
Supplementary material for online publication

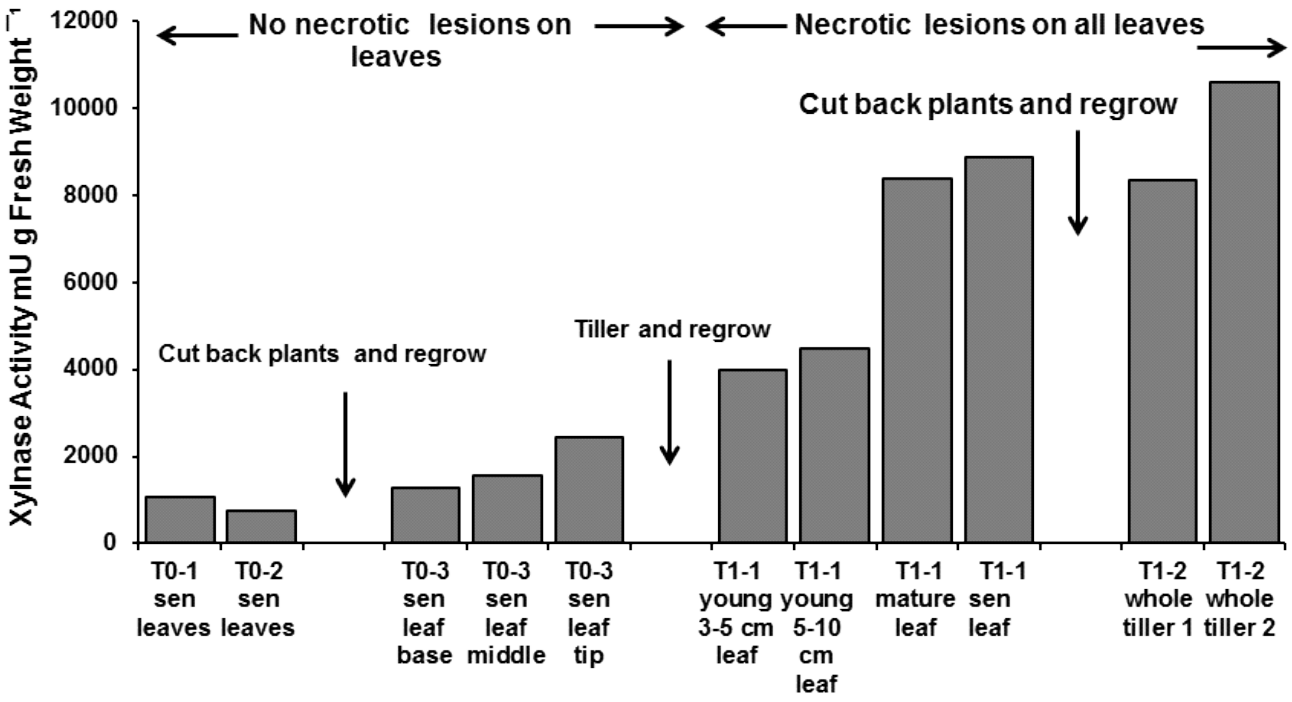
Figures

Fig. S1



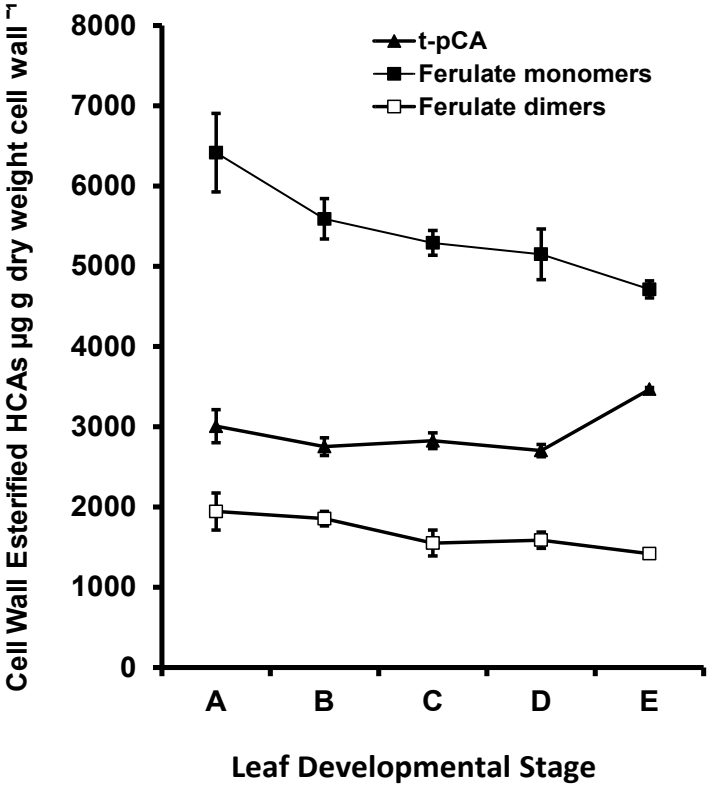
Supplementary Fig. S1. Kinetics of Azo-xylan release by recombinant GC 140 xylanase (XYL2) and leaf extracts of control plant and plant F1 constitutively expressing apoplast targeted xylanase under the actin promoter. Each data point was determined in triplicate. Mean ±sem (n=3).

Fig. S2



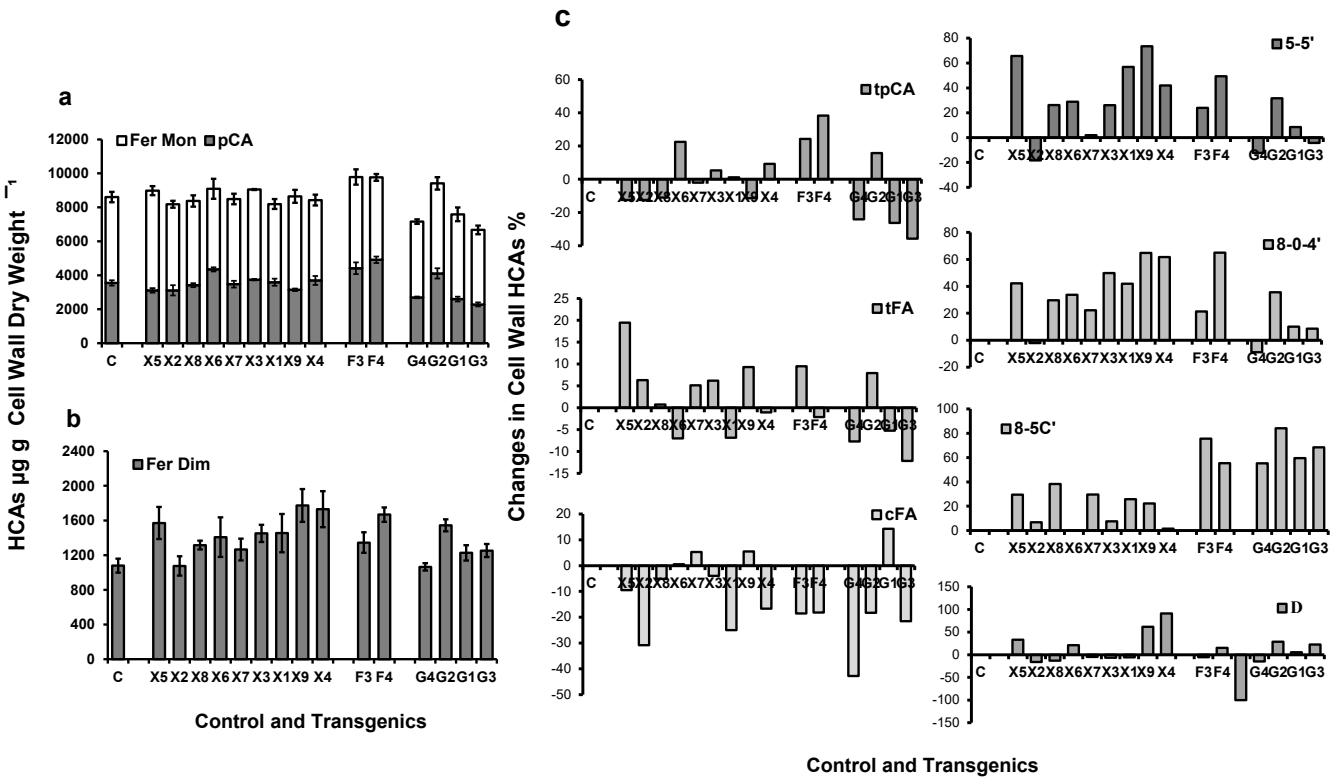
Supplementary Fig. S2. Xylanase activity in senescent leaves of plant X4 following repeated cut back and regrowth and in young and senescent leaves of first generation tillers.

Fig. S3



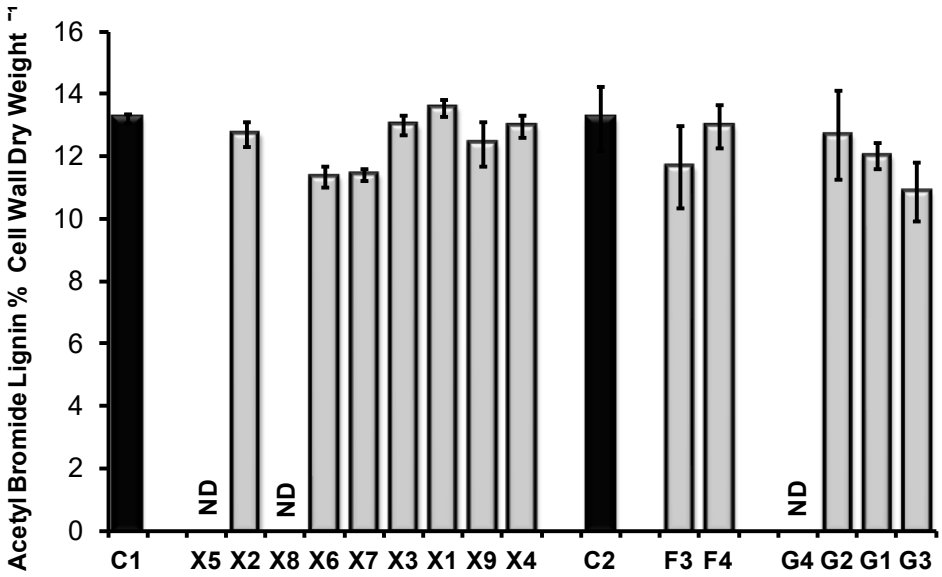
Supplementary Fig. S3. Levels of ester bound HCAs in isolated cell walls from control leaves at different stages of development: A - young leaves (9-14 cm); B - young leaves (20-25 cm); C – mature leaves (27-35 cm); D – fully developed leaves (40-43 cm); E- fully developed leaves with senescing tips. Monomers: Trans *p*-coumaric acid: tpCA; Trans-ferulic acid: tFA; cisferulic acid; Dimers: 8-0-4′-diferulic acid: 8-0-4′ DFA; 5-5′-diferulic acid: 5-5′ DFA; 8-5cyc-diferulic acid benzofuran: 8-5C DFA and unknown ferulate dimer quantified as for ferulic acid:D. Mean ± SEM (n=3).

Fig. S4



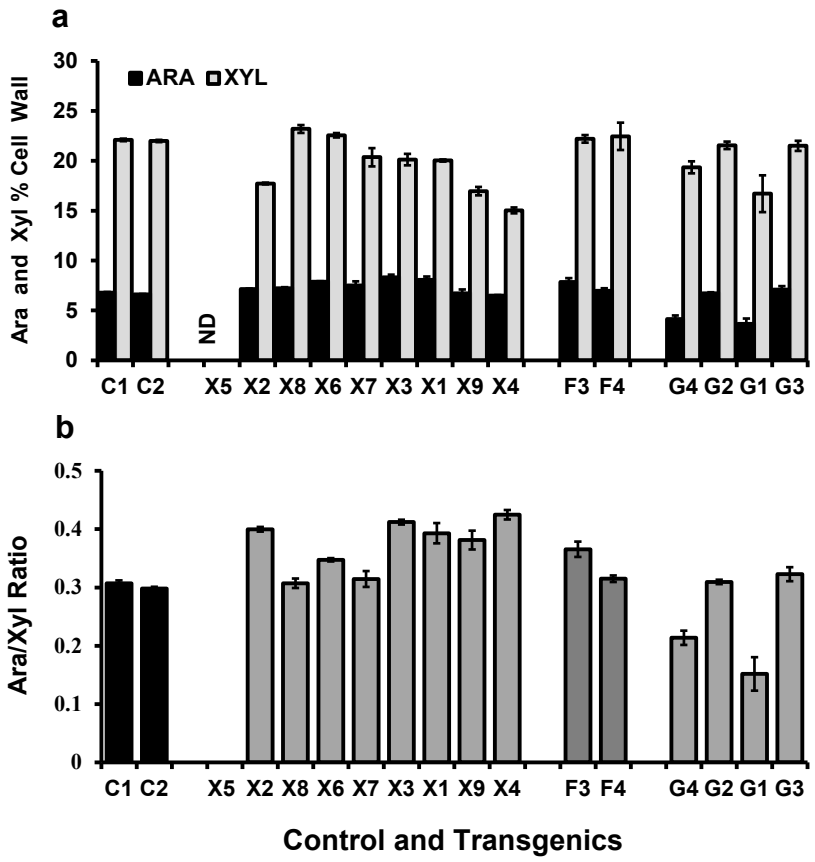
Supplementary Fig. S4. Levels of ester bound monomeric HCAs (a) and dimeric ferulates (b) in isolated cell walls from senescing apoplast targeted XYL2 plants (X1-X9) and (F3-F4) transformed with pIOM6 and pIOF, respectively and Golgi (G1-G4) plants transformed with pGX1, and changes in individual HCAs (c) compared to control plants. Monomers: Trans *p*coumaric acid: tpCA (A) Trans-ferulic acid: tFA (B), cis-ferulic acid (C); Dimers: 8-0-4- diferulic acid: 8-0-4′ DFA (D), 5-5′diferulic acid: 5-5′ DFA (E), 8-5cyc-diferulic acid benzofuran: 8-5C DFA (F) and unknown ferulate dimer quantified as for ferulic acid: D. Mean ± SEM (n=3).

Fig. S5



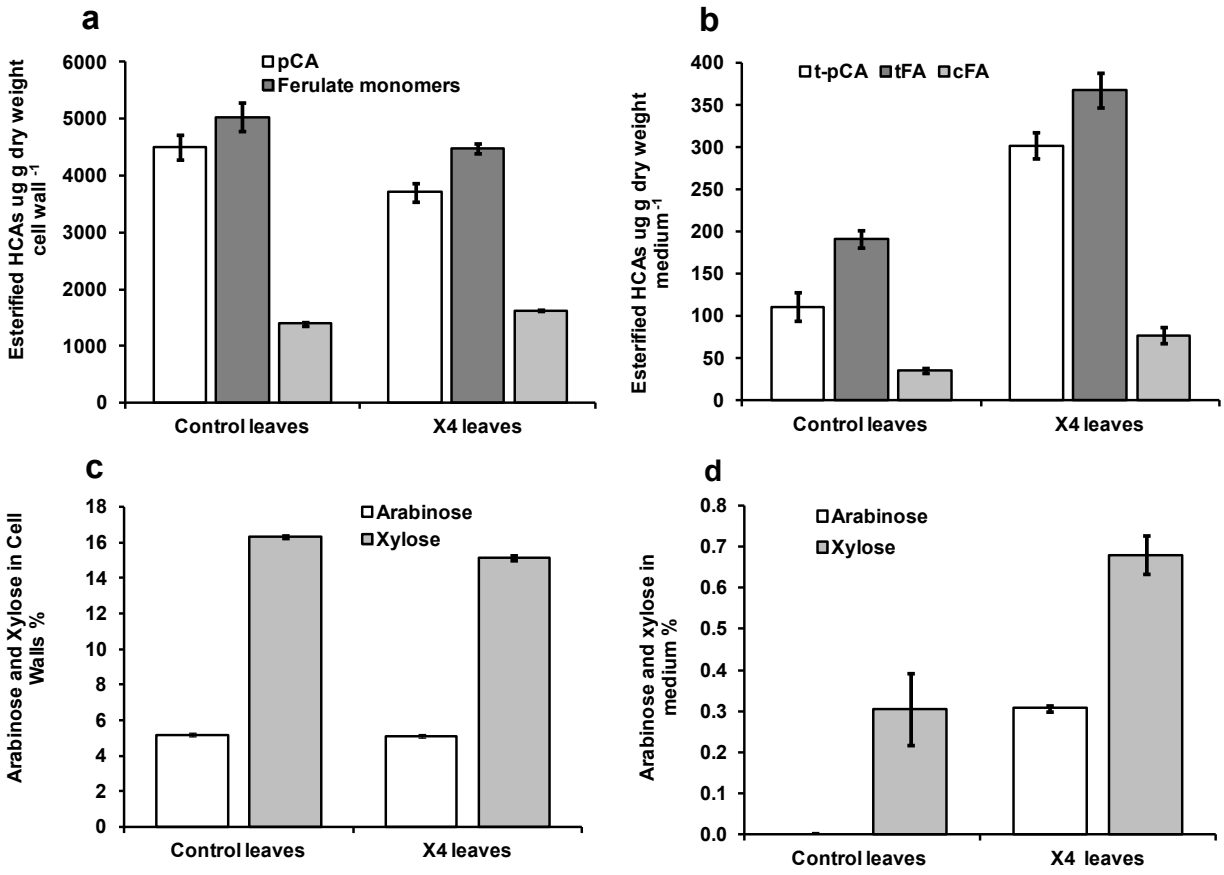
Supplementary Fig. S5. Acetyl bromide determination of the lignin content of leaves of senescing apoplast targeted XYL2 plants transformed with pIOM6 (X1-X9), young leaves of apoplast targeted XYL2 plants transformed with pIOF (F3-F4) and young leaves of Golgi targeted XYL2 plants transformed with pGX (G1-G4), compared to control non-transformed plants (C). Mean ± SEM (n=3) (ND = not determined).

Fig. S6



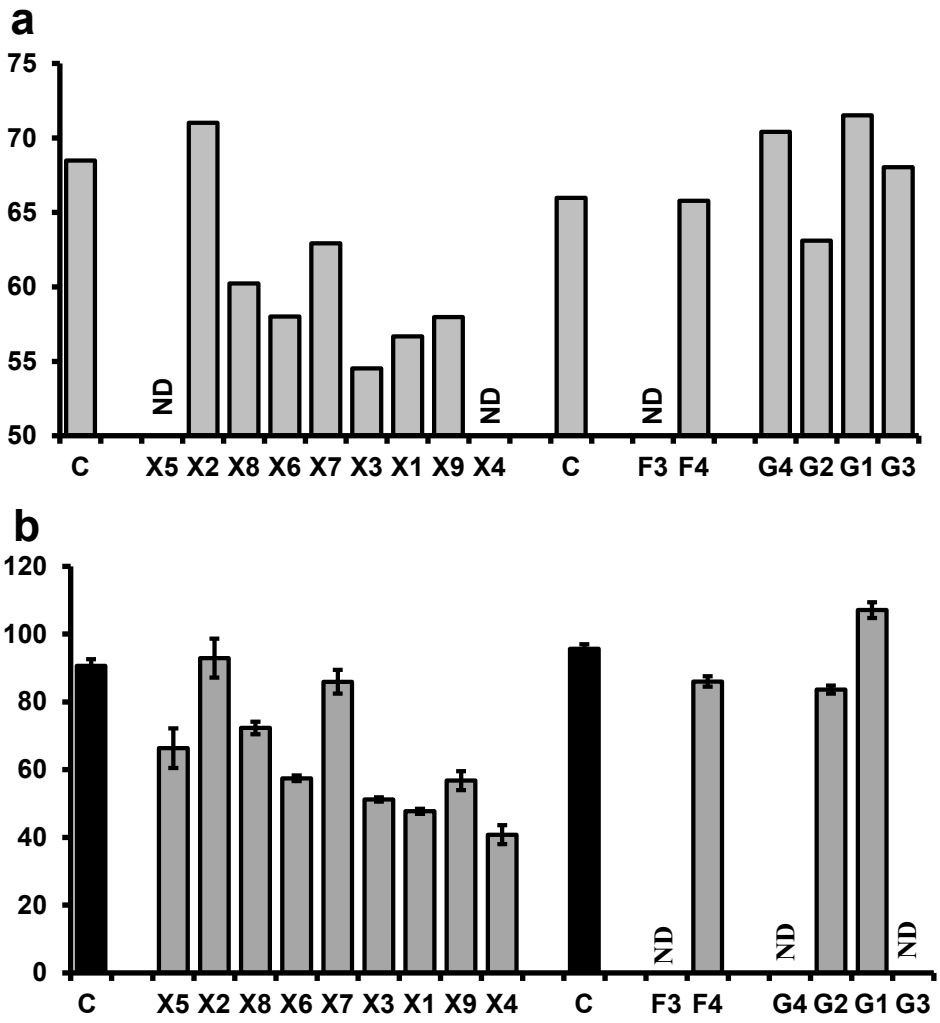
Supplementary Fig. S6. Cell wall arabinose and xylose polysaccharide composition (a) and the ARA/XYL ratio (b) of cell walls isolated from leaves of senescing apoplast targeted XYL2 plants (X1-X9) transformed with pIOM6, constitutive apoplast targeted XYL2 plants (F3-F4) transformed with pIOF and constitutive Golgi targeted XYN2 plants (G1-G4) transformed with pGX1, compared to control non-transformed plants (C). Mean ± SEM (n=3) (ND = not determined).

Fig. S7



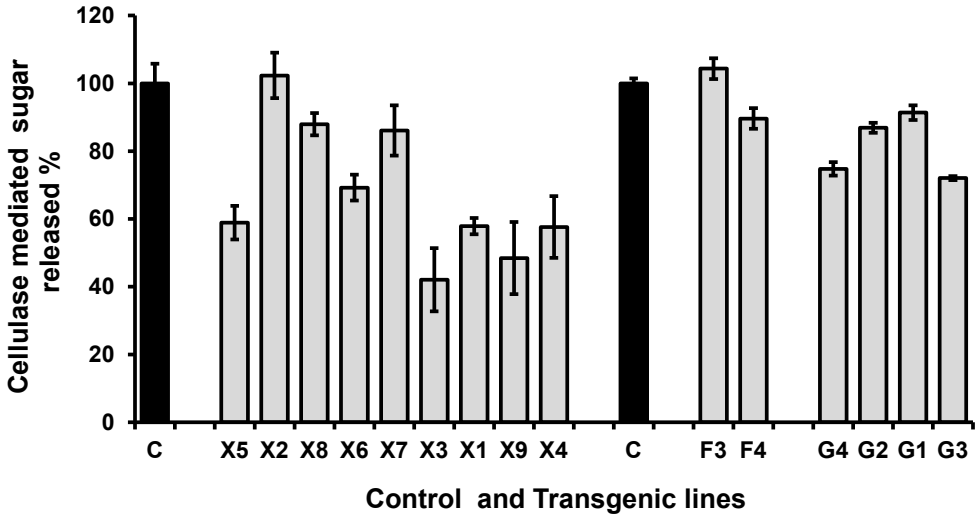
Supplementary Fig. S7 Esterified HCAs (a & b) and arabinoxylans (c & d) either remaining in the cell wall pellet (a & c) or solubilised into the medium (b & d) following auto digestion of leaves of plant X4 in the absence of exogenous FAE, followed by either NaOH hydrolysis (a & b) or TFA hydrolysis (c & d).

Fig. S8



Supplementary Fig. S8 *In vitro* dry matter digestibility (IVDMD) (a) and initial rate of digestion over 6h incubation under rumen conditions (b) of leaves of senescing apoplast targeted XYL2 plants transformed with pIOM6 (X1-X9), mature leaves of apoplast targeted XYL2 plants transformed with pIOF (F3-F4) and mature leaves of Golgi targeted XYL2 plants transformed with pGX (G1-G4), compared to control non-transformed plants (C). (ND = not determined).

Fig. S9



Supplementary Fig. S9 *T. viriee* cellulase-mediated release of sugars (glucose equivalents) (control = 100%) of leaves of senescing apoplast targeted XYL2 plants (X1-X9) and mature leaves of apoplast targeted xylanase plants (F3-F4) transformed with pIOM6 and pIOF and Golgi targeted XYL2 plants (G1-G4) transformed with pGX, compared to control non-transformed plants (C) after 24 h at 37º C [(mean ± SEM (n = 2)]. (ND = not determined).