Supplementary Material

Supplementary table 1. Analysis of CD spectra for Rex8A and Xyn8A wild type and mutant proteins using DICHROWEB[a]

Protein	α-helix (%)	β-sheet (%)	β-turn (%)	unordered (%)
Xyn8A WT	71 ± 6	9 ± 3	9 ± 4	10 ± 3
Xyn8A E104A	73 ± 1	10 ± 2	7 ± 1	8 ± 1
Xyn8A D164A	73 ± 1	12 ± 2	9 ± 2	6 ± 2
Xyn8A D303A	71 ± 3	11 ± 2	9 ± 1	9 ± 1
Rex8A WT	81 ± 2	6 ± 1	6 ± 2	6 ± 2
Rex8A E90A	81 ± 3	5 ± 1	7 ± 1	7 ± 4
Rex8A D148A	82 ± 2	5 ± 1	6 ± 2	5 ± 1
Rex8A D286A	82 ± 2	5 ± 2	6 ± 1	7 ± 1

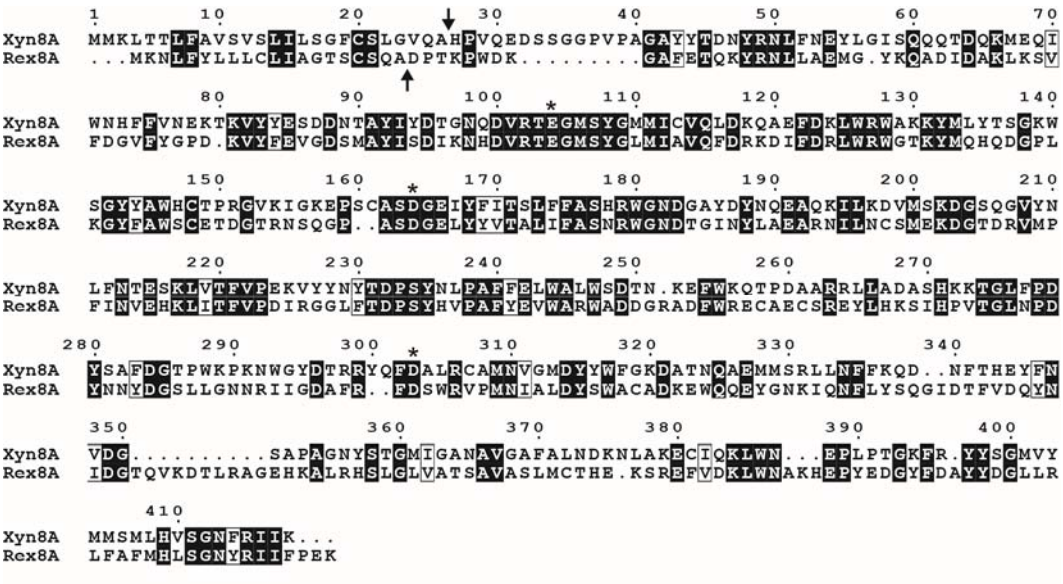
[a] CD spectra were recorded in the far-UV range utilizing a J-815 CD spectropolarimeter. Buffer conditions were 50 mM sodium phosphate, pH 6.0 for Rex8A and mutants, and 50 mM sodium phosphate, pH 6.5 for Xyn8A and mutants. The spectra were recorded from 190 to 260 nm at a scan rate of 50 nm/s and a 1-nm wavelength step with five accumulations. Each reaction was performed three times, and the data are means ± standard deviations of the means. The spectra were uploaded onto the DICHROWEB online server and analyzed as described in Experimental Procedures.

Supplementary figure 1. Amino acid sequence alignment for Rex8A (BACINT_00927) and Xyn8A (BACINT_04210). The amino acid sequences for the two proteins were aligned using ClustalW with the BLOSUM 62 similarity matrix and visualized using ESPript (http://espript.ibcp.fr/ESPript/ESPript/). Black boxes indicate amino acid identity and white boxes indicate amino acid similarity. Amino acids predicted to be important for catalytic activity and subsequently targeted for site-specific mutagenesis are indicated with an asterisk. Arrows indicate predicted signal peptide cleavage sites.

Supplementary figure 2. Optimal parameters for *B. intestinalis* Xyn8A, Rex8A, and Xyl3A activity. (A) To determine the optimal pH (i) for Xyn8A, xylopentaose (2 mM) was incubated

with Xyn8A (25 nM) at 37 °C for 5 minutes in buffers of various pH, ranging from 4.5 to 8.0. To determine the optimal temperature (ii) for Xyn8A, xylopentaose (2 mM) was incubated with Xyn8A (25 nM) at various temperatures ranging from 25 - 65 °C for 5 minutes in phosphate buffer (pH 6.5). (B) To determine the optimal pH (i) for Rex8A, xylotriose (2 mM) was incubated with Rex8A (25 nM) at 37 °C for 5 minutes at various pHs, ranging from 4.5 to 8.0. Buffers were as follows: 50 mM sodium citrate, 150 mM NaCl, pH 4.0 - 6.0; 50 mM sodium phosphate, 150 mM NaCl, pH 6.0 - 8.0; 50 mM Bis-Tris propane, 150 mM NaCl, pH 8.0 - 8.5. To determine the optimal temperature (ii) for Rex8A, xylotriose (2 mM) was incubated with Rex8A (25 nM) at various temperatures ranging from 25 - 65 °C for 5 minutes in phosphate buffer (pH 6.0). Hydrolysis products were separated using high performance anion exchange chromatography (HPAEC) and detected using a pulsed amperometric detector (PAD). The hydrolysis products were quantified by comparing the peak area and retention time to standard curves constructed with known concentrations of commercially available standards. (C) To determine the optimal pH (i) for Xyl3A, *para*-nitrophenol xylopyranoside (2 mM) was incubated with Xyl3A (250 nM) at 37°C for 10 minutes at various pH values, ranging from 4.0 to 8.5. Release of *para*-nitrophenol was monitored at 400 nm continuously using a Synergy II microplate reader. The extinction coefficient for *p*NP was determined at each pH by preparing dilutions of *p*NP in each of the separate buffers and measuring the absorbance at 400 nm. To determine the optimal temperature for Xyl3A, *para*-nitrophenol xylopyranoside (1 mM) was incubated with Xyl3A (250 nM) at various temperatures ranging from 25 - 65 °C for 5 minutes in phosphate buffer (pH 6.5). Release of *para*-nitrophenol was monitored at 400 nm continuously using a Cary UV-Vis spectrophotometer.

Supplementary Fig. 1



Supplementary Fig. 2