## **Supplemental Information**

**Supplemental Figure S1: Thin layer chromatography of EUR\_01860 enzyme hydrolysis products after 2 hours at 37°C.** EUR\_01860 (1µg/ml) hydrolysis of 5mM maltotetraose (G4), 5mM maltohexaose (G6) and 0.3% glycogen (Glyc). Lane C is a TLC control consisting of 2 nmol each of glucose (G1c), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), maltoheptaose (G7).

**Supplemental Figure S2: Sedimentation velocity results for maltooligosaccharide binding proteins from** *Eubacterium rectale*. Shown are van Holde - Weischet diffusion corrected sedimentation coefficient distributions for three mutants in the presence (circles) and absence (triangles) of maltooligosaccharide. Red: EUR\_01830 (maltotriose); blue: EUR\_01240 (glucose); green: EUR\_31480 (maltose). In all cases, the sedimentation coefficient distribution increased when the oligosaccharide was added, suggestive of maltooligosaccharide binding.

**Supplemental Table S1**: Complete list of proteins identified among all samples; ANOVA analysis p-value reflects statistical significance in protein quantities among the three groups of samples (APM, GLU and H7) See excel spreadsheet.

**Supplemental Table S2**: Supplemental Table S2: Complete list of proteins  $\geq$ 3-fold more abundant in APM or H7 compared to GLU ( $\geq$  5\* spectra counts). See excel spreadsheet.

**Supplemental Table S3**: Supplemental Table S3: Complete list of proteins  $\geq$ 3-fold upregulated in GLU compared to APM or H7 ( $\geq$  5\* spectra counts). See excel spreadsheet.

## Supplemental Table S4: Weight - average sedimentation coefficients for each proteins in the apo form and with 1 mM maltooligosaccharide.

Species:	М	s (apo)	s (ligand)	С (µМ)
EUR_01830	42.7	3.37 (3.39)	3.47 (3.51)	17.8 (6.51)
EUR_01240	43.4	3.63 (3.67)	3.89 (3.61)	14.5 (5.09)
EUR_31480	48.4	4.05 (4.07)	4.21 (4.21)	9.71 (3.26)

Values in parentheses reflect the results from the 3-fold lower concentration measurements. The slight increase in s seen in the lower concentration is due to decreased concentration-dependent non-ideality. Molar mass (M, g/mol) estimated from sequence, weight-average sedimentation coefficient (s x  $10^{-13}$ ) obtained from the enhanced van Holde – Weischet distributions.

## Supplemental Table S5: Primers

Name	Sequence	Construct/ purpose
EUR_01830_nhis	5'-CATCATCACCACCATCACGAGAACCTG TACTTCCAGGGCAGCGGCAACGGAGGAAATGGA-3'	EUR_01830 – pETiteNhis
EUR_01830_end	5'-GTGGCGGCCGCTCTATTATTTAAGAGAATCATTAAG-3'	
EUR_31480_nhis	5'- CATCATCACCACCATCACGAGAACCTGTACTTCCAGGGCGG AGACGGAACAGAGAAAGGCTC-3'	EUR_31480- pETiteNhis
EUR_31480_end	5'- GTGGCGGCCGCTCTATTACTGCTCGTTCTGAAGATCCCAG- 3'	
EUR_01240_Nhis	5'-CATCATCACCACCATCACGAGAACCTGTACTTCCAGGGC TCAGATGGGGGTTCTTCTGATAC-3'	EUR_10240- pETiteNhis
EUR_01240_end	5'-GTGGCGGCCGCTCTATTACTGGTTCAAAAGAGCTGTATA AG-3'	
EUR_21010_Nhis	5'-CATCATCACCACCATCACGAGAACCTGTACTTCCAGGGC GATACATCCGACACAGGAAGCTC-3'	EUR_21010- pETiteNhis
EUR_21010_end	5'- GTGGCGGCCGCTCTATTATTCTGAGATGTCAGGATATTTC-3'	
EUR_01860_Nnat	5'-GAAGGAGATATACATATGGGTGGAAAGAATACCGAGACG- 3'	EUR_01860- pETiteChis
EUR_01860_Chis	5'-GTGATGGTGGTGATGATGCTTCAAAACAAGCACCGAATA- 3'	
EUR_21100_130Nnat	5'-GAAGGAGATATACATATGGCTGCGAGCCCGACAATCCG-3'	EUR_21000- pETiteChis
EUR_21100_Chis	5'-GTGATGGTGGTGATGATGAGCTGCTTTTTTAGTGTCATTC- 3'	

EUR_21100_for	5'-GTAGTGAGATGCCGATTGAGC-3'	qPCR
EUR_21100_rev	5'-CGTATGTATCTGTACCACTTGC-3'	
EUR_01860_for	5'-GTGCTGCGGGTGGAAAGAATAC-3'	qPCR
EUR_01860_rev	5'-AATGCCGTCACCGTCACTGTC-3'	
EUR_01830_for	5'-AGCGGCAACGGAGGAAATGGA-3'	qPCR
EUR_01830_rev	5'-TTGCCTTAGCGAATGCCTCACACT-3'	
EUR_31480_for	5'-GGAAGAGAGAAAGGCTCATC-3'	qPCR
EUR_31480_rev	5'-CCATCAGCTGCTGTTACTAC-3'	
EUR_01240_for	5'-TCTTGGAACAGGCGTAGA-3'	qPCR
EUR_01240_rev	5'-CCATGTTCCGATTGCATTTC-3'	
GapA_for	5'-TGGTCGTATCGGACGTCTTGCTTT-3'	qPCR (normalizer)
GapA_rev	5'-TGCAGAAACCTCATCTGCATGCTC-3'	



Supplemental Figure S1



Supplemental Figure S2