

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURE LEGENDS

Suppl. Fig. S1. Wall extension activity of EXLX1 with a variety of substrates (A-D). At the point indicated with the arrow, EXLX1 was added at 200 µg/mL. Two representative responses are shown.

Suppl. Fig. S2. Purity of EXLX1 and variants visualized by Coomassie-stained SDS-PAGE. Ten µg of protein was loaded on each well. All EXLX1 variants were run on 10% acrylamide gels except for domains D1 and D2 which were run on a 15% gel.

Suppl. Fig. S3. EXLX1 binding to cell walls and wall components. A. Effect of alkali pretreatment on EXLX1 binding to wheat coleoptile cell walls. B. Effect of CaCl₂ on EXLX1 binding to insoluble arabinoxylan. In A and B, 12 µg/mL of EXLX1 were incubated in 0.3 mL of HEPES (25 mM), pH 7.5 containing 0.5 mg/mL of cell walls or 1 mg/mL arabinoxylan. Error bars indicate standard errors (3≤N≤6). C. EXLX1 binding to various cellulosic substrates. Fifty µg/mL EXLX1 were incubated in 0.4 mL of NaOAc (25 mM), pH 5.5 containing 25 mg/mL of cellulosic material. PASC: phosphoric acid-swollen Avicel, PASCf: phosphoric acid-swollen fibrous cotton fibers, filter paper: VWR Grade 43 filter paper. The low binding to cotton fibers may be due to differences in accessible surface area and cellulose crystallinity compared to other celluloses used here.

Suppl. Fig. S4. Randomly chosen positively charged amino acids in domain D2 were altered to glutamine and the effects of these changes on EXLX1 binding to cell wall components were studied. Lysines and asparagines in D2 that were altered to glutamine are shown in magenta (K145Q-K171Q-K188Q) and yellow (R173Q-K180Q-K183Q). W125 is shown in blue as a positional reference.

Suppl. Fig. S5. Alignment of domain D2 from EXLX1 with domain D2 from plant expansins and bacterial and fungal expansin-like sequences. The protein alignment and sequence logo were generated by MUSCLE and WebLogo servers respectively. The size of the amino acid letter in the sequence logo indicates the degree of conservation on a logarithmic scale. The GenBank database accession numbers of the protein sequences used in the alignment are as follows: NP_389744 (*Bacillus subtilis*), NP_638881 (*Xanthomonas campestris*), YP_050313 (*Pectobacterium atrosepticum*), YP_001220664 (*Clavibacter michiganensis*), YP_971728 (*Acidovorax citrulli*), XP_001274247 (*Aspergillus clavatus*), XP_367645 (*Magnaporthe grisea*), XP_001799311 (*Phaeosphaeria nodorum*), AAK56124 (*Zea mays*), P43213 (*Phleum pretense*), AAB60916 (*Arabidopsis thaliana* 1), AAB37749 (*Cucumis sativus*), NP_172717 (*Arabidopsis thaliana* 2), and NP_566197 (*Arabidopsis thaliana* 3). PDB numbers are given in parentheses when protein structures are available.

SUPPLEMENTARY TABLES

Suppl. Table S1. Primers used to generate EXLX1 variants.

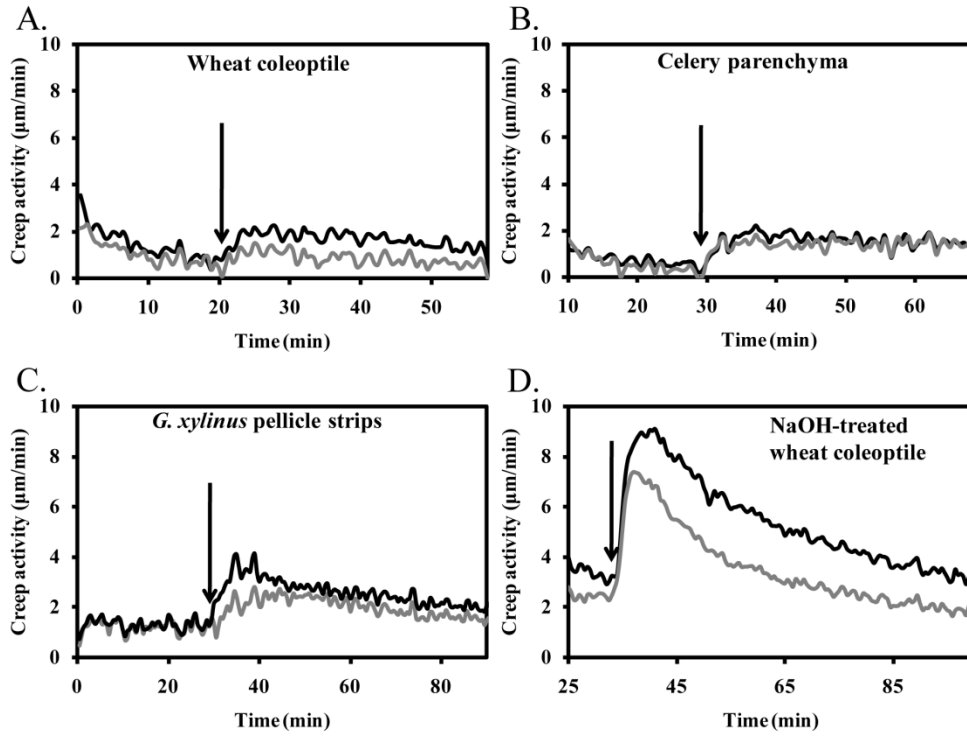
Amino acid change	Primer 1	Primer 2
T12A	5' gaaggttatgcagcgatacagggtcag 3'	5' ctgaccctgtatacgtgcataaccttc 3'
T14A	5' gcaacgtatgcagggtcaggc 3'	5' gcctgaccctgcatacgttgc 3'
S16A	5' caacgtatacagggcaggtattcagg 3'	5' cctgaatagctgccctgtatacgttgc 3'
D71A	5' cgtatatgttactgctctttatcccgaagg 3'	5' ccttcgggataaagagcagtaacatatacgc 3'
D71N	5' cgtatatgttactaatctttatcccgaagg 3'	5' ccttcgggataaagattagtaacatatacgc 3'
Y73A	5' gttactgatcttctcccgaaggcgc 3'	5' gcgccttcgggagcaagatcagtaac 3'
Y73F	5' gttactgatcttttcccgaaggcgc 3'	5' gcgccttcgggaaaaagatcagtaac 3'
E75A	5' gatctttatcccgcaggcgctcggg 3'	5' cccgagcgcctcgggataaagatc 3'
E75Q	5' gatctttatcccgaaggcgcctcggg 3'	5' cccgagcgccttgggataaagatc 3'
D82A	5' cggggagctcttctctgtcacctaag 3'	5' cattaggtgacagagcaagagctccccg 3'
D82E	5' cggggagctcttgaactgtcacctaag 3'	5' cattaggtgacagttcaagagctccccg 3'
D82N	5' cggggagctcttaactgtcacctaag 3'	5' cattaggtgacagattaagagctccccg 3'
K95A	5' cggcaatatggcagacggaaaaatc 3'	5' gattttccgtctgcatattgccg 3'
K98A	5' taaagacggagcaatcaatattaatg 3'	5' catttaataattgattgtcctcttca 3'
K119A	5' gtaccggatcgagaaggcagcagc 3'	5' gctgctgccttctgcgatccggtagc 3'
W125A	5' gcagcagcaggcgtggcgagcaatccaag 3'	5' cttggattgctgccacgcctgctgctgc 3'
W126A	5' gcagcagcagggtggcgagcaatccaag 3'	5' cttggattgctgccgccacctgctgctgc 3'
K145Q	5' atggaatatgaacaggatggttaagt 3'	5' cactaccatctgttcatattccat 3'
K148Q	5' tatgaaaaggatggtcagtgatcaacatggag 3'	5' ctccatgtgatccactgaccatcctttcata 3'
Y157A	5' gagaaaatggacgctaaccattttgtg 3'	5' caaaaatggftagcgtccattttctc 3'
W125A-W126A	5' gcagcagcaggcggcgagcaatccaag 3'	5' cttggattgctgccgccacctgctgctgc 3'
K171Q	5' ggtactggctctctccaagtcagaatgac 3'	5' gtcagtcattctgacttggagagagccagt 3'
K188Q	5' gaaagacaccattccacagctgctgaaagc 3'	5' gttccgctttcaggcagctgtggaatggtgtct 3'
R173Q-K180Q- -K183Q	5' ctctctcaaagtccaatgactgacatccgcg gacaagttgtgaagacaccattc 3'	5' ctttggaatggtgtcttgcacaactgtccgcgg atgtcagtcattggactttgac 3'

Suppl. Table S2. Comparison of the secondary structure of EXLX1 variants to that of wildtype EXLX1, as inferred by circular dichroism spectra. The actual secondary structure of wildtype EXLX1 as well as individual domains D1 and D2 was determined from the full-length EXLX1 structure (PDB #: 3D30).

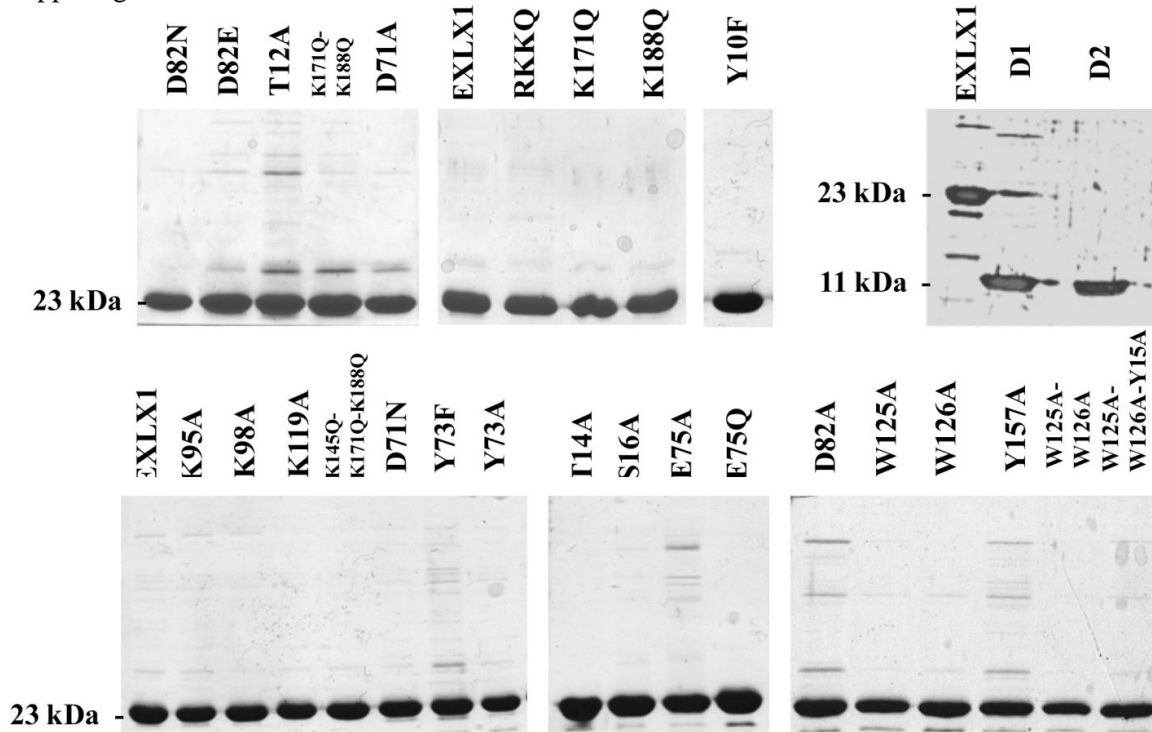
EXLX1 variant	fraction				total
	α -helix	β -sheet	turns	unordered	
EXLX1 (from structure)	0.081	0.408	0.134	0.377	1.000
EXLX1	0.067	0.441	0.111	0.381	1.000
T12A	0.062	0.457	0.110	0.371	1.000
T14A	0.079	0.423	0.110	0.388	1.000
S16A	0.060	0.465	0.106	0.369	1.000
D71A	0.074	0.420	0.115	0.391	1.000
D71N	0.067	0.448	0.110	0.375	1.000
Y73A	0.066	0.439	0.114	0.382	1.001
E75A	0.066	0.446	0.110	0.378	1.000
D82A	0.077	0.414	0.118	0.391	1.000
D82N	0.076	0.419	0.116	0.389	1.000
D82E	0.075	0.426	0.110	0.389	1.000
K119A	0.071	0.438	0.108	0.383	1.000
W125A	0.070	0.437	0.112	0.380	0.999
W126A	0.074	0.429	0.113	0.384	1.000
Y157A	0.073	0.418	0.117	0.392	1.000
W125A-W126A	0.077	0.429	0.116	0.378	1.000
W125A-W126A-Y157A	0.068	0.436	0.115	0.381	1.000
R173Q-K180Q-K183Q	0.071	0.453	0.106	0.370	1.000
D1 (from structure)	0.165	0.360	0.194	0.281	1.000
D1	0.144	0.269	0.147	0.439	0.999
D2 (from structure)	0.000	0.490	0.084	0.426	1.000
D2	0.062	0.422	0.123	0.394	1.001

SUPPLEMENTARY FIGURES

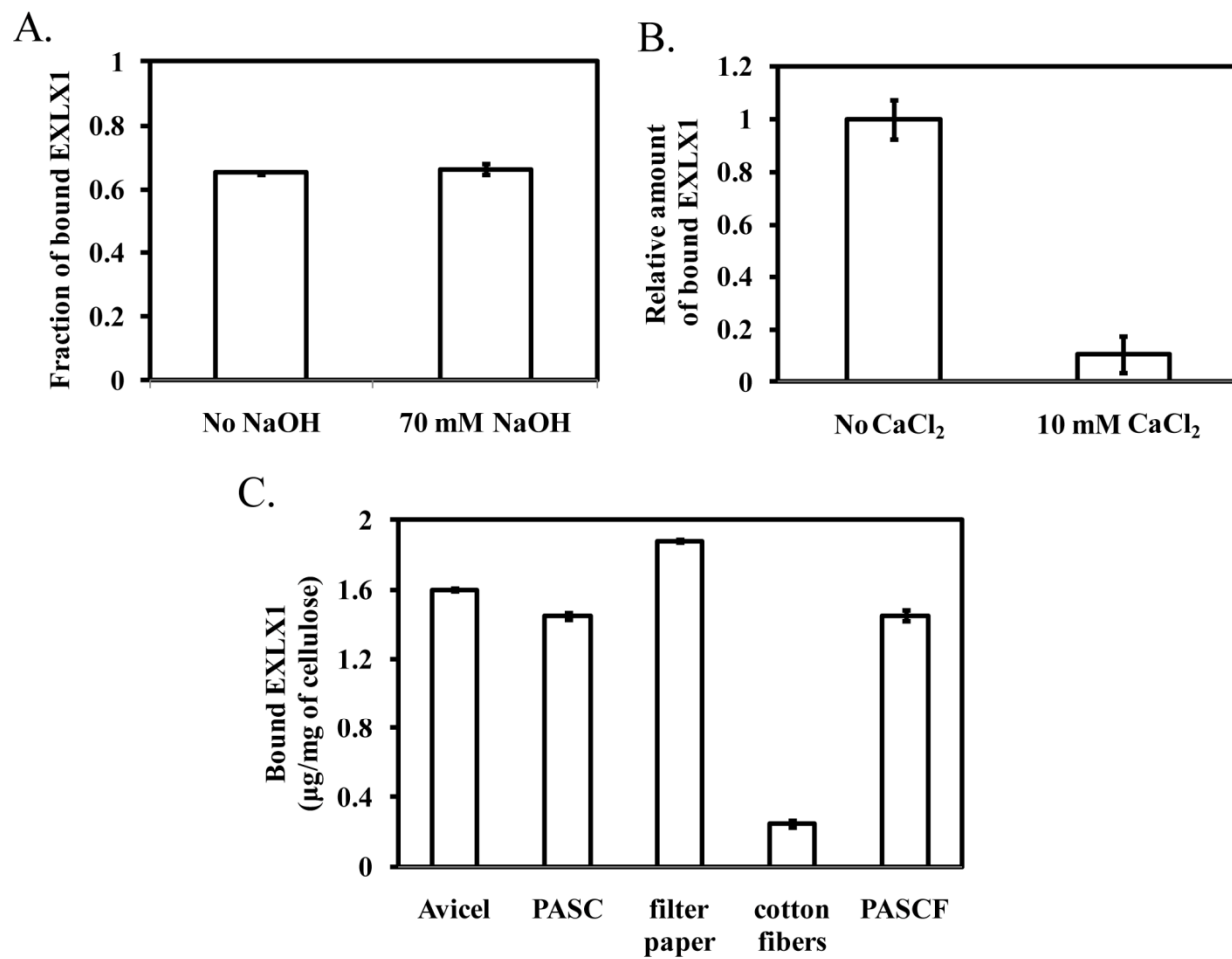
Suppl. Fig. S1.



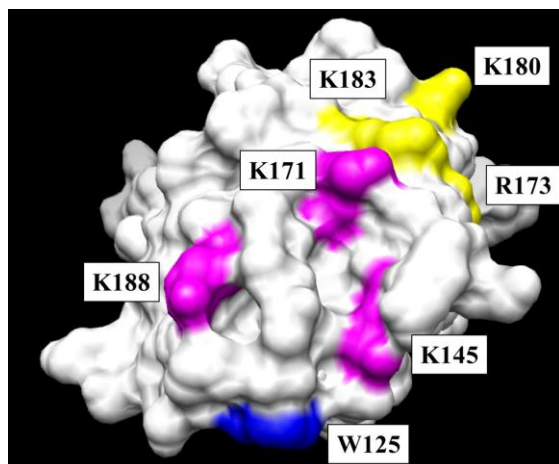
Suppl. Fig. S2.



Suppl. Fig. S3.

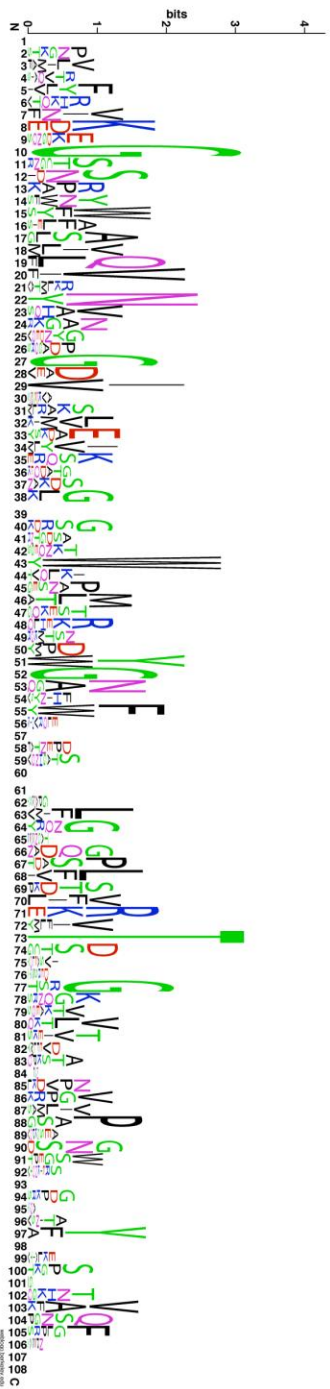


Suppl. Fig. S4.



Bacteria Fungi Plants

Bacillus subtilis (PDB# 3D30)
Xanthomonas campestris
Pectobacterium atrosepticum
Clavibacter michiganensis
Aspidovorax citrulli
Magnaporthe oryzae
Phaeosphaeria nodorum
Zea mays (PDB# 2HCZ)
Pleurotus ostreatus (PDB# 1N10)
Arabidopsis thaliana 1
Cucumis sativus
Arabidopsis thaliana 2
Arabidopsis thaliana 3



Suppl. Fig. S5.