Materials and Methods

Cloning, expression and purification of LysM1-2 of CEBiP

The gene portion encoding the LysM1 and LysM2 domains of CEBiP (LysM1-2, residues 85-192) was cloned in pETM-20 and the pETM-30, which contain the stabilizing Trx and GST fusion tags, respectively. For cloning, two PCR primers containing the *NcoI* (forward) and the *Hind*III (reverse) restriction sites were designed to amplify the *lysM* coding sequence starting at residue Tyr_{85}. Two vectors of pETM series were digested with *Nco*I/*Hind*III and ligated to the digested PCR product by quick-ligation. After plasmid amplification and sequencing, the pETM-20 and pETM-30 expression vectors were used to transform different bacterial host strain (BL21(DE3), BL21(DE3)RP, BL21(DE3)RIL, Rosetta2(DE3), Rosetta(DE3)pLysS). The colonies were inoculated in 10 mL of LB for the small-scale expression screening. For this experiment, bacterial strains were grown at 37° C until they reached an OD_{600} of 0.6-0.7 and then the temperature was either kept to 37 °C or decreased to 22 °C. The recombinant expression was induced with different IPTG concentration (0.2-1 mM). Frozen pellets were lysed in 1.5 mL of 50 mM Tris-HCl buffer (pH 8.0) and 150 mM NaCl or PBS buffer (10 mM phosphate buffer, pH 7.4, 2.7 mM KCl and 137 mM NaCl), containing a cocktail of proteases inhibitor (Roche). Glycerol was added to both buffers to a final concentration of 5 % (v/v). The samples were sonicated for 2 min and the total lysate samples were taken before centrifugation at 13,000 g for 30 min. The resulting supernatants were visualized by SDS-PAGE. The highest yields of protein expression and solubility were obtained using thioredoxin (Trx) as a stabilizing partner (i.e. pETM-20).

Small scale expression experiments allowed us to set best conditions for LysM1-2 expression, as fused to Trx-tag. Starting from this indication, the purification protocol was modified during large-scale purifications. Briefly, pellets from 1L bacterial culture were re-suspended in 40 mL of buffer (PBS, 10 mM Imidazole or 50 mM Tris-HCl buffer, pH 8.0, 200 mM NaCl, 10 mM imidazole) containing 5 % (v/v) of glycerol and 0.01 % (v/v) CHAPS as detergent. A protease-inhibitor cocktail (Roche Diagnostic) was added to the mixture before the sonication which was performed for 10 min in a cold water bath. The lysate was centrifuged at 18,000 rpm for 35 min, and the supernatant filtered and loaded onto a pre-equilibrated Hi-Trap chelating columns (GE Healthcare) charged with $NiCl_2$, connected to a FPLC system. After washing with 15 volumes of binding buffer, a linear gradient of imidazole (20-300 mM) was applied to elute the protein. The fractions containing Trx-LysM protein were pooled and dialyzed against 2

L of PBS, 0.01 % CHAPS and 10 mM DTT (pH 7.2) with one exchange at 4 °C. The purification of the Trx-LysM1-2 was optimized by Superdex 200 gel filtration chromatography (10/30 column, GE Healthcare) equilibrated with the same dialysis buffer. In this step the soluble aggregates were removed from the protein sample. The homogeneity of protein was tested by SDS-PAGE. Fractions of soluble and monodisperse Trx-LysM1-2 were pooled and concentrated to 3 mg/mL for NMR experiments.

To check the correct folding of LysM1-2 domains, the homogenous fractions of Trx-LysM1-2 coming from the gel-chromatography were used for TEV cleavage. TEV protease and Trx-His-tag were removed by applying the protein mixture to a Hi-trap chelating column. The flow through was collected, concentrated and loaded again on S75 gel filtration chromatography equilibrated in 50 mM Tris-HCl, 150 mM NaCl (pH 8.0), 0.01 % (v/v) CHAPS and 10 mM DTT.

Mutant preparation for LysM1-2 of CEBiP.

The plasmid expression vector encoding the $I_{122}A$ mutation was generated by site-directed mutagenesis of wild-type plasmid pETM-20-LysM1-2 using the Stratagene QuikChange kit and the mutagenic primers Fw: 5' CCC GAC CCC AAC AAG GCT AAT GTC AGC CAG ACG 3' and Rv 5' CGT CTG GCT GAC ATT AGC CTT GTT GGG GTC GGG 3'.

Circular dichroism

To analyze the conformational state of LysM1-2 once Trx had been cleaved, far-UV CD spectra were registered at 20 °C. All CD spectra were recorded with a Jasco J-810 spectropolarimeter equipped with a Peltier temperature control system (Model PTC-423-S). Molar ellipticity per mean residue, [θ] in deg $cm^2 \bullet dmol^{-1}$, was calculated from the equation: $[\theta] = [\theta]obs \bullet mrw \bullet (10 \bullet l \bullet C)^{-1}$, where [θ]obs is the ellipticity measured in degrees, mrw is the mean residue molecular mass (108.6 Da), C is the protein concentration in $g \bullet L^{-1}$ and l is the optical path length of the cell in cm. Far-UV measurements (183-250 nm) were carried out at 20 °C using a 0.1 cm optical path length cell and a protein concentration of 0.2 $mg \bullet mL^{-1}$.

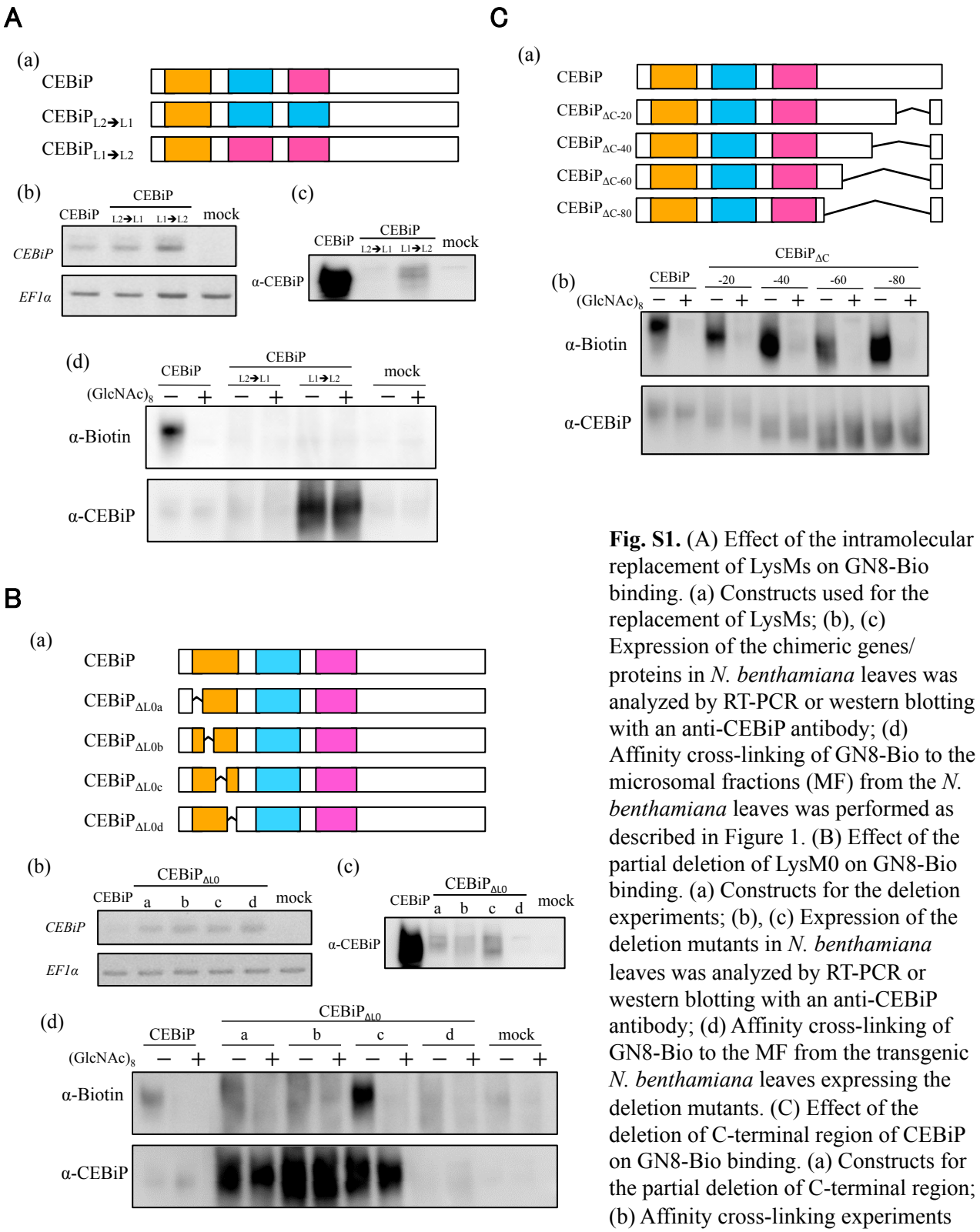

Fig. S1. (A) Effect of the intramolecular replacement of LysMs on GN8-Bio binding. (a) Constructs used for the replacement of LysMs; (b), (c) Expression of the chimeric genes/ proteins in *N. benthamiana* leaves was analyzed by RT-PCR or western blotting with an anti-CEBiP antibody; (d) Affinity cross-linking of GN8-Bio to the microsomal fractions (MF) from the *N. benthamiana* leaves was performed as described in Figure 1. (B) Effect of the partial deletion of LysM0 on GN8-Bio binding. (a) Constructs for the deletion experiments; (b), (c) Expression of the deletion mutants in *N. benthamiana* leaves was analyzed by RT-PCR or western blotting with an anti-CEBiP antibody; (d) Affinity cross-linking of GN8-Bio to the MF from the transgenic *N. benthamiana* leaves expressing the deletion mutants. (C) Effect of the deletion of C-terminal region of CEBiP on GN8-Bio binding. (a) Constructs for the partial deletion of C-terminal region; (b) Affinity cross-linking experiments were performed as described in (A).

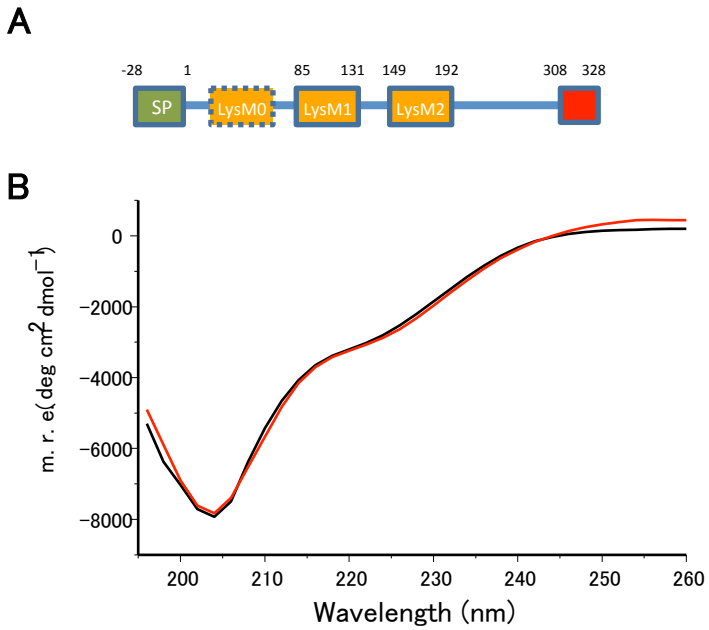

Fig. S2. (A) Domain organization of CEBiP according to the PFAM database (Bateman A, et al. (2004) Nucleic Acids Res 32:D138-141). The C-terminal transmembrane helix was predicted using TMPRED. (B) Superposition of CD spectra of LysM1-2 (black, residues 85-192) and LysM1-2 I122A mutant (red) in 50 mM Tris-HCl, 150 mM NaCl, and 5 mM DTT

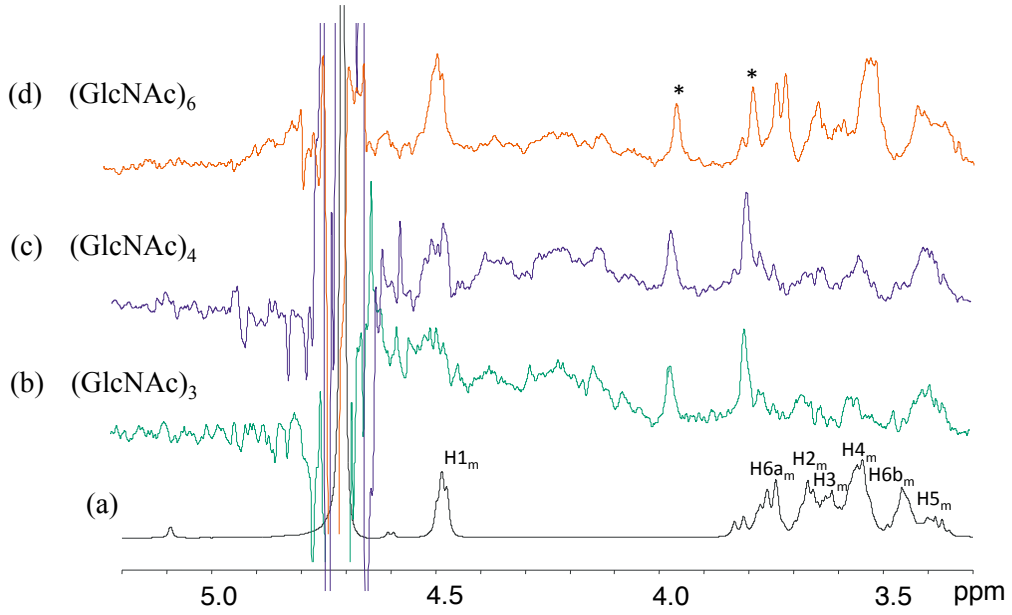

Fig. S3. Reference ^{1}H NMR spectrum (a) and STD 1D NMR spectra of mixture Trx-LysM1-2 : $(GlcNAc)_3$ (b), $(GlcNAc)_4$ (c), $(GlcNAc)_6$ (d) 1:100.
* Residual protein signals.

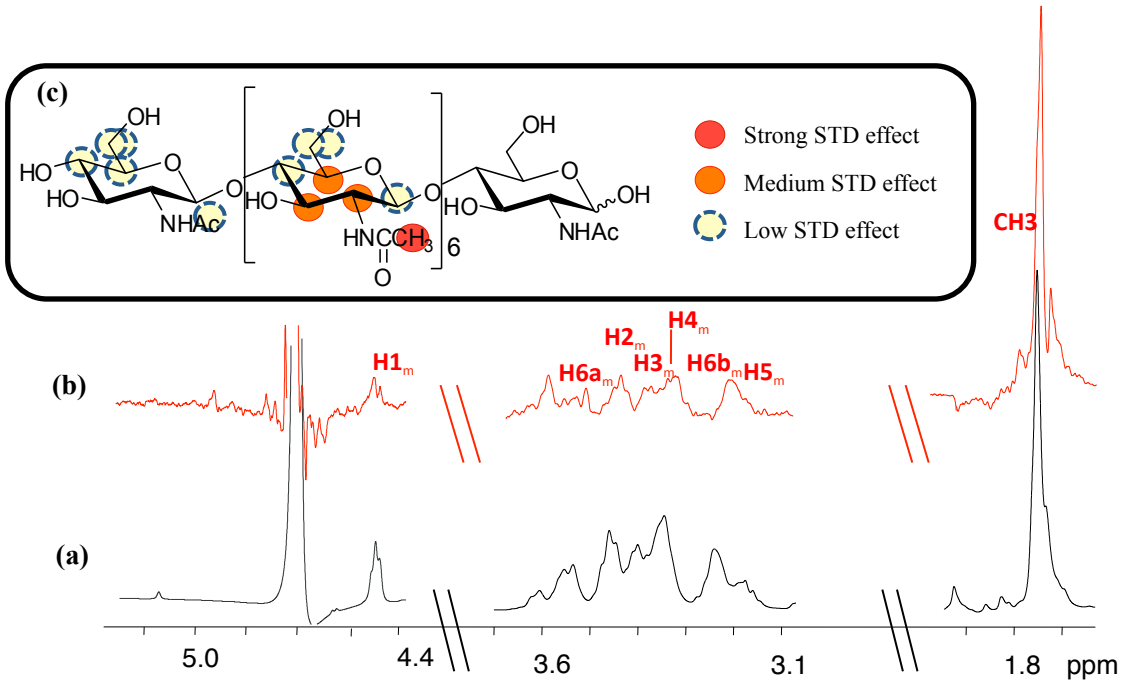

Fig. S4. Reference ^{1}H NMR spectrum (a) and STDD NMR spectrum (b) of mixture Trx-LysM1-2 : $(GlcNAc)_8$ 1:100. (c), Chemical structure and epitope binding of $(GlcNAc)_8$ to Trx-LysM1-2.

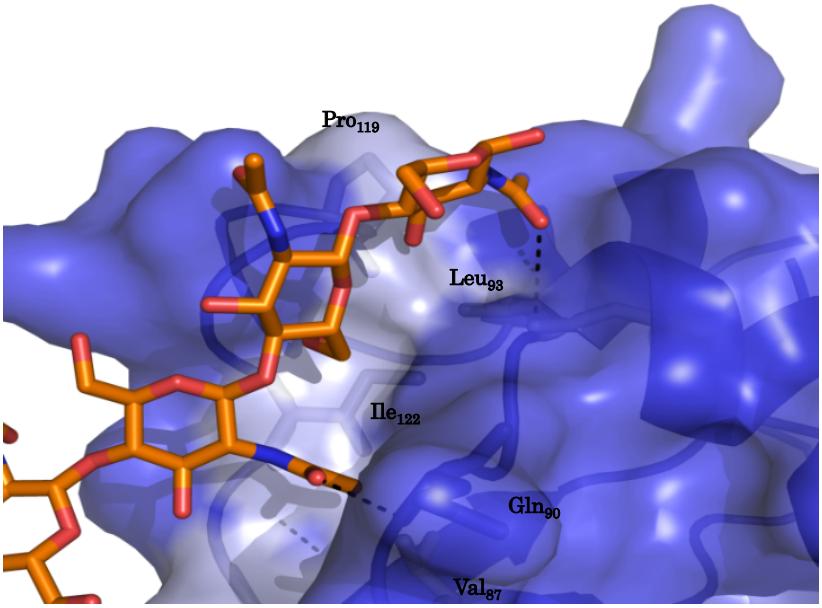

Fig. S5. Details of interactions of $(GlcNAc)_4$ with the LysM1 domain. Hydrogen bonds formed by the acetyl moieties are shown as dashed lines.

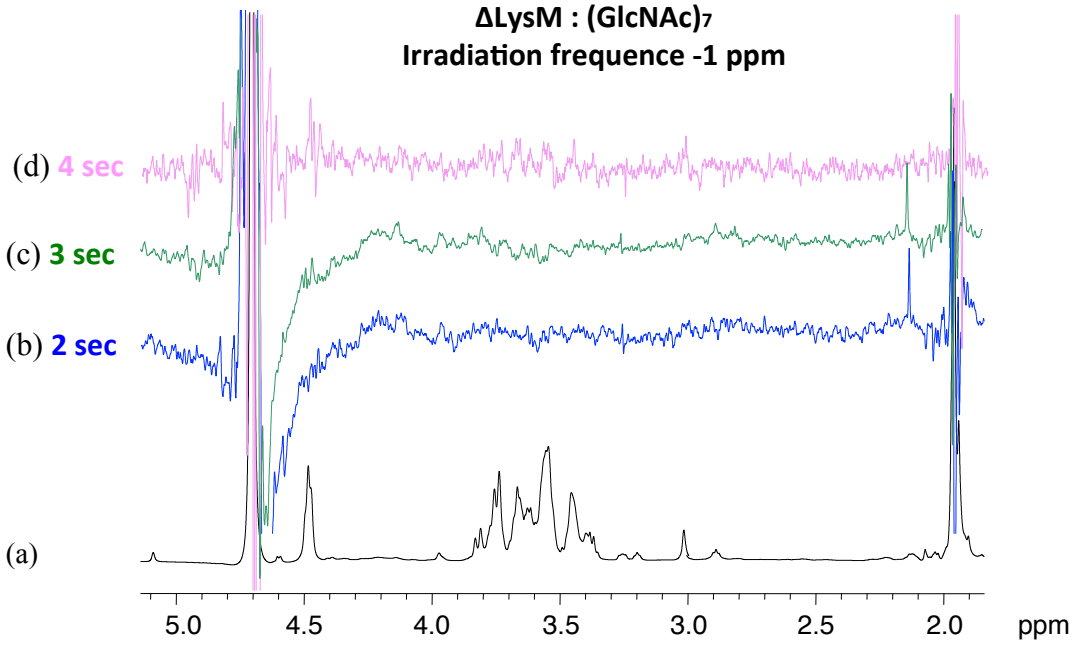

Fig. S6. Reference ^1H NMR spectrum (a) and STDD NMR spectra of mixture Trx-LysM1-2 $I_{122}A$: $(GlcNAc)_7$ at saturation time of 2 sec (b), 3 sec (c), 4 sec (d).

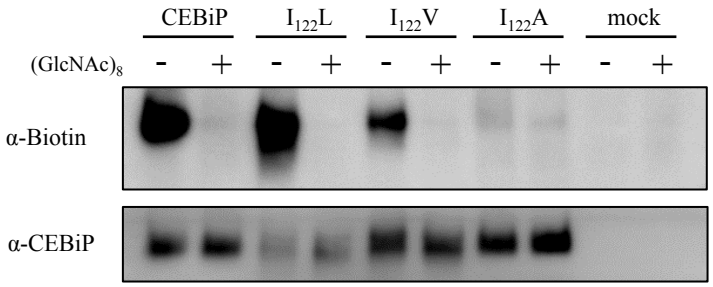

Fig. S7. Binding of GN8-Bio to mutant CEBiP proteins.
CEBiP proteins mutated for the indicated amino acid residues were expressed in *N. benthamiana*, from which the microsomes for affinity labeling were prepared.

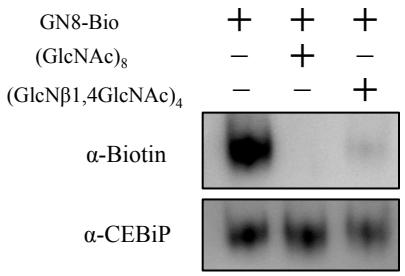

Fig. S8. $(GlcN\beta1,4GlcNAc)_4$ inhibits the binding of GN8 to CEBiP.
GN8-Bio (0.04 μM) was mixed with the MF in the presence (+) or absence (-) of 4 μM $(GlcNAc)_8$ or $(GlcN\beta1,4GlcNAc)_4$ as a competitor.

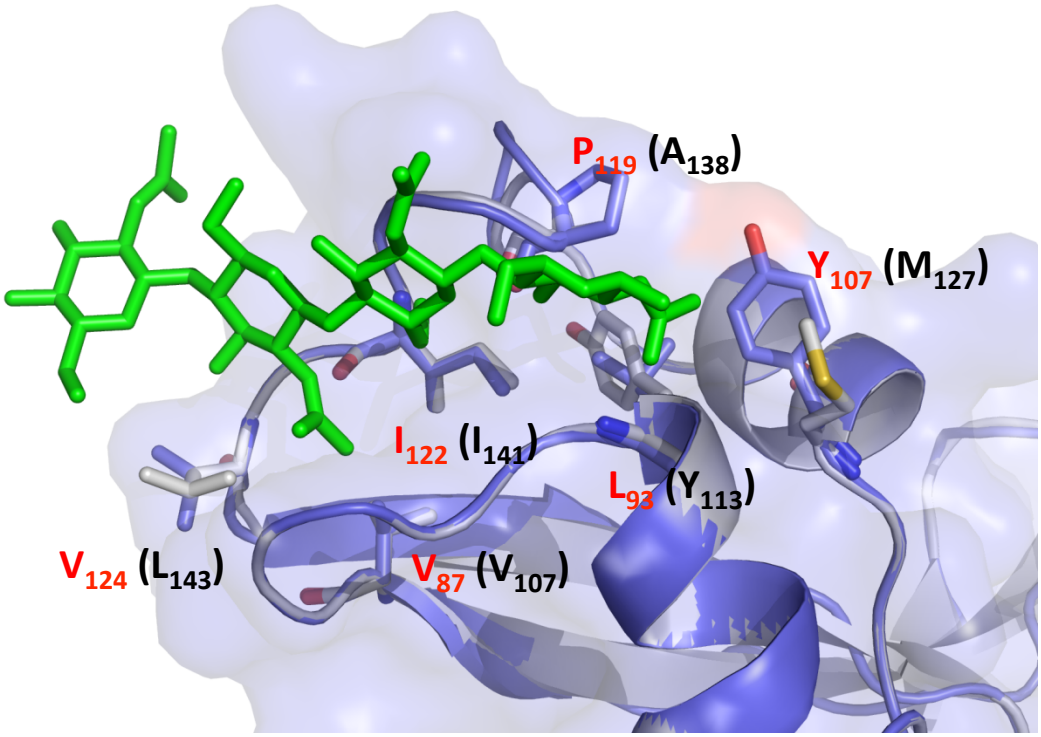

Fig. S9. Residues involved in chitin binding in CEBiP and in CERK1. Superposition of sugar binding sites in CERK1 crystal structure (grey) and CEBiP model (blue). Main residues involved in binding and the sugar ligand are shown in ball-and-stick representation. Red labels represent residue numbers in CEBiP whereas black labels are for those in CERK1.

Table S1. NMR assignments of hepta-chitooligosaccharide in D_2O

Heptamer	$^{1}H1$ /$^{13}C1$	$^{1}H2$ /$^{13}C2$	$^{1}H3$ /$^{13}C3$	$^{1}H4$ /$^{13}C4$	$^{1}H5$ /$^{13}C5$	$^{1}H6$ /$^{13}C6$	CH_3
α	5.09 90.3	3.78 53.7	3.59 72.3	3.53 78.8	3.78 69.8	3.77/3.55 59.86	1.96 22.38
β	4.60 94.6	3.59 56.2	3.58 72.3	3.52 79.1	3.42 74.6	3.75/3.58 59.9	1.97 22.38
m	4.49 101.2	3.64 55.2	3.63 72	3.47 78.8	3.46 74.5	3.84/3.65 60.6	1.97 22.38
n	4.46 100.9	3.68 54.9	3.49 73.3	3.38 69.4	3.40 75.7	3.84/3.65 60.6	1.97 22.38

α or β, reducing end α or β anomer residue; m, middle residue; n, non-reducing end residue.

Table S2. Experimental STD intensities of $(GlcNAc)_7$ bound to LysM at different saturation times

STDmax and Ksat were calculated by fitting the data to a monoexponential equation: $STD = STDmax\ (1 - \exp(-ksat^{t}))$.

Protons of internal GlcNAc	**Experimental STD%**				**STD_{max}**	**K_{sat}**	**STD (fit) Slope of curve at t_{sat}=0**	**STD epitopes (fit)%**
	1sec	**2sec**	**3sec**	**4sec**				
$H1_m$	30	25	40.8	57	0.2905	0.2287	0.066	32.9 %
$H2_m$	43.5	35	61.2	73	0.6243	0.1702	0.106	52.8 %
$H3_m$	51.6	47.5	73.5	80.9	0.5924	0.2258	0.133	66.3 %
$H4_m$	36.6	30	46.9	58.7	0.5259	0.1574	0.082	40.9 %
$H5_m$	48.3	40	57.1	63.5	0.4962	0.2002	0.099	49.3 %
$H6a_m$	34	27.5	40.8	46	0.3524	0.2057	0.072	35.9 %
$H6b_m$	35	27.5	51	58.7	0.5732	0.1387	0.079	39.3 %
CH3	100	100	100	100	0.6315	0.3176	0.2006	100 %

Table S3. Experimental and calculated interproton distances for chito-heptasaccharide

Distance	**Experimental Free state**	**Experimental Bound state**	**Calculated Φ= 56,6° Ψ= 6.9°**
H1-H'4	2.4	2.35	2.4
H1-H'5	3.8	3.79	3.9

Table S4. Sequence alignment of LysM1 region of CEBiP homologs

Receptors	Amino acid sequence	Binding ligand	Ref.
CEBiP	YVVQPQDGLDAIARNVFNAFVTYQEIAAAN--NIPDP-NKINVSQTLWIP	Chitin	(1)
LYM2(AtCEBiP1)	YTIKKDDILSFVATEIFGGLVTYEKISEVN--KIPDP-NKIEIGQKFWIP	Chitin	(2, 3)
MtLYM2	YKIVPGDTLDAIARVRFAGLVKYQQIQTAN--KIPDA-NNITAGATIWIP	Chitin	(4)
LYM1(At1g21880)	YKTRPSDNLGSIADSVYGGLVSAEQIQEAN--SVNDP-SLLDVGTSLVIP	PGN	(2, 5)
LYM3(At1g77630)	YKTRTSDTLGSIADSVYGGLVSPEQIQVAN--SETDL-SVLDVGTKLVIP	PGN	(2, 5)
OsLYP6	YSARPADTLASVADVVFAGLASADQIRTANGLSAEDPDAPLDAGATLVVP	Chitin / PGN	(6)
OsLYP4	YVARPGDTLASVASSVYGGLTTPDWISDSNGILGAKPDAAVDAGTTLFVP	Chitin / PGN	(6)
	* * * * * * *		

The black diamond indicates the position corresponding to Ile_{122} in CEBiP and the asterisk indicates the conserved amino acid residues.

(1) Kaku et al. (2006) *Proc Natl Acad Sci USA* 103:11086-11091; (2) Shinya et al. (2012) *Plant Cell Physiol* 53:1696-1706; (3) Faulkner et al. (2013) *Proc Natl Acad Sci USA* 110:9916-9970; (4) Fliegmann et al. (2011) *Plant Physiol Biochem* 49:709-720; (5) Willmann et al. (2011) *Proc Natl Acad Sci* USA 108:19824-19829; (6) Liu et al. (2012) *Plant Cell* 24:3406-3419.

Table S5. Primers used in this work

(a) Primer sets for deletion experiments of CEBiP

Construct		Forward (5' - 3')	Reverse (5' - 3')	Destination vector
ΔL1		CTGCCCTGCAGCTGCGACAA	GATGGGGAGGCGGTCCGACT	pMDC32
ΔL2		CTCCCTGTGTGCCGTTCATC	CGGGACATCTAGAATCTGTC	pMDC32
Δ L0	a	GTCGCCCGCTTCAACACCAC	GAGGATGGCGGACTTGCAGG	pEAQ-HT-DEST1
	b	GGCGCCAACGGCCTCCCCGA	GAGGTTGCCGTAGGTGGTGG	pEAQ-HT-DEST1
	c	GCCCCCGTCGCCGCCAATTC	GAGGAGGTCGGGGAGGGTGG	pEAQ-HT-DEST1
	d	TTCCGCTGCCGCTGCAACGG	GGAGGAAAGCGTGCCGTCGG	pEAQ-HT-DEST1
Δ L2	a	ATCGCTGCCAAGTACGGGGT	CGGGACATCTAGAATCTGTC	pEAQ-HT-DEST1
	b	CTTCTCACCAGAAATAAGAT	CGCCGACGTGTTCTCCCCTT	pEAQ-HT-DEST1
	c	AAATTGCAGATGGGACAGAT	CGTGGACTCCGTCACCCCGT	pEAQ-HT-DEST1
	d	CTCCCTGTGTGCCGTTCATC	CGTGGGGTCGTCGATCTTAT	pEAQ-HT-DEST1
Δ C	-20	AGCCTTGCAACTAATCAGAC	GCAACCGGTGCCGTTCGTCT	pEAQ-HT-DEST1
	-40	AGCCTTGCAACTAATCAGAC	GCATCCCTTGTTCTGTACTG	pEAQ-HT-DEST1
	-60	AGCCTTGCAACTAATCAGAC	GCGGATGCAGTTTCCTGCGG	pEAQ-HT-DEST1
	-80	AGCCTTGCAACTAATCAGAC	ATCAGCTGAGGTATCGCTGA	pEAQ-HT-DEST1

(b) Primer sets for LysM-swapping experiments (I ; Amplification of inserted fragments, B ; Amplification of backbone vectors)

Construct			Forward (5' - 3')	Reverse (5' - 3')	Destination vector
CEBiP LysM	L2➔L1	I	GTGATGCACCTCGCCTACGTcgtgcagccgcagga	ACGGCACACAGGGAGCGGAAtccacagcgtctggc	pEAQ-HT-DEST1
		B	CTCCCTGTGTGCCGTTCATC	CGGGACATCTAGAATCTGTC	
	L1➔L2	I	GACCGCCTCCCCATCTACAGcgtcggcaaagggga	GCAGCTGCAGGGCAGCGGGAcatctagaatctgtc	pEAQ-HT-DEST1
		B	CTGCCCTGCAGCTGCGACAA	GATGGGGAGGCGGTCCGACT	
LysM0	At2-Os-Os	I	CCTGCAAGTCCGCCATCCTCtactcaagcaagaacgcaac	CCGTTGCAGCGGCAGCGGAAtgggacacgtacgacttgat	pEAQ-HT-DEST1
		B	TTCCGCTGCCGCTGCAACGG	GAGGATGGCGGACTTGCAGG	
	At1-Os-Os	I	CCTGCAAGTCCGCCATCCTCtacacactctacaccgacct	CCGTTGCAGCGGCAGCGGAAtgggattttgaggaagagct	pEAQ-HT-DEST1
		B	TTCCGCTGCCGCTGCAACGG	GAGGATGGCGGACTTGCAGG	
LysM1	Os-At2-Os	I	AGTCGGACCGCCTCCCCATCtacaccatcaagaaagacga	TTGTCGCAGCTGCAGGGCAGagggatccaaaactttgac	pEAQ-HT-DEST1
		B	CTGCCCTGCAGCTGCGACAA	GATGGGGAGGCGGTCCGACT	
	Os-At1-Os	I	AGTCGGACCGCCTCCCCATCtacaaaacccgaccttctga	TTGTCGCAGCTGCAGGGCAGggggataacaagactagtcc	pEAQ-HT-DEST1
		B	CTGCCCTGCAGCTGCGACAA	GATGGGGAGGCGGTCCGACT	
LysM2	Os-Os-At2	I	CTAACGTGATGCACCTCGCCtacgcacatgtagtcaaact	GATGAACGGCACACAGGGAGagggacgtcgagaggtttat	pEAQ-HT-DEST1
		B	CTCCCTGTGTGCCGTTCATC	CGGGACATCTAGAATCTGTC	
	Os-Os-At1	I	CTAACGTGATGCACCTCGCCtatgttgtgaaggagattga	GATGAACGGCACACAGGGAGaggaacagcgagaatatctc	pEAQ-HT-DEST1
		B	CTCCCTGTGTGCCGTTCATC	CGGGACATCTAGAATCTGTC	

(c) Primer sets for site-directed mutagenesis

Construct	Forward (5' - 3')	Reverse (5' - 3')	Destination vector
$L_{93}A$	GCCGACGCCATCGCGCGCAACGT	CCCGTCCTGCGGCTGCACGA	pEAQ-HT-DEST1
$Y_{107}A$	GCCCAGGAGATCGCCGCCGCGAA	GGTGACGAAGGCGTTGAACA	pEAQ-HT-DEST1
$P_{119}A$	GCCAACAAGATAAATGTCAGCCA	GTCGGGGATGTTGTTCGCGG	pEAQ-HT-DEST1
$I_{122}A$	GCCAATGTCAGCCAGACGCTGTG	CTTGTTGGGGTCGGGGATGT	pEAQ-HT-DEST1
$I_{122}L$	CTTAATGTCAGCCAGACGCTGTG	CTTGTTGGGGTCGGGGATGT	pEAQ-HT-DEST1
$I_{122}V$	GTCAATGTCAGCCAGACGCTGTG	CTTGTTGGGGTCGGGGATGT	pEAQ-HT-DEST1
$V_{124}A$	GCCAGCCAGACGCTGTGGATTCC	ATTTATCTTGTTGGGGTCGG	pEAQ-HT-DEST1

(d) Primer sets for gene expression analysis by RT-PCR

	Forward (5' - 3')	Reverse (5' - 3')
CEBiP	gtttgtacaaaaaagcaggc *	agccaccgcgcaggtgaagt
EF1α	tcgccttgtggaagtttgagac	aacattgtcaccagggagtgcc

*This sequence is located attB1 region of destination vectors.