SUPPLEMENTARY INFORMATION

Structural signatures in EPR3 define a unique class of plant carbohydrate receptors

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	EPR3
Data collection	
Space group	P 1 21 1
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	59.82, 36.08, 72.66
α, β, γ (°)	90.00, 93.92, 90.00
Resolution range (Å)	47.7-1.87 (1.94-1.87)*
$R_{\text{meas}}(\%)$	9.8 (94.6)
CC1/2	0.99 (0.82)
Ι/σΙ	15.72 (1.82)
Completeness (%)	96.9 (84.2)
Redundancy	6.3 (4.7)
•	
Refinement	
No. unique reflections	25173 (2151)
Reflections in R _{free} set	2000
$R_{ m work}$ / $R_{ m free}$ (%)	16.3 / 21.0
No. residues	302
No. atoms	
Protein	2362
Glycosylation +	42
small solutes	
Water	170
<i>B</i> -factors (Å ²)	
Protein	39.8
Glycosylation +	83.9
small solutes	
Water	44.4
R.m.s. deviations	
Bond lengths (Å)	0.015
Bond angles (°)	1.17

Supplementary Table 1 Data collection and refinement statistics

Data was collected from one crystal. *Values in parentheses are for highest-resolution shell.

Destain	EDD2	EDD2 + D7A EDC				
Protein	EPK3	EPK3 + K/A EPS				
Data collection						
Beamline	PETRA III P12	PETRA III P12				
Wavelength (nm)	1.24	1.24				
s range (nm ⁻¹)	0.120 - 3.340	0.106 - 3.063				
Exposure time (s)	0.045	0.045				
Concentration range (mg/ml)	0.6 - 22	0.6 - 22				
(data shown for 6.8)						
Ligand concentration (M)	N/A	0.001				
Temperature (°C)	20	20				
Structural parameters						
Rg (nm) from Guinier	2.29 ± 0.2	2.22 ± 0.1				
Rg (nm) from P(r) plot	2.28 ± 0.07	2.02 ± 0.04				
D_{max} (nm)	7.5	7.1				
Porod volume estimate (Å ³)	56.7	52.5				
Estimated molecular mass from Porod						
volume (kDa)	29.0	26.5				
Estimated molecular mass from forward						
scattering I(0) (kDa)	24.7	24.7				
Calculated molecular mass from amino						
acid sequence (kDa)*	23.6	23.6				
Estimated mass of glycosylated EPR3						
from SDS-PAGE (kDa)	25-35	25-35				
Software employed						
Primary data reduction	Primus					
Ab initio analysis	BioXtas RAW					
Rigid body modeling	DAMMIN/DAMMIF					
Visualization	colores/PyMOL					

Supplementary Table 2 SAXS data collection and scattering-derived parameters

* The molecular mass was calculated without considering the contributions from glycosylation.



Supplementary Fig. 1 | Purification of the EPR3-Nb186 complex. a, Overlay of chromatograms from SEC experiments with EPR3, Nb186 and the EPR3-Nb186 complex. b, SDS-PAGE of the EPR3-Nb186 complex used for crystallization and c, a representative image of a single EPR3-Nb186 crystal.



Supplementary Fig. 2 | **The EPR3-Nb186 structure**. Cartoon representation of the EPR3-Nb186 complex crystal structure with Nb186 coloured in blue and EPR3 coloured as in Fig. 1.

а	M1		β1 α1 <mark>β2 β3</mark>	
	Lotus Chickpea Medicago Soybean Phaseolus Populus Malus Vitis Theobroma Ricinus Fragaria Maize Rice Wheat Barley Consensus	42 34 57 41 35 1 31 29 29 47 37 38 36	MEPFNCS-TKIRTONSLIYHISIGLKVEEIARFYSVNLSRIRPITRG-TKQDYLVSVP IEPFKCS-TKIRTONASLYHINYDQKIRQIAHFYSIGVSQIRPIIRS-TKQDYLVKVP MEPYKCS-TKMRTONASLYHINYDQKIRQIAHFYSIGVSQIRPIIRS-TKQDYLVKVP LEPFKCS-FKIRTONASLYHISYGNNIDDIATFYSVSTSQIRPINR-TEQDYLITVP LEPFKCS-SKISTONASLYHISYGNNIDDIATFYSVSTSQIRPINR-TEQDYLITVP VIPLSCS-SKISTONASLYHISYGNNADEIANFYSVHPSQIKPITRG-TKQDYLVVP WYPFSCS-DQVQNDOSYLYHISEGLSIRQIASFYSVKSSNVEPITRG-LKQDYLVSVP VNPLSCS-AKINTONASLYHINIGLARFYSVHPSQIRPITRG-LKQDYLVSVP VNPLSCS-SKISTONASLYHINIGLARGIAFFYSVNRSEITYVN,QDYLVTVP VYPLGCA-SNIPSCNSYLYHISKGLSIEEMANFYSVNTSQIQSITHD-AKLDYLVSVP WIPFTCS-ESS-HVIMONASLYHINIGLQIDQIAYFYNVSITQITPIING-DRKDYIINVP VIPTGSA-SH-HINISSYLYHISKGLSIEEMANFYSVNSKVPVARSTUVVPW WQPLTCNAAVSNN-PSCGSFLYVTPRGRISEVSVFNGNASLIQPIKRLSGSEDLLVVVP WQPMQCD-AASLNPSSCSSILVYTPGGRSLSEIASLFNGSTSLOPIKRLSGSEDLLVVPP MQPMQCD-AASLNPSSCSSYLVYTPQGRSLSEIASLFNGSASRTOPIKRLSGSEDLLVVPP MEPF+CSAAKS+NIRTCNSSLYHISYGL+IEEIASFYSVN+SQIKPI+RGSTKQDYLVSVP	
b	M2			
	Lotus Chickpea Medicago Soybean Phaseolus Populus Malus Vitis Theobroma Ricinus Fragaria Maize Rice Wheat Barley Consensus	101 93 93 116 100 60 94 57 90 91 91 110 99 101 98	P4 CI2 p5 RNTNGL-NGYFYHRSYKVKUNDSYDIJNLFYSGQAWPVNEDLVVPNENTTHIFGG KNTNDL-SGYFYETYKVSPNETSYDIANLFYSGQAWPVNEDLVPNENVTNIFGG NDTNGL-GGYFYDTYKVSPNETSYDIANLFYSGQAWPINGELVDNENVTNIFGG NDTNGL-GGYFYDTYKVKSNDTFVNINNFYSGQAWPINGELDDNEDLAHLFGG KNINGT-GGYFYDTYKVKPNDFVNINNFYSGQAWPINGELDDNEDLAHLFGG KDVNGT-GGYFYDTYKVKQFDIFYNVSGQIYSGQAWPYGQERLFIACDMISVHVGG KNINGT-KAYFYDTYKVKQFDFFNVSGQIYSGQAWPYGQERLFIACDMISVHVGG KNINGT-LGYFYDTYKVKQFDTFVNVSGQIYSGQAWPGESSFFICGUPGK KNINGT-KAYFYDAIYQVKRDDTFVNVSGQIYSGQAWPSGEFSFFICGUPGK KDIDGT-GYPYDTYKVKDGDTYNVSGQISGFYSGQAWFGESSFFICGUPHLGGC KDUNGT-LGYFYDTYKVKDGDTYNVSGQISGFYSGQAWFGESSFFICGUPHLGGC KDIDGT-GMYYDTYNVQVGDTFSVSGFISGLANVG-DGRTLIAGTIAVHLCGC EAINDTHAFFHDTQYEVEGDTADNISNIFSGLANVG-DGTLIAGTIAVHLCGC GAINDTMSCLFHDTYKVSPDDTADSINSN-FSGLAWNVIPTANKTITVHLLGGC GAINDTMSGYFYDTYKVSPDTFVNVSHLYSGQAWPVN+ELSTF+ANETITIHLPGGC KNINGTMSGYFYDTYKVKPNDTFVNVN+L+YSGQAWNVN+ELSTF+ANETITIHLPGGC	
С	LysM3 Lotus Chickpea Medicago Soybean Phaseolus Populus Malus Vitis Theobroma Ricinus Fragaria Maize Rice Wheat Barley Consensus	158 149 172 156 119 153 116 149 150 142 169 158 155 152	β6 α3 α4 β7 SE-SGSQIVVTYTVQRNDTPLSIALLINATVEGMVSVNSVMAPNPTFIDVGMVLYVPRL SE-SGSQIVVTYTVQRNDTPLSIALLINATVDGMVRINQVLGPNFTFIDIGVVLVVPRL SE-FESQIVVTYTVQRNDTPSISLLINATUDGMVRINQULGPNFTFIDIGVVLVVPRL SEKSDSQIVVTYTVQRNDTPSISLLINATLDGMVRINQULGPNFTFIDIGVVLVVPRL SEKNDSQIVVTYTVQRNDTPSISLLINATLDGMVRINQULGPNFTFIDIGVVLVVPRL SEKNDSQIVVTYTVQRNDTPSISLLINATLDGMVRINQULGPNFTFIDIGVVLVVPRL SEKNDSQIVVTYTVQRNDTPSISLINATLDGMVRINGULAQNPSFIDVTVVLVVPRL SEKNDSQIVVTYTVQRNDTPSISLINATLDGMVRINEVLAQNPSFIDVTVVLVVPRL LE-VEAKLIVTYTQRDTLTGIAELLSALISGIENINSTILQNSEYIUVGVLFVPRK VQ-SQSQIVVTYTVQLHDTLSDIGTLISAKISGIESMNSILIQNSEYIUVGVLFVPRK VG-SRSQIVVTYTVQLHDTLSDIATLLSAKISGIESMNSILIQNSEYIDVGVLFVPRK SGVVLFVPRK VS-RKSQUVVTYTVQLHDTLSDIATLSAKISGIESMNSFILRPNFTLDIGVVLFVPRK SGVLFVPRK VS-SSQUVTYTVQLRDTLSDIATLSAKISGIESMNSFILRPNFTHPFNFTLGGVLFVPRK SS-TAPEGVLSYVQURDTLSDIATLSAKISGIESMNSFILRPNFTHPFFF SS-TAPEGVLSYNQEDTLSTASLFSSRUGDILMSFVTKDPNFTRPVVVFKI SS-TAPEGVLSYNQEDTLSNATLFRSSRELSINAGVT-DPDFTKPGWLFPFK+ SS-TAPEGVLSYNQARDTLSNATLFRSSRELSINAGVT-DPDFTKEGWLFFFFF+ SS-TAPEGVLSYNQARDTLSNATLFRSSRELSINAGVT-DPDFTKEGWLFFFF+	

Supplementary Fig. 3 | Sequence alignment of EPR3 homologues. Sequence alignment of the extracellular domain of EPR3 homologues from dicots (legumes and non-legumes) and monocots showing the conserved secondary structure arrangement of **a**, M1 ($\beta\alpha\beta\beta$ fold) **b**, M2 ($\beta\alpha\beta$ fold) and c, LysM3 (baab fold). Lotus (BAI79269.1), Chickpea (XP 004489790.1), Medicago (XP_003613165.1), Soybean (XP_003517716.1), Phaseolus (XP_007157313.1), Populus (XP_008340354.1), (XP 002322185.1), Malus Vitis (XP 002272814.2), Theobroma (XP 007036352.1), Ricinus (XP 002527912.1), Fragaria (XP 004300916.1), Maize (XP 008657477.1), Rice (XP 015628733.1), Wheat (CDM80098.1) Barley and (MLOC 5489.2).



Supplementary Fig. 4 | **Structural modelling of M1 from EPR3 homologues**. **a**, Side-by-side comparison of the EPR3 crystal structure of M1 and an *ab-initio* atomic-level force field model of EPR3 M1. **b**, Modelling of M1 from EPR3 homologues reveals the same overall $\beta\alpha\beta\beta$ arrangement. The molecular fit (RMSD) in Å to the crystal structure of EPR3-M1 is denoted.



Supplementary Fig. 5 | Structural comparison of plant receptors. Cartoon representations of EPS (*Lotus* EPR3) and chitin (*Lotus* CERK6) receptors coloured as indicated. The root mean square deviation (RMSD) of the structural superposition is given in Å (Rigid-body superimposition of 112 equivalent C α atoms).



а

С

β-D-GicNAcp-(1-4)-β-D-GicNAcp-(1-4)-β-D-GicNAcp-(1-4)-β-D-GicNAcp-(1-4)-β-D-GicNAcp-(1-4)-β-D-GicNAcp



Supplementary Fig. 6 | **Carbohydrate ligands used in this study**. The proposed structures of the EPS ligands are shown together with their corresponding MALDI-TOF MS spectrum. **a**, R7A EPS **b**, R7A deOAc-EPS **c**, Chitohexose (CO6) control **d**, *R. leguminosarum* EPS and **e**, *S. meliloti* EPS.



Supplementary Fig. 7 | Binding experiments with de-glycosylated EPR3 and de-glycosylated EPR3-Nb186 complex. a, MST binding experiment of de-glycosylated EPR3 to R7A EPS. b MST binding experiment of de-glycosylated EPR3-Nb186 complex to R7A EPS. The equilibrium dissociation constants (K_d) in the 95% confidence interval, goodness of fit (R^2), and number of independent protein preparations used for the measurements (n) are reported.



Supplementary Fig. 8 | **SAXS data, fits and models**. SAXS analysis of EPR3 showing scattering curve with model fit for EPR3 in the **a**, absence of ligand and **b**, in the presence of R7A EPS ligand. Guinier plots and P(r) distance distribution plot with D_{max} are shown together with docking of EPR3 into the SAXS envelope. EPR3 has an extended stem-like structure in both the absence and presence of ligand. The overall dimensions are shown in angstrom (Å). **c**, **d** Dimensionless Kratky plots of EPR3 with and without R7A EPS. **c**, Vc-based with the volume of an ideal sphere (dotted line, y = 0.82) and **d**, Rg-based with indicated Guinier-Kratky point ($\sqrt{3}/1.1$, dotted lines). Addition of the ligand does not induce changes in either flexibility or globularity of EPR3.