Supporting Information

Substrate preference of an ABC importer corresponds to selective growth on β-(1,6)-galactosides in *Bifidobacterium animalis* subsp. *lactis*

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Table S1. Carbohydrate ligands screened for binding to *Bal*6GBP.

Linkage	Ligand	Structure	Binding
α-(1,4)	Maltose[a]	α-D-Glc*p*-(1,4)-D-Glc*p*	-
α-(1,6)	Melibiose[b]	α-D-Gal*p*-(1,6)-D-Glc*p*	-
β-(1,2)	Sucrose[b]	β-D-Glc*p*-(1,2)-D-Fru*f*	-
β-(1,2)	Sophorose[b]	β-D-Glc*p*-(1,2)-D-Glc*p*	-
β-(1,4)	Cellobiose[b]	β-D-Glc*p*-(1,4)-D-Glc*p*	-
β-(1,4)	Mannobiose[a]	β-D-Man*p*-(1,4)-D-Man*p*	-
β-(1,3)	β-3-Galactobiose (β3Gal2)[c]	β-D-Gal*p*-(1,3)-D-Gal*p*	+
β-(1,3)	Galβ13Glc[d]	β-D-Gal*p*-(1,3)-D-Glc*p*	+
β-(1,4)	Lactulose[b]	β-D-Gal*p*-(1,4)-D-Fru*f*	+
β-(1,4)	β-4-Galactobiose (β4Gal2)[c]	β-D-Gal*p*-(1,4)-D-Gal*p*	+
β-(1,4)	Lactose[b]	β-D-Gal*p*-(1,4)-D-Glc*p*	+
β-(1,4)	Epilactose[e]	β-D-Gal*p*-(1,4)-D-Man*p*	+
β-(1,6)	β-6-Galactobiose (β6Gal2)[c]	β-D-Gal*p*-(1,6)-D-Gal*p*	+
β-(1,6)	Allolactose[f]	β-D-Gal*p*-(1,6)-D-Glc*p*	+
β-(1,3)	Lacto-*N*-biose[c]	β-D-Gal*p*-(1,3)-D-GlcNAc*p*	+
β-(1,4)	β-4-Galactorhamnose[d]	β-D-Gal*p*-(1,4)-L-Rha*p*	+
β-(1,4)	β-4-Galactotriose (β4Gal3)[g]	[β-D-Gal*p*-(1-4)]$_2$-D-Gal*p*	+
β-(1,4)	β-4-Galactotetraose (β4Gal4)[g]	[β-D-Gal*p*-(1-4)]$_3$-D-Gal*p*	+
β-(1,4)	β-4-Galactopentaose (β4Gal5)[g]	[β-D-Gal*p*-(1-4)]$_4$-D-Gal*p*	+
β-(1,6)	β-6-Galactotetraose (β6Gal4)[g]	[β-D-Gal*p*-(1-6)]$_3$-D-Gal*p*	+
Mix	Commercial GOS[h]	Mixture	+

[a]From Megazyme. [b]From Sigma. [c]From Dextra UK. [d]Generously provided by Dr. Motomitsu Kitaoka (Enzyme Laboratory, National Food Research Institute, National Agriculture and Food Research Organization (NARO), Tsukuba, Japan (Nakajima *et al.*, 2009). [e]Generously provided from Ass. Prof. Wataru Saburi (Research Faculty of Agriculture, Hokkaido University, Japan) (Saburi *et al.*, 2010). [f]From Carbosynth (Berkshire, UK). [g]Generously provided by Prof. Mads Hartvig Clausen, DTU-Chemistry (Andersen *et al.*, 2018). [h]From DuPont Nutrition & health (Kantvik, Finland).

Table S2. Polar contacts between *Bal*6GBP and β-(1,6)-galactobiose.

Ligand atom		H_2O/Protein atom	Hydrogen bond distance (Å)	
			Chain A	Chain B
Position 2	O2	Glu98 OE1	2.7	2.7
		Gly310 N	2.9	2.9
	O3	Asp145 OD1	3.1	3.1
		Asp145 OD2	2.8	2.8
		Ser311 OG	2.7	2.6 (2.7)[a]
	O4	Gln96 NE2	2.9	2.9 (2.8)
		H_2O/Asp48 OD2	2.7/2.7	2.7/2.7
	O5	Trp47 NE1	2.8	2.8
	O6	Asn279 ND2	2.7 (2.8)	2.7 (2.8)
		H_2O/Asp48 OD2	2.7 (2.8)/2.9	3.1 (3.2)/2.9
Position 1	O1 α	Asn308 OD1	2.8	2.7
		H_2O/Thr272 OD1	3.0/3.0	N/A
	O1 β	H_2O/Asp196 OD1	2.7/2.7	2.7/2.7
	O2	Asn308 ND2	2.9 (3.1)	2.9 (3.1)
		Asp196 OD2	2.6 (2.7)	2.6 (2.7)
	O3	H_2O/Arg406 NH1	2.6/3.1	2.7 (2.8)/3.1
		H_2O/Arg406 NH1	2.8/3.0	2.8/3.1
	O4	Asn74 ND2	2.8 (2.9)	2.8 (2.9)
		Glu98 OE1	2.6 (2.7)	2.6 (2.8)
	O5	Trp254 NE1	2.9 (3.0)	2.9 (3.0)

[a]Distances in parenthesis indicate the hydrogen bond distances in case of double conformations of either ligand or amino acid side chains.

Fig. S1. Alignment of *Bal*6GBP in the closed ligand-bound form and the open ligand-free form. Domain movements between the forms were analysed with the DynDom server (Hayward & Berendsen, 1998)and the rotation angle was 32.3° (99.9% closure) with 0.1 Å translation vector.

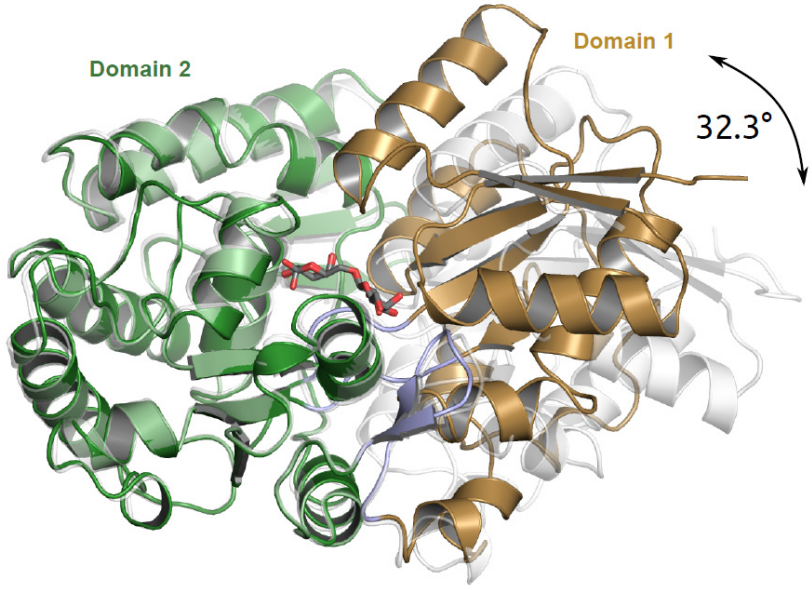


Fig. S2. Surface representations of *Bal*6GBP in complex with β6Gal2 and of GL-BP with GNB, LNB or LNT. The figure depicts the difference in the solvent occluded ligand-binding site in *Bal*6GBP as opposed to the open binding site in GL-BP. Both proteins are shown in the closed bound conformation and for *Bal*6GBP, domain 1 is brown and domain 2 is green, and these are held together by linker regions in light blue. For GL-BP, domain 1 is light green, domain 2 is blue and the linker is light blue.

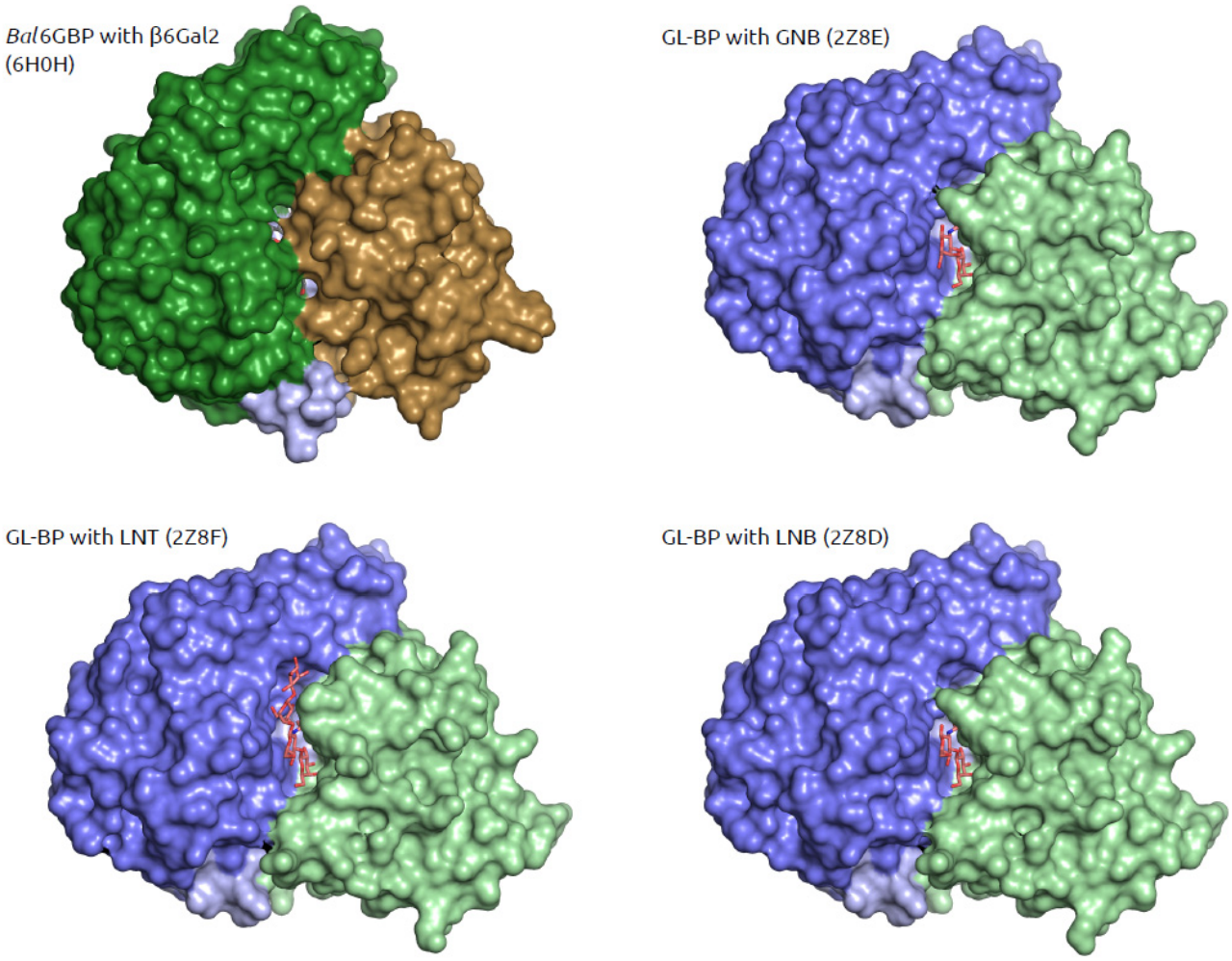
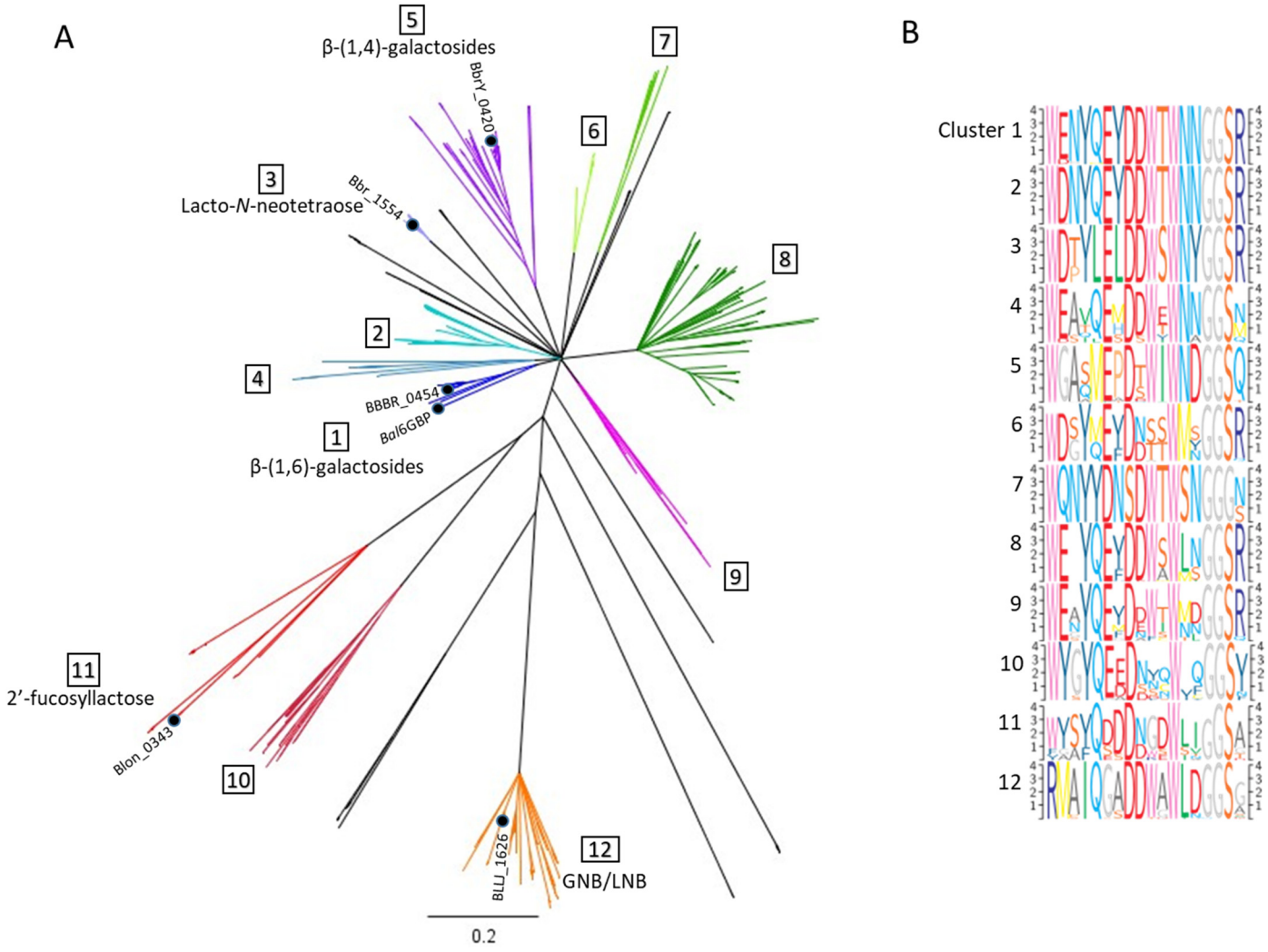


Fig. S3. Phylogenetic tree of bifidobacterial SBP. A, Tentative clades are assigned by their numbers and the position of characterized members and their specificities is indicated. B, Sequence logo signatures of the different clades depicting the sequence spanning the aromatic platform.



Supporting information references

Andersen, M.C.F., Boos, I., Kinnaert, C., Awan, S.I., Pedersen, H.L., Kracun, S.K., Lanz, G., Rydahl, M.G., Kjaerulff, L., Hakansson, M., Kimbung, R., Logan, D.T., Gotfredsen, C.H., Willats, W.G.T., and Clausen, M.H. (2018) Synthesis of branched and linear 1,4-linked galactan oligosaccharides. *Org Biomol Chem* **16**: 1157-1162.

Hayward, S., and Berendsen, H.J.C. (1998) Systematic analysis of domain motions in proteins from conformational change: New results on citrate synthase and T4 lysozyme. *Proteins: Struct Funct Genet* **30**: 144-154.

Nakajima, M., Nishimoto, M., and Kitaoka, M. (2009) Characterization of three β -galactoside phosphorylases from *Clostridium phytofermentans*. Discovery of D-galactosyl- β 1->4-L-rhamnose phosphorylase. *J Biol Chem* **284**: 19220-19227.

Saburi, W., Yamamoto, T., Taguchi, H., Hamada, S., and Matsui, H. (2010) Practical preparation of epilactose produced with cellobiose 2-epimerase from *Ruminococcus albus* NE1. *Biosci Biotechnol Biochem* **74**: 1736-1737.