

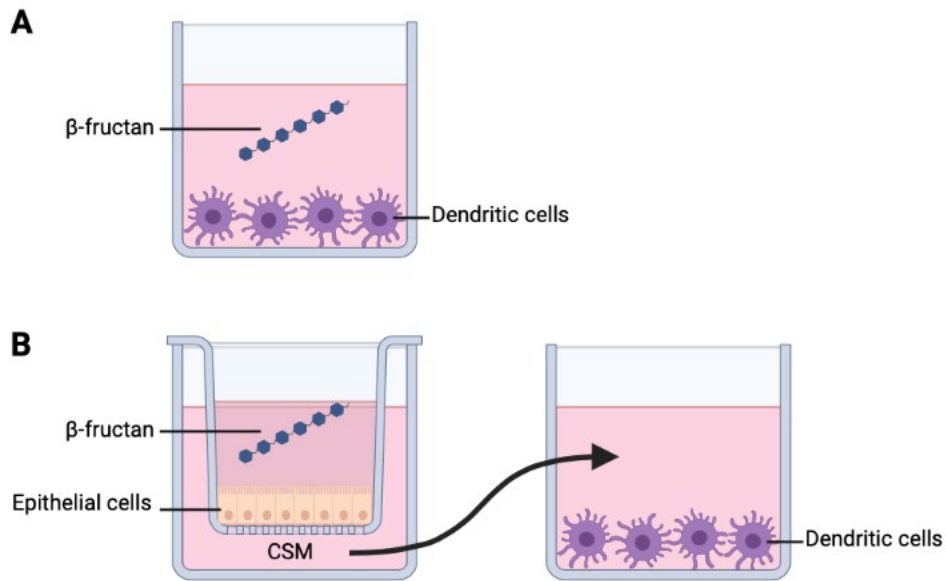
Supplementary tables and figures

Exopolysaccharide β -(2,6)-levan-type fructans have a molecular-weight-dependent modulatory effect on Toll-Like Receptor signalling

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Supplementary table 1. Cell densities and positive controls used in reporter cell stimulation experiments

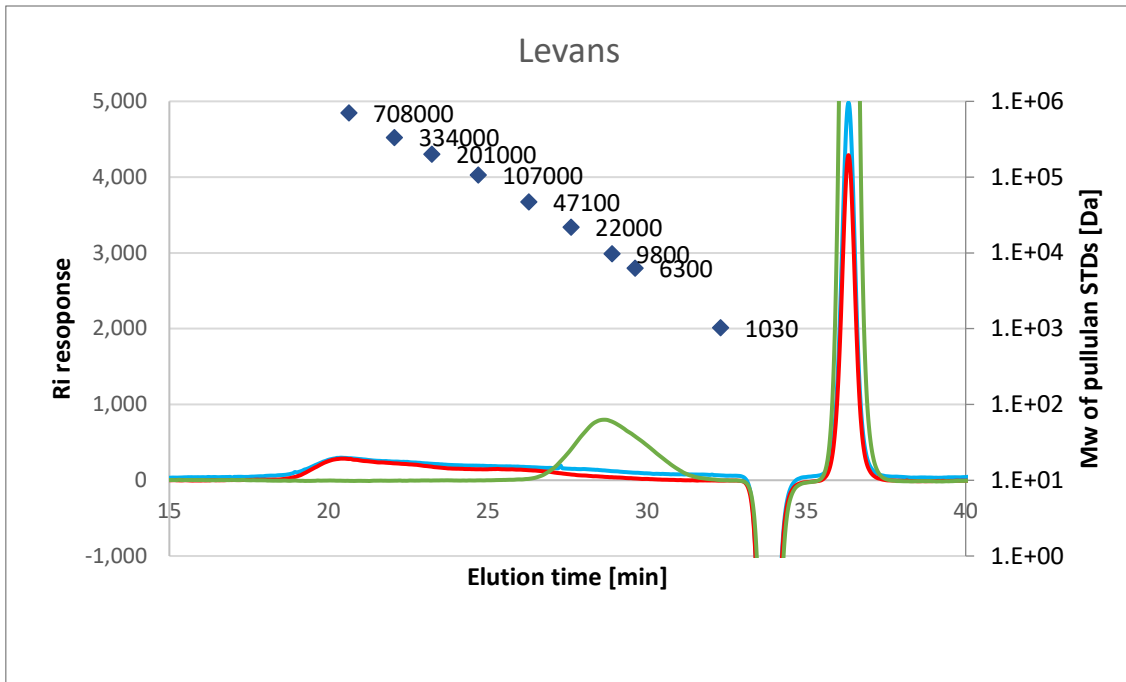
Cell line	Cell Density	Agonist	Agonist concentration
THP-1MD2-CD14	1*10 ⁶ cells/mL	<i>E.coli</i> K12 lipopolysaccharide (LPS) Ultrapure	100 ng/mL
THP-1 DefMyD	2*10 ⁶ cells/mL	L-Ala-gamma-D-Glu-mDAP (TriDAP)	10 µg/mL
HEK-Blue hTLR2	2.8*10 ⁵ cells/mL	Heat-killed <i>Listeria monocytogenes</i> (HKLM)	10 ⁷ cells/mL
HEK-Blue hTLR3	2.8*10 ⁵ cells/mL	Poly(I:C) high molecular weight	1 mg/mL
HEK-Blue hTLR4	1.4*10 ⁵ cells/mL	<i>E.coli</i> K12 lipopolysaccharide (LPS) Ultrapure	10 ng/mL
HEK-Blue hTLR5	1.4*10 ⁵ cells/mL	Recombinant flagellin from <i>S. typhimurium</i> (Rec-FLA-ST)	100 ng/mL
HEK-Blue hTLR7	2.2*10 ⁵ cells/mL	Imiquimod	5 µg/mL
HEK-Blue hTLR8	2.2*10 ⁵ cells/mL	Single stranded RNA (ssRNA40/LyoVec TM)	5 µg/mL
HEK-Blue hTLR9	4.5*10 ⁵ cells/mL	Type B CpG oligonucleotide (ODN 2006)	100 mg/mL



Supplementary figure 1: Design of the *in vitro* DC experiments. (A) DCs were incubated with β -fructans for 48 h to investigate their stimulatory properties and (B) to investigate the effect of soluble epithelial factors in response to incubation with β -fructans on DCs, Caco-2 cells were cultured on trans well inserts and incubated with β -fructans for 24 h on the apical side. Subsequently, Caco-spent medium (CSM) from the basolateral basin was incubated with DCs for another 48 h. DC supernatants were analyzed for cytokine production.

Supplementary table 2: Data under detection limit

Cytokine	Lower detection limit (µg/mL)	Interpretated value (µg/mL)
MCP-1/CCL2	-	-
MIP-1α/CCL3	13,4979	1,34979
IL-1β	2,97668	0,297668
IL-1RA	4645	464,5
IL-6	6,17284	0,617284
IL-10	5,28121	0,528121
TNFα	3,29218	0,329218



Supplementary figure 2: Molecular weight determination of the Levans included in this study measured by gel permeation chromatography (GPC). IMw β -(2,6)-fructan is represented in green. mMw β -(2,6)-fructan is represented in red and the hMw β -(2,6)-fructan is represented in blue. Blue dots on top of the plot represent the pullulan STDs.