Supporting Information

Laser-facilitated epicutaneous immunotherapy with hypoallergenic beta-glucan neoglycoconjugates suppresses lung inflammation and avoids local side effects in a mouse model of allergic asthma

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Supplementary Methods

NMR spectroscopy

All NMR data were measured on a 600 MHz Avance III HD spectrometer (Bruker Biospin) equipped with a $^{1}H/^{13}C/^{15}N/^{31}P$ QXI probe at 298 K. The lyophilized samples were dissolved in D_2O (100%D, Armar, Germany) and measured in 5 mm tubes. Spectra were referenced to 2,2-dimethyl-2-silapentanesulfonic acid (DSS) using an external sample of 2 mM sucrose 0.5 mM DSS in H_2O/D_2O (Bruker Biospin). The ^{13}C axis was indirectly referenced using the recommended scaling factor Ξ of 0.251449530 according to IUPAB [1]. Standard 2D ^{1}H-^{13}C HSQC spectra were recorded with 2018 and 13.9 ppm spectral width for ^{1}H and 250 data points and 82.8 ppm spectral width using 64 scans, a recycle delay of 4 sec resulting in a measurement time of ~19 hours. 2D ^{1}H-^{1}H TOCSY spectra were collected with 2018 and 11.7 ppm spectral width for ^{1}H and 512 data points and 10.4 ppm spectral width using 4 scans, mixing times of 80 ms or 12 ms, a recycle delay of 1 sec resulting in a measurement time of approx. 50 min. 2D ^{1}H-^{1}H NOESY spectra were acquired with 2018 data points and 10.0 ppm spectral width for ^{1}H and 700 data points and 10.0 ppm spectral width using 40 scans, a mixing time of 120 ms, a recycle delay of 1 sec resulting in a measurement time of 11 hours. 2D ^{1}H-^{13}C HMBC spectra were measured using the pulse sequence hmbclpndqf (Bruker Biospin) with 4096 points and 20 ppm spectral width for ^{1}H and 512 data points and 222 ppm spectral width for ^{13}C, 64 scans, optimized for a J_{CH} long range coupling of 8 Hz, a recycle delay of 1.5 sec resulting in a measurement time of 17 hours. 2D ^{1}H-^{13}C HSQC-TOCSY spectra were recorded using the pulse sequence hsqcdietgpsisp.2 (Bruker Biospin) with 1024 and 16 ppm spectral width for ^{1}H and 512 data points and 49.5 ppm spectral width, using 128 scans, a recycle delay of 1.2 sec and mixing times of 12 ms or 82 ms, resulting in a measurement time of ~24 hours Data were processed with Topspin 3.2 (Bruker Biospin, Germany) and the chemical shift assignment was achieved using the software Sparky 3.115 (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco).

Laminarin binding assays

Binding of soluble murine dectin-1a receptor (fused to an IgG1 Fc domain, Invivogen) to plate-bound OVA or LamOVA was assessed by ELISA. Briefly, a 96-well F-bottom plate was coated overnight with 10 µg/mL OVA or LamOVA in PBS. The next day, unbound molecules were removed by washing with PBS, 0.02% Tween followed by blocking with a polymer based blocking reagent (ROTI™Block, Carl Roth) for 1h at room temperature. Subsequently, increasing concentrations of Fc-dectin-1a (Dec-1a) were added starting from 2 ng/mL. The highest concentration was 2 µg/mL. After incubation for 1h

at RT the plate was washed, and HRP-conjugated anti-human IgG1 (Biolegend) was added (dilution 1:500) and incubated for 1h at RT. After washing, the assay was developed by addition a of Chemiluminescence ELISA substrate (Roche).

Interaction of OVA, LamOVA or laminarin with Fc-dectin-1a in solution was measured by Microscale Thermphoresis (MST) using a Monolith NT.115 RED instrument (Nanotemper, Munich, Germany). 50 µg of Dec-1a or soluble human TLR5 fused to an IgG1 Fc domain (TLR5, used as unspecific binding control), were buffer exchanged into 1x PBS buffer. Subsequently, the receptors were covalently labeled with NT-647-NHS dye (Nanotemper) at a molar ratio of 1:3 (protein:dye) in PBS buffer for 30 min at 20 °C in the dark. Unreacted, excess dye was removed using a NAP-5 column (GE Healthcare) preequilibrated in PBS buffer and DOL (degree of labeling) and protein concentration were determined according to the manufacturer's instructions. To determine the binding affinity of LamOVA, OVA, and 4.5kD laminarin, a 1:2 dilution series was prepared in PBST buffer (PBS + 0.05% Tween-20). Based on the assumption that 1 OVA molecule (45 kDa) was modified on average with 6 laminarin moieties (4.5 kDa each), the concentration of the LamOVA stock solution was 2.2 mg/ml (51 µM) based on OVA and 1.3 mg/ml (288 µM) based on laminarin. Labeled Dec-1a or TLR5 were diluted 1:50 in PBST buffer, to reach a final concentration of 4.4 nM. 10 µl of the respective sample dilution were mixed with 10 µl of the labeled receptors and incubated for 15 min at 20 °C in the dark. To remove possible aggregates, the complexes were centrifuged at 13.000 g for 15 min at 4°C. Immediately after centrifugation, the reactions were transferred into standard coated capillaries and MST traces were measured at 25 °C, 50% LED and medium MST power in an NT.115 RED instrument. Data were recorded with the MO.Control 1.5.1 (NanoTemper) software and further analyzed using MO-Affinity Analysis 2.2.6 (NanoTemper). MST traces were processed using an MST on-time of 2.5 sec and fitted using Hill (assuming multiple binding sites) fit model. EC50 values were determined from three independent binding experiments. To confirm that the shift in MST signal was due to complex formation rather than unspecific aggregation, samples were incubated with 10% SDS and 10 mM DTT and heated to 95 °C for 5min before measuring MST traces.

Generation of bone marrow-derived dendritic cells

Bone marrow was harvested from femur and tibia of BALB/c mice. For generation of FL-BMDCs, 3 mL of a $2.5x10^6$ cells/mL bone marrow cell suspension were seeded into non-cell culture treated 6-well plates and stimulated with 200ng/mL human Flt3-L for 9 days at 37°C, 5% CO_2 and 95% humidity. Between days 4 and 6, 1.5 mL of culture medium (RPMI/1640 supplemented with 2.5 mM HEPES, 100 IU/mL penicillin, 100 mg/mL streptomycin, 2 mM L-glutamine, 10% fetal calf serum) were added to each well. For generation of GM-BMDCs, 10 mL of a $2x10^5$ cells/mL bone marrow cell suspension

were plated into non-cell culture treated petri dishes and incubated in the presence of murine GM-CSF for 8 days. On day 3, 10 mL of GM-CSF containing culture medium (RPMI/1640 supplemented with 20 ng/mL GM-CSF, 100 IU/mL penicillin, 100 mg/mL streptomycin, 2 mM L/Glutamine, 10% FCS and 50 µM 2-mercaptoethanol) was added to each well, and on day 5, 10 mL medium was replaced.

Conjugate uptake, BMDC activation and T cell proliferation

To assess uptake of glycoconjugates by BMDCs, their activation, and their capacity to induce T cell proliferation, FL-BMDCs and GM-BMDCs harvested on days 9 and 8, respectively were used.

For uptake analysis, OVA and LamOVA were labeled with Invitrogen pHrodo™ iFL Green STP Ester (ThermoFisher Scientific) according to the manufacturer's protocol. Briefly, pHrodo was resuspended in DMSO to a concentration of 10 mM. For labeling, 50 µL OVA or glycoconjugates were mixed with 5 µL of a 1M $NaHCO_3$ solution (pH 8.3), added to the dye and incubated at RT for 60 min in the dark. The coupling reaction was stopped by addition of 1/10 volume of 1 M Tris pH8 followed by another incubation for 60 min at RT in the dark. Unreacted dye was removed from the samples by running over an Illustra NAP-5 column (GE healthcare). Labeled OVA and conjugates were analyzed for their fluorescence intensity at an excitation wavelength of 488nm and an emission wavelength scan between 520 and 560nm. Exact protein concentrations were determined by amino-acid analysis.

$1.5x10^5$ cells were stimulated overnight at 37°C, 5% CO_2, 95% humidity with 5µg of labeled conjugates or OVA, respectively. Incubation with PBS served as negative control. To analyze CD86 and Dectin-1 expression, $1.5x10^5$ cells were stimulated with 5, 25 or 50 µg/mL OVA or an equivalent amount of LamOVA, 12.5, 62.5 or 125 µg/mL laminarin, 1µg/mL LPS (positive control), or PBS (negative control), respectively, overnight at 37°C, 5% CO_2, 95% humidity. Cells were analyzed by flow cytometry on a Cytoflex S flow cytometer (Beckman Coulter). See suppl. Fig. 11 for gating strategy.

For T cell – BMDC co-cultures, OVA-specific T cells harvested from BALB/c DO11.10 mice were labeled with 1 µM fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE) in PBS. Briefly, lymph node and spleen cell suspensions were incubated for 10 minutes at 37°C in a water bath with CFSE. The reaction was stopped by addition of 10% FCS. After washing, the cells were resuspended in T cell medium (RPMI-1640 supplemented with 25 mM HEPES, 100 IU/mL penicillin, 100 mg/mL streptomycin, 2 mM L-glutamine, 10% FCS) and stained with anti-CD62L – APC-eFluor780 and anti-CD4 – eFluor450 for sorting of naïve T cells on a BD FACS Aria™ III cell sorter (BD Biosciences). Naïve DO11.10 T cells ($1x10^5$ cells/mL) and BMDCs ($0.33x10^5$ cells/mL) were co-cultured in T cell medium in the presence of 50 µg/mL OVA or an equivalent amount of conjugates or medium

for 5 days at 37°C, 5% CO_2, 95% humidity. To determine the percentage of proliferating cells, staining with anti-CD4-PerCP-Cy5.5 and fixable viability dye APC-eFluor 780 was performed and cells were analyzed by flow cytometry. Culture supernatants were used for cytokine secretion analysis by LEGENDPlex assay according to manufacturer's protocol.

BAL

Bronchoalveolar lavages were performed as described previously [2]. Following staining with anti-CD8-FITC, anti-SiglecF-PE, anti-CD45-PerCp-Cy5.5, anti-CD4-BV421, anti-CD19-PE/Cy7, and anti-Ly6G/Ly6C (Gr1)-APC, cells were analyzed by flow cytometry. Leucocytes were gated on a SSC vs. CD45 plot followed by doublets exclusion. Neutrophils were determined as $Gr1^{high}$ Siglec F^{low}, and $Gr1^{low}$ Siglec F^{high} cells were further divided into eosinophils and alveolar macrophages based on autofluorescence in the BV510 channel. $Gr1^{neg}$ Siglec F^{neg} lymphocytes were further separated into CD4 and CD8 T cells. See suppl. Fig. 12 for gating strategy.

Lung digestion

After transcardial perfusion, the right lung lobe was cut into 1x1mm pieces in digestion buffer containing Liberase™ (0.25 mg/mL, Roche), Hyaluronidase (1 mg/mL, Sigma), DNAse I (0.03 mg/mL, Sigma) and Collagenase XI (0.25 mg/mL, Sigma) in RPMI-1640 and incubated on a rotary shaker (300rpm, 30min, 37°C). Thereafter, the cell suspension without visible clumps was placed on ice and the remaining tissue was passed through a 21G needle and combined with 2 mL of fresh digestion buffer. The suspension was incubated for additional 30 min under the same conditions. The cells were pooled and EDTA was added to a final concentration of 10mM. The sample was filtered through a 100µm cell strainer (Greiner Bio One) and labeled (15min, 4°C) with biotin anti-mouse CD45.2 antibody diluted 1:50 in FACS buffer (PBS, 1% BSA, 2mM EDTA) and sorted using BD IMag™ Streptavidin Particles Plus – DM (BD Biosciences). Prior to staining, non-specific binding sites were blocked by resuspending cells in 20µL hybridoma supernatant containing anti-CD16/CD32 for 5 min at 4°C. Finally, the cell pellet was stained using the following anti-mouse antibodies: CD11b-PerCp/Cy5.5, CD45-eFluor506, GR-1-AlexaFluor700, Siglec F-Superbright 600, CD24-PE/Dazzle 594, MHC II-FITC. After incubation for 30 min at 4°C, cells were washed tree times. Finally, the pellet was resuspended in FACS buffer and cells were analyzed on a Cytoflex S flow cytometer (Beckman Coulter). Samples were analyzed according to the gating strategy published by Misharin et al. [3] (Suppl. Fig. 13).

Basophil activation test (BAT)

Cell-bound IgE was analyzed *ex vivo* by basophil activation test [4]. Whole blood was drawn from vena saphena the day after the second aerosol challenge. To prevent coagulation, 10% v/v Li-Heparin (10mg/mL in DPBS) was added. 30µL of heparinized blood was either used untouched or washed three times with 200µL of RPMI (260g, RT, 5min) to remove serum containing blocking IgGs. 30µL of RPMI with or without 2ng/mL OVA was added to the samples and incubated for 2h at 37°C, 5% CO2, 95% humidity. Subsequently, samples were put on ice and all further steps were performed with ice-cold solutions at 4°C. Samples were washed with 120µL FACS buffer and pelleted (5min centrifugation at 260 g). Cells were stained in 30µL of FACS buffer containing anti-mouse IgE-FITC, anti-CD4-PerCp-Cy5.5, anti-CD19-PE/Cy7, and anti-CD200R-APC for 20 min on ice, washed with 200µL FACS buffer, and the pellets were resuspended in red blood cell lysis buffer (eBioscience) and incubated for 5min at RT followed by two washing steps with FACS buffer. Finally, cells were resuspended in 50µL FACS buffer and analyzed by flow cytometry on a FACS Canto II flow cytometer (BD Biosciences). Basophils were gated as IgE^{high} $CD19^{neg}$ $CD4^{neg}$ and the basophil activation status was assessed as median fluorescence intensity of CD200R.

Flow Cytometry of restimulated splenocytes

Cell pellets from restimulated cells were centrifuged for 5min at 400g at 4°C. After one wash with 150µL DPBS, pellets were resuspended in 20 µL hybridoma supernatant containing anti-CD16/32 to block Fc-receptors. After 5min incubation at 4°C, 20 µL of extracellular staining mix was added directly to the samples. Extracellular staining mix contained fixable viability dye eFluor 506, anti-CD44-BV650, anti-CD25-Super Bright 600 anti-CD4-APC-eFluor780 diluted in DPBS. Plates were briefly mixed on an oscillating shaker and incubated for 30 min at 4°C in the dark. Subsequently, 100µL of ice-cold FACS buffer were added, and cells were pelleted (400g, 4 min, 4°C). Cell pellets were resuspended in 150 µL ice-cold Fix/Perm buffer (Foxp3/Transcription Factor Staining Buffer Kit, Tonbo Biosciences) and incubated 60min at 4°C in the dark. After centrifugation (400g, 4min, 4°C), pellets were washed twice in 100 µL Perm buffer from the same kit. Pellets were resuspended in 30 µL of Perm buffer containing anti-FoxP3-APC and GATA3-BV421. Samples were incubated for 30 min at RT in the dark. After one more wash with 100 µL Perm buffer, cell pellets were resuspended in 80 µL FACS buffer and analyzed on a Cytoflex S flow cytometer (Beckman Coulter). See suppl. Fig. 14 for gating strategy.

Supplementary Tables

Supplementary Table 1. Antibodies used in the study.

Marker	Fluorophore	Final Dilution	Clone	Manufacturer
BMDC stimulation, BMDC-DO11 T cell co-culture				
CD11b	PerCP-Cy5.5	1:500	M1/70	BioLegend
CD11c	BV421	1:100	N418	BioLegend
CD86	BV605	1:200	GL-1	BioLegend
Dectin-1	PE	1:100	RH1	BioLegend
Fixable viability dye	APC-eFluor780	1:3000		Invitrogen
Lung digestion				
CD11b	PerCp-Cy5.5	1:200	M1/70	BioLegend
CD24	PE/Dazzle 594	1:600	M1/69	Biolegend
CD45	eFluor 506	1:100	30-F11	Invitrogen
GR-1	Alexa Fluor 700	1:200	RB6-8C5	Biolegend
MHC II	FITC	1:200	M5/114.15.2	BioLegend
Siglec F	Superbright 600	1:200	1RNM44N	Invitrogen
BAL				
CD4	BV421	1:200	RM4-5	Invitrogen
CD45	PerCP-Cy5.5	1:400	A20	Invitrogen
GR-1	APC	1:200	RB6-8C5	Invitrogen
Siglec F	PE	1:200	E50-2440	BD
CD8	FITC	1:100	53-6.7	Invitrogen
CD19	PE-Cy7	1:100	1D3	Invitrogen
Lymphocyte restimulation				
CD4	APC-eFluor780	1:400	RM4-5	Invitrogen
CD25	Superbright 600	1:100	PC61.5	Invitrogen
CD44	BV650	1:50	IM7	Biolegend
FoxP3	APC	1:100	FJK-16s	Invitrogen
GATA3	BV421	1:100	16E10A23	BioLegend
Fixable viability dye	eFluor 506	1:1000		Invitrogen
BAT				
CD4	PerCp-Cy5.5	1:200	GK1.5	BioLegend
CD19	PE-Cy7	1:200	eBio1D3 (1D3)	eBioscience
IgE	FITC	1:200	RME-1	BioLegend
CD200R	APC	1:200	OX110	Invitrogen

Supplementary Table 2: Observed chemical shifts of dialysed laminarin dissolved in D_2O measured at 298 K in comparison to previously reported data (Petersen et al. 2000, Eur. J. Biochem 267, 361)

Moiety		H1	H1'	H2	H3	H4	H5	H6	H6'	C1	C2	C3	C4	C5	C6	
M	Mannitol	4.195	3.847	3.910	3.891	3.801	3.769	3.873	3.681	74.6	72.4	71.7	71.9	73.5	66.0	this work
		4.168	3.850	3.910	3.865	3.820	3.765	3.854	3.684	74.5	72.5	72.8[a]	72.0	71.9[a]	65.9	Petersen et al.[b]
a	3Glc3	4.801	–	3.574	3.793	3.523	3.528	3.932	3.749	105.3	76.0	86.9	70.8	78.4	63.4	this work
		4.780	–	3.573	3.786	3.537	3.515	3.928	3.747	105.4	75.9	87.0	70.8	78.3	63.4	Petersen et al.[b]
b	3Glc1M	4.548	–	3.544	3.775	n.d.[c]	3.518	3.938	n.d.[c]	105.4	75.8	86.9	70.9	78.3	n.d.[c]	this work
		4.544	–	3.544	3.768	3.510	3.510	3.928	3.750	105.4	75.6	87.1	70.9	78.3	63.4	Petersen et al.[b]
c (α)	3Glcα	5.242	–	3.735	3.924	3.530	3.871	n.d.[c]	n.d.[c]	94.8	73.8	85.0	n.d.[c]	74.0	n.d.[c]	this work
		5.242	–	3.727	3.921	3.522	3.886	3.845	3.779	94.8	73.5	85.2	n.d.[c]	n.d.[c]	n.d.[c]	Petersen et al.[b]
c (β)	3Glcβ	4.685	–	3.446	3.754	3.509	n.d.[c]	3.903	n.d.[c]	98.4	76.6	87.2	70.9	78.3	n.d.[c]	this work
		4.673	–	3.440	3.735	3.530	3.898	3.898	3.750	98.4	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]	Petersen et al.[b]
d	Glc3Glc3	4.759	–	3.374	3.529	3.418	3.497	3.926	3.737	105.6	76.2	78.3	72.3	78.3	63.5	this work
		4.740	–	3.380	3.535	3.426	3.491	3.919	3.730	105.6	76.1	78.3	72.3	78.3	63.4	Petersen et al.[b]
e	3(6)Glc3	4.775	–	3.590	3.804	3.604[d]	3.703[d]	4.235	3.898	105.3	76.0	86.7	70.9	77.3	71.6	this work
		4.759	–	3.586	3.795	3.594	3.698	4.209	3.886	105.4	75.8	86.9	70.9	77.2	71.5	Petersen et al.[b]
f	Glc6	4.540	–	3.319	3.500	3.411	3.464	3.919	3.747	105.5	75.9	78.3	72.3	78.6	63.5	this work
		4.520	–	3.319	3.505	3.405	3.455	3.911	3.733	105.6	75.9	78.4	72.3	78.4	63.4	Petersen et al.[b]
g	3Glc6(3)Glc3	4.567	–	3.513	3.745	n.d.[c]	n.d.[c]	3.925	n.d.[c]	105.3	75.7	87.2	70.9	78.3	n.d.[c]	this work
		4.560	–	3.519	3.725	3.490	3.510	3.921	3.729	105.4	75.9	87.3	70.9	78.3	63.4	Petersen et al.[b]
h	Glc3Glc6	4.731	–	3.374	3.532	n.d.[c]	n.d.[c]	3.916	3.727	105.6	76.2	78.3	72.3	78.8	n.d.[c]	this work
		4.709	–	3.378	3.482	3.430	3.533	3.819	3.730	105.7	76.1	78.3	72.3	78.3	63.2	Petersen et al.[b]
i	6Glc3	4.732	–	3.382	3.536	3.486	3.666	4.231	3.877	105.3	76.2	78.3	72.2	77.5	71.5	this work
		4.714	–	3.391	3.540	3.480	3.672	4.203	3.872	105.7	76.0	78.3	70.9	77.5	71.5	Petersen et al.[b]
j	unknown [e]	4.855	–	3.589	3.652	3.521	3.416	3.958	3.755	105.9	n.d.[c]	88.9	70.3	79.6	n.d.[c]	this work

[a] reported values are likely incorrect, chemical shifts of mannitol form the Madison Metabolomics Consortium (entry bmse000099; doi:10.13018/BMSE000099) report 71.9, 71.9, 73.49 and 65.92 ppm for C3, C4, C5 and C6, respectively.

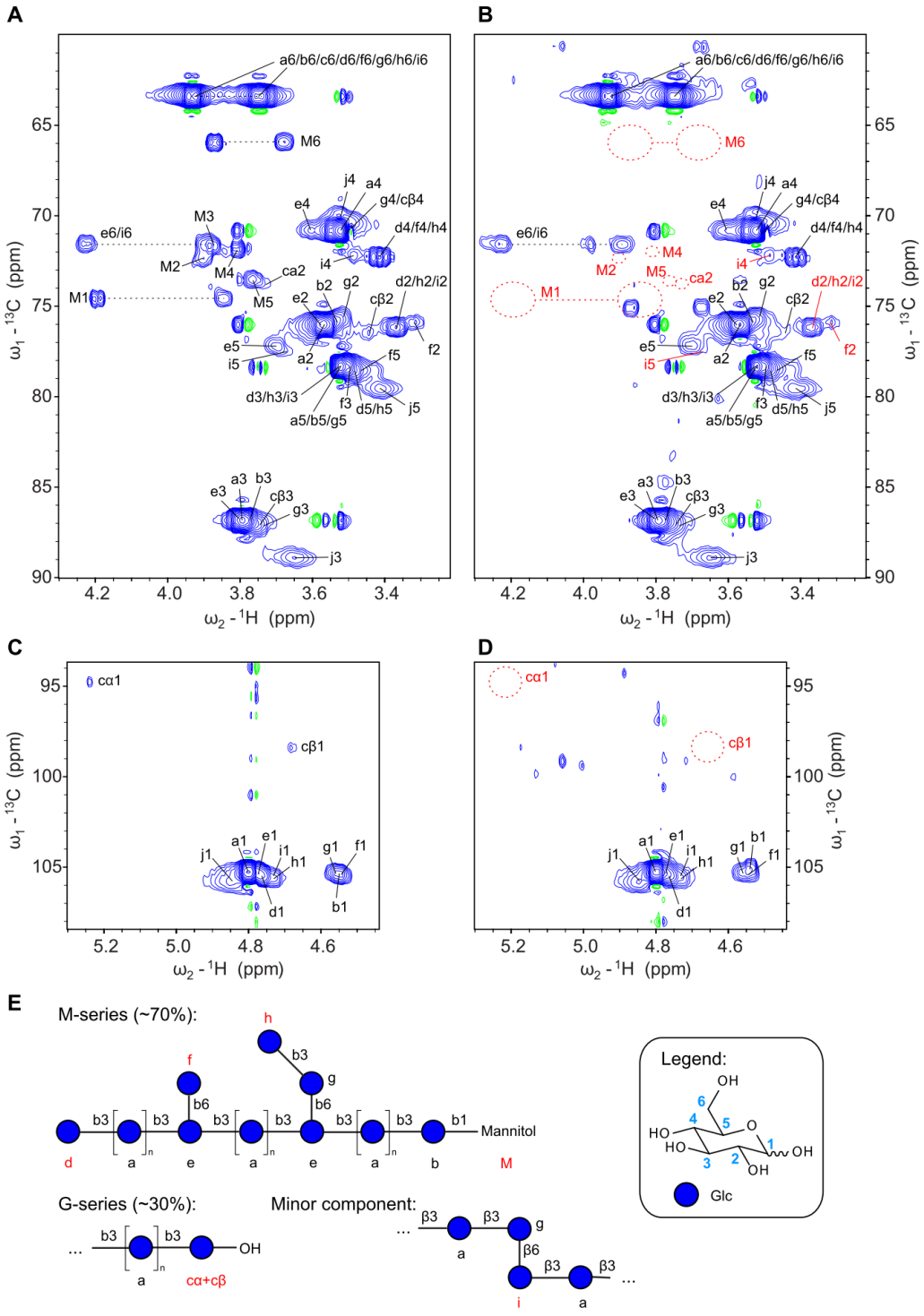
[b] reported chemical shifts of laminarin from *Laminaria digitata* (Sigma), 1.2 ppm were added to the reported ^{13}C chemical shifts to achieved a comparable referencing to DSS

[c] n.d. stands for not determined, mainly due to overlapping chemical shift correlations

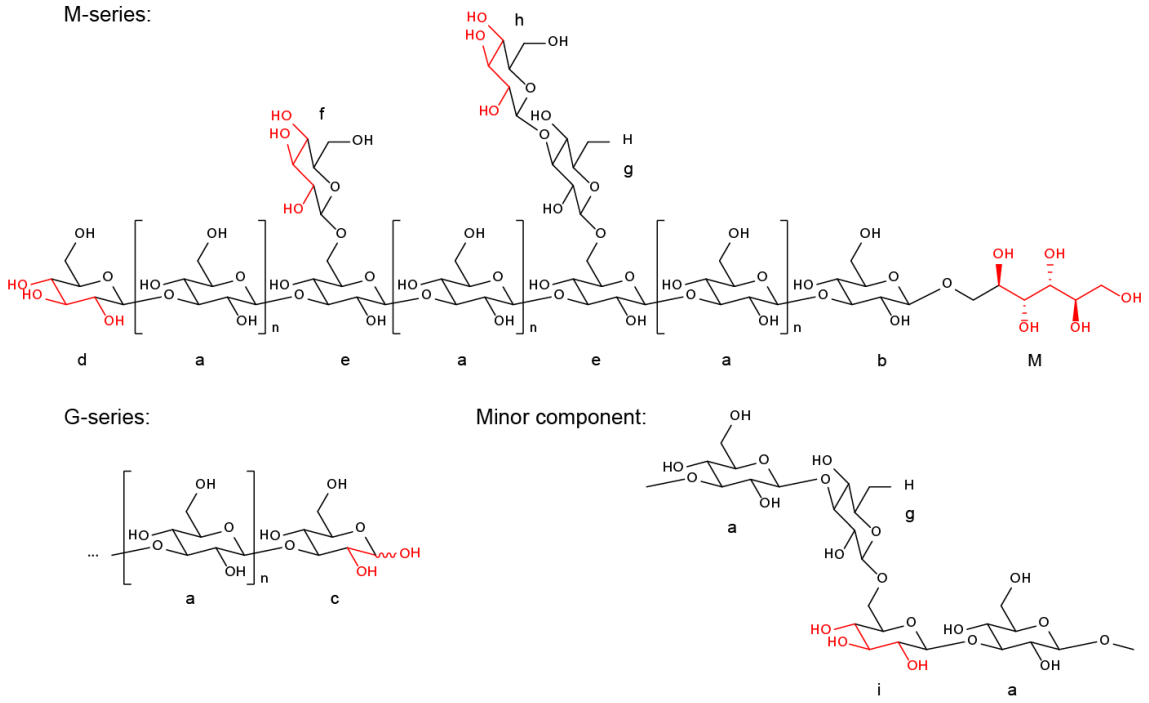
[d] resonances are broadened, likely due to the locally increased tumbling time due to branching or local dynamics

[e] spin system not reported by Petersen et al, the ^{13}C chemical shift of C3 indicates a substitution at O3, the chemical shifts of H6 and H6' point to the lack of substitution at O6, suggesting a backbone moiety with β1,3 linkages on both sides, all signals show line broadening hinting that this moiety is close to a branch side, e.g. 3**Glc**3(6)Glc3 or 3(6)Glc3**Glc**3

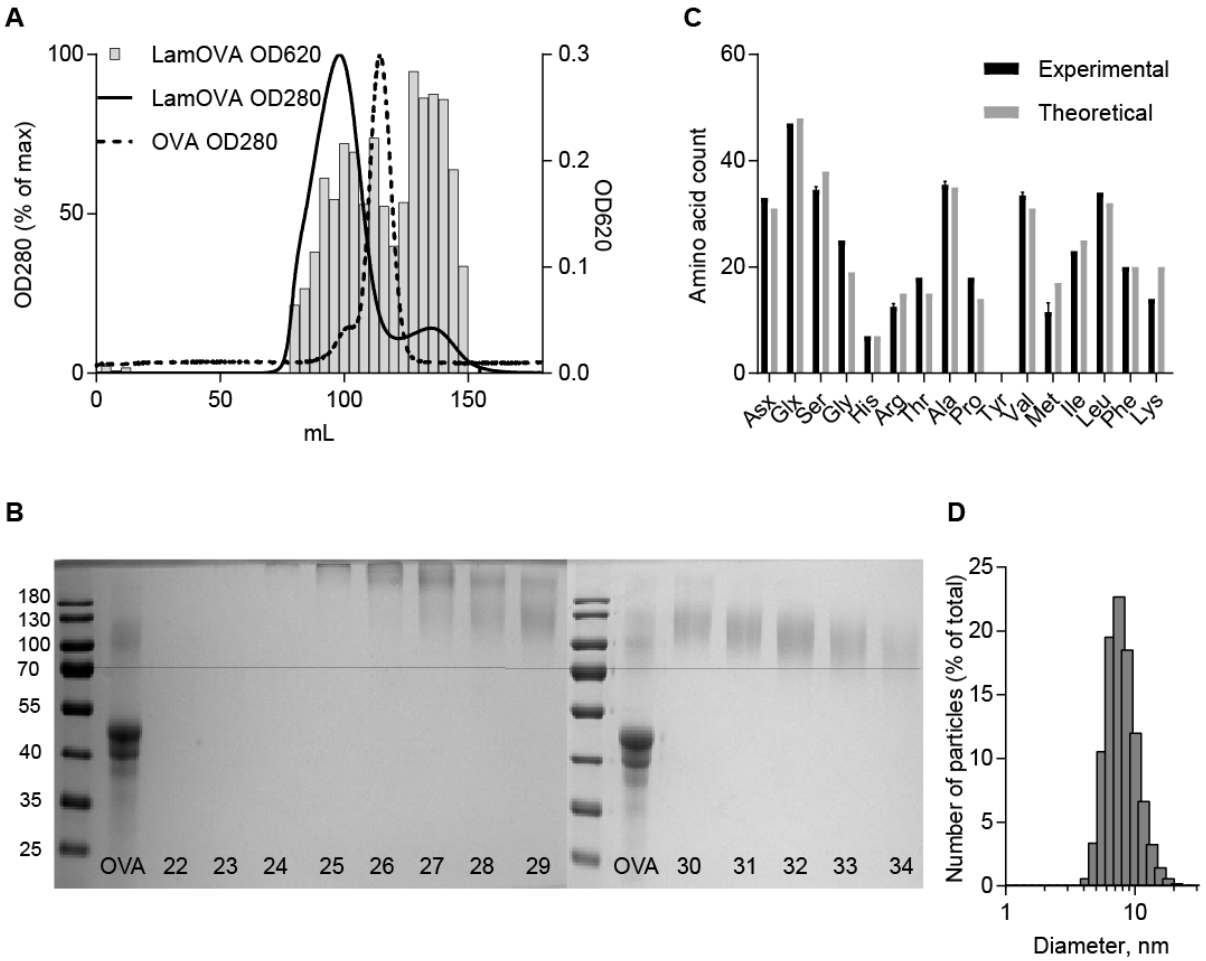
Supplementary Figures



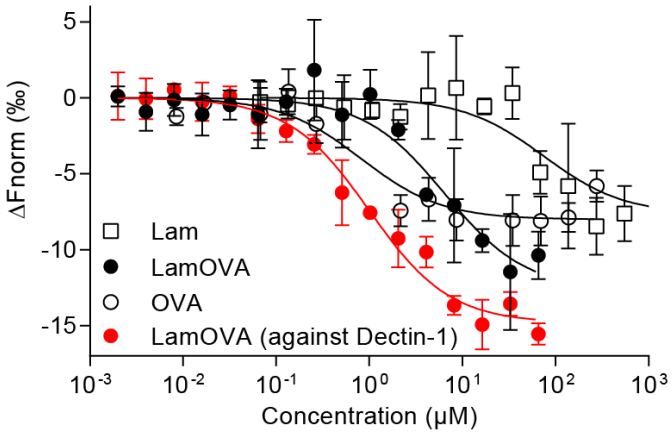
Supplementary Figure 1. Two-dimensional ^{1}H-^{13}C HSQC NMR spectra of untreated laminarin (A and C) and oxidized laminarin (B and D) with schematic structures (E). Panel A and C show the aliphatic region in which each ^{1}H-^{13}C one-bond correlation gives one signal (carbon C2 to C5) and methylene groups give two signals (H6-C6 and H6'-C6 connected by a dotted line). Positive signals are in blue, negative signals in green (only noise, artefacts and antiphase relay signals). Spin systems are labeled with a one-letter code and the number indicates the corresponding carbon. Spin systems in red indicate moieties whose signals either disappear or show reduced signal intensity upon periodate oxidation.



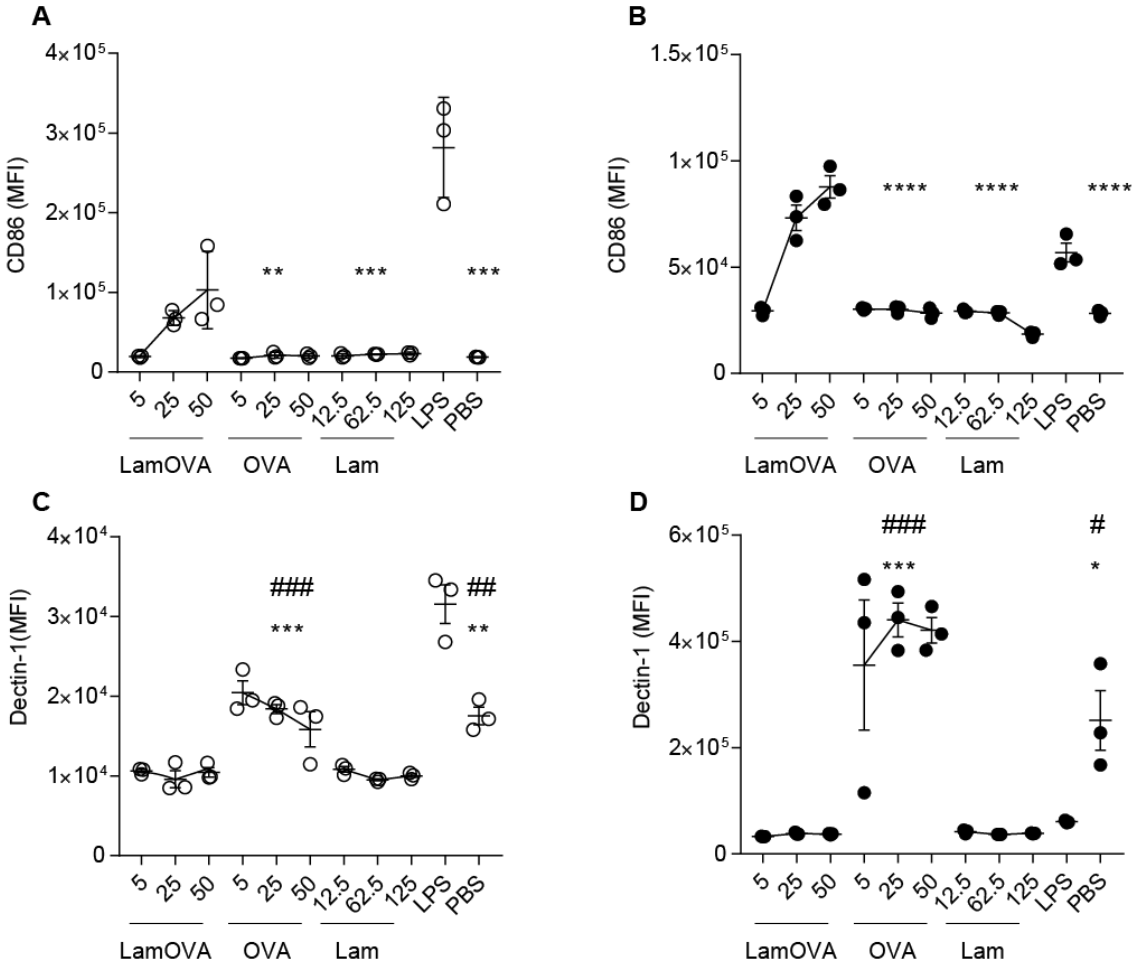
Supplementary Figure 2. Structure of laminarin as determined by NMR. Laminarin is heterogeneous and consists of a mixture of M-series and G-series molecules that end with mannitol (M) or an unprotected glucose (c) at the reducing end, respectively. Molecules vary in length and branching with an average number of 25 glucose moieties. Expected sites for periodate oxidation containing accessible, neighboring OH-groups (vicinal diols) are indicated in red.



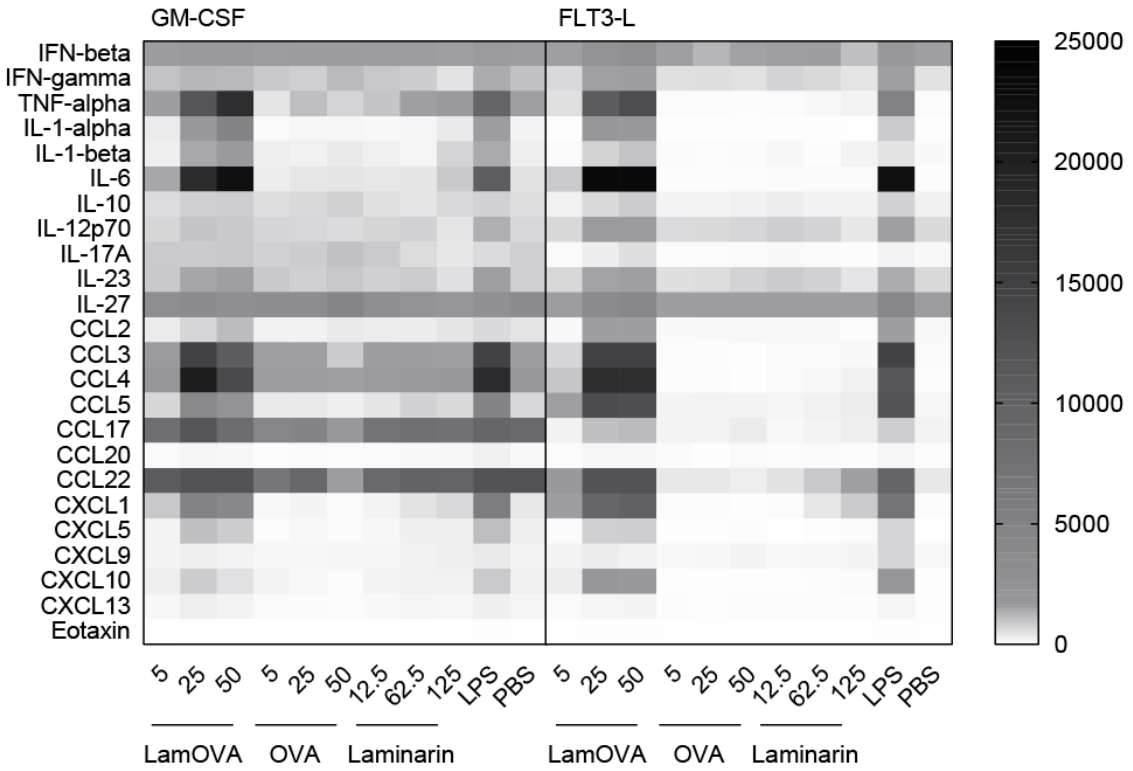
Supplementary Figure 3. Characterization of laminarin OVA conjugates. A) SEC chromatograms for LamOVA and OVA at 280nm. Laminarin concentration in LamOVA fractions was determined by anthrone method (620nm). B) OVA and LamOVA fractions 22-34 were analyzed by 10% SDS-PAGE and coomassie staining. Fractions 24-34 (elution between 70 and 100mL) migrated above 70kDa (threshold indicated by line) and were pooled for further analysis. C) Amino acid analysis. After reductive amination, only 14 of 20 lysine residues were detected. D) LamOVA size distribution (number-weighted) of particles as measured by dynamic light scattering. Mean hydrodynamic diameter was 8.27nm.



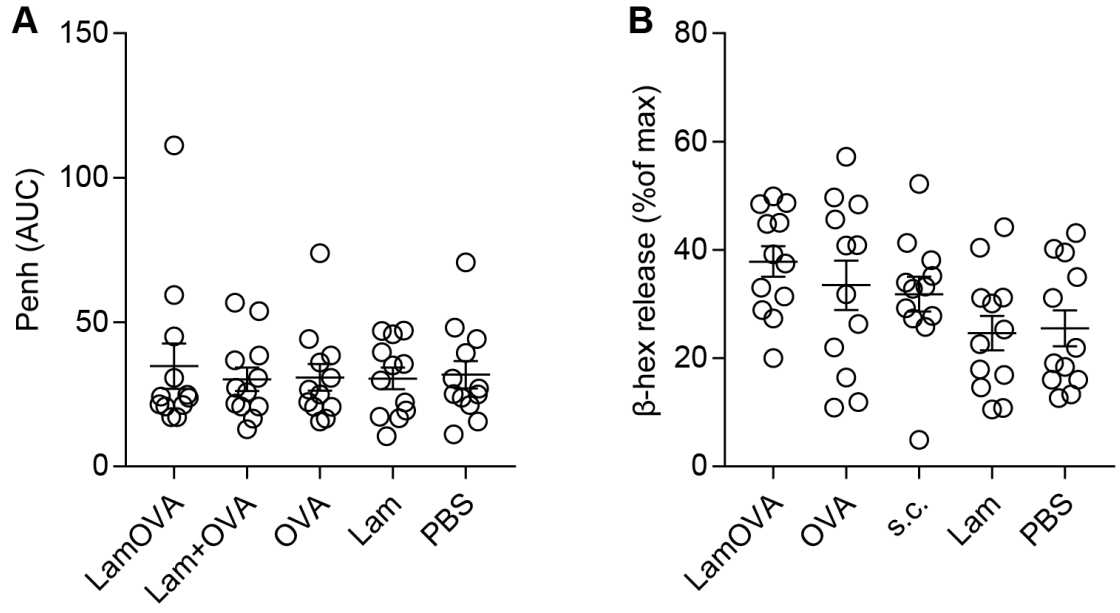
Supplementary Figure 4. Binding of soluble TLR5 receptor (fused to IgG-Fc) to soluble LamOVA, OVA, and laminarin. Data are shown as changes in normalized fluorescence (ΔFnorm) of MST measurements (mean±SD of three independent binding experiments). Binding of LamOVA to soluble Dectin-1 receptor is shown in red for reference.



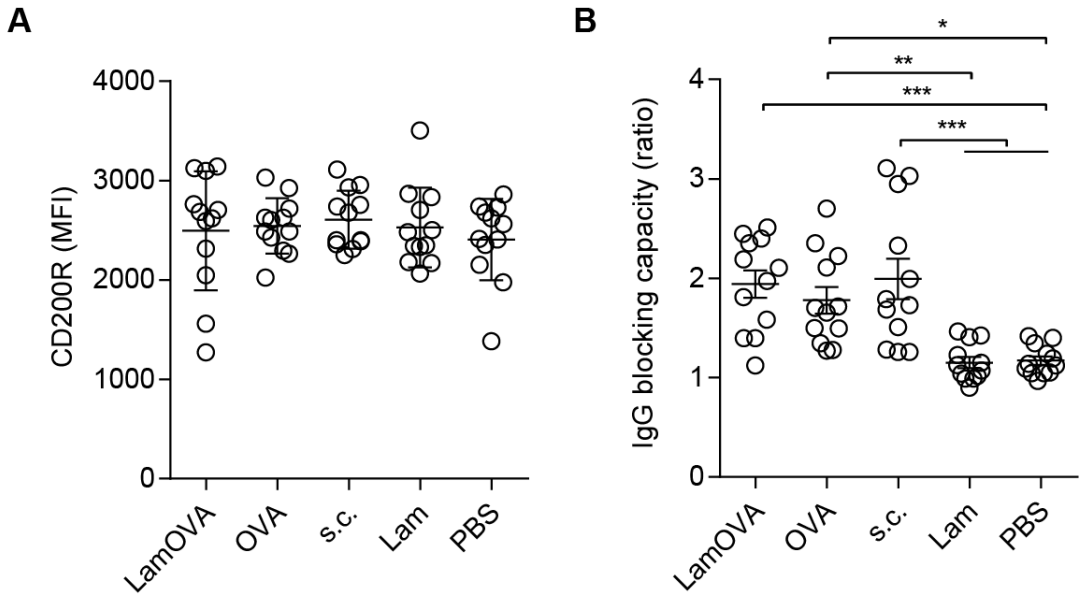
Supplementary Figure 5. CD86 and dectin-1 expression on stimulated BMDCs. GM- and FL-BMDCs were stimulated with LamOVA, OVA, or PBS for 24 hours. Protein concentrations for OVA and LamOVA were 5, 25, and 50µg/mL. Laminarin was used at equivalent doses as those present in LamOVA (12.5, 25, 62.5µg/mL). LPS (1µg/mL) served as positive control. Data are shown as median fluorescence intensity (MFI) of CD86 (A and B) and dectin-1 (C and D) on GM-BMDCs (A and C, white circles) and FL-BMDCs (B and D, black dots). Data are shown as means±SEM and individual replicates (n=3). Statistical significance vs. LamOVA (*), vs. OVA (#), and vs. PBS (§) was analyzed by two-way RM ANOVA followed by Tukey's multiple comparisons test.



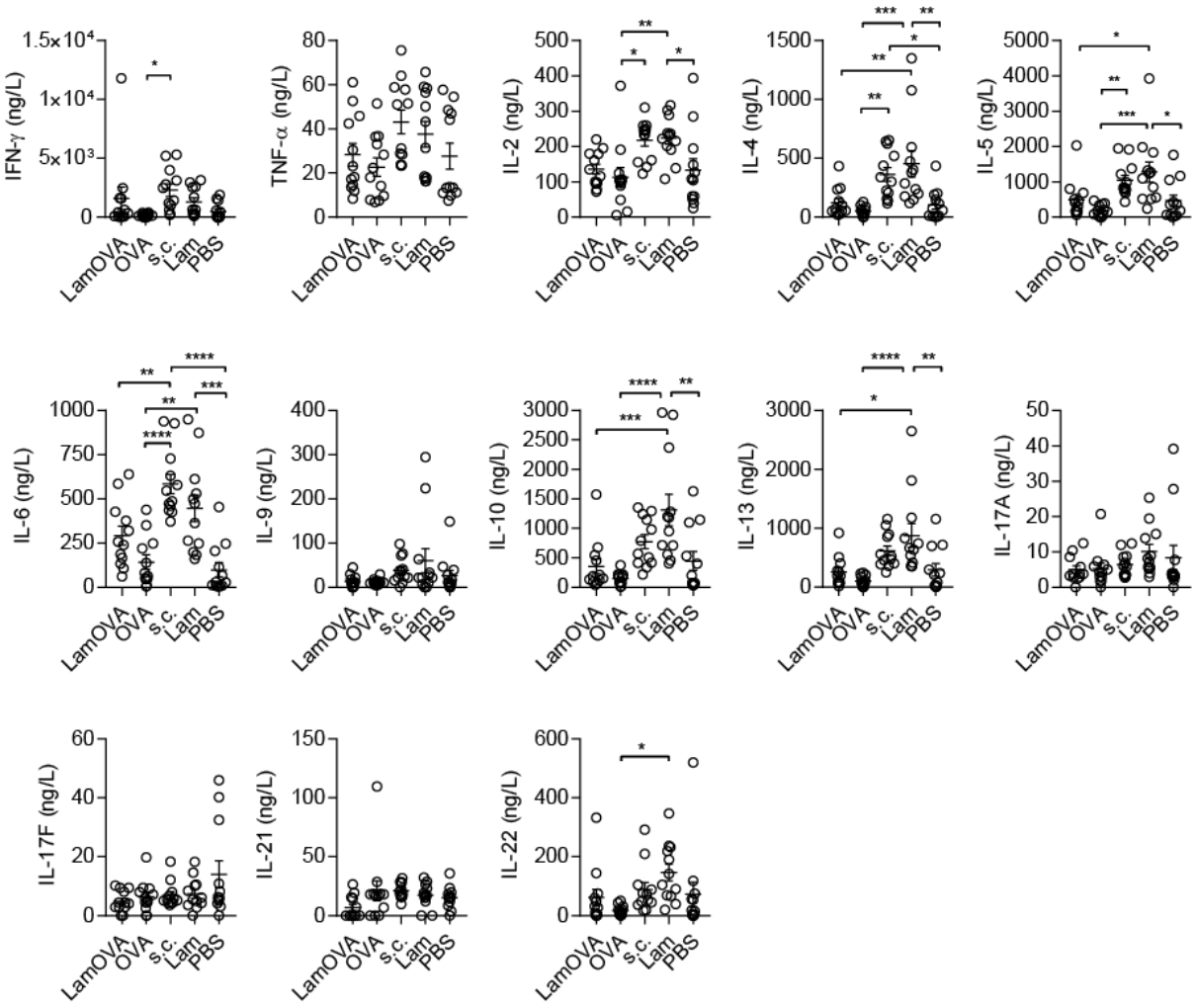
Supplementary Figure 6. Cytokine and chemokine secretion from stimulated BMDCs. GM- and FL-BMDCs were incubated 24h in the presence or absence of increasing doses of OVA, LamOVA or laminarin (shown in µg/mL). Laminarin was used at the same doses as present in LamOVA conjugates. 1 µg/mL LPS served as positive control. Data are shown as pg/mL.



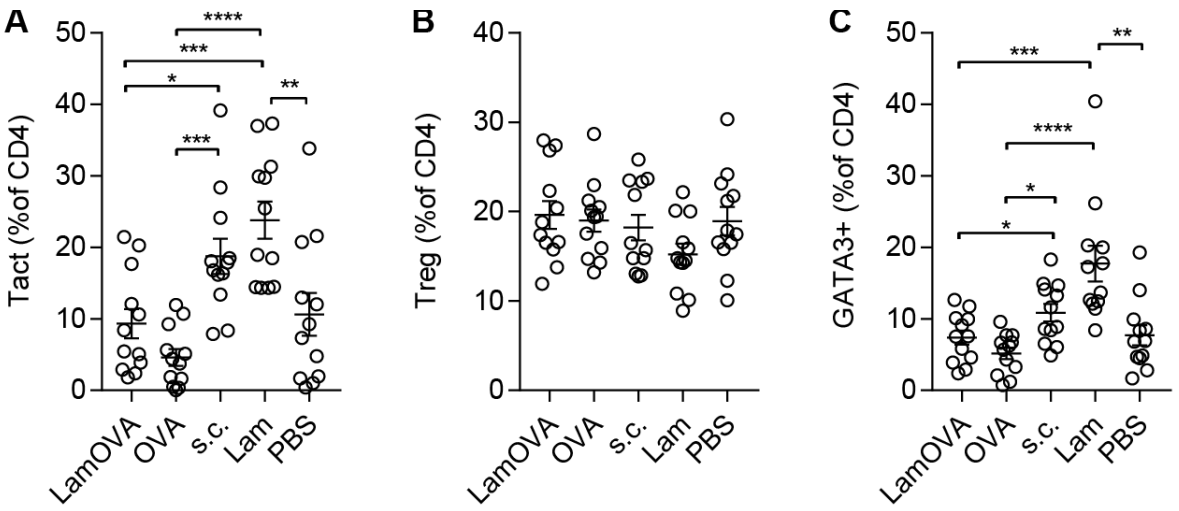
Supplementary Figure 7. Group stratification of sensitized mice before treatment. BALB/c mice were sensitized by two i.p. injections of OVA followed by two i.n challenges. A) Before treatment, airway hyperresponsiveness was assessed by WBP and mice were stratified into treatment groups with similar means and distribution of Penh. Data are shown as area under the curve (AUC) of a methacholine challenge dose response curve. B) Presence of OVA-specific IgE before treatment was confirmed by RBL assay (serum dilution 1:100). Data are shown as percentage of total beta-hexosaminidase release.



Supplementary Figure 8. Cell bound IgE and IgG blocking capacity after treatment and challenge. A) After the second aerosol challenge, blood was drawn and serum was washed away. After 2h *in vitro* restimulation with OVA, basophil activation status was assessed by flow cytometry as upregulation of CD200R. MFI = median fluorescence intensity. B) IgG blocking capacity is shown as the ratio of CD200R expression in basophils in the absence and presence of autologous serum. Data are shown as means±SEM and individual animals (n=12).

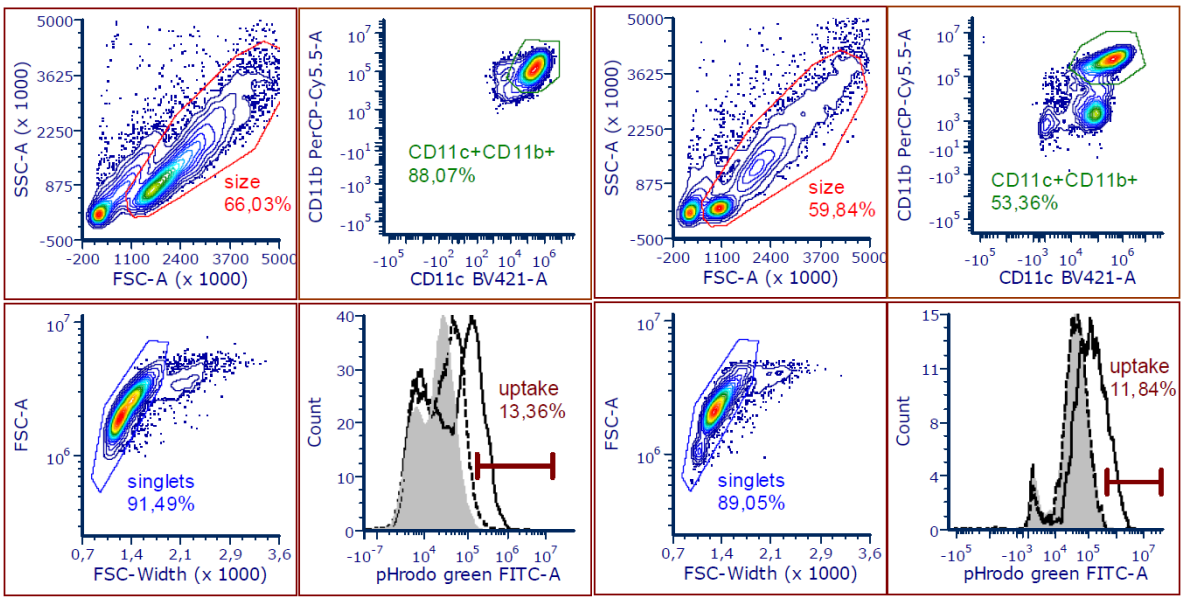


Supplementary Figure 9. Cytokine secretion of splenocytes restimulated with OVA for 3 days. Concentrations of different cytokines in cell culture supernatants was analyzed by flow cytometry using LEGENDplex assay. Data are shown as means±SEM and individual animals (n=12).

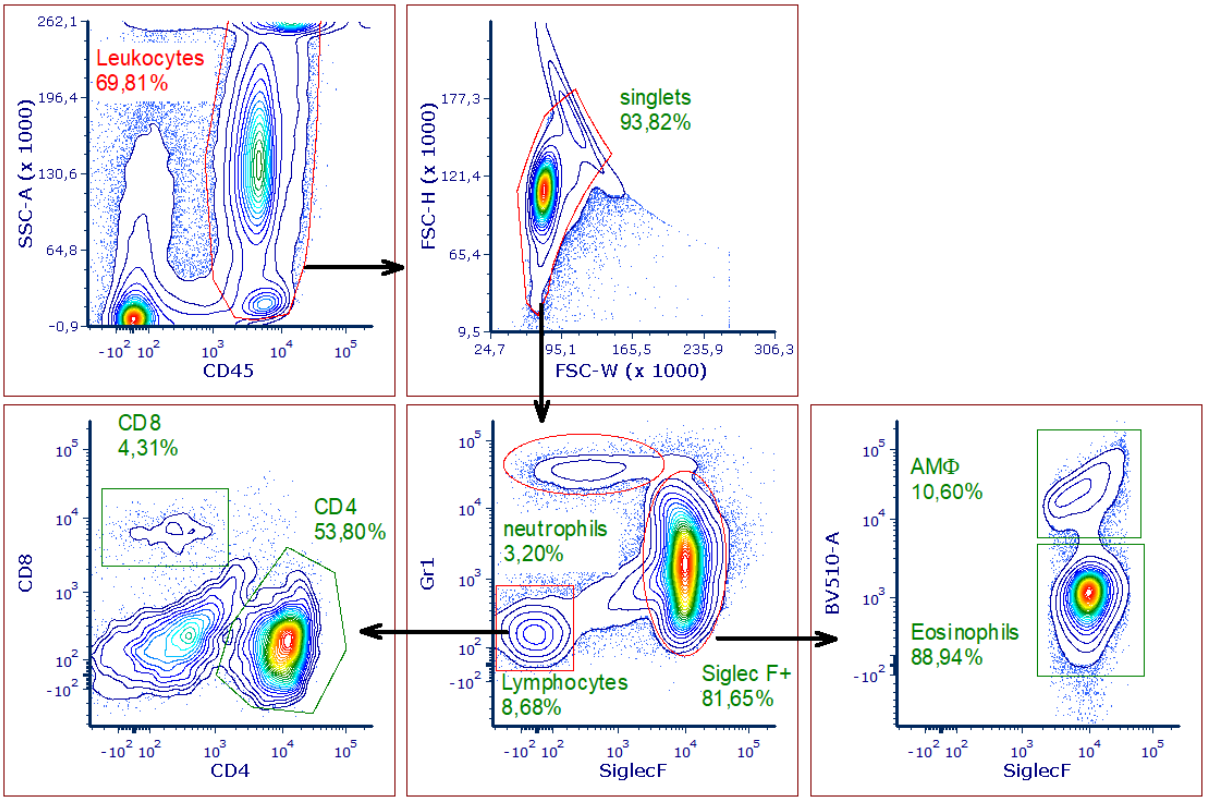


Supplementary Figure 10. Percentage of activated (Tact), regulatory (Treg) and GATA3+ T cells. Splenocytes were restimulated with OVA for 3 days and the percentage of Tact, Treg, and GATA3+ cell was assessed by flow cytometry. Data are shown as means±SEM and individual data points (n=12).

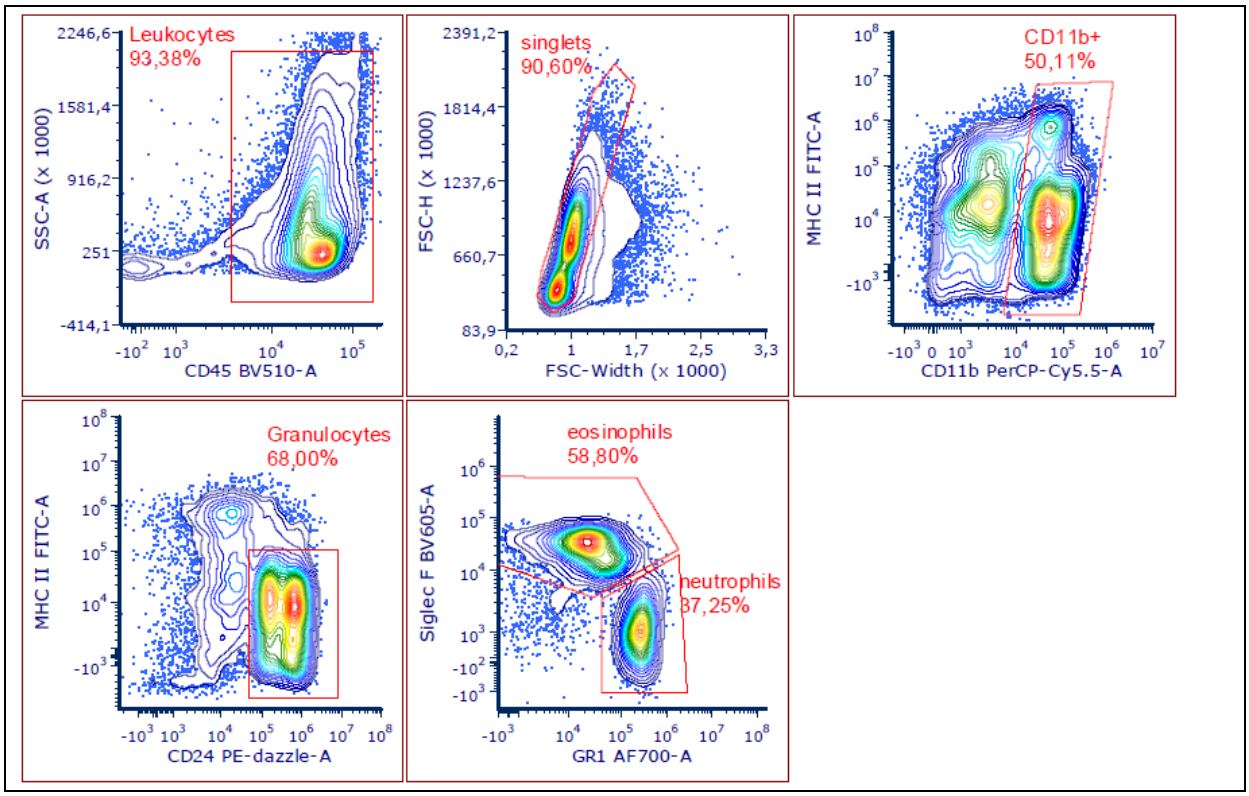
A B



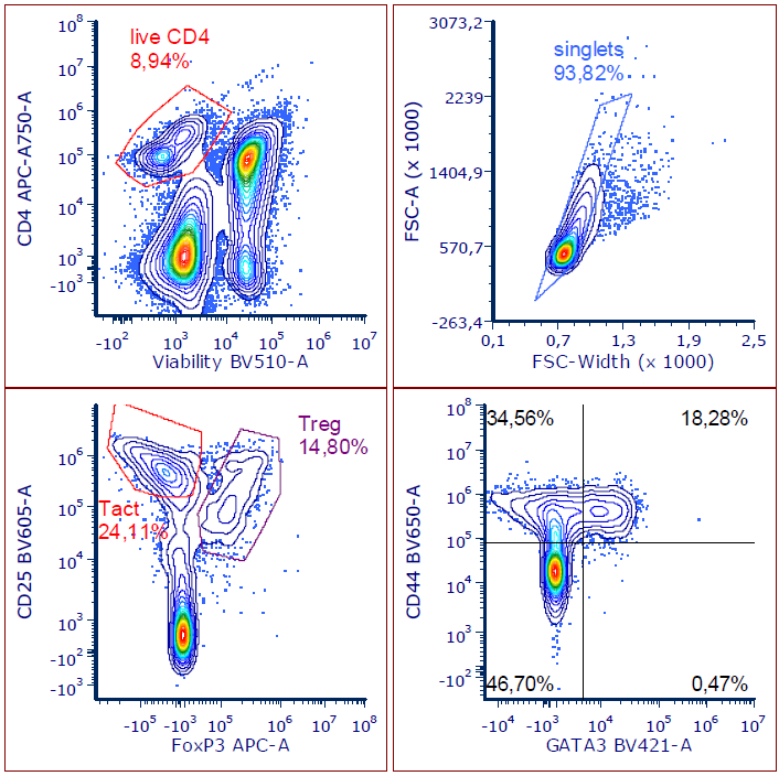
Supplementary Figure 11. Gating strategy for BMDCs. GM-BMDC (A) or FL-BMDCs (B) were first gated according to size to remove debris. Then, CD11c+CD11b+ cells were gated following exclusion of doublets on a FSC-W/FSC-A plot. The gated cells were then further analyzed for antigen uptake or expression of CD86 or dectin-1. Representative density plots and histograms from an uptake experiment are shown. Grey histogram shows PBS control, dotted line indicates cells incubated with pHrodo-OVA and solid line cells incubated with pHrodo-LamOVA.



Supplementary Figure 12. Gating strategy for BAL Leukocytes. Total Leukocytes were gated based upon expression of CD45 followed by doublet exclusion. Neutrophils and lymphocytes were gated on a Siglec-F vs. Gr-1 plot. SiglecF+ cells were discriminated into alveolar macrophages (AMΦ) and eosinophils based on autofluorescence on the BV510 channel. Lymphocytes were further distributed into CD4+ and CD8+ cells.



Supplementary Figure 13. Gating strategy lung digests. Total Leukocytes were gated based upon expression of CD45 followed by doublet exclusion. CD11b+ cells were gated on a MHC-II vs. CD11b plot. Granulocytes were identified as CD24+ MHC-II- cells and further separated into eosinophils and neutrophils based on SiglecF and Gr-1 expression.



Supplementary Figure 14. Gating strategy splenocyte cultures. After in vitro restimulation, live CD4 cells were gated followed by doublet exclusion. Activated T cells (Tact) and regulatory T cells (Treg) were gated based on their expression of CD25 and FoxP3. Th2 cells were identified as CD44+GATA3+.

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