

Microarray investigation of glycan remodeling during macrophage polarization reveals α 2-6 sialic acid as an anti-inflammatory indicator

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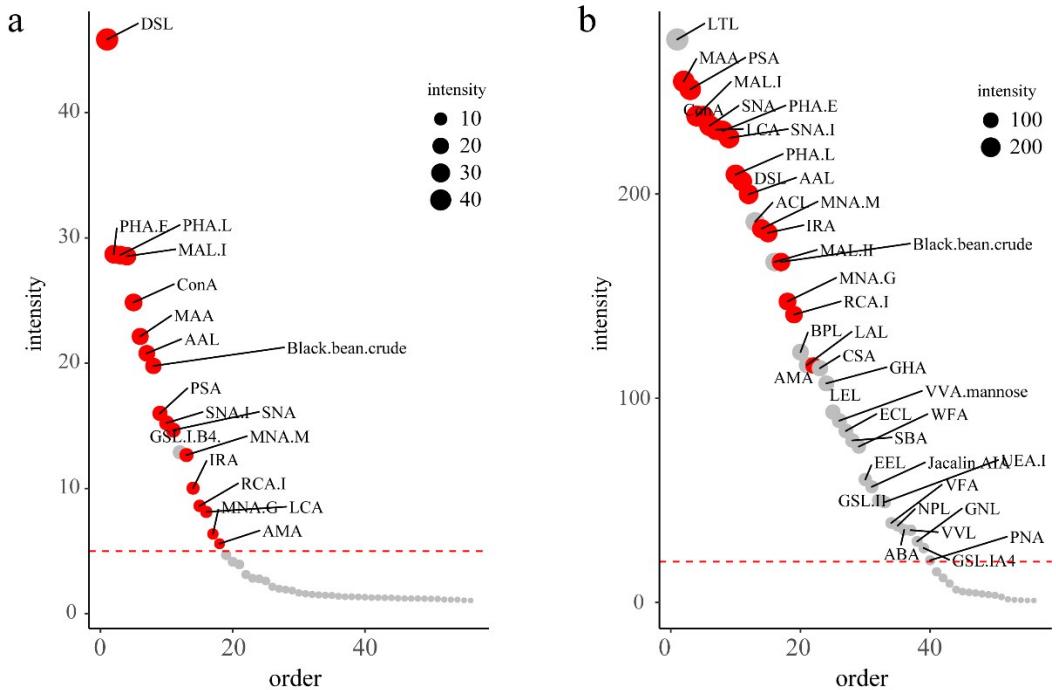


Fig. S1 Fluorescence intensity plot of the lectins to THP-1 (a) and RAW264.7 (b). The size of the dot represent the fluorescence intensity. The signal under the red line was seen as none detected signal in the experiment. The red dot represent the lectins which was both detected in two cell lines.

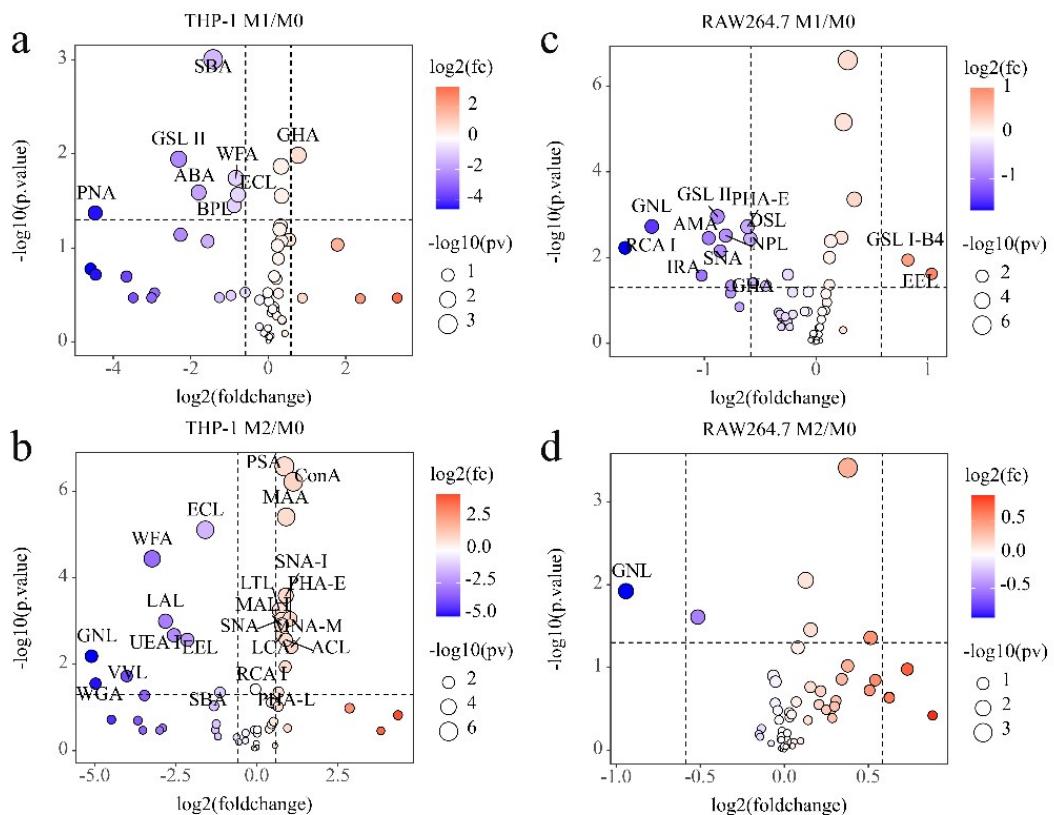


Fig. S2 The volcano plot of variation lectins during M0 differentiation into M1 (a) or M2 (b) in THP-1. The volcano plot of variation lectins during M0 differentiation into M2 (c) or M2 (d) in

RAW264.7.

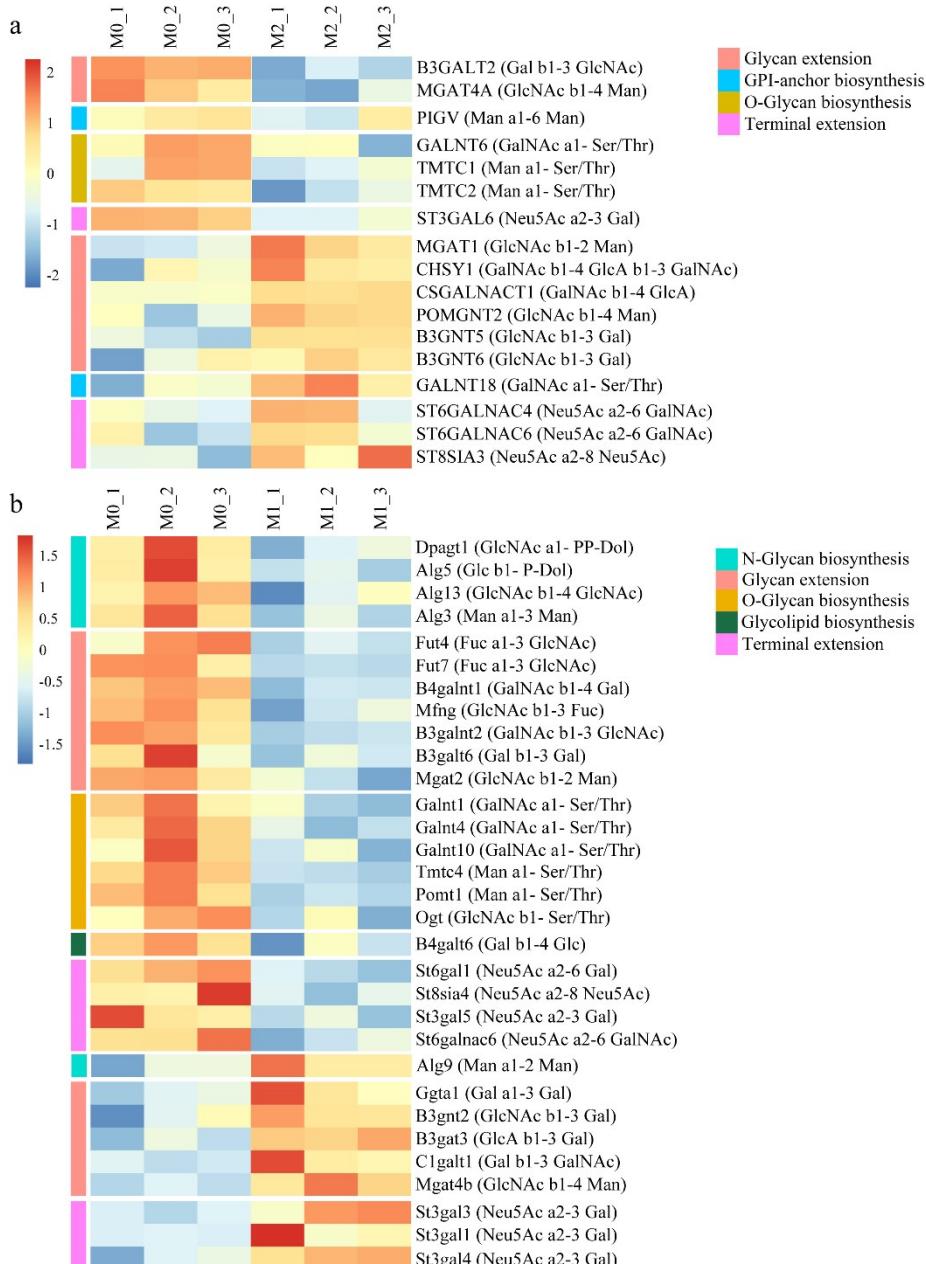


Fig. S3 Fig. S3 Heatmap of the glycosyltransferase gene in THP-1 derived macrophage and M2 subtypes (a); the heatmap of the glycosyltransferase gene in RAW264.7 and its M1 subtypes (b).

Table. S1 List of primer sequences used in qRT-PCR experiments

Species	Genes	Primers	Sequences
human	CD11b	Forward	GTGAAGCCAATAACGCAGC
human	CD11b	Reverse	CTCCCATCCGTGATGACAAC
human	CD36	Forward	TGTAACCCAAGACGCTGAGG
human	CD36	Reverse	GAAGGTTCGAACAGATGGCAC

human	CCL3	Forward	CAGCCAGGTGTCATTTCTT
human	CCL3	Reverse	TCAGGCATTCAAGTCCAGGT
human	TNF- α	Forward	TAGCCCATGTTGTAGCAAACC
human	TNF- α	Reverse	ATGAGGTACAGGCCCTGTGAT
human	CCL17	Forward	ACTGCTCCAGGGATGCCATCGTTTT
human	CCL17	Reverse	ACAAGGGATGGATCTCCCTCACTG
human	CCL22	Forward	TGATTACGATCCGTTACCGTCT
human	CCL22	Reverse	CCTGAAGGTTAGAACACACCAC
human	ACTIN	Forward	CCTAGAAGCATTGCGGTGG
human	ACTIN	Reverse	GAGCTACGAGCTGCCTGACG
mouse	NOS2	Forward	AAACCCCTGTGCTGTTCTC
mouse	NOS2	Reverse	ATACTGTGGACGGGTCGATG
mouse	CXCL10	Forward	AAGTGCTGCCGTCACTTCT
mouse	CXCL10	Reverse	CCTATGGCCCTCATTCTCAC
mouse	Arg-1	Forward	GCAGAGGTCCAGAAGAATGG
mouse	Arg-1	Reverse	AGCATCCACCCAAATGACAC
mouse	Mrc-1	Forward	GGAGGCTGATTACGAGCAGT
mouse	Mrc-1	Reverse	CATAGGAAACGGGAGAACCA
mouse	ACTIN	Forward	GTGCTATGTTGCTCTAGACTTCG
mouse	ACTIN	Reverse	ATGCCACAGGATTCCATACC

Table S2. Table of the lectins spotted on the microarray and their binding glycan linkage.

lectin	names	glycan	THP-1	RAW 264.7
N-glycan				
AMA	Arum maculatum Lectin (Lords and Ladies)	Mannose on N-glycan	✓	✓
ConA	Con A Lectin	Mannose on N-glycan	✓	✓
Complex N-glycan				
CALSEPA	Calystegia sepium Lectin (Hedge Bindweed Rhizomes)	Galactosylated biantennary N-type with bisecting GlcNAc		
PHA-E	Phaseolus vulgaris Erythroagglutinin	complex N-glycan	✓	✓
DSL	Datura stramonium lectin	complex N-glycan	✓	✓
PHA-L	Phaseolus vulgaris Leucoagglutinin	complex N-glycan	✓	✓
High-Mannose N-glycan				
VFA	Wisteria floribunda lectin	High Mannose	✓	
UDA	Urtica dioica Lectin (Stinging Nettle)	High Mannose		
NPL, NPA	Narcissus pseudonarcissus	High Mannose	✓	

	(daffodil) lectin			
GNL	Galanthus nivalis (snowdrop) lectin	High Mannose	✓	
HHL, AL	Hippeastrum hybrid lectin	High Mannose		
MNA-M	Morniga M Lectin (black elderberry)	Mannose	✓	✓
ASA	Allium sativum Lectin (Garlic)	$\alpha(1, 3)$ -linked mannosyl units		
Fuc α 1-6GlcNAc (Core Fuc)				
PSA	Pisum sativum agglutinin	Fuc α 1-6GlcNAc (Core Fuc)	✓	✓
LCA	Lens Culinaris Agglutinin	Fuc α 1-6GlcNAc (Core Fuc)	✓	✓
TL	Tulipa sp. Lectin (Tulip)	Fuc α 1-6GlcNAc (Core Fuc)		
AAL	Aleuria aurantia lectin	Fuc a1, 6 Gal	✓	✓
Fuc α 1, 2 Gal				
UEA I	Ulex europaeus agglutinin I	Fuc a1, 2 Gal	✓	
PTL I, WBA I	Psophocarpus tetragonolobus lectin I	GalNAca1-3(Fuc α 1-2)Gal		
EEL	Euonymus europaeus lectin	Fuc α 1-2Gal β 1-3GlcNAc, Gala1-3Gal β 1-3GlcNAc	✓	
LAL	Laburnum anagyroides Lectin (Gold Chain)	Fuc α 1-2Gal β 1-4Glc (H-type 6)	✓	
α 2, 3 sialic acid				
MAL I	Maackia amurensis lectin I	sialic acid α 2, 3 Gal	✓	✓
MAA	Maackia amurensis Lectin	sialic acid α 2, 3 Gal	✓	✓
α 2, 6 sialic acid				
SNA, EBL	Sambucus nigra (Elderberry Bark)	sialic acid α 2, 6 Gal	✓	✓
SNA-I	Sambucus nigra lectin	sialic acid α 2, 6 Gal	✓	✓
Gal β 1-4GlcNAc				
WFA, WFL	Wisteria floribunda lectin	Gal β 1-4GlcNAc	✓	
ECL	Erythrina cristagalli Lectin	Gal β 1-4GlcNAc	✓	
BPL	Bauhinia Purpurea Lectin	Gal β 1-4GlcNAc	✓	
RCA I, RCA120	Ricinus communis agglutinin I	Galb1-4GlcNAcb or Galb1-3GlcNAcb	✓	✓
STL, PL	Solanum tuberosum lectin	Gal β 1-4GlcNAc, (GlcNAc β 1-4)n		

LTL	Lotus tetragonolobus lectin	Galb1-4(Fuca1-3)GlcNAc	✓	
LEL, TL	Lycopersicon esculentum lectin	Galβ1-4GlcNAc	✓	
GlcNAc terminal				
HMA	Homarus americanus Lectin	N-Acetylneuraminic acid, N-Acetylgalactosamine		
WGA	Triticum vulgare Lectin (Wheat Germ)	GlcNAca1-3Galb1-4GlcNAcb		
GSL II, BSL II	Griffonia (Bandeiraea) simplicifolia lectin II	GlcNAca1-3Galb1-4GlcNAcb		
PWM	Phytolacca americana Lectin (Pokeweed)	(GlcNAcβ1-4)n		
ABA	Agaricus bisporus Lectin (Mushroom)	GlcNAca1-3Galb1-4GlcNAcb		
α -Gal link				
GSL.IA4	Pure Griffonia simplicifolia Lectin	α -Gal, α -GalNAc	✓	
MNA.G	Morniga G Lectin (black elderberry)	Galactose	✓	✓
PNA	Arachis hypogaea Lectin (Peanut)	Gal terminal	✓	
GSL I-B4, BSL I-B4	GSL I - isolectin B4	Gala1-4Gal, Gala1-3Gal		✓
GalNAc terminal link				
GHA	Glechoma hederacea Lectin (ground ivy)	GalNAc	✓	
IAA	Iberis amara Lectin	GalNAc terminal		
IRA	Iris hybrid Lectin (Dutch Iris)	GalNAc terminal	✓	✓
Black.bean. crude	Phaseolus vulgaris sp. Lectin	GalNAc terminal	✓	✓
CSA	Cytisus sessilifolius Lectin (Portugal Broom)	GalNAc terminal	✓	
VVA.mannose	Vicia villosa Lectin (Hairy Vetch, Mannose Specific)	GalNAcβ1-4Gal, GalNAcβ1-3Gal, α -GalNAc	✓	
VVL, VVA	Vicia villosa lectin	GalNAcβ1-4Gal, GalNAcβ1-3Gal, α -GalNAc	✓	
SBA	Glycine max Lectin (Soybean)	α - or β -linked GalNAc, Galα1-4Gal-Glc	✓	
DBA	Dolichos biflorus agglutinin	α - or β -linked GalNAc		

HPA	Helix pomatia Lectin (Snail)	α -GalNAc, α -GlcNAc		
T, Tn antigen				
ACL, ACA	Amaranthus caudatus lectin	Sia α 2-3Gal β 1-3GalNAc (sialyl T)	✓	
MAL II	Maackia amurensis lectin II	Gal β 1-3GalNAc (T-antigen), Sia α 2-3Gal β 1-3GalNAc (sialyl T)	✓	
MPL	Maclura pomifera lectin	α -GalNAc (Tn-antigen), Gal β 1-3GalNAc (T-antigen)		
Jacalin, AIA	Jacalin	GlcNAc β 1-3GalNAc (Core3), Sia α 2-3Gal β 1-3GalNAc (sialyl T), Gal β 1-3GalNAc (T-antigen), α -GalNAc (Tn-antigen)	✓	
SSA	Salvia sclarea	GalNAc α -O-Ser/Thr (Tn antigen)		