frontiers

Supplementary Material

Human B cell differentiation is characterized by progressive remodeling of O-linked glycans

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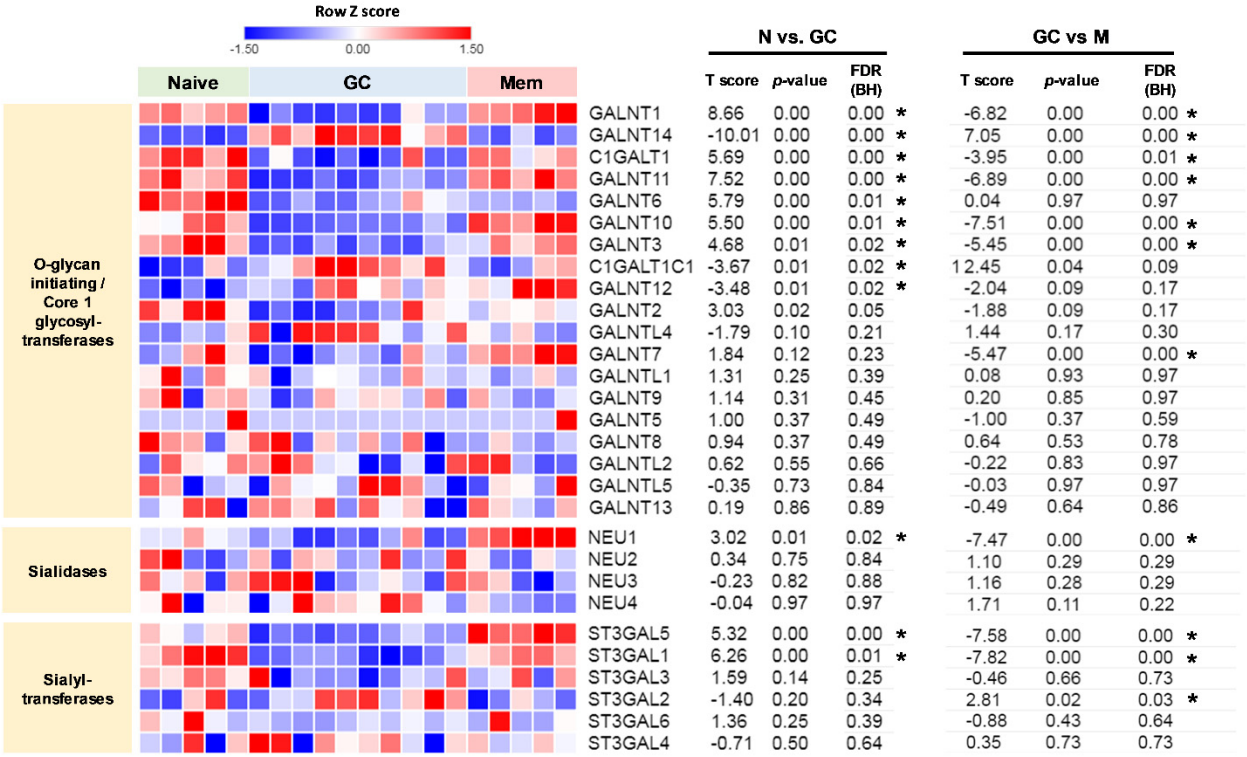
1.1 Supplementary Figures

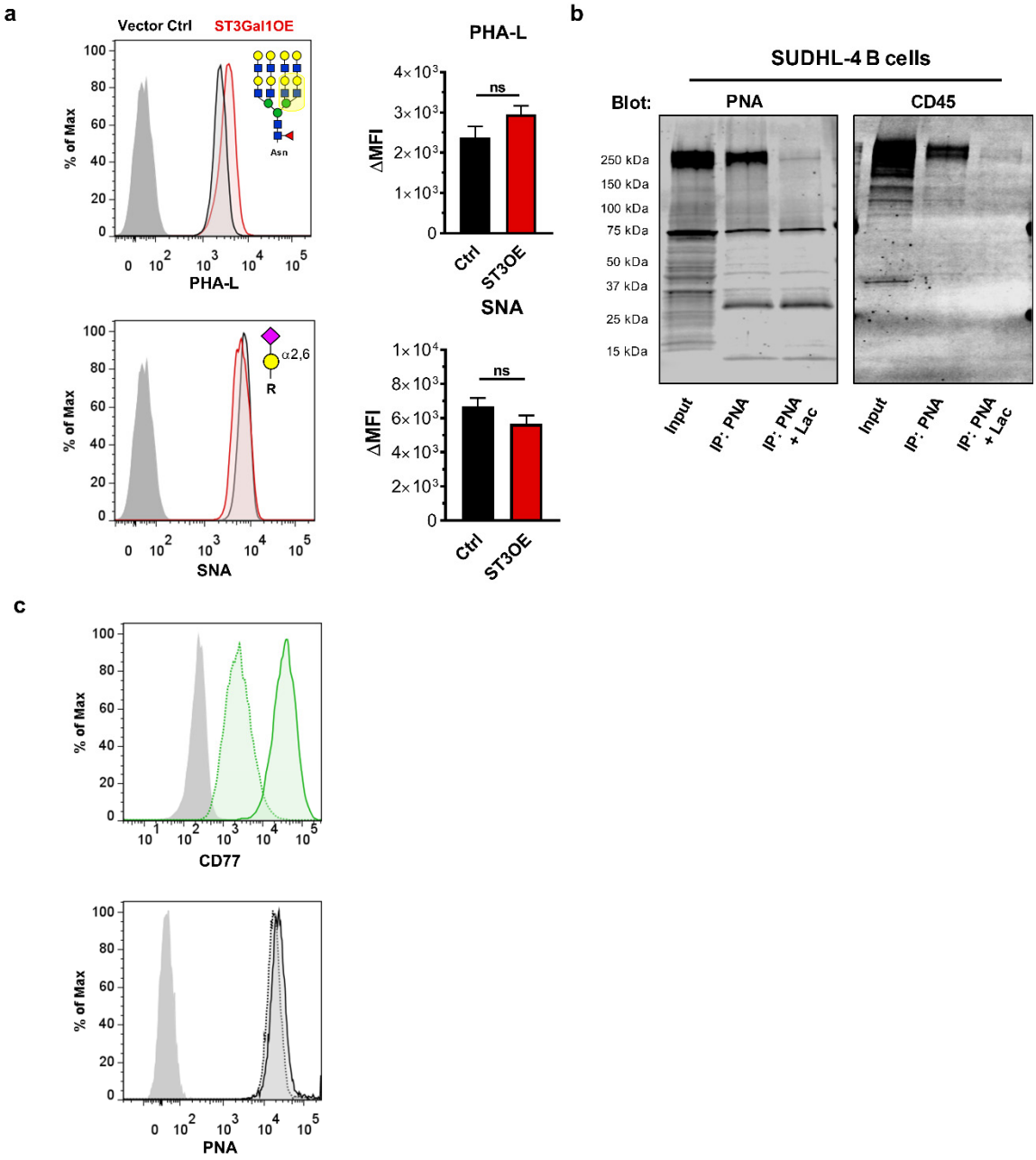
Supplementary Figure 1



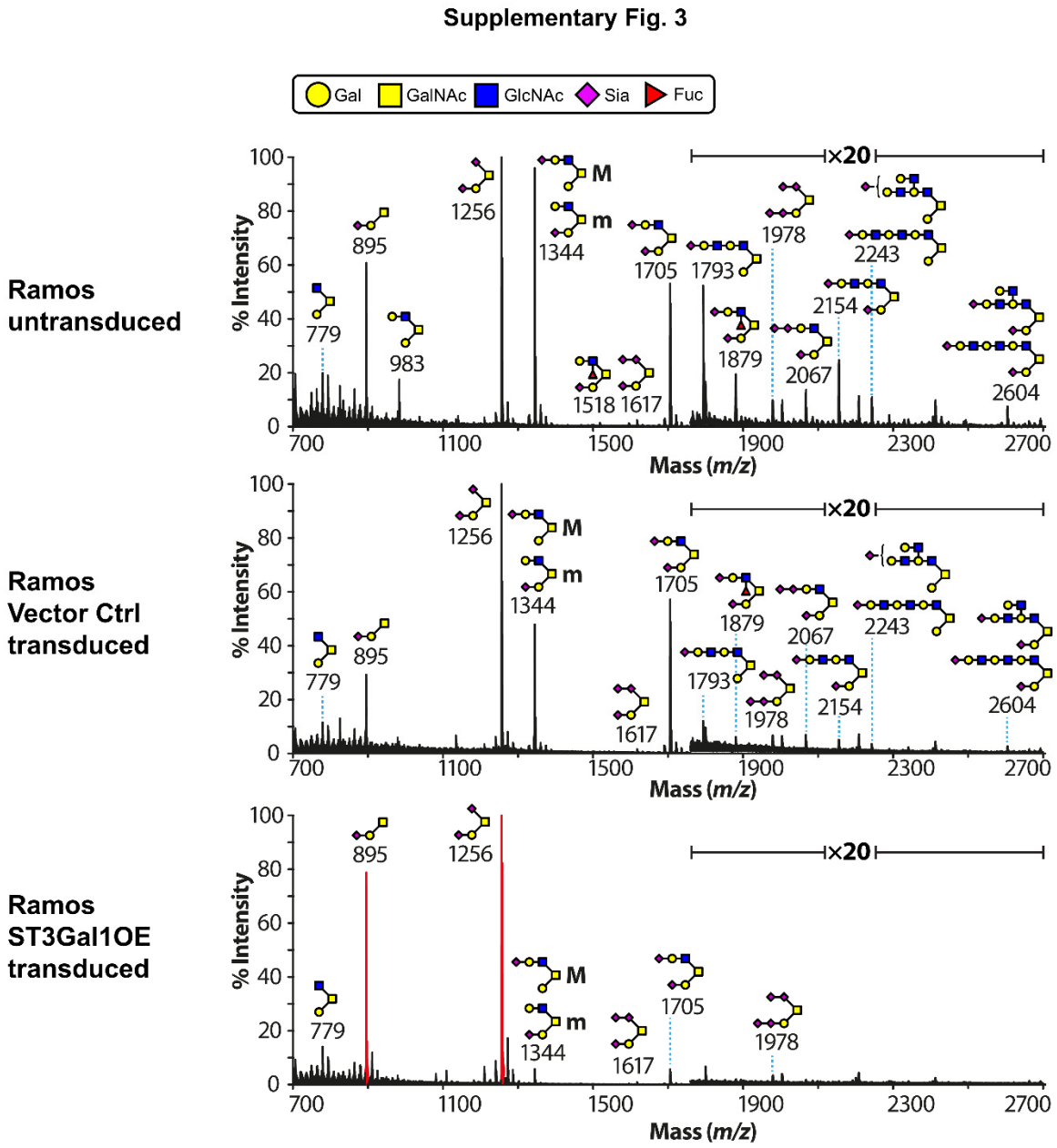
		N vs. GC				GC vs M			
		T score	*p*-value	FDR (BH)		T score	*p*-value	FDR (BH)	
O-glycan initiating / Core 1 glycosyl-transferases	GALNT1	8.66	0.00	0.00	*	-6.82	0.00	0.00	*
	GALNT14	-10.01	0.00	0.00	*	7.05	0.00	0.00	*
	C1GALT1	5.69	0.00	0.00	*	-3.95	0.00	0.01	*
	GALNT11	7.52	0.00	0.00	*	-6.89	0.00	0.00	*
	GALNT6	5.79	0.00	0.01	*	0.04	0.97	0.97	
	GALNT10	5.50	0.00	0.01	*	-7.51	0.00	0.00	*
	GALNT3	4.68	0.01	0.02	*	-5.45	0.00	0.00	*
	C1GALT1C1	-3.67	0.01	0.02	*	12.45	0.04	0.09	
	GALNT12	-3.48	0.01	0.02	*	-2.04	0.09	0.17	
	GALNT2	3.03	0.02	0.05		-1.88	0.09	0.17	
	GALNTL4	-1.79	0.10	0.21		1.44	0.17	0.30	
	GALNT7	1.84	0.12	0.23		-5.47	0.00	0.00	*
	GALNTL1	1.31	0.25	0.39		0.08	0.93	0.97	
	GALNT9	1.14	0.31	0.45		0.20	0.85	0.97	
	GALNT5	1.00	0.37	0.49		-1.00	0.37	0.59	
	GALNT8	0.94	0.37	0.49		0.64	0.53	0.78	
	GALNTL2	0.62	0.55	0.66		-0.22	0.83	0.97	
	GALNTL5	-0.35	0.73	0.84		-0.03	0.97	0.97	
	GALNT13	0.19	0.86	0.89		-0.49	0.64	0.86	
Sialidases	NEU1	3.02	0.01	0.02	*	-7.47	0.00	0.00	*
	NEU2	0.34	0.75	0.84		1.10	0.29	0.29	
	NEU3	-0.23	0.82	0.88		1.16	0.28	0.29	
	NEU4	-0.04	0.97	0.97		1.71	0.11	0.22	
Sialyl-transferases	ST3GAL5	5.32	0.00	0.00	*	-7.58	0.00	0.00	*
	ST3GAL1	6.26	0.00	0.01	*	-7.82	0.00	0.00	*
	ST3GAL3	1.59	0.14	0.25		-0.46	0.66	0.73	
	ST3GAL2	-1.40	0.20	0.34		2.81	0.02	0.03	*
	ST3GAL6	1.36	0.25	0.39		-0.88	0.43	0.64	
	ST3GAL4	-0.71	0.50	0.64		0.35	0.73	0.73	

Supplementary Fig. 1: Analysis of O-glycosylation enzyme expression in tonsillar B cells. Publicly available datasets (GSE12195) were analyzed for expression of O-glycan initiating enzymes (*GALNTs)*, Core 1 synthase (*C1GALT1*), C1GalT1 chaperone (*C1GALT1C1*), sialidases (*NEUs*), and α2,3-sialyltransferases (*ST3GALs*) in human B cell subsets. Each column represents a unique tonsil specimen. Statistics were performed for each row by individual two-tailed, unpaired Student's *t*-test and corrected for multiple comparisons using Benjamini-Hochberg False Discovery procedure. FDR $q < 0.05$ (*) was considered statistically significant.

Supplementary Fig. 2

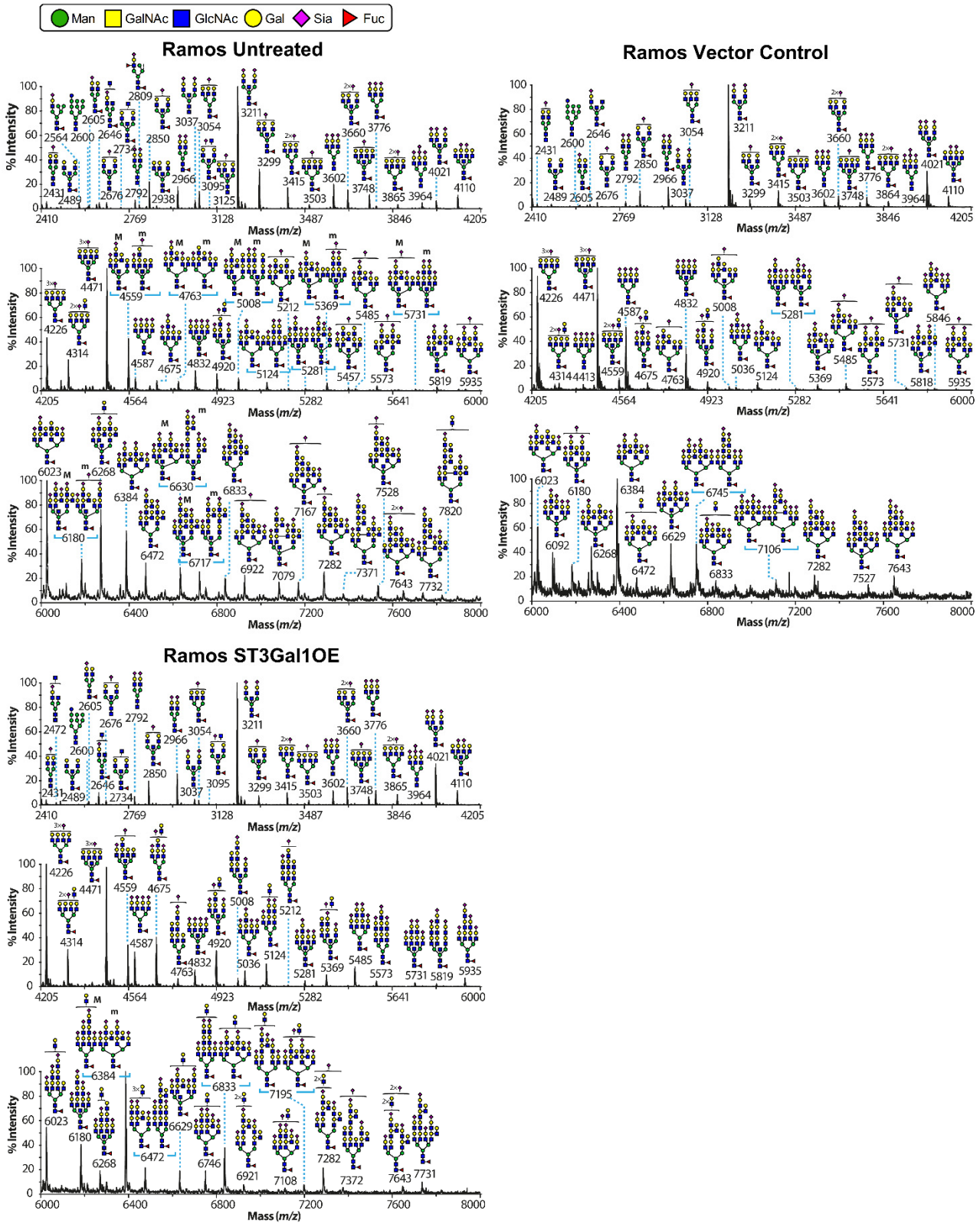


Supplementary Fig. 2: PNA ligands are expressed as O-glycans on CD45 and are not meaningfully expressed on glycolipids of Ramos B cells. (a) Representative histogram (*left*) and quantification (*right*) of PHA-L (tri- and tetra-antennary N-glycans) and SNA (α2,6-sialic acid) plant lectin binding to vector control and ST3Gal1OE Ramos B cells. **(b)** Immunoprecipitation (IP) of PNA-binding proteins from lysates of the GC-derived diffuse large B cell lymphoma (DLBCL) lymphoma cell line SUDHL-4, followed by SDS-PAGE and immunoblot with either PNA (*left*) or total CD45 antibody (*right*). As a negative control for carbohydrate binding, IP was also performed in the presence of a sugar inhibitor, lactose (Lac; right lane). **(c)** Representative histograms depicting CD77 (Gb3 glycolipid) expression and PNA binding by Ramos B cells without (solid line) or with (dotted line) 72hr treatment with D,l-threo-1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol-HCl (PPPP), a Glc-Cer synthase (UCGC) inhibitor that blocks glycolipid synthesis.



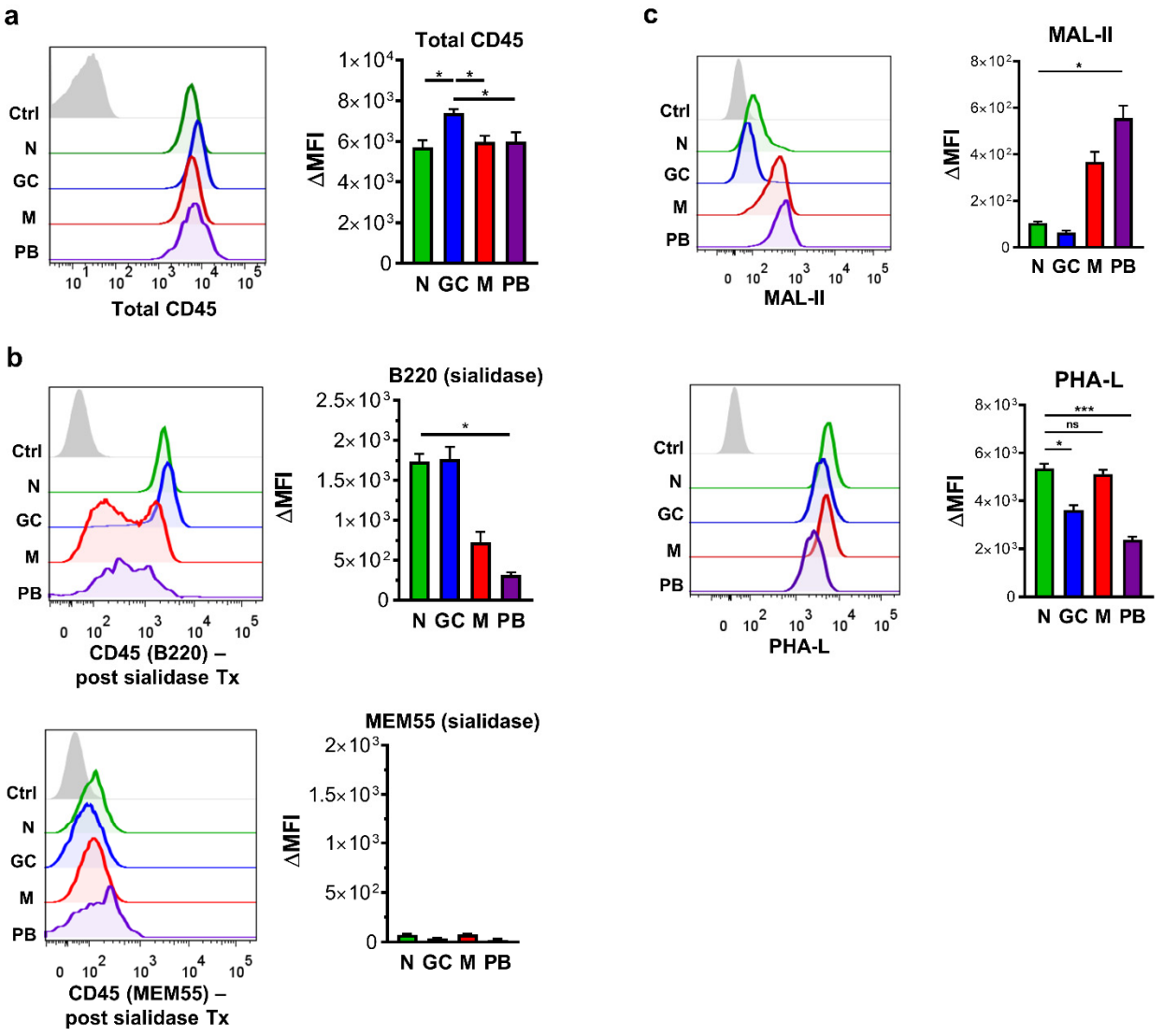
Supplementary Figure 3: O-glycomic analysis of ST3Gal1 variant Ramos B cells. Conventional MALDI-TOF MS analysis of O-glycans from untreated, vector control and ST3Gal1OE Ramos B cells. Structures above a bracket have not been unequivocally defined. Indicated areas in the spectra have a 20-fold magnification. "M" and "m" designations indicate major and minor abundances, respectively. Cartoon structures were drawn according to http://www.functionalglycomics.org guidelines and are representative from repeat experiments on two different biological replicates. Structure assignments are based on composition, tandem mass spectrometry and biosynthetic knowledge. Fuc, fucose; Man, mannose; Gal, galactose; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; Sia, N-acetylneuraminic acid (sialic acid). Full methods can be found in Materials and Methods.

Supplementary Fig. 4



Supplementary Fig. 4: N-glycomic analysis of ST3Gal1 variant Ramos B cells. MALDI-TOF MS analysis of permethylated N-glycans released by PNGase F digestion of untreated, vector control and ST3Gal1OE Ramos B cells. Structures above a bracket have not been unequivocally defined. "M" and "m" designations indicate major and minor abundances, respectively. Cartoon structures were drawn according to http://www.functionalglycomics.org guidelines and are representative from repeat experiments on two different biological replicates. Structure assignments are based on composition, tandem mass spectrometry and biosynthetic knowledge. Fuc, fucose; Man, mannose; Gal, galactose; GlcNAc, N-acetylglucosamine; Sia, N-acetylneuraminic acid (sialic acid). Full methods can be found in Materials and Methods.

Supplementary Fig. 5



Supplementary Fig. 5: Analysis of CD45 glycoform and global glycosylation of primary B cells by CD45 mAb and plant-lectin based flow cytometry. (a) Representative histograms (*left*) and quantification (*right*) of total CD45 expression (HI30 clone) on tonsillar B cell subsets. **(b)** Representative histograms (*left*) and quantification (*right*) of binding of CD45 mAbs B220 and MEM55 to the indicated tonsillar B cell subsets following treatment with *Arthrobacter ureafaciens* sialidase. **(c)** Representative histograms (*left*) and quantification (*right*) of binding of MAL-II (sialylated T-antigen) and PHA-L plant lectins (tri- and tetra-antennary complex N-glycan binding preference) to primary tonsillar B cells by flow cytometry, gated as in Fig.1a. See also Fig. 7a for schematic depicting glycan-binding preferences of MAL-II and PHA-L. For (a) and (b), n=5 distinct tonsil specimens. For (c), n=8 (MAL-II) or n=9 (PHA-L) distinct tonsil specimens. Statistics in (a-c) were calculated using a Kruskal-Wallis test with Dunn's multiple comparisons test. Throughout, bars and error bars depict the mean and SEM, respectively. ns = not significant, *$p \leq 0.05$, ***$p \leq 0.001$. ΔMFI, background subtracted geometric mean fluorescence intensity.

Supplementary Table 1: Oligonucleotide sequences

Target	Application	Forward (5'→3')	Reverse (5'→3')
Hu *ST3GAL1*	cDNA Amplification	cgacgaattcgccacc**atggtgaccctgcggaagagg**	ccgggatcc**tcatctccccttgaagatccggatttt**
Hu *ST3GAL1*	qRT-PCR (60°C)	gcatttctctttcccacagc	ctaattcccagccaccttca
Hu *GCNT1*	qRT-PCR (60°C)	aatttccgatgcccatgat	agggccaagtcctttcaaat
Hu *VCP* (housekeeping)	qRT-PCR (60°C)	aggatgatccagtgcctgag	ggaatctgaagctgccaaag

Supplementary Table 2: Antibodies and reagents

Lectins and glycobiology reagents						
Target	**Conjugate**	**Clone**	**Source**	**Catalog number**	**Concentration / Dilution**	**Incubation time**
Arachis hypogea (PNA)	FITC	-	Sigma	L7381	10μg/mL (FACS)	45min, ice
Jacalin lectin	FITC	-	Vector	FL-1151	5μg/mL (FACS)	45min, ice
Phaseolus vulgaris Leucoagglutinin (PHA-L)	FITC	-	Vector	FL-1111	2μg/mL (FACS)	45min, ice
Solanum Tuberosum Agglutinin (STA)	FITC	-	Vector	FL-1161	2μg/mL (FACS)	45min, ice
Helix pomatia agglutinin	AlexaFluor 488	-	Life Technologies	L11271	5μg/mL (FACS)	45min, ice
Arachis hypogea (PNA)	Biotin	-	Vector	L6135	2μg/mL (FACS)	45min, ice
Sambucus Nigra Agglutinin (SNA)	Biotin	-	Vector	FL-1301	2μg/mL (FACS)	45min, ice
Maackia Amurensis Lectin II (MAL-II)	Biotin	-	Vector	B-1265	0.5μg/mL (FACS)	45min, ice
Phaseolus vulgaris Leucoagglutinin (PHA-L)	Biotin	-	Vector	B-1115	0.1μg/mL (FACS)	45min, ice
Solanum Tuberosum Agglutinin (STA)	Biotin	-	Vector	B-1165	0.1μg/mL (FACS)	45min, ice
Sambucus Nigra Agglutinin (SNA)	Biotin	-	Vector	B-1305	0.25μg/mL (FACS)	45min, ice
Arthrobacter ureafaciens sialidase	-	-	Millipore-Sigma	10269611001	125mU / mL	1hr, RT
D-1-threo-1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol-HCl (PPPP)	-	-	Gift from Dr. Ronald L. Schnaar (Johns Hopkins)	-	2μM	72hr incubation

Flow cytometry antibodies and staining reagents						
Target	**Conjugate**	**Clone**	**Source**	**Catalog number**	**Concentration / Dilution**	**Incubation time**
CD3	APC-Cy7	HIT3a	Biolegend	300318	1:100 (FACS)	45min, ice
CD14	APC-Cy7	HCD14	BIolegend	325620	1:160 (FACS)	45min, ice
CD19	PerCP	HIB19	Biolegend	302228	1:40 (FACS)	45min, ice
CD19	APC	HIB19	Biolegend	302212	1:100 (FACS)	45min, ice
CD19	APC/Fire 750	HIB19	Biolegend	302257	1:40 (FACS)	45min, ice
CD27	PE-Cy7	LG.3A10	Biolegend	124216	1:160 (FACS)	45min, ice
CD38	PE	HB-7	Biolegend	356604	1:160 (FACS)	45min, ice
CD38	PerCP/Cy5.5	HB-7	Biolegend	356613	1:160 (FACS)	45min, ice
CD43 (Core 2 glycoform)	-	1D4	LSBio	LSC179306	1:500 (FACS)	45min, ice
CD45	APC	HI30	Biolegend	304012	1:25 (FACS)	45min, ice
CD45 (B220)	Biotin	RA3-6B2	BD	553086	1:100 (FACS)	45min, ice
CD45RB (MEM55)	-	MEM55	Thermo	MA1-19115	1:500 (FACS)	1hr, RT
CD45RB (MEM55)	FITC	MEM55	Thermo	MA1-19571	1:5 (FACS)	45min, ice
IgD	FITC	IA6-2	Biolegend	348206	1:200 (FACS)	45min, ice
IgD	PE	IA6-2	Biolegend	348203	1:200 (FACS)	45min, ice
Streptavidin	FITC	-	Biolegend	405202	1:1000 (FACS)	30min, ice
Streptavidin	APC	-	Biolegend	405207	1:500 (FACS)	30min, ice
Zombie NIR Fixable Viability Kit	-	-	Biolegend	423106	1:1600 (FACS)	45min, ice
Magnetic sorting antibodies and reagents						
Target	**Conjugate**	**Clone**	**Source**	**Catalog number**	**Concentration / Dilution**	**Incubation time**
Anti-Biotin microbeads	-	-	Miltenyi	130-090-485	Manufact. guidelines	Manufact. guidelines
Anti-FITC microbeads	-	-	Miltenyi	130-048-701	Manufact. guidelines	Manufact. guidelines
IgD	Biotin	IA6-2	Biolegend	348212	1:40 (MACS)	10min, ice
CD77	FITC	5B5	Biolegend	357104	1:20 (MACS)	10min, ice
Western blot and immunoprecipitation reagents						
Target	**Conjugate**	**Clone**	**Source**	**Catalog number**	**Concentration / Dilution**	**Incubation time**
CD45	-	HI30	Biolegend	304002	1μg/mL (WB)	1hr, RT
CD45	-	D9M8I	CST	13917	1:2000	O/N, 4C
CD45RB (MEM55)	-	MEM55	Thermo	MA1-19115	2μg/mL (WB)	1hr, RT
Arachis hypogea (PNA)	Biotin	-	Sigma	L6135	5μg/mL	1hr, RT
Maackia Amurensis Lectin II (MAL-II)	Biotin	-	Vector	B-1265	0.5μg/mL	1hr, RT
Donkey anti-Goat IgG (H+L)	IRDye® 800CW	Polyclonal	Li-Cor	926-32214	1:20,000 (WB)	30min, RT

Goat anti-Rabbit IgG (H+L)	IRDye® 800CW	Polyclonal	Li-Cor	926-32211	1:20,000 (WB)	30min, RT
Goat anti-Mouse IgG (H+L)	IRDye® 800CW	Polyclonal	Li-Cor	926-32210	1:20,000 (WB)	30min, RT
Goat anti-Rabbit IgG (H+L)	IRDye® 680LT	Polyclonal	Li-Cor	926-68023	1:20,000 (WB)	30min, RT
Goat anti-Mouse IgG (H+L)	IRDye® 680RD	Polyclonal	Li-Cor	926-68070	1:20,000 (WB)	30min, RT
Streptavidin	IRDye® 800CW	-	Li-Cor	926-32230	1:10,000 (WB)	30min, RT