Supplementary Material

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

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## 1.1 Supplementary Figures

**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: 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: 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: 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<0.05, ***p<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     | cgacgaattgcaccatgtgacctgcggaa gagg | ccgggatcccatctccccctgaagatccg gatttt |
| Hu ST3GAL1      | qRT-PCR (60°C)         | gcatttctttccacagc                   | ctaattccagccaccttca                 |
| Hu GCNT1        | qRT-PCR (60°C)         | aatccgatccgtcatgat                  | agggcagctctttcaat                   |
| Hu VCP (housekeeping) | qRT-PCR (60°C)     | aggatgatccagtgcctgag               | ggaatctgaagctgcacaaag               |

### Supplementary Table 2: Antibodies and reagents

| Lectins and glyobiology 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-hexadecanoylamoino-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)             |                 |
| 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       |
| Antibody Type                  | Dye     | Source | Cat. No. | Dilution          | Incubation Time |
|--------------------------------|---------|--------|---------|-------------------|-----------------|
| Goat anti-Rabbit IgG (H+L)    | IRDye® 800CW | Polyclonal | Li-Cor | 926-32211         | 1:20,000 (WB)   |
| Goat anti-Mouse IgG (H+L)     | IRDye® 800CW | Polyclonal | Li-Cor | 926-32210         | 1:20,000 (WB)   |
| Goat anti-Rabbit IgG (H+L)    | IRDye® 680LT  | Polyclonal | Li-Cor | 926-68023         | 1:20,000 (WB)   |
| Goat anti-Mouse IgG (H+L)     | IRDye® 680RD  | Polyclonal | Li-Cor | 926-68070         | 1:20,000 (WB)   |
| Streptavidin                  | IRDye® 800CW | -      | Li-Cor | 926-32230         | 1:10,000 (WB)   |