Supporting information for:

Construction of multivalent homo- and hetero-functional ABO blood group glycoconjugates using a trifunctional linker strategy

Gour Chand Daskhan¹, Hanh-Thuc Tran¹, Peter J. Meloncelli¹, Todd L. Lowary^{1,3}, Lori J. West^{1,2,3} and Christopher W. Cairo^{1,3}*

*To whom correspondence should be addressed. Tel.: 780 492 0377; fax: 780 492 8231; e-mail: <u>ccairo@ualberta.ca</u>

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Figure S1. Clustering of CD22 receptors by glycoconjugates and glycoproteins.

A-BCL cells were treated with 25 ng mL⁻¹ PBS, **36**, AGP, PAA, **33**, or **9**, fixed and stained with anti-CD22 (mouse), and visualized using confocal microscopy. (A) Representative cells are shown in transmitted (top), fluorescence (middle), and the mask showing identified pixels counted for the clustering analysis (bottom). (B) Cluster size on 10 individual cells was determined using particle analysis in ImageJ and plotted using the beanplot statistical package in R. Each individual white horizontal line within the beanplot represents one single data point, the black horizontal line indicates the geometric mean for each condition, and the dotted line indicates geometric mean of all conditions within the plot. Populations which were statistically different from control are indicated where p < 0.0001 with ****, as calculated by GraphPad Prism software.



Figure S2. Clustering of CD22 receptors by glycoconjugates 8, 10, 34, and 35.

A-BCL cells were treated with 25 ng mL⁻¹ PBS, **8**, **10**, **34**, or **35**, fixed and stained with anti-CD22 (mouse), and were visualized using confocal microscopy. (A) Representative cells are shown in transmitted (top), fluorescence (middle), and the mask showing identified pixels counted for the clustering analysis (bottom). (B) Cluster size on 10 individual cells was determined using particle analysis in ImageJ and plotted using the beanplot statistical package in R. Each individual white horizontal line within the beanplot represents one single data point, the black horizontal line indicates the geometric mean for each condition, and the dotted line indicates geometric mean of all conditions within the plot. Populations which were statistically different from control are indicated where p < 0.0001 with ****, as calculated by GraphPad Prism software.





A-BCL cells were treated with 25 ng mL⁻¹ PBS, **36**, AGP, PAA, **33**, or **9**, fixed and stained with anti-IgM (mouse), and visualized using confocal microscopy. (A) Representative cells are shown in transmitted (top), fluorescence (middle), and the mask showing identified pixels counted for the clustering analysis (bottom). (B) Cluster size on 10 individual cells was determined using particle analysis in ImageJ and plotted using the beanplot statistical package in R. Each individual white horizontal line within the beanplot represents one single data point, the black horizontal line indicates the geometric mean for each condition, and the dotted line indicates geometric mean of all conditions within the plot. Populations which were statistically different from control are indicated where p < 0.0001 with ****, as calculated by GraphPad Prism software.



Figure S4. Clustering of BCR by glycoconjugates 8, 10, 34, and 35.

A-BCL cells were treated with 25 ng mL⁻¹ PBS, **8**, **10**, **34**, or **35**, fixed and stained with anti-IgM (mouse), and visualized using confocal microscopy. (A) Representative cells are shown in transmitted (top), fluorescence (middle), and the mask showing identified pixels counted for the clustering analysis (bottom). (B) Cluster size on 10 individual cells was determined using particle analysis in ImageJ and plotted using the beanplot statistical package in R. Each individual white horizontal line within the beanplot represents one single data point, the black horizontal line indicates the geometric mean for each condition, and the dotted line indicates geometric mean of all conditions within the plot. Populations which were statistically different from control are indicated where p < 0.0001 with ****, as calculated by GraphPad Prism software.



Figure S5. Co-localization of CD22 and BCR by glyconconjugate 36.

A-BCL cells were treated with 0 or 25 ng mL⁻¹ of **36**, stained and fixed, and then imaged by two-color confocal microscopy (CD22, green; IgM, red) (**A** and **C**). Two separate replicated experiments for each condition were performed, and multiple individual cells were analyzed in two separate runs and analyzed using the co-localization function (coloc2) in imageJ. The results were analyzed and plotted using GraphPad Prism (**B** and **D**).



Figure S6. Wider field images of co-localization of CD22 and BCR by glyconconjugate 36.

A-BCL cells were treated with 0 or 25 ng mL⁻¹ of **36**, stained and fixed, and then imaged by two-color confocal microscopy (CD22, green; IgM, red) following the same protocol used in Fig S5.