

Supplement to:
Restricted processing of CD16a N-glycans from primary human NK cells impacts structure and
function

Kashyap R. Patel⁺⁺, Jacob T. Roberts⁺⁺, Ganesh P. Subedi⁺⁺ and Adam W. Barb
⁺⁺ denotes equal contribution

From the Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology,
Molecular Biology Building, Ames, IA 50011

Running Title: *N-glycans from NK cell CD16a*

To Whom Correspondence Should be Addressed: Adam W. Barb, Roy J. Carver Department of
Biochemistry, Biophysics and Molecular Biology, 2437 Pammel Drive, Molecular Biology
Building, rm 4210, Iowa State University, Ames, IA 50011; Email: abarb@iastate.edu

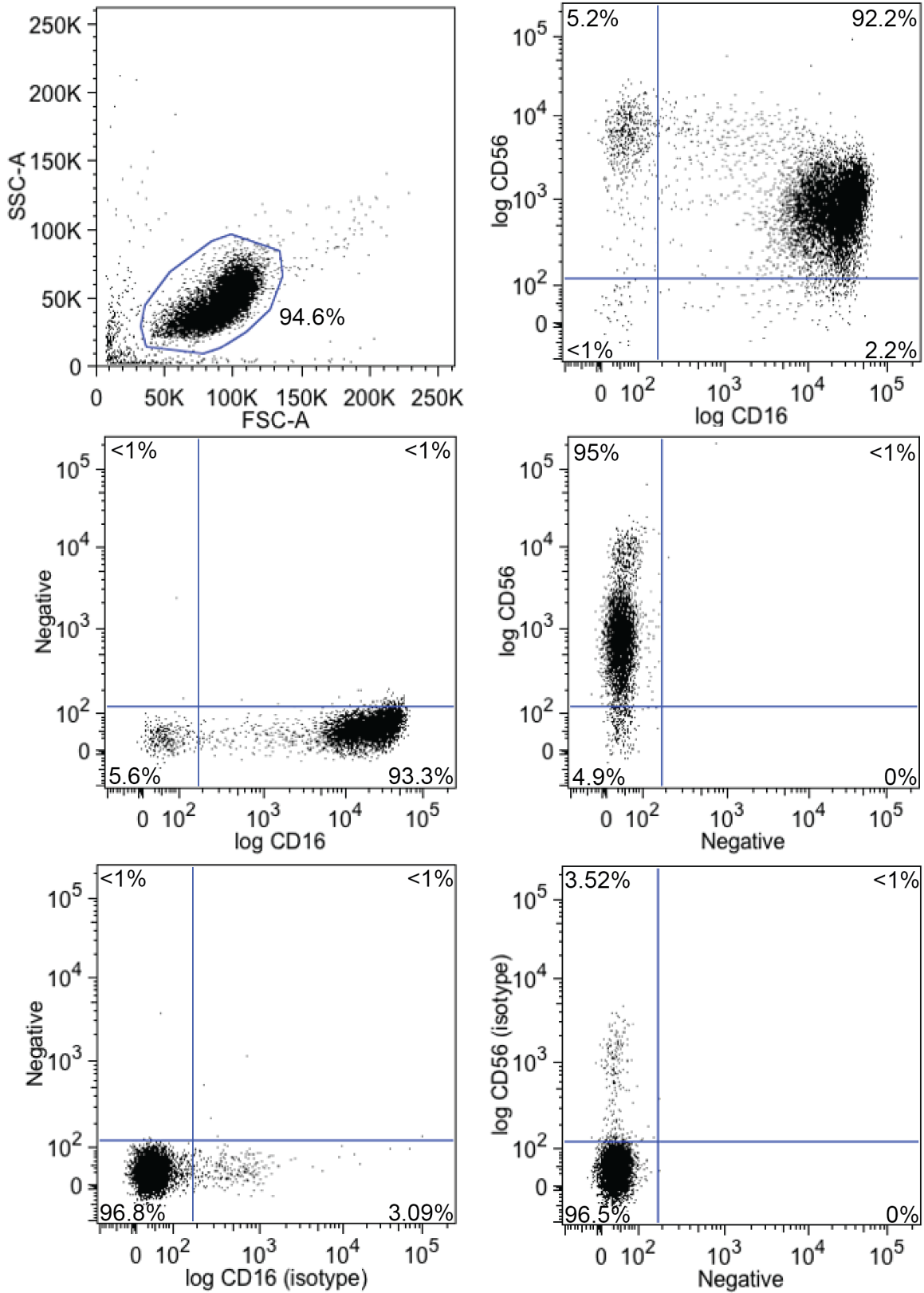


Figure S1. Flow cytometry and controls for NK cells isolated with negative selection.

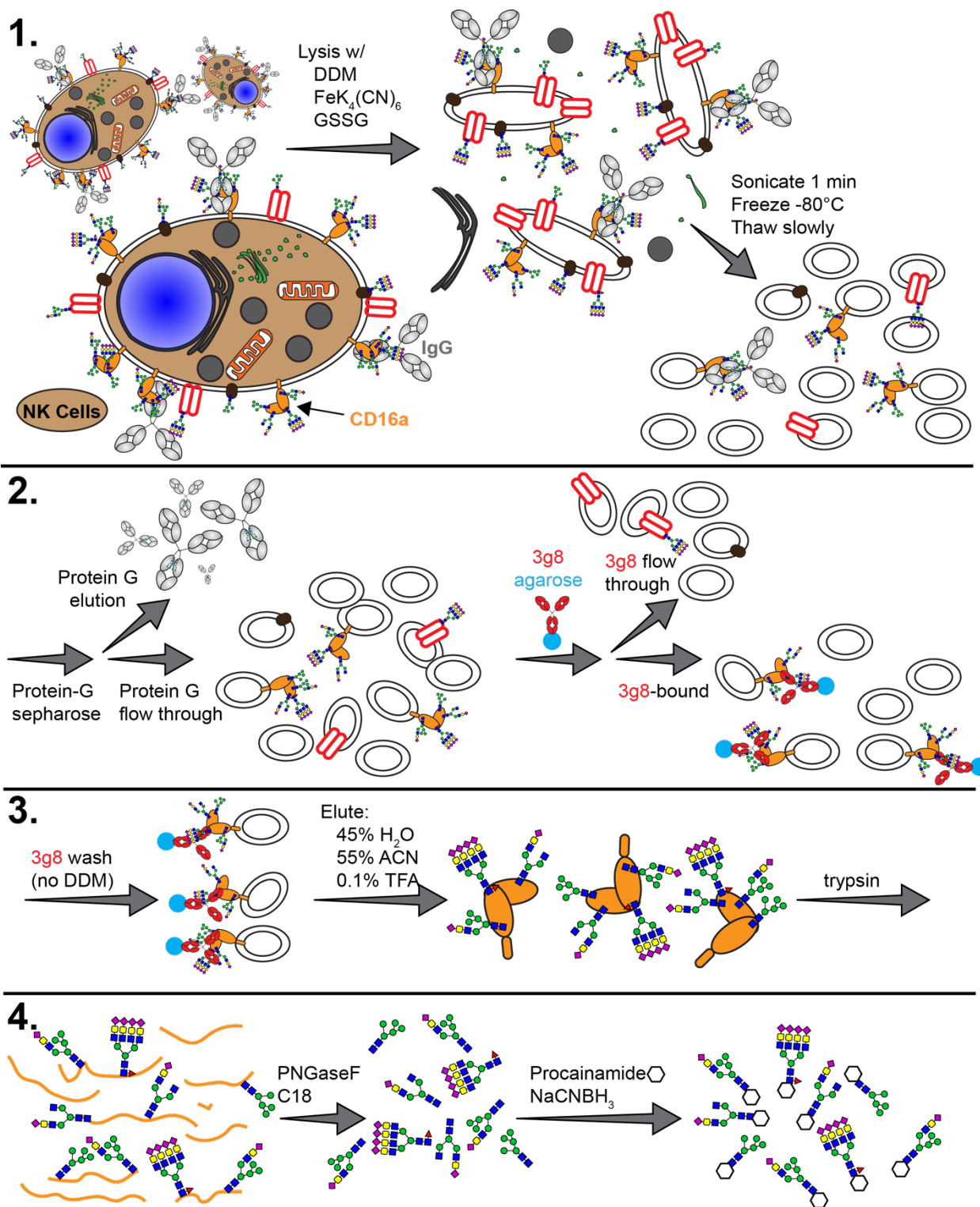


Figure S2. CD16a purification and N-glycan analysis scheme.

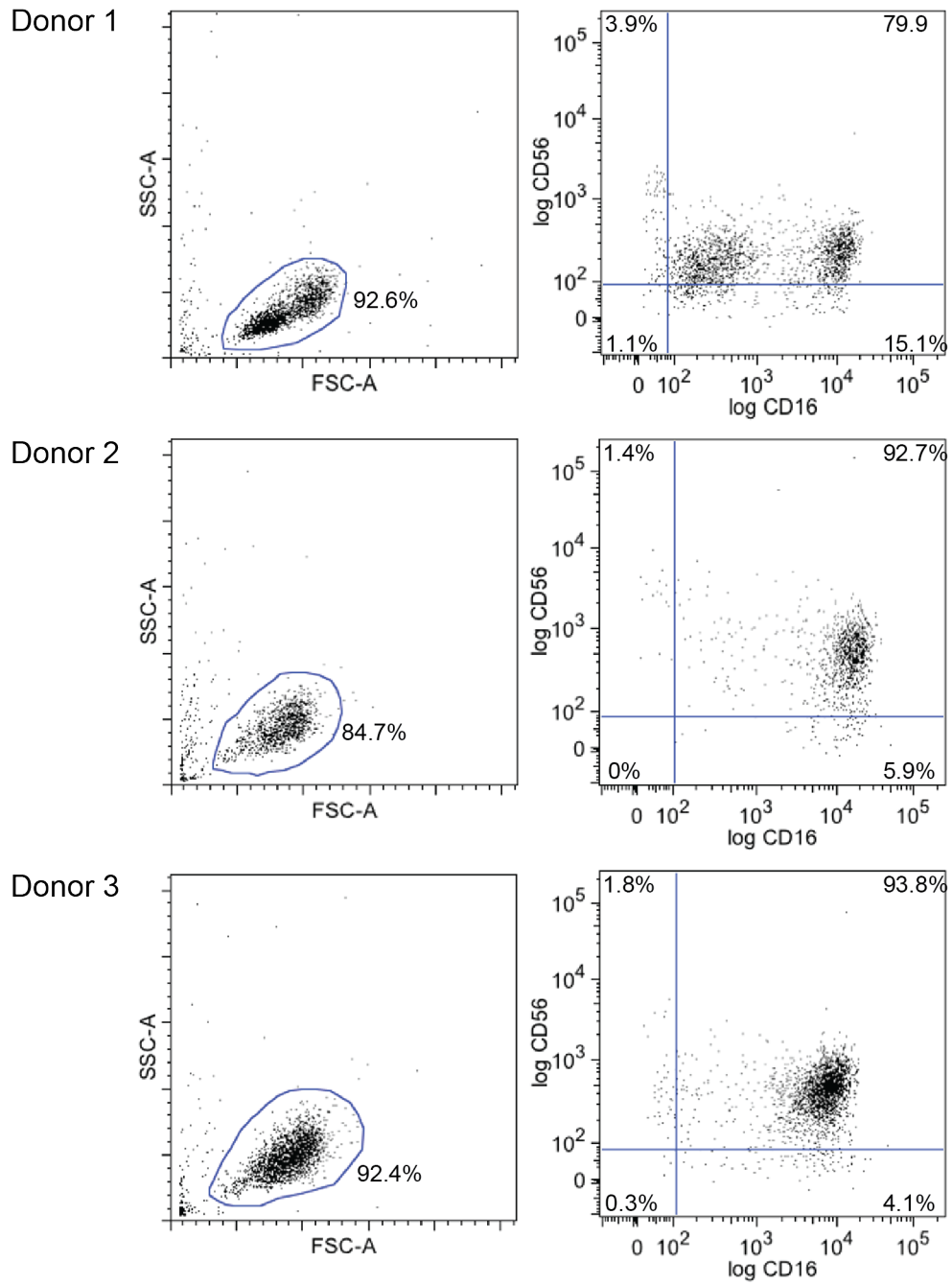


Figure S3. Flow cytometry for NK cells from three donors isolated by negative selection.

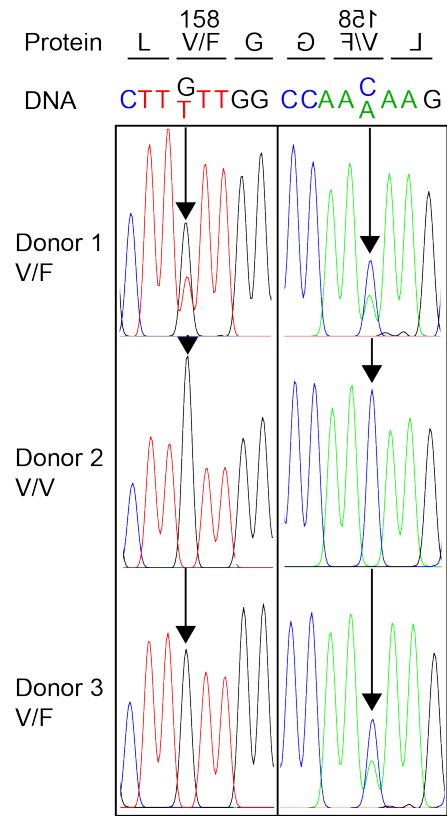


Figure S4. DNA sequencing of CD16a cDNA reveals the genotype of each donor.

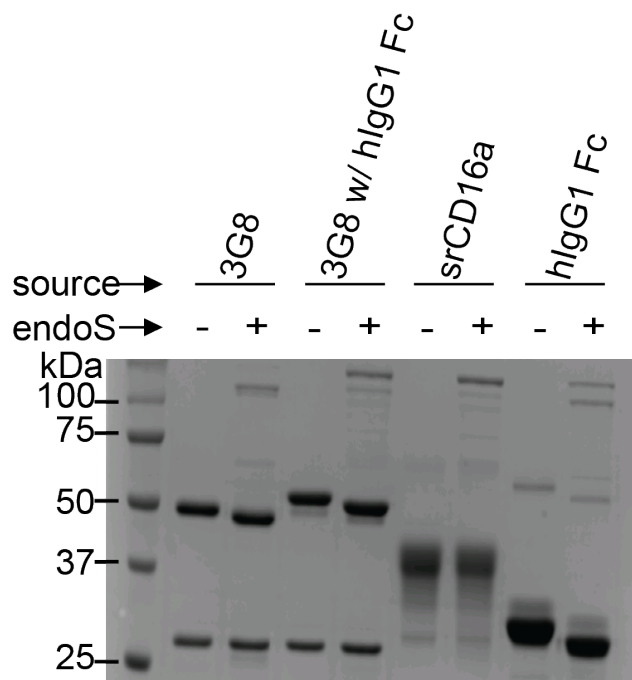


Figure S5. Treating 3G8 with EndoS removes N-glycans that could potentially contaminate the N-glycan analysis.

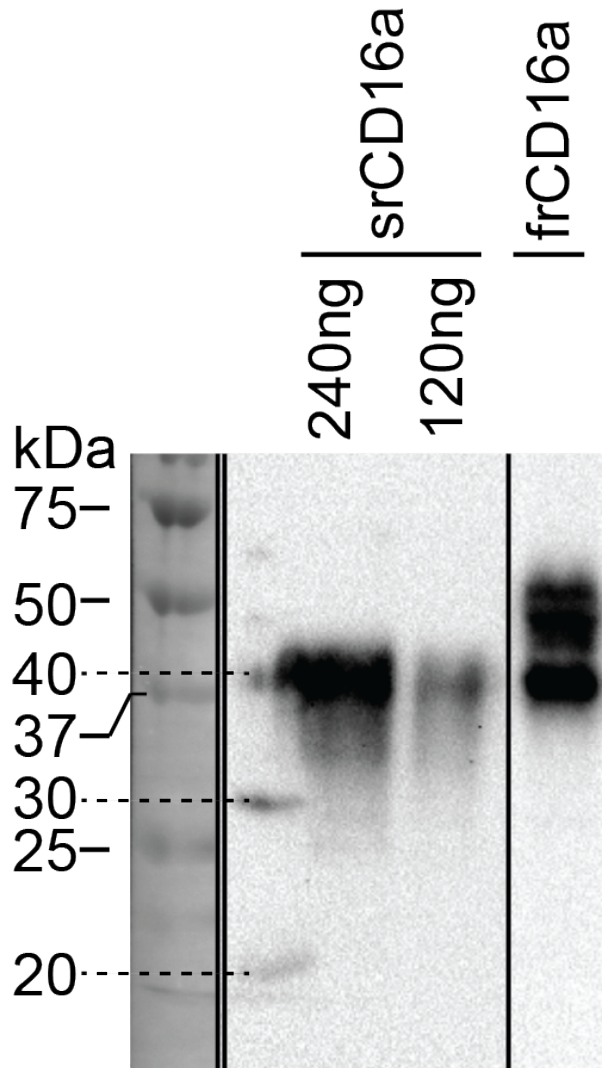


Figure S6. Western blot of soluble recombinant CD16a and full-length recombinant CD16a. All of these images are prepared from one membrane. The ladder on the left is a bright-field image of the membrane showing the transferred SDS-PAGE ladder and is separated from the western blot by a solid double vertical line. The molecular mass of each band is denoted with a solid horizontal line. The right image of the western blot shows a ladder in the left-most lane with molecular mass indicated with dashed horizontal lines. The single solid vertical line indicates a splice between the lanes of the western blot, however, contrast was not altered from the original image.

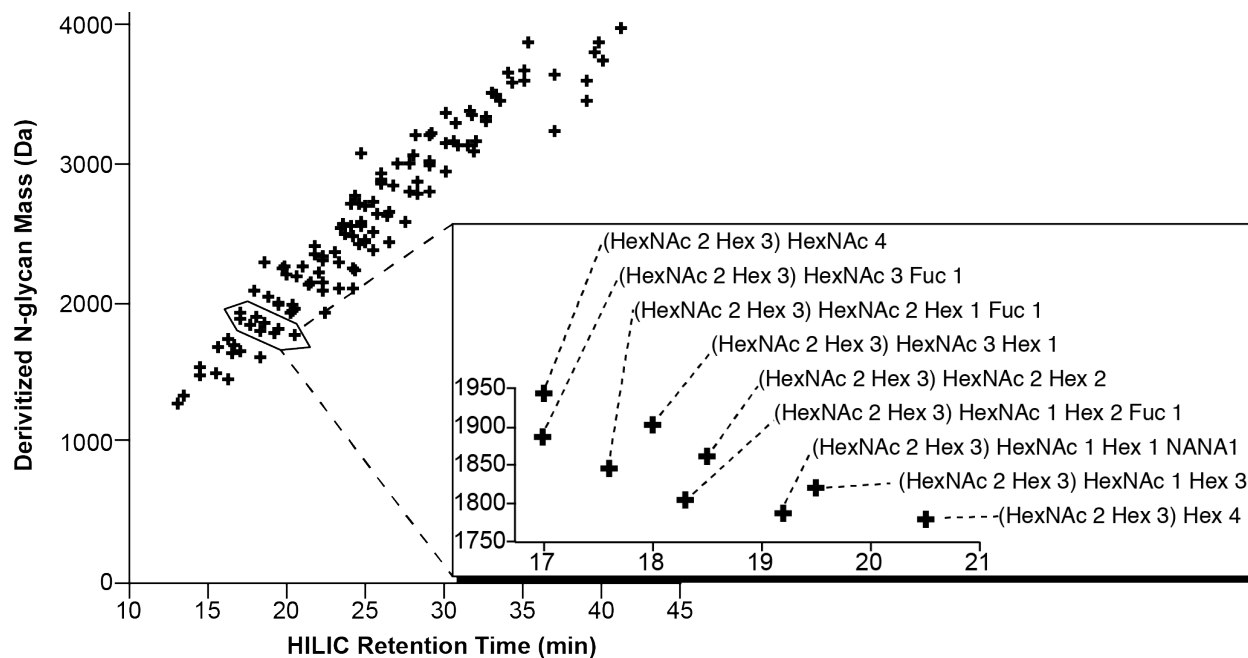


Figure S7. Separation of the recombinant CD16a extracellular domain-only procainamide-derivatized N-glycans as marked by peak elution times from HILIC. The inset shows the identification of N-glycans that form the fine features in this plot. Each N-glycan contains nine residues (with a single exception of the NANA-containing N-glycan that has eight) and shows the more hydrophilic N-glycans elute almost five minutes later.