Supporting Material

Immunoglobulin G glycoprofiles are unaffected by common bottom-up sample processing

Manuela Amez-Martín, Manfred Wuhrer and David Falck*.

Center of Proteomics and Metabolomics, Leiden University Medical Center, P.O. Box 9600, 2300 RC, Leiden, The Netherlands.

*Corresponding author: David Falck. Address: Center of Proteomics and Metabolomics, Leiden University Medical Center, P.O. Box 9600, Postzone P1-Q, 2300 RC, Leiden, The Netherlands. E-mail: d.falck@lumc.nl

Table of Contents

Methods section

LaCyTools settings

Alignment	
Minimum alignment features	5
S/N cut-off	100
Mass window	±0.1 Th
Time window	±6 s

Calibration	
Minimum calibrants	4
S/N cut-off	27
Mass window	±0.2 Da

Extraction	
Mass window	±0.065 Th
Time window	±13 s

Curation criteria

Analyte curation was performed with multiple quality criteria: the S/N of the analyte for 80% of all spectra had to be at least 9, the isotopic pattern quality score for the comparison of the theoretical and observed isotopic envelope of the analyte had to be lower than 0.2 for 80% of all spectra and the ppm-error of the analyte had to be lower than ±10 for the mean of all spectra. The final analyte list can be found in Table S-3.

Calculation of derived glycosylation features

For IgG1:

- Sialylation = 0.5*(G1FS+ G2S+G2FS+G1FSN+G2FSN)+G2FS2
- Galactosylation = 0.5*(G1+G1F+G1FN+G1FS+G1FSN)+G1F-N+G2+G2F+G2S+G2FN+G2FS+G2FSN+ G2FS2
- Fucosylation = G0F-N+G1F-N+G0F+G1F+G0FN+G2F+G1FN+G1FS+G2FN+G2FS+G1FSN+G2FSN+ G2FS2
- Bisecting GlcNAc = G0FN+G1FN+G2FN+ G1FSN+G2FSN

For IgG2:

- Sialylation = 0.5*(G1FS+G2FS+G1FSN+G2FSN)
- Galactosylation = 0.5*(G1+G1F+G1FN+G1FS+G1FSN)+G1F-N+G2+G2F+G2FN+G2FS+G2FSN
- Fucosylation = G0F-N+G1F-N+G0F+G1F+G0FN+G2F+G1FN+G1FS+G2FN+G2FS+G1FSN+G2FSN
- Bisecting GlcNAc = G0FN+G1FN+G2FN+ G1FSN+G2FSN

For IgG4:

- Sialylation = 0.5*G2FS
- Galactosylation = 0.5*(G1F+G1FN)+G2F+G2FS
- Bisecting GlcNAc = G0FN+G1FN

G0N was not included in the bisection formula because not all its homologous structures were part of final analyte list after curation. This prevented changes in bisection to be dependent on fucosylation.

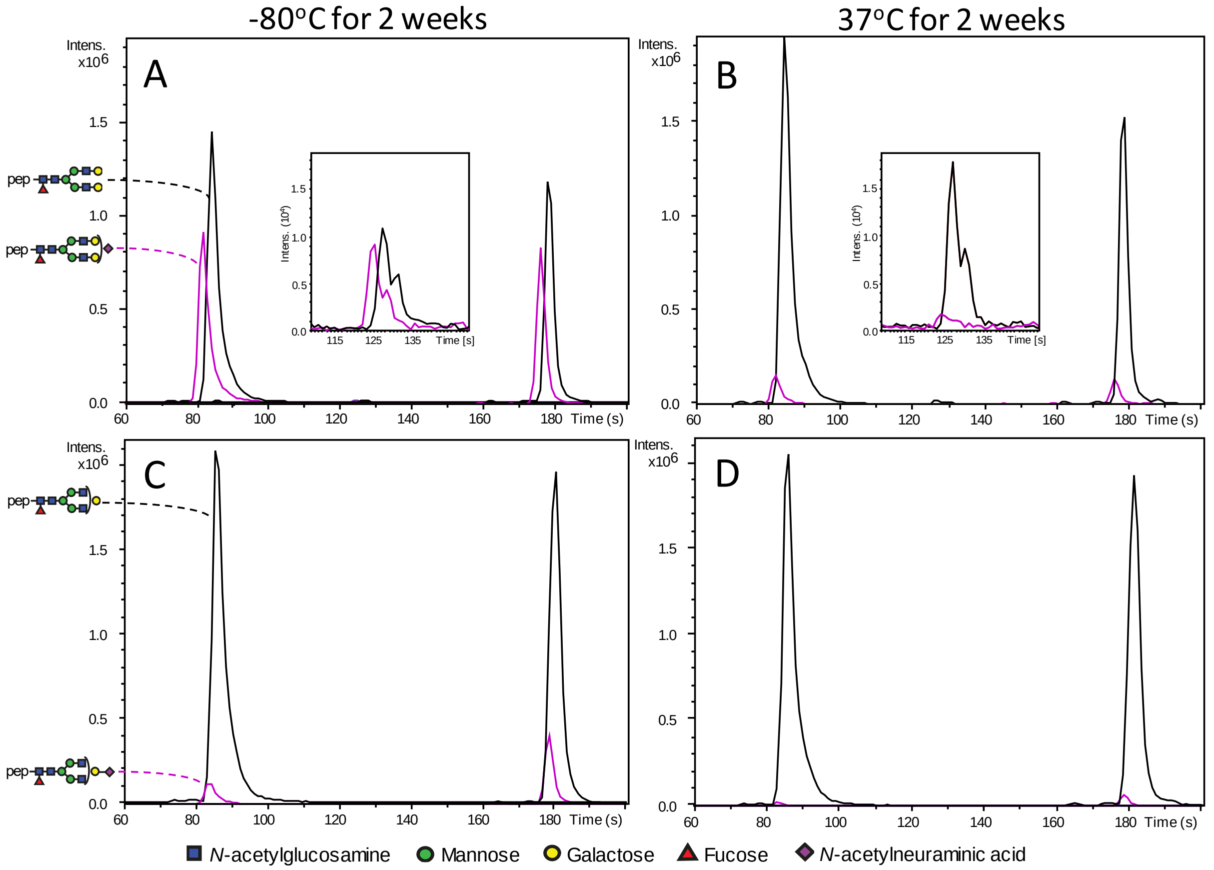

Figure S-1 Extracted Ion Chromatogram (EIC) of sialylated and asialylated IgG1 glycoforms after acid exposure and heat. Sialylation decreases after acid exposure at elevated temperatures. A) and B) EIC of glycoform H5N4F1S1 (purple) and H5N4F1 (black) corresponding to IgG1 and IgG2 tryptic peptide for 2 acidic exposure conditions: 80^{o}C for 2 weeks (A) and 37^{o}C for 2 weeks (B). IgG4 is illustrated as zoom-in due to the difference in intensity among the different IgG subclasses. For all IgG subclasses, acid exposure at elevated temperatures causes the loss of sialylation, which is observed in a decrease of H5N4F1S1 and an increase in H5N4F1. C) and D) EIC of glycoform H4N4F1S1 (purple) and H4N4F1 (black) corresponding to IgG1 and IgG2 tryptic peptides for 2 acidic exposure conditions: 80^{o}C for 2 weeks (C) and 37^{o}C for 2 weeks (D). For these IgG subclasses, a decrease of H4N4F1S1 is observed at acid exposure at elevated temperature.

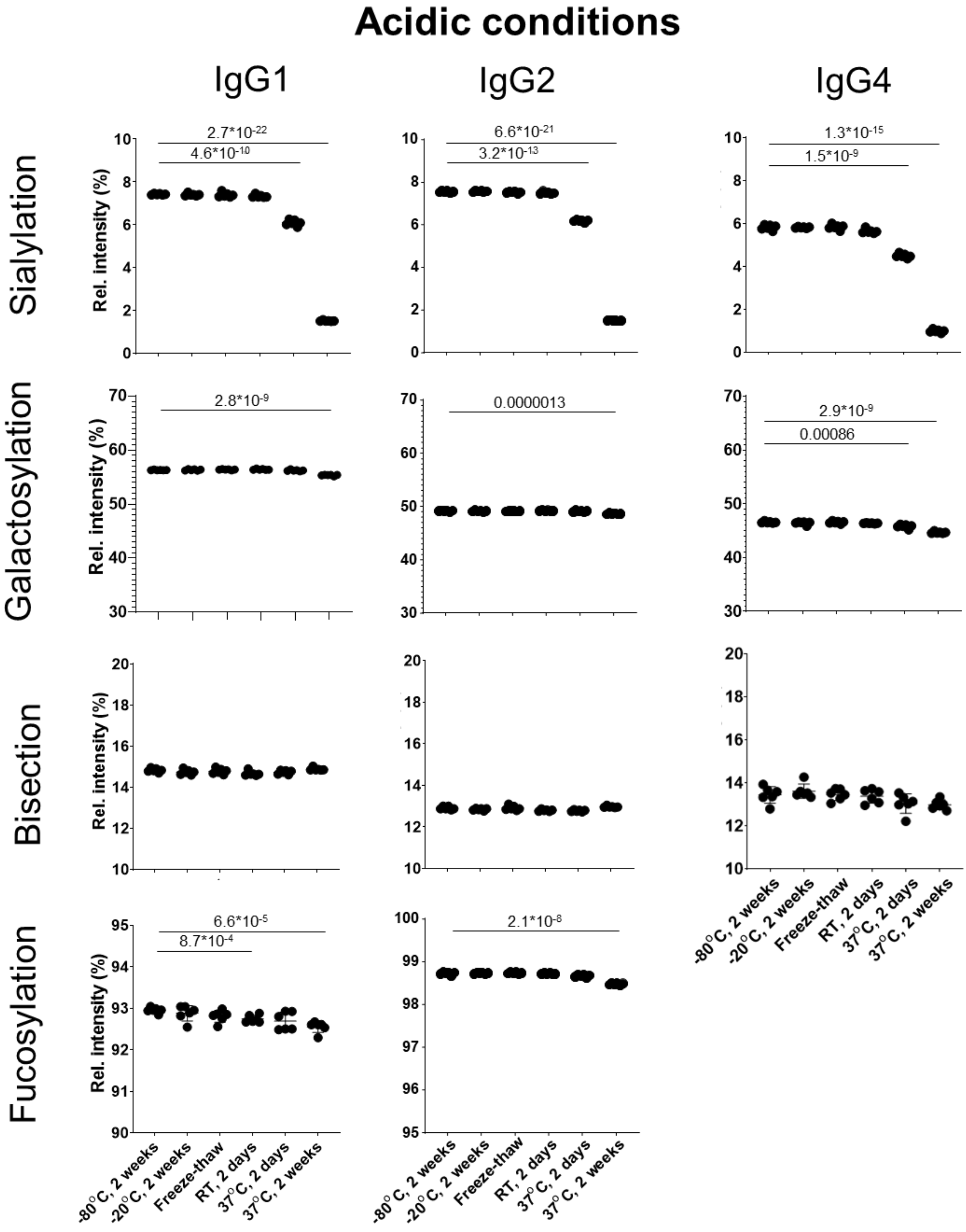

Figure S-2 Graphs comparing acidic conditions. Graphs illustrate the changes regarding glycosylation features (sialylation, galactosylation, bisection and fucosylation) under acidic conditions for three IgG subclasses (IgG1, IgG2 and IgG4). All features stay stable under normal and accidental sample processing conditions. Under stress conditions, sialylation decreased and galactosylation slightly decreased. Bisection and fucosylation do not changed, and fucosylation apparently decreases slightly due to minor interdependencies with sialic acid (p-values of t-test findings are displayed). Lines and error bars indicate the mean and standard deviation for each condition.

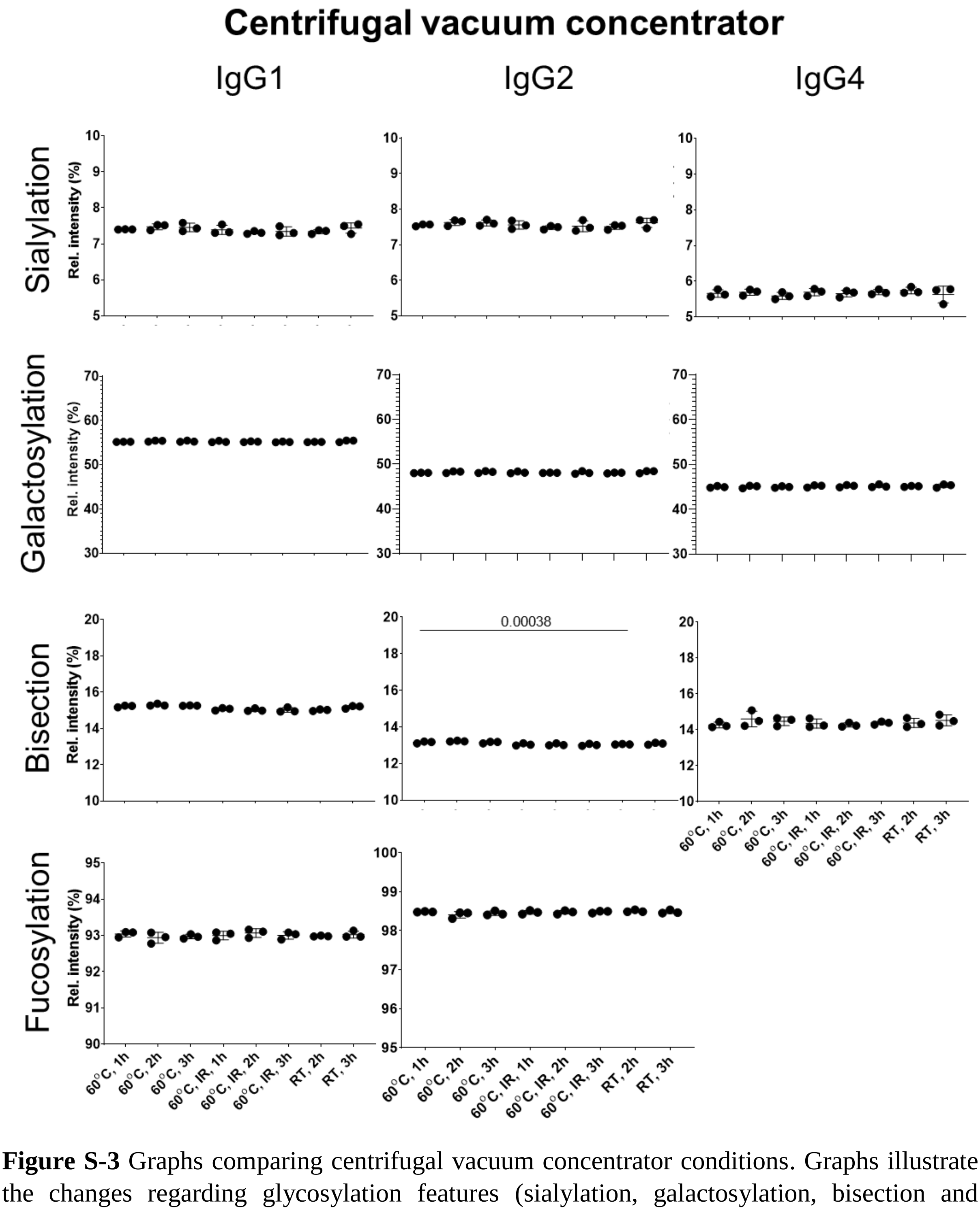

Figure S-3 Graphs comparing centrifugal vacuum concentrator conditions. Graphs illustrate the changes regarding glycosylation features (sialylation, galactosylation, bisection and fucosylation) under centrifugal vacuum concentrator conditions for three IgG subclasses (IgG1, IgG2 and IgG4). Overall, all features do not remarkably change (p-value of t-test finding is displayed). Lines and error bars indicate the mean and standard deviation for each condition.

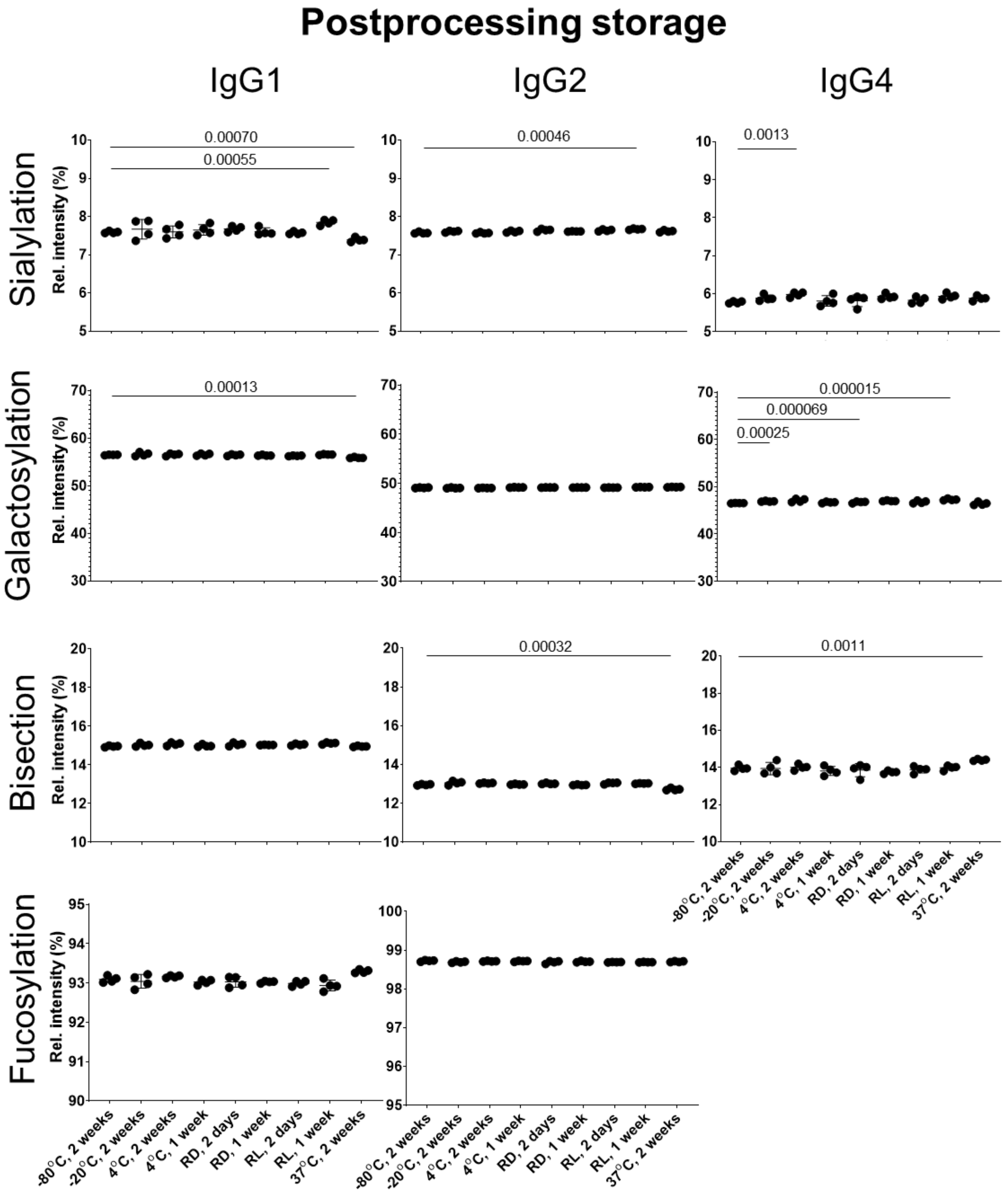

Figure S-4 Graphs comparing post-processing storage conditions. Graphs illustrate the changes regarding glycosylation features (sialylation, galactosylation, bisection and fucosylation) under different post-processing sample storage conditions for three IgG subclasses (IgG1, IgG2 and IgG4). Minor changes of less than 1% occur, except for fucosylation (p-values of t-test findings are displayed). Lines and error bars indicate the mean and standard deviation for each condition.

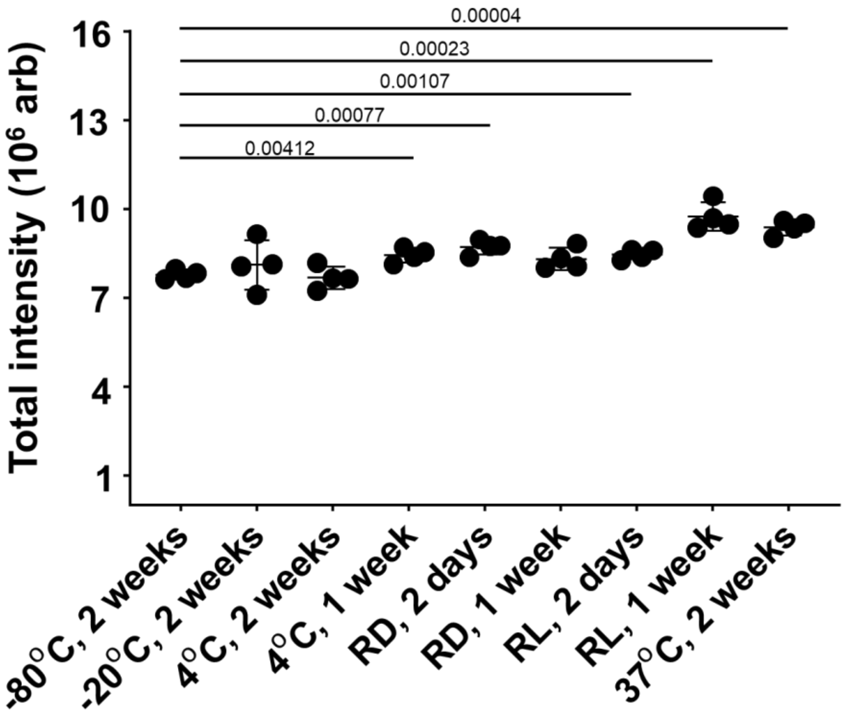

Figure S-5 Total intensity comparing post-processing storage conditions. Total intensity of all IgG1 glycoforms after post-processing sample storage is depicted. Though minor changes were observed, overall total intensities are highly comparable (p-values of t-test findings are displayed). Lines and error bars indicate the mean and standard deviation for each condition.

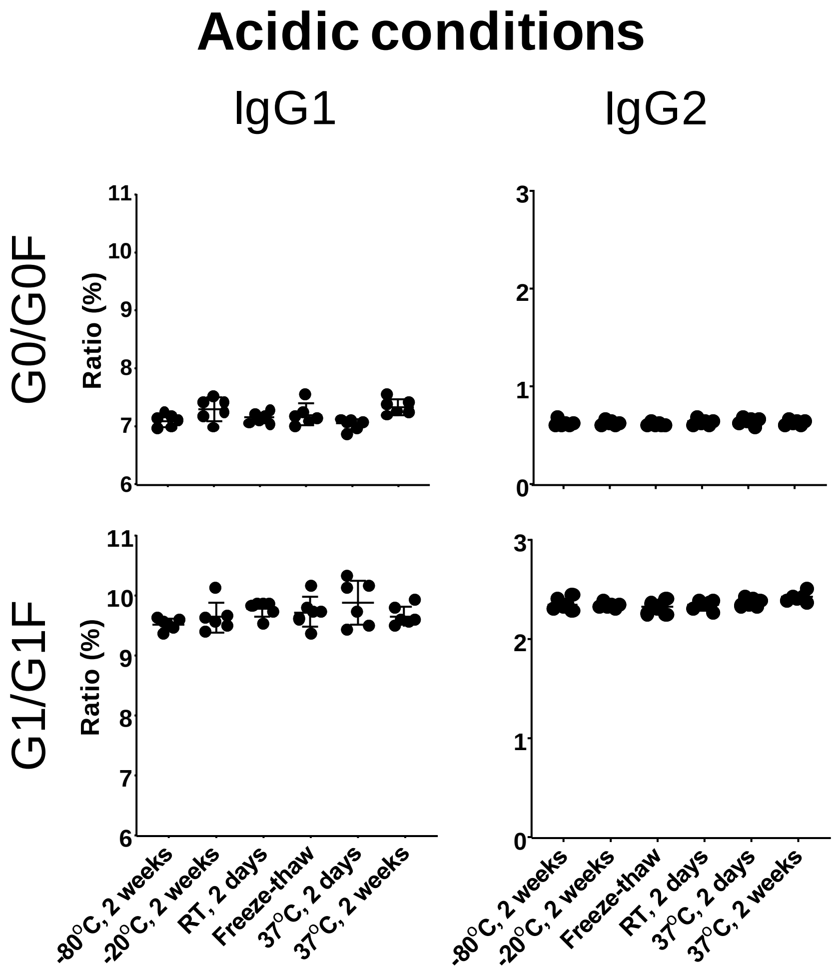

Figure S-6 Graphs comparing fucosylated and non-fucosylated asialylated species under different acidic conditions. The ratio of homologous fucosylated and non-fucosylated asialylated glycopeptides stays stable under acidic exposure in all three IgG subclasses (IgG1, IgG2 and IgG4). Lines and error bars indicate the mean and standard deviation for each condition.