Supporting Information

Serum and plasma immunoglobulin G Fc *N*-glycosylation is stable during storage

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, 2300 RC, Leiden, The Netherlands. E-mail: d.falck@lumc.nl

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LaCyTools settings

Alignment	
Minimum alignment features	6
S/N cut-off	100
Mass window	±0.1 Th
Time window	±5 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 (plasma); ±12 s (serum)
Minimum isotopic fraction	0.9

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) + G2 + G2F + G2FN + G2FS + G2FSN
- Fucosylation = G0F-N + G0F + G1F + G0FN + G2F + G1FN + G1FS + G2FN + G2FS + G1FSN + G2FSN
- Bisecting GlcNAc = G0FN + G1FN + G2FN + G1FSN + G2FSN

For IgG4:

- Sialylation = 0.5 * (G1FS + G2FS)
- Galactosylation = 0.5 * (G1F + G1FN + G1FS) + 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 potential changes in bisection to be dependent on fucosylation.

ESI-MS parameters

The ESI-MS parameters were as follows: positive mode, end plate offset 500 V, capillary voltage 1200 V, nano-Booster™ pressure 0.2 bar, dry gas flow 3.0 L/min, dry temperature 180 °C, mass range *m/z* 550– 1800; spectral acquisition frequency 1 Hz, focus setting active; Collision cell settings: collision energy 5.0 eV, transfer time 110 µs, pre-pulse storage 21 µs.

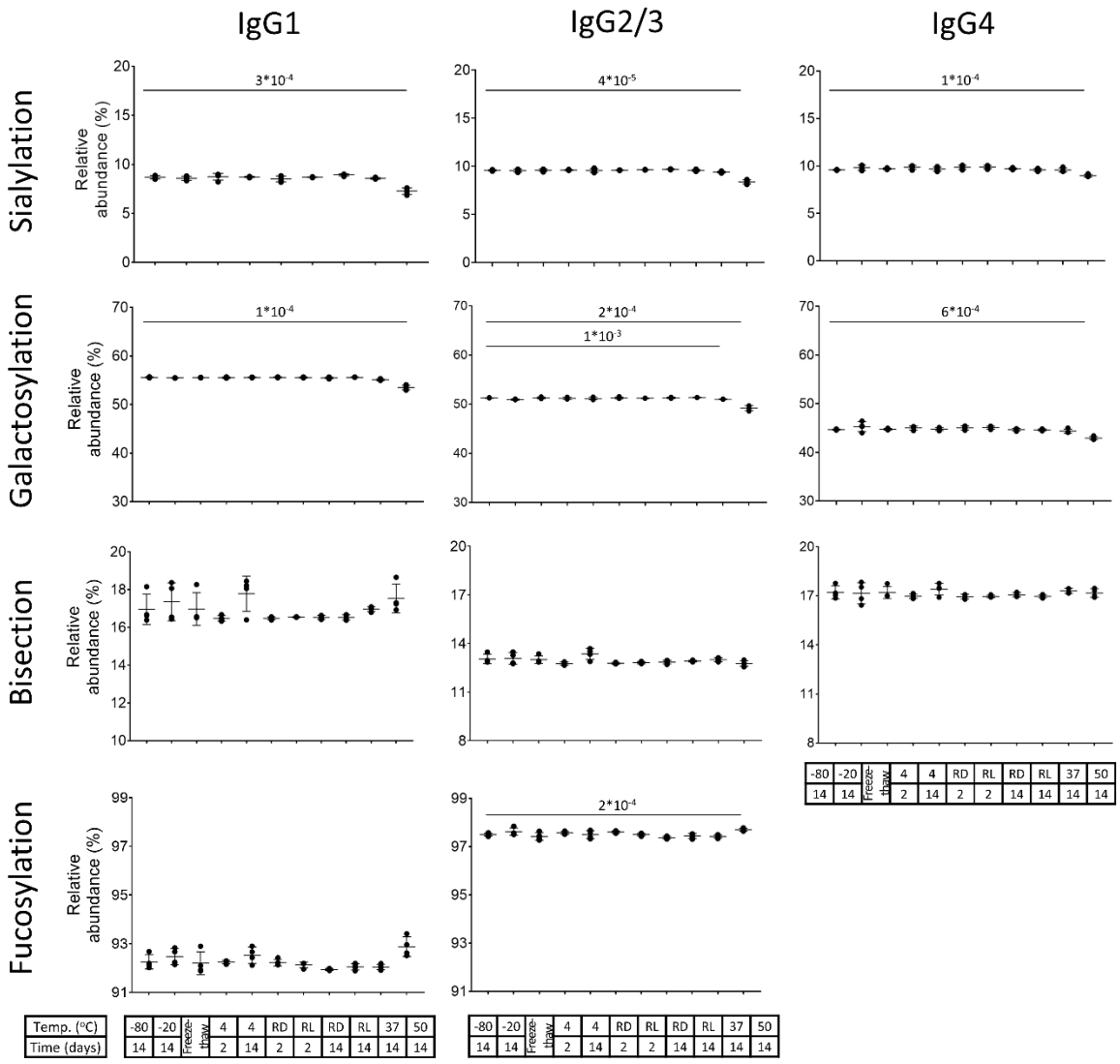

Figure S-1. Serum IgG glycosylation features from donor 1 (sialylation, galactosylation, bisection and fucosylation) of IgG subclasses (IgG1, IgG2/3 and IgG4) after different storage conditions. A decrease in sialylation and galactosylation for all IgG subclasses is consistently observed after sample storage at 50ºC for 2 weeks when compared to the reference condition (-80°C). A smaller decrease in sialylation was also visible for IgG2/3 after storage at 37ºC for 2 weeks, and fucosylation slightly increased after 2 weeks at 50ºC, while bisected *N*-acetylglucosamine remained stable (p-values of t-test findings are displayed). RD: room temperature in the dark, RL: room temperature with exposure to light.

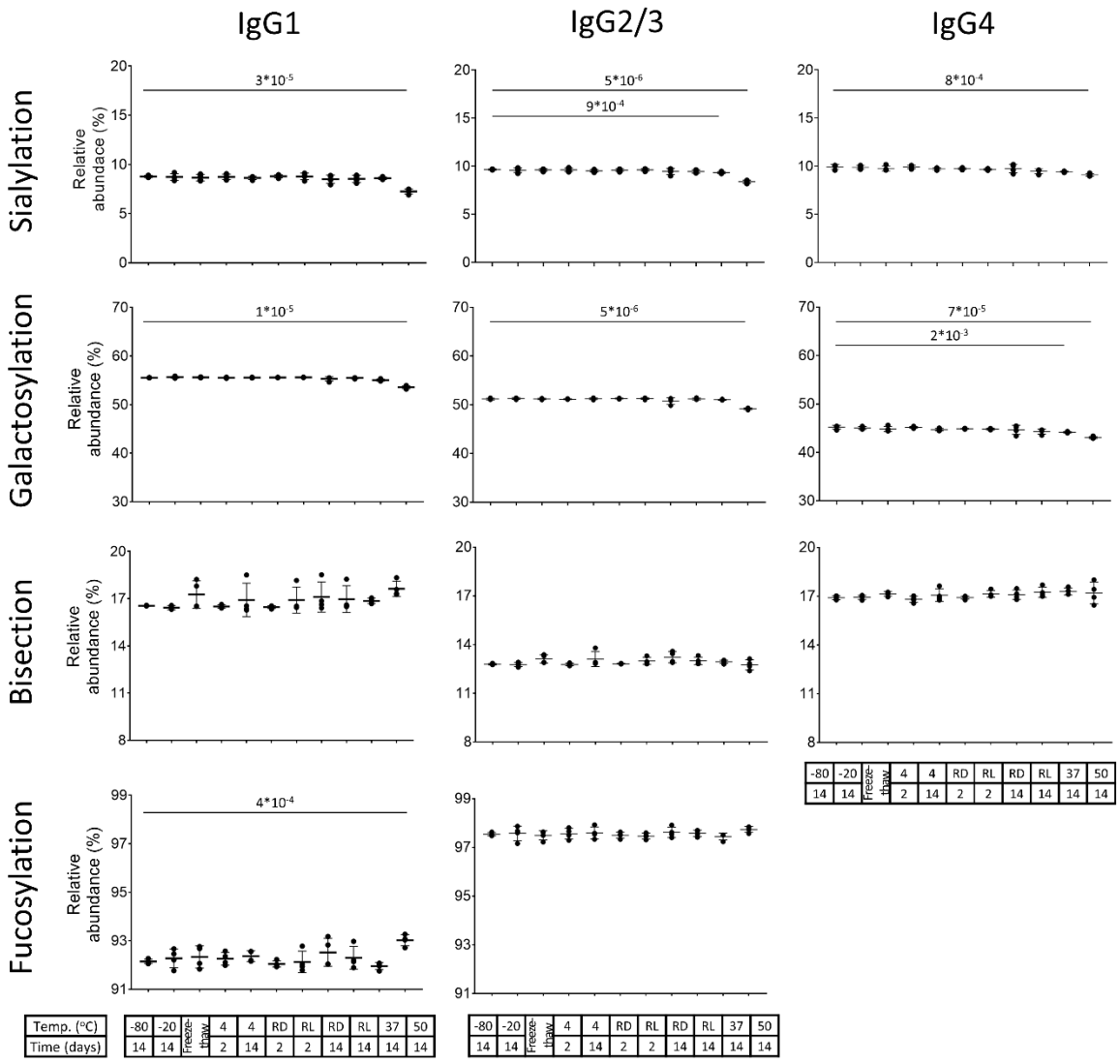

Figure S-2. Plasma IgG glycosylation features from donor 1 (sialylation, galactosylation, bisection and fucosylation) of IgG subclasses (IgG1, IgG2/3 and IgG4) after different storage conditions. A decrease in sialylation and galactosylation for all IgG subclasses is consistently observed after sample storage at 50°C for 2 weeks when compared to the reference condition (-80°C). A smaller decrease in sialylation was also visible for IgG2/3 and IgG4 after storage at 37°C for 2 weeks, as well as a small decrease in galactosylation for IgG4. Fucosylation slightly increased after 2 weeks at 50°C for IgG1 while bisected *N*-acetylglucosamine remained stable (p-values of t-test findings are displayed). RD: room temperature in the dark, RL: room temperature with exposure to light.

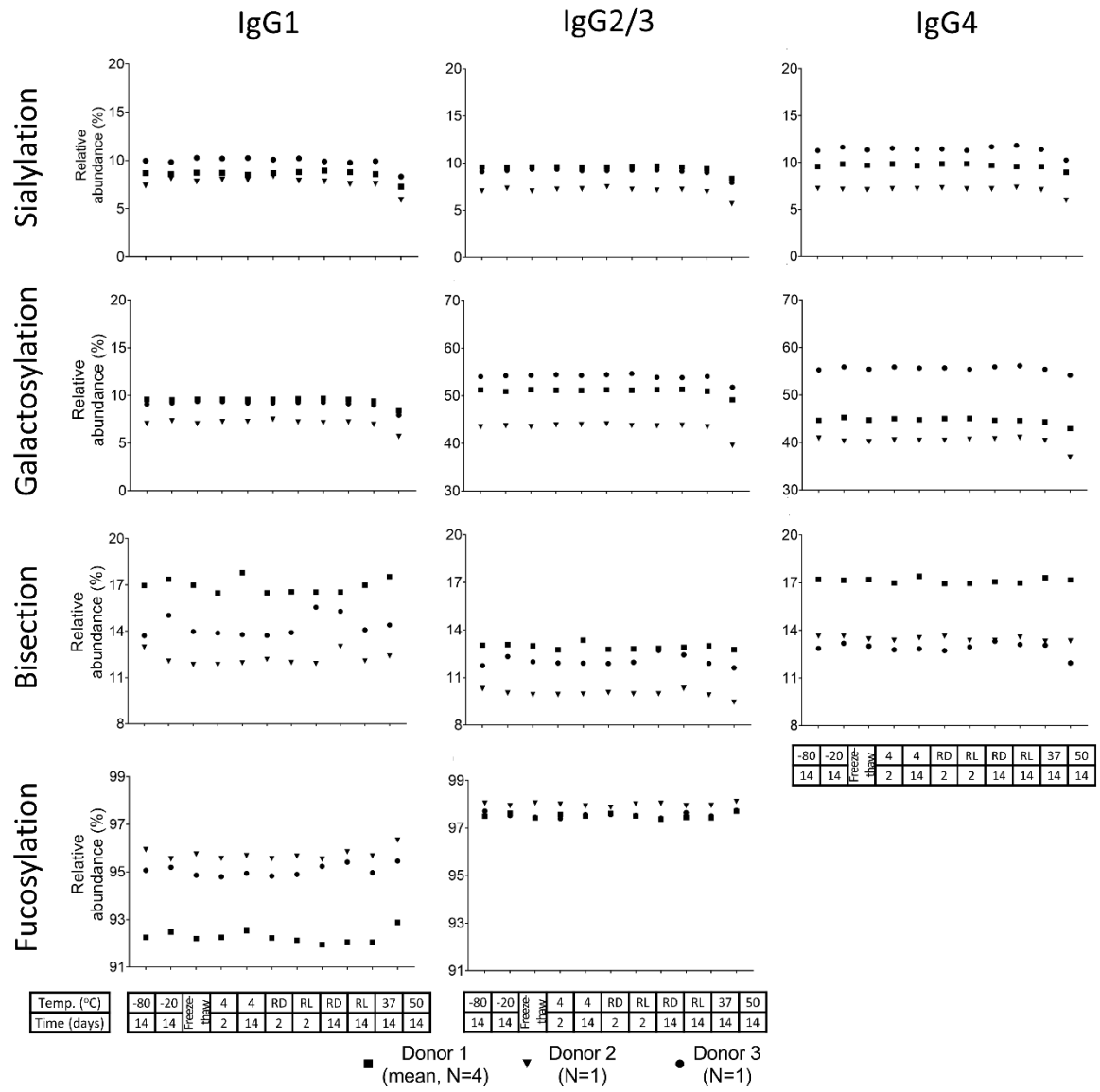

Figure S-3. Serum IgG glycosylation features from 3 donors (sialylation, galactosylation, bi-section and fucosylation) of IgG subclasses (IgG1, IgG2/3 and IgG4) after different storage conditions. A decrease in sialylation for IgG1 and IgG2/3 is observed after sample storage at 50°C for 2 weeks when compared to the reference condition (-80°C). A decreasing trend is also observed in sialylation for IgG4 and galactosylation for all IgG subclasses after storage at 50°C for 2 weeks, as well as a subtle increasing trend in fucosylation after storage at 50°C for 2 weeks when compared to the reference condition (-80°C). RD: room temperature in the dark, RL: room temperature with exposure to light.

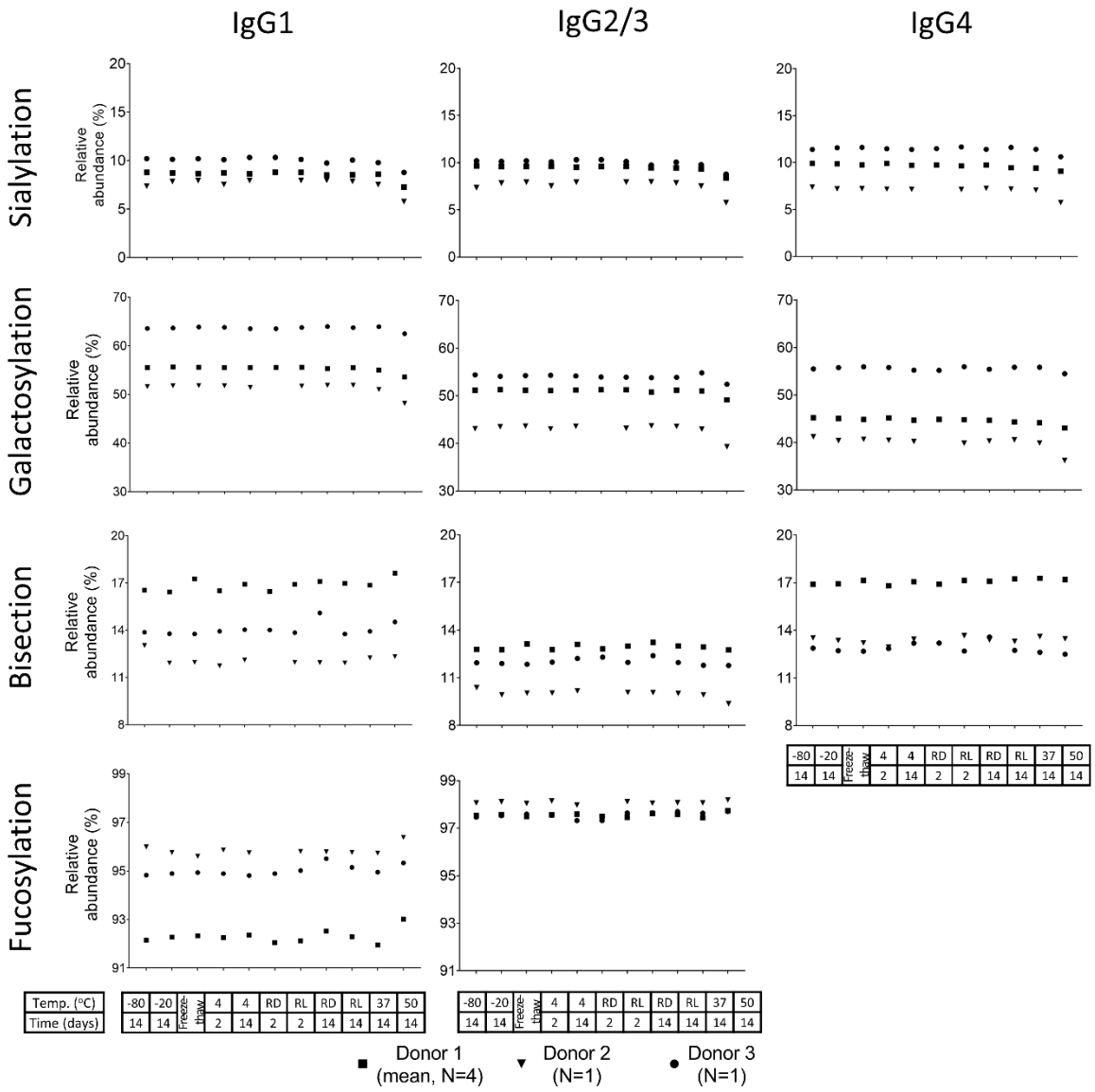

Figure S-4. Plasma IgG glycosylation features from 3 donors (sialylation, galactosylation, bisection and fucosylation) of IgG subclasses (IgG1, IgG2/3 and IgG4) after different storage conditions. All derived traits for all IgG subclasses remained stable, except for a decreasing trend in sialylation and galactosylation for all IgG subclasses is consistently observed after sample storage at 50°C for 2 weeks when compared to the reference condition (-80°C). Fucosylation showed a slightly increasing trend after 2 weeks at 50°C while bisected *N*-acetylglucosamine remained stable. RD: room temperature in the dark, RL: room temperature with exposure to light.

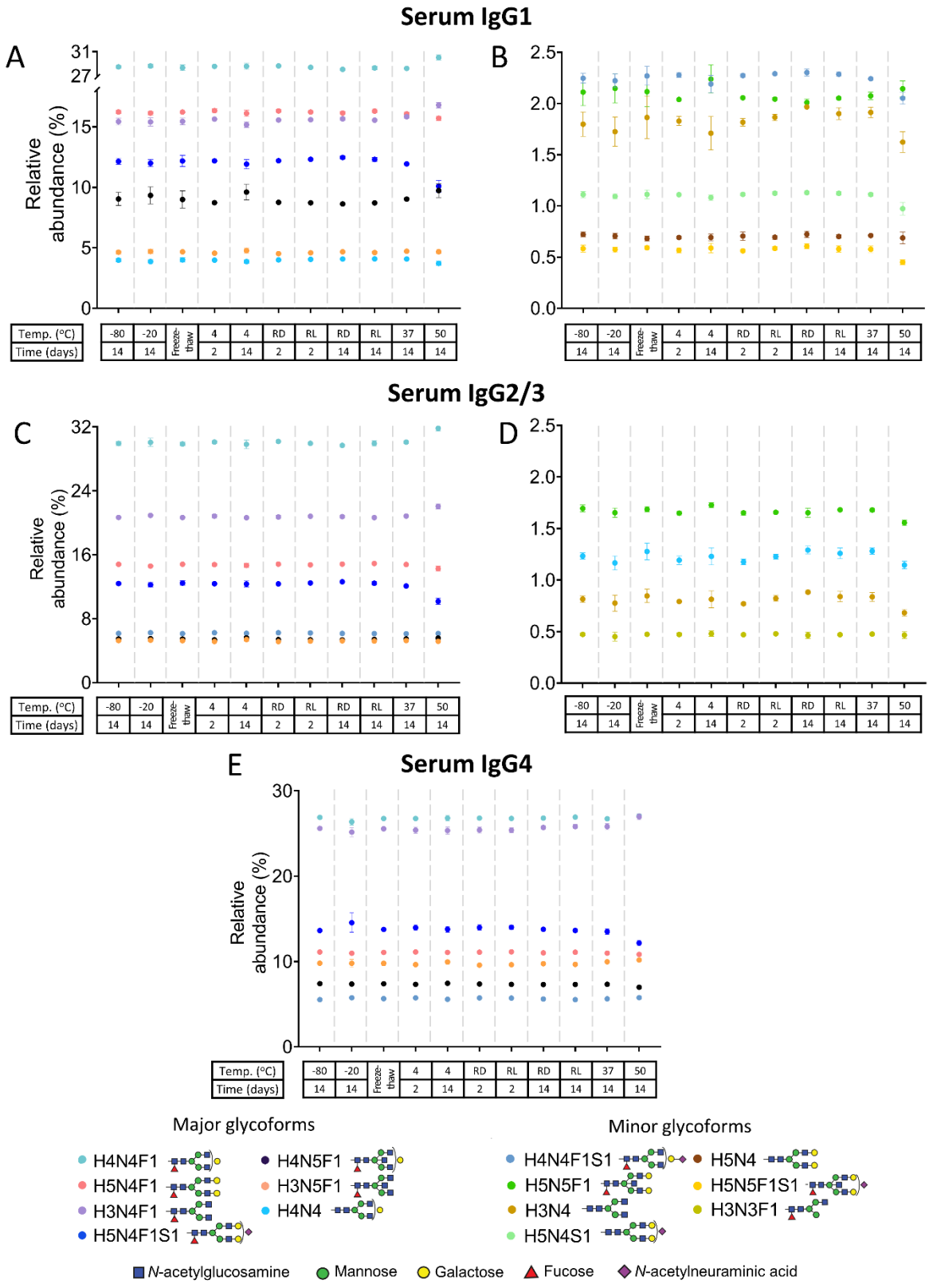

Figure S-5. Serum IgG glycopeptides from donor 1 (mean, SD) at the different studied storage conditions, with a relative abundances above 0.5%. A) and B) IgG1 glycopeptides, C) and D) IgG2/3 glycopeptides and E) IgG4 glycopeptides. A) and C) comprise glycopeptides at a relative abundance above 2.5%, and B) and D) on the right comprise glycopeptides at a relative abundance below 2.5%.

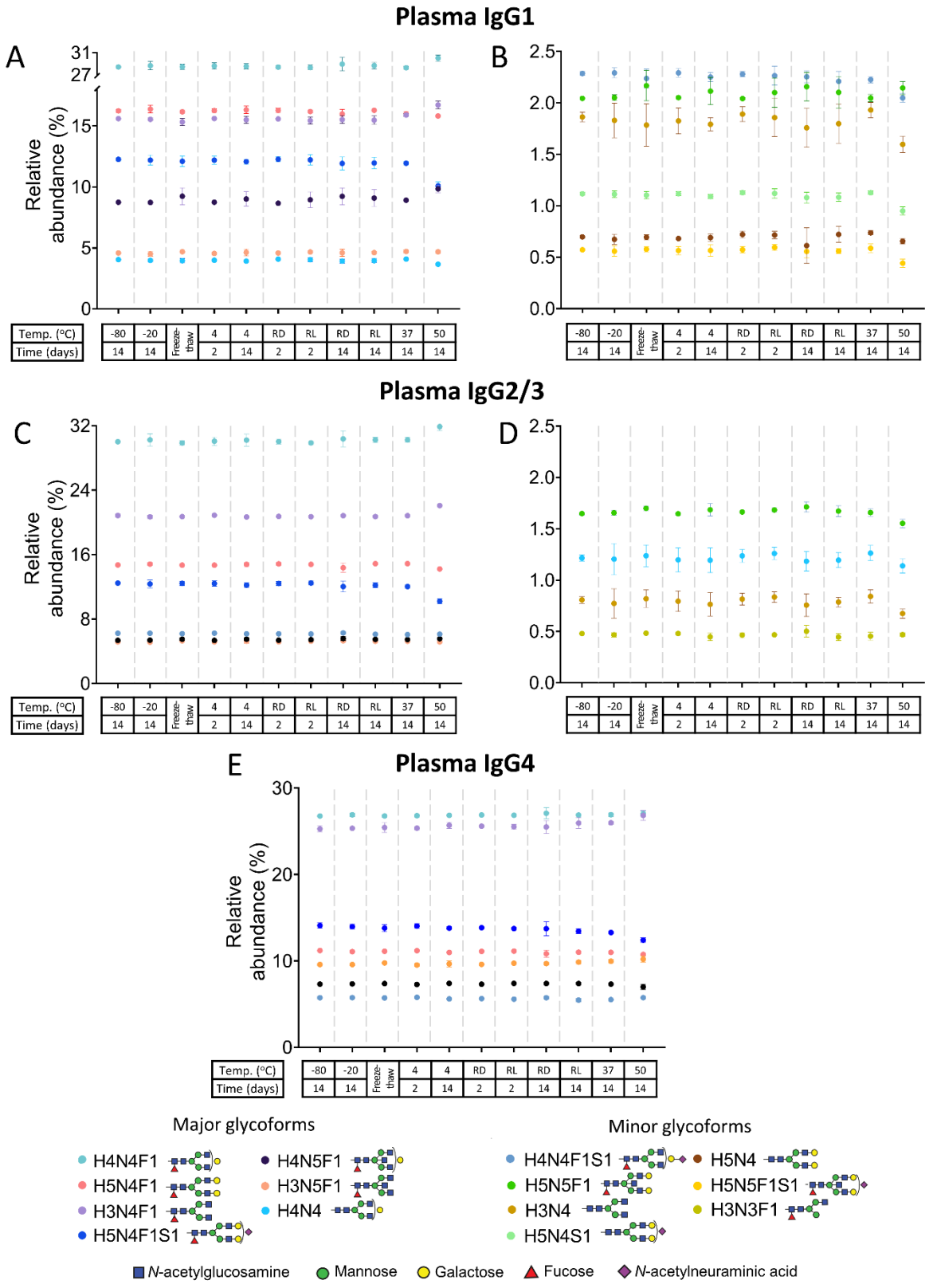

Figure S-6. Plasma IgG glycopeptides from donor 1 (mean, SD) at the different studied storage conditions, with a relative abundances above 0.5%. A) and B) IgG1 glycopeptides, C) and D) IgG2/3 glycopeptides and E) IgG4 glycopeptides. A) and C) comprise glycopeptides at a relative abundance above 2.5%, and B) and D) on the right comprise glycopeptides at a relative abundance below 2.5%.