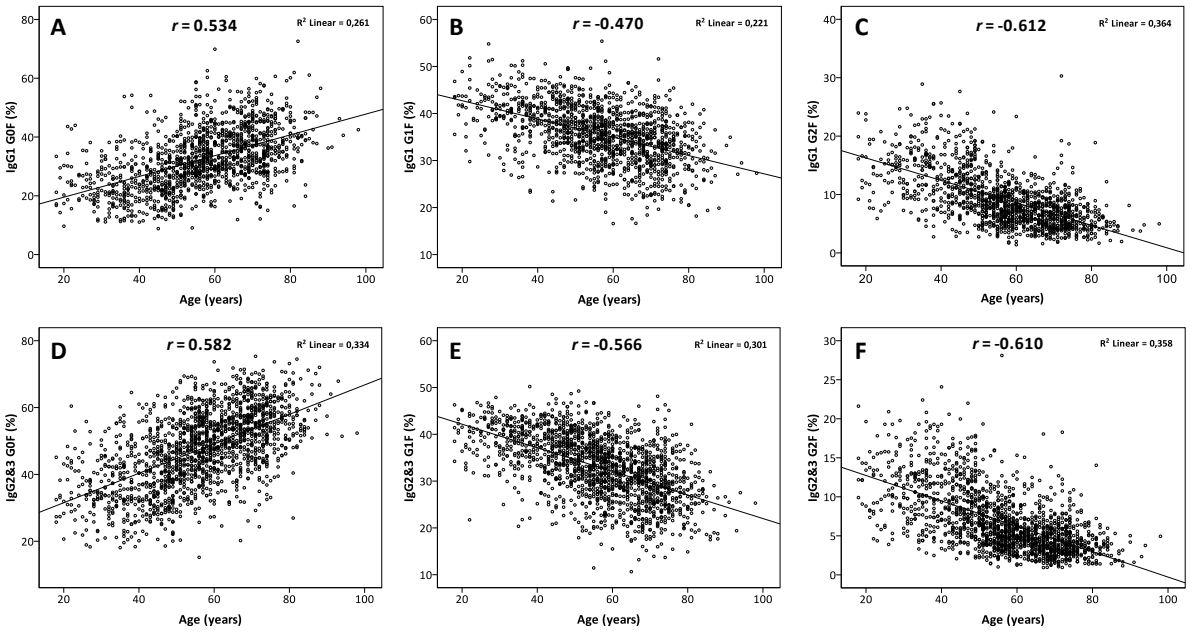
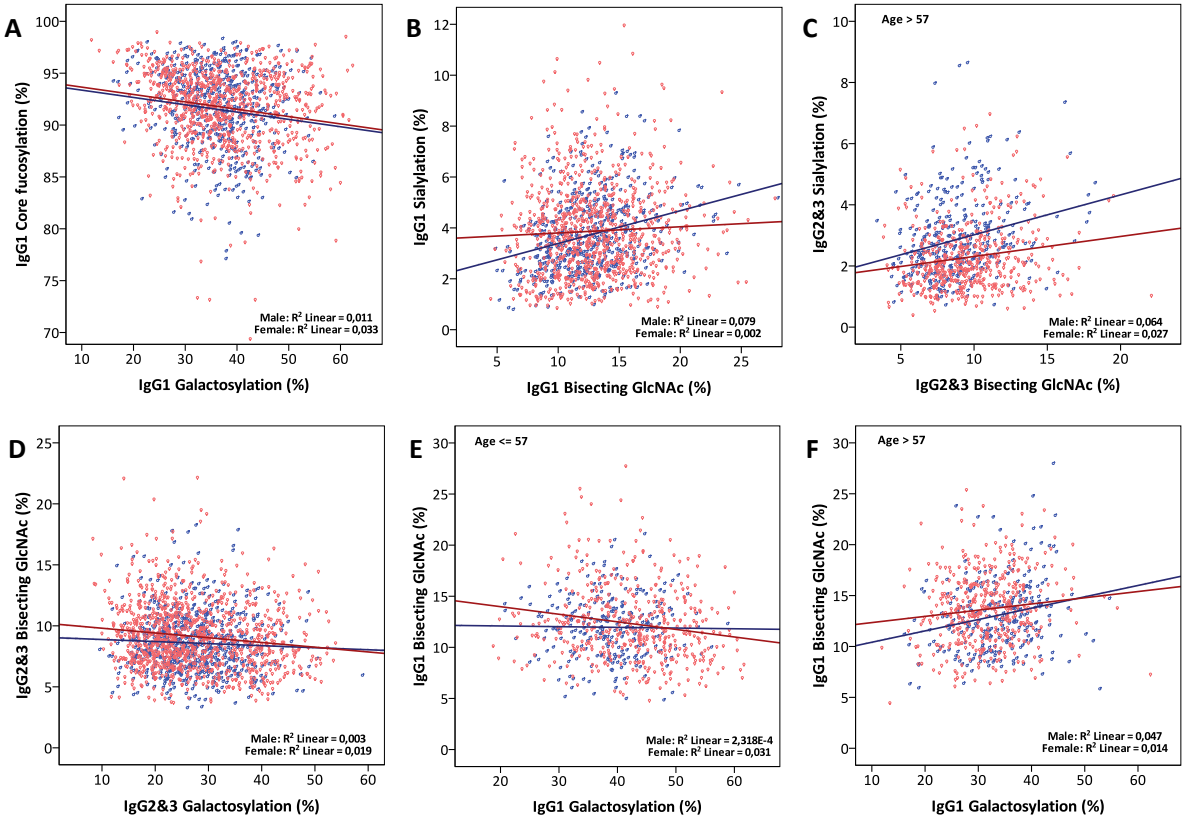
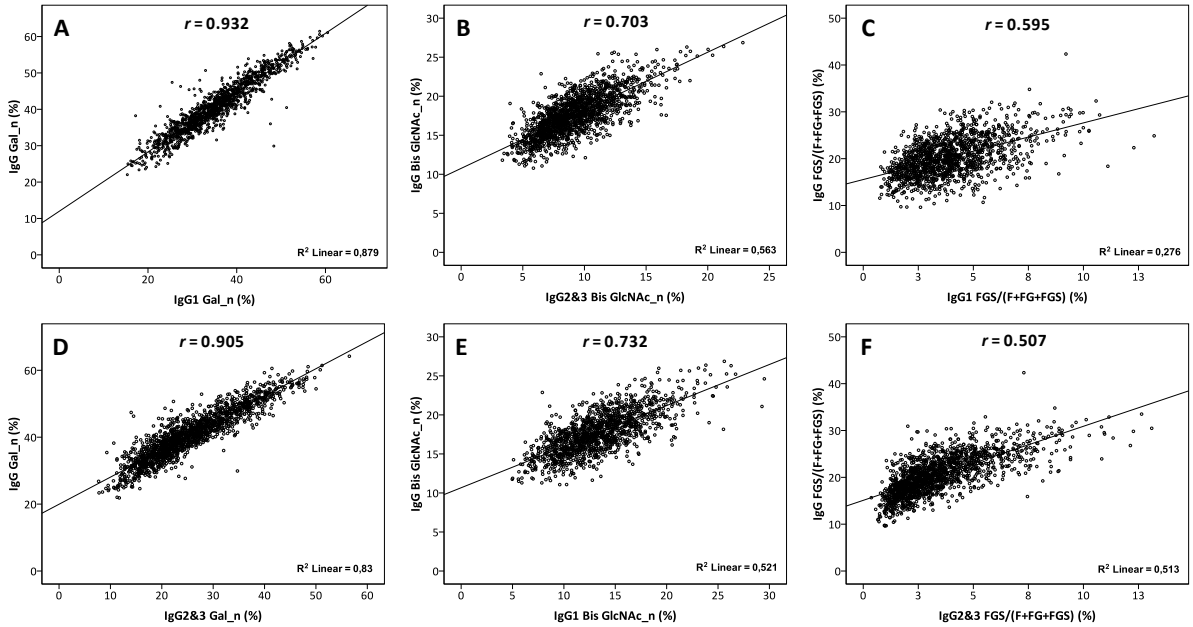


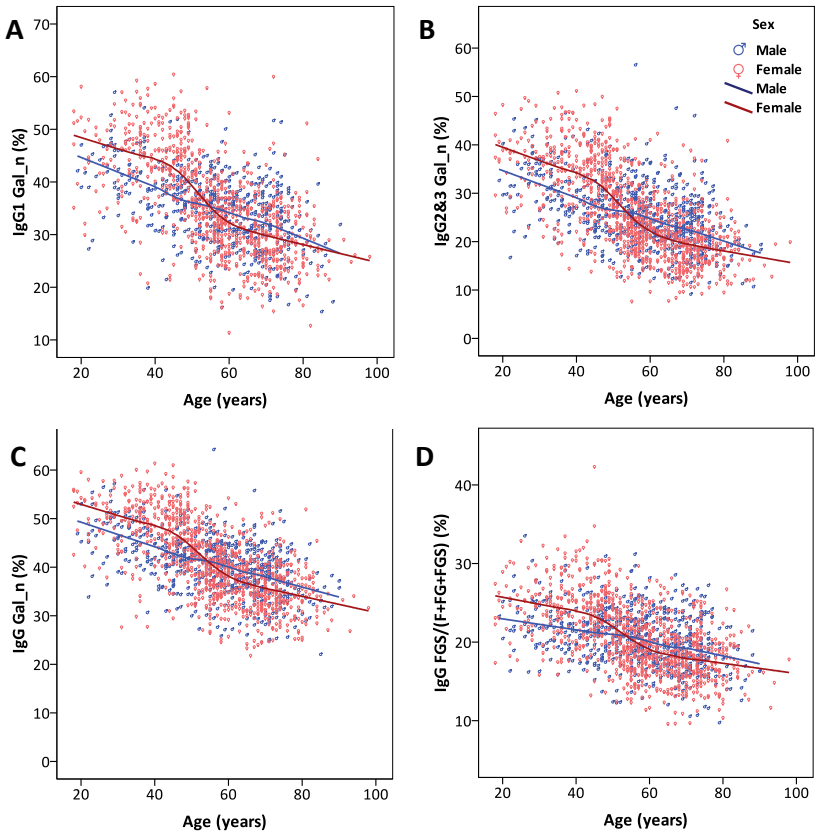
Supplementary Figure 1. Age dependence of IgG1 and IgG2 &3 glycoforms with correlation coefficients (*r*) and coefficients of determination (R^2).



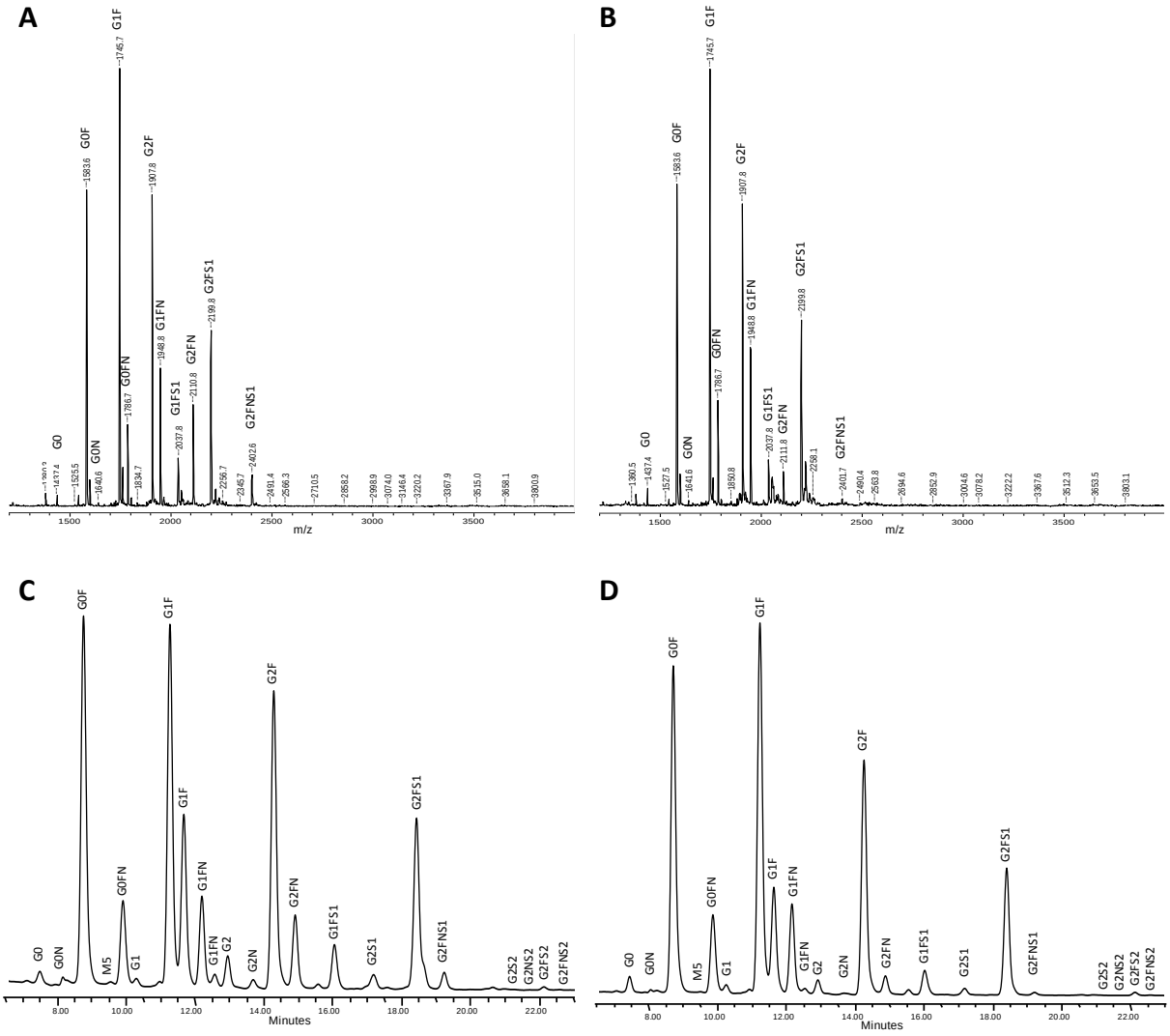
Supplementary Figure 2. Statistically significant correlations between IgG Fc glycosylation features stratified for sex. Females are plotted in red with a fitted line in dark red, while males are plotted in blue with a fitted line in dark blue. R^2 - coefficient of determination.



Supplementary Figure 3. Correlations (*r* - correlation coefficients) between glycosylation features from MALDI-TOF-MS and HILIC profiling. R^2 - coefficient of determination.



Supplementary Figure 4. Age dependence of MS (IgG1 Fc, IgG2&3 Fc) and HILIC (total IgG) galactosylation levels in neutral glycoforms and percentage of sialylation of fucosylated structures. Females are plotted in red with a fitted line in dark red, while males are plotted in blue with a fitted line in dark blue. Both lines were fitted using the loess (locally weighted scatterplot smoothing) method.



Supplementary Figure 5. Linear-negative ion mode MALDI-TOF-MS spectra of 2-AA-labeled *N*-glycan species of IgG standard (A) and IgG Fc standard (B) and HILIC chromatogram of 2-AB *N*-glycan species of IgG standard (C) and IgG Fc standard (D). Glycan species are given as in Table 1.

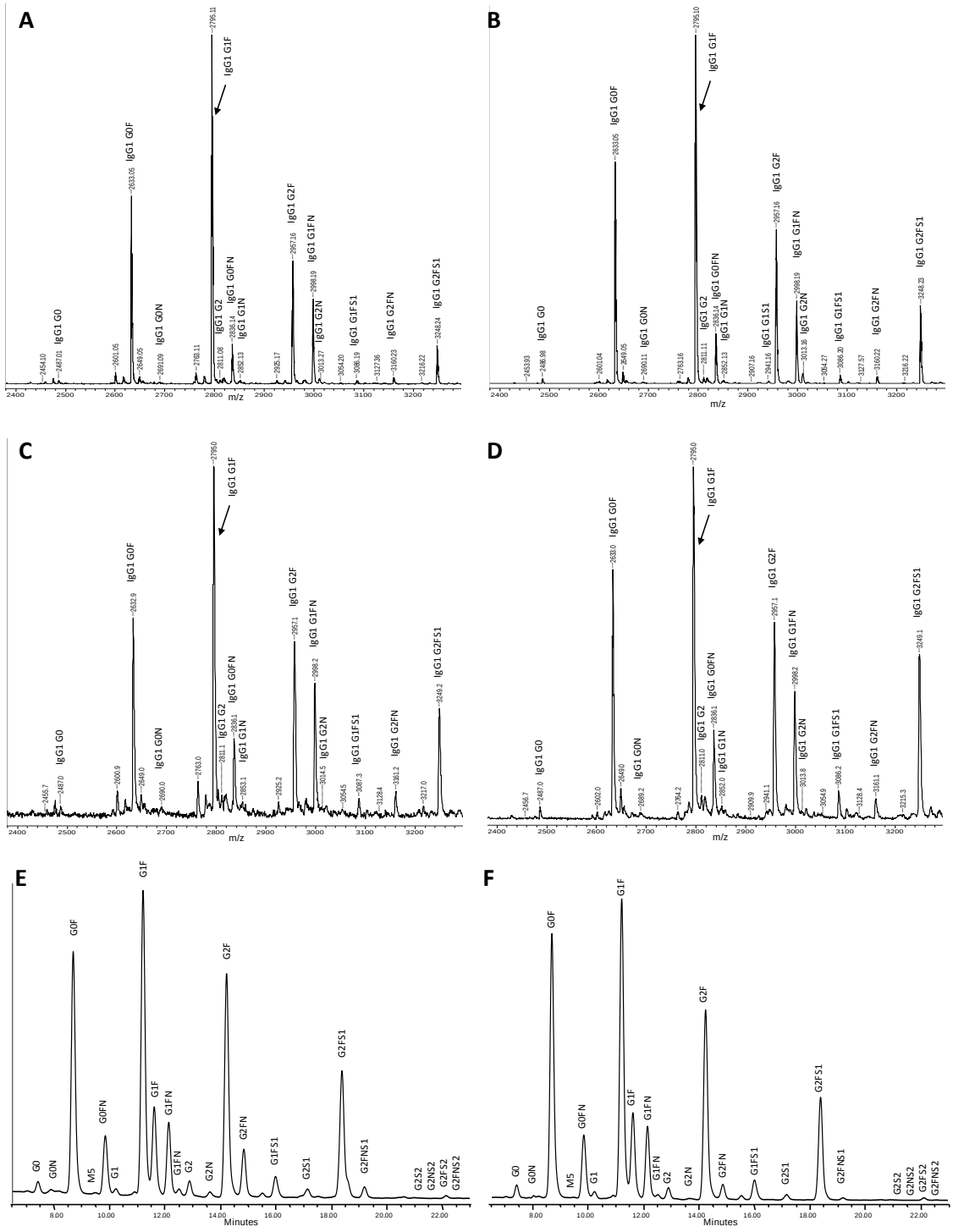
2-AB-labeled *N*-glycans were analyzed by HILIC as described in Experimental section. 2-AA-labeled glycans were prepared and analyzed as follows:

***N*-glycan release and 2-AA labeling of IgG standards.** To 100 µg of dried human IgG and IgG Fc standards 30 µl of 1.33% SDS was added followed by a 10 min incubation at 60°C to allow protein denaturation. Subsequently, 20 µl of a *N*-glycan release mixture was added containing 2% NP-40, 2.5x PBS and 0.5 mU PNGase-F (Roche, Manheim, Germany) followed by an overnight incubation at 37°C. To the released *N*-glycans, 25 µl labeling mixture (0.35M 2-AA in DMSO with 15% glacial acetic acid) and 25 µl reducing agent (1M 2-picoline borane in DMSO) was applied and incubated for 2h at 65°C.

Cotton HILIC microSPE of released *N*-glycans of IgG standards. Cotton HILIC microSPE was performed as described previously (Selman, M.H., *et al.*, Cotton HILIC SPE microtips for microscale

purification and enrichment of glycans and glycopeptides, *Anal. Chem*. 2011, 83(7):2492-9), with minor modifications. Briefly, 5 µl of the 2-AA labeled *N*-glycans from the human IgG and IgG Fc standards was diluted with ACN to a final ACN concentration of 84%. *N*-glycans were adsorbed to the cotton HILIC microSPE by aspirating and dispensing the sample 20x. The *N*-glycans were washed 3x with 84% ACN containing 1% TFA, and eluted directly onto an AnchorChip MALDI target (Bruker Daltonics, Bremen, Germany) with 2 µl ultrapure water. Spotted samples were allowed to dry, overlaid with 1 µL of 5 mg/mL 2,5-dihidroxybenzoic acid (DHB; Bruker-Daltonics) in 50 % acetonitrile and allowed to dry in a controlled manner underneath a pierced cap containing 5 holes of ca. 5 mm.

MALDI-TOF-MS. 2-AA labeled *N*-glycans of the IgG and IgG Fc standards were analyzed on an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) which was operated in the negative-ion linear mode). Ions between *m/z* 1000 and 4500 were recorded. To allow homogeneous spot sampling a random walk laser movement with 50 laser shots per raster spot was applied and each sum mass spectrum was generated by accumulation of 4000 laser shots. Mass spectra were internally calibrated using a list of known 2-AA labeled *N*-glycans. Data processing and evaluation were performed with FlexAnalysis Software (Bruker Daltonics) and Microsoft Excel, respectively. The data were baseline subtracted and the intensities of a defined set of 2-AA labeled N-glycans (47 plasma N-glycans; Supplementary Table 4) were automatically determined for each spectrum.



Supplementary Figure 6. Reflectron-negative (A, B) and linear-negative (C, D) ion mode MALDI-TOF-MS spectra of tryptic glycopeptides, and HILIC chromatograms of 2-AB-labeled *N*-glycan species (E, F). IgG1 standard (A, C, E) and IgG Fc standard (B, D, F) were used for the analyses. Glycan species are given as in Table 1.

2-AB-labeled *N*-glycans were analyzed by HILIC as described in the Experimental section. *N*-glycopeptides were prepared and analyzed as follows:

Trypsin digestion of IgG standards. Twenty µg of a human IgG1 and IgG Fc standard (Athens Research & Technology, Athens, GA) was digested overnight at 37°C with 200 ng sequencing grade trypsin (Promega, Madison, WI).

Reverse-phase solid phase extraction (RP-SPE) of glycopeptides. *N*-glycopeptides were purified and desalted as described in the Experimental section.

MALDI-TOF-MS. *N*-glycopeptides of the IgG1 and IgG Fc standards were spotted onto MTP 384 polished steel target plate (Bruker Daltonics), overlaid with 1 µL of 5 mg/mL 4-chloro-α-cyanocinnamic acid (Cl-CCA; 95% purity; Bionet Research) in 50 % acetonitrile and allowed to dry at room temperature. N-glyopeptides were analyzed on an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics) which was operated in the negative-ion reflectron and leniear mode. Ions between *m/z* 1000 and 4500 were recorded. To allow homogeneous spot sampling a random walk laser movement with 50 laser shots per raster spot was applied and each sum mass spectrum was generated by accumulation of 2000 laser shots. Mass spectra were internally calibrated using a list of known glycopeptides. Data processing and evaluation were performed with FlexAnalysis Software (Bruker Daltonics) and Microsoft Excel, respectively. The data were baseline subtracted and the intensities of a defined set of glycopeptides (16 IgG1 glycoforms) were automatically defined for each spectrum.

Supplementary Table 1. Correlation coefficients of IgG glycosylation features and age stratified for sex for the IgG1 Fc, IgG2&3 Fc and the total IgG.

IgG subclass	Glycosylation feature	All ages		Ages ≤ 57		Ages > 57	
		Female	Male	Female	Male	Female	Male
		r (P)	*r (P)*	*r (P)*	*r (P)*	*r (P)*	*r (P)*
IgG1 Fc	Gal_n	**-0.66** (<0.001)	**-0.45** (<0.001)	**-0.53** (<0.001)	**-0.40** (<0.001)	**-0.21** (<0.001)	**-0.24** (<0.001)
	Bis GlcNAc_n	**0.24** (<0.001)	**0.18** (<0.001)	**0.25** (<0.001)	**0.20** (0.002)	0.04 (0.450)	0.06 (0.306)
	FGS/(F+FG+FGS)	**-0.42** (<0.001)	**-0.15** (0.001)	**-0.37** (<0.001)	-0.11 (0.088)	-0.01 (0.957)	-0.07 (0.252)
IgG2&3 Fc	Gal_n	**-0.69** (<0.001)	**-0.46** (<0.001)	**-0.55** (<0.001)	**-0.34** (<0.001)	**-0.27** (<0.001)	**-0.28** (<0.001)
	Bis GlcNAc_n	**0.13** (<0.001)	**0.12** (0.002)	**0.14** (0.001)	0.14 (0.010)	-0.06 (0.208)	0.03 (0.549)
	FGS/(F+FG+FGS)	**-0.59** (<0.001)	**-0.31** (<0.001)	**-0.47** (<0.001)	**-0.26** (<0.001)	**-0.22** (<0.001)	**-0.15** (0.008)
IgG	Gal_n	**-0.68** (<0.001)	**-0.49** (<0.001)	**-0.56** (<0.001)	**-0.37** (<0.001)	**-0.28** (<0.001)	**-0.26** (<0.001)
	Bis GlcNAc_n	**0.30** (<0.001)	**0.23** (<0.001)	**0.34** (<0.001)	**0.32** (<0.001)	0.03 (0.498)	0.07 (0.201)
	FGS/(F+FG+FGS)	**-0.57** (<0.001)	**-0.34** (<0.001)	**-0.45** (<0.001)	**-0.16** (0.005)	**-0.20** (<0.001)	**-0.16** (0.004)

Positive correlation coefficients (*r*) for age indicate increased levels with increasing age, while negative correlation coefficients indicate decreased levels with increasing age. Correlations found to be significant after Bonferroni correction for gender and glycosylation features ($P \leq 0.008$) are in bold. Gal_n - level of galactosylation in neutral glycoforms, Bis GlcNAc_n - level of bisecting *N*-acetylglucosamine in neutral glycoforms and FGS/(F+FG+FGS) - degree of sialylation of fucosylated glycoforms without bisecting *N*-acetylglucosamine.

Supplementary Table 2. Descriptives of glycosylation features in females and males with statistical significance (*P*) of sex differences and differences between age groups for the IgG1 Fc, IgG2&3 Fc and the total IgG.

IgG subclass	Glycosylation feature	Ages ≤ 57			Ages > 57			Differences between age groups (*P*)	
		Female	Male	Sex differences (*P*)	Female	Male	Sex differences (*P*)		
		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Female	Male
IgG1 Fc	Gal_n	41.4 (12.2)	37.5 (8.4)	**<0.001**	30.0 (8.6)	32.5 (9.3)	**<0.001**	**<0.001**	**<0.001**
	Bis GlcNAc_n	12.4 (4.7)	12.5 (4.3)	0.633	14.2 (4.9)	13.2 (4.6)	0.013	**<0.001**	**0.002**
	FGS/(F+FG+FGS)	4.4 (2.4)	3.8 (2.2)	**<0.001**	3.0 (1.9)	3.3 (2)	**0.003**	**<0.001**	0.159
IgG2&3 Fc	Gal_n	31.0 (13)	28.2 (8.7)	**<0.001**	20.0 (7.3)	22.6 (7.6)	**<0.001**	**<0.001**	**<0.001**
	Bis GlcNAc_n	8.7 (3.5)	8.5 (3.1)	0.028	9.5 (3.7)	8.7 (3.2)	**0.001**	**<0.001**	0.105
	FGS/(F+FG+FGS)	3.9 (2.8)	3.5 (2.0)	**0.002**	2.2 (1.4)	2.7 (1.6)	**<0.001**	**<0.001**	**0.004**
IgG	Gal_n	45.9 (10.9)	43.2 (7.4)	**<0.001**	35.9 (7.7)	38.4 (6.8)	**<0.001**	**<0.001**	**<0.001**
	Bis GlcNAc_n	17.0 (3.4)	16.8 (3.7)	0.139	18.5 (3.5)	17.6 (2.9)	**<0.001**	**<0.001**	0.008
	FGS/(F+FG+FGS)	22.7 (5.9)	21.4 (4.6)	**<0.001**	18.0 (4.5)	19.0 (4.9)	**<0.001**	**<0.001**	**<0.001**

P-values of sex differences ($P \leq 0.006$) and differences between age groups ($P \leq 0.006$) found to be significant after Bonferroni correction for multiple comparisons are in bold. IQR – interquartile range. Gal_n - level of galactosylation in neutral glycoforms, Bis GlcNAc_n - level of bisecting *N*-acetylglucosamine in neutral glycoforms and FGS/(F+FG+FGS) - degree of sialylation of fucosylated glycoforms without bisecting *N*-acetylglucosamine.

Supplementary Table 3. Correlation coefficients of glycosylation features for the IgG1 Fc, IgG2&3 Fc and the total IgG.

IgG subclass	Glycosylation feature	All ages		Ages ≤ 57		Ages > 57	
		Bis GlcNAc_n	FGS/(F+FG+FGS)	Bis GlcNAc_n	FGS/(F+FG+FGS)	Bis GlcNAc_n	FGS/(F+FG+FGS)
		r (P)	*r (P)*	*r (P)*	*r (P)*	*r (P)*	*r (P)*
IgG1 Fc	Gal_n	-0.63 (0.018)	**0.65** (<0.001)	-0.09 (0.013)	**0.63** (<0.001)	**0.15** (<0.001)	**0.57** (<0.001)
	Bis GlcNAc_n		**0.26** (<0.001)		**0.26** (<0.001)		**0.40** (<0.001)
IgG2&3 Fc	Gal_n	**-0.07** (0.003)	**0.80** (<0.001)	-0.04 (0.244)	**0.78** (<0.001)	0.02 (0.965)	**0.70** (<0.001)
	Bis GlcNAc_n		**0.14** (<0.001)		**0.17** (<0.001)		**0.24** (<0.001)
IgG	Gal_n	**-0.22** (<0.001)	**0.82** (<0.001)	**-0.21** (<0.001)	**0.79** (<0.001)	-0.04 (<0.278)	**0.75** (<0.001)
	Bis GlcNAc_n		**-0.19** (<0.001)		**-0.17** (<0.001)		-0.05 (<0.166)

Correlations found to be significant after Bonferroni correction ($P \leq 0.008$) are in bold. Gal_n - level of galactosylation in neutral glycoforms, Bis GlcNAc_n - level of bisecting *N*-acetylglucosamine in neutral glycoforms and FGS/(F+FG+FGS) - degree of sialylation of fucosylated glycoforms without bisecting *N*-acetylglucosamine.

Supplementary Table 4. Calculated monoisotopic *m/z* values of 2-AA labeled glycans.

Glycan species	Glycan composition	$[M-H]^-$
M5	H5N2	1354.4789
FA1	H3N3F1	1379.5106
G0	H3N4	1436.5320
M6	H6N2	1516.5317
M2A1G1S1	H3N3S1	1524.5481
G0F	H3N4F1	1582.5899
G1	H4N4	1598.5848
G0N	H3N5	1639.6114
M7	H7N2	1678.5848
M3A1G1S1	H4N3S1	1686.6009
M5A1G1	H6N3	1719.6111
G1F	H4N4F1	1744.6428
G2	H5N4	1760.6377
G0FN	H3N5F1	1785.6693
G1N	H4N5	1801.6642
FM3A1G1S1	H4N3H1S1	1832.6588
M8	H6N2	1840.6374
M4A1G1S1	H5N3S1	1848.6537
G1S1	H4N4S1	1889.6803
G2F	H5N4F1	1906.6956
G1FN	H4N5F1	1947.7221
G2N	H5N5	1963.7170
M9	H9N2	2002.6902
M5A1G1S1	H6N3S1	2010.7065
G1FS1	H4N4F1S1	2035.7382
G2S1	H5N4S1	2051.7331
G2FN	H5N5F1	2109.7750
G2FS1	H5N4F1S1	2197.7910
G2NS1	H5N5S1	2254.8125
G3F	H6N5F1	2271.8278
G2S2	H5N4S2	2342.8285
G2FNS	H5N5F1S1	2400.8704
G3NS1	H6N6S1	2416.8653
G2FS2	H5N4F1S2	2488.8864
G2NS2	H5N5S2	2545.9079
G3FS1	H6N5F1S1	2562.9232
G2FNS2	H5N5F1S2	2691.9658
G3S2	H6N5S2	2707.9607
G3FS2	H6N5F1S2	2854.0186
G3S3	H6N5S3	2999.0561
G4S2	H7N6S2	3073.0929
G3FS3	H6N5F1S3	3145.1140
G4FS2	H7N6F1S2	3219.1508
G4S3	H7N6S3	3364.1883
G4FS3	H7N6F1S3	3510.2462
G4S4	H7N6S4	3655.2837
G4FS4	H7N6F1S4	3801.3417

Supplementary Table 5. Comparison of relative abundance (%) of *N*-glycan species of IgG and IgG Fc standards measured by MALDI-TOF-MS of 2-AA labeled *N*-glycans and HILIC of 2AB labeled *N*-glycans.

Glycan species	IgG		Fc	
	MALDI-TOF-MS of 2-AA *N*-glycans	**HILIC of 2-AB *N*-glycans**	**MALDI-TOF-MS of 2-AA *N*-glycans**	**HILIC of 2-AB *N*-glycans**
G0F	18.09	20.29	18.87	21.52
G1F	25.06	28.62	26.38	32.60
G2F	17.82	16.14	17.80	16.53
G0FN	4.65	4.84	5.65	5.52
G1FN	7.88	5.62	8.41	6.55
G2FN	5.79	4.10	1.87	1.45
G1FS1	2.74	2.86	2.60	2.29
G2FS1	10.06	10.63	11.60	9.19
G2FNS1	1.78	0.99	0.35	0.22
G2FNS2	0.01	0.06	0.05	0.05
G2FS2	0.04	0.19	0.13	0.23
G2NS2	0.01	0.02	0.06	0.01
G0	0.68	0.71	0.87	0.97
G1	1.50	0.46	1.79	0.60
G2	2.21	1.79	1.42	1.07
G0N	0.20	0.60	0.21	0.32
G2N	0.50	0.49	0.18	0.14
G2S1	0.91	1.16	1.58	0.54
G2S2	0.07	0.12	0.11	0.07
M5	0.03	0.29	0.04	0.12

Glycan species are given as in Table 1.

Supplementary Table 6. Comparison of relative abundance (%) of *N*-glycan species of IgG1 and IgG Fc standards measured by MALDI-TOF-MS of *N*-glycopeptides and HILIC of 2-AB labeled *N*-glycans.

Glycan species	IgG1			Fc		
	RN ion mode MALDI-TOF-MS of glycopeptides	LN ion mode MALDI-TOF-MS of glycopeptides	HILIC of 2-AB *N*-glycans	RN ion mode MALDI-TOF-MS of glycopeptides*	LN ion mode MALDI-TOF-MS of glycopeptides*	HILIC of 2-AB *N*-glycans
G0F	22.27	18.28	18.49	22.68	19.65	21.67
G1F	41.49	32.92	30.45	35.67	27.88	32.83
G2F	14.55	16.00	17.71	15.73	15.40	16.65
G0FN	4.62	6.58	4.75	5.10	6.70	5.56
G1FN	10.01	11.98	6.25	8.43	9.84	6.60
G2FN	0.65	1.51	3.94	0.67	1.21	1.46
G1FS1	0.29	0.82	2.23	0.85	1.84	2.31
G2FS1	4.44	9.50	11.47	7.99	12.82	9.26
G0	0.31	0.06	0.80	0.48	0.55	0.98
G1	0.76	1.18	0.46	1.17	2.02	0.61
G2	0.37	1.12	1.31	0.62	1.48	1.08
G0N	0.09	0.04	0.66	0.13	0.10	0.32
G2N	0.13	0.00	0.43	0.33	0.11	0.14
G2S1	0.03	0.00	1.05	0.16	0.39	0.54

Glycan species are given as in Table 1.

* only IgG1 glycopeptides measured