

Supplemental material

Naegeli et al., <https://doi.org/10.1084/jem.20190293>

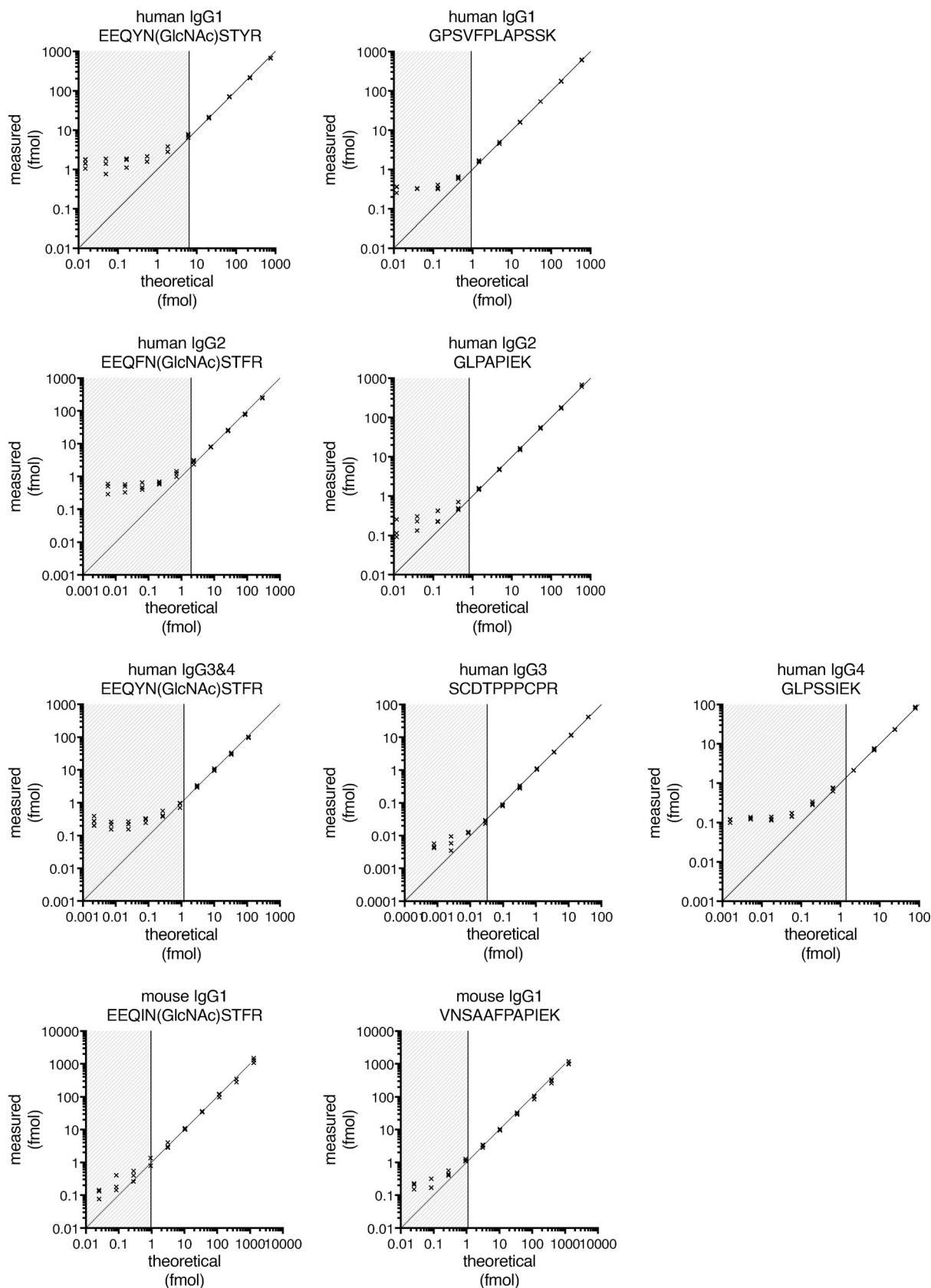


Figure S1. **IgG SRM assay calibration.** Measured amounts of each peptide are plotted against the theoretical amounts. The line and shading mark measurements below the limit of quantification.

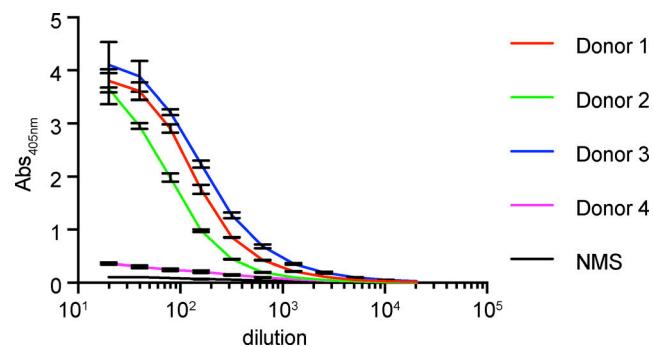


Figure S2. **Anti-M1 IgG response in donor sera for phagocytosis assays.** IgG response to M1 was determined by ELISA, and absorbance (Abs) at 405 nm in respect to serum dilution is shown. Data are presented as mean of triplicates with errors bars representing SEM. Donors 1–3 were classified as high anti-M1 response, and donor 4 as low anti-M1 response. Normal mouse serum (NMS) acted as negative control.

Table S1. SRM assay for human and mouse IgGs

| Protein | Peptide sequence | Isotope label | Precursor m/z | Fragment m/z | Collision energy | Ion |
|------------|----------------------------|---------------|---------------|--------------|------------------|-----------------|
| Human IgG1 | GPSVFPLAPSSK | Light | 593.83 | 846.47 | 26 | y8 |
| | GPSVFPLAPSSK | Light | 593.83 | 699.4 | 18 | y7 |
| | GPSVFPLAPSSK | Light | 593.83 | 489.27 | 20 | y5 |
| | GPSVFPLAPSSK | Light | 593.83 | 418.23 | 20 | y4 |
| | GPSVFPLAPSS <u>K</u> | Heavy | 597.83 | 854.49 | 26 | y8 |
| | GPSVFPLAPSS <u>K</u> | Heavy | 597.83 | 707.42 | 18 | y7 |
| | GPSVFPLAPSS <u>K</u> | Heavy | 597.83 | 497.28 | 20 | y5 |
| | GPSVFPLAPSS <u>K</u> | Heavy | 597.83 | 426.24 | 20 | y4 |
| | EEQYN[GlcNAc]STYR | Light | 696.80 | 204.09 | 21 | GlcNAc |
| | EEQYN[GlcNAc]STYR | Light | 696.80 | 843.38 | 21 | y5 |
| | EEQYN[GlcNAc]STYR | Light | 696.80 | 640.19 | 21 | y5 ^a |
| | EEQYN[GlcNAc]STYR | Light | 696.80 | 526.26 | 21 | y4 |
| | EEQYN[GlcNAc]STY <u>R</u> | Heavy | 701.80 | 204.09 | 21 | GlcNAc |
| | EEQYN[GlcNAc]STY <u>R</u> | Heavy | 701.80 | 853.39 | 21 | y5 |
| | EEQYN[GlcNAc]STY <u>R</u> | Heavy | 701.80 | 650.20 | 21 | y5 ^a |
| | EEQYN[GlcNAc]STY <u>R</u> | Heavy | 701.80 | 536.27 | 21 | y4 |
| Human IgG2 | GLPAPIEK | Light | 412.75 | 654.38 | 15 | y6 |
| | GLPAPIEK | Light | 412.75 | 557.33 | 15 | y5 |
| | GLPAPIEK | Light | 412.75 | 486.29 | 15 | y4 |
| | GLPAPIEK | Light | 412.75 | 276.16 | 15 | y2 |
| | GLPAPIE <u>K</u> | Heavy | 416.75 | 662.40 | 15 | y6 |
| | GLPAPIE <u>K</u> | Heavy | 416.75 | 565.34 | 15 | y5 |
| | GLPAPIE <u>K</u> | Heavy | 416.75 | 494.31 | 15 | y4 |
| | GLPAPIE <u>K</u> | Heavy | 416.75 | 284.17 | 15 | y2 |
| | EEQFN[GlcNAc]STFR | Light | 680.80 | 204.09 | 23 | GlcNAc |
| | EEQFN[GlcNAc]STFR | Light | 680.80 | 974.46 | 15 | y6 |
| | EEQFN[GlcNAc]STFR | Light | 680.80 | 827.39 | 15 | y5 |
| | EEQFN[GlcNAc]STFR | Light | 680.80 | 510.27 | 21 | y4 |
| | EEQFN[GlcNAc]STFR <u>R</u> | Heavy | 685.81 | 204.09 | 23 | GlcNAc |
| | EEQFN[GlcNAc]STFR <u>R</u> | Heavy | 685.81 | 984.47 | 15 | y6 |
| | EEQFN[GlcNAc]STFR <u>R</u> | Heavy | 685.81 | 837.40 | 15 | y5 |
| | EEQFN[GlcNAc]STFR <u>R</u> | Heavy | 685.81 | 520.28 | 21 | y4 |
| Human IgG3 | SCDTPPPCCR | Light | 593.75 | 824.41 | 21 | y7 |
| | SCDTPPPCCR | Light | 593.75 | 723.36 | 21 | y6 |
| | SCDTPPPCCR | Light | 593.75 | 626.31 | 21 | y5 |
| | SCDTPPPCCR | Light | 593.75 | 529.26 | 21 | y4 |
| | SCDTPPPCCR <u>R</u> | Heavy | 598.76 | 834.42 | 21 | y7 |
| | SCDTPPPCCR <u>R</u> | Heavy | 598.76 | 733.37 | 21 | y6 |
| | SCDTPPPCCR <u>R</u> | Heavy | 598.76 | 636.32 | 21 | y5 |
| | SCDTPPPCCR <u>R</u> | Heavy | 598.76 | 539.26 | 21 | y4 |

Table S1. SRM assay for human and mouse IgGs (Continued)

| Protein | Peptide sequence | Isotope label | Precursor m/z | Fragment m/z | Collision energy | Ion |
|------------------|-------------------|---------------|---------------|--------------|------------------|-----------------|
| Human IgG4 | GLPSSIEK | Light | 415.73 | 660.36 | 15 | y6 |
| | GLPSSIEK | Light | 415.73 | 563.30 | 15 | y5 |
| | GLPSSIEK | Light | 415.73 | 476.27 | 15 | y4 |
| | GLPSSIEK | Heavy | 419.74 | 668.37 | 15 | y6 |
| | GLPSSIEK | Heavy | 419.74 | 571.32 | 15 | y5 |
| | GLPSSIEK | Heavy | 419.74 | 484.29 | 15 | y4 |
| Human IgG3 and 4 | EEQYN[GlcNAc]STFR | Light | 688.80 | 204.09 | 25 | GlcNAc |
| | EEQYN[GlcNAc]STFR | Light | 688.80 | 1,118.51 | 14 | y7 |
| | EEQYN[GlcNAc]STFR | Light | 688.80 | 915.32 | 20 | y7 ^a |
| | EEQYN[GlcNAc]STFR | Light | 688.80 | 990.45 | 16 | y6 |
| | EEQYN[GlcNAc]STFR | Heavy | 693.81 | 204.09 | 25 | GlcNAc |
| | EEQYN[GlcNAc]STFR | Heavy | 693.81 | 1,128.52 | 14 | y7 |
| | EEQYN[GlcNAc]STFR | Heavy | 693.81 | 925.33 | 20 | y7 ^a |
| | EEQYN[GlcNAc]STFR | Heavy | 693.81 | 1,000.46 | 16 | y6 |
| Mouse IgG1 | VNSAAFPAPIEK | Light | 622.34 | 872.49 | 20 | y8 |
| | VNSAAFPAPIEK | Light | 622.34 | 801.45 | 16 | y7 |
| | VNSAAFPAPIEK | Light | 622.34 | 654.38 | 20 | y5 |
| | VNSAAFPAPIEK | Light | 622.34 | 486.29 | 32 | y4 |
| | VNSAAFPAPIEK | Heavy | 626.34 | 880.5 | 20 | y8 |
| | VNSAAFPAPIEK | Heavy | 626.34 | 809.46 | 16 | y7 |
| | VNSAAFPAPIEK | Heavy | 626.34 | 662.4 | 20 | y5 |
| | VNSAAFPAPIEK | Heavy | 626.34 | 494.31 | 32 | y4 |
| | EEQIN[GlcNAc]STFR | Light | 663.81 | 204.09 | 20 | GlcNAc |
| | EEQIN[GlcNAc]STFR | Light | 663.81 | 827.39 | 22 | y5 |
| | EEQIN[GlcNAc]STFR | Light | 663.81 | 624.20 | 24 | y5 ^a |
| | EEQIN[GlcNAc]STFR | Light | 663.81 | 510.27 | 24 | y4 |
| | EEQIN[GlcNAc]STFR | Heavy | 668.82 | 204.09 | 20 | GlcNAc |
| | EEQIN[GlcNAc]STFR | Heavy | 668.82 | 837.40 | 22 | y5 |
| | EEQIN[GlcNAc]STFR | Heavy | 668.82 | 634.21 | 24 | y5 ^a |
| | EEQIN[GlcNAc]STFR | Heavy | 668.82 | 520.28 | 24 | y4 |

Table S1. SRM assay for human and mouse IgGs (Continued)

| Protein | Peptide sequence | Isotope label | Precursor m/z | Fragment m/z | Collision energy | Ion |
|-------------|------------------|---------------|---------------|--------------|------------------|-----|
| RT peptides | AGGSSEPVTLADK | Light | 644.82 | 800.45 | 22 | y8 |
| | AGGSSEPVTLADK | Light | 644.82 | 604.33 | 22 | y6 |
| | VEATFGVDESANK | Light | 683.83 | 966.45 | 23 | y9 |
| | VEATFGVDESANK | Light | 683.83 | 819.38 | 23 | y8 |
| | YILAGVESNK | Light | 547.30 | 817.44 | 19 | y8 |
| | YILAGVESNK | Light | 547.30 | 633.32 | 19 | y6 |
| | TPVISGGPYYER | Light | 669.84 | 1,041.50 | 23 | y9 |
| | TPVISGGPYYER | Light | 669.84 | 928.42 | 23 | y8 |
| | TPVITGAPYYER | Light | 683.85 | 956.45 | 23 | y8 |
| | TPVITGAPYYER | Light | 683.85 | 855.40 | 23 | y7 |
| | GDLDAASYYAPVR | Light | 699.34 | 926.47 | 24 | y8 |
| | GDLDAASYYAPVR | Light | 699.34 | 855.44 | 24 | y7 |
| | TGFIIDPGGVIR | Light | 622.85 | 713.39 | 22 | y7 |
| | TGFIIDPGGVIR | Light | 622.85 | 598.37 | 22 | y6 |
| | GTFIIDPAIIVR | Light | 636.87 | 854.51 | 22 | y8 |
| | GTFIIDPAIIVR | Light | 636.87 | 626.40 | 22 | y6 |
| | ADVTPADFSEWSK | Light | 726.84 | 1,066.48 | 25 | y9 |
| | ADVTPADFSEWSK | Light | 726.84 | 387.19 | 25 | b4 |

Overview of all the SRM transitions analyzed in this study. All cysteines are carbamidomethylated. Underlined residues are heavy isotope-labeled (¹³C and ¹⁵N).

^aFragment ions that have undergone a neutral loss of the GlcNAc modification.

Table S2. SRM detection limits and conversion factors

| Protein | Peptide sequence | LLoQ (fmol) | Conversion factor |
|------------------|-------------------|-------------|-------------------|
| Human IgG1 | GPSVFPLAPSSK | 1.62 | 1.74 |
| | EEQYN[GlcNAc]STYR | 6.41 | 2.15 |
| Human IgG2 | GLPAPIEK | 1.45 | 1.76 |
| | EEQFN[GlcNAc]STFR | 1.98 | 1.23 |
| Human IgG3 | SCDTPPPCCR | 0.16 | 1.64 |
| Human IgG4 | GLPSSIEK | 2.38 | 1.72 |
| Human IgG3 and 4 | EEQYN[GlcNAc]STFR | 1.19 | 1.74 |
| Mouse IgG1 | VNSAAFPAPIEK | 1.11 | 1.29 |
| | EEQIN[GlcNAc]STFR | 0.96 | 3.47 |

Lower limits of quantification (LLoQs) and conversion factors are given for all the peptides analyzed by SRM in this study. The conversion factor denotes the ratio between the measured amounts and the amounts of each peptide spiked in.

Table S3 is provided online as a separate Excel file and lists patient and bacterial isolate information.