Short half-life of human IgG3 due to competition for FcRn-mediated transport reveals its therapeutic potential.

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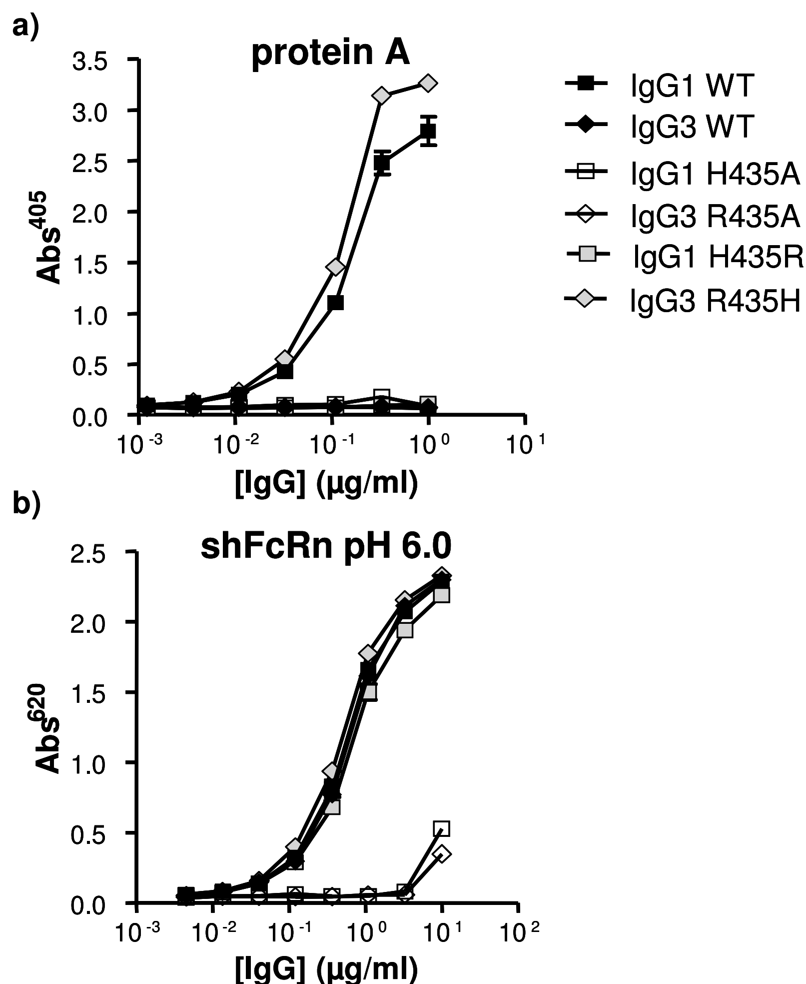
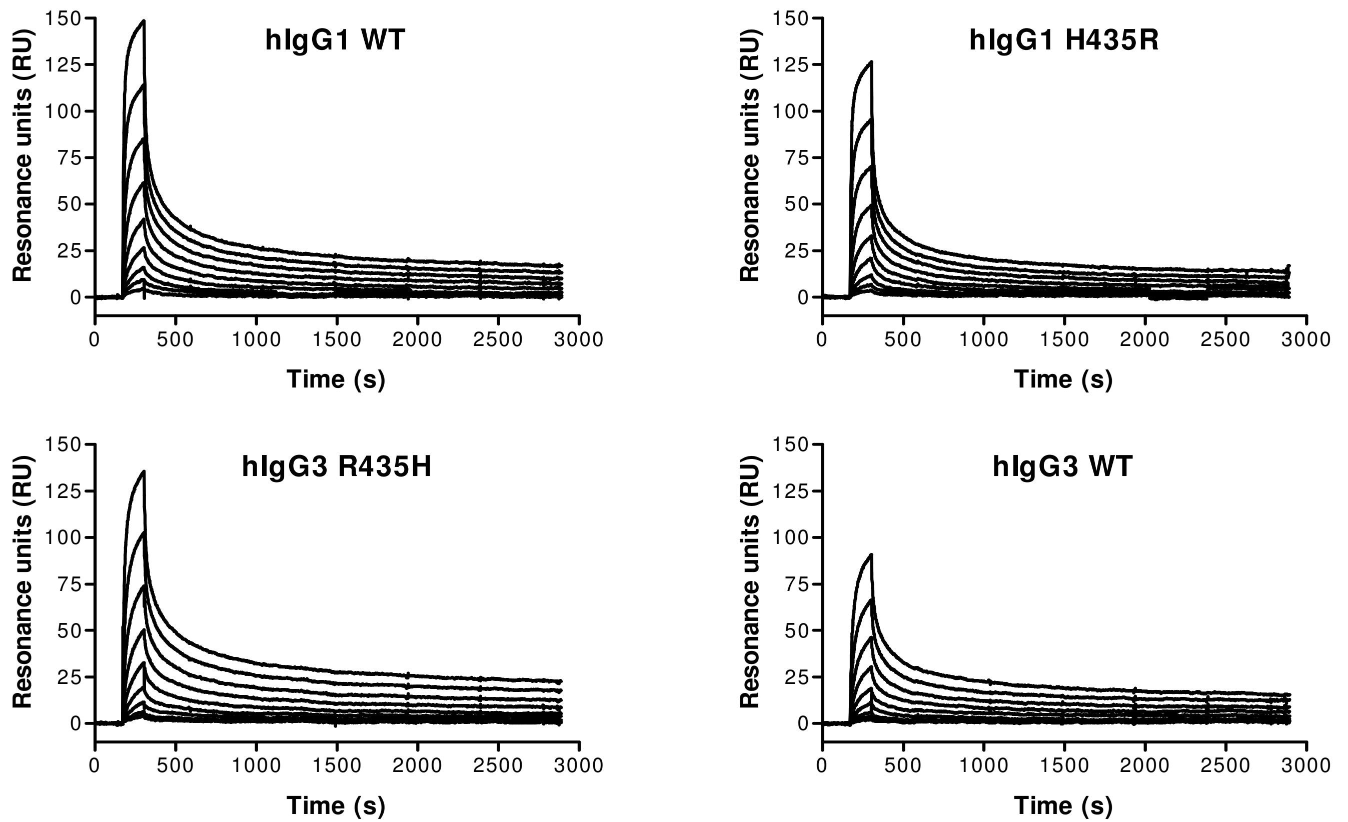


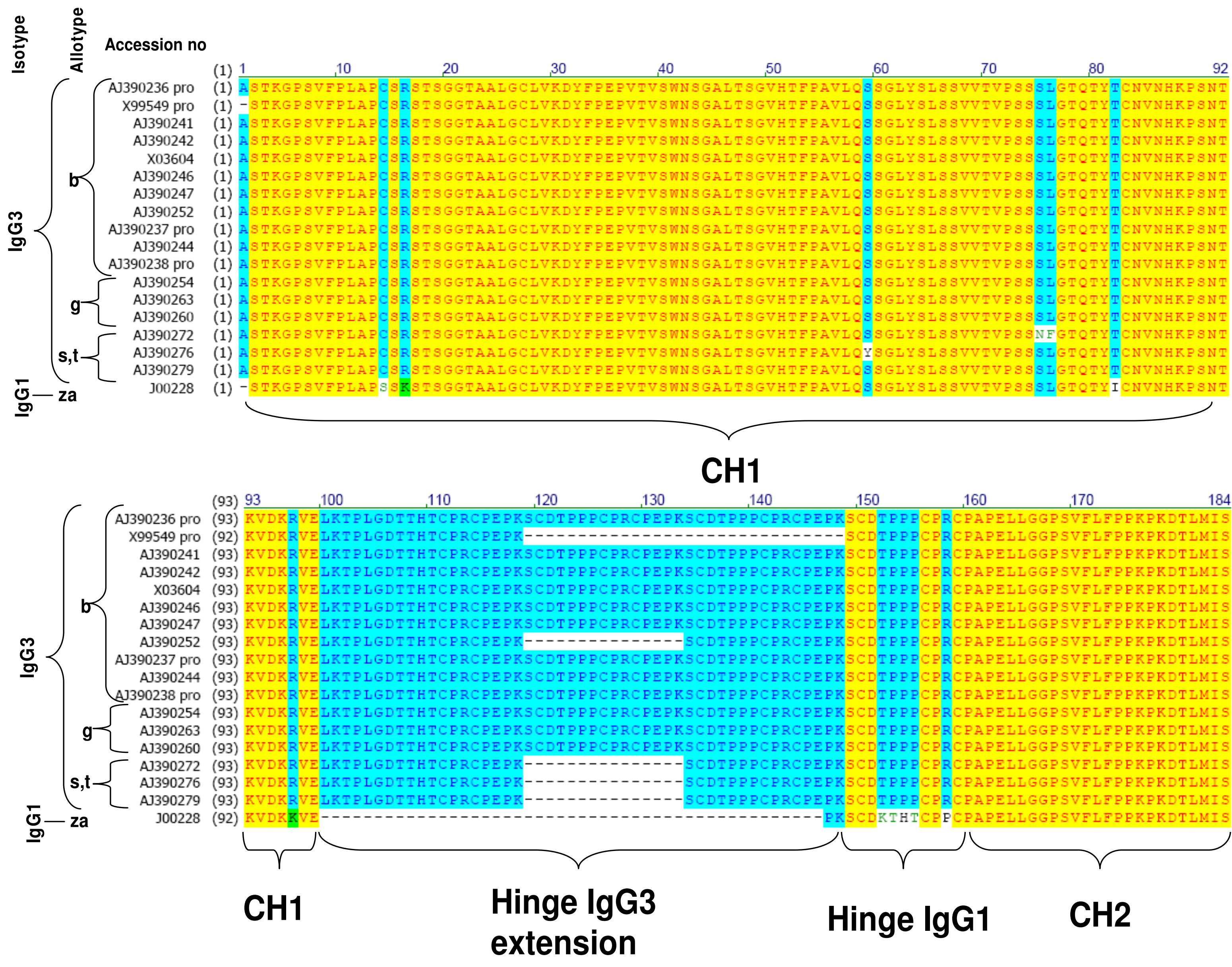
Supplementary Figure S1: Binding of the IgG variants to Protein A and shFcRn. Binding of Protein A to serial dilutions of the IgG variants (A) and shFcRn at pH 6.0 (B). Maxisorp ELISA plates (Nunc, Denmark) were coated with 10.0 µg/ml pneumococcal polysaccharide 6B (ATCC, Manassas, VA) overnight at 4°C. They were then blocked with 4% skimmed milk (Acumedia) for 1 h and washed four times with PBS/0.05% Tween 20 (PBS/T) pH 7.4 (as were all subsequent washing steps or at pH 6.0 as indicated). Serial dilutions of the IgG variants, diluted in PBS/T with 4% skimmed milk at the indicated pH, were added to the wells and incubated for 1.5 h at room temperature. After washing, 0.5 µg/ml GST-fused shFcRn (ref. 42 and 54) (pre-incubated with an HRP conjugated polyclonal anti-GST from goat (GE Healthcare) in 4% skimmed milk PBS/T), or alkaline phosphatese conjugated protein A (Sigma-Aldrich) was incubated at pH 7.4 or at pH 6.0 for 1.5 h at room temperature, then washed. 100 µl of the substrate TMB (Calbiochem) or 1 mg/mL p-nitrophenyl phosphate (NPP) in 10% diethanolamine buffer, pH 9.8 was added to each well, and the absorbance at 620 nm or 405 nm, respectively for shFcRn and Protein A, measured using a Sunrise TECAN spectrophotometer (TECAN, Maennedorf, Switzerland). The data represent mean and standard deviation (not visible when less than the size of the symbol) from 3 independent experiments.



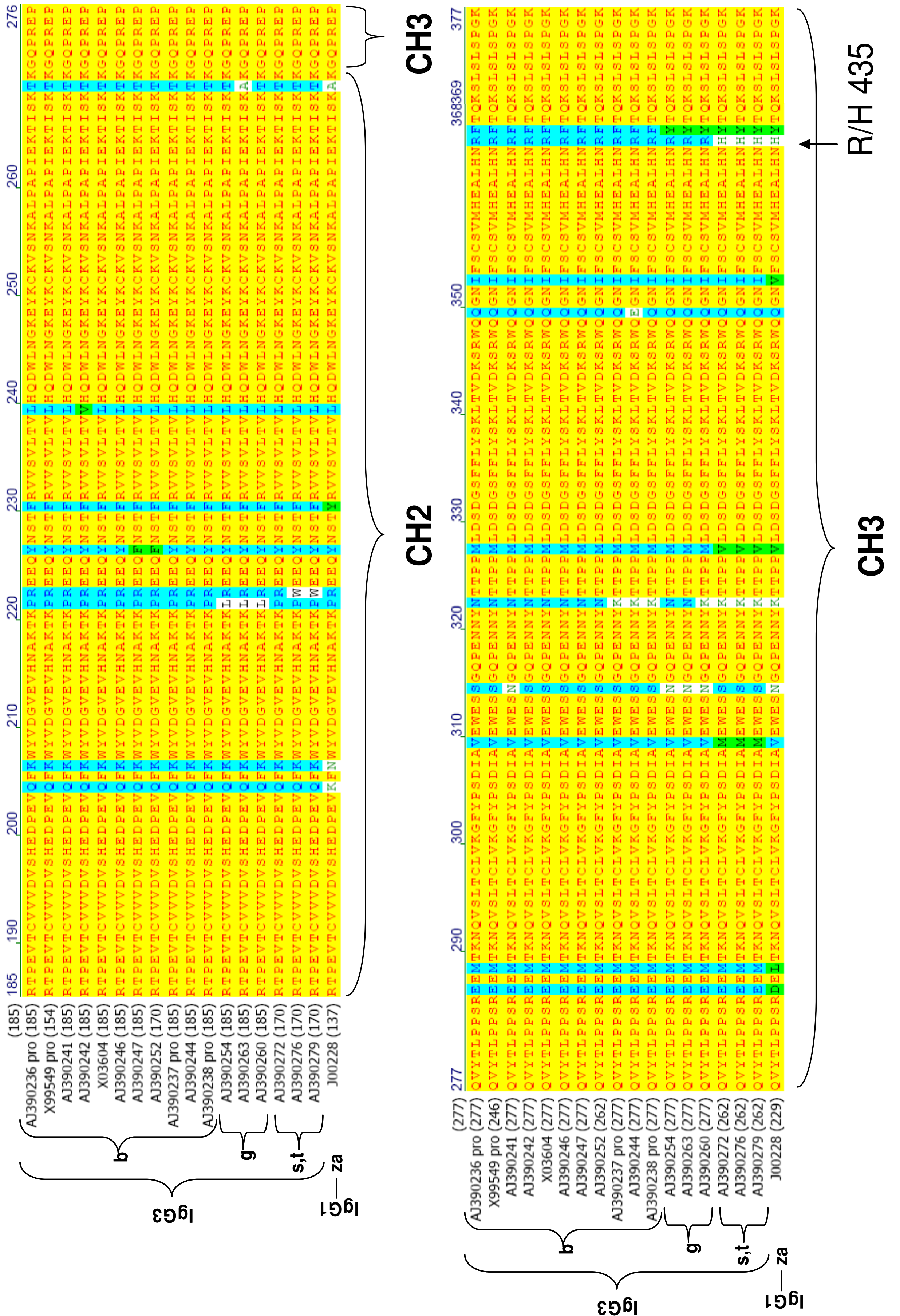
Supplementary Figure S2: Binding of the IgG variants to shFcRn-GST.
shFcRn-GST (~1000 RU) was immobilized to a CM5 sensor chip by amine coupling, and IgG variants were injected at different concentrations (500-2 nM) with a constant flow rate of 60 µl/min at 25°C pH 6.0. HBS-P buffer (GE Healthcare) with pH 7.4 was used to regenerate the flow cells at the end of each dissociation phase. Kinetic constants were calculated from the resulting sensorgrams using the bivalent binding model provided by the BIAevaluation 4.1 software (Table 1).The data are representative of three independent experiments.

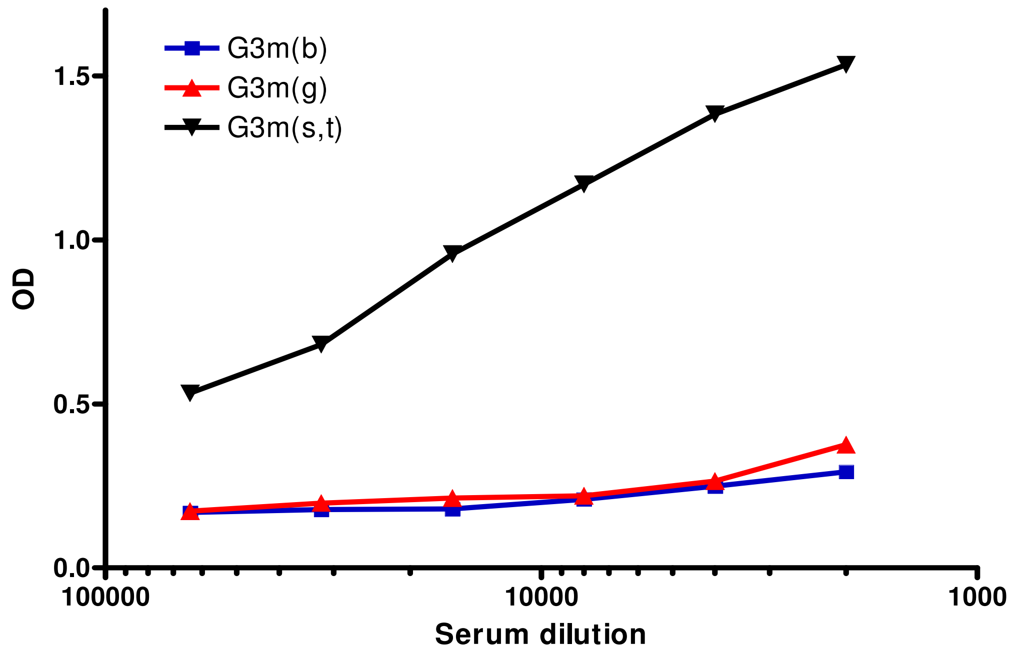
Supplementary Figure S3: The amino acid sequences of all described IgG3 variants. The sequences of IgG3 allotypes and their simplified nomenclature as deduced from Refs 55 and 56 compared to IgG1 (allotype G1m(za)). The definition of the G3m(b) is broadly defined here, but strictly applies only to AJ390247 and AJ390252 as they contain an Phe at position 296 (Ref 55). The hinge regions of the different IgG3 variants varies from two to four exons, three of which are exactly repeated, resulting in a 30-60 amino acid hinge. Numbering of these sequences is according to their occurrence in IgG from the CH1 domain. The standard numbering of amino acids discussed in the text is also indicated where appropriate. The recombinant IgG3 used in this paper (accession number AF237585) corresponds to AF390263 (G3m(g)).

Supplementary Figure. S3

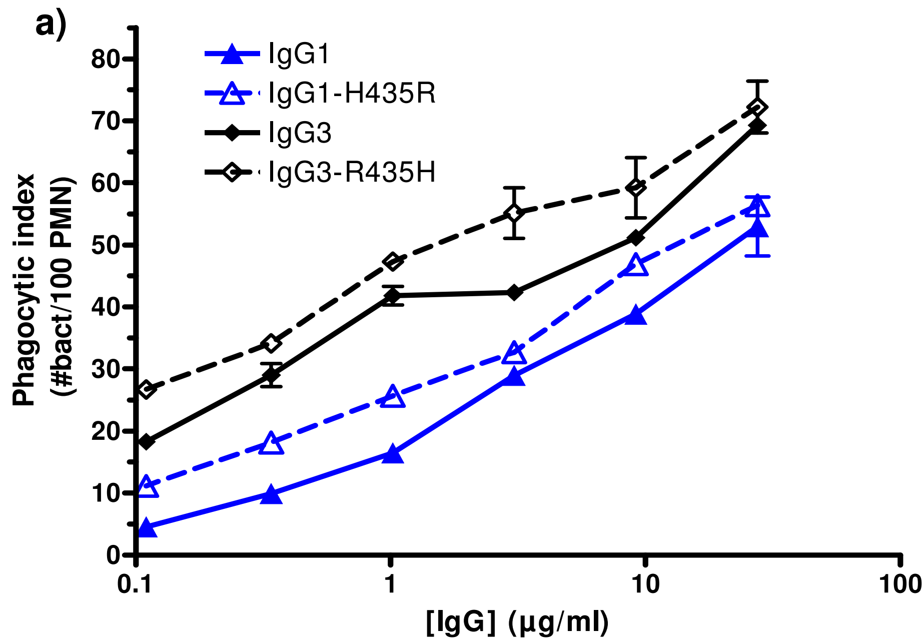


Supplementary Figure. S3 continued.





Supplementary Figure S4: Specificity of the monoclonal 1.5A10 anti G3m(s,t). Serum from homozygous G3m(b) or G3m(g) or heterozygousG3m(s,t) individuals were serially diluted in a 1.5A10 / IgG3-HRP Sandwich ELISA (see materials and methods). The data represent mean and standard deviation from 3 independent experiments.



b)

IgG (µg/ml)	IgG1 vs IgG1-H435R	IgG1 vs IgG3	IgG1 vs IgG3-R435H	IgG1-H435R vs IgG3	IgG1-H435R vs IgG3-R435H	IgG3 vs IgG3-R435H
27.50	NS	P<0.001	P<0.001	P<0.01	P<0.001	NS
9.170	NS	P<0.01	P<0.001	NS	P<0.01	NS
3.060	NS	P<0.001	P<0.001	P<0.05	P<0.001	P<0.01
1.020	P < 0.05	P<0.001	P<0.001	P<0.001	P<0.001	NS
0.3400	NS	P<0.001	P<0.001	P<0.01	P<0.001	NS
0.1100	NS	P<0.001	P<0.001	NS	P<0.01	NS

Supplementary Figure S5: Phagocytosis of *Streptococcus pneumoniae* serotype 6B opsonized with V-gene matched IgG1 and IgG3 demonstrates the superior effector functions of IgG3. A) Swapping the amino acid in position 435 (histidine in IgG1 and ariginine in IgG3) has no significant affect on the phagocytosis efficiency. Ingested bacteria were quantified by FACS as described in ref. 15. The data represent mean and standard deviation from 3 independent experiments. B) The Level of significance as calculated by calculated by two-way Anova with the Bonferroni post-tests.

Supplementary Table S1: Kinetics of the IgG interactions with shFcRn

Analyte	ka1 (10^4/Ms)	kd1(10^{-3}/s)	KD (nM)
IgG1 WT[b]	4.26±0.60	8.61±0.11	201.9
IgG3 WT[b]	1.87±0.10	7.41±0.16	395.7
IgG1 H435R[b]	3.36±0.24	10.08±1.91	321.4
IgG3 R435H[b]	2.35±0.12	7.76±0.20	330.2

a: Dilutions of IgG variants were injected over immobilized shFcRn as shown in Supplementary Fig. S2.b: The kinetic rate constants were obtained using the bivalent ligand model supplied by the BIAevaluation 4.1 software. The kinetic values represent the average of triplicates.

Supplementary References

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