






Supplemental table 1¹

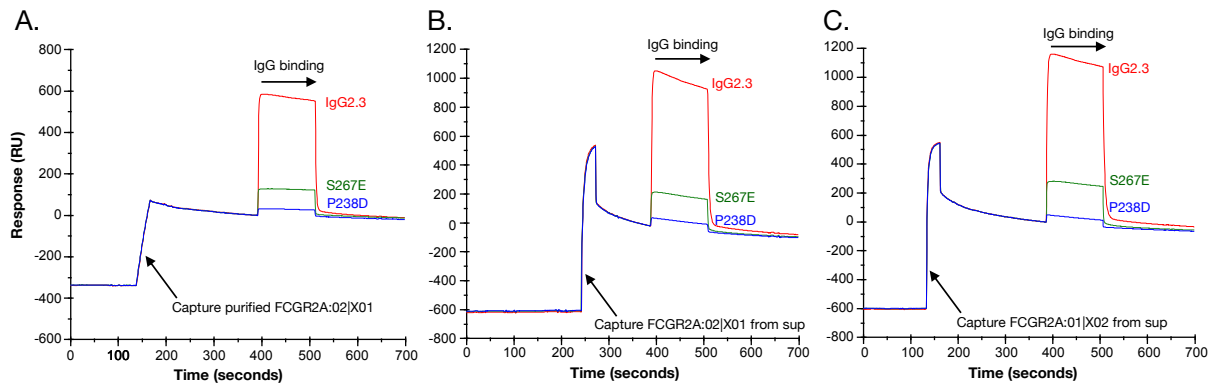
Primers used for PacBio allele discovery				
Gene	Primer name	Primer sequence	Predicted size	
FCGR1A	FCGR1.3_F	[Barcode] + CCACCAGCTTGGAGACAAC	1181 bp	
	FCGR1.3_R	[Barcode] + GACGGTCCAGATCGATGG		
FCGR2A	FCGR2A.3_F	[Barcode] + GACTGGACGTTGGCACAGT	1010 bp	
	FCGR2A.3_R	[Barcode] + TTGTCATCCACTCAGCAAGC		
FCGR2B	FCGR2B.3_F	[Barcode] + GAGAAGGCTGTGACTGCTG	960 bp	
	FCGR2B.3_R	[Barcode] + AAATCCCAAGGCAAGACAATG		
FCGR3A	FCGR3.3_F	[Barcode] + GAACCTGGTGGGTGACAGAG	864 bp	
	FCGR3.3_R	[Barcode] + GGGTTGCAAATCCAGAGAAA		
Primers used for MiSeq genotyping assay				
Gene	Exon	Primer name	Primer sequence	Predicted size
FCGR1A	4	CS1_FCGR1_Ex4_F1	[CS1 Adapter] + TTTTGGGTTCACCTTTTTTCAGA	293 bp
		CS2_FCGR1_Ex4_R1	[CS2 Adapter] + TGACTGTTCTATACGATACCTTTC	
FCGR2A	3	CS1_FCGR2A_Ex3_Fv3	[CS1 Adapter] + CCCTTCAGGGTTATTTATTCACA	325 bp
		CS2_FCGR2A_Ex3_R4	[CS2 Adapter] + AGGGCCTTCCTCCACTGAC	
FCGR2A	4	CS1_FCGR2A_Ex4_Fv2	[CS1 Adapter] + AAAATGAGCTGAAAACTCTTGGA	348 bp
		CS2_FCGR2A_Ex4_Rv2	[CS2 Adapter] + CCCTACATCTTGGCAGATTCC	
FCGR2B	4	CS1_FCGR2B_Ex4_V1_F	[CS1 Adapter] + GACCTCCCGGGTCCTCT	317 bp
		CS2_FCGR2B_Ex4_V1_R	[CS2 Adapter] + TACATCTTGGCAGATTCCCC	
FCGR3A	3	CS1_FCGR3_Exon3_F2	[CS1 Adapter] + CTCACCCCATATTCTCTTGG	347 bp
		CS2_FCGR3_Exon3_R2	[CS2 Adapter] + TTTGAGCACCTTTGTTTCA	

¹ Primers used for PacBio allele discovery and MiSeq genotyping assay.

Human NC000001	T	A	A	T	G	T	C	T	G	T	C	T	T	C	C	C	T	A	G	T	G	C	
Rhesus NC_007858
Mafa-FCGR2A:01	.	.	.	G																			
Mafa-FCGR2A:02	
Mafa-FCGR2A:03	
Mafa-FCGR2A:04	.	.	.	G																			
Mafa-FCGR2A:05	.	.	.	G																			
Mafa-FCGR2A:06	.	.	.	G																			
Mafa-FCGR2A:07	.	.	.	G																			

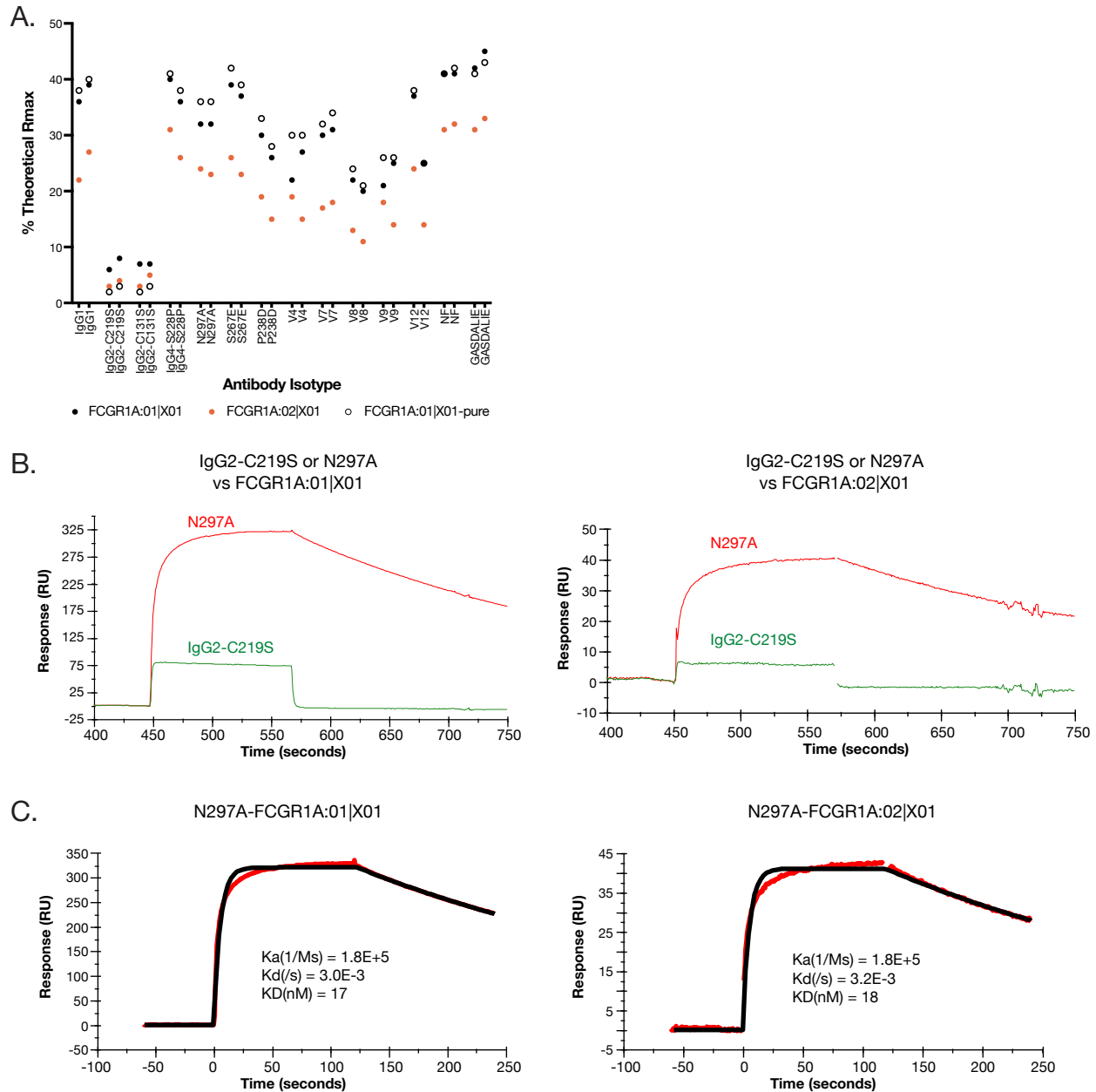
Supplemental figure 1

The presence of an early splice site in FCGR2A:01, 04, 05, 06 and 07 causes exon 5 (colored bars) to be extended compared to FCGR2A:02 and 03, which more closely resemble the human and rhesus FCGR2A alleles.



Supplemental figure 2

Example SPR sensorgram screening assay data for representative IgG binding to (A) purified FCGR2A:02|X01, (B) FCGR2A:02|X01 captured from supernatant, and (C) FCGR2A:01|X02 captured from supernatant.



Supplemental figure 3

FCGR1A binding to fourteen different IgG isotypes, with two different monoclonal antibodies per isotype, was tested by SPR. In the SPR screening data, similar trends in %Rmax were observed for different IgG isotypes binding to the two FCGR1A variants (A). In addition, the two variants showed similar binding kinetics in both the raw SPR sensorgram data (example data in B) and fitted data (example data in C; data, in red, was fitted to a 1:1 Langmuir binding model, in black). The expression and purification of all FCGR2A, FCGR2B, and FCGR3A variants were typical for well-behaved his-tagged proteins, with multi-mg/L yields and monomeric products with low levels of high molecular weight aggregates or degradation products as measured by size-exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALS) detector. In addition, the SPR data for antibody binding was similar for these purified FCGRs and their respective unpurified supernatants (see main text). In contrast, FCGR1A was challenging to produce, had low titers (especially FCGR1A:02|X01), and the purified protein showed high levels of aggregate (>20%) even after preparative size exclusion chromatography purification. Moreover, purified FCGR1A demonstrated poor capture to the anti-His sensor chip surface, and had very low fractional activity as determined by Biacore calibration-free concentration analysis (CFCA). Despite these unique challenges with the FCGR1A extracellular domain protein constructs, the SPR data suggest that the fraction of protein that was active had similar binding activity between the two FCGR1A variants.