Plasmonic Fluor-Enhanced Antigen Arrays for High-Throughput, Serological Studies of SARS-CoV-2

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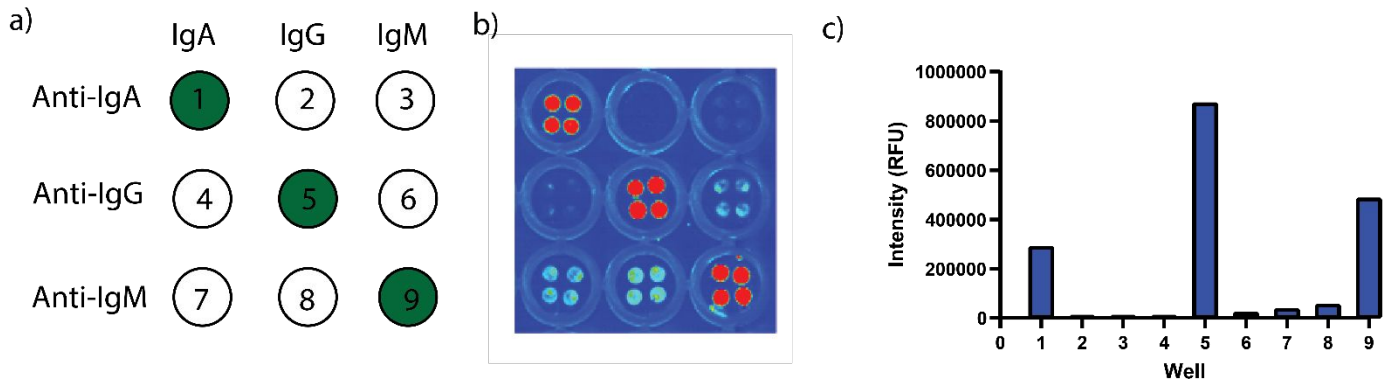
a)

PC	PC	PC	PC	SPIKE RBD	SPIKE S2	IgG
N FL	N FL	N FL	N FL	SPIKE RBD	SPIKE S2	IgG
N NTD	N NTD	N NTD	N NTD	SPIKE RBD	SPIKE S2	IgG
Orf3b	Orf3b	Orf3b	Orf3b	SPIKE RBD	SPIKE S2	IgG
Orf8	Orf8	Orf8	Orf8	IgM	IgM	IgM
SPIKE S1	SPIKE S1	SPIKE S1	SPIKE S1	IgM	EMPTY	IgA
SPIKE NTD	SPIKE NTD	SPIKE NTD	SPIKE NTD	IgA	IgA	IgA

b) c) d)

Figure S1. Specificity of IgG, IgA, and IgM biotinylated-secondary antibodies used in the 7 x 7 array. 7 x 7 array of antigens were exposed to a buffer solution, and subsequently exposed to biotinylated secondary antibodies against (a) IgG, (b) IgA, and (c) IgM. In each of the assays, only the positive controls and the respective immunoglobulin controls reacted with the secondary antibodies.

Figure S2. Cross-reactivity between secondary antibodies against IgA, IgG, and IgM. (a) Schematic demonstrating the spotted antibodies in each well (columns) and secondary antibody-plasmonic fluor conjugate used for readout. Green circles denote on-target interactions. (b) False-color images obtained for each of the wells, with red corresponding to high signal intensity, and dark-blue corresponding to no signal. (c) Intensity values for each of the wells.

Table S1. Related to Figure 3. Intensity readouts for each antigen combination. Average intensities and standard error of the mean (SEM) of (a) positive patient plasma and (b) negative patient plasma.

(a)

	IgM		IgG		IgA	
Antigen	Average (RFU)	SEM (RFU)	Average (RFU)	SEM (RFU)	Average (RFU)	SEM (RFU)
N FL	19,229	468	81,734	1,578	69,921	15,172
N NTD	6,731	311	146,601	2,912	130,289	15,242
ORF3b	3,535	200	18,146	386	34,345	5,561
ORF8	3,756	249	18,965	421	34,406	5,939
Spike S1	5,698	347	88,383	2,031	48,033	1,671
Spike NTD	11,071	354	6,852	204	20,985	3,195
Spike RBD	14,811	365	75,727	1,346	42,781	3,011
Spike S2	7,360	223	130,448	3,014	68,739	2,420

(b)

	IgM		IgG		IgA	
Antigen	Average (RFU)	SEM (RFU)	Average (RFU)	SEM (RFU)	Average (RFU)	SEM (RFU)
N FL	8,379	440	8,282	182	1,139	370
N NTD	623	43	2,225	71	2,977	391
ORF3b	693	43	4,595	137	722	156
ORF8	868	48	5,182	141	759	150
Spike S1	273	32	851	227	1,217	65
Spike NTD	3,699	287	1,965	95	758	103
Spike RBD	1,459	110	973	57	1,227	146
Spike S2	357	38	994	66	2,196	86

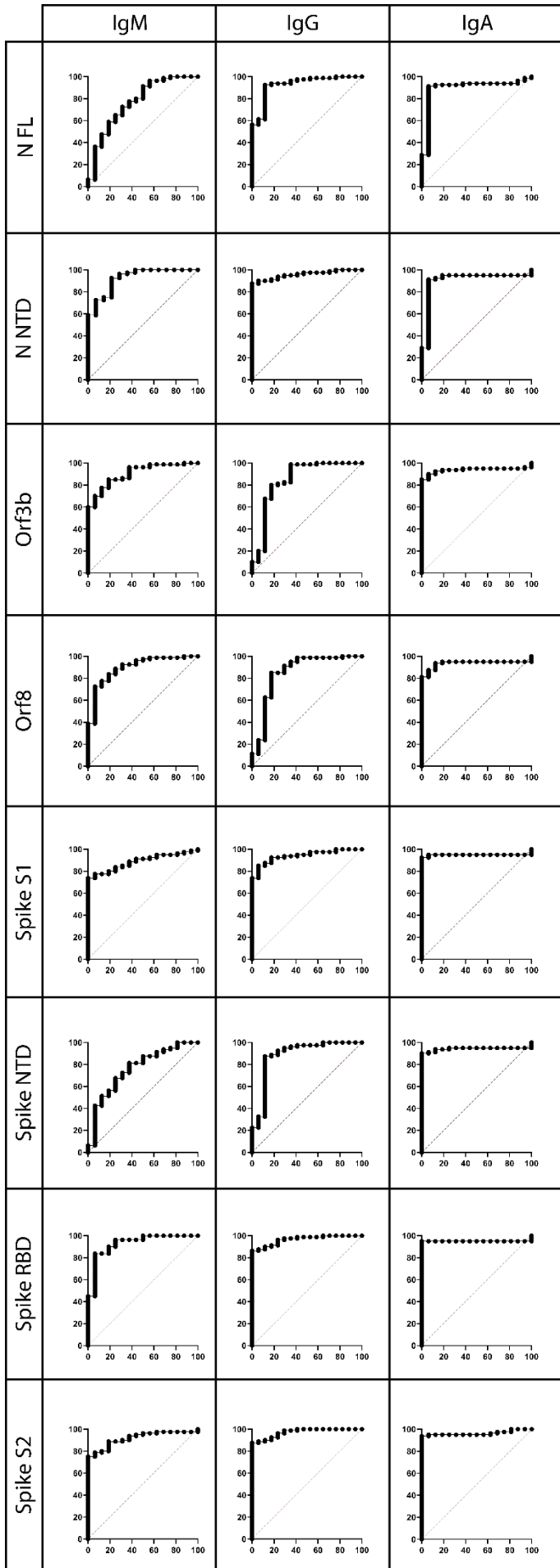|	IgM	IgG	IgA
N FL			
N NTD			
Orf3b			
Orf8			
Spike S1			
Spike NTD			
Spike RBD			
Spike S2			

Figure S3. Related to Table 1. Receiver-Operator Curves (ROC) for each antigen and immunoglobulin response. Each column corresponds to IgM, IgG, and IgA (left to right). Each row represents the serological response towards a specific antigen. Y-axis corresponds to sensitivity (%), and the x-axis corresponds to 1 – specificity (%).
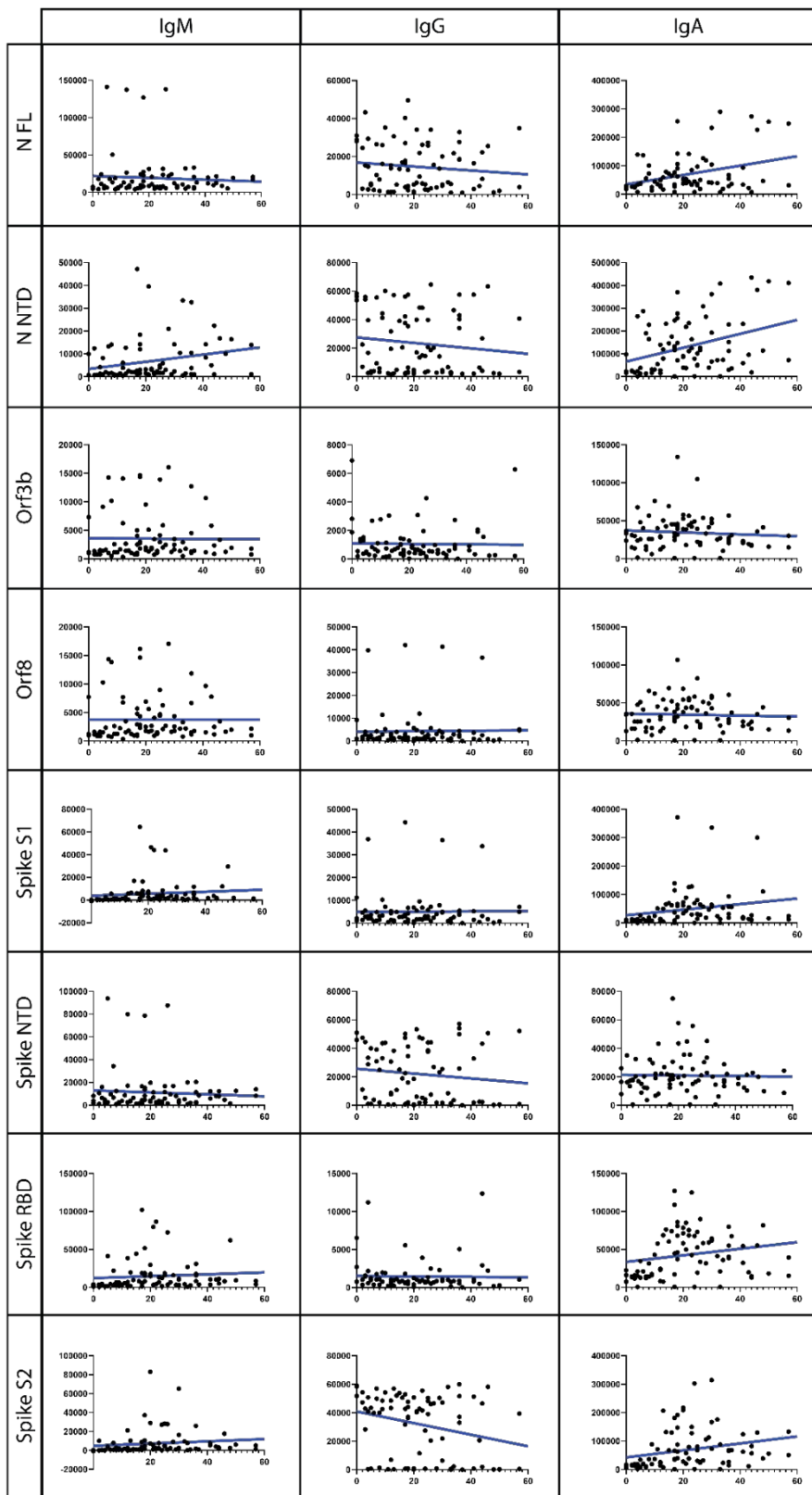
Figure S4. Related to Figure 4. Serological responses for each antigen as a function of time. Vertical columns represent the IgM, IgG, and IgA (left to right) responses for each antigen for 80 separate, positive samples from 0 to 60 days post symptom onset. Each horizontal row represents a specific SARS-CoV-2 antigen. The blue line represents the

linear correlation for each data set, reflected in **Table S2**. Y-axis for each graph is intensity (RFU) and the x-axis days post symptom onset, ranging from 0 to 60.

Table S2. Related to Figure S4. Linear correlation value (R^2), slope, and y-intercepts for linear fits for each antigen and immunoglobulin combination.

Antigen	R^2	Slope	Y-Intercept
IgM			
N FL	0.0038	1,642	34,525
N NTD	0.0573	3,054	64,851
Orf3b	0.0001	-130	37,139
Orf8	0.0000	-65	35,804
Spike S1	0.0115	985	29,936
Spike NTD	0.0042	-22	21,451
Spike RBD	0.0075	433	33,515
Spike S2	0.0151	1,229	42,416
IgG			
N FL	0.0141	-104	16,774
N NTD	0.0154	-191	27,493
Orf3b	0.0003	-2	1,091
Orf8	0.0002	9	4,109
Spike S1	0.0001	6	4,911
Spike NTD	0.0003	-3	1,506
Spike RBD	0.0148	-170	25,683
Spike S2	0.0690	-407	40,846
IgA			
N FL	0.1202	1,652	34,525
N NTD	0.1344	3,054	64,851
Orf3b	0.0072	-130	37,139
Orf8	0.0022	-65	35,804
Spike S1	0.0434	984	26,936
Spike NTD	0.0005	-21	21,451
Spike RBD	0.0418	433	33,515
Spike S2	0.0641	1,229	42,416

Table S3. Related to Figures 4, 5, S3, S4, and Table S1. Patient demographics of positive patient samples used in this study.

| Patient	Gender	Age	Days Post-Symptom Onset			
			Sample #1	Sample #2	Sample #3	Sample #4
1	Female	74	33	44	50	57
2	Male	60	4	17	24	33
3	Male	42	8	18	28	41
4	Male	56	9	17	26	34
5	Male	60	3	12	22	30
6	Male	79	5	20	24	30
7	Female	81	5	12	18	26
8	Male	64	0	13	25	43
9	Male	72	0	9	17	23
10	Male	61	12	20	25	36
11	Male	68	4	18	30	46
12	Male	88	4	10	18	25
13	Female	88	11	21	29	37
14	Male	57	2	8	15	22
15	Female	58	3	20	44	57
16	Female	70	0	16	36	48
17	Female	73	2	13	32	41
18	Female	69	6	14	18	27
19	Male	67	7	17	23	36
20	Male	41	7	17	21	36
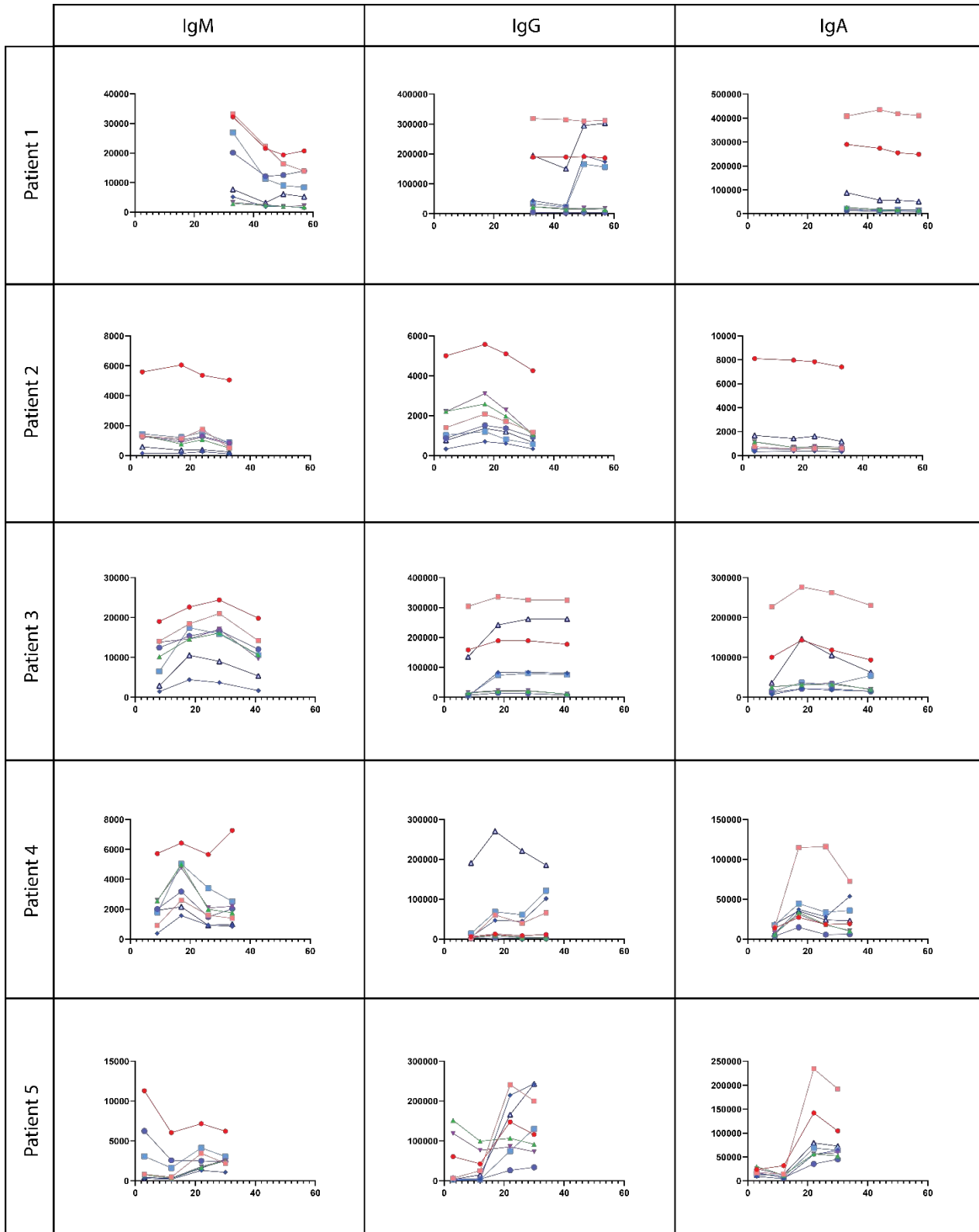
Figure S5. Serological response for 8 SARS-CoV-2 antigens for patients longitudinally. Serological response to 8 separate antigens for IgM, IgG, and IgA assessed during a hospital stay. Each individual row represents a single patient, with each column representing the IgM, IgG, or IgA response (from left to right). Patients #1 through #5.
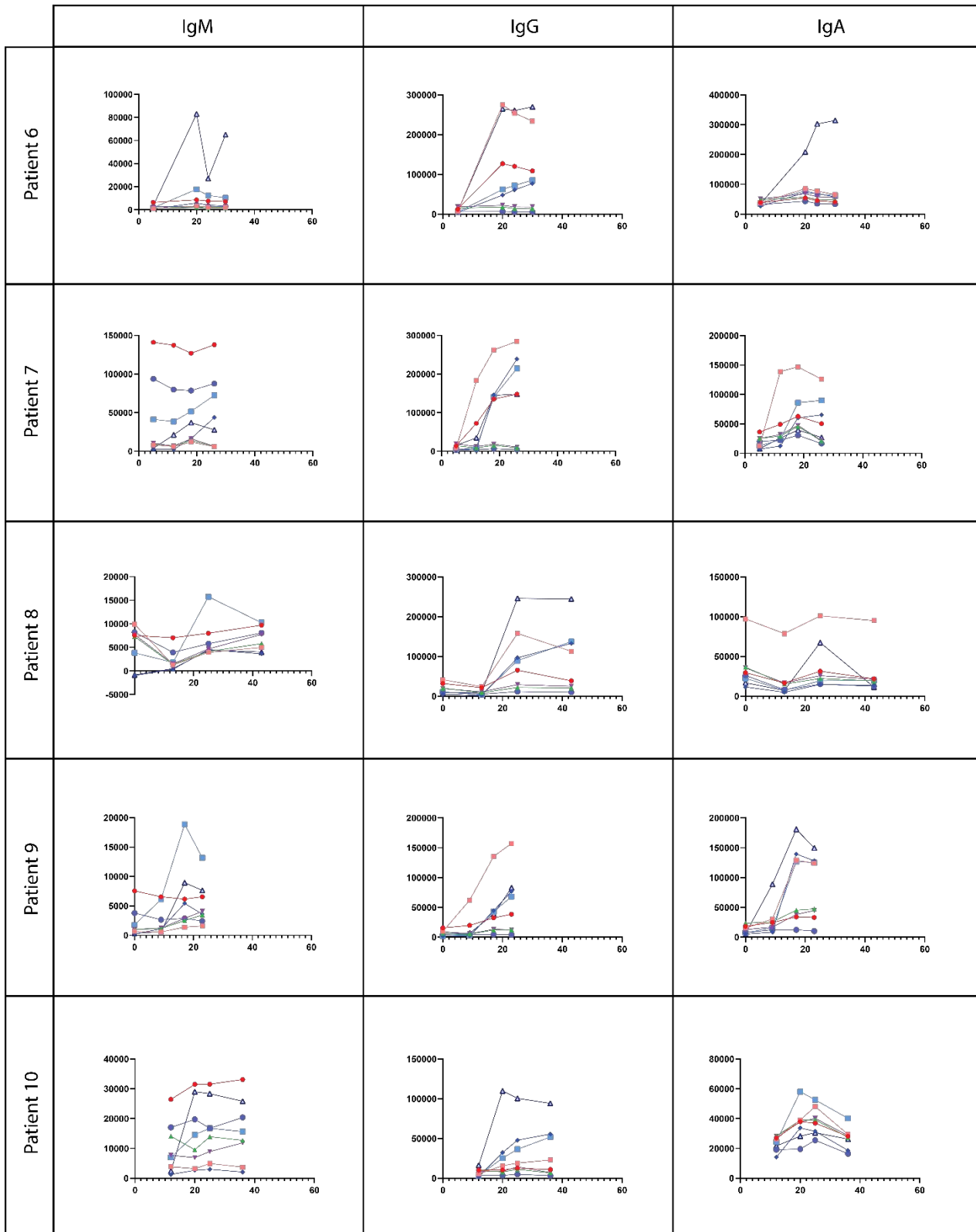
Figure S5 (continued). Serological response for 8 SARS-CoV-2 antigens for patients longitudinally. Serological response to 8 separate antigens for IgM, IgG, and IgA assessed during a hospital stay. Each individual row represents a single patient, with each column representing the IgM, IgG, or IgA response (from left to right). Patients #6 through #10.
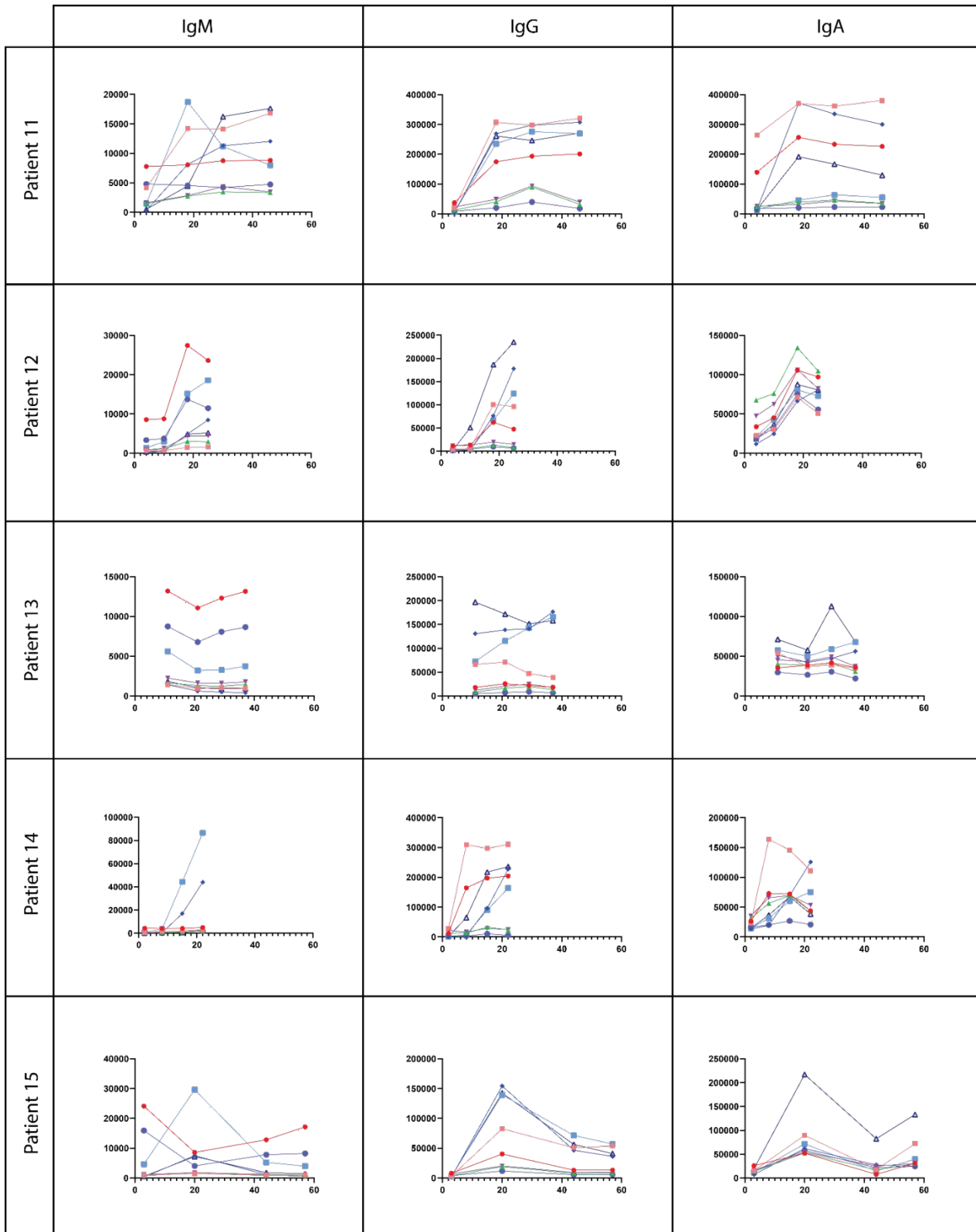
Figure S5 (continued). Serological response for 8 SARS-CoV-2 antigens for patients longitudinally. Serological response to 8 separate antigens for IgM, IgG, and IgA assessed during a hospital stay. Each individual row represents a single patient, with each column representing the IgM, IgG, or IgA response (from left to right). Patients #10 through #15.
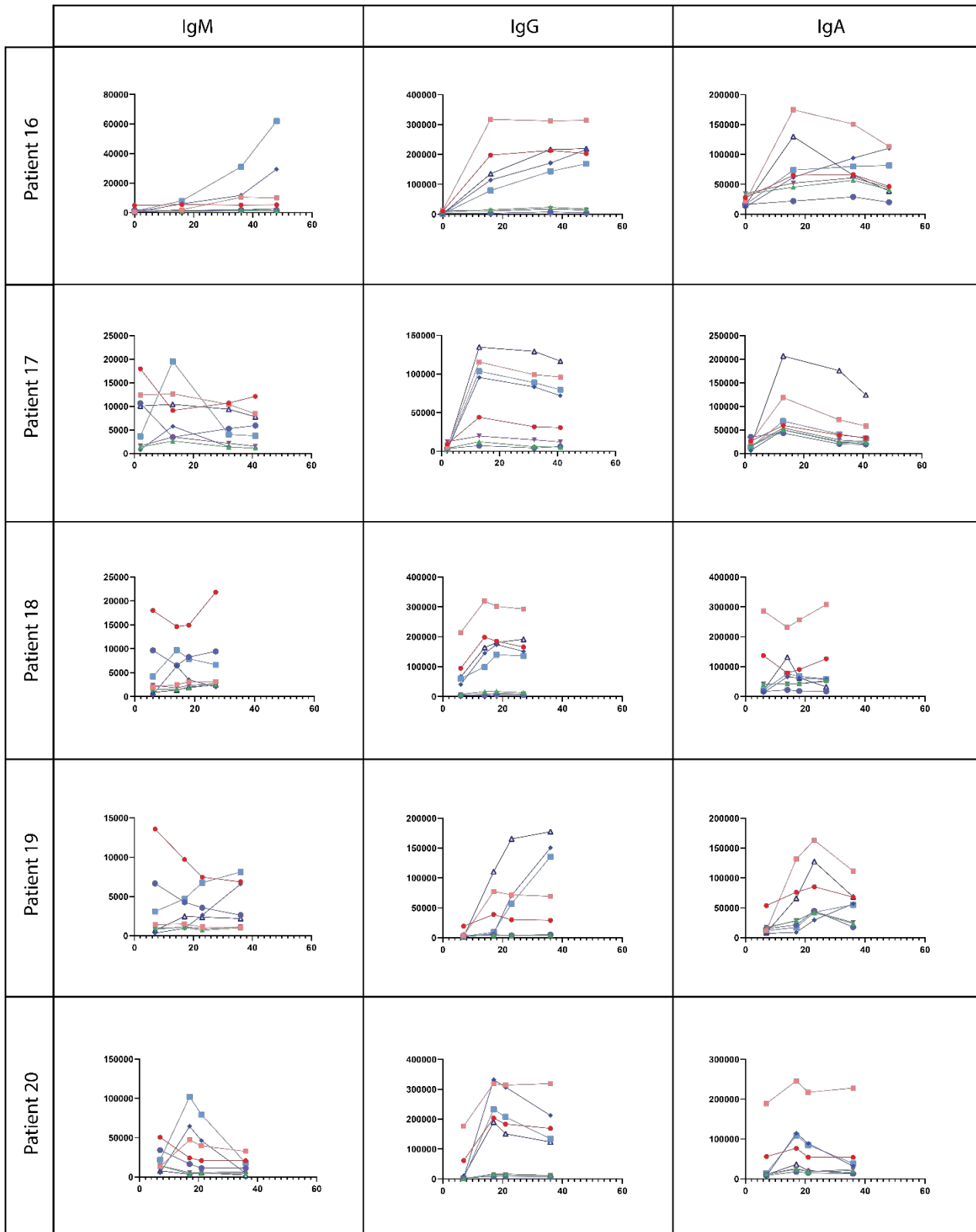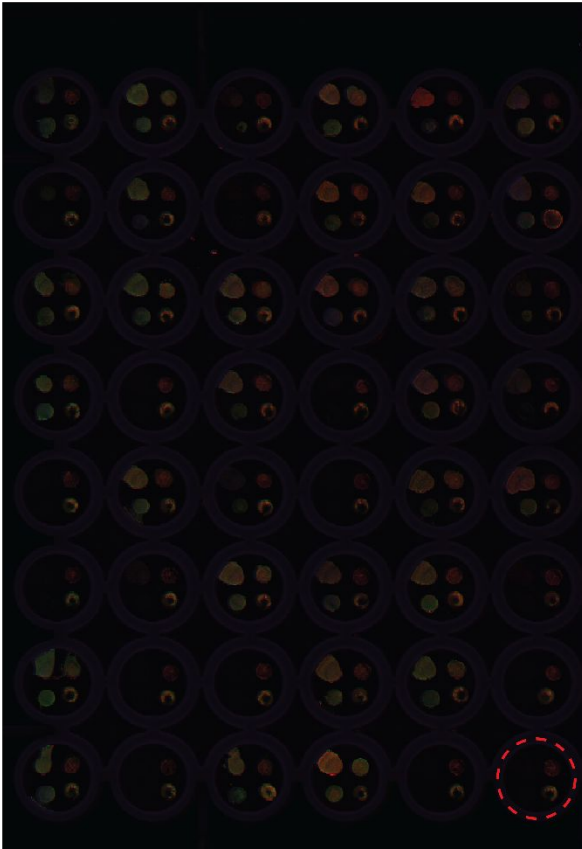
Figure S5 (continued). Serological response for 8 SARS-CoV-2 antigens for patients longitudinally. Serological response to 8 separate antigens for IgM, IgG, and IgA assessed during a hospital stay. Each individual row represents a single patient, with each column representing the IgM, IgG, or IgA response (from left to right). Patients #15 through #20.
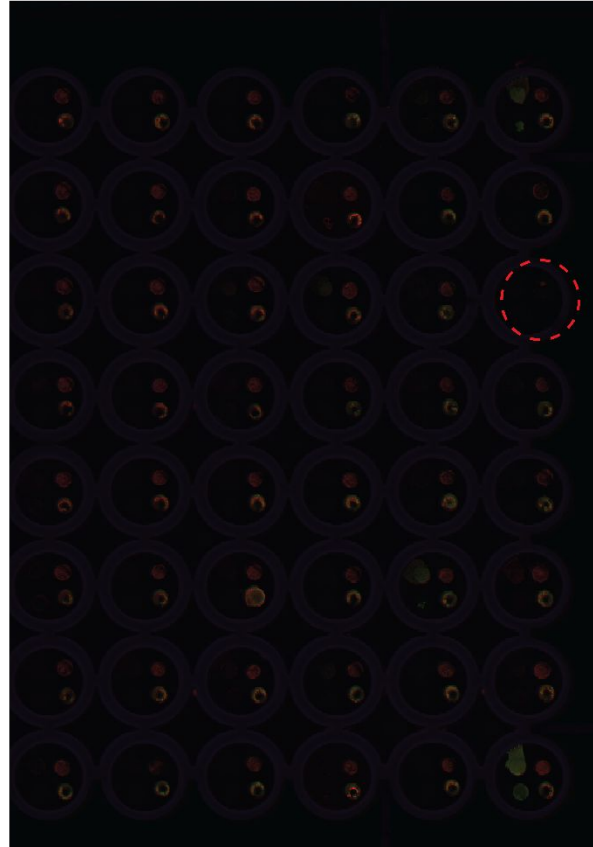
a)

b)

Figure S6. Related to Figure 6. Multiplexed detection of immunoglobulins in a single well and sample. Overlay of the raw intensity data for the detection of IgM (PF$_{650}$), IgG (PF$_{800}$), and IgA (PF$_{550}$). The dashed circles highlight the wells omitted from analysis. (a) Patients positive for SARS-CoV-2 and (b) patients negative for SARS-CoV-2.

Table S4. Related to Figures 6 and S6, and Table S5. Patient demographics of positive patient samples used in this study.

Patient	Gender	Age	Days Post-Symptom Onset	Patient	Gender	Age	Days Post-Symptom Onset
21	Female	74	36	45	Male	35	11
22	Male	60	24	46	Female	15	13
23	Male	41	4	47	Female	72	19
24	Male	55	17	48	Male	76	16
25	Male	75	11	49	Male	84	8
26	Female	73	13	50	Male	67	4
27	Male	60	20	51	Male	69	13
28	Male	50	9	52	Male	64	14
29	Male	74	18	53	Female	92	12
30	Female	45	16	54	Male	72	24
31	Male	65	10	55	Female	74	7
32	Male	64	17	56	Male	60	18
33	Female	23	11	57	Male	69	32
34	Male	70	9	58	Male	67	14
35	Male	61	10	59	Male	72	11
36	Male	80	10	60	Male	82	9
37	Male	58	10	61	Female	90	18
38	Male	79	16	62	Male	60	17
39	Female	60	9	63	Male	80	37
40	Female	80	9	64	Female	84	10
41	Female	56	10	65	Male	56	12
42	Female	54	16	66	Male	66	14
43	Female	62	21	67	Female	70	11
44	Male	51	20				

Table S5. Related to Figure 6. Average intensities and standard deviation (Std. Dev.) of each of antigen. (a) Positive patient plasma and (b) negative patient plasma.

(a)

Antigen	IgM		IgG		IgA		
	Average (RFU)	Std. Dev. (RFU)	Average (RFU)	Std. Dev. (RFU)	Average (RFU)	Std. Dev. (RFU)	n
N FL	2,364	1,297	1,849	1,407	957	828	47
Spike S1	5,631	3,659	3,347	2,447	2,065	1,391	47
Spike S2	4,092	1,095	1,368	1,121	735	389	47

(b)

Antigen	IgM		IgG		IgA		
	Average (RFU)	Std. Dev. (RFU)	Average (RFU)	Std. Dev. (RFU)	Average (RFU)	Std. Dev. (RFU)	n
N FL	958	384	230	436	84	126	47
Spike S1	1,541	950	576	1,132	292	270	47
Spike S2	3,754	1,342	701	158	524	98	47

a)

IgA	IgG
IgM	Comb.

b)

PF800-anti-IgA

PF550-anti-IgG

PF650-anti-IgM

Combination
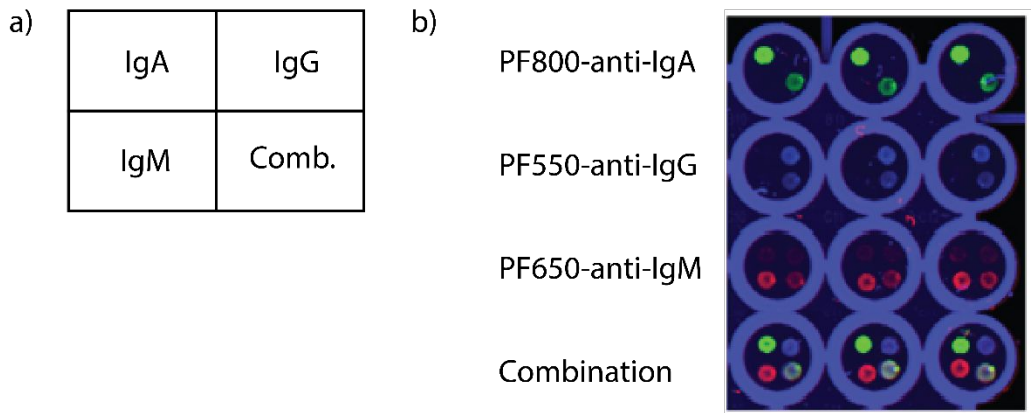
Figure S7. Cross-reactivity between PF$_{550}$, PF$_{650}$, and PF$_{800}$ with measurements in a single-well. (a) Schematic of the spot lay-out within an individual well. Comb. refers to spotting of IgA, IgG, and IgM together. (b) Readout of all three PF signals simultaneously. Each row refers to the secondary antibody and plasmonic fluor used with three wells. The bottom row, "Combination", received all three secondary antibody-plasmonic fluor conjugates.

Table S6. Comparison of SARS-CoV-2 Serology Assays. Several commercial serological assays against SARS-CoV-2 antigens are highlighted, with various performance metrics. In addition to the type of technique utilized by the assay, Assays are grouped by the types of antigens assessed.

Company	Assay Name	Technique	Sensitivity (%)	Specificity (%)	Samples (n)	Antigens	IgM	IgG	IgA	Total	Volume (µL)	Dilution	TAT (min)	Citation
Assays for N-Protein														
Epitope Diagnostics	KT-1034 EDI	ELISA	81	100	43	N	X	X			10	1:100	80	[1]
Abbott Diagnostics	Abbott Diagnostics SARS-CoV-2 Immunoassay	CLIA	81	100	43	N		X			100	None	29	[1]
Biorad	Platelia SARS-CoV-2 Total Ab assay	ELISA	97.4	94.9	208	N				X	100	1:5	90	[1]
Assays with Spike Antigens														
EuroImmun	EuroImmun SARS-CoV-2 ELISA	ELISA	81	92	43	S1		X			100	1:101	120	[2]
Wantai	WANTAI SARS-CoV-2 Ab ELISA	ELISA	99.6	88.8	260	Spike RBD				X	100	None	75	[3]
DiaSorin	LIAISON® SARS-CoV-2 S1/S2 IgG	CLIA	71	96	42	S1/S2	X	X			164	None	35	[2]
Mabtech	ELISA Path: SARS-CoV-2 (RBD) Total Antibody	ELISA	99.6	79.3	278	Spike RBD				X	50	1:2	195	[3]

Table S6 (continued). Comparison of SARS-CoV-2 Serology Assays. Several commercial serological assays against SARS-CoV-2 antigens are highlighted, with various performance metrics. In addition to the type of technique utilized by the assay, Assays are grouped by the types of antigens assessed.

Company	Assay Name	Technique	Sensitivity (%)	Specificity (%)	Samples (n)	Antigens	IgM	IgG	IgA	Total	Volume (µL)	Dilution	TAT (min)	Citation
						**Assays with Mixed Antigens**								
ThermoFisher	Coronavirus Ig Total Human 11-Plex ProcartaPlex Panel	Luminex Bead Assay	See below	See below	See below	4 Antigens				X	25	1:1,000	160	[4]
			93.7	98.2	568	Strimer (FL)								
			84.1	100	568	S1								
			83.4	100	568	RBD								
			91.1	98.2	568	N								
Auragent Bioscience	N/A	Plasmonic Fluors	See below	See below	See below	8 Antigens	X	X	X		100	1:100	80	N/A
			92.5	93.75	80	N FL								
			92.5	100	80	N NTD								
			98.75	100	80	Orf3b								
			93.75	93.75	80	Orf8								
			92.5	100	80	Spike S1								
			90	100	80	Spike NTD								
			95	100	80	Spike RBD								
			93.75	100	80	Spike S2								

References:1.   Tré-Hardy, M.; Wilmet, A.; Beukinga, I.; Favresse, J.; Dogné, J.-M.; Douxfils, J.; Blairon, L., Analytical and clinical validation of an ELISA for specific SARS-CoV-2 IgG, IgA, and IgM antibodies. *J Med Virol* **2021,** *93* (2), 803-811.

2.   Piec, I.; English, E.; Thomas, M. A.; Dervisevic, S.; Fraser, W. D.; John, W. G., Performance of SARS-CoV-2 serology tests: Are they good enough? *PloS one* **2021,** *16* (2), e0245914-e0245914.

3.   Lagerqvist, N.; Maleki, K. T.; Verner-Carlsson, J.; Olausson, M.; Dillner, J.; Wigren Byström, J.; Monsen, T.; Forsell, M.; Eriksson, J.; Bogdanovic, G.; Muschiol, S.; Ljunggren, J.; Repo, J.; Kjerstadius, T.; Muradrasoli, S.; Brytting, M.; Szekely Björndal, Å.; Åkerlund, T.; Nilsson, C.; Klingström, J., Evaluation of 11 SARS-CoV-2 antibody tests by using samples from patients with defined IgG antibody titers. *Scientific Reports* **2021,** *11* (1), 7614.

4.   Borena, W.; Kimpel, J.; Gierer, M.; Rössler, A.; Riepler, L.; Oehler, S.; von Laer, D.; Miholits, M., Characterization of Immune Responses to SARS-CoV-2 and Other Human Pathogenic Coronaviruses Using a Multiplex Bead-Based Immunoassay. *Vaccines* **2021,** *9* (6), 611.