

Prevalence of IgG and IgM to SARS-CoV-2 and other human coronaviruses in Sierra Leone, Democratic Republic of Congo, and Uganda: A Longitudinal Study

Supplementary information

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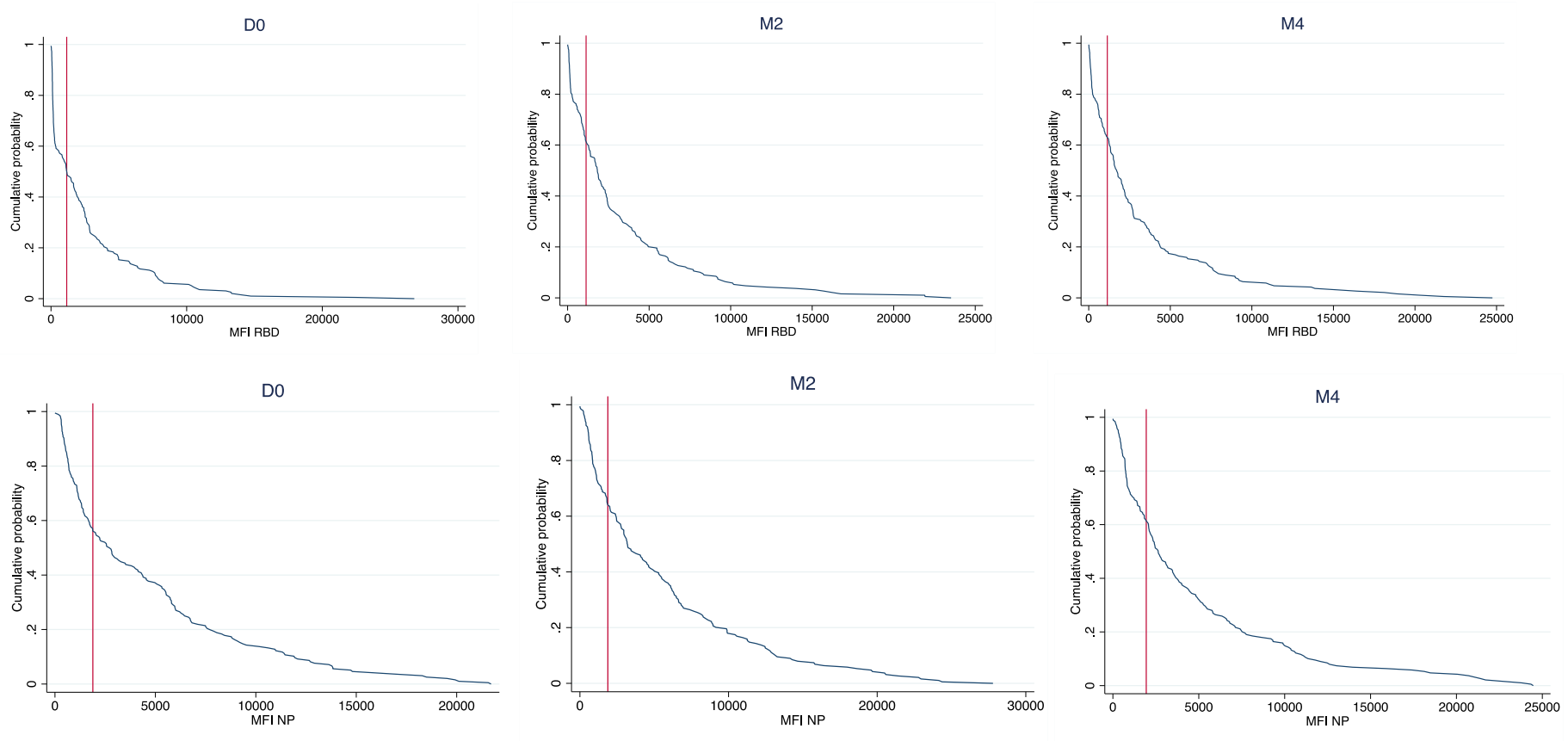
Suppl. Table 1. Serological profiles of unvaccinated participants over time

Luminex IgG/IgM RBD/NP profile	M0	M2	M4	DRC (N=186)		SL (N=62)		UG (N=98)	
				n	%	n	%	n	%
1 – Persistently positive	positive	positive	positive	119	64.0	41	66.1	83	84.7
2 - Seroreversion	positive	positive	negative	5	2.7	8	12.9	2	2.0
3 - Seroreversion	positive	negative	negative	2	1.1	1	1.6	1	1.0
4 – Reinfection ¹	positive	negative	positive	8	4.3	6	9.7	4	4.1
5 – Persistently negative	negative	negative	negative	23	12.4	0	0	4	4.1
6 - Acquisition	negative	negative	positive	4	2.2	0	0	3	3.1
7 - Acquisition	negative	positive	positive	24	12.9	4	6.5	1	1.0
8 – Acquisition ¹	negative	positive	negative	1	0.0	2	3.2	0	0.0

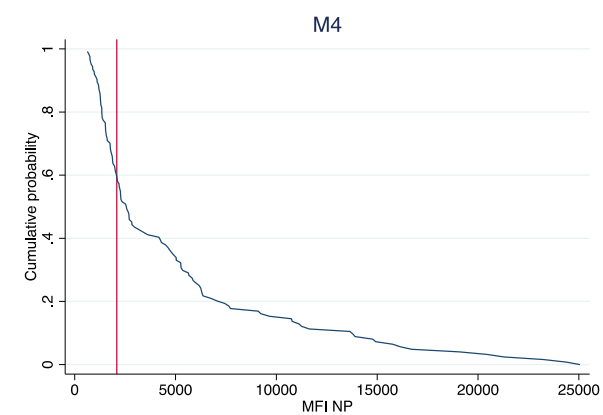
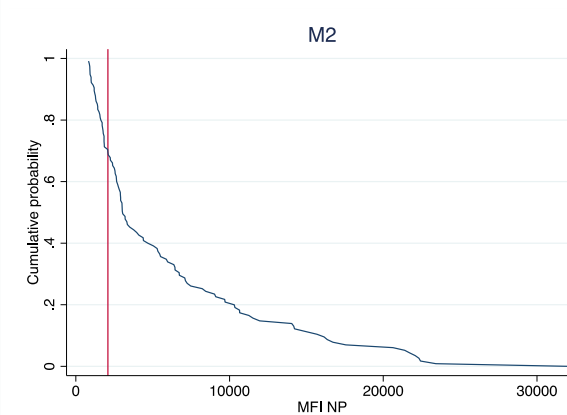
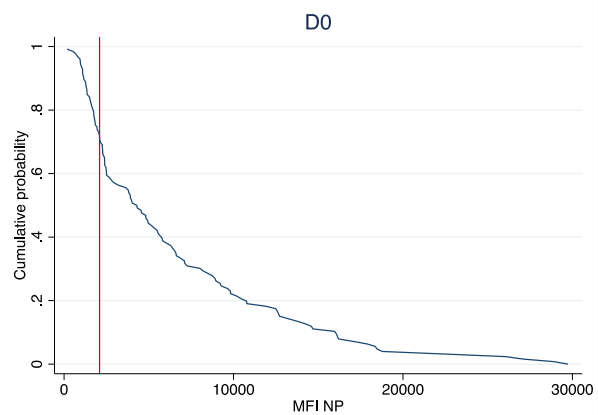
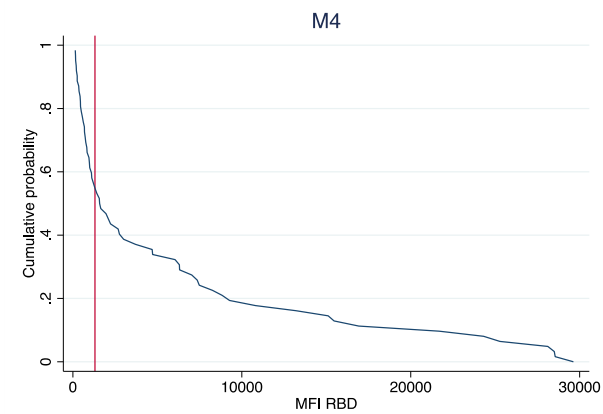
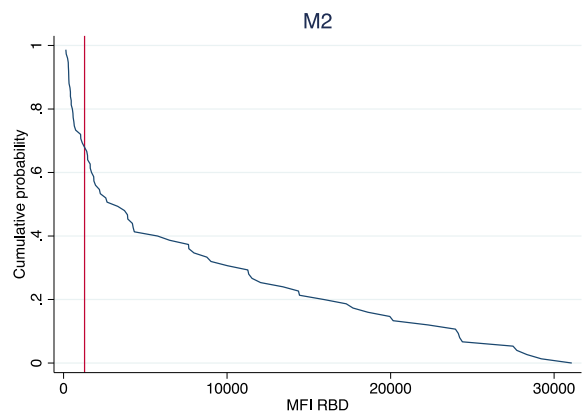
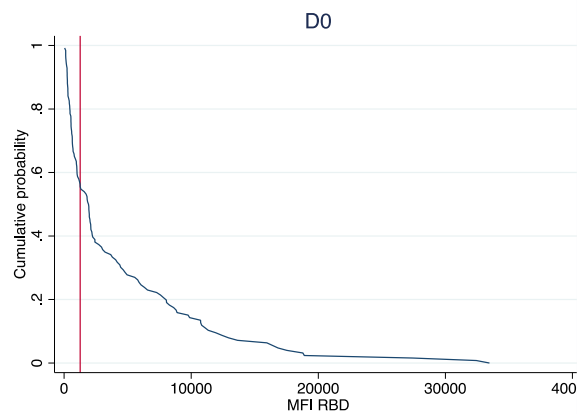
¹This could also represent misclassification of the M2 sample. Participants included in this table had to have available IgG and IgM data for RBD and NP for every timepoint and had to remain unvaccinated for the duration of the study.

Suppl. Figure 1. Reverse Cumulative distribution curves – MFI of IgG to SARS-CoV-2 receptor binding domain (RBD) and nucleocapsid protein (NP) – among unvaccinated

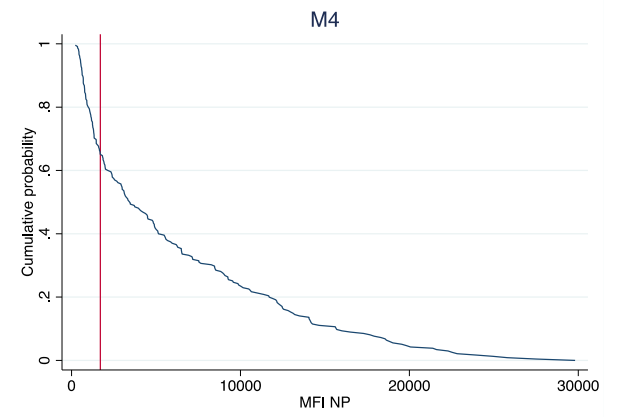
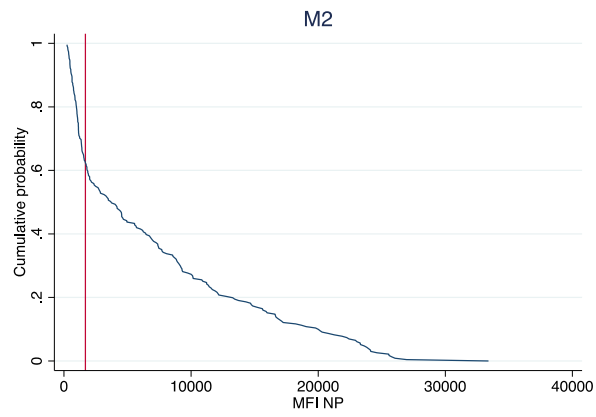
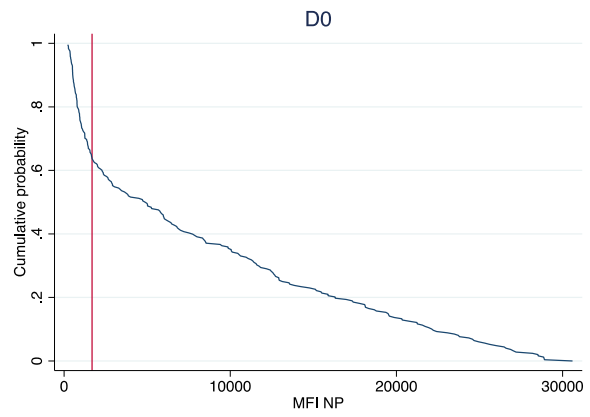
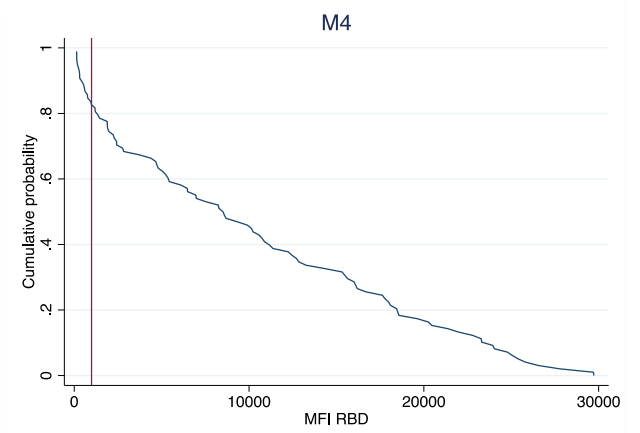
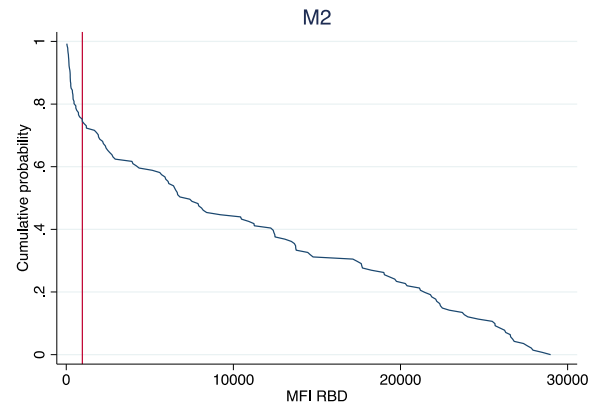
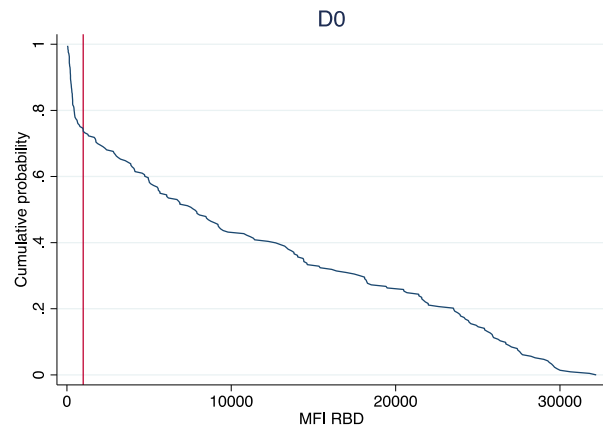
i. DRC



ii. Sierra Leone

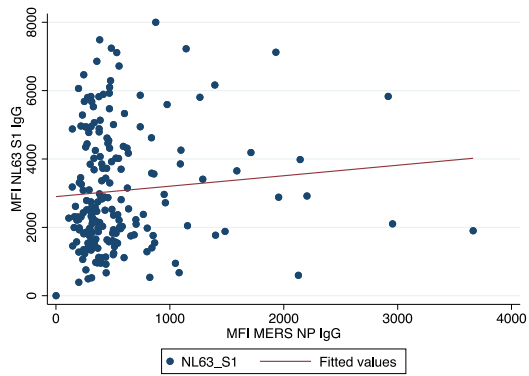
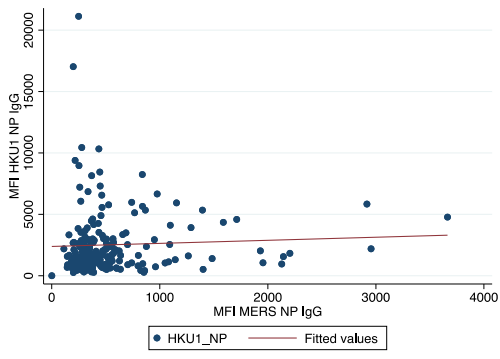
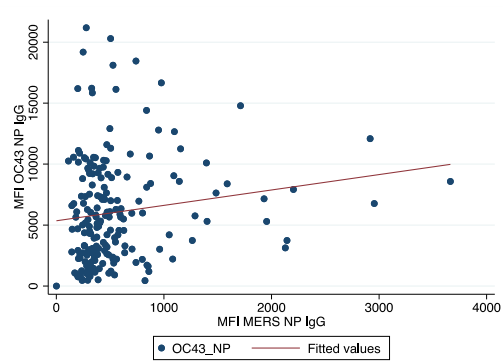
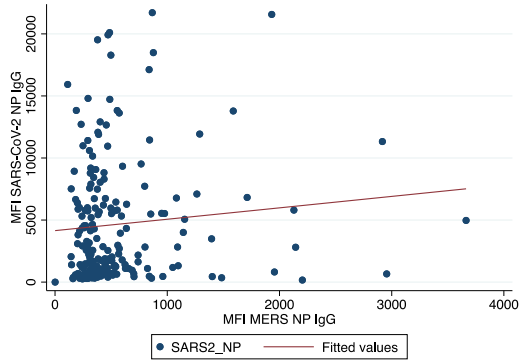


iii. Uganda

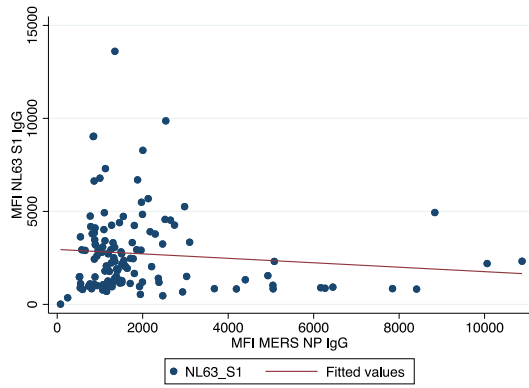
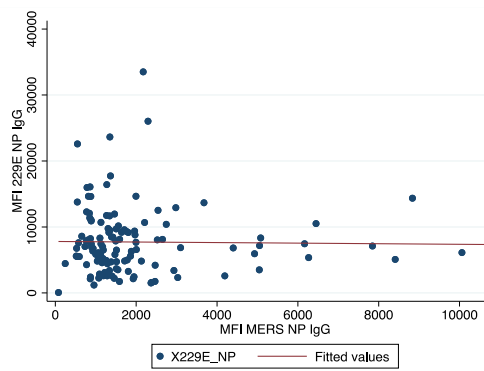
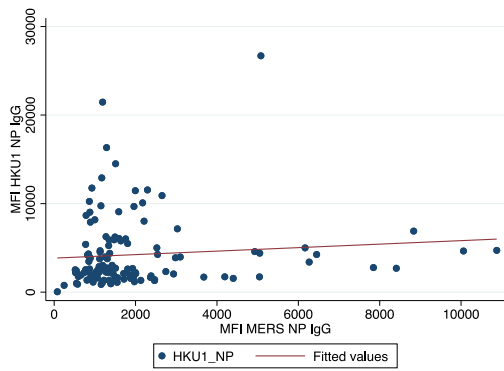
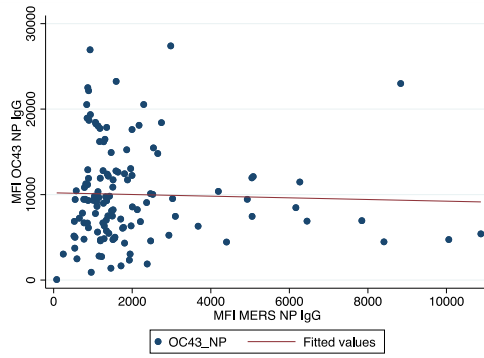
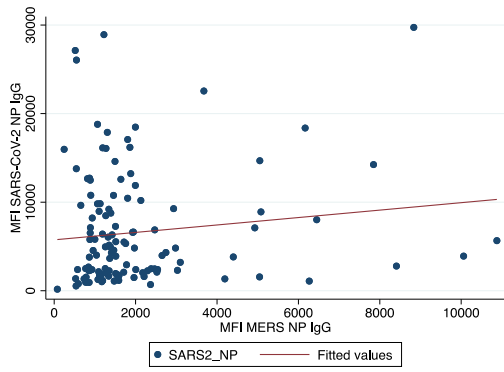


Suppl. Figure 2. Correlation between MERS NP and other HCoV at baseline. Line: line of best fit

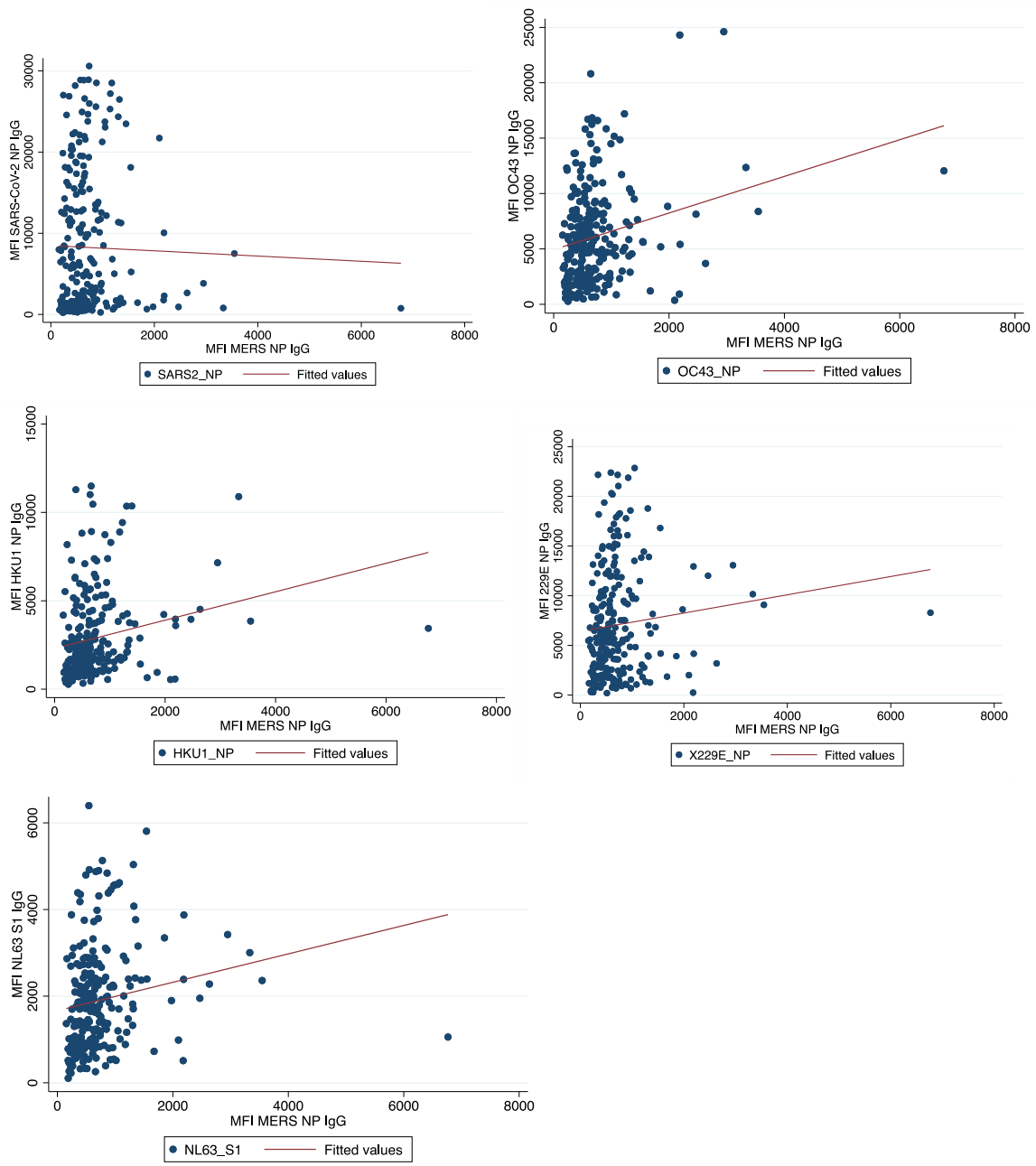
a) DRC *excluding 1 value of MERS NP MFI > 10,000



b) Sierra Leone



c) Uganda

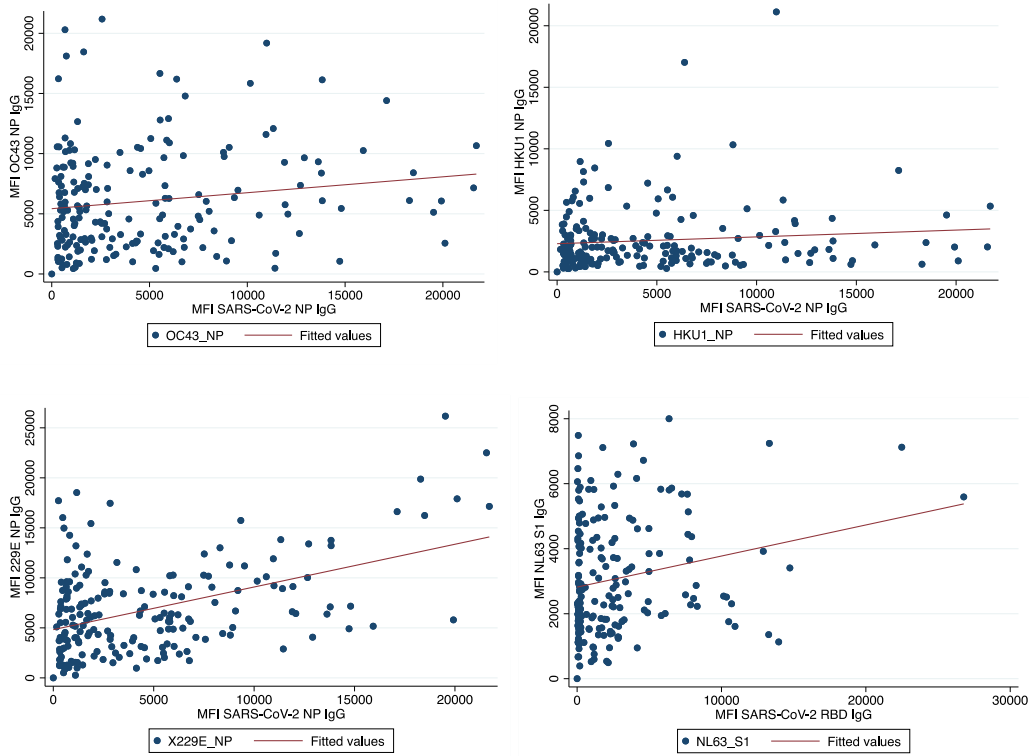


Suppl. Table 2. Correlation coefficients of MFI units of IgG to MERS-NP and HCoV-229E among all samples, and MERS-NP with SARS2 among the unvaccinated

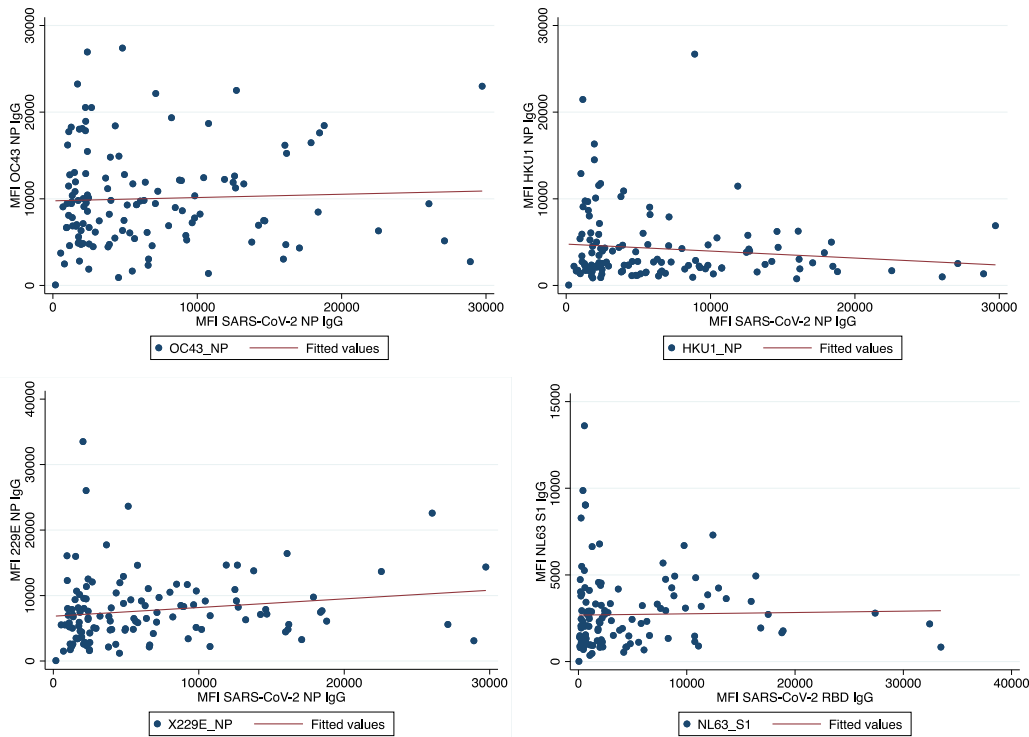
Antigen	Corr coef. IgG to MERS NP MFI	p-value	n
DRC			
SARS-CoV-2 NP	0.093	0.1959	195
OC43 NP	0.148	0.0389	195
HKU1 NP	0.048	0.5088	195
X229E NP	0.158	0.0275	195
NL63 RBD	0.089	0.2161	195
Sierra Leone			
SARS-CoV-2 NP	0.098	0.2751	126
OC43 NP	-0.033	0.7122	126
HKU1 NP	0.0931	0.3	126
X229E NP	-0.016	0.8589	126
NL63 RBD	-0.106	0.239	126
Uganda			
SARS-CoV-2 NP	-0.046	0.503	213
OC43 NP	0.239	0.001	248
HKU1 NP	0.2141	0.0007	248
X229E	0.107	0.0944	248
NL63 RBD	0.1720	0.007	248

Suppl. Figure 3. Correlations between MFI of IgG to SARS-CoV-2 NP/RBD and MFI of IgG to HCoV-2 among the unvaccinated at enrolment

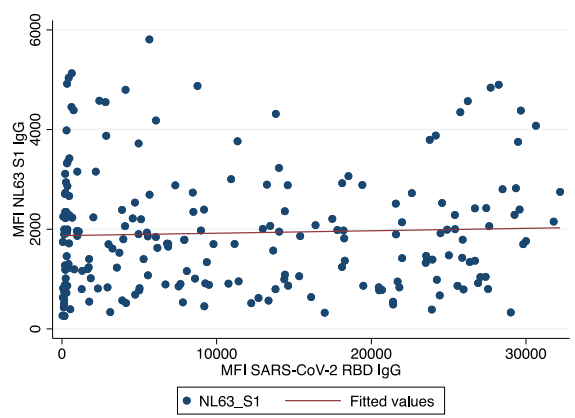
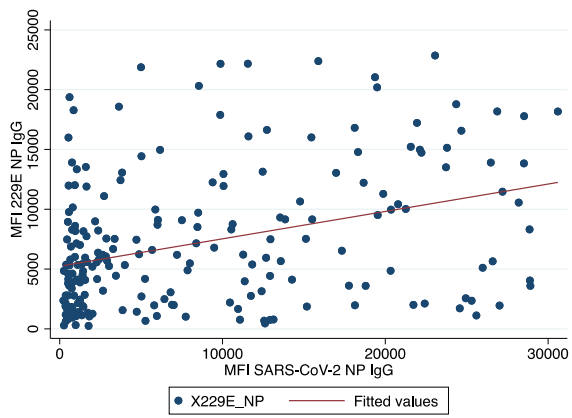
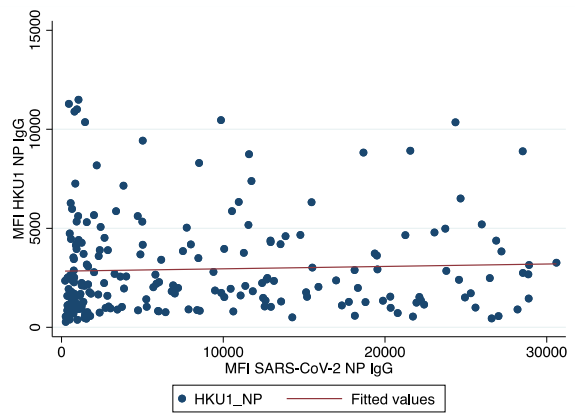
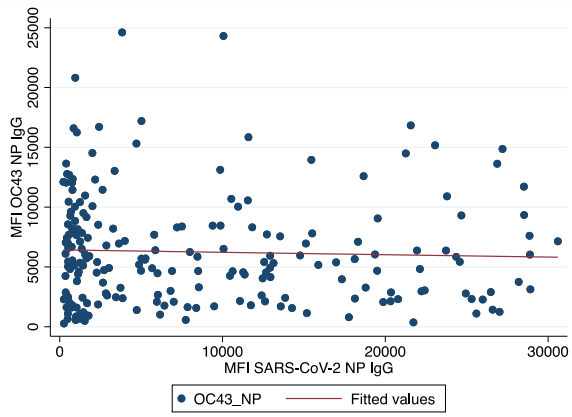
a. DRC



b. Sierra Leone



c. Uganda



Suppl. Table 3. Correlation coefficients of MFI units to SARS-CoV-2 NP / RBD protein and HCoVs, among the unvaccinated, at the time of enrolment

Antigen	HCoV 'seroprevalence' D0 among unvaccinated	Corr coef. IgG to SARS- CoV-2 NP MFI	p-value	n
DRC				
OC43-NP	2.6	0.1513	0.0342	196
X229E- NP	2.6	0.4608	<0.001	196
HKU1- NP	11.2	0.1024	0.1534	196
NL63 – S1*	4.6	0.2120	0.0029	196
MERS-NP	7.14	0.093	0.1959	196
Sierra Leone				
OC43-NP	19.1	0.0426	0.6358	126
X229E- NP	3.2	0.1654	0.0642	126
HKU1- NP	23.0	-0.127	0.1558	126
NL63 – S1*	7.9	0.0214	0.8120	126
MERS-NP	36.5	0.098	0.2751	126
Uganda				
OC43-NP	4.7	-0.039	0.5699	213
X229E- NP	7.5	0.3551	<0.001	213
HKU1- NP	12.7	0.0434	0.5285	213
NL63 – S1*	13.2	0.0419	0.5431	213
MERS-NP	14.6	-0.0481	0.4850	213

*corr coef is against SARS-CoV-2 RBD domain as the NL63 antigen was the S1 protein

Suppl. Table 4. A sensitivity analysis to assess if SARS-CoV-2 seroprevalence (RBD/NP IgG/M) differs in samples with evidence of high MFI to other HCoVs¹

¹ In the table we defined the HCoV ‘seropositive’ column as those with IgG MFI units 3 standard deviations above the mean of European negative controls. The data on other HCoVs are based on IgG to OC43 NP, HKU1 NP, 229E NP, NL63 S1 protein

a) DRC

		OC43 NP IgG			Seroprevalence to OC43	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	56	2	58	3.4%	0.606
	(+)	135	3	138	2.2%	
	Total	191	5	196	2.6%	
	Seroprevalence to SARS2	71%	60%			

		HKU1 NP IgG			Seroprevalence to HKU1	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	51	7	58	12.1%	0.808
	(+)	123	15	138	10.9%	
	Total	174	22	196	11.2%	
	seroprevalence to SARS2	71%	68%			

		229E NP IgG			Seroprevalence to 229E	Chi2 p-value
		(-)	(+)	Total		
Luminex RBD/NP IgG / IgM	(-)	57	1	58	1.7%	0.634
	(+)	134	4	138	2.9%	
	Total	191	5	196	2.6%	
	seroprevalence to SARS2	70%	80%			

		NL63 S1 IgG			Seroprevalence to NL63	Chi2 p-value
		(-)	(+)	Total		
Luminex RBD/NP IgG / IgM	(-)	55	3	58	5.2%	0.801
	(+)	132	6	138	4.3%	
	Total	187	9	196	4.6%	
	seroprevalence to SARS2	71%	67%			

b) Sierra Leone

		OC43 NP IgG			Seroprevalence to OC43	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	10	4	14	28.6%	0.336
	(+)	92	20	112	17.9%	
	Total	102	24	126	19.0%	
	Seroprevalence to SARS2	90%	83%			

		HKU1 NP IgG			Seroprevalence to HKU1	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	10	4	14	28.6%	0.600
	(+)	87	25	112	22.3%	
	Total	97	29	126	23.0%	
	Seroprevalence to SARS2	90%	86%			

		229E NP IgG			Seroprevalence to 229E	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	13	1	14	7.1%	0.369
	(+)	109	3	112	2.7%	
	Total	122	4	126	3.2%	
	Seroprevalence to SARS2	89%	75%			

		NL63 S1 IgG			Seroprevalence to NL63	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	14	0	14	0.0%	0.244
	(+)	102	10	112	8.9%	
	Total	116	10	126	7.9%	
	Seroprevalence to SARS2	88%	100%			

c) Uganda among unvaccinated at baseline only

		OC43 NP IgG			Seroprevalence to OC43	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	21	2	23	8.7%	0.9224
	(+)	182	8	190	4.2%	
	Total	203	10	213	4.7%	
	seroprevalence to SARS2	90%	80%			

		HKU1 NP IgG			Seroprevalence to HKU1	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	20	3	23	13.0%	0.003
	(+)	166	24	190	12.6%	
	Total	186	27	213	12.7%	
	seroprevalence to SARS2	89%	89%			

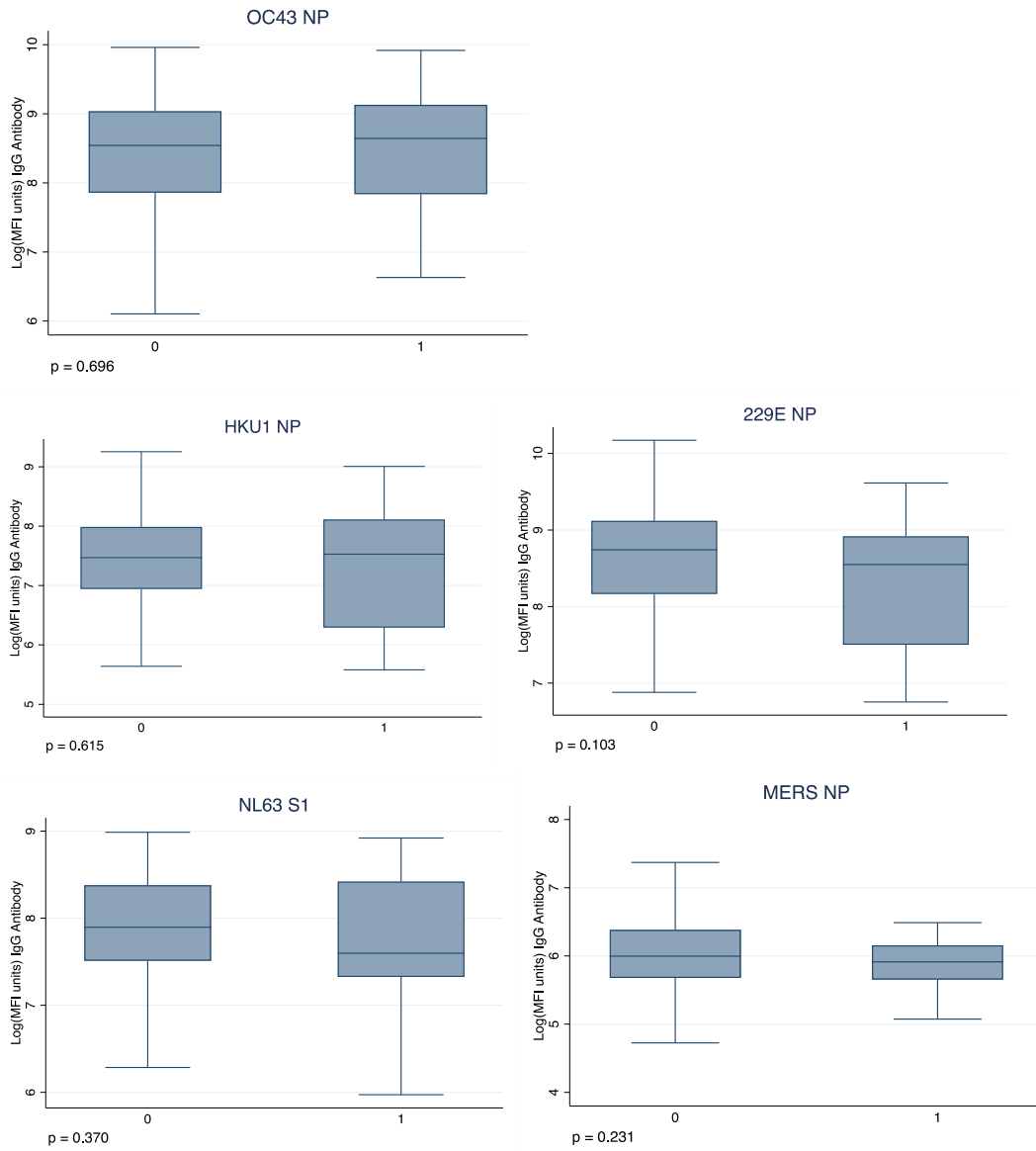
		229E NP IgG			Seroprevalence to HKU1	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	22	1	23	4.4%	0.372
	(+)	175	15	190	7.9%	
	Total	197	16	213	7.5%	
	seroprevalence to SARS2	89%	94%			

		NL63 S1 IgG			Seroprevalence to NL63	Chi2 p-value
		(-)	(+)	Total		
SARS2 RBD/NP IgG / IgM	(-)	20	3	23	13.0%	0.0002
	(+)	165	25	190	13.2%	
	Total	185	28	213	13.2%	
	seroprevalence to SARS2	89%	89%			

Suppl. Table 5. Factors associated with remaining persistently negative throughout follow up in the DRC

DRC	Control		Persistently negative		Total	OR	95%CI	LRT p-value	
	N	% (col)	N	% (col)					
All	163	87.6	23	12.4	186				
Age group									
	<30 yrs	41	25.2	7	30.4	48	1	0.848	
	30-45 yrs	72	44.2	9	39.1	81	0.73		0.25-2.11
	>45yrs	50	30.7	7	30.4	57	0.82		0.27-2.53
Sex									
	Male	62	38.0	18	78.3	80	5.87	2.07-16.6	<0.001
	Female	101	62.0	5	21.7	106	1		
Role ²									
	Doctor, CO, nurse	135	82.8	19	82.6	154	1	0.980	
	Support staff ⁴	28	17.2	4	17.4	32	1.01		0.57-1.79
Highest level of schooling									
	None ⁷						~		
	Complete primary	1	0.6	0	0	1	~	~	
	Incomplete secondary	12	7.4	2	8.7	14	1.18	0.25-5.66	
	Complete secondary & above	149	92.0	21	91.3	170	1		
Smoke once/ week or more									
	No	162	99.4	23	100	185	~		
	Yes	0	0	0	0	0	~		
MUAC									
	Underweight (<24cm)	13	8.0	4	17.4	17	2.19	0.64-7.55	0.287
	Normal	114	69.9	16	69.6	130	1		
	Obese (>31cm)	36	22.1	3	13.0	39	0.59		
Known contact with a confirmed COVID-19 case									
	Yes	42	25.8	5	21.7	47	1	0.673	
	No	121	74.2	18	78.3	139	1.25		0.44-3.57
Use of a mask at work									
	None of the time							0.834	
	Half the time	27	16.6	5	21.7	32	1.38		0.44-4.33
	Most but not all the time	82	50.3	11	47.8	93	1		
	All the time	54	33.1	7	30.4	61	0.97		0.35-2.65
Known Pre-existing conditions									
	Yes	29	17.8	6	26.1	35	1.63	0.59-4.49	0.36
	No	134	82.2	17	73.9	151	1		
COVID-19 vaccination									
	Yes	0	0	0	0	0			
	No	163	100	23	100	186			

Suppl. Figure. 4. HCoV IgG MFI units at baseline, comparing those who were seropositive to SARS-CoV-2 at least once during the study ('0' below, n=163) with those remained persistently seronegative to SARS-CoV-2 IgG/M to RBD/ N ('1' below, n=23), in Goma. P-values are for a Wilcoxon rank-sum test



Suppl. Table 6. Seroprevalence of SARS-CoV-2 in vaccinated¹

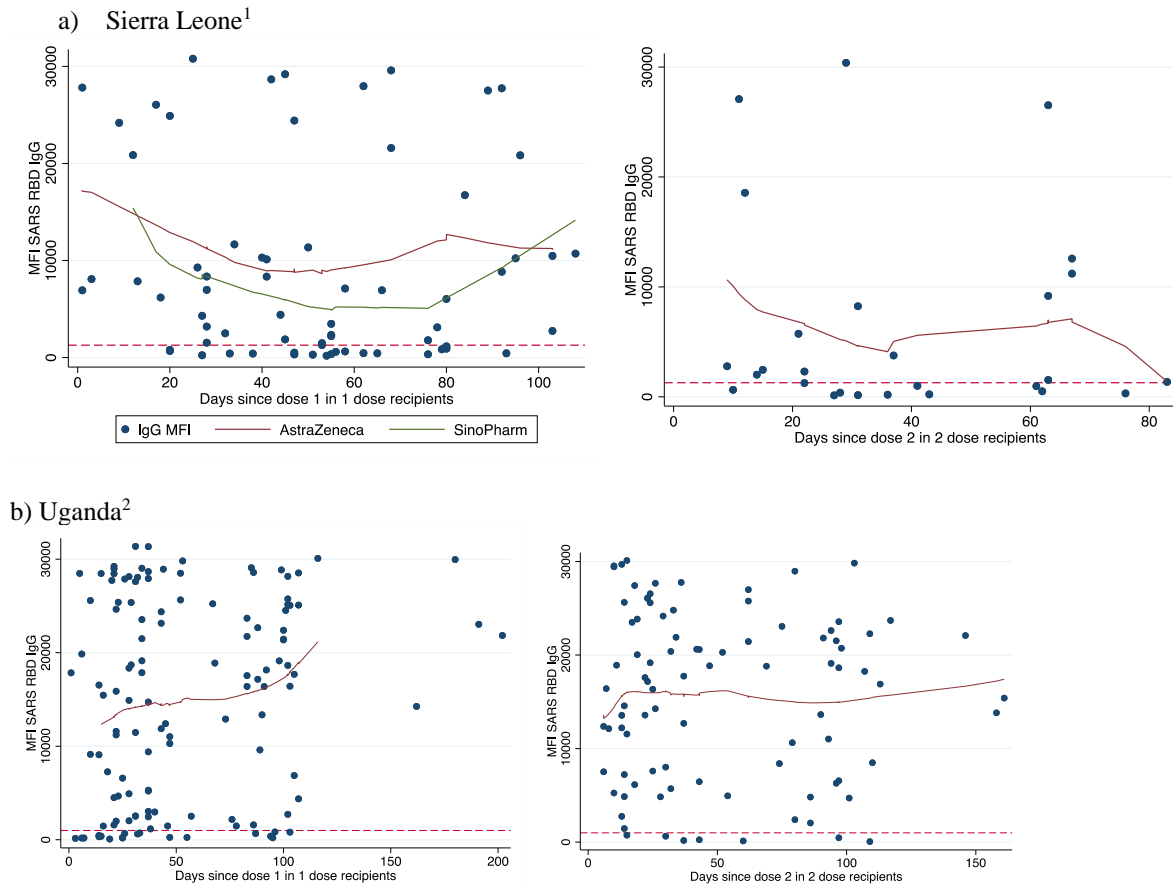
Masaka, Uganda	Among those vaccinated before D0								
	D0			M2			M4		
	N (Total)	n (pos)	%	N (Total)	n (pos)	%	N (Total)	n (pos)	%
SARS2 RBD IgG	35	30	85.7	35	32	91.4	34	32	94.1
SARS2 NP IgG	35	23	65.7	35	22	62.9	34	23	67.6
SARS2 RBD/NP IgG	35	31	88.6	35	33	94.3	34	32	94.1
SARS2 RBD/NP IgG/IgM	35	33	94.3	35	33	94.3	34	34	100.0

Masaka, Uganda	Among those vaccinated between M0 and M2						Among those vaccinated between M2 and M4		
	M2			M4			M4		
	N (Total)	n (pos)	%	N (Total)	n (pos)	%	N (Total)	n (pos)	%
SARS2 RBD IgG	54	47	87.0	51	46	90.2	41	33	80.5
SARS2 NP IgG	54	36	66.7	51	33	64.7	41	28	68.3
SARS2 RBD/NP IgG	54	49	90.7	51	48	94.1	41	33	80.5
SARS2 RBD/NP IgG/IgM	54	49	90.7	51	50	98.0	41	36	87.8

Kambia, Sierra Leone	Among those vaccinated between M0 and M2						Among those vaccinated between M2 and M4		
	M2			M4			M4		
	N (Total)	n (pos)	%	N (Total)	n (pos)	%	N (Total)	n (pos)	%
SARS2 RBD IgG	40	34	85	40	24	60	12	7	58.3
SARS2NP IgG	40	27	67.5	40	29	72.5	12	5	41.7
SARS2 RBD/NP IgG	40	35	87.5	40	32	80	12	8	66.7
SARS2 RBD/NP IgG/IgM	40	37	92.5	40	33	82.5	12	8	66.7

¹In DRC: all participants remained unvaccinated for the duration of the study. The tables are based on timing of first dose, some of these participants may have been given a second dose between month 2 and month 4

Suppl. Figure 5. Trends in IgG MFI to SARS-COV-2 RBD by time since vaccination in Sierra Leone and Uganda. Curves are estimated using LOWESS, locally weighted smoothing of scatterplot data. Dashed line is the cut off for seropositivity in each setting.



¹ In Sierra Leone, all participants had written records of vaccination and product: AstraZeneca dose 1 recipients n=45; Sinopharm dose 1 recipients n=33. Due to small numbers AstraZeneca and Sinopharm recipients were combined for the dose 2 analysis n=33.

² In Uganda 128 participants submitted data on the date of dose 1. Only 14 participants had a written record with product recorded in Uganda, and some of these dates could be mis-remembered as based on recall. A total of 91 participants submitted data on the date of dose 2. Four dates were excluded as outliers when we estimated the trend using LOWESS.

Suppl. Table 7: Analyses and sample sizes

	Research questions	Sample size			Refer to table/ figure
		DRC	SL	UG	
1	Among unvaccinated: Prevalence of IgG/IgM to SARS2 as indicator of natural exposure (extent of transmission)	V1=196 V2=189 V2=189	V1=126 V2=75 V3=62	V1=213 V2=141 V3=98	Table 2
2	Among unvaccinated: Rate of waning of IgG after natural infection? Restricted to those persistently positive over time to attempt to restrict to a group with similar time of infection	n=119	n=41	n=83	Table 3, Fig 2
3	Among vaccinated: seroprevalence over time among those vaccinated before baseline (group 1), vaccinated between V1-V2 (group 2) and vaccinated between V2 and V3 (group 3)	0	Group1=35 group2=54 group3=41	Group 1=0 Group 2=40 Group 3= 12	Table 4
4	Among vaccinated: MFI units by time since vaccination (waning?) and prevalence of hybrid immunity	0	78	128	Supplementary information
5	Among vaccinated: does evidence of prior natural exposure affect vaccine responses?	0	seronegative at baseline=4 vs. seropositive at baseline=44	seronegative at baseline=12 vs seropositive at baseline=96	Figure 3
6	Among unvaccinated: Do responses to HCoV's correlate with SARS2 and do they interfere with seroprevalence estimates?	V1=196 V2=189 V2=189	V1=126 V2=75 V3=62	V1=213 V2=141 V3=98	Supplementary information
7	Among unvaccinated: Does IgG to HCoV's influence subsequent risk of acquisition (among those with no evidence of prior infection)	Remained uninfected=23 vs infected during follow up=28	0	0	Figure 1