Supplementary information

Seasonal coronavirus protective immunity is short-lasting

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Supplementary Materials for

Seasonal coronavirus protective immunity is short-lasting
NMED-BC105995A

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This PDF file includes:

Supplementary Fig. 1. Antibody reactivity to NCt of HCoV-NL63, HCoV-229E, HCoV-OC43, HCoV-HKU1, and full N of SARS-CoV-2.
Red: normalized optical density (OD) for HCoV-NL63, blue: HCoV-229E, green: HCoV-OC43, purple: HCoV-HKU1, orange: SARS-CoV-2, gray: measles virus. Error-bars show standard deviations from two or three replicates. In black font and underlined: OD fold rises ≥1.40 marking an infection for the indicated HCoV. In grey font: OD fold rises ≥1.40 classified as cross-reactivity or when no differentiation could be made between the infecting and the cross-reactive HCoV (when the difference in fold change was <10%).
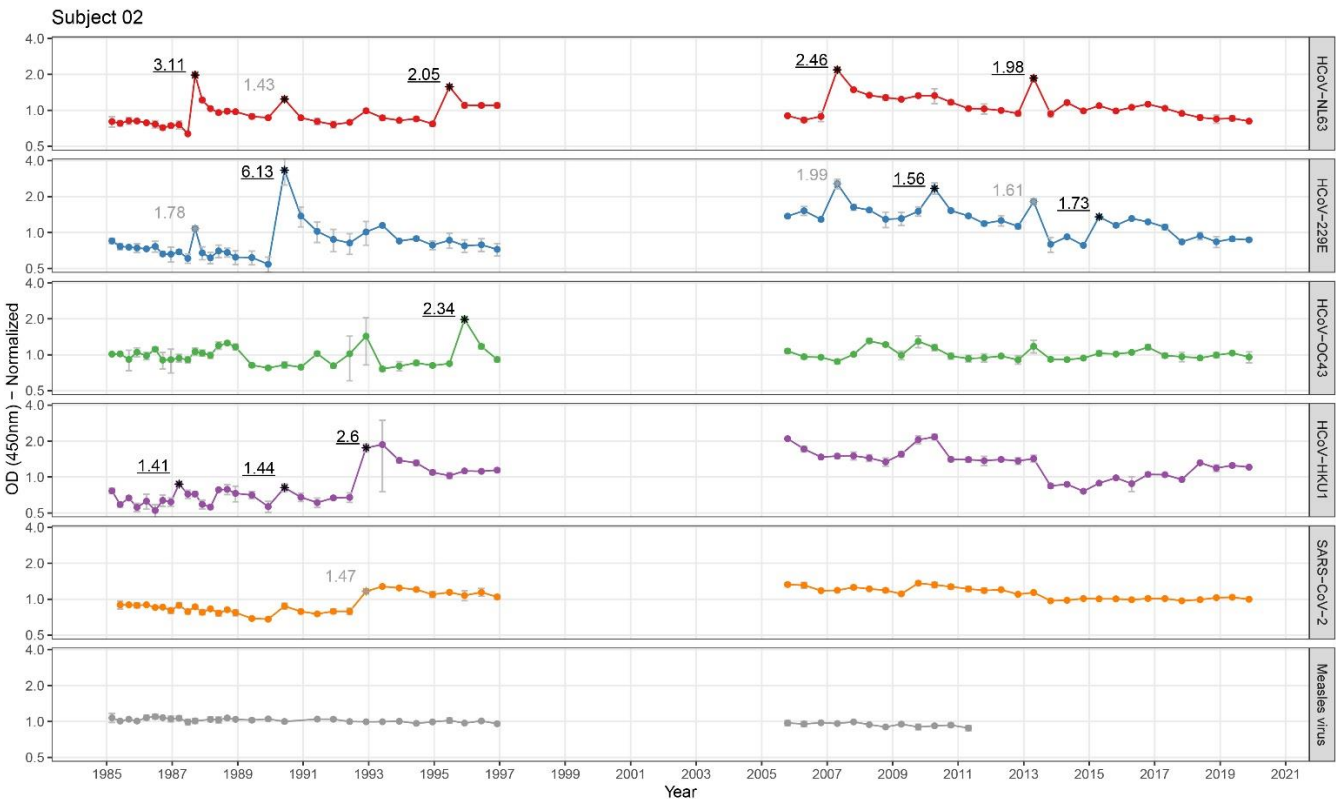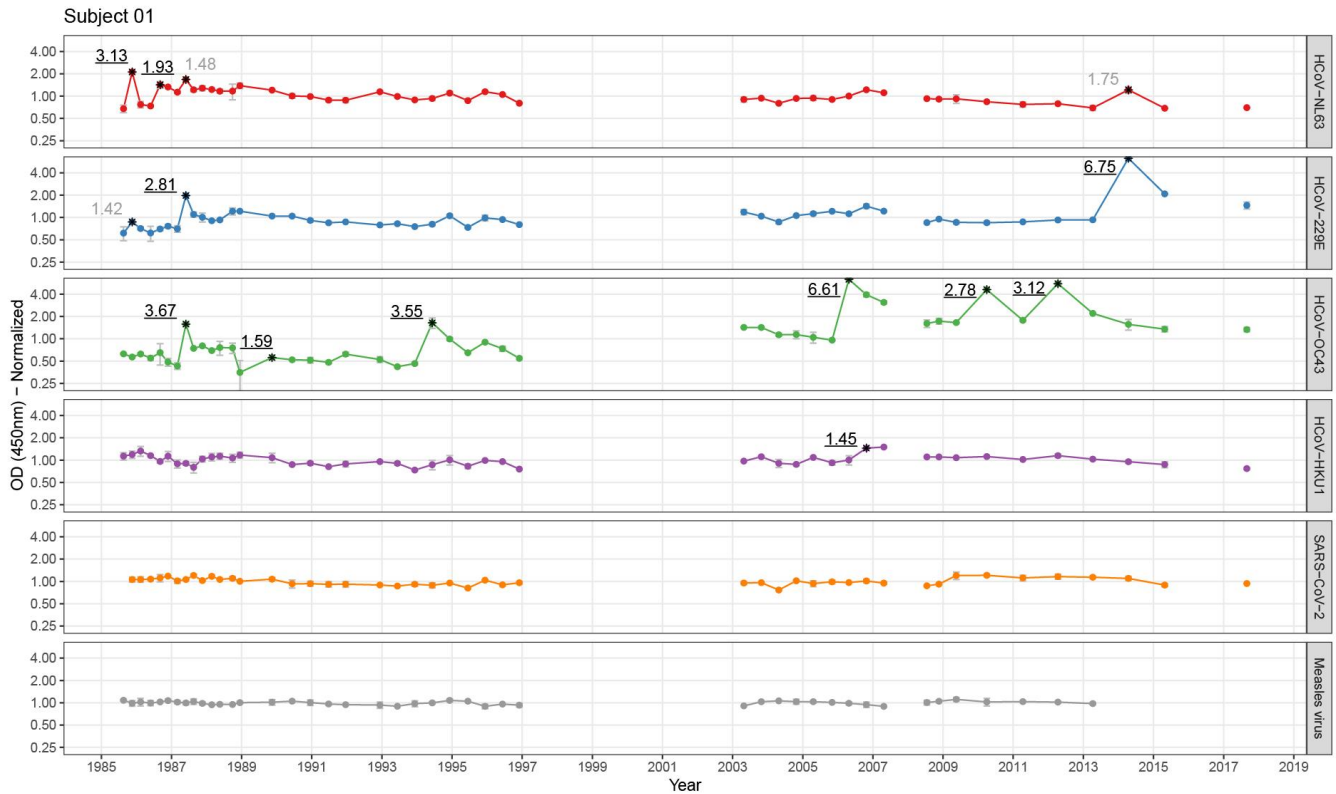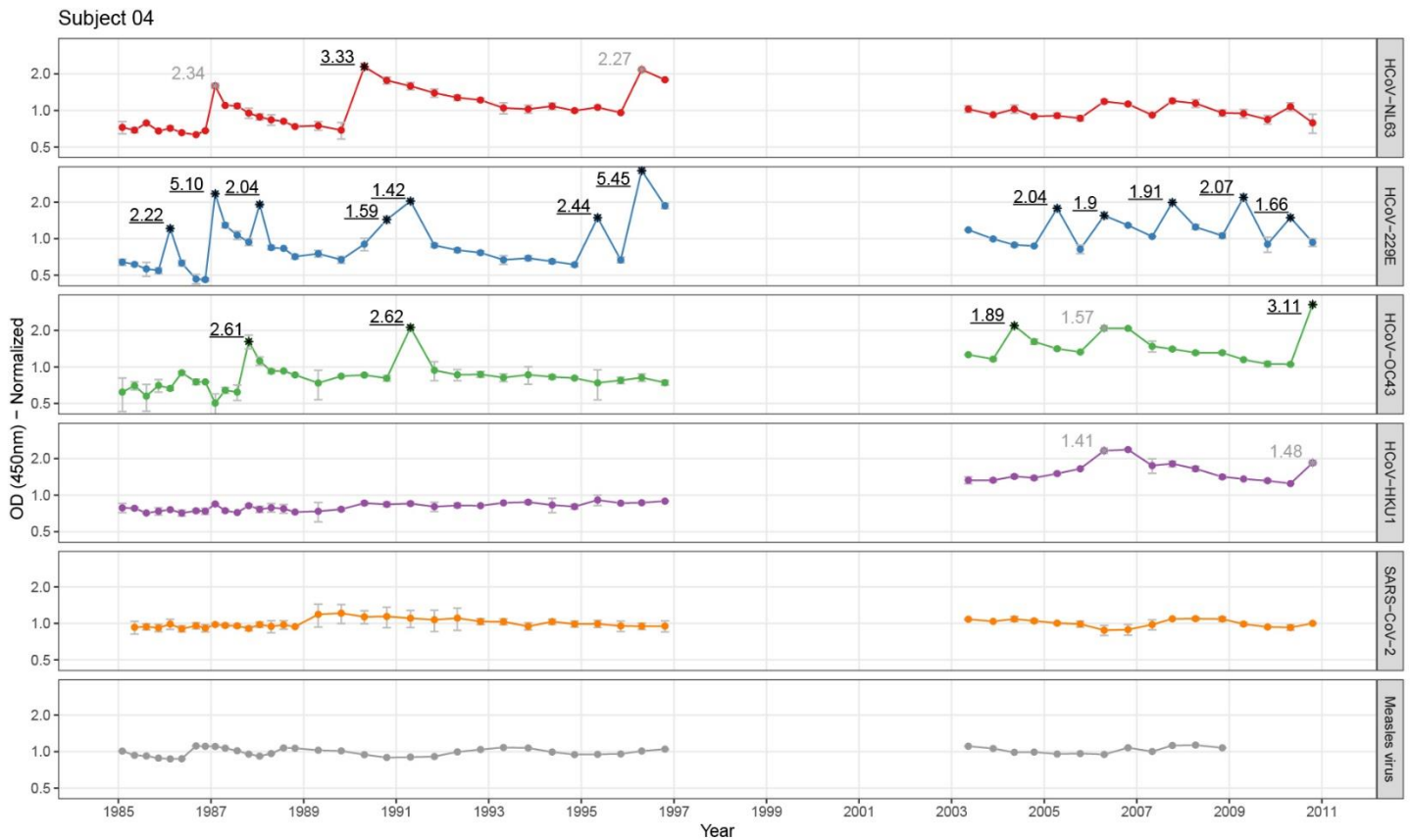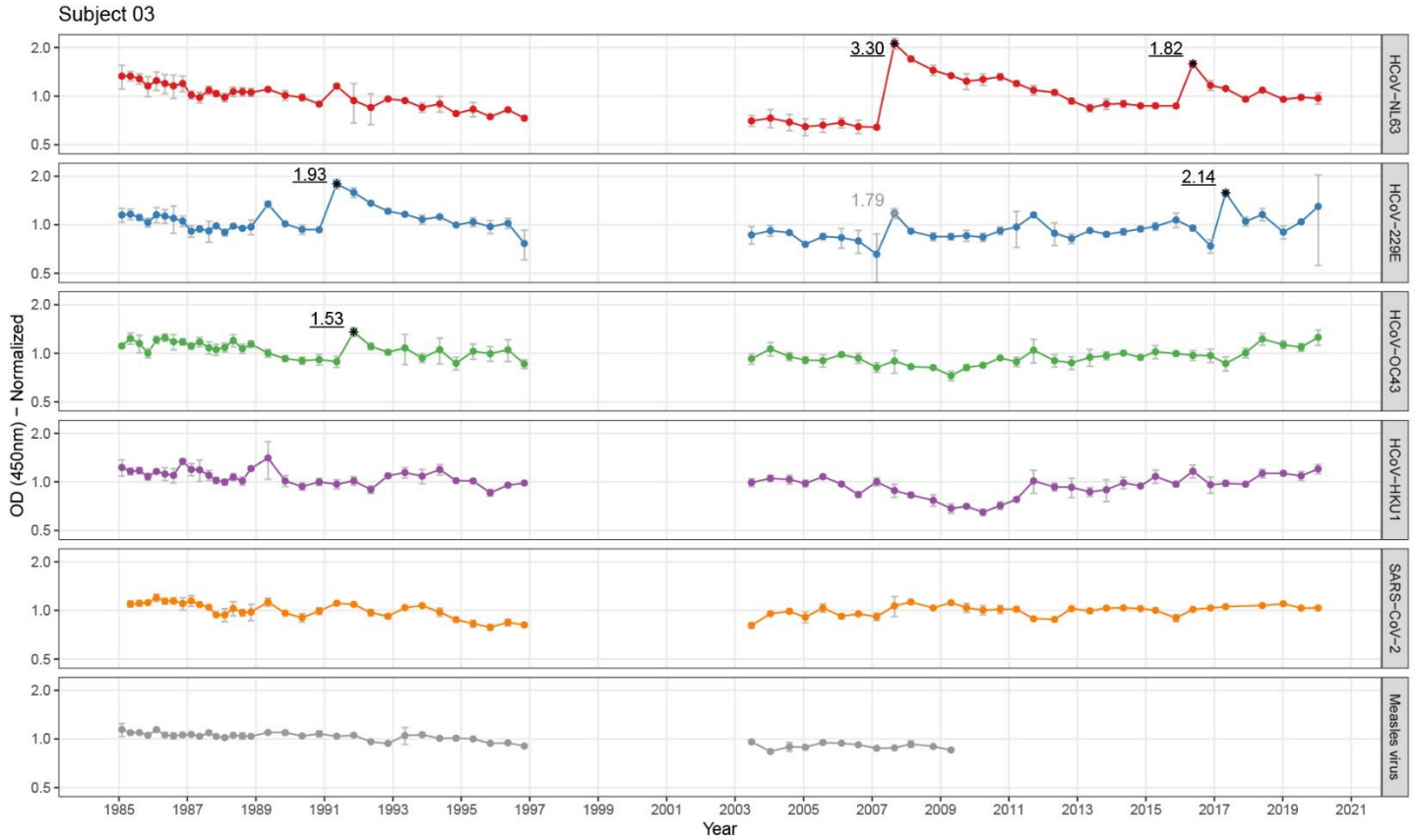
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Supplementary Fig. 1 continued

Subject 03

Subject 04

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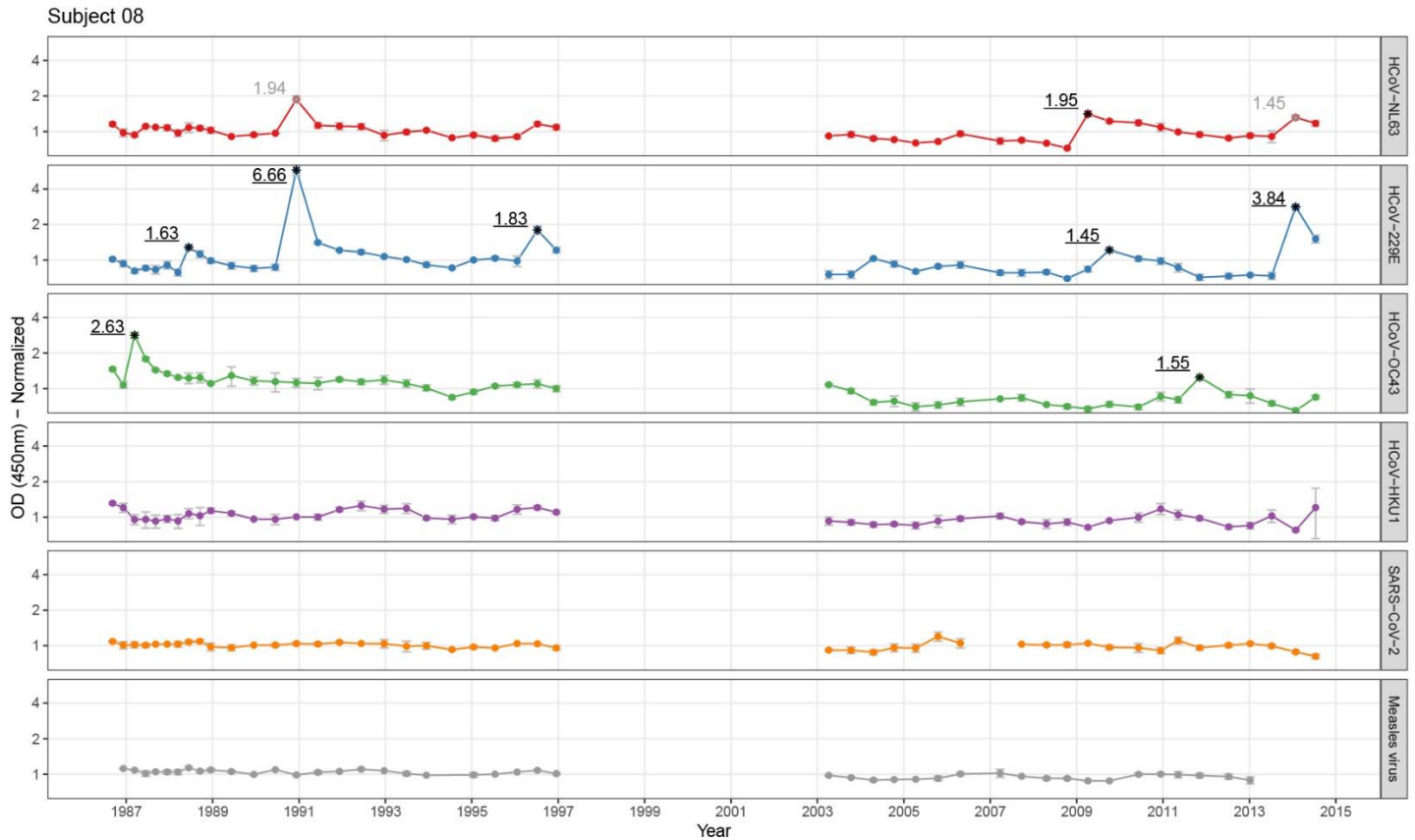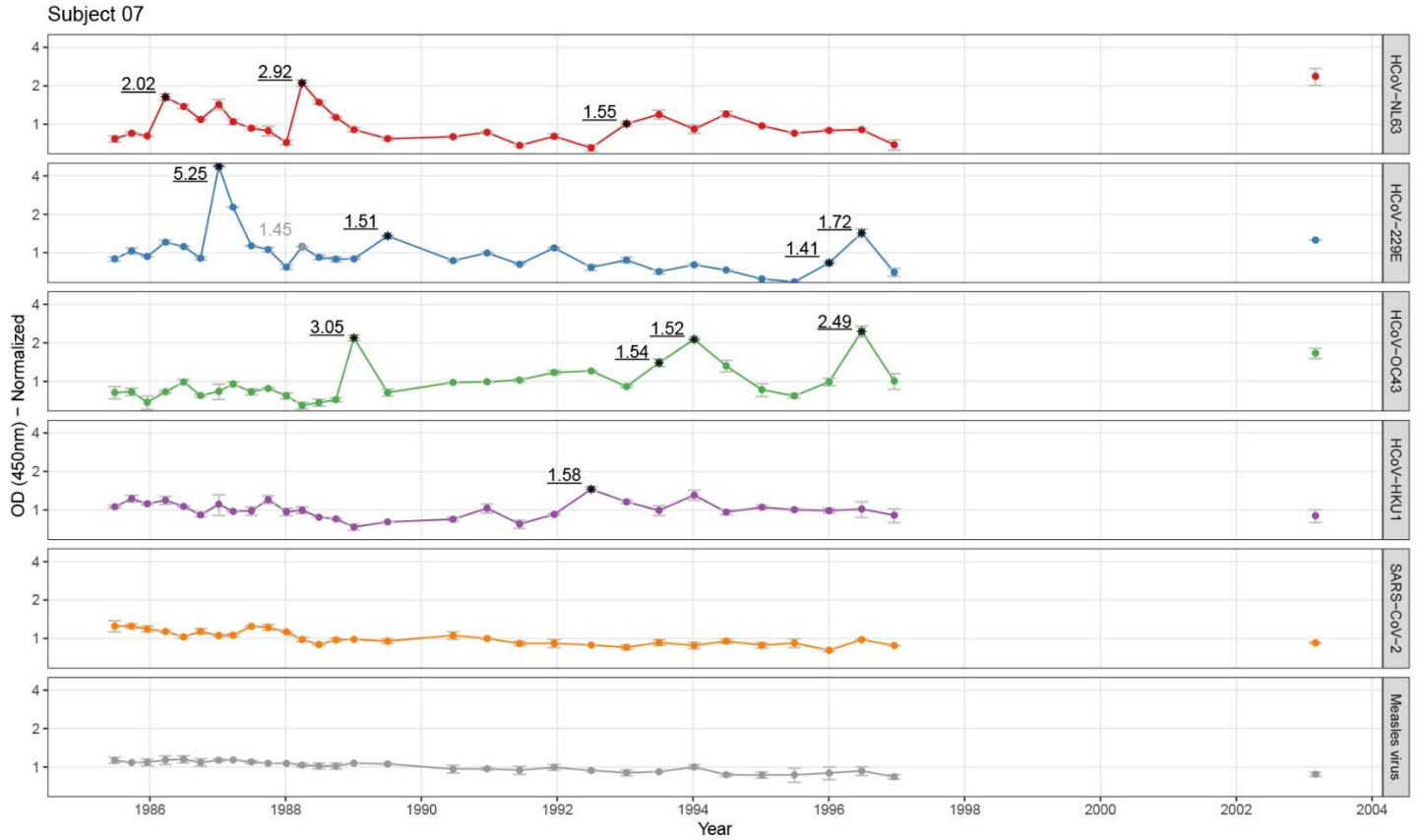**Supplementary Fig. 1 continued**

Subject 05

Subject 06

Supplementary Fig. 1 continued
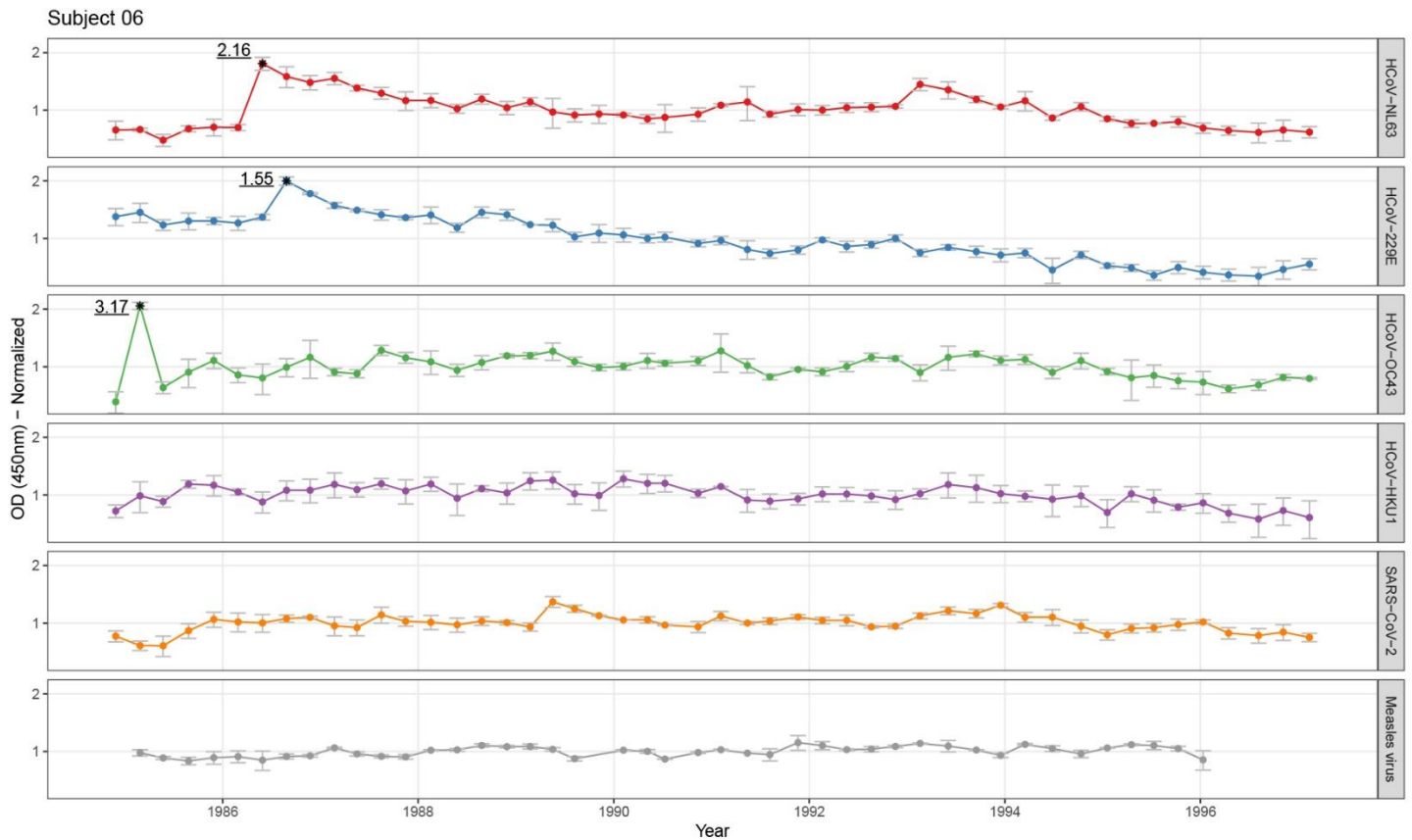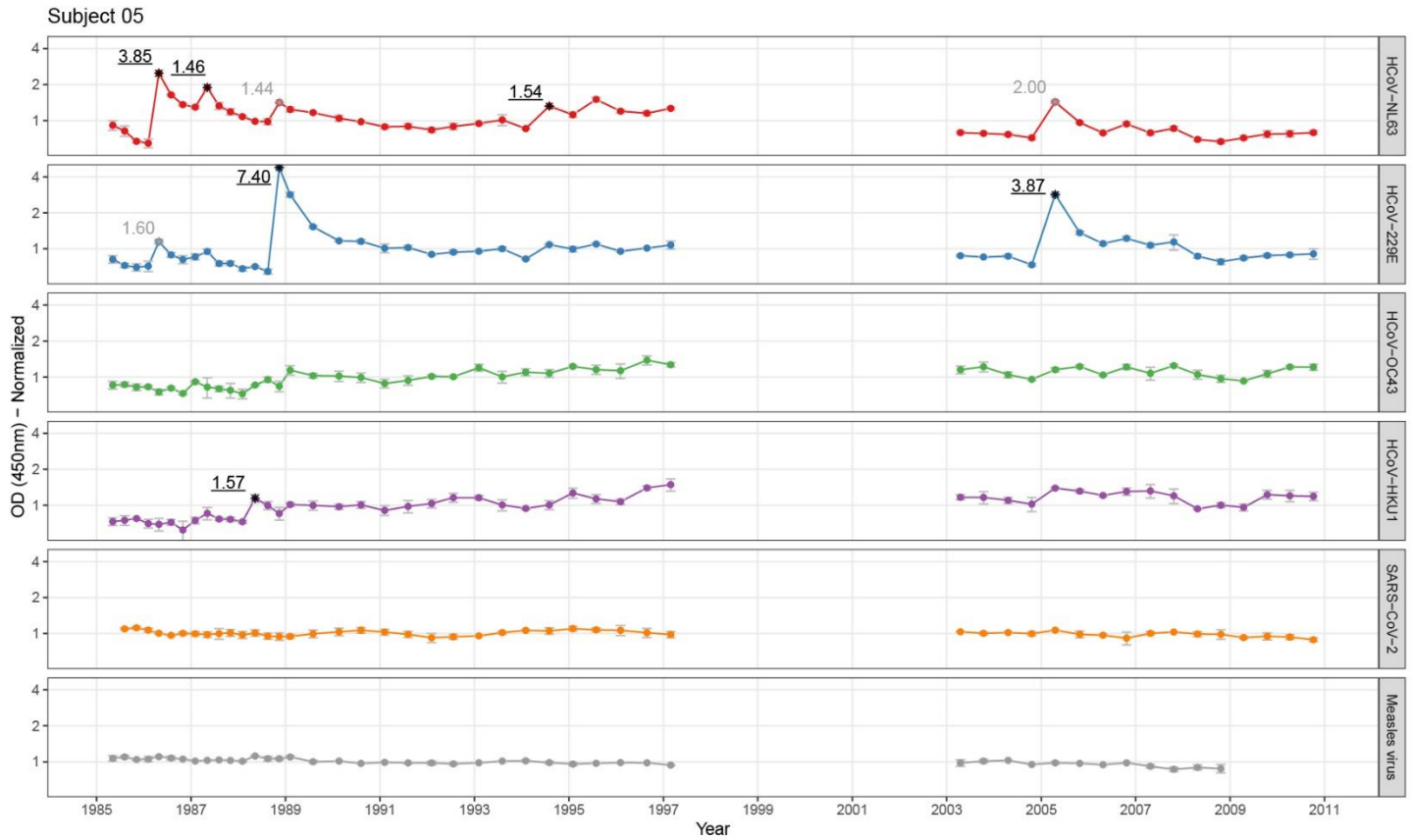
Subject 07

Subject 08
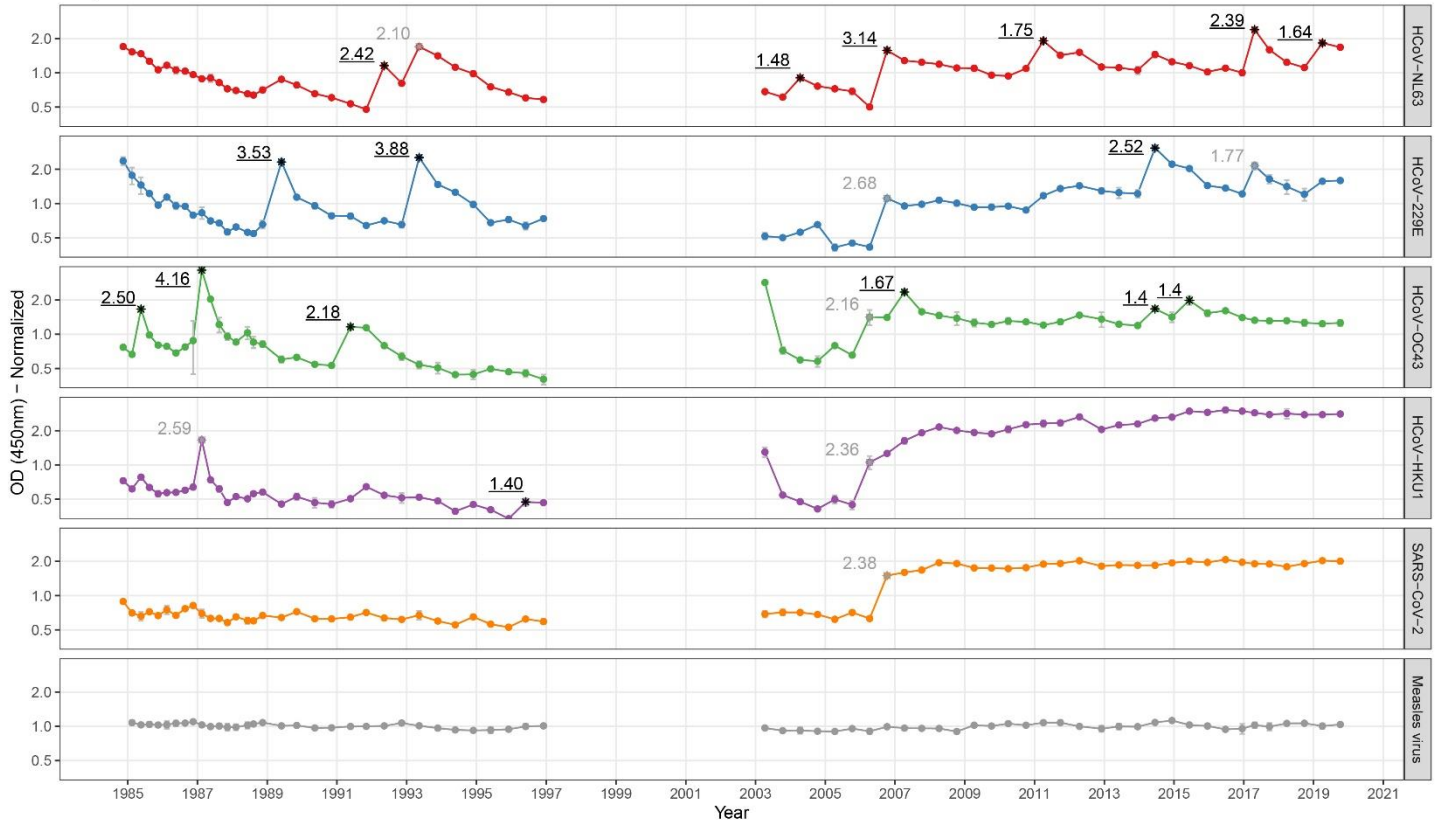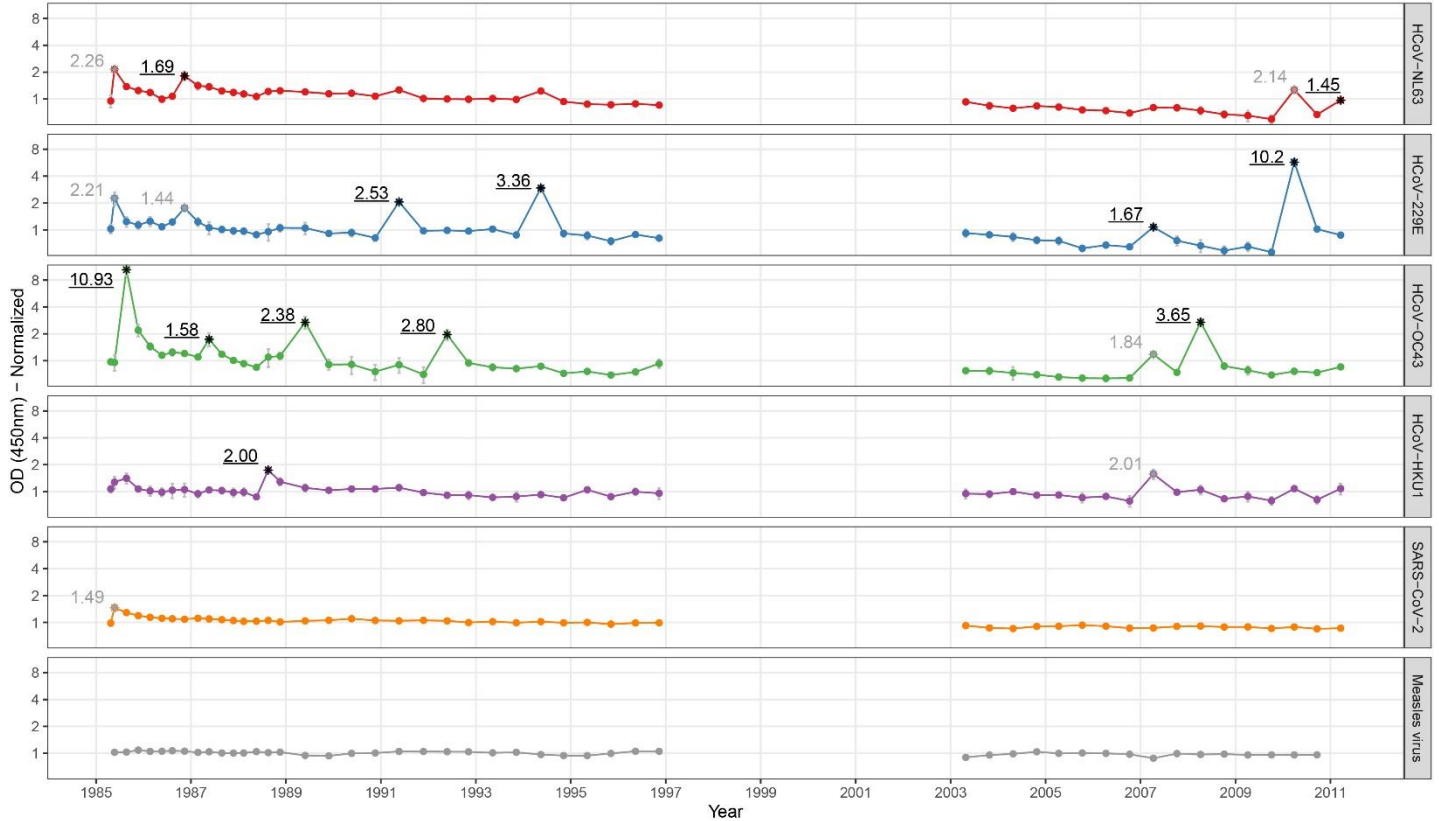
Supplementary Fig. 1 continued

Subject 09

Subject 10

Supplementary Table 1. Identity between coronaviruses for full N-protein and NCt-protein

FULL N-protein	HKU1	OC43	SARS-CoV-2	229E	NL63	Accession
HKU1	100%	-	-	-	-	ADN03343.1
OC43	**65.69%***	100%	-	-	-	AAR01019.1
SARS-CoV-2	32.55%	34.14%	100%	-	-	YP_009724397.2
229E	26.26%	23.65%	23.51%	100%	-	NP_073556.1
NL63	24.44%	24%	25.87%	**46.21%**	100%	YP_003771.1
NCt in this study						
HKU1	100%					
OC43	**49.59%**	100%				
SARS-CoV-2	<20%	<20%	100%			
229E	<20%	<20%	<20%	100%		
NL63	<20%	<20%	<20%	**41.77%**	100%	

* Full N-protein identity HCoV-OC43 vs HCoV-HKU1: 66%, and NCt identity 50%, so NCt as antigen in ELISA is more specific; Full N-protein identity HCoV-NL63 vs HCoV-229E: 46%, and NCt identity 42%, so NCt as antigen in ELISA is more specific

Supplementary Table 2. Within species diversity for the Spike protein and the N-protein. An identity matrix was made from representatives of all variants described in literature. Presented in the table is the identity shared between the most divergent strains within a seasonal coronavirus species.

Spike protein

Intraspecies variation HCoV-NL63:	97.01 % identity
Intraspecies variation HCoV-229E:	94.75 % identity
Intraspecies variation HCoV-OC43:	94.17 % identity
Intraspecies variation HCoV-HKU1:	84.66 % identity

N-protein

Intraspecies variation HCoV-NL63:	97.34 % identity
Intraspecies variation HCoV-229E:	95.39 % identity
Intraspecies variation HCoV-OC43:	97.09 % identity
Intraspecies variation HCoV-HKU1:	90.02 % identity

Supplementary Table 3. Cross-reactivity of antibodies within genera. Results of the anti-NCt ELISA for each seasonal coronavirus on paired sera of the RT-PCR confirmed coronavirus infections. In grey: blocks where cross reactivity within genera is shown. In bold: match between antibody fold change and RT-PCR of the same HCoV.

	Antibody rise (OD fold change >1.4)			
PCR positive	**NL63**	**229E**	**OC43**	**HKU1**
NL63 (n = 11)	**11 (100%)**	4 (36%)	0 (0%)	0 (0%)
229E (n = 16)	9 (56%)	**13 (81%)**	0 (0%)	1 (6%)
OC43 (n = 14)	0 (0%)	0 (0%)	**10 (71%)**	2 (14%)
HKU1 (n = 18)	1 (6%)	1 (6%)	1 (6%)	**4 (22%)**

Supplementary Table 4. Self-reported symptoms since previous visit

		No virus (n=398)	Any virus (n=93)	NL63 (n=23)	229E (n=37)	OC43 (n=29)	HKU1 (N=10)
Any symptom*	n (%)	69 (17.3%)	**26 (28.0%)**	**10 (43.5%)**	9 (24.3%)	6 (20.7%)	3 (30.0%)
	p-value**		**0.028**	**0.006**	0.396	0.811	0.414
Fever (>38 °C, > 3 days)	n (%)	23 (5.8%)	**12 (12,9%)**	**6 (26.1%)**	4 (10.8%)	2 (6.9%)	2 (20.0%)
	p-value		**0.024**	**0.003**	0.325	1.000	0.149
Fatigue	n (%)	20 (5.0%)	4 (4.3%)	2 (8.7%)	2 (5.4%)	0 (0.0%)	1 (10.0%)
	p-value		1.000	0.314	0.701	0.386	0.398
Cough	n (%)	25 (6.3%)	8 (8.6%)	4 (17.4%)	3 (8.1%)	0 (0.0%)	2 (20.0%)
	p-value		0.489	0.061	0.730	0.246	0.141
Sore throat	n (%)	10 (2.5%)	5 (5.4%)	2 (8.7%)	2 (5,4%)	1 (3.4%)	0 (0.0%)
	p-value		0.175	0.154	0.316	0.605	1.000
Headache	n (%)	5 (1.3%)	4 (4.3%)	1 (4.3%)	2 (5.4%)	1 (3.4%)	0 (0.0%)
	p-value		0.070	0.355	0.143	0.425	1.000
Dyspnoea	n (%)	16 (4.0%)	5 (5.4%)	1 (4.3%)	3 (8.1%)	0 (0.0%)	2 (20.0%)
	p-value		0.570	1.000	0.206	0.627	0.064
Myalgia	n (%)	12 (3.0)%)	5 (5.4%)	1 (4.3%)	1 (2.7%)	3 (10.3)	0 (0.0%)
	p-value		0.339	0.566	1.000	0.072	1.000
Nausea	n (%)	5 (1.3%)	2 (2.2%)	0 (0.0%)	1 (2.7%)	0 (0.0%)	1 (10.0%)
	p-value		0.622	1.000	0.426	1.000	0.135
Diarrhea	n (%)	5 (1.3%)	4 (4.3%)	0 (0.0%)	1 (2.7%)	2 (6.9%)	1 (10.0%)
	p-value		0.070	1.000	0.510	0.094	0.153

* Methods of data collection: During the cohort visit, participants were asked whether they had experienced the following symptoms since their last visit: fever (temperature >38 ˚C for at least 3 days), fatigue, cough, sore throat, headache, dyspnea, myalgia, nausea, or diarrhea.
** p-value results from Fisher's exact test. Comparison group = 'no virus (n=398)'