

1 **Typically asymptomatic but with robust antibody formation: Children's unique**
2 **humoral immune response to SARS-CoV-2**

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49 **Running Title:** SARS-CoV-2 serology in children

50

51 **Competing Interests**

52 NSM was a speaker at Luminex user meetings in the past. The Natural and Medical

53 Sciences Institute at the University of Tübingen is involved in applied research projects as a

54 fee for services with Luminex. The other authors report no competing interests.

55

56 **Abstract**

57 *Background:* Long-term persistence of antibodies against SARS-CoV-2, particularly the
58 SARS-CoV-2 Spike Trimer, determines individual protection against infection and potentially
59 viral spread. The quality of children's natural humoral immune response following SARS-
60 CoV-2 infection is yet incompletely understood but crucial to guide pediatric SARS-CoV-2
61 vaccination programs.

62 *Methods:* In this prospective observational multi-center cohort study, we followed 328
63 households, consisting of 548 children and 717 adults, with at least one member with a
64 previous laboratory-confirmed SARS-CoV-2 infection. The serological response was
65 assessed at 3-4 months and 11-12 months after infection using a bead-based multiplex
66 immunoassay for 23 human coronavirus antigens including SARS-CoV-2 and its Variants of
67 Concern (VOC) and endemic human coronaviruses (HCoVs), and additionally by three
68 commercial SARS-CoV-2 antibody assays.

69 *Results:* Overall, 33.76% of SARS-CoV-2 exposed children and 57.88% adults were
70 seropositive. Children were five times more likely to have seroconverted without symptoms
71 compared to adults. Despite the frequently asymptomatic course of infection, children had
72 higher specific antibody levels, and their antibodies persisted longer than in adults (96.22%
73 versus 82.89% still seropositive 11-12 months post infection). Of note, symptomatic and
74 asymptomatic infections induced similar humoral responses in all age groups. In
75 symptomatic children, only dysgeusia was found as diagnostic indicator of COVID-19. SARS-
76 CoV-2 infections occurred independent of HCoV serostatus. Antibody binding responses to
77 VOCs were similar in children and adults, with reduced binding for the Beta variant in both
78 groups.

79 *Conclusions:* The long-term humoral immune response to SARS-CoV-2 infection in children
80 is robust and may provide long-term protection even after asymptomatic infection.

81 (Study ID at German Clinical Trials Register: 00021521)

82 **Introduction**

83 To date, our knowledge of children's immune response to infection with severe acute
84 respiratory syndrome coronavirus type 2 (SARS-CoV-2) remains incomplete. In light of
85 current debates on vaccination strategies and nonpharmaceutical preventative measures
86 (e.g. school closures), a comprehensive understanding of protective immunity after natural
87 infection in children is required. As with other viral infections, immune control of SARS-CoV-2
88 is achieved through a concerted interplay of humoral and cellular immunity (reviewed in ^{1,2}).
89 Neutralizing antibodies in children are of particular interest in this context, given their role in
90 blocking virus entry into cells by inhibiting the interaction between the viral receptor binding
91 domain (RBD) within the S-glycoprotein and the angiotensin-converting enzyme 2 (ACE2)
92 receptor³.

93 Previous longitudinal studies of the humoral response have found that neutralizing antibodies
94 peak within 3-5 weeks post-infection with a calculated half-life of up to 8 months, suggesting
95 long-term protection in convalescent individuals^{1,4-6}. However, most studies only included
96 adults, and longitudinal studies on SARS-CoV-2 infections in children had limited sample
97 size and duration of follow-up post-infection⁷⁻¹⁷. Furthermore, it remains unclear as to
98 whether any form of cross-protection is offered by endemic human coronaviruses (HCoVs)
99 that regularly circulate in the pediatric population, with some studies identifying cross-
100 protection and others not.^{18,19}

101 To provide an in-depth characterization of the humoral response in children, we initiated a
102 multi-center longitudinal study, encompassing 328 households each with at least one SARS-
103 CoV-2-infected member, which were followed for up to 12 months after the first infection in
104 each household. This cohort is unique as the subjects exhibited mainly asymptomatic or mild
105 disease with uninfected family members serving as environmental and age-matched
106 controls. We performed an extensive serological evaluation of SARS-CoV-2 infection in all
107 household members, comprising analyses of production of antibodies against various SARS-
108 CoV-2 antigens, including Variants of Concern (VOCs), production of neutralizing antibodies
109 and the role of endemic human coronaviruses (HCoVs).

110 **Materials and Methods**

111 Cohort

112 This study forms part of a non-interventional, prospective observational national multi-center
113 cohort study, including 553 children and 726 adults from 328 households each with at least
114 one individual with a SARS-CoV-2 reverse-transcriptase polymerase chain reaction (RT-
115 PCR) proven infection and/or a symptomatic and later serologically proven infection.
116 Participants were recruited during the first wave of the pandemic (May to August 2020) via
117 local health authorities and an in-hospital database of households with at least one
118 laboratory-confirmed SARS-CoV-2 infection. A full list of the inclusion and exclusion criteria
119 are in the **Supplementary Appendix**.

120 Study oversight

121 This part of the study was conducted by the University Children's Hospitals in Freiburg,
122 Tübingen and Ulm, Germany. Ethics approval was obtained from the respective Medical
123 Faculties' independent ethics committees (University of Freiburg: 256/20_201553; University
124 of Tübingen: 293/2020BO2; University of Ulm: 152/20). Written informed consent was
125 obtained from adult participants and from parents or legal guardians on behalf of their
126 children at both sampling time points. Children's preferences on whether or not to provide a
127 blood sample were respected throughout. This study was registered at the German Clinical
128 Trials Register (DRKS), study ID 00021521, conducted according to the Declaration of
129 Helsinki, and designed, analyzed and reported according to the Strengthening the Reporting
130 of Observational Studies in Epidemiology (STROBE) reporting guidelines.

131 Sample collection

132 Samples were collected at two separate time points, an early time point (T1) at a median of
133 109 days (IQR 67-122 days) after earliest symptom onset in household and a late time point
134 (T2) at 340 days (IQR 322-356 days) post-symptom onset (**Table 1, Figure S1 in**
135 **Supplementary Appendix**).

136 Serological assays

137 Antibodies against SARS-CoV-2 in 2236 samples were detected using the following four
138 assays: (1) EuroImmun-Anti-SARS-CoV-2 ELISA IgG and IgA (S1), (2) Siemens
139 Healthineers SARS-CoV-2 IgG (RBD), (3) Roche Elecsys Ig (N) and (4) MULTICOV-AB, a
140 previously published bead-based multiplex immunoassay that simultaneously analyses
141 antibody binding to 23 antigens from SARS-CoV-2 (including VOCs)^{20,21}. Seropositivity was
142 defined as any three of the four SARS-CoV-2 assays being positive. The MULTICOV-AB
143 assay also analyses antibody binding to endemic coronavirus antigens (i.e. HCoV-OC43, -
144 NL63, -HKU1 and -229E)^{20,21}. 385 samples were analyzed with the GenScript SARS-CoV-2
145 Surrogate Virus Neutralization Test (sVNT). See the **Supplementary Appendix** for details
146 on the assay methodology.

147 Data collection

148 Children and adults from eligible households were asked to provide information on
149 demography and presence of symptoms (fever, cough, diarrhea or dysgeusia) in temporal
150 relation to RT-PCR-proven or symptomatic and later serologically proven SARS-CoV-2
151 infection within the household. The four symptoms were chosen based on previous research
152 and public health advice at the time. “Symptomatic infections” were defined by seropositivity
153 and a history of at least one of these four symptoms in temporal relationship to exposure.
154 Seropositivity in individuals who did not show any of these four symptoms was defined as
155 “asymptomatic infection”. For most households, the earliest confirmed PCR-positive infection
156 (“index case”) could be determined (see **Supplementary Appendix** for further information).

157 Statistical Analysis

158 All data analysis was performed in RStudio (Version 1.2.5001), running R (version 3.6.1) with
159 the additional packages “beeswarm”, “Rcolorbrewer”, “gplots” and “VennDiagram”. The type
160 of statistical analysis performed and (where appropriate) the subset of the study population
161 used is listed in the figure legends. Between-group differences of continuous endpoints were
162 analyzed using Mann-Whitney-U tests while correlation was analyzed using the Spearman

163 rank. All analyses were exploratory in nature and p-values may not be interpreted as
164 confirmatory. A comprehensive material and methods section including detailed protocols
165 can be found in the **Supplementary Appendix**.

166

167 **Results**

168 548 children and 717 adults from 328 households were examined at T1 and 279 households
169 including 402 children and 569 adults were followed to T2 (see Methods and Appendix for full
170 details, **Table 1** for a description of the study population, **Figure S2** in the **Supplementary**
171 **Appendix** for details on the age structure of the study population). Children were
172 substantially less often seropositive (33.76% at T1, 37.56% at T2) than adults (57.88% at T1,
173 49.56% at T2) (**Table 1**). Seropositive participants were almost exclusively mildly or
174 asymptotically infected. In seropositive individuals, asymptomatic infections were five
175 times more common in children (44.86% T1, 45.70% T2) than in adults (8.67% T1, 10.99%
176 T2) (**Table 1**), with the proportion of asymptomatic infections decreasing with increasing age
177 (**Figure S3**). Overall, hospitalization was rare (3.61% of adults, 0% of children, **Table 1**). The
178 performance of the four serological assays for children and adults at T1 and T2 is shown in
179 **Table S1 and Figure S4**.

180 The detailed humoral immune response against different SARS-CoV-2 antigens, assessed
181 by MULTICOV-AB is shown in **Figure 1**. Children had significantly higher antibody titers
182 against spike ($p < 0.001$), RBD ($p < 0.001$), S1 domain ($p < 0.001$) and nucleocapsid ($p = 0.01$)
183 compared to adults at T1. This increased response was confirmed by the three commercial
184 assays (**Figure S5**). In addition, we observed a large difference in seroreversion, with only
185 3.78% of children, but 17.11% of adults seroreverting between T1 and T2 (**Table 1**).
186 Seroreversion was not associated with the response to particular antigens, although the
187 largest and smallest decay in antibody concentrations were observed for antibodies against
188 the S2 domain and nucleocapsid, respectively, regardless of age (**Figure S6**).

189 For both children and adults, there was no significant difference in antibody response
190 between symptomatic and asymptomatic infections (**Figure 2a and b, Figure S7**). The
191 frequency of reported symptoms differed between adults and children and the predictive
192 value of each symptom varied between both groups (**Figure 2c and d**). While any of the
193 symptoms fever, cough, diarrhea or dysgeusia proved to be a good indicator of infection in

194 adults, dysgeusia was by far the best predictive symptom in children (87.50% of children with
195 dysgeusia were seropositive; 95% CI 71.39%-95.15%, 30.52% of children without dysgeusia
196 were seropositive for SARS-CoV-2, 95% CI 29.74%-31.30% **Figure 2d**). Conversely, cough
197 was a poor predictor of SARS-CoV-2 infection in children (37.37% of children with a cough
198 were seropositive; 95% CI 29.25%-46.28%, 33.04% of children without a cough were
199 seropositive; 95% CI 30.99%-35.15%, **Figure 2d**). Further examination of predictive
200 symptoms among children showed that in contrast to dysgeusia, cough only gained
201 predictive value in children above the age of 12 and the predictive value of fever increased
202 with age (**Table S2**). There was no difference in the humoral response associated with the
203 presence of particular symptoms in either adults or children (**Figure S8**).

204 To further explore differences in the antibodies produced by children and adults, we analyzed
205 their neutralization potential in a surrogate assay (sVNT) as well as their binding towards
206 VOCs. The neutralizing potential of children's sera exceeded that of adults' at T1 ($p < 0.001$)
207 and T2 ($p = 0.02$) (**Figure 3a**). However, this could be attributed to antibody titers, as
208 neutralization in children correlated with the S1-directed antibody response (Spearman's
209 rank 0.86, **Figure 3b**). There was no difference in antibody binding responses to the RBD of
210 Alpha and Beta VOCs between adults and children, with an identical binding for the Alpha
211 variant compared to wild-type (Spearman's rank 0.95, **Figure 3c**) and a reduction in binding
212 for the Beta variant (Spearman's rank 0.69, **Figure 3d**).

213 Seroprevalence against endemic coronaviruses rose sharply with age in early childhood, and
214 was stable in older children, adolescents and adults independent of age (**Figure 4a, Figure**
215 **S9**). In contrast to SARS-CoV-2 seroreversion, HCoV antibody titers decreased faster in
216 younger children than in adults (**Figure S10**). There were HCoV naïve samples in this cohort
217 and some individuals showed a substantial increase in HCoV antibody response indicating
218 exposure towards endemic HCoVs between the two time points (**Figure 4b in red, Figure**
219 **S11**). Amongst SARS-CoV-2 exposed individuals in households with a defined index case
220 (index cases excluded from the analysis, see Methods), there was no difference in HCoV
221 antibody titers between SARS-CoV-2 seropositive and seronegative children or adults

222 (p=0.21, **Figure 4c, Figure S12**). In addition, we assessed whether SARS-CoV-2 infection
223 boosted HCoV antibody responses, however there was no evidence for an association
224 between HCoV antibody responses and SARS-CoV-2 antibody responses in exposed
225 children or adults (Spearman's rank 0.03, **Figure 4d**).

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229 Discussion

230 To our knowledge, this is the largest prospective multi-center study comprehensively
231 comparing the adult and pediatric longitudinal humoral immune response following SARS-
232 CoV-2 household exposure. As the humoral immunity against SARS-CoV-2 is now
233 increasingly accepted as the central correlate of protection²²⁻²⁴, improving our incomplete
234 understanding in children^{25,26} is of considerable value for public health and vaccination
235 strategies. Importantly, our outpatient cohort has high epidemiological relevance, as a mild
236 course is the most frequent outcome of SARS-CoV-2 infection overall²⁷. Our findings identify
237 several unique features of the pediatric serological immune response against SARS-CoV-2.

238 Children had a lower seroprevalence after household exposure and seropositivity followed
239 asymptomatic infection more frequently than in adults. This is in agreement with our previous
240 report of a different cohort consisting of parent-child pairs²⁸. In light of potential pediatric
241 vaccination campaigns, children's humoral response to SARS-CoV-2 is markedly increased
242 in both quantity and longevity, with children seroreverting significantly slower than adults.
243 Children generated a higher titers of SARS-CoV-2 antibodies than their parents after being
244 exposed to the same viral strain, and antibody titers are negatively correlated with age. Of
245 particular interest are the increase in antibodies produced against the S1 domain and RBD,
246 both of which are associated with higher neutralization capacity, indicating that children
247 produce a high quality humoral response against SARS-CoV-2^{3,22,29}. The quality of the
248 pediatric humoral response is further illustrated by the similar binding capacity against the
249 SARS-CoV-2 Alpha and Beta VOCs compared to adults. Children also had significantly
250 higher neutralizing antibody titers than adults, indicating increased protection. This increase
251 in neutralization was directly correlated with higher antibody titers in the other assays, and
252 therefore may not be due to substantial qualitative changes of the pediatric antibody profiles.
253 These findings are in line with one preprinted study³⁰ but in contrast to two previous studies,
254 which found that children generated a lower humoral response to SARS-CoV-2 than adults,
255 with a corresponding reduction in neutralization activity^{11,13}. However, compared to our
256 cohort, all three studies were substantially smaller in sample size and the latter two

257 investigated a different disease spectrum comprising mostly hospitalized children or those
258 diagnosed with hyperinflammatory MIS-C syndrome, and sampled blood at earlier time
259 points after presumed infection.

260 It is striking that antibody levels in seropositive individuals were independent of fever, cough
261 or diarrhea, as clinical proxies for systemic or localized inflammation of the respiratory or
262 gastrointestinal tract, respectively. Previous studies have reported a clear correlation
263 between disease severity and neutralizing antibody titers in adults^{6,31}. At the other end of the
264 disease spectrum with mildly affected younger adults and children, this association was not
265 detectable irrespective of age. This diverges from the classical infection immunology dogma
266 that systemic pathogen-host interaction is required for the generation of robust immune
267 memory. While titers themselves did not differ between asymptomatic and symptomatic
268 infections, we found substantial differences in titers between adults and children. Presence of
269 any symptom was predictive of seropositivity in adults, whereas children showed substantial
270 differences in both the prevalence of symptoms in seropositive individuals and the predictive
271 values of symptoms with respect to SARS-CoV-2 seropositivity. Since cough was a relatively
272 common symptom in children irrespective of seroconversion, it was not useful in predicting
273 SARS-CoV-2 infection. In contrast, dysgeusia, an infrequent symptom among children, was
274 highly accurate in predicting infection. These findings suggest that symptom criteria used for
275 subsequent PCR testing need to be different for children and adults.

276 Similarly to other authors^{32,33}, we identified that exposure to HCoVs, as measured by
277 seropositivity typically happens within the first five years of life. The relatively small decline of
278 HCoV antibody levels during the study period, especially in the adult population, in
279 comparison to the decline in SARS-CoV-2 antibody levels after a single infection suggests
280 that long-term serological immunity against HCoVs may be driven by recurrent exposure. We
281 observed HCoV infections in previously naïve individuals, indicating that endemic HCoVs still
282 circulated between T1 and T2 despite SARS-CoV-2 related distancing and hygiene
283 measures. Although cross-reactivity and/or cross-protection between SARS-CoV-2 and
284 HCoVs have been hypothesized, our analyses did not find evidence for such effects. For

285 both Alpha- and Beta-coronaviruses, HCoV antibody responses were not associated with a
286 lower likelihood of seroconversion following SARS-CoV-2 exposure. Along with frequent
287 HCoV seronegativity in younger childhood, this strongly argues that the lower incidence of
288 SARS-CoV-2 infection in children is not due to HCoV cross-protection. Moreover, there was
289 no evidence for boosting of HCoV titers following SARS-CoV-2 infection. In contrast to other
290 studies which did identify an effect for endemic HCoV infection, our cohort is composed of
291 intensely exposed individuals from within the same households, which is a substantial
292 strength compared to previous studies that have used pre-pandemic sera or indirect control
293 groups^{18,30,34}.

294 Limitations of our study include the potential recall-bias inherent to retrospective self- or
295 parent-reporting of symptoms via questionnaires and physician-interviews. Additionally, PCR
296 tests for SARS-CoV-2 during the first wave in Germany were mostly limited to the household
297 index case, meaning it is possible that infected individuals were not identified as such,
298 despite the multi-assay serological approach. However, the in-depth characterization of the
299 humoral response provides valuable data for clinicians, public health officials and the public,
300 at a time when children are increasingly viewed as a potential viral reservoir due to exclusion
301 of pediatric populations from current vaccination strategies. Similarly, while PCR testing was
302 not available for all individuals, the strength of this cohort comes from the comparatively
303 large number of children, inclusion of children and adults from the same household, the
304 inclusion of seronegative household members as well-matched controls, and the prospective
305 longitudinal analysis of the humoral response in children for up to one year post-infection.
306 The seropositive cohort also comprises almost exclusively individuals with mild or
307 asymptomatic infections and so provides real-world data representative for the majority of
308 SARS-CoV-2 infections in the community.

309 In summary, although children mostly show mild or even asymptomatic clinical courses
310 following SARS-CoV-2 infection, they mount a strong and enduring humoral immune
311 response. This strongly argues for sustained protection after infection, and might inform the
312 design of vaccination strategies for SARS-CoV-2 convalescent children.

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- 404

405 **Figure Legends**

406 **Figure 1: Children have a significantly higher humoral response to SARS-CoV-2 than**
407 **adults.** The humoral response generated following SARS-CoV-2 household exposure with
408 seroconversion was examined using MULTICOV-AB. Children (n=181) produced significantly
409 more antibodies against the Spike (**a**, $p<0.001$), RBD (**b**, $p<0.001$), S1 domain (**c**, $p<0.001$)
410 and nucleocapsid (NC) (**e**, $p=0.01$) than adults (n=414). There was no significant difference
411 for either the S2 domain (**d**, $p=0.66$) or the N-terminal domain of the nucleocapsid (NC NTD)
412 (**f**, $p=0.40$). Only samples from T1 with a seropositive status (see Methods) are shown. Box
413 and whisker plots with the box representing the median, 25th and 75th percentiles, while
414 whiskers show the largest and smallest non-outlier values. Outliers were identified using
415 upper/lower quartile \pm 1.5 times IQR. Statistical significance was calculated using Mann-
416 Whitney-U (two-sided) with significance defined as being $*<0.01$, $***<0.001$

417 **Figure 2: SARS-CoV-2 infections in children are more often asymptomatic than in**
418 **adults, although dysgeusia is a good indicator of SARS-CoV-2 infection in both adults**
419 **and children.** Box and whisker plots showing that there is no difference in antibody
420 response between asymptomatic and symptomatic SARS-CoV-2 infections in adults (**a**,
421 $p=0.684$, $n=415$) or children (**b**, $p=0.712$, $n=185$). Boxes represent the median, 25th and 75th
422 percentiles, while whiskers show the largest and smallest non-outlier values. Outliers were
423 identified using upper/lower quartile \pm 1.5 times IQR. Statistical significance was calculated
424 using Mann-Whitney-U (two-sided). The four symptoms reported in this study were then
425 examined for their frequency within the study population (**c**), with all symptoms more
426 commonly reported in seropositive adults than seropositive children. Each symptom was
427 then examined for its predictive ability to indicate SARS-CoV-2 infection (**d**), with dysgeusia a
428 strong predictor in both adults (84.18%) and children (87.50%). All other symptoms were
429 poor predictors in children (fever 59.46%, cough 37.37%, diarrhea 54.55%) compared to
430 adults (fever 85.77%, cough 75.03%, diarrhea 80.65%). Only samples from T1 were
431 analyzed for this figure (n=717 adults, 548 children). “+” indicates presence of the symptom
432 “-“ indicates absence of the symptom.

433 **Figure 3 - Children and adults produce antibodies with equal neutralizing potential and**
434 **their antibodies offer the same protection against Variants of Concern.** (a) Box and
435 whisker plot showing that antibodies produced by children (n=118) have a significantly higher
436 inhibition of ACE2 binding than those produced by adults (n=267, $p < 0.001$) at T1 and T2
437 ($p = 0.02$, child n=59, adult n=106) as determined by the sVNT assay. Boxes represent the
438 median, 25th and 75th percentiles, while whiskers show the largest and smallest non-outlier
439 values. Outliers were identified using upper/lower quartile ± 1.5 times IQR. Statistical
440 significance was calculated using Mann-Whitney-U (two-sided) with *** indicating a p-value
441 < 0.001 . To determine whether this was due to the higher titers in children, SARS-CoV-2 S1
442 humoral response was determined using MULTICOV-AB for T1 and plotted against the
443 results of the sVNT assay, with Spearman's rank used to determine the correlation (b),
444 confirming that the increase in neutralization is due to higher titers. Protection against the
445 Alpha (c) and Beta (d) VOCs was determined by MULTICOV-AB and plotted as a linear
446 regression against the antibody binding response to the wild-type (wt) RBD, with Spearman's
447 rank used to determine the correlation. There was no difference in antibody response
448 between children (n=166, T1 samples only) and adults (n=381, T1 samples only) for either
449 variant.

450 **Figure 4: HCoV offer no protection against SARS-CoV-2, nor do they show a boost-**
451 **back antibody response following SARS-CoV-2 infection.** Samples from households with
452 a known index case were examined with MULTICOV-AB to determine whether the antibody
453 response to endemic coronaviruses (HCoV) provides any protection against infection with
454 SARS-CoV-2. Initial screening of the population showed that seroprevalence increases with
455 age, although several samples were within the blank range of the HCoV assays, indicating
456 the presence of naïve samples (a). Naïve samples were defined as those having less than
457 one-tenth the mean antibody response (indicated by dotted line), with the majority of these
458 samples occurring in children under the age of 5. (b) Line graph showing the longitudinal
459 response of these naïve samples from T1 to T2, with new infections in HCoV-OC43 shown in
460 red. (c) Box and whisker plot showing there is no significant difference in HCoV-OC43

461 antibody response between SARS-CoV-2 seropositive and seronegative individuals, among
462 either adults (n=440, p=0.974) or children (n=436, p=0.214). Boxes represent the median,
463 25th and 75th percentiles, while whiskers show the largest and smallest non-outlier values.
464 Outliers were identified using upper/lower quartile \pm 1.5 times IQR. Statistical significance
465 was calculated by Mann-Whitney-U (two-sided) with *** indicating a p-value <0.001 and ns
466 indicating a p-value >0.01. (d) When comparing paired samples longitudinally within the
467 SARS-CoV-2 seropositive subgroup, there was no increase in HCoV-OC43 S1 response in
468 either adults (n=76) or children (n=103) following SARS-CoV-2 infection. Change in response
469 is presented as log₂-fold change from T1 to T2 and only samples with either log₂-fold
470 change greater than 1 or smaller than -1 are shown. Spearman's rank was used to calculate
471 any correlation between the change in response for HCoV-OC43 and SARS-CoV-2. The
472 same figures for the endemic coronaviruses HCoV-NL63, HCoV-HKU1 and HCoV-229E can
473 be found as Supplementary figures 10 – 12.

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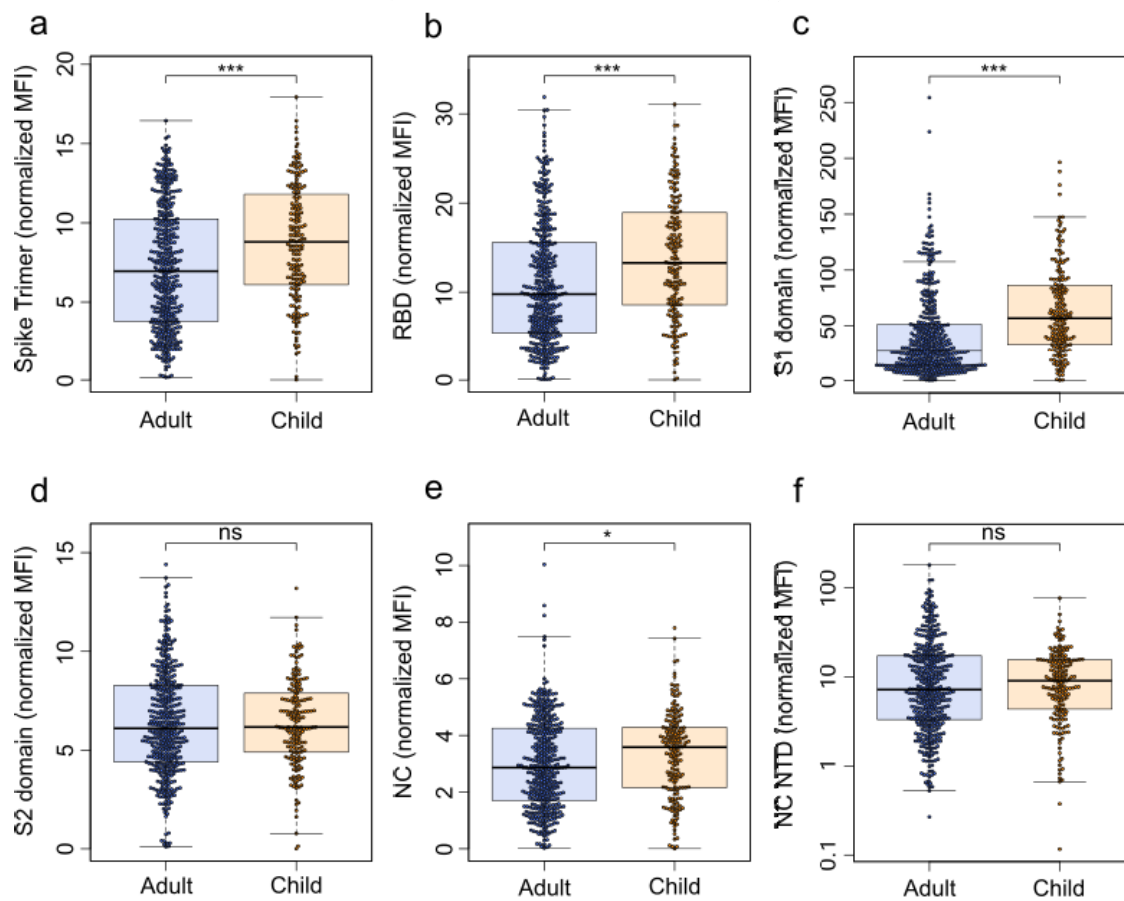
477 **Author Contributions**

478 RE, HR, AJ, DF, PH, ARF and KMD conceived the study. HR, ADu, RE, AJ, PH, ARF, KMD
479 and NSM designed the experiments. RE, HR, AJ, PH, ARF, KMD and NSM procured
480 funding. ADu, MB, DJ, AS, RG, JM, AHi, CL, TG, ADi, DH, HH, AP, TI, TS and H-JG
481 performed experiments. RE, HR, AJ, DF, MZ SB, LF, PF, AHa, JR, E-MJ, CE, MW, TG and
482 MR collected samples or organized their collection. BJ, HS, MS, BT, GFH and BM supported
483 the sample collection and provided key resources. PK, BT and UR produced the RBD
484 mutants. HR, AJ, ADu, MB, DF, AHi, ADi, KK, SW, E-MJ, AP, TI, TS, H-JG, MW, CE, KMD
485 and MR curated the data. MB and ADu performed the data analysis. ADu and MB generated
486 the figures. ADu, HR, AJ and RE wrote the first draft of the manuscript. All authors approved
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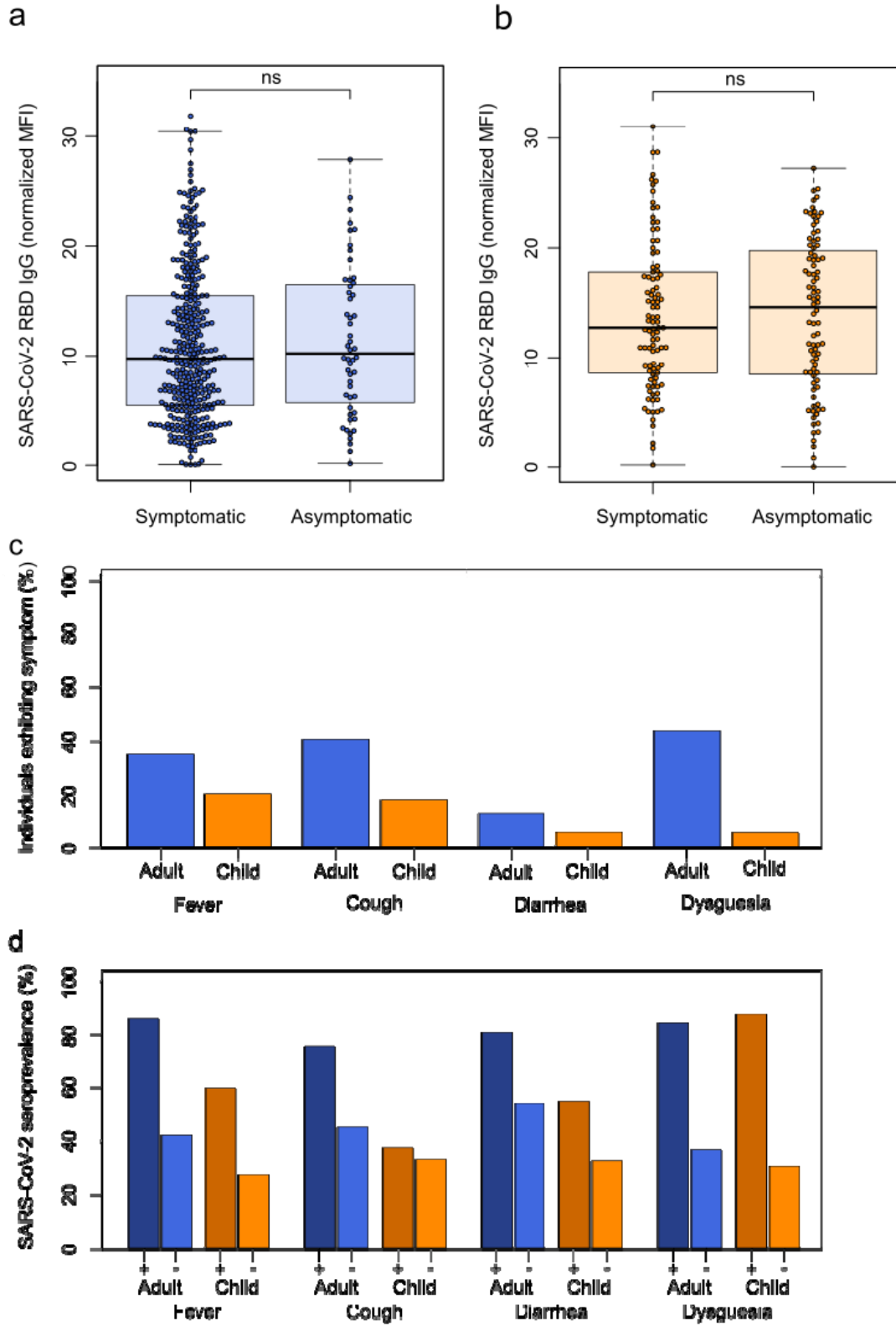
504 **Figure 1**



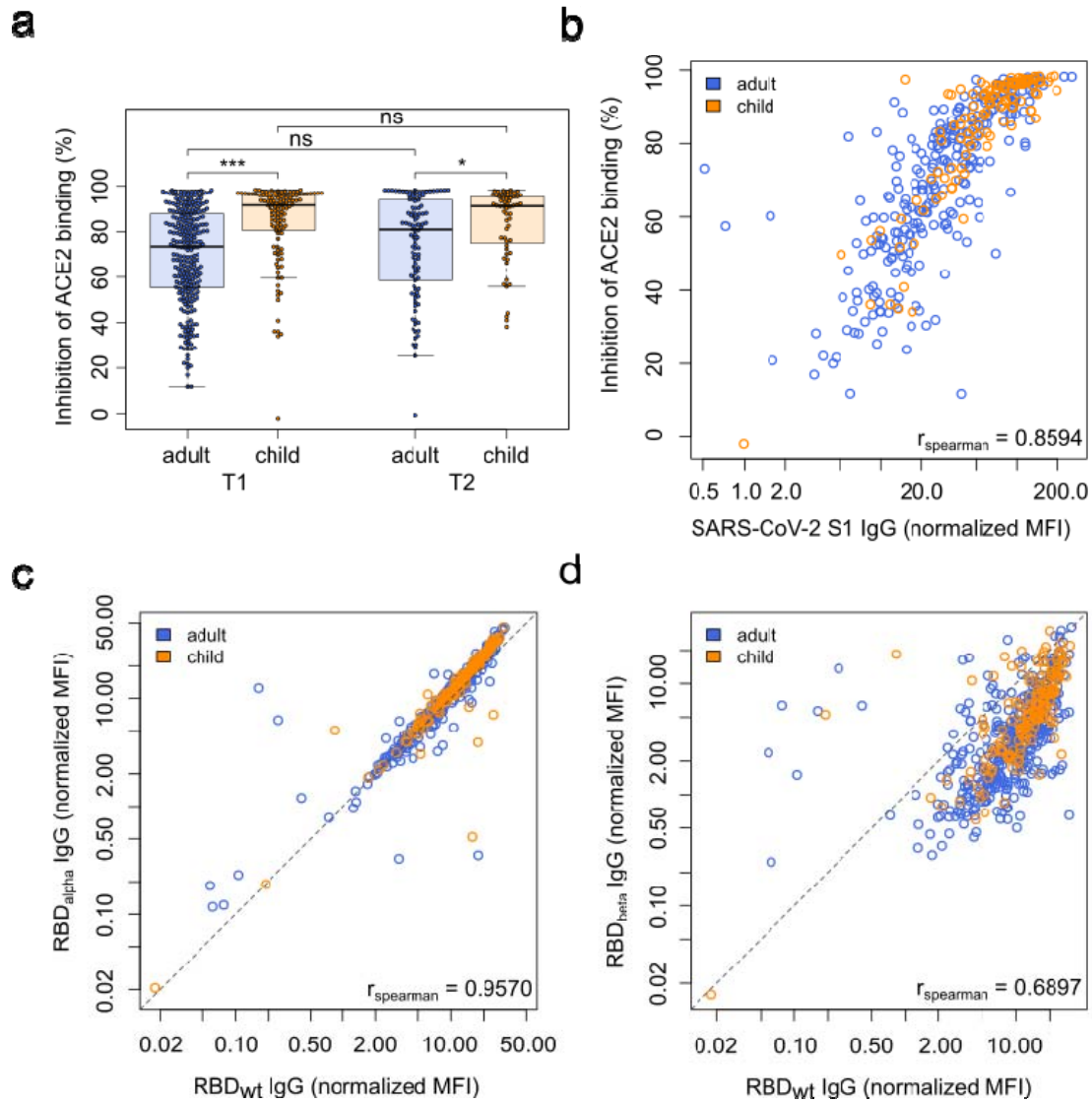
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507 **Figure 2**



509 **Figure 3**

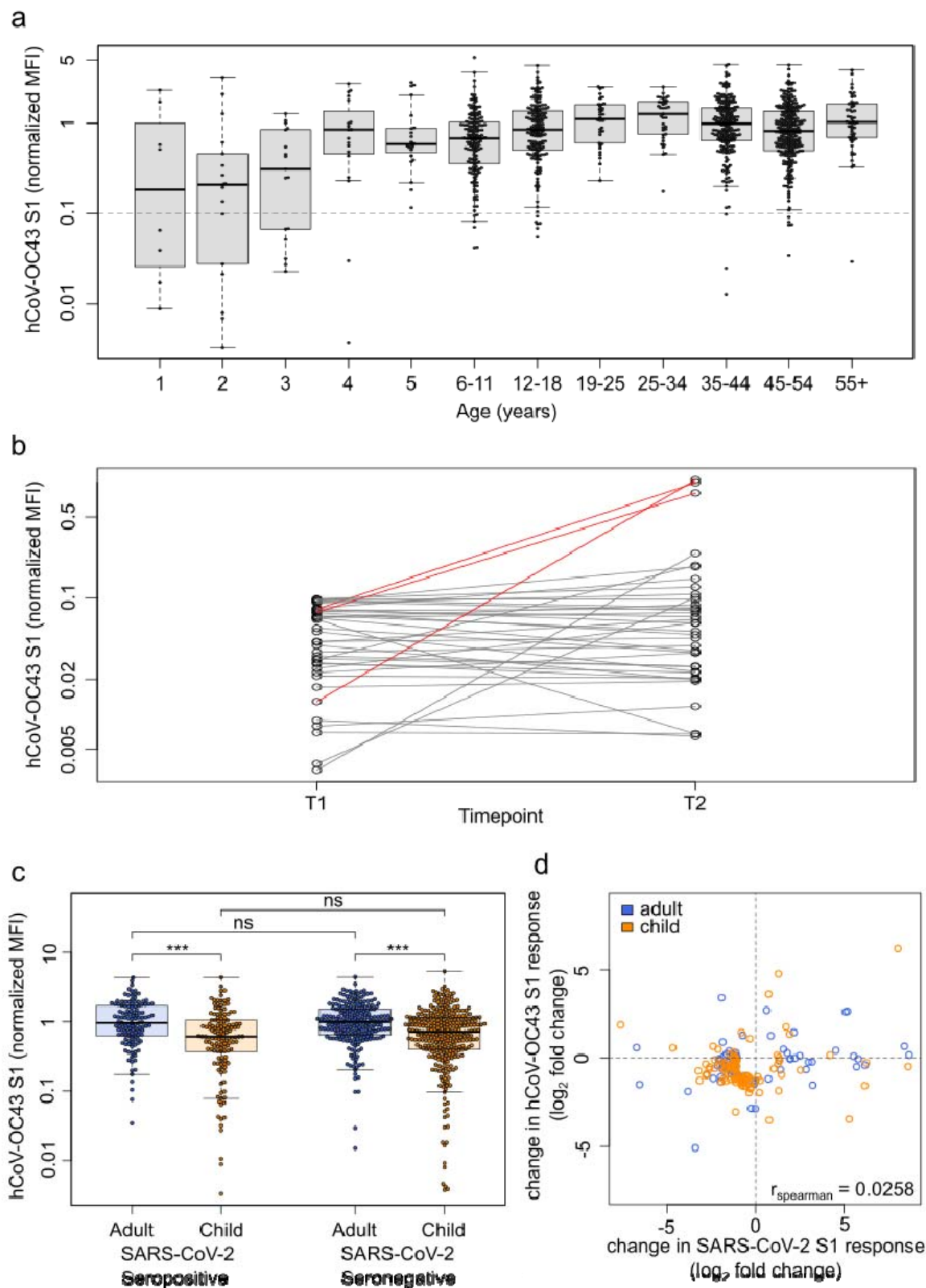


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513 **Figure 4**



516 **Table 1 – Demographics and key information for the study cohort**

Timepoint [⊠]	T1 [⊠]		T2 [⊠]	
Number-of-participants-by-age-group-(n) [⊠]	Adult-(717) [⊠]	Children-(548) [⊠]	Adult-(569) [⊠]	Children-(402) [⊠]
Median-Age-yr-(IQR) [⊠]	44-(37-49) [⊠]	10-(6-13) [⊠]	45-(38-50) [⊠]	10-(6-14) [⊠]
Number-of-females-(%) [⊠]	362-(50.49) [⊠]	277-(50.55) [⊠]	297-(52.20) [⊠]	202-(50.25) [⊠]
Smoker-(%) [⊠]	75-(10.46) [⊠]	2-(0.36) [⊠]	62-(10.90) [⊠]	2-(0.50) [⊠]
BMI-(IQR) [⊠]	25.37-(22.20-27.74) [⊠]	17.41-(14.92-19.46) [⊠]	24.69-(22.34-28.08) [⊠]	16.96-(15.00-19.69) [⊠]
Seropositive-(%) [¶]	415-(57.88) [¶]	185-(33.76) [¶]	282-(49.56) [¶]	151-(37.56) [¶]
- → Asymptomatic-(%) [¶]	36-(8.67) [¶]	83-(44.86) [¶]	31-(10.99) [¶]	69-(45.70) [¶]
- → Symptomatic-(%) [⊠]	379-(91.33) [⊠]	102-(55.14) [⊠]	251-(89.01) [⊠]	82-(54.30) [⊠]
Seroreverted-at-T2-(%) [⊠]	n/a [⊠]	n/a [⊠]	71-(17.11) [⊠]	7-(3.78) [⊠]
Symptoms-at-disease-onset-(of-seropositive) [¶]				
- → Fever-(%) [¶]	217-(52.29) [¶]	66-(35.68) [¶]	151-(53.55) [¶]	49-(32.45) [¶]
- → Cough-(%) [¶]	221-(53.25) [¶]	37-(20.00) [¶]	154-(54.61) [¶]	33-(21.85) [¶]
- → Dysgeusia-(%) [¶]	266-(64.10) [¶]	28-(15.14) [¶]	176-(62.41) [¶]	24-(15.89) [¶]
- → Diarrhea-(%) [⊠]	75-(18.07) [⊠]	18-(9.73) [⊠]	55-(19.50) [⊠]	16-(10.60) [⊠]
Median-(IQR)-days-from-positive-PCR-test-result-to-timepoint [⊠]	96-(63-120) [⊠]		333-(319-353) [⊠]	
Median-(IQR)-days-from-symptoms-onset-to-timepoint-(of-seropositive) [⊠]	109-(67-122) [⊠]		340-(322-356) [⊠]	
Hospitalised-(of-seropositive)-(%) [⊠]	15-(3.61) [⊠]	0-(0.00) [⊠]	n/a [⊠]	
Vaccinated-(%) [⊠]	n/a [⊠]	n/a [⊠]	24-(4.22) [⊠]	1-(0.25) [⊠]
Number-of-households [⊠]	328 [⊠]		279 [⊠]	
Median-(IQR)-number-of-household-members [⊠]	4-(3-4) [⊠]		4-(3-4) [⊠]	

517

518 See methods for definition of how samples were defined as being seropositive,
 519 asymptomatic or symptomatic. Median time from positive PCR test to time point (n=368 at
 520 T1, n=310 at T2) and median time from symptoms onset to time point (n=349 at T1, n=243 at
 521 T2) are calculated using adult samples for which this data was available.

522