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Induction of an early IFN-γ cellular response and high plasma levels of SDF-1α are inversely associated with COVID-19 severity and residence in rural areas in Kenyan patients

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Short Title:	Early IFN-γ response and high SDF-1α levels linked to Lower COVID-19 Severity in rural Kenyan patients
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Keywords:	COVID-19, SARS-CoV-2, IFN-γ-secreting cells, cytokines, chemokines, kinetics, Sub-Saharan Africa.
Abstract:	Introduction COVID-19 was less severe in Sub-Saharan Africa (SSA) compared with Europe and North America. It is unclear whether these differences could be explained immunologically. Here we determined the levels of ex vivo SARS-CoV-2 peptide-specific IFN-γ producing cells, and levels of plasma cytokines and chemokines over the first month of COVID-19 diagnosis among Kenyan COVID-19 patients from urban and rural areas. Methods Between June 2020 and August 2022, we recruited and longitudinally monitored 188 COVID-19 patients from two regions in Kenya, Nairobi (urban, n = 152) and Kilifi (rural, n = 36), with varying levels of disease severity – severe, mild/moderate, and asymptomatic. IFN-γ secreting cells were enumerated at 0-, 7-, 14- and 28-days post diagnosis by an ex vivo enzyme-linked immunospot (ELISpot) assay following in vitro stimulation of peripheral blood mononuclear cells (PBMCs) with overlapping peptides from several SARS-CoV-2 proteins. A multiplexed binding assay was used to measure the levels of 22 plasma cytokines and chemokines. Results Higher frequencies of IFN-γ-secreting cells against the SARS-CoV-2 spike peptides were observed on the day of diagnosis among the asymptomatic compared to the patients with severe COVID-19. Higher concentrations of 17 of the 22 cytokines and chemokines measured were positively associated with severe disease, and in particular interleukin (IL)-8, IL-18 and IL-1ra ($p<0.0001$), while a lower concentration of SDF-1α was associated with severe disease ($p<0.0001$). Concentrations of 8 and 16 cytokines and chemokines including IL-18 were higher among Nairobi asymptomatic and mild patients compared to their respective Kilifi counterparts. Conversely, the concentrations for SDF-1α were higher in rural Kilifi compared to Nairobi ($p=0.012$). Conclusion In Kenya, as seen elsewhere, pro-inflammatory cytokines and chemokines were associated with severe COVID-19, while an early IFN-γ cellular response to overlapping SARS-CoV-2 spike peptides was associated with reduced risk of disease. Living in urban Nairobi (compared with rural Kilifi) was associated with increased levels of pro-inflammatory cytokines/chemokines.
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4 **Short Title**: Early IFN-γ response and high SDF-1α levels linked to Lower COVID-19
5 Severity in rural Kenyan patients

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Abstract

Introduction

COVID-19 was less severe in Sub-Saharan Africa (SSA) compared with Europe and North America. It is unclear whether these differences could be explained immunologically. Here we determined the levels of *ex vivo* SARS-CoV-2 peptide-specific IFN-γ producing cells, and levels of plasma cytokines and chemokines over the first month of COVID-19 diagnosis among Kenyan COVID-19 patients from urban and rural areas.

Methods

Between June 2020 and August 2022, we recruited and longitudinally monitored 188 COVID-19 patients from two regions in Kenya, Nairobi (urban, n = 152) and Kilifi (rural, n = 36), with varying levels of disease severity – severe, mild/moderate, and asymptomatic. IFN-γ secreting cells were enumerated at 0-, 7-, 14- and 28-days post diagnosis by an *ex vivo* enzyme-linked immunospot (ELISpot) assay following *in vitro* stimulation of peripheral blood mononuclear cells (PBMCs) with overlapping peptides from several SARS-CoV-2 proteins. A multiplexed binding assay was used to measure the levels of 22 plasma cytokines and chemokines.

Results

Higher frequencies of IFN-γ-secreting cells against the SARS-CoV-2 spike peptides were observed on the day of diagnosis among the asymptomatic compared to the patients with severe COVID-19. Higher concentrations of 17 of the 22 cytokines and chemokines measured were positively associated with severe disease, and in particular interleukin (IL)-8, IL-18 and IL-1ra ($p<0.0001$), while a lower concentration of SDF-1α was associated with severe disease ($p<0.0001$). Concentrations of 8 and 16 cytokines and chemokines including IL-18 were higher among Nairobi asymptomatic and mild patients compared to their

50 respective Kilifi counterparts. Conversely, the concentrations for SDF-1α were higher in rural
51 Kilifi compared to Nairobi (p=0.012).

52 Conclusion

53 In Kenya, as seen elsewhere, pro-inflammatory cytokines and chemokines were associated
54 with severe COVID-19, while an early IFN-γ cellular response to overlapping SARS-CoV-2
55 spike peptides was associated with reduced risk of disease. Living in urban Nairobi
56 (compared with rural Kilifi) was associated with increased levels of pro-inflammatory
57 cytokines/chemokines.

58 **Keywords:** COVID-19, SARS-CoV-2, IFN-γ-secreting cells, cytokines, chemokines,
59 kinetics, Sub-Saharan Africa.

60

Introduction

Despite widespread transmission of SARS-CoV 2, SSA experienced a reduced burden of severe coronavirus disease 2019 (COVID-19) and associated mortality than North America and Europe (1). This observation is both puzzling (2) and paradoxical (3), because of the relatively weaker and underfunded health systems in SSA. Some of the proposed, but still unproven, explanations for the reduced burden of severe COVID-19 in SSA include under-diagnosis of cases and mortality, a younger population (7), warmer climatic conditions with outdoor living, high levels of pre-existing cross-protective antibodies and T-cells induced from a high prevalence of infectious agents with SARS-CoV-2 like immune determinants, and immune regulation associated with either prior BCG vaccination (5–11) or chronic/repeated infections including helminths (12) and malaria (13). Like other viruses, SARS-CoV-2 induces a plethora of inflammatory host responses, including cytokines and chemokines (14), that play key roles in either protective immunity or immunopathology (15). Notably, the pathogenesis of COVID-19 has been linked to dysregulated and excessive cytokine and chemokine responses, upon SARS-CoV-2 infection (16). Numerous studies have linked increased levels of cytokines and chemokines to severe COVID-19 and associated mortality, including IL-1β, IL-1ra, IL-2, IL-6, IL7, IL-8, IL-18, IFN-γ, TNF-α, IFN-γ-inducible protein 10 (IP-10), granulocyte macrophage-colony stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), and Macrophage inflammatory protein-1 alpha (MIP-1-α) (17–24). Collectively called a “cytokine storm”, a dysregulated cytokine response is implicated as the cause of the multiple organ failures and the acute respiratory distress syndrome (ARDS), which characterise severed COVID-19 and associated fatalities (25).

84 Published data show that the characteristic antibody response to SARS-CoV-2 infection,
85 where levels increase with time, viral loads and COVID-19 severity, were experienced in
86 Kenyan patients (26). Thus, there is no evidence of the primary acute antibody response
87 controlling the infection outcome. Aside from antibodies, T-cell responses can control
88 viremia, either directly by killing virus infected cells or indirectly by providing the relevant
89 co-stimulatory molecules for supporting antibody production by B cells (27). However, there
90 is a paucity of data on the cellular response to SARS-CoV-2 in Kenyan patients, and its
91 possible role in modulating disease severity. Furthermore, it is unclear whether the
92 differences in the rates of severe disease between urban and rural dwellers within SSA, as
93 well as between developed countries and SSA, could be explained immunologically. In this
94 study, we collected longitudinal blood samples from individuals from Nairobi (urban) and
95 Kilifi (rural) with varying degrees of COVID-19 severity (asymptomatic, mild/moderate and
96 severe) and compared levels of their *ex vivo* SARS-CoV-2 Spike peptide-specific IFN-γ
97 producing cells, and levels of plasma cytokines and chemokines over their first month of
98 COVID-19 diagnosis.
99

Methods

Study design, setting and participants

Participant sampling has been described previously (26). Briefly, we included 400 blood samples from a longitudinal cohort study of 188 patients that aimed at understanding the kinetics of naturally acquired immune responses to SARS-CoV-2 among COVID-19 patients from two sites in Kenya: 1) The Aga Khan University Hospital (AKUH) in Nairobi, an urban metropolitan academic medical Centre; and 2) Kilifi County Hospital, a community-based government hospital serving a rural coastal region. The samples were collected during the COVID-19 pandemic between June 2020 and August 2022. At the time of the study, SARS-CoV-2 transmission in Nairobi was higher than in Kilifi (28). Participants were adults, aged ≥18 years old, recruited within seven days of positive diagnosis of COVID-19 by RT-PCR testing. Initial sampling was at day 0 (i.e., day of diagnosis). Follow-up and subsequent samplings were done on days 7, 14 and 28 from a positive SARS-CoV-2 infection diagnosis. We collected 20 mL of venous blood in sodium heparin vacutainer. Additionally, we included residual longitudinal plasma samples from the AKUH biobank for cytokine and chemokine measurements. These samples were collected from COVID-19 patients who consented to this follow-up study on the day of diagnosis (i.e., day 0) and on day 28.

COVID-19 severity classification

We included patients from five COVID-19 severity groups of asymptomatic, mild, moderate, severe, and critical as determined by clinicians at the time of diagnosis following the National Institutes of Health (NIH, USA) guidelines(29). Asymptomatic patients were those who tested positive for SARS-CoV-2 via RT-PCR but did not display any COVID-19 symptoms. Mild cases were SARS-CoV-2 positive and exhibited symptoms such as fever, sore throat, cough, malaise, headache, muscle pain, vomiting, nausea, diarrhoea, anosmia, or

ageusia, without any signs of shortness of breath, dyspnea, or abnormal chest imaging. Moderate cases tested positive for SARS-CoV-2 and showed evidence of lower respiratory tract infection based on clinical examination or imaging, with an oxygen saturation (SpO2) of ≥94% on room air. Severe cases were positive for SARS-CoV-2 with an SpO2 of <94% on room air, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mm Hg, a respiratory rate >30 breaths/minute, and/or lung infiltrates >50%. Critically ill patients were positive for SARS-CoV-2 and experienced respiratory failure, septic shock, and/or multiple organ dysfunction syndrome. Due to the small numbers in the moderate and critical groups, mild cases were lumped together with moderate (mild/moderate), whilst critical were grouped with severe ones (severe). Thus, we studied immune responses among three COVID-19 patient groups: asymptomatic, mild/moderate and severe.

Procedures

Plasma separation and PBMC isolation

Plasma was separated by centrifuging the tubes at 440 g for 10 minutes using an Eppendorf 5810R centrifuge, aliquoted in 2 mL microcentrifuge tubes, and immediately stored in -80°C freezers until the time for laboratory analysis. PBMCs were isolated from the remaining blood component using density gradient centrifugation media (Lymphoprep™ (1.077 g/ml, Stem Cell Technologies), aliquoted to 1.8 mL cryovials, and stored in -196°C liquid nitrogen tanks until usage. Plasma and PBMC samples from AKUH were transported in dry ice and liquid nitrogen respectively, to the KEMRI–Wellcome Trust Research Programme laboratories in Kilifi and stored appropriately for laboratory analyses. Prior to use, PBMCs were thawed and rested at 37°C, 5% CO_2 for 15–16 hours. PBMC counting was done using Vi-CELL XR Cell Viability Analyser or Countess™ Cell Counting Chamber Slides (Thermo Scientific) before assay setup.

149 **SARS-CoV-2 synthetic peptides pools for ELISpot measurements**

150 A total of 641 peptides (15–18-mers with a ten amino acid overlap) were pooled into ten
151 peptide pools, spanning different regions of the virus. The pools covered: the spike protein
152 region (S1: positions 1–93 and S2: positions 94–178), membrane protein (M: positions 1–31),
153 nucleocapsid protein (NP: positions 1–55), non-structural proteins (NSP 3B: positions 207–
154 306, NSP 3C: position307–379, NSP 12B: positions 665–729 and NSP 15–16: positions 886–
155 972) and Open Reading Frame (ORF3: positions 1–37 and ORF8: positions 1–15). These
156 peptides (which were synthesised by Mimotopes Pty Ltd and a kind donation from Professor
157 Susanna Dunachie's laboratory, Oxford University) were used to stimulate PBMCs for *ex*
158 *vivo* IFN-γ ELISpot assay. The peptide sequences and pooling details are provided in Table
159 S1.

160 **Interferon gamma ELISpot assay**

161 To quantify *the ex vivo* interferon gamma (IFN-γ) cellular response to overlapping SARS-
162 CoV-2 peptides from different proteins, we stimulated PBMC with synthetic peptides pools
163 through an *in vitro* IFN-γ ELISpot assay, as previously described (30). Briefly, 5 µg/ml of
164 anti- human IFN-γ antibody clone 1-D1K (Mabtech, AB, Sweden) was used to coat
165 Multiscreen-I 96 ELISpot plates overnight. Individual's PBMC were plated in duplicates at
166 200,000 cells per well for each specific protein, based on pre-prepared plate templates.
167 Peptide pools were then added at a final concentration of 2 µg/mL per wells and incubated
168 for 16–18 hours at 37^0C, 5% CO_2, 95% humidity. Concanavalin A (ConA; Sigma) was used
169 as the positive control at a final concentration of 5 µg/mL per well, while dimethyl sulfoxide
170 (DMSO; Sigma), which was a constituent of the diluent for the peptides and Con A was used
171 at a similar concentration to peptides to serve as the negative control. IFN-γ secreting cells
172 were then detected using an anti-human IFN-γ biotinylated antibody clone 7-B6-1 (Mabtech)
173 at 1 µg/mL and an incubation for 2–4 hours. Thereafter, streptavidin alkaline phosphatase

antibody (Mabtech) was added at 1 µg/mL and incubated for 1–2 hours, and the IFN-γ spots then developed using 1-Step™ NBT/BCI (nitro blue tetrazolium/5-bromo-4-chloro-3-phosphatase) substrate (Thermo Scientific) during a 7-minute incubation in the dark. The enzyme-substrate reaction was stopped by rinsing the plate 3 times under running tap water. Plates were then airdried for at least 2 days on an open lab bench, and spots enumerated on an AID ELISpot Reader version 4.0. Results are hereby reported as spot-forming units (SFU)/10^6 PBMC after subtracting the background (mean SFU from negative control wells). Data from failed individual PBMC tests, defined here as either, an excessive background where the negative control wells had >80 SFU/10^6 PBMCs, or a positive control well with an average of <100 SFU/10^6 PBMCs (too few), were excluded. We also applied the ELISpot assay limit of detection of 10 SFU/10^6 PBMCs, with all wells having values <10 SFU/10^6 PBMCs replaced with 5 SFU/10^6 PBMCs. For the participants who did not have enough PBMC to be tested against all the peptide pools, we prioritised measurements against pools from S1, S2, NP and M proteins. Data are reported only for the individuals whose PBMC were tested against all the available peptide pools for each specific protein segment. We summed the responses from the different pools of the same protein segment, which resulted in different sample sizes for different proteins as follows: spike (171 samples for S1, S2), NP (162 samples), M (136 samples), NSPs (100 samples for NSP 3B, NSP 3C, NSP 12B and NSP 15 -16) and ORF (90 samples for ORF 3 and ORF 8) Table S2.

Luminex assay

Plasma concentrations of 22 cytokines and chemokines were measured using a Human ProcartaPlex™ Human Panel 1A (Thermo Fisher Scientific, Cat. No. EPX010-12010-901, Lot number 316776-000), which consisted of: a) Th1/Th2 specific cytokines: GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-18, TNF-α, IL-9, IL-21; b) Pro-Inflammatory cytokines: IFN-α, IL-1α, IL-1ra, IL-7, TNF-β; and c) Chemokines: Eotaxin, GRO-α, IP-10, MCP-1, MIP-1α,

MIP-1β, SDF-1α, according to the manufacturer's instructions. Briefly,1x capture magnetic beads were added to the plates, and unbound beads were then washed away with 1X wash buffer. Plasma samples were thawed and diluted at 1:2 with 1X universal assay buffer (UAB) before addition to the plates. Standards from the kit at 4-fold serial dilutions of 1:5, 1:20, 1:80, 1:320, 1:1280, 1:5120, 1:20480 as well as a blank (UAB), were also added. The plates were then incubated on a shaker at 600 rpm for 2 hours at room temperature. After incubation, contents were discarded, plates washed, and 1X biotinylated detection antibody added. The plates were then incubated for 30 minutes on an Eppendorf Thermomixer Comfort shaker at room temperature. The plates were then washed, and 1X Streptavidin-PE added and incubated for 30 minutes on an Eppendorf Thermomixer Comfort shaker at room temperature, the plates were then washed before adding 1X reading buffer for 5 minutes on an Eppendorf Thermomixer Comfort shaker at room temperature. All wash steps were performed on an Invitrogen hand-held magnetic plate washer. Data were acquired on the Magpix systems multiplex Luminex machine and concentrations (pg/mL) of the samples calculated in Belysa® Immunoassay Curve Fitting software version 1.1.0 (31) using a 5- or 4-parameter logistic standard curve generated from standards of known concentration.

Statistical analysis

For the ELISpot data, time point specific geometric means (GMs) of IFN-γ secreting cells for each of the different SARS-COV-2 peptides pools were calculated for each severity group and geographical location. For each peptide, variations in ELISpot responses were compared using a linear mixed effects model on log-transformed PBMCs values with patient as a random effect, and time (i.e., day of sampling), severity group and time-by-severity group interaction term as fixed effects (32), followed by Tukey's multiple comparisons. Within each severity group, differences between geographic locations were compared using Kruskal

Wallis paired with a Dunn's multiple comparisons. This analysis set was restricted to 59 patients who had PBMC samples.

Cytokine and chemokine data were first normalised to have a zero mean and a standard deviation of one, and cross-correlations among the cytokines determined using Pearson correlations and principal component analysis (PCA). Principal components (PCs) were extracted based on scree-plot, variance explained and the interpretability of the components. Next, the non-normalised cytokine and chemokine data were log-transformed and fitted into linear mixed effects regression models with age, sex, day of sampling, location or disease severity as fixed effects and patient as a random effect. Interactions were explored and separate models fitted where necessary. For location comparisons, severe and moderate COVID-19 cases were excluded as these categories were only present in the Nairobi data. Results from the regression models were presented using heat maps, in which the effect size (i.e., coefficient) determined the density of colour-shading for each square. TNF-β data were excluded from the analyses as only one patient had a measurable concentration. Thus, we analysed 21 cytokines from all 188 patients. Analyses were performed using R version 4.3.0 (33). The factoextra package (34) was used for PCA. For visualisations, the pheatmap (35) and ggplot2 (36) packages and GraphPad Prism Software version 10.1.2 (37) were used.

Ethical approval

The study obtained ethical approval from the Kenya Medical Research Institute's Scientific and Ethics Review Unit (KEMRI SERU; protocol no. 4081) and the Aga Khan University, Nairobi, Institutional Ethics Review Committee (protocol no. 2020 IERC-55 V5 and 2020 IERC-135 V2). Written informed consent was obtained from all willing patients before their enrolment into the study.

247 # Results

248 ## Participant baseline characteristics

249 Using the NIH clinical guidelines for grading COVID-19 severity of 188 patients, 27 (14%)
250 were asymptomatic, 75 (40%) were mild/moderate and 86 (46%) were severe cases.
251 Collectively, the 188 patients contributed 400 blood samples collected over the first month of
252 diagnosis: day 0 (187 samples), day 7 (50 samples), day 14 (53 samples), and day 28 (110
253 samples). Fewer samples were collected on days 7 and 14 mainly due to design; that is,
254 residual longitudinal plasma samples from AKUH biobank were only collected in day 0 and
255 day 28. Of the 188 patients, 129 (69%) were male, 36 (19%) were from rural Kilifi and 152
256 (81%) from urban Nairobi (Table 1). Their median age at recruitment was 48 years (IQR 37–
257 58), with disease severity increasing with age. All 86 patients with severe COVID-19 were
258 from Nairobi as we were unable to recruit severe patients in Kilifi. Underlying comorbidities
259 such as diabetes, HIV/AIDS, and hypertension were prevalent among the mild/moderate and
260 severe COVID-19 groups. Two participants with severe disease died on day 7 and 28
261 respectively (Table 1).

262 **Table 1.** Participant demographic and clinical characteristics[†]

Characteristic	Asymptomatic (n = 27)	Mild/Moderate (n = 75)	Severe (n = 86)
Median age at recruitment (IQR), years	40 (33, 55)	46 (35, 54)	53 (43, 60)
Male sex	15 (56%)	46 (61%)	68 (79%)
Location			
Kilifi	21 (78%)	15 (20%)	0

Characteristic	Asymptomatic (n = 27)	Mild/Moderate (n = 75)	Severe (n = 86)
Nairobi	6 (22%)	60 (80%)	86 (100%)
Comorbidities			
Diabetic	1 (7%)	28 (42%)	47 (63%)
Hypertensive	0	15 (22%)	33 (44%)
HIV positive	0	5 (8%)	4 (5%)
Had COPD	0	0	1 (1%)
Had Renal disease	0	0	2 (3%)
Had heart disease	0	0	6 (8%)
Patient management			
Were hospital admissions	0	56 (84%)	75 (100%)
In intensive care unit	0	4 (6.0%)	15 (20%)
Clinical Outcome, died	0	0	2 (3%)

263 † Data are median (IQR) or number (%); COPD if Chronic obstructive pulmonary disease.

264 Kinetics and frequencies of *ex vivo* IFN-γ secreting cells by 265 COVID-19 severity and geographical location

266 Kinetics and levels of IFN-γ secreting cell responses were assessed in a subset of 59
267 participants with PBMC samples, contributing 172 samples. At day 0, the frequency of IFN-γ
268 secreting cells to overlapping SARS-CoV-2 spike peptide was significantly higher in
269 asymptomatic patients compared to severe patients (GMs: 117 [95% CI 71–194] vs 32 [95%
270 CI 8–76]; p=0.0366) (Figure 1a, Table S3). For the IFN-γ secreting cells specific to

271 overlapping M peptides, severe patients exhibited significantly higher levels at day 7 than
272 mild/moderate patients (GMs: 90 [95% CI 37–217] vs 19 [95% CI 10–39]; p= 0.0180; Figure
273 1b, Table S3). We did not observe any significant differences in the frequencies of IFN-γ
274 secreting cell responses to NP, NSPs, and ORFs overlapping peptides (Figure 1c–e, Table
275 S3). For all five SARS-CoV-2 peptides we observed temporal differences in IFN-γ secreting
276 cells within each COVID-19 severity group (Figure 1, Table S3). Spike peptide: In the
277 asymptomatic group, IFN-γ secreting cells were significantly elevated on day 7 than on day
278 14 (GMs: 161 [95% CI 86–301] vs 92 [95% CI 44–189]; p=0.0039) and day 28 (102 [95% CI
279 49–209]; p=0.0042). For the mild/moderate group, levels significantly increased from 86
280 (95% CI 53–138) on day 7 to 142 (95% CI 78–257) on day 14 (p=0.0403). In the severe
281 group, significantly higher levels were observed on day 7 (70 [95% CI 29–165]; p=0.0381),
282 day 14 (117 [95% CI 52–260]; p=0.0058), and day 28 (108 [95% CI 33–348]; p=0.0037)
283 relative to day 0 (23 95% CI 7–76).
284 M Peptide: In the asymptomatic patients, IFN-γ secreting cells were significantly higher on
285 day 14 (89 [95% CI 30–258]) compared to day 0 (GMs: 28 [95% CI 13–59]; p=0.0364) and
286 day 7 (GMs: 48 [95% CI 18–129]; p=0.0297). In the mild/moderate group, day 14 levels
287 were significantly higher compared to day 0 (p=0.0039), day 7 (p<0.0001), and day 28
288 (p=0.002). Additionally, day 28 levels were significantly higher than day 7 (p=0.0049). No
289 significant differences were observed between timepoints in the severe group.
290 NP Peptide: Among the asymptomatic group, a significant decline was observed from day 7
291 (71 [95% CI 33–153]) to day 28 (47 [95% CI 24–88]; p=0.0035). In the mild/moderate
292 group, IFN-γ secreting cell levels were significantly higher on day 14 (GMs: 67 [95% CI 34–
293 130]) compared to day 0 (GMs: 35 [95% CI 14–83]; p=0.0037), day 7 (GMs: 44 [95% CI 26–
294 73]; p=0.0178), and day 28 (GMs: 47 [95% CI 24–91]; p=0.0003). No significant differences
295 were observed between timepoints in the severe group. For NSP peptide: a significant decline

296 in IFN-γ secreting cells was observed within the mild/moderate group from 80 (95% CI 33–
297 194) at day 14 to 28 (95% CI 7–106) at day 28 (p=0.0316). For ORF peptide: a significant
298 increase in IFN-γ secreting cells was observed in the mild/moderate group between day 0 (14
299 [95% CI 4–43]) and day 7 (GMs: 38 [95% CI 16–85]; p=0.0242).

300 **Figure 1. Induction of a higher IFN-gamma cellular response to Spike protein on day of**
301 **diagnosis is associated with asymptomatic infection.**

302 Frequencies of ex-vivo IFN-γ secreting cells against SARS-CoV-2 peptide pools spanning (**a**)
303 spike protein, (**b**) M protein, **c** NP protein, (**d**) NSP proteins and (**e**) ORF proteins. Bars
304 represent geometric mean and 95% CI.

305 Linear mixed effects model with Tukey's multiple comparisons, was used, * $P < 0.05$.

306 Number of samples analysed for: spike responses = 171, M responses = 136, NP responses =
307 162, NSP responses = 100 and ORF responses = 90.

308

309 We also assessed whether there were differences in IFN-γ secreting cell responses by location
310 (urban Nairobi and rural Kilifi) among asymptomatic and mild patients. Relative to Nairobi,
311 significantly higher levels of IFN-γ secreting cell responses to the SARS-CoV-2 spike
312 peptide (218 [95% CI 125–381] vs 56 [95% CI 28–110]; p=0.0057; Figure 2a) and NP
313 peptide (94 [95% CI 51–169] vs 19 [95% CI 8–48; p=0.0171; Figure 2c) were observed
314 among the asymptomatic patients in Kilifi on day 0. There were no significant differences
315 between Kilifi and Nairobi in the IFN-γ secreting cell responses to overlapping peptides for
316 the M protein for the asymptomatic patients (Figure 2b), nor for the spike, or NP, and or M
317 peptides among mild patients (Figure 2d–f). Data for severe patients are shown for Nairobi
318 participants only (Figure 2g–i), as we were unable to recruit severe patients in Kilifi.

319 **Figure 2. Induction of a higher IFN-γ cellular response on the day of diagnosis is**
320 **associated with Kilifi participants.**

321 Comparison of IFN-γ cellular responses between Kilifi and Nairobi COVID-19 patients with:
322 asymptomatic disease for (**a**) Spike protein, (**b**) M protein, (**c**) NP protein; Mild disease for
323 (**d**) Spike protein, € M protein, (**f**) NP protein; and Severe disease for (**g**) Spike protein, (**h**) M
324 protein, (**i**) NP protein. Kilifi didn't have severe patients.
325 Bars represent geometric mean and 95% CI. Kruskal–Wallis one-way ANOVA, with Dunn's
326 multiple comparisons test, was performed. * $P < 0.05$, ** $P < 0.01$.
327

328 Kinetics of cytokine and chemokine responses across COVID-19
329 severity groups and geographical location

330 Asymptomatic patients consistently showed elevated levels of SDF-1α at all time-points, but
331 lower levels of all the other cytokines and chemokines measured and no detectable levels of
332 IL-9 (Figure 3a). Similarly, for mild/moderate patients, high levels of SDF-1α (Figure 3b),
333 were observed at all timepoints whereas other cytokines and chemokines were secreted at low
334 to intermediate levels. High cytokine and chemokines levels were seen among the severe
335 patients with IL-Iβ, IL-6, IL-2, IFN-γ, GM-CSF, IFN-α, IL-7 and GRO-α decreasing over
336 time, while MIP-1α, MIP-Iβ, MCP-1, and SDF-1α increased with time. Levels for IL1-ra, and
337 IL-9 were similar at all the time points (Figure 3c).
338 **Figure 3. Kinetics of Cytokine and Chemokine Concentrations in COVID-19 Patients.**
339 Mean of log10-transformed cytokine/chemokine concentrations plotted over time for (**a**)
340 Asymptomatic participants, (**b**) Mild/Moderate participants, and (**c**) Severe participants**.** * -
341 Levels for all participants were below detectable levels.
342

343 We compared asymptomatic against mild/moderate and severe participants to evaluate
344 differences among the clinical phenotypes. Mild/moderate participants had significantly
345 higher levels of IL-18, IL-8, IL-1ra, IL-6, GM-CSF, IP-10, MCP-1, TNF-α, MIP-1α, IFN-γ,

346 IL-2, IL-7, IL-1β, IL-9, Eotaxin and IFN-α than asymptomatic patients. The largest effect
347 sizes were observed with IL-18 (1.107, p<0.0001), IL-8 (1.105, p<0.0001) and IL-1ra,
348 (0.672, p=0.013). On the contrary, levels for SDF-1α were significantly reduced among the
349 mild/moderate patients relative to the asymptomatic (effect size -0.182, p<0.0001) (Figure 4).
350 Severe participants had significantly higher levels for cytokines (IL-8, IL-18, IL-1ra, IL-6,
351 IP-10, MIP-1α, TNF-α, IL-9, IFN-γ, GM-CSF, IL-7, IL-1β, MCP-1, IL-2, GRO-α, Eotaxin
352 and IFN-α) compared to asymptomatic cases, except for IL-1a, IL-21 and MIP-Iβ which had
353 similar levels, and SDF-1α (effect size -0.195, p<0.0001), which was significantly reduced.
354 The largest effect size was observed in IL-8 (1.754, p<0.0001), IL-18 (1.666, p<0.0001) and
355 IL-1ra (1.197, p<0.0001) (Figure 4).
356 For asymptomatic individuals, the cytokines and chemokine levels for IL-6, MIP-1α, IL-18,
357 GRO-α, IL-2, IL-8, TNF-α and GM-CSF were significantly higher among Nairobi than Kilifi
358 patients. Comparisons for IL-6 (1.139, p<0.0001), MIP-1a (1.093, p=0.004) and IL-18
359 (1.025, p=0.002) had the largest effect sizes (Figure 4). For mild patients, all cytokines (IL-8,
360 IL-18, IL-1ra, MIP-1α, IL-6, IP-10, IFN-γ, TNF-α, MCP-1, GM-CSF, IL-9, IL-1β, Eotaxin,
361 IL-7, MIP-Iβ, and IFN-α) were significantly higher among Nairobi patients in comparison to
362 Kilifi patients except for GRO-α, IL-1α, IL-2 and IL-21 which were similar. Notably, the
363 largest effect size for these comparisons was observed for IL-8 (1.69, p<0.0001), IL-18
364 (1.634, p<0.0001) and IL-1ra (1.355, p<0.001). On the other hand, SDF-1α (effect size -
365 0.161, p = 0.017) was significantly lower in Nairobi than in Kilifi patients.

366 **Figure 4. Higher Cytokine and Chemokine concentrations in Asymptomatic and Mild**
367 **Patients from Nairobi Compared to Kilifi, and in Mild/Moderate and Severe Patients**
368 **Compared to Asymptomatic Individuals.**
369 ns – not-significant, *p* value >0.05, empty means it was significant at p<0.05 - <0.0001. * -
370 Levels for all participants were below detectable levels.
371
372

373 **Principal component analysis**

374 The concentrations of the majority of the 21 cytokines and chemokines were positively
375 correlated with each other. However, the levels of SDF-1α were negatively correlated with
376 those of IL-2, IP-10, IL-7, IFN-γ, GM-CSF, and IL-18 (Figure S1). We retained the first three
377 principal components from a principal component analysis, accounting for 62% of the total
378 variability in the 21 cytokines and chemokines (Figure S2a). IL-9, MIP-1α, TNF-α, MCP-1,
379 MIP-Iβ, IL1-ra, IL-6, IL-Iβ, GRO-α and IL-8 were the most strongly associated with the first
380 PC. The second PC was most strongly associated with IP-10, IFN-γ, GM-CSF, IL-2, IL-18,
381 IL-7, IFN-α, Eotaxin and SDF-1α. The third PC was associated with IL-1 α and IL-21 (Figure
382 S3). There was no clear separation among the mild/moderate and severe groups. However,
383 data points for the asymptomatic participants clustered together demonstrating a much-
384 reduced diversity of cytokine and chemokine levels than the other groups (Figure 5), and
385 median PC scores did not change over the first month (Figure S2b).

386 **Figure 5. Scatter plot by Severity at baseline.**

387 **(a)** PC1 vs PC2; **(b)** PC2 vs PC3

388

389 Among the symptomatic groups (mild/moderate/severe), a steady decline of all the PCs over
390 one month was observed, with a steep decline occurring between day 0 and day 7 (Figure
391 S2c). There was no apparent difference of the cytokine and chemokine responses between
392 asymptomatic participants from Kilifi and Nairobi (Figure S4a). For mild cases, Kilifi
393 patients clustered together, although there was a slight overlap with Nairobi participants
394 (Figure S4b). Using biplots, there was no apparent difference between asymptomatic and
395 mild Kilifi patients (Figure S4c). For Nairobi, clinical phenotypes were asymptomatic, mild,
396 moderate, and severe. We combined the mild and moderate, and observed no clear difference

397 between the mild/moderate and severe groups, while the few asymptomatic participants

398 seemed to cluster together (Figure S4d).

399

400

401

402 **Discussion and conclusion**

403 In this study, we measured associations between the acute IFN-γ cellular, and 22 cytokine
404 and chemokine, immune response to SARS-CoV-2 (within a month of diagnosis) with
405 COVID-19 severity and possible modulation by differential environmental exposures in
406 urban and rural areas in Kenya.

407 As had been seen in earlier reports from Singapore, Netherlands, and Italy, which associated
408 increased IFN-γ secreting cells measured by ELISpot assay in the early phase of infection
409 with milder disease (38–40), we found that higher frequencies of an early IFN-γ cellular
410 response to SARS-CoV-2 spike overlapping peptides was associated with asymptomatic,
411 compared to severe SARS-CoV-2 infection outcomes. This observation would suggest either:
412 1) that the IFN-γ cellular response contribute to protection against disease progression, or 2)
413 that developing severe COVID-19 depresses this response. The latter interpretation may be
414 supported by previous reports from China and USA reporting correlations of an early
415 induction of increased basal T-cell specific responses ($CD4^+$ and $CD8^+$ T) in COVID-19
416 patients with mild disease, which was suppressed among their severely sick counterparts
417 (41,42).

418 Whilst the concentrations of the pro-inflammatory cytokines and chemokines IL-6, IL-Iβ,
419 GRO-α, IFN-γ, GM-CSF, IFN-α, IL-7 and IL-2 in severe patients declined to basal levels at
420 day 28 from day of diagnosis, we found that the concentrations for the chemokines MIP-1α,
421 MIP-Iβ, MCP-1, and SDF-1α were increasing with time. This is to be expected as cytokines
422 and chemokines play different roles and at different times of the immune response to
423 infection. MIP-1α, MCP-1 and MIP-Iβ are chemoattractant and play key roles in the
424 recruitments of leukocytes such as monocytes, T-cells, and neutrophil to the sites of infection
425 (43,44). Similarly increasing kinetics for MIP-1α and MCP-1 in severe and fatal patients
426 were reported in Norway and China, respectively (45,46). Notably, we found that higher

427 concentrations of SDF-1α were associated with asymptomatic individuals, hinting at a
428 potential protective role from severe disease progression. SDF-1α (CXCL12), is a
429 chemokine involved in the recruitment of T-cells (47), CD34+ hematopoietic stem/progenitor
430 cells (48), lymphocytes and monocytes (49) to the site of infection further enhancing
431 inflammation. In contrast, studies from China and Bulgaria did not observe significant
432 differences in SDF-1α levels among asymptomatic, mild, moderate, severe or fatal cases
433 (18,46,50). However, others have also implicated SDF-1α in disease severity based on
434 genetic association studies in a single-centre study (51), although this finding was not
435 corroborated in multi-centre genome-wide association studies (52). SDF-1α may aid in the
436 timely recruitment of T and other cells (47), to the sites of infection, enhancing viral
437 elimination, reduction in inflammation, and promotion of recovery.
438 We demonstrate that elevated levels of eighteen cytokines and chemokines were associated
439 with severe COVID-19, in agreement with previous reports linking them to severe lung
440 injury and ARDS (17–22,53). However, the associations with IL-8, IL-18 and IL-1ra, had the
441 strongest effect sizes in the current study. IL-8, has been implicated in the activation and
442 recruitment of neutrophils to the site of infection, thereby promoting inflammation (54). IL-
443 18 amplifies the immune response by inducing the production of IFN-γ by T-cells and natural
444 killer cells (55). Thus, IL-8 and IL-18 could amplify the excessive inflammation that
445 characterises severe COVID-19. On the contrary, IL1-ra is known to suppress the production
446 of proinflammatory cytokines such as IL-1 and TNF-α (56), and probably helps mitigate the
447 effects of excessive inflammation, thus reducing tissue damage and associated mortality.
448 In parallel to the reduced rates of severe COVID-19 and associated deaths in SSA relative to
449 North America and Europe, we and others have suggested higher rates of severe COVID-19
450 in cities compared to rural areas in SSA (28). Whilst this difference could be explained by
451 higher levels of SARS-CoV-2 transmission in busy metropolitan cities, or by more complete

452 reporting of cases (28,57,58), we wondered whether there were also plausible biological
453 explanations. We compared inflammatory cytokine and chemokine levels, and found that the
454 asymptomatic patients from Nairobi had higher levels of 8 cytokines and chemokines than
455 their asymptomatic counterparts from Kilifi, with IL-6, IL-18 and MIP-1α, having the
456 strongest associations. Similarly, levels of 16 cytokines and chemokines were higher among
457 mild- Nairobi, than Kilifi, COVID-19 patients with IL-8, IL-18 and IL-1ra being the most
458 differentially secreted. Moreover, the basal frequencies of IFN-γ secreting cells in
459 asymptomatic patients from Nairobi, relative to those of their Kilifi counterparts, were
460 reduced. Collectively, these findings would suggest that the immune response to SARS-CoV-
461 2 is less inflammatory among residents of rural areas (59).

462 Our study was faced with a few limitations. Firstly, our data are incomplete for some of the
463 patients due to missed follow-ups, or due to unavailability of adequate numbers of PBMCs to
464 quantify IFN-γ cellular responses to the full spectrum of the peptide pools corresponding to
465 all the different SARS-CoV-2 proteins. However, our analyses are assumed valid under the
466 missing at random mechanism given the likelihood approach (60). Secondly, we had
467 difficulties recruiting asymptomatic patients in Nairobi and were unable to recruit severe
468 cases in Kilifi and thus the sample size is relatively small.

469 In conclusion, although severe disease was rare in Kenya, the inflammatory cytokine profile
470 in patients with severe COVID is similar to that of North American and European severe
471 COVID-19 patients. However, just like the early IFN-γ secreting cellular response, increased
472 levels of the chemokine, SDF-1α, were associated with asymptomatic SARS-CoV-2
473 infection, suggesting a potential role of these responses in protection against disease
474 progression. Finally, the differential cytokine and chemokine, and IFN-γ cellular responses
475 between urban Nairobi and rural Kilifi patients suggest a plausible biological explanation for
476 the increased frequency of severe COVID-19 in African SSA cities relative to rural areas.

477 Together, these findings provide insights into potentially COVID-19 protective immune
478 responses, and their modulation by differential environmental exposures. Nonetheless,
479 additional studies are required to extend and replicate these important findings as they could
480 inform future control of COVID-19 and empower the control of similar pandemics.
481
482

483
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486

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682

683 # Supporting information

684 **Figure S1. Correlation matrix of 21 cytokines across all time points from 400 patients.**
685 Pearson's correlation coefficients are visualised, with red indicating positive correlations,
686 blue negative correlations, and white representing no correlation.

687 **Figure S2. Principal component analysis of the cytokines. a)** Scree-plot showing that the
688 first 3 principal components explained 62% of variability in the cytokines data for all
689 participants; **(b)** Line plots for the first 3 principal component illustrating trends over time for
690 asymptomatic participants; and **(c)** Line plots for the first 3 principal component illustrating
691 trends over time for symptomatic (mild, moderate and severe) participants.

692 **Figure S3. Cytokines loading on the first three principal components (PC1–PC3).** The
693 color scale indicates the loading value, with red indicating a higher positive loading, blue a
694 higher negative loading and light-yellow minimal loading.

695 **Figure S4. Scatter plots showing cytokine data for participants grouped by location and**
696 **clinical phenotype. (a)** Asymptomatic participants, **(b)** mild cases, **(c)** participants from
697 Kilifi, and **(d)** participants from Nairobi. Each point represents an individual's cytokine
698 measurement, allowing for a visual assessment of cytokine variability across different groups
699 based on location and clinical presentation.

700 **Table S1. Peptides Sequences.** Each entry represents a distinct peptide along with its
701 associated properties. The pooling strategy for the 10 peptide pools used is shown below.

702 **Table S2. Sample size for each peptide.** Number of participants sampled for each peptide
703 across different clinical phenotypes (asymptomatic, mild/moderate, severe) and time points
704 (Day 0, 7, 14, 28).

705 **Table S3. Associations of SARS-CoV-2 Peptides (Spike, M, NP, NSP, and ORF) with**
706 **asymptomatic, mild/moderate, and severe clinical phenotypes over time.** Tukey's

707 multiple comparisons test was used to assess differences within and between clinical

708 phenotypes across different time points. Bold p values are significant, $p<0.05$.

709

Figure 1

Click here to access/download;Figure;Figure 1.tif

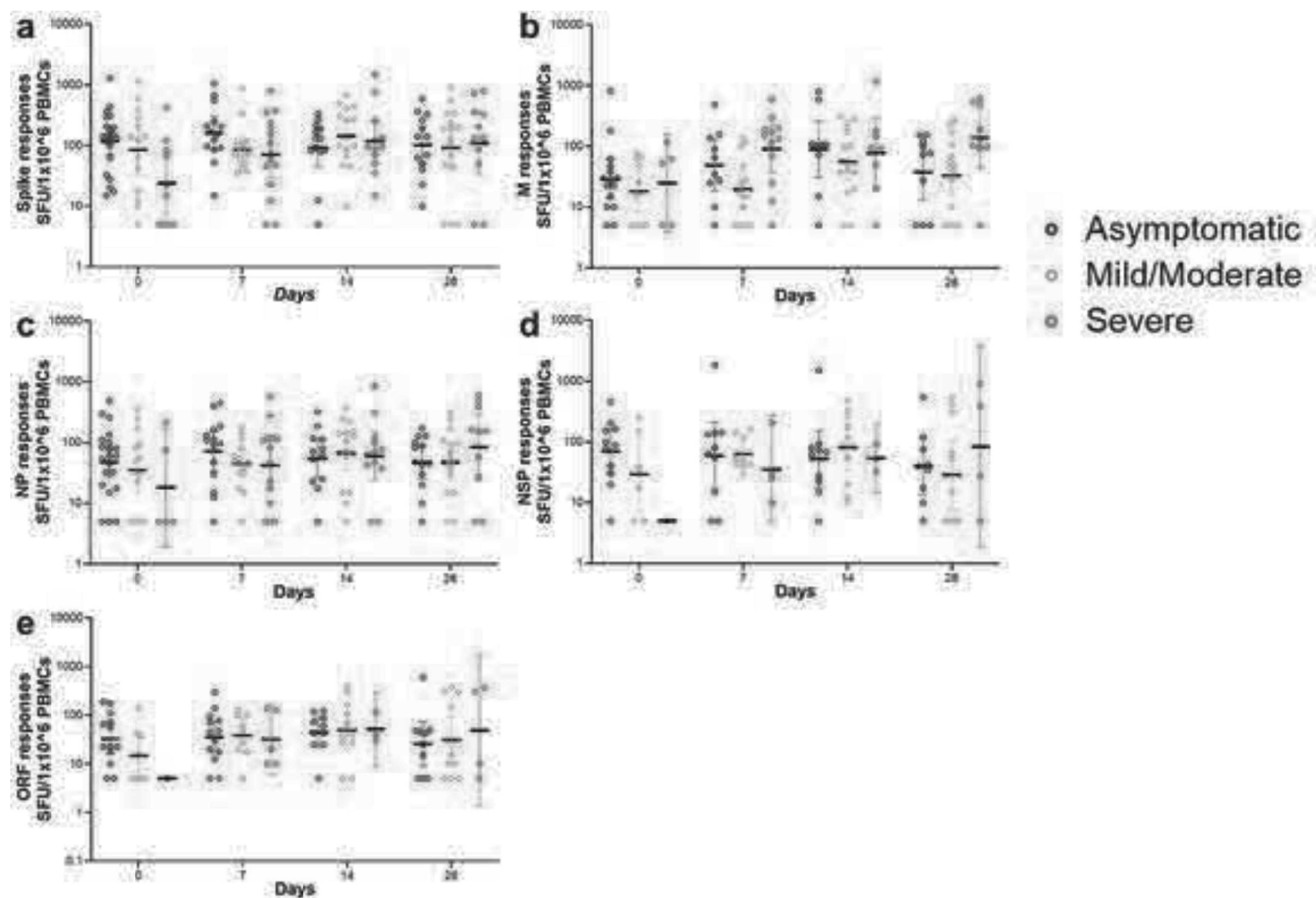

Figure 2

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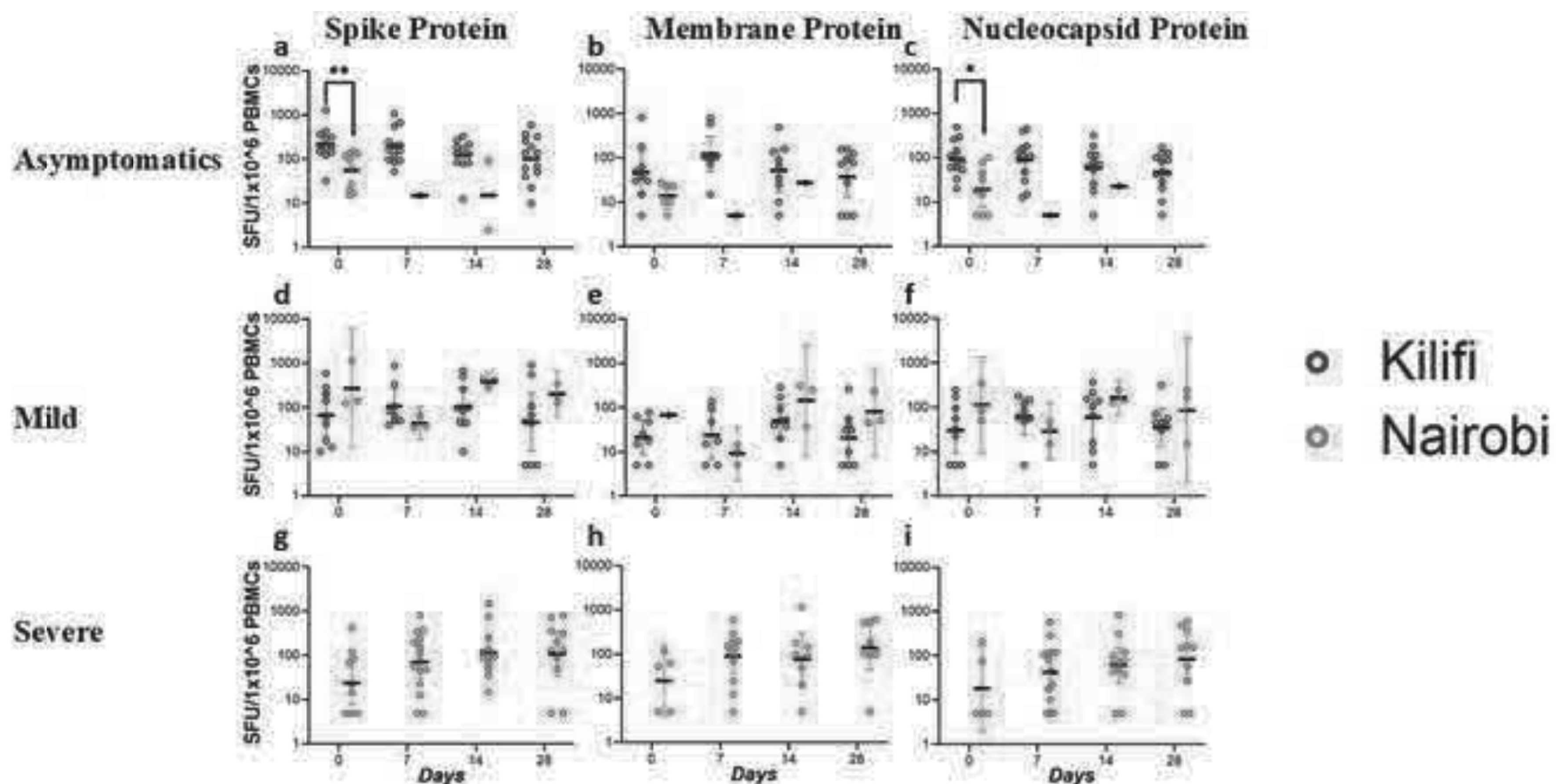

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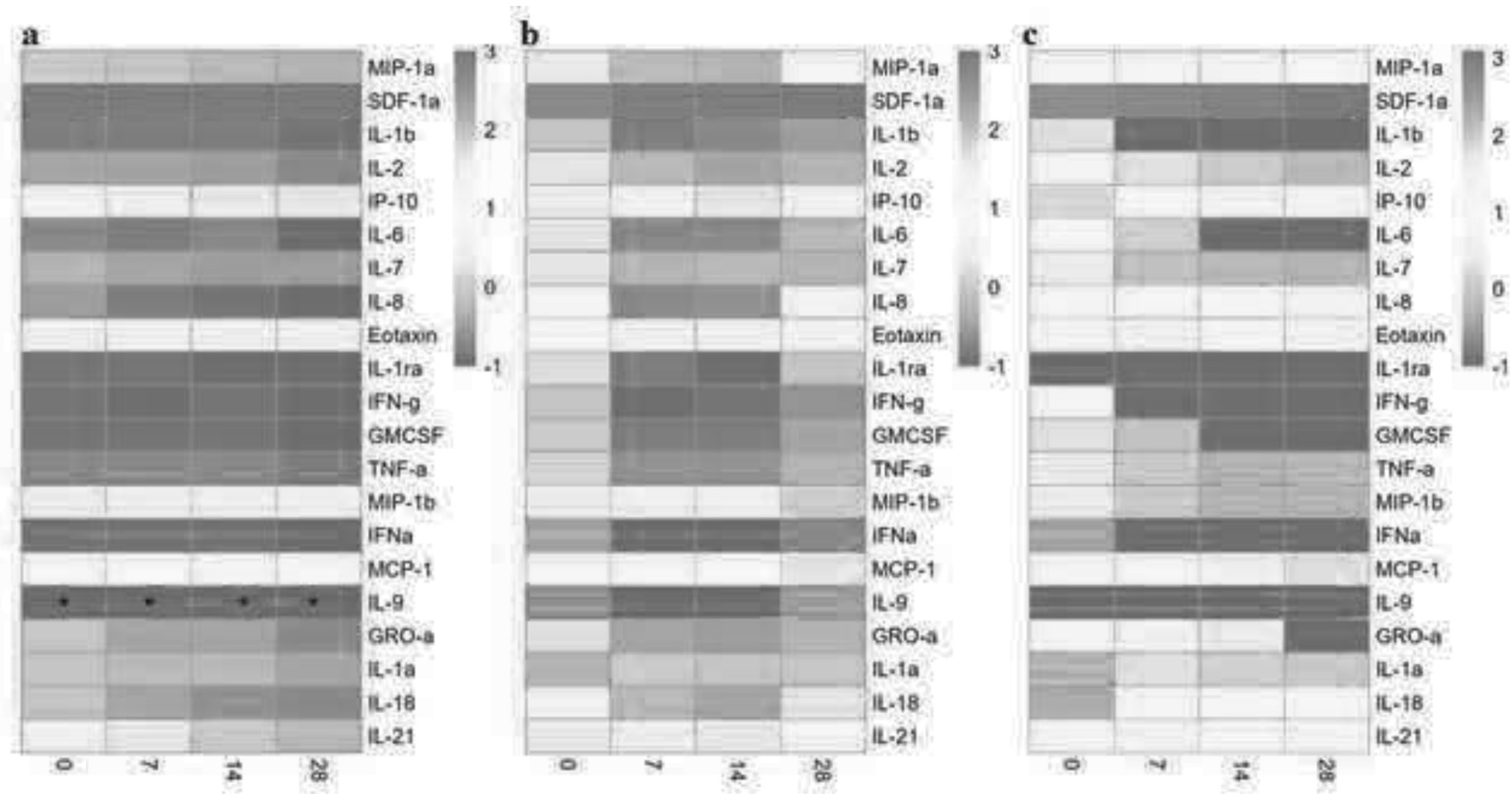

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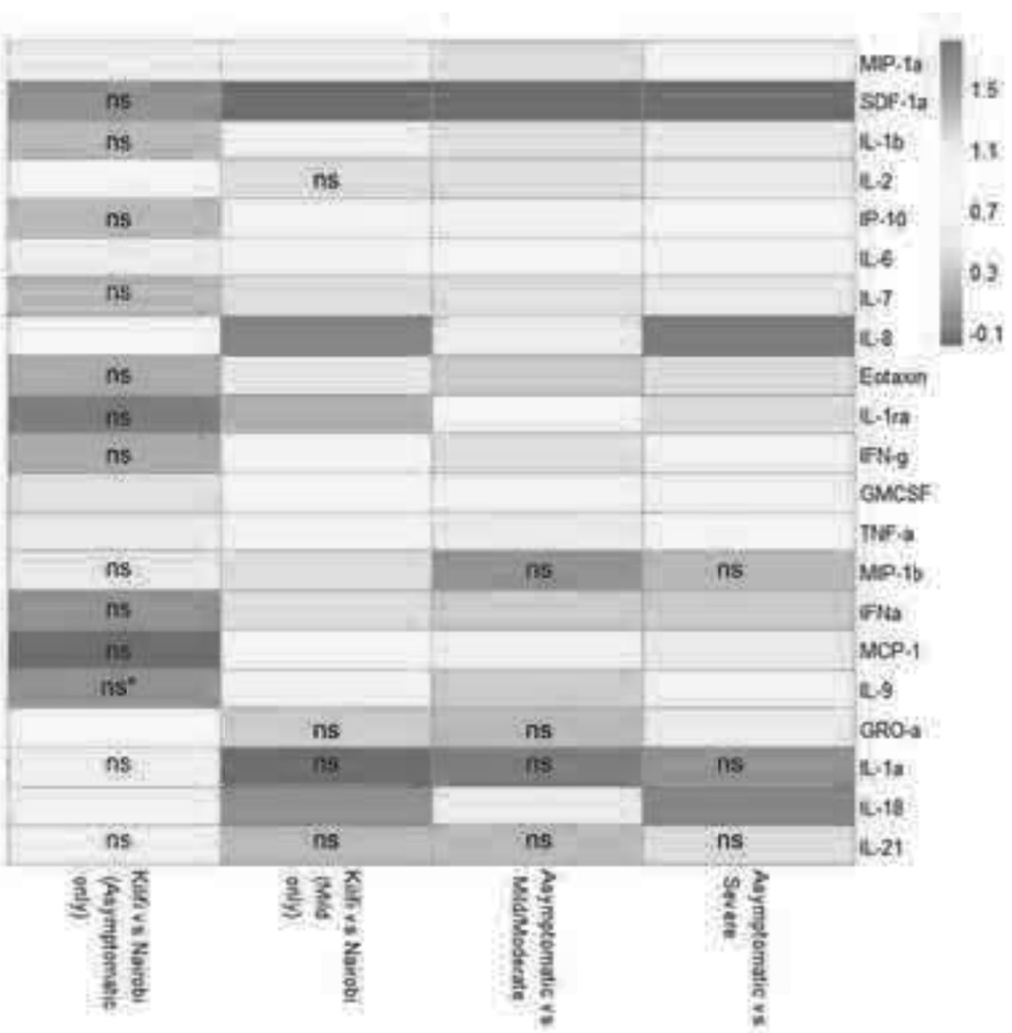

Figure 5

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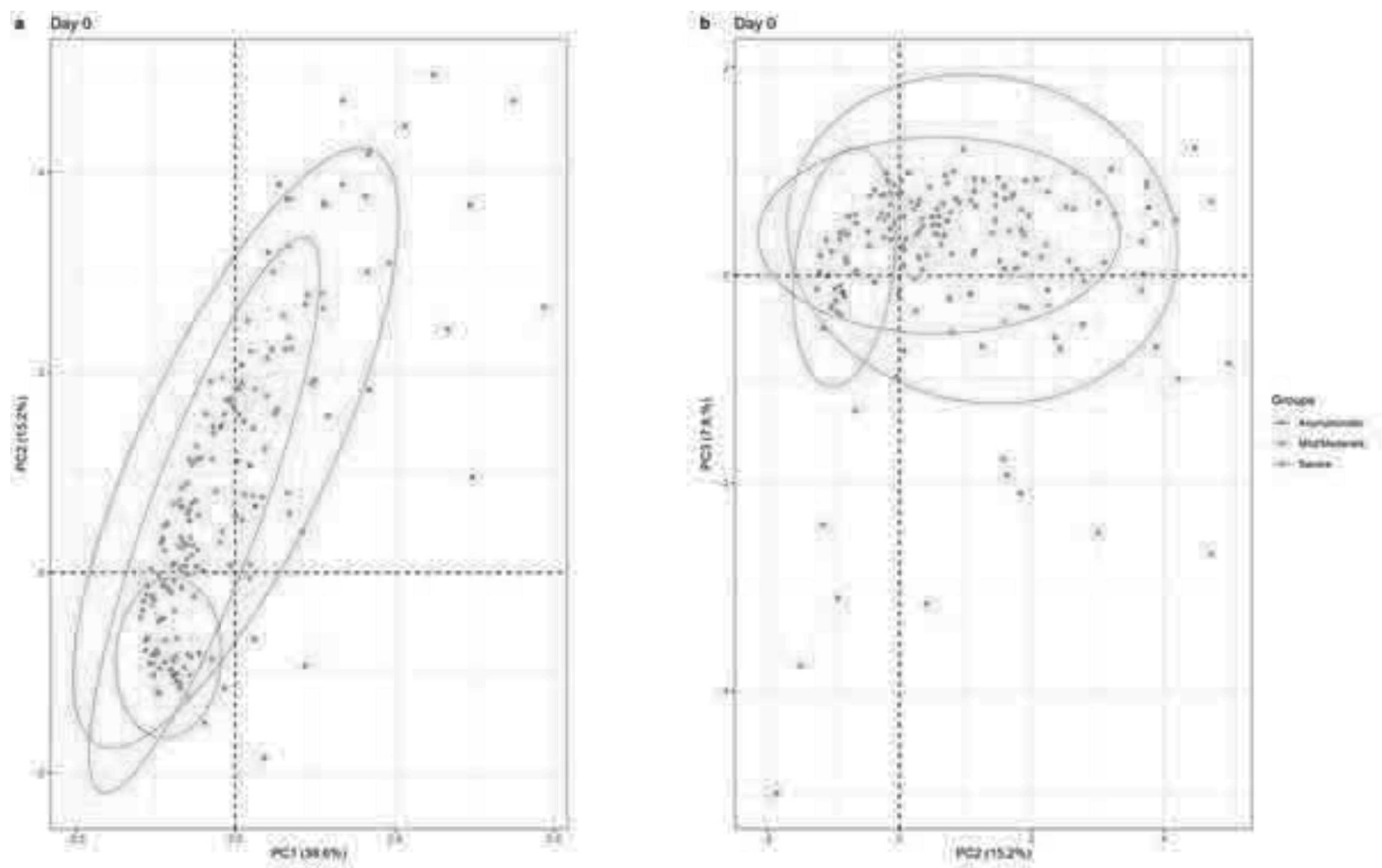

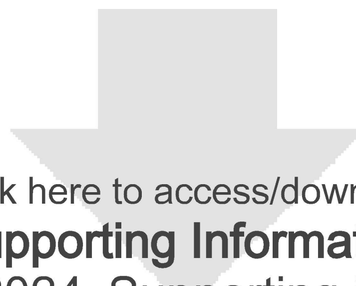

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Supporting Information
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THE AGA KHAN UNIVERSITY

Faculty of Health Sciences
Medical College

Ref: 2020/IERC-55 (v5)
May 19, 2020

Dr. Reena Shah
Faculty and Principal Investigator,
Aga Khan University, Nairobi, Kenya

Dear Dr. Reena and team,

RE: COVID-19 BIOREPOSITORY AND DATA REGISTRY

The Aga Khan University, Nairobi Institutional Ethics Review Committee (IERC), is in receipt of your protocol resubmitted to the Research Office on May 14, 2020 (Protocol Version 5.0). With reference to the IERC letter Ref: 2020/IERC-55 (v4)) dated May 13, 2020, the committee has reviewed the resubmitted protocol and confirms that concerns earlier raised have been addressed.

The IERC endorses the creation of the proposed institutional biorepository of samples and information from patients who have tested positive for COVID-19. This approval is valid from **May 19, 2020** to **May 20, 2021,** and is subject to compliance with the following requirements;

1. The operations of the repository shall be governed at all times by all applicable national and international laws, rules and regulations. IERC guidelines and Aga Khan University Hospital policies shall also apply, including those specific to patient samples and records management.
2. Any **subsequent research study** to be undertaken based on data/samples obtained from the repository must be submitted to the IERC for independent review.
3. As applicable, prior to export of biological specimens/data, the PI will ensure that a Material Transfer Agreement (MTA)/Data Transfer Agreement (DTA) is in place, as well as seek shipment authority/permit from the relevant government ministry. Copies of these must be submitted to the Research Office for record purposes.
4. All consent forms must be filed in the project's study binder and patient's hospital records.
5. Further, you must provide an interim report 60 days before expiration of the validity of this approval and request extension if additional time is required.
6. You should notify the committee of any changes that may affect your project (amendments, deviations, violations, discontinuation) and a final report submitted to the Research Office for record purposes.
7. The hospital management shall be notified of any manuscripts emanating from this work.

If you have any questions, please contact Research Office at research.supportea@aku.edu or 020-366 2148/1136.

With best wishes,

Wangari .

Dr. Wangari Waweru-Siika,
Chair - Institutional Ethics Review Committee (IERC)
Aga Khan University, (Kenya)

Copy – Co Investigators – Drs. Shahin Sayed, Mansoor Saleh and Rodney Adam

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P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **September 21, 2021**

TO: **ISABELLA OYIER**
PRINCIPAL INVESTIGATOR

THROUGH **THE DEPUTY DIRECTOR, CGMR-C**
KILIFI

Dear Madam,

RE: **KEMRI/SERU/CGMRC/200/4081 (*REQUEST FOR ANNUAL RENEWAL AND PROTOCOL DEVIATION*): INTEGRATED STUDIES OF THE NATURAL HISTORY OF SARS-COV-2 INFECTIONS IN KENYA.**

Thank you for the continuing review report for the period **September 10, 2020, to August 30, 2021.**

The Committee noted that a protocol deviation report has been submitted as the request for annual renewal was done later than the KEMRI SERU stipulated submission date.

This is to inform you that the expedited review team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval**.

This approval is valid from **September 21, 2021** through to **September 20, 2022**. Please note that authorization to conduct this study will automatically expire on **September 20, 2022**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the SERU by **August 09, 2022.**

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to the SERU for review prior to initiation. You may continue with the study.

Yours faithfully,

PROF. CHARLES OBONYO,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

THE AGA KHAN UNIVERSITY

Faculty of Health Sciences
Medical College

Ref: 2020/IERC-135 (v3)
December 10, 2021

Dr. Shahin Sayed - Principal Investigator
Chair and Director Laboratory Medicine,
Department of Pathology, Medical College, Aga Khan University, Nairobi

Dear Dr Sayed and team,

Re: Host immune responses to SARS-CoV-2: correlating kinetics with the natural history of infection

The Aga Khan University, Nairobi Institutional Ethics Review Committee (IERC), in receipt of your progress report submitted to the Research Office (RO) on November 12, 2021. With reference to the IERC letter Ref: 2020/IERC-135 (v2) dated December 8, 2020, the committee records that during the reporting period, the PI self-reports that:

1. No changes have been made to the study objectives.
2. No adverse effects/events have been encountered;
3. The study approval duration will expire on December 07, 2021
4. The team is requesting extension of the approval duration to continue with the study.

Based on the above considerations, the IERC has granted an extension approval for a further one year. This approval is valid until **December 06, 2022.** As PI, you are further expected to observe all the guidelines as per the study approval letter Ref: 2020/IERC-135 (v2) dated December 8, 2020

You are also reminded that researchers desiring to reinstate/continue research activities during COVID-19 pandemic must comply with the COVID-19 SOPs for Research. You are also reminded to file a copy of the research licence from the National Commission for Science, Technology and Innovation (NACOSTI) with a RO. Also submit the completed Self-Assessment Tool -Monitoring Ethical Compliance in Research.

If you have any questions, please contact AKU Research Office at AKUKenya.ResearchOffice@aku.edu or 020 366 2148/1136.

With best wishes,

Dr. Christopher Opio,
Chair - Institutional Ethics Review Committee (IERC)
Aga Khan University, (Kenya)

Copy: Co-Investigators

AK 963

3rd Parklands Avenue, off Limuru Road, P. O. Box 30270, GPO 00100, Nairobi, Kenya
Telephone: +254 20 366 2107/2109; Fax: +254 20 374 4035

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IMCA-006	Day 0	AKUH	11/02/2021
IMCA-007	Day 14	AKUH	11-02-2021
IMCA-007A	Day 0	AKUH	02/09/2021
IMCA-007A	Day 7	AKUH	12/09/2021
IMCA-008	Day 0	AKUH	18/02/2021
IMCA-008	Day 14	AKUH	04/03/2021
IMCA-008	Day 7	AKUH	25/02/2021
IMCA-009	Day 0	AKUH	18/02/2021
IMCA-009	Day 14	AKUH	04/03/2021
IMCA-009	Day 28	AKUH	17/03/2021
IMCA-009	Day 7	AKUH	25/02/2021
IMCA-010	Day 0	AKUH	18/02/2021
IMCA-010	Day 14	AKUH	04/03/2021
IMCA-010	Day 28	AKUH	22/03/2021
IMCA-010	Day 7	AKUH	25/02/2021
IMCA-010A	Day 0	AKUH	04/09/2021
IMCA-010A	Day 7	AKUH	11/09/2021
IMCA-012	Day 0	AKUH	03/03/2021
IMCA-012	Day 14	AKUH	19/03/2021
IMCA-012	Day 28	AKUH	01/04/2021
IMCA-013	Day 0	AKUH	03/03/2021
IMCA-013	Day 14	AKUH	18/03/2021
IMCA-013	Day 28	AKUH	01/04/2021
IMCA-032	Day 0	AKUH	25/03/2021
IMCA-032	Day 28	AKUH	22/04/2021
IMCA-032	Day 7	AKUH	01/04/2021
IMCA-033	Day 0	AKUH	25/03/2021
IMCA-033	Day 14	AKUH	13/04/2021
IMCA-033	Day 28	AKUH	22/04/2021
IMCA-033	Day 7	AKUH	01/04/2021
IMCA-038	Day 0	AKUH	26/03/2021
IMCA-038	Day 14	AKUH	13/04/2021
IMCA-038	Day 28	AKUH	22/04/2021
IMCA-046	Day 0	AKUH	14/04/2021

IMCA-046	Day 14	AKUH	05/05/2021
IMCA-046	Day 28	AKUH	18/05/2021
IMCA-046	Day 7	AKUH	23/04/2021
IMCA-050	Day 0	AKUH	01/07/2021
IMCA-050	Day 14	AKUH	16/07/2021
IMCA-050	Day 28	AKUH	27/07/2021
IMCA-050	Day 7	AKUH	07/07/2021
IMCA-052	Day 0	AKUH	08/07/2021
IMCA-052	Day 14	AKUH	03/08/2021
IMCA-052	Day 7	AKUH	16/07/2021
IMCA-053	Day 0	AKUH	08/07/2021
IMCA-053	Day 14	AKUH	03/08/2021
IMCA-053	Day 7	AKUH	16/07/2021
IMCA-055	Day 0	AKUH	03/08/2021
IMCA-055	Day 14	AKUH	19/08/2021
IMCA-055	Day 28	AKUH	07/09/2021
IMCA-055	Day 7	AKUH	10/08/2021
IMCA-056	Day 0	AKUH	03/08/2021
IMCA-056	Day 14	AKUH	16/08/2021
IMCA-056	Day 28	AKUH	31/08/2021
IMCA-056	Day 7	AKUH	11-08-2021
IMCA-057	Day 0	AKUH	16/08/2021
IMCA-057	Day 14	AKUH	26/08/2021
IMCA-057	Day 28	AKUH	14/09/2021
IMCA-057	Day 7	AKUH	23/08/2021
IMCA-058	Day 0	AKUH	19/08/2021
IMCA-058	Day 7	AKUH	23/08/2021
IMCA-059	Day 0	AKUH	23/08/2021
IMCA-059	Day 14	AKUH	31/08/2021
IMCA-059	Day 28	AKUH	16/09/2021
IMCA-059	Day 7	AKUH	26/08/2021
IMC-K002	Day 0	Kilifi	3/24/2021
IMC-K002	Day 14	Kilifi	3/24/2021
IMC-K002	Day 28	Kilifi	3/24/2021
IMC-K002	Day 7	Kilifi	3/24/2021
IMC-K004	Day 0	Kilifi	04/01/2021
IMC-K004	Day 14	Kilifi	04/01/2021
IMC-K004	Day 28	Kilifi	04/01/2021
IMC-K004	Day 7	Kilifi	04/01/2021
IMC-K005	Day 0	Kilifi	04/12/2021
IMC-K005	Day 7	Kilifi	04/12/2021
IMC-K006	Day 0	Kilifi	4/20/2021
IMC-K006	Day 14	Kilifi	4/20/2021
IMC-K006	Day 28	Kilifi	4/20/2021
IMC-K006	Day 7	Kilifi	4/20/2021
IMC-K007	Day 0	Kilifi	4/21/2021
IMC-K007	Day 14	Kilifi	4/21/2021
IMC-K007	Day 28	Kilifi	4/21/2021

IMC-K007	Day 7	Kilifi	4/21/2021
IMC-K008	Day 0	Kilifi	4/26/2021
IMC-K008	Day 14	Kilifi	4/26/2021
IMC-K008	Day 28	Kilifi	4/26/2021
IMC-K008	Day 7	Kilifi	4/26/2021
IMC-K009	Day 0	Kilifi	4/28/2021
IMC-K009	Day 28	Kilifi	4/28/2021
IMC-K009	Day 7	Kilifi	4/28/2021
IMC-K010	Day 0	Kilifi	05/07/2021
IMC-K010	Day 14	Kilifi	05/07/2021
IMC-K010	Day 28	Kilifi	05/07/2021
IMC-K010	Day 7	Kilifi	05/07/2021
IMC-K011	Day 0	Kilifi	05/07/2021
IMC-K011	Day 14	Kilifi	05/07/2021
IMC-K011	Day 7	Kilifi	05/07/2021
IMC-K012	Day 0	Kilifi	5/13/2021
IMC-K012	Day 14	Kilifi	5/13/2021
IMC-K012	Day 28	Kilifi	5/13/2021
IMC-K012	Day 7	Kilifi	5/13/2021
IMC-K014	Day 0	Kilifi	5/21/2021
IMC-K014	Day 14	Kilifi	5/21/2021
IMC-K014	Day 28	Kilifi	5/21/2021
IMC-K014	Day 7	Kilifi	5/21/2021
IMC-K015	Day 0	Kilifi	5/21/2021
IMC-K015	Day 14	Kilifi	5/21/2021
IMC-K015	Day 28	Kilifi	5/21/2021
IMC-K015	Day 7	Kilifi	5/21/2021
IMC-K016	Day 0	Kilifi	06/04/2021
IMC-K016	Day 14	Kilifi	06/04/2021
IMC-K016	Day 28	Kilifi	06/04/2021
IMC-K016	Day 7	Kilifi	06/04/2021
IMC-K017	Day 0	Kilifi	06/04/2021
IMC-K017	Day 14	Kilifi	06/04/2021
IMC-K017	Day 7	Kilifi	06/04/2021
IMC-K019	Day 0	Kilifi	6/17/2021
IMC-K019	Day 7	Kilifi	6/17/2021
IMC-K023	Day 0	Kilifi	6/28/2021
IMC-K023	Day 14	Kilifi	6/28/2021
IMC-K023	Day 28	Kilifi	6/28/2021
IMC-K023	Day 7	Kilifi	6/28/2021
IMC-K024	Day 0	Kilifi	6/30/2021
IMC-K024	Day 14	Kilifi	6/30/2021
IMC-K024	Day 28	Kilifi	6/30/2021
IMC-K024	Day 7	Kilifi	6/30/2021
IMC-K025	Day 0	Kilifi	07/02/2021
IMC-K025	Day 14	Kilifi	07/02/2021
IMC-K025	Day 28	Kilifi	07/02/2021
IMC-K025	Day 7	Kilifi	07/02/2021

IMC-K026	Day 0	Kilifi	07/07/2021
IMC-K026	Day 14	Kilifi	07/07/2021
IMC-K026	Day 7	Kilifi	07/07/2021
IMC-K027	Day 0	Kilifi	7/16/2021
IMC-K027	Day 14	Kilifi	7/16/2021
IMC-K027	Day 28	Kilifi	7/16/2021
IMC-K027	Day 7	Kilifi	7/16/2021
IMC-K029	Day 0	Kilifi	7/23/2021
IMC-K029	Day 14	Kilifi	7/23/2021
IMC-K029	Day 28	Kilifi	7/23/2021
IMC-K029	Day 7	Kilifi	7/23/2021
IMC-K030	Day 0	Kilifi	7/28/2021
IMC-K030	Day 14	Kilifi	7/28/2021
IMC-K031	Day 0	Kilifi	10/14/2021
IMC-K031	Day 14	Kilifi	10/14/2021
IMC-K031	Day 28	Kilifi	10/14/2021
IMC-K031	Day 7	Kilifi	10/14/2021
IMC-K032	Day 0	Kilifi	10/21/2021
IMC-K032	Day 7	Kilifi	10/21/2021
IMC-K033	Day 0	Kilifi	11/04/2021
IMC-K033	Day 14	Kilifi	11/04/2021
IMC-K033	Day 28	Kilifi	11/04/2021
IMC-K033	Day 7	Kilifi	11/04/2021
IMC-K034	Day 0	Kilifi	12/20/2021
IMC-K034	Day 7	Kilifi	12/20/2021
IMC-K035	Day 0	Kilifi	01/10/2022
IMC-K035	Day 14	Kilifi	01/10/2022
IMC-K035	Day 28	Kilifi	01/10/2022
IMC-K035	Day 7	Kilifi	01/10/2022
IMC-K037	Day 0	Kilifi	1/13/2022
IMC-K037	Day 14	Kilifi	1/13/2022
IMC-K037	Day 28	Kilifi	1/13/2022
IMC-K037	Day 7	Kilifi	1/13/2022
IMC-K038	Day 0	Kilifi	1/13/2022
IMC-K038	Day 14	Kilifi	1/13/2022
IMC-K038	Day 28	Kilifi	1/13/2022
IMC-K038	Day 7	Kilifi	1/13/2022
IMC-K039	Day 0	Kilifi	1/19/2022
IMC-K039	Day 14	Kilifi	1/19/2022
IMC-K039	Day 28	Kilifi	1/19/2022
IMC-K039	Day 7	Kilifi	1/19/2022
IMC-K040	Day 0	Kilifi	23-06-2022
IMC-K040	Day 14	Kilifi	07-07-2022
IMC-K040	Day 28	Kilifi	21-07-2022
IMC-K040	Day 7	Kilifi	29-06-2022
IMC-K043	Day 0	Kilifi	04-07-2022
IMC-K043	Day 14	Kilifi	18-07-2022
IMC-K043	Day 28	Kilifi	01-08-2022

IMC-K043	Day 7	Kilifi	12-07-2022
IMC-K044	Day 0	Kilifi	04-07-2022
IMC-K044	Day 14	Kilifi	18-07-2022
IMC-K044	Day 28	Kilifi	01-08-2022
IMC-K044	Day 7	Kilifi	12-07-2022
IMC-K045	Day 0	Kilifi	06-07-2022
IMC-K045	Day 14	Kilifi	21-07-2022
IMC-K045	Day 28	Kilifi	02-08-2022
IMC-K045	Day 7	Kilifi	13-07-2022
IMC-K046	Day 0	Kilifi	08-07-2022
IMC-K046	Day 14	Kilifi	22-07-2022
IMC-K046	Day 28	Kilifi	04-08-2022
IMC-K046	Day 7	Kilifi	15-07-2022
IMC-K047	Day 0	Kilifi	18-07-2022
IMC-K047	Day 14	Kilifi	01-08-2022
IMC-K047	Day 28	Kilifi	12-08-2022
IMC-K047	Day 7	Kilifi	26-07-2022

1. AKU_IERC Approval Letter Biorepository protocol.
    a. Covers samples with sample IDs starting with **BR**.
    b. Samples included in this study were collected within the approved ethics period: **May 19, 2020, to May 19, 2021**.

The remaining samples, with sample IDs starting with **IMCA-** or **IMC-K**, are covered by the following ethics approvals:

EMRI_SERU4081_ImmunoCOV_ApprovalLetter_Del1_1

   a. Ethics approval period: **September 10, 2020, to September 9, 2021**.

3. KEMRI_SERU4081_ImmunoCOV_ethics approval renewal 2021-2022
    a. Ethics approval period: **September 21, 2021, to September 20, 2022**.

KU_IERC_ImmunoCoV_Approval_Letter_Del1_1

   a. Approval period: **December 8, 2020, to December 7, 2021**.

5. AKU_ImmunoCoV_IERC Approval_Renewal_2021_2022
    a. Approval period: **December 8, 2021, to December 6, 2022**.