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# Sustained cellular immunity in adults recovered from mild COVID-19

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## Abstract

Transient lymphocytopenia is frequently observed in acute phase of coronavirus disease 2019 (COVID-19). It remains a concern whether impairment of cellular immunity may be retained after COVID-19. Here, we demonstrate by extensive lymphocyte profiling in 44 adults after mild COVID-19 that cellular immunity is not fundamentally altered in convalescent patients. Except for increased activated CD8+ lymphocytes, total counts of B, T, and NK cells and their subsets did not differ significantly between patients after COVID-19 and healthy controls after a median of 27 days (range 13–45) suggesting no residual cellular immune deficiency after recovery from mild COVID-19.

**KEYWORDS**
cellular immunity, COVID-19, lymphocytopenia, SARS-CoV-2

## 1 | INTRODUCTION

Approximately 20% of patients with coronavirus disease 2019 (COVID-19) have a severe or critical disease with respiratory or multi-organ dysfunction and a high mortality rate. A total of 80% of patients present with mild COVID-19 or non-severe pneumonia according to the World Health Organization (WHO). While pulmonary manifestations dominate the disease phenotype in most severe cases,[1] extent and consequences of impairment of other organ systems including the cellular immunity[2] with subsequent susceptibility to secondary infections are less clear to date.

Transient lymphocytopenia is a common finding in COVID-19 and closely related viral diseases by other *Coronavirinae*, such as SARS and MERS.[3,4] In contrast, lymphocytopenia is generally rare in most other viral diseases and has been reported for only few other viruses in the early phase of infection such as the avian influenza virus H5N1, the respiratory syncytial virus, or the swine foot-and-mouth disease virus.[5–7] Prolonged impaired cellular immunity following COVID-19 has been a concern based on observations from the measle virus disease where decrease of lymphocytes leads to suppression of immune response and enhanced overall childhood infectious disease mortality.[8,9]

In this study, we investigated whether patients who have recovered from COVID-19 retain alterations of their lymphocyte subset composition accompanied by transient immune suppression rendering them susceptible to subsequent infections. We focused on adult convalescents from mild COVID-19 who constitute the vast majority of patients affected from SARS-CoV-2.

Christoph Schmid and Rainer Claus contributed equally to this study.

## 2 | PATIENTS AND METHODS

### 2.1 | Patients

Between April 6 and May 15, 2020, B-, T-, and NK-lymphocyte subsets and SARS-CoV-2 antibody IgG and IgA titers (Euroimmun, Lübeck, Germany) were analyzed in the peripheral blood from 44 adults recovered from COVID-19 and 44 healthy controls. All convalescent COVID-19 patients had been tested positive for SARS-CoV-2 by local health organizations and were recruited from a call for volunteers for plasma donation. At the time of study enrollment, each patient had a negative test for SARS-CoV-2 and had not shown symptoms of COVID-19 for at least 2 weeks. A historical group of healthy controls was built retrospectively from a cohort of voluntary blood donors from 2019 (pre-COVID-19 era), balancing age and gender with the COVID-19 patient group. The study was approved by the institutional ethical committee. Signed informed consent was obtained from all participants.

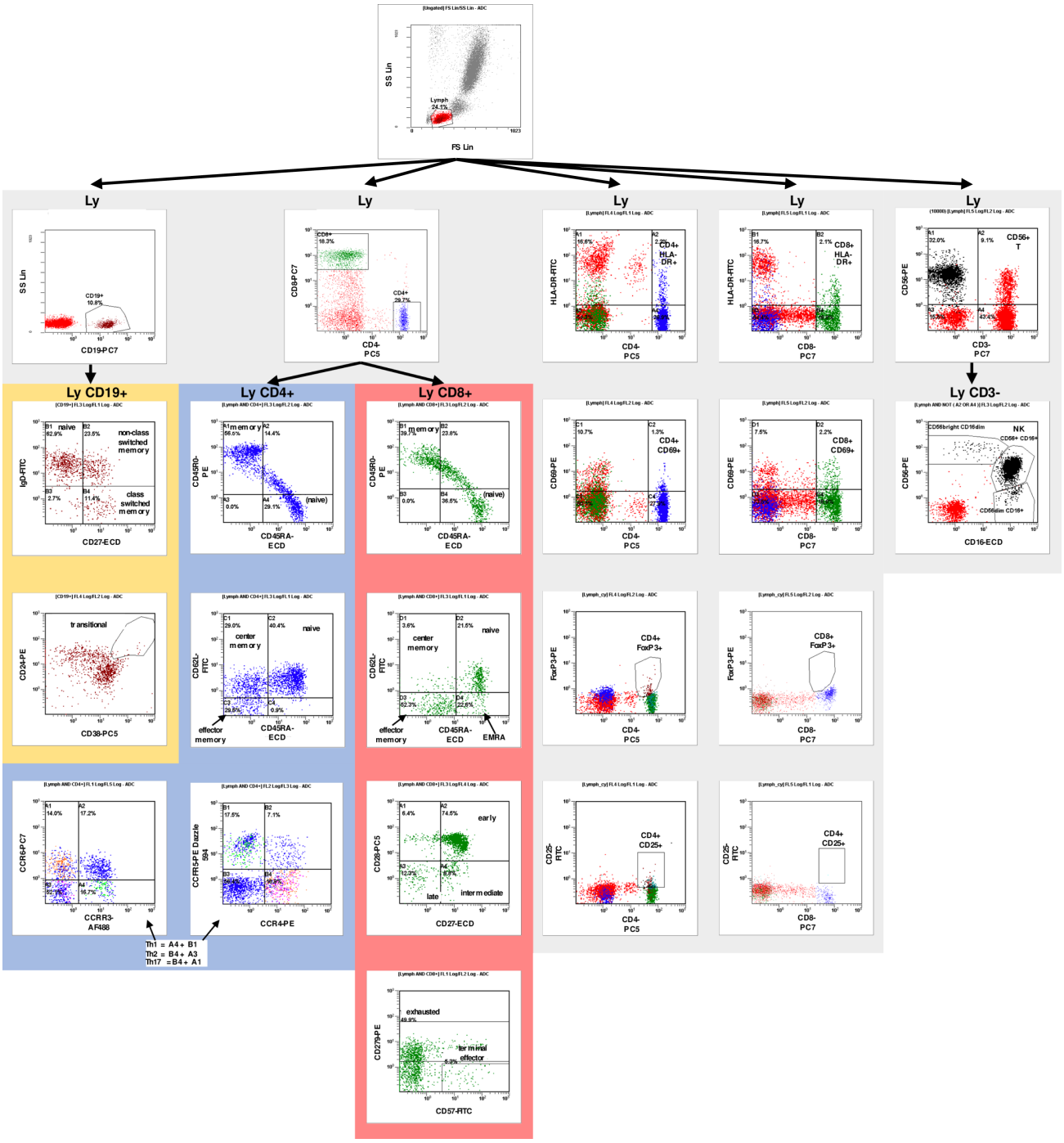


**FIGURE 1** Flowcytometric gating strategy of lymphocyte types and subsets from a healthy donor included in the study. Lymphocytes were identified by using forward and side scatter; B lymphocytes were defined by the presence of CD19 and T helper and cytotoxic lymphocytes by the presence of CD4 and CD8, respectively. Lymphocyte subsets were further divided as described in materials and methods and in Table S2

## 2.2 | Sample processing and flow cytometric analysis

EDTA peripheral blood was collected and processed within a maximum of 24 h. Blood samples were distributed into seven 50 μL aliquots. A total of 10 μL of commercial fluorescein isothiocyanate (FITC-) and phycoerythrin (PE-) as well as 5 μL of phycoerythrin Texas red-X (ECD-) and phycoerythrin-cyanin (PC5- and PC7-) labeled antibodies purchased from Beckman Coulter (Brea, California) and Biolegend (San Diego, California) were added followed by 15 min incubation (Table S1). Erythrocyte lysis was conducted by addition of 500 μL VersaLyse (Beckman Coulter, California) for 15 min followed by centrifugation with 360 g for 5 min. Subsequently, samples were washed with PBS. After cell staining, lymphocyte subsets were analyzed by flow cytometry-based immunophenotyping (FC500, Beckman Coulter, California). Raw data were deposited at http://flowrepository.org/id/FR-FCM-Z343.

To assure data quality, flow rate, laser power calibration, and event dispersion of the FC 500 flow cytometer were checked on a daily basis using Flow-Check Pro Fluorospheres (Beckman Coulter). Autosetups with Cyto-Comp Cells (Beckman Coulter) were routinely repeated for the automated compensation matrices. Voltages and compensation settings of all single channels were tested specifically with beads for the five-color flow cytometry panel of this study.

For correct identification of lymphocyte subsets, plausibility controls with reference material from healthy donors were used repeatedly and identical samples were measured as internal standards on a regular basis.

## 2.3 | Gating strategy and analysis

For all lymphocyte subsets, percentages were determined, and absolute numbers were calculated. Absolute leukocyte counts were measured with Stem-Count (Stem-Kit, Beckman Coulter) using CD45-FITC. A minimum of 10,000 events per aliquot of each sample was analyzed. Lymphocytes were identified by forward and side scatter. To divide lymphocytes into different subsets, established standards and a well-described gating strategy as shown in Figure 1 were used.[10] Detailed information on lymphocyte subset specific immune phenotypes and on fluorochrome-antibody conjugates are listed in Table S2. B lymphocytes were identified by the presence of CD19 (CD19-PC7 IM3628) and were further divided into naïve (IgD+ CD27 –; IgD-FITC B30652, CD27-ECD B26603), non-class-switched (NCS) memory (IgD+ CD27+), class-switched (CS) memory (IgD– CD27+) and transitional (CD24hi CD38hi; CD24-PE IM1428U, CD38-PC5 A07780) subsets. T lymphocytes defined by positivity for CD8 or CD4 were subdivided into naïve (CD62L+ CD45RA+), memory T cells (CD4+ CD45RA– CD45RO+/CD8+ CD45RA– CD45RO+), which were further divided into central memory (CD62L+ CD45RA–), effector memory (CD62L– CD45RA–), effector memory RA+ (EMRA) (CD62L– CD45RA+) and activated memory (HLA-DR+ or CD69+) cells, and FoxP3+ cells. Furthermore, type 1, 2 and 17 CD4+ T helper (Th1/Th2/Th17) cells were identified by using antibodies against CXCR3, CCR4, CCR5, and CCR6. Th1 cells were defined as CD4+ CXCR3+ CCR4– CCR5+ CCR6–, Th2 cells as CD4+ CXCR3– CCR4+ CCR5– CCR6– and Th17 cells as CD4+ CXCR3– CCR4+ CCR5– CCR6+. Within cytotoxic CD8+ T lymphocytes, activated subsets in early (CD28+ CD27+), intermediate (CD28– CD27+) and late (CD28– CD27–) status as well as exhausted (CD279+) and terminal effector (CD279– CD57+) cells were identified. CD56 T cells were defined as CD56+ CD3+. NK lymphocytes were detected as CD56+ CD3– cells and subdivided into three NK subsets (CD56+ CD16+, CD56dim CD16bright, and CD56bright CD16dim).

Wilcoxon test for associated samples (two-tailed) was used to detect significant differences of lymphocyte subsets among convalescent COVID-19 patients and healthy controls. Pre-defined subgroups defined by sex, age (> vs. < median years), duration of COVID-19, time from onset, and end of symptoms of COVID-19 (> vs. < median days) were analyzed within the cohort of patients recovered from COVID-19. A $p$ value <0.05 was regarded as statistically significant.

**TABLE 1** Demographics and characteristics of convalescents from COVID-19 and age and sex balanced controls

| Characteristics | COVID-19 patients ($n$ = 44) | Controls ($n$ = 44) |
|---|---|---|
| Age (years), median (range) | 43 (18–61) | 40 (18–61) |
| Gender, $n$ (%) | | |
| Male | 31 (70.5) | 30 (68) |
| Female | 13 (29.5) | 14 (32) |
| Symptoms during COVID-19, $n$ (%) | | |
| Fever (>38°C) | 27 (61.4) | |
| Myalgia | 19 (43.2) | |
| Cough | 17 (38.6) | |
| Headache | 9 (20.5) | |
| Sorethroat | 6 (13.6) | |
| Dorsal pain | 6 (13.6) | |
| Loss of taste | 4 (9.1) | |
| Dyspnoe | 3 (6.8) | |
| Diarrhoe | 2 (4.5) | |
| Duration of symptoms (days), median (range) | 11 (1–26) | |
| Severity of COVID-19 (according to WHO), $n$ (%) | | |
| Mild illness | 43 (97.7) | |
| Pneumonia | 1 (2.3) | |
| Severepneumonia | 0 | |
| ARDS | 0 | |
| Sepsis | 0 | |
| Time from first symptoms to analysis (days), median (range) | 37 (26–51) | |
| Time from end of symptoms to analysis (days), median (range) | 27 (13–45) | |

## 3 | RESULTS

Median age of patients was 43 years (range 18–61), 70% were male. Basic demographics were well matched between cases and healthy controls ($p$ = 0.930 for age, $p$ = 0.818 for sex). Convalescent COVID-19 patients did not exhibit relevant comorbidities and had suffered from mild COVID-19, defined as uncomplicated upper respiratory tract viral infection or pneumonia without need for supplemental oxygen according to the interim classification and guidance of the WHO. The most frequent COVID-19 associated symptoms included fever >38°C, myalgia, cough, headache, and sore throat (Table 1). One patient had radiographically proven pneumonia. Median symptom duration was 11 days (range 1–26). Median intervals from disease onset and end of symptoms to assessment of the immune status were 37 (range 26–51) and 27 (range 13–45) days, respectively.

Serological testing showed positivity for anti-SARS-CoV-2 IgG in 39 of 44 patients (89%) and for anti-SARS-CoV-2 IgA in 36 of 44 patients (82%). In 4/44 patients (9%) neither IgA nor IgG titres were positive, one patient had IgA but no IgG antibodies, and four patients had IgG but no IgA antibodies. The total numbers of B lymphocytes (median 195/µl [range 61–461] for patients vs. 206/µl [range 65–770] for controls) and their subsets did not differ significantly between both groups (Figure 2, upper panel analyzed data given as lymphocyte subtype per microliter are available in Table S3). Similarly, total CD3+ lymphocytes (median 1244/µl [range 661–2243] vs. 1175/µl [range 475–23,224]), total CD4+ (median 753/µl [range

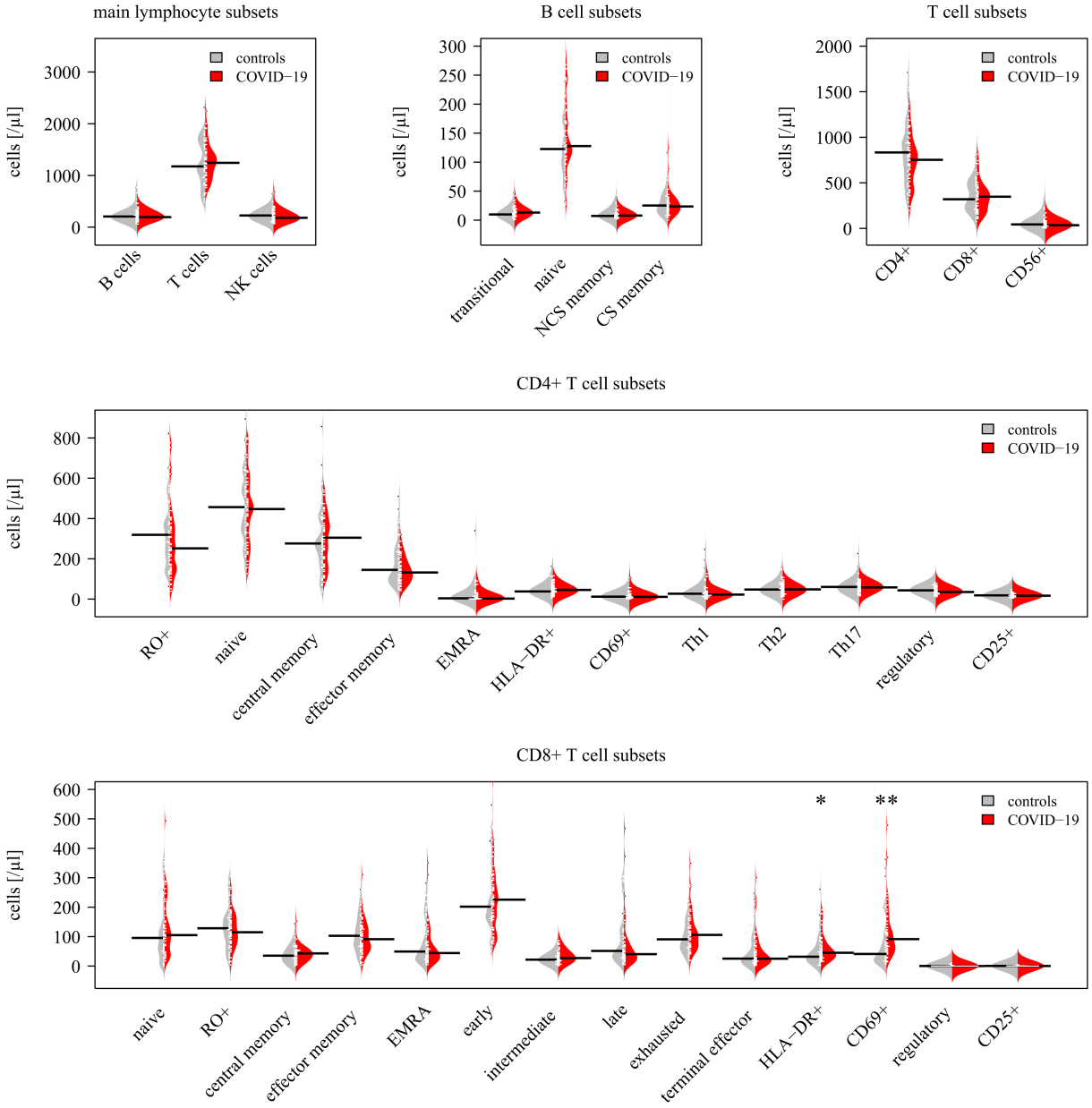


**FIGURE 2** Density distribution of main lymphocyte types and subsets between patients of the control cohort (gray) and the COVID-19 cohort (red) displayed as bean plots. Black bars indicate the median, white ticks indicate individual data points, *$p$ = 0.038, **$p$ = 0.002

196–1365] vs. 833/μl [range 286–1711]) and CD8+ T cells (median 348/μl [range 107–814] vs. 320/μl [range 75–789]) and ratio of CD4+/CD8+ T cells (median 2.13 vs. 2.28) were not significantly different between convalescents from COVID-19 and controls. Except for higher numbers of activated CD8+/HLA-DR+ ($p$ = 0.038) and CD8+/CD69+ lymphocytes ($p$ = 0.002) in COVID-19 patients, no significant differences in any of the T lymphocyte subsets were detectable (Figure 2, lower panel). Subgroup analyses (including patients' age, gender, and latency between onset/end of symptoms and time of analysis) did not reveal associations with the cellular immune status either.

## 4 | DISCUSSION

The observation of transient lymphocytopenia in COVID-19 patients raises the question whether impairment of cellular immune response, followed by increased susceptibility to secondary infections might constitute a concern for convalescents. In this study, no significant differences between cell numbers for B, T, and NK lymphocytes and most of their subsets indicative for acquired immune deficiency could be detected between patients recovered from mild COVID-19 and comparable healthy controls. The only significant lymphocyte subset deviation observed in our study was an increased number of activated T cells (CD8+/HLA-DR+ and CD8+/CD69+) in the patient group. This is most likely due to the recent SARS-CoV-2 infection per se, and timely normalization of these T cell subsets would be expected. Likewise, no alterations of lymphocyte subsets were found in the pre-planned subgroup analyses of COVID-19 patients. The finding of retained cellular immunity especially with regard to normal counts of memory and regulatory T cells is of clinical significance, suggesting that long-term immune deficiency as observed, for example, for measles is unlikely for the large number of patients who recover from mild COVID-19.

Furthermore, a reliable response of the humoral immune system after mild COVID-19 was demonstrated in our study. This result is line with published data of previously reported anti-SARS-CoV-2 antibody testing.[11]

Our study has some limitations: the focus of our investigation was centered on adults recovering from mild COVID-19, as they constitute the vast majority of patients (approximately 80%) affected by SARS-CoV-2. Thus, our results might not be transferable to more severe disease courses, to children or to elderly patients. Furthermore, lymphocyte counts during acute phase of the disease were not available for the COVID-19 patient cohort due to the design and recruitment procedure of the study. However, as intermediate or long-term immunodeficiency might not be reflected by the extent of transient lymphocytopenia but rather the prolonged alteration of lymphocyte subsets, we could demonstrate by extensive immune phenotyping efforts that none of the relevant lymphocyte subpopulations exhibited meaningful differences indicative for impaired cellular immune response.

In summary, our data provide evidence that mild COVID-19 does not lead to detectable cellular immune deficiencies that may cause increased susceptibility for subsequent infections. In a current long-term follow-up, we are assessing the rate of subsequent infections and related complications after COVID-19.

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### AUTHOR CONTRIBUTIONS

**Andreas Rank:** Conceptualization; data curation; formal analysis; investigation; methodology; writing-original draft. **Phillip Löhr:** Formal analysis; investigation; methodology. **Reinhard Hoffmann:** Investigation; resources. **Alanna Ebigbo:** Investigation; resources. **Stefanie Grützner:** Investigation; resources. **Christoph Schmid:** Conceptualization; project administration; supervision; writing-review and editing. **Rainer Claus:** Conceptualization; project administration; supervision; visualization; writing-original draft; writing-review and editing.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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