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LETTER TO THE EDITOR Open Access

Exposure of monocyte-derived macrophages to the UV-inactivated SARS-CoV-2 VOCs shows similar effects on the transcriptomic profile as active virus: a comparative analysis

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Dear Editor,

Our recent publication "Transcriptomic Profiles of Monocyte-Derived Macrophages Exposed to SARS-CoV-2 VOCs Reveal Immune-Evasion Escape Driven by Delta" revealed a transcriptomic alteration and an evolution of the host monocyte-derived macrophages (MDM) in response to the exposure to SARS-CoV-2 VOCs, particularly for Delta variant. We now decided to investigate the effects of inactivated SARS-CoV-2 VOCs exposure on human MDMs, with a particular focus on the immune response key players. Isolated SARS-CoV-2 virus can be completely inactivated by ultraviolet (UV) radiation that has been shown to abolish SARS-CoV-2 infectivity in mice, while preserving the antigenic properties [1]. Thus, we firstly inactivated SARS-CoV-2 VOCs by UV radiation and then we exposed human MDM to the inactivated viral particles, as previously described [2]. Briefly, 10 mL of 10000TCID50/mL viable SARS-CoV-2 were exposed to UV-light for 2 h at room temperature, aliquoted and stored at -80 °C. To assess viral inactivation, 200uL were inoculated on VERO E6 cells, and plates were checked daily. No cytopathic effect was detected up to 10 days post inoculum. After confirmation, MDM were exposed to the UV-inactivated SARS-CoV-2 inoculum at the same conditions. RNA-Seq experiments and analyses were performed as previously described [3]. We performed a comparative analysis between the RNA-Seq data, derived from the MDM exposed to the inactivated SARS-CoV-2 VOCs, and our recently published RNA-Seq data obtained from MDM exposed to the viable SARS-CoV-2 VOCs [3]. Surprisingly, we did not detect significant differences in the transcriptomic profiles between the two datasets, as shown in the Volcano plots (Fig. 1A), despite few coding and non-coding RNAs (Supplementary Table 1).

In our previous study [2], we found that some of the most differentially expressed pathways in the MDM exposed to active SARS-CoV-2 VOCs were the ones related to PARP9 and PARP14 pathways, which are

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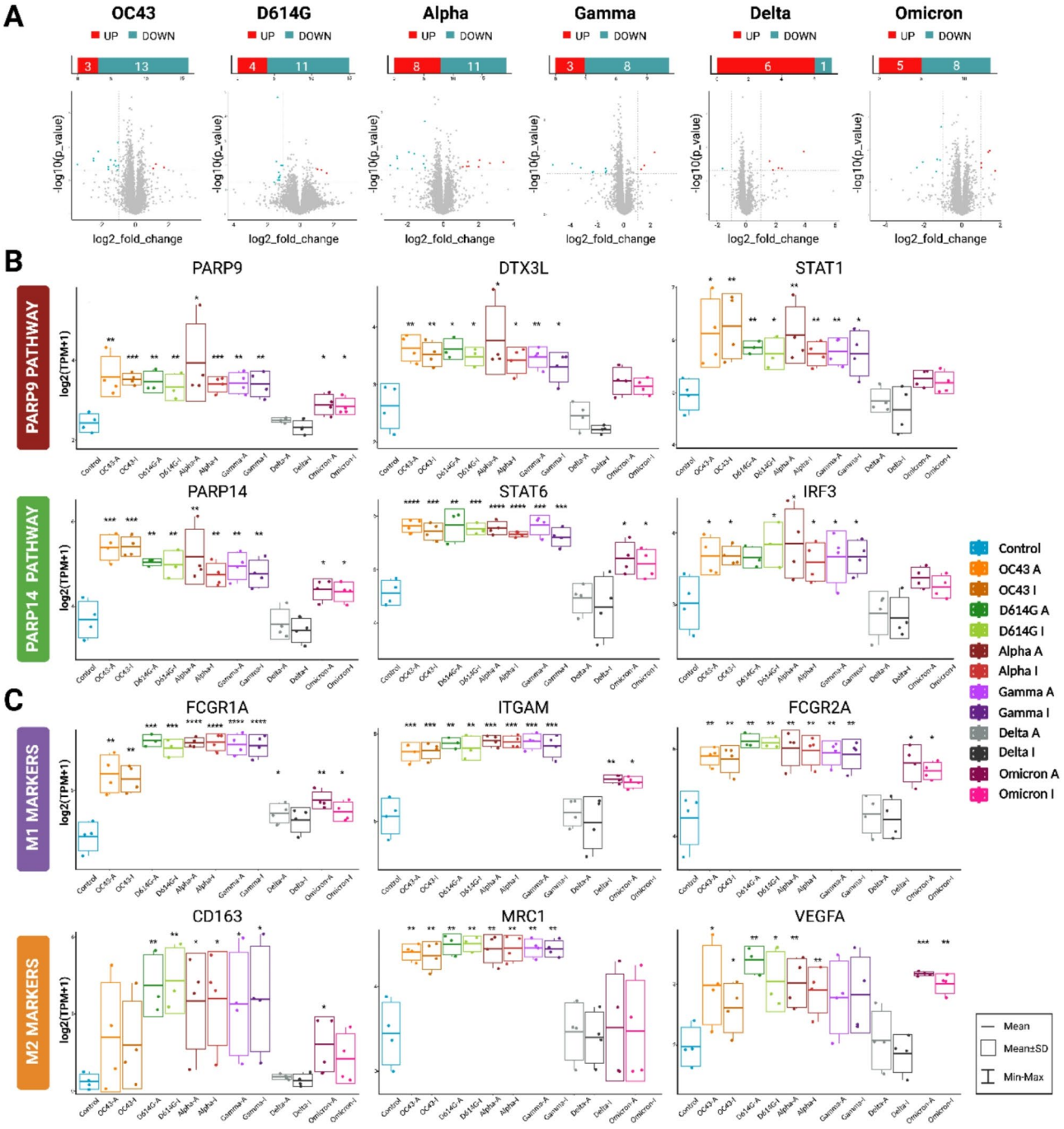

Fig. 1 (**A**) Volcano plots showing the differentially expressed genes (DEGs, 1 < Log2_FC<-1, $p < 0.05$) in macrophages exposed to UV-inactivated OC43, D614G, Alpha, Delta, Gamma, and Omicron variants over each active counterpart. Red dots represent up-regulated genes, and blue dots down-regulated genes; (**B**) Relative gene expression of the transcripts related to PARP9 and PARP14 pathways in macrophages exposed to UV-inactivated OC43, D614G, Alpha, Delta, Gamma, and Omicron variants and each active counterpart. (**C**) Relative gene expression of M1 and M2 representative markers in macrophages exposed to UV-inactivated OC43, D614G, Alpha, Delta, Gamma, and Omicron variants and each active counterpart. Statistical significance of each sample was calculated over the control by using a Student t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. This image was created using BioRender (https://www.biorender.com/)

known to be crucial for macrophages activation [4]. Thus, we wondered whether we could observe the same trend for the MDM exposed to inactivated SARS-CoV-2 VOCs. Strikingly, we found that MDM exposed to inactivated SARS-CoV-2 VOCs behaved exactly as each active counterpart. Indeed, mRNA levels of both the PARP9 axis, including its ligand DTX3L and the downstream transcription factor STAT1, and the PARP14 axis, including

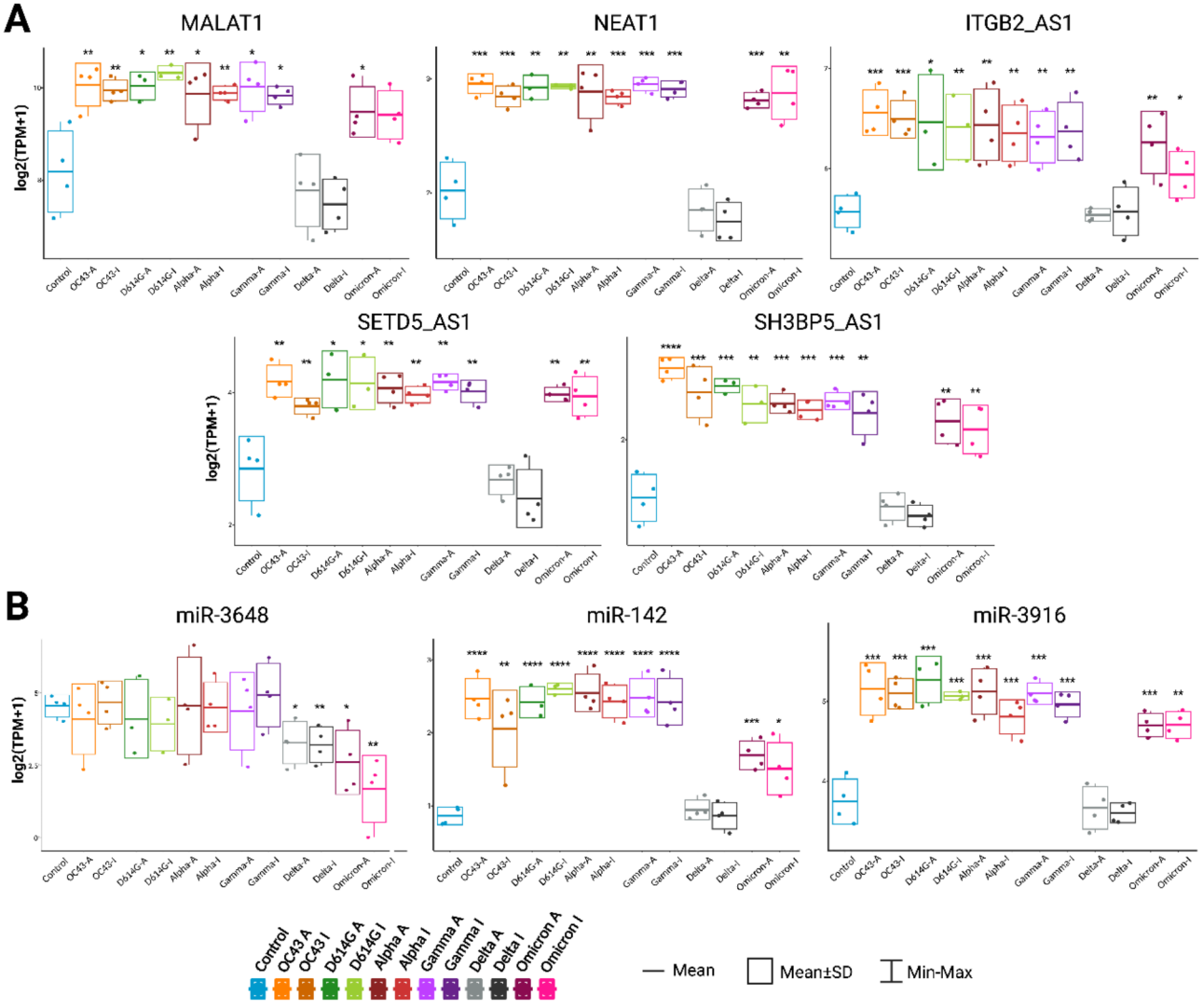

Fig. 2 (**A**) Comparative Relative gene expression of lncRNAs in macrophages exposed to UV-inactivated OC43, D614G, Alpha, Delta, Gamma, and Omicron variants and each counterpart; (**B**) Comparative relative gene expression of miRNAs in macrophages exposed to UV-inactivated OC43, D614G, Alpha, Delta, Gamma, and Omicron variants and each counterpart. Statistical significance of each sample was calculated over the control by using a Student t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. This image was created using BioRender (https://www.biorender.com/)

STAT6 and interferon regulatory factor 3 (IRF3), strongly and significantly increased in macrophages exposed to the inactivated OC43, SARS-CoV-2 D614G, Alpha and Gamma coronavirus variants, compared to the control, with the only exception of Delta (Fig. 1B). We did observe the same trend also for the M1/M2 markers (Fig. 1C). Notably, also in this case, MDM exposed to inactivated Delta variant did not express the M1/M2 markers, suggesting an M0 status of these cells, comparable to the naïve control group. Finally, we observed that the expression pattern of various non-coding RNAs, such as lncRNAs miRNAs and snoRNAs, in MDM exposed to inactivated SARS-CoV-2 VOCs was similar to the one of MDM exposed to active SARS-CoV-2 VOCs (Fig. 2A-B).

Altogether, our findings strongly suggest that the UV-inactivated SARS-CoV-2 VOCs are capable to induce on human monocytes-derived macrophages the same effects as the active VOCs counterparts. Indeed, inactivated viruses are able to induce the same alteration in the expression profiles for both coding RNAs and non-coding RNAs, crucial for macrophages activation and differentiation (M1/M2 status), thus suggesting their potential to trigger essential immune pathways. Importantly, the same immune evasion phenotype assessed for the active Delta variant was also observed in the MDM exposed to UV-inactivated delta variant. These observations open new perspectives on the role of inactivated viruses on the transcriptomic profiles of human macrophages and pave the way for further studies on the role of inactivated

vaccines, especially in immunocompromised individuals with reduced mRNA vaccine efficacy [5].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06264-1.

Supplementary Material 1

Acknowledgements
Figures were created with the licenced version of BioRender as indicated in the figure legends.

Author contributions
AG, IC, DL, PGC and FB designed the project. JCS, RV, CC, RG, SC and NC acquired, analysed, and interpreted the data. AG, IC, VM and MB wrote the manuscript. All authors approved the final manuscript, agreed to be personally accountable for their contribution and agreed to investigate, resolve, and document appropriately in the literature any questions related to the accuracy or integrity of this work that may be raised.

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Data availability
All the data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved (P_175_2021) by the Medical Ethics Committee of IRCCS Policlinico San Matteo of Pavia, and was conducted in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from all patients prior to enrolment.

Consent for publication
The authors have seen and approved the final manuscript.

Competing interests
The authors have declared that they have no competing interests.

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