Supplementary Material

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**Supplementary Figure 1. Peripheral blood T-lymphocyte analysis strategy.** Initially the singlets were selected for FSC and SSC, then the population of live cells. Within the living cells, the lymphocyte population was selected and later the CD3+ population. The CD4+ and CD8+ populations were identified and subsequently evaluated by markers CD38, HLA-DR, and CD28.

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**Supplementary Figure 2. Analysis strategy for evaluating PD-1 expression in PBMC**. Initially the singlets were selected for FSC and SSC, then the population of live cells. Within the living cells, the lymphocyte population was selected, and later the CD56+ and CD3+ populations were identified. Within the CD3+ population, the CD4+ and CD8+ T-cell gates were selected. In the CD4+ and CD8+ populations, the expression of PD-1 was evaluated by SSC-A.



Supplementary Figure 3. Analysis strategy for the functional assessment of CD4+ T-lymphocytes and CD8+ T-lymphocytes. Initially the singlets were selected for FSC and SSC, then the population of live cells. Within the living cells, the lymphocyte population was selected, and later the CD56+ and CD3+ populations were identified. Within the CD3+ population, the CD4+ and CD8+ T-cell gates were selected. In the CD4+ and CD8+ populations, the frequency of IFN-α, Granzyme, Perforin, CD107a, and TNF was evaluated by SSC-A.



**Supplementary Figure 4. Analysis strategy for assessing the purity of CD8+ T-lymphocytes.** Initially, the singlets were selected for FSC and SSC, then the population of live cells. Within the living cells, the lymphocyte population was selected and later the TCD8+ CD3+ lymphocyte populations were identified.



**Supplementary Figure 5. Median Fluorescence Intensity (MFI) of activation markers in T-lymphocytes from individuals with COVID-19.** The graphs show the MFI of (A) CD38+ in CD4+ T-lymphocytes, (B) HLA-DR+ in CD4+ T-lymphocytes, (C) CD28+ in CD4+ T-lymphocytes, (D) CD38+ in CD8+ T-lymphocytes, (E) HLA-DR+ in CD8+, and (F) CD28+ T-lymphocytes on CD8+ T-lymphocytes in uninfected individuals from patients with moderate and severe/critical disease. PD-1 MFI of PBMCs from (G) CD4+ T-lymphocytes, (H) CD8+ T-lymphocytes from uninfected patients with severe/critical COVID-19. The red dots represent patients with the critical infection. The bars represent the median and interquartile range. \*\* P < 0.01, \*\*\* P < 0.001, and \*\*\*\* P < 0.0001.

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**Supplementary Figure 6. Evaluation of the cytotoxic profile of CD4+ and CD8+ T lymphocytes in SARS-CoV-2 infection, considering the double positive.** The graphs represent the cytotoxic profile (granzyme A, perforin, CD107a, IFN-γ, TNF) of CD4+ and CD8+ T lymphocytes from PBMCs from control subjects and those affected with severe/critical COVID-19, considering the double positives. (A) Cytotoxic profile of CD4+ T lymphocytes, from basal levels and (B) with stimulation with PMA and Ionomycin, (C) Cytotoxic profile of CD8+ T lymphocytes, from basal levels and (D) with stimulation with PMA and Ionomycin. Stimulated values were subtracted from baseline values. The red dots represent patients with the critical infection. \* P<0.0001.

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**Supplementary Figure 7. Cluster distribution evaluated by tSNE.** Percentage of clusters evaluated in the tSNE technique of baseline and stimulated groups of uninfected patients with severe/critical COVID-19.