**SUPPLEMENTARY MATERIALS**

**MATHERIAL AND METHODS**

**Study patients**

The two patients were recruited immediately upon arrival to the Emergency Department of La Paz University Hospital (**Figure 1**). As controls, 10 healthy volunteers were recruited (**Table 1**). All patients and healthy controls signed an informed consent, and the data were treated according to the recommended confidentiality criteria and following the ethical guidelines of the 1975 Declaration of Helsinki.

**Retrospective analysis**

Plasma samples and viability frozen peripheral blood mononuclear cells from fifty-seven COVID-19 patients with disparate evolution were included. Blood samples taken on admission and before any treatment were stored in the Biobank of La Paz University Hospital during the first wave of the pandemic. Patients included in the study were classified according to their final outcome as survivors (n=47) or *exitus* (n=10). Additionally, new healthy volunteers (n=15) were also included (**Supplementary Table** **1**). All patients and healthy controls signed an informed consent, and the data were treated according to the recommended confidentiality criteria and following the ethical guidelines of the 1975 Declaration of Helsinki.

**Whole blood sample preparation and plasma collection**

Blood samples were collected in BD Vacutainer® CPT™ Sodium Heparin and BD Vacutainer ® Lithium Heparin PST tubes. For plasma isolation, CPT tubes containing the blood were centrifuged at room temperature for 15 min at a relative centrifugal force of 1800. Plasma samples were stored at -80º C until analysis. For leukocyte cell isolation, 0.5 mL of blood from the lithium heparin PST tube were treated with BD FACS lysing solution (BD Biosciences) for red blood cell lysis. After that, the samples were washed twice with phosphate-buffered saline and stored at 4 ºC until analyzed by flow cytometry.

**Cytokine and soluble immune checkpoints quantification**

The IL-6 and IL-17 levels in the patients and healthy volunteers’ plasma were measured by using the Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Cytokine Kit from BD Biosciences. The levels of soluble Galectin-9, PD-L1, PD-1, LAG-3, CTLA-4, 4-1BB, and CD86 in the patients and healthy volunteers’ plasma were measured by using LEGENDplex™ HU Immune Checkpoint Panel 1 from BioLegend. Samples were acquired in FACSCalibur (BD Biosciences) flow cytometers, and the data was analyzed using FCAP Array Software v3.0 (BD Biosciences) and LEGENDplex™ Software v8 (BioLegend).

**Antibodies and flow spectral cytometry analysis**

The FACS analysis was performed using specific human antibodies to the following surface molecules: CD3-Spark Blue 550, CD4-Brilliant Violet (BV) 570, CD16-BV510, CD14-Alexa Fluor® 488, CD56-BV750, CD3-BV510, CD14-Spark Blue-550, CD27-Allophycocyanin (APC), CD45-Peridin-Chlorophyll-protein (PerCP), CD57-Fluorescein (FITC), CD95-Phycoerythrin (PE)-Cyanine (Cy)-5, HLA-DR-APC/Fire750, PD-1-BV785, PD-L1-PE (all 14 from BioLegend), HLA-DR-Brilliant UltraViolet (BUV) 496, CD8-BUV805, CD16-BUV496, CD45RA-BUV395 and CD56-BUV737 (all 5 from BD Biosciences) and CD4-CF568 (from Cytek Biosciences).

Cells were stained with proper antibodies for 30 min at 4 ºC in the dark and washed once with phosphate buffer saline. Unlabeled cells were used as negative controls. Samples were acquired in Cytek™ Aurora cytometer (Cytek Biosciences), and the data were analyzed with FlowJo, version 10.6.2 software (Flowjo, Ashland, Oregon).

A subset of T cells (CD4+ and CD8+ T cells) were gated using FlowJo FlowJo® software (Flowjo, Ashland, Oregon). Positive gate of each marker was manually defined by CD3+ CD4+ and CD8+. After gating on populations of interest each sample was exported and concatenate for further dimensionality reduction analysis (*t*-SNE) using FlowJo software. General lineage markers were examined by running *t*-SNE with only the following markers: CD3, CD4, CD8.The Barnes-Hut implementation of *t*-SNE with 1,000 iterations, a perplexity parameter of 30, and a trade-off θ of 0.5 was used for applying the dimensionality reduction algorithm. *t*-SNE maps were generated by plotting each event by its *t*-SNE dimensions in a dot-plot. Intensities for marker of TIM-3 were overlaid on the dot-plot to show the expression of this marker on different time-point of patients. These values were normalized using manually set upper and lower limits to account for differing levels of background staining of each marker.

**FIGURE LEGENDS**

**Supplementary Figure 1. Images from patient 1.** A,A chest contrast axial CT scan image on day 7 of the illness, showing bilateral patchy interstitial pneumonia with ground-glass opacities of peripheral and central distribution is shown. B, A chest X-Ray revealing bilateral interstitial pneumonia of peripheral predominance on day 7 of the disease is shown.

**Supplementary Figure 2. Images from patient 2.** A and B, show the chest non-contrast axial CT scan on day 7 of illness, showing interstitial pneumonia of peripheral patchy distribution with both alveolar occupation (arrows indicated with I) and ground-glass opacities (arrows indicated with II). C, A chest X-Ray revealing bilateral interstitial pneumonia on day 7 of the disease is shown.

**Supplementary Figure 3. Immunophenotyping of naïve, central memory and terminally differentiated T cells from patients 1 and 2.** A and B, The analysis of the CD4+ and CD8+ naïve cells (CD45RA+CD27+, TNaïve) is shown. The percentage of TNaïve on gated CD4+ (A) and CD8+ (B) are shown. C and D, The analysis of the CD4+ and CD8+ central memory cells (CD45RA-CD27+, TCM) is shown. The percentages of TCM on gated CD4+ (C) and CD8+ (D) are shown. E and F, The analysis of the terminally differentiated cells (CD45RA+CD27-, TEM-RA) is shown. The percentages of TEM-RA on gated CD4+ (E) and CD8+ (F) are shown. Data from patient 1 are shown in red, and data from patient 2 is shown in green. Dashed lines represent the calculated means from the analysis of a unique sample from 10 HVs (mean ± SD). Arrows indicate important clinical events (patient 1: ICU admission, sepsis and septic shock. Patient 2: discharge).

**Supplementary Figure 4. Gating strategy.**

**Supplementary Figure 5. TIM-3 expression, cell population profile and cytokine levels in patients 1 and 2.** A, The MFI of TIM-3 on gated CD14+ monocytes are shown. B, The percentages of monocytes from total blood cells are shown. C, The MFI of TIM-3 on gated CD3-CD56+ (NK cells) are shown. D, The percentages of CD3-CD56+ (NK cells) from total blood cells are shown. E, The MFI of TIM-3 on gated CD16+ neutrophils are shown. F, The percentages of CD16+ neutrophils from total blood cells are shown. G and H, The cytokine levels quantified on plasma from the patients and HVs are shown. IL6 (G) and IL17 (H). Data from patient 1 are shown in red, and data from patient 2 is shown in green. Dashed lines represent the calculated means from the analysis of a unique sample from 10 HVs (mean ± SD). Arrows indicate important clinical events (patient 1: ICU admission, sepsis and septic shock. Patient 2: discharge).

**Supplementary Table 1**

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|  | Survivors(n=47) | *Exitus*(n=10) | HV(n=15) |
| Age – years | 51.54±12.59 | 69.71±12.61 | 58.5±10.31 |
| Sex, male – n (%) | 24 (51.06) | 8 (80.00) | 10 (66.66) |
| Smoking history – n, (%) |  |  |  |
| Never smoked | 25 (53.19) | 8 (80.00) | 10 (66.66) |
| Current smoker | 1 (2.12) | 1 (10.00) | 5 (33.33) |
| Addicted to alcohol – n, (%) | 0 (0.00) | 0 (0.00) | 0(0.00) |
| Coexisting disorder – n, (%) |  |  |  |
| Hypertension | 7 (14.89) | 7 (70.00) | 0(0.00) |
| Diabetes | 6 (12.76) | 5 (50.00) | 0(0.00) |
| Cardiovascular disease | 4 (8.51) | 1 (10.00) | 0(0.00) |
| Chronic renal disease | 0 (0·00) | 1 (10.00) | 0(0.00) |
| Obesity | 3 (6.38) | 0 (0·00) | 0(0.00) |
| Asthma | 2 (4.25) | 0 (0·00) | 0(0.00) |
| COPD | 2 (4.25) | 3 (30.00) | 0(0.00) |
| Oncologic disease | 3 (6.38) | 0 (0·00) | 0(0.00) |
| Immunodeficiency  | 4 (8.51) | 4 (40.00) | 0(0.00) |

**Supplementary Table 1. Demographics and baseline characteristics of patients included in the validation cohort.** Abbreviations: HV, Healthy Volunteers; COPD, Chronic obstructive pulmonary disease.