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Supplemental information

Mass cytometry reveals a conserved immune trajectory of recovery in hospitalized COVID-19 patients

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Mass cytometry reveals a conserved immune trajectory of recovery in hospitalized COVID-19 patients

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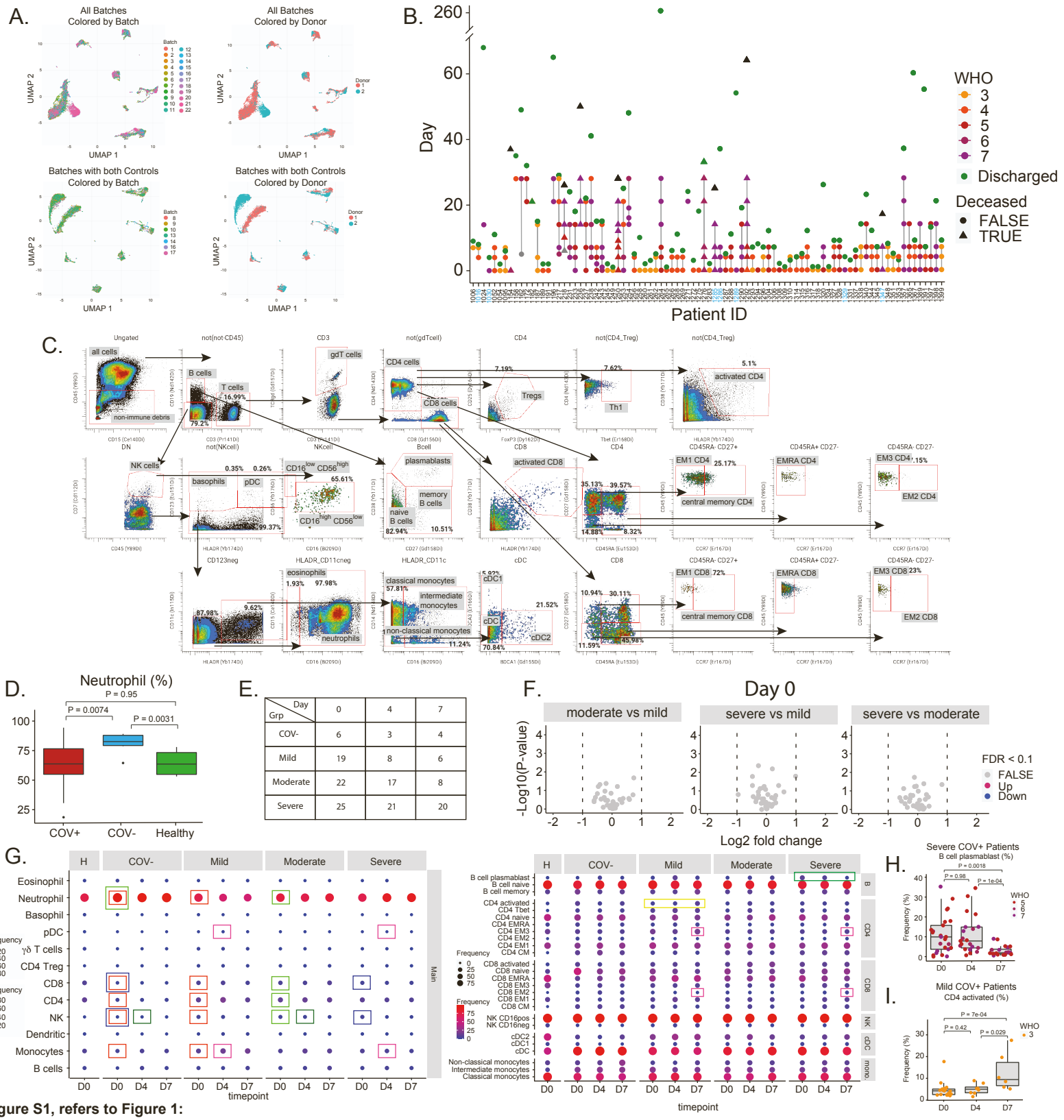

Figure S1, refers to Figure 1:

A) Dimensionality reduction plots by UMAP colored by batch and by reference donor used for data normalization. Two reference donors were used for batch normalization, with seven batches containing both reference donors. Normalization removes any batch effects effectively while preserving their biological differences between donors 1 and 2. (Top) UMAP containing cells from the internal reference donor samples from all batches, colored by batch of origin (left) and colored by donor (right). (Bottom) UMAP containing cells from the internal reference donor samples from the seven batches in which both reference donors were analyzed, colored by batch (left) and colored by donor (right). **B)** All patient samples included in study (219 samples from 88 patients). COVID-19 patients shown in black and COVID-19 negative patients in blue. Points indicate sample timepoint and are coloured according to WHO score. Green points indicate the day of discharge, while triangles indicate patients that died. **C)** Gating strategy for manual immune cell gates. **D)** Frequency of neutrophils in COVID-19 positive (COV+), COVID-19 negative (COV-) patients, and healthy controls at D0. Nominal p-values obtained by Wilcoxon Rank Sum Test. **E)** Number of samples at each time point (D0, D4, and D7) for each COVID-19 severity group and for COVID-19 negative patients. **F)** Differential expression analysis of immune cell populations between COVID-19 severity groups and COVID-19 negative patients at D0. The log2 fold changes are plotted against the negative log10(nominal p-values). Nominal p-values obtained by Wilcoxon Rank Sum Test. Colors indicate if cell populations are significantly down- (blue) or upregulated (purple) or not differentially expressed (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **G)** Immune cell population abundance at D0, D4, and D7 in COVID-19 patients divided into severity groups based on their WHO score, as well as in COVID-19 negative patients, and healthy individuals at D0. Nominal p-values obtained by Wilcoxon Rank Sum Test, followed by Benjamini-Hochberg correction with FDR < 0.1. Immune cell populations that are significantly different after BH correction (across time points within groups or cross-sectional at the same time point between groups) are highlighted with coloured boxes corresponding to the time point and group of comparison. All comparisons between patients and healthy individuals at D0 are illustrated with Nominal p-values in main figure 1C. **H+I)** Frequency of B cell plasmablasts (H) and CD4 activated T cells (I) in patients suffering from severe and mild disease, respectively. Nominal p-values obtained by Wilcoxon Rank Sum Test.

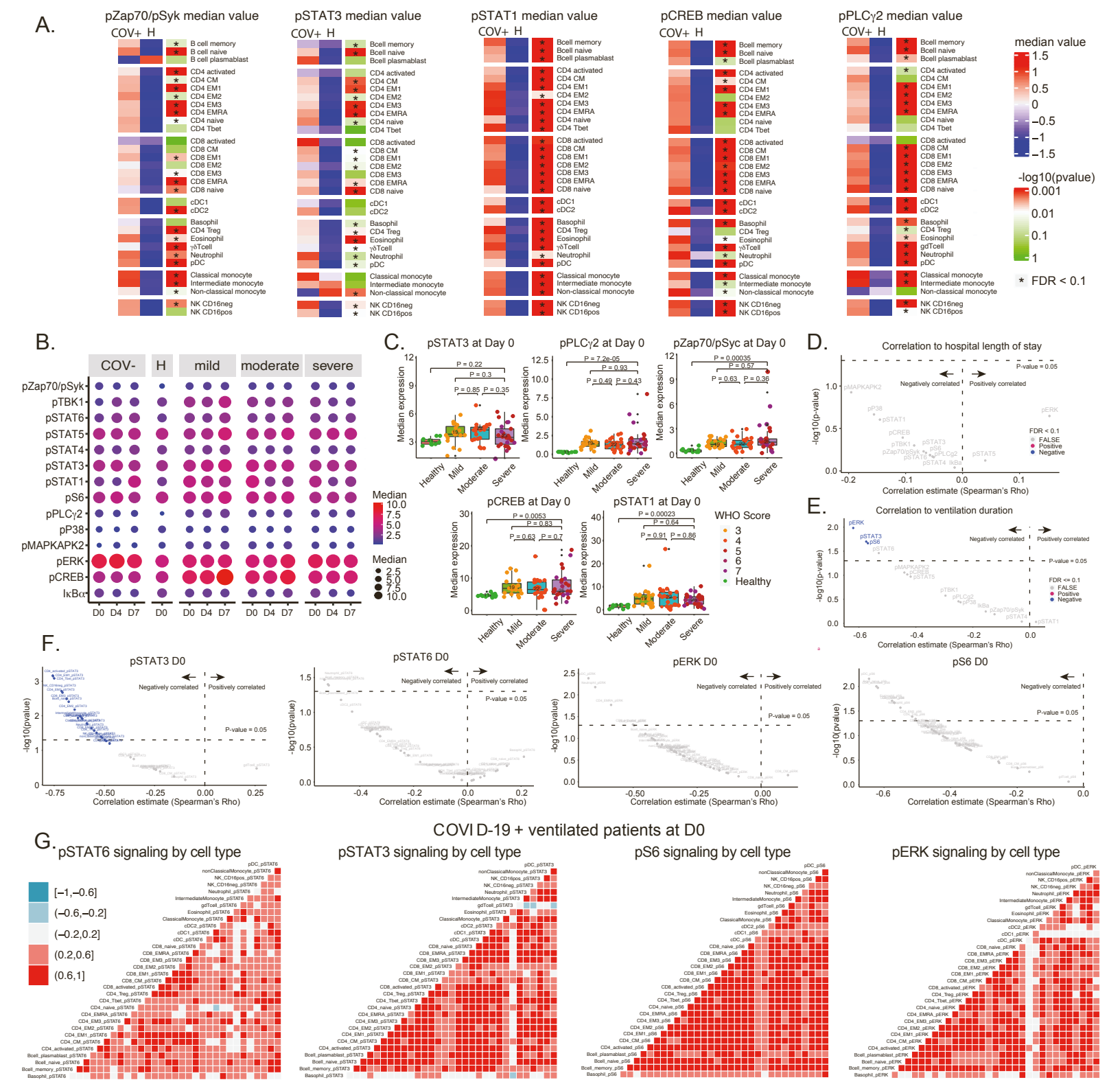

Figure S2, refers to Figure 2:

A) Expression of significant changing signaling molecules from 2B in all CD45+ cell population subsets at D0 in COVID-19 patients (COV+) and healthy individuals (H). Median expression values have been centered on heatmap. Nominal p-values obtained by Wilcoxon Rank Sum Test, followed by Benjamini-Hochberg correction with FDR < 0.1. **B)** Median signaling molecule values at D0, D4, and D7 in COVID-19 patients divided into severity groups based on their WHO score, as well as in COVID-19 negative patients, and healthy individuals at D0. All comparisons had FDR > 0.1. **C)** Specific comparisons from S1B. Nominal p-values obtained by Wilcoxon Rank Sum Test. **D+E)** Correlation between median signaling molecule values at D0 and hospital length of stay (D) for COV+ patients (n = 65, excluding the patient that is hospitalized for 260 days) and ventilation duration (E) for COV+ patients that are ventilated (n = 16). Correlation estimates and nominal p-values are obtained by Spearman correlation, followed by Benjamini-Hochberg correction. Colors indicate if features are significantly negatively (blue) or positively (purple) correlated or not significantly correlated (FALSE, grey) after Benjamini-Hochberg correction, FDR <= 0.1. **F)** Correlation between median signaling molecule values within cell population subtypes at D0 and ventilation duration for COV+ patients that are ventilated (n = 16). Correlation estimates and nominal p-values are obtained by Spearman correlation, followed by Benjamini-Hochberg correction. Colors indicate if features are significantly negatively (blue) or positively (purple) correlated or not significantly correlated (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **G)** Correlation of median signaling molecule values by cell type for COV+ ventilated patients at D0 (n = 16). Correlation estimates are obtained by Spearman correlation.

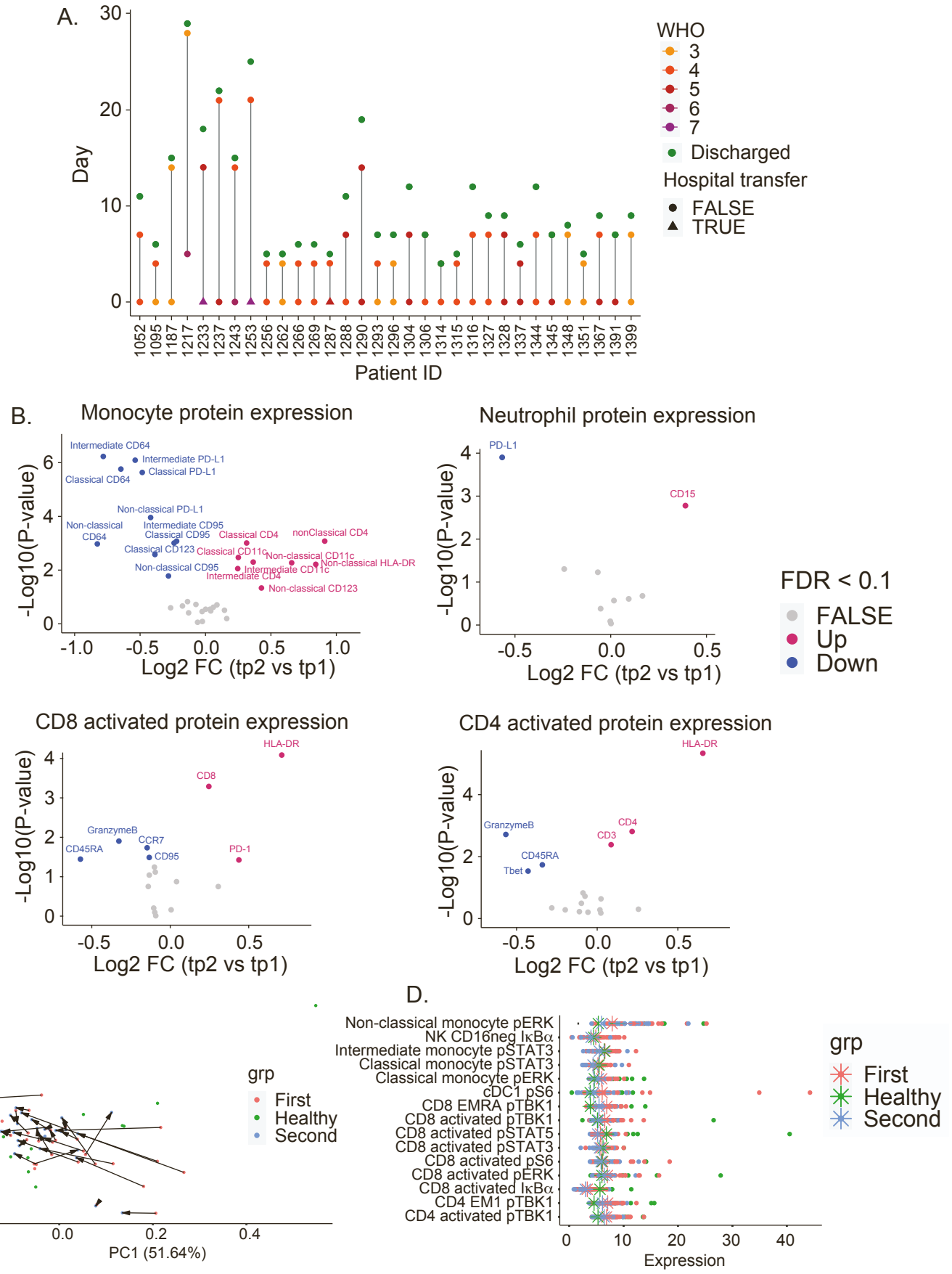

Figure S3, refers to Figure 3:
A) Samples used for intra-patient analysis in Figure 3 of patients that are discharged from the hospital within 30 days of admission (n = 32). Points indicate sample timepoint and are coloured according to WHO score. Green points indicate the day of discharge. **B)** Paired differential expression analysis of protein expression on monocyte subsets, neutrophil, CD8- and CD4 activated T cells between the first (tp1) and second (tp2) timepoints illustrated in 3A (paired Wilcoxon Rank Sum Test). The log2 fold changes (tp2 vs tp1) are plotted against the negative log10(nominal p-values). Colors indicate if cell populations are significantly down- (blue) or upregulated (purple) from tp1 to tp2 or not differentially expressed (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **C)** Principal component analysis of significant signaling molecules in 3I for tp1, tp2, and healthy controls. **D)** Expression of signaling molecules (from 3I and S3C) for tp1, tp2, and healthy controls. Stars indicate median value for each group.

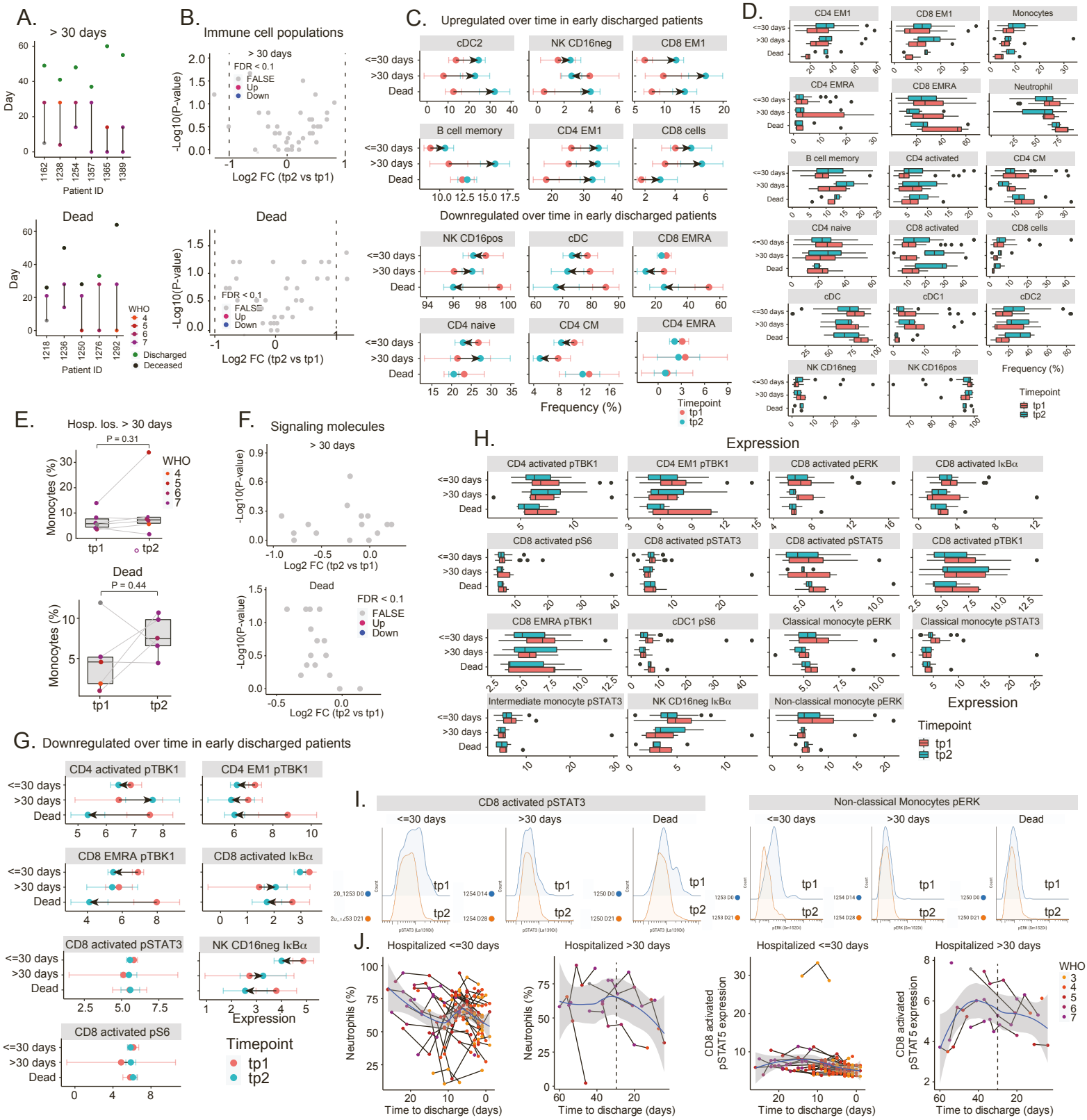

Figure S4, refers to Figure 4:
A) Samples used for intra-patient analysis in Figure 4 of patients that are discharged after 30 days (left, n = 6) and patients that die (right, n = 5). Points indicate sample timepoint and are coloured according to WHO score. Green points indicate the day of discharge. **B)** Paired differential expression analysis of immune cell populations between the first (tp1) and second (tp2) timepoints illustrated in 4A (paired Wilcoxon Rank Sum Test). The log2 fold changes (tp2 vs tp1) are plotted against the negative log10(nominal p-values). Colors indicate if cell populations are significantly down- (blue) or upregulated (purple) from tp1 to tp2 or not differentially expressed (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **C)** Median cell population frequencies at tp1 (red) and tp2 (blue) for patients that are discharged <30 days, >30 days, and deceased. **D)** Cell population frequencies at tp1 (red) and tp2 (blud) for patients that are discharged <=30 days, >30 days, and deceased. **E)** Frequency of monocytes at tp1 and tp2 for patients that are discharged after 30 days or die. Lines connect samples from the same patient. Nominal p-values obtained by paired Wilcoxon Rank Sum Test. **F)** Paired differential expression analysis of signaling molecules between the first (tp1) and second (tp2) timepoints illustrated in 4A (paired Wilcoxon Rank Sum Test). The log2 fold changes (tp2 vs tp1) are plotted against the negative log10(nominal p-values). Colors indicate if signaling molecules are significantly down- (blue) or upregulated (purple) from tp1 to tp2 or not differentially expressed (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **G)** Median expression of signaling molecules at tp1 (red) and tp2 (blue) for patients that are discharged <=30 days, >30 days, and deceased. **H)** Expression of signaling molecules at tp1 (red) and tp2 (blue) for patients that are discharged <=30 days, >30 days, and deceased. **I)** Expression of pSTAT3 in activated CD8 T cells (left) and pERK in non-classical monocytes (right) at tp1 (blue) and tp2 (orange) for representative patients that are discharged <=30 days, >30 days, and deceased. **J)** Neutrophil frequencies (left plots) and CD8 activated pSTAT5 expressions (right plots) relative to time to discharge in all samples from patients who are discharged <=30 days (n = 142 samples) or >30 days (n = 30 samples). Black lines connect samples from the same patient. Blue lines and grey shadow represent the best fitted smooth line and 95% confidence interval.

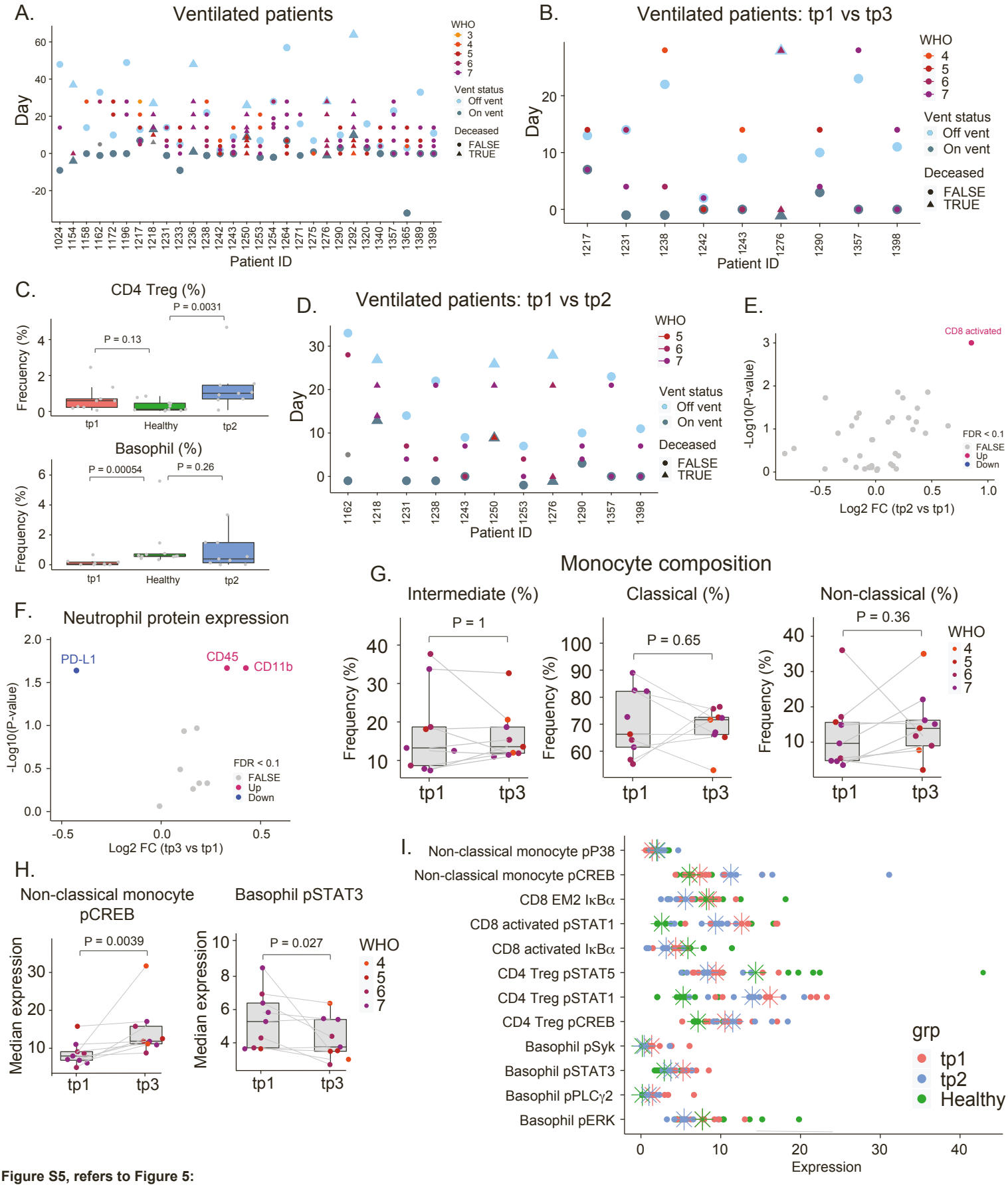

Figure S5, refers to Figure 5:
A) All samples for patients that have been put on a ventilator. Dark blue points indicate when a patient is put on a ventilator. Light blue points indicate when a patient is taken off a ventilator. **B)** Samples used for intra-patient analysis between tp1 and tp3 in Figure 5 of patients that have been put on a ventilator (n = 9). Points indicate sample timepoint and are coloured according to WHO score. Dark blue points indicate when a patient is put on a ventilator. Light blue points indicate when a patient is taken off a ventilator. **C)** Frequencies of CD4 Tregs and Basophils at tp1, tp3, and in healthy controls. Nominal p-values obtained by Wilcoxon Rank Sum Test. **D)** Samples used for intra-patient analysis between tp1 and tp2 in Figure 5 for patients that have been put on a ventilator (n = 11). Points indicate sample timepoint and are coloured according to WHO score. Dark blue points indicate when a patient is put on a ventilator. Light blue points indicate when a patient is taken off a ventilator. **E)** Paired differential expression analysis of immune cell populations between the first (tp1) and second (tp2) timepoints illustrated in 5A (paired Wilcoxon Rank Sum Test). The log2 fold changes (tp2 vs tp1) are plotted against the negative log10(nominal p-values). Colors indicate if cell populations are significantly down- (blue) or upregulated (purple) from tp1 to tp2 or not differentially expressed (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **F)** Paired differential expression analysis of protein expression on neutrophils between the first (tp1) and third (tp3) timepoints illustrated in 5A (paired Wilcoxon Rank Sum Test). The log2 fold changes (tp3 vs tp1) are plotted against the negative log10(nominal p-values). Colors indicate if cell populations are significantly down- (blue) or upregulated (purple) from tp1 to tp3 or not differentially expressed (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **G)** Frequencies of monocyte subsets at tp1 and tp3. Nominal p-values obtained by paired Wilcoxon Rank Sum Test. **H)** Expression of pSTAT3 in Basophils and pCREB in non-classical monocytes at tp1 and tp3. Lines connect samples from the same patient. Nominal p-values obtained by paired Wilcoxon Rank Sum Test. **I)** Expression of signaling molecules in 5M for tp1, tp2, and healthy controls. Stars indicate median value for each group.

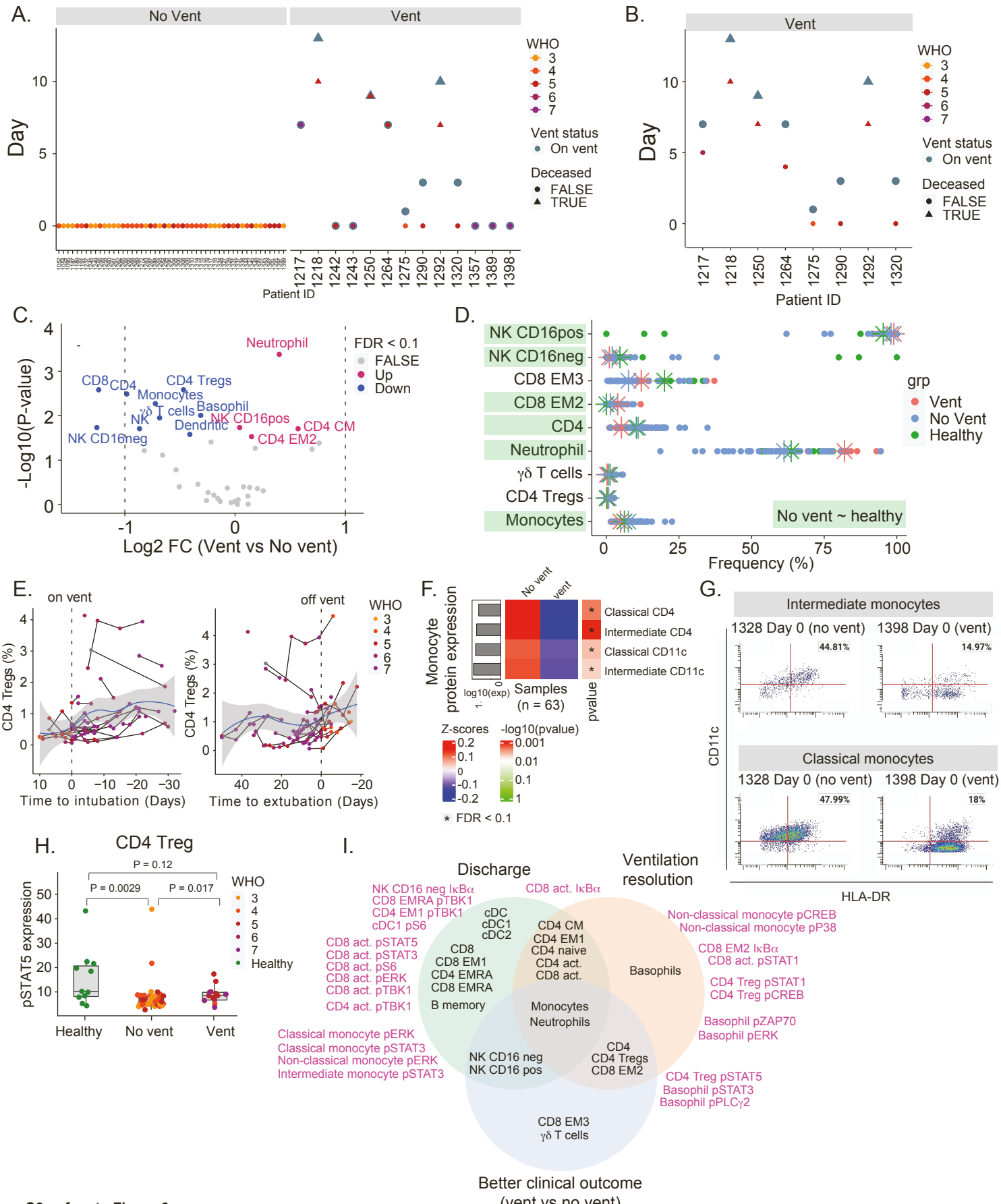

Figure S6, refers to Figure 6:
A) Samples used for inter-patient analysis in Figure 6. For ventilated patients (n = 13), the latest sample before the patient is put on a ventilator or, if available, the sample at the day of ventilation is used. For non-ventilated patients (n = 50), D0 is used. **B)** Samples obtained prior to ventilation (n = 8). **C)** Differential expression analysis of immune cell populations between ventilated (from S6B) and non-ventilated patients (from S6A) (Wilcoxon Rank Sum Test). The log2 fold changes (vent vs no vent) are plotted against the negative log10(nominal p-values). Colors indicate if cell populations are significantly down- (blue) or upregulated (purple) for vent vs no vent or not differentially expressed (FALSE, grey) after Benjamini-Hochberg correction, FDR < 0.1. **D)** Population frequencies of significant immune cell subsets in 6B for ventilated-, non-ventilated patients, and healthy controls. Stars indicate median value for each group. Cell populations are highlighted in green if non-ventilated patients are closer to healthy controls than ventilated patients. **E)** CD4 Treg frequencies relative to intubation / extubation in all samples from ventilated patients. Black lines connect samples from the same patient. Blue lines and grey shadows represent the best fitted smooth line and 95% confidence interval. Dotted lines intersect the x-axis at day of intubation / extubation. **F)** Protein expression on monocyte subsets in ventilated- and non-ventilated patients. Mean protein expression values have been log10 transformed, scaled, and centered on heatmap. Bars indicate mean protein expression across all samples. Only significant proteins are shown (Wilcoxon Rank Sum Test, Benjamini-Hochberg correction with FDR < 0.1). **G)** Scatter plots of CD11c and HLA-DR expression on intermediate (left) and classical monocytes (right) in representative patients. **H)** Expression of pSTAT5 in CD4 Tregs in non-ventilated and ventilated patients as well as healthy individuals. Nominal p-values obtained by Wilcoxon Rank Sum Test. **I)** Significantly changing immune cell populations (black text) and signaling molecules (purple) accompanying discharge (green), ventilation resolution (orange), and better clinical outcome (blue).