iScience, Volume *24*

Supplemental information

Multi-dimensional and longitudinal systems profiling reveals predictive pattern of severe COVID-19

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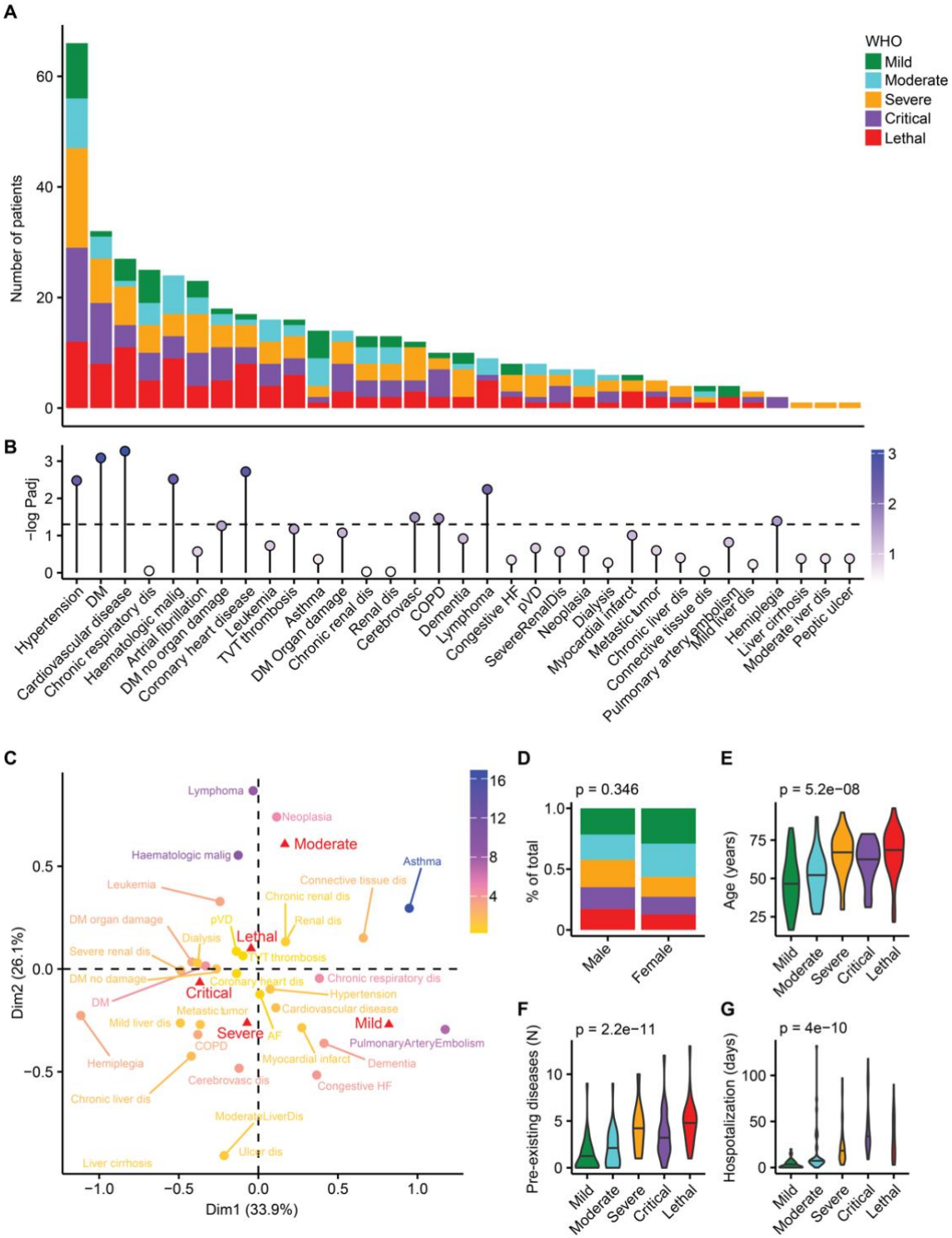

Figure S1. Characteristics of our patient cohort, Related to Figure 1.

(A) Number of patients and relative distribution of WHO disease severity with depicted preconditions.

(B) Negative adjusted log10 P values of impact of respective precondition on WHO disease severity. Significance level of negative adjusted log10 P = 0.05 is shown as dashed line. Color represents significance. Chi-square test was used.

(C) Correspondence analysis of chronic preconditions with WHO disease severities. Color shows contribution to non-uniform distribution. (D) Relative frequency of disease severities in male and female patients.

(E–G) Distribution of Age (E), number of chronic diseases (F) and hospitalization days (G) in patients with different disease severities. One-way-ANOVA was used for statistical significance. Data is represented as violin plots with median.

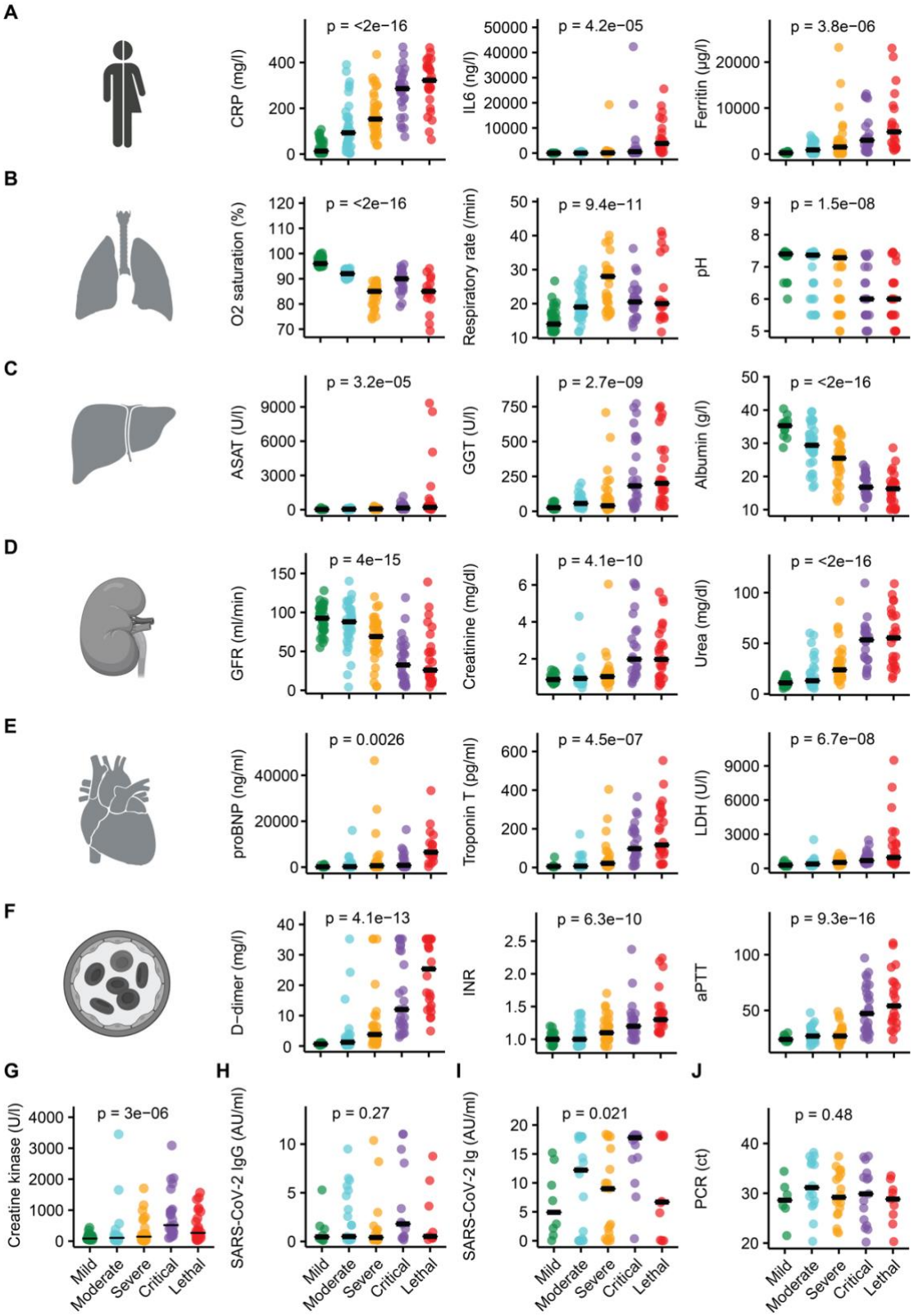

Figure S2. Laboratory markers in COVID-19, Related to Figure 2.

(A–G) Analysis of parameters indicative of inflammation (A) and dysregulation of the respiratory (B), hepatobiliary (C), renal (D), cardiac (E), coagulative (F), and musculoskeletal (G) systems.

(H–J) Analysis of SARS-CoV-2 IgG by Euroimmun test (H), SARS-CoV-2 Ig by Wantei test (I) and PCR ct value (J) of patients with mild, moderate, severe, critical and lethal COVID-19. One-way-ANOVA was used to test statistical significance. Exact n is provided in Table S4. Exact P values are provided in the figure. Individual data points and median are shown.

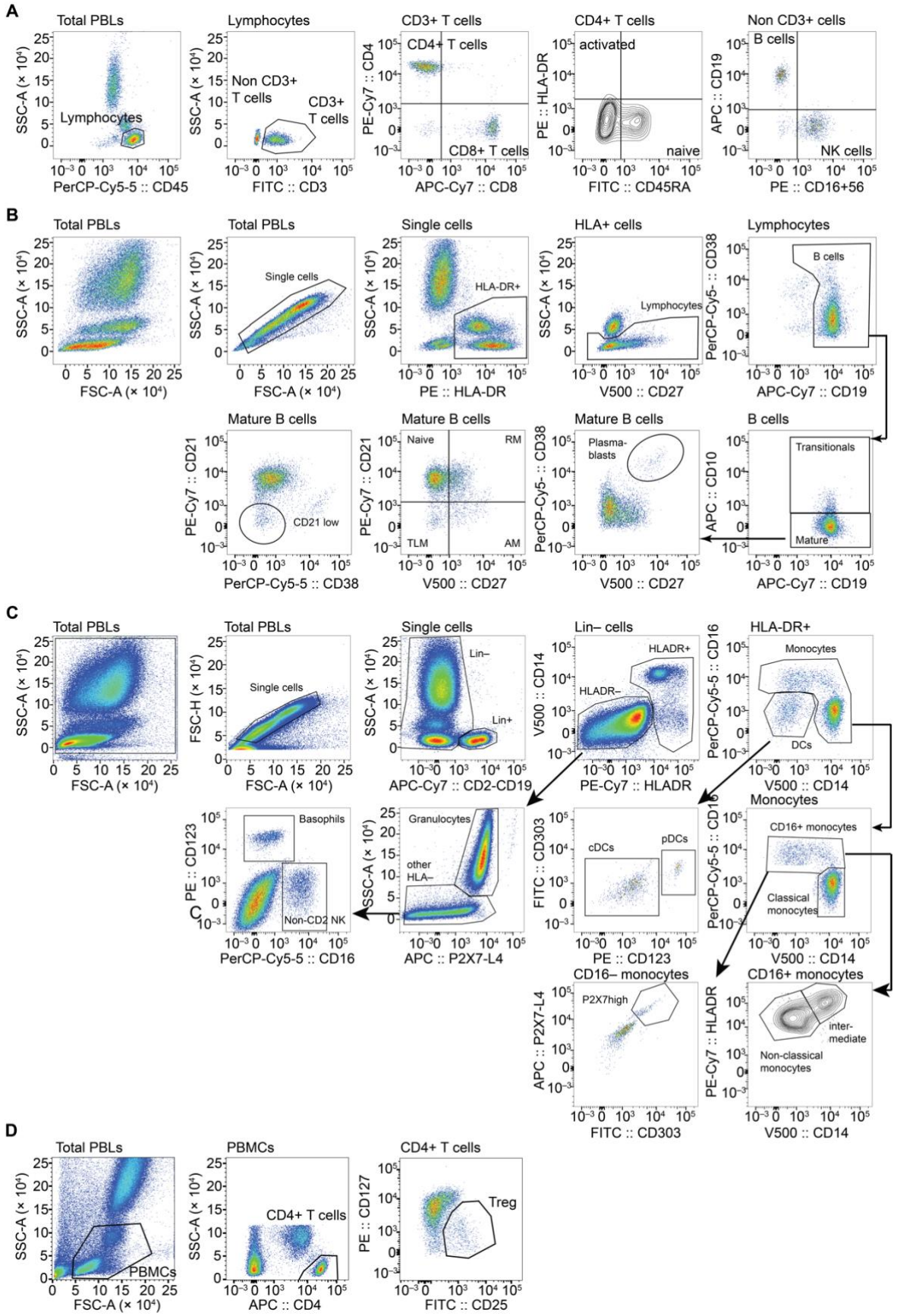

Figure S3. Gating strategies, Related to all Figure including immune cell phenotyping.
(A–D) Gating strategies of lymphocytic (A), B cell (B) and myeloid subsets (C) and regulatory T cells (D). Used antibodies are provided in Table S6.

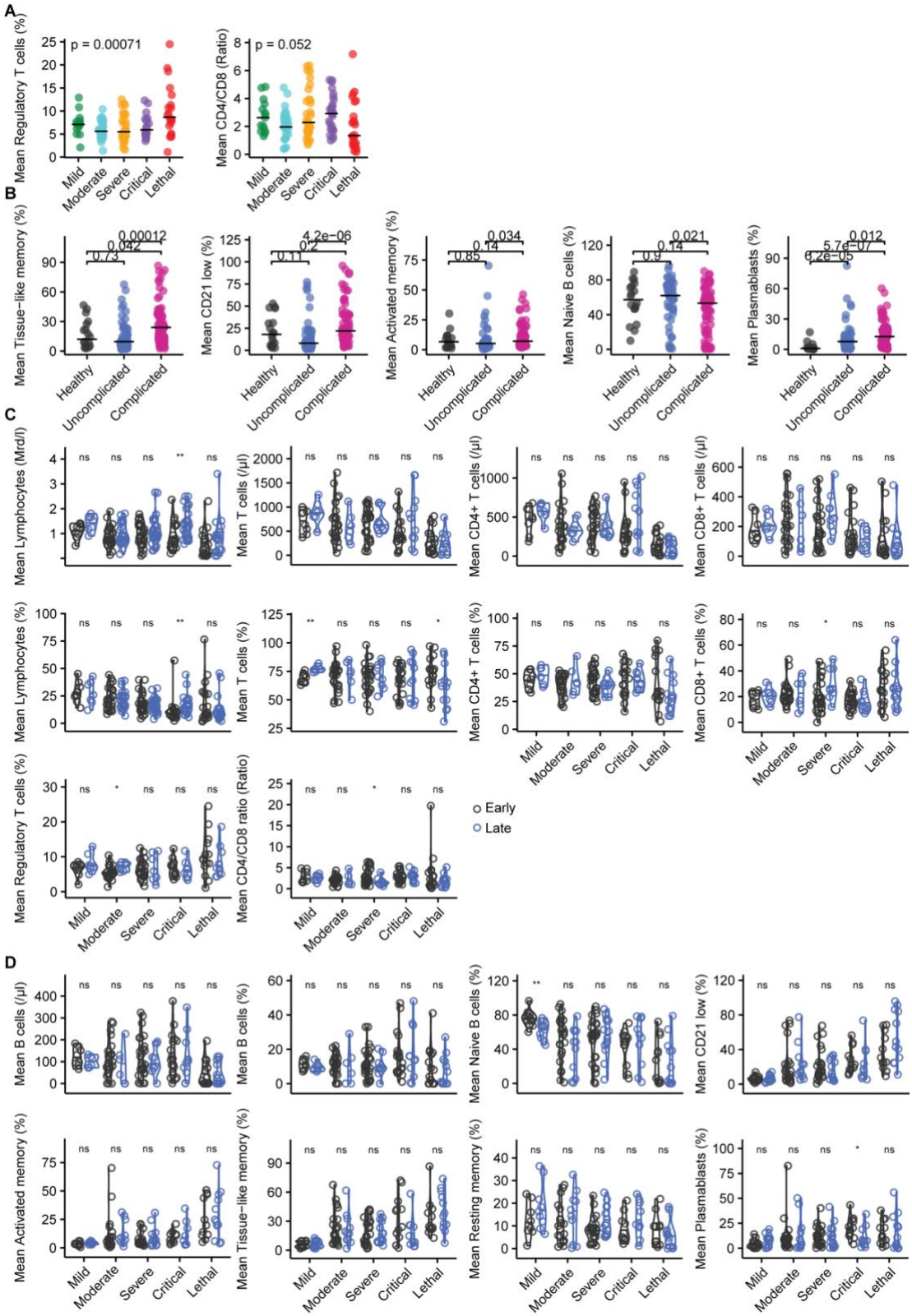

Figure S4. Lymphocyte populations in early and late COVID-19, Related to Figure 4.

(A) Frequency of regulatory T cells and CD4/CD8 ratio across all disease severity classes. One-way-ANOVA was used.

(B) Frequency of the indicated B cell populations in healthy donors and patients with uncomplicated and complicated COVID-19. Exact P values are shown in figure.

(C and D) Comparison of the indicated T cell (C) and B cell (D) populations between early and late COVID-19. Data is shown as violin plot with median. Exact n is provided in Table S4. Significance was calculated by FDR-adjusted Wilcoxon-test. Significant results are indicated by * P < 0.05, ** P < 0.01.

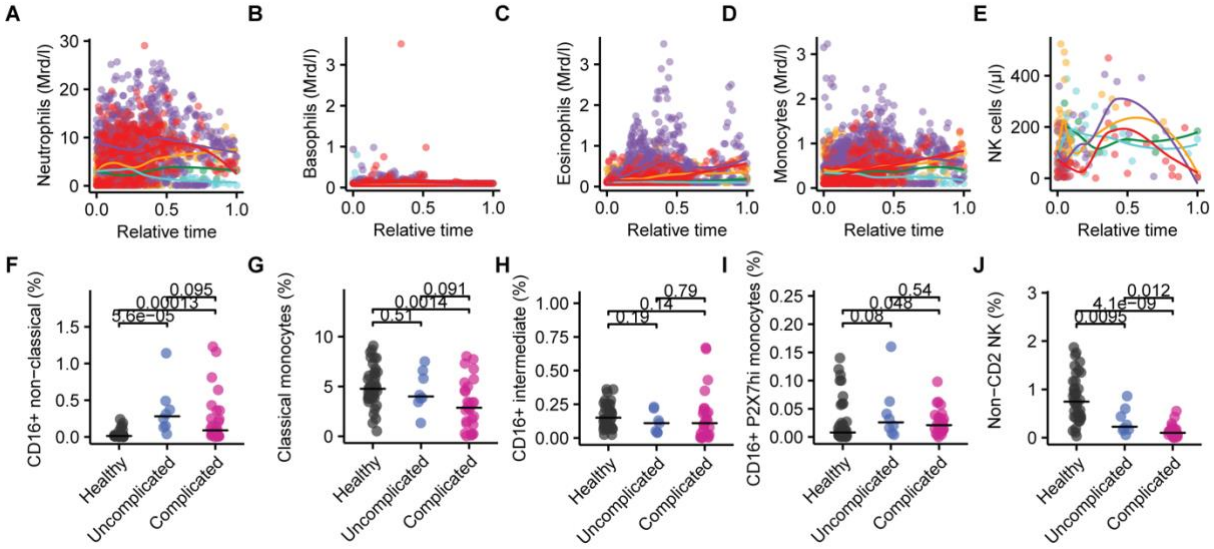

Figure S5. Innate immune subpopulations in COVID-19, Related to Figure 5.

(A–D) Frequency of total monocytes of CD16+ non-classical (A), classical (B), CD16+ intermediate (C), CD16+P2X7high (D) monocytes from healthy donors and patients with uncomplicated and complicated COVID-19, shown as percentage of total peripheral blood leucocytes.

(E) Frequency of non-CD2 NK cells of total NK cells from healthy donors and patients with uncomplicated and complicated COVID-19, shown as percentage of total peripheral blood leucocytes. Exact n is provided in Table S4. Significance was calculated by FDR-adjusted Wilcoxon-test. Significant results are indicated by * P < 0.05, ** P < 0.01.

(F–J) Time course of the absolute counts from all COVID-19 patients of basophils (F), eosinophils (G), monocytes (H), neutrophils (I) and NK cells (J). Trend line for each WHO severity is depicted in respective color.

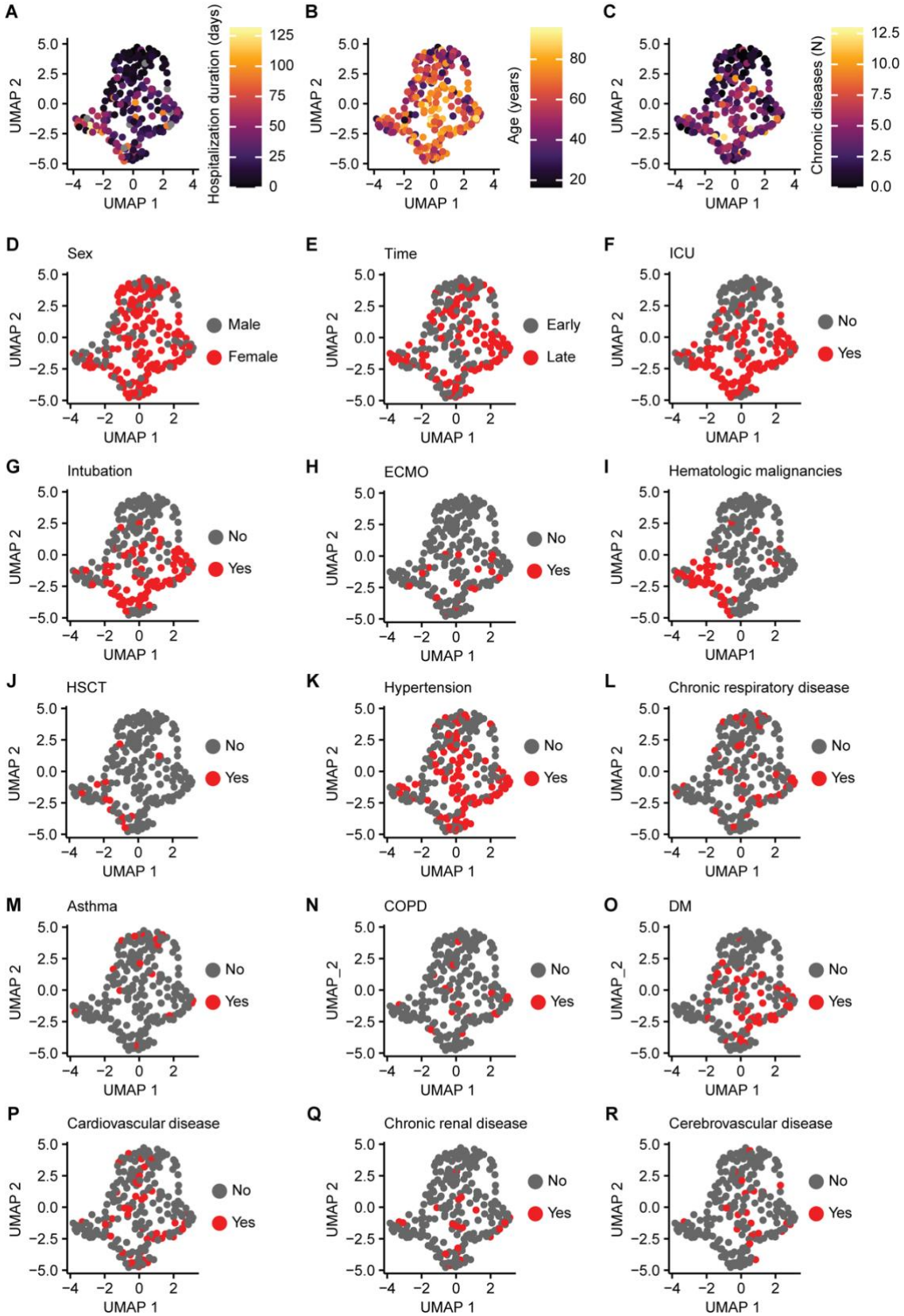

Figure S6. Patient distribution in unbiased clustering, Related to Figure 6.
(A–R) Representation of hospital duration (A), age (B), number of chronic diseases (C), sex (D), early or late timepoint (E), ICU admission (F), intubation (G), ECMO (H), hematologic malignancies (I), hematopoietic stem cell transplantation (J), hypertension (K), chronic respiratory disease (L), asthma (M), chronic obstructive pulmonary disease (N), diabetes mellitus (O), cardiovascular diseases (P), chronic renal diseases (Q), cerebrovascular diseases (R) in UMAP clustering of COVID-19 patients.

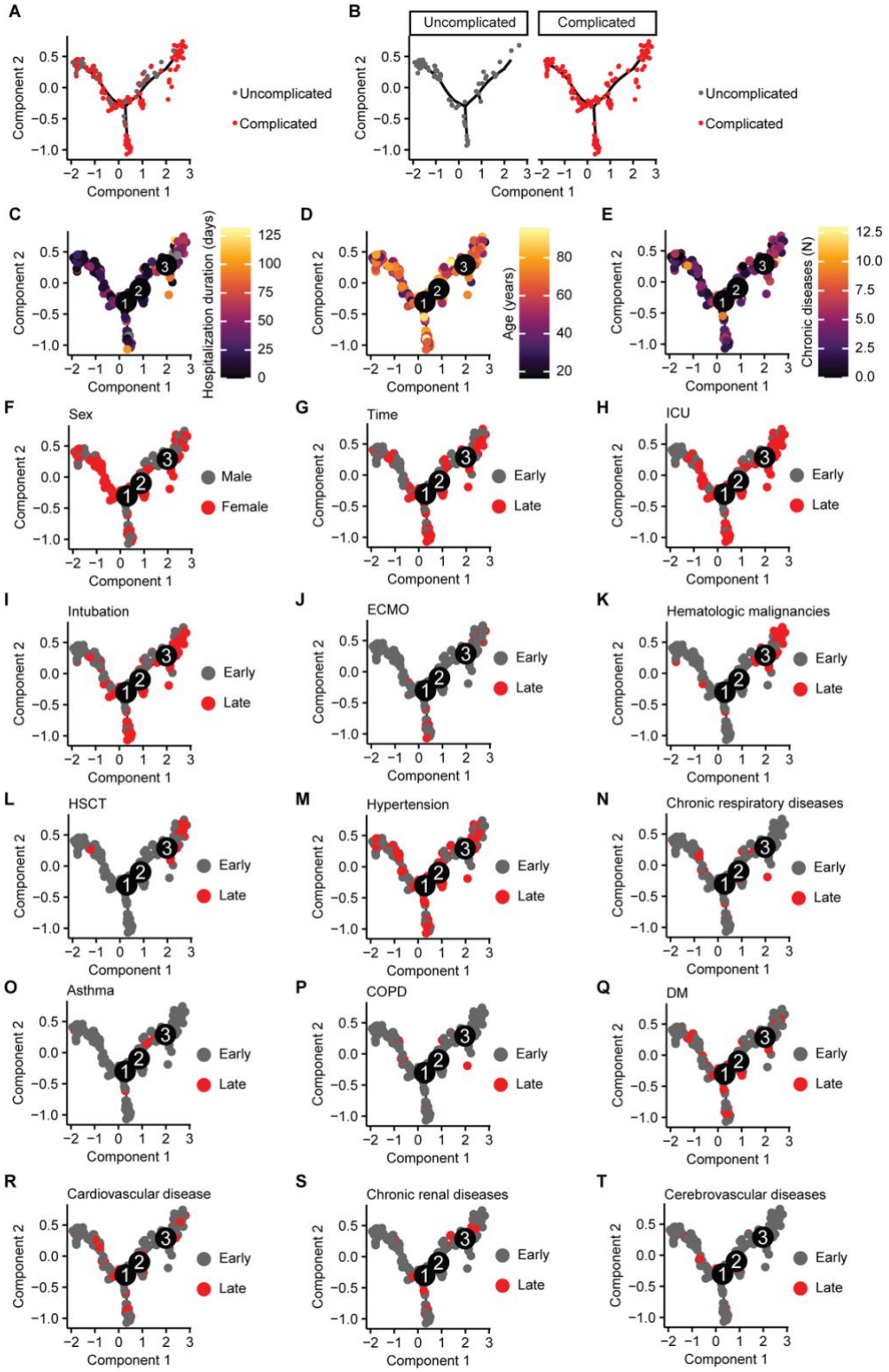

Figure S7. Patient distribution in pseudo-time trajectory, Related to Figure 7.

(A and B) Representation of uncomplicated and complicated disease courses in the pseudo-time trajectory.

(C–T) Representation of hospital duration (C), age (D), number of chronic diseases (E), sex (F), early or late timepoint (G), ICU admission (H), intubation (I), ECMO (J), hematologic malignancies (K), hematopoietic stem cell transplantation (L), hypertension (M), chronic respiratory disease (N), asthma (O), chronic obstructive pulmonary disease (P), diabetes mellitus (Q), cardiovascular diseases (R), chronic renal diseases (S), cerebrovascular diseases (T) in pseudo-time-trajectory of COVID-19 patients.

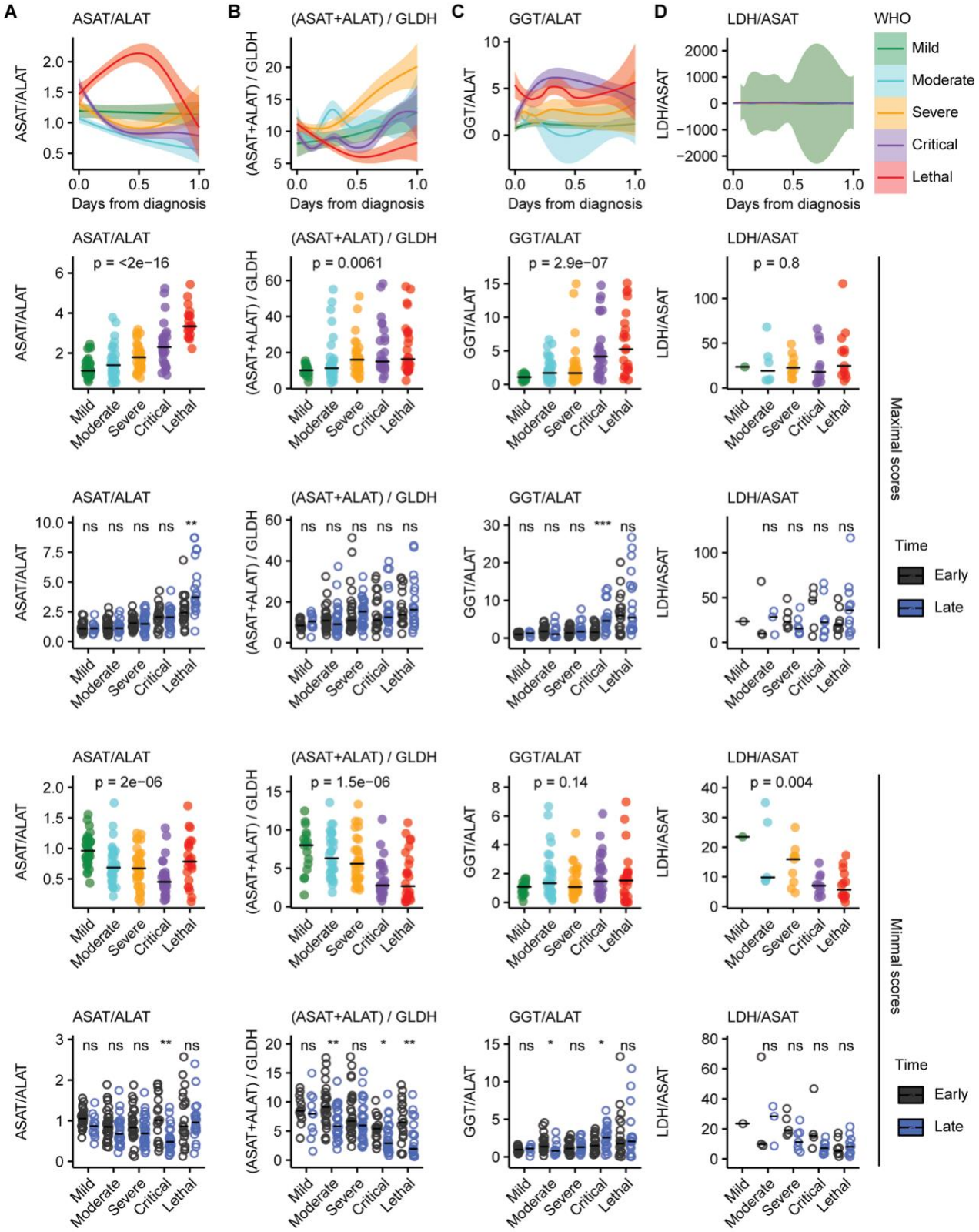

Figure S8. Analysis of liver enzymes indicates hepatocyte destruction, Related to Figure 7.
(A–D) Time course, maximal value across disease severity classes, maximal values in early and late disease, minimal value across all disease severity classes, minimal values in early and late disease (from to bottom) of ASAT/ALAT ratio (A), (ASAT+ALAT)/GLDH ratio (B), GGT/ALAT ratio (C), LDH/ASAT ratio (D). Data points representing individuals and median are shown. For comparison across all time points one-way-ANOVA was used, for comparisons between early and late timepoints unpaired two-tailed Wilcoxon-test was used.

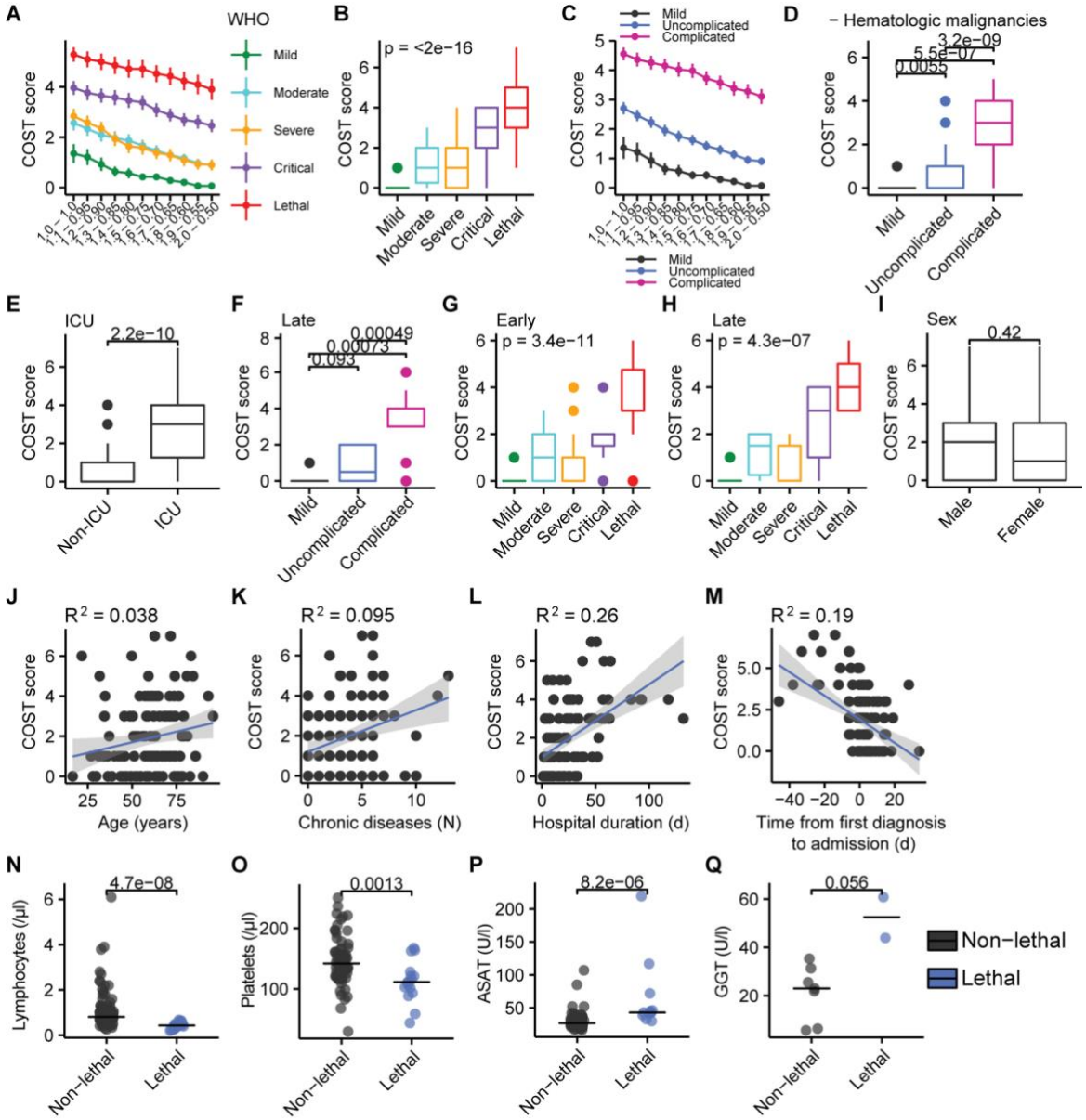

Figure S9. Validation of COVID-19 disease severity (COST) score, Related to Figure 7.

(A) COST score of COVID-19 patients with respective scaling factor for upper and lower limits.

(B) Score of patients with mild, moderate, severe, critical, lethal COVID-19. Significance was calculated by one-way-ANOVA.

(C) Frequency of patients with different disease severities for different scoring results.

(D) Score of patients without hematologic malignancies who suffered from mild, uncomplicated or complicated COVID-19. Significance was calculated by one-way-ANOVA.

(E) Comparison of the scores from patients with (n = 60) or without (n = 62) admission to ICU.

(F–H) Comparison of scores from early and late timepoints. Significance was calculated by one-way-ANOVA.

(I) Comparison of scores from male (n = 80) and female (n = 42) patients with COVID-19.

(J–M) Pearson correlation analysis of Score with patient age (J; n = 122), number of chronic diseases (K; n = 122), hospital duration (L; n = 122) and time from first diagnosis to admission (M; n = 122).

(N–Q) Lower quartile values of lymphocyte count (N) and platelets (O) and median of ASAT (P) and GGT (Q) from studies that exclusively included patients with non-lethal or lethal outcome. Data points from individual studies and median are shown. If not stated otherwise, boxplot with median, IQR and outliers are displayed, and significance was calculated by FDR-adjusted Wilcoxon-test and exact n is provided in Table S4.

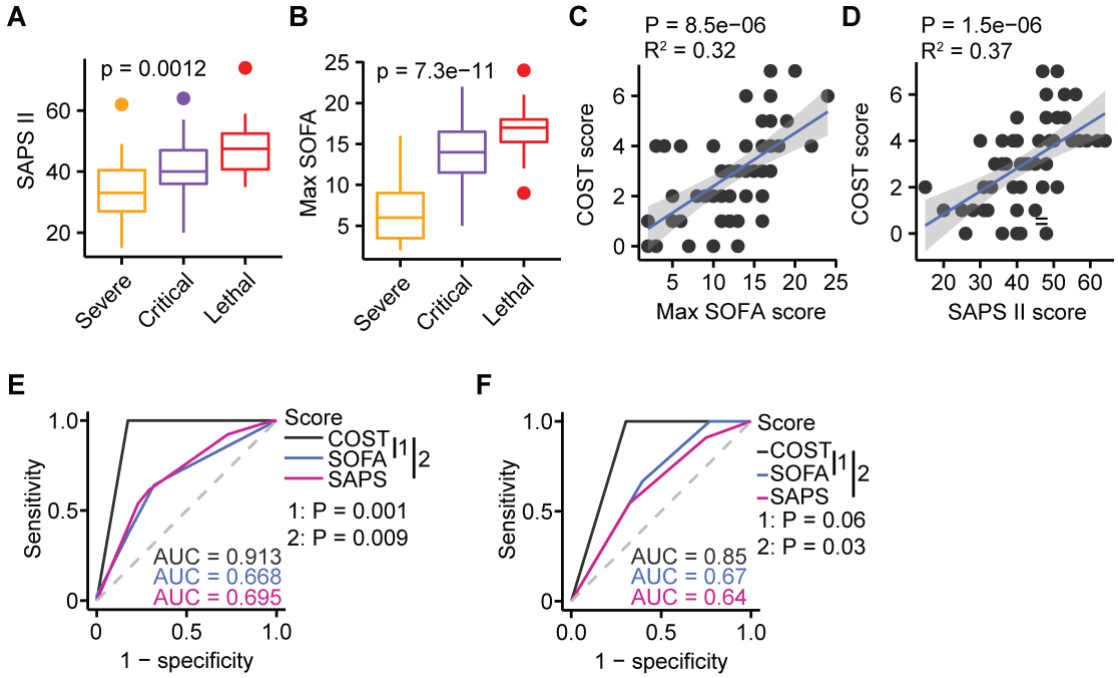

Figure S10. Comparison of COST score with SOFA and SAPS scores, Related to Figure 7.
(A and B) Maximal SAPS II (A) and SOFA (B) score of patients with severe, critical and lethal COVID-19. Data is shown as boxplot and significance was calculated by one-way-ANOVA.
(C and D) Pearson correlation analysis of COST and SOFA (T) or SAPSII (U) score (n = 61).
(E) ROC analysis of maximal COST, SOFA and SAPS score from all timepoints (n = 61).
(F) ROC analysis of COST, SOFA and SAPS score from first available timepoints (n = 61).

	N / Mean	Frequency (%) / SD
Sex (N / Frequency)		
- Male	113	0.65
- Female	60	0.35
WHO (N / Frequency)		
- Mild	48	0.28
- Moderate	37	0.21
- Severe	34	0.20
- Critical	28	0.16
- Lethal	26	0.15
Age (years; Mean / SD)	57.33	17.00
Hospitalization (N / Frequency)		
- Inpatient	148	85.55
- ICU	70	40.46
- Transferal	27	15.61
- Outpatient	25	14.45
Hospitalization time (days; Mean / SD)		
- Total hospitalization	22.07	26.71
- ICU stay	18.69	17.27
Chronic preconditions (Mean / SD)	2.90	2.61
Chronic preconditions (N / Frequency)		
- Chronic respiratory disease	27	15.61
- Diabetes mellitus type 2, no organ damage	18	10.40
- Leukemia	16	9.25
- Diabetes mellitus type 2, with organ damage	16	9.25
- Leukemia	16	9.25
- Cerebrovascular disease	12	6.94
- Dementia	10	5.78
- Lymphoma	9	5.20
- Congestive heart failure	8	4.62
- Peripheral venous disease	8	4.62
- Neoplasia	8	4.62
- Severe renal disease	7	4.05
- Myocardial infarction	6	3.47
- Metastatic malignancy	5	2.89
- Connective tissue disorder	5	2.89
- Hemiplegia	2	1.16
- Moderate liver disease	1	0.58

Table S1. Patient characteristics. Related to Figures 1 and S1.

Parameter	Unit	Range	Higher/Lower	Cut-off
AP	U/l	40 – 129	Higher	232
ASAT	U/l	10 – 50	Higher	90
GGT	U/l	> 65	Higher	117
Lymphocytes	Billion/l	1.1 – 3.4	Lower	0.66
B cells	Cells/µl	80 – 500	Lower	48
CD4+ T cells	Cells/µl	500 – 1350	Lower	300
Platelets	Billion/l	150 – 400	Lower	90

Table S5. COST score. Related to Figure 7.

Table S6. Antibodies used for flowcytometric staining. Related to all Figures including immune cell phenotyping.

Lymphocyte subpopulations:

Fluorochrome	Antigen	Clone	Catalog No.	Supplier
Multitest 6-color TBNK Reagent			644611	BD Biosciences
FITC	CD3	SK7		
PE	CD16/CD56	B73.1 / NCAM 16.2		
PerCP-Cy5.5	CD45	2D1 (HLe-1)		
PE-Cy7	CD4	SK3		
APC	CD19	SJ25C1		
APC-Cy7	CD8	SK1		

Regulatory T cells:

Fluorochrome	Antigen	Clone	Catalog No.	Supplier
FITC	CD25	M-A251	356106	BioLegend
PE	CD127	HIL-7R-M21	557938	BD Biosciences
APC	CD4	SK3	345771	BD Biosciences

B cell subsets:

Fluorochrome	Antigen	Clone	Catalog No.	Supplier
FITC	CXCR3	G025H7	353704	BioLegend
PE	HLA-DR	L243	307606	BioLegend
PerCP-Cy5.5	CD38	HIT2	303522	BioLegend
PE-Cy7	CD21	Bu32	354912	BioLegend
APC	CD10	HI10a	312210	BioLegend
APC-Cy7	CD19	HIB19	302218	BioLegend
BV421	CD73	AD2	562430	BD Biosciences
BV500	CD27	O323	302835	BioLegend

Myeloid subsets:

Fluorochrome	Antigen	Clone	Catalog No.	Supplier
FITC	CD303	201A	354208	BL
PE	CD123	6H6	306006	BL
PerCP-Cy5.5	CD16	B73.1	360712	Biolegend
PE-Cy7	HLA-DR	L243	307616	Biolegend
AF647	P2X7	L4	-/-	own*
APC-Cy7	CD2; CD19	RPA-210; HIB19	300220;302218	Biolegend
BV421	CD11c	3.9	565806	BD
BV500	CD14	MSE2	301842	Biolegend

* purified from hybridoma supernatant