

Supplementary materials

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Fig. S7. Comparison of blood plasma from untreated, rhDNase-treated, and healthy individuals.

Supplementary methods – Detailed methods section

Tables Attached -

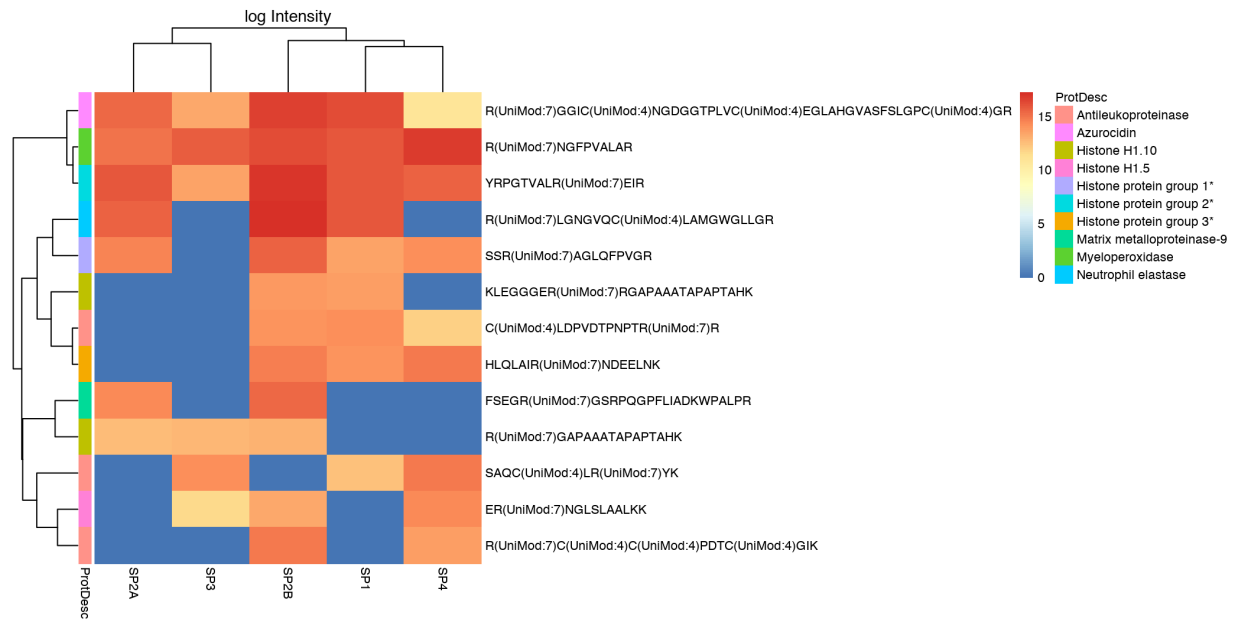
Supplementary table -1. List of proteins found in COVID-19 sputum

Supplementary table-2. Fold changes of proteins found in COVID-19 sputum before and after rhDNase treatment

Supplementary table-3. List of proteins found in blood plasma of COVID-19 patients

Supplementary figures

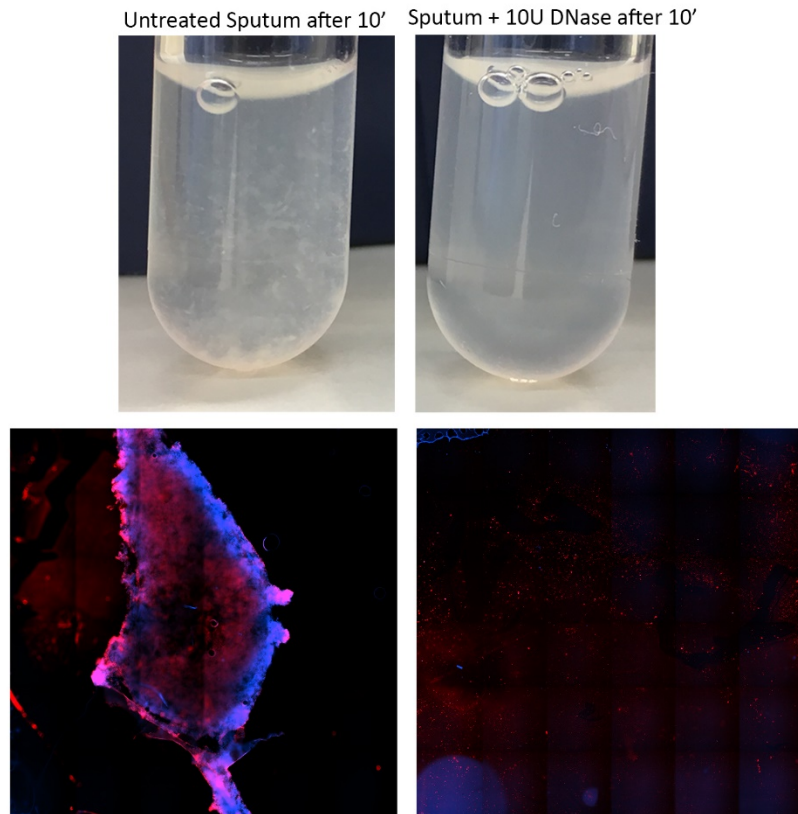
Fig. S1. Citrullinated histones and granule-proteins from neutrophils are present in COVID-19 sputum



Identification of citrullinated peptides derived from Neutrophil NET proteins in Covid-19 lung sputum using DIA-MS.

A spectral library which included citrullinated peptides was built by searching pooled and fractionated Covid-19 lung sputum DDA runs with the variable modification +0.98 Da on arginine residues (UniMod:7) and up to three missed trypsin cleavages. This library was used for searching individual DIA-MS runs of lung sputum (n=5) from 4 Covid-19 patients. The peptide level results were filtered as follows: including NET proteins and including peptides with citrullinated modifications and finally excluding peptides with the citrullinated modification located in the C-terminal position of a peptide. The intensities of the filtered citrullinated peptides are shown in the heatmap. Each row represent a citrullinated peptide with modifications shown on the right side. The mapped proteins and genes are indicated with colours on the left side.

Fig. S2. DNase 1 degrades NETs in COVID-19 sputum



DNase degrades NETs in COVID-19 sputum and reduces viscosity. Sputum from a COVID-19 patient (n =1) was treated with +/- 10 units of DNase (Abcam) for 10 minutes at 37 degrees Celsius. This resulted in clarification of the sputum and reduced viscosity. Immunofluorescence against neutrophil elastase and DNA revealed that DNase treatment degraded NETs in COVID-19 sputum. Whereas large NETs persisted in untreated COVID-19 sputum.

Table S1. Baseline characteristics and clinical parameters of SARS-Cov2 positive patients prior to treatment with rhDNase

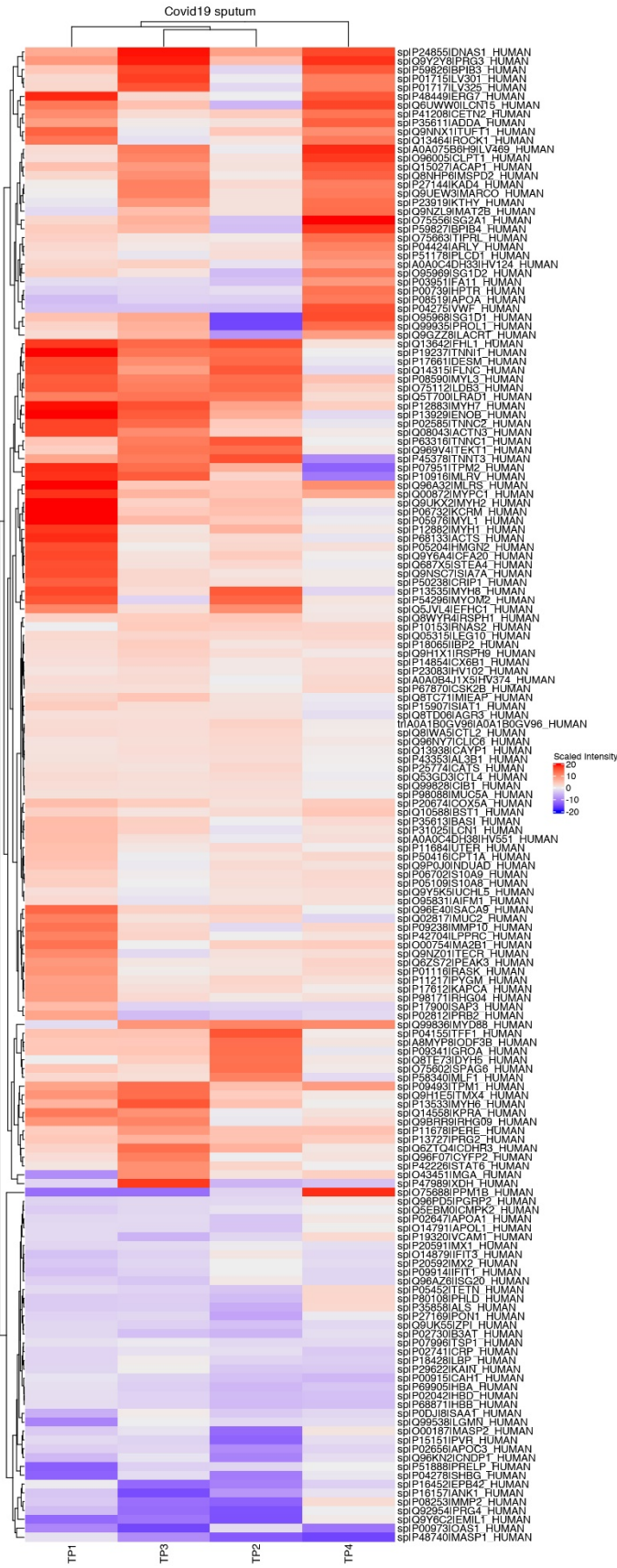
	TP1	TP2	TP3	TP4	TP5
Baseline characteristics					
Age (years)	50s	70s	60s	60s	60s
Gender	Male	Male	Male	Male	Female
Hospital stay (days)	7	26	16	19	8
Concomitant medications					
Antibiotics	Cefotaxime	Cefotaxime	Cefotaxime	Imipenem	Cefotaxime
Chloroquine phosphate(dose)	500mg BD	500mg BD	500mg OD	500mg BD	500mg OD
Anticoagulant		Enoxaparin	Enoxaparin		
Mucolytic		Acetylcysteine			
Anti-inflammatory		Betamethasone			
Clinical parameters (range before rhDNase treatment start)¹					
<i>Vital signs</i>					
Temperature (°C)	35.8-36.9	36.2-38.1	37.9-40	36.8-40.3	35.1-37.7
Respiratory rate (breaths/min)	18-23	22-36	20-30	20-37	18-24
Pulse (beats/min)	82-98	67-100	70-110	85-120	75-97
<i>Blood cell counts</i>					
WBC (x10 ⁹ /L)	6.9-11.4	9.1-19.3	6.7-8.4	3.1-11.9	7-8.4
Neutrophils (x10 ⁹ /L)	6.3-11.1	8.4-17	5.3-7.1	2.7-2.8	5.4-7.2
Lymphocytes (x10 ⁹ /L)	0.3-0.4	0.2-1.2	0.5-0.9	0.3-0.3	0.7-1.1
<i>Severity markers</i>					
CRP (mg/L)	223-231	23-192	224-296	83-211	219-278
PCT (µg/L)	0.48	0.17-3.5	1.6		
LD (µkat/L)	7.3-7.3	6.6-13	4.2-5.4	1.2-9.7	
D-dimer (mg/L)	0.63	2.4-10	0.85-0.9	2-2	1.4-2.9
Ferritin (µg/L)	892-1042	649-3949	1535-1717	8748	1158-1342
Fibrinogen (g/L)	8.8-9	6.6-6.7	8.4		9-9
Triglycerides (mmol/L)	2.1-2.2	0.9-1.7	1.2.1.4	1.6	
NEWS	4-6	6-9	6-9	6-14	4-8
Organ failures and biomarkers (range before rhDNase treatment start)					
Total number of failing organs	1	3	2	2	2
<i>Cognitive</i>	no	no	no	no	no
Glasgow coma score	15-15	15-15	15-15	15-15	15-15
<i>Liver</i>	no	no	no	no	no

Creatinine ($\mu\text{mol/L}$)	67-82	138-318	129-155		62-150
<i>Coagulopathy</i>	no	no	no	no	no
INR	1.1	0.9-1	1	1.1	0.9-1.2
Platelets ($\times 10^9/\text{L}$)	260-314	283-650	214-234	134-162	208-293
<i>Cardiovascular</i>	no	yes	no	yes	no
Estimated MAP (mmHg)	82-102	68-90	73-90	63-93	78-98
<i>Respiratory</i>	yes	yes	yes	yes	yes
O ₂ Saturation (%)	91-98	80-97	89-95	86-96	88-96
SpO ₂ /FiO ₂	192-245	111-381	118-424	171-383	391-447
rhDNase treatment					
Treatment start (days from admission)	3.5	10.5	3.5	4.5	5
Treatment duration (days)	3	5	6	5	3

TP = treated patient; ACE2 = angiotensin converting enzyme-2; BD = bi-daily; OD = once daily; WBC = white blood cell count; CRP = C-reactive protein; PCT = procalcitonin; LD = lactate dehydrogenase; NEWS = national early warning score; ALT = alanine transaminase; AST = aspartate aminotransferase; INR = international normalized ratio; MAP = mean arterial pressure; SpO₂ = peripheral capillary oxygen saturation; = FiO₂ = fraction of inspired oxygen

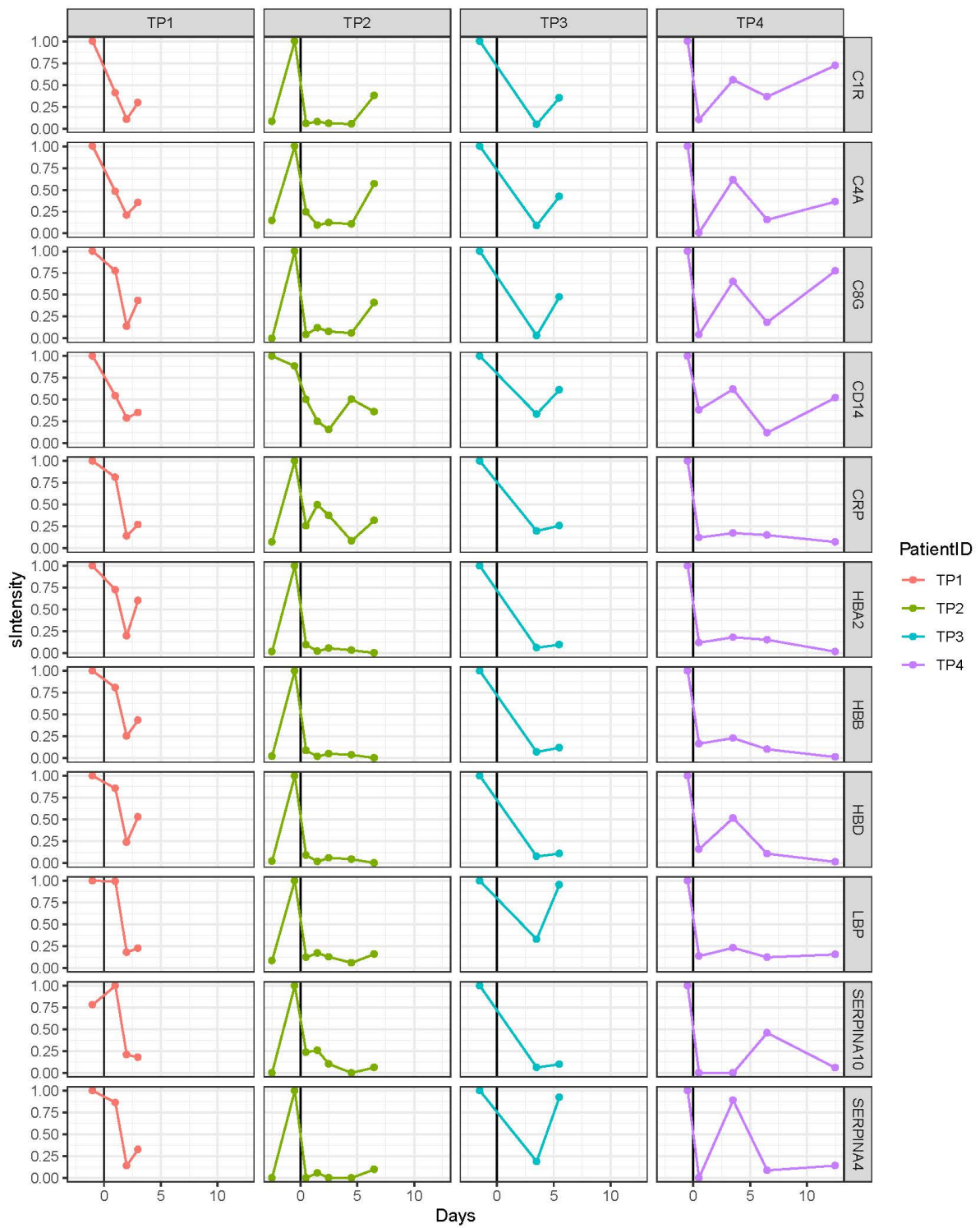
¹Where only one measurement was taken during this time, only one number is reported

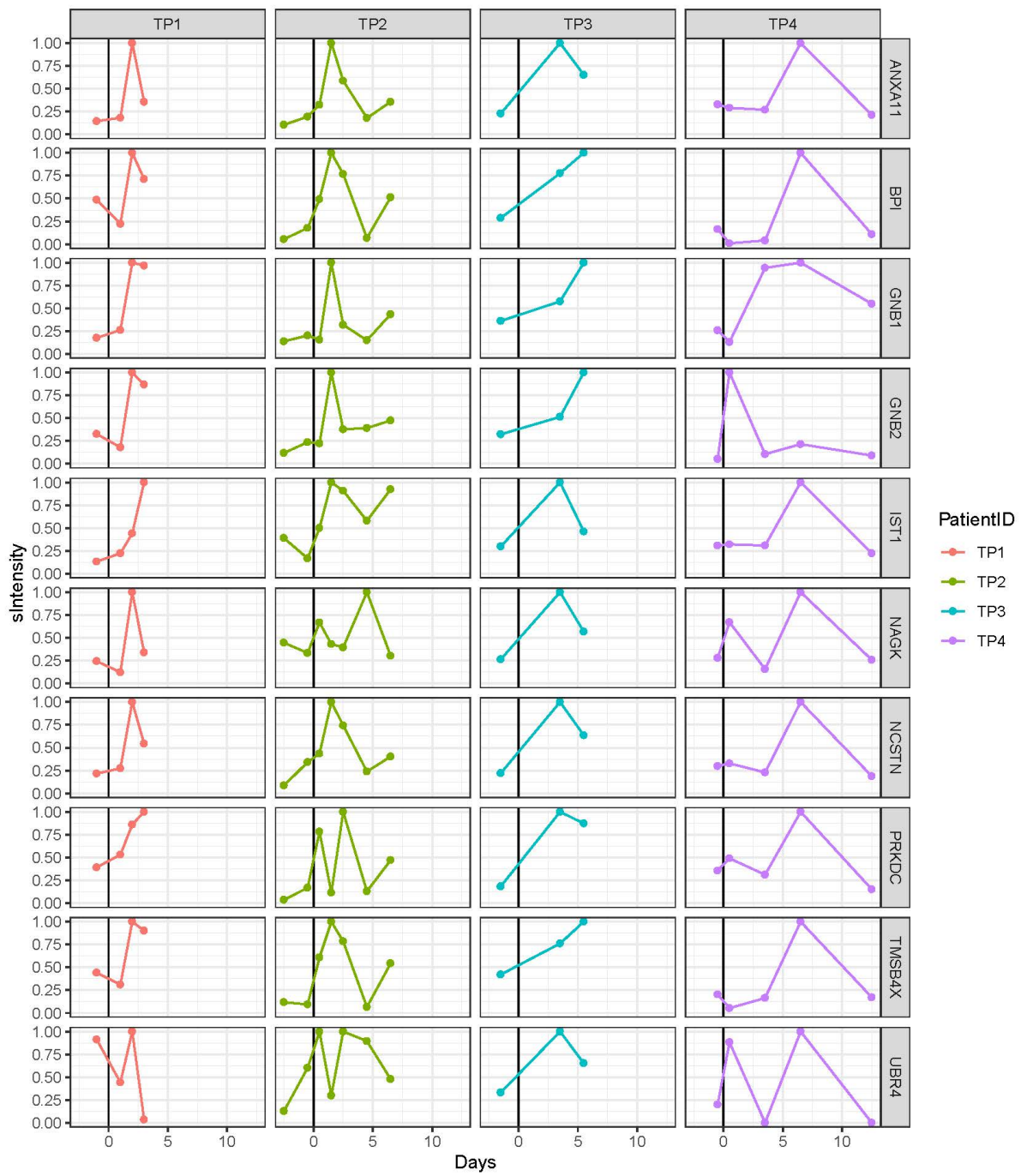
Fig. S3. Heat map of sputum proteome after treatment with rhDNase.

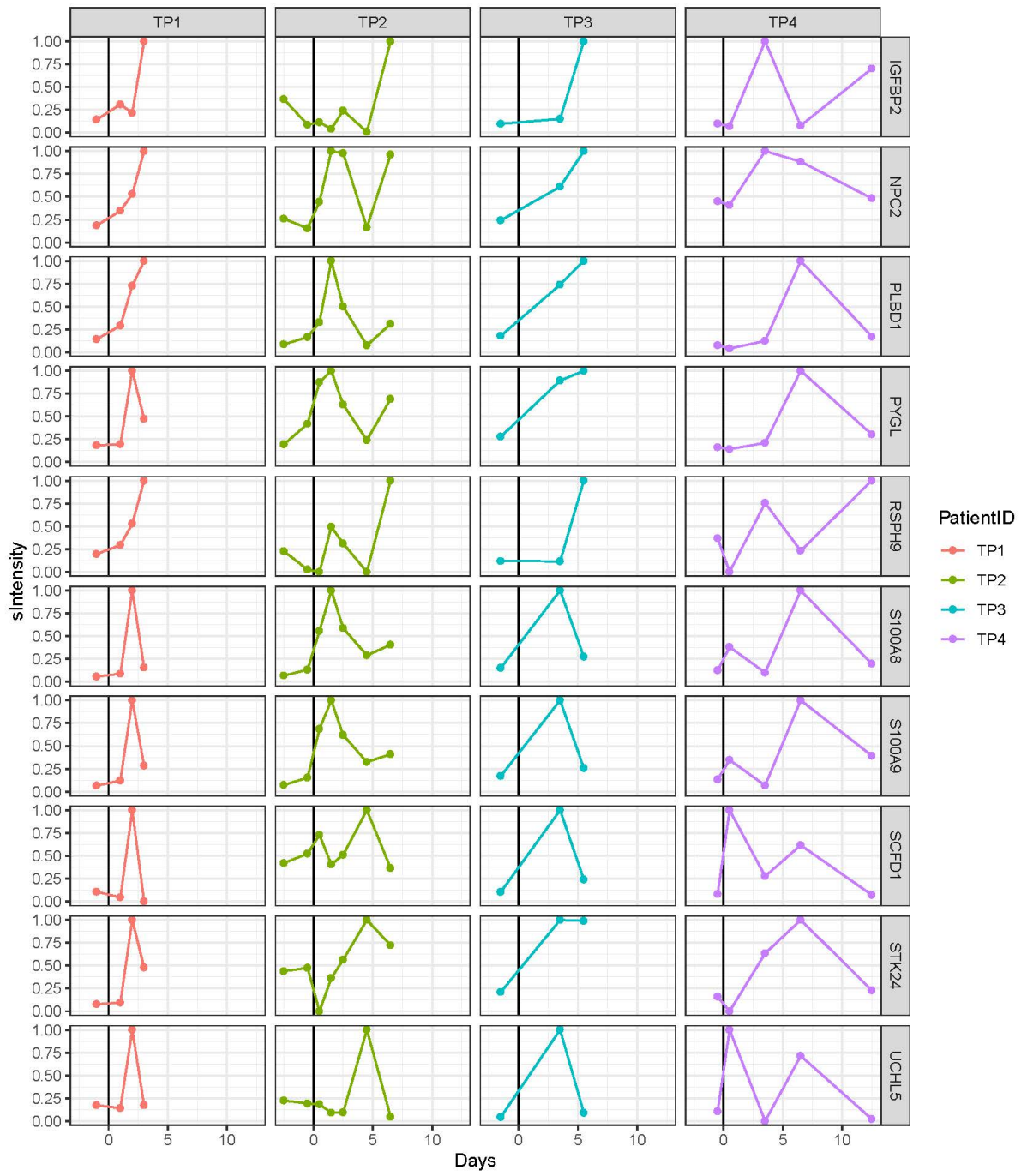


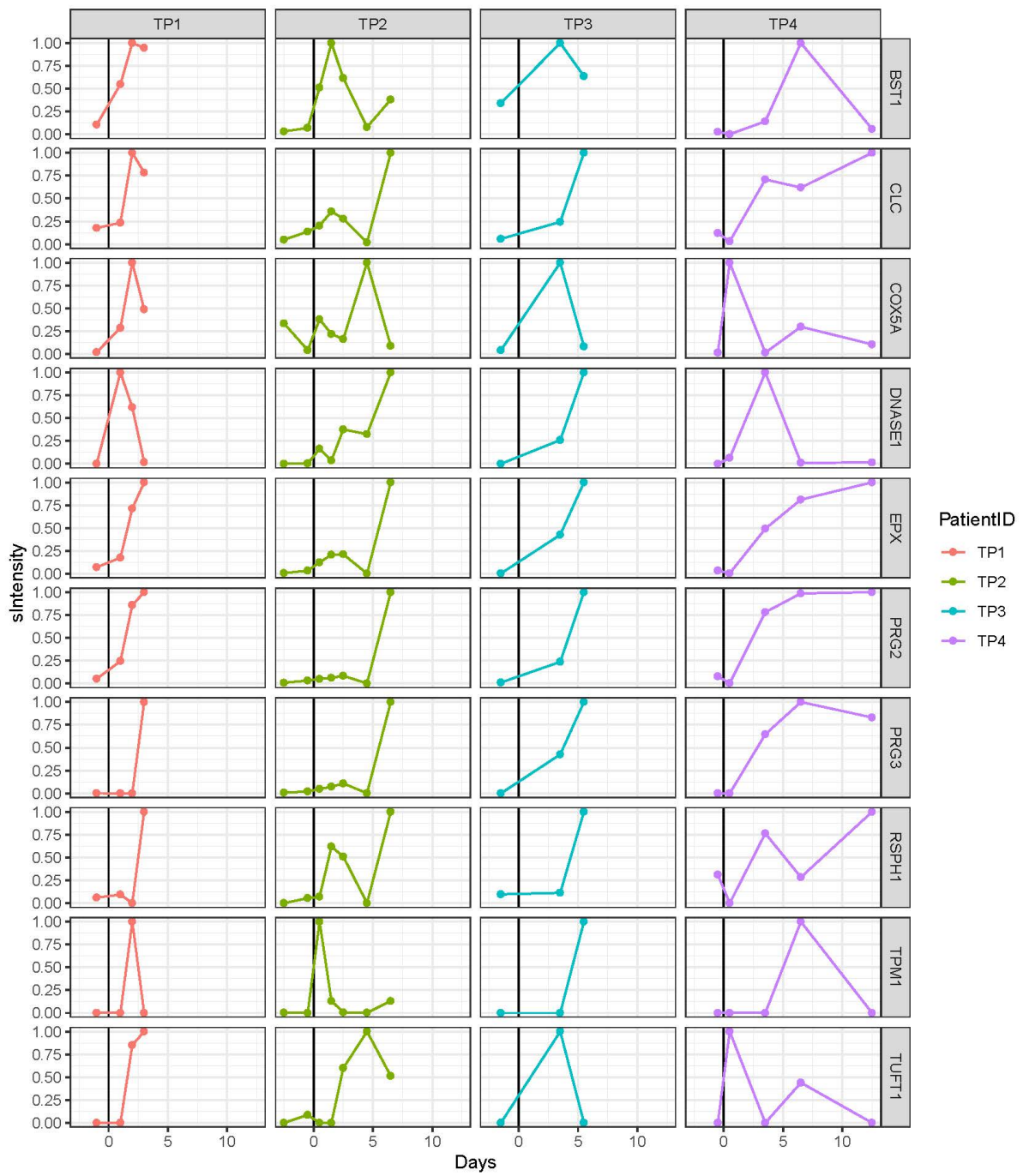
Differential analysis of sputum protein abundance levels between covid-19 patients pre- and post DNase1 treatment. Heatmap of 170 proteins (rows) identified to change with DNase1 treatment (TP1-TP4). The mapped gene name for each protein are indicated to the right. The columns for each patient are the days relative to DNase1 treatment start (Day 0) and are plotted as barplots above the heatmap. Colours in the heatmap represent the the scaled intensity per protein and patient (where maximum intensity is set to 1).

Fig.S4. Sputum proteins over time per patient



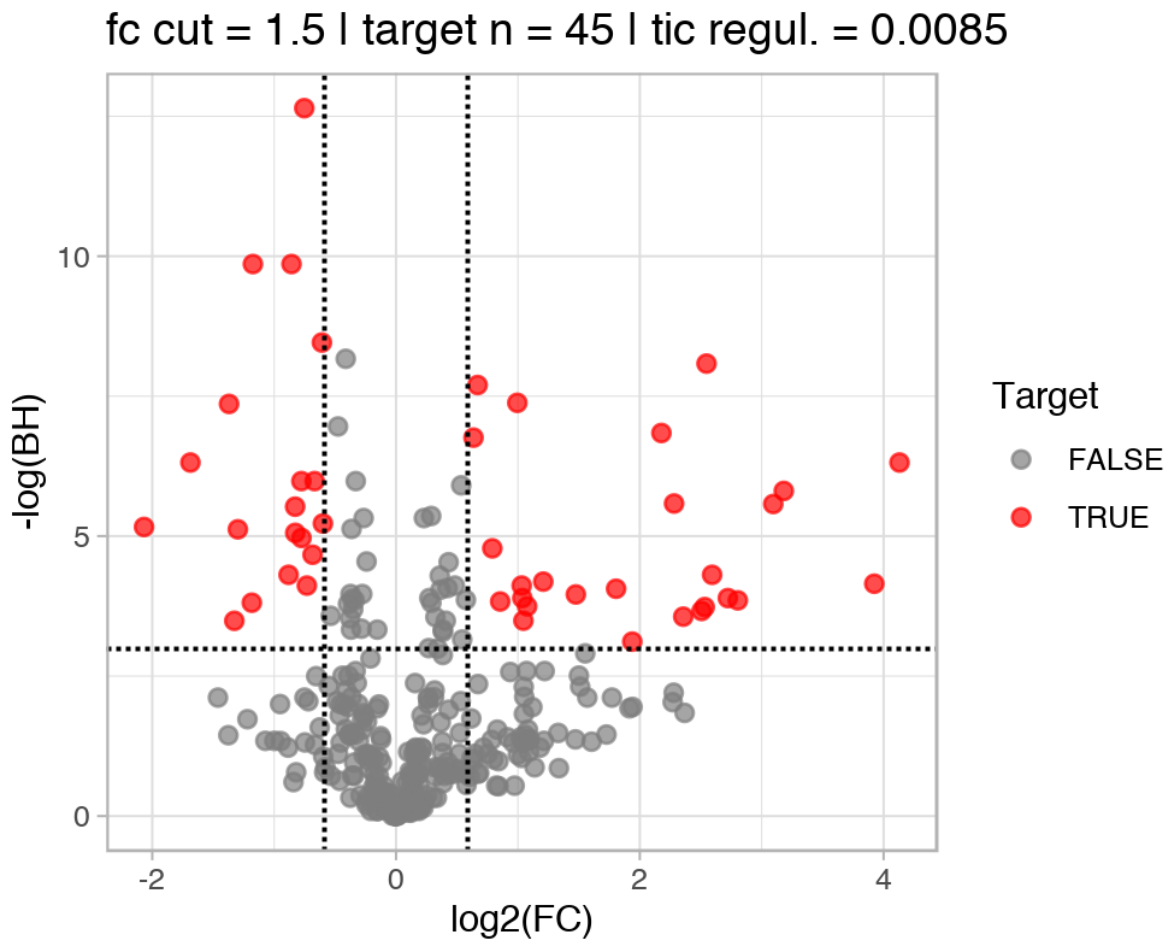






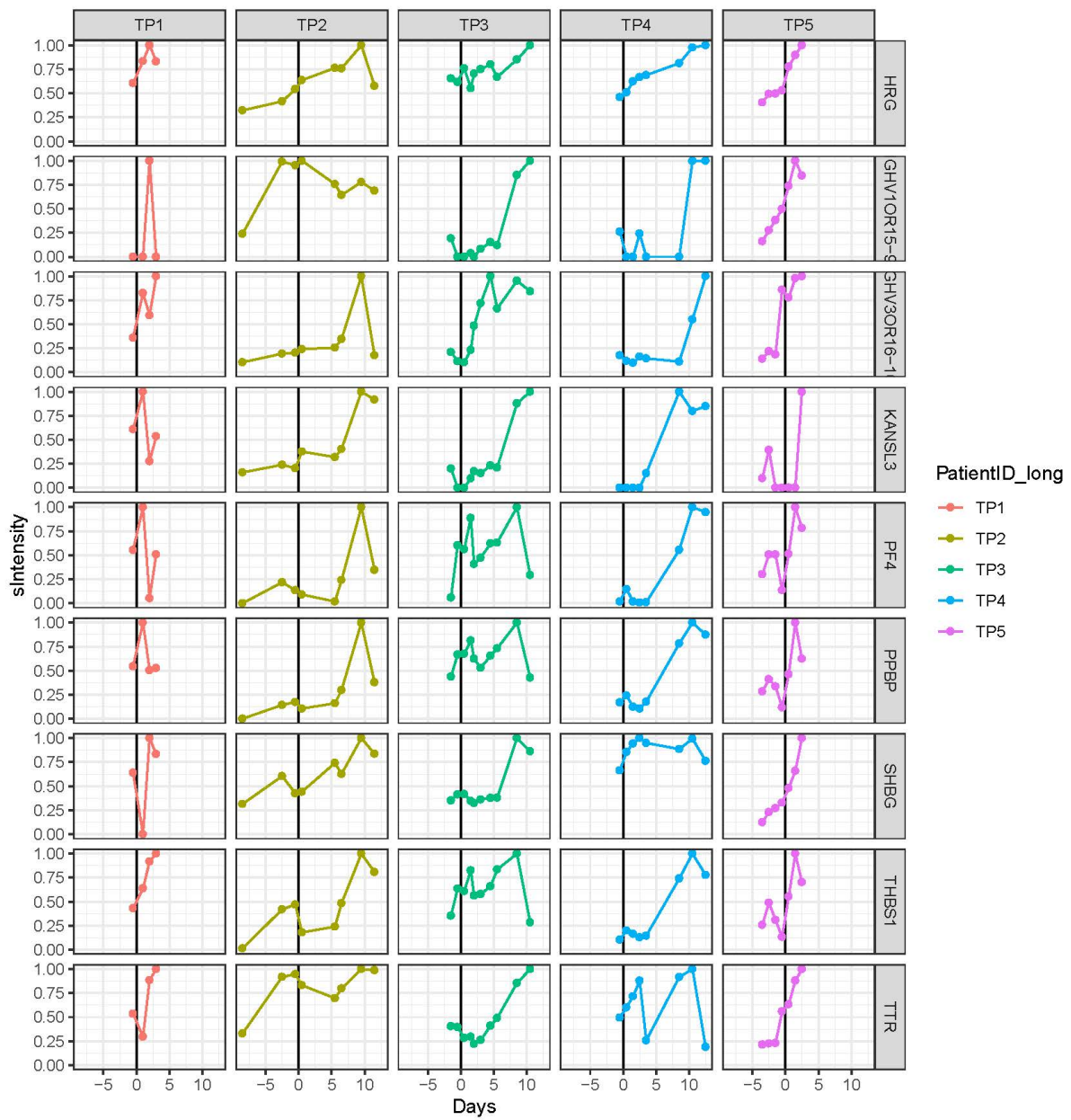
Individual plots of 10 selected proteins from a) (the scaled intensity is the same as in a). The vertical black lines in plots indicate Day 0 (DNase1 treatment start).

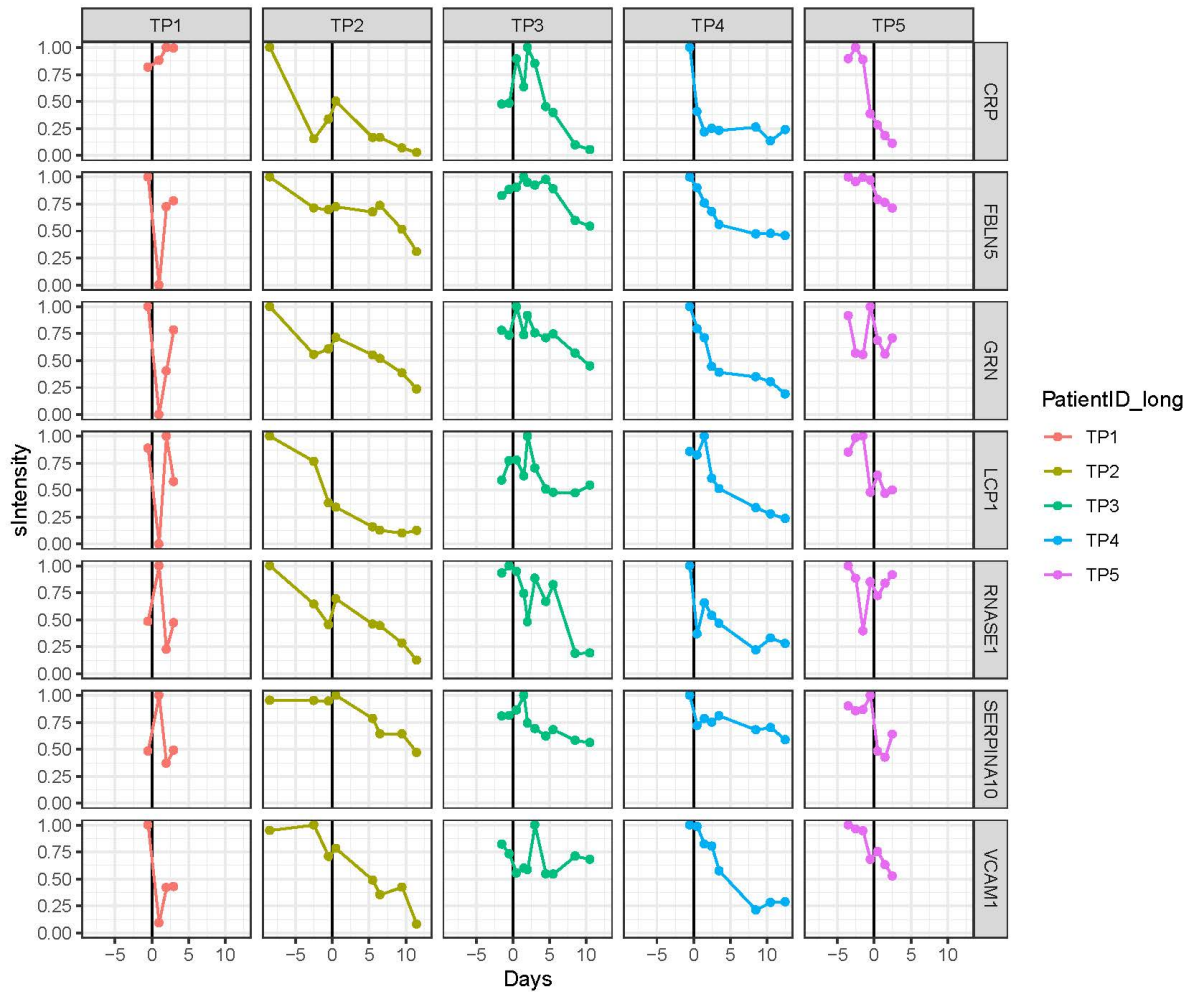
Fig. S5. Volcano plot depicting plasma proteins significantly regulated after rhDNase treatment.

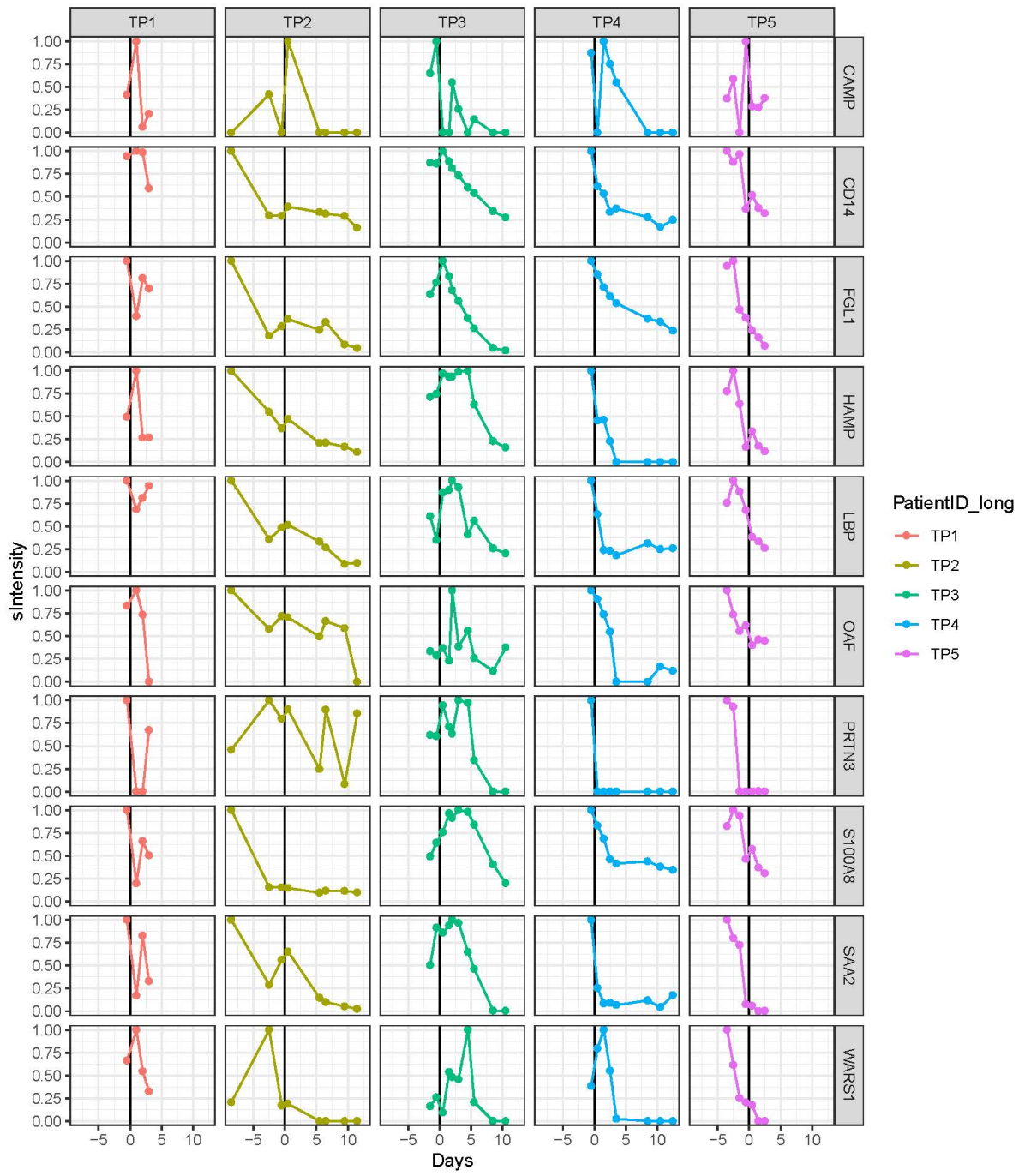


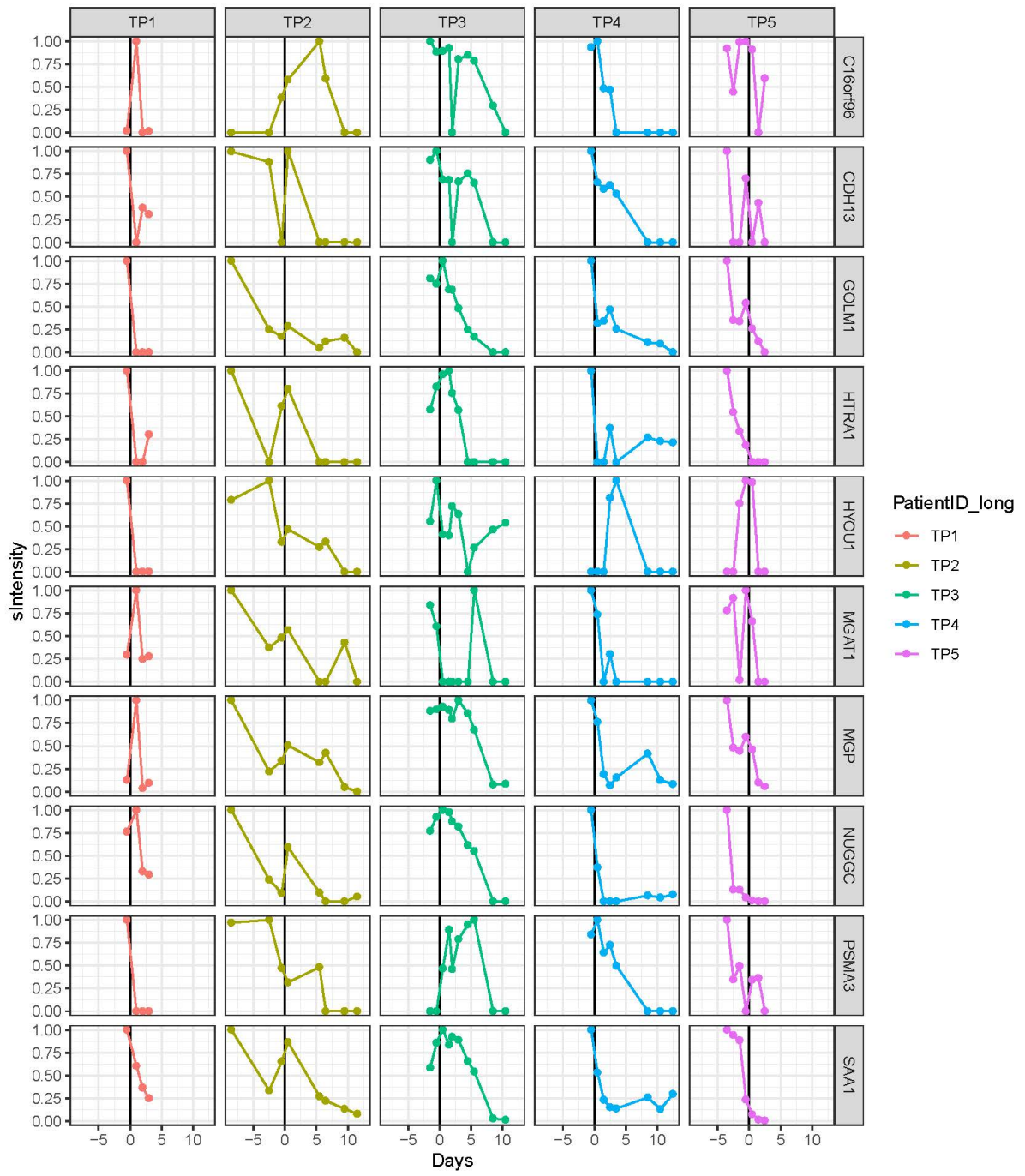
Volcano plot of plasma proteins of covid-19 patients pre- versus post DNase1 treatment. Vertical dotted lines are the foldchange cut offs (-1/1.5 and +1.5). Horizontal dotted line is the adjusted p-value cut off (0.05, Benjamini & Hochberg corrected).

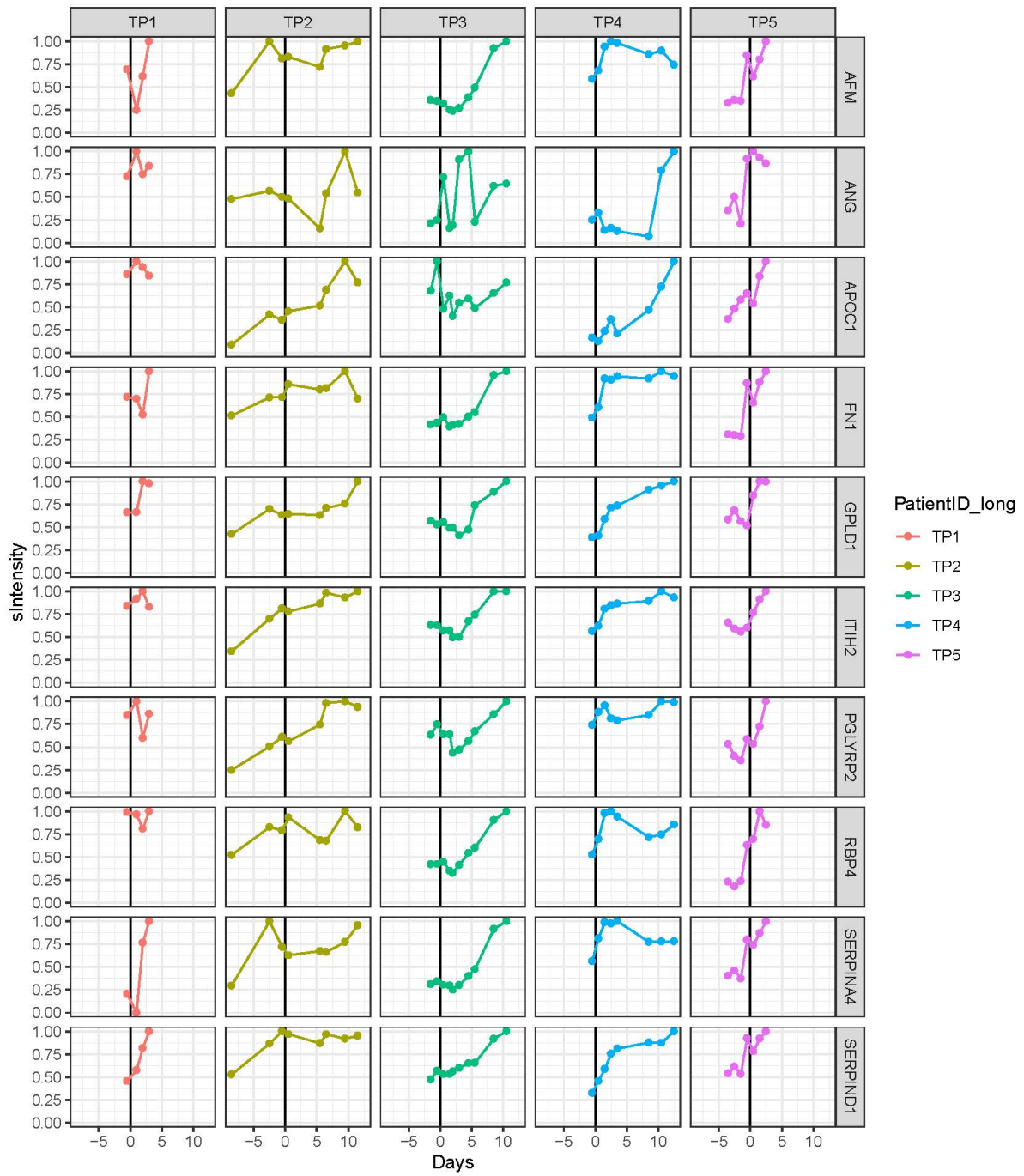
Fig. S6. Plasma proteins intensity plots per patient over time.





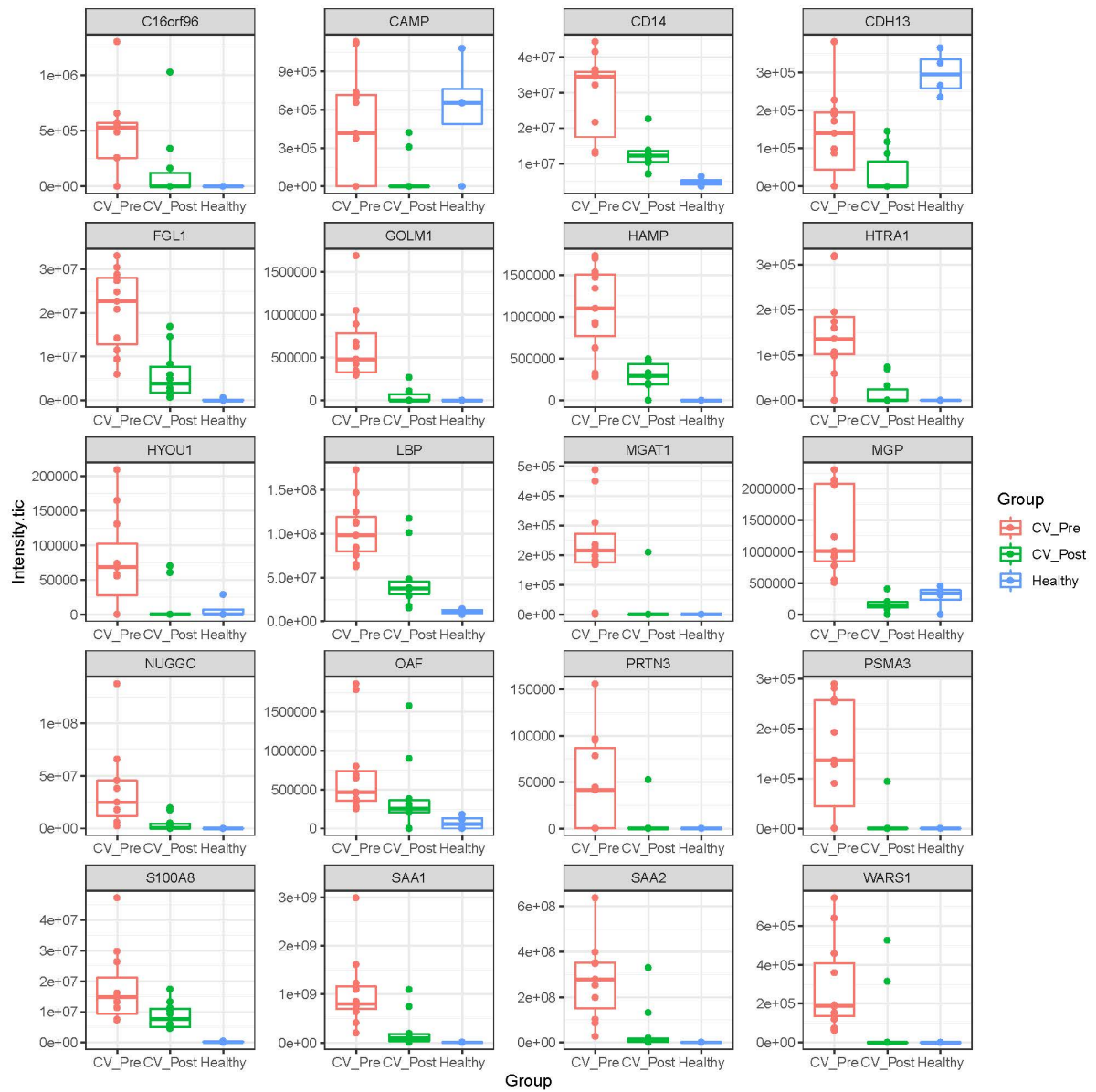


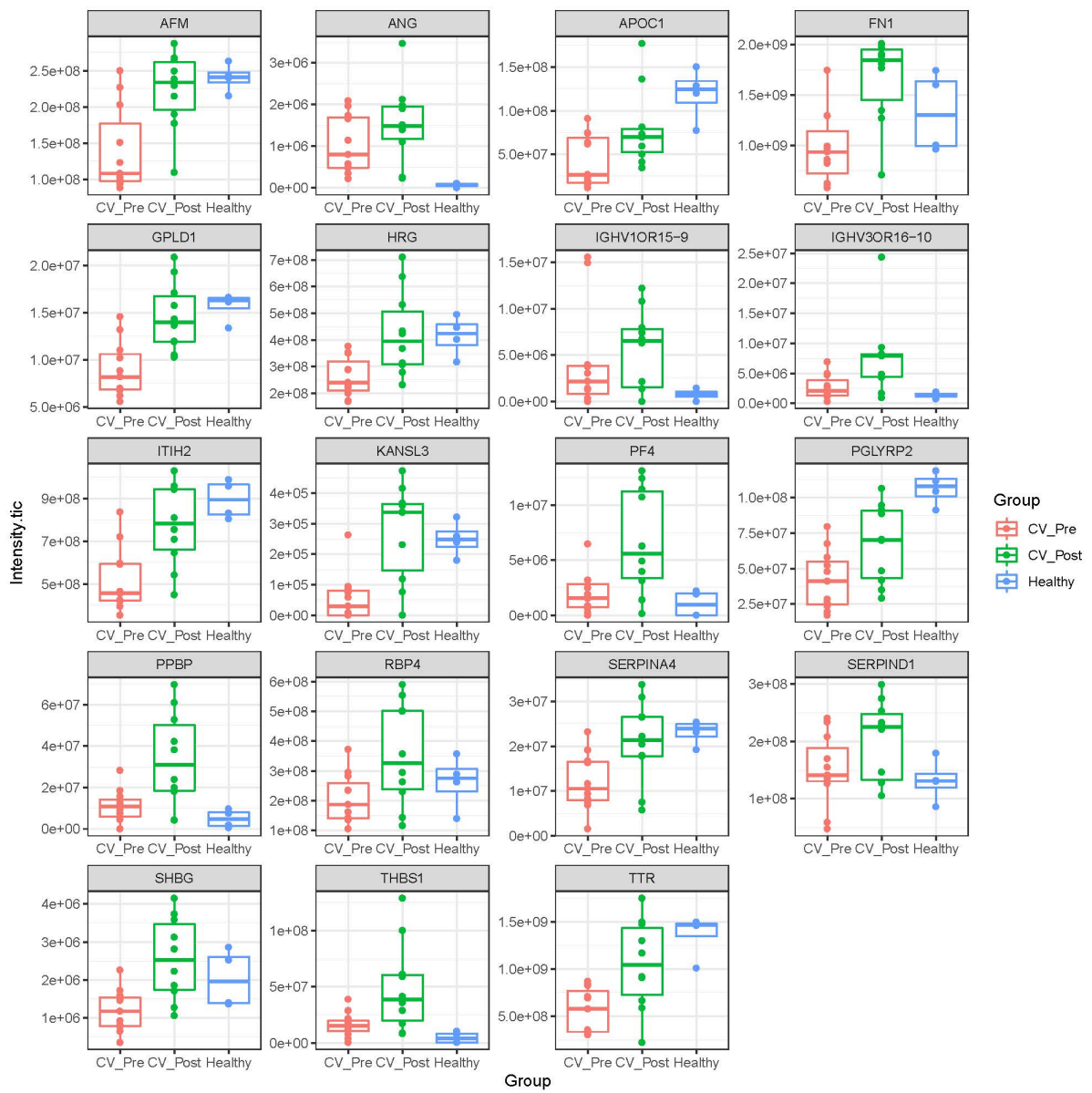
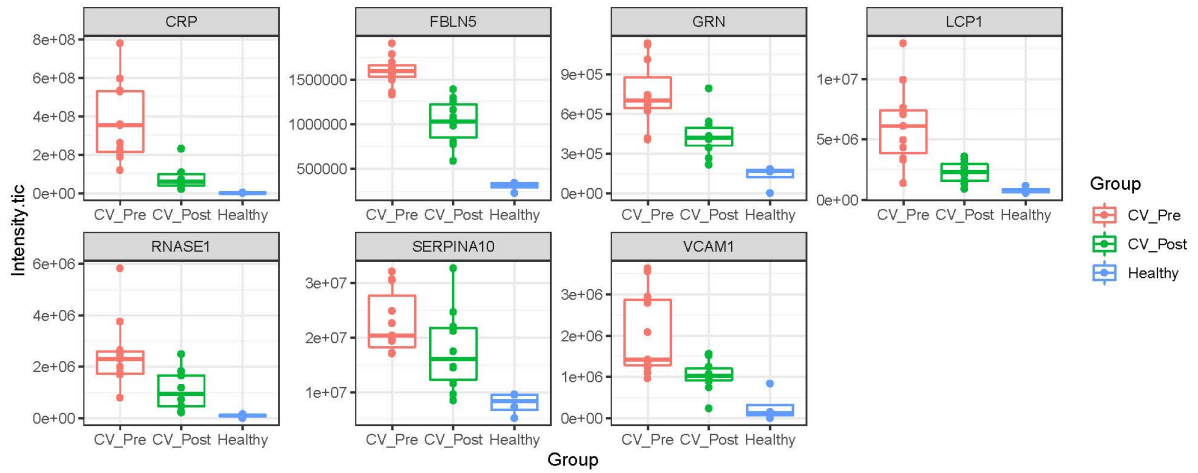




Individual plots of all proteins included in Figure 6. The vertical black lines in plots indicate Day 0 (DNase1 treatment start).

Fig. S7. Comparison of blood plasma from untreated, rhDNase-treated, and healthy individuals.





Boxplots of all proteins included in the heat map presented in Figure 6 A compared with untreated (CV_Pre), rhDNase treated (CV_Post) and healthy blood plasma (Healthy).