

SUPPLEMENTARY APPENDIX**Anti-Platelet Factor 4 Antibodies Causing VITT do not Cross-React with SARS-CoV-2 Spike Protein**

Andreas Greinacher, MD^{1*}, Kathleen Selleng, MD^{1*}, Julia Mayerle, MD², Raghavendra Palankar, PhD^{1*}, Jan Wesche^{1*}, Sven Reiche, PhD³, Andrea Aebischer, VMD³, Theodore E. Warkentin, MD⁴, Maximilian Muenchhoff, MD⁵, Johannes C. Hellmuth, MD⁶, Oliver T. Keppler, MD⁵, Daniel Duerschmied, MD⁷, Achim Lothar, MD^{7,8}, Siegbert Rieg, MD⁹, Meinrad Paul Gawaz, MD¹⁰, Karin Anne Lydia Mueller, MD¹⁰, Christian S. Scheer, MD¹¹, Matthias Napp, MD¹¹, Klaus Hahnenkamp, MD¹¹, Guglielmo Lucchese, MD¹², Antje Vogelgesang, PhD¹², Agnes Flöel, MD¹², Piero Lovreglio, MD¹³, Angela Stufano, MD¹³, Rolf Marschalek, PhD¹⁴, Thomas Thiele, MD¹ and the co-authors in the appendix¹⁵

Table of Contents

Supplementary Material = SM	2
SM1 Recombinant protein cloning and expression	2
SM2 Biotinylation of PF4.....	2
SM3 SARS-CoV-2 spike protein ELISAs	4
SM4.1 S1 domain anti-SARS-CoV-2 IgG ELISA.....	4
SM4.2 Anti-SARS-CoV-2 IgG SARS-CoV-2 spike ectodomain, RBD-SD1, anti-PF4 IgG and anti-PF4/heparin IgG ELISAs	4
SM4 COVID-19 patient cohorts.....	5
Cohort Munich, Germany	5
Cohort Freiburg, Germany	6
Cohort Tuebingen, Germany.....	6
Cohort Greifswald, Germany	7
Cohort Bari, Italy	7
Supplementary Table SM1	8
Supplementary Table SM2: WHO clinical progression scale for COVID-19 ¹	8
References for supplementary materials	16
Supplementary Results	9
SR1 - Identification of immunogenic epitopes and homologies of human PF4 and SARS-CoV-2 spike protein and comparative analysis of their 3D structures	9
Supplementary Table SR1A: Amino acid sequence of PF4 and SARS-CoV-2 Spike protein	12

Supplementary Table S1B: Identified sequences homologies between PF4 and Spike protein	12
Supplementary Figure S2	15
Supplementary Figure S3	15

Supplementary Material (SM)

SM1 Recombinant protein cloning and expression

The SARS-CoV2 Spike ectodomain amino acids 17 – 1213 and the RBD-SD1 domain aa 319 – 519 (based on QHD43416, PMID:33191578) were amplified from a codon-optimized synthetic gene (GeneArt, Thermo Scientific). The constructs were cloned in the expression vector pEXPR103 (IBA Lifesciences, Göttingen, Germany) in frame with an N-terminal modified mouse Ig kappa light chain signal peptide and a c-terminal double Strep tag.

Expi293 cells were grown in Expi293 expression medium (Thermo Scientific) and polycarbonate Erlenmeyer flasks (Corning) at 37°C, 8% CO₂, 125 rpm. For transfection, cell density was adjusted to 2x 10⁶ cells/ml and the cells were transfected with 1 µg/ml of plasmid DNA using the ExpiFectamine293 transfection kit (Thermo Scientific) according to the manufacturer's instructions. The cells were subsequently incubated at 37°C, 8% CO₂, 125 rpm. The supernatant was harvested 5-6 days after transfection by centrifugation at 6000 x g for 20 min at 4°C.

Biotin contained in the expression medium was blocked by addition of BioLock (iba lifesciences) as recommended and the supernatants were purified using Strep-Tactin XT Superflow high capacity resin (IBA Lifesciences) according to the protocol of the manufacturer. The proteins were eluted with 50 mM Biotin (in 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA; pH 8.0) and stored at -80°C until further use.

SM2 Biotinylation of PF4

Biotinylated PF4 (Cat.-No. 006/16, Chromatec, Greifswald, Germany) was commercially obtained and has previously been described.^{1 11} Briefly, heparin sepharose (GE Healthcare, Uppsala, Sweden) was washed 3 times with PBS pH 7.4, mixed with PF4 (Chromatec, Greifswald, Germany) from human platelets for 15 min, and then kept at 4°C overnight. The PF4-heparin-sepharose suspension was mixed and incubated with biotin-XX SE (6-((6-((Biotinoyl)Amino)Hexanoyl)Amino)Hexanoic Acid, Succinimidyl Ester) (Molecular Probes, Eugene, OR, USA) for 1h at RT. Samples were washed (0.8 M NaCl, 20 mM Hepes, 2 mM EDTA, pH 7.4), and biotinylated PF4 was eluted with high salt buffer (2 M NaCl, 20 mM Hepes, 2 mM EDTA, pH 7.4). The concentration of biotinylated PF4 was determined by a bicinchoninic acid protein assay kit using bovine serum albumin as standard (Sigma-Aldrich, Taufkirchen, Germany). Binding of heparin to biotinylated PF4 was not affected since the polyanion binding site of PF4 is protected from alteration by biotin.

For quality control biotinylated PF4 was tested with VITT sera in the washed platelet functional test. VITT antibodies induced platelet activation in the presence of biotinylated PF4 for the same way as in the presence of native PF4 (n=16). SM2 Affinity purification of serum IgG by biotin-PF4 and biotin-PF4/heparin coupled magnetic beads

Coupling of biotinylated PF4 (biotin-PF4) (Cat. No. 006/16; Chromatec, Greifswald, Germany) to streptavidin-conjugated paramagnetic microbeads (Dynabeads-SA) (Cat. No. 65601, Dynabeads MyOne Streptavidin T1, Invitrogen) was performed according to the manufacturer's instructions. Briefly, 250 µL Dynabeads-SA were washed four times with 1 mL PBS (pH 7.4) and resuspended in 250 µL biotin-PF4 (400 µg/mL PBS). For each sample, 250 µL biotin-PF4 and Dynabeads-SA were coincubated for 30 min at room temperature with gentle rotation and washed four times with 500 µL washing-buffer (PBS pH 7.4 supplemented with 0.1% BSA). 200 µL of serum was added and incubated for 90 min at 37°C under gentle rotation. Beads were then washed four times in 500 µL washing-buffer. 400 µL acidic elution-buffer (0.1 M glycine, pH 2.7) was added for 1 min. The eluate was subjected immediately to a 100k-centrifugal filter device (Amicon Ultra-2, Merck Millipore, Darmstadt, Germany) and

centrifuged for 5 min, 4000xg. Samples were washed with an additional 400 μ L elution-buffer, centrifuged again, and the remaining 100-130 μ L supernatant immediately neutralized with 10 μ L Tris-HCl-buffer (1 M, pH 9.0). The protein concentration of each sample was measured at 280 nm on a NanoDrop2000 photo spectrometer (ThermoFisher, Waltham, USA) against the respective blank (TRIS-neutralized glycine-buffer).

For affinity purification of anti-PF4 IgG from biotin-PF4/heparin coupled Dynabeads-SA, PF4/heparin complexes of 1.0 IU/mL unfractionated heparin (Heparin-Natrium 25000 IE/5mL; Ratiopharm) with 40 μ g/mL PF4 (30% biotin-PF4) were formed in 12.5 mL PBS at room temperature for 1 h. The coupling to washed Dynabeads-Streptavidin (250 μ L per sample) was performed consecutively in two steps with 2500 μ L of the complex-solution (2x 1250 μ L) for 30 min each and with subsequent steps performed as described above.

SM3 SARS-CoV-2 spike protein ELISAs

SM4.1 S1 domain anti-SARS-CoV-2 IgG ELISA

Anti-SARS-CoV-2 ELISA was performed in CoV-2 ELISA IgG (recombinant S1-domain; EI 2606-9620 G; EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). The ELISA was performed according to the manufacturer's protocol.

SM4.2 Anti-SARS-CoV-2 IgG SARS-CoV-2 spike ectodomain, RBD-SD1, anti-PF4 IgG and anti-PF4/heparin IgG ELISAs

All in-house ELISAs were performed in flat-bottomed microwell plates (Cat. No. 478042 Thermo Scientific, CovaLink). ELISA plates were coated with 20 μ g/mL (100 μ L/well) in coating buffer (50mM NaH₂PO₄, 0.1% NaN₃) of SARS-CoV-2 full-length spike protein or receptor-binding domain (RBD) for 24h at 4°C. Antigen passivated plates were washed five times with washing buffer (150mM NaCl, 1% Tween20 pH 7.5) followed by blocking with 2% BSA (400 μ L/well) in coating buffer at room temperature for 2h. 100 μ L/microwell of serum samples (diluted to 1:200) and affinity-purified IgG eluates (diluted to 1:20) in sample diluent (0.05 M NaH₂PO₄, 0.15 M NaCl, 7.5% goat normal serum, pH 7.5), respectively and incubated for 60

min at room temperature and then washed five times. Binding of human IgG was detected with chromogenic tetramethylbenzidine substrate (100 μ L/microwell) (Kementec, Taastrup, Denmark) in the presence of horseradish peroxidase-conjugated to goat anti-human IgG (diluted to 1:15,000 in sample diluent) (Jackson ImmunoResearch Europe Ltd, Ely, UK) incubated for 60 min at room temperature and then washed five times. The chromogenic substrate reaction was stopped with 1 M H₂SO₄ (100 μ L/microwell) 10 min post-incubation at room temperature, and optical absorbance was measured at 450 nm (reference: 620 nm) (Tecan, Männedorf, Switzerland) within 10 min. Blank measurements were subtracted from each sample measurement.

For the preparation of PF4 ELISA, microwells were coated with 20 μ g/mL (100 μ L/well) of PF4 (Chromatec, Greifswald, Germany) in coating buffer for 24h at 4°C. For the PF4/heparin ELISA, complexes of 0.5 IU/mL unfractionated heparin (Heparin-Natrium 25000 IE/5mL, Ratiopharm) with 20 μ g/mL PF4 were formed in coating buffer at room temperature for 1 h and incubated for seven days at 4°C and washed five times before use. For some experiments recombinant PF4 produced in *E. coli* (PF4-hr, Chromatec, Greifswald, Germany) was used. Sera or purified anti-PF4 antibodies were tested as described above.

SM4 COVID-19 patient cohorts

Cohort Munich, Germany: From March 2020 to March 2021, patients were prospectively included in the Registry of the LMU Klinikum (CORKUM, WHO trial ID DRKS00021225). The study was approved by the local ethics committee (project no. 20-454). For the serological substudy reported here, 55 patients below the age of 65 with available serum obtained between 5 and 60 days after positive reverse-transcriptase PCR (RT-PCR) testing of SARS-CoV-2 in nasopharyngeal swabs were studied. Clinical outcome was recorded as the maximum WHO Score.² Median age of all patients in this substudy was 47 (range, 20-64),

16 female (29%) and 39 male (71%). Blood was drawn 15.6 (range, 6-54 days) days after the first positive result of PCR testing for SARS-CoV-2. Maximal WHO clinical progression scale in this cohort was 6 (mean 4.0; range, 1-6). Seven patients required intensive care unit (ICU) treatment, none died, although 3 remained in ICU at time of writing.

Cohort Freiburg, Germany: From March 2020 to March 2021, patients were prospectively included in the COVID-19 Registry Freiburg (WHO trial ID DRKS00021206, ethics approval 153/20) and the UKF COVID-19 cohort, and biosamples collected into the FREEZE biobank (ethics approval 383/19). Inclusion was based on the diagnosis of COVID-19 with positive RT-PCR testing of SARS-CoV-2 in nasopharyngeal swabs. For the serological substudy reported here, 42 patients with available serum obtained between 1 and 20 days after positive RT-PCR testing of SARS-CoV-2 in nasopharyngeal swabs, were studied. Median age of all patients was 59 (range, 0.5-81), 18 female (43%) and 24 male (57%). Blood was drawn 4.9 (range, 1-20 days) days after the first positive result of PCR testing for SARS-CoV-2. Maximal WHO clinical progression scale in this cohort was 7 (mean 4.4; range, 1-7).

Cohort Tuebingen, Germany: From February 2020 to March 2021, 261 patients were prospectively included in the SARS CoV-2 registry of the Department of Cardiology and Angiology of the University Hospital, Tübingen, Germany. The study was approved by the local ethics committee (240/2018BO2). Patients over 18 years after PCR-based diagnosis of a SARS-CoV-2 infection were included. Exclusion criteria were other viral or bacterial co-infections and cancer. For the substudy reported here, 32 SARS-CoV-2 positive patients below the age of ≤ 65 years with COVID-19 and positive RT-PCR testing of SARS-CoV-2 in nasopharyngeal swabs with available citrate anticoagulated plasma samples were analysed. Blood samples were obtained within 48 hours of hospital admission and between 0 to 13 days after first symptom onset. Median age of this cohort was 56 years (range, 25-65 years), 17 (53%) were women and 15 (47%) were men. Maximal WHO clinical progression scale in this cohort was 7 (mean 5, range, 3-7).

Cohort Greifswald, Germany: Since April 2020, patients with COVID-19 and other viral-induced pneumonia were prospectively included in the Greifswald VIP (Viral-induced-pneumonia) database (registered DRKS-ID: DRKS00023770). This database was approved by the local ethics committee (identifier: (BB060/20a). Blood samples and clinical data were collected (1) at hospital admission, (2) 5-7 days after admission, (3) at intensive care unit admission, (4) 5-7 days after ICU admission, and (5) at discharge. Patients and clinical data were registered using the CentraXX software; blood samples were stored in the Integrated Research Biobank of the University Medicine Greifswald.³ For the serological substudy reported here 32 patients with COVID-19 and positive RT-PCR testing of SARS-CoV-2 in nasopharyngeal swabs and available serum or citrate anticoagulated plasma samples were included. Median age of all patients was 70 (range, 32-88), 15 female (47%) and 17 male (53%). Blood was drawn 10.3 (mean; range, 5-19 days) days after the first positive result of PCR testing for SARS-CoV-2. Maximal WHO clinical progression scale in this cohort was 10 (mean, 5.7; range, 4-10).

Cohort Bari, Italy: Since October 2020, patients with COVID-19 were prospectively included in a multicenter observational study aimed at characterizing autoimmune phenomena in COVID-19 patients. Approval was obtained by the Ethics Committee, Università di Brescia, Italy (ID Number: NP 4463). For the serological substudy reported here 61 patients with COVID-19 and positive RT-PCR testing of SARS-CoV-2 in nasopharyngeal swabs were included. Median age of all patients was 54 years (range, 26-66), 31 female (51%) and 30 male (49%). Blood was drawn 23 (mean; range, 5-68) days after the first positive result of PCR testing for SARS-CoV-2 and after the last swab PCR testing was already negative. Maximal WHO clinical progression scale in this cohort was 2 (mean 2).

Supplementary Table SM1: Summary of the characteristic of patients selected for the presented study cohorts

Registries	Munich	Freiburg	Tuebingen*	Greifswald	Bari
Consecutive COVID-19 patients, confirmed by Sars-Cov-2 nasopharyngeal swab PCR test	Yes	Yes	Yes	Yes	Yes
Age >18 years	Yes	No [#]	Yes	Yes	Yes
Age <65 years	Yes	No age limit	Yes	No age limit	No age limit
Informed consent	Yes	Yes	Yes	Yes	Yes
Blood drawing after positive SARS-Cov-2 PCR test	5-65 days after positive SARS-Cov-2 PCR test	1-20 days after positive SARS-Cov-2 PCR test	Within 2 days after hospital admission	days 0; 5-7 after hospital admission; 5-7 after ICU admission	At least 5 days after positive SARS-Cov-2 PCR test
Recruitment start	2020/03/01	2020/02/27	2020/02/25	2020/08/17	2020/10/01
Ongoing study	Yes	Yes	No	Yes	Yes
Patients recruited until presented study cohort was collected	419 (as of 24 th of March 2021)	676 (as of 31 th of January 2021)	261 (as of 1 st of December 2020)	153 (as of 7 th of April 2021)	62 (as of 31 th of March 2021)
Study cohort with sufficient serum or citrate plasma volume (1ml)	55	42	32	32	61

*consecutive patients of the department of cardiology and angiology; # two children age 0.5 and 1 year were included

Supplementary Table SM2: WHO clinical progression scale for COVID-19¹

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalized: moderate disease	Hospitalized; no oxygen therapy*	4
	Hospitalized; oxygen by mask or nasal prongs	5
Hospitalized: severe disease	Hospitalized; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation: pO ₂ /FiO ₂ ≥150, SpO ₂ /FiO ₂ ≥200	7

	Mechanical ventilation: $pO_2/FiO_2 < 150$, $SpO_2/FiO_2 < 200$ or vasopressors	8
	Mechanical ventilation: $pO_2/FiO_2 < 150$ and vasopressors, dialysis or ECMO	9
Dead	Dead	10

ECMO=extracorporeal membrane oxygenation; FiO_2 =fraction of inspired oxygen; NIV=non-invasive ventilation; pO_2 =partial pressure of oxygen; SpO_2 =oxygen saturation; *If hospitalized for isolation only, record status as for ambulatory patient.

Supplementary Results (SR)

SR1 Identification of immunogenic epitopes and homologies of human PF4 and SARS-CoV-2 spike protein and comparative analysis of their 3D structures

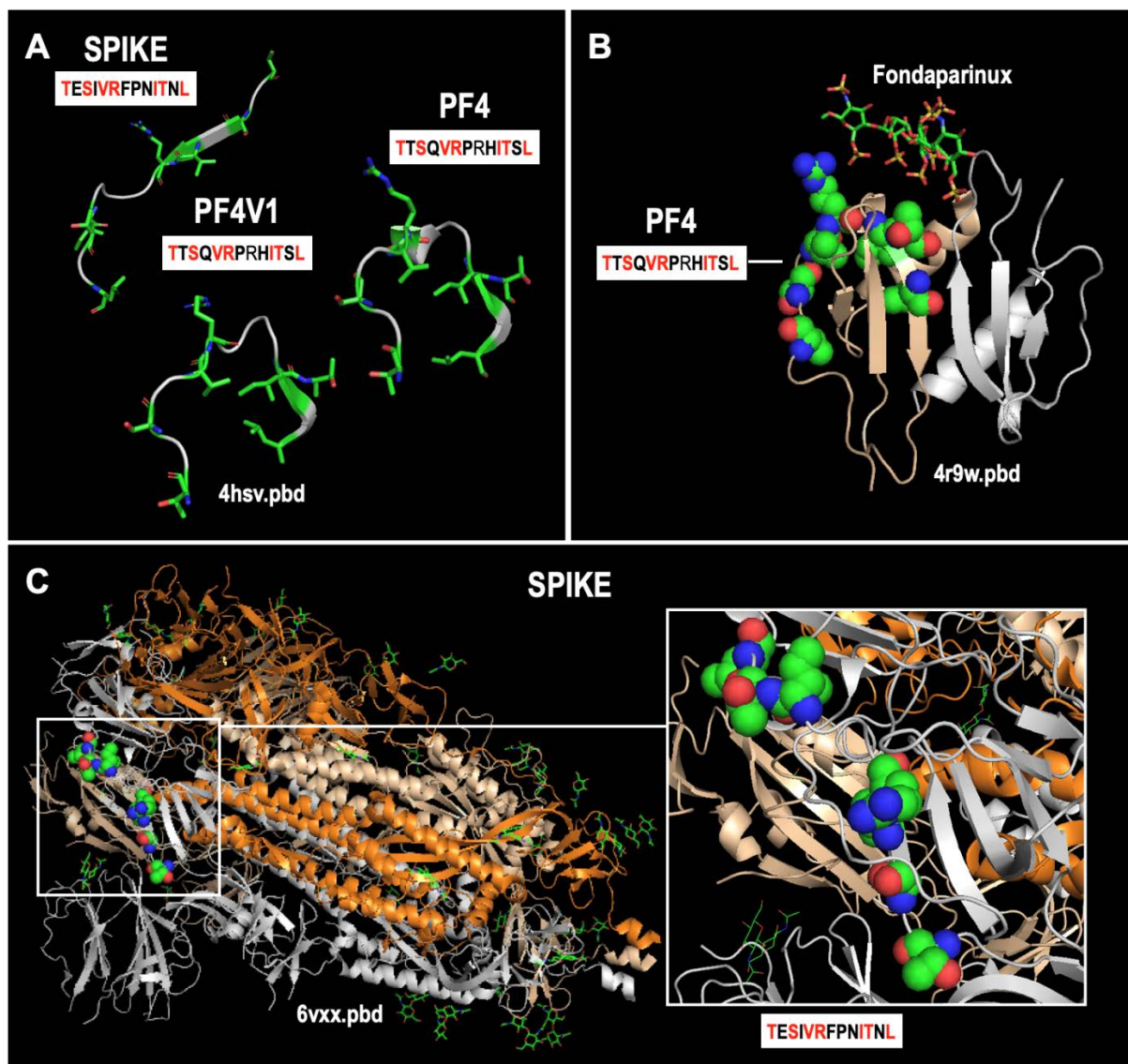
A total of three and 63 potential immunogenic epitopes, respectively, were identified within the 70 amino acid sequence of PF4 (6-21, 23-43 and 49-66) and the 1273 amino acid long sequence of the SARS-CoV-2 spike protein (bold letters in the amino acid sequences, **Supplementary Table SR1A**). The spike protein variants $\Delta H69\Delta V70$, E484K and N501Y (present in B.1.1.7, B.1.351, and P.1, respectively) showed the same immunogenic profile (data not shown).

Both proteins showed sequence homologies between 23.5% and 66.7% (**Supplementary Table SR1B**). Overlapping homologous sequences varied between 5 and 22 residues in length. In addition, sequence identities were manually investigated for identical amino acids that are spaced by 2-3 amino acids to be localized on the same side of a particular motif. Restricting the search to motifs longer than 10 amino acids identified three motifs within the spike protein sequence (145-155, 323-335, and 677-694) that shared a potential immunogenic epitope with PF4. One of them is located in the structure file (6vxx.pdb: 323-335) and displays high similarity to two consecutive epitopes within PF4 (6-21/23-43).

For 3D comparison analysis, we used the following pdb-files: 6vxx.pdb for the trimeric spike protein and 4r9w.pdb for dimeric PF4 bound to fondaparinux. Both the "15-27"- and the

"323-335"-sequences display a β -sheet-flexible loop structure. While the spike epitope resembles a planar configuration, the PF4 structure is more of a pleated sheet (see **Figure S1A**). Of interest, the same motif in PF4 is involved in binding fondaparinux and heparin (see **Figure S1B**). In **Figure S1C**, the surface epitope "323-335" is shown in one subunit of the trimeric SARS-CoV-2 spike protein (left), and the identified epitope is enlarged again in the magnified inset on the right. We assume that part of this epitope is similar in structure and shape surrounding the central Valine-Arginine motif between the spike protein and PF4. Binding of an antibody to this epitope may induce small conformational changes in PF4, similar to what has been observed by heparin binding to PF4. We also performed the structural analysis using the alternative tools <http://sysbio.unl.edu/SVMTriP/>; <https://www.bioinformatics.nl/cgi-bin/emboss/antigenic>; and <http://www.cbs.dtu.dk/services/BepiPred/> , which gave comparable results.

Supplementary Figure S1:



Supplementary Figure S1: Comparison of the 3D-structures of PF4 and SARS-CoV-2 Spike

A. The isolated surface epitopes of Spike (amino acid sequence 323-335: TESTVRFPNITNL) and PF4 and PF4V1 (amino acid sequence 15-27: TTSQVRPRHITSL) are shown in their secondary structures. The red letter are the identical amino acids. All three epitopes are predicted to be immunogenic and reside on the surface of all three protein. The identified linear epitopes share a β -sheet-flexible loop structure that could potentially initiate an unintended cross-reactivity of antibodies, because of their possibility to fold-on-demand.

B. The structure of dimeric PF4 with bound fondaparinux is shown. The identified epitope TTSQVRPRHITSL makes part of the binding pocket for fondaparinux and represents the heparin binding site of PF4.

C. The homologous epitope (amino acid sequence: TESTVRFPNITNL; colored spheres in the 3-dimensional structure of trimeric Spike protein) is shown for one (grey structure) of the three subunits. The highlighted surface epitope of Spike is displayed on the left. As shown

under B and C, the identified linear epitope is present on the surface of both proteins, which make them accessible for potential antibody binding. Any changes in the structure of Spike, like Furin cleavage (at amino acid position 682-685) or conformational changes (amino acids 986/987) is not able to alter the accessibility of this linear Spike epitope.

Supplementary Table SR1A: Amino acid sequence of PF4 and SARS-CoV-2 Spike protein

Protein sequences marked in "grey" are not present in the published protein structures. Protein sequences marked in "bold" were identified as potentially immunogenic peptides sequences. The highest homology between both proteins is depicted in "yellow", and identical amino of that motif acids are marked in "red".

PF4/CXCL4 (motif of interest: aa 15-27 of processed protein)

MSSAAGFCASRPGLLFLGLLLLLPLVVAFAASA | EAEEDGDLQCLCVK**TTSQVRPRHITS**LEVIKAGPHCPTA
QLIATLKNRKRKICLDLQAPLYKKI IKKLLS (x4)

PF4V1/CXCL4L1 (motif of interest: aa 15-27 of processed protein)

MSSAARSRLTRATRQ**EMLFLALLLLPVVAFARA** | EAEEDGDLQCLCVK**TTSQVRPRHITS**LEVIKAGPHC
PTAQLIATLKNRKRKICLDLQALLYKKI I KEHLES

SARS-CoV-2 Spike protein (motif aa 323-335)

MFVFLVLLPLVSSQCVNLTTRTQ**LPP**AYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS
GTNGTKRF**DN**PVLPFNDGVYFAS**TE**KSNIIRGWIFGTTLD**SK**TQ**SL**LIVNNATNVV**IK**VCE**FQ**CND**P**FLG
VYYHKNK**SW**ME**S**FRVYSSANN**CT**FEYVS**Q**PFLMDLE**GK**QGNFKNLREFV**F**KNIDGYFKIY**SK**HTPINLV
RDL**PQ**GFSALEPLVDLPIGINITRFQ**TLL**LALHRSY**LTP**GDSSSGWTAGAAAYYVGYLQ**PRT**FL**LK**YEN**GT**
ITDAVDCALDPLSETK**CT**LK**SFT**VEKGIYQTSNFRV**QP****TESIVRFPNITNL**CP**FGE**VFNATRFASVYAWNR
KRISNCVADYSVL**YNS**AS**SF**STFKCYGV**SPT**KLNDLC**FT**NVYAD**SF**VI**RGE**VRQIAPGQTGKIADYNYKLP
DD**FT**GC**VIA**WNSNNLDSK**VG**GN**Y**NYLYR**LFR**KS**NL**KPFERDI**STE**IYQAG**STP**CNG**VE**GFNCY**FPL**Q**SY**GF
QPT**NG**VG**YQ**PYRVVLS**FEL**LHAPATVCGPKKSTNLVKNKCVNFN**GLT**GT**GV**L**TES**N**KK**FL**PF**Q**Q**FG**RD**
IADTTDAVRDP**Q**T**LE**ILDIT**PCS**FGGVSVITPGTNTSNQ**VAV**LYQ**DVN**CTEV**PVA**IHAD**QLT**P**TWR**VY**STG**
SN**VF**Q**TR**AGCLIGAEHVNN**SY**EC**DI**PIGAGICASYQ**TQ**TNS**PR**RARS**VAS**Q**SII**AY**TMS**LGAEN**SV**AYSNN
SIAIPTN**FT**IS**VT**TEILPV**SMT**K**TS**VDC**TM**YICGD**STE**C**SN**LL**LQ**YGS**FCT**QLNRAL**TGI**AVEQDKNT**Q**EV
FA**QV**K**Q**IYK**TP**PIKDFGGF**NFS**Q**IL**PD**PS**K**PS**KRS**FIE**DL**FN**K**VT**LADAG**FI**K**Y**GD**CL**GD**IA**ARD**LICA**
Q**K**F**NGL**TVLP**PLL**T**DE**MI**AQ**Y**TS**ALL**AG**TITSGWTF**GAGA**L**QIP**FAMQ**MAY**RFNGIG**VTQ**N**VLY**EN**Q**KLI
ANQFNSAIGKI**Q**DSL**S**STASAL**GKL**Q**DVV**NQ**NAQ**ALNTLV**KQL**SS**NFG**AI**SS**V**LN**D**IL**SRLDK**VE**AEV**Q**ID
RLIT**GR**L**Q**SL**Q**TYVT**Q**QLIRAAEIRASANLAAT**KM**SEC**VL**G**Q**SKRVDFCG**KY**H**LMS**FP**Q**SAP**HG**V**FL**HV
TY**VPA**Q**E**K**NFT**T**APA**I**CH**D**GKA**H**F**PRE**GV**F**V**S**NG**TH**W**F**V**T**Q**R**N**F**Y**EP**Q**IIT**TD**NT**F**V**S**GN**CD**V**V**IG**IV**NN**T**
V**YD**PL**Q**PE**LDS**F**KE**ELDKY**F**KN**H**T**SP**D**VD**L**GDI**SGINAS**VV**NI**Q**KEIDRLNE**VAK**N**LN**ES**LID**L**Q**EL**G**K**YE**
Q**Y**IK**WP**W**YI**W**L**GF**IA**GL**IA**IV**M**VT**IM**LC**CM**T**SC**CS**CL**G**CC**SC**G**SC**CK**F**DE**DD**S**EP**VL**K**GV**KL**H**Y**T** (x3)

Supplementary Table SR1B: Identified sequences homologies between PF4 and Spike protein

Identified homologies marked in "grey" are not present in the published protein structures, and thus, could not be analyzed. Bases on the assumption that we were looking for surface epitopes that could potentially be bound by cross-reacting antibodies, all identified motifs not located on the surface of the SRAS-CoV-2 Spike protein were not analyzed.

66.7% identity in 6 residues overlap; Score: 18.0; Gap frequency: 0.0%
 PF4 14 TTSQVR
 Spike 572 TTDAVR
 ** **

60.0% identity in 5 residues overlap; Score: 22.0; Gap frequency: 0.0%
 PF4 9 CLCVK
 Spike 1241 CSCLK
 * * *

53.8% identity in 13 residues overlap; Score: 23.0; Gap frequency: 0.0%
 PF4 14 TTSQVRPRHITSL
 Spike 323 TESIVRFPNITNL
 * * ** ** *

50.0% identity in 8 residues overlap; Score: 21.0; Gap frequency: 0.0%
 PF4 25 SLEVIKAG
 Spike 469 STEIYQAG
 * * **

45.5% identity in 11 residues overlap; Score: 22.0; Gap frequency: 0.0%
 PF4 45 KNGRKICLDLQ
 Spike 1191 KNLNESLIDLQ
 ** ***

45.5% identity in 11 residues overlap; Score: 19.0; Gap frequency: 0.0%
 PF4 59 YKKIIKKLLLES
 Spike 145 YHKNNKSWMES
 * * * **

44.4% identity in 9 residues overlap; Score: 19.0; Gap frequency: 0.0%
 PF4 47 GRKICLDLQ
 Spike 667 GAGICASYQ
 * ** *

44.4% identity in 9 residues overlap; Score: 18.0; Gap frequency: 0.0%
 PF4 61 KIIKKLLES
 Spike 1188 EVAKNLNES
 * * **

42.9% identity in 14 residues overlap; Score: 22.0; Gap frequency: 0.0%
 PF4 5 GDLQCLCVKTTTSQV
 Spike 999 GRLQSLQTYVTQQL
 * ** * * *

36.4% identity in 11 residues overlap; Score: 20.0; Gap frequency: 0.0%
 PF4 36 PTAQLIATLKN
 Spike 295 PLSETKCTLKS
 * ***

36.4% identity in 11 residues overlap; Score: 19.0; Gap frequency: 0.0%
 PF4 37 TAQLIATLKNG
 Spike 875 SALLAGTITSG
 * * * *

35.3% identity in 17 residues overlap; Score: 26.0; Gap frequency: 0.0%
 PF4 13 KTTSQVRPRHITSLEVI
 Spike 677 QTNSPRRARSVASQSII
 * * * * * *

35.3% identity in 17 residues overlap; Score: 22.0; Gap frequency: 0.0%

PF4 27 EVIKAGPHCPTAQLIAT
 Spike 1011 QLIRAAEIRASANLAAT
 * * * * *

33.3% identity in 18 residues overlap; Score: 32.0; Gap frequency: 0.0%

PF4 8 QCLCVKTTTSQVRPRHITS
 Spike 14 QCVNLTTRTQLPPAYTNS
 ** * * * *

33.3% identity in 9 residues overlap; Score: 20.0; Gap frequency: 0.0%

PF4 11 CVKTTTSQVR
 Spike 15 CVNLTTRTQ
 ** *

33.3% identity in 18 residues overlap; Score: 19.0; Gap frequency: 0.0%

PF4 29 IKAGPHCPTAQLIATLKN
 Spike 624 IHADQLTPTWRVYSTGSN
 * * ** * *

33.3% identity in 9 residues overlap; Score: 18.0; Gap frequency: 0.0%

PF4 25 SLEVIKAGP
 Spike 581 TLEILDITP
 ** *

31.8% identity in 22 residues overlap; Score: 22.0; Gap frequency: 0.0%

PF4 46 NGRKICLDLQAPLYKKI IKKLL
 Spike 801 NFSQILPDPSKPSKRSFIEDLL
 * * * * * **

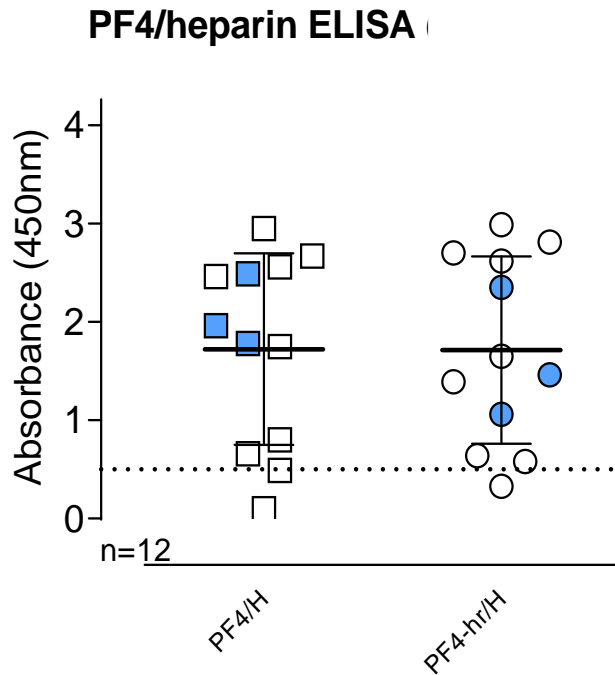
30.0% identity in 10 residues overlap; Score: 19.0; Gap frequency: 0.0%

PF4 53 DLQAPLYKKI
 Spike 53 DLFLPFFSNV
 ** *

23.5% identity in 17 residues overlap; Score: 24.0; Gap frequency: 0.0%

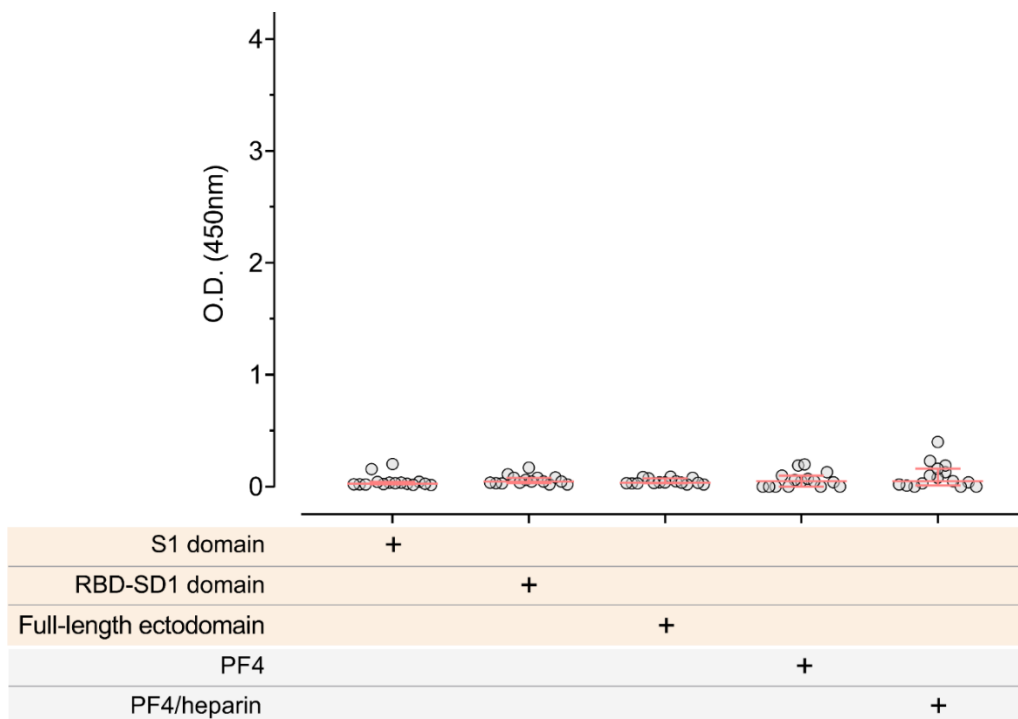
PF4 12 VKTTTSQVRPRHITSLEV
 Spike 720 ISVTTEILPVSMTKTSV
 * * * *

Supplementary Figure S2



Supplementary Figure S2: Comparison of 9 VITT sera (open symbols) in the PF4/heparin ELISA using human platelet derived PF4 and recombinant PF4. The blue closed symbols are three sera of patients with heparin induced thrombocytopenia used for control. (PF4=human platelet derived PF4, PF4-hr=human recombinant PF4)

Supplementary Figure S3



Supplementary Figure S3: Results of control sera (n=15) tested in the S1 domain, RBD-SD1 domain, full-length ectodomain of the spike protein, PF4 and PF4/heparin ELISAs.

References for supplementary materials

1. Newman PM, Swanson RL, Chong BH. Heparin-induced thrombocytopenia: IgG binding to PF4-heparin complexes in the fluid phase and cross-reactivity with low molecular weight heparin and heparinoid. *Thromb Haemost.* 1998;80(2):292-297
2. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis.* 2020;20(8):e192-e197
3. Winter T, Friedrich N, Lamp S, et al. The Integrated Research Biobank of the University Medicine Greifswald. *Open Journal of Bioresources.* 2020;7