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Supplemental information

Soluble ACE2 correlates with severe COVID-19 and can impair antibody responses

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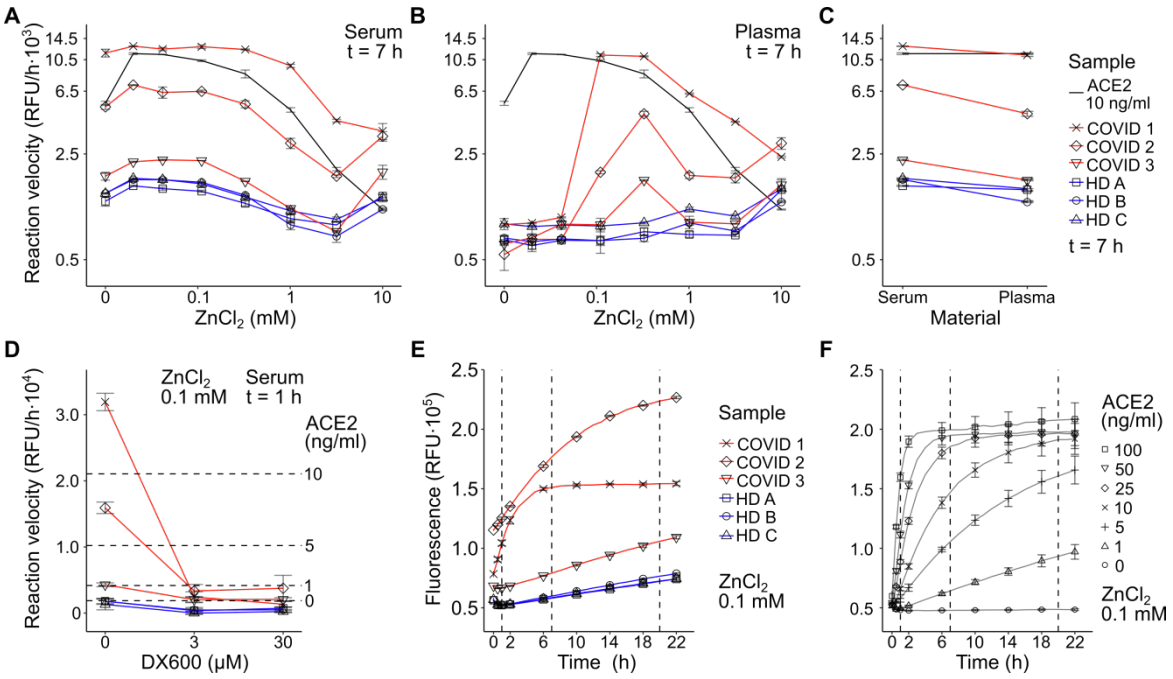

Figure S1. Optimized measurement of ACE2 enzymatic activity in blood samples. Related to Figure 1. Fluorescence release after cleavage of the quenched compound Mca-APK(Dnp) in concurrent **(A)** serum and **(B)** plasma samples of COVID-19 patients (#1-3; red, from heparin-tubes) and healthy donors (HD-A-C; blue; from EDTA-tubes). Initial cleavage velocity (relative fluorescence units (RFU) over time) in presence of increasing concentrations of zinc chloride. **(C)** Maximum reaction velocity detected in serum and plasma at respective zinc optimums. **(D)** Serum cleavage activity in absence or presence (3, 30 mM) of the ACE2 inhibitor DX600. **(E)** Cleavage kinetics of serum samples and **(F)** recombinant ACE2 at indicated concentrations. Dashed lines at 1h, 7h, or 20h indicate optimal time points that were used for consecutive analysis. Measurements were performed in triplicates, mean +/- SD is shown.

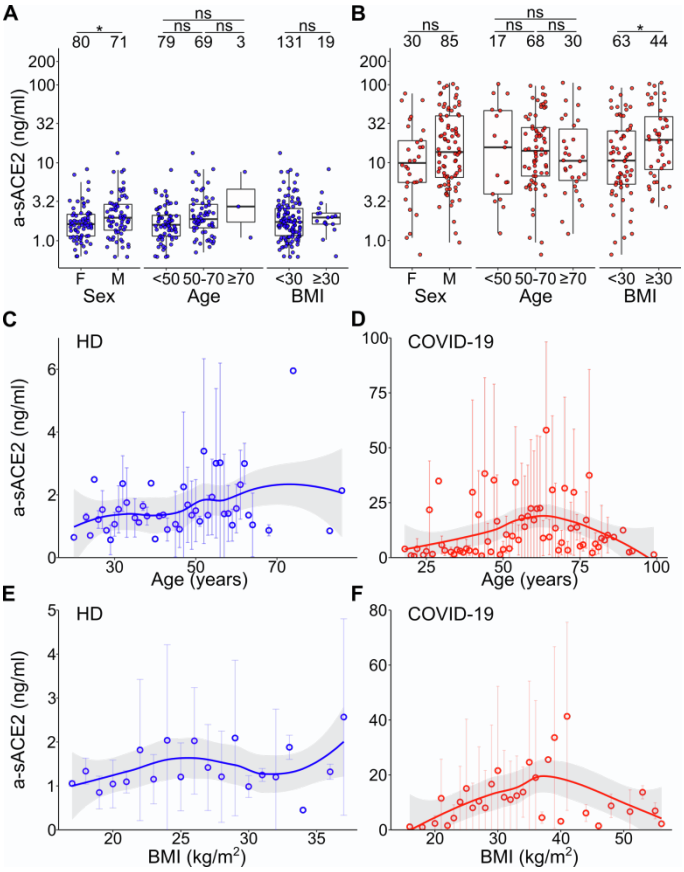

Figure S2. Serum levels of a-sACE2 by age and by gender. Related to Figure 1. (A) Shown is the average a-sACE2 concentration for all tested healthy donors (blue) and **(B)** severe COVID-19 patients (red) by sex, age, and BMI donor groups. **(C-F)** Distribution of a-sACE2 serum concentrations for different age and BMI groups in healthy donors and all COVID-19 patients. Boxplots depict median +/- interquartile range. Whisker length is 1.5 interquartile ranges.

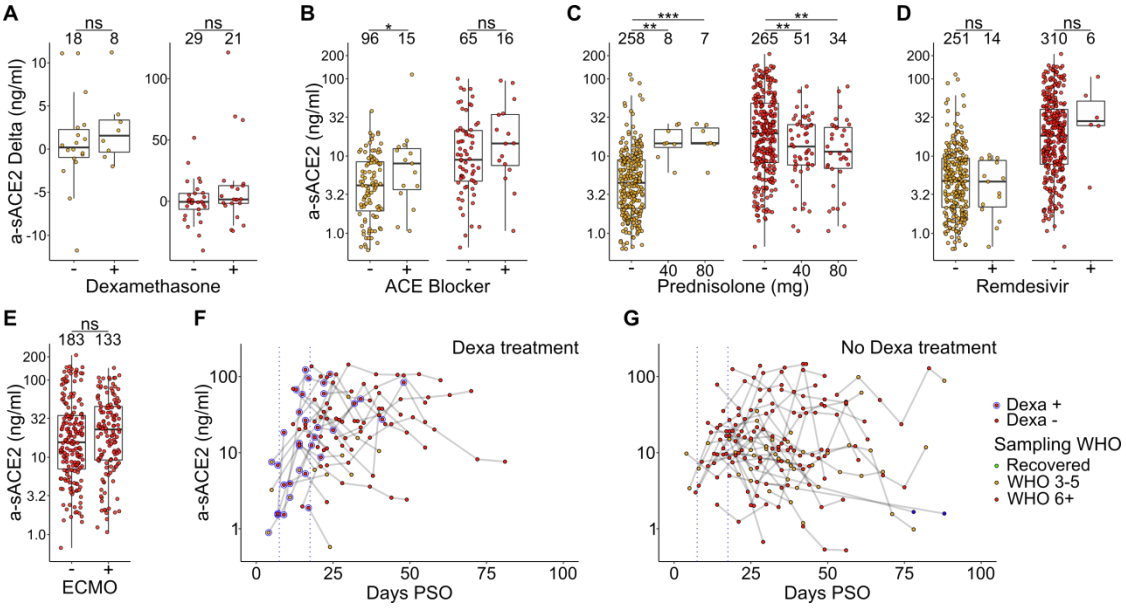

Figure S3. Medical treatment and a-sACE2 serum levels. Related to Figure 1. (A) Relative change (Delta) in a-sACE2 pre- and post-dexamethasone (Dexa) treatment compared to samples taken at respective time points post symptom onset of non-treated donors. Samples were collected during moderate (yellow) and severe (red) disease **(B)** Serum a-sACE2 concentrations of patients with or without ACE-blocker treatment. **(C)** Serum a-sACE2 in blood samples from patients without or with 40 and 80 mg prednisolone, **(D)** remdesivir, and **(E)** ECMO treatment. **(F)** Serum a-sACE2 of blood samples from patients with and **(G)** without dexamethasone (Dexa) treatment. Samples were taken at distinct time points post symptom onset (PSO). Samplings during Dexa administration are marked in blue. Samples were obtained from patients with moderate (WHO3-5, yellow) or severe (WHO6+, red) COVID-19. The mean of a-sACE2 was calculated from technical duplicates and distinct time points for longitudinal samples. Median +/- interquartile range is indicated for each population. Whisker length is 1.5 interquartile ranges. Statistical analyses by two-sided Mann–Whitney test (***p<0.001, **p < 0.01, and *p < 0.05; ns, not significant).

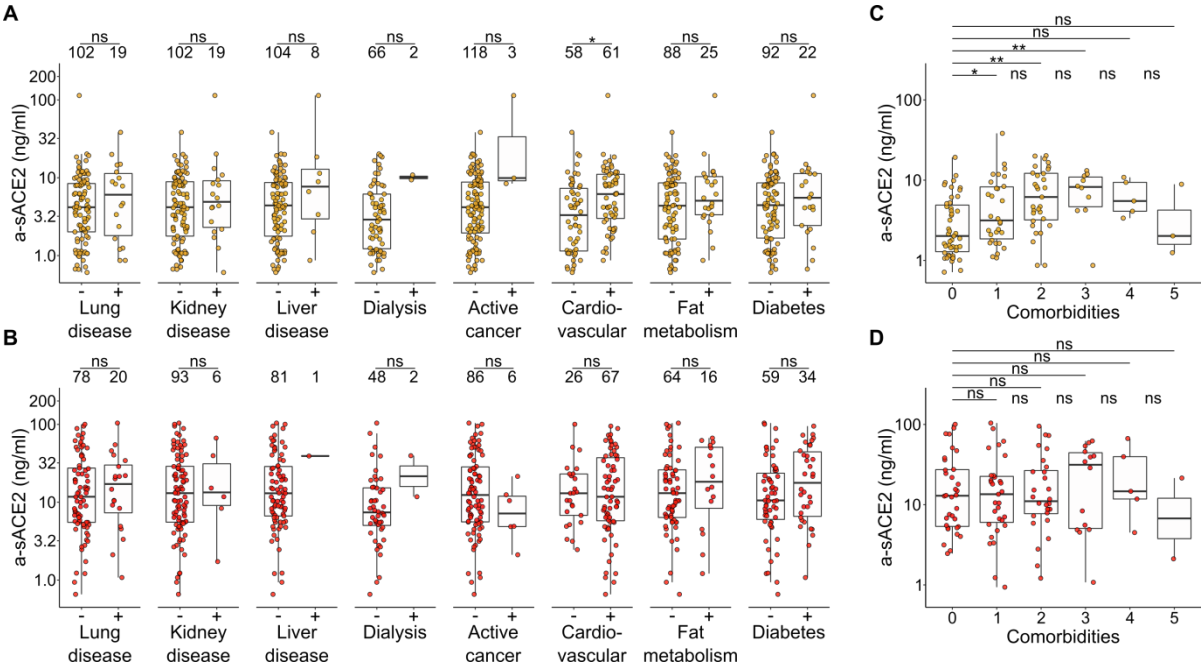

Figure S4. Serum a-sACE2 in COVID-19 patients with distinct comorbidities. Related to Figure 1. Mean serum a-sACE2 of patients with moderate (WHO3-5, yellow) and severe COVID-19 (WHO6+, red). The type **(A-B)**, or number **(C-D)**, of comorbidities is indicated. The mean of a-sACE2 was calculated from technical duplicates and distinct time points for longitudinal samples. Median +/- interquartile range is indicated for each population. Whisker length is 1.5 interquartile ranges. Statistical analyses by two-sided Mann–Whitney test (**p < 0.01 and *p < 0.05; ns, not significant).

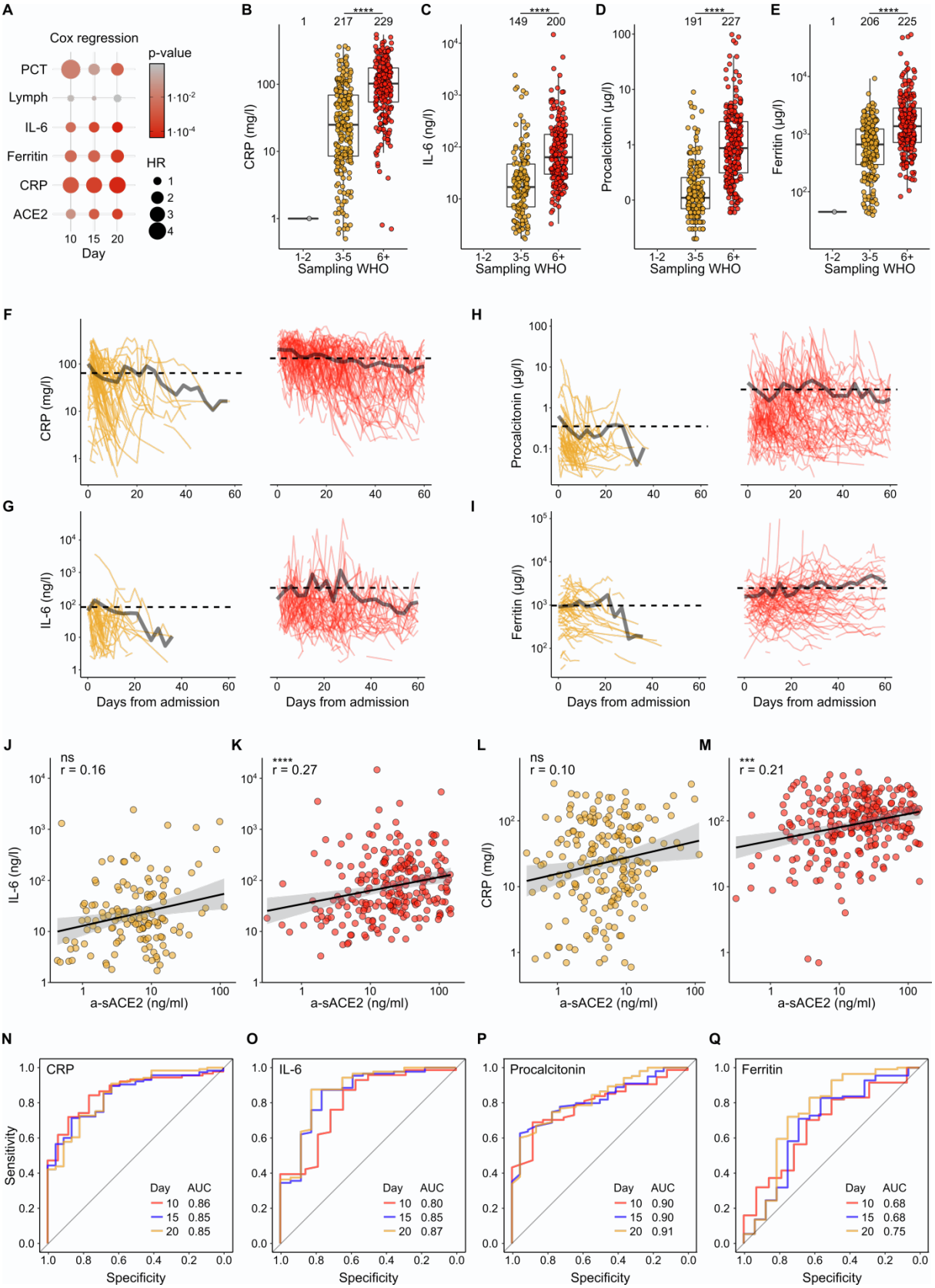

Figure S5. Clinical parameters and survival. **Related to Figure 1. (A)** Summary plot of Cox regression analysis showing p-values and hazard ratios (HR) of indicated parameters. The survival probability over days after admission is based on $\log_2$ maximum marker-

concentrations of all COVID-19 patients within 10, 15, or 20 days post admission. Gender, dexamethasone, cardiovascular disease, and lipid metabolism disorder were used as covariates. **(B)** Levels of C-reactive protein (CRP), **(C)** IL-6, **(D)** procalcitonin, and **(E)** ferritin in blood samples obtained during mild (WHO1-2, grey), moderate (WHO3-5, yellow), and severe COVID-19 disease (WHO6+, red). Median +/- interquartile range is indicated for each population. Whisker length is 1.5 interquartile ranges. Statistical analyses by two-sided Mann–Whitney test (****$p < 0.0001$, ***$p < 0.001$). **(F-I)**, Indicated parameters were measured in serum at designated days after patient admission to hospital. A dashed line indicates an average level over two months from admission. The trend line denotes the average biomarker level every 3 days. **(J-M)** Spearman Correlation of a-sACE2 and IL6 or CRP performed with a 95% confidence interval. **(N)** Receiver operating characteristic (ROC) curve analysis using CRP, **(O)** IL-6, **(P)** procalcitonin, and **(Q)** ferritin levels for predicting death. CRP yielded an area under the curve (AUC) of 0.85 (95% CI: 0.77-0.92), IL-6: 0.85 (95% CI: 0.75-0.96); procalcitonin: 0.82 (0.73-0.90); ferritin: 0.68 (0.52-0.84). For all clinical parameters, the maximum marker-concentration within 10 (red), 15 (blue) and 20 (yellow) days post admission was used. AUC, area under the curve. CI, confidence interval**.**

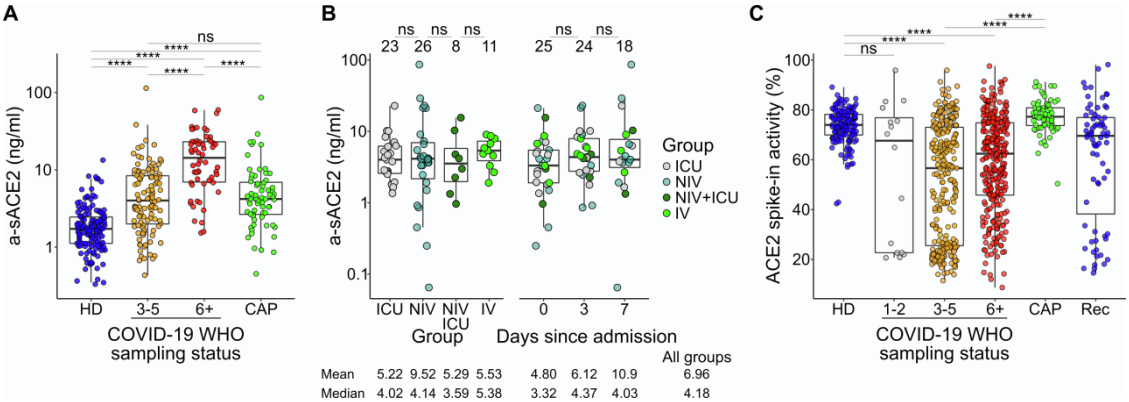

Figure S6. Levels of a-sACE2 in COVID-19 versus patients with SARS-CoV-2 independent pneumonia. **Related to Figure 1. (A)** Concentration of a-sACE2 serum samples in healthy donors (HD, blue), moderate (WHO3-5, yellow), severe (WHO6+, red) COVID-19 patients, and patients with non-COVID lung disease obtained from the Community-Acquired Pneumonia Network (CAP, green). Samples were obtained up to 7 days post admission. **(B)** ACE2 measurements of patients with SARS-COV-2 independent pneumonia cases ordered by the treatment applied (ICU = intensive care unit, NIV = none-invasive ventilation, IV = intubation) and days post admission. **(C)** Percent ACE2 activity of 50 ng/ml recombinant ACE2 after addition to serum (spike-in activity in %). The analysis included samples obtained from recovered COVID-19 patients (Rec, blue). Median +/- interquartile range is indicated for each population. Whisker length is 1.5 interquartile ranges. Statistical analyses by two-sided Mann–Whitney test (****p < 0.0001, ns > 0.05).

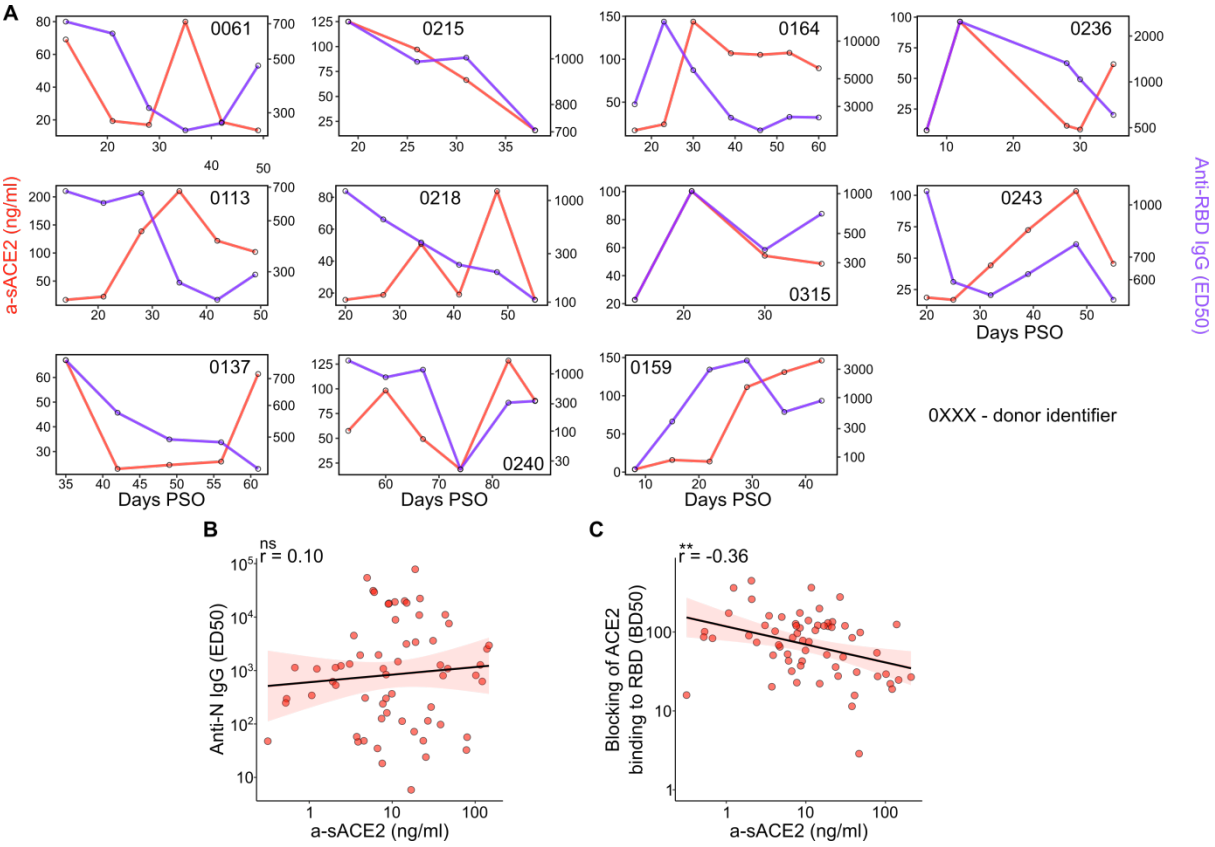

Figure S7. Longitudinal analysis of serum levels of a-sACE2 and RBD-specific IgG in COVID-19 patients. Related to Figure 1 and Figure 3. (A) Shown are the concentrations of a-sACE2 (left y-axis) and 50% of maximum IgG binding to RBD (ED50, right y-axis) for individual patients followed over distinct time points post-symptom onset (PSO). **(B)** Reciprocal serum dilution to achieve 50% of maximal IgG binding (ED50) to nucleocapsid (N) and **(C)** 50% of maximum blocking of ACE2 binding to RBD (BD50) is plotted against a-sACE2 for N = 65 severe COVID-19 patients. Spearman correlation performed with a 95% confidence interval. **(C)** represents the subset of data depicted in Figure 3D, for which anti-N titer was measured.

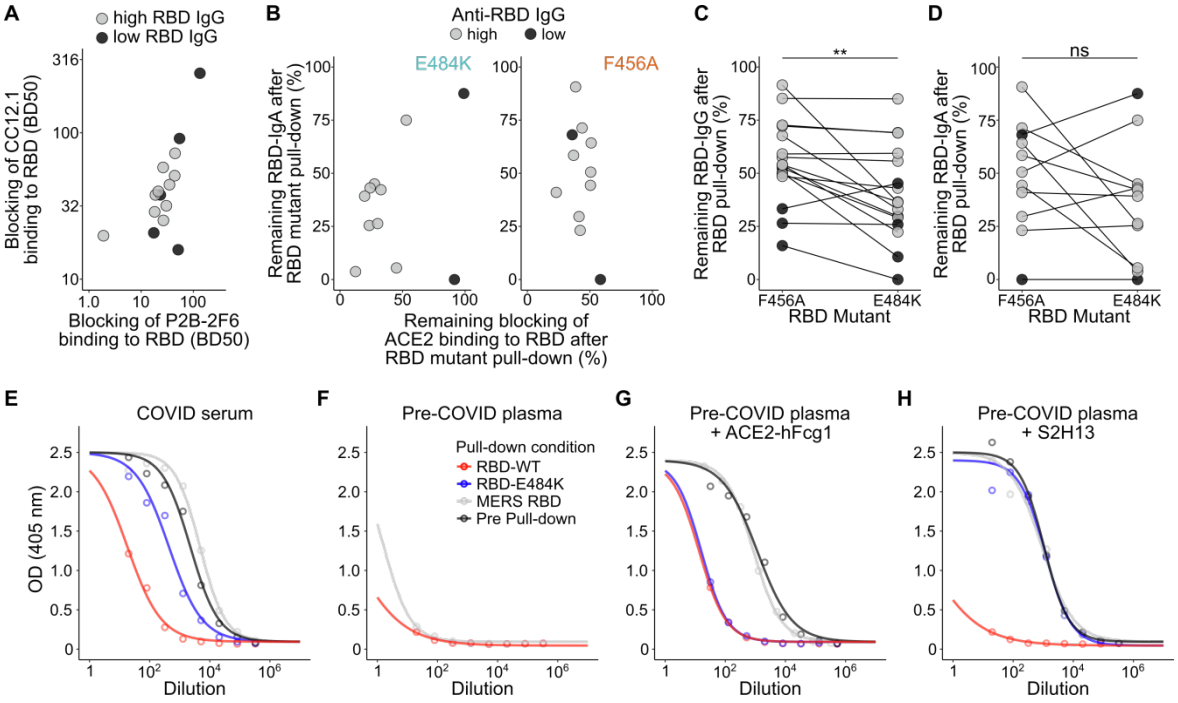

Figure S8. Serum depletion by SARS-CoV-2 RBD. Related to Figure 3. (A) Sera of patients with RBD high versus low antibody titers were depleted by a pull-down with WT and mutant SARS-CoV-2 RBDs, and MERS-CoV as control. Binding of remaining IgA to RBD and ACE2 blocking-of-binding is depicted in % after depletion with RBD-E484K and **(B)** RBD-F456A. **(C)** Binding of remaining IgG and **(D)** IgA to RBD after pull-down with RBD-E484K and RBD-F456A. Statistical analyses by paired Mann-Whitney test (**p < 0.01 and ns, not significant). **(E)** Representative serum depletion of a COVID-19 patient, **(F)** a pre-COVID collected healthy donor plasma, **(G)** an ACE2-hFcg1 spike-in of 2 µg/ml in pre-COVID plasma, **(H)** a spike-in of 2 µg/ml S2H13 class-II nAb (incapable of binding to RBD-E484K) into pre-COVID plasma. Optical densities of reciprocal serum dilutions indicate residual anti-RBD IgG after pull down.

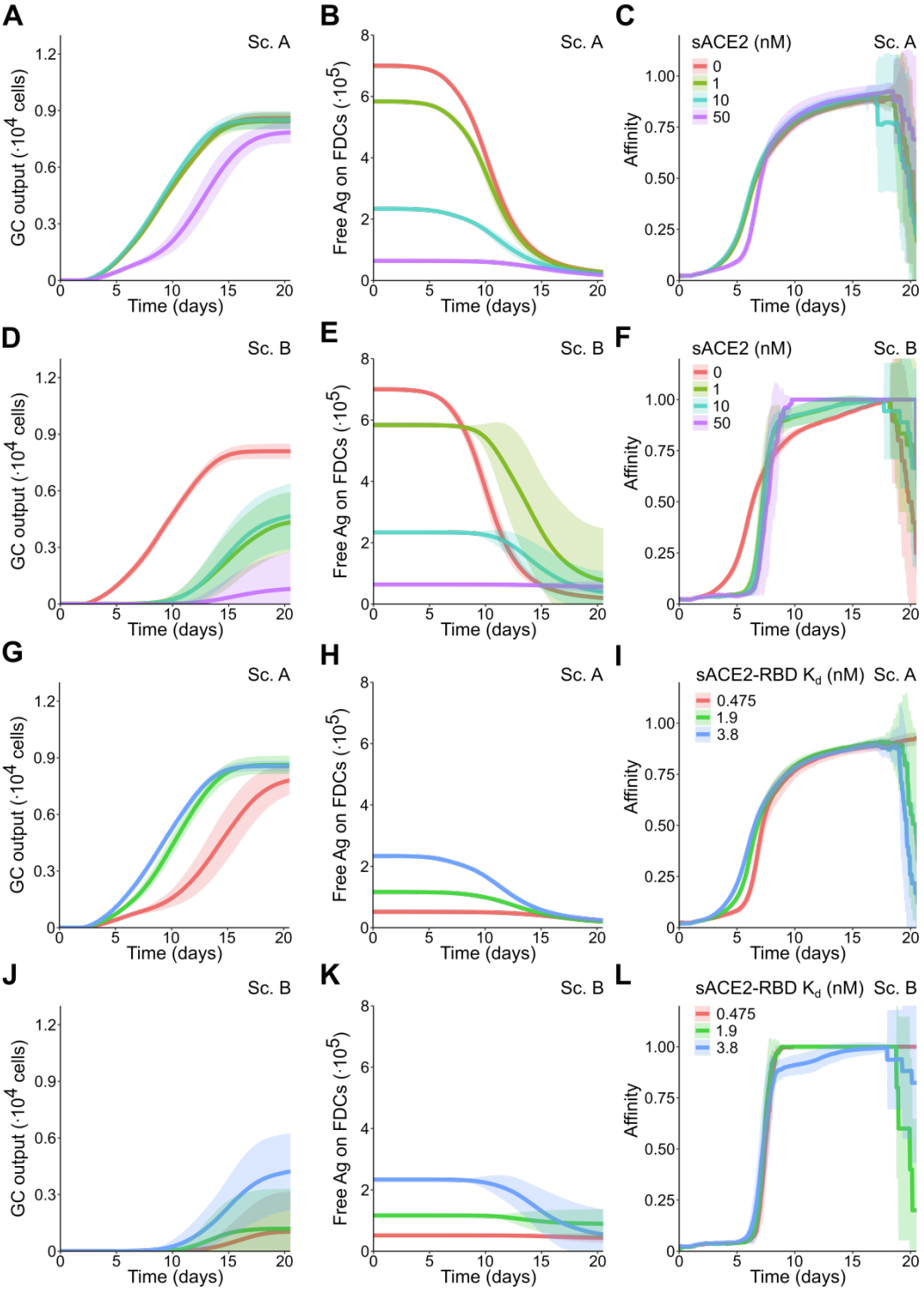

Figure S9. Soluble ACE2 affects the germinal center function in silico. Related to Figure 4. Affinity of GC B cells, free antigen on FDC and GC output are plotted for varying sACE2 concentrations in **(A-C)** epitope masking only, **(D-F)** epitope masking with mean field Ab-B cell competition and for varying sACE2 affinities in **(G-I)** epitope masking only and in **(J-L)** epitope masking with mean field Ab-B cell competition.

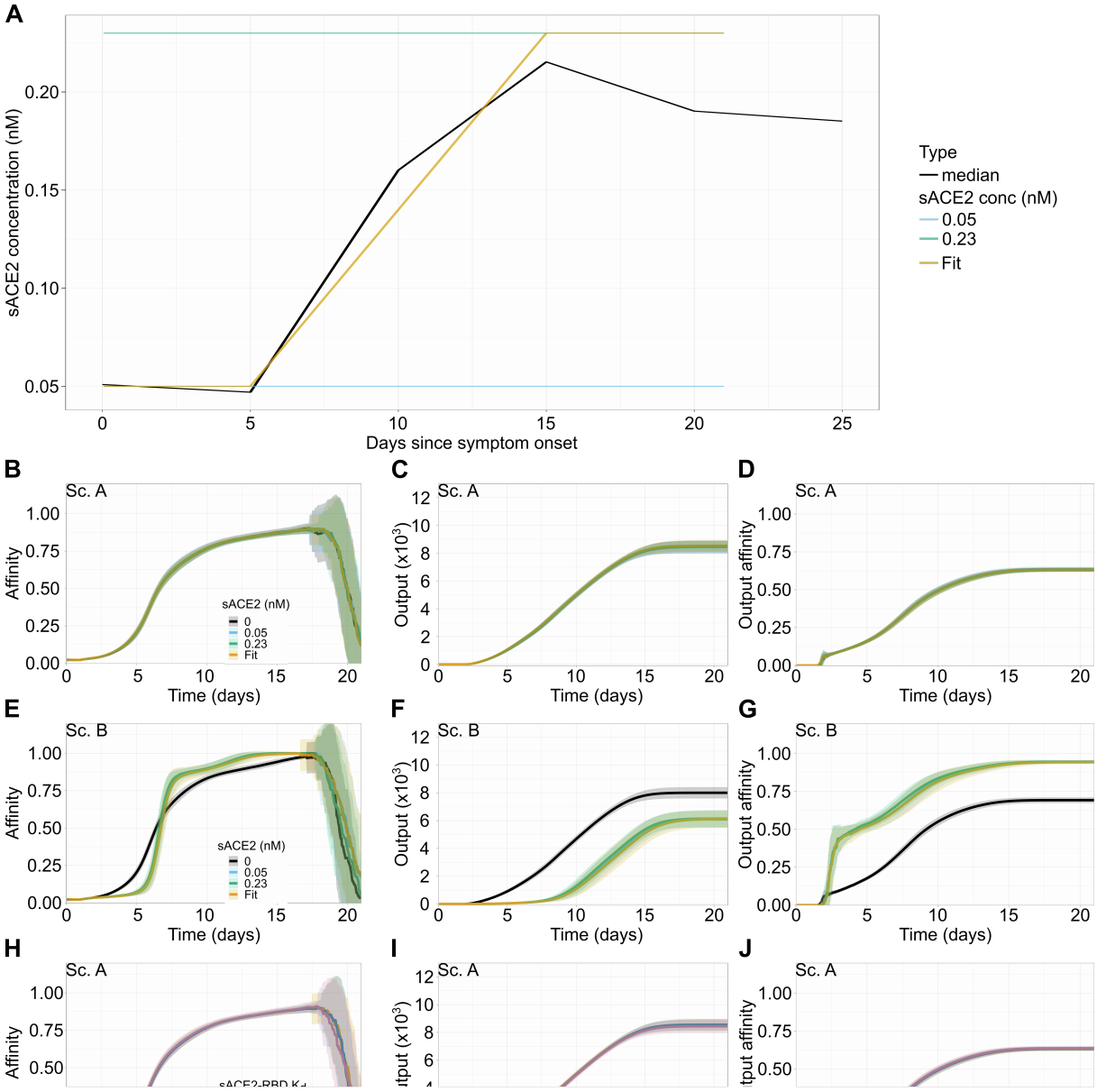

Figure S10. In silico germinal center function is affected by physiological concentrations of sACE2. Related to Figure 4. (A) Trend of median sACE2 concentration observed in severe COVID-19 patients. Affinity of GC B cells, GC output, and output Ab affinity are plotted for varying sACE2 concentrations in **(B-D)** epitope masking only, **(E-G)** epitope

masking with mean field Ab-B cell competition and for varying sACE2 affinities in **(H-J)** epitope masking only and in **(K-M)** epitope masking with mean field Ab-B cell competition.

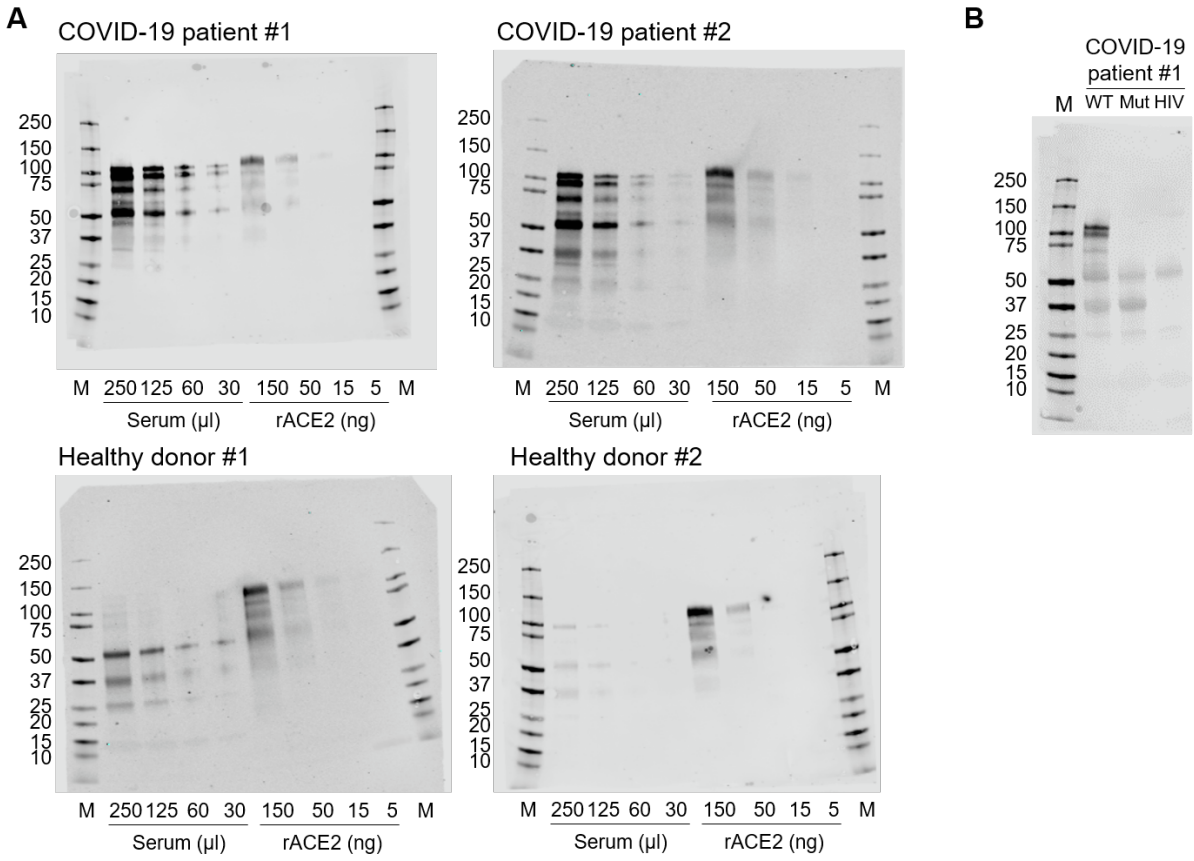

Figure S11. Gel source data for ACE2 pull-down. Related to Figure 2. (A) ACE2 was pulled down with the magnetic beads coupled to SARS-CoV-2 RBD from the indicated volume of COVID-19 or healthy donor sera. Recombinant ACE2 (rACE2) was used as a control. **(B)** ACE2 was pulled down from 100 µl COVID-19 patient #1 serum with the magnetic beads coupled to SARS-CoV-2 RBD wild-type (WT), A475R/G496R mutant (Mut), and HIV gp140 (HIV). Marker (M) size is in kDa.