Persistent low-grade inflammation, SARS-CoV-2 infection and mental health deterioration signs impact on systemic levels of aminoacid neurotransmitter precursors

Manuscript

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## Data and code availability

Anonymized patient data will be made available upon request to the corresponding author. The INCOV cohort data are publicly available at <https://data.mendeley.com/datasets/96v329bg7g/1>. The analysis pipeline code is available at <https://github.com/PiotrTymoszuk/simmun_revision>.

# Tables

Table 1: Characteristic of the local SIMMUN cohort. Numeric variables are presented as medians with interquartile ranges. Categorical variables are presented as percentages and counts within complete observation sets.

| **Variablea** | **Uninfected** | **SARS-CoV-2** | **Test** | **Significanceb** | **Effect size** |
| --- | --- | --- | --- | --- | --- |
| Participants, n | 101 | 64 |  |  |  |
| Sex | female: 64% (65) male: 36% (36) | female: 58% (37) male: 42% (27) | χ² | ns (p = 0.54) | V = 0.066 |
| Age, years | 50 [IQR: 35 - 58] range: 18 - 69 | 50 [IQR: 32 - 56] range: 20 - 68 | Mann-Whitney | ns (p = 0.45) | r = 0.069 |
| Body mass classc | normal: 53% (54) overweight: 32% (32) obesity: 15% (15) | normal: 53% (34) overweight: 27% (17) obesity: 20% (13) | χ² | ns (p = 0.6) | V = 0.079 |
| Somatic comorbidity | 54% (55) | 45% (29) | χ² | ns (p = 0.43) | V = 0.089 |
| Psychiatric comorbidity | 50% (50) | 28% (18) | χ² | p = 0.021 | V = 0.21 |
| HADS anxiety score | < 8: 57% (58) ≥ 8: 43% (43) | < 8: 80% (51) ≥ 8: 20% (13) | χ² | p = 0.013 | V = 0.23 |
| HADS depression score | < 8: 69% (70) ≥ 8: 31% (31) | < 8: 84% (54) ≥ 8: 16% (10) | χ² | ns (p = 0.078) | V = 0.17 |
| Depression or anxiety signs, HADS ≥ 8 | 45% (45) | 20% (13) | χ² | p = 0.0078 | V = 0.25 |
| PSS-4 stress score | 6 [IQR: 3 - 9] range: 0 - 14 | 5 [IQR: 3 - 8] range: 1 - 13 | Mann-Whitney | ns (p = 0.33) | r = 0.095 |
| anti-RBD SARS-CoV-2, IgG, AU | 0.31 [IQR: 0.28 - 0.34] range: 0.21 - 0.99 | 16 [IQR: 14 - 17] range: 0.42 - 20 | Mann-Whitney | p < 0.001 | r = 0.84 |
| COVID-19 severity |  | ambulatory: 73% (47) hospitalized: 27% (17) |  |  |  |
| aHADS: hospital anxiety and depression scale; PSS-4: perceived stress scale, 4 item; RBD: receptor binding domain; IgG: immunoglobulin gamma; AU: arbitrary urnits. | | | | | |
| bcorrected for multiple testing with the false discovery rate method. | | | | | |
| coverweight: body mass index (BMI) 25 - 30 kg/m²; obesity: BMI > 30 kg/m² | | | | | |

Table 2: Characteristic of the external INCOV cohort. Numeric variables are presented as medians with interquartile ranges. Categorical variables are presented as percentages and counts within complete observation sets.

| **Variable** | **Uninfected** | **SARS-CoV-2** | **Test** | **Significance** | **Effect size** |
| --- | --- | --- | --- | --- | --- |
| Participants, n | 27 | 140 |  |  |  |
| Sex | female: 56% (15) male: 44% (12) | female: 41% (58) male: 59% (82) | χ² | ns (p = 0.25) | V = 0.1 |
| Age, yearsa | 56 [IQR: 49 - 60] range: 32 - 70 | 62 [IQR: 48 - 73] range: 26 - 89 | Mann-Whitney | p = 0.044 | r = 0.17 |
| Body mass class | normal: 44% (12) overweight: 22% (6) obesity: 33% (9) | normal: 26% (36) overweight: 36% (51) obesity: 38% (53) | χ² | ns (p = 0.17) | V = 0.16 |
| Ethnicsb | Asian: 0% (0) Black or African-American: 12% (3) White: 88% (21) Other: 0% (0) | Asian: 16% (22) Black or African-American: 9.3% (13) White: 46% (65) Other: 29% (40) | χ² | p = 0.0013 | V = 0.33 |
| COVID-19 severity |  | mild: 2.9% (4) moderate: 59% (83) severe: 25% (35) critical: 13% (18) |  |  |  |
| aoverweight: body mass index (BMI) 25 - 30 kg/mm², obesity: BMI > 30 kg/mm² | | | | | |
| bWHO ordinal scale for clinical improvement; mild: 1 - 2, moderate: 3 - 4, severe: 5 - 6, critical: 7 | | | | | |

# Figures

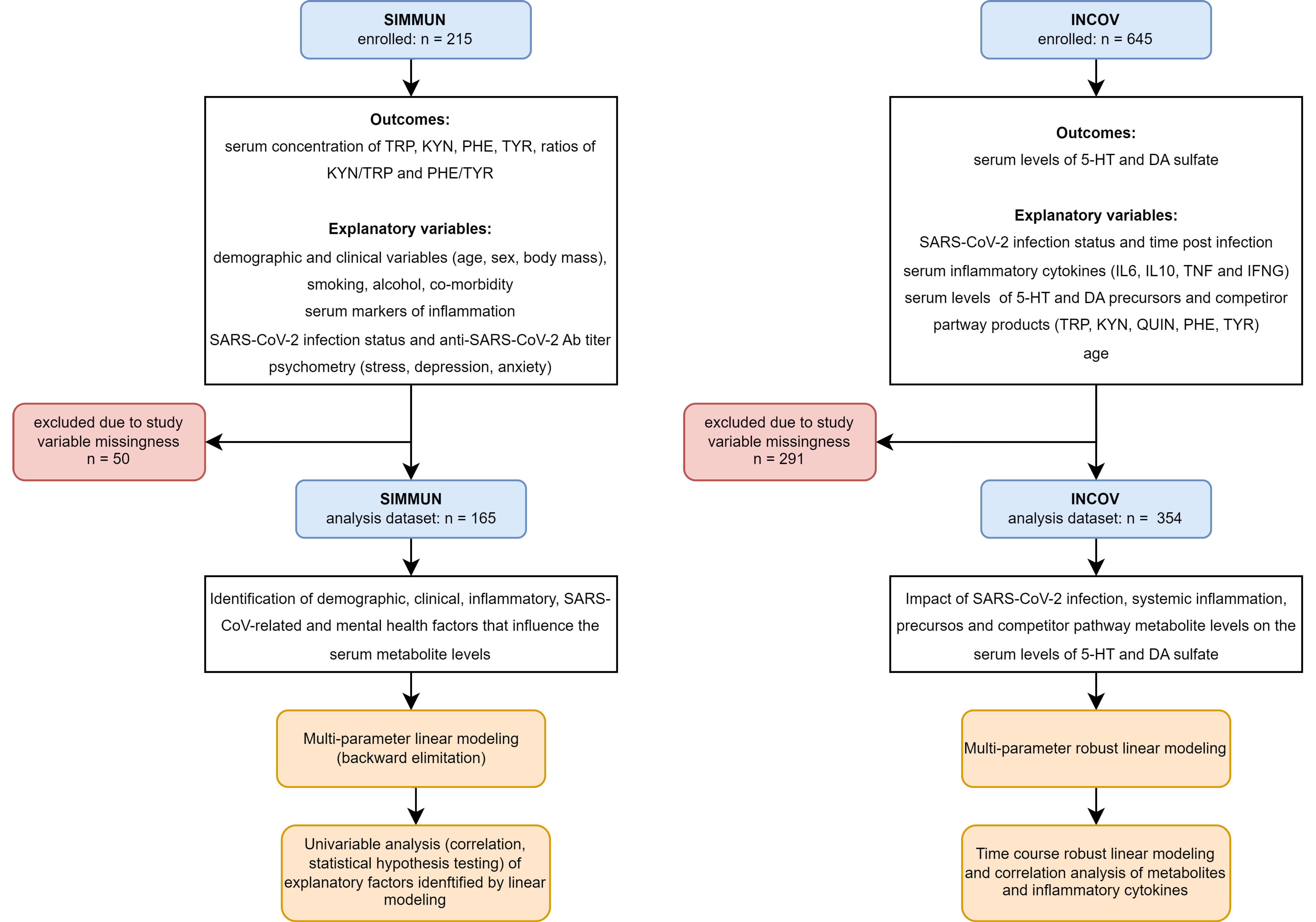


Figure 1: Inclusion scheme for the SIMMUN and INCOV cohorts, and analysis strategy.

**Figure 1. Inclusion scheme for the SIMMUN and INCOV cohorts, and analysis strategy.**

*TRP: tryptophan; KYN: kynurenine; PHE: phenylalanine; TYR: tyrosine; KYN/TRP: kynurenine - tryptophan ratio; PHE/TYR: phenylalanine - tyrosine ratio; QUIN: quinolinate; 5-HT: serotonin; DA: dopamine; Ab: antibody; IL6: interleukin-6; IL10: interleukin-10; TNF: tumor-necrosis factor alpha; IFNG: interferon gamma.*

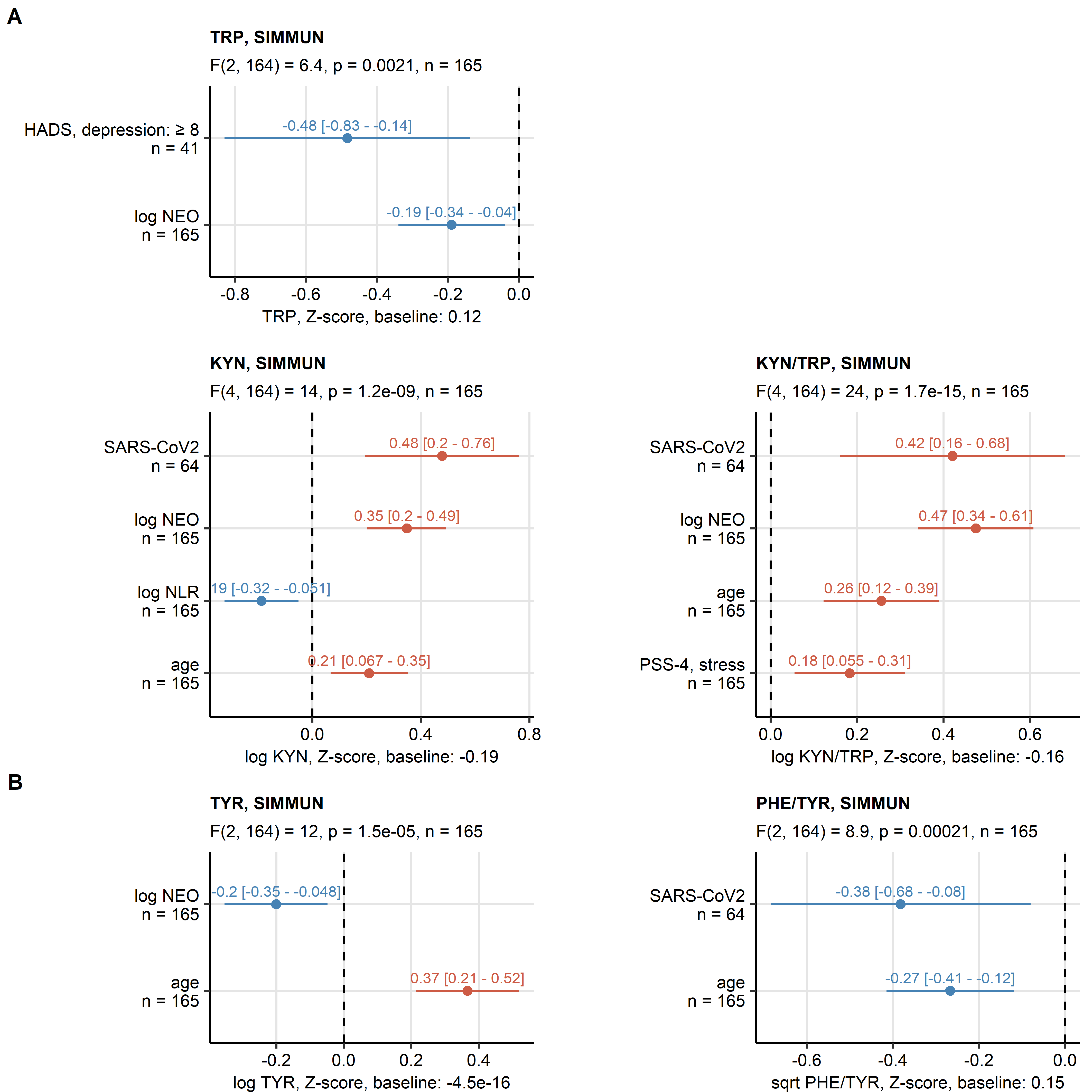


Figure 2: Results of multi-parameter linear modeling of tryptophan, kynurenine, kynurenine/tryptophan ratio, tyrosine and phenylalanine/tyrosine ratio in the SIMMUN cohort.

**Figure 2. Results of multi-parameter linear modeling of tryptophan, kynurenine, kynurenine/tryptophan ratio, tyrosine and phenylalanine/tyrosine ratio in the SIMMUN cohort.**

*Effects of systemic inflammation markers (neopterin [NEO], neutrophil-lymphocyte ratio [NLR]), SARS-CoV-2 infection status, titre of immunoglobulin gamma against the receptor binding domain of the S1 SARS-CoV-2 protein (anti-RBD IgG), scores of anxiety, depression (hospital depression and anxiety scale [HADS]) and mental stress (perceived stress scale, 4 item [PSS4]), age and sex was investigated by multi-parameter linear regression with backward elimination of non-significant terms. Overall model validity was assessed by likelihood-ratio test (LRT). Significant model coefficient estimates with 95% confidence intervals are presented in Forest plots. LRT results (F values, degrees of freedom and p values) and total numbers of complete observations are indicated in the plot captions. Model intercepts (baseline) are indicated in the X axis titles.*

*TRP: tryptophan; KYN: kynurenin; KYN/TRP: kynurenine - tryptophan ratio; PHE: phenylalanine; TYR: tyrosine; PHE/TYR: phenylalanine - tyrosine ratio.*

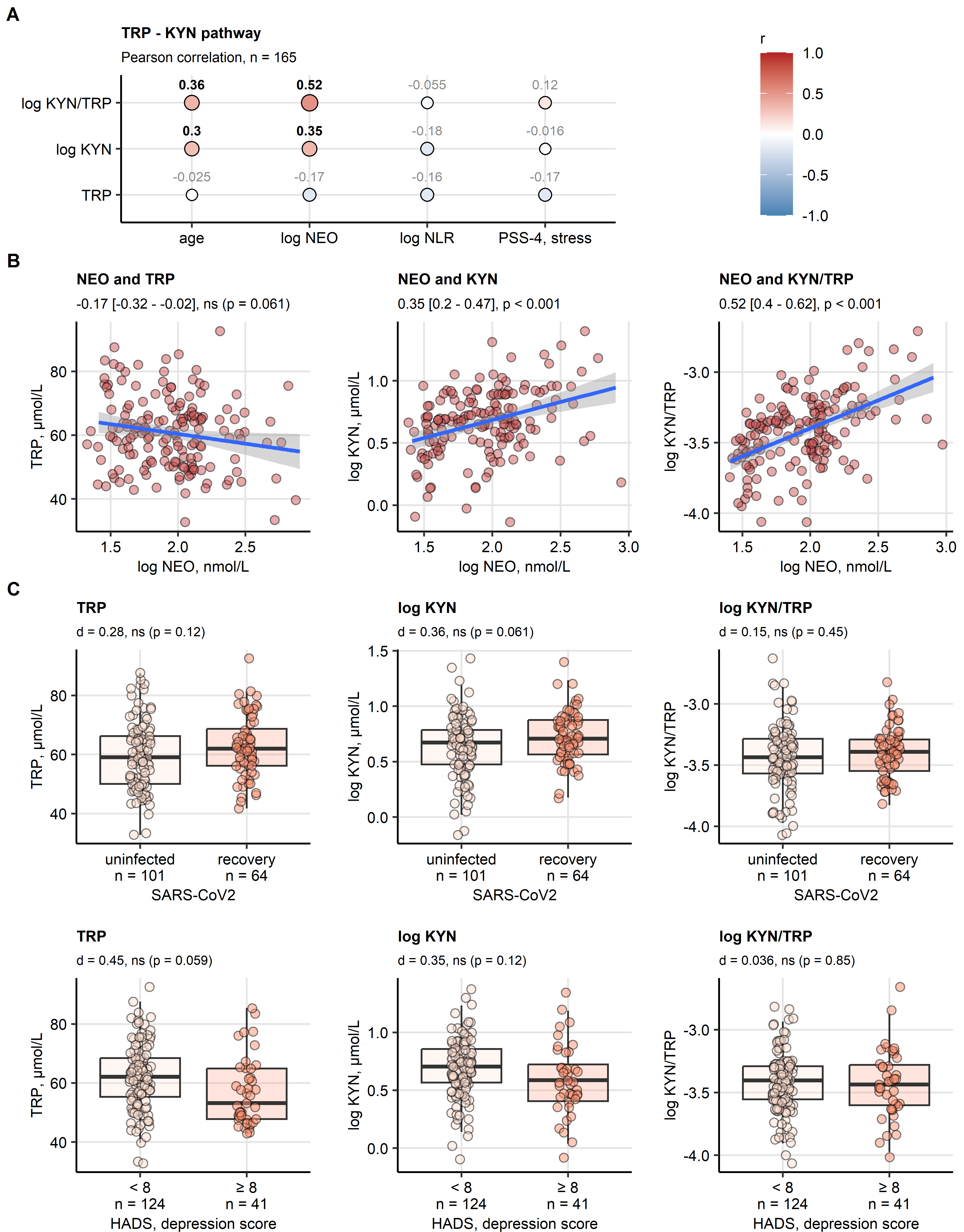


Figure 3: Effects of age, serum inflammatory markers neopterin and neutrophil-lymphocyte ratio, stress, depression and SARS-CoV-2 infection on tryptophan, kynurenine and kynurenine/tryptophan ratio in the SIMMUN cohort.

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*Age, systemic inflammatory markers: neopterin (NEO), neutrophil - lymphocyte ratio (NLR), SARS-CoV-2 infection status, depression signs (hospital anxiety and depression scale [HADS] > 8 points) and mental stress scoring (perceived stress scale, 4 item [PSS-4]) were identified as significant determinants of systemic levels of tryptophan (TRP), kynurenine (KYN) and kynurenine - tryptophan ratio (KYN/TRP). Their association with serum concentrations of these metabolites was investigated by univariable correlation analysis and statistical hypothesis testing.*

*(A, B) Correlation of the metabolite levels and the numeric explanatory factors of interest was assessed by Pearson’s test corrected for multiple testing by the false discovery rate method. (A) Correlation coefficients are presented in a bubble plot with point size and color corresponding to values of correlation coefficients (r); points are labeled with their r values, significant effects are highlighted in bold. (B) Scatter plots of serum concentrations of NEO, TRP, KYN and KYN/TRP. Points represent single observations, fitted linear trends with 95% confidence intervals are visualized as blue lines with gray ribbons. Correlation coefficients with 95% confidence intervals and p values are displayed in the plot captions.*

*(C) Levels of the metabolite levels were compared between infected and uninfected participants, and between participants with and without depression signs by two-tailed T test with Cohen’s d effect size statistic. P values were corrected for multiple testing with the false discovery rate method. Medians with interquartile ranges (IQR) are represented by boxes, whiskers span over 150% IQR, single observations are depicted as points. Effect sizes and p values are displayed in the plot captions. Numbers of observations in the strata are indicated in the X axes.*

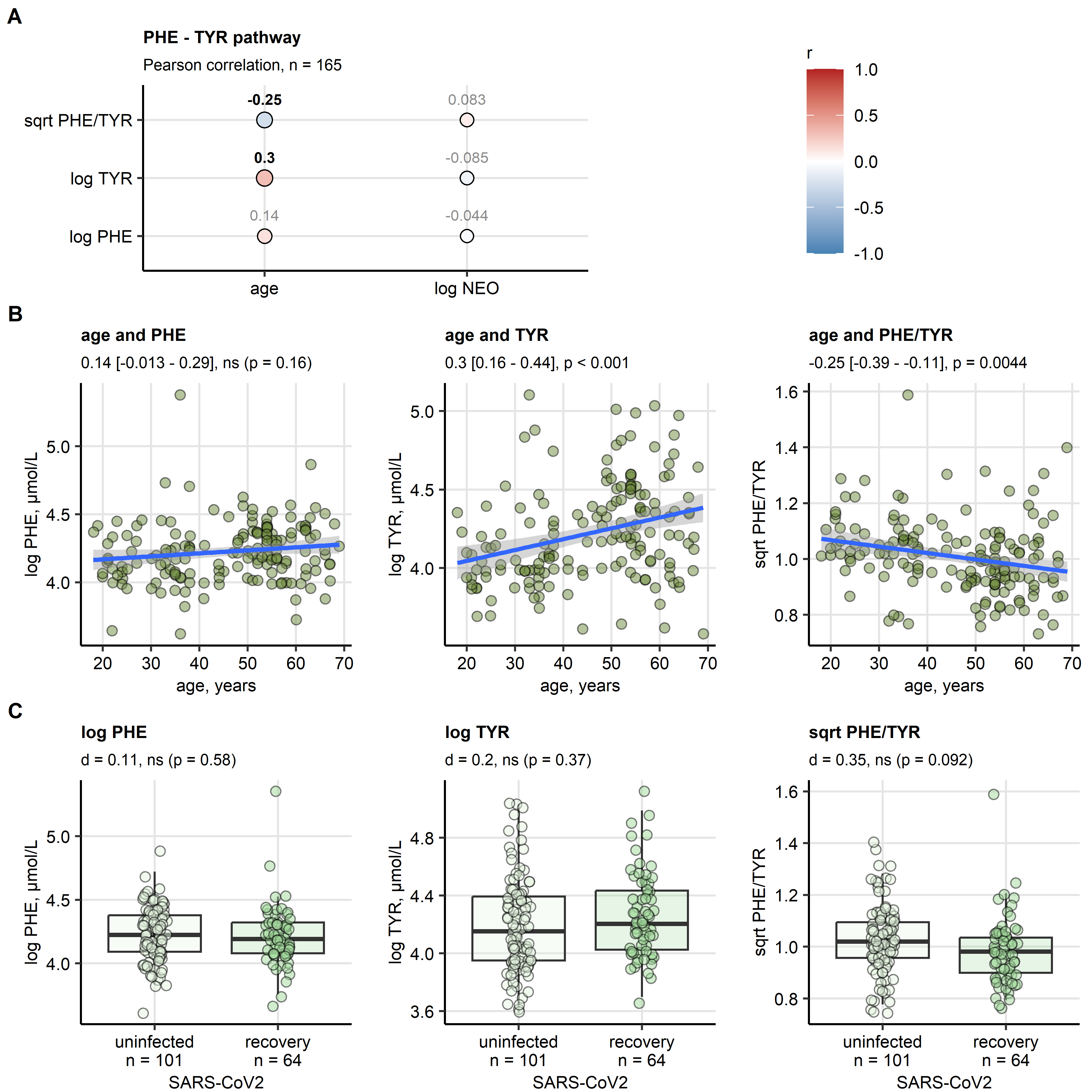


Figure 4: Effects of age, serum inflammatory marker neopterin and SARS-CoV-2 infection on phenylalanine, tyrosine and phenylalanine/tyrosine ratio in the SIMMUN cohort.

**Figure 4. Effects of age, serum inflammatory marker neopterin and SARS-CoV-2 infection on phenylalanine, tyrosine and phenylalanine/tyrosine ratio in the SIMMUN cohort.**

*Age, the systemic inflammatory marker neopterin (NEO), and SARS-CoV-2 infection status were identified as significant determinants of systemic levels of phenylalanine (PHE), tyrosine (TYR) and phenylalanine - tyrosine ratio (PHE/TYR). Their association with serum concentrations of these metabolites was investigated by univariable correlation analysis and statistical hypothesis testing.*

*(A, B) Correlation of the metabolite levels and the numeric explanatory factors of interest was assessed by Pearson’s test corrected for multiple testing by the false discovery rate method. (A) Correlation coefficients are presented in a bubble plot with point size and color corresponding to values of correlation coefficients (r); points are labeled with their r values, significant effects are highlighted in bold. (B) Scatter plots of patient’s age, PHE, TYR and PHE/TYR. Points represent single observations, fitted linear trends with 95% confidence intervals are visualized as blue lines with gray ribbons. Correlation coefficients with 95% confidence intervals and p values are displayed in the plot captions.*

*(C) Levels of the metabolite levels were compared between infected and uninfected participants by two-tailed T test with Cohen’s d effect size statistic. P values were corrected for multiple testing with the false discovery rate method. Medians with interquartile ranges (IQR) are represented by boxes, whiskers span over 150% IQR, single observations are depicted as points. Effect sizes and p values are displayed in the plot captions. Numbers of observations in the strata are indicated in the X axes.*

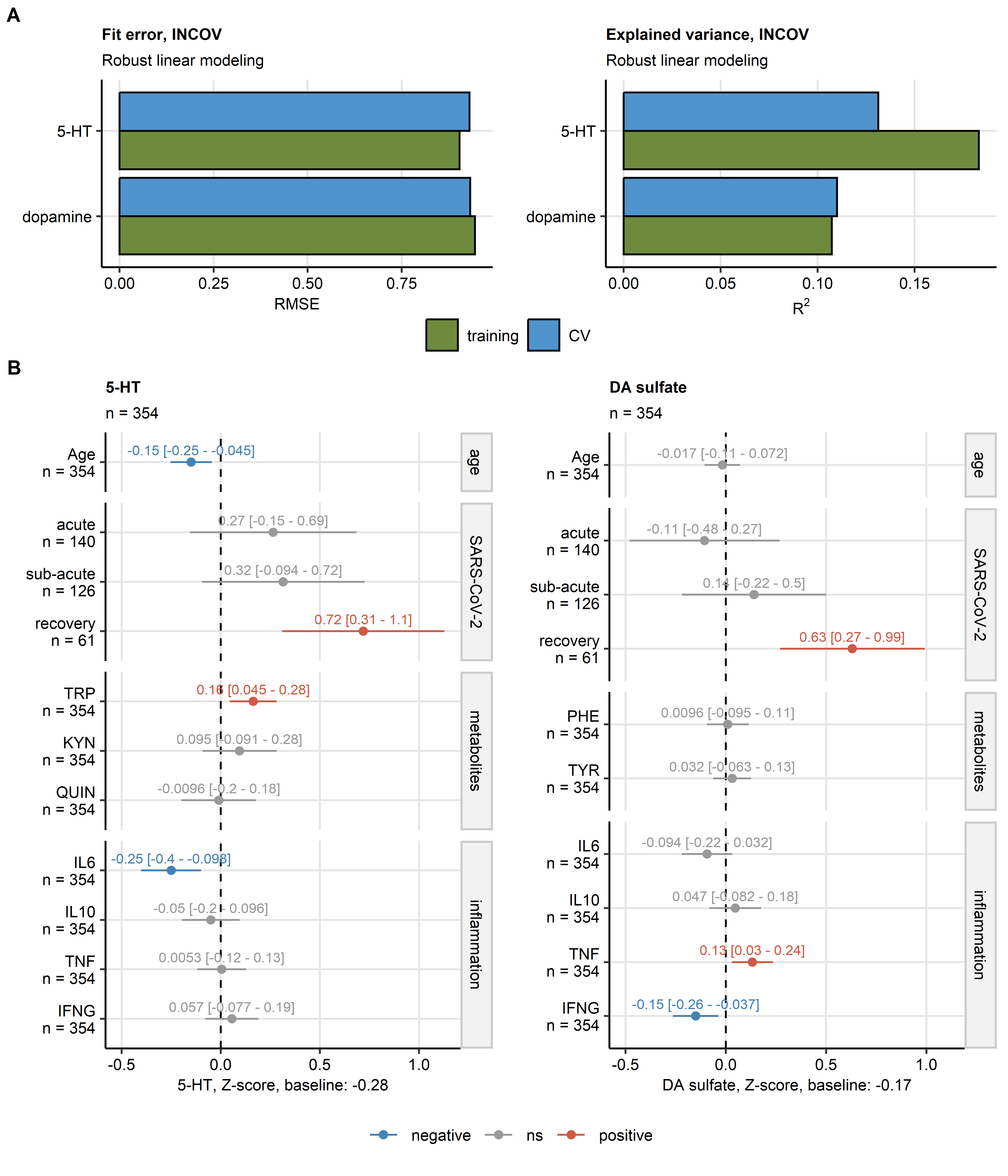


Figure 5: Results of multi-parameter robust linear modeling of serum levels of serotonin and dopamine sulfate in the INCOV cohort.

**Figure 5. Results of multi-parameter robust linear modeling of serum levels of serotonin and dopamine sulfate in the INCOV cohort.**

*Effects of patient’s age, timepoint after SARS-CoV-2 infection (acute: median 10 days, sub-acute: median 14 days, recovery: median 64 days after infection as compared with healthy controls), serum levels of metabolites related to neurotransmitter biosynthesis and competitor pathways (tryptophan [TRP], kynurenine [KYN], quinolinate [QUIN], phenylalanine [PHE] and tyrosine [TYR]), and serum concentrations of inflammatory cytokines (interleukin-6 [IL6], interleukin-10 [IL10], tumor necrosis factor-alpha [TNF] and interferon-gamma [IFNG]) on serum concentrations of serotonin (5-hydroxy tryptophan [5-HT]) and dopamine sulfate (DA sulfate) were modeled by multi-parameter robust linear regression.*

*(A) Model fit error (root mean square error [RMSE]) and fraction of explained variances (R-squared) of the robust linear models assessed in the genuine training dataset and 10-fold cross-validation (CV).*

*(B) Estimated of model coefficients with 95% confidence intervals presented in Forest plots. Numbers of complete observations are indicated in the plot captions. Model intercepts (baseline) are indicated in the X axis titles.*

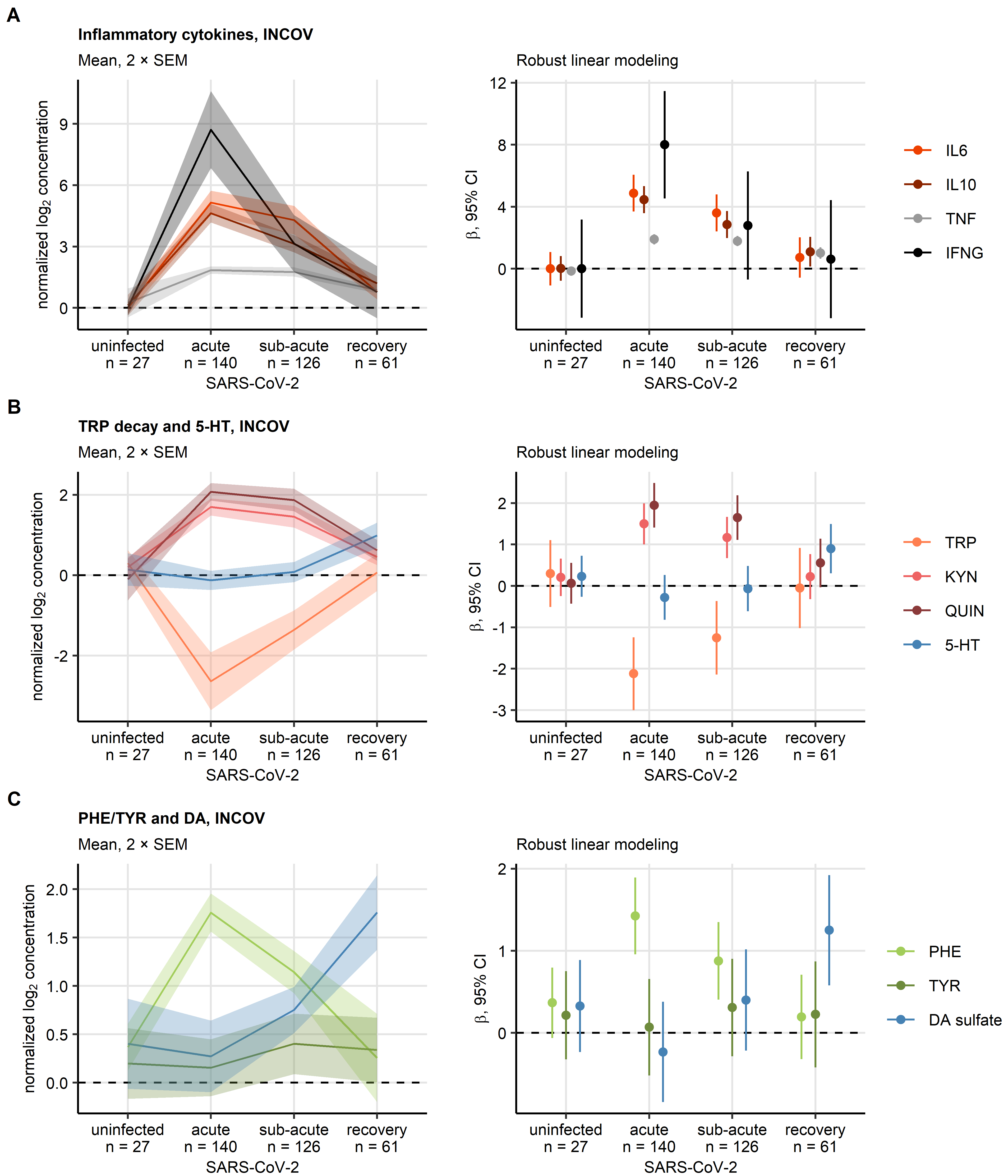


Figure 6: Time course of inflammatory cytokines, serotonin, dopamine sulfate and the neurotransmitter biosynthesis precursors and competitor pathway products during SARS-CoV infection and recovery in the INCOV cohort.

**Figure 6. Time course of inflammatory cytokines, serotonin, dopamine sulfate and the neurotransmitter biosynthesis precursors and competitor pathway products during SARS-CoV infection and recovery in the INCOV cohort.**

*Time courses of normalized log2-transformed serum concentrations of inflammatory cytokines (A, interleukin-6 [IL6], interleukin-10 [IL-10], tumor necrosis factor-alpha [TNF] and interferon gamma [INFG]), metabolites implicated in serotonin synthesis (B, tryptophan [TRP], kynurenine [KYN], quinolinate [QUIN] and serotonin/5-hydroxy tryptophan [5-HT]) and metabolites implicated in dopamine turnover (C, phenylalanine [PHE], tyrosine [TYR] and dopamine sulfate [DA sulfate]). Differences between uninfected controls and acute, sub-acute SARS-CoV-2 infection and recovery (median 10, 14 and 64 days after infection, respectively) were modeled by robust linear regression with the uninfected controls as baseline. Mean normalized concentrations with 2 standard errors of the mean (2 SEM) are depicted as solid lines with tinted ribbons (plots on the left). Robust linear model coefficient estimates with 95% confidence intervals are visualized in Forest plots (right). Numbers of complete observations per timepoint are indicated in the X axes.*

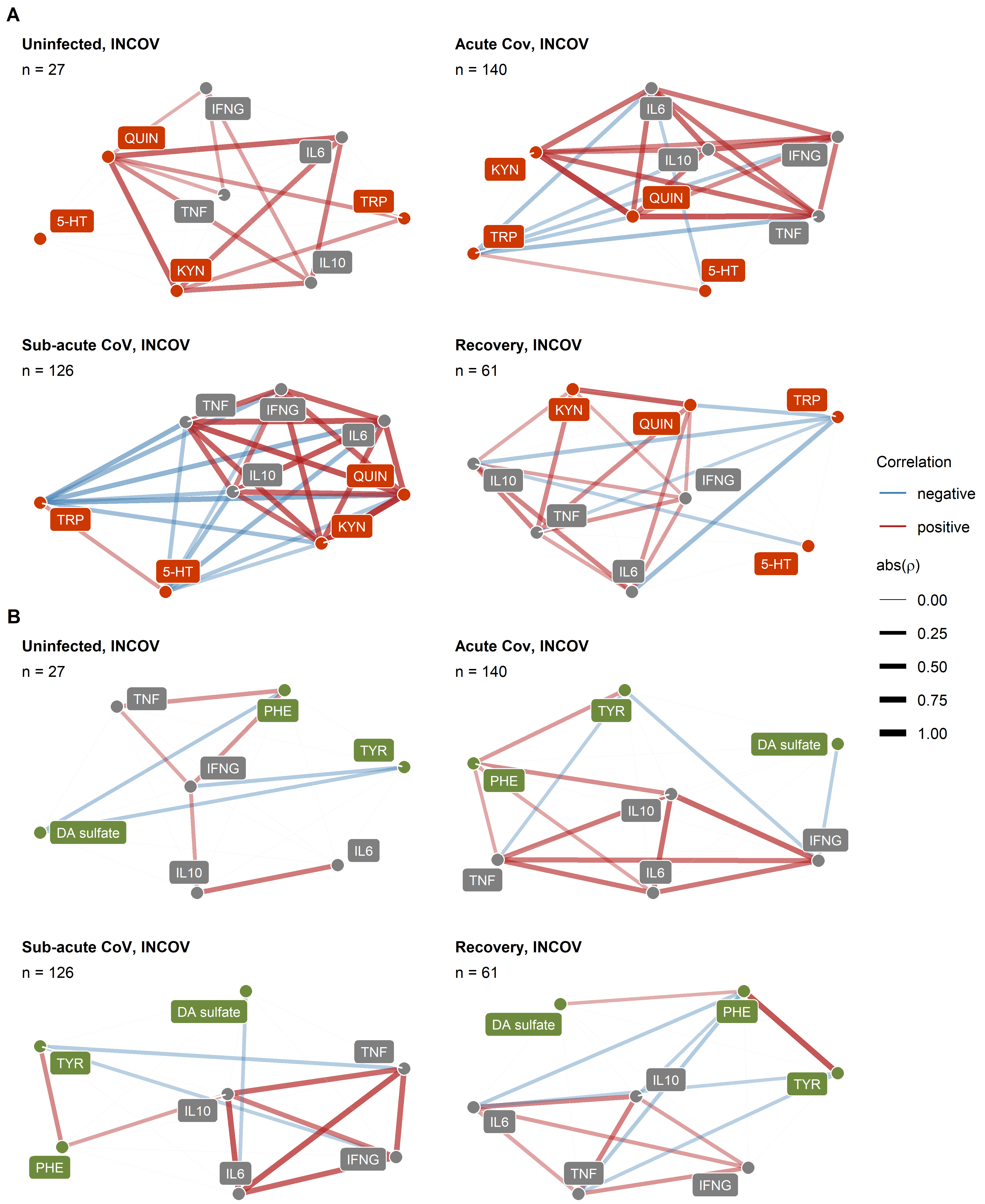


Figure 7: Correlation of serum levels of inflammatory cytokines, serotonin and dopamine sulfate, their precursors and competitor pathway products in healthy controls and SARS-CoV-2 patients of the INCOV cohort.

**Figure 7. Correlation of serum levels of inflammatory cytokines, serotonin and dopamine sulfate, their precursors and competitor pathway products in healthy controls and SARS-CoV-2 patients of the INCOV cohort.**

*Serum levels of metabolites implicated in serotonin synthesis (A, tryptophan [TRP], kynurenine [KYN], quinolinic acid [QUIN], serotonin [5-hydroxy tryptophan, 5-HT]), dopamine turnover (B, phenylalanine [PHE], tyrosine [TYR], dopamine sulfate [DA sulfate]) and inflammatory cytokines (interleukin-6 [IL6], interleukin-10 [IL10], tumor necrosis factor-alpha [TNF], interferon-gamma [INFG]) were subjected to pairwise correlation analysis in uninfected and SARS-CoV-2-infected individuals (acute: median 10 days, sub-acute: median 14 days, recovery: median 64 days after infection). Correlation coefficient matrices for at least moderate effects ( > 0.2) were visualized as force-directed network plots. Node color codes for the parameter type (gray: inflammatory cytokines, orange: serotonin, green: dopamine turnover), edge width and color codes for the value and sign of the correlation coefficient.*

# Acknowledgements

The scheme of report findings was created with bioicons by Simon Duerr. The SARS-CoV-2 virus image was created by Hanna Vega and licensed under the terms of a Creative Commons CC-BY SA 4.0.

# References