SUPPLEMENTARY INFORMATION

Metal-coding assisted serological multi-omics profiling deciphers the role of selenium in COVID-19 immunity

Materials and methods

Reagents, virus and cell lines

The Pierce™ NHS-activated magnetic beads, magnetic stand and borate buffer (pH 8.5) were purchased from Life Technologies; the MaxPAR antibody conjugation kits were bought from Fluidigm. Multielement standard (TraceCERT®) (40 mg/L Al, 40 mg/L As, 100 mg/L B, 100 mg/L 40 mg/L Ba, 10 mg/L Be, 10 mg/L Cd, 10 mg/L Co, 20 mg/L Cr, 20 mg/L Cu, 100 mg/L Fe, 10 mg/L Mn, 20 mg/L Ni, 40 mg/L Pb, 100 mg/L Se, 100 mg/L Tl, 40 mg/L V, 100 mg/L Zn) for ICP-MS was purchased from Sigma. The tuning solution containing 10 μg/l Ce, Co, Li, Tl, and Y was from Agilent Technologies. The cell culture medium (RPMI-1640), Fetal Bovine Serum, Trypsin, Penicillin-Streptomycin, and IL-2 were from life technology. B cell and $CD4^+$ T cell isolation kits were from BioLegend. The deionized water (18.2 MΩ.cm) was used throughout the experiments.

The SARS-CoV-2 (HKU-001a strain) was isolated from the nasopharyngeal aspirate specimen of a patient with laboratory-confirmed COVID-19 in Hong Kong.[1] All experiments with live viruses were conducted using Biosafety Level 3 facilities in Queen Mary Hospital, The University of Hong Kong, as previously described.[2] SARS-CoV-2 strain was a wild-type strain in both patients and the mouse model.

Facilities and Apparatus

A quadrupole-based inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700x, Agilent Technologies, USA), equipped with a glass concentric nebulizer and an impact spray chamber, was used for metal(loid)s profiling in solution. The CyTOF (Helios) was used throughout the single-cell analysis.

Antibody conjugation with metal-polymer tags

All the detection antibodies specific to each inflammation mediator, cell surface marker and intracellular cellular cytokines were labelled with different lanthanide tags individually using the MaxPAR antibody conjugation kits according to the company's recommended protocol. Each lanthanide tag was conjugated on specific antibodies through a reaction with thiol (-SH) groups in the side chain of cysteine. After determining the concentration of metal-labelled antibodies using BCA calibration, the metal-labelled antibodies were preserved in PBS for long-term storage at 4 °C. A summary of all detection antibodies specific to inflammation mediators, cell surface

markers and intracellular cellular cytokines and their corresponding metal tags are shown in Table S3 and S6.

Magnetic beads functionalization with capture antibodies

To conjugate capture antibodies on magnetic beads, N-hydroxysuccinimide (NHS) activated magnetic beads were used to covalently immobilize proteins through reaction with primary amines on proteins. After removing the preservation buffer using a magnetic stand, the NHS-activated magnetic beads were activated in ice-cold hydrochloric acid (1 mM, pH 3). The antibodies (100 μg) remaining labelled were added into magnetic beads (4 mg) immediately after removing hydrochloric acid. The mixture of proteins and magnetic beads were incubated for two hours at room temperature on a rotator. The glycine (0.1 M, pH 2.0) was added after incubation and the antibody-labelled magnetic beads were washed with ultrapure water twice, followed by quenching the reaction in ethanolamine (3 M, pH 9.0). The magnetic beads were then washed with ultrapure water and PBS twice and then resuspended in PBS containing 0.05% sodium azide at a final concentration of 10 mg/mL for the antibody-conjugated magnetic beads. The capture antibodies of inflammation mediators labelled on magnetic beads are shown in Table S4.

Serum samples for multi-omics profiling

Under the approval of the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 13-372 and UW 14-249), archived serum samples of COVID-19 patients and control subjects (non-infected blood donors prior to or during the COVID-19 outbreak) were obtained from Queen Mary Hospital in Hong Kong.[3] A total of 100 non-infected controls, 76 mild COVID-19 patients, and 31 moderate to severe COVID-19 patients were included in this study. None of the COVID-19 patients and controls were vaccinated. The serum samples were heat-inactivated by incubation at 56°C for 30 min before storing at -80°C until further use. Archived serum samples of Generalized Severe Periodontitis patients (the severity and extent: 3/4G+grade C, i.e., generalized, stage III-IV and grade C following the new classification of periodontal diseases and conditions[4]) were obtained from Faculty of Dentistry, the University of Hong Kong (IRB UW 17–303).[5]

Metallome screening of serum samples using ICP-MS

Prior to metallome profiling of serum samples, the detection range of different metal(loid)s (Se, Zn, Ca, Mg, K, Cr, Fe, Cu, Mo, and I) by ICP-MS was determined using multielement standard. The metallome levels of serum samples with different dilution levels were quantified using ICP-MS to optimize the dilution condition for serum metal(loid)s profiling. A dilution factor of 20 was applied, given all the metal(loid)s detected are within the detection range of ICP-MS. All instrument parameters of ICP-MS were optimized before each measurement using a standard tuning solution. To minimize possible polyatomic interferences such as $^{40}Ar^{12}C$, $^{43}Ca^{16}O$, and $^{40}Ar^{19}F$ in the human serum matrix, helium gas was added throughout the

quantification of different elements. Table S2 summarizes the instrument parameters and measurement conditions of ICP-MS for metallome profiling.

Multiplexed proteomic profiling by ICP-MS

In our previous study, we described multiple antibody quantification of COVID-19 patients based on ICP-MS by introducing metal-tagged antibodies.[6] The multiplexed profiling of proteome associated with inflammation mediators was based on the previously reported antibody profiling protocol.[6] Generally, the magnetic beads functionalized with the inflammation mediator-specific antibodies were blocked in blocking buffer (PBS, 0.1% Tween-20, 2.5% BSA) for 30 min at room temperature. The serum samples were diluted (1:10) using assay buffer (PBS contained 2.5% BSA, 0.1% Tween-20, 0.3% Triton-100) and 100 μL of diluted samples were incubated with functionalized magnetic beads for 30 min at room temperature. The magnetic beads were then washed with washing buffer (PBS, 0.1% Tween-20) three times, followed by incubation with metal tag-labelled secondary antibodies for 30 min at room temperature. The magnetic beads were then washed with PBS four times, and 50 μL of elution buffer (1% HNO_3) was added into each tube to release the metal-tagged antibodies. The magnetic stand was used throughout the experiment during the washing and elution step to separate magnetic beads from reaction solution. The released solution was diluted in 500 μL 1% HNO_3 with 0.5 ppb indium (In) as an internal standard for multiplexed metal quantification by ICP-MS. Given the IFN-α and CRP was labelled with the same metal tag (^{159}Tb), CRP was measured separately from other inflammation mediators.

Peripheral blood collection and sample preparation

Fresh peripheral blood (PB) from non-infected donors or COVID-19 patients were collected from Queen Mary Hospital (Hong Kong, China). All PB samples were collected into heparin blood tubes and stored at 4 °C for no more than 12 hours before processing. The lymphocytes were separated by density gradient centrifugation (Ficoll® Paque Plus, GE Healthcare) according to the manufacturers' recommended protocols to remove erythrocytes, platelets, and granulocytes. Briefly, the whole PB was diluted with PBS of equivalent volume, and the diluted sample was gently added on the surface of ficoll solution (1:1) in 50 ml tubes prior to centrifugation at room temperature (30 min, 20 °C, 400 × g). The upper layer containing plasma was removed gently to keep the state of the lymphocyte layer. All the lymphocytes were collected in a new 15 ml tube and washed with 6 ml PBS twice (10 min, 20 °C, 100×g). The isolated PBMCs were used freshly for the following antibody staining or were cryopreserved in cell preservation media (90% heat-inactivated fetal bovine serum (Gibco) +10% DMSO (Sigma), and stored in liquid nitrogen until used in the assays. Before staining the PBMC cells, the number of cells was counted by hemocytometer under an optical microscope and 3×10^6 lymphocytes were used for each staining.

B cell and $CD4^+$ T cell isolation from human PBMC cells

The B cells and $CD4^+$ T cells were purified from separated PBMC according to the manufacturers' recommended protocols based on negative selection. Briefly, the separated PBMC from healthy donors were filtered with 70 um cell strainers to remove cell aggregates. The PBMC was resuspended in an appropriate volume of MojoSort™ buffer to adjust the cell concentration to 1×10^8 cells/mL. About 10^7 cells were aliquoted into a new tube and mixed with 10 μL of the Biotin-Antibody Cocktail specific to capture the non-targeted cells. The cell mixture was incubated on ice for 15 minutes, followed by addition of 10 μL of Streptavidin Nanobeads to remove the captured cells. After incubation on ice for 15 minutes, the captured cells were removed using magnetic separation. The remaining liquid containing targeted cells was transferred to a new tube. The B cells and $CD4^+$ cells were washed and collected by centrifuge.

Cell staining with metal tag-labelled antibodies

Mass cytometry staining was performed as previously reported.[7] Briefly, the fresh isolated mononuclear cells from peripheral blood were resuspended in prewarmed PBS solution. Cisplatin (5 μM) was added to the cell suspension as a viability reagent and incubated for 5 min at room temperature with continuous shaking. Cisplatin staining was quenched by washing with Maxpar Cell Staining Buffer (Fluidigm). The cells were counted by hemocytometer under an optical microscope and resuspended 3 million cells in Maxpar Cell Staining Buffer with a volume of 50 μL in 15 mL polypropylene tubes for the following antibody staining. About 50 μL of antibody cocktail with a mixture of the metal tag-labelled antibodies were prepared for immune cell classification. The antibody cocktail specific to cell surface markers was added into each tube and mixed with the cell suspension for a total volume of 100 μL, then further incubated for 30 min at room temperature. The cells were washed twice using 2 mL Maxpar Cell Staining Buffer by centrifugation (300×g, 5 min) and the supernatant was removed. The cell pellets were disrupted by vortexing. A freshly prepared 1.6 % formaldehyde solution (Sigma) was added (1 mL) into each sample for cell fixing (10 min at room temperature) and the fixing buffer was removed by centrifugation (800×g, 5 min). After that, cells were permeabilized by incubation with Perm buffer (Fluidigm) for 15 min at room temperature. After washing with Maxpar Cell Staining Buffer, about 50 μL intracellular antibody cocktail for intracellular cytokine characterization was added into each tube and incubated for 30 minutes at room temperature. Cells were washed with 2 mL Maxpar Cell Staining Buffer twice and incubated with 125 nM intercalator-Ir in Maxpar Fix and Perm Buffer (Fluidigm) overnight at 4 °C. Cells were washed with 2 mL of Maxpar Cell Staining Buffer twice by centrifugation (800×g, 5 mins) to remove the intercalator buffer and were left pelleted until analysis by a mass cytometer. Prior to detection by mass cytometry, 0.1×EQ beads (3.3×10^4 beads/mL) (Fluidigm) containing Cerium ($^{140/142}$Ce), Europium ($^{151/153}$Eu), Holmium (^{165}Ho), and Lutetium ($^{175/176}$Lu) were added to cell suspension for data calibration and later normalization. The cell density was adjusted to $5.0\text{-}10\times10^5$ cells/mL in deionization water after filtration using cell strainer cap tubes for mass cytometry (Helios) assay.

Mass cytometry assays

The CyTOF (Helios) was started, cleaned and tuned before sample analysis using a wash and tuning solution. Ultrapure argon was used for the whole analysis process. The suspension of antibody staining cells and calibration beads in cell acquisition buffer (Fluidigm) was prepared for mass cytometer assay after filtration by cell strainer cap tubes with an event rate between 200-400 events/s. Calibration bead signals were used as a real-time detector to monitor the machine's performance throughout the whole analysis process. All the data acquired from mass cytometry were exported as an FCS file for further analysis by flowjo.

Cell culture

PBMC, purified B cells, and purified $CD4^+$ T cells were maintained in RPMI 1640 mediums supplied with 10% FBS, 1% Pen-Strep and 100U/mL IL-2.

Western Blotting

After culture, cells were harvested, washed in ice-cold PBS, and lysates immediately extracted, or were kept as pellets at −80 °C until lysis. Whole cell lysates were extracted using protein extraction reagent (Life Technology) with 1× protease and phosphatase inhibitor cocktail. Lysates were resolved on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels, transferred to polyvinylidene fluoride membranes, blocked (5% non-fat milk) and the membranes were probed with incubated with primary antibodies overnight at 4°C. The membranes were washed with a washing buffer and incubated with anti-rabbit-HRP (#7074) or anti-mouse-HRP (#7076) secondary antibody (Cell Signaling). The Immunoblots were visualized using WesternBright ECL kit (Advansta), and imaged using ChemiDoct XRS+ System. All the primary and secondary antibodies were diluted in the ratio 1:1000 and 1:2000, respectively. The antibodies used in this experiment including ERK1/2 (#4695)/Phospho-ERK(#4370) (Cell Signaling), Phospho-STAT3 (690452) (Biolegend), Akt(#9272)/Phospho-Akt(#4060) (Cell Signaling), and Gsk3β(#9832)/Phospho-Gsk3β (#5558) (Cell Signaling). To visualize the proteins and corresponding phosphorylated proteins on the same blot, western blot strip buffer (Abcam) was used to remove the labeled primary (phosphorylated proteins) and secondary antibodies from western blot membrane and restain the other specified antibodies (total form proteins, GAPDH) for visualization. The raw data of western blot was provided as supplementary documents.

RT-PCR assay

Cell or tissue samples were collected and resuspended in Buffer RL. The total RNA was extracted using RNA mini kit (Qiagen ID: 74104 and Takara ID:9767) according to the corresponding manufacturers' protocols. All primers were searched from PrimerBank[8] or designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by Sangon Biotech. The qRT-PCR was performed in 384-well plates by using the Applied Biosystems

QuantStudio 6 Real-Time PCR system (Thermo Fisher). The reaction (10 µl) was performed by using SYBR® Premix Ex Taq™ (TaKaRa, Japan). Each well contained 5 µl of SYBR® Premix Ex Taq™, 0.2 µl of ROX Reference Dye, 3 µl of sterilized ddH_2O, 0.4 µl of each primer (10 µmol L^{-1}), and 1 µl of cDNA template. The reaction conditions were 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 30 s. After PCR amplification, a melting curve was obtained by the following process: 95 °C for 3 s, followed by 60 °C for 30 s.

Evaluation of anti-SARS-CoV-2 activity of selenium in a mouse model

Mice were kept in biosafety level housing and fed with water without or with sodium selenite (0.20 mg/L) for 10 days before experiments. Mice were randomly allocated to experimental groups (n = 5) for antiviral evaluation. All experimental protocols were approved by the Animal Ethics Committee at the University of Hong Kong (CULATR) and were performed according to the standard operating procedures of the Biosafety Level 3 animal facilities (reference code: CULATR 5370-20). After virus infection for 4 days, the lungs, brain, liver, and kidney tissue samples were collected. mRNA level of selenoproteome, cytokine and chemokine in the tissue homogenates was detected by RT–PCR methods as we previously described.[9] The tissue pathology of infected mice were examined by H&E staining in accordance with an established protocol.[10]

Statistical analysis

The comparisons of metal(loid)s and inflammation mediators among groups were performed using U tests for categoric variables or ANOVA/t-test for continuous variables. Benjamini-Hochberg correction was used for all multiple comparisons to control the false discovery rate. False discovery rate–adjusted P values less than 0.05 were considered statistically significant. The Spearman rank correlation coefficient was used for correlation analysis between the expression level of sera metal(loid)s and mediators involved in inflammation and antibody response. All the data were tested for normality before choosing the parametric or nonparametric test for a statistically significant evaluation. All statistical tests were calculated using GraphPad Prism (9.0). The correlation networks were generated using Cytoscape. UMAP was performed on scaled metal(loid)s variables using the umap function of the R package uwot v. 0.1.14 (Melville J (2022). uwot: The Uniform Manifold Approximation and Projection (UMAP) Method for Dimensionality Reduction. R package version 0.1.14, https://CRAN.R-project.org/package=uwot) with PCA as the initiation. The data points in UMAP maps were colorized according to the normalization with sigmoid. The abnormal animal data were removed based on 4d test ($|x_{out}-x_{mean}|>4d$, d=average deviation).

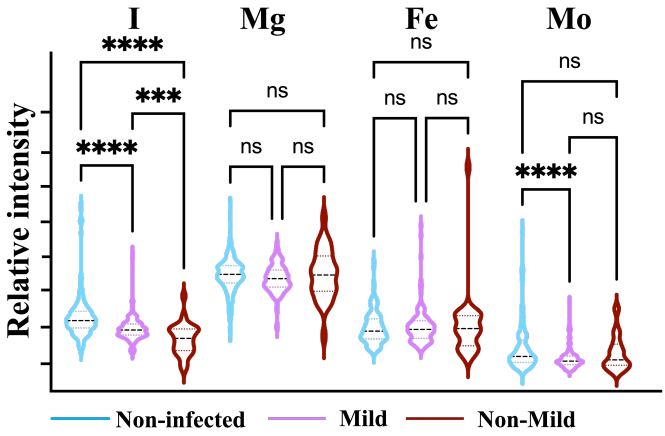


Figure S1 Comparison of different metal(loid)s among the sera from non-infected individuals and COVID-19 patients with different levels of severities. The dashed line denotes the median value of each parameter across all samples used in the plot. 0.0001<(***)P<0 .001, (****)P<0 .0001.

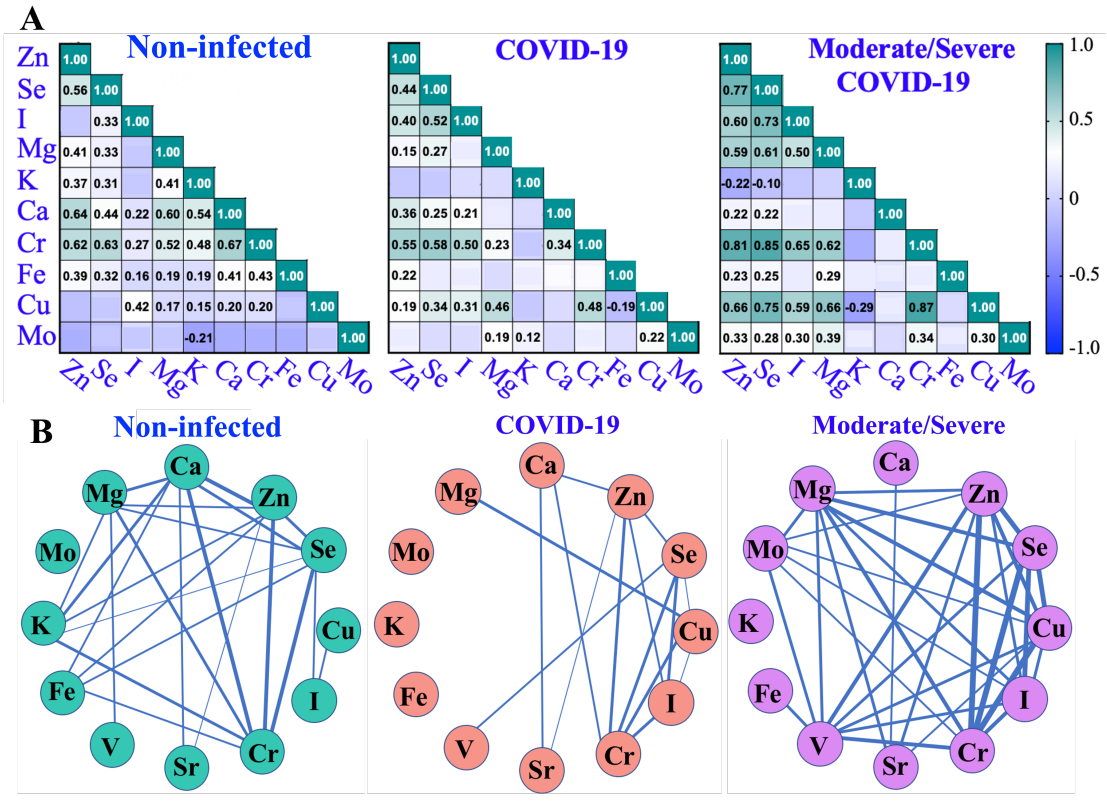


Figure S2 (A) Correlation metrics among metal(loid)s in non-infected individuals (*left*), COVID-19 patients (*mediate*), and COVID-19 patients with more severe symptoms *(right)*. (B) Network of Spearman Rho correlations between metal(loid)s in non-

infected controls (*left*), COVID-19 patients (*middle*), and COVID-19 patients with more severe symptoms *(right*). Only correlation strength with significant correlation (P < 0.05) and ρ value larger than 0.3 was used for the generation of correlation networks. The line thickness shown in the network reflects the value of the correlation coefficient.

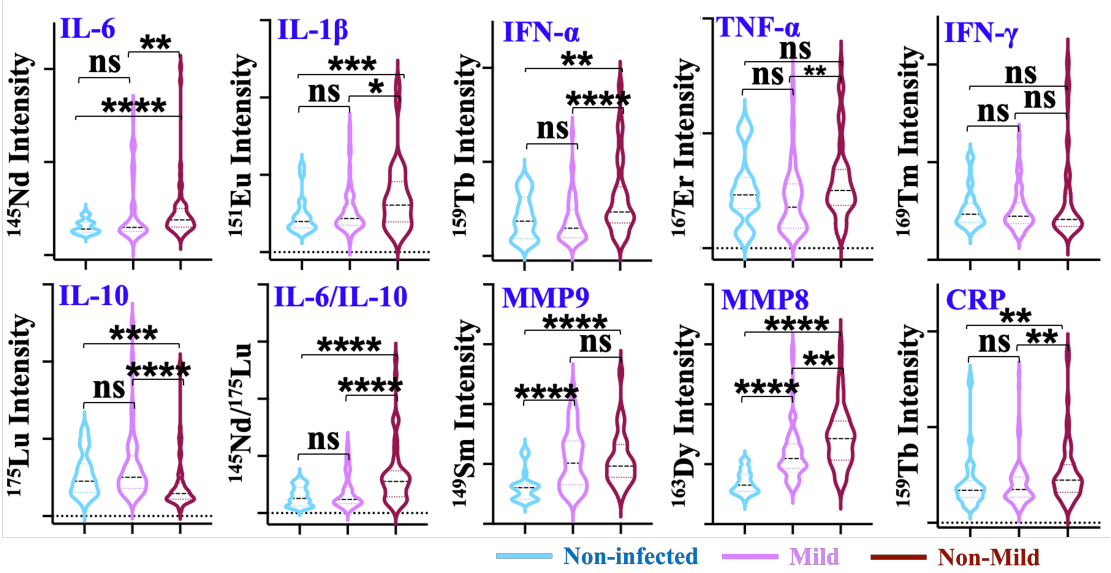


Figure S3 Multiplexed serological profiling of inflammation mediators using ICP-MS. Comparison of serum levels of different inflammation mediators among the sera from non-infected individuals and COVID-19 patients with different levels of severities. The dashed line denotes the median value of each parameter across all samples used in the plot. 0.01<(*)P<0 .05, 0.001<(**)P<0 .01, 0.0001<(***)P<0 .001, (****)P<0 .0001.

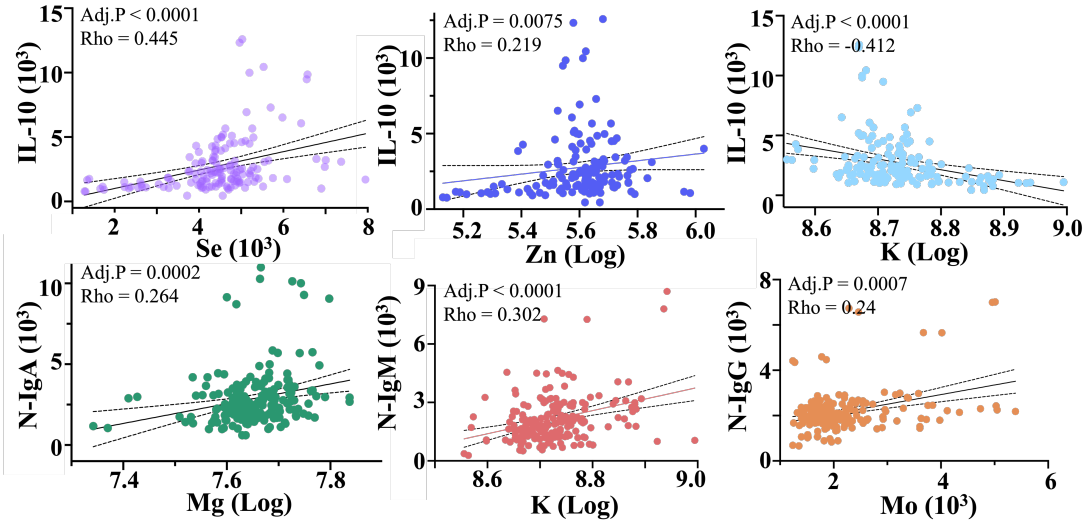


Figure S4 Linear regression between Se, Zn, K and IL-10, Mg and N protein-specific IgA antibody, K and N protein-specific IgM antibody, Mo and N protein-specific IgG antibody. The black line and gray dashed line represent a simple linear regression and its 95% confidence interval, respectively. Rho indicates the Spearman Rho coefficient. Correlation with Adj. P<0.05 is of statistical significance.

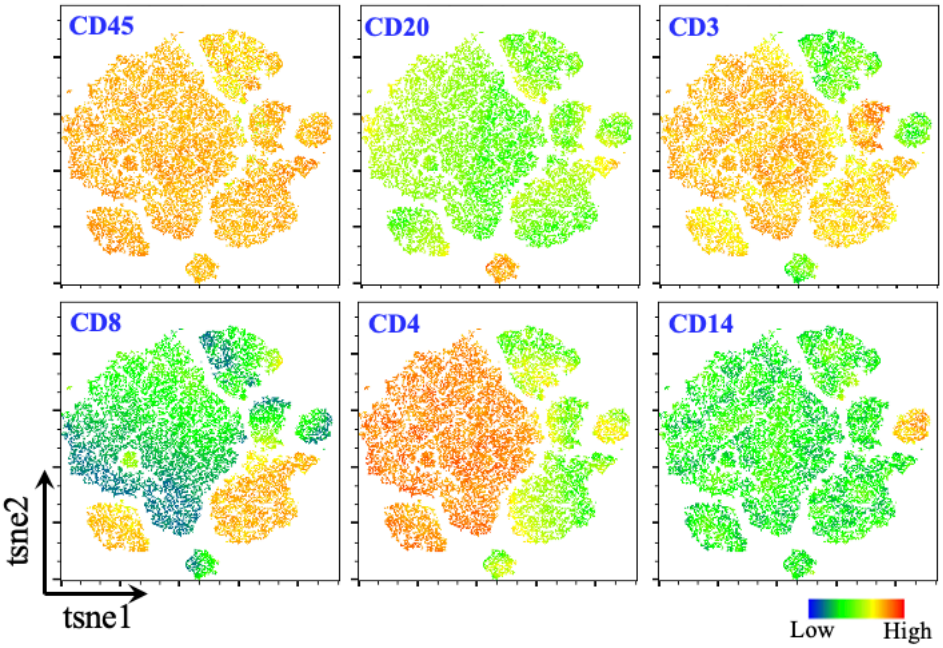


Figure S5 Expression pattern of different cell surface markers across the lymphocytes of PBMC from non-infected individuals and COVID-19 patients.

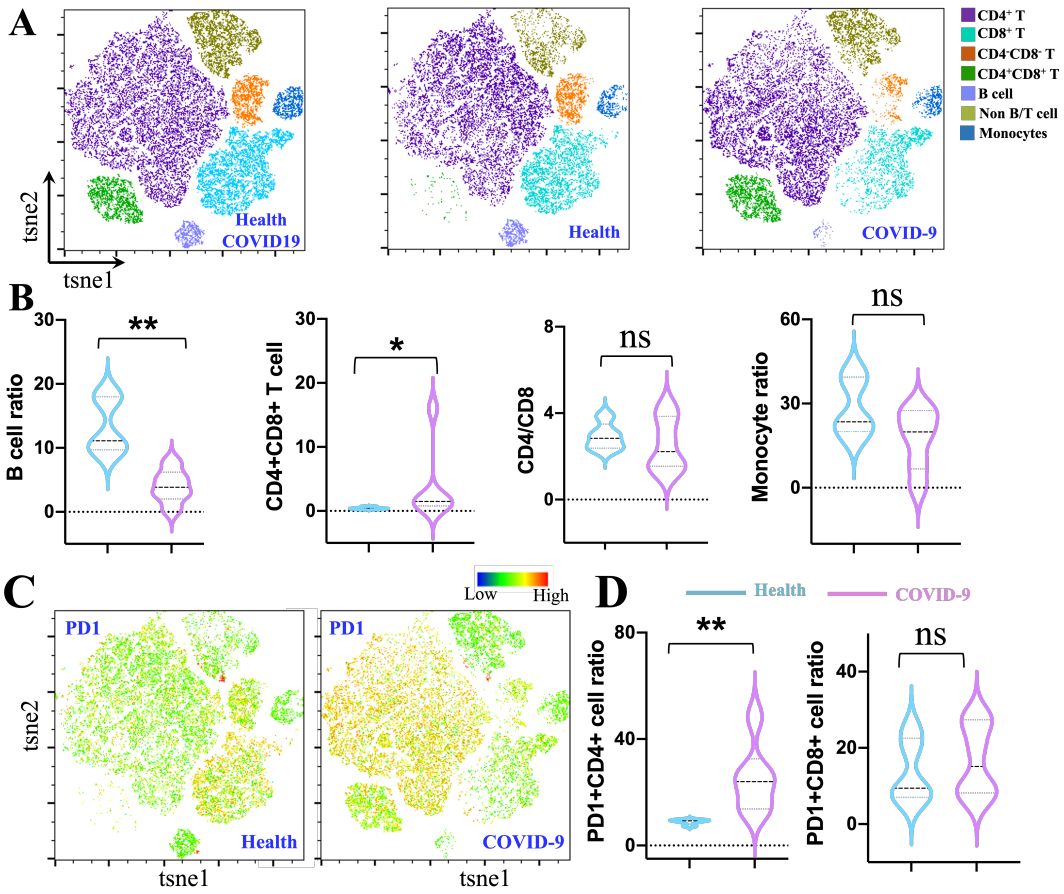


Figure S6 (A) tSNE map of immune cell pattern of PBMC from COVID-19 patients and non-infected controls (The cell classification map was generated by integration of the cell information from both COVID-19 patients and non-infected control). (B) Comparison of immune cell ratios between non-infected controls and COVID-19 patients for B cells, $CD4^+CD8^+$ T cells, monocytes and $CD4^+/CD8^+$ ratios. (C) The expression pattern of PD1 across all the immune cells of COVID-19 patients and non-infected individuals. (D) Comparison of $PD1^+CD4^+$ and $PD1^+CD8^+$ T cells between COVID-19 patients and non-infected control. P values were calculated by a two-tailed t-test to evaluate the significant change between groups. 0.01<(*) P<0.05; 0.001<(**) P < 0.01.

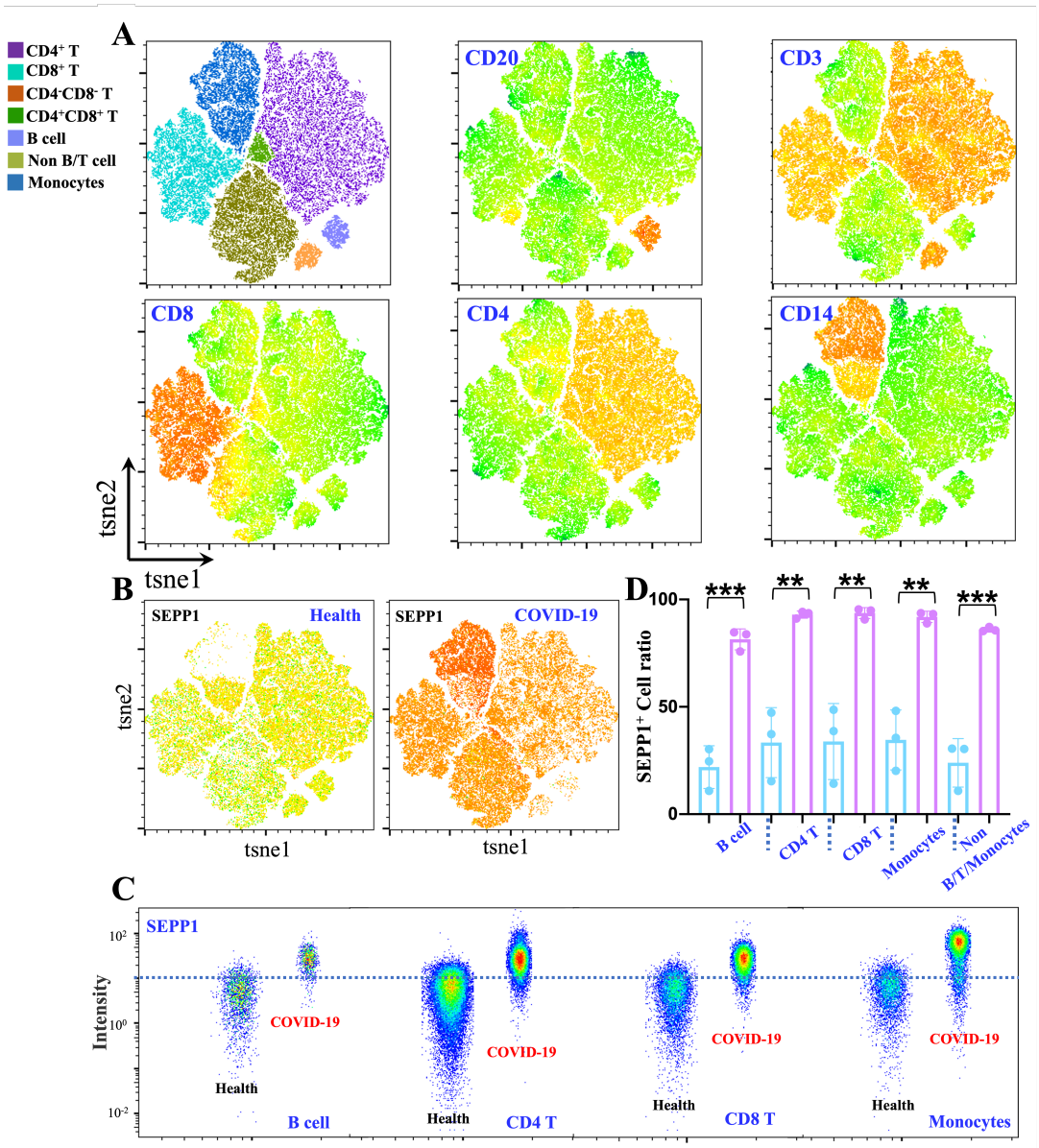


Figure S7 (A) Immune cell pattern of PBMC from COVID-19 patients and non-infected individuals. (B) tSNE map showing the expression pattern of SEPP1 across all the immune cells of COVID-19 patients and non-infected controls. (C) The variation of SEPP1 intensity in different types of immune cells. (D) Quantified $SEPP1^+$ cell ratios in different types of immune cells. P values were calculated by a two-tailed t-test to evaluate the significant change between groups. 0.001<(**) P<0.01; 0.0001<(***) P < 0.001.

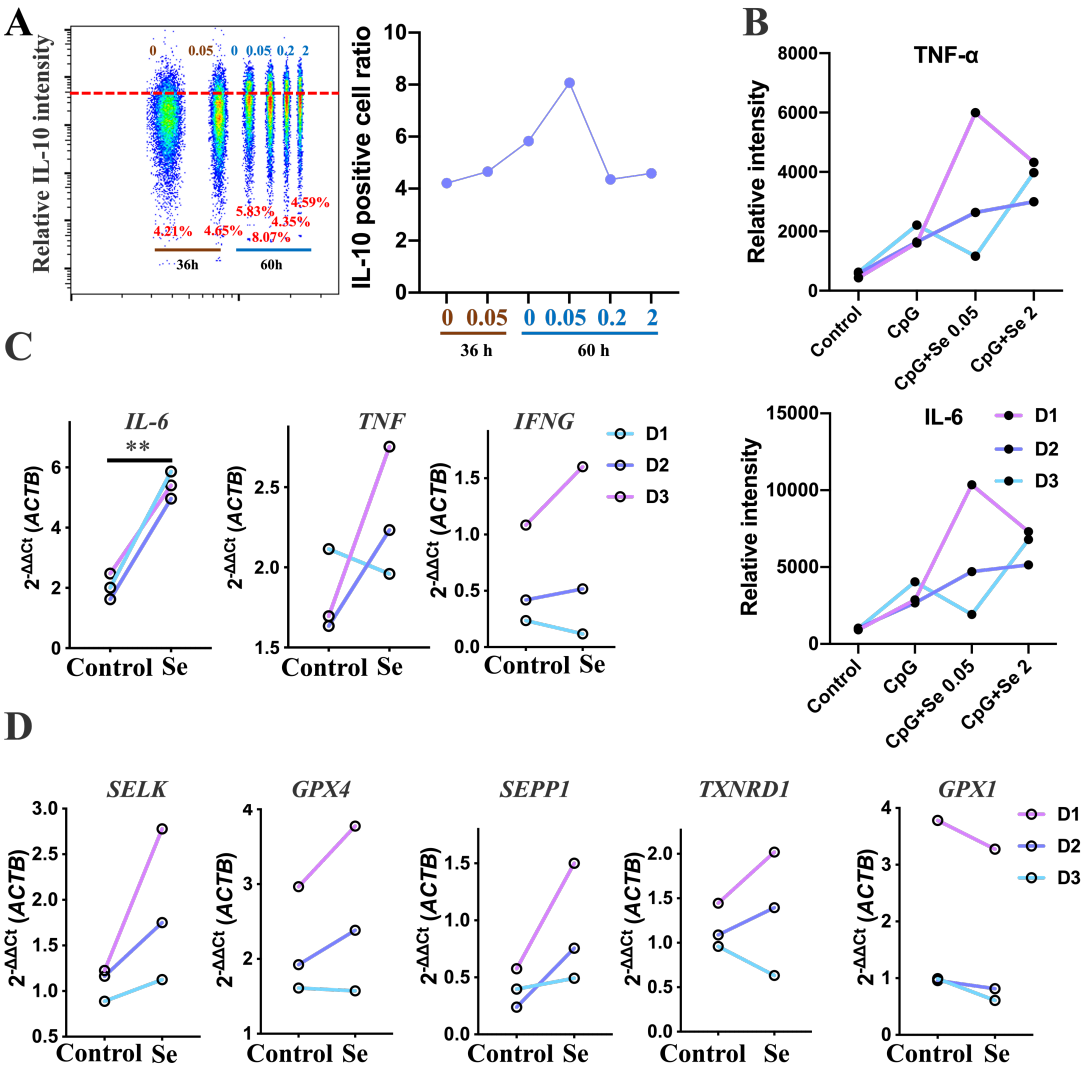


Figure S8 Se modulates immune cells. (A) IL-10 expression of B cells after treatment with different concentrations of Se for different time points. (B) Modulation of IL-6 and TNF-a in the supernatant of B cells upon different treatment conditions. (C) Comparison of mRNA levels of *IL-6*, *TNF* and *IFNG* between activated B cells with or without Se (0.05 μM 60 h) treatment. (D) Comparison of mRNA levels of selenoproteins (SELK, GPX4, SEPP1, TXNRD and GPX1 between activated B cells with or without Se (0.05 μM 60 h) treatment. P values were calculated by a two-tailed t-test to evaluate the significant change between groups. 0.001<(**) P < 0.01.

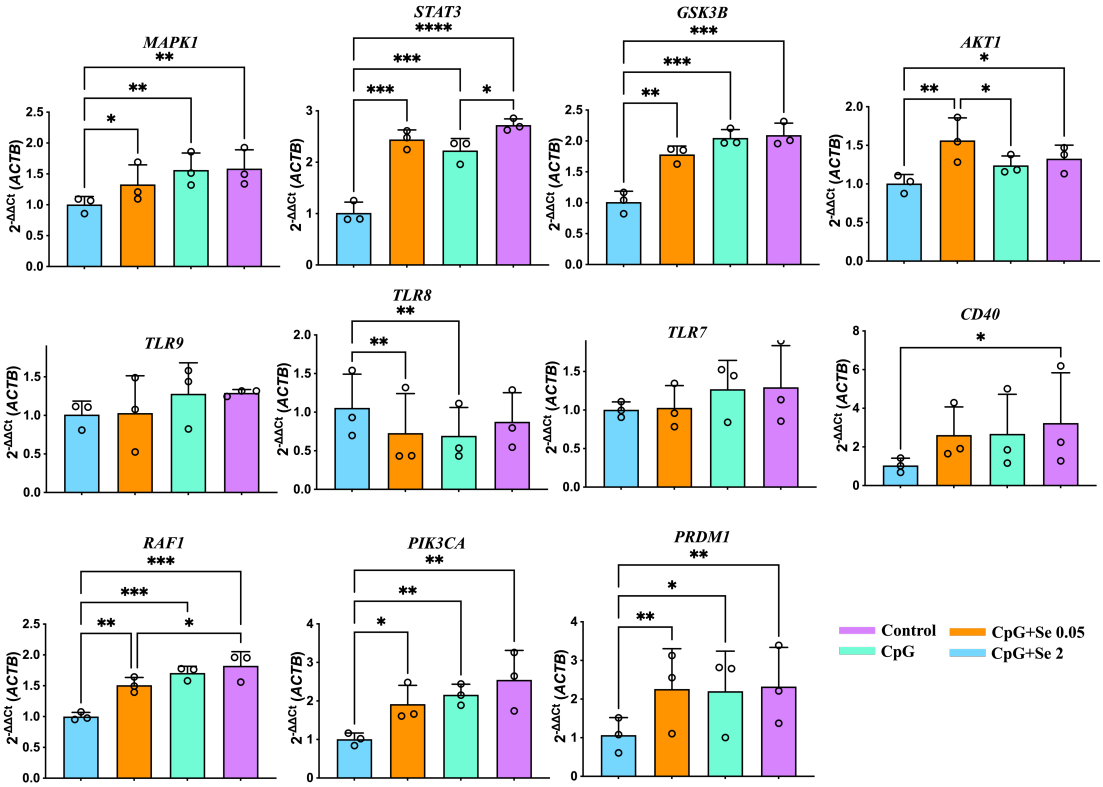
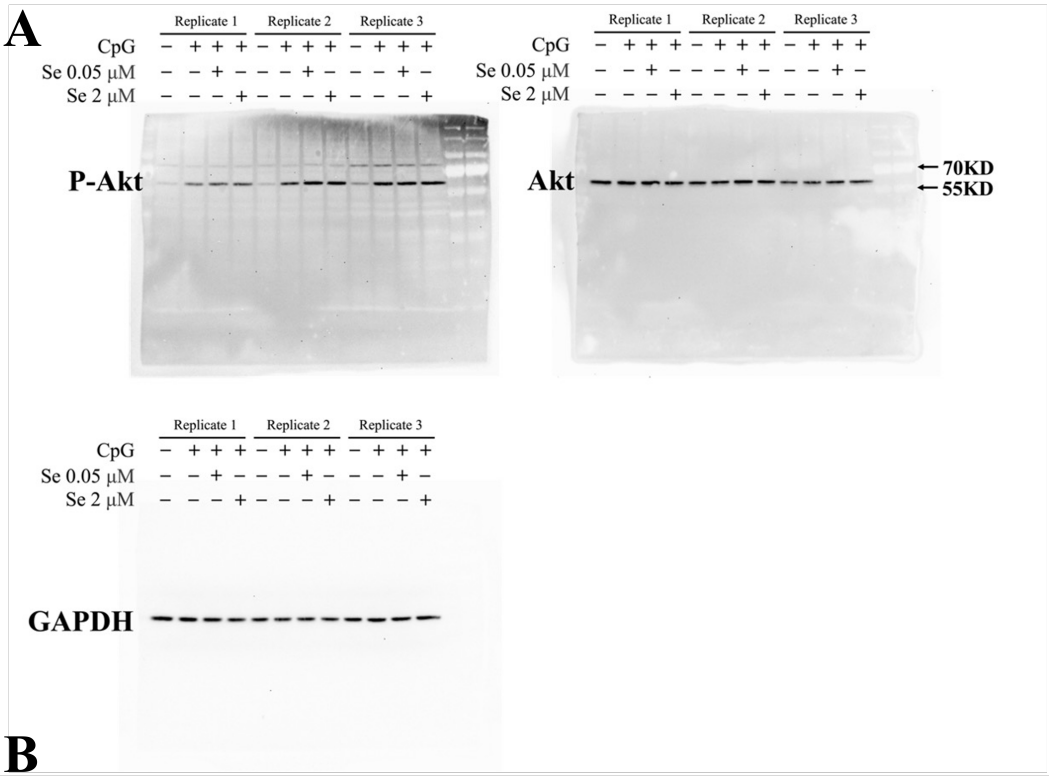


Figure S9 Comparison of mRNA expression of signaling molecules (*MAPK1, STAT3, GSK3B, AKT1, TLR7, TLR8, TLR9, CD40, RAF1, PIK3CA, PRDM1*) involved in MAPK, PI-3K, and STAT3 pathways in B cells upon different treatment conditions. P values were calculated by a one-way ANOVA test to evaluate the significant change among groups. 0.01<(*)P<0.05, 0.001<(**)P<0.01, 0.0001<(***)P<0.001, (****)P<0 .0001.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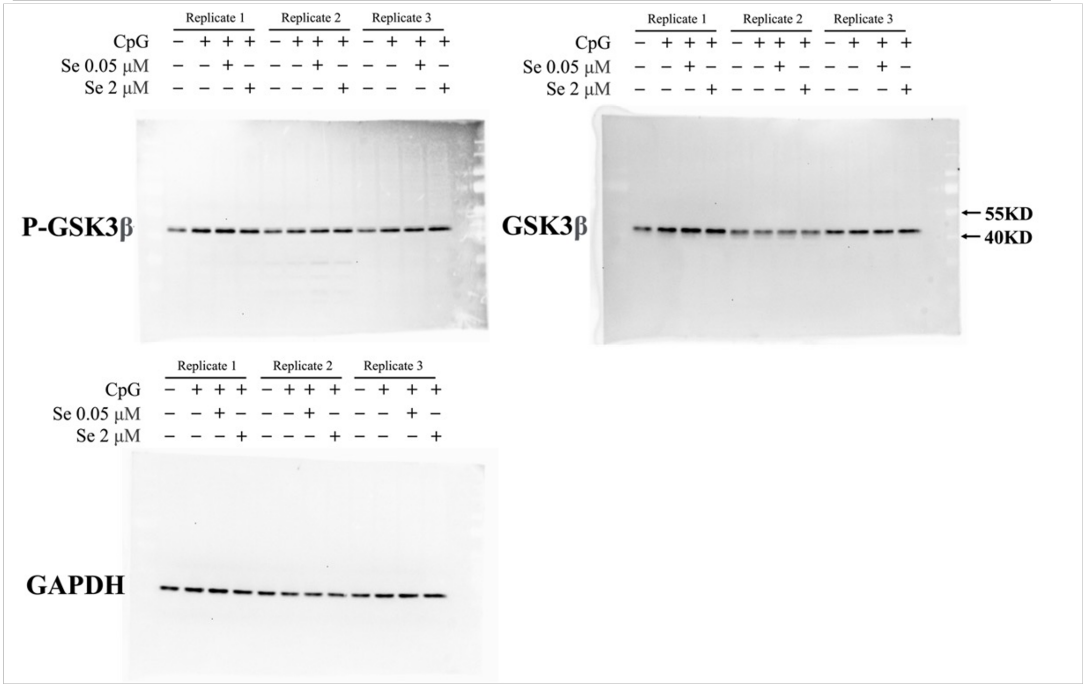
Replicate 2
Replicate 3
CpG
Se 0.05 μM
Se 2 μM
P-Akt
Akt
70KD
55KD
GAPDH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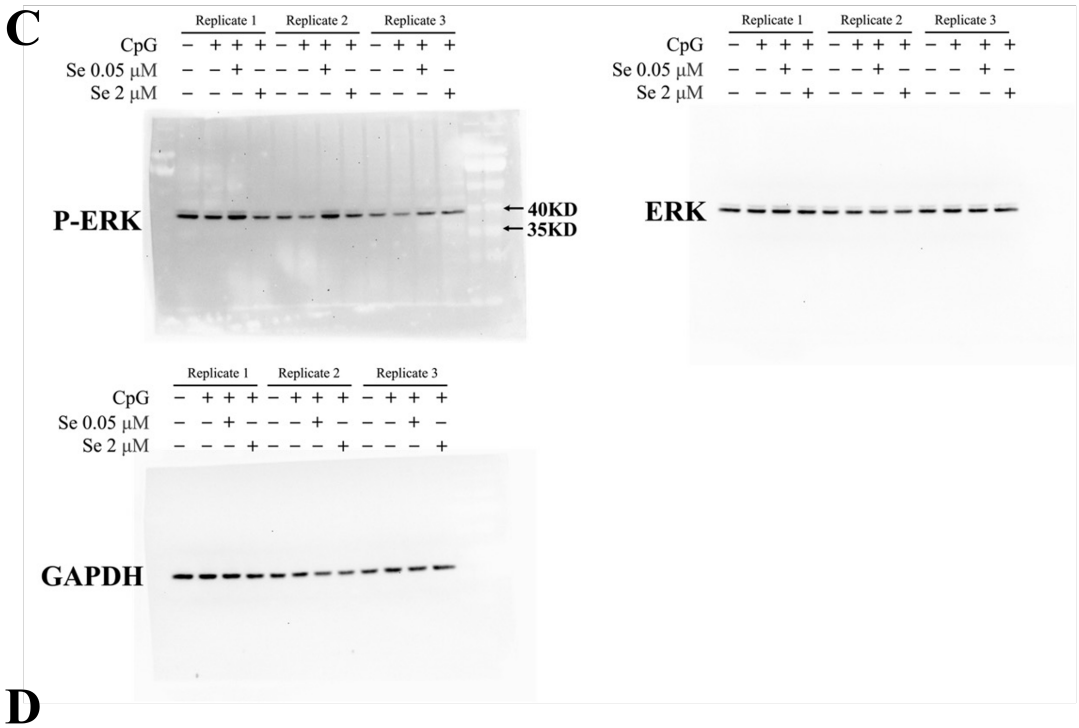

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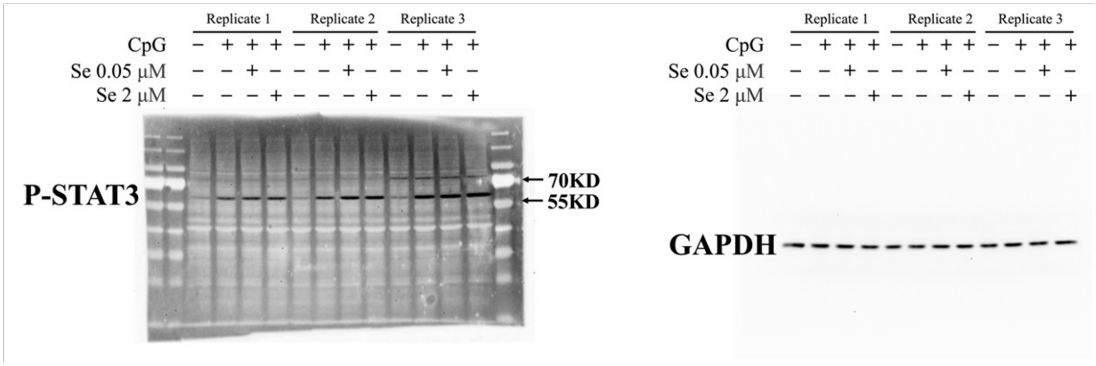


Figure S10 Protein expression of Akt/P-Akt (A), ERK/P-ERK (B), Gsk3β/ P-Gsk3β (C), P-STAT3 (D) and corresponding internal standard (GAPDH) in B cells upon different treatment conditions.

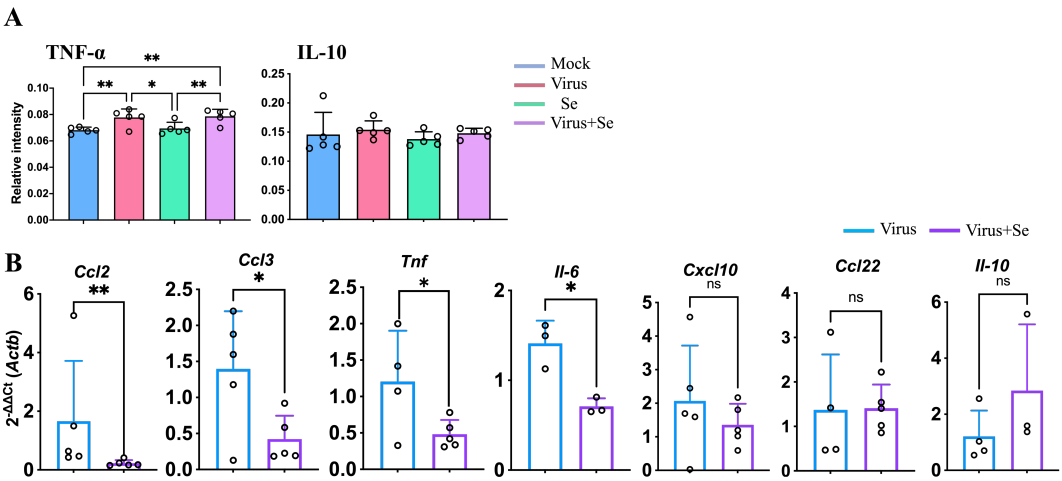
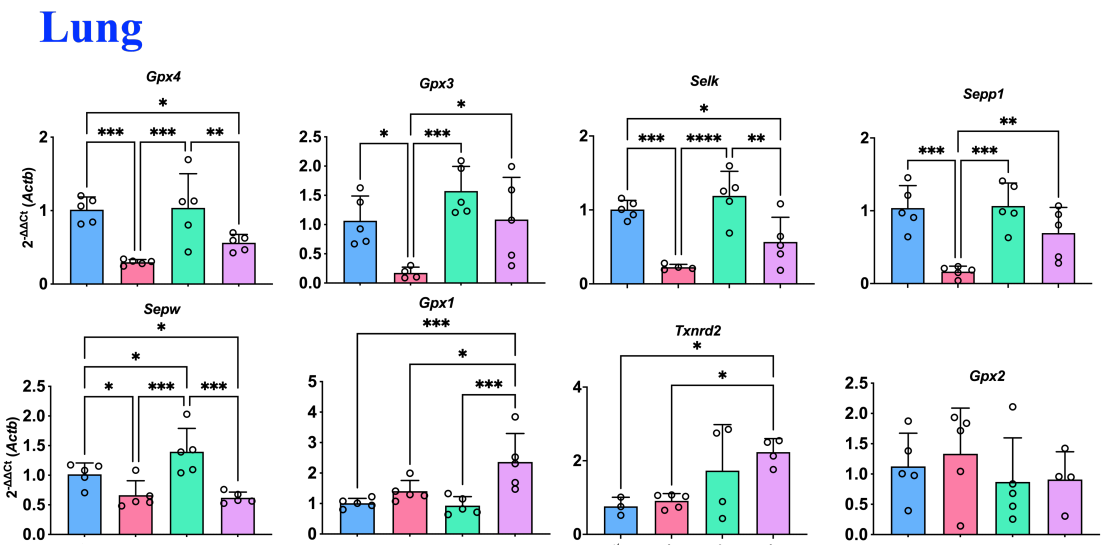
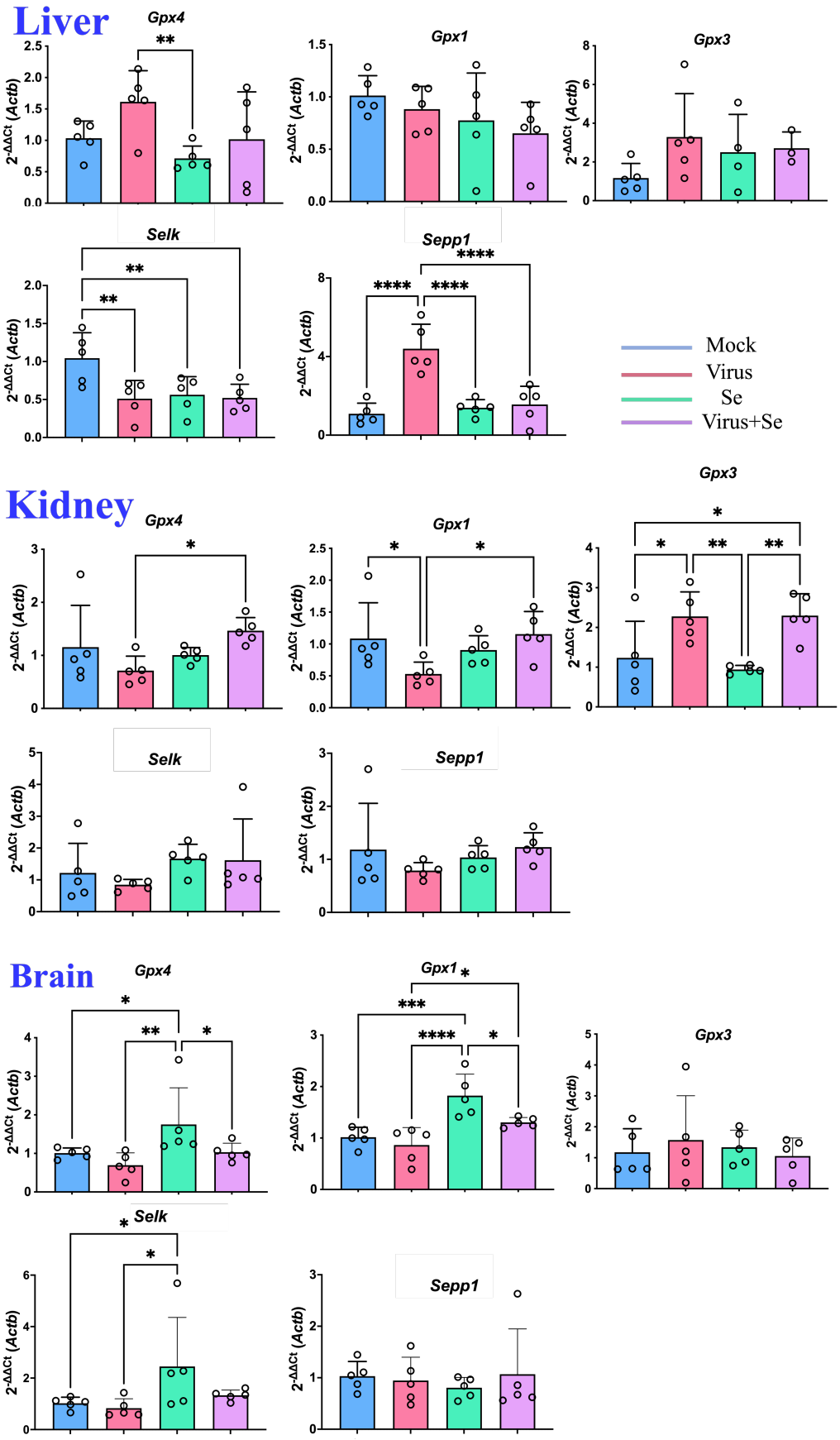


Figure S11 (A) Comparison of the cytokine (TNF-α and IL-10) level in the mouse serum under different treatment conditions. (B) Cytokine and chemokine mRNA levels in lung tissues of virus-infected mice with or without selenium pretreatment. 0.01<(*)P<0.05, 0.001<(**)P<0.01.

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Selk
Sepp1
Mock
Virus
Se
Virus+Se
Kidney
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Sepp1
2-ΔΔCt (Actb)

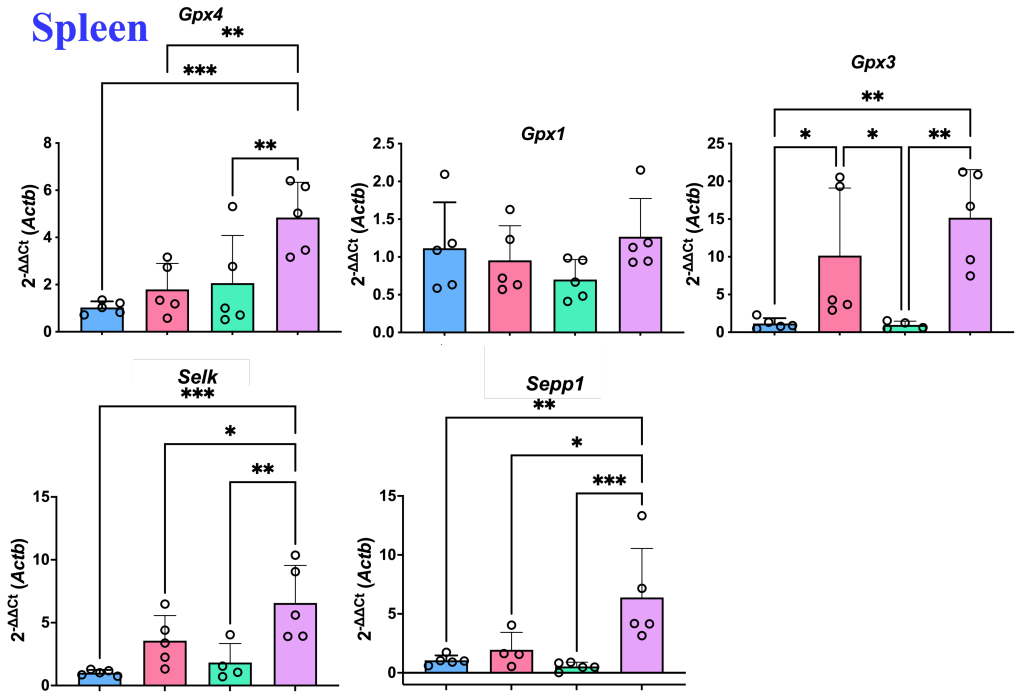

Figure S12 Comparison of mRNA levels of different selenoproteins in the lung, liver, kidney, brain and spleen of mice under different experimental conditions. *Mock*: non-infected control; *Virus*: virus infection without Se treatment; *Se*: treatment with 0.2 mg/kg selenium without virus infection and *Virus+Se*: virus infection with pre-supplement of 0.2 mg/kg selenium. P values were calculated by the ANOVA test to evaluate the statistical significance of variations among groups. (*) 0.01< P < 0.05, (**) 0.001< P< 0.01, (***) 0.0001< P< 0.001, (****) P < 0.0001.

Table S1. The clinical characteristics (gender, age, collection date, infection status) of COVID-19 cases included in this study.

Sex (M: male; F: female)	Age	Day of symptom onset	Severity
M	61	17	Mild
M	62	12	Mild
F	64	10	Moderate
M	81		Mild
M	53		Moderate
M	71	10	Mild
F	51	12	Mild
F	29	14	Mild
M	60	13	Mild
M	66	13	Mild
M	78	13	Death
F	64	12	Mild
M	13	13	Mild
F	75		Mild
F	54	10	Mild
F	40		Mild
F	40	9	Mild
F	32		Mild
M	39	10	Mild
M	74	3	Mild
M	59	14	Moderate
F	60	14	Mild
F	48	11	Mild
M	31		Mild
F	32		Mild
M	55		Moderate
M	25	11	Mild
M	48	14	Moderate
F	54	10	Mild
M	82	22	Mild
M	54	10	Mild
M	31	10	Moderate
M	67	5	Mild
F	44	13	Mild
M	65		Mild
M	59	14	Moderate
M	62	10	Mild

F	20	37	Mild
F	50	11	Mild
M	31		Mild
F	37		Mild
M	37		Mild
F	58	10	Mild
M	43	8	Moderate
M	60	11	Moderate
M	60	17	Severe
M	43	22	Mild
M	45	10	Mild
M	36	12	Severe
M	59	8	Mild
F	30	7	Mild
F	22		Mild
M	69		Mild
F	80	15	Moderate
F	42	10	Mild
M	55	14	Moderate
M	21	8	Mild
M	46	12	Moderate
F	52	13	Moderate
F	73		Mild
F	48	13	Moderate
F	91	16	Mild
M	27	9	Mild
F	11	17	Mild
M	1	27	Mild
F	46		Mild
F	46	3	Mild
M	49		Mild
M	53	13	Mild
M	45	17	Severe
F	66		Mild
F	80	17	Mild
F	44	7	Mild
F	8	23	Mild
F	60	8	Mild
M	63	3	Mild
F	40		Mild
F	55	6	Mild
F	32		Mild
F	41		Mild
F	17		Mild

F	29		Mild
M	30		Mild
F	5		Mild
M	30		Mild
F	49		Mild
M	25		Mild
F	63		Mild
M	59		Mild
F	49		Mild
F	42		Mild
M	78	4	Severe
F	67	2	Severe
F	76	2	Severe
M	79	3	Severe
M	68	2	Severe
F	70	7	Severe
F	66	3	Severe
M	71	2	Severe
M	70	3	Severe
M	72	5	Severe
F	68	4	Severe
M	36	6	Severe
M	78	4	Severe

Table S2. Operating conditions for metal(loid)s quantification by ICP-MS

RF power (W)	1550
RF Matching (V)	1.80
Sampling Depth (mm)	8.0
Carrier Gas (L/min)	1.05
S/C Temp (°C)	2
Makeup Gas (L/min)	0
Extract 1 (V)	0
Extract 1 (V)	-195
Omega Bias (V)	-80
Omega Lens (V)	8.0
Cell Entrance (V)	-30
Cell Exit (V)	-50
Deflect (V)	12.0
Plate Bias (V)	-40
He Gas (mL/min)	1.0
OctP Bias (V)	-8.0
OctP RF (V)	160
Energy Discrimination (V)	4.5

Table S3. Summary of detection antibodies used in this study

Antibody	Metal tags	Clone	Manufacturer
IL-6	^{145}Nd	973115	R&D
MMP8	^{163}Dy	100608	R&D
IFN-γ	^{169}Tm	NA	R&D
IFN-α	^{159}Tb	NA	Abcam
IL-1β	^{151}Eu	NA	Abcam
TNF-α	^{167}Er	NA	Abcam
MMP9	^{149}Sm	NA	Abcam
IL-10	^{175}Lu	NA	Abcam
CRP	^{159}Tb	987313	R&D

Table S4. Summary of capture antibodies

Antibody	Clone	Manufacturer
IL-6	973132	R&D
MMP8	100619	R&D
IFN-γ	K3.53	R&D
IFN-α	NA	Abcam
IL-1β	NA	Abcam
TNF-α	NA	Abcam
MMP9	NA	Abcam
IL-10	NA	Abcam
CRP	987313	R&D

Table S5. Operating conditions for metal quantification by ICP-MS for proteome profiling

RF power (W)	1550
RF Matching (V)	1.80
Sampling Depth (mm)	8.0
Carrier Gas (L/min)	1.05
S/C Temp (°C)	2
Makeup Gas (L/min)	0
Extract 1 (V)	0
Extract 1 (V)	-195
Omega Bias (V)	-80
Omega Lens (V)	8.0
Cell Entrance (V)	-30
Cell Exit (V)	-50
Deflect (V)	12.0
Plate Bias (V)	-40
OctP Bias (V)	-8.0
OctP RF (V)	160
Energy Discrimination (V)	4.5

Table S6. Antibody panel used in mass cytometry

Antibody	Metal tags	Manufacturer
CD45	^{154}Sm	Fludigm
CD3	^{170}Er	Fludigm
CD4	^{174}Yb	Fludigm
CD8	^{168}Er	Fludigm
CD14	^{160}Gd	Fludigm
CD20	^{147}Sm	Fludigm
PD1	^{165}Ho	Biolegend
IL-6	^{172}Yb	Biolegend
IFN-γ	^{155}Gd	Biolegend
TNF-α	^{169}Tm	Biolegend
IL-10	^{145}Nd	Biolegend
SEPP1	^{155}Gd	Life Technology

Table S7. Primer sequences of selected genes studied in RT-PCR (5'-3'). All the primers were searched from the PrimerBank website.

	Species	Forward Primers	Reverse Primers
Gpx1	Mouse	AGTCCACCGTGTATGCCTTCT	GAGACGCGACATTCTCAATGA
Gpx2	Mouse	GCCTCAAGTATGTCCGACCTG	GGAGAACGGGTCATCATAAGGG
Gpx3	Mouse	CCTTTTAAGCAGTATGCAGGCA	CAAGCCAAATGGCCCAAGTT
Gpx4	Mouse	TGTGCATCCCGCGATGATT	CCCTGTACTTATCCAGGCAGA
Sepp1	Mouse	AGCTCTGCTTGTTACAAAGCC	CAGGTCTTCCAATCTGGATGC
Selk	Mouse	GTTTACATCTCGAATGGTCAGGT	CCCTCTTCCATCGTCGTATCTG
Selw	Mouse	GCCGTTCGAGTCGTGTATTGT	CACTTCAAAGAACCCGGTGAC
Txnrd1	Mouse	CCCACTTGCCCCAACTGTT	GGGAGTGTCTTGGAGGGAC
Txnrd2	Mouse	GATCCGGTGGCCTAGCTTG	TCGGGGAGAAGGTTCCACAT
Tnf	Mouse	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Ccl2	Mouse	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Cxcl10	Mouse	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Ccl3	Mouse	TTCTCTGTACCATGACACTCTGC	CGTGGAATCTTCCGGCTGTAG
Il-6	Mouse	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Il-10	Mouse	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Ccl22	Mouse	AGGTCCCTATGGTGCCAATGT	CGGCAGGATTTTGAGGTCCA
IL-6	Human	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
IL-10	Human	GACTTTAAGGGTTACCTGGGTTG	TCACATGCGCCTTGATGTCTG
TNF	Human	GGGTTGGGCAACAAGTATGTC	GGTGTCATCTCGGAGGTAATTCA
IFNG	Human	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
SELK	Human	ATGGTTTACATCTCGAACGGAC	AACTCAGCTATTCCCCAGAAGA
GPX4	Human	GAGGCAAGACCGAAGTAAACTAC	CCGAACTGGTTACACGGGAA
SEPP1	Human	AAAGCTCCTTATGTAAGCAACCC	ACAGTCACTGAACCATTGGAGT
TXNRD1	Human	ATATGGCAAGAAGGTGATGGTCC	GGGCTTGTCCTAACAAAGCTG
GPX1	Human	CAGTCGGTGTATGCCTTCTCG	GAGGGACGCCACATTCTCG
TLR9	Human	CTGCCTTCCTACCCTGTGAG	GGATGCGGTTGGAGGACAA
TLR7	Human	TCCTTGGGGCTAGATGGTTTC	TCCACGATCACATGGTTCTTTG
TLR8	Human	ATGTTCCTTCAGTCGTCAATGC	TTGCTGCACTCTGCAATAACT
CD40	Human	ACTGAAACGGAATGCCTTCCT	CCTCACTCGTACAGTGCCA
GSK3B	Human	GGCAGCATGAAAGTTAGCAGA	GGCGACCAGTTCTCCTGAATC
STAT3	Human	CAGCAGCTTGACACACGGTA	AAACACCAAAGTGGCATGTGA
MAPK1	Human	TACACCAACCTCTCGTACATCG	CATGTCTGAAGCGCAGTAAGATT
AKT1	Human	AGCGACGTGGCTATTGTGAAG	GCCATCATTCTTGAGGAGGAAGT
PIK3CA	Human	CCACGACCATCATCAGGTGAA	CCTCACGGAGGCATTCTAAAGT
PRDM1	Human	AAGCAACTGGATGCGCTATGT	GGGATGGGCTTAATGGTGTAGAA
RAF1	Human	GGGAGCTTGGAAGACGATCAG	ACACGGATAGTGTTGCTTGTC

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