

Divergent responses to SARS-CoV-2 infection reveal brain resilience-associated astrocyte programs in centenarians

Danyllo Oliveira

Universidade de São Paulo

Amanda Assoni

Universidade de São Paulo

Luis Ariel Espinosa-Rodríguez

Universidade Federal do Rio de Janeiro

Mauricio Quiñones-Vega

Universidade Federal do Rio de Janeiro

Joyce Esposito

Universidade de São Paulo

Raul Hernandez Bortolin

Universidade de São Paulo

Ana Paula Paiva

Universidade de São Paulo

Monize Valeria Silva

Universidade de São Paulo

Letícia Rocha da Silva

Universidade de São Paulo

Ma Hui Ling

Universidade de São Paulo

Sabrina Komatsu

Universidade de São Paulo

Lara Pacheco

Universidade de São Paulo

Kayque Telles Silva

Universidade de São Paulo

Fernanda Chianca

Universidade de São Paulo

André Costa

Universidade de São Paulo

Maria Fernanda Castro-Amarante

Universidade de São Paulo

Mariângela Silva

Universidade de São Paulo

Luís Carlos Ferreira

Universidade de São Paulo

Luana Mendonça de Oliveira

Universidade de São Paulo

Leticia Martins

Universidade de São Paulo

Anna Julia Pietrobon

Universidade de São Paulo

Maria Notomi Sato

Universidade de São Paulo

Fábio Nogueira

Universidade de São Paulo

Mateus Vidigal de Castro

mateusvcastro@gmail.com

Universidade de São Paulo

Mayana Zatz

Universidade de São Paulo

Gilberto Domont

Universidade Federal do Rio de Janeiro

Research Article

Keywords: COVID-19, SARS-CoV-2, Astrocytes, Centenarians, Proteomics

Posted Date: August 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10391383/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

**“Divergent responses to SARS-CoV-2 infection reveal
brain resilience-associated astrocyte programs in centenarians”**

Danyllo Oliveira^{1,6+}, Amanda Assoni¹⁺, Luis Ariel Espinosa-Rodríguez²⁺, Mauricio Quiñones-Vega², Joyce Esposito¹, Raul Hernandez Bortolin¹, Ana Paula Paiva¹, Monize Valeria Silva¹, Letícia Rocha da Silva¹, Ma Hui Ling¹, Sabrina Komatsu¹, Lara Pacheco¹, Kayque Telles Silva¹, Fernanda Chianca¹, André Costa¹, Maria Fernanda Castro-Amarante¹, Mariângela Silva³, Luís Carlos Ferreira³, Luana Mendonça de Oliveira⁴, Letícia Martins⁴, Anna Julia Pietrobon⁴, Maria Notomi Sato⁴, Fábio Nogueira^{2,5}, Mateus Vidigal de Castro^{1*}, Mayana Zatz^{1*} and Gilberto Domont^{2*}

+ Contributed equally to the manuscript

* Corresponding Authors: Mateus Vidigal de Castro, PhD. Human Genome and Stem Cell Research Center, University of Sao Paulo, Brazil (mateusvidigal@usp.br). Mayana Zatz, PhD. Human Genome and Stem Cell Research Center, University of Sao Paulo, Brazil (mayazatz@usp.br). Gilberto Domont, PhD. Proteomics Unit, Department of Biochemistry, Institute of Chemistry and Research Center on Precision Medicine, Universidade Federal do Rio de Janeiro, Cidade Universitaria, Rio de Janeiro, Brazil (gilberto@iq.ufRJ.br);

Institutions:

¹Human Genome and Stem Cell Research Center, University of São Paulo, São Paulo, Sao Paulo, Brazil;

²Proteomics Unit, Department of Biochemistry, Institute of Chemistry and Research Center on Precision Medicine, Universidade Federal do Rio de Janeiro, Cidade Universitária, Rio de Janeiro, Brazil;

³Institute of Biomedical Sciences (ICB), University of São Paulo, São Paulo, SP, Brazil;

⁴Laboratory of Dermatology and Immunodeficiency Research, LIM 56, Tropical Medicine Institute, Faculty of Medicine, University of São Paulo, São Paulo, SP, Brazil;

⁵Laboratory of Proteomics (LabProt), LADETEC, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

⁶Department of Pathological Sciences, Santa Casa School of Medical Sciences, Sao Paulo, Sao Paulo, Brazil

ORCID

Luis Ariel Espinosa-Rodríguez: <https://orcid.org/0000-0002-6363-855X>

Fabio C.S. Nogueira: <https://orcid.org/0000-0001-5507-7142>

Gilberto B. Domont: <https://orcid.org/0000-0002-1329-6483>

Summary

Astrocytes were generated from PBMC-derived iPSCs of centenarians (≥ 100 years) who recovered from asymptomatic to moderate COVID-19 and from adults (< 45 years) who experienced critical disease. Cells were infected with either the ancestral Wuhan SARS-CoV-2 or the more virulent Gamma (P.1) variant and analyzed 48 hours post-infection. Despite comparable infection rates across groups, astrocytes infected with the P.1 variant released higher levels of viral particles than the ancestral Wuhan, indicating strain-dependent differences in post-entry viral dynamics. Notably, centenarian-derived astrocytes exhibited a more restrained inflammatory response, with reduced secretion of CXCL8 with Wuhan infection and CXCL10 with P.1 infection. Quantitative proteomic profiling revealed extensive infection-driven remodeling of the astrocyte proteome, with a largely conserved global response across all groups. However, centenarian astrocytes showed reduced detection of antigen presentation-related proteins and distinct regulation of metabolic pathways, whereas astrocytes from severe cases displayed broader dysregulation of stress- and immune-related processes and increased NRP1 abundance without corresponding changes in infection susceptibility. These differences may reflect intrinsic cellular states associated with resilience and contribute to a more controlled response to the viral challenge in centenarians.

Keywords: COVID-19; SARS-CoV-2; Astrocytes; Centenarians; Proteomics.

Introduction

The COVID-19 pandemic has revealed a highly heterogeneous clinical landscape, with outcomes ranging from asymptomatic infections to death [1]. Older adults, particularly those over 65 years of age, have consistently been at the highest risk for severe disease, hospitalization, and mortality[2]. In contrast, younger individuals have generally exhibited milder disease courses, although exceptions with severe cases also occurred[3]. Among the elderly, factors such as the presence of multiple comorbidities, senescence and chronic low-grade inflammation ("inflammaging") are believed to contribute to the heightened vulnerability to SARS-CoV-2 infection [4]. However, some extraordinary cases defy this general trend. A subset of centenarians worldwide — individuals traditionally considered extremely vulnerable — demonstrated unexpected resilience, surviving COVID-19 with mild symptoms or even asymptomatic infections, before the availability of vaccines [5–7]. Understanding the biological mechanisms underlying such resilience could unveil novel protective pathways against viral infections and age-related immune decline.

Apart from typical respiratory alterations, patients affected by SARS-CoV-2 frequently develop neurological symptoms [8]. They might range from mild clinical presentations, such as headache and anosmia to severe phenotypes, notably encephalopathy and seizures. Although the underlying mechanisms associated with them are not completely understood, they are most likely multifactorial and caused by endothelial dysfunction, microvascular thrombosis and blood-brain barrier dysfunction[9].

Interestingly, these alterations give rise to increased vascular permeability and infiltrators of the immune system in the Central Nervous System (CNS), leading to tissue injury and glial stress. In association with hypoxia of brain parenchyma caused by cerebrovascular thrombosis, these pathological events lead to multiple neurological symptoms frequently found in SARS-CoV-2 infection[8–10].

Astrocytes are highly important components of CNS, being associated with brain energetic balance, neuroimmune regulation and, in association with vascular components, the blood brain barrier maintenance. Astrocytes are the first CNS cells to be targeted by viruses during neuro infection being responsible for launching innate immune responses leading to secretion of cytokines and neuroinflammation [11]. Astrocytes undergo activation during such pathological processes, as reflected by elevated circulating levels of glial fibrillary

acidic protein [8,12]. Indeed, recent evidence demonstrated that disruption of astrocyte homeostatic functions by SARSCoV-2 may contribute to the neurological disorder associated with COVID-19 [13].

In this study, we adopted a reverse approach compared with most COVID-19 research: instead of focusing solely on susceptibility, we examined individuals at both extremes of clinical outcomes—resilient long-lived centenarians who recovered from COVID-19 and young adults who experienced severe disease, both unvaccinated. Using SARS-CoV-2 infection in iPSC-derived astrocytes generated from PBMCs of these individuals, we sought to uncover differences in inflammatory responses that may reveal mechanisms underlying healthy aging and resilience to viral insults, using an integrative framework combining viral quantification assay, flow cytometry, chemokine profiling, and quantitative proteomics.

Materials and Methods

Volunteer Recruitment, Blood Collection, and Sample Processing

Induced pluripotent stem cells (iPSCs) used in this study were obtained from a previously established cell biobank derived from the “Resilient Brazilian Super Elderly” cohort[14], a unique resource comprising individuals who recovered from COVID-19 prior to vaccination. These iPSC lines were originally generated from peripheral blood mononuclear cells (PBMCs) collected in 2020, before the implementation of vaccination programs and the emergence of SARS-CoV-2 variants of concern.

For the present study, four iPSC lines derived from centenarians were selected and used to generate astrocytes for downstream functional assays. A comparative group consisting of three iPSC lines derived from unvaccinated adults under 45 years who experienced critical COVID-19 requiring intubation was included. Baseline characteristics of these individuals are presented in **Table 1**.

Human astrocytes were generated from iPSCs following a previously established two-step differentiation protocol over four weeks [15]. Additional methodological details are provided in the **Supplemental Material**. Differentiated cells were subsequently characterized by immunofluorescence and gene expression analysis. Astrocyte identity was confirmed by the expression of the astroglial marker GFAP at both protein and transcript levels. In addition,

the expression of SARS-CoV-2 entry factors, including ACE2 and neuropilin-1 (NRP1), was assessed by immunofluorescence. Representative images and quantitative analyses are shown in **Fig. 1**.

SARS-CoV-2 strains and *in vitro* infection

The SARS-CoV-2 Wuhan strain (2020) and the highly virulent Gamma (P.1) variant were generously provided by Edison L. Durigon and used for *in vitro* infection assays. Viral stocks were propagated in Vero cells as previously described [16], and titers were determined by 50% tissue culture infective dose (TCID₅₀) assays. All experiments involving SARS-CoV-2 were conducted under biosafety level 3 (BSL-3) conditions at the Institute of Biomedical Sciences, University of São Paulo, Brazil, in accordance with WHO Laboratory Biosafety Guidance for novel coronavirus (2019-nCoV). Human iPSC-derived astrocytes were infected with SARS-CoV-2 [Wuhan or Gamma (P.1)] at a fixed multiplicity of infection (MOI) of 1 in all experiments. During the initial adsorption phase (1 hour), a minimal volume of viral inoculum was applied to maximize cell–virus contact, and plates were maintained at room temperature. Following virus adsorption, cells were thoroughly washed and incubated in an astrocyte culture medium for 48 hours under standard conditions (37 °C, 5% CO₂). Mock-infected cells were included as controls. After 48 hours, cells were harvested for downstream analyses.

Cell Staining for Detection of SARS-CoV-2 Spike Protein by Flow Cytometry

After 48 hours of infection, human iPSC-derived astrocytes were dissociated using Accutase (Thermo Fisher, Catalog A1110501) for 5 minutes at 37°C, collected, and washed twice with PBS (pH 7.4). Cells were fixed with 4% paraformaldehyde (Invitrogen) for 15 minutes at room temperature and washed twice with PBS. Permeabilization was performed using PBS containing 0.1% Triton X-100 (Invitrogen) for 15 minutes at room temperature. Cells were then washed with a washing buffer (PBS + 1% BSA, Sigma). To block nonspecific binding, cells were incubated for 30 minutes at room temperature with PBS containing 5% BSA. Staining was performed using a SARS-CoV-2 Spike Protein recombinant rabbit monoclonal antibody (Thermo Fisher, Catalog 703959) diluted 1:500 in PBS + 1% BSA for 1 hour at 4 °C. After three washes with PBS, cells were incubated with an Alexa Fluor 647 goat anti-rabbit IgG secondary antibody (Thermo Fisher, Catalog A32733) diluted 1:1000 for 45 minutes at 4 °C in the dark. Cells were then resuspended in PBS containing 1% BSA and immediately

analyzed using a BD FACSymphony™ A1 flow cytometer (BD Biosciences). A minimum of 10,000 events was acquired per sample. Positivity thresholds were defined using isotype controls and uninfected cells. Data analysis was performed using FlowJo software v10.8 (BD Biosciences), with quantification of spike-positive (Spike+) cells based on fluorescence intensity in the BB515-A channel.

SARS-CoV-2 Viral Particle Quantification by ELISA

The quantification of SARS-CoV-2 viral particles in the culture supernatant of infected astrocytes was performed using a commercial enzyme-linked immunosorbent assay (ELISA) kit targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (Thermo Fisher, Catalog. EH492RB), following the manufacturer's instructions. Briefly, culture supernatants were collected 48 hours post-infection, centrifuged to remove cell debris, and applied to 96-well plates pre-coated with capture antibodies specific for the SARS-CoV-2 RBD. After incubation and washing, detection was performed using an HRP-conjugated secondary antibody and TMB substrate. The reaction was stopped with the provided stop solution, and absorbance was measured at 450 nm using a microplate reader (BioTek Synergy HTX, Agilent). A standard curve generated with known concentrations of recombinant RBD protein was used to quantify viral particle equivalents in each sample. Data represents relative levels of RBD-containing viral particles in the culture supernatants, expressed as pg/mL.

Measurement of chemokines by cytometric bead array

The levels of CCL2/MCP-1, CCL5/RANTES, CXCL8/IL-8, and CXCL10/IP-10 chemokines in the culture supernatants of iPSC-derived human astrocytes were quantified using the Cytometric Bead Array (CBA) with a commercial kit (BD Biosciences, Catalog. 552990), following the manufacturer's instructions. Supernatants were collected 48 hours after SARS-CoV-2 infection (MOI = 1.0). Data acquisition was performed on a BD LSRFortessa™ Cell Analyzer (BD Biosciences), and the results were analyzed using FCAP Array Software v3.0 (BD Biosciences). Data represents the absolute concentrations of chemokines in the culture supernatants, expressed in pg/mL.

Proteomics assay

Proteomics profiling was performed using iPSC-derived human astrocytes, as detailed in the **Supplemental Material**. Cell pellets (1×10^6 cells) were lysed in SDS-based buffer under

reducing and denaturing conditions, followed by sonication, alkylation, and protein digestion with sequence-grade trypsin using S-Trap mini filters. Peptides were desalted, quantified, normalized for the concentration across samples, and pooled for quality control prior to analysis. Equal amounts (250 ng) of peptides were analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a Vanquish Neo (Thermo Scientific) system coupled to an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific), operating in data-independent acquisition (DIA) mode with variable isolation windows. Raw data were processed using DIA-NN in a library-free workflow, followed by the generation of an experimental spectral library and protein inference at a false discovery rate (FDR) of 1%. Downstream statistical and bioinformatic analyses were performed in R using established proteomics pipelines for normalization, differential expression, pathway enrichment, and network analysis. Outlier exclusion criteria were defined based on deviation across multiple Quality Control metrics rather than a single parameter. A detailed description of protein extraction, mass spectrometry acquisition parameters, data processing, and bioinformatic analyses is provided in the **Supplemental Material**.

Results

Astrocyte infection with the virulent P.1 variant leads to increased viral particle release, with differential chemokine responses

To determine whether astrocytes from centenarians and younger severely affected adults differ in their susceptibility to SARS-CoV-2 infection, we quantified infection rates and viral output across tested experimental conditions. Flow cytometry at 48 hours post-infection revealed that iPSC-derived astrocytes were permissive to infection, with a relatively low proportion (~10%) of spike-positive cells. Infection rates were similar across age groups and viral strains (**Fig. 2c, d**), indicating comparable susceptibility to viral entry and initial infection. Despite this, infection with the Gamma (P.1) variant resulted in a significant increase in viral particle release, measured indirectly by the amount of the RBD portion of the Spike protein, compared to the Wuhan strain, irrespective of age group (**Fig. 2e**). These findings suggest that while astrocyte susceptibility is largely conserved, strain-dependent differences emerge for viral replication and/or release, providing a functional basis for downstream host response differences.

Given the similar infection rates observed across groups, we next assessed whether astrocytes differ in their inflammatory responses to SARS-CoV-2 infection. Chemokine quantification revealed no significant changes in CCL2 or CCL5 across conditions (**Fig. 2f**). In contrast, CXCL8 and CXCL10 exhibited selective modulation, particularly in centenarian-derived astrocytes. CXCL8 levels were reduced following Wuhan infection, whereas CXCL10 was decreased after Gamma infection, indicating a context-dependent attenuation of inflammatory signaling. Together, these results indicate that despite comparable infection levels, centenarian astrocytes mount a more restrained and selective inflammatory response, suggesting intrinsic differences in immune regulation that may contribute to resilience.

Proteomics profiling of SARS-CoV-2–infected astrocytes reveals infection-driven clustering independent of age and viral strain

To gain a systems-level understanding of the molecular responses underlying the observed phenotypic differences, we performed quantitative proteomic profiling of SARS-CoV-2–infected astrocytes. A DIA-MS proteomics workflow (**Fig. 3a**) enabled robust quantification of changes in protein abundance associated with infection and age. Protein extracts were processed using S-Trap digestion, analyzed by LC-MS/MS, and computationally processed using DIA-NN [17] and downstream statistical analysis using the MS-DAP pipeline [18] for data filtering, normalization and differential analysis.

During the first analysis using MS-DAP, three control samples were identified as outliers and flagged as “excluded” in the sample metadata (**Supp. Table 1**) based on multiple orthogonal quality control metrics (identifications, abundance distribution, coefficient of variation, PCA, Pearson correlation), where they showed consistent deviation from the main cluster of control samples (**Supp. Fig. S2**). These samples also exhibited an atypically high number of identified protein groups compared to retained controls, suggesting potential technical variability. Importantly, their exclusion improved overall data consistency without affecting the observed main biological trends.

A second analysis in the MS-DAP was performed using 18 samples distributed into five groups (Control or Ctrl, n=4; Gamma_adults or Ga, n=3; Gamma_centenarians or Gc, n=4; Wuhan_adults or Wa, n=3; and Wuhan_centenarians or Wc, n=4). A quality control pool consisting of a peptide mixture of all samples was included throughout the analysis,

demonstrating technical reproducibility with a Pearson correlation from 0.97 to 0.99 (**Supp. Fig. S3**) and low Coefficient of Variation (CoV) at peptide and protein-level (mean CoV 11.7% and 6.7%, **Supp. Fig. S2b**), respectively.

A total of 7,043 protein groups were identified across all samples, with individual identifications ranging from 3,980 to 6,405 protein groups (**Fig. 3b**), corresponding to 19,673 to 53,898 peptides (**Supp. Fig. S3b**). Label-free quantification ranged from 2,892 to 5,209 protein groups (**Fig. 3c**; 5,574 proteins in total), corresponding to 11,857 to 33,984 peptides (**Supp. Fig. S3c**) in 75% of the samples filtered “by contrast” between two analyzed groups. After applying a “global” filtering, we found that 2,724 proteins (**Fig. 3c**), corresponding to 11,026 peptides (**Supp. Fig. S3c**), were quantified across all groups. The coefficient of variation across the five groups ranged from 22.3% to 30.7% for peptides (**Supp. Fig. S3d**) and 22.4% to 25.9% for proteins (**Supp. Fig. S3e**). Principal component analysis (**Figure 3D**) showed that the first component (PC1, 63.0%) accounted for most of the variability, clearly separating the viral infected cells from the Ctrl group. The second component (PC2, 11.6%) did not reveal clear distinctions based on viral strain or patient age. Together, PC1 and PC2 explained 74.6% of the total variance. Pearson correlation of samples (**Supp. Fig. S3f**) confirmed the presence of two distinct clusters corresponding to Ctrl and all infected groups.

The dynamic range of the 2,724 quantified proteins across all samples (**Fig. 3e**) spanned six orders of magnitude, with histones (H2AC4, H2AX), elongation factor 1 (EEF1A2), galectin-1 (LGALS1), the metabolic enzyme lactate dehydrogenase (LDH2), and cytoskeletal proteins such as tubulins (TUBB8, TUBB2B, TUBA1C), actin (ACTG1), and vimentin (VIM) among the 15 most abundant proteins detected. The top 30 quantified proteins contributed to 23% of the total abundance (**Supp. Table 2**). To confirm the purity of the cell culture, common brain cell markers were searched, including glial fibrillary acidic protein (GFAP, astrocytes), macrosialin (CD68, microglia), galactocerebrosidase (GALC, oligodendrocytes), and tubulin β -3 (TUBB3, neurons). An enrichment of 27 common astrocyte markers [19] was observed, corresponding to structural and membrane proteins (VIM, GFAP, NES), glucose metabolism, fatty acid oxidation, and transcription factors. GFAP quantification at the proteomics-level agrees with the immunofluorescence and RT-PCR (**Figure 1**). Fourteen astrogliosis gene markers (**Supp. Fig. S3g**) were also identified, spanning intracellular, secreted, structural, and membrane proteins across three orders of magnitude. These

astrocytes and astrogliosis markers (**Supp. Table 2**) were consistently identified across all “contrast” comparisons in the study (**Supp. Fig. S4-S5**).

Subtle qualitative proteomic differences in infected astrocytes from centenarians and adults highlight reduced MHC class II detection

To determine whether age and clinical outcome influence astrocyte proteomic responses to SARS-CoV-2 infection, we directly compared infected astrocytes from centenarians and adults within each viral strain (Gc vs. Ga or Wc vs. Wa; **Fig. 4a,b**). Surprisingly, despite the large number of quantified proteins, differential expression analysis revealed remarkably minimal differences between groups, with only a single differentially abundant protein (DAP) identified in each comparison. Specifically, one DAP was detected among 4,563 proteins in Gc vs. Ga ($\text{FDR} < 0.01$, $|\log\text{FC}| > 0.57$; **Fig. 4a**) and one among 4,963 proteins in Wc vs. Wa ($\text{FDR} < 0.01$, $|\log\text{FC}| > 0.58$; **Fig. 4b**). Tropomyosin 2 (TPM2) was upregulated in the Gamma strain comparison ($\log\text{FC} = 1.2$), whereas tissue inhibitor of metalloproteinases 2 (TIMP2) was downregulated in the Wuhan strain comparison ($\log\text{FC} = -0.92$).

Together, these findings indicate that SARS-CoV-2 infection elicits highly conserved global proteomic responses in astrocytes, largely independent of age or clinical outcome in the patient background. This apparent lack of differential abundance prompted us to explore complementary analytical strategies to uncover more subtle or qualitative differences between groups.

To capture differences not detected by conventional differential expression analysis, we performed a complementary (1) presence/absence and (2) differential detection analyses. When performing the former analysis comparing the list of identified proteins across the five astrocyte groups, represented by a control defined as Mock-infected (non-infected) samples derived from both centenarian and adult groups (Ctrl); Gamma_adults (Ga); Gamma_centenarians (Gc); Wuhan_adults (Wa); Wuhan_centenarians (Wc). In at least three samples per group, we found eight exclusive proteins shared between Ga and Wa, and 174 exclusives to Gc and Wc (**Supp. Fig. S6a**). Some of the exclusive proteins in Ga and Wa astrocytes (**Supp. Fig. S6b**) correspond to Angiotensin-converting enzyme (ACE), V-type

proton ATPase subunit G2 (ATP6V1G2) and the Betaine-homocysteine S-methyltransferase 1 (BHMT). Among the Gc and Wc groups we found ten hub genes associated with measles virus infection and the IL-1 signaling pathway (**Supp. Fig. S6c**). A total of 1205 proteins were shared among all infected astrocytes (Ga, Gc, Wa and Wc) and hub genes were enriched in organellar large ribosomal subunit (**Supp. Fig. S6d**).

Also, a differential detection analysis [18] within the MS-DAP pipeline was performed on proteins that showed extreme differences in the number of detected peptides between two sample groups (hereafter referred to as up/down-detected). This analysis is useful for peptides that lack data for differential expression analysis and especially for a cell-type proteomic design, such as in the present study. Only proteins consistently detected in at least three samples per group with an absolute z-score cutoff > 6 were study in the centenarians vs. adults-infected astrocyte proteome for both viruses (**Fig. 4c,d**). In the astrocyte proteome modulated by the Gamma strain (**Fig. 4c**), the histogram shows 52 and 9 proteins up- and down-detected, respectively, whereas 46 up- and 13 down-detected proteins were found in the Wuhan strain (**Fig. 4d**). Venn diagrams (**Fig. 4e**) of these results for both viruses show an overlap of four or five genes for down- and up-detected proteins, respectively. Despite the higher number of up-detected proteins, no functional enrichment of Gene Ontology categories was observed. The immune response via antigen processing and presentation was exclusively over-represented for down-detected proteins in both viruses (**Fig. 4f, Supp. Table 3**) and could be explained mainly by four genes encoding components of the MHC class II molecules (CD74, HLA-DRA, HLA-DRB1, HLA-DRB3).

These two approaches revealed a subset of proteins differentially detected in centenarian astrocytes, alongside a consistent reduction in MHC class II components. These findings point to qualitative, rather than quantitative, differences in immune-related processes between centenarian and young adult-infected groups.

SARS-CoV-2 infection vs. Control induces extensive proteomic remodeling in human astrocytes

Given the minimal differences observed between centenarian- and adult-derived astrocytes upon infection, we next examined the overall impact of SARS-CoV-2 infection on the astrocyte proteome. To assess this, we compared infected conditions (Ga, Gc, Wa, Wc) to

the Mock-infected (non-infected) group with cells derived from both centenarian and adult groups. Control samples derived from centenarians and adults were combined after confirming highly similar global proteomic profiles, as evidenced by the absence of significant differences at the protein level and minimal variation at the peptide level (FDR < 0.01, $|\log FC| > 0.725$, **Supp. Fig. S7 and Supp. Table 4**). This approach increased statistical power for downstream comparisons while maintaining biological consistency and enabled a robust assessment of infection-driven proteomic changes. Additionally, PCA (**Fig. 3d**) and Pearson correlation analysis (**Supp. Fig. S3f**) showed no segregation between control samples based on age, further supporting their integration into a single Ctrl group.

Differential expression analyses were then performed separately for each viral strain and age group (Gc/Ctrl, Ga/Ctrl, Wc/Ctrl, and Wa/Ctrl; **Fig. 5a**). Using a 1% FDR threshold and a bootstrap-based fold-change estimation, we identified a total of 1,725 differentially abundant proteins (DAPs) across all comparisons (**Fig. 5b**). Specifically; 1,218 DAPs were detected in Gc/Ctrl (617 downregulated and 601 upregulated); 1,029 in Ga/Ctrl (447 down and 582 up); 1,269 in Wc/Ctrl (532 down and 737 up) and 965 in Wa/Ctrl (419 down and 546 up) indicating a broad proteomic response to infection.

Then, we decided to identify distinct and shared proteomic alterations across all detected DAPs in the four groups of SARS-CoV-2 infected (Gc, Ga, Wc, Wa) vs. Ctrl astrocytes. The upset plot in **Fig. 5b** depicts 634 proteins that were found commonly dysregulated, indicating an overlap of DAPs within all groups, while 224 genes were exclusive to Wc/Ctrl, 129 to Gc/Ctrl, 87 to Ga/Ctrl, and 47 to Wa/Ctrl. A similar overlapping trend was observed in up (**Supp. Fig. S8a**) and down-regulated DAPs (**Supp. Fig. S8b**) showing 388 and 246 common DAPs, respectively.

Infection induced extensive proteomic remodeling across all conditions, and a large proportion of dysregulated proteins was shared across groups, highlighting a conserved core response. These results reinforce the notion that infection-driven changes dominate the astrocyte proteome, potentially masking more subtle group-specific effects.

Conserved and group-specific proteomic responses revealed by fold-change correlation analysis in infected astrocytes

Although global proteomic responses to SARS-CoV-2 infection were largely conserved between centenarian- and adult-derived astrocytes, we next sought to distinguish shared from group-specific alterations using a fold-change correlation analysis. Comparison of infection-induced changes (Gc/Ctrl vs. Ga/Ctrl and Wc/Ctrl vs. Wa/Ctrl; **Fig. 5d,e, Supp. Table 5**) revealed a substantial overlap of DAPs between groups of both viral strains, highlighting a highly conserved core response to infection. In line with this, log₂ fold-change values of shared DAPs showed a strong Pearson correlation ($r = 0.98$), indicating similar directionality and magnitude of proteomic changes across groups.

Despite this overall similarity, contrast-specific filtering revealed subsets of DAPs uniquely detected in each comparison, with centenarian-derived astrocytes consistently showing a larger number of exclusive proteins than adult-derived cells. To enable quantitative comparison across groups, missing values for non-quantified proteins were handled consistently, allowing calculation of Pearson correlations over a shared protein space. Under these conditions, centenarian astrocytes exhibited lower correlations ($r = 0.82\text{--}0.89$), suggesting more heterogeneous or variable proteomic responses not captured in adult-derived cells. Strong correlations confirmed a conserved response; however, centenarian astrocytes displayed a higher number of exclusive proteins. This suggests that resilience may be associated with additional layers of proteomic adaptation superimposed on a shared core response.

To further explore these differences at a functional level, we performed 2D annotation enrichment analysis [20] of DAPs (**Supp. Table 6**) within Gamma (Gc/Ctrl vs. Ga/Ctrl, **Fig. 5e**) and the Wuhan strain (Wc/Ctrl vs. Wa/Ctrl, **Fig. 5f**). This revealed a broadly consistent enrichment pattern across groups, including upregulation of pathways related to viral translation, RNA binding, and ribosome function, alongside downregulation of proteasome activity and metabolic processes. Notably, immune-related pathways, including antigen processing and NF- κ B signaling, showed coordinated downregulation in both strains, although more prominently (higher number of terms) in the Gamma condition. Glycosphingolipid metabolism emerged as one of the few pathways displaying opposite regulation between groups in the Wuhan strain. An orthogonal protein-protein interaction network analysis using STRING [21] further supported these findings by identifying both shared and group-specific functional modules (**Fig. 6, Supp. Table 7**).

In the Gamma strain, common DAPs (Gc/Ctrl and Ga/Ctrl) were predominantly enriched in RNA and nucleotide metabolism, jointly with mitochondrial ATP synthesis, reinforcing the presence of a conserved infection-driven response (**Fig. 6a**). In contrast, DAPs exclusive to centenarian-derived astrocytes (Gc/Ctrl) showed enrichment in cytoplasmic translation, integrin-mediated cell surface interactions, and pathways related to DNA damage recognition, autophagy and ErbB signaling, several of which were downregulated (**Fig. 6b**). Adult-specific DAPs (Ga/Ctrl) were associated with upregulation of translation, ATP synthesis, and fatty acid degradation (**Fig. 6c**).

A similar pattern was observed in the Wuhan strain with common DAPs (Wc/Ctrl and Wa/Ctrl) enriched in RNA metabolism, chaperone complex formation, and tRNA aminoacylation (**Fig. 6d**). Centenarian-specific DAPs (Wc/Ctrl) were associated with viral translation, host-virus receptor interactions, electron transport chain activity, and sphingolipid metabolism (**Fig. 6e**), whereas adult-specific DAPs (Wa/Ctrl) showed enrichment in ribosome assembly, GPI anchor biosynthesis, and NADH dehydrogenase activity (**Fig. 6f**).

Selective up-regulation of NRP1 in adult-derived astrocytes highlights differential regulation of SARS-CoV-2 entry-associated pathways

Given the overall conservation of infection-induced responses, we next asked whether pathway-level analyses could reveal more subtle, functionally relevant differences associated with key host factors involved in SARS-CoV-2 infection. Over-representation analysis of KEGG pathways using all DAPs from infection vs. Ctrl comparisons (Gc/Ctrl, Ga/Ctrl, Wc/Ctrl, and Wa/Ctrl) revealed a consistent enrichment of several pathways across all groups (**Fig. 7a**). These included pathways related to ribosome function, neurodegenerative diseases, and Coronavirus disease, indicating a shared core response to infection. In contrast, pathways associated with amino acid metabolism (e.g., cysteine and methionine metabolism), biosynthesis, and the proteasome were broadly downregulated.

The Coronavirus disease pathway showed a high degree of gene overlap across all conditions (**Fig. 7b**), supporting its central role in the astrocyte response to infection. Closer inspection of this pathway (**Fig. 7c**) revealed differential regulation of specific components. Notably, Neuropilin-1 (NRP1), a key co-receptor implicated in SARS-CoV-2 entry into

astrocytes [10], was detected in all groups but significantly up-regulated exclusively in adult-derived astrocytes (Ga/Ctrl and Wa/Ctrl; **Fig. 7d**). In addition to NRP1, this pathway showed coordinated modulation of multiple host factors, including up-regulation of ribosomal components and dysregulation of signaling molecules such as EGFR, NFKB1, and MAP kinases (**Fig. 7c,d**), indicating broad remodeling of host translational and signaling machinery upon infection.

Enhanced down-regulation of cell cycle, antigen presentation, and DNA repair pathways in adult-derived astrocytes

To further define the functional consequences of the pathway-level differences identified above, we performed gene set enrichment analysis using Reactome pathways across all infection vs. Ctrl comparisons (**Supp. Tables 8-9, Supp. Fig. S9**). This analysis revealed both shared and group-specific pathway alterations, with markedly more extensive dysregulation observed in astrocytes derived from adults than in centenarians.

Across both viral strains, centenarian-derived astrocytes (Gc/Ctrl and Wc/Ctrl; **Supp. Fig. S9a,c**) exhibited relatively limited pathway modulation, predominantly characterized by upregulation of translation-related processes and a modest downregulation of innate immune pathways. In contrast, adult-derived astrocytes (Ga/Ctrl and Wa/Ctrl; **Supp. Fig. S9b,d**) displayed a broader pattern of dysregulation, including widespread downregulation of pathways associated with innate immunity, antigen presentation, and inflammatory signaling. Notably, pathways related to APC-mediated degradation of cell cycle proteins, NF- κ B signaling, stabilization of p53, and DNA damage repair mechanisms were consistently downregulated in adults across both conditions. While minor strain-specific differences were observed, the overall pattern of enhanced pathway suppression in adult-derived astrocytes remained conserved between Gamma and Wuhan infections.

Discussion

Understanding SARS-CoV-2 infection in astrocytes is particularly relevant given their central role in maintaining homeostasis of the central nervous system. Dysfunction of these cells can disrupt metabolic support, neurotransmitter balance, and inflammatory regulation—processes implicated in cognitive impairment and neuropsychiatric manifestations reported in a subset of COVID-19 patients [13]. In this context, postmortem analyses have provided compelling *in vivo* evidence that astrocytes represent a primary cellular target of SARS-CoV-2 within the human brain [10].

Complementary *in vitro* studies further demonstrated that astrocytes are permissive to productive SARS-CoV-2 infection despite negligible ACE2 expression. Andrews et al. [22] identified alternative coreceptors, including CD147 and DPP4, as critical mediators of astrocyte infection, linking viral tropism to astrocyte reactivity, inflammatory signaling, and gliosis-like responses. Moreover, **Kong et al.** [23] showed that neuropilin-1 (NRP1) acts as a major entry receptor in astrocytes. In contrast, a recent report based in iPSC-derived astrocytes showed that these cells are susceptible to infection but not permissive to SARS CoV-2 replication but trigger neuroinflammation, neurotransmission dysregulation and cell death [24]. Notably, even a limited rate of infection—approximately 2–6% of GFAP⁺ cells at 4 days post-infection—is sufficient to trigger robust inflammatory and interferon responses, generating a hostile microenvironment that impairs neuronal viability.

In line with these findings, we observed detectable infection of iPSC-derived astrocytes in our study model (~10%). Indeed, as centenarian donors experienced mild disease whereas adult donors had critical COVID-19, age and disease severity are not independent variables in our study design. Therefore, the observed differences may reflect a combination of age-related and disease severity-associated effects rather than a single isolated factor.

No significant differences in infection rates were detected between astrocytes exposed to the Wuhan strain and those infected with the Gamma (P.1) variant, suggesting comparable efficiency of initial astrocyte entry even for a more virulent variant under these experimental conditions. However, despite similar infection rates, astrocytes infected with the P.1 variant released significantly higher levels of viral proteins (RBD portion of the Spike protein) into the culture supernatant compared with those infected with the Wuhan strain. This observation is consistent with previous studies showing that the P.1 variant displays accelerated replication kinetics and achieves higher infectious titers than the ancestral strain

in human epithelial cell models, including Caco-2 and Calu-3 cells [25]. Together, these findings suggest that the increased virulence of the P.1 variant may be driven not by enhanced cell entry, but rather by differences in intracellular replication dynamics and/or viral egress.

Despite the relatively low infection rate, this level of astrocyte infection was sufficient to elicit a measurable inflammatory response, particularly following exposure to the P.1 variant. In this context, we observed that centenarian-derived astrocytes exhibited significantly reduced secretion of CXCL8 and CXCL10 when compared with adult-derived astrocytes after Wuhan or P.1 infection, respectively. Notably, even a limited proportion of infected astrocytes may be sufficient to modulate the inflammatory milieu through paracrine signaling, whereby infected cells influence the neighboring uninfected astrocytes. This finding is particularly intriguing, as CXCL8 [26] and CXCL10 [27] are key mediators of neuroinflammation and immune cell recruitment, and their dysregulated expression has been implicated in CNS pathology [28]. The attenuated chemokine response observed in centenarian astrocytes may therefore reflect an intrinsic capacity to restrain excessive inflammatory amplification in response to a more virulent viral challenge, potentially contributing to preserved CNS homeostasis in extreme longevity.

To further elucidate the intrinsic cellular alterations associated with SARS-CoV-2 infection in astrocytes, we employed a proteomic approach to comprehensively profile infection-induced molecular changes. Although only a limited number of differentially abundant proteins were identified in SARS-CoV-2-infected astrocytes from centenarians vs. adults within the same virus strain, specific candidates may still provide biological insight. For instance, tropomyosin 2 (TPM2), upregulated in Gamma-infected centenarian astrocytes, has been associated with cytoskeletal organization and may reflect adaptive remodeling processes [29]. Conversely, TIMP2, which was downregulated in centenarian-derived astrocytes infected with Wuhan, is involved in extracellular matrix regulation. While matrix metalloproteinases have been implicated in viral infection processes [30], the functional relevance of TIMP2 modulation in this context remains unclear and warrants further investigation.

Although the differential detection analysis performed by MS-DAP should be interpreted with caution, it suggests an over-representation of proteins associated with antigen processing and presentation, particularly components of the MHC class II pathway. CD74 represents the invariant chain of MHC class II, while HLA-DR molecules play a central

role in antigen presentation to CD4⁺ T cells [31,32]. In our dataset, a higher number of detected peptides corresponding to MHC class II-related proteins was observed in infected astrocytes derived from adults than in centenarians. While the functional implications of this observation remain to be fully elucidated, it may reflect differences in immune activation states between groups rather than differences in infection efficiency, which was comparable across all conditions.

Notably, astrocytes can produce cytokines and chemokines under both basal and stimulated conditions [33] and MHC class II expression has been associated with reactive or inflammatory astrocyte phenotypes. In this context, the reduced detection of MHC class II components in centenarian astrocytes may be consistent with a more restrained immune profile. Supporting this notion, genetic variation within the MHC region has been associated with COVID-19 outcomes, including enrichment of specific HLA alleles in resilient elderly populations [34]. Together, these findings raise the possibility that attenuated antigen presentation capacity in centenarian astrocytes may contribute to limiting excessive neuroinflammatory responses. However, functional validation will be required to determine whether these differences translate into altered antigen presentation capacity.

Presence/absence protein profiling revealed ACE, ATP6V1G2, and BHMT as uniquely detected in infected adult-derived astrocytes. While ACE2 was not detected, the presence of ACE may suggest alterations in components of the renin-angiotensin system, which has been implicated in neuroinflammatory processes [35]. The detection of ATP6V1G2 and BHMT may further point to metabolic and cellular stress-related adaptations in these cells. In contrast, Gamma or Wuhan-infected centenarian-derived astrocytes showed unique detection of proteins associated with IL-1 signaling and antiviral response pathways. While the functional implications of these findings remain to be established, they may reflect differences in the regulation of immune-related pathways between groups [36,37]. Importantly, a large proportion of proteins were shared across all infected conditions, reinforcing the presence of a conserved core astrocytic response to SARS-CoV-2 infection.

Infection with either the Gamma or Wuhan strains vs. Control triggered widespread proteomic alterations across all groups, predominantly affecting ribosomal function, proteasome degradation, amino acid metabolism, and mitochondrial energy production. These findings are consistent with previous reports [38,39], indicating that SARS-CoV-2 extensively remodels host proteomes by co-opting translational machinery while perturbing

proteostasis and cellular metabolism. The activation of stress-response pathways and translational inhibition underscores the substantial burden SARS-CoV-2 imposes on astrocytic homeostasis.

Although the general response patterns of centenarian and adult-derived astrocytes within the same virus strain were highly correlated, specific differences were notable. Differential enrichment analyses revealed that pathways such as gluconeogenesis[40] and sphingolipid[41] metabolism were differentially modulated between Wuhan-infected centenarian and adult-derived astrocytes. These observations suggest that centenarian astrocytes may retain a greater degree of metabolic flexibility under infection-induced stress conditions. Moreover, centenarian-derived astrocytes infected with Gamma or Wuhan showed downregulation of ErbB signaling and DNA damage[42] recognition, potentially indicating a suppression of controlled pathways of cell proliferation to limit viral replication.

Although NRP1, a key co-receptor implicated in SARS-CoV-2 entry into astrocytes, was detected in all groups, its significant upregulation was observed exclusively in astrocytes derived from adults infected with Gamma or Wuhan. This observation is consistent with previous reports supporting a role for NRP1 in facilitating viral entry into neural cells [10]. However, given that infection rates were comparable across all groups in our experimental conditions, the increased abundance of NRP1 in adult-derived astrocytes does not appear to translate into enhanced susceptibility to infection. Instead, this differential regulation may reflect alterations in host cell signaling, viral processing, or post-entry events rather than initial viral entry per se. In this context, NRP1 upregulation may represent a marker of a distinct cellular response to infection or a primed state associated with severe disease, rather than a direct determinant of infection efficiency [43]. These findings highlight the importance of considering host factor regulation beyond its canonical role in viral entry.

The gene set enrichment analyses of infected vs Control revealed that adult-derived astrocytes exhibited a broader and more pronounced downregulation of pathways involved in innate immunity, antigen processing, cell cycle regulation, and p53 stabilization. These alterations, which were less evident in centenarian astrocytes, suggest a more extensive disruption of cellular regulatory programs in adult-derived cells. Such differences may reflect a reduced capacity to maintain cellular homeostasis under infection, potentially contributing to divergent cellular responses to SARS-CoV-2[44].

To sum up, our findings show that SARS-CoV-2 infection elicits a largely conserved astrocytic response, upon which subtle but consistent differences distinguish centenarian- from adult-derived cells, with centenarian astrocytes exhibiting a more restrained inflammatory profile and reduced antigen presentation signatures, while adult severely affected cells display broader dysregulation of immune, host factors such as NRP1 and stress-related pathways. These results suggest that resilience to SARS-CoV-2 is not driven by reduced infection susceptibility, but by a more controlled cellular response associated with intrinsic regulatory programs of extreme longevity.

Declarations

Ethics approval

The study was approved by the Committee for Ethics in Research of the Institute of Biosciences at the University of São Paulo (CAAE 34786620.2.0000.5464) following the Declaration of Helsinki principles, ICH06 Good Clinical Practices, and Brazilian Health Regulatory Agency (ANVISA) resolution number 466 from 2012 that regulates research with humans.

Funding information

This work was supported by the Sao Paulo Research Foundation (FAPESP) [grant numbers 2013/08028-1, 2014/50931-3, 2020/09702-1, 2021/05194-4] and JBS S.A [grant number 69004]. Grants from the Brazilian National Council for Scientific and Technological Development (CNPq) [grant number 404134/2020-3](404140/2020-3, 311735/2023-1), 309373/2025-5], FAPERJ [E-26/210.173/2018, E-26/201.052/2021, 202.781/2023, E-26/201.249/2022 and E-26/201.604/2021 and E-26/200.354/2024, and 381060/2024-1] are acknowledged. The funders were not involved in study design, data collection, analysis, data interpretation, manuscript writing, or the decision to submit the work for publication.

Clinical trial number: not applicable.

References

1. Israfil, S. M. H. *et al.* (2021). Clinical Characteristics and Diagnostic Challenges of COVID-19: An Update From the Global Perspective. *Frontiers in Public Health* vol. 8 Preprint at <https://doi.org/10.3389/fpubh.2020.567395>.
2. Onder, G., Rezza, G. & Brusaferro, S. (2020). Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *JAMA* <https://doi.org/10.1001/jama.2020.4683>
3. Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., *et al.* (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* 395, 1054–1062. [https://doi.org/10.1016/S0140-6736\(20\)30566-3](https://doi.org/10.1016/S0140-6736(20)30566-3)
4. Tizazu, A. M., Mengist, H. M. & Demeke, G. (2022). Aging, inflammaging and immunosenescence as risk factors of severe COVID-19. *Immunity and Ageing* vol. 19 Preprint at <https://doi.org/10.1186/s12979-022-00309-5>.
5. Caruso, C., Accardi, G., Aiello, A., Calabrò, A., Ligotti, M.E., Candore, G. (2023). Centenarians born before 1919 are resistant to COVID-19. *Aging Clin. Exp. Res.* 35, 217–220.
6. de Castro, M. V., Silva, M.R.V., Naslavsky, M.S., Scliar, M.O., Nunes, K., Passos-Bueno, M.R. *et al.* (2022). The oldest unvaccinated Covid-19 survivors in South America. *Immunity and Ageing* 19: 57 <https://doi.org/10.1186/s12979-022-00310-y>.
7. Trombetta, C. M., Accardi, G., Aiello, G., Calabro, A., Caruso, C., Ligotti, M.E., *et al.* (2024). Centenarians, semi and supercentenarians, COVID-19 and Spanish flu: a serological assessment to gain insight into the resilience of older centenarians to COVID-19. *Immunity and Ageing* 21: 44 <https://doi.org/10.1186/s12979-024-00450-3>.
8. Kanberg, N., Ashton, N.J., Andersson, L.M., Ylmaz, A., Lindh, M., Nilsson, S. *et al.* (2020). Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. *Neurology* 95, E1754–E1759. <https://doi.org/10.1212/WNL.00000000000010111>
9. Varga, Z., Flammer, A.J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A.S., *et al.* (2020). Endothelial cell infection and endotheliitis in COVID-19. *The Lancet* 395, 1417–1418. [https://doi.org/10.1016/S0140-6736\(20\)30937-5](https://doi.org/10.1016/S0140-6736(20)30937-5).
10. Crunfli, F., Carregari, V.C., Veras, F.P., Silva, L.S., Nogueira, M.H., Antunes, A.S.L.M., *et al.* (2022). Morphological, cellular, and molecular basis of brain infection in COVID-19 patients. *Proc. Natl. Acad. Sci. U. S. A.* 119,e2200960119. <https://doi.org/10.1073/pnas.2200960119>
11. Jorgačevski, J. & Potokar, M. (2023). Immune Functions of Astrocytes in Viral Neuroinfections. *International Journal of Molecular Sciences* vol. 24 Preprint at <https://doi.org/10.3390/ijms24043514>.
12. Wood, G. K., Sargent, B.F., Ahmad, Z.U.A., Tharmaratnam, K., Dunai, C., Egbe, F.N. *et al.* (2025). Posthospitalization COVID-19 cognitive deficits at 1 year are global and associated with elevated brain injury markers and gray matter volume reduction. *Nat. Med.* 31, 245–257. <https://doi.org/10.1038/s41591-024-03309-8>

13. Steardo, L., Steardo, L. & Scuderi, C. (2023) Astrocytes and the Psychiatric Sequelae of COVID-19: What We Learned from the Pandemic. *Neurochemical Research* 48, 1015–1025 <https://doi.org/10.1007/s11064-022-03709-7>.
14. de Castro, M. V., Sabato, C., Girardi, R.M.D., Silva, M.R.V., Dell'Aquila, L.P., Razuk-Filho, A., et al. (2025). Insights into disease resilience and longevity: Hints from COVID-19 recovered nonagenarians and centenarians. *Gene*. 934,149025. <https://doi:10.1016/j.gene.2024.149025>.
15. Ghirotto, B., Oliveira, D.F., Cipelli, M., Basso, P.J., Lima, J., Breda, C.N.S., et al. (2022). MS-Driven Metabolic Alterations Are Recapitulated in iPSC-Derived Astrocytes. *Ann. Neurol.* 91, 652–669. <https://doi:10.1002/ana.26336>.
16. Ogando, N. S. Dalebout, T. J., Zevenhoven-Dobbe, J.C., Limpens, R.W.A., van der Meer, Y., Caly, Y., et al. (2020). SARS-coronavirus-2 replication in Vero E6 cells: Replication kinetics, rapid adaptation and cytopathology. *Journal of General Virology* 101, 925–940. <https://doi:10.1099/jgv.0.001453>
17. Demichev, V., Messner, C. B., Vernardis, S. I., Lilley, K. S. & Ralser, M. (2020). DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat. Methods* 17, 41–44. <https://doi:10.1038/s41592-019-0638-x>.
18. Koopmans, F., Li, K. W., Klaassen, R. V. & Smit, A. B. (2023). MS-DAP Platform for Downstream Data Analysis of Label-Free Proteomics Uncovers Optimal Workflows in Benchmark Data Sets and Increased Sensitivity in Analysis of Alzheimer's Biomarker Data. *J. Proteome Res.* 22, 374–386. <https://doi:10.1021/acs.jproteome.2c00513>.
19. Jurga, A. M., Paleczna, M., Kadluczka, J. & Kuter, K. Z. (2021) Beyond the GFAP-astrocyte protein markers in the brain. *Biomolecules* 11, 1361 <https://doi.org/10.3390/biom11091361>.
20. Cox, J. & Mann, M. (2012). 1D and 2D annotation enrichment: a statistical method integrating quantitative proteomics with complementary high-throughput data. *BMC Bioinformatics* 13 Suppl 16, <https://doi:10.1186/1471-2105-13-S16-S12>.
21. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., et al. (2023) The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* 51, D638–D646. <https://doi:10.1093/nar/gkac1000>.
22. Andrews, M. G., Mukhtar, T., Eze, U.C., Simoneau, C.R., Ross, J., Parikshak, N., et al. (2022). Tropism of SARS-CoV-2 for human cortical astrocytes. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2122236119. <https://doi:10.1073/pnas.2122236119>
23. Kong, W., Montaro, M., Corley, M.J., Helmy, E., Kobayashi, H., Kinisu, M., et al. (2022). Neuropilin-1 Mediates SARS-CoV-2 Infection of Astrocytes in Brain Organoids, Inducing Inflammation Leading to Dysfunction and Death of Neurons. *mBio* 13, e0230822. <https://doi:10.1128/mbio.02308-22>.
24. Segabinazi, E., Tocantins, F, R., Glaser, T., Maglio, T., Oliveira, N.C., Castanha, A.G., et al. (2025). Astroglia-mediated neuroinflammation as a putative mechanism of neurological

outcomes in COVID-19? Insights from a Brazilian cohort. *Brain Behav. Immun. Health* 49, 101115 [https:// doi: 10.1016/j.bbih.2025.101115](https://doi.org/10.1016/j.bbih.2025.101115)

25. Mautner, L., Hoyos, M., Dangel, A., Berger, C., Ehrhardt, A., Baiker, A. (2022). Replication kinetics and infectivity of SARS-CoV-2 variants of concern in common cell culture models. *Virology Journal* 19, 76. [https:// doi: 10.1186/s12985-022-01802-5](https://doi.org/10.1186/s12985-022-01802-5)

26. Lv, H., Li, J., Che, Y. Q. (2019). CXCL8 gene silencing promotes neuroglial cells activation while inhibiting neuroinflammation through the PI3K/Akt/NF- κ B-signaling pathway in mice with ischemic stroke. *J. Cell. Physiol.* 234, 7341–7355 [https:// doi: 10.1002/jcp.27493](https://doi.org/10.1002/jcp.27493).

27. Liu, M. T., Keirstead, H. S., Lane, T. E. (2001). Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. *J. Immunol.* 167, 4091–4097. [https:// doi: 10.4049/jimmunol.167.7.4091](https://doi.org/10.4049/jimmunol.167.7.4091).

28. Vida, H., Sahar, M., Nikdouz, A. & Arezoo, H. (2025). Chemokines in neurodegenerative diseases. *Immunology and Cell Biology* 103, 275–292. [https:// doi: 10.1111/imcb.12843](https://doi.org/10.1111/imcb.12843).

29. Wen, Z., Zhang, Y., Lin, Z., Shi, K., Jiu, Y. (2020). Cytoskeleton - A crucial key in host cell for coronavirus infection. *Journal of Molecular Cell Biology* 12, 968–979. <https://doi.org/10.1093/jmcb/mjaa042>.

30. Benlarbi, M., Laroche, G., Fink, C., Fu, K., Mulloy, R.P., Phan, A., *et al.* (2022). Identification and differential usage of a host metalloproteinase entry pathway by SARS-CoV-2 Delta and Omicron. *iScience* 25, 105316. [https:// doi: 10.1016/j.isci.2022.105316](https://doi.org/10.1016/j.isci.2022.105316)

31. Wang, N., Waghray, D., Caveney, N. A., Jude, K. M. & Garcia, K. C. (2024). Structural insights into human MHC-II association with invariant chain. *Proc. Natl. Acad. Sci. U. S. A.* 121, e2403031121. [https:// doi: 10.1073/pnas.2403031121](https://doi.org/10.1073/pnas.2403031121).

32. Fleury, S. Thibodeau, J., Croteau, G., Labrecque, N., Aronson, H.E., Cantin, C., *et al.* (1995). HLA-DR polymorphism affects the interaction with CD4. *J. Exp. Med.* 182, 733 [https:// doi: 10.1084/jem.182.3.733](https://doi.org/10.1084/jem.182.3.733)

33. Choi, S. S., Lee, H. J., Lim, I., Satoh, J. I., Kim, S. U. (2014). Human astrocytes: Secretome profiles of cytokines and chemokines. *PLoS One* 9, e92325. [https:// doi: 10.1371/journal.pone.0092325](https://doi.org/10.1371/journal.pone.0092325).

34. Castelli, E. C., Castro M.V., Naslavsky, M.S., Scliar, M.O., Silva, N.S.B., Pereira, R.N., *et al.* (2022). MUC22, HLA-A, and HLA-DOB variants and COVID-19 in resilient super-agers from Brazil. *Front. Immunol.* 13, 975918. [https:// doi: 10.3389/fimmu.2022.975918](https://doi.org/10.3389/fimmu.2022.975918).

35. Porel, P., Hunjan, G., Singh, S. & Aran, K. R. (2025). Is the renin-angiotensin system a friend or foe in neurological diseases? Unveiling its role and therapeutic potential. *Ageing Research Reviews* 112, 102854. <https://doi.org/10.1016/j.arr.2025.102854>.

36. Van Damme, J., de Lay, M., Opdenakker, G., Billiau, A., De Somer, P., van Beeumen J. (1985). Homogeneous interferon-inducing 22K factor is related to endogenous pyrogen and interleukin-1. *Nature* 314, 266–268. [https:// doi: 10.1038/314266a0](https://doi.org/10.1038/314266a0).

37. Randolph-Habecker, J., Iwata, M., Geballe, A. P., Jarrahan, S., Torok-Storb, B. (2002). Interleukin-1-mediated inhibition of cytomegalovirus replication is due to increased IFN- β

- production. *Journal of Interferon and Cytokine Research* 22, 765–772. [https:// doi: 10.1089/107999002320271350](https://doi.org/10.1089/107999002320271350).
38. Carregari, V. C., Reis-de-Oliveira, G., Crunfil, F., Smith, B.J., Souza, G.F., Muraro, S.P., *et al.* (2024). Diving into the proteomic atlas of SARS-CoV-2 infected cells. *Sci. Rep.* 14, 7375. [https:// doi: 10.1038/s41598-024-56328-3](https://doi.org/10.1038/s41598-024-56328-3).
39. Dozio, V., Sanchez, J. C. (2018). Profiling the proteomic inflammatory state of human astrocytes using DIA mass spectrometry 06 Biological Sciences 0601 Biochemistry and Cell Biology. *J. Neuroinflammation* 15, 331. [https:// doi: 10.1186/s12974-018-1371-6](https://doi.org/10.1186/s12974-018-1371-6).
40. Sebastiani, P., Solovieff, N., Dewan, A.T., Walsh, K.M., Puca, A., Hartley, S.W., *et al.* (2012). Genetic Signatures of Exceptional Longevity in Humans. *PLoS One* 7, e29848. [https:// doi: 10.1371/journal.pone.0029848](https://doi.org/10.1371/journal.pone.0029848).
41. Li, S. & Kim, H.-E. (2022) Implications of Sphingolipids on Aging and Age-Related Diseases. *Frontiers in Aging* 2, 797320. [https:// doi: 10.3389/fragi.2021.797320](https://doi.org/10.3389/fragi.2021.797320)
42. Franzke, B., Neubauer, O. & Wagner, K. H. (2015). Super DNAGing-New insights into DNA integrity, genome stability and telomeres in the oldest old. *Mutat. Res. Rev. Mutat. Res.* 766, 48–57. [https:// doi: 10.1016/j.mrrev.2015.08.001](https://doi.org/10.1016/j.mrrev.2015.08.001)
43. Rodrigues, T., Beltrão, G. S., Girardi, H., Pinto, A. R. (2025). Viral reprogramming of glial metabolism as a driver of neuroinflammation. *Front. Immunol.* 16, 1686774. [https:// doi: 10.3389/fimmu.2025.1686774](https://doi.org/10.3389/fimmu.2025.1686774)
44. Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.C., Uhl, S., Hoagland, D., Moller, R., *et al.* (2020). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* 181, 1036-1045.e9 . [https:// doi: 10.1016/j.cell.2020.04.026](https://doi.org/10.1016/j.cell.2020.04.026)

Figure Legends

Figure 1. Characterization of iPSC-derived astrocytes and expression of SARS-CoV-2 entry factors. Astrocytes differentiated from induced pluripotent stem cells (iPSCs) of Brazilian centenarians (100+, IDs 01–04) and younger adults (50–, IDs 05–07) were characterized by immunofluorescence and gene expression analysis. Cells were stained for GFAP (astrocyte marker, green), ACE2 or NRP1 (red), and nuclei were counterstained with DAPI (blue). (A–D, I–K) Representative images showing GFAP and NRP1 expression in astrocytes derived from centenarians and adults, respectively. (E–H, M–O) Representative images showing GFAP and ACE2 expression in astrocytes from both groups. Scale bars represent 20 μ m. (L) Quantification of fluorescence intensity (a.u.) of ACE2 and NRP1 in astrocytes, showing comparable ACE2 levels between groups (ns) and increased NRP1 expression in adults (**p <

0.01). (P) GFAP expression assessed by RT-qPCR ($2^{-\Delta\Delta Ct}$), confirming astrocyte identity in differentiated cells compared to undifferentiated iPSCs. Data are presented as mean $\pm$ SEM.

Figure 2. SARS-CoV-2 infects iPSC-derived human astrocytes with strain-dependent differences in viral release and chemokine responses. iPSC-derived astrocytes from unvaccinated Brazilian centenarians (IDs 1–4) and young adults (IDs 5–7) were infected with SARS-CoV-2 (Wuhan strain or Gamma (P.1) variant; MOI = 1.0) for 48 h. (A–B) Representative immunofluorescence images showing GFAP+ astrocytes (green), SARS-CoV-2 spike protein (red), and nuclei (DAPI, blue). Panel A shows astrocytes derived from a centenarian, and panel B from a young adult. Viral puncta are observed within GFAP+ cells in both groups, indicating that astrocytes are permissive to infection. Scale bars: 5 μ m. (C–D) Flow cytometry analysis shows similar proportions of spike-positive astrocytes across age groups and viral strains, indicating comparable susceptibility to infection. (E) ELISA quantification of viral particles in culture supernatants reveals a marked increase in viral release following P.1 infection compared to the Wuhan strain, in both centenarian- and adult-derived astrocytes. (F) Chemokine levels measured by CBA (CCL2, CCL5, CXCL8, CXCL10) show no significant changes for CCL2 and CCL5, whereas CXCL8 and CXCL10 display selective modulation, particularly in centenarian-derived astrocytes, consistent with a more regulated inflammatory response. Data are presented as mean $\pm$ SEM. * $p < 0.05$.

Figure 3. Proteomics workflow and results obtained in this study. (A) Experimental protocol implemented for astrocyte cells. (B) Number of protein groups identified and (C) quantified across all samples and comparisons. (D) Principal Component Analysis (PCA) of 11,026 peptides from 2,724 proteins across all groups. Numbers indicate paired biological replicates, and conditions are as follows: Ctrl: Control, Ga: Gamma_adults, Gc: Gamma_centenarians, Wa: Wuhan_adults, Wc: Wuhan_centenarians. (E) Ranking plot of the 2,724 quantified proteins, highlighting 27 genes corresponding to astrocyte markers.

Figure 4. Differential expressions or detection analysis of Gc vs. Ga and Wc vs. Wa astrocytes. Volcano plots showing DAPs (FDR < 0.01) in Gc vs. Ga (A) and Wc vs. Wa (B).

Histogram of differential detection analysis displaying proteins with strong z-score differences in observed peptides between Gc vs. Ga (C) and Wc vs. Wa (D). Venn diagram (E) and Gene Ontology Biological Process (GOBP) enrichment analysis (F) of differentially detected proteins (absolute z-score > 6) derived from (C) and (D), showing significant enrichment only for down-detected proteins. Ga: Gamma_adults; Gc: Gamma_centenarians; Wa: Wuhan_adults; Wc: Wuhan_centenarians.

Figure 5. Differential expression analysis of infected vs. Ctrl astrocytes and fold-change correlation between centenarians and adults within each strain (Gamma and Wuhan). (A) Volcano plots of infected groups (Gc, Ga, Wc, Wa) vs. Ctrl. (B) UpSet plot of DAPs found in the four infected groups vs. Ctrl. Fold-change correlation expressed through a logFC-logFC plots (C-D) and 2D enrichment annotation (E-F) of Gc/Ctrl vs Ga/Ctrl and Wc/Ctrl vs Wa/Ctrl astrocytes. Ctrl: Control, Ga: Gamma_adults, Gc: Gamma_centenarians, Wa: Wuhan_adults, Wc: Wuhan_centenarians.

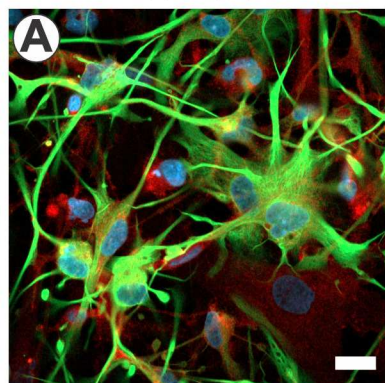
Figure 6. Protein-protein interaction network analysis of common and exclusive DAPs identified in Figure 6C-D. (A) Common in Gc/Ctrl vs. Ga/Ctrl, (B) exclusive in Gc/Ctrl, (C) exclusive in Ga/Ctrl, (D) common in Wc/Ctrl vs. Wa/Ctrl, (E) exclusive in Wc/Ctrl, and (F) exclusive in Wa/Ctrl. Ctrl: Control, Ga: Gamma_adult, Gc: Gamma_centenarian, Wa: Wuhan_adult, Wc: Wuhan_centenarian. Log2 Fold-changes (logFC) corresponds to infected groups (Ga, Gc, Wa or Wc) vs. Ctrl.

Figure 7. Over-representation analysis of DAPs in infected groups vs. Ctrl using KEGG pathways and the representation of the Coronavirus disease pathway. (A) Enrichment analysis of all DAPs found in each contrast of infected groups (Gc, Ga, Wc, Wa) vs. Ctrl for each age (c or a). (B) The Circos plot shows overlapping genes associated with COVID-19 pathways across contrasts. (C) Schematic representation of the Coronavirus disease pathway (image generated by KEGG pathviewer). (D) Box plots of relevant DAPs identified in (C) and involved in COVID-19. Significance thresholds: ns (not significant, FDR > 0.01), ** (significant,

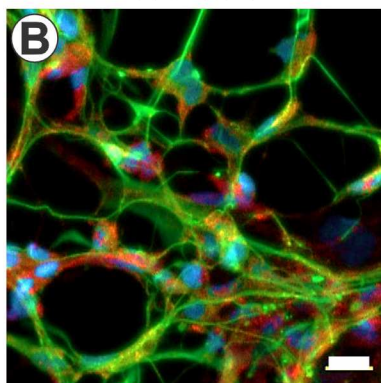
FDR < 0.01). Ctrl: Control, Ga: Gamma_adults, Gc: Gamma_centenarians, Wa: Wuhan_adults, Wc: Wuhan_centenarians.

Centenarians (100+)

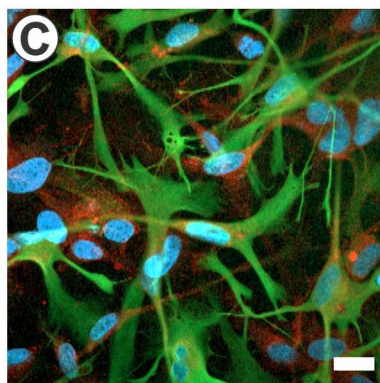
ID 01



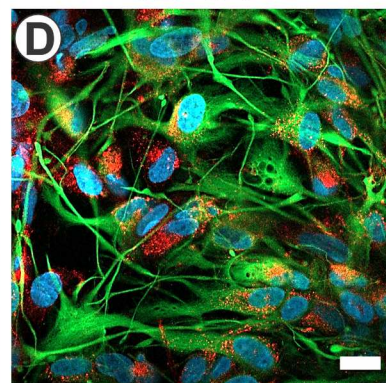
ID 02



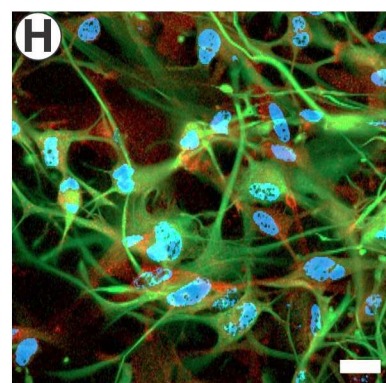
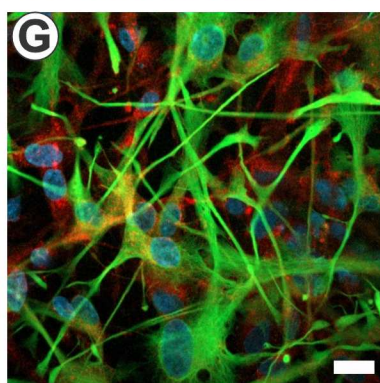
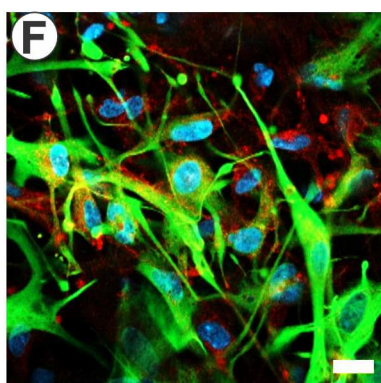
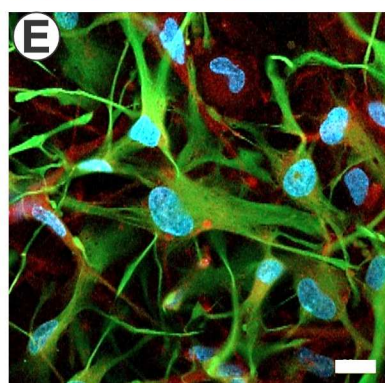
ID 03



ID 04



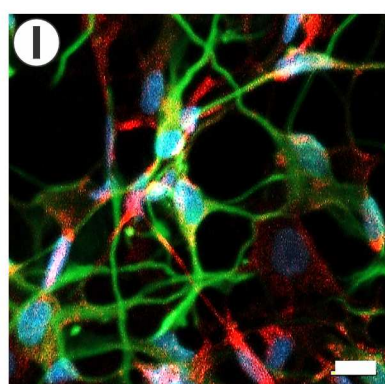
GFAP/NRP1/DAPI



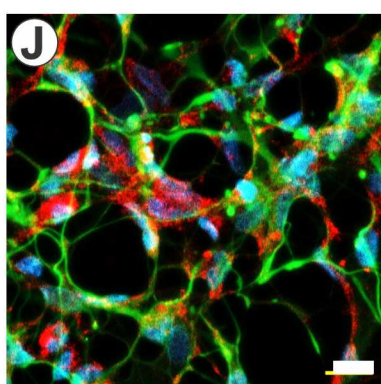
GFAP/ACE2/DAPI

Adults (50-)

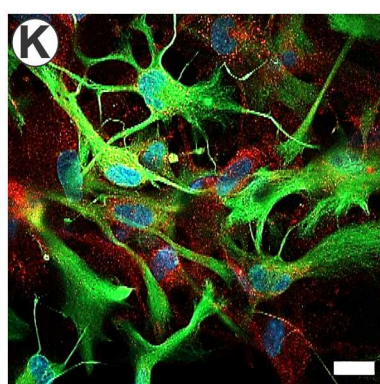
ID 05



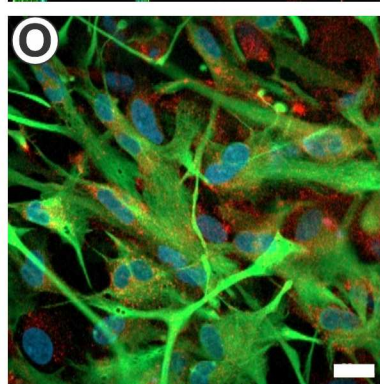
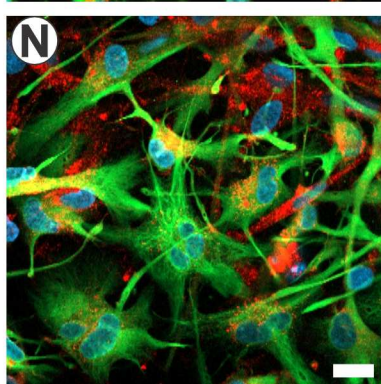
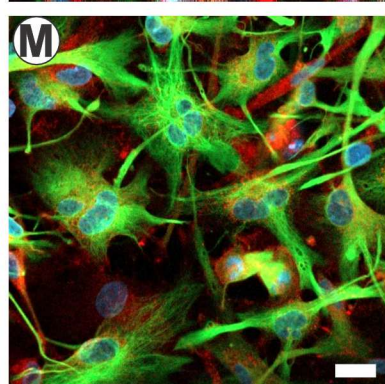
ID 06



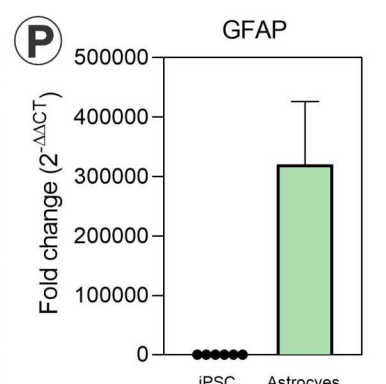
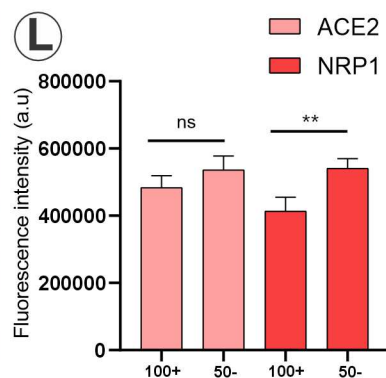
ID 07



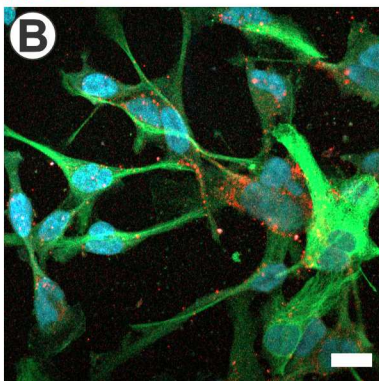
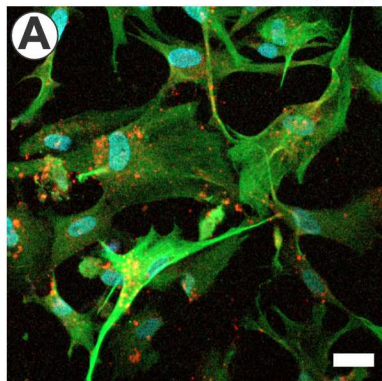
GFAP/NRP1/DAPI



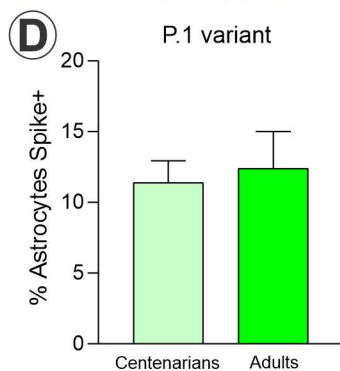
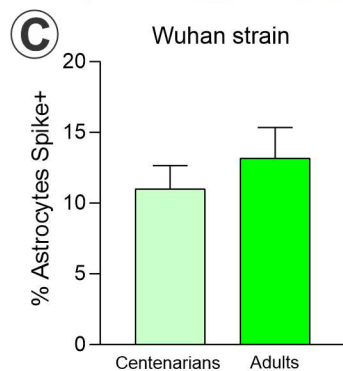
GFAP/ACE2/DAPI



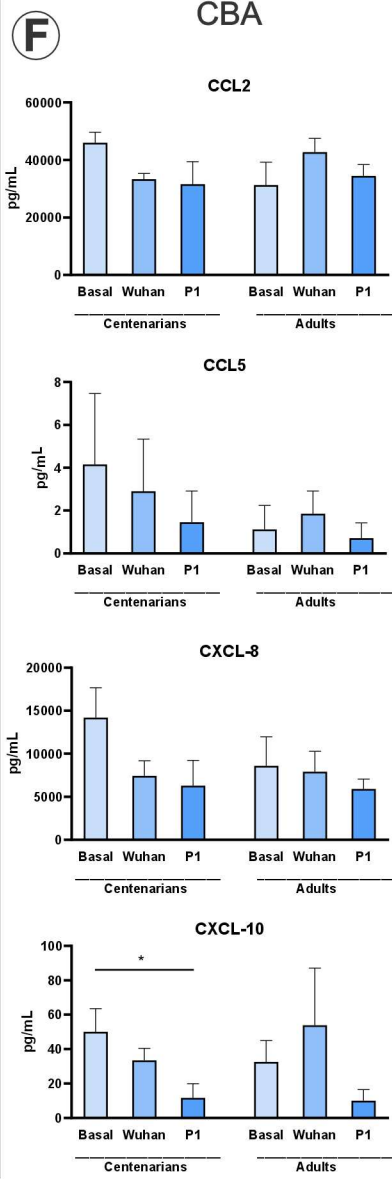
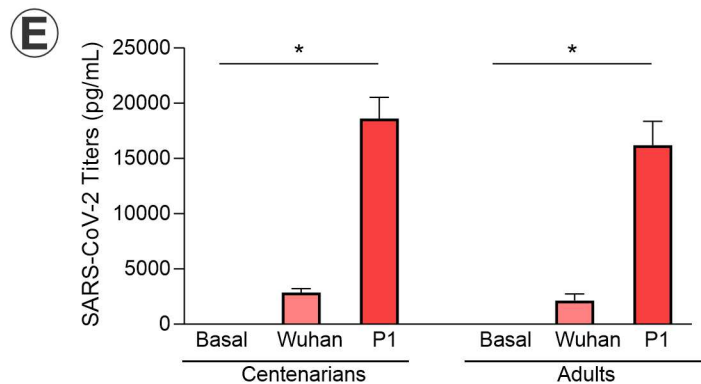
Fluorescence

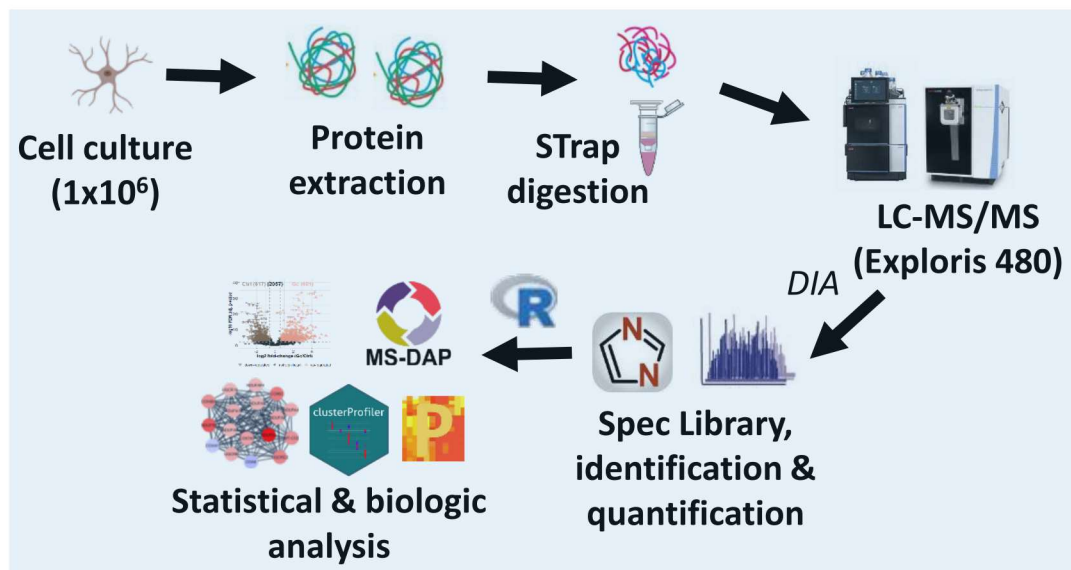
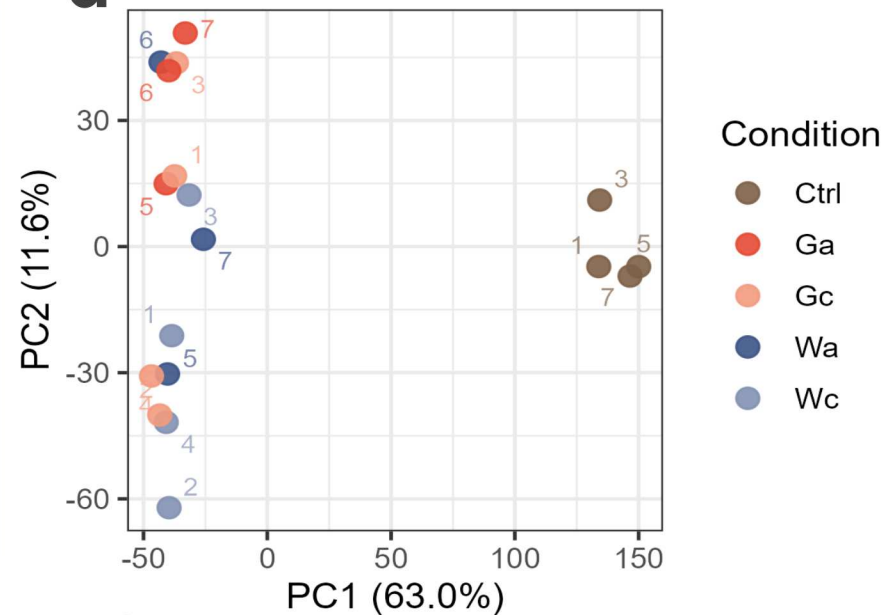
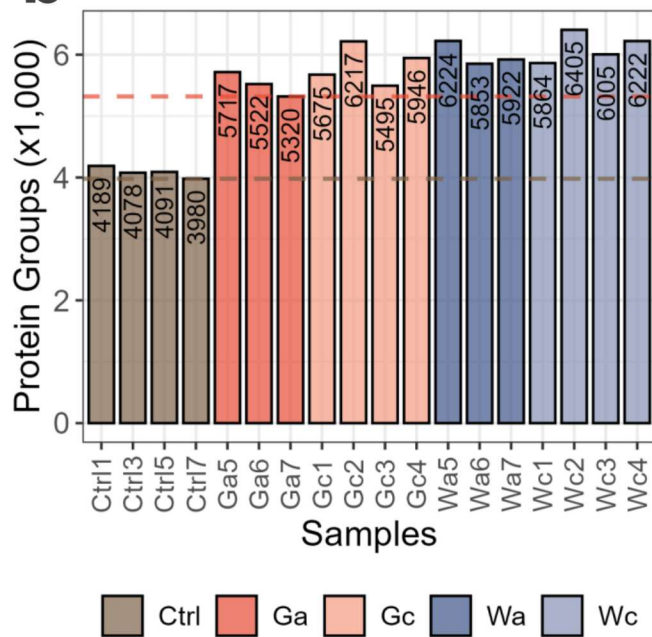
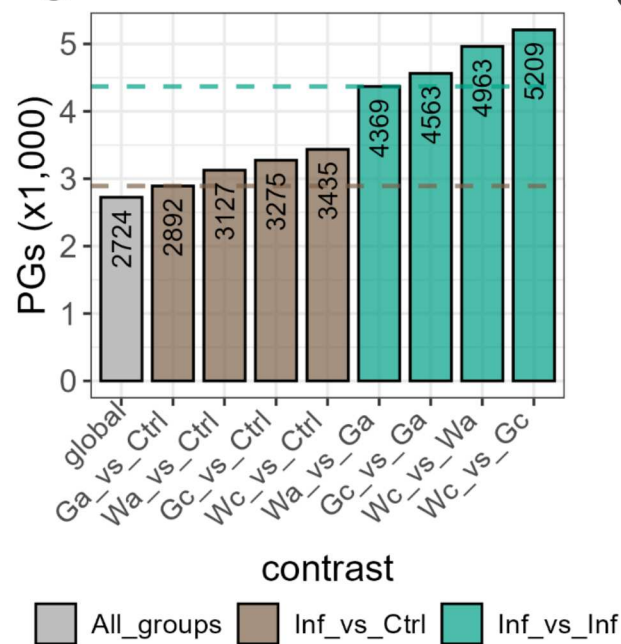
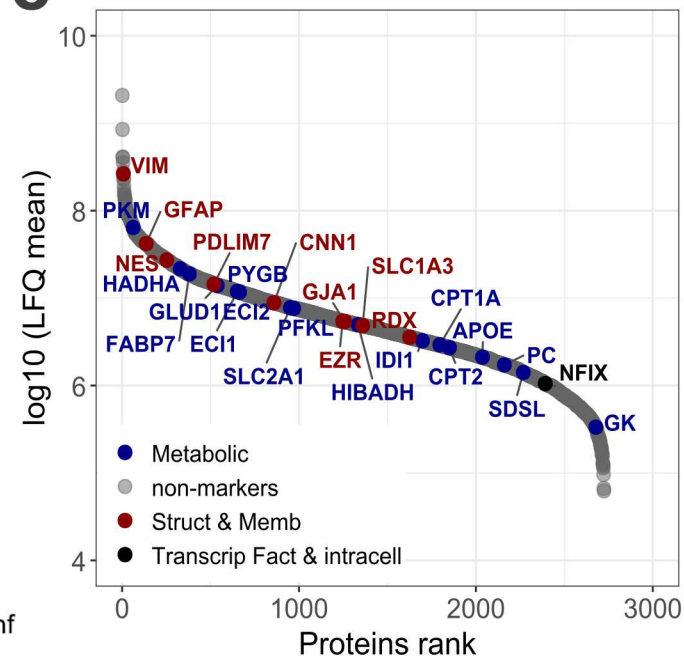


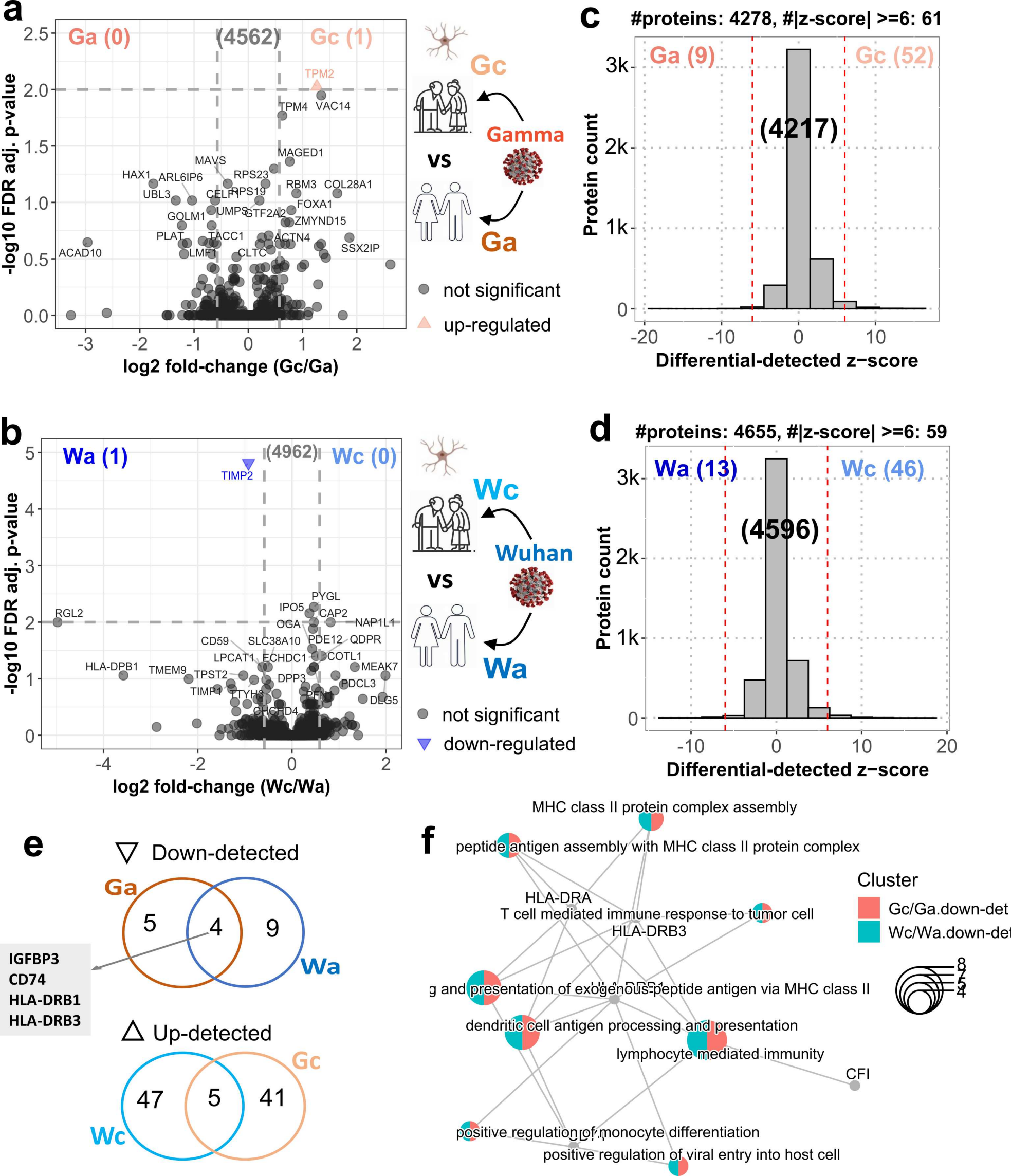
Flow Cytometry

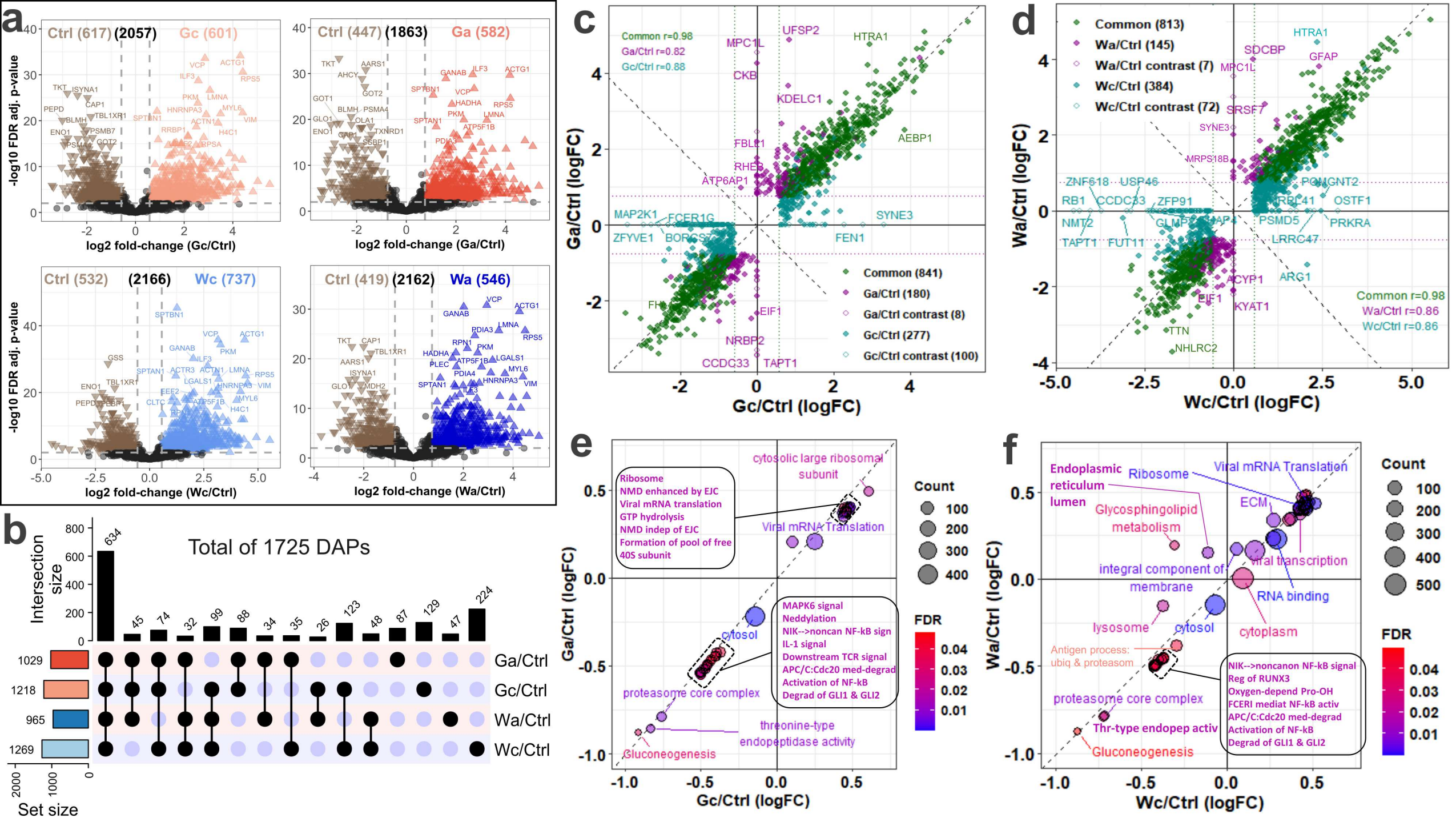


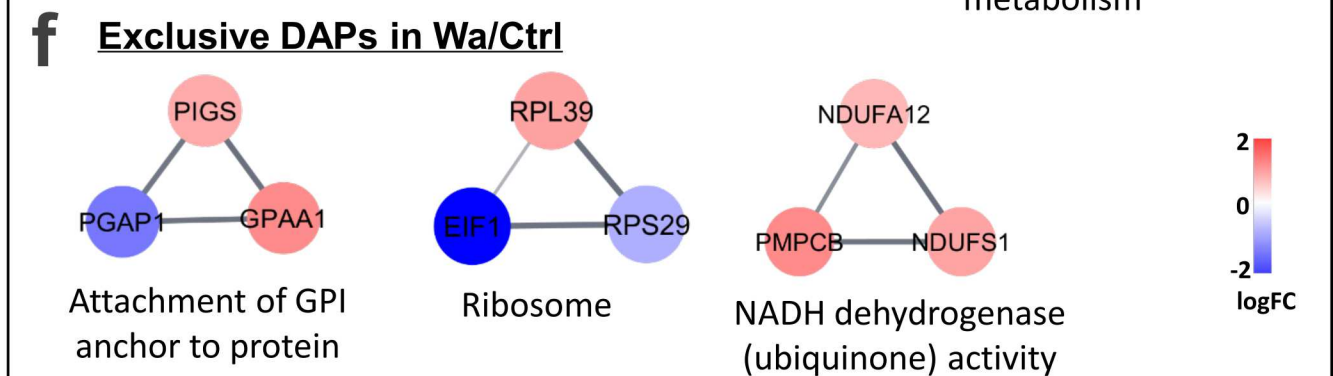
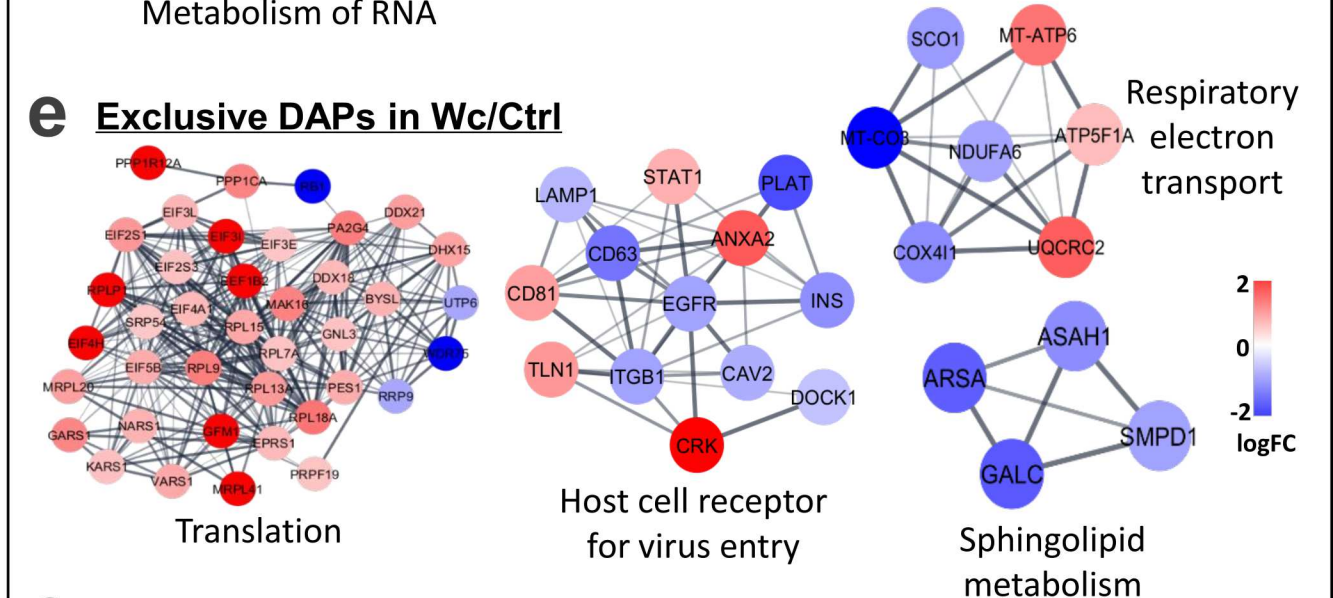
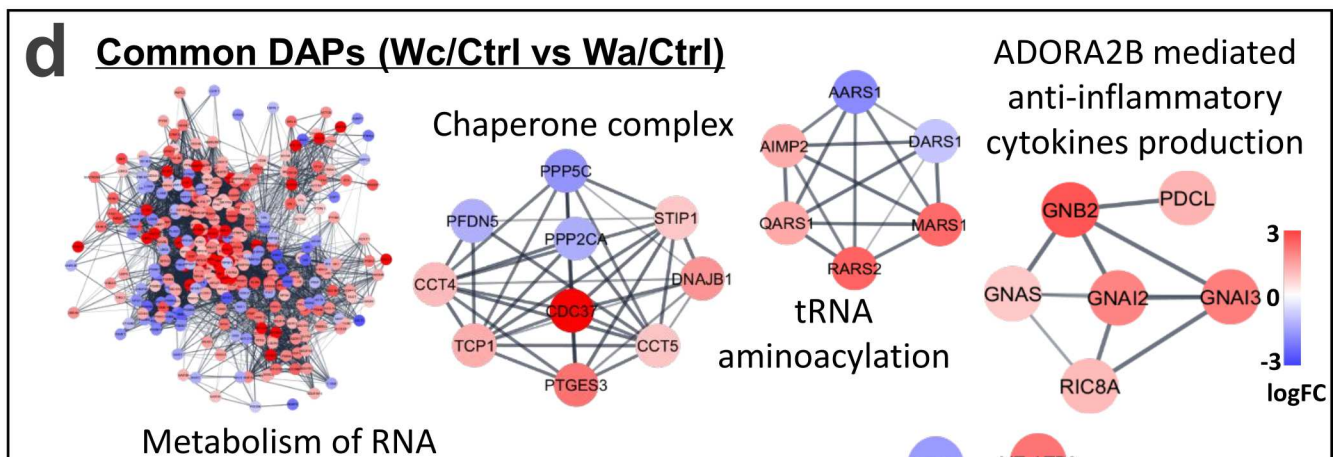
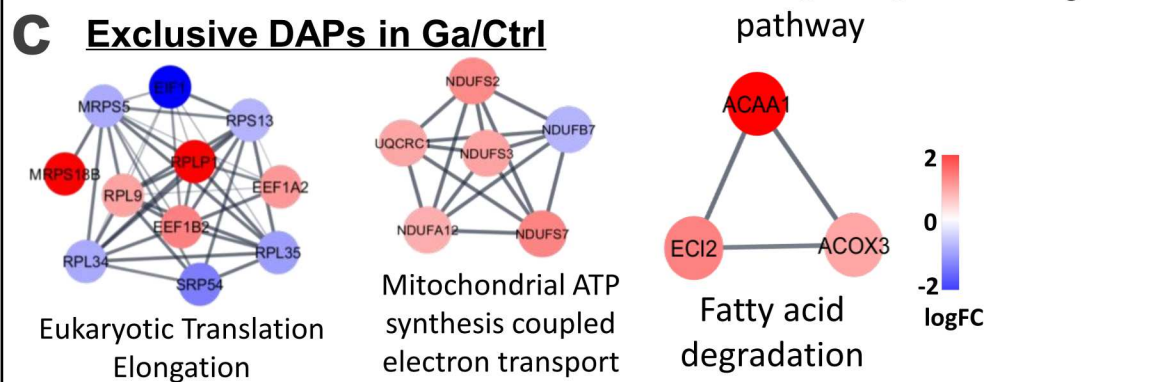
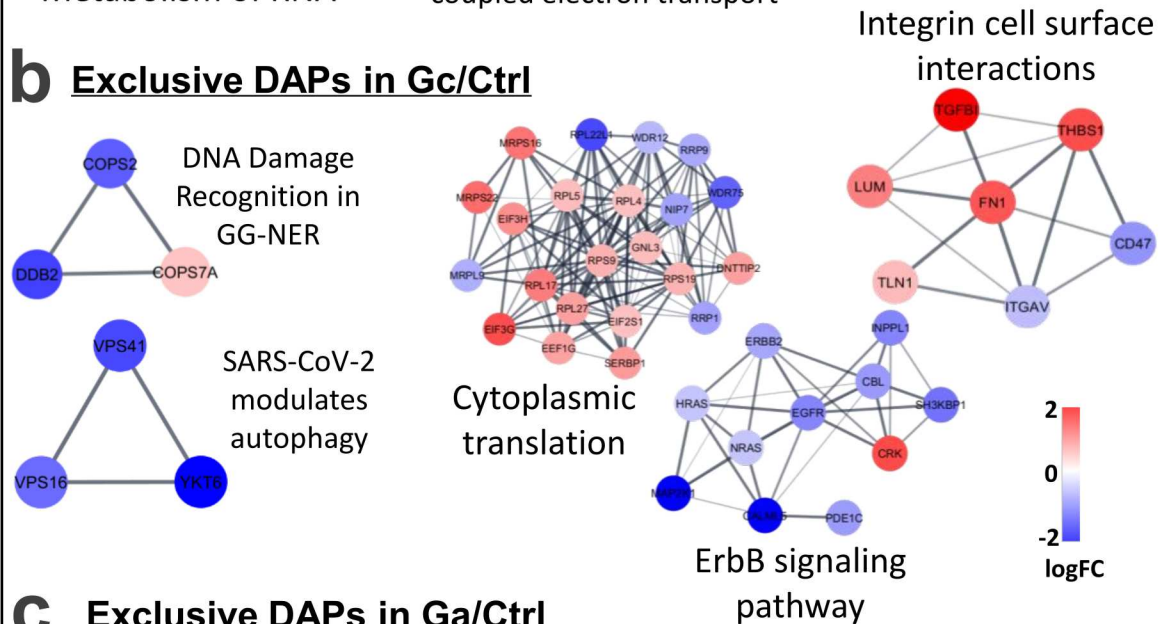
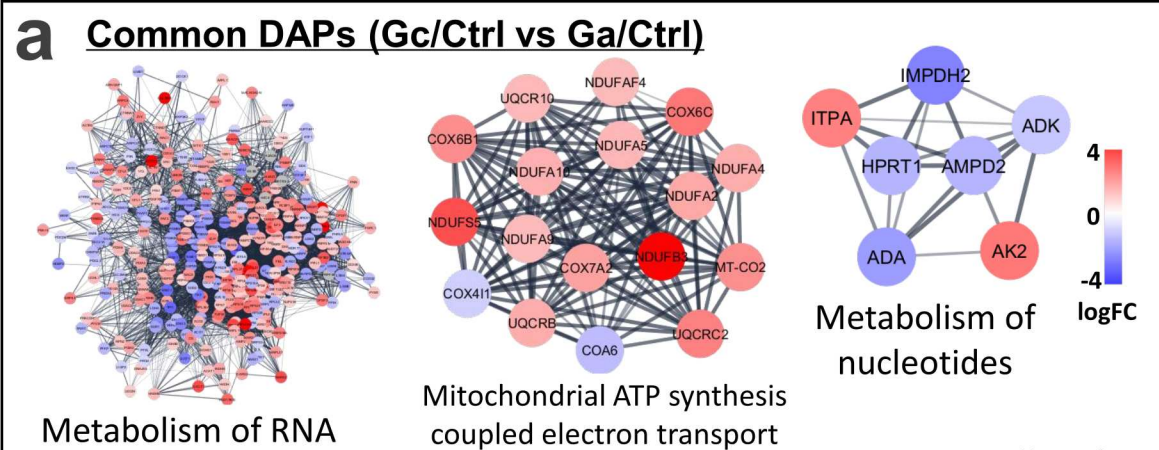
ELISA



a**d****b****c****e**







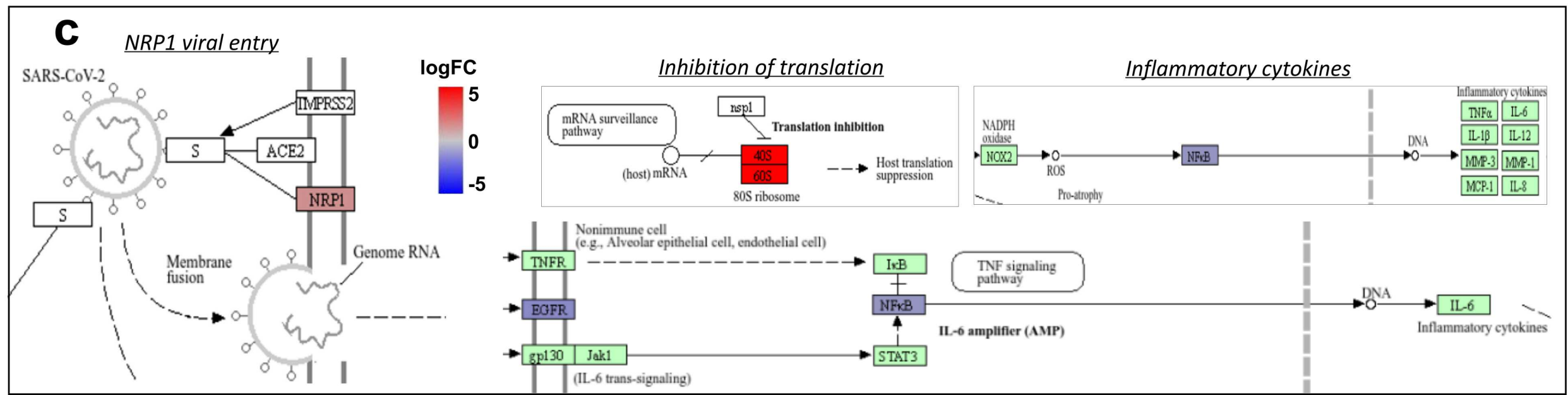
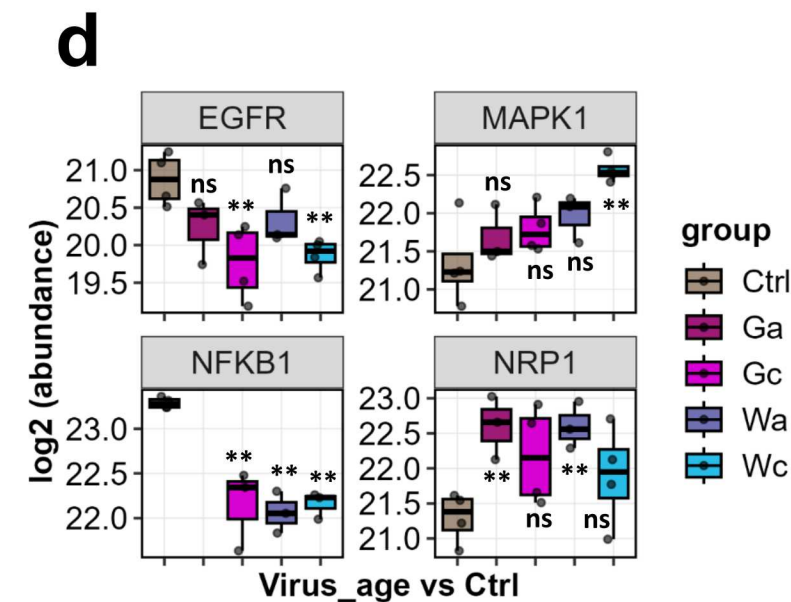
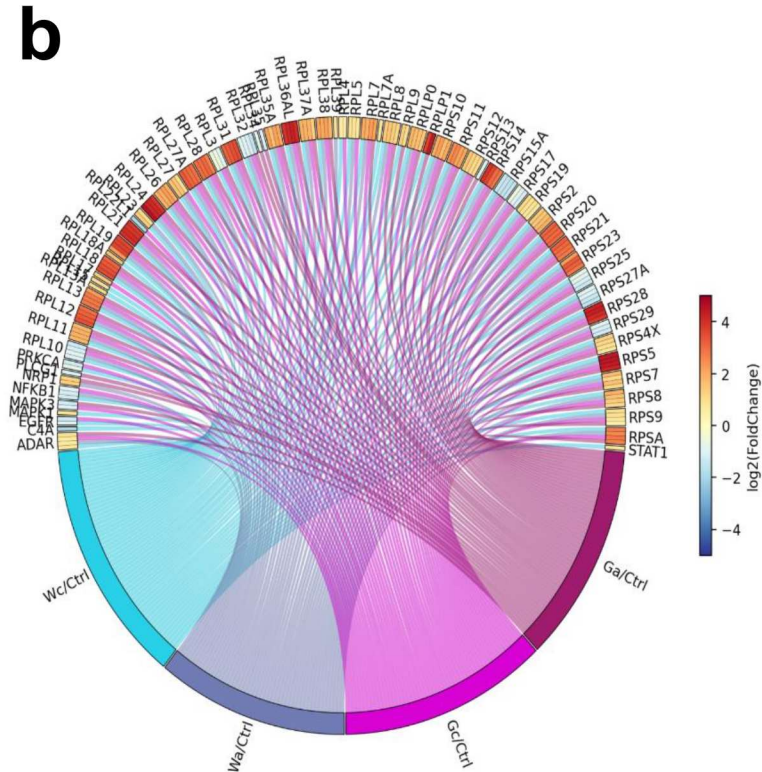
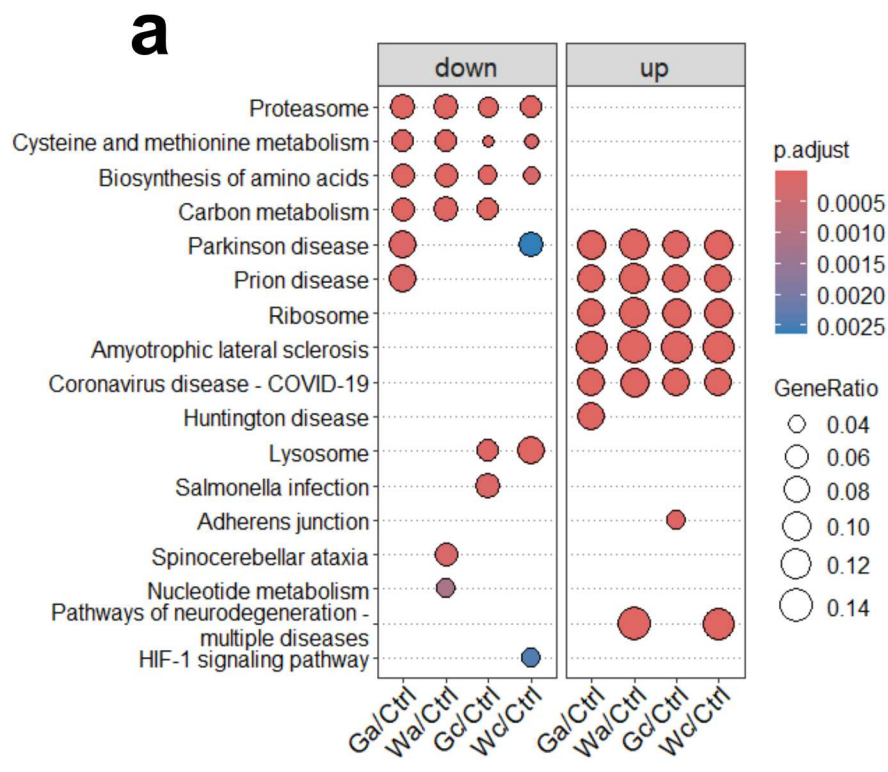


Table 1

Table 1. Demographic and clinical data of the study volunteers.

Info/Group	CENTENARIANS				YOUNG ADULTS		
ID	01	02	03	04	05	06	07
Sex	F	M	M	F	M	F	M
Age*	114	110	106	100	44	26	31
Comorbidities*	Alzheimer; Hypertension; Epilepsy	Hypertension	Benign prostatic hyperplasia	Alzheimer; Hearing loss	None	None	None
COVID severity	Moderate	Moderate	Asymptomatic	Mild	Critical	Critical	Critical

*Age and medical comorbidities were self-reported.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.tiff](#)
- [SM1msdapoutput.xlsx](#)
- [SM2markersliterature.xlsx](#)
- [SM3clusterProfiler.xlsx](#)
- [SM4msdapCtrl.xlsx](#)
- [SM5logFClogFC.xlsx](#)
- [SM62Denrichment.xlsx](#)
- [SM7commonexclusivecytoscape.xlsx](#)
- [SM8deaoutputgoat.xlsx](#)
- [SM9goatoutputreactome.xlsx](#)