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The multiple roles of nsp6 in the molecular pathogenesis of SARS-CoV-2

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to evolve and adapt after its emergence in late 2019. As the causative agent of the coronavirus disease 2019 (COVID-19), the replication and pathogenesis of SARS-CoV-2 have been extensively studied by the research community for vaccine and therapeutics development. Given the importance of viral spike protein in viral infection/transmission and vaccine development, the scientific community has thus far primarily focused on studying the structure, function, and evolution of the spike protein. Other viral proteins are understudied. To fill in this knowledge gap, a few recent studies have identified nonstructural protein 6 (nsp6) as a major contributor to SARS-CoV-2 replication through the formation of replication organelles, antagonism of interferon type I (IFN-I) responses, and NLRP3 inflammasome activation (a major factor of severe disease in COVID-19 patients). Here, we review the most recent progress on the multiple roles of nsp6 in modulating SARS-CoV-2 replication and pathogenesis.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China in late 2019 and quickly spread across the globe to become a major pandemic (Zhu et al., 2020). SARS-CoV-2 has continuously evolved to generate variants of concern (VoCs) with altered viral transmissibility and immune evasion of vaccine- and/or infection-elicited immunity. Different VoCs have led to wave after wave of infections around the world. A large portion of SARS-CoV-2-related research has focused on the spike (S) protein due to its importance in infection and vaccines (Walls et al., 2020; Q. Wang et al., 2020). As a highly immunogenic viral factor, the S protein was an ideal vaccine target for the recently approved vaccines and for the development of effective therapeutic antibodies (Abdolmaleki et al., 2022; Patel et al., 2022; Plante et al., 2021; Walls et al., 2020; Q. Wang et al., 2020). S protein, however, is just one of many viral factors that contribute to the molecular pathogenesis of SARS-CoV-2.

SARS-CoV-2 is an enveloped betacoronavirus containing a single-stranded positive-sense RNA genome (Bar-On et al., 2020). Once the S protein binds to the host receptor angiotensin-converting enzyme 2 (ACE2), the virus can enter the cell by two methods: (i) via the endocytosis pathway whereupon the viral envelope may fuse with the endosomal membrane to release the viral genome into the cytoplasm, or (ii) via the proteolytic cleavage of the S protein by the host factor TMPRSS2, triggering fusion of the viral envelope with the plasma membrane and release of the viral genome directly into the cell (Fig. 1; Hoffmann et al., 2020). The SARS-CoV-2 genome contains a 5′ cap and a 3’ polyadenylated tail that facilitate protein translation by host machinery without activating cellular immune sensors (Kim et al., 2020). Two long open reading frames (ORFs) encoding polyproteins pp1a and pp1ab are co-translationally processed by viral proteases to make 16 individual nonstructural proteins (nsps), which together form the replication complex (Fig. 1; Cui et al., 2019; Kim et al., 2020). Additional ORFs encode 7 accessory proteins, thought to antagonize host immune responses (Y. Liu et al., 2022), and 4 structural proteins that form the virus particle (Fig. 2; Cui et al., 2019).

Replication of the viral genome occurs in double-membrane vesicles (DMVs) that originate from the endoplasmic reticulum (ER) to form replication organelles that protect nascent viral genomic RNA from pattern recognition receptors (PRRs) that might trigger an interferon response (Fig. 1; Knoops et al., 2008; Scutigliani and Kikkert, 2017; Yang et al., 2020). Translation of structural proteins at the ER begins the process of virion assembly (Fig. 1; Fehr and Perlman, 2015; Saraste and Prydz, 2021). Newly replicated viral genomes are coated with

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nucleocapsid (N) proteins that facilitate virion assembly with envelope (E) and membrane (M) proteins in the ER-to-Golgi intermediate compartment (ERGIC; Fig. 1; de Haan and Rottier, 2005; V'kovski et al., 2021). Finally, rather than egress through the conventional biosynthetic secretory exocytosis pathway like most other RNA viruses, betacoronaviruses including SARS-CoV-2 are released from the infected cell through the lysosomal pathway (Fig. 1; Ghosh et al., 2020).

All nonstructural proteins are considered to have crucial roles in replication. While the molecular structure of nsp6 has yet to be solved (Gupta et al., 2020; Mariano et al., 2020), recent studies have suggested that nsp6 contributes to SARS-CoV-2 replication through a variety of mechanisms. Three major functions have been reported for nsp6: (i) It dimerizes to form replication organelles, (ii) it antagonizes host innate immune response by tampering with IFN-I signaling pathways, and (iii) it activates NOD-like receptor (NLR) Family Pyrin Domain Containing 3 (NLRP3) inflammasomes by impeding the acidification of lysosomes (Bills et al., 2023; Ricciardi et al., 2022; Sun et al., 2022a, 2022b). Here, we will review these processes and implications for nsp6 in SARS-CoV-2 molecular pathogenesis.

2. Nsp6 structure

Coronavirus nsp6 is a transmembrane protein with an approximate molecular weight of 34 kDa and localizes to the ER membrane and the perinuclear space (Angelini et al., 2013; Cottam et al., 2011; Lee et al., 2021; Ricciardi et al., 2022; Snijder et al., 2020). Sequence comparisons show SARS-CoV-2 nsp6 shares 87% identity with SARS-CoV nsp6 (Fig. 2A). Alignment with SARS-CoV-2 variants shows that a three amino-acid deletion (ΔSGF) is common to six previous SARS-CoV-2 variants [Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Eta (B.1.525), Iota (B.1.526), and Lambda (C.37)] (Fig. 2A; Peacock et al., 2021; Romero et al., 2021). Phylogenetic analyses using Nextstrain reveal that ΔSGF emerged independently among these variants, suggesting a fitness advantage (Hadfield et al., 2018; Ricciardi et al., 2022). Separately, the Delta variant nsp6 contains a single unique amino acid change V149A. Interestingly, the initial Omicron BA.1 also contains a three amino-acid deletion (ΔLSG) along with a unique amino acid change (I189V), though the triple deletion is shifted to one amino acid upstream. Subsequent Omicron sublineages (BA.2, BA.4, and BA.5) instead contain the original ΔSGF deletion without additional changes (Fig. 2A). Because the shifted ΔLSG from the initial BA.1 was converged to ΔSGF in subsequent Omicron sublineages, it is reasonable to hypothesize that ΔSGF conveys a greater fitness advantage over ΔLSG. Whether V149A and I189V mutations contribute in any way to pathogenesis is unknown. Experiments are needed to test the above hypotheses.

The ΔSGF deletion has been demonstrated to be involved in both the formation of DMVs as well as antagonism of IFN-I signaling pathways and is located in the long lumenal loop (91–112; Fig. 2F; Bills et al., 2023; Ricciardi et al., 2022). Structural simulations of the lumenal loop (91–112) suggest that it is disordered, but experimental evidence under different biological conditions shows that the loop shows partial helicity (Kumar et al., 2021). The lumenal loop (91–112) and C-terminal domain (CTD; 229–290) consist of clusters of multiple aromatic and charged amino acids that would likely drive protein-protein interactions

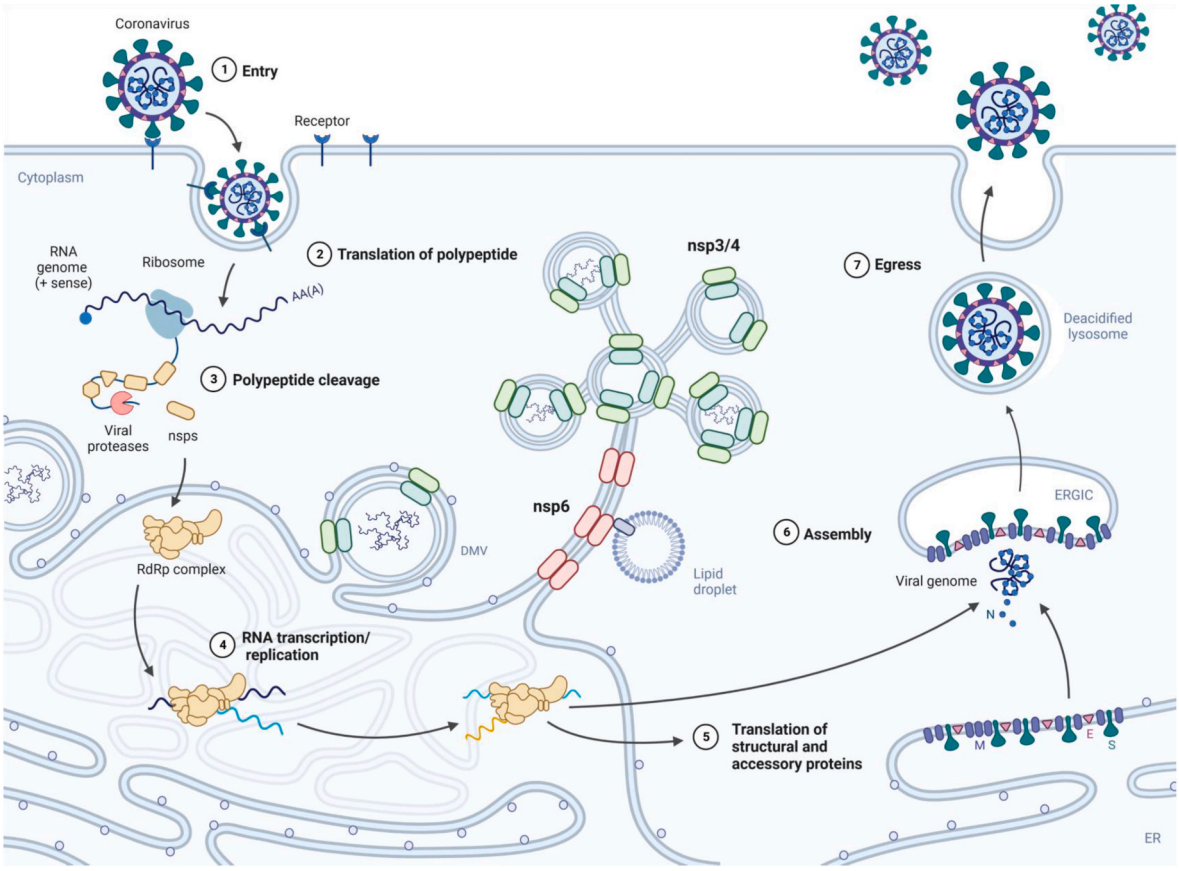

Fig. 1. SARS-CoV-2 Replication Cycle
Schematic of the SARS-CoV-2 replication cycle. Upon entry the nonstructural proteins (nsps) are co-translationally cleaved by viral proteases to form the RNA-dependent RNA polymerase (RdRp) complex. Nsp6 proteins embedded in the ER membrane homodimerize to form linear zippered ER structures that connect with nsp3/nsp4 double membrane vesicles (DMVs) that shield nascent viral RNA (vRNA). Nsp6 recruits lipid droplets to replenish DMVs. Newly synthesized genomes are coated with nucleocapsid (N) and are packaged into viral particles with spike (S), envelope (E), and membrane (M) proteins. Adapted from "Life Cycle of Coronavirus", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

(Ricciardi et al., 2022).

Various predictive software have produced contradictory results: DeepTMHMM predicts 8 transmembrane domains and both N- and C-termini end in the cytoplasm, whereas Protter predicts 7 transmembrane domains with the N-terminus in the cytoplasm and the C-terminus in the lumen (Hallgren et al., 2022; Omasits et al., 2014). Both the N-terminal end and the highly conserved C-terminal end should be in the cytoplasm given that they must be proteolytically processed by the cytosolic protease, nsp5 (Mariano et al., 2020).

Structural predictions using the artificial intelligence program AlphaFold (DeepMind) yield interesting results (Jumper et al., 2021). In contrast to predictions by Protter, but in agreement with the DeepTMHMM software, AlphaFold predicts that SARS-CoV-2 nsp6 has 8 transmembrane domains (Fig. 2B and C; Hallgren et al., 2022; Jumper et al., 2021; Omasits et al., 2014). Accordingly, the N- and C-termini are in the cytoplasm and the structure contains a long lumenal loop (amino acids 91–112) and a structured C-terminal domain. The long lumenal loop, where ΔSGF and ΔLSG occurred in different SARS-CoV-2 variants, shows some degree of helicity and a few non-covalent interactions between the loop and the rest of the protein, suggesting that the loop is flexible to mediate protein-protein interactions. The CTD consists of 2 helices and 2 strands and shows some non-covalent interactions within the CTD and interactions between the CTD and other regions of the protein, suggesting that the CTD maintains a structure to mediate protein-protein interactions. Additionally, the nsp6 structure contains a short lumenal helix between transmembrane domains 1 and 2, and another very short lumenal helix between transmembrane domains 7 and 8 (Fig. 2B and C; Jumper et al., 2021).

AlphaFold also predicts that nsp6 dimerizes through interactions between transmembrane domains 5, 6, and 7 (Fig. 2D; Jumper et al., 2021). This model, however, does not rule out the possibility of end-to-end homodimerization that would mediate ER zippering, a central function of nsp6 as reported in a recent study, which involves the formation of narrow and exclusive membranous channels through the juxtaposition of adjacent ER membranes (Ricciardi et al., 2022). AlphaFold is highly accurate for predicting structures when similar protein structures have been solved (Jumper et al., 2021). However, caution should be taken with the current nsp6 structure model.

Experimental evidence supports predictions that nsp6 has only seven transmembrane domains, the last of which does not traverse the ER membrane (Fig. 2F; Ricciardi et al., 2022). Indeed, immunofluorescence experiments have shown that permeabilization of the plasma membrane allows the detection of N- and C-tagged nsp6 (Ricciardi et al., 2022). Analysis of another coronavirus, mouse hepatitis virus (MHV), experimentally demonstrated that nsp6 has 7 transmembrane domains (Oostra et al., 2008). More recent studies of SARS-CoV-2 nsp6 suggest that the 7th transmembrane domain, having an amphipathic sequence to form a helix, likely associates with the ER membrane rather than traverses the lipid bilayer (Fig. 2D; Ricciardi et al., 2022). Indeed, full-length nsp6 forms puncta within the ER, whereas removing the C-terminus or introducing F220Q and T222W mutations in the amphiphilic helix caused nsp6 to diffuse throughout the ER (Ricciardi et al., 2022). Compared with the AlphaFold model, this model differs in the structure of the C-terminal region after residue 157, which contains just two transmembrane domains (i.e., transmembrane 6 and the ER-associated helix 7; Fig. 2F) rather than 3 transmembrane domains

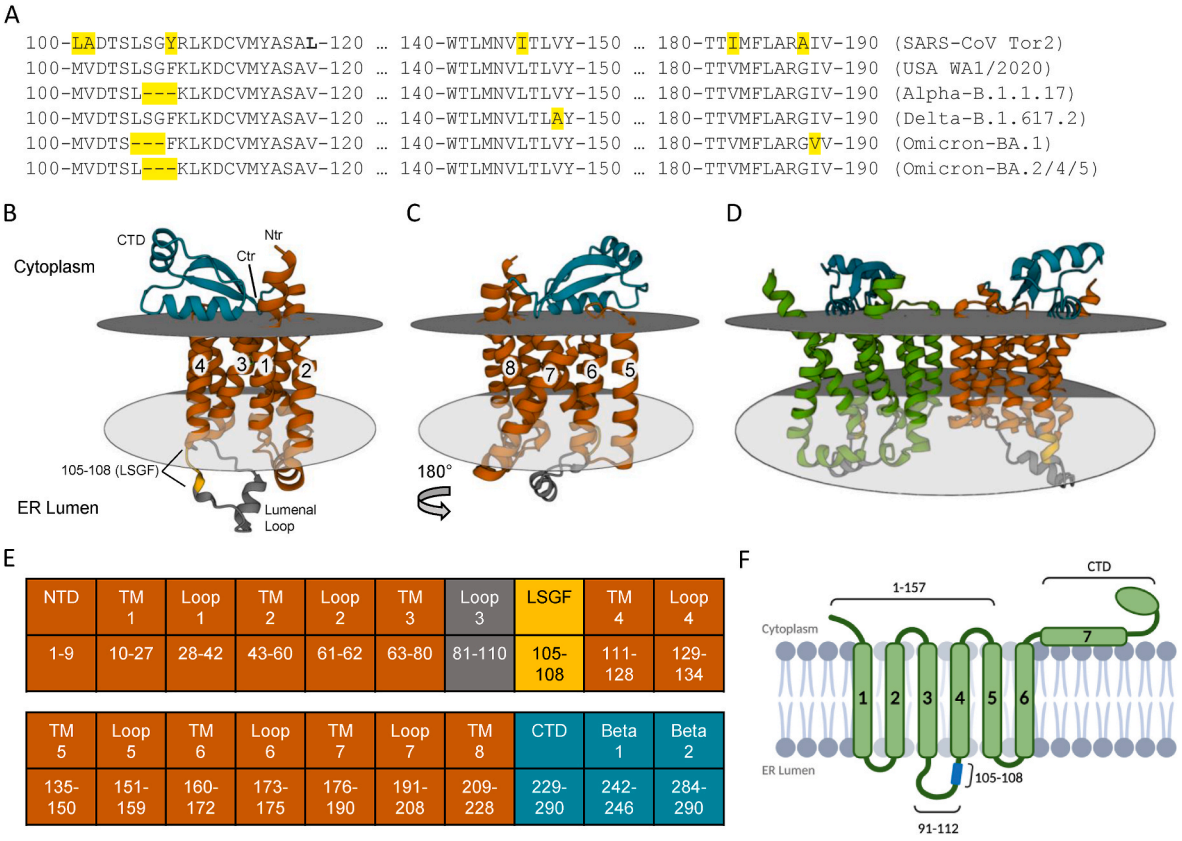

Fig. 2. Nsp6 structure and conserved ΔSGF (A) Alignment of the variants of concern (VoCs) with the USA WA1/2020 strain, isolated from the first COVID-19 case in the USA. Beta, Gamma, Eta, Iota, and Lambda (not shown) are identical to Alpha. (B,C) nsp6 monomers and (D) dimer as predicted by the AI software AlphaFold; The lumenal loop (gray), C-terminal domain (CTD; teal), and the 105–108 region (yellow) are highlighted; gray discs represent membrane layers. (E) Amino acid sequence of nsp6 with secondary structures highlighted according to AlphaFold predictions in (B,C). (F) Schematic of nsp6 structure based on experimental evidence with numbered transmembrane domains and the 105–108 region highlighted (blue). (E–G) 3D structures presented with Mol* 3D. Schematic (F) Created with BioRender.

(transmembranes 6–8 and the two C-terminal β-sheets; Fig. 2B). An atomic structure of nsp6 is required to validate these models.

3. Double membrane vesicle formation

The SARS-COV-2 nsp3, nsp4, and nsp6 proteins have been shown to modify the ER membranes to form DMVs and the same is true for the respective SARS-CoV proteins (Fig. 1; Angelini et al., 2013; Hagemeijer et al., 2014; Klein et al., 2020; Ricciardi et al., 2022). These double membrane structures provide a protective environment where viral RNA replication can occur away from cytoplasmic sensors and defenses (Knoops et al., 2008; Ricciardi et al., 2022; Scutigliani and Kikkert, 2017). Ricciardi et al. demonstrated that C-terminally tagged SARS-CoV-2 nsp6 expressed alone disseminates throughout the ER, while N-terminally tagged and untagged nsp6 form round structures and colocalize with the host protein Cb5, an ER marker, demonstrating a role for the C-terminus in DMV formation (Ricciardi et al., 2022). Furthermore, they demonstrated that nsp6 homodimerizes to form linear and circular zippered ER structures that encapsulate the surrounding cytoplasm but maintain a clear connection to the ER. Nsp6 is capable of restricting access to these nsp6 compartments, allowing entry only to ER membrane proteins with small lumenal domains such as VAP-A, but not ER lumenal proteins, such as calreticulin (Fig. 1; Ricciardi et al., 2022). Interestingly, truncating the C-terminus of nsp6 removed the ability to form zippered ER structures, but the remainder of nsp6 (amino acids 1–157; Fig. 2F) retained the ability to homodimerize with full-length nsp6 (Ricciardi et al., 2022). It seems likely that the long lumenal loop (91–112) of nsp6 plays a key role in facilitating homodimerization given its positioning in the ER lumen. Interestingly, the small molecule K22, a known inhibitor of several coronaviruses, reduces ER zippering and results in zippering of the nuclear envelope (Ricciardi et al., 2022). The authors suggest that replication could occur in the DMVs formed from the nuclear envelope; however, given the preference for ER-derived replication organelles, the nuclear envelope is likely an unfavorable location for viral replication (Ricciardi et al., 2022)

Lipid droplets (LDs) are required for SARS-CoV-2 replication and are thought to replenish lipids in DMV structures (Fig. 1; Gomes Dias et al., 2020; Ricciardi et al., 2022; Twu et al., 2021). The C-terminus of nsp6 recruits Double FYVE-containing protein 1 (DFCP1), a host protein known to complex with RAB18 to anchor LDs to lipid membranes (Gao et al., 2019; Herker et al., 2021; Li et al., 2019; Ricciardi et al., 2022). Nsp6-mediated interactions with LDs promote the growth of DMV structures as the replication organelles form. Additionally, nsp6 zippering controls access to DMVs by blocking entry to any unwelcome ER lumenal proteins or ER membrane proteins with large lumenal domains, while simultaneously maintaining free access to lipids (Ricciardi et al., 2022).

Previous studies showed that co-expression of nsp3/nsp4 without nsp6 is sufficient to generate DMV structures (Ji et al., 2022; Twu et al., 2021). However, Ricciardi et al. demonstrated that nsp3/nsp4/nsp6 co-expression produces tighter clusters of DMVs that are more numerous, more uniformly shaped, and have a smaller average diameter compared to DMVs produced by nsp3/nsp4 expression without nsp6 (Ricciardi et al., 2022). The smaller DMVs are tethered to the ER by nsp6 linear zippered ER structures (Ricciardi et al., 2022). Importantly, nsp6 (ΔSGF) (emerged in SARS-CoV-2 variants) shows higher zippering activity that produces more uniform DMV structures and improved organization of DMVs through a more developed array of zippered ER connections (Ricciardi et al., 2022). It was proposed that the enhanced zippering activity of nsp6(ΔSGF) may be a major contributing factor in the immune evasion of variants containing ΔSGF (Lee et al., 2022; Ricciardi et al., 2022; Thorne et al., 2022).

Of note, SARS-CoV-2 nsp3/4 induce the production of phosphatidylinositol 3-phosphate (PI3P), a component necessary for DMV formation; class III phosphatidylinositol 3-kinase (PI3K) and the PI3P-binding protein DFCP1 are both essential host factors in this process (Twu et al., 2021). TMEM41B and VMP1 are also essential host factors for DMV biogenesis that interact with nsp3/4 complexes and manage phosphatidylserine distribution, an important component for maintaining DMV structures (Ji et al., 2022). Other nonstructural proteins, including nsp2/3 and nsp8, also localize in DMVs during SARS-CoV infection but their role in DMV formation remains unknown (Bost et al., 2000; van der Meer et al., 1999). Therefore, while the evolution of nsp6 enhances DMV formation and organization, other nonstructural proteins are also essential.

4. Modulation of autophagy

Autophagy is a regular process to manage cellular waste and destroy intracellular infectious material (Fan and Zong, 2008). In the case of viral infection, autophagy is important to control infection and prevent widespread dissemination of the virus throughout the host by targeting viral components for lysis, as well as to process antigens for presentation to major histocompatibility (MHC) molecules (Wu and Yue, 2020). Studies have demonstrated that SARS-CoV-2 nsp6 inhibits the formation of hybrid pre-autophagosomal structures (HyPAS), which are derived from fused *cis* Golgi and endosomal membranes (Kumar et al., 2021). This results in smaller autophagosomes that likely degrade viral components less efficiently (Cottam et al., 2014; Gassen et al., 2019). Related coronaviruses, including SARS-CoV, also utilize nsp6 to form smaller ER-derived vesicles with the properties of nascent autophagosomes (Cottam et al., 2011, 2014). At late stages of infection, SARS-CoV-2 nsp6 produces smaller autophagosomes to reduce autophagy overall and, in combination with ORF3a, prevents the fusion of compartments containing viral components with lysosomes (Miao et al., 2021).

5. NLRP3 inflammasome activation and pyroptosis

Rodrigues et al. showed that nsp6 inhibits the lysosome-autophagy system by binding to a lysosomal proton pump component. Nsp6 inhibits lysosomal acidification, resulting in a buildup of non-digestive autophagosomes (Rodrigues et al., 2020). In line with these results, Sun et al. demonstrated that nsp6 binds directly to ATP6AP1, a component of the vacuolar ATPase proton pump, to prevent lysosomal acidification in lung epithelial cells (Fig. 4; Sun et al., 2022b; 2022a). Nsp6 does not, however, block the fusion of autophagosomes with lysosomes; thus, non-digestive lysosomes accumulate and activate the NLRP3 inflammasome, leading to caspase-1-dependent maturation of interleukin-1β (IL-1β) and IL-18 and triggering pyroptosis, an inflammatory form of apoptosis (Bergsbaken et al., 2009; Broz and Dixit, 2016; Sun et al., 2022a, 2022b). In the same study, IL-1β, IL-18, and M65 were identified as markers for severe COVID-19 (Nagy et al., 2021; Sun et al., 2022a). An amino acid substitution of L37F that accumulated in nsp6 of some clinical isolates, which was associated with asymptomatic cases of SARS-CoV-2, reduced nsp6's interactions with ATP6AP1, allowing lysosomal acidification to proceed as normal and consequently failing to stimulate the NLRP3 inflammasome pathway (Sun et al., 2022a, 2022b; R. Wang et al., 2020). Not surprisingly, the L37F variant is not associated with any variants given that it compromises viral fitness in SARS-CoV-2 (R. Wang et al., 2020).

Recent studies have demonstrated the role of inflammasome-activated pyroptosis in lung-resident macrophages and its contribution to severe disease in COVID-19. About 10% of blood monocytes and 8% of lung macrophages in COVID-19 patients were infected with SARS-CoV-2 through the uptake of antibody-opsonized virus particles by Fcγ receptors on the cell surface (Junqueira et al., 2022). SARS-CoV-2 infection thus activated NLRP3 and AIM2 inflammasomes in nearly a quarter of lung macrophages, leading to pyroptosis, which was shown to be important for preventing persistent replication of the virus within monocytes/macrophages while simultaneously triggering an alarm to mobilize an immune response (Junqueira et al., 2022). As an adverse effect, the release of intracellular contents with an increased

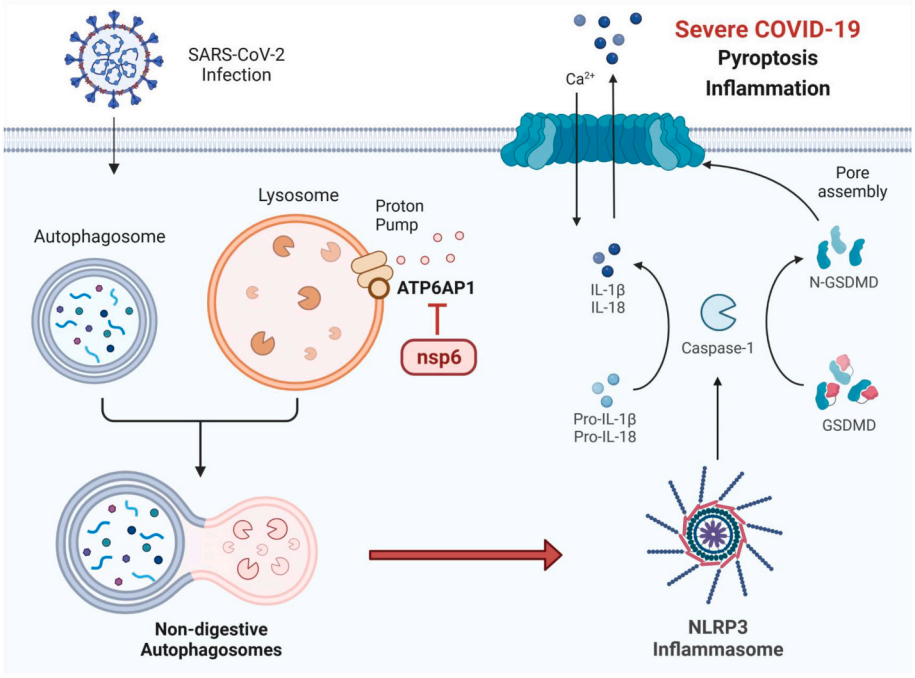

Fig. 3. Nsp6 suppression of lysosomal acidification activates NLRP3 inflammasome
Nsp6 interacts with ATP6AP1 proton pump component to suppress acidification of lysosomes by blocking cleavage activation of ATP6AP1. This leads to accumulation of non-digestive autophagosomes, which activates the NRLP3 inflammasome instigating pyroptosis, a key feature of severe COVID-19. Created with BioRender. Adapted from Sun et al., 2022.

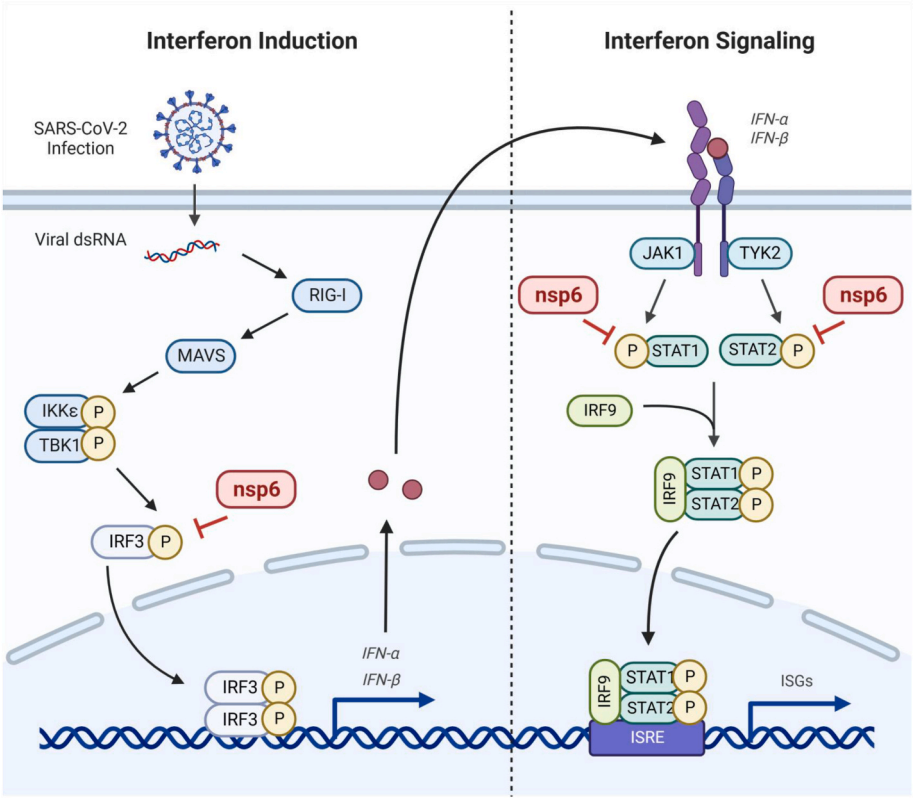

Fig. 4. nsp6 antagonizes interferon pathways
Schematic of interferon induction and interferon signaling pathways and the steps antagonized by nsp6. Nsp6 binds to TBK1 to block phosphorylation of IRF3 and prevent IFN-I induction. Nsp6 blocks phosphorylation of STAT1 and STAT2 to suppress IFN-I signaling. Created with BioRender. Adapted from Xia et al. (2020).

concentration of IL-1β and IL-18 could be the cause of a cytokine storm that can damage organs, and cause vascular leakage and respiratory distress in COVID-19 patients (Junqueira et al., 2022; Rodrigues et al., 2020). A few other studies have similarly shown an increase in IL-1β in the blood and NLRP3 inflammasome activation, which correlate with clinical outcomes in COVID-19 patients (Hadjadj et al., 2020; Hui et al., 2020; Rodrigues et al., 2020; Zheng et al., 2021). These data suggest that the nsp6-mediated activation of NLRP3 inflammasomes and subsequent pyroptosis may be a significant contributor to severe disease in SARS-CoV-2 infected individuals.

Of note, a screen using affinity-purification mass spectrometry identified Sigma-1 receptor (SIGMAR1) as well as 3 components of the vacuolar ATPase involved in ion transport, ATP13A3 ATP5MG, and ATP6AP1, as high-confidence nsp6 interactors, corroborating recent studies (Gordon et al., 2020). A genome-wide CRISPR screen revealed ATP6AP1 as an important host factor for SARS-CoV-2 infection (Hou et al., 2022). ATP6AP1 and the SIGMAR1 are both known drug targets (Gordon et al., 2020). Hydroxychloroquine, an inhibitor of the Sigma-1 receptor, gained much attention at the beginning of the pandemic due to its controversial use despite evidence that the molecule did not offer significant clinical benefits to COVID-19 patients (WHO Solidarity Trial Consortium, 2021). Haloperidol, however, proved more effective at reducing SARS-CoV-2 infection *in vitro*, possibly by inhibiting SIGMAR1 and nsp6 interactions (WHO Solidarity Trial Consortium, 2021).

6. Nsp6 antagonism of IFN-I pathways

The IFN pathway, comprising of type I IFNs (IFNα and IFNβ) and type III IFNs (IFNλ), is part of the first line of defense against viral infections (Hoffmann et al., 2015; Kotenko and Durbin, 2017; Levy et al., 2011). A triggered IFN response culminates in the activation of the innate immune response and promotion of adaptive immunity against future infection by the same pathogen. Treatment with IFNα and IFNβ has already been investigated as a possible therapeutic in multiple trials, but the results were variable (Jhuti et al., 2022).

We previously reported that several SARS-CoV-2 nonstructural and accessory proteins are responsible for antagonizing the IFN-I induction and signaling pathways in infected host cells (Fig. 3; Xia et al., 2020). Nsp6 in particular inhibits IFN-I production by binding to tank-binding kinase 1 (TBK1) to block phosphorylation of interferon regulatory 3 (IRF3), preventing nuclear translocation and subsequent gene activation. To block IFN-I signaling, nsp6 blocks phosphorylation of signal transducer and activator of transcription proteins 1 (STAT1) and STAT2 by receptor-associated Janus kinase 1 (JAK1) and Tyrosine kinase 2 (TYK2), preventing STAT1/2 complexing with interferon regulatory factor 9 (IRF9) and subsequent nuclear translocation to activate interferon-stimulated genes (ISGs; Xia et al., 2020). In support of our findings, another study showed that nsp6 reduced IFN-I induction and IFN-I signaling pathways to a similar degree (Shemesh et al., 2021; Yuen et al., 2020). In contrast, one study similarly reported that nsp6 strongly blocked IFNβ promoter activity as well as suppressed MAVS-induced mRNA expression of IFNβ, IFNλ1, and IFNλ2/3 but had no effect on IFN-I signaling (Shemesh et al., 2021), while another study reported that nsp6 enhanced IFN-I signaling but had no effect on IFN-I induction (Lei et al., 2020). These differences could be due to distinct experimental methods.

The Alpha variant of SARS-CoV-2, one of the first variants that emerged in mid-2020, was shown to be resistant to IFN-I and IFN-III treatment (Guo et al., 2022). Recently, we showed that nsp6 contributes to IFN-I resistance in variants (Bills et al., 2023). When treated with IFNα, an index SARS-CoV-2 isolate USA-WA1/2020 (WA1) containing the Alpha nsp6(ΔSGF) replicated to a higher level in Vero E6-TMPRSS2 cells than the parental WA1 strain (Bills et al., 2023). Furthermore, we showed that expression of variant nsp6(ΔSGF) did not alter IFN-I induction compared to WA1 nsp6; but Alpha, BA.1, and a mutant nsp6 (ΔLSG) reduced the activation of interferon-stimulated regulatory element (ISRE) gene promoter while Delta had no effect (Bills et al., 2023). These results were corroborated by Western blots showing that Alpha, BA.1, and nsp6(ΔLSG) inhibit the phosphorylation of STAT1/2, but not Delta nsp6 (Bills et al., 2023). Further substantiating these results, Omicron BA.1 was recently shown to be more resistant to IFNα treatment compared to WA1 and Delta variant (B.1.617.2; Shalamova et al., 2022). These data suggest that both ΔSGF and ΔLSG mutations in nsp6 variants enhance repression of the host IFN signaling pathway.

The question remains how ΔSGF and ΔLSG impact the overall structure of nsp6. The deletion of three residues shortens the long lumenal loop (91–112), leading to an altered structure of the loop and its interactions with host factors in the IFN signaling pathways. However, the exact mechanism by which nsp6 prevents STAT1/2 phosphorylation is unknown.

On the other hand, enhancement of nsp6 antagonism of IFN-I responses may be due to improved control over DMV formation as discussed above (Ricciardi et al., 2022). Since IFN alpha receptors I and 2 (IFNAR1/2) are heavily glycosylated, it's possible that extensive ER remodeling could cause ER stress that would prevent proper maturation of IFNAR1/2 in the ER/Golgi apparatus network (Ling et al., 1995; Smirle et al., 2013; Sommereyns and Michiels, 2006; Sprooten and Garg, 2020). Indeed, expression of mCherry-tagged nsp6 alone in HEK293T cells was highly cytotoxic, and nsp6 was similarly cytotoxic in an *in vivo Drosophila* model (Lee et al., 2021). Cytotoxicity from nsp6 expression could interfere with IFNAR1/2 trafficking to the plasma membrane, hence, the IFN-I signaling pathway would not be activated. Whether the nsp6-mediated antagonism of IFN-I signaling is due to the direct binding of nsp6 to components of the IFN-I signaling pathway or an indirect effect resulting from improved DMV organization remains to be seen. Further work is required to elucidate the mechanism by which nsp6 suppresses IFN-I responses.

7. Ubiquitination of SARS-CoV-2 nsp6 activates NF-κB expression

As described above in section 6, many studies have demonstrated that nsp6 is involved in antagonism of the IFN-I signaling pathway which would prevent activation of ISG expression and, therefore, limit immune responses and allow propagation of viral replication. Patients who suffer from severe COVID-19, however, tend to have higher levels of proinflammatory cytokines that lead to a cytokine storm, resulting in acute respiratory distress syndrome and organ damage (Blanco-Melo et al., 2020; del Valle et al., 2020; Huang et al., 2020; Karki et al., 2021; Kesmez Can et al., 2021; Nishitsuji et al., 2022; Zhou et al., 2020). Thus, it's possible that SARS-CoV-2 activates the expression of proinflammatory cytokines via an alternative mechanism during the later stages of infection.

Nishitsuji et al. reported that nsp6 interacts with transforming growth factor β-activated kinase 1 (TAK1), a host factor involved in the activation of the canonical NF-κB pathway (Fig. 5; Nishitsuji et al., 2022). NF-κB is considered one of the most important transcription factors for the activation of proinflammatory cytokines during SARS-CoV-2 infection (Neufeldt et al., 2022; Nilsson-Payant et al., 2021). Residue K61 of nsp6 is polyubiquitinated by the E3 ubiquitin ligase tripartite motif-containing 13 (TRIM13), which facilitates complexing of nsp6 and TAK1 with NF-κB essential modulator (NEMO) and the subsequent activation of the NF-κB pathway (Fig. 5; Nishitsuji et al., 2022). Activation of NF-κB signaling leads to increased mRNA expression of IL-8 and IFNγ-induced protein 10 (IP-10), both of which are known to be significantly elevated in serum levels of patients with severe COVID-19 (Blanco-Melo et al., 2020; del Valle et al., 2020; Huang et al., 2020; Karki et al., 2021; Kesmez Can et al., 2021; Nishitsuji et al., 2022; Zhou et al., 2020). It's possible that inhibition of nsp6 or NF-κB signaling may ease the severity of COVID-19 symptoms by suppressing proinflammatory cytokines.

8. NSP6 mutations *in vivo*

In addition to showing that nsp6 mutations enhance suppression of IFN-I signaling pathways, we reported that intranasal infection of mice with a mutant WA1 SARS-CoV-2 containing the ΔSGF nsp6 deletion (ΔSGF-WA1) produces more viral RNA in lungs than those inoculated with the parental WA1 virus (Bills et al., 2023). Consistently, ΔSGF-WA1-infected mice on average began losing weight a day earlier than WA1-infected mice and the disease-state lasted for seven days, a day longer than WA1-infected mice (Bills et al., 2023). ΔSGF-WA1

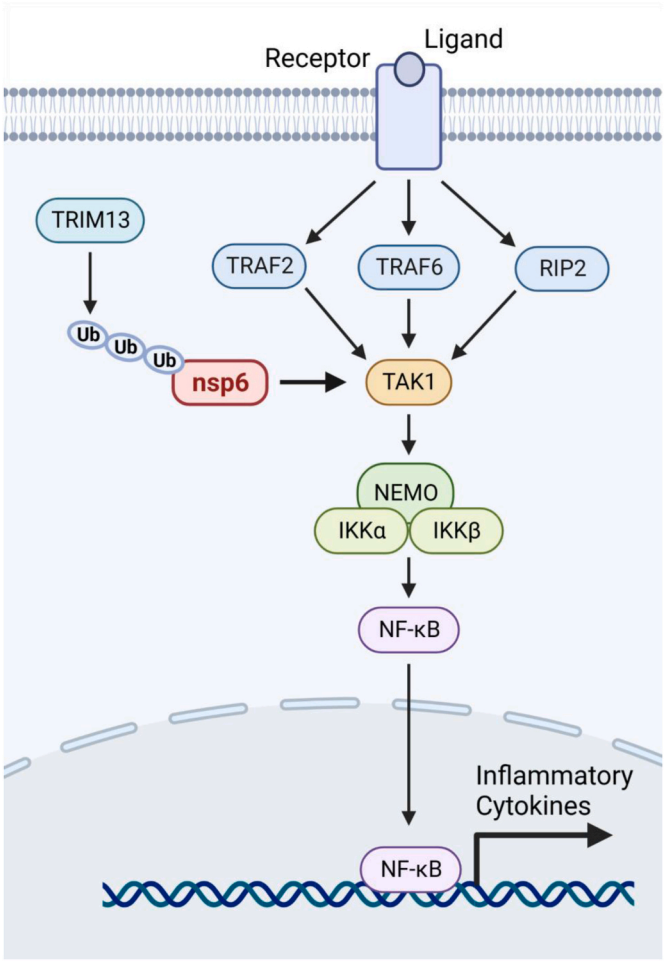

Fig. 5. Nsp6 ubiquitination activates NF-κB
TRIM13 ubiquitination of nsp6 promotes complexing with TAK1 and NEMO, resulting in activation of NF-κB and upregulation of NF-κB-regulated transcripts of inflammatory cytokines. Created using BioRender. Adapted from Nishitsuji et al., 2020.

proved more lethal with a survival rate of 50% compared to 75% for WA1. Surprisingly, analysis of histopathology of infected lung tissues from both groups of mice received similar scores, suggesting ΔSGF-WA1 does not cause greater cytopathic effect in the lungs. Instead, analysis of the host response using the nCounter Analysis System and Ingenuity Pathway Analysis revealed that in both mouse lung tissues and infected primary human airway epithelial cells, cytokine pathways and pathogen-induced cytokine storm pathways were downregulated in the initial stages of infection but by day 4 were significantly upregulated. This suggests that ΔSGF-WA1 efficiently represses early immune responses to allow for viral replication, and the immune system then overcompensates for the overwhelming viral load, causing a cytokine storm that likely results in organ failure and death (Bills et al., 2023). This is a common feature of COVID-19 in humans, where IFN-I response is delayed during the early stages of infection (which is consistent with findings from SARS-CoV infection) (Arunachalam et al., 2020; Channappanavar et al., 2016) followed by a proinflammatory response in the later stages of disease (Arunachalam et al., 2020; Blanco-Melo et al., 2020; Lucas et al., 2020; Ramos-Casals et al., 2021; Vabret et al., 2020). These results are in agreement with the nsp6-mediated activation of the NLRP3 inflammasome and NF-κB as described above in sections 5 and 7 (Nishitsuji et al., 2022; Rodrigues et al., 2020; Sun et al., 2022a).

Notably, a recent study showed that parental SARS-CoV-2 containing BA.1 S gene combined with BA.1 nsp6 (ΔLSG + I189V) was drastically attenuated in recombinant ACE2/TMPRSS2/Caco-2 cells and in K18-hACE2 mice, closely resembling the attenuated phenotype of full-length Omicron BA.1 (Chen et al., 2023). For comparison, only 20% of mice infected with the recombinant BA.1 S virus survived; 71% of mice infected with BA.1 S/nsp6 virus survived; and 100% of full-length BA.1-infected mice survived (Chen et al., 2023). This is in line with previous results that mutations in the 5′-UTR-nsp12 region attenuate SARS-CoV-2 replication in K18-hACE2 mice, while BA.1 S mutations increase virulence (S. Liu et al., 2022). However, this is in contrast to our finding that mice infected with ΔSGF-WA1 increased mortality in K18-hACE2 mice (Bills et al., 2023). The reason for this discrepancy is unclear given that ΔLSG, ΔLSG+I189V, and ΔSGF nsp6 all showed improved suppression of IFN-I signaling *in vitro* (Bills et al., 2023). It was suggested that mutations in BA.1 S alter viral tropism, while nsp6 mutations may function as an adaptation to an altered tissue environment (Chen et al., 2023). Whether BA.1 S and nsp6 work in concert is unknown. Given that infection with mutant ΔSGF-WA1 reduces survival while mutant BA.1 S/nsp6 increases survival, the contradictory results may suggest an epistatic interaction between S and nsp6.

9. Conclusions

The COVID-19 pandemic has ravaged the world and caused unprecedented social and economic damages in the past century (Collaborators, 2022; Lopez-Leon et al., 2021; Rose, 2021). The rapid development and approval of vaccines has changed the course of the pandemic and saved countless lives (Watson et al., 2022). However, future variants remain a threat, as are other coronaviruses with pandemic potential (Gralinski and Menachery, 2020). Thus, it is important to continue studying coronaviruses, especially the roles of nonstructural proteins and accessory proteins. Although several functions of SARS-CoV-2 nsp6 protein have been reported, many questions remain. Solving the atomic structure of the nsp6 protein will provide crucial insights into its molecular mechanism, such as how ΔSGF and ΔLSG alter the nsp6 structure to enhance protein-protein interactions or, in the case of L37F, hinder interactions (Aiewsakun et al., 2021; Bills et al., 2023; R. Wang et al., 2020). The structural information may also improve our understanding of nsp6-mediated DMV formation and designing inhibitors of its function. Additionally, identifying specific interacting partners will be vital to understanding the nsp6 antagonism of IFN-I pathways and other functions.

Declaration of competing interest

X.X. and P.-Y.S. have filed a patent on the reverse genetic system. Other authors declare no competing interests.

Data availability

No data was used for the research described in the article.

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References

Abdolmaleki, G., Taheri, M.A., Paridehpour, S., Mohammadi, N.M., Tabatabaei, Y.A., Mousavi, T., Amin, M., 2022. A comparison between SARS-CoV-1 and SARS-CoV2: an update on current COVID-19 vaccines. DARU. J. Pharmaceut. Sci. https://doi.org/10.1007/s40199-022-00446-8.

Aiewsakun, P., Nilplub, P., Wongtrakoongate, P., Hongeng, S., Thitithanyanont, A., 2021. SARS-CoV-2 genetic variations associated with COVID-19 pathogenicity. Microb. Genom. 7 https://doi.org/10.1099/mgen.0.000734.

Angelini, M.M., Akhlaghpour, M., Neuman, B.W., Buchmeier, M.J., 2013. Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. mBio 4. https://doi.org/10.1128/mBio.00524-13.

Arunachalam, P.S., Wimmers, F., Mok, C.K.P., Perera, R.A.P.M., Scott, M., Hagan, T., Sigal, N., Feng, Y., Bristow, L., Tsang, O.T.Y., Wagh, D., Coller, J., Pellegrini, K.L., Kazmin, D., Alaaeddine, G., Leung, W.S., Chan, J.M.C., Chik, T.S.H., Choi, C.Y.C., Chuerta, H., McCullough, M.P., Lv, H., Anderson, E., Edupuganti, S., Upadhyay, A. A., Bosinger, S.E., Terry, H., Khatri, P., Rouphael, N., Peiris, M., Pulendran, B., 2020. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. Science 369, 1210–1220. https://doi.org/10.1126/SCIENCE.ABC6261, 1979.

Bar-On, Y.M., Flamholz, A., Phillips, R., Milo, R., 2020. SARS-CoV-2 (COVID-19) by the numbers. Elife 9. https://doi.org/10.7326/M20-1190.

Bergsbaken, T., Fink, S.L., Cookson, B.T., 2009. Pyroptosis: host cell death. Nat. Rev. Microbiol. 7, 99–109. https://doi.org/10.1038/nrmicro2070.Pyroptosis.

Bills, C.J., Xia, H., Chen, J.Y.-C., Yeung, J., Kalveram, B., Walker, D., Xie, X., Shi, P.-Y., 2023. Mutations in SARS-CoV-2 variant nsp6 enhance type-I interferon antagonism. ReVision.

Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.C., Uhl, S., Hoagland, D., Møller, R., Jordan, T.X., Oishi, K., Panis, M., Sachs, D., Wang, T.T., Schwartz, R.E., Lim, J.K., Albrecht, R.A., tenOever, B.R., 2020. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell 181, 1036–1045. https://doi.org/10.1016/j.cell.2020.04.026 e9.

Bost, A.G., Carnahan, R.H., Lu, X.T., Denison, M.R., 2000. Four proteins processed from the replicase gene polyprotein of mouse hepatitis virus colocalize in the cell periphery and adjacent to sites of virion assembly. J. Virol. 74, 3379–3387. https://doi.org/10.1128/jvi.74.7.3379-3387.2000.

Broz, P., Dixit, V.M., 2016. Inflammasomes: mechanism of assembly, regulation and signalling. Nat. Rev. Immunol. https://doi.org/10.1038/nri.2016.58.

Channappanavar, R., Fehr, A.R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D.K., Perlman, S., 2016. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe 19, 181–193. https://doi.org/10.1016/j.chom.2016.01.007.

Chen, D., Chin, C.V., Kenney, D., Tavares, A.H., Khan, N., Conway, H.L., Liu, G., Choudhary, M.C., Gertje, H.P., Connell, A.K.O., Adams, S., Kotton, D.N., Herrmann, A., Ensser, A., Connor, J.H., Bosmann, M., Li, J.Z., Gack, M.U., Baker, S. C., Kirchdoerfer, R.N., 2023. Spike and nsp6 are key determinants of SARS-CoV-2 Omicron BA.1 attenuation. Nature. https://doi.org/10.1038/s41586-023-05697-2.

Collaborators, C.-19E.M., 2022. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. Lancet 399, 1513–1536. https://doi.org/10.1016/S0140-6736(21)02796-3.

Cottam, E.M., Maier, H.J., Manifava, M., Vaux, L.C., Chandra-Schoenfelder, P., Gerner, W., Britton, P., Ktistakis, N.T., Wileman, T., 2011. Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. Autophagy 7, 1335–1347. https://doi.org/10.4161/auto.7.11.16642.

Cottam, E.M., Whelband, M.C., Wileman, T., Cottam, E.M., Whelband, M.C., Wileman, T., 2014. Coronavirus NSP6 restricts autophagosome expansion. Autophagy 10, 1426–1441. https://doi.org/10.4161/auto.29309.

Cui, J., Li, F., Shi, Z.L., 2019. Origin and evolution of pathogenic coronaviruses. Nat. Rev. Microbiol. 17, 181–192. https://doi.org/10.1038/s41579-018-0118-9.

de Haan, C.A.M., Rottier, P.J.M., 2005. Molecular interactions in the assembly of coronaviruses. Adv. Virus Res. 64, 165–230. https://doi.org/10.1016/S0065-3527(05)64006-7.

del Valle, D.M., Kim-Schulze, S., Huang, H.H., Beckmann, N.D., Nirenberg, S., Wang, B., Lavin, Y., Swartz, T.H., Madduri, D., Stock, A., Marron, T.U., Xie, H., Patel, M., Tuballes, K., van Oekelen, O., Rahman, A., Kovatch, P., Aberg, J.A., Schadt, E., Jagannath, S., Mazumdar, M., Charney, A.W., Firpo-Betancourt, A., Mendu, D.R., Jhang, J., Reich, D., Sigel, K., Cordon-Cardo, C., Feldmann, M., Parekh, S., Merad, M., Gnjatic, S., 2020. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat. Med. 26, 1636–1643. https://doi.org/10.1038/s41591-020-1051-9.

Fan, Y., Zong, W.X., 2008. The cellular decision between apoptosis and autophagy. Apoptosis: Cellular Outcomes of Cancer Therapy 127–141. https://doi.org/10.3109/9781420020502-9.

Fehr, A.R., Perlman, S., 2015. Coronaviruses: an overview of their replication and pathogenesis. In: Coronaviruses: Methods and Protocols, pp. 1–23.

Gao, G., Sheng, Y., Yang, H., Chua, B.T., Xu, L., 2019. DFCP1 associates with lipid droplets. Cell Biol. Int. 43, 1492–1504. https://doi.org/10.1002/cbin.11199.

Gassen, N.C., Niemeyer, D., Muth, D., Corman, V.M., Martinelli, S., Gassen, A., Hafner, K., Papies, J., Mösbauer, K., Zellner, A., Zannas, A.S., Herrmann, A., Holsboer, F., Brack-Werner, R., Boshart, M., Müller-Myhsok, B., Drosten, C., Müller, M.A., Rein, T., 2019. SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. Nat. Commun. 10, 2–4. https://doi.org/10.1038/s41467-019-13659-4.

Ghosh, S., Dellibovi-Ragheb, T.A., Kerviel, A., Pak, E., Qiu, Q., Fisher, M., Takvorian, P. M., Bleck, C., Hsu, V.W., Fehr, A.R., Perlman, S., Achar, S.R., Straus, M.R., Whittaker, G.R., de Haan, C.A.M., Kehrl, J., Altan-Bonnet, G., Altan-Bonnet, N., 2020. β-Coronaviruses use lysosomes for egress instead of the biosynthetic secretory pathway. Cell 183, 1520–1535. https://doi.org/10.1016/j.cell.2020.10.039 e14.

Gomes Dias, S.S., Cardoso Soares, V., Ferreira, A.C., Sacramento, C.Q., Fintelman-Rodrigues, N., Temerozo, J.R., Teixeira, L., Silva, M.A.N. da, Barreto, E., Mattos, M., Freitas, C.S. de, Azevedo-Quintanilha, I.G., Manso, P.P.A., Miranda, M.D., Siqueira, M.M., Hottz, E.D., Pão, C.R.R., Bou-Habib, D.C., Barreto-Vieira, D.F., Bozza, F.A., Souza, T.M.L., Bozza, P.T., 2020. Lipid droplets fuel SARS-CoV-2 replication and production of inflammatory mediators. PLoS Pathog. 16, e1009127 https://doi.org/10.1371/journal.ppat.1009127.

Gordon, D.E., Jang, G.M., Bouhaddou, M., Xu, J., Obernier, K., White, K.M., O'Meara, M. J., Rezelj, V.v., Guo, J.Z., Swaney, D.L., Tummino, T.A., Hüttenhain, R., Kaake, R.M., Richards, A.L., Tutuncuoglu, B., Foussard, H., Batra, J., Haas, K., Modak, M., Kim, M., Haas, P., Polacco, B.J., Braberg, H., Fabius, J.M., Eckhardt, M., Soucheray, M., Bennett, M.J., Cakir, M., McGregor, M.J., Li, Q., Meyer, B., Roesch, F., Vallet, T., mac Kain, A., Miorin, L., Moreno, E., Naing, Z.Z.C., Zhou, Y., Peng, S., Shi, Y., Zhang, Z., Shen, W., Kirby, I.T., Melnyk, J.E., Chorba, J.S., Lou, K., Dai, S.A., Barrio-Hernandez, I., Memon, D., Hernandez-Armenta, C., Lyu, J., Mathy, C.J.P., Perica, T., Pilla, K.B., Ganesan, S.J., Saltzberg, D.J., Rakesh, R., Liu, X., Rosenthal, S.B., Calviello, L., Venkataramanan, S., Liboy-Lugo, J., Lin, Y., Huang, X.P., Liu, Y.F., Wankowicz, S.A., Bohn, M., Safari, M., Ugur, F.S., Koh, C., Savar, N.S., Tran, Q.D., Shengjuler, D., Fletcher, S.J., O'Neal, M.C., Cai, Y., Chang, J. C.J., Broadhurst, D.J., Klippsten, S., Sharp, P.P., Wenzell, N.A., Kuzuoglu-Ozturk, D., Wang, H.Y., Trenker, R., Young, J.M., Cavero, D.A., Hiatt, J., Roth, T.L., Rathore, U., Subramanian, A., Noack, J., Hubert, M., Stroud, R.M., Frankel, A.D., Rosenberg, O. S., Verba, K.A., Agard, D.A., Ott, M., Emerman, M., Jura, N., von Zastrow, M., Verdin, E., Ashworth, A., Schwartz, O., D'Enfert, C., Mukherjee, S., Jacobson, M., Malik, H.S., Fujimori, D.G., Ideker, T., Craik, C.S., Floor, S.N., Fraser, J.S., Gross, J. D., Sali, A., Roth, B.L., Ruggero, D., Taunton, J., Kortemme, T., Beltrao, P., Vignuzzi, M., García-Sastre, A., Shokat, K.M., Shoichet, B.K., Krogan, N.J., 2020. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature 583, 459–468. https://doi.org/10.1038/s41586-020-2286-9.

Gralinski, L.E., Menachery, V.D., 2020. Return of the Coronavirus: 2019-nCoV. Viruses 12.

Guo, K., Barrett, B.S., Morrison, J.H., Mickens, K.L., Vladar, E.K., Hasenkrug, K.J., Poeschla, E.M., Santiago, M.L., 2022. Interferon resistance of emerging SARS-CoV-2 variants. Proc. Natl. Acad. Sci. U. S. A. 119, e2203760119 https://doi.org/10.1073/pnas.2203760119.

Gupta, R., Charron, J., Stenger, C.L., Painter, J., Steward, H., Cook, T.W., Faber, W., Frisch, A., Lind, E., Bauss, J., Li, X., Sirpilla, O., Soehnlen, X., Underwood, A., Hinds, D., Morris, M., Lamb, N., Carcillo, J.A., Bupp, C., Uhal, B.D., Rajasekaran, S., Prokop, J.W., 2020. SARS-CoV-2 (COVID-19) structural and evolutionary dynamicome: insights into functional evolution and human genomics. J. Biol. Chem. 295, 11742–11753. https://doi.org/10.1074/jbc.ra120.014873.

Hadfield, J., Megill, C., Bell, S.M., Huddleston, J., Potter, B., Callender, C., Sagulenko, P., Bedford, T., Neher, R.A., 2018. NextStrain: real-time tracking of pathogen evolution. Bioinformatics 34, 4121–4123. https://doi.org/10.1093/bioinformatics/bty407.

Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., Péré, H., Charbit, B., Bondet, V., Chenevier-Gobeaux, C., Breillat, P., Carlier, N., Gauzit, R., Morbieu, C., Pène, F., Marin, N., Roche, N., Szwebel, T.A., Merkling, S.H., Treluyer, J.M., Veyer, D., Mouthon, L., Blanc, C., Tharaux, P.L., Rozenberg, F., Fischer, A., Duffy, D., Rieux-Laucat, F., Kernéis, S., Terrier, B., 2020. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 369, 718–724. https://doi.org/10.1126/science.abc6027, 1979.

Hagemeijer, M.C., Monastyrska, I., Griffith, J., van der Sluijs, P., Voortman, J., van Bergen en Henegouwen, P.M., Vonk, A.M., Rottier, P.J.M., Reggiori, F., de Haan, C. A.M., 2014. Membrane rearrangements mediated by coronavirus nonstructural proteins 3 and 4. Virology 458–459, 125–135. https://doi.org/10.1016/j.virol.2014.04.027.

Hallgren, J., Tsirigos, K.D., Damgaard Pedersen, M., Juan, J., Armenteros, A., Marcatili, P., Nielsen, H., Krogh, A., Winther, O., 2022. DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks. bioRxiv, 2022.04.08.487609.

Herker, E., Vieyres, G., Beller, M., Krahmer, N., Bohnert, M., 2021. Lipid droplet contact sites in health and disease. Trends Cell Biol. 31, 345–358. https://doi.org/10.1016/j.tcb.2021.01.004.

Hoffmann, H.H., Schneider, W.M., Rice, C.M., 2015. Interferons and viruses: an evolutionary arms race of molecular interactions. Trends Immunol. 36, 124–138. https://doi.org/10.1016/j.it.2015.01.004.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., Müller, M.A., Drosten, C., Pöhlmann, S., 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181, 271–280. https://doi.org/10.1016/j.cell.2020.02.052 e8.

Hou, J., Wei, Y., Zou, J., Jaffery, R., Liang, S., Zheng, C., Chen, K., 2022. Integrated multi-omics analyses identify key anti-viral host factors and pathways controlling SARS-CoV-2 infection. https://doi.org/10.21203/RS.3.RS-1910932/V1.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., Cao, B., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

Hui, K.P.Y., Cheung, M.C., Perera, R.A.P.M., Ng, K.C., Bui, C.H.T., Ho, J.C.W., Ng, M.M. T., Kuok, D.I.T., Shih, K.C., Tsao, S.W., Poon, L.L.M., Peiris, M., Nicholls, J.M., Chan, M.C.W., 2020. Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. Lancet Respir. Med. 8, 687–695. https://doi.org/10.1016/S2213-2600(20)30193-4.

Jhuti, D., Rawat, A., Guo, C.M., Wilson, L.A., Mills, E.J., Forrest, J.I., 2022. Interferon treatments for SARS-CoV-2: challenges and opportunities. Infect. Dis. Ther. 11, 953–972. https://doi.org/10.1007/s40121-022-00633-9.

Ji, M., Li, M., Sun, L., Zhao, H., Li, Ying, Zhou, L., Yang, Z., Zhao, X., Qu, W., Xue, H., Zheng, Z., Li, Yiming, Deng, H., Zhao, Y.G., 2022. VMP1 and TMEM41B are essential for DMV formation during β-coronavirus infection. JCB (J. Cell Biol.) 221, e202112081. https://doi.org/10.1083/jcb.202112081.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S.A.A., Ballard, A.J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., Senior, A.W., Kavukcuoglu, K., Kohli, P., Hassabis, D., 2021. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589. https://doi.org/10.1038/s41586-021-03819-2.

Junqueira, C., Crespo, Â., Ranjbar, S., de Lacerda, L.B., Lewandrowski, M., Ingber, J., Parry, B., Ravid, S., Clark, S., Schrimpf, M.R., Ho, F., Beakes, C., Margolin, J., Russell, N., Kays, K., Boucau, J., das Adhikari, U., Vora, S.M., Leger, V., Gehrke, L., Henderson, L., Janssen, E., Kwon, D., Sander, C., Abraham, J., Goldberg, M.B., Wu, H., Mehta, G., Bell, S., Goldfeld, A.E., Filbin, M.R., Lieberman, J., 2022. FcγR-mediated SARS-CoV-2 infection of monocytes activates inflammation. Nature. https://doi.org/10.1038/s41586-022-04702-4.

Karki, R., Sharma, B.R., Tuladhar, S., Williams, E.P., Zalduondo, L., Samir, P., Zheng, M., Sundaram, B., Banoth, B., Malireddi, R.K.S., Schreiner, P., Neale, G., Vogel, P., Webby, R., Jonsson, C.B., Kanneganti, T.D., 2021. Synergism of TNF-α and IFN-γ triggers inflammatory cell death, tissue damage, and mortality in SARS-CoV-2 infection and cytokine shock syndromes. Cell 184, 149–168. https://doi.org/10.1016/j.cell.2020.11.025 e17.

Kesmez Can, F., Özkurt, Z., Öztürk, N., Sezen, S., 2021. Effect of IL-6, IL-8/CXCL8, IP-10/CXCL 10 levels on the severity in COVID 19 infection. Int. J. Clin. Pract. 75, e14970 https://doi.org/10.1111/ijcp.14970.

Kim, D., Lee, J.Y., Yang, J.S., Kim, J.W., Kim, V.N., Chang, H., 2020. The architecture of SARS-CoV-2 transcriptome. Cell 181, 914–921. https://doi.org/10.1016/j.cell.2020.04.011 e10.

Klein, S., Cortese, M., Winter, S.L., Wachsmuth-Melm, M., Neufeldt, C.J., Cerikan, B., Stanifer, M.L., Boulant, S., Bartenschlager, R., Chlanda, P., 2020. SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography. Nat. Commun. 11, 1–10. https://doi.org/10.1038/s41467-020-19619-7.

Knoops, K., Kikkert, M., van den Worm, S.H.E., Zevenhoven-Dobbe, J.C., van der Meer, Y., Koster, A.J., Mommaas, A.M., Snijder, E.J., 2008. SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol. 6, 1957–1974. https://doi.org/10.1371/journal.pbio.0060226.

Kotenko, S.v., Durbin, J.E., 2017. Contribution of type III interferons to antiviral immunity: location, location, location. J. Biol. Chem. 292, 7295–7303. https://doi.org/10.1074/jbc.R117.777102.

Kumar, A., Kumar, P., Saumya, K.U., Giri, R., 2021. Investigating the conformational dynamics of SARS-CoV-2 NSP6 protein with emphasis on non-transmembrane 91–112 & 231–290 regions. Microb. Pathog. 161, 105236 https://doi.org/10.1016/j.micpath.2021.105236.

Lee, J.G., Huang, W., Lee, H., van de Leemput, J., Kane, M.A., Han, Z., 2021. Characterization of SARS-CoV-2 proteins reveals Orf6 pathogenicity, subcellular localization, host interactions and attenuation by Selinexor. Cell Biosci. 11, 1–12. https://doi.org/10.1186/s13578-021-00568-7.

Lee, J.Y., Wing, P.A.C., Gala, D.S., Noerenberg, M., Järvelin, A.I., Titlow, J., Zhuang, X., Palmalux, N., Iselin, L., Thompson, M.K., Parton, R.M., Prange-Barczynska, M., Wainman, A., Salguero, F.J., Bishop, T., Agranoff, D., James, W., Castello, A., McKeating, J.A., Davis, I., 2022. Absolute quantitation of individual SARS-CoV-2 RNA molecules provides a new paradigm for infection dynamics and variant differences. Elife 11, e74153. https://doi.org/10.7554/eLife.74153.

Lei, X., Dong, X., Ma, R., Wang, W., Xiao, X., Tian, Z., Wang, C., Wang, Y., Li, L., Ren, L., Guo, F., Zhao, Z., Zhou, Z., Xiang, Z., Wang, J., 2020. Activation and evasion of type I interferon responses by SARS-CoV-2. Nat. Commun. 11, 1–12. https://doi.org/10.1038/s41467-020-17665-9.

Levy, D.E., Marié, I.J., Durbin, J.E., 2011. Induction and function of type i and III interferon in response to viral infection. Curr. Opin. Virol. 1, 476–486. https://doi.org/10.1016/j.coviro.2011.11.001.

Li, Dongfang, Zhao, Y.G., Li, Di, Zhao, H., Huang, J., Miao, G., Feng, D., Liu, P., Li, Dong, Zhang, H., 2019. The ER-localized protein DFCP1 modulates ER-lipid droplet contact formation. Cell Rep. 27, 343–358. https://doi.org/10.1016/j.celrep.2019.03.025 e5.

Ling, L.E., Zafari, M., Reardon, D., Brickelmeier, M., Goelz, S.E., Benjamin, C.D., 1995. Human type I interferon receptor, IFNAR, is a heavily glycosylated 120-130 kD membrane protein. J. Interferon Cytokine Res. 15, 55–61. https://doi.org/10.1089/jir.1995.15.55.

Liu, S., Selvaraj, P., Sangare, K., Luan, B., Wang, T.T., 2022. Spike protein-independent attenuation of SARS- CoV-2 Omicron variant in laboratory mice. Cell Rep. 40, 111359 https://doi.org/10.1016/j.celrep.2022.111359.

Liu, Y., Zhang, X., Liu, J., Xia, H., Zou, J., Muruato, A.E., Periasamy, S., Kurhade, C., Plante, J.A., Bopp, N.E., Kalveram, B., Bukreyev, A., Ren, P., Wang, T., Menachery, V.D., Plante, K.S., Xie, X., Weaver, S.C., Shi, P.Y., 2022. A live-attenuated SARS-CoV-2 vaccine candidate with accessory protein deletions. Nat. Commun. 13 (1 13), 1–14. https://doi.org/10.1038/s41467-022-31930-z, 2022.

Lopez-Leon, S., Wegman-Ostrosky, T., Perelman, C., Sepulveda, R., Rebolledo, P.A., Cuapio, A., Villapol, S., 2021. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Sci. Rep. 11, 1–14. https://doi.org/10.1038/s41598-021-95565-8.

Lucas, C., Wong, P., Klein, J., Castro, T.B.R., Silva, J., Sundaram, M., Ellingson, M.K., Mao, T., Oh, J.E., Israelow, B., Takahashi, T., Tokuyama, M., Lu, P., Venkataraman, A., Park, A., Mohanty, S., Wang, H., Wyllie, A.L., Vogels, C.B.F., Earnest, R., Lapidus, S., Ott, I.M., Moore, A.J., Muenker, M.C., Fournier, J.B., Campbell, M., Odio, C.D., Casanovas-Massana, A., Obaid, A., Lu-Culligan, A., Nelson, A., Brito, A., Nunez, A., Martin, A., Watkins, A., Geng, B., Kalinich, C., Harden, C., Todeasa, C., Jensen, C., Kim, D., McDonald, D., Shepard, D., Courchaine, E., White, E.B., Song, E., Silva, E., Kudo, E., DeIuliis, G., Rahming, H., Park, H.J., Matos, I., Nouws, J., Valdez, J., Fauver, J., Lim, J., Rose, K.A., Anastasio, K., Brower, K., Glick, L., Sharma, L., Sewanan, L., Knaggs, L., Minasyan, M., Batsu, M., Petrone, M., Kuang, M., Nakahata, M., Campbell, M., Linehan, M., Askenase, M.H., Simonov, M., Smolgovsky, M., Sonnert, N., Naushad, N., Vijayakumar, P., Martinello, R., Datta, R., Handoko, R., Bermejo, S., Prophet, S., Bickerton, S., Velazquez, S., Alpert, T., Rice, T., Khoury-Hanold, W., Peng, X., Yang, Y., Cao, Y., Strong, Y., Herbst, R., Shaw, A.C., Medzhitov, R., Schulz, W.L., Grubaugh, N.D., dela Cruz, C., Farhadian, S., Ko, A.I., Omer, S.B., Iwasaki, A., 2020. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 584, 463–469. https://doi.org/10.1038/s41586-020-2588-y.

Mariano, G., Farthing, R.J., Lale-Farjat, S.L.M., Bergeron, J.R.C., 2020. Structural characterization of SARS-CoV-2: where we are, and where we need to Be. Front. Mol. Biosci. https://doi.org/10.3389/fmolb.2020.605236.

Miao, G., Zhao, H., Li, Y., Ji, M., Chen, Y., Shi, Y., Bi, Y., Wang, P., Zhang, H., 2021. ORF3a of the COVID-19 virus SARS-CoV-2 blocks HOPS complex-mediated assembly of the SNARE complex required for autolysosome formation. Dev. Cell 56, 427–442. https://doi.org/10.1016/j.devcel.2020.12.010 e5.

Nagy, Á., Pongor, S., Győrffy, B., 2021. Different mutations in SARS-CoV-2 associate with severe and mild outcome. Int. J. Antimicrob. Agents 57, 106272. https://doi.org/10.1016/j.ijantimicag.2020.106272.

Neufeldt, C.J., Cerikan, B., Cortese, M., Frankish, J., Lee, J.Y., Plociennikowska, A., Heigwer, F., Prasad, V., Joecks, S., Burkart, S.S., Zander, D.Y., Subramanian, B., Gimi, R., Padmanabhan, S., Iyer, R., Gendarme, M., el Debs, B., Halama, N., Merle, U., Boutros, M., Binder, M., Bartenschlager, R., 2022. SARS-CoV-2 infection induces a pro-inflammatory cytokine response through cGAS-STING and NF-κB. Commun. Biol. 5 https://doi.org/10.1038/s42003-021-02983-5.

Nilsson-Payant, B.E., Uhl, S., Grimont, A., Doane, A.S., Cohen, P., Patel, R.S., Higgins, C. A., Acklin, J.A., Bram, Y., Chandar, V., Blanco-Melo, D., Panis, M., Lim, J.K., Elemento, O., Schwartz, R.E., Rosenberg, B.R., Chandwani, R., tenOever, B.R., 2021. The NF-κB transcriptional footprint is essential for SARS-CoV-2 replication. J. Virol. 95 https://doi.org/10.1128/jvi.01257-21.

Nishitsuji, H., Iwahori, S., Ohmori, M., Shimotohno, K., Murata, T., 2022. Ubiquitination of SARS-CoV-2 NSP6 and ORF7a facilitates NF-κB activation. mBio 13, 1–18. https://doi.org/10.1128/mbio.00971-22.

Omasits, U., Ahrens, C.H., Müller, S., Wollscheid, B., 2014. Protter: interactive protein feature visualization and integration with experimental proteomic data. Bioinformatics 30, 884–886. https://doi.org/10.1093/bioinformatics/btt607.

Oostra, M., Hagemeijer, M.C., van Gent, M., Bekker, C.P.J., te Lintelo, E.G., Rottier, P.J. M., de Haan, C.A.M., 2008. Topology and membrane anchoring of the coronavirus replication complex: not all hydrophobic domains of nsp3 and nsp6 are membrane spanning. J. Virol. 82, 12392–12405. https://doi.org/10.1128/jvi.01219-08.

Patel, R., Kaki, M., Potluri, V.S., Kahar, P., Khanna, D., 2022. A comprehensive review of SARS-CoV-2 vaccines: Pfizer, Moderna & Johnson & Johnson. Hum. Vaccines Immunother. https://doi.org/10.1080/21645515.2021.2002083.

Peacock, T.P., Penrice-Randal, R., Hiscox, J.A., Barclay, W.S., 2021. SARS-CoV- 2 one year on: evidence for ongoing viral adaptation. J. Gen. Virol. https://doi.org/10.1099/jgv.0.001584.

Plante, J.A., Mitchell, B.M., Plante, K.S., Debbink, K., Weaver, S.C., Menachery, V.D., 2021. The variant gambit: COVID-19's next move. Cell Host Microbe. https://doi.org/10.1016/j.chom.2021.02.020.

Ramos-Casals, M., Brito-Zerón, P., Mariette, X., 2021. Systemic and organ-specific immune-related manifestations of COVID-19. Nat. Rev. Rheumatol. 17, 315–332. https://doi.org/10.1038/s41584-021-00608-z.

Ricciardi, S., Guarino, A.M., Giaquinto, L., Polishchuk, E.v., Santoro, M., di Tullio, G., Wilson, C., Panariello, F., Soares, V.C., Dias, S.S.G., Santos, J.C., Souza, T.M.L., Fusco, G., Viscardi, M., Brandi, S., Bozza, P.T., Polishchuk, R.S., Venditti, R., de Matteis, M.A., 2022. The role of NSP6 in the biogenesis of the SARS-CoV-2 replication organelle. Nature 606, 761–768. https://doi.org/10.1038/s41586-022-04835-6.

Rodrigues, T.S., de Sá, K.S.G., Ishimoto, A.Y., Becerra, A., Oliveira, S., Almeida, L., Gonçalves, A.V., Perucello, D.B., Andrade, W.A., Castro, R., Veras, F.P., Toller-Kawahisa, J.E., Nascimento, D.C., de Lima, M.H.F., Silva, C.M.S., Caetite, D.B., Martins, R.B., Castro, I.A., Pontelli, M.C., de Barros, F.C., do Amaral, N.B., Giannini, M.C., Bonjorno, L.P., Lopes, M.I.F., Santana, R.C., Vilar, F.C., Auxiliadora-Martins, M., Luppino-Assad, R., de Almeida, S.C.L., de Oliveira, F.R., Batah, S.S., Siyuan, L., Benatti, M.N., Cunha, T.M., Alves-Filho, J.C., Cunha, F.Q., Cunha, L.D., Frantz, F.G., Kohlsdorf, T., Fabro, A.T., Arruda, E., de Oliveira, R.D.R., Louzada-Junior, P., Zamboni, D.S., 2020. Inflammasomes are activated in response to SARS-cov-2 infection and are associated with COVID-19 severity in patients. J. Exp. Med. 218 https://doi.org/10.1084/JEM.20201707.

Romero, P.E., Dávila-Barclay, A., Salvatierra, G., González, L., Cuicapuza, D., Solís, L., Marcos-Carbajal, P., Huancachoque, J., Maturrano, L., Tsukayama, P., 2021. The emergence of sars-CoV-2 variant Lambda (C.37) in south America. Microbiol. Spectr. 9, e0078921 https://doi.org/10.1128/spectrum.00789-21.

Rose, A., 2021. COVID-19 economic impacts in perspective: a comparison to recent U.S. disasters. Int. J. Disaster Risk Reduc. 60, 102317 https://doi.org/10.1016/j.ijdrr.2021.102317.

Saraste, J., Prydz, K., 2021. Assembly and cellular exit of coronaviruses: hijacking an unconventional secretory pathway from the pre-golgi intermediate compartment via the golgi ribbon to the extracellular space. Cells 10, 1–20. https://doi.org/10.3390/cells10030503.

Scutigliani, E.M., Kikkert, M., 2017. Cytokine & Growth Factor Reviews Interaction of the innate immune system with positive-strand RNA virus replication organelles. Cytokine Growth Factor Rev. 37, 17–27. https://doi.org/10.1016/j.cytogfr.2017.05.007.

Shalamova, L., Felgenhauer, U., Wilhelm, J., Schaubmar, A.R., Büttner, K., Schoen, A., Widera, M., Ciesek, S., Weber, F., 2022. Omicron variant of SARS-CoV-2 exhibits an increased resilience to the antiviral type I interferon response. PNAS Nexus 1, 1–4. https://doi.org/10.1093/pnasnexus/pgac067.

Shemesh, M., Aktepe, T.E., Deerain, J.M., McAuley, J.L., Audsley, M.D., David, C.T., Purcell, D.F.J., Urin, V., Hartmann, R., Moseley, G.W., Mackenzie, J.M., Schreiber, G., Harari, D., 2021. SARS-CoV-2 suppresses IFNβ production mediated by NSP1, 5, 6, 15, ORF6 and ORF7b but does not suppress the effects of added interferon. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1009800.

Smirle, J., Au, C.E., Jain, M., Dejgaard, K., Nilsson, T., Bergeron, J., 2013. Cell biology of the endoplasmic reticulum and the golgi apparatus through proteomics. Cold Spring Harbor Perspect. Biol. 5, a015073 https://doi.org/10.1101/CSHPERSPECT.A015073.

Snijder, E.J., Limpens, R.W.A.L., de Wilde, A.H., de Jong, A.W.M., Zevenhoven-Dobbe, J. C., Maier, H.J., Faas, F.F.G.A., Koster, A.J., Bárcena, M., 2020. A unifying structural and functional model of the coronavirus replication organelle: tracking down RNA synthesis. PLoS Biol. 18, 1–25. https://doi.org/10.1371/journal.pbio.3000715.

Sommereyns, C., Michiels, T., 2006. N-glycosylation of murine IFN-β in a putative receptor-binding region. J. Interferon Cytokine Res. 26, 406–413. https://doi.org/10.1089/jir.2006.26.406.

Sprooten, J., Garg, A.D., 2020. Type I interferons and endoplasmic reticulum stress in health and disease. Int. Rev. Cell Mol. Biol. 350, 63–118. https://doi.org/10.1016/bs.ircmb.2019.10.004.

Sun, X., Liu, Y., Huang, Z., Xu, W., Hu, W., Yi, L., Liu, Z., Chan, H., Zeng, J., Liu, X., Chen, H., Yu, J., Chan, F.K.L., Ng, S.C., Wong, S.H., Wang, M.H., Gin, T., Joynt, G.M., Hui, D.S.C., Zou, X., Shu, Y., Cheng, C.H.K., Fang, S., Luo, H., Lu, J., Chan, M.T.V., Zhang, L., Wu, W.K.K., 2022a. SARS-CoV-2 non-structural protein 6 triggers NLRP3-dependent pyroptosis by targeting ATP6AP1. Cell Death Differ. 29, 1240–1254. https://doi.org/10.1038/s41418-021-00916-7.

Sun, X., Yu, J., Wong, S.H., Chan, M.T.V., Zhang, L., Wu, W.K.K., 2022b. SARS-CoV-2 targets the lysosome to mediate airway inflammatory cell death. Autophagy 18, 2246–2248. https://doi.org/10.1080/15548627.2021.2021496.

Thorne, L.G., Bouhaddou, M., Reuschl, A.-K., Zuliani-Alvarez, L., Polacco, B., Pelin, A., Batra, J., Whelan, M.V.X., Hosmillo, M., Fossati, A., Ragazzini, R., Jungreis, I., Ummadi, M., Rojc, A., Turner, J., Bischof, M.L., Obernier, K., Braberg, H., Soucheray, M., Richards, A., Chen, K.-H., Harjai, B., Memon, D., Hiatt, J., Rosales, R., McGovern, B.L., Jahun, A., Fabius, J.M., White, K., Goodfellow, I.G., Takeuchi, Y., Bonfanti, P., Shokat, K., Jura, N., Verba, K., Noursadeghi, M., Beltrao, P., Kellis, M., Swaney, D.L., García-Sastre, A., Jolly, C., Towers, G.J., Krogan, N.J., 2022. Evolution of enhanced innate immune evasion by SARS-CoV-2. Nature 602, 487–495.

Twu, W.I., Lee, J.Y., Kim, H., Prasad, V., Cerikan, B., Haselmann, U., Tabata, K., Bartenschlager, R., 2021. Contribution of autophagy machinery factors to HCV and SARS-CoV-2 replication organelle formation. Cell Rep. 37, 110049 https://doi.org/10.1016/j.celrep.2021.110049.

Vabret, N., Britton, G.J., Gruber, C., Hegde, S., Kim, J., Kuksin, M., Levantovsky, R., Malle, L., Moreira, A., Park, M.D., Pia, L., Risson, E., Saffern, M., Salomé, B., Esai Selvan, M., Spindler, M.P., Tan, J., van der Heide, V., Gregory, J.K., Alexandropoulos, K., Bhardwaj, N., Brown, B.D., Greenbaum, B., Gümüş, Z.H., Homann, D., Horowitz, A., Kamphorst, A.O., Curotto de Lafaille, M.A., Mehandru, S., Merad, M., Samstein, R.M., Agrawal, M., Aleynick, M., Belabed, M., Brown, M., Casanova-Acebes, M., Catalan, J., Centa, M., Charap, A., Chan, A., Chen, S.T., Chung, J., Bozkus, C.C., Cody, E., Cossarini, F., Dalla, E., Fernandez, N., Grout, J., Ruan, D.F., Hamon, P., Humblin, E., Jha, D., Kodysh, J., Leader, A., Lin, M., Lindblad, K., Lozano-Ojalvo, D., Lubitz, G., Magen, A., Mahmood, Z., Martinez-Delgado, G., Mateus-Tique, J., Meritt, E., Moon, C., Noel, J., O'Donnell, T., Ota, M., Plitt, T., Pothula, V., Redes, J., Reyes Torres, I., Roberto, M., Sanchez-Paulete, A.R., Shang, J., Schanoski, A.S., Suprun, M., Tran, M., Vaninov, N., Wilk, C.M., Aguirre-Ghiso, J., Bogunovic, D., Cho, J., Faith, J., Grasset, E., Heeger, P., Kenigsberg, E., Krammer, F., Laserson, U., 2020. Immunology of COVID-19: current state of the science. Immunity 52, 910–941. https://doi.org/10.1016/j.immuni.2020.05.002.

van der Meer, Y., Snijder, E.J., Dobbe, J.C., Schleich, S., Denison, M.R., Spaan, W.J.M., Locker, J.K., 1999. Localization of mouse hepatitis virus nonstructural proteins and RNA synthesis indicates a role for late endosomes in viral replication. J. Virol. 73, 7641–7657. https://doi.org/10.1128/jvi.73.9.7641-7657.1999.

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., Thiel, V., 2021. Coronavirus biology and replication: implications for SARS-CoV-2. Nat. Rev. Microbiol. 19, 155–170. https://doi.org/10.1038/s41579-020-00468-6.

Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T., Veesler, D., 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181, 281–292. https://doi.org/10.1016/j.cell.2020.02.058 e6.

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K. Y., Wang, Qisheng, Zhou, H., Yan, J., Qi, J., 2020. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell 181, 894–904. https://doi.org/10.1016/j.cell.2020.03.045 e9.

Wang, R., Chen, J., Hozumi, Y., Yin, C., Wei, G.W., 2020. Decoding asymptomatic COVID-19 infection and transmission. J. Phys. Chem. Lett. 11, 10007–10015. https://doi.org/10.1021/acs.jpclett.0c02765.

Watson, O.J., Barnsley, G., Toor, J., Hogan, A.B., Winskill, P., Ghani, A.C., 2022. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. Lancet Infect. Dis. 22, 1293–1302. https://doi.org/10.1016/S1473-3099(22)00320-6.

WHO Solidarity Trial Consortium, 2021. Repurposed antiviral drugs for covid-19 — interim WHO solidarity trial results. N. Engl. J. Med. 384, 497–511. https://doi.org/10.1056/nejmoa2023184.

Wu, W.K.K., Yue, J., 2020. Autophagy in host-microbe interactions. Semin. Cell Dev. Biol. 101, 1–2. https://doi.org/10.1016/j.semcdb.2020.02.001.

Xia, H., Cao, Z., Xie, X., Zhang, X., Chen, J.Y.C., Wang, H., Menachery, V.D., Rajsbaum, R., Shi, P.Y., 2020. Evasion of type I interferon by SARS-CoV-2. Cell Rep. 33, 108234 https://doi.org/10.1016/j.celrep.2020.108234.

Yang, H., Lyu, Y., Hou, F., 2020. SARS-CoV-2 infection and the antiviral innate immune response. J. Mol. Cell Biol. 12, 963–967. https://doi.org/10.1093/jmcb/mjaa071.

Yuen, C.K., Lam, J.Y., Wong, W.M., Mak, L.F., Wang, X., Chu, H., Cai, J.P., Jin, D.Y., To, K.K.W., Chan, J.F.W., Yuen, K.Y., Kok, K.H., 2020. SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists. Emerg. Microb. Infect. 9, 1418–1428. https://doi.org/10.1080/22221751.2020.1780953.

Zheng, J., Wang, Y., Li, K., Meyerholz, D.K., Allamargot, C., Perlman, S., 2021. Severe acute respiratory syndrome coronavirus 2-induced immune activation and death of monocyte-derived human macrophages and dendritic cells. JID (J. Infect. Dis.) 223, 785–795. https://doi.org/10.1093/infdis/jiaa753.

Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Qi, Y., Sun, R., Tian, Z., Xu, X., Wei, H., 2020. Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. Natl. Sci. Rev. 7, 998–1002. https://doi.org/10.1093/nsr/nwaa041.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F., Tan, W., 2020. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 382, 727–733. https://doi.org/10.1056/nejmoa2001017.