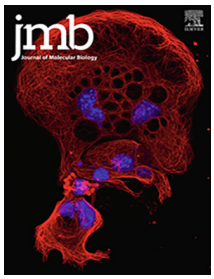
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Review Article



Mechanisms of Antiviral Immune Evasion of SARS-CoV-2

Daniel K. Beyer [1] **and Adriana Forero** [2*]

*1 - **Molecular Genetics,** College of Arts and Sciences, The Ohio State University, Columbus, OH 43210, USA*
*2 - **Department of Microbial Infection and Immunity,** College of Medicine, The Ohio State University, Columbus, OH 43210, USA*

***Correspondence to Adriana Forero:** adriana.forero@osumc.edu (A. Forero), @adforu (A. Forero)*

https://doi.org/10.1016/j.jmb.2021.167265
Edited by Alex Compton

Abstract

Coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is characterized by a delayed interferon (IFN) response and high levels of proinflammatory cytokine expression. Type I and III IFNs serve as a first line of defense during acute viral infections and are readily antagonized by viruses to establish productive infection. A rapidly growing body of work has interrogated the mechanisms by which SARS-CoV-2 antagonizes both IFN induction and IFN signaling to establish productive infection. Here, we summarize these findings and discuss the molecular interactions that prevent viral RNA recognition, inhibit the induction of IFN gene expression, and block the response to IFN treatment. We also describe the mechanisms by which SARS-CoV-2 viral proteins promote host shutoff. A detailed understanding of the host-pathogen interactions that unbalance the IFN response is critical for the design and deployment of host-targeted therapeutics to manage COVID-19.

© 2021 Elsevier Ltd. All rights reserved.

Introduction

The *Coronaviridae* are a family of enveloped, positive single-stranded RNA viruses that encompass the *alpha*-, *beta*-, *gamma*-, and *deltacoronaviruses*. Coronaviruses have a wide host range, but *alpha*- and *betacoronaviruses* predominantly cause mild to moderate respiratory disease in humans. In the 21st century, there have been three major outbreaks of *betacoronaviruses* causing severe disease: the severe acute respiratory syndrome (SARS-CoV) in 2002, Middle East respiratory syndrome (MERS-CoV) in 2012, and coronavirus disease (COVID-19; SARS-CoV-2) in 2019. Severe cases of COVID-19 result in the development of pneumonia and hyperinflammatory responses that promote acute respiratory distress or multi-organ failure that can lead to death. As of September 8th, 2021, almost 222 million individuals have been infected with SARS-CoV-2 world-wide causing approximately 4,582,338 deaths (WHO). The life cycle of coronaviruses, including their mechanism of entry and genome uncoating, viral genome replication, genome translation and processing, virion assembly, and viral shedding has been recently reviewed.[1,2] SARS-CoV-2 has a genome of about 30 kb that shares a high degree of similarity with SARS-CoV (79%) and to a lesser extent MERS-CoV (50%).[3] The genome (Figure 1 (a)), flanked by a 5′ untranslated region (UTR) and a 3′ UTR, encodes for non-structural, structural, and accessory proteins necessary to promote the viral life cycle and evade the host immune response (Table 1)[4]. The open reading frames ORF1a and ORF1b encode for two large polyproteins, pp1a

Please cite tts article as: D.K. Beyer and A. Forero, Mechanisms of Antiviral Immune Evasion of SARS-CoV-2Journal of Molecular Biology, https://doi.org/10.1016/j.jmb.2021.167265

Table 1 Mechanisms for SARS-CoV-2 evasion of IFN responses.

Viral Protein	Known Functions	Host Interactions	Mechanism of IFN Evasion	Reference(s)
NSP1	Host translational shut off	40 s Ribosomal Subunit, NXF1	Blocks host mRNA translation Inhibits mRNA nuclear export	41,42,94–98,109
NSP3	Papain-like proteinase; polyprotein processing	MDA5, IRF3	Targeting of ISGylated proteins: MDA5 and IRF3	41,68,69,70
NSP5	3C-like Protease; polyprotein processing	RIG-I, STAT1	Inhibition of K63 polyubiquitination of RIG-I	66
NSP6	Membrane rearrangements	TBK1	Inhibition of IRF3, STAT1/2 phosphorylation	42
NSP7	RdRp Subunit(non-enzymatic)		Inhibition of type I IFN signaling	42
NSP8	RdRp Subunit(primase)	7SL RNA, SRP19, SRP54, SRP72	Inhibition of protein trafficking	61,72,94
NSP9	non-enzymatic RBP	7SL RNA	Inhibition of protein trafficking	94
NSP10	RNA-capping		RLR evasion Enhancement of NSP14 inhibition	54–56,109,110
NSP12	RNA-dependent RNA polymerase	IRF3	Inhibition of IRF3 nuclear translocation	41,87
NSP13	RNA helicase	TBK1, TBKBP1	RLR evasion Inhibition of TBK1, IRF3, and STAT1/STAT2 phosphorylation Inhibits NF-kB activation	41,42,54–56,61,71,72
NSP14	RNA-capping (3′-5′ exoribonuclease)		RLR evasion Blocks host mRNA translation IFNAR1 Antagonism	40,41,42,54–56,90,109,110
NSP15	Uridylate-specific endoribonuclease	RNF41	RLR evasion	40,59,60,61
NSP16	RNA-capping (2′-O-methyl-transferase)	U1 and U2 snRNA	RLR evasion Spliceosome inhibition	54–56,94
ORF3a	Viroporin	TRIM59	Inhibition of STAT1 phosphorylation	42,61
ORF3b			Inhibition of IRF3 nuclear translocation	86
ORF6		KPNA1/2, Nup98, Rae1	Inhibition of nuclear import and export	41,42,88,61,93
ORF7a			Inhibition of STAT2 phosphorylation	42,100
ORF7b			Inhibition of STAT2 phosphorylation	42,90
ORF9b		NEMO, TOM70, RIG-I, MDA5, MAVS, TBK1, STING, TRIF	Inhibition of K63-linked poly-Ub of NEMO Blocks TOM70-HSP90 interaction Inhibition of TBK1 phosphorylation	61,72,73,75,76,80
M (membrane)	Viral assembly	RIG-I, MDA5, MAVS, TBK1, TRAF3	Block of MAVS aggregation Inhibition of TRAF3-TANK-TBK1-IKKe complex formation	42,81,82
N (nucleocapsid)	Viral capsid	RIG-I	Inhibits RIG-I ubiquitination Inhibition of STAT1/2 phosphorylation	41,63,101
S (spike)	Cell entry	ACE2		
E (envelope)	Forms viral envelope			

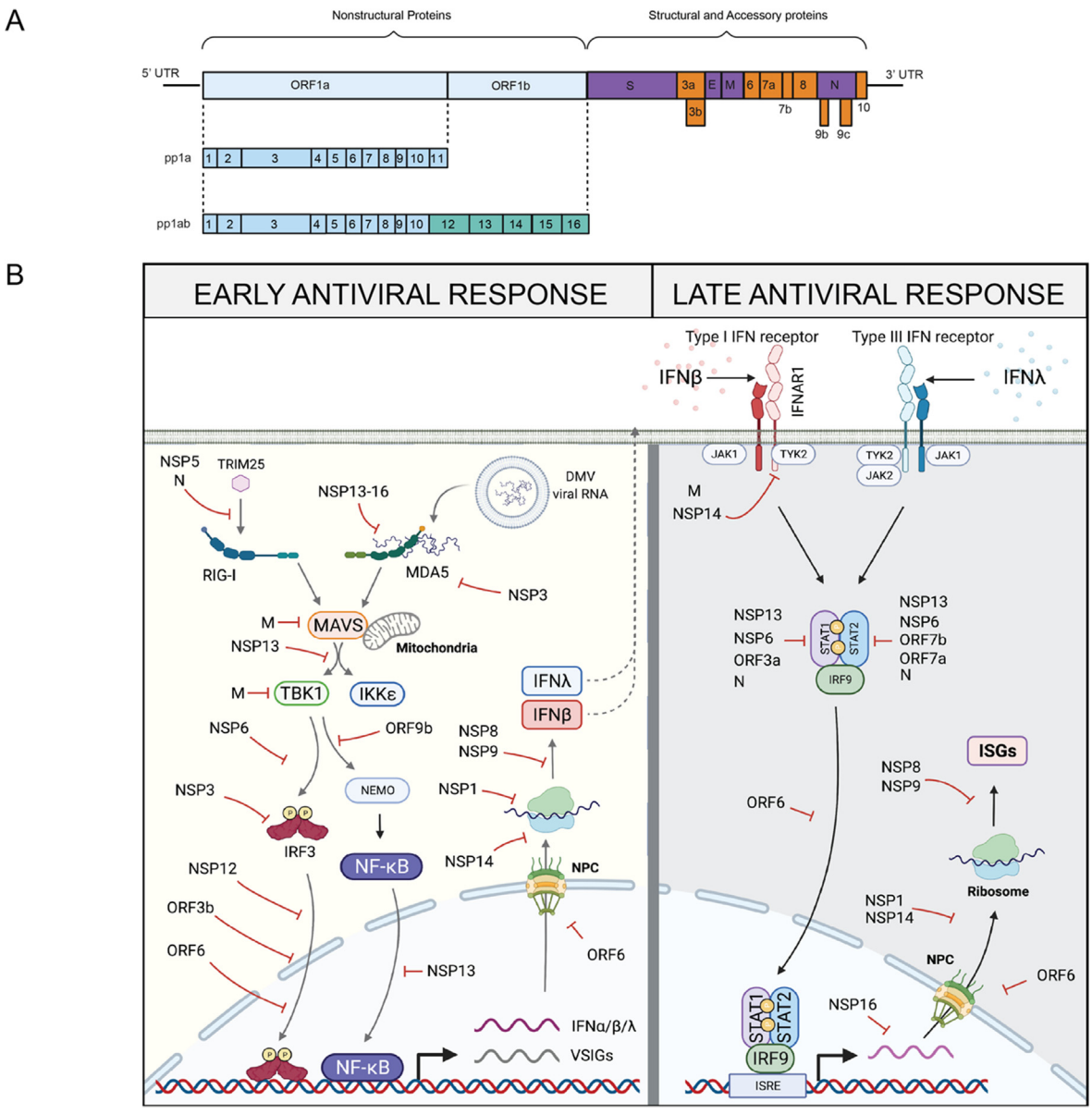


Figure 1. Targeted host innate immune evasion by SARS-CoV-2. (A) Genome Structure of SARS-CoV-2 delineating the open reading frames that encode the non-structural (blue), structural (purple) and accessory (orange) proteins. (B) Interactions leading to the inhibition of early signaling cascades that lead to the induction of interferon (IFN) and virus-stress inducible genes (VSIGs) (right). Viral antagonism of the late antiviral response that follows type I and type III IFN sensing and downstream IFN-stimulated gene (ISG) induction (left). Created with BioRender.com.

and pp1ab. Pp1a and pp1ab are proteolytically cleaved by two viral proteases, the papain-like protease (PLPro; NSP3) and the 3C-like protease (MPro; NSP5), giving rise to individual non-structural proteins (NSP). NSP1 is the major regulator of host and viral RNA translation and NSP2-16 compose the viral replication and transcription complex (RTC). Four structural proteins are encoded in individual ORFs and are interspersed amongst the ORFs of accessory proteins in the genome. The spike (S) protein is a class I fusion glycoprotein and defines viral tropism by engaging the cellular receptor angiotensin-converting enzyme 2 (ACE2)[5–8] through the receptor binding domain (RBD). The nucleocapsid (N) protein coats the viral RNA and associates with the membrane (M) protein to promote packaging of the viral genome. The envelope (E) and membrane (M) proteins are necessary for virion formation and assembly. In the mature virion, the viral RNA is coated by the nucleocapsid (N) protein and enveloped in a lipid bilayer that contains the spike (S), the envelope (E), and membrane (M) proteins. The SARS-CoV-2 accessory proteins are encoded by individual ORFs (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, and ORF10). These proteins display the

least degree of homology across coronaviruses and can be dispensable for viral replication, playing a role in modulating the host response to coronavirus infection.

Cellular infection and detection of pathogen-associated molecular patterns (PAMP) activates pattern recognition receptors (PRR) that initiate signal transduction cascades that culminate in type I and III IFN gene expression. Upon SARS-CoV-2 infection, double-stranded viral RNA (vRNA) is sensed by three major pathways: the RIG-I-like receptors (RLRs) pathway, the 2′,5′-oligoadenylate synthetase (OAS)/RNAseL pathway, and the PKR pathway.[9] RLRs bind RNA through a C-terminal helicase domain which promotes the conformational change that exposes the N-terminal caspase activation and recruitment domain (CARD). RLR CARD interactions with CARD on the mitochondrial antiviral signaling (MAVS) protein promote the oligomerization of MAVS on mitochondrial membranes. Recruitment of TANK binding kinase 1 (TBK1) or inhibitor of κ-B kinase ε (IKKε) to the RLR/MAVS signalosome promotes kinase auto-phosphorylation and downstream activation of the transcription factors, IRF3 and NF-κB.[10] IRF3 phosphorylation leads to homodimerization and nuclear translocation, where IRF3 cooperates with NF-κB to drive the expression of type I and III IFN genes and virus-stress inducible genes (VSIGs) (Figure 1(b), left). Although the OAS/RNAseL and PKR pathways are independent of to the RLR pathways, OAS proteins (OAS1, OAS2, OAS3) and PKR are IFN-stimulated genes (ISGs). Therefore, RLR activation and IFN induction can amplify the alternative host innate immune mechanisms to target viral and host RNA for degradation and promote cell death. The late antiviral response follows the transcriptional activation, translation, and secretion of type I IFNs (IFNα, IFNβ, IFNω, amongst others) and type III IFNs (IFNλ1-4).[11] Binding of type I (IFNα/β) and III (IFNλ) IFNs to cognate cellular receptors (IFNAR1/2 and IFNLR1/IL10R2, respectively) directs the Janus kinases JAK1, TYK2, and JAK2 to phosphorylate the transcription factors STAT1 and STAT2 at tyrosine residues. A heterotrimeric transcriptional complex composed of STAT1, STAT2, and IRF9 (ISGF3) recognizes and binds to IFN-stimulated response elements (ISREs) found in the upstream promoter regulatory regions of hundreds of antiviral effector genes known as IFN-stimulated genes (ISGs) (Figure 1, right). Although either IFNα/β or IFN-λ can confer antiviral protection, the tissue and dose of infection and the abundance and distribution of IFN receptors can further specify unique functions carried out by these two distinct cytokine families.[12–15]

The absence of IFN production or sensing can lead to increased susceptibilities to numerous viral infections.[16] Interestingly, it appears that an imbalance between a strong IFN-mediated antiviral response and heightened inflammatory disease underlies the detrimental outcomes of SARS-CoV-2 viral disease.[17,18] Relative to the robust induction of IFNs that is observed during respiratory viral infections with influenza A virus (IAV), SARS-CoV-2 infection appears to lead to a disruption in IFN responses.[17,19] Deficiencies in the IFN synthesis and response pathways can result in increased COVID-19 severity. Genomic analysis of patients with life-threatening disease revealed genetic lesions in molecular regulators of IFN synthesis (TLR3, UNC93B, TICAM1, TBK1, IRF3 and IRF7) and type I IFN signaling (IFNAR1 and 2).[20] Furthermore, the presence of preexisting neutralizing autoantibodies to type I IFNs, IFN-α2 and IFN-ω also correlate with enhanced disease severity.[21,22] *In vitro* and *in vivo* studies indicate that SARS-CoV-2 is sensitive to the antiviral effects of IFNα/β and IFNλ treatment mediated by ISGs.[23–30] Patient-derived observations suggest that IFN promotes viral clearance during early stages of the infection. The therapeutic use of both type I[31,32] and type III[33] IFNs in COVID-19 patients led to decreases in viral burden in infected individuals and reductions in the time to recovery. The golden hamster model has allowed for further exploration of the therapeutic effect of IFN. Intranasal treatment with type I IFN during early SARS-CoV-2 infection led to decreased viral replication and inflammation and lowered transmission.[34] However, the timing of the IFN response is an important determining factor for disease progression.[35] Murine and hamster models have revealed that at later stages in the infection, IFNs promote noxious inflammatory responses and disrupt repair responses.[36–38]

Interrogating the genetic factors and host-pathogen interactions that shape the host IFN response during infection improves our understanding of the efficacy and therapeutic timing of host-targeted interventions.[39] A rapidly growing body of work has queried viral factors that shape the antiviral response. Timely studies have identified various non-structural, structural, and accessory proteins that antagonize IFN synthesis (early phase) and IFN signaling (late phase) (Table 1) or activate IFN responses.[40–42] This review provides an updated SARS-CoV-2 focused summary of the host-pathogen interactions that dysregulate IFN-mediated antiviral responses. We will discuss the mechanisms of evasion of viral RNA recognition and IFN induction (early antiviral response) and strategies to circumvent the response to IFN treatment (late antiviral response). We will address how SARS-CoV-2 impacts RNA splicing, nuclear transport, and host translational responses, leading to a global inhibition of IFN and ISG gene expression and protein accumulation. This fast-paced area of investigation will drive a better understanding of the uncoupling of antiviral response observed in COVID-19 patients[17,18] and the potential of IFN-based therapies.[31–33]

SARS-CoV-2 evasion of viral RNA recognition:

The antiviral response is initiated after sensing of viral double-stranded RNA by RLRs, RIG-I and MDA5, which culminates in the expression of IFNs and virus-stress inducible genes (VSIGs). Screening of RNA-binding proteins that recognize and antagonize SARS-COV-2 identified MDA5 and LGP2 as the primary sensors of vRNA detection in Calu-3 cells.[43,44] This is in line with evidence of MDA5 recognition of CoV RNA[45,46] and the role LGP2 plays in enhancing MDA5 fiber formation and subsequent interactions with MAVS.[47] Other studies have implicated RIG-I as the sensor of SARS-CoV-2 in A549 and primary human alveolar and bronchial epithelial cells.[48] Further work is necessary to determine whether these RLRs play unique or overlapping roles across time and tissue types. However, coronaviruses employ several strategies to avoid dsRNA recognition by either RIG-I and/or MDA5. NSP3, NSP4, and NSP6 can drive the formation of double-membrane vesicles (DMV), shielding replicating vRNA from RLR recognition.[49]

RIG-I preferentially recognizes short double-stranded RNA (dsRNA) with blunt or exposed 5′ triphosphate (5′-ppp) ends,[50,51] while MDA5 recognizes long, dsRNA.[52] Host mRNA is post-transcriptionally methylated to allow for mRNA translation and avoid self-recognition.[53] The addition of a 7-methylguanylate cap in the 5′ end of vRNA circumvents recognition by both RIG-I and MDA5. This function is carried out by the non-structural proteins NSP13, NSP14, and NSP16 which have a high degree of conservation amongst *betacoronaviruses*.[54–56] NSP13, the RNA triphosphatase, mediates the hydrolysis of the 5′ γ-phosphate in the RNA to generate a 5′-ppN end that is required to transfer a guanidine monophosphate to form the core cap structure, GpppN. NSP14 has N^7-methyl transferase (N^7-MTase) activity and promotes the N^7-methylation of the GpppN guanosine leading to the formation of the cap-0 structure. The cap-0 structure can be recognized by MDA5[57] and antiviral effectors such as the ISG, IFIT1.[58] The viral S-adenosyl-L-methionine (SAM)-dependent 2′-O-methyltransferase (2′-*O*-MTase), NSP16, promotes the formation of the Cap-1 structure though methylation of the ribose in the 2′-*O* position, avoiding vRNA detection. NSP10 can then stabilize the SAM-binding pockets of both NSP14 and NSP16 to complete the vRNA capping process.

MDA5 sensing can be further evaded through processing of vRNA by the highly conserved uridylate-specific endoribonuclease (NendoU), NSP15.[59] NSP15 shortens and prevents the accumulation of 5′-polyU-containing, negative-sense (PUN) coronavirus RNA which is recognized by MDA5.[60] The decisive role of NSP15 in immune evasion is supported by its requirement in IFN-competent cells while being dispensable to support viral growth in IFN-deficient cells.[60] One study suggests that SARS-CoV-2 NSP15 inhibits the activation of an IFNβ promoter reporter following exogenous expression of the N-terminal domain of RIG-I (RIG-I CARD) in 293FT cells.[40] Although the data supports that NSP15-mediated inhibition of RIG-I signaling is independent of dsRNA recognition, the mechanisms of such inhibition remain elusive. NSP15 has also been shown to bind the E3 ligase, RNF41 (NRDP1),[61] which enhances TBK1 and IRF3 activation to promote IFN production.[62] This interaction could potentially account for the decrease in IFNβ-reporter activity.

Modification of RLRs by ubiquitin and ubiquitin-like proteins is necessary to promote the activation of antiviral responses. The structural protein N binds to the DExD/H domain of RIG-I inhibiting the activation of an IFNβ promoter reporter, endogenous IFNβ mRNA expression, and IRF3 activation in response to SeV and transfection with polyI:C (pI:C), a synthetic dsRNA ligand.[41,63] On the other hand, N enhanced reporter activity in response to RIG-I/MDA5 CARD overexpression,[41] suggesting that RLR antagonism could require the C-terminal domains of RIG-I. N-terminal K63 ubiquitination of RIG-I by the ring finger E3 ubiquitin ligase, TRIM25, is necessary for antiviral activity.[64] While the SARS-CoV N protein has been demonstrated to bind TRIM25 and prevent RIG-I ubiquitination,[65] this interaction has not been demonstrated for SARS-CoV-2 N. However, the antagonism of RIG-I K63 polyubiquination could be carried out by NSP5 (Mpro)-mediated inhibition of the TRIM25-RIG-I interaction.[66] Conjugation of proteins with the ubiquitin-like protein, ISG15 (ISGylation), enhances host resistance to viral infection (reviewed in 67). Recent reports have identified MDA5, but not RIG-I, as a target for ISGylation.[68] ISGylation of MDA5 at Lys23 and Lys43 is necessary for MDA5 oligomerization, activation of IRF3 phosphorylation (S396), and downstream IFNβ secretion and ISG expression. The SARS-CoV-2 protease NSP3 (PLpro) preferentially targets ISGylated substrates as determined through fluorescent-based *in vitro* cleavage assays of ubiquitinated substrates.[69] This is in contrast to the substrate preference of SARS-CoV NSP3 which primarily targets K48-Ub2 conjugated substrates. NSP3 prevented MDA5 ISGylation *in vitro* and during SARS-CoV-2 infection. Treatment of infected cells with the coronavirus NSP3 inhibitor GRL-0617 rescued ISG15 conjugation to MDA5.[68] These observations were consistent with reported NSP3-mediated inhibition of IFNβ promoter reporter activity.[41] IRF3 stabilization upon activation can also be directly antagonized through SARS-CoV-2 NSP3. ISGylation of IRF3 supports antiviral signaling by

preventing IRF3 proteolytic degradation upon activation. Cell free assays demonstrated that NSP3, but not NSP5, cleaves IRF3.[70] Treatment of cells with the non-covalent NSP3 inhibitor, GRL-0617, enhanced global ISGylation upon IFNα stimulation, rescued IRF3 ISGylation, and led to a decrease in SARS-CoV-2 infectious virus.[69] NSP3 impairs IFN production by targeting both MDA5 and IRF3, prioritizing it as a therapeutic target to prevent viral dissemination.[68,69]

SARS-CoV-2 evasion of RLR signaling and IFN production:

Following RLR activation, TBK1 is recruited to the RLR/MAVS signalosome to activate the transcription factors (TFs) IRF3 and NF-κB that transactivate IFNβ and IFNλ gene expression. A few SARS-CoV-2 proteins have been identified to antagonize dsRNA recognition by disrupting this signaling complex. NSP6 and NSP13 inhibit IFNβ promoter reporter activity in response to RIG-I CARD in HEK293T cells. This reduction was attributed to a decrease in IRF3 (S396) phosphorylation following pI:C transfection in NSP6 or NSP13 expressing cells.[42] Both proteins could associate with TBK1, but only the association with NSP13 disrupted the activation of TBK1 as determined by S172 phosphorylation and downstream activation of both IRF3 and NF-κB.[71] Comparison between the ability of NSP6 and NSP13 proteins encoded by the highly pathogenic coronaviruses (SARS-CoV, MERS-CoV, and SARS-CoV-2) showed differences only in the level of antagonism of NSP6. MERS-CoV NSP6 did not show inhibition of IFNβ promoter reporter activity, while the level of inhibition was comparable between SARS-CoV and SARS-CoV-2-encoded NSP6. Independent studies also detected NSP13-TBK1 interactions,[61,71,72] but failed to capture the interaction between NSP6-TBK1[61] or the inhibition of IFN induction by NSP6.[41] These discrepancies could be explained by differing expression levels of NSP6 between studies, as the recombinant protein could be robustly detected in studies reporting inhibition while very low protein detection in the latter two studies. These observations suggests that the level of protein expression during infection could be critical to promote immune antagonism.

There is also compelling evidence that ORF9b disrupts the RLR signalosome by localizing to the mitochondria and binding to the mitochondrial outer membrane protein, TOM70. This interaction occurs at critical residues necessary for recognition of the mitochondrial targeting signal within TOM70 target substrates.[61,72,73] TOM70 recruits TBK1 and IRF3 to the mitochondria through interactions with the chaperone protein HSP90.[74] The interaction between SARS-CoV-2 ORF9b and TOM70 lowered the affinity for HSP90 binding,[73] and inhibited IFNβ promoter reporter activity following pI:C stimulation or MAVS overexpression.[75] As expected, exogenous expression of ORF9b antagonizes the induction of type I and III IFNs downstream of RLRs.[76] While the role of cGAS/STING in antiviral responses against SARS-CoV-2 remains to be explored, agonists that trigger IFN induction through this DNA sensing pathway have been used to boost antiviral responses during in vitro and in vivo infections.[77] Interestingly, ORF9b was shown to interact with components of the cGAS-STING DNA sensing pathway, decreasing TBK1 and IRF3 activation in HEK293T cells overexpressing STING.[76] Lastly, the NF-κB essential modulator (NEMO) is an important scaffolding component for the IkB kinase complex that controls the subcellular localization of NF-κB. NEMO is regulated by binding of both Lys-48 (K48) and Lys-63 (K63) polyubiquitin chains (reviewed in 78). NEMO mutants deficient in polyubiquitin-binding have a diminished ability to activate TBK1/IRF3 and downstream IFN production.[79] Exogenous expression of SARS-CoV-2 ORF9b was sufficient to mediate an interaction with NEMO, preventing K63-linked polyubiquitination of NEMO and the activation and nuclear translocation of NF-κB.[80]

Two studies have also suggested that the structural protein, M, can also antagonize RLR signaling[81,82] as is observed for SARS-CoV M protein, but not MERS-CoV-M.[83] Expression of M was sufficient to abrogate induction of IFNβ and IFNλ endogenous gene expression[81] and IFNβ promoter reporter activity after pI:C or RIG-I/MDA5 CARD transfection or infection with Sendai Virus (SeV).[81,82] In vitro, M interacts with RIG-I, MDA5, MAVS, TBK1, TRAF3, but not IRF3 and inhibits MAVS oligomerization. Infection with SeV promotes co-localization between M and TBK1 inhibiting IRF3 phosphorylation (S396) and nuclear translocation, lowering antiviral responses in infected cells.[81] TBK1 is a vital crossroad for multiple host-signaling pathways beyond the regulation of IFN responses. Proinflammatory signaling and autophagy are also coordinated by the activities of TBK1 and thus the extent to which SARS-CoV-2 M could also disrupt these biological processes remains to be determined.[84]

There are other proteins that antagonize IFN production through mechanisms yet to be defined. The ORF3b protein of SARS-CoV and SARS-CoV-related viruses is one such protein.[85] The ORF3b protein of SARS-CoV-2 and SARS-CoV-2 related viruses are remarkably shortened relative to SARS-CoV due to C-terminal truncations.[86] These truncated proteins strongly antagonized IFN responses relative to SARS-CoV ORF3b. The absence of a putative C-terminal nuclear localization signal (NLS) allows for increased cytosolic localization of SARS-CoV-2 and SARS-CoV-2 like ORF3b, correlating with antagonism of IRF3 activation. Naturally occurring ORF3b variants having slightly increased lengths were more efficient at

inhibiting IFN.[86] Another direct regulator of IRF3 is NSP12, the viral RNA-dependent RNA polymerase (RdRp).[41,87] SARS-CoV-2 NSP12 inhibited SeV, RIG-I/MDA5 CARD,[41,87] and pI:C[87] mediated induction of IFNβ promoter activity. Although IRF3 phosphorylation was unaffected, nuclear translocation was impaired. This inhibition was independent of RdRp and nidovirus RdRp-associated nucleotidyltransferase (NiRAN) catalytic activities.[87] NSP12 antagonism of nuclear translocation was specific to IRF3 as the response to IFN stimulation, which also requires nuclear import of TFs, was unaffected.[41] Future mechanistic studies will be necessary to better understand the molecular mechanisms by which SARS-CoV-2 ORF3b and NSP12 antagonizes IRF3 functions.

Late antagonism of IFN responses by SARS-CoV-2:

The secretion of type I (IFNα/β) and III (IFNλ) IFNs activates IFN receptors (IFNα/β; IFNAR and IFNλ; IFNLR) to initiate the late phase of antiviral innate immunity (Figure 1B; right). Both IFNAR and IFNLR signaling leads to STAT1 and STAT2 phosphorylation at tyrosine residues to promote their association with IRF9 to transactivate ISRE-mediated ISG expression. Antagonism of ligand-receptors interactions, receptor expression, and STAT activation/nuclear translocation can readily disarm this powerful antiviral response. Indeed, SARS-CoV-2 infection can inhibit the cellular response to either universal type I treatment as determined by decreased STAT1 and STAT2 phosphorylation, nuclear translocation, ISRE-promoter reporter activity, and endogenous ISG expression.[88] The mechanisms by which SARS-CoV-2 inhibits the response to IFNs are varied. One major SARS-CoV regulator of type I IFN receptor expression is ORF3a, which targets IFNAR1 for lysosomal degradation by promoting its ubiquitination.[89] Although the effect of SARS-CoV-2 ORF3a on IFNAR1 stability has not been addressed, it is shown to interact with antiviral regulatory E3 ligases that could disarm the antiviral response.[61] However, IFNAR1 antagonism appears to be the function of SARS-CoV-2 NSP14. NSP14 expression profoundly inhibited IFNAR1 expression resulting in impaired STAT1 phosphorylation following IFNβ stimulation. The loss of IFNAR1 in NSP14-expressing cells could be rescued by Bafilomycin A1 treatment suggesting that IFNAR1 is targeted for lysosomal degradation.[90] Whether SARS-CoV-2 inhibits the expression and distribution of IFNLR1, which confers antiviral protection in the respiratory epithelium, remains to be addressed.

The ORF6 gene is encoded by the *Sarbecoviruses*, a subgenus of the *Betacoronaviruses*. The SARS-CoV-2 ORF6 protein inhibits the activation of an ISRE promoter reporter following IFN treatment.[41,42,88] The potent antagonism of IFN response by ORF6 is a conserved function across viruses.[91] Interestingly, ORF6 does not impair STAT1 Y701 or STAT2 Y689 phosphorylation upon IFN stimulation.[42] Rather ORF6 inhibits the nuclear translocation of STAT1 and STAT2.[42,88] Nuclear shuttling of cytoplasmic TFs through the nuclear pore complex (NPC) is necessary for DNA binding and gene expression regulation. The karyopherins/importins are tasked with TF docking to the NPC. Given that SARS-CoV ORF6 can antagonize STAT1 nuclear import by sequestering karyopherins in the ER/Golgi,[92] it was proposed that SARS-CoV-2 blocks the translocation of TFs through similar mechanisms. Although SARS-CoV2 ORF6 binds to KPNA2,[42] the overexpression of KPNA1 or KPNA2 did not alleviate the cytoplasmic retention of STAT1. This suggested an alternative mechanism for inhibition of nuclear trafficking. AP-MS[61] and immunoprecipitation assays[88,93] confirmed the interactions between SARS-CoV-2 ORF6 and the NPC Nup98-Rae1 heterodimer that mediates nuclear import/export. Amino acid substitutions in ORF6 (M58R) that abrogate binding to Nup98-Rae1, but allows for KPNA1 and KPNA2 interaction, demonstrate that the blockade of Nup98-Rae1 is necessary to inhibit STAT1 translocation and IFN responses.[88] Whether ORF6 blockade of the NPC allows for selective nuclear import of proteins to promote other pro-viral activities remains to be elucidated.

In vitro experiments have also identified additional antagonists of STAT1/2 activation. In addition to blocking IRF3 activation, exogenous NSP6 expression inhibits STAT1 (Y701) and STAT2 (Y689) phosphorylation and ISRE-reporter activation in IFNα -treated HEK293T cells.[42] Chimeric SARS-CoV-2-luciferase reporter replicons encoding either SARS-CoV or MERS-CoV NSP6 demonstrated the increased antagonistic efficacy of SARS-CoV-2 NSP6. Viruses expressing SARS-CoV or MERS-CoV NSP6 were markedly more sensitive to the dose-dependent antiviral effects of IFNα than the wild-type (WT) replicon.[42] Interestingly, opposing roles for modulation of IFNβ responses have been reported for NSP6. Low levels of SARS-CoV-2 NSP6 expression enhanced ISRE-promoter reporter activity after 24hrs of IFN stimulation.[41] Both studies were carried out in HEK293T cells, highlighting the contribution of viral protein expressions levels to their ability to counter the host response.

Additional non-structural and accessory proteins were found to suppress IFNα (NSP1, NSP7, NSP13, NSP14, ORF3a, ORF7a, and ORF7b)[42] or IFNβ signaling (NSP1, NSP3, NSP13, NSP14, ORF8)[41]. NSP1 inhibits global host translation[94–98] and inhibits IFN responses.[41,42] The detailed mechanisms of host shutoff will be discussed in later sections. The detailed mechanisms of SARS-CoV-2 NSP3 (PLpro)-mediated IFN antagonism

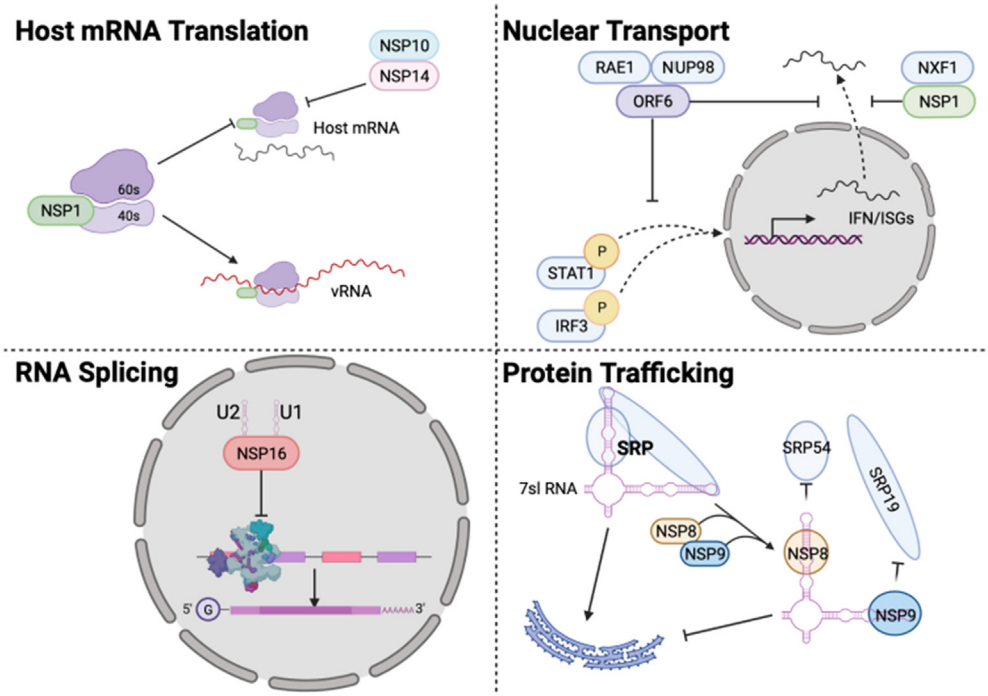


Figure 2. Broad modulation of host cell responses that impair innate immune functions. SARS-CoV-2 mediates host shutoff through the inhibition of messenger RNA (mRNA) translation. Translation of viral RNA (vRNA) is favored in infected cells. Additional inhibition of antiviral responses is due to deficits in RNA maturation. SARS-CoV-2 gene products block the splicing of host RNA. Similarly, the nuclear trafficking of transcription factors involved in the induction and response to interferon (IFN) and IFN-stimulated genes (ISGs) is impaired. Sequestration of subunits of the nuclear pore complex, RAE1 and NUP68 prevent the nuclear import of transcription factors, IRF3 and STAT1. Blockade of nuclear export of IFN and ISG mRNA inhibits antiviral immunity. This can be partly achieved by inhibition of the nuclear RNA export factor 1 (NFX1). Lastly, SARS-CoV-2 blocks the trafficking of proteins through the secretory pathway by inhibiting structural components of the Signal Recognition Particle (SRP). Created with BioRender.com.

have not been explored, but it could likely be attributed to its ability to target ISGylated proteins.[99] ISG15 conjugation is an important regulator of IFNα/β through IFNAR-dependent and independent regulatory mechanisms (summarized in 67). SARS-CoV-2 NSP7 is highly conserved relative to SARS-CoV NSP7,[23] a known IFN antagonist with poorly defined mechanisms.[85,92] NSP7 plays a role in RNA replication, forming complexes with NSP8 to stabilize NSP12. It remains to be determined whether these interactions could affect the efficacy of the ability of NSP7-mediated IFN antagonism in the infected cell. NSP13 has also been shown to consistently inhibit IFN signaling as evidenced by reduced ISRE-reporter activity[41,42] and concomitant inhibition of STAT1/STAT2 phosphorylation. SARS-CoV-2 ORF3a, ORF7a, and ORF7b can also disrupt the phosphorylation of STAT1 and STAT2 after 30 minutes of IFNα treatment.[42,90] ORF7a preferentially inhibited the phosphorylation of STAT2 at Y689, with minimal effects on STAT1 Y701 activation or nuclear translocation. The K63-linked polyubiquitination of ORF7a at Lys119 was necessary for suppression of STAT2 phosphorylation.[100] Like ORF7a, ORF7b also preferentially inhibited STAT2 activation and ISRE-mediated transcriptional activity, but the mechanisms for antagonism are not fully characterized.

Exogenous expression of the structural proteins M and N also results in discrepant IFN inhibitory responses. One study suggests that overexpression of M leads to a decrease in ISRE-reporter activity following IFNα treatment.[42] Two studies have documented N-mediated inhibition of ISRE-activity,[41] STAT1 and STAT2 phosphorylation, and nuclear translocation in response to SeV and SARS-CoV-2 infection or IFNβ treatment, decreasing ISG mRNA accumulation.[101] On the other hand, similar screens failed to identify N as a negative regulator of type I or III IFN signalling.[42,90] Thus, further investigation is still warranted to define whether N targets the late antiviral response or whether its ability to counter RLR signaling and the feedforward IFN responses contributes to these discrepancies. The detailed mechanism by which many of these proteins antag-

onize IFN responses remains to be addressed. Further studies would aid in unveiling the determinants that direct antagonism of defined STAT proteins.[102] Such studies could provide a better understanding of host range or modulation of unique ISG expression profiles which could affect the trajectory of host immune responses.[14]

Global regulation of host immune responses:

The activation and nuclear translocation of cellular TFs is essential to promote the transcription of ISGs. Moreover, for these genes to carry out antiviral functions, their rapid and accurate post-transcriptional, translational, and post-translational processing is necessary to support robust expression levels and proper subcellular localization or secretion.[103–105] Thus, viruses encode various mechanisms to promote the shutoff of host gene expression by abrogating these essential functions.[106] The resolution of the SARS-CoV-2:host protein–protein and RNA-protein interactomes has uncovered numerous interactions that indicate that viral infection could regulate nuclear trafficking, RNA maturation, mRNA translation, and protein trafficking.[61,72,94] The disruption of these global cellular responses by SARS-CoV-2 infection and individual proteins abrogate innate immune signaling (Figure 2) and could have further immunological consequences. NSP1 is amongst the most concordant and better understood SARS-CoV-2 host regulatory proteins. SARS-CoV-2 infection results in a global host mRNA translational shutoff and this function has been largely attributed to NSP1. NSP1 binds to the 18S ribosomal RNA through a 37-nt region that corresponds to helix 18.[94] This interaction nucleates the association with the 40S ribosomal subunit which results in the loss of mRNA translational initiation. While the C-terminal domain of NSP1 contacts the mRNA entry channel, hindering ribosomal access to host mRNA,[95–98] viral RNA translation is unaffected. Access of vRNA to the entry channel is favored by interactions between the first stem loop structure in the viral 5′ cap and the N-terminal domain of NSP1 that releases the C-terminal domain from the mRNA entry channel.[95] Genomic surveillance studies identified SARS-CoV-2 NSP1 variants with N-terminal deletions. The detection of NSP1 deletion variants occurred primarily in non-severe COVID-19 patients, correlating with lower viral loads, erythrocyte sedimentation rates (ESR), IFNβ serum levels, and CD8+ T cell counts in patients. Although NSP1 variants had similar replication kinetics in Calu-3 cells and retained the ability to interact with the 40S ribosomal subunit, viruses carrying these mutations elicited lower IFN responses.[107] These results potentially suggest alternative mechanisms by which NSP1 targets the host antiviral response. NSP1 interacts with the mRNA export receptor, NXF1, preventing its localization to the nuclear pore complex (NPC). This results in the loss of poly-adenylated (poly(A)) export from the nucleus and thus decreased translation.[108] Similarly, ORF6 localization to the NPC results in the accumulation of mRNA in the nucleus and impairs the translation of nascent transcripts.[93]

Like NSP1, NSP14 can also inhibit the translation of ISGs following type I IFN stimulation.[109] NSP14 mediates replication proof-reading through an N-terminal 3′–5′exoribonuclease (ExoN) domain and controls capping of viral RNA through the C-terminal N7-MTase domain. NSP14 requires both ExoN catalytic activity and the N7-MTase domains to inhibit host mRNA translation.[109] Interactions between NSP14 and NSP10, the later required to activate the enzymatic activity of NSP14,[110] enhances translational shutdown. While the exact inhibitory mechanisms have yet to be elucidated, NSP14 does not affect the nuclear/cytoplasmic distribution of cellular RNA. These studies highlight independent strategies by which SARS-CoV-2 dually blocks mRNA trafficking and ribosomal accessibility to inhibit the initiation of host innate immune responses.

In addition to disrupting host mRNA distribution, SARS-CoV-2 infection also hinders host RNA processing. A comprehensive study of viral protein:host RNA interactions identified 10 out of 26 viral proteins that bound both human mRNA and non-coding RNA to perturb innate immune responses. NSP16 localizes to the nucleus where it binds to the small nuclear RNAs (snRNAs) U1 and U2, critical components of the pre-mRNA spliceosome. These snRNAs initiate splicing through recognition of the 5′ splice site at intron–exon junctions (U1) and the intron branchpoint site (U2). The inhibition of ISG-specific RNA splicing by NSP16/U1/U2 was determined by an IRF7 exon–intron–exon minigene reporter and sequencing of metabolically labelled nascent transcripts. These observations were consistent with the increase levels of ISG RNAs exhibiting intron retentions in SARS-CoV-2 infected cells.[94]

The signal recognition particle (SRP) is a ribonucleoprotein that co-translationally mediates the transport proteins through the secretory pathway or membrane targeting. The long noncoding-RNA, 7SL, scaffolds the complex formed by SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72.[111] SARS-CoV-2 impairs protein trafficking through interactions between NSP8 and NSP9 with 7SL.[94] NSP8 binding inhibits the recruitment of SRP54 to 7SL, while NSP9 inhibits the interaction with SRP19. The SRP assembly could be further hindered by direct or indirect interactions between NSP8, SRP19, and SRP72.[61] The disruption of plasma membrane protein targeting during *in vitro* infection with SARS-CoV-2 was demonstrated using an SRP-dependent nerve growth factor receptor (NGFR)-GFP reporter assay

and puromycin-incorporation assays (SUnSET). The expression of either NSP8 or NSP9 alone was sufficient to inhibit the membrane accumulation of NGFR-GFP or puromycin-labelled proteins relative to non-membrane protein accumulation. Interestingly, expression of NSP8/9 inhibited the cellular response to IFNβ treatment although NSP8/9 has not been demonstrated to directly affect IFN receptor expression at the plasma membrane or the integration of signals from membrane-targeted receptors to dampen the response to IFNβ stimulation. Interestingly, 7SL can promote viral assembly of HIV through interactions with Gag and other host proteins.[112,113] Whether NSP8/NSP9 utilizes this mechanism to promote coronavirus virion assembly remains to be addressed.

Future perspectives

Comprehensive and timely studies have uncovered the disarming potential that individual SARS-CoV-2 viral proteins have on the host antiviral response. These studies have revealed the immunoregulatory functions unique to SARS-CoV-2 encoded proteins and those conserved across highly-pathogenic coronaviruses. Although not discussed due to the absence of known mechanisms, a few SARS-CoV-2 proteins were also found to enhance IFN responses.[41,90] Some of these interactions could account for the excessive inflammation observed in COVID-19 patients. Importantly, the host modulatory effect is not restricted to enhancement of IFN-mediated antiviral responses. SARS-CoV-2 can also hijack cytokine signaling pathways to enhance proinflammatory cytokine production.[114–117]

Our current understanding of IFN antagonists is limited by the use of individual viral gene overexpression systems. These systems could result in non-physiological protein expression levels, failing to reflect the magnitude and kinetics of viral gene and protein expression during the course of infection.[118] Importantly, the expression of single viral genes could fail to detect important interactions between viral proteins critical for the regulation of host immune responses. The development and implementation of replication-competent recombinant viruses will continue to strengthen our understanding of the mechanisms by which single proteins,[42] viral protein–protein complexes, or protein-vRNA complexes shape the host antiviral response. As genomic surveillance data continues to reveal naturally occurring mutations in SARS-CoV-2 genes, it is critical to understand how viral evolution impacts mechanisms of viral entry, replication, transmission and evasion of host innate and adaptive immune responses. The use of primary cells and organoids,[119–121] animal models[122] and patient-derived observations have facilitated our understanding of disease progression, immunological outcomes, and the development of vaccines and viral therapies. The integration of these tools will continue to provide a better understanding of how tissue tropism and cell identity shape susceptibility to SARS-CoV-2 infection and immunological outcomes.[9]

CRediT authorship contribution statement

Daniel K. Beyer: Conceptualization, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition, Supervision. **Adriana Forero:** Writing – review & editing, Visualization, Funding acquisition, Supervision.

Acknowledgements

This work was supported by funds from the Pelotonia Undergraduate Fellowship Program (DKB).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received 1 July 2021;
Accepted 14 September 2021;
Available online xxxx

Keywords:
interferons;
ISGs;
immune evasion;
COVID-19;
SARS-CoV-2

References

1. Hartenian, E., Nandakumar, D., Lari, A., Ly, M., Tucker, J. M., Glaunsinger, B.A., (2020). The molecular virology of coronaviruses. *J. Biol. Chem.*, **295**, 12910–12934. https://doi.org/10.1074/jbc.REV120.013930.
2. V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., Thiel, V., (2020). Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Rev. Microbiol.*,, 1–16. https://doi.org/10.1038/s41579-020-00468-6.
3. Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., et al., (2019). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*, **395** (2020), 565–574. https://doi.org/10.1016/S0140-6736(20)30251-8.
4. Arya, R., Kumari, S., Pandey, B., Mistry, H., Bihani, S.C., Das, A., Prashar, V., Gupta, G.D., et al., (2021). Structural insights into SARS-CoV-2 proteins. *J. Mol. Biol.*, **433**, https://doi.org/10.1016/j.jmb.2020.11.024 166725.

5. Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., et al., (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, **579**, 270–273. https://doi.org/10.1038/s41586-020-2012-7.
6. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., et al., (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, **581**, 215–220. https://doi.org/10.1038/s41586-020-2180-5.
7. Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., Li, F., (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature*, **581**, 221–224. https://doi.org/10.1038/s41586-020-2179-y.
8. Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., Zhou, Q., (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, **367**, 1444–1448. https://doi.org/10.1126/science.abb2762.
9. Li, Y., Renner, D.M., Comar, C.E., Whelan, J.N., Reyes, H.M., Cardenas-Diaz, F.L., Truitt, R., Tan, L.H., et al., (2021). SARS-CoV-2 induces double-stranded RNA-mediated innate immune responses in respiratory epithelial-derived cells and cardiomyocytes. *PNAS*, **118** https://doi.org/10.1073/pnas.2022643118.
10. Liu, S., Cai, X., Wu, J., Cong, Q., Chen, X., Li, T., Du, F., Ren, J., et al., (2015). Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. *Science*, **347** https://doi.org/10.1126/science.aaa2630.
11. Onoguchi, K., Yoneyama, M., Takemura, A., Akira, S., Taniguchi, T., Namiki, H., Fujita, T., (2007). Viral Infections Activate Types I and III Interferon Genes through a Common Mechanism. *J. Biol. Chem.*, **282**, 7576–7581. https://doi.org/10.1074/jbc.M608618200.
12. Galani, I.E., Triantafyllia, V., Eleminiadou, E.-E., Koltsida, O., Stavropoulos, A., Manioudaki, M., Thanos, D., Doyle, S.E., et al., (2017). Interferon-λ Mediates Non-redundant Front-Line Antiviral Protection against Influenza Virus Infection without Compromising Host Fitness. *Immunity*, **46**, 875–890.e6. https://doi.org/10.1016/j.immuni.2017.04.025.
13. Davidson, S., McCabe, T.M., Crotta, S., Gad, H.H., Hessel, E.M., Beinke, S., Hartmann, R., Wack, A., (2016). IFNλ is a potent anti-influenza therapeutic without the inflammatory side effects of IFNα treatment. *EMBO Mol. Med.*, **8**, 1099–1112. https://doi.org/10.15252/emmm.201606413.
14. Forero, A., Ozarkar, S., Li, H., Lee, C.H., Hemann, E.A., Nadjsombati, M.S., Hendricks, M.R., So, L., et al., (2019). Differential Activation of the Transcription Factor IRF1 Underlies the Distinct Immune Responses Elicited by Type I and Type III Interferons. *Immunity*, **51**, 451–464.e6. https://doi.org/10.1016/j.immuni.2019.07.007.
15. Pervolaraki, K., Stanifer, M.L., Münchau, S., Renn, L.A., Albrecht, D., Kurzhals, S., Senís, E., Grimm, D., et al., (2017). Type I and Type III Interferons Display Different Dependency on Mitogen-Activated Protein Kinases to Mount an Antiviral State in the Human Gut. *Front. Immunol.*, **8** https://doi.org/10.3389/fimmu.2017.00459.
16. Müller, U., Steinhoff, U., Reis, L.F.L., Hemmi, S., Pavlovic, J., Zinkernagel, R.M., Aguet, M., (1994). Functional role of type I and type II interferons in antiviral defense. *Science*, **264**, 1918–1921. https://doi.org/10.1126/science.8009221.
17. Galani, I.-E., Rovina, N., Lampropoulou, V., Triantafyllia, V., Manioudaki, M., Pavlos, E., Koukaki, E., Fragkou, P. C., et al., (2021). Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. *Nature Immunol.*, **22**, 32–40. https://doi.org/10.1038/s41590-020-00840-x.
18. Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., Péré, H., Charbit, B., et al., (2020). Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science*,. https://doi.org/10.1126/science.abc6027.
19. Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.-C., Uhl, S., Hoagland, D., Møller, R., Jordan, T.X., Oishi, K., et al., (2020). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*, **181** https://doi.org/10.1016/j.cell.2020.04.026. 1036–1045.e9.
20. Zhang, Q., Bastard, P., Liu, Z., Pen, J.L., Moncada-Velez, M., Chen, J., Ogishi, M., Sabli, I.K.D., et al., (2020). Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science*, **370** https://doi.org/10.1126/science.abd4570.
21. Wang, E.Y., Mao, T., Klein, J., Dai, Y., Huck, J.D., Jaycox, J.R., Liu, F., Zhou, T., et al., (2021). Diverse functional autoantibodies in patients with COVID-19. *Nature*,, 1–6. https://doi.org/10.1038/s41586-021-03631-y.
22. Bastard, P., Rosen, L.B., Zhang, Q., Michailidis, E., Hoffmann, H.-H., Zhang, Y., Dorgham, K., Philippot, Q., et al., (2020). Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science*, **370** https://doi.org/10.1126/science.abd4585.
23. Lokugamage, K.G., Hage, A., de Vries, M., Valero-Jimenez, A.M., Schindewolf, C., Dittmann, M., Rajsbaum, R., Menachery, V.D., (2020). Type I Interferon Susceptibility Distinguishes SARS-CoV-2 from SARS-CoV. *J. Virol.*, **94** https://doi.org/10.1128/JVI.01410-20.
24. Vanderheiden, A., Ralfs, P., Chirkova, T., Upadhyay, A. A., Zimmerman, M.G., Bedoya, S., Aoued, H., Tharp, G. M., et al., (2020). Type I and Type III Interferons Restrict SARS-CoV-2 Infection of Human Airway Epithelial Cultures. *J. Virol.*, **94** https://doi.org/10.1128/JVI.00985-20.
25. Stanifer, M.L., Kee, C., Cortese, M., Zumaran, C.M., Triana, S., Mukenhirn, M., Kraeusslich, H.-G., Alexandrov, T., et al., (2020). Critical Role of Type III Interferon in Controlling SARS-CoV-2 Infection in Human Intestinal Epithelial Cells. *Cell Rep.*, **32**, https://doi.org/10.1016/j.celrep.2020.107863 107863.
26. Wang, S., Li, W., Hui, H., Tiwari, S.K., Zhang, Q., Croker, B.A., Rawlings, S., Smith, D., et al., (2020). Cholesterol 25-Hydroxylase inhibits SARS-CoV-2 and other coronaviruses by depleting membrane cholesterol. *EMBO J.*, **39**, e106057. https://doi.org/10.15252/embj.2020106057.
27. Pfaender, S., Mar, K.B., Michailidis, E., Kratzel, A., Boys, I.N., V'kovski, P., Fan, W., Kelly, J.N., et al., (2020). LY6E impairs coronavirus fusion and confers immune control of viral disease. *Nature Microbiol.*, **5**, 1330–1339. https://doi.org/10.1038/s41564-020-0769-y.
28. X. Zhao, S. Zheng, D. Chen, M. Zheng, X. Li, G. Li, H. Lin, J. Chang, et al., LY6E Restricts Entry of Human Coronaviruses, Including Currently Pandemic SARS-CoV-2, J. Virol. 94 (n.d.) e00562–e00520. 10.1128/JVI.00562-20..

29. R. Nchioua, D. Kmiec, J.A. Müller, C. Conzelmann, R. Groß, C.M. Swanson, S.J.D. Neil, S. Stenger, et al., SARS-CoV-2 Is Restricted by Zinc Finger Antiviral Protein despite Preadaptation to the Low-CpG Environment in Humans, MBio 11 (n.d.) e01930–e02020. 10.1128/mBio.01930-20..
30. Shi, G., Kenney, A.D., Kudryashova, E., Zani, A., Zhang, L., Lai, K.K., Hall-Stoodley, L., Robinson, R.T., et al., (2021). Opposing activities of IFITM proteins in SARS-CoV-2 infection. *EMBO J.*, **40**, e106501. https://doi.org/10.15252/embj.2020106501.
31. Peiffer-Smadja, N., Yazdanpanah, Y., (2021). Nebulised interferon beta-1a for patients with COVID-19. *Lancet Respir. Med.*, **9**, 122–123. https://doi.org/10.1016/S2213-2600(20)30523-3.
32. Monk, P.D., Marsden, R.J., Tear, V.J., Brookes, J., Batten, T.N., Mankowski, M., Gabbay, F.J., Davies, D. E., et al., (2021). Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Respir. Med.*, **9**, 196–206. https://doi.org/10.1016/S2213-2600(20)30511-7.
33. Feld, J.J., Kandel, C., Biondi, M.J., Kozak, R.A., Zahoor, M.A., Lemieux, C., Borgia, S.M., Boggild, A.K., et al., (2021). Peginterferon lambda for the treatment of outpatients with COVID-19: a phase 2, placebo-controlled randomised trial. *Lancet Respir. Med.*,. https://doi.org/10.1016/S2213-2600(20)30566-X.
34. Hoagland, D.A., Møller, R., Uhl, S.A., Oishi, K., Frere, J., Golynker, I., Horiuchi, S., Panis, M., et al., (2021). Leveraging the antiviral type I interferon system as a first line of defense against SARS-CoV-2 pathogenicity. *Immunity*, **54**, 557–570.e5. https://doi.org/10.1016/j.immuni.2021.01.017.
35. Park, A., Iwasaki, A., (2020). Type I and Type III Interferons – Induction, Signaling, Evasion, and Application to Combat COVID-19. *Cell Host Microbe*, **27**, 870–878. https://doi.org/10.1016/j.chom.2020.05.008.
36. Broggi, A., Ghosh, S., Sposito, B., Spreafico, R., Balzarini, F., Cascio, A.L., Clementi, N., Santis, M.D., et al., (2020). Type III interferons disrupt the lung epithelial barrier upon viral recognition. *Science*, **369**, 706–712. https://doi.org/10.1126/science.abc3545.
37. Israelow, B., Song, E., Mao, T., Lu, P., Meir, A., Liu, F., Alfajaro, M.M., Wei, J., et al., (2020). Mouse model of SARS-CoV-2 reveals inflammatory role of type I interferon signaling. *J. Exp. Med.*, **217** https://doi.org/10.1084/jem.20201241.
38. Boudewijns, R., Thibaut, H.J., Kaptein, S.J.F., Li, R., Vergote, V., Seldeslachts, L., Van Weyenbergh, J., De Keyzer, C., et al., (2020). STAT2 signaling restricts viral dissemination but drives severe pneumonia in SARS-CoV-2 infected hamsters. *Nature Commun.*, **11**, 5838. https://doi.org/10.1038/s41467-020-19684-y.
39. Menachery, V.D., Baric, R.S., (2013). Bugs in the system. *Immunol. Rev.*, **255**, 256–274. https://doi.org/10.1111/imr.12092.
40. Yuen, C.-K., Lam, J.-Y., Wong, W.-M., Mak, L.-F., Wang, X., Chu, H., Cai, J.-P., Jin, D.-Y., et al., (2020). SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists. *Emerging Microbes Infect.*, **9**, 1418–1428. https://doi.org/10.1080/22221751.2020.1780953.
41. Lei, X., Dong, X., Ma, R., Wang, W., Xiao, X., Tian, Z., Wang, C., Wang, Y., et al., (2020). Activation and evasion of type I interferon responses by SARS-CoV-2. *Nature Commun.*, **11**, 3810. https://doi.org/10.1038/s41467-020-17665-9.
42. 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**, https://doi.org/10.1016/j.celrep.2020.108234 108234.
43. Yin, X., Riva, L., Pu, Y., Martin-Sancho, L., Kanamune, J., Yamamoto, Y., Sakai, K., Gotoh, S., et al., (2020). MDA5 Governs the Innate Immune Response to SARS-CoV-2 in Lung Epithelial Cells. Social Science Research Network, Rochester, NY. 10.2139/ssrn.3682826.
44. A. Rebendenne, A.L. Chaves Valadão, M. Tauziet, G. Maarifi, B. Bonaventure, J. McKellar, R. Planès, S. Nisole, et al., SARS-CoV-2 Triggers an MDA-5-Dependent Interferon Response Which Is Unable To Control Replication in Lung Epithelial Cells, J. Virol. 95 (n.d.) e02415–20. 10.1128/JVI.02415-20..
45. Roth-Cross, J.K., Bender, S.J., Weiss, S.R., (2008). Murine Coronavirus Mouse Hepatitis Virus Is Recognized by MDA5 and Induces Type I Interferon in Brain Macrophages/Microglia. *J. Virol.*, **82**, 9829–9838. https://doi.org/10.1128/JVI.01199-08.
46. Z.B. Zalinger, R. Elliott, K.M. Rose, S.R. Weiss, MDA5 Is Critical to Host Defense during Infection with Murine Coronavirus, J. Virol.. 89 (n.d.) 12330–12340. 10.1128/JVI.01470-15..
47. Duic, I., Tadakuma, H., Harada, Y., Yamaue, R., Deguchi, K., Suzuki, Y., Yoshimura, S.H., Kato, H., Takeyasu, K., Fujita, T., (2020). Viral RNA recognition by LGP2 and MDA5, and activation of signaling through step-by-step conformational changes. *Nucleic Acids Res.*, **48**, 11664–11674. https://doi.org/10.1093/nar/gkaa935.
48. Yamada, T., Sato, S., Sotoyama, Y., Orba, Y., Sawa, H., Yamauchi, H., Sasaki, M., Takaoka, A., (2021). RIG-I triggers a signaling-abortive anti-SARS-CoV-2 defense in human lung cells. *Nature Immunol.*, **22**, 820–828. https://doi.org/10.1038/s41590-021-00942-0.
49. 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. *Nature Commun.*, **11**, 5885. https://doi.org/10.1038/s41467-020-19619-7.
50. Schlee, M., Roth, A., Hornung, V., Hagmann, C.A., Wimmenauer, V., Barchet, W., Coch, C., Janke, M., et al., (2009). Recognition of 5' triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. *Immunity*, **31**, 25–34. https://doi.org/10.1016/j.immuni.2009.05.008.
51. Schmidt, A., Schwerd, T., Hamm, W., Hellmuth, J.C., Cui, S., Wenzel, M., Hoffmann, F.S., Michallet, M.-C., et al., (2009). 5′-triphosphate RNA requires base-paired structures to activate antiviral signaling via RIG-I. *PNAS*, **106**, 12067–12072.
52. Kato, H., Takeuchi, O., Mikamo-Satoh, E., Hirai, R., Kawai, T., Matsushita, K., Hiiragi, A., Dermody, T.S., et al., (2008). Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid–inducible gene-I and melanoma differentiation–associated gene 5.

J. Exp. Med., **205**, 1601–1610. https://doi.org/10.1084/jem.20080091.

53. Hyde, J.L., Diamond, M.S., (2015). Innate immune restriction and antagonism of viral RNA lacking 2-O methylation. *Virology*, **479–480**, 66–74. https://doi.org/10.1016/j.virol.2015.01.019.
54. Menachery, V.D., Debbink, K., Baric, R.S., (2014). Coronavirus non-structural protein 16: Evasion, attenuation, and possible treatments. *Virus Res.*, **194**, 191–199. https://doi.org/10.1016/j.virusres.2014.09.009.
55. Viswanathan, T., Arya, S., Chan, S.-H., Qi, S., Dai, N., Misra, A., Park, J.-G., Oladunni, F., et al., (2020). Structural basis of RNA cap modification by SARS-CoV-2. *Nature Commun.*, **11**, 3718. https://doi.org/10.1038/s41467-020-17496-8.
56. Rosas-Lemus, M., Minasov, G., Shuvalova, L., Inniss, N. L., Kiryukhina, O., Brunzelle, J., Satchell, K.J.F., (2020). High-resolution structures of the SARS-CoV-2 2′-O-methyltransferase reveal strategies for structure-based inhibitor design. *Sci. Signal.*, **13** https://doi.org/10.1126/scisignal.abe1202.
57. Züst, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., et al., (2011). Ribose 2′- O -methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. *Nature Immunol.*, **12**, 137–143. https://doi.org/10.1038/ni.1979.
58. Daffis, S., Szretter, K.J., Schriewer, J., Li, J., Youn, S., Errett, J., Lin, T.-Y., Schneller, S., et al., (2010). 2′- O methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature*, **468**, 452–456. https://doi.org/10.1038/nature09489.
59. M.N. Frazier, L.B. Dillard, J.M. Krahn, L. Perera, J.G. Williams, I.M. Wilson, Z.D. Stewart, M.C. Pillon, et al., Characterization of SARS2 Nsp15 Nuclease Activity Reveals it's Mad About U, BioRxiv (2021) 2021.06.01.446181. 10.1101/2021.06.01.446181..
60. Hackbart, M., Deng, X., Baker, S.C., (2020). Coronavirus endoribonuclease targets viral polyuridine sequences to evade activating host sensors. *PNAS*, **117**, 8094–8103. https://doi.org/10.1073/pnas.1921485117.
61. Gordon, D.E., Jang, G.M., Bouhaddou, M., Xu, J., Obernier, K., White, K.M., O'Meara, M.J., Rezelj, V.V., et al., (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.
62. Wang, C., Chen, T., Zhang, J., Yang, M., Li, N., Xu, X., Cao, X., (2009). The E3 ubiquitin ligase Nrdp1 "preferentially" promotes TLR-mediated production of type I interferon. *Nat. Immunol.*, **10**, 744–752. https://doi.org/10.1038/ni.1742.
63. Chen, K., Xiao, F., Hu, D., Ge, W., Tian, M., Wang, W., Pan, P., Wu, K., Wu, J., (2021). SARS-CoV-2 Nucleocapsid Protein Interacts with RIG-I and Represses RIG-Mediated IFN-β Production. *Viruses*, **13**, 47. https://doi.org/10.3390/v13010047.
64. Gack, M.U., Shin, Y.C., Joo, C.-H., Urano, T., Liang, C., Sun, L., Takeuchi, O., Akira, S., et al., (2007). RIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature*, **446**, 916–920. https://doi.org/10.1038/nature05732.
65. Hu, Y., Li, W., Gao, T., Cui, Y., Jin, Y., Li, P., Ma, Q., Liu, X., Cao, C., (2017). The Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid Inhibits Type I Interferon Production by Interfering with TRIM25-Mediated RIG-I Ubiquitination. *J. Virol.*, **91** https://doi.org/10.1128/JVI.02143-16.
66. Wu, Y., Ma, L., Zhuang, Z., Cai, S., Zhao, Z., Zhou, L., Zhang, J., Wang, P.-H., Zhao, J., Cui, J., (2020). Main protease of SARS-CoV-2 serves as a bifunctional molecule in restricting type I interferon antiviral signaling, Signal Transduction and Targeted. *Therapy*, **5**, 1–3. https://doi.org/10.1038/s41392-020-00332-2.
67. Perng, Y.-C., Lenschow, D.J., (2018). ISG15 in antiviral immunity and beyond. *Nature Rev. Microbiol.*, **16**, 423–439. https://doi.org/10.1038/s41579-018-0020-5.
68. Liu, G., Lee, J.-H., Parker, Z.M., Acharya, D., Chiang, J.J., van Gent, M., Riedl, W., Davis-Gardner, M.E., et al., (2021). ISG15-dependent activation of the sensor MDA5 is antagonized by the SARS-CoV-2 papain-like protease to evade host innate immunity. *Nature Microbiol.*, **6**, 467–478. https://doi.org/10.1038/s41564-021-00884-1.
69. Shin, D., Mukherjee, R., Grewe, D., Bojkova, D., Baek, K., Bhattacharya, A., Schulz, L., Widera, M., et al., (2020). Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. *Nature*, **587**, 657–662. https://doi.org/10.1038/s41586-020-2601-5.
70. Moustaqil, M., Ollivier, E., Chiu, H.-P., Tol, S.V., Rudolffi-Soto, P., Stevens, C., Bhumkar, A., Hunter, D.J.B., et al., (2020). SARS-CoV-2 proteases PLpro and 3CLpro cleave IRF3 and critical modulators of inflammatory pathways (NLRP12 and TAB1): implications for disease presentation across species. *Emerging Microbes Infect.*,, 1–27. https://doi.org/10.1080/22221751.2020.1870414.
71. Vazquez, C., Swanson, S.E., Negatu, S.G., Dittmar, M., Miller, J., Ramage, H.R., Cherry, S., Jurado, K.A., (2021). SARS-CoV-2 viral proteins NSP1 and NSP13 inhibit interferon activation through distinct mechanisms. *PLoS ONE*, **16**, https://doi.org/10.1371/journal.pone.0253089 e0253089.
72. Gordon, D.E., Hiatt, J., Bouhaddou, M., Rezelj, V.V., Ulferts, S., Braberg, H., Jureka, A.S., Obernier, K., et al., (2020). Comparative host-coronavirus protein interaction networks reveal pan-viral disease mechanisms. *Science*, **370** https://doi.org/10.1126/science.abe9403.
73. Gao, X., Zhu, K., Qin, B., Olieric, V., Wang, M., Cui, S., (2021). Crystal structure of SARS-CoV-2 Orf9b in complex with human TOM70 suggests unusual virus-host interactions. *Nature Commun.*, **12**, 2843. https://doi.org/10.1038/s41467-021-23118-8.
74. Liu, X.-Y., Wei, B., Shi, H.-X., Shan, Y.-F., Wang, C., (2010). Tom70 mediates activation of interferon regulatory factor 3 on mitochondria. *Cell Res.*, **20**, 994–1011. https://doi.org/10.1038/cr.2010.103.
75. Jiang, H.-W., Zhang, H.-N., Meng, Q.-F., Xie, J., Li, Y., Chen, H., Zheng, Y.-X., Wang, X.-N., et al., (2020). SARS-CoV-2 Orf9b suppresses type I interferon responses by targeting TOM70. *Cell Mol. Immunol.*, **17**, 998–1000. https://doi.org/10.1038/s41423-020-0514-8.
76. L. Han, M.-W. Zhuang, J. Deng, Y. Zheng, J. Zhang, M.-L. Nan, X.-J. Zhang, C. Gao, et al., SARS-CoV-2 ORF9b antagonizes type I and III interferons by targeting multiple components of the RIG-I/MDA-5–MAVS, TLR3–TRIF, and cGAS–STING signaling pathways, J. Med. Virol. n/a (n.d.). 10.1002/jmv.27050..
77. Li, M., Ferretti, M., Ying, B., Descamps, H., Lee, E., Dittmar, M., Lee, J.S., Whig, K., et al., (2021). Pharmacological activation of STING blocks SARS-CoV-

2 infection. *Sci. Immunol.*, **6** https://doi.org/10.1126/sciimmunol.abi9007.

78. Clark, K., Nanda, S., Cohen, P., (2013). Molecular control of the NEMO family of ubiquitin-binding proteins. *Nature Rev. Mol. Cell Biol.*, **14**, 673–685. https://doi.org/10.1038/nrm3644.
79. Zhao, T., Yang, L., Sun, Q., Arguello, M., Ballard, D.W., Hiscott, J., Lin, R., (2007). The NEMO adaptor bridges the nuclear factor-κB and interferon regulatory factor signaling pathways. *Nature Immunol.*, **8**, 592–600. https://doi.org/10.1038/ni1465.
80. Wu, J., Shi, Y., Pan, X., Wu, S., Hou, R., Zhang, Y., Zhong, T., Tang, H., et al., (2021). SARS-CoV-2 ORF9b inhibits RIG-I-MAVS antiviral signaling by interrupting K63-linked ubiquitination of NEMO. *Cell Rep.*, **34** https://doi.org/10.1016/j.celrep.2021.108761.
81. Zheng, Y., Zhuang, M.-W., Han, L., Zhang, J., Nan, M.-L., Zhan, P., Kang, D., Liu, X., et al., (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) membrane (M) protein inhibits type I and III interferon production by targeting RIG-I/MDA-5 signaling, Signal Transduction and Targeted. *Therapy*, **5**, 1–13. https://doi.org/10.1038/s41392-020-00438-7.
82. Fu, Y.-Z., Wang, S.-Y., Zheng, Z.-Q., Yi Huang, W.-W., Li, Z.-S., Xu, Y.-Y. Wang, (2021). SARS-CoV-2 membrane glycoprotein M antagonizes the MAVS-mediated innate antiviral response. *Cell. Mol. Immunol.*, **18**, 613–620. https://doi.org/10.1038/s41423-020-00571-x.
83. Siu, K.-L., Chan, C.-P., Kok, K.-H., Chiu-Yat Woo, P., Jin, D.-Y., (2014). Suppression of innate antiviral response by severe acute respiratory syndrome coronavirus M protein is mediated through the first transmembrane domain. *Cell. Mol. Immunol.*, **11**, 141–149. https://doi.org/10.1038/cmi.2013.61.
84. Louis, C., Burns, C., Wicks, I., (2018). TANK-Binding Kinase 1-Dependent Responses in Health and Autoimmunity. *Front. Immunol.*, **9** https://doi.org/10.3389/fimmu.2018.00434.
85. Kopecky-Bromberg, S.A., Martínez-Sobrido, L., Frieman, M., Baric, R.A., Palese, P., (2007). Severe Acute Respiratory Syndrome Coronavirus Open Reading Frame (ORF) 3b, ORF 6, and Nucleocapsid Proteins Function as Interferon Antagonists. *J. Virol.*, **81**, 548–557. https://doi.org/10.1128/JVI.01782-06.
86. Konno, Y., Kimura, I., Uriu, K., Fukushi, M., Irie, T., Koyanagi, Y., Sauter, D., Gifford, R.J., et al., (2020). SARS-CoV-2 ORF3b Is a Potent Interferon Antagonist Whose Activity Is Increased by a Naturally Occurring Elongation Variant. *Cell Rep.*, **32**, https://doi.org/10.1016/j.celrep.2020.108185 108185.
87. Wang, W., Zhou, Z., Xiao, X., Tian, Z., Dong, X., Wang, C., Li, L., Ren, L., et al., (2021). SARS-CoV-2 nsp12 attenuates type I interferon production by inhibiting IRF3 nuclear translocation. *Cell. Mol. Immunol.*,, 1–9. https://doi.org/10.1038/s41423-020-00619-y.
88. Miorin, L., Kehrer, T., Sanchez-Aparicio, M.T., Zhang, K., Cohen, P., Patel, R.S., Cupic, A., Makio, T., et al., (2020). SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. *PNAS*, **117**, 28344–28354. https://doi.org/10.1073/pnas.2016650117.
89. Minakshi, R., Padhan, K., Rani, M., Khan, N., Ahmad, F., Jameel, S., (2009). The SARS Coronavirus 3a protein causes endoplasmic reticulum stress and induces ligand-independent downregulation of the type 1 interferon receptor. *PLoS ONE*, **4**, https://doi.org/10.1371/journal.pone.0008342 e8342.
90. Hayn, M., Hirschenberger, M., Koepke, L., Nchioua, R., Straub, J.H., Klute, S., Hunszinger, V., Zech, F., et al., (2021). Systematic functional analysis of SARS-CoV-2 proteins uncovers viral innate immune antagonists and remaining vulnerabilities. *Cell Rep.*,. https://doi.org/10.1016/j.celrep.2021.109126 109126.
91. Kimura, I., Konno, Y., Uriu, K., Hopfensperger, K., Sauter, D., Nakagawa, S., Sato, K., (2021). Sarbecovirus ORF6 proteins hamper induction of interferon signaling. *Cell Rep.*,. https://doi.org/10.1016/j.celrep.2021.108916 108916.
92. Frieman, M., Yount, B., Heise, M., Kopecky-Bromberg, S. A., Palese, P., Baric, R.S., (2007). Severe Acute Respiratory Syndrome Coronavirus ORF6 Antagonizes STAT1 Function by Sequestering Nuclear Import Factors on the Rough Endoplasmic Reticulum/Golgi Membrane. *J. Virol.*, **81**, 9812–9824. https://doi.org/10.1128/JVI.01012-07.
93. Addetia, A., Lieberman, N.A.P., Phung, Q., Hsiang, T.-Y., Xie, H., Roychoudhury, P., Shrestha, L., Loprieno, M.A., et al., (2021). SARS-CoV-2 ORF6 Disrupts Bidirectional Nucleocytoplasmic Transport through Interactions with Rae1 and Nup98. *MBio*, **12** https://doi.org/10.1128/mBio.00065-21.
94. Banerjee, A.K., Blanco, M.R., Bruce, E.A., Honson, D.D., Chen, L.M., Chow, A., Bhat, P., Ollikainen, N., et al., (2020). SARS-CoV-2 Disrupts Splicing, Translation, and Protein Trafficking to Suppress Host Defenses. *Cell*, **183**, 1325–1339.e21. https://doi.org/10.1016/j.cell.2020.10.004.
95. Schubert, K., Karousis, E.D., Jomaa, A., Scaiola, A., Echeverria, B., Gurzeler, L.-A., Leibundgut, M., Thiel, V., et al., (2020). SARS-CoV-2 Nsp1 binds the ribosomal mRNA channel to inhibit translation. *Nature Struct. Mol. Biol.*, **27**, 959–966. https://doi.org/10.1038/s41594-020-0511-8.
96. Yuan, S., Peng, L., Park, J.J., Hu, Y., Devarkar, S.C., Dong, M.B., Shen, Q., Wu, S., et al., (2020). Nonstructural Protein 1 of SARS-CoV-2 Is a Potent Pathogenicity Factor Redirecting Host Protein Synthesis Machinery toward Viral RNA. *Mol. Cell*, **80**, 1055–1066.e6. https://doi.org/10.1016/j.molcel.2020.10.034.
97. Thoms, M., Buschauer, R., Ameismeier, M., Koepke, L., Denk, T., Hirschenberger, M., Kratzat, H., Hayn, M., et al., (2020). Structural basis for translational shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2. *Science*, **369**, 1249–1255. https://doi.org/10.1126/science.abc8665.
98. Lapointe, C.P., Grosely, R., Johnson, A.G., Wang, J., Fernández, I.S., Puglisi, J.D., (2021). Dynamic competition between SARS-CoV-2 NSP1 and mRNA on the human ribosome inhibits translation initiation. *Proc. Natl. Acad. Sci. U. S. A.*, **118** https://doi.org/10.1073/pnas.2017715118.
99. Mielech, A.M., Kilianski, A., Baez-Santos, Y.M., Mesecar, A.D., Baker, S.C., (2014). MERS-CoV papain-like protease has delSGylating and deubiquitinating activities. *Virology*, **450–451**, 64–70. https://doi.org/10.1016/j.virol.2013.11.040.
100. Cao, Z., Xia, H., Rajsbaum, R., Xia, X., Wang, H., Shi, P.-Y., (2021). Ubiquitination of SARS-CoV-2 ORF7a promotes antagonism of interferon response. *Cell. Mol.*

Immunol., **18**, 746–748. https://doi.org/10.1038/s41423-020-00603-6.

101. Mu, J., Fang, Y., Yang, Q., Shu, T., Wang, A., Huang, M., Jin, L., Deng, F., et al., (2020). SARS-CoV-2 N protein antagonizes type I interferon signaling by suppressing phosphorylation and nuclear translocation of STAT1 and STAT2. *Cell Discovery*, **6**, 1–4. https://doi.org/10.1038/s41421-020-00208-3.
102. Chowdhury, F.Z., Farrar, J.D., (2013). Stat2. *JAK-STAT*, **2**, https://doi.org/10.4161/jkst.23633 e23633.
103. Rath, S., Prangley, E., Donovan, J., Demarest, K., Wingreen, N.S., Meir, Y., Korennykh, A., (2019). Concerted 2–5A-Mediated mRNA Decay and Transcription Reprogram Protein Synthesis in the dsRNA Response. *Mol. Cell.*, **75**, 1218–1228.e6. https://doi.org/10.1016/j.molcel.2019.07.027.
104. Schwerk, J., Soveg, F.W., Ryan, A.P., Thomas, K.R., Hatfield, L.D., Ozarkar, S., Forero, A., Kell, A.M., et al., (2019). RNA-binding protein isoforms ZAP-S and ZAP-L have distinct antiviral and immune resolution functions. *Nature Immunol.*, **20**, 1610–1620. https://doi.org/10.1038/s41590-019-0527-6.
105. Yount, J.S., Karssemeijer, R.A., Hang, H.C., (2012). S-Palmitoylation and Ubiquitination Differentially Regulate Interferon-induced Transmembrane Protein 3 (IFITM3)-mediated Resistance to Influenza Virus*. *J. Biol. Chem.*, **287**, 19631–19641. https://doi.org/10.1074/jbc.M112.362095.
106. Rivas, H.G., Schmaling, S.K., Gaglia, M.M., (2016). Shutoff of Host Gene Expression in Influenza A Virus and Herpesviruses: Similar Mechanisms and Common Themes. *Viruses*, **8**, 102. https://doi.org/10.3390/v8040102.
107. Lin, J., Tang, C., Wei, H., Du, B., Chen, C., Wang, M., Zhou, Y., Yu, M., et al., (2021). Genomic monitoring of SARS-CoV-2 uncovers an Nsp1 deletion variant that modulates type I interferon response. *Cell Host Microbe*, **29**, 489–502.e8. https://doi.org/10.1016/j.chom.2021.01.015.
108. Zhang, K., Miorin, L., Makio, T., Dehghan, I., Gao, S., Xie, Y., Zhong, H., Esparza, M., et al., (2021). Nsp1 protein of SARS-CoV-2 disrupts the mRNA export machinery to inhibit host gene expression, Science. *Advances.*, **7**, eabe7386. https://doi.org/10.1126/sciadv.abe7386.
109. Hsu, J.C.-C., Laurent-Rolle, M., Pawlak, J.B., Wilen, C.B., Cresswell, P., (2021). Translational shutdown and evasion of the innate immune response by SARS-CoV-2 NSP14 protein. *PNAS*, **118** https://doi.org/10.1073/pnas.2101161118.
110. Bouvet, M., Imbert, I., Subissi, L., Gluais, L., Canard, B., Decroly, E., (2012). RNA 3'-end mismatch excision by the severe acute respiratory syndrome coronavirus nonstructural protein nsp10/nsp14 exoribonuclease complex. *PNAS*, **109**, 9372–9377.
111. Faoro, C., Ataide, S.F., (2021). Noncanonical Functions and Cellular Dynamics of the Mammalian Signal Recognition Particle Components. *Front. Mol. Biosci.*, **8** https://doi.org/10.3389/fmolb.2021.679584.
112. Khan, M.A., Goila-Gaur, R., Opi, S., Miyagi, E., Takeuchi, H., Kao, S., Strebel, K., (2007). Analysis of the contribution of cellular and viral RNA to the packaging of APOBEC3G into HIV-1 virions. *Retrovirology*, **4**, 48. https://doi.org/10.1186/1742-4690-4-48.
113. Wang, T., Zhang, W., Tian, C., Liu, B., Yu, Y., Ding, L., Spearman, P., Yu, X.-F., (2008). Distinct viral determinants for the packaging of human cytidine deaminases APOBEC3G and APOBEC3C. *Virology*, **377**, 71–79. https://doi.org/10.1016/j.virol.2008.04.012.
114. Lin, X., Fu, B., Yin, S., Li, Z., Liu, H., Zhang, H., Xing, N., Wang, Y., et al., (2021). ORF8 contributes to cytokine storm during SARS-CoV-2 infection by activating IL-17 pathway. *IScience*, **24**, https://doi.org/10.1016/j.isci.2021.102293 102293.
115. Ferreira, A.C., Soares, V.C., de Azevedo-Quintanilha, I.G., Dias, S. da S.G., Fintelman-Rodrigues, N., Sacramento, C.Q., Mattos, M., de Freitas, C.S., et al., (2021). SARS-CoV-2 engages inflammasome and pyroptosis in human primary monocytes. *Cell Death Discov.*, **7**, 1–12. https://doi.org/10.1038/s41420-021-00428-w.
116. Siu, K.-L., Yuen, K.-S., Castano-Rodriguez, C., Ye, Z.-W., Yeung, M.-L., Fung, S.-Y., Yuan, S., Chan, C.-P., et al., (2019). Severe acute respiratory syndrome Coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. *FASEB J.*, **33**, 8865–8877. https://doi.org/10.1096/fj.201802418R.
117. Rodrigues, T.S., de Sá, K.S.G., Ishimoto, A.Y., Becerra, A., Oliveira, S., Almeida, L., Gonçalves, A.V., Perucello, D.B., et al., (2021). 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.
118. Finkel, Y., Mizrahi, O., Nachshon, A., Weingarten-Gabbay, S., Morgenstern, D., Yahalom-Ronen, Y., Tamir, H., Achdout, H., et al., (2021). The coding capacity of SARS-CoV-2. *Nature*, **589**, 125–130. https://doi.org/10.1038/s41586-020-2739-1.
119. Stanifer, M.L., Pervolaraki, K., Boulant, S., (2019). Differential Regulation of Type I and Type III Interferon Signaling. *Int. J. Mol. Sci.*, **20**, 1445. https://doi.org/10.3390/ijms20061445.
120. Tiwari, S.K., Wang, S., Smith, D., Carlin, A.F., Rana, T.M., (2021). Revealing Tissue-Specific SARS-CoV-2 Infection and Host Responses using Human Stem Cell-Derived Lung and Cerebral Organoids. *Stem Cell Rep.*, **16**, 437–445. https://doi.org/10.1016/j.stemcr.2021.02.005.
121. Fiege, J.K., Thiede, J.M., Nanda, H.A., Matchett, W.E., Moore, P.J., Montanari, N.R., Thielen, B.K., Daniel, J., et al., (2021). Single cell resolution of SARS-CoV-2 tropism, antiviral responses, and susceptibility to therapies in primary human airway epithelium. *PLoS Pathog.*, **17**, https://doi.org/10.1371/journal.ppat.1009292 e1009292.
122. Muñoz-Fontela, C., Dowling, W.E., Funnell, S.G.P., Gsell, P.-S., Riveros-Balta, A.X., Albrecht, R.A., Andersen, H., Baric, R.S., et al., (2020). Animal models for COVID-19. *Nature*, **586**, 509–515. https://doi.org/10.1038/s41586-020-2787-6.