JANNE TYNELL

Virus-Host Interactions of Emerging Respiratory Pathogens
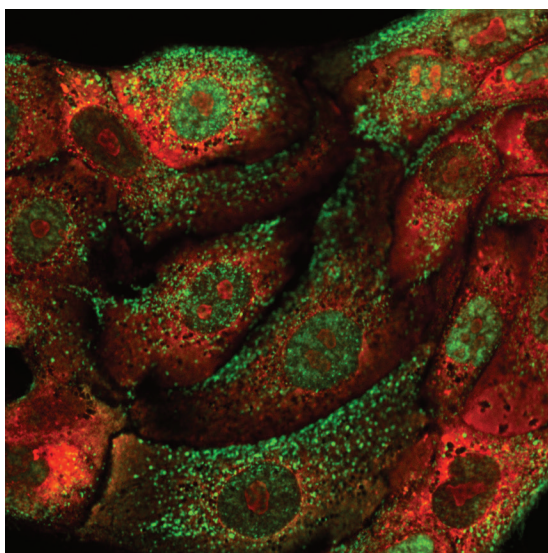
NATIONAL INSTITUTE FOR HEALTH AND WELFARE AND
FACULTY OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES
DOCTORAL PROGRAMME IN INTEGRATIVE LIFE SCIENCE
UNIVERSITY OF HELSINKI

Virus-host interactions of emerging respiratory pathogens

Janne Tynell

Viral Infections Unit, Department of Infectious Diseases

National Institute for Health and Welfare

and

Division of General Microbiology, Department of Biosciences

Faculty of Biological and Environmental Sciences

University of Helsinki

and

Doctoral Programme in Integrative Life Science

University of Helsinki

ACADEMIC DISSERTATION

Cover photo: Intracellular localization of influenza A NS1 and NP proteins within A/Udorn/72 –infected MDCK cells

Supervisors
Krister Melén, PhD
National Institute for Health and Welfare
Helsinki, Finland

Professor Ilkka Julkunen, MD, PhD
National Institute for Health and Welfare
Helsinki, Finland
and
Department of Virology
University of Turku, Finland

Thesis Committee members
Professor Olli Vapalahti, MD
Department of Virology, Haartman Institute
University of Helsinki, Finland

Docent Tero Ahola, PhD
Department of Food and Environmental Sciences
University of Helsinki, Finland

Reviewers
Docent Anita Huovilainen, PhD
Finnish Food Safety Authority Evira
Helsinki, Finland

Professor Ari Hinkkanen, PhD
Department of Biotechnology and Molecular Medicine
University of Eastern Finland, Kuopio, Finland

Custos
Professor Sarah Butcher, PhD
Institute of Biotechnology
University of Helsinki, Finland

Opponent
Docent Maija Vihinen-Ranta, PhD
Department of Biological and Environmental Science and Nanoscience Center
University of Jyväskylä, Finland

Dedicated to Anniina

Abstract

Respiratory virus infections are a major cause of morbidity and mortality worldwide. Decades of research have yielded many breakthroughs in our understanding of virus-host interactions, but many aspects of viral pathogenesis still remain unresolved. Vaccines and antiviral treatments have been developed, but they are imperfect or completely lacking for many viral agents. Novel emerging viruses form an additional challenge, which can only be overcome by proper preparation and quick response against these unexpected infectious threats. Understanding virus-host interactions is critical for elucidating aspects of viral pathogenesis and devising better treatment strategies against viral pathogens.

Our research has focused on the virus-host interactions of two major respiratory pathogens with a recent history of outbreaks by a novel viral agent, the influenza A virus and coronavirus. Influenza A virus has plagued humankind throughout human history and continues to cause annual epidemics and occasional pandemics associated with significant mortality. NS1 protein is one of the major virulence factors of influenza A virus. It has a multitude of different interactions with host cell components that either aid viral replication or hamper the antiviral response exhibited by the host cell. These interactions are located both in the host cell nucleus and the cytoplasm, and three signals regulating the intracellular localization of NS1 protein have been identified. While the critical impact of nuclear localization signal 1 is well known, the other two localization signals have remained poorly characterized.

In this work we provide a detailed description of the NS1 nuclear export signal (NES), showing that the NES region is well conserved within different influenza A strains and that certain mutations within the region cause attenuation of virus replication. Through the use of different mutant viruses, we show that the attenuated phenotype is not due to impaired localization alone, but rather involves defects in the functions of NS1. We also show that NS1 is not exported through the classical CRM1-dependent pathway and we establish the nucleolar proteins that bind NS1 and thus guide its nucleolar localization.

Human coronaviruses are a major cause of the common cold. For a long time coronaviruses were thought to cause only mild upper respiratory tract infections in humans. However, this view changed with the emergence of the highly lethal SARS coronavirus in 2002 and the identification of MERS coronavirus in 2012. While the SARS outbreak was efficiently contained, MERS coronavirus continues to circulate in camels and causes repeated introductions into the human population in the Middle East. Our MERS coronavirus research concentrated on characterizing MERS

infection of human macrophages and dendritic cells, two important cell types of the innate immune system. We show that MERS coronavirus does not replicate productively in these leucocytes, but a significant innate immune response is generated.

Altogether this work identifies important aspects of virus-host interaction of two important respiratory pathogens. We provide new information on the mechanisms and impact of influenza A virus NS1 intracellular localization and we characterize MERS coronavirus infection in primary human leucocytes as well as highlight important differences in the host cell responses between MERS and SARS coronaviruses.

Keywords: Influenza A virus, NS1, intracellular localization, MERS coronavirus, macrophage, dendritic cell

Tiivistelmä

Hengitystievirusten aiheuttamat infektiot ovat maailmanlaajuisesti merkittävä sairastavuutta ja kuolleisuutta aiheuttava haaste kansanterveydelle. Vuosikymmenten tutkimustyö on tuottanut lukuisia läpimurtoja hengitystievirusinfektioiden mekanismien tuntemuksessa, mutta monet virusten taudinaiheutuskyvyn piirteet ovat yhä epäselviä. Rokotusten ja antiviraaliyhdisteiden kehitykseen suunnatut ponnistelut ovat tuottaneet lukuisia rokotteita ja lääkkeitä hengitystieviruksia vastaan, mutta ne ovat usein suojausteholtaan puutteellisia tai puuttuvat kokonaan tiettyjä virusryhmiä vastaan. Erilaisista eläinperäisistä lähteistä ilmaantuvat uudenlaiset virukset muodostavat erityisen uhkan, johon varautuminen edellyttää valmistautumista sekä valmiutta nopeisiin vastatoimiin. Viruksen ja isäntäsolun välisten vuorovaikutusten ymmärtäminen on keskeisessä asemassa kehitettäessä uusia parempia hoitomenetelmiä sekä valmistauduttaessa uusiin virusuhkiin.

Viruksen ja isäntäsolun vuorovaikutuksiin keskittyvä tutkimuksemme käsittelee kahta merkittävää hengitystievirusta, influenssa A -virusta sekä MERS-koronavirusta. Jo antiikin ajoista lähtien ihmiskunnan vitsauksena ollut influenssa A -virus aiheuttaa vuosittaisia talviaikaan sijoittuvia epidemioita sekä ajoittaisia maailmanlaajuisia pandemioita. NS1-proteiini on merkittävä influenssa A -viruksen virulenssitekijä, jolla on lukuisia infektiota edistäviä tai isäntäsolun immuunivastetta estäviä vuorovaikutuksia isäntäsolun proteiinien kanssa. Nämä vuorovaikutukset sijoittuvat sekä tumaan että solulimaan. NS1-proteiinista on tunnistettu kolme solunsisäistä paikantumista ohjaavaa signaalia, joista yhden tiedetään olevan tärkeä virusinfektiolle, mutta kahden muun merkitys on jäänyt epäselväksi.

Tässä työssä luomme seikkaperäisen kuvauksen NS1-proteiinin tumasta solulimaan kulkeutumisen välittävän signaalin toiminnasta. Näytämme että kyseinen paikantumissignaali on hyvin säilynyt eri viruskantojen välillä ja osoitamme, että tietyt signaaliin kohdistuvat mutaatiot aiheuttavat viruksen replikaatiokyvyn huomattavan heikentymisen. Osoitamme erilaisten mutanttivirusten avulla, että heikentynyt fenotyyppi ei johdu ainoastaan NS1-proteiinin virheellisestä paikantumisesta, vaan aiheutuu NS1-proteiinin toiminnallisista vioista. Osoitamme myös, että NS1-proteiinin kulkeutuminen tumasta solulimaan ei ole CRM1-välitteinen, sekä tunnistamme NS1-proteiinin kanssa vuorovaikuttavat tumajyväsproteiinit.

Koronavirukset tunnetaan merkittävinä flunssan aiheuttajina ja niitä pidettiin pitkään lähinnä lievien hengitystieinfektioiden aiheuttajina ihmisissä, mutta tämä näkemys on muuttunut merkittävää kuolleisuutta aiheuttaneiden SARS- ja MERS-

epidemioiden myötä. SARS-koronavirus ilmaantui vuonna 2002 ja saatiin nopeasti taltutettua, mutta vuonna 2012 havaittu MERS-koronavirus kiertää yhä kameleissa aiheuttaen toistuvia ihmisinfektioita Lähi-Idässä. MERS-koronavirusta käsittelevä osa työstämme keskittyi analysoimaan MERS-koronaviruksen vuorovaikutusta ihmisen makrofagien ja dendriittisolujen kanssa. Osoitamme, että MERS-koronavirusinfektio ei johda merkittävään virusreplikaatioon näissä soluissa, mutta huomattava immuunivaste syntyy siitä huolimatta.

Kaiken kaikkiaan työmme tuo ilmi tärkeitä piirteitä näiden merkittävien hengitystievirusten vuorovaikutussuhteista isäntäsolujen kanssa. Tuotamme uutta tietoa influenssa A -viruksen NS1-proteiinin solunsisäisen paikantumisen mekanismeista ja merkityksestä sekä analysoimme MERS-koronavirusinfektion piirteet ihmisen valkosoluissa korostaen samalla MERS- ja SARS-koronavirusten välisiä eroja.

Avainsanat: Influenssa A -virus, NS1-proteiini, solunsisäinen paikantuminen, MERS-koronavirus, makrofagi, dendriittisolu

Contents

List of original papers

I   Tynell, J., K. Melen and I. Julkunen. Mutations within the conserved NS1 nuclear export signal lead to inhibition of influenza A virus replication. Virol J. 2014 Jul 14;11:128, PMID: 25023993

JT, KM and IJ designed the study. JT generated the recombinant viruses, performed most of the experiments and analyzed the results. JT and IJ wrote the manuscript.

II   Melen, K., J. Tynell, R. Fagerlund, P. Roussel, D. Hernandez-Verdun and I. Julkunen. Influenza A H3N2 subtype virus NS1 protein targets into the nucleus and binds primarily via its C-terminal NLS2/NoLS to nucleolin and fibrillarin. Virol. J. 2012 Aug 21;9:167. PMID: 22909121.

JT participated in the design of the study, performed immunofluorescence experiments, analyzed the results and contributed to the writing of the manuscript. KM and IJ wrote the manuscript.

III Tynell, J., V. Westenius, E. Rönkkö, V. J. Munster, K. Melén, P. Österlund, I. Julkunen. Middle East respiratory syndrome coronavirus (MERS-CoV) shows poor replication but significant induction of antiviral responses in human monocyte-derived macrophages and dendritic cells. J Gen Virol. 2016 Feb;97(2):344-55. PMID: 26602089.

JT, PÖ and IJ designed the study. JT performed most of the experiments and analyzed the results. JT and IJ wrote the manuscript.

These articles are reproduced with the kind permission of their copyright holders.

Abbreviations

ACE2	angiotensin converting enzyme 2
ADAR	adenosine deaminase acting on RNA
ARDS	acute respiratory distress syndrome
CPSF	cleavage and polyadenylation specificity factor
CRM1	chromosome region maintenance 1 protein
CXCL10	C-X-C motif ligand 10
DC	dendritic cell
DPP4	dipeptidyl peptidase 4
dsRNA	double-stranded RNA
FCS	fetal calf serum
FRET	fluorescence (or Förster) resonance energy transfer
GST	glutathione S-transferase
GFP	green fluorescent protein
IFN	interferon
IKK	inhibitory κB kinase
IL	interleukin
IRF	interferon regulatory transcription factor
kDa	kilodalton
LMB	leptomycin B
MARCO	macrophage receptor with collagenous structure
MDCK	Madin Darby canine kidney

MDDC	monocyte-derived dendritic cell
MDM2	mouse double minute 2 homolog
MERS-CoV	Middle East respiratory syndrome coronavirus
MOI	multiplicity of infection
mRNA	messenger RNA
MxA	myxovirus resistance gene product
NEP	nuclear export protein
NES	nuclear export signal
NF-κB	nuclear factor kappa-B
NLS	nuclear localization signal
NoLS	nucleolar localization signal
NP	nucleoprotein
NS1	non-structural protein 1
ORF	open reading frame
PABII	poly-A binding protein II
PAF1	polymerase associated factor 1
pDC	plasmacytoid dendritic cell
PgD	prostaglandin
PI3K	phosphoinositide 3-kinase
PKR	protein kinase R
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RdRp	RNA-dependent RNA polymerase

RIG-I	retinoic acid-inducible gene I
RSV	respiratory syncytial virus
SARS-CoV	Severe acute respiratory syndrome coronavirus
TGF-β	transforming growth factor beta
Th	T helper cell
TLR	Toll-like receptor
TMPRSS2	transmembrane protease, serine 2
TNF-α	tumor necrosis factor alfa
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand
TRIM25	tripartite motif-containing protein 25

1 Review of the literature

1.1 Lungs as an interface for host-pathogen interaction

Because of its role as an organ of gas exchange, the lungs become regularly in direct interaction with the outside environment. Every day up to 9000 liters of air go through the lungs, providing a constant challenge of airborne particles, allergens and microbes (Kopf, Schneider & Nobs 2015). Appropriate responses to these different challenges are vital to the proper functioning of the lungs. Inappropriate responses against harmless agents can lead to severe complications like asthma, but at the same time invading pathogens must be stopped to maintain the integrity of the lungs. Various mechanisms of the innate and adaptive immune systems have evolved to protect the lungs against environmental challenges.

A mechanical barrier consisting of the tightly junctioned lung epithelium and mucus secreted by goblet cells and other epithelial cells represents the simplest pulmonary defense against respiratory pathogens and harmful particles (Whitsett, Alenghat 2015). Mucins comprising the gel-like mucus entangle microbes and prevent their adhesion to the epithelial cell surface (Voynow, Rubin 2009). The action of cilia on the epithelial cell surfaces moves the mucus towards the upper respiratory tract and thus works to remove particles and microbes from the lungs. The mucins themselves also have direct antimicrobial properties. Lung epithelial cells also secrete various kinds of antimicrobial peptides, defensins and surfactants and other host-defense proteins and influence the function of pulmonary macrophages and dendritic cells through cytokine production (Whitsett, Wert & Weaver 2010, Tecle, Tripathi & Hartshorn 2010).

Macrophages and dendritic cells (DCs) are key constituents of the host immune system. Macrophages reside in tissues throughout the body and often act as first responders to microbial invasion (Gordon 2002). Besides their ability to phagocytose microbes and infected cells, macrophages also regulate inflammatory responses through production of inflammatory cytokines. DCs are antigen presenting cells and act as initiators and regulators of the adaptive immune response (Mellman, Steinman 2001). They form an important link between the innate and adaptive immune systems. In the lungs macrophages and dendritic cells are located near the epithelium and constantly sample the airway lumen for foreign material (Kopf, Schneider & Nobs 2015). Pulmonary macrophages and DCs form an interplay of interactions with each other and with the alveolar epithelial cells to regulate homeostasis and inflammatory responses against invading pathogens.

1.1.1 Lung-resident macrophages

Lung-resident macrophages can be grouped into interstitial macrophages, bronchial macrophages and alveolar macrophages (Kopf, Schneider & Nobs 2015). Interstitial macrophages reside in the space between alveoli, where they interact with DCs and present antigens to interstitial T cells. Alveolar macrophages are located in the lumen of the alveoli attached to alveolar epithelial cells. A major function of alveolar macrophages is inhibiting inflammatory responses in the lung (Holt et al. 1993). This is achieved by inhibition of T cell activation by DCs and secretion of TGF-β, which induces suppression of Th2 response by regulatory T cells (Josefowicz et al. 2012). Alveolar macrophages are also major producers of type I interferons (Kumagai et al. 2007). Close contact and interaction with alveolar epithelial cells is important in regulating the functions of alveolar macrophages (Westphalen et al. 2014). Integrins expressed on the surface of alveolar epithelial cells are essential for the activation of TGF-β secreted by alveolar macrophages (Munger et al. 1999). Alveolar epithelial cells also express CD200, which inhibits TLR activation in alveolar macrophages (Snelgrove et al. 2008). Activation of Toll-like receptors in response to pathogen associated molecular patterns causes detachment of alveolar macrophages from the epithelium, resulting in waning of the suppressive properties of the macrophages and activation of inflammatory responses (Soroosh et al. 2013). The suppressive functions of alveolar macrophages are also important in restoring the normal state after clearance of infection, thus avoiding extensive immunopathological damage resulting from prolonged inflammation. Clearance of apoptotic cells is also an important function of alveolar macrophages during infection and has been shown to be critical in the host defence against influenza A virus infection (Hashimoto et al. 2007, Watanabe et al. 2005).

1.1.2 Lung-resident dendritic cells

The lung DC population consists of plasmacytoid dendritic cells (pDCs), monocyte-derived dendritic cells (MDDCs) and conventional dendritic cells (cDCs), which can be further divided into CD11b+ DCs and CD103+ DCs based on integrin expression (Kopf, Schneider & Nobs 2015). Lung DCs are mainly localized just next to the epithelium on the basolateral side, where they sample the airway lumen with their tendril-like protrusions. MDDCs are known to stimulate Th1 responses in infected lung tissue and are potent inducers of pro-inflammatory cytokines (Iijima, Mattei & Iwasaki 2011). Plasmacytoid DCs have a role in preventing unnecessary responses to harmless inhaled antigens, can act as antigen presenting cells and are known as very strong producers of type I interferons in response to virus infection (de Heer et al. 2004, Colonna, Trinchieri & Liu 2004). Conventional DCs and especially CD103+ DCs are important in activating T cells through antigen presentation (del Rio et al. 2007). Upon phagocytosis, processing of antigen and maturation, DCs migrate into the draining lymph nodes and present antigens to CD8+ T cells

(Mellman, Steinman 2001). CD103+ DCs favor induction of effector CD8+ T cells, whereas CD11b+ DCs seem to favor induction of memory CD8+ T cells because of differential CD24 expression (Kim et al. 2014).

1.2 Respiratory viruses

According to WHO, lower respiratory tract infections cause 3.1 million deaths each year, thus accounting for 5.5% of all annual deaths (WHO, Fact sheet N°310, 2014). Bacterial pathogens, especially Streptococcus pneumoniae, are the leading cause of community-acquired pneumonia, but a third of adult pneumonia cases are estimated to be caused by viruses (File 2003, Ruuskanen et al. 2011). Influenza viruses, respiratory syncytial virus, rhinoviruses, human metapneumovirus and coronaviruses are the most common viral causes of pneumonia and altogether they account for roughly 200 million viral pneumonia cases each year (Ruuskanen et al. 2011). Influenza viruses are one of the most important respiratory pathogens due to annual epidemics resulting in up to 500 000 deaths and up to five million severe infections each year (WHO, Fact sheet N°211, 2014). In addition, influenza A virus causes occasional pandemics with potentially very high mortality (Elderfield, Barclay 2011). The most lethal pandemic to date was the Spanish flu pandemic of 1918, causing up to 50 million casualties (Taubenberger, Morens 2006). Other influenza A pandemics of the last century include the Asian pandemic of 1957, the Hong Kong pandemic of 1968 and the recent swine flu pandemic of 2009 (Elderfield, Barclay 2011). Vaccines and antiviral drugs are available against influenza viruses, but otherwise only supportive treatment is mostly available against viral pneumonia (Ruuskanen et al. 2011).

Besides a hefty death toll, respiratory infections inflict a large economic burden on the society through lost working hours and increasing health care costs. One study estimated the annual cost of community-acquired pneumonia in the United States to be as high as 17 billion dollars (File, Marrie 2010). Adults are estimated to suffer 2-4 common colds each year leading to millions of lost working days in the developed countries (Heikkinen, Jarvinen 2003). Rhinoviruses are the most common cause of the common cold with an estimated proportion of 30-50 % of all cases (Heikkinen, Jarvinen 2003). Coronaviruses and influenza viruses are also common causes with proportions of 10-15 % and 5-15 %, respectively. Other causative agents of the common cold include respiratory syncytial virus, metapneumovirus, parainfluenza viruses, adenoviruses and enteroviruses (Heikkinen, Jarvinen 2003). Rhinoviruses alone harbor a diversity of over 160 different serotypes and thus the sheer number of different causative agents makes the concept of a cure for the common cold an unrealistic notion. There are however various kinds of treatments available for the different symptoms associated with the common cold (Heikkinen, Jarvinen 2003).

1.3 Influenza viruses

Influenza viruses belong to the Orthomyxoviridae family and are divided into three genera of influenza A, influenza B and influenza C viruses (Fields Virology 2012). Influenza A viruses are further classified into subtypes according to the antigenic properties of the surface glycoproteins hemagglutinin and neuraminidase. Currently 18 classes of hemagglutinin and 11 classes of neuraminidase are known (Tong et al. 2012, Tong et al. 2013). Influenza B viruses are divided into Victoria-like and Yamagata-like lineages based on the phylogeny of hemagglutinin and neuraminidase genes. Influenza viruses are enveloped RNA viruses harboring a segmented single-stranded negative sense RNA genome of approximately 10-13 kb in size (Fields Virology 2012). They code for up to 16 proteins depending on the genus and specific strain (Chen et al. 2001, Wise et al. 2009, Jagger et al. 2012, Muramoto et al. 2013). Replication of influenza viruses takes place inside the host cell nucleus (Herz et al. 1981, Jackson et al. 1982). The segmented genome of influenza viruses enables genetic reassortment between different viral strains, allowing efficient exchange of large amounts of genetic information.

Influenza C virus infects pigs and humans and causes mild upper respiratory tract infections (Guo et al. 1983). Influenza B virus has been isolated from seals in addition to humans and causes a similar disease as influenza A virus, characterized as an acute viral infection ranging from mild upper respiratory tract infections to severe viral pneumonia (Osterhaus et al. 2000). Influenza A virus infects birds, pigs, horses and other mammals in addition to humans (Yoon, Webby & Webster 2014). Wild aquatic birds are considered the natural reservoir of influenza A virus (Yoon, Webby & Webster 2014). In wild birds influenza A virus infects the respiratory and intestinal tracts and is typically asymptomatic, but outbreaks causing high mortality and economic losses in domestic poultry occur regularly (Franca, Brown 2014). Highly pathogenic avian strains are characterized by an insertion of basic amino acids in the hemagglutinin cleavage site, allowing cleavage by furin-like proteases and systemic spread of the infection in the host (Bottcher-Friebertshauser et al. 2014). Avian influenza A strains bind to α2,3-linked sialic acid receptors as opposed to the α2,6-linked sialic acid receptors used by human strains (Rogers, Paulson 1983). Human lungs contain α2,3-linked sialic acid receptors only in the lower respiratory tract, resulting in poor transmissibility of avian strains to humans but severe disease in the case of a successful transmission (Shinya et al. 2006). Pandemic influenza A strains arise from genetic reassortment between avian, human and/or swine strains, and pigs are thought to function as mixing vessels for pandemic strains, since they readily express both α2,3-linked and α2,6-linked sialic acid receptors in their airways (Elderfield, Barclay 2011). Currently two influenza A subtypes, H1N1 and H3N2, circulate in the human population causing annual

epidemics. Also avian subtypes H5N1 and H7N9 continue to circulate in poultry and cause outbreaks in humans.

1.3.1 Pathogenesis of influenza A virus

The clinical picture of influenza A varies from a mild upper respiratory tract infection to severe viral pneumonia. Common symptoms include sudden development of high fever, headache, cough, fatigue and malaise (Taubenberger, Morens 2008). Severe cases are associated with bronchitis, diffuse alveolar damage and pneumonia leading to acute respiratory distress syndrome (ARDS) (Taubenberger, Morens 2008). Death occurs due to respiratory and/or multiorgan failure. Fatal cases are often associated with secondary bacterial infection, especially Streptococcus pneumoniae -induced pneumonia (Madhi, Klugman & Vaccine Trialist Group 2004, Morens, Taubenberger & Fauci 2008). Young children and the elderly are particularly susceptible to severe influenza and many underlying medical conditions are also known to predispose to an adverse outcome (Thompson et al. 2003, Thompson et al. 2004). Several different influenza A virus-specific antiviral drugs and biannually updated vaccines against seasonal influenza are available as treatment and prevention options (Tsiodras, Nikolopoulos & Bonovas 2012, Fiore, Bridges & Cox 2009).

Influenza A virus can infect epithelial cells throughout the lungs, but severe infections are especially associated with infection of pneumocytes of the alveolar epithelium (Shinya et al. 2006, Taubenberger, Morens 2008). The more abundant type I pneumocytes express predominantly the α2,6-linked sialic acid receptors, whereas type II pneumocytes express the α2,3-linked sialic acid receptors (Shinya et al. 2006, van Riel et al. 2006, van Riel et al. 2007). Thus, the targeted cell type in the alveolus depends on the virus strain. Infection of the alveolar pneumocytes by influenza A virus damages the epithelium through virus-induced apoptosis or necrosis and causes production of proinflammatory cytokines, which results in early recruitment of neutrophils and monocytes to the alveolar lumen (Perrone et al. 2008, Short et al. 2014). Neuthrophils and monocyte-derived macrophages produce more proinflammatory cytokines further enhancing the inflammatory response and recruiting even more neutrophils and monocytes into the alveoli (Short et al. 2014). Neutrophils also produce reactive oxygen species and neutrophil extracellular traps, which damage and trap invading pathogens, but also have cytotoxic properties (Narasaraju et al. 2011, Amulic et al. 2012). Macrophages induce the formation of cytotoxic nitric oxide and peroxynitrite compounds through expression of nitric oxide synthase and induce apoptosis of alveolar epithelial cells by expressing tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) (Fang 2004, Herold et al. 2008). Extensive tissue damage resulting from the actions of neutrophils and macrophages is a major contributor to the development of ARDS

during influenza A virus infection (Short et al. 2014). Magnitude of the host inflammatory response and neutrophil infiltration into the lungs have been found to be better indicators for the severity of disease than virus-induced tissue damage (Narasaraju et al. 2011, Brandes et al. 2013, Weiland et al. 1986). This excessive inflammatory response during influenza A infection has been termed 'cytokine storm' and is especially prominent in human infections with highly pathogenic avian strains (Cheung et al. 2002, Yuen, Wong 2005). Consistent with the immunopathologic features of influenza A virus infection, depletion of the immunosuppressive alveolar macrophages results in a fatal infection even with a low viral dose (Schneider et al. 2014). Infection of alveolar macrophages by influenza A virus has also been shown and might contribute to pathogenesis (van Riel et al. 2011). Despite the apparent detrimental effects imposed by the host innate immune responses, immunosuppressive compounds alone do not protect mice from lethal H5N1 infection and also depletion of neutrophils worsens rather than improves the disease outcome (Salomon, Hoffmann & Webster 2007, Tate et al. 2011).

Secondary bacterial infections are present in 30-90% of fatal influenza cases and bacterial pneumonia is a major cause of death of influenza patients (Mina, Klugman 2014). Several influenza-induced effects have been identified that promote bacterial invasion (Mina, Klugman 2014). A major synergistic contributor seems to be excessive type I interferon production during co-infection (Shahangian et al. 2009). Type I interferons block Nod2-mediated recruitment of monocytes, reduce type 17 T-helper cell polarization and inhibit CXCL1 and MIP-2 production (Nakamura, Davis & Weiser 2011, Kudva et al. 2011, Li, Moltedo & Moran 2012). Other synergistic aberrant immunomodulary effects include increased productions of IL-10, IL-12 and IFN-γ, which cause inhibition of neutrophil recruitment and reduce the expression of MARCO in alveolar macrophages (van der Sluijs et al. 2004, Sun, Metzger 2008). Influenza A infection also enhances bacterial carriage density and duration and has several other local and systemic effects that might contribute to secondary bacterial infection (Mina, Klugman 2014).

Several influenza A viral factors have been identified as contributors towards severe disease. Most notable of these is the surface protein hemagglutinin (HA), which determines receptor specificity and is the major antigenic determinant and a decisive factor in the division of avian strains into highly pathogenic and low pathogenic strains based on the presence of a polybasic insertion near the HA cleavage site (Bottcher-Friebertshauser et al. 2014). The viral neuraminidase is indicated in enhancing bacterial adherence and replication through cleavage of sialic acids and exposure of bacterial receptors on the lung epithelium (McCullers, Bartmess 2003). Similarly, the influenza A virus protein PB1-F2 promotes viral pathogenesis and also contributes to secondary bacterial infection (McAuley et al. 2007). Other

established influenza A virus virulence factors include proteins of the polymerase complex and the non-structural protein 1 (NS1), which modulates a multitude of different host cell functions (Gabriel, Fodor 2014, Hale et al. 2008).

1.3.2 NS1, a multifunctional antagonist of innate immune responses

NS1 is considered one of the major virulence factors of influenza A due to its function as an antagonist of host innate immune responses (Hale et al. 2008, Garcia-Sastre et al. 1998). NS1 prevents interferon production by the host cell by several mechanisms. Inhibition of mRNA processing and transport by NS1 through interaction with CPSF30 and PABII in the nucleus causes a general inhibition of all host cell protein expression (Nemeroff et al. 1998, Chen, Li & Krug 1999). NS1 interaction with TRIM25 blocks the activation of the cytoplasmic RNA sensor RIG-I (Gack et al. 2009). Other suggested targets for NS1 interaction within the RIG-I pathway include RIG-I itself and the ubiquitin E3 ligase Riplet, but these interactions might only represent co-binding to RNA as indicated by the requirement of a functional NS1 RNA-binding motif for these interactions (Pichlmair et al. 2006, Mibayashi et al. 2007, Rajsbaum et al. 2012). NS1 inhibits IKK-mediated NF-κB activation through interaction with IKK (Gao et al. 2012). NS1 also inhibits the action of IFN-induced antiviral proteins PKR and 2'-5'-oligoadenylate synthetase by direct interaction and sequestration of dsRNA, respectively (Min, Krug 2006, Min et al. 2007).

Another recognized function of NS1 is activation of the PI3K pathway through binding to the p85β subunit of PI3K (Hale et al. 2006, Ehrhardt et al. 2006, Shin et al. 2007). PI3K is involved in many cellular processes, but activation of the downstream effector Akt and its suppressive function on apoptosis are thought to be the main target of NS1 PI3K activation (Ehrhardt et al. 2007). Direct binding of NS1 to Akt has also been proposed (Matsuda et al. 2010). In addition, NS1 inhibits apoptosis by direct interaction and inhibition of the tumor suppressor p53 and by inhibition of Jun N-terminal kinase, which is involved in death receptor-mediated apoptosis signaling (Ludwig et al. 2002, Terrier et al. 2013, Wang et al. 2010). In some experimental settings NS1 has been found to promote apoptosis, but this has not been verified in the context of an infection (Lam et al. 2008). Besides the anti-apoptotic functions of NS1, several influenza A proteins are known to induce host cell apoptosis (Chen et al. 2001, Wang et al. 2012). Thus, the control of apoptosis during influenza A infection appears to be a complex process probably leading to different outcomes with different virus strains and in different hosts.

NS1 also has numerous other interactions with host cell proteins. NS1 promotes virus protein translation through interactions with viral mRNA, hStaufen and with

translation initiation factors eIF4GI and PABI (Park, Katze 1995, Falcon et al. 1999, Aragon et al. 2000, Burgui et al. 2003). NS1 affects splicing of viral mRNA through interaction with NS1-binding protein (Fortes, Beloso & Ortin 1994, Garaigorta, Ortin 2007, Tsai et al. 2013). NS1 interacts with and inhibits the host cell mRNA export machinery (Fortes, Beloso & Ortin 1994, Satterly et al. 2007). NS1 is also involved in temporal regulation of the viral infection (Min et al. 2007). NS1 targets and inhibits the human PAF1 transcription elongation complex through histone mimicry (Marazzi et al. 2012). The NS1 C-terminus of many avian strains forms a PDZ domain ligand, which allows interaction with many host proteins involved in various cellular processes, but the exact significance of these interactions for the virus infection is unclear (Obenauer et al. 2006, Jackson et al. 2008). NS1 interaction with the cellular adapter proteins Crk and CrkL further enhance NS1-mediated PI3K activation (Heikkinen et al. 2008, Ylosmaki et al. 2015).

The many functions of NS1 are critical for influenza A virus infection. Studies with recombinant viruses lacking the NS1 gene have shown severe attenuation of the virus, but normal replication in IFN deficient systems (Garcia-Sastre et al. 1998). Similar studies using mutant viruses with specific NS1 interactions knocked out have indicated important roles for at least RNA binding, CPSF30 binding, PI3K binding and PAF1 binding (Min, Krug 2006, Noah, Twu & Krug 2003, Shin et al. 2007, Marazzi et al. 2012). There is however a lot of strain and host dependent variation in the importance of any given NS1 function.

1.3.3 Structure of NS1

NS1 is a 26 kDa protein consisting of an N-terminal RNA-binding domain, a C-terminal effector domain and a short linker region connecting the two domains (Hale et al. 2008). Both domains independently form homodimers through self-association and the full length NS1 also exists as a dimer both *in vitro* and *in vivo* (Nemeroff, Qian & Krug 1995, Liu et al. 1997, Bornholdt, Prasad 2006). Dimerization is essential for certain NS1 functions and lack of NS1 dimerization causes attenuation of the virus (Ayllon et al. 2012, Wang et al. 1999). NS1 has also been shown to form oligomers in structural studies and is speculated to form a tubular structure around dsRNA to prevent activation of cellular dsRNA sensors (Bornholdt, Prasad 2008). The length of NS1 varies from 202 to 237 amino acids and shows strain specificity with certain deletions or insertions often being associated with particular host ranges (Hale et al. 2008). Examples include a five amino acid deletion in the linker region of avian H5N1 strains, the seven amino acid C-terminal extension in human strains isolated between 1950 and 1989 and the 11 amino acid C-terminal deletion found on swine origin strains (Dundon, Capua 2009, Melen et al. 2007). The NS1 C-terminal region beyond amino acid 202 has been termed the "disordered tail" due to

difficulties in obtaining a structure for this region in crystallographic studies (Hale et al. 2008). This is speculated to be a consequence of intrinsic flexibility within the region and binding to a specific ligand might be needed to obtain an ordered conformation (Hale et al. 2008).

Increasing evidence indicates that NS1 can adopt multiple different conformations to facilitate its many interactions (Hale 2014). This is made possible by the heterogeneity of the helix-helix interface of the effector domain dimer and by the flexibility of the linker region (Kerry et al. 2011, Aramini et al. 2014, Carrillo et al. 2014). A model based on positioning of individual effector domains in relation to each other at the dimeric interface divides NS1 structures into 'helix-closed' and 'helix-open' conformations depending on the availability of tryptophan 187 (Kerry et al. 2011). A similar division into 'open', 'semi-open' and 'closed' conformations can be made based on the length and flexibility of the linker region (Carrillo et al. 2014). Additional conformations are formed upon NS1 binding to CPSF30 and PI3K (Das et al. 2008, Hale et al. 2010). An alternative strand-strand interface for the effector domain dimer observed in structural studies may offer still more conformational options (Bornholdt, Prasad 2006). Thus, current evidence depicts NS1 as a dynamic multiform protein that adopts a certain conformation based on the availability of ligands, NS1 concentration and possible specific regulatory mechanisms. No such regulatory mechanisms have been proven as of yet, but post translational modifications such as phosphorylation and SUMOylation are intriguing possibilities as structural regulators (Privalsky, Penhoet 1978, Xu et al. 2011). Evidence of NS1 conformation during infection obtained through the use of an antibody specific for 'helix-open' conformation showed only nuclear localization during the first 8 hours of infection, but ubiquitous localization throughout the cell at late stages of infection, suggesting that conformational prevalence of NS1 might be controlled temporally during infection (Kerry et al. 2011).

Functional epitopes of NS1 are scattered throughout the protein and in many cases coincide with one another (Figure 1). Besides binding dsRNA, the RNA-binding domain contains a nuclear localization signal and interacts with TRIM25 (Gack et al. 2009). The effector domain contains a nuclear export signal and interacts with TRIM25, PKR, CPSF30, PABII, p85β and many other host proteins (Hale et al. 2008). There is a lot of NS1 sequence variation between strains and based on that variation NS1 proteins can be divided into alleles A and B, with most avian viruses grouping into allele B and human viruses into allele A (Treanor et al. 1989, Ludwig et al. 1991). Some of the regions of NS1 are more conserved than others and thus are thought to be structurally important (Darapaneni et al. 2009).
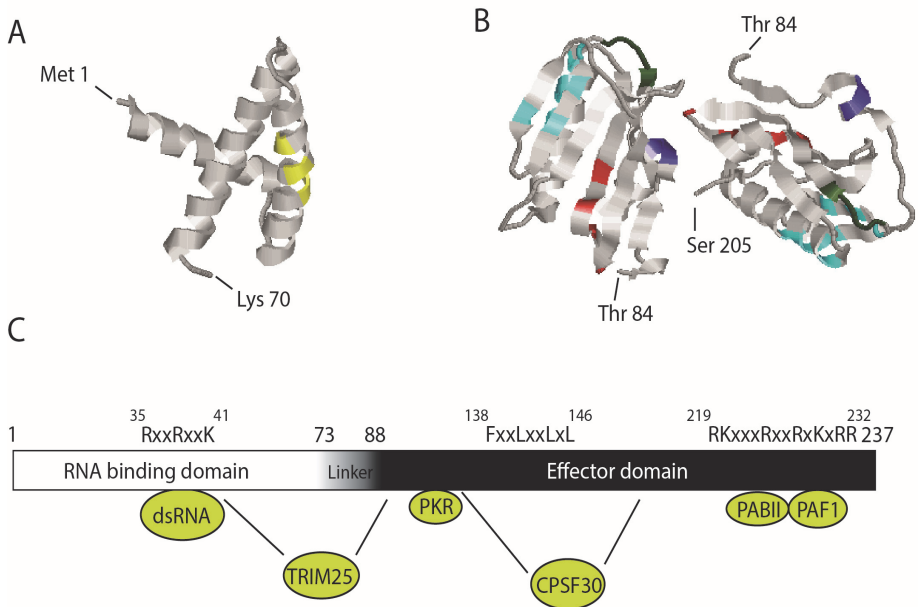
Figure 1. **The structure of NS1**. A) A cartoon depiction of the A/Udorn/72 NS1 RNA binding domain as a monomer. PDB ID 1AIL, source (Liu et al. 1997). B) A cartoon depiction of the A/Udorn/72 NS1 effector domain as a dimer. The C-terminal "tail" beyond amino acid 205 is not modeled in the structure due to its disordered nature. PDB ID 3EE8, source (Xia, Monzingo & Robertus 2009) C) A schematic representation of the A/Udorn/72 NS1 protein with the intracellular localization signals and some of the major interactions highlighted. Cartoon figures were generated using RasMol 2.7.5 software (www.rasmol.org). Colours in the cartoon structures depict NS1 interaction epitopes; NLS1/dsRNA binding – yellow, NES – red, TRIM25 – blue, CPSF30 – cyan.

1.3.4 Intracellular localization of NS1

Different functions of NS1 both in the nucleus and the cytoplasm necessitate a mechanism for nucleo-cytoplasmic transport. This is facilitated by the existence of a nuclear localization signal (NLS1), a nuclear export signal (NES) and a concurrent nuclear/nucleolar localization signal (NLS2/NoLS) in the NS1 structure (Melen et al. 2007, Greenspan, Palese & Krystal 1988, Li, Yamakita & Krug 1998). The NLS1 is well conserved within different influenza A strains and comprises amino acids R35, R38 and K41 (Melen et al. 2007, Greenspan, Palese & Krystal 1988). The C-terminal NLS2 is a classical bipartite NLS involving basic amino acids at positions 219, 220, 224, 229, 231 and 232 (Melen et al. 2007). Unlike NLS1, NLS2/NoLS is only found in some strains and is not necessary for efficient nuclear import (Melen et al. 2007). The NS1 NES is a classical hydrophobic leucine-rich nuclear export

signal consisting of the amino acids F138, L141, L144 and L146 (Li, Yamakita & Krug 1998). During infection NS1 mostly localizes into the nucleus, but it is also found in the cytoplasm, especially at early and late stages of infection. Constant shuttling between the nucleus and cytoplasm is one possible mechanism for NS1 localization, but the apparent temporal variation suggests an activating event for NS1 NES at a late stage of infection. Li and coworkers identified the NS1 amino acid region adjacent to NES as an inhibitory element and suggested binding to a viral protein to be necessary for NES activation, since NS1 in transfected cells remains entirely nuclear (Li, Yamakita & Krug 1998). Table 1 indicates the conservation rates of each NS1 localization signals within avian and human strains. While the NLS and NES signals are well conserved, the NLS2/NoLS is found fully functional in only a small percentage of strains.

Table 1. Conservation rates of NS1 localization signals

Amino acid position	Localiz. signal sequence	Avian		Human H1N1		Human H3N2	
		Consensus	Prevalence	Consensus	Prevalence	Consensus	Prevalence
NLS		Avian		H1N1		H3N2	
34	x	Asp	9997/10000	Asp	8103/8107	Asp	7078/7079
35	**Arg**	**Arg**	9995/10000	**Arg**	8104/8107	**Arg**	7077/7079
36	x	Leu	9937/10000	Leu	8084/8107	Leu	7011/7082
37	x	Arg	9985/10000	Arg	8099/8107	Arg	7072/7082
38	**Arg**	**Arg**	9997/10000	**Arg**	8104/8107	**Arg**	7083/7083
39	x	Asp	9996/10000	Asp	8101/8107	Asp	7079/7083
40	x	Gln	9995/10000	Gln	8105/8107	Gln	7081/7083
41	**Lys**	**Lys**	9886/10000	**Lys**	8096/8107	**Arg**	6721/7083
42	x	Ser	7900/10000	Ser	8104/8107	Ser	7002/7083
NES		Avian		H1N1		H3N2	
137	x	Ile	7509/10000	Ile	8120/8138	Ile	7012/7087
138	**Phe**	**Phe**	9599/10000	**Phe**	8127/8137	**Phe**	7075/7087
139	x	Asp	7497/10000	Asn	6367/8138	Asp	4451/7087
140	x	Arg	7820/10000	Arg	8133/8139	Arg	7072/7087
141	**Leu**	**Leu**	9816/10000	**Leu**	8137/8140	**Leu**	6994/7087
142	x	Glu	9869/10000	Glu	8130/8141	Glu	7071/7087

143	x	Thr	8163/10000	Thr	6772/8141	Thr	6726/7087
144	**Leu**	**Leu**	9997/10000	**Leu**	8133/8139	**Ile**	6179/7087
145	x	Ile	7593/10000	Ile	6634/8138	Val	6722/7087
146	**Leu**	**Leu**	7889/10000	**Leu**	8131/8138	**Leu**	7075/7087
147	x	Leu	9965/10000	Leu	8134/8138	Leu	7077/7087
148	x	Arg	9991/10000	Arg	8121/8138	Arg	7075/7087
149	x	Ala	9991/10000	Ala	8137/8139	Ala	7079/7087

NoLS		Avian		H1N1		H3N2	
219	**Lys**	**Lys**	7849/8100	**Lys**	8072/8093	**Lys**	6998/7031
220	**Arg**	**Arg**	7765/8071	**Arg**	1759/1768	**Arg**	6958/7000
221	x	Lys	5907/8057	Lys	1546/1768	Glu	4213/6989
222	x	Met	7642/8057	Met	1760/1768	Met	6982/6989
223	x	Ala	7800/8057	Ala	1763/1768	Ala	6963/6989
224	**Arg**	**Arg**	7839/8057	**Gly**	1616/1768	**Arg**	6826/6989
225	x	Thr	5717/7975	Thr	1713/1768	Thr	6974/6989
226	x	Ile	5809/7975	Ile	906/1768	Ala	6651/6989
227	**Arg**	**Glu**	7674/7975	**Arg**	1753/1768	**Arg**	6966/6989
228	x	Ser	7821/7975	Ser	1753/1768	Ser	6962/6986
229	**Lys**	**Glu**	7541/7938	**Glu**	1755/1767	**Lys**	4870/6985
230	x	Val	7711/7936	Val	1644/1767	Val	6937/6985
231	**Arg**	**Arg**	184/188	**Arg**	156/156	**Arg**	331/332
232	**Arg**	**Gly**	172/188	**Arg**	156/156	**Arg**	332/332
233	x	Asn	186/188	Asn	156/156	Asp	331/332

Consensus sequences for NS1 regions were calculated by analyzing up to 10000 avian and human NS1 sequences from the Influenza Research Database –website (http://www.fludb.org) with the Analyze Sequence Variation (SNP) tool available on the website (Squires et al. 2012).

1.4 Coronaviruses

Coronaviruses belong to the Nidovirales order and can be further divided into four genera of alpha-, beta-, gamma and deltacoronaviruses (Perlman, Netland 2009, Adams, Carstens 2012). Coronaviruses are enveloped RNA viruses harboring a single-stranded positive sense RNA genome of 27-32 kb in size (Woo et al. 2009). The large genome of coronaviruses is made possible by the action of a virus-encoded exoribonuclease, which provides proofreading activity to the replication of the coronavirus genome (Denison et al. 2011). Two thirds of the coronavirus genome is composed of an open reading frame (ORF) coding for the replicase polyprotein 1a/1b and the rest contains ORFs coding for the structural proteins S, E, M and N, and a variable collection of accessory proteins (Figure 2) (Woo et al. 2009). The 3'-proximal ORFs are expressed through the generation of a nested set of subgenomic RNAs to serve as templates for mRNA production (Fehr, Perlman 2015). This is enabled by a mechanism where the coronavirus RNA-dependent RNA polymerase (RdRp) slows down at specific transcriptional regulatory sequences in the genome and either continues elongation or switches templates to the 5′ end of the genome. The template switching property of the coronavirus RdRp makes coronaviruses prone for recombination, resulting in large variations in accessory gene composition between different coronaviruses and thought to make coronaviruses particularly adept at adapting to new host species (Graham, Baric 2010).
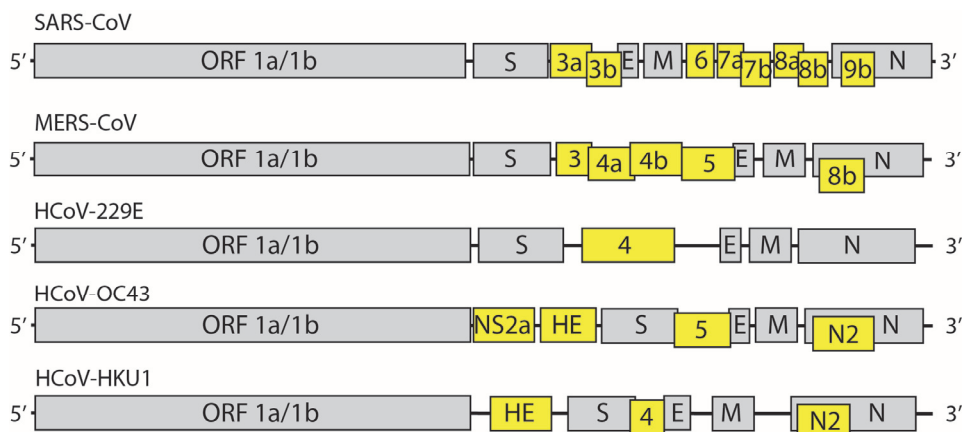

Figure 2. Genome organization of human coronaviruses. All coronaviruses encode for the replicase ORF 1a/1b and structural proteins spike (S), envelope (E), membrane (M) and nucleoprotein (N) (marked in gray). In addition, each strain encodes for several accessory proteins (yellow). Gene sizes are not drawn to precise scale. Adapted from (Liu et al. 2014).

Coronaviruses are the causative agents of gastrointestinal and respiratory tract infections in cows, pigs, chickens, dogs, cats and many other animal species (Fehr, Perlman 2015, Saif 2004). Coronaviruses impose a significant burden on the livestock industry with viruses like bovine CoV, infectious bronchitis virus and porcine hemagglutinating encephalomyelitis virus causing significant mortality in cattle, poultry and pigs. Bats are known to harbor most of the coronavirus diversity and they are suspected to be the origin of many human coronaviruses (Hu et al. 2015). Six different human coronaviruses are currently known, including HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1 and MERS-CoV (Hamre, Procknow 1966, McIntosh, Becker & Chanock 1967, Peiris et al. 2003b, van der Hoek et al. 2004, Woo et al. 2005, Zaki et al. 2012). In humans coronaviruses mostly cause only mild upper respiratory tract infections and are estimated to account for 10-15% of all cases of common cold (Heikkinen, Jarvinen 2003). Notable exceptions are SARS-CoV and MERS-CoV, which both cause severe respiratory tract infections with high mortality (Peiris et al. 2003a, Assiri et al. 2013).

1.4.1 SARS-coronavirus

SARS-CoV emerged in 2002, causing a worldwide epidemic with over 8000 infected and almost 800 fatalities in 26 countries (WHO 2004). The first recorded incident of SARS-CoV infection was in November 2002, when a man from Foshan, China, was hospitalized due to atypical pneumonia (Zhong et al. 2003). SARS-CoV emerged as a global epidemic in March 2003, after a SARS-CoV infected man residing in a hotel in Hong Kong transmitted the disease to 16 other residents of the hotel and these individuals then spread the virus to Vietnam, Singapore and Canada (Centers for Disease Control and Prevention (CDC) 2003). Global public health efforts concentrated on rapid identification and isolation of new cases and proper infection control methods at hospitals successfully ended the outbreak by July 2003 (Riley et al. 2003, WHO 2003a). The source of the virus was tracked to Chinese animal markets and specifically to Himalayan palm civets and raccoon dogs (Guan et al. 2003). A SARS-like coronavirus re-emerged in December 2003 causing four more human cases (Liang et al. 2004), which prompted mass cullings of civets and other animals in Chinese animal markets in January 2004 (Normile 2004). The virus has not been detected in palm civets or raccoon dogs after the cullings and no more human SARS-CoV cases have been recorded since 2004 (Kan et al. 2005). Bats are considered to be the natural reservoir of SARS-like coronaviruses based on isolations of very closely related viruses from Chinese horseshoe bats (Li et al. 2005, Ge et al. 2013).

SARS-CoV is classified as a lineage b betacoronavirus and harbors a genome of almost 30 kb in size (Rota et al. 2003, Marra et al. 2003, Snijder et al. 2003). The genome organization of SARS-CoV resembles that of other coronaviruses, containing open reading frames for the 1a/1b polyprotein, the structural S, E, M and N proteins as well as eight accessory proteins. The eight accessory proteins of SARS-CoV are involved in a multitude of different host cell functions, including apoptosis, cell cycle regulation, DNA synthesis and activation of the innate immune response (Liu et al. 2014). None of the accessory proteins are absolutely required for SARS-CoV replication, but the IFN antagonist Orf6 is a known virulence factor (Pewe et al. 2005, Kopecky-Bromberg et al. 2007).

1.4.2 Pathogenesis of SARS-CoV

SARS-CoV is the causative agent of a condition termed severe acute respiratory syndrome (SARS), which manifests itself as viral pneumonia featuring a rapid development of abnormalities in chest radiography (Peiris et al. 2003a). Typical clinical symptoms include fever, dry cough, myalgia, malaise and chills (Peiris et al. 2003a, Peiris, Guan & Yuen 2004, Hui, Memish & Zumla 2014). There is a high prevalence of lymphopenia and some patients develop watery diarrhea during the illness (Peiris et al. 2003a). Severe cases develop into acute respiratory distress syndrome (ARDS) often requiring mechanical ventilation. The main cause of death is respiratory failure due to extensive alveolar damage (Nicholls et al. 2003). The mean incubation time is 4.6 days with a range of 2 to 14 days (Hui, Memish & Zumla 2014). Viral load remains relatively low for the first few days of infection and peaks at 10 days after the onset of symptoms (Peiris et al. 2003a). Due to the lack of coronavirus specific drugs, treatment of SARS was mostly restricted to supportive treatment. Some patients were treated with the purine nucleoside analogue ribavirin, but no clinical benefits were found in a retrospective study (Leong et al. 2004). Treatment with lopinavir and ritonavir appeared to have some benefit as an initial treatment (Chan et al. 2003). Corticosteroids were used widely in Asia as a treatment for SARS and reportedly contributed to lower mortality in some cases, but they also caused long term complications in treated patients (Chen et al. 2006, Lv et al. 2009). In the end, not enough clinical evidence accumulated during the epidemic to suggest beneficial effects for any of the aforementioned treatments (Stockman, Bellamy & Garner 2006).

The receptor for SARS-CoV is the metallopeptidase ACE2, which is expressed in various human tissues (Li et al. 2003, Hamming et al. 2004). The SARS-CoV S protein needs activation by host cell proteases to facilitate virion-cell fusion and thus protease expression by the target cell is also a factor in SARS-CoV tissue tropism (Millet, Whittaker 2015). The transmembrane protease TMPRSS2 is considered the

principal protease involved in SARS-CoV S protein activation, although there is also evidence suggesting the involvement of cathepsin L and HAT (Simmons et al. 2005, Matsuyama et al. 2010, Bertram et al. 2011). Both ACE2 and TMPRSS2 are widely expressed in human lung tissue, including type II pneumocytes (Hamming et al. 2004, Bertram et al. 2012).

Type II pneumocytes of the alveolar epithelium are considered the primary target of SARS-CoV infection in the lungs based on data from immunohistochemical analysis of patient samples and *in vitro* infection experiments (Tse et al. 2004, Nicholls et al. 2006, Mossel et al. 2008). SARS-CoV infection of type II pneumocytes *in vitro* results in a robust innate immune response and similar strong cytokine responses have also been detected in patient samples (Jiang et al. 2005, Qian et al. 2013). Efficient migration of macrophages to the alveoli has been detected in post mortem tissue samples of patients and there is some evidence of infection of alveolar macrophages by SARS-CoV in patient samples, but monocyte-derived macrophages and dendritic cells do not support productive replication of SARS-CoV *in vitro* (Nicholls et al. 2003, Nicholls et al. 2006, Ziegler et al. 2005). Reported extrapulmonary targets for SARS-CoV include tissues of the gastrointestinal system, kidney, skin, liver, spleen, lymph nodes, pancreas and brain, but diarrhea and lymphopenia are the only consistently observed major pathological features outside the lungs (Peiris et al. 2003a, Ding et al. 2004, Gu et al. 2005, Li et al. 2003). Extensive T cell lymphopenia is strongly associated with the acute phase of SARS-CoV infection in patients (Li et al. 2004). Infection of T cells by SARS-CoV has been suggested as the mechanism for the lymphopenia, but evidence supporting this conclusion is controversial (Gu et al. 2005, Chan, Chen 2008). Instead, lymphopenia during SARS-CoV infection might be a consequence of stress-mediated induction of blood cortisol levels further amplified by glucocorticoid treatment, which is known to cause lymphopenia (Panesar et al. 2004). Since many SARS patients were treated with glucocorticoids, it is difficult to estimate how much of the lymphopenia observed in SARS patients is due to glucocorticoid treatment and how much due to direct or indirect influence by SARS-CoV (Sung et al. 2004). Inefficient activation of dendritic cells in the lungs and subsequent weak migration to lymph nodes and lack of a T cell response have been shown to result in a severe disease in SARS-CoV infected mice and a similar mechanism has also been suggested for humans (Zhao et al. 2009). This hypothesis is supported by the findings of Yoshikawa and coworkers, who suggested that cytokine production of lung epithelial cells in response to SARS-CoV infection causes inhibition of T cell priming ability of monocyte-derived dendritic cells (Yoshikawa et al. 2009).

A defining feature of the SARS epidemic was the notable association of severe disease with old age. In Hong Kong, where there were 1755 cases and 298 deaths during the 2002-2003 epidemic, no casualties under the age of 25 were recorded,

whereas death rates of over 50% were observed in patients over 65 years old (Peiris, Guan & Yuen 2004, WHO 2003b). The same phenomenon is also seen in experimental animals, where mice and macaques show similar age-associated increase of disease severity (Roberts et al. 2005, Smits et al. 2010). Age-related loss of naive T cells and changes in their function and repertoire are well known factors in respiratory infections of the elderly, but an additional mechanism affecting dendritic cell migration during SARS-CoV infection has been suggested (Zhao et al. 2011, Goronzy et al. 2015). Studies in mice have attributed age-related association of severe SARS to increased expression of prostaglandin PGD2 in the lungs of old mice, which results in impaired migration of dendritic cells from the lungs to the lymph nodes and a weakened T cell response (Zhao et al. 2011). Such a mechanism could be a factor in SARS-CoV pathogenesis in humans as well.

These observations suggest a model of SARS-CoV pathogenesis involving infection of type II pneumocytes of the alveolar epithelium and replication to high titers in the lung (Tse et al. 2004, Nicholls et al. 2006). SARS-CoV induces early production of IL-6, IL-8, CXCL10 and other cytokines, but inhibits IFN production (Tang et al. 2005, Yoshikawa et al. 2009). Lung resident macrophages and dendritic cells are not productively infected by SARS-CoV, but further contribute to inflammation by producing inflammatory cytokines and chemokines (Ziegler et al. 2005, Law et al. 2005, Yoshikawa et al. 2009). Dendritic cells are poorly activated, resulting in weak migration to the lymph nodes and a weak T cell response (Yoshikawa et al. 2009). This is especially emphasized in elderly patients with their already weakened T cell response and enhanced PGD2 expression in the lung (Zhao et al. 2011, Goronzy et al. 2015). Lymphopenia, whether drug- or virus-induced, further diminishes the T cell response (Panesar et al. 2004). A weak T cell response leads to delayed virus clearance from the lungs and continued replication and inflammation. ARDS develops due to diffuse alveolar damage induced by viral replication in the alveolar epithelium and neutrophil and T cell mediated destruction of alveolar epithelial cells (Perlman, Netland 2009, Nicholls et al. 2003).

1.4.3 MERS-coronavirus

The Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in Saudi-Arabia in 2012 from a man suffering from atypical pneumonia (Zaki et al. 2012). Since then over 1600 infections and over 500 deaths have been reported (WHO, 2016). The ongoing MERS-CoV epidemic is mostly concentrated in the Middle East and Saudi-Arabia, but cases have been exported by travelers to over 25 countries causing occasional secondary spread. Most notable outbreak outside Saudi Arabia took place in South Korea in 2015, resulting in 186 reported cases (Korea Centers for Disease Control and Prevention 2015). Dromedary camels

are the zoonotic source of human infections and MERS-CoV has reportedly been circulating in camels for decades (Azhar et al. 2014, Corman et al. 2014b, Reusken et al. 2013, Raj et al. 2014). The exact transmission route from dromedary camels to humans is still unclear, and besides direct contact might include consumption of raw camel milk and meat (Reusken et al. 2014, Hemida et al. 2015). Evidence for a bat reservoir of MERS-CoV has also been detected, suggesting a possible bat origin of MERS-CoV (Memish et al. 2013, Corman et al. 2014a, Ithete et al. 2013, Wang et al. 2014). Human to human transmission of MERS-CoV is not efficient and the R0 has been estimated to be 0.7 (Chowell et al. 2014). The epidemic persists due to repeated introductions to the human population from dromedary camels.

MERS-CoV is taxonomically a betacoronavirus like SARS-CoV, but is classified in a different lineage (Zaki et al. 2012). The closest known relatives to MERS-CoV are bat coronaviruses BatCoV-HKU4 and BatCoV-HKU5. The MERS-CoV genome is 30 kb in size and contains ORFs coding for five accessory proteins in addition to the typical coronavirus ORFs for the 1a/1b polyprotein and the structural proteins (van Boheemen et al. 2012). Accessory proteins ORF4a, ORF4b and ORF5 have known roles in blocking the host innate immune response, but the functions of ORF3 and ORF8b remain presently unknown (Yang et al. 2013, Niemeyer et al. 2013). No sequence similarity is shared between the accessory proteins of MERS-CoV and SARS-CoV. Studies concerning the evolution of MERS-CoV indicate no significant changes in the MERS-CoV genome during this epidemic (Cotten et al. 2014).

1.4.4 Pathogenesis of MERS-CoV

The clinical picture of MERS has many similarities with SARS (Hui, Memish & Zumla 2014). Most patients present with mild to severe respiratory disease accompanied with fever, chills, cough, shortness of breath and abnormal chest radiography (Assiri et al. 2013). Other common symptoms include myalgia, malaise, diarrhea and lymphopenia (Assiri et al. 2013). A strikingly large proportion of patients have been reported to suffer from some kind of underlying medical condition, most commonly diabetes or renal disease. The disease is typically milder and even asymptomatic in patients without comorbidities (WHO Mers-Cov Research Group 2013). Severe disease is associated with rapid progression into pneumonia requiring mechanical ventilation. Renal failure and thrombocytopenia are common extrapulmonary complications in severe cases (Arabi et al. 2014). Death results from ARDS, septic shock or multi-organ failure (Who Mers-Cov Research Group 2013). The overall mortality rate is over 30%, but this figure might be inflated due to asymptomatic cases going undetected. The mean incubation time is 5.2 days with a range of 2 to 13 days (Assiri et al. 2013). Like with SARS, the treatment of MERS patients is largely concentrated on supportive treatment. Some

patients have been treated with corticosteroids, but with little benefit. Ribavirin and interferon-α2b have been reported to be effective in treatment of MERS-CoV-infected rhesus macaques, but so far convincing clinical evidence supporting their use is missing (Falzarano et al. 2013).

The receptor for MERS-CoV is dipeptidyl peptidase 4 (DPP4) (Raj et al. 2013). DPP4 is expressed in many parts of the lung and especially in the alveolar regions, and can also be found in many other human tissues (Meyerholz, Lambertz & McCray 2016, Lambeir et al. 2003). MERS-CoV S protein can be cleaved by TMPRSS2, which is expressed throughout the lung (Bertram et al. 2012, Gierer et al. 2013). In addition, several other host cell proteases have been shown to cleave MERS-CoV S protein (Shirato, Kawase & Matsuyama 2013, Burkard et al. 2014, Millet, Whittaker 2014, Zmora et al. 2014).

A recently published autopsy study of a lethal case of MERS identified type II pneumocytes and epithelial syncytial cells as the main targets for MERS-CoV infection (Ng et al. 2016). Infection of bronchial submucosal glands was also observed and suggested to contribute to MERS-CoV transmission as a means to access respiratory secretions. The major pathological feature observed in the lungs was diffuse alveolar damage, suggesting a similar mechanism to SARS-CoV of ARDS formation due to viral replication in the alveolar epithelium. No infiltration of neutrophils or macrophages were reported, indicating that excessive inflammation might not be a feature of MERS-CoV pathogenesis. Pulmonary macrophages were not infected by MERS-CoV. Despite previous observations of MERS-CoV RNA in the blood and urine of patients and prevalence of renal failure in fatal cases suggesting systemic infection, no evidence of extrapulmonary infection was found in the brains, kidney, liver, spleen, lymph nodes, bone marrow, small intestine or colon (Ng et al. 2016, Poissy et al. 2014, Drosten et al. 2013). The exact cause of death was not mentioned, but the patient developed hypotension and renal failure and required mechanical ventilation before death.

The median age of MERS-CoV patients is 50 years, making old age a significant risk factor for MERS-CoV infection (WHO, 2015). This might reflect a similar scenario hypothesized for SARS-CoV, where the host T cell response has a critical role in viral pathogenesis. MERS-CoV is able to infect T cells *in vitro*, but the autopsy study by Ng and coworkers reported no infection of lymph nodes or spleen (Ng et al. 2016, Chu et al. 2016). Conflicting results have been published on dendritic cell permissibility for MERS-CoV infection (Chu et al. 2014, Scheuplein et al. 2015).

A clear model of MERS-CoV pathogenesis cannot be currently formed. The autopsy report by Ng and coworkers is in many aspects contradictory to previous data from animal models of MERS-CoV infection. Infection of common marmosets causes moderate to severe disease characterized by infection of type I pneumocytes and alveolar macrophages, strong inflammation of the lung and widespread systemic infection (Falzarano et al. 2014, Baseler et al. 2016). Similar results are seen in *ex vivo* infection of human lung tissue (Hocke et al. 2013). Analysis of a bronchoalveolar lavage sample from a MERS patient showed infection of lung epithelial cells and apoptosis (Hocke et al. 2013). While the autopsy data presented by Ng and coworkers is the most convincing evidence on MERS-CoV pathogenesis so far, it is still based on only one autopsy. Large individual variations are seen in the clinical manifestations and in activation of innate immune responses between patients (Faure et al. 2014). Therefore, more evidence is needed to clarify the pathogenesis of MERS-CoV.

2 Aims of the study

The specific aims of this study were:

1. To determine the critical amino acids for influenza A NS1 nuclear export signal (NES) function during infection

2. To study the importance of NS1 NES for influenza A pathogenesis

3. To investigate the mechanics of NS1 interaction with the host cell nuclear export machinery and the nucleolus

4. To determine susceptibility of primary human leukocytes to MERS-CoV infection and to compare innate immune responses caused by MERS-CoV to those induced by SARS-CoV

3 Materials and Methods

3.1 Cells

Differentiation of macrophages and DCs from monocytes derived from voluntary blood donors was done as previously described (Osterlund et al. 2005, Pirhonen et al. 1999). Macrophages and DCs were identified by their typical morphology. Macrophage–serum-free substitution medium supplemented with 0.6 µg/ml penicillin, 60 µg/ml streptomycin and 10 ng/ml human granulocyte–macrophage colony-stimulating factor (GM-CSF) and RPMI medium supplemented with 0.6 µg/ml penicillin, 60 µg/ml streptomycin, 2 mM L-glutamine, 20 mM HEPES, 10% fetal calf serum (FCS), 10 ng/ml GM-CSF and 20 ng/ml IL-4 were used in the maintainance of macrophages and MDDCs, respectively. Macrophages were used at 7 days and MDDCs at 6 days after cultivation.

Cell lines used in this study were maintained in Eagle minimum essential medium supplemented with 0.6 µg/ml penicillin, 60 µg/ml streptomycin, 2 mM L-glutamine, 20 mM HEPES and 10% FCS at 37°C in 5% CO_2. All the used cell lines are listed in Table 2.

Table 2. Cell lines used in the study.

Cell line	Source	Study
A549	ATCC CCL-185	I, II, III
Calu-3	ATCC HTB-55	III
HEK293	ATCC CRL-1573	I
HuH7	Nakabayashi et al.	II
MDCK	ATCC CCL-34	I, II, III
Vero	ATCC CCL-81	III
Vero E6	ATCC CRL-1586	III

3.2 Viruses

Mutant influenza A viruses were generated using the reverse genetics system described by Neumann and coworkers and passaged twice on MDCK cells (Neumann et al. 1999). Propagation of final stocks of influenza A viruses used in this study was done in 11-day old embryonated chicken eggs at +34 °C for 3 days. Stock titers were determined by plaque titration on MDCK cells. Correct sequences of mutant virus stocks were verified by sequencing. Coronaviruses were propagated for two passages on Vero E6 cells and stock titers were determined by end-point

dilution assay. Propagation of and all experiments with coronaviruses were done in biosafety level 3 conditions. All the viruses used in this study are listed in Table 3.

Table 3. Viruses used in the study.

Virus	Subtype	Titer (pfu/ml)	Study
A/Udorn/72	H3N2	2×10^7	I, II
A/Udorn/72 NS1 (F138A, L141A)	H3N2	1.5×10^8	I
A/Udorn/72 NS1 (L141A)	H3N2	1×10^7	I
A/Udorn/72 NS1 (L144A, L146A)	H3N2	4×10^6	I
A/Udorn/72 NS1 (L146A)	H3N2	2×10^7	I
A/Udorn/72 NS1 (A149V)	H3N2	1.6×10^7	I
A/WSN/33	H1N1	2×10^7	II
A/WSN/33 (A/Brevig Mission/1/18 segm 8)	H1N1	$1\text{-}4 \times 10^7$	II
A/Fin/554/09	H1N1	$1\text{-}4 \times 10^7$	II
A/mallard/Netherlands/12/2000	H7N3	$1\text{-}4 \times 10^7$	II
A/Beijing/89	H3N2	6×10^7	III
SARS-CoV	HKU-39849	2.5×10^7 TCID$_{50}$/ml	III
MERS-CoV	GenBank JX869059	1.5×10^6 TCID$_{50}$/ml	III

3.3 Antibodies

All the antibodies used in this study for either immunofluorescence or Western blot are listed in Table 4.

Table 4. Antibodies used in the study.

Antibody	Source	Study
Guinea pig anti-NS1	Melen et al. 2007	I, II
Rabbit anti-NP	Ronni et al. 1995	I
Rhodamine Red X-goat anti-guinea pig Ig	Jackson ImmunoResearch	I
FITC-anti-rabbit Ig	Jackson ImmunoResearch	I
FITC- goat anti-guinea pig Ig	Jackson ImmunoResearch	II
Rabbit anti-Actin	sc-10731, Santa Cruz Biotechnology	I, III

Rabbit anti-MxA	Ronni et al. 1993	I, III
Rabbit anti-p-IRF3	#4947, Cell Signaling Technology	I
Rabbit anti-p-Akt	#9271, Cell Signaling Technology	I
Rabbit anti-M1	housemade	I
Rabbit anti-NEP	housemade	I
HRP-conjugated rabbit anti-guinea pig	Dako	I, III
HRP-conjugated goat anti-rabbit	Dako	I, II, III
HRP-conjugated goat anti-mouse	Dako	II
Mouse anti-nucleolin	sc-8031, Santa Cruz Biotechnology	II
Mouse anti-B23	sc-32256, Santa Cruz Biotechnology	II
Rabbit anti-fibrillarin	#2639, Cell Signaling	II
Rabbit anti-HIV-1 Rev	Malim, Cullen 1991	II
Guinea pig anti-SARS-CoV N	Ziegler et al. 2005	III
Rabbit anti-MERS-CoV N	Adney et al. 2014	III
Rabbit anti-MERS-CoV N	Sino Biological	III
Rabbit anti-human DPP4	AbCam	III

3.4 qRT-PCR primers

Primers used in the qRT-PCR assays of the study are listed in Table 5.

Table 5. qRT-PCR primers used in the study

Primer target	Source	Study
SARS-CoV ORF1b	Drosten et al. 2003	III
MERS-CoV ORF1b	Corman et al. 2012	III
IFN-beta	Hs00277188_s1, Life Technologies	III
IFN-lambda1	Hs00601677_g1, Life Technologies	III
CXCL10	Hs00171042_m1, Life Technologies	III
TNF-alfa	Hs01113624_g1, Life Technologies	III
MxA	Hs00182073_m1, Life Technologies	III
Human DPP4	10031225, BioRad	III
18S rRNA	4308329, Life Technologies	III

3.5 Methods

All the methods used in this study are listed in Table 6. Specific details for each method are provided in the materials and methods sections of the individual publications.

Table 6. Methods used in the study

Method	Study
Cell culture	I, II, III
Virus propagation in embryonated chicken eggs	I, II, III
Reverse genetics	I, II
Site-directed mutagenesis	I, II
Plasmid isolation	I, II, III
Transfection	I, II
Transformation	I, II
Plaque titration	I, II
Sequencing	I, II
Immunofluorescence	I, II
Protein expression in E. coli	I, II
GST-pulldown	I, II
SDS-PAGE	I, II, III
Western blot	I, II, III
Virus infection experiments	I, II, III
Endpoint dilution assay	I, III
ELISA	I, III
Confocal laser microscopy	I, II
In vitro translation	I, II
Isolation and differentiation of monocytes	III
RNA isolation	III
qRT-PCR	III
cDNA synthesis	III
Student's t-test.	I, III

4 Results and discussion

4.1 Elucidating the significance of NS1 intracellular localization for influenza A virus infection

Besides influenza A virus, several other viruses are known to encode proteins that are targeted into the nucleus as part of their function as antagonizers of host cell transcription and anti-viral response. Examples include respiratory syncytial virus matrix protein and dengue virus nonstructural protein 5 (Ghildyal et al. 2003, Brooks et al. 2002). Inhibition of either nuclear import or export through mutation or inhibitors has identified nucleo-cytoplasmic transport of these proteins to be critical for virulence and virus production (Ghildyal et al. 2009, Pryor et al. 2007, Rawlinson et al. 2009). Nuclear localization of influenza A virus NS1 protein is known to be critical for inhibition of IFN-β mRNA production, but studies on NS1 nuclear export are largely missing (Min, Krug 2006, Donelan, Basler & Garcia-Sastre 2003). In study I we focused on the NS1 NES with the aims of determining the critical amino acids needed for nuclear export of NS1 during infection and studying the impact of NS1 NES abolishment on influenza A virus infection.

The NS1 NES was identified more than 15 years ago, but since then its functions have not been studied much further (Li, Yamakita & Krug 1998). The study by Li et al. used transfected fusion constructs of GST fused to NS1 fragments to identify the NES sequence as well as two critical amino acids (L144 and L146) and an adjacent inhibitory region. We wanted to extend these observations by taking a detailed look at NS1 NES function during infection. To that end we generated recombinant A/Udorn/72 viruses harboring different point mutations in the NS1 NES sequence. Analysis of NS1 localization in infected A549 cells 8-24h post infection by immunofluorescence revealed that the L146A mutation by itself was not sufficient to abolish NES function (I, Fig. 2 and 3). Localization of NS1 L146A was mostly similar to wild-type NS1 with NS1 L146A localizing both in the nucleus and the cytoplasm throughout infection. When combined with L144A, the resulting double mutation L144A, L146A inactivated the NES efficiently resulting in almost exclusively nuclear localization of NS1 at later stages of infection (I, Fig. 2 and 3). The other introduced mutations (L141A; F138A, L141A and A149V) were similarly effective in inactivating the NS1 NES, indicating that leucines 141 and 144 are both critical for NS1 NES function. Interestingly, our data did not support the finding of Li et al. on the critical nature of leucine 146 for NS1 NES function (Li, Yamakita & Krug 1998). An obvious explanation for this discrepancy could be the differences in experimental settings, as Li et al. based their observation on transfection experiments using only the NES region fragment of NS1 bound to GST. Our data is

more likely to reflect the actual mechanism since it was obtained using full length NS1 proteins in the context of an infection. Differences in virus strains and cell lines is also a possible reason for the different observations as many NS1 functions have been shown to be strain and host specific. Our observation that the point mutation A149V also inactivates the NS1 NES supports the description by Li et al. of the region adjacent to NES acting as a regulatory element. The alanine 149 is not part of the NS1 NES sequence as shown by Li et al., but our data fits well with the idea of a regulatory function for the region adjacent to NES since a mutation within this region could have either activating or inhibiting consequences for the NES function depending on the mutation.

The NS1 NES region is highly conserved within all influenza A isolates (I, Table 1), suggesting structural or functional importance for the region. Thus, we expected several of the mutant viruses to display an attenuated phenotype in infection experiments. Growth kinetic experiments done in MDCK cells at MOI 0,001 showed impaired growth properties for the mutant viruses (L144A, L146A) and (A149V), whereas all the other mutant viruses grew to similar titers as the wild-type virus (I, Fig. 4). Similarly, in A549 cells the same viruses stood out as the only ones to cause significant production of IFN-beta and IFN-lambda 1 (I, Fig. 6A and 6B). Further examination of the infections on protein level revealed some variation in the expression kinetics of viral proteins, but overall the differences remained minor (I, Fig. 5). Some increase in MxA production and phosphorylation of IRF3 as compared to the wild-type virus was seen with all the mutant viruses with the most pronounced increases being associated with mutations (L144A, L146A) and (A149V) (I, Fig. 6C). Interestingly, a decrease in phosphorylation of Akt was seen with the mutant viruses (L144A, L146A) and (A149V), indicating an impairment of PI3K activation by NS1 (I, Fig. 6C). Overall, introduction of point mutations into the conserved NS1 NES region caused several changes in phenotype, but the changes correlated poorly with NS1 localization. Most clear changes were seen with the mutant viruses (L144A, L146A) and (A149V), which both also displayed a mostly nuclear NS1, but the changes in phenotype have to be attributed to other factors besides intracellular localization of NS1 because the mutations (F138A, L141A) and (L141A) also caused nuclear retention of NS1 without affecting viral growth properties or IFN antagonism.

Besides the NES, several other functional roles have been assigned to the 138-150 amino acid region of NS1. NS1-mediated inhibition of CPSF by binding to its 30 kDa subunit (CPSF30) is considered one of the main mechanisms for inhibiting host cell protein production during influenza A virus infection. The NS1 NES region has been suggested to be involved in CPSF30 binding with the amino acid 144 playing a critical role in the binding (Twu et al. 2006). Impairment of CPSF inhibition by NS1 could very well explain many phenotypic features of our NES mutant viruses, as the

loss of NS1 CPSF binding function has been shown to cause attenuation of influenza A virus (Noah, Twu & Krug 2003). However, a crystal structure published on the NS1-CPSF complex does not support the idea of the NES region being involved in the binding (Das et al. 2008). To analyze whether our NES mutations affected CPSF binding, we introduced the same mutations into GST-NS1 expression plasmids. A pull-down assay using [35S]-labeled CPSF30 showed similar binding with the mutant NS1 proteins and the wild-type NS1, indicating that CPSF binding was not impaired (I, Fig. 7). Inhibition of mRNA export is another feature attributed to the NS1 NES region (Qian, Alonso-Caplen & Krug 1994). Qian et al. reported the loss of mRNA export inhibition by NS1 when either leucine 144 or leucine 146 was mutated into an alanine. Also mutation of NS1 amino acids 143, 145 and 147 abolished mRNA export inhibition, but mutation L141A had no effect (Qian, Alonso-Caplen & Krug 1994). A later study showed that NS1 binds four different proteins of the mRNA nuclear export machinery, NXF1, p15, Rae1 and E1B-AP5, of which p15 specifically interacts with the NS1 effector domain (Satterly et al. 2007). Along with CPSF binding, inhibition of mRNA export is a way for NS1 to down-regulate host gene expression. Loss of this inhibition through the use of a quinoline carboxylic acid has been shown to cause significant reduction in influenza A virus replication (Zhang et al. 2012). Thus, interaction with the mRNA nuclear export machinery may play a role in the observed NES mutant phenotypes.

NS1 activation of PI3K through binding to the inter-SH2 domain of the p85β subunit of PI3K is also connected to the NES region. Li et al. indicated the NS1 amino acid region 137-142 in NS1-p85β interaction and showed interference of the interaction by mutation of the NS1 amino acids 141 and 142 (Li et al. 2008). In other studies the mutation of NS1 amino acid 138 has been shown to influence the NS1-p85β interaction (Fan et al. 2013, Li et al. 2012). A structural study of the p85β iSH2-NS1 –complex showed that the entire 142-148 amino acid region lies at the NS1-p85β interface and discussed the possibility of salt bridge formation between p85β and residues of the NES region (Hale et al. 2010). Moreover, several studies have shown reductions in growth and IFN antagonistic properties associated with impairment of the NS1-p85β interaction (Hale et al. 2006, Shin et al. 2007, Hale et al. 2010), indicating that loss of p85β binding could explain the observed NES mutant phenotypes. The clear reduction in phosphorylation of Akt by mutant viruses (L144A, L146A) and (A149V) suggests that PI3K activation is indeed impaired in these viruses (I, Fig. 6C).

Finally, consideration should be given to the possibility of changes in NS1 conformation caused by the NES mutations. NS1 is a very multifunctional protein and has been shown to adopt several different quaternary conformations to facilitate different interactions (Carrillo et al. 2014, Das et al. 2008, Hale et al. 2010). Any mutation that restricts NS1 conformational plasticity might knock out functions

dependent on a given conformation. At least the linker region has been suggested to play an important role in NS1 conformational plasticity (Carrillo et al. 2014), but additional sites may also exist. Interestingly, mutations introduced within the NS1 linker region have been shown to result in a phenotype remarkably similar to the one exhibited by the mutant virus (L144A, L146A) (Li, Noah & Noah 2011). The impact of a given protein region for conformational stability is difficult to speculate, but one approach has been to combine structural information with estimations of the degree of conservation of different regions (Darapaneni, Prabhaker & Kukol 2009). Darapaneni et al. described several conserved sites of probable structural importance on NS1 and suggested that both leucine 144 and alanine 149 are involved in stabilizing NS1 structure. Quantitation of NS1 expression during infection showed that NS1 (L144A, L146A) expression was reduced approximately 50% compared to wild-type NS1 expression (I, Fig. S3). A reduction of 50% in NS1 amount is unlikely to cause defects in influenza A virus replication, because even reductions as large as 90% have been found to be insufficient for influencing replication (Chua et al. 2013). However, such a reduction does suggest some structural defect, and thus influence on NS1 conformation by the NES mutations cannot be ruled out.

4.2 Mechanisms of NS1 intracellular localization

Because of its importance for the function of many viral proteins, nucleo-cytoplasmic transport has been suggested as a target for antiviral drug development (Caly, Wagstaff & Jans 2012). In order for that approach to work, it is essential to understand how viral proteins are shuttling between the nucleus and cytoplasm. NS1 nuclear import has been well studied and found to be facilitated by importin α (Melen et al. 2007). However, when this work was started no studies on the mechanism of NS1 nuclear export had been done. Also, interaction of NS1 with host nucleolar proteins remained unresolved. In the second part of our work we focused on the mechanisms of NS1 nuclear export and the interaction of NS1 with the host cell nucleolus.

The NES of influenza A virus NS1 protein is a classical hydrophobic leucine-rich nuclear export signal, which usually facilitates transport across the nuclear membrane through interaction with the nuclear transport receptor CRM1 (Fornerod et al. 1997, Fukuda et al. 1997, Ossareh-Nazari, Bachelerie & Dargemont 1997). There is evidence however, that NS1 is exported independently of CRM1 (Han et al. 2010). Han et al. used transfected NS1 constructs and FRET techniques to show that NS1 did not interact with CRM1. These results should be interpreted with caution however, since the NS1 NES has been reported to remain inactive in transfected cells (Li, Yamakita & Krug 1998). Therefore NS1 CRM1 dependence remains to be verified during an actual infection. To that end we infected A549 cells with

A/Udorn/72 wild-type virus in the presence or absence of Leptomycin B (LMB), a known inhibitor of CRM1 (Nishi et al. 1994). Analysis of NS1 localization showed no significant differences between treated and untreated cells (I, Fig. 7), indicating that NS1 export is independent of CRM1 or possibly that NS1 can be exported by several alternative mechanisms. By what mechanism is NS1 exported then? Examples of leucine rich hydrophobic nuclear export signals functioning independently of CRM1 include protein kinase inhibitor (PKI) and glucocorticoid receptor, which are both exported by the Ca2+-binding protein calreticulin through a mechanism similar to CRM1-mediated export (Holaska et al. 2001, Holaska et al. 2002). Some effort was put into trying to knock down calreticulin protein expression by siRNA to check for a possible impact on NS1 export, but sufficient silencing was not achieved (data not shown). Another possibility is the export of NS1 through the NXF1-p15 pathway, which is involved in mRNA transport. Some viral proteins, including Epstein-Barr virus EB2 and hepatitis B virus core protein, have been shown to rely on the NXF1-p15 pathway for nuclear export (Juillard et al. 2009, Yang et al. 2014). This is an intriguing possibility for NS1 also and should be looked into further, since NS1 has been shown to interact directly with both NXF1 and p15 (Satterly et al. 2007).

The nucleolus is a dynamic, complex, non-membranous structure within the nucleus involved in ribosome biogenesis and a multitude of other functions, including cell cycle regulation, signal recognition particle assembly, apoptosis and stress responses (Boisvert et al. 2007). The nucleolus is also a target for many viral proteins either as a means to perturb some of the functions mediated by the nucleolus or to recruit nucleolar proteins for the benefit of virus infection (Hiscox 2007). Some examples include West Nile virus capsid protein, which activates p53-mediated apoptosis (Yang et al. 2008) and hepatitis C virus Core protein, which targets several nucleolar proteins to induce apoptosis and regulate transcriptional pathways (Kao, Chen & Lee 2004, Otsuka et al. 2000). The function of influenza A NS1 in the nucleolus has not been studied much and thus remains unclear.

Unlike NLS and NES signals, which must mediate translocation across a membrane, nucleolar localization signals are more like retention signals, mediating nucleolar localization by binding to a nucleolar protein (Emmott, Hiscox 2009). Thus, to understand NS1 nucleolar localization mechanics is to understand which nucleolar proteins are bound by NS1. In our second study we concentrated on defining which nucleolar proteins are bound by NS1. To that end we created expression plasmids coding for either full-length or fragmental NS1 proteins from different strains fused to either GFP or GST (II, Fig. 2 and 3). Immunofluorescence analysis confirmed our previous observation (Melen et al. 2007) of NS1 C-terminus and specifically the NoLS sequence directing NS1 nucleolar localization (II, Fig. 2). NS1 was also found to colocalize with the major nucleolar proteins nucleolin, B23 and fibrillarin (II, Fig.

5). However, GST pull-down assays using *in vitro* –translated nucleolin, B23 and fibrillarin proved direct interactions with only nucleolin and fibrillarin (II, Fig. 4). NS1 proteins from strains lacking the C-terminal NoLS still exhibited binding to nucleolar proteins and/or nucleolar localization to some extent, but the interaction/localization was much weaker than with A/Udorn/72 NS1, which contains a functional NoLS (II, Fig. 1, 2, 3 and 4). Interestingly, the N-terminal 1-73 amino acid fragment of A/Udorn/72 also showed binding to *in vitro* –translated nucleolin and fibrillarin, albeit to a lesser degree than the C-terminal fragment (II, Fig. 4). This binding was abolished upon mutating NS1 amino acids R38 and K41, which are critical for NLS1 and RNA-binding functions, indicating that RNA-binding might be involved in the interaction (II, Fig. 4). Since nucleolin and fibrillarin are both RNA-binding proteins, the observed interaction with NS1 RNA-binding domain might not be a true interaction, but rather could represent both NS1 and nucleolin/fibrillarin binding to the same RNA molecule and thus clumping together in the pull-down assays. Such a dependency on RNA for protein-protein interaction in a similar setting has been shown for fibrillarin binding of porcine reproductive and respiratory syndrome virus N protein (Yoo et al. 2003). RNAse treatment of the samples would have clarified this issue, but unfortunately it did not occur to us at the time of experimentation. Our results are consistent with a previous study on nucleolin NS1 binding by Murayama and co-workers, however they failed to observe nucleolin binding by the NS1 C-terminus (Murayama et al. 2007).

Volmer and coworkers studied the nucleolar localization of NS1 proteins from various avian and human strains in different cell types, including human, murine and avian cells (Volmer et al. 2010). Similarly to our results, nucleolar localization in human and murine cells required a functional C-terminal NoLS, but in avian cells all the tested NS1 proteins localized to the nucleolus despite the lack of a C-terminal NoLS in most of them (Volmer et al. 2010). Volmer and coworkers suggested a conserved NS1 "avian NoLS", but did not speculate on the specific sequence of such a functional element. In the light of our own results and those by Murayama and coworkers, it is tempting to speculate that perhaps the NS1 NLS1 also functions as a NoLS in avian cells by binding to avian nucleolar proteins, but is only partially effective in binding mammalian ones, thus leading to the observed weak interactions in binding experiments. Additional binding experiments using avian nucleolar proteins could yield interesting results.

Since NS1 localizes to the nucleolus, there should also be a specific nucleolar function for NS1. Obvious candidates for functional significance are the NS1 interaction partners nucleolin and fibrillarin. Nucleolin is an abundant and multifunctional protein found mostly in the nucleolus, but also in the nucleoplasm, cytoplasm and cell membrane (Abdelmohsen, Gorospe 2012). The main function of nucleolin is considered to be ribosome biogenesis, where it is involved in several

early steps, but nucleolin is also known to play a part in DNA metabolism, transcription and nucleo-cytoplasmic transport (Abdelmohsen, Gorospe 2012). Fibrillarin is a small nucleolar ribonucleoprotein functioning in ribosomal RNA modification during processing of pre-rRNA (Reichow et al. 2007). Interactions with nucleolin by other viral proteins are known to promote viral replication, stimulate translation and facilitate virion entry into the host cell (Hirano et al. 2003, Izumi et al. 2001, Su et al. 2015). The porcine reproductive and respiratory syndrome virus N protein has been shown to bind fibrillarin, but the functional significance of this interaction is unclear (Yoo et al. 2003). Nucleolin and fibrillarin do not have any recognized roles in influenza A virus replication, so it is difficult to speculate on their possible functional significance without further experiments.

One possible explanation for NS1 nucleolar localization is the inhibition of tumor suppressor p53, which is a major factor in cell cycle regulation and apoptosis. p53 is transiently expressed in human cells and its levels are normally kept low by ubiquitination-directed proteolysis (Lee, Gu 2010). Besides ubiquitination, p53 is also a target for acetylation, methylation, phosphorylation, neddylation and sumoylation, leading to a complex regulation system which enables responses to various kinds of cellular stress (Lee, Gu 2010). The nucleolus is involved in p53 regulation by harboring proteins that inhibit the function of E3 ubiquitin ligase MDM2, which is considered the major inducer of p53 proteolysis through ubiquitination (James et al. 2014). Also, p53 reportedly accumulates in the nucleolus after certain kinds of cellular stress (Klibanov, O'Hagan & Ljungman 2001). Influenza A virus NS1 has been shown to interact directly with p53and inhibit its function (Terrier et al. 2013, Wang et al. 2010). This contradicts with reports on p53 activation during influenza A virus infection (Turpin et al. 2005, Zhirnov, Klenk 2007), which is explained by another influenza A virus protein, NP, acting as an activator of p53 (Wang et al. 2012). NP also localizes to the nucleolus and causes an impairment of the MDM2-p53 interaction, thus increasing the stability of p53 (Wang et al. 2012). This sets up an interesting regulatory mechanism, where NS1 and NP exert competing pressures towards p53, possibly resulting in temporal regulation of p53 activity. This view is supported by a study on p53 activation during influenza A infection, where p53 was found to follow a biphasic pattern, activating first at an early stage of infection and then again at the late stage of infection (Shen et al. 2009). It is however doubtful whether nucleolar localization would be essential for p53 interaction, since p53 mostly resides in the nucleoplasm.

Another clue to the nucleolar function of NS1 might be the similarity with the HIV-1 Rev protein. In transfected HuH7 cells NS1 was found to colocalize with Rev and overexpression of Rev even caused displacement of NS1 from the nucleolus (II, Fig. 6), indicating that NS1 and Rev might bind to the same nucleolar structures. Rev is known to bind to B23 (Fankhauser et al. 1991), and while NS1 did not show binding

to *in vitro* translated B23 (II, Fig. 4), there was some evidence of NS1 induced displacement of B23 from the nucleolus to the nucleoplasm in infected A549 cells (II, Fig. 5B). B23 is involved in a wide variety of different cellular processes, including cell cycle regulation, ribosome biogenesis and apoptosis. B23 also regulates p53 stability by interacting with ARF, MDM2 and p53. ARF, a nucleolar inhibitor of MDM2, is stabilized by B23 interaction, but the interaction also prevents ARF from interacting with MDM2 (Korgaonkar et al. 2005). B23-MDM2 interaction causes inhibition of MDM2 (Kurki et al. 2004), while p53 is stabilized by B23 (Colombo et al. 2002). The nucleolar B23 thus normally inhibits ARF, allowing MDM2 to keep p53 levels low, but nucleolar disruption in response to various stress events causes displacement of B23 to the nucleoplasm leading to inhibition of MDM2, stabilization of p53 and apoptosis (Rubbi, Milner 2003). The idea of B23 displacement by NS1 affecting p53 regulation during influenza A infection is an intriguing one, but the observed displacement effect is so small that it is unlikely to have a large impact (II, Fig. 5).

Besides ribosome biogenesis, the nucleolus is also a site for RNA editing. ADAR1 and ADAR2 are RNA editing enzymes that normally shuttle in and out of the nucleolus (Desterro et al. 2003). Their substrates are long dsRNA duplexes and pre-mRNA transcripts, where they function to deaminate adenosine to inosine. Such a function would not be expected to have a role during influenza A virus infection, but interestingly NS1 was found to interact with ADAR1 in a yeast two-hybrid screening assay (de Chassey et al. 2013). Moreover, ADAR1 was found to be a pro-viral host factor and influenza A virus infection caused colocalization of ADAR1 with NS1 inside the nucleolus (de Chassey et al. 2013). NS1 interaction also caused enhancement of ADAR1 editing activity (de Chassey et al. 2013). It is unclear what benefit this might incur for influenza A virus infection, but ADAR1 interaction with NS1 clearly happens in the nucleoli, and thus it is an example of an interaction that might be enforced by the NoLS-directed nucleolar localization.

The NS1 NLS2/NoLS is strain specific, appearing only in human strains isolated between 1950 and 1987 (Melen et al. 2007). It is generated by a single nucleotide mutation changing a stop codon into an arginine codon and causing a seven amino acid extension to the NS1 protein. There must be a beneficial effect for influenza A virus infection conferred by the mutation, since it evolved and lasted for so long, but then again the benefit is probably a small one since it was lost in 1987. The impact of the extension on viral replication was studied by Lohrmann and coworkers and found to be very small (Lohrmann et al. 2013). Using recombinant viruses, Lohrmann and coworkers did not find any differences in replication kinetics or interferon antagonistic properties in cell lines between the wild-type and recombinant viruses (Lohrmann et al. 2013). A slight growth benefit in mice and in competition experiments in cell lines were the only observed differences (Lohrmann

et al. 2013), indicating a minor functional significance for NS1 nucleolar localization.

4.3 Assessing susceptibility of human monocyte-derived macrophages and dendritic cells for MERS-CoV infection

In order to successfully invade the host and start an infection, respiratory viruses entering the lungs have to overcome several obstacles, including mechanical barriers, cytokine production by the airway epithelial cells and the actions of the cells of the innate immune system present in the lung (Whitsett, Alenghat 2015). Lung resident macrophages and dendritic cells play a critical role in infection control and their interaction with invading respiratory viruses can have strong consequences for the outcome of an infection (Kopf, Schneider & Nobs 2015). Understanding this interaction is important for understanding the pathogenesis of a respiratory virus. In the final part of our work we concentrated on the interaction of the novel coronavirus MERS-CoV with human monocyte-derived macrophages and dendritic cells (MDDCs).

Many respiratory viruses are known to infect and replicate in macrophages or dendritic cells. Some examples include influenza A virus (Rodgers, Mims 1982, Bender et al. 1998), rhinovirus (Laza-Stanca et al. 2006) and also human coronaviruses OC43 and 229E (Collins 1998, Funk et al. 2012). Besides the obvious benefit of replication, some viruses use DC infection as a means to invade further into the body by taking advantage of DC migration through the lymphatic system (Rinaldo, Piazza 2004). Our laboratory has previously shown that SARS-CoV is unable to productively replicate in human monocyte-derived macrophages and MDDCs (Ziegler et al. 2005). In study III of the present thesis work we analyzed the replication and induction of innate immune responses by MERS-CoV in human monocyte-derived macrophages and MDDCs and compared the responses to those induced by SARS-CoV. Assays measuring replication at the RNA level and at the level of viable virion production both showed no evidence of efficient MERS-CoV or SARS-CoV replication in either cell type, with no or only a very weak increase in viral RNA levels and a steady decline in viral supernatant titers throughout the infection (III, Fig. 1). A similar infection of susceptible Vero E6 and Calu-3 cell lines proved the viability of our virus stocks and experimental setting (III, Fig. 2). As still further confirmation, Western blot analysis of macrophage and MDDC cell lysates showed no increase in viral N-protein expression during infection (III, Fig. 3). qRT-PCR measurement of IFN-β, IFN-λ1, CXCL10, TNF-α and MxA mRNA levels showed mostly similar mRNA production between virus-infected macrophages and MDDCs, but a clear difference between MERS-CoV and SARS-CoV was observed (III, Fig. 4 & 5). Whereas SARS-CoV only induced a slight increase in IFN-λ1 mRNA expression in MDDCs, significant inductions of CXCL10

and MxA as well as some induction of IFN-β and IFN-λ1 was seen in response to MERS-CoV infection (III, Fig. 4 & 5). Curiously, this difference between the viruses was not evident in Calu-3 cells, where both viruses induced a strong response (III, Fig. 6). As a control, influenza A (A/Beijing/89) infection was included in the MDDC experiments to estimate the MERS-CoV response in the context of a known strong inducer of innate immune responses. MERS-CoV – induced cytokine responses were rather weak in comparison to A/Beijing/89 virus, which induced approximately 100- to 1000-fold higher levels as compared to MERS-CoV (III, Fig. 5), but that should also be expected since no efficient replication was observed for MERS-CoV in MDDCs (III, Fig. 1). MxA expression, however, reached levels comparable to those induced by A/Beijing/89 virus (III, Fig. 5), indicating a relatively strong innate immune response against MERS-CoV.

Efficient mRNA production does not always correspond to efficient protein translation and/or secretion. Therefore, to further characterize the innate immune responses induced by MERS-CoV, we performed ELISA analysis of cell culture supernatants from the macrophage, MDDC and Calu-3 infection experiments to quantitate the production of IFN-λ1, TNF-α and CXCL10. No measurable amounts of IFN-λ1 or TNF-α were detected (data not shown), suggesting inefficient translation or secretion despite the observed increases in mRNA production. This was true even for Calu-3 cells, where up to a 1000-fold increase in IFN-λ1 mRNA expression in relation to uninfected cells was measured, highlighting the importance of confirming gene expression data at the protein level. CXCL10 concentrations of up to 10000 pg/ml were measured in supernatants of MERS-CoV infected macrophages and MDDCs, in contrast to no detectable CXCL10 in response to SARS-CoV infection (III, Fig. 7). A notable feature were the striking differences between donors, as half of the analyzed donors gave strong CXCL10 responses against MERS-CoV, while the other half gave no response at all (III, Fig. 7). In Calu-3 cells, SARS-CoV was a better inducer of CXCL10 than MERS-CoV (III, Fig. 7). Western blot analysis of MxA protein levels in infected macrophages and MDDCs showed that the observed increases in mRNA expression also resulted in increased MxA protein production (III, Fig. 3). In MERS-CoV infected macrophages strong MxA bands were seen from 24 h post infection onwards, whereas in MDDCs no production was detected until the 72 h time point (III, Fig. 3). SARS-CoV infection was also found to result in MxA production in macrophages at the 24 h time point (III, Fig. 3). In an effort to evaluate the biological significance of the observed cytokine production, we used UV-inactivated supernatants from the infection experiments to prime A549 cells followed by an infection with A/Beijing/89 virus. Measurement of influenza A virus M1 mRNA expression levels at 6 h post infection showed that supernatants from MERS-CoV infected macrophages and MDDCs and A/Beijing/89 infected MDDCs were the only ones to result in significant reduction of M1 expression levels (III, Fig. S1). All

this indicates that there is a significant difference in blocking host cell innate immune responses between SARS-CoV and MERS-CoV.

Determinants of SARS-CoV virulence have been well studied during the past 10 years and as a result the roles of several SARS-CoV proteins in blocking host cell innate immune responses have been elucidated (DeDiego et al. 2014). Since all coronaviruses share the polyprotein encoding 1a/1b gene, the different host cell responses to SARS-CoV and MERS-CoV are probably due to different compositions of accessory genes. For SARS-CoV, the accessory proteins Orf3a, Orf3b and Orf6 have all been shown to inhibit interferon signaling by different mechanisms (Kopecky-Bromberg et al. 2007, Minakshi et al. 2009, Spiegel et al. 2005, Frieman et al. 2007). In addition, the structural proteins M and N are known to inhibit type I interferon production (Kopecky-Bromberg et al. 2007, Siu et al. 2009). For MERS-CoV, many functions of the accessory proteins still remain unclear, but a few recent studies have identified the accessory proteins Orf4a, Orf4b and Orf5 as well as the structural and non-structural M and nsp3 proteins as IFN antagonists (Yang et al. 2013, Niemeyer et al. 2013, Matthews et al. 2014, Yang et al. 2014). The mechanisms of function have been solved for Orf4a and Orf4b, which elicit their inhibitory effects through suppression of PACT-induced activation of RIG-I (Siu et al. 2014) and through interaction with TBK1 and IKKε and inhibition of IRF3 and IRF7 (Yang et al. 2015). These differing sets of antagonistic proteins are likely the major reason for the observed variation in host response, but other factors might be involved as well.

Besides harboring different sets of IFN antagonistic proteins, another major difference between MERS-CoV and SARS-CoV is different receptor usage. ACE2, the receptor of SARS-CoV (Li et al. 2003), is widely expressed in many different tissues (Hamming et al. 2004), but reportedly not in monocytes or MDDCs (Law et al. 2005). SARS-CoV has been shown to bind to DC-SIGN, which is expressed in DCs, but binding to DC-SIGN does not facilitate entry inside the cell (Yang et al. 2004). Thus, the lack of a suitable receptor might be a factor both in the lack of SARS-CoV replication and in the lack of a SARS-CoV induced host response in macrophages and MDDCs. DPP4, the receptor for MERS-CoV (Raj et al. 2013), is also expressed in many different tissues and reportedly also in monocyte-derived macrophages and MDDCs (Lambeir et al. 2003, Zhong et al. 2013). We verified the expression of DPP4 in the cells used in our experiments by qRT-PCR and Western blot and found all of them to express DPP4, although there were differences in the expression levels between different cell types (III, Fig. 8). The lack of a receptor is therefore not an explanation for the poor replication of MERS-CoV in macrophages and MDDCs. However, the lack of N protein expression and viral RNA production in MERS-CoV infected macrophages and MDDCs (III, Fig. 1 & 3) suggests an early block in the viral replication cycle. Besides receptor availability, an alternative

reason for failed virion internalization might be deficient viral spike protein cleavage. The coronavirus S protein is a class I viral fusion protein and it requires cleavage for efficient internalization into a host cell (Bosch et al. 2003). The activity of host cell surface proteases is essential for this cleavage and thus the complement of cell surface proteases should be considered a factor affecting coronavirus host cell tropism (Millet, Whittaker 2015). MERS-CoV has been shown to utilize several different host cell proteases for S protein cleavage, including TMPRSS2 (Gierer et al. 2013), cathepsin L (Shirato, Kawase & Matsuyama 2013), furin (Burkard et al. 2014, Millet, Whittaker 2014) and DESC1 and MSPL (Zmora et al. 2014). It is unclear whether any of these can be considered the "primary" protease used by MERS-CoV for S protein cleavage, but at least the overexpression of TMPRSS2 and furin has been shown to increase host cell susceptibility to MERS-CoV infection (Shirato, Kawase & Matsuyama 2013, Millet, Whittaker 2014). It is likely that MERS-CoV can utilize different proteases in different cell types (Shirato, Kawase & Matsuyama 2013). Studies on the specific content of cell surface proteases on monocyte-derived macrophages and MDDCs are not available, but some studies on the tissue distribution of TMPRSS2 have been made. Vaarala and coworkers reported the absence of TMPRSS2 expression in peripheral leukocytes (Vaarala et al. 2001), while Bertram and coworkers found TMPRSS2 expression in alveolar macrophages and some other leukocytes (Bertram et al. 2012). An additional infection experiment with added trypsin or a similar serine protease would have helped clarify the issue of whether cell surface protease composition plays a role in MERS-CoV infection of monocyte-derived macrophages and MDDCs.

A notable aspect when dealing with primary human macrophages and dendritic cells is the possibility of large variations between individual donors. Responses to infection can have significant differences between donors and even susceptibility to infection can vary with some viruses (Bol et al. 2009). Such variability was evident in our experiments also, as striking differences were observed in CXCL10 production between donors (III, Fig. 7). Interestingly, comparison of MERS patients has revealed similar large variations between patients in their immune response to MERS-CoV (Faure et al. 2014). Clearly, individual attributes can have a large impact on the course of MERS-CoV infection and deciphering these features will help us better understand the pathogenesis of MERS-CoV. Conflicting reports have also been published on MERS-CoV replication in macrophages and MDDCs. Like us, Scheuplein and coworkers found monocyte-derived macrophages and MDDCs unpermissive for MERS-CoV replication (Scheuplein et al. 2015), while Chu and Zhou and their coworkers reported productive infection in both macrophages and MDDCs (Chu et al. 2014, Zhou et al. 2013). Whether this represents donor variability or differences in experimental settings is unclear, but our results push the consensus towards monocyte-derived macrophages and MDDCs being unsusceptible towards MERS-CoV infection.

5 Conclusions and future prospects

The influenza A virus NS1 is an incredibly multifunctional protein, having a multitude of different interactions with host cell components both in the nucleus and in the cytoplasm. Temporal and spatial regulation of the many functions of NS1 are still poorly understood, but intracellular localization undoubtedly plays a significant role in the regulation. Accordingly, nuclear localization of NS1 has been shown to be crucial for inhibiting the production of IFN-β mRNA (Min, Krug 2006, Donelan, Basler & Garcia-Sastre 2003). Despite being described over 15 years ago, the significance of the NS1 nuclear export signal for influenza A virus infection had not been analyzed. Also the mechanisms involved in NS1 nuclear export and in nucleolar localization remained poorly described.

In the present thesis work, we set out to investigate the mechanisms and impact of NS1 nuclear export and nucleolar localization. We found nuclear export of NS1 to not only be non-essential for virus infection, but we failed to see any consistent effects in growth or IFN antagonistic properties resulting from NS1 nuclear retention in mutant viruses harboring abolished NES signals. This was surprising considering the conserved nature of the NS1 NES. We did see increased IFN production and diminished growth properties resulting from certain NES mutations, but we had to assign these effects to something aside from localization since these phenotypes were not shared by all mutant viruses with inactivated NES signals. This raises additional questions on the possibility of other functions coinciding with the NS1 NES. Based on current knowledge, the most plausible explanation for the observed phenotypes is the impairment of p85β interaction, but other possibilities are sure to exist and should be looked into further. Supporting evidence for the disturbance of NS1 activation of PI3K by the NES mutations could be gained by additional experiments assessing induction of apoptosis by the mutant viruses. One should also bear in mind that these experiments were done in cell lines and therefore the results might not be an accurate representation of the situation in living animals. An obvious next step would be to conduct animal experiments using virus strains suitable for animal infections harboring the same NES mutations. There is already evidence showing a reduction in influenza A H5N1 pathogenesis in chickens upon introduction of the NS1 A149V mutation (Li et al. 2006). The same mutation does not seem to have an impact on virus replication in mammals however (Jiao et al. 2008), suggesting either strain or host specific variation in the importance of the NS1 NES region.

In the mechanistic part of our study we were unable to establish the exact mechanism of export used by NS1, but our results suggest the independence of NS1 nuclear export from CRM1. This was also somewhat surprising considering that the literature on classical leucine-rich nuclear export signals indicates that a vast majority of them mediate nuclear export through interaction with CRM1. Export through interaction with calreticulin is one possibility, but unfortunately we were unsuccessful in trying to knock down calreticulin in our experimental settings and thus cannot speculate further. Perhaps a more interesting alternative is export through the NXF1-p15 pathway. NS1 is known to interact with both NXF1 and p15 and examples from other viruses support the feasibility of this idea. More effort should be put into knocking down these host cell proteins with siRNA or other methods in order to gain a better understanding of NS1 nuclear export. Another direction for future studies is the dynamic analysis of NS1 localization kinetics using advanced microscopic methods like fluorescence recovery after photobleaching (FRAP) or fluorescence loss in photobleaching (FLIP). Such analysis would answer the question whether NS1 NES is only activated at a certain point of infection or rather upholds constant shuttling between the nucleus and cytoplasm.

In our second study we were able to elucidate the nucleolar proteins which interact with NS1 and thus drive its nucleolar localization. Our results also confirm the C-terminal NoLS as the NS1 region responsible for nucleolar localization. The functional aspect of NS1 nucleolar localization still remains elusive, as the recognized interaction partners nucleolin and fibrillarin have not been assigned any known roles in influenza A virus replication, but they make good focal points for future research aimed at solving the nucleolar function of NS1. Regulation of apoptosis through p53 interaction has been previously suggested to be the nucleolar function of NS1, but sufficient evidence is lacking to make such a statement. The reported interaction of NS1 with ADAR1 is an interesting observation and a good target for future studies.

Historically regarded only as a minor threat to public health, the human coronaviruses have gained newfound attention upon first the emergence of SARS-CoV in 2002 and now again with the emergence of MERS-CoV in 2012. While considerable effort has been put in elucidating the aspects of MERS-CoV pathogenesis, much of it still remains unclear. In the present study we showed that infection of human monocyte-derived macrophages and MDDCs with MERS-CoV does not result in productive replication. Despite the lack of replication, a significant induction of antiviral genes in response to MERS-CoV infection was measured, marking a clear difference to SARS-CoV, which barely induced any measurable increase in antiviral gene expression. Our results clarify the current consensus on MERS-CoV infection of leukocytes, since conflicting reports on macrophage and MDDC permissibility to MERS-CoV infection have been published previously. Our

observation of large differences in CXCL10 production between donors also highlights the impact of individual variation in MERS-CoV response, which is an aspect of MERS-CoV infection also reported in patients. Future studies might focus on the exact mechanisms that cause the observed differences between MERS-CoV and SARS-CoV.

6 Acknowledgements

I would like to thank Dr. Denis Kainov and the former members of his group, Dr. Maria Anastasina, Dr. Oxana Denisova and Docent Laura Kakkola for fruitful collaboration during this study.

The support of friends and family has been essential for the success of this project. I want to thank all my friends at Eteläsuomalainen osakunta for the laughter and the songs. A special thank you goes to the members of our weekly gaming group, Petri and Maiju, for the countless contests decided on a roll of a die and for sharing the troubles of PhD life over a few pints. Members of LRP and Masa, thank you for the lifetime of adventures experienced during our gaming nights.

Finally, I want to thank my parents for raising me up to trust my own skills and judgement and for always believing in me. Your love and unwavering support have encouraged me to pursue a career in science. Anniina, my beloved wife, thank you for always being there for me and for supporting me even through difficult times. Sharing the joys and sorrows of life with someone has made it all the more meaningful and I look forward to sharing the rest of my life with you.

Helsinki, May 2016

Janne Tynell

References

Abdelmohsen, K. & Gorospe, M. 2012, "RNA-binding protein nucleolin in disease", RNA biology, vol. 9, no. 6, pp. 799-808.

Adams, M.J. & Carstens, E.B. 2012, "Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2012)", Archives of Virology, vol. 157, no. 7, pp. 1411-1422.

Adney, D.R., van Doremalen, N., Brown, V.R., Bushmaker, T., Scott, D., de Wit, E., Bowen, R.A. & Munster, V.J. 2014, "Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels", Emerging infectious diseases, vol. 20, no. 12, pp. 1999-2005.

Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D. & Zychlinsky, A. 2012, "Neutrophil function: from mechanisms to disease", Annual Review of Immunology, vol. 30, pp. 459-489.

Arabi, Y.M., Arifi, A.A., Balkhy, H.H., Najm, H., Aldawood, A.S., Ghabashi, A., Hawa, H., Alothman, A., Khaldi, A. & Al Raiy, B. 2014, "Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection", Annals of Internal Medicine, vol. 160, no. 6, pp. 389-397.

Aragon, T., de la Luna, S., Novoa, I., Carrasco, L., Ortin, J. & Nieto, A. 2000, "Eukaryotic translation initiation factor 4GI is a cellular target for NS1 protein, a translational activator of influenza virus", Molecular and cellular biology, vol. 20, no. 17, pp. 6259-6268.

Aramini, J.M., Hamilton, K., Ma, L.C., Swapna, G.V., Leonard, P.G., Ladbury, J.E., Krug, R.M. & Montelione, G.T. 2014, "(19)F NMR reveals multiple conformations at the dimer interface of the nonstructural protein 1 effector domain from influenza A virus", Structure (London, England : 1993), vol. 22, no. 4, pp. 515-525.

Assiri, A., Al-Tawfiq, J.A., Al-Rabeeah, A.A., Al-Rabiah, F.A., Al-Hajjar, S., Al-Barrak, A., Flemban, H., Al-Nassir, W.N., Balkhy, H.H., Al-Hakeem, R.F., Makhdoom, H.Q., Zumla, A.I. & Memish, Z.A. 2013, "Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study", The Lancet.Infectious diseases, vol. 13, no. 9, pp. 752-761.

Ayllon, J., Russell, R.J., Garcia-Sastre, A. & Hale, B.G. 2012, "Contribution of NS1 effector domain dimerization to influenza A virus replication and virulence", Journal of virology, vol. 86, no. 23, pp. 13095-13098.

Azhar, E.I., El-Kafrawy, S.A., Farraj, S.A., Hassan, A.M., Al-Saeed, M.S., Hashem, A.M. & Madani, T.A. 2014, "Evidence for camel-to-human transmission of MERS coronavirus", The New England journal of medicine, vol. 370, no. 26, pp. 2499-2505.

Baseler, L.J., Falzarano, D., Scott, D.P., Rosenke, R., Thomas, T., Munster, V.J., Feldmann, H. & de Wit, E. 2016, "An Acute Immune Response to Middle East Respiratory Syndrome Coronavirus Replication Contributes to Viral Pathogenicity", The American journal of pathology, vol. 186, no. 3, pp. 630-638.

Bender, A., Albert, M., Reddy, A., Feldman, M., Sauter, B., Kaplan, G., Hellman, W. & Bhardwaj, N. 1998, "The distinctive features of influenza virus infection of dendritic cells", Immunobiology, vol. 198, no. 5, pp. 552-567.

Bertram, S., Glowacka, I., Muller, M.A., Lavender, H., Gnirss, K., Nehlmeier, I., Niemeyer, D., He, Y., Simmons, G., Drosten, C., Soilleux, E.J., Jahn, O., Steffen, I. & Pohlmann, S. 2011, "Cleavage and activation of the severe acute respiratory syndrome coronavirus spike protein by human airway trypsin-like protease", Journal of virology, vol. 85, no. 24, pp. 13363-13372.

Bertram, S., Heurich, A., Lavender, H., Gierer, S., Danisch, S., Perin, P., Lucas, J.M., Nelson, P.S.,

Pohlmann, S. & Soilleux, E.J. 2012, "Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts", PloS one, vol. 7, no. 4, pp. e35876.

Boisvert, F.M., van Koningsbruggen, S., Navascues, J. & Lamond, A.I. 2007, "The multifunctional nucleolus", Nature reviews.Molecular cell biology, vol. 8, no. 7, pp. 574-585.

Bol, S.M., van Remmerden, Y., Sietzema, J.G., Kootstra, N.A., Schuitemaker, H. & van't Wout, A.B. 2009, "Donor variation in in vitro HIV-1 susceptibility of monocyte-derived macrophages", Virology, vol. 390, no. 2, pp. 205-211.

Bornholdt, Z.A. & Prasad, B.V. 2008, "X-ray structure of NS1 from a highly pathogenic H5N1 influenza virus", Nature, vol. 456, no. 7224, pp. 985-988.

Bornholdt, Z.A. & Prasad, B.V. 2006, "X-ray structure of influenza virus NS1 effector domain", Nature structural & molecular biology, vol. 13, no. 6, pp. 559-560.

Bosch, B.J., van der Zee, R., de Haan, C.A. & Rottier, P.J. 2003, "The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex", Journal of virology, vol. 77, no. 16, pp. 8801-8811.

Bottcher-Friebertshauser, E., Garten, W., Matrosovich, M. & Klenk, H.D. 2014, "The hemagglutinin: a determinant of pathogenicity", Current topics in microbiology and immunology, vol. 385, pp. 3-34.

Brandes, M., Klauschen, F., Kuchen, S. & Germain, R.N. 2013, "A systems analysis identifies a feedforward inflammatory circuit leading to lethal influenza infection", Cell, vol. 154, no. 1, pp. 197-212.

Brooks, A.J., Johansson, M., John, A.V., Xu, Y., Jans, D.A. & Vasudevan, S.G. 2002, "The interdomain region of dengue NS5 protein that binds to the viral helicase NS3 contains independently functional importin beta 1 and importin alpha/beta-recognized nuclear localization signals", The Journal of biological chemistry, vol. 277, no. 39, pp. 36399-36407.

Burgui, I., Aragon, T., Ortin, J. & Nieto, A. 2003, "PABP1 and eIF4GI associate with influenza virus NS1 protein in viral mRNA translation initiation complexes", The Journal of general virology, vol. 84, no. Pt 12, pp. 3263-3274.

Burkard, C., Verheije, M.H., Wicht, O., van Kasteren, S.I., van Kuppeveld, F.J., Haagmans, B.L., Pelkmans, L., Rottier, P.J., Bosch, B.J. & de Haan, C.A. 2014, "Coronavirus cell entry occurs through the endo-/lysosomal pathway in a proteolysis-dependent manner", PLoS pathogens, vol. 10, no. 11, pp. e1004502.

Caly, L., Wagstaff, K.M. & Jans, D.A. 2012, "Nuclear trafficking of proteins from RNA viruses: potential target for antivirals?", Antiviral Research, vol. 95, no. 3, pp. 202-206.

Carrillo, B., Choi, J.M., Bornholdt, Z.A., Sankaran, B., Rice, A.P. & Prasad, B.V. 2014, "The influenza A virus protein NS1 displays structural polymorphism", Journal of virology, vol. 88, no. 8, pp. 4113-4122.

Centers for Disease Control and Prevention (CDC) 2003, "Update: Outbreak of severe acute respiratory syndrome--worldwide, 2003", MMWR.Morbidity and mortality weekly report, vol. 52, no. 12, pp. 241-6, 248.

Chan, K.S., Lai, S.T., Chu, C.M., Tsui, E., Tam, C.Y., Wong, M.M., Tse, M.W., Que, T.L., Peiris, J.S., Sung, J., Wong, V.C. & Yuen, K.Y. 2003, "Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study", Hong Kong medical journal = Xianggang yi xue za zhi / Hong Kong Academy of Medicine, vol. 9, no. 6, pp. 399-406.

Chan, P.K. & Chen, G.G. 2008, "Mechanisms of lymphocyte loss in SARS coronavirus infection", Hong Kong medical journal = Xianggang yi xue

za zhi / Hong Kong Academy of Medicine, vol. 14 Suppl 4, pp. 21-26.

Chen, R.C., Tang, X.P., Tan, S.Y., Liang, B.L., Wan, Z.Y., Fang, J.Q. & Zhong, N. 2006, "Treatment of severe acute respiratory syndrome with glucosteroids: the Guangzhou experience", Chest, vol. 129, no. 6, pp. 1441-1452.

Chen, W., Calvo, P.A., Malide, D., Gibbs, J., Schubert, U., Bacik, I., Basta, S., O'Neill, R., Schickli, J., Palese, P., Henklein, P., Bennink, J.R. & Yewdell, J.W. 2001, "A novel influenza A virus mitochondrial protein that induces cell death", Nature medicine, vol. 7, no. 12, pp. 1306-1312.

Chen, Z., Li, Y. & Krug, R. 1999, "Influenza A virus NS1 protein targets poly(A)-binding protein II of the cellular 3 '-end processing machinery", Embo Journal, vol. 18, no. 8, pp. 2273-2283.

Cheung, C.Y., Poon, L.L., Lau, A.S., Luk, W., Lau, Y.L., Shortridge, K.F., Gordon, S., Guan, Y. & Peiris, J.S. 2002, "Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease?", Lancet (London, England), vol. 360, no. 9348, pp. 1831-1837.

Chowell, G., Blumberg, S., Simonsen, L., Miller, M.A. & Viboud, C. 2014, "Synthesizing data and models for the spread of MERS-CoV, 2013: key role of index cases and hospital transmission", Epidemics, vol. 9, pp. 40-51.

Chu, H., Zhou, J., Wong, B.H., Li, C., Chan, J.F., Cheng, Z.S., Yang, D., Wang, D., Lee, A.C., Li, C., Yeung, M.L., Cai, J.P., Chan, I.H., Ho, W.K., To, K.K., Zheng, B.J., Yao, Y., Qin, C. & Yuen, K.Y. 2016, "Middle East Respiratory Syndrome Coronavirus Efficiently Infects Human Primary T Lymphocytes and Activates the Extrinsic and Intrinsic Apoptosis Pathways", The Journal of infectious diseases, vol. 213, no. 6, pp. 904-914.

Chu, H., Zhou, J., Wong, B.H., Li, C., Cheng, Z.S., Lin, X., Poon, V.K., Sun, T., Lau, C.C., Chan, J.F., To, K.K., Chan, K.H., Lu, L., Zheng, B.J. & Yuen, K.Y. 2014, "Productive replication of

Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response", Virology, vol. 454-455, pp. 197-205.

Chua, M.A., Schmid, S., Perez, J.T., Langlois, R.A. & Tenoever, B.R. 2013, "Influenza A virus utilizes suboptimal splicing to coordinate the timing of infection", Cell reports, vol. 3, no. 1, pp. 23-29.

Collins, A.R. 1998, "Human macrophages are susceptible to coronavirus OC43", Advances in Experimental Medicine and Biology, vol. 440, pp. 635-639.

Colombo, E., Marine, J.C., Danovi, D., Falini, B. & Pelicci, P.G. 2002, "Nucleophosmin regulates the stability and transcriptional activity of p53", Nature cell biology, vol. 4, no. 7, pp. 529-533.

Colonna, M., Trinchieri, G. & Liu, Y.J. 2004, "Plasmacytoid dendritic cells in immunity", Nature immunology, vol. 5, no. 12, pp. 1219-1226.

Corman, V.M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bludau, M., van Boheemen, S., Gopal, R., Ballhause, M., Bestebroer, T.M., Muth, D., Mueller, M.A., Drexler, J.F., Zambon, M., Osterhaus, A.D., Fouchier, R.M. & Drosten, C. 2012, "Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction", Eurosurveillance, vol. 17, no. 39, pp. 3-8.

Corman, V.M., Ithete, N.L., Richards, L.R., Schoeman, M.C., Preiser, W., Drosten, C. & Drexler, J.F. 2014a, "Rooting the phylogenetic tree of middle East respiratory syndrome coronavirus by characterization of a conspecific virus from an African bat", Journal of virology, vol. 88, no. 19, pp. 11297-11303.

Corman, V.M., Jores, J., Meyer, B., Younan, M., Liljander, A., Said, M.Y., Gluecks, I., Lattwein, E., Bosch, B.J., Drexler, J.F., Bornstein, S., Drosten, C. & Muller, M.A. 2014b, "Antibodies against MERS coronavirus in dromedary camels,

Kenya, 1992-2013", Emerging infectious diseases, vol. 20, no. 8, pp. 1319-1322.

Cotten, M., Watson, S.J., Zumla, A.I., Makhdoom, H.Q., Palser, A.L., Ong, S.H., Al Rabeeah, A.A., Alhakeem, R.F., Assiri, A., Al-Tawfiq, J.A., Albarrak, A., Barry, M., Shibl, A., Alrabiah, F.A., Hajjar, S., Balkhy, H.H., Flemban, H., Rambaut, A., Kellam, P. & Memish, Z.A. 2014, "Spread, circulation, and evolution of the Middle East respiratory syndrome coronavirus", mBio, vol. 5, no. 1, pp. 10.1128/mBio.01062-13.

Darapaneni, V., Prabhaker, V.K. & Kukol, A. 2009, "Large-scale analysis of influenza A virus sequences reveals potential drug target sites of non-structural proteins", Journal of General Virology, vol. 90, pp. 2124-2133.

Das, K., Ma, L., Xiao, R., Radvansky, B., Aramini, J., Zhao, L., Marklund, J., Kuo, R., Twu, K.Y., Arnold, E., Krug, R.M. & Montelione, G.T. 2008, "Structural basis for suppression of a host antiviral response by influenza A virus", Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 35, pp. 13093-13098.

de Chassey, B., Aublin-Gex, A., Ruggieri, A., Meyniel-Schicklin, L., Pradezynski, F., Davoust, N., Chantier, T., Tafforeau, L., Mangeot, P.E., Ciancia, C., Perrin-Cocon, L., Bartenschlager, R., Andre, P. & Lotteau, V. 2013, "The interactomes of influenza virus NS1 and NS2 proteins identify new host factors and provide insights for ADAR1 playing a supportive role in virus replication", PLoS pathogens, vol. 9, no. 7, pp. e1003440.

de Heer, H.J., Hammad, H., Soullie, T., Hijdra, D., Vos, N., Willart, M.A., Hoogsteden, H.C. & Lambrecht, B.N. 2004, "Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen", The Journal of experimental medicine, vol. 200, no. 1, pp. 89-98.

DeDiego, M.L., Nieto-Torres, J.L., Jimenez-Guardeno, J.M., Regla-Nava, J.A., Castano-Rodriguez, C., Fernandez-Delgado, R., Usera, F. & Enjuanes, L. 2014, "Coronavirus virulence genes with main focus on SARS-CoV envelope gene", Virus research, vol. 194, pp. 124-137.

del Rio, M.L., Rodriguez-Barbosa, J.I., Kremmer, E. & Forster, R. 2007, "CD103- and CD103+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4+ and CD8+ T cells", Journal of immunology (Baltimore, Md.: 1950), vol. 178, no. 11, pp. 6861-6866.

Denison, M.R., Graham, R.L., Donaldson, E.F., Eckerle, L.D. & Baric, R.S. 2011, "Coronaviruses: an RNA proofreading machine regulates replication fidelity and diversity", RNA biology, vol. 8, no. 2, pp. 270-279.

Desterro, J.M., Keegan, L.P., Lafarga, M., Berciano, M.T., O'Connell, M. & Carmo-Fonseca, M. 2003, "Dynamic association of RNA-editing enzymes with the nucleolus", Journal of cell science, vol. 116, no. Pt 9, pp. 1805-1818.

Ding, Y., He, L., Zhang, Q., Huang, Z., Che, X., Hou, J., Wang, H., Shen, H., Qiu, L., Li, Z., Geng, J., Cai, J., Han, H., Li, X., Kang, W., Weng, D., Liang, P. & Jiang, S. 2004, "Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways", The Journal of pathology, vol. 203, no. 2, pp. 622-630.

Donelan, N.R., Basler, C.F. & Garcia-Sastre, A. 2003, "A recombinant influenza A virus expressing an RNA-binding-defective NS1 protein induces high levels of beta interferon and is attenuated in mice", Journal of virology, vol. 77, no. 24, pp. 13257-13266.

Drosten, C., Gunther, S., Preiser, W., van der Werf, S., Brodt, H., Becker, S., Rabenau, H., Panning, M., Kolesnikova, L., Fouchier, R., Berger, A., Burguiere, A., Cinatl, J., Eickmann, M., Escriou, N., Grywna, K., Kramme, S., Manuguerra, J., Muller, S., Rickerts, V., Sturmer, M., Vieth, S., Klenk, H., Osterhaus, A., Schmitz, H. & Doerr,

H. 2003, "Identification of a novel coronavirus in patients with severe acute respiratory syndrome", New England Journal of Medicine, vol. 348, no. 20, pp. 1967-1976.

Drosten, C., Seilmaier, M., Corman, V.M., Hartmann, W., Scheible, G., Sack, S., Guggemos, W., Kallies, R., Muth, D., Junglen, S., Muller, M.A., Haas, W., Guberina, H., Rohnisch, T., Schmid-Wendtner, M., Aldabbagh, S., Dittmer, U., Gold, H., Graf, P., Bonin, F., Rambaut, A. & Wendtner, C.M. 2013, "Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection", The Lancet.Infectious diseases, vol. 13, no. 9, pp. 745-751.

Dundon, W.G. & Capua, I. 2009, "A Closer Look at the NS1 of Influenza Virus", Viruses, vol. 1, no. 3, pp. 1057-1072.

Ehrhardt, C., Marjuki, H., Wolff, T., Nuernberg, B., Planz, O., Pleschka, S. & Ludwig, S. 2006, "Bivalent role of the phosphatidylinositol-3-kinase (PI3K) during influenza virus infection and host cell defence", Cellular microbiology, vol. 8, no. 8, pp. 1336-1348.

Ehrhardt, C., Wolff, T., Pleschka, S., Planz, O., Beermann, W., Bode, J.G., Schmolke, M. & Ludwig, S. 2007, "Influenza A virus NS1 protein activates the PI3K/Akt pathway to mediate antiapoptotic signaling responses", Journal of virology, vol. 81, no. 7, pp. 3058-3067.

Elderfield, R. & Barclay, W. 2011, "Influenza pandemics", Advances in Experimental Medicine and Biology, vol. 719, pp. 81-103.

Emmott, E. & Hiscox, J.A. 2009, "Nucleolar targeting: the hub of the matter", EMBO reports, vol. 10, no. 3, pp. 231-238.

Falcon, A.M., Fortes, P., Marion, R.M., Beloso, A. & Ortin, J. 1999, "Interaction of influenza virus NS1 protein and the human homologue of Staufen in vivo and in vitro", Nucleic acids research, vol. 27, no. 11, pp. 2241-2247.

Falzarano, D., de Wit, E., Feldmann, F., Rasmussen, A.L., Okumura, A., Peng, X., Thomas, M.J., van Doremalen, N., Haddock, E., Nagy, L., LaCasse, R., Liu, T., Zhu, J., McLellan, J.S., Scott, D.P., Katze, M.G., Feldmann, H. & Munster, V.J. 2014, "Infection with MERS-CoV causes lethal pneumonia in the common marmoset", PLoS pathogens, vol. 10, no. 8, pp. e1004250.

Falzarano, D., de Wit, E., Rasmussen, A.L., Feldmann, F., Okumura, A., Scott, D.P., Brining, D., Bushmaker, T., Martellaro, C., Baseler, L., Benecke, A.G., Katze, M.G., Munster, V.J. & Feldmann, H. 2013, "Treatment with interferon-alpha2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques", Nature medicine, vol. 19, no. 10, pp. 1313-1317.

Fan, S., Macken, C.A., Li, C., Ozawa, M., Goto, H., Iswahyudi, N.F., Nidom, C.A., Chen, H., Neumann, G. & Kawaoka, Y. 2013, "Synergistic effect of the PDZ and p85beta-binding domains of the NS1 protein on virulence of an avian H5N1 influenza A virus", Journal of virology, vol. 87, no. 9, pp. 4861-4871.

Fang, F.C. 2004, "Antimicrobial reactive oxygen and nitrogen species: concepts and controversies", Nature reviews.Microbiology, vol. 2, no. 10, pp. 820-832.

Fankhauser, C., Izaurralde, E., Adachi, Y., Wingfield, P. & Laemmli, U.K. 1991, "Specific complex of human immunodeficiency virus type 1 rev and nucleolar B23 proteins: dissociation by the Rev response element", Molecular and cellular biology, vol. 11, no. 5, pp. 2567-2575.

Faure, E., Poissy, J., Goffard, A., Fournier, C., Kipnis, E., Titecat, M., Bortolotti, P., Martinez, L., Dubucquoi, S., Dessein, R., Gosset, P., Mathieu, D. & Guery, B. 2014, "Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside?", PloS one, vol. 9, no. 2, pp. e88716.

Fehr, A.R. & Perlman, S. 2015, "Coronaviruses: an overview of their replication and pathogenesis", Methods in molecular biology (Clifton, N.J.), vol. 1282, pp. 1-23.

File, T.M. 2003, "Community-acquired pneumonia", Lancet (London, England), vol. 362, no. 9400, pp. 1991-2001.

File, T.M.,Jr & Marrie, T.J. 2010, "Burden of community-acquired pneumonia in North American adults", Postgraduate medicine, vol. 122, no. 2, pp. 130-141.

Fiore, A.E., Bridges, C.B. & Cox, N.J. 2009, "Seasonal influenza vaccines", Current topics in microbiology and immunology, vol. 333, pp. 43-82.

Fornerod, M., Ohno, M., Yoshida, M. & Mattaj, I.W. 1997, "CRM1 is an export receptor for leucine-rich nuclear export signals", Cell, vol. 90, no. 6, pp. 1051-1060.

Fortes, P., Beloso, A. & Ortin, J. 1994, "Influenza-Virus Ns1 Protein Inhibits Premessenger Rna Splicing and Blocks Messenger-Rna Nucleocytoplasmic Transport", Embo Journal, vol. 13, no. 3, pp. 704-712.

Franca, M.S. & Brown, J.D. 2014, "Influenza pathobiology and pathogenesis in avian species", Current topics in microbiology and immunology, vol. 385, pp. 221-242.

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", Journal of virology, vol. 81, no. 18, pp. 9812-9824.

Fukuda, M., Asano, S., Nakamura, T., Adachi, M., Yoshida, M., Yanagida, M. & Nishida, E. 1997, "CRM1 is responsible for intracellular transport mediated by the nuclear export signal", Nature, vol. 390, no. 6657, pp. 308-311.

Funk, C.J., Wang, J., Ito, Y., Travanty, E.A., Voelker, D.R., Holmes, K.V. & Mason, R.J. 2012, "Infection of human alveolar macrophages by human coronavirus strain 229E", Journal of General Virology, vol. 93, pp. 494-503.

Gabriel, G. & Fodor, E. 2014, "Molecular determinants of pathogenicity in the polymerase complex", Current topics in microbiology and immunology, vol. 385, pp. 35-60.

Gack, M.U., Albrecht, R.A., Urano, T., Inn, K., Huang, I., Carnero, E., Farzan, M., Inoue, S., Jung, J.U. & Garcia-Sastre, A. 2009, "Influenza A Virus NS1 Targets the Ubiquitin Ligase TRIM25 to Evade Recognition by the Host Viral RNA Sensor RIG-I", Cell Host & Microbe, vol. 5, no. 5, pp. 439-449.

Gao, S., Song, L., Li, J., Zhang, Z., Peng, H., Jiang, W., Wang, Q., Kang, T., Chen, S. & Huang, W. 2012, "Influenza A virus-encoded NS1 virulence factor protein inhibits innate immune response by targeting IKK", Cellular microbiology, vol. 14, no. 12, pp. 1849-1866.

Garaigorta, U. & Ortin, J. 2007, "Mutation analysis of a recombinant NS replicon shows that influenza virus NS1 protein blocks the splicing and nucleo-cytoplasmic transport of its own viral mRNA", Nucleic acids research, vol. 35, no. 14, pp. 4573-4582.

Garcia-Sastre, A., Egorov, A., Matassov, D., Brandt, S., Levy, D.E., Durbin, J.E., Palese, P. & Muster, T. 1998, "Influenza A virus lacking the NS1 gene replicates in interferon-deficient systems", Virology, vol. 252, no. 2, pp. 324-330.

Ge, X.Y., Li, J.L., Yang, X.L., Chmura, A.A., Zhu, G., Epstein, J.H., Mazet, J.K., Hu, B., Zhang, W., Peng, C., Zhang, Y.J., Luo, C.M., Tan, B., Wang, N., Zhu, Y., Crameri, G., Zhang, S.Y., Wang, L.F., Daszak, P. & Shi, Z.L. 2013, "Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor", Nature, vol. 503, no. 7477, pp. 535-538.

Ghildyal, R., Baulch-Brown, C., Mills, J. & Meanger, J. 2003, "The matrix protein of Human respiratory syncytial virus localises to the nucleus of infected cells and inhibits transcription", Archives of Virology, vol. 148, no. 7, pp. 1419-1429.

Ghildyal, R., Ho, A., Dias, M., Soegiyono, L., Bardin, P.G., Tran, K.C., Teng, M.N. & Jans, D.A. 2009, "The respiratory syncytial virus matrix protein possesses a Crm1-mediated nuclear export mechanism", Journal of virology, vol. 83, no. 11, pp. 5353-5362.

Gierer, S., Bertram, S., Kaup, F., Wrensch, F., Heurich, A., Kramer-Kuhl, A., Welsch, K., Winkler, M., Meyer, B., Drosten, C., Dittmer, U., von Hahn, T., Simmons, G., Hofmann, H. & Pohlmann, S. 2013, "The spike protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralizing antibodies", Journal of virology, vol. 87, no. 10, pp. 5502-5511.

Gordon, S. 2002, "Pattern recognition receptors: doubling up for the innate immune response", Cell, vol. 111, no. 7, pp. 927-930.

Goronzy, J.J., Fang, F., Cavanagh, M.M., Qi, Q. & Weyand, C.M. 2015, "Naive T cell maintenance and function in human aging", Journal of immunology (Baltimore, Md.: 1950), vol. 194, no. 9, pp. 4073-4080.

Graham, R.L. & Baric, R.S. 2010, "Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission", Journal of virology, vol. 84, no. 7, pp. 3134-3146.

Greenspan, D., Palese, P. & Krystal, M. 1988, "Two nuclear location signals in the influenza virus NS1 nonstructural protein", Journal of virology, vol. 62, no. 8, pp. 3020-3026.

Gu, J., Gong, E., Zhang, B., Zheng, J., Gao, Z., Zhong, Y., Zou, W., Zhan, J., Wang, S., Xie, Z., Zhuang, H., Wu, B., Zhong, H., Shao, H., Fang, W., Gao, D., Pei, F., Li, X., He, Z., Xu, D., Shi, X., Anderson, V.M. & Leong, A.S. 2005, "Multiple organ infection and the pathogenesis of SARS", The Journal of experimental medicine, vol. 202, no. 3, pp. 415-424.

Guan, Y., Zheng, B.J., He, Y.Q., Liu, X.L., Zhuang, Z.X., Cheung, C.L., Luo, S.W., Li, P.H., Zhang, L.J., Guan, Y.J., Butt, K.M., Wong, K.L., Chan, K.W., Lim, W., Shortridge, K.F., Yuen, K.Y., Peiris, J.S. & Poon, L.L. 2003, "Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China", Science (New York, N.Y.), vol. 302, no. 5643, pp. 276-278.

Guo, Y.J., Jin, F.G., Wang, P., Wang, M. & Zhu, J.M. 1983, "Isolation of influenza C virus from pigs and experimental infection of pigs with influenza C virus", The Journal of general virology, vol. 64 (Pt 1), no. Pt 1, pp. 177-182.

Hale, B.G. 2014, "Conformational plasticity of the influenza A virus NS1 protein", The Journal of general virology, vol. 95, no. Pt 10, pp. 2099-2105.

Hale, B.G., Barclay, W.S., Randall, R.E. & Russell, R.J. 2008, "Structure of an avian influenza A virus NS1 protein effector domain", Virology, vol. 378, no. 1, pp. 1-5.

Hale, B.G., Jackson, D., Chen, Y., Lamb, R.A. & Randall, R.E. 2006, "Influenza A virus NS1 protein binds p85 beta and activates phosphatidylinositol-3-kinase signaling", Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 38, pp. 14194-14199.

Hale, B.G., Kerry, P.S., Jackson, D., Precious, B.L., Gray, A., Killip, M.J., Randall, R.E. & Russell, R.J. 2010, "Structural insights into phosphoinositide 3-kinase activation by the influenza A virus NS1 protein", Proceedings of the National Academy of Sciences of the United States of America, vol. 107, no. 5, pp. 1954-1959.

Hale, B.G., Randall, R.E., Ortin, J. & Jackson, D. 2008, "The multifunctional NS1 protein of influenza A viruses", Journal of General Virology, vol. 89, pp. 2359-2376.

Hamming, I., Timens, W., Bulthuis, M.L., Lely, A.T., Navis, G. & van Goor, H. 2004, "Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in

understanding SARS pathogenesis", The Journal of pathology, vol. 203, no. 2, pp. 631-637.

Hamre, D. & Procknow, J.J. 1966, "A new virus isolated from the human respiratory tract", Proceedings of the Society for Experimental Biology and Medicine.Society for Experimental Biology and Medicine (New York, N.Y.), vol. 121, no. 1, pp. 190-193.

Han, H., Cui, Z., Wang, W., Zhang, Z., Wei, H., Zhou, Y. & Zhang, X. 2010, "New regulatory mechanisms for the intracellular localization and trafficking of influenza A virus NS1 protein revealed by comparative analysis of A/PR/8/34 and A/Sydney/5/97", Journal of General Virology, vol. 91, pp. 2907-2917.

Hashimoto, Y., Moki, T., Takizawa, T., Shiratsuchi, A. & Nakanishi, Y. 2007, "Evidence for phagocytosis of influenza virus-infected, apoptotic cells by neutrophils and macrophages in mice", Journal of immunology (Baltimore, Md.: 1950), vol. 178, no. 4, pp. 2448-2457.

Heikkinen, L.S., Kazlauskas, A., Melen, K., Wagner, R., Ziegler, T., Julkunen, I. & Saksela, K. 2008, "Avian and 1918 Spanish influenza a virus NS1 proteins bind to Crk/CrkL Src homology 3 domains to activate host cell signaling", The Journal of biological chemistry, vol. 283, no. 9, pp. 5719-5727.

Heikkinen, T. & Jarvinen, A. 2003, "The common cold", Lancet (London, England), vol. 361, no. 9351, pp. 51-59.

Hemida, M.G., Elmoslemany, A., Al-Hizab, F., Alnaeem, A., Almathen, F., Faye, B., Chu, D.K., Perera, R.A. & Peiris, M. 2015, "Dromedary Camels and the Transmission of Middle East Respiratory Syndrome Coronavirus (MERS-CoV)", Transboundary and emerging diseases, .

Herold, S., Steinmueller, M., von Wulffen, W., Cakarova, L., Pinto, R., Pleschka, S., Mack, M., Kuziel, W.A., Corazza, N., Brunner, T., Seeger, W. & Lohmeyer, J. 2008, "Lung epithelial apoptosis in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing ligand", The Journal of experimental medicine, vol. 205, no. 13, pp. 3065-3077.

Herz, C., Stavnezer, E., Krug, R. & Gurney, T.,Jr 1981, "Influenza virus, an RNA virus, synthesizes its messenger RNA in the nucleus of infected cells", Cell, vol. 26, no. 3 Pt 1, pp. 391-400.

Hirano, M., Kaneko, S., Yamashita, T., Luo, H., Qin, W., Shirota, Y., Nomura, T., Kobayashi, K. & Murakami, S. 2003, "Direct interaction between nucleolin and hepatitis C virus NS5B", The Journal of biological chemistry, vol. 278, no. 7, pp. 5109-5115.

Hiscox, J.A. 2007, "RNA viruses: hijacking the dynamic nucleolus", Nature reviews.Microbiology, vol. 5, no. 2, pp. 119-127.

Hocke, A.C., Becher, A., Knepper, J., Peter, A., Holland, G., Tonnies, M., Bauer, T.T., Schneider, P., Neudecker, J., Muth, D., Wendtner, C.M., Ruckert, J.C., Drosten, C., Gruber, A.D., Laue, M., Suttorp, N., Hippenstiel, S. & Wolff, T. 2013, "Emerging human middle East respiratory syndrome coronavirus causes widespread infection and alveolar damage in human lungs", American journal of respiratory and critical care medicine, vol. 188, no. 7, pp. 882-886.

Holaska, J., Black, B., Love, D., Hanover, J., Leszyk, J. & Paschal, B. 2001, "Calreticulin is a receptor for nuclear export", Journal of Cell Biology, vol. 152, no. 1, pp. 127-140.

Holaska, J., Black, B., Rastinejad, F. & Paschal, B. 2002, "Ca2+-dependent nuclear export mediated by calreticulin", Molecular and cellular biology, vol. 22, no. 17, pp. 6286-6297.

Holt, P.G., Oliver, J., Bilyk, N., McMenamin, C., McMenamin, P.G., Kraal, G. & Thepen, T. 1993, "Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells in vivo by resident alveolar macrophages", The Journal of experimental medicine, vol. 177, no. 2, pp. 397-407.

Hu, B., Ge, X., Wang, L.F. & Shi, Z. 2015, "Bat origin of human coronaviruses", Virology journal, vol. 12, no. 1, pp. 221-015-0422-1.

Hui, D.S., Memish, Z.A. & Zumla, A. 2014, "Severe acute respiratory syndrome vs. the Middle East respiratory syndrome", Current opinion in pulmonary medicine, vol. 20, no. 3, pp. 233-241.

Iijima, N., Mattei, L.M. & Iwasaki, A. 2011, "Recruited inflammatory monocytes stimulate antiviral Th1 immunity in infected tissue", Proceedings of the National Academy of Sciences of the United States of America, vol. 108, no. 1, pp. 284-289.

Ithete, N.L., Stoffberg, S., Corman, V.M., Cottontail, V.M., Richards, L.R., Schoeman, M.C., Drosten, C., Drexler, J.F. & Preiser, W. 2013, "Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa", Emerging infectious diseases, vol. 19, no. 10, pp. 1697-1699.

Izumi, R.E., Valdez, B., Banerjee, R., Srivastava, M. & Dasgupta, A. 2001, "Nucleolin stimulates viral internal ribosome entry site-mediated translation", Virus research, vol. 76, no. 1, pp. 17-29.

Jackson, D., Hossain, M.J., Hickman, D., Perez, D.R. & Lamb, R.A. 2008, "A new influenza virus virulence determinant: the NS1 protein four C-terminal residues modulate pathogenicity", Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 11, pp. 4381-4386.

Jackson, D.A., Caton, A.J., McCready, S.J. & Cook, P.R. 1982, "Influenza virus RNA is synthesized at fixed sites in the nucleus", Nature, vol. 296, no. 5855, pp. 366-368.

Jagger, B.W., Wise, H.M., Kash, J.C., Walters, K.A., Wills, N.M., Xiao, Y.L., Dunfee, R.L., Schwartzman, L.M., Ozinsky, A., Bell, G.L., Dalton, R.M., Lo, A., Efstathiou, S., Atkins, J.F., Firth, A.E., Taubenberger, J.K. & Digard, P. 2012, "An overlapping protein-coding region in influenza A virus segment 3 modulates the host response", Science (New York, N.Y.), vol. 337, no. 6091, pp. 199-204.

James, A., Wang, Y., Raje, H., Rosby, R. & DiMario, P. 2014, "Nucleolar stress with and without p53", Nucleus (Austin, Tex.), vol. 5, no. 5, pp. 402-426.

Jiang, Y., Xu, J., Zhou, C., Wu, Z., Zhong, S., Liu, J., Luo, W., Chen, T., Qin, Q. & Deng, P. 2005, "Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome", American journal of respiratory and critical care medicine, vol. 171, no. 8, pp. 850-857.

Jiao, P., Tian, G., Li, Y., Deng, G., Jiang, Y., Liu, C., Liu, W., Bu, Z., Kawaoka, Y. & Chen, H. 2008, "A single-amino-acid substitution in the NS1 protein changes the pathogenicity of H5N1 avian influenza viruses in mice", Journal of virology, vol. 82, no. 3, pp. 1146-1154.

Josefowicz, S.Z., Niec, R.E., Kim, H.Y., Treuting, P., Chinen, T., Zheng, Y., Umetsu, D.T. & Rudensky, A.Y. 2012, "Extrathymically generated regulatory T cells control mucosal TH2 inflammation", Nature, vol. 482, no. 7385, pp. 395-399.

Juillard, F., Hiriart, E., Sergeant, N., Vingtdeux-Didier, V., Drobecq, H., Sergeant, A., Manet, E. & Gruffat, H. 2009, "Epstein-Barr virus protein EB2 contains an N-terminal transferable nuclear export signal that promotes nucleocytoplasmic export by directly binding TAP/NXF1", Journal of virology, vol. 83, no. 24, pp. 12759-12768.

Kan, B., Wang, M., Jing, H., Xu, H., Jiang, X., Yan, M., Liang, W., Zheng, H., Wan, K., Liu, Q., Cui, B., Xu, Y., Zhang, E., Wang, H., Ye, J., Li, G., Li, M., Cui, Z., Qi, X., Chen, K., Du, L., Gao, K., Zhao, Y.T., Zou, X.Z., Feng, Y.J., Gao, Y.F., Hai, R., Yu, D., Guan, Y. & Xu, J. 2005, "Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms", Journal of virology, vol. 79, no. 18, pp. 11892-11900.

Kao, C.F., Chen, S.Y. & Lee, Y.H. 2004, "Activation of RNA polymerase I transcription by hepatitis C virus core protein", Journal of Biomedical Science, vol. 11, no. 1, pp. 72-94.

Kerry, P.S., Ayllon, J., Taylor, M.A., Hass, C., Lewis, A., Garcia-Sastre, A., Randall, R.E., Hale, B.G. & Russell, R.J. 2011, "A transient homotypic interaction model for the influenza A virus NS1 protein effector domain", PloS one, vol. 6, no. 3, pp. e17946.

Kim, T.S., Gorski, S.A., Hahn, S., Murphy, K.M. & Braciale, T.J. 2014, "Distinct dendritic cell subsets dictate the fate decision between effector and memory CD8(+) T cell differentiation by a CD24-dependent mechanism", Immunity, vol. 40, no. 3, pp. 400-413.

Klibanov, S.A., O'Hagan, H.M. & Ljungman, M. 2001, "Accumulation of soluble and nucleolar-associated p53 proteins following cellular stress", Journal of cell science, vol. 114, no. Pt 10, pp. 1867-1873.

Kopecky-Bromberg, S.A., Martinez-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", Journal of virology, vol. 81, no. 2, pp. 548-557.

Kopf, M., Schneider, C. & Nobs, S.P. 2015, "The development and function of lung-resident macrophages and dendritic cells", Nature immunology, vol. 16, no. 1, pp. 36-44.

Korea Centers for Disease Control and Prevention 2015, "Middle East Respiratory Syndrome Coronavirus Outbreak in the Republic of Korea, 2015", Osong public health and research perspectives, vol. 6, no. 4, pp. 269-278.

Korgaonkar, C., Hagen, J., Tompkins, V., Frazier, A.A., Allamargot, C., Quelle, F.W. & Quelle, D.E. 2005, "Nucleophosmin (B23) targets ARF to nucleoli and inhibits its function", Molecular and cellular biology, vol. 25, no. 4, pp. 1258-1271.

Kudva, A., Scheller, E.V., Robinson, K.M., Crowe, C.R., Choi, S.M., Slight, S.R., Khader, S.A., Dubin, P.J., Enelow, R.I., Kolls, J.K. & Alcorn, J.F. 2011, "Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice", Journal of immunology (Baltimore, Md.: 1950), vol. 186, no. 3, pp. 1666-1674.

Kumagai, Y., Takeuchi, O., Kato, H., Kumar, H., Matsui, K., Morii, E., Aozasa, K., Kawai, T. & Akira, S. 2007, "Alveolar macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses", Immunity, vol. 27, no. 2, pp. 240-252.

Kurki, S., Peltonen, K., Latonen, L., Kiviharju, T.M., Ojala, P.M., Meek, D. & Laiho, M. 2004, "Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation", Cancer cell, vol. 5, no. 5, pp. 465-475.

Lam, W.Y., Tang, J.W., Yeung, A.C., Chiu, L.C., Sung, J.J. & Chan, P.K. 2008, "Avian influenza virus A/HK/483/97(H5N1) NS1 protein induces apoptosis in human airway epithelial cells", Journal of virology, vol. 82, no. 6, pp. 2741-2751.

Lambeir, A.M., Durinx, C., Scharpe, S. & De Meester, I. 2003, "Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV", Critical reviews in clinical laboratory sciences, vol. 40, no. 3, pp. 209-294.

Law, H.K., Cheung, C.Y., Ng, H.Y., Sia, S.F., Chan, Y.O., Luk, W., Nicholls, J.M., Peiris, J.S. & Lau, Y.L. 2005, "Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells", Blood, vol. 106, no. 7, pp. 2366-2374.

Laza-Stanca, V., Stanciu, L.A., Message, S.D., Edwards, M.R., Gern, J.E. & Johnston, S.L. 2006, "Rhinovirus replication in human macrophages induces NF-kappaB-dependent tumor necrosis factor alpha production", Journal of virology, vol. 80, no. 16, pp. 8248-8258.

Lee, J.T. & Gu, W. 2010, "The multiple levels of regulation by p53 ubiquitination", Cell death and differentiation, vol. 17, no. 1, pp. 86-92.

Leong, H.N., Ang, B., Earnest, A., Teoh, C., Xu, W. & Leo, Y.S. 2004, "Investigational use of ribavirin in the treatment of severe acute respiratory syndrome, Singapore, 2003", Tropical medicine & international health : TM & IH, vol. 9, no. 8, pp. 923-927.

Li, T., Qiu, Z., Han, Y., Wang, Z., Fan, H., Lu, W., Xie, J., Ma, X. & Wang, A. 2003, "Rapid loss of both CD4+ and CD8+ T lymphocyte subsets during the acute phase of severe acute respiratory syndrome", Chinese medical journal, vol. 116, no. 7, pp. 985-987.

Li, T., Qiu, Z., Zhang, L., Han, Y., He, W., Liu, Z., Ma, X., Fan, H., Lu, W., Xie, J., Wang, H., Deng, G. & Wang, A. 2004, "Significant changes of peripheral T lymphocyte subsets in patients with severe acute respiratory syndrome", The Journal of infectious diseases, vol. 189, no. 4, pp. 648-651.

Li, W., Moltedo, B. & Moran, T.M. 2012, "Type I interferon induction during influenza virus infection increases susceptibility to secondary Streptococcus pneumoniae infection by negative regulation of gammadelta T cells", Journal of virology, vol. 86, no. 22, pp. 12304-12312.

Li, W., Moore, M.J., Vasilieva, N., Sui, J., Wong, S.K., Berne, M.A., Somasundaran, M., Sullivan, J.L., Luzuriaga, K., Greenough, T.C., Choe, H. & Farzan, M. 2003, "Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus", Nature, vol. 426, no. 6965, pp. 450-454.

Li, W., Noah, J.W. & Noah, D.L. 2011, "Alanine substitutions within a linker region of the influenza A virus non-structural protein 1 alter its subcellular localization and attenuate virus replication", The Journal of general virology, vol. 92, no. Pt 8, pp. 1832-1842.

Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J.H., Wang, H., Crameri, G., Hu, Z., Zhang, H., Zhang, J., McEachern, J., Field, H., Daszak, P., Eaton, B.T., Zhang, S. & Wang, L.F. 2005, "Bats are natural reservoirs of SARS-like coronaviruses", Science (New York, N.Y.), vol. 310, no. 5748, pp. 676-679.

Li, W., Wang, G., Zhang, H., Shen, Y., Dai, J., Wu, L., Zhou, J., Jiang, Z. & Li, K. 2012, "Inability of NS1 protein from an H5N1 influenza virus to activate PI3K/Akt signaling pathway correlates to the enhanced virus replication upon PI3K inhibition", Veterinary research, vol. 43, pp. 36-9716-43-36.

Li, Y., Yamakita, Y. & Krug, R.M. 1998, "Regulation of a nuclear export signal by an adjacent inhibitory sequence: the effector domain of the influenza virus NS1 protein", Proceedings of the National Academy of Sciences of the United States of America, vol. 95, no. 9, pp. 4864-4869.

Li, Y., Anderson, D.H., Liu, Q. & Zhou, Y. 2008, "Mechanism of influenza A virus NS1 protein interaction with the p85 beta, but not the p85 alpha, subunit of phosphatidylinositol 3-kinase (PI3K) and up-regulation of PI3K activity", Journal of Biological Chemistry, vol. 283, no. 34, pp. 23397-23409.

Li, Z., Jiang, Y., Jiao, P., Wang, A., Zhao, F., Tian, G., Wang, X., Yu, K., Bu, Z. & Chen, H. 2006, "The NSI gene contributes to the virulence of H5N1 avian influenza viruses", Journal of virology, vol. 80, no. 22, pp. 11115-11123.

Liang, G., Chen, Q., Xu, J., Liu, Y., Lim, W., Peiris, J.S., Anderson, L.J., Ruan, L., Li, H., Kan, B., Di, B., Cheng, P., Chan, K.H., Erdman, D.D., Gu, S., Yan, X., Liang, W., Zhou, D., Haynes, L., Duan, S., Zhang, X., Zheng, H., Gao, Y., Tong, S., Li, D., Fang, L., Qin, P., Xu, W. & SARS Diagnosis Working Group 2004, "Laboratory diagnosis of four recent sporadic cases of community-acquired SARS, Guangdong Province, China", Emerging infectious diseases, vol. 10, no. 10, pp. 1774-1781.

Liu, D.X., Fung, T.S., Chong, K.K., Shukla, A. & Hilgenfeld, R. 2014, "Accessory proteins of SARS-CoV and other coronaviruses", Antiviral Research, vol. 109, pp. 97-109.

Liu, J., Lynch, P.A., Chien, C.Y., Montelione, G.T., Krug, R.M. & Berman, H.M. 1997, "Crystal structure of the unique RNA-binding domain of the influenza virus NS1 protein", Nature structural biology, vol. 4, no. 11, pp. 896-899.

Lohrmann, F., Dijkman, R., Stertz, S., Thiel, V., Haller, O., Staeheli, P. & Kochs, G. 2013, "Emergence of a C-terminal seven-amino-acid elongation of NS1 in around 1950 conferred a minor growth advantage to former seasonal influenza A viruses", Journal of virology, vol. 87, no. 20, pp. 11300-11303.

Ludwig, S., Schultz, U., Mandler, J., Fitch, W.M. & Scholtissek, C. 1991, "Phylogenetic relationship of the nonstructural (NS) genes of influenza A viruses", Virology, vol. 183, no. 2, pp. 566-577.

Ludwig, S., Wang, X., Ehrhardt, C., Zheng, H., Donelan, N., Planz, O., Pleschka, S., Garcia-Sastre, A., Heins, G. & Wolff, T. 2002, "The influenza A virus NS1 protein inhibits activation of Jun N-terminal kinase and AP-1 transcription factors", Journal of virology, vol. 76, no. 21, pp. 11166-11171.

Lv, H., de Vlas, S.J., Liu, W., Wang, T.B., Cao, Z.Y., Li, C.P., Cao, W.C. & Richardus, J.H. 2009, "Avascular osteonecrosis after treatment of SARS: a 3-year longitudinal study", Tropical medicine & international health : TM & IH, vol. 14 Suppl 1, pp. 79-84.

Madhi, S.A., Klugman, K.P. & Vaccine Trialist Group 2004, "A role for Streptococcus pneumoniae in virus-associated pneumonia", Nature medicine, vol. 10, no. 8, pp. 811-813.

Malim, M.H. & Cullen, B.R. 1991, "HIV-1 structural gene expression requires the binding of multiple Rev monomers to the viral RRE: implications for HIV-1 latency", Cell, vol. 65, no. 2, pp. 241-248.

Marazzi, I., Ho, J.S., Kim, J., Manicassamy, B., Dewell, S., Albrecht, R.A., Seibert, C.W., Schaefer, U., Jeffrey, K.L., Prinjha, R.K., Lee, K., Garcia-Sastre, A., Roeder, R.G. & Tarakhovsky, A. 2012, "Suppression of the antiviral response by an influenza histone mimic", Nature, vol. 483, no. 7390, pp. 428-433.

Marra, M.A., Jones, S.J., Astell, C.R., Holt, R.A., Brooks-Wilson, A., Butterfield, Y.S., Khattra, J., Asano, J.K., Barber, S.A., Chan, S.Y., Cloutier, A., Coughlin, S.M., Freeman, D., Girn, N., Griffith, O.L., Leach, S.R., Mayo, M., McDonald, H., Montgomery, S.B., Pandoh, P.K., Petrescu, A.S., Robertson, A.G., Schein, J.E., Siddiqui, A., Smailus, D.E., Stott, J.M., Yang, G.S., Plummer, F., Andonov, A., Artsob, H., Bastien, N., Bernard, K., Booth, T.F., Bowness, D., Czub, M., Drebot, M., Fernando, L., Flick, R., Garbutt, M., Gray, M., Grolla, A., Jones, S., Feldmann, H., Meyers, A., Kabani, A., Li, Y., Normand, S., Stroher, U., Tipples, G.A., Tyler, S., Vogrig, R., Ward, D., Watson, B., Brunham, R.C., Krajden, M., Petric, M., Skowronski, D.M., Upton, C. & Roper, R.L. 2003, "The Genome sequence of the SARS-associated coronavirus", Science (New York, N.Y.), vol. 300, no. 5624, pp. 1399-1404.

Matsuda, M., Suizu, F., Hirata, N., Miyazaki, T., Obuse, C. & Noguchi, M. 2010, "Characterization of the interaction of influenza virus NS1 with Akt", Biochemical and biophysical research communications, vol. 395, no. 3, pp. 312-317.

Matsuyama, S., Nagata, N., Shirato, K., Kawase, M., Takeda, M. & Taguchi, F. 2010, "Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2", Journal of virology, vol. 84, no. 24, pp. 12658-12664.

Matthews, K.L., Coleman, C.M., van der Meer, Y., Snijder, E.J. & Frieman, M.B. 2014, "The ORF4b-encoded accessory proteins of Middle East respiratory syndrome coronavirus and two related bat coronaviruses localize to the nucleus and inhibit innate immune signalling", The

Journal of general virology, vol. 95, no. Pt 4, pp. 874-882.

McAuley, J.L., Hornung, F., Boyd, K.L., Smith, A.M., McKeon, R., Bennink, J., Yewdell, J.W. & McCullers, J.A. 2007, "Expression of the 1918 influenza A virus PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia", Cell host & microbe, vol. 2, no. 4, pp. 240-249.

McCullers, J.A. & Bartmess, K.C. 2003, "Role of neuraminidase in lethal synergism between influenza virus and Streptococcus pneumoniae", The Journal of infectious diseases, vol. 187, no. 6, pp. 1000-1009.

McIntosh, K., Becker, W.B. & Chanock, R.M. 1967, "Growth in suckling-mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease", Proceedings of the National Academy of Sciences of the United States of America, vol. 58, no. 6, pp. 2268-2273.

Melen, K., Kinnunen, L., Fagerlund, R., Ikonen, N., Twu, K.Y., Krug, R.M. & Julkunen, I. 2007, "Nuclear and nucleolar targeting of influenza A virus NS1 protein: Striking differences between different virus subtypes", Journal of virology, vol. 81, no. 11, pp. 5995-6006.

Mellman, I. & Steinman, R.M. 2001, "Dendritic cells: specialized and regulated antigen processing machines", Cell, vol. 106, no. 3, pp. 255-258.

Memish, Z.A., Mishra, N., Olival, K.J., Fagbo, S.F., Kapoor, V., Epstein, J.H., Alhakeem, R., Durosinloun, A., Al Asmari, M., Islam, A., Kapoor, A., Briese, T., Daszak, P., Al Rabeeah, A.A. & Lipkin, W.I. 2013, "Middle East respiratory syndrome coronavirus in bats, Saudi Arabia", Emerging infectious diseases, vol. 19, no. 11, pp. 1819-1823.

Meyerholz, D.K., Lambertz, A.M. & McCray, P.B.,Jr 2016, "Dipeptidyl Peptidase 4 Distribution in the Human Respiratory Tract: Implications for the Middle East Respiratory

Syndrome", The American journal of pathology, vol. 186, no. 1, pp. 78-86.

Mibayashi, M., Martinez-Sobrido, L., Loo, Y.M., Cardenas, W.B., Gale, M.,Jr & Garcia-Sastre, A. 2007, "Inhibition of retinoic acid-inducible gene I-mediated induction of beta interferon by the NS1 protein of influenza A virus", Journal of virology, vol. 81, no. 2, pp. 514-524.

Millet, J.K. & Whittaker, G.R. 2015, "Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis", Virus research, vol. 202, pp. 120-134.

Millet, J.K. & Whittaker, G.R. 2014, "Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein", Proceedings of the National Academy of Sciences of the United States of America, vol. 111, no. 42, pp. 15214-15219.

Min, J.Y. & Krug, R.M. 2006, "The primary function of RNA binding by the influenza A virus NS1 protein in infected cells: Inhibiting the 2'-5' oligo (A) synthetase/RNase L pathway", Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 18, pp. 7100-7105.

Min, J.Y., Li, S., Sen, G.C. & Krug, R.M. 2007, "A site on the influenza A virus NS1 protein mediates both inhibition of PKR activation and temporal regulation of viral RNA synthesis", Virology, vol. 363, no. 1, pp. 236-243.

Mina, M.J. & Klugman, K.P. 2014, "The role of influenza in the severity and transmission of respiratory bacterial disease", The Lancet.Respiratory medicine, vol. 2, no. 9, pp. 750-763.

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, vol. 4, no. 12, pp. e8342.

Morens, D.M., Taubenberger, J.K. & Fauci, A.S. 2008, "Predominant role of bacterial pneumonia

as a cause of death in pandemic influenza: implications for pandemic influenza preparedness", The Journal of infectious diseases, vol. 198, no. 7, pp. 962-970.

Mossel, E.C., Wang, J., Jeffers, S., Edeen, K.E., Wang, S., Cosgrove, G.P., Funk, C.J., Manzer, R., Miura, T.A., Pearson, L.D., Holmes, K.V. & Mason, R.J. 2008, "SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells", Virology, vol. 372, no. 1, pp. 127-135.

Munger, J.S., Huang, X., Kawakatsu, H., Griffiths, M.J., Dalton, S.L., Wu, J., Pittet, J.F., Kaminski, N., Garat, C., Matthay, M.A., Rifkin, D.B. & Sheppard, D. 1999, "The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis", Cell, vol. 96, no. 3, pp. 319-328.

Muramoto, Y., Noda, T., Kawakami, E., Akkina, R. & Kawaoka, Y. 2013, "Identification of novel influenza A virus proteins translated from PA mRNA", Journal of virology, vol. 87, no. 5, pp. 2455-2462.

Murayama, R., Harada, Y., Shibata, T., Kuroda, K., Hayakawa, S., Shimizu, K. & Tanaka, T. 2007, "Influenza A virus non-structural protein 1 (NS1) interacts with cellular multifunctional protein nucleolin during infection", Biochemical and biophysical research communications, vol. 362, no. 4, pp. 880-885.

Nakabayashi, H., Taketa, K., Miyano, K., Yamane, T. & Sato, J. 1982, "Growth of human hepatoma cells lines with differentiated functions in chemically defined medium", Cancer research, vol. 42, no. 9, pp. 3858-3863.

Nakamura, S., Davis, K.M. & Weiser, J.N. 2011, "Synergistic stimulation of type I interferons during influenza virus coinfection promotes Streptococcus pneumoniae colonization in mice", The Journal of clinical investigation, vol. 121, no. 9, pp. 3657-3665.

Narasaraju, T., Yang, E., Samy, R.P., Ng, H.H., Poh, W.P., Liew, A.A., Phoon, M.C., van Rooijen, N. & Chow, V.T. 2011, "Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis", The American journal of pathology, vol. 179, no. 1, pp. 199-210.

Nemeroff, M.E., Qian, X.Y. & Krug, R.M. 1995, "The influenza virus NS1 protein forms multimers in vitro and in vivo", Virology, vol. 212, no. 2, pp. 422-428.

Nemeroff, M., Barabino, S., Li, Y., Keller, W. & Krug, R. 1998, "Influenza virus NS1 protein interacts with the cellular 30 kDa subunit of CPSF and inhibits 3 ' end formation of cellular pre-mRNAs", Molecular cell, vol. 1, no. 7, pp. 991-1000.

Neumann, G., Watanabe, T., Ito, H., Watanabe, S., Goto, H., Gao, P., Hughes, M., Perez, D., Donis, R., Hoffmann, E., Hobom, G. & Kawaoka, Y. 1999, "Generation of influenza A viruses entirely from cloned cDNAs", Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 16, pp. 9345-9350.

Ng, D.L., Al Hosani, F., Keating, M.K., Gerber, S.I., Jones, T.L., Metcalfe, M.G., Tong, S., Tao, Y., Alami, N.N., Haynes, L.M., Mutei, M.A., Abdel-Wareth, L., Uyeki, T.M., Swerdlow, D.L., Barakat, M. & Zaki, S.R. 2016, "Clinicopathologic, Immunohistochemical, and Ultrastructural Findings of a Fatal Case of Middle East Respiratory Syndrome Coronavirus Infection in the United Arab Emirates, April 2014", The American journal of pathology, vol. 186, no. 3, pp. 652-658.

Nicholls, J.M., Butany, J., Poon, L.L., Chan, K.H., Beh, S.L., Poutanen, S., Peiris, J.S. & Wong, M. 2006, "Time course and cellular localization of SARS-CoV nucleoprotein and RNA in lungs from fatal cases of SARS", PLoS medicine, vol. 3, no. 2, pp. e27.

Nicholls, J.M., Poon, L.L., Lee, K.C., Ng, W.F., Lai, S.T., Leung, C.Y., Chu, C.M., Hui, P.K., Mak, K.L., Lim, W., Yan, K.W., Chan, K.H., Tsang, N.C., Guan, Y., Yuen, K.Y. & Peiris, J.S. 2003, "Lung pathology of fatal severe acute respiratory syndrome", Lancet (London, England), vol. 361, no. 9371, pp. 1773-1778.

Niemeyer, D., Zillinger, T., Muth, D., Zielecki, F., Horvath, G., Suliman, T., Barchet, W., Weber, F., Drosten, C. & Muller, M.A. 2013, "Middle East respiratory syndrome coronavirus accessory protein 4a is a type I interferon antagonist", Journal of virology, vol. 87, no. 22, pp. 12489-12495.

Nishi, K., Yoshida, M., Fujiwara, D., Nishikawa, M., Horinouchi, S. & Beppu, T. 1994, "Leptomycin-B Targets a Regulatory Cascade of Crm1, a Fission Yeast Nuclear-Protein, Involved in Control of Higher-Order Chromosome Structure and Gene-Expression", Journal of Biological Chemistry, vol. 269, no. 9, pp. 6320-6324.

Noah, D.L., Twu, K.Y. & Krug, R.M. 2003, "Cellular antiviral responses against influenza A virus are countered at the posttranscriptional level by the viral NS1A protein via its binding to a cellular protein required for the 3' end processing of cellular pre-mRNAS", Virology, vol. 307, no. 2, pp. 386-395.

Normile, D. 2004, "Infectious diseases. Viral DNA match spurs China's civet roundup", Science (New York, N.Y.), vol. 303, no. 5656, pp. 292.

Obenauer, J.C., Denson, J., Mehta, P.K., Su, X., Mukatira, S., Finkelstein, D.B., Xu, X., Wang, J., Ma, J., Fan, Y., Rakestraw, K.M., Webster, R.G., Hoffmann, E., Krauss, S., Zheng, J., Zhang, Z. & Naeve, C.W. 2006, "Large-scale sequence analysis of avian influenza isolates", Science (New York, N.Y.), vol. 311, no. 5767, pp. 1576-1580.

Ossareh-Nazari, B., Bachelerie, F. & Dargemont, C. 1997, "Evidence for a role of CRM1 in signal-mediated nuclear protein export", Science (New York, N.Y.), vol. 278, no. 5335, pp. 141-144.

Osterhaus, A.D., Rimmelzwaan, G.F., Martina, B.E., Bestebroer, T.M. & Fouchier, R.A. 2000, "Influenza B virus in seals", Science (New York, N.Y.), vol. 288, no. 5468, pp. 1051-1053.

Osterlund, P., Veckman, V., Siren, J., Klucher, K., Hiscott, J., Matikainen, S. & Julkunen, I. 2005, "Gene expression and antiviral activity of alpha/beta interferons and interleukin-29 in virus-infected human myeloid dendritic cells", Journal of virology, vol. 79, no. 15, pp. 9608-9617.

Otsuka, M., Kato, N., Lan, K., Yoshida, H., Kato, J., Goto, T., Shiratori, Y. & Omata, M. 2000, "Hepatitis C virus core protein enhances p53 function through augmentation of DNA binding affinity and transcriptional ability", The Journal of biological chemistry, vol. 275, no. 44, pp. 34122-34130.

Panesar, N.S., Lam, C.W., Chan, M.H., Wong, C.K. & Sung, J.J. 2004, "Lymphopenia and neutrophilia in SARS are related to the prevailing serum cortisol", European journal of clinical investigation, vol. 34, no. 5, pp. 382-384.

Park, Y.W. & Katze, M.G. 1995, "Translational control by influenza virus. Identification of cis-acting sequences and trans-acting factors which may regulate selective viral mRNA translation", The Journal of biological chemistry, vol. 270, no. 47, pp. 28433-28439.

Peiris, J.S., Chu, C.M., Cheng, V.C., Chan, K.S., Hung, I.F., Poon, L.L., Law, K.I., Tang, B.S., Hon, T.Y., Chan, C.S., Chan, K.H., Ng, J.S., Zheng, B.J., Ng, W.L., Lai, R.W., Guan, Y., Yuen, K.Y. & HKU/UCH SARS Study Group 2003, "Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study", Lancet (London, England), vol. 361, no. 9371, pp. 1767-1772.

Peiris, J.S., Guan, Y. & Yuen, K.Y. 2004, "Severe acute respiratory syndrome", Nature medicine, vol. 10, no. 12 Suppl, pp. S88-97.

Peiris, J.S., Lai, S.T., Poon, L.L., Guan, Y., Yam, L.Y., Lim, W., Nicholls, J., Yee, W.K., Yan, W.W., Cheung, M.T., Cheng, V.C., Chan, K.H., Tsang, D.N., Yung, R.W., Ng, T.K., Yuen, K.Y. & SARS study group 2003, "Coronavirus as a possible cause of severe acute respiratory syndrome", Lancet (London, England), vol. 361, no. 9366, pp. 1319-1325.

Perlman, S. & Netland, J. 2009, "Coronaviruses post-SARS: update on replication and pathogenesis", Nature reviews.Microbiology, vol. 7, no. 6, pp. 439-450.

Perrone, L.A., Plowden, J.K., Garcia-Sastre, A., Katz, J.M. & Tumpey, T.M. 2008, "H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice", PLoS pathogens, vol. 4, no. 8, pp. e1000115.

Pewe, L., Zhou, H., Netland, J., Tangudu, C., Olivares, H., Shi, L., Look, D., Gallagher, T. & Perlman, S. 2005, "A severe acute respiratory syndrome-associated coronavirus-specific protein enhances virulence of an attenuated murine coronavirus", Journal of virology, vol. 79, no. 17, pp. 11335-11342.

Pichlmair, A., Schulz, O., Tan, C.P., Naslund, T.I., Liljestrom, P., Weber, F. & Reis e Sousa, C. 2006, "RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates", Science (New York, N.Y.), vol. 314, no. 5801, pp. 997-1001.

Pirhonen, J., Sareneva, T., Kurimoto, M., Julkunen, I. & Matikainen, S. 1999, "Virus infection activates IL-1 beta and IL-18 production in human macrophages by a caspase-1-dependent pathway", Journal of Immunology, vol. 162, no. 12, pp. 7322-7329.

Poissy, J., Goffard, A., Parmentier-Decrucq, E., Favory, R., Kauv, M., Kipnis, E., Mathieu, D., van der Werf, S., Guery, B. & MERS-CoV Biology Group 2014, "Kinetics and pattern of viral excretion in biological specimens of two MERS-CoV cases", Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology, vol. 61, no. 2, pp. 275-278.

Privalsky, M.L. & Penhoet, E.E. 1978, "Influenza virus proteins: identity, synthesis, and modification analyzed by two-dimensional gel electrophoresis", Proceedings of the National Academy of Sciences of the United States of America, vol. 75, no. 8, pp. 3625-3629.

Pryor, M.J., Rawlinson, S.M., Butcher, R.E., Barton, C.L., Waterhouse, T.A., Vasudevan, S.G., Bardin, P.G., Wright, P.J., Jans, D.A. & Davidson, A.D. 2007, "Nuclear localization of dengue virus nonstructural protein 5 through its importin alpha/beta-recognized nuclear localization sequences is integral to viral infection", Traffic (Copenhagen, Denmark), vol. 8, no. 7, pp. 795-807.

Qian, X.Y., Alonso-Caplen, F. & Krug, R.M. 1994, "Two functional domains of the influenza virus NS1 protein are required for regulation of nuclear export of mRNA", Journal of virology, vol. 68, no. 4, pp. 2433-2441.

Qian, Z., Travanty, E.A., Oko, L., Edeen, K., Berglund, A., Wang, J., Ito, Y., Holmes, K.V. & Mason, R.J. 2013, "Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome-coronavirus", American journal of respiratory cell and molecular biology, vol. 48, no. 6, pp. 742-748.

Raj, V.S., Farag, E.A., Reusken, C.B., Lamers, M.M., Pas, S.D., Voermans, J., Smits, S.L., Osterhaus, A.D., Al-Mawlawi, N., Al-Romaihi, H.E., AlHajri, M.M., El-Sayed, A.M., Mohran, K.A., Ghobashy, H., Alhajri, F., Al-Thani, M., Al-Marri, S.A., El-Maghraby, M.M., Koopmans, M.P. & Haagmans, B.L. 2014, "Isolation of MERS coronavirus from a dromedary camel, Qatar, 2014", Emerging infectious diseases, vol. 20, no. 8, pp. 1339-1342.

Raj, V.S., Mou, H., Smits, S.L., Dekkers, D.H.W., Mueller, M.A., Dijkman, R., Muth, D.,

Demmers, J.A.A., Zaki, A., Fouchier, R.A.M., Thiel, V., Drosten, C., Rottier, P.J.M., Osterhaus, A.D.M.E., Bosch, B.J. & Haagmans, B.L. 2013, "Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC", Nature, vol. 495, no. 7440, pp. 251-254.

Rajsbaum, R., Albrecht, R.A., Wang, M.K., Maharaj, N.P., Versteeg, G.A., Nistal-Villan, E., Garcia-Sastre, A. & Gack, M.U. 2012, "Species-specific inhibition of RIG-I ubiquitination and IFN induction by the influenza A virus NS1 protein", PLoS pathogens, vol. 8, no. 11, pp. e1003059.

Rawlinson, S.M., Pryor, M.J., Wright, P.J. & Jans, D.A. 2009, "CRM1-mediated nuclear export of dengue virus RNA polymerase NS5 modulates interleukin-8 induction and virus production", The Journal of biological chemistry, vol. 284, no. 23, pp. 15589-15597.

Reichow, S.L., Hamma, T., Ferre-D'Amare, A.R. & Varani, G. 2007, "The structure and function of small nucleolar ribonucleoproteins", Nucleic acids research, vol. 35, no. 5, pp. 1452-1464.

Reusken, C.B., Farag, E.A., Jonges, M., Godeke, G.J., El-Sayed, A.M., Pas, S.D., Raj, V.S., Mohran, K.A., Moussa, H.A., Ghobashy, H., Alhajri, F., Ibrahim, A.K., Bosch, B.J., Pasha, S.K., Al-Romaihi, H.E., Al-Thani, M., Al-Marri, S.A., AlHajri, M.M., Haagmans, B.L. & Koopmans, M.P. 2014, "Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014", Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, vol. 19, no. 23, pp. 20829.

Reusken, C.B., Haagmans, B.L., Muller, M.A., Gutierrez, C., Godeke, G.J., Meyer, B., Muth, D., Raj, V.S., Smits-De Vries, L., Corman, V.M., Drexler, J.F., Smits, S.L., El Tahir, Y.E., De Sousa, R., van Beek, J., Nowotny, N., van Maanen, K., Hidalgo-Hermoso, E., Bosch, B.J., Rottier, P., Osterhaus, A., Gortazar-Schmidt, C.,

Drosten, C. & Koopmans, M.P. 2013, "Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study", The Lancet.Infectious diseases, vol. 13, no. 10, pp. 859-866.

Riley, S., Fraser, C., Donnelly, C.A., Ghani, A.C., Abu-Raddad, L.J., Hedley, A.J., Leung, G.M., Ho, L.M., Lam, T.H., Thach, T.Q., Chau, P., Chan, K.P., Lo, S.V., Leung, P.Y., Tsang, T., Ho, W., Lee, K.H., Lau, E.M., Ferguson, N.M. & Anderson, R.M. 2003, "Transmission dynamics of the etiological agent of SARS in Hong Kong: impact of public health interventions", Science (New York, N.Y.), vol. 300, no. 5627, pp. 1961-1966.

Rinaldo, C.R.,Jr & Piazza, P. 2004, "Virus infection of dendritic cells: portal for host invasion and host defense", Trends in microbiology, vol. 12, no. 7, pp. 337-345.

Roberts, A., Paddock, C., Vogel, L., Butler, E., Zaki, S. & Subbarao, K. 2005, "Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans", Journal of virology, vol. 79, no. 9, pp. 5833-5838.

Rodgers, B.C. & Mims, C.A. 1982, "Influenza virus replication in human alveolar macrophages", Journal of medical virology, vol. 9, no. 3, pp. 177-184.

Rogers, G.N. & Paulson, J.C. 1983, "Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin", Virology, vol. 127, no. 2, pp. 361-373.

Ronni, T., Melen, K., Malygin, A. & Julkunen, I. 1993, "Control of Ifn-Inducible Mxa Gene-Expression in Human-Cells", Journal of Immunology, vol. 150, no. 5, pp. 1715-1726.

Ronni, T., Sareneva, T., Pirhonen, J. & Julkunen, I. 1995, "Activation of Ifn-Alpha, Ifn-Gamma, Mxa, and Ifn Regulatory Factor-1 Genes in Influenza-a Virus-Infected Human Peripheral-

Blood Mononuclear-Cells", Journal of Immunology, vol. 154, no. 6, pp. 2764-2774.

Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., Penaranda, S., Bankamp, B., Maher, K., Chen, M.H., Tong, S., Tamin, A., Lowe, L., Frace, M., DeRisi, J.L., Chen, Q., Wang, D., Erdman, D.D., Peret, T.C., Burns, C., Ksiazek, T.G., Rollin, P.E., Sanchez, A., Liffick, S., Holloway, B., Limor, J., McCaustland, K., Olsen-Rasmussen, M., Fouchier, R., Gunther, S., Osterhaus, A.D., Drosten, C., Pallansch, M.A., Anderson, L.J. & Bellini, W.J. 2003, "Characterization of a novel coronavirus associated with severe acute respiratory syndrome", Science (New York, N.Y.), vol. 300, no. 5624, pp. 1394-1399.

Rubbi, C.P. & Milner, J. 2003, "Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses", The EMBO journal, vol. 22, no. 22, pp. 6068-6077.

Ruuskanen, O., Lahti, E., Jennings, L.C. & Murdoch, D.R. 2011, "Viral pneumonia", Lancet (London, England), vol. 377, no. 9773, pp. 1264-1275.

Saif, L.J. 2004, "Animal coronaviruses: what can they teach us about the severe acute respiratory syndrome?", Revue scientifique et technique (International Office of Epizootics), vol. 23, no. 2, pp. 643-660.

Salomon, R., Hoffmann, E. & Webster, R.G. 2007, "Inhibition of the cytokine response does not protect against lethal H5N1 influenza infection", Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 30, pp. 12479-12481.

Satterly, N., Tsai, P.L., van Deursen, J., Nussenzveig, D.R., Wang, Y., Faria, P.A., Levay, A., Levy, D.E. & Fontoura, B.M. 2007, "Influenza virus targets the mRNA export machinery and the nuclear pore complex", Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 6, pp. 1853-1858.

Scheuplein, V.A., Seifried, J., Malczyk, A.H., Miller, L., Hocker, L., Vergara-Alert, J., Dolnik, O., Zielecki, F., Becker, B., Spreitzer, I., Konig, R., Becker, S., Waibler, Z. & Muhlebach, M.D. 2015, "High Secretion of Interferons by Human Plasmacytoid Dendritic Cells upon Recognition of Middle East Respiratory Syndrome Coronavirus", Journal of virology, vol. 89, no. 7, pp. 3859-3869.

Schneider, C., Nobs, S.P., Heer, A.K., Kurrer, M., Klinke, G., van Rooijen, N., Vogel, J. & Kopf, M. 2014, "Alveolar macrophages are essential for protection from respiratory failure and associated morbidity following influenza virus infection", PLoS pathogens, vol. 10, no. 4, pp. e1004053.

Shahangian, A., Chow, E.K., Tian, X., Kang, J.R., Ghaffari, A., Liu, S.Y., Belperio, J.A., Cheng, G. & Deng, J.C. 2009, "Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice", The Journal of clinical investigation, vol. 119, no. 7, pp. 1910-1920.

Shen, Y., Wang, X., Guo, L., Qiu, Y., Li, X., Yu, H., Xiang, H., Tong, G. & Ma, Z. 2009, "Influenza A virus induces p53 accumulation in a biphasic pattern", Biochemical and biophysical research communications, vol. 382, no. 2, pp. 331-335.

Shin, Y., Liu, Q., Tikoo, S.K., Babiuk, L.A. & Zhou, Y. 2007, "Effect of the phosphatidylinositol 3-kinase/Akt pathway on influenza A virus propagation", Journal of General Virology, vol. 88, pp. 942-950.

Shinya, K., Ebina, M., Yamada, S., Ono, M., Kasai, N. & Kawaoka, Y. 2006, "Avian flu: influenza virus receptors in the human airway", Nature, vol. 440, no. 7083, pp. 435-436.

Shirato, K., Kawase, M. & Matsuyama, S. 2013, "Middle East respiratory syndrome coronavirus infection mediated by the transmembrane serine protease TMPRSS2", Journal of virology, vol. 87, no. 23, pp. 12552-12561.

Short, K.R., Kroeze, E.J., Fouchier, R.A. & Kuiken, T. 2014, "Pathogenesis of influenza-induced

acute respiratory distress syndrome", The Lancet.Infectious diseases, vol. 14, no. 1, pp. 57-69.

Simmons, G., Gosalia, D.N., Rennekamp, A.J., Reeves, J.D., Diamond, S.L. & Bates, P. 2005, "Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry", Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 33, pp. 11876-11881.

Siu, K.L., Kok, K.H., Ng, M.H., Poon, V.K., Yuen, K.Y., Zheng, B.J. & Jin, D.Y. 2009, "Severe acute respiratory syndrome coronavirus M protein inhibits type I interferon production by impeding the formation of TRAF3.TANK.TBK1/IKKepsilon complex", The Journal of biological chemistry, vol. 284, no. 24, pp. 16202-16209.

Siu, K.L., Yeung, M.L., Kok, K.H., Yuen, K.S., Kew, C., Lui, P.Y., Chan, C.P., Tse, H., Woo, P.C., Yuen, K.Y. & Jin, D.Y. 2014, "Middle east respiratory syndrome coronavirus 4a protein is a double-stranded RNA-binding protein that suppresses PACT-induced activation of RIG-I and MDA5 in the innate antiviral response", Journal of virology, vol. 88, no. 9, pp. 4866-4876.

Smits, S.L., de Lang, A., van den Brand, J.M., Leijten, L.M., van IJcken, W.F., Eijkemans, M.J., van Amerongen, G., Kuiken, T., Andeweg, A.C., Osterhaus, A.D. & Haagmans, B.L. 2010, "Exacerbated innate host response to SARS-CoV in aged non-human primates", PLoS pathogens, vol. 6, no. 2, pp. e1000756.

Snelgrove, R.J., Goulding, J., Didierlaurent, A.M., Lyonga, D., Vekaria, S., Edwards, L., Gwyer, E., Sedgwick, J.D., Barclay, A.N. & Hussell, T. 2008, "A critical function for CD200 in lung immune homeostasis and the severity of influenza infection", Nature immunology, vol. 9, no. 9, pp. 1074-1083.

Snijder, E.J., Bredenbeek, P.J., Dobbe, J.C., Thiel, V., Ziebuhr, J., Poon, L.L., Guan, Y., Rozanov,

M., Spaan, W.J. & Gorbalenya, A.E. 2003, "Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage", Journal of Molecular Biology, vol. 331, no. 5, pp. 991-1004.

Soroosh, P., Doherty, T.A., Duan, W., Mehta, A.K., Choi, H., Adams, Y.F., Mikulski, Z., Khorram, N., Rosenthal, P., Broide, D.H. & Croft, M. 2013, "Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance", The Journal of experimental medicine, vol. 210, no. 4, pp. 775-788.

Spiegel, M., Pichlmair, A., Martinez-Sobrido, L., Cros, J., Garcia-Sastre, A., Haller, O. & Weber, F. 2005, "Inhibition of Beta interferon induction by severe acute respiratory syndrome coronavirus suggests a two-step model for activation of interferon regulatory factor 3", Journal of virology, vol. 79, no. 4, pp. 2079-2086.

Squires, R.B., Noronha, J., Hunt, V., Garcia-Sastre, A., Macken, C., Baumgarth, N., Suarez, D., Pickett, B.E., Zhang, Y., Larsen, C.N., Ramsey, A., Zhou, L., Zaremba, S., Kumar, S., Deitrich, J., Klem, E. & Scheuermann, R.H. 2012, "Influenza Research Database: an integrated bioinformatics resource for influenza research and surveillance", Influenza and Other Respiratory Viruses, vol. 6, no. 6, pp. 404-416.

Stockman, L.J., Bellamy, R. & Garner, P. 2006, "SARS: systematic review of treatment effects", PLoS medicine, vol. 3, no. 9, pp. e343.

Su, P.Y., Wang, Y.F., Huang, S.W., Lo, Y.C., Wang, Y.H., Wu, S.R., Shieh, D.B., Chen, S.H., Wang, J.R., Lai, M.D. & Chang, C.F. 2015, "Cell surface nucleolin facilitates enterovirus 71 binding and infection", Journal of virology, vol. 89, no. 8, pp. 4527-4538.

Sun, K. & Metzger, D.W. 2008, "Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza

infection", Nature medicine, vol. 14, no. 5, pp. 558-564.

Sung, J.J., Wu, A., Joynt, G.M., Yuen, K.Y., Lee, N., Chan, P.K., Cockram, C.S., Ahuja, A.T., Yu, L.M., Wong, V.W. & Hui, D.S. 2004, "Severe acute respiratory syndrome: report of treatment and outcome after a major outbreak", Thorax, vol. 59, no. 5, pp. 414-420.

Tang, N.L., Chan, P.K., Wong, C.K., To, K.F., Wu, A.K., Sung, Y.M., Hui, D.S., Sung, J.J. & Lam, C.W. 2005, "Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome", Clinical chemistry, vol. 51, no. 12, pp. 2333-2340.

Tate, M.D., Ioannidis, L.J., Croker, B., Brown, L.E., Brooks, A.G. & Reading, P.C. 2011, "The role of neutrophils during mild and severe influenza virus infections of mice", PloS one, vol. 6, no. 3, pp. e17618.

Taubenberger, J.K. & Morens, D.M. 2008, "The pathology of influenza virus infections", Annual review of pathology, vol. 3, pp. 499-522.

Taubenberger, J.K. & Morens, D.M. 2006, "1918 Influenza: the mother of all pandemics", Emerging infectious diseases, vol. 12, no. 1, pp. 15-22.

Tecle, T., Tripathi, S. & Hartshorn, K.L. 2010, "Review: Defensins and cathelicidins in lung immunity", Innate immunity, vol. 16, no. 3, pp. 151-159.

Terrier, O., Diederichs, A., Dubois, J., Cartet, G., Lina, B., Bourdon, J.C. & Rosa-Calatrava, M. 2013, "Influenza NS1 interacts with p53 and alters its binding to p53-responsive genes, in a promoter-dependent manner", FEBS letters, vol. 587, no. 18, pp. 2965-2971.

Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Bridges, C.B., Cox, N.J. & Fukuda, K. 2004, "Influenza-associated hospitalizations in the United States", Jama, vol. 292, no. 11, pp. 1333-1340.

Thompson, W.W., Shay, D.K., Weintraub, E., Brammer, L., Cox, N., Anderson, L.J. & Fukuda, K. 2003, "Mortality associated with influenza and respiratory syncytial virus in the United States", Jama, vol. 289, no. 2, pp. 179-186.

Tong, S., Li, Y., Rivailler, P., Conrardy, C., Castillo, D.A., Chen, L.M., Recuenco, S., Ellison, J.A., Davis, C.T., York, I.A., Turmelle, A.S., Moran, D., Rogers, S., Shi, M., Tao, Y., Weil, M.R., Tang, K., Rowe, L.A., Sammons, S., Xu, X., Frace, M., Lindblade, K.A., Cox, N.J., Anderson, L.J., Rupprecht, C.E. & Donis, R.O. 2012, "A distinct lineage of influenza A virus from bats", Proceedings of the National Academy of Sciences of the United States of America, vol. 109, no. 11, pp. 4269-4274.

Tong, S., Zhu, X., Li, Y., Shi, M., Zhang, J., Bourgeois, M., Yang, H., Chen, X., Recuenco, S., Gomez, J., Chen, L.M., Johnson, A., Tao, Y., Dreyfus, C., Yu, W., McBride, R., Carney, P.J., Gilbert, A.T., Chang, J., Guo, Z., Davis, C.T., Paulson, J.C., Stevens, J., Rupprecht, C.E., Holmes, E.C., Wilson, I.A. & Donis, R.O. 2013, "New world bats harbor diverse influenza A viruses", PLoS pathogens, vol. 9, no. 10, pp. e1003657.

Treanor, J.J., Snyder, M.H., London, W.T. & Murphy, B.R. 1989, "The B allele of the NS gene of avian influenza viruses, but not the A allele, attenuates a human influenza A virus for squirrel monkeys", Virology, vol. 171, no. 1, pp. 1-9.

Tsai, P.L., Chiou, N.T., Kuss, S., Garcia-Sastre, A., Lynch, K.W. & Fontoura, B.M. 2013, "Cellular RNA binding proteins NS1-BP and hnRNP K regulate influenza A virus RNA splicing", PLoS pathogens, vol. 9, no. 6, pp. e1003460.

Tse, G.M., To, K.F., Chan, P.K., Lo, A.W., Ng, K.C., Wu, A., Lee, N., Wong, H.C., Mak, S.M., Chan, K.F., Hui, D.S., Sung, J.J. & Ng, H.K. 2004, "Pulmonary pathological features in coronavirus associated severe acute respiratory

syndrome (SARS)", Journal of clinical pathology, vol. 57, no. 3, pp. 260-265.

Tsiodras, S., Nikolopoulos, G. & Bonovas, S. 2012, "Antivirals used for influenza chemoprophylaxis", Current medicinal chemistry, vol. 19, no. 35, pp. 5947-5956.

Turpin, E., Luke, K., Jones, J., Tumpey, T., Konan, K. & Schultz-Cherry, S. 2005, "Influenza virus infection increases p53 activity: role of p53 in cell death and viral replication", Journal of virology, vol. 79, no. 14, pp. 8802-8811.

Twu, K., Noah, D., Rao, P., Kuo, R. & Krug, R. 2006, "The CPSF30 binding site on the NS1A protein of influenza A virus is a potential antiviral target", Journal of virology, vol. 80, no. 8, pp. 3957-3965.

Vaarala, M.H., Porvari, K.S., Kellokumpu, S., Kyllonen, A.P. & Vihko, P.T. 2001, "Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues", The Journal of pathology, vol. 193, no. 1, pp. 134-140.

van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T.M., Raj, V.S., Zaki, A.M., Osterhaus, A.D., Haagmans, B.L., Gorbalenya, A.E., Snijder, E.J. & Fouchier, R.A. 2012, "Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans", mBio, vol. 3, no. 6, pp. 10.1128/mBio.00473-12.

van der Hoek, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J., Wolthers, K.C., Wertheim-van Dillen, P.M., Kaandorp, J., Spaargaren, J. & Berkhout, B. 2004, "Identification of a new human coronavirus", Nature medicine, vol. 10, no. 4, pp. 368-373.

van der Sluijs, K.F., van Elden, L.J., Nijhuis, M., Schuurman, R., Pater, J.M., Florquin, S., Goldman, M., Jansen, H.M., Lutter, R. & van der Poll, T. 2004, "IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection", Journal of immunology (Baltimore, Md.: 1950), vol. 172, no. 12, pp. 7603-7609.

van Riel, D., Leijten, L.M., van der Eerden, M., Hoogsteden, H.C., Boven, L.A., Lambrecht, B.N., Osterhaus, A.D. & Kuiken, T. 2011, "Highly pathogenic avian influenza virus H5N1 infects alveolar macrophages without virus production or excessive TNF-alpha induction", PLoS pathogens, vol. 7, no. 6, pp. e1002099.

van Riel, D., Munster, V.J., de Wit, E., Rimmelzwaan, G.F., Fouchier, R.A., Osterhaus, A.D. & Kuiken, T. 2007, "Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals", The American journal of pathology, vol. 171, no. 4, pp. 1215-1223.

van Riel, D., Munster, V.J., de Wit, E., Rimmelzwaan, G.F., Fouchier, R.A., Osterhaus, A.D. & Kuiken, T. 2006, "H5N1 Virus Attachment to Lower Respiratory Tract", Science (New York, N.Y.), vol. 312, no. 5772, pp. 399.

Volmer, R., Mazel-Sanchez, B., Volmer, C., Soubies, S.M. & Guerin, J.L. 2010, "Nucleolar localization of influenza A NS1: striking differences between mammalian and avian cells", Virology journal, vol. 7, pp. 63-422X-7-63.

Voynow, J.A. & Rubin, B.K. 2009, "Mucins, mucus, and sputum", Chest, vol. 135, no. 2, pp. 505-512.

Wang, Q., Qi, J., Yuan, Y., Xuan, Y., Han, P., Wan, Y., Ji, W., Li, Y., Wu, Y., Wang, J., Iwamoto, A., Woo, P.C., Yuen, K.Y., Yan, J., Lu, G. & Gao, G.F. 2014, "Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26", Cell host & microbe, vol. 16, no. 3, pp. 328-337.

Wang, W., Riedel, K., Lynch, P., Chien, C.Y., Montelione, G.T. & Krug, R.M. 1999, "RNA binding by the novel helical domain of the influenza virus NS1 protein requires its dimer structure and a small number of specific basic amino acids", RNA (New York, N.Y.), vol. 5, no. 2, pp. 195-205.

Wang, X., Deng, X., Yan, W., Zhu, Z., Shen, Y., Qiu, Y., Shi, Z., Shao, D., Wei, J., Xia, X. & Ma, Z. 2012, "Stabilization of p53 in influenza A virus-infected cells is associated with compromised MDM2-mediated ubiquitination of p53", The Journal of biological chemistry, vol. 287, no. 22, pp. 18366-18375.

Wang, X., Shen, Y., Qiu, Y., Shi, Z., Shao, D., Chen, P., Tong, G. & Ma, Z. 2010, "The non-structural (NS1) protein of influenza A virus associates with p53 and inhibits p53-mediated transcriptional activity and apoptosis", Biochemical and biophysical research communications, vol. 395, no. 1, pp. 141-145.

Watanabe, Y., Hashimoto, Y., Shiratsuchi, A., Takizawa, T. & Nakanishi, Y. 2005, "Augmentation of fatality of influenza in mice by inhibition of phagocytosis", Biochemical and biophysical research communications, vol. 337, no. 3, pp. 881-886.

Weiland, J.E., Davis, W.B., Holter, J.F., Mohammed, J.R., Dorinsky, P.M. & Gadek, J.E. 1986, "Lung neutrophils in the adult respiratory distress syndrome. Clinical and pathophysiologic significance", The American Review of Respiratory Disease, vol. 133, no. 2, pp. 218-225.

Westphalen, K., Gusarova, G.A., Islam, M.N., Subramanian, M., Cohen, T.S., Prince, A.S. & Bhattacharya, J. 2014, "Sessile alveolar macrophages communicate with alveolar epithelium to modulate immunity", Nature, vol. 506, no. 7489, pp. 503-506.

Whitsett, J.A. & Alenghat, T. 2015, "Respiratory epithelial cells orchestrate pulmonary innate immunity", Nature immunology, vol. 16, no. 1, pp. 27-35.

Whitsett, J.A., Wert, S.E. & Weaver, T.E. 2010, "Alveolar surfactant homeostasis and the pathogenesis of pulmonary disease", Annual Review of Medicine, vol. 61, pp. 105-119.

WHO, Fact sheet N°211. 2014. www.who.int/mediacentre/factsheets/fs211/en/

WHO. Fact sheet N°310. 2014. www.who.int/mediacentre/factsheets/fs310/en/

WHO Mers-Cov Research Group 2013, "State of Knowledge and Data Gaps of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Humans", PLoS currents, vol. 5, pp. 10.1371/currents.outbreaks.0bf719e352e7478f8a d85fa30127ddb8.

WHO. Middle East respiratory syndrome coronavirus (MERS-CoV). 2016. www.who.int/emergencies/mers-cov/en/

WHO. SARS outbreak contained worldwide. 2003a. www.who.int/mediacentre/news/releases/2003/pr 56/en/

WHO. Summary of Current Situation, Literature Update and Risk Assessment. 2015. www.who.int/csr/disease/coronavirus_infections/ risk-assessment-7july2015/en/

WHO. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. 2004. www.who.int/csr/sars/country/table2004_04_21/ en/

WHO. Update 96 - Taiwan, China: SARS transmission interrupted in last outbreak area. 2003b. www.who.int/csr/don/2003_07_05/en/

Wise, H.M., Foeglein, A., Sun, J., Dalton, R.M., Patel, S., Howard, W., Anderson, E.C., Barclay, W.S. & Digard, P. 2009, "A complicated message: Identification of a novel PB1-related protein translated from influenza A virus segment 2 mRNA", Journal of virology, vol. 83, no. 16, pp. 8021-8031.

Woo, P.C., Lau, S.K., Chu, C.M., Chan, K.H., Tsoi, H.W., Huang, Y., Wong, B.H., Poon, R.W., Cai, J.J., Luk, W.K., Poon, L.L., Wong, S.S., Guan, Y., Peiris, J.S. & Yuen, K.Y. 2005, "Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia", Journal of virology, vol. 79, no. 2, pp. 884-895.

Woo, P.C., Lau, S.K., Huang, Y. & Yuen, K.Y. 2009, "Coronavirus diversity, phylogeny and

interspecies jumping", Experimental biology and medicine (Maywood, N.J.), vol. 234, no. 10, pp. 1117-1127.

Xia, S., Monzingo, A.F. & Robertus, J.D. 2009, "Structure of NS1A effector domain from the influenza A/Udorn/72 virus", Acta crystallographica.Section D, Biological crystallography, vol. 65, no. Pt 1, pp. 11-17.

Xu, K., Klenk, C., Liu, B., Keiner, B., Cheng, J., Zheng, B.J., Li, L., Han, Q., Wang, C., Li, T., Chen, Z., Shu, Y., Liu, J., Klenk, H.D. & Sun, B. 2011, "Modification of nonstructural protein 1 of influenza A virus by SUMO1", Journal of virology, vol. 85, no. 2, pp. 1086-1098.

Yang, C.C., Huang, E.Y., Li, H.C., Su, P.Y. & Shih, C. 2014, "Nuclear export of human hepatitis B virus core protein and pregenomic RNA depends on the cellular NXF1-p15 machinery", PloS one, vol. 9, no. 10, pp. e106683.

Yang, M.R., Lee, S.R., Oh, W., Lee, E.W., Yeh, J.Y., Nah, J.J., Joo, Y.S., Shin, J., Lee, H.W., Pyo, S. & Song, J. 2008, "West Nile virus capsid protein induces p53-mediated apoptosis via the sequestration of HDM2 to the nucleolus", Cellular microbiology, vol. 10, no. 1, pp. 165-176.

Yang, X., Chen, X., Bian, G., Tu, J., Xing, Y., Wang, Y. & Chen, Z. 2014, "Proteolytic processing, deubiquitinase and interferon antagonist activities of Middle East respiratory syndrome coronavirus papain-like protease", The Journal of general virology, vol. 95, no. Pt 3, pp. 614-626.

Yang, Y., Ye, F., Zhu, N., Wang, W., Deng, Y., Zhao, Z. & Tan, W. 2015, "Middle East respiratory syndrome coronavirus ORF4b protein inhibits type I interferon production through both cytoplasmic and nuclear targets", Scientific reports, vol. 5, pp. 17554.

Yang, Y., Zhang, L., Geng, H., Deng, Y., Huang, B., Guo, Y., Zhao, Z. & Tan, W. 2013, "The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory

syndrome coronavirus (MERS-CoV) are potent interferon antagonists", Protein & cell, vol. 4, no. 12, pp. 951-961.

Yang, Z.Y., Huang, Y., Ganesh, L., Leung, K., Kong, W.P., Schwartz, O., Subbarao, K. & Nabel, G.J. 2004, "pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN", Journal of virology, vol. 78, no. 11, pp. 5642-5650.

Ylosmaki, L., Schmotz, C., Ylosmaki, E. & Saksela, K. 2015, "Reorganization of the host cell Crk(L)-PI3 kinase signaling complex by the influenza A virus NS1 protein", Virology, vol. 484, pp. 146-152.

Yoo, D., Wootton, S.K., Li, G., Song, C. & Rowland, R.R. 2003, "Colocalization and interaction of the porcine arterivirus nucleocapsid protein with the small nucleolar RNA-associated protein fibrillarin", Journal of virology, vol. 77, no. 22, pp. 12173-12183.

Yoon, S.W., Webby, R.J. & Webster, R.G. 2014, "Evolution and ecology of influenza A viruses", Current topics in microbiology and immunology, vol. 385, pp. 359-375.

Yoshikawa, T., Hill, T., Li, K., Peters, C.J. & Tseng, C.T. 2009, "Severe acute respiratory syndrome (SARS) coronavirus-induced lung epithelial cytokines exacerbate SARS pathogenesis by modulating intrinsic functions of monocyte-derived macrophages and dendritic cells", Journal of virology, vol. 83, no. 7, pp. 3039-3048.

Yuen, K.Y. & Wong, S.S. 2005, "Human infection by avian influenza A H5N1", Hong Kong medical journal = Xianggang yi xue za zhi / Hong Kong Academy of Medicine, vol. 11, no. 3, pp. 189-199.

Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D. & Fouchier, R.A. 2012, "Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia", The New

England journal of medicine, vol. 367, no. 19, pp. 1814-1820.

Zhang, L., Das, P., Schmolke, M., Manicassamy, B., Wang, Y., Deng, X., Cai, L., Tu, B.P., Forst, C.V., Roth, M.G., Levy, D.E., Garcia-Sastre, A., de Brabander, J., Phillips, M.A. & Fontoura, B.M. 2012, "Inhibition of pyrimidine synthesis reverses viral virulence factor-mediated block of mRNA nuclear export", The Journal of cell biology, vol. 196, no. 3, pp. 315-326.

Zhao, J., Zhao, J., Legge, K. & Perlman, S. 2011, "Age-related increases in PGD(2) expression impair respiratory DC migration, resulting in diminished T cell responses upon respiratory virus infection in mice", The Journal of clinical investigation, vol. 121, no. 12, pp. 4921-4930.

Zhao, J., Zhao, J., Van Rooijen, N. & Perlman, S. 2009, "Evasion by stealth: inefficient immune activation underlies poor T cell response and severe disease in SARS-CoV-infected mice", PLoS pathogens, vol. 5, no. 10, pp. e1000636.

Zhirnov, O.P. & Klenk, H. 2007, "Control of apoptosis in influenza virus-infected cells by up-regulation of Akt and p53 signaling", Apoptosis, vol. 12, no. 8, pp. 1419-1432.

Zhong, J., Rao, X., Deiuliis, J., Braunstein, Z., Narula, V., Hazey, J., Mikami, D., Needleman, B., Satoskar, A.R. & Rajagopalan, S. 2013, "A potential role for dendritic cell/macrophage-expressing DPP4 in obesity-induced visceral inflammation", Diabetes, vol. 62, no. 1, pp. 149-157.

Zhong, N.S., Zheng, B.J., Li, Y.M., Poon, Xie, Z.H., Chan, K.H., Li, P.H., Tan, S.Y., Chang, Q., Xie, J.P., Liu, X.Q., Xu, J., Li, D.X., Yuen, K.Y., Peiris & Guan, Y. 2003, "Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003", Lancet (London, England), vol. 362, no. 9393, pp. 1353-1358.

Zhou, J., Chu, H., Li, C., Wong, B.H., Cheng, Z.S., Poon, V.K., Sun, T., Lau, C.C., Wong, K.K., Chan, J.Y., Chan, J.F., To, K.K., Chan, K.H.,

Zheng, B.J. & Yuen, K.Y. 2013, "Active Replication of Middle East Respiratory Syndrome Coronavirus Replication and Aberrant Induction of Inflammatory Cytokines and Chemokines in Human Macrophages: Implications for Pathogenesis", The Journal of infectious diseases, .

Ziegler, T., Matikainen, S., Ronkko, E., Osterlund, P., Sillanpaa, M., Siren, J., Fagerlund, R., Immonen, M., Melen, K. & Julkunen, L. 2005, "Severe acute respiratory syndrome coronavirus fails to activate cytokine-mediated innate immune responses in cultured human monocyte-derived dendritic cells", Journal of virology, vol. 79, no. 21, pp. 13800-13805.

Zmora, P., Blazejewska, P., Moldenhauer, A.S., Welsch, K., Nehlmeier, I., Wu, Q., Schneider, H., Pohlmann, S. & Bertram, S. 2014, "DESC1 and MSPL activate influenza A viruses and emerging coronaviruses for host cell entry", Journal of virology, vol. 88, no. 20, pp. 12087-12097.

Recent Publications in this Series