UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

THE ROLE OF THE NUCLEAR PORE COMPLEX IN THE CGAS-STING PATHWAY

MÉMOIRE PRÉSENTÉ COMME EXIGENCE PARTIELLE POUR L'OBTENTION DE LA MAÎTRISE EN BIOLOGIE MOLÉCULAIRE ET CELLULAIRE

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Abstract

The innate immune system is a vital part of the defense mechanism of our body, it serves as the first line of defense against infections and pathogens, providing a rapid, non-specific response to recognize and eliminate foreign invaders. It includes physical barriers, such as the skin and mucous membranes, as well as cellular and chemical components like phagocytes and inflammation. The inflammatory response of the body is crucial for the body's defense against infections, injuries, and harmful stimuli. While essential for protection, an uncontrolled or chronic inflammatory response can contribute to various diseases, highlighting the delicate balance required for maintaining health. One of the pathways involved in this response is the cGAS-STING pathway, a crucial pathway responsible for the protection of our organism against bacterial infection, viral infection and DNA damage. The cGAS protein serves a detector of dsDNA in the cytoplasm to then activate a cascade of events resulting in the secretion of type-I interferon and pro-inflammatory cytokines. It has been shown that the dysfunction of the cGAS-STING pathway can be involved in pathologies like auto-immune diseases. To prevent sustained activation, the nuclear membrane and its nuclear pore complex are the main components involved in preventing DNA from escaping the nucleus. The objective of this project is to determine whether the depletion of nucleoporins has an impact on the nuclear integrity of cells and its effect on the cGAS-STING pathway by using siRNA in Mouse Embryonic Fibroblast cells (MEFs) to deplete various nucleoporins. Our results have shown that the depletion of three nucleoporins, TPR, NUP160 and NUP358, induces the activation of the cGAS-STING pathway. Also, an increase in the frequency of Nuclear Envelope Budding (NEB) events, as observed by confocal microscopy, suggests the possibility that the depletion of certain nucleoporins may lead to a breach in the nuclear membrane and potentially release chromatin in the cytoplasm. Characterization of the impact of the deregulation of the nuclear pore complex could provide key insights to better understand the functions of this very crucial complex present in every living cells.

Keywords: Immune response, Inflammation, NPC, TPR, NUP160, NUP358, Lamina, Nuclear Envelope Budding, cGAS-STING, Interferon

Résumé

Le système immunitaire inné est un élément essentiel du mécanisme de défense de notre corps. Il constitue la première ligne de défense contre les infections et les agents pathogènes, fournissant une réponse rapide et non spécifique pour reconnaître et éliminer les envahisseurs étrangers. Il comprend des barrières physiques, telles que la peau et les muqueuses, ainsi que des composants cellulaires et chimiques comme les phagocytes et les cytokines. La réponse inflammatoire est cruciale pour la défense de l'organisme contre les infections, les blessures et les stimuli nocifs. Bien qu'essentielle à la protection, une réponse inflammatoire incontrôlée ou chronique peut contribuer à diverses maladies, mettant en évidence l'équilibre délicat nécessaire au maintien de la santé. L'une des voies impliquées dans cette réponse est la voie cGAS-STING, une voie cruciale responsable de la protection de notre organisme contre les infections bactériennes, les infections virales et les dommages à l'ADN. La protéine cGAS sert de détecteur d'ADNdb dans le cytoplasme pour activer ensuite une cascade d'événements aboutissant à la sécrétion d'interféron de type I et de cytokines pro-inflammatoires. Il a été démontré que le dysfonctionnement de la voie cGAS-STING peut être impliqué dans certaines pathologies comme les maladies auto-immunes. Pour empêcher une activation chronique, la membrane nucléaire et son complexe de pores nucléaires sont les principaux composants impliqués dans la prévention de la fuite de l'ADN du noyau. . L'objectif de ce projet est de déterminer si une déficience de nucléoporines a un impact sur l'intégrité nucléaire et son effet sur la voie cGAS-STING et en utilisant des siRNA dans des cellules de fibroblastes embryonnaires de souris (MEF) pour dépléter diverses nucléoporines. Nos résultats ont montré que la déplétion de trois nucléoporines, TPR, NUP160 et NUP358, induit une activation de la voie cGAS-STING. En outre, une augmentation de la fréquence des événements de bourgeonnement de l'enveloppe nucléaire (BEN), comme le montre la microscopie confocale, suggère la possibilité que la déplétion de certaines nucléoporines puisse conduire à une brèche dans la membrane nucléaire et éventuellement libérer de la chromatine dans le cytoplasme. La caractérisation de l'impact de la dérégulation du complexe des pores nucléaires pourrait fournir des informations clés pour mieux comprendre les fonctions sophistiquées de ce complexe crucial présent dans toutes les cellules vivantes.

Mots clés : Réponse immunitaire, Inflammation, NPC, TPR, NUP160, NUP358, Lamine, Bourgeonnement de l'enveloppe nucléaire, cGAS-STING, Interféron

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List of abbreviations and acronyms

PRR	Pattern Recognition Receptors
PAMPS	Pathogen-Associated Molecular Patterns
DNA	Deoxyribonucleic acid
DAMP	Damage associated molecular pattern
TLR	Toll-like receptors
NLR	NOD-like receptors
cGAS	Cyclic GMP-AMP Synthase
TLR9	Toll-like receptor 9
ALR	AIM2-like receptor
cGAMP	2′3′ cyclic GMP–AMP
STING	Stimulator of Interferon Genes
TBK1	TANK-Binding Kinase 1
IKK	Nuclear Factor Kappa Kinase
IRF3	Interferon Regulatory Factor 3
NF-κB	Nuclear Factor Kappa B
IFN-I	Type I interferons
ER	Endoplasmic reticulum
NUP	Nucleoporin
mtDNA	Mitochondrial DNA

SASP	Senescence-associated secretory phenotype
NPC	Nuclear Pore Complex
dsDNA	Double stranded DNA
TREX	Three prime repair exonuclease
JAK	Janus Kinase
STAT	Signal transducer and activator of transcription proteins
MAD	Mitotic Arrest Deficient
CXCL10	C-X-C motif chemokine ligand 10
RNP	Ribonucleoprotein
NEB	Nuclear Envelope Budding
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
RSAD2	Radical S-adenosyl methionine domain containing 2
IL6	Interleukin 6
tRNA	Transfer RNA
IL1	Interleukin 1
TNF-α	Tumour Necrosis Factor alpha
pH2AX	Phospho-histone H2AX
NK	Natural Killer cells
SCI	Systemic chronic inflammation
DNAse	Deoxyribonucleases
IFN-λ	Interferon lambda

1.Introduction

1.1 The innate immune system

The immune system serves as the body's initial defense against a variety of pathogens, such as bacteria, viruses, and fungi. It offers immediate and non-specific protection by recognizing common features shared by different invaders. This defense mechanism involves several components working together to identify and respond to these common pathogenic traits.[1] First, there are physical barriers, including the skin, mucous membranes, and the epithelial cells lining the respiratory and gastrointestinal tracts. These barriers act as the body's first line of defense, preventing pathogens from entering the body.[1]

Phagocytic cells, like macrophages and neutrophils, play a crucial role in the innate immune response. Although these cells are the most important and active in this process, most cells are also equipped with pattern recognition receptors (PRRs) and they can detect pathogen-associated molecular patterns (PAMPs) on the surface of pathogens and microbes. When these PAMPs are recognized, phagocytic cells engulf and digest the invading pathogens. This process is an essential part of the immune response.[2]

Natural Killer (NK) cells are another component of the innate immune system. They are specialized lymphocytes responsible for identifying and eliminating infected or abnormal host cells, such as virus-infected or cancerous cells. NK cells achieve this by recognizing the absence of specific host proteins on these cells, leading to their elimination through apoptosis.[3]

Additionally, the complement system is a group of proteins that, when activated, enhances the overall immune response. It can tag pathogens for destruction, trigger inflammation, and directly attack the pathogens. This systemic response helps coordinate the immune system's reaction to the threat.[4]

The innate immune response includes the release of inflammatory mediators, such as histamines and cytokines, like TNFα and various interleukins which initiate the inflammatory response. This occurs after cells recognize damage-associated molecular patterns (DAMPs) from damaged cells or dying cells as well as PAMPs in infected cells.[5] This response serves to recruit more immune cells to the site of infection, increase blood flow, and enhance the activity of immune cells. Moreover, the innate immune system can induce fever in response to infection. This increase in body temperature helps inhibit pathogen replication and enhances the overall function of immune cells.[5]

While the innate immune system plays a crucial role in the body's defense, it has certain limitations. It lacks the specificity of the adaptive immune system and relies on recognizing general pathogen patterns and it does not provide long-lasting immunity. Some pathogens have evolved strategies to evade the innate immune system, making them harder to detect and eliminate.[6] Such pathogens include viruses like HIV, which attacks $CD4^+$ T cells, macrophages, monocytes and microglial cells.[7] Bacteria such as *Pseudomonas aeruginosa*, who can secrete proteases that degrades the host proinflammatory cytokines such as the interferon lambda (IFN-λ).[8]

The innate immune system also plays a vital role in the coordination of the overall immune response. It serves as the first responder, and its activation is a crucial step in initiating the adaptive immune response.[9] By alerting the adaptive immune system to the presence of pathogens and microbes, it facilitates a more targeted and specific immune response. The immune system uses a cell to cell communication to coordinate an effective response depending on which pathogen or problem there is.[10]

In summary, the innate immune system is a fundamental component of the body's defense against a wide array of pathogens. Its components work in concert to provide

immediate protection, recognizing common pathogenic features and initiating a coordinated response that contributes to overall immune system function and host defense.

1.2 The inflammatory response: A fundamental process

Inflammation, the body's innate response to injury, infection, or irritation, is a fundamental and complex biological process that plays a pivotal role in maintaining overall health and well-being. At its core, inflammation is a natural defense mechanism that the body deploys to combat harmful stimuli, a critical part of the immune response designed to restore tissue homeostasis and promote healing.[11]

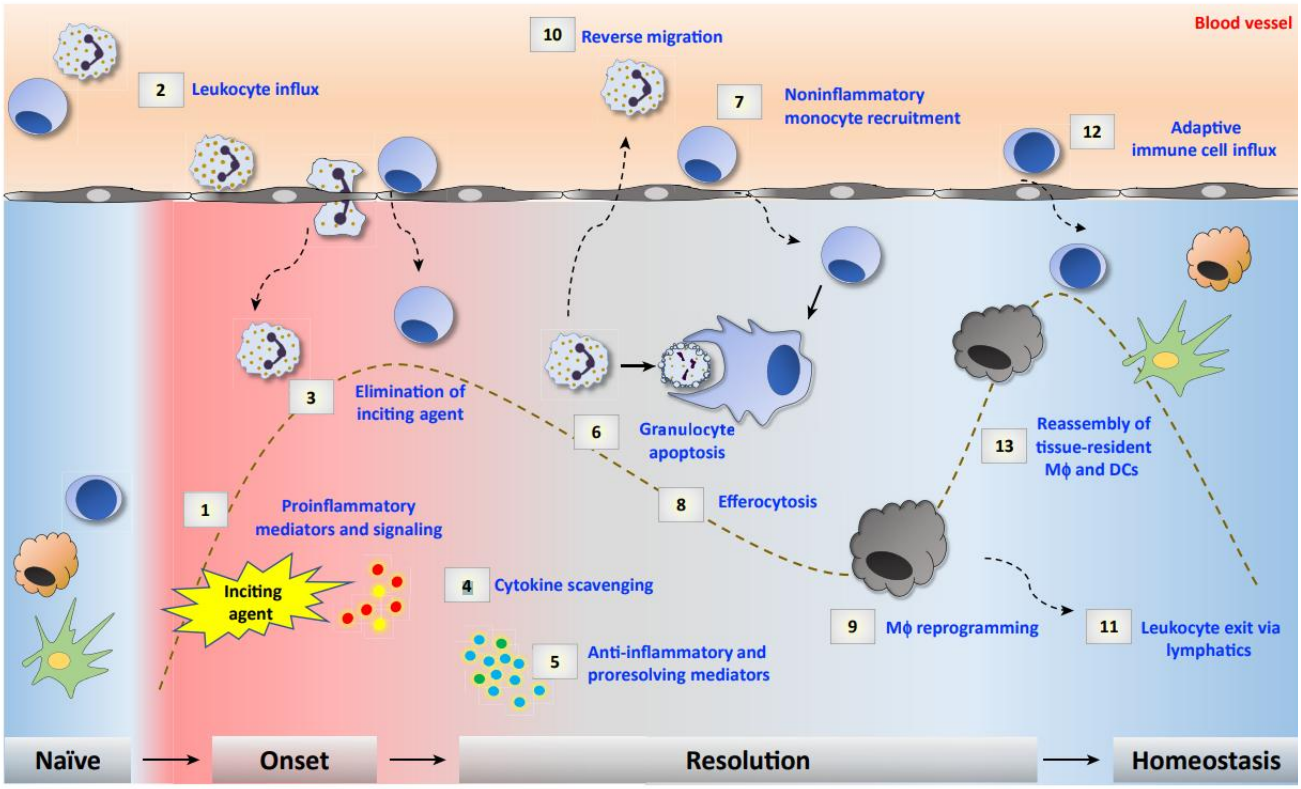


Figure 1: Coordination of the inflammatory response

Source : Sugimoto, Michelle A.; Vago, Juliana P.; Perretti, Mauro; Teixeira, Mauro M. (2019). Mediators of the Resolution of the Inflammatory Response. Trends in Immunology, S1471490619300079

While inflammation serves as a protective mechanism under normal circumstances, when it becomes chronic or excessive, it can give rise to a myriad of health issues and is increasingly recognized as a driver of several chronic diseases.[12] The primary purpose of inflammation is to protect the body from these threats and restore tissue integrity. It operates through a complex cascade of cellular and molecular events, involving various immune cells, signaling molecules, and changes in blood flow. The initial phase of inflammation, termed acute inflammation, is characterized by the recruitment of immune cells to the site of injury or infection.

Neutrophils, macrophages and lymphocytes, three key players in the immune system, are mobilized to eliminate pathogens and debris, while also initiating tissue repair processes. These immune cells release cytokines, chemical messengers that orchestrate the inflammatory response, and other molecules that help to kill invading microorganisms. This phase is essential for preventing the spread of infection and facilitating wound healing. A normal inflammatory response will temporarily upregulate the immune system activity to then resolves to its normal level once the threat or pathogen has passed.[12]

While acute inflammation is a protective mechanism, it can become a double-edged sword when it transforms into chronic inflammation. Chronic inflammation is characterized by a slow and long-term inflammation that lasts for months to years. The extent and effects of this chronic inflammation depends on the cause of the inflammation and on how a person's system is able to repair and overcome this inflammation.

1.3 The two faces of the inflammatory response

The inflammatory response is often compared to a double-edged sword, as it serves as a vital and protective component of the immune system while also harboring potential drawbacks if not appropriately controlled. This dual nature can be elucidated through examining both the advantages and disadvantages associated with the inflammatory process.

On the positive side, inflammation plays a pivotal role in the body's defense against infections. By isolating and eradicating pathogens, it effectively curtails the spread of infections.[13] Additionally, inflammation contributes significantly to the repair and healing of damaged tissues, expediting debris removal and initiating the regeneration process. Furthermore, the release of inflammatory signals serves to summon immune cells to the infection or injury site, thereby bolstering the immune response and aiding in the elimination of harmful agents.[13]

Conversely, there are notable drawbacks to the inflammatory response. Prolonged or chronic inflammation can lead to various chronic conditions such as autoimmune diseases, allergies, and certain cancers. Unregulated inflammatory responses may result in collateral damage to healthy tissues due to the release of enzymes and reactive oxygen species by immune cells. Autoimmune disorders can arise when the immune system mistakenly targets and attacks the body's own tissues, turning the immune response detrimental. Excessive inflammation, exemplified by a cytokine storm in severe infections or certain diseases, can have systemic effects and cause organ damage.[13-15]

As shown in **Figure 2,** SCI, systemic chronic inflammation, can be serious and encompass an elevated risk of developing the metabolic syndrome. This syndrome involves a trio of health issues: hypertension, hyperglycemia, and dyslipidemia.

Furthermore, SCI-driven damage is associated with an increased likelihood of developing type 2 diabetes, non-alcoholic fatty liver disease (NAFLD),[16, 17] hypertension, cardiovascular disease (CVD), chronic kidney disease, various types of cancer, depression, neurodegenerative and autoimmune diseases,[18] osteoporosis, and sarcopenia.[12] The

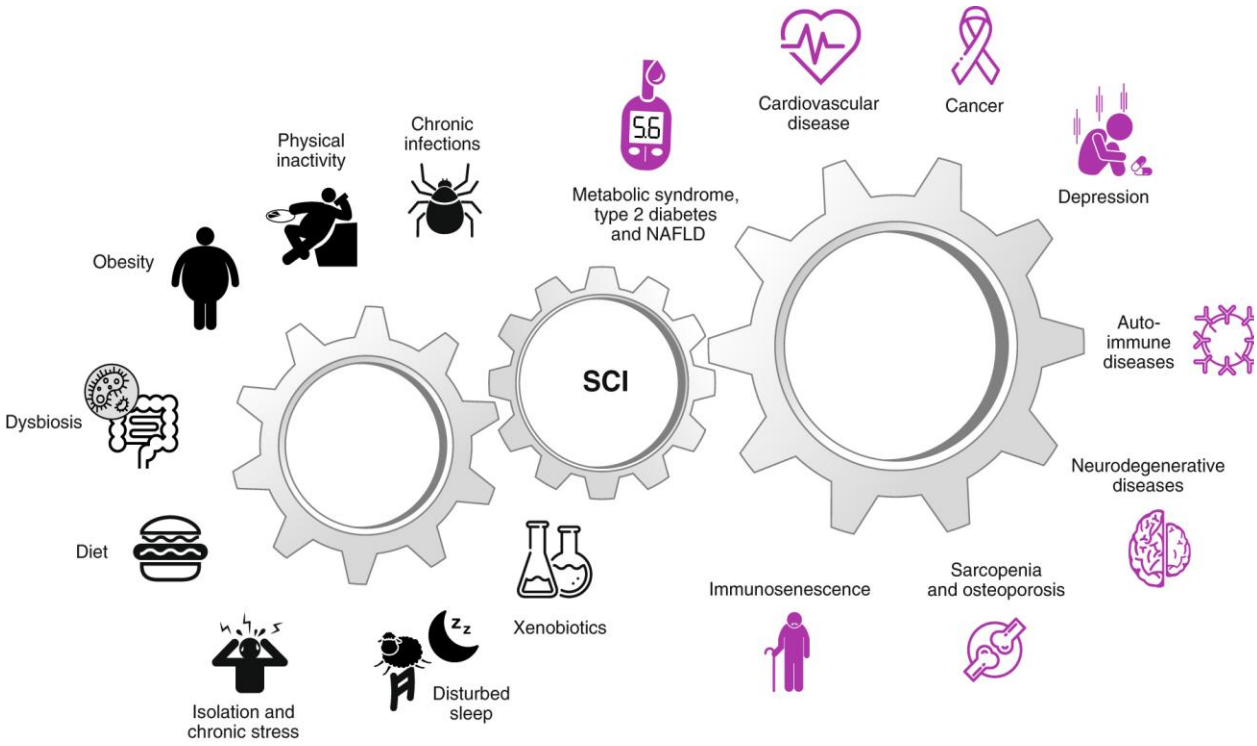


Figure 2: Causes and consequences of low-grade systemic chronic inflammation
Source: Furman, D., Campisi, J., Verdin, E. et al. Chronic inflammation in the etiology of disease across the life span. Nat Med 25, 1822–1832 (2019)

connection between inflammation and the onset or progression of these conditions is most robustly established for metabolic syndrome, type 2 diabetes, and CVD. It is noteworthy that patients with autoimmune diseases like rheumatoid arthritis, characterized by systemic inflammation, often exhibit insulin resistance, dyslipidemia, and hypertension, leading to higher rates of metabolic syndrome, type 2 diabetes, and CVD, particularly ischemic heart disease and stroke.[12] As the acute inflammatory response is normally activated by PAMPs, chronic inflammation is typically triggered by DAMPs in the absence of an infection.

Maintaining a delicate balance in the inflammatory response is crucial for overall health. While the immune system is adept at initiating inflammation, when necessary, it also possesses mechanisms to regulate and resolve inflammation once the threat has been neutralized. Dysregulation of this equilibrium can lead to either an inadequate response, allowing infections to persist, or an excessive response, resulting in tissue damage and the development of chronic conditions.[19] Consequently, understanding and modulating the

inflammatory response are focal points of research in medicine, aiming to develop targeted therapies for disorders linked to inflammation.

1.4 The cGAS-STING pathway

In the field of immunology, the cGAS-STING pathway, named after the cyclic GMP-AMP synthase (cGAS) and the Stimulator of Interferon Genes (STING) and the sensing of self-DNA stand as crucial component in the protection of an organism's health. The relationship between these two entities has become a great point of interest for researchers and clinicians around the world. At the intersection of immunity and autoinflammation, the cGAS-STING pathway and its interaction with the body's own DNA , called self-DNA, roles play crucial roles in coordinating immune responses that protect against pathogens while preventing harmful attacks on the body's own tissues. The cGAS-STING pathway emerged as an important player in innate immunity, responsible for recognizing cytoplasmic dsDNA, including self-DNA, and initiating a cascade of immune responses.

cGAS is an important cytoplasmic dsDNA sensor but there are other main receptors involved in the recognition of nucleic acids. For instance, there are toll-like receptors (TLR) and RIG-I-like receptors (RLR). TLRs recognize nucleic acid motifs that are extracellular or located in endosomes (such as CpG DNA for TLR9 or double-stranded RNA for TLR3)[20], while RLRs detect nucleic acids (DNA or RNA) in the cytoplasm.[21] Other cytosolic DNA sensors are AIM2-like receptors (ALRs), which activates the inflammasome to trigger inflammation via the release of pro-inflammatory cytokines like IL-1β and IL-18. AIM2-like receptors need a specific length of 70-base pairs (bp) of dsDNA as the minimum length necessary to activate the AIM2 inflammasome, but 200-bp of dsDNA allows for optimal AIM2 activation.[22] Unlike TLRs which are transmembrane proteins, cGAS is a protein located mainly in the cytoplasm and in the nucleus.[23]

The cGAS protein belongs to the nucleotidyltransferase family and is a sensor of cytosolic dsDNA.[24] As it binds to the DNA, it synthesizes a second messenger called cyclic GMP-AMP, cGAMP, which will activate the protein STING, a protein residing in the endoplasmic reticulum membrane. There exist two distinct types of cGAMP which have different phosphodiester linkages, one between 2′-OH of GMP and 5′-phosphate of AMP, and the other between 3′-OH of AMP and 5′-phosphate of GMP. This molecule, termed 2′3′-cGAMP, is unique in that it binds to the adaptor protein STING with a much greater affinity than cGAMP molecules containing other combinations of phosphodiester linkages. [25] Only the 2′3′-cGAMP is produced by mammals.

As shown in **Figure 3** Cyclic GMP-AMP, in turn, serves as a second messenger that activates the protein STING, once activated, STING initiates a signaling cascade culminating in the production of type I interferons (IFN-I) and other proinflammatory cytokines. These signaling molecules mobilize the immune system, recruiting immune cells to the site of infection, and enhancing the overall antiviral response. Therefore, the cGAS-STING pathway stands as crucial component of the immune system.[26]

Historically, the cGAS-STING pathway was primarily associated with its role in antiviral defense. Its activation in response to cytoplasmic DNA, such as viral DNA, was a well-established phenomenon. However, the revelation that the pathway could also recognize self-DNA introduced an intriguing layer of complexity to its functions. Self-DNA encompasses the genetic material originating from the host organism itself. [27] It resides within the cell's nucleus, encapsulated, and safeguarded against the cytoplasm, where the cGAS-STING pathway operates. The pathway's ability to sense self-DNA, an integral component of the host, necessitates a finely tuned regulatory mechanism to prevent unwarranted immune activation.[26] Self-DNA can also come from the mitochondria, which has its own DNA and can activate the cGAS-STING pathway.[28]

Failure in maintaining this balance can have profound consequences, ranging from

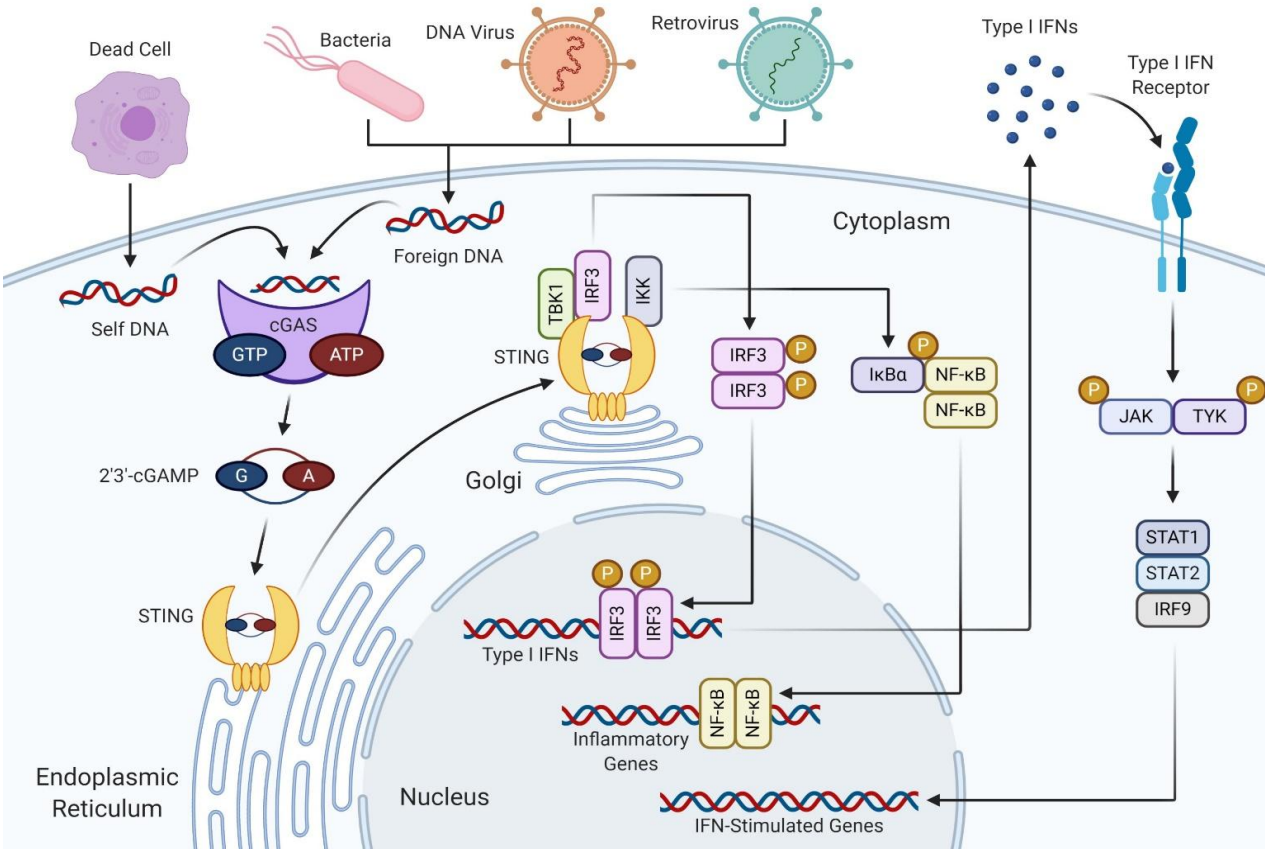


Figure 3: The cGAS-STING pathway and the IFN signaling pathway

Source: Ma et al., The cGAS-STING pathway: The role of self-DNA sensing in inflammatory lung disease, FASEB J, 2020

autoimmune disorders to inflammatory pathologies. Hence, understanding how the cGAS-STING pathway is able to activate in presence of foreign DNA but restrains to activate in the presence of self-DNA is important. It has been shown that cGAS does not discriminate self-DNA to non self-DNA. It will recognise any dsDNA that has a certain length, which is a minimum of 40nt long in mice and 70nt long in humans and will recognise any sequence of dsDNA as it binds to the DNA backbone.[29] Furthermore, it has been previously shown that cGAS can recognise DNA:RNA hybrids and that it can activate the pathway.[30] In addition to DNA:RNA hybrids, unpaired DNA nucleotides surrounding short double-stranded regions—such as those found in stem-loop structures of single-stranded HIV-1 DNA—can activate the type I interferon–inducing DNA sensor cGAS in a sequence-dependent way. In particular, DNA structures with unpaired guanosines

flanking short (12- to 20-base pair) double-stranded segments, known as Y-form DNA, strongly stimulated cGAS and significantly enhanced its enzymatic activity.[31]

The activation of cGAS occurs when dsDNA or other substrates mentioned previously is found in the cytoplasm. Although some research has shown that cGAS is also present in the nucleus, it is not activated in the nucleus. [32] It has been shown that other molecules in the nucleus, such as BAF, will outcompete cGAS for DNA binding, therefore restricting the activation of the cGAS-STING pathway. [33] There is still no consensus on whether cGAS activates at the ends of DNA or not as some studies have shown that it can activate on a undigested plasmid.[29, 34 35]

The way the cGAS-STING pathway doesn't always activate in the presence of self-DNA is a fascinating matter. Indeed, several mechanisms are in place to ensure that the pathway remains silent in the presence of self-DNA while responding robustly to foreign invaders. One critical factor is compartmentalization. Only under exceptional circumstances, such as during cell division, does the nuclear envelope allows the exposure of the DNA to the rest of the cell.

Although, to this date, it is now globally accepted that cGAS is also located at the nucleus for potentially different purposes.[36] Post-translational modifications were shown to be made on cGAS such as phosphorylation to inhibit its activation. [37] The nucleus is guarded by the nuclear envelope, a double membrane structure that acts as a barrier against the escape of self-DNA into the cytoplasm. The DNA in the nucleus is also much longer than the 70nt previously mentioned and offers a great affinity on the nucleosomes for cGAS in a way that cGAS cannot form dimers to activate .[38]

Another layer of defense is the presence of nuclear DNA-binding proteins, such as histones, which package the DNA into a condensed form and lowers the affinity of cGAS.

This packaging effectively conceals self-DNA from cGAS and other cytoplasmic sensors. A cryo-electron microscopy study from Kujirai, T. et al. (2020) showed that nucleosomes themselves inhibit the stimulation of cGAS as the nucleosome complex inhibits cGAS by binding the acidic patch of the histone H2A-H2B dimer and nucleosomal DNA.[39] Additionally, DNA methylation patterns play a role in distinguishing self-DNA from foreign DNA, providing yet another level of specificity in the discrimination process.[40] It has been shown that the phosphorylation of CDK1 impairs cGAS of sensing self-DNA in mitosis for example.[41, 42]

The presence of deoxyribonucleases (DNase) is critical for maintaining a low level of DNA in the cytoplasm. At least three DNases have been identified in the past to degrade self-dsDNA : DNAse I, DNAse II and TREX1 (also called DNAse III). These DNase maintain a level of cytosolic DNA under the threshold of cGAS activation to stop the cell from having an immune response.[34]

Despite these safeguards, the cGAS-STING pathway can be activated in response to self-DNA under specific conditions, such as during cellular stress, genomic instability or cell damage.[43,44] Such activation can lead to the production of IFN-I and other proinflammatory cytokines.[43, 44] Along with the functions previously mentioned, the cGAS/STING pathway is also involved in various functions such as in the non-canonical inflammasome formation.[45] The cGAS-STING pathway has a role in the regulation of the calcium hemostasis and in the endoplasmic reticulum (ER) stress response.[46] It plays a crucial role in regulating the TRPV2 calcium channel to rescue replication forks.[47] cGAS regulation by PIP2, a major regulator of $Ca2^{+}$ signaling through the ion channels and the endoplasmic reticulum (ER) stores linked $Ca2^{+}$ -sensor, STIM1, is crucial for cGAS sensing of self-DNA.[48]

The detection of leaking mitochondrial DNA (mtDNA) is also a role of the cGAS-STING pathway.[46] In addition, it has been shown to be responsible of the autophagy induction,

or the cellular senescence and including in the senescence-associated secretory phenotype (SASP) production.[49] Indeed, the SASP is an inflammatory response associated with the senescence of cells and one of the pathways responsible for the induction is the cGAS-STING pathway.[50] Senescence is a stable cell cycle arrest that can be triggered by cells in response to various stimuli including DNA damage, telomere shortening and numerous stress signals.[51] It is an important part of the body's defense to prevent dysfunctional cells from further causing damage or becoming cancerous but also to simply dispense of old cells. There has been evidence that self-derived DNA fragments activate the SASP.[49] It has been shown that there is an activation of cGAS as deoxyribonucleases are downregulated in senescent cells which will increase the amount of DNA present in the cytoplasm.[52] Yang, H., et al. (2017) showed that MEF and human BJ fibroblasts cGAS KO cells were used and treated with etoposide to induce DNA damage. They discovered that cGAS was essential for the SASP as the expression of the genes IL6 and IL1β were significantly reduced in the cGAS KO cells. The same was done with ionizing radiation and the result was similar.[53]

The cGAS-STING pathway has a very important and beneficial role in our system's immunity. It helps our body to detect danger within a cell and alert surrounding cells. Dysregulation of this balance can lead to autoimmune and inflammatory diseases, as self-DNA leakage or DNase deficiencies can aberrantly trigger the pathway. Beyond antiviral defense and self-DNA detection, it also has a role in autophagy, ER stress, calcium homeostasis and cellular senescence, linking it to aging, genomic stability, and inflammation control.

1.5 The diseases associated with the cGAS-STING pathway and Interferonopathies

The intricate interplay between the cGAS-STING pathway and self-DNA has far-reaching implications for human health and disease. On one hand, the pathway's ability to detect and respond to foreign DNA is crucial for antiviral defense and immune surveillance

against cancer cells, highlighting its role as a guardian of the good health of the entire system. On the other hand, dysregulation of the cGAS-STING pathway, leading to the activation of immune responses against self-DNA, has been implicated in a range of autoimmune and autoinflammatory disorders.

Conditions such as systemic lupus erythematosus (SLE), Aicardi-Goutières syndrome (AGS), and STING-associated vasculopathy with onset in infancy (SAVI) are linked to aberrant activation of the pathway, resulting in chronic inflammation and tissue damage.[54] Furthermore, recent research has unveiled the role of the cGAS-STING pathway in diseases including neurodegenerative disorders like Alzheimer's disease and metabolic conditions such as obesity.[55] These findings suggest that the pathway's involvement in immune responses may extend beyond the traditional knowledge of infection and autoimmunity.

The cGAS-STING pathway, by initiating inflammation, promotes tumorigenesis in certain types of cancer. Chronic inflammation caused by SASP via the cGAS-STING pathway is crucial for the development of hepatocellular carcinoma (HCC).[49] Cancerous cells with high genome instability can generate micronuclei or release fragmented DNA in cytoplasm and activate the cGAS-STING pathway. [56] The involvement of cGAS or STING in cancer has also been reported in many previous studies as it is becoming a therapeutic target for therapy against cancer.[49, 57, 58]

In chronic obstructive pulmonary disease (COPD), a severe chronic inflammatory disease that causes lung functional loss with chronic lung inflammation, it has been shown that the cGAS-STING pathway is activated as one of the pathways in the immune response by epithelial and inflammatory cells of smokers.[59] By using genetic knockout mice, it was found that lung inflammation was alleviated in the absence of cGAS or STING. This indicates that the pathway might be a potential therapeutic target for controlling lung inflammation caused by cigarette smoking.[60]

In summary, the innate immune system constitutes the body's primary defense mechanism, employing physical barriers, phagocytic and natural killer cells, and inflammatory mediators to provide rapid, non-specific protection while initiating adaptive immune responses. Inflammation is a fundamental biological process essential for pathogen clearance and tissue repair, yet its persistence leads to chronic inflammatory states that underlie numerous autoimmune, metabolic, and cardiovascular disorders. The cGAS–STING pathway functions as a key sensor of cytosolic DNA, activating type I interferon and proinflammatory cytokine signaling to coordinate antiviral and antitumor immunity while maintaining tolerance to self-DNA. Aberrant activation or defective regulation of this pathway contributes to the pathogenesis of autoimmune and autoinflammatory diseases such as systemic lupus erythematosus, Aicardi-Goutières syndrome, and STING-associated vasculopathy, as well as certain cancers and neurodegenerative disorders.

2.0 The nuclear membrane

The cell nucleus serves as a guardian of genetic integrity, protected by a structure known as the nuclear membrane. The nucleus, a vital compartment in eukaryotic cells, contains the blueprint of life in the form of DNA. The nuclear membrane's core responsibility is to secure and protect the everything inside the nucleus but also to regulate the cell's functions.

The nuclear membrane, often referred to as the nuclear envelope, is a double-layered structure that encapsulates the nucleus, effectively segregating its contents from the rest of the cell. The outer nuclear membrane is continuous with the endoplasmic reticulum, while the inner nuclear membrane is associated with various nuclear components.[61]

The nuclear membrane is not just a physical structure; it plays an active role in numerous cellular functions. One of its paramount functions is the regulation of nucleocytoplasmic transport. Small molecules and ions can pass through the nuclear pores freely, while macromolecules, including RNA and proteins, must adhere to intricate transport mechanisms. This strict control ensures that only specific proteins or genetic material can enter or leave the nucleus. This regulation is pivotal for gene expression, DNA replication, and numerous other nuclear processes. Furthermore, the nuclear membrane houses a diverse array of proteins that contribute to its structural integrity, such as lamina proteins, which provide mechanical support. Additionally, the inner nuclear membrane interacts with chromatin, the complex of DNA and proteins that form chromosomes, impacting gene regulation and epigenetic processes.[62]

There has been reports of senescent cells exhibiting a loss of their nuclear envelope integrity and a formation of CCFs (cytoplasmic chromatin fragments). These CCFs once in the cytoplasm are then detected by the cGAS-STING pathway and will then proceed to induce an inflammatory response.[63] Some researchers have found nuclear 'blebbing' associated with the formation of CCFs in senescent cells. They found this event to be associated with a depletion of lamina A/C and B1.[64] Some pathologies have also been associated with CCFs and a constant expression of inflammatory genes such as Louis-Bar Syndrome.[65] Indeed, the Louis-Bar Syndrome occurs when a mutation occurs on the ATM gene which leads to defective DNA repair, especially double-stranded-DNA. The lack of DNA repair increased the genome instability of the cell and leads to CCFs. It also be previously discussed that the ATM gene is involved in preventing mitochondrial disorders from oxidative stress which could lead to mitochondrial DNA being released in the cell.[66]

Integral to the integrity of the nuclear membrane are the nuclear pore complexes (NPCs), which act as controllers at the gates of the nucleus. At first glance, the nuclear membrane may appear as a passive physical barrier, but its dynamism and selectivity in molecular transport have made it a focal point of investigation. Indeed, even though the entire structure itself is anchored in the nuclear membrane, individual nucleoporins can be very

dynamic[67, 68]. It has been shown that the NUP107-160 complex for instance is very stable but nucleoporins forming the nuclear basket are very dynamic.[69]

2.1 Lamina

Within the nucleus and its membrane, there is a vital structural component known as the nuclear lamina that plays a central role in maintaining the integrity and the functionality of the cell. The laminas, composed of lamina proteins, form a dynamic scaffold beneath the inner nuclear membrane and act as guardians of the genetic information within the nucleus. Their multifaceted functions encompass structural support, gene regulation, and cellular responses to mechanical and environmental cues.[62] Lamin proteins, the building blocks of the nuclear lamina, are fibrous proteins that provide structural stability to the nucleus. The lamina primarily consists of two major classes of lamin proteins: A-type and B-type lamins. A-type lamins, represented by lamin A and lamin C, are generated from the LMNA gene and are localized to the nuclear lamina, while B-type lamins, such as lamin B1 and lamin B2, are encoded by the LMNB1 and LMNB2 genes and are distributed throughout the nucleoplasm.[70]

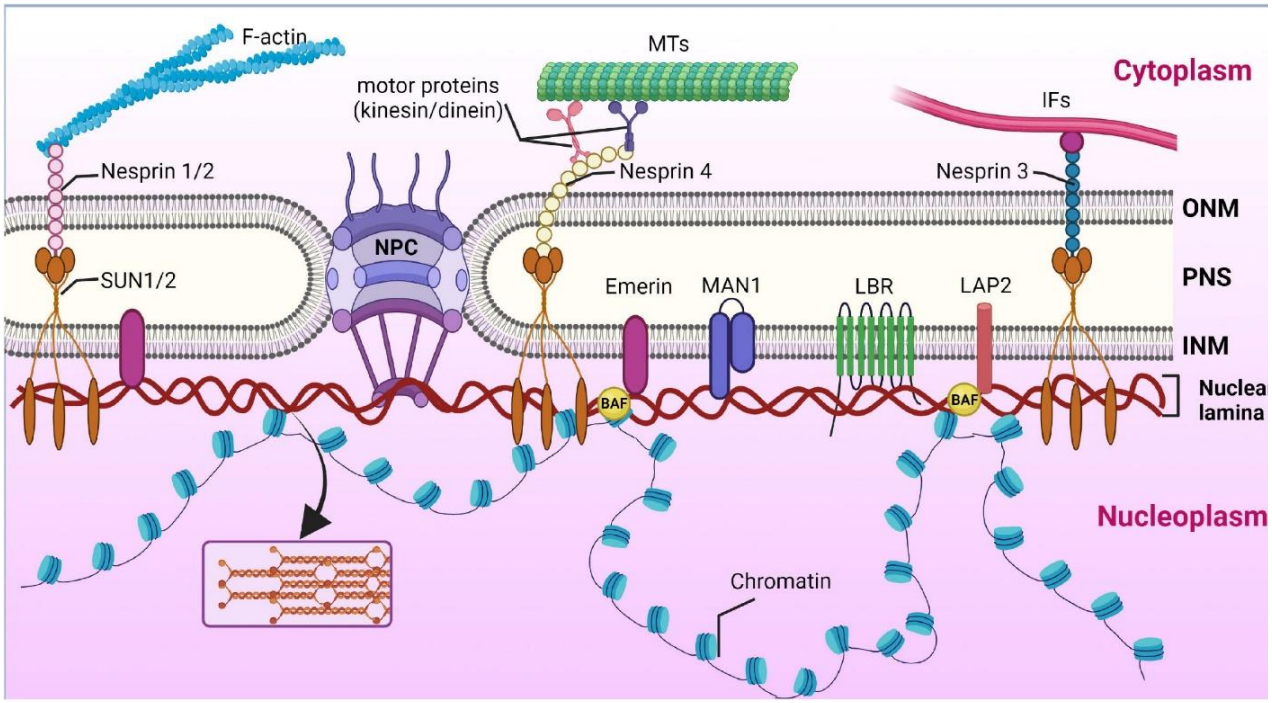


Figure 4: The lamina network and the nuclear membrane

Source : Malashicheva, A. & Perepelina, K. Diversity of Nuclear Lamin A/C Action as a Key to Tissue-Specific Regulation of Cellular Identity. Frontiers in Cell and Developmental Biology 9 (2021).

This structural integrity is critical for protecting the genetic material within the nucleus from external forces and mechanical stresses. The lamina's role as a guardian of nuclear integrity is particularly vital during processes such as cell division and mechanotransduction, where the nucleus must resist deformation and retain its structural integrity.[71]

Beyond its structural role, the nuclear lamina is deeply involved in gene regulation and chromatin organization. Lamins interact with various proteins and chromatin regions, influencing gene expression and epigenetic modifications. The nuclear periphery, where the lamina is situated, is enriched with factors that contribute to transcriptional regulation and chromatin organization. [72]

Nuclear lamina can sequester specific chromatin regions, influencing the spatial organization of the genome. Changes in lamina-chromatin interactions can impact gene expression patterns and contribute to cellular differentiation and development. [72]

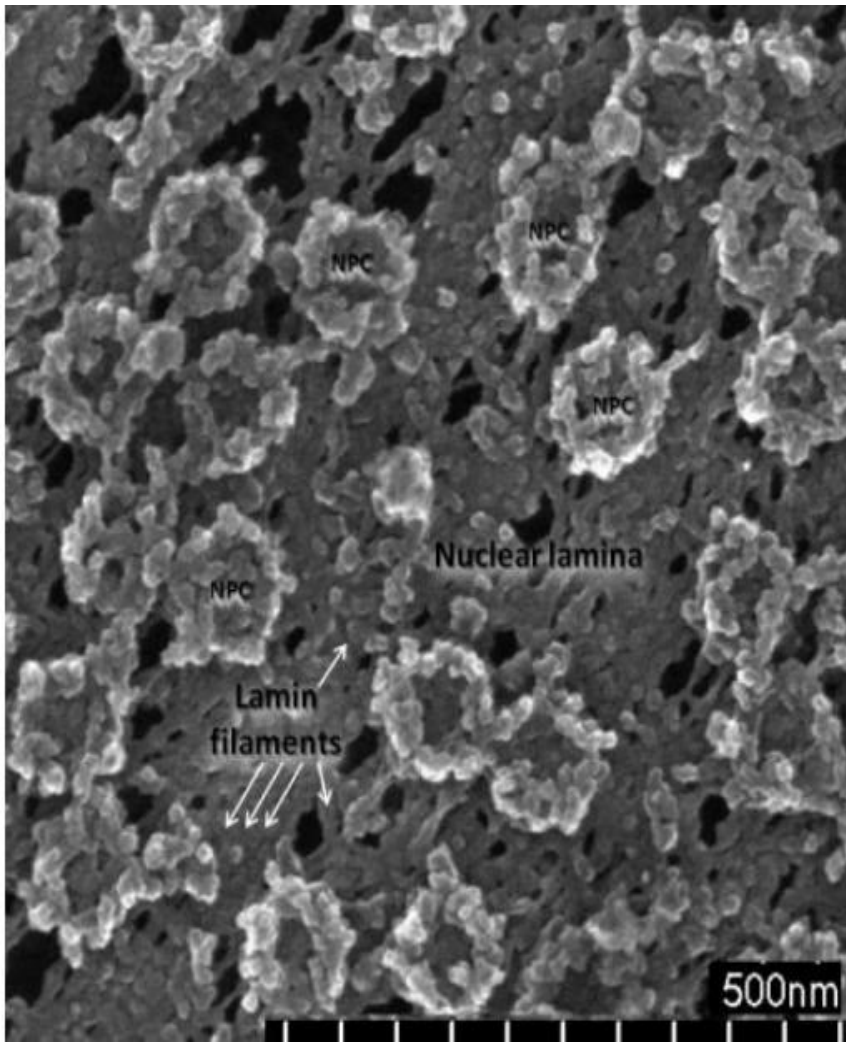


Figure 5 : Lamin filaments by electron microscopy

Source: Martin W. Goldberg, Nuclear pore complex tethers to the cytoskeleton, Seminars in Cell & Developmental Biology, Volume 68, 2017,Pages 52-58, ISSN 1084-9521

2.2 Diseases associated with the lamina

The significance of the nuclear lamina becomes particularly apparent when considering diseases linked to mutations or dysregulation of lamin proteins. Mutations in the LMNA gene, which encodes lamin A and lamin C, are associated with a group of disorders collectively referred to as laminopathies. These disorders can affect various tissues and systems, resulting in conditions such as muscular dystrophy, lipodystrophy, and premature aging syndromes.[73-76] Laminopathies lead to genomic instability and an accumulation of DNA damage. This instability and DNA damage can lead to the activation of the cGAS-STING pathway and to major disruption in the nuclear envelope and potentially cause leakage of DNA in the cytosol.[77]

The Emery-Dreifuss muscular dystrophy is also caused by mutations of the gene LMNA that regulates the A-type lamins.[78] It is a rare genetic muscle disease that slowly aggravates the muscle condition of shoulders, arms and calves of the patient and also causes stiff joints. One of the most famous laminopathy known today is the Hutchinson-Gilford Progeria Syndrome, it is characterized by features that develop in the childhood and can be perceived as accelerated aging. The characteristics of the syndrome includes narrow nose, small mouth, micrognathia, alopecia, atherosclerosis and more.[79] Mutations in the Lamina has also been found in dilated cardiomyopathies even though multiple genes can be attributed to those pathologies. There also been cases of patient with Charcot-Marie-Tooth Type 2B1, a disease that affects both sensory and motor nerves in the body, which were found to have LMNA mutations The Werner Syndrome is also caused by LMNA mutations.[80],[81],[82] Understanding the impact of lamin mutations on cellular and tissue function is an active area of research in medical genetics.

Beside the Lamina A,B and C, other lamina-associated proteins are the culprits of several pathologies as shown in **figure 6**. For example, mutations encoding for LBR gene, a receptor of the Lamina B protein are found in Pelger-Huet Anomaly and Greenberg

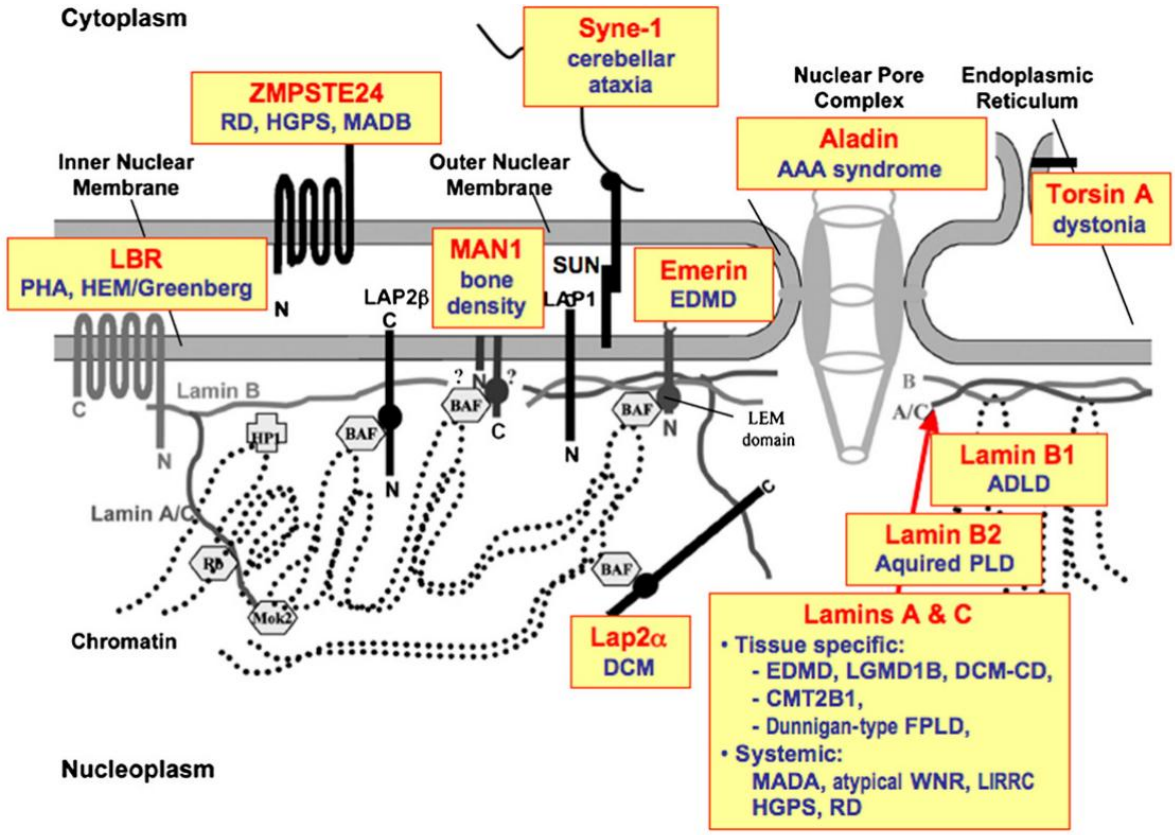


Figure 6: Laminopathies and other lamina-associated pathologies

Source: Howard J. Worman, Gisèle Bonne,"Laminopathies": A wide spectrum of human diseases, Experimental Cell Research ,Volume 313, Issue 10, 2007 ,Pages 2121-2133, ISSN 0014-

dysplasia.[83] Mutations of the Emerin gene, a protein that helps tether chromatin in the nucleus, are responsible for Emery-Dreifuss muscular dystrophy.[83] Furthermore, mutations in the gene coding for the ZMPSTE24 protein, an important metallopeptidase crucial for the maturation of Lamina A, are found in diseases such as Hutchingson-Gilford progeria syndrome and mandibuloacral dysplasia.[83]

2.3 The nuclear pore complex (NPC)

The Nuclear Pore Complex (NPC) is a critical structure in eukaryotic cells that regulates the passage of molecules between the nucleus and cytoplasm. Comprising multiple protein components called nucleoporins (Nups), the NPC forms a cylindrical structure within the nuclear envelope. It operates with eightfold rotational symmetry, allowing for

precise control over the movement of macromolecules while safeguarding the integrity of the nucleus. The central channel of the NPC serves as a gateway for nucleocytoplasmic transport and is vital for gene expression and cellular responses.[84] The NPC acts as a selective gatekeeper, ensuring that only authorized molecules are granted access, thus maintaining the integrity of the nuclear environment. Small molecules and ions can diffuse through the NPC, macromolecules, including RNA and proteins, require specific transport proteins to facilitate their passage.[85]

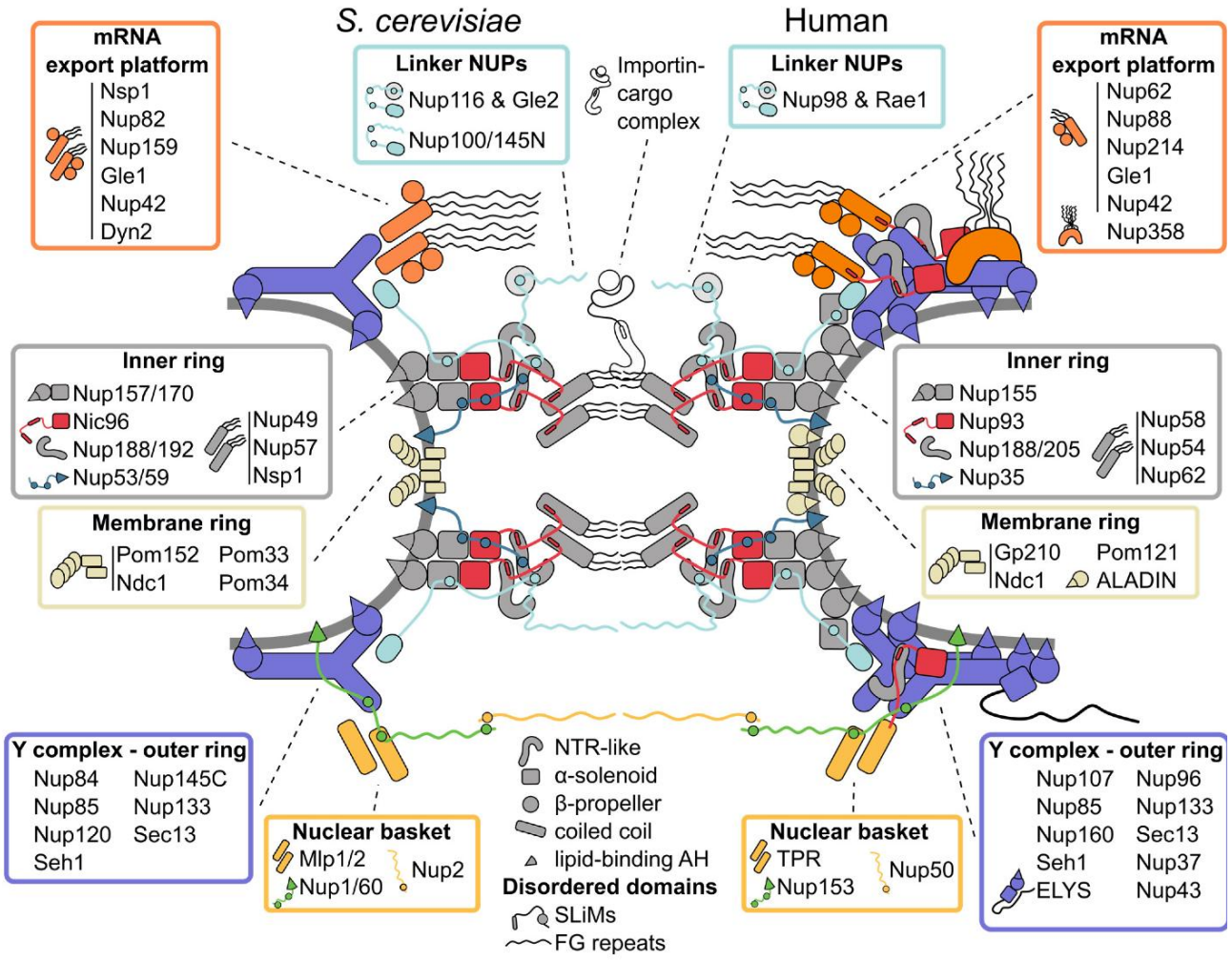


Figure 7: Inventory of the nuclear pore complex

Source: Dultz, E.; Wojtynek, M.; Medalia, O.; Onischenko, E. The Nuclear Pore Complex: Birth, Life, and Death of a Cellular Behemoth. Cells 2022, 11, 1456

The NPC is composed of 30 different nucleoporins conserved between all animal species that are present in multiple of 8 since it is a symmetric structure.[86] These nucleoporins have distinct functions and they will form protein complexes of the NPC called subcomplexes. The four major subcomplexes are: the outer ring, the inner ring, the cytoplasmic filaments and the nuclear basket which resides at the nuclear portion of the NPC. As shown in **figure 7**, the outer ring is composed of 10 nucleoporins, the inner ring is composed of 5 plus 3 associated nucleoporins, the cytoplasmic filaments are composed of 8 nucleoporins and the nuclear basket is composed of 3. Some nucleoporins like NUP98 and NUP62 are components of multiple subcomplexes and thought to act as bridges between subcomplexes. Each subcomplex has been attributed specific functions and are dependent on their position within the nuclear pore complex. For instance, the

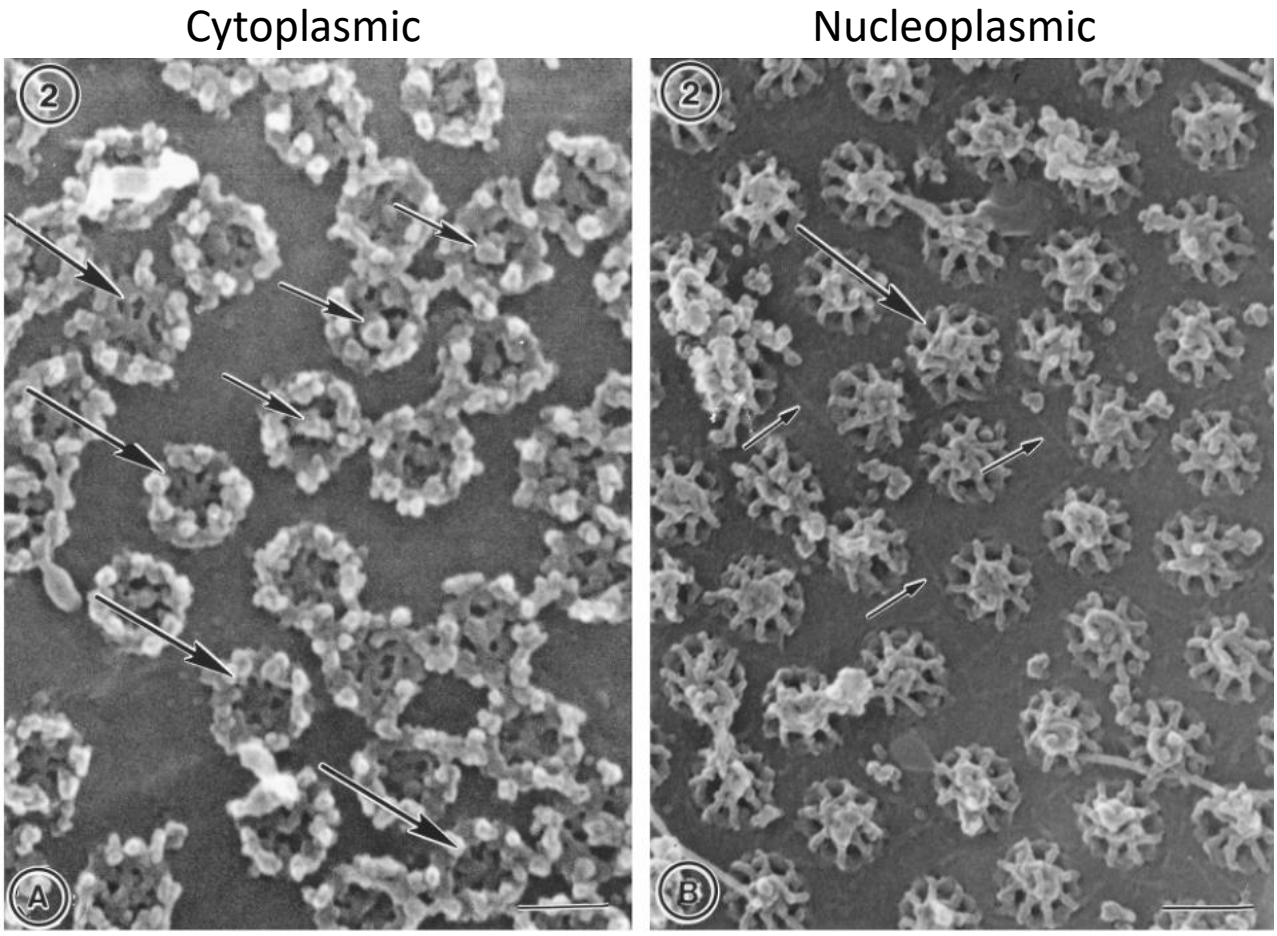


Figure 8: Electron microscopy of the Nuclear Pore Complex

Source: Martin W. Goldberg, Nuclear pore complex tethers to the cytoskeleton, Seminars in Cell & Developmental Biology, Volume 68, 2017,Pages 52-58, ISSN 1084-9521,

Y complex (outer ring) and the inner ring complex are known for their structural attributes and are essential to this formation of the NPC.[87]

The structure of nuclear pore complexes (NPCs) in vertebrates, as observed through electron microscopy reconstructions, reveals that they have a central framework that has an octagonal shape and is approximately 40 nanometers long. This framework has an internal diameter of about 50 nanometers and an external diameter of around 120 nanometers. On the side facing the cell's interior (cytoplasm), there is a ring-like structure with filaments extending about 50 nanometers, which is attached to the central framework. The nuclear side of the NPC consists of two components: the nuclear ring and the distal ring, together forming the nuclear basket of the NPC, which is approximately 75 nanometers in length. This nuclear basket is connected to the central framework on the nuclear side of the NPC.[88] Approximately two-thirds of these Nups are classified as structural or scaffold Nups and together form the predominant physical structure of the pore. The remaining one-third component proteins are classified as phenylalanine-glycine-repeat Nups (FG-Nups) that form the selectively permeable barrier within the nuclear pore.[89] It has been shown that aging mitotic cells are struggling with the assembly of large nuclear pore complexes the older they get. A loss of quality control of the nuclear pore complex assembly and a loss of the protein complex stoichiometry seems to be a prominent phenotype in aging cells.[90] The assembly complex is efficient and operates at a very high rate. When macromolecules are translocated through the NPCs, it is a very rapid process, occurring at a rate of about 1000 translocations per second.[87]

Beyond its role as a molecular gate, the NPC plays a critical role in the regulation of gene expression. It influences the spatial distribution of genes within the nucleus, which, in turn, affects the timing and efficiency of transcription and gene regulation. Furthermore, the NPC is intricately involved in cellular responses to environmental cues, allowing the cell to sense and adapt to changes in its surroundings.

Dysfunction in the NPC can lead to a range of diseases and disorders. In cancer, for example, alterations in NPC functionality can disrupt the transport of essential molecules, contributing to uncontrolled cell growth and proliferation. Additionally, genetic mutations in nucleoporins or defects in nucleocytoplasmic transport have been linked to neurodegenerative disorders and other diseases.[91]

As shown in **Figure 9** The NUPs seed the formation of NPCs by interacting with the re-forming NE Early during mitotic exit, NPC assembly initiates with chromatin-bound NUP assemblies which are integrated into membrane fenestrae of the reforming NE. In contrast, NPC assembly into a sealed NE during late mitosis and in interphase occurs via an "inside-out" mechanism that initiates with the deformation of the inner nuclear membrane and ultimately requires fusion of the inner and outer nuclear membranes.[92, 93 94] It requires fusion of the inner and outer nuclear membranes by a poorly understood mechanism. The membrane fusion involves phosphatidic acid (PA) rich membranes and the transmembrane protein torsin AAA+ ATPases in vertebrates.[95]

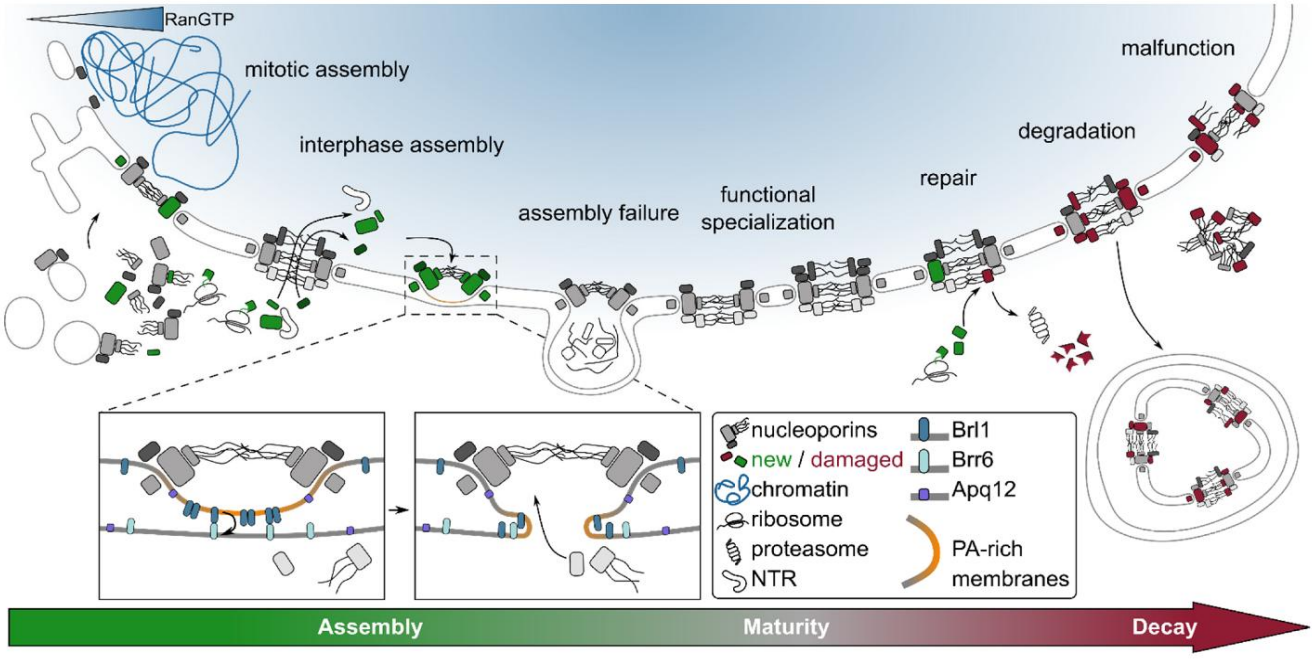


Figure 9: The lifecycle of the nuclear pore complex

Source: Dultz, E.; Wojtynek, M.; Medalia, O.; Onischenko, E. The Nuclear Pore Complex: Birth, Life, and Death of a Cellular Behemoth. *Cells* 2022, *11*, 1456.

inner nuclear membrane enclosed by the NE and deprived of cytoplasmic NUPs. NPCs mature into compositionally and functionally different sub-populations, e.g., the budding yeast NPC can vary in the content of nuclear basket proteins or the number of nuclear Y rings. Damaged NPCs can be repaired by proteasomal degradation of individual NUPs without the requirement for complete NPC disassembly. Entire NPCs can be degraded by the autophagy machinery. NPCs can accumulate damage in old age or disease, such as oxidative damage, loss of NUPs or phase-transition of FG NUPs in the cytoplasm, which leads to NPC malfunction and impaired transport.[96]

2.4 NPC-associated diseases

As previously mentioned, dysfunction in the NPC can lead to a range of diseases and disorders. One well-known example is cancer, where alterations in the NPC's functionality can affect the transport of proteins and RNAs, leading to uncontrolled cell growth and proliferation.[97,98] Additionally, genetic mutations in NUPs or defects in nucleocytoplasmic transport can result in a multitude of diseases. Here are some examples of diseases associated with the dysfunction of the NPC.

Nup98 is involved in at least 14 translocations, mainly associated with hematological malignancies including myelodysplastic syndrome (MDS), acute myelogenous leukemia (AML), and chronic myelogenous leukemia (CML).[99] Studies been reported in which NUP point mutations were shown to cause diseases: a highly specific kidney disease, the early childhood-onset disease steroid-resistant nephrotic syndrome (SRNS), and an XX gonadal dysgenesis disease. [100] Both diseases are caused by mutations in human scaffold NUPs — NUP93, NUP205 and NUP107 which are 'housekeeping' nuclear pore components and have key scaffolding functions.[101]

Another instance of a disease associated with nucleoporin involvement, affecting the development of female gonads, is attributed to a recessive mutation in NUP107. The D447N mutation changes the negative charge into a neutral one, which abolishes salt

bridges, therefore presumably a destabilization of the overall NUP107 protein fold in this region occurs and possible impairment of NUP107's interaction with other subunits in the NPC.[100] Patients with this illness exhibit its manifestation when carrying homozygous diploid traits. The conjecture involves the idea that such distortions might compromise the meiotic DNA-damage response, considering the functional associations of the Y-complex with chromatin. The mutation could lead to nuclear transport defects of specific signaling molecules crucial for ovary development, such as bone morphogenetic protein 15 (BMP15), a member of the TGF-β superfamily. These disruptions collectively may result in oocyte death. They also found that the knockdown of NUP107 in the gonads of female Drosophila melanogaster resulted in sterility.[100]

Examples of pathologies associated with the decrease of nucleoporins expression include ALS (myotrophic lateral sclerosis), which Workman, M.J. et al (2023) showed that the levels of NUP188 was decreased in patients with severe ALS.[102] Similarly, researchers have shown that Nup50 levels are lower in ALS patient samples and in iPSC-derived neurons from ALS patients relative to controls.[103]

Furthermore, in some diseases the expression level of some nucleoporins has been found to be altered. These differences in expression are not necessarily the cause of these diseases but are often correlated to pathologies and can be used as biomarkers. For instance, Nup88 is overexpressed in ovarian, breast, mesothelioma, colon, and prostate cancer patient specimens, and its overexpression is typically associated with advanced tumors.[104] Nup214 translocations are present in rare forms of AML and acute non-lymphoblastic leukemias.[105] [99]

The nuclear pore complex has been associated with a great number of diseases. It is not surprising as it is vital component of the cell and is involved in a myriad of functions and pathways.

2.5 TPR

TPR, translocated promoter region, is a nucleoporin located in the nuclear basket section of the NPC. It is part of the 3 nucleoporins forming this very dynamic subcomplex and has been linked to a multitude of cellular functions. There is a total of 8 copies of TPR in each NPC.[88] It is a coiled-coil structure with a FG repeat at the end of it. The nuclear basket nucleoporins directly interact with the nucleocytoplasmic transport machinery, provide anchoring sites for mRNA export and quality control factors, and link the NPC to the transcriptional regulatory machinery.[106, 107] It has been studied for various pathologies and conditions such as cancer.[108-111] The nuclear basket and TPR have been identified to be regulators of gene expression, to interact with the nuclear lamina, act as sentinels in quality control of misfolded proteins or RNA that try to transit through the NPC. [108] It is also involved in the signal recognition of proteins presenting nuclear localization signals (NLS) or nuclear export signals (NES).[112]

Li, Y. et al. (2021) has shown that in the absence of TPR in the NPC, it was noticed that 75% of the incoming mRNPs from the nucleus failed to dock at the nuclear basket. It was also suggested that TPR's function was not compensated by NUP153 or NUP50 even if they possessed their full copies. They also found that the absence of TPR slightly altered the nuclear export route after the mRNPS had entered the nuclear pore basket.[88] TPR is also involved in the quality control of the unspliced mRNA in the nucleus.[88, 112]

TPR has been shown to protect from RNA-mediated replication stress, symmetry of the replication forks and interplay with the GANP protein, a component of the TREX-2 complex.[108] The TREX-2 complex is an evolutionarily conserved multiprotein complex that acts as a platform to which many of the components of the nuclear mRNA processing machinery bind, it is important for the mRNA biogenesis, nuclear export, facilitating integration of this phase of the gene expression pathway, as well as mediating the re-positioning of highly regulated actively transcribing genes (such as GAL1) to the NPCs. [113] Studies have shown that TPR inhibits the tRNA nuclear export and cell growth in cancer

cells. Indeed, Chen, M. et al. (2021) has shown that TPR is in bigger quantity in various lung cancer cells in comparison to normal human lung tissue by Western Blot. Also, they have measured an increased nuclear accumulation of tRNAs in lung cancer cells which had a TPR knockdown as well as a slower growth.[110]

The nucleoporin TPR has been linked to events affecting the P53 signaling, an important coordinator of the stress caused by DNA damage and key tumor suppressor. [111] P53, which is involved in the regulation of exonucleases, has also been linked to an activation of the cGAS-STING pathway by degrading TREX1 which leads to an accumulation of cytosolic DNA.[114, 115] In contrast, it also has been demonstrated that mutations of P53 suppresses the STING/TBK1/IRF3 pathway.[115]

TPR–Met fusions, where Met is a receptor tyrosine kinase that controls morphogenesis, proliferation, survival, and migration, are found in gastric carcinomas. [107] Typically, the functions of the fusion protein are not related to transport and could be the driving feature in terms of cancer.

Very interestingly, Boumendil, C., et al (2019) has demonstrated that TPR is necessary for the SASP as well as senescence-associated heterochromatin foci (SAHF). [116] Indeed, when IMR-90 cells were transfected with an siRNA against TPR and senescence was induced by an oncogene. In depleted cells of TPR, no SASP was observed following the induction of senescence in comparison to their control. This result is similar to the results previously mentioned in an experiment where cGAS was removed from cells and the same phenotype was observed.[116] This research played a major role for this project as it served as the main starting point for this project. Indeed, this phenotype observed following the depletion of TPR having an effect on an inflammatory response such as the SASP made us think about its potential role in another inflammatory response pathway such as the cGAS-STING pathway.

2.6 NUP160

Nup160 is a nucleoporin located in the Y complex of the outer ring of the nuclear pore complex and it is a β-propeller domain with a α-helical solenoid structure.[106 117, 118] This complex, often called the NUP107-160 complex, forms the outer ring, located on both sides of the nuclear pore complex. Because of its shape, this complex is also called the Y complex. This complex has been known to relate to vesicle coats and potentially stabilizes the curved pore membrane of the NPC.[119] The Y complex is recruited to kinetochores at the onset of mitosis. It is also required for the recruitment of Crm1 and RanGAP1-RANBP2.

The Y complex has been identified to be a critical part of the NPC assembly as it decreased the NPC density and prevented associated of FG-repeat nucleoporins with chromatin. [120] Zuccolo, M. et al. 2007 demonstrated that an efficient knockdown of the NUP107-160 complex has been shown to induce a mitotic delay.[121]

Ryan, K.J. et al (2017) has demonstrated that the NUP160 gene has been identified as one of the six neurodegenerative disease–associated loci with genotype-driven gene expression differences in their cell model system, human monocyte-derived microglia-like (MDMi) cells .[122] NUP160 has also been shown to be implicated in the kidneys as monogenic steroid-resistant nephrotic syndrome (SRNS) and proteinuria in nonconsanguineous family were found to carry heterozygous mutations of the NUP160 gene.[123]

As an individual nucleoporin, NUP160 remains largely unexplored, with limited information currently available.

2.7 NUP358

Nup358, also known as RanBP2, is a sizable nucleoporin in mammals that possesses several distinct domains. These include a cyclophilin homology domain, a domain that acts as a ligase for modifiers similar to ubiquitin within the SUMO family, domains responsible for binding to both GTP- and GDP-bound states of the Ran GTPase, a leucine-rich region (LRR), and a region containing numerous motifs for binding to nuclear transport receptors.

Nup358 can be found at metaphase spindles and kinetochores in a manner dependent on microtubules. Its presence in these locations plays a vital role in facilitating interactions between microtubules and kinetochores. Additionally, during interphase, Ran governs the dynamics of mitotic microtubules and the assembly of the spindle by controlling the binding and release of spindle assembly factors from proteins that bind Ran-GTP and function as nuclear transport receptors. Also, the RANBP2 nucleoporin has also been associated with functions in chromosome segregation and miRSIC-mediated mRNA silencing.[124, 125]

Nup358 plays a role in modulating neuronal excitability by functionally stabilizing the electrical characteristics of the neuronal membrane. [126] It also has been a nucleoporin that has vastly diverged over evolution in many species has it is a very dynamic nucleoporin. [124]

This nucleoporin has been linked with multiple diseases such as colorectal cancer, autoimmune myositis, multiple myeloma, acute myeloid leukemia, myeoproliferative neoplasm and more.[127] RANBP2 mediate nucleocytoplasmic transport of AIRE, a crucial transcriptional regulator that acts on gene expression and influences clonal deletion of differentiating T cells in the thymus.[127] The gene encoding for RANBP2 has been mutated

and linked as the main cause of acute necrotizing encephalopathy (ANE) as shown in an article in 2009.[128]

Interestingly, studies have shown that patients with acute necrotizing encephalopathy, a disease associated with RANBP2 mutations, have been reported to have elevated levels of I IL1-β and IL-6 serum levels which suggests an inflammatory response following a mutation of NUP358.[129, 130]

In summary, the nuclear membrane, or nuclear envelope, is a double-layered barrier that preserves nuclear compartmentalization and controls molecular exchange through selective nucleocytoplasmic transport mediated by nuclear pore complexes (NPCs). Beneath the inner membrane, the nuclear lamina, composed of A- and B-type lamins, provides mechanical stability, organizes chromatin, and regulates gene expression and cellular differentiation.

Mutations or defects in lamins or lamina-associated proteins compromise nuclear integrity and are associated with laminopathies, including muscular dystrophies, premature aging syndromes, and cardiomyopathies, which can trigger cGAS–STING activation through cytosolic DNA leakage. The NPC, a dynamic structure built from nucleoporins such as the NUP107–160 complex, NUP98, NUP358, and TPR, governs bidirectional molecular transport and participates in gene regulation and mitotic progression; its dysfunction has been implicated in such as cancer and neurodegenerative diseases.

Together, alterations in the nuclear lamina and NPC disrupt genome organization, cellular signaling, and inflammatory homeostasis, underscoring their central roles in maintaining nuclear architecture and overall cellular health.

What is the role of nucleoporins in cytosolic DNA accumulation and cGAS activation?

Nucleoporins seem to play a role in the integrity of the nuclear membrane; this suggests that their depletion could lead to release and accumulation of nuclear DNA in the cytosol and therefore activation of the cGAS-STING pathway

3. Hypothesis and objectives

Our hypothesis is that the depletion of the nucleoporin TPR will negatively affect the nuclear integrity, which will result in an accumulation of DNA and cGAS activation

Objective 1: Determine if the depletion of TPR has an effect on the cGAS-STING pathway and the innate immune response

Objective 2: Determine if the effect of the depletion of TPR is specific to TPR or other nucleoporins

Objective 3: Determine if the nuclear integrity is compromised following a depletion of the nucleoporins TPR, NUP160 and NUP358

4. Materials and methods

4.1 Cell culture

Mouse Embryonic Fibroblast wild type (wt) immortalized with SV40T, Mouse Embryonic Fibroblast STING KO immortalized with SV40T and HeLa cells were cultured in Falcon® cell culture dish at 37°C , 5% CO_2 in DMEM medium with 4.5g/L glucose, L-glutamine supplementation, and sodium pyruvate additive accompanied with 10% (v/v) heat-inactivated foetal bovine serum and 1% antimycotic-antimycin. The MEFs STING KO used for these experiments were BALB/c-*Sting1*^{em3Vnce}/J and the MEFs wild type (wt) are BALB/*c-WT* constructed in laboratory.[131]

4.2 Cell transfection

MEFs and HeLa cells were transfected using the transfection reagent Lipofectamine™ RNAiMAX by Fisher at a concentration of 1:600 v/ combined with the siRNA duplex against TPR, NUP160 and NUP358 at a concentration of 10nM diluted in Opti-MEM™ in a 48 wells plate. The cells were first transfected at the initial time of the incubation and then transfected again at 5nM after 48 hours for a total of 72 hours. siRNAS from IDT (*Integrated DNA Technologies*) :

2 siRNAS against TPR were pooled (13.1 and 13.2)

mm.Ri.Tpr.13.1-SEQ1 rGrGrArGrCrUrUrGrArGrArArGrCrUrArArArUrArArCrCAA,

mm.Ri.Tpr.13.1-SEQ2 rUrUrGrGrUrUrArUrUrUrArGrCrUrUrCrUrCrArArGrCrUrCrCrArG

mm.Ri.Tpr.13.2-SEQ1 rGrCrUrUrCrArArGrUrUrUrArGrArUrGrArUrArUrUrUrCTC

mm.Ri.Tpr.13.2-SEQ2 rGrArGrArArArArUrArUrCrArUrCrUrArArArCrUrUrGrArArGrCrUrU

mm.Ri.Nup160.13.1-SEQ1 rArArGrCrArUrGrGrArGrGrCrUrArUrUrGrUrCrArArCrUTA

mm.Ri.Nup160.13.1-SEQ2 rUrArArGrUrUrGrArCrArArUrArGrCrCrUrCrCrArUrGrCrUrUrCrA

mm.Ri.Ranbp2.13.1-SEQ1 rCrGrGrArUrArArArUrCrArUrGrGrArArUrUrArUrUrUrUrCAA

mm.Ri.Ranbp2.13.1-SEQ2 rUrUrGrArArArArUrArArUrUrCrCrArUrGrArUrUrArUrCrCrGrUrC

Scrambled Negative Control DsiRNA

4.3 Gene expression analysis

RNA was extracted from lysed MEFs cells using the GENEzol™ RNA extraction kit by *Genaid* according to the manufacturer's protocol. RNA was reverse transcribed using Applied Biosystems High-Capacity cDNA Reverse. Quantitative PCR reaction was performed using SensiFAST SYBR No-ROX Kit (*Bioline*) and specific primers for genes of interest were used. 14 QPCR was performed with a CFX Connect (*Bio-Rad*) qPCR instrument and a QuantStudio 5 from Thermo Fisher Scientific. Results were normalized on the housekeeping gene TBP, GAPDH and 18S using the ddCT method.[132]

4.4 List of primers

Name of primer	**Sequence of primer**
mGAPDH_FWD	GCC TGC TTC ACC ACC TTC T
mGAPDH_REV	CCC CAA TGT GTC CGT CGT G
HGAPDH_FWD	GCA GGG GGG AGC CAA AAG GGT
HGAPDH_REV	TGG GTG GCA GTG ATG GCA TGG
TBP-mouse_F1	AAT AAG AGA GCC ACG GAC AAC
TBP-mouse_R1	TCT GGA TTG TTC TTC ACT CTT GG
hIFIT1-FWD	TCA CCA GAT AGG GCT TTG CT
hIFIT1-REV	CAC CTC AAA TGT GGG CTT TT
hTBP-FWD	GCA CAG GAG CCA AGA GTG AA
hTBP-REV	TTG TTG GTG GGT GAG CAC AA
hCXCL10-FWD	GGT GAG AAG AGA TGT CTG AAT CC
hCXCL10-REV	GTC CAT CCT TGG AAG CAC TGC A
hISG15-FWD	CTC TGA GCA TCC TGG TGA GGA A
hISG15-REV	AAG GTC AGC CAG AAC AGG TCG T
hIL6-FWD	AGA CAG CCA CTC ACC TCT TCA G
hIL6-REV	TTC TGC CAG TGC CTC TTT GCT G
mTPR FWD Set 4	TTG TCT GAA GTC CGT CTT TCT C
mTPR REV Set 4	GCC CTG GAT TGT CTG TAG ATT AG

hTPR fwd	CTG GTC TTC GCC TTT CTT CT
hTPR rev	CAA AAT ACT GTT GTT CGC TCT CC
mRSAD2_FWD	CTG TGC GCT GGA AGG TTT
mRSAD2_REV	ATT CAG GCA CCA AAC AGG AC

4.5 ELISA

Mouse CXCL10 (IP10) was measured using the mouse CXCL10 ELISA kit (*R&D Sytems*) according to the manufacturer's protocol. The supernatant used in all experiments was collected from MEFs (wt) cells after 72 hours following the depletion of TPR, NUP160 and NUP358.

4.6 Microscopy

Mouse embryonic fibroblasts wild type (wt) and *STING KO* (MEFs) were grown at 37°C 5% CO2 on sterile microscopy coverslips for 72 hours and then fixed in 4% PFA for 20 minutes. The fixed cells were permeabilized using 0.2% Triton in PBS 1X. Permeabilized cells were blocked using the blocking solution of (0.1% Triton with 1% BSA in PBS 1X) for 15 minutes prior to their incubation with specific antibodies. After multiple wash steps and antibody incubation, the cells were stained with DAPI for 15 minutes and then mounted on microscopy slides using ProLong™ Gold Antifade mounting reagent (*ThermoFisher*).

Images were taken using a Leica SP8 laser scanning fluorescence confocal and analysed with the LAS X software from Leica. The Leica SP8 is equipped with 405 nm, 488 nm, 552 nm and 638 nm lasers.

Antibodies used : ProLong Gold Antifade Reagent with DAPI #8961 was excited with the 405 nm laser and detected with PMT 1 configured at : 439 nm – 477 nm (peak excitation : 358 nm,peak emission : 461 nm), Cell signalling Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 647 Conjugate) #9720 was excited with the 638 nm laser and detected with HyD 3 configured at : 654 nm – 684 nm (peak excitation : 650

nm, peak emission : 668 nm), Cell signalling cGAS (D3O8O) Rabbit mAb #31659 , Cell signalling Lamin A/C (4C11) Mouse mAb (Alexa Fluor® 488 Conjugate) #8617 was excited with the 488 nm laser and detected with PMT 1 configured at : 498 nm – 542 nm (peak excitation : 494 nm ,peak emission : 520 nm), Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 555 Conjugate) #4413 was excited with the 552 nm laser and detected with PMT 2 configured at : PMT 2 (554 nm – 583 nm) (peak excitation :555 nm , peak emission : 565 nm) and Abcam Anti TPR antibody (ab84516).

4.7 Statistical analyses

Statistical analyses were carried out using Prism 7 (*GraphPad Software Inc.*).

One-way ANOVA, paired T-tests and two-way ANOVA were performed depending on the data sets. Tukey's HSD was used a post-hoc test following significant ANOVA results.

Statistical analysis of confocal images was performed manually after 10 images were taken at 40x per condition in areas with high density of cells on the slides.

5. Results

To test whether the depletion of TPR has a negative effect on the cGAS-STING pathway, we performed a knockdown of TPR using a siRNA duplex against TPR and the transfection reagent Lipofectamine RNAiMAX in MEFs cells to first test the model of our study and to test how efficient the knockdown of TPR is. After a transfection of 48 hours, we could see a reduction of 67% of the mRNA expression of the gene TPR in MEFs cells transfected with the siRNA as seen in **figure 10**.

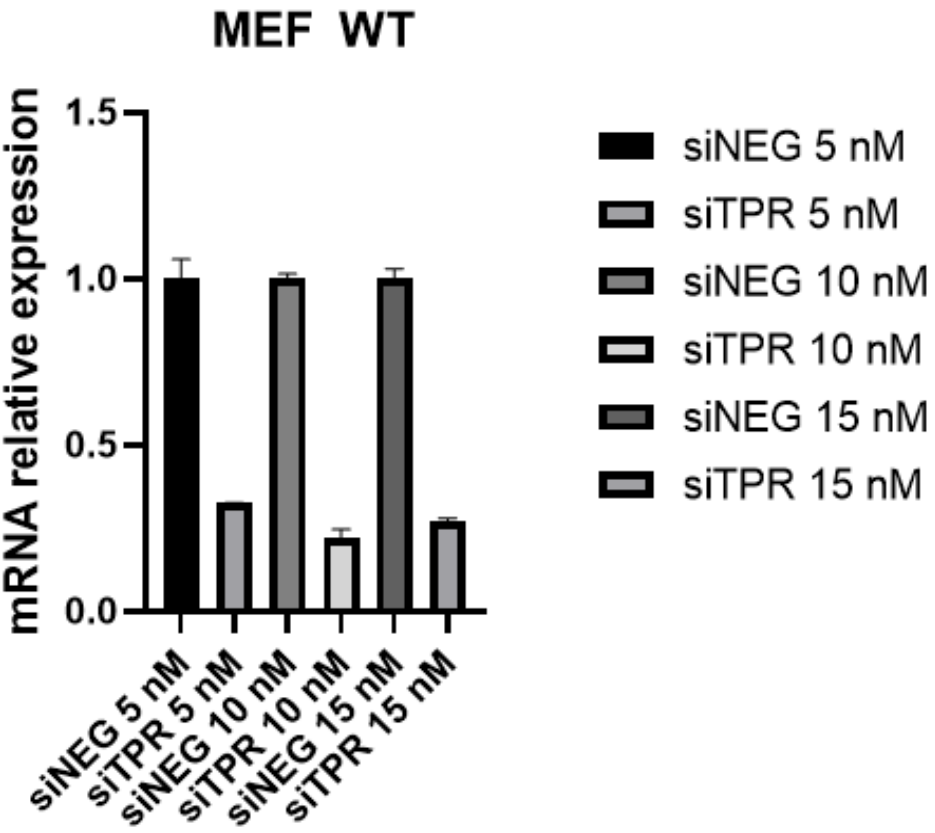


Figure 10: Validation of TPR mRNA levels following transfection of siRNAs

TPR mRNA relative expression at 5, 10 and 15 nM following transfection of siRNA in MEF (wt) cells after 48 hours with Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v

The result suggests a significant decrease in the TPR mRNA even though further test need to be done to determine if the mRNA levels translate to the protein levels of TPR. The knockdown was also tested using immunofluorescence with an antibody against TPR in MEF cells as shown in **figure 11** and we could see a complete depletion of TPR at the protein levels.

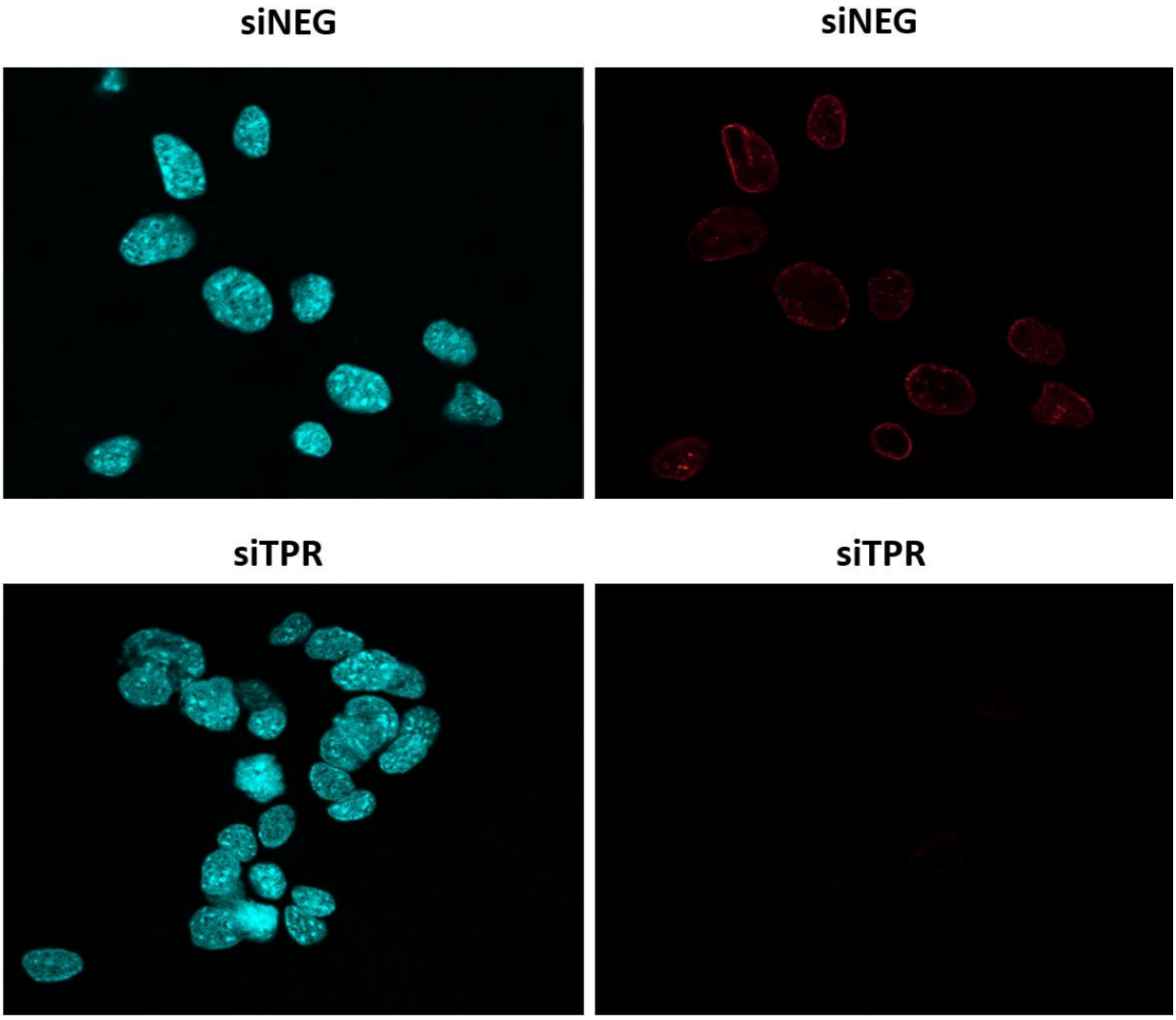


Figure 11: Knockdown of TPR observed by immunofluorescence.

MEFs cells transfected for 72 hours with siRNA against TPR and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. Immunofluorescence done using an primaryantibody against TPR and alexa-555 as the secondary antibody (red), DAPI (blue). N=3

5.1 Knockdown of TPR induces the expression of interferon-stimulated genes

Once we measured the decrease in the mRNA levels of TPR in transfected MEFs model, we then tested whether the genes stimulated by the interferon (ISGs) were upregulated or downregulated. We hypothesize the depletion of TPR will have an effect on the cGAS-STING pathway, therefore inducing an IFN-I response which we can measure looking at ISGs. To our surprise, all of the genes associated with the IFN-I response were upregulated as shown in **Figure 12.** These genes are CXCL10, RSAD2, IFIT1-P56 and ISG15 . There was also an increase in the mRNA expression levels of the IL6 gene, a pro-inflammatory cytokine. There was no increase in the expression of the cGAS gene following the depletion of although cGAS is also an ISG. These results suggest that there

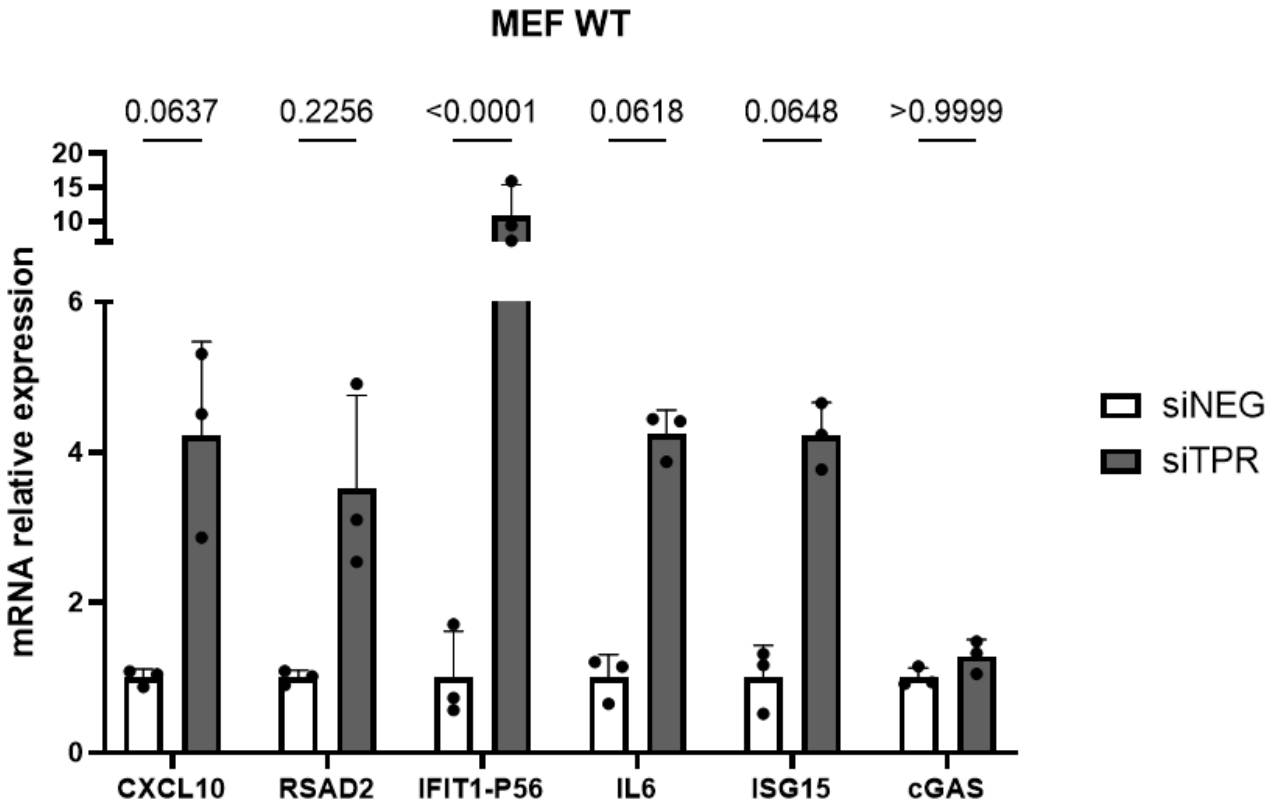


Figure 12: **Upregulation of Interferon Stimulated Genes (ISGs)**

mRNA relative expression of CXCL10, RSAD2, IFIT1, IL6, ISG15 and cGAS following a depletion of TPR in a double transfection of 72 hours in MEFs (wt) cells using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=3 Paired t-test

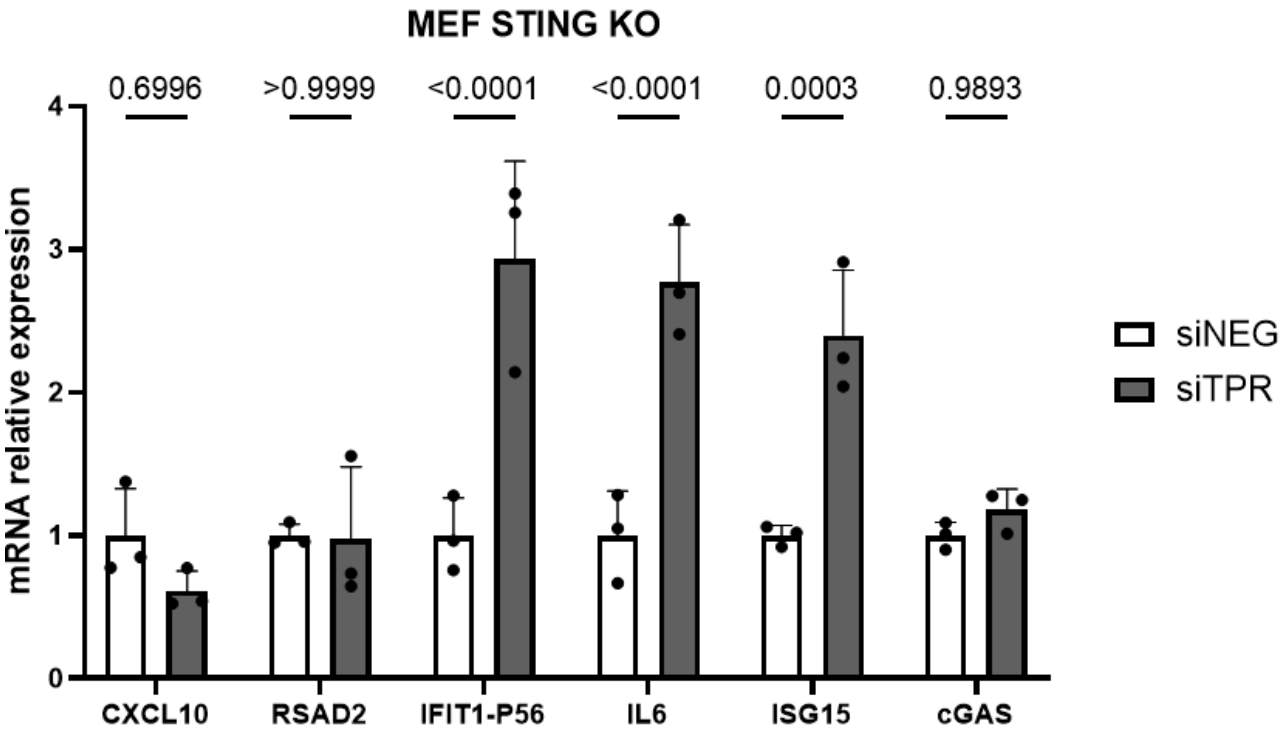


Figure 13 : **Significant reduction of the upregulation of Interferon Stimulated Genes**

mRNA relative expression of CXCL10, RSAD2, IFIT1, IL6, ISG15 and cGAS following a depletion of TPR in STING KO cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600. Paired t-test

is an activation of the IFN-I response and potentially the activation of the cGAS-STING pathway.

We conducted three experiments of 72 hours and obtained similar results in all those experiments showing increase of the mRNA expression of 4 ISGs. To determine whether the upregulation of ISGs were cGAS-STING dependant, we repeated the same experiment using STING KO MEFs cells that to test whether the inflammatory response observed in the data of **Figure 12** is dependent of the cGAS-STING pathway or not. By removing STING in cells, we can test if this increase in the production of IFN-I following the depletion of TPR is dependent of the STING protein or not.

After repeating the protocol used in the experiment of **figure 12**, we observed very different results in the STING KO cells. As shown in **figure 13,** the upregulation of the ISGs was quite different than the results previously obtained. There were no changes in the STING KO for the genes CXCL10 and RSAD2 and a small increase in the genes IFIT1, ISG15 and IL6. These results suggests that the response observed after 72 hours of transfection seem to highly dependent on the cGAS-STING pathway.

5.2 Gene expression level analyses of genes associated with the interferon response in HeLa cells

The same experiment was performed in HeLa cells to verify if the same phenotype as MEFs cells was observed in human cells but also cancerous cells. To our surprise, the increase mRNA expression of the ISGs was much higher in comparison to their control than in MEFs cells as shown in **figure 14 and 15.**

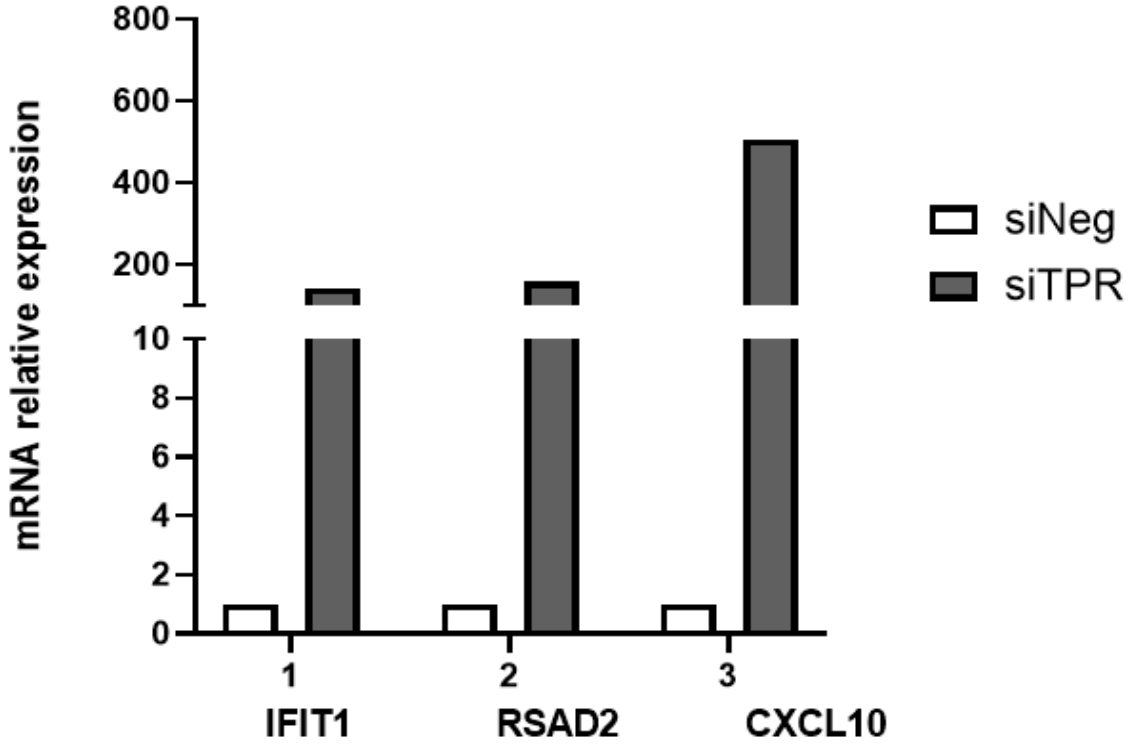


Figure 14: **Relative expression of IFIT1, RSAD2 and CXCL10 following TPR depletion**

mRNA relative expression levels of IFIT1, RSAD2 and CXCL10 in HeLa (wt) cells over a double transfection of 72 hours using 10nM of siRNAand Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=1

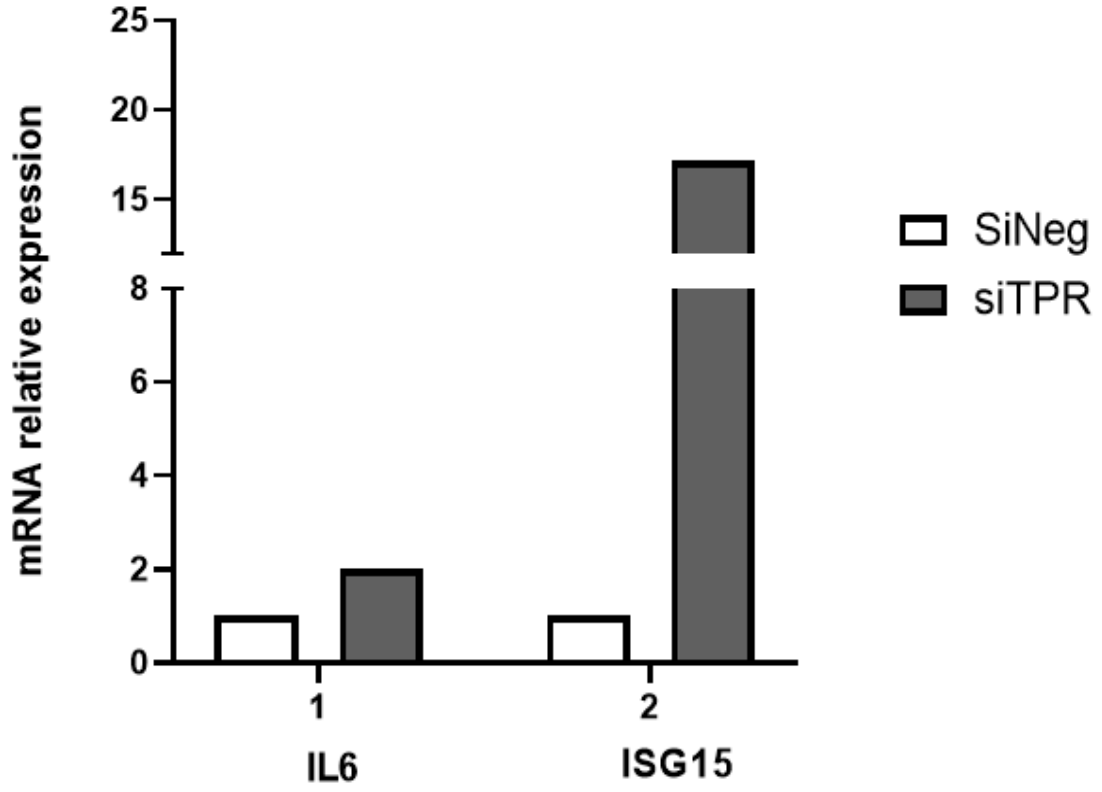


Figure 15: **mRNA levels of ISG15 following a depletion of TPR**

mRNA relative expression levels of IL6 and ISG15 in HeLa (wt) cells over a double transfection of 72 hours using 10nM of siRNA against TPR and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=1

Indeed, in both figures, we can observe a high increase of the mRNA levels of ISGs such as IFIT1, RSAD2, CXCL10 and ISG15. CXCL10 even reached sometimes above 500 folds increase compared to its control. This result, if repeated, would suggest that the depletion of TPR causes an inflammatory response in both human and mouse cell lines that have been immortalised or transformed. A small increase of IL6 is to be expected since these cells already have a constant inflammatory state.

An ELISA of Human IL-6 was performed after the depletion of TPR in HeLa cells but the negative control was already at a very high level of produced IL-6 by the HeLa cells that it was impossible to measure the differences accurately following the depletion of TPR and there's only a slight increase in the IL-6 gene expression compared to the genes stimulated by IFN-I.

5.3 Gene expression analyses of ISGs following HU treatment

For this experiment, we used a drug called hydroxyurea to increase DNA damage in MEFs (wt) cells. Hydroxyurea, HU, is a ribonucleotide reductase inhibitor which decreases the production of deoxyribonucleotides by scavenging tyrosyl free radicals as they are involved in the reduction of nucleoside diphosphates (NDPs).[133] It will inhibit the repair of DNA and will lead to an increase of DNA damage in a cell exposed over time cell death in healthy cells. It induces replication stress and will normally arrest cells in the S-phase of the cycle, although there might be other effects.[134] By using HU, we were able to observe that cells that were depleted of TPR had a greater increase in the expression of the 4 ISGs tested, especially IFIT1, CXCL10 and RSAD2 as shown in **figure 16**.

This result suggests that TPR is potentially involved in protecting against DNA damage and helps the DNA repair system to function properly. This could lead to an accumulation of DNA in the cytosol which could activate cGAS. It has been previously shown that TPR protects against replication stress.[108]

We did not quantify the amount of DNA damage yet. This figure is also only from one experiment and will need to be repeated a near future. Surprisingly, the activation of IL6, ISG15 and CXCL10 was not seen in the samples with siTPR without HU, unlike in **figure 12**.

This experiment has been performed only once, so errors could occur. Supplementary experiments need to be done to better analyze the data. As expected following our other experiments, the most activated genes were IFIT1 and RSAD2 which concords with previous data.

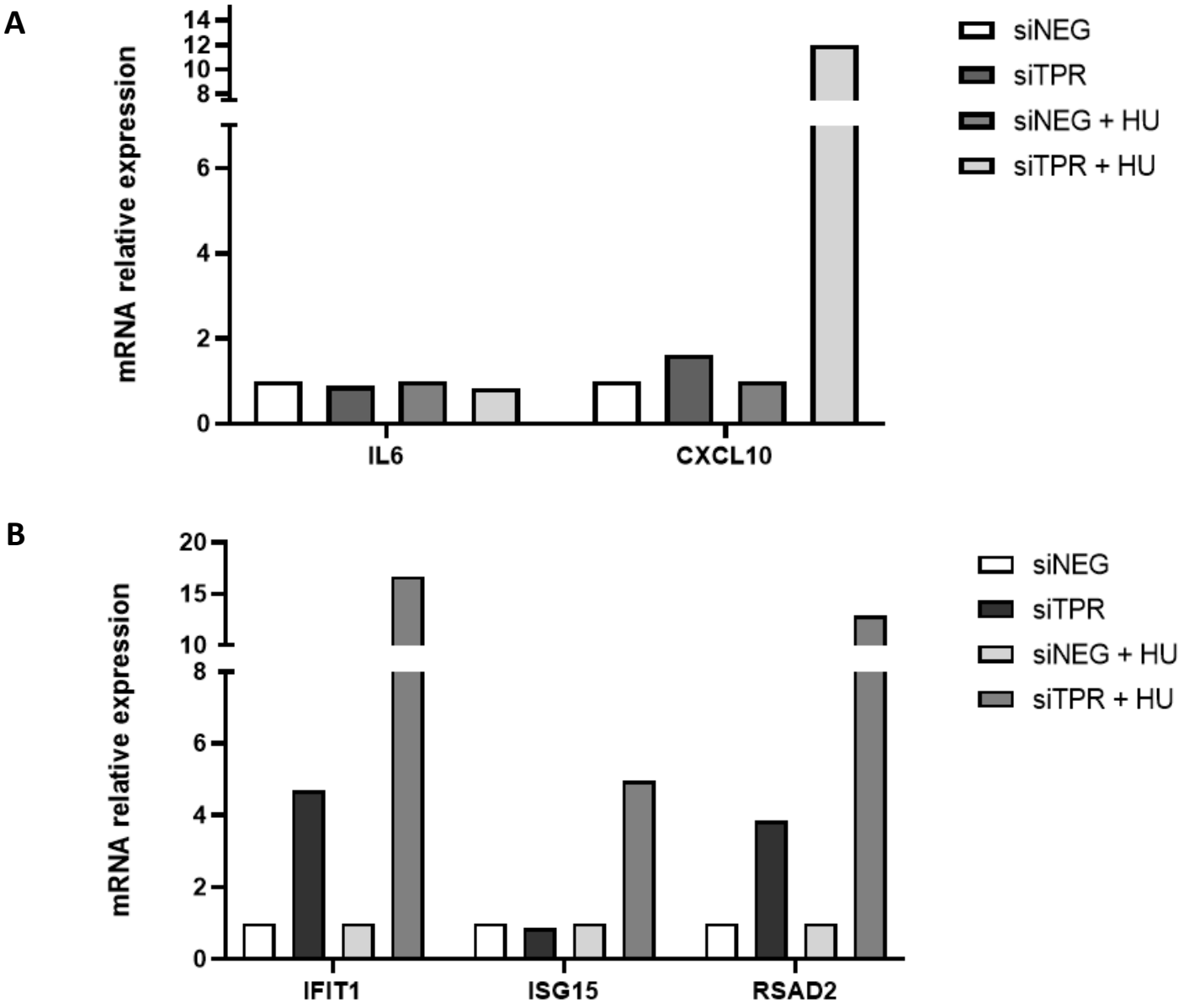


Figure 16: Change in mRNA levels of 4 ISGs following a depletion of TPR and HU treatment

mRNA expression levels of IFIT1, CXCL10, ISG15 and RSAD2 observed following depletion of TPR and use of HU for 48 hours at 10uM in MEFs (wt) cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=1

5.4 Gene expression analyses of ISGs following the depletion of NUP358 and NUP160

To determine whether the effect observed following the depletion of TPR was observed using other nucleoporins, we decided to repeat the experiments by doing a knockdown the effects observed would be different as the TPR, NUP160 and NUP 358 are in different NUP subcomplexes and have not been shown to interact directly with each other.

As shown in **figure 17,** we proceeded to the depletion of NUP160 and NUP358, two nucleoporins situated in 2 different subcomplexes of the NPC. NUP358 is positioned at the opposite of TPR, on the cytoplasmic border and NUP160 is located in the middle of the Y complex, a structure forming a ring that accounts for a majority of the structural components of the NPC and is crucial for its assembly.[135] We first thought that the depletion of NUP358 wouldn't affect the cell as much as NUP160 and TPR since it has been shown in that it assembles at the last part of the process of the nuclear pore complex assembly[86].

The depletion of NUP358 seems to upregulate the expression of CXCL10, IFIT1 and ISG15 with a similar intensity than the depletion of TPR as observed in **figure 17**. We can observe as well, a significant increase in expression of CXCL10 mRNA levels after the depletion of NUP160. using siRNA against the nucleoporin NUP160 and NUP358. We wanted to know whether this mRNA relative expression increase could be measurable by measuring the protein CXCL10 by ELISA.

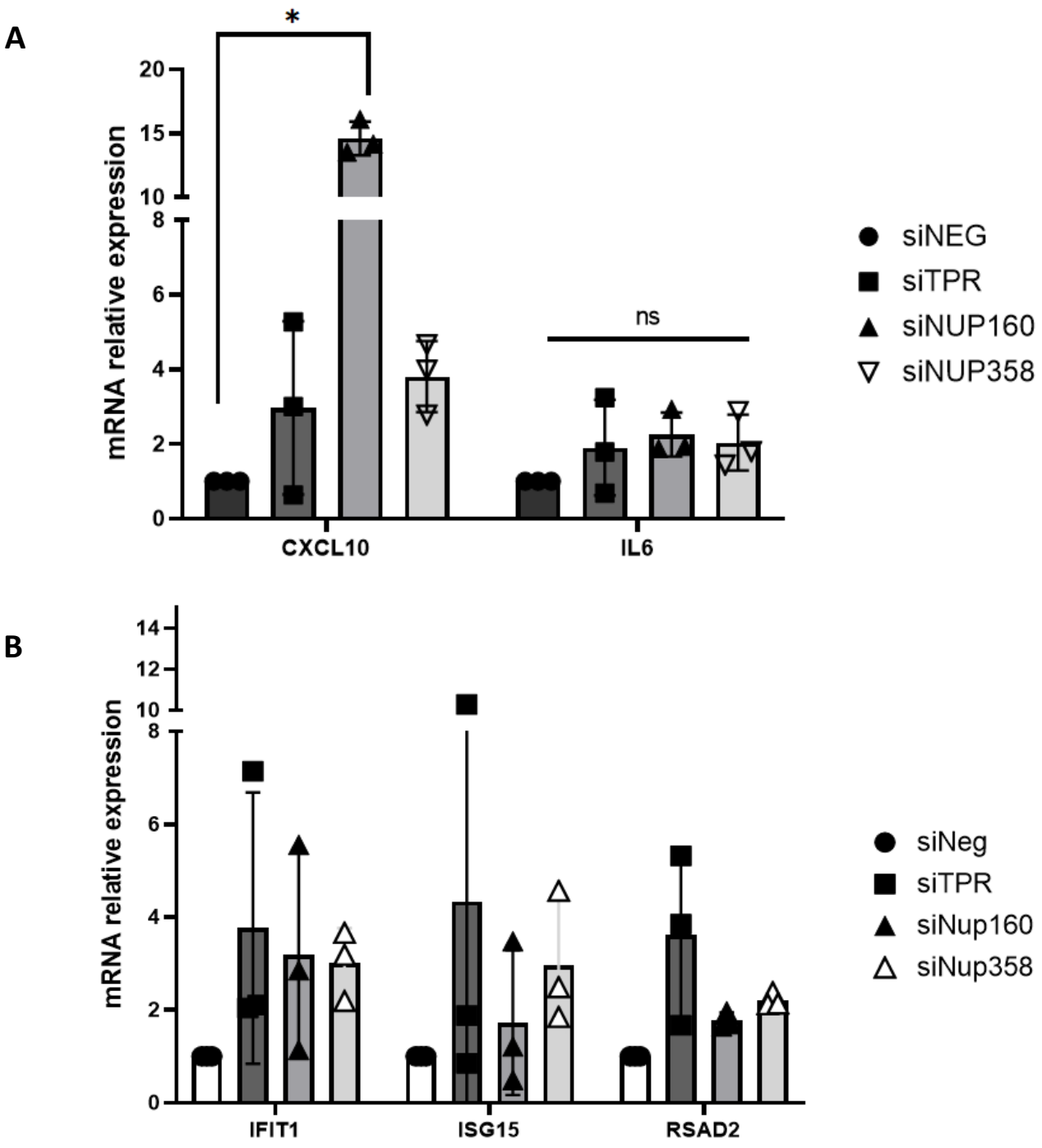


Figure 17: **mRNA levels of 4 ISGs following a depletion of NUP160, NUP358 and TPR**

mRNA levels of CXCL10, IL6, IFIT1, ISG15 and RSAD2 following NUP depletion of MEFs (wt) cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=3 Statistical analysis :Two-way ANOVA, post-hoc test : Tukey's HSD

5.5 Expression of mRNA levels of CXCL10 translates to the production of the CXCL10 protein

Following the result obtained in **figure 17** and the possibility that the mRNA export system has been impaired, hence the increase levels of the mRNA levels of CXCL10, we then performed an ELISA to validate if the mRNA levels were correlated to the production of the chemokine CXCL10 or if some of the mRNA was being blocked at the nuclear pore complex of the cells. Firstly, we checked if after a depletion with siRNA of TPR in MEFs cells, we could observe a similar increase in the production of CXCL10 compared to its increase in the mRNA levels as shown in **figure 12** and **figure 17.** A shown in **figure 18**, there is a similar increase of the production of CXCL10 by MEFs cells after a 72 hours double transfection with siRNA against TPR than there was by RT-qPCR. In mRNA levels, there was an increase on average of 3-4 folds and a similar increase was observed following an ELISA test.

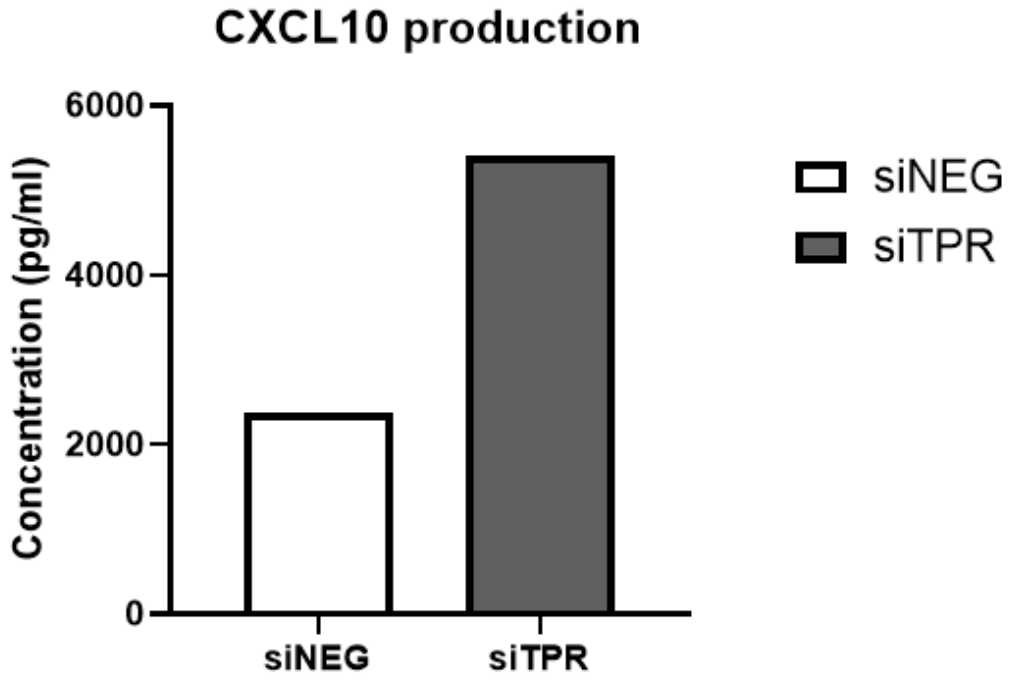


Figure 18: **Concentration of CXCL10 following depletion of TPR**

Tested by ELISA following a depletion of TPR in MEFs (wt) cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=1

This result demonstrate that the mRNA export system is still functioning for this specific mRNA, and mRNA can be transported to the ER to get translated and finally secreted by the cell. There shouldn't be an accumulation of mRNA in the nucleus following a blockage at the NPC but we cannot currently prove it with certitude.

5.6 Expression of mRNA levels correlates also with the CXCL10 production following depletion of NUP160 and NUP358

We then proceeded by repeating the experiment previously done by depleting NUP160 and NUP358 alongside of TPR in MEFs WT cells. The results were very similar to the results obtained after the depletion of TPR, there was a significant increase of CXCL10 production following the depletion of TPR, NUP160 and NUP358 after a 72 hours

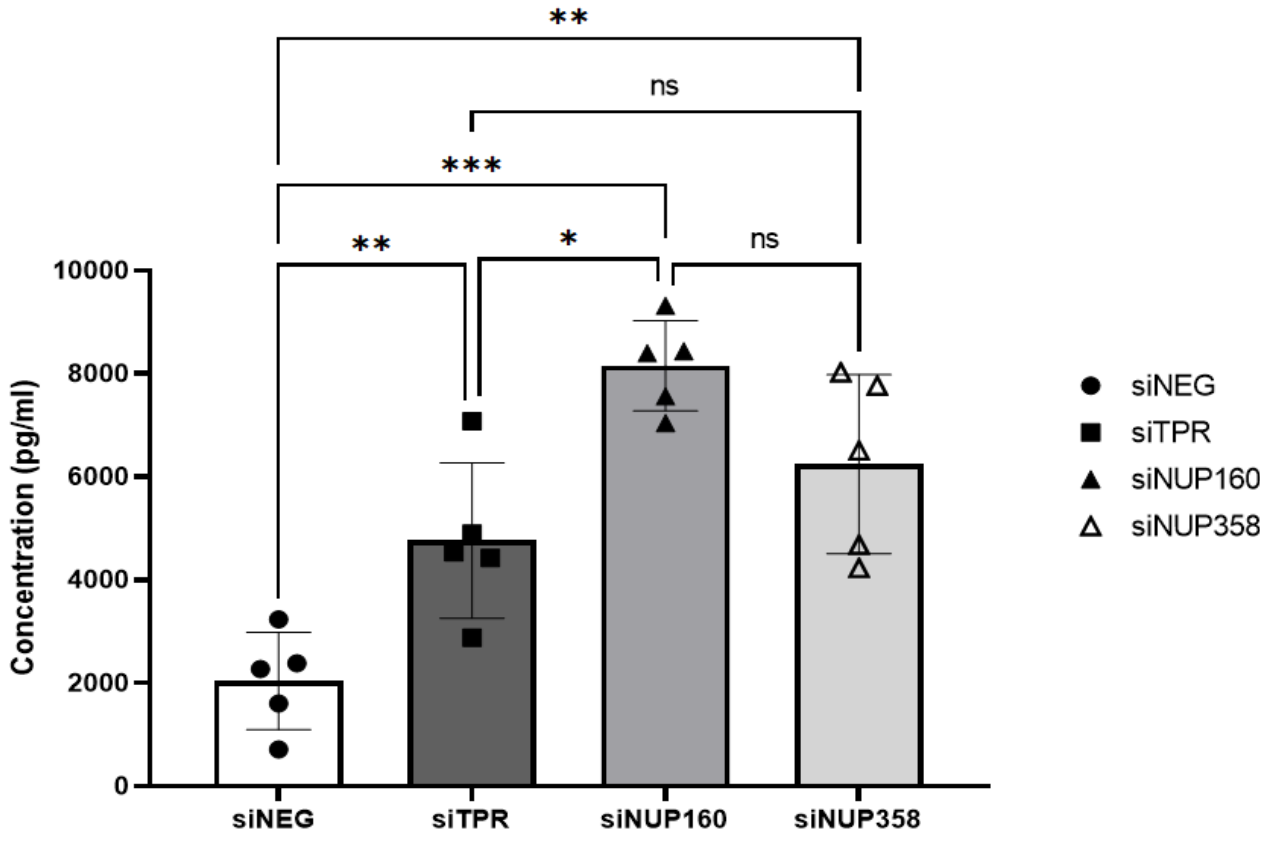


Figure 19: **Increase of the production of CXCL10 following nucleoporin knockdown**

ELISA test was performed following a depletion of TPR, NUP160 and NUP358 in MEFs (wt) cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=5 One-way ANOVA, Post-Hoc test : Tukey's HSD

transfection. It follows the same pattern as the increase shown by RT-qPCR for the CXCL10 gene. As shown in **figure 19**, over 5 experiments of 72 hours, there was a significant increase of CXCL10 following the knockdown of all 3 nucleoporins but most remarkably following the NUP160 depletion. It is no surprise since it was the nucleoporin that affected the most the mRNA expression of CXCL10 following the transfection. It is intriguing that the decrease of NUP358 level leads to an even bigger production of CXCL10 than TPR considering it has a role as a docking platform in the NPC for nucleocytoplasmic transport. [136] We thought it would be less likely to affect the structure of the NPC and alter its function or cause DNA damage. It is possible that because it is part of a complex, its depletion leads to depletion of the entire MUP358-NUP88-NUP214 complex.

5.7 CXCL10 production following depletion of nucleoporins is STING dependent

The next step was to test whether this activation following the decrease of the levels of NUP160 and NUP358 was also involving the cGAS-STING pathway just like it was demonstrated when we did a knockdown of TPR in previous experiment. We then proceeded to test by ELISA the production of CXCL10 in MEFs (wt) without any treatment, in MEFs (wt) treated with H151, a sting antagonist widely used to block STING from being functional, as well in MEFs STING KO. H151 is a molecule which covalently and irreversibly binds to human and murine STING to block the activation-induced palmitoylation and clustering of STING, ultimately inhibiting the downstream signaling pathway.[137] This would allow us to verify if it is STING dependent and solidify that the cGAS-STING pathway is involved by using the same cells, the MEFs (wt), to validate if STING is involved.

As shown in **figure 20**, we observed a decrease in CXCL10 production following the knockdown of all 3 nucleoporins in the STING KO cells in comparison to their non-treated controls. The same was observed for cells treated with H151, although the decrease was less significant relative to the control. It is important to note that using a drug will never

be the same as having a knock out in another cell line and the drug was used for 48 hours over the 72 hours total time of transfection. The cells were treated with 20ug/ml of H151 for the last 48 hours of the experiment, leading us to believe that there's a portion of the activation occurring in the first 24 hours following the transfection with siRNA.

We can also note that H151 reduces slightly the production of CXCL10 in non-treated cells suggesting that the model used, the MEFs, have a baseline level of CXCL10 secreted. This has been shown with the same cell lines in a previous research.[138] This could potentially reduce the increase of ISGs and the activation of the cGAS-STING pathway.

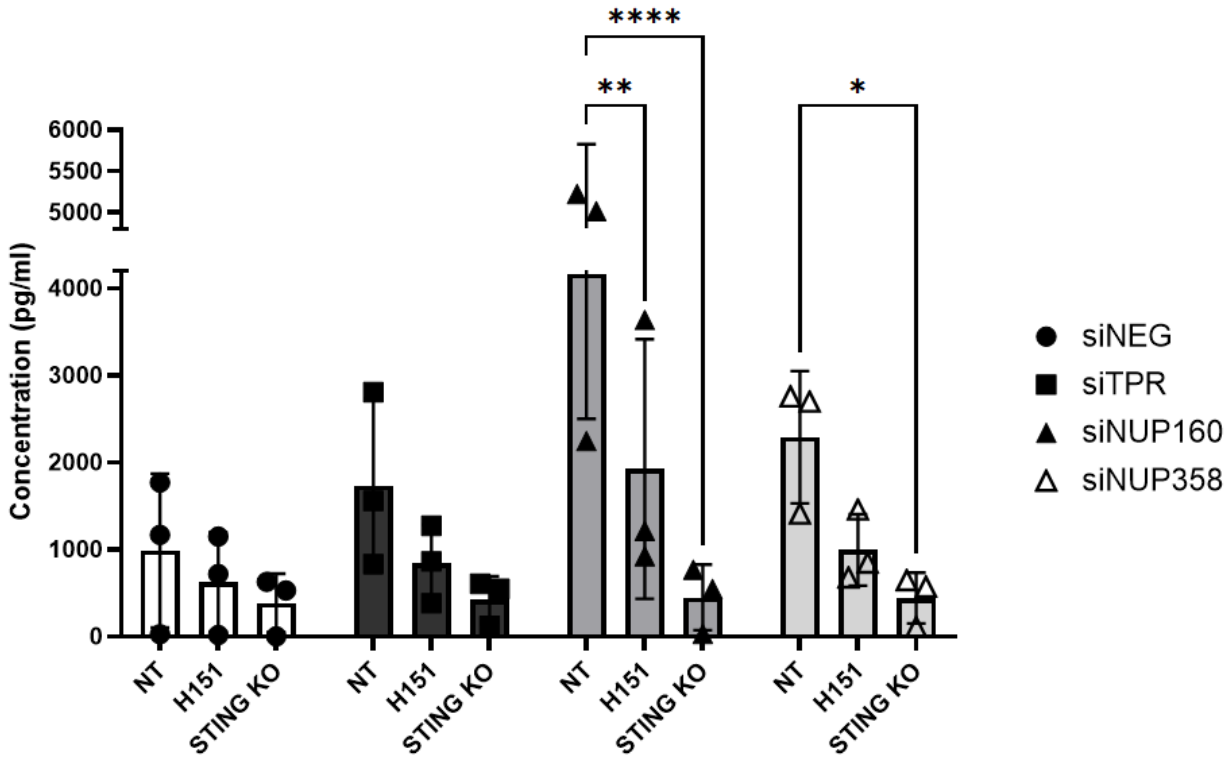


Figure 20: **Decrease of the production of CXCL10 in STING KO cells and H151 treated cells**

CXCL10 concentration levels following a depletion of TPR, NUP160 and NUP358 in MEFs (wt) and MEF STING KO cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. MEFs (wt) cells were treated withH1515 used at 20ug/ml for 48 hours before stopping collecting the supernatant. N=3 *Statistical analysis* : two-way ANOVA,

5.8 cGAS foci observed colocalizing with lamina protrusion

After having observed the immune response by the cells following depletion of various nucleoporins, we then pursued our third objective which is to determine if the nuclear integrity is compromised following depletion of various NUPs, as it could be the cause of

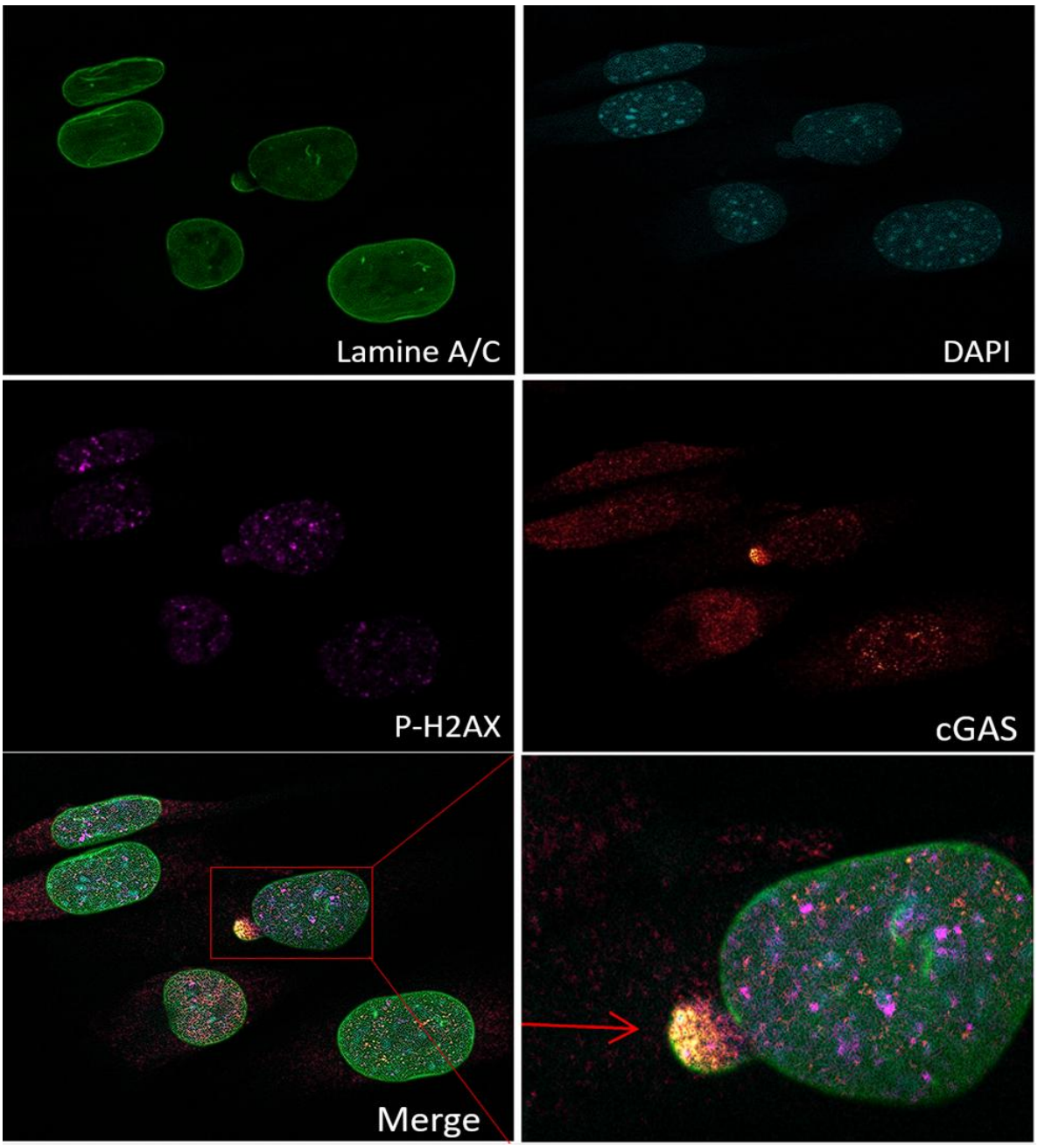


Figure 21: **cGAS foci colocalizes with lamina protrusion.**

Lamina A/C (green), DAPI (blue), p-h2AX, (purple), cGAS (red)**.** Representative image taken by confocal microscopy of cells depleted of TPR cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX.

the observed cGAS-STING activation. We hypothesised that the nuclear membrane integrity could be at fault. We used confocal microscopy to focus on whether the cells have different nuclear membrane shapes and if the overall shape of the cell is affected by the deregulation of their nuclear pore complex components. If there was to be a break in the nuclear membrane, this could release chromatin into the cytosol and therefore activate cGAS. As lamina is a key component of the nuclear membrane and we wanted to verify nuclear integrity, we decided to stain the lamina A/C of cells, DNA, TPR, cGAS and P-H2AX, a marker of DNA damage.[139]

We observed the presence of micronuclei in depleted cells as well as irregularities of the lamina A/C overall structure. These anomalies of the lamina come in various forms; some could be classified as lamin protrusion or lamin budding but also as chromatin bridges or lamina extension as seen in **supplementary figure 1**. We observed foci of cGAS in a following experiment at the location of some of these irregularities. As shown in **figure 21,** cGAS signal is colocalizing with lamina protrusion in some events and indicating us that if these events are happening more frequently in cells depleted of TPR, it could be the source of the activation of the cGAS-STING pathway demonstrated in previous results.

We then investigated if the frequency of these events, especially the ones commonly observed in transfected cells, was higher in depleted cells in comparison to the negative control. Firstly, we classified the events as lamina protrusion, or budding in one category. The criteria used for this classification were that the lamina had a deformation and a protuberance at one part of the lamina structure pointing outward. Whether these protrusions were considered late or early, they were classified in the same category. Examples of these events are demonstrated in **figure 22 A and B**. Furthermore, we classified any events involving a small, closed lamina structure within the cell's cytoplasm as a micronuclei, see example in **figure 22 C.**

We hypothesise that protrusions categorized as ‘’early’’, (A) will eventually lead to the ‘’late’’ (B) stage and then to a formation of a micronuclei (C) after cell division and separation of the protrusion.

A. Early protrusion B.Late protrusion C. Micronuclei

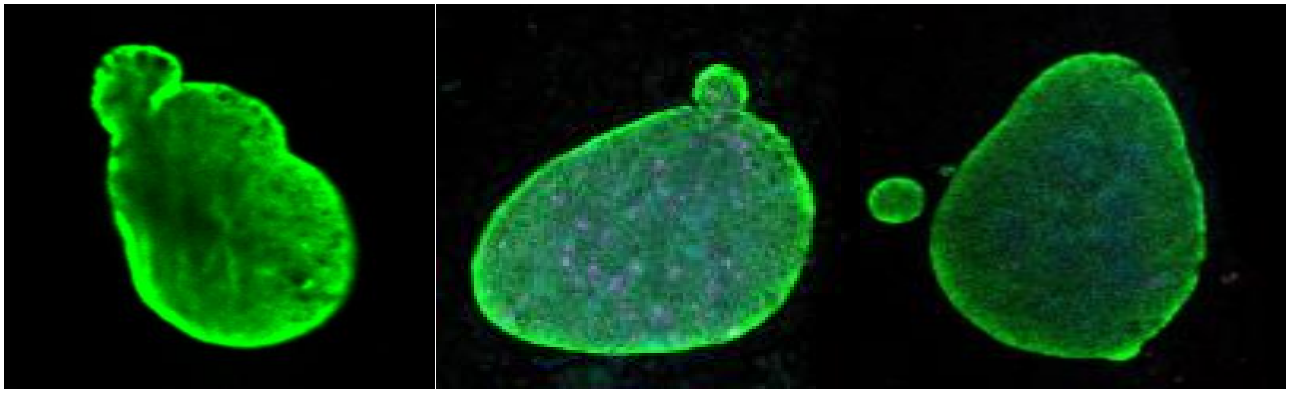


Figure 22: **Example of protrusion of lamina and micronuclei**

Lamina A/C (green), DAPI (blue) p-h2AX (purple)**.** Confocal microscopy of MEFs (wt) cells depleted of TPR cells over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX.

5.9 Analyses of lamina protrusion and micronuclei formation in transfected cells with siRNA against TPR

By confocal microscopy, a multitude of images were taken of MEFs cells transfected with scramble negative siRNA, siRNA against TPR and siRNA against NUP160. Following this, an analysis of the cells and their lamina structure was performed. Three separate experiments were done where 369, 234 and 295 cells were analyzed for the cells treated with a scramble negative siRNA and where 266, 542 and 249 cells were analyzed for the cells depleted of TPR respectively .The results indicate that over 3 experiments where a total 2129 cells were accounted for, there is a trend showing an increase of the formation of lamina protrusion as well as the formation of micronuclei in TPR-depleted cells.

All data points on the graph of **figure 23 A and B** are the percentage of the number of cells in each experiment depending on their treatment.

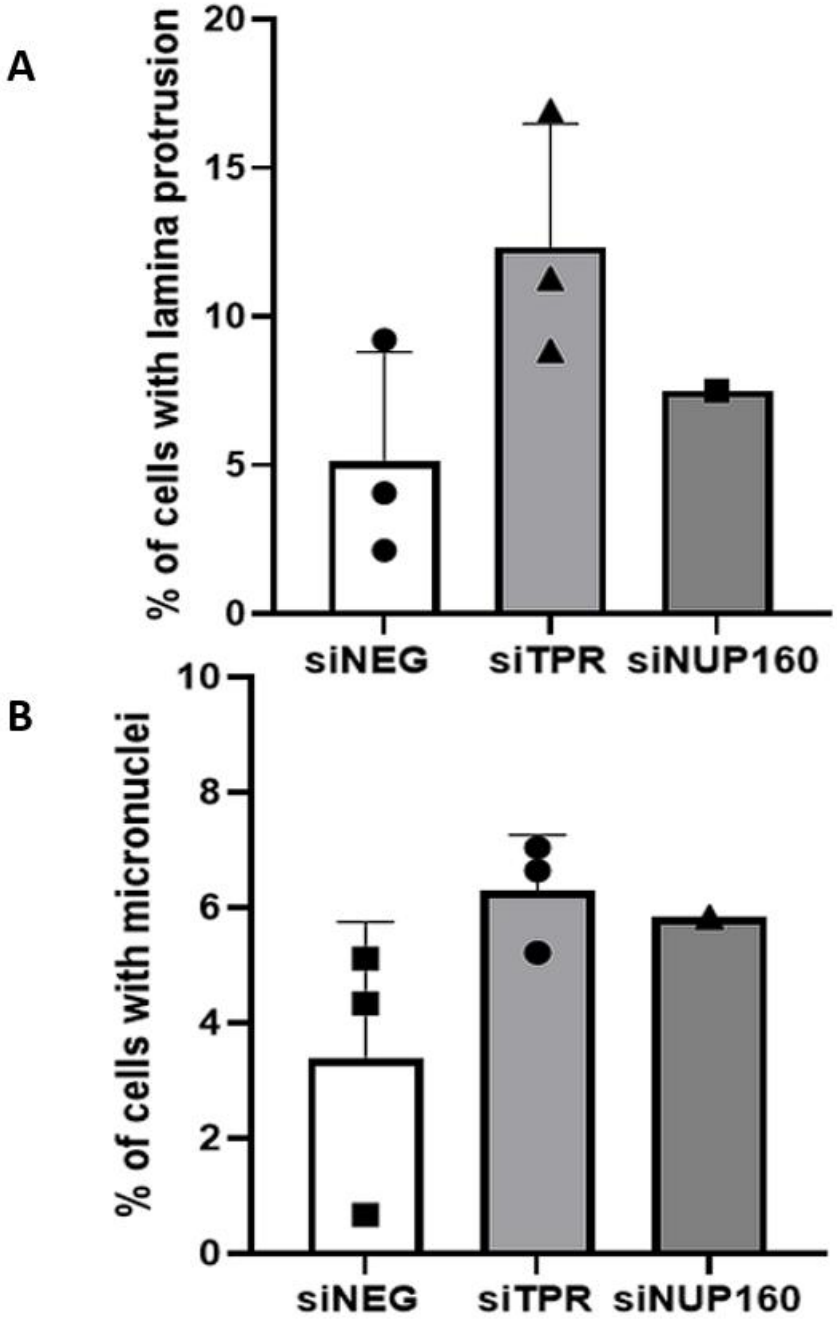


Figure 23: Frequency of lamina protrusion and micronuclei.

Percentage of cells analyzed with lamina protrusion and micronuclei in transfected cells with siRNA against TPR and NUP160. Cells analysed for siNEG : 898 Cells analyzed for siTPR : 1231 cells over 3 experiments. N=3 for siNEG, siTPR and N=1 for siNUP160**

In the first experiment, we saw 4% of the control cells versus 7% of cells depleted of TPR with the presence of micronuclei and 9.2% of the control cells versus 16.6% of cells depleted of TPR presenting a lamina protrusion or Nuclear Envelope Budding (NEB) . The two following experiments showed an increase in the presence of micronuclei as results were 5% versus 6% and 0.67% versus 5.22% , control versus TPR depletion respectively, of cells with micronuclei. The increase of NEB of the lamin A/C was even greater as difference of control versus TPR-depleted cells were 2.13% versus 11.25% for the second experiment and 4% versus 8.9%. We are planning to repeat this experiment in hope for a significant different following statistical analysis after more data has been collected. We performed the same experiment with cells treated with the siRNA against NUP160 and observed similar results as the cells depleted of TPR. This experiment as only done once and will need to be repeated. This preliminary result is suggesting that equivalent results will be seen after depleting NUP160 in comparison of depleting of TPR.

5.10 The depletion of NUP160 also lowers the levels of TPR

Following the knockdown of NUP160 in MEFs cells by transfection of siRNA, a noticeable difference was observed by confocal microscopy in the mean fluorescence of the antibody against TPR between the negative control and cells depleted of NUP160.

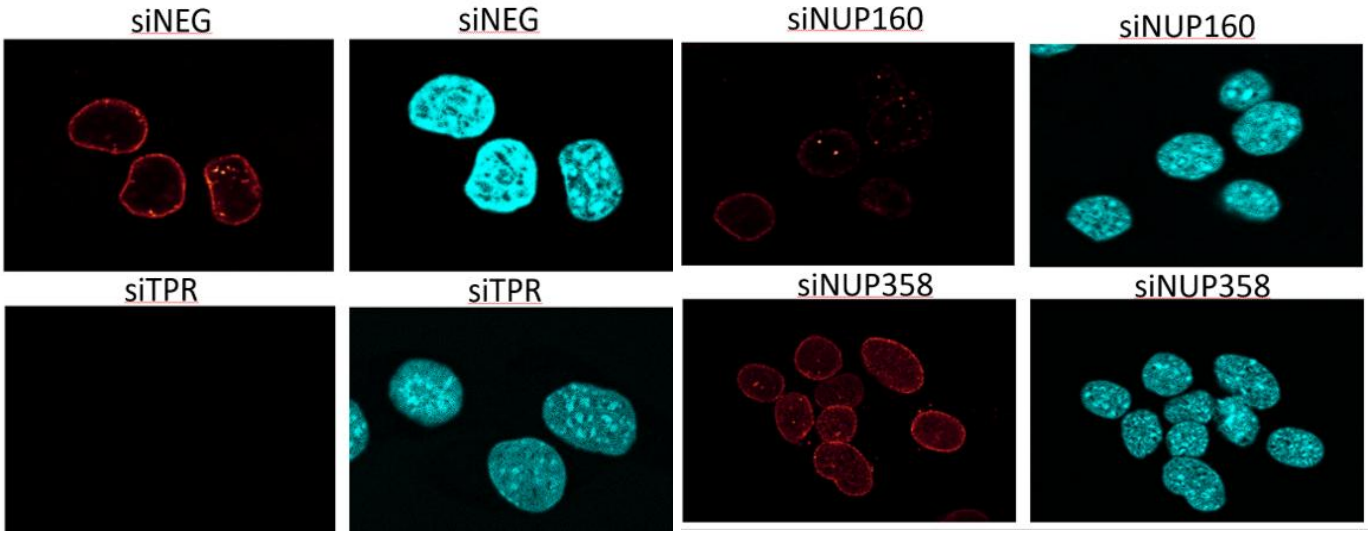


Figure 24: Decrease of the fluorescent intensity of TPR in cells transfected with siNUP160 and siTPR.

Fluorescence of TPR in cells depleted of NUP358 compared to the negative control. The slides were prepared after a depletion of TPR, NUP160 and NUP358 in MEFs (wt) over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=3 TPR (red), DAPI (blue)

As shown in **figure 24,** we can see that the fluorescence of TPR, in red, is lower than the negative control but also of the cells in which NUP358 was depleted. We then proceeded to analyse 25 cells of each condition by measuring their mean fluorescence over the total

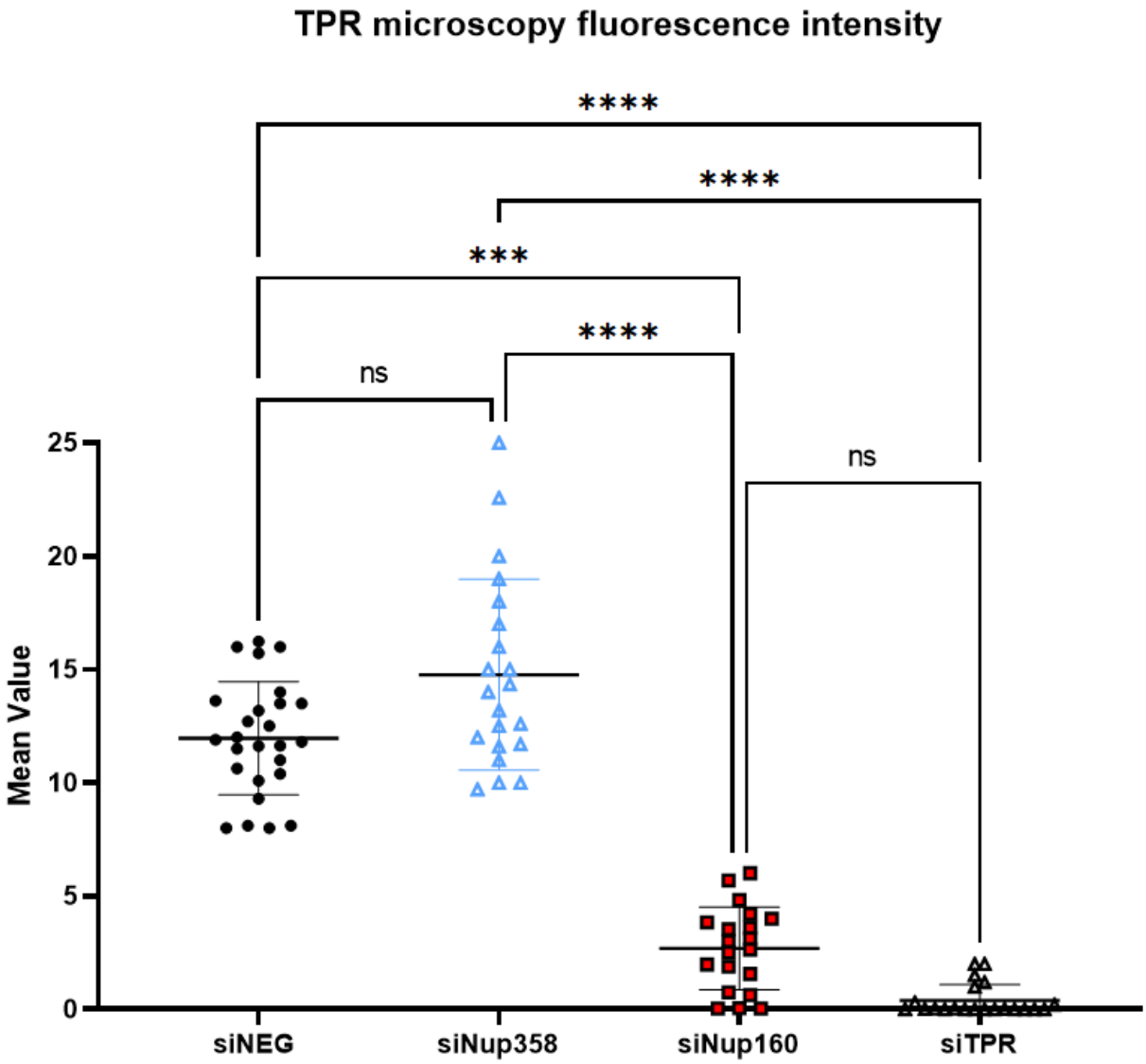


Figure 25: **Decrease of the mean fluorescence value of TPR in cells treated with siTPR and siNUP160.**

Significant decrease of the mean value of TPR in fluorescence in cells depleted of NUP160 and TPR in opposite of the depletion of NUP358. The test was performed following a depletion of TPR, NUP160 and NUP358 in MEFs (wt) over a double transfection of 72 hours using 10nM of siRNA and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. N=3 *Statistical analysis* : two-way ANOVA, Tukey's HSD

surface of the nucleus of each cell. As shown in **Figure 25,** there is a significant reduction of the mean value of fluorescence of TPR in NUP160 depleted cells as well as TPR depleted cells. For the cells with siRNA against NUP358 there was no decrease of the signal of TPR.

This data suggests that affecting the Y complex and the NUP160 in cells would also affect the nuclear basket and TPR simultaneously. Even though NUP160 cells still had a weak signal for TPR, it is still not as much of a decrease compared to cells treated with siRNA against TPR. This might be because the decrease the nucleoporin of NUP160 is not as efficient as the transfection with siRNA against TPR. We could test that by western blot in the near future and more effectively quantify the presence of both proteins.

6. Discussion

6.1 Model

We used SV40T-immortalized MEFs (wt) and STING KO cells because these fibroblasts grow rapidly, do not need any additives to grow properly but mainly because they have the cGAS-STING pathway and can induce an inflammatory response. They facilitate research but come limitations. They resemble cancerous cells and will not truly represent healthy primary cells. These murine cells have been used across the globe in many research laboratories for DNA damage, inflammation, cGAS-STING response and cancer for instance.[53, 140]

We used transfection of siRNA against NUPs with the transfection agent Lipofectamine™ RNAiMAX by Fisher as it has been used in our laboratory and has been proven to be effective. For nucleoporins like TPR, it is necessary to perform a knockdown as a knockout would not allow the cells to survive and grow as the nuclear pore complex is an essential component for the cell's survival.[141] Transfection with siRNA has its advantages and downsides, for instance, it is easy to achieve and repeat but it is also vulnerable to RNases

in the cell. Its effects last between 3 to 7 days depending on the mRNA targeted according to companies like Fisher and Sigma-Aldrich. siRNA can also potentially induce off-target effects following some mismatches. shRNA(small hairpin RNA) is a another potential way to induce a knockdown in cells and can be incorporated via plasmid vectors and will provide a long-term stable expression once integrated into genomic DNA.[142]

The measured levels of ISGs would be significantly higher if we could have knocked outs of TPR, NUP160 and NUP358 without the cells dying. A complete knockout of these genes would kill the cells. The consequences of these knockdowns that we observed are thought to be amplified in a situation where a mutation of the genes encoding for TPR, NUP160 and NUP358 would be permanent.

These results are also limited by its model's own limitations, the MEFs cells and the HeLa cells are both immortal cells that will divide indefinitely and already have DNA damage as a baseline. The HeLa have very high amount of CXCL10 without any treatment and represent a poor model for testing the upregulation of the cGAS-STING pathway for that reason but despite having very high baseline of CXCL10 produced, we were able to see the a significant difference when we analyzed the mRNA levels by RT-qPCR which has not the same limitations as an ELISA. Our results represent the most likely outcome in the context of cancerous cells, but results could differ in healthy cells.

6.2 Inflammation and cGAS-STING

Results obtained in **figures 12 and 13** suggest that there are potentially other pathways involved in this total inflammatory response. It is also suggesting that other pathways might have a small impact on the interferon response. These pathways could be an activation of the NF-κB/TNF-α pathway and the inflammasome's IL1 production. The JAK/STAT pathway could also be responsible of some measured upregulation of ISGs. For example, ISG15 can be transcribed following the detection of IFN I by neighbouring cells via the JAK/STAT pathway.[143]

The NF-κB pathway is a proinflammatory pathway based on the expression of proinflammatory genes including cytokines and chemokines once activated. It has been a great target for new anti-inflammatory drugs but also could be a weak target for chronic inflammation pathologies.[144] It is a pathway than can be activated by many different stimuli, such as PAMPs, bacteria, DAMPs, cytokines such as IL-6, IL-1, IL-8 ,TNF-α and IFN-I are produced upon the activation of the NF-κB pathway. The NF-κB pathway is activated when IκB proteins are degraded after being phosphorylated by the IκB kinase (IKK) complex, which is triggered by signals from outside the cell. This allows NF-κB to move into the nucleus and activate genes involved in immune responses, inflammation, cell survival, and proliferation. NF-κB also activates the production of IκBα, which re-inhibits NF-κB, creating a feedback loop that regulates its activity.[145] It has an important role in the autoimmune diseases and even cancer.[146]

IFN-I and the NF-κB pathway can be activated independently of cGAS. Indeed, an IFN-I response following the detection of dsDNA can also come from TLR9 activation. TLR9 is a pattern recognition receptor that detects unmethylated CpG motifs commonly found in viral DNA from pathogens like EBV, HPV16, and HSV-2.[147] It can also detect mitochondrial DNA in the cytosol. While initially studied in immune cells, TLR9 is also active in epithelial cells such as keratinocytes. Upon activation, TLR9 recruits the adaptor protein MyD88, initiating a signaling cascade involving IRAK1, IRAK4, TRAF6, TAK1, and IKK. This leads to the nuclear translocation of NF-κB subunits p50 and p65, which activate proinflammatory genes like TNF-α and pro–IL-1β, with gene specificity regulated by posttranslational modifications.[147]

Although the results obtained in **figure 10** after testing the transfection of siRNA against TPR for 48 hours were never tested again for the 72 hours transfection, it was assumed that the knockdown was efficient. Even though it was not quantified by western blot, we confirmed the knockdown by microscopy and RT-qPCR. We have done the same

experiment for the duplex of siRNAs against NUP160 and NUP358 and to determine which siRNA to use for the following experiments. Even though the intensity of the protein knockdown has only been quantified by immunofluorescence in confocal microscopy, it is clear that there's a significant reduction in the protein levels. Being informed of the intensity of the knockdown could allow us to better correlate the results shown most experiments as the knockdown of NUP160 has been showing similar results as the knockdown of TPR but at an increase level regarding the activation of the cGAS-STING pathway. Results shown in **figure 25,** regarding the decrease of the levels of TPR following the depletion of NUP160 could be correlated better if we knew how much of a knockdown has been happening in those cells. It would be normal to observe the same kind of results by ELISA, RT-qPCR and even in quantifying the events of NEB following the depletion of NUP160 knowing that it also affects equally the presence of TPR. In **figure 17**, we observed that NUP160 depletion markedly upregulated CXCL10 mRNA expression levels, whereas TPR depletion had only a modest effect. However, when assessing CXCL10 protein production following knockdown, TPR depletion produced a significant increase, and NUP160 depletion produced an even greater effect. This suggests a potential additive phenotype, consistent with the observed transcriptional findings.

The results show that the trigger of the innate immune response following a mutation of the NPC would cause inflammation in the surrounding microenvironment and would be threatening to its surroundings in a case of chronic inflammation but mainly for cancerous cells. Possible mutations of nucleoporins include the loss of function of a nucleoporin, misfolding of the protein, loss of affinity to its anchoring site and over and under expression of the nucleoporin. Indeed, it has previously been shown that some cancer cells will quickly invade its surrounding tissues if a chronic inflammation is present. For instance, in murine and human hepatocellular carcinomas (HCC), NUP98 expression was reduced simultaneously with p21 as the 3′UTR of NUP98 was shown to protect P21 mRNA from exosome-mediated degradation.[148]

6.3 Nuclear pore complex

We chose to test the depletion of TPR, NUP160 and NUP358 for different reasons. TPR was first chosen because of its implication in the loss of SASP reported in IMR90 cells and we thought that this phenotype could be associated with the fact that it affects the cGAS-STING pathway who has been demonstrated to be greatly involved in the production of SASP.[116] Following the results obtained that the depletion of TPR in MEFs cells and HeLa cells induces the activation of ISGs, we then proceeded to identify if this consequence was unique to the loss of TPR and potentially the nuclear pore basket or if it was due to loss of the NPC overall structural integrity and other complexes associated with it.

Sakuma, S. et al. (2021) has shown that the knockdown using siRNA of various nucleoporins affected the signal intensity of Mab414, antibody which links to the FG-repeats of all nucleoporins presenting an FG-repeat, in immunofluorescence at different degrees depending on the depleted nucleoporin.[97] This suggests that the knockdown of some nucleoporins influences the structure and viability of the nuclear pore complex more than others. The nucleoporin they tested that had the greatest loss of signal was NUP160. We also tested by immunofluorescence mab414 signal, as shown in **supplementary figure 2**. Surprisingly, the lowest variation in signal and even an increase in the signal of Mab414 was observed, was the knockdown of NUP358. A significant reduction in Mab414 signal was noticed in cells transfected with siRNAs against TPR and NUP160, which is consistent with previous studies.[97, 149] It could be possible that TPR regulates the number of nucleoporins in each subcomplexes in the assembly phase and its knockdown would affect the proper number of nucleoporins in each section. This result suggests that these nucleoporin have a great effect on the loss of the NPC structural integrity and could be a positive control for our hypothesis that the overall NPC structure instability could be the cause of the activation of cGAS-STING and not only specific to the depletion of TPR or the nuclear pore basket. It is also important to mention that the nucleoporin NUP160 is part of a different subcomplex of the NPC and technically it is hard to say if its depletion would affect the NPC the same way as a depletion of nuclear pore basket NUPs.

Secondly, one research group demonstrated that within the 10 nucleoporins tested, NUP358 was the last nucleoporin to be added to the formation of the NPC. This was valid in both postmitotic assembly and in interphase assembly as NUP358 would assemble 10 to 15 minutes later than the other nucleoporins tested : NUP153, Pom121, NUP107, Seh1, NUP205, NUP93, NUP62, NUP214, and TPR. TPR and Nup153, both nuclear basket nucleoporins were the 2nd and 3rd nucleoporins recruited in the assembly of the NPC during the interphase assembly stage.[86] They also showed that the recruitment of TPR precedes the recruitment of NUP62 in the interphase nuclear pore complex assembly.[86] This means that TPR affects at least one other nucleoporin to be correctly assembled in the nuclear pore complex. By choosing the nucleoporin NUP358, we hypothesised that it would act as a negative control for TPR for various reasons. Firstly, because it is the latest NUP to be assembled, it highly likely that is not essential for most of the structural assembly, and it potentially doesn't have a major structural role. Secondly, it is located at the opposite of TPR, it is a cytoplasmic NUP and is separated by the outer ring, inner ring, scaffold and the nuclear envelope from TPR. By affecting NUP358, we can hypothesise than the cellular consequences resulting from its depletion are mainly independent of a depletion of any NUP in the nuclear pore basket.

Although, following the results in **figure 24 and 25,** there seems to be a potential affiliation of TPR or the nuclear basket with the Y complex. It is impossible to determine with these experiments whether there's a direct or indirect interaction but there seems to be nucleoporins connected between these two subcomplexes. It has been shown that NUP93 could be a common link between multiple subcomplexes of the NPC as its depletion affects all subcomplexes compared to other NUPs could be the potential cause of the indirect reduction of TPR following NUP160 depletion.[150]

This study has been on the depletion of various nucleoporins and their effect on the NPC. It would be interesting to observe if the same phenotype can be found when overexpressing the same nucleoporins as tested in this research. We would expect a different phenotype but possibly similar activation of the cGAS-STING pathway. Nuclear

envelope budding could also be potentially observed since the imbalance of the nuclear membrane would be affected as much in an overexpression than a depletion. It is possible that the lamina network would also be affected. An increase in the number of nucleoporins could restrict the spatial availability for the lamina to be abundant and well anchored into the NPCs.

Results obtained in **figure 18** and **figure 19** prove that the production of proteins induced by the IFN-I response such as CXCL10 in this case, is still functional despite the nuclear pore complex being affected by the depletion of NUP160, TPR and NUP358. An accumulation of mRNA at the NPC could be the source of upregulation of some ISGs genes since they are blocked at the nuclear pore and the cell would keep creating mRNA until the conceiving of the functional proteins as the normal immune response. By measuring levels of CXCL10 secreted by MEFs cells and successfully compare different conditions, we can hypothesise that the mRNA transport could still be affected following the depletion of various nucleoporins but also we can attest that the nucleo-cytoplasmic transport system is still functional even though it could be partially functional.

There has been research on the minimum number of nucleoporins in the nuclear basket for the nuclear pore complex to still be functioning, from 1 protein unit to 8.[151] This provides information that a knockdown of half of the nucleoporins of the NPC basket would maybe not affect their functions as much and that some NPC who have 0 NUP from the nuclear basket will have functional problems and could lead to some of the NEB events or membrane loss of integrity. This would probably happen more frequently using a longer knockdown from an shRNA for instance.

6.4 Nuclear envelope integrity

It has been reported in the past that TREX1 is potentially responsible for the inhibition of cGAS as it reduces the presence of ligands in normal conditions where cGAS would be constantly activated at any DNA ending in the cytoplasm. TREX1 degrades cytosolic DNA

to suppress inflammation is reported to be localized at the DNA bridges following a nuclear rupture where is creates ssDNA via exonucleolytic resection.[152] By creating ssDNA, it reduces cGAS activity since it can only detect dsDNA. In senescent cells, there a decrease of DNase II and TREX1 which leads to an increased activation of cGAS.[153] The onset of SASP is believed to be involving an increase presence of cytosolic DNA to then activate the cGAS-STING pathway. It has previously been shown that TPR is necessary for the SASP production in senescent cells and such do not undergo cell cycle anymore.[116] We were expecting comparable results in our study following this previous research, but it turned out that the depletion of TPR activates the cGAS-STING pathway instead of inhibiting it. The cells used in our experiments are not senescent and are highly proliferative. They induced senescence with an oncogene, so the cell state is quite different. They observed a drastic decrease of the production of IL1α, IL1β, IL6 and IL8 which are traditionally secreted after a cell becomes senescent. Because senescent cells do not divide anymore and will secrete pro-inflammatory cytokines, their investigation on TPR seems mainly valid in the context of senescent cells. Senescent cells are likely to have suffered from DNA damage in the past and are already undergoing a stress response. Our results were made in cells that can highly proliferate and therefore can also accumulate DNA damage during mitosis. Therefore, it is possible that our results differ as the context of these different studies is different. The underlying mechanism of the cGAS-STING pathway activation in our research seems to be present because of overtime nuclear instability which would occur in proliferative functioning cells, healthy or cancerous.

The results shown in **figure 15** are very intriguing. They suggest that TPR is involve in possibly DNA repair. Because hydroxyurea inhibits DNA repair and a higher activation of ISGs was observed following treatment of HU in cells depleted of TPR in comparison to their control, we can assume that our cellular model, the MEFs, which are immortalized have a lot of ongoing DNA repair since they divide rapidly and often and carry out some mutations since they can bypass some systems that would normally turn them senescent.

This is compatible with research that has been realized in the past where TPR has been found to be responsible for genome stability.[108]

Denais, C.M. et al. (2016) observed that following breaks of the nuclear membrane in cells, the nuclear envelope rapidly reseals itself and that a cell can repair multiple nuclear breaks in a short amount of time.[154] They observed an accumulation of Lamina A within 2 minutes after observing a rupture of the nuclear envelope. This process seems to require members of the endosomal sorting complexes required for transport III (ESCRT III). After a depletion of ESCRT III, the time in which the re-compartmentalization of the nucleus increased drastically. They also discovered that under normal circumstances, 90% of cells survive after experiences repeated nuclear membrane ruptures.[154]

6.5 Nuclear Envelope Budding

In **figure 21**, we can observe the accumulation of cGAS at what seems to be a NEB event. This has been reported in the past in multiple studies. Halfmann, C.T. et al (2019) observed BAF and cGAS to rapidly appear on nuclear rupture site following a NE-rupture technique in live-imaging. Within minutes, both proteins diffused into the nucleoplasm.[155] cGAS also has been known to accumulate at the membrane of micronuclei and following its degradation.[156] The protein cGAS is found in micronuclei formed in a cell with genome instability and activates the innate immune system.[157]

A way of transport out of the nucleus was first discussed in the 1950s by researchers working on the D*rosophila melanogaster* when a form of nuclear 'budding' was described and their first thought was that the out pocketing of this membrane is most likely a normally cellular function involved in some sort of cargo transportation.[158] In 2012, a group of researchers tried to elucidate these budding events of the nuclear membrane and characterized the cargo transported in these NEB events.[159] They proposed that the contents of these events were large ribonucleoprotein (RNP) granules which were too

large to go through the NPC and then could be transported to the specific location they needed to go.

In Huntington's disease, studies reported interesting results and similar observations about the nuclear membrane deformation. The results show that in mice, iPSNs, and post-mortem human brain tissue, mutant huntingtin (mHTT) co-aggregates with NUP62, NUP88, Gle1, and RanGAP1 and causes nuclear envelope abnormalities, altered subcellular distribution of Ran GTPase, nuclear mRNA accumulation and deficiencies in active nucleocytoplasmic transport.[160-162]

The use of electron microscopy would increase the accuracy of the images taken and we could see if the NEB observed in confocal microscopy in **Figure 22** with the Lamin A and C network having budding-like behavior if other proteins and the whole structure of the nuclear membrane is budding also.

As previously discussed, there has been reports of CCFs in senescent cells, these fragments of chromatin that can be detected by cGAS and induces an inflammatory response. It is possible that these fragments are the initial cause of the inflammatory response and leads to the SASP. In our results, we did not test if our cells were senescent, it is a possibility even though our cells have been immortalized using SV40, which inhibits P53, Rb and other tumor suppressor genes which technically prevents cells to become senescent.[163] It is possible that the loss of integrity of the membrane imitates what would happen if the cells became senescent and would release chromatin fragments similar to CCFs associated with senescent cells.

6.6 Lamina

As shown in **supplementary figure 3,** a decrease of the lamina network intensity was detected in a lot of cells which had a knockdown of TPR. These events were not quantified

as it is highly dependent on the accuracy staining of the antibody between each experiment. This phenotype has also been seen by Kittisopikul, M. et al (2021) where they analyzed the lamin meshwork by immunofluorescence following a depletion of TPR, NUP153 but also Lmna -/- and Lmnb1 -/-cells.[164] A very similar loss of density was observed in both Lmna -/- and Lmnb1 -/- and the MEFs we transfected.

The activation of the cGAS-STING pathway through lamina and nuclear protrusion was hypothesised in an article published in 2020.[165] They suggested that by affecting the lamina of myocytes by knocking out the gene LMNA, they would observe lamina and chromatin protrusion when the myonuclei would migrate and a mechanical force would be applied. This leak of chromatin in the cytoplasm would lead to an accumulation and binding of cGAS to the site of breakage and then activate the cGAS-STING pathway.

The observations and quantitative analyses regarding the proportion of cells exhibiting anomalies detected with the lamin A/C antibody are constrained by the intrinsic stability of the nuclear pore complex (NPC). This structure is highly stable and does not require frequent synthesis of new nucleoporins, except during cell growth or division.[106] Consequently, it is plausible that non-dividing cells present prior to transfection remain largely unaffected, whereas actively dividing cells engaged in NPC biogenesis are more susceptible to transfection-induced alterations.

7. Conclusion

The depletion of TPR induces an inflammatory response which is characterized by an upregulation of ISGs such as CXCL10, RSAD2, IFIT1 and ISG15. Additionally, a similar effect has been observed following the depletion of NUP160 and NUP358. The response has been observed to be majorly dependent of the cGAS-STING pathway after comparing results with MEFs STING KO cells and MEFs (wt) treated with H151, a STING antagonist.

This response has also been observed in HeLa cells, showcasing that is also occurring in other cell types.

The knockdown of NUP160 also affects the levels of TPR and suggests that the knockdown of nucleoporins in the Y-complex impacts the overall quantity of TPR. This result demonstrates that the results obtained following the knockdown of one NUP is not necessarily specific and might affect the presence of other NUPs.

TPR protects DNA damage from damage induced by hydroxyurea, a drug affecting DNA repair. The upregulation of the gene CXCL10 also translated to an increase of production of CXCL10 as demonstrated by measuring CXCL10 by ELISA in supernatant of transfected cells.

An increase of protrusions and micronuclei was observed in cells depleted of TPR in comparison to their control. This result suggests that the rupture of the nuclear membrane following a NEB or a micronuclei degradation could be the source of the activation of cGAS-STING. The depletion of NUP160 also reduces the levels of TPR as observed by immunofluorescence.

It is clear that many factors can be in play when it comes to the activation of the cGAS-STING pathway following a depletion of diverse nucleoporins as the NPC is a very dynamic and complex component of our cells and any event of a loss of the nuclear membrane integrity can lead to various consequences within a cell.

8. Perspectives

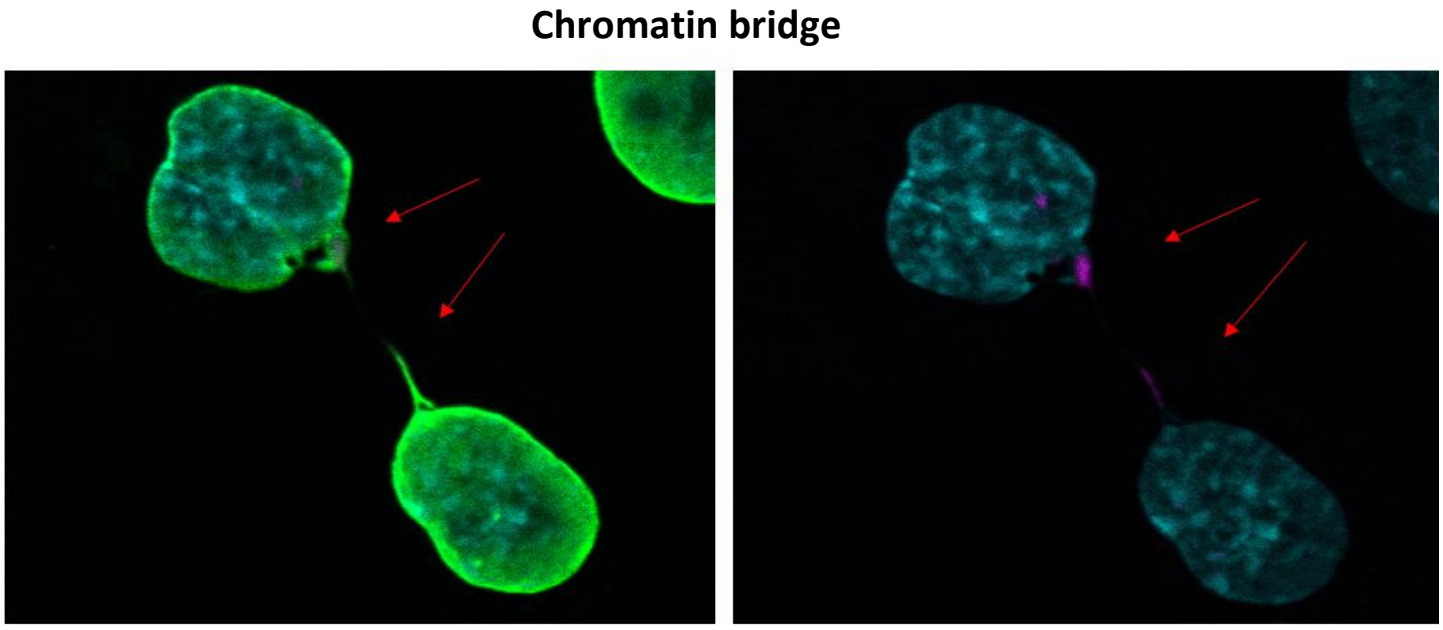
The findings of this projects sheds light on a potential new source of activation of the cGAS-STING pathway which could be involved in several autoimmune diseases or any

pro-inflammatory-associated pathologies. There is also a multitude of pathologies associated with DNA damage, lamina damage and malfunctioning mRNA export in which the initial cause is still unclear and could be associated to the nuclear pore complex. Understanding the molecular mechanism of our organism is key to better understand any pathologies associated with its dysfunction.

By studying potential causes of chronic inflammation, it can allow other researchers to identify biomarkers which could lead to an early disease detection, monitoring the progression of an ongoing disease. Identifying new targets for therapy could lead to the development of novel therapeutic interventions in certain diseases. Better understanding the nuclear-cytoplasmic transport gene expression regulation by the NPC is key to the potential development of therapeutic agents for a multitude of known and unknown diseases.

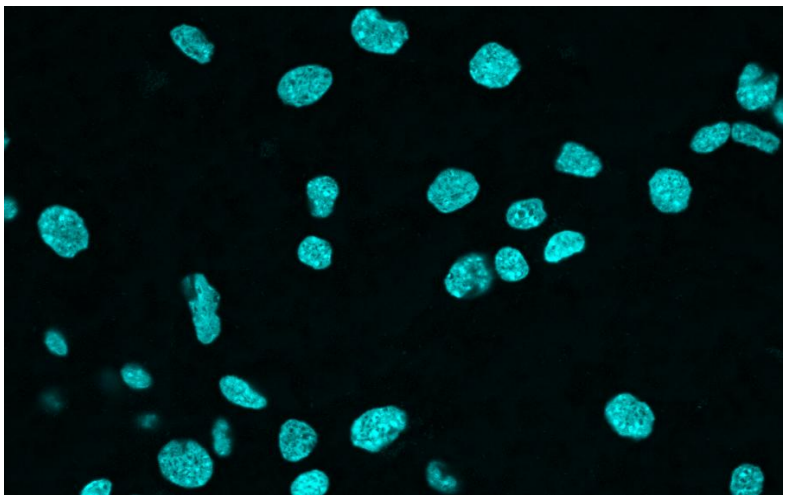
Since all cells of the body possess a nuclear pore complex, it is of high importance to understand its in and outs to better understand vital parts of an organism's well being and proper functioning. Research on the nuclear pore complex can also benefit other fields of research other than medical since all eukaryotic organisms possess nuclear pores.

9. Supplementary figures



Supplementary figure 1: Observation of chromatin bridges in TPR-depleted MEFs. MEFs cells transfected for 72 hours with siRNA against TPR and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. Immunofluorescence done using a primary antibody against TPR and alexa-555 as the secondary antibody. Lamina (green), DAPI (blue), P-H2AX (purple).

siNEG



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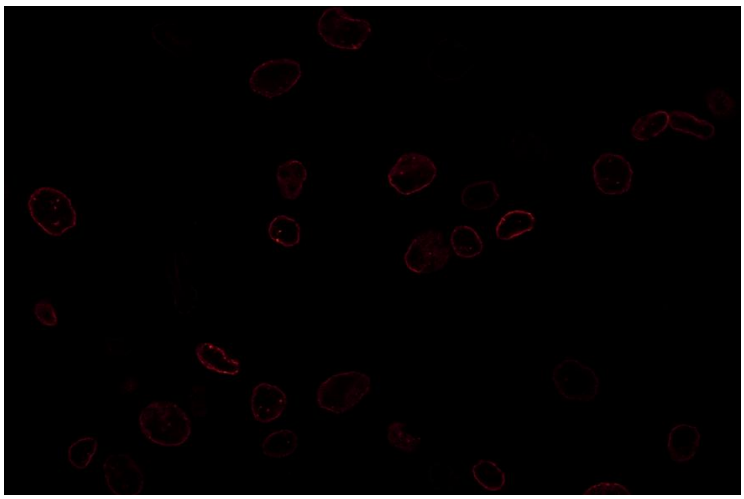
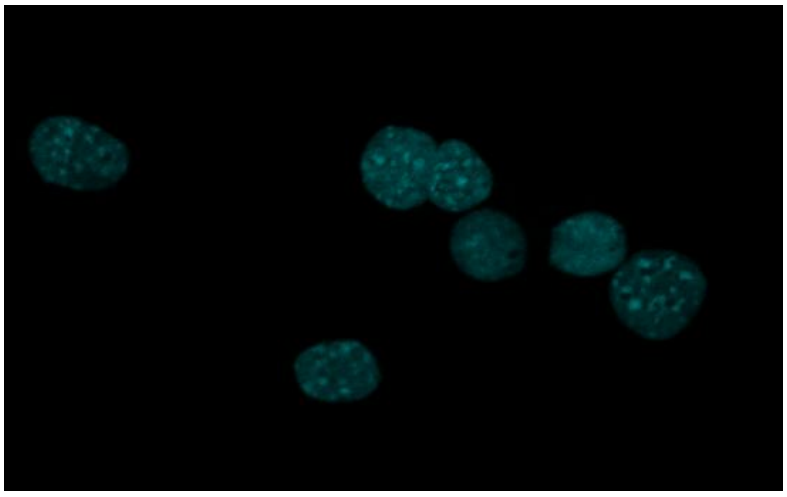


NUP358



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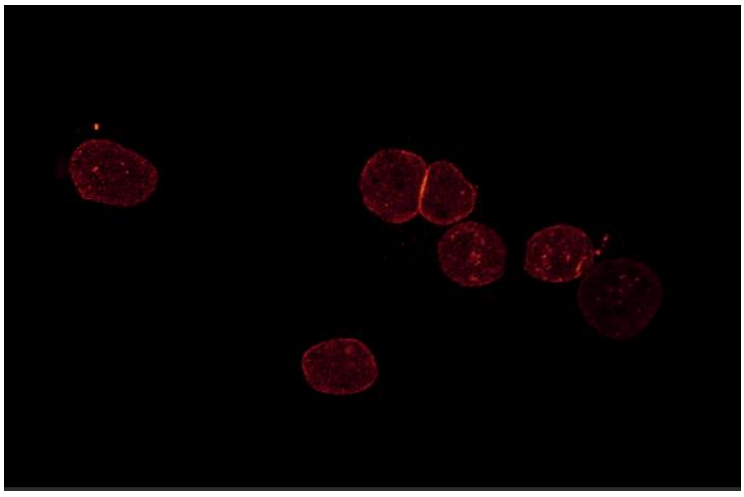

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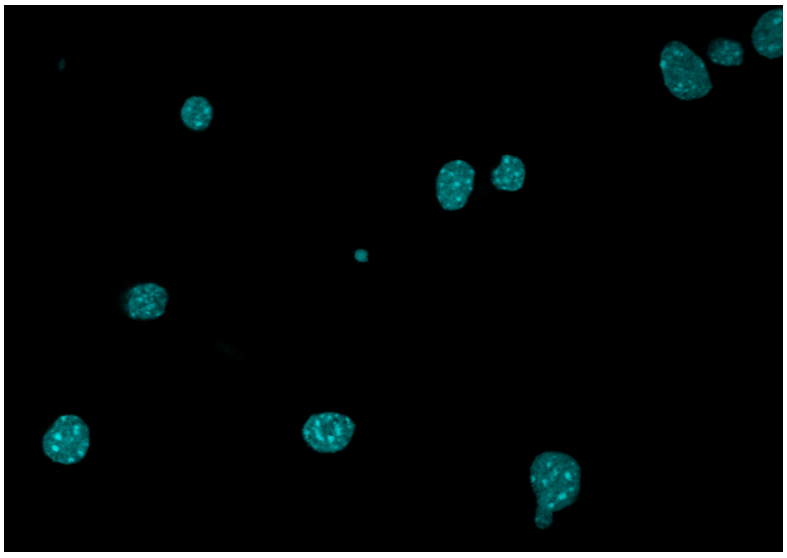

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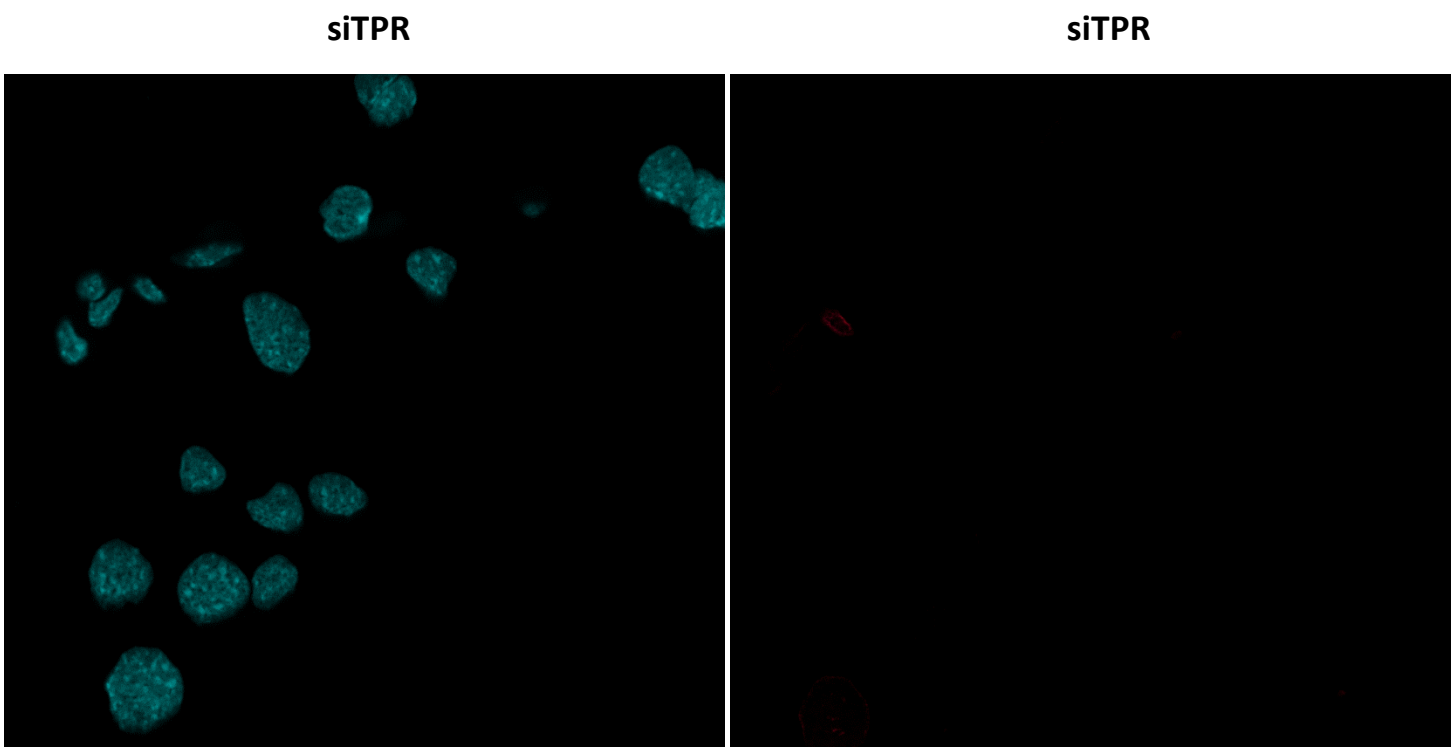
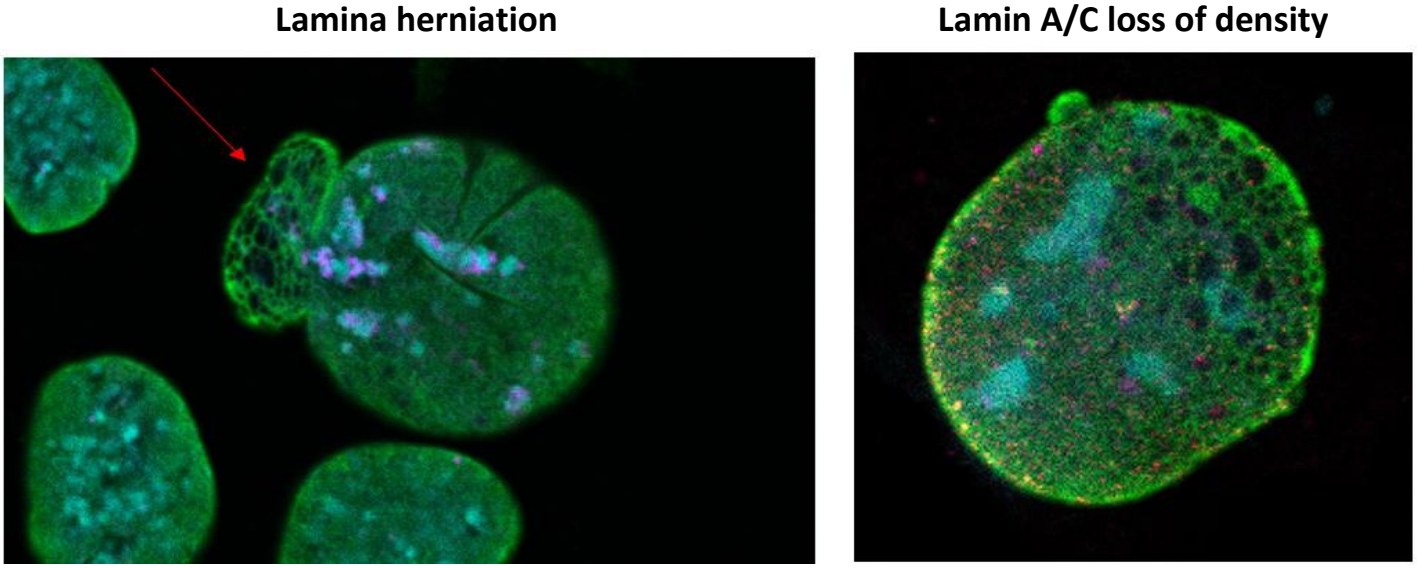
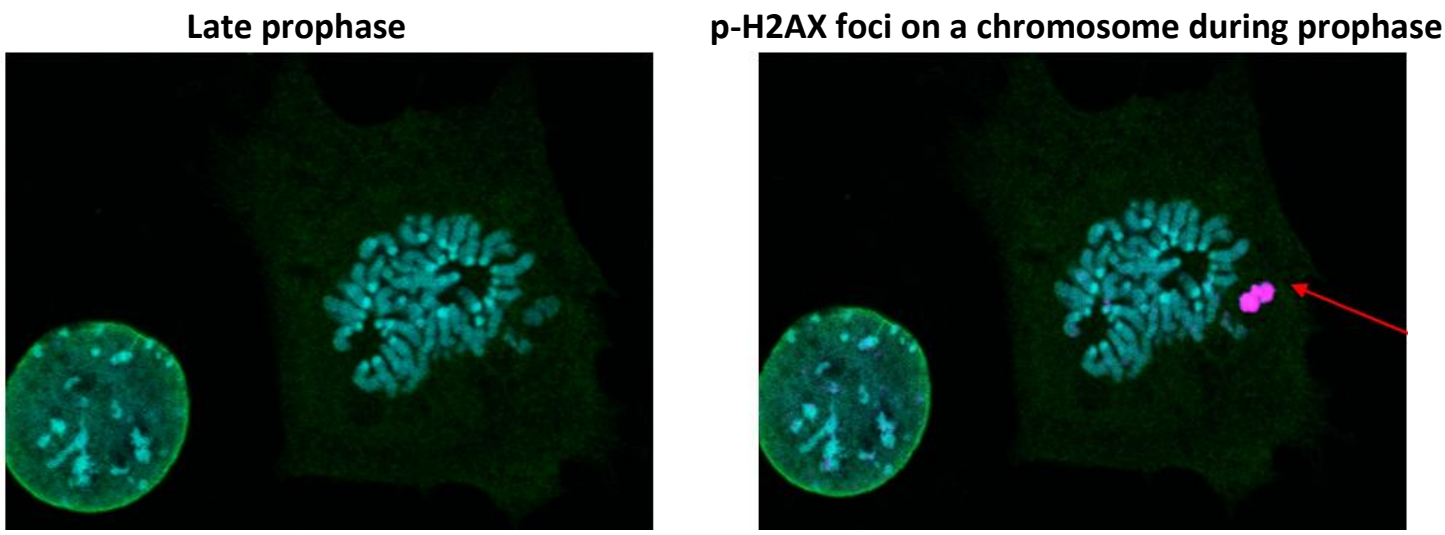


Supplementary figure 2: Mab414 fluorescence following TPR, NUP358 and NUP160 depletion. MEFs cells transfected for 72 hours with siRNA against TPR and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. Immunofluorescence done by confocal microscopy using a primary antibody against Mab414 and alexa-555 as the secondary antibody (red) . DAPI (blue). N=1

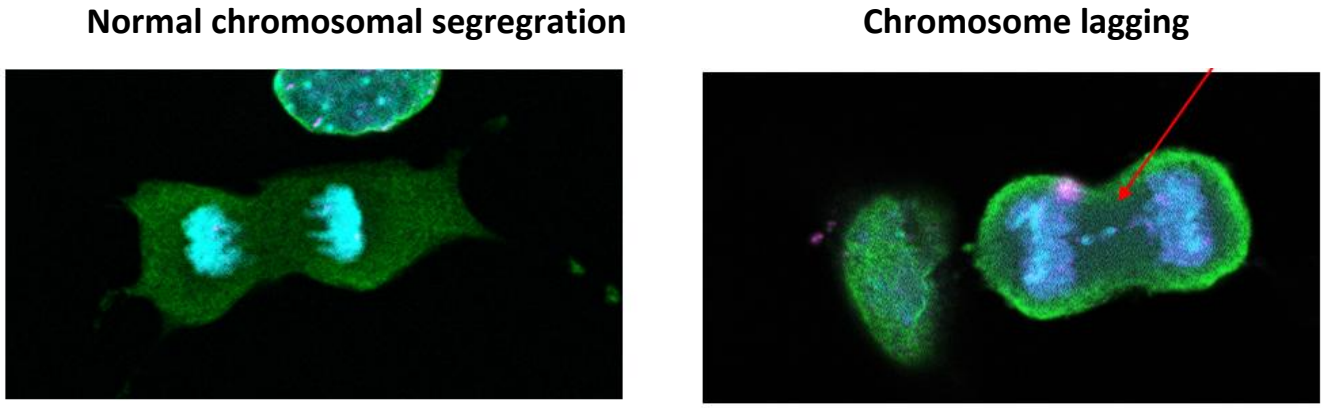


Supplementary figure 3: Lamina herniation and loss of density of the Lamin A/C meshwork. MEFs cells transfected for 72 hours with siRNA against TPR and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. Immunofluorescence done using a primary antibody against TPR and alexa-555 as the secondary antibody.

Lamina, (green). DAPI (blue). P-H2AX (purple).



Supplementary figure 4: p-H2AX foci detected in late prophase chromosome defect. MEFs cells transfected for 72 hours with siRNA against TPR and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. Immunofluorescence done using a primary antibody against TPR and alexa-555 as the secondary antibody. Lamina, green DAPI, blue. P-H2AX, purple.



Supplementary figure 5: Chromosome lagging observed in TPR-depleted MEFs.

MEFs cells transfected for 72 hours with siRNA against TPR and Lipofectamine™ RNAiMAX at a concentration of 1:600 v/v. Immunofluorescence done using an primary antibody against TPR and alexa-555 as the secondary antibody. Lamina (green), DAPI (blue), P-H2AX (purple).

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