From Department of Medicine, Solna
Karolinska Institutet, Stockholm, Sweden

MECHANISMS OF TYPE I INTERFERONS IN AUTOIMMINUTY AND CANCER

William A. Nyberg

Karolinska Institutet

Stockholm 2019

Cover by Sofie Önnestam.
Illustrations in the thesis were made using Servier Medical Art.
All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by US-AB.

ISBN 978-91-7831-288-7

Mechanisms of type I interferons in autoimmunity and cancer
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

William A. Nyberg

Principal Supervisor:
Dr. Alexander Espinosa
Department of Medicine, Solna
Karolinska Institutet

Co-supervisor(s):
Professor Marie Wahren-Herlenius
Department of Medicine, Solna
Karolinska Institutet

Opponent:
Professor Jonas Nilsson
Institute of Clinical Science
Sahlgrenska Cancer Center
Göteborgs Universitet

Examination Board:
Professor Martin Bergö
Department of Bioscience and Nutrition
Karolinska Institutet

Associate Professor Andor Pivarcsi
Department of Medicine, Solna
Karolinska Institutet

Professor Ingileif Jónsdóttir
Faculty of Medicine
University of Iceland (Háskóli Íslands)

The public defense will take place on **Friday 22nd February** at **9.00** in the CMM lecture hall (L8:00) at the Karolinska University Hospital, Solna.

ABSTRACT

Interferons (IFNs) were first described in the 1950's and has since been characterized as potent inducers of pathogen defense mechanisms. Since then, researchers have uncovered a depth of mechanisms in the IFN system extending beyond immune modulation of pathogen defense. IFNs have been described to play a role in the control of cell homeostasis as well as contributing to both the natural defense against cancer and cancer progression.

The objective of the work performed in this thesis was to understand how type I IFNs are able to contribute to such a wide range of biological processes and to investigate metastatic progression of malignant melanoma. We first performed screens of miRNAs on sorted immune cells from patients treated with IFNβ. We identified miR-31-5p to be downregulated in T cells of patients treated with IFNβ. miR-31-5p was also decreased in patients with autoimmune disease, characterized by an IFN signature, further proving type I IFN regulation of miR-31-5p expression levels. We were able to show that miR-31-5p regulate metabolism of CD4+ T cells. In an attempt to get new insights into IFNAR1 signaling, we applied proximity proteomics techniques to IFNAR1. Labeled proteins were extracted and subsequently analyzed using quantitative mass spectrometry. We successfully generated interactome data that revealed insights to a possible nuclear translocation mechanism of ligand bound IFNAR1.

Bromodomain (BRD) proteins are chromatin bound proteins that regulate a multitude of processes important in transcriptional regulation and epigenetic regulation of gene expression. Several BRD proteins regulate the expression of type I IFN genes and are emerging targets for new cancer drugs. We therefore aimed to identify BRD proteins that play a role in metastatic progression and plasticity of malignant melanoma. We identified that the BRD protein TRIM28 controls a plasticity switch of melanoma cells through expression of JunB. Plasticity switching of melanoma cells represent a model of metastatic progression.

Recently, the role of ER stress in both protecting and killing tumors has been highlighted. We therefore aimed to identify new small molecules capable of inducing strong ER stress. We identified the IFN-inducing small molecule IMQ as a potent ER stress inducer in melanoma cells independently of TLR7/8 expression. ER stress induced by IMQ in melanoma cells lead to cell death and represents a possible pharmaceutical strategy to target and kill cancer cells.

In summary, our studies provide insights to type I IFN driven mechanisms with implications for autoimmune disease and cancer.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Interferon (IFN) är en grupp av signaleringsmolekyler som kallas för cytokiner och upptäcktes för första gången på 1950-talet. Upptäckten att denna grupp proteiner kunde hjälpa celler att bekämpa infektioner gav denna grupp namnet interferon, efter det engelska ordet ”interfere”. Forskare har under åren som gått sedan den första upptäckten karaktäriserat IFN-familjen och dess funktioner. Sammanfattningsvis så fungerar denna familj av cytokiner som en potent signaleringsmolekyl för immunförsvaret vid upptäckten av en infektion. Immunceller svarar på IFN genom att producera faktorer som hjälper immunförsvaret att bekämpa infektionen. Denna grupp av cytokiner kan delas in i tre grupper baserat på deras funktion och struktur, typ I, II och III. Typ I IFN kan produceras av de allra flesta celler i kroppen som en respons av virus- och bakterieinfektioner. Stora mängder typ I IFN kan även produceras av specialiserade immunceller. Dessa cytokiner fungerar alltså som immunreglerande signaleringsmolekyler. Forskning på IFN har visat att deras roll i människans biologi sträcker sig längre än immunreglering. Det har visat sig att cytokinerna både bekämpar och i viss mån bidrar till utvecklingen av vissa cancerformer, det har även visat sig att de bidrar till uppkomsten av autoimmuna sjukdomar och reglerar viktiga cellulära funktioner.

Målet med forskningen i denna avhandling var att kartlägga mekanismer bakom typ I IFN som bidrar till dess komplexa funktioner, samt att studera den metastatiska processen av malignt melanom.

I den första studien studerade vi patienter behandlade med IFN, före och efter behandling. Celler från dessa patienter isolerades och analyserades för deras uttryck av mikro RNA molekyler. Vi fann att T celler från patienter behandlade med IFN uttrycker lägre nivåer av mikro RNA-31 efter behandling. Vidare identifierade vi att mikro RNA-31 reglerar energimetabolismen i T celler, vilket har stor betydelse för T cellernas kapacitet att bekämpa infektioner. I den andra studien studerade vi receptorn för typ I IFN med avsikt att kartlägga alla de protein-interaktioner som uppstår intra-cellulärt efter att receptor bundit till IFN. Vi utvecklade nya metoder för identifiering av protein-interaktioner för receptorer och identifierade tidigare okända interaktionspartners för IFN-receptorn.

Malignt melanom kopplades tidigt ihop med IFN där cytokinerna har använts i behandlingssyfte. I den fjärde studien studerade vi den metastatiska processen för malignt melanom. Vi upptäckte att höga nivåer av proteiner TRIM28 kunde kopplas till kortare överlevnad för patienter diagnostiserade med metastatisk melanom. Vi visar även att TRIM28 kontrollerar kritiska mekanismer för genuttryck i melanom-celler. Genreglering av TRIM28 i melanom-celler kontrollerar cellernas förmåga att metastasera och växa. Slutligen så visar vi i den tredje studien hur den IFN-inducerande molekylen, imiquimod, har förmågan att binda till melanom-celler och döda cellerna genom att inducera intracellulär stress.

Sammanfattningsvis så presenterar arbetet i denna avhandling nya insikter i hur typ I IFN kan påverka autoimmuna sjukdomar samt cancer.

LIST OF SCIENTIFIC PAPERS

I. **miR-31 regulates metabolism and is suppressed in T cells from patients with Sjögren's syndrome**
Alina Johansson, William A. Nyberg, Maria Sjöstrand, Noah Moruzzi, Petra Bergman, Mohsen Khademi, Magnus Andersson, Fredrik Piehl, Per-Olof Berggren, Ruxandra Covacu, Maja Jagodic, Alexander Espinosa.
European Journal of Immunology, 2018 (Epub ahead of print).

II. **Mapping interactions of the type I interferon receptor using proximity proteomics labeling**
William A. Nyberg, Tianlin He, Alexander Espinosa.
Manuscript.

III. **Imiquimod induces ER stress and Ca^{2+} influx independently of TLR7 and TLR8**
William A. Nyberg, Alexander Espinosa.
Biochemical and Biophysical Research Communications, 2016, 473, 789-794.

IV. **TRIM28 controls a JunB dependent switch between tumor growth and invasiveness in melanoma**
William A. Nyberg, Tianlin He, Maria Sjöstrand, Lucia Pellé, Ruxandra Covacu, Alexander Espinosa.
Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

IFN	Interferon
TLR	Toll-like receptor
PRR	Pattern recognition receptor
PAMP	Pathogen-associated molecular pattern
IRF	Interferon regulatory factor
JAK1	Janus kinase 1
TYK2	Tyrosine kinase 2
STAT	Signal transducer and activator of transcription
ISRE	Interferon-stimulated response element
MAPK	Mitogen-activated protein kinase
IFNAR	IFNα receptor
ISG	Interferon stimulated gene
BRD	Bromodomain
miRNA	Micro RNA
TCGA	The cancer genome atlas
ERK1/2	Extracellular signal-regulated kinases 1/2
CSC	Cancer stem cell
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum
XBP1	Xbox-binding protein 1
TRIM	Tripartite-motif family
RNAPII	RNA polymerase II
ERV	Endogenous retrovirus
OCR	Oxygen consumption rate
ECAR	Extracellular acidification rate
Co-IP	Co-immunoprecipitation
LC-MS	Liquid chromatography-mass spectrometry
shRNA	Short hairpin RNA
KD	Knockdown
KO	Knockout

ChIP	Chromatin immunoprecipitation
IMQ	Imiquimod

1 INTRODUCTION

Interferons (IFN) were first reported in 1954 as an "inhibitory factor" by Nagano and Kojima, discovered in the skin of inoculated rabbits (Nagano and Kojima, 1954). The cytokines were first named by Isaacs and Lindenmann in 1957 for their capacity to "interfere" with viral infections (Isaacs and Lindenmann, 1957). Over several decades, researchers have uncovered a depth of mechanisms in the IFN system. Different types of IFNs have been characterized and their regulation and role in immunity have been elucidated. The role of IFNs in modulating immunity and contributing to disease have been studied for a long time. In later years, emerging studies have revealed complex mechanisms of interferons describing their roles in early development and controlling cell homeostasis.

1.1 TYPE I INTERFERONS

IFNs are divided in to three types (I-III). The type I IFN gene family consist of IFNα, IFNβ, IFNε, IFNκ and IFNω. The IFNα family consist of at least 13 genes in humans while only one single gene exist for IFNβ (Honda et al., 2006). Type II IFNs consist of IFNγ and are produced mainly by NK cells and T cells in response to viral and bacterial infections and activate a broad range of immune cells. Type III IFNs consist of IFNλ1, IFNλ2 and IFNλ3. They act mainly on the mucosal epithelial cells to protect maintain healthy mucosal surfaces (Wack et al., 2015). The type I IFN system has developed evolutionary as a natural defense mechanism against viral infections. All vertebrates have genes encoding both IFNα and IFNβ in their genome (Stetson and Medzhitov, 2006). The existence of type I IFNs throughout the vertebrate lineage signify the evolutionary importance of the type I IFN system.

1.1.1 Production and regulation of type I IFNs

Type I IFNs can be produced and secreted by all nucleated cells. However, the main producers of IFNα are plasmacytoid dendritic cells (pDCs) and they are often referred to as natural IFN producers. Type I IFNs are produced in response to microbial infection mainly through recognition of pathogen-associated molecular patterns (PAMPs) by the pattern recognition receptors (PRRs).

Toll-like receptors (TLRs)

TLRs are PRRs mainly expressed by specialized immune cells and recognize different types of extracellular PAMPs. All TLRs recognize extracellular PAMPs, either on the cell surface or in endosomes. Some TLRs are expressed mainly in endosomes such as TLR3, TLR7 and TLR9 while other are expressed on cell surfaces, such as TLR4. However, TLR4 is also found in endosomes after internalization upon lipopolysaccharide (LPS) recognition (Kagan et al., 2008). TLR4 is expressed in conventional DCs and macrophages (Takeuchi et al., 1999). TLR3 responds to double-stranded RNA while TLR7 and TLR8 recognizes single-

stranded RNA (Alexopoulou et al., 2001; Heil et al., 2004). TLR9 recognizes unmethylated CpG motifs of DNA and is expressed by pDCs, together with TLR7 they represent the main sensory mechanisms for the natural IFN producers (Lund et al., 2003). All TLRs contain an intracellular Toll-IL-1 receptor (TIR) domain. The TIR domain signals via recruitment of differentiation primary response gene 88 (MyD88) for all TLRs except for TLR3 which signals via TIR domain containing adaptor-inducing IFN (TRIF). TLR4 signals via both MyD88 and TRIF (Akira et al., 2006). Activation of MyD88 by TLR4 occurs at the cell surface while TRIF activation by TLR4 occurs upon internalization. Signaling by TLRs via either TRIF or MyD88 leads to activation of interferon regulatory factors (IRF). The IRFs are a family of transcription factors with well conserved DNA binding sites recognizing interferon-stimulated response elements (ISRE) (Darnell et al., 1994; Honda and Taniguchi, 2006). MyD88 activates IRF7 and TRIF activates IRF7 via TANK-binding kinase 1 (TBK1). Activated IRF3 and IRF7 lead to expression of type I IFNs and they are key transcription factors for type I IFN production (Honda et al., 2005; Sakaguchi et al., 2003).

Cytosolic PRRs

As opposed to TLRs, that recognize extracellular PAMPs, cytosolic recognition of PAMPs occur through cytosolic PRRs. While TLRs are mainly expressed by specialized cells, cytosolic PRRs are expressed by all nucleated cell. Of the cytosolic PRRs leading to type I IFN production, two well described RNA sensors are retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5). Both contain caspase activation and recruitment domains (CARD). Both receptors are activated by viral nucleic acids but recognizes different types of dsRNA (Kato et al., 2006). Upon activation, the RIG-I and MDA5 recruits mitochondrial antiviral signaling (MAVS) that leads to a signaling cascade resulting in activation of mainly IRF3 or nuclear factor κB (NF-κB) (Yoneyama and Fujita, 2009). In addition to cytosolic RNA sensors, pathogen-derived cytosolic DNA is identified by cyclic GMP/AMP synthase (cGAS). Activation of cGAS leads to production of cytosolic guanosine monophosphate-adenosine monophosphate (cGAMP), in the presence of GTP and ATP, which in turn triggers stimulator of interferon genes (STING). Activation of STING results in either IRF3 or NF-κB activation (Ishikawa and Barber, 2008; Sun et al., 2013). IFNα expression can only be induced through IRF activation while IFNβ expression can be induced by both IRFs and NF-κB (Ryals et al., 1985; Sato et al., 1998a; Sato et al., 1998b). IFNα production is therefore predominantly produced by pDCs that express high levels of IRF7 and TLR7 while IFNβ production is more widespread.

1.1.2 Type I IFN signaling

IFNα and IFNβ signals via the same cell surface receptor, the IFNα receptor (IFNAR). IFNAR is a heterodimeric receptor that consist of the subunits IFNAR1 and IFNAR2 (Novick et al., 1994; Uze et al., 1990). Type I IFN engage with IFNAR, leading to dimerization of the receptor subunits and activation of Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2),

resulting in phosphorylation of the receptor (Wilks, 1989). Signal transducer and activator of transcription (STAT) proteins are recruited and phosphorylated by the receptor (Fu et al., 1992; Schindler et al., 1992). STAT phosphorylation leads to dimerization and translocation to the nucleus (Levy and Darnell, 2002; Stark and Darnell, 2012). STAT dimerization can differ and the combinations result in separate gene activation patterns. Canonical type I IFN signaling results in STAT1-STAT2 heterodimerization and complex formation with IRF9 to generate the interferon-stimulated gene factor 3 (ISGF3) (Levy et al., 1988). ISGF3 recognizes and bind genes with ISRE sites. In contrast, STAT1 homodimers bind and induces expression of genes containing gamma-activated sequences (GAS), typically a hallmark of IFNγ signaling. STAT3 homodimers are also known to be formed as well as activation more STATs (Darnell et al., 1994; Ivashkiv and Donlin, 2014). The components of canonical type I IFN signaling are broadly expressed and most cells can mount a response to type I IFNs. The STAT1 and IRF9 genes contain ISRE sites and are therefore induced by ISGF3 formation. Increased expression of STAT1 and IRF9 induces the response to type I IFNs through positive feed-back and cells are able to rapidly respond IFNs (Gough et al., 2012; Levy et al., 1990; Wong et al., 2002).

In addition to canonical IFNAR signaling via the JAK-STAT pathway, IFNAR also activates p38 mitogen-activated protein kinase (MAPK) (Li et al., 2004; Ramsauer et al., 2002) and phosphatidylinositol 3-kinase (PI3K) (Platanias et al., 1996; Uddin et al., 1997; Uddin et al., 1995). It is clear that activation of more than one signaling pathway is critical for the type I IFN response, as no single signaling cascade is sufficient to mount a full antiviral response (Platanias, 2005). The context-dependent nature of the signaling cascades downstream of IFNAR is still not fully elucidated. Extensive research efforts will be required to understand the complexity of the type I IFN signaling and the downstream events that occur upon IFNAR activation.

1.1.3 Regulation of IFNAR signaling

The multiple signaling cascades activated by IFNAR upon ligand binding results in a diverse cellular response. Many response factors are regulators of the signaling activity, resulting in positive or negative feedback control.

Positive regulation

Augmented signaling was briefly mentioned in the previous section by IFN-induced expression of STAT1 and IRF9. Many more regulatory mechanisms of IFNAR signaling have been demonstrated. STAT1 and IRF9 expression is also induced by other immunomodulatory cytokines such as tumor necrosis factor (TNF), interleukin-6 (IL-6) and IFNγ (Hu et al., 2002; Mitani et al., 2001; Venkatesh et al., 2013). These mechanisms allow responding cells to be primed for type I IFN response and augments the signal intensity. Signal augmentation also occurs via spleen tyrosine kinase (SYK) that mediates STAT1 phosphorylation, an event that is dependent on activation of immunoreceptor tyrosine-based

activation motifs (ITAMs) (Tassiulas et al., 2004). These mechanisms are implemented to either amplify an existing signal or to prime cells for robust type I IFN responses.

Negative regulation

Type I IFN signaling induces expression of a number of negative regulators that limit the extent of IFNAR signaling in a negative feedback loop. Suppressor of cytokine signaling (SOCS) proteins 1 and 3 are IFN induced genes that bind to IFNAR to suppress activity of JAK (Yoshimura et al., 2007). Ubiquitin carboxy-terminal hydrolase 18 (USP18) is also an IFN induced gene and is capable of binding to IFNAR2, replacing JAK1 and reducing STAT1 phosphorylation (Sarasin-Filipowicz et al., 2009). IFNAR activity is also regulated by lysosomal degradation and endocytosis of IFNAR1 and IFNAR2 (Chmiest et al., 2016; Claudinon et al., 2007). Identification of new regulatory mechanisms of IFNAR signaling presents opportunities for pharmaceutical intervention to limit pathogenic IFNAR signaling.

1.1.4 Type I IFN-induced transcription

Type I IFN signaling leads to induction of a large set of genes. These are collectively referred to as IFN stimulated genes (ISGs). The Interferome (v2.01) classifies up to 2000 genes as ISGs while more stringent methods to identify ISG normally refer to >300 genes (Rusinova et al., 2013; Schoggins et al., 2011). These genes are involved in a wide range of biological functions such as anti-viral and anti-bacterial defense, cell proliferation, immune regulation and apoptosis.

Transcriptional regulation

Genomic accessibility and the epigenetic landscape are crucial for gene expression, and the expression of ISGs is no different. Induction of ISGs require genomic accessibility of the ISGF3. IRFs recruit histone acetyltransferases (HATs) that acetylates histones resulting in the opening up of nucleosome structures (Bell et al., 2011). This is performed by recruitment of the BRG1- or HBRM-associated factors (BAF) chromatin remodeling complex (Liu et al., 2002). The bromodomain (BRD) containing protein SMARCA4 is recruited by ISGF3 upon type I IFN stimulation (Ni et al., 2005). The induction of ISGs at the transcriptional level is highly complex, SMARCA4 regulates only a subset of ISGF3-mediated expression of ISGs (Huang et al., 2002). Regulation of ISGs by SMARCA4 at the transcriptional level is only one of many mechanisms that control expression of ISGs. Acetylation of histones lead to recruitment of BRD4 that recruits the positive transcription elongation factor b (pTEFb) complex. The pTEFb complex is critical for transcription and elongation of RNA and can be potentially be targeted to reduce expression of inflammatory genes (Nicodeme et al., 2010; Patel et al., 2013). With the development of potent BRD inhibitors such as JQ-1 for BRD4 (Meng et al., 2014). BRDs have emerged as lucrative targets to alter expression of ISGs in inflammatory diseases (Klein et al., 2016).

microRNAs (miRNA) are small non-coding RNAs that function as post-transcriptional regulators of gene expression. Many miRNAs regulate the type I IFN response (David, 2010). Reduction miR-155 leads to induced responsiveness of CD8+ T cells to type I IFN by regulating STAT1 mRNA (Gracias et al., 2013). Another miRNA with suppressive functions is miR-146a that downregulates STAT1 in Th1 cells (Lu et al., 2010). Responsiveness to type I IFN can also be regulated by miR-29a mediated degradation of IFNAR1 mRNA (Papadopoulou et al., 2011). In all, the role of miRNAs in the type I IFN system is a growing field and can help to explain the context dependent-differences in type I IFN signaling.

1.1.5 Autoimmunity and interferons

Autoimmune diseases constitute a large family of complex diseases including systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS). A common trait amongst these two diseases is the upregulation of ISGs, commonly referred to as an IFN signature (Baechler et al., 2003; Wildenberg et al., 2008). Strong evidence exists for the involvement of type I IFNs in systemic autoimmune diseases, e.g. treatment of patients with type I IFN against chronic viral infections and neoplasias can lead to the development of symptoms of autoimmune disease (Ronnblom et al., 1990; Schilling et al., 1991). Chronic exposure of high type I IFN levels shifts the immune system towards pathological functions that contribute to autoimmune diseases (Crow et al., 2015). Blocking IFNAR1 using the antibody Anifrolumab to reduce type I IFN signaling is an approach currently being assessed in clinical trials for SLE patients (Furie et al., 2017). Results from the phase II clinical trial of Anifrolumab were promising, although, early reports from the phase III clinical trial are less encouraging. The role of type I IFNs in systemic autoimmune diseases is complex. Even though strong evidence exists for their contribution to the diseases, it remains unclear if therapeutic intervention in the signaling pathways will be successful. To understand the biology of type I IFN driven disease, further research must be performed on the underlying mechanism contributing to pathogenesis.

1.2 CANCER

The immune system has been known to play a role in cancer for a long time. German researcher Wilhelm Busch discovered as early as 1868 that infection with erysipelas caused tumor shrinkage. Further evidence was reported in 1893 in a case report of 10 patients treated with erysipelas infections (Coley, 1991). These findings led to experiments where patients with bladder cancer were treated intradermally with Bacillus Calmette-Guerin in 1976 (Morales et al., 2017). The concept of immune surveillance of tumors was proposed in 1970 (Morales et al., 2017) and has been widely accepted and included as a hallmark of cancer (Hanahan and Weinberg, 2011). IFNs were reported in 1984 to have anti-neoplastic effects after intra muscular injections to patients with hairy-cell leukemia (Quesada et al., 1984). Clinical trials to treat metastatic melanoma patients with recombinant IFNα showed moderate overall response rates (Creagan et al., 1986). IFNα was then used in clinics as an adjuvant for patients with malignant melanoma post-surgery showing improved overall survival (Tarhini et al., 2012).

1.2.1 Malignant melanoma

Cutaneous malignant melanoma is a cancer originating from melanocytes in the skin. Mutations in malignant melanoma are mainly caused by UV radiation. The high exposure to UV in the skin makes malignant melanoma the most mutated form of cancer (Alexandrov et al., 2013; Lawrence et al., 2013). Some of the most common mutations in melanoma are mutations in the *BRAF*, *NRAS* and *PTEN* genes. A study performing whole-exome sequencing of 121 melanoma tumors identified *BRAF* mutations in 90/121 samples, *NRAS* mutations in 33/121 samples and *PTEN* mutations in 16/121 samples. Only 16/121 samples were free of either *BRAF* or *NRAS* mutations (Hodis et al., 2012). The high mutation frequency of *BRAF* and *NRAS* is a common recurrence in melanoma studies (Krauthammer et al., 2012; Zhang et al., 2016). *BRAF* mutations were reported in >50% of all melanoma patients from the large genome-wide sequencing effort of The Cancer Genome Atlas (TCGA) (Cancer Genome Atlas, 2015; Guan et al., 2015).

The *BRAF* and *NRAS* genes encode the Raf and Ras kinases, both critical for MAPK signaling. Ras acts upstream of Raf and is activated by many receptor tyrosine kinases (RTKs). Raf activation by Ras results in phosphorylation, activation and nuclear translocation of extracellular signal–regulated kinases 1/2 (ERK1/2) (Mendoza et al., 2011; Vojtek et al., 1993; Zhang et al., 1993). The common *BRAF* and *NRAS* are activating mutations resulting in constitutive active MAPK signaling. The most common alteration to *BRAF* is the V600E mutation (Cancer Genome Atlas, 2015; Hodis et al., 2012). Phosphatase and tensin homolog (PTEN) is the protein encoded by the third most common mutation gene mutation in melanoma. PTEN acts as a negative regulator of PI3K signaling and loss-of-function mutations results in increased PI3K signaling. The identification of mutations in these

pathways have led to major efforts in identifying potent inhibitors by the pharmaceutical industry.

Clinical overview of malignant melanoma

According to statistics from the American Cancer Society the 5-year survival rate for patients diagnosed with stage I malignant melanoma is >90%. The survival rates for patients with metastatic melanoma is much worse with estimates as low 5-19% 5-year survival for stage IV patients (Sandru et al., 2014). The introduction of kinase inhibitors targeting the Ras-Raf pathway have been shown to be effective in targeting melanoma tumors. However, tumors develop resistance already within 6-8 months of the initiation of the treatment (Long et al., 2014; Long et al., 2015). Combination of multiple inhibitors increases efficacy but can only delay the onset of resistance temporarily (Robert et al., 2015a). The discovery of immune checkpoint blockade inhibitors offers further treatment options for patients with metastatic melanoma. Many patients do not respond to immune checkpoint blockade therapy with response rates reported between 40-65% (Larkin et al., 2017; Robert et al., 2015b; Schachter et al., 2017). Like with kinase inhibitors, melanoma tumors develop resistance to checkpoint blockade therapy (Gide et al., 2018; Zaretsky et al., 2016). Another promising group of inhibitors for melanoma are the bromodomain (BRD) inhibitors with multiple clinical trials undergoing (Perez-Salvia and Esteller, 2017). These will undoubtedly add to the growing number of treatment options for malignant melanoma.

The recent discoveries of novel inhibitors and therapies have improved the outlook for patients with metastatic malignancies. But moderate response rates and resistance mechanisms present major challenges to overcome metastatic progression of the disease.

1.2.2 Phenotype switching of melanoma

Survival rates for patients with melanoma decrease significantly for patients with metastatic disease. The main cause of death of melanoma is not the primary tumor but dissemination of metastatic tumors to distal organs. For cancer cells to disseminate from a primary tumor to form a metastasis, cells need to invade local tissue at the primary tumor site to access either blood or lymph vessels. This process is referred to as epithelial-mesenchymal transition (EMT) (Thiery et al., 2009). Metastatic cells need to survive in the circulation and extravasate from the blood vessel into new tissue. Finally, metastatic cells need to adapt to the new environment and proliferate. The reversed EMT process is usually referred to as mesenchymal-epithelial transition (MET). The metastatic process is initiated in melanoma as the primary tumor begins a vertical growth phase (Clark et al., 1984).

EMT in epithelial cancers is driven by the expression of proteins such as TWIST, SNAIL, ZEB1/2 that repress E-cadherin expression and promote a mesenchymal-like cell state (Thiery et al., 2009). With regards to expression of EMT factors, research groups have shown conflicting results in melanoma (Caramel et al., 2013; Denecker et al., 2014). Melanoma is

not an epithelial cancer and is not driven by classical EMT. Furthermore, the metastatic process of melanoma is not driven by acquisition of new mutations but rather seem to be driven by a high degree of plasticity (Falletta et al., 2017). It has been suggested that metastases of melanoma are formed by seeding of rare cancer stem cells (CSC) (Nguyen et al., 2012). Reports of subpopulations of melanoma with stem cell like properties first emerged in 2005 (Fang et al., 2005; Frank et al., 2005). However, these early reports have later been questioned and the proposed model of tumors seeded by CSC seem to not play a major role in metastatic formation of melanoma (Brinckerhoff, 2017; Cheli et al., 2014; Croteau et al., 2013).

Melanoma plasticity

Tumor heterogeneity can be described by several models. The CSC model where a small amount of stem-cell like cells in a tumor has the capacity to drive tumor progression (Nguyen et al., 2012; Reya et al., 2001). The CSC maintain self-renewal capabilities and the bulk of the tumor is made up of differentiated CSC that make up the intra-tumoral heterogeneity. Metastases are formed by seeding of CSC making up the inter-tumoral heterogeneity. Another model described is the clonal evolution model which is driven by genetic mutations and a clonal selection driving tumor progression (Nowell, 1976). Both these heterogeneity models represent unidirectional tumor progression and genetic heterogeneity. A third model is the bidirectional phenotypic plasticity model which suggests that cancers cells can undergo rapid phenotypic switching driven by reversible transcriptional and epigenetic changes. It has become apparent that melanoma cells are characterized by a high degree of plasticity and alternate between invasive and proliferative states (Hoek et al., 2008; Kim et al., 2017). To fully understand the metastatic process of melanoma, it is crucial to identify the factors controlling the phenotypic plasticity.

1.2.3 ER stress and cancer

Endoplasmic reticulum (ER) stress is a physiological response to events such as abundance of misfolded proteins or protein overload in the ER. To restore the protein homeostasis, ER stress triggers activation of the unfolded protein response (UPR) pathway (Maurel et al., 2015). The UPR pathway is mediated by three ER membrane bound sensory proteins, inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase RNA-like ER kinase (PERK) (Maurel et al., 2015). ER stress leads to IRE1-dependent splicing of Xbox binding protein 1 (XBP1), as well as phosphorylation of PERK and proteolytic cleavage of ATF6. These events lead to expression of ER stress induced genes such as DDIT3, ERDJ4, GRP78 and EPT1 (Walter and Ron, 2011).

Moderate levels of ER stress have been shown to protect cancer cells and increase their capacity to survive in nutrient deprived environments, mainly through XBP1 promoted expression of hypoxia driven gene signatures (Chen et al., 2014). ER stress has also been shown to negatively impact the anti-tumor immune response in T cells and DCs (Cubillos-

Ruiz et al., 2015; Song et al., 2018). Meaning that moderate levels of ER stress lead to increased survival of cancer cells and a reduced anti-tumor immune response. However, high levels of ER stress have been shown to induce cell death (Maurel et al., 2015). The apoptosis-inducing potency of high ER stress can be exploited to target and kill tumor cells (Fribley et al., 2004). A search for additional potent ER stress inducers that are effective against cancers is therefore lucrative to identify therapies.

1.3 TRIM28/KAP1/TIF1β

The tripartite-motif is a superfamily of proteins known as TRIMs that can be found in all metazoan animals (Ozato et al., 2008). The TRIM family contains 75 proteins (Versteeg et al., 2013) in humans and is characterized by the presence of the tripartite-motif that contains a RING domain, one or two B-box domains and a coiled coil domain, also referred to as the RBCC domain (Ozato et al., 2008). TRIM28, also known as KRAB domain-associated protein 1 (KAP1) or transcriptional intermediary factor 1 β (TIF1β), belong to a subclass of TRIMs together with TRIM24 (TIF1α), TRIM33 (TIF1γ) and TRIM66 (TIF1δ). This subclass of TRIMs is characterized by their specific C-terminal motif that contains a PHD-Bromodomain (PB) (Peng et al., 2002). BRD containing proteins are classified as epigenetic readers that typically bind to acetylated lysine on histones (Dhalluin et al., 1999; Haynes et al., 1992). BRDs represent a family of epigenetic proteins that have emerged as lucrative drug targets in cancer (Filippakopoulos and Knapp, 2014; Fujisawa and Filippakopoulos, 2017). We identified TRIM28 as a repressed gene in immune cells from patients with pSS (data not shown) and subsequently identified that TRIM28 plays a major role in malignant melanoma.

1.3.1 Immune regulation by TRIM28

It has been suggested that TRIM28 modulates innate immune signaling responses by interacting with multiple transcription factors involved in immune system regulation. One such report suggests that TRIM28 act as a negative regulator on IRF7 to modulate the type IFN and antiviral response (Liang et al., 2011), another study discovered that TRIM28 bind and interact in a similar fashion with IRF5 (Eames et al., 2012). TRIM28 has also been reported to interact with members of the STAT transcription factor family.

In a screen of TRIM28-STAT interactions it was reported that TRIM28 binds with high affinity to STAT3 but also with lower affinity to STAT1. The same study showed that siRNA knockdown (KD) of TRIM28 potentiated the effect STAT3 mediated IL-6 stimulation of human cells in vitro (Tsuruma et al., 2008). STAT1 association to TRIM28 has also been reported in another study performed in human cells in vitro where KD of TRIM28 again potentiated the effect of type I IFN signaling (Kamitani et al., 2008). Interestingly it has been reported that closely related proteins TRIM24 and TRIM33 are associated with immune phenotypes related to the type I IFN system in mouse models (Ferri et al., 2015; Tisserand et al., 2011). Taken together, these reports indicate a role for TRIM28 in regulating innate immune functions. We have observed a reduction of TRIM28 expression in both patients with systemic autoimmune diseases, and in IFNβ treated multiple sclerosis patients. Therefore, the regulatory system involving type I IFN and TRIM28 requires further investigation.

1.3.2 TRIM28 in development and differentiation

TRIM28 is a critical regulator of development and differentiation, however TRIM28 does not interact directly to DNA but through interaction with DNA-binding KRAB-domain containing zinc finger (ZNF) transcription factors as shown in 1996 by several independent reports (Friedman et al., 1996; Kim et al., 1996; Moosmann et al., 1996). Strong evidence for the critical role of TRIM28 in regulation of development was shown in 2000 by Cammas and Losson when they established that mice deficient in TRIM28 did not undergo gastrulation and that no surviving embryos was found at E8.5 (Cammas et al., 2000). Further evidence of TRIM28 importance in development comes from conditional knockout (cKO) mouse models where it has been shown that inactivation of Trim28 in the forebrain causes heightened levels of anxiety and alterations in learning (Jakobsson et al., 2008). It has also been shown that TRIM28 controls erythropoiesis by inactivating Trim28 in hematopoietic cells of adult mice and observing severe anemia (Hosoya et al., 2013). Studies of TRIM28 have also demonstrated a function of maintaining pluripotency of embryonic stem cells (ESCs) as well as being required for ESCs differentiation (Cheng et al., 2014; Rowe et al., 2013; Seki et al., 2010). It is clear that TRIM28 plays a very important role in cell differentiation and proliferation and affects many aspects of cellular physiology and although exact mechanisms of how TRIM28 influences these functions is unclear there have been numerous findings over the past decade giving insight in to the functions of TRIM28. In the coming sections I will summarize some of the most important findings regarding the functions of TRIM28 that emphasizes how this protein is able to influence such wide range of cellular processes.

1.3.3 TRIM28 as an epigenetic regulator

Histone modifications and chromatin remodeling

One of the most well described mechanisms of TRIM28 is the coordinated interaction with SET domain Bifurcated 1 (SETDB1) which is a methyltransferase that specifically trimethylates (me3) Lys-9 of histone H3 (H3K9). H3K9me3 functions as a tag to recruit the binding of HP-1 which in turns acts as epigenetic transcriptional repressor thereby silencing euchromatin regions (Schultz et al., 2002). The recruited HP-1 also binds directly to a HP-1 binding domain on TRIM28 to form a highly effective euchromatin transcriptional repressive complex (Iyengar et al., 2011; Sripathy et al., 2006). In addition to trimethylating H3K9, the PB domain of TRIM28 can also interact with Mi2α that can be found in the nucleosome remodeling and histone deacetylation (NuRD) complex that effectively recruits histone deacetylases (HDACs) (Schultz et al., 2002; Schultz et al., 2001). Acetylated histones are a mark of euchromatin and active transcription, HDACs effectively remove the charged acetyl group of the histone and heterochromatin is eventually formed.

DNA methylation

In addition to histone modifications and chromatin remodeling TRIM28 has also been reported to be associated with genomic imprinting of DNA during embryogenesis by controlling DNA methylation. Studies have shown that TRIM28, through KRAB-ZNF recruitment, is able to induce cytosine methylation in murine ESCs. Interestingly, the same study shows that KRAB/TRIM28 methylation does not occur in differentiated cells but is established during early embryogenesis and maintained through development (Quenneville et al., 2012). Other studies have shown that TRIM28 interacts through specific KRAB/ZFP, ZFP57, with the DNA methyltransferase (DNMT) recruiting protein NP95 to maintain genomic imprinting (Zuo et al., 2012). However, it is unclear what role TRIM28 plays in recruitment of DNMTs and since histone lysine methylations are involved in this process it will require further investigation to determine exactly how TRIM28 contributes to genomic imprinting.

Transcriptional elongation

One of the most important processes to affect gene expression is the control of transcription by the RNA polymerase II (RNAPII) complex. Classically there are three stages of transcription by RNAPII: initiation, elongation and termination. RNAPII promoter-proximal pausing is a mechanism where RNAPII is already recruited to the transcription start site (TSS) but remains paused in activity before initiating processive elongation (Bunch et al., 2014). This mechanism enables RNAPII being able to be released and activated with unprecedented rate upon an activating signal. Investigation in to the regulatory mechanisms of RNAPII-pausing has identified a number of important factors of the RNAPII complex including NELF (negative elongation factor), POLR2M (DNA directed RNA polymerase II subunit), MYC and P-TEFb (positive transcription elongation factor)/CDK9 (Bunch and Calderwood, 2015). Intriguingly, a role for TRIM28 in control of RNAPII-pausing has been uncovered by screening for proteins bound to the pause site of the human gene HSPA1B in vitro (Bunch et al., 2014). Other researchers have shown that TRIM28 is crucial in recruiting 7SK small nuclear ribonucleoprotein complex and delivering inactive P-TEFb kinase to RNAPII-paused promoters that are needed for RNAPII activation and elongation (McNamara et al., 2016). While TRIM28 seem to play a role in RNAPII-pausing, contradictory reports lead to questions on the exact function TRIM28 exerts on RNAPII-pausing.

Repression of endogenous retroviruses

Around 8-10% of the human and mouse genome consists of mobile genetic elements knows as endogenous retroviruses (ERVs) which are retroviruses that have been integrated in to germline cells of the infected host and are passed on to the offspring. Many ERVs contains sequences that can act as regulatory element and thus altering gene expression of the host genome (Jern and Coffin, 2008). TRIM28 has been shown to play an important role in silencing ERV expression during embryogenesis by inducing repressive histone marks as previously described. Recent research has demonstrated that ERV repression mediated by histone modifications by TRIM28 in murine neuronal progenitor cells (NPCs) effectively leads to altered transcriptional dynamics (Fasching et al., 2015). The report also showed that

depletion of TRIM28 in ESCs causes activation of gene promoters nearby ERVs. The repression of ERVs by TRIM28 is effectively altering the expression dynamics of nearby genes and long noncoding RNAs (lncRNAs), which illustrates both the importance of ERVs in transcriptional regulation of embryogenesis but also the multitude of effects that TRIM28 plays in development.

1.3.4 TRIM28 in cancer

With their broad range of cellular functions, it is not surprising that many TRIM proteins are associated to cancer progression. Some TRIMs, like TRIM19, are even involved directly to carcinogenesis by translocating to other genes generating fusion proteins that drive tumor progression (Cambiaghi et al., 2012).

TRIM28 is associated to various forms of cancer

TRIM28 is highly expressed in many forms of cancer and in many cases associated with more severe disease. Reports have linked high TRIM28 levels to poor outcome in both gastric and thyroid cancer (Martins et al., 2013; Yokoe et al., 2010) as well as being associated to aggressive forms of ovarian cancer and metastatic cervical cancer (Cui et al., 2014; Lin et al., 2013). TRIM28 has also been shown to promote cell growth and proliferation of breast cancer (Addison et al., 2015). Adding to previous reports that TRIM28 mediates degradation of p53 through E3 ligase activity (Doyle et al., 2010; Wang et al., 2005), recent reports have identified TRIM28 as an E3 ligase for ubiquitination of AMP activated proteinase kinase (AMPK) (Pineda and Potts, 2015). The complex formation is stabilized by MAGE proteins and subsequently leads to proteasome degradation of AMPK. TRIM28 mediated degradation of AMPK was shown to regulate mTOR signaling as well as autophagy. Interestingly autophagy can also be induced by endoplasmic reticulum (ER) stress (Lee et al., 2015) and it has been shown that certain cancer drugs such as bortezomib and nelfinavir induce ER stress (Mathur et al., 2014; Takenokuchi et al., 2015). This raises the question whether or not TRIM28 mediated regulation of autophagy plays any role in sensitivity to such drugs. We have identified TRIM28 as highly expressed and associated to poor outcome in malignant melanoma. Taken together with previous reports of TRIM28 in tumor biology, it becomes clear that research is needed to determine the role that TRIM28 plays in melanoma progression and to evaluate its potential as a drug target for novel cancer therapies.

2 AIMS

The objective of the work performed in this thesis was to understand how type I IFNs are able to contribute to such a wide range of biological processes and to investigate metastatic progression of malignant melanoma.

2.1 SPECIFIC AIMS

Project I: To identify miRNAs regulated by type I IFNs and elucidate their role in immune cells.

Project II: To get new insights into IFNAR1 signaling by interactome analysis.

Project III: To identify TLR agonists capable of inducing ER stress in melanoma cells.

Project IV: To understand the role of BRDs in metastatic progression and plasticity of malignant melanoma.

3 METHODOLOGICAL CONSIDERATIONS

In the following section advantages and limitations of the methods used in project I-IV will be presented. For more detailed description of the methods used, please refer to the methods sections of each project.

3.1 METABOLIC MEASUREMENTS IN HUMAN CD4+ T CELLS

In project I, we first analyzed public RNA-seq data from mouse *miR-31*$^{-/-}$ CD8+ T cells and identified an altered expression of metabolic gene signatures. To determine the role miR-31 plays in metabolism of human CD4+ T cells we performed metabolic measurements using a Seahorse XFe24 Extracellular Flux Analyzer. The measurements were performed on human CD4+ T cells transfected with a miR-31-5p inhibitor or a negative control inhibitor. Measurements were also performed on untransfected CD4+ T cells stimulated with IFNα. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured in all experiments and normalized to cell count. All cells were activated using anti-CD3/anti-C28 beads and supplemented with IL-2 for the duration of the experiments.

The Seahorse XFe24 is well established for T cell work and is efficient in collecting data from multiple measurements from each well. However, the protocol used for metabolic measurements can be optimized for either OCR or ECAR quantification. In our setup we collected data from both OCR and ECAR measured simultaneously in each well using published protocols for OCR (Wu et al., 2016). The experiment therefor lacked optimal conditions for ECAR quantification. A limitation when performing Seahorse measurements is the large number of cells needed for each well and the need to include at least five technical replicates. Therefore we collected as much data as possible from each experiment with less consideration for optimized conditions. An alternative method to measure metabolic activity is to use an Oroboros oxygraphy. However, it restrict users by requiring even more cells per measurement compared to a Seahorse and is less established for us of primary cells. Less sensitive colorimetric assays to measure glycolysis may also be used.

3.2 PROXIMITY PROTEOMICS USING BIOTIN LIGASES

In project II and IV we aimed to identify the interactomes and IFNAR1 and TRIM28 respectively. The most commonly used method to identify protein-protein interactions is co-immunoprecipitation (Co-IP). When performing Co-IP, a cell lysate is prepared and often crosslinked before the addition of an antibody targeting your protein of interest. Subsequently, the antibodies bound to the protein of interest and its crosslinked interactors are then pulled down and washed for further analysis. In project II and IV we chose to use an antibody-free proximity labeling method using biotin ligases. To perform biotin ligase proximity labeling we cloned the cDNA sequence of IFNAR1 and TRIM28 in to retroviral expression vectors containing either the biotin ligase BioID2 or hBASU (Ramanathan et al.,

2018; Roux et al., 2012). Cells were then transduced with the fusion protein expression vector and selected using puromycin. To perform the proximity labeling, biotin was added to the culture medium prior to cell lysis and streptavidin-biotin pulldown. Stringent washing was followed by analysis using LC-MS.

All labeling of interacting protein by biotin ligases occurs in living cells, the protein being studied leaves a trace throughout the cell of biotin labeled interactors. This allows you to perform dynamic mapping of a full protein interactome compared to Co-IP which will only give you a snapshot of protein interactions. Some interactions identified by biotin ligases might only occur transiently and those will not be identified by Co-IP. To capture a large protein complex when performing Co-IP, it is often critical to crosslink cells prior to lysis. However, these protein complexes are very fragile and might be disrupted during antibody pulldown and subsequent washing steps. When using biotin ligases, we can determine the labeling radius of the ligase by introducing a linker of varying size, this allows the ligase to tag interactors of varying degree of proximity to the fusion protein. Once tagged all biotinylated proteins are bound using streptavidin-coated beads. Because the affinity between streptavidin and biotin is so high it allows for very stringent washing procedures minimizing any background proteins that may be bound to the beads. When performing Co-IP, you are relying on to the antibody specificity and affinity to its target to reduce background which is a major contributor to background noise.

Finally, a major advantage using biotin ligases over Co-IP is the reduced impact of cellular localization to determine the interactome. For example, membrane bound proteins can be hard to extract using regular cell lysis methods and therefore performing Co-IP on such proteins represents a major challenge. Using biotin ligases, we were able to tag membrane bound receptor IFNAR1 with either BioID or hBASU and successfully identify its intracellular interactors.

Using biotin ligase also comes with certain limitations compared to previous established methods. First and foremost a fusion protein is generated by adding a ligase of 233 aa in the case of BioID2 or 260 aa in the case of hBASU. It is possible that this fusion protein is limited in functions compared to the endogenous protein and it is therefore important to test functionality of the fusion protein where possible, and to make sure that the cellular localization is not altered. While biotin ligase labeling presents major advantages to identify large set of interactors it might be harder to distinguish between direct and transient protein interactions. Stable isotope labeling in cell culture (SILAC) was introduced to the culture medium prior to the experiments to label the protein fractions with either heavy or light isotopes of Lysine and Arginine. This allowed for quantification of the labeled protein fractions and to distinguish between common and rare interactions.

3.3 MEASURING ER STRESS

In project III we screened a panel of TLR and NOD agonists to identify those able to induce ER stress in melanoma cells. To identify ER stress induced agonists, we used unconventional splicing of XBP1 mRNA as a marker for ER stress. During ER stress, 26 nucleotides (nt) are excised from XBP1 by activated IRE1. Spliced XBP1s can be discriminated from unspliced XBP1u by PCR on cDNA. Gel separation of the PCR product generates two bands with a 26nt size difference, XBP1s and XBP1u. While PCR on XPB1 cDNA is an efficient method for screening purposes it does not allow for precise quantification of ER stress.

An alternative method to identify ER stress is to quantify gene expression of ER stress target genes by qPCR. In project III we use *CHOP*, *ERDJ5* and *BIP* as target genes for ER stress quantification. Other genes containing ER stress response elements or unfolded protein response elements in their promoter regions may also be used (Alger et al., 2011). As an alternative strategy to qPCR, induced ER target genes can be confirmed at the protein level by immunoblotting. Immunoblotting can also be used to detect phospho-eIF2a or XPB1s.

3.4 GENOME WIDE CRISPR/CAS9 SCREENING

As part of project III, we used a genome-wide CRISPR/Cas9 screen to try to identify the mechanisms whereby IMQ induces ER stress in cancer cells. We used A375 melanoma cells that lack expression of TLR7/TLR8 to identify an unknown IMQ receptor.

To circumvent issues with fading Cas9 expression levels when using lentiviral vectors we first generated Cas9 expressing A375 cells using transposon integration. Stable Cas9 expression was confirmed prior to lentiviral transduction of a genome-wide gRNA library (Sanjana et al., 2014). IMQ was added to the cells in an attempt to identify surviving clones of cells, surviving cells would be sequenced to identify any genes critical for IMQ actions. No surviving clones of cells were obtained, likely due to rapid shift in pH of the culturing medium caused by addition of IMQ. To circumvent the limitations of using IMQ in cell culture we planned to use the fluorescent properties to IMQ to perform a FACS based screen. Clones with lower IMQ intensity would represent a fraction of cells with reduced levels of IMQ receptors.

3.5 GENE PERTURBATION WITH SHRNA AND CRISPR/CAS9

In project IV we aimed to introduce genetic perturbations to examine the effect of TRIM28 on melanoma cells. We decided to mainly use short hairpin RNA (shRNA) mediated KD. Using shRNA packed in to lentiviruses allows for efficient KD in cells generated in as little as 7 days post transduction, limiting any deviation to the parental cell line introduced by selection pressure. shRNAs are easily used in a different cell lines and allows for a comparison of KD effects in a multitude of cell lines efficiently. shRNAs also provide partial

loss-of function, similar to the effects of pharmaceutical inhibition. KO leads to complete loss-of function, which is hard to mimic using inhibitors and might not be suitable for studies to identify novel drug targets.

The major limitations using shRNA KD is the possibilities of non-specific RNA degradation leading to unwanted off-target effect. To circumvent this, we used at least two different shRNA constructs to study the effect of TRIM28 KD. Alternative gene perturbation methods used in this project include CRISPR/Cas9 KO in A375 cells. KO clones were generated using lentiCRISPR_V2 (Sanjana et al., 2014) and single-cell sorted. KO were confirmed by immunoblotting. These cells were used to confirm key findings from shRNA experiments. However, by single-cell sorting cancer cells and expanding a single clone we risk introducing clonal differences compared to the parental cell line. In an attempt to avoid this, we performed CRISPR interference (Thakore et al., 2015) targeting TRIM28. We were not able to identify gRNAs with targeting efficiency comparable to the shRNA constructs used for TRIM28.

3.6 CANCER MODELS

To study the effect of TRIM28 on tumor growth *in vivo* in project IV we decided to use xenograft engraftment models. Immuno-compromised mice are engrafted with tumor cells of human origin most commonly through either *subcutaneous* or *intravenous* injection. Xenografts are widely used due to efficient engraftment of human cancer cells that can be treated with inhibitors or genetically modified prior to injections. Initial experiments were performed using a syngeneic mouse model. *Trim28* KD B16.F10 were injected *subcutaneously* in to C57BL/6J mice and tumor size was measured. We proceeded to focus our efforts on xenograft models.

To assess the effect of TRIM28 on both primary tumor growth and lung colonization we performed both *subcutaneous* and *intravenous* engraftments using *TRIM28* KD cells. These experiments were complemented *in vitro* with a Matrigel transwell migration assay to examine the effect of TRIM28 on the migratory capacity of melanoma cell. With these three models we were able to study primary growth, colonization and migration of tumor cells upon *TRIM28* KD.

However, to study the role of TRIM28 in metastatic progression, a model is required where a primary tumor disseminates in to metastatic tumor growth. A model to study this is the tamoxifen induced BRafCA, PtenloxP, Tyr::CreERT2 (Dankort et al., 2009) crossed with *Trim28* KO mice. A limitation is the timeframe of the extensive breeding required to succeed.

Experiments to assess the clinical relevance of targeting TRIM28 in metastatic tumors would ideally be performed on established tumors. It is possible to use inducible shRNA constructs that would be induced when metastatic tumors are fully formed. This approach mimics

clinical settings where pharmaceutical intervention usual occurs after the formation of metastases.

3.7 RNAPII-PAUSING BY CHIP-QPCR

Proximity proteomics in melanoma cells identified multiple TRIM28 interactors that together form the RNAPII-pause complex. Previous reports have linked TRIM28 to RNAPII-pausing (Bunch et al., 2014; McNamara et al., 2016), however it remains unclear which role TRIM28 plays in RNAPII-pausing. To study the role of TRIM28 in RNAPII-pausing in project IV, ChIP-qPCR was performed targeting RNAPII in A375 cells transduced with shSCRAMBLED or shTRIM28 lentiviruses. A375 cells were starved for 48 hours using serum free DMEM. Cells were then collected without or 30 minutes after addition of 10% serum followed by lysis and ChIP against RNAPII.

By starving the cells of serum, RNAPII is accumulated at the TSS of RNAPII-pause regulated genes. The addition of serum then releases the complex and activates transcription. A pausing index was calculated by first performing qPCR targeting the TSS and gene body of RNAPII-pause regulated genes to quantify the occupancy of RNAPII. The abundance of RNAPII at the TSS was then divided by the abundance in the gene body (pause index). This method allows for measurement of RNAPII location along the gene, more RNAPII in the gene body indicates more transcription and less RNAPII-pausing. However, to accurately quantify transcribing RNAPII these experiments can be complemented by performing ChIP against phosphorylated RNAPII (pRNAPII).

These methods are very efficient when studying selected genes in a multitude of cell conditions. For global gene analysis of RNAPII pausing it is possible to use global run-on sequencing (GRO-seq). In GRO-seq, the addition of sarkosyl inhibits the recruitment of additional RNAPII to TSSs, and the addition of labeled nucleotides (BrUTP) enables nascent RNA to be isolated using anti-BrdU antibodies. GRO-seq therefore allows for identification of recently transcribed RNA and makes it possible to map the position of transcriptionally engaged RNAPII (Core et al., 2008). By using GRO-seq in combination with inhibitors of RNAPII transcription initiation or elongation, it is possible to track the activity of RNAPII along the genome (Jonkers et al., 2014). Performing GRO-seq on cells with *TRIM28* KD would have allowed for a global view of the effect of TRIM28 on RNAPII activity.

4 RESULTS AND DISCUSSION

4.1 PROJECT I: MIR-31 REGULATES CD4+ T CELL METABOLISM

Systemic autoimmune diseases are characterized by the upregulation of a large set of type I IFN regulated genes. It is still poorly understood how these genes contribute to autoimmune diseases such as pSS. In project I, we aimed to identify miRNAs regulated by type I IFNs and explore their role in autoimmunity. To identify miRNAs regulated by acute type I IFN treatment we used blood samples collected from patients with multiple sclerosis (MS) before and 18 hours after treatment with IFNβ (Avonex). By isolating major immune cell populations and performing gene expression analysis, we identified a T cell specific reduction of miR-31-5p and a monocyte specific reduction of miR-150-5p. Using IFNβ treated MS patients is a unique opportunity to study the type I IFN system in humans. It allowed us to study systemic regulation of miRNA *in vivo* to identify relevant miRNAs. To verify our findings, we treated blood samples from healthy donors with IFNβ and analyzed expression of miR-31-5p and miR-150-5p. However, only miR-31-5p was verified. A limitation of studying systemic regulation is that we were not able exclude that the monocyte specific reduction of miR-150-5p was caused by cell interactions extrinsic to monocytes. Due to the fast rate of IFNβ action, a shorter time interval than 18 hours might have proved more optimal to study direct regulation by IFNβ on specific cell types. Furthermore, we verified miR-31-5p reduction in T cells chronically exposed to IFNβ by collecting samples from patients with pSS and comparing to healthy controls. We concluded that miR-31-5p is reduced in T cells both after acute and chronic IFNβ exposure.

To further understand what role miR-31-5p plays in modulating T cell activity, we explored publicly available data sets. Interestingly, analysis of gene expression data of T cells from miR-31$^{-/-}$ animals (GSE98615) identified altered expression of genes involved in energy metabolism, suggesting a role for miR-31-5p in metabolism. Previous studies have reported increased glycolysis in T cells from SLE patients (Yin et al., 2015). We hypothesized that miR-31-5p regulates metabolism in human T cells.

To test our hypothesis, we transfected human T cells with a miR-31-5p inhibitor or a control inhibitor and measured metabolism. Indeed, we identified increased glycolysis in T cells with reduced miR-31-5p levels. Our data show that miR-31-5p negatively regulates metabolism in T cells. We can hypothesize that the reduced levels of miR-31-p caused by type IFNs in patients with autoimmune disease is driving increased metabolic rates. We also observed that addition of IFNα to CD4+ T cells increased the energy metabolism, a mechanism we hypothesize to be regulated via miR-31-5p reduction. However, the cells were only treated with IFNα for 24 hours prior to analysis and exhibited limited increases in glycolysis. In this project we were able to show that miR-31-5p regulated metabolism in CD4+ T cells but we can only hypothesize what role miR-31-5p plays in the type I IFN induced metabolic increases. Metabolism plays a major role in modulating T cells in immunity, it is possible that reduced levels of miR-31-5p contribute to autoimmunity by making autoreactive T cells less prone to exhaustion.

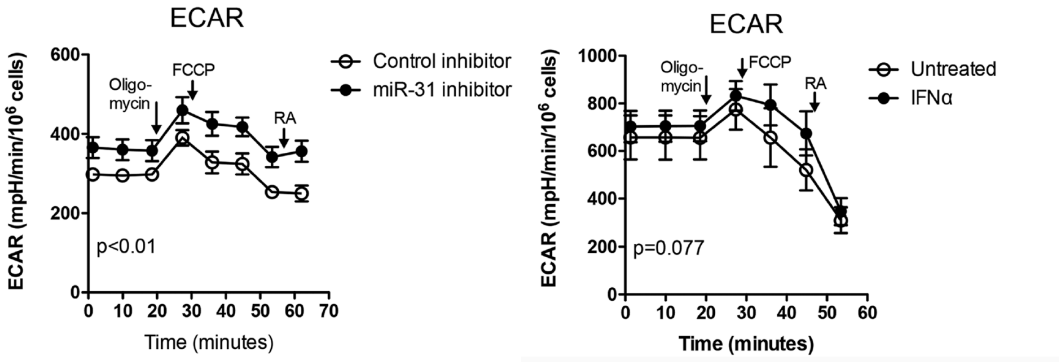

Figure 1. ECAR measured in CD4+ T cells upon miR-31 inhibition (left) and 24 hours of IFNα stimulation (right).

4.2 PROJECT II: REVEALING IFNAR1 PROTEIN INTERACTIONS

In project II we aimed to develop techniques to identify protein interactions of a transmembrane protein. Recent development in of proximity labeling techniques using bacterial biotin ligase (BirA) allows for protein labeling in living cells without the need to extract the protein of interest from the cell lysate, circumventing issues with solubilization of transmembrane proteins. In project II we used multiple variants of BirA to reveal protein interactions of the transmembrane receptor IFNAR1.

Two different BirA ligases were used, modified from *Aquifex aeolicus* (BioID2) and human codon optimized *Bacillus subtilis* BirA (hBASU). We synthesized the two enzyme and compared their kinetics. hBASU showed significant labeling already after 1 minute of biotin exposure while no labeling was observed by BioID2. The standard range of labeling times for BioID2 is 16-24 hours (Roux et al., 2013), signifying a huge improvement in kinetics for the hBASU. The faster kinetics can be utilized to identify the rapid protein-protein interactions occurring downstream of IFNAR1 after IFNβ stimulation. The extended biotin exposure when using BioID2 also lead to a much larger portion of labeled proteins that can be classified as background, these are mainly protein interactions occurring during protein synthesis in the ribosome.

We then cloned the cDNA sequence of IFNAR1 in to both BioID2 and hBASU vectors and performed quantitative mass spectrometry on labeled proteins. As expected the interactome using hBASU labeling was much smaller than that of BioID2, mainly due to the shorter incubation time with biotin. However, the two data sets generated an overlapping (>90%) set of interactors validating the two methods. In addition, we identified a number of IFN-stimulated proteins (IFIT1, HERC5 and EIF2AK2) as interactors after stimulation with IFNβ further validating the system. Rather surprisingly we did not identify some of the known key interactors of IFNAR1 such as JAK1, TYK2 and IFNAR2. This could be explained by the design of the IFNAR1-BirA fusion protein, where we introduced a flexible linker between the proteins. It is possible that by doing so the BirA is out of range of the closest interactors. It is also possible that steric hindrance prohibits the enzyme to reach the closes interactors.

Interestingly, among the interactors we identified was a large number of nuclear proteins. This indicates that IFNAR1 translocates to the nucleus following stimulation, a claim that is supported by previous reports (Lubick et al., 2015; Subramaniam and Johnson, 2004). Many cell surface receptors are known to translocate to the nucleus suggesting that this might occur also during type I IFN signaling.

In project II, we present methods to identify interactomes of challenging transmembrane proteins. Using these techniques, we successfully presented interactome data for IFNAR1.

4.3 PROJECT III: IMQ INDUCES ER STRESS IN MELANOMA CELLS

Moderate levels of ER stress have been shown to protect cancer cells from hypoxia. However, high levels of ER stress induce apoptosis of cancer cells and potentially provides an alternative route to target and kill tumors (Maurel et al., 2015). In project III, we screened a number of TLR and NOD agonists in melanoma cells to identify potent inducers of ER stress. TLR agonists have previously been shown to induce ER stress (Martinon et al., 2010). We used splicing of XBP1 to indicate ER stress and identified imiquimod (IMQ) as a potent ER stress inducer. We confirmed the findings by gene expression analysis of ER stress target genes.

IMQ is known to induce type I IFNs via TLR7/TLR8 activation. However, melanoma cells do not express neither TLR7 or TLR8 indicating an alternative ER stress inducing mechanism. IMQ belongs to a group of small purine analogs referred to as imidazoquinolines. When studying properties of other purine containing molecules we verified that ATP is a potent inducer of cytosolic Ca^{2+} (Bandyopadhyay et al., 2000). Since depletion of Ca^{2+} from ER stores is known to induce ER stress (Mekahli et al., 2011), we hypothesized that IMQ induced ER stress through release of ER Ca^{2+}. We measured Ca^{2+} influx by Fluo-3-AM. IMQ treatment of melanoma cells did indeed induce a rapid increase in cytosolic Ca^{2+}. Ionomycin was used as a positive control. We tested imidazoquinolines RSQ and CL075 but none of them induced influx of Ca^{2+}, further validating that IMQ act through a separate mechanism to TLR7. We also found that buffering extracellular Ca^{2+}, or blocking calcium release activated channel (CRAC) Ca^{2+} channels, still led to release of ER Ca^{2+} stores and ER stress after IMQ treatment. It has long been believed that the anti-cancer effects of IMQ are driven by TLR-activation of tumor infiltrating immune cells, leading to production of IFNs and increased anti-tumor effects (Schon and Schon, 2007). While this might still be the case, our data suggest that IMQ also acts directly on cancer cells independent of immune cell activation. At this stage we concluded that IMQ triggers ER stress via release of Ca^{2+} from ER stores independently of TLR7 and TLR8.

We hypothesized that IMQ bound an unknown cell surface receptor which induced release of ER Ca^{2+} and a secondary cytosolic Ca^{2+} influx. Since IMQ is a purine analog, we further hypothesized that it would bind a G protein-coupled receptor (GPCR). Similar to purines. By identifying the receptor, we aimed to possibly of design more specific and potent receptor

ligands that could potentially be used to target and kill melanoma cells. We decided to attempt genome-wide CRISPR/Cas9 screening using the GeCKO library to identify the receptor (Sanjana et al., 2014). Using transposon systems, we generated Cas9 expressing A375 cells and validated stable expression. We then introduced a genome-wide gRNA library and treated cells with IMQ, untreated cells were used as control. Surviving cells are sequenced by NGS and the gRNA work as a barcode to identify the perturbated gene underlying survival. We did not succeed in identifying surviving cell clones. During the course of the experiments we observed a rapid shift in pH of the culture medium causing all cells to die. The pH change occurred 4-5 days after addition of IMQ. We were not able to circumvent this issue. Instead, we devised a strategy to use the fluorescent properties of IMQ perform a FACS based screen. Unstained cells upon addition of IMQ and transduction of the gRNA library would represent a population of cell deficient in the IMQ binding receptor.

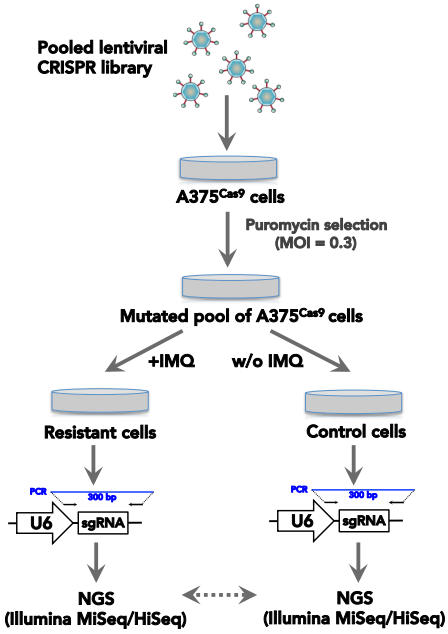

Figure 2. Overview of the study design to identify an unknown IMQ receptor by genome wide-CRISPR/Cas9 screening. The genome-wide library of gRNAs are added to Cas9-expressing A375 cells. Resistant cells are then sequenced and compared to control cells.

In conclusion, we have identified that small molecule IMQ is a potent inducer of ER stress in melanoma cells through release of Ca^{2+}, independently of TL7 and TLR8. Our data suggest that IMQ binds an unknown cell surface receptor that potentially could be targeted for therapeutic purposes.

4.4 PROJECT IV: TRIM28 DRIVES A PHENOTYPIC SWITCH IN MELANOMA BY CONTROLING JUNB EXPRESSION

During metastatic progression in malignant melanoma, unlike epithelial cancers such as breast cancer, cells typically do not undergo canonical EMT. It has become apparent that melanoma cells are prone to phenotype switching, and the plasticity is seemingly not driven

by mutations (Brinckerhoff, 2017; Cheli et al., 2014; Croteau et al., 2013; Falletta et al., 2017; Hoek et al., 2008; Kim et al., 2017). We hypothesized that metastatic progression in malignant melanoma is driven by transcriptional regulation of a phenotype switch. Therefore, we set out to identify which transcriptional regulators were controlling this switch and the underlying mechanisms.

In an attempt to identify genes associated to metastatic progression of melanoma, we analyzed RNA-seq data from 367 metastatic melanoma patients available from The Cancer Genome Atlas (TCGA). By performing Principle Component Analysis (PCA) and partitioning around medoids clustering we identified two clusters of patients, cluster 1 (C1) and cluster 2 (C2). Survival analysis of these patient clusters revealed that patients in C2 had significantly shorter overall survival. We identified a strong repression of BRDs in cluster C2. Interestingly, four BRDs were instead significantly upregulated in C2 compared to C1; *BRPF1*, *BRD4*, *SMARCA4* and *TRIM28*. We were intrigued by the BRD signature since BRDs are epigenetic regulators that are highly involved in cancers (Andrieu et al., 2016; Fujisawa and Filippakopoulos, 2017; Jain and Barton, 2017) and comprise a family of proteins that can be targeted therapeutically (Filippakopoulos et al., 2010). Amongst the four upregulated genes *TRIM28* was the most upregulated in C2. Survival analysis based on *TRIM28* high or low expression showed significantly shorter survival for stage III melanoma patients with high *TRIM28* expression. Previous studies have highlighted the importance of TRIM28 multiple cancer forms (Fong et al., 2018; Li et al., 2017; Wei et al., 2016). At this stage we hypothesized that TRIM28 was the discriminating factor of C2 and furthermore was a driving factor for metastatic progression of disease.

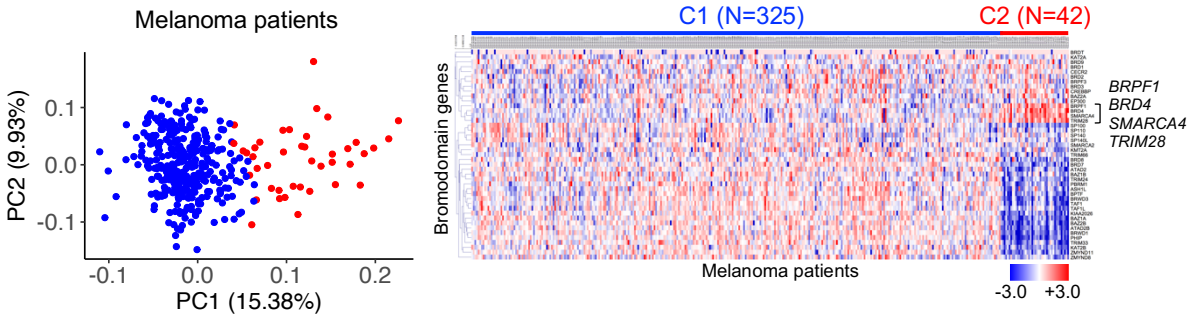

Figure 3. PCA plot of 367 melanoma patients showing C1 in blue and C2 in red (left). Heatmap of BRD genes showing BRD signature in C2 (right).

To test this hypothesis *TRIM28* KD cells were generated. We identified a repressed metastatic gene signature (Winnepenninckx) in *TRIM28* KD cells as well as a repressed YAP1 signature. YAP1 is a part of Hippo signaling that control cell homeostasis and organ growth during development (Piccolo et al., 2014). Previous reports link YAP1 to the development of melanoma metastases (Lamar et al., 2012; Nallet-Staub et al., 2014) and taken together with the reduced metastatic signature we hypothesized that TRIM28 drives expression of important metastatic gene signatures. By performing *intravenous* xenograft engraftments in to BALBc nu/nu animals we identified that *TRIM28* KD cells colonized significantly less in the lung compared to NTC cells. We also observed that *TRIM28* KD cells

were significantly less invasive *in vitro* compared to control cells. We were able to show that TRIM28 regulates expression of metastatic gene signatures and that TRIM28 is necessary for invasiveness and colonization of melanoma cells.

Interestingly, we also observed increased MAPK signatures in *TRIM28* KD cells, independent of ERK1/2 phosphorylation. Amongst the genes upregulated were *CXCL8* and *CXCL1*. We were surprised because MAPK signaling and *CXCL8* expression is associated to increase tumor growth (Liu et al., 2016a). No increase in pERK1/2 was observed, based on these results we hypothesized that regulation of MAPK signature genes occurred at transcriptional level. Upon *subcutaneous* xenograft engraftments, we observed significantly increased tumor growth in *TRIM28* KD cells. This data was in concurrence with the MAPK data. These results were surprising since TRIM28 reduction has been associated to reduced tumor growth in breast cancer and prostate cancer (Fong et al., 2018; Li et al., 2017; Wei et al., 2016). We hypothesize that the increased tumor growth is driven by pro-tumorigenic factors such as *CXCL8*. It is possible that epithelial cancers respond differently to *CXCL8* or that TRIM28 acts via a mechanism specific for melanoma cells. These results further elucidate the differences between epithelial cancers and malignant melanoma.

To identify the mechanisms underlying TRIM28 regulation of MAPK and YAP1 signatures we established the TRIM28 interactome in melanoma cells using BioID proximity proteomics (Roux et al., 2012). Our initial hypothesis was that TRIM28 interacts with components of Hippo or MAPK signaling thereby altering the activity of YAP1. However, no known interactors involved in either YAP1 or MAPK signaling were identified. Instead, we identified several key components of the RNAPII-pause complex including CD9, CDK11, CDK12 and HEXIM1. Previous reports identified TRIM28 as a part of the RNAPII-pause complex, but the studies reported conflicting mechanisms (Bunch et al., 2014; McNamara et al., 2016). The study by McNamara et al. identified TRIM28 as a recruiter for the RNAPII initiation complex, while the study by Bunch et al. identified TRIM28 as a negative regulator of RNAPII transcriptional initiation. To elucidate the role of TRIM28 in RNAPII-pausing in melanoma we combined ChIP-qPCR with analysis of ChIP-seq data. Global ChIP signals of TRIM28, CDK9 and HEXIM1 overlapped to a great degree, indicating co-occupancy at TSS. By ChIP-qPCR, we confirmed TRIM28 as a negative regulator of RNAPII pausing on the *JUNB* and *FOSL1* genes.

The role and function of TRIM28 on the RNAPII-pausing complex seem similar to that of BRD4 (Patel et al., 2013). However, BRD4 is not identified as an interacting partner to TRIM28 in our BioID experiments. It is possible that TRIM28 and BRD4 are mutually exclusive in their functions and regulate separate sets of genes, both through the RNAPII-pause complex. It leads to an interesting question whether or not KD of BRD4, or inhibition by JQ-1, in melanoma cells induce a similar phenotypic switch as KD of TRIM28.

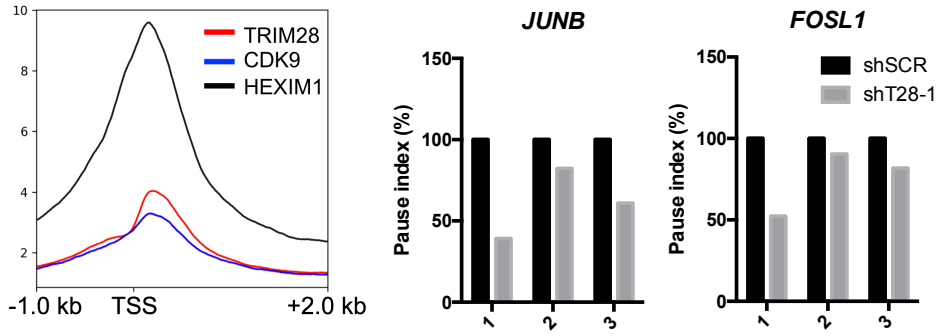

Figure 4. Overlap of ChIP-seq signals from TRIM28, CDK9 and HEXIM1 (left). Pause index on JUNB and FOSL1 in control SCRAMBLED control cells and TRIM28 KD cells (right).

FOSL1 and JunB are both part of the AP-1 transcription factor complex which acts downstream of ERK1/2 in the MAPK signaling cascade. This led us to hypothesize that increased levels of JunB and FOSL1 after TRIM28 KD explained the observed increase in expression of MAPK signature genes. In addition, FOSL1 has been to shown interact with TEAD4 and activate transcription of YAP1 target genes (Liu et al., 2016b; Zanconato et al., 2015). Since we observed decreased expression of YAP1 target genes, we instead hypothesized that JunB simultaneously drove the expression of MAPK signatures (including *CXCL8* expression) and suppressed the expression of YAP1 target genes.

We generated *JUNB* overexpressing cells and indeed they had strongly repressed expression of YAP1 target genes expression as well as increased *CXCL8* expression. In contrast, when overexpressing *FOSL1* we observed increased YAP1 target gene expression as expected but no differences in *CXCL8* expression.

In conclusion, we have demonstrated that TRIM28 is a negative regulator of RNA-pausing. TRIM28 regulates expression of *JUNB* through RNAPII-pausing and increased expression of *JUNB* in melanoma leads to a phenotype switching.

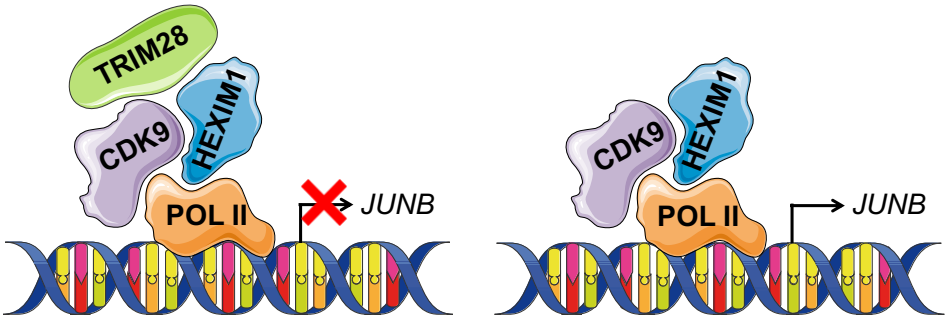

Figure 5. Proposed model of the role of TRIM28 in RNAPII pausing. TRIM28 interacts with CDK9 and HEXIM1 of the RNAPII pausing complex. KD of TRIM28 releases the pause complex and induces JUNB transcription.

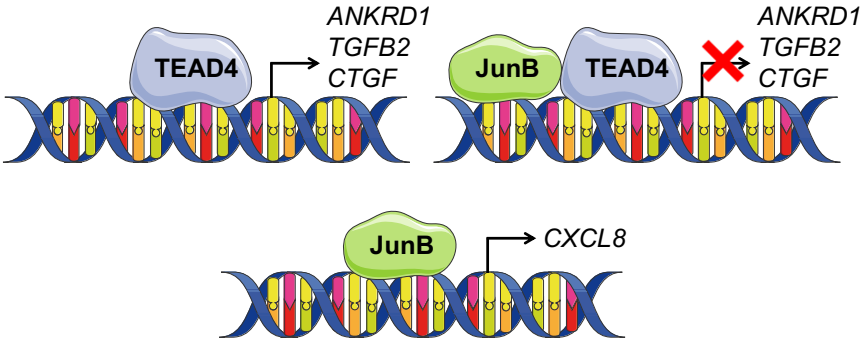

Figure 6. Proposed model by which JunB inhibits the expression of TEAD4 activated genes while activating CXCL8 expression. JunB expression leads to a phenotypic switch in melanoma cells.

5 FUTURE PERSPECTIVES

How does regulation of T cell metabolism contribute to T cell function?

After discovering suppressed levels of miR-31-5p in T cells of patients with pSS, as well as in patients treated with IFNβ, we found that miR-31-5p negatively regulates the energy metabolism of CD4+ T cells. It remains to elucidate if this mechanism contributes to autoimmune diseases and if this mechanism can be targeted in any way to reduce disease burden. This finding also suggests that reduced miR-31-5p levels during chronic type I IFN exposure serves to avoid T cell exhaustion. This could potentially be a mechanism both in systemic autoimmune diseases and chronic viral infections. Indeed, miR-31 knockout CD8+ T cells respond better to viral infections with reduced signs of exhaustion (Moffett et al., 2017).

Does IFNAR1 translocate to the nucleus and is it of functional importance?

By developing a method to identify protein interactions of transmembrane proteins we were able to generate a protein interactome for IFNAR1. By analyzing the interactome data we identified a number of unexpected nuclear proteins upon receptor activation. This suggest that IFNAR1 translocate to the nucleus upon activation. However, at this stage we lack definitive proof that nuclear translocation occurs.

If what we identified is true, the major question is whether or not nuclear translocation of IFNAR1 has functional implications to the cell. Many receptors are known to translocate to the nucleus with functional implication. It is possible that nuclear translocation of IFNAR1 signify additional features of the type I IFNA system not yet known to researchers. The role of the type I IFN system in autoimmune disease is poorly understood. IFNAR1 nuclear translocation represents an exciting question that could contribute further to our understanding of the diseases.

Can we use IMQ as a model to develop ER stress-inducing cancer therapies?

We show that IMQ trigger ER stress in cancer cells independently of TLR7 and TLR8. By doing so inducing cell death. We also show that IMQ in fact bind to the cell surface of melanoma cells despite lack of TLR7/TLR8 expression. The challenge moving forward will be to identify cell surface receptors able to induce ER stress. In doing so researchers might be able to engineer ligands targeting the specific receptor to induce cell death via ER stress. We attempted to identify the receptor by performing genome-wide perturbation screens with moderate success. It is important to ask whether or not the effects observed are caused by a single receptor or mechanism, or whether IMQ acts on multiple pathways triggering ER stress in cells. By performing smaller screens against selected targets such as cell surface receptors only, we ca reduce background in the experiments and can study exactly what we

are looking for. Increasing chances of determining the exact ER stress inducing mechanisms by IMQ.

Is TRIM28 a suitable target to treat patients with metastatic melanoma?

We have identified that TRIM28 is a regulator of a phenotypic switch in melanoma cells that regulate invasiveness and growth of melanoma cells. The major questions are now whether or not this mechanism can be targeted therapeutically and if it would be beneficial for patients.

TRIM28 contains a BRD and is part of the BRD protein family. Many inhibitors targeting BRDs are currently being developed and evaluated by academic researcher groups and the pharmaceutical industry. Typical BRDs bind acetylated lysine through a structure referred to as a Z-loop that contains two tyrosine residues and a more distal asparagine residue. Researchers have taken advantage of this binding pocket to develop a range of BRD inhibitors with specificity towards different classes of BRDs. However, TRIM28 belongs to a class of atypical BRD proteins. The BRD of TRIM28 does not contain the critical tyrosine and asparagine residues commonly used to develop inhibitors against and is therefore unaffected by existing BRD inhibitors.

To develop an inhibitor against TRIM28 we must first study the binding pocket of TRIM28 in order to identify inhibitors that can possibly bind and inhibit TRIM28. This represent a major future challenge. An alternative approach would be to inhibit other proteins in the RNAPII pausing complex such as CDK9. Inhibitors for CDK9 are currently in clinical trials (Morales and Giordano, 2016). However, specificity of current CDK-inhibitors is an issue.

The second question is whether or not targeting TRIM28 would be beneficial for patients. As we have shown, metastatic melanoma patients with high expression of TRIM28 have a shorter survival. We have also shown that TRIM28 drives a metastatic phenotype of melanoma cells. We reason that targeting of TRIM28 would limit metastatic progression of the disease which could prolong survival of melanoma patients. However, in addition to the discovery that reduced levels of TRIM28 limited the metastatic capabilities of melanoma cells, we also identified that proliferation increased. This means that targeting TRIM28 as a therapy might lead to increased growth of existing tumors. Because the metastatic progression of melanoma to distal organs represent the major cause of death for melanoma patients, therapies that specifically target the metastatic progression is of great need. Therefore, TRIM28 could be targeted to specifically inhibit metastatic progression in combination with pre-existing treatment options.

6 ACKNOWLEDGEMENTS

During my PhD I have had the pleasure to work with and getting to know some fantastic people. I will try to thank as many of you as possible.

I would like to start by thanking my supervisor **Alex,** it has truly been a pleasure working with you. I could not have asked for a better mentor and teacher during these years. Thank you for always being available and willing to help, no matter how small of an issue or insignificant of a question I might have had.

To my co-supervisor **Marie**, thank you for supporting my work and for creating the platform that made a lot of my work possible. You have created a fantastic research environment that I am glad to have been a part of.

To the people who have been a part of the **Espinosa team** over the years. Thank you **Maria** for supervising me when I just started in the lab as a master student, for creating a very friendly and intellectually stimulating environment. **Alina**, thank you for all the help with animals over the years and for your work that contributed to my thesis. **Tianlin**, thank you for all the hard work you put in to my projects and good luck with your PhD. I would also like to thank **Lucia** for your effort and help with my projects. **Diego**, we've had a lot of fun in the lab and you have brought new thinking and new ideas. Thank you and I look forward to seeing your project finished.

I want to thank all past and present members of the **MWH group**, thank you for making everyday life in the lab so much easier. **Nikos** and **Lauro**, thanks for all those hours spent chatting football memories… I mean working late… in the lab. **Guðny**, **Jorge**, **Rita**, **Lara M, Albin**, thank you guys for all the help over the years in the lab and for all the fun times. Whether it be video game nights, making fun videos in the lab or just having a beer after work. **Amina** and **Vijole**, thank you for all the work you have done over the years to help the lab and just to make it a nice environment. **Malin**, **Sabrina**, **Johannes**, **Tilen, Lara A**, **Hanna**, thank you guys for your contributing to the great environment in the lab over the years.
I wish all of you the best for the future and don't be strangers!

I would also like to take the opportunity to thank **Noah M** for your contributions with metabolic measurements and **Rux** for helping with pathway analysis. **Kunal**, thank you for helping me with the migration assays. I also want to thank all the people behind the scenes who work at the administration, animal house facility and the IT department for making life easier for us PhD students.

All the people working at **CMM** and the **Rheumatology unit**, thank you for these years. The guys from the CMM pub crew, **Eliane, Michael, Susi, Melanie, Natalie, Matthias, John, Sharan, April, Miguel**, I had some really good times and some that I can't remember that well… Thank you guys.

And of course to all my friends outside of work, thank you guys! Special thanks to **Axel** and **Sofie** for offering to help out with my dissertation party. **Axel**, Thanks for all the years of scientific discussions, not-so scientific discussion and all the late night beers, you're up next! **Sofie**, you're a star for also helping me with the cover!

To the most important people in my life, **my family**. Thank you for always being there. None of this would have been possible without the unconditional support of my parents. **My father** for always showing interest in what I do and **my mother** for always encouraging me and pushing me to go further.

Finally, **Yolanda**. Thank you for always being there for me and for bringing balance to my life. For always supporting me in whatever I want to do and for telling me when I need to stop talking about my work. Thank you, I love you.

7 REFERENCES

Addison, J.B., Koontz, C., Fugett, J.H., Creighton, C.J., Chen, D., Farrugia, M.K., Padon, R.R., Voronkova, M.A., McLaughlin, S.L., Livengood, R.H.*, et al.* (2015). KAP1 promotes proliferation and metastatic progression of breast cancer cells. Cancer Res *75*, 344-355.

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell *124*, 783-801.

Alexandrov, L.B., Nik-Zainal, S., Wedge, D.C., Aparicio, S.A., Behjati, S., Biankin, A.V., Bignell, G.R., Bolli, N., Borg, A., Borresen-Dale, A.L.*, et al.* (2013). Signatures of mutational processes in human cancer. Nature *500*, 415-421.

Alexopoulou, L., Holt, A.C., Medzhitov, R., and Flavell, R.A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature *413*, 732-738.

Alger, H.M., Rayavarapu, S., and Nagaraju, K. (2011). Measurement of activation of the endoplasmic reticulum stress response in autoimmune myositis. Methods Enzymol *489*, 207-225.

Andrieu, G., Tran, A.H., Strissel, K.J., and Denis, G.V. (2016). BRD4 Regulates Breast Cancer Dissemination through Jagged1/Notch1 Signaling. Cancer Res *76*, 6555-6567.

Baechler, E.C., Batliwalla, F.M., Karypis, G., Gaffney, P.M., Ortmann, W.A., Espe, K.J., Shark, K.B., Grande, W.J., Hughes, K.M., Kapur, V.*, et al.* (2003). Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A *100*, 2610-2615.

Bandyopadhyay, A., Shin, D.W., and Kim, D.H. (2000). Regulation of ATP-induced calcium release in COS-7 cells by calcineurin. Biochem J *348 Pt 1*, 173-181.

Bell, O., Tiwari, V.K., Thoma, N.H., and Schubeler, D. (2011). Determinants and dynamics of genome accessibility. Nat Rev Genet *12*, 554-564.

Brinckerhoff, C.E. (2017). Cancer Stem Cells (CSCs) in melanoma: There's smoke, but is there fire? J Cell Physiol *232*, 2674-2678.

Bunch, H., and Calderwood, S.K. (2015). TRIM28 as a novel transcriptional elongation factor. BMC Mol Biol *16*, 14.

Bunch, H., Zheng, X., Burkholder, A., Dillon, S.T., Motola, S., Birrane, G., Ebmeier, C.C., Levine, S., Fargo, D., Hu, G.*, et al.* (2014). TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release. Nat Struct Mol Biol *21*, 876-883.

Cambiaghi, V., Giuliani, V., Lombardi, S., Marinelli, C., Toffalorio, F., and Pelicci, P.G. (2012). TRIM proteins in cancer. Adv Exp Med Biol *770*, 77-91.

Cammas, F., Mark, M., Dolle, P., Dierich, A., Chambon, P., and Losson, R. (2000). Mice lacking the transcriptional corepressor TIF1beta are defective in early postimplantation development. Development *127*, 2955-2963.

Cancer Genome Atlas, N. (2015). Genomic Classification of Cutaneous Melanoma. Cell *161*, 1681-1696.

Caramel, J., Papadogeorgakis, E., Hill, L., Browne, G.J., Richard, G., Wierinckx, A., Saldanha, G., Osborne, J., Hutchinson, P., Tse, G.*, et al.* (2013). A switch in the expression of embryonic EMT-inducers drives the development of malignant melanoma. Cancer Cell *24*, 466-480.

Cheli, Y., Bonnazi, V.F., Jacquel, A., Allegra, M., De Donatis, G.M., Bahadoran, P., Bertolotto, C., and Ballotti, R. (2014). CD271 is an imperfect marker for melanoma initiating cells. Oncotarget *5*, 5272-5283.

Chen, X., Iliopoulos, D., Zhang, Q., Tang, Q., Greenblatt, M.B., Hatziapostolou, M., Lim, E., Tam, W.L., Ni, M., Chen, Y.*, et al.* (2014). XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature *508*, 103-107.

Cheng, B., Ren, X., and Kerppola, T.K. (2014). KAP1 represses differentiation-inducible genes in embryonic stem cells through cooperative binding with PRC1 and derepresses pluripotency-associated genes. Mol Cell Biol *34*, 2075-2091.

Chmiest, D., Sharma, N., Zanin, N., Viaris de Lesegno, C., Shafaq-Zadah, M., Sibut, V., Dingli, F., Hupe, P., Wilmes, S., Piehler, J.*, et al.* (2016). Spatiotemporal control of interferon-induced JAK/STAT signalling and gene transcription by the retromer complex. Nat Commun *7*, 13476.

Clark, W.H., Jr., Elder, D.E., Guerry, D.t., Epstein, M.N., Greene, M.H., and Van Horn, M. (1984). A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. Hum Pathol *15*, 1147-1165.

Claudinon, J., Monier, M.N., and Lamaze, C. (2007). Interfering with interferon receptor sorting and trafficking: impact on signaling. Biochimie *89*, 735-743.

Coley, W.B. (1991). The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. Clin Orthop Relat Res, 3-11.

Core, L.J., Waterfall, J.J., and Lis, J.T. (2008). Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science *322*, 1845-1848.

Creagan, E.T., Ahmann, D.L., Frytak, S., Long, H.J., and Itri, L.M. (1986). Recombinant leukocyte A interferon (rIFN-alpha A) in the treatment of disseminated malignant melanoma. Analysis of complete and long-term responding patients. Cancer *58*, 2576-2578.

Croteau, W., Jenkins, M.H., Ye, S., Mullins, D.W., and Brinckerhoff, C.E. (2013). Differential mechanisms of tumor progression in clones from a single heterogeneous human melanoma. J Cell Physiol *228*, 773-780.

Crow, M.K., Olferiev, M., and Kirou, K.A. (2015). Targeting of type I interferon in systemic autoimmune diseases. Transl Res *165*, 296-305.

Cubillos-Ruiz, J.R., Silberman, P.C., Rutkowski, M.R., Chopra, S., Perales-Puchalt, A., Song, M., Zhang, S., Bettigole, S.E., Gupta, D., Holcomb, K.*, et al.* (2015). ER Stress Sensor XBP1 Controls Anti-tumor Immunity by Disrupting Dendritic Cell Homeostasis. Cell *161*, 1527-1538.

Cui, Y., Yang, S., Fu, X., Feng, J., Xu, S., and Ying, G. (2014). High levels of KAP1 expression are associated with aggressive clinical features in ovarian cancer. Int J Mol Sci *16*, 363-377.

Dankort, D., Curley, D.P., Cartlidge, R.A., Nelson, B., Karnezis, A.N., Damsky, W.E., Jr., You, M.J., DePinho, R.A., McMahon, M., and Bosenberg, M. (2009). Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet *41*, 544-552.

Darnell, J.E., Jr., Kerr, I.M., and Stark, G.R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science *264*, 1415-1421.

David, M. (2010). Interferons and microRNAs. J Interferon Cytokine Res *30*, 825-828.

Denecker, G., Vandamme, N., Akay, O., Koludrovic, D., Taminau, J., Lemeire, K., Gheldof, A., De Craene, B., Van Gele, M., Brochez, L.*, et al.* (2014). Identification of a ZEB2-MITF-ZEB1 transcriptional network that controls melanogenesis and melanoma progression. Cell Death Differ *21*, 1250-1261.

Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. Nature *399*, 491-496.

Doyle, J.M., Gao, J., Wang, J., Yang, M., and Potts, P.R. (2010). MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. Mol Cell *39*, 963-974.

Eames, H.L., Saliba, D.G., Krausgruber, T., Lanfrancotti, A., Ryzhakov, G., and Udalova, I.A. (2012). KAP1/TRIM28: an inhibitor of IRF5 function in inflammatory macrophages. Immunobiology *217*, 1315-1324.

Falletta, P., Sanchez-Del-Campo, L., Chauhan, J., Effern, M., Kenyon, A., Kershaw, C.J., Siddaway, R., Lisle, R., Freter, R., Daniels, M.J., *et al.* (2017). Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. Genes Dev *31*, 18-33.

Fang, D., Nguyen, T.K., Leishear, K., Finko, R., Kulp, A.N., Hotz, S., Van Belle, P.A., Xu, X., Elder, D.E., and Herlyn, M. (2005). A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res *65*, 9328-9337.

Fasching, L., Kapopoulou, A., Sachdeva, R., Petri, R., Jonsson, M.E., Manne, C., Turelli, P., Jern, P., Cammas, F., Trono, D., *et al.* (2015). TRIM28 represses transcription of endogenous retroviruses in neural progenitor cells. Cell Rep *10*, 20-28.

Ferri, F., Parcelier, A., Petit, V., Gallouet, A.S., Lewandowski, D., Dalloz, M., van den Heuvel, A., Kolovos, P., Soler, E., Squadrito, M.L., *et al.* (2015). TRIM33 switches off Ifnb1 gene transcription during the late phase of macrophage activation. Nat Commun *6*, 8900.

Filippakopoulos, P., and Knapp, S. (2014). Targeting bromodomains: epigenetic readers of lysine acetylation. Nat Rev Drug Discov *13*, 337-356.

Filippakopoulos, P., Qi, J., Picaud, S., Shen, Y., Smith, W.B., Fedorov, O., Morse, E.M., Keates, T., Hickman, T.T., Felletar, I., *et al.* (2010). Selective inhibition of BET bromodomains. Nature *468*, 1067-1073.

Fong, K.W., Zhao, J.C., Song, B., Zheng, B., and Yu, J. (2018). TRIM28 protects TRIM24 from SPOP-mediated degradation and promotes prostate cancer progression. Nat Commun *9*, 5007.

Frank, N.Y., Margaryan, A., Huang, Y., Schatton, T., Waaga-Gasser, A.M., Gasser, M., Sayegh, M.H., Sadee, W., and Frank, M.H. (2005). ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. Cancer Res *65*, 4320-4333.

Fribley, A., Zeng, Q., and Wang, C.Y. (2004). Proteasome inhibitor PS-341 induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in head and neck squamous cell carcinoma cells. Mol Cell Biol *24*, 9695-9704.

Friedman, J.R., Fredericks, W.J., Jensen, D.E., Speicher, D.W., Huang, X.P., Neilson, E.G., and Rauscher, F.J., 3rd (1996). KAP-1, a novel corepressor for the highly conserved KRAB repression domain. Genes Dev *10*, 2067-2078.

Fu, X.Y., Schindler, C., Improta, T., Aebersold, R., and Darnell, J.E., Jr. (1992). The proteins of ISGF-3, the interferon alpha-induced transcriptional activator, define a gene family involved in signal transduction. Proc Natl Acad Sci U S A *89*, 7840-7843.

Fujisawa, T., and Filippakopoulos, P. (2017). Functions of bromodomain-containing proteins and their roles in homeostasis and cancer. Nat Rev Mol Cell Biol.

Furie, R., Khamashta, M., Merrill, J.T., Werth, V.P., Kalunian, K., Brohawn, P., Illei, G.G., Drappa, J., Wang, L., Yoo, S., *et al.* (2017). Anifrolumab, an Anti-Interferon-alpha Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. Arthritis Rheumatol *69*, 376-386.

Gide, T.N., Wilmott, J.S., Scolyer, R.A., and Long, G.V. (2018). Primary and Acquired Resistance to Immune Checkpoint Inhibitors in Metastatic Melanoma. Clin Cancer Res *24*, 1260-1270.

Gough, D.J., Messina, N.L., Clarke, C.J., Johnstone, R.W., and Levy, D.E. (2012). Constitutive type I interferon modulates homeostatic balance through tonic signaling. Immunity *36*, 166-174.

Gracias, D.T., Stelekati, E., Hope, J.L., Boesteanu, A.C., Doering, T.A., Norton, J., Mueller, Y.M., Fraietta, J.A., Wherry, E.J., Turner, M., *et al.* (2013). The microRNA miR-155 controls CD8(+) T cell responses by regulating interferon signaling. Nat Immunol *14*, 593-602.

Guan, J., Gupta, R., and Filipp, F.V. (2015). Cancer systems biology of TCGA SKCM: efficient detection of genomic drivers in melanoma. Sci Rep *5*, 7857.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646-674.

Haynes, S.R., Dollard, C., Winston, F., Beck, S., Trowsdale, J., and Dawid, I.B. (1992). The bromodomain: a conserved sequence found in human, Drosophila and yeast proteins. Nucleic Acids Res *20*, 2603.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., and Bauer, S. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science *303*, 1526-1529.

Hodis, E., Watson, I.R., Kryukov, G.V., Arold, S.T., Imielinski, M., Theurillat, J.P., Nickerson, E., Auclair, D., Li, L., Place, C.*, et al.* (2012). A landscape of driver mutations in melanoma. Cell *150*, 251-263.

Hoek, K.S., Eichhoff, O.M., Schlegel, N.C., Dobbeling, U., Kobert, N., Schaerer, L., Hemmi, S., and Dummer, R. (2008). In vivo switching of human melanoma cells between proliferative and invasive states. Cancer Res *68*, 650-656.

Honda, K., Takaoka, A., and Taniguchi, T. (2006). Type I interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors. Immunity *25*, 349-360.

Honda, K., and Taniguchi, T. (2006). IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat Rev Immunol *6*, 644-658.

Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N., Ohba, Y., Takaoka, A., Yoshida, N.*, et al.* (2005). IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature *434*, 772-777.

Hosoya, T., Clifford, M., Losson, R., Tanabe, O., and Engel, J.D. (2013). TRIM28 is essential for erythroblast differentiation in the mouse. Blood *122*, 3798-3807.

Hu, X., Herrero, C., Li, W.P., Antoniv, T.T., Falck-Pedersen, E., Koch, A.E., Woods, J.M., Haines, G.K., and Ivashkiv, L.B. (2002). Sensitization of IFN-gamma Jak-STAT signaling during macrophage activation. Nat Immunol *3*, 859-866.

Huang, M., Qian, F., Hu, Y., Ang, C., Li, Z., and Wen, Z. (2002). Chromatin-remodelling factor BRG1 selectively activates a subset of interferon-alpha-inducible genes. Nat Cell Biol *4*, 774-781.

Isaacs, A., and Lindenmann, J. (1957). Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci *147*, 258-267.

Ishikawa, H., and Barber, G.N. (2008). STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature *455*, 674-678.

Ivashkiv, L.B., and Donlin, L.T. (2014). Regulation of type I interferon responses. Nat Rev Immunol *14*, 36-49.

Iyengar, S., Ivanov, A.V., Jin, V.X., Rauscher, F.J., 3rd, and Farnham, P.J. (2011). Functional analysis of KAP1 genomic recruitment. Mol Cell Biol *31*, 1833-1847.

Jain, A.K., and Barton, M.C. (2017). Bromodomain Histone Readers and Cancer. J Mol Biol *429*, 2003-2010.

Jakobsson, J., Cordero, M.I., Bisaz, R., Groner, A.C., Busskamp, V., Bensadoun, J.C., Cammas, F., Losson, R., Mansuy, I.M., Sandi, C.*, et al.* (2008). KAP1-mediated epigenetic repression in the forebrain modulates behavioral vulnerability to stress. Neuron *60*, 818-831.

Jern, P., and Coffin, J.M. (2008). Effects of retroviruses on host genome function. Annu Rev Genet *42*, 709-732.

Jonkers, I., Kwak, H., and Lis, J.T. (2014). Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. Elife *3*, e02407.

Kagan, J.C., Su, T., Horng, T., Chow, A., Akira, S., and Medzhitov, R. (2008). TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. Nat Immunol *9*, 361-368.

Kamitani, S., Ohbayashi, N., Ikeda, O., Togi, S., Muromoto, R., Sekine, Y., Ohta, K., Ishiyama, H., and Matsuda, T. (2008). KAP1 regulates type I interferon/STAT1-mediated IRF-1 gene expression. Biochem Biophys Res Commun *370*, 366-370.

Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., Uematsu, S., Jung, A., Kawai, T., Ishii, K.J., *et al.* (2006). Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature *441*, 101-105.

Kim, I.S., Heilmann, S., Kansler, E.R., Zhang, Y., Zimmer, M., Ratnakumar, K., Bowman, R.L., Simon-Vermot, T., Fennell, M., Garippa, R., *et al.* (2017). Microenvironment-derived factors driving metastatic plasticity in melanoma. Nat Commun *8*, 14343.

Kim, S.S., Chen, Y.M., O'Leary, E., Witzgall, R., Vidal, M., and Bonventre, J.V. (1996). A novel member of the RING finger family, KRIP-1, associates with the KRAB-A transcriptional repressor domain of zinc finger proteins. Proc Natl Acad Sci U S A *93*, 15299-15304.

Klein, K., Kabala, P.A., Grabiec, A.M., Gay, R.E., Kolling, C., Lin, L.L., Gay, S., Tak, P.P., Prinjha, R.K., Ospelt, C., *et al.* (2016). The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. Ann Rheum Dis *75*, 422-429.

Krauthammer, M., Kong, Y., Ha, B.H., Evans, P., Bacchiocchi, A., McCusker, J.P., Cheng, E., Davis, M.J., Goh, G., Choi, M., *et al.* (2012). Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. Nat Genet *44*, 1006-1014.

Lamar, J.M., Stern, P., Liu, H., Schindler, J.W., Jiang, Z.G., and Hynes, R.O. (2012). The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. Proc Natl Acad Sci U S A *109*, E2441-2450.

Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Rutkowski, P., Grob, J.J., Cowey, C.L., Lao, C.D., Schadendorf, D., Ferrucci, P.F., Smylie, M., *et al.* (2017). Overall survival (OS) results from a phase III trial of nivolumab (NIVO) combined with ipilimumab (IPI) in treatment-naive patients with advanced melanoma (CheckMate 067). Cancer Research *77*.

Lawrence, M.S., Stojanov, P., Polak, P., Kryukov, G.V., Cibulskis, K., Sivachenko, A., Carter, S.L., Stewart, C., Mermel, C.H., Roberts, S.A., *et al.* (2013). Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature *499*, 214-218.

Lee, W.S., Yoo, W.H., and Chae, H.J. (2015). ER Stress and Autophagy. Curr Mol Med *15*, 735-745.

Levy, D.E., and Darnell, J.E., Jr. (2002). Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol *3*, 651-662.

Levy, D.E., Kessler, D.S., Pine, R., Reich, N., and Darnell, J.E., Jr. (1988). Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. Genes Dev *2*, 383-393.

Levy, D.E., Lew, D.J., Decker, T., Kessler, D.S., and Darnell, J.E., Jr. (1990). Synergistic interaction between interferon-alpha and interferon-gamma through induced synthesis of one subunit of the transcription factor ISGF3. EMBO J *9*, 1105-1111.

Li, J., Xi, Y., Li, W., McCarthy, R.L., Stratton, S.A., Zou, W., Li, W., Dent, S.Y., Jain, A.K., and Barton, M.C. (2017). TRIM28 interacts with EZH2 and SWI/SNF to activate genes that promote mammosphere formation. Oncogene *36*, 2991-3001.

Li, Y., Sassano, A., Majchrzak, B., Deb, D.K., Levy, D.E., Gaestel, M., Nebreda, A.R., Fish, E.N., and Platanias, L.C. (2004). Role of p38alpha Map kinase in Type I interferon signaling. J Biol Chem *279*, 970-979.

Liang, Q., Deng, H., Li, X., Wu, X., Tang, Q., Chang, T.H., Peng, H., Rauscher, F.J., 3rd, Ozato, K., and Zhu, F. (2011). Tripartite motif-containing protein 28 is a small ubiquitin-related modifier E3 ligase and negative regulator of IFN regulatory factor 7. J Immunol *187*, 4754-4763.

Lin, L.F., Li, C.F., Wang, W.J., Yang, W.M., Wang, D.D., Chang, W.C., Lee, W.H., and Wang, J.M. (2013). Loss of ZBRK1 contributes to the increase of KAP1 and promotes KAP1-mediated metastasis and invasion in cervical cancer. PLoS One *8*, e73033.

Liu, H., Kang, H., Liu, R., Chen, X., and Zhao, K. (2002). Maximal induction of a subset of interferon target genes requires the chromatin-remodeling activity of the BAF complex. Mol Cell Biol *22*, 6471-6479.

Liu, Q., Li, A., Tian, Y., Wu, J.D., Liu, Y., Li, T., Chen, Y., Han, X., and Wu, K. (2016a). The CXCL8-CXCR1/2 pathways in cancer. Cytokine Growth Factor Rev *31*, 61-71.

Liu, X., Li, H., Rajurkar, M., Li, Q., Cotton, J.L., Ou, J., Zhu, L.J., Goel, H.L., Mercurio, A.M., Park, J.S.*, et al.* (2016b). Tead and AP1 Coordinate Transcription and Motility. Cell Rep *14*, 1169-1180.

Long, G.V., Fung, C., Menzies, A.M., Pupo, G.M., Carlino, M.S., Hyman, J., Shahheydari, H., Tembe, V., Thompson, J.F., Saw, R.P.*, et al.* (2014). Increased MAPK reactivation in early resistance to dabrafenib/trametinib combination therapy of BRAF-mutant metastatic melanoma. Nat Commun *5*, 5694.

Long, G.V., Stroyakovskiy, D., Gogas, H., Levchenko, E., de Braud, F., Larkin, J., Garbe, C., Jouary, T., Hauschild, A., Grob, J.J.*, et al.* (2015). Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. Lancet *386*, 444-451.

Lu, L.F., Boldin, M.P., Chaudhry, A., Lin, L.L., Taganov, K.D., Hanada, T., Yoshimura, A., Baltimore, D., and Rudensky, A.Y. (2010). Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell *142*, 914-929.

Lubick, K.J., Robertson, S.J., McNally, K.L., Freedman, B.A., Rasmussen, A.L., Taylor, R.T., Walts, A.D., Tsuruda, S., Sakai, M., Ishizuka, M.*, et al.* (2015). Flavivirus Antagonism of Type I Interferon Signaling Reveals Prolidase as a Regulator of IFNAR1 Surface Expression. Cell Host Microbe *18*, 61-74.

Lund, J., Sato, A., Akira, S., Medzhitov, R., and Iwasaki, A. (2003). Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. J Exp Med *198*, 513-520.

Martinon, F., Chen, X., Lee, A.H., and Glimcher, L.H. (2010). TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. Nat Immunol *11*, 411-418.

Martins, M.B., Marcello, M.A., Morari, E.C., Cunha, L.L., Soares, F.A., Vassallo, J., and Ward, L.S. (2013). Clinical utility of KAP-1 expression in thyroid lesions. Endocr Pathol *24*, 77-82.

Mathur, A., Abd Elmageed, Z.Y., Liu, X., Kostochka, M.L., Zhang, H., Abdel-Mageed, A.B., and Mondal, D. (2014). Subverting ER-stress towards apoptosis by nelfinavir and curcumin coexposure augments docetaxel efficacy in castration resistant prostate cancer cells. PLoS One *9*, e103109.

Maurel, M., McGrath, E.P., Mnich, K., Healy, S., Chevet, E., and Samali, A. (2015). Controlling the unfolded protein response-mediated life and death decisions in cancer. Semin Cancer Biol *33*, 57-66.

McNamara, R.P., Reeder, J.E., McMillan, E.A., Bacon, C.W., McCann, J.L., and D'Orso, I. (2016). KAP1 Recruitment of the 7SK snRNP Complex to Promoters Enables Transcription Elongation by RNA Polymerase II. Mol Cell *61*, 39-53.

Mekahli, D., Bultynck, G., Parys, J.B., De Smedt, H., and Missiaen, L. (2011). Endoplasmic-reticulum calcium depletion and disease. Cold Spring Harb Perspect Biol *3*.

Mendoza, M.C., Er, E.E., and Blenis, J. (2011). The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends Biochem Sci *36*, 320-328.

Meng, S., Zhang, L., Tang, Y., Tu, Q., Zheng, L., Yu, L., Murray, D., Cheng, J., Kim, S.H., Zhou, X.*, et al.* (2014). BET Inhibitor JQ1 Blocks Inflammation and Bone Destruction. J Dent Res *93*, 657-662.

Mitani, Y., Takaoka, A., Kim, S.H., Kato, Y., Yokochi, T., Tanaka, N., and Taniguchi, T. (2001). Cross talk of the interferon-alpha/beta signalling complex with gp130 for effective interleukin-6 signalling. Genes Cells *6*, 631-640.

Moffett, H.F., Cartwright, A.N.R., Kim, H.J., Godec, J., Pyrdol, J., Aijo, T., Martinez, G.J., Rao, A., Lu, J., Golub, T.R.*, et al.* (2017). The microRNA miR-31 inhibits CD8(+) T cell function in chronic viral infection. Nat Immunol *18*, 791-799.

Moosmann, P., Georgiev, O., Le Douarin, B., Bourquin, J.P., and Schaffner, W. (1996). Transcriptional repression by RING finger protein TIF1 beta that interacts with the KRAB repressor domain of KOX1. Nucleic Acids Res *24*, 4859-4867.

Morales, A., Eidinger, D., and Bruce, A.W. (2017). Intracavitary Bacillus Calmette-Guerin in the Treatment of Superficial Bladder Tumors. J Urol *197*, S142-S145.

Morales, F., and Giordano, A. (2016). Overview of CDK9 as a target in cancer research. Cell Cycle *15*, 519-527.

Nagano, Y., and Kojima, Y. (1954). [Immunizing property of vaccinia virus inactivated by ultraviolets rays]. C R Seances Soc Biol Fil *148*, 1700-1702.

Nallet-Staub, F., Marsaud, V., Li, L., Gilbert, C., Dodier, S., Bataille, V., Sudol, M., Herlyn, M., and Mauviel, A. (2014). Pro-invasive activity of the Hippo pathway effectors YAP and TAZ in cutaneous melanoma. J Invest Dermatol *134*, 123-132.

Nguyen, L.V., Vanner, R., Dirks, P., and Eaves, C.J. (2012). Cancer stem cells: an evolving concept. Nat Rev Cancer *12*, 133-143.

Ni, Z., Karaskov, E., Yu, T., Callaghan, S.M., Der, S., Park, D.S., Xu, Z., Pattenden, S.G., and Bremner, R. (2005). Apical role for BRG1 in cytokine-induced promoter assembly. Proc Natl Acad Sci U S A *102*, 14611-14616.

Nicodeme, E., Jeffrey, K.L., Schaefer, U., Beinke, S., Dewell, S., Chung, C.W., Chandwani, R., Marazzi, I., Wilson, P., Coste, H.*, et al.* (2010). Suppression of inflammation by a synthetic histone mimic. Nature *468*, 1119-1123.

Novick, D., Cohen, B., and Rubinstein, M. (1994). The human interferon alpha/beta receptor: characterization and molecular cloning. Cell *77*, 391-400.

Nowell, P.C. (1976). The clonal evolution of tumor cell populations. Science *194*, 23-28.

Ozato, K., Shin, D.M., Chang, T.H., and Morse, H.C., 3rd (2008). TRIM family proteins and their emerging roles in innate immunity. Nat Rev Immunol *8*, 849-860.

Papadopoulou, A.S., Dooley, J., Linterman, M.A., Pierson, W., Ucar, O., Kyewski, B., Zuklys, S., Hollander, G.A., Matthys, P., Gray, D.H.*, et al.* (2011). The thymic epithelial microRNA network elevates the threshold for infection-associated thymic involution via miR-29a mediated suppression of the IFN-alpha receptor. Nat Immunol *13*, 181-187.

Patel, M.C., Debrosse, M., Smith, M., Dey, A., Huynh, W., Sarai, N., Heightman, T.D., Tamura, T., and Ozato, K. (2013). BRD4 coordinates recruitment of pause release factor P-TEFb and the pausing complex NELF/DSIF to regulate transcription elongation of interferon-stimulated genes. Mol Cell Biol *33*, 2497-2507.

Peng, H., Feldman, I., and Rauscher, F.J., 3rd (2002). Hetero-oligomerization among the TIF family of RBCC/TRIM domain-containing nuclear cofactors: a potential mechanism for regulating the switch between coactivation and corepression. J Mol Biol *320*, 629-644.

Perez-Salvia, M., and Esteller, M. (2017). Bromodomain inhibitors and cancer therapy: From structures to applications. Epigenetics *12*, 323-339.

Piccolo, S., Dupont, S., and Cordenonsi, M. (2014). The biology of YAP/TAZ: hippo signaling and beyond. Physiol Rev *94*, 1287-1312.

Pineda, C.T., and Potts, P.R. (2015). Oncogenic MAGEA-TRIM28 ubiquitin ligase downregulates autophagy by ubiquitinating and degrading AMPK in cancer. Autophagy *11*, 844-846.

Platanias, L.C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol *5*, 375-386.

Platanias, L.C., Uddin, S., Yetter, A., Sun, X.J., and White, M.F. (1996). The type I interferon receptor mediates tyrosine phosphorylation of insulin receptor substrate 2. J Biol Chem *271*, 278-282.

Quenneville, S., Turelli, P., Bojkowska, K., Raclot, C., Offner, S., Kapopoulou, A., and Trono, D. (2012). The KRAB-ZFP/KAP1 system contributes to the early embryonic establishment of site-specific DNA methylation patterns maintained during development. Cell Rep *2*, 766-773.

Quesada, J.R., Reuben, J., Manning, J.T., Hersh, E.M., and Gutterman, J.U. (1984). Alpha interferon for induction of remission in hairy-cell leukemia. N Engl J Med *310*, 15-18.

Ramanathan, M., Majzoub, K., Rao, D.S., Neela, P.H., Zarnegar, B.J., Mondal, S., Roth, J.G., Gai, H., Kovalski, J.R., Siprashvili, Z.*, et al.* (2018). RNA-protein interaction detection in living cells. Nat Methods *15*, 207-212.

Ramsauer, K., Sadzak, I., Porras, A., Pilz, A., Nebreda, A.R., Decker, T., and Kovarik, P. (2002). p38 MAPK enhances STAT1-dependent transcription independently of Ser-727 phosphorylation. Proc Natl Acad Sci U S A *99*, 12859-12864.

Reya, T., Morrison, S.J., Clarke, M.F., and Weissman, I.L. (2001). Stem cells, cancer, and cancer stem cells. Nature *414*, 105-111.

Robert, C., Karaszewska, B., Schachter, J., Rutkowski, P., Mackiewicz, A., Stroiakovski, D., Lichinitser, M., Dummer, R., Grange, F., Mortier, L.*, et al.* (2015a). Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med *372*, 30-39.

Robert, C., Schachter, J., Long, G.V., Arance, A., Grob, J.J., Mortier, L., Daud, A., Carlino, M.S., McNeil, C., Lotem, M.*, et al.* (2015b). Pembrolizumab versus Ipilimumab in Advanced Melanoma. New Engl J Med *372*, 2521-2532.

Ronnblom, L.E., Alm, G.V., and Oberg, K.E. (1990). Possible induction of systemic lupus erythematosus by interferon-alpha treatment in a patient with a malignant carcinoid tumour. J Intern Med *227*, 207-210.

Roux, K.J., Kim, D.I., and Burke, B. (2013). BioID: a screen for protein-protein interactions. Curr Protoc Protein Sci *74*, Unit 19 23.

Roux, K.J., Kim, D.I., Raida, M., and Burke, B. (2012). A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J Cell Biol *196*, 801-810.

Rowe, H.M., Kapopoulou, A., Corsinotti, A., Fasching, L., Macfarlan, T.S., Tarabay, Y., Viville, S., Jakobsson, J., Pfaff, S.L., and Trono, D. (2013). TRIM28 repression of retrotransposon-based enhancers is necessary to preserve transcriptional dynamics in embryonic stem cells. Genome Res *23*, 452-461.

Rusinova, I., Forster, S., Yu, S., Kannan, A., Masse, M., Cumming, H., Chapman, R., and Hertzog, P.J. (2013). Interferome v2.0: an updated database of annotated interferon-regulated genes. Nucleic Acids Res *41*, D1040-1046.

Ryals, J., Dierks, P., Ragg, H., and Weissmann, C. (1985). A 46-nucleotide promoter segment from an IFN-alpha gene renders an unrelated promoter inducible by virus. Cell *41*, 497-507.

Sakaguchi, S., Negishi, H., Asagiri, M., Nakajima, C., Mizutani, T., Takaoka, A., Honda, K., and Taniguchi, T. (2003). Essential role of IRF-3 in lipopolysaccharide-induced interferon-beta gene expression and endotoxin shock. Biochem Biophys Res Commun *306*, 860-866.

Sandru, A., Voinea, S., Panaitescu, E., and Blidaru, A. (2014). Survival rates of patients with metastatic malignant melanoma. J Med Life *7*, 572-576.

Sanjana, N.E., Shalem, O., and Zhang, F. (2014). Improved vectors and genome-wide libraries for CRISPR screening. Nat Methods *11*, 783-784.

Sarasin-Filipowicz, M., Wang, X., Yan, M., Duong, F.H., Poli, V., Hilton, D.J., Zhang, D.E., and Heim, M.H. (2009). Alpha interferon induces long-lasting refractoriness of JAK-STAT signaling in the mouse liver through induction of USP18/UBP43. Mol Cell Biol *29*, 4841-4851.

Sato, M., Hata, N., Asagiri, M., Nakaya, T., Taniguchi, T., and Tanaka, N. (1998a). Positive feedback regulation of type I IFN genes by the IFN-inducible transcription factor IRF-7. FEBS Lett *441*, 106-110.

Sato, M., Tanaka, N., Hata, N., Oda, E., and Taniguchi, T. (1998b). Involvement of the IRF family transcription factor IRF-3 in virus-induced activation of the IFN-beta gene. FEBS Lett *425*, 112-116.

Schachter, J., Ribas, A., Long, G.V., Arance, A., Grob, J.J., Mortier, L., Daud, A., Carlino, M.S., McNeil, C., Lotem, M.*, et al.* (2017). Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet *390*, 1853-1862.

Schilling, P.J., Kurzrock, R., Kantarjian, H., Gutterman, J.U., and Talpaz, M. (1991). Development of systemic lupus erythematosus after interferon therapy for chronic myelogenous leukemia. Cancer *68*, 1536-1537.

Schindler, C., Fu, X.Y., Improta, T., Aebersold, R., and Darnell, J.E., Jr. (1992). Proteins of transcription factor ISGF-3: one gene encodes the 91-and 84-kDa ISGF-3 proteins that are activated by interferon alpha. Proc Natl Acad Sci U S A *89*, 7836-7839.

Schoggins, J.W., Wilson, S.J., Panis, M., Murphy, M.Y., Jones, C.T., Bieniasz, P., and Rice, C.M. (2011). A diverse range of gene products are effectors of the type I interferon antiviral response. Nature *472*, 481-485.

Schon, M.P., and Schon, M. (2007). Imiquimod: mode of action. Br J Dermatol *157 Suppl 2*, 8-13.

Schultz, D.C., Ayyanathan, K., Negorev, D., Maul, G.G., and Rauscher, F.J., 3rd (2002). SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev *16*, 919-932.

Schultz, D.C., Friedman, J.R., and Rauscher, F.J., 3rd (2001). Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. Genes Dev *15*, 428-443.

Seki, Y., Kurisaki, A., Watanabe-Susaki, K., Nakajima, Y., Nakanishi, M., Arai, Y., Shiota, K., Sugino, H., and Asashima, M. (2010). TIF1beta regulates the pluripotency of embryonic stem cells in a phosphorylation-dependent manner. Proc Natl Acad Sci U S A *107*, 10926-10931.

Song, M., Sandoval, T.A., Chae, C.S., Chopra, S., Tan, C., Rutkowski, M.R., Raundhal, M., Chaurio, R.A., Payne, K.K., Konrad, C.*, et al.* (2018). IRE1alpha-XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. Nature *562*, 423-428.

Sripathy, S.P., Stevens, J., and Schultz, D.C. (2006). The KAP1 corepressor functions to coordinate the assembly of de novo HP1-demarcated microenvironments of heterochromatin required for KRAB zinc finger protein-mediated transcriptional repression. Mol Cell Biol *26*, 8623-8638.

Stark, G.R., and Darnell, J.E., Jr. (2012). The JAK-STAT pathway at twenty. Immunity *36*, 503-514.

Stetson, D.B., and Medzhitov, R. (2006). Type I interferons in host defense. Immunity *25*, 373-381.

Subramaniam, P.S., and Johnson, H.M. (2004). The IFNAR1 subunit of the type I IFN receptor complex contains a functional nuclear localization sequence. FEBS Lett *578*, 207-210.

Sun, L., Wu, J., Du, F., Chen, X., and Chen, Z.J. (2013). Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science *339*, 786-791.

Takenokuchi, M., Miyamoto, K., Saigo, K., and Taniguchi, T. (2015). Bortezomib Causes ER Stress-related Death of Acute Promyelocytic Leukemia Cells Through Excessive Accumulation of PML-RARA. Anticancer Res *35*, 3307-3316.

Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, H., Ogawa, T., Takeda, K., and Akira, S. (1999). Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity *11*, 443-451.

Tarhini, A.A., Gogas, H., and Kirkwood, J.M. (2012). IFN-alpha in the treatment of melanoma. J Immunol *189*, 3789-3793.

Tassiulas, I., Hu, X., Ho, H., Kashyap, Y., Paik, P., Hu, Y., Lowell, C.A., and Ivashkiv, L.B. (2004). Amplification of IFN-alpha-induced STAT1 activation and inflammatory function by Syk and ITAM-containing adaptors. Nat Immunol *5*, 1181-1189.

Thakore, P.I., D'Ippolito, A.M., Song, L., Safi, A., Shivakumar, N.K., Kabadi, A.M., Reddy, T.E., Crawford, G.E., and Gersbach, C.A. (2015). Highly specific epigenome editing by CRISPR-Cas9 repressors for silencing of distal regulatory elements. Nat Methods *12*, 1143-1149.

Thiery, J.P., Acloque, H., Huang, R.Y., and Nieto, M.A. (2009). Epithelial-mesenchymal transitions in development and disease. Cell *139*, 871-890.

Tisserand, J., Khetchoumian, K., Thibault, C., Dembele, D., Chambon, P., and Losson, R. (2011). Tripartite motif 24 (Trim24/Tif1alpha) tumor suppressor protein is a novel negative regulator of interferon (IFN)/signal transducers and activators of transcription (STAT) signaling pathway acting through retinoic acid receptor alpha (Raralpha) inhibition. J Biol Chem *286*, 33369-33379.

Tsuruma, R., Ohbayashi, N., Kamitani, S., Ikeda, O., Sato, N., Muromoto, R., Sekine, Y., Oritani, K., and Matsuda, T. (2008). Physical and functional interactions between STAT3 and KAP1. Oncogene *27*, 3054-3059.

Uddin, S., Fish, E.N., Sher, D.A., Gardziola, C., White, M.F., and Platanias, L.C. (1997). Activation of the phosphatidylinositol 3-kinase serine kinase by IFN-alpha. J Immunol *158*, 2390-2397.

Uddin, S., Yenush, L., Sun, X.J., Sweet, M.E., White, M.F., and Platanias, L.C. (1995). Interferon-alpha engages the insulin receptor substrate-1 to associate with the phosphatidylinositol 3'-kinase. J Biol Chem *270*, 15938-15941.

Uze, G., Lutfalla, G., and Gresser, I. (1990). Genetic transfer of a functional human interferon alpha receptor into mouse cells: cloning and expression of its cDNA. Cell *60*, 225-234.

Venkatesh, D., Ernandez, T., Rosetti, F., Batal, I., Cullere, X., Luscinskas, F.W., Zhang, Y., Stavrakis, G., Garcia-Cardena, G., Horwitz, B.H.*, et al.* (2013). Endothelial TNF receptor 2 induces IRF1 transcription factor-dependent interferon-beta autocrine signaling to promote monocyte recruitment. Immunity *38*, 1025-1037.

Versteeg, G.A., Rajsbaum, R., Sanchez-Aparicio, M.T., Maestre, A.M., Valdiviezo, J., Shi, M., Inn, K.S., Fernandez-Sesma, A., Jung, J., and Garcia-Sastre, A. (2013). The E3-ligase TRIM family of proteins regulates signaling pathways triggered by innate immune pattern-recognition receptors. Immunity *38*, 384-398.

Vojtek, A.B., Hollenberg, S.M., and Cooper, J.A. (1993). Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell *74*, 205-214.

Wack, A., Terczynska-Dyla, E., and Hartmann, R. (2015). Guarding the frontiers: the biology of type III interferons. Nat Immunol *16*, 802-809.

Walter, P., and Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. Science *334*, 1081-1086.

Wang, C., Ivanov, A., Chen, L., Fredericks, W.J., Seto, E., Rauscher, F.J., 3rd, and Chen, J. (2005). MDM2 interaction with nuclear corepressor KAP1 contributes to p53 inactivation. EMBO J *24*, 3279-3290.

Wei, C., Cheng, J., Zhou, B., Zhu, L., Khan, M.A., He, T., Zhou, S., He, J., Lu, X., Chen, H.*, et al.* (2016). Tripartite motif containing 28 (TRIM28) promotes breast cancer metastasis by stabilizing TWIST1 protein. Sci Rep *6*, 29822.

Wildenberg, M.E., van Helden-Meeuwsen, C.G., van de Merwe, J.P., Drexhage, H.A., and Versnel, M.A. (2008). Systemic increase in type I interferon activity in Sjogren's syndrome: a putative role for plasmacytoid dendritic cells. Eur J Immunol *38*, 2024-2033.

Wilks, A.F. (1989). Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc Natl Acad Sci U S A *86*, 1603-1607.

Wong, L.H., Sim, H., Chatterjee-Kishore, M., Hatzinisiriou, I., Devenish, R.J., Stark, G., and Ralph, S.J. (2002). Isolation and characterization of a human STAT1 gene regulatory element. Inducibility by interferon (IFN) types I and II and role of IFN regulatory factor-1. J Biol Chem *277*, 19408-19417.

Wu, D., Sanin, D.E., Everts, B., Chen, Q., Qiu, J., Buck, M.D., Patterson, A., Smith, A.M., Chang, C.H., Liu, Z., *et al.* (2016). Type 1 Interferons Induce Changes in Core Metabolism that Are Critical for Immune Function. Immunity *44*, 1325-1336.

Yin, Y., Choi, S.C., Xu, Z., Perry, D.J., Seay, H., Croker, B.P., Sobel, E.S., Brusko, T.M., and Morel, L. (2015). Normalization of CD4+ T cell metabolism reverses lupus. Sci Transl Med *7*, 274ra218.

Yokoe, T., Toiyama, Y., Okugawa, Y., Tanaka, K., Ohi, M., Inoue, Y., Mohri, Y., Miki, C., and Kusunoki, M. (2010). KAP1 is associated with peritoneal carcinomatosis in gastric cancer. Ann Surg Oncol *17*, 821-828.

Yoneyama, M., and Fujita, T. (2009). RNA recognition and signal transduction by RIG-I-like receptors. Immunol Rev *227*, 54-65.

Yoshimura, A., Naka, T., and Kubo, M. (2007). SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol *7*, 454-465.

Zanconato, F., Forcato, M., Battilana, G., Azzolin, L., Quaranta, E., Bodega, B., Rosato, A., Bicciato, S., Cordenonsi, M., and Piccolo, S. (2015). Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nat Cell Biol *17*, 1218-1227.

Zaretsky, J.M., Garcia-Diaz, A., Shin, D.S., Escuin-Ordinas, H., Hugo, W., Hu-Lieskovan, S., Torrejon, D.Y., Abril-Rodriguez, G., Sandoval, S., Barthly, L., *et al.* (2016). Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. N Engl J Med *375*, 819-829.

Zhang, T., Dutton-Regester, K., Brown, K.M., and Hayward, N.K. (2016). The genomic landscape of cutaneous melanoma. Pigment Cell Melanoma Res *29*, 266-283.

Zhang, X.F., Settleman, J., Kyriakis, J.M., Takeuchi-Suzuki, E., Elledge, S.J., Marshall, M.S., Bruder, J.T., Rapp, U.R., and Avruch, J. (1993). Normal and oncogenic p21ras proteins bind to the amino-terminal regulatory domain of c-Raf-1. Nature *364*, 308-313.

Zuo, X., Sheng, J., Lau, H.T., McDonald, C.M., Andrade, M., Cullen, D.E., Bell, F.T., Iacovino, M., Kyba, M., Xu, G., *et al.* (2012). Zinc finger protein ZFP57 requires its co-factor to recruit DNA methyltransferases and maintains DNA methylation imprint in embryonic stem cells via its transcriptional repression domain. J Biol Chem *287*, 2107-2118.