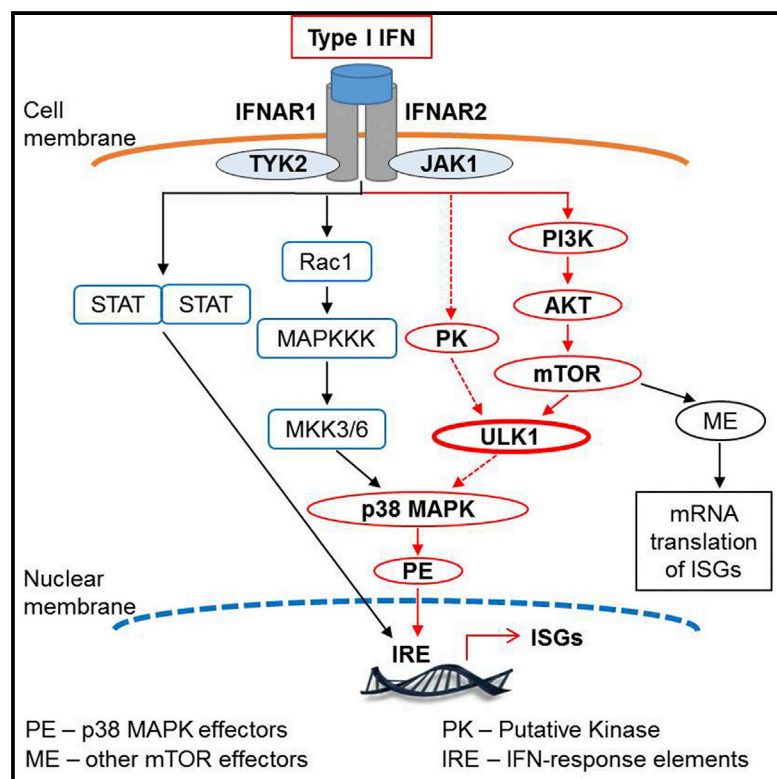


Central Role of ULK1 in Type I Interferon Signaling

Graphical Abstract



Authors

Diana Saleiro, Swarna Mehrotra, ...,
Amit K. Verma, Leonidas C. Platanias

Correspondence

l-platanias@northwestern.edu

In Brief

Saleiro et al. demonstrate that ULK1 mediates type I IFN-inducible activation of p38 MAPK, transcriptional activation of IFN-regulated genes, generation of type I IFN-mediated antiviral responses, and antineoplastic effects in myeloproliferative neoplasms.

Highlights

- Type I IFNs induce phosphorylation of ULK1 at serine 757
- ULK activity mediates transcriptional activation of ISGs and activation of p38 MAPK
- IFN-dependent activation of ULK1/2 is essential for generation of antiviral responses
- ULK1 is required for IFN-induced antineoplastic effects in MPN progenitors

Accession Numbers

GSE60778



Central Role of ULK1 in Type I Interferon Signaling

Diana Saleiro,¹ Swarna Mehrotra,¹ Barbara Kroczyńska,¹ Elspeth M. Beauchamp,^{1,2} Pawel Lisowski,^{3,4} Beata Majchrzak-Kita,⁵ Tushar D. Bhagat,⁶ Brady L. Stein,¹ Brandon McMahon,¹ Jessica K. Altman,^{1,2} Ewa M. Kosciuczuk,¹ Darren P. Baker,⁷ Chunfa Jie,¹ Nadereh Jafari,¹ Craig B. Thompson,⁸ Ross L. Levine,⁹ Eleanor N. Fish,⁵ Amit K. Verma,⁶ and Leonidas C. Platanias^{1,2,*}

¹Robert H. Lurie Comprehensive Cancer Center and Division of Hematology-Oncology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

²Division of Hematology-Oncology, Department of Medicine, Jesse Brown Veterans Affairs Medical Center, Chicago, IL 60612, USA

³Department of Molecular Biology, Institute of Genetics and Animal Breeding, 05-552 Jastrzebiec n/Warsaw, Poland

⁴iPS Cell-Based Disease Modeling Group, Max-Delbrück-Center for Molecular Medicine (MDC) in the Helmholtz Association, 13092 Berlin, Germany

⁵Toronto General Research Institute, University Health Network and Department of Immunology, University of Toronto, Toronto, ON M5G 2M1, Canada

⁶Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461, USA

⁷Biogen Idec Inc., 14 Cambridge Center, Cambridge, MA 02142, USA

⁸Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

⁹Human Oncology and Pathogenesis Program, and Leukemia Service, Memorial Sloan Kettering Cancer Center; and Weill Cornell Medical College, New York, NY 10065, USA

*Correspondence: l-platanias@northwestern.edu

<http://dx.doi.org/10.1016/j.celrep.2015.03.056>

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

SUMMARY

We provide evidence that the Unc-51-like kinase 1 (ULK1) is activated during engagement of the type I interferon (IFN) receptor (IFNR). Our studies demonstrate that the function of ULK1 is required for gene transcription mediated via IFN-stimulated response elements (ISRE) and IFN γ activation site (GAS) elements and controls expression of key IFN-stimulated genes (ISGs). We identify ULK1 as an upstream regulator of p38 α mitogen-activated protein kinase (MAPK) and establish that the regulatory effects of ULK1 on ISG expression are mediated possibly by engagement of the p38 MAPK pathway. Importantly, we demonstrate that ULK1 is essential for antiproliferative responses and type I IFN-induced antineoplastic effects against malignant erythroid precursors from patients with myeloproliferative neoplasms. Together, these data reveal a role for ULK1 as a key mediator of type I IFNR-generated signals that control gene transcription and induction of antineoplastic responses.

INTRODUCTION

Type I interferons (IFNs) are cytokines with important antitumor, antiviral, and immunomodulatory properties (González-Navajas et al., 2012; Bekisz et al., 2013; Platanias, 2005). These cytokines have clinical activity against viral infections and several human malignancies (Hervas-Stubbs et al., 2011; Bekisz et al., 2013;

Kotredes and Gamero, 2013; Platanias, 2013; Stein and Tiu, 2013). Despite continuing efforts to define the precise mechanisms by which IFNs generate antineoplastic responses, the sequence of events and the specific coordination of different IFN-activated signaling cascades required for such responses remain incompletely defined (Platanias, 2013).

All type I IFNs bind to type I IFN receptor (IFNR), the engagement of which activates JAK-STAT (Janus-activated kinase-signal transducer and activator of transcription) signaling pathways (Platanias, 2005; Stark and Darnell, 2012; Ivashkiv and Donlin, 2014). Beyond these pathways, activation of several other IFN-signaling cascades occurs during engagement of IFN receptors, including the p38 mitogen-activated protein kinase (MAPK) pathway (Uddin et al., 1999; Li et al., 2004), the phosphatidylinositol 3-kinase (PI3K)-AKT pathway (Kaur et al., 2008a, 2008b), and the mammalian target of rapamycin complex 1 (mTORC1) and mTORC2 signaling cascades (Kaur et al., 2007, 2012, 2014). The functions of these pathways are essential for optimal transcription and/or mRNA translation of various interferon-stimulated genes (ISGs) that are needed for the induction of IFN-responses (Kaur et al., 2007, 2008b, 2014).

Although the relevance and functional importance of mTORC1 signals in promoting functional IFN responses is well established (Kaur et al., 2007), the precise mechanisms and distinct roles of downstream mTORC1 effectors in the process remain to be defined. Previous work has demonstrated that activated mTORC1 prevents autophagy by phosphorylation of serine 757 (Ser757) of Unc-51-like kinase 1 (ULK1) and by disrupting the interaction between ULK1 and AMP-activated protein kinase (AMPK) (Kim et al., 2011). ULK1 and ULK2 are the closely related mammalian homologs of the

serine/threonine autophagy-related (ATG) protein kinase ATG1, the first identified ATG product in yeast, and both are involved in the regulation of autophagy (Alers et al., 2012). In the present study, we examined whether ULK1 is engaged in IFN signaling and what role it plays in the induction of type I IFN-mediated responses. Our studies provide evidence implicating ULK1 in type I IFN signaling and transcriptional activation of ISGs and define a mechanism by which such ULK1-mediated activity occurs in the IFN system, possibly involving regulation/activation of p38 MAPK.

RESULTS

Type I IFN-Induced Phosphorylation of ULK1 on Serine 757 Is AKT Dependent

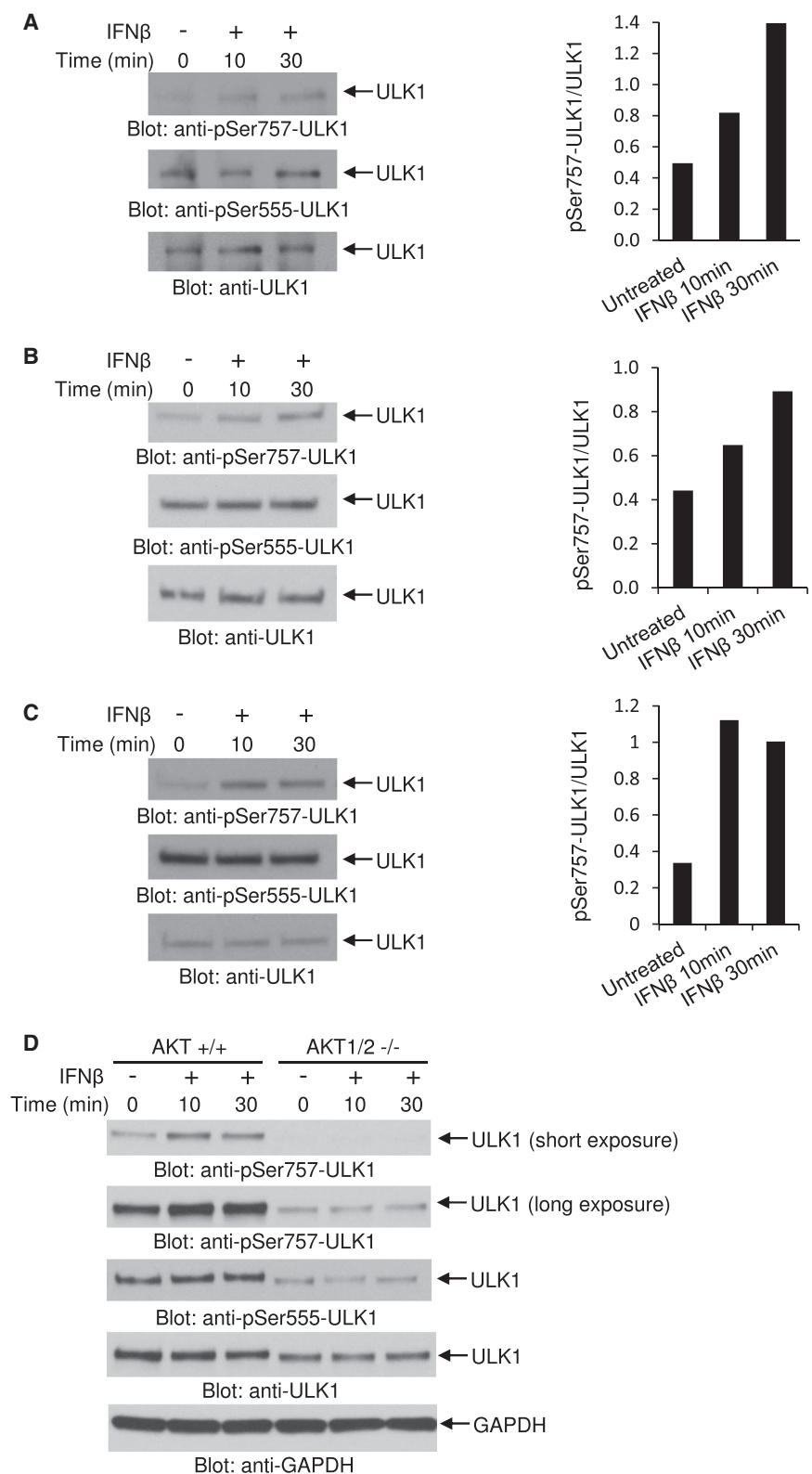
In initial studies, we examined whether type I IFN treatment induces phosphorylation of ULK1 in IFN-sensitive cells. Treatment of different IFN-sensitive malignant hematopoietic cell lines (U937, KT-1, and U266) with human IFN β induced phosphorylation of ULK1 at the mTORC1 phosphorylation site (Kim et al., 2011), Ser757 (Figures 1A–1C). In contrast, there was no IFN β -dependent induction of phosphorylation of ULK1 at Ser555 (Figures 1A–1C), the amino acid residue phosphorylated by AMPK (Bach et al., 2011). Previous studies have established that the serine/threonine protein kinase AKT is activated downstream of PI3K (Kaur et al., 2008a) and mTORC2 (Kaur et al., 2012) during engagement of the type I IFNR and regulates downstream engagement of mTORC1 (Kaur et al., 2008b). We examined whether engagement of ULK1 in IFN-signaling requires upstream AKT activity. For this, we determined the effects of IFN β treatment on the phosphorylation of ULK1 using Akt1/2 double-knockout (Akt1/2 $^{-/-}$) mouse embryonic fibroblasts (MEFs) (Peng et al., 2003). Treatment of Akt1/2 $^{+/+}$ MEFs with mouse IFN β resulted in phosphorylation of ULK1 on Ser757 (Figure 1D). However, IFN β -induced phosphorylation of ULK1 on Ser757 was defective in Akt1/2 $^{-/-}$ MEFs (Figure 1D). In contrast, there was no IFN β -dependent induction of phosphorylation of ULK1 at Ser555 in both Akt1/2 $^{+/+}$ and Akt1/2 $^{-/-}$ MEFs (Figure 1D). Together, these data suggest that upstream AKT activity is essential for regulation of type I IFN-induced phosphorylation of ULK1 on Ser757.

Requirement of ULK1/2 Activity for Transcriptional Activation of Type I IFN-Stimulated Genes

Our data establish that ULK1 is activated via the type I IFNR. As the generation of IFN responses depends on expression of ISGs and their protein products (Darnell et al., 1994; Stark and Darnell, 2012; Cheon et al., 2014), we initiated studies to determine whether ULK1 controls type I IFN-dependent gene transcription. Initially, we determined whether ULK1/2 activity is required for transcriptional activation via IFN-stimulated response elements (ISRE) or IFN γ activation site (GAS) elements in luciferase reporter assays, using MEFs with targeted disruption of both the *Ulk1* and *Ulk2* genes. For these studies, we used Ulk1/2 $^{+/+}$ and Ulk1/2 $^{-/-}$ MEFs (Cheong et al., 2011), as ULK1 and ULK2 kinases were previously shown to have at least partially redundant functions in fibroblasts (Kundu et al., 2008; Lee and Tournier, 2011). IFN β -dependent transcriptional activa-

tion via either ISRE or GAS elements was significantly reduced in the absence of Ulk1 and Ulk2 expression (Figures 2A and 2B). To further define the role of ULK1/2 in ISG regulation, we sought to identify IFN-inducible genes differentially expressed in Ulk1/2 $^{+/+}$ and Ulk1/2 $^{-/-}$ MEFs, using genome Illumina microarrays. Using principal component analysis (PCA) of differentially expressed genes, we found that the three biological replicates of gene expression profiles cluster together and that the control and IFN β -treated Ulk1/2 $^{+/+}$ and Ulk1/2 $^{-/-}$ cells represent separated groups (Figure S1A). Comparison of the transcriptomic profiles revealed IFN-inducible expression of 356 genes in Ulk1/2 $^{+/+}$ MEFs (Figure 2C), whereas only 264 genes were inducible in Ulk1/2 $^{-/-}$ MEFs (Figure 2D). Notably, although 225 genes were induced in both Ulk1/2 $^{+/+}$ and Ulk1/2 $^{-/-}$ MEFs (Figures 2E and 2F), the expression of 84 of these genes was significantly higher in the Ulk1/2 $^{+/+}$ MEFs compared to the Ulk1/2 $^{-/-}$ MEFs (Figure 2F and Table S1, genes highlighted in red). 131 genes were found to be induced only in the Ulk1/2 $^{+/+}$ MEFs (Figures 2E and 2G; Table S2), whereas 39 unique genes were induced in the Ulk1/2 $^{-/-}$ MEFs (Figures 2E and 2H; Table S3). The differentially expressed genes between Ulk1/2 $^{+/+}$ and Ulk1/2 $^{-/-}$ MEFs were classified among biochemical pathways using the KEGG database (Tables S4, S5, and S6). Most of the genes whose transcriptional induction by IFN β treatment was defective or decreased in Ulk1/2 $^{-/-}$ MEFs could be classified among biochemical pathways that regulate adaptive and innate immunity, as well as antiviral, antiproliferative, and pro-apoptotic responses (Table S4, genes highlighted in red and green; Table S5). In contrast, genes induced by IFN β only in Ulk1/2 $^{-/-}$ MEFs could be classified among biochemical pathways that are involved in cell adhesion and DNA transcription (Table S6). A functional gene network, generated using IPA 2014 software, is shown in Figure S1B and demonstrates relationships among the 215 genes whose expression is defective or decreased in the absence of Ulk1/2.

In further studies, we confirmed the requirement for ULK1/2 activity in the expression of several key ISGs using qRT-PCR (Figures 3A–3I). Among the genes whose expression was found defective in the absence of Ulk1/2 were *Cxcl10* (Zhang et al., 2005) and *Eif2ak2* (García et al., 2006; McAllister and Samuel, 2009), both of which are involved in the induction of antiviral effects and control of apoptosis. The induction of several other genes whose function was necessary for generation for IFN biological responses was also defective in Ulk1/2 $^{-/-}$ cells, including *Irgm2* (Hunn et al., 2008), *Gch1* (Rani et al., 2007; Alp and Channon, 2004), *Ifit3* (Schmeisser et al., 2010; Liu et al., 2011); *Oasl2* (Zhu et al., 2014), *Irf7* (Sharma et al., 2003; Honda et al., 2005; Colina et al., 2008), *Irf9* (Darnell et al., 1994; van Boxel-Dezaire et al., 2006), and *Isg54/Ifit2* (Yang et al., 2012) (Figures 3A–3I). To determine whether ULK1 expression is required for transcriptional activation of IFN-induced genes in other cell types, studies were performed with human U937 cells in which ULK1 was knocked down using specific small interfering RNAs (siRNAs) (Figure 3J). We found decreased IFN-inducible mRNA expression of *ISG15* and *ISG54* (Figures 3K and 3L), genes with crucial roles in the induction of IFN responses (Lenschow et al., 2007; Yang et al., 2012), further establishing a key role for ULK1 in type I IFN signaling.



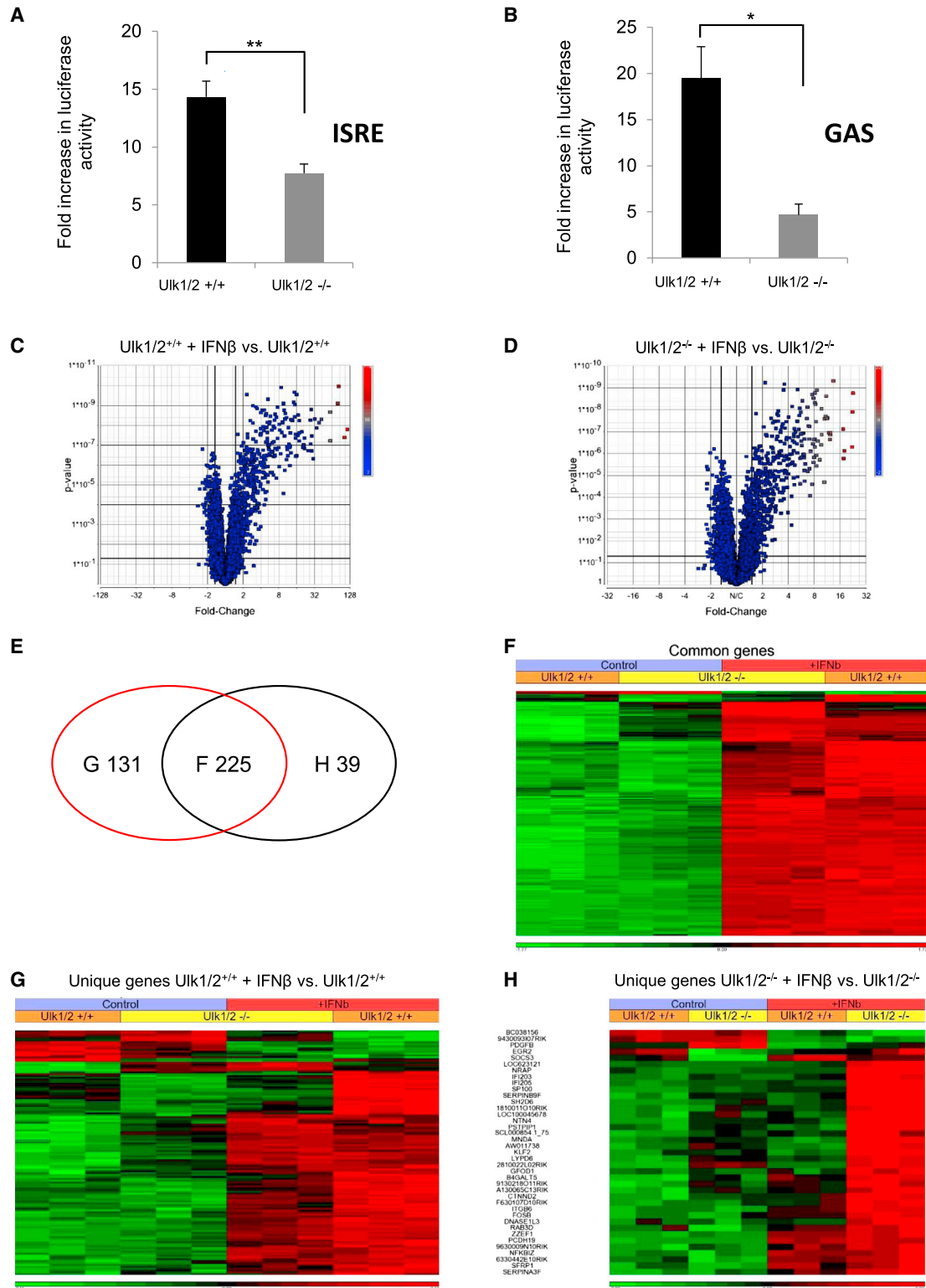


Figure 2. Targeted Disruption of *Ulk1/2* Gene Expression Impairs IFN β -Dependent Gene Transcription

(A and B) *Ulk1/2*^{+/+} and *Ulk1/2*^{-/-} MEFs were transfected with an ISRE-luciferase construct (A) or an 8 \times GAS-luciferase construct (B). 42 hr after transfection, the cells were incubated for 6 hr in the presence or absence of mouse IFN β , and luciferase activity was measured. Data are expressed as fold increase of luciferase activity in response to IFN β treatment over control untreated samples for each condition. Bar graphs show means \pm SE of four independent experiments for (A)

(legend continued on next page)

It has been extensively established that ULK1 regulates the induction of autophagy (Kim et al., 2011; Russell et al., 2013). In addition, there is also evidence for IFN-dependent induction of autophagy (Ambjørn et al., 2013; Schmeisser et al., 2014). We determined whether inhibition of autophagy modulates IFN-dependent transcriptional activation. The effects of siRNA-mediated knockdown of ATG5, a protein required in the early stages of autophagosome formation (Mizushima et al., 2001), were initially determined. No significant differences in IFN-dependent *Isg15*, *Isg54*, and *Irf9* mRNA expression were observed between control cells and cells in which ATG5 was knocked down (Figures 4A–4D). Consistent with this, treatment of cells with the autophagy inhibitors chloroquine or bafilomycin A1 (Klionsky et al., 2008) did not significantly affect ISG mRNA expression (Figures 4E–4G), further establishing that ULK1 promotes type I IFN-dependent transcriptional activation of key target genes in an autophagy-independent manner.

ULK1 Mediates Type I IFN-Dependent Activation of p38 MAPK

To define the mechanisms by which ULK1 activity may regulate type I IFN-dependent transcriptional activation, we examined whether it is required for activation of pathways that control type I IFN-dependent transcriptional activation of sensitive genes. As activation of Stat1 is essential for transcriptional induction of genes that contain ISRE or GAS elements in their promoters (Stark and Darnell, 2012), we first determined if phosphorylation/activation of Stat1 is Ulk1/2 dependent in MEFs. IFN β -dependent phosphorylation of Stat1 on serine 727 and tyrosine 701 was inducible in both Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs (Figure 5A), indicating that the functions of ULK1/2 are not required for type I IFN-induced activation of Stat1. As Stat1 is a key type I IFN-regulated protein involved in complexes that control both ISRE- and GAS-dependent transcription, these studies suggested that the effects of ULK1/2 on type I IFN-inducible transcriptional activation are independent of modulation of the classical STAT pathways.

Previous studies have demonstrated that the p38 MAPK pathway complements the function of STAT pathways and plays a critical role in type I IFN-induced transcriptional activation via both ISRE and GAS elements (Uddin et al., 1999, 2000; Li et al., 2004). We examined the possibility that the effects of ULK1/2 on ISG transcription are mediated by effects on p38

MAPK activity. We found that IFN β -induced phosphorylation of p38 MAPK was substantially decreased in Ulk1/2^{-/-} MEFs as compared to Ulk1/2^{+/+} MEFs (Figure 5B). Additionally, this defective p38 MAPK phosphorylation could be rescued by ectopic re-expression of wild-type ULK1 (ULK1 WT), but not a kinase-inactive ULK1 mutant (ULK1 K46I) (Egan et al., 2011) (Figure 5C). Complementation of Ulk1/2^{-/-} MEFs with ULK1 WT also restored IFN-induced transcriptional activation via GAS elements (Figure 5D). Moreover, we found that p38 α MAPK is phosphorylated by ULK1 kinase in *in vitro* assays (Figures 5E and S2), further suggesting that p38 MAPK mediates the regulatory effects of Ulk1 in type I IFN-dependent transcriptional activity.

ULK1/2 Activity Is Required for Induction of Type I IFN-Dependent Antiviral and Antiproliferative Effects

To define whether the defective type I IFN-dependent gene transcription seen in Ulk1/2^{-/-} MEFs has consequences in the generation of antiviral responses by type I IFNs, the ability of mouse IFN α to protect cells from encephalomyocarditis virus (EMCV) infection was compared in Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs. Ulk1/2^{-/-} MEFs were much more sensitive to EMCV infection compared to Ulk1/2^{+/+} MEFs (Figure S3). Specifically, at least a 50-fold reduction in infective dose was required to induce comparable EMCV-induced cytopathic effects (CPE) in the Ulk1/2^{-/-} MEFs compared with the Ulk1/2^{+/+} MEFs (Figure S3). Moreover, IFN α -induced antiviral dose-response data indicated that Ulk1/2^{-/-} cells are also less responsive to mouse IFN α treatment compared with Ulk1/2^{+/+} cells (Figure 6A). Together, these data show that Ulk1/2^{-/-} MEFs are more sensitive to viral infection and less sensitive to the antiviral effects of IFN α compared with Ulk1/2^{+/+} MEFs, establishing that engagement of Ulk1/2 is required for the control of type I IFN-generated antiviral responses in MEFs.

We also determined whether ULK1 is required for the generation of type I IFN-antiproliferative responses. For this purpose, we performed studies involving siRNA-mediated knockdown of ULK1 in U937 cells, followed by assessment of IFN β -inhibitory responses on leukemic blast colony-forming unit (CFU-L) colony growth. As shown in Figure 6B, inhibition of expression of ULK1 partially reversed suppression of CFU-L colony formation by IFN β treatment, implicating ULK1 as a signaling element required for the generation of type I IFN-antiproliferative effects.

and three independent experiments for (B), using technical triplicates in each experiment. Statistical analyses were performed using Student's t test (*p < 0.05; **p < 0.01).

(C–H) Differential expression of ISGs in Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs. Cells were incubated in the presence or absence of mouse IFN β for 6 hr. The gene expression profiles of untreated MEFs were compared with those of IFN β -treated MEFs in three independent experiments using MouseWG-6 v2.0 Expression BeadChips and Illumina iScan. (C and D) Volcano plots of differentially expressed genes after IFN β treatment are shown for (C) Ulk1/2^{+/+} and (D) Ulk1/2^{-/-} MEFs. 356 genes were differentially expressed between untreated and IFN β -treated Ulk1/2^{+/+} cells (C), whereas 264 genes were differentially expressed between untreated and IFN β -treated Ulk1/2^{-/-} cells (D). (E) Venn diagram showing the gene expression overlap existing between differentially expressed genes in Ulk1/2^{+/+} MEFs (red ellipse) and Ulk1/2^{-/-} MEFs (black ellipse) after treatment with IFN β . (F) Hierarchical clustering of differentially expressed genes in both Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs upon IFN β treatment. Differences in the effects of IFN β treatment between Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs are seen for 87 genes; 84 of these genes are characterized by a less efficient IFN β -driven transcription in Ulk1/2^{-/-} MEFs (see list of genes in Table S1).

(G) Hierarchical clustering of differentially expressed genes only in Ulk1/2^{+/+} MEFs (see list of genes in Table S2).

(H) Hierarchical clustering of differentially expressed genes only in Ulk1/2^{-/-} MEFs (see list of genes in Table S3).

All annotations presented here are based on statistical analyses and are presented with p values after false discovery rate correction. Only annotations with p values < 0.05 are shown. See also Tables S4, S5, and S6 and Figure S1.

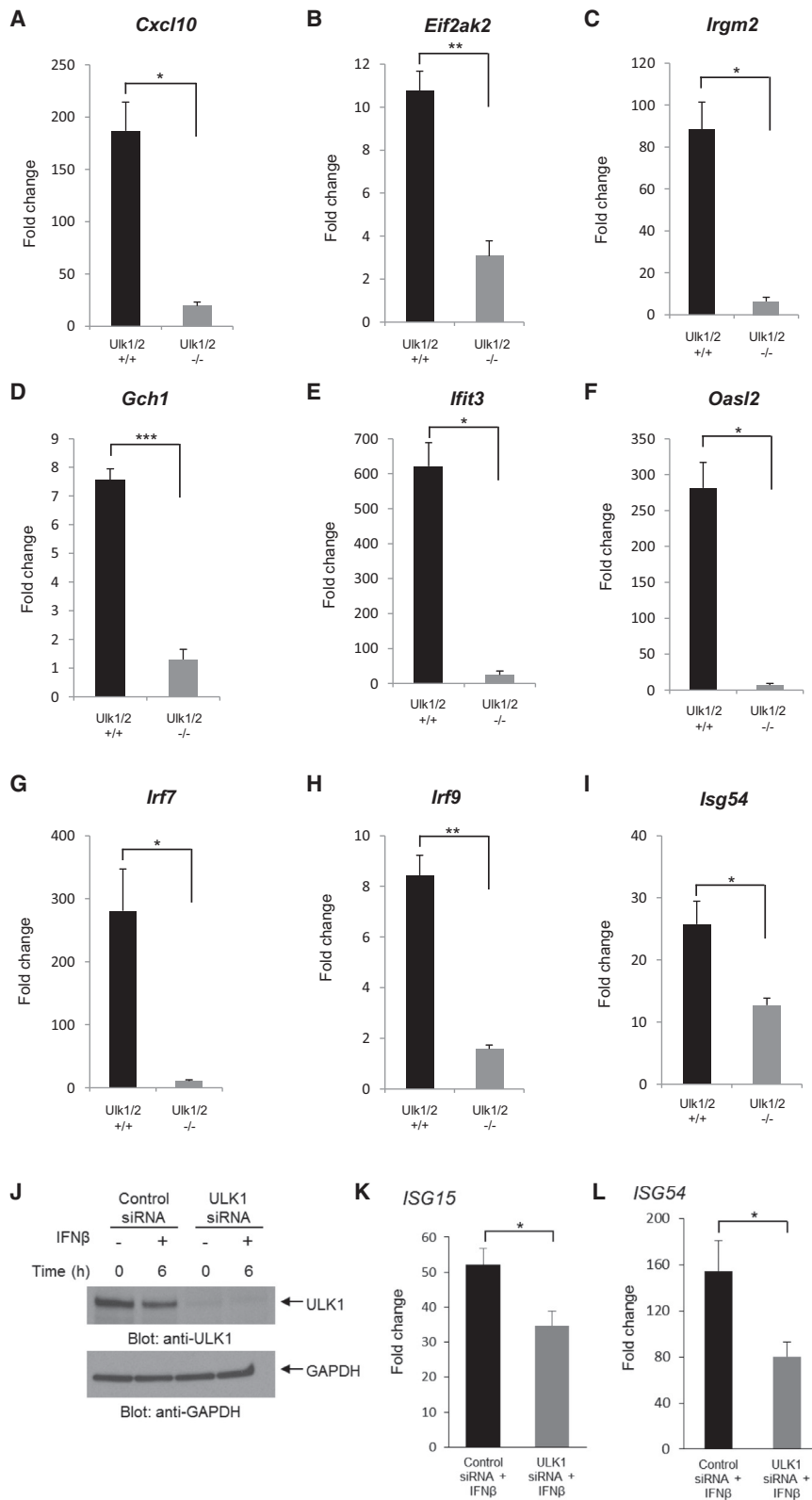


Figure 3. Requirement of ULK1/2 Activity for IFN β -Dependent Transcription

(A–I) Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs were left untreated or were treated with mouse IFN β for 6 hr. (J–L) U937 human leukemia cells were transfected with either control or ULK1 siRNAs. 24 hr after transfection, the cells were either left untreated or were incubated with human IFN β for 6 hr. (J) Levels of ULK1 protein expression are shown, using western immunoblotting, probing with ULK1-specific antibody. The immunoblot was also probed for GAPDH as a loading control. (A–I, K, and L) qRT-PCR analyses of the relative mRNA expression of ISGs after IFN β stimulation in (A–I) Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs and (K and L) U937 cells after siRNA transfection are shown. Expression levels of the indicated genes were determined using GAPDH for normalization. Data are expressed as fold change over untreated samples (A–I) or control siRNA untreated samples (K and L) and bar graphs represent means \pm SE of three independent experiments for (A)–(F) and (L) and four independent experiments for (G)–(I) and (K). Statistical analyses were performed using Student's t test between treated groups (*p < 0.05; **p < 0.01; ***p < 0.001).

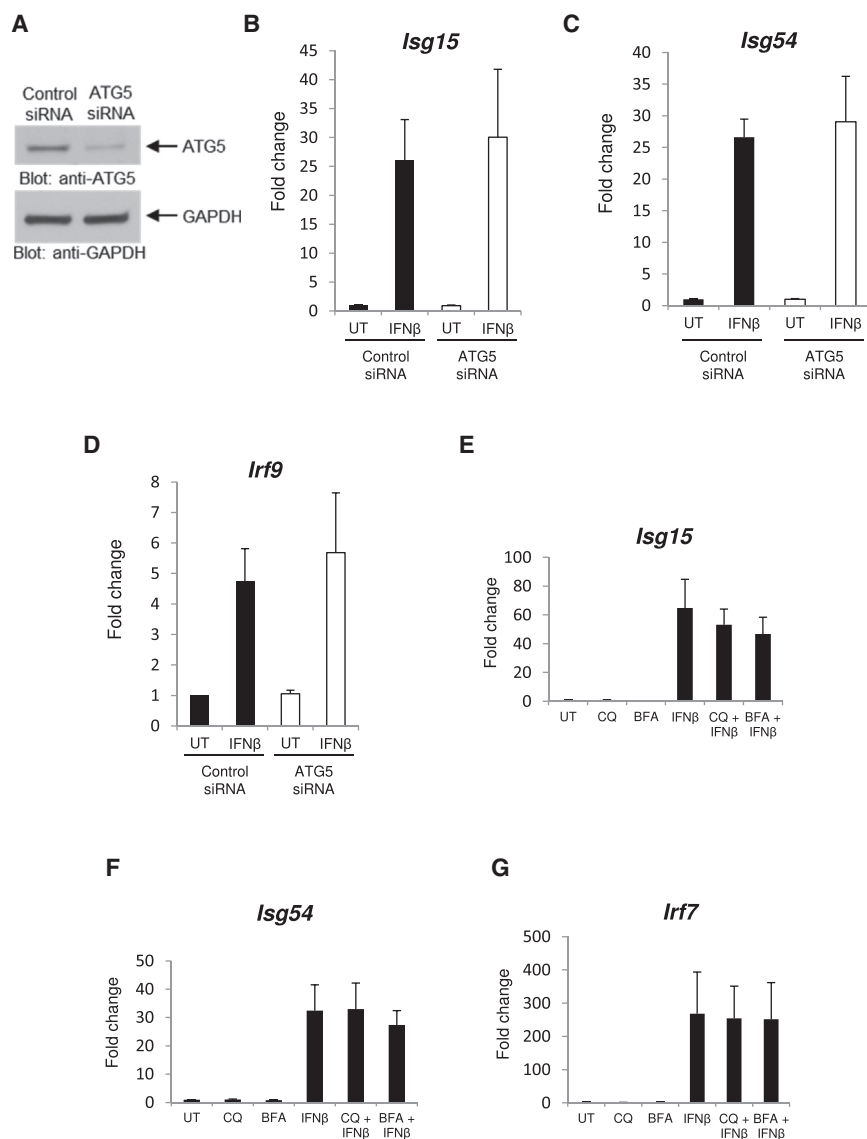


Figure 4. ULK1/2 Activity Regulates ISG Transcription in an Autophagy-Independent Manner

(A) Total cell lysates from MEFs transfected with either control siRNA or ATG5-specific siRNA were resolved by SDS-PAGE and immunoblotted with anti-ATG5 or anti-GAPDH-specific antibodies, as indicated.

(B–D) MEFs transfected with control siRNA or ATG5-specific siRNA were treated with mouse IFN β for 6 hr, and mRNA expression for the indicated genes was assessed by qRT-PCR, using GAPDH for normalization. Data are expressed as fold change over control siRNA untreated (UT) samples, and bar graphs represent means \pm SE of three independent experiments.

(E–G) MEFs were treated with chloroquine (CQ), bafilomycin A1 (BFA), and/or mouse IFN β . mRNA expression for the indicated genes was assessed by qRT-PCR, using GAPDH for normalization. Data are expressed as fold change over control untreated (UT) cells, and bar graphs represent means \pm SE of five independent experiments for (E) and (G) and three independent experiments for (F).

ULK1 Is Critical for IFN Regulation of Normal Hematopoiesis and the Generation of IFN Responses in Myeloproliferative Neoplasms

As type I IFNs are potent regulators of normal hematopoiesis (Platanias, 2005), in subsequent studies, we determined if engagement of ULK1 activity is necessary for the generation of growth-inhibitory responses on normal CD34⁺-derived hematopoietic precursors. For this purpose, we used specific siRNAs to knock down ULK1 expression in primary normal human bone marrow progenitors and examined the effects of this knockdown on the inhibitory effects of IFN α on CD34⁺-derived erythroid and myeloid precursors. As expected, treatment with human IFN α suppressed the growth of normal myeloid (granulocyte-macrophage colony-forming unit [CFU-GM]) and early erythroid (erythroid burst-forming unit [BFU-E]) progenitors in clonogenic assays in methylcellulose (Figure 7A). However, these suppressive effects were reversed by ULK1 knockdown (Figure 7A), indi-

cating key and essential roles for ULK1 in the control of normal hematopoiesis by type I IFNs.

In further studies, we examined whether the engagement of ULK1 by the type I IFNR is essential for generation of antineoplastic responses. It is well established that polycythemia vera (PV) and other Philadelphia-chromosome-negative myeloproliferative neoplasms (MPNs) are sensitive to type I IFN therapy, and type I IFN treatment is currently used for the treatment of such neoplasms (Tefferi and Vainchenker, 2011; Kiladjian et al., 2011; Cassinat et al., 2014). We determined whether ULK1 is required

for generation of type I IFN-dependent growth-inhibitory effects on malignant erythroid progenitors from patients with PV. When primary peripheral blood mononuclear PV cells were treated with IFN β , we observed induction of ULK1 phosphorylation on serine 757 (Figure 7B). Importantly, when the effects of type I IFN treatment on malignant erythroid progenitors from five different MPN patients were assessed, we found that siRNA-mediated targeted inhibition of ULK1 expression reversed the suppressive effects of IFN β on primitive malignant erythroid precursors in vitro (Figure 7C). Thus, ULK1 engagement via the type I IFNR appears to be essential for generation of antineoplastic effects in MPNs.

Next, we determined whether ULK1 expression is upregulated in the peripheral blood of MPN patients. Specific analysis for ULK1 gene expression from a previously reported microarray profiling study in neutrophils from a cohort of patients with chronic MPNs (Rampal et al., 2014) showed that ULK1

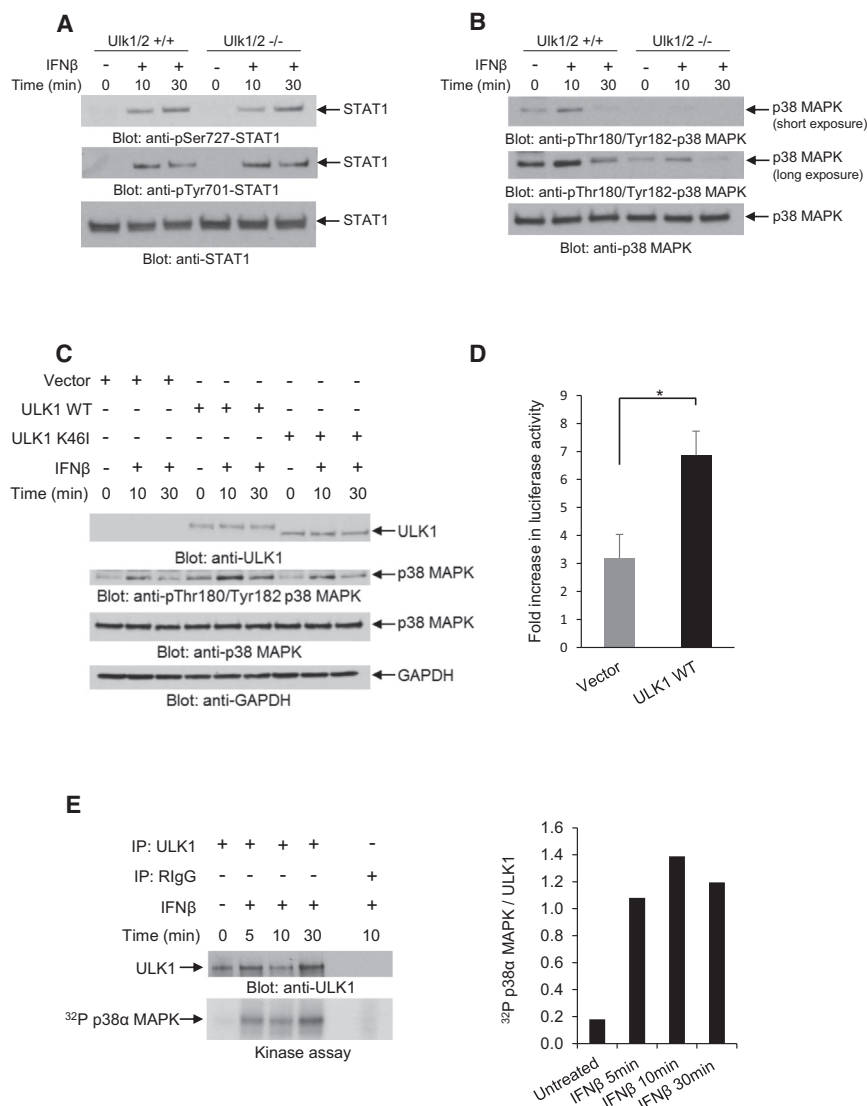


Figure 5. ULK1/2 Activity Is Required for Type I IFN-Dependent Activation of the p38 MAPK

(A) Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs were treated with mouse IFN β as indicated. Equal amounts of total cell lysates were resolved by SDS-PAGE and consecutively immunoblotted with antibodies against pSer727-STAT1, pTyr701-STAT1, and STAT1, as indicated.

(B) Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs were treated with mouse IFN β for 10 or 30 min, as indicated. Equal amounts of total cell lysates were resolved by SDS-PAGE and consecutively immunoblotted with antibodies against the phosphorylated form of p38 MAPK on Thr180/Tyr182 and against p38 MAPK. Short and longer exposures of p-Thr180/Tyr182 p38 MAPK from the same blot are shown.

(C) Ulk1/2^{-/-} MEFs were transfected with pcDNA6.2 empty vector (Vector) or ULK1 WT or ULK1-K46I (kinase inactive) plasmids, as indicated. 48 hr after transfection, the cells were treated with mouse IFN β for 10 and 30 min, as indicated. Equal amounts of total cell lysates were resolved by SDS-PAGE and consecutively immunoblotted with the indicated antibodies.

(D) Ulk1/2^{-/-} MEFs were transfected with pcDNA6.2 empty vector (Vector), or ULK1 WT plasmids, as indicated. 24 hr after transfection, these cells were transfected with an 8 \times GAS-luciferase construct. 42 hr later, the cells were incubated for 6 hr in the presence or absence of mouse IFN β , and luciferase activity was measured. Data are expressed as fold increase of luciferase activity in response to IFN β treatment over control untreated samples for each condition. Bar graphs show means \pm SE of three independent experiments using technical triplicates in each experiment. Statistical analyses were performed using Student's t test (*p < 0.05).

(E) U937 cells were starved overnight prior to IFN β treatment and then treated with human IFN β for 5, 10, and 30 min, as indicated. After cell lysis, equal amounts of protein were immunoprecipitated with either ULK1-specific antibody or control non-immune rabbit IgG (RlgG). In vitro kinase assays to

detect ULK1 activity were subsequently performed on the immunoprecipitates, using p38 α MAPK recombinant inactive protein as an exogenous substrate. (Left and top) Immunoblot demonstrating total immunoprecipitated ULK1 expression used in each condition for the in vitro kinase assay. (Left and bottom) Autoradiography film demonstrating ULK1-induced phosphorylation of p38 α MAPK after IFN β treatment is shown. Note: a lane between ULK1 and RlgG immunoprecipitates was loaded with 1 \times loading dye for best separation between the wells. (Right) Bands were quantified by densitometry using ImageJ software, and data are expressed as ratios of ³²P p38 α MAPK over total immunoprecipitated ULK1. See also Figure S2.

expression is increased in different groups of MPN patients, including patients with PV, essential thrombocythemia (ET), and myelofibrosis (MF) (Figure 7D). The upregulation of expression of ULK1 was also seen in another independent group of MPN patients (Figure 7E) using RT-PCR analysis for ULK1 mRNA expression, establishing upregulation of ULK1 expression in MPNs and suggesting a mechanism to explain the unique sensitivity of these neoplasms to the effects of type I IFNs.

DISCUSSION

Type I IFNs are cytokines with important biological effects in vitro and in vivo and have been used extensively in the treatment of

various malignancies, viral syndromes, and autoimmune disease in humans (Borden et al., 2007; Cheon et al., 2014; Plataniias, 2013). The important biological and therapeutic properties of type I IFNs reflect the induction of expression of key genes via the type I IFNR that mediate diverse biological responses, including antineoplastic, immune modulatory, and antiviral effects (Cheon et al., 2014; Kroczyńska et al., 2014). The precise mechanisms accounting for the transcriptional activation and mRNA translation of such ISGs have been the focus of extensive work that led to the original discovery of JAK-STAT pathways (Darnell et al., 1994; Plataniias, 2005; Stark and Darnell, 2012). Besides the classical JAK-STAT signaling cascades, the type I IFNR engages several other cellular pathways in normal and

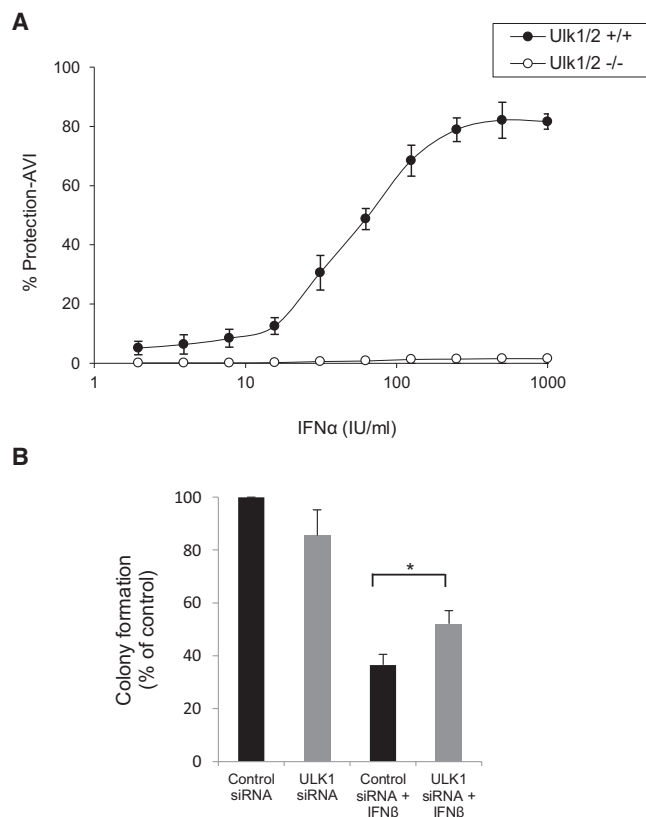


Figure 6. Regulation of Type I IFN Responses by ULK1/2 Activity

(A) Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs were seeded in quadruplicate in individual wells of 96-well plates and then treated with mouse IFN α for 16 hr, as indicated. Ulk1/2^{+/+} cells were subsequently challenged with a 1:2 \times 10⁴ dilution of encephalomyocarditis virus (EMCV) and the Ulk1/2^{-/-} cells with a 1:10⁶ dilution of EMCV. EMCV-induced cytopathic effects (CPEs) were determined 24 hr later. The data are expressed as percent protection from CPEs adjusted to viral infective dose (% protection-AVI). Values shown represent means \pm SD of two independent experiments. See also Figure S3.

(B) U937 cells were transfected with either control siRNA or ULK1 siRNA, and leukemic CFU-L colony formation was assessed in clonogenic assays in methylcellulose in the presence or absence of human IFN β , as indicated. Data are expressed as percent colony formation of control siRNA-transfected untreated cells, and bar graphs represent means \pm SE of five independent experiments. Statistical analysis was performed using Student's t test (* p < 0.05).

malignant cells (Platanias, 2005; van Boxel-Dezaire et al., 2006; González-Navajas et al., 2012), the functions of which are required for the generation of type I IFN-dependent biological responses (Platanias, 2005). Among them, the p38 MAPK cascade appears to act as an auxiliary pathway, necessary for optimal transcription of ISGs, without modulating elements of the STAT-pathway (Platanias, 2005). Despite a better understanding of the mechanisms of type I IFN signaling in the past decades, several questions have remained unanswered, particularly the mechanisms that define signaling specificity. Although, in some cases, selective use of the type I IFNR subunits may account for differential gene expression among distinct type I IFNs (de Weerd et al., 2013), the precise effectors of such

specific pathways and the potential interactions with other cytokine receptors remain to be defined (Kaur and Platanias, 2013). Also, there is a need to identify cellular elements linking pathways that control IFN-dependent gene transcription to the ones that regulate subsequent mRNA translation of ISGs, as this should allow better understanding of the mechanisms that account for specificity of expression of ISG products.

In the present study, we provide evidence that the kinase ULK1 is phosphorylated by engagement of the type I IFNR at serine 757, an mTORC1 phosphorylation site known to inhibit ULK1 in pathways that control the initiation of autophagy (Kim et al., 2011). Furthermore, our data show that ULK1 is activated after engagement of the type I IFNR and that its activated form can, either directly or through intermediate kinases, phosphorylate the p38 MAPK in immune complex kinase assays *in vitro*. This suggests that during type I IFN treatment, the pro-autophagic functions of ULK1 are blocked, and instead, ULK1 activity is possibly re-directed toward regulation of the p38 MAPK pathway. Consistent with this, type I IFN-inducible activation of the p38 MAPK pathway is defective in cells with targeted disruption of the Ulk1/2 genes and appears to result in defective downstream ISG transcription. Moreover, type I IFN-induced activation of p38 MAPK and transcriptional activation via GAS elements is restored by ectopic expression of ULK1 WT protein in Ulk1/2^{-/-} MEFs. Hence, it is possible that for optimal activation of p38 MAPK pathway in the type I IFN system, the activities of both ULK1 and MKK3/6 (Li et al., 2005) are required. Notably, the effects of ULK1 on ISG expression appear to reflect selective regulation of the p38 MAPK pathway, as functional engagement of STAT1 is intact in Ulk1/2^{-/-} cells.

In previous studies, we had demonstrated that AKT is required for mRNA translation of ISGs, but not ISG transcription (Kaur et al., 2008b). In the present study, we provide evidence that Akt1/2 activity is required for type I IFN-induced ULK1 phosphorylation at serine 757. Furthermore, given that transcription of ISGs is defective in Ulk1/2^{-/-} MEFs and in U937 cells in which ULK1 has been knocked down, it is possible that another type I IFN-activated kinase(s) act(s) upstream of ULK1 during engagement of the type I IFNR, and concomitant regulation of ULK1 by such kinase may be necessary for the transcriptional activity of ULK1. Some examples of possible kinases are PKC- δ and ERK1 kinases, which have been identified as potential kinases of ULK1 (Mack et al., 2012) and are activated downstream of type I IFNR (Platanias, 2005), but this remains to be determined in future studies.

The potential engagement of ULK1 in type I IFN signaling has important functional implications for the generation of the effects of type I IFNs. Our studies provide evidence for an involvement of ULK1 in the induction of both type I IFN-antiviral responses and growth-inhibitory activities. They also suggest key and essential roles for ULK1 in the generation of the suppressive regulatory effects of type I IFNs on normal hematopoiesis, by demonstrating that ULK1 knockdown reverses suppression of myeloid (CFU-GM) and early erythroid (BFU-E) hematopoietic progenitors. Most importantly, our data identify ULK1 as an essential element for the generation of the antineoplastic effects of type I IFNs on primitive malignant hematopoietic precursors from patients with PV, an MPN where IFN treatment has major

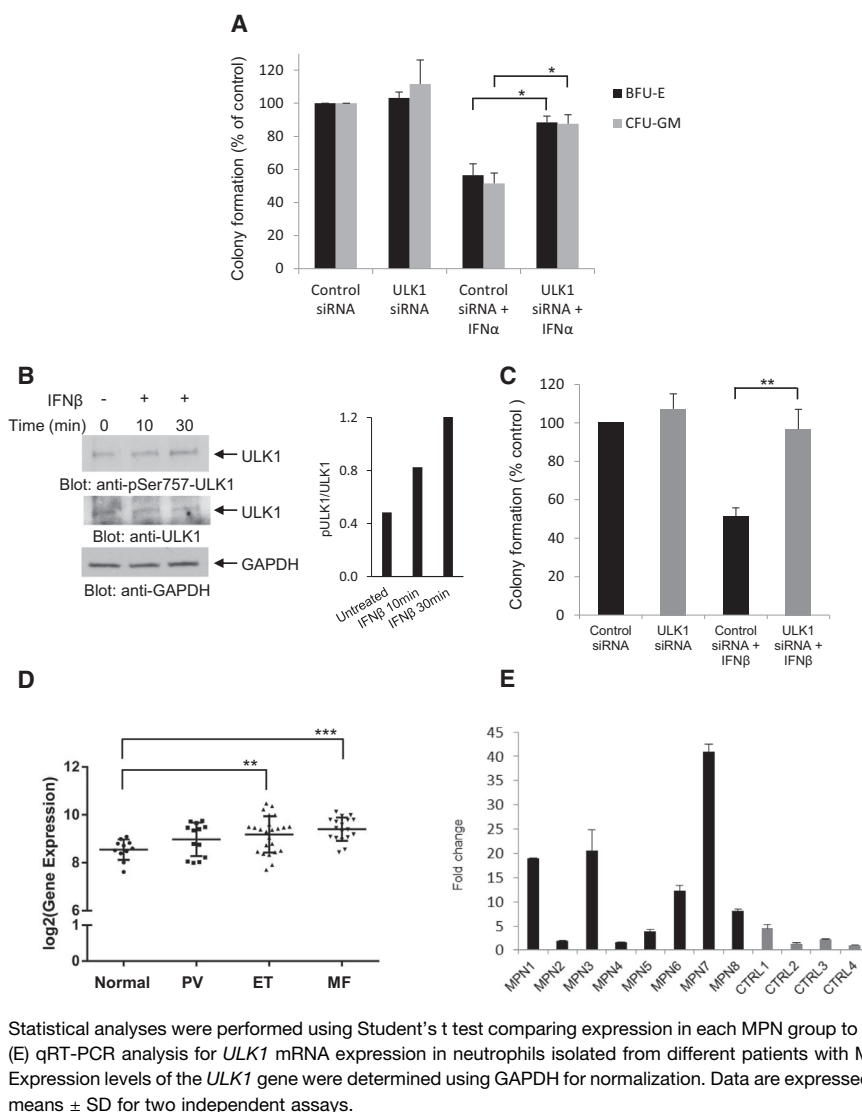


Figure 7. Requirement of ULK1 for the Regulatory Effects of Type I IFNs on Normal and Malignant Human Hematopoiesis

(A) Normal human bone marrow-derived CD34⁺ cells were transfected with either control siRNA or ULK1 siRNA and incubated in clonogenic assays in methylcellulose in the presence or absence of human IFN α , and myeloid (CFU-GM) and erythroid (BFU-E) progenitor colony formation was assessed. Data are expressed as percent colony formation of control siRNA-transfected untreated cells and represent means \pm SE of three independent experiments. Statistical analysis was performed using Student's t test (* $p < 0.05$).

(B) (Left) Serum-starved circulating primary peripheral blood mononuclear cells from a patient with PV were treated with human IFN β for 10 or 30 min, as indicated. Total cell lysates were resolved by SDS-PAGE and consecutively immunoblotted with p-S757 ULK1 and ULK1 antibodies. The immunoblot was also probed for GAPDH as a loading control. (Right) Bands were quantified by densitometry using ImageJ software, and data are expressed as ratios of p-ULK1/ULK1.

(C) Peripheral blood mononuclear cells from patients with PV were transfected with either control siRNA or ULK1 siRNA, and the effects of human IFN β on malignant erythroid (BFU-E) colony formation were assessed by clonogenic assays in methylcellulose. Data are expressed as percent colony formation of control siRNA-transfected untreated cells and represent means \pm SE of five independent experiments, using cells from five different patients with PV.

(D) Boxplot shows gene expression of *ULK1* in neutrophils in a large independent cohort of normal individuals (normal, $n = 11$) and patients with PV ($n = 13$), ET ($n = 24$), and MF ($n = 18$).

Statistical analyses were performed using Student's t test comparing expression in each MPN group to the normal group (** $p < 0.01$; *** $p < 0.001$). (E) qRT-PCR analysis for *ULK1* mRNA expression in neutrophils isolated from different patients with MPNs (MPN1-8) and age-matched controls (CTRL1-4). Expression levels of the *ULK1* gene were determined using GAPDH for normalization. Data are expressed as fold change over CTRL4, and bar graphs represent means \pm SD for two independent assays.

clinical activity (Tefferi and Vainchenker, 2011). Remarkably, when expression of *ULK1* mRNA was specifically analyzed in a large cohort of patients with different MPNs, we found significant increases in *ULK1* expression in different subtypes of MPNs, including PV, essential thrombocytosis, and myelofibrosis. There is prior evidence that the p38 MAPK pathway is involved in the generation of type I IFN-antileukemic effects (Mayer et al., 2001), while other studies have shown that p38 MAPK activation is essential for the generation of the inhibitory effects of type I IFNs on JAK2V617F-positive hematopoietic progenitor cells from MPN patients (Lu et al., 2010). Our findings suggest a mechanism by which *ULK1* and p38 MAPK are engaged in type I IFN-signaling in MPNs and, most importantly, provide an explanation for the unique sensitivity of these malignancies to the effects of type I IFNs, due to overexpression of *ULK1*.

It should be noted that in a recent study, *ULK1* was shown to inhibit STING activity, leading to inhibition of IRF3, and consequent suppression of type I IFN production (Konno et al., 2013).

These events appear to function as a negative-feedback control mechanism to prevent sustained transcription of ISGs (Konno et al., 2013) and limit development of IFN-dependent autoimmune inflammatory disorders (Gall et al., 2012). The results of our studies, taken in context with the report of Konno et al. (2013), suggest a dual regulatory role for *ULK1* in the control of type I IFN responses, acting as a "molecular switch" in the IFN system that regulates the balance and duration of IFN biological responses. In this model, *ULK1* appears to regulate directly early signals that control ISG expression and induction of type I IFN responses. At the same time, a more delayed *ULK1*-mediated effect appears to be the suppression of type I IFN production by suppressing STING activity, thus limiting/optimizing the response. The recognition of this unique role for *ULK1* should have important clinical-translational implications, as modulation of *ULK1* activity may be used as an approach to selectively enhance the activity of type I IFNs on MPN cells.

EXPERIMENTAL PROCEDURES

Materials and some of the methods can be found in the [Supplemental Experimental Procedures](#).

Cells and Cell Culture

U937, U266, and KT-1 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics. The immortalized Akt1/2^{+/+} and Akt1/2^{-/-} MEFs were kindly provided by Dr. Nissim Hay (University of Illinois at Chicago) (Peng et al., 2003). The immortalized Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs have been described previously (Cheong et al., 2011). MEFs were cultured in DMEM medium supplemented with 10% FBS and antibiotics. Peripheral blood from patients with PV was collected after obtaining consent approved by the institutional review board of Northwestern University. Additionally, blood samples were collected from patients with MPNs and controls at Albert Einstein School of Medicine, under an institutional review board-approved study.

Immunoblotting

Cells were treated, transfected, and lysed as described in [Supplemental Experimental Procedures](#). Equal amounts of total cell lysates were resolved by SDS-PAGE and processed for immunoblotting essentially as in our previous studies (Uddin et al., 1999; Kaur et al., 2007; Kroczyńska et al., 2009).

Luciferase Reporter Assays

Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs were co-transfected with a β -galactosidase expression vector and either an ISRE-luciferase or 8X GAS-luciferase construct. Luciferase activities were measured and normalized to β -galactosidase activity as in previous studies (Uddin et al., 1999). See [Supplemental Experimental Procedures](#) for a detailed description.

Microarray Analysis

Total RNA was isolated from Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs untreated or treated with IFN β (n = 3), and labeled cRNA was hybridized to MouseWG-6 v2.0 Expression BeadChips. See [Supplemental Experimental Procedures](#) for a detailed description.

qRT-PCR

qRT-PCR was carried out using commercially available 6-carboxyfluorescein-labeled probes and primers (Applied Biosystems). The mRNA amplification was calculated as described previously (Kaur et al., 2007), and the data were plotted as the increase of fold change as compared with control samples. See the [Supplemental Experimental Procedures](#) for a detailed description.

Immunoprecipitations and In Vitro Kinase Assays

In vitro kinase assays to detect ULK1 kinase activity in cells treated with IFN β were performed essentially as in previous studies (Kroczyńska et al., 2009). MAPK14 (p38 α MAPK) recombinant human inactive protein was used as an exogenous substrate. See the [Supplemental Experimental Procedures](#) for a detailed description.

Antiviral Assays

The antiviral effects of mouse IFN α on Ulk1/2^{+/+} and Ulk1/2^{-/-} MEFs were determined in assays using ECMV as the challenge virus, as in previous studies (Kaur et al., 2012).

Hematopoietic Cell Progenitor Assays

The effects of ULK1 knockdown were assessed in leukemic (CFU-L), erythroid (BFU-E), or myeloid (CFU-GM) colony formation using clonogenic assays in methylcellulose (STEMCELL Technologies) in the absence or presence of type I IFNs, as in previous studies (Mayer et al., 2001; Joshi et al., 2009; Kroczyńska et al., 2012; Mehrotra et al., 2013; Kaur et al., 2014). See the [Supplemental Experimental Procedures](#) for a detailed description.

Statistical Analyses

Student's t test was used for comparison of one observation between two groups. One-way ANOVA was used to compare more than two groups

followed by Tukey's test. Differences were considered statistically significant when p values were less than 0.05.

ACCESSION NUMBERS

The NCBI GEO accession number for the microarray data reported in this paper is GSE60778.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and six tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.03.056>.

AUTHOR CONTRIBUTIONS

D.S. and L.C.P. designed research; D.S., S.M., B.K., T.D.B., B.L.S., B.M., J.K.A., and B.M.-K. performed research; D.S., S.M., E.M.B., B.K., E.M.K., P.L., C.J., N.J., E.N.F., A.K.V., and R.L.L. analyzed data; D.P.B. and C.B.T. provided key materials; D.S. and L.C.P. wrote the manuscript; and L.C.P. conceived the project.

ACKNOWLEDGMENTS

We thank Dr. Nissim Hay (University of Illinois at Chicago) for the Akt1/2^{+/+} and Akt1/2^{-/-} MEFs. We also thank Dr. Reuben Shaw (The Salk Institute for Biological Studies, La Jolla, CA) for providing the ULK1 plasmids through Addgene. This work was supported by grants CA77816, CA155566, CA161196, and CA121192 from the NIH, by a Merit review grant from the Department of Veterans Affairs, and by grant DPN/MOB109/II/2012. D.P.B. is an employee of BiogenIdec and owns BiogenIdec stock.

Received: October 3, 2014

Revised: February 16, 2015

Accepted: March 25, 2015

Published: April 16, 2015

REFERENCES

- Alers, S., Löffler, A.S., Wesselborg, S., and Stork, B. (2012). The incredible ULKs. *Cell Commun. Signal.* 10, 7.
- Alp, N.J., and Channon, K.M. (2004). Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. *Arterioscler. Thromb. Vasc. Biol.* 24, 413–420.
- Ambjørn, M., Ejlerskov, P., Liu, Y., Lees, M., Jäättelä, M., and Issazadeh-Navikas, S. (2013). IFN β /interferon- β -induced autophagy in MCF-7 breast cancer cells counteracts its proapoptotic function. *Autophagy* 9, 287–302.
- Bach, M., Larance, M., James, D.E., and Ramm, G. (2011). The serine/threonine kinase ULK1 is a target of multiple phosphorylation events. *Biochem. J.* 440, 283–291.
- Bekisz, J., Sato, Y., Johnson, C., Husain, S.R., Puri, R.K., and Zoon, K.C. (2013). Immunomodulatory effects of interferons in malignancies. *J. Interferon Cytokine Res.* 33, 154–161.
- Borden, E.C., Sen, G.C., Uze, G., Silverman, R.H., Ransohoff, R.M., Foster, G.R., and Stark, G.R. (2007). Interferons at age 50: past, current and future impact on biomedicine. *Nat. Rev. Drug Discov.* 6, 975–990.
- Cassinat, B., Verger, E., and Kiladjian, J.J. (2014). Interferon alfa therapy in CALR-mutated essential thrombocythemia. *N. Engl. J. Med.* 371, 188–189.
- Cheon, H., Borden, E.C., and Stark, G.R. (2014). Interferons and their stimulated genes in the tumor microenvironment. *Semin. Oncol.* 41, 156–173.
- Cheong, H., Lindsten, T., Wu, J., Lu, C., and Thompson, C.B. (2011). Ammonia-induced autophagy is independent of ULK1/ULK2 kinases. *Proc. Natl. Acad. Sci. USA* 108, 11121–11126.

- Colina, R., Costa-Mattioli, M., Dowling, R.J., Jaramillo, M., Tai, L.H., Breitbach, C.J., Martineau, Y., Larsson, O., Rong, L., Svitkin, Y.V., et al. (2008). Translational control of the innate immune response through IRF-7. *Nature* **452**, 323–328.
- 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.
- de Weerd, N.A., Vivian, J.P., Nguyen, T.K., Mangan, N.E., Gould, J.A., Braniff, S.J., Zaker-Tabrizi, L., Fung, K.Y., Forster, S.C., Beddoe, T., et al. (2013). Structural basis of a unique interferon- β signaling axis mediated via the receptor IFNAR1. *Nat. Immunol.* **14**, 901–907.
- Egan, D.F., Shackelford, D.B., Mihaylova, M.M., Gelino, S., Kohnz, R.A., Mair, W., Vasquez, D.S., Joshi, A., Gwinn, D.M., Taylor, R., et al. (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* **331**, 456–461.
- Gall, A., Treuting, P., Elkon, K.B., Loo, Y.M., Gale, M., Jr., Barber, G.N., and Stetson, D.B. (2012). Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. *Immunity* **36**, 120–131.
- García, M.A., Gil, J., Ventoso, I., Guerra, S., Domingo, E., Rivas, C., and Esteban, M. (2006). Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action. *Microbiol. Mol. Biol. Rev.* **70**, 1032–1060.
- González-Navajas, J.M., Lee, J., David, M., and Raz, E. (2012). Immunomodulatory functions of type I interferons. *Nat. Rev. Immunol.* **12**, 125–135.
- Hervas-Stubbs, S., Perez-Gracia, J.L., Rouzaut, A., Sanmamed, M.F., Le Bon, A., and Melero, I. (2011). Direct effects of type I interferons on cells of the immune system. *Clin. Cancer Res.* **17**, 2619–2627.
- Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N., Ohba, Y., Takaoka, A., Yoshida, N., and Taniguchi, T. (2005). IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* **434**, 772–777.
- Hunn, J.P., Koenen-Waisman, S., Papic, N., Schroeder, N., Pawlowski, N., Lange, R., Kaiser, F., Zerrahn, J., Martens, S., and Howard, J.C. (2008). Regulatory interactions between IRG resistance GTPases in the cellular response to *Toxoplasma gondii*. *EMBO J.* **27**, 2495–2509.
- Ivashkiv, L.B., and Donlin, L.T. (2014). Regulation of type I interferon responses. *Nat. Rev. Immunol.* **14**, 36–49.
- Joshi, S., Kaur, S., Redig, A.J., Goldsborough, K., David, K., Ueda, T., Watanabe-Fukunaga, R., Baker, D.P., Fish, E.N., Fukunaga, R., and Platanius, L.C. (2009). Type I interferon (IFN)-dependent activation of Mnk1 and its role in the generation of growth inhibitory responses. *Proc. Natl. Acad. Sci. USA* **106**, 12097–12102.
- Kaur, S., and Platanius, L.C. (2013). IFN- β -specific signaling via a unique IFNAR1 interaction. *Nat. Immunol.* **14**, 884–885.
- Kaur, S., Lal, L., Sassano, A., Majchrzak-Kita, B., Srikanth, M., Baker, D.P., Petroulakis, E., Hay, N., Sonenberg, N., Fish, E.N., and Platanius, L.C. (2007). Regulatory effects of mammalian target of rapamycin-activated pathways in type I and II interferon signaling. *J. Biol. Chem.* **282**, 1757–1768.
- Kaur, S., Sassano, A., Joseph, A.M., Majchrzak-Kita, B., Eklund, E.A., Verma, A., Brachmann, S.M., Fish, E.N., and Platanius, L.C. (2008a). Dual regulatory roles of phosphatidylinositol 3-kinase in IFN signaling. *J. Immunol.* **181**, 7316–7323.
- Kaur, S., Sassano, A., Dolniak, B., Joshi, S., Majchrzak-Kita, B., Baker, D.P., Hay, N., Fish, E.N., and Platanius, L.C. (2008b). Role of the Akt pathway in mRNA translation of interferon-stimulated genes. *Proc. Natl. Acad. Sci. USA* **105**, 4808–4813.
- Kaur, S., Sassano, A., Majchrzak-Kita, B., Baker, D.P., Su, B., Fish, E.N., and Platanius, L.C. (2012). Regulatory effects of mTORC2 complexes in type I IFN signaling and in the generation of IFN responses. *Proc. Natl. Acad. Sci. USA* **109**, 7723–7728.
- Kaur, S., Kroczyńska, B., Sharma, B., Sassano, A., Arslan, A.D., Majchrzak-Kita, B., Stein, B.L., McMahon, B., Altman, J.K., Su, B., et al. (2014). Critical roles for Rictor/Sin1 complexes in interferon-dependent gene transcription and generation of antiproliferative responses. *J. Biol. Chem.* **289**, 6581–6591.
- Kiladjian, J.J., Mesa, R.A., and Hoffman, R. (2011). The renaissance of interferon therapy for the treatment of myeloid malignancies. *Blood* **117**, 4706–4715.
- Kim, J., Kundu, M., Viollet, B., and Guan, K.L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132–141.
- Klionsky, D.J., Elazar, Z., Seglen, P.O., and Rubinsztein, D.C. (2008). Does bafilomycin A1 block the fusion of autophagosomes with lysosomes? *Autophagy* **4**, 849–850.
- Konno, H., Konno, K., and Barber, G.N. (2013). Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. *Cell* **155**, 688–698.
- Kotredes, K.P., and Gamera, A.M. (2013). Interferons as inducers of apoptosis in malignant cells. *J. Interferon Cytokine Res.* **33**, 162–170.
- Kroczyńska, B., Kaur, S., Katsoulidis, E., Majchrzak-Kita, B., Sassano, A., Kozma, S.C., Fish, E.N., and Platanius, L.C. (2009). Interferon-dependent engagement of eukaryotic initiation factor 4B via S6 kinase (S6K)- and ribosomal protein S6K-mediated signals. *Mol. Cell. Biol.* **29**, 2865–2875.
- Kroczyńska, B., Sharma, B., Eklund, E.A., Fish, E.N., and Platanius, L.C. (2012). Regulatory effects of programmed cell death 4 (PDCD4) protein in interferon (IFN)-stimulated gene expression and generation of type I IFN responses. *Mol. Cell. Biol.* **32**, 2809–2822.
- Kroczyńska, B., Mehrotra, S., Arslan, A.D., Kaur, S., and Platanius, L.C. (2014). Regulation of interferon-dependent mRNA translation of target genes. *J. Interferon Cytokine Res.* **34**, 289–296.
- Kundu, M., Lindsten, T., Yang, C.Y., Wu, J., Zhao, F., Zhang, J., Selak, M.A., Ney, P.A., and Thompson, C.B. (2008). Ulk1 plays a critical role in the autophagic clearance of mitochondria and ribosomes during reticulocyte maturation. *Blood* **112**, 1493–1502.
- Lee, E.J., and Tournier, C. (2011). The requirement of uncoordinated 51-like kinase 1 (ULK1) and ULK2 in the regulation of autophagy. *Autophagy* **7**, 689–695.
- Lenschow, D.J., Lai, C., Frias-Staheli, N., Giannakopoulos, N.V., Lutz, A., Wolff, T., Osiak, A., Levine, B., Schmidt, R.E., Garcia-Sastre, A., et al. (2007). IFN-stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. *Proc. Natl. Acad. Sci. USA* **104**, 1371–1376.
- Li, Y., Sassano, A., Majchrzak, B., Deb, D.K., Levy, D.E., Gaestel, M., Nebreda, A.R., Fish, E.N., and Platanius, L.C. (2004). Role of p38alpha Map kinase in Type I interferon signaling. *J. Biol. Chem.* **279**, 970–979.
- Li, Y., Batra, S., Sassano, A., Majchrzak, B., Levy, D.E., Gaestel, M., Fish, E.N., Davis, R.J., and Platanius, L.C. (2005). Activation of mitogen-activated protein kinase kinase (MKK) 3 and MKK6 by type I interferons. *J. Biol. Chem.* **280**, 10001–10010.
- Liu, X.Y., Chen, W., Wei, B., Shan, Y.F., and Wang, C. (2011). IFN-induced TPR protein IFIT3 potentiates antiviral signaling by bridging MAVS and TBK1. *J. Immunol.* **187**, 2559–2568.
- Lu, M., Zhang, W., Li, Y., Berenzon, D., Wang, X., Wang, J., Mascarenhas, J., Xu, M., and Hoffman, R. (2010). Interferon-alpha targets JAK2V617F-positive hematopoietic progenitor cells and acts through the p38 MAPK pathway. *Exp. Hematol.* **38**, 472–480.
- Mack, H.I., Zheng, B., Asara, J.M., and Thomas, S.M. (2012). AMPK-dependent phosphorylation of ULK1 regulates ATG9 localization. *Autophagy* **8**, 1197–1214.
- Mayer, I.A., Verma, A., Grumbach, I.M., Uddin, S., Lekmine, F., Ravandi, F., Majchrzak, B., Fujita, S., Fish, E.N., and Platanius, L.C. (2001). The p38 MAPK pathway mediates the growth inhibitory effects of interferon-alpha in BCR-ABL-expressing cells. *J. Biol. Chem.* **276**, 28570–28577.
- McAllister, C.S., and Samuel, C.E. (2009). The RNA-activated protein kinase enhances the induction of interferon-beta and apoptosis mediated by cytoplasmic RNA sensors. *J. Biol. Chem.* **284**, 1644–1651.

- Mehrotra, S., Sharma, B., Joshi, S., Kroczyńska, B., Majchrzak, B., Stein, B.L., McMahon, B., Altman, J.K., Licht, J.D., Baker, D.P., et al. (2013). Essential role for the Mnk pathway in the inhibitory effects of type I interferons on myeloproliferative neoplasm (MPN) precursors. *J. Biol. Chem.* **288**, 23814–23822.
- Mizushima, N., Yamamoto, A., Hatano, M., Kobayashi, Y., Kabeya, Y., Suzuki, K., Tokuhiwa, T., Ohsumi, Y., and Yoshimori, T. (2001). Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J. Cell Biol.* **152**, 657–668.
- Peng, X.D., Xu, P.Z., Chen, M.L., Hahn-Windgassen, A., Skeen, J., Jacobs, J., Sundararajan, D., Chen, W.S., Crawford, S.E., Coleman, K.G., and Hay, N. (2003). Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. *Genes Dev.* **17**, 1352–1365.
- Platanias, L.C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat. Rev. Immunol.* **5**, 375–386.
- Platanias, L.C. (2013). Interferons and their antitumor properties. *J. Interferon Cytokine Res.* **33**, 143–144.
- Rampal, R., Al-Shahrouf, F., Abdel-Wahab, O., Patel, J.P., Brunel, J.P., Mermel, C.H., Bass, A.J., Pretz, J., Ahn, J., Hricik, T., et al. (2014). Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood* **123**, e123–e133.
- Rani, M.R., Shrock, J., Appachi, S., Rudick, R.A., Williams, B.R., and Ransohoff, R.M. (2007). Novel interferon-beta-induced gene expression in peripheral blood cells. *J. Leukoc. Biol.* **82**, 1353–1360.
- Russell, R.C., Tian, Y., Yuan, H., Park, H.W., Chang, Y.Y., Kim, J., Kim, H., Neufeld, T.P., Dillin, A., and Guan, K.L. (2013). ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat. Cell Biol.* **15**, 741–750.
- Schmeisser, H., Mejido, J., Balinsky, C.A., Morrow, A.N., Clark, C.R., Zhao, T., and Zoon, K.C. (2010). Identification of alpha interferon-induced genes associated with antiviral activity in Daudi cells and characterization of IFIT3 as a novel antiviral gene. *J. Virol.* **84**, 10671–10680.
- Schmeisser, H., Bekisz, J., and Zoon, K.C. (2014). New function of type I IFN: induction of autophagy. *J. Interferon Cytokine Res.* **34**, 71–78.
- Sharma, S., tenOever, B.R., Grandvaux, N., Zhou, G.P., Lin, R., and Hiscott, J. (2003). Triggering the interferon antiviral response through an IKK-related pathway. *Science* **300**, 1148–1151.
- Stark, G.R., and Darnell, J.E., Jr. (2012). The JAK-STAT pathway at twenty. *Immunity* **36**, 503–514.
- Stein, B.L., and Tiu, R.V. (2013). Biological rationale and clinical use of interferon in the classical BCR-ABL-negative myeloproliferative neoplasms. *J. Interferon Cytokine Res.* **33**, 145–153.
- Tefferi, A., and Vainchenker, W. (2011). Myeloproliferative neoplasms: molecular pathophysiology, essential clinical understanding, and treatment strategies. *J. Clin. Oncol.* **29**, 573–582.
- Uddin, S., Majchrzak, B., Woodson, J., Arunkumar, P., Alsayed, Y., Pine, R., Young, P.R., Fish, E.N., and Platanias, L.C. (1999). Activation of the p38 mitogen-activated protein kinase by type I interferons. *J. Biol. Chem.* **274**, 30127–30131.
- Uddin, S., Lekmine, F., Sharma, N., Majchrzak, B., Mayer, I., Young, P.R., Bokoch, G.M., Fish, E.N., and Platanias, L.C. (2000). The Rac1/p38 mitogen-activated protein kinase pathway is required for interferon alpha-dependent transcriptional activation but not serine phosphorylation of Stat proteins. *J. Biol. Chem.* **275**, 27634–27640.
- van Boxel-Dezaire, A.H., Rani, M.R., and Stark, G.R. (2006). Complex modulation of cell type-specific signaling in response to type I interferons. *Immunity* **25**, 361–372.
- Yang, Z., Liang, H., Zhou, Q., Li, Y., Chen, H., Ye, W., Chen, D., Fleming, J., Shu, H., and Liu, Y. (2012). Crystal structure of ISG54 reveals a novel RNA binding structure and potential functional mechanisms. *Cell Res.* **22**, 1328–1338.
- Zhang, H.M., Yuan, J., Cheung, P., Chau, D., Wong, B.W., McManus, B.M., and Yang, D. (2005). Gamma interferon-inducible protein 10 induces HeLa cell apoptosis through a p53-dependent pathway initiated by suppression of human papillomavirus type 18 E6 and E7 expression. *Mol. Cell. Biol.* **25**, 6247–6258.
- Zhu, J., Zhang, Y., Ghosh, A., Cuevas, R.A., Forero, A., Dhar, J., Ibsen, M.S., Schmid-Burgk, J.L., Schmidt, T., Ganapathiraju, M.K., et al. (2014). Antiviral activity of human OASL protein is mediated by enhancing signaling of the RIG-I RNA sensor. *Immunity* **40**, 936–948.