The SKIV2L RNA exosome limits activation of the RIG-I-like receptors

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Sensors of the innate immune system that detect intracellular nucleic acids must be regulated to prevent inappropriate activation by endogenous DNA and RNA. The exonuclease Trex1 regulates the DNA-sensing pathway by metabolizing potential DNA ligands that trigger it. However, an analogous mechanism for regulating the RIG-I-like receptors (RLRs) that detect RNA remains unknown. We found here that the SKIV2L RNA exosome potently limited the activation of RLRs. The unfolded protein response (UPR), which generated endogenous RLR ligands through the cleavage of cellular RNA by the endonuclease IRE-1, triggered the production of type I interferons in cells depleted of SKIV2L. Humans with deficiency in SKIV2L had a type I interferon signature in their peripheral blood. Our findings reveal a mechanism for the intracellular metabolism of immunostimulatory RNA, with implications for specific autoimmune disorders.

Antiviral immunity is initiated in virus-infected cells by sensors of the innate immune system that detect viral nucleic acids. These sensors must detect viral RNA or DNA among a vast excess of cellular RNA and DNA, which presents the challenge of discriminating self from non-self and the risk of inappropriate immune responses to self nucleic acids. The specificity of these innate antiviral sensors is achieved mainly by the detection of unique structural features that distinguish viral nucleic acids. For example, the RNA helicase RIG-I binds to 5′-triphosphate RNA that is present in the genomes of many classes of RNA viruses but is scarce in host cells. Similarly, the RNA helicase Mda5 is activated by long, double-stranded viral RNA that is not routinely formed in host cells. For the sensing of intracellular DNA by the interferon-stimulatory DNA (ISD) pathway, the mechanisms for the discrimination of self from non-self are less clear, because the known sensors of DNA are activated in a sequence-independent fashion by nearly any double-stranded DNA. Indeed, published crystal structures of several intracellular DNA receptors have revealed that most molecular contacts with immunostimulatory DNA are made with the sugar-phosphate backbone and not with specific bases.

The metabolism of intracellular nucleic acids has been identified as an essential mechanism for limiting activation of the ISD pathway. The 3′-repair exonuclease Trex1 was identified in a biochemical screen for ISD-binding proteins. Loss-of-function mutations in the human gene TREX1 cause Aicardi-Goutières syndrome (AGS), a severe type I interferon–associated autoimmune disease. Studies of Trex1-deficient mice as a model of AGS have defined Trex1 as an essential negative regulator of the ISD pathway. Moreover, reverse-transcribed cDNAs of endogenous retroelements accumulate in Trex1-deficient cells, and Trex1 is a potent antiretroviral enzyme. Such studies have provided a framework for understanding the pathogenic mechanisms of AGS and related diseases and have revealed an important source of endogenous intracellular nucleic acids that, if they are not properly metabolized, can trigger sensors of the innate immune system that detect DNA.

Given those findings, we sought to determine whether an analogous mechanism exists to metabolize intracellular RNA for regulation of the RIG-I-like receptors (RLRs) and whether there is a source of relevant endogenous immunostimulatory RNAs that, when they accumulate, trigger the RLRs. We found that the cytosolic 3′-to-5′ RNA exosome, defined by the presence of the RNA helicase SKIV2L, was an important negative regulator of the RLR-mediated antiviral response. We identified the RNA-cleavage products of the endonuclease IRE-1 (‘inositol-requiring enzyme 1’) as immunostimulatory RNAs that, upon activation of the unfolded protein response (UPR), activated the RLR pathway in cells depleted of SKIV2L. We found that type I interferon signature in the peripheral blood cells of SKIV2L-deficient humans. Our findings reveal a mechanism that might contribute to interferon-associated autoimmune diseases.

RESULTS

SKIV2L limits the RLR antiviral response

We began our exploration of potential negative regulators of the RNA-activated antiviral response by considering the ubiquitous pathways for RNA degradation that mediate the turnover of mRNAs and elimination of incompletely spliced RNA transcripts. These pathways are...
often initiated by endonucleolytic cleavage of the RNA, followed by degradation of the two resulting products by distinct enzyme complexes. The exonuclease XRN1 metabolizes RNA in the 5′-to-3′ direction, and the RNA exosome degrades RNA in the 3′-to-5′ direction. The RNA exosome is a multiprotein complex composed of several core factors associated with key accessory proteins that determine its subcellular localization and RNA substrate specificity. The RNA exosome responsible for the turnover of RNA in the cytoplasm of human cells is formed by the RNA helicase SKIV2L together with additional subunits that are well characterized in yeast (SKI3 and SKI8) but remain poorly defined in humans. A published study has identified SKIV2L as a gene potentially linked to susceptibility to the autoimmune disorder systemic lupus erythematosus in humans. We established stable, robust knockdown of SKIV2L or XRN1 in primary mouse bone marrow–derived macrophages (BMDMs) through the use of lentivirus-delivered short hairpin RNA (shRNA) and confirmed depletion of these proteins by immunoblot analysis (Fig. 1a). We stimulated these cells by transfection of a pure triphosphate RNA ligand (h) or poly(I:C) (d) with a triphosphate RNA ligand of RIG-I (b) or poly(I:C) (c); results are presented relative to those of untreated cells. (d–f) Quantitative RT-PCR analysis of IFN-β mRNA in primary mouse macrophages stably transfected with shRNA as in a (key) and stimulated for various times (horizontal axis) with a triphosphate RNA ligand of RIG-I (d) or poly(I:C) (e); results presented as in a. *P < 0.05, **P < 0.001, ***P < 0.0005 and ****P < 0.0001 (two-way analysis of variance (ANOVA)). Data are representative of at least three independent experiments with biological triplicates (mean and s.d. in b–h).

These data suggested that SKIV2L was a specific negative regulator of the RNA-activated RLR response.

The UPR triggers interferons in cells depleted of SKIV2L

We considered the potential existence of endogenous immunostimulatory RNAs that, after they accumulate in cells, might trigger the RLRs. We hypothesized that the UPR might provide a source of such RNAs. The UPR is a stress response that is activated when the burden of newly synthesized polypeptides in the endoplasmic reticulum (ER) exceeds its protein-folding capacity. Activation of the UPR induces the expression of genes that encode ER protein chaperones, ER membrane–biosynthetic enzymes and the ER-associated protein-degradation machinery and thus restores homeostasis by increasing the size and functional capacity of the ER. One key component of the UPR is the ER-resident transmembrane kinase and endoribonuclease IRE-1 (refs. 23,24). The ER-luminal domain of IRE-1 is activated directly by misfolded proteins, which leads to the activation of its cytosolic RNA-endonuclease domain. The main role of IRE-1 in induction of the UPR is to catalyze the removal of a small intron from mRNA encoding the transcription factor XBP1 by precisely cleaving this mRNA at each end of the intron. The resulting exons are then spliced by tRNA ligase; this newly spliced mRNA encodes functional XBP1 protein, which migrates to the nucleus and induces the expression of dozens of UPR-inducible genes. In addition to its role in splicing XBP1 mRNA, IRE-1 in metazoan organisms also mediates the proteosome degradation of many mRNAs localized near the ER. This regulated IRE-1-dependent RNA decay transiently reduces the burden of newly synthesized proteins entering the ER and protects it from further proteotoxic stress. Endonuclease cleavage by IRE-1...
generates RNAs with a 3′ cyclic phosphate moiety, and these RNAs are efficiently cleared by the SK12 RNA exosome30. Notably, RNAs containing 3′ cyclic phosphates are able to activate RLRs32,33. Moreover, a published study has found that activation of the UPR by cholera toxin triggers an IRE-1-dependent, RIG-I-mediated inflammatory response34. On the basis of such findings, we reasoned that activation of the UPR in cells depleted of SKIV2L might result in the accumulation of endogenous RNA-cleavage products generated by IRE-1 that could elicit an ectopic type I interferon response.

We knocked down SKIV2L or XRN1 in immortalized mouse embryonic fibroblasts (MEFs) and then stimulated these cells with thapsigargin, a chemical inhibitor of the sarcoplasmic reticulum-endoplasmic reticulum calcium ATPase SERCA, which depletes the ER of its calcium stores and triggers a potent UPR. Upon induction of the UPR, we found that cells depleted of SKIV2L activated substantial production of IFN-β that we did not observe in either cells depleted of XRN1 or cells transduced with control shRNA with a scrambled guide RNAs targeting Ern1 (above lanes). (b) RT-PCR analysis of splicing of XBP-1 mRNA (right margin; as in Fig. 2c) in immortalized MEFs transduced as in (a) (left margin) and treated for various times with thapsigargin (above lanes) with thapsigargin or tunicamycin (TM). Below (and right margin), PstI digestion site in the intron of unspliced XBP-1 mRNA, used to distinguish spliced lanes) with thapsigargin or tunicamycin (TM). Below (and right margin), PstI digestion site in the intron of unspliced XBP-1 mRNA, used to distinguish spliced (right margin) from unspliced cDNA (U1 and U2). bp, base pairs. (d,e) Quantitative RT-PCR analysis of IFN-β mRNA in primary mouse macrophages transduced with shRNA (key) and treated with thapsigargin (d) or tunicamycin (e); results presented as in a. *P < 0.05 and **P < 0.0001 (two-way ANOVA). Data are representative of three experiments (a; mean and s.d.) or at least three independent experiments with biological triplicates (b–e; mean and s.d. in d,e).

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the UPR by causing the accumulation of unglycosylated proteins in the ER\(^{21}\). We treated macrophages with thapsigargin or tunicamycin and monitored the kinetics of activation of the UPR by tracking the IRE-1-dependent splicing of XBP1 mRNA\(^{35}\). We found that thapsigargin-induced splicing of XBP1 mRNA was nearly complete at 2 h after treatment and returned to baseline by 24 h (Fig. 2c). In contrast, tunicamycin-induced splicing of XBP1 mRNA occurred with delayed kinetics, commencing at 6 h and continuing through 24 h (Fig. 2c). Notably, both thapsigargin and tunicamycin activated IFN-β production in cells depleted of SKIV2L with kinetics that precisely mirrored the activation of IRE-1 (Fig. 2d,e). Thus, two mechanistically distinct activators of the UPR triggered a substantial antiviral response in cells depleted of SKIV2L.

We next investigated whether the enhanced UPR-induced interferon response in cells depleted of SKIV2L required IRE-1 activity. To do this, we used the genome-editing approach based on clustered regularly interspaced short palindromic repeats (CRISPR) and the endonuclease Cas9 (refs. 36,37) to target the endogenous gene Ern1\(^{38}\). We developed a construct similar to one described in a published report\(^{39}\) that enables coexpression of a guide RNA and Cas9 from a single lentiviral vector. We targeted coding exons of Ern1 in immortalized MEFs with two different guide RNAs and found nearly complete modification of the target sequences in these cells several days after selection for transduced cells (Supplementary Fig. 1). The targeted cells had reduced expression of IRE-1α protein and had severely compromised UPR-induced splicing of XBP1 mRNA compared with that of control cells transduced to express Cas9 alone (Fig. 3a,b), which demonstrated extensive loss of IRE-1 function in the targeted cells. We next knocked down SKIV2L in the control cells transduced to express Cas9 alone and in the cells in which Ern1 was targeted and stimulated these cells with thapsigargin to induce the UPR. Depletion of SKIV2L in the control cells resulted in an enhanced UPR-induced interferon response; however, this enhancement was significantly blunted in both lines of cells in which Ern1 was targeted (Fig. 3c).

To formally establish a role for signaling via RLRs in the enhanced antiviral response in cells depleted of SKIV2L, we compared the induction of IFN-β expression in wild-type BMDMs with that of BMDMs deficient in the signaling adaptor MAVS, which lack RIG-I and Mda5-induced production of type I interferons\(^{39}\). As expected, the interferon response to transfection of RIG-I ligand in wild-type cells, as well as the enhanced interferon response in cells depleted of SKIV2L, was entirely dependent on MAVS (Fig. 3d). Similarly, the enhanced response to transfection of poly(I:C) in cells depleted of SKIV2L required MAVS, with the residual induction of interferon in MAVS-deficient cells probably mediated by the Toll-like receptor 3–TRIF pathway\(^{40}\) (Fig. 3e). Notably, we found that the induction of interferon during activation of the UPR in cells depleted of SKIV2L was also dependent on MAVS (Fig. 3f). Together these data revealed SKIV2L as an important negative regulator of the RLR response to exogenous RNA ligands. Moreover, we identified the UPR as a source of endogenous RNA ligands that triggered an ectopic antiviral response in SKIV2L-deficient cells through an IRE-1α- and MAVS-dependent pathway.

**SKIV2L-deficient humans have a type I interferon signature**

We next explored a role for SKIV2L in regulating the type I interferon response in humans. Loss-of-function mutations in the human gene SKIV2L cause trichohepatoenteric syndrome (THES; OMIM entry number 222470)\(^{41}\). THES is an extremely rare disease characterized by growth retardation, facial dysmorphism, immunodeficiency, abnormalities of the liver and intestines and intractable diarrhea that...
Different roles for SKIV2L and TTC37 in RLR regulation

Given the presence of an interferon signature in SKIV2L-deficient patients with THES and its absence in TTC37-deficient patients with the same disease, we sought to determine whether SKIV2L has a unique function in the regulation of RLRs that is distinct from its role in the classic cytosolic RNA exosome that contains both SKIV2L and TTC37. We therefore established stable and efficient knockdown of TTC37 in primary mouse macrophages by lentivirus-delivered shRNA (Fig. 5a). We found that depletion of SKIV2L markedly enhanced the interferon response to transfected RLR ligand. In contrast, macrophages depleted of TTC37 exhibited an interferon response similar to that of cells transduced with control shRNA with a scrambled sequence (Fig. 5b). Similarly, knockdown of SKIV2L enhanced the interferon response to thapsigargin-induced activation of the UPR, but knockdown of TTC37 had no effect (Fig. 5c). Together with the ISG data presented above from patients with THES (Fig. 4), these findings revealed a unique role for SKIV2L in the regulation of RLR responses distinct from its role in the SKIV2L-TTC37 cytosolic RNA exosome.

DISCUSSION

Our data have extended the paradigm of negative regulation of cell-intrinsic innate antiviral responses first described for the DNA-sensing pathway by identifying a key negative regulator of RLRs that detect RNA. We found that the cytosolic RNA exosome, defined by the presence of the RNA helicase SKIV2L, was important for limiting the activation of RLRs. We identified the UPR as a cellular stress response that generated endogenous RLR ligands, and we showed that cells undergoing a UPR triggered an aberrant interferon-mediated antiviral response if these endogenous RNAs were not metabolized. We found that humans with deficiency in SKIV2L had a strong type I interferon signature in their peripheral blood, indicative of a chronic antiviral response that may be relevant to human autoimmune disease. Finally, we presented evidence obtained from human patients and mouse cells that the role of SKIV2L in the regulation of RLRs was independent of another exosome protein, TTC37. Together these findings reveal a previously unknown mechanism for the regulation of RLRs and emphasize the importance of intracellular metabolism of nucleic acids for preventing aberrant innate immune responses.

We found that cells depleted of SKIV2L had an enhanced IFN-β response to RLR ligands, but cells depleted of XRN1 did not. Perhaps more relevant to autoimmune disease, we identified the RNA-degradation products of IRE-1 generated during the UPR as a source of endogenous immunostimulatory RNAs that triggered a MAVS-dependent interferon response in cells depleted of SKIV2L but not in wild-type cells. Notably, this finding revealed a mechanism by which a ‘sterile’ stress response might be misinterpreted as a viral infection if cells fail to metabolize the RNA-degradation products formed as a result of the stress. The UPR-activated RLR response in cells depleted of SKIV2L was robust and significant but was much less potent than the response to transfected triphosphate RNA. This probably reflected the number of RIG-I ligands introduced by transfection: 1 μg of transfected RIG-I ligand contains approximately $1 \times 10^{13}$ molecules, which would amount to $\sim 10 \times 10^6$ molecules delivered per cell in our experimental system. In contrast, IRE-1-mediated cleavage of cellular mRNAs would generate far fewer endogenous RIG-I ligands. Moreover, it is also likely that 3′-cyclic-phosphate RNAs are less efficient activators of RLRs than are 5′-triphosphate RNAs. Despite the relatively weaker interferon response triggered by the UPR, we propose that such an interferon response in a chronic setting would be sufficient to drive substantial pathology.

Our findings suggest a potential previously unknown mechanism for the pathogenesis of certain autoimmune diseases that affect highly secretory cells that routinely undergo ER stress, including Sjögren’s syndrome (which affects salivary and lacrimal glands) and type 1 diabetes (which affects insulin-secreting beta cells of the exocrine pancreas). We propose a simple model in which regular, episodic...

Figure 5 Different roles for SKIV2L and TTC37 in the regulation of RLRs. (a) Immunoblot analysis of TTC37 in lysates of primary BMDMs treated with control or TTC37-specific shRNA. (b,c) Quantitative RT-PCR analysis of IFN-β mRNA in cells transduced with lentivirus-delivered shRNA (key) and treated for 2 h with thapsigargin (b) or for 4 h with RIG-I ligands (c); results are presented relative to those of untreated cells. *P < 0.01 (one-way ANOVA). Data are representative of two independent experiments (a) or two independent experiments with biological triplicates (b,c; mean and s.d.).
activation of the UPR would generate endogenous RIG-I ligands. In people with deficiencies in their ability to metabolize these RNAs, this UPR would trigger a chronic, inappropriate antiviral response, leading to activation of the immune system and eventual destruction of the cells over time. Notably, our model emphasizes the role of the secretory cells themselves as active participants in the autoimmune process. By activating the cell-intrinsic antiviral response, these cells become the targets of an adaptive immune response, similar to the manner in which virus-infected cells are targeted for elimination by cytotoxic T cells.

We showed that human patients with deficiency in SKIV2L had a potent interferon signature in their peripheral blood cells and have thus provided evidence for the relevance of this regulatory mechanism to human disease. Our findings raise two questions. First, does this interferon signature contribute to the pathogenesis of THES? Given the lack of an interferon signature in TTC37-deficient patients with THES, we propose that most of the symptoms of this severe disease are the consequence of loss of the "housekeeping" function of the cytosolic RNA exosome in the general turnover of RNA rather than the aberrant interferon response found only in SKIV2L-deficient patients. Second, given the strong interferon signature, why do SKIV2L-deficient patients not develop autoimmunity? Notably, some patients with THES also exhibit immunodeficiency and require supplemental immunoglobulins, which suggests an essential role for the RNA exosome in lymphocyte function. Indeed, we found that the survival of cells in which SKIV2L was knocked down was compromised after stimulation, which precluded several experiments we attempted, including infection with RNA viruses (data not shown). We suggest that defective lymphocyte function in patients with THES prevents overt autoimmune disease despite the potent interferon signature evident in their peripheral blood cells. However, the immunological aspects of THES have thus far been incompletely defined, in large part because of the extreme rarity of this disease and the severity of the other symptoms. It will be informative to further explore potential autoimmune phenotypes in THES and their different manifestations in patients with SKIV2L mutations and those with TTC37 mutations, given the findings presented above.

We have presented evidence that SKIV2L contributed to the regulation of RLRs but the other defined component of the cytosolic RNA exosome, TTC37, did not. The subcellular localization and RNA substrate specificity of the RNA exosome are determined by its associated cofactors, such that the exosome associated with SKIV2L and TTC37 is involved mainly in cytosolic RNA decay. Moreover, the RNA exonucleases that also associate with the core exosome components can each mediate degradation of unique subsets of RNAs in cells. We propose that the role for SKIV2L identified here is distinct from its involvement in the conventional cytosolic RNA exosome, and future studies should determine whether there are proteins that partner with SKIV2L that are uniquely involved in the regulation of RLRs. Such proteins may serve to target SKIV2L specifically to immunostimulatory RNAs.

In summary, we have identified a regulatory mechanism in which the metabolism of intracellular RNA ligands limited the activation of RLRs. We propose that deficient function of this regulatory mechanism would predispose a person to autoimmune diseases, particularly those that affect highly secretory cells. The identification of other components of this pathway might provide insight into the pathogenesis of certain interferon-associated autoimmune disorders.

METHODS

Methods and any associated references are available in the online version of the paper.
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ONLINE METHODS

Mice and cells. All mice used were on an inbred C57BL/6 background, and C57BL/6 mice were from the Jackson Laboratories. Mavs−/− mice (provided by M. Gale, Jr.) were generated on a C57BL/6 background as described 13. BMDMs were harvested from leg bones and were cultured for 8 d in complete RPMI medium supplemented with macrophage colony-stimulating factor. Wild-type controls for all experiments were age-matched C57BL/6 mice. Mice used were 4–20 weeks of age, and litters were used when available. All mice were maintained in accordance with guidelines of the University of Washington Institutional Animal Care and Use Committee. HEK293T human embryonic kidney cells were obtained from the American Type Culture Collection. Fibroblasts immortalized with SV40 large T antigen were generated by retroviral transduction of primary MEFs. All cell lines tested negative for mycoplasma contamination.

Cell treatments and analysis. RIG-I ligand was transfected and purified in vitro as described with the T7 Megashortscript kit (Ambion) 17. The PU/UC A template sequence is in Supplementary Table 1. BMDMs were plated at a density of 0.7 × 10^6 in 12-well plates for transfection. For transfection, 5 µg poly(I:C) or 1 µg RIG-I ligand were made into a complex with Lipofectamine 2000 (Life Technologies) at a ratio of 1 µg nucleic acid to 1 µl lipid in a final volume of 1 ml. Final concentrations of 30 µg/ml DMXAA (Sigma-Aldrich), 10 ng/ml lipopolysaccharide (Sigma-Aldrich), 500 nM thapsigargin (Life Technologies) and 2.5 µg/ml tunicamycin (Santa Cruz Biotechnology) were added directly to the culture medium. For quantitative RT-PCR analysis of IFN-β mRNA, cells were harvested into RNA-Bee isolation reagent (Tel-Test). RNA was treated with DNase (Ambion) and primed with oligo(dT), then was reverse-transcribed with Superscript III (Life Technologies). cDNA was used for PCR with Eva Green reagents (Bio-Rad Laboratories) on a Bio-Rad CFX96 Real-Time System. The abundance of each cytokine mRNA was normalized to that of HPRT mRNA and results were compared with those of untreated cells transduced with the same shRNA for calculation of relative induction. Primers are in Supplementary Table 1.

Knockdown with lentivirus-delivered shRNA and CRISPR. SKIV2L-specific, TTC37-specific and control shRNA constructs were designed and then were cloned into the plKO.1 vector. XRN1-specific shRNA was designed and then was cloned into the retroviral MSCV-LMP vector. SKIV2L was targeted by the sense sequence 5′-GGCGATATGGGATGTATCTGAAT-3′. TTC37 was targeted by a combination of two plasmids, 5′-AGAAGATTATGTGCGCTGCCTT-3′ (sense) and 5′-TCAGAGATATCCGCAGCTTCAATT-3′ (sense). XRN1 was targeted by the sense sequence 5′-GAGTAGCTTCTAGAGATAA-3′. The control shRNA targeted sequence encoding enhanced green fluorescent protein (5′-CAACAGATGGAGGACCCAA-3′). Knockdown of protein was confirmed by immunoblot analysis of whole-cell extracts with mouse monoclonal antibodies according to standard techniques. The antibodies were as follows: anti-SKIV2L (11462-1-AP; Proteintech Group), anti-TTC37 (ab122421; Abcam), anti-XRN1 (sc-98459; Santa Cruz Biotechnology) and anti-actin (AC-74; Sigma).

For knockdown of SKIV2L, XRN1 and TTC37 in BMDMs, lentivirus pseudotyped with vesicular stomatitis virus envelope glycoprotein was produced by transfection of 2.5 × 10^6 HEK293T cells for 48 h in 10-cm plates with 10 µg of the shRNA knockdown construct, 9 µg pSPAX-2 (a lentiviral packaging plasmid with a gene encoding a protein for resistance to puromycin) and 1 µg pSVSV-G (plasmid encoding vesicular stomatitis virus envelope glycoprotein). 4 × 10^6 BMDMs were transduced with HEK293T viral supernatants on days 3 and 4 after harvest, then were selected for 3 d with 5 µg/ml puromycin (Life Technologies) and then plated at equal numbers for treatments (described above).

For targeting of Ern1 with CRISPR-Cas9, we developed a lentivirus vector similar to one already published 18 in which an RNA polymerase III promoter–driven guide RNA and an RNA polymerase II promoter–driven Cas9-T2A cassette (including sequence encoding a protein for resistance to blasticidin) were constitutively expressed from a single, self-inactivating lentivirus upon integration into the host cell genome. Immortalized MEFs were transduced and selected as described above, and targeting of the Ern1 locus via CRISPR was evaluated by restriction fragment length polymorphism with a restriction site that overlapped the CRISPR targeting site (Ern1 guide 1, BsaJ; Ern1 guide 2, MluI). Products were separated by electrophoresis through a 3% MetaPhor agarose gel (Lonza; Supplementary Fig. 1). The sequences of the guide RNA target sequences were as follows: Ern1 RNA guide 1 (sense), 5′-GCTTTGTTTGGTTCTCAGCCC-3′; Ern1 guide RNA 2 (sense), 5′-GGGGAGGCTTGAACCAATT-3′.

Splicing assay. RT-PCR analysis of Xbp1 splicing was done as described 35. cDNA was obtained as described above. Products were amplified with sense primer mXBP1.3S (5′-AAACAGAGTTAGCGGCAGACTG-3′) and antisense primer mXBP1.2AS (5′-GGATCTCTAAAACCTAGAGCCTTCTT-3′). PCR products were then digested with PstI and separated by electrophoresis through a 3% MetaPhor agarose gel (Lonza).

Human interferon signature scores. Peripheral blood samples were obtained from Humans who provided informed, written consent, then the samples were processed and analyzed for the expression of six human ISGs as described 44. The collection of samples from patients with AGS was approved by the Leeds (East) Research Ethics Committee (10/H1307/132). The collection of samples from patients with THES was approved by the South Birmingham Research Ethics Committee, and by the French ministry of Health (AC-2011-1312).

Statistical analysis. Quantitative RT-PCR data were analyzed with Graphpad Prism software by two-way ANOVA. Differences with a P value of <0.05 were considered significant.