The RNA binding protein La promotes RIG-I-mediated type I and type III IFN responses following Sendai viral infection

Title

The RNA binding protein La/SSB promotes RIG-I-mediated type I and type III IFN responses following Sendai viral infection

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Conflict of Interest Statement

The authors have no financial conflict of interest with the work and results presented herein.

Abstract

La/SS-B (or La) is a 48 kDa RNA-binding protein and an autoantigen in autoimmune disorders such as systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS). La involvement in regulating the type I interferon (IFN) response is controversial - acting through both positive and negative regulatory mechanisms; inhibiting the IFN response and enhancing viral growth, or directly inhibiting viral replication. We therefore sought to clarify how La regulates IFN production in response to viral infection. ShRNA knockdown of La in HEK 293T cells increased Sendai virus infection efficiency, decreased IFN-β, IFN-λ1, and interferon-stimulated chemokine gene expression. In addition, knockdown attenuated CCL-5 and IFN-λ1 secretion. Thus, La has a positive role in enhancing type I and type III IFN production. Mechanistically we show that La directly binds RIG-I and have mapped this interaction to the CARD domains of RIG-I and the N terminal domain of La. In addition, we showed that this interaction is induced following RIG-I activation and that overexpression of La enhances RIG-I-ligand binding. Together, our results demonstrate a novel role for La in mediating RIG-I-driven responses downstream of viral RNA detection, ultimately leading to enhanced type I and III IFN production and positive regulation of the anti-viral response.

Introduction

Host viral detection systems rely mostly on recognition of viral nucleic acids by pattern recognition receptors (PRRs) including, RNA and DNA-sensing Toll-like receptors (TLR-3, -7, -8, -9), DNA receptors (DAI, AIM2, IFI16, DDX41) and RIG-I-like receptors (RLRs). RIG-I is an essential type I and type III IFN-inducing receptor required for the detection of negative-sense single stranded RNA viruses such Sendai virus, a member of the *Paramyxoviridae* family, in addition to *Rhabdoviridae* and *Orthomyxoviridae* family members [1] [2,3]. Upon recognition of pathogenic RNA, an ATP-dependent conformational change is triggered in RIG-I exposing the activatory CARD domains. This allows interaction between the second CARD domain of the receptor and the CARD domain of downstream mitochondrial-associated adaptor, IPS-1 [4-6]. This interaction leads to assembly and activation of downstream IKK-related kinases TBK-1 and IKK-ε, that subsequently phosphorylate IRF-3 and IRF-7 [7,8]. This ultimately results in transcriptional induction of both type I and type III IFNs, which in turn leads to robust expression of IFN-stimulated genes (ISGs) [9,10].

Type I IFNs, including IFN-α, -β, -ω, -κ and -ε, act on cells via binding to the IFN-α receptor (IFNαR), comprised of an IFNαR1 and IFNαR2 heterodimer [11,12]. Type I IFN synthesis occurs in virtually all cell types downstream of anti-viral PRR recognition of viral RNA/DNA. Once secreted by the virally-infected cell, type I IFNs bind and activate IFNαR, leading to induction of interferon stimulated genes (ISGs) through activation of JAK1 and Tyk2, followed by phosphorylation of signal-transducing activators of transcription (STAT) proteins STAT1 and STAT2 [13-16]. ISGs, including RIG-I, TLR-3, OAS1 and OAS2, are expressed following STAT1/STAT2 activation, leading to the inhibition of transcription and translation of viral proteins [17,18], along with induction and synthesis of MHC class I expression. This makes the cell more susceptible to $CD8^+$ cytotoxic T cells [19,20], activates NK cells which selectively kill virus-infected cells [21,22], and leads to maturation of DCs [23] and B cell responses [20,24].

Functional members of the Type III IFN family, including IFN-λ1 (IL-29), IFN-λ2 (IL-28A) and IFN-λ3 (IL-28B), are induced downstream of TLR-3 and RLR signalling [25,26] but signal through an independent cell-surface receptor complex, consisting of IL10R2 (also called CRF2-4) and IFN-λR1 (also called IL-28RA) [27,28]. While the type I IFN receptor is ubiquitously expressed, the expression of the IFN-

λR1 component of the type III IFN receptor complex appears to be more limited and restricted to cells of epithelial origin, plasmacytoid DCs, macrophages, monocyte-derived DCs and intra-hepatic natural killer cells (NKs) [29]. Upon type III IFN binding to the receptor, a signal transduction cascade ensues involving activation of JAK1, JAK2 and Tyk2, followed by STATs activation and ISG expression, almost identical to that induced by type I IFN receptor [27,30].

Whilst anti-viral TLRs and RLRs are well recognised for their role in inducing type I and type III IFNs, more recently RNA polymerase III (RNA pol III), an enzyme involved in the transcription of non-coding RNA, was reported to act as an anti-viral PRR by regulating type I IFN induction through generation of a RIG-I ligand [31,32]. RNA pol III is able to transcribe AT-rich dsDNA into the 5'ppp-dsRNA format required for recognition by RIG-I and subsequent IFN induction [32]. Interestingly, an autoantigen associated with systemic autoimmune disease, La/SSB (La), binds to RNA pol III transcripts and stabilises newly-synthesised RNAs [33-38]. In addition to its interaction with a large variety of newly-formed RNAs, La binds a number of virus-encoded RNAs, such as adenovirus VA RNA I and VA RNA II, EBV EBER 1 & 2 RNA, and leader RNA of negative strand RNA viruses [39-42]. Because La can interact with viral RNA, studies have sought to clarify its role in anti-viral immunity. Some studies proposed that La is manipulated by viruses in an attempt to block the anti-viral response, which it reportedly achieves by binding and sequestering the dsRNA ligand for RIG-I, thus preventing activation of the pathway [43-45]. On the other hand, La was also shown to promote an anti-viral response to *flock house* virus (FHV), although the mechanism involved was unclear [46]. Thus the role of La in regulating anti-viral immune responses is not well understood.

Our work described herein demonstrates a novel positive role for La in regulating type I and type III IFN responses downstream of Sendai virus infection. Our results show that knockdown of La severely impairs the ability of cells to mount an anti-viral response to Sendai virus infection, resulting in enhanced infectivity, as a result of reduced type I and III IFN production. We observed that La bound RIG-I in a ligand-inducible manner and that the CARD domains of RIG-I and RNA-binding domain of La are required for this interaction. The association between La and RIG-I promotes the interaction of RIG-I with dsRNA, thereby enhancing RIG-I-driven type I and type III IFN induction. Thus, La is required for an optimum IFN response to

Sendai virus infection by binding to the anti-viral RIG-I receptor and promoting its interaction with its cognate ligand.

Results

La depletion results in enhanced Sendai virus infection efficiency and decreased Type I and Type III Interferon responses

Sendai virus (SeV) strains are enveloped paramyxoviruses with single-stranded, non-segmented, negative sense RNA genomes. SeV strains may vary significantly in their degrees of virulence. A number of virulence factors have been mapped to either structural proteins associated with differential virus attachment and entry into host cells, or non-structural proteins implicated in immune modulation that includes antagonism of interferon signalling [47-50]. This study utilized a recombinant SeV expressing eGFP (rSeV/eGFP) and SeV *Cantell* strain, both capable of inducing type III IFN responses, while only the *Cantell* strain induces an additional robust type I IFN response [51,52]. Evidence suggests that this differential induction of type I IFN is due to the presence of defective-interfering (DI) particles in the *Cantell* strain [53,54].

To investigate the effect La knockdown on Sendai infectivity, HEK 293T cells infected with rSeV/eGFP and transfected with either a La-specific or scrambled shRNA were evaluated by UV microscopy, using areas of comparable monolayer confluency for image analyses (Figure 1). Supplemental Figure 1 demonstrates successful La depletion in HEK 293T cells, both at gene (Supplemental Figure 1a & b) and protein (Supplemental Figure 1c & d) levels, validating the La shRNA construct used throughout this work. Analysis of fluorescence (using Image J software) demonstrated higher eGFP coverage following rSeV/eGFP infection in cells depleted of La compared with those transfected with the scrambled control, suggesting that knockdown of La enhanced viral infectivity (Figure 1a and b). Importantly, the increased eGFP coverage seen in La-depleted monolayers was reflected in an increase in viral titres released from these cells, compared with control cells (Figure 1c). As La has been published to be involved in a number of cellular processes, including RNAi processing, the viability of cells transfected with scrambled or La shRNA was compared in order to ensure that La knockdown did not affect cell viability. As shown in Figure 1d, cell viability was equivalent across the 2 different experimental conditions.

We next investigated the effect of La knockdown in HEK 293T cells on type I and type III IFN induction by quantitative PCR (qPCR) following rSeV/eGFP or SeV

Cantell infection. As stated above, rSeV/eGFP induced a strong type III IFN response but no type I IFN, whereas the SeV *Cantell* induced a robust type I IFN response in addition to type III IFNs [51,52]. La knockdown resulted in significant reduction of both IFN-β and IFN-λ1 mRNA levels following SeV *Cantell* infection (Figure 2a and b), whereas a reduction in only IFN-λ1 expression was observed in La-depleted cells following infection with rSeV/eGFP, albeit at the later time point of 48 hours post-infection (hpi) (Figure 2e and f). Importantly, La knockdown had no effect on housekeeping gene expression as shown in supplemental Figure 1e. Interestingly, expression of CXCL-10 (IP-10) was significantly attenuated by La depletion following infection with both SeV strains, compared with scrambled controls (Figure 2d and h). CXCL-11 was only impaired in La-depleted cells following *Cantell* infection (Figure 2c and g), suggesting that CXCL-11 induction is specifically regulated by La in the context of a type I IFN response. In contrast, the induction of CXCL-11 in response to rSeV/eGFP was poor, with La depletion having no effect on the cells' ability to mount a response.

Analysis of cytokine release following SeV infection demonstrated that CCL-5 (RANTES, a type I and III-regulated chemokine) production was impaired following SeV *Cantell* infection in La-depleted cells (Figure 3a). Unsurprisingly, given that ISG expression was only induced at mRNA level following 48 hpi, no CCL-5 release was detected following infection with rSeV/eGFP strain across the 48 hour time course of infection, nor was any significant difference observed in La-depleted cells, compared with scrambled controls (Figure 3c). IFN-λ1 release was completely abrogated in La-depleted cells, compared with controls, following infection with either SeV strain, further supporting our findings suggesting that La is crucial for the IFN response downstream of viral infection (Figure 3b and d). Assessing the effect of La knockdown on proinflammatory cytokine production in response to SeV *Cantell* infection demonstrated that La knockdown had little or no effect on the ability of the *Cantell* strain to induce IL-8, IL-6 or TNF-α (Figure 3e-g). Importantly, our results demonstrate not only that La positively regulates type I IFN responses downstream of SeV infection, but that it also has a novel role in promoting type III I IFN induction downstream of SeV infection.

La enhances RIG-I binding to RNA ligand via direct interaction with the CARD-domain of RIG-I

SeV *Cantell* has been reported to rely entirely on RIG-I to elicit an anti-viral immune response [55]. Having demonstrated that La is required for type I and III IFN production in response to SeV challenge, and given its ability to bind RNA, we hypothesised that La may directly regulate RIG-I activation through regulation of RNA binding. To test this hypothesis, HEK 293T cells were transfected with FLAG-tagged RIG-I and increasing concentrations of La from 0-2 µg. Cell lysates were incubated with 1 µg biotin-labelled poly(I:C), and poly(I:C)-binding proteins were subsequently isolated. The ability of RIG-I to bind poly(I:C) was determined by western blotting using anti-FLAG antibody. As Figure 4a shows, increasing concentrations of La enhanced the ability of RIG-I to bind poly(I:C). A faint lower band on the gel (corresponding to La) indicates, as would be expected from previous reports, that La was also capable of direct interaction with the RNA ligand. The lower panel of Figure 4a demonstrates total RIG-I and La expression in the lysates; endogenous La can be observed strongly in the lane without FLAG-tagged La overexpressed, due to blotting with anti-La antibody. However, transfection with increasing concentrations of La (lanes 2-5) dose-dependently increases expression, as expected. Full blots as well as statistical analysis of corresponding optical densitometry across three individual experiments are shown in Supplemental Figure 2.

Having observed enhanced binding between RIG-I and its RNA ligand in the presence of over-expressed La, we hypothesised that La may achieve this through direct binding to RIG-I. Co-immunoprecipitation studies demonstrated an inducible interaction between La and RIG-I following stimulation of RIG-I overexpressing HEK 293T cells with the RIG-I agonist, 5'ppp-dsRNA (Figure 4b, Supplemental Figure 3). This inducible interaction was statistically significant across three independent experiments (Supplemental Figure 3d). In addition, HeLa cells over-expressing GFP-La and flag-tagged RIG-I were stimulated with 5'ppp-dsRNA, which induces La translocation from the nucleus to the cytoplasm where it can co-localise and interact with RIG-I (Figure 4c). Additional confocal images demonstrating this pattern are shown in Supplemental Figure 4 and overlap coefficients for each image are given in Supplemental Table S4.1. The translocation of La from the nucleus to the cytoplasm is consistent with data from previous studies that demonstrate similar translocation of La following viral infection [44,45,56]. In addition, cells depleted of La had a reduced

response to 5'ppp-dsRNA in their ability to induce IFN-β, confirming the ability of La to directly promote RIG-I induced IFN-β expression (Supplemental Figure 3c).

In order to determine the domains responsible for this interaction, either full-length or N-terminal-only (aa 1-204) His-tagged recombinant La was incubated with lysates from HEK 293T cells over-expressing full-length RIG-I, the CARD domain-only of RIG-I, or the helicase domain-only of RIG-I. Potential interactions were analysed by western blotting. As Figure 4d (upper panel) demonstrates, an interaction was observed between full-length La and full-length RIG-I, as expected, but also with the RIG-I deletion mutant expressing only the CARDs. No interaction was observed with helicase-only mutant of RIG-I. This indicates that the activatory CARD domains (spanning amino acids 1-284) of RIG-I are necessary and sufficient for the interaction between La and RIG-I to occur. Further analysis demonstrated that the N-terminal but not the C-terminal domain of La is required for interaction with both full length and CARD domains of RIG-I (Figure 4d, lower panel).

Collectively, our results indicate a novel role for La as a positive regulator of type I and type III IFN production in response to SeV infection. We demonstrate that the mechanism of this regulation occurs through a direct interaction between La and RIG-I, which promotes RIG-I binding to its cognate ligand. Our findings not only contribute to the understanding of molecular mechanisms behind RIG-I-mediated regulation of IFN induction, but also provide valuable insight into the potential that dysregulation of La activity may contribute to over-activation of RIG-I and hence dysregulated IFN production, as observed in autoimmune diseases such as SLE.

Discussion:

The induction of IFN expression is a crucially important part of the innate anti-viral immune response, not only for destruction of viral RNA and limitation of viral spread, but also for activation of adaptive immunity and selective killing of virally-infected host cells. With this work we have demonstrated a novel interaction between La and RIG-I, which results in enhanced RIG-I-RNA association. Knockdown of La resulted in increased Sendai viral infection efficiency, decreased IFN-β, IFN-λ1 and ISG mRNA expression and attenuated CCL-5 and IFN-λ1 release, compared with control cells. Overall, these findings highlight an essential and novel role for La in mediating optimal type I and type III IFN responses following viral challenge in order to protect the host by both limiting viral replication and promoting the clearance of the pathogen.

Type I IFNs are the first line of defence against most types of viral infection, including the murine pathogen, SeV (DI^+). They induce an anti-viral state in host cells. This is achieved by JAK/STAT pathway-mediated activation of interferon-stimulated genes (ISGs), such as RIG-I, CXCL-10, CXCL-11, OAS1 and OAS2 [57]. Our findings demonstrated a significant decrease in the induction of IFN-β, CXCL-10, and CXCL-11 following SeV infection upon depletion of La. While type III IFNs are structurally and genetically distinctive from type I IFNs and act through a separate receptor system, they have similar mechanisms of induction, signal transduction and biological function [26,58]. SeV is a potent inducer of type III IFN responses [27,59]. Our study identifies La as a novel positive regulator of IFN-λ1 induction downstream of SeV infection. Collectively our results indicate an important role for La in inhibiting SeV replication by promoting both type I (as seen following *Cantell* infection) and type III (as seen following *Cantell* and rSeV/eGFP) IFN responses.

RIG-I is central to the regulation of both type I and type III IFN production as it is responsible for detection of SeV infection within cells. Regarding regulation of RIG-I activity, a number of proteins have been identified to play a role through post-translational modifications. For example, TRIM25 and Riplet/RNF135/REUL induce K63-linked ubiquitination within the CARD domains of RIG-I following viral infection, a modification which is necessary for interaction with IPS-1 [60-62]. In addition, CK2-mediated phosphorylation of RIG-I at Thr 770 and Ser 854 inhibits the anti-viral

response to both hepatitis C virus and SeV and renders RIG-I inactive [63]. This prevents TRIM25-mediated ubiquitination of RIG-I, thereby negatively regulating the IFN response [60]. With this work, we have identified a novel function for the autoantigen La in enhancement of anti-viral responses. Specifically, it binds directly to the RIG-I receptor in an inducible manner and strengthens RIG-I binding to its RNA ligand, making it unique in its mechanism of action from other known RIG-I regulators, such as TRIM25 and CK2. Thus La positively regulates type I and type III IFN responses by augmenting stable RNA-RIG-I complex formation, which results in robust pathway activation. As RIG-I can also drive inflammatory gene expression through interaction with a IPS-1-CARD9-Bcl-10 complex and activation of NFκB [64], it would appear that the interaction between La and RIG-I is able to enhance the IFN-β response (presumably via enhancing interaction of IPS-1 with TBK-1) possibly independent of the ability of RIG-I to drive NFκB activation. This is supported by the fact that La knockdown has no effect on inflammatory gene expression downstream of RIG-I interaction whereas IFN-β expression is severely reduced.

Regarding a potential role for La in regulating assembly of RNA-binding complexes, Liu and colleagues [46] demonstrated a role for La in RNAi processing. They reported that La associated with Ago2 of the RISC complex in an RNA-dependent manner, thereby promoting RISC complex catalysis and RNAi processing. This finding is consistent with our data which show that La augments RIG-I binding to poly I.C. Importantly, deletion of the RNA-binding domain of La blocks interaction with RIG-I, underlining the RNA-binding role of La in driving RIG-I activity. In keeping with our findings that La is a positive regulator of viral-induced type I IFN, Liu *et al* showed that La could promote the anti-viral response to *flock house* virus (FHV) in Drosophilia S2 cells. Indeed, La depletion resulted in increased FHV infectivity, which supports our findings that La promotes anti-viral responses to Sendai virus in HEK 293T cells. In contrast, Bitko and colleagues demonstrated enhanced IFN-β mRNA levels and decreased viral titres upon siRNA depletion of La [43]. In addition, Domitrovich *et al.* argued a role for La as a negative regulator of IFN production in the context of HCV replication, based on a 63-67% reduction in RNA replication in the absence of La in Huh7 cells and increased IFN-β mRNA 10 hours post-RSV infection in the absence of La [44]. However, similar to our findings, both of these studies also observed an overall decrease in IFN-β production 24 hours post-

infection in La-depleted cells, suggesting that La may play a dual role in regulating anti-viral responses. Indeed, La may play a role in maintaining homeostasis in cells with respect to IFN-β production, as evidenced by the enhanced IFN-β expression in unstimulated cells depleted of La (Supplementary Figure 3c), whereas when RIG-I is activated, depletion of La results in substantial reduction in IFN-β production. This indicates that the loss of La may somehow disrupt homeostatic mechanisms to maintain appropriate IFN-β levels or indeed may inhibit viral-specific evasion mechanisms. For example, the RSV-derived NS2 protein binds to RIG-I and blocks its interaction with IPS-1, thereby preventing IRF-3 activation [65,66]. Thus the loss of La may disrupt the negative function of NS2 on this system, thereby contributing to the enhanced IFN-β observed in similar studies. Extensive studies would be required to address these questions, which are outside the scope of this manuscript.

With this work, we identify role for La in regulating IFN responses by promoting the RIG-I-mediated anti-viral response through direct association with RIG-I and enhancing RIG-I binding to its viral agonist. Importantly, our study is the first to assess the role for La in regulating type III IFN responses, with all previous studies focusing on type I IFN only. SeV infection experiments support these findings, with depletion of La resulting in increased viral infectivity and decreased type I and type III IFN responses, compared with controls. These findings highlight an important and novel role for La in the promotion of optimal type I and type III IFN responses following SeV challenge, serving to protect the host through limiting viral replication.

Methods

Materials

The SW5 monoclonal La antibody was generated by Professor Michael Bachmann at the Technical University of Dresden and was a kind gift from Dr. JS Maier-Moore [67]. All flag-tagged RIG-I plasmid constructs were a kind gift from Dr. Kate Fitzgerald (UMASS Med School, Worcester, MA). The GFP-tagged La construct was a gift from Dr. Karl Albert Brokstad (University of Bergen, Germany). Monoclonal M2 Flag antibody was purchased from Santa Cruz, pcDNA3.1 empty vector control from Invitrogen and biotin-labelled poly(I:C) from Cayla-Invivogen. A Mission® shRNA construct specific to human La, as well as a scrambled control, were purchased from Sigma.

Cell Culture

HEK 293T and HeLa cell lines were cultured in Dulbecco's Modified Essential Medium (DMEM) containing stable 2 mM L-glutamine, 10% (v/v) foetal calf serum (FCS), 100 units/ml Penicillin, 100 µg/ml Streptomycin and 100 µg/ml gentamicin. LLC-MK2 cells (ECACC 85062804) were grown in minimum Eagle's medium (MEM) containing 40 g/ml non-essential amino acids and supplemented with 10% heat inactivated FCS, 2 mM L-glutamine, 2 mg/ml sodium carbonate, 100 g/ml gentamicin and 1.25 mg/ml Fungizone. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

La Knockdown

HEK 293T cells were seeded at 5×10^4 cells per ml and transfected the following day with 500 ng of scrambled or La-specific shRNA (Sigma). Following 48 hr, cells were washed with PBS prior to viral infection as detailed below.

Viral Infection

The rescue and characterisation of recombinant Sendai virus expressing eGFP (rSeV/eGFP) was previously described [68]. The SeV Cantell strain, a wild type strain containing defective interfering particles, comes originally from Charles River Laboratories. Media was discarded and replaced with fresh pre-warmed DMEM supplemented with antibiotic only (no FCS), in order to limit cell growth. Cells were

then infected with either rSeV/eGFP or SeV *Cantell* at a multiplicity of infection (MOI) of 0.1 or 10, as indicated in figure legends. One hour post-infection, inocula were removed by discarding media and replaced with DMEM supplemented with antibiotic and 1% FCS to ensure cell survival while maintaining limited growth. At indicated time points, media was carefully removed and retained for subsequent cytokine analysis, cells were gently re-suspended in ice-cold PBS and centrifugation was carried out at 400×*g* for 5 min to pellet cells for subsequent analysis. Cells were then re-suspended in Trizol reagent for gene expression analysis or SDS sample buffer supplemented for protein expression analysis.

SeV titrations

For SeV/eGFP, a 1:10 dilution series of the sample was added to LLC-MK2 cells in MEM 1% FBS. At 24 hpi fluorescent foci were counted. The titer is calculated as fluorescent forming units (FFU) by multiplying the average number of foci by the dilution factor at a given dilution. The dilution at which the foci were counted is equal to the inverse of the exponent of the final FFU. The titer of SeV Cantell stock was determined by plaque assay as previously described [69].

Real-time polymerase chain reaction (qPCR)

RNA was extracted from cell cultures using Trizol™ (Sigma) and reverse transcribed to complementary DNA using the GoScript Reverse Transcription kit (Promega), as per manufacturer's instructions. Real-time quantitative PCR investigating gene expression was performed using primers listed in Table 1, with SYBR Green Taq ReadyMix (Sigma) according to manufacturer's recommendations. Data were analyzed using an ABI Prism 7900 system (Applied Biosystems) and were normalized to 18s RNA. Real-time PCR data were analyzed using the $2^{-\Delta\Delta ct}$ method [70].

Western blotting

To prepare whole cell lysates, cells were lysed in SDS buffer (250 mM Tris-HCl, pH 6.8, 10% SDS, 0.5% Bromophenol blue, 50% Glycerol, 50 nM DTT) and boiled at 95°C for 10 min. Equal quantities of whole cell lysates were resolved by electrophoresis on a denaturing SDS–polyacrylamide gel according to the method of Laemmli [71] and transferred to a nitrocellulose membrane. Following immunoblotting, the membrane was developed using enhanced chemiluminescent horse radish peroxidase (HRP) substrate (Millipore).

Co-immunoprecipitation

Cells were treated as described in the figure legends, lysed in EBC lysis buffer (Deionised water containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% Nonidet P40, 0.5% (w/v) sodium deoxycholate and 0.1% SDS containing 1 mM Sodium orthovanadate (Na_3VO_4), 1 mM Phenylmethylsulfonylfluoride (PMSF), 1 mM Potassium fluoride (KF),) and incubated with SW5 anti-La antibody coupled to protein A sepharose beads. Thereafter, immune complexes were washed and re-suspended in SDS sample buffer for western blot analysis.

Recombinant protein pull-downs

Following a gentle wash with ice-cold PBS, lysates were prepared by addition of EBC buffer. After sonication and centrifugation, the supernatant was incubated with 50 µl Nickel agarose beads coupled to approximately 1 µg either full length (8A) or N-terminal truncated (7A) recombinant La (Dr. J. Maier-Moore), for 2 h on rotation at 4°C. After incubation, nickel agarose was washed three times with EBC buffer by gentle inversion and centrifugation at 5,000 × *g.* Beads were then re-suspended in SDS sample buffer for western blot analysis.

Enzyme-linked Immunosorbance Assay (ELISA)

ELISAs were carried out using DuoSet® ELISA Development Kit for human CCL-5 (Rantes) or human IFN-λ1 (IL-29) (eBioscience) as per the manufacturer's instructions.

RNA Immunoprecipitation

Cells were seeded and transfected as indicated in figure legends. Cells were lysed for 20 min at 4°C on rotation in freshly prepared sterile RNA Immunoprecipitation (RIP) buffer (150 mM KCl, 25 mM Tris pH 7.4, 5 mM EDTA, 0.5 mM DTT, 0.5% NP-40) supplemented with protease inhibitors and SUPERase RNAse inhibitor (Sigma). Samples were sonicated for 15 sec, cell debris was pelleted by centrifugation and cell supernatants were transferred to fresh tubes. One µg of biotin-labelled poly(I:C) (Invivogen) was added, followed by incubation at 4°C for 1-2 h. The samples were then added to 50 µl of pre-washed UltraLink NeutrAvidin beads (Pierce) and incubated for 1 h at 4°C. The resulting immune complexes were then washed with RIPA buffer and re-suspended in 30 µl SDS sample buffer, prior to western blot analysis.

Immunofluorescence

HeLa cells were seeded at 1×10^5/well on coverslips, transfected and stimulated as indicated in figure legends. Cells were fixed and permeabilised with 4% paraformaldehyde and 0.2% (v/v) Triton X-100 in PBS. After washing, cells were blocked in PBS with 1.2% (w/v) Fish Gelatin and 100 mM glycine and then incubated at 37°C for 1 h with the primary antibody of interest at 1:100 dilution in blocking buffer, followed by detection with the appropriate fluorescently labelled secondary antibody at 1:200 dilution. Cells were mounted and nuclei stained using ProLong® Gold anti-fade reagent with DAPI. Cells were imaged using the LSM 710 System (Carl Zeiss) and analysed for co-localisation using Zen 9 software.

Statistical analysis

All data was analysed using GraphPad Prism (version 7) statistical software package, as specified. Statistical comparison between groups was carried out using tests described in figure legends. Data is graphically represented as mean +/- standard error of the mean (SEM). *P* values less than or equal to 0.05 were considered significant.

Data Availability

• No datasets were generated or analysed during the current study.

• All data generated or analysed during this study are included in this published article (and its Supplementary files).

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Author Contributions

RM was responsible for study design, data collection, data analysis and drafting of the manuscript. LB was responsible for data collection, data analysis and manuscript revision. UFP was involved in the coordination of the project, data analysis and critical revision of the manuscript. JMM supplied reagents, helpful discussion and critical review of the manuscript. CAJ was responsible for initial conception and design of the study, data analysis and critical revision of the manuscript.

Additional information

The authors declare no competing financial interests.

References

1 Yoneyama, M. *et al.* Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J Immunol* **175**, 2851-2858, (2005).

2 Kato, H. *et al.* Cell type-specific involvement of RIG-I in antiviral response. *Immunity* **23**, 19-28, (2005).

3 Loo, Y. M. *et al.* Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. *J Virol* **82**, 335-345, (2008).

4 Kawai, T. *et al.* IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nature immunology* **6**, 981-988, (2005).

5 Meylan, E. *et al.* Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* **437**, 1167-1172, (2005).

6 Kumar, H. *et al.* Essential role of IPS-1 in innate immune responses against RNA viruses. *J Exp Med* **203**, 1795-1803, (2006).

7 Sharma, S. *et al.* Triggering the interferon antiviral response through an IKK-related pathway. *Science* **300**, 1148-1151, (2003).

8 Fitzgerald, K. A. *et al.* IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. *Nature immunology* **4**, 491-496, (2003).

9 Lin, R., Mamane, Y. & Hiscott, J. Structural and Functional Analysis of Interferon Regulatory Factor 3: Localization of the Transactivation and Autoinhibitory Domains. *Molecular and Cellular Biology* **19**, 2465-2474, (1999).

10 Kumar, K. P., McBride, K. M., Weaver, B. K., Dingwall, C. & Reich, N. C. Regulated Nuclear-Cytoplasmic Localization of Interferon Regulatory Factor 3, a Subunit of Double-Stranded RNA-Activated Factor 1. *Molecular and Cellular Biology* **20**, 4159-4168, (2000).

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

12 Isaacs, A., Lindenmann, J. & Valentine, R. C. Virus interference. II. Some properties of interferon. *Proc R Soc Lond B Biol Sci* **147**, 268-273, (1957).

13 O'Shea, J. J., Notarangelo, L. D., Johnston, J. A. & Candotti, F. Advances in the understanding of cytokine signal transduction: the role of Jaks and STATs in immunoregulation and the pathogenesis of immunodeficiency. *J Clin Immunol* **17**, 431-447, (1997).

14 Horvath, C. M., Stark, G. R., Kerr, I. M. & Darnell, J. E., Jr. Interactions between STAT and non-STAT proteins in the interferon-stimulated gene factor 3 transcription complex. *Mol Cell Biol* **16**, 6957-6964, (1996).

15 Sato, M. *et al.* Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. *Immunity* **13**, 539-548, (2000).

16 Honda, K. *et al.* IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* **434**, 772-777, (2005).

17 Clemens, M. J. & Elia, A. The double-stranded RNA-dependent protein kinase PKR: structure and function. *J Interferon Cytokine Res* **17**, 503-524, (1997).

18 Stark, G. R., Kerr, I. M., Williams, B. R. G., Silverman, R. H. & Schreiber, R. D. How cells respond to interferons. *Annual Review of Biochemistry* **67**, 227-264, (1998).

19 Le Bon, A. *et al.* Direct Stimulation of T Cells by Type I IFN Enhances the CD8+ T Cell Response during Cross-Priming. *The Journal of Immunology* **176**, 4682-4689, (2006).
20 Le Bon, A. *et al.* Cutting Edge: Enhancement of Antibody Responses Through Direct Stimulation of B and T Cells by Type I IFN. *The Journal of Immunology* **176**, 2074-2078, (2006).
21 Trinchieri, G., Santoli, D., Granato, D. & Perussia, B. Antagonistic effects of interferons on the cytotoxicity mediated by natural killer cells. *Fed Proc* **40**, 2705-2710, (1981).
22 Salazar-Mather, T. P., Ishikawa, R. & Biron, C. A. NK cell trafficking and cytokine expression in splenic compartments after IFN induction and viral infection. *J Immunol* **157**, 3054-3064, (1996).
23 Le Bon, A. *et al.* Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. *Nature immunology* **4**, 1009-1015, (2003).
24 Le Bon, A. *et al.* Type i interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. *Immunity* **14**, 461-470, (2001).
25 Zhou, L. *et al.* Activation of toll-like receptor-3 induces interferon-lambda expression in human neuronal cells. *Neuroscience* **159**, 629-637, (2009).
26 Onoguchi, K. *et al.* Viral infections activate types I and III interferon genes through a common mechanism. *J Biol Chem* **282**, 7576-7581, (2007).
27 Kotenko, S. V. *et al.* IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nature immunology* **4**, 69-77, (2003).
28 Sheppard, P. *et al.* IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nature immunology* **4**, 63-68, (2003).
29 Sommereyns, C., Paul, S., Staeheli, P. & Michiels, T. IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. *PLoS Pathog* **4**, e1000017, (2008).
30 Dumoutier, L., Lejeune, D., Hor, S., Fickenscher, H. & Renauld, J. C. Cloning of a new type II cytokine receptor activating signal transducer and activator of transcription (STAT)1, STAT2 and STAT3. *Biochem J* **370**, 391-396, (2003).
31 Chiu, Y. H., Macmillan, J. B. & Chen, Z. J. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* **138**, 576-591, (2009).
32 Ablasser, A. *et al.* RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nature immunology* **10**, 1065-1072, (2009).
33 Maraia, R. J. Transcription termination factor La is also an initiation factor for RNA polymerase III. *Proc Natl Acad Sci U S A* **93**, 3383-3387, (1996).
34 Gottlieb, E. & Steitz, J. A. The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription in vitro. *EMBO J* **8**, 841-850, (1989).
35 Gottlieb, E. & Steitz, J. A. Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. *EMBO J* **8**, 851-861, (1989).
36 Stefano, J. E. Purified lupus antigen La recognizes an oligouridylate stretch common to the 3' termini of RNA polymerase III transcripts. *Cell* **36**, 145-154, (1984).

37 Maraia, R. J. & Intine, R. V. A. Recognition of Nascent RNA by the Human La Antigen: Conserved and Divergent Features of Structure and Function. *Mol. Cell. Biol.* **21**, 367-379, (2001).
38 Fairley, J. A. *et al.* Human La is found at RNA polymerase III-transcribed genes in vivo. *Proc Natl Acad Sci U S A* **102**, 18350-18355, (2005).
39 Kurilla, M. G. & Keene, J. D. The leader RNA of vesicular stomatitis virus is bound by a cellular protein reactive with anti-La lupus antibodies. *Cell* **34**, 837-845, (1983).
40 Kurilla, M. G., Cabradilla, C. D., Holloway, B. P. & Keene, J. D. Nucleotide sequence and host La protein interactions of rabies virus leader RNA. *J Virol* **50**, 773-778, (1984).
41 Wilusz, J. & Keene, J. D. Interactions of plus and minus strand leader RNAs of the New Jersey serotype of vesicular stomatitis virus with the cellular La protein. *Virology* **135**, 65-73, (1984).
42 Wilusz, J., Kurilla, M. G. & Keene, J. D. A host protein (La) binds to a unique species of minus-sense leader RNA during replication of vesicular stomatitis virus. *Proc Natl Acad Sci U S A* **80**, 5827-5831, (1983).
43 Bitko, V., Musiyenko, A., Bayfield, M. A., Maraia, R. J. & Barik, S. Cellular La protein shields nonsegmented negative-strand RNA viral leader RNA from RIG-I and enhances virus growth by diverse mechanisms. *J Virol* **82**, 7977-7987, (2008).
44 Domitrovich, A. M., Diebel, K. W., Ali, N., Sarker, S. & Siddiqui, A. Role of La autoantigen and polypyrimidine tract-binding protein in HCV replication. *Virology* **335**, 72-86, (2005).
45 Costa-Mattioli, M., Svitkin, Y. & Sonenberg, N. La Autoantigen Is Necessary for Optimal Function of the Poliovirus and Hepatitis C Virus Internal Ribosome Entry Site In Vivo and In Vitro. *Mol. Cell. Biol.* **24**, 6861-6870, (2004).
46 Liu, Y. *et al.* Autoantigen La Promotes Efficient RNAi, Antiviral Response, and Transposon Silencing by Facilitating Multiple-Turnover RISC Catalysis. *Mol Cell* **44**, 502-508, (2011).
47 Garcin, D., Itoh, M. & Kolakofsky, D. A Point Mutation in the Sendai Virus Accessory C Proteins Attenuates Virulence for Mice, but Not Virus Growth in Cell Culture. *Virology* **238**, 424-431, (1997).
48 Itoh, M., Shibuta, H. & Homma, M. Single amino acid substitution of Sendai virus at the cleavage site of the fusion protein confers trypsin resistance. *J Gen Virol* **68 (Pt 11)**, 2939-2944, (1987).
49 Yamaguchi, R., Iwai, H. & Ueda, K. Variation of virulence and other properties among Sendai virus strains. *Microbiol Immunol* **32**, 235-240, (1988).
50 Mochizuki, Y., Tashiro, M. & Homma, M. Pneumopathogenicity in mice of a Sendai virus mutant, TSrev-58, is accompanied by in vitro activation with trypsin. *J Virol* **62**, 3040-3042, (1988).
51 Lopez, C. B., Garcia-Sastre, A., Williams, B. R. & Moran, T. M. Type I interferon induction pathway, but not released interferon, participates in the maturation of dendritic cells induced by negative-strand RNA viruses. *J Infect Dis* **187**, 1126-1136, (2003).
52 Lopez, C. B. *et al.* TLR-independent induction of dendritic cell maturation and adaptive immunity by negative-strand RNA viruses. *J Immunol* **173**, 6882-6889, (2004).
53 Baum, A. & García-Sastre, A. Differential recognition of viral RNA by RIG-I. *Virulence* **2**, 166-169, (2011).

54 Baum, A., Sachidanandam, R. & García-Sastre, A. Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 16303-16308, (2010).
55 Melchjorsen, J. *et al.* Activation of innate defense against a paramyxovirus is mediated by RIG-I and TLR7 and TLR8 in a cell-type-specific manner. *J Virol* **79**, 12944-12951, (2005).
56 Meerovitch, K. *et al.* La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. *J Virol* **67**, 3798-3807, (1993).
57 Brierley, M. M., Marchington, K. L., Jurisica, I. & Fish, E. N. Identification of GAS-dependent interferon-sensitive target genes whose transcription is STAT2-dependent but ISGF3-independent. *FEBS J* **273**, 1569-1581, (2006).
58 Ank, N. *et al.* Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. *J Virol* **80**, 4501-4509, (2006).
59 Coccia, E. M. *et al.* Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur J Immunol* **34**, 796-805, (2004).
60 Gack, M. U., Nistal-Villan, E., Inn, K.-S., Garcia-Sastre, A. & Jung, J. U. Phosphorylation-Mediated Negative Regulation of RIG-I Antiviral Activity. *J. Virol.* **84**, 3220-3229, (2010).
61 Gao, D. *et al.* REUL Is a Novel E3 Ubiquitin Ligase and Stimulator of Retinoic-Acid-Inducible Gene-I. *PLoS ONE* **4**, e5760, (2009).
62 Oshiumi, H., Matsumoto, M., Hatakeyama, S. & Seya, T. Riplet/RNF135, a RING finger protein, ubiquitinates RIG-I to promote interferon-beta induction during the early phase of viral infection. *J Biol Chem* **284**, 807-817, (2009).
63 Sun, Z., Ren, H., Liu, Y., Teeling, J. L. & Gu, J. Phosphorylation of RIG-I by Casein Kinase II Inhibits Its Antiviral Response. *J. Virol.* **85**, 1036-1047, (2011).
64 Poeck, H. *et al.* Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nature immunology* **11**, 63-69, (2010).
65 Ling, Z., Tran, K. C. & Teng, M. N. Human respiratory syncytial virus nonstructural protein NS2 antagonizes the activation of beta interferon transcription by interacting with RIG-I. *J Virol* **83**, 3734-3742, (2009).
66 Goswami, R. *et al.* Viral degradasome hijacks mitochondria to suppress innate immunity. *Cell Res* **23**, 1025-1042, (2013).
67 Pruijn, G. J., Thijssen, J. P., Smith, P. R., Williams, D. G. & Van Venrooij, W. J. Anti-La monoclonal antibodies recognizing epitopes within the RNA-binding domain of the La protein show differential capacities to immunoprecipitate RNA-associated La protein. *Eur J Biochem* **232**, 611-619, (1995).
68 Villenave, R. *et al.* Cytopathogenesis of Sendai Virus in Well-Differentiated Primary Pediatric Bronchial Epithelial Cells. *Journal of Virology* **84**, 11718-11728, (2010).
69 Touzelet, O. *et al.* De novo generation of a non-segmented negative strand RNA virus with a bicistronic gene. *Virus research* **140**, 40-48, (2009).
70 Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408, (2001).

71 Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685, (1970).

Figure legends

Figure 1: Depletion of La in HEK 293T results in an increase in Sendai viral infection efficiency. HEK 293T cells were transfected with 500 ng of La-specific or scrambled Mission® shRNA (Sigma) for 48 h. Cells were then infected with rSeV/eGFP at an MOI of 0.1. (a) GFP positivity was visualised using a Nikon Eclipse TE2000-U and a Hamamatsu ORCA ER camera. (b) % monolayer GFP-positive analysis was carried out using Image J software. (c) rSeV/eGFP titrations (FFU/mL) were carried out on LLC-MK2 cells. Areas under the curves were calculated and compared. Results are from two independent experiments carried out in duplicate. (d) 48h post transfection, a well from each condition (Scrambled or La) was trypsinised, following which a trypan blue cell count was performed to determine the number of viable cells prior to infection. Data shown is combined average cell counts from two independent experiments.

Figure 2: IFN-β IFN-λ and ISG mRNA expression is attenuated in La-depleted cells following SeV infection. HEK 293T cells were transfected with 500 ng of either La-specific or scrambled Mission® shRNA (Sigma) for 48 h after which they were infected with SeV *Cantell* (a-d) or rSeV/eGFP (e-h) at an MOI of 10 and incubated for the indicated time points. IFN-β (a, e), IFN-λ1 (b, f), CXCL11 (c, g), CXCL10 (d, h) expression was determined by RT-qPCR. Data shown are a representative of three independent experiments in each case. *$p<0.05$, **$p< 0.01$, ***$p<0.001$ and ***$p<0.0001$, as determined by unpaired *t*-test, comparing scrambled to La shRNA at each time point.

Figure 3: CCL5 & IFN-λ1 release is decreased in La-depleted cells following SeV infection. HEK 293T cells were transfected with 500 ng of either La-specific or scrambled Mission® shRNA (Sigma) for 48 h after which they were infected with SeV *Cantell* (a-b) or rSeV/eGFP (c-d) at an MOI of 10. CCL5 (a, c) and IFN-λ1 (b, d)

cytokine release was determined by ELISA. IL-8 (e), IL-6 (f) and TNF-α (g) was measured from cells supernatants using a multi-plex human pro-inflammatory 7-spot assay (MSD). Data shown is the combined average of three independent experiments. *p<0.05, **p<0.01 and ****p<0.0001, as determined by unpaired *t*-tests, comparing scrambled to La shRNA at each time point.

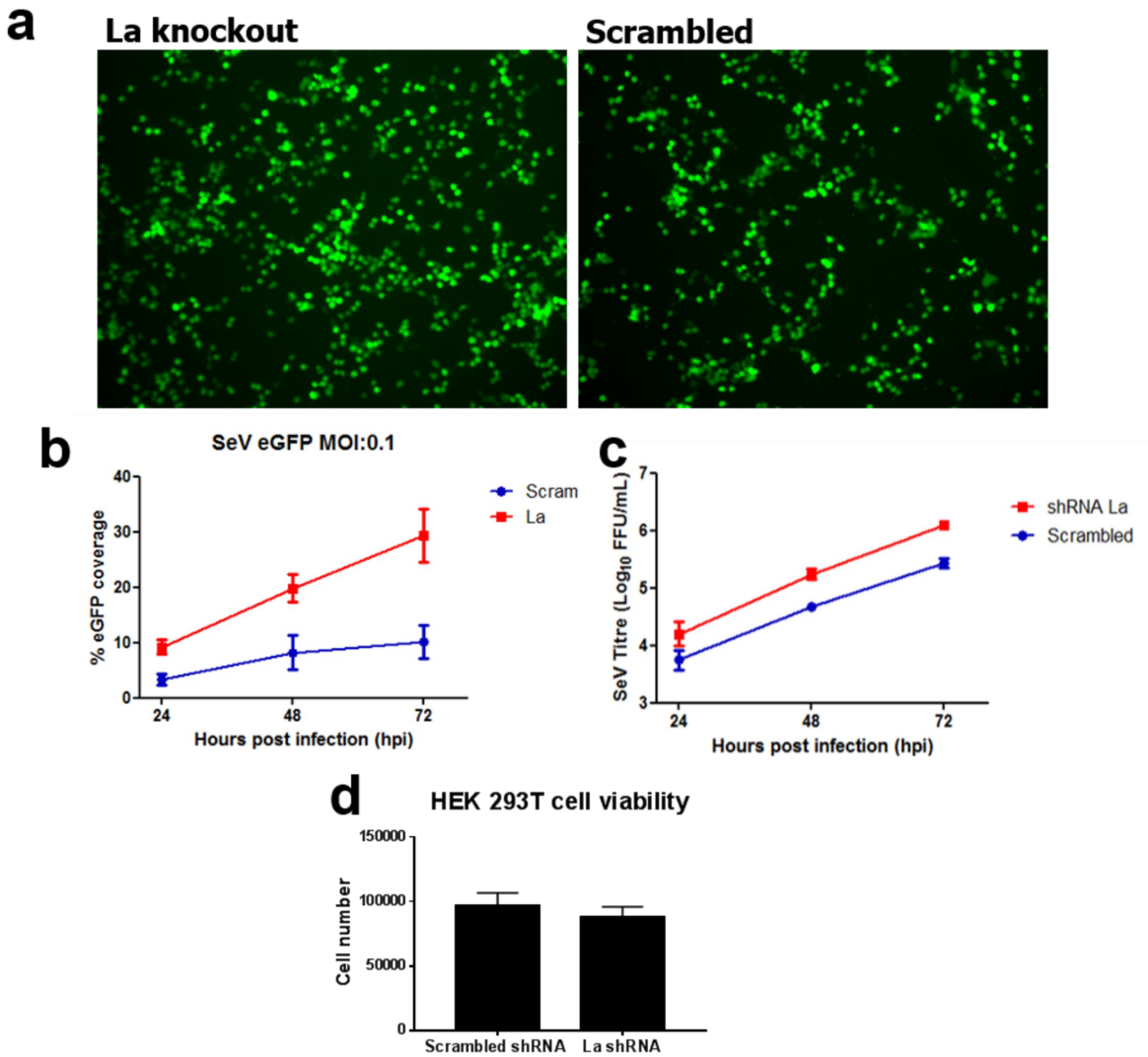
Figure 4: La enhances RIG-I binding to RNA ligand and interacts with RIG-I following 5'ppp-dsRNA stimulation. (a) HEK 293T cells were transfected with 4 µg EV or 2 µg FLAG-tagged RIG-I with increasing FLAG-tagged La, as indicated. Analysis of the ability of FLAG-tagged La or RIG-I to bind biotinylated poly(I:C) was assessed by western blotting with anti-FLAG antibody. Expression of FLAG-tagged or RIG-I constructs in whole cell lysates was determined by western blotting with either anti-La or anti-RIG-I antibodies, as appropriate. (b) HEK 293T cells were transfected as indicated and stimulated for 1, 3, or 6 h with 1 µg 5'ppp-dsRNA (Invivogen). Following immunoprecipitation of La-containing complexes with a La-specific antibody, the ability of over-expressed RIG-I to interact with endogenous La was determined by western blotting using anti-FLAG antibody. (c) HeLa cells were seeded on UV-irradiated coverslips, transfected with 2 µg of GFP-tagged La and 2 µg FLAG-tagged RIG-I, following which they were stimulated with 1 µg 5'ppp-dsRNA for 6 h. Immunostaining with anti-RIG-I antibody indicates that La and RIG-I co-localise following stimulation with 5'ppp-dsRNA. Both images are at 63X magnification. (d) Recombinant La was incubated with lysates prepared from HEK 293T cells overexpressing FLAG-tagged RIG-I, FLAG-tagged RIG-I-CARD, or FLAG-tagged RIG-I-Helicase (Heli), as indicated. The ability of RIG-I and La to interact was analysed by western blotting. In all cases images are representative of three independent experiments.

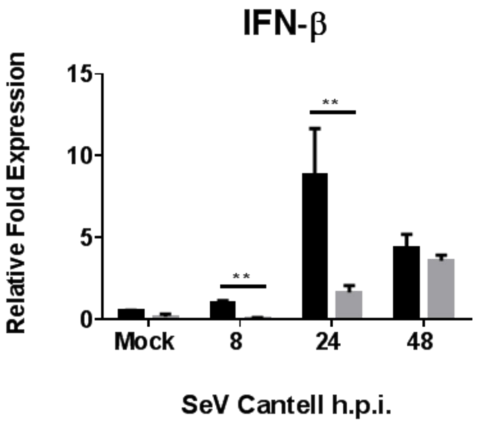
Table 1: Human primers used in this study;

Primer Name	Primer Sequence	Product Size(bp)
La sense	GAAGGAGAGGTGGAAAAAG	372
La anti-sense	AAGCCCCGCAAACAAAAG	
IFN-β sense	CTAGCACTGGCTGGAATGAGA	217

IFN-β anti-sense	CTGACTATGGTCCAGGCACA	
18S sense	TTGACGGAAGGGCACCACCA	131
18S anti-sense	GCACCACCACCCACGGAATCG	
IFN-λ1 sense	GGACGCCTTGGAAGAGTCACT	84
IFN-λ1 anti-sense	AGAAGCCTCAGGTCCCAATTC	
CXCL-10 sense	GGAAGCACTGCATCGATTTTG	519
CXCL-10 anti-sense	CAGAATCGAAGGCCATCAAGA	
CXCL-11 sense	GCCTTGGCTGTGATATTGTGTG	686
CXCL-11 anti-sense	CACTTTCACTGCTTTTACCCCAG	

1
2


a
La knockout
Scrambled
b
SeV eGFP MOI:0.1
% eGFP coverage
Hours post infection (hpi)
Scram
La
c
SeV Titre (Log10 FFU/mL)
Hours post infection (hpi)
shRNA La
Scrambled
d
HEK 293T cell viability
Cell number
Scrambled shRNA
La shRNA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**
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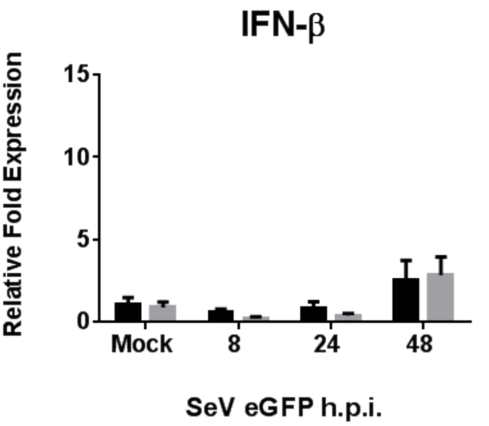
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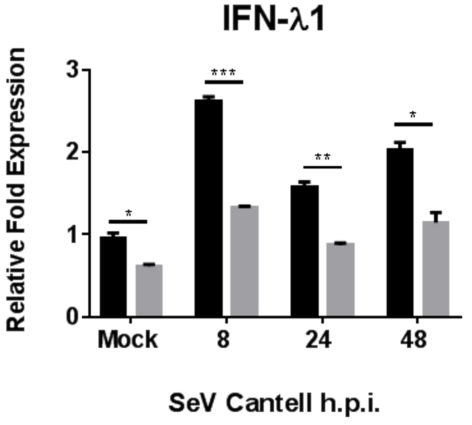
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*
***
**
*
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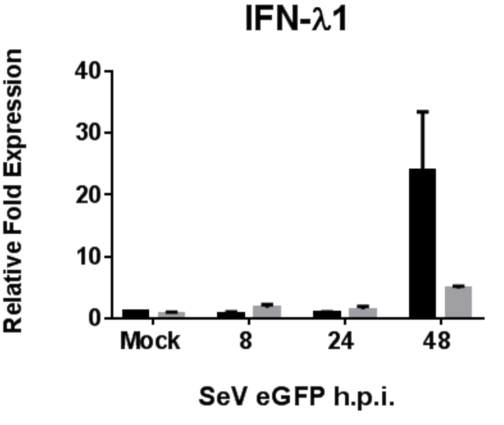
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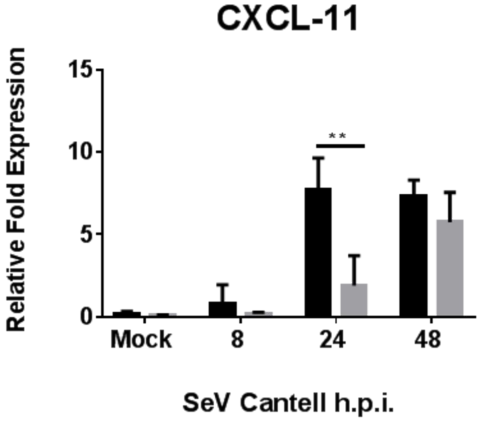
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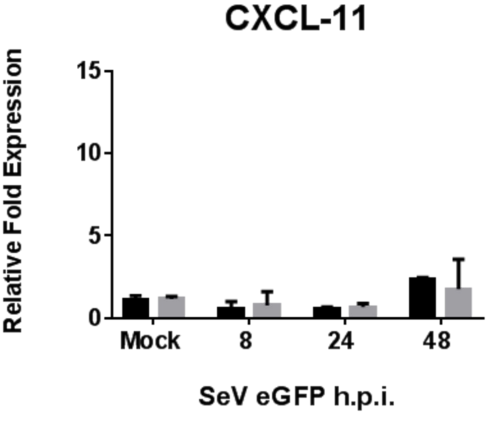
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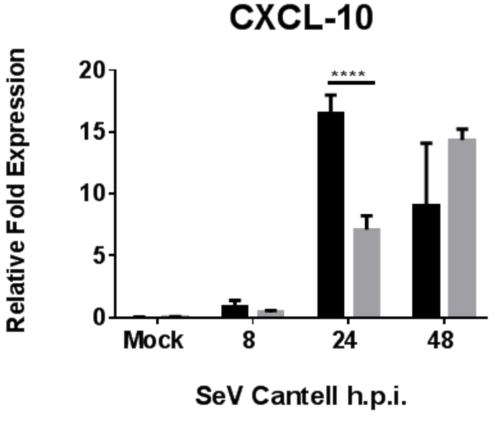
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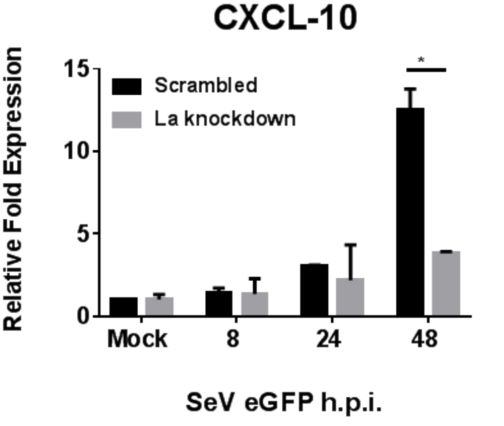
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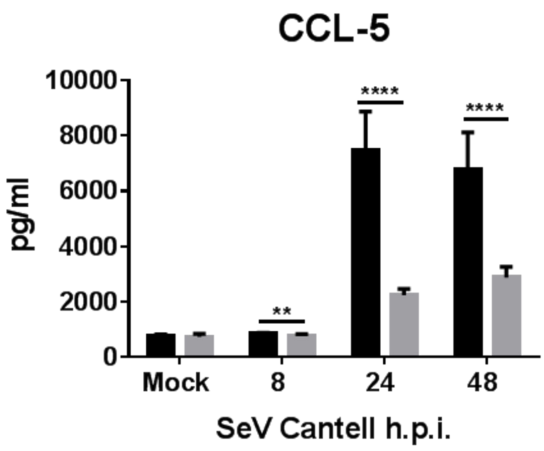
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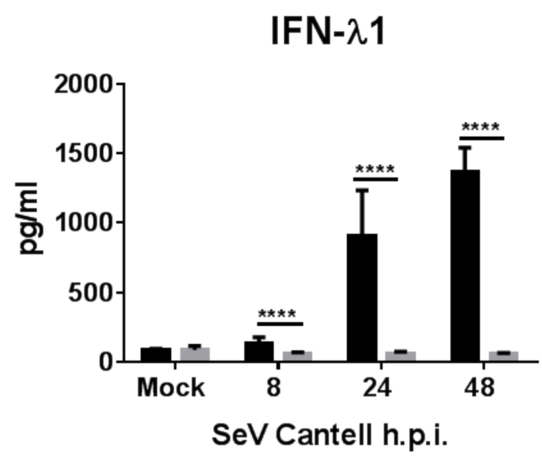
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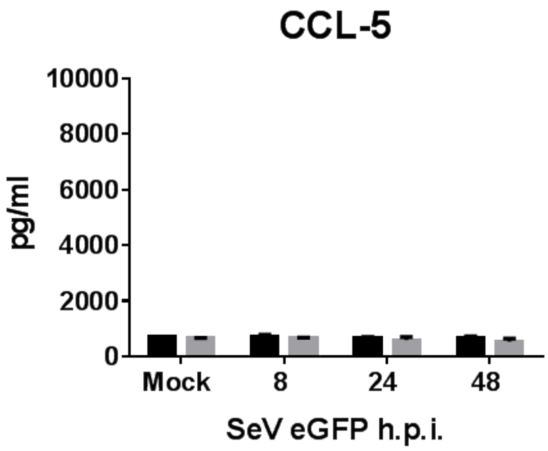
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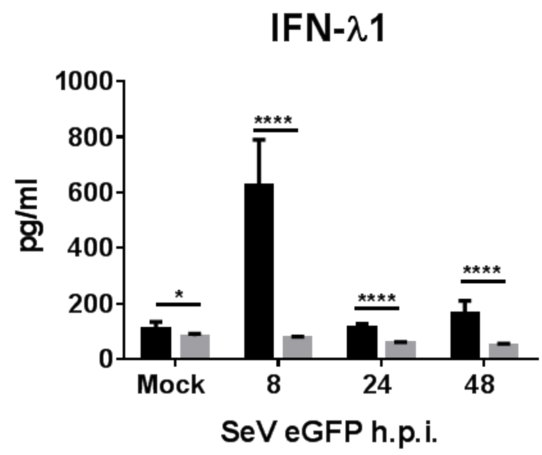
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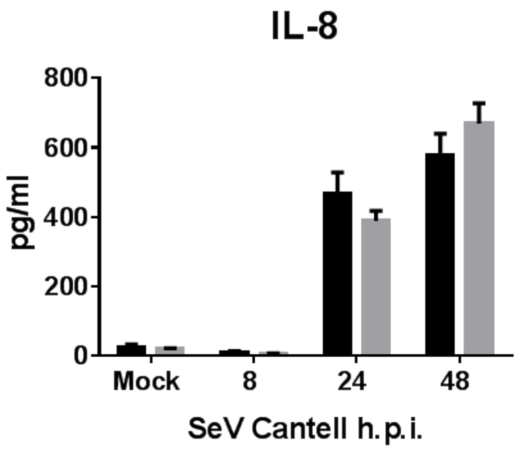
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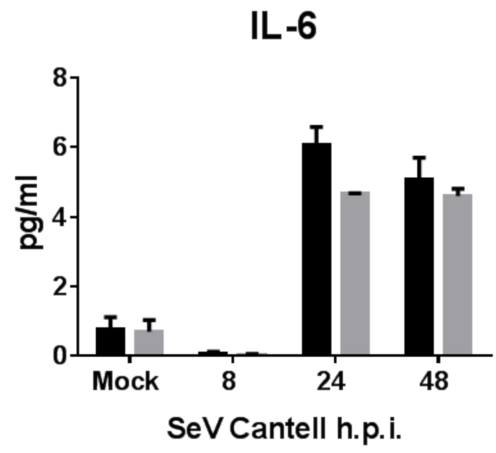
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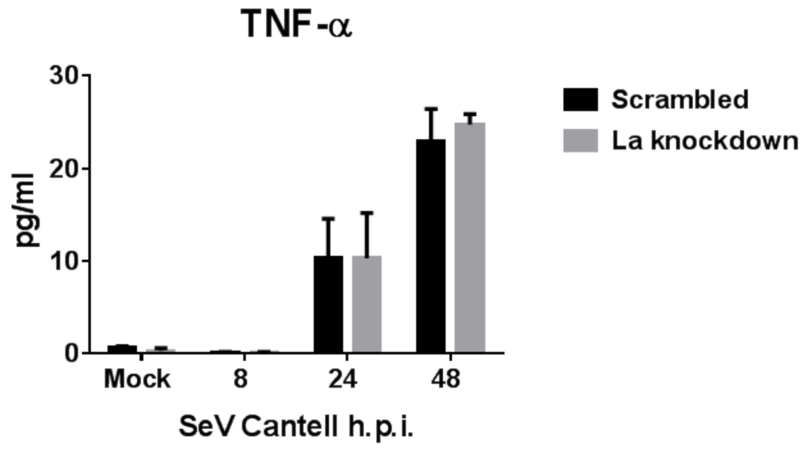
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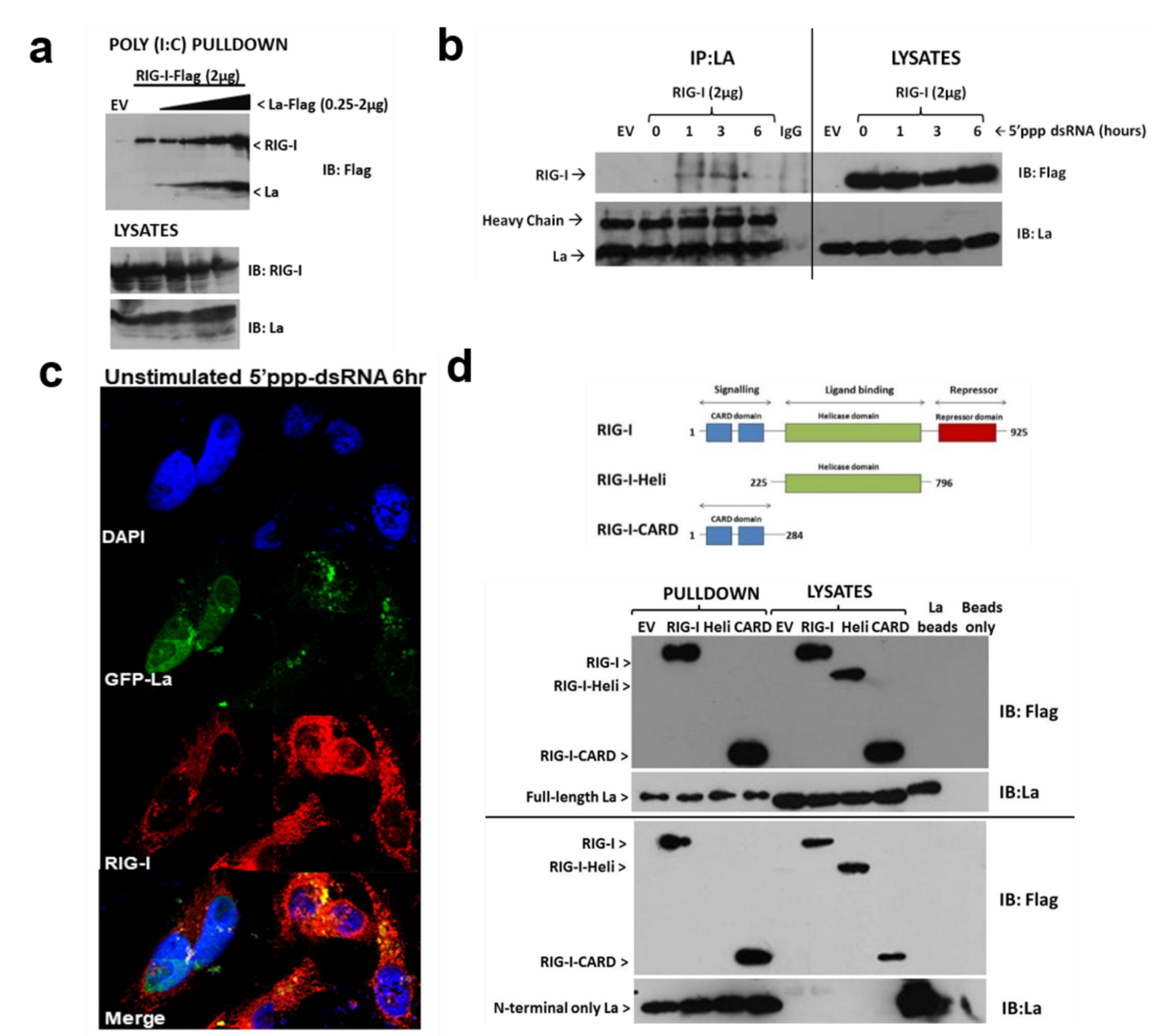
Scrambled
La knockdown


a
POLY (I:C) PULLDOWN
RIG-I-Flag (2µg)
EV
< La-Flag (0.25-2µg)
< RIG-I
IB: Flag
< La
LYSATES
IB: RIG-I
IB: La
b
IP:LA
LYSATES
RIG-I (2µg)
RIG-I (2µg)
EV 0 1 3 6 IgG
EV 0 1 3 6 ← 5'ppp dsRNA (hours)
RIG-I →
Heavy Chain →
La →
IB: Flag
IB: La
c
Unstimulated 5'ppp-dsRNA 6hr
DAPI
GFP-La
RIG-I
Merge
d
Signalling
Ligand binding
Repressor
CARD domain
Helicase domain
Repressor domain
RIG-I
1
925
Helicase domain
RIG-I-Heli
225
796
CARD domain
RIG-I-CARD
1
284
PULLDOWN
LYSATES
La beads
Beads only
EV RIG-I Heli CARD EV RIG-I Heli CARD
RIG-I >
RIG-I-Heli >
RIG-I-CARD >
IB: Flag
Full-length La >
IB:La
RIG-I >
RIG-I-Heli >
RIG-I-CARD >
IB: Flag
N-terminal only La >
IB:La